Sequential One-Pot Glycosylations Using 1-Hydroxyl and 1-Thiodonors

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General Procedures. All reactions were performed under argon atmosphere. Dichloromethane was refluxed with P₂O₅ and distilled before use. TTBP was synthesized as described by Crich *et al.*¹ Traces of water in the donor and acceptor glycosides, diphenylsulfoxide and TTBP were removed by coevaporation with toluene and dichloroethane. Molecular sieves (3Å) were flame dried before use. ¹H and ¹³C NMR spectra were recorded with a Bruker DPX 300 (300 and 75.1 MHz) or a Bruker AV 400 (400 and 100 MHz). ¹H NMR chemical shifts (δ) in CDCl₃ are reported relative to tetramethylsilane. Mass spectra were recorded on a PE/SCIEX API 165 equipped with an Electrospray Interface (Perkin-Elmer). Optical rotations were recorded on a Propol automatic polarimeter in CHCl₃.

General procedure for the dehydrative condensations of 1-hydroxyl donors and thioglycosides:

To a solution of the 1-hydroxyl donor (1.2-1.5 equiv to acceptor, 0.02 M in DCM), Ph₂SO (2.2 equiv to donor) and TTBP in DCM at -60°C was added Tf₂O (1.05 equiv to donor). The temperature was raised to -40°C and stirred at this temperature for one hour. Then a solution of acceptor in DCM (0.1 M) was added and the reaction mixture was allowed to warm to room temperature. Dry Et₃N (10 equiv to donor) was added and the

reaction mixture was washed with saturated NaHCO₃ and water. After drying (MgSO₄) and concentration the residue was purified by column chromatography (ethyl acetate/petroleum ether or ethyl acetate/toluene).

Ethyl 4-*O*-(2,3,4,6-tetra-*O*-benzyl- α/β -D-glucopyranosyl)-2,3,6-tri-*O*-benzyl-1-thio- β -D-glucopyranoside (16): 2,3,4,6-tetra-*O*-benzyl- α/β -D-glucopyranose **8** (97 mg, 0.18 mmol) was activated with Ph₂SO (80 mg, 0.40 mmol) and Tf₂O (33 μ L, 0.20 mmol) in the presence of TTBP (112 mg, 0.45 mmol) as described above. Condensation with ethyl 2,3,6-tri-*O*-benzyl-1-thio- β -D-glucopyranoside **12** (74 mg, 0.15 mmol) gave disaccharide **16** (88 mg, α/β 3:1, 87%) as an oil.²

3,4-di-*O*-benzoyl-6-*O*-tert-butyldimethylsilyl- α -D-glucopyranose 1,2-[phenyl phenyl (3-*O*-benzyl-2,3-*O*-isopropylidene-1-thio- α -D-mannopyranosid-6-yl) orthoacetate] (17): 2,3,4-tri-*O*-benzoyl-6-*O*-tert-butyldimethylsilyl- α -D-glucopyranose **9** (124 mg, 0.20 mmol) was activated with Ph₂SO (115 mg) and Tf₂O (35 μ L) in the presence of TTBP (152 mg) as described above. Condensation with phenyl 4-*O*-benzyl-2,3-*O*-isopropylidene-1-thio- α -D-mannopyranoside **13** (69 mg, 0.17 mmol) gave after basic work-up (drying with Na₂SO₄) and purification using neutralized silicagel (2% Et₃N added to the eluent) orthoester **17** (136 mg, 81%) as an oil. R_f 0.70 (10% EtOAc in toluene). ¹H NMR (400 MHz, CDCl₃): 8.08-7.13 (m, 25H, CH_{arom}), 5.81 (d, 1H, *J* = 5.3 Hz, H-1'), 5.73 (s, 1H, H-1), 5.62 (dd, 1H, *J* = 1.5 Hz, *J* = 2.3 Hz, H-3'), 5.35 (d, 1H, *J* = 8.6 Hz, H-4'), 4.81 (d, 1H, *J* = 11.5 Hz, CHH Bn), 4.47 (m, 2H, H-2', CHH Bn), 4.31 (m, 2H, H-2, H-3), 4.24 (m, 1H, H-5), 3.82 (m, 1H, H-5'), 3.72 (m, 2H, H-6', H-6'), 3.56 (dd, 1H, *J* = 2.0 Hz, *J* = 10.6 Hz, H-6), 3.43 (m, 2H, H-4, H-6), 1.47 (s, 3H, CH₃ isoprop), 1.34 (s, 3H, CH₃ isoprop), 0.79 (s, 9H, CH₃ *t*Bu TBS), -0.05 (s, 3H, CH₃ TBS), -0.06 (s, 3H, CH₃ TBS); ¹³C NMR (100 MHz, CDCl₃): δ = 165.0, 164.4, 137.9, 135.2, 133.4, 133.2, 132.0, 130.0, 129.8, 129.5, 129.4, 129.3, 128.8, 128.4, 128.3, 128.2, 127.7, 127.55, 127.48, 126.6, 120.9, 109.5, 97.6, 83.9, 78.3, 76.3, 76.2, 72.7, 71.9, 69.5, 69.2, 69.1, 68.2, 63.7, 63.1, 27.9, 26.3, 25.7, 18.1, -5.5; ES-MS: *m/z* 1013.6 [M + Na]⁺.

