Supporting Information

for

Exploring the Effect of Sialic acid Orientation on Ligand-Receptor Interactions

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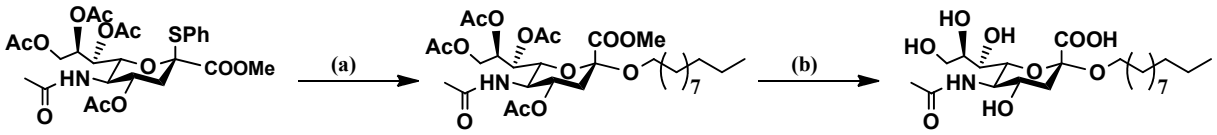
1. General Information

All chemicals used were reagent grade and used as supplied except where noted. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in CAN solution followed by heating. Column chromatography was carried out using force flow of the indicated solvent on Fluka Kieselgel 60 (230–400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on Bruker 500 MHz (or 125 MHz for 13C) and Jeol 400 MHz (or 100 MHz for 13C) using residual solvents signals as an internal reference ($CDCl_3$ δH, 7.26 ppm, δc 77.3 ppm and CD_3OH δH 3.31 ppm, δc

49.0 ppm). The chemical shifts (δ) are reported in *ppm* and coupling constants (J) in Hz. IR spectra were recorded on a Perkin Elmer 1600 FTIR spectrometer. Optical rotation measurements were conducted using a Perkin- Elmer 241 polarimeter.

2. Synthesis of compound 3 and 4



5 **3**

***Scheme S1*: Reagents and conditions :** (a) 1-undecanol, NIS, Mol sieve 4 Å, TfOH; (b) NaOMe, MeOH

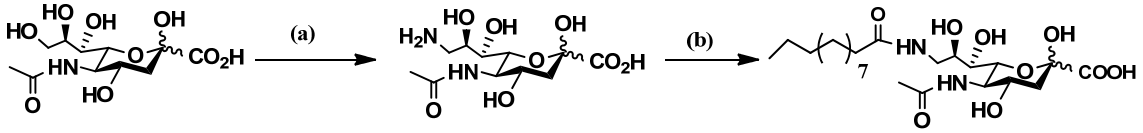
Methyl (undecyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-_-D*galacto*-2-nonulopyranosid)onate.

Thiophenyl-sialic acid glycoside (300 mg, 0.51 mmol) and 1-undecanol (177 mg, 1.02 mmol) were dissolved in anhydrous dichloromethane (10.0 mL) under an atomosphere of argon; 4 Å molecular sieves (500 mg) was added. The temperature was cooled to -40 °C and *N*-iodosuccinimide (172 mg, 0.77 mmol) and catalytic amount trifluoromethanesulfonic acid (15 µL, 0.15 mmol)of was added. After stirring for 3 h, the reaction mixture was diluted with DCM (20 mL) and filtered through a pad of celite. After filtration, the organic solution was washed with a 1 : 1 mixture of 10% $Na_2S_2O_3$ and sat. $NaHCO_3$, dried and evaporated. The residue was purified by column chromatography on silica gel using DCM : MeOH (95: 5) yield (178 mg , 53.6%) of compound 3 (α/β 3/1) and further purified by a mixture of acetone : CH_2Cl_2 (10 : 90) as eluent to afford sequentially the pure 3 (90.8 mg, yield 28%). R_f = 0.5 (DCM : MeOH, 96: 4); $[\alpha]_D^{r.t}$ = +26.1 (c = 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.38-5.20 (m, 3H), 4.83-4.54 (m 1H), 4.31 (dd, 1H, J = 12.4, 2.6 Hz), 4.13-4.01 (m, 3H), 3.87 (dd, 1H, J = 9.3, 6.6 Hz), 3.73 (s, 3H), 3.29 (m, 1H), 2.45 (dd, 1H, J = 12.8, 4.8 Hz), 2.13 (s, 3H), 2.12 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.93 (dd, 1H, J = 12.6, 12.6 Hz), 1.86 (s, 3H), 1.46- 1.55 (m, 2H), 1.19-1.34 (m, 16H), 0.86 (t, 3H, J = 6.6 Hz). ^{13}C-NMR ($CDCl_3$, 100 MHz): δ 175.0, 174.6, 173.2, 172.1, 169.6, 98.7,

75.1, 72.3, 71.9, 71.5, 70.1 68.8, 67.5, 65.1, 63.4, 52.9, 41.4, 32.1, 29.7, 29.6, 25.9, 23.2 22.7, 14.1. Maldi-ToF m/z calc'd for $C_{31}H_{51}NO_{13}Na$ ($M+Na^+$): 668.3256; found: 668.3411.

Undecyl 5-acetamido-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosylonic acid (1)

Methyl (undecyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-*glycero*-_-D*galacto*-2-nonulopyranosid)onate (100.0 mg, 0.12 mmol) was dissolved in methanol (10 mL), and a sodium methoxide (25 mg) was added. The mixture was stirred for 30 minutes and neutralized with amberlite H^+ resin. The residue was finally purified by column chromatography using a gradient of DCM:MeOH (1:1) as eluent to afford the desired 1 as a white solid (75 mg, 93% yield).). $[\alpha]_D^{r.t}$ = +11.9 (c = 1.0, H_2O); ^{1}H-NMR (400 MHz, CD_3OD): δ 3.87-3.83 (m, 2H); 3.79-3.71 (m, 3H); 3.71-3.61(m, 3H); 3.6-3.4 (m, 2H); 2.74 (dd, 1H, J = 4.1, 12.0 Hz); 2.02 (s, 3H); 1.48-1.56 (m, 2H); 1.17-1.38 (m, 16H); 0.87 (t, 3H, J = 6.3 Hz). ^{13}C NMR (CD_3OD, 100 MHz): 174.6, 98.8, 77.3, 77.1. 75.4, 72.6, 72.2, 71.6, 71.2, 53.3, 40.8, 33.1, 30.1, 29.9, 25.7, 22.6, 13.5. HRMS m/z calc'd for $C_{22}H_{41}NO_9$ $(M-H)^-$: 462.2691; found:462.2698.



