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Synthesis of heptasaccharide and nonasaccharide analogues of the lentinan repeating unit

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Abstract—The allyl glycoside 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*-(1→6)]-α-D-Glc*p* (**18**) and the acetonyl glycoside of 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*-(1→6)]-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→6)]-α-D-Glc*p* (**28**) were synthesized as analogues of the lentinan heptaose repeating unit. 4,6-*O*-Benzylidenated monosaccharide donor **3** and 4,6-*O*-benzylidenated tetrasaccharide acceptor **14** were used to ensure the β-linkage in the synthesis of **18**, while 4,6-*O*-benzylidenated disaccharide acceptor **20**, and 4,6-*O*-benzylidenated disaccharide donors **21** and **24** were used to ensure the β-linkage in the synthesis of **28**.

Keywords: Trichloroacetimidate; β-(1→6)-Branched β-(1→3)-glucan; Synthesis

1. Introduction

Polysaccharides with antitumor activity separated from fungi 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] Some physicochemical and immunopharmacological investigations showed that the antitumor activity of these glucans may be related to the triple-helix structures of the β-(1→3)-linked backbone chains,[2a,b] and some biological aspects of β-glucans have been reported.[2c–e] 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.[3] However, an interesting result in our research revealed that a synthetic allyl glycoside of 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*-(1→6)]-β-D-Glc*p*-(1→3)-α-D-Glc*p*[4] at a dose of 5 mg/kg effectively inhibited the U_{14} tumor (58.4%).[5] Encouraged by the bioassay results, we are trying to synthesize more structurally diverse 3,6-branched glucans and investigate structure–activity relationships. The major structure of lentinan consists of a glucoheptaose repeating unit as shown in Figure 1, and its synthesis was reported by our group.[4]

Heptaoside analogues with the β-(1→3)-linked pentaose backbone and C-6, C-6‴ branches, and C-6 disaccharide branch were also synthesized.[6] We present

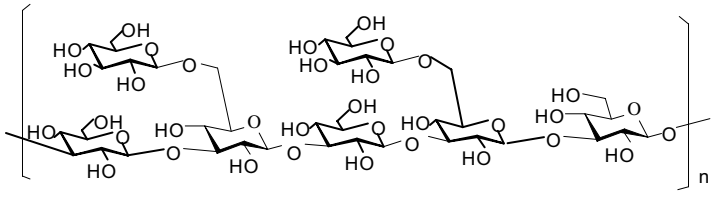

Figure 1. Structure of lentinan.

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herein the syntheses of allyl glucoheptaoside **18** consisting of the β-(1→3)-linked pentaose backbone with β-(1→6)-linked glucosyl side chains attached at C-6 and C-6″ of the backbone, and acetonyl glucononaoside **28** consisting of a β-(1→3)-linked hexaose backbone with glucosyl side chains linked at C-6, C-6″, and C-6‴, respectively.

2. Results and discussion

Scheme 1 shows the synthesis of the glucoheptaoside **18**. Allyl 3-*O*-chloroacetyl-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside[4] (**1**), allyl 4,6-di-*O*-acetyl-3-*O*-chloroacetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside[4] (**4**), and 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyltrichloroacetimidate[7] (**9**) were used as the key synthons. Deallylation of **1** with $PdCl_2$ in acetic acid containing sodium acetate gave **2**, and the benzylidene group was not affected under the mild conditions. Subsequent trichloroacetimidate formation with trichloroacetonitrile in the presence of DBU afforded the glycosyl donor **3**. Meanwhile, removal of the benzylidene group of **4** in glycol–acetonitrile with toluenesulfonic acid furnished the diol acceptor **5**, while subsequent acetylation of **5**, deallylation, and trichloroacetimidate formation produced the disaccharide donor **8**. Condensation of the diol **5** with **9** selectively yielded (1→6)-linked trisaccharide **10**, then acetylation and dechloroacetylation with thiourea gave the trisaccharide acceptor **12**. Consequently, coupling of **12** with the donor **3**, followed by dechloroacetylation, furnished the tetrasaccharide acceptor **14**, and subsequent coupling of **14** with the disaccharide donor **8** gave the hexasaccharide **15**. Debenzylidenation of **15** followed by selective coupling with **9** afforded the heptasaccharide **17**. Finally, deprotection produced the target heptaoside **18**.

Scheme 2 shows the synthesis of glucononaoside **28**. First, acetonyl 4,6-di-*O*-acetyl-3-*O*-chloroacetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**19**) was obtained by oxidation of the corresponding allyl glycoside of disaccharide **4** with $PdCl_2$. Actually, our original goal was to remove the allyl group of **4** to obtain the corresponding hemiacetal. However, it was found that treatment of **4** in tetrahydrofuran, which was not purged with nitrogen to remove the air, with $PdCl_2$ gave **19** and the hemiacetal in almost equal amount, and the two products were easily separated. Similar oxidation of the allyl group to the acetonyl group was reported many years ago.[8] The disaccharide acceptor **20** was readily obtained by dechloroacetylation of **19** with thiourea. Condensation of **20** with 4,6-di-*O*-acetyl-3-*O*-chloroacetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranosyl trichloroacetimidate[4] (**21**), followed by dechloroacetylation, afforded the tetrasaccharide acceptor **23**. Subsequent coupling of **23** with the disaccharide donor **24**[6] produced the hexasaccharide **25** with benzylidene groups at the 4,6-, 4″,6″-, and 4‴,6‴-positions, respectively. Removal of the benzylidene groups of **25**, followed by selective coupling with **9**, and then deacylation, gave the target nonaoside **28**.

In summary, with benzylidenated monosaccharide and disaccharide donors, and benzylidenated oligosaccharide acceptors, β-(1→3)-linked glucans were readily obtained, and the formation[9] of α-linkages was restrained.

Preliminary bioassays for **18** and **28** revealed that **18** had activity similar to the lentinan heptaoside,[4,5] while **28** showed even better activity.

