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Note

Preparation of orthogonally protected chitosan oligosaccharides: observation of an anomalous remote substituent effect

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Abstract

Orthogonally protected chitosan tetrasaccharides were synthesized in a convergent fashion by trichloroacetimidate activation. The anomeric substituent at the reducing end of the disaccharide acceptor has a remarkably strong influence on glycosidic coupling; a thiophenyl-substituted disaccharide was observed to be an unusually poor glycosyl acceptor in comparison with the corresponding allyloxy-substituted disaccharide. © 2002 Elsevier Science Ltd. All rights reserved.

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Repeating-unit oligosaccharides and their esters have been identified as signaling elements for a broad spectrum of biological processes, such as elicitors in plant defense mechanisms,[1] nodulation factors of leguminous roots,[2] or regulators of fibroblast growth factor-mediated cellular processes.[3] In several cases there is strong evidence that the positional distribution of acyl or sulfate groups on the carbohydrate repeat units has a critical role in determining the specificity of biological action. For example, chitosan sulfated mostly at the O-2 and O-3 positions has demonstrated potent in vitro activity against HIV-1 infection of T-lymphocytes, whereas chitin sulfated at the O-6 position has exhibited strong anticoagulatory properties.[4] However, the intrinsic polydispersity of fractionated or semisynthetic carbohydrates introduces ambiguity in determining the structural basis for biological activity.

Systematic studies with repeating-unit oligosaccharides of defined sizes and substitution patterns can be useful for establishing unambiguous structure–activity relationships. To this end we are developing protecting group systems whose cleavage conditions are compatible with sensitive functional groups such as sulfates.[5] Here we present a concise synthesis of an orthogonally protected chitosan tetrasaccharide with an allyl group at the reducing end as a functionalizable tether.[6] In the course of this work, we have determined that remote substituents can have surprisingly dramatic effects on reactivity during glycosidic coupling.

Glucosamine-derived acceptor and donor **1** and **2** were synthesized using known procedures[6,7] and coupled in the presence of catalytic trimethylsilyl trimethanesulfonate (TMSOTf) to yield disaccharide **3** on a multigram scale (see Scheme 1). Trichloroacetimidate-activated glycosylation gave consistently better yields in our hands than direct activation of the thiophenyl glycoside.[8] It should be mentioned that oxidative cleavage of the anomeric thiophenyl group with *N*-bromosuccinimide (NBS) was accompanied by partial bromination of the *p*-methoxybenzyl (PMB) protecting group at O-6; however, this did not appear to interfere with subsequent transformations.[9] The protected disaccharide **3** was converted straightforwardly into glycosyl acceptors **4** and **6** and into glycosyl donor **5**, but attempts to couple disaccharides **4** and **5** were unsuccessful despite numerous variations in reaction conditions. Increasing the amount of TMSOTf to 1.3–1.5 equiv did not improve the yield of coupling reactions involving **4**, indicating that coordination of the Lewis acid by the thiophenyl group was not a rate-determining factor. However, substituting the anomeric *S*-thiophenyl unit with an *O*-allyl group greatly in-

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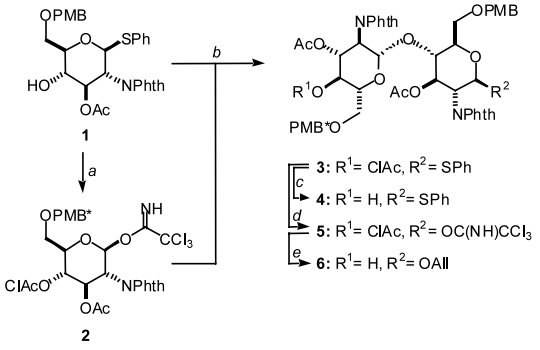
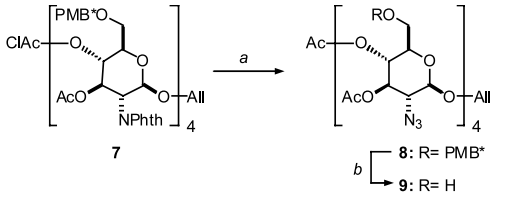
creased the reactivity of the nucleophile, enabling tetrasaccharide **7** to be obtained in 70% yield. Intermediate **7** was then transformed into orthogonally protected tetrasaccharide **8** by aminolysis of phthalimide and acetate groups with ethylenediamine,[10] followed by reprotection as a tetraazide (see Scheme 2).[11] The partially brominated PMB groups were cleaved to yield the corresponding tetraol **9**, whose chemical composition was confirmed by elemental analysis.

