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Synthesis of a mannose heptasaccharide of the pathogenic yeast, *Candida glabrata* IFO 0622 strain

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Abstract

An effective synthesis of the mannose heptasaccharide existing in the pathogenic yeast, *Candida glabrata* IFO 0622 strain was achieved via TMSOTf-promoted condensation of a tetrasaccharide donor **13** with a trisaccharide acceptor **16**, followed by deprotection. The tetrasaccharide **13** was constructed by coupling of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**7**) with allyl 3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (**10**), followed by deallylation and trichloroacetimadation. The trisaccharide **16** was obtained by coupling of 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate with **10**, and subsequent 6-O-deacetylation. The disaccharide **7** was prepared through coupling of perbenzoylated mannosyl trichloroacetimidate with 4,6-*O*-benzylidene-1,2-*O*-ethylidene-β-D-mannopyranose, then simultaneous debenzylidenation and deethylidenation, and subsequent acetylation, selective 1-O-deacetylation, and trichloroacetimidation. The disaccharide **10** was obtained by self-condensation of 3,4,6-tri-*O*-benzoyl-1,2-*O*-allyloxyethylidene-β-D-mannopyranose, followed by selective 2-O-deacetylation.

Keywords: Mannose oligosaccharides; Trichloroacetimidates; Regio- and stereoselective synthesis

1. Introduction

Candida species are opportunistic pathogens of humans that frequently cause severe systemic infections in patients with AIDS,[1] cancer,[2] and burns[3] as well as in those under immunosuppressive or radiation therapy.[4] *Candida glabrata* as a medically important fungus has been steadily attracting attention from microbiologists interested in infectious disease research.[5] A structural analysis of the cell wall mannan isolated from *C. glabrata* IFO 0622 strain was carried out by Suzuki's group.[6] Three novel oligosaccharides, i.e., a tetraose, a hexaose, and a heptaose, were obtained from mild acetolysis of acid- and alkali-stable mannan moiety. The tetraose and hexaose were known α-(1→2)- and α-(1→3)-linked structures, while the heptaose was identified as 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*-(1→2)-D-Man. To the best of our knowledge, there have been no reports dealing with the synthesis of the heptasaccharide. For an investigation of structure–function relationships of mannan, we present herein a facile and convergent synthesis of the mannose heptasaccharide.

2. Results and discussion

Retrosynthetic analysis indicated that the mannose heptamer can be obtained with a (1→6) linkage by condensation of two moieties, i.e., a mannose tetramer donor and a mannose trimer acceptor. The tetraasaccharide then can be constructed from a (1→3)-linked disaccharide donor and a (1→2)-linked disaccharide acceptor, while the trisaccharide can be built from the same (1→2)-linked disaccharide acceptor and a mannose donor.

Our synthetic route is shown in Scheme 1. Coupling of perbenzoylated mannosyl trichloroacetimidate[7] **1** with 4,6-*O*-benzylidene-1,2-*O*-ethylidene-β-D-mannopy-

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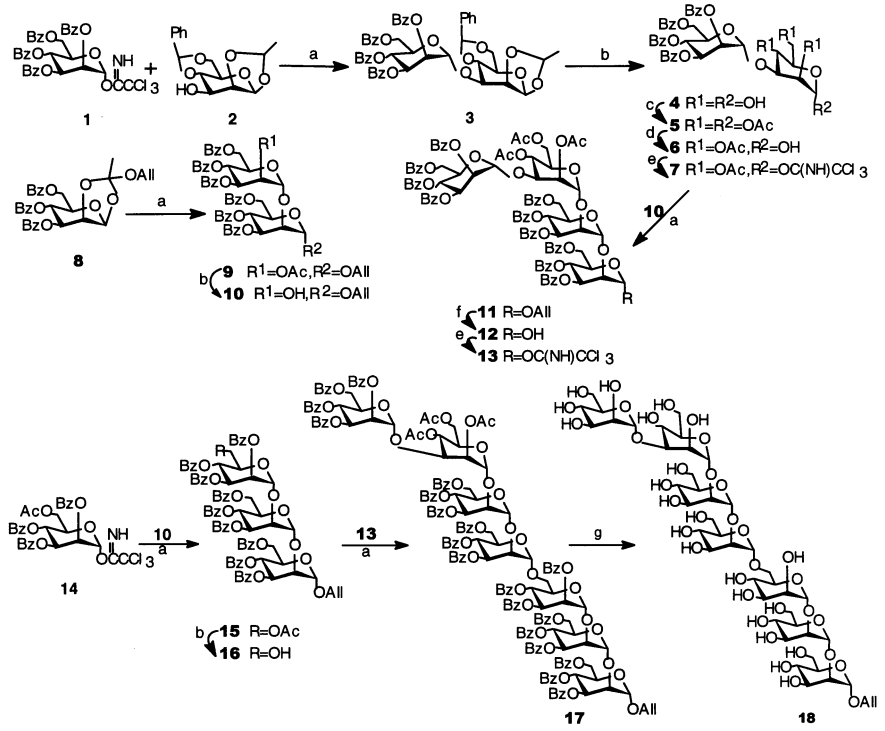

