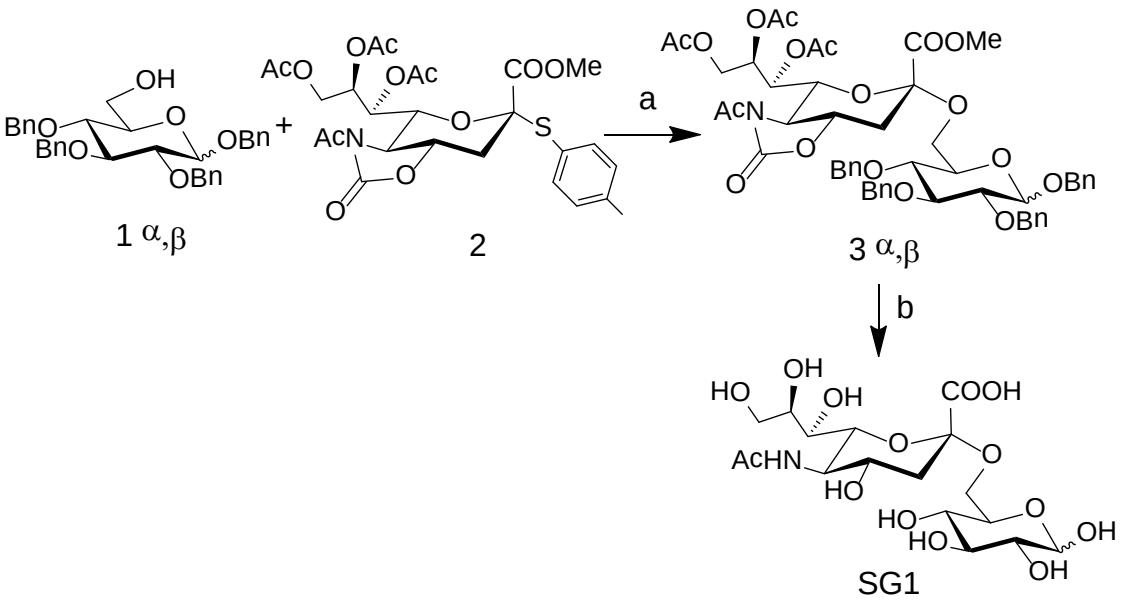
I. Chemical Synthesis.

General:

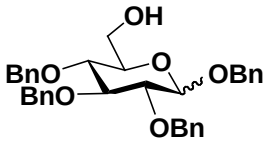
Glycosylation reactions were performed under argon with solvents dried using a solvent purification system (Innovative Technology Inc., Amesbury, MA, USA). Other chemical reagents were of analytical grade, used as supplied, without further purification unless indicated. The acidic ion exchange resin used was Amberlite® IR-120 (H^+) resin. Analytical thin layer chromatography (TLC) was performed on silica gel 230-400 mesh (Silicycle, Quebec City, Canada). Plates were visualized under UV light, and/or by staining with acidic $CeH_8Mo_3N_2O_{12}$, followed by heating. ^1H and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer. Chemical shifts are reported in δ (ppm) units using ^{13}C and residual ^1H signals from deuterated solvents as references. Spectra were analyzed with MNova® (Mestrelab Research, Escondido, CA, USA). Electrospray ionization mass spectra were recorded on a Micromass QT 2 (Waters) and data were analyzed with MassLynx® 4.0 (Waters, Milford, MA, USA) software. Reported yields refer to spectroscopically and chromatographically pure compounds that were dried under high vacuum (10^{-2} mbar) before analytical characterization, unless otherwise specified.

Abbreviations: N, N Dimethyl formamide, DMF; Ethyl acetate, EtOAc; Dichloromethane, DCM; Thin layer chromatography, TLC; Methanol, MeOH; Ethanol, EtOH; Sodium hydride, NaH; Benzyl bromide, BnBr; Triethylamine, NEt_3; *tert*-Butyldimethylsilyl chloride, TBSCl; 4-Dimethylaminepyridine, DMAP; Sulphuric acid, H_2SO_4,; Trifluoromethanesulfonic acid, TfOH; *N*-Iodosuccinimide, NIS; Sodium methoxide, NaOMe; Palladium hydroxide, $Pd(OH)_2$; Sodium hydroxide, NaOH; Sodium thiosulfate, $Na_2S_2O_3$; Hydrochloric acid, HCl; Glucose oxidase, GOD.



Scheme 1. *Reagents and Conditions*: a) TfOH, NIS, DCM, -60 °C, 2h, 75%; b) i) NaOMe, MeOH, rt, 30 min.; ii) $Pd(OH)_2/C/H_2$, EtOH, rt, 12h.; iii) 0.05 N NaOH in H_2O, rt, 4h, 93% over three steps.

Benzyl 2,3,4-tri-O-benzyl-α,β-D-glucopyranoside:

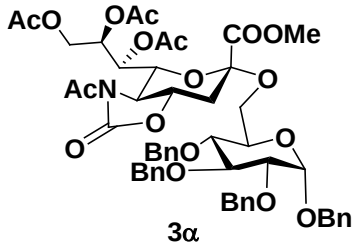


1α,β

To a solution of compound Benzyl α,β-D-glucopyranoside [1] (1.0 g, 3.7 mmol) in pyridine (0.010 L), TBSCl (0.85 g, 5.5 mmol) was added, followed by addition of DMAP (0.04 g, 0.37 mmol). The reaction mixture was stirred overnight at rt. After completion of reaction as monitored by TLC, the solvent was removed using a rotary evaporator and the crude compound was used directly without further purification. To a stirred suspension of NaH (0.89 g, 18 mmol, prewashed with dry hexane to remove the oil present in 60% suspension of NaH) in anhydrous DMF (0.010 L), the crude TBS compound in DMF (0.010 L) was added at 0 °C. The reaction mixture was stirred under argon atmosphere for 30 min. BnBr (2.2 ml, 18 mmol) was added dropwise via syringe and reaction mixture was stirred for 3 h. The reaction mixture was quenched with 1M HCl (10 mL), diluted with EtOAc (20 mL) and washed with water (2 x 10 mL) and brine (2 x 10 mL). The organic layer was dried over $NaSO_4$ and concentrated under reduced pressure to obtain the tetra benzyl protected compound. The crude compound was used directly without further purification. To a stirred solution of the crude compound in MeOH (0.020 L), H_2SO_4 in MeOH (1M, 0.50 mL) was added. The reaction mixture was stirred at rt and

the reaction progress was monitored by TLC using two solvent systems: EtOAc–hexanes (1:9) and EtOAc–hexanes (1:1). After completion of reaction as monitored by TLC, the reaction mixture was diluted with EtOAc (20 mL) and washed with saturated $NaHCO_3$ (2 x 20 mL) and brine (2 x 20 mL). The organic layer was dried over $NaSO_4$, concentrated under reduced pressure and subjected to flash chromatography to give **1α**(0.55 g, 27% over three steps) and **1β** (0.40 g, 20% over three steps). Spectral data is in complete agreement with reported value.[2]

