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Supporting Information

69451 Weinheim, Germany

A Bioorthogonal Raman Reporter Strategy for SERS Detection of Glycans on Live Cells**

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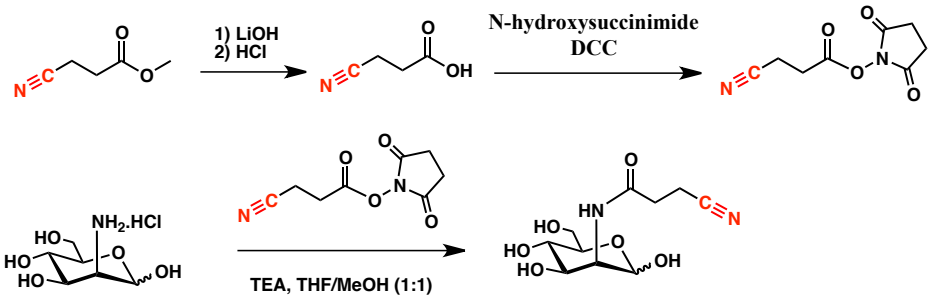
anie_201301387_sm_miscellaneous_information.pdf

Experimental Procedure

General methods and materials. All chemical reagents were obtained from Sigma-Aldrich and J&K and used without further purification. Analytical thin layer chromatography (TLC) was performed on glass-backed Analtech Uniplate silica gel plates, and compounds were visualized by staining with Ceric ammonium molybdate (CAM) or 50% H_2SO_4 in MeOH. Flash chromatography was performed using Merck 60 Å 200-300 mesh silica gel. ^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AVANCE III 500 MHz or 300 MHz spectrometer in CD_3OH or D_2O using tetramethylsilane (TMS) as an internal standard. Electrospray ionization (ESI) mass spectra and high resolution electrospray ionization mass spectra (HR-ESI-MS) were obtained on a Bruker APEX IV Fourier transform ion cyclotron resonance mass spectrometer at the Analytical Instrumentation Center at Peking University.

Synthesis of alkynl and azido sugars. ManNAl,[1] Ac_4ManNAl,[1] SiaNAl,[1] ManNAz,[2] Ac_4ManNAz,[2] and SiaNAz[3] were synthesized as previously described.

Synthesis of ManNCy



Cyanopropionic acid was derived from hydrolysis of methyl 3-cyanopropanoate. Methyl 3-cyanopropanoate (4.52 g, 30.0 mmol) was dissolved in 60 mL THF/H_2O (1:1), followed by addition of LiOH (864 mg, 36.0 mmol). The mixture was stirred at rt overnight, and the pH was adjusted to 1.0 by con. HCl. Then the mixture was extracted with 100 mL ethyl acetate for three times. The combined organic layer was washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give cyanopropionic acid (2.24 g, 75%) as white solid, which was

used directly without further purification.

To a solution of cyanopropionic acid (1.98 g, 20.0 mmol) in 50 mL anhydrous THF, N-hydroxysuccinimide (2.53 g, 22.0 mmol) and DCC (4.95 g, 24.0 mmol) were added at 0 ˚C and the mixture was stirred for 30 min. The mixture was allowed to warm to rt and stirred for additional 8 h. The precipitate was removed by filtration, and the filtrate was concentrated to give a white solid, which was used directly for the next step.

Mannosamine hydrochloride (2.15g, 10.0 mmol) and the crude product of cyanopropionic acid succinimidyl ester obtained from the previous step were dissolved in 100 mL anhydrous THF/MeOH (1:1) under N_2 atmosphere, and the mixture was cooled to 0 ˚C in an ice-water bath. Anhydrous TEA (1.0 mL, 7.0 mmol) was added and the solution was stirred for 1 h at 0 ˚C. The reaction was then allowed to warm to rt, and stirred for additional 12 h. After removal of the solvent, the residue was purified by flash chromatography on silica gel, eluted with CH_2Cl_2 and gradually increasing the polarity to CH_2Cl_2:MeOH (4:1) to give the product (1.82 g, 70%) as a white foam.

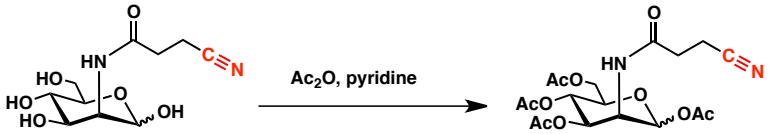
TLC (R_f=0.4, CH_2Cl_2:MeOH =4:1, stained by 50% H_2SO_4 in MeOH).

^{1}H NMR (500 MHz, D_2O) δ 2.69-2.76 (m, 4H), 3.46-3.51 (m, 1H), 3.61-3.63 (m, 0.5H), 3.73-3.92 (m, 3H), 4.06 (dd, *J*=5.0, 10.0 Hz, 0.5H), 4.35 (d, *J*=5.0Hz, 0.5H), 4.49 (d, *J*=5.0Hz, 0.5H), 5.02-5.20 (m, 1H).

^{13}C NMR (125 MHz, D_2O) δ 12.8, 12.9, 13.0, 30.5, 30.6(d), 30.9, 53.4, 54.2(d), 56.8, 60.5, 60.7, 66.6, 66.8, 68.8, 70.0, 70.2, 70.7, 71.6, 72.0(d), 73.8, 76.0, 76.4, 90.9, 93.0, 93.2, 94.9, 120.8, 173.3, 174.2.

HRMS (ESI): Calcd for $C_{10}H_{16}N_2O_6Na$ $[M+Na]^+$ 283.0901, found 283.0903.

Synthesis of Ac_4ManNCy



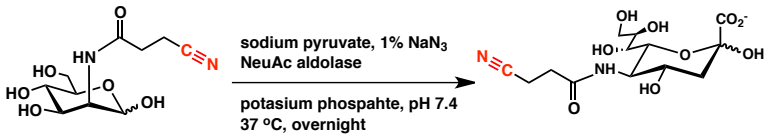
Acetic anhydride (1.02 g, 10.0 mmol) was added to a stirred solution of ManNCy (260 mg, 1.0 mmol) in 30 mL dehydrate pyridine under N_2 atmosphere. The reaction mixture was stirred at rt overnight and the solvent was removed *in vacuo*. The residue was re-dissolved in 20 mL of CH_2Cl_2 and was washed with 2 mol/L HCl (20 mL), saturated $NaHCO_3$ (20 mL) and brine (20 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was obtained after evaporation of the solvent and further purified by column chromatography (hexanes: ethyl acetate, 1:1) to give the product (362 mg, 85%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.95-2.12 (m, 12H), 2.59-2.65 (m, 4H), 3.66-3.68 (m, 0.5H), 3.98-4.01 (m, 1.5H), 4.19-4.21 (m, 1H), 4.61-4.62 (m, 0.5H), 4.74-4.76 (m, 0.5H), 5.03-5.13 (m, 1.5H), 5.26-5.29 (m, 0.5H), 5.81-5.95 (m, 1H), 6.17 (d, *J*=10.0Hz, 0.5H), 6.36 (d, *J*=10.0Hz, 0.5H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 12.0, 12.2, 19.6(d), 19.7(d), 19.8(d), 30.2, 30.4, 48.6, 48.8, 61.3, 61.5, 64.5, 65.0(d), 67.7, 69.2, 70.1, 72.5, 89.6, 90.6, 117.9, 118.0, 167.3, 167.4, 168.5, 168.8(d), 169.1(d), 169.7(d).

HRMS (ESI): Calcd for $C_{18}H_{24}N_2O_{10}Na$ $[M+Na]^+$ 451.1329, found 451.1332.

Synthesis of SiaNCy



ManNCy (1.30 g, 5.0 mmol) was dissolved in 80 mL of 0.050 M potassium phosphate buffer (pH = 7.4), followed by addition of sodium pyruvate (2.75 g, 25.0 mmol), NaN_3 (final concentration of 1% (w/v)) and NeuAc aldolase. The reaction mixture was placed in a shaking incubator at 37 ˚C for 24 h, after which TLC analysis indicated the completion of the reaction. The reaction mixture was then purified by anion-exchange chromatography using AG1-X2 resin, formate form (Bio-Rad). The product was eluted with a gradient of 1.0 to 1.5 M formic acid. Fractions were analyzed for the presence of the product using TLC (R_f= 0.1, MeOH:CH_2Cl_2 = 1:1).

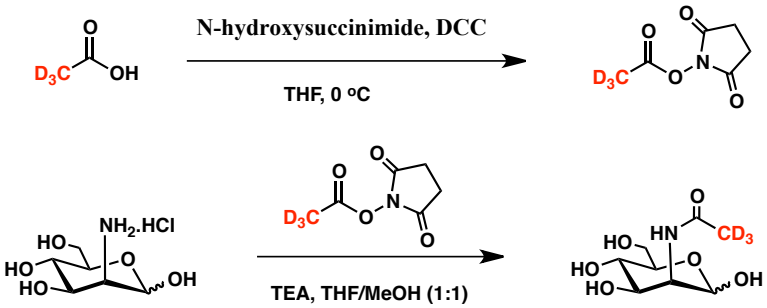
The fractions containing the desired product were combined and concentrated *in vacuo* to give the product (1.13 g, 65%) as a white solid.

^{1}H NMR (500 MHz, D_2O) δ 1.84 (t, *J*=10.0Hz, 1H), 2.28 (dd, *J*=5.0, 10.0Hz, 1H), 2.64-2.75 (m, 4H), 3.55-3.60 (m, 2H), 3.71-3.74 (m, 1H), 3.78-3.81 (dd, *J*=5.0, 15.0Hz, 1H), 3.92-3.96 (app t, 1H), 4.02-4.06 (m, 2H).

