Rationally Designed Peptidomimics Biosensor for Sialic acid on Cell Surfaces

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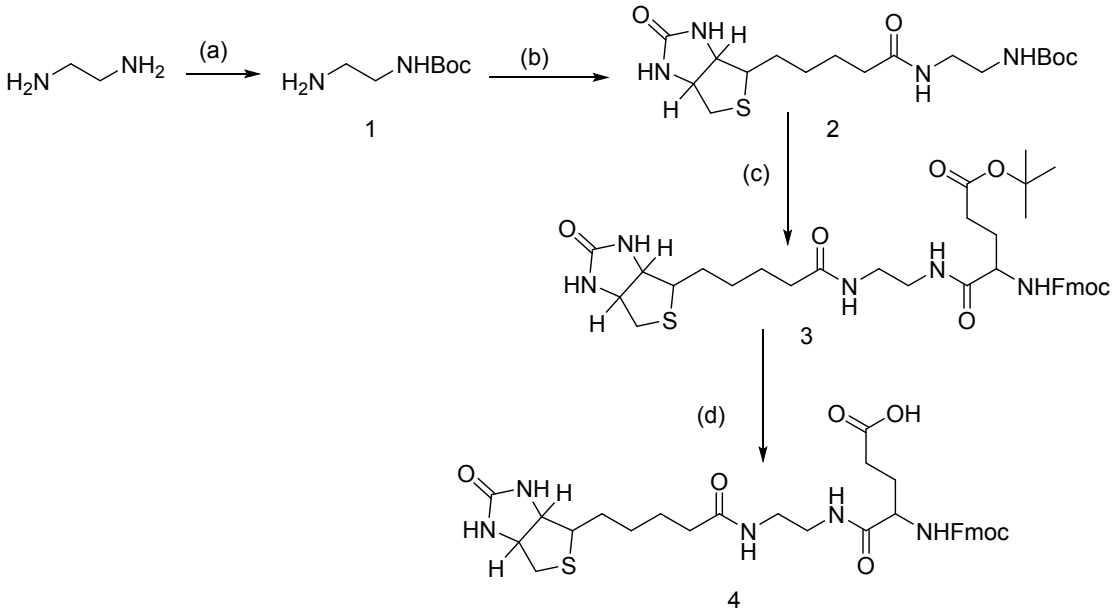
1. General Methods

General Experimental Details. Commercial grade reagents and solvents were used without further purification except as indicated below. Deionized water was obtained from an inhouse purification system. 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 CAM 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 Jeol 400 MHz (or 100 MHz for 13C) using residual solvents signals as an internal reference ($CDCl_3$ δ H, 7.26 ppm, δ c 77.3 ppm and CD_3OH δ H 3.31 ppm, δc 49.0 ppm). The chemical shifts (δ) are reported in *ppm* and coupling constants (J) in Hz. DMEM media was

purchased from Invitrogen. HeLa, MCF-7, MDA-MB, and NIH-3T3 cells were obtained from the National Centre for Cell Science (NCCS), Pune. High resolution mass spectra (HRMS) were recorded with an Agilent 6210 ESI-TOF mass spectrometer. University of Pune, Zoology department microarray scanner facility was used to read the microarray plates. Biotinylated-SNA, FITC-conjugated ConA, PNA and UEA1 was purchased from vector labs. Energy-minimized structures were calculated by molecular mechanics using MM2 in CS Chem 3D.[1]

2. Synthesis of Fmoc-biotin precursor.



Scheme S1. Synthesis of **4** : (a) Boc_2O, DCM 0 °C-RT, 12 h, 80%; (b) Biotin, EDC, HOBt, DMF, 12 h, 80%; (c) 40%TFA, DCM, 2 h, 90%; FmocGlu(O^{t}Bu)OH, EDC, HOBt, DMF, 12 h, 73%; (d) 25% TFA, DCM, 2h, 98%.

Synthesis of **1**. Synthesis of mono Boc protected ethylene diamine was carried out as reported in the literature.[2]

Synthesis of **2.** Biotin (1 g, 4.09 mmol), HOBt (0.66 g, 4.91 mmol) and EDCI (0.93 g, 4.91 mmol) were stir in anhydrous DMF at ice cold codition for 15 min. To this solution mono-Boc protected ethylene diamine (0.65 g, 4.09 mmol), dissolved in anhydrous DMF was slowly added. The corresponding reaction mixture was allowed to stir for 12 h. After completion of reaction (monitored by TLC) water (100 mL) was added and the aqueous layer was extracted with DCM (3 x 80 mL). The combined organic layer was washed with $NaHCO_3$ (50 mL) to remove the

unreacted free acid followed by washing with $KHSO_4$ (50 mL) to remove the unreacted free amine. The combined organic layer was washed with $NaHCO_3$ (50 mL) followed by water (3 x 50 mL) and brine. The organic layer was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product obtained was purified by silica gel chromatography (with 6% MeOH in DCM) to afford compound **2** as a white solid. Yield: 1.2 g, (80 %). Molecular Formula, $C_{17}H_{30}N_4SO_4$. [Mass: calculated 386.1988, observed (M + Na) 409.1890].

^{1}H NMR (CD3OD, 400 MHz) δ_H: 4.47 (dd, *J* = 7.8, 4.3 Hz, 1H), 4.28 (dd, *J* = 7.9, 4.5 Hz, 1H), 3.75-3.64 (m, 1H), 3.34 – 3.30 (m, 1H), 3.26 – 3.15 (m, 4H), 3.12 (t, *J* = 6.0 Hz, 2H), 2.95 – 2.86 (m, 1H), 2.68 (d, *J* = 12.7 Hz, 1H), 2.24 – 2.13 (m, 2H), 1.76 – 1.51 (m, 4H), 1.40 (s, 9H).

Synthesis of 3.

Compound 2 (1.3 g, 3.4 mmol) was dissolved in dichloromethane/trifluoroacetic acid (3:1, 10 mL) and stirred at room temperature for 2 h. The solvent was evaporated in vacuo, the residue was re-dissolved in anhydrous DMF (20 mL) and the pH adjusted to 8 using triethylamine. FmocGlc(O^{t}Bu)OH (1 g, 2.3 mmol), HOBT (0.47 g, 3.5 mmol) and EDCI (0.67 g, 3.5 mmol) were added to the above mixture and stirred for 12 h at rt. Finally, the mixture was concentrated in vacuo and purified by flash chromatography to afford **3** as a thick liquid Yield: 1.58 g, (73 %). Molecular Formula, $C_{36}H_{47}N_5SO_7$. [Mass: calculated 693.3196, observed (M + H^+) 694.3271].

^{1}H NMR (CD_3OD, 400 MHz) δ_H: 7.77 (d, *J* = 7.5 Hz, 2H), 7.64 (t, *J* = 6.5 Hz, 2H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.33 – 7.25 (m, 2H), 4.47 – 4.29 (m, 3H), 4.21 (t, *J* = 6.2 Hz, 2H), 4.02 (dd, *J* = 9.2, 5.1 Hz, 1H), 3.34 – 3.31 (m, 1H), 3.26 – 3.21 (m, 2H), 3.14 – 3.06 (m, 1H), 2.85 (dd, *J* = 12.7, 4.9 Hz, 1H), 2.64 (d, *J* = 12.7 Hz, 1H), 2.28 (t, *J* = 7.5 Hz, 2H), 2.20 – 2.09 (m, 2H), 2.08 – 1.97 (m, 1H), 1.81 (dt, *J* = 21.2, 7.2 Hz, 1H), 1.71 – 1.47 (m, 4H), 1.42 (s, 9H), 1.39 – 1.28 (m, 2H).

^{13}C NMR (100 MHz, Methanol D4) δ_C: 175.06, 173.21, 172.45, 164.73, 165.05, 143.59, 141.19, 127.50, 126.87, 124.92, 119.63, 80.50, 66.64, 61.87, 60.28, 55.54, 54.66, 53.46, 39.68, 38.81, 38.56, 35.48, 31.34, 28.33, 28.07, 27.03, 25.37.

Synthesis of **4**.

Compound **3** (1 g, 1.4 mmol) was dissolved in dichloromethane/trifluoroacetic acid (4:1, 10 mL) and stirred at room temperature for 2 h. The solvent was evaporated in vacuo, the residue was coevaporated with DCM.

3. Solid state synthesis of P-1 and P-2 peptide.

The readily available Rink amide resin with loading value 0.5-0.6 mmol/g was used and standard Fmoc chemistry was employed. The resin bound Fmoc group was first deprotected with 20% piperidine in DMF and the coupling reactions were carried out using *in situ* active ester method, using HBTU as a coupling reagent and HOBt as a recemization suppresser and DIPEA as a catalyst. All the materials used were of peptide synthesis grade (Sigma-Aldrich) and was used without further purification. Analytical grade DMF was purchased from Merck (India) and was distilled over P_2O_5 under vacuum at 45^oC, stored over 4Å molecular sieves for 2 days before using for peptide synthesis.

(a) Synthesis protocol for solid phase synthesis

The resin was pre-swollen overnight and the following steps were performed for each cycle.

- Wash with DMF 4 x 5 mL.
- 20 % piperidine in DMF 2 x 5 mL (15 min for each) for deprotection of Fmoc group.
- Wash with DMF 3 x 5 mL, MeOH 3 x 5 mL and DCM with 3 x 5 mL.
- Test for complete deprotection **(**chloranil test).
- Coupling reaction with amino acid, DIPEA, HOBt and HBTU (3 eq.) in DMF (1 mL).
- Repeat of the coupling reaction in NMP for better yield.
- Test for completion of coupling reaction (chloranil test).

This cycle was repeated for every amino acid.

General procedure for Fmoc deprotection

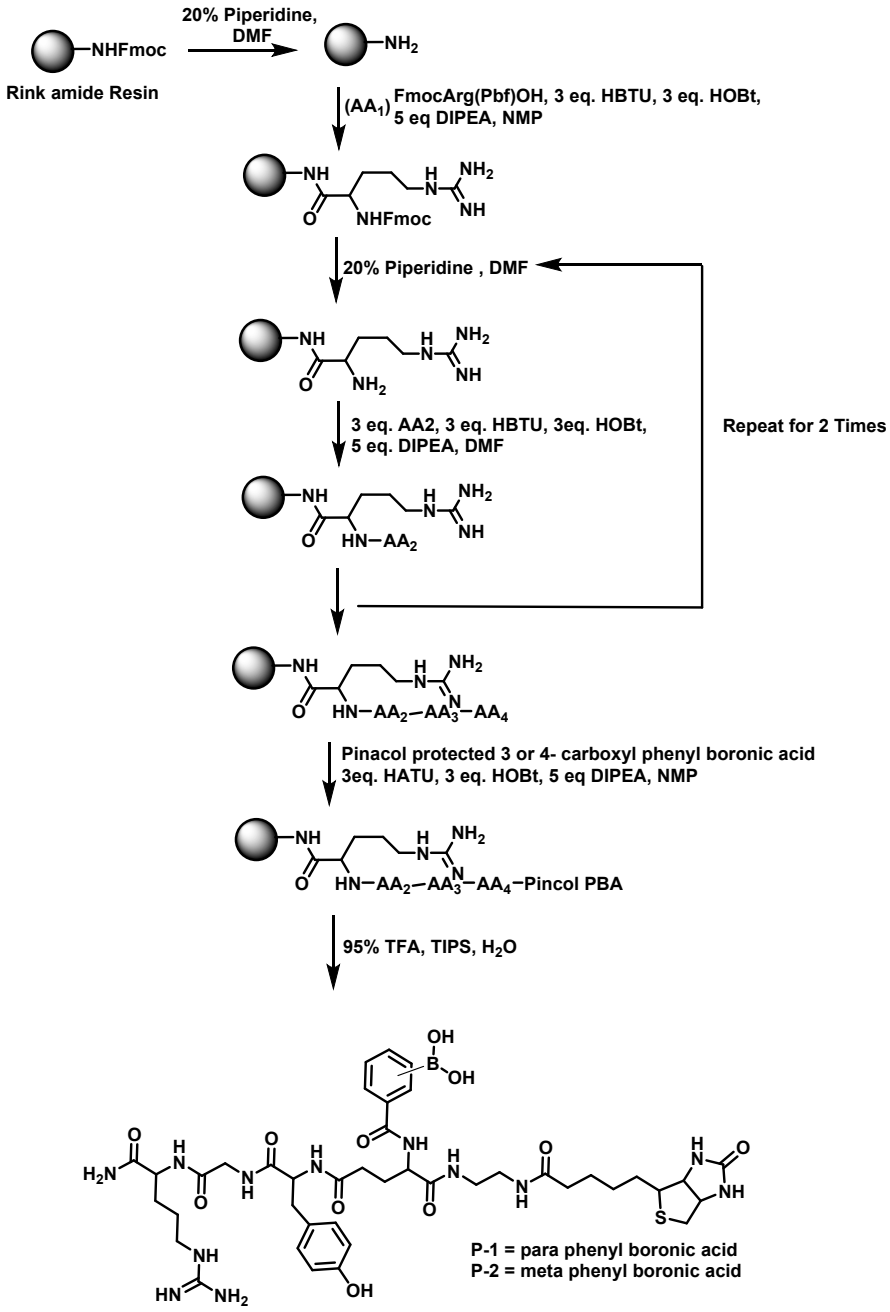
20% piperidine in DMF was added to the resin and the reaction mixture was kept for 15 min, drained and the piperidine treatment was repeated 3 times. Finally the resin was washed with DMF (3x), MeOH (3x) and DCM (3x).

(a) General procedure for peptide couplings on Rink Amide Resin

Fmoc-AA-OH (3 eq), HBTU (3 eq) and HOBT (3 eq) dissolved in DMF/NMP followed by DIPEA (7-8 eq) were added to the amino-functionalized resin in DMF. The mixture was kept for 2 h and last 5 min bubbled with N_2 and washed with DMF (3x), MeOH (3x) and DCM (3x). The loading value for peptide synthesis is taken as 0.5~0.6. The coupling reaction was repeated in NMP for better yield.

(b) General procedure for cleavage of peptides from the solid support

The dry peptide-resin (20 mg) was taken in round-bottomed flask to which of 95% TFA in H_2O and Triisopropylsilane (as scavengers, 2-3 drops) were added. The resulting mixture was kept for 2 h by gentle shaking. The mixture was filtered through a sintered funnel and the resin was washed with 3 x 5 mL of above solution. The filtrate was collected in pear shape round-bottom flak and evaporated under reduced pressure. The resin was washed with MeOH (3 X 5 mL) and the washings were evaporated to dryness. The residue obtained was dissolved in anhydrous methanol (0.1 mL) and to it anhydrous diethyl ether (4 x 1.5 mL) was added. The off-white precipitate obtained was centrifuged. The precipitation procedure was repeated twice to obtained peptide as a colourless powder.