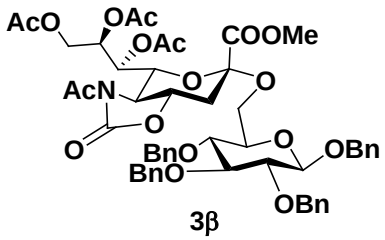
Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate-(2,6)-1,2,3,4-tetra-*O*-benzyl-α-D-glucopyranoside (3α) :



3α

To the stirred solution of acceptor **1α** (0.20 g, 0.35 mmol) and donor **2** [3] (0.24 g, 0.38 mmol) in anhydrous DCM (4.0 ml) under an argon atmosphere at -40 °C, NIS (0.19 g, 0.84 mmol) was added followed by TfOH (0.52 ml, 0.35 mmol, 10% in anhydrous DCM). The reaction mixture was stirred at this temperature for ~2h until complete disappearance of the acceptor as determined by TLC. NEt_3 (0.50 mL) was added to quench the reaction and warmed to rt. The reaction mixture was diluted with DCM (20 mL), washed with aqueous solution of $Na_2S_2O_3$ (2 x 10 mL) dried over Na_2SO_4 and concentrated under reduced pressure. The residue was subjected to flash silica gel column chromatography eluting with (hexanes:EtOAc 7:3) to afford the **3α** (0.27 g, 75 %) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.45 – 7.26 (m, 20H), 5.58 (dd, *J* = 8.9, 1.7 Hz, 1H), 5.45 (ddd, *J* = 8.5, 6.1, 2.5 Hz, 1H), 4.97 (d, *J* = 10.8 Hz, 1H), 4.86 – 4.78 (m, 3H), 4.76 (d, *J* = 6.1 Hz, 1H), 4.73 – 4.65 (m, 2H), 4.56 (dd, *J* = 15.0, 12.2 Hz, 2H), 4.26 (dd, *J* = 12.4, 2.7 Hz, 1H), 4.20 (dd, *J* = 10.8, 4.5 Hz, 1H), 4.14 (d, *J* = 7.2 Hz, 1H), 4.08 – 3.98 (m, 2H), 3.98 – 3.84 (m, 2H), 3.73 (s, 3H), 3.66 (dd, *J* = 11.2, 9.4 Hz, 1H), 3.60 (d, *J* = 9.4 Hz, 1H), 3.58 – 3.52 (m, 1H), 3.49 (dd, *J* = 10.6, 2.0 Hz, 1H), 2.94 (dd, *J* = 12.1, 3.4 Hz, 1H), 2.49 (s, 3H), 2.15 (s, 3H), 2.12 – 2.06 (m, 1H), 2.04 (s, 3H), 1.81 (s, 3H). 13C NMR (100 MHz, CDCl3) : δ 171.8, 170.6, 169.9, 169.9, 168.4, 153.6, 138.8, 138.4, 138.2, 137.1, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7, 127.5, 99.2, 95.5, 81.9, 79.6, 77.5, 75.7, 75.2, 75.0, 74.9, 73.0, 71.4, 69.9, 69.0, 68.4, 64.7, 62.8, 59.1, 52.9, 36.6, 24.7, 21.1, 20.7, 20.5. HRMS (ESI): Calculated for $C_{53}H_{59}NO_{18}Na$ [M+ Na] 1020.3630; Found 1020.3629.

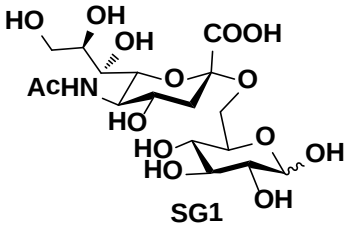
Methyl 5-acetamido-7,8,9-tri-*O*-acetyl-5-*N*,4-*O*-carbonyl-3,5-dideoxy-D-glycero-α-D galacto-non-2-ulopyranosylonate-(2,6)-1,2,3,4-tetra-*O*-benzyl-β -D-glucopyranoside (3β):



3β

3β was synthesized in a manner from donor **2** [3] (0.24 g, 0.38 mmol) and **1β** (0.20 g, 0.35 mmol) similar to that of **3α** and the product was purified using flash silica gel column chromatography eluting with hexanes:Et_2OAc (7:3) to afford the compound **3β** (0.27 g, 75 %) as a yellowish solid.^{1}H NMR (400 MHz, $CDCl_3$) : δ 7.46 – 7.24 (m, 20H), 5.65 (d, *J* = 8.6 Hz, 1H), 5.52 (dt, *J* = 8.7, 4.3 Hz, 1H), 4.97 (d, *J* = 11.5 Hz, 2H), 4.91 (d, *J* = 10.9 Hz, 1H), 4.82 (s, 2H), 4.76 (t, *J* = 11.0 Hz, 2H), 4.68 (dd, *J* = 10.8, 5.5 Hz, 2H), 4.51 (d, *J* = 7.9 Hz, 1H), 4.37 (dd, *J* = 12.4, 2.8 Hz, 1H), 4.21 (dd, *J* = 11.2, 4.6 Hz, 1H), 4.11 – 3.95 (m, 2H), 3.76 (s, 3H), 3.75 – 3.60 (m, 4H), 3.56 – 3.44 (m, 2H), 2.99 (dd, *J* = 12.3, 3.2 Hz, 1H), 2.51 (s, 3H), 2.16 (s, 3H), 2.11 (s, 3H), 2.12-2.01 (m, 1H), 1.91 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) : δ 177.5, 171.8, 170.6, 170.0, 168.3, 153.7, 138.6, 138.4, 138.3, 137.3, 128.4, 128.4, 128.4, 128.3, 128.1, 128.1, 127.9, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.6, 102.3, 99.3, 84.5, 82.0, 75.7, 75.20, 75.1, 74.8, 73.9, 71.5, 71.0, 68.6, 64.6, 62.9, 59.1, 52.9, 36.6, 24.7, 21.2, 20.8, 20.6. HRMS (ESI): Calcd for $C_{53}H_{59}NO_{18}Na$ [M+ Na] 1020.3630; Found 1020.3629.

5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosylonate-(2,6)-α,β-D-gluco pyranoside (SG1) :



SG1

3α or **3β** (0.060 g, 0.060 mmol) was dissolve in MeOH (0.010 L) and treated with a 30% solution of NaOMe (75 µl) in CH_3OH and stirred at rt for 1 h. The solution was neutralized with Amberlite® IR 120 (H^+) resin, filtered and concentrate to dryness. The dried compound was treated with $Pd(OH)_2/C$ (0.010 g) in absolute EtOH was stirred for 12h at rt under H_2 at 1 atmosphere. After completion of reaction as monitored by TLC, reaction mixture was filtered using celite pad, washed with EtOH and combined solvent was concentrate to dryness. The dried compound was treated with NaOH (0.05 N, 3.0 mL) and reaction was stirred for 4h. After

completion of reaction, as monitored by TLC (DCM:MeOH:NH_4OH) (8:2:1), reaction was neutralized using Amberlite® IR 120 (H^+) resin, filter, concentrate and subjected to P-2 gel column to furnish **SG1**. (0.013 g, 93 % over three steps) (54:46, ratio determined by ^1H NMR spectroscopy). ^1H NMR (400 MHz, D_2O) δ 5.12 (d, *J* = 3.2 Hz, 1H, H1 α), 4.54 (d, *J* = 7.8 Hz, 1H, H1 β), 4.07 – 3.86 (m, 2H), 3.86 – 3.69 (m, 7H), 3.68-3.53 (m, 5H), 3.51 – 3.41 (m, 2H), 3.40 – 3.33 (m, 1H), 3.19-3.14 (m, 1H), 2.63 (dd, *J* = 12.5, 4.2 Hz, 1H), 1.95 (s, 3H), 1.74-1.68 (m, 1H) ^{13}C NMR (101 MHz, D_2O) δ 177.7, 176.8, 174.7, 95.9, 92.1, 75.6, 74.4, 73.9, 72.3, 72.8, 71.4, 71.8, 69.5, 69.45, 6.2, 68.2, 67.9, 63.1, 62.9, 62.7, 51.8, 39.7, 22.0. HRMS (ESI): Calcd for $C_{17}H_{29}NO_{14}Na$ $[M+ Na]^+$ 494.1486, found 494.1497.

