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Synthesis of two oligosaccharides, the GPI anchor glycans from *S. cerevesiae* and *A. fumigatus*

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Abstract—Two oligosaccharides, α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→6)-α-D-Man*p*-(1→4)-α-D-Glc*p*NAc (**I**) and 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→4)-α-D-Glc*p*NAc (**II**), the glycosylphosphatidylinositol (GPI) anchor glycans from *S. cerevesiae* and *A. fumigatus* were synthesized as their methyl glycosides in a regio- and stereoselective manner. The pentasaccharide **I** was obtained from 6-*O*-selective glycosylation of methyl 2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (**8**) with 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**9**), followed by benzoylation, deacetylation, and mannosylation, and then by deprotection. The hexasaccharide (**II**) was obtained via condensation of allyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (**17**) with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**16**), followed by deallylation, trichloroacetimidation, and coupling with acceptor (**8**), and finally by deprotection.

Keywords: Oligosaccharide; Mannose; Glucosamine

1. Introduction

Numerous membrane glycoproteins of eukaryotic cells are anchored in the lipid bilayer by a glycosylphosphatidylinositol (GPI) membrane anchor. GPI-anchored proteins are involved in many physiological processes including cell wall organization and biosynthesis. In fungi, structural and biosynthetic studies of GPI are mostly restricted to the model yeasts, *S. cerevesiae* and *A. fumigatus*, which are human pathogens. The GPI molecules of the former have a glycan with mainly four mannose residues, Manα1-2Manα1-2Manα1-6Manα1-4GlcNα1-6myo-inositol-1-PO_4,[1] while the GPI glycan moiety of the latter is mainly a linear pentamannose structure linked to a glucosamine residue: Manα1-3Manα1-2Manα1-2Manα1-6Manα1-4GlcN.[2] Several reports on the synthesis of GPI anchor have appeared.[3] In order to provide a detailed knowledge of chemical behavior and the structure–bioactivity relationships among these oligosaccharides, we present herein an unambiguous synthesis of the two inositol-omitted GPI glycans.

2. Results and discussion

As shown in Scheme 1, methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (**1**)[4] was chosen as the starting material. 4,6-*O*-Benzylidenation of **1**, followed by benzoylation with benzoyl chloride in pyridine and debenzylidenation with 90% HOAc, afforded the crude product **4**, which was directly successfully monobenzoylated to give methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside[5] (**5**) (82.2%, for four steps). Coupling of the mannose donor **6**[6] with the acceptor **5** in the presence of a catalytic amount TMSOTf furnished the (1→4)-linked disaccharide **7**. The 4,6-*O*-acetyl groups of **7** were successfully removed in 2–4% CH_3COCl–methanol[7] without affecting the acetamido and any benzoyl groups to give the common disaccharide acceptor **8** (77.7%). Condensation of

