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

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Abstract

α-D-Man*p*-(1→3)-[α-D-Man*p*-(1→6)]-α-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-[α-D-Man*p*-(1→6)]-D-Glc*p* and α-D-Man*p*-(1→3)-[β-D-Glc*p*-(1→6)]-α-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)[-α-D-Man*p*-(1→6)]-D-Glc*p* were synthesized in a regio- and stereoselective way as the mannose-containing analogues of the immunomodulating 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*.

Keywords: Oligosaccharide; Mannose; Glucose

1. Introduction

Many biologically active polysaccharides from traditional herbal medicines that are derived from sources such as *Ganoderma lucidum*, *Schizophyllum commune* and *Lentinus edodes* have a β-(1→3)-linked glucosyl backbone with β-(1→6)-branched glucosyl side chains.[1] Of them, polysaccharides from *L. edodes*, called lentinan, have the strongest antitumor effect, particularly, for Sarcoma-180 (S_{180}) in mice and have been used as an antitumor agent as an immunostimulant in Japan and China for many years. Clinically, lentinan has proved effective with chemotherapeutic agents for patients with recurrent gastric and colorectal cancer. Recent studies revealed that α-(1→3)-linked glucans also exist in some medically important fungi such as *Cryphonectrini parasitica* and *G. lucidum*.[2] Some physicochemical and immunopharmacological investigations show that the antitumor activity of these glucans may be closely related to the triplet-helix structures of the β-(1→3)-linked backbone chains.[3] It was also reported that only higher molecular-weight fractions (MW > 16,000) obtained from partial hydrolysis of lentinan with formic acid showed antitumor activity.[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*, in combination with the chemotherapeutic agent cyclophosphamide (CPA), at a dose of 0.5–1 mg/kg substantially increased the inhibition of S_{180} for CPA, but decreased the toxicity caused by CPA. It was noted that this hexasaccharide was not fully β-linked like the repeating unit of letinan[6] but contained one α-likage between the two trisaccharide moieties. This inspired us to carry out more research regarding the study on structure–function relationships of oligosaccharides. We present herein the synthesis of mannose-containing analogues of the active glucose hexasaccharide.

2. Results and discussion

To investigate the immunomodulation mechanism induced by the active glucohexaose, β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→6)]-α-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-[-D-Glc*p*-(1→6)]-D-Glc*p*, a series of structurally different analogues was needed. We first tried to replace the nonreducing end β-D-Glc*p* of the (1→3)-linked backbone with an α-D-Man*p*, and also replace the two or one β-D-Glc*p* branches with α-D-Man*p*, respectively.

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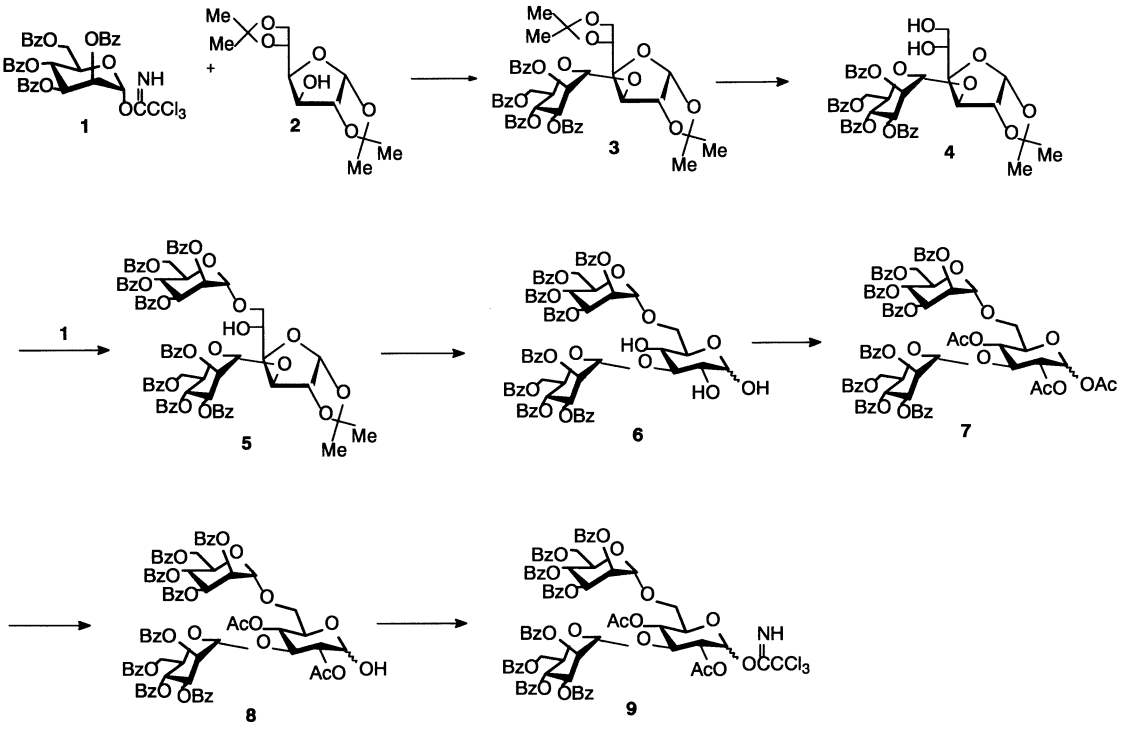


Scheme 1.