3,4,6-tri-*O*-pivaloyl- α -D-glucopyranose 1,2-[*tert*-butyl phenyl (3-*O*-benzyl-2,3-*O*-isopropylidene-1-thio- α -D-mannopyranosid-6-yl) orthoacetate] (18): 2,3,4,6-tetra-*O*-pivaloyl- α -D-glucopyranose **10** (77 mg, 0.15 mmol) was condensed with phenyl 3-*O*-benzyl-2,3-*O*-isopropylidene-1-thio- α -D-mannopyranoside (**13**) as described for the condensation of **9** and **13** to give orthoester **18** (85%). R_f 0.85 (25% EtOAc in PE). $[\alpha]_D^{25} +41.0$ ($c = 0.50$, CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.47\text{--}7.27$ (m, 10H, CH_{arom}), 5.66 (s, 1H, H-1), 5.51 (d, 1H, $J = 5.6$ Hz, H-1'), 5.17 (dd, 1H, $J = 3.3$ Hz, $J = 4.2$ Hz, H-3'), 4.89 (m, 2H, H-4', CHH Bn), 4.62 (d, 1H, $J = 11.2$ Hz, CHH Bn), 4.30 (m, 2H, H-2, H-3), 4.29 (dd, 1H, $J = 3.2$ Hz, $J = 5.6$ Hz, H-2'), 4.14 (m, 4H, H-5, H-5', H-6', H-6'), 3.75 (m, 3H, H-4, H-6, H-6'), 1.50 (s, 3H, CH_3 isoprop), 1.38 (s, 3H, CH_3 isoprop), 1.24 (s, 9H, CH_3 Piv), 1.21 (s, 18H, 2 x CH_3 Piv), 1.03 (s, 9H, CH_3 Piv); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 177.5, 176.8, 176.3, 138.1, 133.1, 131.8, 129.1, 128.3, 128.0, 127.9, 127.7, 124.6, 109.6, 96.9, 84.0, 78.4, 76.3, 75.6, 74.3, 73.3, 70.8, 69.9, 67.8, 67.3, 63.4, 62.7, 39.4, 38.8, 38.64, 38.55, 28.0, 27.1, 27.0, 26.5, 25.7$; ES-MS: m/z 923.5 $[\text{M} + \text{Na}]^+$.