A systematic investigation of the glycosylation step with different coupling partners revealed thiophenyl disaccharide **4** to be a singularly poor glycosyl acceptor (see Table 1), encouraging us to examine the influence of the remote anomeric substituent in further detail. Chromatographic recovery and analysis of the components from the attempted coupling of acceptor **4** with donor **5** revealed a significant amount of TMS-protected acceptor, whereas relatively little TMS ether was recovered from the coupling reaction between **6** and **5**. Acceptors **4** and **6** were treated with TMSOTf at −78 °C in CH_2Cl_2 in the presence of tetramethylurea as a proton scavenger to determine their relative rates of TMS ether formation, but no significant differences were observed. We next considered the possibility of the thiophenyl moiety having a role as a trimethylsilyl transfer agent; however, adding one molar equivalent of thiophenyl disaccharide **3** to the coupling reaction between **6** and **5** affected neither the yield of the tetrasaccharide **7** nor the amount of TMS-protected side product.

Substituent effects on glycosylation rates have been extensively investigated,[12,13] but to the best of our knowledge remote substituent effects of this nature have not been documented despite the widespread use of thioarylglycosides as intermediates in oligosaccharide synthesis.[8,10] Although the basis for the deactivating influence of the remote thiophenyl group on glycosyl acceptors remains undetermined, the anomalous effect may have some broader implications, e.g., in the design of solid-phase syntheses for constructing oligosaccharide libraries.[14,15]

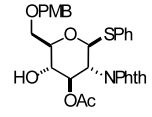
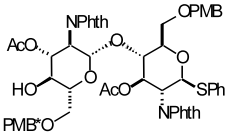
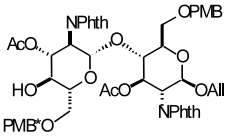


Scheme 1. Synthesis of chitosan disaccharide intermediates. All = allyl, Phth = phthalimido, PMB(*) = *p*-methoxybenzyl (or *m*-bromo-*p*-methoxybenzyl). Reagents and conditions: a. (i) $ClCH_2COCl$, pyridine, Et_2O, −20 °C; (ii) NBS, 9:1 CH_3COCH_3–water, −20 to 0 °C; (iii) CCl_3CN, DBU, CH_2Cl_2, 0 °C (70% over three steps). b. **2** (1.5 equiv), TMSOTf (0.3 equiv), 4 Å mol. sieves, CH_2Cl_2, −20 °C (78%). c. Thiourea, 1:1 MeOH–CH_2Cl_2, 40 °C (95%). d. Same as a, ii and iii (77% over two steps). e. (i) allyl alcohol, 4 Å mol. sieves, TMSOTf, CH_2Cl_2, −20 °C to rt; (ii) thiourea, 1:1 MeOH–CH_2Cl_2, 40 °C (95% over two steps).



Scheme 2. Synthesis of orthogonally protected chitosan tetrasaccharide. All = allyl, Phth = phthalimido, PMB(*) = *p*-methoxybenzyl (or *m*-bromo-*p*-methoxybenzyl). Reagents and conditions: a. (i) ethylenediamine, *n*-BuOH, 100 °C; (ii) TfN_3, $CuSO_4$, K_2CO_3, 10:9:1 CH_2Cl_2–MeOH–water; (iii) Ac_2O, pyridine (39% yield over three steps). b. DDQ, 4:1 CH_2Cl_2–water, rt (60%).

Table 1
TMSOTf-mediated coupling of mono- and disaccharide trichloroacetimidates with different glycosyl acceptors[a]

Glycosyl acceptor	Coupling yield[b] (%)	
	Monosaccharide **2**	Disaccharide **5**
 **1**	78	60
 **4**	15	0
 **6**	56	50 (70[c])

[a] All = allyl, Phth = phthalimido, PMB(*) = *p*-methoxybenzyl (or *m*-bromo-*p*-methoxybenzyl).

[b] Reaction conditions: 1.0 equiv glycosyl acceptor (0.1 M), 1.5 equiv glycosyl donor, 0.3 equiv TMSOTf, 4 Å mol. sieves, CH_2Cl_2, −20 °C to rt.

