Supporting Information

Chemoenzymatic synthesis of the oligosaccharide moiety of the tumor-associated antigen disialosyl globopentaosylceramide

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1. Chemical synthesis

1.1 General procedures

All chemicals were purchased from commercial sources. NMR spectra (\(^1\)H, \(^{13}\)C, COSY, HSQC) were obtained on an Agilent 400-MR DD2 or Bruker 750 MHz. Chemical shifts are reported in part per million (ppm) relative to CDCl\(_3\) (7.26 ppm), TMS (0.00 ppm) or D\(_2\)O (4.79 ppm). NMR data is presented as: chemical shift, multiplicity (where s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet) and the coupling constant in Hertz (Hz). Mass spectra were obtained on a Shimadzu ESI LC-MS QP8000 or Kratos Analytical Maxima-CFR MALDI-TOF system (using 2,5-dihydroxybenzoic acid matrix). Reported HRMS data was obtained on an Agilent technologies 6560 Ion mobility Q-TOF. Semi-preparative HPLC was performed on an Applied Biosystems 400 solvent delivery system and 757 Absorbance Detector (UV absorbance set on 214 nm) using HILIC column (XBridge\textsuperscript{\textregistered} Amide 5 µm, 4.6 mm x 250 mm column, Waters). The mobile phase for analytical and semi-preparative HPLC runs consisted of buffers A and B. For C\(_{18}\) columns buffer A is 0.1 % TFA in H\(_2\)O and buffer B is 10 % A + 90 % CH\(_3\)CN and a gradient was used. For HILIC column chromatography buffer A is 10 mM NH\(_4\)COOH in H\(_2\)O (pH = 4) and B is 10 % A + 90 % CH\(_3\)CN at isocratic conditions. Size exclusion chromatography was performed on Bio-Gel P-2 (45-90 µm) with water as the eluent. Column chromatography was performed on silica gel G60 (Silicycle 60 – 200 µm, 60 Å). TLC analysis was conducted on silica gel 60 F254 (EMD Chemicals Inc.) with detection by UV light (254 nm) and staining by 10 % H\(_2\)SO\(_4\) in EtOH or p-anisaldehyde solution, followed by heating for visualization. Molecular sieves (4 Å) were flame-dried prior to use.

1.2 NMR nomenclature

The monosaccharides of glycan DSGb5 have been labeled as shown in Figure S1. Starting from the reducing end of the pentasaccharide core Gb5, these were labeled as Glc-I, Gal-II, Gal-III, GalNAc-IV, Gal-V. The sialosides were named Neu5Ac-VI for the \(\alpha\)2,3-linked sialic acid and Neu5Ac-VII for the \(\alpha\)2,6-linked sialic acid.

Figure S1. Monosaccharide labeling system for DSGb5
1.3 Experimental procedures

2,2,2-Trichloroacetimidate 2,3,4,6-O-acetyl-α-D-galactopyranoside (9). Compound 9 was synthesized according to previous synthesis.¹ NMRS of the α-anomer of the title compound are described below. ¹H NMR (400 MHz, CDCl₃) δ 8.66 (1H, s, NH), 6.58 (1H, d, J = 3.1 Hz, H-1), 5.55 (1H, d, J = 2.6 Hz, H-4), 5.45 – 5.31 (2H, m, H-3, H-2), 4.43 (1H, t, J = 6.6 Hz, H-5), 4.20 – 4.01 (2H, m, H-6), 2.15 (3H, s, OAc), 2.03 – 1.98 (9H, m, 3x OAc). ¹³C NMR (101 MHz, CDCl₃) δ 170.2 (C, OAc), 170.0 (C, OAc), 170.0 (C, OAc), 169.9 (C, OAc), 160.8 (C=NH), 93.4 (C-1), 68.9 (C-5), 67.40, 67.27, 66.80, 61.2 (C-6), 20.6 (CH₃, OAc), 20.5 (CH₃, OAc), 20.5 (CH₃, OAc), 20.4 (CH₃, OAc).

Scheme S1. Synthesis of GalNHTroc acceptor 10 (similar to the procedure as described for GlcNH₂).²

1,3,4,6-tetra-O-acetyl-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (22). NaHCO₃ (23 g, 278 mmol) was added to Galactosamine·HCl (20 g, 93 mmol) in H₂O (180 mL) and after 30 min 2,2,2-trichloroethyl chloroformate (15.3 mL, 111 mmol) was added. After overnight stirring the white solids were filtered off, washed with H₂O and dried under high vacuum overnight. The solids were dissolved in pyridine (100 mL) and acetic anhydride (80 mL) was added. The reaction mixture was stirred for 3 h and concentrated in vacuo. The obtained oil was dissolved in DCM, washed with 1M HCl (2x), H₂O, sat. aq. NaHCO₃, filtered and concentrated in vacuo. The resulting crude was dissolved in DCM (80 mL) and imidazole (7.4 g, 109 mmol) was added. When all imidazole was dissolved, tert-hexyldimethylsilyl chloride (8.6 mL, 44 mmol) was added. The mixture was stirred overnight, washed with 1 M HCl, sat. aq. NaHCO₃, dried (Na₂SO₄), filtered and concentrated in vacuo to afford 22 (19 g, 40%, over two steps). ESI HRMS (m/z): [M + Na]⁺ calcd for C₁₇H₂₂Cl₃NO₁₁, 544.0156; found 544.0155. [α]²⁵/₅₈⁹ = 326.1° (C = 0.1; CHCl₃).

Dimethylhexylsilyl 3,4,6-tri-O-acetyl-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (23). Hydrazine acetate (3.7 g, 40 mmol) was added to a solution of compound 22 (19 g, 36 mmol) in DMF (60 mL). The mixture was stirred overnight, concentrated in vacuo, dissolved in EtOAc, washed with sat. aq. NaHCO₃, H₂O and dried (Na₂SO₄), filtered and concentrated in vacuo. The resulting crude was dissolved in DCM (80 mL) and imidazole (7.4 g, 109 mmol) was added. When all imidazole was dissolved, tert-hexyldimethylsilyl chloride (8.6 mL, 44 mmol) was added. The mixture was stirred overnight, washed with 1 M HCl, sat. aq. NaHCO₃, dried (Na₂SO₄), filtered and concentrated in vacuo. The obtained residue was purified by silica column chromatography using Toluene:EtOAc (1:0 to 6:4 v/v) as the eluent to afford 23 (17.2 g, 76%, over two steps). ¹H NMR (400 MHz, CDCl₃) δ 5.35 (1H, d, J = 3.2 Hz, H-4), 5.16 (1H, d, J = 9.9 Hz, H-3), 4.95 (1H, d, J = 8.3 Hz, NH), 4.84 – 4.68 (1H, m, H-1; CHH, Troc), 4.62 (1H, d, J = 11.7 Hz, CHH, Troc), 4.20 – 4.05 (2H, m, H-6), 3.89 (1H, t, J = 6.6 Hz, H-5), 3.78 (1H, dd, J = 18.4, 9.2 Hz, H-2), 2.16 (2H, s,
OAc), 2.04 (3H, s, OAc), 1.99 (3H, s, OAc), 1.66 – 1.58 (1H, m, CH, TDS), 0.95 – 0.76 (12H, m, 4x CH₃, TDS), 0.17 (3H, s, CH₃-Si), 0.14 (3H, s, CH₃-Si). ¹³C NMR (101 MHz, CDCl₃) δ 170.5 (C, OAc), 170.4 (C, OAc), 170.3 (C, OAc), 154.0 (C=O, Troc), 96.4 (C-1), 95.3 (C₅Cl₃), 74.5 (CH₂, Troc), 70.7 (C-5), 69.9 (C-3), 66.9 (C-4), 61.8 (C-6), 54.7 (C-2), 33.9 (CH, TDS), 24.8 (C, TDS), 20.7 (CH₃, OAc), 20.6 (CH₃, OAc), 20.6 (CH₃, OAc), 19.9 (2x CH₃, TDS), 18.5 (2x CH₃, TDS), -1.9 (CH₃-Si), -3.4 (CH₃-Si). ESI HRMS (m/z): [M + NH₄]+ calcd for C₃₂H₇₈Cl₃NO₁₀Si, 639.1669; found 639.1675. [α]²⁵/⁵⁸₉ = -32.7° (C = 0.1; CHCl₃).

Dimethylthexylsilyl 2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (24). Freshly prepared NaOMe was added to compound 23 (17.3 g, 28 mmol) in MeOH (50 mL). After 2 h the reaction was quenched by addition of Amberlite H⁺ resin, filtered and concentrated in vacuo to afford 24 (13 g, 95%). This product was then used in the next step without additional purification. ESI HRMS (m/z): [M + Na]+ calcd for C₁₇H₃₂Cl₃NO₇Si, 518.0911; found 518.0913. [α]²⁵/⁵₈₉ = -77.0° (C = 0.1; CHCl₃).

