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Synthesis of mannose-containing analogues of (1 → 6)-branched (1 → 3)-glucohexaose

Zicheng Wu and Fanzuo Kong*

Research Center for Eco-Environmental Sciences, Academia Sinica, P.O. Box 2871, Beijing 100085, China

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Abstract—Coupling of the trisaccharide acceptor 2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**2**) with the trisaccharide donor 2,3,4,6-tetra-*O*-benzoyl-α-D-annopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**1**) gave an α-linked hexasaccharide **3**, while coupling of **2** with the trisaccharide donor 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 (**7**) produced α- **8** and β-linked **12** hexasaccharides in a ratio of 3:2. Deprotection of **3**, **8**, and **12** afforded the analogues of the immunomodulator β-D-Glc*p*-(1 → 3)-[β-D-Glc*p*-(1 → 6)]-α-D-Glc*p*-(1 → 3)-β-D-Glc*p*-(1 → 3)-[β-D-Glc*p*-(1 → 6)]-D-Glc*p* (**A**).

Keywords: Oligosaccharide; Mannose; Glucose

1. Introduction

β-(1 → 6)-Branched β-(1 → 3)-glucans are the common structures of biologically active polysaccharides from *Ganoderma lucidum*, *Schizophyllum commune*, and *Lentinus edodes*.[1] Recent studies revealed that α-(1 → 3)-linked glucans also exist in some medically important fungi such as *Cryphonectrini parasitica* and *Ganoderma lucidum*.[2] Some scientists show that the antitumor activity of these glucans may be closely related to the triple-helix structures of the glucans[3] with certain molecular weights (MW > 16,000).[4] However, an interesting result in our research revealed[5] that a synthetic hexasaccharide, β-D-Glc*p*-(1 → 3)-[β-D-Glc*p*-(1 → 6)]-α-D-Glc*p*-(1 → 3)-β-D-Glc*p*-(1 → 3)-[β-D-Glc*p*-(1 → 6)]-D-Glc*p* (**A**) has similar or even better activity compared to that of lentinan.[5] The synthesis of two mannose-containing analogues of **A** has been reported.[6] We present herein the synthesis of three additional mannose-containing analogues of the active glucose hexasaccharide.

2. Results and discussion

As shown in Scheme 1, condensation of the trisaccharide acceptor 2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**2**)[5a] with the trisaccharide donor 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**1**)[5a] afforded an α-linked hexasaccharide **3** in a high yield (84%), and no β-linked product was detected. O-Deisopropylidenation of **3** in 90% CF_3CO_2H smoothly offered the hemiacetal **4** (93%), and subsequent acetylation (95%) and deacylation yielded the target hexaose **6** (87%).

However, when the trisaccharide acceptor **2** was coupled with the trisaccharide donor 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 (**7**),[5a] a mixture

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* Corresponding author. Tel.: +86-10-62936613; fax: +86-10-629235-63; e-mail: fzkong@mail.rcees.ac.cn

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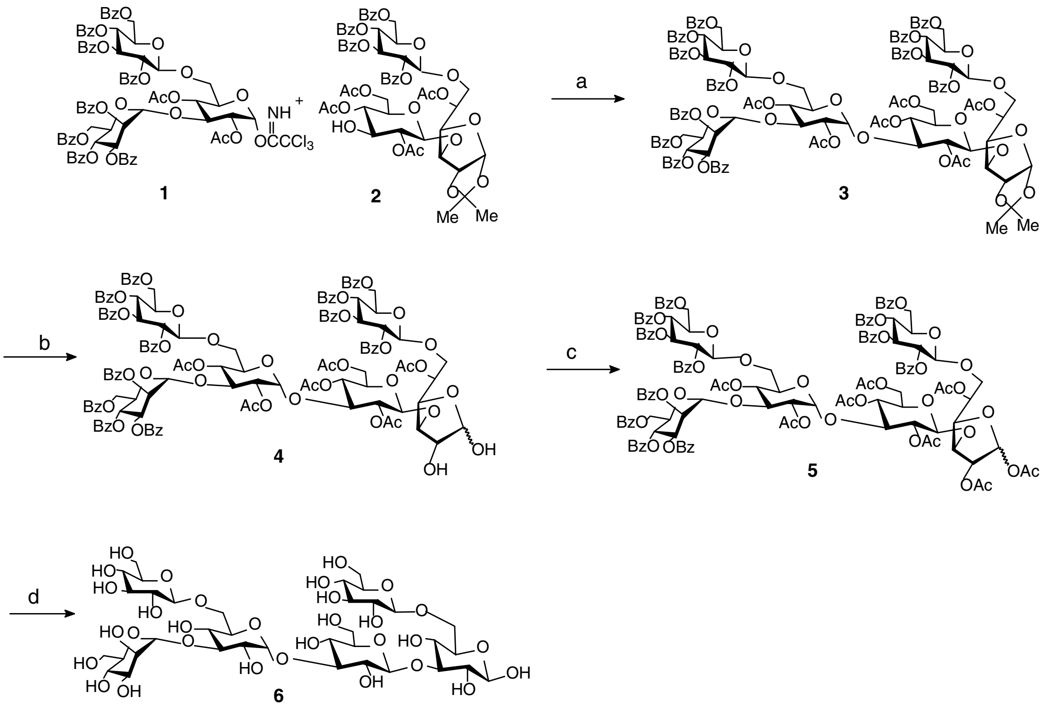


