Highly Efficient Syntheses of the Phytoalexin-Elicitor Active β-(1→3)-Branched β-(1→6)-Linked Heptaglucose and Its Dodecyl Glycoside

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Abstract: A highly efficient method for the synthesis of 3,6-branched gluco-oligosaccharides was developed by using 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose, 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate, and 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate through a regio- and stereoselective manner. The β-(1→3)-branched β-(1→6)-linked heptaglucose **1** and its dodecyl glycoside **2** having phytoalexin-elicitor activity were prepared using the developed strategy. Bioassays showed that the phytoalexin-elicitor activity of the dodecyl β-(1→3)-branched β-(1→6)-linked hexaglucoside **2** is slightly more than that of the corresponding reducing end free heptaglucose **1**.

Key words: oligosaccharides, phytoalexin-elicitor, synthesis, glycosides, glycosylations

A central problem in carbohydrate chemistry is how to prepare oligosaccharides efficiently and simply. In the last decades, much effort has been paid to the oligosaccharide synthesis. However, up to date, there are no general applicable methods or strategies for oligosaccharide synthesis, and consequently the preparation of oligosaccharides is very time consuming compared with the synthesis of other biopolymers such as peptides and nucleic acids. Generally speaking, production of a complex oligosaccharide on an industrial scale is very difficult if not impossible so far. We always ask the question as to which method is the most suitable in carbohydrate synthesis. Does a single powerful method or strategy in the synthesis of oligosaccharides really exist? Maybe, owing to this structural complexity, the preparation of oligosaccharides will never achieve the same levels as the preparation of peptides and nucleic acids, but we can create relatively general procedures which are peculiarly effective for certain types of oligosaccharides.

3,6-Branched gluco-oligosaccharides are a common structure characteristic of many biologically active polysaccharides such as the phytoalexin-elicitor β-glucan and antitumor polysaccharides from schizophyllan, sceroglucan, and lentinan.[1] The β- (1→3)-branched β-(1→6)-linked glucose oligomers isolated from mycelial walls of the fungus *Phytophthora megasperma* f. sp. *Glycinea* can induce the formation of phytoalexins in soybean.[2] The most active heptasaccharide **1** (Figure1) is effective in very low doses, approximately 0.1 pmol per cotyledon.[3] It should be noted that, although much of this work was done with soybean cotyledons, it was established that the glucan-elicitor also elicited the synthesis of different phytoalexins in a wide range of other plant species.[4] These important discoveries stimulated the interest of scientists. Since their isolation and identification, the glucan-elicitors have been prepared by different groups,[5] and various methods and strategies have been used including the very elegant solid-phase strategies.[5k,l] Recently, in a preliminary communication, we have disclosed a newly efficient method suitable for large scale synthesis of 3,6-branched β-linked gluco-oligosaccharids using 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose as the starting material, and the β-(1→3)-branched β-(1→6)-linked glucohexaose phytoalexin-elicitor on a 100 g scale was achieved in our laboratory.[6]

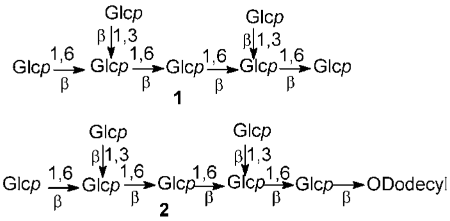

Figure 1 Glucose heptasaccharide **1** and its dodecyl glycoside **2**

Some research reports show that modification of oligosaccharides with hydrophobic long alkyls at the reducing terminal can increase their biological activity.[7] The reason is that the newly formed compounds are composed both of a hydrophilic oligosaccharide portion and a hydrophobic long alkyl portion, and have the characteristic of a surface-active agent able to coagulate together. In search of higher phytoaleaxin-elicitor active oligosaccharides, a dodecyl β-(1→3)-branched β-(1→6)-linked heptaglucoside **2** was synthesized in our laboratory. In this paper, we present in detail the syntheses of the heptaglucose **1** and its dodecyl glycoside **2**. Also the bioassay results of the compounds **1** and **2** as the phytoaleaxin-elicitors are given.

