Supporting information

Laser cleavable probes-based cell surface engineering for *in-situ* sialoglycoconjugates profiling by LDI mass spectrometry

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Experimental section.

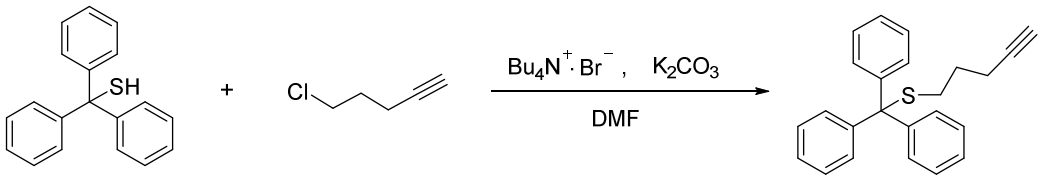
Section I: reagents/materials/cells

Reagents and materials. Tris (1-benzyl-1H-1, 2, 3-triazol-4-yl) met (TBTA)[1], acetylated N-azidomannosamine, 5-chloro-1-pentyne (98%) and triphenylmethanethiol (97%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). (+)-Sodium L-ascorbate (99%) and tetrabutylammonium bromide were purchased from J&K Scientific. Chloroform-d for NMR was purchased from Beijing Innochem co., LTD. Tunicamycin was bought from Aladdin reagent. N-Azidoacetyl-Mannosamine tetraacylated ($Ac_4ManNAz$, 99%) and the DIBO-Alexa Fluor 647 (99%) were purchased from Thermo Scientific (Waltham, Massachusetts, USA).

Cells. Hela human cervical carcinoma cells, HepG-2 human liver carcinoma cells, and 293 human embryonic kidney cells were obtained from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). These cells were cultured in the humidified incubator at 37℃ with 5% CO_2. The culture medium was DMEM (Jiangsu KeyGEN BioTECH Corp., Ltd., China) with 10% fetal bovine serum (Zhejiang Tianhang Biotechnology Co. Ltd., China), 100 units/mL penicillin and 100 μg/mL streptomycin. All the three kinds of cells are adherent cells.

Section II: synthesis and characterization of Tphsene

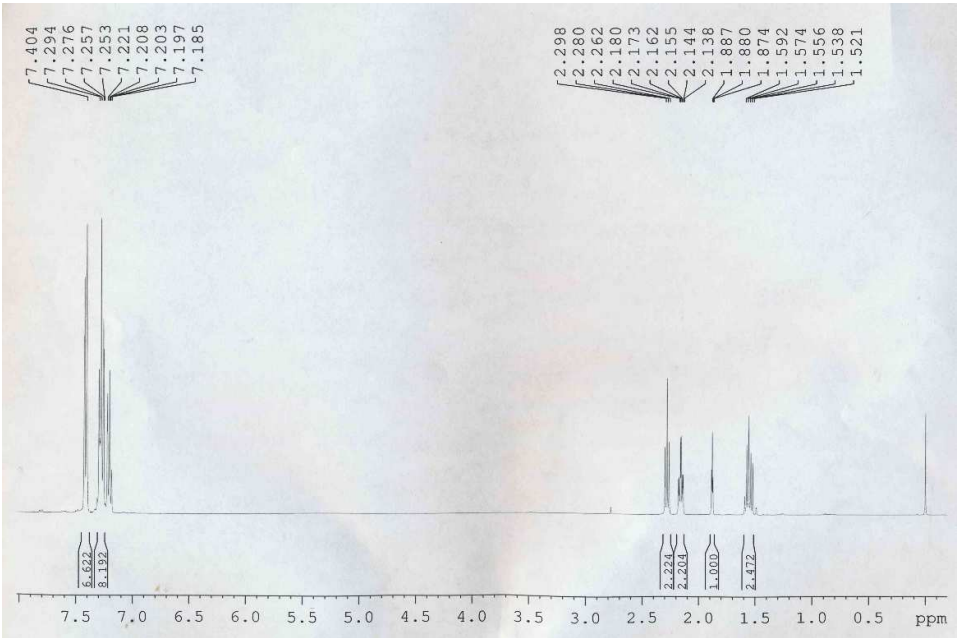
Synthesis of tag (Tphsene):



