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Note

A facile synthesis of the tetrasaccharide repeating unit of mannoglucan from *Microellobosporia grisea*

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Abstract

A facile synthesis of the tetrasaccharide repeating unit of mannoglucan from *Microellobosporia grisea* was achieved through coupling of 4,6-*O*-benzylidene-1,2-*O*-ethylidene-α-D-glucopyranose with per-O-benzoylated mannopyranosyl trichloroacetimidate, followed by debenzylidenation, selective 6-O-mannopyranosylation, then hydrolysis of the 1,2-*O*-ethylidene group, 1,2-O-acetylation and conversion to the 1-trichloroacetimidate, and subsequent condensation of the activated trisaccharide with allyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside.

Keywords: Mannoglucan; Oligosaccharides; Regio- and stereoselective synthesis

A D-manno-D-glucan from *Microellobosporia grisea*, an actinomycete, has a high degree of antitumor activity and consists of tetrasaccharide repeating units having the structure shown in Fig. 1 [1]. For study on the relationship of structure and antitumor activity, it was deemed to be helpful to synthesize the repeating unit of the mannoglucan.

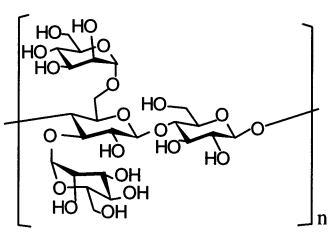

Fig. 1. The D-manno-D-galactan from *Microellobosporia grisea*.

Although the repeating unit only contains four sugar residues, its heterogeneity of composition and its nonlinear structure make the synthesis troublesome. However, using appropriate starting materials in combination with a selective glycosylation technique, the target compound can be synthesized in a facile and effective way. Herein we report the synthesis of the repeating tetrasaccharide unit.

As outlined in Scheme 1, 1,2-*O*-ethylidene-α-D-glucopyranose (**1**), a readily prepared compound [2], was converted into 4,6-*O*-benzylidene-1,2-*O*-ethylidene-α-D-glucopyranose (**2**) [3] with diethoxytoluene in the presence of zinc chloride. Coupling of **2** with the donor 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate afforded 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-benzylidene-1,2-*O*-ethylidene-α-D-glucopyran-

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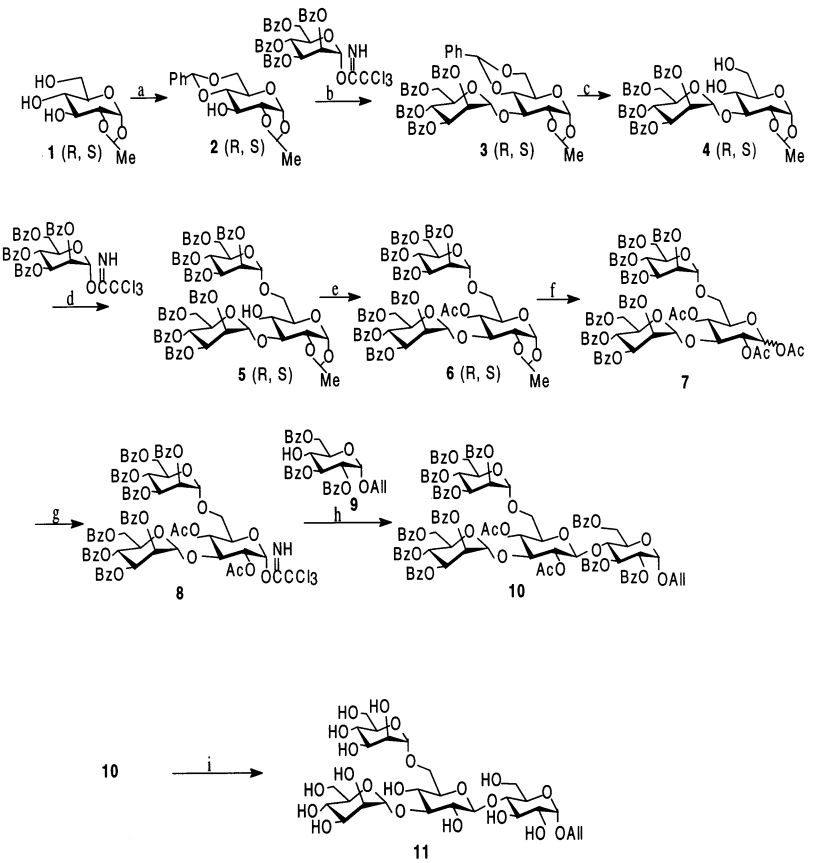