6 4

***Scheme S2*: Reagents and conditions :** (a) TsOH, Py; NaN_3, Acetone/H_2O; Pd-C, H_2, MeOH; (b) dodecanoic acid, DIC, NHS, dioxane/H_2O.

Compound 6 was synthesized using published procedure.

5-Acetamido-9- (dodecanyl) -3,5,9-trideoxy-D-glyceo-D-galacto-2-nonulosonic acid (4).

Dodecanoic acid (300 mg, 1.5 mmol) in N-hydroxysuccinimide (172 mg, 1.7 mmol) and DMAP (183 mg, 1.7 mmol) were dissolved in DCM (10 mL) and stirred at RT. After stirring for 4 h, the reaction mixture was filtered to remove urea and purified by colum chromatography PE : EA (1:1) to yield dodcanoic acid active ester. This was dissolved in dioxane (7 mL) and mixed with 5-Acetamido-9-amino-3,5,9-trideoxy-D-glyceo-D-galacto-2-nonulosonic acid **6** (120 mg, 0.5 mmol) with pH maintained between 8-9 with sodium biocarbohydrate solution. The mixture was stirred at RT for 48 h. The solvent was concentrated and the residue was purified by flash chromatography (DCM:MeOH 1:1) to afford the white solid (85 mg , 38.6%).$[\alpha]_D^{r.t}$ = -2.3 (c = 1.0, H_2O); ^{1}H-NMR (400 MHz, CD_3OD): δ 3.98-3.80 (m, 4H); 3.71 (t, 1H, J = 4.0 Hz);

3.56 (dd, 1H, *J* = 4.4 Hz); 3.15(dd, 1H, *J* = 4.0 Hz); 2.18 (t, 2H, *J* = 8.0 Hz); 2.11 (dd, 1H, *J* = 4.0, 2.8 Hz); 1.97 (s, 3H), 1.82 (t, 1H, *J* = 8.8 Hz), 1.59-1.54 (m, 2H), 1.37-1.18 (m, 16H) 0.87 (t, 3H, *J* = 10.0 Hz); ^{13}C NMR (CD_3OD, 100 MHz): 176.6, 174.7, 96.8, 71.3, 71.1. 70.4, 69.6, 53.3, 43.8, 40.4, 36.1, 31.1, 29.8, 29.5, 25.7, 23.7, 22.6, 13.5. HRMS m/z calc'd for $C_{23}H_{42}N_2O_9$ $(M-H)^+$: 488.2890; found:488.2808.

3. Synthesis of micelles 1 and 2

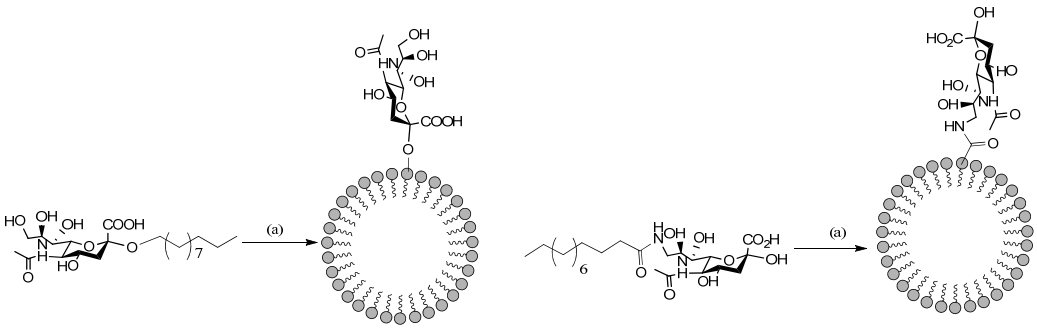


Figure S1. Synthesis of sialic acid micelles: (a) water

The amphiphiles **3** and **4** (5 mg) were dissolved in 1mL of milli-Q water respectively and sonicated for 30 mins at RT. The solution was then filtered using whatman polydisc 0.45 µm pore size nylon membrane filters to remove dust and large non-micellar aggregates.