Scheme 1. Reagents and conditions: a. TMSOTf, CH_2Cl_2, N_2, -15 °C to rt, 4 h; b. CH_2Cl_2, CH_3OH–CH_3COCl, rt; c. Ac_2O/py (dry), rt, 6 h; d. CH_2Cl_2, NH_3–MeOH, rt; e. CCl_3CN, DBU, CH_2Cl_2, rt, 8 h; f. $PdCl_2$, CH_2Cl_2, rt, 2 h; g. NH_3–MeOH, rt.

ranose (**2**) gave 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-benzylidene-1,2-*O*-ethylidene-β-D-mannopyranose (**3**) in high yield (83%). Removal of the benzylidene and ethylidene groups was readily achieved simultaneously with 0.3:50:10 acetyl chloride–methanol–dichloromethane within 1 h, giving the disaccharide **4** as a white solid in high yield (90%) after purification. This reaction was smooth and easily controlled. Acetylation of **4** with acetic anhydride in pyridine, followed by selective 1-O-deacetylation with M solution of ammonia in 1:1 methanol–dichloromethane, and then trichloroacetimidation with trichloroacetonitrile in the presence of DBU furnished 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (**7**) in 61% yield (for three steps). The disaccharide **10** was prepared by TMSOTf-promoted self condensation of 3,4,6-tri-*O*-benzoyl-1,2-*O*-allyloxyethylidene-β-D-mannopyranose,[8] followed by selective 2-O-deacetylation with 0.5:100:50 acetyl chloride–methanol–dichloromethane.[8,9] Condensation of the disaccharide donor **7** with the disaccharide acceptor **10** gave the tetrasaccharide **11** in 61% yield. Deallylation[10] of **11** with $PdCl_2$ in dichloromethane furnished the tetrasaccharide hemiacetal **12**, and subsequent trichloroacetimidation produced the tetrasaccharide donor **13**. Coupling of 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate[11] (**14**) with the disaccharide acceptor **10**, followed by selective 6-O-deacetylation produced the trisaccharide acceptor **16**. Finally, condensation of the tetrasaccharide donor **13** with **16** gave the heptasaccharide **17**, and subsequent deacylation in ammonia-saturated methanol yielded the target mannose heptasaccharide **18**, whose bioassay is in progress.

Compared to the previously reported syntheses[10,12] of complex mannans containing (1→2), (1→3), and (1→6) linkages, the method presented herein is simpler and convergent, owing to the sole use of acyl protection groups. It should be possible to carry out large-scale synthesis employing the method as described.

3. Experimental

General methods.—Optical rotations were determined at 25 °C with a Perkin–Elmer model 241-Mc automatic polarimeter. ^1H, ^{13}C NMR and ^1H–^{13}C COSY spectra were recorded with Bruker ARX 400 spectrometers (400 MHz for ^1H, 100 MHz for ^{13}C) at 25 °C for solutions in $CDCl_3$ or D_2O as indicated. Mass spectra were measured with MALDITOF-MS spectrometer or recorded with a VG PLATFORM mass spectrometer using the ESI mode. Thin-layer chromatography (TLC) was performed on Silica Gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV 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. The pure R-isomer[13] of 1,2-*O*-ethylidene-β-D-mannopyranose was used for the synthesis of **2**, giving predominantly the R-isomer of **2**, and subsequently **3** as well.