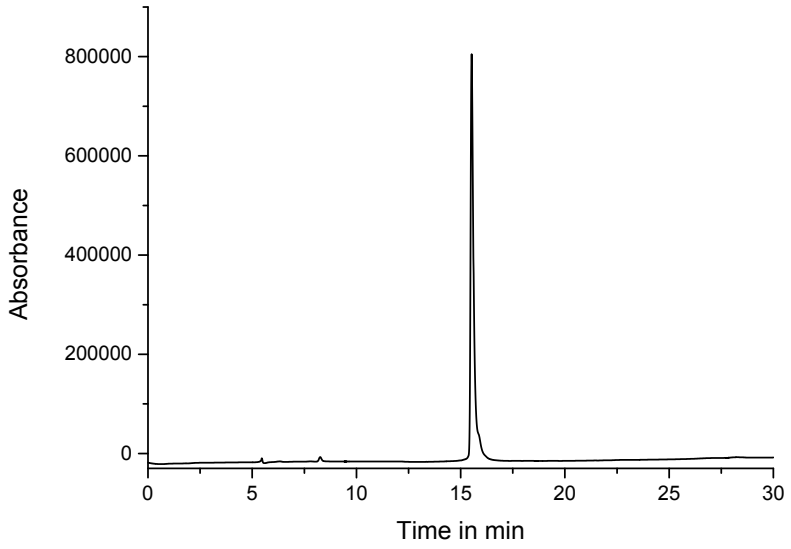
Scheme S2. Solid phase peptide synthesis of **P-1** and **P-2**.

4. HPLC Characterization.

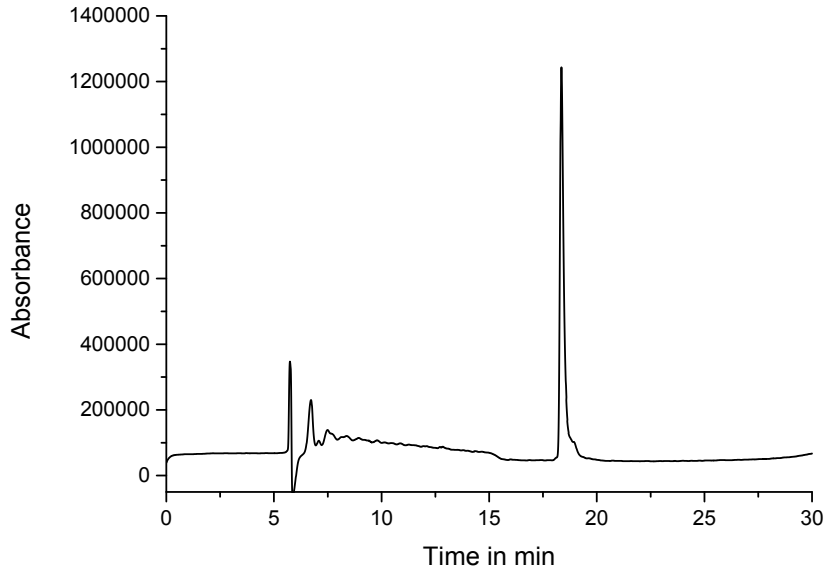
Peptides **P-1** and **P-2** were purified by semi preparative HPLC on reversed phase column using Waters 600 device equipped with a 2998-Photodiode array detector (PDA). The solvent system was 0.1% TFA in acetonitrile:water (5:95) - solution A and 0.1% TFA in acetonitrile:water (50:50) - solution B. A gradient of 0-100% A-B (in 30 min) and a flow rate of 3 mL/min were

used and the eluting solution was monitored at 220 nm. The fractions corresponding to the emergence of the peptide were collected and concentrated using a speed vacuum device to afford the neat peptides. The purity of the final products were further analyzed by analytical HPLC using a gradient of 0 to 100% B in 20 min at a flow rate of 1.5 mL/min. The peptides were found to be more than 95% pure.

HPLC chromatogram for Peptide **P-1**



HPLC chromatogram for Peptide **P-2**



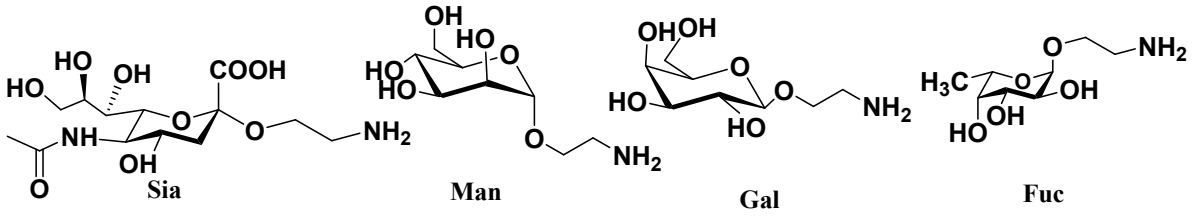
Peptide P-1 ^{1}H NMR (500 MHz, D_2O): 7.86 (d, *J* = 7.6Hz, 2H), 7.78 (d, *J* = 7.6Hz, 2H), 7.06 (d, *J* = 8.2Hz, 2H), 6.75 (d, J = 8.2Hz, 2H), 4.50-4.44 (m, 2H), 4.35-4.32 (m, 1H), 4.26-4.21(m, 2H), 3.84-3.70 (m, 2H), 3.45-3.40 (m, 1H), 3.37-3.32 (m, 1H), 3.29-3.24 (m, 2H), 3.10-3.07 (m, 3H), 3.03-2.99 (m, 1H), 2.89-2.84 (m, 2H), 2.68 (d, *J*=10Hz, 1H), 2.44-2.32 (m, 2H), 2.10-2.04 (m, 3H), 2.01-1.94 (m, 1H), 1.85-1.79 (m, 1H), 1.70-1.63 (m, 1H), 1.58-1.50 (m, 2H), 1.49-1.30 (m, 4H), 1.18-1.13 (m, 2H).

^{13}C NMR (500 MHz, D_2O): 177.02, 176.15, 175.00, 174.14, 173.57, 171.21, 170.05, 165.23, 156.60, 154.47, 134.42, 130.00, 130.41, 128.04, 128.61, 115.38, 61.85, 60.16, 55.54, 55.19, 54.10, 52.92, 42.47, 40.42, 39.66, 38.82, 38.45, 35.99, 35.52, 31.59, 28.03, 27.83, 27.46, 26.63, 25.07, 24.31.

Peptide P-2 ^{1}H NMR (500 MHz, D_2O):8.12 (s, 1H), 7.95 (d, *J* = 8.2Hz, 1H), 7.85 (d, *J* = 8.2Hz, 1H), 7.54 (t, *J* = 10Hz, 1H), 7.06 (d, *J* = 8.2Hz, 2H), 6.75 (d, J = 8.2Hz, 2H), 4.49-4.45 (m, 2H), 4.34-4.32 (m, 1H), 4.25-4.21(m, 2H), 3.83-3.70 (m, 2H), 3.45-3.40 (m, 1H), 3.37-3.32 (m, 1H), 3.29-3.24 (m, 2H), 3.10-3.07 (m, 3H), 3.01-2.99 (m, 1H), 2.88-2.83 (m, 2H), 2.68 (d, *J*=10Hz, 1H), 2.44-2.32 (m, 2H), 2.10-2.04 (m, 3H), 2.01-1.94 (m, 1H), 1.85-1.79 (m, 1H), 1.70-1.63 (m, 1H), 1.58-1.50 (m, 2H), 1.49-1.30 (m, 4H), 1.18-1.13 (m, 2H).

^{13}C NMR (500 MHz, D_2O): 177.00, 176.14, 174.98, 174.14, 173.62, 171.20, 170.29, 166.22, 163.07, 162.79, 156.69, 154.45, 137.76, 132.44, 130.38, 129.60, 128.45, 128.02, 115.38, 61.87, 60.18, 55.50, 55.17, 54.08, 52.92, 42.47, 40.42, 39.64, 38.88, 38.44, 35.96, 35.50, 31.57, 28.03, 27.82, 27.46, 26.66, 25.06, 24.30.

5. Microarray studies.



For the synthesis of **Sia**, **Man**, **Gal** and **Fuc** previously reported protocol was used.[3,4]

N-hydroxyl succinimide coated microarray plate was obtained from Arrayit company. Monosaccharides Sia, Man, Gal and Fuc monosaccharides at different concentrated (50, 10, 5, 2, 1, 0.5, 0.2 and 0.1 μM) were imprinted of volumn 1 μL with five replicates vertically places in each sub-array. After 1 h incubation, slides were washed with blocking buffer containing (ethanol amine (10 mM) in PBS (PH 7.8, 0.01 mM) for 1 h and washed several times with PBS (0.05% Tween-20). After drying, slides were incubated with 100 μM of peptide (**P-1**/**P-2)** (volume 1 nL) for 2 h followed by 0.1 μg of streptavidin-Cy3. In case of **SNA**, 0.1 μg (7.5 μM) of **SNA** in PBS (PH 7.8, 0.01 mM) was added and incubated for 1 h. Finally, the images were obtained using Array Worx microarray reader exciting 550 nm and emission 570 nm.

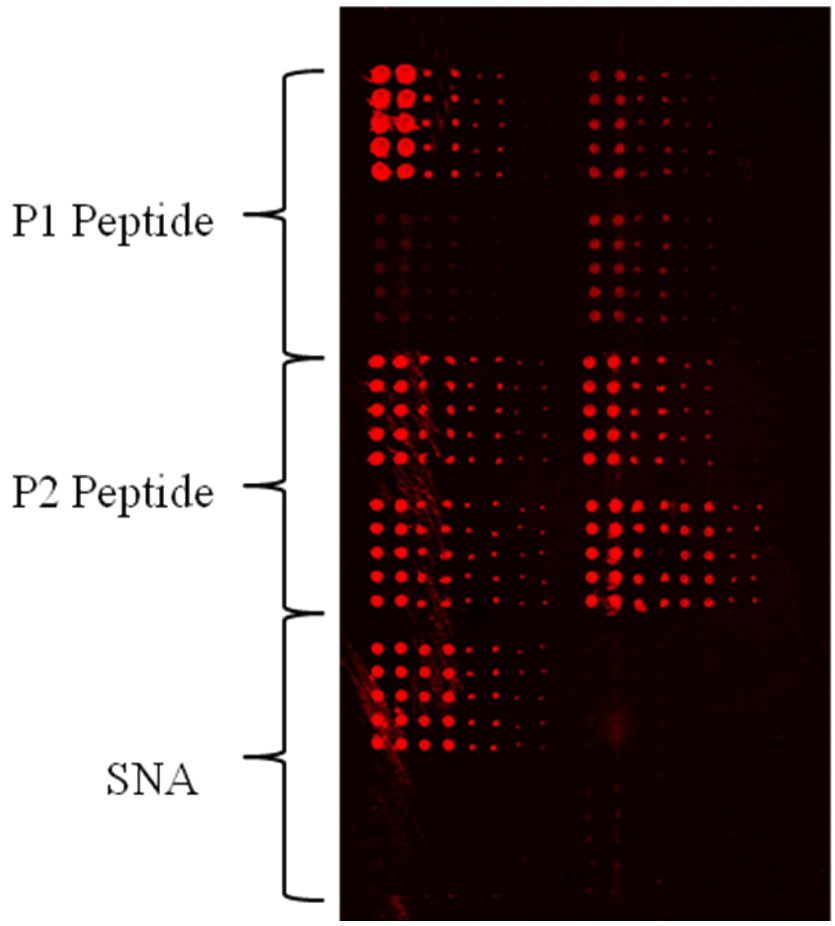


Figure S1. Microarray profile : **Sia**, **Man**, **Gal** and **Fuc** with **P-1**, **P-2** and **SNA** lectin. Sia (1, 3, 5); Man(2, 4, 6,); Fuc (7, 9, 11); Gal(8, 10, 12)

Determination of K_d of P-1/P-2 peptide Protocol.

N-hydroxyl succinimide coated microarray plate was obtained from Arrayit company and sialic acid at different concentrated of (50, 10, 5, 2, 1, 0.5, 0.2 and 0.1 μM) were imprinted with five replicates. After 1h incubation, slides were washed with blocking buffer. Slides were imprinted with **P-1**/**P-2** peptide at different concentration (5000, 1000, 500, 100 nM) Followed by streptavidin-Cy3 (0.1 μg). Finally, slides were washed with PBS (0.05% Tween-20). The images were obtained using Array Worx microarray reader exciting 550 nm and emission 570 nm K_d was calculated by equation 1. F_{max} represent maximum fluorescent intensity and [P] is the total peptide concentration.

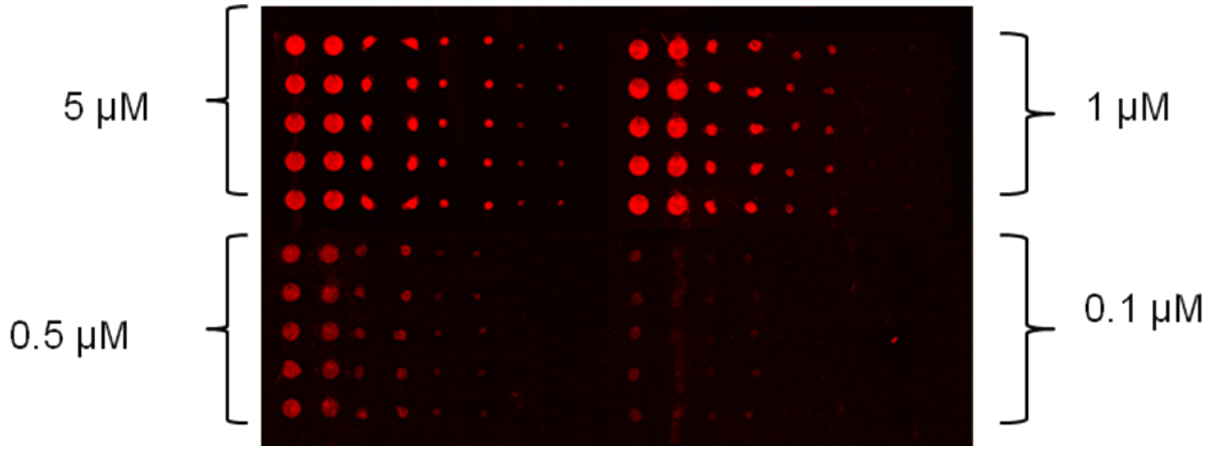


Figure S2. **Sia** binding affinity with different concentration of **P-1** peptide.