Scheme 1. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −20 °C to rt, 3–5 h; (b) 90% CF_3CO_2H, rt; (c) Ac_2O, Pyr. rt 12 h; (d) satd NH_3/MeOH, rt, 7 d.

of α- and β-linked hexasaccharide in a ratio of 3:2 was obtained. The mixture was well separated giving α-linked hexasaccharide **8** (48.0%) and β-linked hexasaccharide **12** (30.9%), respectively. It was noted that coupling of the acceptor **2** with the trisaccharide donor **1** solely gave the α-linked hexasaccharide, while coupling of the same donor **7** with the trisaccharide acceptor 2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)]-5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose also furnished the α-linked hexasaccharide.[6] These results indicated that any subtle changes of the structures in either the donor or the acceptor could vary the stereoselectivity in glycosylation (Scheme 2).

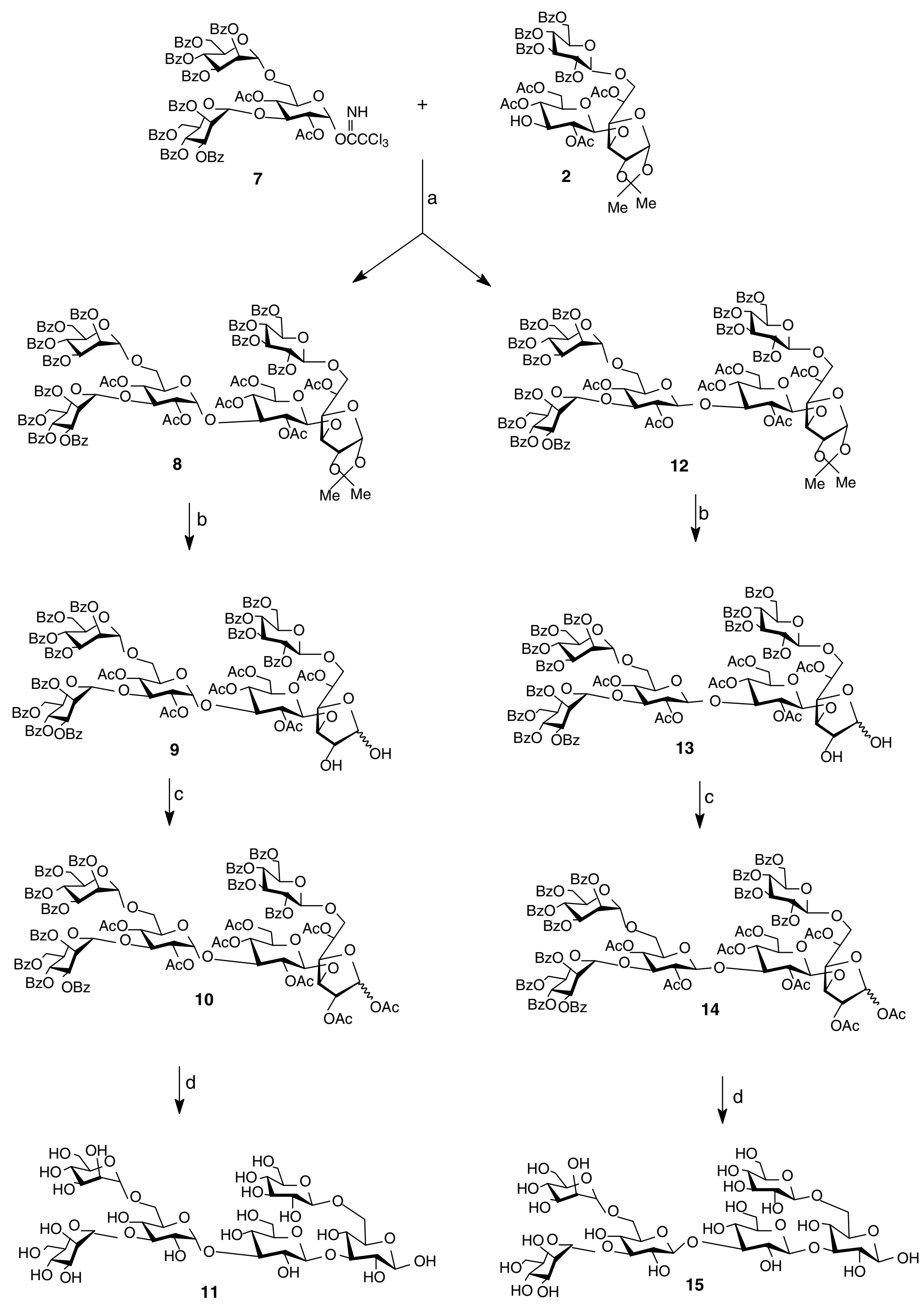
The target hexaoses **11** and **15** were obtained by O-deisopropylidenation of **8** and **12**, subsequent acetylation, and deacylation, respectively. The ^{1}H NMR of **11** showed characteristic signals at δ 5.28 with $J_{1,2}$ 4.0 Hz for α-H-1 of Glc*p*, 5.15 and 4.82 for α-H-1 of Man*p*, and 4.58, 4.43, 4.42 with J 8.0 Hz for 3β-H-1 of Glc*p*, while the ^{1}H NMR of **15** gave characteristic signals at δ 5.15 and 4.84 for α-H-1 of Man*p*, and 4.62, 4.58, 4.43, and 4.42 with J 8.0 Hz for 4β-H-1 of Glc*p*.