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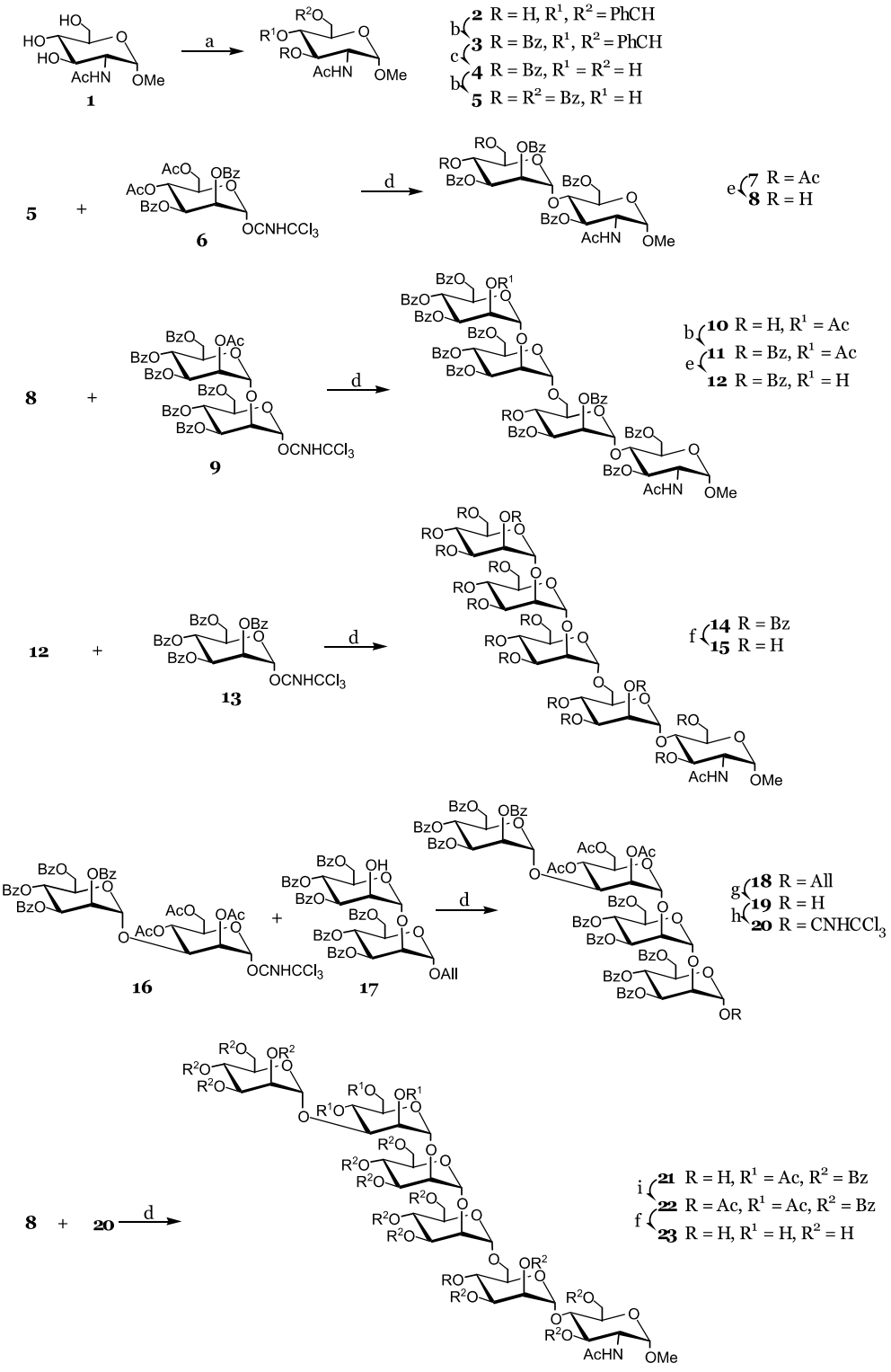

Scheme 1. Reagents and conditions: (a) PhCHO, triethyl orthoformate, CH_3CN, TsOH, rt, 2 h; (b) BzCl–pyridine (dry), rt, 12 h; 96.2% for **11**; (c) 90% HOAc, reflux, 6 h; (d) TMSOTf (0.01 equiv), CH_2Cl_2, −20 to 0 °C, 2–4 h; 80.4% for **7**, 90.5% for **10**, 75.6% for **14**, 66.3% for **18**, and 82.9% for **21**, respectively; (e) 2–4% CH_3COCl in CH_2Cl_2–CH_3OH, rt, 12 h; 77.7% for **8**, 74.5% for **12**; (f) Satd NH_3–MeOH, rt, 7 days; 92.2% for **15**, 86.0% for **23**; (g) $PdCl_2$, MeOH, rt, 2 h; 82.4%; (h) Cl_3CN, DBU, CH_2Cl_2, rt, 3h; 92.1%; (i) Ac_2O–pyridine (dry), rt, 12 h; 97.4%.

8 with 2-*O*-acetyl-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate[8] (**9**) selectively then gave tetrasaccharide **10** in high yield (90.5%). Keeping the temperature below −20 °C during the addition of TMSOTf was necessary in order to avoid byproduct formation. The

(1 → 6)-linkage was confirmed by benzoylation of **10** to give **11**, whereby the ^{1}H NMR spectrum of **11** showed a newly emerged downfield characteristic signal at δ 6.03 ppm (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz) for H-4′, compared to that of **10**. Consecutive deacetylation of **11** with 2–4% CH_3COCl–methanol afforded the tetrasaccharide acceptor **12** in satisfactory yield (74.5%). Subsequent glycosylation of **12** with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**13**) furnished the protected pentasaccharide **14** in good yield (75.6%). Finally deacylation of **14** in ammonia–methanol gave the target pentasaccharide **15**.