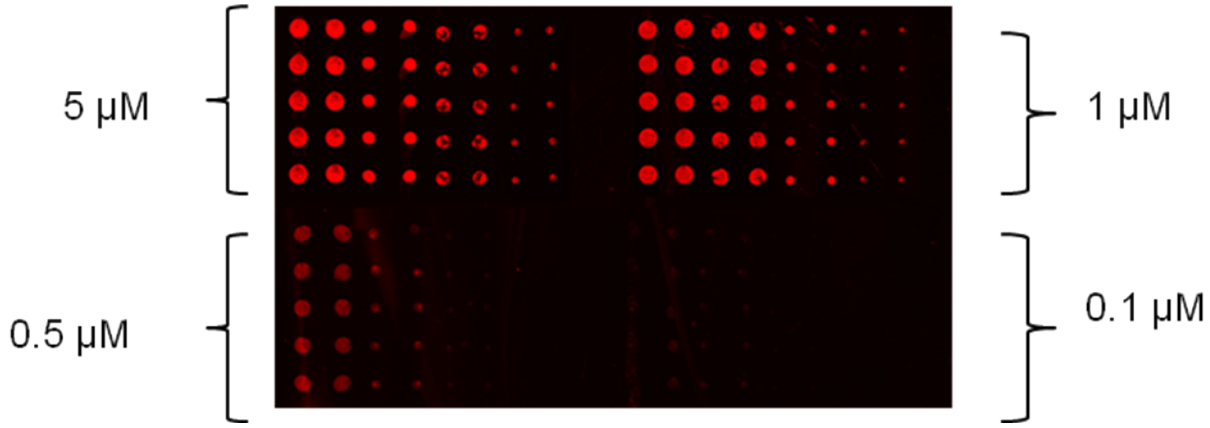


Figure S3. **Sia** binding affinity with different concentration of **SNA** lectin.

$$\mathbf{F} = \frac{\mathbf{F_{max}\,[P]}}{\mathbf{[P]} + \mathbf{K_d}} \qquad \text{------ (1)}$$

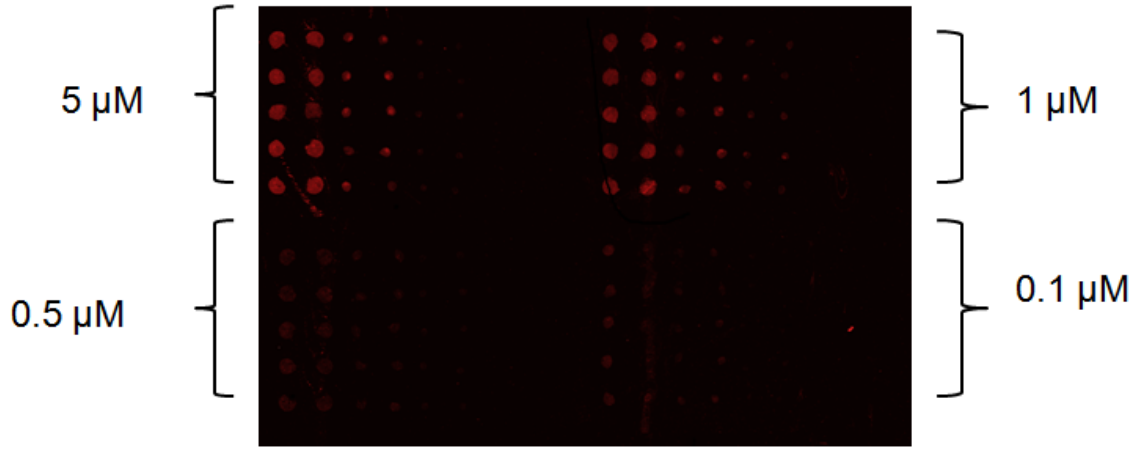


Figure S4. **Fuc** binding affinity with different concentration of **P-1** peptide.

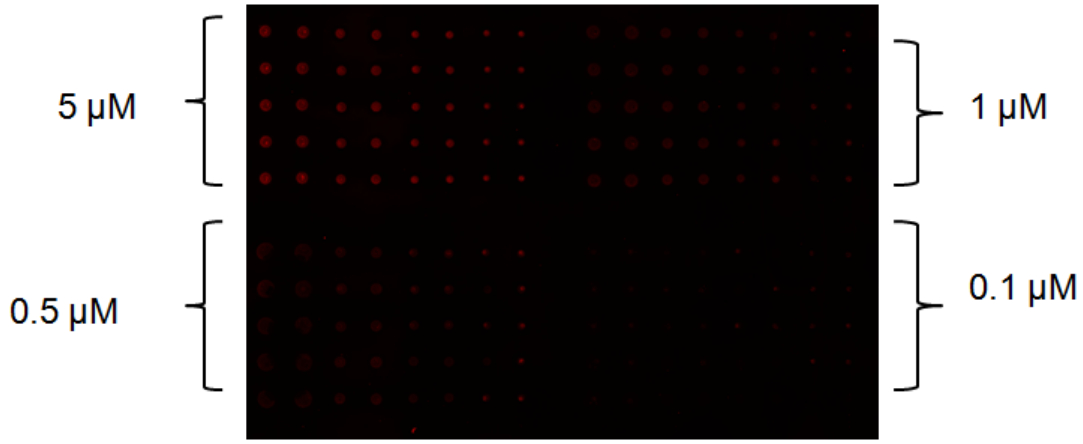


Figure S5. **Man** binding affinity with different concentration of **P-1** peptide.

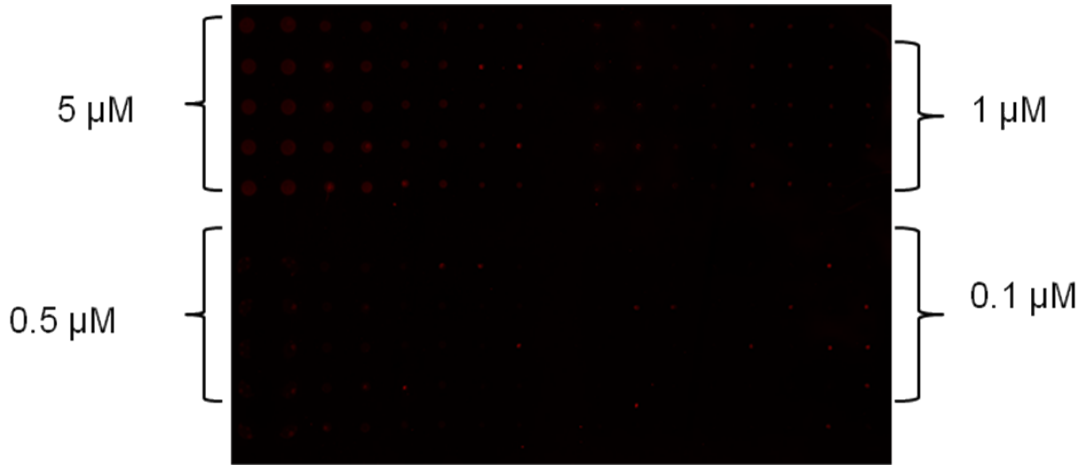


Figure S6. **Gal** binding affinity with different concentration of **P-1** peptide.

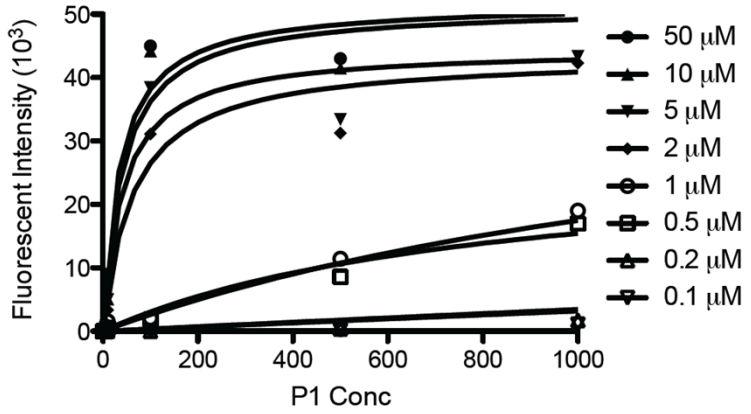


Figure S7. Binding curve of **P-1** with different concentration of Sia.

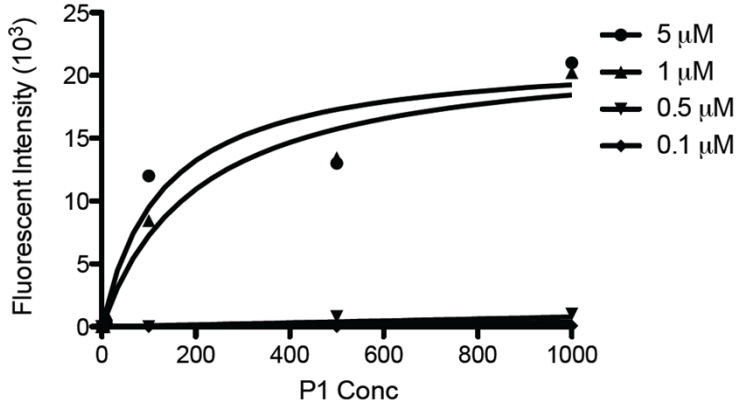


Figure S8. Binding curve of **P-1** with different concentration of Man.

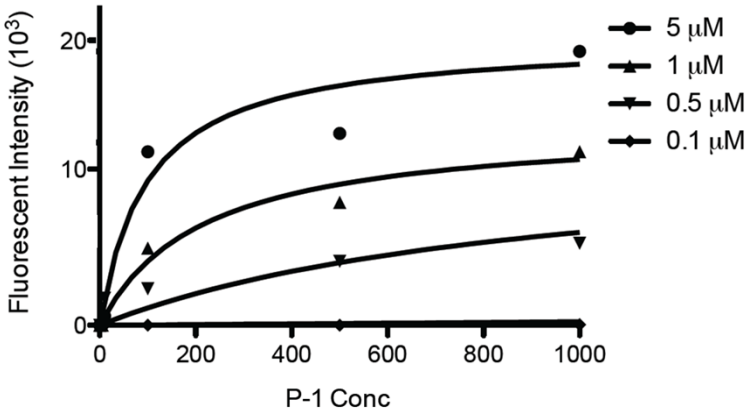


Figure S9. Binding curve of **P-1** with different concentration of Fuc.

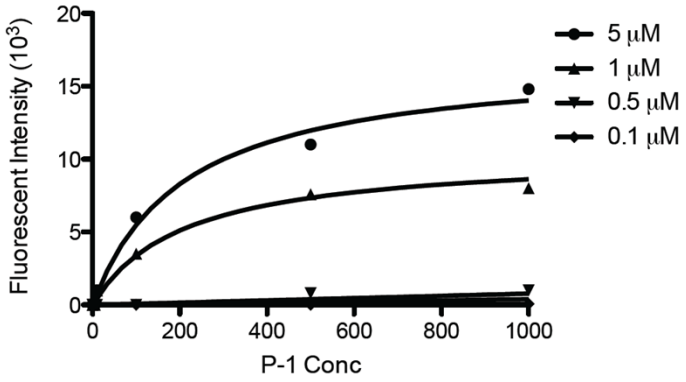


Figure S10. Binding curve of **P-1** with different concentration of Gal.

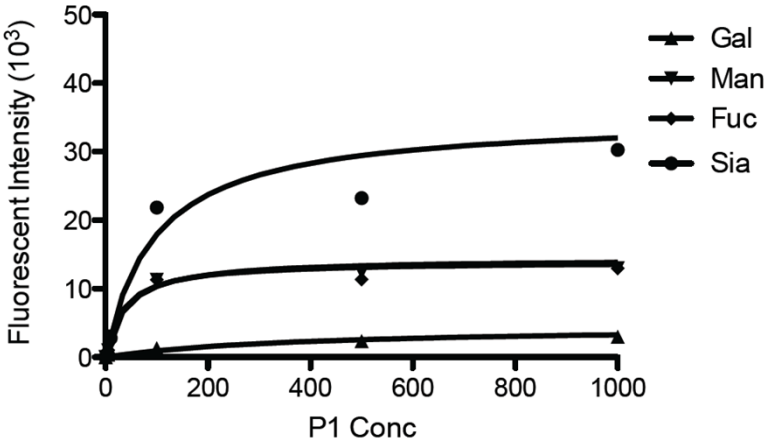


Figure S11. Binding curve of **P-1** with different concentration of sugars.

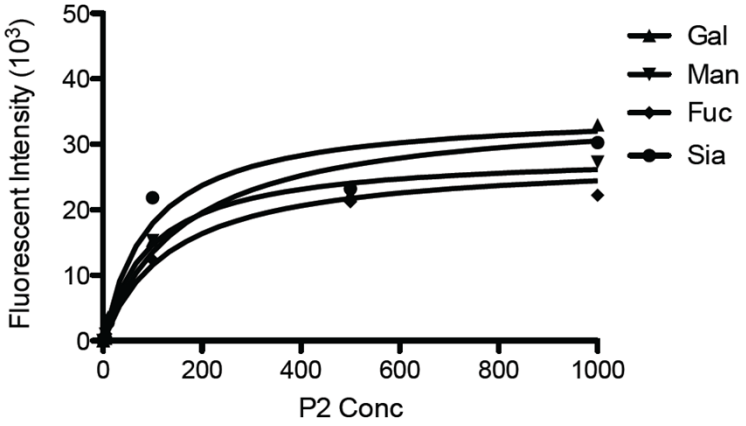


Figure S12. Binding curve of **P-2** with different concentration of sugars.

Sugar	P-1 (μM)	P-2 (μM)	SNA (μM)
Sialic acid (Sia)	0.0754	0.0786	0.0705
Mannose (Man)	10.23	0.104	-
Galactose (Gal)	2030	0.0753	-
Fucose (Fuc)	10.56	0.127	

Table S1. K_d values of **P-1, P-2 and SNA** with different sugar substrates.

6. Streptavidin-Peptidomimic conjugations.

Streptavidin-Peptidomimic complexation was performed by using standard streptavidin-biotin conjugation. Briefly, **P-1** or **P-2** (300 μM) and streptavidin (10 ug/ml) were dissolved in PBS (pH 7.4) and vertex together for 10 min at RT. The complex was kept as such for another 2 h at RT and used as such for biological studies.