Scheme 1. Reagents and conditions: (a) $PhCH(OEt)_2$, $ZnCl_2$, rt, 4 h, 88%. (b) CH_2Cl_2, Me_3SiOTf, −42 °C to rt, 90%. (c) AcOH, 4:1 $Cl_2CHCOOH$–water (v/v), rt, 6 h, 91%. (d) CH_2Cl_2, Me_3SiOTf, −42 °C to rt, 85%. (e) Ac_2O/Pyr, 98%. (f) F_3CCOOH (90%), rt, 1 h, then Ac_2O/Pyr, rt, 3 h, 70%. (g) K_2CO_3, DMF, rt, 12 h, 95%, then CH_2Cl_2, CCl_3CN, DBU, rt, 2 h, 90%. (h) CH_2Cl_2, Me_3SiOTf, −42 °C to rt, 90%. (I). MeONa/MeOH, rt, 85%.

ose (**3**). Debenzylidenation with 4:1 $Cl_2CHCOOH$–water at room temperature smoothly yielded 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-1,2-*O*-ethylidene-α-D-glucopyranose (**4**). In our preceding papers [3,4], selective 6-O-glycosylation using per-*O*-acetyl glycosyl bromides as the donors and unprotected or partially protected glycosides as the acceptors was successfully achieved via orthoester formation–rearrangement. It was reported that with sugar trichloroacetimidates as the glycosyl donors under kinetic control conditions, orthoesters were the intermediates that were converted into the corresponding glycosidic linkage in situ by simple elongation of the reaction time [5,6]. Thus, in the present research, selective 6-O-glycosylation of **4** was carried out at −40 °C using 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate as the donor to give the trisaccharide, 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-1,2-*O*-ethylidene-α-D-glucopyranose (**5**) in satisfactory yield (85%). Acetylation of **5** then gave **6**.

Removal of the 1,2-*O*-ethylidene group from **6** with 90% trifluoroacetic acid, followed by acetylation with acetic anhydride in pyridine, furnished 3,6-di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-1,2,4-tri-*O*-acetyl-D-glucopyranose (**7**) in 70% yield. Selective 1-O-deacetylation with potassium carbonate in DMF [7], followed by treatment with trichloroacetonitrile and DBU [8], gave the trisaccharide trichloroacetimidate **8** in high yield (90%). Condensation of the trisaccharide donor **8** with the monosaccharide acceptor allyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**9**) afforded the tetrasaccharide allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-

(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**10**) in high yield (90%). Deacylation at room temperature with saturated ammonia in methanol afforded the unprotected tetrasaccharide allyl glycoside **11**.

In summary, herein we present a facile and effective synthesis of the targeted tetrasaccharide. Most of the reactions involved in the synthesis were readily carried out with satisfactory yields, whereas the use of acyl protective groups substantially simplified the reaction routes.

1. Experimental

General methods.—Melting points were determined with a 'Mel–Temp' apparatus. Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter for solutions in a 1 dm jacketed cell. ^{1}H NMR spectra were recorded with Varian XL-400 and XL-200 spectrometers, for solutions in $CDCl_3$ with tetramethylsilane (Me_4Si) as the internal standard. Chemical shifts are expressed in ppm downfield from the internal Me_4Si absorption. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the ESI technique to introduce the sample. Thin-layer chromatography (TLC) was performed on silica gel HF, detection being affected by charring with 30% (v/v) H_2SO_4 in MeOH or sometimes by UV detection. Column chromatography was conducted by elution of a column (8/100 mm, 16/240 mm, 18/300 mm, 35/400 mm) of silica gel (100–200 mesh) and EtOAc–petroleum ether (bp 60–90 °C) as the eluent. Analytical LC was performed with a Gilson HPLC consisting of a pump (model 306), stainless steel packed with silica gel (Spherisorb SiO_2, 10 × 300 or 4.6 × 250 mm), differential refractometer (132-RI Detector), UV–vis detector (model 118), and EtOAc–petroleum ether (bp 60–90 °C) was used as the eluent at a flow rate of 1–4 mL/min. Solutions were concentrated at a temperature < 60 °C under diminished pressure. Compounds **3**, **4**, **5**, and **6** containing 1,2-*O*-ethylidenated sugar residues were obtained as R, S mixtures that were used directly in the next reaction. Purification of the mixtures usually gave one isomer (R or S), which was subjected to NMR spectroscopic analysis, but isomerization of the pure isomer occurred upon a long-term standing and during NMR determination.