[c] Same as b, but with 1.2 equiv of glycosyl donor, −20 to 4 °C.

1. Experimental

General methods.—All chemicals were obtained from Aldrich Chemical Co. and used as received. All experiments were conducted under an atmosphere of dry argon unless otherwise noted. IR spectra were acquired with a Nicolet Nexus 670 FTIR spectrometer. Optical rotations were measured at 20–25 °C with a Rudolph Research AUTOPOL® III polarimeter. ^{1}H and ^{13}C spectra were recorded on a Varian Unity Inova NMR spectrometer operating at 300 and 75 MHz, respectively, or on a Bruker DRX 500 operating at 500 and 125 MHz, respectively. Chemical shifts are referenced to the solvent used (7.27 and 77.23 ppm for $CDCl_3$, 3.31 and 49.15 ppm for CD_3OD, 7.16 and 128.39 ppm for C_6D_6) unless otherwise stated. Electrospray-ionization mass spectra (ESIMS) were acquired using either a Hewlett–Packard 5989B or a Finnigan 4000 mass spectrometer. The chromatographic purity of the products was assessed using TLC on Silica Gel 60 F_{254} (E. Merck) with either ethanolic *p*-anisaldehyde or ninhydrin staining solutions. Chromatographic separations were performed using silica gel (ICN SiliTech 32–63D). Elemental analysis was performed in the Department of Chemistry, Purdue University. In cases where compounds were partially brominated, molar equivalents and yields were determined using the relative intensities of the ^{1}H NMR peaks in the aromatic region to estimate the fraction of brominated product.

3-O-Acetyl-4-O-chloroacetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**2**).—Toluene (3 × 25 mL) was evaporated from thiophenyl glycoside **1** (2.3 g, 4.08 mmol), and the residue was dissolved in anhyd Et_2O (38 mL). The solution was cooled to −15 °C, and pyridine was added (1.65 mL, 20.42 mmol) followed by dropwise addition of chloroacetyl chloride (1.3 mL, 16.33 mmol). The resulting mixture was stirred for 1 h from −15 to −10 °C. Saturated aq $NaHCO_3$ was added, and the mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were evaporated to dryness, and the resulting residue was dissolved in 9:1 CH_3COCH_3–water (60 mL) and cooled to −20 °C, followed by addition of NBS (2.78 g, 15.64 mmol). After stirring for 1 h from −20 to −10 °C, the reaction was quenched with satd aq $NaHCO_3$ (20 mL) and diluted with water (20 mL), followed by extraction with dichloromethane (3 × 50 mL). The combined organic extract was dried over $MgSO_4$, filtered and concentrated in vacuo. Chromatography on silica gel (20–40% EtOAc–hexanes gradient elution) afforded 1.87 g of the intermediate lactol as a white solid. This product was azeotropically dried with toluene (3 × 50 mL) and dissolved in dry CH_2Cl_2 (35 mL), followed by addition of 4 Å molecular sieves (3.5 g). The mixture was stirred at room temperature for 30 min, then cooled to 0 °C and treated with CCl_3CN (6.85 mL, 68.4 mmol) and DBU (153 μL, 1.03 mmol). After 2 h the reaction mixture was concentrated in vacuo and purified by flash chromatography (10–20% EtOAc–toluene, +1% Et_3N) to yield trichloroacetimidate **2** (1.98 g, 70% over three steps) as a white solid with a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers. $[\alpha]_D$ +58.6° (*c* 1.03, CH_2Cl_2); IR (neat): 1747, 1719, 1385, 1221 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.71 (1H, d, *J* 6.3 Hz), 7.89–7.74 (4H, m), 7.27 (2H, dd, *J* 2.4, 9.0 Hz), 6.92 (2H, d, *J* 8.7 Hz), 6.66 (1H, dd, *J* 1.8, 9.0 Hz), 5.96 (1H, dt, *J* 4.5, 9.9 Hz), 5.42 (1H, dt, *J* 2.4, 9.9 Hz), 4.71–4.42 (3H, m), 4.13–3.63 (8H, m), 1.93 (3H, d, *J* 1.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.49, 167.65, 166.44, 160.86, 159.67, 155.84, 134.74, 133.49, 131.46, 131.33, 130.08, 129.71, 128.80, 123.97, 114.08, 112.07, 93.85, 73.88, 73.84, 73.43, 72.72, 71.44, 71.37, 70.54, 68.26, 67.99, 56.56, 55.55, 53.82, 40.75, 20.75. ESIMS: *m*/*z* 713 $[M + Na]^+$, 792, 794 $[M + Br + Na]^+$.