Phenyl 6-*O*-(2,3,4,6-tetra-*O*-pivaloyl- α -D-glucopyranosyl)-3-*O*-benzyl-2,3-*O*-isopropylidene-1-thio- α -D-mannopyranoside (19): 2,3,4,6-tetra-*O*-pivaloyl- α -D-glucopyranose **10** (77 mg, 0.15 mmol) was activated with Ph_2SO (66 mg) and Tf_2O (28 μL) in the presence of TTBP (34 mg, 1.0 equiv to donor) and condensed with phenyl 3-*O*-benzyl-2,3-*O*-isopropylidene-1-thio- α -D-mannopyranoside (**13**) to give disaccharide **19** in 80% yield. R_f 0.80 (25% EtOAc in PE). $[\alpha]_D^{25} +56.4$ ($c = 0.83$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): 7.53-7.26 (m, 10 H, CH_{arom}), 5.76 (s, 1H, H-1), 5.12 (t, 1H, $J = 9.4$ Hz, H-3'), 4.94 (m, 2H, H-2', H-4'), 4.88 (d, 1H, $J = 11.6$ Hz, CHH Bn), 4.58 (d, 1H, $J = 11.6$ Hz, CHH Bn), 4.43 (d, 1H, $J = 8.0$ Hz, H-1'), 4.34 (m, 2H, H-2, H-3), 4.20 (m, 1H, H-5), 4.13 (dd, 1H, $J = 1.6$ Hz, $J = 12.2$ Hz, H-6'), 3.96 (m, 2H, H-6, H-6'), 3.83 (dd, 1H, $J = 2.5$ Hz, $J = 11.9$ Hz, H-4), 3.40 (m, 1H, H-5'), 1.49 (s, 3H, CH_3 isoprop), 1.37 (s, 3H, CH_3 isoprop), 1.18 (s, 9H, CH_3 Piv), 1.16 (s, 9H, CH_3 Piv), 1.13 (s, 9H, CH_3 Piv), 1.10 (s, 9H, CH_3 Piv); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 178.9, 178.0, 177.2, 138.8, 134.4, 131.9, 130.2, 129.2, 128.7, 128.6, 128.5, 110.4, 101.0, 84.5, 79.0, 77.1, 76.8, 73.5, 73.1,$

72.9, 71.9, 71.8, 68.83, 68.80, 62.8, 39.6, 39.5, 28.7, 28.0, 27.92, 27.87, 27.1; ES-MS: m/z 923.6 $[M + Na]^+$.

Ethyl 6-*O*-(2,3,5,6-tetra-*O*-benzoyl- α -D-galactofuranosyl)-2,3,4-tri-*O*-benzyl-1-thio- β -D-glucopyranoside (20): Condensation of **11** and **14** was executed as described for the condensation of **8** and **12** to give disaccharide **20** (68% yield). R_f 0.85 (40% EtOAc in PE). $[\alpha]_D^{25} +3.8$ ($c = 1.0$, $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$): 8.08-7.22 (m, 35H, CH_{arom}), 6.17 (m, 1H, H-5'), 5.61 (d, 1H, $J = 4.9$ Hz, H-3'), 5.47 (d, 1H, $J = 0.7$ Hz, H-2'), 5.35 (s, 1H, H-1'), 4.92 (d, 1H, $J = 10.7$ Hz, CHH Bn), 4.89 (d, 1H, $J = 10.5$ Hz, CHH Bn), 4.86 (d, 1H, $J = 10.8$ Hz, CHH Bn), 4.79 (m, 4H, H-4', H-6', H-6', CHH Bn), 4.58 (d, 1H, $J = 10.2$ Hz, CHH Bn), 4.46 (d, 1H, $J = 9.8$ Hz, CHH Bn), 4.04 (dd, 1H, $J = 1.5$ Hz, $J = 11.1$ Hz, H-6), 3.79 (dd, 1H, $J = 4.6$ Hz, $J = 11.1$ Hz), 3.67 (m, 2H, H-3, H-4), 3.48 (m, 1H, H-5), 3.22 (m, 1H, H-2), 2.67 (m, 2H, CH_2 SEt), 1.21 (t, 3H, $J = 7.4$ Hz, CH_3 SEt); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.1, 165.8, 165.5, 165.2, 138.5, 137.93, 137.88, 133.4, 133.2, 133.1, 132.9, 129.71, 129.67, 129.5, 129.0, 128.8, 128.4, 128.2, 128.0, 127.6, 105.2, 86.4, 84.8, 81.8, 81.7, 81.6, 78.3, 77.6, 75.7, 75.4, 75.0, 70.4, 65.3, 64.0, 24.9, 15.0$; ES-MS: m/z 1095.3 $[M + Na]^+$.