Preliminary bioassay of the synthetic samples revealed that replacement of the branch glucose of **A** with mannose showed similar activity.[5b] However, study of the stimulatory effects of **6**, **11**, and **15** on the mouse spleen[5b] showed almost no activity, indicating that replacement of the backbone glucose of **A** with mannose abolished the activity completely. Detailed studies for elucidation of structure–stimulatory effects for the synthetic oligosaccharides are still in progress.

3. Experimental

3.1. 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 and ^{13}C NMR spectra were recorded with Varian XL-400 spectrometers for solutions in $CDCl_3$ or in D_2O as indicated. Individual resonances could not identified with the specific sugar residues. Chemical shifts are expressed in ppm downfield from the Me_4Si signal. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the ESI mode. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) sulfuric acid in methanol or 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) with EtOAc–petroleum ether (bp 60–90 °C) as the



Scheme 2. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −20 °C to rt, 3–5 h; (b) 90% CF_3CO_2H, rt; (c) Ac_2O, Pyr. rt 12 h; (d) satd NH_3/MeOH, rt, 7 d.

eluent. Analytical LC was performed with a Gilson HPLC consisting of a pump (model 306), stainless steel column packed with silica gel (Spherisorb SiO_2, 10 × 300 mm or 4.6 × 250 mm), differential refractometer

(132-RI Detector), UV/vis detector (model 118). 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.

3.2. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (3)

Compound **1** (500 mg, 0.320 mmol) and **2** (350 mg, 0.311 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (10 mL). TMSOTf (5 μL, 0.044 mmol) 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 with 1:2 petroleum ether–EtOAc as the eluent give the product **3** (663 mg, 84.2%) as a syrup: $[\alpha]_D$ +32° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.02–7.22 (m, 60H, 12Bz-*H*), 6.13 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.88 (dd, 2H, $J_{3,4} = J_{4,5} = 9.7$ Hz, 2H-4), 5.70–5.67 (m, 3H, H-3, 2H-2), 5.66 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.57 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.49–5.47 (m, 1H, H-5), 5.46 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.40 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.24 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.04 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.99 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.93 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 4.91 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.90 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.83 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 4.77 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.74 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.73–4.63 (m, 3H), 4.53 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.51 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.50–4.45 (m, 2H), 4.36 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.25–4.19 (m, 2H), 4.15–4.02 (m, 7H), 3.94–3.74 (m, 5H), 3.55–3.48 (m, 2H), 2.72–2.70 (m, 1H), 2.20, 2.10, 2.05, 2.00, 1.94, 1.76 (6s, 18H, 6C*H*$_3$CO), 1.37, 1.35 (2s, 6H, 2C*H*$_3$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.1, 170.6, 170.5, 169.4, 169.2, 168.9 (6C, 6*C*OCH$_3$), 166.2, 166.1, 165.8, 165.7, 165.6, 165.4, 165.4, 165.3, 165.3, 165.3, 165.1, 165.1 (12C, 12*C*OPh), 105.2 (α-C-1 of Glc*f*, $J_{C\text{–}H}$ 182.8 Hz), 101.1, 101.1, 98.7 (3β-C-1 of Glc*p*, $J_{C\text{–}H}$ 162.0–163.2 Hz), 98.5 (α-C-1 of Man*p*, $J_{C\text{–}H}$ 171.9 Hz), 94.5 (α-C-1 of Glc*p*, $J_{C\text{–}H}$ 172.3 Hz), 82.3, 79.5, 75.6, 74.4, 73.1, 73.1, 73.1, 73.0, 72.4, 72.4, 72.2, 72.2, 72.1, 72.0, 71.9, 71.8, 70.8, 70.5, 70.5, 70.5, 70.2, 69.8, 69.8, 69.6, 69.6, 69.2, 69.2, 69.0, 68.4, 67.8, 66.5, 63.2, 63.2, 62.5, 62.3, 61.8 (C-2–6), 26.9, 26.5, 21.1, 20.8, 20.6, 20.5. Anal. Calcd for $C_{135}H_{126}O_{49}$: C, 64.03; H, 5.01. Found: C, 64.31; H, 4.89.

