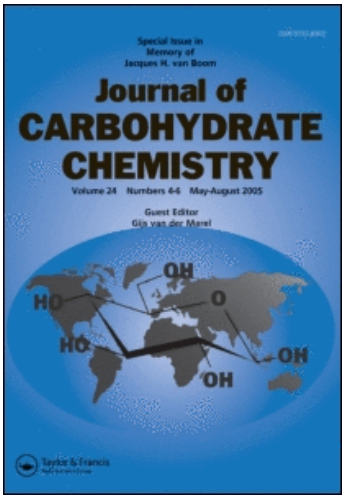

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Synthesis of 3,6-Branched β-D-Glucose Oligosaccharides

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ABSTRACT

A glucohexasaccharide, β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→6)]-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-β-D-Glc*p* was synthesized as its 4-methoxyphenyl glycosidevia 2 + 2 + 2 strategy with benzylidenated glucose mono- and disaccharides as the key intermediates.

Key Words: β-D-Glucans; Trichloroacetimidates; Regio- and stereoselective synthesis.

INTRODUCTION

Glucans consisting of a β-(1→3)-linked backbone with various β-(1→6)-linked mono-, di-, and trisaccharide branches having 0, 1, or 2 (1→3) linked β-D-glucose residues (Figure 1), were found in fruiting bodies and culture broths of *Phytophthora parasitica*.[1] Similarly, the glucans from spores of *Ganoderma lucidum* (Fr.) Karst were shown to have a β-(1→3)-linked glucose backbone with branches of mono-, di-, and oligosaccharide side chains substituted at the C-6 of the glucosyl residues.[2] As these glucans show antitumor activity, chemists are interested in synthesizing the

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minimum active fragments of the polysaccharides, so that they can investigate their structure-activity relationship and develop new antitumor medicines.[3–7] There have been several reports dealing with the syntheses of linear and branched glucans.[3–8] We present herein the stereoselective synthesis of the 4-methoxyphenyl glycoside of β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→6)-]β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-β-D-Glc*p*. with 4,6-benzylidenated glucose derivatives as the key intermediates.

RESULTS AND DISCUSSION

Retrosynthetic analysis suggests that the best way to assemble the target hexaose is to first construct a β-(1→3)-linked tetrasaccharide backbone and a β-(1→3)-linked disaccharide side chain, then couple them at the C-6″-position of the tetrasaccharide backbone. It is well known that the presence of a C-2 ester group capable of neighboring group participation is necessary for the formation of the β-glucosyl linkage. However, our previous work[9] revealed that in (1→3)-glucosylation, the glycosyl bond originally present in either donor or acceptor controlled the stereoselectivity of the forthcoming bond, i.e., the newly formed glycosidic linkage had the opposite anomeric configuration of that of either the donor or acceptor. With acylated β-(1→3)-linked disaccharides as the acceptors and acylated β-(1→3)-linked disaccharide trichloroacetimidates as the donors, α-linked tetrasaccharides are always obtained in spite of the presence of a C-2 ester group. This was troublesome for the synthesis of β-(1→3)- linked tetrasaccharides. However, some reports[4,7] revealed that with 4,6-benzylidenated glucose derivatives as either donor or acceptor, β-linked oligosaccharides are readily obtained.

Therefore, in the present research, benzylidenated glucose mono- or disaccharide derivatives were used as the key intermediates as outlined in Scheme 1. First, condensation of the acceptor **2**,[10] obtained by coupling of 2,4,6-tri-*O*-acetyl-3-*O*-allyl-α-D-glucopyranosyl trichloroacetimidate[11] with 4-methoxyphenol and followed by deallylation, with 3-*O*-allyl-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl trichloroacetimidate (**1**)[4] afforded a β-(1→3)-linked disaccharide **3**. Debenzylidenation, benzoylation, and deallylation furnished the disaccharide acceptor **6**. The disaccharide donor **12** was similarly prepared, i.e., condensation of fully benzoylated glucosyl trichloroacetimidate **7**[12] with isopropyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**8**)[3] selectively offered β-(1→3)-linked disaccharide **9**. The use of isopropyl thioglycoside **8** as the donor rather than methyl or ethyl thioglycosides ensured good reactivity of the donor, but avoided the operation difficulty caused by low boiling points of methanethiol or ethanethiol.

