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A practical synthesis of α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-[α-D-Glc*p*-(1→3)]-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*, an O-specific heterohexasaccharide fragment of *Citrobacter braakii* O7a, 3b, 1c

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Abstract

An O-specific heterohexasaccharide fragment of *Citrobacter braakii* O7a, 3b, 1c, α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-[α-D-Glc*p*-(1→3)]-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p* was synthesized as its methyl glycoside. Acetylation of allyl 4,6-*O*-benzylidene-α-D-mannopyranoside, followed by debenzylidenization and benzoylation gave allyl 2,3-di-*O*-acetyl-4,6-di-*O*-benzoyl-α-D-mannopyranoside (**3**), and subsequent deacetylation of **3** with CH_3COCl–MeOH gave the monosaccharide acceptor **4**. Condensation of isopropyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**6**) with **4** selectively afforded the α-(1→3)-linked disaccharide **7**. Condensation of **7** with the (1→3)-linked disaccharide donor **9**, followed by deallylation and trichloroacetimidation, afforded the tetrasaccharide donor **12**. Coupling of **12** with disaccharide acceptor **13**, followed by debenzylation and deacylation, furnished the target heterohexasaccharide **16**.

Keywords: Oligosaccharide; Glucose; Mannose; Regio- and stereoselective synthesis

1. Introduction

A heterohexasaccharide fragment,[1a] →3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-[α-D-Glc*p*-(1→3)]-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→, which was only slightly different from a pentasaccharide fragment[1b] →3)-α-D-Manp-(1→3)-α-D-Manp-(1→2)-α-D-Manp-(1→2)-α-D-Manp-(1→2)-α-D-Manp-(1→ obtained[1b] from *Hafnia alvei* (strain PCM 1223), was elucidated by Knirel's group as a new structure of the O-specific polysaccharide (O-antigen) from *Citrobacter braakii* O7a, 3b, 1c (strain Be 59/57). Although there was the same pentasaccharide main chain in the structures of the two O-antigens, no cross-reactivity was observed in serological immunoblotting between the lipopolysaccharide of *C. braakii* O7a, 3b, 1c and anti-*H. alvei* PCM 1223 O-serum.[1b,1c] This could be expounded by masking of potentially cross-reactive epitope(s) within the D-mannan chain by the lateral glucose residue in the polysaccharide of *C. braakii*. Synthesis of this fragment is helpful for investigation of structure–bioactivity relationships of oligosaccharides, and this paper will describe a facile preparation of the hexasaccharide repeating unit.

2. Results and discussion

Retrosynthetic analysis (Scheme 1) indicated that the heterohexasaccharide could be built from three disaccharide fragments, i.e., the downstream end disaccharide, the upstream end disaccharide, and the inner disaccharide. The downstream- and upstream end disaccharides could be readily obtained by reported methods,[2,3] whereas the inner disaccharide is the most difficult one to be prepared. The key point is to

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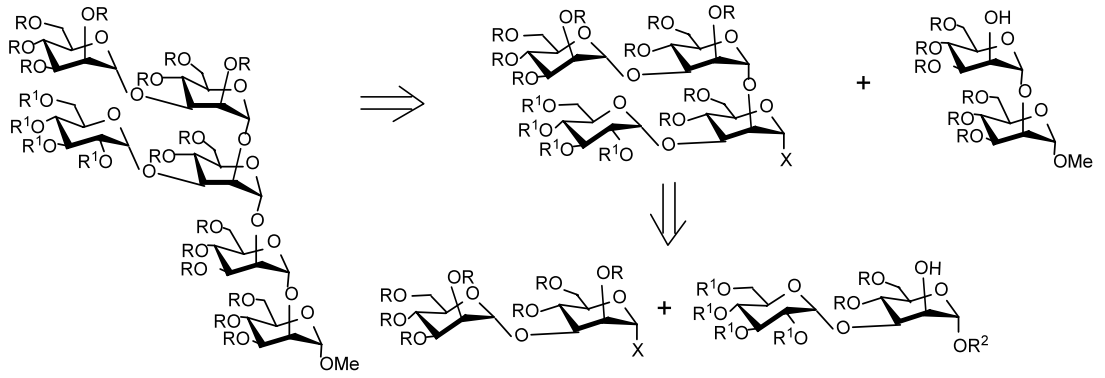


Scheme 1. Retrosynthetic analysis of the heterohexasaccharide.

