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A highly efficient synthesis of an octasaccharide, the repeating unit of the cell-wall mannan of *Trichophyton mentagrophytes* and *T. rubrum*

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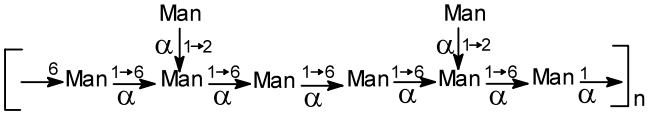
Abstract

A highly concise and effective synthesis of the mannose octasaccharide repeating unit of the cell-wall mannan of *Trichophyton mentagrophytes* and *T. rubrum* was achieved via 6-*O*-glycosylation of a tetrasaccharide acceptor with a tetrasaccharide donor, followed by deprotection. The key tetrasaccharide (**11**) was constructed by selective 6-*O*-glycosylation of allyl 3,4-di-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside with 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate, then with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate. The tetrasaccharide acceptor (**13**) was obtained by selective 6-*O*-deacetylation of **11**, while the tetrasaccharide donor **12** was obtained by deallylation of **11**, followed by trichloroacetimidation.

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

1. Introduction

The anthropophilic dermatophytes, *Trichophyton mentagrophytes* and *T. rubrum*, cause chronic, relatively uninflamed, skin infections of the feet (tinea pedis) and body (tinea corporis), often associated with infection of the nails (onychoycosis). About 90% of chronic dermatophyte infections are caused by *T. mentagrophytes* and *T. rubrum*,[1] in part because these organisms can suppress inflammation and cell-mediated immunity.[2] The cell-wall polysaccharides of these fungi are known to be the major immunologically active substances. There are mainly two kinds of polysaccharides present in the cell-wall, i.e., mannan and galactomannan.[3] The mannan has a linear backbone consisting of α-(1→6)-linked mannose units, with α-(1→2)-linked mannose units as side chains, and its structure[3] is shown as follows:



Blake et al. examined the ability of a cell-wall mannan isolated from *T. rubrum* to suppress cell-mediated immune reactions[4] and found that the *T. rubrum* mannan inhibits the lymphoproliferative response of human mononuclear leukocytes to a variety of antigenic and mitogenic stimuli in vitro. Furthermore, Grando et al. demonstrated that the target cells for this effect of the mannan are monocytes and keratinocytes, by monitoring the binding and uptake of FITC-mannan using flow cytometry and fluorescence microscopy.[5,6] To further elucidate the molecular structure responsible for the immunoinhibitory activity of the fungi mannan, it will be very useful to synthesize the repeating unit of the mannan. We report herewith a highly efficient and concise synthesis of the octasaccharide **1**, the repeating unit of the cell-wall mannan of *T. mentagrophytes* and *T. rubrum*.

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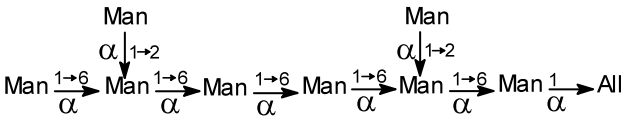
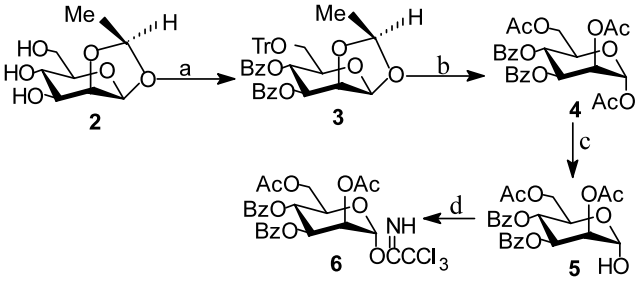


2. Results and discussion

First we synthesized the key synthon **6** (Scheme 1). Thus, tritylation of 1,2-*O*-(*R*-ethylidene)-β-D-mannopyranose[7] (**2**), followed by benzoylation in a one-pot manner, gave the 3,4-di-*O*-benzoyl-1,2-*O*-(*R*-ethylidene)-6-*O*-trityl-D-mannopyranose (**3**). Acetolysis of **3** with CH_2Cl_2–AcOH–Ac_2O–H_2SO_4 in a ratio of 1:1:0.6:0.18 afforded the 1,2,6-tri-*O*-acetyl-3,4-di-*O*-benzoyl-α-D-mannopyranose (**4**). The triacetate **4** was selectively deacetylated at the anomeric position with benzylamine in THF in high yield to give the corresponding 2,6-di-*O*-acetyl-3,4-di-*O*-benzoyl-D-mannopyranose (**5**). Subsequent reaction of **5** with CCl_3CN–DBU in dichloromethane afforded the glycosyl donor **6**.