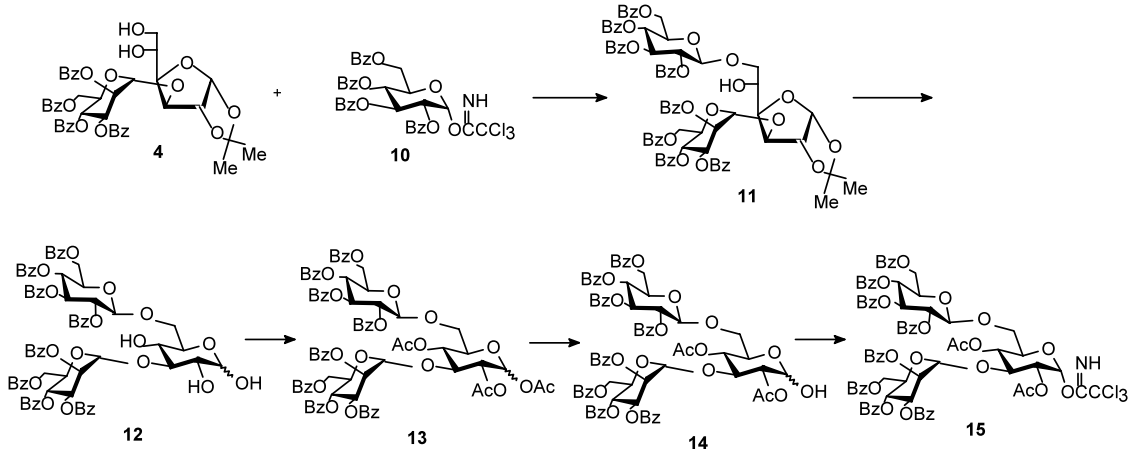
As shown in Scheme 1, a 3,6-mannose branched trisaccharide donor **9** was obtained in a facile way. Thus, reaction of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**2**) with 1,2,3,4-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**1**)[7] smoothly afforded α-(1→3)-linked disaccharide **3** (81.3%). Selective removal of the 5,6-*O*-isopropylidene group of **3** gave the disaccharide diol acceptor **4** in high yield (96.5%). Subsequent coupling of **4** with the donor **1** furnished the trisaccharide **5** (73.1%). Hydrolysis to remove the 1,2-*O*-isopropylidene group was accompanied by ring expansion to give the trisaccharide **6**, and subsequent acetylation yielded the trisaccharide **7** (88.1% for two steps). Selective 1-O-deacetylation of **7** (82.2%), followed by trichloroacetimidation[7] with trichloroacetonitrile in the presence of potassium carbonate, produced the trisaccharide donor **9**.

Scheme 2 outlines the synthesis of the trisaccharide donor **15** with the only mannose substitution at the nonreducing end of (1→3)-linked backbone. Thus, condensation of **4** with perbenzoylated glucopyranosyl trichloroacetimidate **10** gave the trisaccharide **11** in satisfactory yield (81.5%). Hydrolysis, acetylation (87.7% for two steps), selective 1-O-deacetylation, and trichloroacetimidation (94.7 for two steps) affords the another trisaccharide donor **15**.

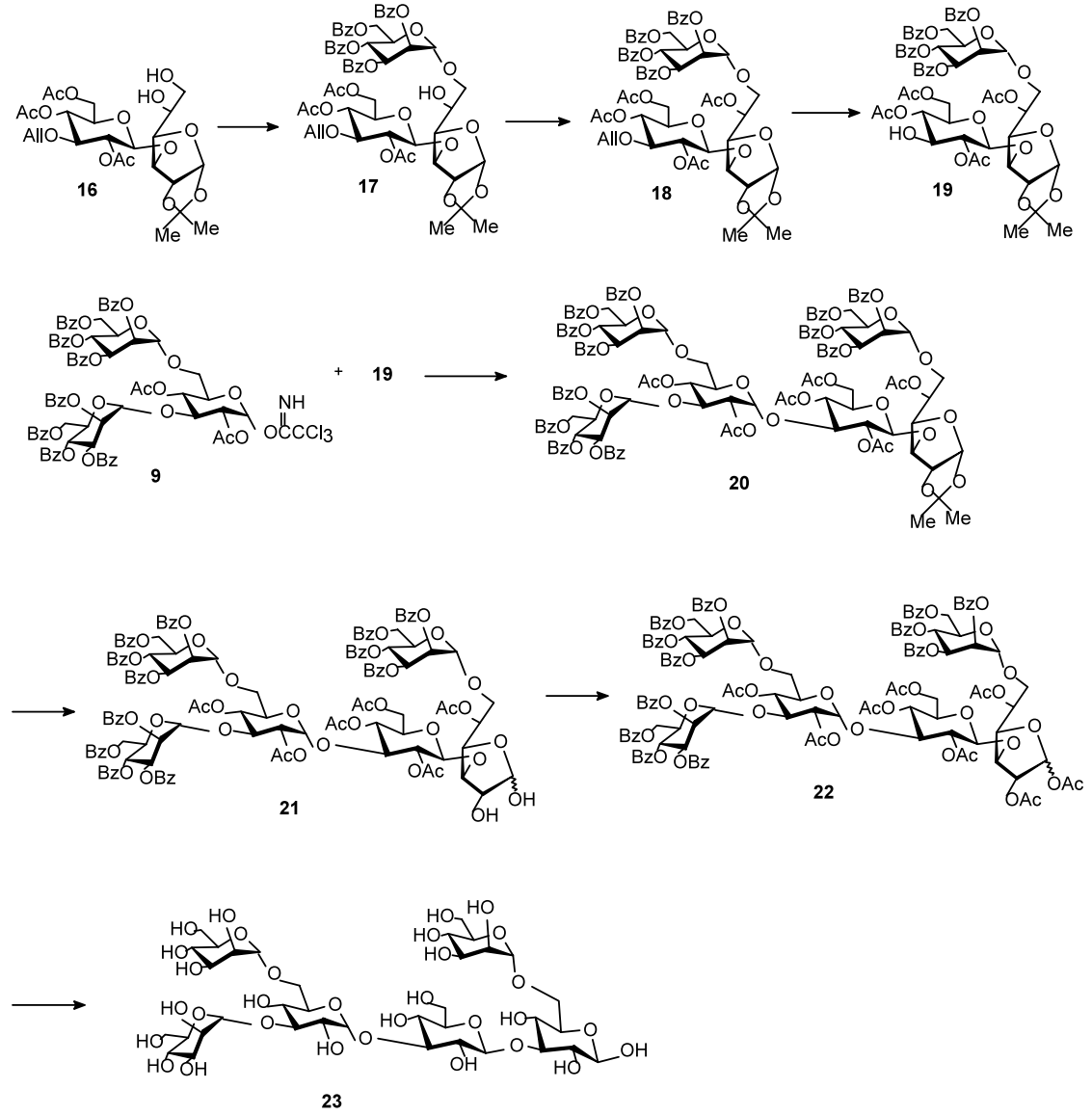
A co-used trisaccharide acceptor for both the donors **9** and **15** was synthesized as shown in Scheme 3. Thus, selective coupling of the disaccharide acceptor **16**[5] with the donor **1** furnished the trisaccharide **17** (63.0%). Acetylation (80.5%), followed by deallylation with $PdCl_2$, gave the trisaccharide acceptor **19** (77.8%). The acetylation of the 5-OH of glucofuranose was necessary, otherwise the subsequent deallylation gave a diol acceptor whose coupling with **9** did not show regioselectivity.

