Supporting information for:

Sialoside anolog arrays for rapid identification of high affinity siglec ligands.

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I. Chemo-enzymatic synthesis of sialoside analog library:

General

Glycosyltransferases were produced as previously reported[1, 2]. Neu5Ac-aldolase (Nal-311) was from Toyobo. Co Ltd, Osaka, Japan. Oligosaccharide substrates and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO). Chinese hamster ovary cell lines producing Siglec-Fc chimeras were obtained from Dr. Paul Crocker and produced as described previously[2]. CMP-9-azido-9-deoxy-Neu5Ac was prepared as previously described[3]. Synthetic reactions were monitored by thin layer chromatography (TLC) performed on Silica Gel 60F pre-coated TLC plates (EMD Chemicals Inc., Gibbstown, New Jersey, USA). Compounds were visualized by UV light (for nucleotides) and/or dipping in 5% sulfuric acid in ethanol, followed by charring. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker DRX-600 MHz instruments at 25 °C and were referenced to acetone δ 2.225 (^{1}H in D_2O) and δ 29.9 (^{13}C in D_2O). (MS) ESI-TOF high-accuracy mass spectrometry (MS) spectra was recorded with an LC MSD TOF (Agilent Technologies, Foster City, California, USA) or a MALDI-TOF (Applied Biosystems, DE) using dihydroxybenzoic acid as matrix. Water was purified by NanoPure Infinity Ultrapure water system (Barnstead/Thermolyne, Dubuque, Iowa, USA) and degassed by vacuum treatment before use.

Synthesis of 4-Pentenyloxyaminoethyl-β-D-2-acetamido-2-deoxy--O-D-galactopyranosyl-β-(1-4)-O-D-glucopyranoside (A)

2-Azidoethyl-*N*-acetyllactoside [1] (2.0g, 3.9 mmol) was first dissolved in MeOH (20 mL) and hydrogenated with Pd/C (10%) and hydrogen (H_2). After removal of the catalyst diisopropylamine (3.9 g, 39 mmol, 10 eq) and 4-pentenyl anhydride (3.5 g, 19.5 mmol, 5 eq) dissolved in methanol (10 mL) was added to the amine. When TLC indicated complete reaction (ethylacetate:acetic acid:methanol:water, 6:3:3:2 by volume) the mixture was concentrated by evaporation and further purified by size exclusion chromatography (Sephadex-G15, 5 x 160 cm, equilibrated with 5% nBuOH). Appropriate fractions were collected and lyophilized to give 3.12 g, 3.5 mmol, 89% yield. Selected ^{1}H NMR (D_2O, 600 MHz) δ 5.87 (m, 1H), 5.15 (d, 1H, J = 11.4 Hz), 5.07 (d, 1H, J = 10.8 Hz), 4.56 (d, 1H, J = 8.0 Hz), 4.50 (d, 1H, J = 8.0 Hz), 4.02 (dd, 1H, J =12.4, 2.0 Hz), 3.96 - 3.91 (m, 2H), 3.88 (dd, 1H, J = 12.5 5.0 Hz), 3.81 – 3.69 (m, 8H), 3.61 (m, 1H), 3.57 (dd, 1H, J = 12.4, 8.0Hz), 3.40 (m, 1H), 2.40 (s, 4H), 2.02 (s, 3H). HRMS expected: *m/z* = 509.2341 (M+H), found: *m/z* = 509.2346.

Synthesis of 4-Pentenyloxyaminoethyl β-5-acetamido-9-azide-3,5,9-trideoxy-D-glycero-D-galacto-2-nonulo-pyranosyl-onate-α-(2-3)-O-D-galactopyranosyl-β-(1-4)-D-2-acetamido-2-deoxy-glucopyranoside (C)

Routine preparation of compound **C** was produced as follows. The disaccharide acceptor **A** (1 eq.) and donor analog CMP-9-azido-9-deoxy-Neu5Ac (2-3 eq.) were dissolved in Tris-HCl buffer (100mM, pH 7.0, 40 mL/mmol acceptor) containing $MnCl_2$ (20mM). The ST3Gal-CMPNeu5Ac synthetase-fusion protein (20 U/ mmol was added and the reaction was allowed to proceed at 37°C. The pH was carefully monitored and adjusted (to pH 6.5-7.0) as needed. After 14 hrs the product was purified by size exclusion chromatography (Sephadex G15) and isolated in 60-70% with a purity > 90%. Selected ^{1}H NMR (D_2O, 600 MHz) δ 5.89 (m, 1H), 5.13 (d, 1H, J = 11.4 Hz), 5.07 (d, 1H, J = 10.8 Hz), 4.57 (d, 1H, J = 7.2 Hz), 4.56 (d, 1H, J = 7.8 Hz), 4.16 - 4.12 (m, 2H), 4.06 4.03 (m, 2H), 3.98 -3.92 (m, 3H), 3.89 - 3.66 (m, 14H), 3.65 – 3.58 (m, 3 H0, 3.54 – 3.51

(dd, 1H, J = 11.2, 7.8 Hz), 2.79 (dd, 1H, J = 4.8, 12.6 Hz), 2.83 (s, 4H), 2.07 (s, 6H), 1.82 (dd, 1H, J = 3.0, 12.0 Hz). Selected ^{13}C NMR (D_2O, 175 MHz) 177.08, 175.79, 175.74, 175.34, 174.64, 137.75, 116.43, 103.41, 101.90, 100.66, 79.10, 76.37, 76.00, 75.60, 73.58, 73.53, 72.21, 71.46, , 70.21, , 69.38, 69.16, 68.24, 63.30, 61.85, 55.87, 53.90, 49.68, 40.53, 35.83, 30.22, 23.03, 22.87. HRMS expected: *m/z* = 8254.3287 (M+H), found: *m/z* = 825.3350.

General procedures for library of 9-N-acyl-substituted analogs of the α2-3 sialoside E.
For the initial screens on sialoside analog glycan arrays, 9-N-acyl substituted sialic acid analogs **E** were made as follows. 9-Azido-9-deoxy sialoside pentenyl protected **C**, (0.17 mmol) was dissolved in methanol/water (3mL, 9:1) and a solution of triphenylphosphine (177 mg, 0.67 mmol) in THF/water (3.25 mL, 1:1). The solution was stirred at room temperature for 16 h and then the solvent was removed. The residue was suspended in water (10 mL) and extracted with ethyl acetate (3x30 mL). The aqueous solution was then lyophylized to afford 9-amino-9-deoxy substituted analog of C (0.16 mmol). This compound was then taken up in methanol (2ml) containing triethylamine (36 uL) and portioned into 20 smaller reactions (5 mg each) and the selected acyl chloride (3-5 eqv. of compounds **1**-**4**, **7**-**16** and **18** was added until the reaction was complete as monitored by TLC (ethylacetate : acetic acid : methanol : water = 10 : 3 : 3 :3 by vol.). Water (100 uL) was added to quench excess acyl chloide and the mixture was left at oom temperatur for 18 hrs. Iodine (10 eqv.) dissoved in methanol (100 uL) and water (50 uL) were added and the reaction mixture was stirred at room temperature for 1 hr. After TLC showed a full conversion to the desired product, the solvent was then removed under reduced pressure and the residue was re-suspended into printing buffer and used for microarray printing without further purification.

Procedures for re-synthesis of 9-N-acyl-substituted sialoside analogs (E)
Re-synthesis **7E**, **8E**, and **10E** was accomplished essentially as described above. After acylation was complete, excess sodium thiosulfate solution (5%) was added to quench the reaction. The solvent was then removed under reduced pressure and the residue was subjected to size exclusion chromatography in water (Sephadex G15) and isolated in 60-70% yield for each of the 9-substituted sialoside analogs. Characterization data for these compounds are:

2-aminoethyl β-5-acetamido-9-benzoylamino-3,5,9-trideoxy-D-glycero-D-galacto-2-nonulo-pyranosyl-onate-α-(2-3)-O-D-galactopyranosyl-β-(1-4)-O-D-2-acetamido-2-deoxy-glucopyranoside (7E) Selected ^{1}H NMR (D_2O, 600 MHz) δ 7.69 (d, 2H, J = 7.8 Hz), 7.51 (t, 1H, J = 7.8, 7.8 Hz), 7.43 (t, 2H, J = 7.8, 7.8 Hz), 4.42 (d, 1H, J = 8.4 Hz), 4.39 (d, 1H, J = 7.8 Hz), 3.99 (dd, 1H, J = 3.0, 9.6 Hz), 3.93 (m, 2H), 3.83 (br s, 1H), 3.79 – 3.57 (m, 17 H), 3.45 (m, 3H), 3.09 (m, 2H), 2.64 (dd, 1H, J = 4.8, 12.6 Hz), 1.93 (s, 3H), 1.88 (s, 3H), 1.69 (t, 1H, J = 12.0, 12.0 Hz) ^{13}C NMR (D_2O, 175 MHz) 175.80, 175.73, 174.57, 172.08, 134.48, 132.98, 129.62, 127.94, 103.38, 101.78, 100.76, 78.86, 76.47, 76.06, 75.50, 73.67, 73.04, 71.10, 70.55, 70.20, 69.07, 68.30, 66.48, 61.87, 60.65, 55.66, 52.54, 43.53, 40.56, 40.25, 22.99, 22.83. HRMS expected: *m/z* = 821.3304 (M+H), found: *m/z* = 821.3298.

