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Facile syntheses of the disaccharide repeating unit of the O-antigenic polysaccharide of *Burkholderia pseudomallei* strain 304b and its dimer and trimer

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ABSTRACT

A highly efficient strategy for the preparation of a disaccharide-repeating unit of the O-antigenic polysaccharide of *Burkholderia pseudomallei* strain 304b, and its dimer and trimer, has been developed through a regio- and stereoselective manner using *p*-methoxylphenyl 2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside and 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl trichloroacetimidate as the key synthons. The target molecules were equipped with a *p*-methoxylphenyl handle at the reducing terminus to allow for their further functionalization and attachment to a carrier protein.

1. Introduction

Lipopolysaccharides (LPSs), a glycoconjugate containing three structural domains: lipid A, a core oligosaccharide, and the O-polysaccharides (O-PS),[1] are ubiquitous components of the outer cell membranes of Gram-negative bacteria, and are thought to be involved in host-pathogen cross-talk. The O-PS, which are exposed towards the external environment, play essential roles in this course.[2–4] The O-PS, normally contains multiple copies of repeating units with typically 1–4 monosaccharide residues, is antigenic, and a number of these glycans have been studied as vaccine candidates.[5]

Melioidosis, or *Burkholderia pseudomallei* infection, is an important cause of mortality in Thailand and other parts of Southeast Asia and Northern Australia.[6] In Northeastern Thailand alone, a population of 7 million people is at risk.[7] In addition to its virulence, *B. pseudomallei* is also intrinsically resistant to penicillin and gentamicin, the usual empirical treatment for suspected septicaemia in many parts of the developing world.[8] Since the small carbohydrates can provoke the formation of antibodies,[9–11] intensified efforts by biochemists to detect *B. pseudomallei* identified unique molecules on the surface of the bacteria. In *B. pseudomallei* strain 304b, a unique disaccharide **1** (Fig. 1) was discovered[8] containing glucose and 6-deoxytalose. In our previous work, in a collaborative project for an investigation of novel vaccines against *B. pseudomallei* infection, we have synthesized the monomer (**2**) and dimer (**3**) of this repeating unit.[12] Here, in order to compare the different effects on pathogen detection caused by the difference in oligosaccharide structure at the terminal position, we designed and synthesized another three oligosaccharides: the monomer (**4**), dimer (**5**) and trimer (**6**) (Fig. 2) of the frame shifted disaccharide repeating unit with glucose at the terminal position. All the synthesized oligosaccharides contain a terminal *p*-methoxylphenyl glycoside, which was necessary for further functionalization and attachment to a carrier protein.[13]

2. Results and discussion

The synthesis of the target oligosaccharides followed a convergent approach. As shown in Scheme 1 and 1,2,4,6-tetra-*O*-acetyl-3-*O*-allyl-D-glucose (**7**),[14] readily prepared from D-glucose (four steps, 70%), was chosen as the starting material. It was converted into the *p*-methoxyphenyl α-D-glucoside (**8**) by treating **7** with *p*-methoxyphenol and boron trifluoride diethyl etherate (70%). Deacetylation of the compound **8**, followed by perbenzoylation with 4 equiv of benzoyl chloride, provided the *p*-methoxylphenyl 3-*O*-allyl-2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (**10**) in 90% yield over two steps. Selective cleavage of the 3-*O*-allyl group was then achieved under the action of $PdCl_2$ in a mixed solvent of $MeOH/CH_2Cl_2$ (2/1) furnishing the key synthon **11**, which is a ready acceptor for further glycosylation reactions. We also acquired the X-ray crystal structure of **10** (Fig. 3); further confirming its structure.

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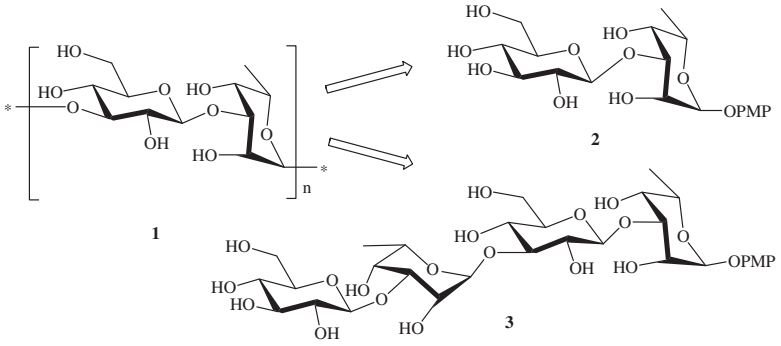


Figure 1. O-Chain repeating unit of LPS from *B. pseudomallei* strain 304b (**1**) and the synthesized oligosaccharides **2** and **3**.

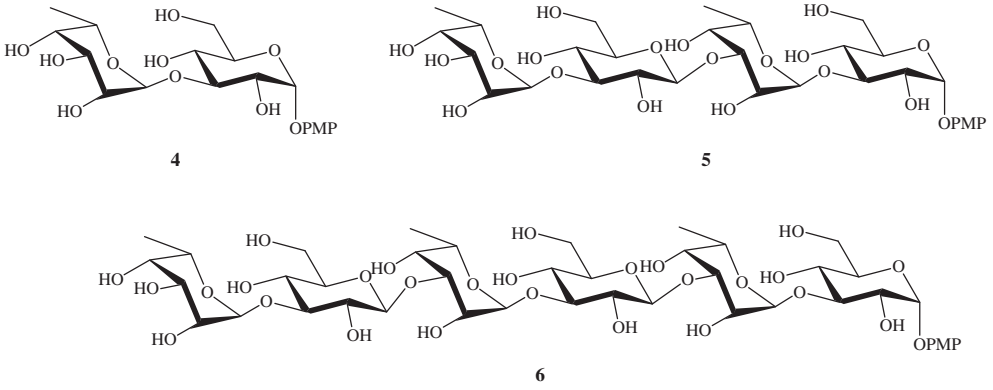
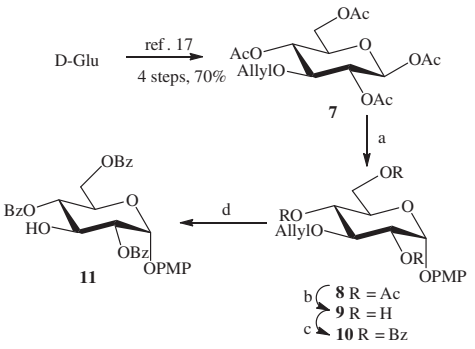


Figure 2. Synthesized O-chain repeating unit of LPS from *B. pseudomallei* strain 304b.