With the two donors **9** and **15**, and the acceptor **19** in hand, the hexasaccharides **23** and **27** were readily assembled (Schemes 3 and 4). Therefore, coupling of **19** with **9** and **15** afforded the hexasaccharides **20** (63.2%) and **24** (76.3%), respectively. It was noted that both of the couplings showed abnormal[8] stereoselectivity affording α-linked hexasaccharides as indicated from the C-1^{III} chemical shifts (94.0 ppm) and $J_{C1\text{-}H1}$ values (172 and 174 Hz, respectively), in spite of the presence of C-2 neighboring group participation of the donors. This was the same as we previously reported[5] for the coupling of a 3,6-β-linked glucose trisaccharide acceptor with a trisaccharide donor with a C-2 ester group, although the 3- or 3,6-glucosyl residues in the donor were replaced with mannosyl residues in the present case. Hydrolysis to remove the 1,2-*O*-isopropylidene group gave **21** and **25**, and subsequent acetylation, and deacylation smoothly furnished the target hexasaccharides **23** and **27**. Compounds **23** and **27** were identified by ^{1}H and ^{13}C NMR spectroscopy and mass spectrometry, showing all of the characteristic signals. Bioassay of the samples is in progress.

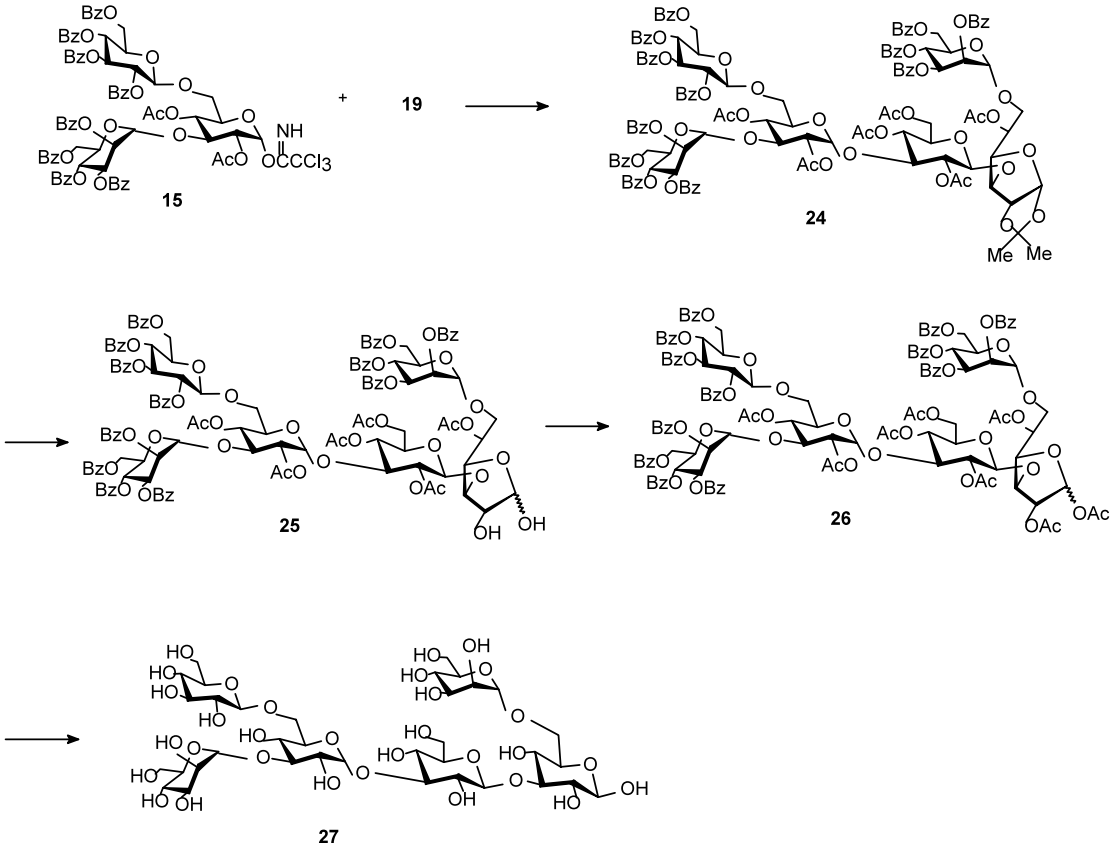
In summary, analogues of the active glucohexaose bearing two and three mannose units were synthesized



Scheme 2.



Scheme 3.



Scheme 4.

in an efficient way. Large-scale preparations should be possible with this method.

3. Experimental

3.1. General methods

Melting points (mp) 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, ^{13}C, and 2D NMR spectra were recorded with Varian XL-400 spectrometers, for solutions in $CDCl_3$ or in D_2O as indicated. Chemical shifts are expressed in ppm downfield from the Me_4Si absorption. Mass spectra were recorded with a VG PLATFORM mass spectrometer operating in the electrospray-ionization (ESI) mode. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) sulfuric acid in MeOH or by UV detection. Column chromatography was conducted by elution of a column (8×100, 16×240, 18×300, 35×400 mm) of silica gel (100–200 mesh) with petroleum ether–EtOAc (bp 60–90 °C) as the 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 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)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (3)

2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**1**) (499 mg, 0.674 mmol) and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**2**) (174 mg, 0.669 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (10.0 mL). TMSOTf (10.0 μL, 0.087 mmol) was added dropwise at −20 °C with N_2 protection. The reaction mixture was stirred for 3 h, during which time the temperature was gradually raised to ambient temperature. The mixture was then neutralized with Et_3N. Concentration of the reaction mixture, followed by purification on a silica gel column with 3:1 petroleum ether–EtOAc as the eluent gave the product **3** (561 mg, 81.3%) as a syrup: $[\alpha]_D$ +15° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.12–7.26 (m,