2-aminoethyl β-5-acetamido-9-(2-chloro-benzoylamino)-3,5,9-trideoxy-D-glycero-D-galacto-2-nonulo-pyranosyl-onate-α-(2-3)-O-D-galactopyranosyl-β-(1-4)-O-D-2-acetamido-2-deoxy-glucopyranoside (8E) Selected ^{1}H NMR (D_2O, 600 MHz) δ 7.43 (d, 2H, J = 7.8 Hz), 7.39 (t, 1H, J = 6.6, 7.2 Hz), 7.32 (t, 1H, J = 7.8, 7.8 Hz), 4.41 (d, 2H, J = 8.4 Hz), 3.99 (dd, 1H, J = 3.0, 10.2 Hz), 3.97 – 3.90 (m, 2H), 3.85 (m, 2H), 3.78 (m, 2H), 3.79 – 3.54 (m, 8H), 3.52 (d, 1H, J = 9 Hz), 3.46 (m, 3H),2.98 (m, 3H) 2.65 (dd, 1H, J = 4.2, 12.0 Hz), 1.93 (s, 3H), 1.92 (s, 3H), 1.69 (t, 1H, J = 12.0, 12.0 Hz) ^{13}C NMR (D_2O, 175 MHz) 135.6, 132.5, 130.7, 129.3, 128.1, 103.4, 101.8, 100.6, 79.2, 76.4, 76.2, 76.0, 73.6, 73.0, 70.9, 70.2, 70.2, 68.5, 68.3, 66.5, 65.6, 61.9, 60.8, 57.3, 55.6, 52.6, 43.1, 40.2, 23.0, 22.9 HRMS expected: *m/z* = 855.2914 (M+H), found: *m/z* = 855.2905.

2-aminoethyl β-5-acetamido-9-(4-chloro-benzoylamino)-3,5,9-trideoxy-D-glycero-D-galacto-2-nonulo-pyranosyl-onate-α-(2-3)-O-D-galactopyranosyl-β-(1-4)-O-D-2-acetamido-2-deoxy-glucopyranoside (10E)

Selected ^{1}H NMR (D_2O, 600 MHz) δ 7.62 (d, 2H, J = 8.0 Hz), 7.38 (d, 2H, J = 8.0 Hz), 4.45 (d, 2H, J = 8.4 Hz), 4.38 (d, 1H, J = 7.8 Hz), 3.99 (dd, 1H, J = 3.0, 10.2 Hz), 3.92 (m, 2H), 3.83 (m, 1H), 3.78 (m, 2H), 3.79 – 3.73 (m, 3H), 3.69 (dd, 1H, J = 3.0, 13.8 Hz), 3.67 – 3.55 (m, 9H), 3.43 (m, 4H), 2.99 (q, 1H, J = 7.2, 14.4 Hz), 2.53 (dd, 1H, J = 4.8, 12.6 Hz), 1.93 (s, 3H), 1.89 (s, 3H), 1.69 (t, 1H, J = 12.0, 12.0 Hz) ^{13}C NMR (D_2O, 175 MHz) 175.8, 175.7, 171.0, 138.4, 133.0, 103.4, 101.8, 100.7, 78.8, 76.5, 76.1, 75.5, 73.7, 73.0, 71.1, 70.6, 70.2, 69.0, 68.3, 66.4, 61.9, 60.6, 55.7, 52.5, 47.5, 43.6, 43.1, 40.5, 40.3, 23.0, 22.8. HRMS expected: *m/z* = 855.2914 (M+H), found: *m/z* = 855.2912.

Synthesis of 2-Cbz-aminoethyl-β-O-D-2-acetamido-2-deoxy-glucopyranoside (B)
Aminoethyl-β-D-2-acetamido-2-deoxy-glucopyranoside (2.2 g, 10 mmol)[1] was dissolved in methanol (30ml) containing triethylamine (2ml). To the solution was added Benzyl Chloroformate dropwise until the reaction was complete as monitored by TLC (CH_2Cl_2 : MeOH = 1 : 1). The mixture was evaporated to remove all the solvents and the residue was subjected to a silica gel chromatography eluted with CH_2Cl_2 : MeOH (2:1 to 1:2) to afford a pale yellow solid as the desired product(2.5g, 70% yield). ^{1}H MHR (CD_3OD) δ 7.27 (m, 5H) 5.02 (s, 1H) 4.40 (d, J = 8.4 Hz), 3.84 (m, H), 3.70 (m, 1H) 3.60 (m, 1H), 3.49 (t, J = 10.2 Hz, 1 H), 3.28 (m, 4H), 1.93 (S, 3H). ^{13}C NMR (CD_3OD) δ 173.98, 158.55, 137.90, 129.34, 128.89, 128.70, 102.56, 77.57, 75.66, 71.73, 69.31, 67.34, 62.44, 56.99, 41.67, 23,11, 23.06. HRMS expected: *m/z* = 583.2109 (M+H), found: *m/z* = 583.2100.

Synthesis of 2-Cbz-aminoethyl β-5-acetamido-9-azide-3,5,9-trideoxy-D-glycero-D-galacto-2-nonulo-pyranosyl-onate-α-(2-6)-O-D-galactopyranosyl-β-(1-4)-D-2-acetamido-2-deoxy-glucopyranoside (D)
9-azido-NeuAc (0.3g, 0.9mmol), compound **B** (0.27g, 0.8mmol), UDP-glucose (0.6g, 1mmol) and CTP (0.7g,1.2mmol) were dissolved in Tris-HCl (100 mM, pH 9.5) containing $MgCl_2$ (20 mM),$MnCl_2$ ((20 mM)), *N. meningitidis* ST3Gal-CMP-Neu5Ac synthetase fusion protein (20 U), betaGalT-GalE(40U) and hST6Gal I(4U). The solution was stirred at 37°C and the pH was adjusted with 1 M NaOH to pH 8.3–9.0. After 30 h, the reaction was lypholized and the residue was suspended in MeOH (100mL). The mixture was filtered and the organic solution was evaporated. The residue was purified by size exclusion chromatography (P2, 1 x 90 cm, equilibrated with water) to afford the desired product in 29% yield (0.2g, 0.23 mmol). ^{1}H MHR (CD_3OD) δ 7.23 (d, J= 4.2 Hz, 4 H), 7.20 (m, 1H), 4.42 (d, J = 8.4Hz, 1H), 4.21 (d, J = 7.2 Hz), 3.90-3.40 (m, 10H), 3.19 (m, 4H), 2.85-3.0 (m, 3H), 2.64(dd, J= 4.5, 12.3 Hz), 1.89 (s, 3H), 1.85 (s, 3H), 1.55 (t, J= 14.7Hz, 1H); ^{13}C NMR (CD_3OD) δ 174.96, 174.45, 174.36, 158.73, 138.18, 129.59, 129.49, 128.99, 128.94, 128.83, 105.15, 102.47, 101.46, 81.86, 75.30, 75.53, 74.53, 73.90, 73.75, 72.36, 71.21, 70.18, 69.73, 69.59, 67.45, 61.92, 56.92, 54.79, 53.82, 52.97, 51.50, 44.54, 42.31, 42.28, 41.82, 31.10, 23.29, 22.81. HRMS expected *m/z*=(M+Na) 899.3128, found *m/z*= 899.3113

General procedures for small scale synthesis of 9-N-acyl-substituted analogs of the α2-6 sialoside F
Compound **D** (300 mg) was dissolved in methanol/water (25ml, 10:0.5) containing triphenylphosphine (800mg, mmol). The mixture was stirred until the TLC showed that no starting material was left. The mixture was evaporated to remove the solvents and The residue was suspended in water (10 mL) and extracted with ethyl acetate (3x10 ml). The aqueous solution was then lypholized to afford 9-amino-NeuAcα2-6Galβ1-4GlcNAcβ-O-ethlamine-Cbz in quantitative yield. An aliquot of this compound (15 mg) was then dissolved in methanol (2ml) with triethylamine (200 uL). To the solution was added dropwise selected of one of the acyl chlorides (**1**, **3**, **4**, **6**, **8-11**, **13**, **15**, **18-34**, **see Table 1S)** until the reaction was complete as monitored by TLC ($CHCl_3$: MeOH = 4 : 6). The compound was loaded to a G-25 gel (superfine, column sized 1cm x 90cm) eluted with water to afford the corresponding 9-acylated compound **F** in about 50-65% yield. A portion of each acylated sialoside (8mg) was dissolved in methanol (2 mL) with acetic acid (100 uL) and to the solution was added Pd/C (10 mg) and the reaction mixture was stirred under H_2 atmosphere at room temperature for 2 days. After TLC showed a full conversion to the desired product, the reaction mixture was filtered, concentrated under reduced pressure followed by purification on a size exclusion chromatography (P-2, 0.4cmx40cm) eluted with 5% n-butanol in distilled water to afford the corresponding products in 60-70% yield.