2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-O-benzylidene-1,2-O-(R,S)-ethylidene-α-D-glucopyranose (**3**).—2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (741 mg, 1 mmol) and 4,6-*O*-benzylidene-1,2-*O*-(*R*,*S*)-ethylidene-α-D-glucopyranose (**2**) (293 mg, 1 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (60 mL). Trimethylsilyltriflate (20 μL, 0.10 equiv) was added dropwise at −42 °C with N_2 protection. The reaction mixture was stirred for 3 h, at the end of which time thin-layer chromatography (TLC) (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. Then the mixture was neutralized with triethylamine and concentrated under reduced pressure to dryness. Further purification by column chromatography (3:1 petroleum ether–EtOAc) gave **3** (785 mg, 90%) as a colorless solid: mp 140–145 °C; $[\alpha]_D$ + 13.0° (*c* 1.1, $CHCl_3$); ^{1}H NMR: δ 8.12–7.25 (m, 20 H, Bz-H), 6.23 (t, 1 H, $J_{3',4'}$ 10.2 Hz, H-4′), 5.95 (dd, 1 H, $J_{2',3'}$ 3.2 Hz, H-3′), 5.84 (dd, 1 H, $J_{1',2'}$ 1.6 Hz, H-2′), 5.62 (s, 1 H, Ph–C–H), 5.56 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1), 5.52 (d, 1 H, H-1′), 5.21 (dd, 1 H, CH_3CH), 4.72–4.67 (m, 2 H), 4.50–4.40 (m, 2 H), 4.20–4.16 (m, 2 H), 4.00–3.97 (m, 1 H), 3.83–3.74 (m, 2 H), 1.49 (d, 3 H, CH_3CH). Anal. Calcd for $C_{49}H_{44}O_{15}$: C, 67.43; H, 5.05. Found: C, 67.45; H, 5.03.

2,3,4,6-Tetra-O-benzoyl-α-D-mannopyranosyl-(1→3)-1,2-O-(R,S)-ethylidene-α-D-glucopyranose (**4**).—2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-4,6-*O*-benzylidene-1,2-*O*-(*R*,*S*)-ethylidene-α-D-glucopyranose (**3**) (872 mg, 1 mmol) was dissolved in AcOH (10 mL) and treated with 4:1 $Cl_2CHCOOH$–water (v/v) (0.8 mL) at rt for 6 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was diluted with water, extracted with CH_2Cl_2 (3 × 30 mL), and the organic phase was washed with satd aq Na_2CO_3 (3 × 50 mL) and

water (2 × 50 mL), dried, and concentrated to yield crude **4** (R, S mixture) (713 mg, 91%) as crystals. Further purification by column chromatography (1:1 petroleum ether–EtOAc) gave fine needles: mp 150–152 °C; $[\alpha]_D$ −24.0° (*c* 1.3, $CHCl_3$); ^1H NMR: δ 8.12–7.25 (m, 20 H, Bz-H), 6.22 (t, 1 H, $J_{3',4'}$ 10.0 Hz, H-4′), 5.93 (dd, 1 H, $J_{2',3'}$ 3.3 Hz, H-3′), 5.81 (dd, 1 H, $J_{1',2'}$ 1.4 Hz, H-2′), 5.56–5.54 (m, 2 H, H-1′, H-1), 5.52 (dd, 1 H, CH_3CH), 4.71–4.68 (m, 2 H), 4.50–4.46 (m, 1 H), 4.37–4.15 (m, 1 H), 3.99–3.90 (m, 4 H), 3.70–3.68 (m, 1 H), 1.35 (d, 3 H, CH_3CH). Anal. Calcd for $C_{42}H_{40}O_{15}$: C, 64.29; H, 5.10. Found: C, 64.26; H, 5.12.

