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Supplementary Information for

Nano-vesicles displaying functional linear and branched oligomannose self-assembled from sequence-defined Janus glycodendrimers

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Supplementary text
Figures S1 to S37
SI References

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Part 1. Solution Synthesis of Azido and Amino-Containing Sugars, and ITC-Containing Janus Dendrimers

1.1 Materials

All reagents were obtained from commercial sources and used without purification unless otherwise stated. CH_2Cl_2 (DCM) was dried over CaH_2 and freshly distilled before use. THF was distilled over Na/benzophenone immediately before use. DMF was dried from CaH_2, distilled, and kept over molecular sieves prior to use. Solvents and reagents were deoxygenated when necessary by purging with nitrogen. ^1H and ^{13}C NMR spectra were recorded at 500 MHz and 126 MHz respectively, on a Bruker DRX (500 MHz) NMR spectrometer. All NMR spectra were measured at 23 °C. Chemical shifts (δ) are reported in ppm and coupling constants (J) are reported in Hertz (Hz). The resonance multiplicities in the ^1H NMR spectra are described as "s" (singlet), "d" (doublet), "t" (triplet), "quint" (quintet) and "m" (multiplet) and broad resonances are indicated by "br". Residual protic solvent of $CDCl_3$ (^1H, δ 7.26 ppm; ^{13}C, δ 77.16 ppm), and tetramethylsilane (TMS, δ 0 ppm) were used as the internal reference in the ^1H- and ^{13}C-NMR spectra. The absorptions are given in wavenumbers (cm^{-1}). Evolution of the reaction was monitored by thin-layer chromatography (TLC) using silica gel 60 F_{254} precoated plates (E. Merck) and compounds were visualized by UV light with a wavelength of 254 or 356 nm. Purifications by flash column chromatography were performed using flash silica gel from Silicycle (60 Å, 40–63 μm) with the indicated eluent. The purity of the products was determined by a combination of TLC and high-pressure liquid chromatography (HPLC) was carried out using Shimadzu LC-20AD high-performance liquid chromatograph pump, a PE Nelson Analytical 900 Series integration data station, a Shimadzu SPD-10A VP (UV-*vis*, λ = 254 nm) and three AM gel columns (a guard column, two 500 Å, 10 μm columns). THF was used as solvent at the oven temperature of 23 ºC. Detection was done by UV absorbance at 254 nm. MALDI-TOF mass spectrometry was performed on a PerSeptive Biosystem-Voyager-DE (Framingham, MA) MALDI-TOF mass spectrometer equipped with nitrogen laser (337 nm) and operating in linear mode. Internal calibration was performed using Angiotensin II and Bombesin as standards. The analytical sample was obtained by mixing the THF solution of the sample (5–10 mg/mL) and THF solution of the matrix (2,5-dihydroxybenzoic acid, 10 mg/mL) in a 1/5 (v/v) ratio. The prepared solution of the sample and the matrix (0.5 μL) was loaded on the MALDI plate and allowed to dry at 23 ºC before the plate was inserted into the vacuum chamber of the MALDI instrument. The laser steps and voltages applied were adjusted depending on both the molecular weight and the nature of each analyzed compound.

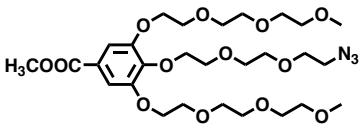
1.2 Synthesis of Azido and Amino-Containing Lactose

Compounds **1, 3–10** were synthesized and characterized according to the literatures (S1, S2).

1.3 Synthesis of ITC-Containing Janus Dendrimers

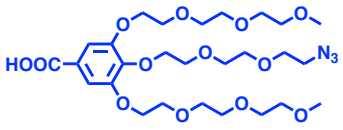
Compounds **14** and **15** were synthesized and characterized according to the literature (S3).

Compounds **16–19** (S4), **22** (S5), **23** (S4), **27** (S5), and amphiphilic Janus dendrimer **JD-3** (S4) were synthesized and characterized according to the publications by Percec laboratory.



20

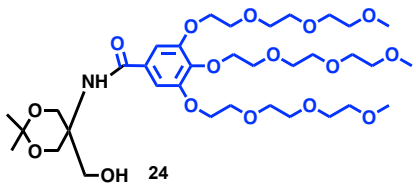
Compound **20**: To a DMF solution (50 mL) of compound **19** (2.7 g, 5.7 mmol) and compound **15** (1.3 g, 6.8 mmol) were successively added K_2CO_3 (2.6 g, 18.8 mmol) and 18-C-6 (130 mg, 0.5 mmol) and the mixture was stirred at 80 ºC for 48 h under nitrogen atmosphere. Then, the reaction mixture was poured into 100 mL water and the mixture was extracted with 3×100 mL of DCM. An organic extract was dried over Na_2SO_4 and concentrated to dryness. The crude product was further purified by column chromatography on silica gel with a mobile phase of EtOAc/MeOH = 20/1 (v/v) to yield compound **20** as a colorless oily liquid (2.8 g, 90%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.29 (s, 2H, 2×Ar*H*), 4.21–4.23 (t, 2H, *J* = 4.5 Hz, OC*H*$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$N$_3$), 4.18–4.20 (t, 4H, *J* = 4.5 Hz, 2×OC*H*$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_3$), 3.85–3.88 (m, 7H, Ar-COOC*H*$_3$ and 2×OCH$_2$C*H*$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_3$), 3.79–3.81 (t, 2H, *J* = 4.5 Hz, OCH$_2$C*H*$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$N$_3$), 3.71–3.74 (m, 6H, 3×C*H*$_2$), 3.63–3.67 (m, 12H, 6×C*H*$_2$), 3.53–3.55 (m, 4H, 2×OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$C*H*$_2$OCH$_3$), 3.36–3.38 (m, 8H, OC*H*$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$C*H*$_2$N$_3$ and 2×OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OC*H*$_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 166.7, 152.4, 142.7, 125.1, 109.1, 72.6, 72.1, 70.9, 70.9, 70.8, 70.7, 70.7, 70.2, 69.7, 69.0, 59.1, 52.3, 50.8.



21

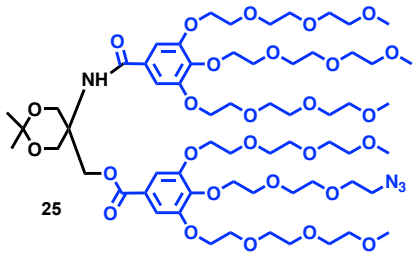
Compound **21**: To an EtOH solution (50 mL) of compound **20** (2.8 g, 4.4 mmol) was added KOH (1.24 g, 22 mmol). The reaction mixture was refluxed for 2 h. After cooling to 23 ºC, 2 mol/L HCl aqueous solution was added until pH = 1. The reaction mixture was then extracted with 3×100 mL of DCM. The organic extract was dried over Na_2SO_4 and evaporated to dryness under reduced pressure to yield compound **21** as a colorless oily liquid (2.8 g, 100 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.35 (s, 2H, 2×Ar*H*), 4.23–4.25 (t, 2H, *J* = 4.5 Hz, OC*H*$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$N$_3$), 4.19–4.21 (t, 4H, *J* = 4.5 Hz, 2×OC*H*$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_3$), 3.86–3.88 (t, 4H, *J* = 4.5 Hz, 2×OCH$_2$C*H*$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_3$), 3.79–3.81 (t, 2H, *J* = 4.5 Hz, OCH$_2$C*H*$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$N$_3$), 3.71–3.74 (m, 6H, 3×C*H*$_2$), 3.64–3.69 (m, 12H, 6×C*H*$_2$), 3.53–3.55 (m, 4H, 2×OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$C*H*$_2$OCH$_3$), 3.36–3.38 (m, 8H,

OC*H*$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$C*H*$_2$N$_3$ and 2×OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OC*H*$_3$). ^{13}C NMR (126 MHz, CDCl$_3$) δ = 170.6, 152.4, 143.3, 124.3, 109.7, 72.6, 72.1, 70.9, 70.8, 70.7, 70.7, 70.2, 69.8, 69.0, 59.1, 50.8.



24

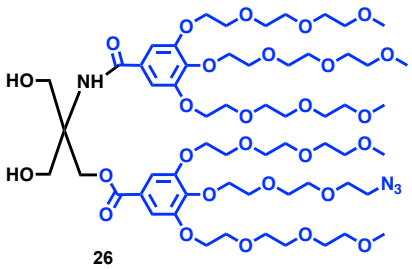
Compound **24**: To a THF (10 mL) solution of compound **22** (1.0 g, 1.64 mmol) and compound **23** (292 mg, 1.81 mmol) was added 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT, 318 mg, 1.81 mmol). The mixture was cooled to 0 ºC and *N*-methylmorpholine (NMM, 415 mg, 4.10 mmol) was added under N_2. The reaction mixture was allowed to stir at 23 ºC for 12 h. The precipitate was then filtered, and the crude product was further purified by column chromatography on silica gel with a mobile phase of DCM/MeOH = 20/1 (v/v) to yield to compound **24** as a colorless oily liquid (1.22 g, 93%). ^{1}H NMR (500 MHz, CDCl$_3$) δ = 7.07 (s, 2H, 2×Ar*H*), 6.91 (s, 1H, N*H*), 4.99 (br, 1H, CH$_2$O*H*), 4.19–4.22 (m, 6H, 3×OC*H*$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_3$), 4.00–4.02 (d, 2H, *J* = 12 Hz, 2×OC*H*H), 3.88–3.91 (d, 2H, *J* = 12 Hz, 2 × OC*H*H), 3.84–3.86 (t, 4H, *J* = 4.5 Hz, 2 × OCH$_2$C*H*$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_3$), 3.78–3.80 (m, 4H, C*H*$_2$OH and OCH$_2$C*H*$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_3$), 3.70–3.73 (m, 6H, 3×OCH$_2$CH$_2$OC*H*$_2$CH$_2$OCH$_2$CH$_2$OCH$_3$), 3.62–3.66 (m, 12H, 3 × OCH$_2$CH$_2$OCH$_2$C*H*$_2$OC*H*$_2$CH$_2$OCH$_3$), 3.51–3.54 (m, 6H, 3 × OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$C*H*$_2$OCH$_3$), 3.36 (m, 9H, 3×OCH$_2$CH$_2$OCH$_2$CH$_2$OCH$_2$CH$_2$OC*H*$_3$), 1.47 (s and s, 6H, 2×CH$_3$). ^{13}C NMR (126 MHz, CDCl$_3$) δ = 168.2, 152.5, 141.7, 129.6, 107.4, 98.9, 72.4, 71.9, 70.7, 70.6, 70.5, 69.7, 69.1, 64.1, 63.9, 59.0, 55.3, 26.9, 20.5.



25

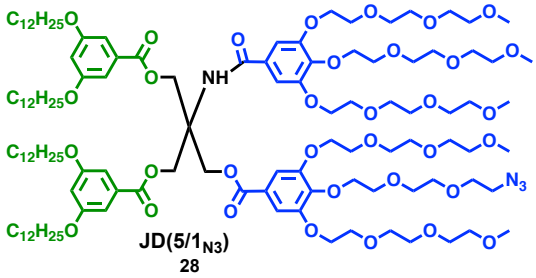
Compound **25**: To a DCM (10 mL) solution of compound **24** (1.10 g, 1.46 mmol), compound **21** (0.91 g, 1.46 mmol) and DPTS (430 mg, 1.46 mmol), was added DCC (600 mg, 2.92 mmol). The mixture was allowed to stir at 23 ºC for 24 h. The precipitate was filtered, and the filtrate was concentrated to dryness. The crude product was further purified by column chromatography with a mobile phase of DCM/MeOH = 10/1 (v/v) to yield compound **25** as a colorless oily liquid (1.58 g, 80%). ^{1}H NMR (500 MHz, CDCl$_3$) δ = 7.30 (s, 2H, 2×Ar*H*), 7.10 (s, 1H, N*H*), 7.03 (s, 2H, 2×

Ar*H*), 4.81 (s, 2H, COCH_2), 4.64–4.66 (d, 2H, *J* = 12 Hz, 2×OC*H*H), 4.15–4.23 (m, 12H, 6×CH_2), 3.83–3.86 (m, 10H, 5×CH_2), 3.77–3.80 (m, 4H, 2×CH_2), 3.70–3.72 (m, 12H, 6×CH_2), 3.61–3.65 (m, 24H, 12×CH_2), 3.50–3.54 (m, 10H, 5×CH_2), 3.35–3.37 (m, 17H, CH_2N$_3$ and 5×OCH_3), 1.61 (s, 3H, CH_3), 1.47 (s, 3H, CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* = 167.4, 167.0, 152.5, 152.5, 143.4, 141.5, 129.4, 124.0, 109.4, 106.7, 98.7, 72.5, 72.4, 71.9, 70.8, 70.7, 70.6, 70.6, 70.5, 69.6, 69.1, 69.0, 65.0, 61.5, 59.0, 54.0, 50.7, 25.3, 22.3.



26

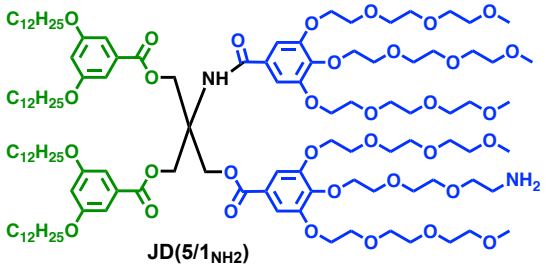
Compound **26**: To a MeOH solution (50 mL) of compound **25** (1.58 g, 1.17 mmol) was added 10 mL of HCl aqueous solution (2M). The mixture was allowed to stir at 23 ºC for 2h and water (50 mL) was added. Then, the mixture was extracted with DCM for 3 times. An organic extract was dried over Na_2SO_4 and evaporated to dryness under reduced pressure to yield compound **26** as a colorless viscous liquid (1.50 g, 98%). ^{1}H NMR (500 MHz, $CDCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) *δ* =7.31 (s, 2H, 2×Ar*H*), 7.21 (s, 1H, N*H*), 7.09 (s, 2H, 2×Ar*H*), 4.63 (s, 2H, COCH_2), 4.49–4.52 (t, 1H, *J* = 6.2 Hz, CH_2O*H*), 4.17–4.24 (m, 12H, 6×CH_2), 3.83–3.87 (m, 10H, 5×CH_2), 3.77–3.81 (m, 4H, 2×CH_2), 3.70–3.73 (m, 14H, 7×CH_2), 3.62–3.66 (m, 24H, 12×CH_2), 3.51–3.54 (m, 10H, 5×CH_2), 3.35–3.38 (m, 17H, CH_2N$_3$ and 5×OCH_3). ^{13}C NMR (126 MHz, $CDCl_3$) *δ* = 168.2, 167.0, 152.6, 152.4, 143.3, 142.0, 129.0, 124.1, 109.5, 107.5, 72.5, 72.5, 72.0, 70.8, 70.7, 70.7, 70.6, 70.6, 70.6, 70.5, 69.8, 69.7, , 69.2, 69.0, 63.2, 62.0, 59.0, 50.7.



28

Compound **28**: To a DCM (10 mL) solution of compound **26** (1.49 g, 1.13 mmol), compound **27** (1.33 g, 2.70 mmol) and DPTS (800 mg, 2.70 mmol), was added DCC (1.16 g, 5.60 mmol). The mixture was allowed to stir at 23 ºC for 24 h. The precipitate was filtered, and the filtrate was concentrated to dryness. The crude product was further purified by column chromatography with a mobile phase of DCM/MeOH = 20/1 (v/v) to yield compound **28** as a colorless oily liquid (2.37

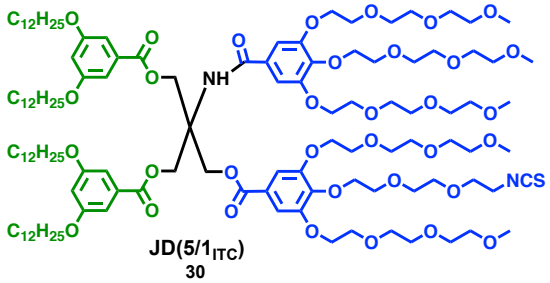
g, 93%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.40 (s, 1H, N*H*), 7.24 (s, 2H, 2×Ar*H*), 7.08 (d, 4H, *J* = 2.5 Hz, 4×Ar*H*), 7.04 (s, 2H, 2×Ar*H*), 6.59–6.60 (t, 2H, *J* = 2.5 Hz, 2×Ar*H*), 4.95–4.97 (m, 6H, 3×CH_2), 4.16–4.21 (m, 8H, 4×CH_2), 4.10–4.12 (m, 4H, 2×CH_2), 3.87–3.90 (t, 8H, *J* = 6.5 Hz, 4×O*C*$H_2$$CH_2CH_2(CH_2)_8CH_3$), 3.78–3.85 (m, 12H, 6×$CH_2$), 3.69–3.72 (m, 12H, 6×$CH_2$), 3.60–3.66 (m, 24H, 12×CH_2), 3.50–3.54 (m, 10H, 5×CH_2), 3.34–3.36 (m, 17H, CH_2N_3 and 5×OCH_3), 1.72–1.75 (m, 8H, 4×$OCH_2C$$H_2CH_2(CH_2)_8CH_3$), 1.40–1.43 (m, 8H, 4×$OCH_2CH_2C$$H_2(CH_2)_8CH_3$), 1.35–1.32 (m, 64H, 4×$OCH_2CH_2CH_2(C$$H_2)_8CH_3$), 0.86–0.89 (m, 12H, 4×$OCH_2CH_2CH_2(CH_2)_8C$$H_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 167.4, 166.5, 166.2, 160.2, 152.5, 152.4, 143.1, 141.4, 130.9, 129.0, 124.0, 109.1, 107.8, 106.7, 106.4, 72.5, 72.4, 71.9, 70.8, 70.8, 70.7, 70.7, 70.6, 70.6, 70.5, 70.5, 70.1, 69.6, 68.9, 68.8, 68.3, 64.1, 60.0, 59.0, 50.7, 31.9, 29.7, 29.7, 29.6, 29.5, 29.4, 29.1, 26.1, 22.7, 14.1.



JD(5/1$_{NH2}$)
29

Compound **29**: To a mixed DCM (20 mL) and MeOH (10 mL) solution of compound **28** (1.81 g, 0.80 mmol) was added palladium on carbon (10% Pd/C, 180 mg). The mixture was bubbling with H_2 for 30 min and then allowed to stir at 23 ºC for 12 h under H_2 atmosphere. The mixture was then filtered through Celite®. The filtrate was concentrated to dryness and was further purified by column chromatography with a mobile phase of DCM/MeOH = 5/1 (v/v) to yield compound **29** as a colorless oily liquid (1.72 mg, 96%, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.88 (br, 2H, NH_2), 7.65 (s, 1H, N*H*), 7.27 (s, 2H, 2×Ar*H*), 7.12 (s, 2H, 2×Ar*H*), 7.09 (d, 4H, *J* = 2.5 Hz, 4×Ar*H*), 6.60 (t, 2H, *J* = 2.5 Hz, 2×Ar*H*), 4.99 (s, 4H, 2×COOCH_2), 4.95 (s, 2H, COOCH_2), 4.32–4.33 (m, 2H, CH_2), 4.15–4.19 (m, 10H, 5×CH_2), 3.88–3.91 (t, 8H, *J* = 6.5 Hz, 4×O*C*$H_2$$CH_2CH_2(CH_2)_8CH_3$), 3.81–3.84 (m, 10H, 5×$CH_2$), 3.77–3.78 (t, 2H, *J* = 5.0 Hz, CH_2), 3.73–3.75 (m, 2H, CH_2), 3.68–3.71 (m, 10H, 5×CH_2), 3.60–3.65 (m, 24H, 12×CH_2), 3.50–3.54 (m, 10H, 5×CH_2), 3.34–3.36 (m, 15H, 5×OCH_3), 3.17–3.18 (m, 2H, C$H_2$$NH_2$), 1.72–1.75 (m, 8H, 4×$OCH_2C$$H_2CH_2(CH_2)_8CH_3$), 1.40–1.43 (m, 8H, 4×$OCH_2CH_2C$$H_2(CH_2)_8CH_3$), 1.25–1.29 (m, 64H, 4×$OCH_2CH_2CH_2(C$$H_2)_8CH_3$), 0.86–0.89 (m, 12H, 4×$OCH_2CH_2CH_2(CH_2)_8C$$H_3$). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 167.2, 166.5, 166.1, 160.3, 152.5, 152.0, 143.2, 141.4, 131.1, 129.2, 124.4, 109.0, 107.9, 106.7, 72.5, 72.1, 72.0, 72.0, 71.9, 70.9, 70.8, 70.7, 70.7, 70.6, 70.6, 70.5, 70.5, 70.4, 70.2, 70.0, 69.8, 69.4, 68.9, 68.4, 68.4, 67.0, 64.0,

60.0, 59.1, 59.1, 59.1, 40.3, 32.0, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 26.2, 22.8, 14.1. MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{121}H_{206}N_2NaO_{34}$, 2256.0; found 2256.6.



Compound **30**: To a THF (10 mL) solution of compound **29** (480 mg, 0.21 mmol) and Et_3N (72 mg, 0.71 mmol) cooled with an ice bath under N_2 atmosphere. CS_2 (24 mg, 0.31mmol) in 0.2 mL THF was added by syringe. Once conversion of the salt was completed by TLC, the mixture was cooled with an ice bath and TsCl (59 mg, 0.31 mmol) was added. The reaction was stirred at 23 °C for 1 h. The crude product was further purified by column chromatography with a mobile phase of DCM/MeOH = 10/1 (v/v) to yield compound **30** as a colorless oily liquid (450 mg, 95%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.39 (s, 1H, N*H*), 7.24 (s, 2H, 2×Ar*H*), 7.08 (d, 4H, *J* = 2.5 Hz, 4×Ar*H*), 7.04 (s, 2H, 2×Ar*H*), 6.60 (t, 2H, *J* = 2.5 Hz, 2×Ar*H*), 4.84–4.99 (m, 6H, 3×COOCH_2), 4.21–4.23 (t, 2H, J = 4.8 Hz, CH_2), 4.16–4.19 (m, 6H, 3×CH_2), 4.10–4.12 (t, 4H, 2×CH_2), 3.87–3.90 (t, 8H, *J* = 6.5 Hz, 4×OCH_2CH$_2$CH$_2$(CH$_2$)$_8$CH$_3$), 3.78–3.85 (m, 12H, 12×CH$_2$), 3.69–3.73 (m, 12H, 12×CH$_2$), 3.61–3.67 (m, 26H, 13×CH$_2$), 3.50–3.54 (m, 10H, 5×CH$_2$), 3.34–3.36 (m, 15H, 5×OCH_3), 1.71–1.77 (m, 8H, 4×OCH$_2$CH_2CH$_2$(CH$_2$)$_8$CH$_3$), 1.39–1.43 (m, 8H, 4×OCH$_2$CH$_2$CH_2(CH$_2$)$_8$CH$_3$), 1.26–1.29 (m, 64H, 4×OCH$_2$CH$_2$CH$_2$(CH_2)$_8$CH$_3$), 0.86–0.89 (m, 12H, 4×OCH$_2$CH$_2$CH$_2$(CH$_2$)$_8$CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 167.0, 166.5, 166.2, 160.2, 152.5, 152.4, 143.1, 141.5, 130.0, 129.0, 127.0 (-N=*C*=S), 124.0, 109.1, 107.8, 106.7, 106.5, 72.5, 72.4, 72.0, 70.8, 70.8, 70.8, 70.7, 70.7, 70.5, 70.5, 69.6, 69.4, 68.9, 68.9, 68.3, 64.1, 60.0, 59.0, 45.3, 31.9, 29.7, 29.6, 29.6, 29.4, 29.3, 29.2, 2602, 22.7, 14.1. MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{122}H_{204}N_2NaO_{34}S$, 2298.0; found 2301.2.