selectively couple a mannose acceptor at C-3 with a glucose donor by an α-linkage. For this purpose, a glucosyl donor with C-2 possessing non-neighboring group participation and a mannose acceptor with 2,3-OHs were needed. Initially, we tried to use the method[4] that is very effective for selective coupling of a 4,6-*O*-benzylidenated mannose acceptor with acylated mannosyl donors to get α-(1→3)-linked oligosaccharides. However, it was found that using the same acceptor **1**,[5] the coupling with isopropyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**6**) gave an inseparable complex mixture (Scheme 2). To solve this problem, an alternative mannose acceptor was prepared. Thus, allyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**1**)[5] was acetylated with acetic anhydride in pyridine to obtain **2** (95%). Debenzylidenation of **2** and subsequent benzoylation afforded allyl 2,3-di-*O*-acetyl-4,6-di-*O*-benzoyl-α-D-mannopyranoside (**3**). Selective deacetylation of **3** with CH_3COCl–MeOH[6] furnished the alternative acceptor **4**. It was interesting to find that condensation of **6** with **4** selectively gave the required α-(1→3)-linked disaccharide **7** in acceptable yield (55%). The 3-*O*-glycosylation was confirmed by benzoylation of **7** to give allyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (**8**), and the ^{1}H NMR spectrum of **8** showed a doublet at δ 4.90 ppm with $J_{1',2'}$ 3.4 Hz, the salient feature for H-1′ of an α-D-glucopyranoside, and a newly emerged doublet of doublets at δ 5.51 ppm with $J_{1,2}$ 1.8 and $J_{2,3}$ 3.2 Hz, the salient feature for H-2 of an α-D-mannopyranoside. Coupling of the acceptor **7** with the donor, 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**9**),[3] gave tetrasaccharide **10** (75%). Deallylation of **10** with $PdCl_2$ in CH_3OH[7] gave the tetrasaccharide hemiacetal **11** (90%), and subsequent trichloroacetimidation[8] with trichloroacetonitrile in the presence of potassium carbonate readily afforded the tetrasaccharide donor **12** in high yield (90%). Coupling of **12** with the acceptor, methyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (**13**),[9] gave hexasaccharide **14** (70%). Catalytic debenzylation of **14** with hydrogen in the presence of Pd/C afforded **15**, and finally deacylation in ammonia-saturated methanol furnished the target hexasaccharide **16**. The ^{1}H and ^{13}C NMR spectra of **16** showed some characteristic signals such as at δ 5.16, 5.03, 4.89 for 5 H-1 of Man*p*, and δ 5.15 for H-1 of Glc*p*, and δ 102.19, 101.58, 100.72, 100.71, 100.69 for 5 C-1 of Man*p*, and δ 99.37 for C-1 of Glc*p*.

In summary, a facile synthesis of the heterohexasaccharide was achieved in a regio- and stereoselective way.