With the monosaccharide building block **11** in hand, synthesis of the target oligosaccharides was accomplished as shown in Schemes 2 and 3. In our previous work, an efficient and large-scale procedure for the preparation of *p*-methoxylphenyl 4-*O*-benzoyl-6-deoxy-α-L-talopyranoside from L-rhamnose was developed.[15] Another key synthon **12**[15] could be easily obtained with this procedure in 28% yield using L-rhamnose as the starting material. Glycosylation between glucosyl acceptor **11** and 6-deoxytalosyl donor **12** was accomplished using TMSOTf as the catalyst in the presence of 4 Å molecular sieves to afford the disaccharides **13** in 82% yield. The configuration of the glycosyl bond formed in the product was deduced from the corresponding coupling constants ($J_{1',2'}$ 1.2 Hz). Deallyloxycarbonylation of **13** was successfully achieved in MeOH–THF[16] in the presence of CH_3COONH_4, $Pd[P(C_6H_5)_3]_4$ and $NaBH_4$, within 4 min without affecting any of the other protecting groups, giving the desired acceptor **14** in 85% yield. Coupling of the monosaccharide donor **15**,[17] which was obtained from L-rhamnose in 10 steps with a total yield of 30%, with the glucosyl acceptor **11** afforded the expected (1→3)-α-disaccharide **16** in 89% yield. Cleavage of the 4-methoxyphenyl group of **16** with ceric ammonium nitrate (CAN), followed by the reaction with trichloroacetonitrile and DBU,[18] gave the trichloroacetimidate **17** in 75% yield over two steps. Similar to the preparation of **13** and **16**, condensation of the disaccharide donor **17** with the disaccharide acceptor **14** furnished the corresponding β-linked tetrasaccharide **18** in 77% yield. The structure of **18** was confirmed from its ^1H NMR and ^{13}C NMR spectra, and complete assignment of the ^1H and ^{13}C signals was achieved by HSQC experiments (Fig. 4), showing the characteristic signals, such as δ 5.73 (J 3.6 Hz, α-configuration), 4.90 (J 7.9 Hz, β-configuration) for the two H-1's of glucose, and δ 5.40, 5.20 for the two H-1's of talose. Removal of the anomeric methoxylphenyl group of **18** with CAN in 80% $CH_3CN–H_2O$, followed by trichloroacetimidate formation, provided tetrasaccharide donor **19** in 70% yield over two steps.

Finally, condensation of the disaccharide acceptor **14** with the tetrasaccharide donor **19**, following the same glycosylation strategy as mentioned above, gave the fully protected hexasaccharide **20** (65%) whose ^1H NMR spectrum showed a signal at 4.65 ppm (J 7.9 Hz), confirming the β glycosylation. The ^{13}C NMR spectrum of **20** showed six C-1 signals (δ 99.9, 99.7, 99.7, 98.5, 98.1 and 95.7), and the complete assignment of the ^1H and ^{13}C signals of **20** was achieved by HSQC experiments (Fig. 5). Deacylation of the disaccharide **16**, the tetrasaccharide **18** and the hexasaccharide **20** with ammonia-saturated methanol afforded the target disaccharide **4** (88%), tetrasaccharide **5** (84%) or hexasaccharide **6** (83%), respectively. The complete assignment of the ^1H and ^{13}C signals of **5** and **6** was achieved by HSQC experiments.



Scheme 1. Synthesis of key synthon **11**. Reagents and conditions: (a) 4-Methoxyphenol, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0–25 °C, 3 h; (b) MeOH–MeONa, rt, 1 h; (c) BzCl, Py, CH_2Cl_2, −10 °C, 3 h, 90% for **10** over two steps; (d) $PdCl_2$, 2:1 MeOH–CH_2Cl_2, rt, overnight, 75% for **11**.

