Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Chemoselective ligation reaction of N-acetylglucosamine (NAG) with hydrazide functional probes to determine galactosyltransferase activity by MALDI mass spectrometry

Hyojik Yang, Quan Cheng*

Department of Chemistry, University of California,
Riverside, California 92521, United States

*Corresponding author. Tel.: +(1) 951-827-2702
Fax: +(1) 951-827-4713
e-mail: quan.cheng@ucr.edu.

1. General Information for NMR

NMR ^{1}H spectra were recorded on the Varian Inova 400 NMR spectrometer. Proton (^{1}H) chemical shifts are reported in parts per million (δ) with respect to tetramethylsilane (TMS, δ=0), and referenced internally with respect to the proton solvent impurity. ^{13}C spectra were recorded on Varian Inova 400 NMR spectrometer. Deuterium oxide was obtained from Sigma Aldrich, (St. Louis, MO) and used without further purification for all NMR experiments. All other materials were obtained from Aldrich Chemical Company (St. Louis, MO). Solvents were dried through a commercial solvent purification system (SG Water, Inc.).

2. Synthesis of 3,3'-dithiodipropionic acid dihydrazide (DTP).

DTP was synthesized according to a reported procedure[1]. 1 mL of 3-MPA and 1 mL of DMSO were mixed in a 25 mL round bottom flask. The reagents were allowed to stir under atmosphere for 24 hours. Then 3 drops of sulfuric acid and 10 mL of methanol were added to the mixture and refluxed for two hours. The mixture was cooled to room temperature and methanol was removed with reduced pressure. The product was extracted with diethyl ether (10 mL) and water (10 mL). The organic layer was collected and dried with $MgSO_4$. The solution was evaporated with reduced pressure. The pale amber oil product, dimethyl 3,3'-dithiopropionate, was used without purification for the next step. One gram of the product was added to 20 mL of methanol and 1.2 g of hydrazine monohydrate. The mixture was stirred over 18 hours at room temperature. The product is a suspension of white solid, which was filtered and washed with methanol and water. The collected sample was dried in vacuum overnight, yielding a white solid product (520 mg, 52%). NMR (300 MHz, DMSO-d_6) : δ = 9.05 (s, 2H; $NHNH_2$), δ = 4.20 (s, 4H; NH_2NH), δ = 2.88 (t, J = 7.2 Hz, 4H; CH_2CH_2S). δ = 2.40 (t, J = 7.2 Hz, 4H; CH_2SS).