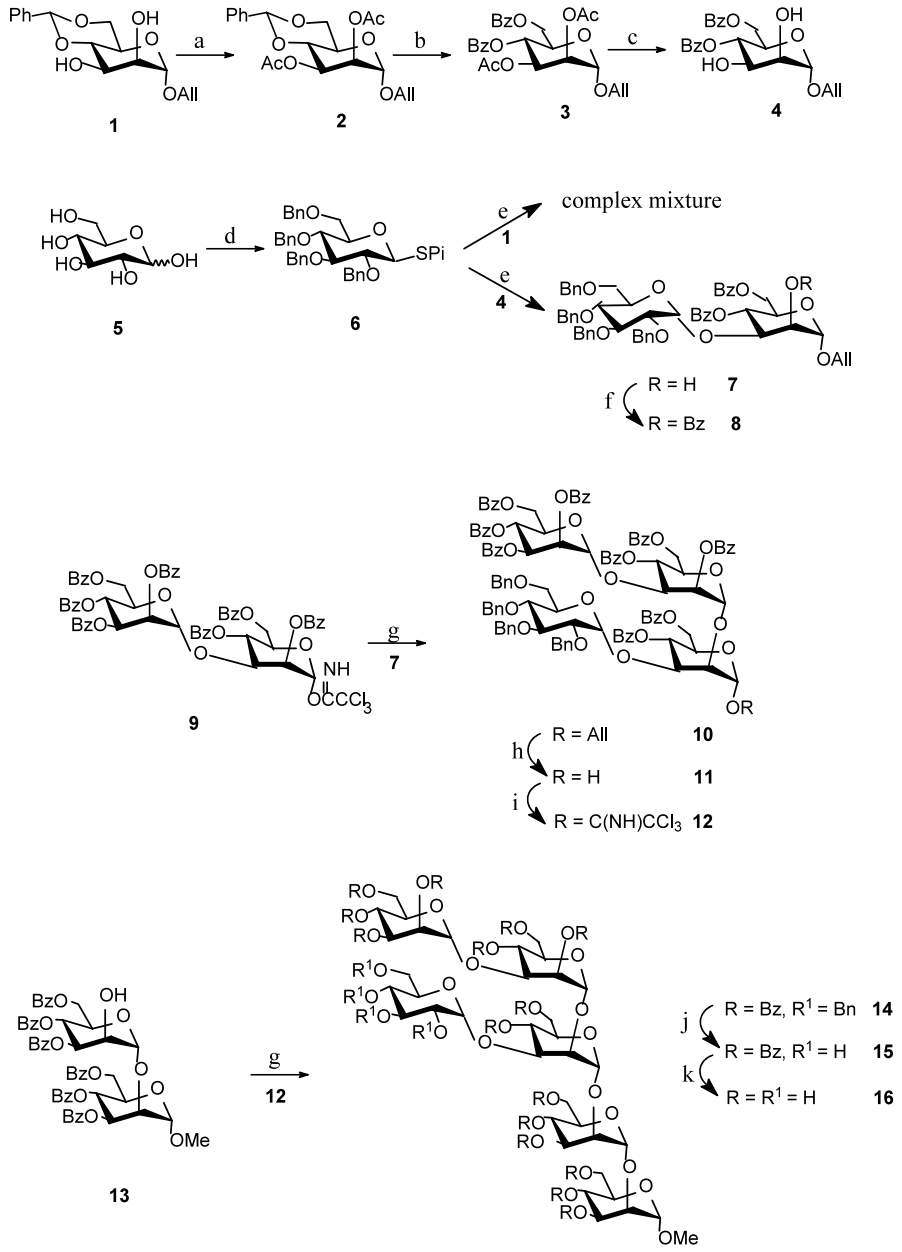
3. Experimental

3.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-MC automatic polarimeter. Melting points were determined with a 'Mel-Temp' apparatus. ^{1}H and ^{13}C NMR spectra were recorded with Bruker ARX 400 spectrometers for solutions in $CDCl_3$ or D_2O as indicated, and individual resonances could not identified with the specific sugar residues. Chemical shifts are given in parts per million (ppm) downfield from internal Me_4Si. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the electrospray-ionization 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 UV detection. Column chromatography was conducted by elution of a column (16 × 240, 18 × 300, 35 × 400 mm) of silica gel (100–200 mesh). Solutions were concentrated at <60 °C under diminished pressure.

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

To a solution of **1**[5] (3.08 g, 10.0 mmol) in Py (10 mL), Ac_2O (2.37, 25.1 mmol) was added dropwise, and the mixture was stirred overnight at room temperature (rt). TLC (2:1 petroleum ether–EtOAc) indicated that the



Scheme 2. Scheme and conditions: (a) Ac_2O, Py, rt. (b) 80% HOAc–H_2O, reflux, 2 h; then PhCOCl–Py, rt. (c) 5% CH_3COCl, CH_3OH, 40 °C, 4 h. (d) i. Ac_2O, NaOAc, reflux, 10 min; ii. $HSCH(CH_3)_2$, $BF_3 \cdot Et_2O$, rt, 1 h; iii. NaOMe, MeOH, rt; iv. $PhCH_2Br$, NaH, DMF. (e) NIS, TMSOTf, CH_2Cl_2, −25 °C. (f) PhCOCl–Py, rt. (g) TMSOTf, CH_2Cl_2, −25 °C. (h) $PdCl_2$, CH_3OH, 40 °C, 4 h. (i) CCl_3CN, CH_2Cl_2, K_2CO_3, rt. (j) H_2, Pd/C, CH_3OH–EtOAc, 40 °C. (k) NH_3–CH_3OH, rt.