7. Confocal laser scanning microscope images.

Cell lines	Growth Media
HeLa	Cells were grown at 37 °C and 5% CO_2 in DMEM medium containing 10% fetal bovine serium and 0.1% stretomycine.
NIH-3T3	Cells were grown at 37 °C and 5% CO_2 in DMEM medium containing 10% fetal bovine serium and 0.1% stretomycine
MDA-MB	Cells were grown at 37 °C and 5% CO_2 DMEM medium containing 10% fetal bovine serium and 0.1% stretomycine

All cells were detached by treating with 0.05% trypsin/EDTA solution. FITC-conjugated **P-1**, **P-2** and **FITC-SNA** were feeded for 2 h and cells were washed twice with PBS and fixed with 500 μL paraformaldehyde (3.7% in PBS, pH = 7.4) by incubating for 10 min at 4 °C. The paraformaldehyde was aspirated and cells were washed with PBS to remove the excess of paraformaldehyde. The cells were then treated with 2 μg mL^{-1} Hoechst 33342 to stain nuclei. Then cells were washed three times with PBS buffer and mounted on a glass slide using 5 μL antifed-mounting medium. The fluorescence of Hoechst 33342 and FITC complexes were excited with an argon laser at 405 nm and 450 nm, and the emission was collected through 403–452 nm and 500-530 nm filters, respectively.

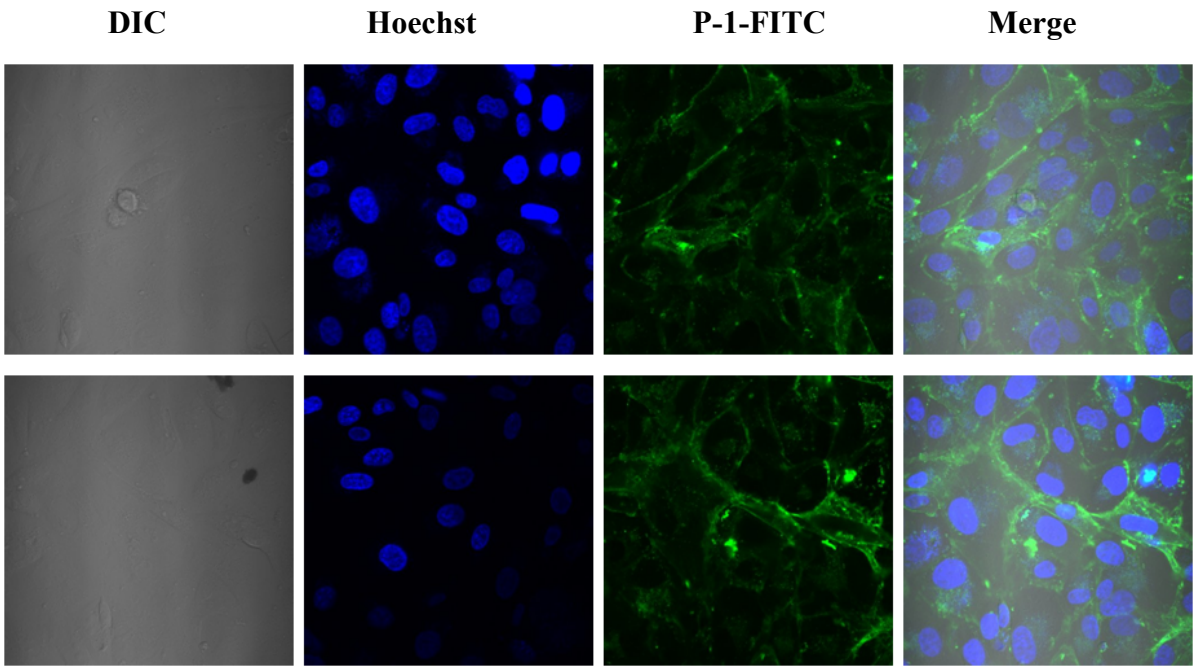


Figure S13. Fluorescence images of NIH-3T3 cell type with **P-1** after 30 mins incubation

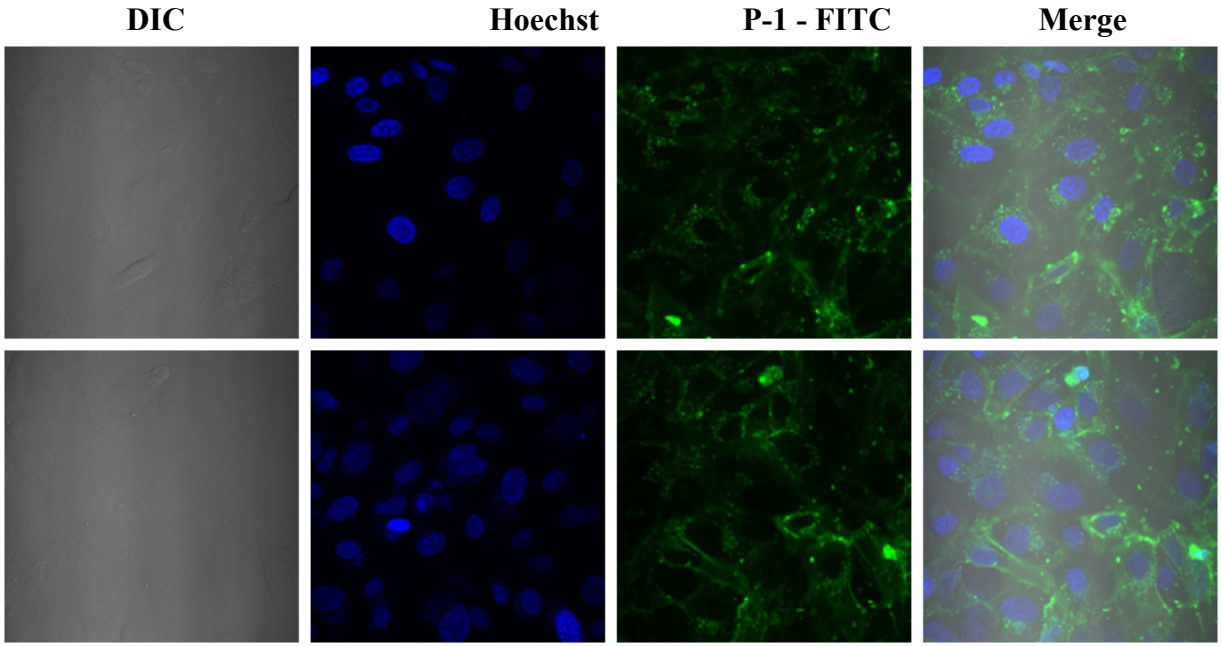


Figure S14. Fluorescence images of NIH-3T3 cell type with **P-1** after 120 mins incubation

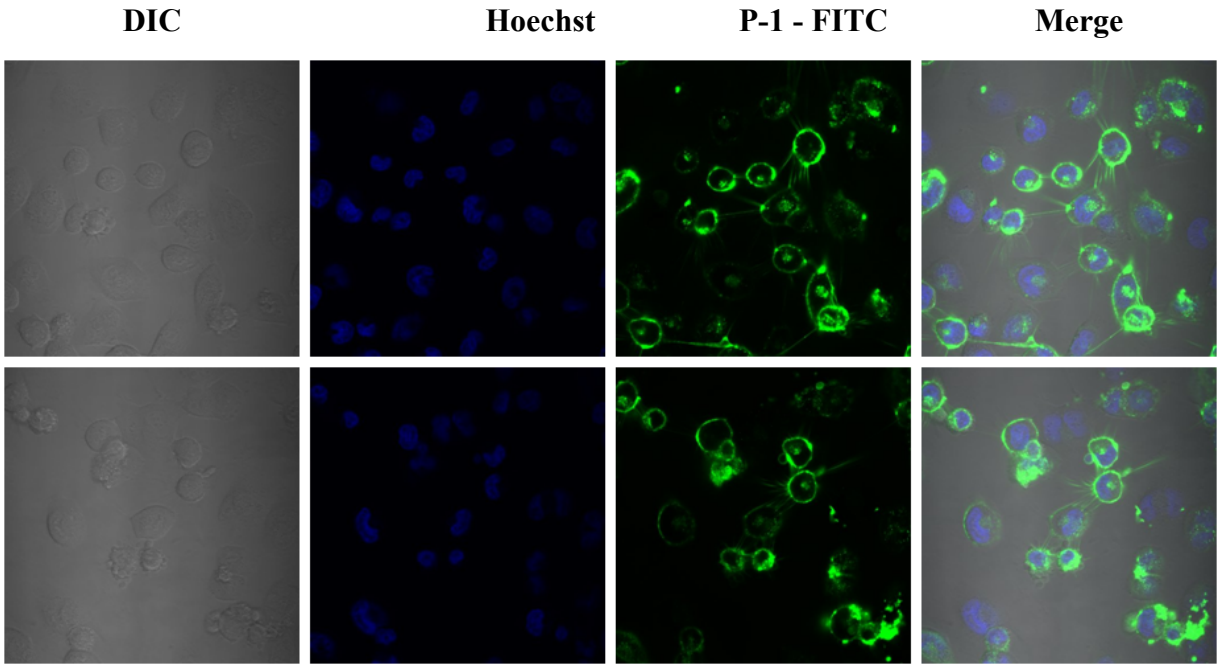


Figure S15. Fluorescence images of HeLa cell type with **P-1** after 30 mins incubation

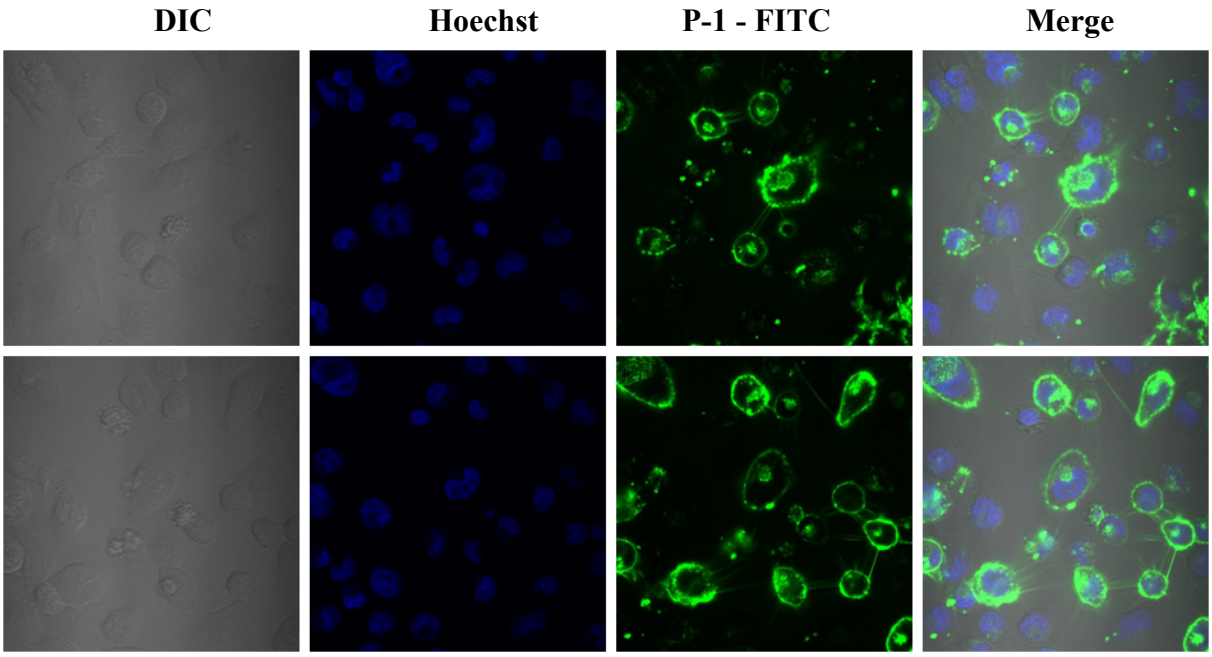


Figure S16. Fluorescence images of HeLa cell type with **P-1** after 120 mins incubation

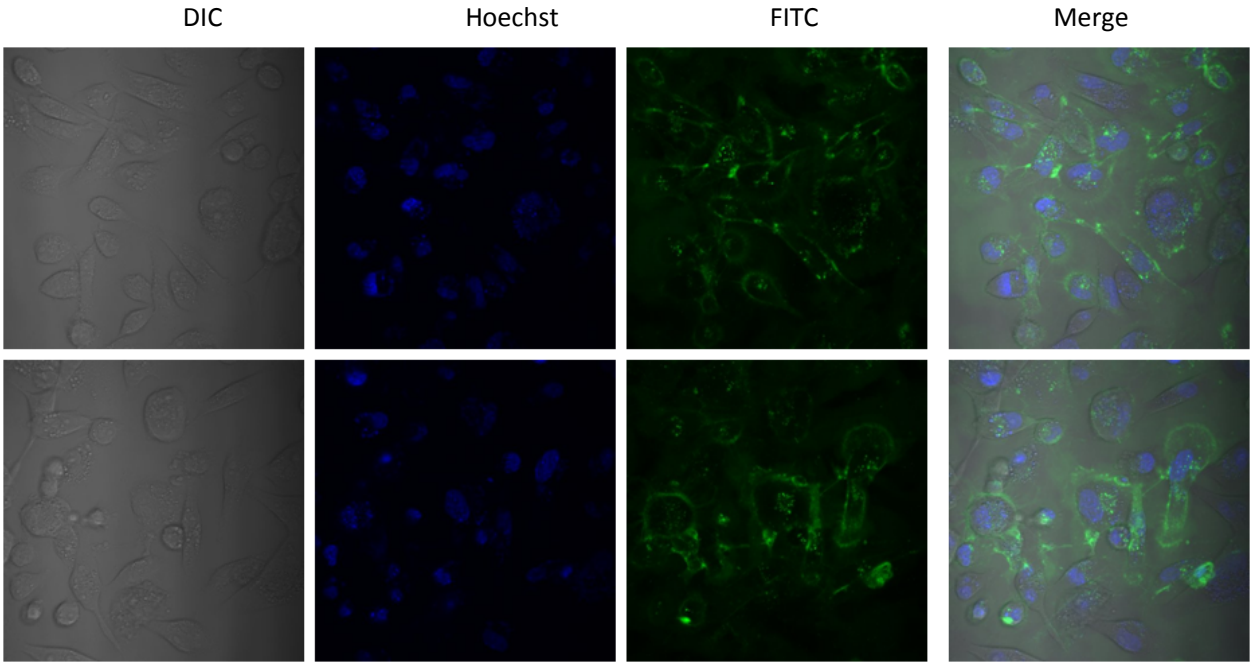


Figure S17. Fluorescence images of MDA-MB-231 cell type with **P-1** after 30 mins incubation