Procedures for re-synthesis of 9-N-acyl-substituted sialoside α2-6 analogs of compound F.
An aliquot of 9-amino-NeuAcα2-6Galβ1-4GlcNAcβ-O-ethlamine-Cbz (20 mg) was dissolved in methanol (2ml) with triethylamine (50 uL) and the selected acyl chloride (**7**, **8**, **10**, or **15**) was added dropwise until the reaction was complete as monitored by TLC ($CHCl_3$: MeOH = 4 : 6). The corresponding product was then purified by fast silica gel column chromatography using the same solvent. Each product was dissolved in methanol (2 mL) with acetic acid (100 uL), Pd/C (10 mg) was added to the solution, and the reaction mixture was stirred under H_2 atmosphere at room temperature for 2 days. After TLC showed a full conversion to the desired product, the reaction mixture was filtered, concentrated under reduced pressure, and purified on a size exclusion chromatography in water (1x 46 cm Sephadex G25) to afford the corresponding products in 60-70% yield. Characterization data for these compounds are:

2-aminoethyl α-5-acetamido-9-benzoylamino-3,5,9-trideoxy-D-glycero-D-galacto-2-nonulo-pyranosyl-onate-(2-6)-[β-D-galactopyranosyl-(1-4)]- β-D-2-acetamido-2-deoxy-glucopyranoside (7F) Selected ^{1}H NMR (D_2O, 600 MHz) δ 7.67 (d, 2H, J = 7.2 Hz), 7.51 (t, 1H, J = 7.2, 7.8 Hz), 7.42 (t, J = 7.8, 7.8 Hz, 2H), 4.47 (d, 1H, J = 7.8 Hz), 4.32 (d, 1H, J = 7.8 Hz), 4.07 (m, 1H), 3.95 (m, 1H), 3.90 – 3.80 (m, 4H), 3.74 – 3.65 (m, 7H), 3.57 – 3.40 (m, 9H), 3.26 (m, 2H), 1.96 (s, 3H), 1.93 (s, 3H), 1.58 (t, 1H, J = 12.6, 12.6 Hz) ^{13}C NMR (D_2O, 175 MHz) 175.7, 134.5, 132.9, 129.6, 127.9, 104.3, 101.4, 101.0, 81.4, 75.2, 74.6, 73.3, 73.1, 73.0, 71.5, 70.9, 70.8, 69.3, 69.0, 64.4, 63.9, 61.0, 57.5, 55.4, 52.7, 40.9, 22.8. HRMS expected: *m/z* =821.3298, found: *m/z* =821.3297.

2-aminoethyl α-5-acetamido-9-(2-chloro-benzoylamino)-3,5,9-trideoxy-D-glycero-D-galacto-2-nonulo-pyranosyl-onate-(2-6)-[β-D-galactopyranosyl-(1-4)]- β-D-2-acetamido-2-deoxy-glucopyranoside (8F) Selected ^{1}H NMR (D_2O, 600 MHz) δ 7.42 – 7.31 (m, 3H), 4.48 (d, 1H, J = 7.8 Hz), 4.32 (d, 1H, J = 7.8 Hz), 3.95 (m, 1H), 3.90 – 3.85 (m, 2H), 3.80 (m, 2H), 3.74 – 3.60 (m, 8H), 3.57 – 3.47 (m, 8H), 3.45 – 3.42 (m, 3H), 3.08 (m, 3H), 2.54 (dd, 1H, J = 4.8, 12.6 Hz, 1H), 1.96 (s, 3H), 1.91 (s, 3H), 1.59 (t, 1H, J = 12.6, 12.6 Hz) ^{13}C NMR (D_2O, 175 MHz) 175.7, 132.4, 130.7, 129.6, 129.2, 128.1, 127.9, 104.3, 101.6, 81.5, 75.2, 74.6, 73.2, 73.1, 72.9, 71.5, 70.7, 70.3, 69.3, 69.0, 66.6, 64.4, 63.3, 61.0, 55.5, 52.8, 48.2, 43.0, 40.9, 40.3, 22.9. HRMS expected: *m/z* =855.2909, found: *m/z* =855.2892.

2-aminoethyl α-5-acetamido-9-(4-chloro-benzoylamino)-3,5,9-trideoxy-D-glycero-D-galacto-2-nonulo-pyranosyl-onate-(2-6)-[β-D-galactopyranosyl-(1-4)]- β-D-2-acetamido-2-deoxy-glucopyranoside (10F) Selected ^{1}H NMR (D_2O, 600 MHz) δ 7.67 (m, 2H), 7.51 (m, 1H), 7.42 (m, 2H), 4.47 (d, 2H, J = 7.8 Hz), 4.38 (d, 1H, J = 7.8 Hz), 3.95 (m, 1H), 3.88 (m, 1H), 3.80 (m, 2H), 3.73 – 3.64 (m, 4H), 3.58 – 3.40 (m, 6H), 3.18 (m, 3H), 2.54 (dd, 1H, J = 4.8, 12.0 Hz), 1.96 (s, 3H), 1.88 (s, 3H), 1.60 (t, 1H, J = 12.6, 12.6 Hz) ^{13}C NMR (D_2O, 175 MHz) 175.8, 175.7, 132.9, 129.7, 127.9, 104.3, 101.6, 101.1, 81.4, 75.2, 74.6, 73.3, 73.1, 71.5, 70.9, 70.7, 70.6, 69.3, 69.0, 66.4, 64.4, 62.0, 61.0, 55.5, 52.7, 47.5, 43.6, 43.5, 40.9, 40.2, 23.1, 22.8. HRMS expected: *m/z* =855.2909, found: *m/z* =855.2903.

2-aminoethyl α-5-acetamido-9-(biphenylcarboxyl)-3,5,9-trideoxy-D-glycero-D-galacto-2-nonulo-pyranosyl-onate-(2-6)-[β-D-galactopyranosyl-(1-4)]- β-D-2-acetamido-2-deoxy-glucopyranoside (15F) ^{1}H MHR (CD_3OD) δ 7.82 (d, J = 8.4Hz, 2H), 7.61 (d, J = 8.4Hz, 2H), 7.55 (d, 2H, J = 7.2 Hz), 7.36 (t, J= 7.8Hz, 2H), 7.27 (m, 1H), 4.46 (d, J = 8.4 Hz, 1H), 4.23 (d, J = 7.2 Hz, 1H), 3.56-4.1 (m, 10H), 3.30 (d, J =7.8 Hz, 1H), 3.20 (m, 4H), 3.01 (m, 1H), 2.64 (dd, 1H), 1.91 (S, 3H), 1.88 (s, 3H), 1.57 (t, J= 12Hz, 1H); ^{13}C NMR (CD_3OD) δ: 174.87, 174.63, 169.83, 145.41, 141.18, 134.42, 130.07, 129.11, 129.04, 128.11, 128.02, 105.04, 102.36, 101.54, 81.80, 76.47, 75.67, 74.66, 74.07, 73.42, 72.57, 72.33, 71.65, 70.32, 69.77, 66.51, 64.91, 62.01, 59.60, 52.28, 56.73, 53.93, 52.80, 51.23, 44.64, 42.35, 40.9823.49, 22.88, 17.66, 15.89**;** HRMS expected *m/z* =919.3436, found *m/z* =919.3473.