The title hexasaccharide was also synthesized in a concise way. α-(1 → 3)-Linked disaccharide donor **16**[9] and α-(1 → 2)-linked disaccharide acceptor **17**[8] were used as the key intermediates. Condensation of **17** with **16** gave tetrasaccharide **18** in acceptable yield (66.3%). Deallylation[10] of **18** with $PdCl_2$ in methanol, followed by trichloroacetimidation[11] with trichloroacetonitrile in the presence of potassium carbonate, produced the tetrasaccharide donor **20** (75.9% for two steps). Selective 6-*O*-glycosylation of disaccharide **8** with tetrasaccharide donor **20** gave the hexasaccharide **21** in high yield (82.9%). The ^{1}H and ^{13}C NMR spectra of **21** showed the characteristic peaks, such as at δ 5.90 ppm ($J_{H-C-N-H}$ 9.4 Hz) for N*H*Ac, 5.46, 5.35, 5.30, 5.28, 5.18 ppm for five Man*p* H-1, and 4.81 ppm ($J_{1,2}$ 3.4 Hz) for Glc*p* H-1; δ 100.5, 99.7, 99.6, 98.6, 98.3, 98.2 ppm for six C-1. The 6-*O*-glycosylation was verified by acetylation of **21** to furnish **22**, and the ^{1}H NMR spectrum of **22** showed a characteristic signal for H-4′ at δ 5.64 ppm with $J_{3,4} = J_{4,5} = 9.8$ Hz. Subsequent deacylation of **22** in ammonia–methanol furnished target hexasaccharide **23**.

In summary, two linear oligosaccharides, containing D-Glc*p*NAc and mannose were synthesized in an efficient way. Large-scale preparations should be possible with this method.

3. Experimental

3.1. General methods

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with Bruker ARX 400 spectrometers (400 MHz for ^{1}H, 100 MHz for ^{13}C) at 25 °C for solutions in $CDCl_3$ or D_2O as indicated, and individual resonances could not be identified with the specific sugar residues. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the ESI mode. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV lamp. Column chromatography was conducted by elution of a column (16 × 240 mm, 18 × 300 mm, 35 × 400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at <60 °C under reduced pressure.

3.2. General procedure for the glycosylations

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

3.3. Methyl 2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (5)

2-Acetamido-2-deoxy-D-glucose (2.00 g, 9.05 mmol) was added to MeOH (10 mL), then AcCl (0.5 mL) was added. The mixture was heated under reflux for 5 h, then evaporated to dryness. Purification of the residue by silica gel chromatography (10:1 EtOAc–MeOH) gave compound **1** (1.35 g, 63.5%). The physical data of the product were the same as that reported.[4] To a solution of **1** (197 mg, 0.84 mmol) in CH_3CN (5 mL) was added PhCHO (0.25 mL), triethyl orthoformate (0.34 mL) and TsOH (18 mg). After stirring at rt for 2 h, a white solid started to precipitate, which was collected by filtration and dried to give compound **2**. To a solution of **2** in pyridine (5 mL) was added benzoyl chloride (98.0 μL, 0.84 mmol) dropwise over 30 min. After stirring the mixture overnight at rt, methanol (2 drops) was added to the reaction mixture, and stirring was continued for 10 min. Water (10 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 × 10 mL). The extracts were washed with 1 M HCl and satd aq $NaHCO_3$, dried (Na_2SO_4), and concentrated to afford crude product **3**, which was directly added to 90% HOAc (10 mL). The mixture was heated under reflux for 6 h. Then the reaction mixture was concentrated and dried in high vacuum to give crude product **4**. Subsequent monobenzoylation of **4** with BzCl (5 mL) in pyridine (98.0 μL, 0.84 mmol) under the same conditions as described above gave the crude product, which was purified by silica gel column chromatography with 2:3 petroleum ether–EtOAc as the eluent to give **5**[5] (305 mg, 82.2% for four steps) as a foamy solid.