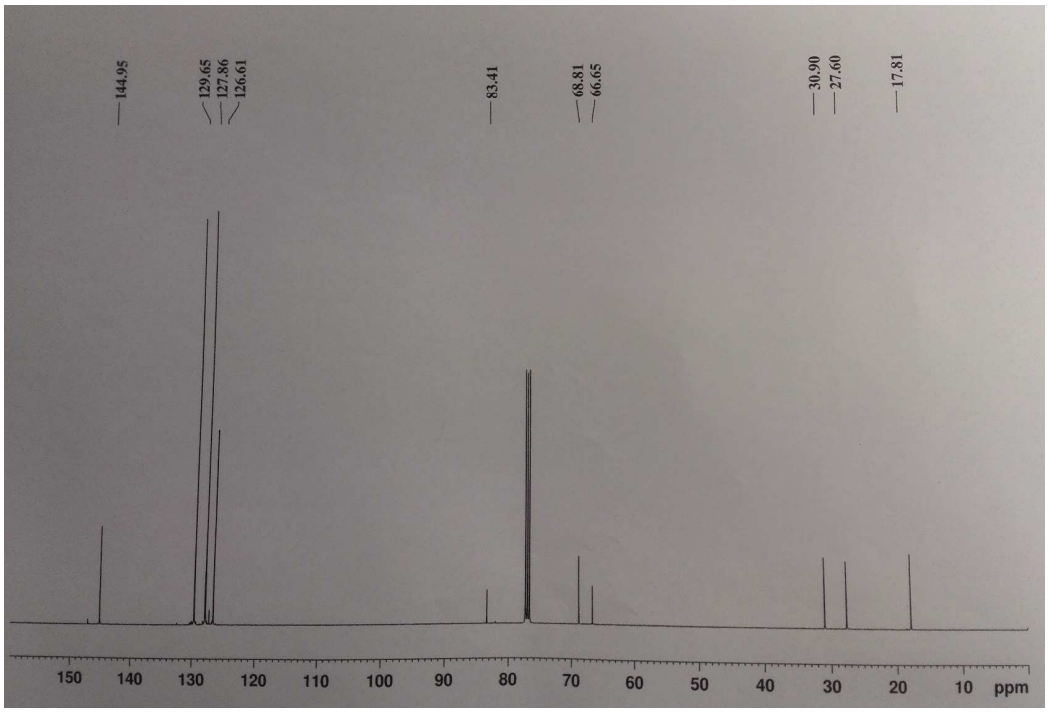
To a solution of 5-chloropent-1-yne (0.8 g, 7.54 mmol) in 10 mL DMF triphenylmethanethiol (2.16g, 7.9mmol), tetrabutylammonium bromide (0.49g, 1.52mol) and potassium carbonate (3.44g, 24.9mmol) were added. The mixture was stirred at room temperature for 5h. After that, 20mL of water was added to the reaction vessel. The target product was extracted with dichloromechane for three times (3×10mL). The organic layer was evaporated under vacuum. The product was purified with silica gel chromatography (petroleum ether: dichloromethane=5:1) and recrystallized to afford a white needle crystal (2g, 78%).

Characterization of Tphsene:

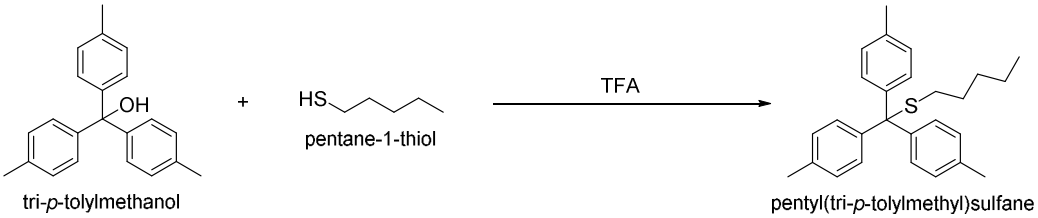
^{1}H NMR ($CDCl_3$, 400MHz):