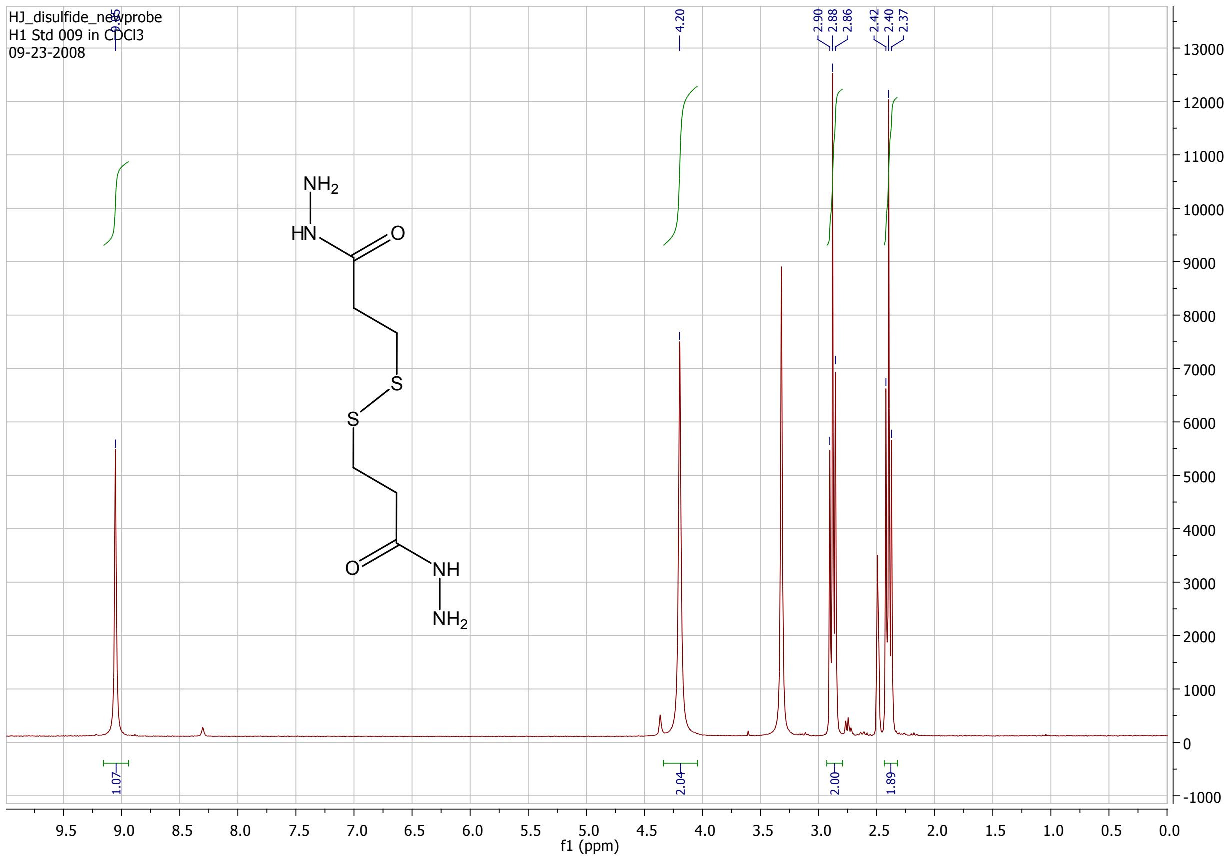

HJ_disulfide_newprobe
H1 Std 009 in CDCl3
09-23-2008
9.05
4.20
2.90
2.88
2.86
2.42
2.40
2.37
NH2
HN
O
S
S
O
NH
NH2
1.07
2.04
2.00
1.89
13000
12000
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
-1000
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Figure S1

