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Synthesis of a mannose heptasaccharide existing in baker's yeast, *Saccharomyces cerevisiae* X2180-1A wild-type strain

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Abstract

A mannose heptasaccharide existing in baker's yeast, *Saccharomyces cerevisiae* X2180-1A wild-type strain, was effectively synthesized as its allyl glycoside via TMSOTf-promoted condensation of a disaccharide donor **13** with a pentasaccharide acceptor **12**, followed by deprotection. The pentasaccharide **12** was constructed by coupling of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-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-mannopyranosyl trichloroacetimidate (**9**) with allyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside (**10**), followed by deacetylation. The tetrasaccharide **9** was obtained by coupling of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**5**) with allyl 3,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (**6**), followed by deallylation and trichloroacetimidation. The disaccharides **6** and **13** were readily obtained by known methods.

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

1. Introduction

α-Linked oligo-D-mannosyl side chains of cell-wall D-mannan of the pathogenic yeast *Candida* species plays important roles in the binding of yeast cells to the marginal zone of mouse spleen[1] and in the mechanism of several types of yeast flocculation.[2] These facts seem of interest from the viewpoints of both host–parasite interactions and the biological roles of mannan. Mild acetolysis and NMR studies[3] of the D-mannan of *Saccharomyces cerevisae* X2180-1A wild-type strain revealed that 1A have a highly branched structure, and a D-mannoheptaose containing α-(1→3)-, α-(1→2)-, α-(1→6)-linkages, was isolated after mild acetolysis (Fig. 1). To the best of our knowledge, there have been no reports dealing with the synthesis of this branched heptasaccharide. For an investigation of structure–function relationships of mannan, we present herein a facile and convergent synthesis of the mannose heptasaccharide.

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2. Results and discussion

Structural analysis indicated that the mannose heptamer can be constructed with a (1→6)-linkage by condensation of two moieties, i.e., a mannose dimer donor **13** and a mannose pentamer acceptor **12**. The pentasaccharide then can be constructed from a mannose acceptor **10** and a tetrasaccharide donor **9**, which can be built from a (1→3)-linked disaccharide donor **5** and a (1→2)-linked disaccharide acceptor **6**. The disaccharide acceptor **6** can be obtained by self condensation of 1,2-*O*-allyloxyethylidene-3,4,6-tri-*O*-benzoyl-β-D-mannopyranose, followed by selective 2-O-deacetylation.

Our synthetic route is shown in Scheme 1. Removal of benzylidene and ethylidene groups of compound **1**[4] was readily achieved simultaneously with 90% aq acetic acid under reflux within 2 h, giving the disaccharide **2** as a white solid in high yield (96%) after purification. Benzoylation of **2** with benzoyl chloride in pyridine, followed by selective 1-*O*-debenzoylation with M solution of ammonia in 1:2 methanol–tetrahydrofuran, and then trichloroacetimidation[5] with trichloroacetonitrile in the presence of DBU furnished 2,3,4,6-tetra-*O*-ben-