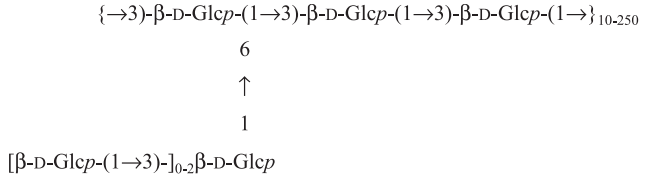

Figure 1. Polysaccharide structure of *Phytophthora parasitica*.

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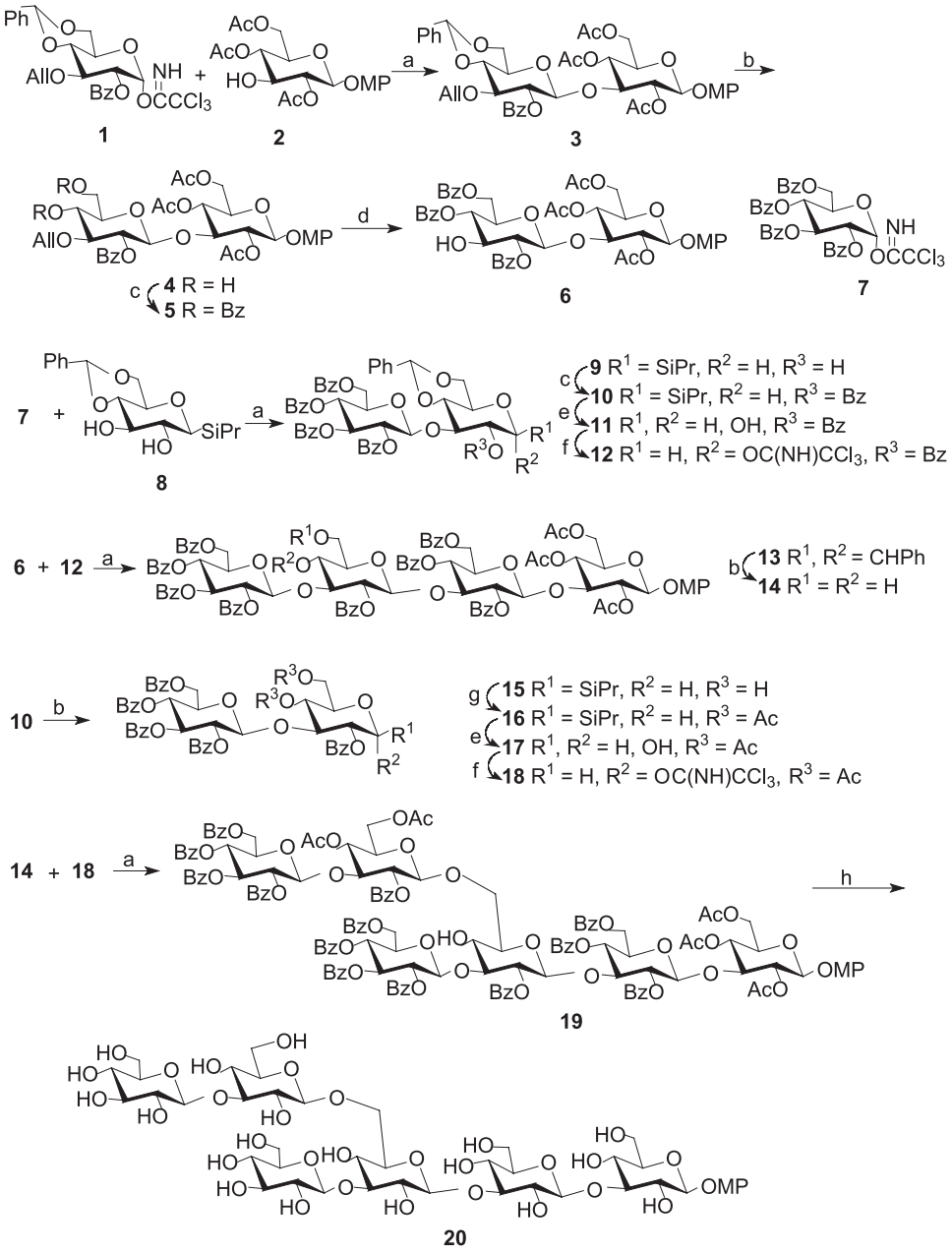

Scheme 1. Conditions and reagents: a: TMSOTf, CH_2Cl_2, −20°C to rt; b: 90% HOAc-H_2O, 80°C, 3 h; c: BzCl, pyridine, rt; d: $PdCl_2$, CH_2Cl_2, MeOH, rt; e: NIS, TMSOTf, CH_2Cl_2, 3 h; f: CCl_3CN, CH_2Cl_2, DBU, rt; g: Ac_2O, pyridine, rt,12 h; h: MeOH, NH_3, rt, two weeks.