2,3,4,6-Tetra-O-*benzoyl*-α-D-*mannopyranosyl*-(1→3)-*4,6*-O-*benzylidene*-*1,2*-O-*ethylidene*-β-D-*mannopyranose* (**3**).—To a cooled solution (0 °C) of **1** (7.40 g, 10 mmol) and **2** (R-form, 2.94 g, 10 mmol) in anhyd CH_2Cl_2 (50 mL) was added TMSOTf (50 μL, 0.28 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 disaccharide **3** as white foam (4.72 g, 83%): For R-isomer: $[\alpha]_D$ −80.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07–7.37 (m, 25 H, Ph*H*), 6.07 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4′), 6.01 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.8 Hz, H-3′), 5.86 (dd, 1 H, $J_{1,2}$ 1.0, $J_{2,3}$ 3.0 Hz, H-2′), 5.66 (s, 1 H, PhC*H*), 5.59 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1′), 5.41 (q, 1 H, *J* 4.7 Hz, MeC*H*), 5.07 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.69 (dd, 1 H, $J_{2,3}$ 2.0, $J_{3,4}$ 9.7 Hz, H-3), 4.62 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6'a}$ 4.2, $J_{5,6'b}$ 2.6 Hz, H-5′), 4.70 (dd, 1 H, $J_{5,6'}$ 4.5, $J_{6,6'}$ 13.5 Hz, H-6′), 4.34–4.23 (m, 4 H, H-2, H-6′ and 2 H-6), 3.81 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 3.36 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{5,6a}$ 4.5, $J_{5,6b}$ 4.3 Hz, H-5), 1.58 (d, 3 H, *J* 4.7 Hz, *Me*CH). Anal. Calcd for $C_{49}H_{44}O_{15}$: C 67.42; H 5.08. Found: C 67.21; H 5.06.

2,3,4,6-Tetra-O-*benzoyl*-α-D-*mannopyranosyl*-(1→3)-*2,4,6-tri*-O-*acethyl*-α-D-*mannopyranosyl trichloroacetimidate* (**7**).—To a solution of **3** (7.58 g, 10.0 mmol) in anhyd MeOH (250 mL) and CH_2Cl_2 (50 mL) was added AcCl (1.5 mL). The flask was stoppered, and the solution was stirred at rt for 1 h, at the end of which time TLC (1:2 petroleum ether–EtOAc) showed that the starting material had disappeared. The solution was neutralized with Et_3N, then concentrated to dryness. The residue was washed with water and extracted 3–4 times with CH_2Cl_2. The organic phase was dried over anhyd Na_2SO_4, then concentrated to dryness. The residue was passed through a short silica gel column (1:1.5 petroleum ether–EtOAc) to give **4** (6.86g, 90%) as a white solid. The white solid was dissolved in pyridine (40 mL), and then Ac_2O (20 mL) was added. After stirring the mixture at rt for 12 h, TLC (3:2 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated to dryness. The resultant crude product **5** was dissolved in a M solution of NH_3–MeOH (400 mL) and stirred at rt until TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was concentrated to give compound **6** as a syrup. A mixture of **6**, trichloroacetonitrile (3.2 mL, 15 mmol), and 1,8-diazabicyclo[5.4.0]undecene (DBU) (0.50 mL, 4.04 mmol) in dry CH_2Cl_2 (50 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 **7** (5.55 g, 61% for three steps from **5** to **7**) as a white foam: $[\alpha]_D$ −8.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.79 (s, 1 H, N*H*), 8.15–7.21 (m, 20 H, 4 BzH), 6.35 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 6.20 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4′), 5.78 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.9 Hz, H-3′), 5.59 (dd, 1 H, $J_{1,2}$ 1.4, $J_{2,3}$ 3.2 Hz, H-2′), 5.54 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 5.48 (dd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 3.2 Hz, H-2), 5.37 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1′), 4.64–4.56 (m, 2 H, 2 H-6′), 4.47 (dd, 1 H, $J_{5,6}$ 2.6, $J_{6,6}$ 12.1 Hz, H-6), 4.42 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3), 4.27 (dd, 1 H, $J_{5,6}$ 5.2, $J_{6,6}$ 12.4 Hz, H-6), 4.21–4.10 (m, 2 H, H-5, H-5′), 2.39 (s, 3 H, *Me*CO), 2.24 (s, 3 H, *Me*CO), 2.10 (s, 3 H, *Me*CO). Anal. Calcd for $C_{48}H_{44}Cl_3NO_{18}$: C, 56.01; H, 4.31. Found: C, 55.88; H, 4.35.