In our synthesis, 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**4**), 3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (**5**), 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopy-

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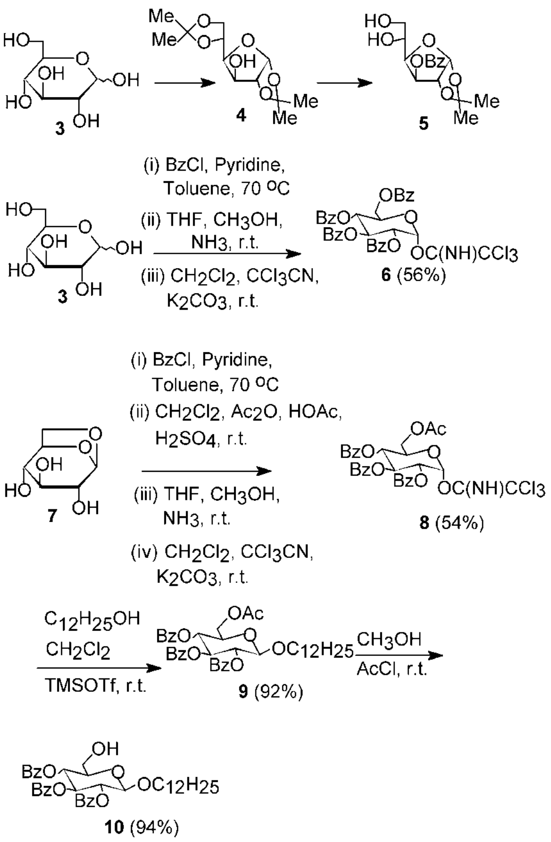
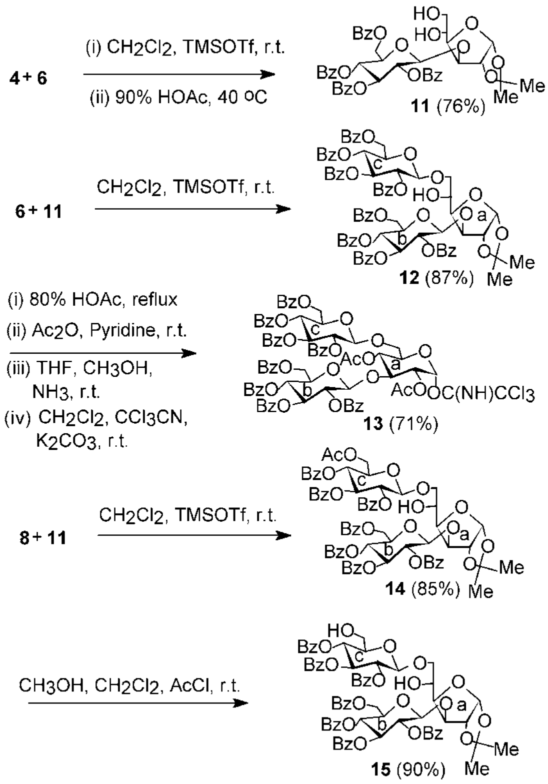
ranosyl trichloroacetimidate (**6**), 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**8**) and dodecyl 2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (**10**) were the basic building materials. Compound **4** was obtained according to the standard method.[8] Compound **5** was prepared from **4**.[9] Compound **6** was obtained as fine crystals from benzoylation of D-glucose, followed by 1-*O*-debenzoylation with ammonium in THF–MeOH and trichloroacetimidation (Scheme 1). Compound **8** was prepared as crystals by the benzoylation of 1,6-anhydro-β-D-glucopyranose (**7**, levoglucosan), a cheap material obtained from the pyrolysis of cellulose,[10] followed by acetolysis, 1-*O*-deacetylation, and trichloroacetimidation. Coupling of **8** with dodecyl alcohol gave compound **9** which was selectively converted to **10** using MeOH containing 0.3% HCl. The solution of MeOH containing HCl was formed in situ by adding acetyl chloride to MeOH.



Scheme 1 Preparation of building materials **4**, **5**, **6**, **8**, and **10**

Coupling of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**4**) with perbenzoylglucosyl trichloroacetimidate **6** in the presence of TMSOTf as catalyst, followed by selective 5,6-*O*-deacetonation afforded β-(1→3)-linked disaccharide **11** as crystals in a high yield (76% for two steps) (Scheme 2). Condensation of **11** with **6** catalyzed by TMSOTf gave regio- and stereoselectively the 3,6-branched trisaccharide **12** in 87% yield. Removal of the 1,2-*O*-isopropylidene group of **12** in 80% HOAc followed by acetylation with acetic anhydride in pyridine, selective 1-*O*-deacetylation with ammonia in THF–MeOH, and subsequent treatment with trichloroacetonitrile in the presence of K_2CO_3 afforded the desired trisaccharide glycosyl donor **13** in 71% yield (for four steps). Condensation of **11** with **8** afforded the 3,6-branched trisaccharide **14** in 85% yield. Selective 6-*O*-deacetylation of **14** in CH_2Cl_2–MeOH containing 0.3% HCl gave the trisaccharide acceptor **15** in 90% yield.



