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Efficient synthesis of a 3,6-branched mannose hepta- and octasaccharide

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Abstract

α-D-Man*p*-(1→3)-[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→6)]-D-Man*p* and α-D-Man*p*-(1→2)-α-D-Man*p*-(1→3)-[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→6)]-D-Man*p*, were synthesized as their methyl glycosides in a regio- and stereoselective way.

Keywords: Oligosaccharide; Mannose; Synthesis

1. Introduction

Asparagine-linked glycoprotein oligosaccharides play a vital role in fundamental biological processes such as cell differentiation, malignant transformation, viral, bacterial and parasitic infections and protein transportations.[1] They are usually divided into three major subgroups, i.e., high-mannose type, complex type and hybrid type, and each of them may consist of diverse structures in living cells.[2] A 3,6-branched mannose nonasaccharide of the N-glycan is expressed on the HIV gp 120 glycoprotein,[3] whereas a 3,6-branched mannose octasaccharide of the N-glycan exists in the adhesion domain of human CD2 (Scheme 1).[4]

As an ongoing project on the research on structure–bioactivity relationship among oligosaccharides, we have synthesized (Scheme 2) a variety of naturally occurring mannose oligosaccharides, such as (1→2)-branched (1→6)-linked mannans[5] and 3,6-branched mannans,[6] and also a series of artificially designed oligosaccharides.[7] It was interesting to find that some synthetic glucose oligosaccharides showed excellent bioactivity compared to the natural ones.[7] In continuation of our studies on oligosaccharides, we present herein syntheses of the mannose hepta- and octasaccharide analogues of the N-glycan mannans.

2. Results and discussion

Our previous communication[8] described an efficient synthesis of the hexasaccharide repeating unit of the exopolysaccharide (GXM) from *Cryptococcus neoformans* serovar A with 4,6-*O*-isopropylidenated mannose derivatives as the key synthons. We found that the 4,6-*O*-isopropylidenated mannose derivatives were also appropriate intermediates in the present research for construction of the required mannose hepta- and octasaccharides. As outlined in Scheme 3, 1,2,3-tri-*O*-acetyl-4,6-*O*-isopropylidene-D-mannopyranose (**1**), obtained from selective 4,6-*O*-isopropylidenation of mannose[9] with 2-methoxypropene, followed by acetylation, was chosen as the starting material. Selective 1-*O*-deacetylation with ammonia in THF–MeOH, followed by trichloroacetimidation[10] with trichloroacetonitrile in the presence of potassium carbonate, gave the donor 2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-α-D-mannopyranosyl trichloroacetimidate (**2**). Condensation of **2** with the acceptor, methyl 4,6-*O*-isopropylidene-α-D-mannopyranoside (**3**), selectively afforded the (1→3)-linked disaccharide **4** (74%). The regioselectivity of the coupling was confirmed by acetylation of **4** to give **5**, and

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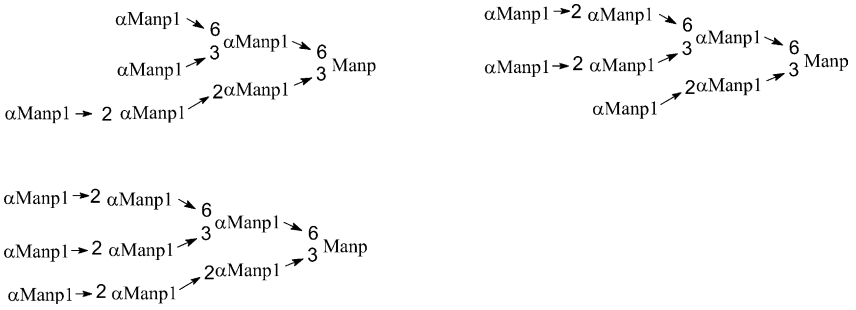


Scheme 1. Examples of naturally occurring N-glycans Man7, Man8, and Man9.

the ^{1}H NMR spectrum of **5** showed a newly emerged downfield doublet of doublets at δ 5.34 ppm with $J_{1,2} =$ 1.5 and $J_{2,3} = 3.0$ Hz for H-2 compared to that of **4**. Deacetylation of **4** or **5** in a solution of ammonia in methanol furnished the disaccharide triol acceptor **6** in high yield. Again, 3-*O*-selective glycosylation of **6** with the donor, 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate[11] (**7**), yielded (1→3)-linked tetrasaccharide **8** (71%). The 3-*O*-glycosylation was identified by acetylation to give **9**, and the ^{1}H NMR spectrum of **9** showed three characteristic signals at δ 5.66 ppm with $J_{1,2} = 0.7$ and $J_{2,3} = 2.7$ Hz, 5.41 ppm with $J_{1,2} = 0.8$ and $J_{2,3} = 3.0$ Hz, and 5.11 ppm with $J_{1,2} = 1.0$ and $J_{2,3} = 3.0$ Hz for H‴-2, H′-2, and H-2, respectively. Removal of the *O*-isopropylidene groups was smoothly carried out with 90% TFA giving the tetrasaccharide acceptor **10** (83%). Selective 6-*O*-glycosylation[12] of **10** with the disaccharide donor **7** afforded octasaccharide **11** (61%), and subsequent acetylation gave **12**. The ^{1}H NMR spectrum of **12** showed eight-proton signals for H-4 at the region of δ 5.2–6.2, indicating the 6-*O*-selective glycosylation. Deacylation of **12** in a saturated solution of ammonia in methanol furnished the target octasaccharide. The heptasaccharide **20** was obtained in a similar way except that, instead of the disaccharide donor **7**, 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**14**) was used to couple **6**. The rest of the steps were exactly the same as those used for the preparation of **13** from **8**. Bioactivity tests for **13** and **20** are in progress.