20 H, Bz–*H*), 6.06 (dd, 1 H, $J_{3',4'} = J_{4',5'} = 10.0$ Hz, H-4′), 6.02 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1), 5.88 (dd, $J_{2',3'}$ 3.3 Hz, $J_{3',4'}$ 10.0 Hz, H-3′), 5.76 (dd, 1 H, $J_{1',2'}$ 1.7 Hz, $J_{2',3'}$ 3.3 Hz, H-2′), 5.41 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1′), 4.73–4.69 (m, 2 H, H-6′, H-3), 4.56–4.51 (m, 2 H, H-6′, H-5), 4.44 (d, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 4.35–4.30 (m, 1 H, H-5), 4.23 (dd, 1 H, *J* 6.2, *J* 8.6 Hz), 4.11 (dd, 1 H, *J* 2.8, *J* 8.8 Hz), 4.01 (dd, 1 H, *J* 4.0, *J* 8.6 Hz, H-6), 1.50, 1.38, 1.31, 1.28 (s, 12 H, 4 C*H*$_3$). Anal. Calcd for $C_{46}H_{46}O_{15}$: C, 65.87; H, 5.49. Found: C, 65.91; H, 5.44.

3.3. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-1,2-*O*-isopropylidene-α-D-glucofuranose (4)

To a solution of 90% HOAc (20 mL) was added **3** (1.20 g, 1.43 mmol), and the mixture was stirred at 40 °C overnight, then concentrated to dryness. The residue was passed through a short silica column (1:1 petroleum ether–EtOAc) to give **4** (1.10 g, 93%) as crystals: mp 144–146 °C; $[\alpha]_D$ +17° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09–7.26 (m, 20 H, Bz–*H*), 6.06 (dd, 1 H, $J_{3',4'} = J_{4',5'} = 9.9$ Hz, H-4′), 6.02 (d, 1 H, $J_{1,2}$ 2.7 Hz, H-1), 5.86 (dd, $J_{2',3'}$ 3.0 Hz, $J_{3',4'}$ 9.9 Hz, H-3′), 5.76 (m, 1 H, H-2′), 5.41 (s, 1 H, H-1′), 4.72–4.67 (m, 2 H, H-6′, H-3), 4.55–4.49 (m, 3 H, H-6′, H-5′, H-2), 4.24 (m, 1 H, H-5), 4.15–4.11 (m, 1 H, H-6), 4.00–3.98 (m, 1 H, H-6), 3.80 (dd, 1 H, *J* 10.0, *J* 4.8 Hz), 1.49, 1.32 (s, 6 H, 2 C*H*$_3$). Anal. Calcd for $C_{43}H_{42}O_{15}$: C, 64.66; H, 5.30. Found: C, 64.92; H, 5.18.

3.4. 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*-isopropylidene-α-D-glucofuranose (5)

Compound **5** was prepared by coupling of **1** (800 mg, 1.08 mmol) with **4** (860 mg, 1.08 mmol) under the same conditions as described for the synthesis of **3** by coupling of **1** with **2**. Concentration of the reaction mixture, followed by purification on a silica gel column with 2:1 petroleum ether–EtOAc as the eluent, gave the product **5** (1.10 g, 73.1%) as a syrup: $[\alpha]_D$ −7° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.09–7.22 (m, 40 H, Bz–*H*), 6.17 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 6.10 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 6.04 (d, 1 H, *J* 3.7 Hz, H-1), 5.96–5.90 (m, 2 H, 2 H-3), 5.81 (dd, 1 H, *J* $_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.2 Hz, H-2), 5.74 (dd, 1 H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.2 Hz, H-2), 5.53 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.21 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.77–4.65 (m, 4 H), 4.58–4.51 (m, 4 H), 4.40 (dd, 1 H, $J_{5,6}$ 2.6 Hz, $J_{6,6}$ 9.6 Hz, H-6), 4.34–4.30 (m, 1 H, H-5), 4.17–4.11 (m, 1 H, H-6), 3.97 (dd, 1 H, $J_{5,6}$ 2.6 Hz, $J_{6,6}$ 10.1 Hz, H-6), 1.51, 1.25 (s, 6 H, 2 C*H*$_3$). Anal. Calcd for $C_{77}H_{68}O_{24}$: C, 67.15; H, 4.98. Found: C, 66.92; H, 5.13.

3.5. 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-D-glucopyranoside (7)

A solution of **5** (3.00 g, 2.15 mmol) in 90% CF_3COOH (20 mL) was stirred for 2 h at room temperature (rt), then concentrated to dryness. The residue was dissolved in Py (30 mL), and then Ac_2O (6 mL) was added. After stirring the mixture at rt for 12 h, TLC (2:1 petroleum ether–EtOAc) indicated 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 gel column chromatography (2:1 petroleum ether–EtOAc) gave **7** (2.80 g, 88.1% for two steps) as a syrupy anomeric mixture. The α-anomer was the major product isolated in pure form and characterized: $[\alpha]_D$ −1.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15–7.26 (m, 40 H, Bz–*H*), 6.39 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 6.30–6.25 (m, 2 H, 2 H-4), 6.15–6.05 (m, 2 H, 2 H-3), 5.94–5.86 (m, 2 H, 2 H-2), 5.32 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.27–5.15 (m, 1 H), 5.10 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.84–4.81 (m, 1 H), 4.53–4.42 (m, 2 H), 4.39–4.31 (m, 2 H), 4.16–4.11 (m, 1 H), 4.06–4.02 (m, 1 H), 3.97–3.88 (m, 3 H), 3.77–3.68 (m, 1 H), 2.34, 2.24, 2.15 (s, 9 H, 3 C*H*$_3$C=O). Anal. Calcd for $C_{80}H_{70}O_{27}$: C, 65.66; H, 4.79. Found: C, 66.92; H, 4.57.