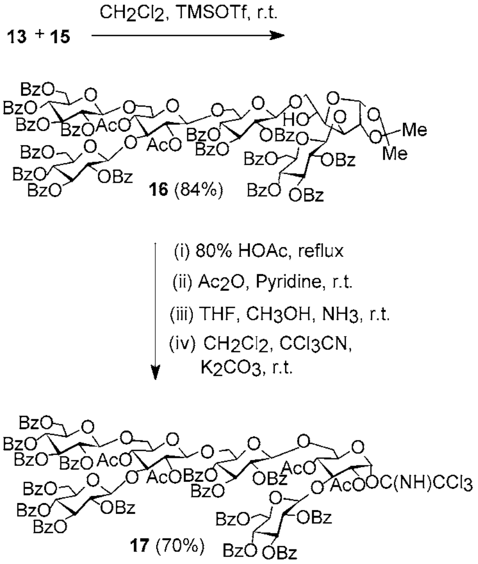
Scheme 2 Preparation of the trisaccharide donor **13** and acceptor **15**

Coupling of **13** with **15** with TMSOTf as the catalyst afforded regio- and stereoselectively the blocked hexasaccharide **16** in a high yield (84%) (Scheme 3). Using the same operations for the preparation of the trisaccharide glycosyl donor **13**, the hexasaccharide glycosyl donor **18** was obtained from **17**.

Coupling of **17** with **5** and **10** afforded the blocked heptasaccharides **18** and **19**, respectively (Scheme 4). Deisopropylidenation of **18** in 80% HOAc, followed by deacetylation in an ammonia-saturated solution of 1:9 CH_2Cl_2–MeOH, furnished the free heptasaccharide **1** as an amorphous white solid in 90% yield. Deprotection of **19** gave **2**.

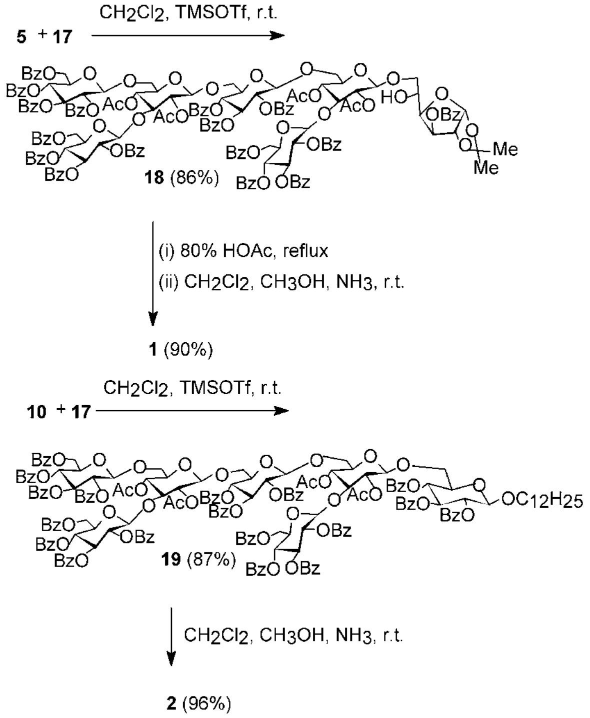
In all of the synthesis, very easily accessible materials and cheap reagents were used and the reactions were carried out smoothly in high yields and in large scales. In the synthesis, several intermediates were not separated and used directly for the further reaction simplifying the operation substantially. The sole use of acyl groups in the synthesis further simplified the procedure.

The bioassay was carried out according to the method devised by Ayers et al.[11] The phytoalexin-elicitor sugars can



Scheme 3 Preparation of the hexasaccharide donor **17**

stimulate glyceollin accumulation in cotyledons of soybean seedlings. The activities of the synthesized oligosaccharides were determined by their concentrations required to elicit half-maximal accumulation of glyceollin (C_{50}). The test results showed that C_{50} of the dodecyl β-(1→3)-branched β-(1→6)-linked hexaglucoside **2** and its underivatized **1** are about 6 nM and 9 nM respectively, indicating that modification of the reducing-end of this oligosaccharide does not have a significant effect on its biological activity. Similar conclusion was reported by others.[5h]



Scheme 4 Preparation of heptasaccharide **1** and its dodecyl glycoside **2**

Optical rotations were determined at 20 °C with a Perkin-Elmer Model 241-Mc automatic polarimeter. Melting points were determined with a Mel-Temp apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker ARX 400 spectrometers (400 MHz for ^{1}H, 100 MHz for ^{13}C) for solutions in $CDCl_3$ or D_2O as indicated. Chemical shifts are given in ppm downfield from internal Me_4Si. TLC was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV detector. Column chromatography was conducted by elution of a column (3 × 30 cm, 6.5 × 50 cm, 9 × 60 cm, 12 × 80 cm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (bp 60–90 °C) as eluent. Solutions were concentrated at <60 °C under reduced pressure.