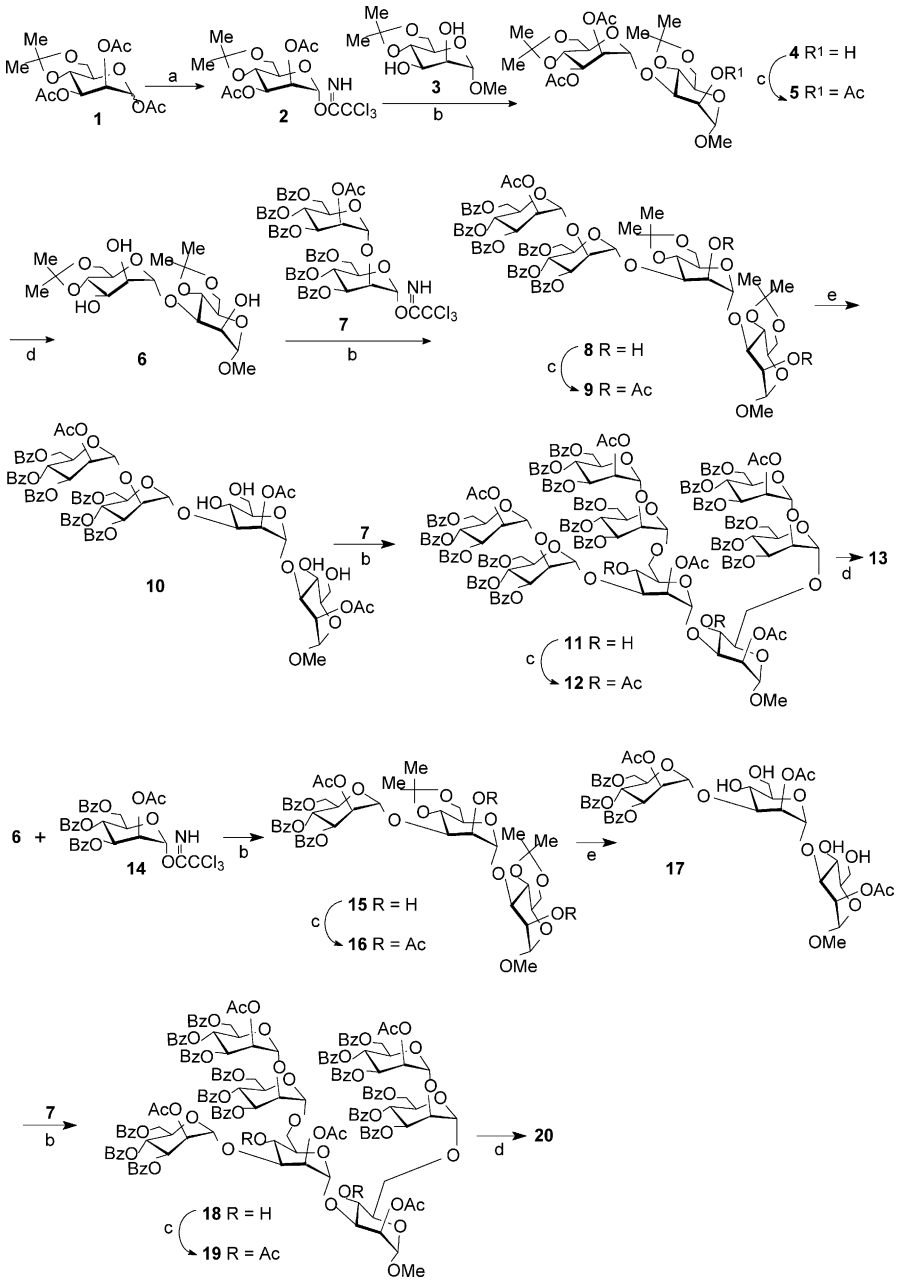
In summary, 3,6-branched mannose hepta- and octasaccharide analogues of the N-glycan mannans were synthesized in a highly regio- and steroselective way with a quite simple procedure. Large-scale preparations should be possible with this method.