With the synthon **6** in hand, construction of the target compound was readily carried out. As shown in Scheme 2, the disaccharide **7** was prepared using **6** as the glycosyl donor and allyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside as the acceptor.[8] Selective removal of the acetyl groups of **7** in methanol solution containing 0.5% HCl gave the diol glycosyl acceptor **8**.[8] Coupling of **8** with 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate[8] as the glycosyl donor selectively gave the (1→6)-linked trisaccharide **9**. Acetylation of **9** confirmed the 6-*O*-glycosylation as the ^{1}H NMR spectrum of acetylated trisaccharide **10** showed a newly emerged doublet of doublets at δ 5.67 ppm for H-2. Condensation of **9** with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate[8] using TMSOTf as catalyst afforded the tetrasaccharide **11**. The ^{1}H NMR spectrum of **11** showed one acetyl signal (δ 1.96), allyl signals (5.47–5.30), and four H-1 signals (5.33, 5.20, 5.05, 4.91) characteristic of the structure of the tetrasaccharide **11**. Selective removal of the 6-*O*-acetyl group of the tetrasaccharide **11** gave the glycosyl acceptor **13**. Deallylation[9] of **11** with $PdCl_2$, followed by activation with CCl_3CN in the presence of K_2CO_3, gave the tetrasaccharide donor **12**. The fully protected octasaccharide **14** was smoothly obtained by coupling **13** with **12**. The ^{13}C NMR spectrum of **14** gave eight signals for C-1 (100.08, 100.06, 98.9, 98.7, 98.1, 97.8, 97.6, 96.8 (8 C-1). Finally, deacylation of **14** in ammonia–saturated methanol gave the target octasaccharide **1**. A bioassay of **1** for its immunoinhibitory activity is in progress.



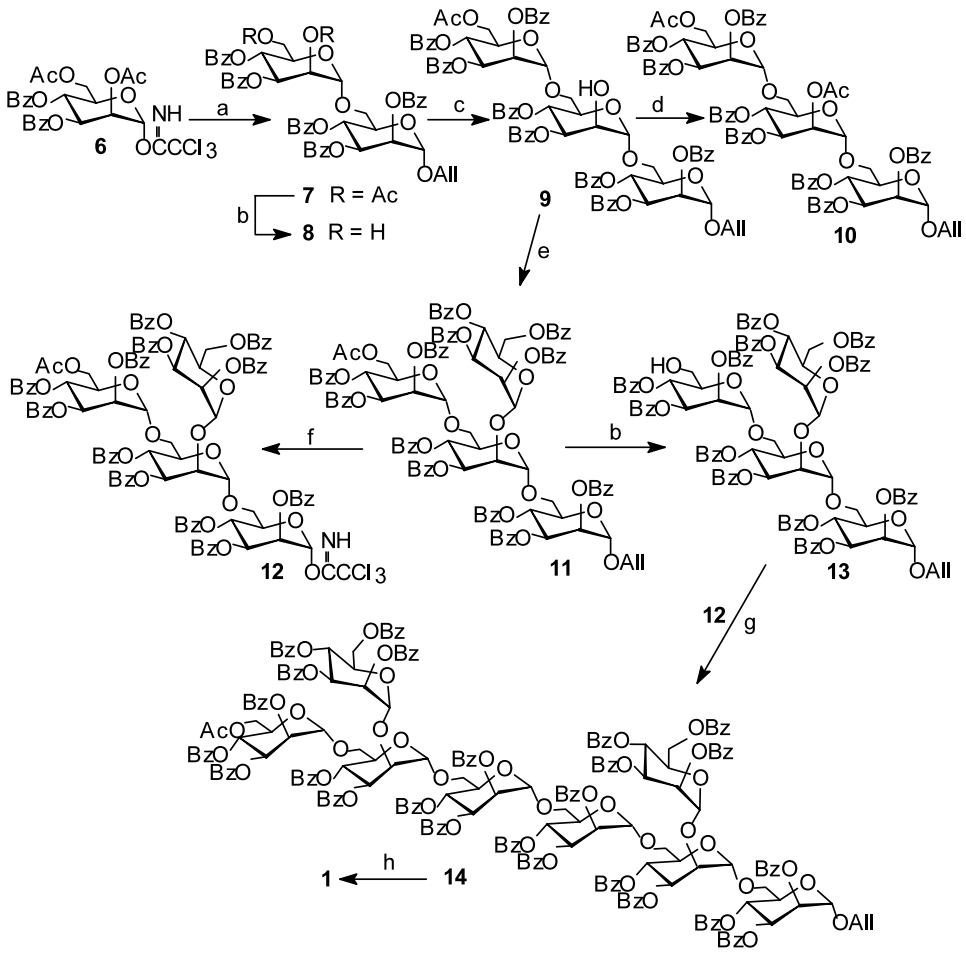
Scheme 1. Conditions and reagents: (a) i. trityl chloride (1.2 equiv), pyridine, 50 °C, 32 h; ii. PhCOCl (4.8 equiv), < 40 °C, 24 h (71% for two steps); (b) 1:1:0.6:0.18 CH_2Cl_2–HOAc–Ac_2O–H_2SO_4, rt, 20 h (83%); (c) benzylamine (4.0 equiv), THF, rt, 24 h (88%); (d) CCl_3CN (3.3 equiv), DBU (0.3 equiv), rt, 5 h (86%).