2,3,4,6-Tetra-*O*-benzoyl-α-D-glucopyranosyl Trichloroacetimidate (6)

BzCl (680 mL, 5.83 mol) was added under vigorous stirring to a solution of D-glucose (**3**; 200 g, 1.11 mol) in toluene (2500 mL) and pyridine (473 mL, 5.85 mol) over 1 h at 70 °C, and then the mixture was stirred at 70 °C for further 7 h. Filtration of pyridine hydrochloride salt and concentration of the filtrate gave a residue which was directly dissolved in a solution of NH_3 (1.5 N) in 2:1 THF–MeOH (5600 mL). The solution was kept at r.t. for 12 h, at the end of which time TLC (petroleum ether–EtOAc, 3:1) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (1000 mL), and then CCl_3CN (120 mL, 1.2 mol) and K_2CO_3 (300 g, 2.17 mol) were added. The reaction mixture was stirred for 24 h at r.t., at the end of which time TLC (petroleum ether–EtOAc, 3:1) indicated that the reaction was complete. The mixture was filtered, the solution was concentrated under reduced pressure, and the residue was decolorized by passing through a short column (12 × 80 cm) packed with silica gel (2500 mL) using petroleum ether–EtOAc (3:1) as eluent. The product **6** was further purified by crystallization from petroleum ether–EtOAc, 3:1; white crystals; yield: 461 g (56% for three steps).[12]

6-*O*-Acetyl-2,3,4-tri-*O*-benzyol-α-D-glucopyranosyl Trichloroacetimidate (8)

BzCl (223 mL, 1.92 mol) was added under vigorous stirring to a solution of **7** (100 g, 0.62 mmol) in toluene (700 mL) and pyridine (158 mL, 1.95 mmol) over 1 h. The mixture was warmed to 70 °C, and then stirred at 70 °C for 7 h. Filtration of pyridine hydrochloride salt and concentration of the filtrate gave a residue which was directly dissolved in a mixture of CH_2Cl_2 (230 mL), Ac_2O (230 mL, 2.44 mol), and AcOH (230 mL). Then H_2SO_4 (23 mL) was added dropwise. This reaction mixture was kept for 20 h at r.t., then poured into ice water. After stirring for 15 min, the mixture was extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were washed with 10% aq $NaHCO_3$, and then concentrated to a syrup. The resulting residue was added to a solution of NH_3 (1.5 N) in THF–MeOH, 2:1 (2400 mL). The solution was kept at room temperature for 3 h, at the end of which time TLC (petroleum ether–EtOAc, 3:1) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was dissolved in CH_2Cl_2 (500 mL), and then CCl_3CN (86 mL, 0.85 mol) and K_2CO_3 (200 g, 1.45 mol) were added. The reaction mixture was stirred for 24 h at r.t., at the end of which time TLC (petroleum ether–EtOAc, 3:1) indicated that the reaction was complete. The mixture was filtered, the solution was concentrated under reduced pressure, and the residue was purified on a column (9 × 60 cm) packed with silica gel (3000 mL) using petroleum ether–EtOAc (3:1) as eluent to give **8** as white crystals; yield: 226.3 g (54% for four steps); mp 81–83 °C; $[\alpha]_D$ +9.0 (c = 1.3, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.65 [s, 1 H, OC(NH)CCl_3], 7.96–7.26 (m, 15 H, 3 PhH), 6.82 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 6.24 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 5.74 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.8 Hz, H-4), 5.59 (dd, 1 H, $J_{1,2}$ = 3.6, $J_{2,3}$ = 9.8 Hz, H-2), 4.50 (m, 1 H, H-5), 4.30 (m, 2 H, H-6,6′), 2.07 (s, 3 H, $COCH_3$).

Anal. Calcd for $C_{31}H_{26}Cl_3NO_{10}$: C, 54.84; H, 3.86. Found: C, 54.72; H, 3.90.