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The regioselectivity was confirmed by benzoylation to give **10**, whose 2D ^{1}H NMR spectrum showed H-2 at δ 5.31 ppm with $J_{1,2}$ = 8.9 Hz and $J_{2,3}$ = 9.7 Hz. It was reported[13] that transformation of thioglycosides to the corresponding hemiacetals was achieved by treating the thioglycosides with water, *N*-iodosuccinimide (NIS) and catalytic TMSOTf in acetone. It was found in our research, that treatment of **10** with NIS and catalytic TMSOTf in reagent grade dichloromethane without addition of water gave better results, affording the hemiacetal **11** smoothly (85%). Subsequent trichloroacetimidation of **11** furnished the disaccharide donor **12** (93%). Coupling of **12** with **6** gave β-(1→3)-linked tetrasaccharide **13** in acceptable yield (51%). This is different from the coupling that gives completely α-linked tetrasaccharide[10] with acylated β-(1→3)-linked disaccharides as the donors and acylated β-(1→3)-linked disaccharides as the acceptors, indicating the important role of the 4,6-*O*-benzylidene group in controlling stereoselectivity of glycosylation. Debenzylidenation afforded the tetrasaccharide acceptor **14** (92%). The branch disaccharide donor **18** was prepared from **10** by debenzylidenation, acetylation, dethiopropylation, and trichloroacetimidation (69% for four steps). Glycosylation of the tetrasaccharide acceptor **14** with the disaccharide donor **18** selectively gave β-(1→6)-linked hexasaccharide **19** (80%), and subsequent deacylation in saturated ammonia-methanol solution yielded the target hexaoside **20** (95%). The ^{1}H and ^{13}C NMR spectra of **20** showed some characteristic signals such as at δ 4.88 (1 H, $J_{1,2}$ 7.6, H-1), 4.70 (1 H, $J_{1,2}$ 8.2, H-1), 4.66 (1 H, $J_{1,2}$ 8.0, H-1), 4.65 (1 H, $J_{1,2}$ 8.0, H-1), 4.60 (1 H, $J_{1,2}$ 7.6, H-1), 4.43 (1 H, $J_{1,2}$ 8.0, H-1), 103.0, 102.8, 102.6, 102.6, 102.6, 101.0 (6 C-1, $J_{C\text{-}1,H\text{-}1}$ = 164.2, 165.5, 165.0, 164.7, 164.9, 165.3 Hz), 84.8, 84.6, 84.2, 84.2 (4 C, glycosylated C-3), 68.0 (1 C, glycosylated C-6). The C-3 data also confirmed the C-6″-selective glycosylation of **14** with **18**, otherwise, if C-4″ was glycosylated, one more signal at ∼80 ppm would appear.

In summary, a convergent synthesis of glucans consisting of a (1→3)-β-D-linked tetrasaccharide backbone with a (1→6)-β-D-linked disaccharide branch having one (1→3)-linked β-D-glucose residue was achieved. The method is simple and practical, and should be possible for large-scale synthesis.

EXPERIMENTAL

Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^{1}H NMR, ^{13}C NMR and ^{1}H–^{1}H, ^{1}H–^{13}C COSY spectra were recorded with Bruker ARX 400 spectrometers (400 MHz for ^{1}H, 100 MHz for ^{13}C) at 25°C for solutions in $CDCl_3$ or D_2O as indicated. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the ESI mode. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH or in some cases by a UV lamp. Column chromatography was conducted by elution of a column (16 × 240 mm, 18 × 300 mm, 35 × 400 mm) of silica gel (100–200 mesh) with EtOAc-petroleum ether (60–90°C) as the eluent. Solutions were concentrated at <60 °C under reduced pressure.

General procedure for glycosylations. A mixture of donor and acceptor was dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2. TMSOTf

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(0.05 equiv) was added dropwise at −20 °C under nitrogen. 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, gave the desired products.