II. Assays:

Materials. MDCK (Madin-Darby canine kidney) cells were purchased from ATCC® (CCL-34™, Manassas, VA) and maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco, Grand Island NY) supplemented with 10% Fetal Bovine Serum (FBS Gibco, Grand Island NY). Influenza A virus strains used in this study were obtained from BEI Resources (Manassas, VA). 2'-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid (MUNANA) was purchased from Sigma Aldrich, St. Louis, MO for the fluorescence experiments.

Nasal and Throat Sample Collection. Nasal and throat samples were collected from four healthy volunteers after obtaining institutional review board and informed consent from the volunteers. Dry sterile cotton tipped swabs were used for each sample collection. Samples were stored in phosphate buffered saline (PBS) at 4 °C until subsequent use.

Plaque Assays. Virus titers of different influenza strains were determined using standard plaque assays in MDCK cells to quantify the amount of virus. MDCK cells were grown to confluency in 6-well plates. Once confluent, media was removed from the cells and were washed three times with plain DMEM to remove residual FBS. Virus suspension was serially diluted 10-fold and added to duplicate wells at 400 µL/ well. Virus was adsorbed for 1 h at 37 °C in a 5.0% CO_2 incubator. One-hour post-adsorption, virus suspension was removed and Avicel (1.2%, 2.0 mL, FMC Biopolymer) supplemented with 2 µg/mL TPCK-trypsin (Sigma-Aldrich, St. Louis, MO) was added to each well. Avicel was prepared as described previously. [4] Plates were incubated for 5 days to allow for plaque formation. On day 5, the Avicel overlay was removed carefully from each well and the wells were washed two times with 1X PBS followed by methanol (100%) fixation. Fixed wells were stained with 0.2% crystal violet and plaques were counted to determine virus titers.

Real-time RT-PCR. Viral RNA was extracted according to manufacturer's instructions using MagMAX viral isolation kit (Life Technologies, Grand Island NY). cDNA was synthesized from the viral RNA using High Capacity cDNA Reverse Transcription Kit purchased from Applied

Biosystems (Grand Island, NY). Real time PCR was performed using primers and probe for matrix gene, as described previously. [5] The oligonucleotide probe sequence is 5`CTCAGTTATTCTGCTGGTGCACTTGCCA consisting of 5` reporter dye 6-carboxyfluorescein (FAM) and 3` quencher dye 6-carboxytetramethylrhodamine (TAMRA). The forward primer sequence (5` GGACTGCAGCGTAGACGCTT) and two reverse primers sequences (5` CATCCTGTTGTATATGAGGCCCAT and 5` CATTCTGTTGTATATGAGGCCCAT) were used to facilitate amplification of all the strains. PCR mix of 30 µL was prepared using 15 µL TaqMan Universal PCR mix (Applied Biosystems, Grand Island NY), 1µM primers, 0.5 µM probe, and 5 µL of cDNA. Real time PCR amplification and detection was conducted using Applied Biosystems 7500 Fast Real-Time PCR System.

Electrochemical Assay using the 3 electrode system. The initial proof of principle studies were performed using three-electrode system.[6] (**Figure S1, Supplementary materials**) Briefly, a glassy carbon working electrode (GCE, 3.0 mm in diameter), a saturated calomel reference electrode, and a platinum counter electrode. Fabrication of the electrode was performed by polishing the surface of the glassy carbon electrode on the alumina slurries to form a clear mirror followed by washing with distilled water and drying with N_2. The electrode was coated with coating solution achieved by mixing 1ml of solution A (2 mM Bi (NO_3). $5H_2O$ and 3mM SeO_2 in 1:20 diluted nitric acid) and 2 ml of solution B (2mM $K_3Fe(CN)_6$, 2mM $FeCl_3$, 0.1M KCl and 10mM HCl) followed by cyclic voltammetry scanning from +0.60 V to -0.20 V at 20 mVs^{-1} for 30 cycles. After the coating, the electrode was activated by cycling from 0.35 V to -0.05 V at 50 mVs^{-1} for 30 times in solution C (0.1M KCl and 10mM HCl). 5.0 mg of Glucose oxidase (GOD) was dissolved in 1 mL of 0.5% chitosan solution (prepared in 2% acetic acid) and the resulting GOD (10 µL) solution was dropped on the surface of the electrode to form a GOD layer. The GOD layer was air dried to form a firm coated layer on the working electrode. To detect the neuraminidase activity of influenza virus, 100-200 µL of virus stock (PFU determined by plaque assays) was incubated with **SG1** (500 µL, $1x10^{-3}$ M) for 1 h. To detect the Amperometric response, final solution volume was adjusted to 1 mL. Virus was inactivated using UV light or 1% Triton X-100 (Sigma Aldrich, St. Louis MO). The amount of glucose released by NA cleavage of **SG1** was detected by glassy carbon electrode using electrochemical analyzer (CH Instruments Ltd, China) at rt. An amperometric i-t curve at a working potential of 0.00 V was recorded and the current at 100 seconds (when stable) is reported. Increase in peak current correlates with increased glucose concentration, which in turn, reflects the activity of influenza virus neuraminidase.

Drug susceptibility studies. 10 ng of Zanamivir or Oseltamivir carboxylate was premixed with the virus for 30 min. Next, the solution was incubated with **SG1** for 1 h at rt. and glucose released was measured as described in the previous section.

Electrochemical assay using printed electrodes. Printed electrodes were purchased from CH instruments, Austin, Texas, USA, (Figure S4) and coated and activated the surface using the solutions as described above. GOD was coated on the surface using chitosan as previously described. Next, 100 µL of virus containing solution was mixed with **SG1** (100 µL, 2 $x10^{-3}$ M) for 1 h at 37^{o}C. 20 µL of this solution was dropped onto the surface of the test area of printed electrode to test for the presence of glucose as described previously.