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. Individual resonances could not identified with the specific sugar residues. Chemical shifts are expressed in ppm downfield from the Me_4Si absorption. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the ESI mode. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) sulfuric acid in methanol 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

Scheme 2. Synthetic mannose hepta- and octasaccharides.



Scheme 3. Reagents and conditions: (a) (i) THF–CH_3OH, 1.5 N NH_3, rt, 2–3 h; (ii) CH_2Cl_2, CCl_3CN (2.0 equiv), K_2CO_3 (2.0 equiv), rt, 12 h, 71%. (b) TMSOTf (0.05–0.10 equiv), 4 Å MS, CH_2Cl_2, −20 °C, 2–4 h (74% for **4**, 71% for **8**, 61% for **11**, 67% for **15**, 75% for **18**). (c) Ac_2O–Pyridine, 81–93%. (d) CH_3OH saturated with ammonia, rt, 12–36 h, 73–96%. (e) 90% TFA, rt, 2 h, 83% for **10**, 80% for **17**.

flow rate of 1–4 mL/min. Solutions were concentrated at a temperature <60 °C under diminished pressure.

3.2. 2,3-Di-*O*-acetyl-4,6-*O*-isopropylidene-α-D-mannopyranosyl trichloroacetimidate (2)

Compound **1** (6.90 g, 20.0 mmol) was dissolved in 1 M solution of ammonia–methanol (100 mL) and stirred for 4 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was concentrated and dried under high vacuum giving a white foamy solid. This foamy solid was dissolved in dry dichloromethane (50 mL), then trichloroacetonitrile (6.3 mL, 30 mmol) and 1,8-diazabicyclo[5.4.0]undecene (DBU) (0.50 mL, 4.04 mmol) was added subsequently. The reaction mixture was stirred under nitrogen for 3 h and then concentrated. The residue was purified by chromatography (4:1

petroleum ether–EtOAc) to give **2** (7.40 g, 82.2%) as a syrup: $[\alpha]_D$ +42.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1 H, CN*H*CCL$_3$), 6.17 (s, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.50 (dd, 1 H, $J_{1,2}$1.6 Hz, $J_{2,3}$ 3.4 Hz, H-2), 5.30 (dd, 1 H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 10.3 Hz, H-3), 4.11 (dd, 1 H, $J_{3,4}$ 10.3 Hz, $J_{4,5}$ 10.0 Hz, H-4), 3.98–3.85 (m, 3 H), 2.20 (s, 3 H, C*H*$_3$CO), 2.04 (s, 3 H, C*H*$_3$CO), 1.55 (s, 3 H, isopropylidene), 1.42 (s, 3 H, isopropylidene). Anal. Calcd for $C_{15}H_{20}Cl_3NO_8$: C, 40.12; H, 4.49. Found: C, 40.40; H, 4.73.

3.3. Methyl 2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-α-D-mannopyranosyl-(1→3)-4,6-*O*-isopropylidene-α-D-mannopyranoside (4)

To a cooled solution (−20 °C) of **3** (1.17 g, 5.0 mmol) and **2** (2.46 g, 5.5 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 (two 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 disaccharide **4** (1.93 g, 74.2%) as a syrup: $[\alpha]_D$ + 43.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.36 (dd, 1 H, $J_{1,2}$ 1.0 Hz, $J_{2,3}$ 3.2 Hz, H-2′), 5.21 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.9 Hz, H-3′), 5.20 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1′), 4.73 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.14 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 4.06–4.02 (m, 2 H), 3.95 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.0 Hz, H-3), 3.87–3.76 (m, 5 H), 3.60 (m, 1 H, H-5), 3.36 (s, 3 H, OC*H*$_3$), 2.17 (s, 3 H, C*H*$_3$CO), 2.15 (s, 3 H, C*H*$_3$CO), 1.52 (s, 6 H, isopropylidene), 1.40 (s, 3 H, isopropylidene), 1.29 (s, 3 H, isopropylidene); ^{13}C NMR (100 MHz, $CDCl_3$): 170.0, 169.6 (2 C, 2 *C*OCH$_3$), 101.4 (1 C, Me$_2$*C*O$_2$), 100.1, 100.0 (2 C, 2 C-1), 99.5 (1 C, Me$_2$*C*O$_2$), 74.1, 71.6, 71.1, 70.0, 68.9, 68.6, 65.4, 64.2, 62.4, 62.2 (10 C, C2 ~ 6, C2′ ~ 6′), 54.9 (O*C*H$_3$), 29.1, 29.0, 20.8, 20.7 [4 C, 2 (*C*H$_3$)$_2$CO$_2$], 19.3, 19.1 (2 C, 2 CO*C*H$_3$). Anal. Calcd for $C_{23}H_{36}O_{13}$: C, 53.07; H, 6.97. Found: C, 53.32; H, 6.70.