^{13}C NMR (125 MHz, D_2O) δ 13.0, 25.2, 28.8, 30.9, 39.0, 52.2, 63.2, 63.7, 68.4, 70.4(d), 95.6, 120.8, 173.3, 173.9, 176.5.

HRMS (ESI): Calcd for $C_{13}H_{20}N_2O_9$ Na $[M+Na]^+$ 371.1061, found 371.1066

Synthesis of [D_3]ManNAc



To a solution of [2,2,2-D_3]acetic acid (128 mg, 2.00 mmol) in 10 mL anhydrous THF, N-hydroxysuccinimide (230 mg, 2.00 mmol) and DCC (495 mg, 2.40 mmol) were added at 0 ˚C and the mixture was stirred for 30 min. The mixture was allowed to warm to rt and stirred for additional 6 h. The precipitate was removed by filtration, and the filtrate was concentrated to give a white solid, which was used directly for the next step.

Mannosamine hydrochloride (299 mg, 1.40 mmol) and the crude product of [D_3]acetic acid succinimidyl ester obtained from the previous step were dissolved in 12 mL anhydrous THF/MeOH (1:1) under N_2 atmosphere, and the mixture was cooled to 0 ˚C in an ice-water bath. Anhydrous TEA (1.0 mL, 7.0 mmol) was added and the solution was stirred for 30 min at 0 ˚C. The reaction was then allowed to warm to rt and stirred for additional 12 h. After removal of the solvent, the residue was purified by flash chromatography on silica gel, eluted with CH_2Cl_2 and gradually increasing the polarity to CH_2Cl_2:MeOH (4:1) to give the product (259 mg, 83%) as a

white foam.

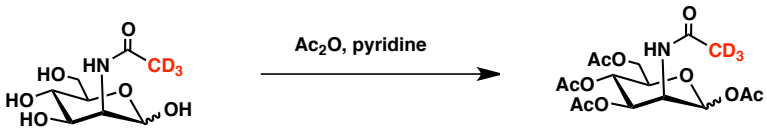
TLC (R_f=0.4, CH_2Cl_2:MeOH =4:1, stained by 50% H_2SO_4 in MeOH).

^{1}H NMR (300 MHz, D_2O) δ 3.41-3.45 (m, 0.5H), 3.53 (t, J=9.9Hz, 0.5H), 3.63-3.66 (m, 0.8H), 3.80-3.93 (m, 3H), 4.06 (dd, J=4.8, 9.9Hz, 0.5H), 4.33 (d, J=4.5Hz, 0.5H), 4.46 (d, J=4.5Hz, 0.4H), 5.03 (s, 0.4H), 5.13 (s, 0.5H)

^{13}C NMR (125 MHz, D_2O) δ 23.2, 46.7, 48.8, 53.1, 54.0, 60.4, 66.5, 66.8, 68.8, 71.9, 72.0, 76.3, 92.9, 93.0, 174.8, 175.7

HRMS (ESI): Calcd for $C_8H_{12}D_3NO_6Na$ $[M+Na]^+$ 247.1088, found 247.0976

Synthesis of [D_3]Ac_4ManNAc



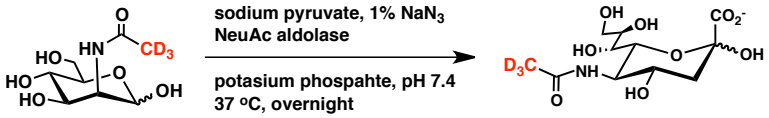
To a solution of [D_3]ManNAc (259 mg, 1.16 mmol) in 2 mL pyridine, was added 0.5 mL acetic anhydride. The reaction mixture was stirred at rt overnight and the solvent was removed *in vacuo*. The residue was re-dissolved in 10 mL of CH_2Cl_2 and was washed with 10% $NaHSO_4$ (10 mL), saturated $NaHCO_3$ (10 mL) and brine (10 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude product was obtained after evaporation of the solvent and further purified by column chromatography (hexanes: ethyl acetate, 1:1) to give the product (368 mg, 81%) as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.66 (s, 1H), 2.01 (s, 3H), 2.07 (s, 3H), 2.10 (s, 3H), 2.18 (s, 3H), 4.02-4.08 (m, 2H), 4.29 (dd, J=5.1, 12.9Hz, 1H), 4.62-4.67 (m, 1H), 5.17 (t, J=10.2Hz, 1H), 5.34 (dd, J=4.5, 9.9Hz, 1H), 5.71 (d, J=9.0Hz, 1H), 6.03 (d, J=1.8Hz, 1H).

^{13}C NMR (125 MHz, D_2O) δ 18.8, 47.5, 60.2, 63.5, 66.9, 68.2, 69.4, 71.6, 88.8, 89.9, 166.3, 167.9, 168.1, 168.4, 168.7, 168.9.

HRMS (ESI): Calcd for $C_{16}H_{20}D_3NO_{10}Na$ $[M+Na]^+$ 415.1510, found 415.1408.

Synthesis of [D_3]Sia

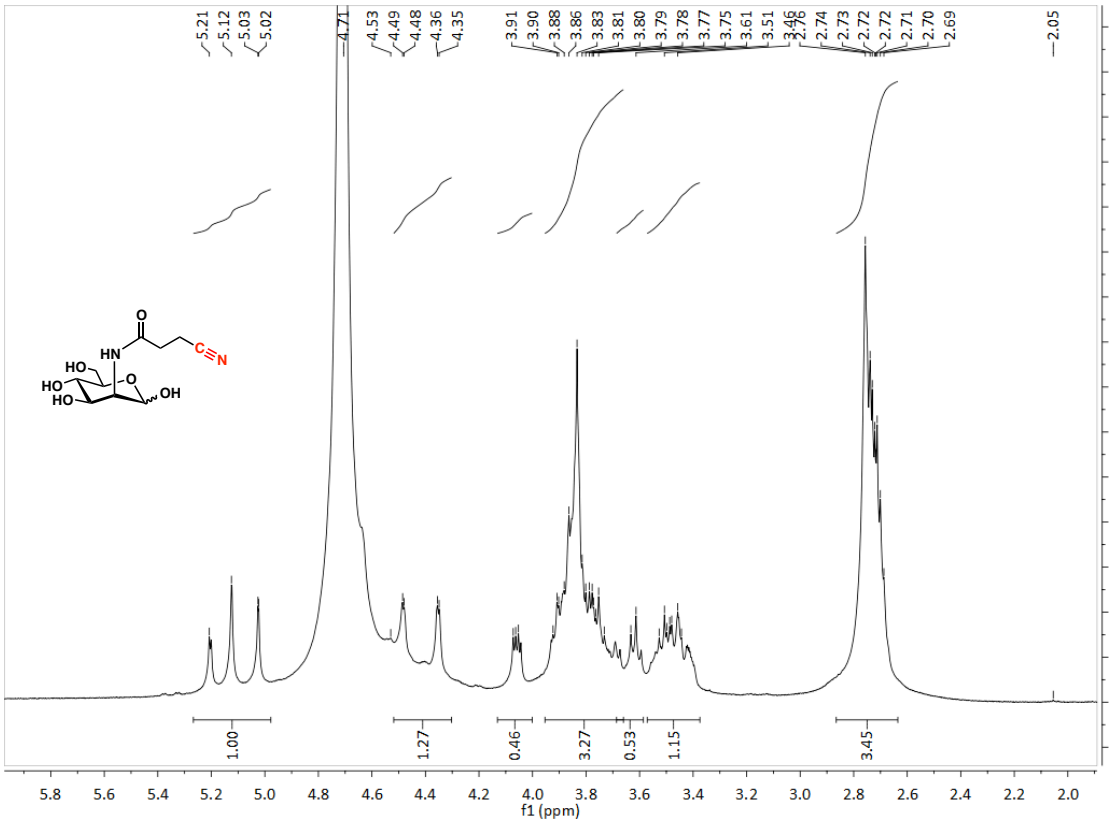


[D_3]ManNAc (0.86 g, 3.84 mmol) was dissolved in 50 mL of 0.050 M potassium phosphate buffer (pH = 7.4), followed by addition of sodium pyruvate (2.11 g, 19.2 mmol), NaN_3 (final concentration of 1% (w/v)) and NeuAc aldolase. The reaction mixture was placed in a shaking incubator at 37 ˚C for 14 h, after which TLC analysis indicated the completion of the reaction. The reaction mixture was then purified by anion-exchange chromatography using AG1-X2 resin, formate form (Bio-Rad). The product was eluted with a gradient of 1.0 to 1.5 M formic acid. Fractions were analyzed for the presence of the product using TLC (R_f= 0.1, MeOH:CH_2Cl_2 = 1:1). The fractions containing the desired product were combined and concentrated *in vacuo* to give the product (0.85 g, 71%) as a white solid.

^{1}H NMR (500 MHz, D_2O) δ 1.70-1.75 (dd, *J*=12.0Hz, 1H), 2.14-2.18 (dd, *J*=4.5, 13.0Hz, 1H), 3.40-3.42 (d, *J*=9.0Hz, 1H), 3.45-3.49 (dd, *J*=6.0Hz, 1H), 3.58-3.61(m, 1H), 3.67-3.70 (dd, *J*=2.5, 9.0Hz, 1H), 3.75-3.79 (app t, 1H), 3.89-3.94 (m, 2H).

^{13}C NMR (125 MHz, D_2O) δ 21.3, 38.9, 52.1, 63.2, 66.8, 68.3, 70.2, 70.5, 95.4, 173.4, 175.0.