Phenyl 3-*O*-(2,3,5,6-tetra-*O*-benzoyl- α -D-galactofuranosyl)-2-azido-4,6-*O*-benzylidene-2-deoxy-1-seleno- α -D-galactopyranoside (21): Condensation of **11** and **15** was executed as described for the condensation of **8** and **12** to give disaccharide **21** (56% yield). R_f 0.55 (20% EtOAc in PE). $[\alpha]_D^{25} +83.6$ ($c = 1.0$, $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$): 8.01-7.03 (m, 30H, CH_{arom}), 6.08 (m, 1H, H-5'), 6.07 (d, 1H, $J = 5.2$ Hz, H-1), 5.69 (s, 1H, CH Ph), 5.67 (d, 1H, $J = 5.0$ Hz, H-3'), 5.58 (d, 1H, $J = 0.9$ Hz, H-2'), 5.53 (s, 1H, H-1'), 4.72 (m, 3H, H-4', H-6', H-6'), 4.50 (m, 1H, H-4), 4.47 (dd, 1H, $J = 5.2$ Hz, $J = 10.4$, H-2), 4.04 (m, 3H, H-3, H-5, H-6), 3.91 (d, $J = 11.1$ Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 166.2, 165.6, 165.2, 137.3, 133.9, 133.4, 133.3, 133.2, 129.9, 129.8, 129.7, 129.4, 129.3, 129.1, 129.0, 128.81, 128.75, 128.2, 128.0, 127.7, 125.8, 107.5, 100.6, 85.0, 82.5, 81.9, 78.4, 77.3, 75.2, 70.2, 68.9, 64.9, 63.4, 59.7$. ES-MS: m/z 1034.5 $[M + Na]^+$.

Phenyl 3-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio- β -D-galactopyranoside (24): Activation of **22** was executed as described for the activation of **8**. Acceptor **23** was added and the reaction mixture was kept at -40°C for 1 hour, after which it was slowly warmed to 0°C. Standard work-up and purification gave disaccharide **24** in 64%. R_f 0.80 (33% EtOAc in PE). $[\alpha]_D^{25} + 90.8$ ($c = 1.0$, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 8.12-7.14 (m, 35 H, CH_{arom}), 5.67 (d, 1H, $J = 2.8$ Hz, H-4), 5.61 (t, 1H, $J = 9.9$ Hz, H-2), 5.22 (d, 1H, $J = 3.2$ Hz, H-1'), 4.81 (d, 1H, $J = 10.1$ Hz, H-1), 4.76 (d, 1H, $J = 11.4$ Hz, CHH Bn), 4.65 (s, 2H, CH₂ Bn), 4.64 (d, 1H, $J = 12.2$ Hz, CHH Bn), 4.48 (m, 2H, H-6, CHH Bn), 4.36 (m, 4 H, H-6, 3xCHH Bn), 4.16 (dd, 1H, $J = 3.0$ Hz, $J = 9.7$ Hz, H-3), 3.93 (m, 3H, H-2', H-5, H-5'), 3.75 (dd, 1H, $J = 2.6$ Hz, $J = 10.1$ Hz, H-3'), 3.44 (dd, 1H, $J = 7.3$ Hz, $J = 9.6$ Hz, H-6'), 3.23 (bs, 1H, H-4'), 3.20 (dd, 1H, $J = 5.2$ Hz, $J = 9.6$ Hz, H-6'), 1.89 (s, 3H, CH₃ Ac); ¹³C NMR (100 MHz, CDCl₃): δ = 170.3, 165.9, 164.8, 138.6, 138.5, 138.3, 138.2, 133.4, 133.2, 131.8, 129.8, 129.7, 129.5, 129.3, 128.7, 128.4, 127.8, 127.6, 127.5, 127.4, 93.3, 87.0, 78.7, 75.5, 74.8, 74.7, 74.3, 73.21, 73.15, 73.0, 72.1, 69.9, 69.4, 68.9, 65.1, 62.7, 20.4; ES-MS: m/z 1067.5 [M + Na]⁺, 1083.4 [M+K]⁺.