2,3,4,6-Tetra-O-*benzoyl*-α-D-*mannopyranosyl*-*(1→3)*-*[2,3,4,6-tetra*-O-*benzoyl*-α-D-*mannopyranosyl*-*(1→6)]*-*1,2*-O-(R,S)-*ethylidene*-α-D-*glucopyranose* (**5**).—2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (741 mg, 1 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-1,2-*O*-(*R*,*S*)-ethylidene-α-D-glucopyranose (**4**) (784 mg, 1 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (60 mL). Trimethylsilyltriflate (20 μL, 0.10 equiv) was added dropwise at −42 °C with N_2 protection. The reaction mixture was stirred for 3 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was then neutralized with triethylamine and concentrated under reduced pressure to dryness. Further purification by column chromatography (3:1 petroleum ether–EtOAc) gave **5** (1158 mg, 85%) as a colorless solid: mp 131–134 °C; $[\alpha]_D$ −1.5° (*c* 1.0, $CHCl_3$); ^1H NMR: δ 8.12–7.25 (m, 40 H, Bz-H), 6.27 (t, 1 H, *J* 10.2 Hz), 6.13 (t, 1 H, *J* 10.1 Hz), 5.97–5.93 (m, 2 H), 5.87 (dd, 1 H, *J* 1.5, *J* 3.3 Hz), 5.85 (dd, 1 H, *J* 1.4, *J* 3.2 Hz), 5.80–5.76 (m, 3 H), 5.25 (dd, 1 H, CH_3CH), 4.74–4.67 (m, 3 H), 4.55–4.43 (m, 3 H), 4.25–4.20 (m, 3 H), 4.03–4.00 (m, 1 H). 3.96–3.89 (m, 2 H), 1.42 (d, 3 H, CH_3CH). Anal. Calcd for $C_{76}H_{66}O_{24}$: C, 66.96; H, 4.85. Found: C, 66.99; H, 4.82.

2,3,4,6-Tetra-O-*benzoyl*-α-D-*mannopyranosyl*-*(1→3)*-*[2,3,4,6-tetra*-O-*benzoyl*-α-D-*mannopyranosyl*-*(1→6)]*-*4*-O-*acetyl*-*1,2*-O-(R,S)-*ethylidene*-α-D-*glucopyranose* (**6**).—To the solution of 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-1,2-*O*-(*R*,*S*)-ethylidene-α-D-glucopyranose (**5**) (1362 mg, 1 mmol) in pyridine (25 mL) was added Ac_2O (0.5 mL, 5 mmol), and the mixture was stirred overnight at rt. TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. Ice water was added, and the mixture was diluted with CH_2Cl_2, washed with 1 N HCl, water, and satd aq sodium bicarbonate. The organic layer was combined, dried, and concentrated. Further purification by column chromatography (3:1 petroleum ether–EtOAc) gave **6** (1376 mg, 98%) as colorless crystals: mp 137–140 °C; $[\alpha]_D$ −2.5° (*c* 1.1, $CHCl_3$); ^1H NMR: δ 8.12–7.26 (m, 40 H, Bz-H), 6.26 (t, 1 H, *J* 10.0 Hz), 6.15 (t, 1 H, *J* 10.2 Hz), 5.99–5.95 (m, 2 H), 5.89 (dd, 1 H, *J* 1.4, *J* 3.2 Hz), 5.86 (dd, 1 H, *J* 1.5, *J* 3.2 Hz), 5.82–5.75 (m, 4 H), 5.28 (dd, 1 H, CH_3CH), 4.77–4.64 (m, 4 H), 4.60–4.40 (m, 3 H), 4.22–4.15 (m, 2 H), 3.99–3.95 (m, 1 H), 3.90–3.85 (m, 1 H), 2.04 (s, 3 H, CH_3CO), 1.38 (d, 3 H, CH_3CH). Anal. Calcd for $C_{78}H_{68}O_{25}$: C, 66.67; H, 4.84. Found: C, 66.69; H, 4.82.