II. Printing and analysis of sialoside analog glycan array

Quantitation of glycans for printing

Prior to printing, glycans were quantified using the periodate resorcinol assay for sialic acids [4] with the Amplex Red Galactose/Galactose Oxidase Kit (Invitrogen, Carlsbad, CA) for galactose. The periodate resourcinol assay was carried out as described for glycosidically bound sialic acids, except that the periodate oxidation was done at 37° C instead of 4° C to ensure cleavage of the 9-substituted sialic acids between the C7-C8 vicinal hydroxyl groups. For quantitation of galactose, the Galactose Assay of the protocol was used. Terminal sialic acids were hydrolysed chemically with 1 M hydrochloric acid at 80˚C for 1 hr. The pH was then neutralized with 1M sodium hydroxide. N-acetyl-lactosamineβ-propylamine was used as a standard (125mM – 0.24mM). Two serial dilutions were prepared per glycan, where the starting concentration was assumed to be in the range of the standard curve concentrations. Fluorescence was measured with the Perkin Elmer Victor fluorescence plate reader (Perkin Elmer, Waltham, MA) using excitation at 530 nm and fluorescence detection at 590 nm. The median analog concentrations were calculated from the standard curve.

Printing the glycan array

Analog glycan arrays were printed by robotic contact arraying as described[5]. Approximately 0.6 nL of analog in print buffer (300 mM phosphate, 0.005% Tween-20, pH 8.5) was printed onto NHS-activated microscope glass slides per spot (Blixt *et al.*, 2004). Each analog structure (**1–34**) was printed at 10 different concentrations in 2-fold dilutions (100–0.2 μM), and each dilution was deposited 8 times. Immediately after the print, slides were placed in a chamber at 80% humidity for 30 min. The remaining NHS groups were blocked by immersing the slides in blocking buffer (50 mM ethanolamine in 50 mM borate buffer, pH 9.2) for 1 h. Slides were rinsed in water, dried by low-speed centrifugation, and stored in desiccators at room temperature before use.

Analysis of glycan binding protein to the array

Biotinylated plant lectins and recombinant Siglec-Fc chimera were screened on the printed analog glycan array according to described procedures (Blixt PNAS 2004). Briefly, glycan array slides were re-hydrated for 2 minutes in PBS followed by incubation with 1 mL of biotinylated plant lectin or Siglec-Fc chimera (10ug/mL) pre-complexed (2:1 molar ratio) with Alexa Flour-488 labeled anti-human or anti-mouse IgG, Fc fragment specific secondary antibody (Jackson ImmunoResearch Laboratories Inc.). Samples were incubated for 1 hour at room temperature in a hydrated chamber with gentle rocking. All incubations are performed likewise with 1 mL volumes prepared in 3% BSA/0.05% Tween/PBS (dilution buffer). Sample was removed and slides were washed by four successive rinses in 0.05% Tween/PBS, PBS and dH2O. Biotinylated lectins were detected with a subsequent incubation (1 hr at room temperature) with Alexa Flour-488 labeled streptavidin (1:5000; 0.4 μg/ml) incubated in the dark. The slides were washed a final time by four successive rinses each in 0.05% Tween/PBS and PBS followed by 3 successive washes in separate reservoirs of distilled H_2O. The slides were then dried by low-speed centrifugation prior to image acquisition. Fluorescence intensity was measured using a ScanArray 5000 (Perkin Elmer) confocal scanner and image analyses were performed using IMAGENE image analysis software (BioDiscovery, El Segundo, CA) as described previously. The average over the printed replicates and the SEM for selected glycans was calculated using GraphPad Prism software.

Supporting Figure S1

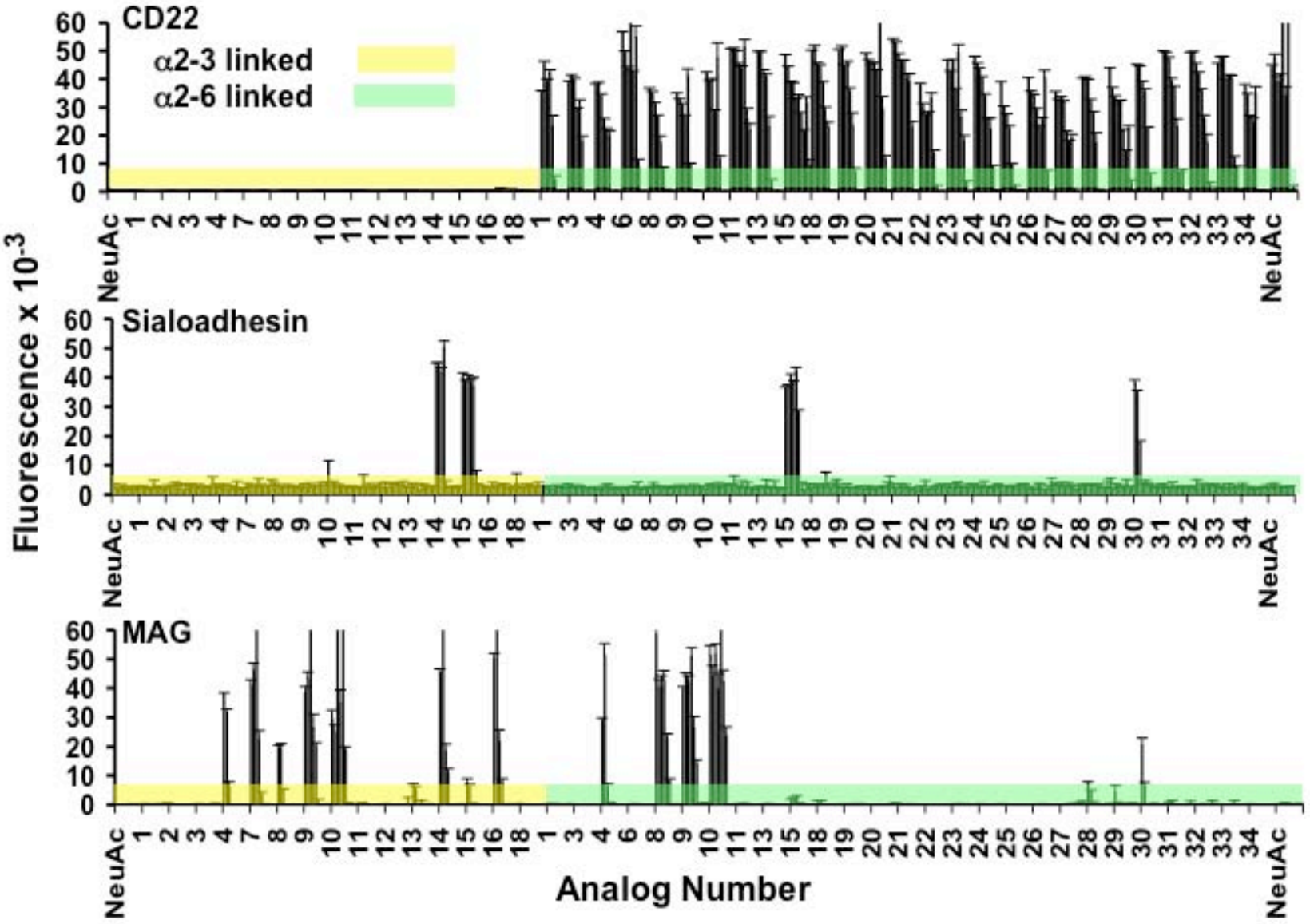


Figure S1. Binding of Siglec-Fc chimeras to the sialoside analog array. Human CD22-Fc chimera (*top*), mouse sialoadhesin-Fc chimera (*middle*) and mouse MAG-Fc chimera (*bottom*) were complexed with goat anti-human IgG-FITC (2:1; 50 μg/mL total protein), overlayed onto the printed analog array, washed and scanned for fluorescence (see Supplementary material and the legend for Figure 2). Acyl substituents **1-34** of **E** (yellow) and/or **F** (green) are found in Table S1. Shown is relative binding to each glycan printed at 10 serial dilutions starting at 100 μM. 'NeuAc' labeled lanes are sialosides with unsubstituted Neu5Ac in α2-3 (**H**) and α2-6 (**I**) linkage to N acetyl-lactosamine.

