Synthesis of β-(1→6)-Branched (1→3)-Glucononaoside with Alternate β- and α-Bonds in the Backbone

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Lauryl glycoside of 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)-] α-*D*-Glc*p*-(1→3)-β-*D*-Glc*p*-(1→3)-[β-*D*-Glc*p*-(1→6)-] β-*D*-Glc*p* was synthesized through 3+3+3 strategy. 3-*O*-Allyl-2,4,6-tri-*O*-benzoyl-β-*D*-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-*D*-glucopyranosyl-(1→6)-] 1,2-*O*-isopropylidene-α-*D*-glucofuranose was used as the key intermediate which was converted to the corresponding trisaccharide donor and acceptor readily.

Keywords oligosaccharide, synthesis, glucose

Introduction

As part of an ongoing project for studying and developing new immunoboosting reagents, the heptasaccharide[1] repeating unit of lentinan and its anologues[2] and the pentasaccharide fragments[3] of Epicoccum nigrum Ehrenb. ex Schlecht have been synthesized. It was interesting to find that not only the heptasaccharide shows strong antitumor activity as we expected, but also a 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)-[β-*D*-Glc*p*-(1→6)-] *D*-Glc*p*, has good immunoregulating activity.[2] Bioassay showed that in combination with the chemotherapeutic agent cyclophosphamide (CPA), the glucohexaose at a dose of 0.5 mg/kg to 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[4] but contained one α-likage between the two trisaccharide moieties. For a detailed study on the action mechanism of the glucose hexasaccharide, a variety of model compounds was needed. Herein the synthesis of a glucononaose containing three trisaccharide units was presented.

Results and discussion

As shown in Scheme 1, trisaccharide **6** obtained by condensation of 3-*O*-allyl-2,4,6-tri-*O*-benzoyl-α-*D*-glucopyranosyl trichloroacetimidate (**1**)[5] with diacetone glucofuranose followed by selective removal of 5,6-*O*-isopropylidene group and then coupling with perbenzoylated glucopyranosyl trichloroacetimidate,[6] was used as the key intermediate since it could transform to a trisaccharide donor **7** and acceptor **9** readily. Hydrolysis to remove 1,2-*O*-isopropylidene of **6** was accompanied by ring expansion, subsequent acetylation with acetic anhydride, selective 1-*O*-deacetylation in a solution of benzylamine in THF, and then trichloroacetimidation with trichloroacetonitrile in the presence of K_2CO_3 afforded the trisaccharide donor **7**. Reaction of **7** with lauryl alcohol promoted by catalytic TMSOTf gave trisaccharide **8** and deallylation with $PdCl_2$[7] furnished the trisaccharide acceptor **9**. Condensation of donor **7** with acceptor **9** yielded hexasaccharide **10** in satisfactory yield (73.2%) with an α-bond[2,5] between the two trisaccharide moieties as indicated from the ^{13}C NMR spectral data showing C-1 signals at δ 101.1, 101.0, 100.8, 100.4 and 100.2 for β bonds with $J_{C1,H1}$ 163.0—164.8 Hz, and δ 93.1 for α bond with $J_{C1,H1}$ 174.2 Hz, respectively. Deallylation of **10** gave the hexasaccharide acceptor **11**, and subsequent coupling with the trisaccharide donor **12**[8] readily afforded the nonasaccharide **13** (64.8%) that also showed an α-linkage between the trisaccharide and the hexasaccharide as indicated from the NMR data (2 α-C-1 at δ 93.7 and 93.3). Finally, deacylation of **13** in a saturated solution of ammonia in methanol gave the target glucononaoside **14** in high yield (89.6%). The ^1H NMR and ^{13}C NMR data of **14** showed all of the characteristic signals including 2α H-1 at δ 5.22 and 5.19, 7β C-1 at δ 102.9, 102.8, 102.7, 102.7, 102.6, 102.5 and 102.3 and 2α C-1 at δ 99.1 and 99.1, respectively. Bioactivity test of **14** is in progress and the results will be reported in due course.

In summary, a concise and efficient method for construction of β-(1→6)-branched (1→3)-linked glucononaose with alternate β- and α-linkages in the backbone was achieved using trisaccharide as the building block. This method will be suitable for synthesis of high oligosaccharides of similar structure.