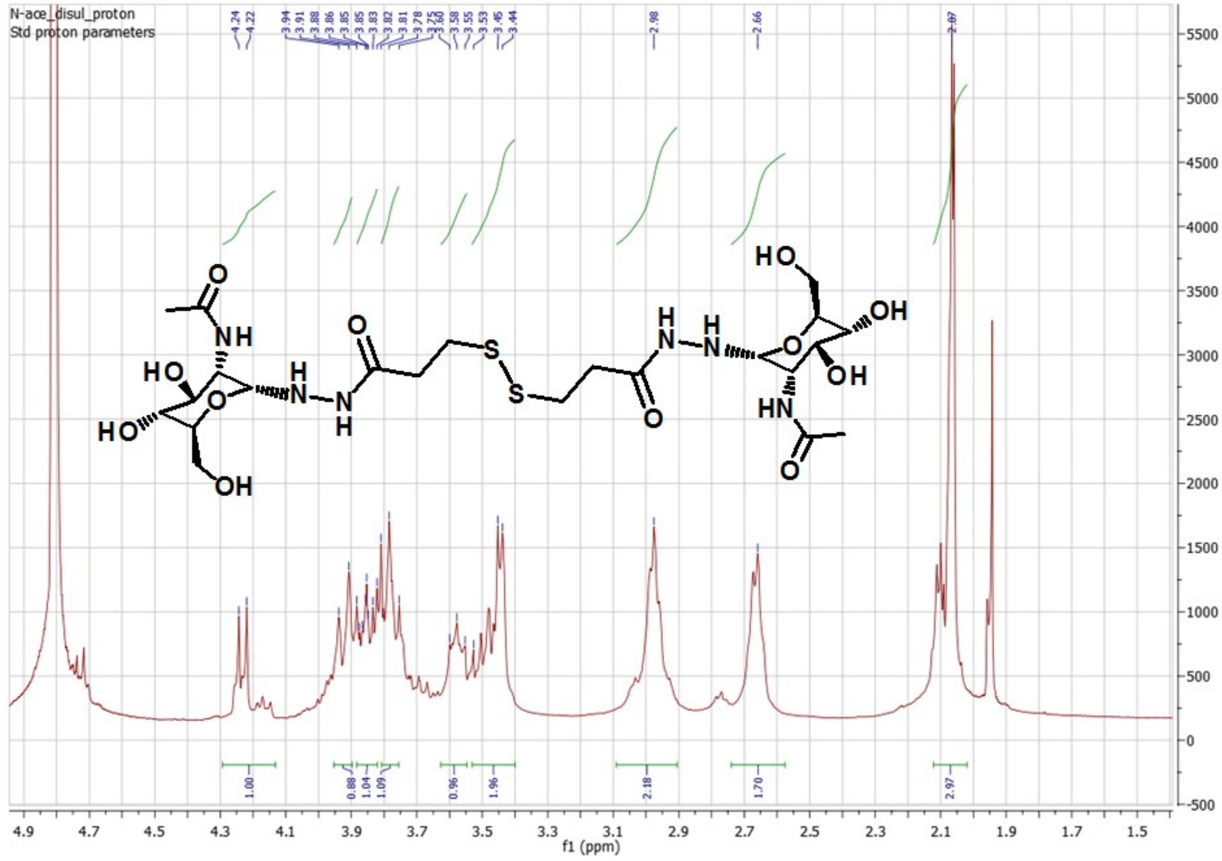


Figure S2.

^{1}H NMR (400 MHz, D_2O) δ 4.24 (d, 1H, J = 9.6 Hz), 3.94 (d, 1H, J = 12.4 Hz), 3.88-3.75 (m, 2H), 3.60 (t, 1H, J = 8.4 Hz and 10.4 Hz), 3.53–3.44 (m, 2H), 2.98(m, 2H), 2.66(m, 4H), 2.07(s, 3H, Ac)

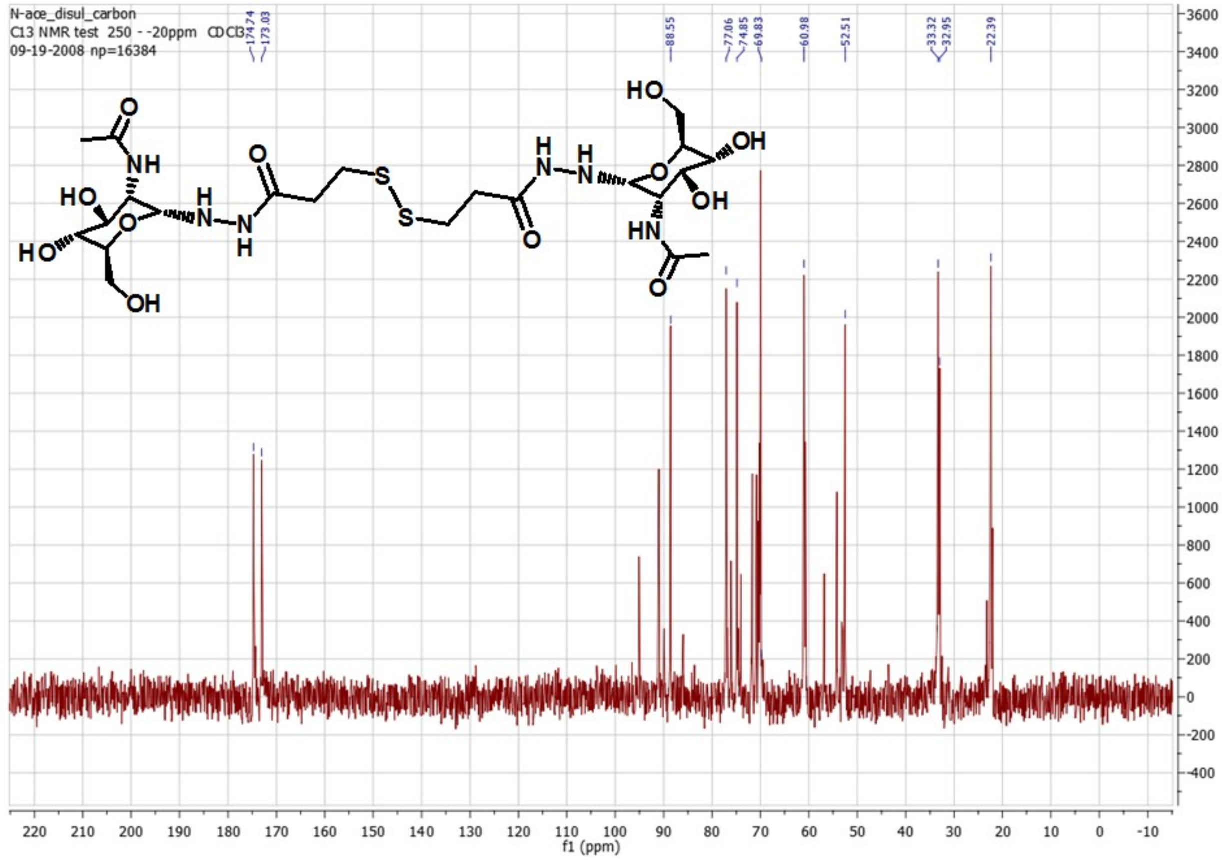
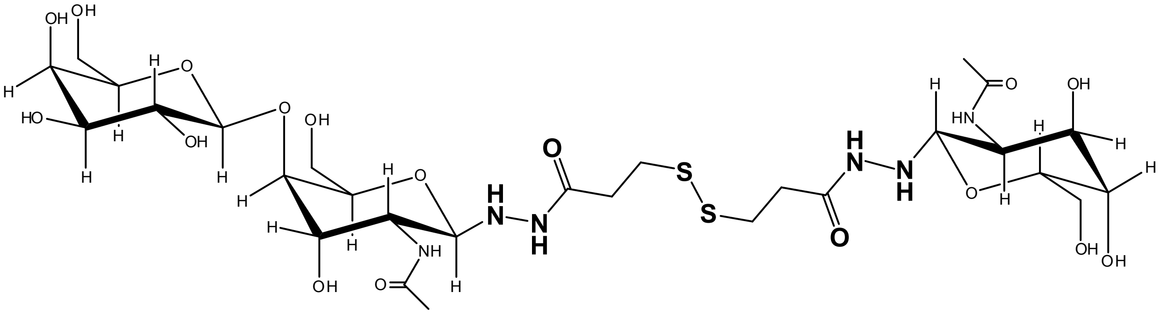