reaction was complete. The mixture was diluted with CH_2Cl_2, washed with 1 N HCl, water, and satd aq $NaHCO_3$. The organic layer was combined, dried, and concentrated. Purification by column chromatography (2:1 petroleum ether–EtOAc) gave **2** as a syrup (3.72 g, 95%): $[\alpha]_D^{20}$ +38.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.48–7.35 (m, 5 H, PhH), 5.89 (m, 1 H, CH_2=C*H*–CH_2–), 5.58 (s, 1 H, PhCH=), 5.44 (dd, 1 H, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.7 Hz, H-3), 5.37 (dd, 1 H, $J_{1,2}$ 1.6 Hz, H-2), 5.34–5.23 (m, 2 H, C*H*$_2$=CH–CH_2–), 4.82 (s, 1 H, H-1), 4.28 (dd, 1 H, $J_{5,6}$ 4.4 Hz, $J_{6,6'}$ 10.0 Hz, H-6), 4.23–3.99 (m, 2 H, CH_2=CH–C*H*$_2$–), 4.05 (dd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 4.02 (m, 1 H, H-5), 3.85 (dd, 1 H, $J_{5,6'}$ 10.0 Hz, H-6′), 2.17 (s, 3 H, CH_3), 2.03 (s, 3 H, CH_3). Anal. Calcd for $C_{20}H_{24}O_8$: C, 61.22; H, 6.12. Found: C, 60.98; H, 6.15.

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

Compound **2** (3.92 g, 10.0 mmol) was dissolved in 80% HOAc (50 mL), and the mixture was stirred under reflux

for 2 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated to dryness under reduced pressure. To a solution of the residue in Py (8 mL), BzCl (2.5 mL, 21.6 mmol) was added dropwise, and the mixture was stirred overnight at rt. TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH_2Cl_2, washed with 1 N HCl, water, and satd aq $NaHCO_3$. The organic layer was combined, dried, and concentrated. Purification by column chromatography (3:1 petroleum ether–EtOAc) gave **3** as a syrup (4.35 g, 85%): $[\alpha]_D^{20}$ +49.4° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.05–7.38 (m, 10 H, PhH), 5.92 (m, 1 H, CH_2=C*H*–CH_2–), 5.71 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.64 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3), 5.33 (dd, 1 H, $J_{1,2}$ 1.7 Hz, H-2), 5.34–5.22 (m, 2 H, C*H*$_2$=CH–CH_2–), 4.93 (d, 1 H, H-1), 4.56 (dd, 1 H, $J_{5,6}$ 2.9 Hz, $J_{6,6'}$ 12.0 Hz, H-6), 4.44 (dd, 1 H, $J_{5,6'}$ 5.4 Hz, H-6′), 4.31 (m, 1 H, H-5), 4.28–4.06 (m, 2 H, CH_2=CH–C*H*$_2$–), 2.16 (s, 3 H, CH_3), 1.91 (s, 3 H, CH_3). Anal. Calcd for $C_{27}H_{28}O_{10}$: C, 63.28; H, 5.47. Found: C, 63.47; H, 5.44.

3.4. Allyl 4,6-di-*O*-benzoyl-α-D-mannopyranoside (4)

To a solution of **3** (2.56 g, 5.0 mmol) in anhyd CH_3OH (60 mL) was added CH_3COCl (3.0 mL), and the mixture was stirred for 3–4 h at 40 °C, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, then passed through a silica-gel column with 1:1 petroleum ether–EtOAc as the eluent to give **4** as a syrup (1.71 g, 80%): $[\alpha]_D^{20}$ +85.7° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.06–7.38 (m, 10 H, PhH), 5.92 (m, 1 H, CH_2=C*H*–CH_2–), 5.40 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.31–5.20 (m, 2 H, C*H*$_2$=CH–CH_2–), 5.01 (s, 1 H, H-1), 4.59 (dd, 1 H, $J_{5,6}$ 2.6 Hz, $J_{6,6'}$ 11.9 Hz, H-6), 4.45 (dd, 1 H, $J_{5,6'}$ 5.9 Hz, H-6′), 4.28–4.04 (m, 5 H), 3.27 (s, 1 H, OH), 2.76 (s, 1 H, OH). Anal. Calcd for $C_{23}H_{24}O_8$: C, 64.49; H, 5.61. Found: C, 64.80; H, 5.58.