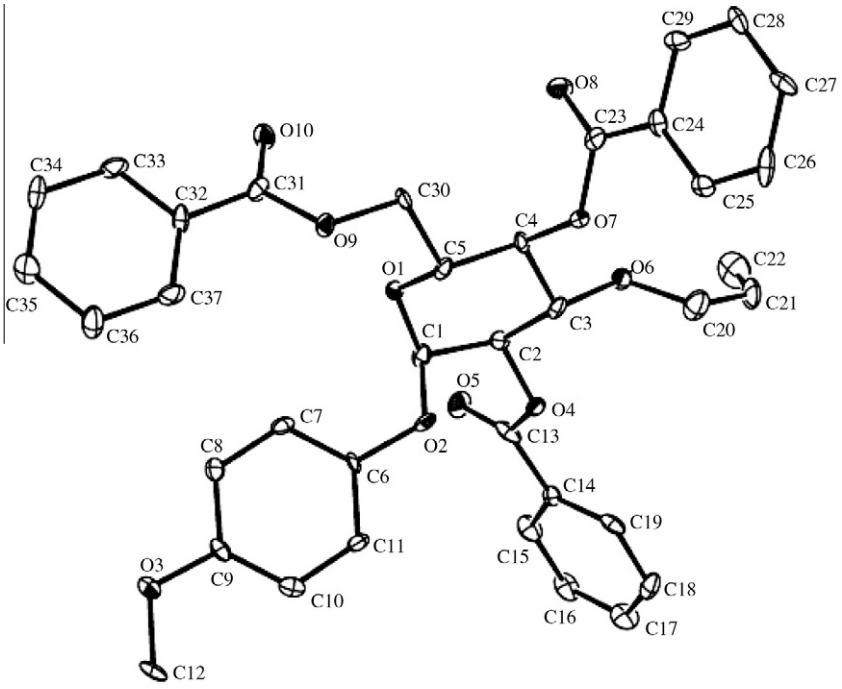
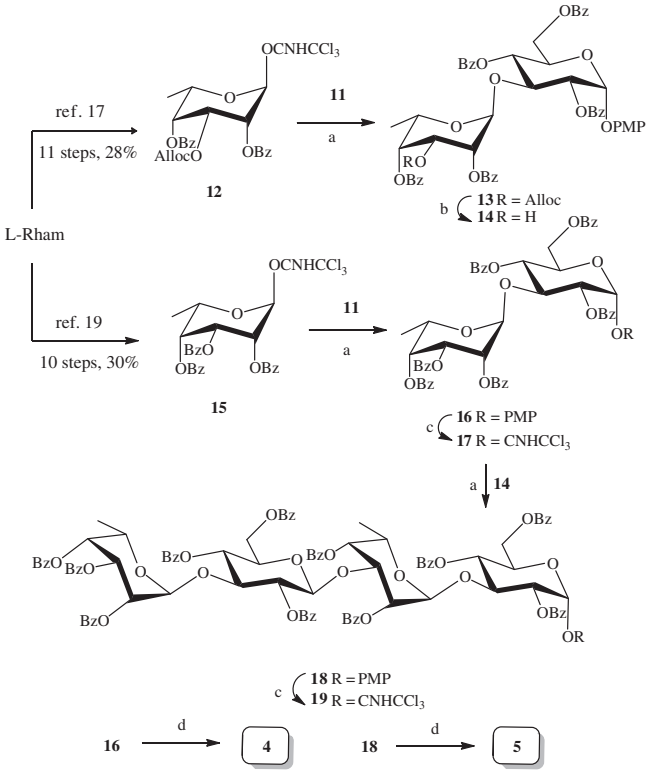


Figure 3. X-ray crystal structure of compound **10**.



Scheme 2. Synthesis of the target disaccharide **4** and tetrasaccharide **5**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −10 °C to rt, 2 h, 82% for **13**, 89% for **16**, 77% for **18**; (b) CH_3COONH_4, $Pd[P(C_6H_5)_3]_4$, $NaBH_4$, MeOH–THF, −10 °C, 4 min, 85% for **14**; (c) 4:1 CH_3CN–H_2O, CAN, 30 °C, then CCl_3CN, DBU, CH_2Cl_2, rt, 0.5 h, 75% over two steps for **17**, 70% over two steps for **19**; (d) satd NH_3–MeOH, rt, 96 h, 88% for **4**, 84% for **5**.

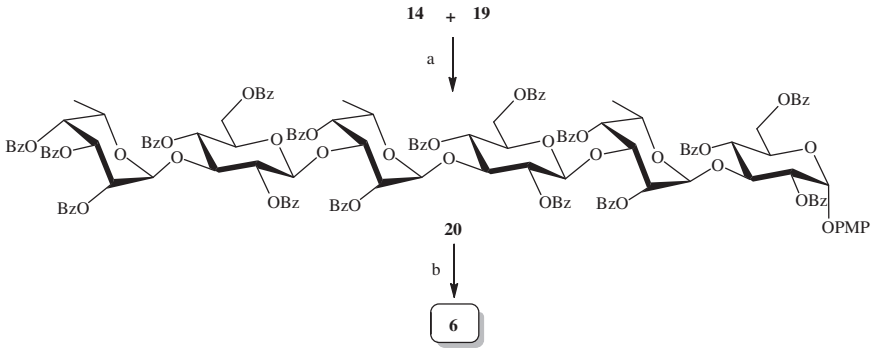
3. Conclusion

In conclusion, a convergent total synthesis of a disaccharide-repeating unit of the O-antigenic polysaccharide of *B. pseudomallei* strain 304b, and its dimer and also trimer, was achieved through a regio- and stereoselective manner with suitably synthesized building blocks. The target molecules were functionalized with a *p*-methoxylphenyl group at the reducing end of the glucoside, which could be easily removed for subsequent attachment to a carrier protein. The described method can be used for construction of higher oligosaccharides with similar structures. Immunological investigations of the resultant disaccharide **4**, tetrasaccharide **5** and hexasaccharide **6** are in progress and the results will be reported in due course.

4. Experimental procedures

4.1. General methods

Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter for soln in a 1-dm, jacketed cell. ^1H and ^{13}C NMR spectra were recorded with Bruker DPX300 and Bruker AVANCE600 spectrometers in $CDCl_3$ or D_2O solns. Internal references: TMS (δ 0.000 ppm for ^1H), $CDCl_3$ (δ 77.00 ppm for ^{13}C), HOD (δ 4.700 for ^1H). Elemental analysis was performed on a Yanaco CHN Corder MF-3 automatic elemental analyzer. High-resolution mass spectra (HRMS) was performed by the Peking University, and electrospray-ionization mass spectra (ESIMS) was performed by the China Agricultural University. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) H_2SO_4 in MeOH or by UV detection. Column chromatography was conducted by elution of a column of silica gel (200–300 mesh) with EtOAc/petroleum ether (bp 60–90 °C) as the eluent. Solns were concd at a temperature <60 °C under diminished pressure.



Scheme 3. Synthesis of the target hexasaccharide **6**. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −10 °C to rt, 2 h, 65% for **20**; (b) satd NH_3–MeOH, rt, 96 h, 83% for **6**.