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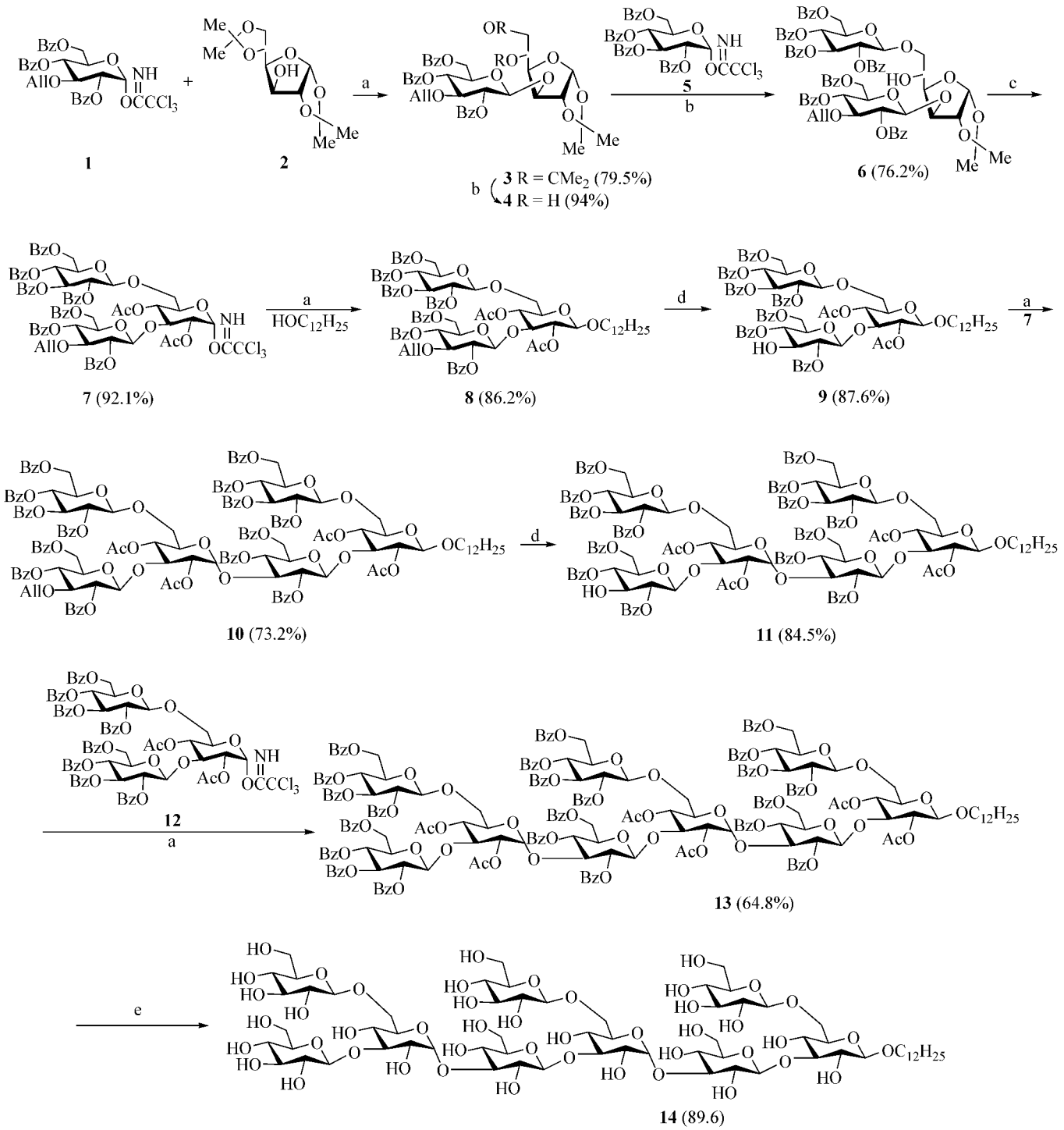
Scheme 1



Reagents and conditions: (a) TMSOTf, CH_2Cl_2, room temperature, 3 h; (b) 90% HOAc, 40 ℃, 24 h; (c) (i) 90% CF_3COOH, 2 h; (ii) Ac_2O-Pyridine, room temperature, 2 h; (iii) THF, benzyl amine, room temperature, 5 h; (iv) Cl_3CCN, K_2CO_3, CH_2Cl_2, 8 h; (d) $PdCl_2$ in CH_2Cl_2-CH_3OH, room temperature, 3 h; (e) saturated NH_3/MeOH, room temperature, 7 d.