3.5. Isopropyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (6)

To a solution of per-*O*-acetyl-D-glucopyranoside (63.9 g, 164 mmol) from D-glucose in CH_2Cl_2 was added isopropyl mercaptan (21.0 mL) and a solution of $BF_3 \cdot Et_2O$ (45 mL, 47% in Et_2O). After stirring the mixture at rt for 1 h, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH_2Cl_2 and washed with satd aq $NaHCO_3$. The organic layer was dried, and concentrated. To a solution of the residue in MeOH (200 mL) was added 4.0 M NaOMe–MeOH solution dropwise to pH 10. After stirring the mixture at rt for 5 h, TLC (3:1 EtOAc–MeOH) indicated that the reaction was complete. The reaction mixture was neutralized with 1:10 HOAc–MeOH, then the mixture was concentrated, and the residue was purified by column chromatography (3:1 EtOAc–MeOH) to give a solid (23.4 g, 60%). To a solution of the solid (2.38 g, 10.0 mmol) in dry DMF (80 mL) were added $PhCH_2Br$ (7.0 mL, 58.9 mmol) and NaH (1.15 g) at 0 °C. After stirring the mixture overnight at rt, TLC (4:1 petroleum–EtOAc) indicated that, the reaction was complete. Water (10 mL) was added to the reaction mixture, and then the mixture was diluted with EtOAc and washed with water. The organic layer was dried and concentrated. Purification by column chromatography (3:1 petroleum ether–EtOAc) gave an amorphous solid **6** (5.68 g, 95%): $[\alpha]_D^{20}$ +6.2° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.14 (m, 20 H, PhH), 4.92 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1), 4.94–4.52 (m, 8 H, PhC*H*$_2$), 3.73 (dd, 1 H, $J_{5,6}$ 1.8 Hz, $J_{6,6'}$ 10.9 Hz, H-6), 3.69 (dd, $J_{2,3} = J_{3,4} = 9.3$ Hz, H-3), 3.64 (dd, 1 H, $J_{5,6'}$ 5.0 Hz, H-6′), 3.59 (dd, 1 H, $J_{4,5}$ 9.3 Hz, H-4), 3.47 (m, 1 H, H-5), 3.43 (dd, 1 H, H-2), 3.25 (m, 1 H, (CH_3)C*H*S), 1.38–1.34 (dd, 6 H, (C*H*$_3$)CHS). Anal. Calcd for $C_{37}H_{42}O_5S$: C, 74.25; H, 7.02. Found: C, 74.61; H, 6.98.

3.6. Allyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-4,6-di-*O*-benzoyl-α-D-mannopyranoside (7)

Donor **6** (2.99 g, 5.0 mmol) and acceptor **4** (2.14 g, 5.0 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (70 mL). TMSOTf (15 μL, 0.08 mmol) and NIS (0.112 g, 0.5 mmol) were added at −25 °C with nitrogen protection. The reaction mixture was stirred for 3 h, at the end of which time TLC indicated that the reaction was complete. Then the mixture was neutralized with Et_3N and concentrated under reduced pressure to dryness. Purification by column chromatography (3:1 petroleum ether–EtOAc) gave **7** as a syrup (2.61 g, 55%): $[\alpha]_D^{20}$ +29.0° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.05–7.13 (m, 30 H, PhH), 5.93 (m, 1 H, CH_2=C*H*–CH_2–), 5.75 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4), 5.32–5.21 (m, 2 H, C*H*$_2$=CH–CH_2–), 4.86 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1′), 4.83 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 4.79–4.01 (m, 15 H), 3.89 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3′), 3.69–3.57 (m, 3 H), 3.41–3.32 (m, 3 H), 3.25 (dd, 1 H, $J_{4,5}$ 9.6 Hz, H-4′); ^{13}C NMR ($CDCl_3$): δ 166.42, 165.60 (2 Ph*C*O), 138.73, 138.25, 138.12, 137.46, 133.76, 133.00, 132.93, 129.92, 129.83, 128.54, 128.46, 128.40, 128.28, 128.15, 128.10, 127.96, 127.77, 127.65 (Ph–C), 117.89 (*C*H_2=CH–CH_2–), 99.47, 97.76 (2 C-1), 81.75, 79.58, 79.14, 77.98, 75.63, 74.94, 73.67, 72.57, 71.15, 69.43, 69.30, 68.93, 68.46, 68.24, 64.16. Anal. Calcd for $C_{57}H_{58}O_{13}$: C, 72.00; H, 6.11. Found: C, 71.75; H, 6.14.