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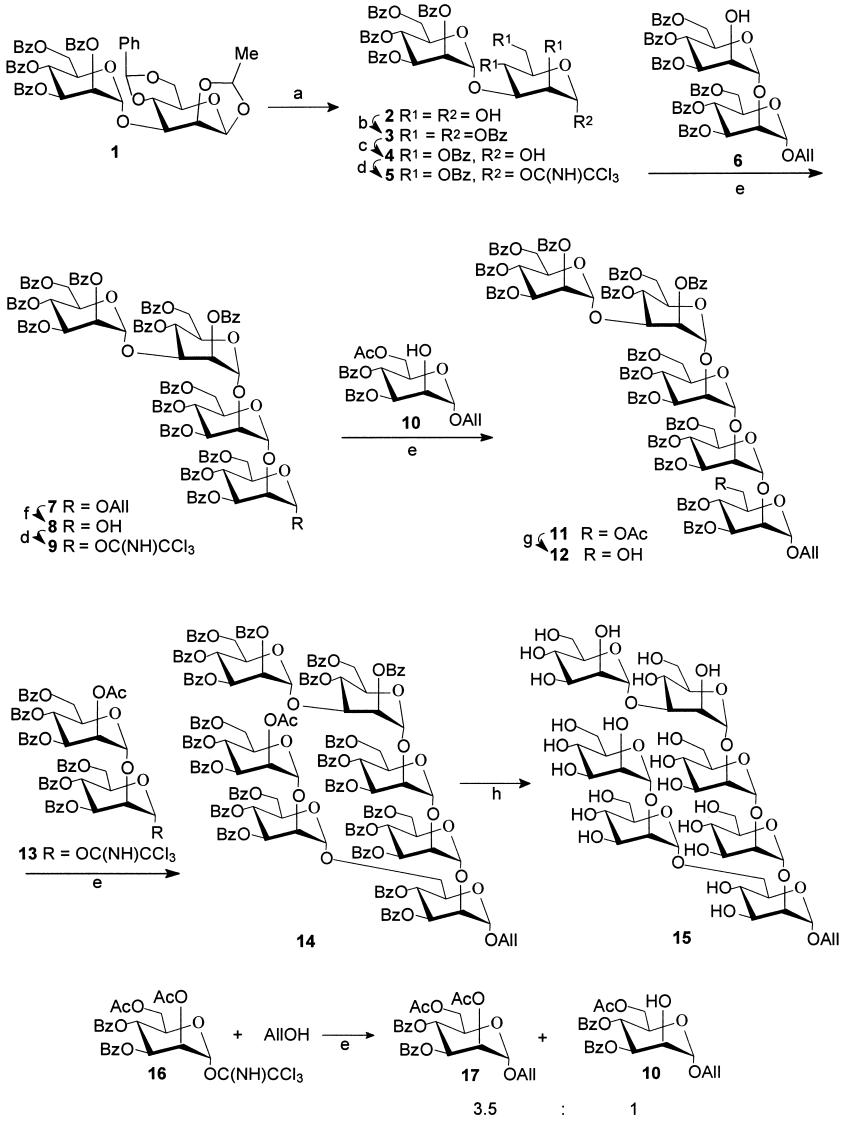
α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*-(1→2)-α-D-Man*p*
6
↑
1
α-D-Man*p*-(1→2)-α-D-Man*p*

Fig. 1. Structure of the isolated mannoheptaose.

zoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**5**) in 65% yield (for three steps). Condensation of the disaccharide donor **5** with the disaccharide acceptor **6**[6,7] gave the tetrasaccharide **7** in 61% yield. Deallylation[8] of **7** with $PdCl_2$ in dichloromethane furnished the tetrasaccharide hemiacetal **8**, and subsequent trichloroacetimidation produced the tetrasaccharide donor **9** (81% for two steps). The monosaccharide acceptor, allyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside (**10**), was obtained as a byproduct of the coupling reaction of 2,6-di-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**16**) with allyl alcohol. This coupling gave allyl 2,6-di-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside (**17**) and **10** in a ratio of 3.5:1. When compound **17**, which is used as a basic material in the synthesis of 2-branched (1→6)-linked mannans,[9] was prepared in large quantities, **10** was obtained in sub-



Scheme 1. Conditions and reagents: a: 90% HOAc–H_2O, reflux, 2 h; b: BzCl, pyridine, RT; c: THF, MeOH, NH_3, RT; d: CCl_3CN, DBU, CH_2Cl_2, RT, 8 h; e: TMSOTf, CH_2Cl_2, N_2, −15 °C to RT, 4 h; f: $PdCl_2$, CH_2Cl_2, RT, 2 h. g: CH_2Cl_2, MeOH–AcCl, RT; h: MeOH, NH_3, RT, 7 d.

stantial amounts. Compound **16** was prepared by 1-O-deacetylation of 1,2,6-tri-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranose, followed by trichloroacetimidation. The use of the acceptor **10** made the synthesis simple and practical, since it allowed one to build a difficultly accessible (1→2)-linkage first, then build an easily accessible (1→6)-linkage. Therefore, condensation of the tetrasaccharide donor **9** with **10**, followed by selective 6-O-deacetylation, produced the pentasaccharide acceptor **12**. Finally, condensation of the pentrasacchride acceptor **12** with disaccharide donor **13**[6] furnished the heptasaccharide **14** (82%), and subsequent deacylation in ammonia-saturated methanol yielded the target allyl mannoheptaoside **15**, whose bioassay is in progress.

In summary, a convergent and facile synthesis of branched mannoheptaose containing (1→2)-, (1→3)-, and (1→6)-linkages was achieved. The method presented is simpler and more practical compared to those for the synthesis of mannans with similar structures. It should be possible to carry out a large-scale synthesis employing the described 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 NMR ^{13}C and 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. Mass spectra were recorded with a VG PLATFORM mass spectrometer in the electrospray-ionization (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 on columns (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. 2,3,4,6-Tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-*D*-mannopyranosyl trichloroacetimidate (5)