3.4. Methyl 2,3-di-*O*-acetyl-4,6-*O*-isopropylidene-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-*O*-isopropylidene-α-D-mannopyranoside (5)

To a solution of **4** (104 mg, 0.2 mmol) in pyridine (5 mL) was added acetic anhydride (2.0 mL, 2 mmol). The reaction mixture was stirred at room temperature (rt) for 12 h, at the end of which time TLC (3:1 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 (3:1 petroleum ether–EtOAc) to give compound **5** (100 mg, 89.3%) as a foamy solid: $[\alpha]_D$ +38.9° (*c* 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.37 (dd, 1 H, $J_{1,2}$ 1.2 Hz, $J_{2,3}$ 3.3 Hz, H-2′), 5.34 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.0 Hz, H-2), 5.26 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.8 Hz, H-3′), 5.12 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1′), 4.70 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.20 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 4.11–3.87 (m, 8 H), 3.66 (m, 1 H, H-5), 3.34 (s, 3 H, OC*H*$_3$), 2.21 (s, 3 H, C*H*$_3$CO), 2.19 (s, 3 H, C*H*$_3$CO), 2.14 (s, 3 H, C*H*$_3$CO), 1.55 (s, 3 H, isopropylidene), 1.53 (s, 3 H, isopropylidene),1.38 (s, 3 H, isopropylidene), 1.31 (s, 3 H, isopropylidene). Anal. Calcd for $C_{25}H_{38}O_{14}$: C, 53.37; H, 6.81. Found: C, 53.41; H, 6.62.

3.5. Methyl 4,6-*O*-isopropylidene-α-D-mannopyranosyl-(1→3)-4,6-*O*-isopropylidene-α-D-mannopyranoside (6)

Disaccharide **4** (2.60 mg, 5.0 mmol) was dissolved in a satd solution of ammonia in methanol (25 mL). After 2 h at rt, the reaction mixture was concentrated, and the residue was purified by flash column chromatography on a silica gel column (EtOAc) to give compound **6** (2.10 g, 96.3%) as a foamy solid: $[\alpha]_D$ +90.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.21 (s, 1 H, H-1′), 4.69 (s, 1 H, H-1), 3.34 (s, 3 H, C*H*$_3$CO), 1.52 (s, 3 H, isopropylidene), 1.49 (s, 3 H, isopropylidene), 1.43 (s, 3 H, isopropylidene), 1.37 (s, 3 H, isopropylidene); ^{13}C NMR (100 MHz, $CDCl_3$): δ 101.4, 101.3 (2 C, 2 Me$_2$*C*O$_2$), 100.2, 99.8 (2 C, 2 C-1), 73.6, 71.4, 71.3, 71.2, 70.9, 68.9, 64.7, 64.3, 62.3, 62.2 (10 C, C2 ~ 6, C2′ ~ 6′), 54.9 (O*C*H$_3$), 29.3, 29.2, 19.4, 19.3 [4 C, 2 (*C*H$_3$)$_2$CO$_2$]. Anal. Calcd for $C_{19}H_{32}O_{11}$: C, 52.28; H, 7.39. Found: C, 52.51; H, 7.67.

3.6. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-isopropylidene-α-D-mannopyranosyl-(1→3)-4,6-*O*-isopropylidene-α-D-mannopyranoside (8)

To a cooled solution (−20 °C) of **6** (2.18 g, 5 mmol) and **7** (6.34 g, 5.5 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 (two drops). 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 trisaccharide **8** (5.08 g, 71.2%) as a syrup: $[\alpha]_D$ + 41.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.05–7.33 (m, 30 H, 6 Ph*H*), 5.98 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.8 Hz, H-3), 5.96 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.88 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.9 Hz, H-3), 5.85 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.66 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.64 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.1 Hz, H-2), 5.36 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 5.13 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.72 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 3.37 (s, 3 H, OC*H*$_3$), 2.03 (s, 3 H, COC*H*$_3$), 1.49, 1.46, 1.39, 1.34 (4 s, 12 H, isopropylidene); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3 (*C*OCH$_3$), 166.5, 166.1, 165.6, 165.4,165.2, 164.9 (6 C, 6 *C*OPh), 101.2, 100.6 (2 C, 2 Me$_2$*C*), 99.8, 99.5, 99.3, 99.1 (4 C, 4 C-1), 54.8 (O*C*H$_3$), 29.2, 29.1 (2 C, *C*H$_3$C*C*H$_3$), 20.5

(COCH_3), 19.3, 19.2 (2 C, CH_3CCH_3). Anal. Calcd for $C_{75}H_{78}O_{28}$: C, 63.10; H, 5.51. Found: C, 63.35; H, 5.69.