4-Methoxyphenyl 3-*O*-allyl-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranoside (3). Donor **1** (3.67 g, 6.59 mmol) and acceptor **2** (2.42 g, 6.09 mmol) were coupled as described in the general procedure. Purification by chromatography with 3:1 petroleum ether-EtOAc as the eluent gave disaccharide **3** (3.63 g, 74%): $[\alpha]_D$ + 21.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.10–6.75 (m, 14H, Bz-*H*, Ph-*H*, Mp-*H*), 5.55 (m, 1H, $CH_2-CH{=}CH_2$), 5.48 (s, 1H, PhC*H*), 5.12 (dd, 1H, J 1.3 Hz, J 17.2 Hz, $CH_2-CH{=}CH_2$), 5.10 (dd, 1H, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 9.5 Hz, H-2′), 5.01 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 4.99 (dd, 1H, J 1.3 Hz, J 10.2 Hz, $CH_2-CH{=}CH_2$), 4.97 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.83 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1′), 4.69 (d, 1H, $J_{1,2}$ 7.6 Hz, H-1), 4.40 (dd, 1H, $J_{5,6'e}$ 5.7 Hz, $J_{6'a,6'e}$ 11.2 Hz, H-6′e), 4.19–4.09 (m, 5H, H-6′a, H-6e, H-6a, $CH_2-CH{=}CH_2$), 3.81 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4′), 3.78 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3′), 3.74 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3), 3.62 (s, 3H, OCH_3), 3.62 (ddd, 1H, $J_{4,5}$ 9.5 Hz, $J_{5,6'e}$ 5.7 Hz, $J_{5,6'a}$ 4.2 Hz, H-5′), 3.54 (ddd, 1H, $J_{4,5}$ 9.5 Hz, $J_{5,6e}$ 5.8 Hz, $J_{5,6a}$ 4.6 Hz, H-5), 2.08, 2.05, 1.90 (3s, 9H, 3 *Me*CO).

Anal. Calcd for $C_{42}H_{46}O_{16}$: C 62.52; H 5.75. Found: C 62.37; H 5.67.

4-Methoxyphenyl 2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranoside (6). A mixture of **3** (3.39 g, 4.21 mmol) and 90% HOAc-H_2O (100 mL) was refluxed for 2 h. The reaction mixture was concentrated to a syrup, and then co-evaporated with toluene (10 mL) three times. The residue was purified by chromatography with 1:1 petroleum ether-EtOAc as the eluent to give compound **4** (2.90 g, 93%). Benzoyl chloride (1.50 mL, 12.8 mmol) was added to the solution of **4** in pyridine (10 mL). The reaction mixture was stirred at rt for 4 h, the excess benzoyl chloride was destroyed by MeOH, then the mixture was concentrated. The residue was dissolved in CH_2Cl_2 (50 mL) and washed with 0.2 M HCl, saturated aqueous sodium bicarbonate, water and then concentrated. The residue was purified by chromatography with 2:1 petroleum ether-EtOAc as the eluent to give **5** (3.34 g, 90%). To a solution of **5** (3.12 g, 3.28 mmol) in CH_2Cl_2 (60 mL) and CH_3OH (30 mL) was added $PdCl_2$ (75 mg, 0.42 mmol), the reaction mixture was stirred at rt until TLC (2:1 petroleum ether-EtOAc) suggested that the reaction was complete. Then the mixture was filtered, the solution was concentrated to dryness, and the residue was purified by flash chromatography with 2:1 petroleum ether-EtOAc as the eluent to give **6** (2.75 g, 92%): $[\alpha]_D$ + 6.1 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.10–6.75 (m, 19H, 3 Bz-*H*, Mp-*H*), 5.41 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4′), 5.19 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.5 Hz, H-2′), 5.09 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4), 5.06 (dd, 1H, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.90 (s, 1H, $J_{1,2}$ 7.8 Hz, H-1′), 4.76 (s, 1H, $J_{1,2}$ 7.7 Hz, H-1), 4.61 (dd, 1H, $J_{5',6'e}$ 12.2 Hz, $J_{6'e,\ 6'a}$ 4.2 Hz, H-6′e), 4.48 (dd, 1H, $J_{5',6'a}$ 12.1 Hz, $J_{6'e,\ 6'a}$ 4.2 Hz, H-6′a), 4.18–4.39 (m, 5H, H-3′, H-3, H-5′, H-6e, H-6a), 3.74 (s, 3H, OCH_3), 3.61 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6e}$ 12.3 Hz, $J_{5,6a}$ 12.1 Hz, H-5), 2.04, 2.02, 1.94 (3s, 9H, 3 *Me*CO).

Anal. Calcd for $C_{46}H_{46}O_{18}$: C 62.30; H 5.23. Found: C 62.37; H 5.20.