Supporting Figure S2

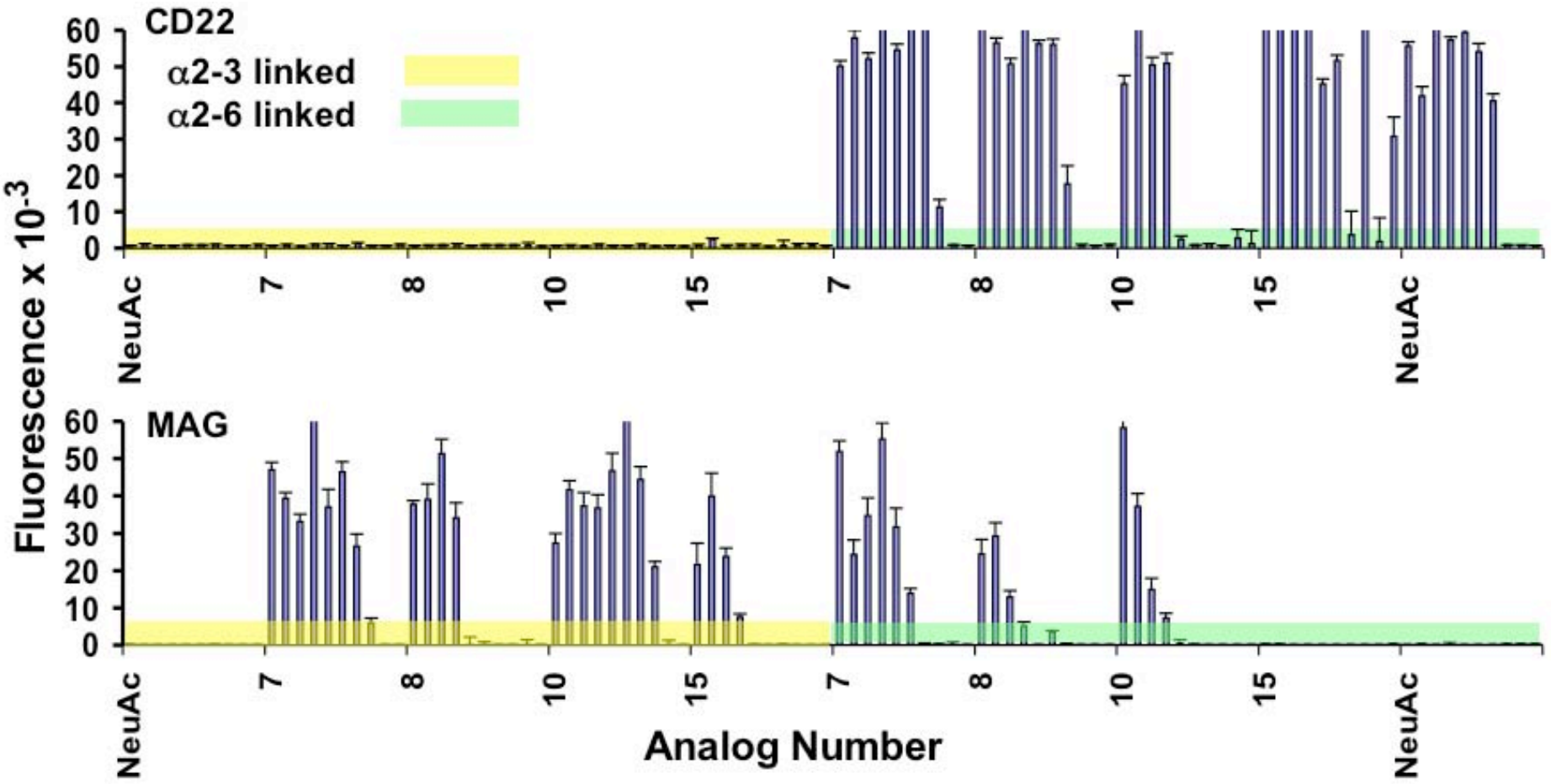
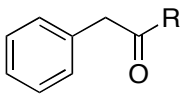
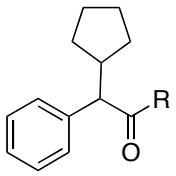
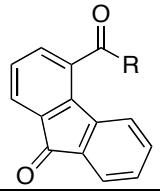
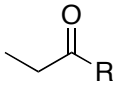
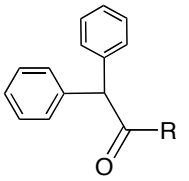
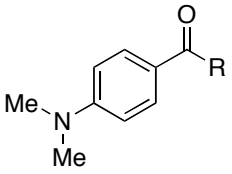
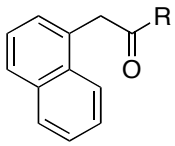
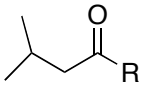
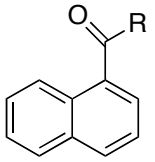
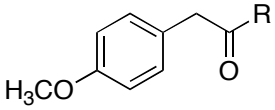
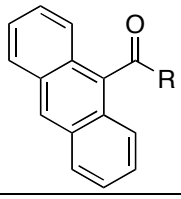
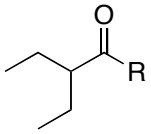
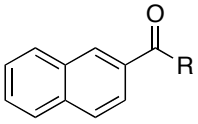
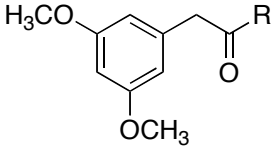
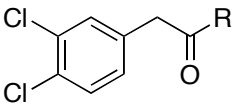
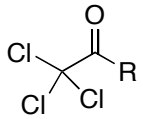
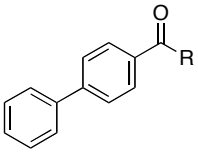
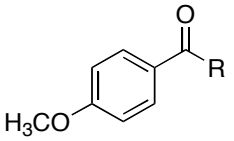
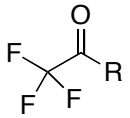
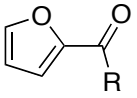
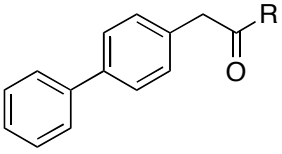
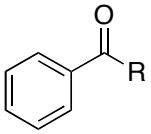
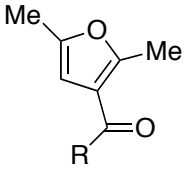
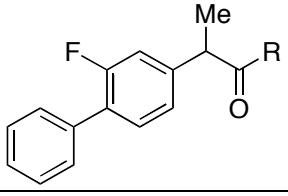
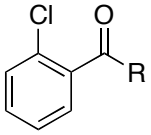
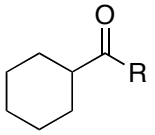
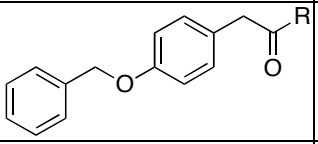
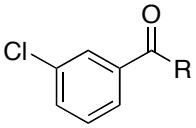
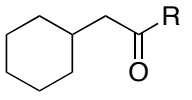
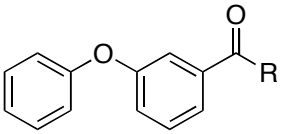
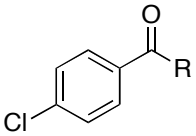
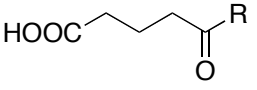
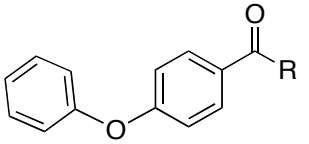


Figure S2. Binding of CD22-Fc and MAG-Fc chimeras to re-synthesized sialoside analogs. Selected acyl analogs (**8-10,15**) of **E** (yellow) or **F** (green) that showed increased binding to CD22 or MAG in the initial screens were synthesized, purified and printed on slides to confirm reactivity (see legend of Figure 3 and experimental procedures for details).

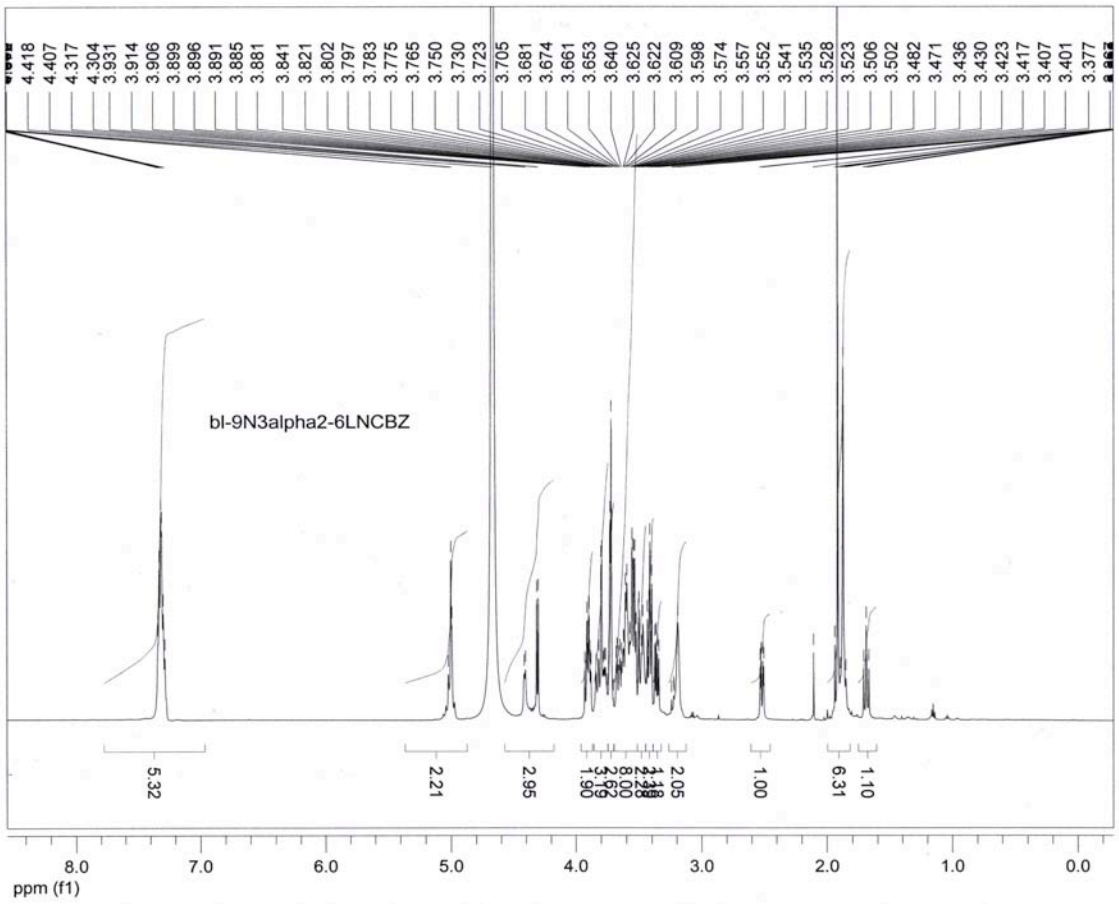
Supporting Table S1:

Table S1. Sialoside analog library. Shown are the alternative acyl groups **1-34** substituted at R_2 on **E** and/or **F** comprising the sialoside analog library. Also shown are the control glycans representing non-sialylated N-acetyl-lactosamine precursor (**G**) and corresponding Neu5Ac derivatives in α2-3 (**H**) and α2-6 linkage (**I**).

#	Acyl group	#	Acyl group	#	Acyl group	#	Acyl group
1		**11**		**21**		**31**	
2		**12**		**22**		**32**	
3		**13**		**23**		**33**	
4		**14**		**24**		**34**	
5		**15**		**25**		**Cpd**	**Structure**
						G	Galβ1-4GlcNAcβ-sp
6		**16**		**26**		**H**	Neu5Acα2-3Galβ1-4GlcNAcβ-sp
7		**17**		**27**		**I**	Neu5Acα2-6Galβ1-4GlcNAcβ-sp
8		**18**		**28**		**J**	Neu5Acα2-6Galβ1-4[6S]GlcNAcβ-sp
9		**19**		**29**			
10		**20**		**30**			

^{1}H NMR of B and C and resynthesized acyl-substituted analogs 7E, 8E, 10E, 7F, 8F and 10F.

B



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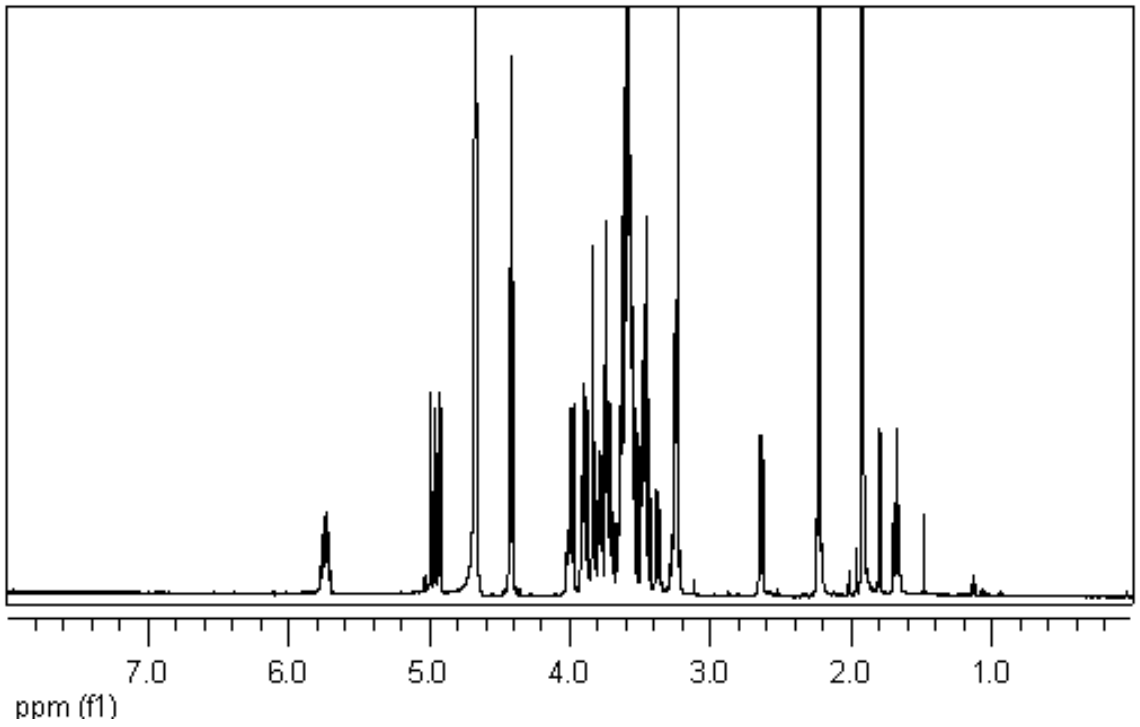

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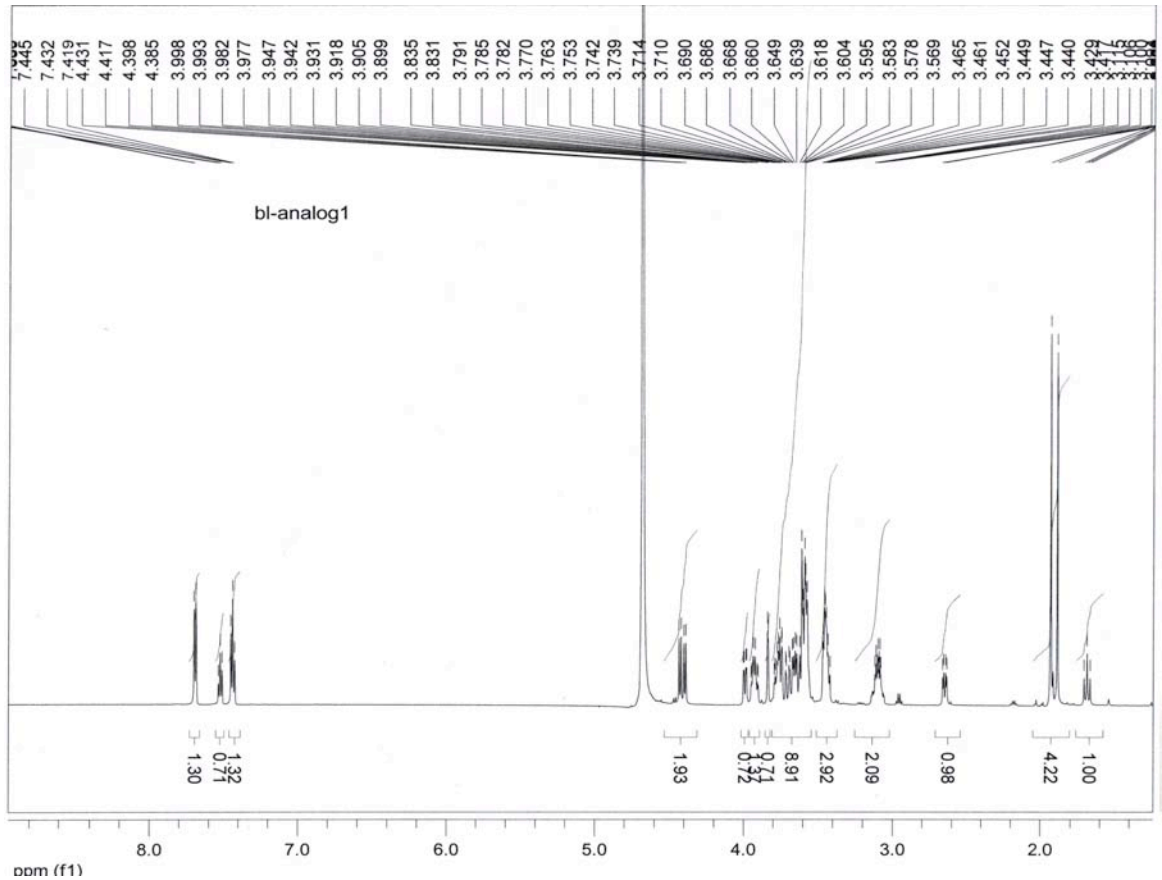