3.3. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-5-*O*-acetyl-D-glucofuranose (4)

A solution of **3** (600 mg, 0.237 mmol) in 90% CF_3CO_2H (5 mL) was stirred at rt until TLC (1:1 petroleum ether–EtOAc) indicted that the reaction was complete, then concentrated to dryness. Purification by silica column chromatography (2:1 petroleum ether–EtOAc) gave **4** (550 mg, 93%) as a syrupy anomeric mixture, and the α-anomer was isolated in pure form and characterized: $[\alpha]_D^{25}$ +40° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.03–7.26 (m, 60H, 12Bz-*H*), 6.12 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.91 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.83 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.76 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.73 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.69 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.65 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.59–5.53 (m, 1H, H-5), 5.50 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.46 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.23 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.04 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.97 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.94 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 4.92 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.90 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.86 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.79 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 4.74 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.70 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.68–4.62 (m, 4H), 4.52 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.49–4.41 (m, 6H), 4.29–4.15 (m, 6H), 4.08–4.04 (m, 3H), 3.96–3.72 (m, 6H), 3.58–3.55 (m, 2H), 2.75–2.72 (m, 1H), 2.17, 2.14, 2.09, 2.02, 1.92, 1.84 (s, 18H, 6C*H*$_3$CO). Anal. Calcd for $C_{132}H_{122}O_{49}$: C, 63.61; H, 4.90. Found: C, 63.92; H, 4.81.

3.4. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-1,2,5-tri-*O*-acetyl-D-glucofuranose (5)

Compound **4** (500 mg, 0.201 mmol) was dissolved in pyridine (10 mL), and then Ac_2O (5 mL) was added. After stirring the mixture at rt for 12 h, TLC (2:1 petroleum ether–EtOAc) indicted that the reaction was complete. The reaction mixture was extracted with CH_2Cl_2 (50 mL), washed with dil HCl and satd aq $NaHCO_3$. The organic phase was dried over anhyd Na_2SO_4, then concentrated to dryness. Purification by silica column chromatography (2:1 petroleum ether–EtOAc) gave **5** (490 mg, 95%) as a syrupy anomeric mixture, and α-anomer was isolated in pure form and characterized: $[\alpha]_D$ +29° (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.02–7.22 (m, 60H, 12Bz-H), 6.28 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 6.21 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 6.19 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.91 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.86 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.75–5.74 (m, 1H, H-5), 5.72–5.66 (m, 3H, 2H-3, H-2), 5.54 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.53 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.27 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.12 (dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.06 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.04 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.01 (dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.90 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.83 (dd, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.75 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 4.72 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.62 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.59–4.51 (m, 5H), 4.49 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.40 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.33–4.25 (m, 2H), 4.24–4.06 (m, 7H), 3.94–3.73 (m, 6H), 3.64–3.63 (m, 1H), 2.30, 2.21, 2.20, 2.11, 1.95, 1.94, 1.92, 1.83 (s, 24H, 8CH_3CO); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.6, 170.52, 169.8, 169.6, 169.3, 169.2, 169.2, 168.7 (8C, 8COCH$_3$), 166.1, 166.1, 165.9, 165.8, 165.5, 165.5, 165.4, 165.4, 165.2, 165.2, 165.2, 165.0 (12C, 12COPh), 101.2, 100.9, 100.6 (3β-C-1 of Glc$p$, $J_{C\text{–}H}$ 162.0–163.2 Hz), 98.3 (α-C-1 of Man$p$, $J_{C\text{–}H}$ 172.0 Hz), 96.8 (α-C-1 of Glc$f$, $J_{C\text{–}H}$ 172.1 Hz), 94.6 (α-C-1 of Glc$p$, $J_{C\text{–}H}$ 172.3 Hz), 83.6, 78.5, 76.2, 73.0, 73.0, 73.0, 72.3, 72.3, 72.1, 72.1, 72.1, 72.0, 72.0, 72.0, 71.9, 71.8, 70.8, 70.5, 70.5, 70.5, 70.2, 70.1, 70.0, 70.0, 69.8, 69.8, 69.6, 69.6, 69.2, 69.2, 69.0, 68.2, 68.2, 68.0, 67.8, 66.9, 65.6, 65.6, 62.5, 63.1, 61.6 (C-2–6). Anal. Calcd for $C_{136}H_{126}O_{51}$: C, 63.40; H, 4.90. Found: C, 63.69; H, 4.81.