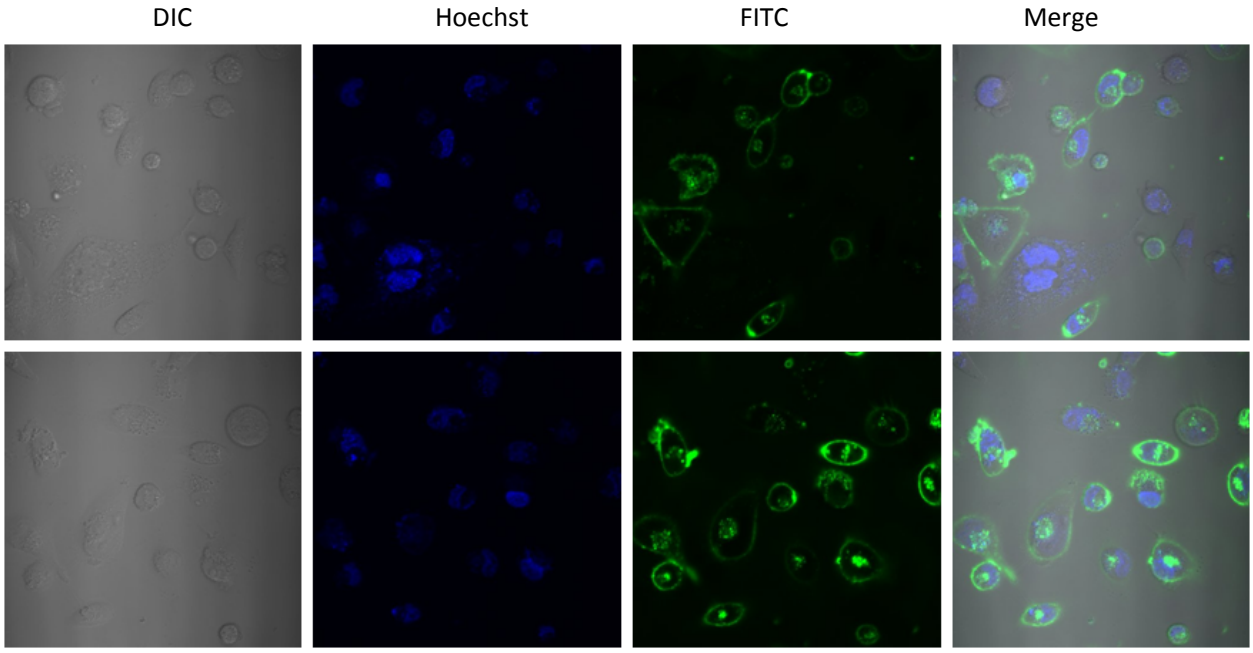


Figure S18. Fluorescence images of MDA-MB-231 cell type with **P-1** after 120 mins incubation

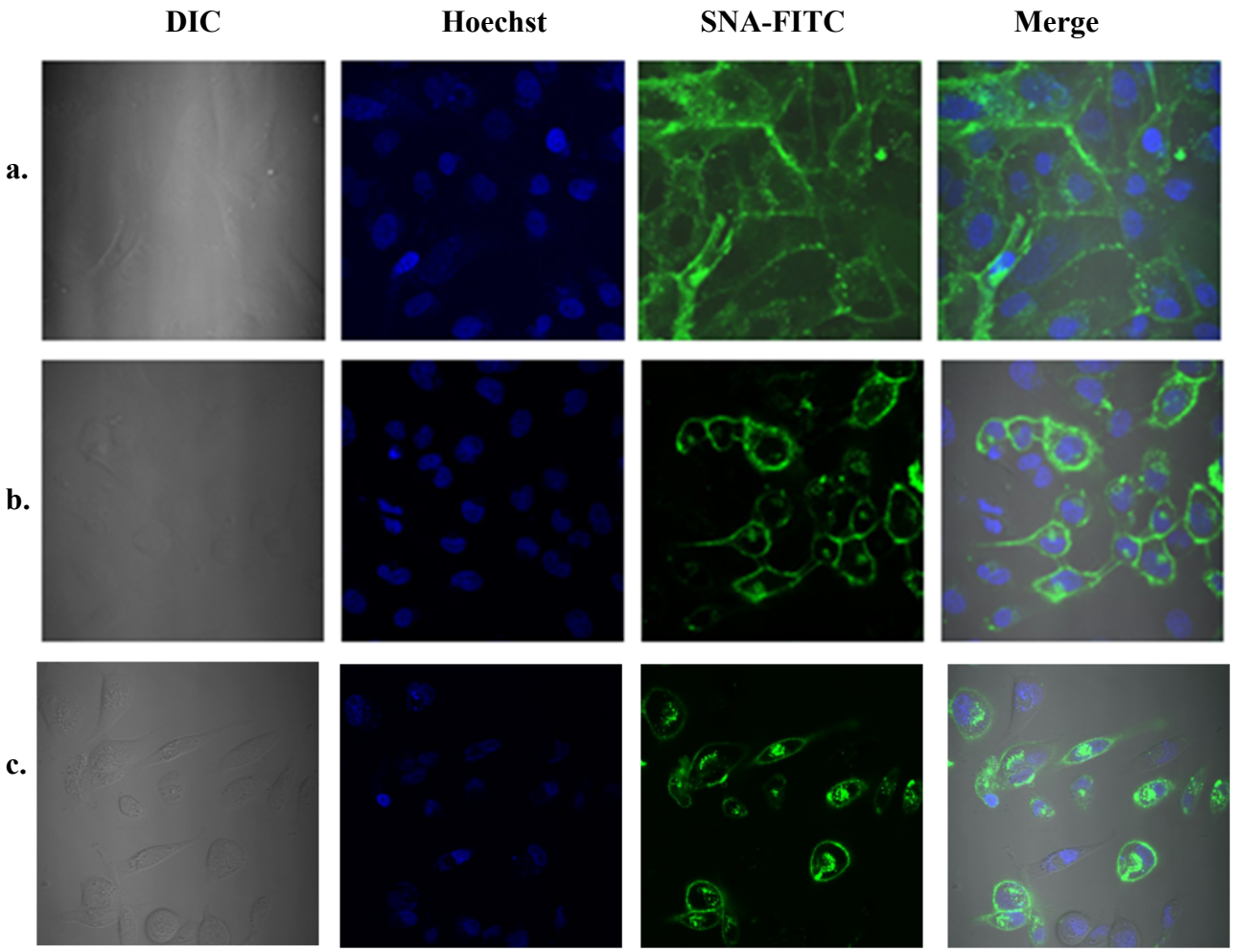


Figure S19. Fluorescent images of different cell lines with **SNA: (**a) NIH-3T3; (b) HeLa; (c) MDA-MB-231

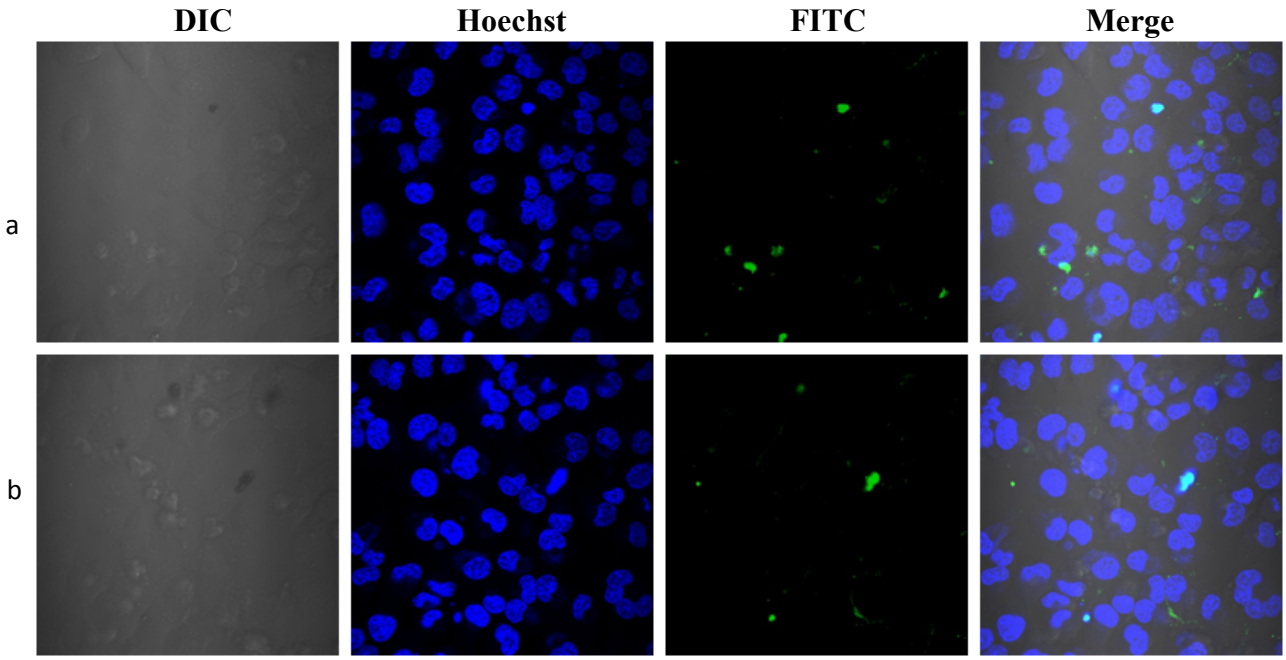


Figure S20. Fluorescence images of sialidase treated HeLa cells with (a) **P-1** and (b) **SNA** after 30 mins incubation.

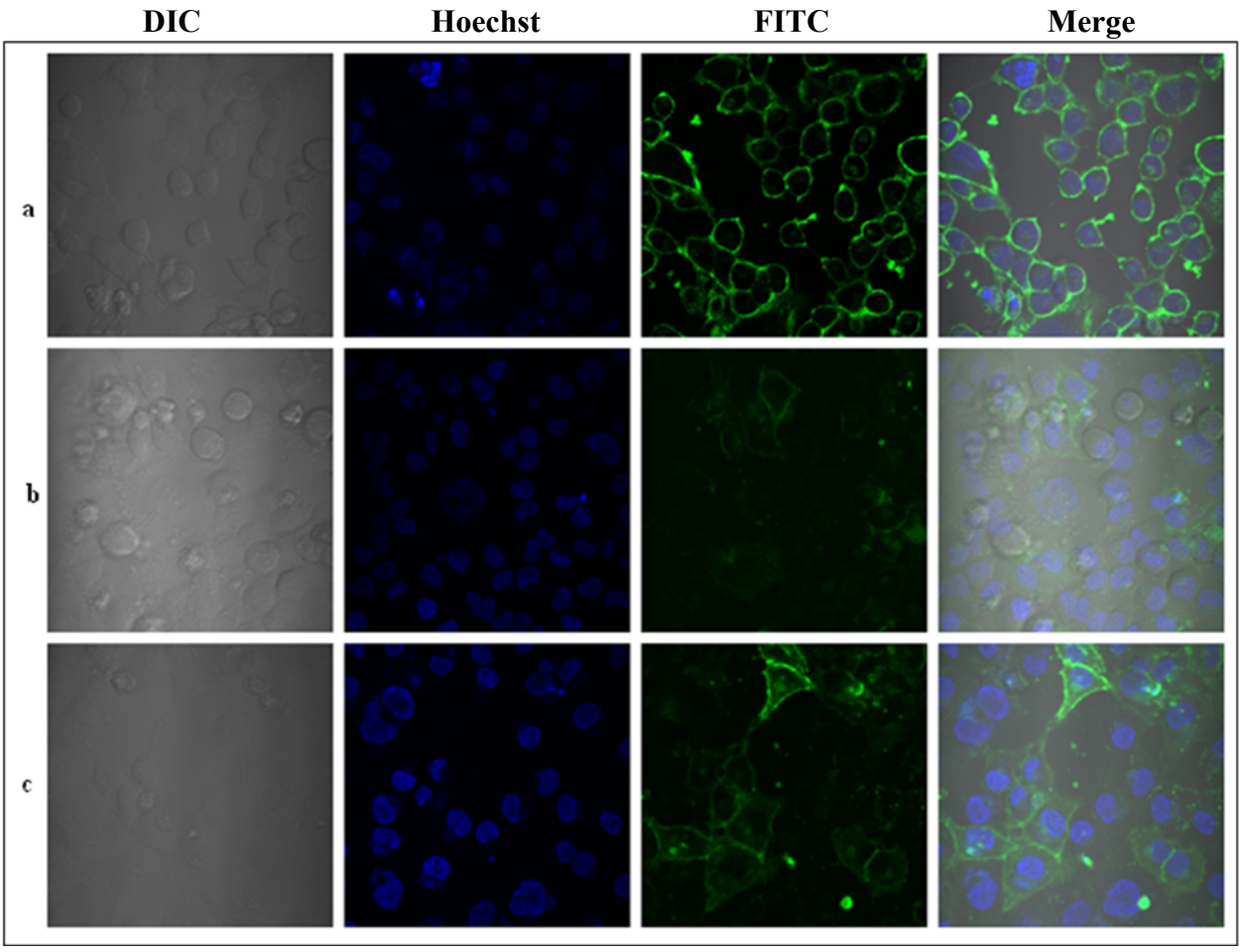


Figure S21. Fluorescence images of HeLa cell type with (a)**ConA,** (b) **PNA** and (c)**UEA1** after 30 mins incubation.