Allyl 3,4,6-tri-O-*benzoyl*-α-D-*mannopyranosyl*-(1→2)-*3,4,6-tri*-O-*benzoyl*-α-D-*mannopyranoside* (**10**).—To a solution of **9** (8.67 g, 10.0 mmol) in anhyd MeOH (100 mL) and CH_2Cl_2 (50 mL) was added AcCl (0.5 mL). The flask was stoppered, and the solution was stirred at rt until TLC (3:1 petroleum ether–EtOAc) showed that the starting material disappeared (about 0.5–1 h). The solution was neutralized with Et_3N, then concentrated to dryness. The residue was passed through a short silica gel column (3:1 petroleum ether–EtOAc) to give **10** (6.69 g, 80%) as a white solid: $[\alpha]_D$ −14.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14–7.24 (m, 30 H, 6 BzH), 5.97 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4′), 5.90 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 5.88 (m, 1 H, C*H*=CH$_2$), 5.82 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.9 Hz, H-3′), 5.79 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 10.0 Hz, H-3), 5.26 (dd, 1 H, *J* 1.5, *J* 17.2 Hz, CH=C*H*$_2$), 5.18 (dd, 1 H, *J* 1.5, *J* 10.6 Hz, CH=C*H*$_2$), 5.17 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1′), 5.14 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.63–4.35 (m, 8 H, H-2′, H-2, H-5′, H-5, 2 H-6′, 2 H-6), 4.18 (dd, 1 H, *J* 5.4, *J* 12.7 Hz, C*H*$_2$–CH=CH$_2$), 3.92 (dd, 1 H, *J* 6.0, *J* 12.7 Hz, C*H*$_2$–CH=CH$_2$). Anal. Calcd for $C_{57}H_{50}O_{17}$: C, 67.99; H, 5.00. Found: C, 68.12; H, 5.03.

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* (**13**).—Compound **11** (3.25 g, 61%) was prepared by coupling of **7** (2.91 g, 2.83 mmol) with **10** (2.84 g, 2.82 mmol) under the same conditions as described for the synthesis of **3** by coupling of **1** with **2**. To a solution of **11** (0.8 g, 0.43 mmol) in anhyd MeOH (20 mL) was added $PdCl_2$ (50 mg). After stirring the mixture at rt for 2 h, TLC (3:2 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, and the solution was concentrated to dryness. The resultant residue was purified by flash chromatography

(2:1 petroleum ether–EtOAc) to give **13** (0.6 g, 70% for two steps) as a white foam: $[\alpha]_D$ −19.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.69 (s, 1 H, NH), 8.15–7.21 (m, 50 H, 10 BzH), 6.59 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1), 6.24 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4‴), 6.05 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.3 Hz, H-4′), 5.97 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4″), 5.81 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.1 Hz, H-3‴), 5.78 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 10.3 Hz, H-3′), 5.73 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.1 Hz, H-3″), 5.47–5.39 (m, 3 H, H-1‴, H-1″, H-3), 5.30 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1′), 5.23 (dd, 1 H, $J_{3,4} = J_{4,5}$ 12.0 Hz, H-4), 4.71–4.34 (m, 13 H, H-2‴, H-2″, H-2′, H-2, H-5‴, 2 H-6‴, 2 H-6″, 2 H-6′, 2 H-6), 3.96 (m, 3 H, H-5″, H-5′, H-5), 2.22 (s, 3 H, *Me*CO), 2.04 (s, 3 H, *Me*CO), 2.02 (s, 3 H, *Me*CO). Anal. Calcd for $C_{102}H_{88}Cl_3NO_{34}$: C, 61.93; H, 4.48. Found: C, 61.78; H, 4.43.