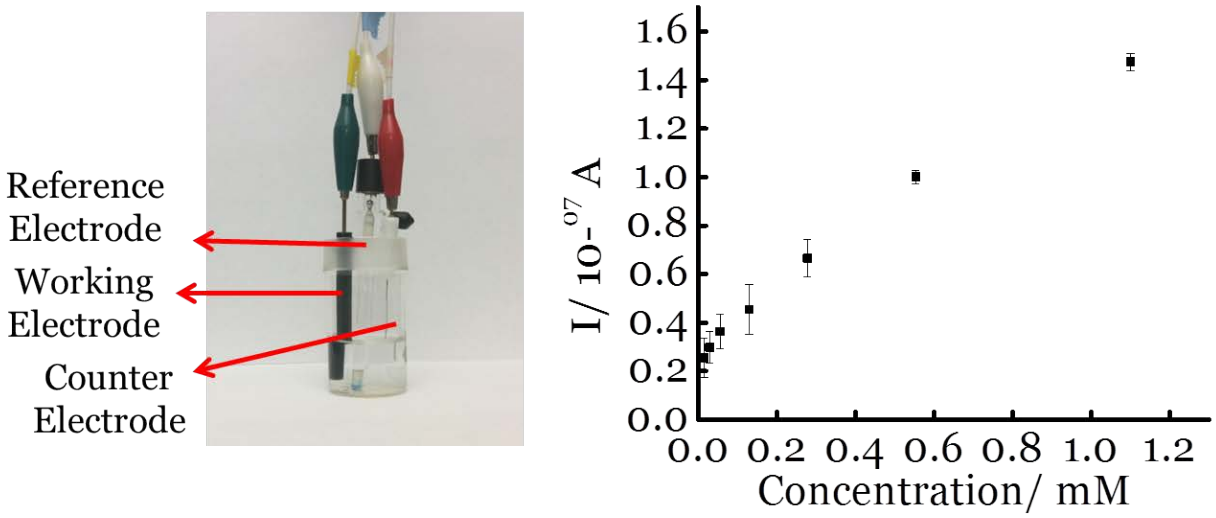


Figure S1. Left. Image of the electrochemical cell. **Right:** Standard curve glucose concentration versus current using the electrochemical cell. Amperometric i-t curve was recorded using different concentrations of glucose and the current at 100 seconds is reported.

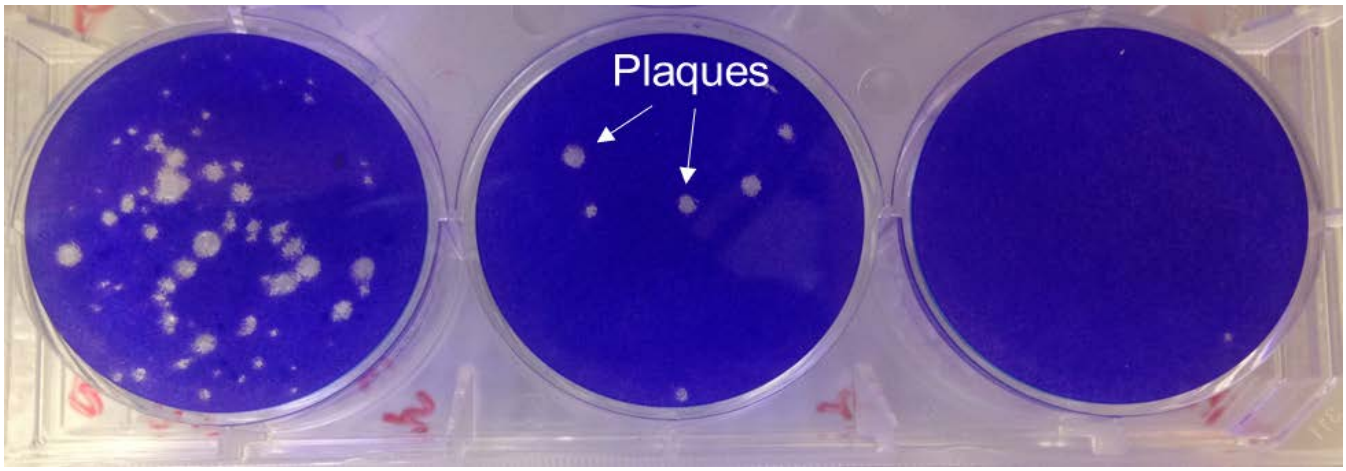


Figure S2. Images of plaque assay for one strain. Confluent MDCK cells were infected with approximately 60, 6 and 0 viral particles (A/Brisbane/59/2007) and incubated for 5 days with a semi-solid media overlay. Plaque forming units (pfu) were counted after fixing and staining the cells.

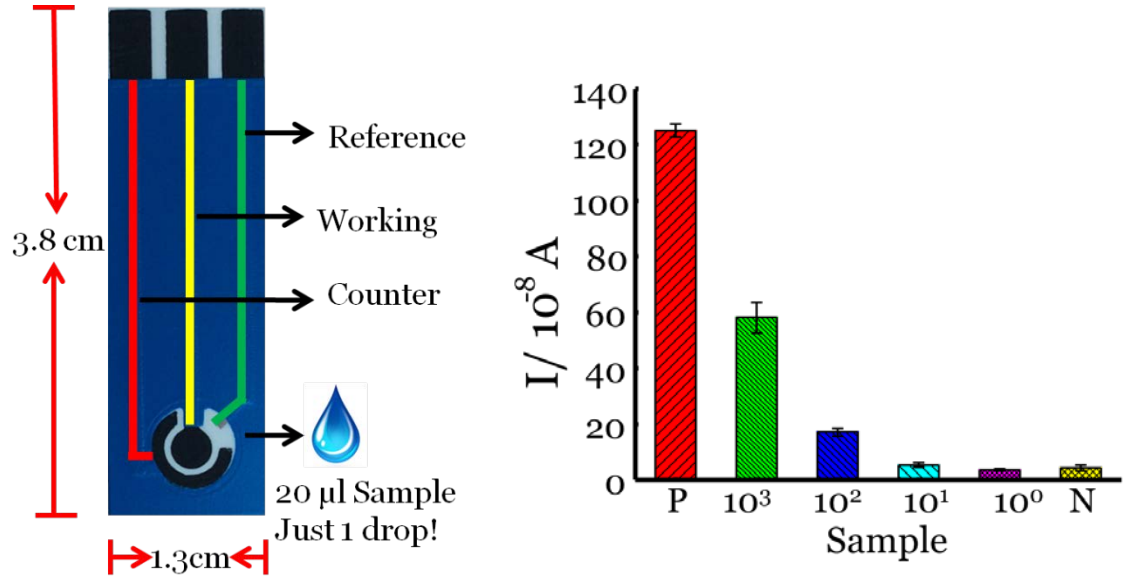


Figure S3. Left: Image of the printed disposable electrode to improve performance and user friendliness. **Right:** Analytical sensitivity studies using A/HongKong/8/1968 (H3N2) strain. P is positive sample, which is 1 mM glucose, N is negative control (**SG1** but no virus), and the numbers represent the number of viral particles in the sample. 100 µL of virus containing solution was mixed with **SG1** (100 µL, $2x10^{-3}$ M) for 1 h. 20 µL of this solution was used to test for the presence of glucose. The y axis represents current, **I**, in amperes measured after 100 s using an amperometric i-t curve at a working potential of 0.00 V, and the x-axis represents different samples.

