Supporting Information

Chemoenzymatic Synthesis of Sialooligosaccharides on Arrays for Studies of Cell Surface Adhesion

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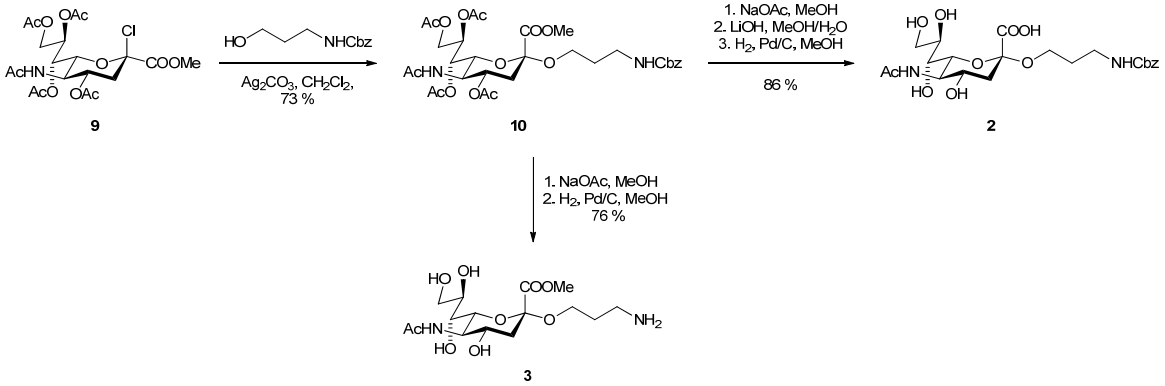
Experimental Section

General

All chemicals and solvents were of analytical grade and if not otherwise stated purchased from Sigma-Aldrich or Fisher Scientific and used as delivered. Alkanethiol linkers [HS-$(CH_2)_{17}$-EG_3-OH (**8**) and HS-$(CH_2)_{17}$-EG_6-OCH_2COOH] were purchased from Prochimia Surfaces (Poland). Aminoethyl glycosides **1**, **4-6** are common building blocks for array fabrication and are easily available *via* chemical synthesis as described previously.[1,2] Briefly, *O*-acetylated glycosyl donors (acetate, trichloroacetimidate or bromide) were coupled with *N*-benzyloxycarbonylethanolamine under standard glycosylation conditions and purified by flash chromatography. Acetyl groups were removed by treatment with sodium methoxide in methonal, methyl ester hydrolysed with lithium hydroxide and *N*-Cbz group cleaved by hydrogenolysis to afford the desired glycosides. The sialic acid derivatives **2** and **3** were prepared as shown in Scheme S1.



Scheme S1: Synthesis of sialic acid derivatives **1**-**3**.

Synthesis of methyl 5-acetamido-2-*O*-(*N*-benzyloxycarbonyl)aminopropyl-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (10):