Part 2. Building Blocks for Automated Glycan Assembly Automated Glycan Assembly

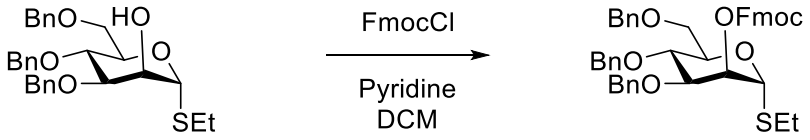
2.1 Materials and Methods

All chemicals used were reagent grade and used as supplied unless otherwise noted. The automated syntheses were performed on a home-built synthesizer developed at the Max Planck Institute of Colloids and Interfaces. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in a *p*-anisaldehyde (PAA) solution. Flash column chromatography was carried out by using forced flow of the indicated solvent on Fluka Kieselgel 60 M (0.04 – 0.063 mm). Analysis and

purification by normal and reverse phase HPLC was performed by using an Agilent 1200 series. Products were lyophilized using a Christ Alpha 2-4 LD plus freeze dryer. ^{1}H, ^{13}C and HSQC NMR spectra were recorded on a Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), or Bruker Biospin AVANCE700 (700 MHz) spectrometer. Spectra were recorded in CDCl3 by using the solvent residual peak chemical shift as the internal standard (CDCl3: 7.26 ppm ^{1}H, 77.0 ppm ^{13}C) or in D_2O using the solvent as the internal standard in ^{1}H NMR (D2O: 4.79 ppm ^{1}H) and a D6-acetone spike as the internal standard in ^{13}C NMR (acetone in D2O: 30.89 ppm ^{13}C) unless otherwise stated. High resolution mass spectra were obtained using a 6210 ESI-TOF mass spectrometer (Agilent) and a MALDI-TOF autoflex^{TM} (Bruker). MALDI and ESI mass spectra were run on IonSpec Ultima instruments. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer. Optical rotations were measured by using a Perkin-Elmer 241 and Unipol L1000 polarimeter.

2.2 Synthesis of Building Blocks (BB) for Automated Glycan Assembly

Ethyl 3,4,6-O-benzyl-2-O-fluorenylcarboxymethyl-thio-α-D-mannopyranoside, S1



S1 **S2**

S1 was prepared according to previously established procedures. (S6)

Ethyl 3,4,6-O-benzyl-thio-α-D-mannopyranoside **S1** (9.1 g, 18.5 mmol) was dissolved in DCM (120 mL). Pyridine (6.0 mL, 74 mmol) and (9H-fluoren-9-yl)methyl carbonochloridate (12.0 g, 46.2 mmol) were added. The yellow solution was stirred at rt until completion (3 h). The reaction was diluted with DCM and washed with 1 M HCl, then saturated aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, filtered and evaporated. The resulting yellow oil was purified by column chromatography (Hexanes: EtOAc = 4:1) to give **S2** as a pale sticky oil (13.7 g, 74%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.80 – 7.72 (m, 2H), 7.67 – 7.58 (m, 2H), 7.43 – 7.15 (m, 19H), 5.44 (d, *J* = 1.3 Hz, 1H), 5.29 (dd, *J* = 3.0, 1.6 Hz, 1H), 4.89 (d, *J* = 10.8 Hz, 1H), 4.76 – 4.67 (m, 2H), 4.60 – 4.50 (m, 3H), 4.43 (dd, *J* = 10.1, 7.2 Hz, 1H), 4.35 – 4.29 (m, 1H), 4.26 (t, *J* = 7.5 Hz, 1H), 4.20 (ddd, *J* = 9.4, 4.5, 1.6 Hz, 1H), 4.00 (t, *J* = 9.4 Hz, 1H), 3.94 (dd, *J* = 9.3, 3.0 Hz, 1H), 3.85 (dd, *J* = 10.9, 4.7 Hz, 1H), 3.72 (dd, *J* = 10.9, 1.8 Hz, 1H), 2.73 – 2.55 (m, 2H), 1.29 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, Chloroform-*d*) δ 154.91, 143.65, 143.39, 141.42, 141.34, 138.46, 138.38, 137.84, 128.47, 128.45, 128.07, 128.05, 127.99, 127.95, 127.91, 127.85, 127.77, 127.69, 127.30, 125.57, 125.35, 120.14, 120.10, 82.19, 78.75, 75.41, 74.76, 74.74, 73.55, 72.14, 72.05, 70.40, 69.06, 46.77, 25.63, 15.03; m/z (HRMS+) 739.2722 $[M + Na]^+$ ($C_{44}H_{44}O_7SNa$ requires

739.2700); R_f = 0.31 (silica, Hex : EtOAc 4 : 1). $[\alpha]_D^{20}$ +27.91 (*c* 1, $CHCl_3$); IR (neat) ν_{max} = 1259, 728, 680 cm^{-1}.

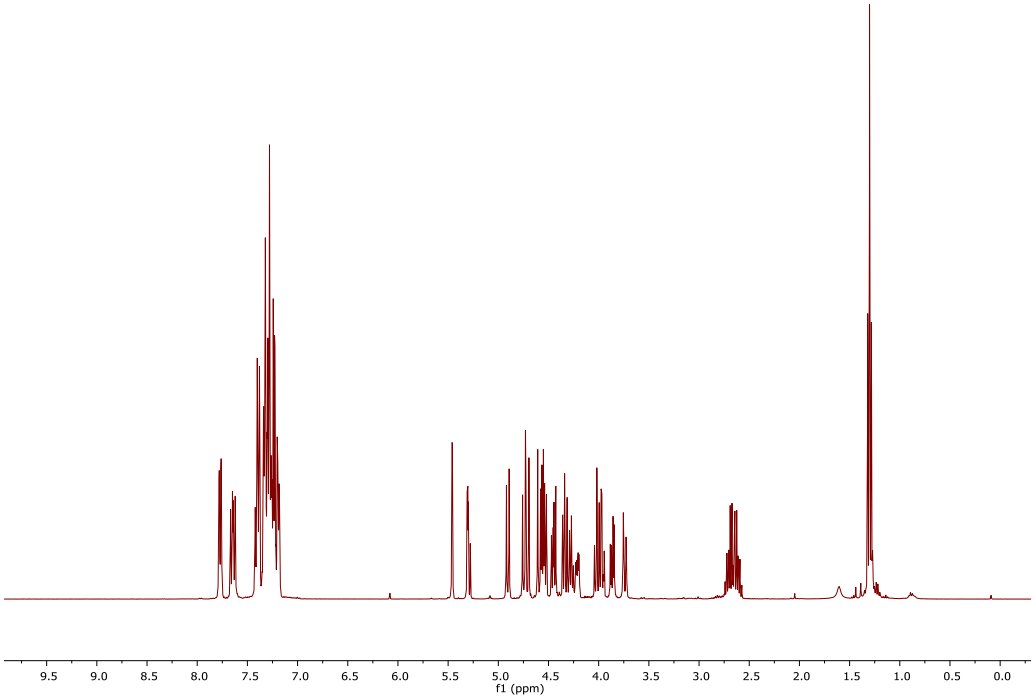


Fig. S1. ^1H NMR of S2 (400 MHz, $CDCl_3$).

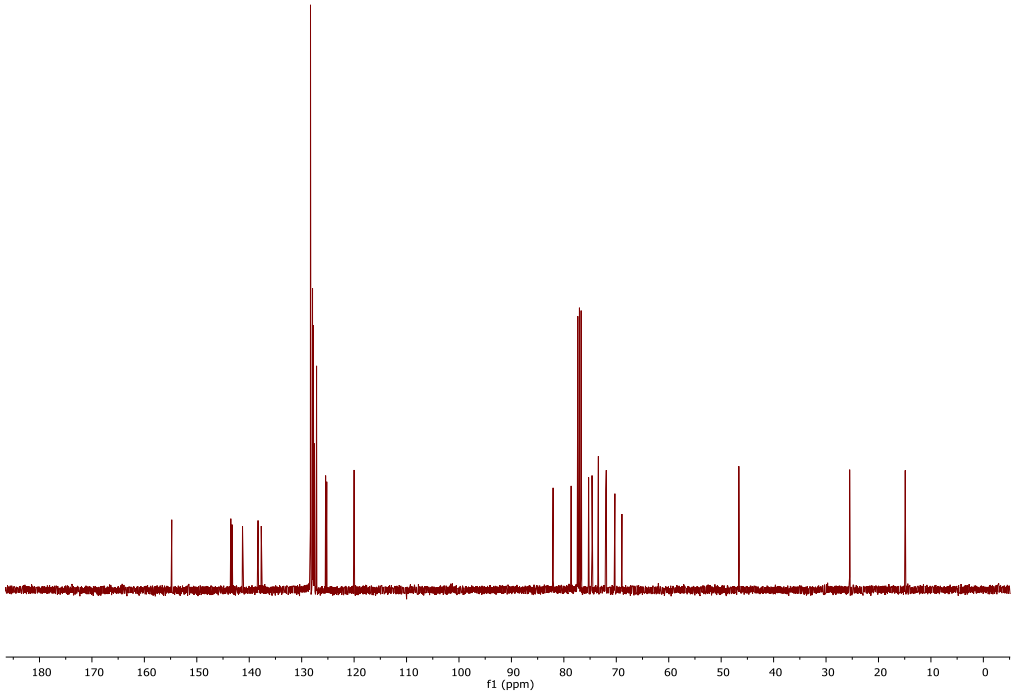


Fig. S2. ^{13}C NMR of S2 (101 MHz, $CDCl_3$).

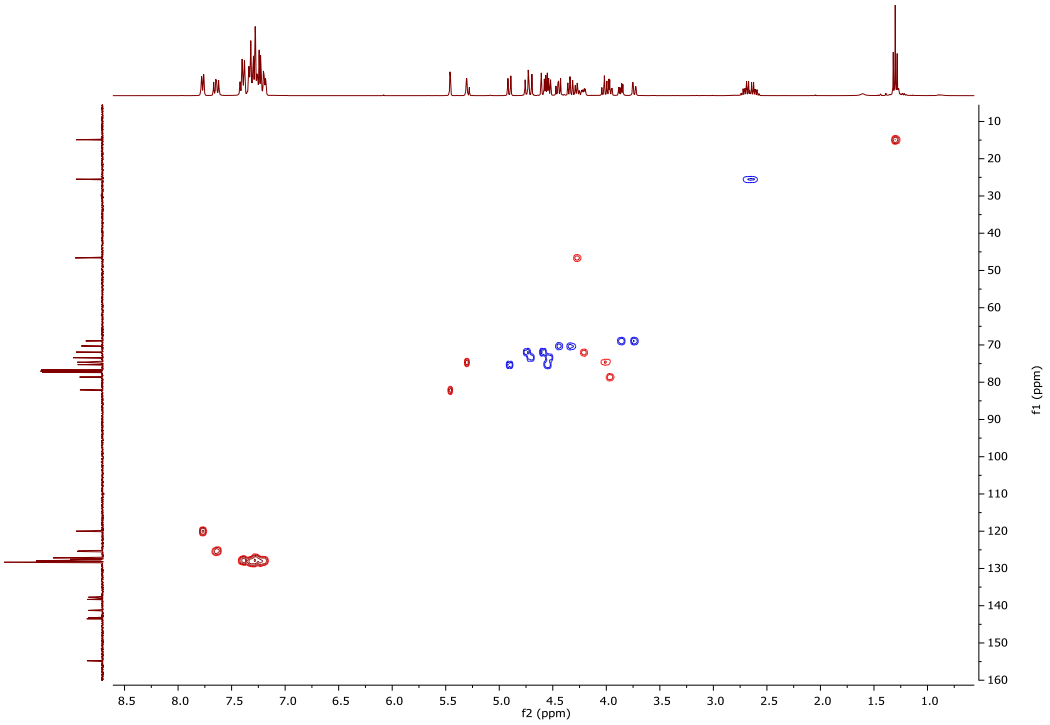
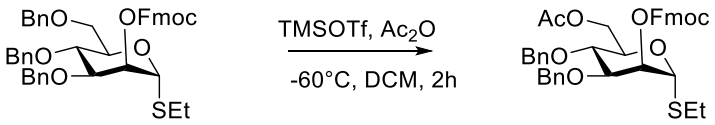


Fig. S3. HSQC NMR of S2 ($CDCl_3$).

Ethyl 6-O-acetyl-3,4,6-O-benzyl-2-O-fluorenylcarboxymethyl-thio-α-D-mannopyranoside, 31



S2 **31**

Ethyl 3,4,6-O-benzyl-2-O-fluorenylcarboxymethyl-thio-α-D-mannopyranoside **S2** (1.8 g, 2.5 mmol) was dissolved in 5mL of dry DCM, followed by Ac_2O (5.9 mL, 62.5 mmol). The mixture was cooled to -60 °C and stirred for 10 min. A solution of 1:1 TMSOTf–DCM (0.45 mL, 1.2 mmol) was added dropwise over 10 min. After the reaction was finished (3 hours), a 1:1 mixture of sat $NaHCO_3$–DCM (10 mL) was added and the mixture was stirred for additional 30 min. The organic layer was separated and washed with $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4 and concentrated to afford a pale yellow oil. The residue was purified by flash chromatography using 20% EtOAc–hexane to obtain **31** as a colorless oil (1.15 g, 68%). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.78 (dd, *J* = 7.6, 2.3 Hz, 2H), 7.63 (dd, *J* = 11.8, 7.5 Hz, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.36 – 7.21 (m, 12H), 5.40 (s, 1H), 5.28 (br s, 1H), 4.93 (d, *J* = 10.8 Hz, 1H), 4.74 (d, *J* = 11.3 Hz, 1H), 4.60 (dd, *J* = 15.3, 11.1 Hz, 2H), 4.46 (dd, *J* = 10.4, 7.2 Hz, 1H), 4.36 (ddd, *J* = 18.3, 12.6, 7.8 Hz, 3H), 4.29 – 4.20 (m, 2H), 3.95 (dd, *J* = 9.3, 3.2 Hz, 1H), 3.86 (t, *J* = 9.5 Hz, 1H), 2.63 (m, Hz, 2H), 2.06 (s, 3H), 1.30 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR (151 MHz, cdcl$_3$) δ 170.93, 154.80, 143.62,

143.33, 141.47, 141.40, 138.09, 137.65, 128.60, 128.53, 128.26, 128.16, 128.07, 128.03, 127.99, 127.29, 125.47, 125.27, 120.22, 120.19, 82.31, 78.70, 75.47, 74.50, 74.33, 72.04, 70.40, 63.39, 46.80, 25.79, 20.97, 15.08; m/z (HRMS+) 691.2341 $[M + Na]^+$ ($C_{39}H_{40}O_8SNa$ requires 691.2336); $[\alpha]_D^{20}$ +42.59 (*c* 1, $CHCl_3$); IR (neat) ν_{max} = 744, 1215 cm^{-1}.

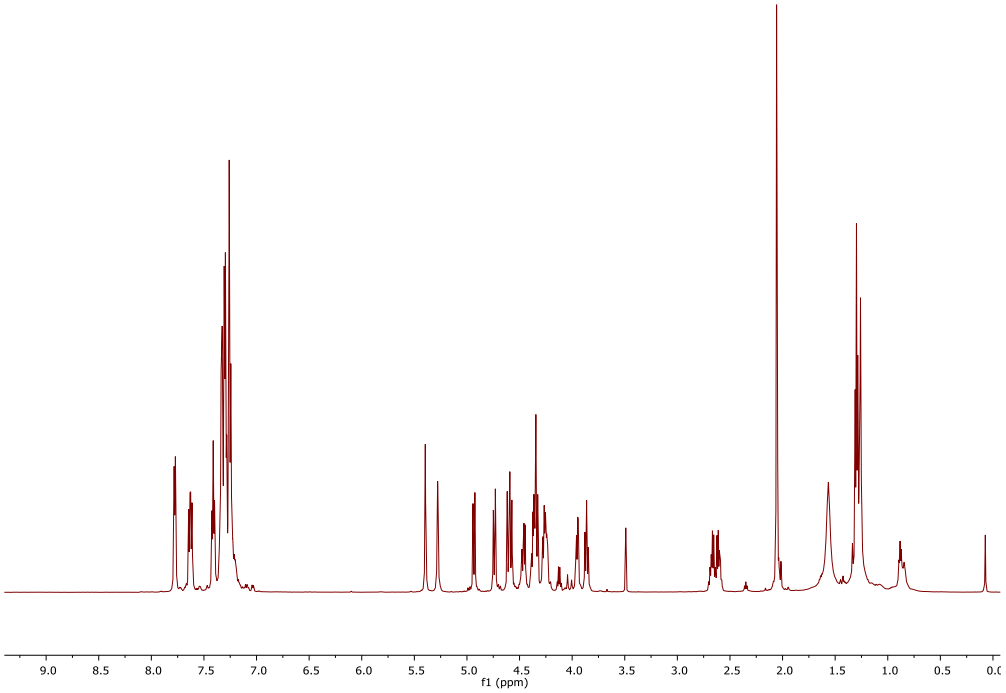


Fig. S4. ^1H NMR of 31 (600 MHz, $CDCl_3$).

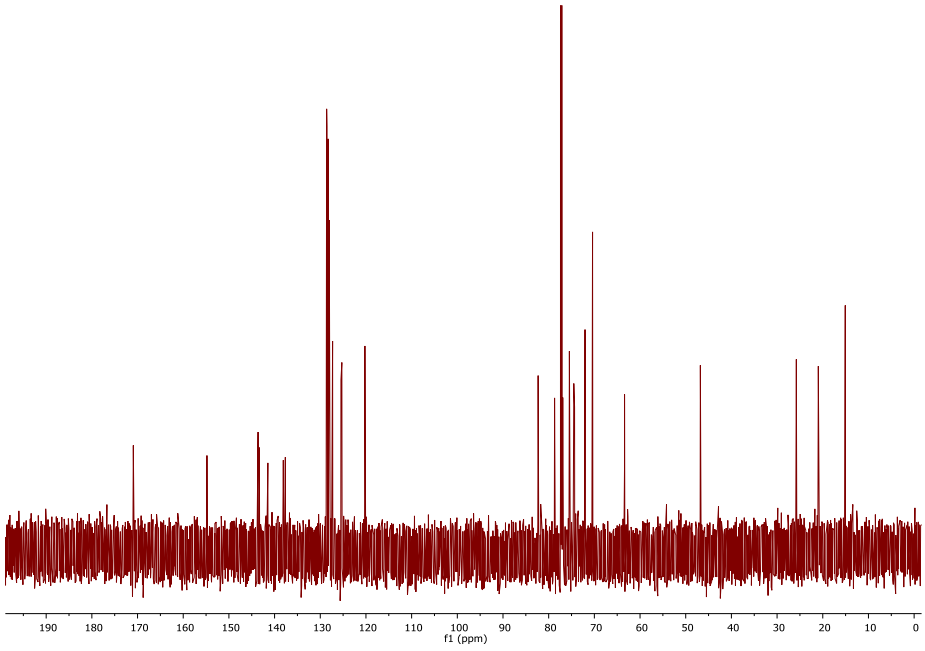


Fig. S5. ^{13}C NMR of 31 (151 MHz, $CDCl_3$).

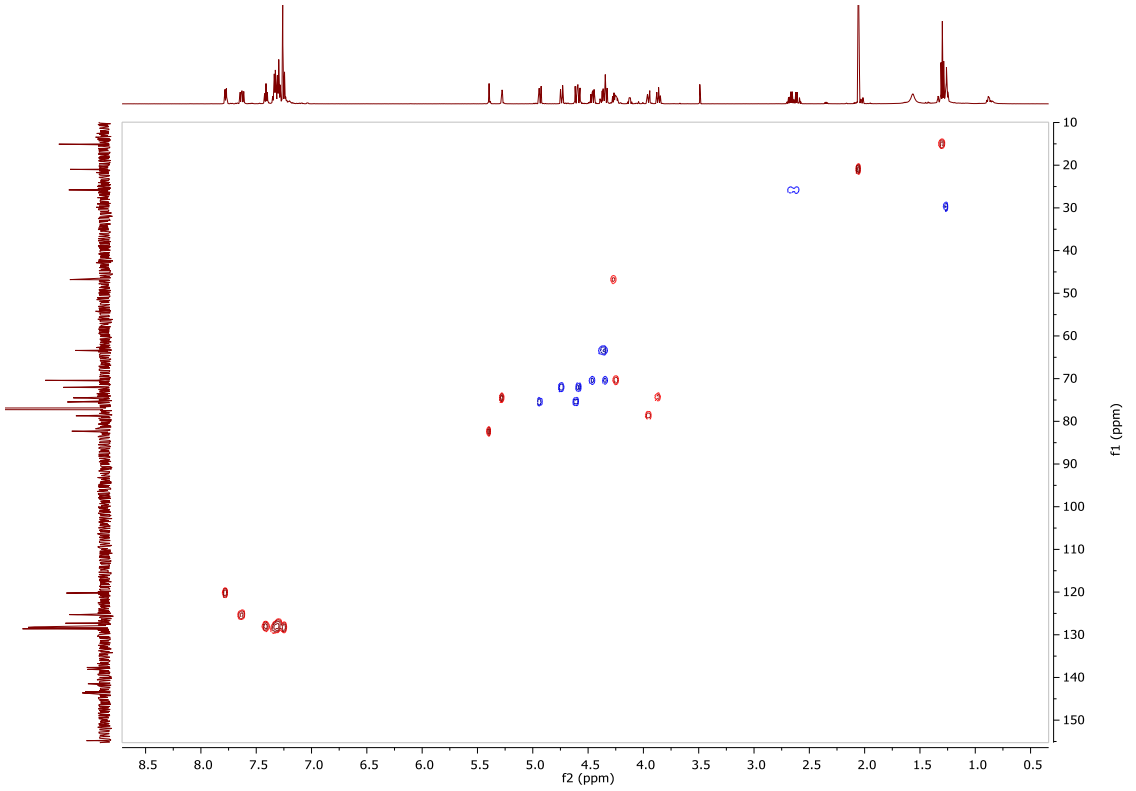
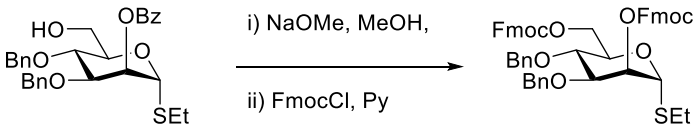


Fig. S6. HSQC NMR of 31 ($CDCl_3$).