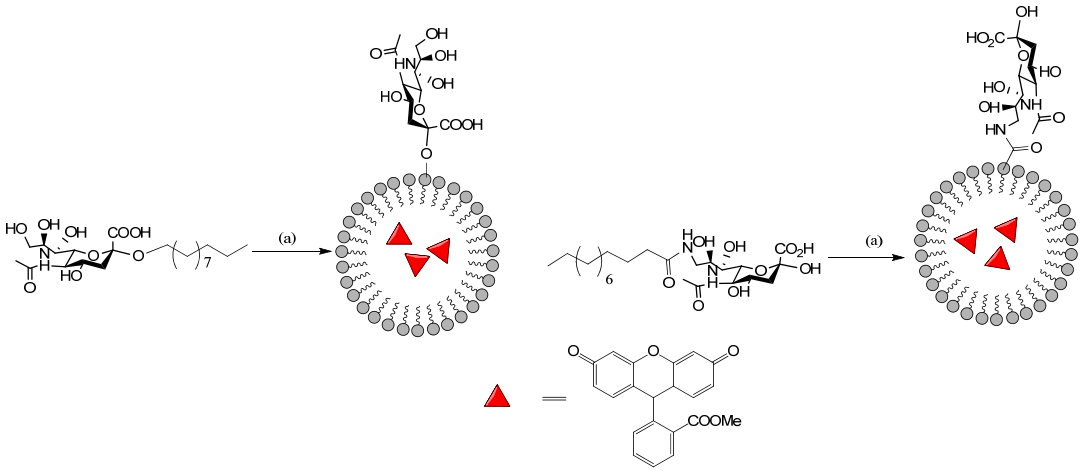


Figure S2. Synthesis of sialic acid micelles: (a) water/fluorescein

Fluorescent hosted micelles : 5 mg of amphiphiles **3** or **4** and 3 mg of fluorescein methylester were dissolved in 1 mL of milli-Q water and sonicated for 30 mins at RT. The fluorescein hosted

micelles were purified by filtering with a microcon centrifugal filter device with a cutoff range of 100 KD. Then filtrate was dissolved in 1 mL of milli-Q water and used as such for further experiment. The concentration of sialic acid was established by DMB-HPLC method. The concentration of fluorescein was determined by the O.D of the fluorescein.

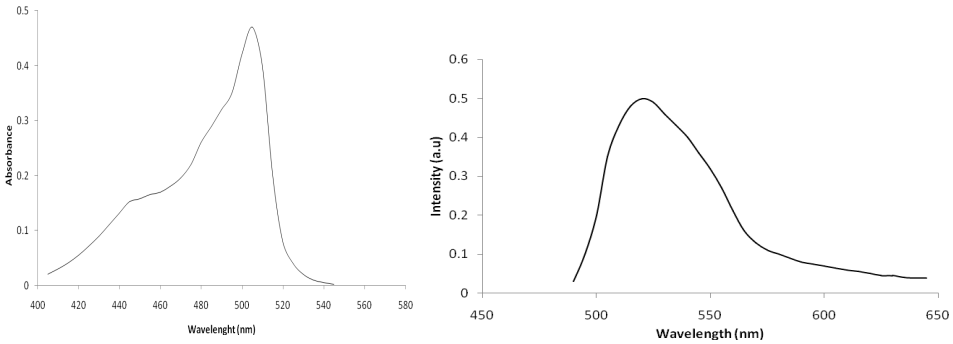


Figure S3. UV-visible and fluorescent spectra of the fluorescein hosted sialic acid micelles

4. **Dynamic light scattering (DLS).** The size of the micelles was assesed using DLS measurements. The experiments were performed using Malvern Zetasizer Nano ZS-90 apparatus utilizing 633 nm red laser. The samples were kept at 25^{O}C. All samples were systematically studied at 90 degree. The solutions were put in ordinary 10 mm in diameter glass cells. The minimum sample volume required for an experiment was 1 mL.

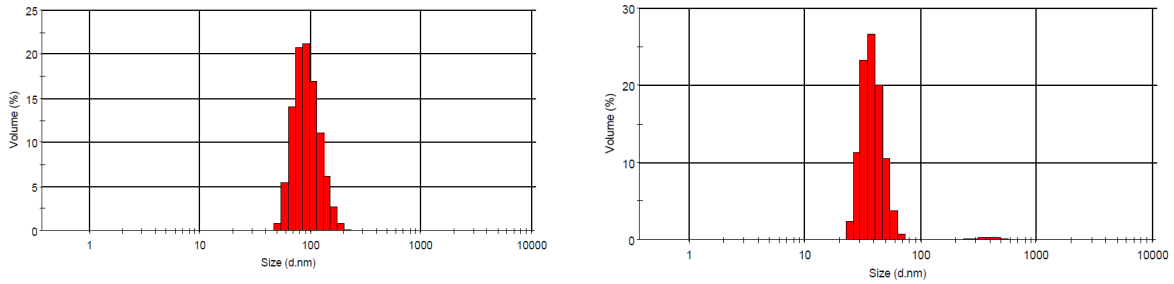


Figure S4. DLS of compound **1** (a) and **2** (b)

Atomic Force Microscopy (AFM) Measurement.

Topology of the micelles were investigated in the dry state with a AFM-JPK instrument with nanowizard-II setup. AFM is also attached with Zeiss inverted optical microscope.

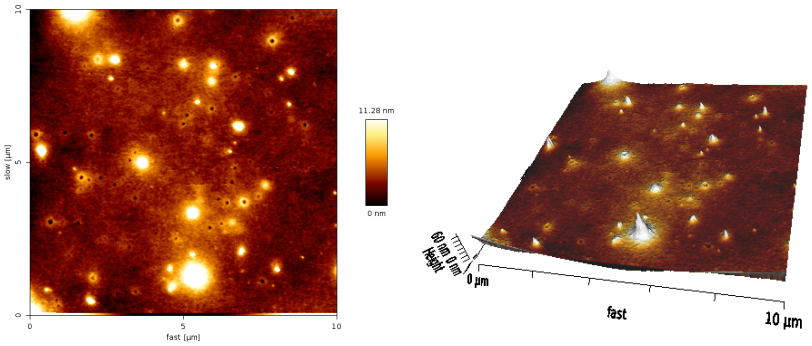


Figure S5. AFM topographical images of (a) compound **2** with 200 mM of $CaCl_2$ solution, (b) topology. The image scale is in micrometers.