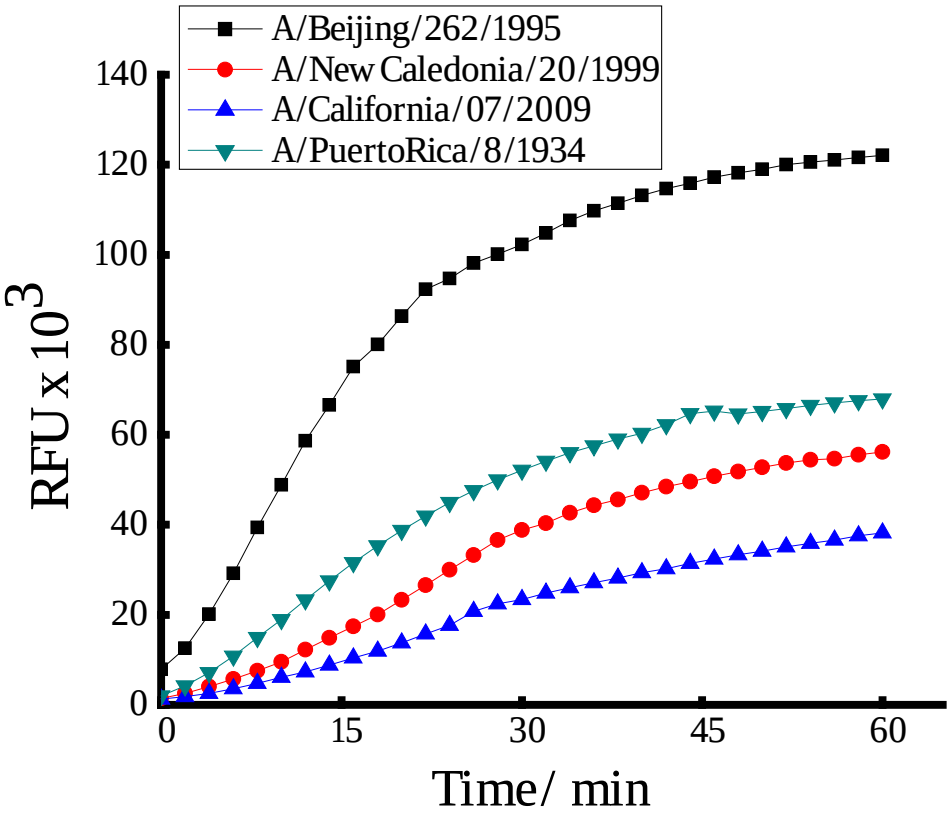


Figure S4. *Fluorescence experiments to demonstrate relative NA activity of four different strains.* MUNANA (10 µL, 1mM) was added to 100 µL of virus strains. Fluorescent intensity was monitored using a microplate reader (Synergy 2, Biotek, Inc. Winooski, VT) at 2 min intervals for 1 hour at 37 ^{o}C after the addition of the substrate. Fluorescent intensity was read at 460 nm with an excitation at 360 nm.

References:

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[3] X. T. Zhang, Z. Y. Gu, G. W. Xing, *Carbohydr Res* **2014**, *388*, 1.

[4] M. Matrosovich, T. Matrosovich, W. Garten, H. D. Klenk, *Virol J* **2006**, *3*, 63.

[5] L. J. Van Elden, M. Nijhuis, P. Schipper, R. Schuurman, A. M. Van Loon, *J Clin Microbiol* **2001**, *39*, 196.