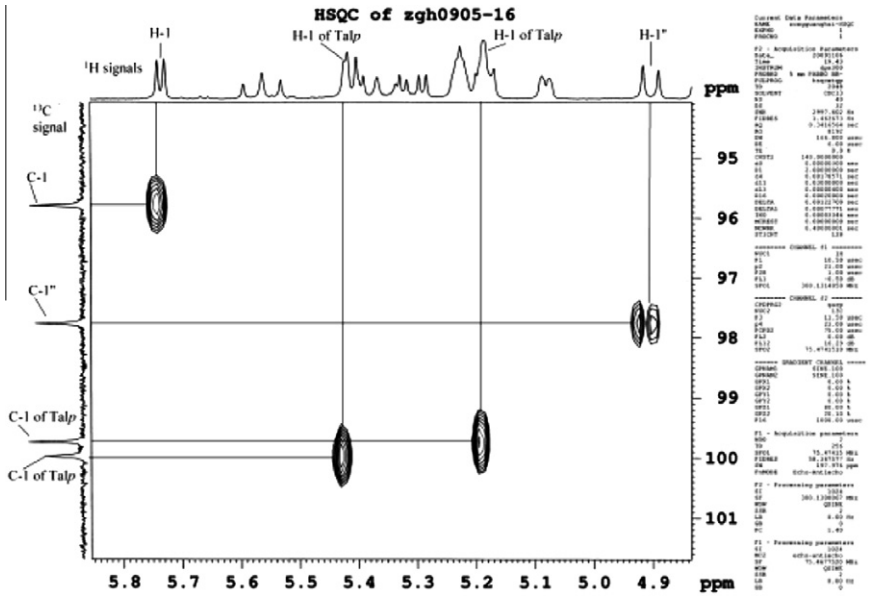


Figure 4. Partial HSQC spectra of the fully protected hexasaccharide **18**.

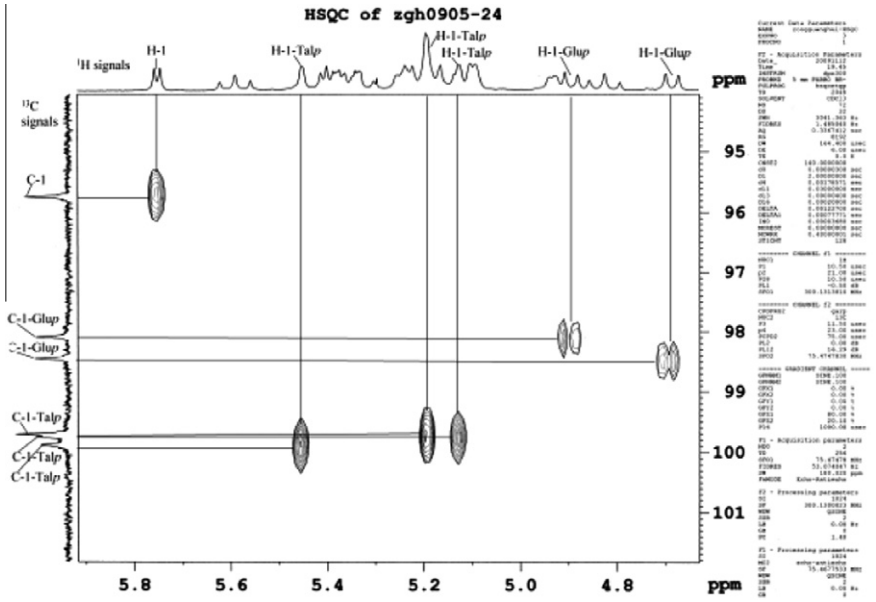


Figure 5. Partial HSQC spectra of the fully protected tetrasaccharide **20**.

4.2. *p*-Methoxylphenyl 3-*O*-allyl-2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (10)

Compound **8**[19] (9.0 g, 20 mmol) was dissolved in 150 mL absolute MeOH, 200 mg MeONa was added to the reaction mixture and stirred at rt for 3 h. Afterwards, neutralization of the reaction mixture with acidic ion exchange resin [Amberlite IR-120 (H+), Alfa Aesar] was conducted and after filtration, the organic phase was concentrated in vacuo, and then co-evaporated with toluene, providing compound **9** as a syrup. Then to a cold (0 °C) solution of the syrup in dry pyridine (100 mL) was added benzoyl chloride (2.8 mL, 24 mmol). The reaction mixture was slowly raised to room temperature and stirred for 2 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was diluted with CH_2Cl_2 (100 ml), washed with 1 M HCl (50 mL), water (2 × 50 mL) and dried (Na_2SO_4). The solvent was evaporated under vacuum and the residue was purified by column chromatography (petroleum ether–EtOAc 4:1) to afford compound **10** (11.5 g, 90%) over two steps as a white solid. $[\alpha]_D^{25}$ +89.6 (*c* 1.0 $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13–7.36 (m, 15H, Bz-*H*), 7.03–7.00 (m, 2H, Ar-*H*), 6.70–6.67 (m, 2H, Ar-*H*), 5.76 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.67 (m, 1H, CH_2=C*H*CH_2), 5.53 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.3 Hz, H-4), 5.29 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.9 Hz, H-2), 5.14–4.94 (m, 2H, C*H*$_2$=CHCH_2), 4.56–4.37 (m, 4H, H-3, H-5, CH_2=CHC*H*$_2$), 4.22–4.16 (m, 1H, H-6a), 4.13–4.07 (m, 1H, H-6b), 3.70 (s, 3H, $C_6H_4OCH_3$). ESI-MS *m/z* calcd for $C_{37}H_{34}O_{10}Na$ $(M+Na)^+$ 661.2. Found: 661.2. Anal. Calcd for $C_{37}H_{34}O_{10}$: C, 69.58; H, 5.37. Found: C, 69.47; H, 5.31.