Dimethylthexylsilyl 4,6-O-benzylidene-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (10). Benzaldehyde dimethyl acetal (4.57 mL, 30.4 mmol) and pTsOH·H₂O (1.05 g, 5.5 mmol) were added to a solution of compound 24 (13 g, 26 mmol) in CH₂CN (90 mL). After 1 h the mixture was quenched with Et₃N, concentrated in vacuo and the obtained residue was purified by silica column chromatography using Hexane:EtOAc (1:0 to 8.5:1.5 v/v) as the eluent to obtain compound 10 (4.6 g, 30%). ¹H NMR (400 MHz, CDCl₃) δ 7.55 – 7.48 (2H, m, H-Ar), 7.43 – 7.35 (3H, m, H-Ar), 5.57 (1H, s, CH₂-C₇H₅), 5.05 (1H, s, CH₂, TDS), 4.80 (1H, d, J = 7.8 Hz, H-1), 4.69 (2H, s, CH₂, Troc), 4.28 (1H, d, J = 12.3, 1.3 Hz, H-6α), 4.20 (1H, d, J = 3.6 Hz, H-4), 4.07 (1H, d, J = 12.4, 1.8 Hz, H-6b), 3.92 (1H, d, J = 8.9 Hz, H-3), 3.63 (1H, d, J = 9.4 Hz, H-2), 3.47 (1H, s, H-5), 2.73 (1H, d, J = 9.0 Hz, OH), 1.66 – 1.58 (1H, m, CH, TDS), 0.94 – 0.78 (12H, m, 4x CH₃, TDS), 0.22 (3H, s, CH₃-Si), 0.17 (3H, s, CH₃-Si). ¹³C NMR (101 MHz, CDCl₃) δ 154.7 (C=O, Troc), 137.5 (C, Ar), 129.3, 128.5, 128.3, 126.4, 101.4 (CH₂-C₇H₅), 95.7 (C-1), 75.0 (C-4), 74.7 (CH₂, Troc), 70.4 (C-3), 69.3 (C-6), 66.5 (C-5), 57.8 (C-2), 34.0 (CH, TDS), 20.1 (CH₃, TDS), 20.1 (CH₃, TDS), 18.5 (CH₃, TDS), 18.5 (CH₃, TDS), -1.7 (CH₃-Si), -2.9 (CH₃-Si). ESI HRMS (m/z): [M + Na]+ calcd for C₂₃H₃₈Cl₃NO₇Si, 606.1224; found 606.1227. [α]²⁵/⁵₈₉ = -22.5° (C = 1; CHCl₃).

Scheme S2. Chemical glycosylation of donor 9 and acceptor 10 and formation of disaccharide donor 7a.

Dimethylthexylsilyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-4,6-O-benzylidene-2-[(2,2,2-trichloromethoxy)carbonylamino]-β-D-galactopyranoside (13). A mixture of acceptor 10 (1.0 g, 1.7 mmol), donor 9 (1.3 g, 2.6 mmol) and 4 Å molecular sieves was stirred in DCM (5 mL) for 2 h. The reaction mixture was cooled to -35°C and TMSOTf (62 μL, 0.3 mmol) was added. After 30 min the reaction was quenched with Et₃N, filtered over a pad of Celite and concentrated in vacuo. The obtained residue was purified by silica column chromatography using Toluene:EtOAc (1.0 to 8.5:1.5 v/v) as the eluent to afford compound 13 (878 mg, 56%). ¹H NMR (400 MHz, CDCl₃) δ 7.58 – 7.52 (2H, m,
H-Ar), 7.42 – 7.31 (3H, m, H-Ar), 5.55 (1H, s, CH-C6H5), 5.36 (1H, dd, J = 3.4, 0.8 Hz, H-4, Gal-V), 5.32 – 5.26 (1H, m, NH), 5.21 (1H, d, J = 7.9 Hz, H-1, GalNAc-IV), 5.13 (1H, d, J = 7.7 Hz, H-2, Gal-V), 4.96 (1H, dd, J = 10.4, 3.5 Hz, H-3, Gal-V), 4.78 (1H, d, J = 7.9 Hz, H-1, Gal-V), 4.75 – 4.60 (2H, m, CH2, Troc), 4.46 (1H, dd, J = 11.1, 2.7 Hz, H-3, GalNAc-IV), 4.29 (1H, d, J = 3.3 Hz, H-4, GalNAc-IV), 4.25 (1H, d, J = 12.2, 1.1 Hz, H-6a, GalNAc-IV), 4.22 – 4.07 (2H, m, H-6, Gal-V), 4.03 (1H, d, H-6b, GalNAc-IV), 3.88 (1H, t, J = 6.3 Hz, H-5, Gal-V), 3.54 – 3.36 (2H, m, H-2, GalNAc-IV; H-5, GalNAc-IV), 2.15 (3H, s, OAc), 2.04 (3H, s, OAc), 1.97 (3H, s, OAc), 1.93 (1H, p, J = 13.7, 6.9 Hz, CH, TDS), 0.92 – 0.78 (12H, m, 4x CH3, TDS), 0.19 (3H, s, CH3-Si), 0.13 (3H, s, CH3-Si). 

13C NMR (101 MHz, CDCl3) δ 170.2 (C, OAc), 170.0 (C, OAc), 169.9 (C, OAc), 169.3 (C, OAc), 153.8 (C=O, Troc), 138.0 (C, C6H5), 129.0, 128.2, 126.3, 101.6 (C-1, Gal-V), 100.7 (CH-C6H5), 95.20 (CCl3), 94.5 (C-1, GalNAc-IV), 76.1 (C-4, GalNAc-IV), 75.7 (C-3, GalNAc-IV), 74.5 (CH2, Troc), 70.8 (C-5, Gal-V), 70.8 (C-3, Gal-V), 69.3 (C-6, GalNAc-IV), 68.8 (C-2, Gal-V), 67.0 (C-4, Gal-V), 66.4 (C-5, GalNAc-IV), 61.6 (C-6, Gal-V), 55.9 (C-2, GalNAc-IV), 34.0 (CH, TDS), 24.8 (C, TDS), 20.7 (CH3, OAc), 20.7 (CH3, OAc), 20.5 (CH3, OAc), 20.1 (CH3, TDS), 18.6 (CH3, TDS), 18.5 (CH3, TDS), -1.8 (CH3-Si), -3.1 (CH3-Si). 

ESI HRMS (m/z): [M + NH4]+ calcd for C38H54Cl3NO16Si, 931.2616; found 931.2634. [α]25°250 = 223.8° (C = 0.1; CHCl3).

Scheme S3. Formation of disaccharide donor 7b from disaccharide 13.
2,2,2-Trichloroacetimidate 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-4,6-di-O-acetyl-2-([2,2,2-trichloromethoxy]carbonylamino)-β-D-galactopyranoside (7b). A solution of disaccharide 13 (350 mg, 0.38 mmol) in 80% aq AcOH (4 mL) was heated to 80°C for 4 h. The mixture was allowed to cool to room temperature (RT) and was diluted with EtOAc, washed with H₂O, sat aq. NaHCO₃ (3x), dried (Na₂SO₄), filtered, and concentrated in vacuo. The obtained crude was dissolved in pyridine (3 mL) and Ac₂O (2 mL) was slowly added, followed by DMAP (cat.). After 1 h, the reaction showed complete conversion by TLC and the mixture was concentrated in vacuo. The crude was dissolved in pyridine (3.5 mL) and transferred to a plastic round bottom flask. HF·Pyridine (70% HF, 350 µL) was added and the mixture was stirred overnight. The reaction mixture was diluted with EtOAc, washed with sat. aq. NaHCO₃ (3x), dried (Na₂SO₄), filtered, concentrated in vacuo and co-evaporated with toluene. Quick silica column purification Hexane:EtOAc (1:0 to 1:3 v/v) provided the intermediate in 81% yield. The obtained intermediate (237 mg, 0.31 mmol) was dissolved in DCM and stirred with 4 Å molecular sieves at 0°C. 2,2,2-trichloroethyl chloroformate (297 µL, 2.96 mmol) and Cs₂CO₃ (301 mg, 0.92 mmol) were added after 30 min. After 22 h the reaction mixture was concentrated in vacuo and the obtained residue was purified by silica column chromatography using Hexane:EtOAc (1:0 to 1:1 v/v) as the eluent to isolate the α-anomer of the title compound (143 mg, 53%).

Scheme S4. Synthesis of disaccharide acceptors 12a and 12b from protected lactose 25.