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-D-glucopyranose (8)

Compound **7** (2.50 g, 1.69 mmol) was dissolved in THF (30 mL), and then benzyl amine (1 mL) was added. The mixture was stirred at rt until TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The 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 gel column chromatography (2:1 petroleum ether–EtOAc) gave **8** (2.00 g, 82.2%) as a syrupy anomeric mixture, of which the major α-anomer was characterized: $[\alpha]_D$ +12.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.14–7.26 (m, 40 H, Bz–*H*), 6.21 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 6.06 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 5.93 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.80 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.2 Hz, H-3), 5.73–5.71 (m, 1 H, H-2), 5.56 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.55 (m, 1 H, H-2), 5.34 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.16–5.11 (m, 2 H, H-1, H-4), 4.99 (dd, 1 H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 9.9 Hz, H-2), 4.91–4.80 (m, 2 H), 4.76–4.73 (m, 1 H), 4.64–4.60 (m, 1 H), 4.56–4.55 (m, 1 H), 4.52–4.34 (m, 3 H), 4.34–4.32 (m, 1 H), 4.20–4.09 (m, 1 H), 4.00–3.90 (m, 1 H), 3.76–3.70 (m, 1 H), 2.29, 2.20 (s, 6 H, 2 C*H*$_3$C=O); ^{13}C NMR: δ 169.64, 169.56 (2

CH_3C=O), 166.3, 165.9, 165.6, 165.5, 165.1, 165.0, 164.9, 164.7 (8 C, 8 *C*OPh), 98.7, 98.3, 97.4 (C-1^{I-III}), 89.8 (C-3), 72.7, 72.2, 70.7, 70.4, 70.2, 69.9, 69.7, 69.4, 69.2, 68.66, 67.9, 66.8, 62.5, 62.2 (C-2,-3,-4,-5,-6^{I-III}). Anal. Calcd for $C_{78}H_{68}O_{26}$: C, 65.45; H, 5.45. Found: C, 65.27; H, 5.64.

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 trichloroacetimidate (9)

Compound **8** (2.00 g, 1.39 mmol) was dissolved in CH_2Cl_2 (20 mL), then CCl_3CN (0.1 mL, 2.0 mmol) and K_2CO_3 (1.0 g, 7.0 mmol) was added. The reaction mixture was stirred for 10 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (3:1 petroleum ether–EtOAc) to give **9** (2.00 g, 92.1%) as a syrup: $[\alpha]_D$ +2.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.90 (s, 1 H, C=N*H*), 8.33–7.29 (m, 40 H, Bz–*H*), 6.56 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1), 6.35 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 6.09 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 5.88 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.2 Hz, H-3), 5.78 (dd, 1 H, $J_{2,3}$ 3.1 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.68 (dd, 1 H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.3 Hz, H-2), 5.60 (dd, 1 H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.1 Hz, H-2), 5.32 (d, 1 H, *J* 1.6 Hz, H-1), 5.24–5.20 (m, 2 H), 5.10 (d, 1 H, *J* 1.6 Hz, H-1), 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, 2.16 (s, 6 H, 2 C*H*$_3$C=O). Anal. Calcd for $C_{80}H_{68}Cl_3NO_{26}$: C, 61.37; H, 4.38. Found: C, 61.53; H, 4.51.

3.8. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-1,2-*O*-isopropylidene-α-D-glucofuranose (11)

2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate **10** (3.00 g, 4.05 mmol) and **4** (3.23 g, 4.05 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (50 mL). TMSOTF (15 μL, 0.131 mmol) was added dropwise at −20 °C with N_2 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 2:1 petroleum ether–EtOAc as the eluent, gave the product **11** (4.60 g, 81.5%) as a syrup: $[\alpha]_D$ +24° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.98–7.25 (m, 40 H, Bz–*H*), 6.03 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.99 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.91 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.86 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.2 Hz, H-3), 5.73 (dd, 1 H, $J_{1,2}$ 1.2 Hz, $J_{2,3}$ 4.8 Hz, H-2), 5.67 (dd, 1 H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 5.57 (dd, 1 H, $J_{1,2}$ 8.9 Hz, $J_{2,3}$ 10.2 Hz, H-2), 5.45 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.00 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1), 4.76 (dd, 1 H, $J_{5,6}$ 2.6 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.70–4.67 (m, 2 H), 4.55–4.46 (m, 3 H), 4.40 (dd, 1 H, $J_{5,6}$ 5.0 Hz, $J_{6,6}$ 12.4 Hz, H-6), 4.27–4.25 (m, 1 H, H-5), 4.18–4.16 (m, 3 H), 3.99–3.95 (m, 1 H), 1.43, 1.26 (s, 6 H, 2 C*H*$_3$C=O). Anal. Calcd for $C_{77}H_{68}O_{24}$: C, 67.15; H, 4.98. Found: C, 67.39; H, 5.23.

3.9. 2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl-(1 → 3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1 → 6)]-1,2,4-tri-*O*-acetyl-D-glucopyranose (13)

A solution of **11** (3.00 g, 2.15 mmol) in 90% CF_3COOH (20 mL) was stirred for 2 h at rt, then concentrated to dryness. The residue was dissolved in Py (30 mL), and then Ac_2O (6 mL) was added. After stirring the mixture at rt for 12 h, TLC (2:1 petroleum ether–EtOAc) indicated 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 gel column chromatography (2:1 petroleum ether–EtOAc) gave **13** (2.70 g, 84.7% for two steps) as a syrupy anomeric mixture, of which the major α-anomer was characterized: $[\alpha]_D$ +35° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.99–7.27 (m, 40 H, Bz–*H*), 6.35 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 6.25 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 5.76 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 10.3 Hz, H-3), 5.70–5.53 (m, 3 H), 5.38 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz), 5.32–5.18 (m, 3 H), 4.83–4.77 (m, 1 H), 4.43–4.25 (m, 5 H), 4.16–4.01 (m, 4 H), 2.29, 2.15, 2.14 (s, 9 H, 3 C*H*$_3$C=O). Anal. Calcd for $C_{80}H_{70}O_{27}$: C, 65.66; H, 4.79. Found: C, 66.85; H, 4.52.