Calcium mediated carbohydrate-carbohydrate interactions.

As shown in the figure 4a-4d, after each addition of calcium chloride (0, 100, 200, 300 mM) to **2**, a large aggregates of size 5000-9000 nm was observed. This is due to specific carbohydrate-carbohydrate interaction between sialic acid moiety of **2**. Similar experiment with **1**, didn't show any aggregation with calcium chloride (data not shown). Taken together, our data suggests that spatial orientation finely tunes sialic acid mediated carbohydrate-carbohydrate interactions.

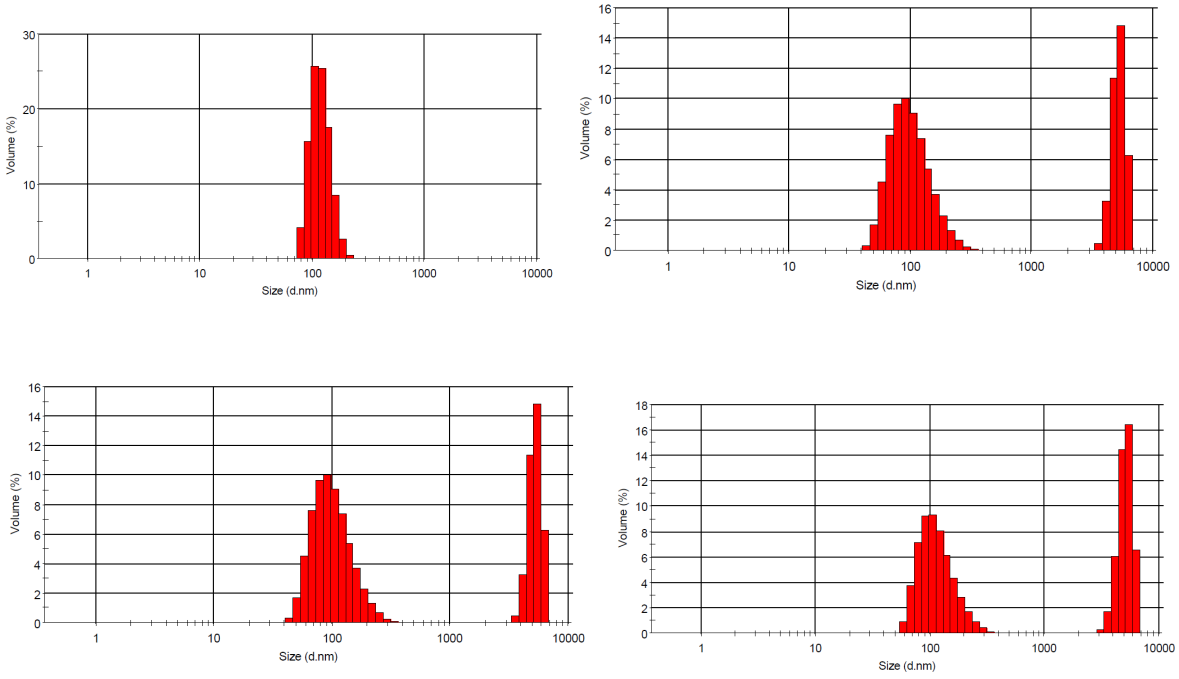


Figure S6. DLS of compound 2 in presence of different concentration of calcium chloride solution (a) no $CaCl_2$; (b) 100 mM; (c) 200 mM and (d) 300 mM respectively

5. **Surface plasmon resonance (SPR).** Binding kinetics were determined by SPR using a BIACORE 300 biosensor instrument (GE Biosystems). *Sambucus Nigra* agglutinin (SNA) and *Limax flavus* agglutinin (LFS) was purchased from Vector lab and E- and P-selectin were purchased from Calbiochem (EMD Biosciences, Darmstadt, Germany). CD22-Fc was gifted by Prof. Ajit varki. The CM5 chip and different running buffer (Table 1) were obtained from GE Healthcare Life Science (India). Neu5Acα(2-6)gal, neuraminic acid and lactose were as an inhibitor. All SPR experiments were performed using Biacore 3000 (Australian biobest biotechnology service, Australia). For the preparation of lectin/protein-coated surfaces, CM5 chip was activated with EDC and NHS followed by injecting 25 $\mu g \cdot mL^{-1}$ of lectin at a flow rate of 10 $\mu l \cdot min^{-1}$for 10 mins. The unreacted carboxylic active ester were neutralized with 1 M ethanolamine for 10 mins. As a control, cell was treated with EDC/NHS as described above.[1] This followed by injecting 1 and 2 (5, 10, 20, 30, 40 and 50 μM) for 60 s at 10 $\mu L \cdot min^{-1}$, followed by regeneration using respective inhibitor at 30 $\mu L \cdot min^{-1}$ for 300 s. The equilibrium dissociation constant (K_D) was determined globally by fittin to the kinetic simultaneous K_a/K_d model, assuming Langmuir (1:1) binding, using BIAevaluation software (BIAcore). The surfaces were strictly regenerated with multiple pulses of 2 M NaCl, 1.5 M glycine-HCl, pH 2.0 followed by an extensive wash procedure using running buffer.