In summary, a highly efficient and concise synthesis of the mannose octasaccharide repeating unit of the mannans of the cell-walls of some fungi was achieved by regio- and stereoselective glycosylation using glycosyl trichloroacetimidates as the donors and partially protected sugars as the acceptors. The sole use of acyl groups in the synthesis substantially simplified the procedure. This method should also be useful for the synthesis of high-mannose oligosaccharides.

3. Experimental

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 NMR and ^{13}C NMR spectra were recorded with Bruker ARX 400 spectrometers (400 MHz for ^{1}H, 100 MHz for ^{13}C) for solutions in $CDCl_3$ or D_2O as indicated. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were recorded with a VG PLATFORM mass spectrometer in 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 detector. 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.

*3,4-Di-*O*-benzoyl-1,2-*O*-[(*R*)-ethylidene]-6-*O*-trityl-β-D-mannopyranose* (**3**).—A solution of 1,2-*O*-[(*R*)-ethylidene]-β-D-mannopyranose (**2**)[7] (6.4 g, 31.1 mmol) and trityl chloride (9.8 g, 35.0 mmol) in pyridine (100 mL) was stirred at 50 °C for 32 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was cooled to 0 °C, and then benzoyl chloride (7.9 mL, 68 mmol) was added dropwise within 30 min to keep the reaction temperature under 40 °C. After 24 h, water (300 mL) was added to the reaction mixture, and stirring was continued for 30 min. The mixture was extracted with CH_2Cl_2 (3 × 100 mL), and the combined



Scheme 2. Conditions and reagents: (a) allyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (1 equiv), TMSOTf (0.26 equiv), CH_2Cl_2, rt, 3 h (88%); (b) MeOH–0.5% HCl, rt, 12–14 h (93–96%); (c) 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (1.0 equiv), CH_2Cl_2, TMSOTf (0.08 equiv), rt, 3 h (86%); (d) $(Ac)_2O$, pyridine, rt, 5 h (100%); (e) 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (1.4 equiv), CH_2Cl_2, TMSOTf (0.16 equiv), rt, 3 h (85%); (f) i. $PdCl_2$, CH_3OH–CH_2Cl_2, 2 h; ii: CCl_3CN, DBU, CH_2Cl_2 8 h (78%); (g) **12** (1.2 equiv), CH_2Cl_2, TMSOTf (0.3 equiv), rt, 3 h (80%); (h) CH_3OH satd with dry NH_3, rt, 72 h (98%).

extracts were washed with 1 N HCl and satd aq $NaHCO_3$, dried (Na_2SO_4) and concentrated to a syrup that was subjected to column chromatography with 4:1 petroleum ether–EtOAc as the eluent. Compound **3** (14.5 g, 71%) was obtained as a syrup: $[\alpha]_D$ −37.2° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.01, 7.90 (2 d, 4 H, Bz*H*), 7.65–7.08 (m, 21 H, Ph*H*), 5.97 (dd, 1 H, $J_{3,4} = J_{5,4}$ 10.0 Hz, H-4), 5.48 (dd, 1 H, $J_{2,3}$ 3.6 Hz, $J_{4,3}$ 10.0 Hz, H-3), 5.43 (d, 1 H, $J_{2,1}$ 2.0 Hz, H-1), 5.37 (q, *J* 4.8 Hz, C*H*-CH_3), 4.49 (dd, $J_{1,2}$ 2.0 Hz, $J_{3,2}$ 3.6 Hz, H-2), 3.79 (m, 1 H, $J_{6a,5}$ 2.8 Hz, $J_{6b,5}$ 4.4 Hz, $J_{4,5}$ 10.0 Hz, H-5), 3.37 (dd, 1 H, $J_{6a,6b}$ 10.2 Hz, $J_{5,6a}$ 2.8 Hz, H-6a), 3.22 (dd, 1 H, $J_{6a,6b}$ 10.2 Hz, $J_{5,6b}$ 4.4 Hz, H-6b), 1.60 (d, 3 H, *J* 4.8 Hz, CH-CH_3). Anal. Calcd for $C_{41}H_{36}O_8$: C, 74.99; H, 5.52. Found: C, 75.16; H, 5.55.