HRMS (ESI): Calcd for $C_{11}H_{17}D_3NO_9$ $[M+H]^+$ 313.1248, found 313.1319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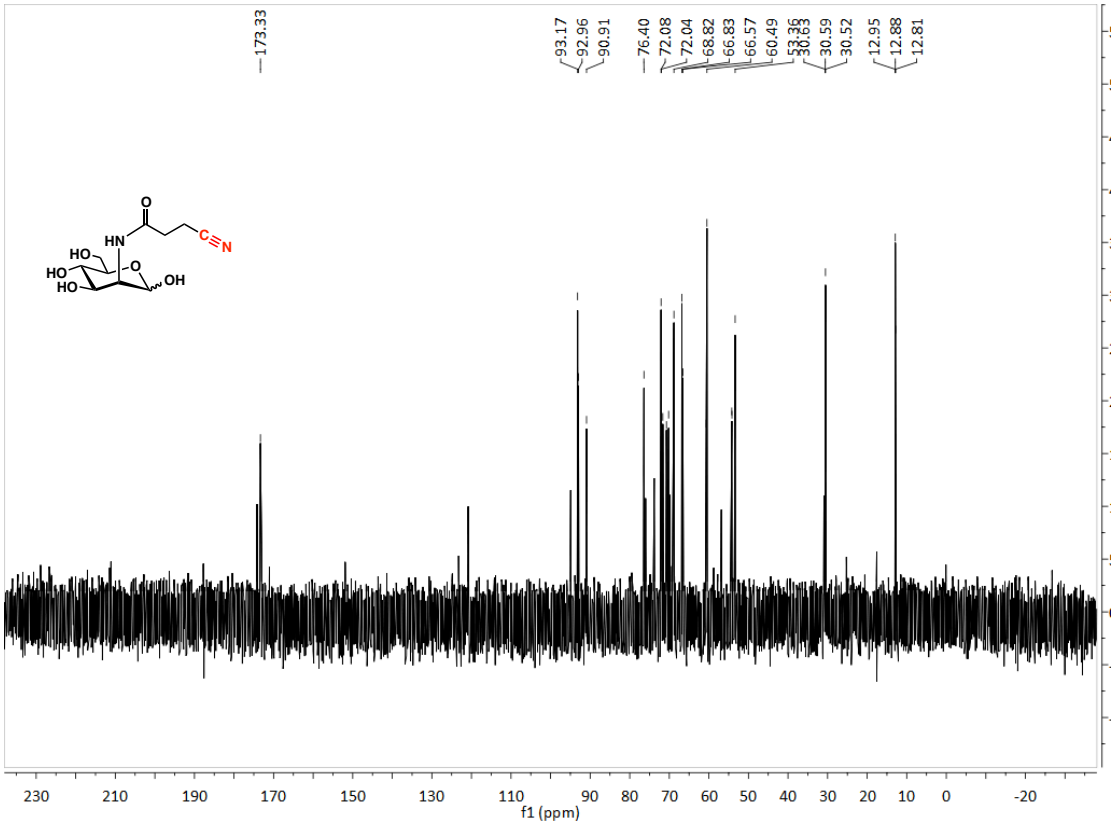
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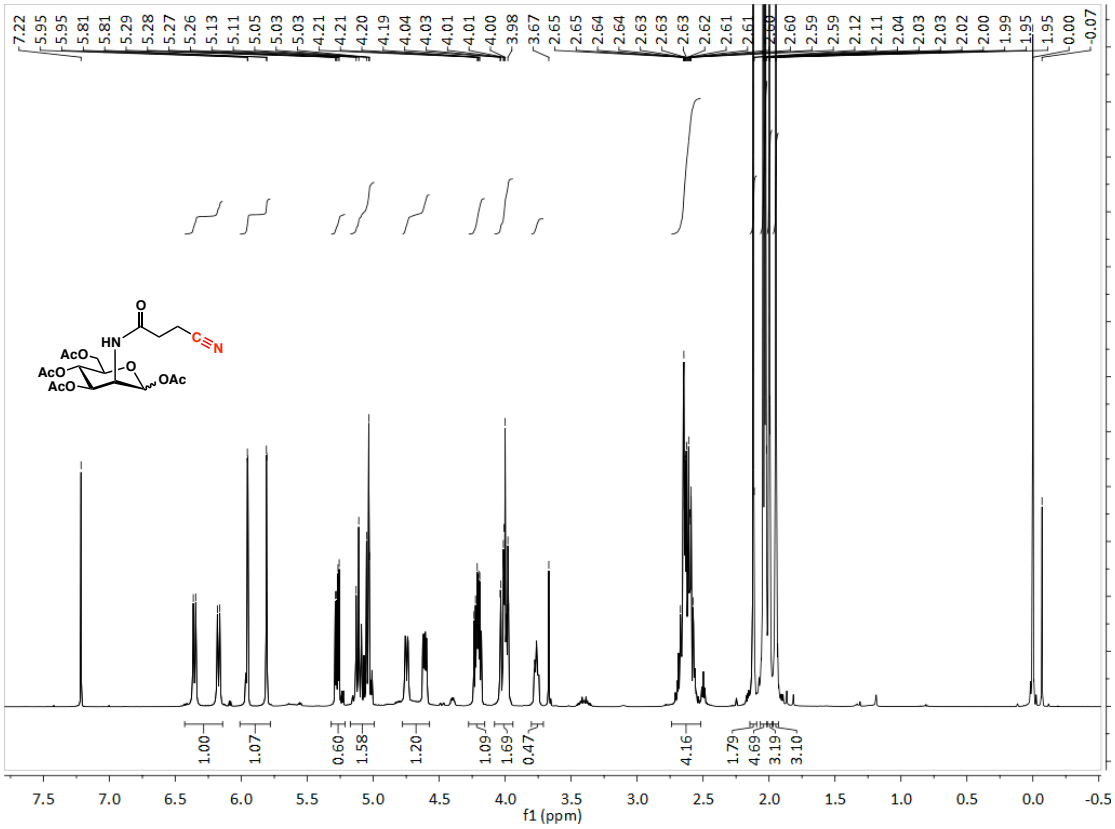
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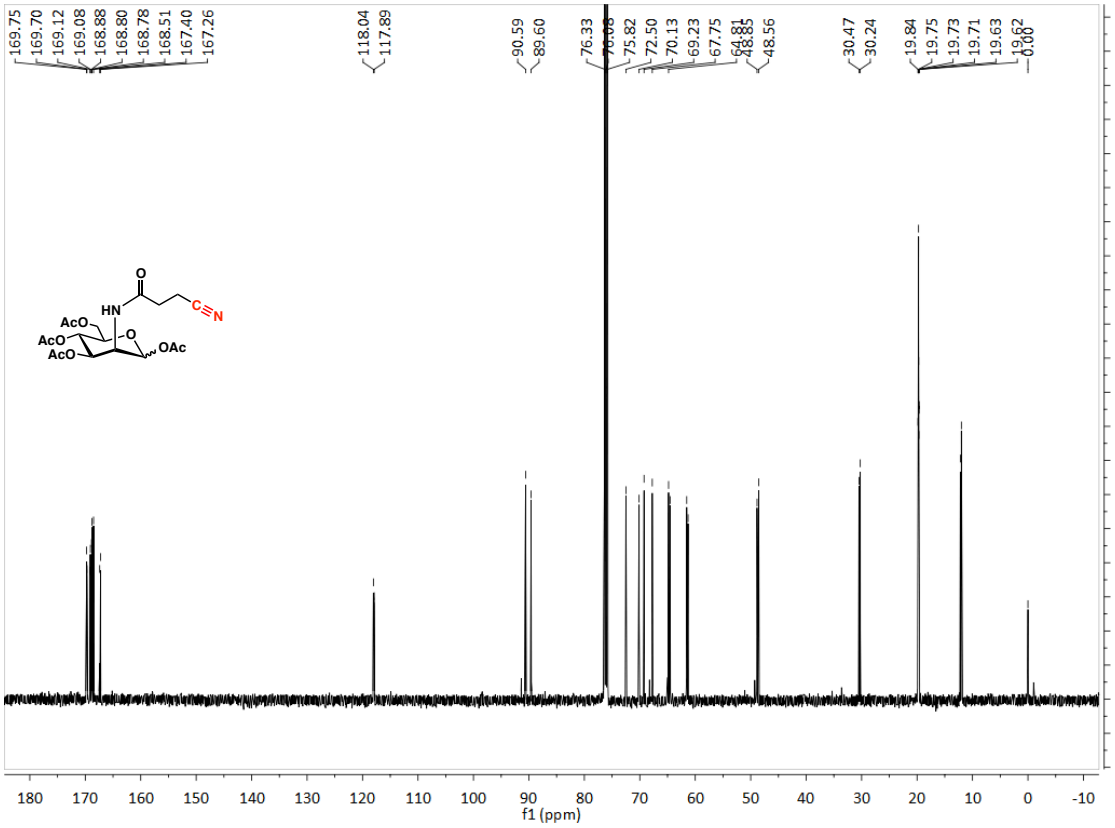
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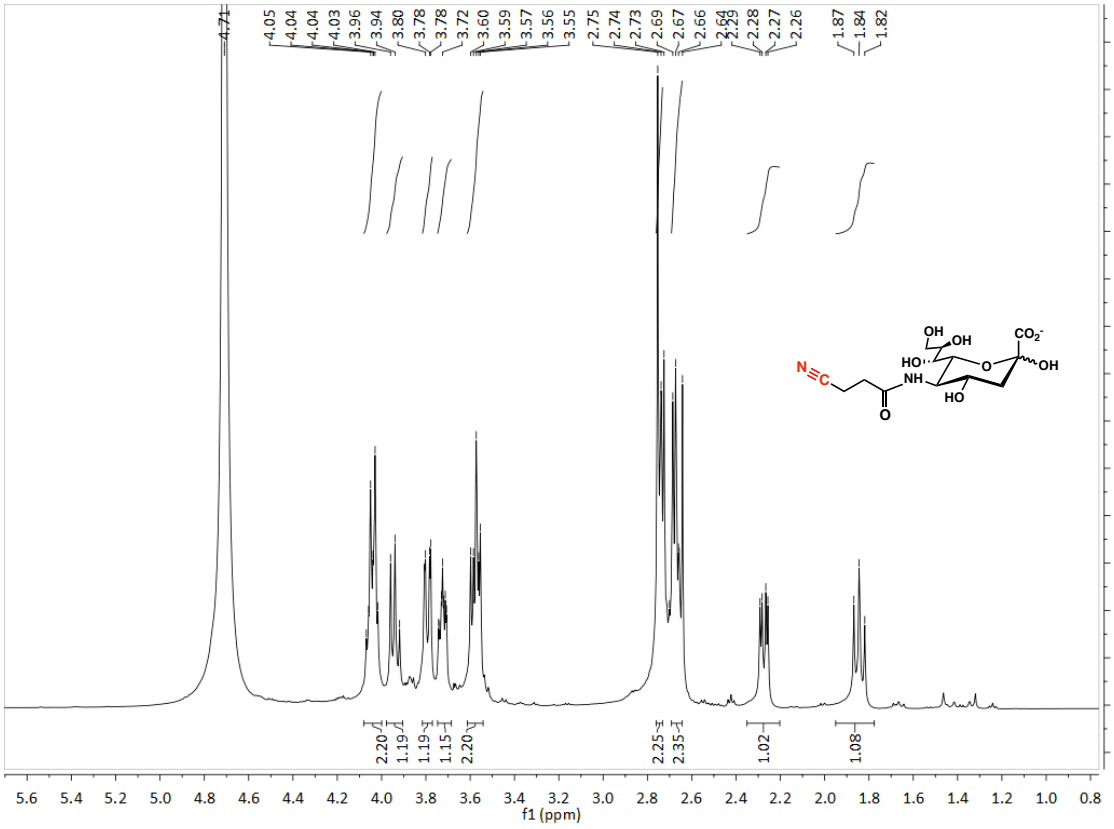
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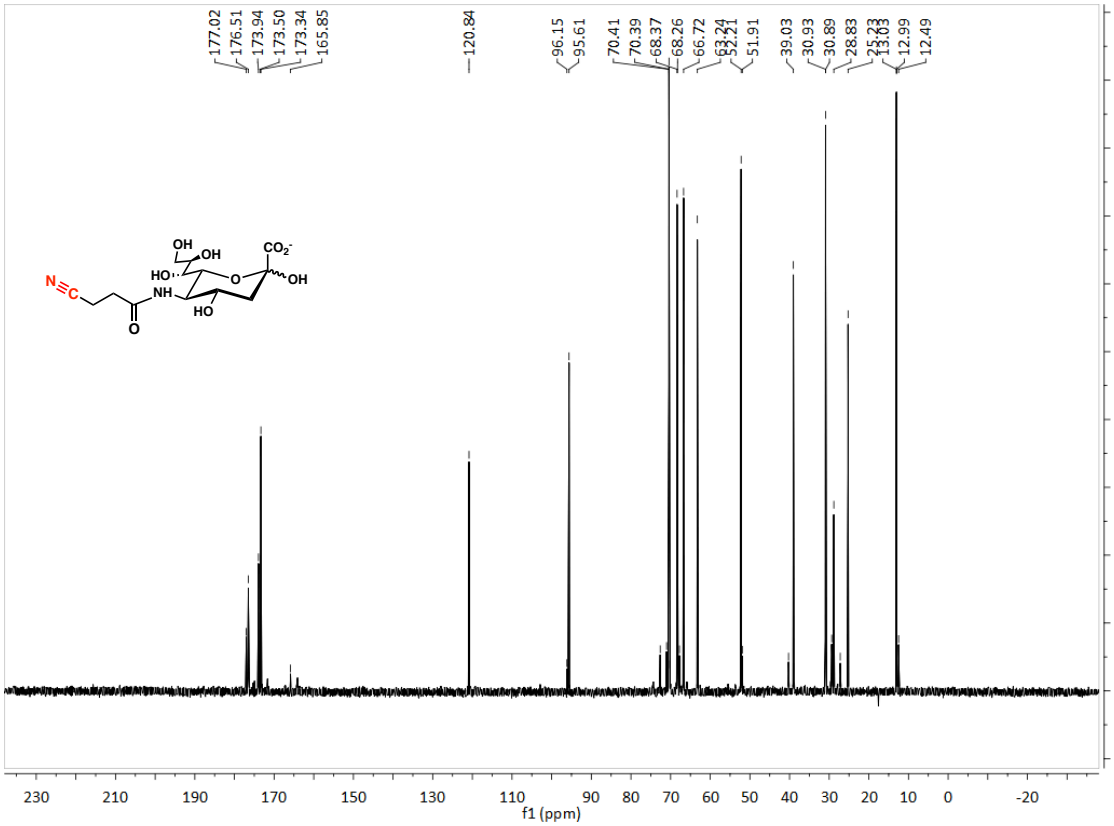
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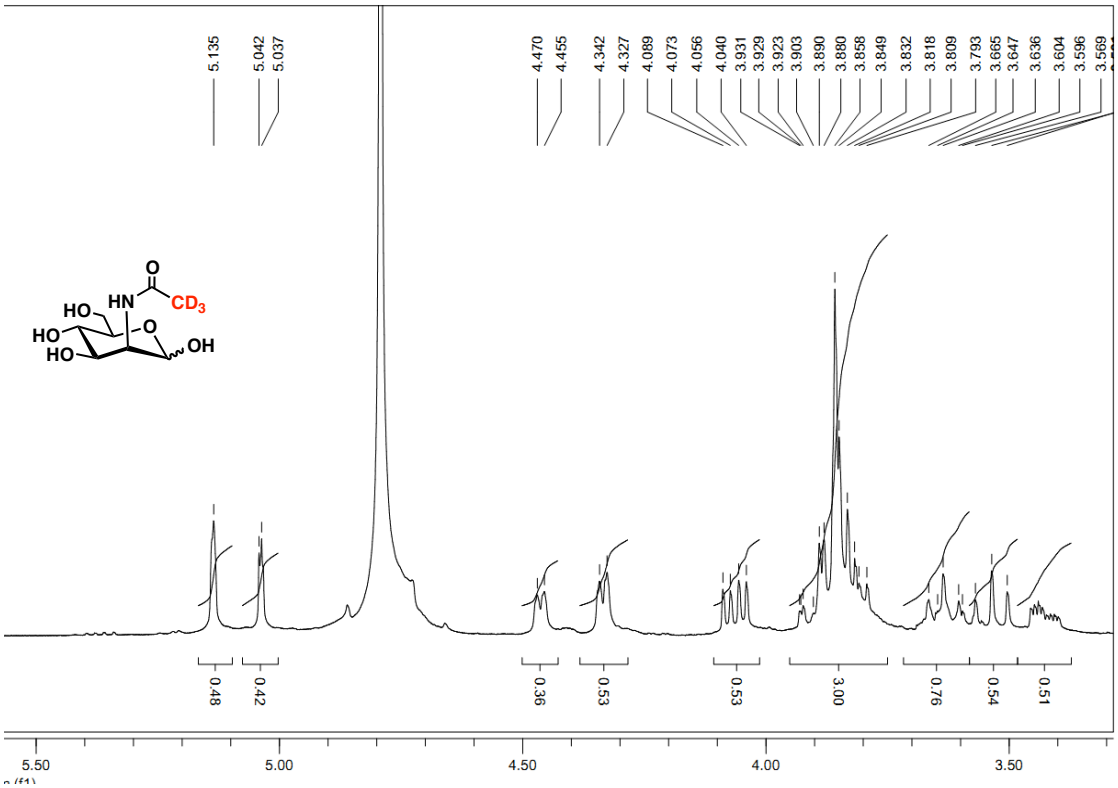