Following lit.[2], to a solution of chloride **9** (510 mg, 1.00 mmol) and *N*-Cbz-aminopropanol (628 mg, 3 mmol, 3 eq.) in 7 mL abs. CH_2Cl_2 were added 500 mg 4 Å molecular sieves and the solution was stirred at room temperature. After 1 h Ag_2CO_3 (552 mg, 2 mmol, 2 eq.) was added and the solution was stirred for 16 h in dark. The solid was filtered over Celite, washed with CH_2Cl_2 and the filtrate evaporated to dryness. Column chromatography (EtOAc/hexane 80:20) yield 498 mg (0.73 mmol, 73 %) of glycoside **10** as colourless foam.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.39 – 7.29 (m, 5H, C_6H_5), 5.43 (d, *J*=9.9, 1H, NH), 5.40 – 5.34 (m, 2H, 8-H, NH), 5.32 (dd, *J*=8.6, *J*=1.8 Hz, 1H, 7-H), 5.13, 5.07 (AB, *J*=12.3, 2H, Ph*C*H_2), 4.86 (ddd, *J*=12.2, *J*=10.0, *J*=4.7 Hz, 1H, 4-H), 4.27 (dd, *J*=12.4, *J*=2.3 Hz, 1H, 9-H_b), 4.13 (dd, *J*=10.7, *J*=2.0 Hz, 1H, 6-H), 4.10 – 4.00 (m, 2H, 5-H, 9-H_a), 3.89 – 3.78 (m, 1H, 1'-H_b), 3.77 (s, 3H, OCH_3), 3.41 – 3.21 (m, 3H, 1'-H_a, 3'-H_aH_b), 2.56 (dd, *J*=12.9, *J*=4.6 Hz, 1H, 3-H_e), 2.13, 2.05, 2.03, 2.01 (4 s, 12H, 4 $COCH_3$), 1.94 ("t", *J*=12.6 Hz, 1H, 3-H_a), 1.88 (s, 3H, $NCOCH_3$), 1.77 (m, 2H, 2'-H_aH_b). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 171.0, 170.7, 170.3, 170.11, 170.06 (5 s, 4 *C*OCH_3, *C*$OOCH_3$), 168.4 (s, NH*C*OCH_3), 156.5 (s, NH*C*OO), 136.73 (s, *i*-C from C_6H_5), 128.5, 128.2, 128.1 (3 d, *o*-, *m*-, *p*-C from C_6H_5), 98.5 (s, C-1), 72.4 (d, C-6), 69.0 (d, C-4), 68.4 (d, C-8), 67.2 (d, C-7), 66.5 (t, O*C*H_2Ph), 62.7, 62.3 (2 t, C-9, C-1'), 52.8 (q, OCH_3), 49.4 (d, C-5), 38.2 (t, C-3'), 38.0 (t, C-3), 29.3 (t, C-2'), 23.2 (q, NHCO*C*H_3), 21.1, 20.9, 20.8, 20.75, 20.72 (4 q, 4 OCO*C*H_3), HRMS (ESI+) *m/z* calculated for $[C_{31}H_{43}O_{15}N_2]^+$ 683.2663: , found 683.2678

Synthesis of 5-acetamido-2-*O*-aminopropyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (2):

Aminopropyl glycoside **10** (205 mg, 0.30 mmol) was dissolved in 5 mL methanol and 16.0 mg (0.30 mmol) sodium methoxide, dissolved in 1 mL methanol were added. After stirring at room temperature for 2 h, the solution was neutralised with Dowex 50WX8-100 (H^+) resin, filtered and concentrated. The residue was dissolved in 5 mL water/methanol (4:1), treated with 21 mg (0.9 mmol) LiOH and the solution was stirred overnight at room temperature. After neutralisation with Dowex 50WX8-100, the resin was filtered off and washed with methanol/water (1:1). The filtrate was concentrated in vacuum to remove methanol and then freeze dried. The sialic acid obtained (white solid, 136 mg, 91 %) was dissolved in 10 mL methanol, treated with 50 mg palladium on carbon (10 %) and the solution was stirred under hydrogen for 4 h. The catalyst was filtered off over Celite, washed with methanol and the filtrate concentrate. The product was re-dissolved in water, treated with activated charcoal and filtered. Lyophilisation of the filtrate yielded 95 mg (0.26 mmol, 86 % starting from **10**) of deprotected sialic acid **2** as white solid.
^{1}H NMR (400 MHz, D_2O) δ = 3.88 – 3.77 (m, 4H), 3.73 – 3.51 (m, 5H), 3.09 (t, 2H, 3'-H_aH_b), 2.68 (dd, *J*=12.5, 4.7, 1H, 3-H_e), 2.00 (s, 3H, $COCH_3$), 1.91 (m, 2H, 2-H_aH_b), 1.65 (t, *J*=12.2, 1H, 3-H_a). ^{13}C NMR (101 MHz, D_2O) δ = 175.1, 173.8, 100.5, 72.6, 71.7, 68.0, 62.6, 62.4, 51.8, 40.2, 37.9, 26.6, 21.9. HRMS (ESI+) *m/z* calculated for $[C_{14}H_{27}O_9N_2]^+$ 367.1711: , found 367.1712

Synthesis of methyl 5-Acetamido-2-*O*-aminopropyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulo-pyranosonate (3):