1,2,6-Tri-O-*acetyl-3,4-di*-O-*benzoyl*-α-D-*mannopyranose* (**4**).—Compound **3** (11 g, 16.77 mol) was dissolved in a mixture of CH_2Cl_2 (50 mL), Ac_2O (50 mL) and AcOH (30 mL), the solution was cooled to 10 °C in an ice bath, and H_2SO_4 (8.8 mL) was added dropwise over 20 min. After the addition was complete, the ice bath was removed and the reaction was allowed to continue for 20 h at ambient temperature. The reaction solution was poured into ice water (400 mL), stirring was continued for an additional 15 min, and the mixture was extracted with $CHCl_3$ (3 × 100 mL). The combined extracts were washed with 10% aq $NaHCO_3$ (3 × 60 mL), dried over Na_2SO_4, and concentrated to a syrup that was subjected to column chromatography with 4:1 petroleum ether–EtOAc as the eluent. Compound **4** (7.2 g, 83%) was obtained as a syrup: $[\alpha]_D$ −28.2° (*c* 1.0, $CHCI_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.95, 7.89 (2 d, 4 H, Bz*H*), 7.53–7.34 (m, 6 H, Bz*H*), 6.21 (d, 1 H, $J_{2,1}$ 1.9 Hz, H-1), 5.84 (dd, 1 H, $J_{3,4} = J_{5,4}$ 9.9 Hz, H-4), 5.75 (dd, 1 H, $J_{2,3}$ 4.7 Hz, $J_{4,3}$ 9.9 Hz, H-3), 5.48 (dd, 1 H, $J_{1,2}$ 1.9 Hz, $J_{3,2}$ 4.7 Hz, H-2), 4.36–4.20 (m, 3 H, H-5, H-6a, H-6b), 2.24, 2.18, 2.05 (3 s, 9 H, 3 COCH_3). Anal. Calcd for $C_{26}H_{26}O_{11}$: C, 60.70; H, 5.09. Found: C, 60.98; H, 5.15.

2,6-Di-O-*acetyl-3,4-di*-O-*benzoyl*-α-D-*mannopyranose* (**5**).—A solution of compound **4** (5 g, 9.73 mmol) and benzylamine (3 mL, 27.4 mmol) in anhyd THF (30 mL) was stirred at rt for 24 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was then concentrated. Purification by flash column chromatography on silica gel (3:1 petroleum ether–EtOAc) gave compound **5** as a syrup (3.96 g, 88%): $[\alpha]_D$ −37.4° (*c* 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95, 7.89 (2 d, 4 H, Bz*H*), 7.52–7.32 (m, 6 H, Bz*H*), 5.84–5.76 (m, 2 H, H-3, H-4), 5.50 (dd, 1 H, $J_{1,2}$ 1.8 Hz, $J_{3,2}$ 2.9 Hz, H-2), 5.38 (d, 1 H, $J_{2,1}$ 1.8 Hz, H-1), 4.51–4.24 (m, 3 H, H-5, H-6a, H-6), 2.17, 2.06 (2 s, 6 H, 2 COCH_3). Anal. Calcd for $C_{24}H_{24}O_{10}$: C, 61.01; H, 5.12. Found: C, 61.29; H, 5.19.

2,6-Di-O-*acetyl-3,4-di*-O-*benzoyl*-α-D-*mannopyranosyl trichloroacetimidate* (**6**).—A mixture of **5** (4.0 g, 8.47 mmol), CCl_3CN (2.1 mL, 20.9 mmol), and 1,8-diazabicyclo[5.4.0]undecene (DBU) (0.25 mL, 1.67 mmol) in dry CH_2Cl_2 (25 mL) was stirred under N_2 for 5 h and then concentrated. The residue was purified by flash chromatography (4:1 petroleum ether–EtOAc) to give **6** (4.5 g, 86%) as a syrup: $[\alpha]_D$ −19.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.84 (s, 1 H, N*H*), 7.96, 7.89 (2 d, 4 H, Bz*H*), 7.53–7.34 (m, 6 H, Bz*H*), 6.41 (d, 1 H, $J_{2,1}$ 1.8 Hz, H-1), 5.89 (dd, 1 H, $J_{3,4} = J_{5,4}$ 10.0 Hz, H-4), 5.80 (dd, 1 H, $J_{2,3}$ 3.4 Hz, $J_{4,3}$ 10.0 Hz, H-3), 5.70 (dd, 1 H, $J_{1,2}$ 1.8 Hz, $J_{3,2}$ 3.4 Hz, H-2), 4.46–4.26 (m, 3 H, H-5, H-6a, H-6b), 2.18, 2.04 (2 s, 6 H, 2 COCH_3). Anal. Calcd for $C_{26}H_{24}Cl_3NO_{10}$: C, 50.62; H, 3.92. Found: C, 50.79; H, 3.85.