Figure S3.

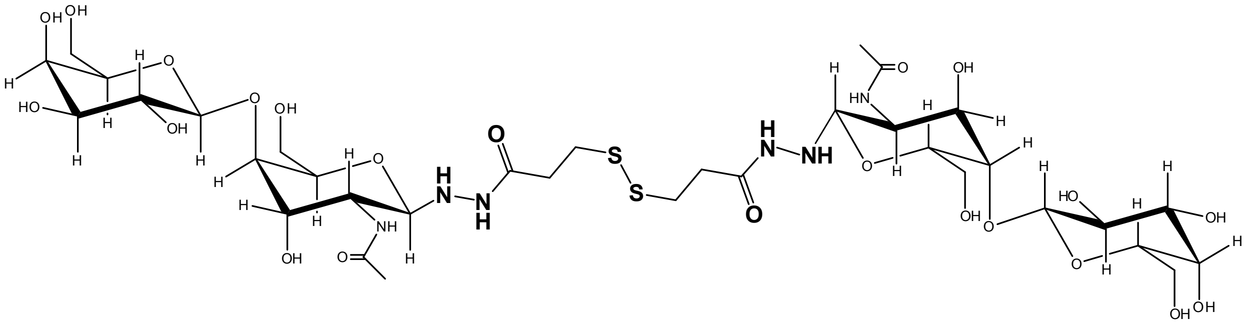
^{13}CNMR (100 MHz, D_2O) δ 174.7, 173.0, 88.6, 77.1, 74.9, 69.8, 60.1, 52.5, 33.3, 33.0, 22.4

(a)



Chemical Formula: C28H50N6O17S2
Exact Mass: 806.27

(b)



Chemical Formula: $C_{34}H_{60}N_6O_{22}S_2$
Exact Mass: 968.32

Figure S4. The chemical structure, formula, and exact mass of the products from the β-1, 4-GT reaction. (a) the monovalent N-acetylactosamine conjugated DTP-β-NAG. (b) the divalent DTP-β-N-acetyllactosamine.