3.7. Allyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (8)

To a solution of compound **7** (0.19 g, 0.2 mmol) in Py (2 mL), BzCl (0.03 mL, 0.26 mmol) was added dropwise, and the mixture was stirred overnight at rt, 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, washed with 1 N HCl, water, and satd aq $NaHCO_3$. The organic layer was combined, dried, and concentrated. Purification by column chromatography (3:1 petroleum ether–EtOAc) gave **8** as a syrup (0.19 g, 90%): $[\alpha]_D^{20}$ +35.9° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.10–7.05 (m, 35 H, PhH), 6.02 (dd, 1 H, $J_{3,4}=J_{4,5}=$ 9.5 Hz, H-4), 5.60 (m, 1 H, CH_2=C*H*–CH_2–), 5.51 (dd, 1 H, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.2 Hz, H-2), 5.39–5.27 (m, 2 H, C*H*$_2$=CH–CH_2–), 5.15 (d, 1 H, H-1), 4.90 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1′), 4.70–4.03 (m, 14 H), 3.80 (dd, 1 H, $J_{2,3}=J_{3,4}=$ 9.5 Hz, H-3′), 3.72 (m, 1 H, H-5′), 3.51 (dd, 1 H, $J_{4,5}=$ 9.5 Hz, H-4′), 3.32–3.24 (m, 3 H). Anal. Calcd for $C_{64}H_{62}O_{14}$: C, 72.87; H, 5.88. Found: C, 73.08; H, 5.85.

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

Donor **9**[3] (1.21 g, 1.0 mmol) and acceptor **7** (0.95 g, 1.0 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (70 mL). TMSOTf (20 μL, 0.10 mmol) was added at −25 °C with nitrogen protection. The reaction mixture was stirred for 3 h, at the end of which time TLC indicated that the reaction was complete. Then the mixture was neutralized with Et_3N and concentrated under reduced pressure to dryness. Purification by column chromatography (2:1 petroleum ether–EtOAc) gave **10** as a syrup (1.50 g, 75%): $[\alpha]_D^{20}$ −30.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.19–7.10 (m, 65 H, PhH), 6.15 (dd, 1 H, $J_{3,4}=J_{4,5}=$ 10.1 Hz, H-4 of Man*p*), 6.05 (dd, 1 H, $J_{3,4}=J_{4,5}=$ 9.9 Hz, H-4 of Man*p*), 5.95–5.83 (m, 3 H, CH_2=C*H*–CH_2–, H-4, H-2 of Man*p*), 5.75 (dd, 1 H, $J_{2,3}$ 2.6 Hz, $J_{3,4}$ 10.1 Hz, H-3 of Man*p*), 5.48 (s, 1 H, H-1 of Man*p*), 5.39 (m, 2 H, H-1, H-2 of Man*p*), 5.27–5.18 (m, 2 H, C*H*$_2$=CH–CH_2–), 5.13 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1 of Man*p*), 4.92 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1 of Glc*p*), 4.82–3.86 (m, 24 H), 3.60–3.28 (m, 4 H); ^{13}C NMR ($CDCl_3$): δ 166.21, 166.07, 165.60, 165.37, 165.13, 165.07, 164.81, 164.67, 164.35 (9 Ph*C*O), 138.88, 138.40, 138.10, 137.70, 133.30, 133.05, 132.86, 132.46, 129.88, 129.73, 129.61, 129.49, 128.33, 128.13, 128.03, 127.94, 127.71, 127.66, 127.34 (Ph–C), 117.82 (*C*H_2=CH–CH_2–), 99.73, 99.20, 98.94, 97.73 (4 C-1), 81.81, 78.49, 75.17, 74.93, 74.22, 73.18, 71.92, 71.48, 71.43, 70.10, 69.93, 69.54, 69.44, 69.20, 68.85, 68.56, 68.41, 66.00, 63.90, 62.95, 61.88. Anal. Calcd for $C_{118}H_{106}O_{30}$: C, 70.73; H, 5.29. Found: C, 70.49; H, 5.43.

3.9. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)]-4,6-di-*O*-benzoyl-α-D-mannopyranose (11)

To a solution of **10** (1.00 g, 0.5 mmol) in anhyd CH_3OH (50 mL) was added $PdCl_2$ (0.1 g), and the mixture was stirred at 40 °C for 4 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, the filtrate was concentrated, and the residue was passed a silica-gel column with 2:1 petroleum ether–EtOAc as the eluent to give **11** as a syrup (0.88 g, 90%): $[\alpha]_D^{20}$ −73.1° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.17–6.95 (m, 65 H, PhH), 6.15 (dd, 1 H, $J_{3,4}=J_{4,5}=$10.1 Hz, H-4 of Man*p*), 6.02 (m, 2 H, H-4 of Man*p*), 5.90 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2 of Man*p*), 5.75 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-3 of Man*p*), 5.52 (dd, 1 H, H-2 of Man*p*), 5.46 (s, 1 H, H-1 of Man*p*), 5.39 (m, 2 H, H-1 of Man*p*), 4.95 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1 of Glc*p*), 4.82–3.90 (m, 22 H), 3.60–3.29 (m, 4 H), 2.85 (bs, 1 H, OH). Anal. Calcd for $C_{115}H_{102}O_{30}$: C, 70.34; H, 5.20. Found: C, 70.12; H, 5.24.