Allyl 2,6-di-O-*acetyl-3,4-di*-O-*benzoyl*-α-D-*mannopyranosyl-(1→6)-2,3,4-tri*-O-*benzoyl*-α-D-*mannopyranoside* (**7**).—A solution of **6** (2.5 g, 4.06 mmol) and allyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (2.16 g, 4.0 mmol) in dry CH_2Cl_2 (40 mL) was stirred with dried molecular sieves (4 Å, 1 g) under N_2 for 15 min, and then TMSOTf (0.2 mL, 1.1 mmol) was added dropwise. After 1 h the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with satd aq $NaHCO_3$ (15 mL). The organic layer was dried over Na_2SO_4 and concentrated. Purification of the residue by flash chromatography (3:1 petroleum ether–EtOAc) gave **7** as a syrup (3.47 g, 88%): $[\alpha]_D$ −51.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14, 7.99, 7.97, 7.89, 7.84 (5 d, 10 H, Bz*H*), 7.55–7.27 (m, 15 H, Bz*H*), 5.99 (m, 1 H, C*H*=CH_2), 5.97–5.73 (m, 5 H, 2 H-4, 2 H-3, H-2), 5.54 (dd, 1 H, H-2), 5.53 (dd, 1 H, ^2J 1.3 Hz, $^3J_{trans}$ 17.1 Hz, CH=CH_2), 5.35 (dd, 1 H, ^2J 1.3 Hz, $^3J_{cis}$ 10.4 Hz, CH=CH_2), 5.16 (d, 1 H, $J_{2',1'}$ 1.5 Hz, H-1′), 4.97 (d, 1 H, $J_{2,1}$ 1.5 Hz, H-1), 4.40–3.70 (m, 8 H, CH_2CH=CH_2, 2 H-5, 4 H-6), 2.13, 1.88 (2 s, 6 H, 2 COCH_3). Anal. Calcd for $C_{54}H_{50}O_{18}$: C, 65.72; H, 5.10. Found: C, 65.94; H, 5.07.

Allyl 3,4-di-O-*benzoyl*-α-D-*mannopyranosyl-(1→6)-2,3,4-tri*-O-*benzoyl*-α-D-*mannopyranoside* (**8**).—A solution of **7** (1.6 g, 1.62 mmol) in aq MeOH (80 mL) containing 0.5% HCl was stirred at rt for 12 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the starting material had disappeared. The mixture was neutralized with Et_3N and then concentrated to dryness. The residue was partitioned between water and CH_2Cl_2, then the organic layer was dried over Na_2SO_4 and concentrated to a syrup. Purification of the residue by flash chromatography (2:1 petroleum ether–EtOAc) gave **8** as a syrup (1.36 g, 93%): $[\alpha]_D$ −8.8° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15, 8.05, 8.00, 7.96, 7.89 (5 d, 10 H, Bz*H*), 7.53–7.25 (m, 15 H, Bz*H*), 6.06–5.71 (m, 6 H, C*H*=CH_2, 2 H-4, 2 H-3, 1 H-2), 5.48 (dd, 1 H, ^2J 1.4 Hz, $^3J_{trans}$ 17.2 Hz, CH=CH_2), 5.38 (dd, 1 H, ^2J 1.4 Hz, $^3J_{cis}$ 10.4 Hz, CH=CH_2), 5.15 (d, 1 H, $J_{2,1}$ 1.5 Hz, H-1), 5.05 (d, 1 H, $J_{2',1'}$ 1.4 Hz, H-1′), 4.41–3.46 (m, 9 H, CH_2CH=CH_2, 2 H-5, 4 H-6, 1 H-2). Anal. Calcd for $C_{50}H_{46}O_{16}$: C, 66.51; H, 5.13. Found: C, 66.80; H, 5.19.