2,3,4,6-Tetra-O-*benzoyl*-α-D-*mannopyranosyl*-*(1→3)*-*[2,3,4,6-tetra*-O-*benzoyl*-α-D-*mannopyranosyl*-*(1→6)]*–*1,2,4-tri*-O-*acetyl*-*α,β*-D-*glucopyranose* (**7**).—2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-4-*O*-acetyl-1,2-*O*-(*R*,*S*)-ethylidene-α-D-glucopyranose (**6**) (1404 mg, 1 mmol) was treated with 90% F_3CCOOH (5 mL) at rt for 1 h, and the solution was concentrated and co-concentrated with toluene. The residue was dissolved in pyridine (5 mL) and treated with Ac_2O (3 mL) for 2 h. After conventional workup, the residue was subjected to column chromatography (3:1 petroleum ether–EtOAc) to yield the title compound **7** as a solid consisting predominantly of the α anomer (1023 mg, 70%): mp 135–138 °C; $[\alpha]_D$ −5.1° (*c* 1.1, $CHCl_3$); ^1H NMR: δ 8.12–7.26 (m, 40 H, Bz-H), 6.37 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 6.27 (t, 1 H, *J* 10.2 Hz), 6.11 (t, 1 H, *J* 10.1 Hz), 5.86 (dd, 1 H, *J* 10.1, *J* 3.1 Hz), 5.76 (dd, 1 H, *J* 10.2, *J* 3.4 Hz), 5.73 (dd, 1 H, *J* 1.7, *J* 3.4 Hz), 5.57 (dd, 1 H, *J* 1.5, *J* 3.1 Hz), 5.30 (d, 1 H, *J* 1.7 Hz), 5.23–5.19 (m, 2 H), 5.09 (s, 1 H), 4.80–4.72 (m, 2 H), 4.52–4.32 (m, 5 H), 4.14–4.11 (m, 1

H), 3.94–3.92 (m, 1 H), 3.70–3.68 (m, 1 H), 2.34 (s, 3 H, CH_3CO), 2.23 (s, 3 H, CH_3CO), 2.15 (s, 3 H, CH_3CO). ^{13}C NMR: δ 170.06, 169.81, 168.87 (3 CH_3CO), 166.09, 165.97, 165.53, 165.45, 165.43, 165.36, 165.33, 165.17 (8 C_6H_5CO), 100.02, 99.22, 97.38 (C-1$^{I–III}$), 89.12 (C-3), 70.73, 70.53, 70.47, 70.19, 70.19, 69.77, 69.63, 69.62, 68.87, 66.83, 66.46, 66.00, 62.70, 61.93 (C-2,3,4,5,6$^{I–III}$). Anal. Calcd for $C_{80}H_{70}O_{27}$: C, 65.66; H, 4.79. Found: C, 65.63; H, 4.81.

*2,3,4,6-Tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)]–2,4-di-*O*-acetyl-α-*D*-glucopyranosyl trichloroacetimidate* (**8**).—A solution of 3,6-di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-1,2,4-tri-*O*-acetyl-D-glucopyranose (**7**) (731 mg, 0.5 mmol) and K_2CO_3 (69 mg, 0.5 mmol) in DMF was stirred for 12 h at rt, at the end of which time TLC (2:1 petroleum ether–EtOAc) showed that the reaction to be complete. Water was added, and the mixture was diluted with CH_2Cl_2, washed with 1 N HCl, water, and satd aq sodium bicarbonate. The organic layer was combined, dried, and concentrated. The residue thus obtained was passed through a short silica gel column with 2:1 petroleum ether–EtOAc as the eluent to give crude 3,6-di-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl)-2,4-di-*O*-acetyl-D-glucopyranose (675 mg, 95%), which was dissolved in CH_2Cl_2 (20 mL) to which CCl_3CN (0.1 mL, 1 mmol) and DBU (14 μL, 0.1 mmol) were added. The reaction mixture was stirred for 2 h, at the end of which time 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 2:1 petroleum ether–EtOAc as the eluent furnished the trisaccharide donor **8** as crystals in a good yield (669 mg, 90%): mp 123–126 °C; $[\alpha]_D$ +1.5° (*c* 1.1, $CHCl_3$); ^{1}H NMR: δ 8.92 (s, 1 H, C=N*H*), 8.15–7.25 (m, 40 H, Bz-H), 6.55 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 6.29 (t, 1 H, *J* 10.2 Hz), 6.09 (t, 1 H, *J* 10.1 Hz), 5.88 (dd, 1 H, *J* 10.2, *J* 3.3 Hz), 5.78 (dd, 1 H, *J* 10.1, *J* 3.1 Hz), 5.68 (dd, 1 H, *J* 1.8, *J* 3.3 Hz), 5.60 (dd, 1 H, *J* 1.4, *J* 3.1 Hz), 5.32 (d, 1 H, *J* 1.8 Hz), 5.24–5.20 (m, 2 H), 5.10 (d, 1 H, *J* 1.4 Hz), 4.78–4.74 (m, 2 H), 4.64–4.58 (m, 1 H), 4.56–4.52 (m, 1 H), 4.44–4.37 (m, 3 H), 4.29–4.22 (m, 1 H), 3.98–3.95 (m, 1 H), 3.73–3.70 (m, 1 H), 2.34 (s, 3 H, CH_3CO), 2.16 (s, 3 H, CH_3CO). Anal. Calcd for $C_{80}H_{68}Cl_3O_{26}N$: C, 61.34; H, 4.35. Found: C, 61.32; H, 4.36.