To a solution of 90% HOAc (250 mL) was added **1** (7.58 g, 8.69 mmol), and the mixture was refluxed for 2.5 h and then concentrated to dryness. The residue was passed through a short silica gel column (1:1.5 petroleum ether–EtOAc) to give **2** (6.32 g, 96%) as a white solid. The white solid was dissolved in pyridine (40 mL), and then benzoyl chloride (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 washed with dilute hydrochloric acid and extracted with CH_2Cl_2. The organic phase was dried over anhyd Na_2SO_4, then concentrated to dryness. The resultant crude product **3** was dissolved in a M solution of NH_3 in 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 **4** as a syrup. A mixture of **4**, 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 **5** (6.58 g, 65% for three steps from **2** to **5**) as a white foam: $[\alpha]_D$ −49.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (s, 1 H, N*H*), 8.11–7.20 (m, 35 H, 7 Bz–H), 6.60 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 6.15 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4′), 6.06 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.87 (dd, 1 H, $J_{1,2}$ 1.4, $J_{2,3}$ 2.8 Hz, H-2′), 5.72 (dd, 1 H, $J_{2,3}$ 2.8, $J_{3,4}$ 9.8 Hz, H-3′), 5.43–5.33 (m, 2 H, H-1′, H-2), 4.76–4.70 (m, 2 H, H-3, H-6′), 4.58–4.47 (m, 4 H, 2 H-5, 2 H-6), 4.35 (dd, 1 H, $J_{5,6}$ 2.7, $J_{6,6}$ 12.3 Hz, H-6). ^{13}C NMR (100 MHz, $DCCl_3$): δ 165.7, 165.7, 165.5, 165.0, 164.8, 164.5, 164.4 (7 C, 7 Ph*C*O), 159.4 (1 C, *C*(NH)CCl_3), 133.5–132.6, 130.0–127.7 (*Ph*CO), 99.5, 94.3 (2 C, 2 C-1), 90.4 (1 C, C(NH)*C*Cl_3), 75.6, 71.4, 70.1, 69.9, 69.6, 69.1, 67.5, 66.1, 62.2, 62.1 (C-2–6). Anal. Calcd for $C_{63}H_{50}Cl_3NO_{18}$: C, 62.26; H, 4.15. Found: C, 62.06; H, 4.20.

3.3. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosy-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (9)

To a cooled solution (0 °C) of **5** (1.70 g, 1.40 mmol) and **6** (1.34 g, 1.33 mmol) in anhyd CH_2Cl_2 (50 mL) was added TMSOTf (5 μL, 0.02 mmol). The mixture was stirred at this temperature 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 (3:2 petroleum ether–EtOAc) to give tetrasaccharide **7** as a foamy solid (1.76 g, 61%). To a solution of **7** (1.71 g, 0.82 mmol) in anhyd MeOH (20 mL) was added $PdCl_2$ (80 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, the solution was concentrated to dryness, and the resultant residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **8** as a white foam. A mixture of **8**, trichloroacetonitrile (0.6 mL, 2.79 mmol) and 1,8-diazabicyclo[5.4.0]-undecene (DBU) (0.06 mL, 0.39 mmol) 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 **9** (1.44 g, 81% for two steps) as a white foam: $[\alpha]_D$ −41.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.74 (s, 1 H, N*H*), 8.18–7.15 (m, 65 H, 13 Bz–H), 6.58 (d, 1 H, $J_{1,2}$ 2.2 Hz, H-1), 6.08 (dd, 1 H, $J_{3,4} = J_{4,5} =$ 10.0 Hz, H-4‴), 6.06 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4″), 6.02 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4′), 5.95 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.88 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3‴), 5.81 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3′), 5.71 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.2 Hz, H-2‴), 5.68 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.0 Hz, H-3), 5.53 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1‴), 5.38 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.2 Hz, H-2″), 5.35 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1″), 5.05 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1′), 4.70–4.13 (m, 14 H, 2 H-2, H-3″, 4 H-5, 8 H-6). ^{13}C NMR (100 MHz, $DCCl_3$): δ 166.0, 166.0, 165.8, 165.7, 165.3, 165.2, 165.2, 165.1, 165.1, 165.0, 164.8, 164.6, 164.4 (13 C, 13 Ph*C*O), 159.8 (1 C, *C*(NH)CCl_3), 133.5–132.6, 130.0–127.7 (*Ph*CO), 99.6, 99.6, 99.2, 96.1 (4 C, 4 C-1), 90.4 (1 C, C(NH)*C*Cl_3), 75.4, 73.8, 71.4, 71.3, 70.4, 70.4, 69.9, 69.9, 69.8, 69.6, 69.6, 69.4, 67.8, 66.8, 66.8, 66.0, 63.3, 63.0, 62.8, 61.9 (C-2–6). Anal. Calcd for $C_{117}H_{94}Cl_3NO_{34}$: C, 64.93; H, 4.38. Found: C, 64.73; H, 4.47.