Allyl 6-O-*acetyl-2,3,4-tri*-O-*benzoyl*-α-D-*mannopyranosyl-(1→6)-3,4-di*-O-*benzoyl*-α-D-*mannopyranosyl-(1→6)-2,3,4-tri*-O-*benzoyl*-α-D-*mannopyranoside* (**9**).—A solution of **8** (0.37 g, 0.41 mmol) and TMSOTf (10 μL, 0.055 mmol) in dry CH_2Cl_2 (6 mL) was stirred with dried molecular sieves (4 Å, 0.4 g) under N_2 for 15 min, and then 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (0.28 g, 0.41 mmol) in CH_2Cl_2 (4 mL) was added dropwise within 20 min. After 3 h the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with satd aq $NaHCO_3$ (5 mL). The organic layer was dried over Na_2SO_4 and concentrated. Purification of the residue by flash chromatography (2:1 petroleum ether–EtOAc) gave **9** as a syrup (0.50 g, 86%): $[\alpha]_D$ −51.4° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.25 (m, 40 H, 8 BzH), 6.26–5.77 (m, 9 H, CH=CH_2, 3 H-4, 3 H-3, 2 H-2), 5.41 (dd, 1 H, ^2J 1.4 Hz, $^3J_{trans}$ 17.2 Hz, CH=CH_2), 5.25 (dd, 1 H, ^2J 1.4 Hz, $^3J_{cis}$ 10.4 Hz, CH=CH_2), 5.20 (d, 1 H, $J_{2,1}$ 1.4 Hz, H-1), 5.14 (d, 1 H, $J_{2,1}$ 1.3 Hz, H-1), 4.84 (d, 1 H, *J* 1.4 Hz, H-1), 4.47–3.37 (m, 13 H, CH_2CH=CH_2, 1 H-2, 3 H-5, 6 H-6, OH), 2.00 (s, 3 H, $COCH_3$). Anal. Calcd for $C_{79}H_{70}O_{25}$: C, 66.85; H, 4.97. Found: C, 66.98; H, 4.86.

Allyl 6-O-*acetyl-2,3,4-tri*-O-*benzoyl*-α-D-*mannopyranosyl-(1→6)-2*-O-*acetyl-3,4-di*-O-*benzoyl*-α-D-*mannopyranosyl-(1→6)-2,3,4-tri*-O-*benzoyl*-α-D-*mannopyranoside* (**10**).—To a solution of **9** (1.20 g, 0.84 mmol) in pyridine (20 mL), Ac_2O (1 mL, 10 mmol) was added dropwise, and the mixture was stirred overnight at rt. TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH_2Cl_2 and sequentially washed with 1 N HCl, water, and satd aq $NaHCO_3$. The organic layers were combined, dried, and concentrated. Purification by column chromatography (2:1 petroleum ether–EtOAc) quantitatively gave **10** as a syrup: $[\alpha]_D$ −99.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.24 (m, 40 H, 8 BzH), 6.21–5.79 (m, 9 H, CH=CH_2, 3 H-4, 3 H-3, 2 H-2), 5.67 (dd, 1 H, H-2), 5.41 (dd, 1 H, ^2J 1.4 Hz,

$^3J_{trans}$ 17.2 Hz, CH=CH_2), 5.25 (dd, 1 H, ^2J 1.4 Hz, $^3J_{cis}$ 10.4 Hz, CH=CH_2), 5.20 (d, 1 H, $J_{2,1}$ 1.4 Hz, H-1), 5.06 (d, 1 H, $J_{2,1}$ 1.3 Hz, H-1), 4.87 (d, 1 H, $J_{2,1}$ 1.4 Hz, H-1), 4.42–3.42 (m, 11 H, CH_2CH=CH_2, 3 H-5, 6 H-6), 2.21, 2.00 (2 s, 6 H, 2 $COCH_3$). Anal. Calcd for $C_{81}H_{72}O_{26}$: C, 66.57; H, 4.96. Found: C, 66.31; H, 5.03.

*Allyl 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1→2)]-3,4-di-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranoside* (**11**).—A solution of **9** (0.37 g, 0.26 mmol) and TMSOTf (10 μL, 0.055 mmol) in dry CH_2Cl_2 (6 mL) was stirred with dried molecular sieves (4 Å, 0.4 g) under N_2 for 15 min, and then 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (0.26 g, 0.35 mmol) in CH_2Cl_2 (4 mL) was added dropwise within 20 min. After 3 h the reaction mixture was diluted with CH_2Cl_2 (30 mL) and washed with satd aq $NaHCO_3$ (5 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Purification of the residue by flash chromatography (2:1 petroleum ether–EtOAc) gave **11** as a syrup (0.45 g, 85%): $[\alpha]_D$ −72.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–7.02 (m, 60 H, 12 BzH), 6.20–5.67 (m, 12 H, CH=CH_2, 4 H-4, 4 H-3, 3 H-2), 5.47 (dd, 1 H, ^2J 1.5 Hz, $^3J_{trans}$ 17.1 Hz, CH=CH_2), 5.33 (d, 1 H, $J_{2,1}$ 1.2 Hz, H-1), 5.30 (dd, 1 H, ^2J 1.5 Hz, $^3J_{cis}$ 10.4 Hz, CH=CH_2), 5.20 (d, 1 H, *J* 1.4 Hz, H-1), 5.05 (d, 1 H, $J_{2,1}$ 1.4 Hz, H-1), 4.91 (d, 1 H, $J_{2,1}$ 1.4 Hz, H-1), 4.77–3.49 (m, 15 H, CH_2CH=CH_2, H-2, 4 H-5, 8 H-6), 1.96 (s, 3 H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5 (1 C, $COCH_3$), 166.0, 165.7, 165.7, 165.6, 165.5, 165.5, 165.4, 165.3, 165.1, 164.9, 164.8, 164.7 (12 C, 12 COPh), 133.3–127.7 (73 C, 12 Ph, CH_2CH=CH_2), 118.2 (1 C, CH_2CH=CH_2), 100.0, 98.8, 97.3, 96.8 (4 C-1), 20.5 (1 C, $COCH_3$). Anal. Calcd for $C_{113}H_{96}O_{34}$: C, 67.93; H, 4.84. Found: C, 67.78; H, 4.89.