Lectin	Specificity	Inhibitor used	Running buffer
Sambucus nigra agglutinin (SNA)	Sialic acid	200 mM neuraminic acid	HBS-EP; 10 mM HEPES, pH 7.5, 10 mM NaCl, 10 mM $CaCl_2$, 0.005% surfactant P20
Limax flavus agglutinin (LFA)	Sialic acid	200 mM neuraminic acid	HBS-EP; PBS, 10 mM $CaCl_2$ and 0.005% surfactant P20
E - selectin	Sialyl lewisX	200 mM Neu5Acα(2-6)gal	HBS-EP; 10 mM PBS, 0.005% surfactant P20
P - selectin	Sialyl lewisA	200 mM Neu5Acα(2-6)gal	HBS-EP; 10 mM PBS 0.005% surfactant P20

CD22-Fc	Neu5Ac/Gcα(2-6)gal	200 mM Neu5Acα(2-6)gal	HBS-EP; 10 mM PBS 0.005% surfactant P20

Table S1. Different sialic acid binding proteins and its specificities.

Lectins	**Compound 1**			**Compound 2**		
	K_a	K_d	K_D (μM)	K_a	K_d	K_D (μM)
Sambucus nigra agglutinin (SNA)	(0.42 ± 0.05) X 10^5	(0.6 ± 0.01) X 10^{-2}	0.146	(0.35 ± 0.05) X 10^5	(0.45 ± 0.01) X 10^{-2}	0.129
Limax flavus agglutinin (LFA)	(0.37 ± 0.02) X 10^5	(0.55 ± 0.03) X 10^{-2}	0.149	(1.12 ± 0.03) X 10^4	(0.15 ± 0.07) X 10^{-2}	0.134
E-selectin	(0.44 ± 0.15) X 10^4	(0.14 ± 0.03) X 10^{-2}	0.32	(0.39 ± 0.15) X 10^4	(0.12 ± 0.03) X 10^{-2}	0.31
P-selectin	(0.56 ± 0.1) X 10^4	(0.19 ± 0.03) X 10^{-2}	0.35	(1.2 ± 0.1) X 10^4	(0.43 ± 0.03) X 10^{-2}	0.36
CD22-Fc	(0.73 ± 0.13) X 10^4	(0.31 ± 0.07) X 10^{-2}	0.43	-	-	-

Table S2. Kinetic parameters for the different SBM interaction with compound **1** and **2**.

6. **Fluorescence-activated cell sorting assay.** CHO cells stably transfected with full-length human CD22 cDNA were cultured in α-MEM supplemented with 10% fetal calf serum and L-glutamine. Cells were incubated with a solution of fluorescein labelled compound **1** and **2** (50 μM in PBS, pH 7.4). After 1 h incubation at 4^0C, cells were washed with phosphate buffered saline (PBS). The cells were then collected with PBS containing 1% FCS by shearing force. Binding of micelles and CD22 protein on CHO cells were measured by flow cytometry using a FACSCantoTM II flow cytometer (Becton Dickinson and Co., Mountain View. CA). Cells were gated on living cells and fluorescence channel FL-2 was used to detect CHO cells that had micelles on it. All data were analyzed with the FlowJo software.

References .

(a) J. H. Myung, K. A. Gajjar, R. M. Pearson, C. A. Launiere, D. T. Eddington, S. Hong, *Anal. Chem*., **2011**, 83, 1078; (b) P. Anton van der Merwe, P. R. Crocker, M. Vinson, A. N. Barclay, R. Schauer, S. Kelm, *J. Bio. Chem*. **1996**, 271, 9273; (c) J. A. W. Halliday, A. H Franks, T. E. Ramsdale, R. Martin, E. Palant, *Glycobiology*, **2001**, 11, 557; (c) G. Mondal,

U. Chatterjee, H. R. Das, B. P. Chatterjee, *Glycoconj. J., 2009*, 26, 1225; (d) H. Ueda, H. Matsumoto, N. Takahashi, H. Ogawa, *J. Bio. Chem*. **2002**, 277, 24916; (e) R. karlsson, A. michaelsson, L. Mattsson, *J. Immunol. Methods*. **1991**, 15, 229.