3.4. Allyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside (10)

Selective 1-O-deacetylation of 1,2,6-tri-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-*mannopyranose*[9] (5.14 g, 10 mmol) in a M solution of NH_3 in MeOH and then trichloroacetimidation with CCl_3CN in the presence of DBU furnished 2,6-di-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**16**) (5.06 g, 82%, for two steps). Coupling of **16** (2.00 g, 3.24 mmol) with allyl alcohol (0.5 mL, 7.21 mmol) gave allyl 2,6-di-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside (**17**, 1.28 g, 2.28 mmol, 70%) as the major product and allyl 6-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranoside (**10**, 304 mg, 0.65 mmol, 20%) as the minor one, the ratio of **17** to **10** is 3.5. For **10**: $[\alpha]_D$ −32.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97–7.26 (m, 10 H, 2 Bz–H), 5.98 (m, 1 H, C*H*=CH_2), 5.85 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.65 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 10.0 Hz, H-3), 5.37 (dd, 1 H, *J* 1.5, *J* 17.2 Hz, CH=CH_2), 5.27 (dd, 1 H, *J* 1.5, *J* 10.4 Hz, CH=CH_2), 5.00 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.34–4.19 (m, 5 H, H-2, H-5, H-6, 2 CH_2–CH=CH_2), 4.10 (dd, 1 H, $J_{5,6'}$ 6.14, $J_{6,6'}$ 12.8 Hz, H-6′), 2.05 (s, 3 H, *Me*CO). Anal. Calcd for $C_{25}H_{26}O_9$: C, 63.82; H, 5.57, Found: C, 63.71; H, 5.56. For **16**: $[\alpha]_D$ −19.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.85 (s, 1 H, N*H*), 7.98–7.26 (m, 10 H, 2 Bz–H), 6.40 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.90 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.80 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 10.0 Hz, H-3), 5.70 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 3.4 Hz, H-2), 4.45 (ddd, 1 H, $J_{4,5}$ 10.0, $J_{5,6} = J_{5,6'} = 12.3$ Hz, H-5), 4.34 (dd, 1 H, $J_{5,6} = 12.3$, $J_{6,6'} = 4.8$ Hz, H-6), (dd, 1 H, $J_{5,6'} = 12.3$, $J_{6,6'} = 4.8$ Hz, H-6′). Anal. Calcd for $C_{26}H_{24}Cl_3NO_{10}$: C, 50.59; H, 4.24, Found: C, 50.74; H, 4.35.

3.5. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosy-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→2)-3,4-di-*O*-benzoyl-α-D-mannopyranoside (12)

Compound **11** (234 mg, 59%) was prepared by coupling of **9** (347 mg, 0.16 mmol) with **10** (75 mg, 0.16 mmol) under the same conditions as described for the synthesis of **7** by coupling of **5** with **6**. Compound **11** was dissolved in anhyd MeOH (20 mL) and CH_2Cl_2 (10 mL), and to the mixture was added AcCl (0.12 mL). The flask was stoppered, and the solution was stirred at rt until TLC (3:2 petroleum ether–EtOAc) showed that the starting material had disappeared (2 h). The solution was neutralized with Et_3N, then concentrated to dryness. The residue was passed through a short silica gel column (3:2 petroleum ether–EtOAc) to give **12** (220 mg, 95%) as a white solid: $[\alpha]_D$ −65.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14–7.10 (m, 75 H, 15 Bz–H), 6.12 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.98$ Hz, H-4), 6.04–5.69 (m, 9 H, H-2, 4 H-3, 4 H-4, C*H*=CH_2), 5.41–5.18 (m, 7 H, 4 H-1, 4 H-3, H-2, C*H*=CH_2), 4.95 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.85–3.73 (m, 21 H, 3 H-2, H-3, 5 H-5, 10 H-6, –CH_2–; CH=CH_2). ^{13}C NMR (100 MHz, $DCCl_3$): δ 166.5, 165.8, 165.7, 165.7, 165.7, 165.5, 165.4, 165.3, 165.3, 165.2, 165.1, 165.0 164.8, 164.6, 164.4 (15 C, 15 Ph*C*O), 133.4–132.6, 130.0–127.8 (–CH_2–*C*H=CH_2, *Ph*CO), 117.2 (1 C, –CH_2–CH=*C*H_2), 100.2, 99.8, 99.4, 99.2, 97.5 (5 C, 5 C-1), 75.4, 71.2, 71.2, 71.0, 70.6, 70.4, 69.9, 69.9, 69.6, 69.6, 69.4, 69.4, 69.3, 69.3, 68.3, 68.3, 67.7, 67.2, 67.2, 66.0, 64.0, 63.5, 62.4, 61.8, 61.2, 60.2 (C-2–6, –*C*H_2–CH=CH_2). Anal. Calcd for $C_{138}H_{116}O_{41}$: C, 68.20; H, 4.81. Found: C, 67.93; H, 4.87.