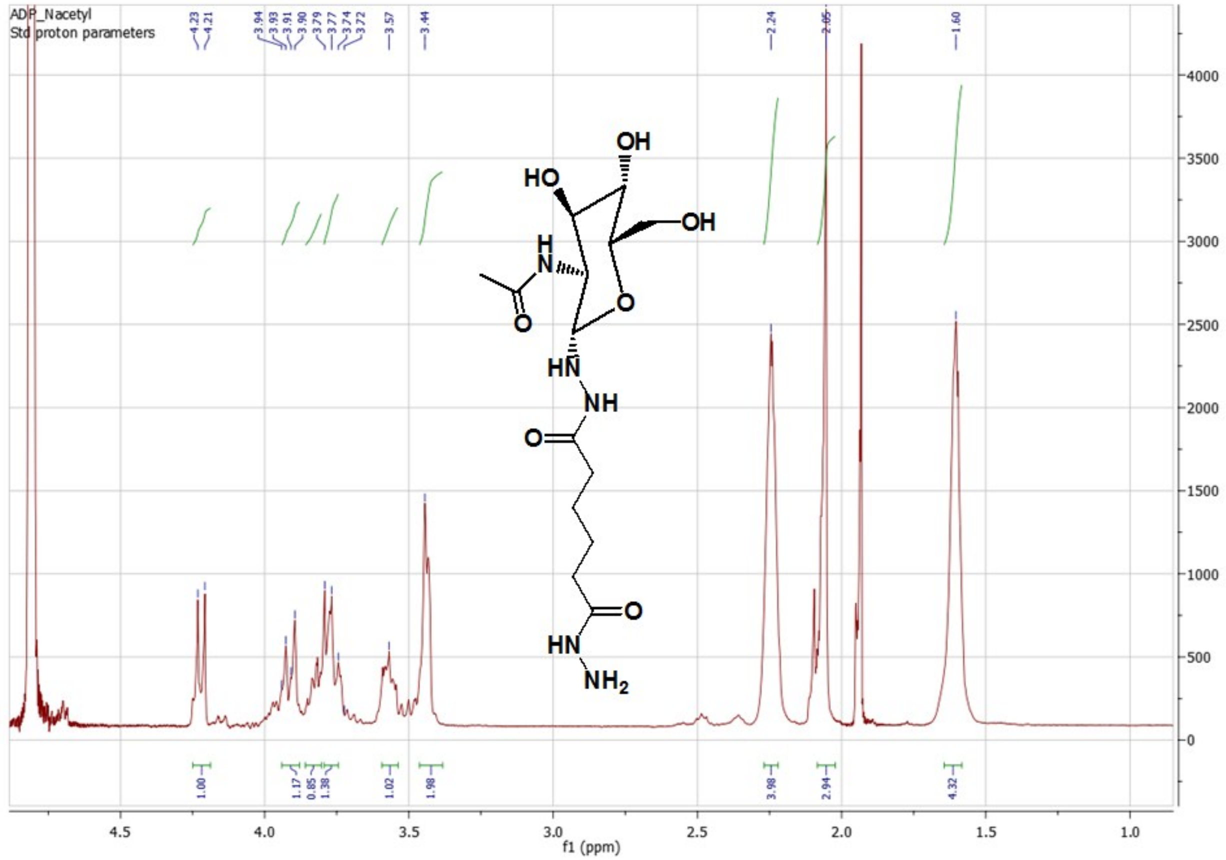


Figure S5.

^{1}H NMR (400 MHz, D_2O) δ 4.23 (d, 1H, J = 9.6 Hz), 3.94 (d, 1H, J = 12.4 Hz), 3.86-3.74 (m, 2H), 3.59 (t, 1H, J = 8.4 Hz and 10.4 Hz), 3.44–3.43 (m, 2H), 2.24 (m, 4H), 2.05 (s, 3H, Ac), 1.60 (m, 4H)