*Allyl 2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1→2)]-3,4-di-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranoside* (**13**).—A solution of **11** (1.6 g, 0.80 mmol) in MeOH (80 mL) containing 0.5% HCl was stirred at rt for 12 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the starting material had disappeared. The mixture was neutralized with Et_3N and then concentrated to dryness. The residue was partitioned between water and CH_2Cl_2, then the organic layer was dried over Na_2SO_4, and concentrated to a syrup. Purification of the residue by flash chromatography (1:1 petroleum ether–EtOAc) gave **13** (1.50 g, 96%): $[\alpha]_D$ −79.6° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.11–7.08 (m, 60 H, 12 BzH), 6.21–5.60 (m, 12 H, CH=CH_2, 4 H-4, 4 H-3, 3 H-2), 5.47 (dd, 1 H, ^2J 1.4 Hz, $^3J_{trans}$ 17.2 Hz, CH=CH_2), 5.32 (d, 1 H, $J_{2,1}$ 1.2 Hz, H-1), 5.30 (dd, 1 H, ^2J 1.4 Hz, $^3J_{cis}$ 10.4 Hz, CH=CH_2), 5.23 (d, 1 H, $J_{2,1}$ 1.3 Hz, H-1), 5.05 (d, 1 H, *J* 1.4 Hz, H-1), 4.90 (d, 1 H, $J_{2,1}$ 1.2 Hz, H-1), 4.71–3.48 (m, 15 H, CH_2CH=CH_2, H-2, 4 H-5, 8 H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.6, 166.0, 165.7, 165.6, 165.5, 165.5, 165.4, 165.4, 165.2, 164.9, 164.9, 164.7 (12 C, 12 COPh), 133.4–127.8 (73 C, 12 Ph, CH_2CH=CH_2), 118.2 (1 C, CH_2CH=CH_2), 99.9, 98.7, 97.3, 96.8 (4 C-1). Anal. Calcd for $C_{111}H_{94}O_{33}$: C, 68.17; H, 4.84. Found: C, 68.44; H, 4.78.

*6-*O*-Acetyl-2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1→2)]-3,4-di-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranosyl trichloroacetimidate* (**12**).—A mixture of compound **11** (2.24 g, 1.12 mmol) and $PdCl_2$ (50 mg) in dry MeOH (50 mL) (**Caution!** Extreme fire hazard.) was stirred vigorously for 4 h at rt, TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was filtered through Celite, and the filtrate was concentrated to dryness. The resulting compound was dissolved in CH_2Cl_2 (20 mL), then CCl_3CN (0.3 mL, 3 mmol) and DBU (42 μL, 0.3 mmol) were added. The reaction mixture was stirred for 2 h, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. Concentration of the reaction mixture, followed by purification on a silica gel column with 1:1 petroleum ether–EtOAc as the eluent, furnished the tetrasaccharide donor **12** in good yield (1.84 g, 78% two steps): $[\alpha]_D$ −71.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.99 (s, 1 H, NH), 8.09–7.03 (m, 60 H, 12 BzH), 6.64 (d, 1 H, $J_{2,1}$ 1.5 Hz, H-1), 6.35–5.90 (m, 11 H, 4 H-4, 4 H-3, 3 H-2), 5.54 (d, 1 H, $J_{2,1}$ 1.2 Hz, H-1), 5.31 (d, 1 H, $J_{2,1}$ 1.3 Hz, H-1), 5.12 (d, 1 H, $J_{2,1}$ 1.4 Hz, H-1), 4.88 (d, 1 H, $J_{2,1}$ 1.5 Hz, H-1), 4.78–3.47 (m, 13 H, H-2, 4 H-5, 8 H-6), 2.04 (s, 3 H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4 (1 C, $COCH_3$), 165.9, 165.6, 165.6, 165.5, 165.4, 165.3, 165.3, 165.2, 165.0, 164.9, 164.8, 164.7 (12 C, 12 COPh), 159.7 (1 C, CCl_3), 133.5–127.7 (72 C, 12 Ph), 99.9, 98.7, 97.1, 94.6 (4 C-1), 90.1 (1 C, C(NH)CCl_3), 20.4 (1 C, $COCH_3$). Anal. Calcd for $C_{112}H_{92}Cl_3NO_{34}$: C, 63.99; H, 4.41. Found: C, 63.71; H, 4.48.