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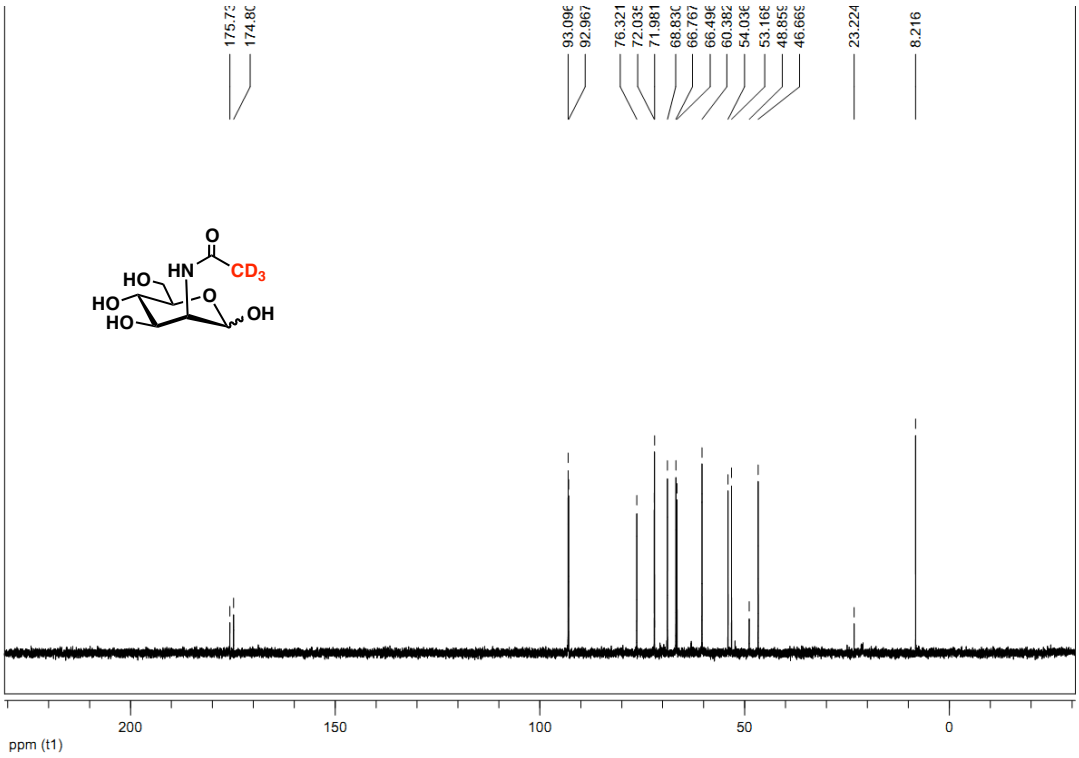
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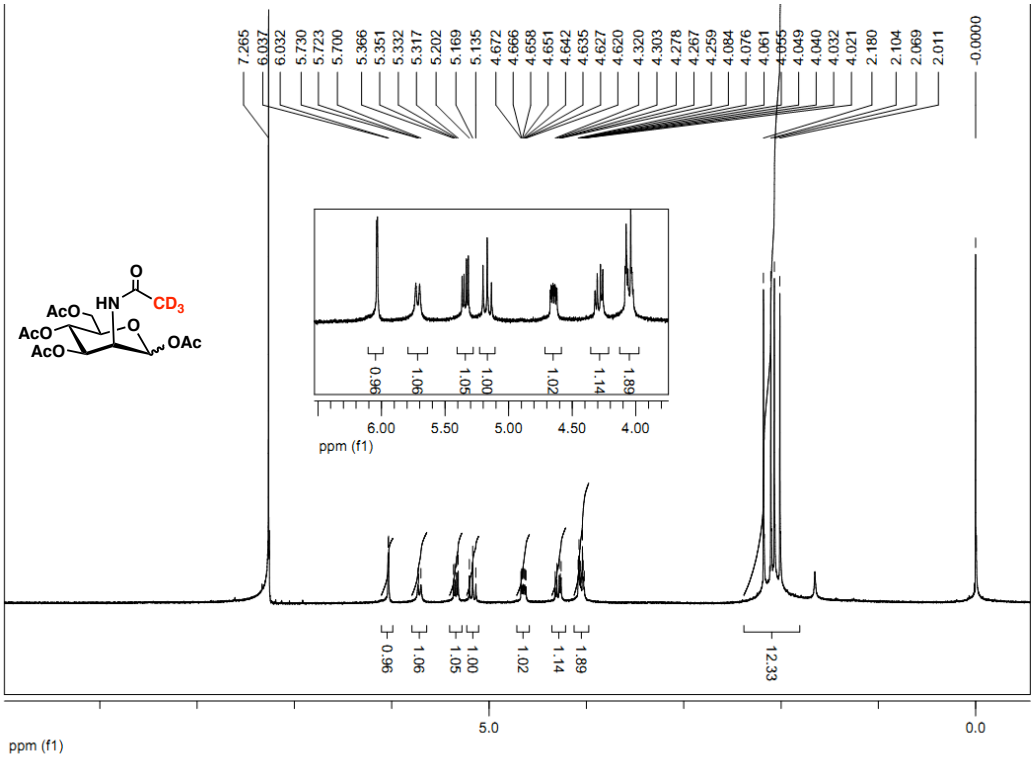
ppm (t1)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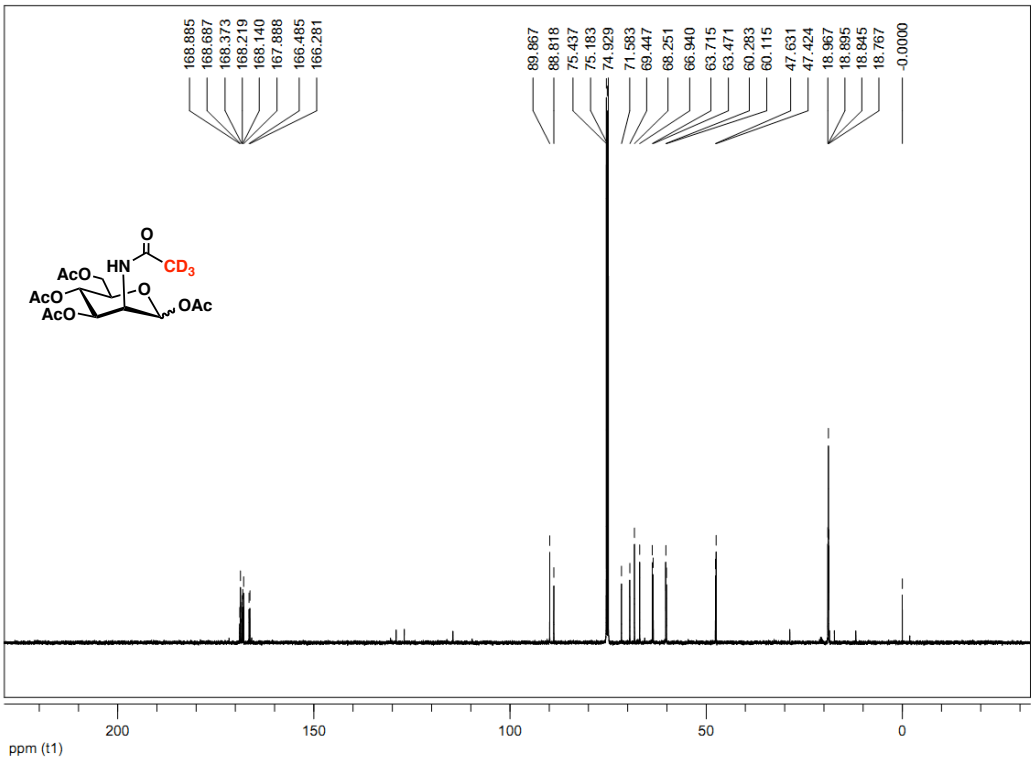
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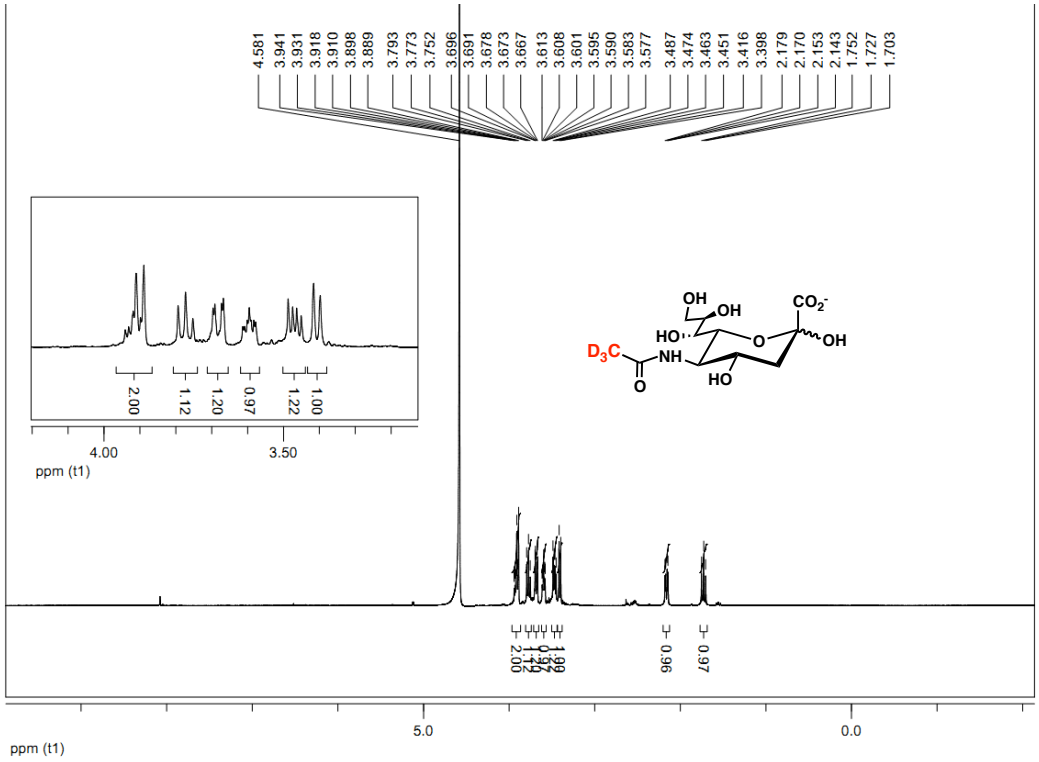
166.485
166.281
89.867
88.818
75.437
75.183
74.929
71.583
69.447
68.251
66.940
63.715
63.471
60.283
60.115
47.631
47.424
18.967
18.895
18.845
18.767
-0.0000
200
150
100
50
0
ppm (t1)
AcO
AcO
AcO
HN
O
CD3
OAc