3.4. Methyl 4,6-di-*O*-acetyl-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (7)

As described in the general procedure, **6** (474 mg, 0.77 mmol) and **5** (284 mg, 0.64 mmol) were coupled, and the product was purified by silica gel column

chromatography with 2:1 petroleum ether–EtOAc as the eluent to give **7** (462 mg, 80.4%) as a foamy solid: $[\alpha]_D$ −12.0 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.14–7.19 (m, 20H, 4*Ph*), 5.88 (d, 1H, $J_{H\text{–}C\text{–}N\text{–}H}$ 9.5 Hz, N*H*Ac), 5.72 (dd, 1H, $J_{2,3}$ 8.8 Hz, $J_{3,4}$ 10.0 Hz, H-3), 5.58–5.53 (m, 2H, H-3′, H-4′), 5.38 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1′), 5.34 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 2.5 Hz, H-2′), 4.80 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 4.73 (dd, 1H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.64 (dd, 1H, $J_{5,6b}$ 4.0 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b), 4.47 (m, 1H, H-2), 4.32 (dd, 1H, $J_{5,6b}$ 4.1 Hz, $J_{6a,6b}$ 12.4 Hz, H-6′b), 4.26 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 4.21–4.17 (m, 2H, H-5, H-5′), 3.97 (dd, 1H, $J_{5,6a}$ 2.1 Hz, $J_{6a,6b}$ 12.4 Hz, H-6′a), 3.48 (s, 3H, CH_3O), 2.06 (s, 3H, CH_3CO), 1.91 (s, 3H, CH_3CO), 1.83 (s, 3H, CH_3CONH). Anal. Calcd for $C_{47}H_{47}NO_{17}$: C, 62.87; H, 5.28. Found: C, 62.59; H, 5.15.

3.5. Methyl 2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (8)

To a solution of **7** (433 mg, 0.48 mmol) in anhyd CH_2Cl_2 (10 mL) was added anhyd MeOH (50 mL), and then acetyl chloride (1.5 mL) was added to the reaction mixture at 0 °C. The solution was stoppered in a flask and stirred at rt until TLC (1:1 petroleum ether–EtOAc) showed that the starting material disappeared. The solution was neutralized with Et_3N, then concentrated to dryness. The residue was passed through a short silica gel column to give **8** (305 mg, 77.7%) as a foamy solid: $[\alpha]_D$ +5.0 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.12–7.18 (m, 20H, 4*Ph*), 5.94 (d, 1H, $J_{H\text{–}C\text{–}N\text{–}H}$ 9.6 Hz, N*H*Ac), 5.68 (dd, 1H, $J_{2,3}$ 8.9 Hz, $J_{3,4}$ 10.9 Hz, H-3), 5.44 (dd, 1H, $J_{2,3}$ 2.5 Hz, $J_{3,4}$ 9.8 Hz, H-3′), 5.29–5.27 (m, 2H, H-1′, H-2′), 4.80 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.72 (dd, 1H, $J_{5,6a}$ 1.9 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.65 (dd, 1H, $J_{5,6b}$ 3.7 Hz, $J_{6a,6b}$ 12.3 Hz, H-6b), 4.41–4.51 (m, 1H, H-2), 4.25–4.11 (m, 3H, H-4, H-5), 3.92–3.75 (m, 4H, H-4′, H-5′, H-6′a, H6′b), 3.48 (s, 3H, CH_3O), 1.83 (s, 3H, CH_3CONH). Anal. Calcd for $C_{43}H_{43}NO_{15}$: C, 63.46; H, 5.33. Found: C, 63.68; H, 5.44.

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 → 6)-2,3-di-*O*-benzoyl-α-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (10)

Donor **9** (213 mg, 0.18 mmol) was coupled with acceptor **8** (125 mg, 0.15 mmol) as described in the general procedure, and the product was purified by chromatography with 1:1 petroleum ether–EtOAc as the eluent to give **10** (251 mg, 90.5%) as a foamy solid: $[\alpha]_D$ −8.6 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.06–7.18 (m, 50H, 10*Ph*), 5.95–5.90 (m, 4H, N*H*Ac, Man*p* H-3, 2 H-4), 5.86 (dd, 1H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.0 Hz, Man*p* H-3), 5.71 (dd, 1H, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 10.9 Hz, Glc*p* H-3), 5.64 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3.2 Hz, Man*p* H-2), 5.48 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 10.5 Hz, Man*p* H-3), 5.41 (d, 1H, $J_{1,2}$ 1.5 Hz, Man*p* H-1), 5.37 (dd, 1H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.4 Hz, Man*p* H-2), 5.17 (d, 1H, $J_{1,2}$ 1.7 Hz, Man*p* H-1), 4.93 (d, 1H, $J_{1,2}$ 1.9 Hz, Man*p* H-1), 4.81 (d, 1H, $J_{1,2}$ 3.4 Hz, Glc*p* H-1), 3.47 (s, 3H, CH_3O), 2.02 (s, 3H, CH_3CO), 1.83 (s, 3H, CH_3CONH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.2, 169.2 (CH_3CO, CH_3CONH), 166.8, 166.6, 166.5, 166.2, 166.1, 165.6, 165.5, 165.3, 165.1, 164.5 (Ph*C*O), 133.4–132.9, 130.0–128.2 (*Ph*CO), 99.8, 99.6, 98.7, 98.3 (C-1), 76.1, 73.9, 73.4, 72.7, 70.8, 70.4, 69.8, 69.6, 69.5, 68.7, 68.3, 68.0, 67.1, 66.2, 66.0, 63.9, 63.5, 63.1, 54.4, 52.1 (C-2–C-6), 55.4 (*C*H_3O), 23.1, 20.6 (*C*H_3CONH, *C*H_3CO). Anal. Calcd for $C_{99}H_{89}NO_{32}$: C, 65.88; H, 4.97. Found: C, 65.71; H, 5.03.