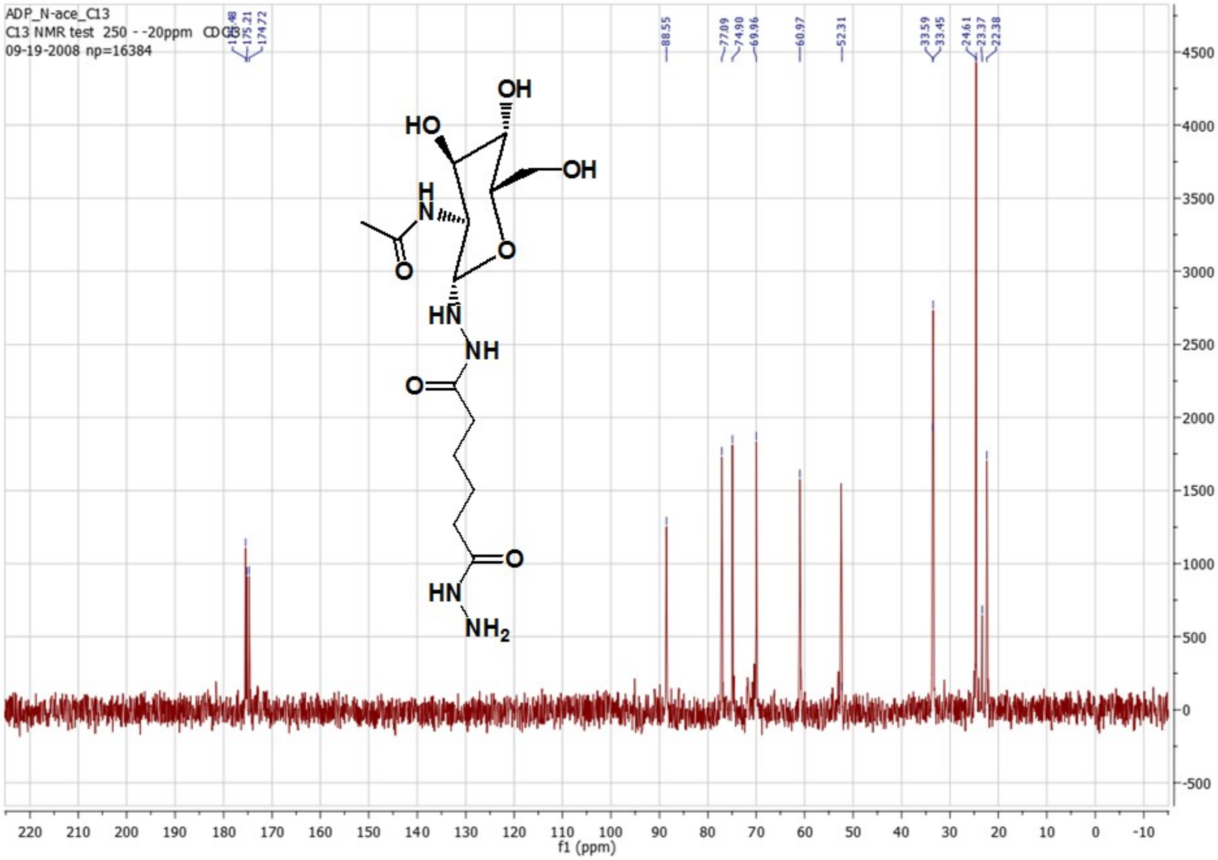


Figure S6.

^{13}C NMR (100 MHz, D$_2$O): d 175.5, 175.2, 174.7, 88.6, 77.1, 74.9, 70.0, 60.1, 52.3, 33.6, 33.5, 24.6, 23.4, 22.4.

3. Surface Coverage Calculation

The surface coverage of DTP-β-NAG was calculated according to the previous literature[2]. Briefly, the surface coverage of adsorbates on the gold film can be estimated via Jung's formula (Eq 1) when the adsorbate layer is very thin as compared to the plasmon decay length[3]. DTP-β-NAG surface coverage (in unit of molecules/cm^2) was estimated with the SPR response:

$$\theta = N\times(I_d/2)\times(R/[m(\eta_a - \eta_s)]) \qquad (1)$$

where N is the bulk number density of the adsorbate; I_d is roughly estimated to be 0.37 for the light wavelength (670 nm) used here; R is SPR response (in the unit of degrees) via binding; *m* is an instrument constant (determined experimentally by calibrating the measured sensor response to changes in refractive index); η_a is the refractive index of adsorbed molecule and η_s is the refractive index of bulk solution (1.33 for water). I_d is larger than the thickness of DTP-β-NAG (~2.2 nm), allowing application of Eq 1[3].