Allyl 2,3,4-tri-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* (**16**).—To a cooled solution (0 °C) of **10** (1.01 g, 1 mmol) and **14** (0.81g, 1.2 mmol) in anhyd CH_2Cl_2 (50 mL) was added TMSOTf (30 μL, 0.12 mmol). The mixture was stirred at 0 °C for 2 h and then quenched with Et_3N (2 drops). The solvent was evaporated to give a residue, which was purified by silica gel column chromatography (2:1 petroleum ether–EtOAc) to give trisaccharide **15** as a foamy solid (1.24 g, 82%). Compound **15** was dissolved in anhyd MeOH (200 mL) and CH_2Cl_2 (100 mL), and to the mixture was added AcCl (1.2 mL). The flask was stoppered, and the solution was stirred at rt until TLC (1:1 petroleum ether–EtOAc) showed that the starting material disappeared (2 h). The solution was neutralized with Et_3N, then concentrated to dryness. The residue was passed through a short silica gel column (1:2 petroleum ether–EtOAc) to give **16** (810 mg, 67% for two steps) as a white solid: $[\alpha]_D$ −71.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14–7.24 (m, 45 H, 9 BzH), 6.04–5.86 (m, 5 H, H-3″, H-4, H-4′, H-4″, C*H*=CH_2), 5.71–5.65 (m, 3 H, H-3, H-3′,H-2″), 5.39 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1″), 5.27 (dd, 1 H, *J* 1.4, *J* 17.2 Hz, CH=*C*H_2), 5.18 (dd, 1 H, *J* 1.4, *J* 10.3 Hz, CH=*C*H_2), 5.14 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1′), 4.73 (d, 1 H, $J_{1,2}$ 1.0 Hz, H-1), 4.63–4.50 (m, 6 H, H-5″, 2 H-6″, 2 H-6′, H-6),. 4.41–4.36 (m, 2 H, H-5′, H-6), 4.18 (dd, 1 H, *J* 6.0, *J* 12.7 Hz, C*H*$_2$–CH=CH_2), 4.11 (ddd, 1 H, $J_{4,5}$ 11.3, $J_{5,6a}$ 8.8, $J_{5,6b}$ 4.4 Hz, H-5), 3.96 (dd, 1 H, *J* 6.0, *J* 12.7 Hz, C*H*$_2$–CH=CH_2), 3.52 (m, 2 H, H-2′, H-2). ^{13}C NMR (100 MHz, $DCCl_3$): δ 166.2, 166.2, 166.0, 166.0, 165.4, 165.2, 165.1, 164.9, 164.7 (9 C, 9 PhCO), 133.5–132.6, 130.0–128.2 (PhCO), 118.0 (1 C, –CH_2–CH=*C*H_2), 100.4, 99.7, 98.0 (3 C, 3 C-1), 71.9, 71.9, 70.3, 70.0, 69.6, 69.3, 68.7, 68.7, 68.6, 67.3, 67.0, 66.7, 63.5, 63.5, 61.6, 61.4 (C-2–C-6, –*C*H_2–CH=CH_2). Anal. Calcd for $C_{84}H_{72}O_{25}$: C, 68.10; H, 4.90. Found: C, 67.90; H, 4.87.

Allyl 2,3,4,6-tetra-O-*benzoyl*-α-D-*mannopyranosyl*-(1→3)-*2,4,6-tri*-O-*acethyl*-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→2)-*3,4,6-tri*-O-*benzoyl*-α-D-*mannopyranosyl*-(1→2)-*3,4,6-tri*-O-*benzoyl*-α-D-*mannopyranoside* (**17**).—Under the same conditions as described for the synthesis of **3** by coupling of **1** with **2**, heptasaccharide **17** (94 mg, 51%) was obtained from coupling of **13** (150 mg, 0.076 mmol) with **16** (100 mg, 0.067 mmol): $[\alpha]_D$ −19.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19–7.20 (m, 95 H, 9 BzH), 6.31–6.18 (m, 2 H, 2 H-4), 6.08–5.63 (m, 11 H, H-2, 4 H-3, 5 H-4, C*H*=CH_2), 5.51–5.15 (m, 10 H, 4 H-1, H-2, 3 H-3, 2 CH=C*H*$_2$), 4.81–4.33 (m, 22 H, 3 H-1, 3 H-2, 4 H-5, 12 H-6), 4.30–4.08 (m, 5 H, 2 H-5, 2 H-6, C*H*$_2$–CH=CH_2), 4.06–3.68 (m, 4 H, 2 H-2, H-5, C*H*$_2$–CH=CH_2), 2.19 (s, 3 H, *Me*CO), 1.94 (s, 3 H, *Me*CO), 1.85 (s, 3 H, *Me*CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.8, 169.6 (3 C, 3 Me*C*O), 166.2, 165.9, 165.8, 165.7, 165.6, 165.5, 165.3, 165.2, 165.1, 165.0, 164.9 (19 C, Ph*C*O), 133.7–132.2, 130.2–129.2, 129.1–127.9 (*Ph*CO, –CH_2–*C*H=CH_2), 118.0 (1 C, –CH_2–CH=*C*H_2), 100.6, 99.8, 99.8, 99.7, 99.0, 98.3, 98.0 (7 C-1), 77.3 (C-3⁗), 73.4, 72.2, 71.3, 70.8, 70.6, 70.3, 70.1, 70.0, 69.7, 69.6, 69.4, 69.2, 68.7, 68.5, 67.2, 66.3, 65.9, 65.8, 63.6, 63.5, 62.9, 62.8, 62.2, 62.0 (C-2–C-6, –*C*H_2–CH=CH_2), 21.4, 20.7, 20.5 (3 *Me*CO). Anal. Calcd for $C_{184}H_{158}O_{58}$: C, 67.03; H, 4.83. Found: C, 67.10; H, 4.82.