3-azido-1-*O*-(4-*O*-[3-*O*-(2,3,4,6-tetra-*O*-benzyl- α -D-galactopyranosyl)-4-*O*-acetyl-2,6-di-*O*-benzoyl- β -D-galactopyranosyl]-3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl}propanol (26):

From **24** and **25**: Disaccharide **24** (0.02 M in DCM) was activated with Ph₂SO (2.0 equiv to donor) and Tf₂O (1.1 equiv to donor) in the presence of TTBP (2.5 equiv to donor) for 10 min at -60°C. Acceptor **25** (1.5 equiv to donor) was added and the reaction mixture was slowly warmed to 0°C. Standard work-up and purification gave trisaccharide **26** (69% yield).

One-pot procedure: 2,3,4,6-Tetra-*O*-benzyl- α -D-galactopyranose **22** (68 mg, 0.125 mmol), Ph₂SO (51 mg, 0.250 mmol), and TTBP (93 mg, 0.375 mmol) were dissolved in DCM (5 mL) and the reaction mixture was cooled to -60°C. Tf₂O (22 μ L, 0.13 mmol) was added and the reaction mixture was brought to -40°C. Stirring was continued for 1

hour at this temperature, after which phenyl 4-*O*-acetyl-2,6-di-*O*-benzoyl-1-thio- β -D-galactopyranoside **23** (52 mg, 0.100 mmol in 1 mL DCM) was added. The reaction mixture was kept at -40°C for 1 hour after which it was slowly warmed to 0°C. After 30 min at 0°C the reaction mixture was cooled to -60°C and a Tf₂O (20 μ L, 0.12 mmol) was added. After the reaction was kept at -60°C for 10 min, 3-azido-1-*O*-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)propanol **25** (86 mg, 0.15 mmol) was added. The reaction was slowly warmed to 0°C after which it was quenched with Et₃N (0.2 mL). Standard work-up and purification gave the α -Gal epitope **26** (121 mg, 0.082 mmol, 80%). R_f 0.60 (33% EtOAc in PE). [α]_D²⁵ +39.2 (*c* = 0.75, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 8.10-6.83 (m, 44H, H_{arom}), 5.54 (m, 2H, H-2', H-4'), 5.14 (d, 1H, *J* = 3.3 Hz, H-1"), 5.00 (d, 1H, *J* = 8.5 Hz, H-1), 4.91 (d, 1H, *J* = 12.4 Hz, CHH Bn), 4.78 (d, 1H, *J* = 8.1 Hz, H-1'), 4.74 (d, 1H, *J* = 11.4 Hz, CHH Bn), 4.63 (s, 2H, CH₂ Bn), 4.62 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.55 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.51 (d, 1H, *J* = 12.4 Hz, CHH Bn), 4.44 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.42 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.35 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.29 (m, 3H, CHH Bn, CHH BN, H-3), 4.22 (dd, 1H, *J* = 6.5 Hz, *J* = 11.3 Hz, H-6'), 4.13 (m, 2H, H-6', H-2), 4.05 (dd, 1H, *J* = 8.5 Hz, *J* = 9.9 Hz, H-4), 3.99 (dd, 1H, *J* = 3.4 Hz, *J* = 10.2 Hz, H-3'), 3.91 (dd, 1H, *J* = 3.3 Hz, *J* = 10.2 Hz, H-2"), 3.85 (bt, 1H, *J* = 6.9 Hz, H-5"), 3.75 (m, 1H, O-CH₂-CH₂), 3.67 (m, 2H, H-5', H-6), 3.58 (m, 2H, H-6, H-3"), 3.41 (m, 1H, H-5), 3.38 (m, 2H, H-6", O-CH₂-CH₂), 3.25 (dd, 1H, *J* = 1.2 Hz, *J* = 2.6 Hz, H-4"), 3.21 (dd, 1H, *J* = 5.9 Hz, *J* = 9.4 Hz, H-6"), 3.08 (m, 2H, CH₂N₃), 1.81 (s, 3H, CH₃ Ac), 1.64 (m, 2H, CH₂CH₂CH₂); ¹³C NMR (100 MHz, CDCl₃): δ = 170.2, 166.0, 164.6, 138.7, 138.4, 138.2, 138.1, 133.7, 133.3, 133.2, 131.5, 129.8, 129.7, 129.3, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.6, 127.5, 127.4, 127.3, 126.9, 123.2, 100.8, 98.3, 94.1, 78.8, 78.3, 76.9, 75.5, 74.8, 74.7, 74.5, 73.5, 73.3, 73.2, 73.1, 72.3, 71.5, 71.0, 69.8, 69.2, 67.8, 65.9, 65.0, 61.7, 55.7, 48.0, 28.8, 20.4; ES-MS: *m/z* 1529.8 [M + Na]⁺.