Dodecyl 6-*O*-Acetyl-2,3,4-tri-*O*-benzyol-β-D-glucopyranoside (9)

To a stirred solution of **8** (4 g, 5.89 mmol) and dodecyl alcohol (1.9 g, 10.2 mmol) in anhyd CH_2Cl_2 (80 mL) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf, 40 μL) at r.t. After 3 h, Et_3N was added to the solution to quench the reaction. The solution was concentrated, the residue was subjected to column chromatography on silica gel (3 × 30 cm, 150 mL) using petroleum ether–EtOAc (3:1) as eluent to give the trisaccharide **9**; 3.8 g (92%); $[\alpha]_D$ +34.2 (c = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.98–7.24 (m, 15 H, 3 PhH), 5.91 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3), 5.63 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4), 5.50 (dd, 1 H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.7 Hz, H-2), 4.82 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1), 4.36 (dd, 1 H, $J_{5,6}$ = 4.8, $J_{6,6'}$ = 12.2 Hz, H-6), 4.29 (dd, 1 H, $J_{5,6'}$ = 3.5, $J_{6,6'}$ = 12.2 Hz, H-6′), 4.06 (m, 1 H, H-5), 3.95–3.55 (m, 2 H, $CH_2C_{11}H_{23}$), 2.03 (s, 3 H, $COCH_3$), 1.54–0.86 (m, 23 H, $CH_2C_{11}H_{23}$).

Anal. Calcd for $C_{41}H_{50}O_{10}$: C, 70.07; H, 7.17. Found: C, 70.36; H, 7.28.

Dodecyl 2,3,4-Tri-*O*-benzyol-β-D-glucopyranoside (10)

Acetyl chloride (0.6 mL) was added to a solution of **9** (3 g, 4.27 mmol) in MeOH (100 mL), and the mixture was kept at r.t. for 20 h. After neutralization with Et_3N and concentration, the residue was subjected to column chromatography on silica gel (3 × 30 cm, 150 mL) using petroleum ether–EtOAc (2:1) as eluent to give **10**; yield: 2.65 g (94%); $[\alpha]_D$ +38.6 (c = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.88–7.15 (m, 15 H, 3 Ph*H*), 5.86 (t, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.8 Hz, H-3), 5.45–5.40 (m, 2 H, H-2, 4), 4.75 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1), 3.87–3.44 (m, 5 H, H-5, 6, 6′, $CH_2C_{11}H_{23}$), 1.45–0.77 (m, 23 H, $CH_2C_{11}H_{23}$).

Anal. Calcd for $C_{39}H_{48}O_9$: C, 70.89; H, 7.32. Found: C, 70.51; H, 7.39.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-1,2-*O*-isopropylidene-α-D-glucofuranose (11)

To a stirred solution of **4** (100 g, 0.38 mol) and **6** (260 g, 0.35 mol) in anhyd CH_2Cl_2 (3000 mL) was added TMSOTf (700 μL, 35 mmol) at r.t. After 3 h, Et_3N was added to the solution to quench the reaction. The solution was concentrated, and the residue was added to a 90% aq AcOH (5000 mL). The mixture was kept at 40 °C for 20 h and then concentrated under reduced pressure. The residue was subjected to column chromatography on silica gel (9 × 60 cm, 2500 mL) using petroleum ether–EtOAc, (1:1) as eluent to give **11** as white crystals; yield: 213 g (76% for two steps); mp 121–123 °C; $[\alpha]_D$ +34.0 (c = 2.5, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.11–7.28 (m, 20 H, 4 PhH), 5.94 (dd, 1 H, $J_{2',3'}$ = $J_{3',4'}$ = 9.7 Hz, H-3′), 5.72 (dd, 1 H, $J_{3',4'}$ = $J_{4',5'}$ = 9.7 Hz, H-4′), 5.54 (dd, 1 H, $J_{1',2'}$ = 7.9, $J_{2',3'}$ = 9.7 Hz, H-2′), 5.53 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1), 5.03 (d, 1 H, $J_{1',2'}$ = 7.9 Hz, H-1′), 4.84 (dd, 1 H, $J_{5',6'a}$ = 3.6, $J_{6'a,6'b}$ = 11.9 Hz, H-6′a), 4.42 (dd, 1 H, $J_{5',6'b}$ = 4.3, $J_{6'a,6'b}$ = 11.9 Hz, H-6′b), 4.41 (d, 1 H, $J_{3,4}$ = 2.6 Hz, H-3), 4.24–4.23 (m, 2 H, H-2,5′), 4.16 (dd, 1 H, $J_{3,4}$ = 2.6, $J_{4,5}$ = 8.8 Hz, H-4), 4.02 (m, 1 H, H-5), 3.83 (dd, 1 H, $J_{5,6a}$ = 3.2, $J_{6a,6b}$ = 11.4 Hz, H-6a), 3.67 (dd, 1 H, $J_{5,6b}$ = 6.0, $J_{6a,6b}$ = 11.4 Hz, H-6b), 1.44, 1.09 [2 s, $C(CH_3)_2$].

Anal. Calcd for $C_{43}H_{42}O_{15}$: C, 64.66; H, 5.30. Found: C, 64.79; H, 5.25.