3.5. α-D-Mannopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-α-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranose (6)

Compound **5** (450 mg, 0.175 mmol) was dissolved in a satd solution of NH_3 in MeOH (20 mL). After a week at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **6** (151.1 mg, 87%) as a foamy solid: $[\alpha]_D^{25}$ +17° (c 1.0, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ 5.25 (d, 1H, J 3.6 Hz, α-H-1 of Glc$p$), 5.19 (s, 1H, α-H-1 of Man$p$), 4.65 (d, 1H, J 8.0 Hz, β-H-1 of Glc$p$), 4.63 (d, 1H, J 8.0 Hz, β-H-1 of Glc$p$), 4.43 (d, 1H, J 8.0 Hz, β-H-1 of Glc$p$), 4.42 (d, 1H, J 8.0 Hz, β-H-1 of Glc$p$), 4.12–3.99 (m, 6H), 3.87–3.78 (m, 10H), 3.68–3.57 (m, 12H), 3.44–3.25 (m, 8H); ^{13}C NMR (D_2O, 100 MHz): δ 102.8, 102.8, 102.8, 101.1 (4β-C-1 of Glc$p$, $J_{C\text{–}H}$ 162.4–163.8 Hz), 101.1 (α-C-1 of Man$p$, $J_{C\text{–}H}$ 172.4 Hz), 99.4 (α-C-1 of Glc$p$, $J_{C\text{–}H}$ 173.6 Hz), 79.6, 75.9, 75.9, 75.9, 75.6, 75.6, 73.1, 73.1, 73.1, 72.9, 72.9, 72.2, 70.8, 70.3, 70.3, 70.3, 69.8, 69.6, 69.6, 69.6, 68.8, 68.7, 68.2, 66.7, 61.7, 61.0, 60.8, 60.8, 60.6 (C-2–6). Anal. Calcd for $C_{36}H_{62}O_{31}$: C, 43.64; H, 6.26. Found: C, 43.86; H, 6.17.

3.6. 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-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (8)

Compound **7** (900 mg, 0.576 mmol) and **2** (648 mg, 0.576 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (20 mL). TMSOTf (5 μL, 0.044 mmol) 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 with 1:2 petroleum ether–EtOAc as the eluent gave two products as a 3:2 mixture. Product **8** (700 mg, 48%) with larger R_f was obtained as a syrup: $[\alpha]_D^{25}$ +28° (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.16–7.25 (m, 60H, 12Bz-H), 6.16 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 6.07 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.95 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.84 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.74–5.69 (m, 3H, H-3, 2H-2), 5.65 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.55 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.50–5.49 (m, 1H, H-5), 5.38 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.26 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.19 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.09 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.08–5.06 (m, 1H), 5.03 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 4.93 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-3), 4.90 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.79 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.73–4.63 (m, 2H), 4.61 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.58 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.49 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.48–4.42 (m, 5H), 4.38 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.26–4.20 (m, 4H), 4.14–4.08 (m, 3H), 3.99–3.93 (m, 2H), 3.89 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6}$ 12.4 Hz, H-6), 3.76–3.72 (m, 1H), 3.51–3.49 (m, 1H), 2.25, 2.19, 2.14, 2.12, 2.00, 1.79 (s, 18H, 6CH_3CO), 1.26, 1.11 (s, 6H, 2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 171.0, 170.7, 170.2, 169.8, 169.4, 168.4 (6C, 6COCH$_3$), 166.2, 166.2, 166.1, 166.1, 165.9, 165.8, 165.7, 165.7, 165.4, 165.4, 165.3, 165.1 (12C, 12COPh), 105.2 (α-C-1 of Glc$f$, $J_{C\text{–}H}$ 183.4 Hz), 101.1, 98.5 (2β-C-1 of Glc$p$, $J_{C\text{–}H}$ 161.2–163.6 Hz), 98.2, 97.5 (2α-C-1 of Man$p$, $J_{C\text{–}H}$ 172.2–173.6 Hz), 94.1 (α-C-1 of Glc$p$, $J_{C\text{–}H}$ 172.6 Hz), 82.2, 78.9, 76.0, 74.3, 73.1, 73.1, 72.4, 72.4, 72.0, 72.0, 72.0, 71.9, 71.9, 71.4, 70.9, 70.5, 70.5, 70.3, 70.3, 70.3, 70.3, 69.9, 69.9, 69.9, 69.3, 69.2, 68.2, 67.9, 67.0, 66.8, 66.4, 63.2, 62.9, 62.4, 61.9 (C-2–6), 26.8, 25.8. Anal. Calcd for $C_{135}H_{126}O_{49}$: C, 64.03; H, 5.01. Found: C, 64.33; H, 4.89.

3.7. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-5-*O*-acetyl-D-glucofuranose (9)

A solution of **8** (600 mg, 0.237 mmol) in 90% CF_3CO_2H (5 mL) was stirred at rt until TLC (1:1 petroleum ether–EtOAc) indicted that the reaction was complete. The mixture was then concentrated to dryness. Purification by silica column chromatography (2:1 petroleum ether–EtOAc) gave **9** (550 mg, 93%) as a syrupy anomeric mixture, and the α-anomer was isolated in pure form and characterized: $[\alpha]_D^{25}$ +35° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.13–7.26 (m, 60H, 12Bz-*H*), 6.27 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 6.19 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.94 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.89 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.73–5.64 (m, 3H, H-3, 2H-2), 5.55 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.54 (dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.50–5.49 (m, 1H, H-5), 5.27 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.20 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.11 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.04 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.03–4.90 (m, 4H), 4.87 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.82 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.76–4.65 (m, 3H), 4.60 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.53–4.42 (m, 5H), 4.28–4.07 (m, 7H), 3.95–3.77 (m, 5H), 3.66–3.63 (m, 2H), 3.51–3.49 (m, 1H), 2.28, 2.19, 2.16, 2.14, 1.93, 1.86 (s, 18H, 6CH_3CO). Anal. Calcd for $C_{132}H_{122}O_{49}$: C, 63.61; H, 4.90. Found: C, 63.96; H, 4.83.