Aminopropyl glycoside **10** (300 mg, 0.44 mmol) was dissolved in 5 mL methanol and 24.0 mg (0.44 mmol) sodium methoxide, dissolved in 2 mL methanol were added. After stirring at room temperature for 4 h, the solution was neutralised with Dowex 50WX8-100 (H^+) resin, filtered and concentrated. The residue (188 mg) was dissolved in 20 mL methanol, 97 mg palladium on carbon (10 %) were added and the solution was stirred under hydrogen for 4 h. The catalyst was filtered off over Celite, washed with methanol and the filtrate concentrated. The product was re-dissolved in water, treated with activated charcoal and filtered. Lyophilisation of the filtrate yielded 128 mg (0.37 mmol, 76 % starting from **10**) of ester **3** as a white solid, suitable for printing onto microarrays.
HRMS (ESI+) *m/z* calculated for $[C_{15}H_{28}O_9N_2]^+$ 381.1873: , found 381.1873

Preparation of SAMs on gold plates

A disposable 64-well gold plate (Applied Biosystems) was cleaned with Piranha solution (3:1 H_2SO_4/H_2O_2) for 30 min, rinsed with distilled water, ethanol and dried in a stream of nitrogen. A DMSO solution of carboxylic acid-terminated and tri(ethylene glycol) alkanethiols (final concentration 0.2 mg/mL, ratio varies from 1:1 up to 1:20) was applied on the plate and left overnight at r.t. to form a mixed SAM. The plate was washed with ethanol and dried under nitrogen. The carboxylic group was activated by immersing of the plate into a solution of EDC and NHS (final concentrations 0.1 M and 0.025M, respectively) in dry DMF for 2 h, followed by washing with water and ethanol and drying as above. For immobilisation of saccharides on the surface, a solution of aminoethyl or aminopropyl glycoside in PBS (100 mM, pH 7.4) was spotted onto the freshly activated monolayer, allowed to react for 4 h at r.t., than rinsed and dried as above.

On-chip enzymatic sialylation with *trans*-sialidase from *Trypanosoma cruzi*

A solution prepared from purified TcTS (10 μL) and fetuin (10 mg) in 100 mL phosphate buffer (50 mM, pH 7) was applied on the glycan-coated plates (ca. 1 μL per well) and the slides were incubated overnight at 37 °C. After washing with PBS saline (pH 7.4) containing 0.5 % Tween-20, water and ethanol they were dried under nitrogen.

On-chip methyl esterification of sialic acid[3]

For the conversion of immobilised sialic acid to its sodium salt the gold plate was immersed into water containing washed and activated Dowex 50WX8. After shaken for 30 min the plate was removed, washed with water, ethanol and dried in a steam of nitrogen. After this the plate was covered with a 50% solution of MeI in dry DMSO and incubated at r. t. Finally the plate is washed with water, ethanol and dried with nitrogen. The progress of the reaction can be easily monitored by MALDI-ToF MS. Generally, no free acid could be detected after 2 h reaction time.

On-chip permethylation

To a gold plate immersed in 7 mL dry DMSO was added ca. 2 mL sodium sodium dimsyl solution, prepared as described in the lit[4] followed by 1.5 mL MeI. After 15 min the plate was removed, washed with water, ethanol and dried.

MALDI-ToF MS analysis

All MALDI-Tof MS experiments were carried out on an Ultraflex II instrument (Bruker Daltonics) in positive reflectron mode. A solution of matrix (2,4,6-trihydroxyacetophenone, 15 mg/mL in acetonitrile) was applied on the gold and allowed to dry before analysis. If not otherwise stated the peaks correspond to sodium adduct $[M+Na]^+$ of the substrates.