Para-methoxyphenyl 2,3,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (12a). Compound 25 (8.1 g, 8.2 mmol) was stirred in DCM (50 mL) with 4 Å molecular sieves for 3 h. The mixture was cooled to -78°C and after adding Et₂SiH (6.5 mL, 41 mmol) stirring was continued for another 30 min. TfOH (1.45 mL, 16.4 mmol) was introduced and after 2.5 h, the reaction mixture was quenched with Et₃N. The resulting mixture was filtered over Celite and the filtrate
was washed with H₂O, sat. aq. NaHCO₃, dried (Na₂SO₄), filtered and concentrated in vacuo. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 20:1 v/v) as the eluent to afford compound **12a** (2.28g, 64%). ¹H NMR (400 MHz, CDCl₃) δ 7.54 – 7.14 (30H, m, Ar-H), 7.02 (2H, d, J = 9.1 Hz, OMP), 6.79 (2H, d, J = 9.1 Hz, OMP), 5.00 (2H, t, J = 10.7 Hz, CH₂, Bn), 4.85 (1H, d, J = 7.4 Hz, H-1, Glc-I), 4.83 – 4.64 (6H, m, 3x, CH₂, Bn), 4.53 – 4.35 (5H, m, 2x CH₂, Bn; H-1, Gal-II), 4.06 – 3.96 (2H, m, H-4, Gal-II; H-5 Gal-II), 3.82 – 3.74 (4H, m, CH₃, OMP; H-6 Glc-I), 3.72 – 3.57 (4H, m, H-4, Glc-I; H-6a, Gal-II; H-2, Glc-I; H-2, Gal-II), 3.52 – 3.45 (2H, m, H-5, Glc-I; H-6b, Gal-II), 3.40 (1H, dd, J = 9.3, 3.4 Hz, H-3, Gal-II), 3.35 (1H, t, J = 3.0 Hz, OH). ¹³C NMR (101 MHz, CDCl₃) δ 155.2 (C, OMP), 151.6 (C, OMP), 139.0 (C, OBN), 138.5 (C, OBN), 138.4 (C, OBN), 138.3 (C, OBN), 138.2 (C, OBN), 137.9 (C, OBN), 128.43, 128.34, 128.28, 128.25, 128.20, 128.08, 127.83, 127.78, 127.73, 127.62, 127.59, 127.54, 127.40, 127.25, 118.4 (2x CH, OMP), 114.5 (2x CH, OMP), 102.8 (C-1, Glc-I), 102.6 (C-1, Gal-II), 82.9 (C-4, Glc-I), 81.6 (C-2, Glc-I), 79.4 (C-2, Gal-II), 77.2 (C-5, Gal-II), 75.4 (CH₂), 75.3 (2x CH₂), 75.1 (C-5, Glc-I), 73.5 (CH₂), 73.1 (CH₂), 72.8 (C-3, Glc-I), 72.0 (CH₂), 68.4 (C-6, Glc-I), 68.3 (C-6, Gal-II), 66.1 (C-4, Gal-II), 55.6 (CH₃, OMP). ESI HRMS (m/z): [M + Na⁺] calcd for C₅₁H₆₀O₁₂, 1006.4736; found 1006.4750.

**N-(Benzy1)-benzoylcarbonyl-5-aminopentan-1-ol** (28). The protected aminopentanol linker was synthesized as described before.⁴

**N-(Benzy1)-benzoylcarbonyl-5-aminopentyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside** (27). Oven-dried (90°C, 1.5 h) ceric ammonium nitrate (1.67 g, 3.0 mmol) was added to a stirring solution of compound **25** (2.0 g, 2.0 mmol) in CH₂CN/H₂O (40/10 mL) at 0°C. After 30 min, the mixture was diluted with DCM, washed with sat. aq. NaHCO₃ (2 x), dried, filtered and concentrated in vacuo. Purification by silica column chromatography using Hexane:EtOAc (1:0 to 1:1 v/v) as the eluent provided the product, which was directly used in the next step.

2,2,2-Trichloroacetonitrile (848 µL, 8.5 mmol) and DBU (51 µL, 0.3 mmol) were added to the intermediate (1.49 g, 1.7 mmol) in DCM (3 mL) with 4 Å molecular sieves at 0°C. After 15 min the reaction mixture was concentrated in vacuo and the crude product was directly purified by silica column chromatography using Toluene:EtOAc (1:0 to 8:2 v/v) as the eluent. The obtained compound **26** was directly used in the next step. A mixture of acceptor **28** (100 mg, 0.3 mmol), donor **26** (468 mg, 0.46 mmol) and 4 Å molecular sieves was stirred in CH₂CN (5 mL) for 1 h. The mixture was cooled to -30°C and TMSCl (1.1 µL, 0.06 mmol) was added. The reaction mixture was allowed to warm to 15°C over 2 h. The reaction mixture was with Et₃N, filtered over a pad of Celite and concentrated in vacuo. The obtained product was dried with NaHCO₃, filtered and concentrated in vacuo. The obtained crude was purified by silica column chromatography using Toluene:EtOAc (1.0 to 7:3 v/v) as the eluent to afford compound **27** (305 mg, 84%). ¹H NMR (400 MHz, CDCl₃) δ 7.56 – 7.07 (40H, m, Ar-H), 5.45 (1H, s, CH-C₆H₅), 5.22 – 5.09 (3H, m), 4.93 – 4.67 (6H, m), 4.54 (1H, d), 4.50 – 4.31 (3H, m, H-1, Glc-I; CH₂, penty), 4.39 – 4.14 (4H, m, H-1, Gal-II), 4.01 (1H, d, d, J = 3.5 Hz, H-4, Gal-II), 3.97 (1H, dd, J = 11.6, 7.1 Hz, H-5, Glc), 3.93 – 3.66 (5H, m, H-2, Glc-I), 3.66 – 3.58 (1H, m, H-3, Gal-II), 3.57 – 3.31 (4H, m, H-2, Gal-II; H-3, Glc-I; H-4, Glc-I), 3.28 – 3.10 (2H, m, CH₂, penty), 2.92 (1H, s, H-5, Gal-II), 1.74 – 1.42 (4H, m, 2x CH₂, penty), 1.41 – 1.17 (2H, m, CH₂, penty). ¹³C NMR (101 MHz, CDCl₃) δ 163.5 (C=O, Cbz), 138.9, 138.8, 138.7, 138.5, 138.3, 138.1, 129.0, 128.8, 128.6, 128.5, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 128.0, 127.8, 127.8, 127.7, 127.6, 127.5, 127.4, 126.6, 103.6 (C-1, Glc-I), 102.9 (C-1, Gal-II), 101.4 (CH-C₆H₅), 92.1, 83.0 (C-3, Gal-II), 81.8 (C-2, Gal-II), 79.6, 78.9 (C-2, Glc-I), 77.6, 7578, 75.3, 75.0, 75.0, 73.7 (C-4, Gal-II), 73.0, 71.7, 69.9, 69.0, 68.3, 67.2, 66.3 (C-5, Gal-II), 50.6, 50.3, 47.2, 46.3, 29.5, 28.0, 23.4.
**N-(Benzyl)-benzylloxycarbonyl-5-aminopentyl 2,3,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (12b).** Compound 27 (300 mg, 0.25 mmol) was stirred in DCM with 4 Å molecular sieves for 2 h. The mixture was cooled to -78°C and after adding Et₃SiH (201 µL, 1.26 mmol) stirring was continued for another 30 min. TFOH (45 µL, 0.50 mmol) was introduced. More Et₃SiH (200 µL) and TFOH (70 µL, 0.8 mmol) were added over time and after 2.5 h the reaction mixture was quenched with Et₃N. The quenched mixture was filtered over Celite, washed with H₂O, sat. aq. NaHCO₃, dried (Na₂SO₄), filtered and concentrated *in vacuo*. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 5:1 v/v) as the eluent to afford compound 12b (191 mg, 64 %). ¹H NMR (400 MHz, CDCl₃) δ 7.59 – 6.95 (40H, m, Ar-H), 5.15 (2H, d, J = 6.7 Hz), 4.89 – 4.61 (6H, m), 4.61 – 4.27 (8H, m, H-1 Glc-I; H-1, Gal-II), 4.01 (1H, s, H-7), 3.99 – 3.91 (1H, m), 3.91 – 3.28 (13H, m, H-2, Glc-I; H-2, Gal-II), 3.28 – 3.10 (2H, m, CH₂ pentyl), 2.40 (1H, s, OH), 1.71 – 1.42 (4H, m, 2x CH₂, pentyl), 1.42 – 1.17 (2H, m, CH₂, pentyl). ¹³C NMR (101 MHz, CDCl₃) δ 139.1, 138.7, 138.6, 138.3, 138.2, 137.9, 128.5, 128.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 127.5, 127.2, 103.6 (C-1, Glc-I), 102.5 (C-1, Gal-II), 82.9, 81.8 (C-2, Glc-I), 81.1, 79.4 (C-2, Gal-II), 76.6 (C-3, Gal-II), 75.3, 75.2, 75.1, 74.9, 73.5, 73.1, 72.7, 72.0, 68.4, 68.3, 67.1, 66.1 (C-4, Gal-II), 50.5, 50.2, 47.2, 46.2, 29.4, 28.0, 27.5, 23.4.