3.8. 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-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-1,2,5-tri-*O*-acetyl-D-glucofuranose (10)

Compound **9** (500 mg, 0.201 mmol) was dissolved in pyridine (10 mL), and then Ac_2O (5 mL) was added. After stirring the mixture at rt for 12 h, TLC (2:1 petroleum ether–EtOAc) indicted that the reaction was complete. The reaction mixture was extracted with CH_2Cl_2 (50 mL), washed with dil HCl and satd aq $NaHCO_3$. The organic phase was dried over anhyd Na_2SO_4, then concentrated to dryness. Purification by silica column chromatography (2:1 petroleum ether–EtOAc) gave **10** (480 mg, 93%) as a syrupy anomeric mixture, and the α-anomer was isolated in pure form and characterized: $[\alpha]_D^{25}$ +42° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.16–7.25 (m, 60H, 12Bz-*H*), 6.21 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 6.19 (dd, 1H, $J_{3,4} = J_{4,5} =$ 9.7 Hz, H-4), 6.05 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.93 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.84 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.74–5.71 (m, 3H, H-3, 2H-2), 5.66 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.52 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.50–5.49 (m, 1H, H-5), 5.25 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.19 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.16 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.13–5.11 (m, 1H), 5.06 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.01 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-3), 4.95 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.90 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.85–4.81 (m, 3H), 4.71 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.65 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.63 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.60 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.56 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.48–4.42 (m, 3H), 4.39 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.26–4.17 (m, 5H), 4.14–4.08 (m, 4H), 3.97–3.92 (m, 2H), 3.88–3.82 (m, 2H), 3.56–3.54 (m, 1H), 2.25, 2.21, 2.18, 2.17, 2.13, 1.99, 1.86, 1.84 (s, 24H, 8CH_3CO); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.9, 170.7, 170.4, 169.4, 169.3, 169.3, 169.3, 169.1 (8C, 8*C*OCH$_3$), 166.1, 166.1, 166.0, 166.0, 165.9, 165.8, 165.8, 165.5, 165.5, 165.3, 165.2, 165.0 (12C, 12*C*OPh), 101.3, 101.1 (2β-C-1 of Glc*p*, $J_{C\text{–}H}$ 161.5–162.9 Hz), 99.2, 98.5 (2α-C-1 of Man*p*, $J_{C\text{–}H}$ 172.0–172.7 Hz), 94.8 (α-C-1 of Glc*f*, $J_{C\text{–}H}$ 172.5 Hz), 94.2 (α-C-1 of Glc*p*, $J_{C\text{–}H}$ 172.6 Hz), 79.1, 76.9, 75.9, 75.8, 73.5, 73.1, 73.1, 73.0, 72.4, 72.3, 72.0, 71.9, 71.8, 71.7, 70.7, 70.6, 70.5, 70.4, 70.4, 70.4, 70.3, 69.7, 69.7, 69.6, 69.6, 69.6, 68.4, 69.4, 69.2, 68.6, 67.9, 66.4, 63.1, 63.0, 62.2, 61.6 (C-2–6). Anal. Calcd for $C_{136}H_{126}O_{51}$: C, 63.40; H, 4.90. Found: C, 63.75; H, 4.78.

3.9. α-D-Mannopyranosyl-(1 → 3)-[α-D-mannopyranosyl-(1 → 6)]-α-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranose (11)

Compound **10** (450 mg, 0.175 mmol) was dissolved in a satd solution of NH_3 in MeOH (20 mL). After a week at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **27** (150 mg, 87%) as a foamy solid: $[\alpha]_D^{25}$ +23° (*c* 1.0, D_2O); ^1H NMR (D_2O, 400 MHz): δ 5.28 (d, 1H, *J* 4.0 Hz, α-H-1 of Glc*p*), 5.15 (s, 1H, α-H-1 of Man*p*), 4.82 (s, 1H, α-H-1 of Man*p*), 4.58 (d, 1H, *J* 8.0 Hz, β-H-1 of Glc*p*), 4.43 (d, 1H, *J* 8.0 Hz, β-H-1 of Glc*p*), 4.42 (d, 1H, *J* 8.0 Hz, β-H-1 of Glc*p*), 4.14–3.98 (m, 8H), 3.86–3.59 (m, 2 2H), 3.43–3.18 (m, 6H); ^{13}C NMR (D_2O, 100 MHz): δ 102.8, 102.8, 101.2 (3β-C-1 of Glc*p*, $J_{C\text{–}H}$ 161.5–162.6 Hz), 101.2, 99.6 (2α-C-1 of Man*p*, $J_{C\text{–}H}$ 172.0–172.6 Hz), 99.3 (α-C-1 of Glc*p*, $J_{C\text{–}H}$ 172.5 Hz), 82.3, 80.1, 75.9, 75.9, 75.6, 75.6, 75.6, 75.6, 74.6, 73.2, 73.1, 73.1, 72.9, 72.9, 72.7, 72.7, 72.2, 71.0, 70.6, 70.4, 70.3, 70.1, 69.9, 69.8, 69.2, 69.2, 68.8, 67.9, 66.8, 66.8, 66.7, 66.7, 61.7, 61.0, 61.0, 60.7, 60.6 (C-2–6). Anal. Calcd for $C_{36}H_{62}O_{31}$: C, 43.64; H, 6.26. Found: C, 43.82; H, 6.15.

