Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

for

Synthesis and evaluation of N^{α},N^{ε}-diacetyl-L-lysine-inositol conjugates as cancer-selective probes for metabolic engineering of GPIs and GPI-anchored proteins

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I. Supplementary Flow Cytometry Data

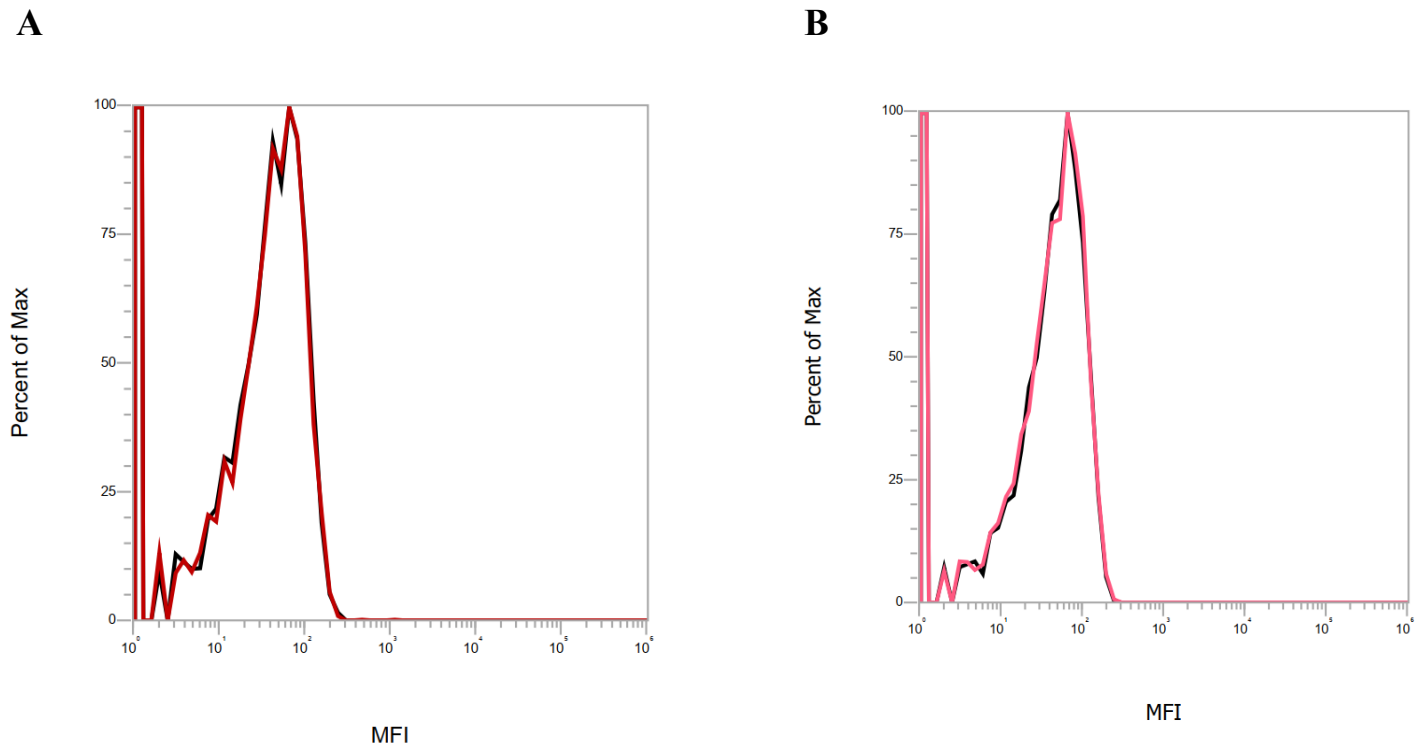


Figure S1: Flow cytometry results of HEK293 cell treated with 200 µM of **1** (A) or **2** (B) (red) against cell treated with PBS (control group, black) for 48 h, followed by click reaction with DBCO-Cy5 (50 µM, 37 ºC, 1 h). More than 10,000 events were recorded during each experiment and three replicate experiments were performed.