Experimental

General methods

Melting points were determined with a "Mel-Temp" apparatus. Optical rotations were determined with a Perkin-Elmer model 241-MC automatic polarimeter for solutions in a 1-dm jacketed cell. ^1H NMR, ^{13}C NMR 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 downfield from the Me_4Si absorption. Mass spectra were recorded with a VG PLAT-

FORM mass spectrometer using the ESI mode. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with sulfuric acid (30%, *V/V*) in methanol or by UV detection. Column chromatography was conducted by elution of a column (8 mm × 100 mm, 16 mm × 240 mm, 18 mm × 300 mm, 35 mm × 400 mm) of silica gel (100—200 mesh) with EtOAc/petroleum ether (b.p. 60—90 ℃) 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 mm × 300 mm or 4.6 mm × 250 mm), differential refractometer (132-RI Detector), UV/vis detector (model 118). EtOAc-petroleum ether (b.p. 60—90 ℃) was used as the eluent at a flow rate of 1—4 mL/min. Solutions were concentrated at a temperature < 60 ℃ under diminished pressure.

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

3-*O*-Allyl-2,4,6-tri-*O*-benzoyl-*α*-*D*-glucopyranosyl trichloroacetimidate (**1**) (5.0 g, 7.40 mmol) and 1,2:5,6-di-*O*-isopropylidene-*α*-*D*-glucofuranose (**2**) (1.75 g, 6.73 mmol) were dried together under high vacuum for 2 h, then dissolved in anhydrous CH_2Cl_2 (120 mL). TMSOTf (50.0 μL, 0.435 mmol) was added dropwise at −20℃ with N_2 protection. The reaction mixture was stirred for 3 h, and 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 petroleum ether-EtOAc (3∶1, *V/V*) as the eluent gave the disaccharide (**3**) (4.14 g, 79.5%) as a syrup. To a solution of 90% HOAc (50 mL) was added **3** (4.00 g, 5.17 mmol), and the mixture was stirred at 40 ℃ overnight, then concentrated to dryness. The residue was passed through a short silica column [petroleum ether-EtOAc (1∶1, *V/V*)] to give **4** (3.56 g, 94%) as a foamy solid. Compound **6** was prepared by coupling of **5** (3.30 g, 4.34 mmol) with **4** (3.50 g, 4.77 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 petroleum ether-EtOAc 2∶1, (*V/V*) as the eluent gave the product **6** (4.34 g, 76.2%) as a syrup∶ $[\alpha]_D$ + 25 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*∶8.16—7.30 (m, 35H, 7Bz-**H**), 5.97 (dd, $J_{3,4} = J_{4,5}$ = 9.6 Hz, ^{1}H, H-4), 5.77 (dd, $J_{3,4} = J_{4,5}$ = 9.7 Hz, 1H, H-4), 5.66—5.63 (m, 1H, CH_2 = C**H**CH_2O), 5.62 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 5.54 (dd, $J_{2,3} = J_{3,4}$ = 9.7 Hz, 1H, H-3), 5.51 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.33 (dd, $J_{1,2} = J_{2,3}$ = 9.7 Hz, 1H, H-2), 5.16—5.12 (m, 1H, C**H**$_2$ = $CHCH_2O$), 5.06 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 5.05—5.03 (m, 1H), 4.87 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.79—4.73 (m, 2H), 4.55 (dd, $J_{5,6}$ = 4.9 Hz, $J_{6,6}$ = 12.2 Hz, 1H, H-6), 4.40—4.36 (m, 2H), 4.26 (d, *J* = 3.6 Hz, 1H), 4.21—4.04 (m, 8H), 3.90—3.87 (m, 1H), 1.33, 1.07 (s, 6H, $2CH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz)∶ *δ*∶166.1, 166.1, 165.7, 165.1, 165.1, 164.4 (7C, 7**C**OPh), 104.9, 101.4, 100.9 (C-$1^{I\text{-}III}$), 82.9, 82.4, 79.1, 78.9, 73.0, 73.0, 72.6, 72.0, 71.9, 71.3, 70.5, 69.6 (C-2, 3, 4, 5, $6^{I\text{-}III}$), 26.5, 25.8 (2C, 2 CO**C**H_3). Anal. calcd for $C_{73}H_{68}O_{23}$∶C 66.77, H 5.18; found C 67.02, H 5.28.

3-*O*-*Allyl*-2,4,6-*tri*-*O*-*benzoyl*-*β*-*D*-*glucopyranosyl*-(1→3)-[2,3,4,6-*tetra*-*O*-*benzoyl*-*β*-*D*-*glucopyranosyl*-(1→6)-]2,4-*di*-*O*-*acetyl*-*α*-*D*-*glucopyranosyl trichloroacetimidate* (**7**)