4.581
3.941
3.931
3.918
3.910
3.898
3.889
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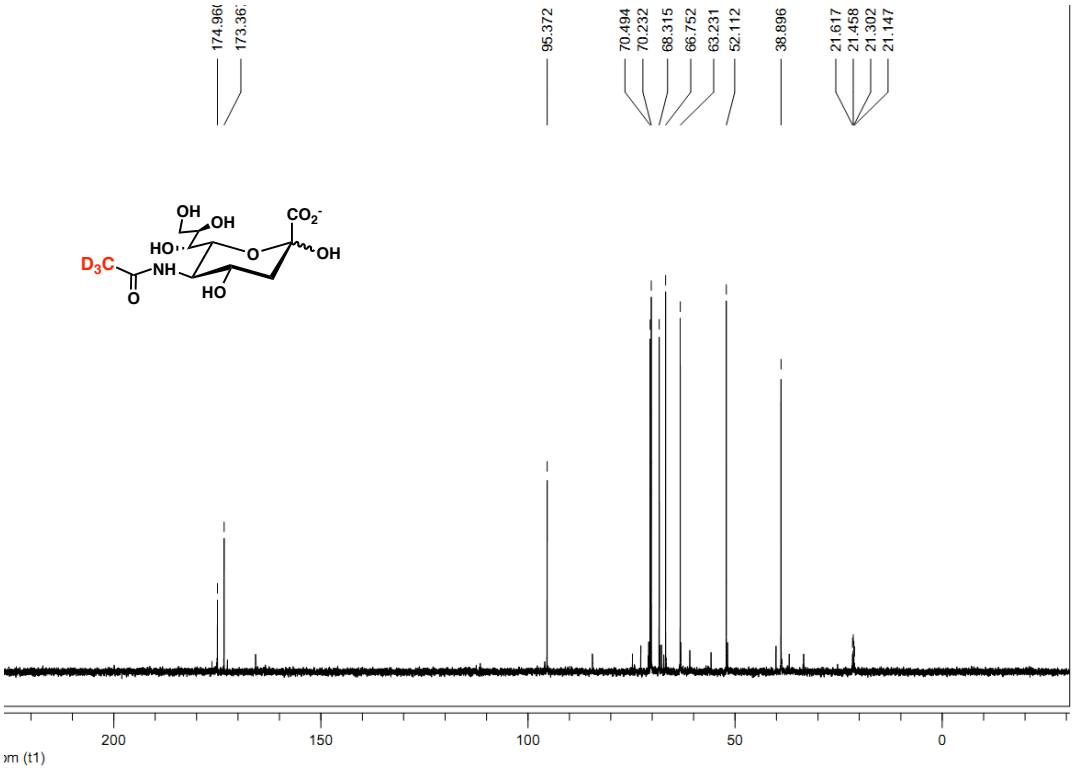
3.773
3.752
3.696
3.691
3.678
3.673
3.667
3.613
3.608
3.601
3.595
3.590
3.583
3.577
3.487
3.474
3.463
3.451
3.416
3.398
2.179
2.170
2.153
2.143
1.752
1.727
1.703
2.00
1.11
1.20
0.97
1.22
1.00
4.00
3.50
ppm (t1)
OH
OH
CO2-
HO
O
OH
D3C
NH
HO
O
2.00
0.96
0.97
5.0
0.0
ppm (t1)