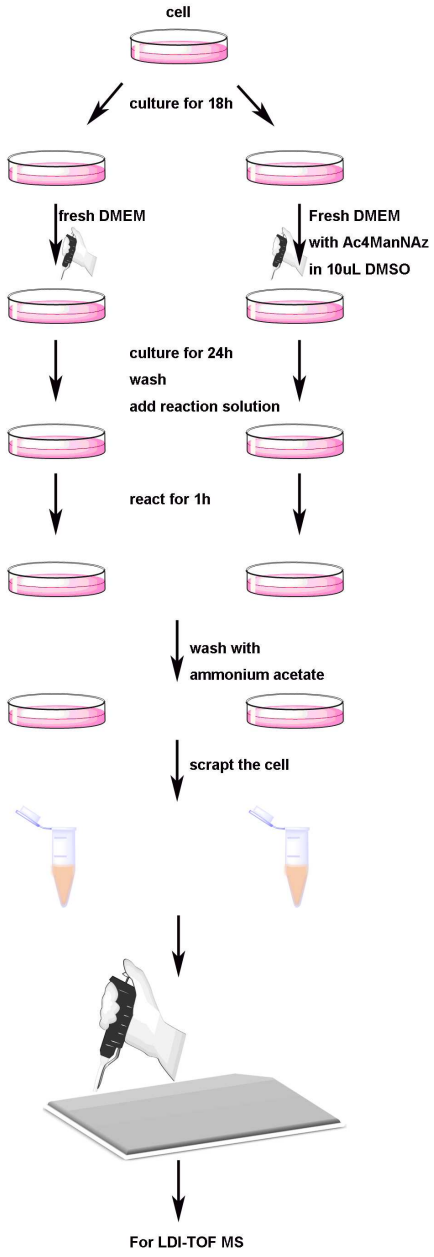
^{13}C NMR ($CDCl_3$, 400MHz):



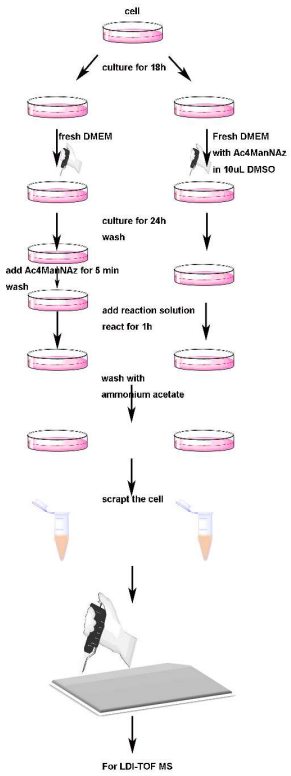
Section III: Synthesis of the internal label PhTsane.



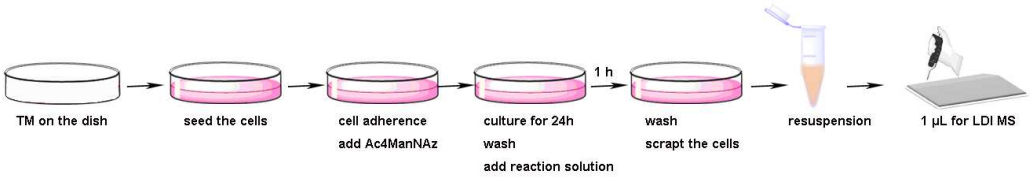
To a solution of trifluoroacetic acid (TFA, 7.14mL), tri-p-tolylmethanol (1.5g, 5mmol) and pentane-1-thiol (0.52g, 5mmol) were added. The mixture solution was stirred at room temperature for 10 minutes. Then, TFA was removed by rotary evaporation. The crude product was washed by diethyl ether and filtered. Then the product was washed by water and a white product was obtained (1.7g). The product was separated by column chromatography (petroleum ether : dichloromethane: 10:1).



Scheme S1. Experiment flow of cell treatment.



Scheme S2. Control group to exclude the nonspecific adsorption of Ac_4ManNAz. The left column is the control group. In the control group, we added the Ac_4ManNAz for 5 minutes. In this short time the Ac_4ManNAz can adsorb to the cell surface, but it have not enough time to be internalized into the cells. Then we do the washing step to remove the adsorbed Ac_4ManNAz.