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 → 6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (11)

To a solution of **10** (241 mg, 0.13 mmol) in pyridine (2 mL) was added benzoyl chloride (23.4 μL, 0.20 mmol). After stirring the mixture overnight at rt, TLC (2:3 petroleum ether–EtOAc) indicated that the reaction was complete. Methanol (2 drops) was added to the reaction mixture, and stirring was continued for 10 min. Water (10 mL) was added, the mixture was extracted with CH_2Cl_2 (3 × 10 mL), and the extracts were washed with 1 M HCl and satd aq $NaHCO_3$, dried (Na_2SO_4), and concentrated. Purification by flash chromatography (1:1 petroleum ether–EtOAc) gave **11** as a foamy solid (245 mg, 96.2%): $[\alpha]_D$ −3.5 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.03–7.12 (m, 55H, 11*Ph*), 6.03 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 5.99–5.76 (m, 7H, N*H*Ac, Man*p* 4 H-3, 2 H-4), 5.58 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 3.0 Hz, Man*p* H-2), 5.45–5.43 (m, 2H, Man*p* H-1, H-2), 5.06 (d, 1H, $J_{1,2}$ 1.6 Hz, Man*p* H-1), 4.91 (d, 1H, $J_{1,2}$ 1.5 Hz, Man*p* H-1), 4.83 (d, 1H, $J_{1,2}$ 3.4 Hz, Glc*p* H-1), 3.52 (s, 3H, CH_3O), 2.01 (s, 3H, CH_3CO), 1.86 (s, 3H, CH_3CONH). Anal. Calcd for $C_{106}H_{93}NO_{33}$: C, 66.70; H, 4.91. Found: C, 66.58; H, 4.80.

3.8. Methyl 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-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (12)

Compound **11** (228 mg, 0.12 mmol) were deacetylated under the same conditions as that used for preparation of **8** from **7**, giving **12** (166 mg, 74.5%) as a foamy solid: $[\alpha]_D$ −12.5 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.02–7.14 (m, 55H, 11*Ph*), 6.05–5.72 (m, 8H, N*H*Ac,

Man*p* 4 H-3, 3 H-4), 5.45 (d, 1H, $J_{1,2}$ 1.2 Hz, Man*p* H-1), 5.43 (m, 1H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.2 Hz, Man*p* H-2), 5.09 (d, 1H, $J_{1,2}$ 1.3 Hz, Man*p* H-1), 4.95 (d, 1H, $J_{1,2}$ 1.6 Hz, Man*p* H-1), 4.85 (d, 1H, $J_{1,2}$ 3.1 Hz, Glc*p* H-1), 3.52 (s, 3H, CH_3O), 1.86 (s, 3H, CH_3CONH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1 (CH_3*C*ONH), 166.6, 166.0, 165.9, 165.8, 165.4, 165.2, 165.1, 165.0, 164.4 (Ph*C*O), 133.0–128.0 (*Ph*CO), 101.8, 99.6, 98.8, 98.1 (C-1), 76.4, 73.7, 72.3, 71.2, 70.8, 70.3, 69.4, 69.3, 69.1, 68.5, 66.7, 66.4, 63.5, 63.1, 60.2, 52.3, 51.9 (C-2–C-6), 55.2 (*C*H_3O), 22.8 (*C*H_3CONH). Anal. Calcd for $C_{104}H_{91}NO_{32}$: C, 66.91; H, 4.91. Found: C, 67.15; H, 4.85.