3.7. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-*O*-isopropylidene-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-4,6-*O*-isopropylidene-α-D-mannopyranoside (9)

To a solution of **8** (2.85 g, 2.0 mmol) in pyridine (30 mL) was added acetic anhydride (20 mL, 20 mmol). The reaction mixture was stirred at rt for 12 h, at the end of which time TLC (3:1 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 (3:1 petroleum ether–EtOAc) to give compound **9** (2.81 g, 93.7%) as a foamy solid: $[\alpha]_D$ +42.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.13–7.29 (m, 30 H, 6 Ph*H*), 6.14 (dd, 1 H, $J_{3,4}=J_{4,5}=9.9$ Hz, H-4), 5.99 (dd, 1 H, $J_{3,4}=J_{4,5}=9.9$ Hz, H-4), 5.83 (dd, 1 H, $J_{2,3}$ 2.7, $J_{3,4}$ 9.9 Hz, H-3), 5.72 (dd, 1 H, $J_{2,3}$ 2.4, $J_{3,4}$ 9.9 Hz, H-3), 5.66 (dd, 1 H, $J_{1,2}$ 0.7, $J_{2,3}$ 2.7 Hz, H-2), 5.49 (d, 1 H, $J_{1,2}$ 0.7, H-1), 5.41 (dd, 1 H, $J_{1,2}$ 0.8, $J_{2,3}$ 3.0 Hz, H-2), 5.22 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 5.11 (dd, 1 H, $J_{1,2}$ 1.0, $J_{2,3}$ 3.0 Hz, H-2), 5.10 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.64 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 3.36 (s, 3 H, OCH_3), 2.32, 2.18, 2.01 (3 s, 9 H, 3 COCH_3), 1.53, 1.49, 1.38, 1.32 (4 s, 12 H, isopropylidene); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 170.2, 169.9 (3 C, 3 *C*OCH_3), 166.4, 165.8, 165.5, 165.3,165.0, 164.9 (6 C, 6 *C*OPh), 99.9, 99.7 (2 C, 2 Me_2C), 99.8, 99.4, 99.3, 99.0 (4 C, 4 C-1), 55.0 (OCH_3), 29.1, 29.0 (2 C, CH_3CCH_3), 20.9, 20.9, 20.5 (3 C, 3 COCH_3), 19.4, 19.2 (2 C, CH_3CCH_3). Anal. Calcd for $C_{79}H_{82}O_{30}$: C, 62.77; H, 5.47. Found: C, 62.48; H, 5.33.

3.8. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2-*O*-acetyl-α-D-mannopyranoside (10)

Compound **9** (1.51 g, 1.0 mmol) was dissolved in 90% TFA (20 mL) and stirred for 2 h, at the end of which time the reaction mixture was poured directly into toluene (100 mL), and then the mixture was concentrated. The residue was purified by flash chromatography (EtOAc) to give **10** (1.19 g, 83.2%) as a syrup: $[\alpha]_D$ +30.1° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.95–7.25 (m, 30 H, 6 Ph*H*), 5.95 (dd, 1 H, $J_{3,4}=J_{4,5}=$ 9.7 Hz, H-4), 5.88 (dd, 1 H, $J_{3,4}=J_{4,5}=9.8$ Hz, H-4), 5.84 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.8 Hz, H-3), 5.68 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.7 Hz, H-3), 5.63 (dd, 1 H, $J_{1,2}$ 1.4, $J_{2,3}$ 3.1 Hz, H-2), 5.56 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 5.50 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.1 Hz, H-2), 5.20 (dd, 1 H, $J_{1,2}$ 1.0, $J_{2,3}$ 3.1 Hz, H-2), 5.12 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.09 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 4.67 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 3.36 (s, 3 H, OCH_3), 2.15, 2.11, 2.00 (3 s, 9 H, 3 COCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.2, 169.2 (3 C, 3 *C*OCH_3), 167.2, 166.8, 165.5, 165.4,165.3, 165.0 (6 C, 6 *C*OPh), 100.5, 99.9, 98.7, 98.4 (4 C, 4 C-1), 55.0 (OCH_3), 21.0, 20.8, 20.5 (3 C, 3 COCH_3). Anal. Calcd for $C_{73}H_{74}O_{30}$: C, 61.25; H, 5.21. Found: C, 61.45; H, 5.40.

3.9. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2-*O*-acetyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2-*O*-acetyl-α-D-mannopyranoside (11)