Phenyl 3-O-acetyl-4-O-chloroacetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside (**3**).—A solution of trichloroacetimidate **2** (4.05 g, 5.86 mmol, a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers) and thiophenyl glycoside **1** (2.2 g, 3.91 mmol) in dry CH_2Cl_2 (43 mL) was stirred under argon at room temperature with 4 Å molecular sieves (4.3 g) for 30 min. The mixture was cooled to −20 °C, then treated with TMSOTf (212 μL, 1.173 mmol). After 5 h, the reaction was quenched with Et_3N and concentrated to an oil. The residue was purified by flash chromatography (10–20% EtOAc–toluene) to yield thiophenyl disaccharide **3** (3.37 g, 78%) as a white solid with a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers. $[\alpha]_D$ +20.9° (*c* 1.03, CH_2Cl_2); IR (neat): 1777, 1747, 1715, 1385, 1229 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.87–7.74 (8H, m), 7.38–7.16 (13H, m), 5.79–5.68 (2H, m), 5.61 (1H, d, *J* 10.5 Hz) 5.45 (1H, d, *J* 8.4 Hz), 5.29 (1H, t, *J* 9.3 Hz), 4.46–3.40 (21H, m), 1.88 (3H, s), 1.85 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.56, 170.40, 168.01, 167.51, 166.35, 134.62, 134.38, 133.23, 131.98, 131.72, 131.64, 131.51, 130.62, 129.95, 129.68, 129.44, 129.06, 128.34, 123.91, 114.05, 113.93, 112.17, 97.37, 83.14, 78.78, 74.28, 73.27, 72.71, 72.41, 72.31, 71.73, 70.75, 68.24, 67.77, 56.55, 55.55, 55.17, 54.20, 40.78, 20.81, 20.69. ESIMS: *m*/*z* 1115 $[M + Na]^+$ 1194, 1196 $[M + Br + Na]^+$.

Phenyl 3-O-acetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-1-thio-β-D-glucopyranoside (**4**).—A mixture of thiophenyl disaccharide **3** (500 mg, 0.458 mmol, a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers) and thiourea (175 mg, 2.29 mmol) in 1:1 MeOH–CH_2Cl_2 (25 mL) was stirred at 40 °C for 14 h, then concentrated to an

oil. The residue was purified by flash chromatography (0–2% MeOH–CH_2Cl_2) to yield disaccharide acceptor **4** (442 mg, 95%) as a white solid with a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers. $[\alpha]_D$ +5.2° (*c* 1.03, CH_2Cl_2); IR (neat): 2941, 1777, 1746, 1715, 1384, 1225, 1049 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.86–7.74 (8H, m), 7.50–6.87 (13H, m), 5.73 (1H, t, *J* 0.5 Hz), 5.63–5.57 (2H, m), 5.47 (1H, d, *J* 8.1 Hz), 4.53–3.42 (20H, m), 3.00 (1H, s), 1.92 (3H, s), 1.86, (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 171.23, 171.09, 170.18, 167.95, 167.44, 159.60, 159.27, 134.90, 134.54, 134.42, 134.31, 133.13, 132.99, 131.68, 131.47, 130.54, 129.64, 129.56, 129.35, 128.99, 128.42, 128.34, 128.24, 124.10, 123.85, 123.71, 117.73, 116.08, 114.13, 113.84, 113.10, 112.11, 111.76, 110.51, 109.34, 100.99, 97.46, 83.01, 78.81, 74.39, 73.89, 73.53, 73.38, 73.21, 72.65, 71.78, 71.34, 70.20, 70.04, 67.72, 65.01, 56.50, 55.49, 55.11, 54.17, 52.46, 20.81; ESIMS: *m*/*z* 1039 $[M + Na]^+$, 1118, 1120 $[M + Br + Na]^+$.