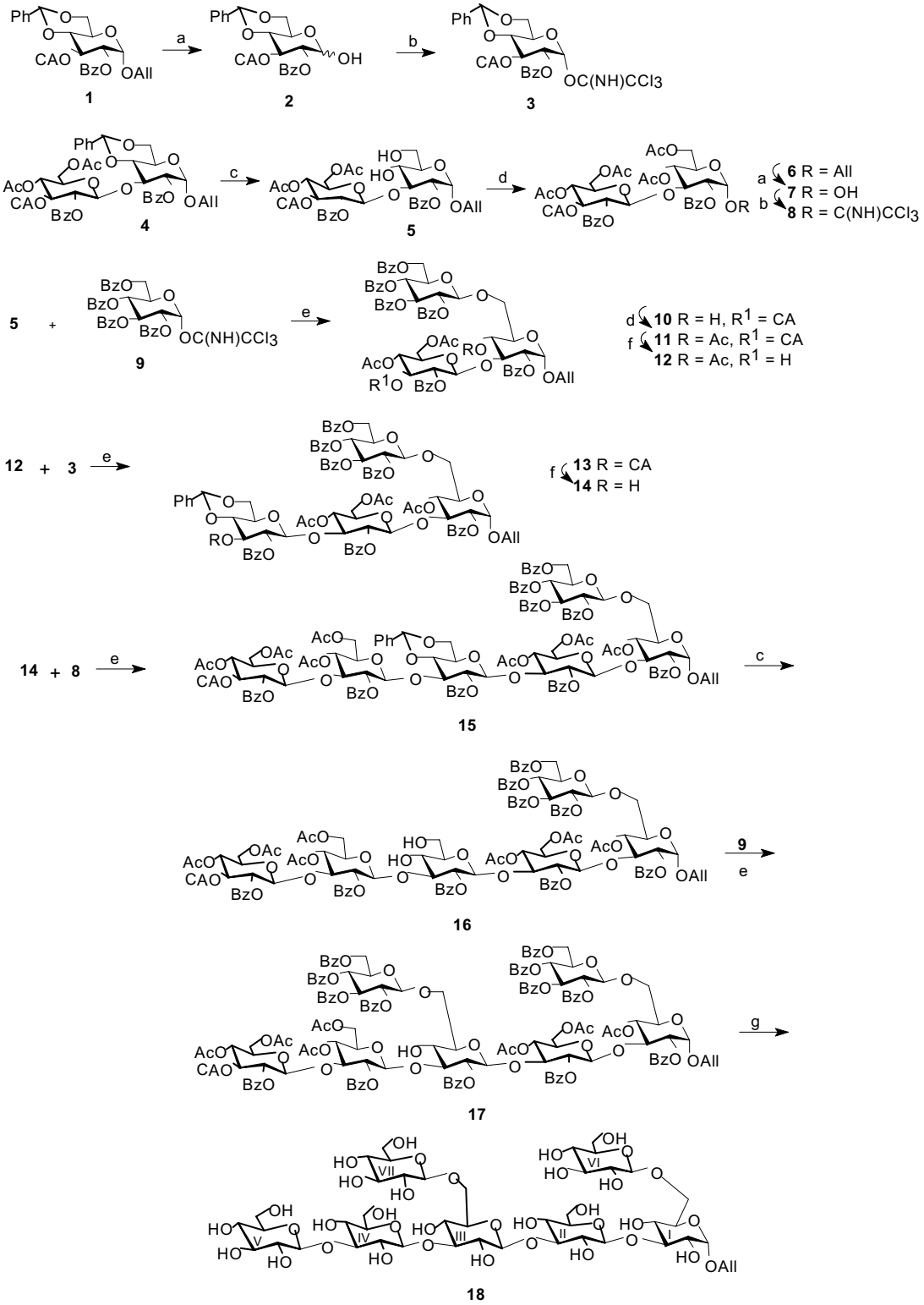
3. Experimental

3.1. General methods

Melting points were determined with a 'Mel-Temp' apparatus. Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter for solutions in a 1 dm, jacketed cell. ^1H NMR and ^{13}C NMR spectra were recorded with Varian XL-400 spectrometers, for solutions in $CDCl_3$ or in D_2O as indicated, and individual resonances could not be identified with the specific sugar residues using 1D techniques. 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 ESI mode. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) sulfuric acid in methanol or by UV detection. Column chromatography was conducted by elution of a column (8×100 mm, 16×240 mm, 18×300 mm, 35×400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (bp 60–90 °C) as the eluent. Analytical LC was performed with a Gilson HPLC consisting of a pump (model 306), stainless steel column packed with silica gel (Spherisorb SiO_2, 10×300 mm or 4.6×250 mm), differential refractometer (132-RI Detector), UV/vis detector (model 118). EtOAc–petroleum ether (bp 60–90 °C) was used as the eluent at a flow rate of 1–4 mL/min. Solutions were concentrated at a temperature <60 °C under diminished pressure.

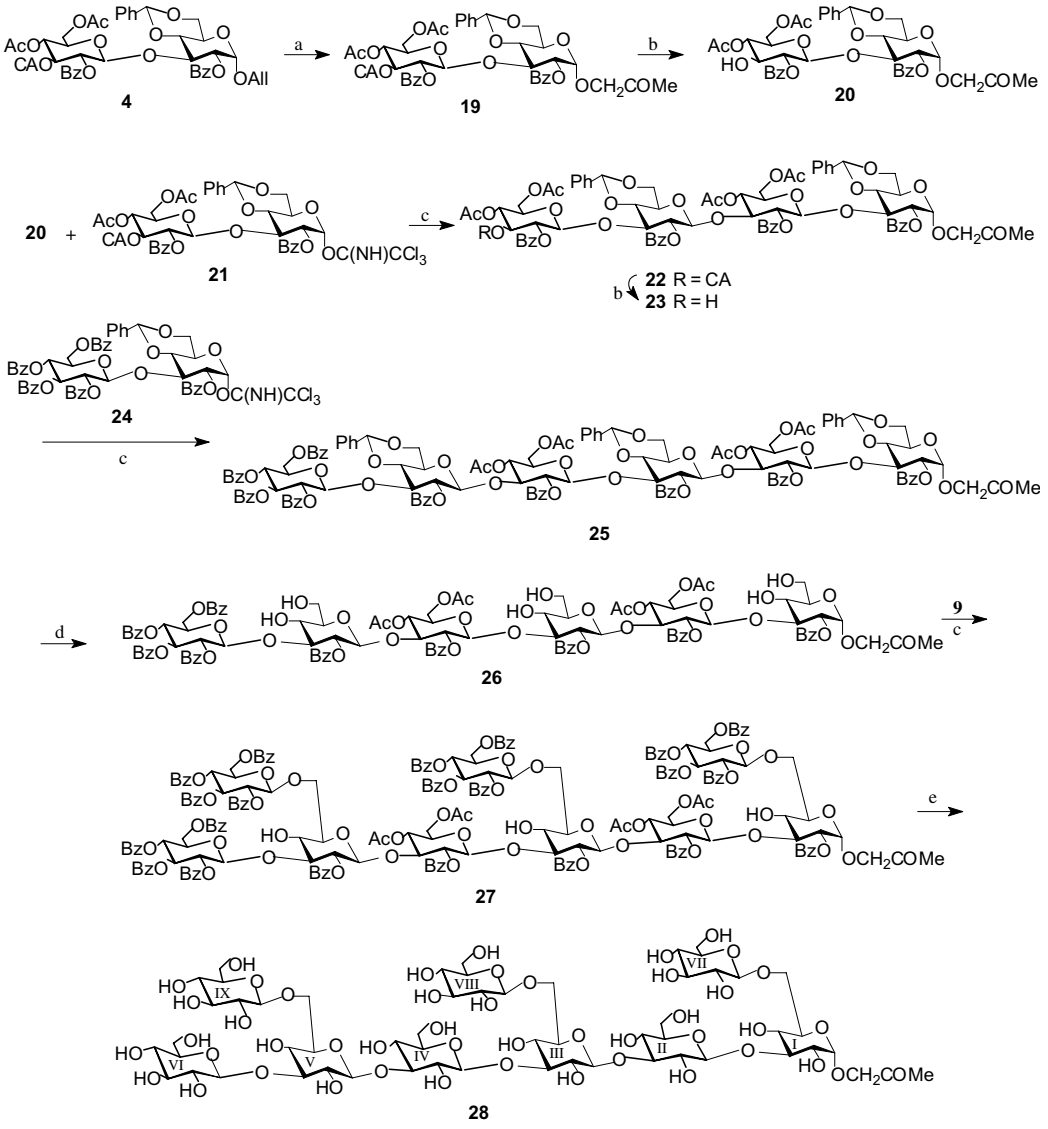
3.2. General procedure for trichloroacetimidate formation