3.10. 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-D-glucopyranose (14)

Compound **13** (2.5 g, 1.69 mmol) was dissolved in THF (30 mL), and then benzyl amine (1 mL) was added. The mixture was stirred at rt until TLC (2:1 petroleum ether ether–EtOAc) indicated that the reaction was complete. The 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 gel column chromatography (2:1 petroleum ether–EtOAc) gave **14** (2.00 g, 82.2%) as a syrupy anomeric mixture, of which the major α-anomer was characterized: $[\alpha]_D$ +30.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.25–7.26 (m, 40 H, Bz–*H*), 6.22 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 6.12 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.95 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.0 Hz, H-3), 5.82 (m, 1 H, H-3), 5.73 (m, 1 H, H-2), 5.57 (m, 2 H, H-1, H-2), 5.41 (d, 1 H, $J_{1,2} = 1.6$ Hz, H-1), 5.16 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.91

–4.28 (m, 7 H), 4.03–3.93 (m, 1 H), 3.81–3.70 (m, 1 H), 2.32, 2.25 (s, 6 H, 2 CH_3C=O); ^{13}C NMR: δ 170.2, 170.0 (2 CH_3C=O), 166.2, 166.1, 166.1, 165.7, 165.6, 165.5, 165.4, 165.3 (8 C, 8 *C*OPh), 100.1, 99.4, 97.6 (C-1^{I-III}), 89.3 (C-3), 73.7, 71.4, 70.9, 70.7, 70.3, 69.9, 69.8, 69.5, 69.1, 66.9, 66.7, 66.2, 62.9, 62.1 (C-2,-3,-4,-5,-6^{I-III}). Anal. Calcd for $C_{80}H_{68}Cl_3NO_{26}$: C, 61.37; H, 4.38. Found: C, 61.27; H, 4.54.

3.11. 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 (15)

Compound **14** (3.00 g, 2.09 mmol) was dissolved in CH_2Cl_2 (30 mL), then CCl_3CN (0.5 mL, 5 mmol) and K_2CO_3 (1.00 g, 7.00 mmol) was added. The reaction mixture was stirred for 10 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (3:1 petroleum ether ether–EtOAc) to give **15** (3.10 g, 94.7%) as a syrup: $[\alpha]_D$ + 40° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.39 (s, 1 H, C=N*H*), 8.11–7.27 (m, 40 H, Bz–*H*), 6.40 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 6.23 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.88 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 5.72–5.63 (m, 2 H, 2 H-3), 5.53–5.50 (m, 2 H, 2 H-2), 5.17 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.10 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 5.03 (dd, 1 H, *J* 3.5, *J* 9.9 Hz), 4.97 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 5.05–4.95 (m, 2 H), 4.73–4.65 (m, 2 H), 4.52–4.42 (m, 2 H), 4.52–4.26 (m, 2 H), 4.00 (d, 1 H, $J_{5,6}$ 7.9 Hz, H-6), 3.74 (dd, 1 H, $J_{5,6}$ 5.4 Hz, H-6), 2.25, 2.07 (s, 6 H, 2 CH_3C=O). Anal. Calcd for $C_{80}H_{68}Cl_3NO_{26}$: C, 61.37; H, 4.38. Found: C, 61.55; H, 4.22.

3.12. 3-*O*-Allyl-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl-(1→6)]-1,2-*O*-isopropylidene-α-D-glucofuranose (17)

Compound **17** was prepared by coupling of **1** (4.70 g, 6.35 mmol) with **16** (3.48 g, 6.35 mmol) under the same conditions as described for the synthesis of **8** by coupling of **7** with **3**. Concentration of the reaction mixture, followed by purification on a silica gel column with 2:1 petroleum ether–EtOAc as the eluent, gave the product **17** (4.50 g, 63.0%) as a syrup: $[\alpha]_D$ −17° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15–7.27 (m, 20 H, Bz–*H*), 6.17 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 5.91–5.83 (m, 1 H, CH_2=C*H*–CH_2O), 5.79 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 5.76 (d, 1 H, $J = 3.7$ Hz, H-1), 5.68 (dd, 1 H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.62–5.58 (m, 2 H, 2 H-3), 5.36–5.20 (m, 1 H, CH_2=CHCH_2O), 5.17 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 5.11–5.00 (m, 1 H, CH_2=CHCH_2O), 4.73–4.59 (m, 4 H), 4.58 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.54–4.42 (m, 3 H), 4.26–4.23 (m, 1 H, CH_2=CHCH_2O), 4.21–4.08 (m, 5 H), 3.92–3.82 (m, 1 H, CH_2=CHCH_2O), 3.72–3.59 (m, 2 H), 2.17, 2.13, 2.11, 1.56, 1.27 (s, 15 H, 5 CH_3C=O). Anal. Calcd for $C_{58}H_{62}O_{23}$: C, 61.81; H, 5.54. Found: C, 61.51; H, 5.72.

3.13. 3-*O*-Allyl-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 (18)

Compound **17** (3.00 g, 2.66 mmol) was dissolved in Py (50 mL), and then Ac_2O (15 mL) was added. After stirring the mixture at 60–70 °C for 24 h, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was extracted with CH_2Cl_2 (50 mL) and then 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 gel column chromatography (2:1 petroleum ether–EtOAc) gave **18** (2.50 g, 80.5%) as a syrup: $[\alpha]_D$ −1.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.15–7.26 (m, 20 H, Bz–*H*), 6.16 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 5.93 (d, 1 H, *J* 3.2 Hz, H-1), 5.85 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.4 Hz, H-3), 5.77–5.75 (m, 1 H, CH_2=C*H*–CH_2O), 5.51 (dd, 1 H, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.3 Hz, H-2), 5.32 (dd, 1 H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.10 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.97–4.93 (dd, 1 H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 4.87–4.82 (dd, 1 H, $J_{4,5} = J_{5,6} = 9.5$ Hz, H-5), 4.80 (m, 1 H, CH_2=CHCH_2O), 4.74–4.68 (m, 2 H), 4.66–4.62 (m, 1 H, CH_2=CHCH_2O), 4.58 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.57–4.48 (m, 2 H), 4.39–4.30 (m, 1 H), 4.28–4.19 (m, 1 H, CH_2=CHCH_2O), 4.18–4.06 (m, 2 H), 3.93–3.85 (m, 1 H), 3.71–3.68 (m, 1 H), 3.68–3.56 (m, 1 H, CH_2=CHCH_2O), 2.15, 2.12, 2.10, 2.03 (s, 12 H, 4 CH_3C=O), 1.62, 1.43 (s, 6 H, 2 CH_3). Anal. Calcd for $C_{60}H_{64}O_{24}$: C, 61.64; H, 5.48. Found: C, 61.53; H, 5.71.