![Scheme S55. Synthesis of galactosyl donor 11 from compound 29.](image)

**Phenyl 3-O-(2-naphthyl)methyl-4,6-O-di-tert-butylsilanediyl-1-thio-β-D-galactopyranoside (30).** Bu₂SnO (5.78 g, 23 mmol) was added to compound 29 (7.98 g, 19 mmol) in toluene (100 mL) and the suspension was heated under reflux for 3 h. The resulting clear solution was cooled to 90 °C and after the addition of 2-(bromomethyl)naphthalene (4.70 g, 21 mmol), tetrabutylammonium iodide (7.86 g, 21 mmol) was added portionwise over 1 h. After overnight stirring at 90 °C, the reaction mixture was concentrated *in vacuo*. The obtained crude was dissolved in DCM, washed with sat. aq. NaHCO₃, extracted with DCM (2x), washed with brine, dried (Na₂SO₄) and filtered over a pad of Celite. The concentrated crude was purified by silica column chromatography using Toluene:EtOAc (1:0 to 10:1 v/v) as the eluent to afford compound 30 (5.88 g, 58 %). ¹H NMR (400 MHz, CDCl₃) δ 7.88 – 7.75 (4H, m, Ar-H), 7.58 – 7.52 (3H, m, Ar-H), 7.51 – 7.43 (2H, m, Ar-H), 7.34 – 7.22 (3H, m, Ar-H), 4.96 (1H, d, J = 11.9 Hz, CHH, Nap), 4.81 (1H, d, J = 11.9 Hz, CHH, Nap), 4.61 – 4.54 (2H, m, H-1, H-4), 4.23 (2H, dd, J = 12.5, 1.9 Hz, H-6), 4.06 (1H, dd, J = 9.5, 1.9 Hz, H-2), 3.41 (1H, dd, J = 9.1, 3.0 Hz, H-3), 3.35 (1H, s, H-5), 2.60 (1H, d, J = 1.9 Hz, OH, H-2), 1.08 (9H, s, t-Bu), 1.07 (9H, s, t-Bu). ¹³C NMR (101 MHz, CDCl₃) δ 135.6, 133.60, 133.25, 133.09, 132.53, 128.85, 128.37, 127.87, 127.72, 127.67, 126.61, 126.19, 125.99, 125.85, 89.2 (C-1), 81.8 (C-3), 75.1 (C-5), 70.5 (CH₂, Nap), 69.5 (C-4), 68.6 (C-2), 67.4 (C-6), 27.7 (3x CH₃, t-Bu), 27.6 (3x CH₃, t-Bu), 23.4 (C, t-Bu), 20.7 (C, t-Bu). ESI HRMS (m/z): [M + Na]⁺ calcd for C₃₁H₃₀O₅S₂Si, 575,2263; found 575.2260. [α]₂₅° = -10.0° (C = 0.01; CHCl₃).

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S8
Phenyl 2-O-benzyl-3-O-(2-naphthyl)methyl-4,6-O-di-tert-butylsilylatediol-1-thio-β-D-galactopyranoside (11). A mixture of compound 30 (1.9 g, 3.4 mmol) and NaH (60% dispersion in oil; 275 mg, 6.9 mmol) in DMF (10 mL) was stirred at 0°C for 10 min. BenzyI bromide (612 μL, 5.2 mmol) was added dropwise and the mixture was stirred at RT for another 30 min before quenching with AcOH in MeOH. The mixture was concentrated in vacuo, diluted with DCM and washed with 1M HCl and water. The organic phase was dried (Na2SO4), filtered and concentrated in vacuo. Crystallization from MeOH provided compound 11 (1.39 g, 63%). 1H NMR (400 MHz, CDCl3) δ 7.86 – 7.77 (3H, m, Ar-H), 7.76 – 7.69 (1H, m, Ar-H), 7.57 – 7.50 (3H, m, Ar-H), 7.50 – 7.40 (3H, m, Ar-H), 7.38 – 7.18 (7H, m, Ar-H), 4.98 – 4.82 (4H, m, 2x CH2, Nap), 4.66 (1H, d, J = 9.8 Hz, H-1), 4.52 (1H, d, J = 2.7 Hz, H-4), 4.18 (2H, dd, J = 12.4, 1.9 Hz, H-6), 3.88 (3H, t, J = 9.4 Hz, H-2), 3.52 (1H, dd, J = 9.1, 3.0 Hz, H-3), 3.26 (1H, s, H-5), 1.15 (9H, s, t-Bu), 1.09 (9H, s, t-Bu). 13C NMR (101 MHz, CDCl3) δ 138.37, 135.83, 134.82, 133.21, 133.01, 132.02, 128.70, 128.41, 128.29, 128.19, 127.84, 127.69, 127.66, 127.22, 126.44, 126.07, 125.86, 88.7 (C-1), 82.6 (C-3) 77.3 (C-2), 75.9, 74.7 (C-5), 71.1, 70.1 (C-4), 67.3 (C-6), 27.7 (CH3, t-Bu), 27.6 (CH3, t-Bu), 23.4 (C, t-Bu), 20.7 (C, t-Bu). ESI HRMS (m/z): [M + Na]+ calcd for C38H46O5S2Si, 665.2733; found 665.2731. [α] 25 °C = 4.5° (C = 0.1; CHCl3).

Para-methoxyphenyl 2-O-benzyl-3-O-(2-naphthyl)methyl-4,6-O-di-tert-butylsilylatediol-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (15a). A mixture of acceptor 12a (1.54 g, 1.56 mmol), donor 11 (1.20 g, 1.87 mmol) and 4 Å molecular sieves was stirred in DCM (15 mL) for 1 h. The reaction mixture was cooled to -30°C and N-iodosuccinimide (700 mg, 3.1 mmol) and triflic acid (14 μL, 0.16 mmol) were added. After 35 min the reaction was quenched with Et3N, filtered over a pad of Celite and concentrated in vacuo. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 20:1 v/v) as the eluent to afford compound 15a (2.13 g, 90%). 1H NMR (400 MHz, CDCl3) δ 7.84 – 7.64 (4H, m, Ar-H), 7.49 – 7.39 (4H, m, Ar-H), 7.36 – 7.13 (34H, m, Ar-H), 7.01 (2H, d, J = 9.0 Hz, OMP), 6.78 (2H, d, J = 9.0 Hz, OMP), 5.12 – 5.07 (1H, d, J = 11.3 Hz, CHH), 5.01 – 4.92 (2H, m, H-1, Gal-II; CHF), 4.85 (1H, d, J = 7.3 Hz, H-1, Glc-I), 4.83 – 4.64 (8H, m, 4x CH2), 4.62 – 4.52 (2H, m, 2x CH2), 4.52 – 4.41 (3H, m, CH2H, H-1, Gal-II; H-4, Gal-III), 4.40 – 4.24 (3H, m, CH2; CHH), 4.17 – 3.87 (6H, m, H-6A, Gal-II; H-4, Gal-III), 3.76 – 3.60 (2H, m, H-2, Glc-I; H-5, Gal-III), 3.59 – 3.43 (3H, m, H-2, Gal-II; H-6B, Gal-II; H-5, Glc-I), 3.35 (1H, dd, J = 8.1, 5.5 Hz, H-3, Glc-I), 3.29 (1H, dd, J = 10.0, 2.7 Hz, H-3, Gal-II), 1.09 (9H, s, t-Bu), 0.99 (9H, s, t-Bu). 13C NMR (101 MHz, CDCl3) δ 155.2 (C, OMP), 151.6 (C, OMP), 139.2 (C, Ar), 138.7 (C, Ar), 138.6 (C, Ar), 138.4 (C, Ar), 138.3 (C, Ar), 138.2 (C, Ar), 136.7 (C, Ar), 133.2, 132.9, 129.0, 128.5, 128.4, 128.4, 128.3, 128.2, 128.2, 128.2, 128.1, 127.9, 127.8, 127.7, 127.7, 127.6, 127.6, 127.5, 127.5, 127.4, 127.4, 126.0, 125.9, 125.9, 125.7, 118.5 (2x CH, OMP), 114.5 (2x CH, OMP), 103.1 (C-1, Glc-I), 102.7 (C-1, Gal-II), 100.2 (C-1, Gal-III), 82.5 (C-5, Gal-III), 81.5 (C-2, Glc-I), 81.1 (C-3, Gal-II), 79.1 (C-2, Gal-II), 78.0 (C-3, Gal-III), 77.2 (C-5, Gal-II), 75.3 (C-5, Glc-I), 75.2, 75.1, 74.9, 74.3 (C-2, Gal-III), 73.7, 73.5 (C-4, Glc-I), 73.4 (C-3, Glc-I), 73.2, 73.1, 72.2, 71.2 (C-4, Gal-III), 70.6, 68.4 (C-6, Glc-I), 67.7 (C-6, Gal-II), 67.5 (C-4, Gal-II), 67.1 (C-6, Gal-III), 55.6 (CH3, OMP), 27.7 (CH3, t-Bu), 27.3 (CH3, t-Bu), 23.3 (C, t-Bu), 20.7 (C, t-Bu). ESI HRMS (m/z): [M + NH4]+ calcd for C93H104O17Si, 1538.7381; found 1538.7403. [α] 25 °C = 357.2° (C = 0.1; CHCl3).
Para-methoxyphenyl 2-O-benzyl-4,6-O-di-tert-butylsilylated β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (8a). DDQ (377 mg, 1.7 mmol) was added to a stirring solution of compound 15a (2.1 g, 1.4 mmol) in DCM (120 mL) and PBS buffer (pH 7.4, 5 mL) and the reaction mixture was kept in darkness. After 3 h, the mixture was diluted with DCM, washed with sat. aq. NaHCO₃ (2 x), H₂O, dried (Na₂SO₄), filtered and concentrated in vacuo. Purification by silica column chromatography using Toluene:EtOAc (1:0 to 4:1 v/v) as the eluent provided compound 8a (987 mg, 52 %). ¹H NMR (400 MHz, CDCl₃) δ 7.42 (2H, d, J = 7.0 Hz, Ar-H), 7.36 – 7.17 (33H, m, Ar-H), 7.01 (2H, d, J = 9.0 Hz, OMP), 6.78 (2H, d, J = 9.1 Hz, OMP), 5.09 (1H, d, J = 11.6 Hz, CHH), 5.01 – 4.94 (2H, m, H-1, Gal-III; CHH), 4.86 (1H, d, J = 7.5 Hz, H-1, Glc-I), 4.81 – 4.56 (8H, m, 4x CH₂), 4.51 – 4.42 (2H, m, H-1, Gal-II; CHH), 4.40 – 4.27 (3H, m, CH₂; CHH), 4.24 (1H, d, J = 3.2 Hz, H-4, Gal-III), 4.09 – 3.95 (5H, m, H-4, Gal-II; H-4, Glc-I; H-6a, Gal-II; H-3, Gal-III; H-5, Gal-II), 3.79 (2H, d, J = 3.1 Hz, H-6, Glc-I), 3.76 (3H, s, CH₃, OMP), 3.72 (2H, s, H-6, Gal-III), 3.70 – 3.61 (3H, m, H-2, Glc-I; H-5, Gal-III; H-2, Gal-II), 3.56 (1H, dd, J = 9.9, 7.2 Hz, H-2, Gal-I), 3.51 – 3.43 (2H, m, H-6b, Gal-II; H-5, Glc-I), 3.34 (1H, dd, J = 8.3, 5.5 Hz, H-3, Glc-I), 3.29 (1H, dd, J = 10.0, 2.7 Hz, H-3, Gal-II), 2.40 – 2.31 (1H, m, OMe), 0.97 (9H, s, t-Bu), 0.90 (9H, s, t-Bu). ¹³C NMR (101 MHz, CDCl₃) δ 155.2 (C, OMP), 151.6 (C, OMP), 139.3 (C, Ar), 138.4 (C, Ar), 138.4 (2 C, Ar), 138.2 (C, Ar), 138.1 (C, Ar), 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.6, 127.6, 127.5, 127.4, 127.3, 127.1, 118.4 (2x CH, OMP), 114.5 (2x CH, OMP), 103.1 (C-1, Gal-II), 102.7 (C-1, Glc-I), 99.4 (C-1, Gal-III), 82.7 (C-5, Gal-III), 81.6 (C-2, Glc-I), 81.1 (C-3, Gal-II), 79.0 (C-2, Gal-II), 77.3 (C-5, Gal-II), 75.5 (C-2, Gal-III), 75.3 (C-5, Glc-I), 75.1, 75.0, 75.0, 73.9 (C-4, Gal-III), 73.2 (C-4, Glc-I), 73.1 (C-3, Glc-I), 73.0, 72.3, 70.1 (C-3, Gal-III), 68.4 (C-6, Glc-I), 67.5 (C-6, Gal, II), 67.1 (C-4, Gal-II), 66.7 (C-6, Gal-III), 55.6 (CH₃, OMP), 27.5 (CH₃, t-Bu), 27.2 (CH₃, t-Bu), 23.2 (C, t-Bu), 20.6 (C, t-Bu). ESI HRMS (m/z): [M + NH₄]⁺ calc for C₈₉H₆₉O₁₇Si, 1398.6755; found 1398.6760. [α]²⁵³₈⁰⁹ = 54.2° (C = 0.05; CHCl₃).