4.3. *p*-Methoxylphenyl 2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (11)

To a solution of **10** (9.2 g, 14.4 mmol) in MeOH (100 mL) and CH_2Cl_2 (50 mL) was added $PdCl_2$ (0.20 g, 1.2 mmol), and the mixture was stirred at rt overnight, at the end of which time TLC (3:1 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 3:1 petroleum ether–EtOAc as the eluent afforded compound **11** (6.5 g, 75%) as a white foam. $[\alpha]_D^{25}$ +102.1 (*c* 1.0 $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.12–7.38 (m, 15H, Bz-*H*), 7.05–7.03 (m, 2H, Ar-*H*), 6.73–6.70 (m, 2H, Ar-*H*), 5.74 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.44 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.4 Hz, H-4), 5.26 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.9 Hz, H-2), 4.70–4.62 (m, 1H, H-5), 4.60–4.50 (m, 2H, H-3, H-6a), 4.47–4.40 (m, 1H, H-6b), 3.71 (s, 3H, $C_6H_4OCH_3$), 2.73 (d, 1H, *J* 5.3 Hz, O*H*). ESI-MS *m/z* calcd for $C_{34}H_{30}O_{10}Na$ $(M+Na)^+$ 621.2. Found: 621.2. Anal. Calcd for $C_{37}H_{34}O_{10}$: C, 68.22; H, 5.05. Found: C, 68.31; H, 5.23.

4.4. *p*-Methoxylphenyl 3-*O*-allyloxycarbonyl-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (13)

Compound **11** (3.3 g, 5.5 mmol), **12** (4.0 g, 6.6 mmol) and 4 Å molecular sieves (4 g) were dried together under high vacuum for 2 h, then dissolved in anhydrous, redistilled CH_2Cl_2 (100 mL). TMSOTf (54 μL, 0.3 mmol) was added dropwise at −10 °C under an N_2 atmosphere. The reaction mixture was stirred for 0.5 h, during which time the mixture was allowed to gradually warm to ambient temperature. TLC (4:2:1 petroleum ether–toluene–EtOAc) indicated that the reaction was complete. Then the reaction mixture was neutralized with triethylamine and filtrated, and the filtrate was concentrated. Purification of the residue by column chromatography (6:3:1 petroleum ether–toluene–EtOAc) gave **13** (4.7 g, 82%) as a white foam. $[\alpha]_D^{25}$ +5.9 (*c* 1.0 $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.14–7.11 (m, 25H, Bz-*H*), 7.02–6.99 (m, 2H, Ar-*H*), 6.71–6.68 (m, 2H, Ar-*H*), 5.85 (m, 1H, CH_2=C*H*CH_2OCO), 5.78 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.67 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 5.53 (d, 1H, $J_{1',2'}$ 1.2 Hz, H-1′), 5.44 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.36–5.19 (m, 4H, H-2′, H-4′, C*H*$_2$=CHCH_2OCO), 5.16 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 3.9 Hz, H-3′), 4.87 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 4.65–4.48 (m, 4H, H-5, H-6, CH_2=CHC*H*$_2$OCO), 4.42 (m, 1H, H-6), 4.04 (m, 1H, H-5′), 3.70 (s, 3H, $C_6H_4OCH_3$), 0.74 (d, 3H, *J* 6.5 Hz, C-C*H*$_3$). ESI-MS *m/z* calcd for $C_{58}H_{52}O_{18}Na$ $(M+Na)^+$ 1059.3. Found: 1059.3. Anal. Calcd for $C_{58}H_{52}O_{18}$: C, 67.18; H, 5.05. Found: C, 67.43; H, 5.20.

4.5. *p*-Methoxylphenyl 2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (14)

To a cooled (−5 °C) solution of compound **13** (2.1 g, 2.0 mmol) in 1:1 MeOH–THF (60 mL) in 250 mL flask was added CH_3COONH_4 (1.6 g, 20 mmol). With vigorous stirring, $NaBH_4$ (18 mg, 0.5 mmol), $Pd[P(C_6H_5)_3]_4$ (92 mg, 0.08 mmol), and then additional $NaBH_4$ (93 mg, 2.5 mmol) was added in 3 portions immediately one after another. One min after the addition of the second portion of $NaBH_4$, TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated under diminished pressure, the residue was dissolved in CH_2Cl_2 (150 mL) and washed with water (50 mL), then the organic phase was dried over Na_2SO_4. Evaporation and purification by flash column chromatography (petroleum ether–EtOAc 3:1) afforded compound **14** as a foamy solid (1.65 g, 85%). $[\alpha]_D^{25}$ +20.4 (*c* 1.0 $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13–7.17 (m, 25H, Bz-*H*), 7.03–7.00 (m, 2H, Ar-*H*), 6.72–6.69 (m, 2H, Ar-*H*), 5.76 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.65 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 5.53 (s, 1H, H-1′), 5.45 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.09–5.07 (m, 2H, H-2′, H-4′), 4.86 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 4.58–4.51 (m, 2H, H-5, H-6), 4.42 (m, 1H, H-6), 4.24 (m, 1H, H-3′), 3.95 (m, 1H, H-5′), 3.70 (s, 3H, $C_6H_4OCH_3$), 2.67 (d, 1H, *J* 5.8 Hz, O*H*), 0.74 (d, 3H, *J* 6.5 Hz, C-C*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 167.0, 166.1, 166.0, 165.5, 165.1 (5 × *C*OPh), 155.5, 150.3, 118.3(2), 114.6(2) ($C_6H_4OCH_3$), 99.6, 95.7 (2 × C-1), 75.5, 73.9, 71.7, 70.4, 69.6, 68.5, 66.0, 65.3, 63.0, 55.5 ($C_6H_4OCH_3$), 16.0 (C-CH_3). ESI-MS *m/z* calcd for $C_{54}H_{48}O_{16}Na$ $(M+Na)^+$ 975.3. Found: 975.3. Anal. Calcd for $C_{54}H_{48}O_{16}$: C, 68.06; H, 5.08. Found: C, 67.88; H, 5.05.