Scheme S3. Experimental flow for drug inhibition study.

Supplementary figures

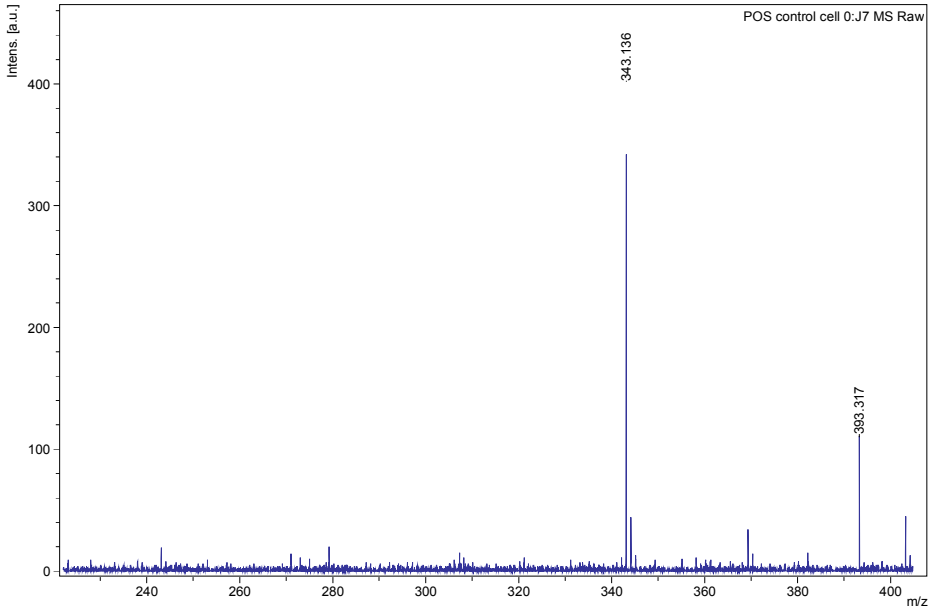


Figure S1. The LDI-MS results (the control group of Scheme S2) indicated that the Ac4ManNAz has been washed away.

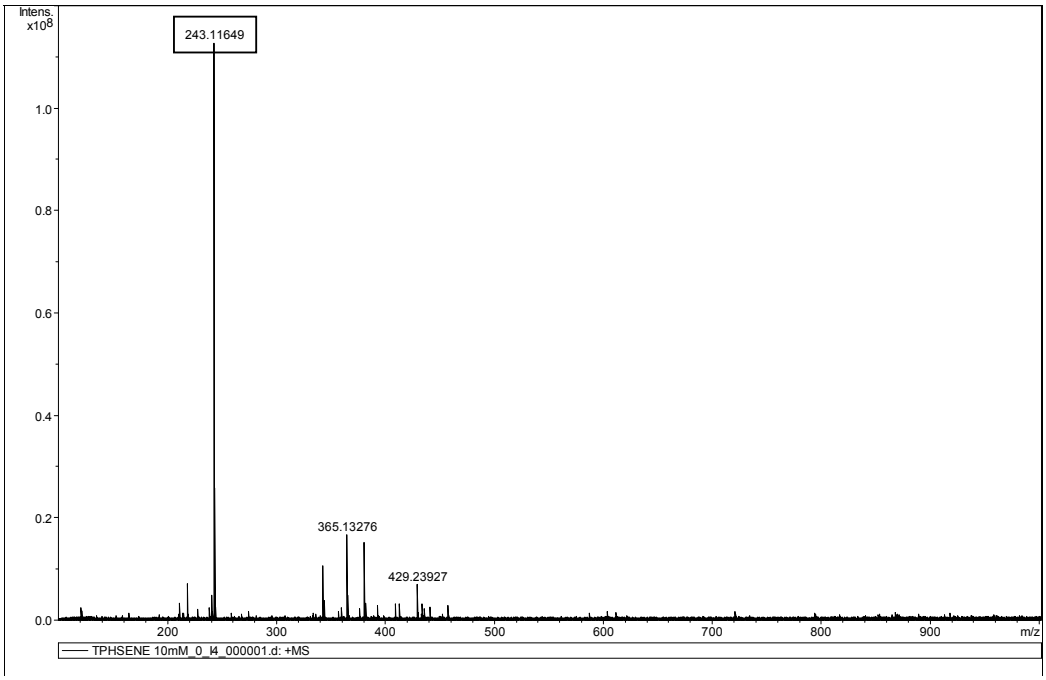


Figure S2. Accurate mass measurement of Tphsene using LDI-FTICR MS.