To calculate the surface coverage of DTP-β-NAG, refractive index of N-acetyl-D-glucosamine (1.580) was used as bulk refractive index, and 1.50 g/cm^3 as bulk density. The technical information was obtained from the commercial vendor (the link shown below). From the SPR signal increase upon DTP-β-NAG binding (0.12 ± 0.01 degree), θ was determined to be $5.90 \pm 0.70 \times 10^{12}$ molecules/cm^2.

https://www.scbt.com/scbt/product/n-acetyl-d-glucosamine-7512-17-6

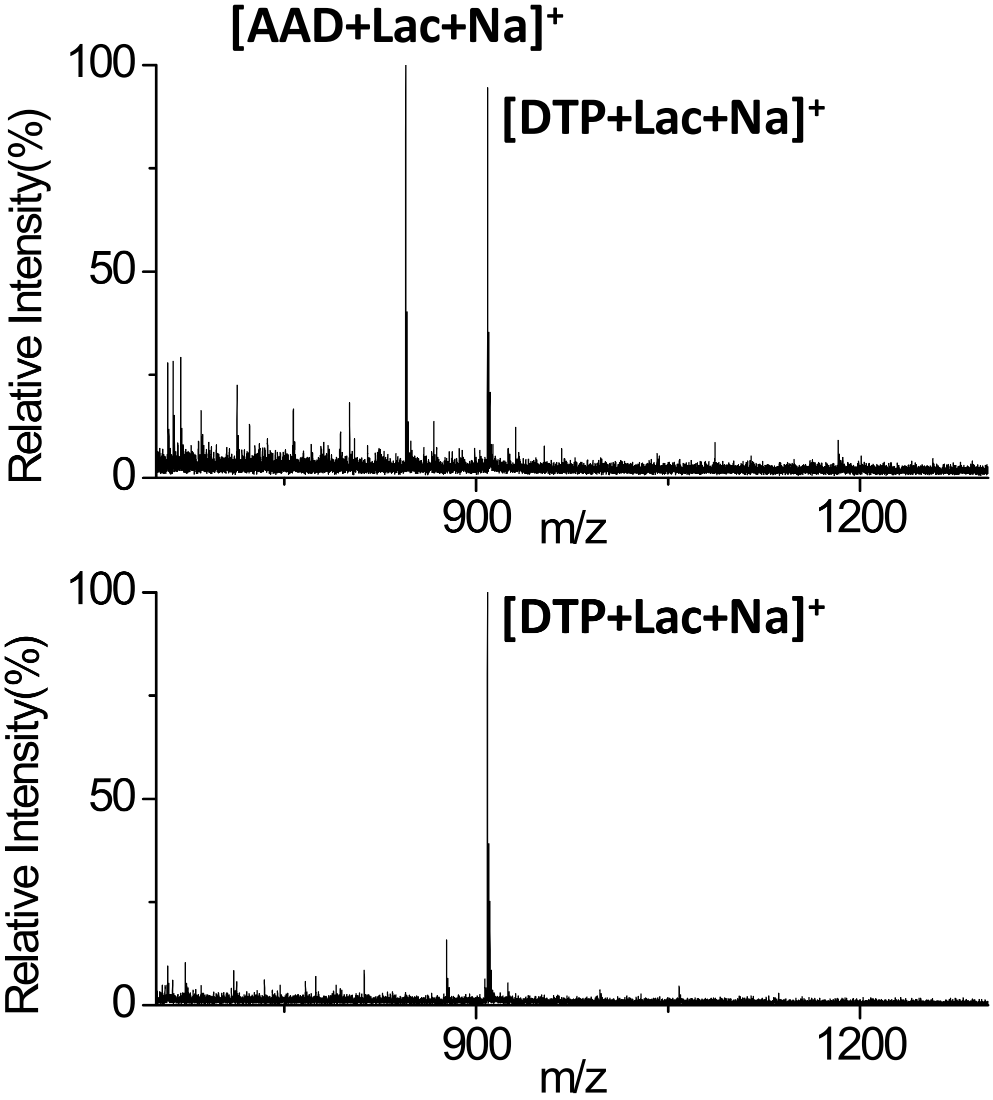
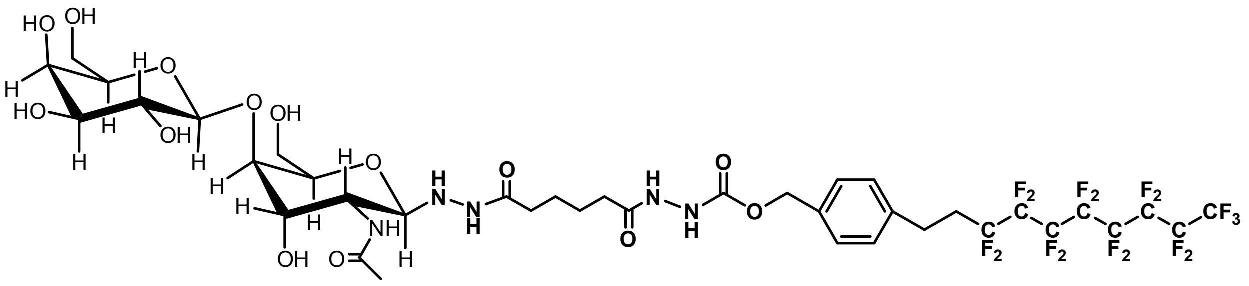