A solution of **6** (3 g, 2.15 mmol) in 90% CF_3COOH (20 mL) was stirred for 2 h at room temperature, then concentrated to dryness. The residue was dissolved in pyridine (30 mL), and then Ac_2O (6 mL) was added. After stirring the mixture at room temperature for 12 h, TLC [petroleum-EtOAc (2∶1, *V/V*)] indicted that the reaction was completed. The reaction mixture was extracted with CH_2Cl_2 (50 mL), washed with dilute HCl and saturated aqueous $NaHCO_3$. The organic phase was dried over anhydrous Na_2SO_4, then concentrated to dryness. Purification by silica column chromatography [petroleum ether-EtOAc (2∶1, *V/V*)] gave 3-*O*-allyl-2,4,6-tri-*O*-benzoyl-*β*-*D*-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-*β*-*D*-glucopyranosyl-(1→6)-]1,2,4-di-*O*-acetyl-*D*-glucopyranose (2.8 g, 88.1% for two steps) as a syrup, which was dissolved in THF (30 mL), and then benzyl amine (1 mL) was added. The mixture was stirred at room temperature until TLC [petroleum-EtOAc (2∶1, *V/V*)] indicted that the reaction was complete. The mixture was extracted with CH_2Cl_2 (50 mL), washed with dilute HCl and saturated aqueous $NaHCO_3$. The organic phase was dried over anhydrous Na_2SO_4, then concentrated to dryness. Purification by silica column chromatography [petroleum ether-EtOAc (2∶1, *V/V*)] gave 3-*O*-allyl-2,4,6-tri-*O*-benzoyl-*β*-*D*-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-*β*-*D*-glucopyranosyl-(1→6)-]2,4-di-*O*-acetyl-*D*-glucopyranose (2 g, 73.5%) as a syrup. The hemiacetal was dissolved in CH_2Cl_2 (20 mL), then CCl_3CN (0.1 mL, 2 mmol) and K_2CO_3 (1 g, 7 mmol) was added. The reaction mixture was stirred for 10 h, at the end of time TLC [petroleum ether-EtOAc (3∶1, *V/V*)] indicted that the reaction was complete. The mixture was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography [petroleum ether-EtOAc (3∶1, *V/V*)] to give **7** (2 g, 92.1%) as a syrup∶ $[\alpha]_D$ + 18 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*∶8.31 (s, 1H, C=NH), 8.06—7.24 (m, 35H, 7Bz-**H**), 6.19 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 5.86 (dd, $J_{3,4} = J_{4,5}$ = 9.7 Hz, 1H, H-4), 5.62 (dd, $J_{3,4} = J_{4,5}$ = 9.7 Hz, 1H, H-4), 5.57—5.49 (m, 1H, CH_2 = C**H**CH_2O), 5.46 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 5.42 (dd, $J_{2,3} = J_{3,4}$ = 9.7 Hz, 1H, H-3), 5.18 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 5.02—4.98

(m, 1H, CH_2 = C**H**CH_2O), 4.98 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.91—4.89 (m, 1H), 4.85 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.82 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.4 Hz, 1H, H-3), 4.65—4.57 (m, 2H), 4.46 (dd, $J_{5,6}$ = 6.4 Hz, $J_{6,6}$ = 12.1 Hz, 1H, H-6), 4.32 (dd, $J_{5,6}$ = 6.4 Hz, $J_{6,6}$ = 12.1 Hz, 1H, H-6), 4.16—4.07 (m, 2H), 4.05—3.99 (m, 5H), 3.94—3.89 (m, 1H), 3.68 (dd, $J_{5,6}$ = 6.4 Hz, $J_{6,6}$ = 12.1 Hz, 1H, H-6), 1.92, 1.82 (s, 6H, $2CH_3C$=O); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.3, 168.9 (2C, 2**C**OCH_3), 166.1, 166.0, 165.6, 165.1, 165.0, 164.9, 164.6 (7C, 7**C**OPh), 101.0, 100.5, 92.4 (C-1^{I-III}), 79.4, 75.7, 73.0, 72.9, 72.9, 72.7, 72.7, 72.1, 72.0, 71.9, 71.8, 71.7, 71.0, 69.7, 67.7, 67.5, 63.3, 62.9 (C-2, 3, 4, 5, 6). Anal. calcd for $C_{76}H_{68}Cl_3NO_{25}$: C 60.78, H 4.53; found C 61.02, H 4.61.