4.6. *p*-Methoxylphenyl 2,3,4-tri-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (16)

Compound **11** (1.79 g, 3.0 mmol) and **15** (2.3 g, 3.7 mmol) were coupled in the presence of catalytic TMSOTf (54 μL, 0.3 mmol) under the same conditions as described above for the coupling of **11** with **12**. Purification by silica gel chromatography with 6:3:1 petroleum ether–toluene–EtOAc as the eluent gave disaccharide **16** (2.8 g, 89%) as a foamy solid. $[\alpha]_D^{25}$ +8.8 (*c* 1.0 $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17–7.13 (m, 30H, Bz-*H*), 7.03–7.00 (m, 2H, Ar-*H*), 6.72–6.69 (m, 2H, Ar-*H*), 5.79 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.71 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.5 Hz, H-4), 5.57 (s, 1H, H-1′), 5.56 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 3.9 Hz, H-3′), 5.47 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.35 (m, 1H, H-2′), 5.31 (m, 1H, H-4′), 4.91 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 4.61–4.53 (m, 2H, H-5, H-6), 4.43 (m, 1H, H-6), 4.14 (m, 1H, H-5′), 3.70 (s, 3H, $C_6H_4OCH_3$), 0.76 (d, 3H, *J* 6.5 Hz, C-C*H*$_3$). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1(2), 165.4, 165.1, 165.0, 164.8 (6 × *C*OPh), 155.5, 150.3, 118.3(2), 114.7(2) ($C_6H_4OCH_3$), 100.0, 95.7 (2 × C-1), 76.1, 73.9, 69.6, 69.2, 68.5, 67.7, 66.3, 65.9, 63.0, 55.5 ($C_6H_4OCH_3$), 15.9 (C-CH_3). ESI-MS *m/z* calcd for $C_{61}H_{52}O_{17}Na$ $(M+Na)^+$ 1079.3. Found: 1079.3. Anal. Calcd for $C_{61}H_{52}O_{16}$: C, 69.31; H, 4.96. Found: C, 69.48; H, 4.70.

4.7. 2,3,4-Tri-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (17)

To a solution of compound **16** (2.5 g, 2.4 mmol) in acetonitrile (80 mL) and water (20 mL) was added ceric ammonium nitrate (CAN) (5.2 g, 9.6 mmol). The mixture was stirred for 20 min at 30 °C, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solvent was evaporated under diminished pressure at 50 °C to give a residue, which was diluted with CH_2Cl_2 and washed with water. The organic phase was dried and concentrated. Purification by silica gel chromatography with 3:1 petroleum ether–EtOAc as the eluent afforded 2,3,4-tri-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranose. A mixture of this compound, trichloroacetonitrile (2 mL, 20 mmol), and 1,8-diazabicyclo [5.4.0] undecene (DBU) (0.2 mL, 1.6 mmol) in dry CH_2Cl_2 (50 mL) was stirred for 0.5 h and then concentrated. The residue was purified by chromatography with 4:1 petroleum ether–EtOAc as the eluent to give **17** (1.95 g, 75%) as a white foam. $[\alpha]_D^{25}$ +19.2 (*c* 1.0 $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60 (s, 1H, CN*H*CCl_3), 8.17–7.10 (m, 30H, Bz-*H*), 5.76 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.79 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.8 Hz, H-4), 5.62 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.54 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 3.9 Hz, H-3′), 5.50 (s, 1H, H-1′), 5.32–5.29 (m, 2H, H-2′, H-4′), 4.77 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.6 Hz, H-3), 4.65 (dd, 1H, *J* 2.5 Hz, 12.2 Hz, H-6), 4.56 (m, 1H, H-5), 4.43 (dd, 1H, *J* 4.5 Hz, 12.2 Hz, H-6), 4.11 (m, 1H, H-5′), 0.74 (d, 3H, *J* 6.5 Hz, C-*CH*$_3$). ESI-MS *m/z* calcd for $C_{56}H_{46}Cl_3NO_{16}Na$ (M+Na)$^+$ 1116.2. Found: 1116.2. Anal. Calcd for $C_{56}H_{46}Cl_3NO_{16}$: C, 61.41; H, 4.23; N, 1.28. Found: C, 61.56; H, 4.37; N, 1.43.

4.8. *p*-Methoxylphenyl 6-deoxy-α-L-talopyranosyl-(1→3)-α-D-glucopyranoside (4)

Compound **16** (0.15 g, 0.14 mmol) was dissolved in satd NH_3–MeOH (40 mL). After 120 h at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **4** (0.054 g, 88%) as a white foamy solid. $[\alpha]_D^{25}$ −46.5 (*c* 0.5 H_2O). ^{1}H NMR (300 MHz, D_2O): δ 6.95 (dd, 4H, Ar-*H*), 5.37 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.17 (s, 1H, H-1′), 4.26 (m, 1H, H-5′), 3.90 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 3.89 (m, 1H, H-2′), 3.85 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 3.3 Hz, H-3′), 3.75–3.66 (m, 8H, H-2, H-4′, H-5, 2 × H-6, $C_6H_4OCH_3$), 3.44 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.6 Hz, H-4), 1.16 (d, 3H, *J* 6.6 Hz, C-*CH*$_3$). ^{13}C NMR (75 MHz, D_2O): δ 154.6, 150.3, 118.7(2), 115.0(2) ($C_6H_4OCH_3$), 101.8, 98.2 (2 × C-1), 79.9, 72.5, 72.2, 71.6, 69.9, 67.8, 67.5, 65.6, 60.3, 55.8 ($C_6H_4OCH_3$), 15.5 (C-*CH*$_3$). HRMS calcd for $C_{19}H_{28}O_{11}Na$ (M+Na)$^+$ 455.1529. Found: 455.1512.