To a solution of the hemiacetal (0.4–2.2 mmol) in dry CH_2Cl_2 (10–25 mL) were added CCl_3CN (3 equiv) and DBU (10–55 μL). The mixture was stirred at rt for 2 h, at the end of which time TLC (3:1 petroleum ether–



Scheme 1. Reagents and conditions: (a) $PdCl_2$, 75% HOAc (75%)–NaOAc (or MeOH), rt, 80% for **2**, 82% for **7**; (b) DBU, Cl_3CCN, CH_2Cl_2, rt, 82% for **3**, 87% for **8**; (c) $C_2H_4(OH)_2$, MeCN, TsOH, rt, 91% for **5**, 79% for **16**; (d) Ac_2O, Pyr, (or AcCl, Pyr, CH_2Cl_2), rt, 98% for **6**, 90% for **11**; (e) TMSOTf, CH_2Cl_2, −10 °C, 4 h, 91% for **10**, 79% for **13**, 75% for **15**, 80% for **17**; (f) thiourea, 2,4-lutidine, CH_2Cl_2–MeOH, reflux, overnight, 89% for **12**, 71% for **14**; (g) satd NH_3–MeOH, rt, 48 h, 98%.

EtOAc) indicated that the reaction was complete. The mixture was concentrated, and the residue so obtained was subjected to column chromatography with 3:1 petroleum ether–EtOAc as the eluent to give the product.



Scheme 2. Reagents and conditions: (a) $PdCl_2$, THF, HOAc (75%)–NaOAc, rt, overnight, 36%; (b) thiourea, 2,4-lutidine, CH_2Cl_2–MeOH, reflux, overnight, 87% for **20**, 81% for **23**,; (c) TMSOTf, CH_2Cl_2, −10 °C, 2–4 h; 71% for **22**, 50% for **25**, 55% for **27**; (d) TsOH, $CH_2(OH)_2$, MeCN, rt, overnight, 69%; (e) satd NH_3–MeOH, rt, 48 h, 98%.

3.3. General procedure for the coupling reaction

To a solution of almost equivalent amounts of glycosyl acceptor and donor in CH_2Cl_2 (15–25 mL) was added 4 Å molecular sieves (0.5–0.8 g). The mixture was stirred and cooled to −10 °C under N_2 protection, and TMSOTf (5–10% equiv) was added. Stirring was continued at low temperature (−10 °C) for 4 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. Et_3N was added to stop the reaction, the mixture was filtered, and the filter cake was washed with CH_2Cl_2. The combined filtrate and washings were washed with satd aq $NaHCO_3$ and water, then dried and concentrated. Purification by column chromatography with 3:1 petroleum ether–EtOAc as the eluent afforded the product.

3.4. 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-α-D-glucopyranose (2)

To a solution of NaOAc (980 mg, 11.95 mmol) and $PdCl_2$ (1.06 g, 5.98 mmol) in HOAc (75%, 5 mL) was added compound **1** (1.5 g, 3.07 mmol). The mixture was stirred at rt overnight under the N_2 protection, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, and the filter cake was washed with CH_2Cl_2. The organic phase was washed with satd aq $NaHCO_3$ and water, then dried and concentrated. Purification by column chromatography with 2:1 petroleum ether–EtOAc as the eluent afforded compound **2** (1.1 g, 80%) as a syrup: $[\alpha]_D^{20}$ +90.0 (*c* 0.7, $CHCl_3$); ^1H NMR: δ 8.04–7.36 (m, 10H, Bz–*H*, Ph–*H*), 5.90 (dd, 1H, $J_{2,3}$

9.8 Hz, $J_{3,4}$ 9.8 Hz, H-3), 5.65 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.55 (s, 1H, PhC*H*), 5.12 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.8 Hz, H-2), 4.33 (dd, 1H, $J_{5,6a}$ 4.9 Hz, $J_{6a,6b}$ 10.1 Hz, H-6a), 4.33–4.23 (m, 1H, H-5), 4.02, 3.97 (ABq, 2H, *J* 14.7 Hz, ClCH_2CO), 3.81 (dd, 1H, $J_{5,6b}$ 10.1 Hz, $J_{6a,6b}$ 10.1 Hz, H-6b), 3.80 (dd, 1H, $J_{3,4}$ 9.8 Hz, $J_{4,5}$ 9.8 Hz, H-4). Anal. Calcd for $C_{22}H_{21}ClO_8$: C, 58.87; H, 4.72. Found: C, 58.76; H, 4.64.

3.5. 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-α-D-glucopyranosyl trichloroacetimidate (3)

Compound **3** was obtained as a syrup (1.09 g, 82.5%) from compound **2** (1.0 g, 2.23 mmol), CCl_3CN (0.66 mL, 6.6 mmol), and DBU (55 μL) according to the general procedure for trichloroacetimidate formation: $[\alpha]_D^{20}$ +78.0 (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 8.60 (s, 1H, N*H*), 8.01–7.37 (m, 10H, Bz–*H*, Ph–*H*), 6.71 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 5.94 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.8 Hz, H-3), 5.59 (s, 1H, PhC*H*), 5.40 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.8 Hz, H-2), 4.41 (dd, 1H, $J_{5,6a}$ 4.9 Hz, $J_{6a,6b}$ 10.4 Hz, H-6a), 4.25–4.18 (m, 1H, H-5), 4.03, 4.00 (ABq, 2H, *J* 14.7 Hz, ClCH_2CO), 3.93 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 3.85 (dd, 1H, $J_{5,6}$ 10.4 Hz, $J_{6a,6b}$ 10.4 Hz, H-6). Anal. Calcd for $C_{24}H_{21}Cl_4NO_8$: C, 48.59; H, 3.57. Found: C, 48.76; H, 3.62.

3.6. Allyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-α-D-glucopyranoside (5)

To a solution of compounds **4** (2 g, 2.38 mmol) and ethylene glycol (1.0 mL, 17.97 mmol) in MeCN (20 mL) was added *p*-toluenesulfonic acid·H_2O (50 mg). The mixture was stirred at rt overnight. TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was neutralized with Et_3N, concentrated and extracted with CH_2Cl_2, and the organic phase was washed with water, then dried and concentrated. Purification by column chromatography with 1:1 petroleum ether–EtOAc as the eluent afforded compound **5** (1.63 g, 91%) as a syrup: $[\alpha]_D^{20}$ +87.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.71–7.01 (m, 10H, 2Bz–*H*), 5.78–5.68 (m, 1H, CH_2=C*H*–CH_2–), 5.44 (dd, 1H, $J_{3,4} = J_{2,3}$ = 9.6 Hz, H-3′), 5.31 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.6 Hz, H-2′), 5.20–5.05 (m, 2H, CH_2=CH–CH_2–), 5.17 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4′), 5.10 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.90 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.8 Hz, H-2), 4.88 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1′), 4.28 (d, 2H, $J_{5,6}$ 4.0 Hz, H-6′), 4.16–3.76 (m, 6H, CH_2=CH–CH_2–, H-5, 5′, 6), 3.85, 3.78 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 3.94 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.8 Hz, H-3), 3.71 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4), 2.13, 2.05 (2s, 6H, 2CH_3CO). Anal. Calcd for $C_{35}H_{39}ClO_{16}$: C, 55.96; H, 5.23. Found: C, 55.84; H, 5.12.