3.9. Methyl 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-tri-*O*-benzoyl-α-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (14)

Compounds **12** (158 mg, 0.09 mmol) and **13** (125 mg, 0.17 mmol) were coupled under the same conditions as described in the general procedure. Purification of the product by chromatography with 2:3 petroleum ether–EtOAc as the eluent afforded **14** (156 mg, 75.6%) as a foamy solid: $[\alpha]_D$ −2.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.04–7.14 (m, 75H, 15*Ph*), 6.08–5.70 (m, 12H, N*H*Ac, Man*p* 5 H-3, 4 H-4, 2 H-2), 5.46–5.45 (m, 2H, Man*p* H-1, H-2), 5.31 (d, 1H, $J_{1,2}$ 1.3 Hz, Man*p* H-1), 5.02 (d, 1H, $J_{1,2}$ 1.1 Hz, Man*p* H-1), 4.85 (d, 1H, $J_{1,2}$ 3.1 Hz, Glc*p* H-1), 4.79 (d, 1H, $J_{1,2}$ 1.5 Hz, Man*p* H-1), 3.53 (s, 3H, CH_3O), 1.86 (s, 3H, CH_3CONH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1 (CH_3*C*ONH), 166.7, 166.1, 166.0, 165.7, 165.4, 165.3, 165.2, 165.1, 165.0, 164.9, 164.6, 164.5 (Ph*C*O), 133.1–128.0 (*Ph*CO), 100.6, 99.7, 99.6, 99.1, 98.3 (C-1), 76.4, 73.7, 72.1, 70.9, 70.4, 70.3, 69.9, 69.7, 69.6, 69.5, 69.3, 68.6, 67.1, 66.4, 66.3, 66.1, 63.5, 63.3, 63.1, 62.4, 60.2, 52.4 (C-2–C-6), 55.4 (*C*H_3O), 23.0 (*C*H_3CONH). Anal. Calcd for $C_{138}H_{117}NO_{41}$: C, 67.78; H, 4.82. Found: C, 67.88; H, 4.81.

3.10. Methyl α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 4)-2-acetamido-2-deoxy-α-D-glucopyranoside (15)

Pentasaccharide **14** (156 mg, 0.06 mmol) was dissolved in satd NH_3–MeOH (20 mL). After 7 days at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **15** (52 mg, 92.2%) as a foamy solid: $[\alpha]_D$ +28.5 (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 5.30 (s, 1H, Man*p* H-1), 5.24 (s, 1H, Man*p* H-1), 5.14 (s, 1H, Man*p* H-1), 5.05 (s, 1H, Man*p* H-1), 4.75 (d, 1H, $J_{1,2}$ 3.4 Hz, Glc*p* H-1), 3.38 (s, 3H, CH_3O), 2.04 (s, 3H, CH_3CONH); ^{13}C NMR (100 MHz, D_2O): δ 172.9 (CH_3*C*ONH), 102.0, 101.7, 100.4, 98.1, 97.7 (C-1), 78.6, 78.2, 77.2, 73.0, 72.5, 72.0, 71.3, 70.2, 70.1, 70.0, 69.8, 69.7, 66.8, 66.7, 66.3, 66.1, 60.9, 60.8, 60.7, 60.6, 54.9, 53.5 (C-2–C-6, *C*H_3O), 21.7 (*C*H_3CONH). Anal. Calcd for $C_{33}H_{57}NO_{26}$: C, 44.85; H, 6.50. Found: C, 44.97; H, 6.54.

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

Donor **16** (333 mg, 0.32 mmol) was coupled with acceptor **17** (296 mg, 0.29 mmol) as described in the general procedure, and the product was purified by chromatography with 2:1 petroleum ether–EtOAc as the eluent to give **18** (365 mg, 66.3%) as a foamy solid: $[\alpha]_D$ −23.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.12–7.24 (m, 50H, 10*Ph*), 6.25 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 6.00–5.72 (m, 6H, 3 H-3, 2 H-4, CH_2–C*H*=CH_2), 5.47–5.46 (m, 1H, H-2), 5.40 (m, 1H, H-2), 5.32 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1), 5.31 (s, 1H, H-1), 5.29–5.19 (m, 3H, CH_2–CH=CH_2, H-4), 5.17 (d, 1H, $J_{1,2}$ 1.3 Hz, H-1), 4.64 (s, 1H, H-1), 2.22, 2.07, 2.05 (3s, 9H, CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.1, 169.9 (CH_3*C*O), 166.3, 166.1, 165.9, 165.7, 165.3, 163.4 (Ph*C*O), 133.6–132.9, 130.1–128.2 (*Ph*CO), 100.3, 99.8, 98.4, 98.0 (C-1), 73.4, 71.4, 70.9, 70.7, 70.4, 69.7, 69.4, 68.9, 68.8, 67.8, 67.3, 66.7, 66.1, 63.6, 63.4, 62.8, 62.0 (C-2–C-6), 20.9, 20.7, 20.6 (*C*H_3CO). Anal. Calcd for $C_{103}H_{92}O_{34}$: C, 66.02; H, 4.95. Found: C, 66.18; H, 4.89.