Ethyl 3,4,-O-benzyl-2,6-O-fluorenylcarboxymethyl-thio-α-D-mannopyranoside, 32



S3 **32**

S3 was prepared according to previously established procedures. (S7)

Ethyl 3,4,6-O-benzyl-thio-α-D-mannopyranoside **S3** (1.4 g, 2.8 mmol) was dissolved in 30 mL of a mixture of DCM–MeOH 1:1, then 5 ml of a solution of NaOMe in MeOH (0.5 M, 3.10 mmol) were added and stirred for 16 h at room temperature. After the reaction was complete, the mixture was neutralized using Amberlite IR-120 ion-exchange resin, filtered and concentrated. The mixture was further purified by a quick filtration on silica gel (DCM:EtOAc:MeOH, 50:40:10) and dried under high vacuum to obtain a clear oil. The product was dissolved in DCM (25 mL), Pyridine (0.6 mL, 5.9 mmol) and (9H-fluoren-9-yl)methyl carbonochloridate (1.28 g, 4.95 mmol) was added. The solution was stirred at rt until completion for 3 hours. The mixture was diluted with DCM and washed with 1 M HCl, saturated aqueous $NaHCO_3$ and water. The organic layer was dried over $MgSO_4$, filtered and evaporated. The resulting yellow oil was purified by column chromatography (Hexanes: EtOAc = 4:1) to give **32** as a white foam (1.5 g, 86%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.87

– 7.81 (m, 4H), 7.77 – 7.63 (m, 4H), 7.50 – 7.32 (m, 18H), 5.55 (d, *J* = 1.5 Hz, 1H), 5.42 (dd, *J* = 3.1, 1.6 Hz, 1H), 5.07 (d, *J* = 10.9 Hz, 1H), 4.85 (d, *J* = 11.3 Hz, 1H), 4.71 (dd, *J* = 20.8, 11.1 Hz, 2H), 4.59 (d, *J* = 4.0 Hz, 2H), 4.51 – 4.40 (m, 4H), 4.34 (td, *J* = 7.5, 2.2 Hz, 2H), 4.12 – 4.00 (m, 3H), 2.83 – 2.64 (m, 2H), 1.39 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.21, 154.85, 144.50, 143.53, 143.35, 143.26, 141.56, 141.38, 141.36, 141.32, 141.30, 138.04, 137.59, 128.59, 128.51, 128.18, 128.15, 128.11, 128.01, 127.97, 127.65, 127.30, 127.28, 127.24, 127.15, 125.47, 125.36, 125.28, 125.25, 124.86, 120.17, 120.14, 82.13, 78.67, 75.48, 74.43, 74.42, 72.00, 70.41, 70.34, 70.11, 66.93, 65.21, 50.41, 46.79, 46.69, 25.68, 15.03; (HRMS+) 887.2669 $[M + K]^+$ ($C_{52}H_{48}O_9SK$ requires 887.2656);); $[\alpha]_D^{20}$ +32 (*c* 1, $CHCl_3$); IR (neat) ν_{max} = 755, 950, 1128,2972 cm^{-1}.

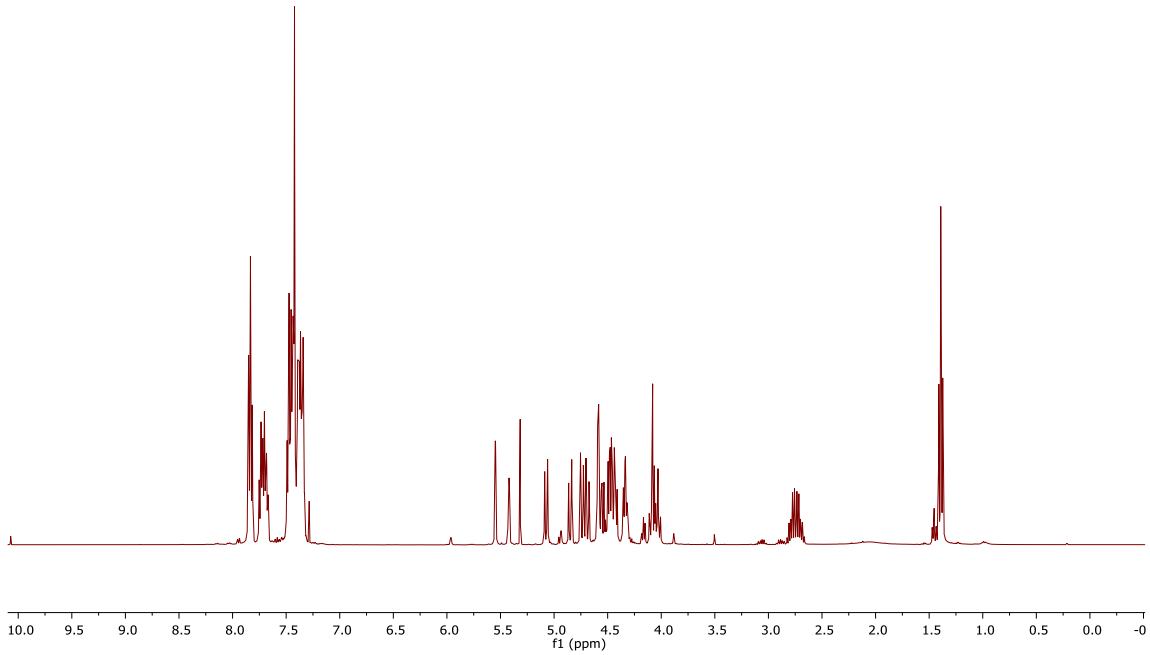


Fig. 7. ^{1}H NMR of 32 (400 MHz, $CDCl_3$).

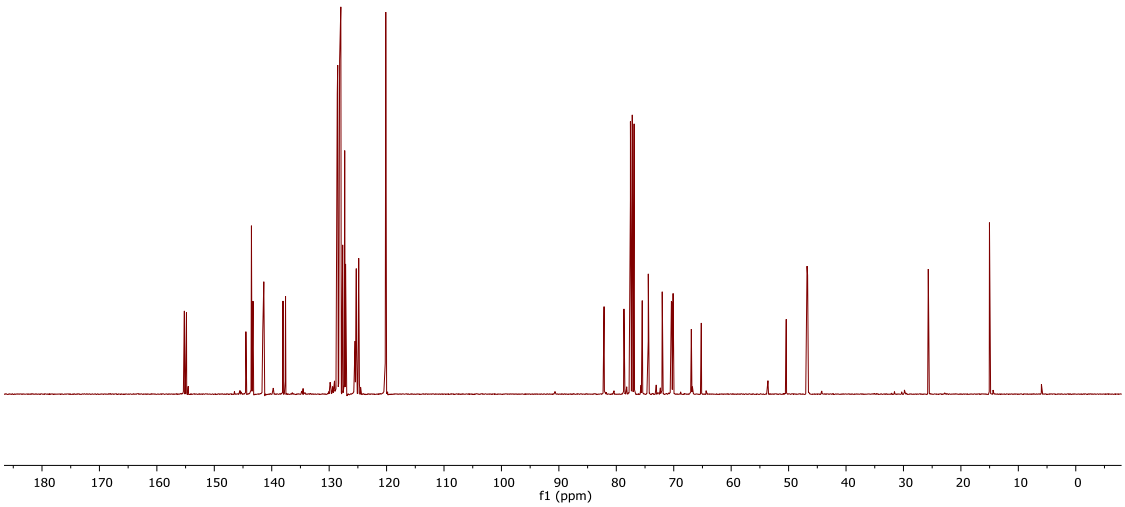


Fig. S8. ^{13}C NMR of 32 (101 MHz, $CDCl_3$).

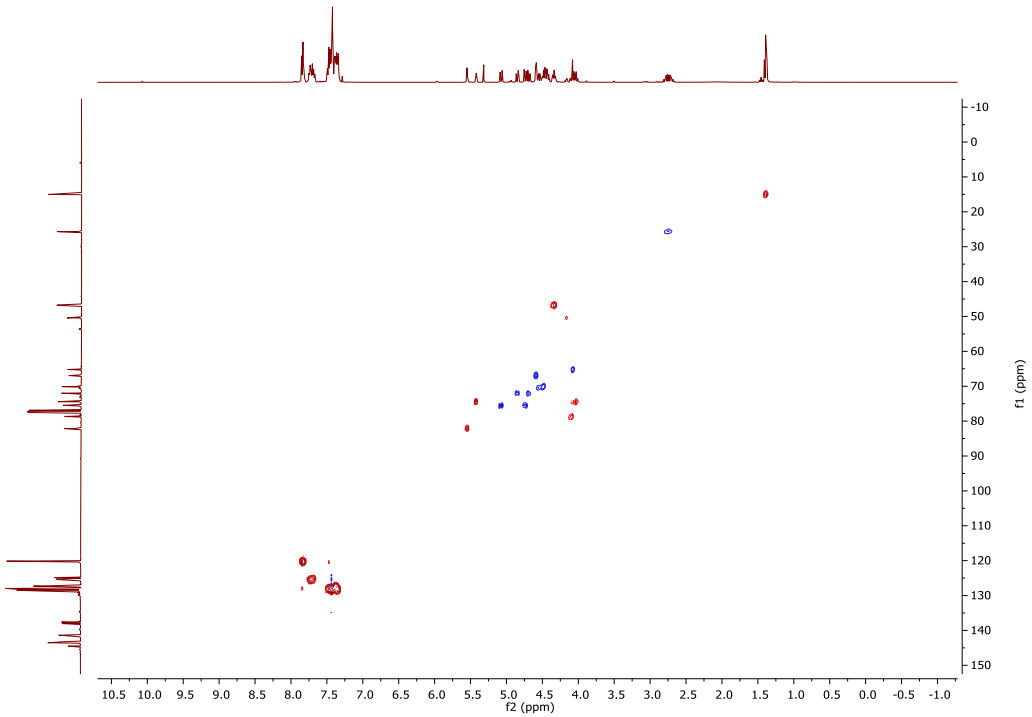
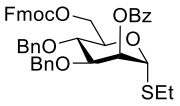
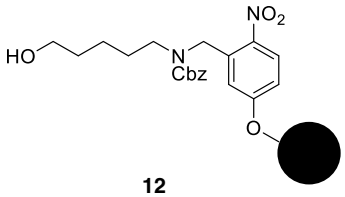


Fig. S9. HSQC NMR of 32 ($CDCl_3$).



11



12

BB 11 and photo-cleavable linker **12** were prepared according to previously established procedures. (S7–S9)

Part 3. Automated Glycan Assembly

3.1 General materials and methods

All solvents used were HPLC-grade. The solvents used for the building block, activator, TMSOTf and capping solutions were taken from an anhydrous solvent system (jcmeyer-solvent systems). The building blocks were co-evaporated three times with chloroform and dried for 1 h on high vacuum before use. Activator, deprotection, acidic wash and building block solutions were freshly prepared and kept under argon during the automation run. All yields of products obtained by AGA were calculated on the basis of resin loading. Resin loading was determined by performing one glycosylation (Module C) with 10 equiv. of **11** followed by DBU promoted Fmoc-cleavage and determination of dibenzofulvene production by measuring its UV absorbance.

3.2 Preparation of stock solutions

- **Building Block**: between 0.06 and 0.08 mmol of building block (depending on BB) was dissolved in 1 mL of DCM.
- **Activator solution**: 1.35 g of recrystallized NIS was dissolved in 40 mL of a 2:1 mixture of anhydrous DCM and anhydrous dioxane. Then triflic acid (55 µL) was added. The solution is kept at 0°C for the duration of the automation run.
- **Fmoc deprotection solution**: A solution of 20% piperidine in DMF (v/v) was prepared.
- **TMSOTf Solution**: TMSOTf (0.45 mL) was added to DCM (40 mL).

3.3 Modules for automated synthesis

Module A: Resin Preparation for Synthesis (20 min)

All automated syntheses were performed on 0.0125 mmol scale. Resin was placed in the reaction vessel and swollen in DCM for 20 min at room temperature prior to synthesis. During this time, all reagent lines needed for the synthesis were washed and primed. Before the first glycosylation, the resin was washed with the DMF, THF, and DCM (three times each with 2 mL for 25 s).

Module B: Acidic Wash with TMSOTf Solution (20 min)

The resin was swollen in 2 mL DCM and the temperature of the reaction vessel was adjusted to -20 °C. Upon reaching the low temperature, TMSOTf solution (1 mL) was added drop wise to the reaction vessel. After bubbling for 3 min, the acidic solution was drained and the resin was washed with 2 mL DCM for 25 s.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Cooling	-	-	-	-20	(15 min)*
Deliver	1	DCM	2 mL	-20	-
Deliver	1	TMSOTf solution	1 mL	-20	3 min
Wash	1	DCM	2 mL	-20	25 s

*Time required to reach the desired temperature.

Module C: Thioglycoside Glycosylation (35 min)

The building block solution (0.08 mmol of BB in 1 mL of DCM per glycosylation) was delivered to the reaction vessel. After the set temperature was reached, the reaction was started by drop wise addition of the activator solution (1.0 mL, excess). The glycosylation conditions are building block dependent (we report the most common set of conditions). After completion of the reaction, the solution is drained and the resin was washed with DCM, DCM:dioxane (1:2, 3 mL for 20 s) and DCM (two times, each with 2 mL for 25 s). The temperature of the reaction vessel is increased to 25 °C for the next module.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Cooling	-	-	-	-20	-
Deliver	1	BB solution	1 mL	-20	-
Deliver	1	Activator solution	1 mL	-20	-
Reaction time (BB dependent)	1			-20 to 0	5 min 20 min
Wash	1	DCM	2 mL	0	5 s
Wash	1	DCM : Dioxane (1:2)	2 mL	0	20 s
Heating	-	-	-	25	-
Wash	2	DCM	2 mL	> 0	25 s

Module D: Fmoc Deprotection (14 min)

The resin was washed with DMF (three times with 2 mL for 25 s) and the temperature of the reaction vessel was adjusted to 25 °C. 2 mL of Fmoc deprotection solution was delivered into the reaction vessel. After 5 min, the reaction solution was drained and the resin washed with DMF (three times with 3 mL for 25 s) and DCM (five times each with 2 mL for 25 s). The temperature of the reaction vessel is decreased to -20 °C for the next module.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Heating	-	-	-	25	(5 min)*
Wash	3	DMF	2 mL	25	25 s
Deliver	1	Fmoc depr. solution	2 mL	25	5 min
Wash	1	DMF	2 mL		
Cooling	-	-	-	-20	-
Wash	3	DMF	2 mL	< 25	25 s
Wash	5	DCM	2 mL	< 25	25 s

*Time required to reach the desired temperature. With capping, this time is not needed.

3.4 Post-synthesizer manipulations

Cleavage from Solid Support

After automated synthesis, the oligosaccharides were cleaved from the solid support using a continuous-flow photoreactor as described previously.[3a]

Purification

Solvent is evaporated *in vacuo* and the crude products were analyzed and purified using analytical and preparative HPLC (Agilent 1200 Series spectrometer).

- **Method A:** (YMC-Diol-300 column, 150 x 4.6 mm) flow rate of 1.0 mL / min with Hex – 20% EtOAc as eluent [isocratic 20% EtOAc (5 min), linear gradient to 55% EtOAc (35 min), linear gradient to 100% EtOAc (5 min)].

- **Method B:** (YMC-Diol-300 column, 150 x 20 mm) flow rate of 15 mL / min with Hex – 20% EtOAc as eluents [isocratic 20% EtOAc (5 min), linear gradient to 55% EtOAc (35 min), linear gradient to 100% EtOAc (5 min)].

3.5 Modules for oligosaccharide deprotection

Module F: Methanolysis

The protected oligosaccharide was dissolved in MeOH:DCM (1.5 mL,1:1). NaOMe in MeOH (0.5 M, 3eq per benzoyl ester) was added and the solution was stirred at room temperature for 12 h, neutralized with Amberlite IR-120 (H^+ form) resin, filtered and concentrated *in vacuo.* The crude compound was used for hydrogenolysis without further purification.

Module G: Hydrogenolysis

The crude compound obtained from *Module F* was dissolved in 2 mL of DCM:*t*BuOH:H_2O (1:0.5:0.5). 100% by weight Pd-C (10%) was added and the reaction was stirred in H_2 bomb with 60 psi pressure for 16 hours. The reactions were filtered through celite, washed with DCM, *t*BuOH and H_2O. The filtrates were concentrated *in vacuo.*

Purification

Following hydrogenolysis, crude products were analyzed/purified using analytical/preparative HPLC (Agilent 1200 Series spectrometer) or reversed phase SPE (Waters Sep-Pak®, C18) column.

- **Method C:** (Hypercarb column, 150 x 4.6 mm) flow rate of 0.7 mL / min with H_2O (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 30% ACN (30 min), linear gradient to 100% ACN (5 min)].

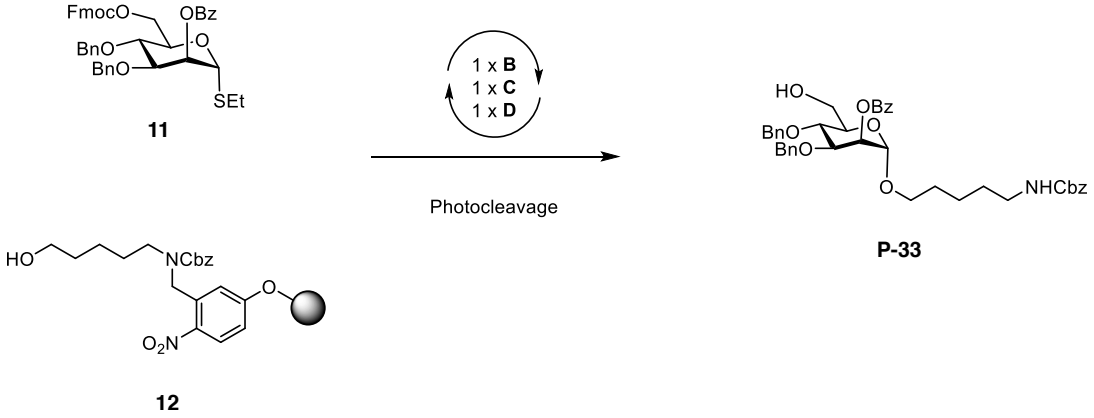
- **Method D:** (Hypercarb column, 150 x 10 mm) flow rate of 1.3 mL / min with H_2O (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 30% ACN (30 min), linear gradient to 100% ACN (5 min)].

- **Method E:** Reversed phase (RP) SPE (Waters Sep-Pak®, C18) column, (H_2O: MeOH = 1:0 to 0:1).

Following purification all products were lyophilized on a Christ Alpha 2-4 LD plus freeze dryer prior to characterization.

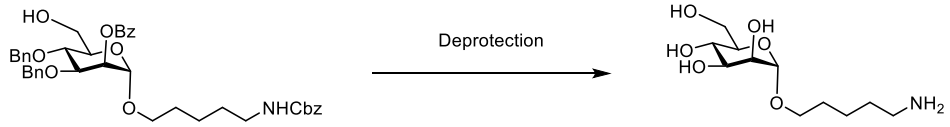
3.6 AGA

3.6.1. Synthesis of monomannoside, 33



	Module	Conditions
	A: Resin Preparation for Synthesis	
1	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Fmoc Deprotection	BB **11,** 6.5 equiv. (-20° for 5 min, 0° for 20 min)

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC (Method B, t_R = 15.0 min) afforded compound **P-34**.



P-33 **33**

Deprotection of **P-33** as reported is Section 3.5 (Module F and G) followed by purification using preparative HPLC (Method E) afforded compound **33** (2.0 mg, 60% based on resin loading). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.74 (d, *J* = 1.8 Hz, 1H), 3.81 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.76 (dd, *J* = 12.2, 1.8 Hz, 1H), 3.68 – 3.57 (m, 3H), 3.53 – 3.38 (m, 3H), 2.88 (t, *J* = 7.6 Hz, 2H), 1.63 – 1.50 (m, 4H), 1.33 (dtd, *J* = 15.6, 7.4, 5.7 Hz, 2H). NMR data were in good agreement with those previously reported. (S10)

Monomannoside, **33**, can be organic solution synthesized according to literature (S1).

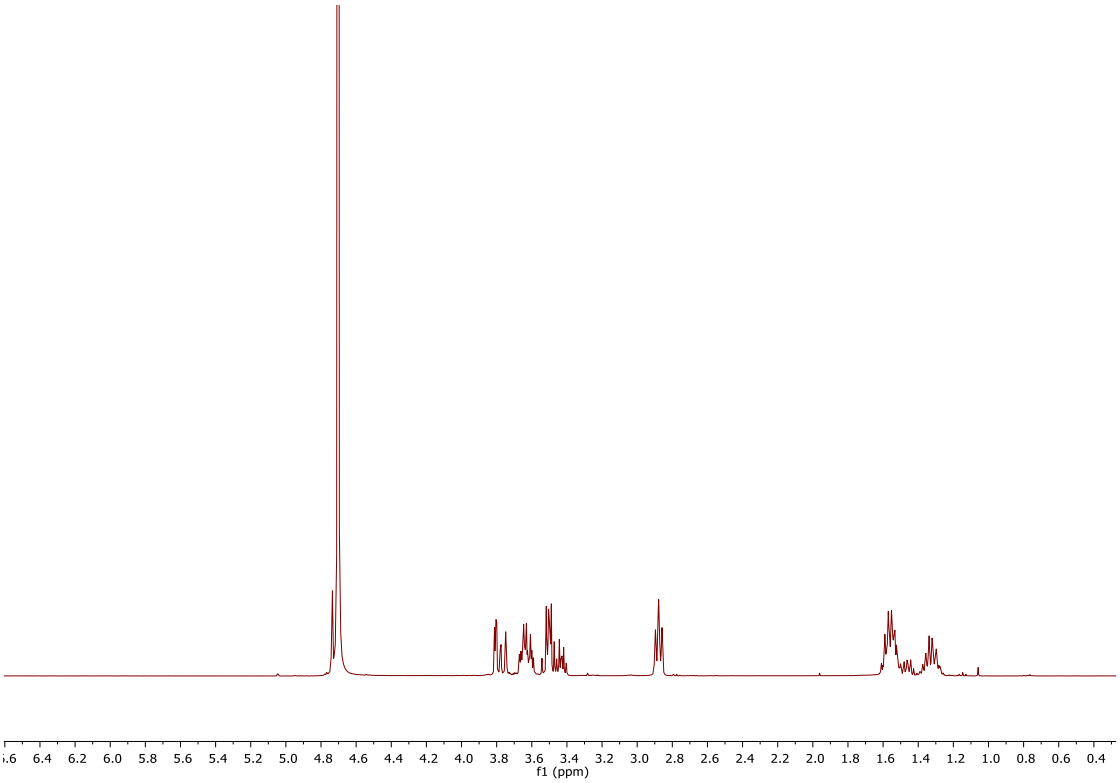
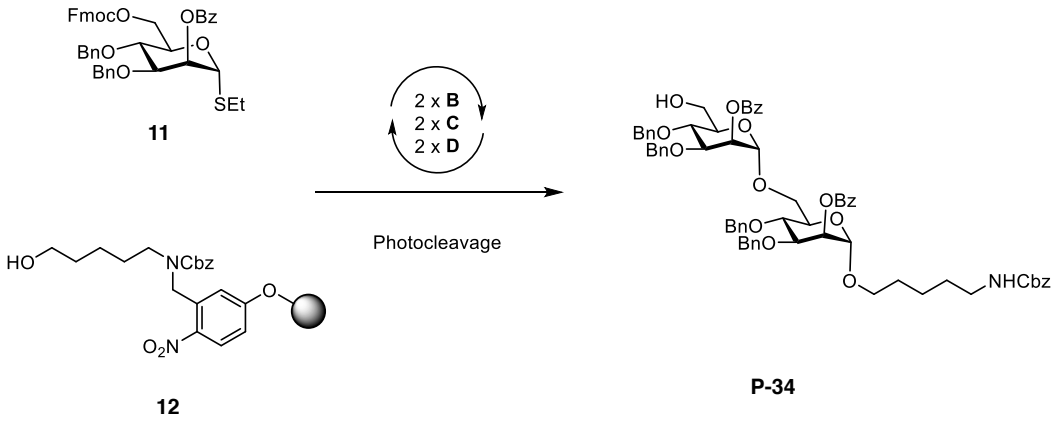


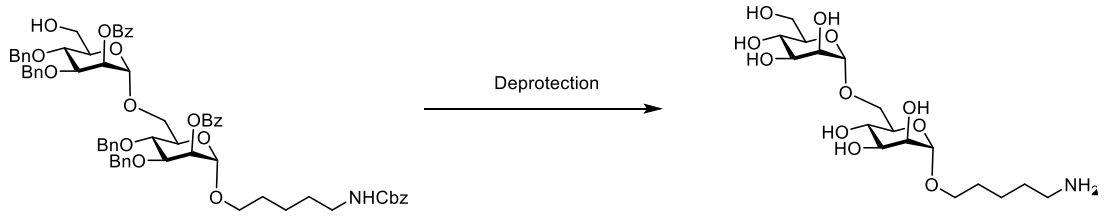
Fig. S10. ^{1}H NMR of 33 (400 MHz, D_2O).