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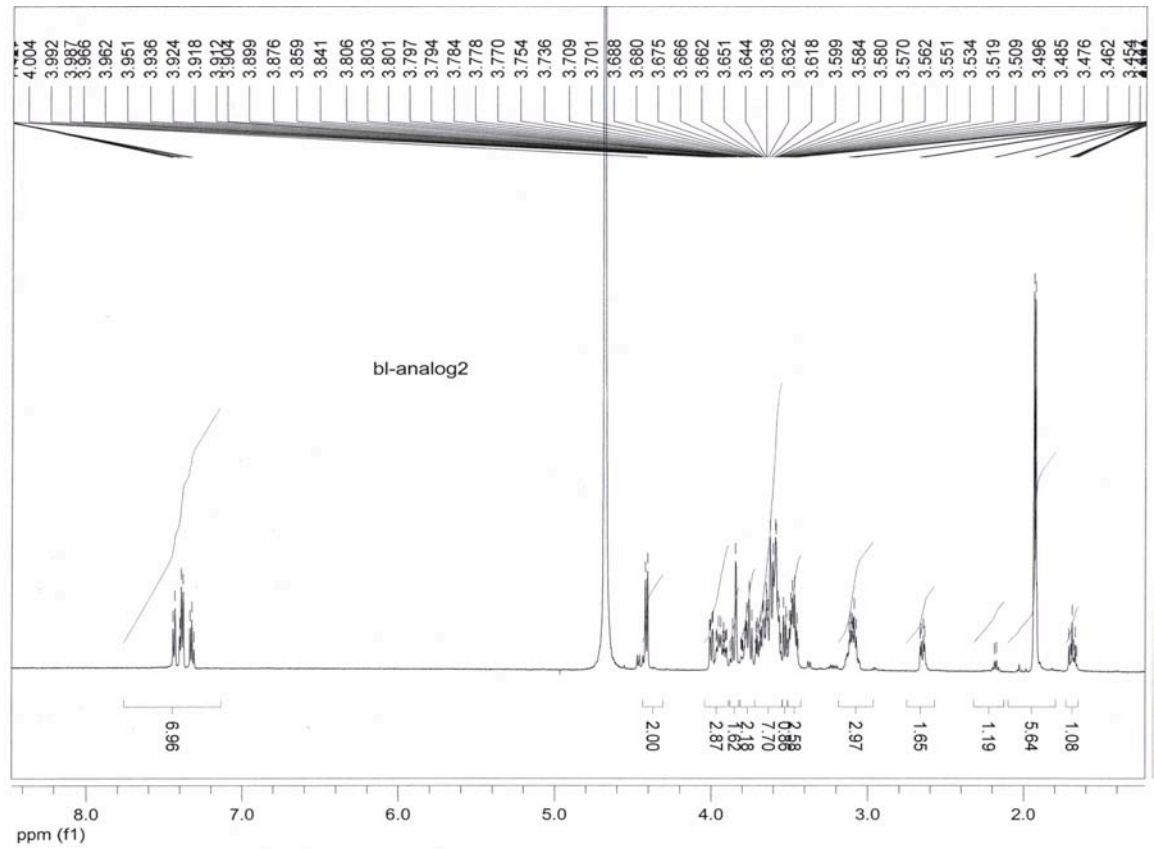

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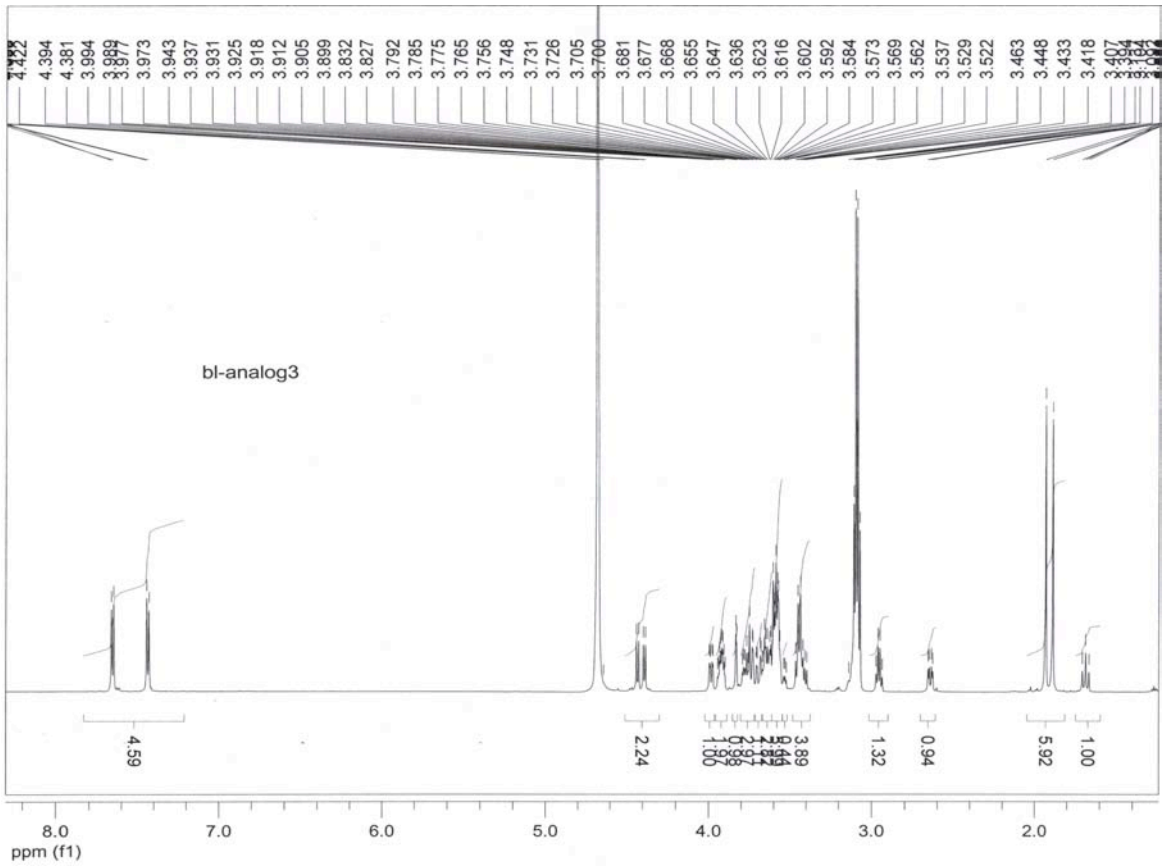
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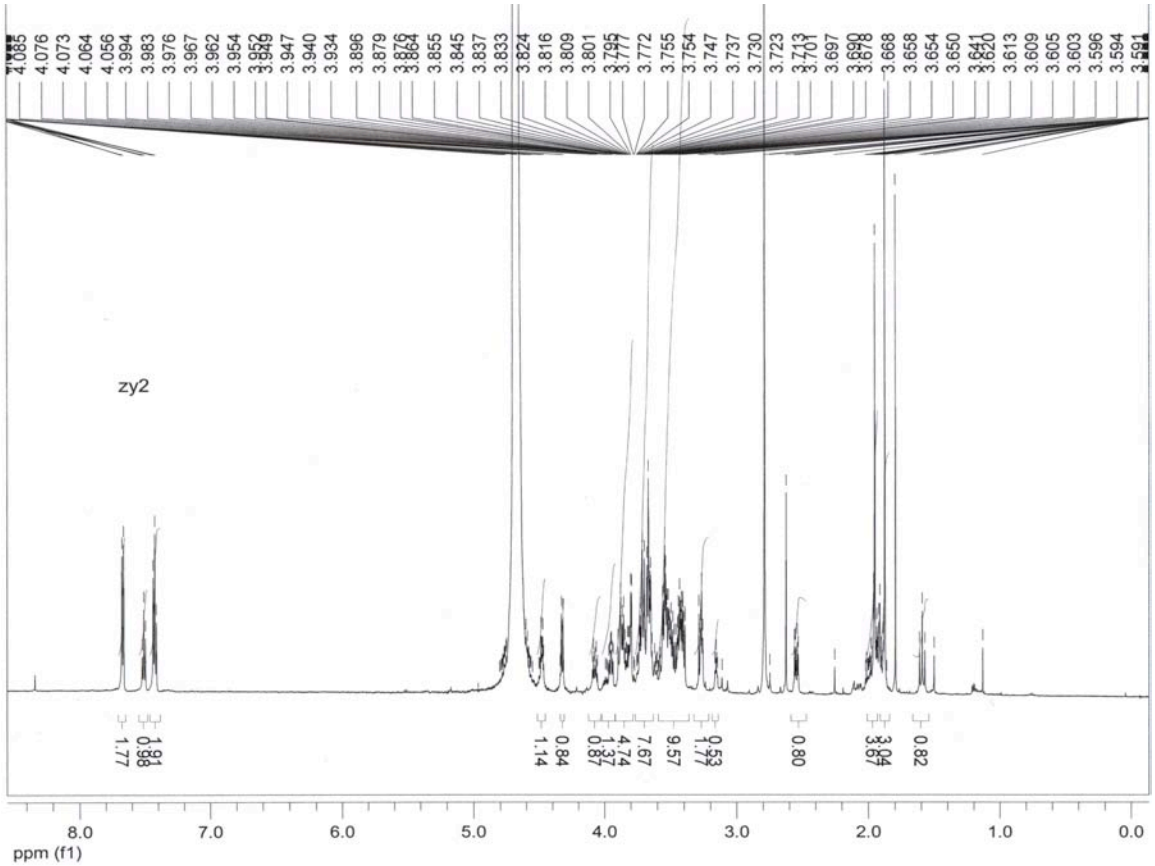
4.0
3.0
2.0
ppm (f1)