174.96
173.36
95.372
70.494
70.232
68.315
66.752
63.231
52.112
38.896
21.617
21.458
21.302
21.147
OH
OH
CO2-
HO
O
OH
D3C
NH
HO
O
200
150
100
50
0
ppm (t1)

Synthesis of MPBA-AuNPs. Gold nanoparticles (AuNPs) with a diameter of ~120 nm were synthesized using a two-step seed-mediated method that was previously described,[4,5] with minor modifications. Briefly, to a boiling aqueous solution of $HAuCl_4$ (0.01%, w/v), was added 1 mL sodium citrate solution (1% in H_2O, w/v) under stirring. The solution was kept boiling for 15 min, followed by cooling to rt to result in the seed nanoparticles with a diameter of ~ 40 nm. 4 mL of the seed nanoparticles solution was diluted to 60 mL with distilled H_2O, to which was added 0.9 mL sodium citrate solution (1% in H_2O, w/v), 0.9 mL $HAuCl_4$ (1% in H_2O, w/v), and 1.4 mL $HONH_3Cl$ solution (10 mM in H_2O). The solution was stirred for 1 h at rt to give AuNPs with a diameter of ~120 nm.

The resulting AuNPs solution was centrifuged at 3000 rpm for 10 min to remove the supernatant, followed by re-suspending AuNPs in 2 mM 4-Mercaptophenylboronic acid (MPBA) in ethanol. The solution was stirred at rt overnight, followed by centrifugation at 3000 rpm for 10 min to remove the excess MPBA. The obtained MPBA-AuNPs were washed with ethanol for 3 times and distilled water for 3 times before use.

SEM and TEM Characterization. SEM images of MPBA-AuNPs were obtained on a Hitachi S-4800 SEM operated at 30 KeV. TEM images of MPBA-AuNPs were obtained on a JEM 2100 microscope operating at an electron energy of 200 keV.

Cell culture. HeLa cells were cultured in DMEM media supplemented with 10% FBS，100 units/mL penicillin and 100 μg/mL streptomycin. CHO cells were cultured in the DMEM/F12 (1:1) media supplemented with 10% FBS，100 units/mL penicillin and 100 μg/mL streptomycin. The cells were maintained at 37 ^{0}C and 5% CO_2 in a water-saturated incubator.

Unnatural sugar incorporation. $Ac_4ManNAz$, $Ac_4ManNAl$, $Ac_4ManNCy$, or $[D_3]Ac_4ManNAc$ was maintained as a 10 mM stock solution in ethanol, which was allowed to evaporate in the Lab-Tek^{TM} 8-well chamber slides prior to the addition of

cells. The cells were seeded in the Lab-Tek^{TM} 8-well chamber slides at the density of 5 x 10^4 cells/mL and incubated for 3 days in medium containing the indicated unnatural sugar at 50 μM concentration, except that desired concentrations of Ac_4ManNAl were used in the experiment for evaluating the sensitivity of SERS detection of SiaNAl. The cells were then washed thoroughly using PBS (pH = 7.4) for 3 times before SERS measurement.

Raman and SERS measurements of unnatural sugar compounds. The Raman and SERS spectral measurements were performed on a confocal Raman microscopy system (InVia, Renishaw, UK; HORIBA JY LABRAM ARAMIS).

The Raman spectra of compounds were obtained on powder samples using a 532 nm laser operating at intensity of 5 mW/μm^2 power and exposure time of 10 s.

For SERS measurements on compounds, the MPBA-AuNPs were coated on the surface of Si wafer, which was then immersed into the solution of unnatural sugars (50 μM in PBS, pH=7.4) for 3 h at 37 ^{0}C. The SERS spectra were then taken using a He-Ne 633 nm laser with intensity of 0.6 mW/μm^2 and exposure time of 10 s.

SERS measurement on live cells. The unnatural sugar-treated cells were incubated with MBPA-AuNPs in PBS for 30 min at 37 ^{0}C, followed by washing with PBS for 3 times. The SERS measurements were then performed using a He-Ne 633 nm laser with intensity of 0.6 mW/μm^2 and exposure time of 10 s. A 100× objective lens and 1800 grooves/mm grating were used for SERS measurements on live cells. Dark-field images of MPBA-AuNPs attached to the cell surface were obtained on the same microscope equipped with a dark-field condenser using a 100× objective lens.

Supporting Reference:

(1) Chang, P. V.; Chen, X.; Smyrniotis, C.; Xenakis, A.; Hu, T.; Bertozzi, C. R.; Wu, P. *Angew. Chem. Int. Ed.* **2009**, *48*, 4030.
(2) Saxon, E.; Bertozzi, C. R. *Science* **2000**, *287*, 2007.
(3) Luchansky, S. J.; Goon, S.; Bertozzi, C. R. *ChemBioChem* **2004**, *5*, 371.
(4) Brown, K. R.; Natan, M. J. *Langmuir* **1998**, *14*, 726.

(5) Fang, P. P.; Li, J. F.; Yang, Z. L.; Li, L. M.; Ren, B.; Tian, Z. Q. *J. Raman Spectrosc.* **2008**, *39*, 1679.

Supporting Figures

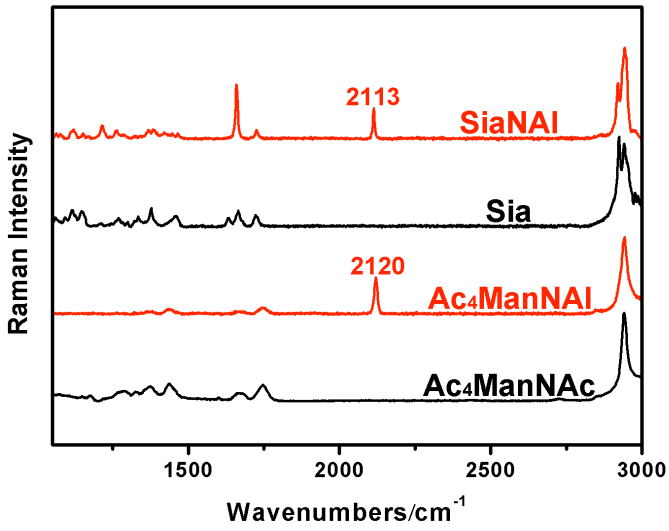
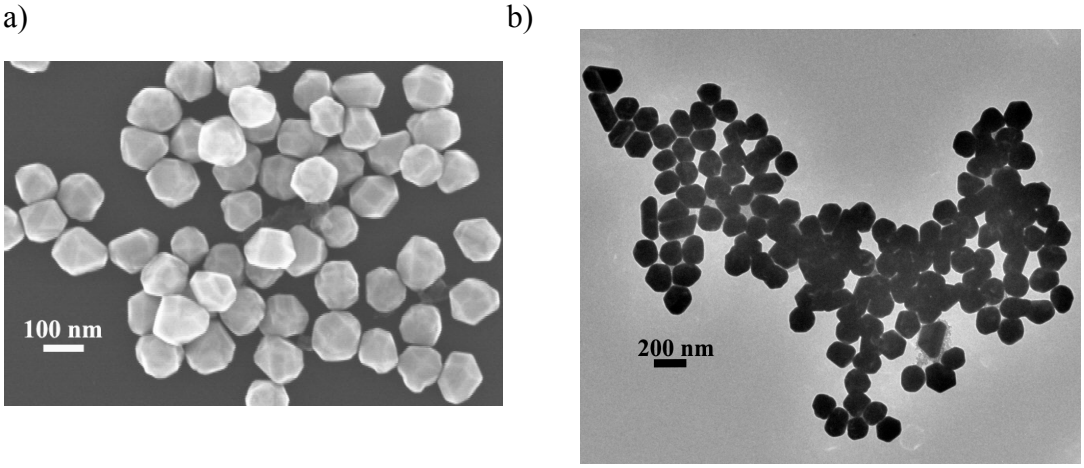