Compound **10** (715 mg, 0.5 mmol) and **7** (1.15 g, 1.0 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (50 mL). TMSOTf (18.0 μL, 0.10 mmol) was added dropwise at −20 °C with N_2 protection. The reaction mixture was stirred for 2 h, during which time the temperature was gradually warmed to ambient temperature. Then the mixture was neutralized with Et_3N and concentrated to dryness under reduced pressure. Purification of the residue by column chromatography on a silica gel column (1:1 petroleum ether–EtOAc) furnished the octasaccharide **11** (1.03 g, 60.9%) as a syrup: $[\alpha]_D$ +28.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09–7.25 (m, 90 H, 18 Ph*H*), 6.04–5.84 (m, 10 H), 5.73–5.67 (m, 5 H), 5.61 (dd, 1 H, $J_{1,2}$ 1.0, $J_{2,3}$ 3.1 Hz, H-2), 5.34 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 5.30 (dd, 1 H, $J_{1,2}$ 1.1, $J_{2,3}$ 3.2 Hz, H-2), 5.27 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 5.24 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 5.14 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.08 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.07 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 4.86–4.75 (m, 2 H), 4.70 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 4.61 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 3.35 (s, 3 H, OCH_3), 2.16, 2.12, 2.01, 1.99, 1.96 (5 s, 15 H, 5 COCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.2, 169.2, 169.2, 169.1 (5 C, 5 *C*OCH_3), 167.2, 166.5, 165.4, 165.3,165.2, 165.9, 165.6, 165.6, 165.5, 165.5, 165.4, 165.3, 165.3, 165.2, 165.2, 165.1, 165.0, 164.9 (18 C, 18 *C*OPh), 100.6, 100.0, 99.8, 99.7, 99.4, 98.9, 98.6, 98.3 (8 C, 8 C-1), 54.9 (OCH_3), 21.0, 20.8, 20.5 (5 C, 5 COCH_3). Anal. Calcd for $C_{185}H_{166}O_{64}$: C, 65.09; H, 4.90. Found: C, 65.25; H, 4.79.

3.10. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-acetyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-acetyl-α-D-mannopyranoside (12)

To a solution of **11** (1.02 g, 0.3 mmol) in pyridine (20 mL) was added acetic anhydride (10 mL, 10 mmol). The reaction mixture was stirred at rt for 12 h, at the end of which time TLC (1:1 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 (1:1 petroleum ether–EtOAc) to give compound **12** (964 mg, 91.8%) as a foamy solid: $[\alpha]_D$ +33.0° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 6.14 (dd, 1 H, $J_{3,4}=J_{4,5}=9.8$ Hz, H-4), 6.05 (dd, 1 H, $J_{3,4}=J_{4,5}=9.9$ Hz, H-4), 6.01 (dd, 1 H, $J_{3,4}=J_{4,5}=9.8$ Hz, H-4), 5.99 (dd, H, $J_{3,4}=J_{4,5}=9.8$ Hz, H-4), 5.96 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 5.91 (dd, H, $J_{3,4}=J_{4,5}=9.9$ Hz, H-4), 5.90–5.80 (m, 5 H), 5.75 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.7 Hz, H-3), 5.68–5.64 (m, 3 H), 5.38 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 5.38 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 5.33 (dd, 1 H, $J_{1,2}$ 1.0, $J_{2,3}$ 3.1 Hz, H-2), 5.30 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 5.24 (d, 2 H, $J_{1,2}$ 1.4, 2 H-1), 5.19 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 5.14–5.08 (m, 4 H, 3 H-1, H-2), 4.73 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 3.33 (s, 3 H, OCH_3), 2.24, 2.16, 2.08, 2.06, 1.99, 1.98, 1.96 (7 s, 21 H, 7 $COCH_3$). Anal. Calcd for $C_{189}H_{170}O_{66}$: C, 64.90; H, 4.90. Found: C, 64.75; H, 4.81.

3.11. Methyl α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-α-D-mannopyranoside (13)

Octasaccharide **12** (700 mg, 0.20 mmol) was dissolved in a saturated solution of ammonia in MeOH (30 mL). After 96 h at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (H_2O) to afford **13** (193 mg, 72.6%) as a foamy solid: ^1H NMR (D_2O, 400 MHz): δ 5.24 (s, 1 H, H-1), 5.02 (s, 2 H, 2 H-1), 4.91 (d, 2 H, $J_{1,2}$ 1.2 Hz, 2 H-1), 4.90 (s, 1 H, H-1), 4.87 (s, 1 H, H-1), 4.61 (s, 1 H, H-1), 3.29 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, D_2O): δ 105.1, 105.1, 104.9, 104.9, 104.9, 103.6, 103.4, 100.6, 100.5 (8 C, 8 C-1), 57.5 (OCH_3). MALDI-TOF MS Calcd for $C_{49}H_{84}O_{41}$: 1329.2 [M]. Found: 1352.1 [M+Na^+].

3.12. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-isopropylidene-α-D-mannopyranosyl-(1→3)-4,6-*O*-isopropylidene-α-D-mannopyranoside (15)