3.7. Allyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranoside (6)

To a solution of compound **5** (600 mg, 0.80 mmol) in CH_2Cl_2 (10 mL) was added pyridine (4 mL) and Ac_2O (3 mL). The mixture was stirred at rt for 4 h, at which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. Concentration of the mixture in vacuo and purification the residue by column chromatography with 3:1 petroleum ether–EtOAc as the eluent afforded compound **6** (654 mg, 98%) as a syrup: $[\alpha]_D^{20}$ +55.9 (*c* 0.9, $CHCl_3$); ^{1}H NMR: δ 7.89–7.07 (m, 10H, 2Bz–*H*), 5.80–5.70 (m, 1H, CH_2=C*H*–CH_2–), 5.32 (dd, 1H, $J_{3,4} = J_{2,3}$ = 9.5 Hz, H-3′), 5.23–5.17 (m, 3H, CH_2=CH–CH_2–, H-2′), 5.15 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.12 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.8 Hz, H-4′), 5.09 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.91 (d, 1H, $J_{1,2}$ 7.7 Hz, H-1′), 4.88 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.8 Hz, H-2), 4.47 (dd, 1H, $J_{5,6a}$ = 4.2 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.36 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.8 Hz, H-3), 4.21 (dd, 1H, $J_{5,6b}$ 4.5 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b), 4.15–3.82 (m, 6H, CH_2=CH–CH_2–, H-5, 5′, 6′), 3.84, 3.78 (ABq, 2H, *J* 14.9 Hz, ClCH_2CO), 2.11, 2.11, 2.07, 2.04 (4s, 12H, 4CH_3CO). Anal. Calcd for $C_{39}H_{43}ClO_{18}$: C, 56.08; H, 5.19. Found: C, 56.16; H, 5.32.

3.8. 4,6-Di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranose (7)

To a solution of compound **6** (550 mg, 0.66 mmol) in MeOH (10 mL) was added $PdCl_2$ (20 mg, 0.11 mmol), and the mixture was stirred at rt for 8 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, the filter cake was washed with CH_2Cl_2, and the combined filtrate and washings was concentrated. Purification by column chromatography with 2:1 petroleum ether–EtOAc as the eluent afforded compound **7** (430 mg, 82%) as a syrup: $[\alpha]_D^{20}$ +30.8 (*c* 0.7, $CHCl_3$); ^{1}H NMR: δ 7.90–7.08 (m, 10H, 2Bz–*H*), 5.54 (br s, 1H, H-1), 5.33 (dd, 1H, $J_{3,4} = J_{2,3}$ = 9.5 Hz, H-3′), 5.22 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.5 Hz, H-2′), 5.17 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.6 Hz, H-4′), 5.09 (dd, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 4.93 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1′), 4.88 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.47 (dd, 1H, $J_{5,6a}$ 4.2 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.41 (dd, 1H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3), 4.25–4.20 (m, 1H, H-5′), 4.17 (d, 2H, $J_{5,6}$ 3.3 Hz, H-6′), 4.13 (dd, 1H, $J_{5,6b}$ 2.1 Hz, $J_{6a,6b}$ 12.5 Hz, H-6b), 3.87–3.84 (m, 1H, H-5), 3.85, 3.78 (ABq, 2H, *J* 14.9 Hz, ClCH_2CO), 2.11, 2.11, 2.08, 2.04 (4s, 12H, 4CH_3CO). Anal. Calcd for $C_{36}H_{39}ClO_{18}$: C, 54.38; H, 4.94. Found: C, 54.22; H, 4.78.

3.9. 4,6-Di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (8)

Compound **8** was obtained as a syrup (340 mg, 87%) from compound **7** (330 mg, 0.42 mmol), CCl_3CN (0.12 mL, 1.22 mmol), and DBU (10 μL) according to the general procedure for trichloroacetimidate formation: $[\alpha]_D^{20}$ +54.3 (*c* 1.5, $CHCl_3$); ^{1}H NMR: δ 8.54 (s, 1H, N*H*), 7.80–7.12 (m, 10H, 2Bz–*H*), 6.55 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.31 (dd, 1H, $J_{3,4} = J_{2,3} = 9.4$ Hz, H-3′), 5.24–5.12 (m, 4H, H-2, 2′, 4, 4′), 4.87 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1′), 4.44 (dd, 1H, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 12.5 Hz, H-6a), 4.38 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3), 4.23–3.85 (m, 5H, H-5, 5′, 6b, 6′), 3.82, 3.76 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 2.11, 2.08, 2.07, 2.02 (4s, 12H, 4CH_3CO). Anal. Calcd for $C_{38}H_{39}Cl_4NO_{18}$: C, 48.58; H, 4.18. Found: C, 48.26; H, 4.25.

3.10. Allyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2-*O*-benzoyl-α-D-glucopyranoside (10)

Compound **10** (806 mg, 91%) was obtained as an amorphous solid from compounds **5** (500 mg, 0.67 mmol) and **9** (600 mg, 0.80 mmol) according to the general procedure for the coupling reaction: $[\alpha]_D^{20}$ +49.9 (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 8.04–6.98 (m, 30H, 6Bz–*H*), 5.93 (dd, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3″), 5.70 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4″), 5.57 (dd, 1H, $J_{1,2}$ 7.9 Hz, $J_{2,3}$ 9.6 Hz, H-2″), 5.56–5.46 (m, 1H, CH_2=CH–CH_2–), 5.39 (dd, 1H, $J_{3,4} = J_{2,3} = 9.6$ Hz, H-3′), 5.25 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2′), 5.13 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4′), 5.06–4.93 (m, 2H, CH_2=CH–CH_2–), 4.97 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1′), 4.84 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.78 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1″), 4.64 (dd, 1H, $J_{5,6a}$ 2.8 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a″), 4.63 (dd, 1H, $J_{1,2}$ 3.8 Hz, $J_{2,3}$ 9.5 Hz, H-2), 4.51 (dd, 1H, $J_{5,6b}$ 4.6 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b″), 4.31 (bd, 1H, $J_{6a,6b}$ 11.6 Hz, H-6a), 4.22 (d, 2H, $J_{5,6}$ 4.0 Hz, H-6′), 4.22–3.72 (m, 5H, CH_2=CH–CH_2–, H-5, 5′, 5″), 3.99 (dd, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.85, 3.78 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 3.54 (dd, 1H, $J_{5,6b}$ 4.7 Hz, $J_{6a,6b}$ 11.6 Hz, H-6b), 3.44 (dd, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4), 2.11, 2.06 (2s, 6H, 2CH_3CO). Anal. Calcd for $C_{69}H_{65}ClO_{25}$: C, 62.32; H, 4.93. Found: C, 62.16; H, 4.80.