3.6.2. Synthesis of α(1-6) dimannoside, 34



	Module	Conditions
	A: Resin Preparation for Synthesis	
2	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Fmoc Deprotection	BB **11,** 6.5 equiv. (-20° for 5 min, 0° for 20 min)

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC (Method B, t_R = 19.5 min) afforded compound **P-34**.



P-34 **34**

Deprotection of **P-33** as reported is Section 3.5 (Module F and G) followed by purification using preparative HPLC (Method E) afforded compound **33** (2.7 mg, 50% based on resin loading). ^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.76 (d, *J* = 1.7 Hz, 1H), 4.71 (s, 2H), 3.81 (ddd, *J* = 12.2, 3.2, 1.7 Hz, 3H), 3.75 (dd, *J* = 11.8, 1.7 Hz, 1H), 3.69 (dd, *J* = 9.1, 3.4 Hz, 1H), 3.67 – 3.48 (m, 8H), 3.42 (dt, *J* = 9.9, 6.1 Hz, 1H), 2.90 – 2.82 (m, 2H), 1.61 – 1.47 (m, 4H), 1.41 – 1.25 (m, 2H). NMR data were in good agreement with those previously reported. (S11)

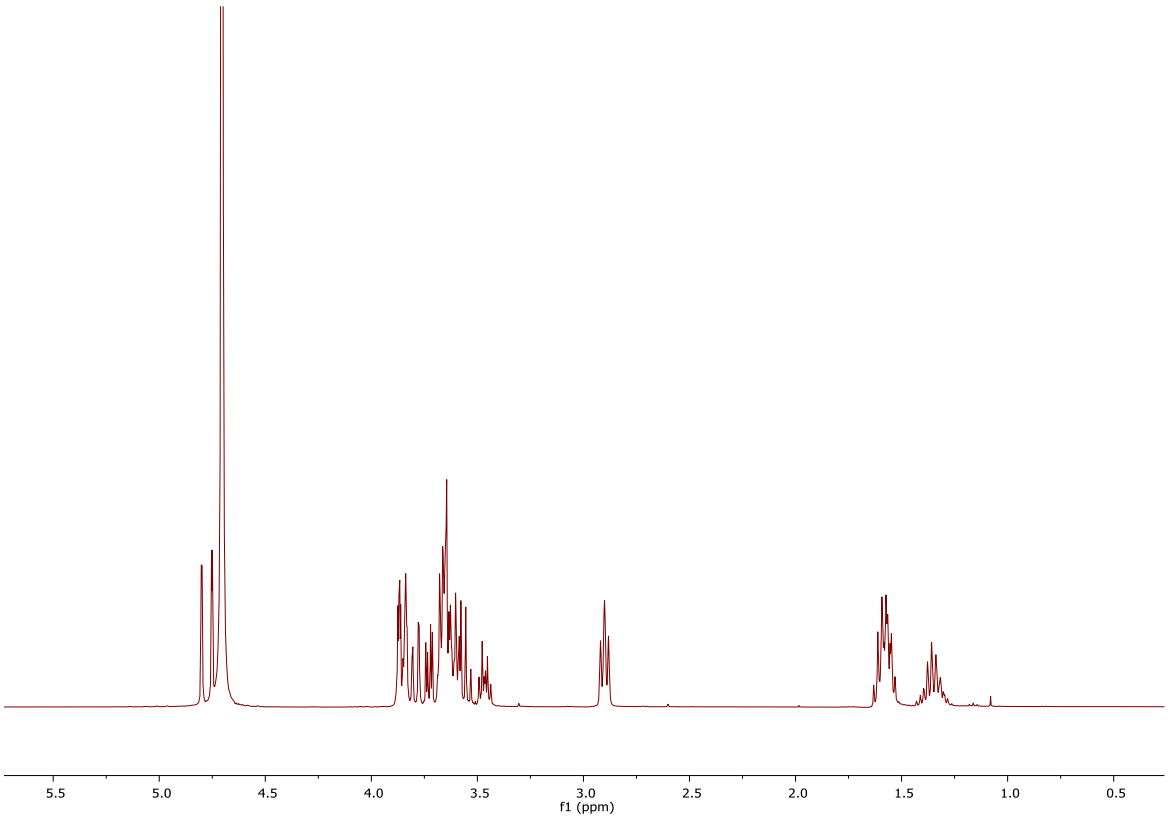
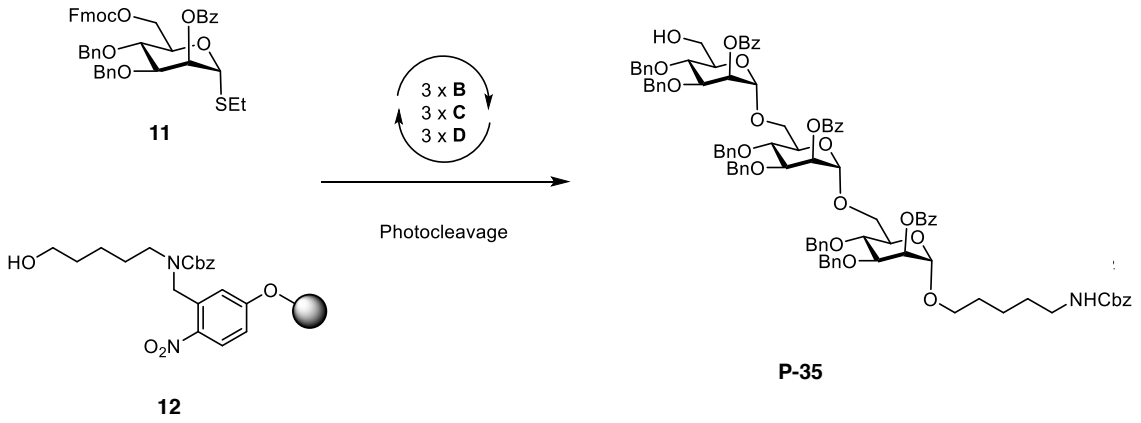


Fig. S11. ^{1}H NMR of 34 (400 MHz, D_2O).

3.6.3. Synthesis of α(1-6) trimannoside, 35



	Module	Conditions
	A: Resin Preparation for Synthesis	
3	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	BB **11,** 6.5 equiv. (-20° for 5 min, 0° for 20 min)
	D: Fmoc Deprotection	

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC (Method B, t_R = 20.7 min) afforded compound **P-35**.

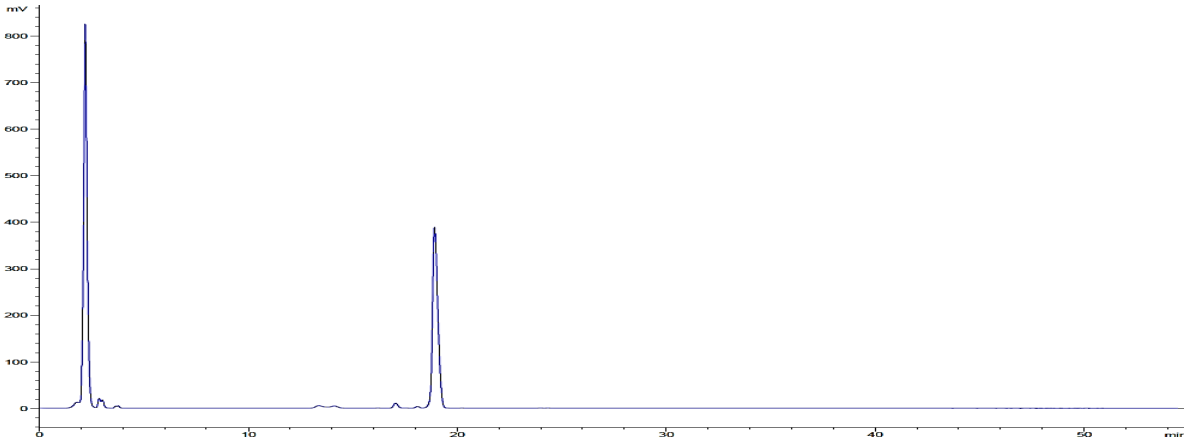
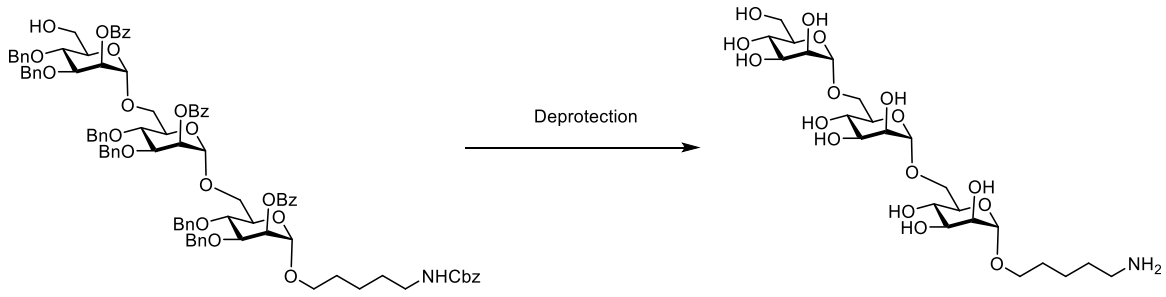


Fig. S12. Crude NP-HPLC of P-35 (ELSD trace, Method A, t_R = 19.3 min).



P-35 **35**

Deprotection of **P-36** as reported is Section 3.5 (Module F and G) followed by purification using preparative HPLC (Method E) afforded compound **36** (3.6 mg, 51% based on resin loading).

Analytical data for **36**: ^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.77 (d, *J* = 1.7 Hz, 1H), 4.74 (d, *J* = 1.7 Hz, 1H), 4.72 (s, 1H), 3.86 – 3.76 (m, 6H), 3.76 – 3.49 (m, 13H), 3.43 (dt, *J* = 9.9, 6.1 Hz, 1H), 2.91 – 2.82 (m, 2H), 1.53 (ddd, *J* = 13.8, 9.1, 7.1 Hz, 4H), 1.42 – 1.22 (m, 2H); ^{13}C NMR (101MHz, Deuterium Oxide) δ 99.79, 99.28, 99.15, 72.62, 70.83, 70.75, 70.70, 70.61, 70.45, 69.98, 69.88, 69.85, 67.54, 66.66, 66.50, 65.51, 60.85, 39.30, 27.94, 26.48, 22.45; m/z (HRMS+) 590.2668[M + H]+ ($C_{23}H_{44}NO_{16}$ requires 590.2655).

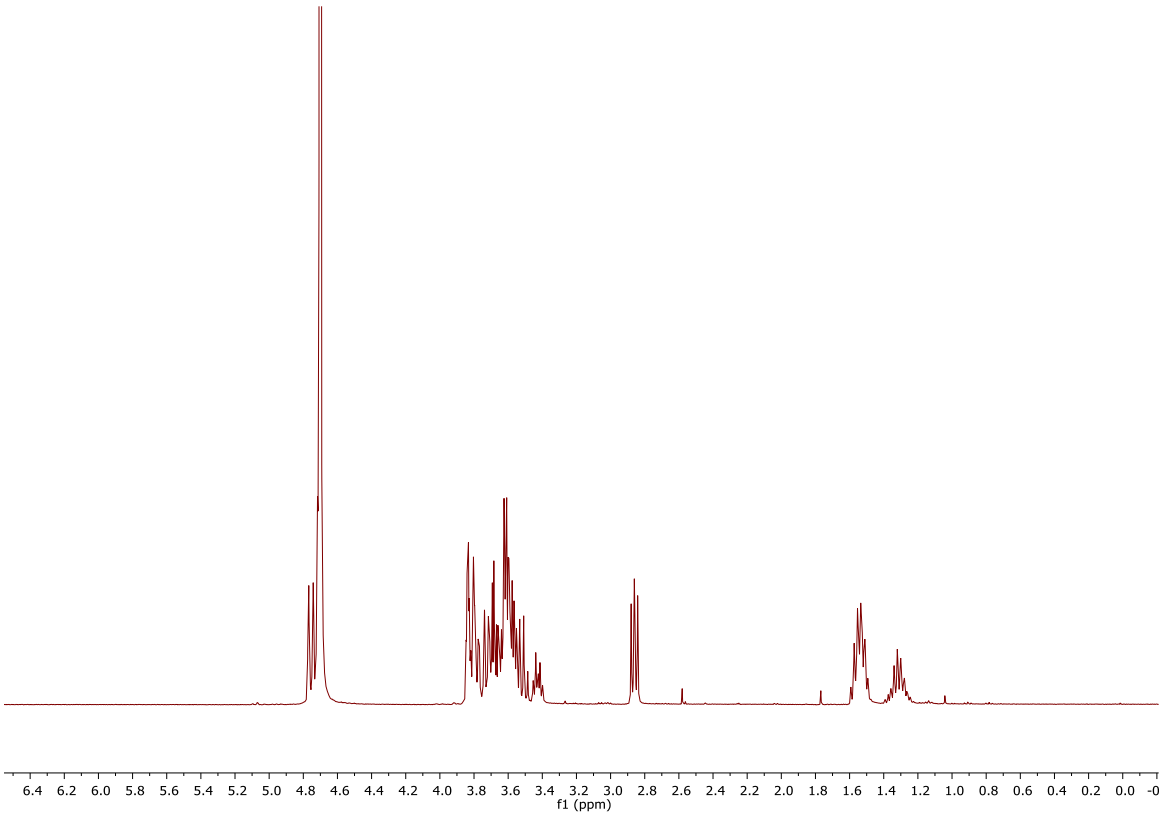


Fig. S13. ^{1}H NMR of 35 (400 MHz, D_2O).

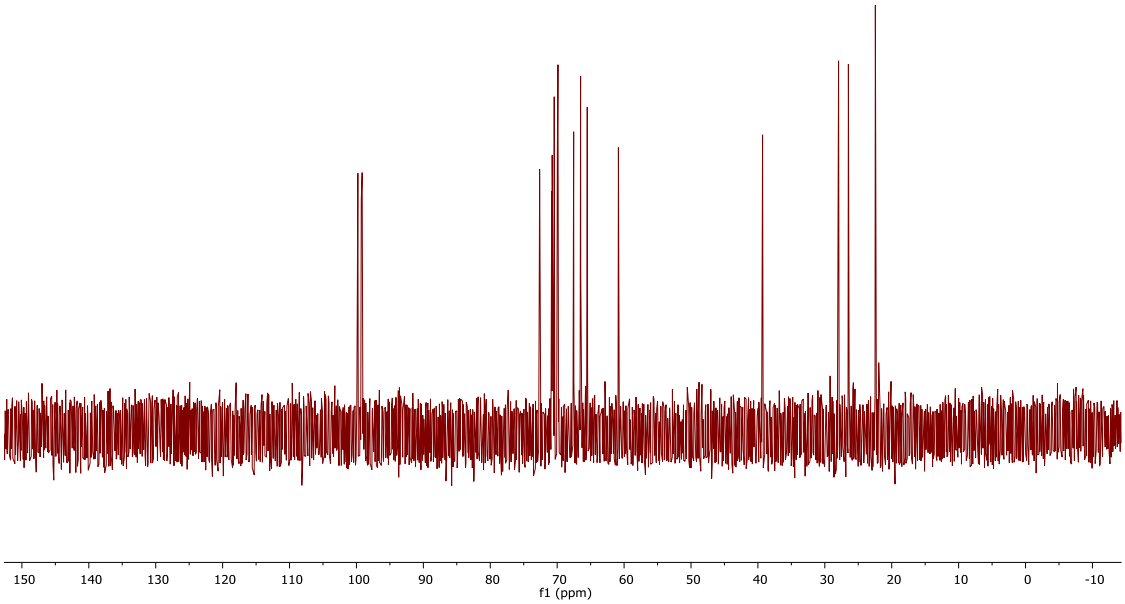


Fig. S14. ^{13}C NMR of 35 (101 MHz, D_2O).

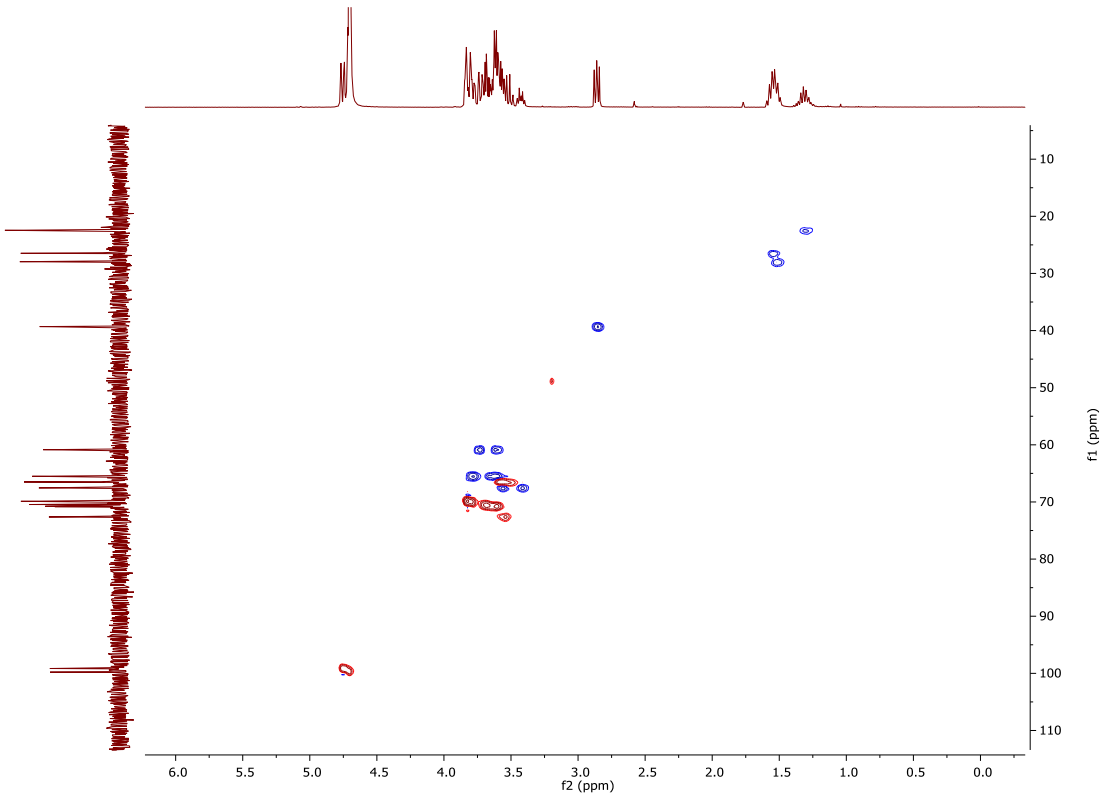
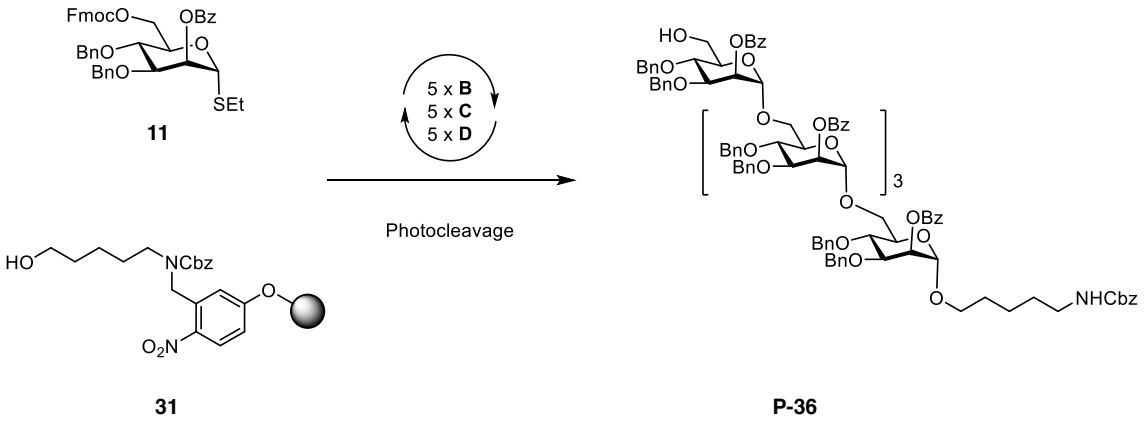


Fig. S15. HSQC NMR of 35 (D_2O).

3.6.4. Synthesis of α(1-6) pentamannoside, 36



	Module	Conditions
	A: Resin Preparation for Synthesis	
5	**B**: Acidic Wash with TMSOTf Solution	
	C: Thioglycoside Glycosylation	BB **11,** 6.5 equiv. (-20° for 5 min, 0° for 20 min)
	D: Fmoc Deprotection	

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC (Method B, t_R = 23.3 min) afforded compound **P-36**.

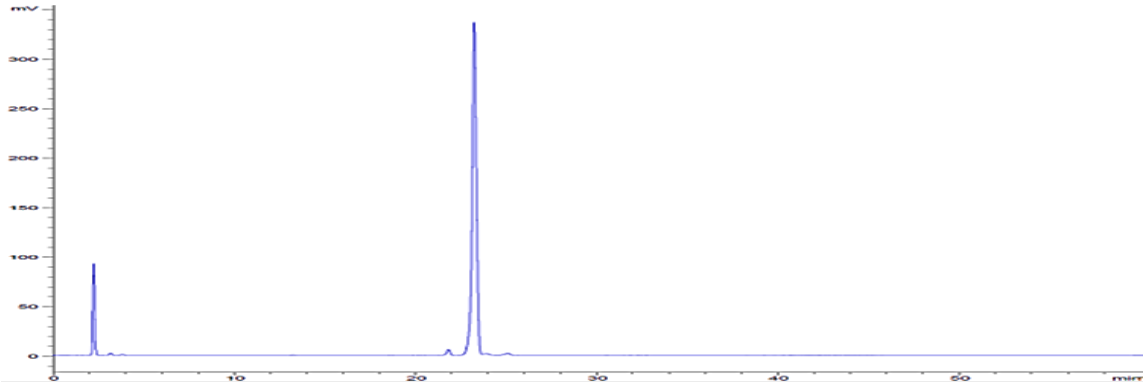
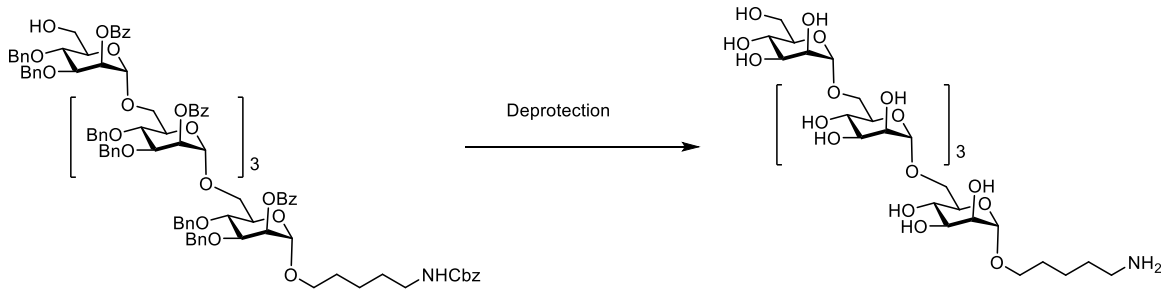


Fig. S16. Crude NP-HPLC of P-36 (ELSD trace, Method A, t_R = 23.2 min).