3-O-Acetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-4-O-chloroacetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**5**).—To a solution of thiophenyl disaccharide **3** (340 mg, 0.311 mmol, a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers) in 9:1 CH_3COCH_3–water (5 mL) at −20 °C was added NBS (333 mg, 1.868 mmol). After stirring for 1 h from −20 to 0 °C, the reaction was quenched with satd aq $NaHCO_3$ (1 mL) and diluted with water (1 mL), followed by extraction with CH_2Cl_2 (3 × 5 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated to an oil. Chromatography on silica gel (20–40% EtOAc–hexanes) afforded 280 mg of the intermediate lactol as a white solid. This was azeotropically dried with toluene (3 × 5 mL), and the residue was dissolved in dry CH_2Cl_2 (7 mL), followed by addition of 4 Å molecular sieves (700 mg). The mixture was stirred at room temperature for 30 min, then cooled to 0 °C. Cl_3CCN (421 μL, 4.2 mmol) and DBU (13 μL, 0.084 mmol) were added. After 5 h the reaction mixture was concentrated and purified by flash chromatography (10–15% EtOAc–toluene, +1% Et_3N) to yield disaccharide donor **5** (288 mg, 75% over two steps) as a white solid with a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers. $[\alpha]_D$ +21.8° (*c* 1.03, CH_2Cl_2); IR (neat): 1742, 1715, 1493, 1388, 1225, 1054 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 8.59 (1H, s), 7.90–7.72 (8H, m), 7.52–6.88 (8H, m) 6.53 (1H, d, *J* 8.7 Hz), 5.86–5.74 (2H, m), 5.51 (1H, d, *J* 8.4 Hz), 5.32 (1H, t, *J* 9.3 Hz), 4.57–3.49 (21H, m), 1.93 (3H, s), 1.87 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.44, 170.21, 167.72, 166.28, 160.75, 155.77, 155.61, 134.62, 134.49, 133.21, 131.91, 131.58, 131.24, 129.88, 128.71, 128.56, 123.77, 114.02, 113.90, 112.15, 111.94, 111.64, 97.12, 93.79, 75.37, 73.66, 72.55, 71.96, 71.46, 70.97, 68.43, 67.29, 56.50, 55.48, 55.09, 54.11, 40.69, 20.78, 20.61. ESIMS: *m*/*z* 1166 $[M+Na]^+$, 1245, 1247 $[M + Br + Na]^+$, 1324, 1326, 1328 $[M + 2\ Br + Na]^+$.

Allyl 3-O-acetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranoside (**6**).—A solution of disaccharide donor **5** (20 mg, 0.0175 mmol, a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers) and allyl alcohol (2.5 μL, 0.035 mmol) in dry CH_2Cl_2 (0.5 mL) was stirred under argon at room temperature with 4 Å molecular sieves (50 mg) for 30 min. The mixture was then cooled to −20 °C, followed by addition of TMSOTf (3.2 μL, 17.5 μmol). After 3 h, the reaction was quenched with Et_3N, filtered and evaporated to dryness. The remaining residue was dissolved in 1:1 MeOH–CH_2Cl_2 (1 mL). Thiourea (6.7 mg, 87.5 μmol) was added, and the mixture was stirred at 40 °C for 14 h, then concentrated to an oil. The residue was purified by flash chromatography (0–2% MeOH–CH_2Cl_2) to yield disaccharide acceptor **6** (16 mg, 95%) as a white solid with a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers.

Compound **6** was also prepared in 63% yield with a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers by coupling trichloroacetimidate **2** (7.31 g, 11.55 mmol, a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers) with allyl 3-*O*-acetyl-4-*O*-chloroacetyl-2-deoxy-6-*O*-*p*-methoxybenzyl-2-phthalimido-β-D-glucopyranoside[6b] (6.54 g, 13.87 mmol). $[\alpha]_D$ −21.0° (*c* 1.03, CH_2Cl_2); IR (neat): 2867, 1773, 1746, 1715, 1610, 1509, 1384, 1240, 1042 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.86–7.72 (8H, m), 7.30–6.85 (8H, m), 5.77–5.51 (3H, m), 5.48 (1H, d, *J* 8.4 Hz), 5.28 (1H, d, *J* 8.4 Hz), 5.05 (2H, dq, *J* 1.2, 17.1 Hz), 4.54–3.44 (21H, m), 3.09 (1H, dd, *J* 3.3, Hz), 1.91 (3H, s), 1.88 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.30, 171.17, 168.05, 159.63, 159.32, 155.77, 134.49, 133.83, 133.01, 131.76, 131.48, 130.55, 130.06, 129.77, 129.61, 129.41, 128.43, 123.72, 117.70, 114.18, 113.98, 113.88, 112.19, 111.75, 97.39, 97.20, 77.56, 74.70, 74.63, 74.05, 73.63, 73.53, 73.46, 72.75, 72.64, 71.91, 71.69, 71.24, 70.14, 70.01, 67.63, 56.56, 55.53, 55.27, 55.21, 20.93, 20.87; ESIMS: *m*/*z* 987 $[M + Na]^+$, 1066, 1068 $[M + Br + Na]^+$.