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Isopropyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6–*O*-benzylidene-1-thio-β-D-glucopyranoside (10). Fully benzoylated glucosyl trichloroacetimidate **7** (7.41 g, 10.0 mmol) and isopropyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside **8** (2.84 g, 8.86 mmol) were coupled as described in the general procedure. The product was purified by chromatography with 3:1 petroleum ether-EtOAc as the eluent to yield disaccharide **9** (7.68 g, 95%). Benzoyl chloride (1.30 mL, 11.1 mmol) was added to the solution of **9** (7.50 g, 8.24 mmol) in pyridine (30 mL). The reaction mixture was stirred at rt for 4 h, excess benzoyl chloride was destroyed by MeOH. The mixture was concentrated. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with 0.2 M HCl, saturated aqueous sodium bicarbonate, water, and then concentrated. The residue was purified by chromatography with 2:1 petroleum ether-EtOAc as the eluent to give **10** (7.64 g, 92%), which is a mixture of *R*,*S* isomers. The mixture was directly used for further reaction without separation, but one isomer was isolated in pure form for spectral analysis: $[\alpha]_D$ + 17.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.03–7.18 (m, 30H, 5 Bz-*H*, Ph-*H*), 5.70 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4′), 5.63 (s, 1H, PhC*H*), 5.59 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3′), 5.46 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.5 Hz, H-2′), 5.31 (dd, 1H, $J_{1,2}$ 8.9 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.01 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1′), 4.66 (d, 1H, $J_{1,2}$ 8.9 Hz, H-1), 4.47 (dd, 1H, $J_{5,6'e}$ 12.1 Hz, $J_{6'e,\ 6'a}$ 4.1 Hz, H-6′e), 4.36 (dd, 1H, $J_{5,6e}$ 12.1 Hz, $J_{6e,\ 6a}$ 4.8 Hz, H-6e), 4.27 (dd, 1H, $J_{5,6'a}$ 12.1 Hz, $J_{6'e,6'a}$ 4.1 Hz, H-6′a), 4.21 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 3.91 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 3.87–3.82 (m, 2H, H-5′, H-6a), 3.55 (ddd, 1H, $J_{4,5}$ 9.7 Hz, $J_{5,6e}$ 12.1 Hz, $J_{5,6a}$ 12.1 Hz, H-5), 3.10 (m, 1H, C*H*$(CH_3)_2$), 1.20 (s, 3H, CH(C*H*$_3)_2$), 1.18 (s, 3H, CH(C*H*$_3)_2$).

Anal. Calcd for $C_{57}H_{52}O_{15}S$: C 67.84; H 5.19. Found: C 67.71; H 5.09.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl trichloroacetimidate (12). A solution of **10** (3.00 g, 2.97 mmol) and NIS (0.82 g, 3.56 mmol) in CH_2Cl_2 was cooled to −20 °C, then TMSOTf (55.0 μL, 0.30 mmol) was added dropwise under nitrogen. The solution was stirred for 3 h, during which time the temperature was gradually raised to ambient temperature. The reaction mixture was neutralized with Et_3N, then concentrated, and the residue was purified by chromatography with 2:1 petroleum ether-EtOAc as the eluent to give **11** (2.44 g, 85%). A mixture of **11**, trichloroacetonitrile (1.2 mL, 5.63 mmol), and DBU (0.2 mL, 1.62 mmol) in dry CH_2Cl_2 (20 mL) was stirred for 3 h and then concentrated. The residue was purified by flash chromatography with 2:1 petroleum ether-EtOAc to give donor **12** (2.59 g, 93%) as a foamy solid: $[\alpha]_D$ + 39.8 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.45 (s, 1H, CN*H*CCl_3), 7.95–7.08 (m, 30H, 5 Bz-*H*, Ph-*H*), 6.50 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.76 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.5, H-4′), 5.65 (s, 1H, PhC*H*), 5.60 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3′), 5.50 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.5 Hz, H-2′), 5.30 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.11 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1′), 4.55 (dd, 1H, $J_{5,6'e}$ 12.0 Hz, $J_{6'e,\ 6'a}$ 3.4 Hz, H-6′e), 4.48 (dd, 1H, $J_{5,6'a}$ 12.0 Hz, $J_{6'e,\ 6'a}$ 3.4 Hz, H-6′a), 4.38–4.31 (m, 2H, H-6e, H-6a), 4.11–4.03 (m, 2H, H-5, H-5′), 3.93 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.7 Hz, H-3), 3.81 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 1.67 (s, 3H, *Me*CO).

Anal. Calcd for $C_{56}H_{46}Cl_3NO_{16}$: C 61.41; H 4.23. Found: C 61.23; H 4.19.