P-36 **36**

Deprotection of **P-36** as reported is Section 3.5 (Module F and G) followed by purification using preparative HPLC (Method E) afforded compound **36** (4.8 mg, 42% based on resin loading). Analytical data for **36**: ^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.73 (s, 1H), 4.71 (s, 1H), 4.67 (s, 1H), 3.80 (m, 2H), 3.78 – 3.71 (m, 10H), 3.71 – 3.49 (m, 15H), 2.82 (t, *J* = 7.7 Hz, 2H), 1.56 – 1.43 (m, 4H), 1.27 (h, *J* = 6.5, 6.0 Hz, 2H). ^{13}C NMR (101 MHz, d_2o) δ 99.76, 99.28, 99.13, 72.58, 70.77, 70.63, 70.53, 70.39, 69.83, 67.49, 66.60, 66.43, 65.38, 60.79, 39.23, 27.91, 26.46, 22.42. m/z (HRMS+) 914.3621 $[M + H]^+$ ($C_{35}H_{64}NO_{26}$ requires 914.3711).

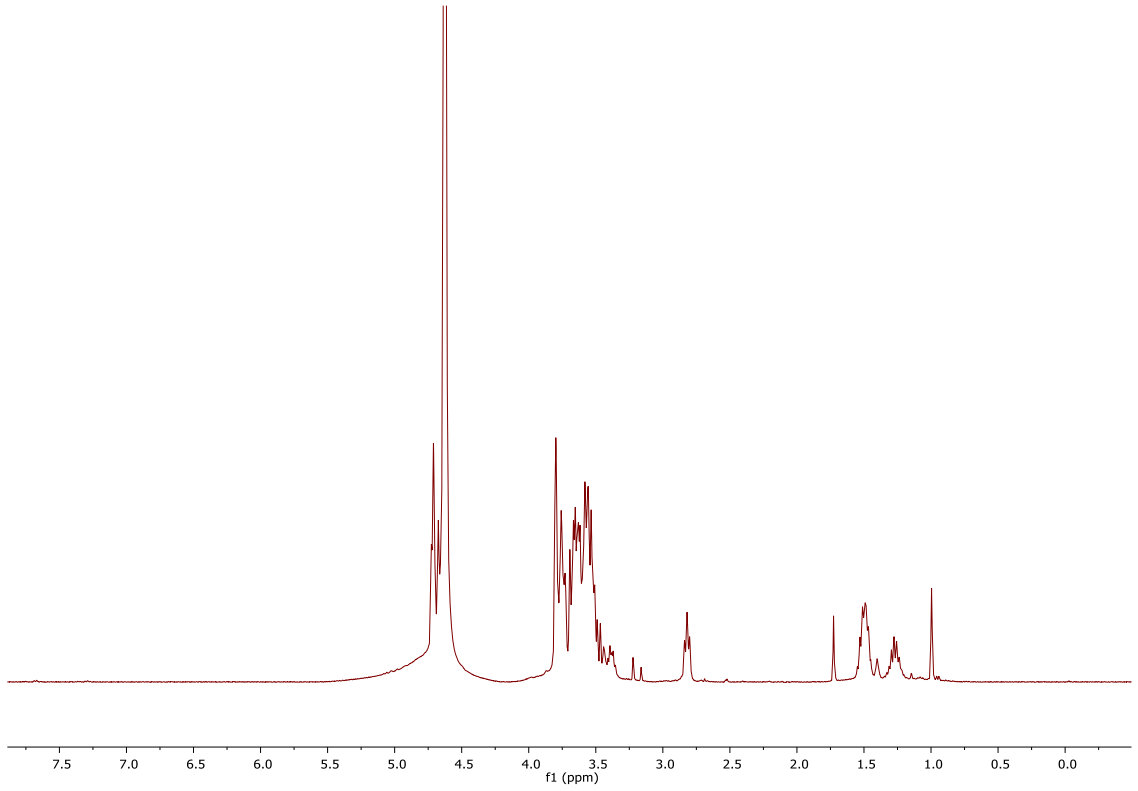


Fig. S17. ^{1}H NMR of 36 (400 MHz, D_2O).

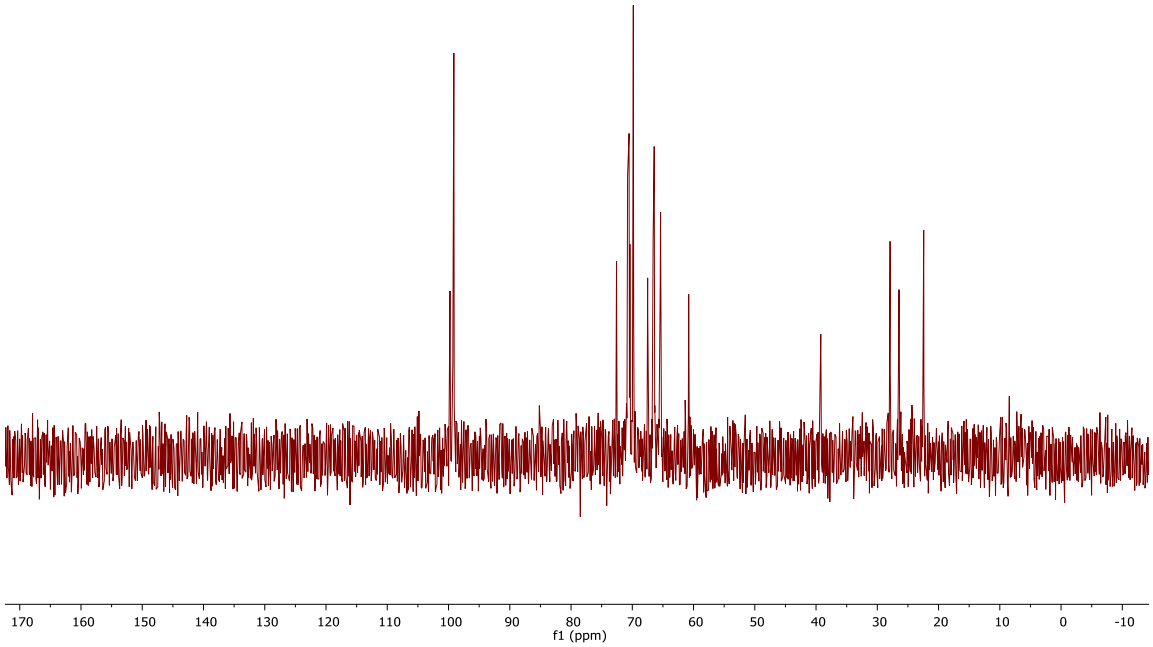


Fig. S18. ^{13}C NMR of 36 (101 MHz, D_2O).

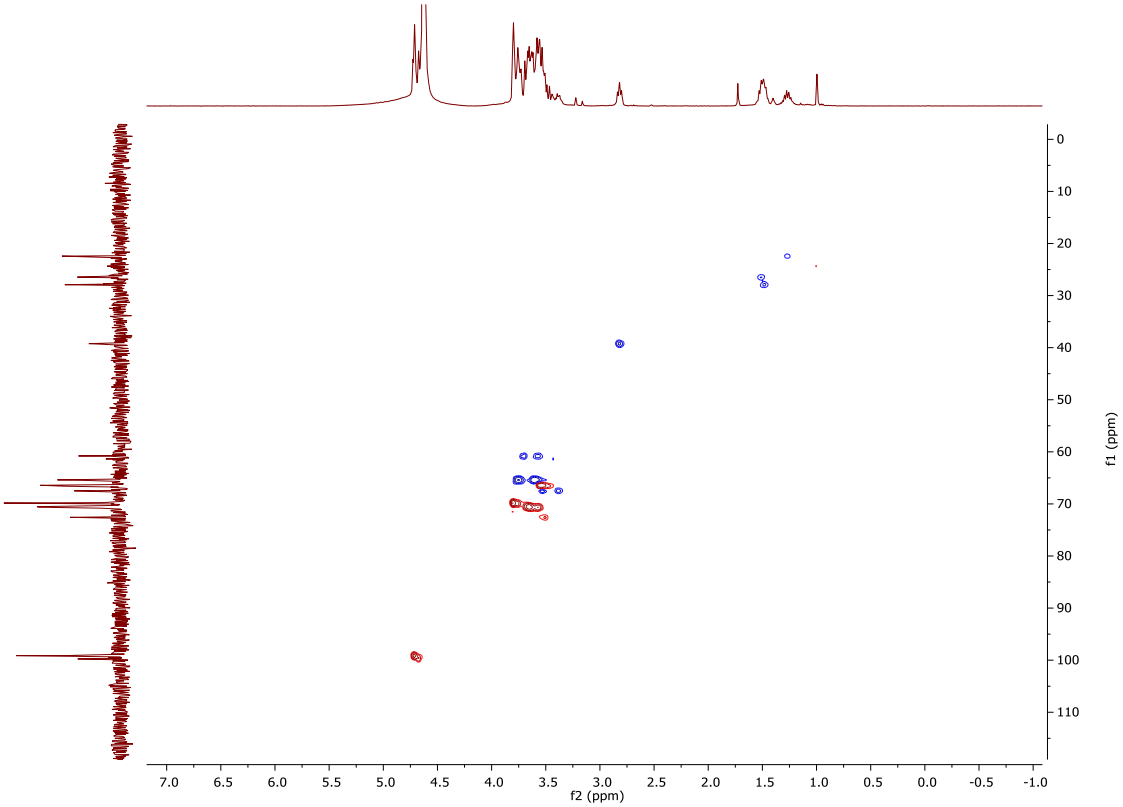
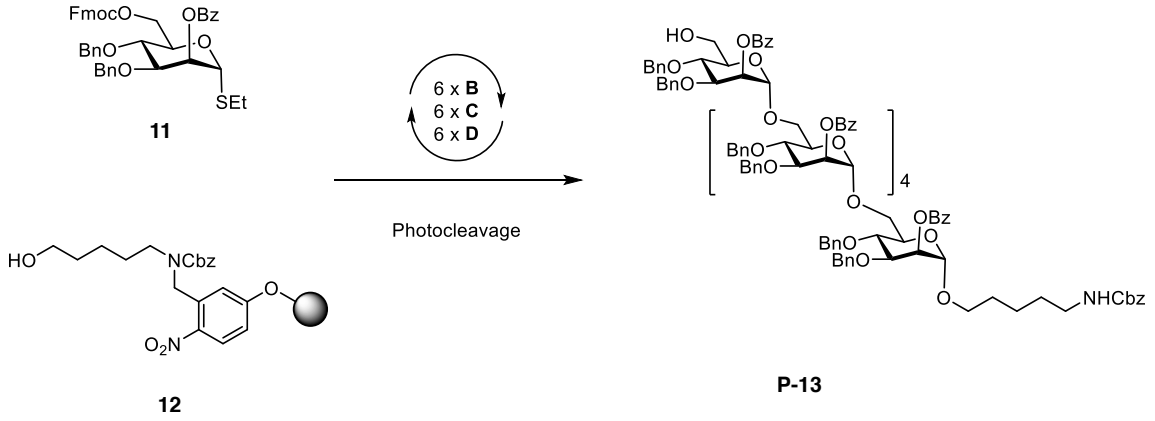


Fig. S19. HSQC NMR of 36 (D_2O).

3.6.5. Synthesis of α(1-6) hexamannoside, 13



	Module	Conditions
	A: Resin Preparation for Synthesis	
6	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Fmoc Deprotection	BB **11,** 6.5 equiv. (-20° for 5 min, 0° for 20 min)

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC (Method B, t_R = 25.2 min) afforded compound **P-13**.

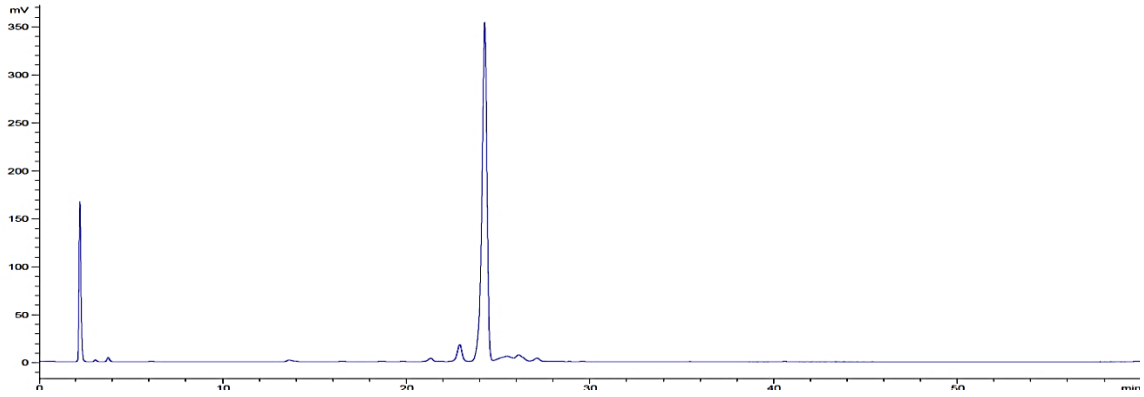
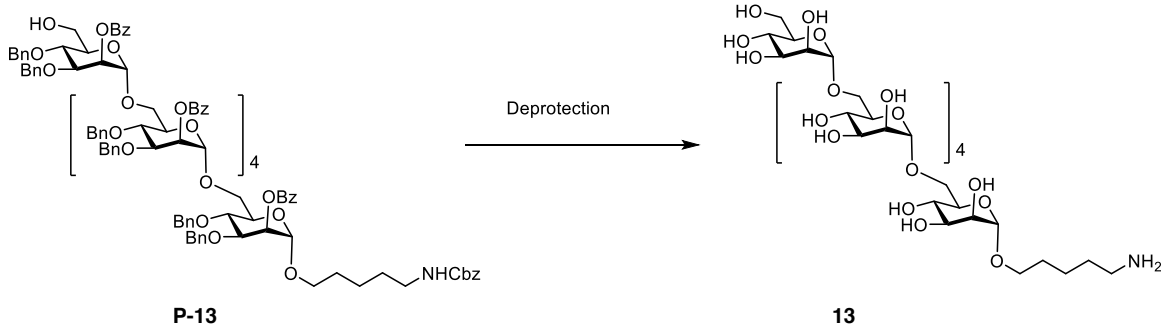


Fig. S20. Crude NP-HPLC of P-13 (ELSD trace, Method A, t_R = 24.1 min).



Deprotection of **P-13** as reported is Section 3.5 (Module F and G) followed by purification using preparative HPLC (Method D, t_R = 24.0 min) afforded compound **13** (5.2 mg, 39% based on resin loading).

Analytical data for **13**: ^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.80 (d, *J* = 1.8 Hz, 1H), 4.80 – 4.77 (m, 4H), 4.75 (d, *J* = 1.7 Hz, 1H), 3.90 – 3.86 (m, 5H), 3.86 – 3.79 (m, 6H), 3.77 (d, *J* = 1.7 Hz, 2H), 3.76 – 3.68 (m, 11H), 3.68 – 3.60 (m, 10H), 3.60 – 3.57 (m, 2H), 3.55 (d, *J* = 9.3 Hz, 1H), 3.46 (dt, *J* = 9.9, 6.1 Hz, 1H), 2.93 – 2.85 (m, 2H), 1.63 – 1.50 (m, 4H), 1.35 (hept, *J* = 6.8 Hz, 2H). ^{13}C NMR (176 MHz, Deuterium Oxide) δ 99.89, 99.42, 99.32, 99.29, 72.72, 70.92, 70.84, 70.80, 70.77, 70.72, 70.69, 70.55, 70.07, 69.99, 69.94, 67.63, 66.75, 66.61, 66.57, 65.62, 65.58, 65.53, 61.32, 60.94, 39.38, 30.65, 28.04, 27.99, 26.57, 26.46, 22.54, 22.03; m/z (HRMS+) 1076.425 $[M + H]^+$ ($C_{41}H_{74}NO_{31}$ requires 1076.424). NMR data were in good agreement with those previously reported. (S12)

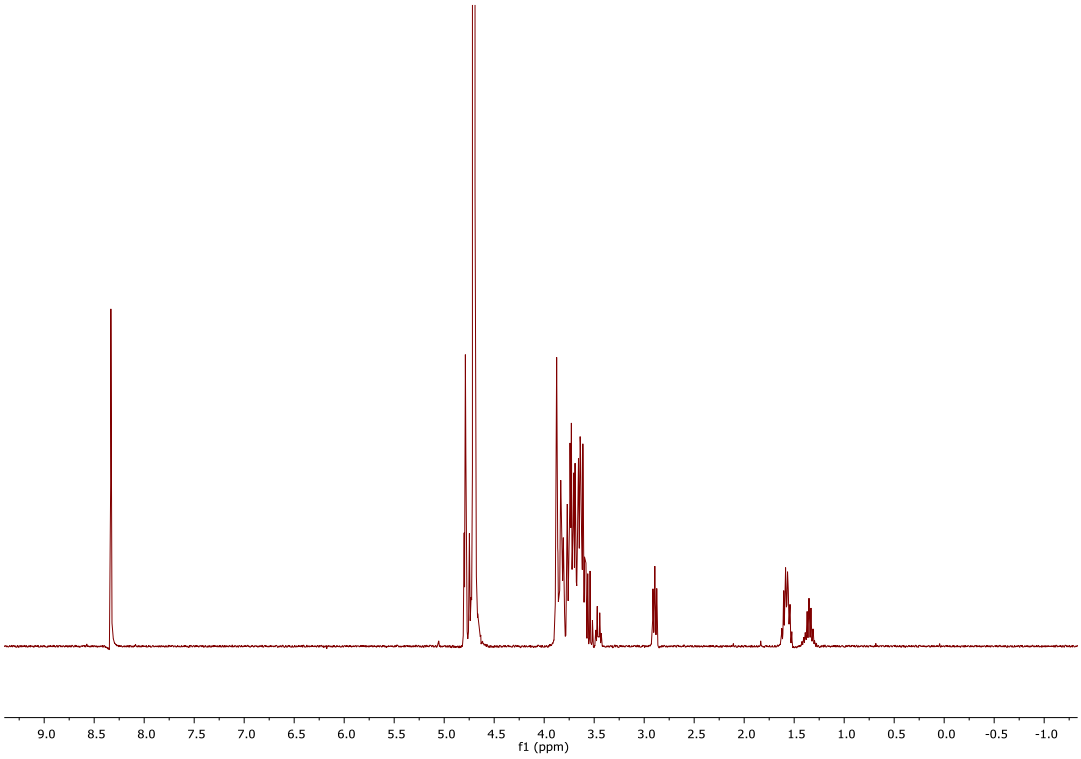


Fig. S21. ^{1}H NMR of 12 (400 MHz, D_2O).

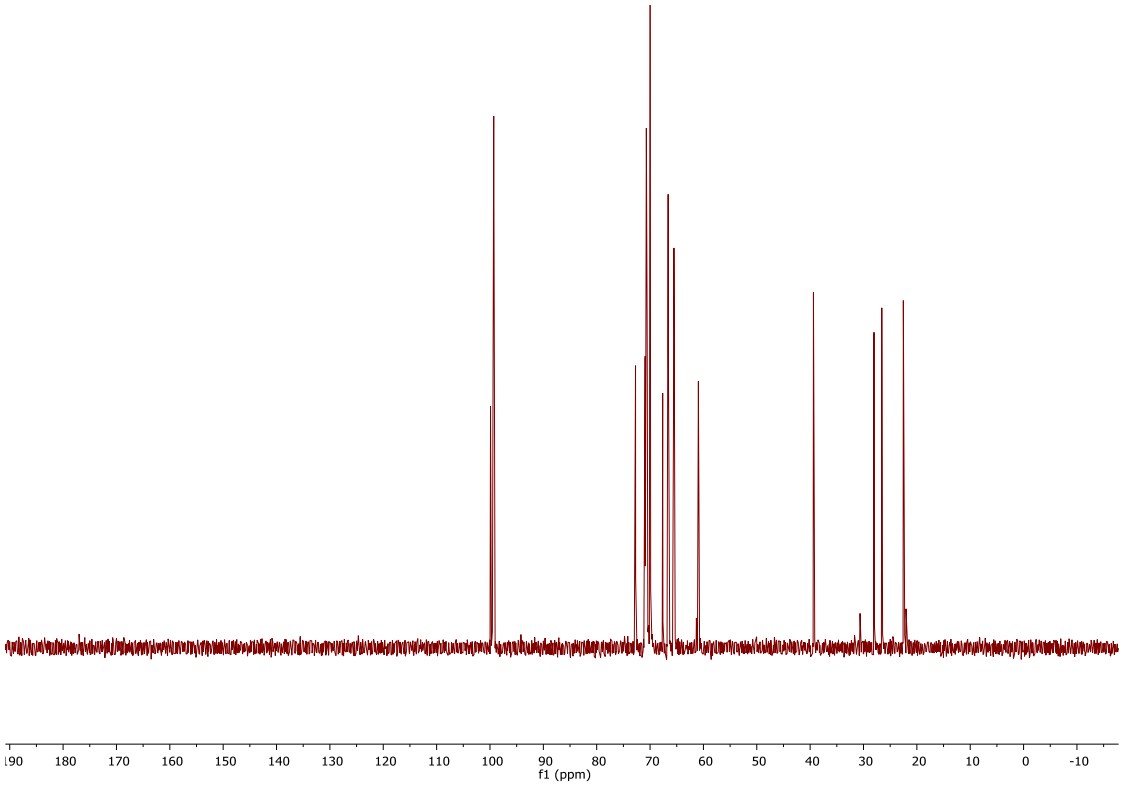


Fig. S22. ^{13}C NMR of 12 (176 MHz, D_2O).