3.10. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)]-4,6-di-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (12)

Compound **11** (0.82 g, 0.42 mmol) was dissolved in CH_2Cl_2 (40 mL), then CCl_3CN (3 mL) and anhyd K_2CO_3 (0.82 g) were added. The reaction mixture was stirred overnight at rt. TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. Concentration of the reaction mixture followed by purification on a silica-gel column with 3:1 petroleum ether–EtOAc as the eluent gave the tetrasaccharide donor **12** as an amorphous solid (0.79 g, 90%): $[\alpha]_D^{20}$ −30.7° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.61 (s, 1 H, HN=), 8.16–6.99 (m, 65 H, PhH), 6.61 (s, 1 H, H-1), 6.15 (m, 3 H), 5.96 (dd, 1 H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.2 Hz, H-2 of Man*p*), 5.77 (dd 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.8 Hz, H-3 of Man*p*), 5.54 (dd, 1 H, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.2 Hz, H-2 of Man*p*), 5.39 (m, 2 H, H-1 of Man*p*), 4.89 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1 of Glc*p*), 4.83–3.92 (m, 22 H), 3.46–3.28 (m, 4 H). Anal. Calcd for $C_{117}H_{102}Cl_3NO_{30}$: C, 66.65; H, 4.84. Found: C, 66.96; H, 4.91.

3.11. Methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→3)]-4,6-di-*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-mannopyranoside (14)

Donor **12** (0.63 g, 0.30 mmol) and acceptor **13**[9] (0.29 g, 0.30 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (40 mL). TMSOTf (10 μL, 0.05 mmol) was added at −25 °C with nitrogen protection. The reaction mixture was stirred for 3 h, at the end of which time TLC indicated that the reaction was complete. Then the mixture was neutralized with Et_3N and concentrated under reduced pressure to dryness. Purification by column chromatography (2:1 petroleum ether–EtOAc) gave **14** as a syrup (0.61 g, 70%): $[\alpha]_D^{20}$ −47.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.14–7.02 (m, 95 H, PhH), 6.14 (dd, 1 H, $J_{3,4} = J_{4,5} =$ 10.0 Hz, H-4 of Man*p*), 6.02 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.9$ Hz, H-4 of Man*p*), 5.96–5.90 (m, 4 H), 5.80 (dd, 1 H, $J_{2,3}$ 2.8 Hz, H-3 of Man*p*), 5.74 (m, 2 H, H-3 of Man*p*), 5.37 (m, 3 H, 2 H-1 and H-2 of Man*p*), 5.28 (s, 1 H, H-1 of Man*p*), 5.25 (d, 1 H, $J_{1,2}$ 2.9 Hz, H-1 of Glc*p*), 5.08 (s, 1 H, H-1 of Man*p*), 5.00 (s, 1 H, H-1 of Man*p*), 4.76–3.79 (m, 32 H), 3.68 (dd, 1 H), 3.36 (m, 1 H), 3.33 (s, 3 H, CH_3O); ^{13}C NMR ($CDCl_3$): δ 166.18, 166.01, 165.87, 165.77, 165.53, 165.50, 165.25, 165.25, 164.04, 165.04, 164.98, 164.82, 164.82, 164.62, 164.33 (15 Ph*C*O), 138.79, 138.49, 138.06, 137.84, 133.23, 133.03, 132.88, 132.78, 129.79, 129.71, 129.56, 129.44, 129.33, 129.26, 128.31, 128.20, 128.07, 127.90, 127.84, 127.66, 127.42 (Ph–C), 101.55, 100.54, 99.61, 99.37, 99.09, 97.52 (6 C-1), 81.59, 78.28, 78.12, 77.88, 77.19, 75.21, 74.77, 74.54, 73.05, 71.83, 71.42, 71.21, 71.04, 70.93, 70.61, 70.08, 69.90, 69.36, 69.01, 68.86, 68.49, 68.29, 67.82, 67.70, 67.01, 65.95, 63.88, 63.69, 61.80, 60.22 (C-2–6), 54.93 (CH_3). Anal. Calcd for $C_{170}H_{148}O_{46}$: C, 69.77; H, 5.06. Found: C, 70.01; H, 5.13.