3,6-Di-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-1,2-*O*-isopropylidene-α-D-glucofuranose (12); Typical Procedure

To a stirred solution of **11** (80 g, 0.1 mol) and **6** (80 g, 0.108 mol) in anhyd CH_2Cl_2 (400 mL) was added TMSOTf (200 μL, 1.0 mmol) at r.t. After 3 h, Et_3N was added to the solution to quench the reaction. The solution was concentrated and the residue was subjected to column chromatography on silica gel (9 × 60 cm, 3000 mL) using petroleum ether–EtOAc (1.5:1) as eluent to give the trisaccharide **12** as a white amorphous solid; yield: 119.8 g (87%); $[\alpha]_D$ +25.3 (c = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.06–7.28 (m, 40 H, 8 PhH), 5.88 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3[b]), 5.87 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.7 Hz, H-3[c]), 5.69 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4[b]), 5.64 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4[c]), 5.53 (dd, 1 H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.7 Hz, H-2[b]), 5.43 (dd, 1 H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.7 Hz, H-2[c]), 5.41 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1[a]), 4.96 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1[b]), 4.93 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1[c]), 4.68 (dd, 1 H, $J_{5,6}$ = 3.4, $J_{6,6'}$ = 12.2 Hz, H-6[b]), 4.48 (dd, 1 H, $J_{5,6'}$ = 4.9, $J_{6,6'}$ = 12.2 Hz, H-6[b]′), 4.67 (dd, 1 H, $J_{5,6}$ = 3.4, $J_{6,6'}$ = 12.2 Hz, H-6[c]), 4.35 (dd, 1 H, $J_{5,6}$ = 4.9, $J_{6,6}$ = 12.2 Hz, H-6[c]′), 4.34–3.65 (m, 8 H), 1.26, 1.03 [2 s, 6 H, $C(CH_3)_2$].

Anal. Calcd for $C_{77}H_{68}O_{24}$: C, 67.15; H, 4.98. Found: C, 67.29; H, 5.02.

2,4-Di-*O*-acetyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-α-D-glucopyranosyl Trichloroacetimidate (13); Typical Procedure

Compound **12** (50 g, 0.036 mol) was added to 80% aq AcOH (500 mL) and the mixture was refluxed for 4 h. The mixture was concentrated and the residue was acetylated by stirring with Ac_2O (250 mL) in pyridine (280 mL) for 2 h at r.t. The resultant trisaccharide was added to a solution of ammonia (1.5 N) in THF–MeOH, 3:1 (500 mL) and the solution was stirred at r.t. for 3 h. The solution was concentrated and the residue was dissolved in CH_2Cl_2 (200 mL). To the solution were added K_2CO_3 (10 g, 0.072) and CCl_3CN (8 mL, 0.072 mol), and the mixture was stirred at r.t. for 12 h. The mixture was filtered and the solids were washed with CH_2Cl_2. The filtrate and the washings were concentrated, and the residue was subjected to column chromatography on silica gel (6.5 × 50 cm, 1300 mL) using petroleum ether–EtOAc (2:1) as eluent to give the trisaccharide donor **13** as a white amorphous solid; yield: 40.4 g (71% for four steps); $[\alpha]_D$ +23.3 (c = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.33 [s, 1 H, $O(NH)CCl_3$], 8.07–7.19 (m, 40 H, 8 PhH), 6.19 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1[a]), 5.91 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3[b]), 5.85 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3[c]), 5.62 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4[b]), 5.61 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4[c]), 5.46 (dd, 1 H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.6 Hz, H-2[b]), 5.42 (dd, 1 H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.6 Hz, H-2[c]), 4.97 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1[b]), 4.96 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1[c]), 4.85 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.5 Hz, H-4[a]), 4.67–4.59 (m, 3 H), 4.50–4.37 (m, 2 H), 4.19–4.02 (m, 4 H), 3.91(dd, 1 H), 3.69 (dd, 1 H), 1.94, 1.78 (2 s, 6 H, 2 CH_3CO).

Anal. Calcd for $C_{80}H_{68}Cl_3NO_{26}$: C, 61.37; H, 4.38. Found: C, 61.53; H, 4.41.