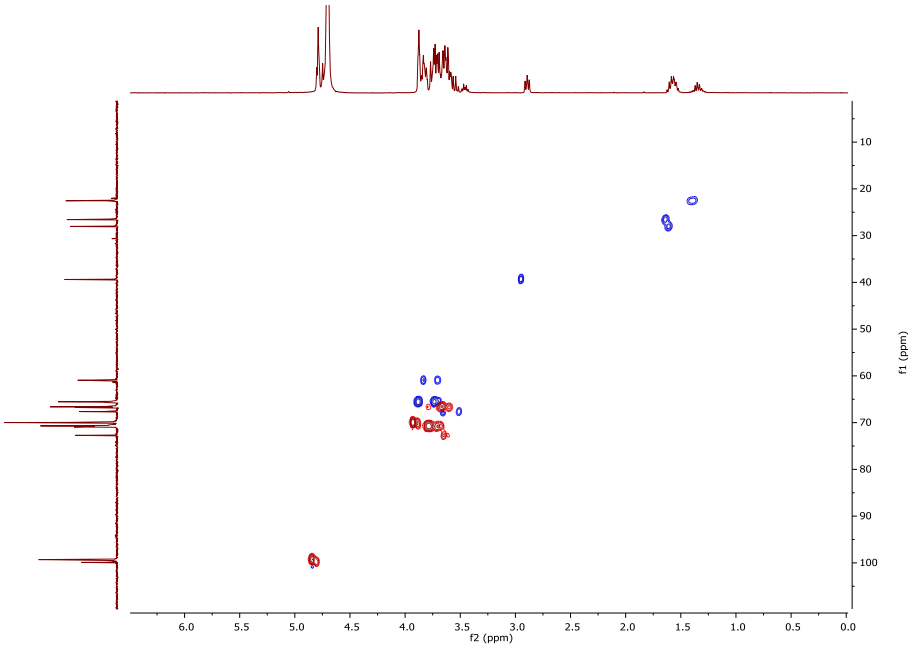
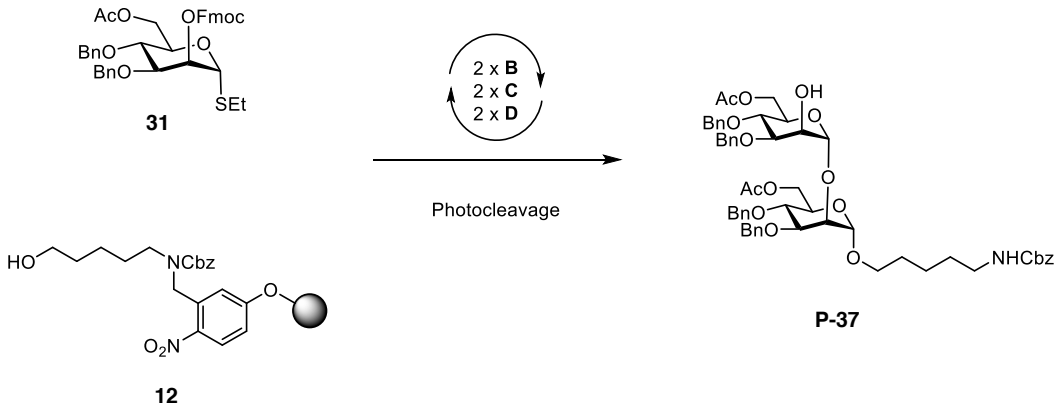


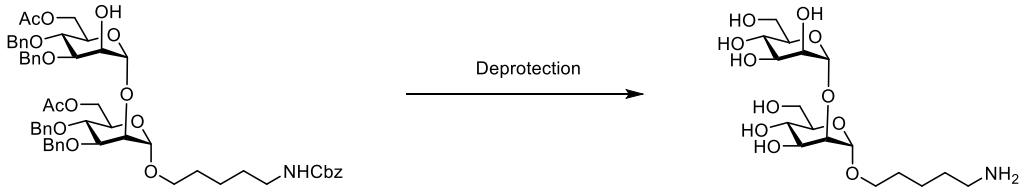
Fig. S23. HSQC NMR of 12 (D_2O).

3.6.6. Synthesis of α(1-2) dimannoside, 37



	Module	Conditions
	A: Resin Preparation for Synthesis	
2	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Fmoc Deprotection	BB **31,** 6.5 equiv. (-20° for 5 min, 0° for 20 min)

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC (Method B, t_R = 20.5 min) afforded compound **P-37**.



Deprotection of **P-37** as reported is Section 3.5 (Module F and G) followed by purification using preparative HPLC (Method E) afforded compound **37** (2.2 mg, 41% based on resin loading). ^{1}H NMR (400 MHz, Deuterium Oxide) ^{1}H NMR (400 MHz, Deuterium Oxide) δ 4.97 (d, *J* = 1.7 Hz, 1H), 4.88 (d, *J* = 1.8 Hz, 1H), 3.94 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.85 – 3.68 (m, 5H), 3.68 – 3.37 (m, 9H), 2.87 (t, *J* = 7.6 Hz, 3H), 1.62 – 1.45 (m, 4H), 1.40 – 1.24 (m, 2H). NMR data were in good agreement with those previously reported. (S9)

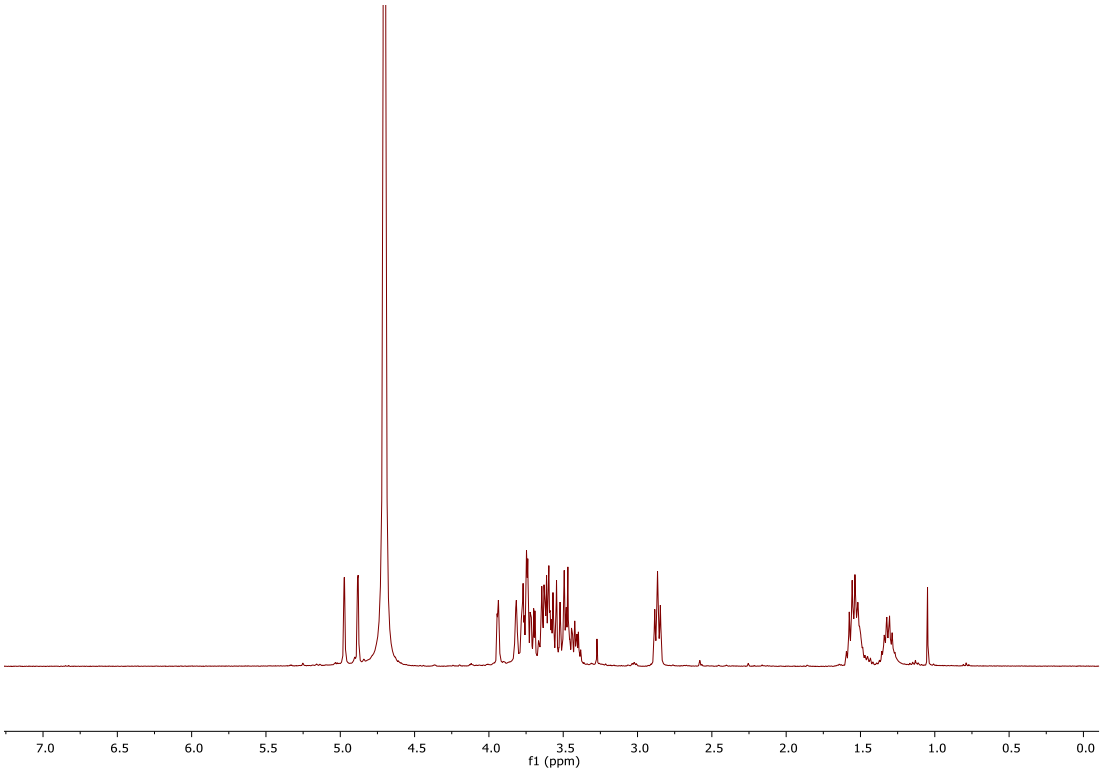
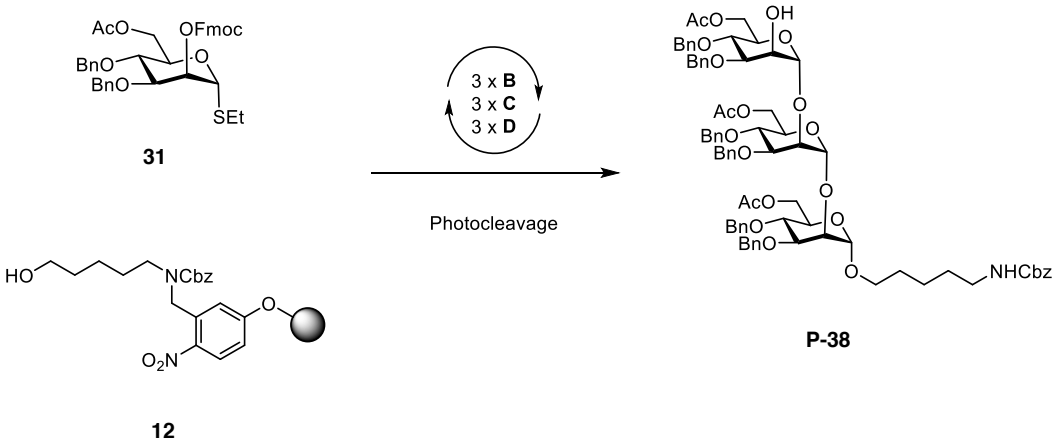


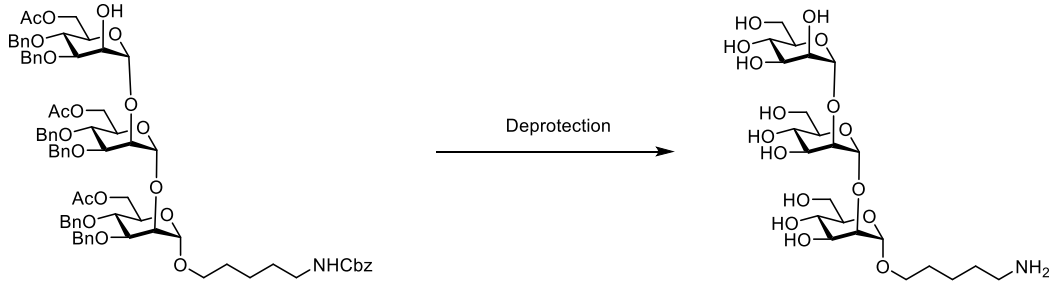
Fig. S24. ^{1}H NMR of 37 (400 MHz, D_2O).

3.6.7. Synthesis of α(1-2) trimannoside, 38



	Module	Conditions
	A: Resin Preparation for Synthesis	
3	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **D**: Fmoc Deprotection	BB **31,** 6.5 equiv. (-20° for 5 min, 0° for 20 min)

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC (Method B, t_R = 21.2 min) afforded compound **P-38**.



P-38 **38**

Deprotection of **P-38** as reported is Section 3.5 (Module F and G) followed by purification using preparative HPLC (Method E) afforded compound **38** (4.1 mg, 55% based on resin loading). ^{1}H NMR (600 MHz, Deuterium Oxide) δ 5.15 (s, 1H), 4.96 (s, 1H), 3.97 (d, *J* = 2.9 Hz, 1H), 3.93 (dd, *J* = 3.4, 1.9 Hz, 1H), 3.84 – 3.73 (m, 6H), 3.70 (dd, *J* = 9.6, 3.2 Hz, 1H), 3.66 – 3.38 (m, 12H), 2.86 (t, *J* = 8.0 Hz, 2H), 1.53 (m, 4H), 1.37 – 1.21 (m, 2H). NMR data were in good agreement with those previously reported. (S12)

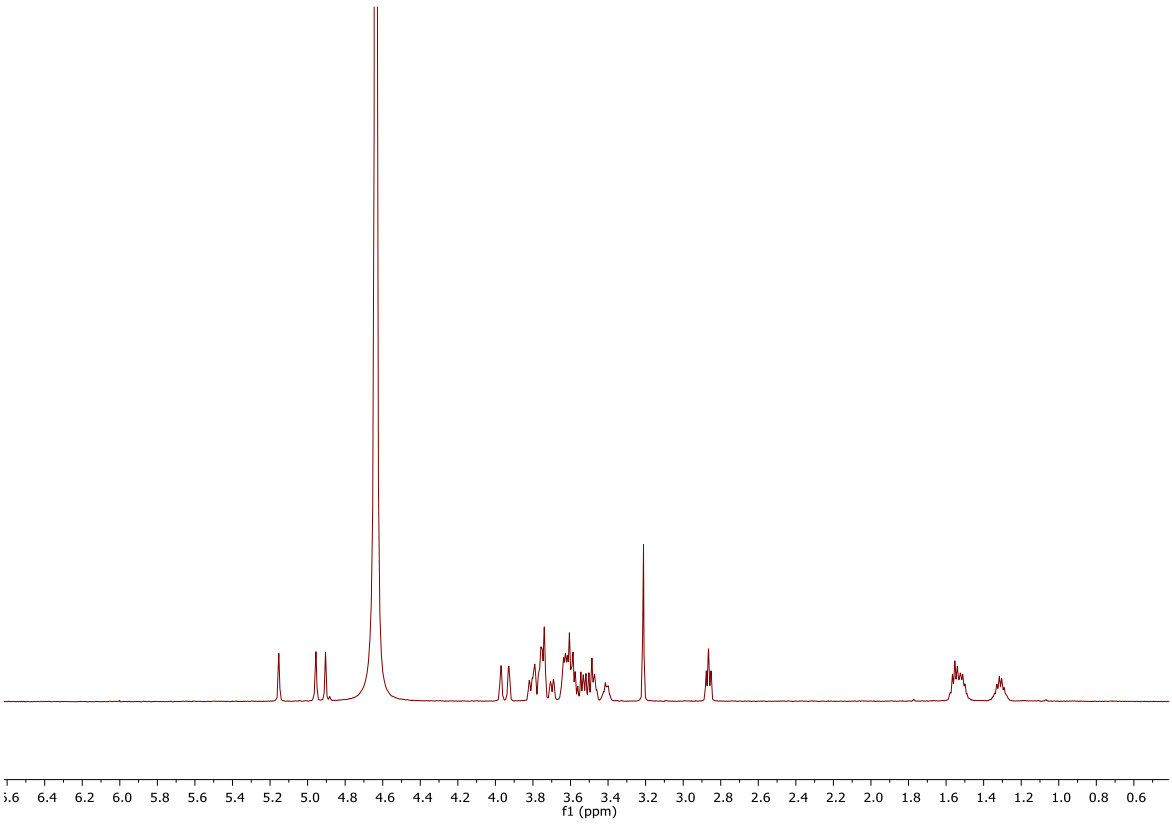
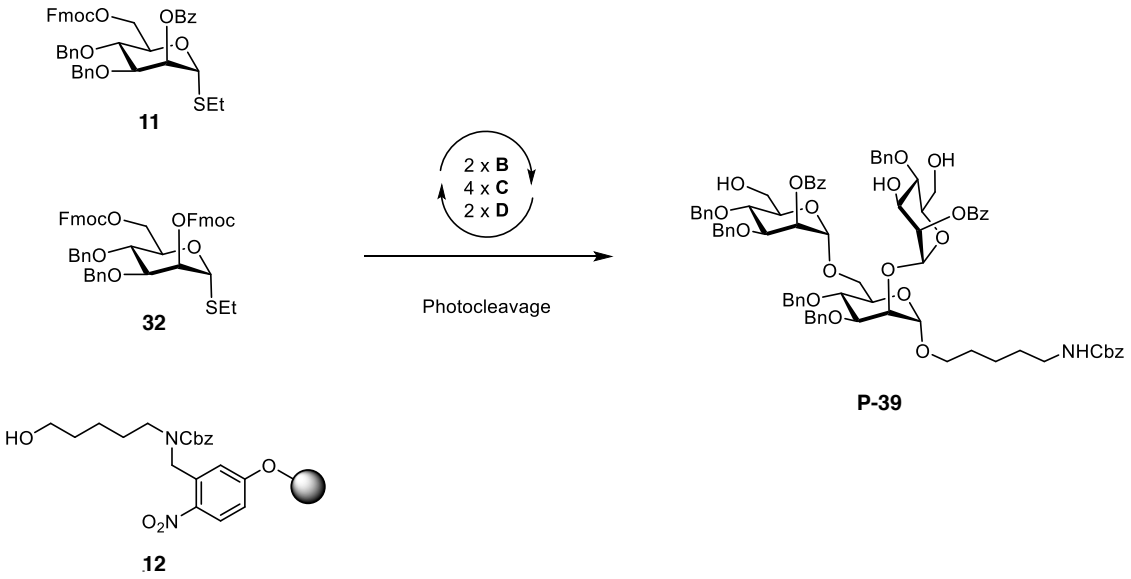


Fig. S25. ^{1}H NMR of 38 (400 MHz, D_2O).

3.6.8. Synthesis of α(1-6) α(1-2) branched trimannoside, 39



	Module	Conditions
	A: Resin Preparation for Synthesis	
1	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **C**: Thioglycoside Glycosylation **D**: Fmoc Deprotection	BB **32,** 8 equiv. (-20° for 5 min, 0° for 20 min)
1	**B**: Acidic Wash with TMSOTf Solution **C**: Thioglycoside Glycosylation **C**: Thioglycoside Glycosylation **D**: Fmoc Deprotection	BB **11,** 6.5 equiv. (-20° for 5 min, 0° for 20 min)

Cleavage from the solid support as described in *Post-synthesizer manipulation* followed by purification using preparative HPLC (Method B, t_R = 26.7 min) afforded compound **P-39**.

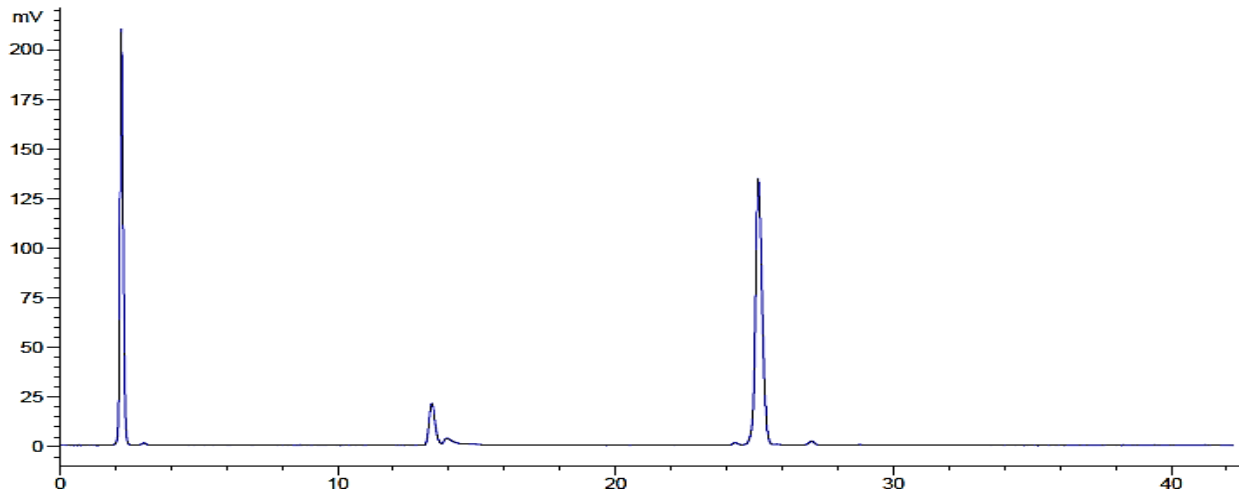
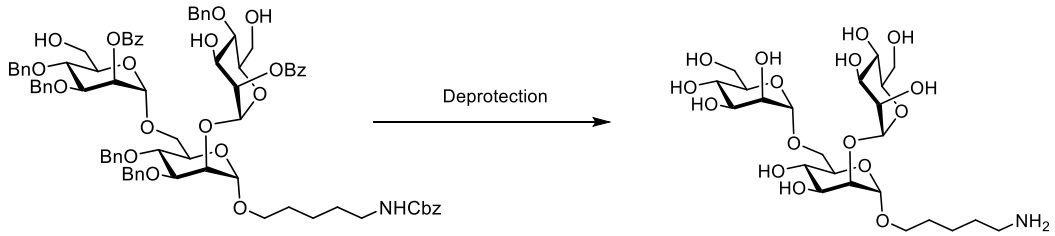


Fig. S26. SCrude NP-HPLC of P-39 (ELSD trace, Method A, t_R = 25.3 min).



P-39 **39**

Deprotection of **P-39** as reported is Section 3.5 (Module F and G) followed by purification using preparative HPLC (Method E) afforded compound **39** (1.8 mg, 25% based on resin loading). Analytical data for **39**: ^{1}H NMR (600 MHz, Deuterium Oxide) δ 4.95 (d, *J* = 1.8 Hz, 1H), 4.88 (d, *J* = 1.8 Hz, 1H), 4.78 (d, *J* = 1.8 Hz, 1H), 3.94 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.87 – 3.83 (m, 2H), 3.81 (dd, *J* = 3.4, 1.8 Hz, 1H), 3.78 – 3.72 (m, 4H), 3.70 – 3.38 (m, 15H), 2.86 (t, *J* = 7.7 Hz, 2H), 1.58 – 1.49 (m,4H), 1.38 – 1.24 (m, 2H). ^{13}C NMR (151 MHz, Deuterium Oxide) δ 102.34, 99.38, 98.14, 78.88, 73.28, 72.74, 71.11, 70.60, 70.35, 70.30, 69.95, 69.90, 67.65, 66.70, 66.57, 66.52, 65.15, 61.24, 61.02, 60.89, 60.85, 39.30, 27.92, 26.47, 22.41; m/z (HRMS+) 590.2667 [M + H]+ ($C_{23}H_{44}NO_{16}$ requires 590.2655).

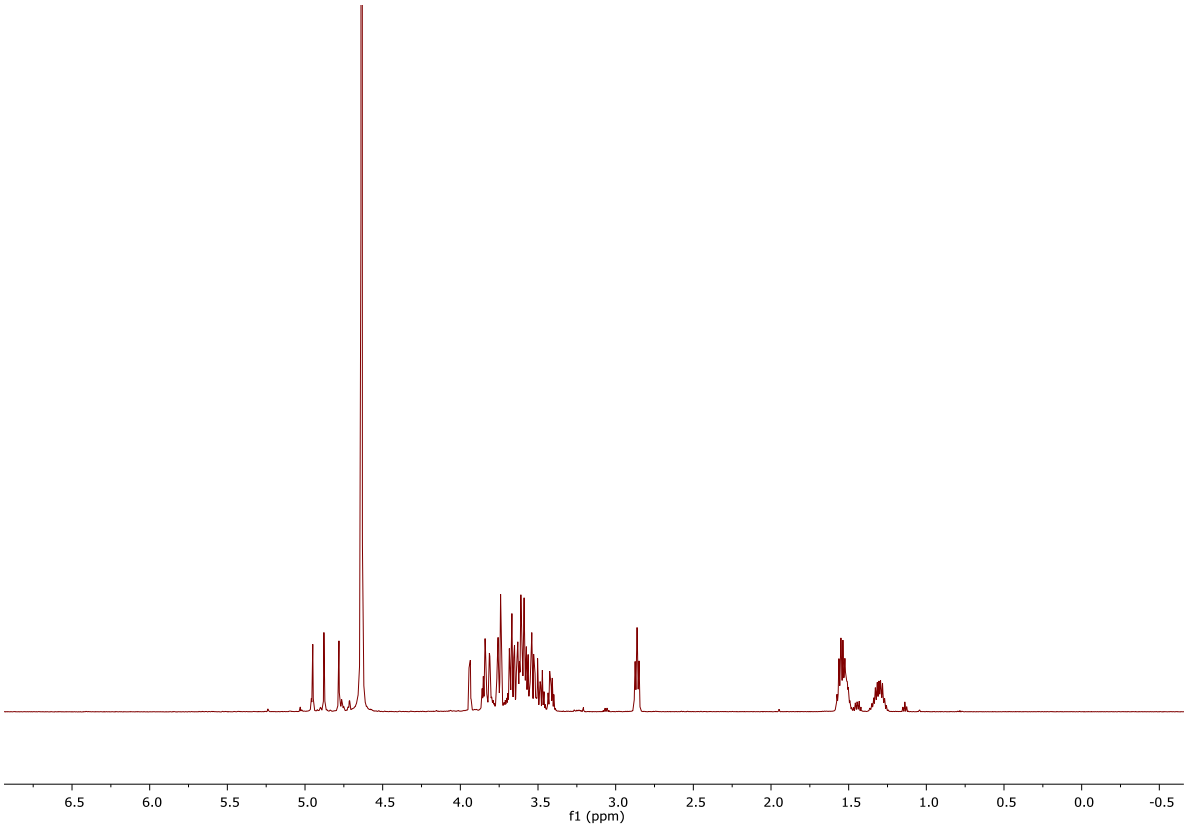


Fig. S27. ^{1}H NMR of 39 (600 MHz, D_2O).