*Allyl 2,3,6-tri-*O*-benzoyl-α-*D*-glucopyranoside* (**9**).—Allyl 4,6-*O*-benzylidene-α-D-glucopyranoside [9] (616 mg, 2 mmol) was dissolved in pyridine (15 mL), and BzCl (0.69 mL, 6 mmol) was added dropwise under stirring. The reaction was monitored by TLC (2:1 petroleum ether–EtOAc). After 3 h at rt, the reaction was complete, and the reaction mixture was worked up in the conventional way. The crude product was dissolved in tetrahydrofuran (20 mL), and H_2SO_4 (1 M, 5 mL) was added under stirring. The reaction was monitored by TLC (1:1 petroleum ether–EtOAc). After 2 h at 70 °C, the reaction was shown to be complete, and the reaction mixture was washed with water, then satd aq sodium bicarbonate. The organic layer was combined, dried, and concentrated. Further purification by column chromatography (1:1 petroleum ether–EtOAc) gave allyl 2,3-di-*O*-benzoyl-α-D-glucopyranoside (762 mg, 89%) as a colorless solid. The solid was dissolved in pyridine (15 mL), and BzCl (0.25 mL, 2.1 mmol) was added dropwise under stirring. The reaction was monitored by TLC (2:1 petroleum ether–EtOAc). After 3 h at rt, the reaction was complete, and the reaction mixture was worked up in the conventional way. Further purification by column chromatography (2:1 petroleum ether–EtOAc) gave allyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (928 mg, 98%) as a colorless solid: mp 148–151 °C; $[\alpha]_D$ +4.5° (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 8.12–7.26 (m, 15 H, Bz-H), 5.91–5.79 (m, 1 H, CH_2=C*H*–CH_2), 5.80 (t, 1 H, $J_{3,4}$ 9.3 Hz, H-3), 5.32–5.30 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 9.3 Hz, H-2), 5.28 (s, 1 H, H-1), 5.29–5.26 (dd, 1 H, CH_2=CH–CH_2), 5.17–5.14 (dd, 1 H, CH_2=CH–CH_2), 4.81 (dd, 1 H, H-6e), 4.62–4.59 (dd, 1 H, H-6a), 4.29–4.24 (m, 1 H, CH_2=CH–CH_2), 4.19–4.16 (m, 1 H, CH_2=CH–CH_2), 4.11–4.06 (m, 1 H, H-5), 3.88 (t, 1 H, H-4). Anal. Calcd for $C_{30}H_{28}O_9$: C, 67.67; H, 5.26. Found: C, 67.63; H, 5.29.