N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 2-O-benzyl-3-O-(2-naphthyl)methyl-4,6-O-di-tert-butylsilylated β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (15b). A mixture of acceptor 12b (189 mg, 0.19 mmol), donor 11 (147 mg, 0.23 mmol) and 4 Å molecular sieves was stirred in DCM (1.5 mL) for 1 h. The reaction mixture was cooled to -30°C and N-iodosuccinimide (86 mg, 0.38 mmol) and triflic acid (1.7 µL, 0.02 mmol) were added. After 20 min the reaction was quenched with Et₃N, filtered over a pad of Celite and concentrated in vacuo. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 5:1 v/v) as the eluent to afford compound 15b (197 mg, 60%). ¹H NMR (400 MHz, CDCl₃) δ 7.95 – 7.64 (m, 4H, Ar-H), 7.54 – 6.79 (48H, m, Ar-H), 5.15 (2H, d, J = 6.3 Hz), 5.06 (1H, d, J = 11.1 Hz), 4.97 (1H, d, J = 3.4 Hz, H-1, Gal-III), 4.87 – 4.62 (7H, m), 4.62 – 4.38 (7H, m, H-1, Gal-II), 4.37 – 4.17 (4H, m, H-1, Glc-I), 4.16 – 4.07 (1H, m), 4.06 – 3.98 (2H, m, H-2, Gal-III), 3.98 – 3.63 (10H, m), 3.62 – 3.07 (10H, m, H-2, Gal-II, H-2, Glc-I), 1.68 – 1.40 (4H, m, 2x CH₂, pentyl), 1.40 – 1.16 (2H, m, CH₂, pentyl), 1.00 (9H, s, t-Bu), 0.97 (9H, s, t-Bu). ¹³C NMR (101 MHz, CDCl₃) δ 139.3, 138.7, 138.7, 138.5, 138.3, 138.2, 136.7, 133.2, 129.0, 129.0, 128.5, 128.5, 128.4, 128.3, 128.2, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.4, 127.3, 126.0, 125.9, 125.9, 125.6, 125.3, 103.5 (C-1, Glc-I), 102.9 (C-1, Gal-II), 100.1 (C-1, Gal-III), 82.5, 81.7 (C-2, Glc-I), 81.1, 79.0 (C-2, Gal-II), 78.0, 77.2, 75.1, 75.0, 74.9, 74.9, 74.2, 73.6 (C-2, Gal-III), 73.1, 73.1, 72.1, 72.1, 70.6, 70.1, 69.7, 68.3, 67.6, 67.5, 67.1, 67.1, 50.2, 47.2, 46.2, 29.4, 27.6 (CH₃, t-Bu), 27.6, 27.3 (CH₃, t-Bu), 23.3 (C, t-Bu), 20.7 (C, t-Bu). [α]²⁵³₈⁰⁹ = -23.5° (C = 0.02; CHCl₃).
N-(Benzyl)-benzyloxycarbonyl-5-aminopentyl 2-O-benzyl-4,6-O-di-tert-butylsilenediyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-glucopyranoside (8b). β-Pinene (71 µL, 0.45 mmol) and DDQ (51 mg, 0.22 mmol) were added to a stirring solution of compound 15b (2.1 g, 1.4 mmol) in DCM/H₂O (9/1 mL). The reaction mixture was kept in darkness and stirred overnight. The mixture was diluted with DCM, washed with sat. aq. NaHCO₃ (2 x), dried (Na₂SO₄), filtered and concentrated in vacuo. Purification by silica column chromatography using Toluene:EtOAc (1:0 to 10:1.2 v/v) as the eluent provided compound 8b (105 mg, 59 %).

1H NMR (400 MHz, CDCl₃) δ 7.42 – 7.09 (45H, m, Ar-H), 5.15 (2H, d, J = 7.0 Hz, CH₂), 5.05 (1H, d, J = 11.6 Hz, H-1, Gal-III), 4.89 – 4.80 (1H, m, C-H), 4.80 – 4.26 (16H, m, H-1, Gal-II; H-1, Glc-I), 4.23 (1H, d, J = 2.8 Hz, H-4, Gal-III), 4.09 – 3.68 (10H, m, H-3, Gal-II; H-2, Glc-I), 3.65 (1H, dd, J = 9.9, 3.2 Hz, H-2, Gal-III), 3.61 – 3.09 (10H, m, H-2, Gal-II; H-2, Glc-I), 2.39 – 2.31 (1H, m, OH), 1.78 – 1.42 (4H, m, 2x CH₂, pentyl), 1.38 – 1.18 (2H, m, CH₂, pentyl), 0.97 (9H, s, t-Bu), 0.90 (9H, s, t-Bu).

13C NMR (101 MHz, CDCl₃) δ 139.4, 138.6, 138.4, 138.4, 138.2, 128.5, 128.4, 128.3, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.5, 127.4, 127.3, 127.1, 103.5 (C-1, Gal-I), 103.0 (C-1, Gal-II), 99.4 (C-1, Gal-III), 82.6, 81.7 (C-2, Glc-I), 81.0, 78.9 (C-2, Gal-II), 77.2, 75.5 (C-2, Gal-III), 75.1, 75.0, 74.9, 73.9 (C-4, Gal-III), 73.2, 73.1, 73.1, 73.0, 72.2, 70.1 (C-3, Gal-III), 68.4, 67.5, 67.1, 66.7, 29.4, 27.5 (CH₃, t-Bu), 27.2 (CH₃, t-Bu), 23.4, 23.2 (C, t-Bu), 20.6 (C, t-Bu). [α]₂⁵ = -53° (C = 0.01; CHCl₃).