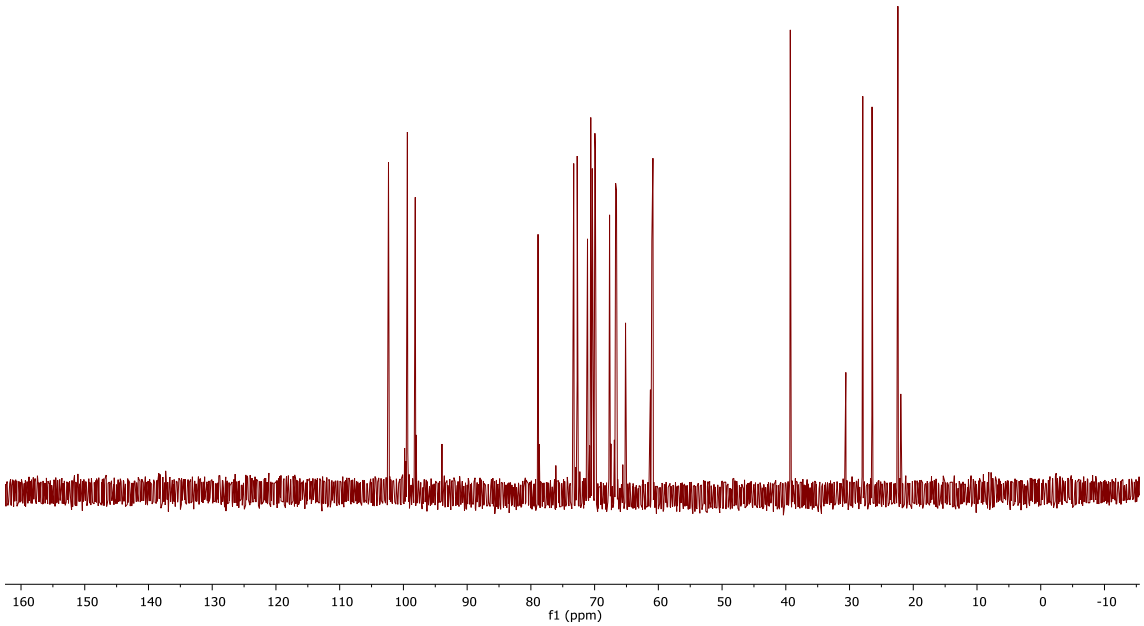


Fig. S28. ^{13}C NMR of 39 (151 MHz, D_2O).

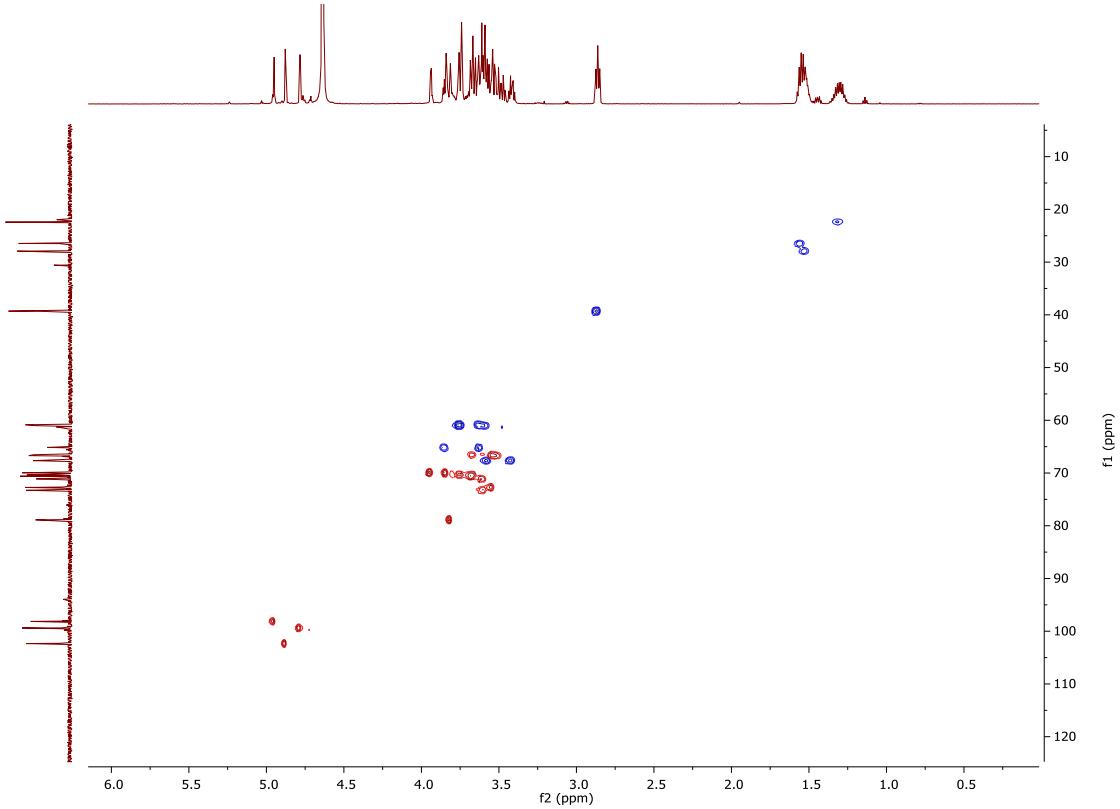
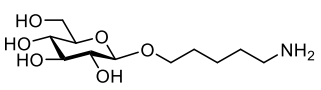


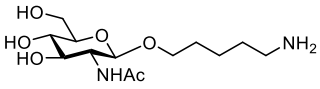
Fig. S29. HSQC NMR of 39 (D_2O).

3.7. Additional structures

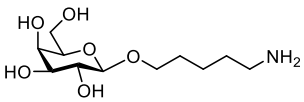
Compounds 40, 41, 42 were prepared according to previously established procedures. (S13, S14)



40



41



42

Analytical data for **40**: ^1H NMR (400 MHz, Deuterium Oxide) δ 4.32 (d, *J* = 7.9 Hz, 1H), 3.84 – 3.73 (m, 2H), 3.63 – 3.49 (m, 2H), 3.38 – 3.17 (m, 3H), 3.17 – 3.05 (m, 1H), 2.87 (t, *J* = 7.5 Hz, 2H), 1.53 (ddd, *J* = 15.3, 9.6, 7.1 Hz, 4H), 1.39 – 1.25 (m, 2H). NMR data were in good agreement with those previously reported. (S13)

Analytical data for **41**: ^1H NMR (400 MHz, Deuterium Oxide) δ 4.44 (d, *J* = 8.5 Hz, 1H), 3.91 – 3.80 (m, 2H), 3.68 (dd, *J* = 12.3, 5.2 Hz, 1H), 3.62 (dd, *J* = 10.4, 8.5 Hz, 1H), 3.54 (dt, *J* = 10.2, 6.3 Hz, 1H), 3.47 (dd, *J* = 10.4, 8.0 Hz, 1H), 3.41 – 3.33 (m, 2H), 2.93 (t, *J* = 7.7 Hz, 2H), 1.98 (s, 3H), 1.61 (q, *J* = 7.7 Hz, 2H), 1.53 (q, *J* = 6.9 Hz, 2H), 1.34 (td, *J* = 7.7, 3.5 Hz, 2H). NMR data were in good agreement with those previously reported. (S14)

Analytical data for **42**: ^{1}H NMR (400 MHz, Deuterium Oxide) ^{1}H NMR (700 MHz, Deuterium Oxide) δ 4.33 (d, *J* = 8.0 Hz, 1H), 3.88 (dt, *J* = 16.6, 5.0 Hz, 2H), 3.71 (qd, *J* = 11.6, 6.1 Hz, 3H), 3.66 – 3.55 (m, 4H), 3.44 (dd, *J* = 9.8, 8.0 Hz, 1H), 2.95 (t, *J* = 7.5 Hz, 2H), 1.62 (dp, *J* = 14.6, 7.3 Hz, 4H), 1.40 (p, *J* = 7.8 Hz, 2H). NMR data were in good agreement with those previously reported. (S13)

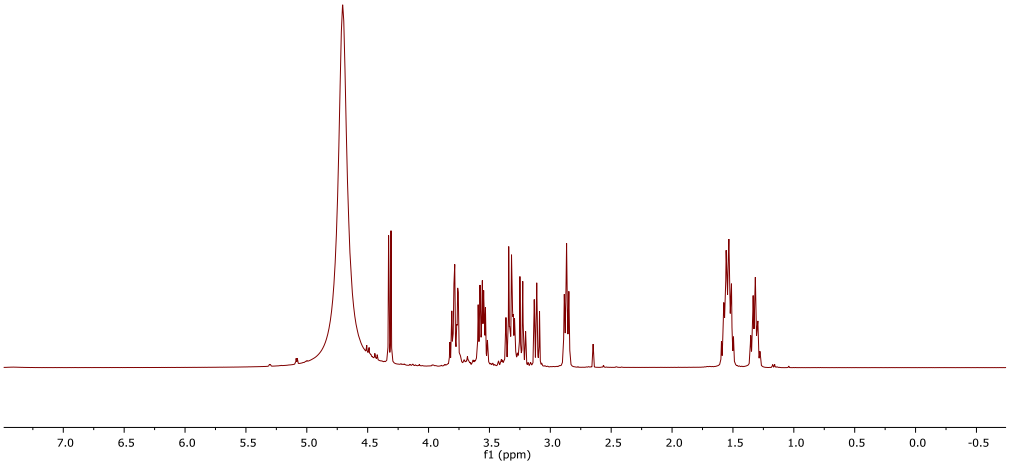


Fig. S30. ^{1}H NMR of 40 (400 MHz, D_2O).

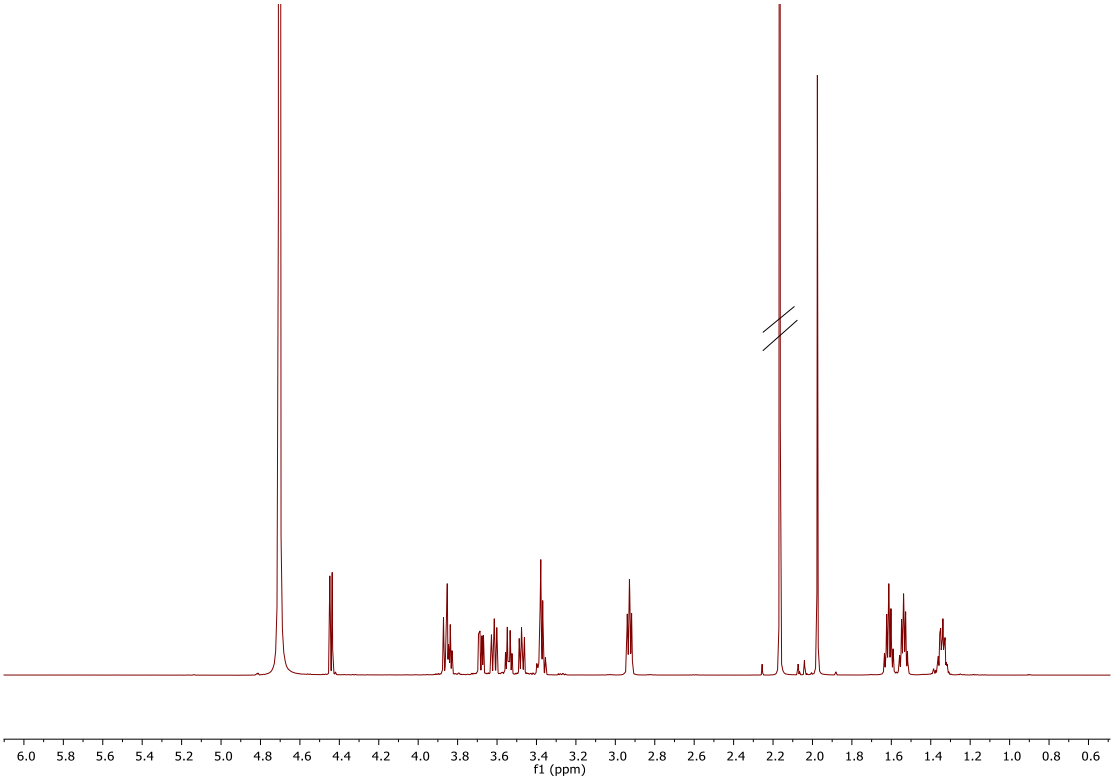


Fig. S31. ^{1}H NMR of 41 (400 MHz, D_2O).

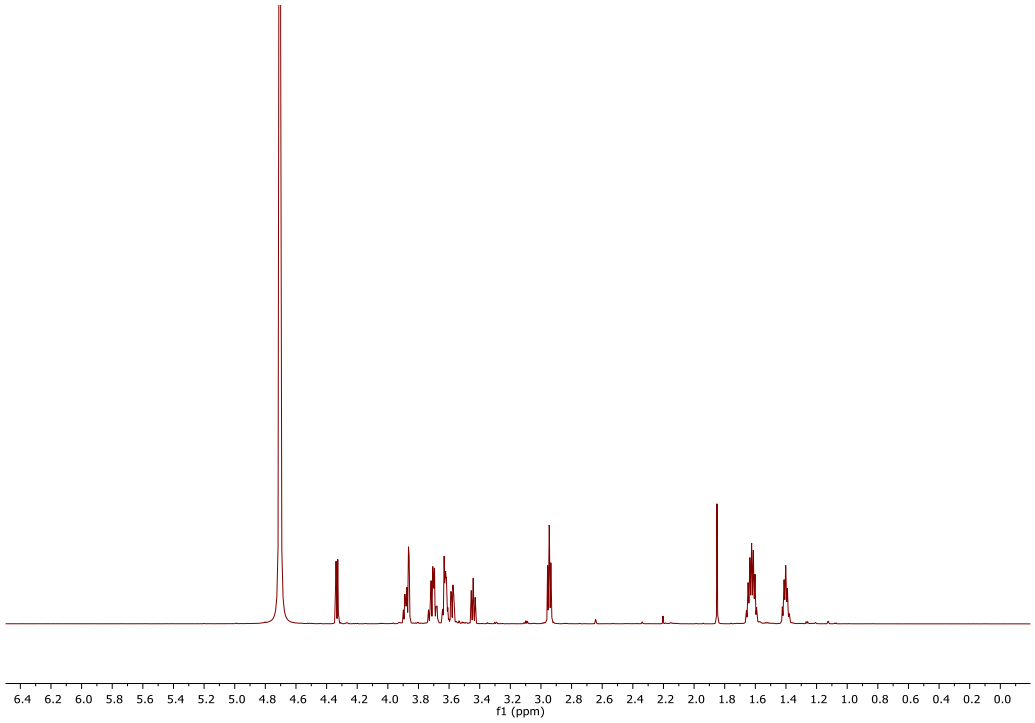


Fig. S32. ^{1}H NMR of 42 (400 MHz, D_2O).

Part 3. Synthesis of Oligosaccharides-Containing Janus Dendrimers

3.1 MALDI-TOF spectra of the amino and ITC containing Janus dendrimers 29 and 30

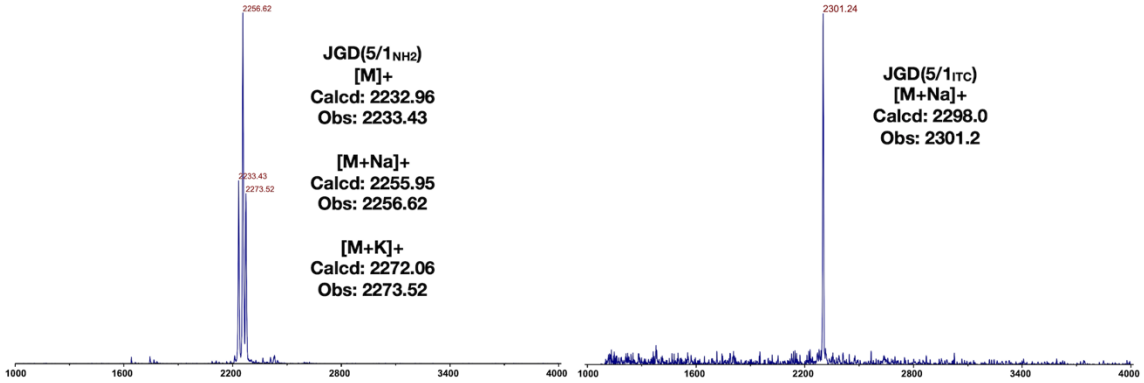


Fig. S33. MALDI-TOF of **29 JD(5/1$_{NH2}$) and 30 JD(5/1$_{ITC}$).**

3.2. Synthesis of Oligosacchrides-Containing Janus Dendrimers via ITC-amine Conjugation.

3.2.1. Synthesis of JD(5/1$_{Lac}^{C5}$)

To a DMF (3 mL) solution of compound **30** (60 mg, 0.026 mmol) and compound **8** (11 mg, 0.026 mmol) was added NEt_3, (10 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH = 10/1 to 5/1 (v/v) to yield to **JD(5/1$_{Lac}^{C5}$)** as a white solid (33 mg, 46%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.43 (s, 1H, N*H*), 7.25 (s, 2H, 2×Ar*H*), 7.08 (d, 4H, *J* = 2.5 Hz, 4×Ar*H*), 6.90 (s, 2H, 2×Ar*H*), 6.59 (t, 2H, *J* = 2.5 Hz, 2 ×Ar*H*), 4.92–4.99 (m, 6H, 3×COOCH_2), 4.42 (br, 1H, (1)-sugar), 4.39 (s, 1H, (1)-sugar), 4.24–3.34 (m, 106H), 1.71–1.77 (m, 8H, 4 × $OCH_2CH_2(CH_2)_8CH_3$), 1.39–1.43 (m, 8H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$,), 1.26–1.29 (m, 64H, 4×$OCH_2CH_2CH_2(CH_2)_8CH_3$), 0.86–0.89 (m, 12H, 4×$OCH_2CH_2CH_2(CH_2)_8CH_3$). MALDI-TOF (m/z): $[M+K]^+$ calcd for $C_{139}H_{237}N_3KO_{45}S$, 2739.6; found 2742.3.

3.2.2. Synthesis of JD(5/1$_{Lac}^{3EO}$)

To a DMF (3 mL) solution of compound **30** (60 mg, 0.035 mmol) and compound **10** (15 mg, 0.035 mmol) was added NEt_3 (20 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH = 10/1 to 5/1 (v/v) to yield to **JD(5/1$_{Lac}^{C5}$)** as a white solid (50 mg, 57%). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.43 (s, 1H, N*H*), 7.25 (s, 2H, 2×Ar*H*), 7.08 (d, 4H, *J* = 2.5 Hz, 4×Ar*H*), 6.90 (s, 2H, 2×Ar*H*), 6.59 (t, 2H, *J* = 2.5 Hz, 2 ×Ar*H*), 4.92–4.99 (m, 6H, 3×COOCH_2), 4.42 (br, 1H, (1)-sugar), 4.39 (s, 1H, (1)-sugar), 4.24–3.34 (m, 114H), 1.71–1.77 (m, 8H, 4 × $OCH_2CH_2(CH_2)_8CH_3$), 1.39–1.43 (m, 12H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$, and $NCH_2CH_2CH_2CH_2CH_2O$), 1.26–1.29 (m, 66H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$ and $NCH_2CH_2CH_2CH_2CH_2O$), 0.86–0.89 (m, 12H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{140}H_{239}N_3NaO_{47}S$, 2769.6; found 2770.5.

3.2.3. Synthesis of JD(5/1$_{Man}^{C5}$)

To a DMF (3 mL) solution of compound **30** (70 mg, 0.035 mmol) and compound **33** (8.2 mg, 0.035 mmol) was added NEt_3 (20 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH = 15/1 to 8/1 (v/v) to yield to **JD(5/1$_{Man}^{C5}$)** as a white solid (35 mg, 45%).^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.43 (s, 1H, N*H*), 7.25 (s, 2H, 2×Ar*H*), 7.09 (d, 4H, *J* = 2.5 Hz, 4×Ar*H*), 7.05 (s, 2H, 2×Ar*H*), 6.60 (t, 2H, *J* = 2.5 Hz, 2 ×Ar*H*), 4.84–4.99 (m, 6H, 3×COOCH_2), 4.71 (br, 1H, (1)-sugar), 4.24–3.34 (m, 103H), 1.71–1.77

(m, 8H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$), 1.39–1.43 (m, 12H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$, and $NCH_2CH_2CH_2CH_2CH_2O$), 1.26–1.29 (m, 66H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$ and $NCH_2CH_2CH_2CH_2CH_2O$), 0.86–0.89 (m, 12H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{133}H_{227}N_3NaO_{40}S$, 2561.5; found 2563.5.

3.2.4. Synthesis of JD[5/1$_{Glc}^{C5}$]

To a DMF (3 mL) solution of compound **30** (51 mg, 0.023 mmol) and compound **40** (6 mg, 0.023 mmol) was added NEt_3 (20 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH = 15/1 to 8/1 (v/v) to yield to **JD(5/1$_{Glc}^{C5}$)** as a white solid (22 mg, 39%). δ = 7.43 (s, 1H, N*H*), 7.25 (s, 2H, 2×Ar*H*), 7.09 (d, 4H, *J* = 2.5 Hz, 4×Ar*H*), 7.05 (s, 2H, 2×Ar*H*), 6.60 (t, 2H, *J* = 2.5 Hz, 2×Ar*H*), 4.84–4.99 (m, 6H, 3×$COOCH_2$), 4.45 (br, 1H, (1)-sugar), 4.24–3.34 (m, 103H), 1.71–1.77 (m, 8H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$), 1.39–1.43 (m, 12H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$, and $NCH_2CH_2CH_2CH_2CH_2O$), 1.26–1.29 (m, 66H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$ and $NCH_2CH_2CH_2CH_2CH_2O$), 0.86–0.89 (m, 12H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{133}H_{227}N_3NaO_{40}S$, 2561.5; found 2562.8.

3.2.5. Synthesis of JD[5/1$_{Gal}^{C5}$]

To a DMF (3 mL) solution of compound **30** (51 mg, 0.023 mmol) and compound **42** (6 mg, 0.023 mmol) was added NEt_3 (20 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH = 10/1 (v/v) to yield to **JD(5/1$_{Gal}^{C5}$)** as a white solid (22 mg, 39%). δ = 7.43 (s, 1H, N*H*), 7.25 (s, 2H, 2×Ar*H*), 7.09 (d, 4H, *J* = 2.5 Hz, 4×Ar*H*), 7.05 (s, 2H, 2×Ar*H*), 6.60 (t, 2H, *J* = 2.5 Hz, 2×Ar*H*), 4.84–4.99 (m, 6H, 3×$COOCH_2$), 4.35 (br, 1H, (1)-sugar), 4.24–3.34 (m, 103H), 1.71–1.77 (m, 8H, 4×$OCH_2CH_2CH_2(CH_2)_8CH_3$), 1.39–1.43 (m, 12H, 4×$OCH_2CH_2CH_2(CH_2)_8CH_3$, and $NCH_2CH_2CH_2CH_2CH_2O$), 1.26–1.29 (m, 66H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$ and $NCH_2CH_2CH_2CH_2CH_2O$), 0.86–0.89 (m, 12H, 4 × $OCH_2CH_2CH_2(CH_2)_8CH_3$). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{133}H_{227}N_3NaO_{40}S$, 2561.5; found 2562.6.