MALDI-ToF MS spectra

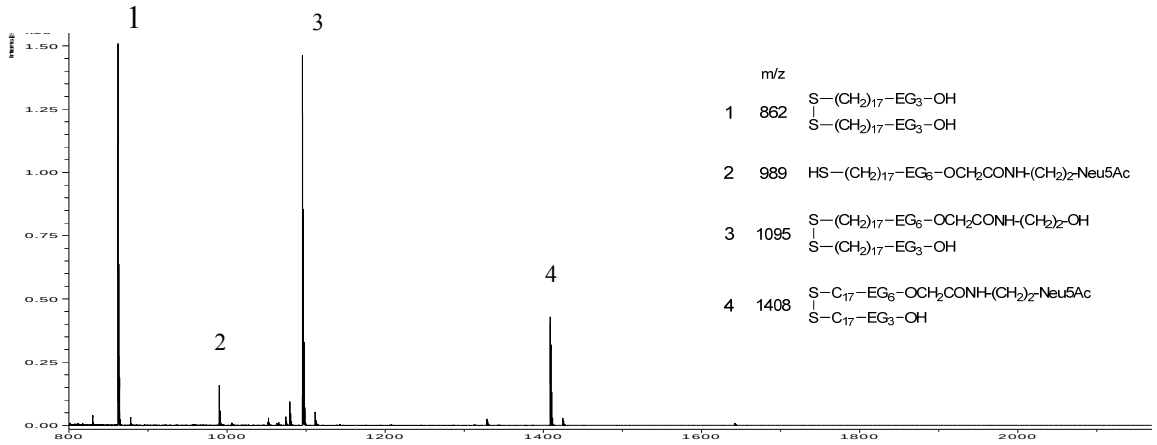


Fig. S1: MALDI-ToF MS spectrum of immobilised aminoethyl glycoside **1** on gold

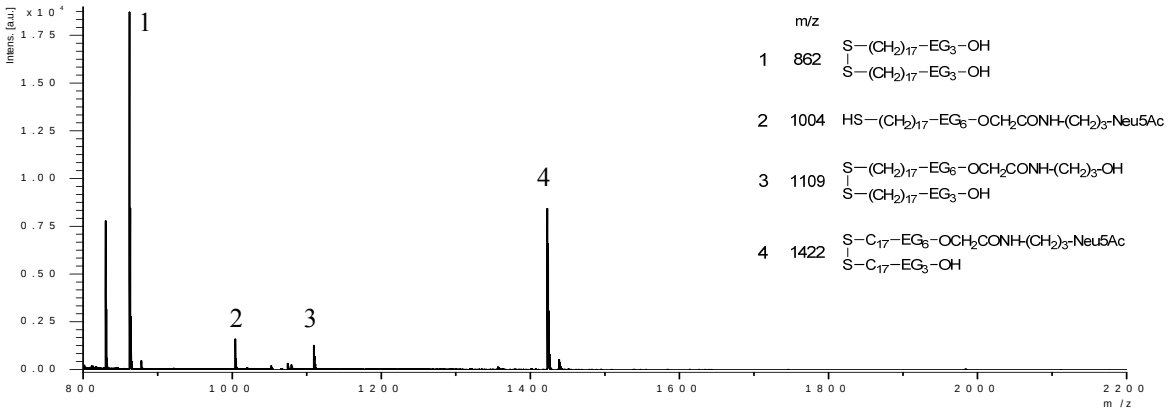


Fig. S2: MALDI-ToF MS spectrum of immobilised aminopropyl glycoside **2** on gold