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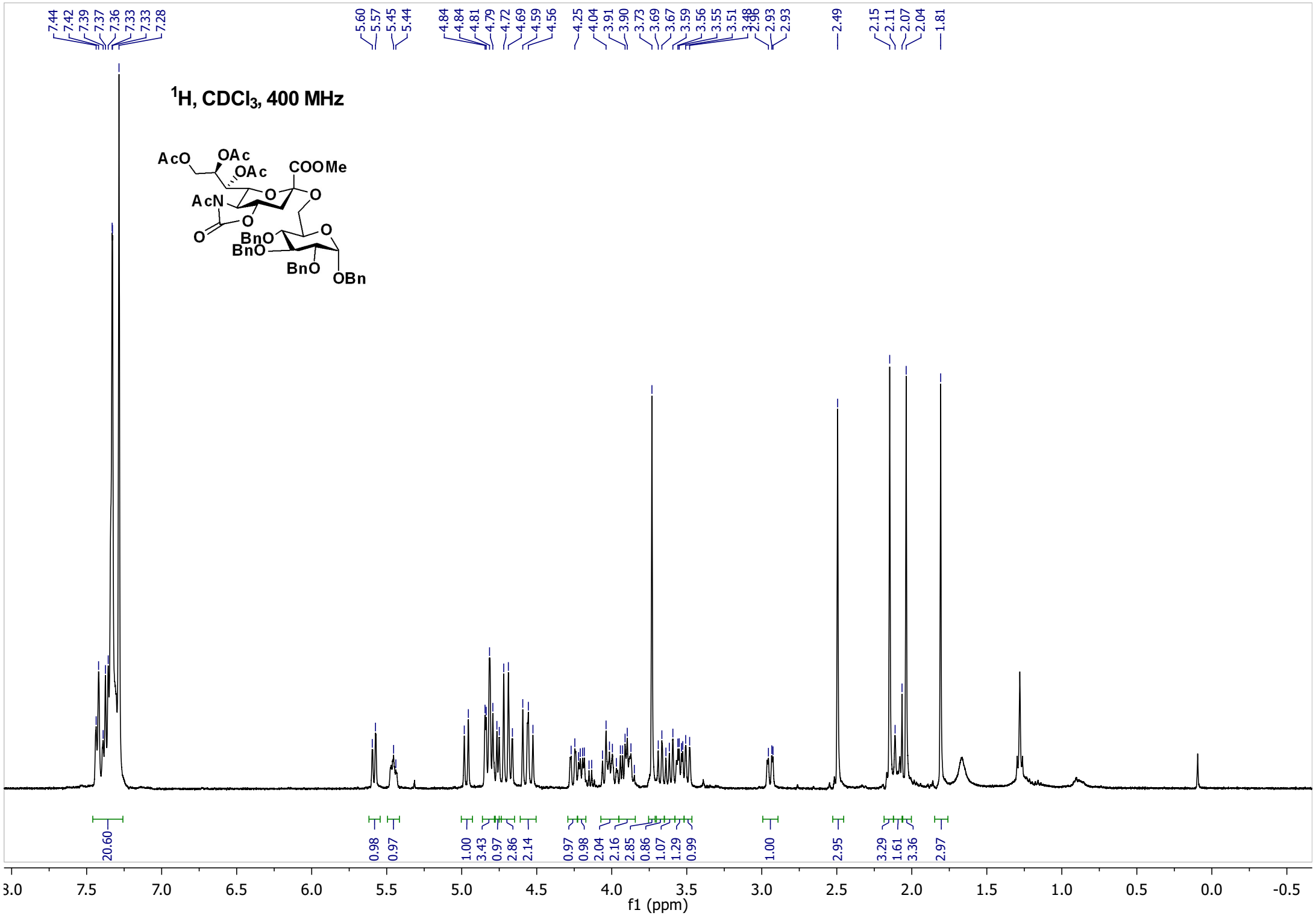
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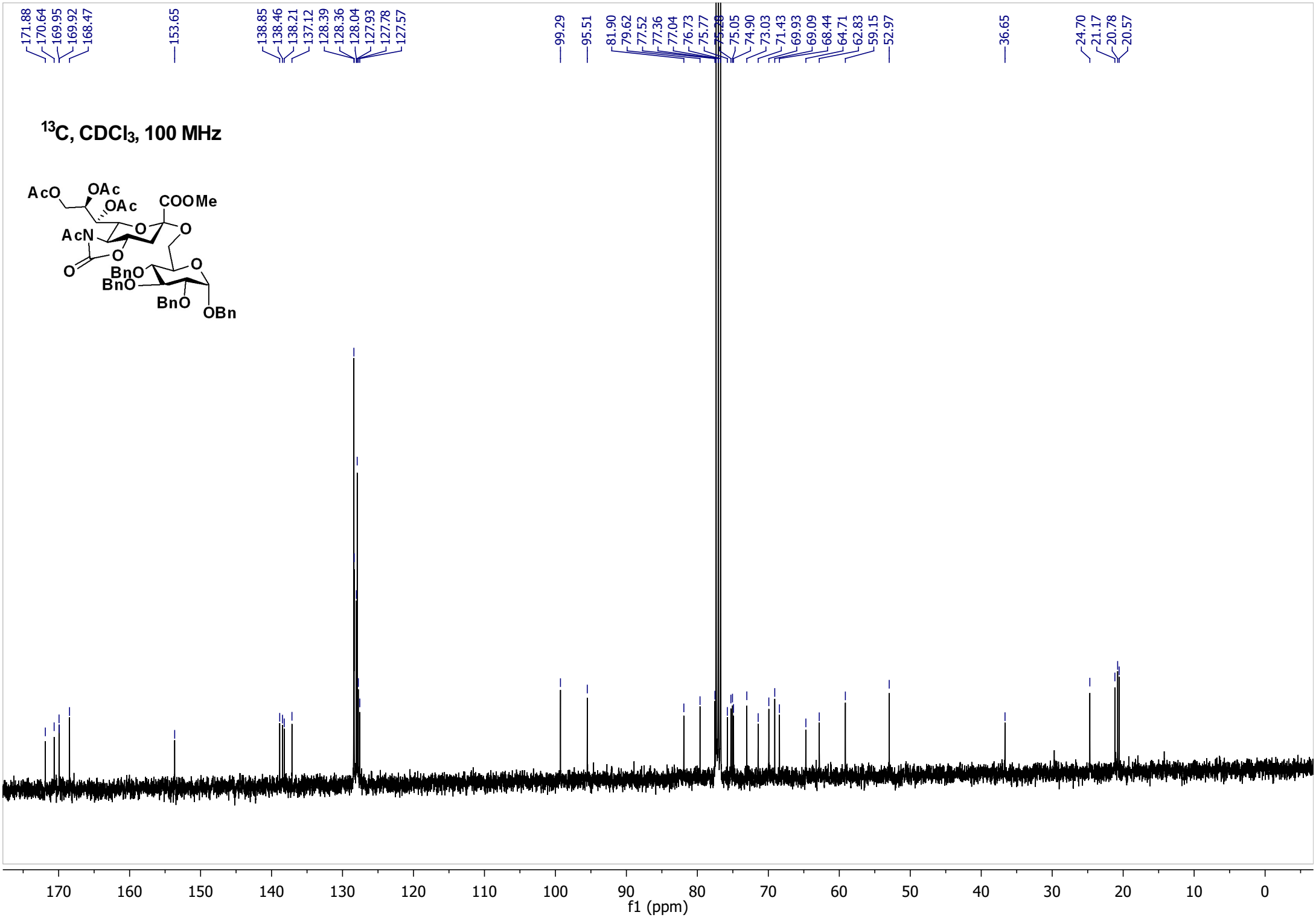
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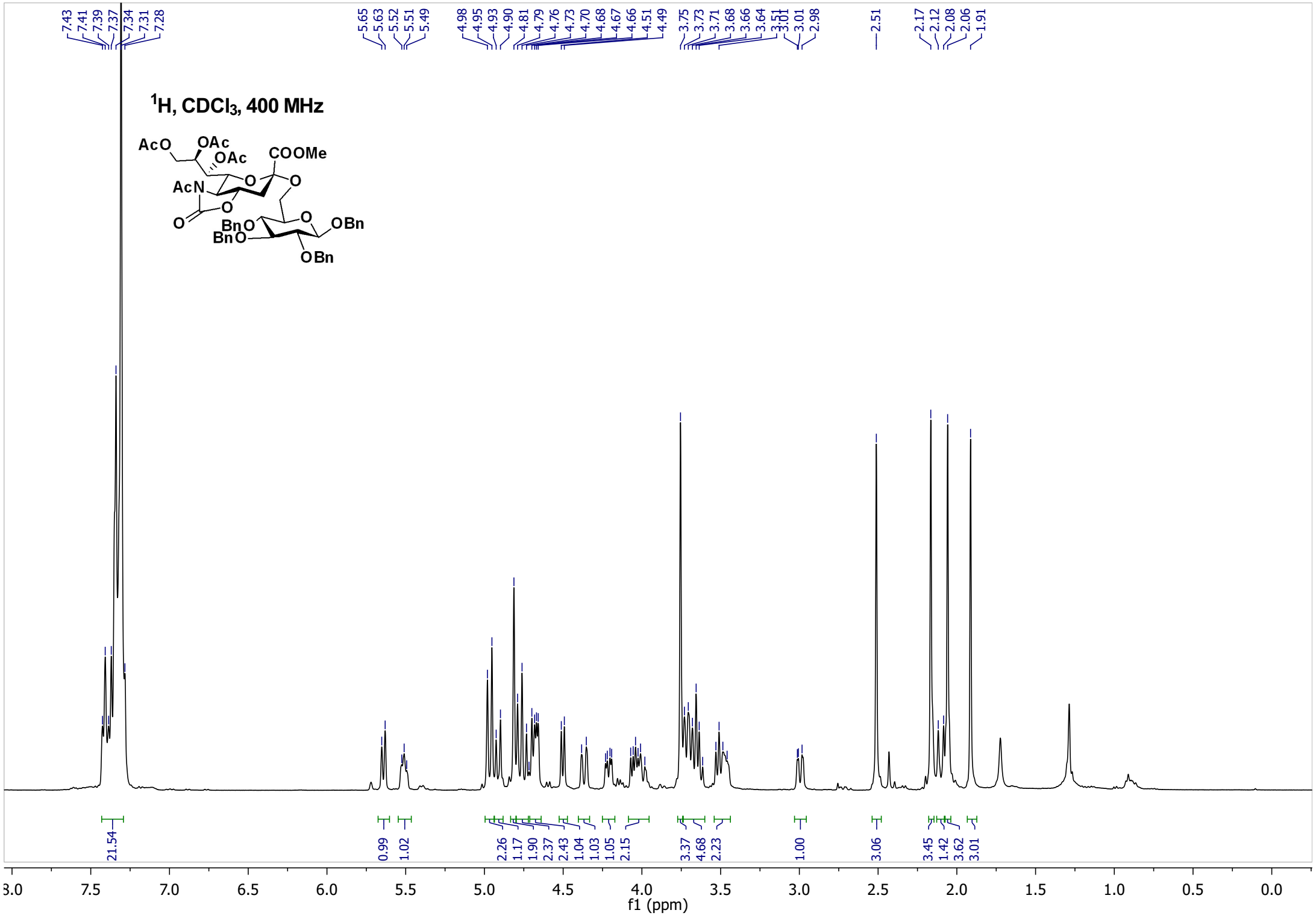
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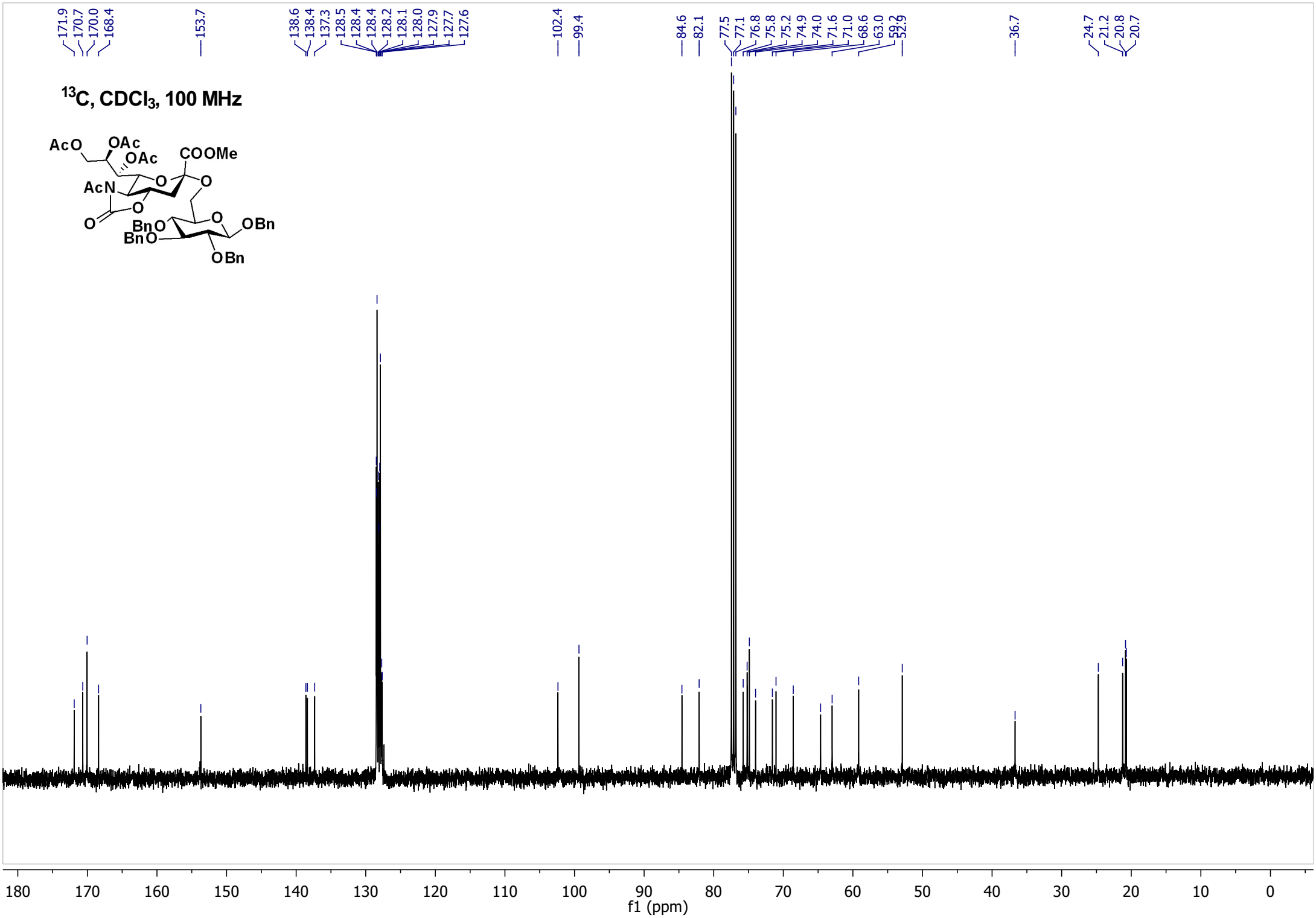