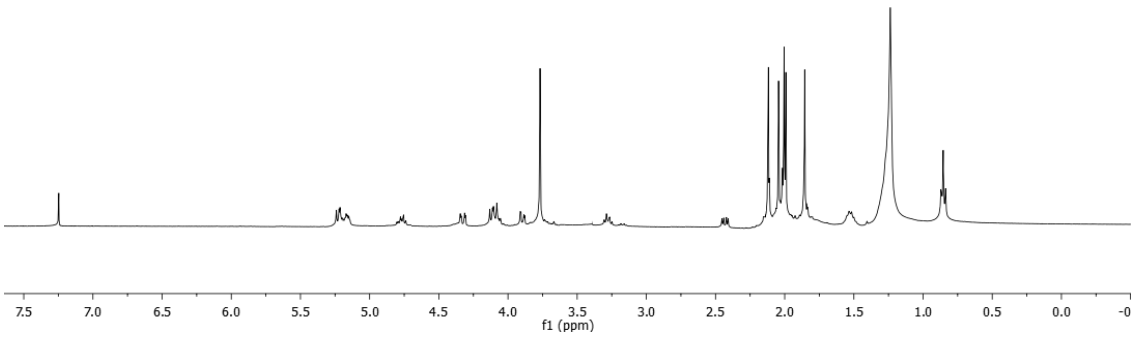


Figure S7. ^{1}H-NMR of **5**

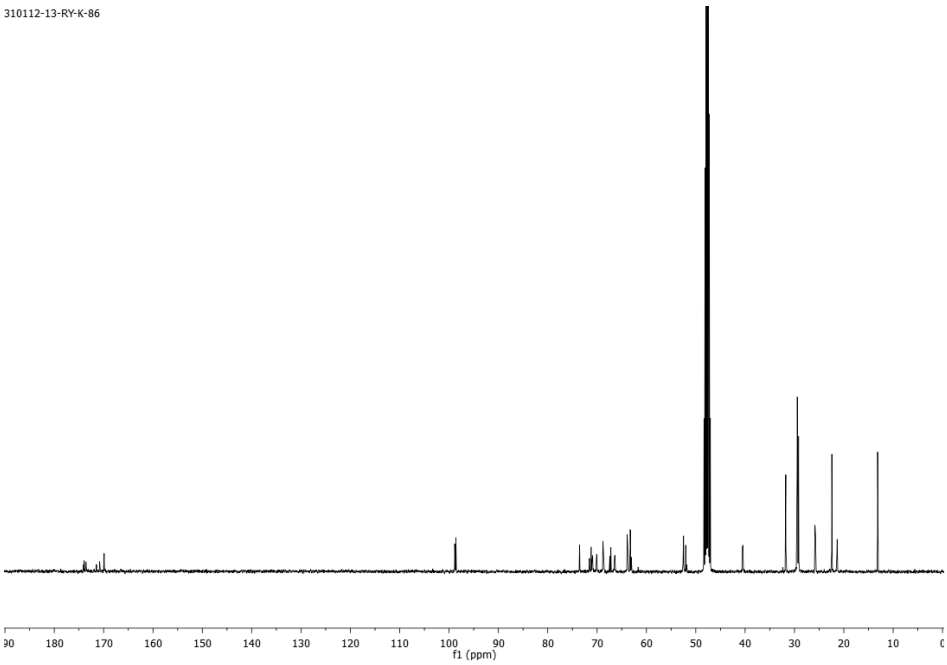


Figure S8. ^{13}C-NMR of **5**

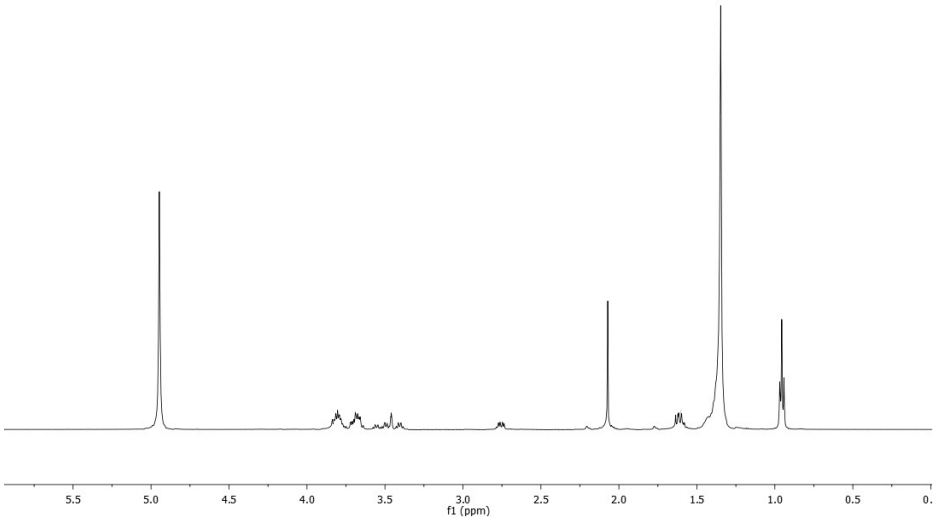