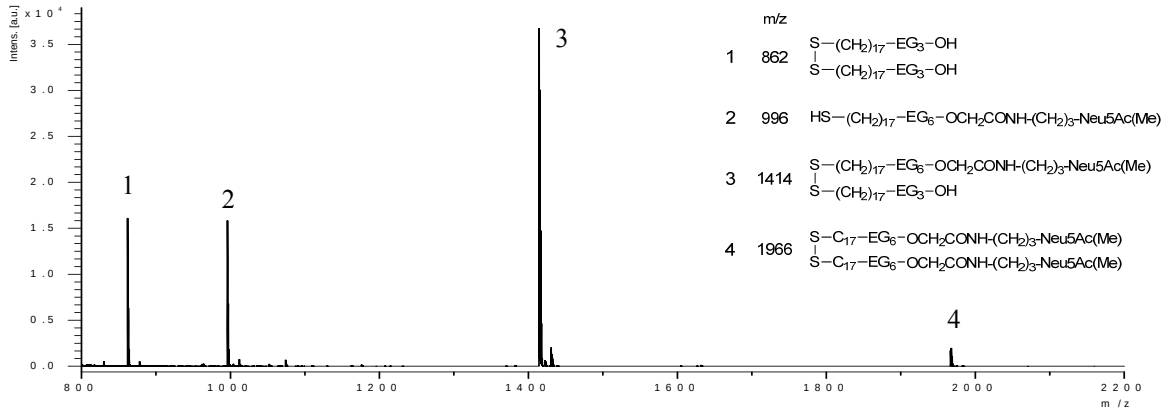


Fig. S3: MALDI-ToF MS spectrum of immobilised methyl ester **3** on gold

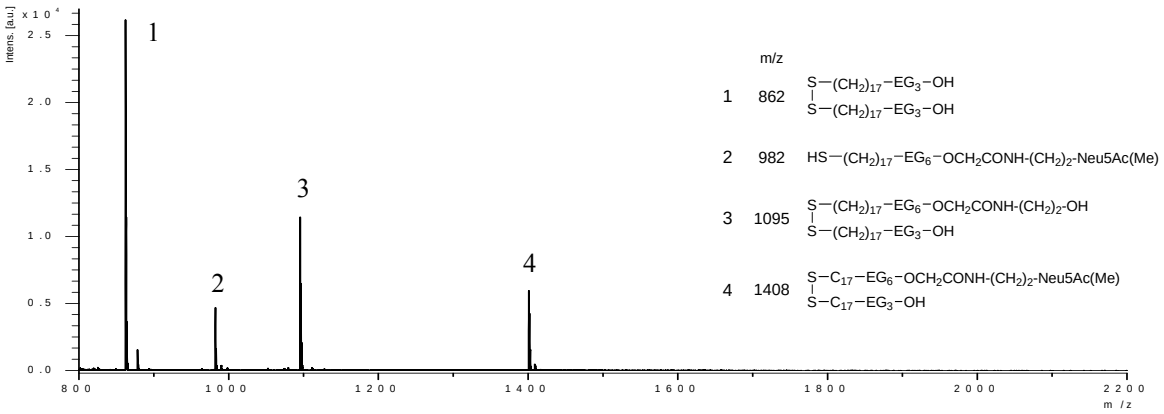


Fig. S4: MALDI-ToF MS spectrum of immobilised sialic acid **1** on gold after methyl esterification

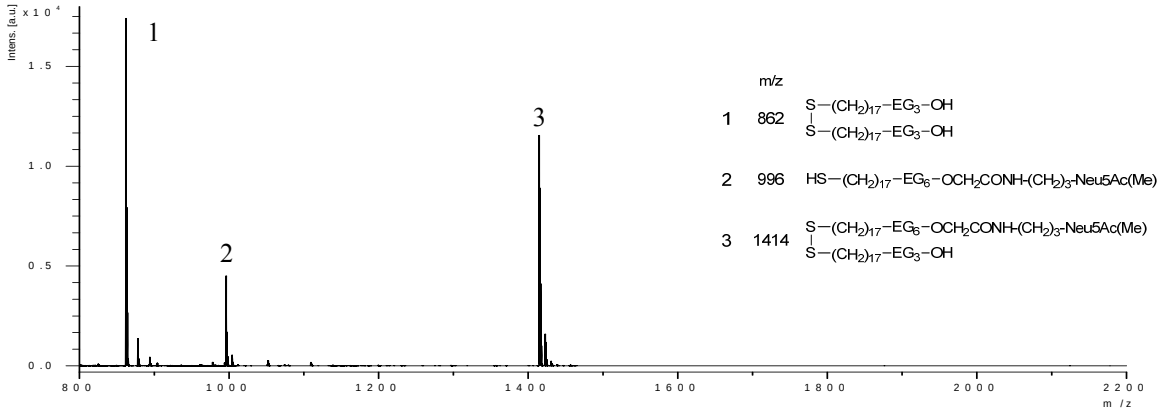


Fig. S5: MALDI-ToF MS spectrum of immobilised aminopropyl glycoside **2** on gold after methyl esterification