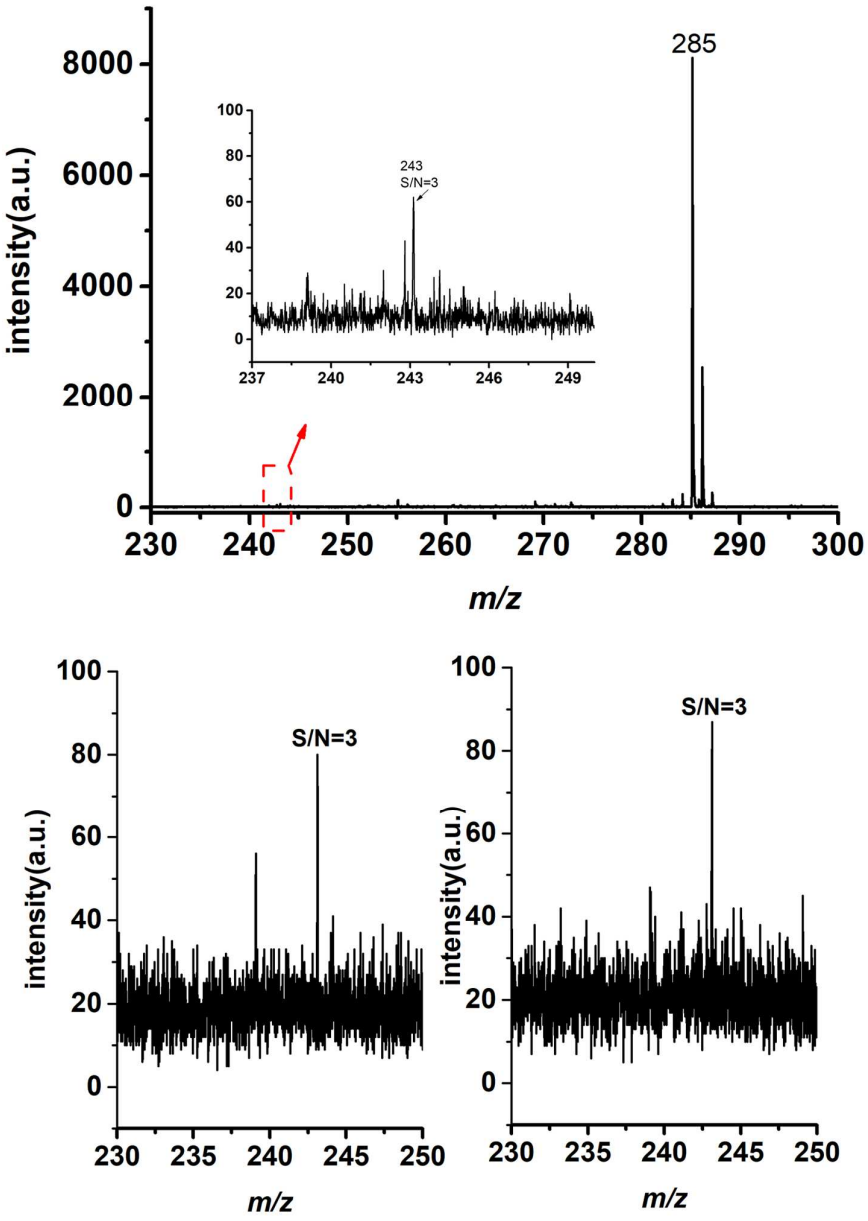


Figure S3. Mass spectra of Tphsene with addition of the internal label. Three repeated experiments are showed. The insert chart is an enlargement of the red box. The amount of the Tphsene is 0.05 μM (5 fmol, 0.1 μL) and the amount of internal label is 1 mM (100 pmol, 0.1 μL). Laser intensity: 20%. Ion mode: positive.