3.6. Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-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-mannopyranosyl-(1→2)-[2,3,4-tri-*O*-benzoyl-6-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-]3,4-di-*O*-benzoyl-α-D-mannopyranoside (14)

Under the same conditions as described for the synthesis of **7** by coupling of **5** with **6**, heptasaccharide **14** (129 mg, 82%) was obtained from coupling of **13** (58 mg, 0.050 mmol) with **12** (113 mg, 0.046 mmol): $[\alpha]_D$ −31.2° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19–6.90 (m, 105 H, 21 Bz–H), 6.20–5.74 (m, 17 H, 3

H-2, 6 H-3, 7 H-4, C*H*=CH_2), 5.60 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.52 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.47–5.31 (m, 5 H, 3 H-2, *CH*=*C*H_2), 5.40 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.32 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.24 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.13 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.92 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.77–4.02 (m, 25 H, H-2, H-3, 7 H-5, 14 H-6, CH_2–CH=CH_2), 1.91 (s, 3 H, *Me*CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.0 (1 C, Me*C*O), 166.1, 166.0, 165.9, 165.8, 165.8, 165.7, 165.7, 165.6, 165.5, 165.4, 165.3, 165.3, 165.2, 165.2, 165.1, 165.1, 165.0, 164.9, 164.7, 164.5 (21 C, Ph*C*O), 133.4–132.5, 130.2–127.9 (*Ph*CO, –CH_2–*C*H=CH_2), 117.7 (1 C, –CH_2–CH=*C*H_2), 100.5, 100.0, 99.9, 99.4, 99.3, 98.7, 98.0 (7 C-1, $J_{C\text{-}1,H\text{-}1}$ = 175.1 to 177.3 Hz), 77.7, 75.8, 75.6, 71.3, 71.1, 71.0, 70.9, 70.5, 70.0, 69.8, 69.7, 69.6, 69.5, 68.8, 68.7, 67.6, 67.5, 67.4, 67.0, 66.3, 66.1, 63.8, 63.5, 63.4, 63.3, 61.9 (C-2–6, –*C*H_2–CH=CH_2), 20.4 (1 *Me*CO). Anal. Calcd for $C_{194}H_{162}O_{58}$: C, 68.10; H, 4.77. Found: C, 67.97; H, 4.69.

3.7. Allyl α-D-mannopyranosyl-(1 → 3)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 2)-[α-D-mannopyranosyl-(1 → 2)-α-D-mannopyranosyl-(1 → 6)-]α-D-mannopyranoside (15)

Compound **14** (129 mg, 0.0377 mmol) was dissolved in a satd solution of NH_3 in MeOH (10 mL). After two weeks at rt, the reaction solution was concentrated, and the residue was purified on a Bio-Gel P-2 column with MeOH–water as the eluent to afford **15** (42 mg, 93%) as a pulverous crystalloid: $[\alpha]_D$ + 82.6° (*c* 1.0, H_2O); ^{1}H NMR (400 MHz, D_2O): δ 5.87 (m, 1 H, C*H*=CH_2), 5.25 (dd, 1 H, *J* 1.2, *J* 16.6 Hz, CH=CH_2), 5.20, 5.19, 5.05, 5.04, 5.03, 4.94 (8 H, 7 H-1, CH=CH_2), 4.15–3.45 (m, 44 H, CH_2–CH=CH_2, H-2–6). ^{13}C NMR (100 MHz, D_2O): δ 133.3 (1 C, –CH_2–*C*H=CH_2), 118.5 (1 C, –CH_2–CH=*C*H_2), 102.2, 102.1, 102.0, 100.6, 100.6, 98.1, 97.5 (7 C-1, $J_{C\text{-}1,H\text{-}1}$ = 173.0 to 176.5 Hz), 78.9, 78.7, 78.6, 78.4, 77.9, 73.3, 73.3, 73.3, 73.3, 73.3, 73.2, 71.2, 70.4, 70.4, 70.4, 70.1, 70.0, 70.0, 70.0, 69.6, 68.4, 67.0, 66.9, 66.9, 66.9, 66.9, 66.9, 66.7, 66.2, 65.7, 61.1, 61.1, 61.1, 60.9, 60.9, 60.9 (C-2–6, –*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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