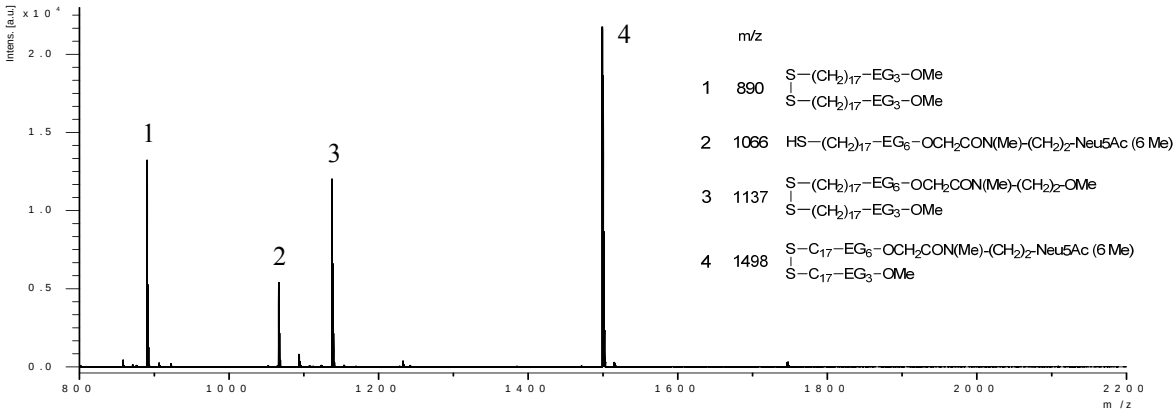


Fig. S6: MALDI-ToF MS spectrum of immobilised sialic acid **1** on gold after permethylation

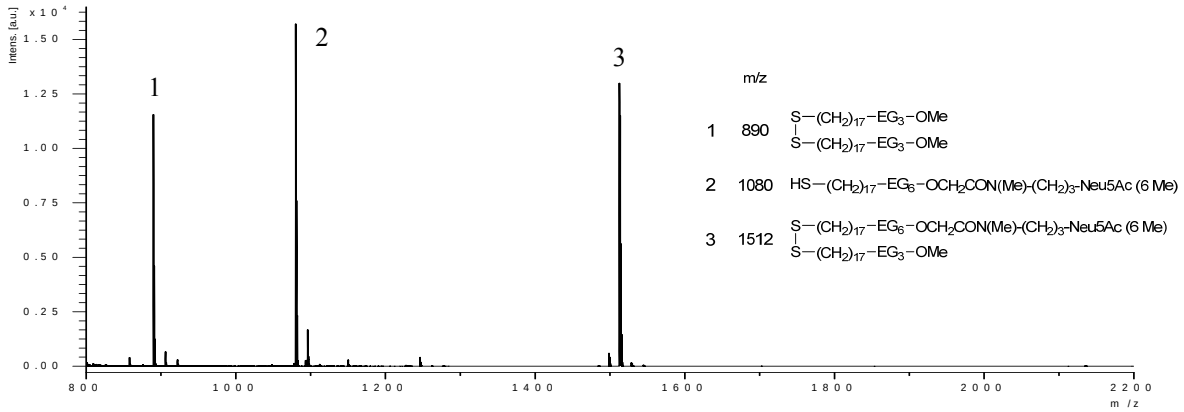


Fig. S7: MALDI-ToF MS spectrum of immobilised aminopropyl glycoside **2** on gold after permethylation