3.14. 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 (19)

To a solution of **18** (2.00 g, 1.71 mmol) in MeOH (20 mL) was added $PdCl_2$ (150 mg, 0.847 mmol). After stirring the mixture for 3 h at rt, 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 (1:1 petroleum ether–EtOAc) to give **19** (1.50 g, 77.8%) as a syrup: $[\alpha]_D$ −15° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.16–7.25 (m, 20 H, Bz–*H*), 6.14 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 5.89 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.6 Hz, H-3), 5.85 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1), 5.76 (dd, 1 H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.1 Hz, H-2), 5.29 (dd, 1 H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 10.2 Hz, H-2), 5.26–5.23 (m, 1 H, H-5), 5.18 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.95

(dd, 1 H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 4.85 (dd, 1 H, $J_{4,5} = J_{5,6} = 9.5$ Hz, H-5), 4.80–4.71 (m, 2 H), 4.66–4.62 (m, 1 H), 4.63 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.57–4.48 (m, 2 H), 4.39 (d, 1 H, $J_{5,6}$ 5.2 Hz, H-6), 4.28–4.19 (m, 1 H), 4.18–4.06 (m, 2 H), 3.93–3.85 (m, 1 H, H-6), 3.73–3.70 (m, 1 H), 3.68–3.56 (m, 1 H), 2.12, 2.10, 2.10, 2.09 (s, 12 H, 4 CH_3C=O), 1.55, 1.33 (s, 6 H, 2 CH_3); ^{13}C NMR: δ 170.6, 170.3, 169.9, 169.7 (4 CH_3C=O), 166.2, 165.4, 165.4, 165.3 (4 C, 4 *C*OPh), 105.1, 98.3, 97.0 (3 C,C-1^{I-III}), 82.5, 79.2, 73.8, 73.6, 72.2, 70.7, 70.3, 68.8, 68.4, 66.6, 65.9, 62.7, 62.2, 60.3 (C-2,-3,-4,-5,-6^{I-III}). Anal. Calcd for $C_{57}H_{60}O_{24}$: C, 60.64; H, 5.36. Found: C, 60.38; H, 5.57.

3.15. 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-α-D-mannopyranosyl-(1→6)]-5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (20)

Compound **9** (550 mg, 0.351 mmol) and **19** (400 mg, 0.355 mmol) were dried together under high vacuum for 2 h and then dissolved in anhyd CH_2Cl_2 (10 mL). TMSOTF (5 μL, 0.044 mmol) was added dropwise at −20 °C with N_2 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 1:1 petroleum ether–EtOAc as the eluent gave the product **20** (561 mg, 63.2%) as a syrup: $[\alpha]_D$ +47° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.07–7.26 (m, 60 H, Bz–*H*), 6.21 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.2$ Hz, H-4), 6.14 (dd, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.1 Hz, H-3), 6.11 (dd, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.2 Hz, H-3), 6.00 (dd, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.89–5.83 (m, 2 H, 2 H-4), 5.79 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 5.78–5.68 (m, 2 H, 2 H-2), 5.52 (d, 1 H, $J_{1,2}$ 2.2 Hz, H-1), 5.44 (dd, 1 H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.5 Hz, H-2), 5.30–5.23 (m, 3 H), 5.13–5.08 (m, 4 H), 4.93 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 4.85–4.61 (m, 8 H), 4.53–4.41 (m, 7 H), 4.32–4.24 (m, 2 H), 4.20–3.95 (m, 7 H), 3.85–3.53 (m, 2 H, H-6), 2.26, 2.23, 2.15, 2.12, 2.11, 1.95 (s, 18 H, 6 CH_3C=O), 1.30, 1.26 (s, 6 H, 2 CH_3); ^{13}C NMR: δ 170.8, 170.6, 170.1, 169.7, 169.5, 168.3 (6 CH_3C=O), 166.2, 166.1, 166.0, 165.8, 165.6, 165.6, 165.4, 165.3, 165.3, 165.3, 165.2, 165.2 (12 *C*OPh), 105.2 (C-1 for glucofuranose α bond, J_{C-H} = 182.5 Hz), 101.2 (C-1 for β bonds, $J_{C-H} = 163.0$ Hz), 98.0, 97.37, 97.2 (3 C-1 for mannose α bond, J_{C-H} = 172.9–174.5 Hz), 94.0 (C-1 for glucose α bond, J_{C-H} = 172 Hz), 82.3, 78.8, 77.2, 76.2, 75.8, 74.1, 74.0, 74.0, 72.0, 71.8, 71.2, 70.9, 70.4, 70.4, 70.3, 70.2, 69.7, 69.49, 69.2, 68.6, 68.5, 68.1, 66.8, 66.7, 66.3, 63.0, 62.8, 62.8, 62.2, 61.9 (C-2,-3,-4,-5,-6^{I-VI}). Anal. Calcd for $C_{135}H_{126}O_{49}$: C, 64.03; H, 5.01. Found: C, 64.31; H, 4.84.

3.16. α-D-Mannopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-β-D-glucopyranose (23)

A solution of **20** (500 mg, 0.198 mmol) in 90% CF_3COOH (5 mL) was stirred for 2 h at rt until TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was then concentrated to dryness. The residue was dissolved in Py (10 mL) and Ac_2O (5 mL) was added. After stirring the mixture at rt for 12 h, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was extracted with CH_2Cl_2 (50 mL) and then 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 gel column chromatography (2:1 petroleum–EtOAc) gave **22** (420 mg, 77.9% for two steps), which was dissolved in a satd soln 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 **23** (140 mg, 86.5%) as foamy solid: $[\alpha]_D$ +15° (*c* 1.0, H_2O); ^{1}H NMR (D_2O, 400 MHz): δ 5.31 (d, 1 H, *J* 3.3 Hz, H-1), 5.28 (s, 1 H, H-1), 5.16 (s, 1 H, H-1), 4.86 (s, 1 H, H-1), 4.56 (d, 1 H, *J* 7.6 Hz, H-1), 4.43 (d, 1 H, *J* 7.8 Hz, H-1), 4.10–3.45 (m, 36 H); ^{13}C NMR: δ 102.3, 100.7 (2 C-1 for β bonds, $J_{C-H} = 163.1–163.7$ Hz), 99.1, 98.9, 98.6 (3 C-1 for mannose α bond, $J_{C-H} = 173.0–174.5$ Hz), 94.0 (C-1 for glucose α bond, $J_{C-H} = 172$ Hz), 82.4, 79.6, 75.4, 74.9, 74.8, 72.7, 72.6, 72.5, 72.4, 72.2, 70.1, 70.0, 69.9, 69.8, 69.6, 69.4, 69.2, 66.3, 66.2, 60.5, 60.3, 60.1 (C-2,-3,-4,-5,-6^{I-VI}). Anal. Calcd for $C_{36}H_{62}O_{31}$: C, 43.64; H, 6.30. Found: C, 43.24; H, 6.58. ESIMS for $C_{36}H_{62}O_{31}$ (990.87): 989.75 $[M-1]^+$.