Figure S9. ^{1}H-NMR of **3**

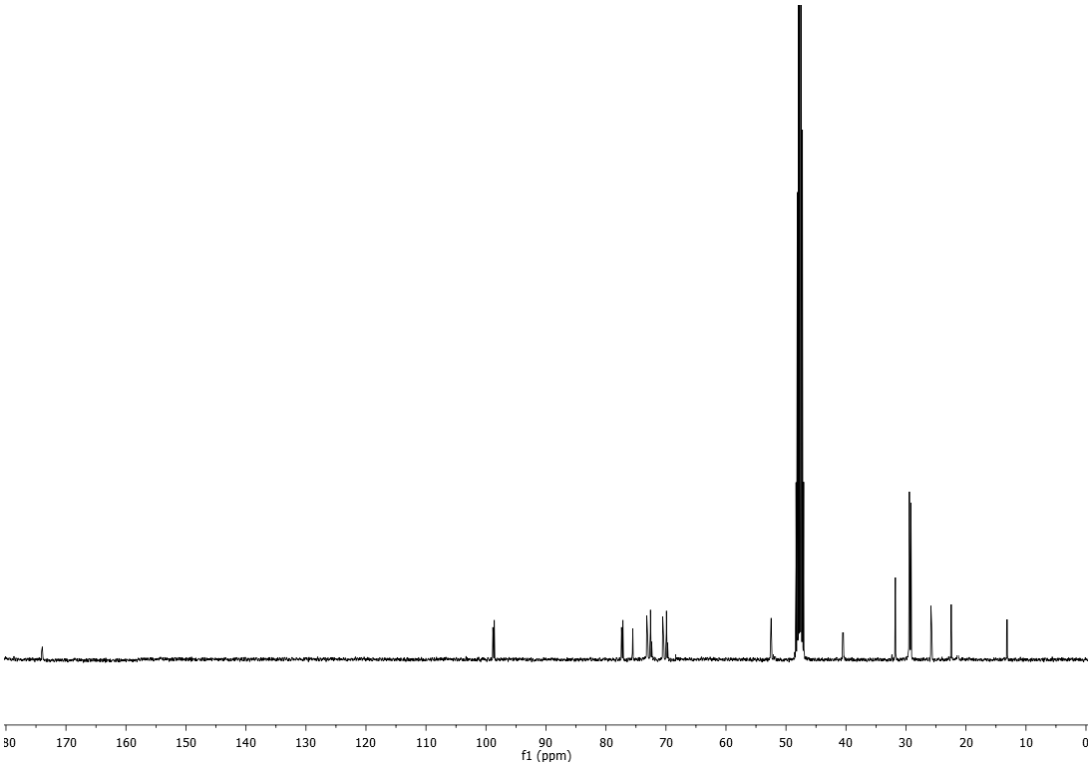


Figure S10. ^{13}C-NMR of **3**

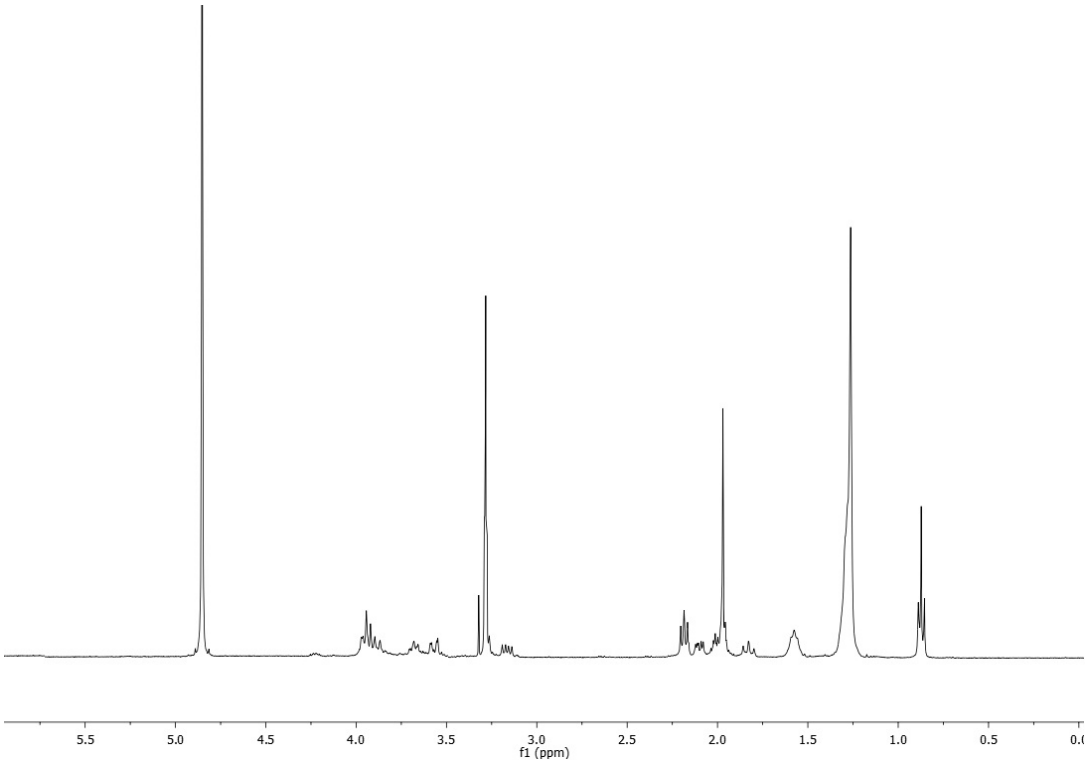


Figure S11. ^{1}H-NMR of **4**