3.12. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-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 trichloroacetimidate (20)

To a solution of **18** (352 mg, 0.19 mmol) in anhyd MeOH (10 mL) was added $PdCl_2$ (10 mg). After stirring the mixture at rt for 2 h, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, the solution was concentrated to dryness, and the resultant residue was purified by flash chromatography (5:3 petroleum ether–EtOAc) to give **19** (284 mg, 82.4%) as a white foam. A mixture of **19** (284 mg, 1.02 mmol), trichloroacetonitrile (28.8 μL, 0.29 mmol), and 1,8-diazabicyclo[5.4.0]-undecene (DBU) (8 μL) in dry CH_2Cl_2 (10 mL), was stirred under nitrogen for 3 h and then concentrated. The residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **20** (282 mg, 92.1%) as a foamy solid: $[\alpha]_D$ −24.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.72 (s, 1H, CN*H*CCl_3), 8.13–7.24 (m, 50H, 10*Ph*), 6.59

(d, 1H, $J_{1,2}$ 1.9 Hz, H-1), 6.24 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 6.08–5.72 (m, 5H, 3 H-3, 2 H-4), 5.46 (dd, 1H, $J_{1,2}$ 1.9 Hz, $J_{2,3}$ 2.9 Hz, H-2), 5.43 (d, 1H, $J_{1,2}$ 1.9 Hz, H-1), 5.39 (dd, 1H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 2.7 Hz, H-2), 5.30 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 5.23 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 4.69 (s, 1H, H-1), 2.22, 2.03, 2.02 (3s, 9H, CH_3CO). Anal. Calcd for $C_{102}H_{88}Cl_3NO_{34}$: C, 61.93; H, 4.48. Found: C, 62.22; H, 4.53.

3.13. Methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-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-di-*O*-benzoyl-α-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (21)

Compound **21** (268 mg, 82.9%) was obtained as a foamy solid by coupling of the donor **20** (268 mg, 0.14 mmol) with the acceptor **8** (100 mg, 0.12 mmol) as described in the general procedure: $[\alpha]_D$ −19.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09–7.18 (m, 70H, 14*Ph*), 6.25 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, Man*p* H-4), 6.01–5.98 (m, 2H, Man*p* 2 H-4), 5.90 (d, $J_{H\text{–}C\text{–}N\text{–}H}$ 9.4 Hz, N*H*Ac), 5.85–5.69 (m, 4H, Man*p* 3 H-3, Glc*p* H-3), 5.46 (d, 1H, $J_{1,2}$ 1.2 Hz, Man*p* H-1), 5.35 (d, 1H, $J_{1,2}$ 1.6 Hz, Man*p* H-1), 5.30 (d, 1H, $J_{1,2}$ 1.9 Hz, Man*p* H-1), 5.24 (dd, 1H, $J_{3,4} = J_{4,5} = 10.0$ Hz, Glc*p* H-4), 5.28 (d, 1H, $J_{1,2}$ 1.5 Hz, Man*p* H-1), 5.18 (d, 1H, $J_{1,2}$ 1.3 Hz, Man*p* H-1), 4.81 (d, 1H, $J_{1,2}$ 3.4 Hz, Glc*p* H-1), 3.49 (s, 3H, CH_3O), 2.21, 2.05, 2.04 (3s, 9H, CH_3CO), 1.84 (s, 3H, CH_3CONH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.0, 169.9, 169.6 (CH_3CO), 166.6, 166.5, 166.4, 166.0, 165.8, 165.4, 165.3, 165.2, 164.4 (Ph*C*O), 133.2–128.2 (*Ph*CO), 100.5, 99.7, 99.6, 98.6, 98.3, 98.2 (C-1), 76.0, 73.7, 73.4, 73.0, 72.6, 71.4, 70.8, 70.6, 70.3, 70.2, 69.6, 69.5, 69.4, 69.2, 68.6, 68.1, 67.6, 67.5, 66.7, 66.6, 66.3, 66.0, 63.7, 63.4, 63.2, 62.5, 62.0, 60.3, 52.3 (C-2–C-6), 55.3 (CH_3O), 23.0, 20.8, 20.5 (CH_3CO, CH_3CONH). Anal. Calcd for $C_{143}H_{129}NO_{48}$: C, 65.32; H, 4.94. Found: C, 65.65; H, 5.02.