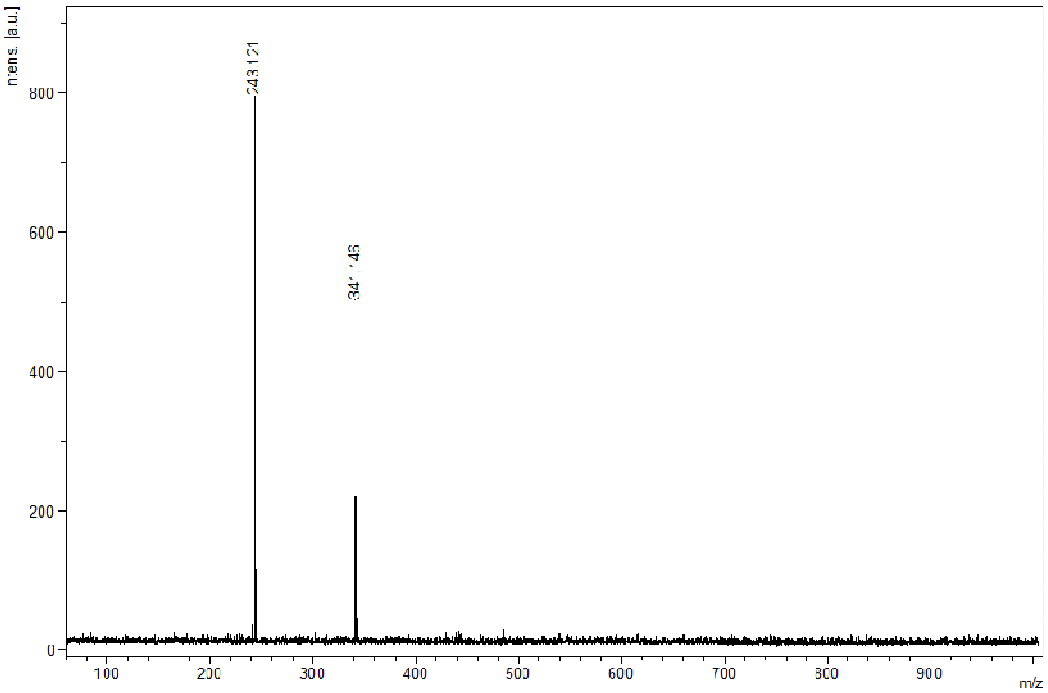


Figure S4. LDI Mass spectrum of tagged HepG2 cells in full mass range. Laser intensity: 20%. Ion mode: positive.