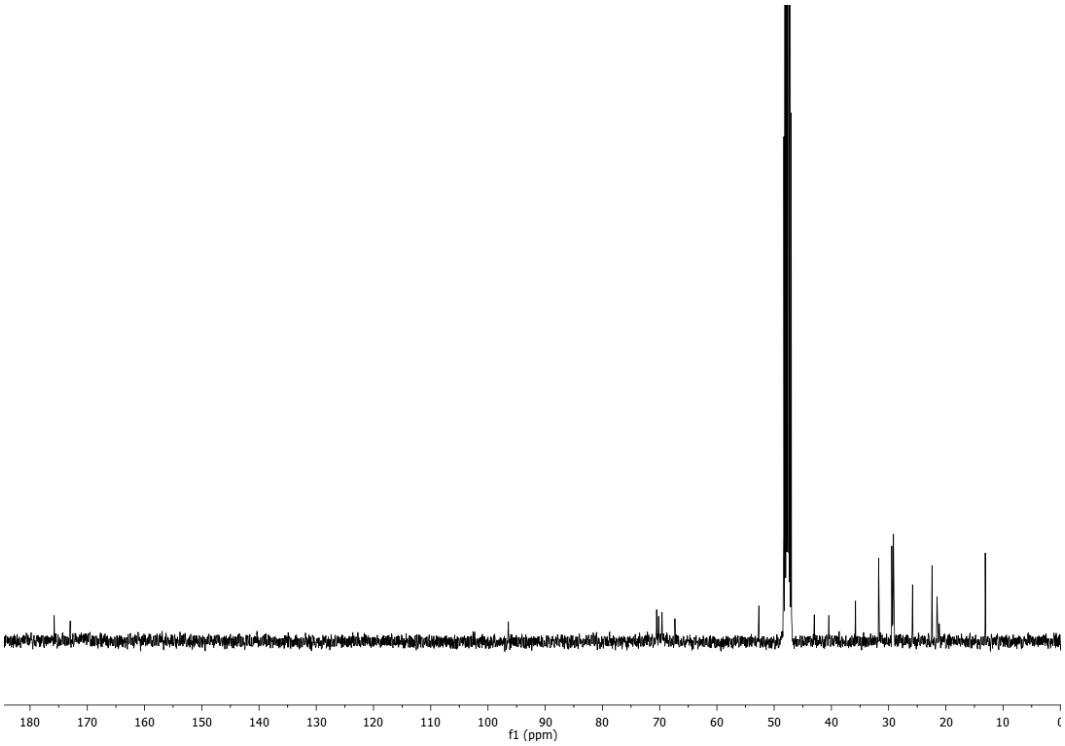


Figure S12. ^{13}C-NMR of **4**

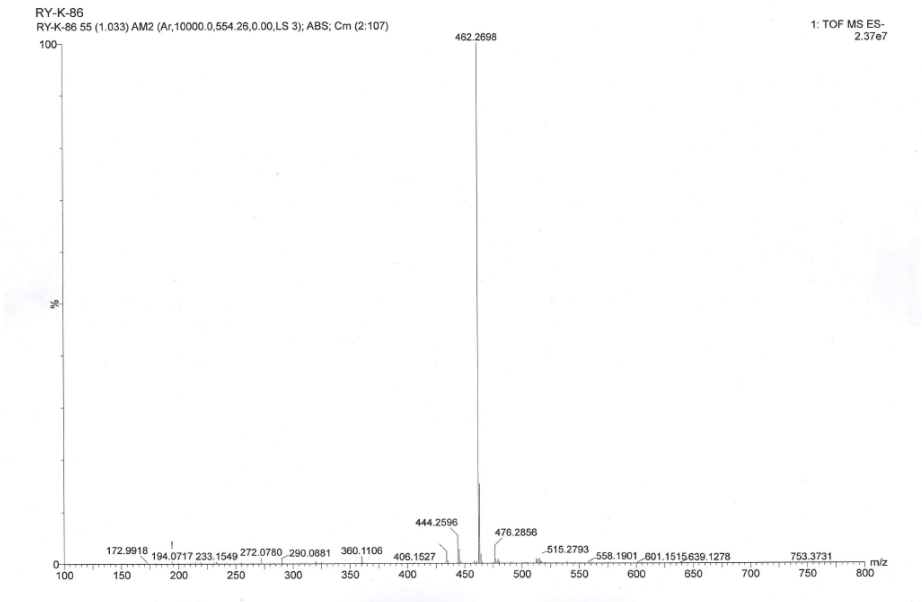


Figure S13. HRMS of **3**

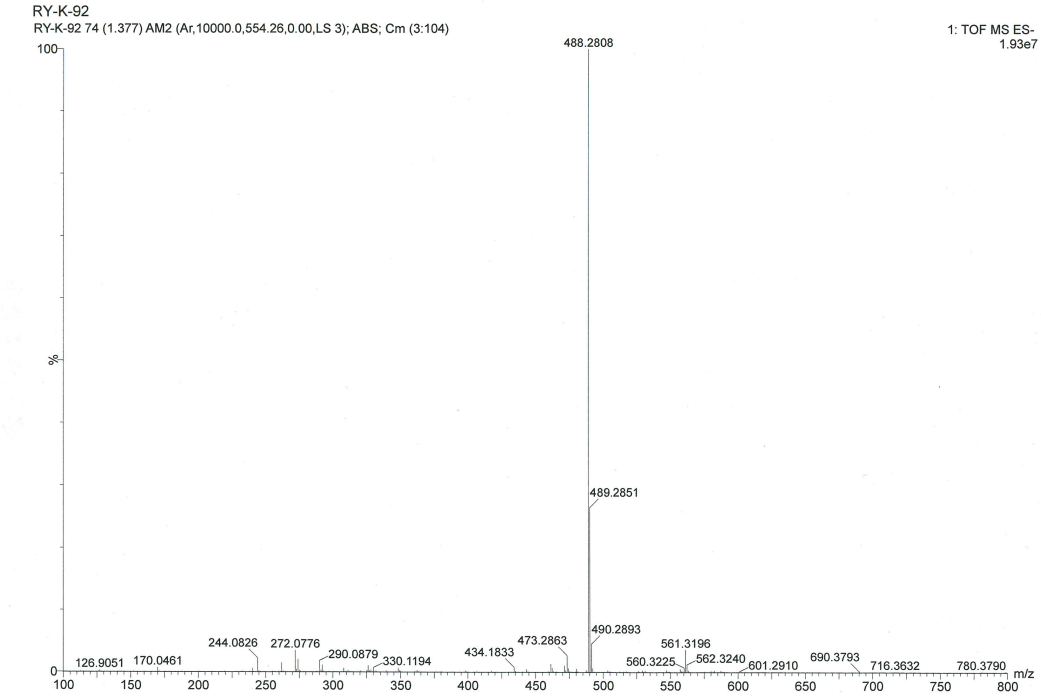


Figure S14. HRMS of **4**