Table S1. Glycosylation conditions of trisaccharide acceptor 8a and disaccharide donor 7a or 7b to afford protected Gb5 (16a, 16b).

<table>
<thead>
<tr>
<th>Donor</th>
<th>Activator</th>
<th>Temperature</th>
<th>Total product</th>
<th>Isolated β-product*</th>
</tr>
</thead>
<tbody>
<tr>
<td>8a</td>
<td>TMSOTf (0.2eq)</td>
<td>-30°C</td>
<td>56-59%</td>
<td>37-42%</td>
</tr>
<tr>
<td>8a</td>
<td>TMSOTf (0.2-0.3eq)</td>
<td>-60°C</td>
<td>72-76%</td>
<td>42-45%</td>
</tr>
<tr>
<td>8a</td>
<td>TfOH (0.1eq)</td>
<td>-10°C</td>
<td>32%</td>
<td>32%</td>
</tr>
<tr>
<td>8a</td>
<td>TfOH (0.1eq)</td>
<td>-50°C</td>
<td>43%</td>
<td>24-28%</td>
</tr>
<tr>
<td>8b</td>
<td>TfOH (0.1eq)</td>
<td>-10°C</td>
<td>19-44%</td>
<td>19-44%</td>
</tr>
<tr>
<td>8b</td>
<td>TfOH (0.1eq)</td>
<td>-50°C</td>
<td>52%</td>
<td>52%</td>
</tr>
</tbody>
</table>

The highest overall yield of the glycosylation was obtained at the coldest activation temperature. However, changes in temperature or activator did not improve β/α selectivities when donor 8a was used. The
glycosylation proved most successful with donor 7b and acceptor 8a in DCM with molecular sieves at -50 °C, since no α-product was formed.

Para-methoxyphenyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-4,6-O-benzylidene-2-[(2,2,2-trichloromethoxy)carbonyl]amino]-β-D-galactopyranosyl-(1→3)-2-O-benzyl-4,6-O-di-tert-butylsilanediyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (16a). A mixture of acceptor 8a (987 mg, 0.71 mmol), donor 7a (845 mg, 0.92 mmol) and 4 Å molecular sieves was stirred in DCM (9 mL) for 1 h. The mixture was cooled to -35°C and TMSOTf (26 µL, 0.14 mmol) was added. After 5 min the reaction was quenched by addition of Et3N. The mixture was filtered over Celite and concentrated in vacuo. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 8:2 v/v) as the eluent to give compound 16a as an oil. The β-anomer of the title pentasaccharide (562 mg, 37 %), (total isolated α/β yield: 59 %). 

1H NMR (400 MHz, CDCl3) δ 7.58 – 7.09 (40H, m, H-Ar), 7.00 (2H, d, J = 8.8 Hz, OMP), 6.78 (2H, d, J = 8.8 Hz, OMP), 5.47 (1H, s, CH-C6H5), 5.36 (1H, s, 5.16 (1H, dd), 5.09 – 4.94 (3H, m), 4.93 – 3.95 (32H, m, 5x H-1), 3.95 – 3.70 (10H, m), 3.70 – 3.55 (3H, m), 3.53 – 3.42 (2H, m), 3.37 – 3.27 (2H, m), 2.90 (1H, s), 2.14 (3H, s, OAc), 2.06 (3H, s, OAc), 2.01 (3H, s, OAc). 1.95 (3H, s, OAc). 1.03 – 0.85 (18H, m, 2x t-Bu). 13C NMR (101 MHz, CDCl3) δ 170.3 (C, OAc), 170.1 (C, OAc), 169.4 (C, OAc), 155.2 (C, OMP), 153.7 (C=O, Troc), 151.5 (C, OMP), 139.4 (C, Ar), 138.5 (C, Ar), 138.4 (C, Ar), 138.3 (C, Ar), 138.1 (C, Ar), 138.0 (C, Ar), 137.9 (C, Ar), 129.0, 129.0, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.7, 127.6, 127.5, 127.5, 127.4, 126.5, 125.3, 118.4 (2x CH, OMP), 114.5 (2x CH, OMP), 103.1 (C-1, Gal-II), 102.7 (C-1, Glc-I), 101.5 (C-1, Gal-V), 101.4 (C-1, GalNAc-IV), 100.8 (CH-C6H5), 99.9 (C-1, Gal-III), 95.3 (CCLS), 81.6, 81.2, 79.3, 78.9, 77.2, 75.9, 75.5, 75.3, 75.1, 74.9, 74.3, 74.2, 73.8, 73.5, 73.3, 73.1, 73.0, 72.1, 70.8, 68.9, 68.7, 68.4, 67.8, 67.5, 67.0, 66.0, 61.4, 55.6 (CH3, OMP), 53.7, 27.5 (CH3, t-Bu). 27.4 (CH3, t-Bu). 23.30, 20.8 (CH3, OAc), 20.7 (CH3, OAc), 20.7 (CH3, OAc). 20.5 (CH3, OAc). ESI HRMS (m/z): [M + Na]+ calcd for C112H130Cl3NO32Si 2156.7309; 2156.7454 found. [α]25^205 = 55.0° (C = 0.05; CHCl3).

N-(Benzyloxy)carbonyl-5-aminopentyl 2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→3)-4,6-O-benzylidene-2-[(2,2,2-trichloromethoxy)carbonyl]amino]-β-D-galactopyranosyl-(1→3)-2-O-benzyl-4,6-O-di-tert-butylsilanediyl-α-D-galactopyranosyl-(1→4)-2,3,6-tri-O-benzyl-β-D-galactopyranoside (16c). A mixture of acceptor 8b (100 mg, 0.063 mmol), donor 7a (75 mg, 0.082 mmol) and 4 Å molecular sieves was stirred in DCM (9 mL) for 1 h. The mixture was cooled to -30°C and TMSOTf (2 µL, 0.013 mmol) was added. After 20 min the reaction was quenched by addition of Et3N. The mixture was filtered over Celite and concentrated in vacuo. The obtained residue was purified by silica gel chromatography using Toluene:EtOAc (1:0 to 8:2 v/v) as the eluent to give compound 16c as an oil. The β-anomer of the title pentasaccharide (62 mg, 42 %). 1H NMR (400 MHz, CDCl3) δ 7.61 – 6.95 (50H, m, Ar-H), 5.47 (1H, s, CH-C6H5), 5.37 (1H, d, J = 3.1 Hz), 5.20 – 5.11 (3H, m), 5.00 (1H, d, J = 11.2 Hz), 4.94 – 4.41 (19H, m, H-1, Gal-
ll steps were monitored by TLC and (Na\textsubscript{2}, GalN\textsubscript{2}C\textsubscript{I}), 1, Gal\textsubscript{1} (0.5H, t, J = 4.50 (1H, d, B) with UV detection (210 nm) affords analytically pure glycan. Bio-Gel P-2 size exclusion chromatography to give the title compound as a white amorphous solid (131 mg, 70 %, over three steps) Additional purification by HPLC with a semi-preparative HILIC column (XBridge\textsuperscript{®} Amide 5 μm, 4.6 mm x 250 mm column, Waters) under isocratic conditions (74% B) with UV detection (210 nm) affords analytically pure glycan. \textsuperscript{1}H NMR (400 MHz, D\textsubscript{2}O) δ 5.21 (0.5H, d, J = 3.7 Hz, H-1α, Glc-I), 4.90 (1H, d, J = 3.8 Hz, H-1, Gal-III), 4.70 – 4.61 (1.5H, m, H-1, GalNAc-IV; H-1β Glc-I), 4.50 (1H, d, J = 7.7 Hz, H-1, Gal-II), 4.45 (1H, d, J = 7.7 Hz, H-1, Gal-V), 4.38 (1H, t, J = 6.3 Hz, H-5, Gal-III), 4.24 (1H, d, J = 2.3 Hz, H-4, Gal-III), 4.17 (1H, d, J = 2.9 Hz, H-4, GalNAc-IV), 4.10 – 4.01 (2H, m, H-2, GalNAc-IV; H-4, Gal-II), 3.99 – 3.54 (23H, m, H-2, Gal-III; H-2, Gal-II), 3.50 (1H, dd, J = 9.8, 7.8 Hz, H-2, Gal-V), 3.27 (0.5H, t, J = 8.4 Hz, H-2, Gal-I), 2.02 (3H, s, NHAc). \textsuperscript{13}C NMR (101 MHz, D\textsubscript{2}O) δ 175.0 (C-1, Gal-I), 79.5, 78.6, 78.6, 77.1, 75.4, 74.8, 74.5, 74.4, 73.8 (C-2, Glc-I), 72.4, 72.0, 71.4, 71.1 (C-2, Gal-III), 70.81 (C-2, Gal-II), 70.51 (C-2, Gal-V), 70.2, 70.1, 68.9, 68.5, 67.9, 67.5, 60.9, 60.9, 60.3, 60.3, 60.3, 51.4 (C-2, GalNAc-IV), 22.2 (CH\textsubscript{3}, NHAc). ESI HRMS (m/z): [M + Na]\textsuperscript{+} calcld for C\textsubscript{32}H\textsubscript{55}NO\textsubscript{26}, 892.2910; found 892.2912.
5-aminopentyl β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4b). Pentasaccharide 16c was deprotected in a total of 5 steps, all steps were the same as for Gb5-OMP, without the CAN reaction. (4.57 mg, 19 %, over 5 steps). 1H NMR (400 MHz, D2O) δ 4.89 (1H, d, J = 3.5 Hz, H-1, Gal-III), 4.67 (1H, d, J = 8.5 Hz, H-1, GalNAc-IV), 4.54 – 4.40 (3H, m, H-1, Gal-III; H-1, Glc-I; H-1, Gal-V), 4.37 (1H, t, J = 6.3 Hz, H-5, Gal-III), 4.23 (1H, s, H-4, Gal-III) 4.16 (1H, d, J = 2.4 Hz, H-4, GalNAc-IV), 4.10 – 3.46 (28H, m), 3.28 (1H, t, J = 8.2 Hz, H-2, Glc-I), 3.03 – 2.94 (2H, m, CH2, penty), 2.01 (3H, s, NHAc), 1.76 – 1.58 (4H, m, 2x CH2, penty), 1.50 – 1.38 (2H, m, CH2 pentyl). 13C NMR (101 MHz, D2O) δ 175.0 (C=O, NHAc), 104.7 (C-1, Gal-V), 103.2 (C-1, Gal-II), 102.8 (C-1, GalNAc-IV), 101.9 (C-1, Glc-I), 100.3 (C-1, Gal-III), 79.5, 78.7, 78.6, 77.1, 75.3, 74.9, 74.7, 74.5, 74.4, 72.8 (C-2, Glc-I), 72.3, 72.0, 70.8 (C-2, Gal-II), 70.5 (C-2, Gal-I), 70.2, 70.0, 68.8 (C-2, Gal-III), 68.5, 67.9, 67.5, 60.9, 60.8, 60.2, 60.2, 59.9, 51.4 (C-2, GalNAc-IV), 39.2 (CH3), 28.1 (CH3), 26.3 (CH2), 22.2 (CH3, NHAc), 22.0 (CH2). ESI HRMS (m/z): [M + H]+ calcd for C37H60N2O26, 955.3977; found 955.3979.