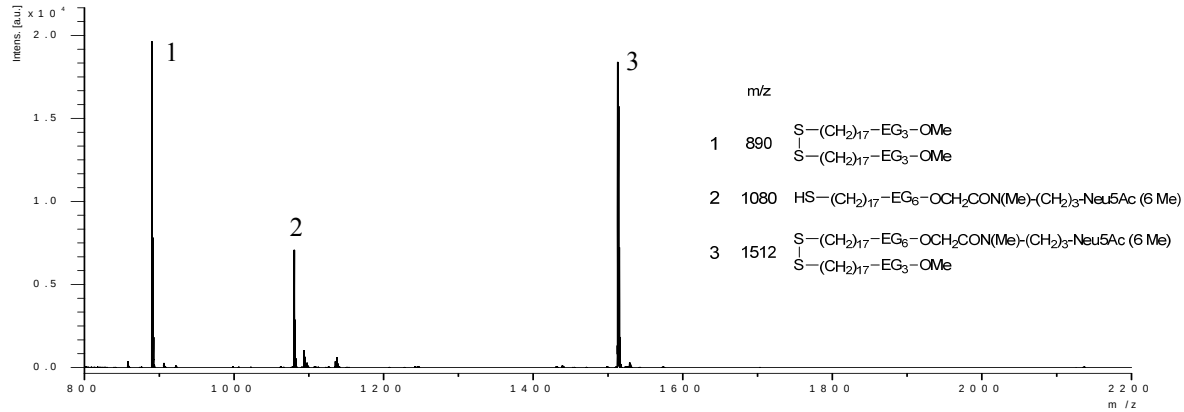


Fig. S8: MALDI-ToF MS spectrum of immobilised methyl ester **3** on gold after permethylation

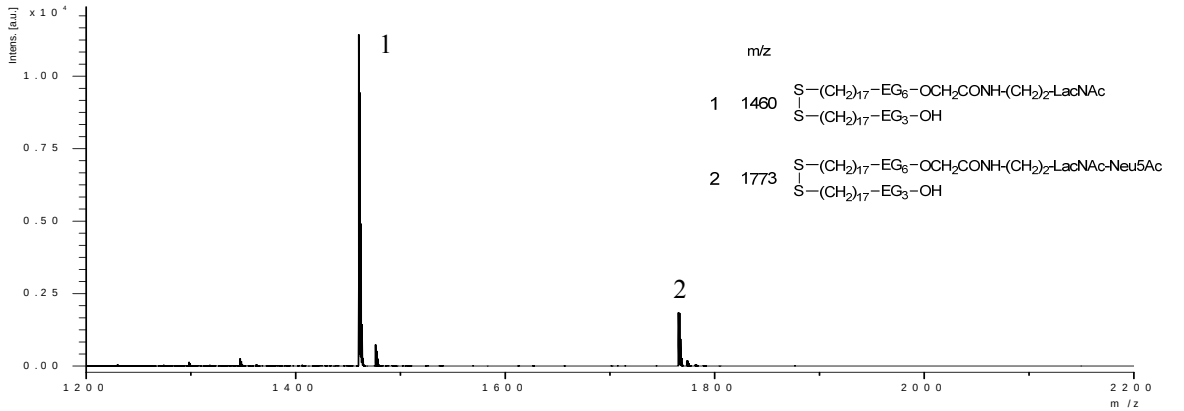


Fig. S9: MALDI-ToF MS spectrum of sialo lactosamine generated from LacNAc with TcTS and fetuin on gold

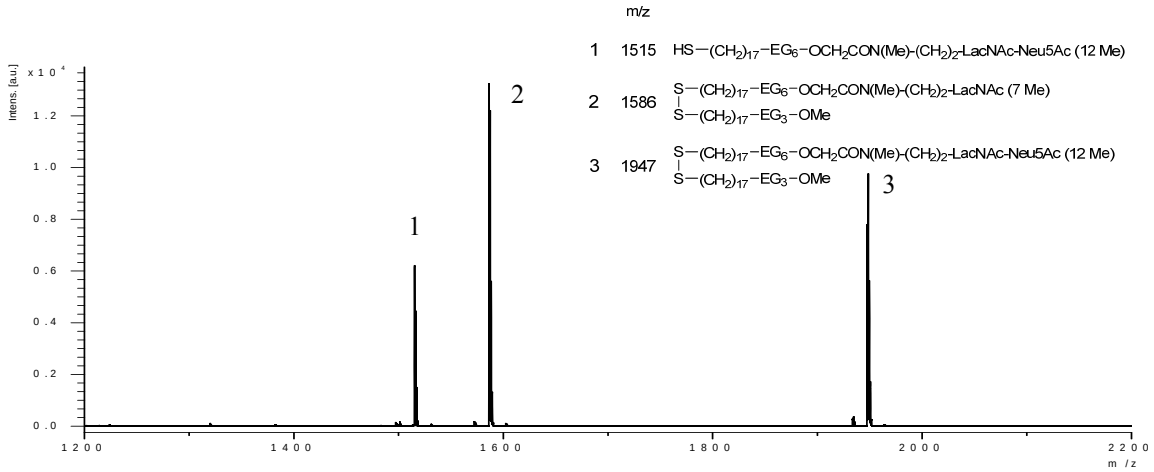


Fig. S10: MALDI-ToF MS spectrum of sialo lactosamine generated from LacNAc with TcTS and fetuin on gold after permethylation