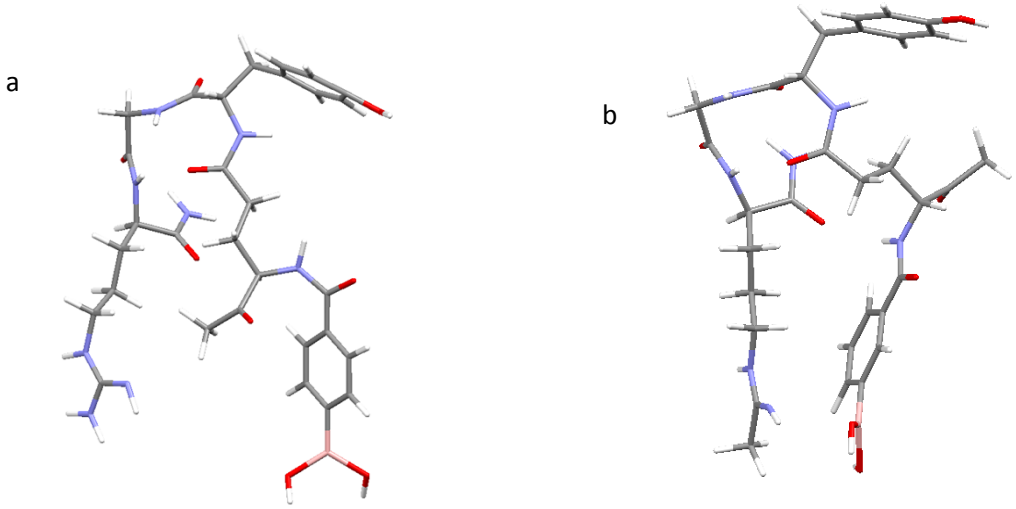


Figure S22. MM2 calculation and Mimimum energy structure of (a) **P-1** and (b) **P-2** peptides

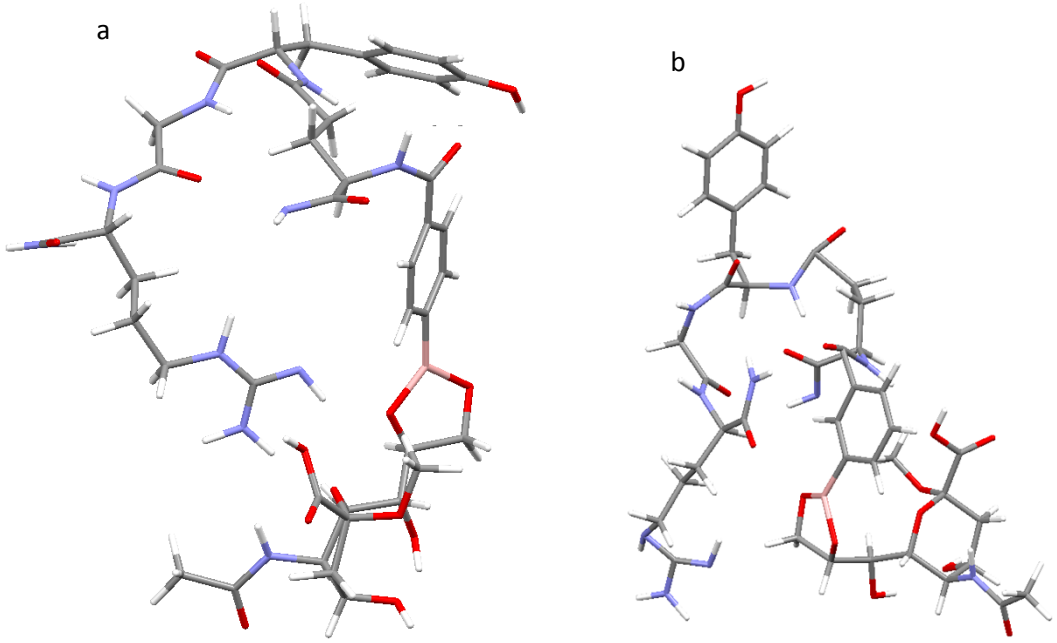


Figure S23. MM2 calculation and Mimimum energy structures of (a) **P-1**-sialic acid and (b) **P-2**-sialic acid.

Energy profile	P-1	P-2	P-1-Sialic acid	P-2-sialic acid
Stretch	6.6347	2.0976	10.4375	20.6588
Bend	68.9118	99.7194	61.9501	114.401
Stretch-bend	-0.891	0.0867	-5.399	-4.5596
Torsion	1.1337	-1.5872	36.9363	22.3342
Non-1,4 VDW	-22.997	-40.7525	-46.994	-20.8856
1,4 VDW	23.885	24.7815	38.3387	34.533
Dipole/Dipole	-45.61	-44.3142	-30.642	-51.7216
Total Energy (Kcal/mol)	31.0681	40.0313	64.6271	114.7603

Table S2. MM2 minimization energy profile.

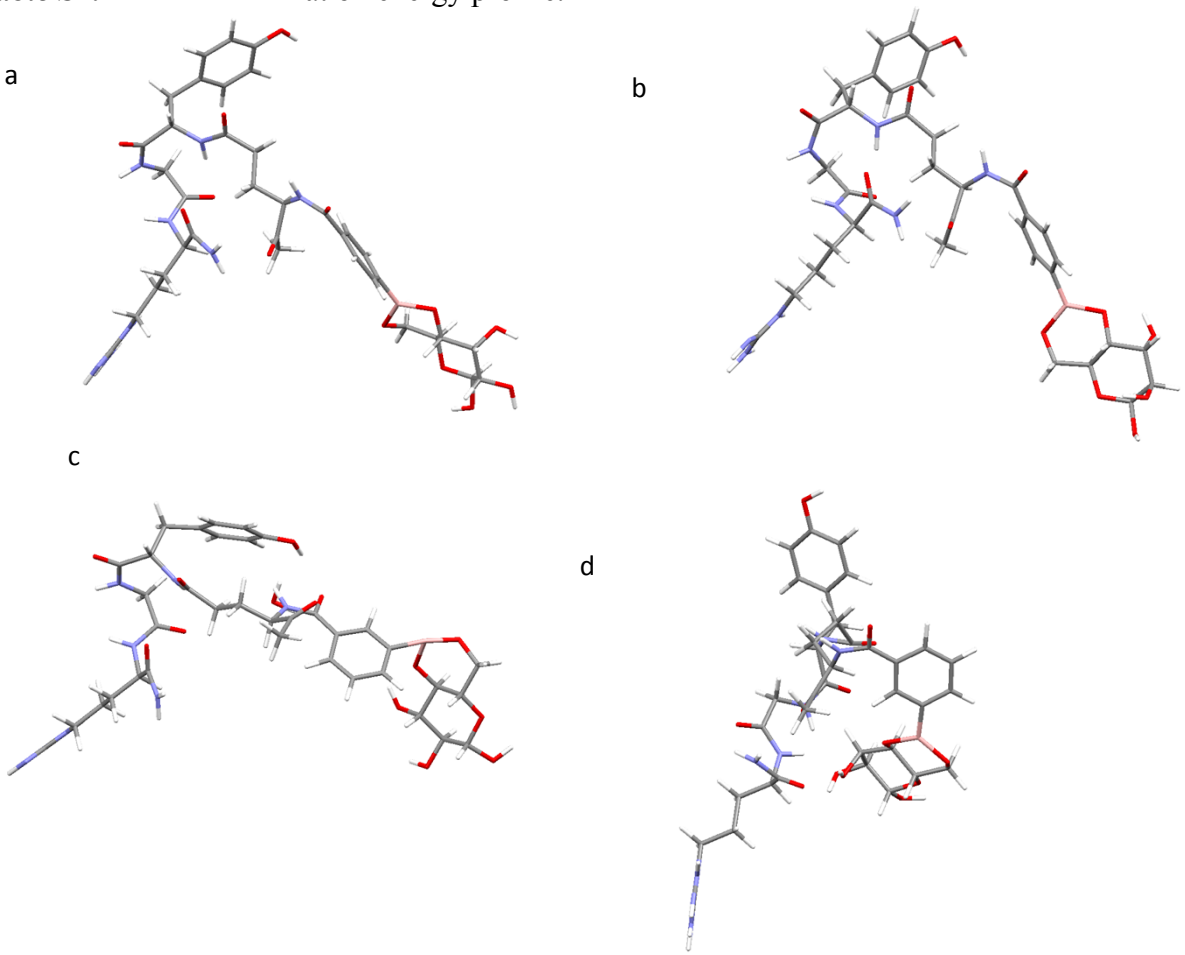


Figure S24. MM2 calculation and minimum energy structure of (a) **P-1-Man**; (b) **P-1-Gal**; (c) **P-2-Man** and (d) **P-2-Gal**

Energy profile	P-1-Man (Kcal/mol)	P-2-Man (Kcal/mol)	P-1-Gal (Kcal/mol)	P-2-Gal (Kcal/mol)
Stretch	4.9807	6.7314	5.1304	13.7922
Bend	92.6822	89.6786	104.0241	219.0013
Stretch-bend	-0.7185	-1.5952	-2.7531	-3.7635
Torsion	13.0224	10.5996	12.7606	13.1582
Non-1,4 VDW	-30.9997	-45.9468	-39.2876	-37.0391
1,4 VDW	32.0866	36.7615	31.8095	45.7523
Dipole/Dipole	-37.4689	-44.8948	-37.9325	-45.7573
Total Energy	73.5848	51.3343	73.7514	205.1441

Table S3. MM2 minimization energy profile.

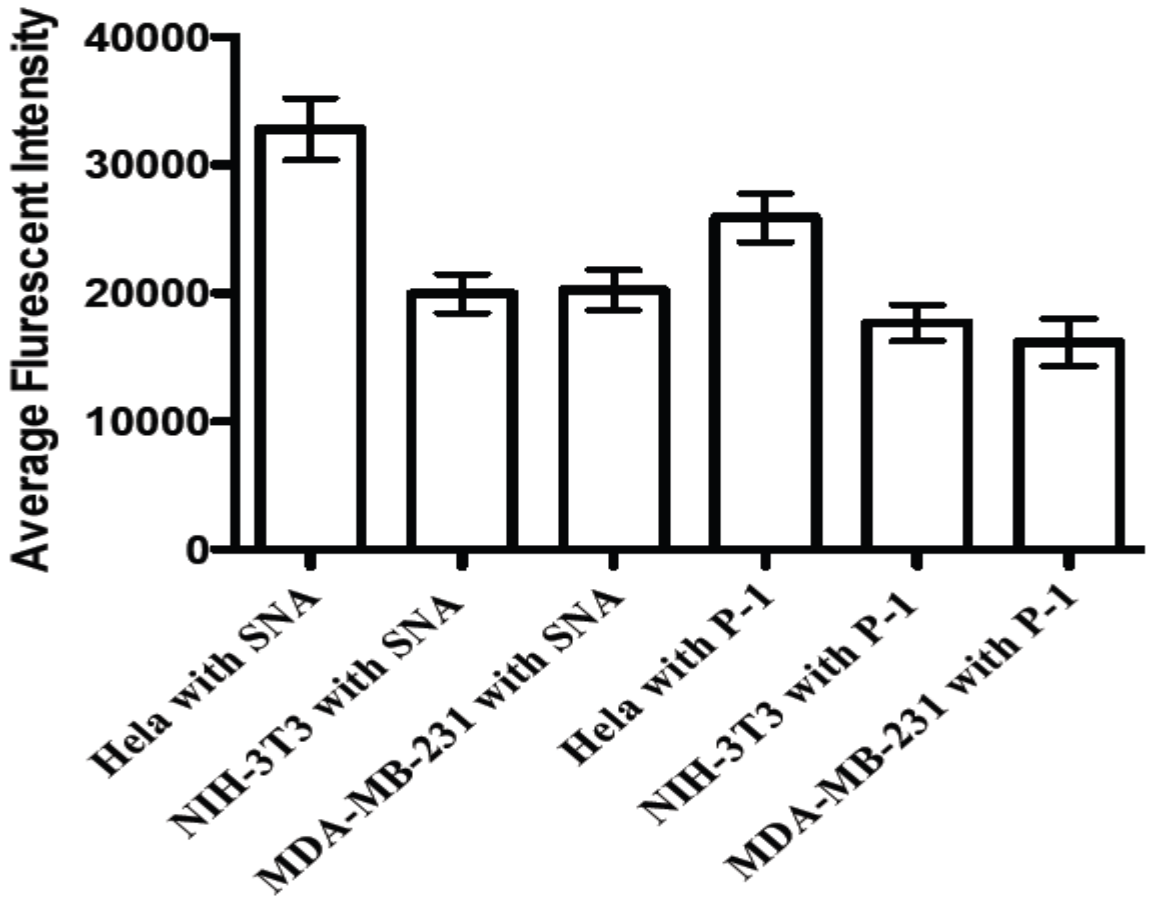
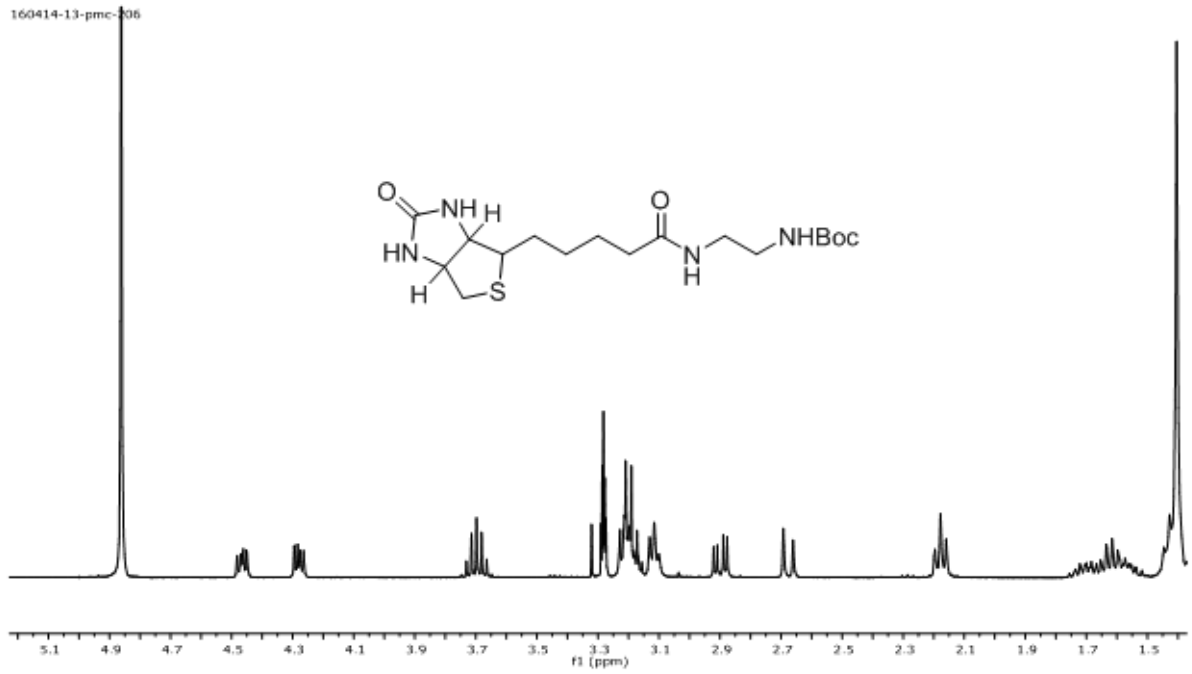
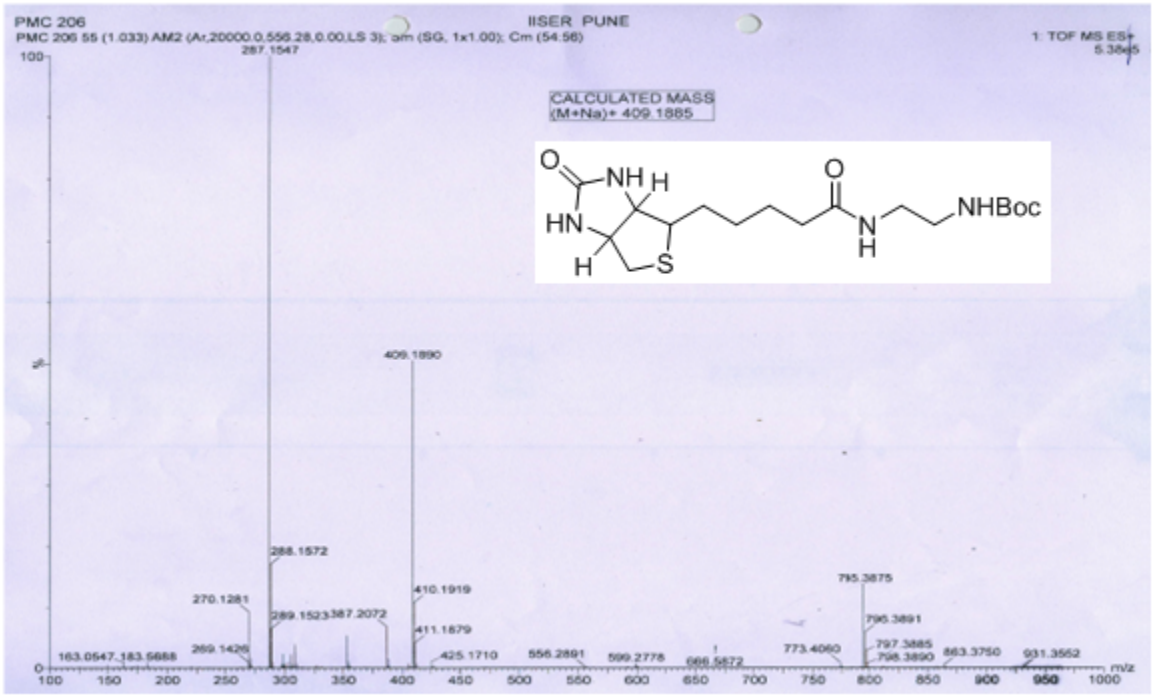
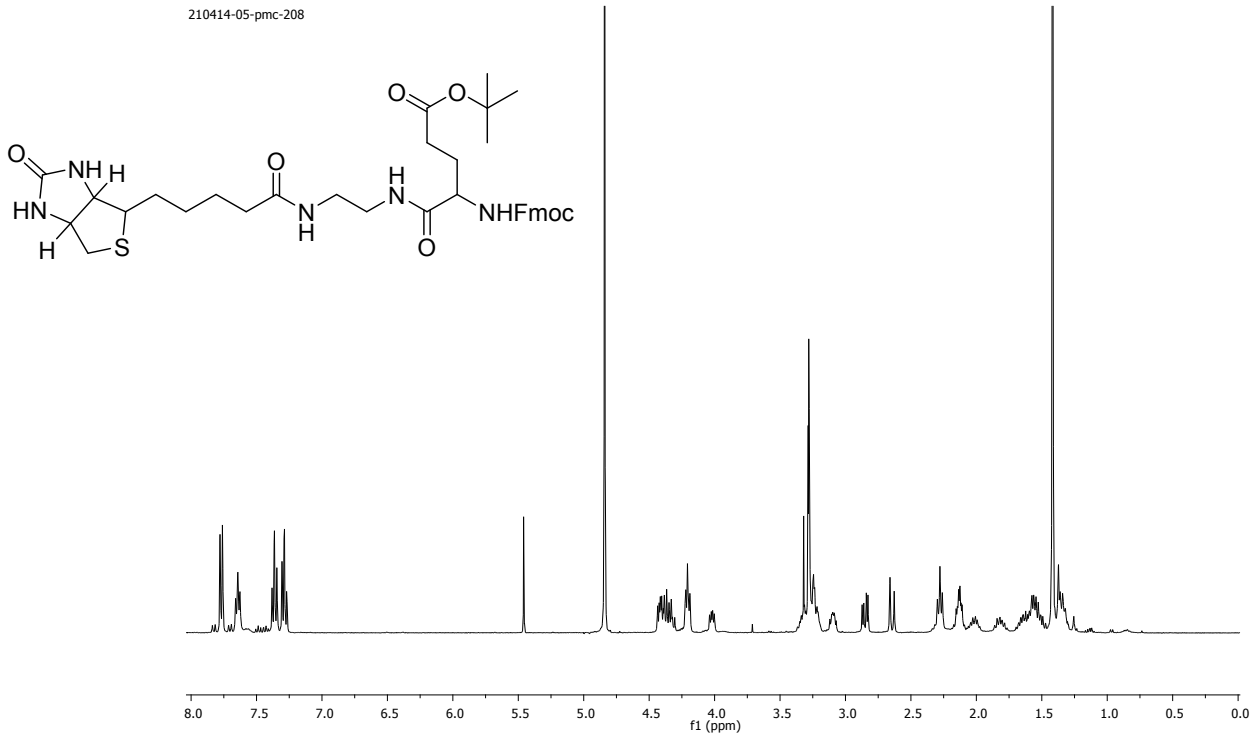