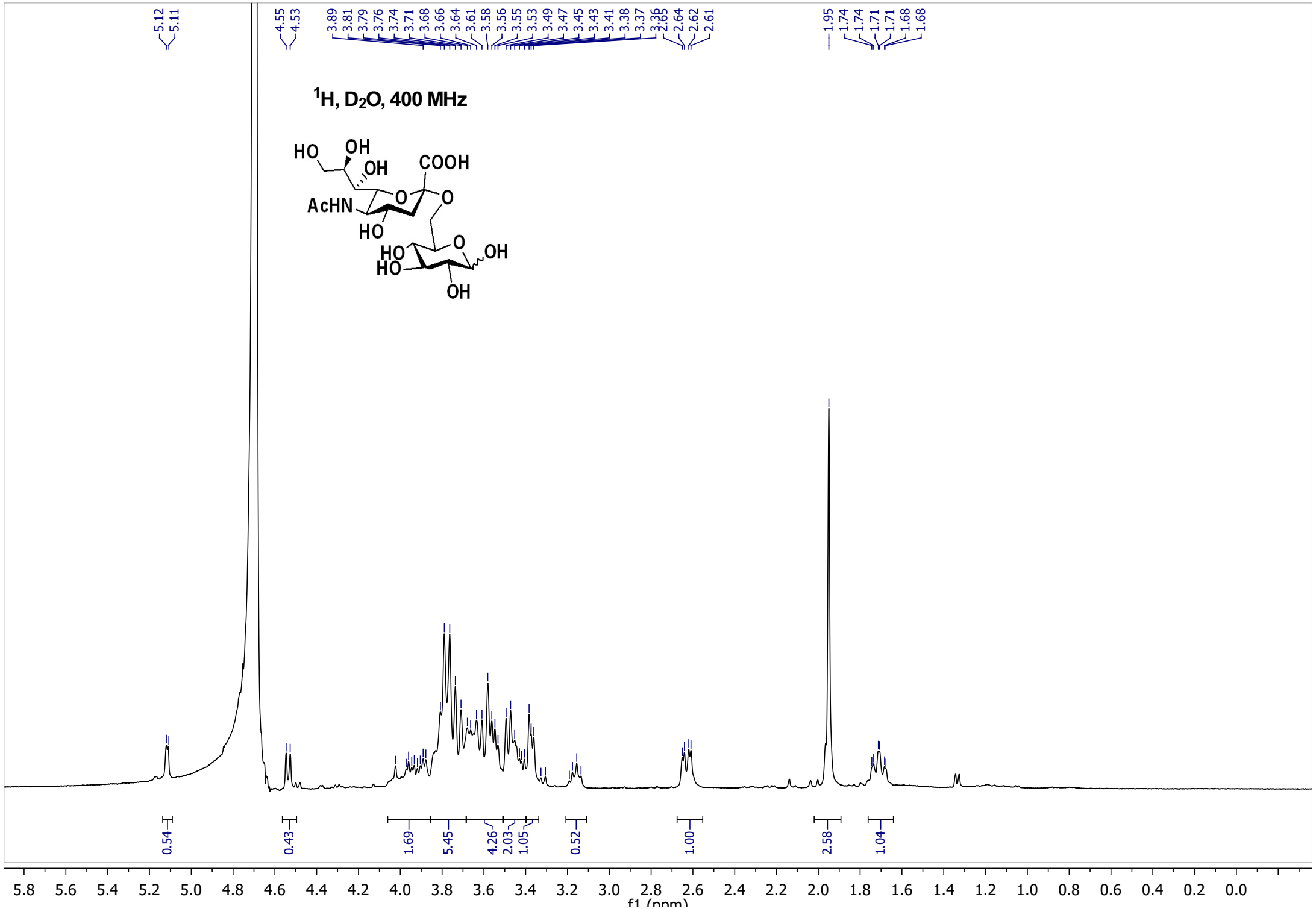
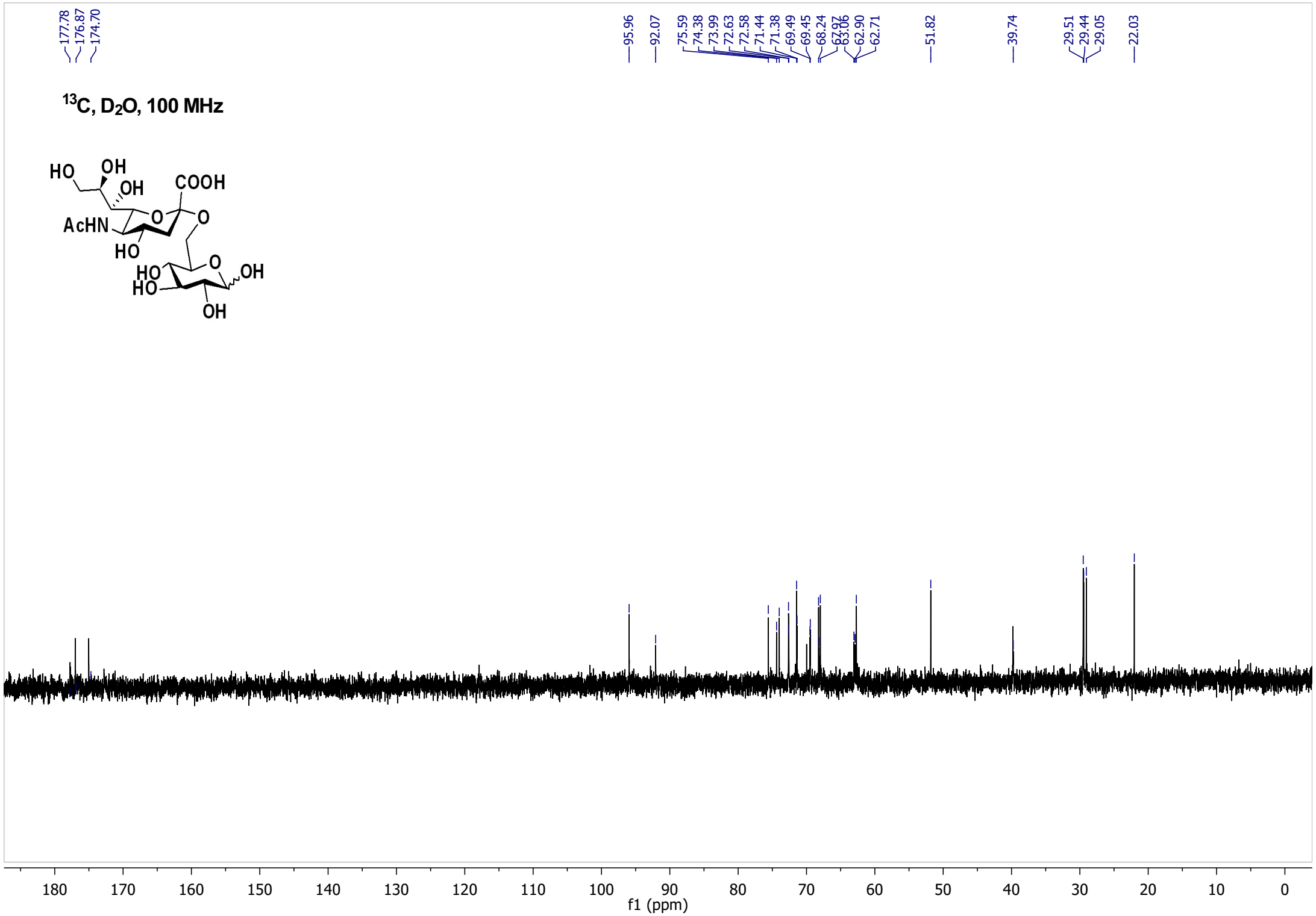
2.12
2.08
2.06
1.91
^{1}H, CDCl$_3$, 400 MHz
AcO
OAc
OAc
COOMe
AcN
O
O
O
O
O
BnO
BnO
OBn
OBn
21.54
0.99
1.02
2.26
1.17
1.90
2.37
2.43
1.04
1.03
1.05
2.15
3.37
4.68
2.23
1.00
3.06
3.45
1.42
3.62
3.01
3.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