3.11. Allyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-4-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranoside (11)

To a solution of compound **10** (700 mg, 0.53 mmol) in CH_2Cl_2 (10 mL) containing pyridine (0.4 mL) was cooled −10 °C, was added a solution of AcCl (0.2 mL, 2.80 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred for 4 h at rt. TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was diluted with CH_2Cl_2 and poured into ice water, then the organic phase was separated, successively washed with N HCl, satd aq $NaHCO_3$, and water, dried, and concentrated. Purification by column chromatography with 3:1 petroleum ether–EtOAc as the eluent afforded compound **11** (650 mg, 90%) as an amorphous solid: $[\alpha]_D^{20}$ +31.9 (*c* 0.5, $CHCl_3$); ^{1}H NMR: δ 8.04–7.07 (m, 30H, 6Bz–*H*), 5.90 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3″), 5.67 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4″), 5.53 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.7 Hz, H-2″), 5.52–5.42 (m, 1H, CH_2=C*H*–CH_2–), 5.27 (dd, 1H, $J_{3,4} = J_{2',3'} = 9.4$ Hz, H-3′), 5.19 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.4 Hz, H-2′), 5.14 (dd, 1H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4′), 5.03–4.93 (m, 2H, CH_2=CH–CH_2–), 4.90 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1′), 4.84 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1″), 4.80 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.79 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4), 4.70 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.5 Hz, H-2), 4.63 (dd, 1H, $J_{5,6a}$ 2.8 Hz, $J_{6a,6b} = 12.2$ Hz, H-6a″), 4.48 (dd, 1H, $J_{5,6b}$ 4.5 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b″), 4.46–3.37 (m, 9H, CH_2=CH–CH_2–, H-5, 5′, 5″, 6, 6′), 4.27 (dd, 1H, $J_{2,3} = J_{3,4} = 9.8$ Hz, H-3), 3.83, 3.76 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 2.08, 2.02, 2.00 (3s, 9H, 3CH_3CO). Anal. Calcd for $C_{71}H_{67}ClO_{26}$: C, 62.16; H, 4.92. Found: C, 61.86; H, 5.02.

3.12. Allyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-4-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranoside (12)

Compound **10** (550 mg, 0.40 mmol) was dissolved in mixed solvents of CH_2Cl_2 (4 mL) and MeOH (6 mL). To the solution were added thiourea (152 mg, 2.00 mmol) and 2,4-lutidine (45 μL, 0.41 mmol), and the reaction mixture was boiled under reflux for 16 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated and extracted with CH_2Cl_2, the organic phase was successively washed with N HCl, satd aq $NaHCO_3$, and water, dried, and concentrated. Purification by column chromatography with 2:1 petroleum ether–EtOAc as the eluent afforded **12** (460 mg, 89%) as an amorphous solid: $[\alpha]_D^{20}$ +41.9 (*c* 1.5, $CHCl_3$); ^{1}H NMR: δ 8.03–7.16 (m, 30H, 6Bz–*H*), 5.90 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3″), 5.67 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4″), 5.53 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.7 Hz, H-2″), 5.55–5.45 (m, 1H, CH_2=C*H*–CH_2–), 5.06–4.98 (m, 4H, CH_2=CH–CH_2–, H-2′, 4′), 4.92 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1′), 4.87 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 4.83 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1″), 4.77 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4), 4.74 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.6 Hz, H-2), 4.64 (dd, 1H, $J_{5,6a}$ 2.9 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a″), 4.48 (dd, 1H, $J_{5,6b}$ 4.7 Hz, $J_{6a,6b}$ 12.3 Hz,

H-6b″), 4.45–3.42 (m, 9H, CH_2=CH–CH_2–, H-5, 5′, 5″, 6, 6′), 4.30 (dd, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 3.70 (dd, 1H, $J_{2,3} = J_{3,4} = 9.4$ Hz, H-3′), 2.09, 2.09, 2.01, 2.00 (3s, 9H, 3CH_3CO). Anal. Calcd for $C_{69}H_{66}O_{25}$: C, 63.98; H, 5.14. Found: C, 63.76; H, 5.12.

3.13. Allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-4-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranoside (13)

Compound **13** (380 mg, 79%) was obtained as an amorphous solid from compounds **12** (360 mg, 0.28 mmol) and **3** (200 mg, 0.34 mmol) according to the general procedure for the coupling reaction: $[\alpha]_D^{20}$ +13.5 (*c* 0.5, $CHCl_3$); ^{1}H NMR: δ 8.10–7.00 (m, 40H, 7Bz–*H*, Ph–*H*), 5.88 (dd, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3^{IV}), 5.65 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4^{IV}), 5.50 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2^{IV}), 5.47 (s, 1H, PhC*H*), 5.48–5.38 (m, 1H, CH_2=C*H*–CH_2–), 5.21 (dd, 1H, $J_{2,3} = J_{3,4} = 9.2$ Hz, H-3^{III}), 5.07 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.6 Hz, H-2^{II}), 5.02 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4^{II}), 5.02 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.2 Hz, H-2^{III}), 4.99–4.80 (m, 2H, CH_2=CH–CH_2–), 4.87 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1^{IV}), 4.70–4.60 (m, 5H, H-1$^{I–III}$, 2, 4), 4.56 (dd, 1H, $J_{5,6}$ 3.1 Hz, $J_{6,6}$ 12.4 Hz, H-6^{IV}), 4.47 (dd, 1H, $J_{5,6}$ 4.7 Hz, $J_{6,6'}$ 12.4 Hz, H-6′^{IV}), 4.38 (dd, 1H, $J_{5,6}$ 4.9 Hz, $J_{6,6}$ 10.2 Hz, H-6^{III}), 4.33 (dd, 1H, $J_{5,6}$ 4.2 Hz, $J_{6,6'}$ 12.4 Hz, H-6^{II}), 4.20–3.31 (m, 13H, CH_2=CH–CH_2–, H-3^{I,II}, 4^{III}, 5$^{I–V}$, 6^{I}, 6′^{I,II,III}), 3.83, 3.76 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 2.08, 2.02, 1.94 (3s, 12H, 3CH_3CO). Anal. Calcd for $C_{91}H_{85}ClO_{32}$: C, 63.32; H, 4.96. Found: C, 63.26; H, 5.00.

3.14. Allyl 2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-4-*O*-acetyl-2-O-benzoyl-α-D-glucopyranoside (14)

Compound **13** (280 mg, 0.16 mmol) was dissolved in mixed solvents of CH_2Cl_2 (4 mL) and MeOH (6 mL). To the solution were added thiourea (62 mg, 0.82 mmol) and 2,4-lutidine (18 μL, 0.16 mmol), and the reaction mixture was boiled under reflux for 16 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated and extracted with CH_2Cl_2, the organic phase was washed with N HCl, satd aq $NaHCO_3$, and water, dried, and concentrated. Purification by column chromatography with 2:1 petroleum ether–EtOAc as the eluent afforded **14** (190 mg, 71%) as an amorphous solid: $[\alpha]_D^{20}$ +14.7 (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 8.02–7.30 (m, 40H, 7Bz–*H*, Ph–*H*), 5.88 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3^{IV}), 5.66 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4^{IV}), 5.51 (s, 1H, PhC*H*), 5.51 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.7 Hz, H-2^{IV}), 5.50–5.40 (m, 1H, CH_2=C*H*–CH_2–), 5.10 (dd, 1H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4^{II}), 5.04–4.87 (m, 4H, CH_2=CH–CH_2–, H-1^{I}, 2^{II}), 4.88 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1^{IV}), 4.73–4.55 (m, 6H, H-1^{II,III}, 2^{I,III}, 4, 6^{IV}), 4.50–3.33 (m, 17H, CH_2=CH–CH_2–, H-3$^{I–III}$, 4^{III}, 5$^{I–IV}$, 6′^{IV}, 6$^{I–III}$), 2.07, 2.01, 1.94 (3s, 12H, 3CH_3CO). Anal. Calcd for $C_{89}H_{84}O_{31}$: C, 64.80; H, 5.13. Found: C, 64.76; H, 5.02.