Lauryl 3-*O*-*allyl*-2,4,6-*tri*-*O*-*benzoyl*-β-D-*glucopyranosyl*-(1→3)-[2,3,4,6-*tetra*-*O*-*benzoyl*-β-D-*glucopyranosyl*-(1→6)-]2,4-*di*-*O*-*acetyl*-β-D-*glucopyranoside* (**8**)

Compound **7** (5 g, 3.33 mmol) and lauryl alcohol (0.74 g, 3.97 mmol) were dried together under high vacuum for 2 h, then dissolved in anhydrous CH_2Cl_2 (50 mL). TMSOTf (50 μL, 0.044 mmol) was added dropwise at −20 ℃ with N_2 protection. The reaction mixture was stirred for 3 h, and 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 petroleum ether-EtOAc (1∶1, *V*/*V*) as the eluent gave the product **8** (4.37 g, 86.2%) as a syrup: $[\alpha]_D$ + 10 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.09—7.22 (m, 35H, 7Bz-**H**), 5.96 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, 1H, H-4), 5.75 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 5.64—5.60 (m, 1H, CH_2 = C**H**CH_2O), 5.58 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 10.1 Hz, 1H, H-2), 5.53 (dd, $J_{2,3}$ = J 3,4 = 9.7 Hz, 1H, H-3), 5.21 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 5.11—5.06 (m, 1H, CH_2 = C**H**CH_2O), 5.01 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 5.00—4.97 (m, 1H), 4.91 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.85 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.4 Hz, 1H, H-2), 4.81 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, 1H, H-3), 4.76—4.64 (m, 2H), 4.56 (dd, $J_{5,6}$ = 6.4 Hz, $J_{6,6}$ = 12.1 Hz, 1H, H-6), 4.50 (dd, $J_{5,6}$ = 6.4 Hz, $J_{6,6}$ = 12.1 Hz, 1H, H-6), 4.26—4.20 (m, 1H, H-5), 4.14 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.12—4.02 (m, 4H), 3.97 (dd, $J_{5,6}$ = 2.6 Hz, $J_{6,6}$ = 12.9 Hz, 1H, H-6), 3.92 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 3.77—3.70 (m, 1H), 3.60—3.55 (m, 1H), 3.52—3.46 (m, 1H), 3.03—3.00 (m, 1H), 2.00, 1.98 (s, 6H, $2CH_3CO$), 1.40—1.14 (m, 20 H), 0.99 (dd, J = 6.8 Hz, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.5, 168.0 (2C, 2**C**OCH_3), 166.1, 166.0, 165.6, 165.1, 165.0, 164.9, 164.8 (7C, 7**C**OPh), 101.1, 100.6, 100.4 (C-1^{I-III}), 79.6, 76.8, 73.5, 72.9, 72.7, 72.6, 72.2, 71.9, 71.8, 69.4, 69.2, 68.7, 67.7, 67.5, 63.3, 62.9 (C-2, 3, 4, 5, 6^{II-III}), 29.6, 29.6, 29.5, 29.4, 29.2, 29.1, 25.7, 22.6, 20.7, 20.5 (lauryl). Anal. calcd for $C_{86}H_{92}O_{25}$: C 67.72, H 6.04; found C 67.99, H 6.12.

Lauryl 2,4,6-*tri*-*O*-*benzoyl*-β-D-*glucopyranosyl*-(1→3)-[2,3,4,6-*tetra*-*O*-*benzoyl*-β-D-*glucopyranosyl*-(1→6)-]-2,4-*di*-*O*-*acetyl*-β-D-*glucopyranoside* (**9**)

To a solution of **8** (2 g, 1.31 mmol) in MeOH (30 mL) was added $PdCl_2$ (150 mg). After stirring for 3 h at room temperature, TLC [petroleum ether-EtOAc (1.5∶1, *V*/*V*)] indicated that the reaction was complete. The mixture was filtered and the solution was concentrated to dryness, and the resultant residue was purified by flash chromatography [petroleum ether-EtOAc (1∶1, *V*/*V*)] to give **9** (1.7 g, 87.6%) as a syrup: $[\alpha]_D$ − 1.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.95—7.19 (m, 35H, 7Bz-**H**), 5.79 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 5.58 (dd, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, 1H, H-4), 5.42 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 5.29 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, 1H, H-3), 4.93 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 4.82 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.76 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.75—4.73 (m, 1H), 4.62 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 4.57—4.52 (m, 2H), 4.40—4.34 (m, 2H), 4.06—4.02 (m, 2H), 4.00 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 3.82—3.77 (m, 2H), 3.56—3.51 (m, 1H), 3.42—3.40 (m, 1H), 3.34—3.32 (m, 1H), 2.92—2.86 (m, 1H), 1.90, 1.81 (s, 6 H, $2CH_3CO$), 1.21—1.06 (m, 20 H), 0.81 (dd, J = 6.8 Hz, J = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.5, 168.3 (2C, 2**C**OCH_3), 166.6, 166.0, 166.0, 165.8, 165.6, 165.1, 165.0 (7C, 7**C**OPh), 101.1, 100.4, 100.2 (C-1^{I-III}), 77.8, 75.2, 74.1, 73.5, 72.8, 72.7, 72.2, 71.7, 71.7, 69.4, 69.3, 68.8, 63.1, 62.9 (C-2, 3, 4, 5, 6), 31.8, 29.6, 29.6, 29.3, 29.2, 29.1, 25.7, 22.6, 20.8, 20.5, 14.1 (lauryl). Anal. calcd for $C_{83}H_{88}O_{25}$: C, 67.12; H, 5.93; found C, 67.36; H, 5.85.