2. Enzymatic synthesis

2.1 Human glycosyl transferase expression

The catalytic domains of human glycosyl transferases (see Table S2 below) were expressed as soluble, secreted fusion proteins by transient transfection of HEK293 suspension cultures. The coding regions were amplified from Mammalian Gene Collection clones using primers that appended a tobacco etch virus protease cleavage site to the NH2-terminal end of the coding region and attL1 and attL2 Gateway adaptor sites to the 5′ and 3′ terminal ends of the amplimer products. The amplimers were recombined via BP clonase reaction into the pDONR221 vector and the DNA sequences were confirmed. The pDONR221 clone was then recombined via LR clonase reaction into a custom Gateway adapted version of the pGen2 mammalian expression vector to assemble a recombinant coding region comprised of a 25 amino acid NH2-terminal signal sequence from the T. cruzi lysosomal α-mannosidase followed by an 8xHis tag, 17 amino acid AviTag, “superfolder” GFP, the nine amino acid sequence encoded by attB1 recombination site, followed by the TEV protease cleavage site and the respective glycosyltransferase catalytic domain coding region.

Suspension culture HEK293 cells (Freestyle 293-F cells, Life Technologies, Grand Island, NY) were transfected as previously described and the culture supernatant was subjected to Ni-NTA superflow chromatography (Qiagen, Valencia, CA). Enzyme preparations eluted with 300 mM imidazole were concentrated to ~1 mg mL−1 using an ultrafiltration pressure cell membrane (Millipore, Billerica, MA) with a 10 kDa molecular weight cutoff.

Table S2. Enzyme expression details.

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<th>Enzyme</th>
<th>Amino Acid Residues</th>
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<td>ST3GAL1</td>
<td>52 - 340</td>
<td>Q11201</td>
</tr>
<tr>
<td>ST6GALNAC5</td>
<td>50 - 336</td>
<td>Q9BVH7</td>
</tr>
<tr>
<td>ST6GALNAC6</td>
<td>31 - 333</td>
<td>Q969X2</td>
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</table>
2.2 Experimental procedures for enzymatic synthesis

αNeu5Ac-(2→3)-β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-α/β-D-glucopyranose (5a).

ST3Gal1 and CIAP were added to compound 4a (6.5 mg, 10 mM final concentration) in H2O with CMP-Neu5Ac (15 mM), MgCl2 (20 mM), sodium cacodylate buffer (50 mM, pH 7.5). The mixture was shaken at 37°C for 94 h and monitored by TLC (EA:MeOH:H2O:HOAc 4:3:2:1). More enzymes were added until no more starting material could be observed. Purification by Bio-Gel P-2 size exclusion chromatography and semi-preparative HILIC column (XBridge® Amide 5 μm, 4.6 mm x 250 mm column, Waters, 70% B isocratic) provided compound 5a (4.57 mg, 53%). 1H NMR (750 MHz, D2O) δ 5.24 (0.5H, d, J = 3.7 Hz, H-1α, Glc-I), 4.93 (1H, d, J = 3.9 Hz, H-1, Gal-III), 4.70 (1H, d, J = 8.5 Hz, H-1, GalNAc-IV), 4.68 (0.5H, d, J = 8.0 Hz, H-1β, Glc-I), 4.55 – 4.51 (2H, m, H-1, Gal-V; H-1, Gal-II), 4.41 – 4.37 (1H, m, H-5, Gal-III), 4.26 (1H, s, H-4, Gal-III), 4.19 (1H, d, J = 3.0 Hz, H-4, GalNAc-IV), 4.08 (2H, dd, J = 9.9, 3.2 Hz, H-2, GalNAc-IV; H-3, Gal-V), 4.05 (1H, d, J = 2.9 Hz, H-4, Gal-II), 4.01 – 3.57 (29.5H, m), 3.55 (1H, dd, H-2, Gal-V), 3.29 (0.5H, t, J = 8.6 Hz, H-2, Glc-I), 2.76 (1H, dd, J = 12.4, 4.6 Hz, H-3eq, Neu5Ac-VI), 2.04 (6H, s, 2x NHAc), 1.79 (1H, t, J = 12.1 Hz, H-3ax, Neu5Ac-VI). ESI HRMS (m/z): [M + Na]+ calcd for C53H72N2O34, 1183.3864; found 1183.3861.

5-amino-pentyl αNeu5Ac-(2→3)-β-D-Galactopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-β-D-glucopyranose (5b).

ST3Gal1 and CIAP were added to compound 4b (2.0 mg, 10 mM final concentration) in H2O with CMP-Neu5Ac (15 mM), MgCl2 (20 mM), sodium cacodylate buffer (50 mM, pH 7.5). The mixture was shaken at 37°C for 24 h and monitored by TLC (EA:MeOH:H2O:HOAc 4:3:3:3). Purification by Bio-Gel P-2 size exclusion chromatography provided compound 5b (1.8 mg, 69%). 1H NMR (600 MHz, D2O) δ 4.92 (1H, d, J = 3.9 Hz, H-1, Gal-III), 4.71 (1H, d, J = 8.5 Hz, H-1, GalNAc-IV), 4.56 – 4.49 (3H, m, H-1 Gal-V; H-1, Gal-II; H-1, Glc-I), 4.39 (1H, t, J = 6.4 Hz, H-5, Gal-III), 4.26 (1H, d, J = 2.4 Hz, H-4, Gal-III), 4.19 (1H, d, J = 2.8 Hz, H-4, GalNAc-IV), 4.13 – 3.48 (34H, m), 3.36 – 3.27 (1H, m, H-2, Glc-I), 3.02 (2H, t, J = 7.5 Hz, CH2), 2.76 (2H, dd, J = 12.4, 4.6 Hz, H-3eq, Neu5Ac-VI), 2.04 (6H, s, 2x NHAc), 1.79 (1H, t, J = 12.1 Hz, H-3ax, Neu5Ac-VI), 1.75 – 1.65 (4H, m, 2x CH2, pentyl), 1.52 – 1.43 (2H, m, CH2, pentyl). ESI HRMS (m/z): [M + H]+ calcd for C46H63N3O34, 1246.4858; found 1246.4970.

αNeu5Ac-(2→3)-β-D-Galactopyranosyl-(1→3)-[αNeu5Ac-(2→6)]-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-galactopyranosyl-(1→4)-α/β-D-glucopyranose (6a).