Figure S7. MALDI mass spectra of (a) mixture of AAD and DTP conjugated lactose (b) after enrichment of DTP conjugated lactose using AuNPs.



Chemical Formula: $C_{38}H_{46}F_{17}N_5O_{14}$
Exact Mass: 1119.28

Figure S8. The chemical structure, formula, and exact mass of the product from the β-1, 4-GT reaction on the PFDT-coated gold surface.

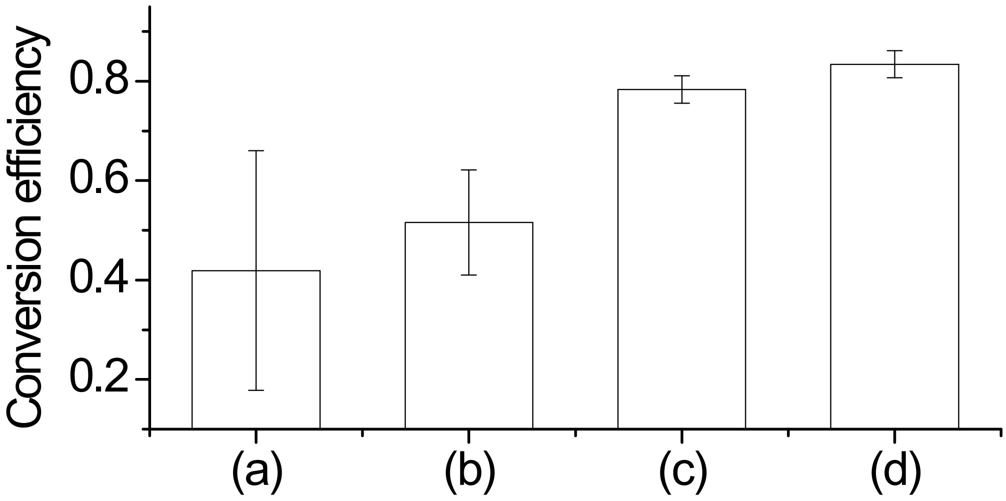


Figure S9. Enzymatic conversion efficiency under different experimental conditions determined with PFDT-modified substrates. Enzymatic reaction conditions are: (a) 3 hrs with 2 μL of UDP-gal, (b) 3 hrs with 4 μL of UDP-gal, (c) 6 hrs with 2 μL of UDP-gal, and (d) 6 hrs with 4 μL of UDP-gal. All data points were obtained with triplicate experiments.

4. Preparation of the perfluorinated surface.

The gold films were prepared according to a previous report.[4] Briefly, Au surface was fabricated by e-beam deposition of a 46-nm thick gold layer onto pre-cleaned glass slides. Cleaned gold substrates were immersed in 1 mM 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiol (PFDT) in ethanol solution overnight, followed by extensive rinsing with ethanol and DI water. The contact angle was measured with a 1μl water droplet on the perfluorinated surface to confirm the substrate hydrophobicity.

Reference:

(1) Halacheva, S. S.; Adlam, D. J.; Hendow, E. K.; Freemont, T. J.; Hoyland, J.; Saunders, B. R. *Biomacromolecules* 2014, *15*, 1814.

(2) Liu, Y.; Liao, P.; Cheng, Q.; Hooley, R. J. *J Am Chem Soc* 2010, *132*, 10383.

(3) Jung, L. S.; Campbell, C. T.; Chinowsky, T. M.; Mar, M. N.; Yee, S. S. *Langmuir* 1998, *14*, 5636.

(4) Linman, M. J.; Yu, H.; Chen, X.; Cheng, Q. *ACS Appl Mater Interfaces* 2009, *1*, 1755.