Figure S1. Raman spectra of Ac_4ManNAl and SiaNAl, which showed an alkyne peak at 2120 cm^{-1} for Ac_4ManNAl and 2113 cm^{-1} for SiaNAl. The natural monosaccharides Ac_4ManNAc and Sia were also measured, which did not show any peak in the silent region.

a) b)



c)

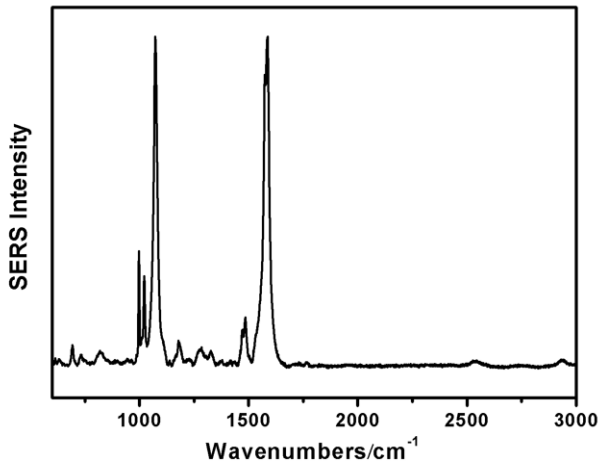
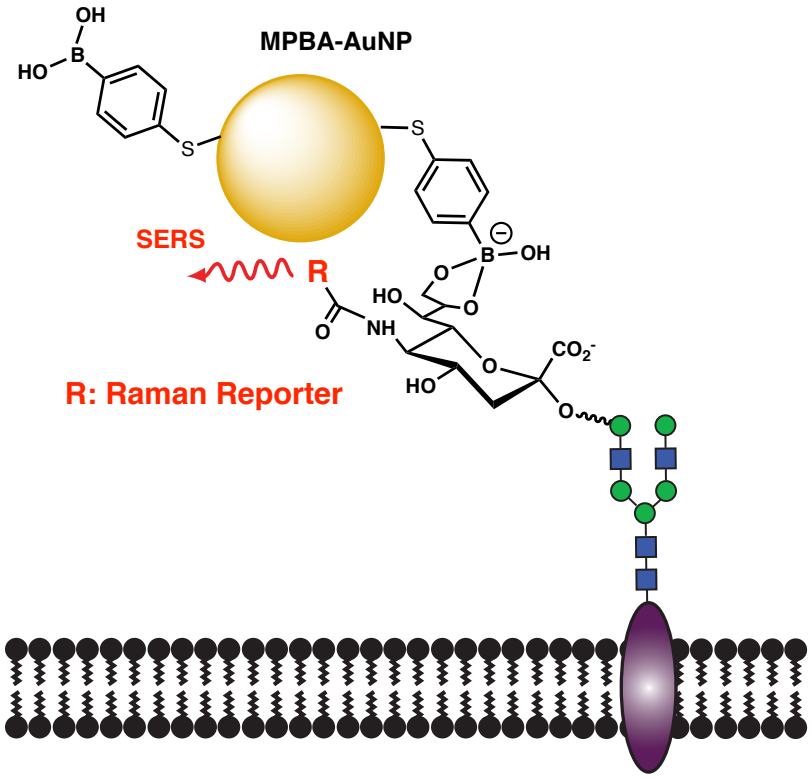
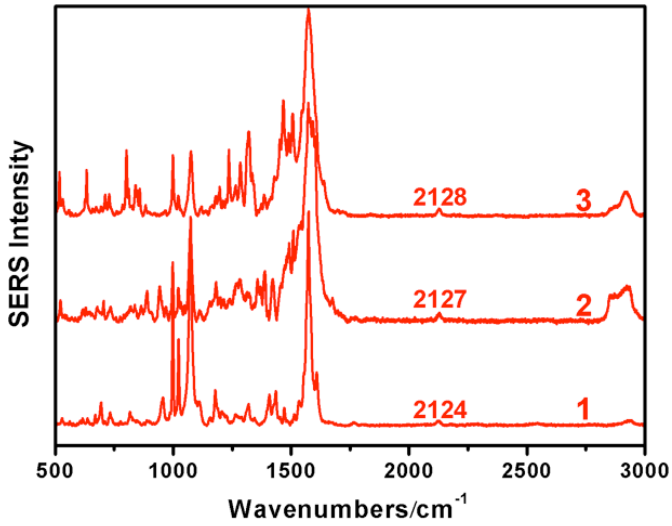


Figure S2. a) Scanning electron micrograph (SEM) of MPBA-AuNPs. b) Transmission electron micrograph (TEM) of MPBA-AuNPs. c) SERS spectrum of MPBA-AuNPs alone. The MPBA molecule does not have Raman peaks in the silent region.



Scheme S1. Schematic of SERS detection of cell-surface sialylated glycans using MPBA-AuNPs as the Raman signal amplifier. The phenylboronic acid moiety forms boronate esters with 1,2- or 1,3- diols of sugars and has higher affinity to sialic acid than other sugars, which ensures both the preferential binding to sialic acids and the efficient Raman signal amplification.

a)



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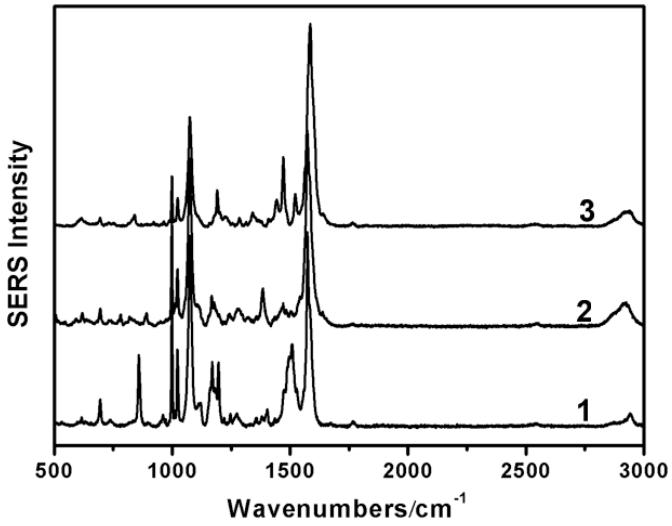
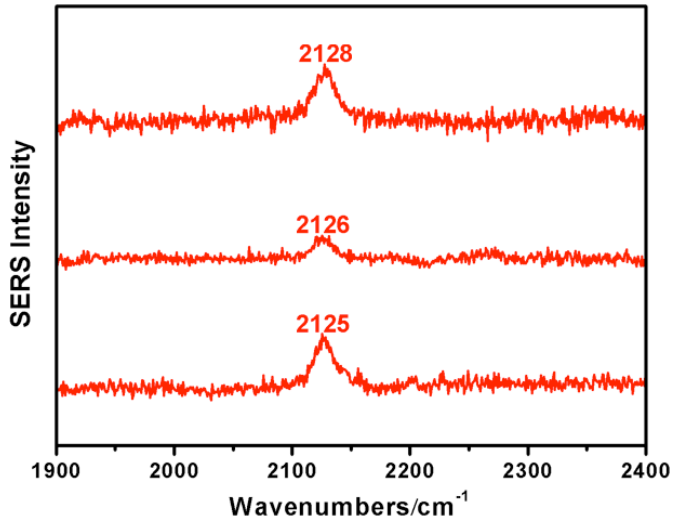


Figure S3. Full SERS spectra (500~3000 cm^{-1}) of HeLa cells treated with a) $Ac_4ManNAl$ and b) $Ac_4ManNAc$. The blow-up between 1900 and 2400 cm^{-1} is shown in Figure 1 in the main text. The SERS data shown are representative of at least 100 measurments.

a)



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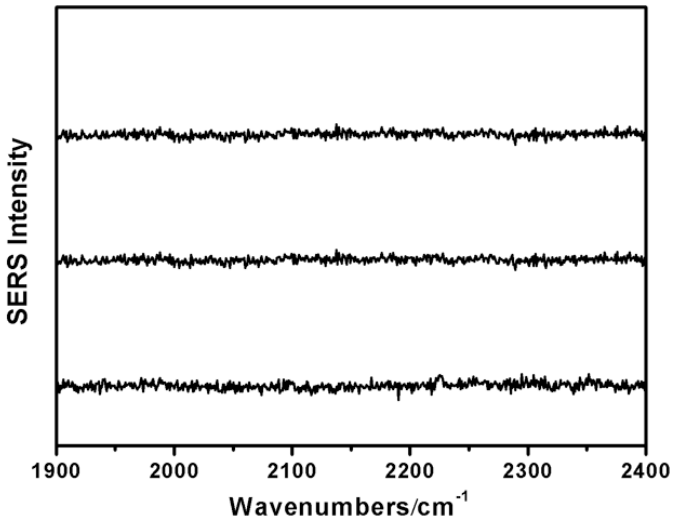

Figure S4. SERS spectra of CHO cells treated with a) $Ac_4ManNAl$ and b) $Ac_4ManNAc$, using MPBA-AuNPs as the signal amplifier. Three representative spectra taken at various cell-surface locations are shown. The SERS data shown are representative of at least 100 measurments.