Allyl 2,3,4,6-tetra-O-*benzoyl*-α-D-*mannopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzoyl*-α-D-*mannopyranosyl-(1→6)]–2,4-di*-O-*acetyl*-β-D-*glucopyranosyl-(1→4)-2,3,6-tri*-O-*benzoyl*-α-D-*glucopyranoside* (**10**).—2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]–2,4-di-*O*-acetyl-α,β-D-glucopyranosyl trichloroacetimidate (**8**) (782 mg, 0.5 mmol) and allyl 2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**9**) (266 mg, 0.5 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (20 mL). Trimethylsilyltriflate (10 μL, 0.10 equiv) was added dropwise at −42 °C with N_2 protection. The reaction mixture was stirred for 3 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. Then the mixture was neutralized with triethylamine and concentrated under reduced pressure to dryness. Further purification by column chromatography (2:1 petroleum ether–EtOAc) gave **10** (870 mg, 90%) as a colorless solid: mp 133–136 °C; $[\alpha]_D$ +38.5° (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 8.09–7.26 (m, 55 H, Bz-H), 6.26 (t, 1 H, *J* 10.1 Hz), 6.12 (t, 1 H, *J* 10.2 Hz), 6.08 (t, 1 H, *J* 9.80 Hz), 5.89 (dd, 1 H, *J* 10.1, *J* 3.30 Hz) 5.86–5.75 (m, 1 H, CH_2=C*H*–CH_2), 5.68 (dd, 1 H, *J* 10.2, *J* 2.60 Hz), 5.65 (dd, 1 H, *J* 2.60, *J* 1.60 Hz), 5.53 (dd, 1 H, *J* 2.60, *J* 1.60 Hz), 5.30 (d, 1 H, *J* 1.6 Hz), 5.28 (d, 1 H, *J* 1.6 Hz),5.27–5.22 (dd, 1 H, C*H*$_2$=CH–CH_2), 5.16–5.06 (m, 4 H), 5.05 (d, 1 H, *J* 3.5 Hz), 4.89–4.86 (m, 2 H), 4.77–4.68 (m, 2 H), 4.65 (d, 1 H, *J* 7.8 Hz), 4.57 (dd, 1 H), 4.50 (dd, 1 H), 4.43 (dd, 1 H), 4.39–4.25 (m, 3 H), 4.10 (t, 1 H, *J* 10.0 Hz), 4.05 (dd, 1 H), 3.80 (t, 1 H, *J* 10.0 Hz), 3.40–3.30 (m, 2 H), 2.26 (s, 3 H, C*H*$_3$CO), 2.18 (s, 3 H, C*H*$_3$CO). ^{13}C NMR: δ 170.08, 169.55, (2 CH_3CO), 166.12, 166.03, 165.92, 165.80, 165.48, 165.38, 165.34, 165.34, 165.23, 165.16, 165.08 (11 C_6H_5CO), 117.84 (*C*H_2=CH–CH_2), 100.52, 100.40, 97.75, 94.93 (C-1$^{I–IV}$), 82.81 (C-3), 77.32 (C-4), 72.74, 71.95, 71.84, 70.81, 70.43, 70.25, 70.03, 70.03, 69.96, 69.91, 69.06, 68.76, 68.66, 67.86, 66.83, 65.71, 62.96, 62.84, 61.62 (CH_2–CH–*C*H_2–, C-2,3,4,5,6$^{I–IV}$). Anal. Calcd for $C_{108}H_{94}O_{34}$: C, 67.01; H, 4.86. Found: C, 67.03; H, 4.85.

Allyl α-D-*mannopyranosyl-(1→3)-[*α-D-*mannopyranosyl-(1→6)]–*β-D-*glucopyranosyl-(1→4)-*α-D-*glucopyranoside* (**11**).—Allyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]–2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranoside (**10**) (77 mg, 0.04 mmol) was suspended in freshly distilled MeOH (5 mL), a solution of CH_3ONa (2 M, 0.1 mL) was added, and the mixture was stirred overnight at rt. TLC showed that the reaction to be complete, and the resulting solution was deionized with Amberlite IR-120 (H^+) anion-exchange resin, filtered, and concentrated. The tetrasaccharide **11** was finally obtained after chromatography on a Sephadex G-25 column (water) as an amorphous powder (24 mg, 85%) after freeze-drying; mp 154–160 °C; $[\alpha]_D$ −6.9° (*c* 0.8, water); ^{1}H NMR: δ 6.02–5.97 (m, 1 H, CH_2=C*H*–CH_2), 5.39–5.28 (m, 2 H, C*H*$_2$=CH–CH_2), 4.90 (d, *J* 7.8 Hz, 1 H), 4.86 (s, 1 H), 4.79 (s, 1 H), 4.50 (d, *J* 7.9 Hz, 1 H), 4.21–3.66 (m, 26 H). MS Calcd for $C_{27}H_{45}O_{21}$: 705.2 [M]. Found: 705.4. Anal. Calcd for $C_{27}H_{46}O_{21}$: C, 45.89; H, 6.51. Found: C, 45.80; H, 6.55.

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