7F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1.14
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0.80
3.84
3.87
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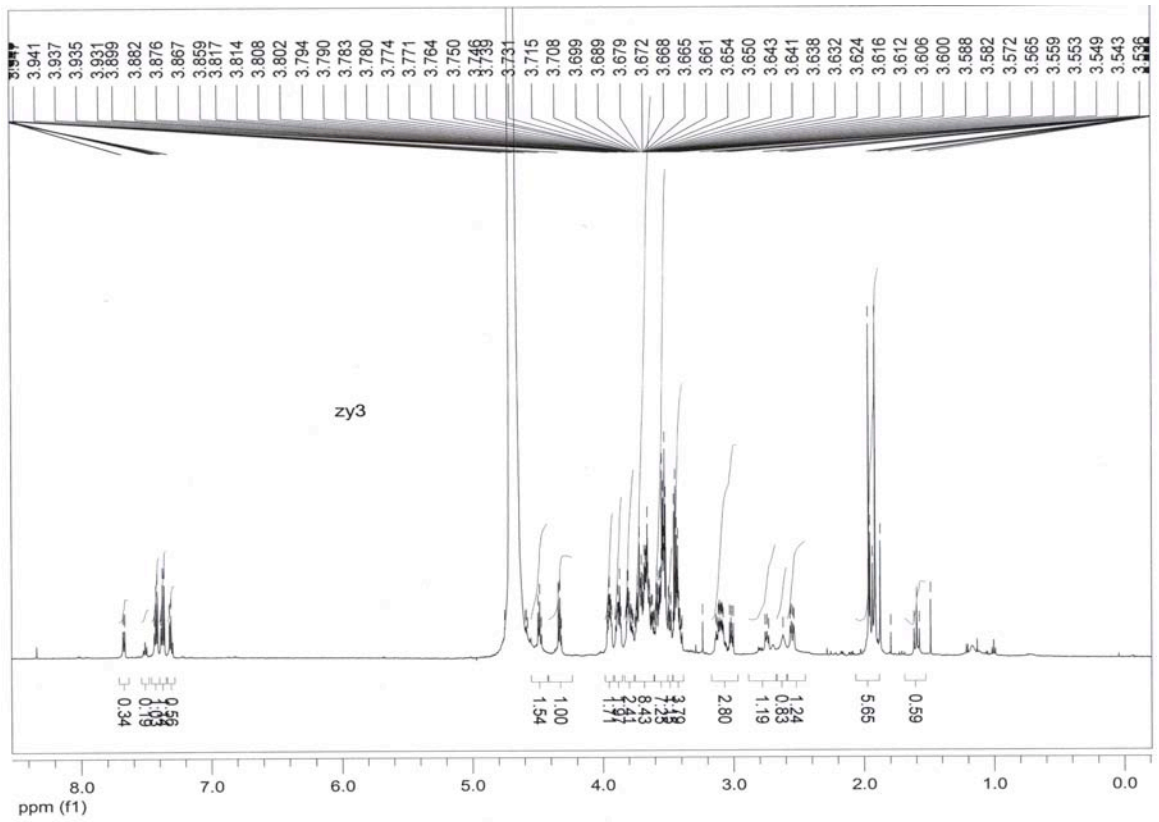
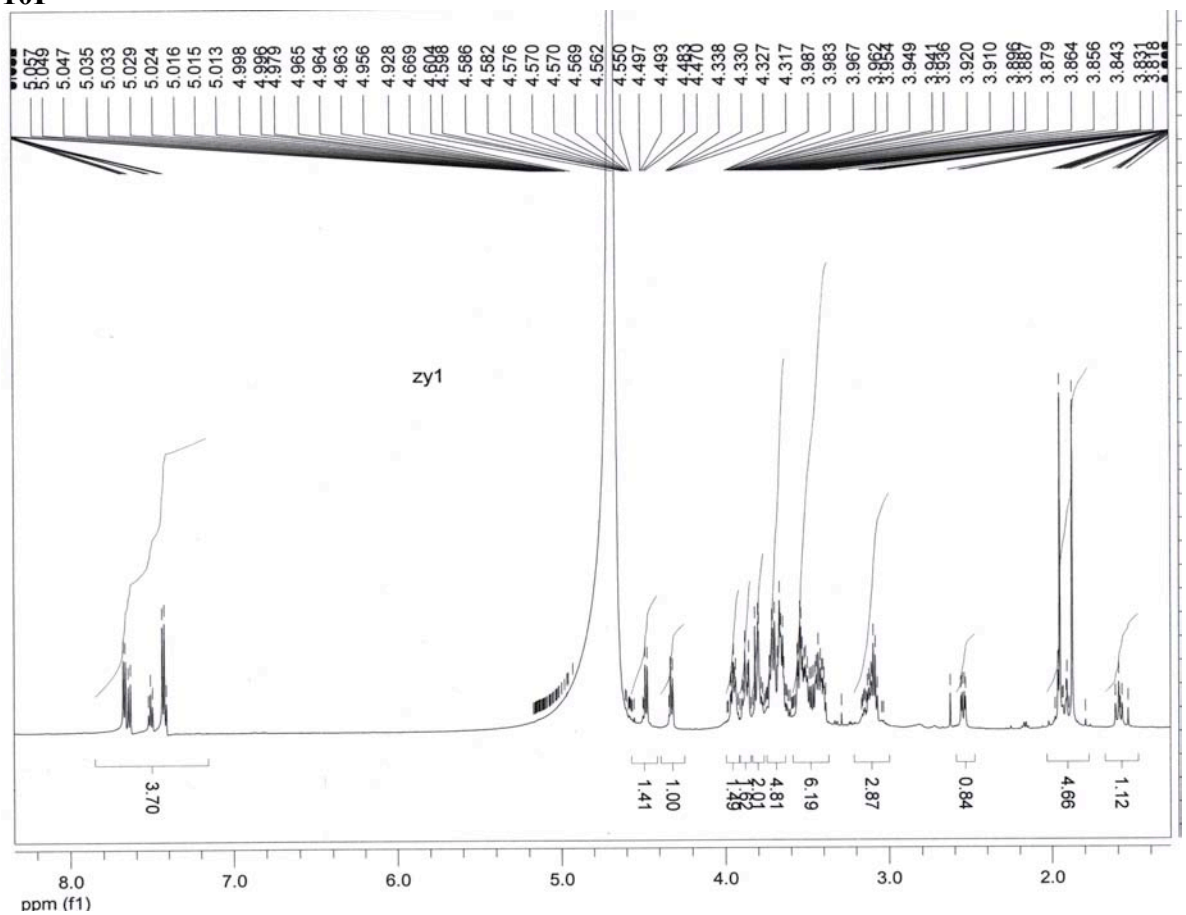
ppm (f1)

8F



10F



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4. Jourdian, G. W.; Dean, L.; Roseman, S., The sialic acids. XI. A periodate-resorcinol method for the quantitative estimation of free sialic acids and their glycosides. *J Biol Chem* **1971,** 246, (2), 430-5.
5. Blixt, O.; Head, S.; Mondala, T.; Scanlan, C.; Huflejt, M. E.; Alvarez, R.; Bryan, M. C.; Fazio, F.; Calarese, D.; Stevens, J.; Razi, N.; Stevens, D. J.; Skehel, J. J.; van Die, I.; Burton, D. R.; Wilson, I. A.; Cummings, R.; Bovin, N.; Wong, C. H.; Paulson, J. C., Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. *Proc Natl Acad Sci U S A* **2004,** 101, (49), 17033-8.