3.10. 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-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (12)

Another product **12** (450 mg, 30.9%) with smaller R_f was obtained as a syrup from the coupling of **2** with **7**: $[\alpha]_D^{25}$ +34° (*c* 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.16–7.26 (m, 60H, 12Bz-*H*), 6.28 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 6.20 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.94 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.86 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.76–5.75 (m, 1H, H-5), 5.69–5.64 (m, 3H, H-3, 2H-2), 5.56 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.54 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.38 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.24 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.10 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.08–5.01 (m, 5H), 4.90 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.83 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.73 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 4.68 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.62 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.55 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.44–4.39 (m, 7H), 4.28 (dd, 1H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.22–4.11 (m, 5H), 3.97–3.86 (m, 3H), 3.80–3.69 (m, 3H), 2.28, 2.22, 2.20, 2.10, 1.86, 1.77 (s, 18H, 6C*H*$_3$CO), 1.34, 1.29 (s, 6H, 2C*H*$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz): δ 170.6, 170.3, 169.9, 169.2, 169.2, 168.4 (6C, 6*C*OCH$_3$, 166.2, 166.2, 166.0, 166.0, 165.8, 165.8, 165.7, 165.4, 165.4, 165.3, 165.3, 165.1 (12C, 12*C*OPh), 105.2 (α-C-1 of Glc*f*, $J_{C\text{–}H}$ 182.5 Hz), 101.0, 101.0, 100.6 (3β-C-1 of Glc*p*, $J_{C\text{–}H}$ 161.4–163.2 Hz), 98.3, 96.8 (2α-C-1 of Man*p*, $J_{C\text{–}H}$ 172.0–173.2 Hz), 83.5, 82.4, 78.9, 78.1, 73.1, 73.1, 72.4, 72.4, 72.1, 72.1, 72.1, 71.4, 71.4, 71.4, 70.8, 70.5, 70.5, 70.2, 70.2, 70.1, 70.1, 69.8, 69.8, 69.7, 69.0, 69.0, 67.9, 67.9, 67.7, 67.5, 66.9, 65.8, 63.3, 63.1, 61.9, 61.7 (C-2–6), 27.0, 26.6. Anal. Calcd for C$_{135}$H$_{126}$O$_{49}$: C, 64.03; H, 5.01. Found: C, 64.28; H, 4.93.

3.11. 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 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-5-*O*-acetyl-D-glucofuranose (13)

A solution of **12** (400 mg, 0.158 mmol) in 90% CF$_3$CO$_2$H (5 mL) was stirred at rt until TLC (1:1 petroleum–EtOAc) indicted that the reaction was complete. The mixture was then concentrated to dryness. Purification by silica column chromatography (2:1 petroleum ether–EtOAc) gave **13** (360 mg, 92%) as a syrupy anomeric mixture, and the α-anomer was isolated in pure form and characterized: $[\alpha]_D^{25}$ +36° (*c* 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.16–7.26 (m, 60H, 12Bz-*H*), 6.28 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 6.19 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.96 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.88 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.76–5.75 (m, 1H, H-5), 5.73–5.64 (m, 3H, H-3, 2H-2), 5.57 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.53 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.26 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.09 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.06–4.92 (m, 5H), 4.89 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.82 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.78–4.63 (m, 5H), 4.61 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.55–4.42 (m, 6H), 4.32–4.10 (m, 3H), 3.95–3.77 (m, 5H), 3.69-3.62 (m, 3H), 2.28, 2.20, 2.12, 2.10, 1.88, 1.79 (s, 18H, 6C*H*$_3$CO). Anal. Calcd for C$_{132}$H$_{122}$O$_{49}$: C, 63.61; H, 4.90. Found: C, 63.90; H, 4.82.