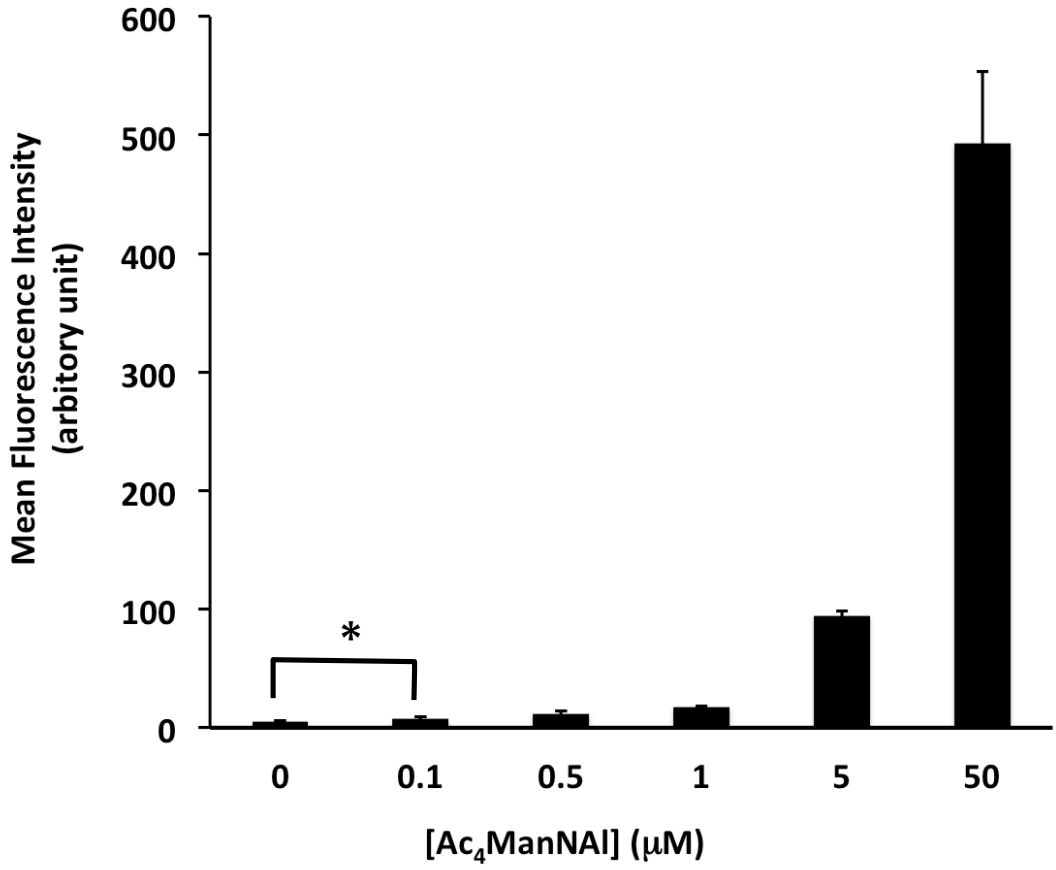

Figure S5. Evaluation of the sensitivity of flow cytometry detection of cell-surface SiaNAl. CHO cells were treated with Ac_4ManNAl at concentrations indicated. The treated cells were reacted with 50 μM biotin-azide using CuAAC assisted by BTTPS ligand, followed by incubation with 2 μg/mL Alexa Flour 647-streptavidin, and analyzed by flow cytometry. Error bars represent the standard deviation from three replicate experiments. The fluoresence intensity detected on cells treated with 0.1 μM Ac_4ManNAl is not distinguishable from the background. *T-test $P > 0.05$ (statistically not significant).

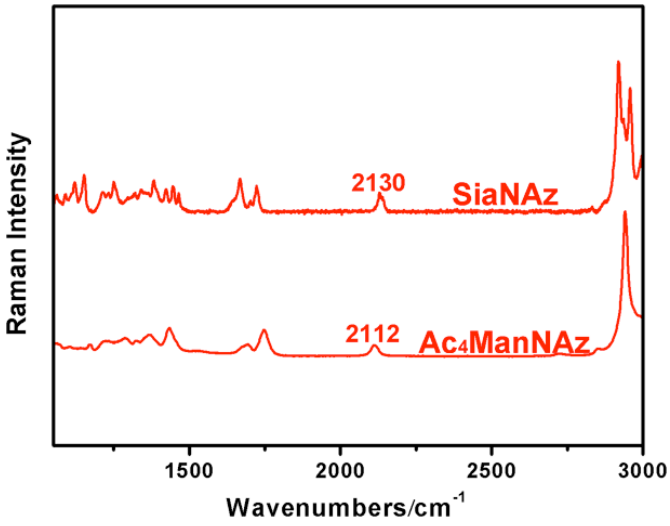

Figure S6. Raman spectra of $Ac_4ManNAz$ and SiaNAz, which showed an azide peak at 2112 cm^{-1} for $Ac_4ManNAz$ and 2130 cm^{-1} for SiaNAz.

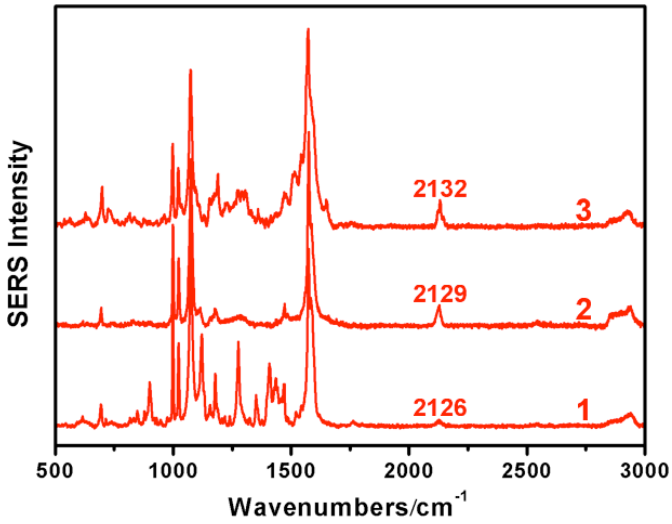

Figure S7. Full SERS spectra (500~3000 cm^{-1}) of HeLa cells treated with Ac_4ManNAz. The blow-up between 1900 and 2400 cm^{-1} is shown in Figure 3 in the main text. The SERS data shown are representative of at least 100 measurments.

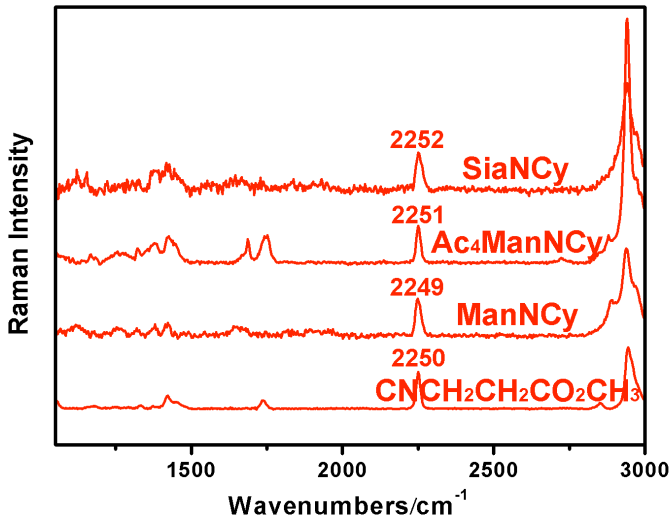

Figure S8. Raman spectra of ManNCy, Ac_4ManNCy and SiaNCy. Methyl 3-cyanopropanoate was also measured to show the peak position of nitrile.

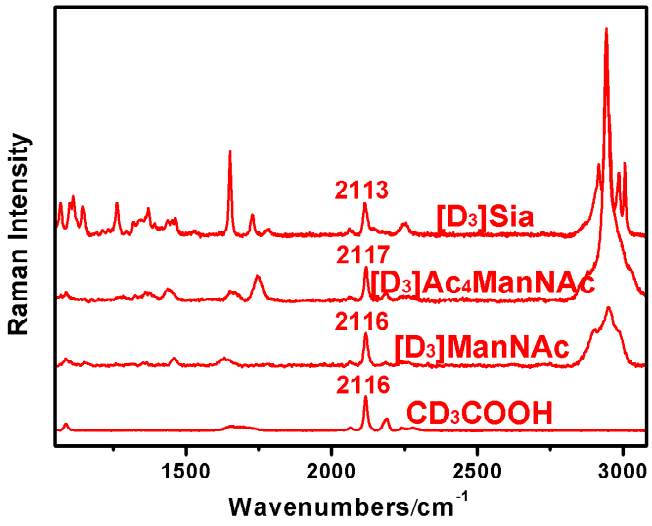

Figure S9. Raman spectra of [D_3]ManNAc, [D_3]Ac_4ManNAc, and [D_3]Sia. [D_3]acetic acid was also measured to show the peak position of C-D.