3.12. Methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-[α-D-glucopyranosyl-(1→3)]-4,6-di-*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-mannopyranoside (15)

To a solution of **14** (0.40 mg, 0.14 mmol) in 1:4 MeOH–EtOAc (40 mL) was added Pd/C (5%, 40 mg). The reaction mixture was stirred for 3 days at rt in a hydrogen atmosphere, at the end of which time TLC indicated that the debenzylation of **14** was complete. Then the mixture was filtered, and the filtrate was concentrated under reduced pressure to dryness. Purification by column chromatography (1:3 petroleum ether–EtOAc) gave **15** as an amorphous solid (0.30 g, 85%): $[\alpha]_D^{20}$ −26.7° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.25–7.05 (m, 75 H, PhH), 6.14 (dd, 1 H, $J_{3,4} = J_{4,5} =$ 9.9 Hz, H-4 of Man*p*), 6.02–5.90 (m, 3 H, H-4 of Man*p*), 5.80 (dd, 1 H, $J_{2,3}$ 3.3 Hz, H-3 of Man*p*), 5.68 (m, 2 H), 5.61 (dd, 1 H, H-2 of Man*p*), 5.45 (dd, 1 H, H-2 of Man*p*), 5.23 (s, 1 H, H-1 of Man*p*), 5.21 (s, 1 H, H-1 of Man*p*), 5.18 (s, 1 H, H-1 of Man*p*), 5.10 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1 of Man*p*), 5.08 (s, 1 H, H-1 of Man*p*), 4.94 (dd, 1 H, H-3 of Man*p*), 4.90 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1 of Glc*p*), 4.63–3.87 (m, 22 H), 3.93 (s, 3 H, CH_3O), 3.42–3.34 (m, 4 H), 1.80 (bs, 4 H, OH); ^{13}C NMR ($CDCl_3$): δ 166.78, 166.73, 166.45, 166.39, 166.37, 166.31, 165.98, 165.80, 165.80, 165.50, 165.46, 165.42, 164.99, 164.99, 164.83 (15 Ph*C*O), 133.71, 133.63, 133.39, 133.25, 133.13, 130.31, 130.17, 130.05, 129.96, 129.85, 129.80, 129.20, 128.71, 128.53, 128.46, 128.26 (Ph–C), 102.05, 100.92, 100.92, 100.11, 100.03, 99.49 (6 C-1), 81.49, 78.79, 75.35, 74.11, 71.11, 72.65, 72.29, 71.92, 70.77, 69.93, 69.70, 69.47, 69.32, 68.75, 68.30, 67.86, 66.95, 66.77, 66.02, 63.95, 63.42, 63.10, 61.59 (C-2–6), 55.35 (CH_3O). Anal. Calcd for $C_{142}H_{124}O_{46}$: C, 66.46; H, 4.84. Found: C, 66.68; H, 4.93.

3.13. Methyl α-D-mannopyranosyl-(1→3)-α-D-mannopyranosyl-(1→2)-[α-D-glucopyranosyl-(1→3)]-α-D-mannopyranosyl-(1→2)-α-D-mannopyranosyl-(1→2)-α-D-mannopyranoside (16)

Compound **15** (0.23 g, 0.09 mmol) was dissolved in a satd solution of NH_3 in anhyd CH_3OH (10 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 **16** as a syrup (77 mg, 85%): $[\alpha]_D^{20}$ +29.6° (*c* 0.9, $CHCl_3$); ^{1}H NMR (D_2O): δ 5.16 (2 s, 2 H, H-1 of Man*p*), 5.15 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1 of Glc*p*), 5.03 (2 s, 2 H, H-1 of Man*p*), 4.89 (s, 1 H, H-1 of Man*p*), 4.16–4.07 (m, 3 H), 3.99–3.46 (m, 32 H), 3.30 (m, 1 H), 3.29 (s, 3 H, CH_3O); ^{13}C NMR (D_2O): δ 102.19, 101.58, 100.72, 100.71, 100.69 (5 C-1 of Man*p*), 99.37 (C-1 of Glc*p*), 78.82, 78.66, 77.89, 77.69, 77.29, 73.56, 73.39, 73.07, 72.79, 72.64, 72.15, 71.68, 70.46, 70.27, 70.13, 70.04, 69.84, 69.73, 67.18, 67.03, 66.95, 66.78, 66.31, 62.58, 61.17, 61.00, 60.79 (C-2–6), 54.93 (CH_3). MALDI-TOFMS Calcd for $C_{37}H_{64}O_{31}$ [M]: 1004.3. Found: 1027.4 [M+Na].

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