To a cooled solution (−20 °C) of **6** (2.18 g, 5 mmol) and **14** (3.73 g, 5.5 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 (two drops). 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 trisaccharide **15** (3.20 g, 67.2%) as a syrup: $[\alpha]_D$ +91.7° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400MHz): δ 7.95–7.34 (m, 15 H, 3 Ph*H*), 5.94 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 5.75 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.0 Hz, H-3), 5.53 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.3 Hz, H-2), 5.43 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.31 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.71 (d, 1 H, $J_{2,1}$ 1.0 Hz, H-1), 4.66–4.58 (m, 3 H), 4.26–4.18 (m, 4 H), 4.04–3.99 (m, 2 H), 3.90–3.82 (m, 4 H), 3.68–3.63 (m, 2 H), 3.36 (s, 3 H, OCH_3), 2.17 (s, 3 H, $COCH_3$), 1.56, 1.45 (2 s, 6 H, isopropylidene), 1.37 (s, 6 H, isopropylidene); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5 ($COCH_3$), 166.3, 165.6, 163.6 (5 C, 5 *C*OPh), 101.2, 100.8, (2 C, 2 Me_2C) 99.9, 99.4, 98.3 (3 C, 3 C-1), 54.8 (OCH_3), 29.1, 28.9 (2 C, CH_3CCH_3), 20.6 ($COCH_3$), 19.2, 19.0 (2 C, CH_3CCH_3). Anal. Calcd for $C_{48}H_{56}O_{20}$: C, 60.50; H, 5.92. Found: C, 60.45; H, 5.66.

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

To a solution of **15** (950 mg, 1.0 mmol) in pyridine (50 mL) was added acetic anhydride (20 mL, 20 mmol). The reaction mixture was stirred at rt for 12 h, at the end of which time TLC (3:1 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 (3:1 petroleum ether–EtOAc) to give compound **16** (830 mg, 80.6%) as a foamy solid: $[\alpha]_D$ +82.6° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.10–7.23 (m, 65 H, 13 Ph*H*), 6.04 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 5.64 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3), 5.45 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 2.9 Hz, H-2), 5.40 (dd, 1 H, $J_{1,2}$ 1.3, $J_{2,3}$ 3.4 Hz, H-2), 5.32 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.22 (dd, 1 H, $J_{1,2}$ 1.3, $J_{2,3}$ 2.9 Hz, H-2), 5.10 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 4.67 (dd, 1 H, $J_{2,3}$ 2.9, $J_{3,4}$ 12.1 Hz, H-3), 4.63 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 4.46–4.38 (m, 2 H), 4.09–4.05 (m, 4 H), 3.87–3.81 (m, 4 H), 3.70–3.60 (m, 2 H), 3.36 (s, 3 H, OCH_3), 2.31, 2.18, 2.07 (3 s, 9 H, 3 $COCH_3$), 1.56, 1.52 (2 s, 6 H, isopropylidene), 1.37 (s, 6 H, isopropylidene). Anal. Calcd for $C_{52}H_{60}O_{22}$: C, 60.22; H, 5.83. Found: C, 60.47; H, 5.61.

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

Compound **16** (1.04 g, 1 mmol) was dissolved in 90% TFA (20 mL) and stirred for 2 h, at the end of which time the reaction mixture was poured directly into toluene (100 mL), and then the mixture was concentrated. The residue was purified by flash chromatography (EtOAc) to give **17** (765 mg, 80.1%) as a syrup: $[\alpha]_D$ +35.3° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.97–7.26 (m, 10 H, 2 Ph*H*), 5.94 (dd, 1 H, $J_{3,4}=J_{4,5}=$ 9.8 Hz, H-4), 5.61 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.8 Hz, H-3), 5.51 (dd, 1 H, $J_{1,2}$ 1.0, $J_{2,3}$ 3.2 Hz, H-2), 5.13 (dd, 1 H, $J_{1,2}$ 0.7, $J_{2,3}$ 3.0 Hz, H-2), 5.35 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 5.19 (dd, 1 H, $J_{1,2}$ 0.8, $J_{2,3}$ 3.1 Hz, H-2), 5.13 (d, 1 H, $J_{1,2}$ 0.7 Hz, H-1), 4.66 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 3.36 (s, 3 H, OC*H*$_3$), 2.20, 2.12, 2.09 (3 s, 9 H, 3 COC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): 170.7, 170.5, 170.0 (3 C, 3 *C*OCH$_3$), 166.8, 165.5 (2 C, 2 *C*OPh), 99.9, 98.8, 98.5 (3 C, 3 C-1), 55.0 (O*C*H$_3$), 20.9, 20.8, 20.6 (3 C, 3 CO*C*H$_3$). Anal. Calcd for $C_{46}H_{52}O_{22}$: C, 57.73; H, 5.48. Found: C, 57.57; H, 5.62.