3.2.6. Synthesis of JD[5/1$_{GlcNAc}^{C5}$]

To a DMF (1 mL) solution of compound **30** (15 mg, 0.0065 mmol) and compound **41** (2.0 mg, 0.0065 mmol) was added NEt_3 (5 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH = 20/1 to 5/1 (v/v) to yield to

JD(5/1$_{GlcNAc}^{C5}$) as a white solid (7.4 mg, 44%). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{135}H_{230}N_4NaO_{40}S$, 2602.6; found 2604.0.

3.2.7. Synthesis of JD[5/1$_{\alpha(1-6)diMan}^{C5}$]

To a DMF (3 mL) solution of compound **30** (32 mg, 0.014 mmol) and compound **34** (5.5 mg, 0.014 mmol) was added NEt_3 (10 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH = 10/1 to 5/1 (v/v) to yield to **JD[5/1$_{\alpha(1-6)diMan}^{C5}$]** as a white solid (18 mg, 49%). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{139}H_{237}N_3NaO_{45}S$, 2723.6; found 2725.2.

3.2.8. Synthesis of JD[5/1$_{\alpha(1-6)triMan}^{C5}$]

To a DMF (3 mL) solution of compound **30** (22 mg, 0.009 mmol) and compound **35** (5.0 mg, 0.009 mmol) was added NEt_3 (5 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH = 10/1 to 5/1 (v/v) to yield to **JD[5/1$_{\alpha(1-6)triMan}^{C5}$]** as a white solid (15 mg, 56%). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{145}H_{247}N_3NaO_{50}S$, 2885.6; found 2882.1.

3.2.9. Synthesis of JD[5/1$_{\alpha(1-6)pentaMan}^{C5}$]

To a DMF (3 mL) solution of compound **30** (25 mg, 0.010 mmol) and compound **36** (9.0 mg, 0.010 mmol) was added NEt_3 (5 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH/water = 100/50/5 (v/v) to yield to **JD[5/1$_{\alpha(1-6)pentaMan}^{C5}$]** as a white solid (15 mg, 56%). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{157}H_{267}N_3NaO_{60}S$, 3209.8; found 3204.4.

3.2.10. Synthesis of JD[5/1$_{\alpha(1-6)hexaMan}^{C5}$]

To a DMF (3 mL) solution of compound **30** (15 mg, 0.006 mmol) and compound **13** (6.4 mg, 0.006 mmol) was added NEt_3 (5 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH/water = 100/50/5 (v/v/v) to yield to **JD[5/1$_{\alpha(1-6)hexaMan}^{C5}$]** as a white solid (9.0 mg, 45%). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{163}H_{2277}N_3NaO_{65}S$, 3371.8; found 3368.5.

3.2.11. Synthesis of JD[5/1$_{\alpha(1-2)diMan}^{C5}$]

To a DMF (3 mL) solution of compound **30** (27 mg, 0.006 mmol) and compound **38** (5.0 mg, 0.012 mmol) was added NEt_3 (10 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH/water = 20/1 (v/v) to yield to **JD[5/1$_{\alpha(1-2)diMan}^{C5}$]** as a white solid (20 mg, 63%). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{139}H_{237}N_3NaO_{45}S$, 2723.6; found 2724.9.

3.2.12. Synthesis of JD[5/1$_{\alpha(1-2)triMan}^{C5}$]

To a DMF (3 mL) solution of compound **30** (22 mg, 0.010 mmol) and compound **38** (5.2 mg, 0.009 mmol) was added NEt_3 (10 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH/water = 100/30/2 (v/v/v) to yield to **JD[5/1$_{\alpha(1-2)triMan}^{C5}$]**as a white solid (15 mg, 59%). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{145}H_{247}N_3NaO_{50}S$, 2885.6; found 2882.4.

3.2.13. Synthesis of JD[5/1$_{\alpha(1-2)\alpha(1-6)branch\text{-}triMan}^{C5}$]

To a DMF (3 mL) solution of compound **30** (40 mg, 0.018 mmol) and compound **39** (7.0 mg, 0.012 mmol) was added NEt_3 (10 uL). The reaction mixture was allowed to stir at 23 ºC for 12 h. The reaction mixture was concentrated to dryness. The crude product was further purified by silica column chromatography with a mobile phase of DCM/MeOH/water = 100/30/3 (v/v/v) to yield to **JD[5/1$_{\alpha(1-2)\alpha(1-6)branch\text{-}triMan}^{C5}$]** as a light yellow solid (14 mg, 45%). MALDI-TOF (m/z): $[M+Na]^+$ calcd for $C_{145}H_{247}N_3O_{50}S$, 2862.7; found 2832.5 (M–30).

3.3. Representative MALDI-TOF Mass Spectra for JGDs

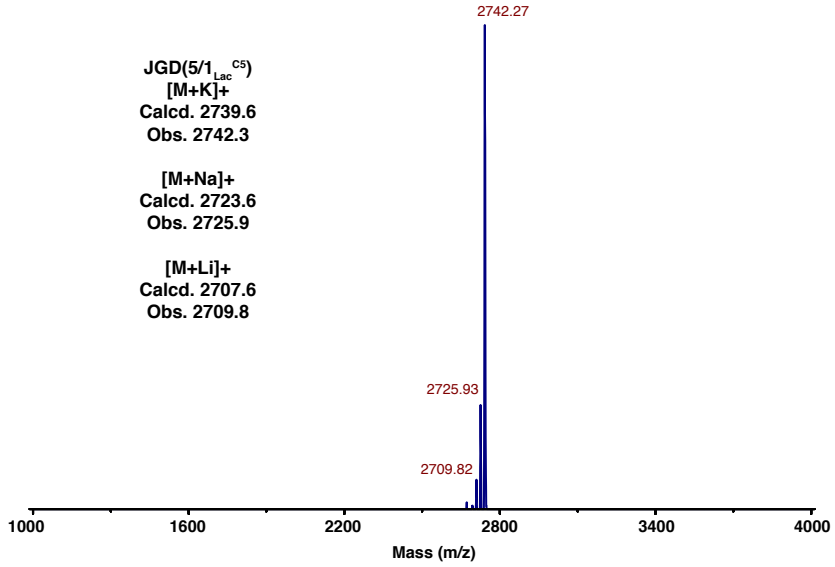


Fig. S34. MALDI-TOF of **JGD(5/1$_{Lac}^{C5}$)**.

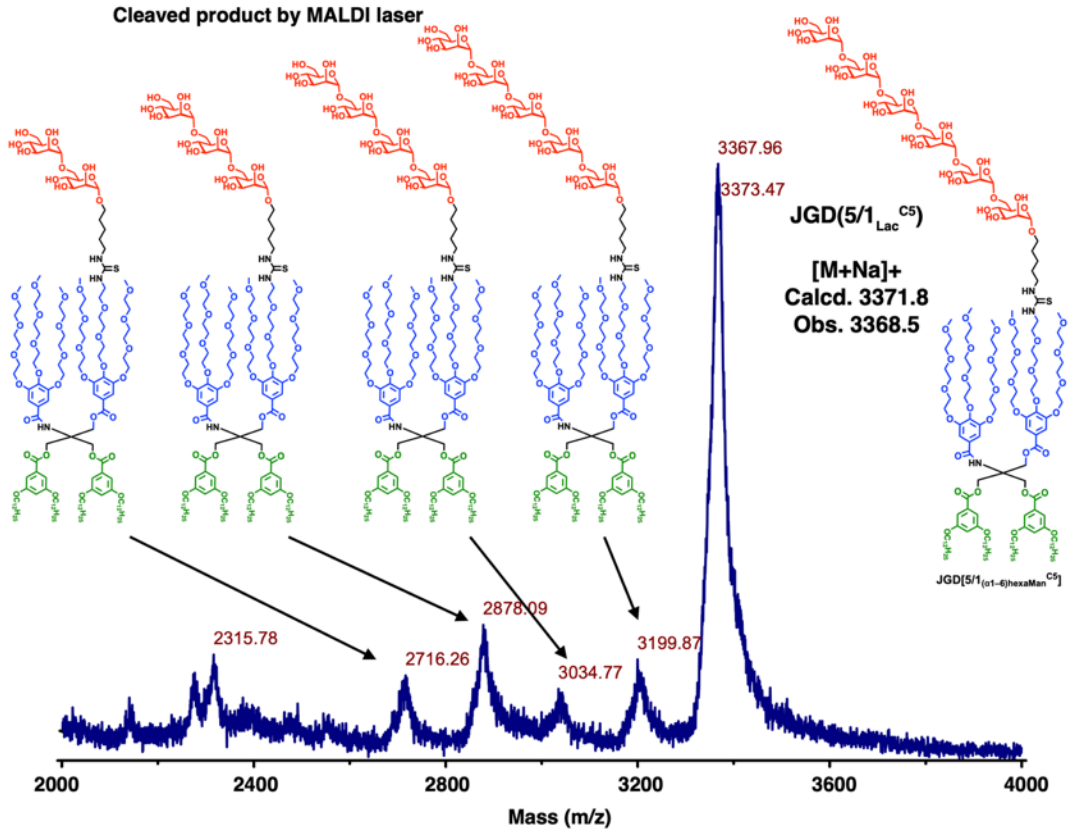


Fig. S35. MALDI-TOF of **JGD[5/1$_{hexaMan}^{C5}$]**.

Part 4. Self-Assembly of Oligosaccharides-Containing Janus Dendrimers

4.1. Preparation of Nanoscale GDSs by Injection. Milli-Q water obtained by Milli-Q UV plus with the resistivity 18.2 MΩ·cm was used for the preparation of phosphate-buffered saline (PBS) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer. PBS (1×) was obtained by dissolving 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4 and 0.24 g of KH_2PO_4 in 800 mL of Milli-Q water, adjusted to pH = 7.4 and diluted to 1,000 mL. HEPES buffer solution (10 mM) was obtained by dissolving 2.38 g of HEPES in 80 mL of Milli-Q water and the pH adjusted to 7.4 with NaOH pellets. 111 mg of $CaCl_2$ and 126 mg of $MnCl_2$ were then added, and the solution diluted to 1,000 mL. A stock solution was prepared by dissolving the required amount of amphiphilic JGDs in THF. GDSs were then generated by injection of 50 μL of the stock solution into 1.0 mL PBS or HEPES buffer, followed by 5-s vortexing.

4.2. Preparation of Giant GDSs by Hydration. A solution of JGDs in THF (100 μL) was deposited on the top surface of a roughened Teflon sheet (1 cm^2), placed in a flat-bottom vial, followed by evaporation of the solvent for 2 h. The Teflon sheet was dried in vacuo for an additional 12 h. Milli-

Q water (1.0 mL) was added to submerge the film on the Teflon sheet, and the vial was placed in a 60 °C oven for 12 h for hydration. The sample was then mixed using a vortex mixer for 30 s.

4.3. Cryogenic transmission electron microscopy

Cryogenic transmission electron microscopy (cryo-TEM) micrographs were taken by two models of cryo-TEM.

a) a JEOL 2100 microscope with an acceleration voltage of 200 kV. Nanoscale GDSs was prepared by Injection method. Cryo-TEM samples by was blotted by hand, then quickly plunged into liquefied ethane (~90 K) cooled by a reservoir of liquid nitrogen to ensure the vitrification of water. The vitrified samples were transferred to a Gatan CT3500TR single tilt cryo-transfer holder in a cryotransfer stage immersed in liquid nitrogen. During the imaging, the cryo-holder was kept below –170 °C to prevent sublimation of vitreous solvent.

b) a Carl Zeiss Libra 120 microscope with an acceleration voltage of 120 kV. Cryo-TEM samples were prepared by plunge freezing of aqueous dispersion on plasma-treated lacey grids. The vitrified specimens were transferred to a Gatan-910 cryoholder. During the imaging, the cryo-holder was kept below –170 °C to prevent sublimation of vitreous solvent.

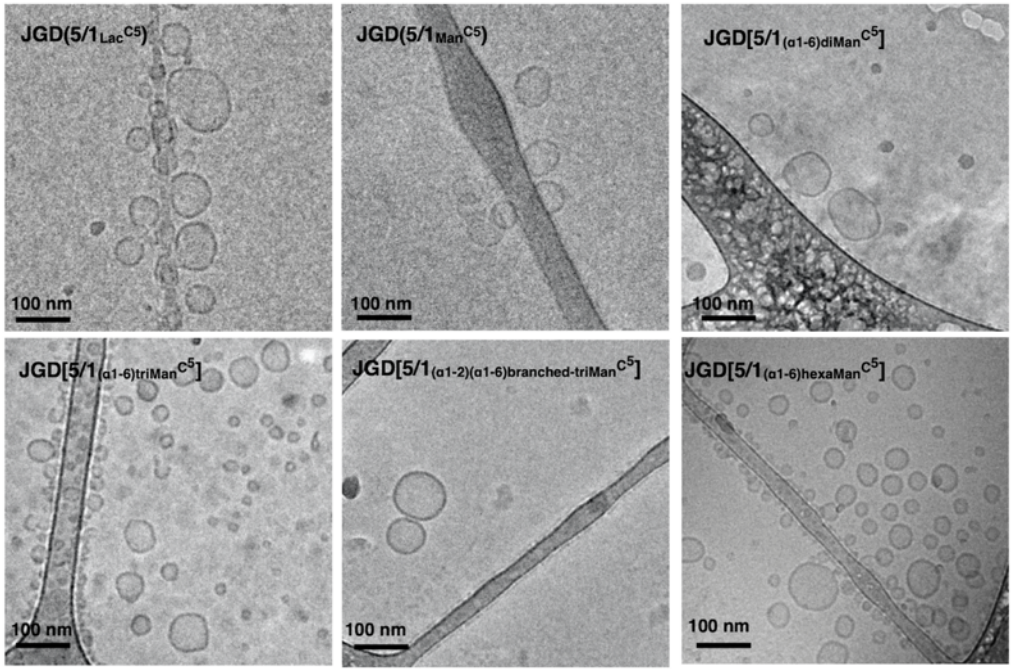


Fig. S36. Representative Cryo-TEM images of GDSs self-assembled from JGDs prepared by ITC-amine “click”-like reaction.

4.5. Aggregation Assays

Aggregation assays of GDSs with lectins were monitored in semimicro disposable cuvettes at 23 °C at wavelength λ = 450 nm by using a Shimadzu UV-vis spectrophotometer UV1601 with Shimadzu/UV Probe software in kinetic mode.

Nanosized GDSs were prepared by injection methods. For Gal and Lac GDSs, PBS solution of galectin (100 μL) was injected into PBS solution (1X, pH = 7.4) of GDSs (900 μL). For oligoMan GDSs, HEPES (with Ca^{2+} and Mn^{2+}) of ConA (100 μL) was injected into HEPES solution (10 mM, pH = 7.4, with Ca^{2+} and Mn^{2+}) of GDSs (900 μL). The cuvette was shaken by hand for 1 s to 2 s before data collection was started. The same GDSs solution was used as a reference. PBS solutions of galectin or HEPES solution (with Ca^{2+} and Mn^{2+}) of ConA were prepared before the aggregation assays and were maintained at 0 °C (ice bath) before data collection. ConA and human galectins were provided by H.-J. Gabius laboratory (Ludwig-Maximilians-University, Germany).

4.4. Atomic Force Microscope

The giant GDSs prepared by hydration in water (~0.3 $mg \cdot mL^{-1}$) were deposited and slowly dried on the freshly peeled mica. All images were acquired with a Multimode AFM NanoScope V (Digital Instruments) as topological scans in tapping mode in air, using silicon probes OTESPA-R3 (Bruker) with a nominal spring constant of 26 $N \cdot m^{-1}$ and a tip radius of 7 nm. The phase images were obtained by monitoring the phase lag of the cantilever vibration compared with the z-piezo-drive voltage while the probe scans the surface with a preset constant amplitude of vibration. The phase data contain additional information about the tip–sample interactions resulting from adhesion, surface stiffness, and viscoelastic effects. The AFM scans including FFT were analyzed using Gwyddion software.

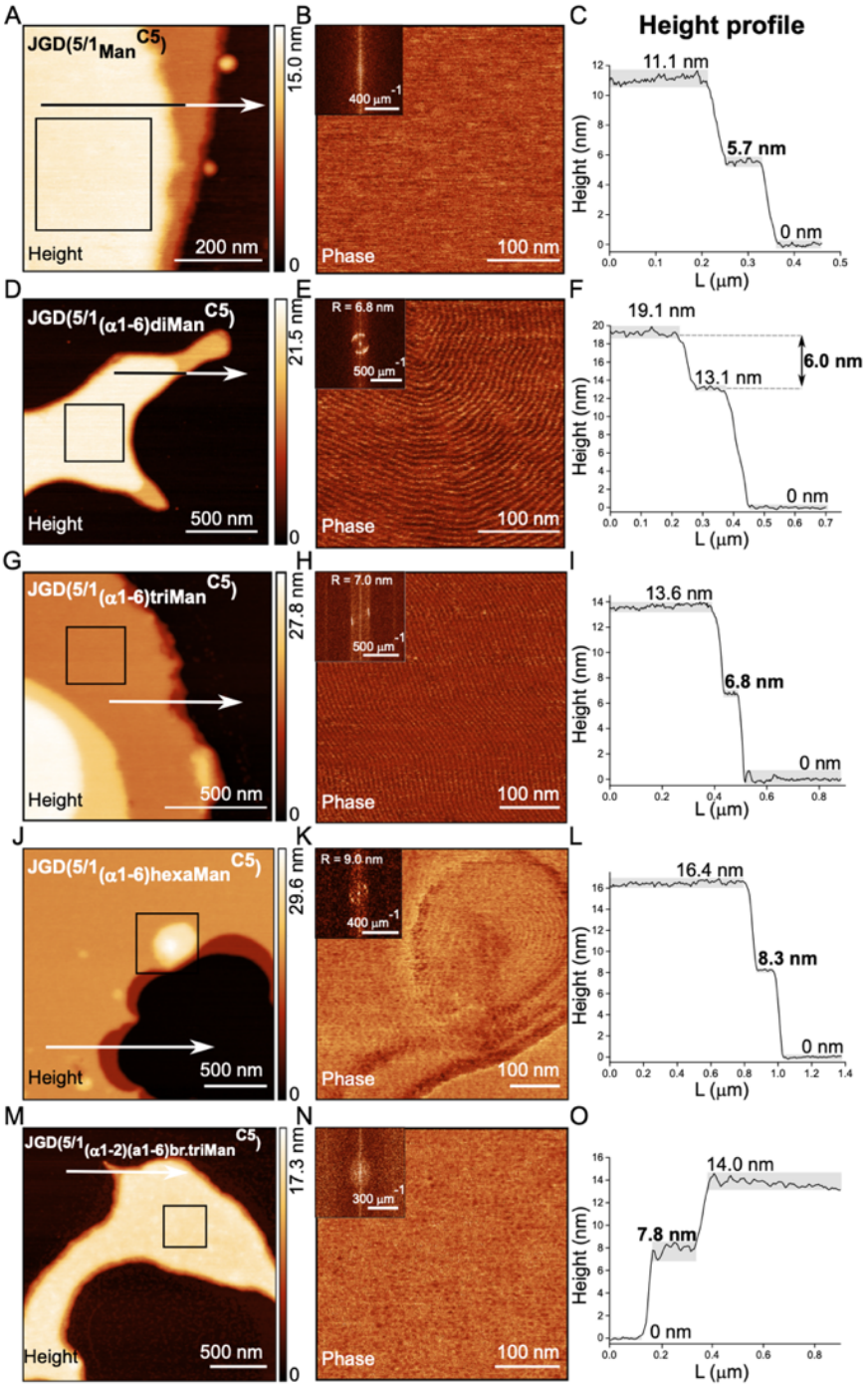


Fig. S37. Surface topography of GDSs formed by self-organization of oligoMan-containing sequence-defined JGDs. AFM height images (A, D, G, J, M), phase images (B, E, H, K, N) with inserted fast Fourier transform (FFT), and the corresponding height profiles (C, F, I, L, O) of semidried GDSs on mica were presented. Arrows in panels (A, D, G, J, M) indicate the directions for analysis of the height profiles in panels (C, F, I, L, O).

SI References

S1. C. Wendeln, A. Heile, H. F. Arlinghaus, B. J. Ravoo, Carbohydrate microarrays by microcontact printing. *Langmuir* **26**, 4933–4940 (2010).

S2. H. Kato, et al.; Preparation and evaluation of lactose-modified monoliths for the adsorption and decontamination of plant toxins and lectins. *Carbohydr. Res.* **346**, 1820–1826 (2011).

S3. P. Amaral, M. Fernandez-Villamarin, J. Correa, R. Riguera, E. Fernandez-Megia, Efficient multigram synthesis of the repeating unit of gallic acid-triethylene glycol dendrimers. *Org. Lett.* **13**, 4522–4525 (2011).

S4. V. Percec, et al., Modular synthesis of amphiphilic Janus glycodendrimers and their self-assembly into glycodendrimersomes and other complex architectures with bioactivity to biomedically relevant lectins. *J. Am. Chem. Soc.* **135**, 9055–9077 (2013).

S5. V. Percec, et al., Self-assembly of Janus dendrimers into uniform dendrimersomes and other complex architectures. *Science* **328**, 1009–1014 (2010).

S6. R. Saksena, J. Zhang, P. Kováč, Synthesis of 2-(trimethylsilyl)ethyl α-d-mannopyranosides revisited. *J. Carbohydr. Chem.* **21**, 453-470 (2002).

S7. K. Naresh, F. Schumacher, H. S. Hahm, P. H. Seeberger, Pushing the limits of automated glycan assembly: synthesis of a 50mer polymannoside. *Chem. Commun.* **53**, 9085-9088 (2017).

S8. L. Krock, et al. Streamlined access to conjugation-ready glycans by automated synthesis. *Chem. Sci.* **3**, 1617-1622 (2012).

S9. S. Eller, M. Collot, J. Yin, H. S. Hahm, P. H., Seeberger, Automated solid-phase synthesis of chondroitin sulfate glycosaminoglycans. *Angew. Chem., Int. Ed.* **52**, 5858-5861 (2013).

S10. M. K. Patel, et al. Analysis of the dispersity in carbohydrate loading of synthetic glycoproteins using MALDI-TOF mass spectrometry. *Chem. Commun.* **46**, 9119-9121 (2010).

S11. O. Calin, S. Eller, P. H. Seeberger, Automated polysaccharide synthesis: assembly of a 30mer mannoside. *Angew. Chem., Int. Ed.* **52**, 5862-5865 (2013).

S12. M. Delbianco, et al., Well-defined oligo- and polysaccharides as ideal probes for structural studies. *J. Am. Chem. Soc.* **140**, 5421–5426 (2018).

S13. C. Wendeln, A. Heile, H. F. Arlinghaus, B. J. Ravoo, Carbohydrate microarrays by microcontact printing. *Langmuir* **26**, 4933-4940 (2010).

S14. T. Ziegler, S. Gerling, M. Lang, Preparation of bioconjugates through an Ugi reaction. *Angew. Chem. Int. Ed.* **39**, 2109-2112 (2000).