Allyl 3-O-acetyl-4-O-chloroacetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranosyl-(1→4)-3-O-acetyl-2-deoxy-6-O-p-methoxybenzyl-2-phthalimido-β-D-glucopyranoside (**7**).—A solution of disaccharide donor **5** (288 mg, 0.252 mmol, a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers) and disaccharide acceptor **6** (202 mg, 0.210 mmol, a 4:1 mixture of non- and mono-3-brominated-

PMB-protected ethers) in dry CH_2Cl_2 (2 mL) was stirred under argon at room temperature with 4 Å molecular sieves (200 mg) for 30 min. The reaction mixture was then cooled to −20 °C, followed by addition of TMSOTf (11.5 μL, 0.063 mmol). The reaction was stirred from −20 °C to 4 °C over a period of 4 h, then quenched with Et_3N and concentrated to an oil. The residue was purified by flash chromatography (10–60% THF–hexanes) to yield tetrasaccharide **7** (289 mg, 70%) as a white solid with an approximately 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers. $[\alpha]_D$ −7.7° (*c* 1.03, CH_2Cl_2); IR (neat): 1777, 1746, 1711, 1610, 1497, 1384, 1225, 1046 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.85–7.69 (16H, m), 7.45–6.75 (16H, m), 5.75–5.22 (10H, m), 5.02 (2H, t, *J* 11.7 Hz), 4.42–3.21 (43H, m), 1.85 (3H, s), 1.83 (3H, s), 1.77 (3H, s), 1.72 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.49, 170.30, 168.34, 167.62, 166.30, 159.26, 155.78, 155.59, 155.45, 134.66, 134.37, 133.81, 133.23, 132.83, 132.55, 131.95, 131.58, 131.26, 130.42, 130.30, 130.03, 129.31, 129.06, 128.78, 128.34, 128.15, 123.66, 117.67, 113.95, 113.84, 113.79, 112.17, 112.04, 111.52, 97.03, 77.54, 74.61, 74.38, 74.25, 73.63, 72.61, 72.55, 72.41, 71.72, 71.40, 71.30, 70.84, 70.11, 68.36, 67.79, 67.49, 67.35, 56.56, 55.55, 55.50, 55.20, 40.76, 20.80, 20.66. ESIMS: *m*/*z* 1969 $[M + Na]^+$, 2048, 2050 $[M + Br + Na]^+$, 2104, 2106, 2108 $[M + 2\ Br + Na]^+$.