6-*O*-Acetyl-2,3,4-tri-*O*-benzoyl-D-glucopyranosyl-(1→6)-[2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-1,2-*O*-isopropylidene-α-D-glucofuranose (14)

Using the same procedure as described for the preparation of **12** from **6** and **11**, the trisaccharide **14** was prepared from **8** (73.3 g, 0.108 mol) and **11** (80 g, 0.1 mol); white amorphous solid; yield: 111.8 g (85%); $[\alpha]_D$ +18.6 (c = 1.1, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05–7.26 (m, 35 H, 7 PhH), 5.87 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3[b]), 5.84 (dd, 1 H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3[c]), 5.65 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4[b]), 5.59 (dd, 1 H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4[c]), 5.51 (dd, 1 H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.6 Hz, H-

2[b]), 5.43 (dd, 1 H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.6 Hz, H-2[c]), 5.42 (d, 1 H, $J_{1,2}$ = 3.6 Hz, H-1[a]), 4.96 (d, 1 H, $J_{1,2}$ = 9.6 Hz, H-1[b]), 4.93 (d, 1 H, $J_{1,2}$ = 9.6 Hz, H-1[c]), 4.71–3.79 (m, 12 H), 2.05 (s, 3 H, CH_3CO), 1.33, 1.05 [2 s, 6 H, $C(CH_3)_2$].

Anal. Calcd for $C_{72}H_{66}O_{24}$: C, 65.75; H, 5.06. Found: C, 66.00; H, 5.03.

2,3,4-Tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl(1→3)]-1,2-*O*-isopropylidene-α-D-glucofuranose (15)

AcCl (6 mL) was added to a solution of **14** (100 g, 0.076 mol) in MeOH–CH_2Cl_2 (1:1, 1000 mL), and the mixture was kept at r.t. for 20 h. After neutralization and concentration, the residue was subjected to column chromatography on silical gel (9 × 60 cm, 3000 mL) using petroleum ether–EtOAc (1.5:1) as eluent to give compound **15** as a white amorphous solid; yield: 87.1 g (90%); $[\alpha]_D$ +22.6 (*c* = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.05–7.26 (m, 35 H, 7 PhH), 5.91 (dd, 1 H, $J_{2,3} = J_{3,4}$ = 9.8 Hz, H-3[b]), 5.90 (dd, 1 H, $J_{2,3} = J_{3,4}$ = 9.8 Hz, H-3[c]), 5.73 (dd, 1 H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4[b]), 5.56 (dd, 1 H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4[c]), 5.54–5.42 (m, 3 H), 4.99 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1[b]), 4.95 (d, 1 H, $J_{1,2}$ = 7.9 Hz, H-1[c]), 4.75–3.77 (m, 12 H), 1.33, 1.05 [2 s, 6 H, $C(CH_3)_2$].

Anal. Calcd for $C_{70}H_{64}O_{23}$: C, 66.03; H, 5.07. Found: C, 66.24; H, 5.10.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-1,2-*O*-isopropylidene-α-D-glucofuranose (16)

The hexasaccharide **16** was prepared by coupling **13** (50 g, 0.032 mol) with **15** (40.7 g, 0.032 mol) under the same conditions as described for the preparation of **12** from **6** and **11**; yield: 71.9 g (84%); $[\alpha]_D$ +26.6 (*c* = 1.0, $CHCl_3$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.4, 168.2 (2 CH_3CO), 112.2 [$C(CH_3)_2$], 105.0, 101.5, 101.1, 101.0, 100.9, 100.2 (6 C-1), 82.9, 82.5 (2 C-3), 26.6, 25.9 [$C(CH_3)_2$], 20.85, 20.51 (2 CH_3CO).

Anal. Calcd for $C_{148}H_{130}O_{48}$: C, 66.41; H, 4.90. Found: C, 66.51; H, 4.86.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl Trichloroacetimidate (17)

The hexasaccharide glycosyl donor **17** was prepared from **16** (18.1 g, 6.76 mmol) under the same conditions as described for the preparation of the trisaccharide donor **13** from **12**; yield: 13.6 g (70% for four steps); $[\alpha]_D$ +33.1 (*c* = 1.0, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.29 [s, 1 H, $O(NH)CCl_3$], 7.90–7.26 (m, 75 H, 15 PhH), 6.24 (d, 1 H, $J_{1,2}$ = 3.5 Hz, H-1), 6.06, 5.92, 5.88, 5.71, 5.70, 5.69, 5.68, 5.47, 5.46, 5.45, 5.39, 5.37 (12 dd, 12 H), 5.01, 4.97, 4.88, 4.75 4.53 (5 d, $J_{1,2}$ = 7.9 Hz, 5 H-1), 1.97, 1.96, 1.85, 1.77 (4 s, 12 H, 4 CH_3CO).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.84, 169.66 169.44, 169.04 (4 CH_3CO), 166.04–163.25 (15 COPh), 160.16 (CCl_3), 133.40–127.44 (90 C, 15 C_6H_5), 101.34, 100.96, 100.96, 100.65, 100.17, 95.1 (6 C-1), 90.2 [$O(NH)CCl_3$], 20.77, 20.48, 20.44, 20.21 (4 CH_3CO).