Allyl 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-*mannopyranosyl*-(1→2)-α-D-*mannopyranoside* (**18**).—Compound **17** (75 mg, 0.0275 mmol) was dissolved in satd NH_3–MeOH (10 mL). After 2 weeks at rt, the reaction solution was concentrated, and the residue was purified on a BioGel P2 column with MeOH–water as the eluent to afford **18** (24 mg, 80%) as a pulverous crystalloid: $[\alpha]_D$ +52.6° (*c* 1.0, water); ^{1}H NMR (400 MHz, D_2O): δ 5.87 (m, 1 H, C*H*=CH_2), 5.25 (dd, 1 H, *J* 1.6, *J* 17.2 Hz, CH=C*H*$_2$), 5.20, 5.18, 5.10, 5.05, 5.04 (5 H, H-1), 5.01 (dd, 1 H, *J* 1.6, *J* 10.8 Hz, CH=C*H*$_2$), 4.94, 4.94 (2 H, H-1), 4.18–3.40 (m, 44 H, C*H*$_2$–CH=CH_2, H-2–H-6). ^{13}C NMR (100 MHz, D_2O): δ 132.7 (1 C, –CH_2–*C*H=CH_2), 117.9 (1 C, –CH_2–CH=*C*H_2), 101.7, 101.7, 101.6, 100.3, 100.0, 97.7, 96.9 (7 C-1, $J_{C\text{-}1,H\text{-}1}$ 172.0–173.5 Hz), 78.2, 78.1, 78.0, 77.8, 77.4, 75.4, 75.2, 75.0, 73.6, 72.9, 72.8, 72.8, 72.4, 72.3, 72.2, 71.5, 71.1, 70.2, 70.0, 70.0, 69.8, 69.7, 69.5, 69.4, 69.1, 67.6, 66.5, 66.4, 66.3, 65.8, 65.6, 61.9, 60.5, 60.4, 60.2, 60.2 (C-2–C-6, –*C*H_2–CH=CH_2). Anal. Calcd for $C_{45}H_{76}O_{36}$: C, 45.30; H, 6.42. Found: C, 45.17; H, 6.47.

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References

1. Goodman, D. S.; Teplitz, E. D.; Wishner, A.; Klein, R. S.; Burk, P. G.; Hershenbaum, E. *J. Am. Acad. Dermatol.* **1987**, *17*, 210–218.
2. Bodey, G. P. *Am. J. Med.* **1984**, *77*, 13–22.
3. Spebar, M. J.; Pruitt, B. A. *J. Trauma* **1981**, *21*, 237–243.
4. Silverman, S., Jr.; Luangjarmekorn, L.; Greenspan, D. *J. Oral Med.* **1984**, *39*, 194–201.
5. Goldman, W. E. *Science* **1999**, *285*, 539–541.
6. Kobayashi, H.; Mitobe, H.; Takahashi, K.; Yamamoto, T.; Shibata, N.; Suzuki, S. *Arch. Biochem. Biophys.* **1992**, *294*, 662–669.
7. Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21–125.
8. (a) Zhu, Y.; Kong, F. *Synlett* **2000**, 1783–1788;
   (b) Zhu, Y.; Kong, F. *Chin. J. Chem.* **2001**, *19*, 119–123.
9. (a) Auzanneau, F.-I.; Forooghian, F.; Pinto, B. M. *Carbohydr. Res.* **1996**, *291*, 21–40;
   (b) Wang, W.; Kong, F. *Carbohydr. Res.* **1999**, *315*, 128–135.
10. Ogawa, T.; Yamamoto, H. *Carbohydr. Res.* **1985**, *137*, 79–87.
11. Heng, L.; Ning, J.; Kong, F. *J. Carbohydr. Chem.* **2001**, *20*, 285–296.
12. (a) Nakahara, Y.; Shibayama, S.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1996**, *280*, 67–84;
   (b) Grice, P.; Ley, S. V.; Pietrunzka, J. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 197–200;
   (c) Ogawa, T.; Sasajima, K. *Tetrahedron* **1981**, *37*, 2787–2792.
13. Betaneli, V. I.; Ovchinnikov, M. V.; Kochetkov, N. K. *Carbohydr. Res.* **1982**, *107*, 285–291.