4-Methoxyphenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4, 6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranoside (13). Donor **12** (1.34 g, 1.22

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mmol) and acceptor **6** (965 mg, 1.06 mmol) were coupled as described in the general procedure. The product was purified by chromatography with 2:1 petroleum ether-EtOAc as the eluent to give tetrasaccharide **13** (1.02 g, 51%): $[\alpha]_D$ − 11.2 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.05–6.70 (m, 49H, 8 Bz-*H*, Mp-*H*, Ph-*H*), 5.54 (dd, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 5.51 (dd, 1H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 5.32 (dd, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 5.27 (dd, 1H, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 9.5 Hz, H-2), 5.22 (s, 1H, PhC*H*), 5.11 (dd, 1H, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 9.5 Hz, H-2), 4.95–4.87 (m, 3H, 2 H-1, H-4), 4.79 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.4 Hz, H-2), 4.78 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.4 Hz, H-2), 4.66 (s, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.65 (s, 1H, $J_{1,2}$ 7.8 Hz, H-1), 4.54 (dd, 1H, $J_{5,6e}$ 2.7, $J_{6e,6a}$ 12.2, H-6e), 4.44–4.31(m, 3H, 3 H-6), 4.25 (dd, 1H, $J_{5,6a}$ 3.6, $J_{6e,6a}$ 12.0, H-6a), 4.15–4.07 (m, 3H, H-3, 2 H-6), 3.97–3.80 (m, 3H, 2 H-3, H-6), 3.73 (m, 2H, H-5, OCH_3), 3.68–3.24 (m, 3H, 3 H-5), 2.74 (dd, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 1.99, 1.88, 1.86 (3s, 9H, 3 *Me*CO). ^{13}C NMR (100 MHz, $DCCl_3$): δ 170.6, 169.3, 168.3 (3C, 3 CH_3CO), 166.2, 166.1, 165.7, 165.1, 164.9, 164.8, 164.4, 164.2 (8C, 8 Ph*C*O), 155.6, 151.2 (2C, Mp-1, Mp-4), 136.9, 134.6, 133.7, 133.6, 133.5, 133.4, 133.3, 133.2, 133.1, 132.8, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.2, 128.9, 128.7, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 126.0 (Ph-*C*, some signals overlapped), 118.3, 114.6 (4C, Mp-2, Mp-3, Mp-5, Mp-6), 101.4 (Ph*C*H), 101.2, 100.6, 100.6, 100.2 (4 C-1), 78.8, 78.3, 77.8, 74.4, 73.2, 73.0, 72.6, 72.4, 72.3, 72.0, 71.8, 71.7, 71.4, 71.0, 69.9, 69.7, 69.3, 68.2, 68.0, 66.3, 63.5, 63.2, 62.4, 62.3, 55.8 (C-2 ∼ 6, O*C*H3, some signals overlapped), 21.1, 20.8, 20.7 (3C, CH_3CO).

Anal. Calcd for $C_{100}H_{90}O_{33}$: C 66.00; H 4.98. Found: C 65.89; H 4.90.

4-Methoxyphenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranoside (14). A mixture of **13** (1.02 g, 0.56 mmol) and 90% HOAc-H_2O (40 mL) were refluxed for 2 h, and then concentrated to dryness and co-evaporated with toluene (10 mL) three times. The residue was purified by chromatography with 1:1 petroleum ether-EtOAc as the eluent to give **14** (860 mg, 92%): $[\alpha]_D$ + 19.8 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.25–6.75 (m, 44H, 8 Bz-*H*, Mp-*H*), 5.69 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 5.51 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4), 5.40 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.13–4.87 (m, 5H, 3 H-2, H-3, H-4), 4.75–4.53 (m, 8H, 4 H-1, 4 H-6), 4.45–4.27 (m, 3H, 3 H-6), 4.15–3.87 (m, 5H, 3 H-3, H-4, H-6), 3.74 (s, 3H, OCH_3), 3.61–3.25 (m, 4H, 4 H-5), 2.01, 1.93, 1.87 (3s, 9H, 3 *Me*CO).

Anal. Calcd for $C_{93}H_{86}O_{33}$: C 64.50; H 5.01. Found: C 64.37; H 4.98.

Isopropyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-1-thio-β-D-glucopyranoside (15). A mixture of **10** (5.52 g, 5.43 mmol) and 90% HOAc-H_2O (100 mL) were refluxed for 2 h, and then concentrated to dryness and co-evaporated with toluene (10 mL) three times. The residue was purified by chromatography with 1:1 petroleum ether-EtOAc as the eluent to afford **15** (4.49 g, 90%): $[\alpha]_D$ + 24.5 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.13–7.09 (m, 20H, 4 Bz-*H*), 5.82 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4′), 5.58 (dd, 1H, $J_{2,3} = J_{3,4} = 9.8$ Hz, H-3′), 5.24 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.8 Hz, H-2′), 5.17 (dd, 1H, $J_{1,2}$ 10.9 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.01 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1′), 4.79 (dd, 1H, $J_{5,6'e}$ 12.2 Hz, $J_{6'e,\ 6'a}$ 3.2 Hz, H-6′e), 4.56 (d, 1H, $J_{1,2}$ 10.9 Hz, H-1), 4.40 (dd, 1H, $J_{5,6e}$ 12.0 Hz, $J_{6e,\ 6a}$ 3.2 Hz, H-6e), 4.26–4.20 (m,

2H, H-3, H-6′a), 3.96 (ddd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6e}$ 12.2 Hz, $J_{5,6a}$ 12.0 Hz, H-5′), 3.91 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.7 Hz, H-4), 3.75 (dd, 1H, $J_{5,6a}$ 12.0 Hz, $J_{6e,\ 6a}$ 3.2 Hz, H-6a), 3.43 (ddd, 1H, $J_{4,5}$ 9.7 Hz, $J_{5,6e}$ 12.1 Hz, $J_{5,6a}$ 12.0 Hz, H-5), 3.07 (m, 1H, C*H*$(CH_3)_2$), 1.16 (s, 3H, CH(C*H*$_3)_2$), 1.15 (s, 3H, CH(C*H*$_3)_2$).

Anal. Calcd for $C_{50}H_{48}O_{15}S$: C 65.21; H 5.25. Found: C 65.12; H 5.18.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (18). Acetic anhydride (2 mL, 21.2 mmol) was added to a solution of **15** (4.49 g, 4.89 mmol) in pyridine (10 mL), the mixture was stirred at rt for 3 h, at which time TLC (2:1 petroleum ether-EtOAc) suggested the reaction was finished. The mixture was concentrated and purified by chromatography with 2:1 petroleum ether-EtOAc as the eluent to afford **16** (4.67 g, 95%). To a mixture of **16** (4.51g, 4.42 mmol) and NIS (1.22 g, 5.30 mmol) in CH_2Cl_2 (100 mL) was added TMSOTf (80 μL, 0.44 mmol) dropwise at − 20°C under nitrogen. The reaction mixture was stirred for 3 h, during which time the temperature was gradually raised to ambient temperature. The mixture was neutralized with Et_3N, then concentrated, and the residue was purified by chromatography with 2:1 petroleum ether-EtOAc as the eluent to afford **17**. A mixture of **17**, trichloroacetonitrile (2.0 mL, 9.38 mmol), and DBU (0.25 mL, 2.03 mmol) in dry CH_2Cl_2 (40 mL) was stirred for 3 h and then concentrated. The residue was purified by flash chromatography with 2:1 petroleum ether-EtOAc to give donor **18** (3.90 g, 81% for two steps) as a foamy solid: $[\alpha]_D$ + 43.9 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.51 (s, 1H, CN*H*CCl_3), 8.07–7.05 (m, 25H, 5 Bz-*H*), 6.56 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.80 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3′), 5.84 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.6, H-4′), 5.48 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2′), 5.28 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 5.19 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.0 Hz, H-2), 5.05 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1′), 4.71 (dd, 1H, $J_{5,6'e}$ 12.2 Hz, $J_{6'e,\ 6'a}$ 3.2 Hz, H-6′e), 4.47 (dd, 1H, $J_{5,6'a}$ 12.2 Hz, $J_{6'e,\ 6'a}$ 3.2 Hz, H-6′a), 4.41 (dd, 1H, $J_{2,3} = J_{3,4}$ = 10.0 Hz, H-3), 4.23 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6e} = J_{5,6a}$ = 12.2 Hz, H-5′), 4.20–4.11 (m, 3H, H-5, H-6e, H-6a) , 2.09 (s, 3H, *Me*CO), 2.04 (s, 3 H, *Me*CO).

Anal. Calcd for $C_{53}H_{46}Cl_3NO_{18}$: C 58.33; H 4.25. Found: C 58.11; H 4.21.