4.9. *p*-Methoxylphenyl 2,3,4-tri-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (18)

To a cooled (−10 °C) solution of **14** (0.85 g, 0.89 mmol) and **17** (1.07 g, 0.98 mmol) in anhydrous, redistilled CH_2Cl_2 (40 mL) was added 4 Å molecular sieves (1.5 g) and the mixture was stirred under an N_2 atmosphere for 30 min. Then TMSOTf (27 μL, 0.15 mmol) was added to the mixture. The reaction mixture was stirred at −10 °C for 0.5 h, during which time the mixture was allowed to gradually warm to ambient temperature. TLC (petroleum ether–EtOAc 3:1) indicated that the reaction was complete. Then the reaction mixture was quenched with Et_3N (2 drops) and filtered. The filtrate was evaporated in vacuo to give a residue, which was purified by silica gel column chromatography (petroleum ether–EtOAc 3:1) to give tetrasaccharide **18** (1.3 g, 77%) a white foam. $[\alpha]_D^{25}$ −60.0 (*c* 1.0 $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06–6.68 (m, 59H, Ar-*H*), 5.73 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.56 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.6 Hz, H-4), 5.41–5.38 (m, 2H, H-1′, H-3′), 5.36 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.8 Hz, H-4″), 5.30 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 9.8 Hz, H-2), 5.22–5.16 (m, 5H, H-1‴, H-2′, H-4′, H-3‴, H-4‴), 5.08 (m, 1H, H-2‴), 4.90 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1″), 4.80 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 4.51–4.26 (m, 7H, H-5, 4 × H-6, H-2″, H-3″), 4.00–3.85 (m, 3H, 3 × H-5), 3.70 (s, 3H, $C_6H_4OCH_3$), 0.68, 0.64 (2d, 6H, *J* 6.4 Hz, 2 × C-*CH*$_3$). ^{13}C NMR (75 MHz, D_2O): δ 166.2, 166.1, 166.0(3), 165.7, 165.2, 164.8, 164.7, 164.6, 163.8 (11 × *C*OPh), 155.4, 150.4, 118.4(2), 114.6(2) ($C_6H_4OCH_3$), 100.0, 99.7, 97.8, 95.8 (4 × C-1), 78.7, 73.6, 73.2, 71.9, 70.2, 69.8, 69.1, 69.0, 68.3, 67.6, 67.5, 66.1, 66.0, 65.7, 63.1, 62.9, 55.5 ($C_6H_4OCH_3$), 15.8, 15.7 (2 × C-*CH*$_3$). ESI-MS *m/z* calcd for $C_{108}H_{92}O_{31}Na$ (M+Na)$^+$ 1907.6. Found: 1907.6. Anal. Calcd for $C_{108}H_{92}O_{31}$: C, 68.78; H, 4.92. Found: C, 68.93; H, 4.75.

4.10. *p*-Methoxylphenyl 6-deoxy-α-L-talopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-6-deoxy-α-L-talopyranosyl-(1→3)-α-D-glucopyranoside (5)

Compound **18** (0.25 g, 0.13 mmol) was dissolved in satd NH_3–MeOH (40 mL). After 1 week at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **5** (82 mg, 84%) as a foamy solid. $[\alpha]_D^{25}$ +35.6 (*c* 0.5 $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.99, 6.82 (2d, 4H, *J* 9.0 Hz, $C_6H_4OCH_3$), 5.34 (d, 1H, $J_{1,2}$ 3.4 Hz, H-1), 5.15 (s, 2H, H-1′, H-1‴), 4.55 (d, 1H, $J''_{1,2''}$ 3.4 Hz, H-1″), 4.27–4.17 (m, 2H, 2 × H-5), 4.05 (m, 1H, H-2‴), 3.99 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 3.0 Hz, H-3′), 3.88 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.4 Hz, H-3), 3.85–3.82 (m, 2H, H-4′, H-4‴), 3.80–3.77 (m, 2H), 3.72–3.62 (m, 9H), 3.51 (m, 1H), 3.45–3.38 (m, 2H), 3.38–3.33 (m, 2H), 1.14, 1.10 (2d, 6H, *J* 6.6 Hz, 2 × C-*CH*$_3$). ^{13}C NMR (75 MHz, D_2O): δ 154.6, 150.4, 118.8(2), 115.1(2) ($C_6H_4OCH_3$), 101.6, 101.5 (2 × C-1-Tal*p*), 101.1 (C-1″), 98.2 (C-1), 81.9, 79.9, 75.9, 73.8, 73.7, 72.5, 72.2, 71.7, 70.0, 69.9, 69.6, 68.0, 67.9, 67.5(2), 65.6, 60.7, 60.3, 55.8 ($C_6H_4OCH_3$), 15.5, 15.4 (2 × C-*CH*$_3$). HRMS calcd for $C_{31}H_{48}O_{20}Na$ (M+Na)$^+$ 763.2637. Found: 763.2624.

4.11. 2,3,4-Tri-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (19)

To a solution of compound **18** (1.0 g, 0.53 mmol) in acetonitrile (40 mL), and water (10 mL) was added CAN (1.2 g, 2.2 mmol). The mixture was stirred for 20 min at 30 °C, at the end of which time TLC (petroleum ether–EtOAc 1:1) indicated that the reaction was complete. The solvent was evaporated under diminished pressure to give a residue, which was dissolved in CH_2Cl_2, and washed with water. The organic phase was dried (Na_2SO_4) and concd. Purification by silica gel chromatography with petroleum ether–EtOAc 3:1 as the eluent afforded 2,3,4-tri-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2, 4 - d i - *O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranose as a slight yellow foamy solid. The foamy solid was dried under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (30 mL) and trichloroacetonitrile (0.5 mL, 5 mmol) and DBU (0.03 mL, 0.3 mmol) were added successively under an N_2 atmosphere. The mixture was stirred for 0.5 h and then concd. The residue was purified by chromatography with petroleum ether–EtOAc 4:1 to give **19** (0.71 g, 70%) as a foamy solid. $[\alpha]_D^{25}$ −40.0 (*c* 0.7 $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.57 (s, 1H, CN*H*CCl_3), 8.09–6.86 (m, 55H, Ar-*H*), 6.73 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.65 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.7 Hz, H-4), 5.41 (dd, 1H, $J_{1,2}$ 3.7 Hz, $J_{2,3}$ 9.7 Hz, H-2), 5.39 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 3.8 Hz, H-3′), 5.36–5.33 (m, 2H, H-1′, H-4″), 5.22–5.19 (m, 2H, H-2′, H-4′), 5.17 (d, 1H, $J_{1,2}$ 1.2 Hz,