3.17. 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-α-D-mannopyranosyl-(1→6)]-5-*O*-acetyl-1,2-*O*-isopropylidene-α-D-glucofuranose (24)

Compound **15** (250 mg, 0.160 mmol) and **19** (180 mg, 0.160 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (10 mL). TMSOTF (5.0 μL, 0.044 mmol) was added dropwise at −20 °C with N_2 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 the

product **24** (350 mg, 76.3%) as a syrup: $[\alpha]_D$ +18° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.13–7.26 (m, 60 H, Bz–*H*), 6.27 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.1$ Hz, H-4), 6.11 (dd, 1 H, $J_{3,4} = J_{4,5} = 10.0$ Hz, H-4), 5.87 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.81 (d, 1 H, *J* 3.5 Hz, H-1), 5.76 (dd, 1 H, $J_{1,2}$ 1.2 Hz, $J_{2,3}$ 2.9 Hz, H-2), 5.66 (dd, 1 H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 10.1 Hz, H-3), 5.51 (dd, 1 H, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.3 Hz, H-2), 5.27–5.21 (m, 2 H, H-5, H-2), 5.09 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.07 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.97 (m, 1 H), 4.97–4.91 (m, 2 H, 2 H-2), 4.83 (dd, 1 H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10.7 Hz, H-2), 4.75 (dd, 1 H, *J* 3.1 Hz, *J* 9.4 Hz), 4.53–4.44 (m, 4 H), 4.42–4.35 (m, 3 H), 4.31–4.26 (m, 1 H), 4.25–4.13 (m, 4 H), 4.03–3.78 (m, 3 H), 3.76–3.69 (m, 1 H, H-5), 3.66–3.58 (m, 1 H, H-5), 2.26, 2.19, 2.14, 2.13, 2.10, 2.09 (s, 18 H, 6 CH_3C=O) 1.32, 1.26 (s, 6 H, 2 CH_3); ^{13}C NMR: δ 170.6, 170.6, 169.8, 169.7, 169.3, 169.1 (6 C, CH_3C=O, 6 *C*OPh), 166.2, 166.1, 166.0, 165.8, 165.6, 165.6, 165.4, 165.4, 165.3, 165.3, 165.3, 165.3 (12 *C*OPh), 105.2 (C-1 for glucofuranose α bond, $J_{C-H} = 182.4$ Hz), 101.2 (C-1 for β bonds, $J_{C-H} = 163.2$ Hz), 99.4 (C-1 for β bonds, $J_{C-H} = 163.0$ Hz), 98.3, 97.4, 94.0 (3 C-1 for α bond, $J_{C-H} = 173.0$–174.5 Hz), 83.2, 82.5, 79.1, 78.1, 76.3, 72.5, 72.3, 72.1, 71.9, 70.4, 70.1, 69.9, 68.9, 68.7, 68.4, 68.3, 66.7, 65.9, 65.6, 62.8, 62.2, 61.9, 61.6 (C-2,-3,-4,-5,-6^{I-VI}). Anal. Calcd for $C_{135}H_{126}O_{49}$: C, 64.03; H, 5.01. Found: C, 64.26; H, 5.35.

3.18. α-D-Mannopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[α-D-mannopyranosyl-(1→6)]-β-D-glucopyranose (27)

A solution of **24** (350 mg, 0.138 mmol) in 90% CF_3COOH (5 mL) was stirred for 2 h at rt until TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete, then concentrated to dryness. The residue was dissolved in Py (10 mL) and Ac_2O (5 mL) was added. After stirring the mixture at rt for 12 h, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was extracted with CH_2Cl_2 (50 mL) and then 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 gel column chromatography (2:1 petroleum ether–EtOAc) gave **26** (300 mg, 84.9% for two steps) as a syrup, which was dissolved in a satd soln 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 **27** (100 mg, 86.3%) as a foamy solid: $[\alpha]_D$ +30° (*c* 1.0, H_2O); ^1H NMR (D_2O, 400 MHz): δ 5.28 (d, 1 H, *J* 3.2 Hz, H-1), 5.22 (s, 1 H, H-1), 5.11 (s, 1 H, H-1), 4.86 (d, 1 H, *J* 7.8 Hz, H-1), 4.56 (d, 1 H, *J* 7.6 Hz, H-1), 4.50 (d, 1 H, *J* 7.8 Hz, H-1), 4.12–3.44 (m, 36 H); ^{13}C NMR: δ 103.1, 101.2, 100.6 (3 C-1 for β bonds, $J_{C-H} = 163.0$–164.7 Hz), 98.4, 97.2, 95.2 (3 C-1 for α bond, $J_{C-H} = 172.3$–174.6 Hz), 82.5, 79.1, 78.1, 76.4, 76.3, 75.9, 72.6, 72.3, 72.1, 72.0, 70.5, 70.2, 70.0, 69.9, 68.8, 68.4, 68.38, 66.8, 66.0, 65.7, 62.9 (C-2,-3,-4,-5,-6^{I-VI}). Anal. Calcd for $C_{36}H_{62}O_{31}$: C, 43.64; H, 6.30. Found: C, 43.36; H, 6.61. ESIMS for $C_{36}H_{62}O_{31}$ (990.87): 989.72 $[M-1]^+$.

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