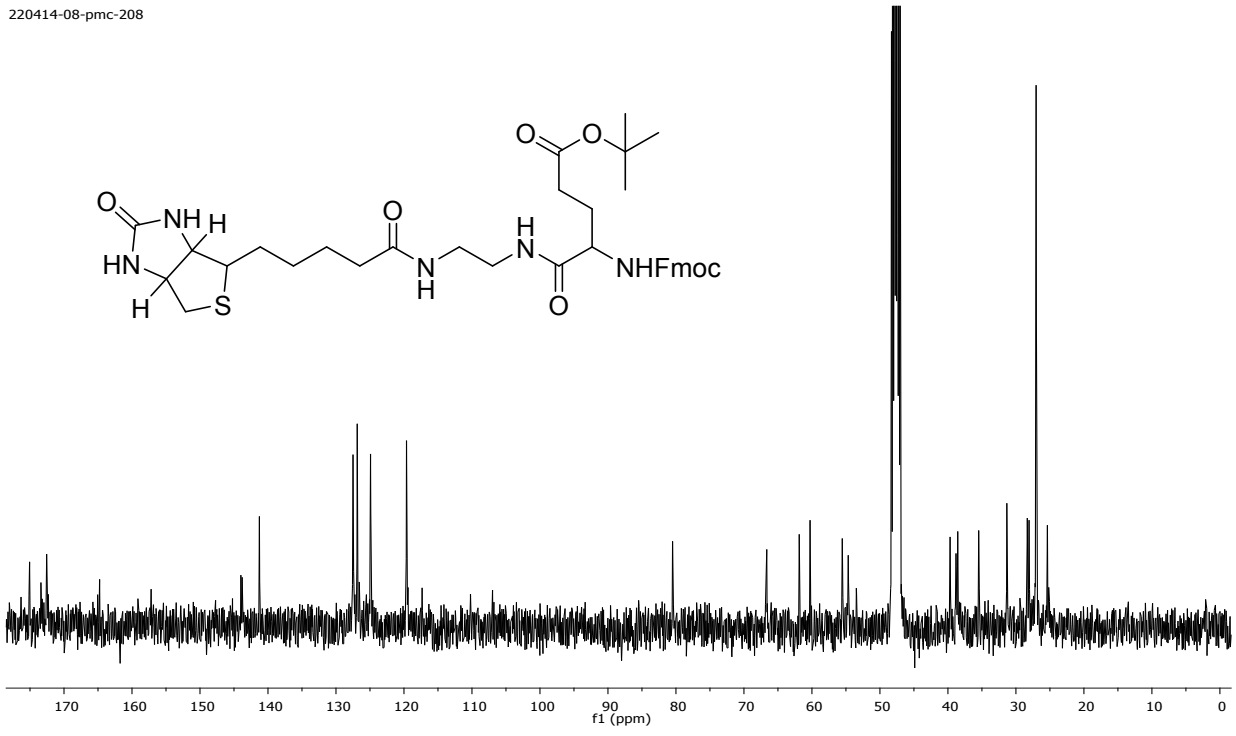
Figure S25. Determination of Sia composition on cell surface by measuring the average fluorescence intensity.

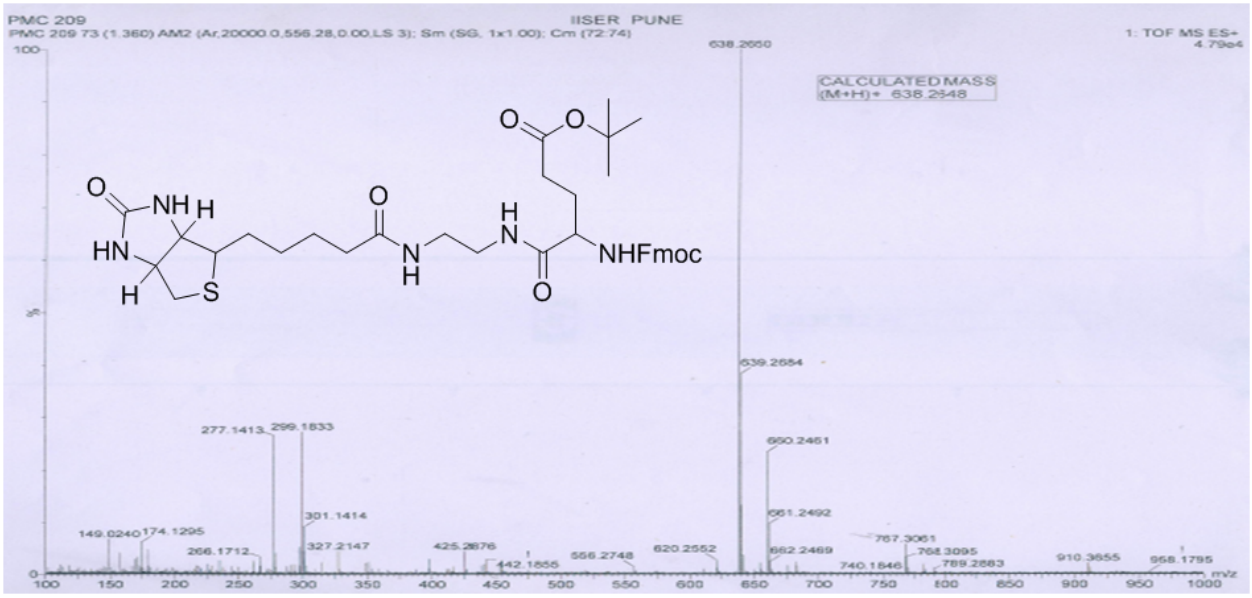

160414-13-pmc-206
O
NH H
HN
H
S
O
N
H
NHBoc
5.1
4.9
4.7
4.5
4.3
4.1
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
2.3
2.1
1.9
1.7
1.5
f1 (ppm)