Ethyl 3-*O*-[methyl (2,3,4-tri-*O*-benzoyl- α -D-glucopyranosyluronate)]-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranoside (29**):** Methyl (2,3,4-tri-*O*-benzoyl- α -D-glucopyranosyluronate) **27** was activated with Ph₂SO and Tf₂O in the

presence of TTBP (1.0 equiv to donor) as described above. Condensation with ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranoside **28** gave after work-up and purification disaccharide **29** in 58% yield. R_f 0.60 (20% EtOAc in toluene). $[\alpha]_D^{25} +14.4$ ($c = 0.50$, CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.80\text{--}7.15$ (m, 24 H, CH_{arom}), 5.64 (t, 1H, $J = 9.4$ Hz, H-3'), 5.57 (s, 1H, CH Ph), 5.44 (t, 1H, $J = 9.4$ Hz, H-4'), 5.32 (dd, 1H, $J = 8.0$ Hz, $J = 9.5$ Hz, H-2'), 5.27 (d, 1H, $J = 10.7$ Hz, H-1), 4.98 (d, 1H, $J = 8.0$ Hz, H-1'), 4.83 (dd, 1H, $J = 8.8$ Hz, $J = 9.8$ Hz, H-3), 4.47 (t, 1H, $J = 10.0$ Hz, H-2), 4.38 (dd, 1H, $J = 4.7$ Hz, $J = 10.4$ Hz), 4.01 (t, 1H, $J = 9.1$ Hz, H-4), 3.86 (t, 1H, $J = 10.3$ Hz, H-6), 3.78 (d, 1H, $J = 9.7$ Hz, H-5'), 3.73 (m, 1H, H-5), 3.49 (s, 3H, $\text{CH}_3 \text{ OMe}$), 2.64 (m, 2H, $\text{CH}_2 \text{ Set}$), 1.14 (t, 3H, $J = 7.4$ Hz, $\text{CH}_3 \text{ SET}$); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 166.9$, 165.4, 165.3, 164.2, 137.0, 133.7, 133.3, 133.2, 132.8, 131.2, 130.9, 129.6, 129.4, 128.7, 128.5, 1128.4, 128.3, 128.0, 126.0, 123.6, 122.7, 101.8, 100.0, 81.4, 81.3, 77.1, 72.3, 72.0, 71.9, 70.5, 70.0, 68.7, 53.7, 52.5, 23.5, 14.7. ES-MS: m/z 966.3 $[\text{M} + \text{Na}]^+$.

Methyl (methyl 4-*O*-{3-*O*-[methyl (2,3,4-tri-*O*-benzoyl- α -D-glucopyranosyluronate)]-4,6-*O*-benzylidene-2-deoxy-2-phthalimido- β -D-glucopyranosyl}-2,3-*O*-benzyl- α -D-glucopyranosyluronate) (31):

Trisaccharide **31** was synthesized from **29** and **30** as described for **26**.