3.14. Methyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-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-di-*O*-benzoyl-4-*O*-acetyl-α-D-mannopyranosyl-(1 → 4)-2-acetamido-3,6-di-*O*-benzoyl-2-deoxy-α-D-glucopyranoside (22)

To a solution of compound **21** (253 mg, 0.10 mmol) in pyridine (5 mL) was added Ac_2O (3 mL, 3 mmol). The reaction mixture was stirred at rt for 12 h and concentrated to give the crude product that was purified by silica gel column chromatography (2:3 petroleum ether–EtOAc) to give hexasaccharide **22** (250 mg, 97.4%) as a foamy solid: $[\alpha]_D$ −26.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11–7.24 (m, 70H, 14*Ph*), 6.25 (dd, 1H, $J_{3,4} = J_{4,5} = 10.1$ Hz, Man*p* H-4), 6.06–5.98 (m, 2H, Man*p* 2 H-4), 5.92 (d, $J_{H\text{–}C\text{–}N\text{–}H}$ 9.5 Hz, N*H*Ac), 5.84–5.72 (m, 4H, Man*p* 3 H-3, Glc*p* H-3), 5.64 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.45 (dd, 1H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.1 Hz, Man*p* H-2), 5.38 (d, 1H, $J_{1,2}$ 1.6 Hz, Man*p* H-1), 5.36 (d, 1H, $J_{1,2}$ 1.6 Hz, Man*p* H-1), 5.29 (d, 1H, $J_{1,2}$ 1.5 Hz, Man*p* H-1), 5.28 (d, 1H, $J_{1,2}$ 1.5 Hz, Man*p* H-1), 5.21 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.9 Hz, Glc*p* H-4), 5.11 (d, 1H, $J_{1,2}$ 1.2 Hz, Man*p* H-1), 4.83 (d, 1H, $J_{1,2}$ 3.4 Hz, Glc*p* H-1), 3.51 (s, 3H, CH_3O), 2.21, 2.02, 1.96, 1.86, 1.85 (5s, 15H, 4CH_3CO, CH_3CONH). Anal. Calcd for $C_{145}H_{131}NO_{49}$: C, 65.19; H, 4.94. Found: C, 65.10; H, 4.87.

3.15. Methyl α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 4)-2-acetamido-2-deoxy-α-D-glucopyranoside (23)

Hexasaccharide **22** (243 mg, 0.09 mmol) was dissolved in satd NH_3–MeOH (30 mL). After 7 days at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **23** (82 mg, 86.0%) as a foamy solid: $[\alpha]_D$ −22.5 (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 5.29 (s, 1H, Man*p* H-1), 5.22 (s, 1H, Man*p* H-1), 5.14 (s, 2H, Man*p* 2 H-1), 5.03 (s, 1H, Man*p* H-1), 4.73 (d, 1H, $J_{1,2}$ 3.2 Hz, Glc*p* H-1), 3.36 (s, 3H, CH_3O), 2.03 (s, 3H, CH_3CONH); ^{13}C NMR (100 MHz, D_2O): δ 172.8 (CH_3CONH), 101.9, 101.8, 101.6, 100.4, 98.1, 97.7 (C-1), 78.6, 78.2, 77.6, 77.2, 76.4, 73.5, 73.1, 73.0, 72.5, 72.0, 71.3, 70.2, 70.1, 70.0, 69.9, 69.8, 69.4, 66.8, 66.7, 66.3, 66.1, 66.0, 60.9, 60.7, 60.6, 60.4, 54.9, 53.5 (C-2–C-6, CH_3O), 21.7 (CH_3CONH). Anal. Calcd for $C_{39}H_{67}NO_{31}$: C, 44.78; H, 6.46. Found: C, 44.89; H, 6.41.

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