Lauryl 3-*O*-*allyl*-2,4,6-*tri*-*O*-*benzoyl*-β-D-*glucopyranosyl*-(1→3)-[2,3,4,6-*tetra*-*O*-*benzoyl*-α-D-*glucopyranosyl*-(1→3)-]2,4-*di*-*O*-*acetyl*-β-D-*glucopyranosyl*-(1→3)-2,4,6-*tri*-*O*-*benzoyl*-β-D-*glucopyranosyl*-(1→3)-[2,3,4,6-*tetra*-*O*-*benzoyl*-β*glucopyranosyl*-(1→6)-]2,4-*di*-*O*-*acetyl*-β-D-*glucopyranoside* (**10**)

Compound **7** (1.34 g, 0.893 mmol) and **9** (1.20 g, 0.809 mmol) were dried together under high vacuum for 2 h, then dissolved in anhydrous CH_2Cl_2 (10 mL). TMSOTf (40 μL, 0.352 mmol) was added dropwise at −20 ℃ with N_2 protection. The reaction mixture was stirred for 3 h, and 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 petroleum ether-EtOAc (1:1, *V/V*) as the eluent gave the product **10** (1.67 g, 73.2%) as a syrup: $[\alpha]_D$ + 25 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.17—7.14 (m, 70H, 14Bz-**H**), 5.96 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.88 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.69—5.66 (m, 1H, $CH_2 = CHCH_2O$), 5.62 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.7$ Hz, 1H, H-2), 5.55—5.49 (m, 5H), 5.38 (dd, $J_{2,3} = J_{3,4} = 9.7$ Hz, 1H, H-3), 5.32 (dd, $J_{2,3} = J_{3,4} = 9.47$ Hz, 1H, H-3), 5.02 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.7$ Hz, 1H, H-2), 4.98 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.96 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.95—4.93 (m, 2H), 4.90 (d, $J_{1,2} = 3.2$ Hz, 1H, H-1), 4.83—4.75 (m, 4H), 4.72 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.65—4.62 (m, 3H), 4.56—4.39 (m, 5H), 4.37—4.32 (m, 2H), 4.30 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.17—4.14 (m, 3H), 4.07 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.06—3.98 (m, 3H), 3.94—3.80 (m, 7H), 3.66—3.62 (m, 2H), 3.52—3.50 (m, 1H), 3.42—3.40 (m, 1H), 3.31—3.24 (m, 1H), 2.97—2.94 (m, 1H), 2.25, 1.93, 1.85, 1.04 (s, 12H, $4CH_3CO$), 1.26—1.14 (m, 20H), 0.85 (dd, $J = 6.8$ Hz, $J = 7.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.6, 169.5, 169.0, 167.6 (4C, 4**C**OCH_3), 166.1, 166.0, 166.0, 166.0, 165.6, 165.5, 165.3, 165.1, 165.0, 165.0, 164.9, 164.7, 164.6, 164.5 (14C, 14**C**OPh), 101.1, 101.0, 100.8, 100.4, 100.1, 93.1 (C-1^{I-VI}), 79.8, 76.8, 73.7, 73.7, 73.2, 73.0, 72.9, 72.9, 72.8, 72.8, 72.7, 72.6, 72.5, 72.2, 72.0, 71.9, 71.8, 69.6, 69.2, 68.7, 67.7, 67.5, 63.3, 63.0 (C-2, 3, 4, 5, 6^{I-VI}), 31.8, 29.6, 29.6, 29.5, 29.3, 29.2, 25.8, 22.5, 21.0, 20.6, 20.3, 19.7, 14.0 (lauryl). Anal. calcd for $C_{157}H_{154}O_{49}$: C 66.76, H 5.46; found C 67.01, H 5.56.