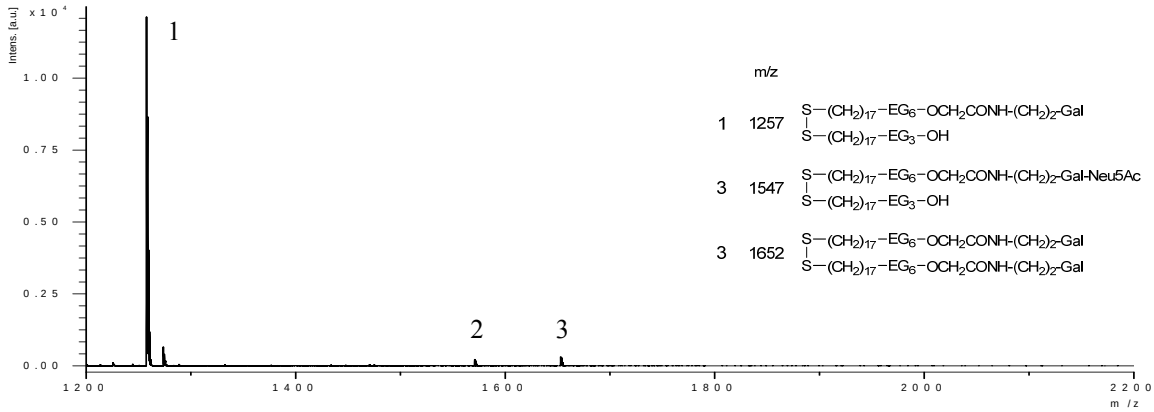


Fig. S11: MALDI-ToF MS spectrum of sialo galactose generated from Gal with TcTS and fetuin on gold

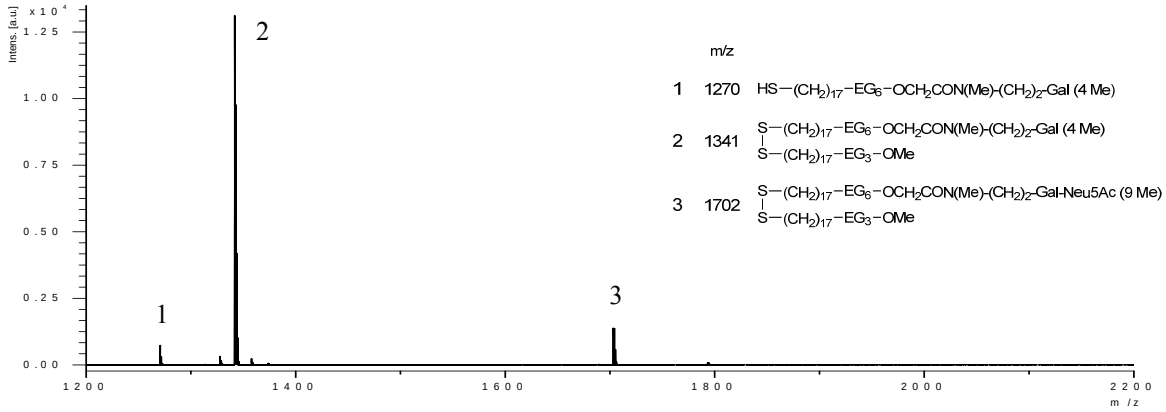


Fig. S12: MALDI-ToF MS spectrum of sialo galactose generated from Gal with TcTS and fetuin on gold after permethylation

Binding of Cells

Chinese Hamster Ovary cells expressing full-length murine sialoadhesin[5] were labelled with 0.5uM CFSE in phosphate buffered saline (PBS) and after washing suspended in PBS containing 0.1% bovine serum albumin (PBA). The cells were resuspended at 6 x106/ml in 5 ml of PBA and gently loaded onto the gold chips to cover the entire surface. The chips were incubated at 37° C for 1 h in the dark and then gently washed in PBA + 2mM EDTA and examined by fluorescent microscopy.

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