171.9
170.7
170.0
168.4
153.7
138.6
138.4
137.3
128.5
128.4
128.4
128.2
128.1
128.0
127.9
127.7
127.6
102.4
99.4
84.6
82.1
77.5
77.1
76.8
75.8
75.2
74.9
74.0
71.6
71.0
68.6
63.0
59.2
52.9
36.7
24.7
21.2
20.8
20.7
13C, CDCl3, 100 MHz
AcO
OAc
OAc
COOMe
AcN
O
O
O
O
O
BnO
BnO
O
OBn
OBn
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


5.12
5.11
4.55
4.53
3.89
3.81
3.79
3.76
3.74
3.71
3.68
3.66
3.64
3.61
3.58
3.56
3.55
3.53
3.49
3.47
3.45
3.43
3.41
3.38
3.37
3.36
2.65
2.64
2.62
2.61
1.95
1.74
1.74
1.71
1.71
1.68
1.68
^{1}H, D$_2$O, 400 MHz
HO
OH
OH
COOH
AcHN
O
O
HO
O
HO
HO
OH
OH
0.54
0.43
1.69
5.45
4.26
2.03
1.05
0.52
1.00
2.58
1.04
5.8
5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
f1 (ppm)


177.78
176.87
174.70
95.96
92.07
75.59
74.38
73.99
72.63
72.58
71.44
71.38
69.49
69.45
68.24
67.97
63.06
62.90
62.71
51.82
39.74
29.51
29.44
29.05
22.03
13C, D2O, 100 MHz
HO
OH
OH
COOH
AcHN
O
O
HO
O
HO
HO
OH
OH
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)