Anal. Calcd for $C_{151}H_{130}Cl_3NO_{50}$: C, 63.30; H, 4.57. Found: C, 63.21; H, 4.61.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→6)-3-*O*-benzoyl-1,2-*O*-isopropylidene-α-D-glucofuranose (18)

The heptasaccharide **18** was prepared by coupling **17** (5 g, 1.77 mmol) with 3-*O*-benzoyl-1,2-di-*O*-isopropylidene-α-D-glucofuranose (0.69 g, 3.09 mmol) under the same conditions as described for the preparation of **12** from **6** and **11**; yield: 4.6 g (86%); $[\alpha]_D$ +34.3 (*c* = 1.0, $CHCl_3$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.58, 169.29 168.23, 168.15 (4 CH_3CO), 165.95, 165.94, 165.94, 165.76, 165.72, 165.60, 165.55,165.50, 165.28, 165.25, 165.10, 165.07, 165.04, 165.01, 165.00, 164.93 (16 PhCO), 112.05 [$C(CH_3)_2$], 105.04, 101.23, 101.05, 100.96, 100.95, 100.58, 100.38 (7 C-1), 83.38, 82.20 (2 C-3), 26.57, 26.11 [$C(CH_3)_2$], 20.78, 20.60, 20.50, 20.42 (4 CH_3CO).

Anal. Calcd for $C_{165}H_{148}O_{56}$: C, 65.47; H, 4.93. Found: C, 65.31; H, 4.86.

β-D-Glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→6)-D-glucopyranose (1)

Compound **18** (3.5 g, 1.16 mmol) was dissolved in 80% AcOH (100 mL) and the mixture was refluxed for 6 h. Concentration of the mixture followed by deacylation in an ammonia-saturated solution of CH_2Cl_2–MeOH, 1:9 (200 mL) at r.t. After 24 h, the mixture was concentrated to about 20 mL, then CH_2Cl_2 (150 mL) was added. The resultant precipitate was filtered and washed with CH_2Cl_2 (4 ×) to give the free heptasaccharide **1** as a white powder; yield: 1.2 g (90% for two steps); $[\alpha]_D$ –25.0 (*c* = 0.1, H_2O).

^{13}C NMR (100 MHz, D_2O): δ = 102.7, 102.6, 102.6, 102.4, 102.4, 102.1, 102.1 (7 C-1), 84.5, 84.3 (2 C-3).

ESMS for $C_{42}H_{72}O_{36}$ (1153.01): 1152.00 $[M – 1]^+$.

Dodecyl 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-acetyl-β-D-glucopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-β-D-glucopyranoside (19)

The heptasaccharide **19** was prepared by coupling **17** (3.4 g, 1.87 mmol) with **10** (1.65 g, 2.5 mmol) under the same conditions as described for the preparation of **12** from **6** and **11**; yield: 3.67 g (87%); $[\alpha]_D$ +30.7 (*c* = 1.0, $CHCl_3$).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 169.66, 169.48, 168.15, 168.09 (4 CH_3CO), 101.27, 101.19, 101.04, 101.04, 100.97, 100.38, 100.38 (7 C-1), 78.51, 78.25 (2 C-3), 31.87, 29.61, 29.57, 29.53, 29.45, 29.38, 29.33, 29.32, 25.84, 22.64, 20.81, 20.73, 20.52, 20.52 (4 CH_3CO), 14,08.

Anal. Calcd for $C_{188}H_{176}O_{58}$: C, 67.14; H, 5.27. Found: C, 67.36; H, 5.36.

Dodecyl β-D-Glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-[β-D-glucopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (2)

Compound **19** (2.2 g, 0.97 mmol) was dissolved in an ammonia-saturated solution of 1:9 CH_2Cl_2–MeOH (100 mL) at r.t. After 24 h, the mixture was concentrated to about 10 mL, and then CH_2Cl_2 (100 mL) was added. The resultant precipitate was filtered and washed with CH_2Cl_2 (4 ×) to afford **2** as a white solid; yield: 1.24 g (96%); $[\alpha]_D$ –19.5 (*c* = 0.1, H_2O).

^{13}C NMR (100 MHz, D_2O): δ = 105.67, 105.64, 105.45, 105.41, 105.31, 105.21, 104.94 (7 C-1), 87.17, 87.03 (2 C-3), 34.20, 32.72, 32.34, 31.96, 31.68, 28.05, 24.94, 16.49.

ESMS for $C_{54}H_{96}O_{36}$ (1321.33): 1320.2 $[M - 1]^+$.

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