Lauryl 2,4,6-*tri-O-benzoyl-β-D-glucopyranosyl-*(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-benzoyl-β-D-glucopyranosyl-*(1→3)-[2,3,4,6-*tetra-O-benzoyl-β-D-glucopyranosyl-*(1→6)-]2,4-*di-O-acetyl-β-D-glucopyranoside* (**11**)

To a solution of **10** (2 g, 0.709 mmol) in MeOH (30 mL) was added $PdCl_2$ (150 mg). After stirring for 3 h at room temperature, TLC [petroleum ether-EtOAc (1.5:1, *V/V*)] indicated that the reaction was complete. The mixture was filtered and the solution was concentrated to dryness, and the resultant residue was purified by flash chromatography [petroleum ether-EtOAc (1:1, *V/V*)] to give **11** (1.67 g, 84.5%) as a syrup: $[\alpha]_D$ − 12 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.01—7.17 (m, 70H, 14Bz-**H**), 5.95 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.87 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.68 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.66 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.52 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.7$ Hz, 1H, H-2), 5.50 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.7$ Hz, 1H, H-2), 5.44—5.41 (m, 2H), 5.38 (dd, $J_{2,3} = J_{3,4} = 9.7$ Hz, 1H, H-3), 4.96 (dd, $J_{1,2} = 3.6$ Hz, $J_{2,3} = 9.7$ Hz, 1H, H-2), 4.93 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.89 (dd, $J_{1,2} = 7.9$ Hz, $J_{2,3} = 9.7$ Hz, 1H, H-2), 4.79 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.77 (d, $J_{1,2} = 3.6$ Hz, 1H, H-1), 4.76—4.75 (m, 2H), 4.64—4.57 (m, 4H), 4.53—4.43 (m, 4H), 4.41 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.38 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.32—4.19 (m, 3H), 4.18—4.10 (m, 2H), 4.08 (d, $J_{1,2} = 7.9$ Hz, 1H, H-1), 4.06—3.98 (m, 4H), 3.96—3.87 (m, 3H), 3.66—3.60 (m, 2H), 3.52—3.48 (m, 1H), 3.42—3.38 (m, 1H), 3.36—3.21 (m, 1H), 2.96—2.92 (m, 1H), 2.24, 1.92, 1.92, 1.04 (s, 12H, $4CH_3CO$), 1.28—1.17 (m, 20H), 0.85 (dd, $J = 6.8$, 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.5, 169.4, 169.1, 167.7 (4C, 4**C**OCH_3), 166.3, 166.1, 166.0, 165.9, 165.8, 165.8, 165.6, 165.5, 165.3, 165.1, 165.0, 165.0, 164.7, 164.7 (14C, 14**C**OPh), 101.1, 101.1, 100.5, 100.4, 100.1, 93.2 (6C-1), 79.8, 77.8, 75.3, 74.4, 73.9, 73.7, 72.8, 72.7, 72.5, 72.3, 72.2, 71.9, 71.7, 71.5, 71.0, 69.6, 69.3, 69.2, 69.0, 68.8, 67.9, 67.5, 63.0, 62.8 (C-2, 3, 4, 5, 6), 31.8, 29.6, 29.6, 29.5, 29.3, 29.2, 25.8, 22.5, 20.8, 20.6, 20.4, 19.7, 14.0 (lauryl). Anal. calcd for $C_{154}H_{150}O_{49}$: C 66.43, H 5.39; found C 66.68, H 5.30.

Lauryl 2,3,4,6-*tetra-O-benzoyl-β-D-glucopyranosyl-*(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-benzoyl-β-D-glucopyranosyl-*(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-benzoyl-β-D-glucopyranosyl-*(1→3)-[2,3,4,6-*tetra-O-benzoyl-β-D-glucopyranosyl-*(1→6)-]2,4-*di-O-acetyl-β-D-glucopyranoside* (**13**)

Compound **12** (406 mg, 0.259 mmol) and **11** (600 mg, 0.216 mmol) were dried together under high vacuum for 2 h, then dissolved in anhydrous CH_2Cl_2 (10 mL). TMSOTf (15 μL, 0.132 mmol) was added dropwise at − 20 ℃ 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 petroleum ether-EtOAc (1:1, *V/V*) as the eluent gave the product **13** (586 mg, 64.8%) as a syrup: $[\alpha]_D$ + 25 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.17—7.17 (m, 110H, 22Bz-**H**), 5.97 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.93 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.85 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.80 (dd, $J_{3,4} = J_{4,5} = 9.7$ Hz, 1H, H-4), 5.72 (dd, $J_{3,4} = J_{4,5} = 9.6$ Hz, 1H, H-4), 5.68—5.62 (m,