3.12. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 6)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-1,2,5-tri-*O*-acetyl-D-glucofuranose (14)

Compound **13** (320 mg, 0.129 mmol) was dissolved in pyridine (10 mL), and then Ac$_2$O (5 mL) was added. After stirring the mixture at rt for 12 h, TLC (2:1 petroleum ether–EtOAc) indicted that the reaction was complete. The reaction mixture was extracted with CH$_2$Cl$_2$ (50 mL), washed with dil HCl and satd aq NaHCO$_3$. The organic phase was dried over anhyd Na$_2$SO$_4$, then concentrated to dryness. Purification by silica gel column chromatography (2:1 petroleum–EtOAc) gave **14** (300 mg, 90%) as a syrupy anomeric mixture, and the α-anomer was isolated in pure form and characterized: $[\alpha]_D^{25}$ +38° (*c* 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 400 MHz): δ 8.03–7.25 (m, 60H, 12Bz-*H*), 6.27 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 6.15 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.91 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.87 (dd, H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.79 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.74–5.71 (m, 2H, H-3, H-2), 5.59 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.55 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.48–5.47 (m, 1H, H-5), 5.28 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.22 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 5.15–5.12 (m, 1H), 5.01 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.00–4.87 (m, 4H), 4.86 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.69 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.68–4.65 (m, 4H), 4.57 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.52 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.50 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.48–4.39 (m, 5H), 4.24 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 4.18–4.15 (m, 2H), 4.09–3.83 (m, 5H), 3.56–3.52 (m, 1H), 3.43–3.40 (m, 1H), 2.13, 2.12, 2.06, 2.04, 2.03, 2.02, 1.91, 1.86 (s, 24H, 8C*H*$_3$CO); ^{13}C NMR (CDCl$_3$, 100 MHz): δ 170.8, 170.7, 170.0, 169.7, 169.4, 169.0, 168.9, 168.8 (8C, 8*C*OCH$_3$), 166.1, 166.1, 166.0, 166.0, 165.8, 165.6, 165.6, 165.6, 165.4, 165.3, 165.2, 165.0 (12C, 12*C*OPh), 101.2, 101.2, 98.9 (3β-C-1 of Glc*p*, $J_{C\text{–}H}$ 161.2–163.4 Hz), 98.4, 97.1 (2α-C-1 of Man*p*, $J_{C\text{–}H}$ 172.0–173.1 Hz), 94.5 (α-C-1 of Glc*f*, $J_{C\text{–}H}$ 172.4 Hz), 82.1, 76.91, 75.5, 74.3, 73.1, 73.1, 73.1, 72.3, 72.3, 72.0, 72.0, 71.8, 71.8, 71.0, 70.6, 70.6, 70.4, 70.4, 70.3, 70.1, 70.1, 69.7, 69.7, 69.6, 69.6, 69.6, 68.5, 69.5, 69.2, 69.0, 68.0, 67.0, 66.3, 63.1, 62.9, 62.2, 61.7 (C-2–6). Anal. Calcd for $C_{136}H_{126}O_{51}$: C, 63.40; H, 4.90. Found: C, 63.67; H, 4.78.

3.13. α-D-Mannopyranosyl-(1 → 3)-[α-D-mannopyranosyl-(1 → 6)]-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl-(1 → 3)-[β-D-glucopyranosyl-(1 → 6)]-β-D-glucopyranose (15)

Compound **14** (280 mg, 0.109 mmol) was dissolved in a satd solution of NH_3 in MeOH (15 mL). After a week at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **15** (90 mg, 83%) as a foamy solid: $[\alpha]_D^{25}$ +24° (*c* 1.0, D_2O); ^1H NMR (D_2O, 400 MHz): δ 5.15 (s, 1H, α-H-1 of Man*p*), 4.84 (s, 1H, α-H-1 of Man*p*), 4.62 (d, 1H, *J* 8.0 Hz, β-H-1 of Glc*p*), 4.58 (d, 1H, *J* 8.0 Hz, β-H-1 of Glc*p*), 4.43 (d, 1H, *J* 8.0 Hz, β-H-1 of Glc*p*), 4.42 (d, 1H, *J* 8.0 Hz, H-1), 4.10–3.96 (m, 10H), 3.86–3.60 (m, 20H), 3.45–3.22 (m, 6H); ^{13}C NMR (D_2O, 100 MHz): δ 103.0, 102.8, 101.0, 101.0 (4β-C-1 of Glc*p*, J_{C-H} 161.5–163.6 Hz), 99.8, 99.8 (2α-C-1 of Man*p*, J_{C-H} 172.0–173.4 Hz), 82.0, 82.0, 80.1, 75.9, 75.9, 75.6, 75.6, 75.6, 75.5, 75.5, 73.8, 73.2, 73.2, 72.7, 72.7, 72.1, 72.1, 71.1, 70.5, 70.5, 70.4, 70.3, 70.0, 69.8, 69.8, 69.6, 68.2, 66.8, 66.8, 66.7, 66.7, 65.6, 61.7, 60.9, 60.9, 60.7, 60.7 (C-2–6). Anal. Calcd for $C_{36}H_{62}O_{31}$: C, 43.64; H, 6.26. Found: C, 43.76; H, 6.18.

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