H-1‴), 5.15 (m, 1H, H-3‴), 5.07 (m, 1H, H-2‴), 4.89 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1″), 4.67 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.5 Hz, H-3), 4.59–4.78 (m, 2H, H-5, H-6), 4.44–4.25 (m, 5H, H-2″, H-3″, 3 × H-6), 4.00–3.88 (m, 3H, 3 × H-5), 3.70 (s, 3H, $C_6H_4OCH_3$), 0.68, 0.64 (2d, 6H, *J* 6.4 Hz, 2 × C-CH_3). ESI-MS *m/z* calcd for $C_{103}H_{86}Cl_3NO_{30}Na$ $(M+Na)^+$ 1944.4. Found: 1944.8. Anal. Calcd for $C_{103}H_{86}Cl_3NO_{30}$: C, 64.29; H, 4.51; N, 0.73. Found: C,64.44; H, 4.68; N, 0.79.

4.12. *p*-Methoxylphenyl 2,3,4-tri-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-6-deoxy-α-L-talopyranosyl-(1→3)-2,4,6-tri-*O*-benzoyl-α-D-glucopyranoside (20)

Glycosylation between disaccharide acceptor **14** (0.14 g, 0.15 mmol) and tetrasaccharide donor **19** (0.30 g, 0.16 mmol) was accomplished by following the same procedure as described above for the preparation of tetrasaccharide **18**. After purification, hexasaccharide **20** (0.26 g, 65%) was afforded as a white foamy solid. $[\alpha]_D^{25}$ −59.1 (*c* 1.0 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 8.03–6.68 (m, 84H, Ar-*H*), 5.72 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.56 (t, 1H, $J_{3,4}$, $J_{4,5}$ 9.6 Hz, H-4), 5.42 (s, 1H, H-1-Tal*p*), 5.37 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.5 Hz, H-3-Glu*p*), 5.35–5.27 (m, 3H, H-2, 2 × H-4-Glu*p*), 5.22–5.09 (m, 7H, 2 × H-1-Tal*p*, 2 × H-3-Tal*p*, 3 × H-4-Tal*p*), 5.08–5.06 (m, 2H, 2 × H-2-Tal*p*), 4.90 (m, 1H, H-2-Tal*p*), 4.86 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1-Glu*p*), 4.80 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.4 Hz, H-3), 4.65 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1-Glu*p*), 4.52–4.08 (m, 11H, 2 × H-2-Glu*p*, 2 × H-3-Glu*p*, 6 × H-6-Glu*p*, H-5), 3.97–3.93 (m, 2H, 2 × H-5), 3.85–3.73 (m, 2H, 2 × H-5), 3.70 (s, 3H, $C_6H_4OCH_3$), 3.47 (m,1H, H-5), 0.67, 0.62, 0.58 (3d, 9H, *J* 6.4 Hz, 3 × C-CH_3). ^{13}C NMR (75 MHz, D_2O): δ 166.1(2), 166.0(2), 165.9(4), 165.6, 165.2, 164.9, 164.7, 164.6, 164.5, 164.4, 163.8 (16 × *C*OPh), 155.4, 150.4, 118.4(2), 114.6(2) ($C_6H_4OCH_3$), 99.9, 99.7(2), 98.5, 98.1, 95.7 (6 × C-1), 55.5 ($C_6H_4OCH_3$), 15.7, 15.5, 14.1 (3 × C-CH_3). ESI-MS *m/z* calcd for $C_{155}H_{132}O_{45}Na$ $(M+Na)^+$ 2735.8. Found: 2735.9. Anal. Calcd for $C_{155}H_{132}O_{45}$: C, 68.58; H, 4.90. Found: C, 68.49; H, 4.77.

4.13. *p*-Methoxylphenyl 6-deoxy-α-L-talopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-6-deoxy-α-L-talopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-6-deoxy-α-L-talopyranosyl-(1→3)-α-D-glucopyranoside (6)

Hexasaccharide **20** (0.18 g, 0.066 mmol) was dissolved in satd NH_3–MeOH (40 mL). After 1 week at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **6** (57 mg, 83%) as a foamy solid. $[\alpha]_D^{25}$ +67.0 (*c* 0.5 $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.02, 6.85 (2d, 4H, *J* 9.0 Hz, $C_6H_4OCH_3$), 5.36 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.16–5.14 (m, 3H, 3 × H-1-Tal*p*), 4.57, 4.55 (2d, 2H, *J* 7.8 Hz, H-1-Glu*p*), 4.27–4.18 (m, 3H, 3 × H-5-Tal*p*), 4.05–4.97 (m, 4H), 3.88 (t, 1H, $J_{2,3}$, $J_{3,4}$ 9.8 Hz, H-3), 3.85–3.78 (m, 6H), 3.72–3.59 (m, 10H), 3.56–3.35 (m, 10H), 1.14 (d, 1H, *J* 6.4 Hz, C-CH_3), 1.12 (d, 1H, *J* 6.2 Hz, C-CH_3), 1.10 (d, 1H, *J* 6.4 Hz, C-CH_3). ^{13}C NMR (75 MHz, D_2O): δ 154.7, 150.3, 118.8(2), 115.1(2) ($C_6H_4OCH_3$), 101.6, 101.5, 101.4 (3 × C-1-Tal*p*), 101.1, 101.1, 98.2 (3 × C-1-Glu*p*), 55.9 ($C_6H_4OCH_3$), 15.5(2), 15.4 (3 × C-CH_3). HRMS for $C_{43}H_{68}O_{29}Na$ $(M+Na)^+$ 1071.3744. Found: 1071.3719.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2011.09.001.

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