3.15. Allyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-4-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranoside (15)

Compound **15** (110 mg, 75%) was obtained as an amorphous solid from compounds **14** (100 mg, 0.061 mmol) and **8** (75 mg, 0.080 mmol) according to the general procedure for the coupling reaction. However, purification was carried out with 2:1 petroleum ether–EtOAc as the eluent: $[\alpha]_D^{20}$ +66.0 (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 8.02–7.05 (m, 50H, 9Bz–*H*, Ph–*H*), 5.87 (dd, 1H, $J_{2,3} = J_{3,4} = 9.8$ Hz, H-3^{VI}), 5.65 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4^{VI}), 5.50 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.8 Hz, H-2^{VI}), 5.47–5.37 (m, 3H, CH_2=C*H*–CH_2–, PhC*H*, H-3^{V}), 5.07–4.78 (m, 13H, CH_2=CH–CH_2–, H-1^{I,VI}, 2$^{I–V}$, 4^{I,II,IV,V}), 4.68–3.18 (m, 29H, H-1$^{II–V}$, 3$^{I–IV}$, 4^{III}, 5$^{I–VI}$, 6$^{I–VI}$), 3.77, 3.71 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 2.05, 2.01, 1.96, 1.91, 1.91, 1.91, 1.91 (7s, 21H, 7CH_3CO). ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.7, 169.2, 169.1, 168.4, 168.2, 168.1 (7C, 7CH_3*C*O–), 166.2, 166.1, 165.9, 165.7, 165.3, 165.2, 165.0, 164.9, 164.7 (9C, 9*C*OPh), 134.3 (1C, CH_2=*C*H–CH_2), 118.1 (1 C, *C*H_2=CH–CH_2), 101.2 (–*C*HPh), 101.0, 100.8, 100.7, 100.6, 99.9 (5C, βC-1), 95.8 (1C, αC-1). Anal. Calcd for $C_{125}H_{121}ClO_{48}$: C, 61.86; H, 5.03. Found: C, 61.95; H, 5.08.

3.16. Allyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-4-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranoside (16)

To a solution of compounds **15** (70 mg, 0.029 mmol) and ethylene glycol (0.1 mL, 1.80 mmol) in MeCN (5 mL) was added *p*-toluenesulfonic acid·H_2O (10 mg). The mixture was stirred at rt overnight, at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was made neutral with Et_3N, concentrated and extracted with CH_2Cl_2 and the organic phase was washed with water, then

dried and concentrated. Purification by column chromatography with 1:1 petroleum ether–EtOAc as the eluent afforded compound **16** (53 mg, 79%) as an amorphous solid: $[\alpha]_D^{20}$ +6.4 (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 8.01–7.11 (m, 45H, 9Bz-*H*), 5.87 (dd, 1H, $J_{2,3} = J_{3,4} = 9.7$ Hz, H-3^{VI}), 5.65 (dd, 1H, $J_{3,4} = J_{4,5} = 9.7$ Hz, H-4^{VI}), 5.49 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.7 Hz, H-2^{VI}), 5.46–5.37 (m, 1H, CH_2=C*H*–CH_2–), 5.09–3.14 (m, 43H, CH_2=CH–CH_2–, H-$1^{I–VI}$, $2^{II–V}$, $3^{I–V}$, $4^{I–V}$, $5^{I–VI}$, $6^{I–VI}$), 3.77, 3.71 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 2.06, 2.05, 2.03, 1.98, 1.97, 1.90, 1.84 (7s, 21H, 7CH_3CO). Anal. Calcd for $C_{118}H_{117}ClO_{48}$: C, 60.60; H, 5.04. Found: C, 60.72; H, 5.12.

3.17. Allyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-4-*O*-acetyl-2-*O*-benzoyl-α-D-glucopyranoside (17)

Compound **17** (30 mg, 80%) was obtained as an amorphous solid from compounds **16** (30 mg, 0.013 mmol) and **9** (12 mg, 0.16 mmol) according to the general procedure for the coupling reaction. However, purification was carried out with 1:1 petroleum ether–EtOAc as the eluent: $[\alpha]_D^{20}$ +19.8 (*c* 0.1, $CHCl_3$); ^{1}H NMR: δ 8.02–7.08 (m, 65H, 13Bz–*H*), 5.93, 5.87 (dd, 2H, $J_{2,3} = J_{3,4} = 9.8$ Hz, H-$3^{VI,VII}$), 5.74, 5.64 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-$4^{VI,VII}$), 5.57–5.35 (m, 3H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.8 Hz, CH_2=C*H*–CH_2–, H-$2^{VI,VII}$), 5.16–3.18 (m, 47H, CH_2=CH–CH_2–, H-$1^{I–VII}$, $2^{I–V}$, $3^{I–V}$, $4^{I–V}$, $5^{I–VII}$, $6^{I–VII}$), 3.72, 3.66 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 2.05, 2.04, 2.00, 1.99, 1.96, 1.86, 1.77 (7s, 21H, 7CH_3CO). ^{13}C NMR: δ 170.6, 170.6, 170.4, 170.2, 169.8, 169.3, 169.2 (7C, 7CH_3*C*O), 166.1, 165.9, 165.7, 165.5, 165.2, 165.2, 165.1, 164.9, 164.8, 164.7, 163.7, 163.6, 163.4 (13C, 13Bz*C*O), 117.30 (s, 1C, *C*H_2=CH–CH_2), 102.0, 101.6, 101.3, 101.2, 100.8, 100.6 (6C, β-C-1), 94.3 (1C, α-C-1), 85.4, 84.5, 83.4, 82.3 (4C, 4C-3). Anal. Calcd for $C_{152}H_{143}ClO_{57}$: C, 62.58; H, 4.94. Found: C, 62.72; H, 5.02.

3.18. Allyl 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-glucopyranosyl-(1→6)]-α-D-glucopyranoside (18)

Satd NH_3 in MeOH (5 mL) was added to compound **17** (30 mg, 10.3 μmol) in MeOH (4 mL). After 48 h at rt, the reaction mixture was concentrated, and the residue was purified by Sephadex LH-20 chromatography (MeOH) to afford **18** (12 mg, 98%) as an amorphous solid: $[\alpha]_D^{20}$ +2.0 (*c* 0.4, H_2O); ^{1}H NMR (D_2O): δ 6.05–5.95 (m, 1H, CH_2=C*H*–CH_2–), 5.40–5.24 (m, 2H, CH_2=CH–CH_2–), 4.96 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^I), 4.84, 4.81, 4.75, 4.74 (4d, 4H, $J_{1,2}$ 8.0 Hz, H-1), 4.51, 4.48 (2d, 2H, $J_{1,2}$ 8.0 Hz, H-1), 4.28–3.28 (m, 44H, CH_2=CH–CH_2–, H-$2^{I–VII}$, $3^{I–VII}$, $4^{I–VII}$, $5^{I–VII}$, $6^{I–VII}$). ^{13}C NMR (D_2O): δ 133.2 (s, 1C, CH_2=*C*H–CH_2), 118.1 (s, 1C, *C*H_2=CH–CH_2), 102.4, 102.3, 102.3, 102.2, 102.2, 102.1 (6C, β-C-1), 94.1 (1C, α-C-1), 84.2, 84.1, 83.9, 83.7, 75.6, 75.2, 75.2, 73.1, 72.9, 72.8, 72.4, 69.2, 67.7, 60.4, 60.3, 60.2, (C-2 to C-6). Anal. Calcd for $C_{45}H_{76}O_{36}$: C, 45.30; H, 6.42. Found: C, 45.16; H, 6.32.