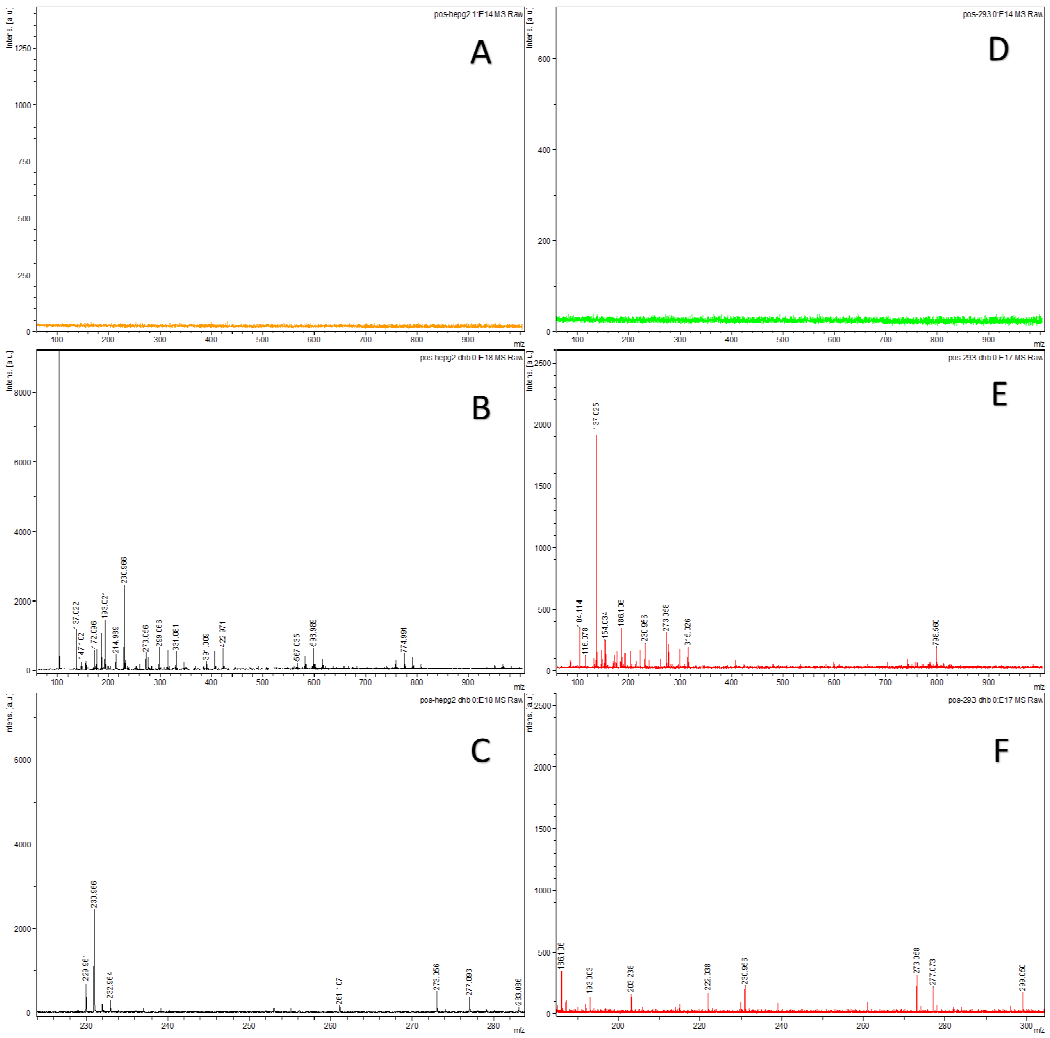


Figure S5. Mass spectra of control group cells (without tag labelling). A: HepG2 cells; B: HepG2 cells with addition of DHB matrix; C: enlarged view of B; D: 293 cells; E: 293 cells with addition of DHB matrix; F: enlarged view of E. Laser intensity: 20%. Ion mode: positive.

Supplementary tables

Concentration of Tphsene (mM)	MS signal intensity ratio of Tphsene to phTsane			Mean ratio	RSD
1	1.0180	0.9545	1.0420	1.00483	0.04499
0.5	0.5280	0.5160	0.5100	0.51800	0.01769
0.1	0.1060	0.1078	0.1200	0.11127	0.06845
0.01	0.03084	0.03526	0.02716	0.03109	0.13046
0.001	0.00954	0.01021	0.01226	0.01067	0.13280

Table S1. Detailed data of standard calibration curve. The results were obtained from three parallel experiments.

Ratio of MS signal intensity			Calculated concentration of sialoglycoconjugates (mM)			Mean concentration (mM)	RSD
0.222	0.205	0.23	0.23565	0.21844	0.24375	0.23262	0.05558
0.08505	0.08708	0.08421	0.09698	0.09904	0.09613	0.09738	0.0153
0.04348	0.0452	0.04047	0.05489	0.05663	0.05184	0.05445	0.04444
0.034	0.0371	0.032	0.04529	0.04843	0.04326	0.04566	0.05694
0.02416	0.02506	0.023	0.03532	0.03624	0.03415	0.03524	0.0298

Table S2. Detailed data of cell-surface sialoglycoconjugates quantitative determination under drug stimuli.

References

1. T. U. Connell, C. Schieber, I. P. Silvestri, J. M. White, S. J. Williams and P. S. Donnelly, *Inorganic chemistry*, 2014, **53**, 6503-6511.