One-pot procedure: Methyl (2,3,4-tri-*O*-benzoyl- α -D-glucopyranosyluronate) **27** (78 mg, 0.15 mmol), Ph_2SO (67 mg, 0.33 mmol), and TTBP (37 mg, 0.15 mmol) were dissolved in DCM (5 mL) and the reaction mixture was cooled to -60°C . Tf_2O (30 μL , 0.16 mmol) was added and the reaction mixture was slowly brought to -20°C in 1 hour, after which ethyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-1-thio- β -D-glucopyranoside **28** (44 mg, 0.10 mmol in 1 mL DCM) was added. The reaction mixture was slowly warmed to room temperature. After 1 hour at room temperature the reaction mixture was cooled to -60°C and a solution of TTBP (124 mg, 0.50 mmol) and Tf_2O (20 μL , 0.12 mmol) were added. After the reaction was kept at -60°C for 10 min, methyl (methyl 2,3-*O*-benzyl- α -D-glucopyranosyluronate) **30** was added. The reaction was warmed to room temperature and stirred overnight, after which it was quenched with Et_3N (0.2 mL). Standard work-up

and purification gave the hyaluronan trisaccharide **31** (40 mg, 0.032 mmol, 32%). R_f 0.50 (50% EtOAc in PE). $[\alpha]_D^{25} + 4.6$ ($c = 0.70$, CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.94\text{--}7.18$ (m, 34H, CH_{arom}), 5.60 (t, 1H, $J = 9.4$ Hz, H-3"), 5.41 (t, 1H, $J = 9.4$ Hz, H-4"), 5.41 (s, 1H, CH Ph), 5.27 (dd, 1H, $J = 8.1$ Hz, $J = 9.4$ Hz, H-2"), 5.20 (d, 1H, $J = 8.4$ Hz, H-1'), 4.93 (d, 1H, $J = 8.8$ Hz, CHH Bn), 4.91 (d, 1H,), 5.27 (dd, 1H, $J = 8.1$ Hz, $J = 9.4$ Hz, H-2"), 5.20 (d, 1H, $J = 8.4$ Hz, H-1'), 4.93 (d, 1H, $J = 8.8$ Hz, CHH Bn), 4.89 (d, 1H, $J = 7.7$ Hz, H-1"), 4.77 (dd, 1H, $J = 8.9$ Hz, $J = 10.2$ Hz, H-3'), 4.72 (d, 1H, $J = 12.2$ Hz, CHH Bn), 4.54 (d, 1H, $J = 12.2$ Hz, CHH Bn), 4.42 (d, 1H, $J = 3.5$ Hz, H-1), 4.28 (dd, 1H, $J = 8.4$ Hz, $J = 10.2$ Hz, H-2'), 4.02 (m, 1H, H-6'), 3.88 (m, 2H, H-4, H-4'), 3.79 (m, 2H, H-3, H-5), 3.74 (d, 1H, $J = 9.6$ Hz, H-5"), 3.49 (s, 3H, $\text{CH}_3 \text{ OMe}$), 3.47 (m, 1H, H-5'), 3.43 (m, 2H, H-2, H-6'), 3.39 (s, 3H, $\text{CH}_3 \text{ OMe}$), 3.25 (s, 3H, $\text{CH}_3 \text{ OMe}$); ^{13}C NMR (100 MHz, CDCl_3): $\delta = 169.8, 167.8, 166.2, 165.9, 165.0, 140.1, 138.8, 137.9, 134.3, 134.1, 134.0, 133.6, 132.2, 130.2, 129.6, 129.4, 129.3, 128.7, 128.1, 102.5, 100.7, 99.5, 99.0, 81.8, 80.2, 79.3, 78.8, 78.1, 76.9, 76.0, 74.5, 73.3, 72.8, 72.7, 72.5, 70.8, 70.5, 69.4, 66.6, 56.5, 56.2, 53.4, 53.2$; ES-MS: m/z 1306.5 $[\text{M} + \text{Na}]^+$.

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