4-Methoxyphenyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-glucopyranoside (19). Donor **18** (204 mg, 0.19 mmol) was coupled with acceptor **14** (300 mg, 0.17 mmol) as described in the general procedure. The product was purified by chromatography with 1:1 petroleum ether-EtOAc as the eluent to give hexsaccharide **19** (361 mg, 80%): $[\alpha]_D$ + 25.6 (*c* 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.10–6.74 (m, 69 H, 13 Bz-*H*, Mp-*H*), 5.65–5.40 (m, 5H, 2 H-3, 3 H-4), 5.40 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.0 Hz, H-2), 5.28 (dd, 1H, $J_{1,2}$ 7.7 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.16–4.74 (m, 7H, H-1, 4 H-2, 2 H-4), 4.75–4.48 (m, 9H, 5 H-1, 4 H-6), 4.34–3.97 (m, 12H, 4 H-3, 8 H-6), 3.90 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.3 Hz, H-4), 3.74 (s, 3H, OC*H*$_3$), 3.73–3.01 (m, 6H, 6 H-5), 2.06, 2.01, 2.01, 1.93, 1.78 (5s, 15H, 5 *Me*CO). ^{13}C NMR (100 MHz, $DCCl_3$): δ 170.5, 170.5, 169.3, 169.2, 168.2 (5C, 5 CH_3*C*O), 166.1, 166.0, 165.9, 165.6, 165.5, 165.0, 164.9, 164.8, 164.6, 164.4, 164.1, 164.0, 163.8 (13C, 13 Ph*C*O), 156.7, 150.9 (2C, Mp-1, Mp-4), 133.4, 133.2, 132.7, 130.0, 129.9, 129.8, 129.7, 129.6, 129.5, 129.4, 129.3, 129.2, 129.1, 128.7,

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128.5, 128.4, 128.3, 128.2, 128.0, 127.8 (72C, 12 Bz-*C*, some signals overlapped), 118.3, 114.4 (4C, Mp-2, Mp-3, Mp-5, Mp-6), 101.4, 101.2, 101.1, 100.7, 100.3, 99.9 (6 C-1), 85.9, 85.7, 78.6, 77.8, 77.4, 77.3, 77.1, 76.7, 76.3, 75.1, 73.6, 73.5, 73.4, 72.9, 72.4, 72.1, 71.9, 71.6, 71.1, 69.6, 68.9, 68.7, 68.5, 68.4, 68.3, 68.1, 63.3, 63.1, 62.4, 62.3, 60.3, 55.6 (C-2 ∼ 6, O*C*H$_3$, some signals overlapped).

Anal. Calcd for $C_{144}H_{130}O_{50}$: C 65.01; H 4.93. Found: C 64.75; H 4.89.

4-Methoxyphenyl β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (20). Compound **19** (350 mg, 0.13 mmol) was dissolved in a saturated solution of ammonia in MeOH (10 mL). After two weeks at rt, the reaction solution was concentrated, and the residue was purified on a Biogel P2 column with MeOH-water as the eluent to afford **20** (135 mg, 95%) as an amorphous solid: $[\alpha]_D$ + 21.2 (*c* 1.0, H_2O); ^{1}H NMR (400 MHz , D_2O): 7.00–6.80 (m, 4H, Mp-H), 4.88 (1H, $J_{1,2}$ 7.6 Hz, H-1), 4.70 (1H, $J_{1,2}$ 8.2 Hz, H-1), 4.66 (1H, $J_{1,2}$ 8.0 Hz, H-1), 4.65 (1H, $J_{1,2}$ 8.0 Hz, H-1), 4.60 (1H, $J_{1,2}$ 7.6 Hz, H-1), 4.43 (1H, $J_{1,2}$ 8.0 Hz, H-1), 4.12–3.26 (m, 36H, H-2 ∼ 6). ^{13}C NMR (100 MHz, D_2O): δ 154.7, 150.9 (2C, Mp-1, Mp-4), 118.3, 115.0 (4C, Mp-2, Mp-3, Mp-5, Mp-6), 103.0, 102.8, 102.6, 102.6, 102.6, 101.0 (6C-1, $J_{C\text{-}1,H\text{-}1}$ = 164.2, 165.5, 165.0, 164.7, 164.9, 165.3 Hz), 84.8, 84.6, 84.2, 84.2 (4C, glycosylated C-3), 76.6, 75.7, 75.6, 75.5, 74.5, 73.5, 73.1, 73.0, 72.9, 72.8, 72.7, 69.6, 68.8, 68.2, 68.1, 68.0, 60.8, 60.6 (C-2 ∼ 6, some signals overlapped).

Anal. Calcd for $C_{43}H_{68}O_{32}$: C, 47.08; H, 6.25. Found: C, 47.11; H, 6.21.

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