*Allyl 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1→2)]-3,4-di-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl-(1→2)]-3,4-di-*O*-benzoyl-α-*D*-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-*D*-mannopyranoside* (**14**).—The tetrasaccharide donor **12** (1.19 g, 0.57 mmol) and the tetrasaccharide acceptor **13** (1.07 g, 0.55 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (30 mL). TMSOTf (30 μL) was added dropwise at −20 °C with N_2 protection. The reaction mixture was stirred for 3 h, during which time the

reaction temperature gradually raised to ambient temperature. Then the mixture was neutralized with Et_3N and concentrated under reduced pressure to an oily residue. Purification by column chromatography (1:1 petroleum ether–EtOAc) gave **14** (1.72 g, 80%) as a syrup: $[\alpha]_D$ −66.2° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.11–6.88 (m, 120 H, 24 BzH), 6.24–5.76 (m, 23 H, $CH{=}CH_2$, 8 H-4, 8 H-3, 6 H-2), 5.46 (dd, 1 H, ^2J 1.5 Hz, $^3J_{trans}$ 17.1 Hz, $CH{=}CH_2$), 5.41 (d, 1 H, $J_{2,1}$ 1.2 Hz, H-1), 5.28 (dd, 1 H, ^2J 1.5 Hz, $^3J_{cis}$ 10.4 Hz, $CH{=}CH_2$), 5.25 (d, 1 H, $J_{2,1}$ 1.3 Hz, H-1), 5.17 (d, 1 H, $J_{2,1}$ 1.3 Hz, H-1), 5.09 (d, 1 H, $J_{2,1}$ 1.2 Hz, H-1), 5.02 (d, 1 H, $J_{2,1}$ 1.2 Hz, H-1), 5.00 (m, 2 H, 2 H-1), 4.95 (d, 1 H, $J_{2,1}$ 1.2 Hz, H-1), 4.79–3.38 (m, 28 H, $CH_2CH{=}CH_2$, 2 H-2, 8 H-5, 16 H-6), 2.03 (s, 3 H, $COCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.3 (1 C, $COCH_3$), 166.0–164.6 (24 C, 24 COPh), 133.3–127.3 (145 C, 24 Ph, $CH_2CH{=}CH_2$), 118.1 (1 C, $CH_2CH{=}CH_2$), 100.08, 100.06, 98.9, 98.7, 98.1, 97.8, 97.6, 96.8 (8 C-1), 20.3 (1 C, $COCH_3$). Anal. Calcd for $C_{221}H_{184}O_{66}$: C, 68.13; H, 4.76. Found: C, 68.41; H, 4.70.

Allyl α-D-*mannopyranosyl-(1→6)-[*α-D-*mannopyranosyl-(1→2)]-*α-D-*mannopyranosyl-(1→6)-*α-D-*mannopyranosyl-(1→6)-*α-D-*mannopyranosyl-(1→6)-[*α-D-*mannopyranosyl-(1→2)]-*α-D-*mannopyranosyl-(1→6)-*α-D-*mannopyranoside* (**1**).—A satd solution of ammonia in MeOH (40 mL) was added to a solution of **14** (811 mg, 0.21 mmol) in CH_2Cl_2 (4 mL). After a week at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on a Sephadex LH-20 (column 2.0 × 30 cm, flow 5 mL/min, about 300 mL MeOH) to afford the single octasaccharidic product **1** as a white amorphous powder (274 mg, 98%): $[\alpha]_D$ +47.1° (*c* 1.0, H_2O); ^1H NMR (D_2O, 400 MHz): δ 5.87 (m, 1 H, $CH{=}CH_2$), 5.24–5.14 (2 H, $CH{=}CH_2$), 5.00–4.79 (8 H-1); ^{13}C NMR (100 MHz, D_2O): 133.3 (1 C, $CH_2CH{=}CH_2$), 118.2 (1 C, $CH_2CH{=}CH_2$), 100.2, 100.1, 99.2, 99.17, 99.10, 99.0, 97.8, 96.8 (8 C-1). ES-IMS: Calcd for $C_{51}H_{86}O_{41}$, 1355.22 [M]. Found, 1378.1 $(M + Na)^+$.

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