3.19. Acetonyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (19)

To a solution of compound **4** (1.2 g, 1.48 mmol) in THF (5 mL) was added HOAc (75%, 5 mL) containing NaOAc (500 mg, 6.10 mmol), then $PdCl_2$ (525 mg, 2.96 mmol) was added. The mixture was stirred at rt overnight, and TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, the filter cake was washed with CH_2Cl_2, and the combined filtrate and washings were concentrated. Purification by column chromatography with 2:1 petroleum ether–EtOAc as the eluent afforded compound **19** (440 mg, 36%) as a syrup: $[\alpha]_D^{20}$ +32.4 (*c* 0.8, $CHCl_3$); ^{1}H NMR: δ 7.82–7.13 (m, 15H, 2Bz–*H*, Ph–*H*), 5.61 (s, 1H, PhC*H*), 5.34 (dd, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3′), 5.29 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.6 Hz, H-2′), 5.17 (dd, 1H, $J_{3,4} = J_{4,5} = 9.8$ Hz, H-4′), 5.13 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.01 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.8 Hz, H-2), 4.98 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1′), 4.67 (dd, 1H, $J_{2,3} = J_{3,4} = 9.8$ Hz, H-3), 4.27–3.67 (m, 7H, H-4, 5, 5′, 6, 6′), 4.15, 4.00 (ABq, 2H, *J* 17.3 Hz, CH_2COCH_3), 3.83, 3.77 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 2.01, 2.01, 2.00 (3s, 9H, 3CH_3CO). Anal. Calcd for $C_{42}H_{43}ClO_{17}$: C, 58.98; H, 5.07. Found: C, 59.06; H, 5.12.

3.20. Acetonyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (20)

Compound **19** (400 mg, 0.485 mmol) was dissolved in mixed solvents of CH_2Cl_2 (4 mL) and MeOH (6 mL). To the solution were added thiourea (185 mg, 2.43 mmol) and 2,4-lutidine (54 μL, 0.49 mmol), and the reaction mixture was boiled under reflux for 16 h, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated, and extracted with CH_2Cl_2, and the organic phase was washed with N HCl, satd aq $NaHCO_3$, and water, dried and concentrated. Purification by column chromatography with 2:1 petroleum ether–EtOAc as the eluent afforded **20** (330 mg, 87%) as an amorphous solid: $[\alpha]_D^{20}$ +50.4 (*c* 1.0, $CHCl_3$); ^{1}H

NMR: δ 7.92–7.21 (m, 15H, 2Bz–*H*, Ph–*H*), 5.60 (s, 1H, PhC*H*), 5.17 (d, 1H, $J_{1,2}$ 3.9 Hz, H-1), 5.09 (dd, 1H, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.4 Hz, H-2′), 5.03 (dd, 1H, $J_{1,2}$ 3.9 Hz, $J_{2,3}$ 9.6 Hz, H-2), 5.01 (dd, 1H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4′), 4.98 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1′), 4.51 (dd, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3), 4.27-3.60 (m, 8H, H-3′, 4, 5, 5′, 6, 6′), 4.16, 4.05 (ABq, 2H, *J* 17.3 Hz, CH_2COCH$_3$), 2.07, 2.01, 2.01 (3s, 9H, 3CH_3CO). Anal. Calcd for $C_{40}H_{42}O_{16}$: C, 61.69; H, 5.44. Found: C, 61.76; H, 5.34.

3.21. Acetonyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-3-*O*-chloroacetyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (22)

Compound **22** (400 mg, 71%) was obtained as an amorphous solid from compounds **20** (280 mg, 0.36 mmol) and **21** (400 mg, 0.43 mmol) according to the general procedure for the coupling reaction. However, purification by column chromatography was carried out with 2:1 petroleum ether–EtOAc as the eluent: $[\alpha]_D^{20}$ +11.1 (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 7.79–7.13 (m, 30H, 4 Bz–*H*, 2Ph–*H*), 5.52, 5.48 (2s, 2H, 2PhC*H*), 5.12–4.92 (m, 6H, H-2$^{II–IV}$, 3^{IV}, 4^{II,IV}), 5.05 (d, 1H, d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{I}), 4.79, 4.64, 4.60 (3d, 3H, $J_{1,2}$ 7.8 Hz, H-1$^{II–IV}$), 4.71 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.6 Hz, H-2^{I}), 4.36–3.30 (m, 17H, H-3$^{I–III}$, 4^{I,III}, 5$^{I–IV}$, 6$^{I–IV}$), 4.08, 3.98 (ABq, 2H, *J* = 17.3 Hz, CH_2COCH$_3$), 3.75, 3.68 (ABq, 2H, *J* 14.8 Hz, ClCH_2CO), 1.96, 1.96, 1.95, 1.93, 1.91 (5s, 15H, 5CH_3CO). Anal. Calcd for $C_{79}H_{79}ClO_{31}$: C, 60.83; H, 5.10. Found: C, 60.76; H, 5.04.

3.22. Acetonyl 4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (23)

Compound **22** (350 mg, 0.22 mmol) was dissolved in mixed solvents of CH_2Cl_2 (4 mL) and MeOH (6 mL). To the solution were added thiourea (85 mg, 1.04 mmol) and 2,4-lutidine (25 μL, 0.23 mmol), and the reaction mixture was boiled under reflux for 16 h, at the end of which time TLC (1.5:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated and extracted with CH_2Cl_2, and the organic phase was washed with N HCl, satd aq $NaHCO_3$, and water, dried, and concentrated. Purification by column chromatography with 2:1 petroleum ether–EtOAc as the eluent afforded **23** (270 mg, 81%) as an amorphous solid: $[\alpha]_D^{20}$ +20.4 (*c* 0.9, $CHCl_3$); ^{1}H NMR: δ 7.79–7.13 (m, 30H, 4Bz–*H*, 2Ph–*H*), 5.52, 5.48 (2s, 2H, 2PhC*H*), 5.10–4.86 (m, 5H, H-2$^{II–IV}$, 4^{II,IV}), 5.05 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{I}), 4.80, 4.64, 4.62 (3d, 3H, $J_{1,2}$ 7.8 Hz, H-1$^{II–IV}$), 4.73 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.6 Hz, H-2^{I}), 4.35–3.30 (m, 18H, H-3$^{I–IV}$, 4^{I,III}, 5$^{I–IV}$, 6$^{I–IV}$), 4.09, 4.01 (ABq, 2H, *J* 17.3 Hz, CH_2COCH$_3$), 2.01, 1.96, 1.96, 1.94, 1.92 (5s, 15H, 5CH_3CO). Anal. Calcd for $C_{77}H_{78}O_{30}$: C, 62.34; H, 5.30. Found: C, 62.16; H, 5.14.