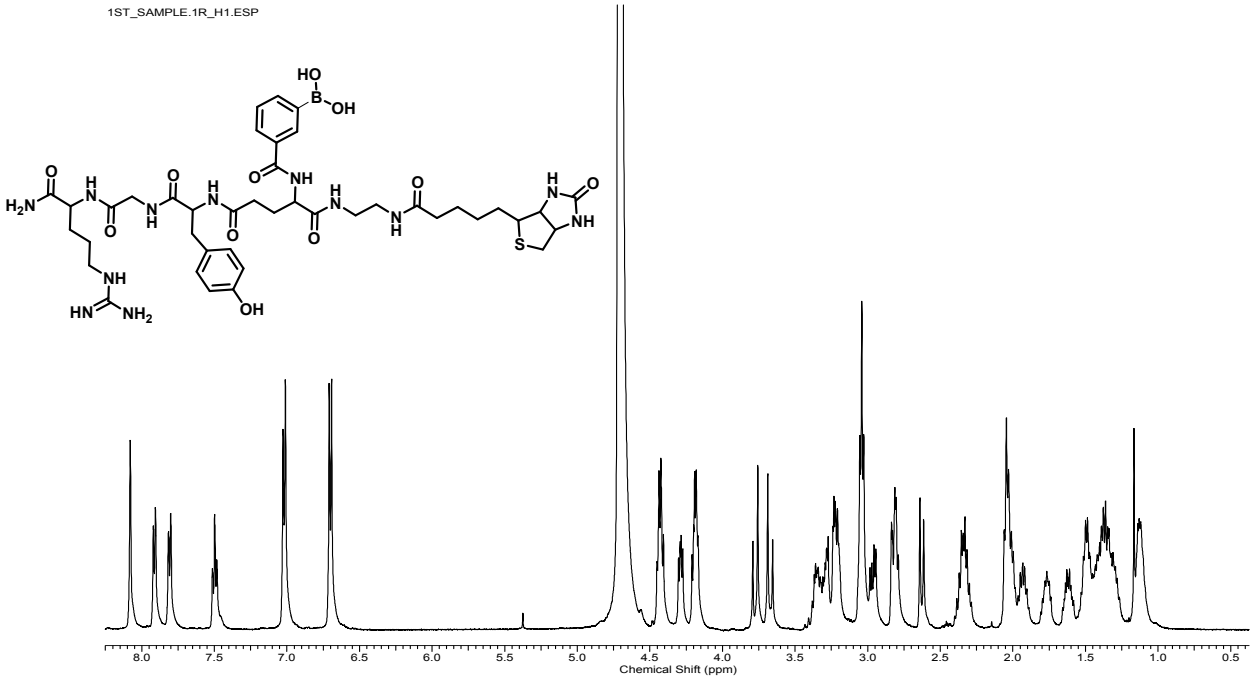
SPECTRA

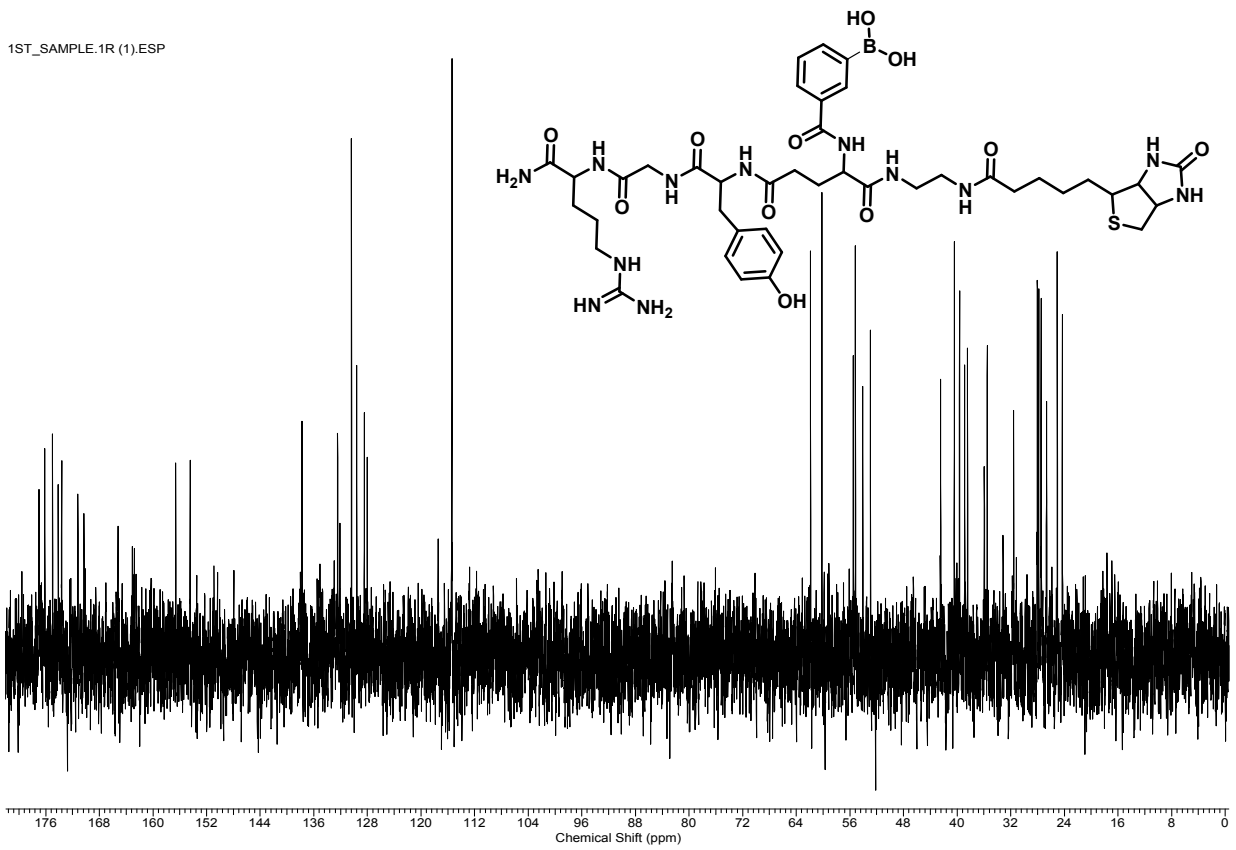

PMC 206
IISER PUNE
PMC 206 55 (1.033) AM2 (Ar,20000.0,556.28,0.00,LS 3); sm (SG, 1x1.00); Cm (54:56)
1: TOF MS ES+
5.38e5
100
%
0
CALCULATED MASS
(M+Na)+ 409.1885
O
NH H
HN
H
S
O
N
H
NHBoc
287.1547
409.1890
288.1572
410.1919
270.1281
289.1523
387.2072
411.1879
269.1426
163.0547
183.0688
425.1710
556.2891
599.2778
666.5872
773.4060
795.3875
796.3891
797.3885
798.3890
863.3750
931.3552
100
150
200
250
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
m/z

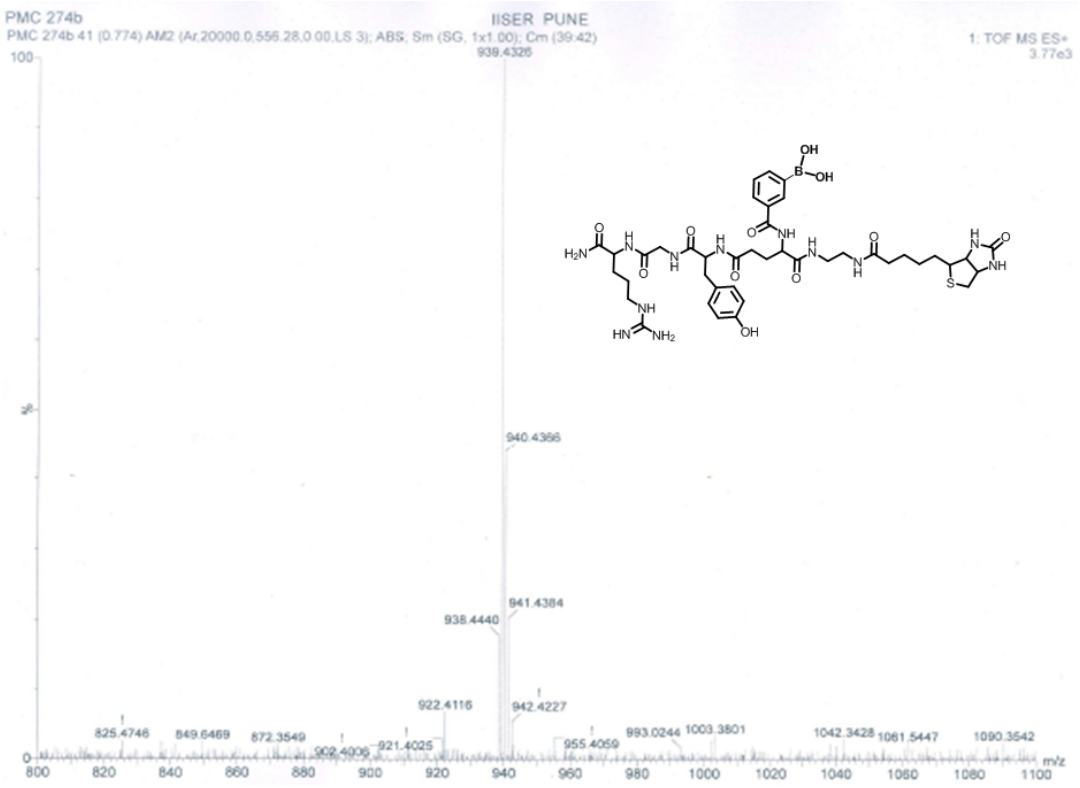

210414-05-pmc-208
O
O
O
NH H
HN
H
S
O
N
H
H
N
O
NHFmoc
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

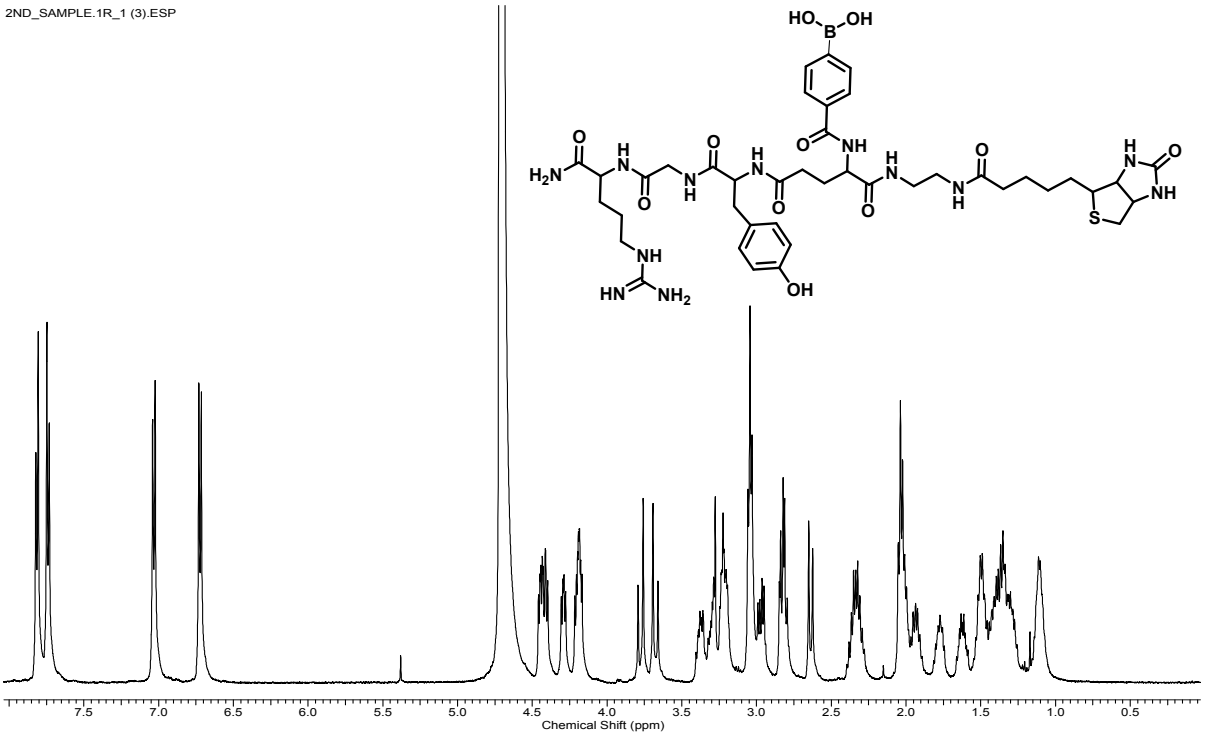

220414-08-pmc-208
O
O
O
NH H
HN
H
S
O
N
H
H
N
O
NHFmoc
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

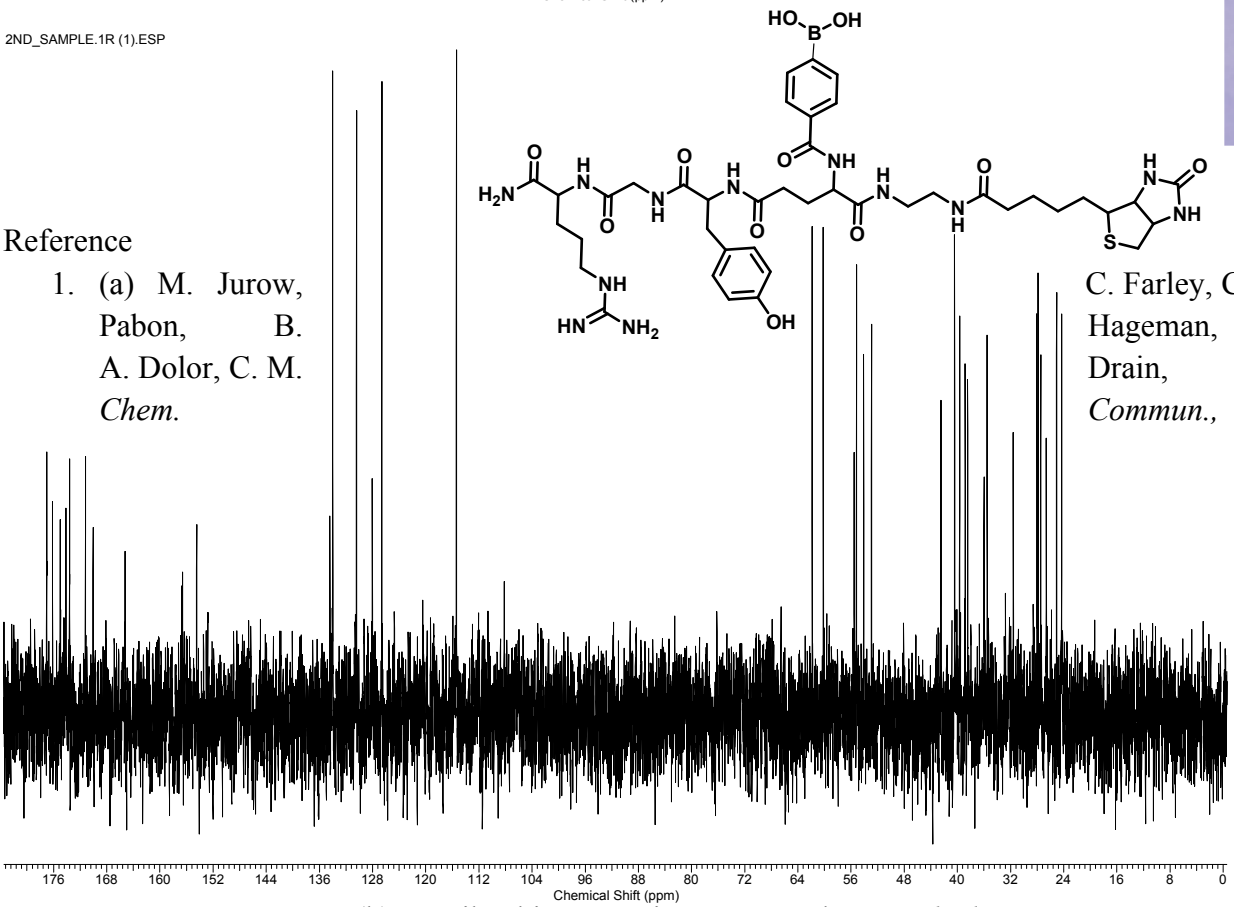

PMC 209
IISER PUNE
PMC 209 73 (1.360) AM2 (Ar,20000.0,556.28,0.00,LS 3); Sm (SG, 1x1.00); Cm (72:74)
1: TOF MS ES+
4.79e4
638.2650
CALCULATED MASS
(M+H)+ 638.2548
NHFmoc
539.2654
277.1413
299.1833
650.2461
301.1414
661.2492
149.0240
174.1295
266.1712
327.2147
425.2876
442.1855
556.2748
620.2552
682.2469
740.1846
767.3061
784.3095
789.2883
910.3655
958.1795
m/z


1ST_SAMPLE.1R_H1.ESP
Chemical Shift (ppm)


1ST_SAMPLE.1R (1).ESP
Chemical Shift (ppm)


PMC 274b
IISER PUNE
PMC 274b 41 (0.774) AM2 (Ar,20000.0,556.28,0.00,LS 3); ABS, Sm (SG, 1x1.00); Cm (39:42)
1: TOF MS ES+
3.77e3
938.4326
940.4366
941.4384
938.4440
922.4116
942.4227
825.4746
849.6469
872.3549
902.4006
921.4025
955.4059
993.0244
1003.3801
1042.3428
1061.5447
1090.3542
m/z





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4. (a) A. Y. Chernyak, G. V. M. Sharma, L. O. Kononov, P. R. Krishna, A. B. Levinsky, N. K. Kochetkov and A. V. R. Rao, *Carbohydrate Research* 1992, **223**, 303-309. (b) J. Ni, S. Singh, L. -X. Wang, *Bioconjugate Chem*. 2003, **14**, 232-238.