ST6GalNAC5 and CIAP were added to a mixture of compound 5a (4.2 mg, 10 mM final concentration) in H2O with CMP-Neu5Ac (15 mM), MgCl2 (20 mM), sodium cacodylate buffer (50 mM, pH 7.5). The mixture was shaken at 37°C overnight and monitored by TLC (EA:MeOH:H2O:HOAc 3:3:3:2). More enzymes were added until no more starting material could be observed. Purification by Bio-Gel
P-2 size exclusion chromatography and semi-preparative HILIC column (XBridge® Amide 5 µm, 4.6 mm x 250 mm column, Waters, 70% B isocratic) provided compound 6a (2.64 mg, 50%). 1H NMR (750 MHz, D$_2$O) δ 5.24 (0.5H, d, J = 3.7 Hz, H-1α, Glc-I), 4.93 (1H, d, J = 3.8 Hz, H-1, Gal-III), 4.70 – 4.65 (1.5H, m, H-1β, Glc-I; H-1, GalNAc-IV), 4.54 – 4.50 (2H, m, H-1, Gal-II, H-1, Gal-V), 4.43 – 4.39 (1H, m, H-5, Gal-III), 4.28 (1H, s, H-4, Gal-III), 4.20 (1H, d, J = 3.0 Hz, H-4, GalNAc-IV), 4.10 – 4.04 (3H, m, H-2, GalNAc-IV; H-3, Gal-V), 4.02 – 3.57 (36.5H, m), 3.55 (1H, dd, J = 9.6, 8.1 Hz, H-2, Gal-V), 3.29 (0.5H, dd, J = 9.0, 8.2 Hz, H-2, Glc-I), 2.79 – 2.70 (2H, m, H-3eq, Neu5Ac-VI; H-3eq, Neu5Ac-VII), 2.07 – 2.01 (9H, m, 3x NHAc), 1.80 (1H, t, J = 12.2 Hz, H-3ax, Neu5Ac-VI), 1.66 (1H, t, J = 12.2 Hz, H-3ax, Neu5Ac-VI). ESI HRMS (m/z): [M + Na]$^+$ calcd for C$_{54}$H$_{88}$N$_3$O$_{42}$, 1474.4818; found 1474.4807.

5-aminopentyl αNeu5Ac-(2→3)-β-D-Galactopyranosyl-(1→3)-[ αNeu5Ac-(2→6)]-2-acetamido-2-deoxy-β-D-galactopyranosyl-(1→3)-α-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (6b). ST6GalNAc5 and CIAP were added to a mixture of compound 5b (1.0 mg, 10 mM final concentration) in H$_2$O with CMP-Neu5Ac (15 mM), MgCl$_2$ (20 mM), sodium cacodylate buffer (50 mM, pH 7.5). The mixture was shaken at 37°C for 4 h and monitored by TLC (EA:MeOH:H$_2$O:HOAc 3:3:3:2). Purification by Bio-Gel P-2 size exclusion chromatography provided compound 6b (0.7 mg, 57%). 1H NMR (600 MHz, D$_2$O) δ 4.92 (1H, d, J = 4.0 Hz, H-1, Gal-III), 4.69 (1H, d, J = 8.5 Hz, H-1, GalNAc-IV), 4.55 – 4.49 (3H, m, H-1, Gal-II; H-1, Gal-V; H-1, Glc-I), 4.40 (1H, t, J = 6.5 Hz, H-5, Gal-III), 4.28 (1H, d, J = 2.7 Hz, H-4, Gal-III), 4.20 (1H, d, J = 3.0 Hz, H-4, GalNAc-IV), 4.11 – 4.04 (3H, m, H-2, GalNAc-IV; H-3, Gal-V, H-4, Gal-II), 4.03 – 3.53 (39H, m), 3.31 (1H, t, J = 8.5 Hz, H-2, Glc-I), 3.06 – 2.99 (2H, m, CH$_2$, pentyl), 2.80 – 2.70 (2H, m, H-3eq, Neu5Ac-VI; H-3eq, Neu5Ac-VII), 2.07 – 2.00 (9H, m, 3x NHAc), 1.79 (1H, t, J = 12.1 Hz, H-3ax, Neu5Ac-VI), 1.75 – 1.63 (5H, m, 2x CH$_2$, pentyl; H-3ax, Neu5Ac-VII ), 1.51 – 1.43 (2H, m, CH$_2$, pentyl). ESI HRMS (m/z): [M + H]$^+$ calcd for C$_{59}$H$_{100}$N$_3$O$_{42}$, 1537.5812; found 1537.5874.

3. Microarray
3.1 Experimental procedures

The synthetic glycans (100 µM in sodium phosphate (250 mM), pH 8.5 buffer) were printed on activated glass slides (Nexterion Slide H, Schott Inc) by piezoelectric non-contact printing (sciFLEXARRAYER S3, Scienion Inc) with a drop volume of ~400 pl and 1 drop per spot at 50% relative humidity. The compounds were printed as replicates of 6 with on each slide 24 subarrays (3x8). The slides were incubation overnight in a saturated NaCl chamber (providing a 75% relative humidity environment), after which the remaining activated esters were quenched with ethanolamine (50 mM) in TRIS (100 mM), pH 9.0. Slides were rinsed with DI water, dried by centrifugation, and stored in a desiccator at RT.

Sub-arrays were incubated with biotinylated lectins (Maackia amurensis leukagglutinin (MAL-II), Soybean agglutinin (SBA) and Wheat Germ agglutinin (WGA); from Vector Labs) at 10 µg/mL premixed with Streptavidin-AlexaFluor635 (5 µg/mL; ThermoFisher Scientific, S32364) in TSM binding buffer (20 mM Tris Cl, pH 7.4, 150 mM NaCl, 2 mM CaCl$_2$, 2 mM MgCl$_2$, 0.05% Tween, 1% BSA) for 1 h followed by washing. Wash steps involved 4 successive washes with each 5 min soak time with 1) TSM wash buffer (20 mM Tris
Cl, pH 7.4, 150 mM NaCl, 2 mM CaCl$_2$, 2 mM MgCl$_2$, 0.05% Tween-20); 2) TSM buffer (20 mM Tris Cl, pH 7.4, 150 mM NaCl, 2 mM CaCl$_2$, 2 mM MgCl$_2$); 3) deionized H$_2$O; and 4) deionized H$_2$O.

Biotin-conjugated ganglioside GM1 polyclonal antibody (2 µg/mL; Bioss, bs-2367R-Biotin) in TSM binding buffer was incubated for 1 h followed by washing as described above. Next the subarray was incubated with Streptavidin-AlexaFluor635 (5 µg/mL) for 1 h followed by washing.

Using the same buffers as above, recombinant human Siglec-7 comp (a gift from Dr. R.L. Schnaar, Johns Hopkins University School of Medicine, Baltimore, MD, USA) was assayed at 50 µg/mL premixed with 6x-His Tag monoclonal antibody-AlexaFluor647 (5 µg/mL; ThermoFisher Scientific MA1-135-A647) with an incubation for 2 h.

All incubation and wash steps were performed at RT. Washed arrays were dried by centrifugation and immediately scanned for fluorescence on a GenePix 4000 B microarray scanner (Molecular Devices) using a detection gain adjusted to avoid saturation of the signal. The data were processed with GenePix Pro 7 software and further analyzed using our home written Microsoft Excel macro. The lowest and highest value of the 6 replicates were excluded, after which the mean fluorescence intensities (corrected for mean background) and standard deviations (SD) were calculated (n=4). Data were fitted using Prism software (GraphPad Software, Inc).

### 3.2 Results and discussion printing controls

The printing of the synthetic compounds was validated by the plant lectins MAL II, SBA and WGA and a GM1 antibody.

MAL-II binds the terminal trisaccharide sequence Neu5Ac(α2-3)Gal(β1–4)GlcNAc/Glc.$^{12}$ Compounds 17 and 20 (Neu5Ac(α2-8)-Neu5Ac(α2-3)Gal(β1–4)Glc) have three terminal intact sugars, and as expected binds to MAL II. Compounds 5b and 6b, with the Neu5Ac(α2-3)Gal-β1,3-GalNAc epitope at the terminal end, are not recognized by MAL II. Similarly, GT1b (21) with the same terminal epitope as 5b and 6b also did not show binding to MAL II.

SBA preferentially binds GalNAc, and also recognizes Gal residues although at much lower affinity.$^{13}$ Binding to SBA was observed for compounds with either a GalNAc or Gal at the terminal residue: 18 (GM2; with GalNAc at the terminal residue) and 4b (Gb5; with Gal at terminal residue). As expected sialylated compounds 5b and 6b (sialylated Gb5; no Gal at the terminal residue) didn’t show any binding. Also compound 19 (GM1a; with Gal at the terminal residue) didn’t show any binding, due to the inhibition effect of Neu5Ac.

WGA preferentially binds GlcNAc moieties, and also interacts with some glycoproteins via terminal sialic acid residues. Indeed the terminal sialylated compound 21 (GT1b) and sialylated Gb5 (5b and 6b) showed binding, while the non-terminal sialylated compounds 18 (GM2), 19 (GM1a) and 4b (Gb5) did not bind. Compound 20 (GD3; with terminal α2,8-Neu5Ac-a2,3-Neu5Ac) also did not bind to WGA, apparently WGA does not recognize this sialylated epitope.

As expected only GM1a (19) showed binding to the GM1 antibody.
4. References

The image contains an NMR spectrum with a 2D representation of the proton-proton correlation spectrum (2D-COSY) for compound 12b. The spectrum shows various peaks at different ppm values along the f1 and f2 axes, indicating the chemical shifts of protons in the compound. The structure of 12b is also depicted with benzoate (BnO) and other functional groups, suggesting the presence of multiple chemical environments within the molecule.
15a