3.23. Acetonyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (25)

Compound **25** (180 mg, 50%) was obtained as an amorphous solid from compounds **23** (220 mg, 0.148 mmol) and **24** (210 mg, 0.192 mmol) according to the general procedure for the coupling reaction. However, purification was carried out with 2:1 petroleum ether–EtOAc as the eluent: $[\alpha]_D^{20}$ +46.9 (*c* 1.0, $CHCl_3$); ^{1}H NMR: δ 7.92–7.07 (m, 60H, 9Bz–*H*, 3Ph–*H*), 5.50, 5.50, 5.37 (3s, 3H, 3PhC*H*), 5.54 (dd, 1H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{VI}), 5.50 (dd, 1H, $J_{3,4} = J_{4,5} = 9.5$ Hz, H-4^{VI}), 5.33 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.5 Hz, H-3^{VI}), 5.09–4.62 (m, 7H, H-2$^{I–V}$, 4^{II,IV}), 5.04 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{I}), 4.79, 4.75, 4.51, 4.50, 4.43 (5d, 5H, $J_{1,2}$ 8.0 Hz, H-1$^{II–VI}$), 4.36–3.24 (m, 26H, H-3$^{I–V}$, 4^{I,III,V}, 5$^{I–VI}$, 6$^{I–VI}$), 4.09, 3.97 (ABq, 2H, *J* 17.3 Hz, CH_2COCH$_3$), 1.96, 1.96, 1.95, 1.89, 1.84 (5s, 15H, 5CH_3CO). Anal. Calcd for $C_{131}H_{122}O_{45}$: C, 65.11; H, 5.09. Found: C, 65.26; H, 5.24.

3.24. Acetonyl 2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2-*O*-benzoyl-α-D-glucopyranoside (26)

To a solution of compounds **25** (130 mg, 0.054 mmol) and ethylene glycol (0.1 mL, 1.80 mmol) in MeCN (5 mL) was added *p*-toluenesulfonic acid·H_2O (10 mg). The mixture was stirred at rt overnight at the end of which time TLC (1:1 petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was made neutralized with Et_3N, concentrated, and extracted with CH_2Cl_2, the organic phase was washed with water, then dried and concentrated. Purification by column chromatography with 1:1 petroleum ether–EtOAc as the eluent afforded compound **26** (80 mg, 69%) as an amorphous solid: $[\alpha]_D^{20}$ +16.3 (*c* 0.8, $CHCl_3$); ^{1}H NMR: δ 8.08–6.97 (m, 45H, 9Bz–*H*), 5.68 (dd, 1H, $J_{2,3} = J_{3,4} = 9.6$ Hz, H-3^{VI}), 5.51 (dd, 1H, $J_{3,4} = J_{4,5} = 9.6$ Hz, H-4^{VI}), 5.38 (dd, 1H, $J_{1,2}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-3^{VI}), 5.06 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1^{I}), 5.03–4.62 (m, 7H, H-2$^{I–V}$, 4^{II,IV}), 4.77, 4.61, 4.59, 4.48, 4.46 (5d, 5H, $J_{1,2}$ 8.0 Hz, H-1$^{II–VI}$), 4.35–3.14 (m, 28H, CH_2COCH$_3$, H-3$^{I–V}$, 4^{I,III,V}, 5$^{I–VI}$, 6$^{I–VI}$), 2.08, 2.04, 1.88, 1.88, 1.82 (5s,

15H, 5CH_3CO). Anal. Calcd for $C_{110}H_{110}O_{45}$: C, 61.39; H, 5.15. Found: C, 61.56; H, 5.24.

3.25. Acetonyl 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-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-acetyl-2-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2-*O*-benzoyl-α-D-glucopyranoside (27)

Compound **27** (60 mg, 55%) was obtained as an amorphous solid from compounds **26** (60 mg, 0.0279 mmol) and **9** (75 mg, 0.1 mmol) according to the general procedure for the coupling reaction. However, purification was carried out with 1:1.5 petroleum ether–EtOAc as the eluent: $[\alpha]_D^{20}$ +46.9 (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 8.05–6.87 (m, 105H, 21Bz–*H*), 5.95–5.29 (m, 12H, H-$2^{VI–IX}$, $3^{VI–IX}$, $4^{VI–IX}$), 4.97–4.37 (m, 15H, H-$1^{II–IX}$, $2^{I–V}$, $4^{II,IV}$), 4.78 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{I}), 4.26–3.10 (m, 37H, CH_2COCH_3, H-$3^{I–V}$, $4^{I,III,V}$, $5^{I–IX}$, $6^{I–IX}$), 2.03, 2.03, 1.99, 1.86, 1.81 (5s, 15H, 5CH_3CO). ^{13}C NMR: δ 170.5, 170.4, 170.4, 169.3, 169.2 (5C, 5CH_3*C*O), 166.1, 166.0, 165.9, 165.7, 165.7, 165.7, 165.6, 165.5, 165.2, 165.2, 165.1, 165.1, 165.0, 164.9, 164.9, 164.8, 164.6, 163.8, 163.5, 163.3, 163.1 (21s, 21C, Bz*C*O), 102.1, 102.1, 101.5, 101.4, 101.4, 100.9, 100.7, 100.5 (8C, β-C-1), 95.3 (1C, α-C-1), 22.65, 21.62, 20.63, 20.45, 20.41 (5C, 5*C*H_3CO). Anal. Calcd for $C_{212}H_{188}O_{72}$: C, 65.49; H, 4.87. Found: C, 65.66; H, 4.74.

3.26. Acetonyl β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-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-glucopyranosyl-(1→6)]-α-D-glucopyranoside (28)

Satd NH_3 in MeOH (5 mL) was added to compounds **27** (40 mg, 10.29 μmol) in MeOH (4 mL). After 48 h at rt, the reaction mixture was concentrated, and the residue was purified by Sephadex LH-20 chromatography (MeOH) to afford **28** as an amorphous solid (15.5 mg, 98%): $[\alpha]_D^{20}$ +2.9 (*c* 1.0, H_2O); ^{1}H NMR (D_2O): δ 4.95 (d, 1H, $J_{1,2}$ 3.9 Hz, H-1^{I}), 4.77, 4.77, 4.76, 4.76, 4.53, 4.53, 4.49, 4.49 (8d, 8H, $J_{1,2}$ 8.0 Hz, H-$1^{II–IX}$), 4.07 (s, 2H, CH_3COCH_2), 4.23–3.30 (m, 54H, H-$2^{I–IX}$, $3^{I–IX}$, $4^{I–IX}$, $5^{I–IX}$, $6^{I–IX}$), 1.99 (s, 3H, CH_3CO). ^{13}C NMR (D_2O): δ 172.10 (1C, CH_3COCH_2), 102.6, 102.6, 102.6, 102.5, 102.3, 102.3, 102.2, 102.2 (8C, β-C-1), 97.98 (1C, α-C-1), 84.57, 84.50, 84.00, 83.80, 81.55 (5C, C-$3^{A–E}$), 21.03 (s, 1C, CH_3COCH_2). Anal. Calcd for $C_{57}H_{96}O_{47}$: C, 44.65; H, 6.31. Found: C, 44.76; H, 6.44.

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