3.15. Methyl 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2-*O*-acetyl-α-D-mannopyranosyl-(1→3)-[2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2-*O*-acetyl-α-D-mannopyranoside (18)

Compound **17** (478 mg, 0.5 mmol) and **7** (1.15 g, 1.0 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (50 mL). TMSOTf (18.0 μL, 0.10 mmol) was added dropwise at −20 °C with N_2 protection. The reaction mixture was stirred for 2 h, and was neutralized with Et_3N. Then the mixture was concentrated to dryness under reduced pressure. Purification of the residue by column chromatography on a silica gel column (1:1 petroleum ether–EtOAc) furnished the heptasaccharide **18** (1.10 g, 74.8%) as a syrup: $[\alpha]_D$ +24.5° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.03–7.26 (m, 75 H, 15 Ph*H*), 6.00–5.83 (m, 9 H), 5.69 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 10.1 Hz, H-3), 5.67–5.66 (m, 2 H), 5.59 (dd, 1 H, $J_{1,2}$ 1.2, $J_{2,3}$ 2.9 Hz, H-2), 5.56 (dd, 1 H, $J_{1,2}$ 0.8, $J_{2,3}$ 2.7 Hz, H-2), 5.41 (d, 1 H, $J_{1,2}$ 0.8, H-1), 5.33 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 5.28 (dd, 1 H, $J_{1,2}$ 1.3, $J_{2,3}$ 3.1 Hz, H-2), 5.28 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 5.26 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 5.10 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 5.07 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 4.71 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 3.33 (s, 3 H, OC*H*$_3$), 2.17, 2.11, 2.09, 2.01, 2.00 (5 s, 15 H, 5 COC*H*$_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.3, 169.7, 169.2, 169.2 (5 C, 5 *C*OCH$_3$), 166.8, 166.5, 166.3, 166.2,165.9, 165.6, 165.6, 165.5, 165.4, 165.4, 165.3, 165.3, 165.2, 165.0, 164.9 (15 C, 15 *C*OPh), 100.1, 99.8, 99.7, 98.9, 98.7, 98.6, 98.3 (7 C, 7 C-1), 54.9 (O*C*H$_3$), 21.8, 20.7, 20.6, 20.5, 20.5 (5 C, 5 CO*C*H$_3$). Anal. Calcd for $C_{158}H_{144}O_{56}$: C, 64.57; H, 4.94. Found: C, 64.30; H, 4.88.

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

To a solution of **18** (880 mg, 0.3 mmol) in pyridine (20 mL) was added acetic anhydride (10 mL, 10 mmol). The reaction mixture was stirred at rt for 12 h, at the end of which time TLC (1:1 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 (1:1 petroleum ether–EtOAc) to give compound **19** (729 mg, 80.5%) as a foamy solid: $[\alpha]_D$ +23.2° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.08–7.25 (m, 75 H, 15 Ph*H*), 6.06 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 6.05 (dd, 1 H, $J_{3,4}=J_{4,5}=9.9$ Hz, H-4), 6.00–5.85 (m, 6 H), 5.82 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3), 5.69 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.8 Hz, H-3), 5.66 (dd, 1 H, $J_{1,2}$ 1.1, $J_{2,3}$ 3.2 Hz, H-2), 5.64 (dd, 1 H, $J_{1,2}$ 1.0, $J_{2,3}$ 3.0 Hz, H-2), 5.43 (dd, 1 H, $J_{3,4}=J_{4,5}=10.0$ Hz, H-4), 5.34 (dd, 1 H, $J_{1,2}$ 1.3, $J_{2,3}$ 3.1 Hz, H-2), 5.31 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.2 Hz, H-2), 5.30 (dd, 1 H, $J_{3,4}=J_{4,5}=10.2$ Hz, H-4), 5.23 (d, 2 H, $J_{1,2}$ 1.3 Hz, 2 H-1), 5.20 (d, 2 H, $J_{1,2}$ 1.4 Hz, 2 H-1), 5.09 (dd, 1 H, $J_{1,2}$ 1.1, $J_{2,3}$ 3.0 Hz, H-2), 5.09 (d, H, $J_{1,2}$ 1.3 Hz, H-1), 5.06 (d, H, $J_{1,2}$ 1.1 Hz, H-1), 4.74 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 3.30 (s, 3 H, OC*H*$_3$), 2.23, 2.16, 2.14, 2.14, 2.09, 2.00, 1.98 (7 s, 21 H, 7 COC*H*$_3$). Anal. Calcd for $C_{162}H_{148}O_{58}$: C, 64.36; H, 4.94. Found: C, 64.50; H, 5.13.

3.17. Methyl α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-α-D-mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→6)]-α-D-mannopyranoside (20)

Octasaccharide **19** (604 mg, 0.20 mmol) was dissolved in a saturated solution of ammonia in MeOH (30 mL). After 96 h at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (H_2O) to afford **20** (173 mg, 74.6%) as a foamy solid: ^1H NMR (D_2O, 400 MHz): δ 5.01 (s, 2 H, 2 H-1), 4.99 (d, 1 H, $J_{1,2}$ 0.6 Hz, H-1), 4.91 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 4.89 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 4.86 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 4.59 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1),

3.28 (s, 3 H, OCH_3); ^{13}C NMR (100 MHz, D_2O): δ 105.1, 105.1, 105.0, 104.9, 103.6, 100.6, 100.5 (7 C, 7 C-1), 57.5 (OCH_3). MALDI-TOF MS Calcd for $C_{43}H_{74}O_{36}$: 1167.0 [M]. Found: 1190.0 [$M+Na^+$].

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