3H), 5.57 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.8 Hz, 1H, H-2), 5.53 (dd, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 5.51—5.47 (m, 2H), 5.40 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, 1H, H-3), 5.35 (dd, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, 1H, H-3), 5.23 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 5.19 (dd, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, 1H, H-2), 4.96 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.92 (d, $J_{1,2}$ = 3.6 Hz, 1H, H-1), 4.90 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.86 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.85—4.70 (m, 6H), 4.69 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.67—4.51 (m, 8H), 4.46—4.37 (m, 4H), 4.36—4.31 (m, 3H), 4.29—4.18 (m, 5H), 4.14 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.07 (d, $J_{1,2}$ = 7.9 Hz, 1H, H-1), 4.05—4.00 (m, 3H), 3.96—3.82 (m, 6H), 3.76—3.73 (m, 1H), 3.63—3.58 (m, 2H), 3.51—3.38 (m, 3H), 3.28—3.16 (m, 2H), 2.97—2.94 (m, 1H), 2.26, 2.20, 2.04, 1.89, 1.75, 1.03 (s, 18H, 6CH_3CO), 1.26—1.07 (m, 20 H), 0.84 (dd, J = 6.8, 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 169.6, 169.4, 169.4, 169.1, 169.1, 167.7 (6C, 6**C**OCH_3), 166.1, 166.1, 166.0, 166.0, 165.9, 165.9, 165.8, 165.8, 165.6, 165.5, 165.4, 165.1, 165.1, 165.0, 165.0, 164.9, 164.8, 164.8, 164.7, 164.6, 164.6, 164.5 (22C, 22**C**OPh), 101.4, 101.3, 101.1, 100.8, 100.5, 100.2, 99.9 (7C-1 for β bonds, $J_{C\text{-}H}$ = 163.0—164.8 Hz), 93.7, 93.3 (2C-1 for α bond, $J_{C\text{-}H}$ = 172.0, 174.6 Hz), 79.8, 74.1, 73.7, 73.3, 73.2, 73.1, 72.8, 72.7, 72.5, 72.4, 72.3, 72.2, 72.1, 72.0, 71.9, 71.8, 71.7, 71.6, 71.0, 70.6, 69.6, 69.5, 69.4, 69.3, 69.1, 69.0, 68.9, 68.6, 68.4, 68.3, 68.2, 67.8, 67.5, 67.4, 63.2, 63.1, 62.9, 62.8, 60.3 (C-2, 3, 4, 5, 6), 31.8, 29.6, 29.6, 29.5, 29.3, 29.2, 25.8, 22.6, 21.0, 20.6, 20.4, 19.8, 14.1 (lauryl). Anal. calcd for $C_{232}H_{216}O_{74}$: C 66.54, H 5.16; found C 66.82, H 5.23.

Lauryl β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)-]α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)-]α-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[α-D-glucopyranosyl-(1→6)-]β-D-glucopyranoside (**14**)

Compound **13** (500 mg, 0.120 mmol) was dissolved in a saturated solution of NH_3 in MeOH (10 mL). After a week at room temperature, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **14** (157 mg, 89.6%) as a foamy solid: $[\alpha]_D$ + 18 (*c* 1.0, H_2O); ^{1}H NMR (D_2O, 400 MHz) δ: 5.22 (d, J = 3.2 Hz, 1H, H-1), 5.19 (d, J = 3.2 Hz, 1H, H-1), 4.68 (d, J = 8.0 Hz, 1H, H-1), 4.62 (d, J = 8.0 Hz, 1H, H-1), 4.40 (d, J = 8.0 Hz, 1H, H-1), 4.36 (d, J = 8.0 Hz, 1H, H-1), 4.32 (d, J = 8.0 Hz, 1H, H-1), 4.11—4.02 (m, 6H), 3.83—3.07 (m, 52H), 1.38—1.16 (m, 20H), 0.82—0.78 (m, 3H); ^{13}C NMR (D_2O, 100 MHz) δ: 102.9, 102.8, 102.7, 102.7, 102.6, 102.5, 102.3 (7C-1 for β bonds, $J_{C\text{-}H}$ = 163.0—164.3 Hz), 99.1, 99.1 (2C-1 for α bond, $J_{C\text{-}H}$ = 174.1Hz) 81.8, 75.9, 75.8, 775.5, 73.4, 73.1, 72.1, 71.0, 70.6, 69.5, 69.5, 69.4, 69.3, 69.1, 69.0, 68.9, 68.6, 68.4, 68.2, 67.8, 67.6 (C-2, 3, 4, 5, 6), 31.7, 29.5, 29.2, 25.5, 22.5, 13.9 (lauryl). Anal. calcd for $C_{66}H_{116}O_{46}$: C 48.18, H 7.06; found C 48.44, H 7.15. ESMS for $C_{66}H_{116}O_{46}$(1645.6): 1667.9 $[M + Na]^+$.

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