Allyl 3,4-di-O-*acetyl-2-azido-2-deoxy-6*-O-p-*methoxybenzyl-β-*D-*glucopyranosyl-(1→4)-3*-O-*acetyl-2-azido-2-deoxy-6*-O-p-*methoxybenzyl-β*-D-*glucopyranosyl-(1→4)-3*-O-*acetyl-2-azido-2-deoxy-6*-O-p-*methoxybenzyl-β*-D-*glucopyranosyl-(1→4)-3*-O-*acetyl-2-azido-2-deoxy-6*-O-p-*methoxybenzyl-β*-D-*glucopyranoside* (**8**).—Tetrasaccharide **7** (100 mg, 0.051 mmol, a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers) in 1:1 *n*-BuOH–$H_2N(CH_2)_2NH_2$ (16 mL) was stirred at 100 °C in a sealed vessel for 24 h. The reaction mixture was then cooled to room temperature and transferred to a round-bottomed flask, followed by azeotropic removal of all volatiles with toluene (5 × 25 mL) to afford the crude amine as a yellow solid. The solid was redissolved with 90% MeOH (20 mL) and treated with freshly prepared TfN_3 in CH_2Cl_2 (20 mL), $CuSO_4$ (1 mg), and K_2CO_3 (3 mg). After 24 h at room temperature, the mixture was concentrated to dryness and passed through a short silica gel column (2:1 EtOAc–hexanes). The partially purified tetraazide was treated with 3:2 pyridine–Ac_2O (4 mL). After 2 days at room temperature, the reaction was poured into cold water, followed by extraction with CH_2Cl_2 (3 × 10 mL). The extracts were washed with satd aq $NaHCO_3$ (2 × 20 mL), dried, concentrated, and purified by flash chromatography (10–40% EtOAc–hexanes) to afford tetraazide **8** (30 mg, 39% over three steps) as a white solid with a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers. $[\alpha]_D$ −30.3° (*c* 1.03, CH_2Cl_2); IR (neat): 2112, 1750, 1606, 1497, 1365, 1228, 1053 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.51–6.87 (16H, m), 5.98 (1H, sp, *J* 5.4 Hz), 5.33 (2H, dq, *J* 1.5, 17.4 Hz), 5.06–3.07 (50H, m), 2.13 (3H, s), 2.11 (3H, s), 2.06 (3H, s), 1.96 (3H, s), 1.64 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.55, 170.45, 170.35, 170.30, 170.23, 169.71, 159.73, 156.07, 155.83, 133.50, 133.40, 133.26, 131.11, 131.02, 130.84, 130.04, 129.98, 129.86, 129.31, 128.78, 128.68, 128.56, 120.77, 119.91, 118.23, 116.82, 114.25, 114.13, 113.94, 112.12, 112.04, 111.93, 111.76, 110.55, 107.63, 105.05, 103.07, 101.19, 101.09, 100.93, 100.87, 74.75, 74.58, 74.24, 74.11, 73.50, 73.37, 73.31, 73.09, 72.73, 72.52, 72.41, 72.25, 70.64, 68.75, 68.16, 67.98, 67.30, 64.27, 64.21, 64.15, 64.10, 64.04, 56.52, 56.47, 55.46, 21.03, 20.92. ESIMS: *m*/*z* 1574, 1576 $[M + Br + Na]^+$, 1675, 1677, 1679 $[M + 2\ Br + Na]^+$.

Allyl 3,4-di-O-*acetyl-2-azido-2-deoxy-β*-D-*glucopyranosyl-(1→4)-3*-O-*acetyl-2-azido-2-deoxy-β*-D-*glucopyranosyl-(1→4)-3*-O-*acetyl-2-azido-2-deoxy-β*-D-*glucopyranosyl-(1→4)-3*-O-*acetyl-2-azido-2-deoxy-β*-D-*glucopyranoside* (**9**).—To a solution of tetraazide **8** (35 mg, 0.023 mmol, a 4:1 mixture of PMB- and (3-bromo)-PMB-protected ethers) in 4:1 CH_2Cl_2–water (5 mL) at room temperature was added DDQ (160 mg, 0.701 mmol). After stirring for 21 h, the reaction was quenched with satd aq $NaHCO_3$ (5 mL), followed by extraction with CH_2Cl_2 (3 × 5 mL). The combined extracts were dried over $MgSO_4$, filtered and concentrated to an oil. Chromatography on silica gel (20–25% CH_3COCH_3–toluene) yielded tetraol **9** (15 mg, 60%) as a white solid: $[\alpha]_D$ −2.2° (*c* 0.45, CH_2Cl_2); IR (neat): 3517, 2111, 1751, 1368, 1228, 1156, 1038 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 5.98 (1H, sp, *J* 5.4 Hz), 5.33 (2H, dq, *J* 1.5, 17.4 Hz), 5.06–3.38 (34H, m), 2.28–2.05 (15H, m). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.90, 170.51, 170.17, 170.06, 133.27, 118.59, 101.28, 101.17, 101.07, 101.02, 75.34, 75.28, 75.23, 74.80, 74.71, 74.44, 72.84, 72.70, 72.59, 72.44, 70.98, 68.97, 64.41, 64.31, 64.26, 64.20, 61.31, 60.93, 60.84, 60.65, 21.16, 21.11, 20.99, 20.87, 20.83. Anal. calcd for $C_{37}H_{52}N_{12}O_{22}$: C, 43.70; H, 5.15; Found: C, 43.70; H, 5.22%.

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