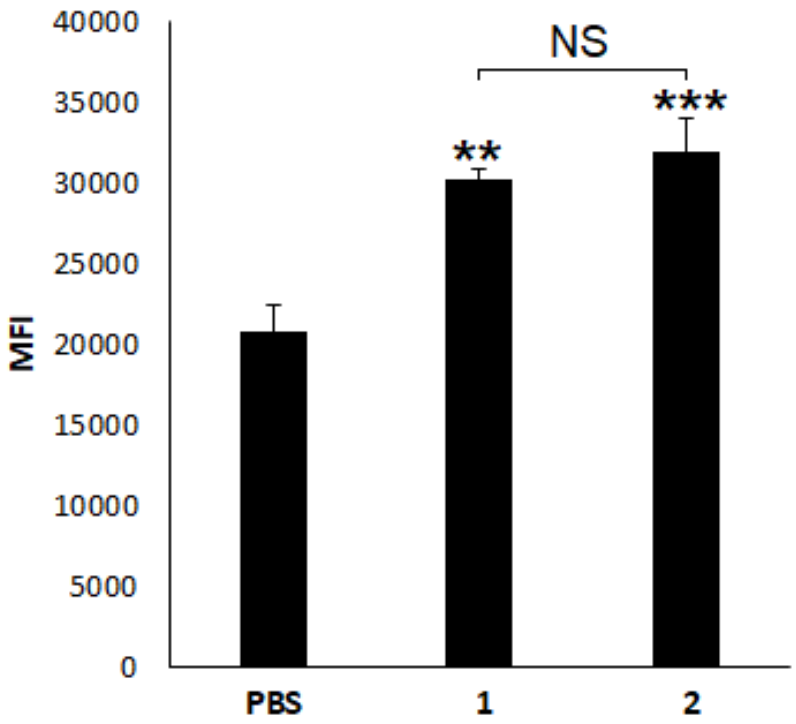


Figure S2: Flow cytometry results of MCF-7 cell treated with PBS (control) or with 200 μM of **1** or **2** for 48 h and then with DBCO-Cy5 (50 μM, 37 °C, 1 h). Data are presented as mean ± standard deviation of three parallel experiments and analyzed by two-tailed student's *t* test: **statistically very different ($P \leq 0.01$) from the PBS group; ***statistically extremely different ($P \leq 0.001$) from the PBS group; NS, no significant difference ($P > 0.05$).

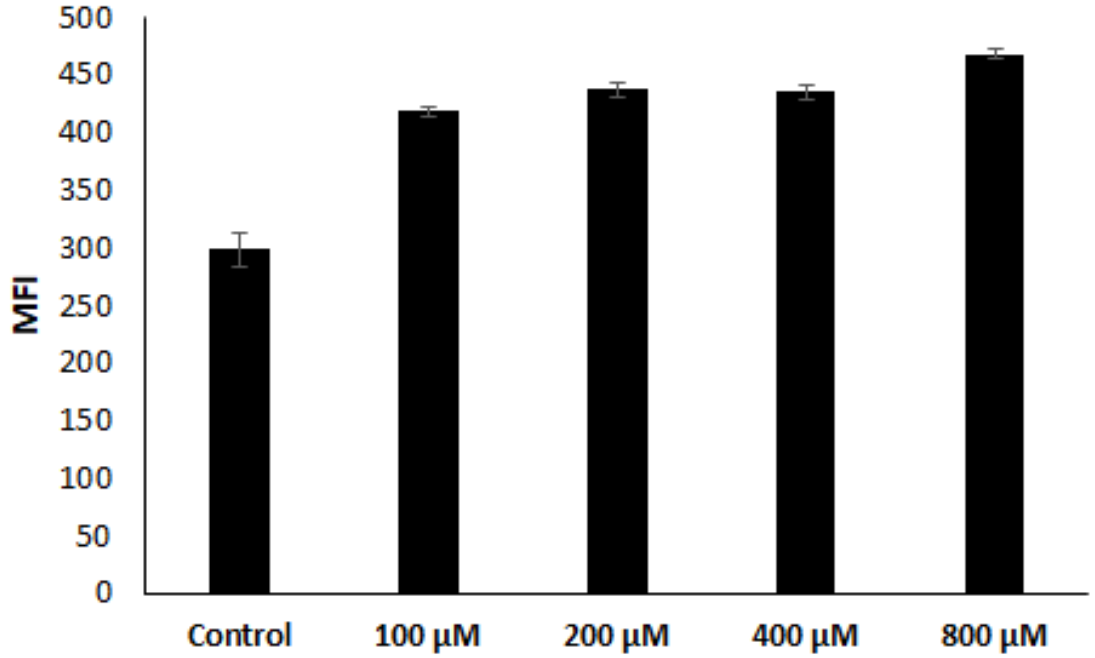


Figure S3: Flow cytometry results of MCF-7 cell treated with PBS (control) or with various concentrations (100-800 µM) of **1** for 48 h and then with DBCO-Cy5 (50 µM, 37 °C, 1 h). Data are presented as mean ± standard deviation of three parallel experiments. Difference of the treatment groups with the control (PBS) group was all statistically significant ($P \leq 0.05$).

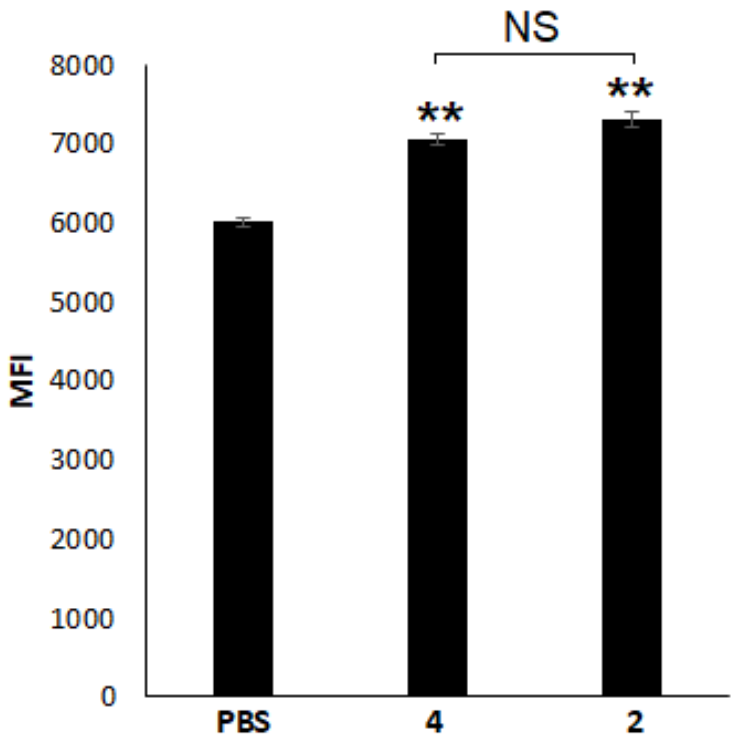
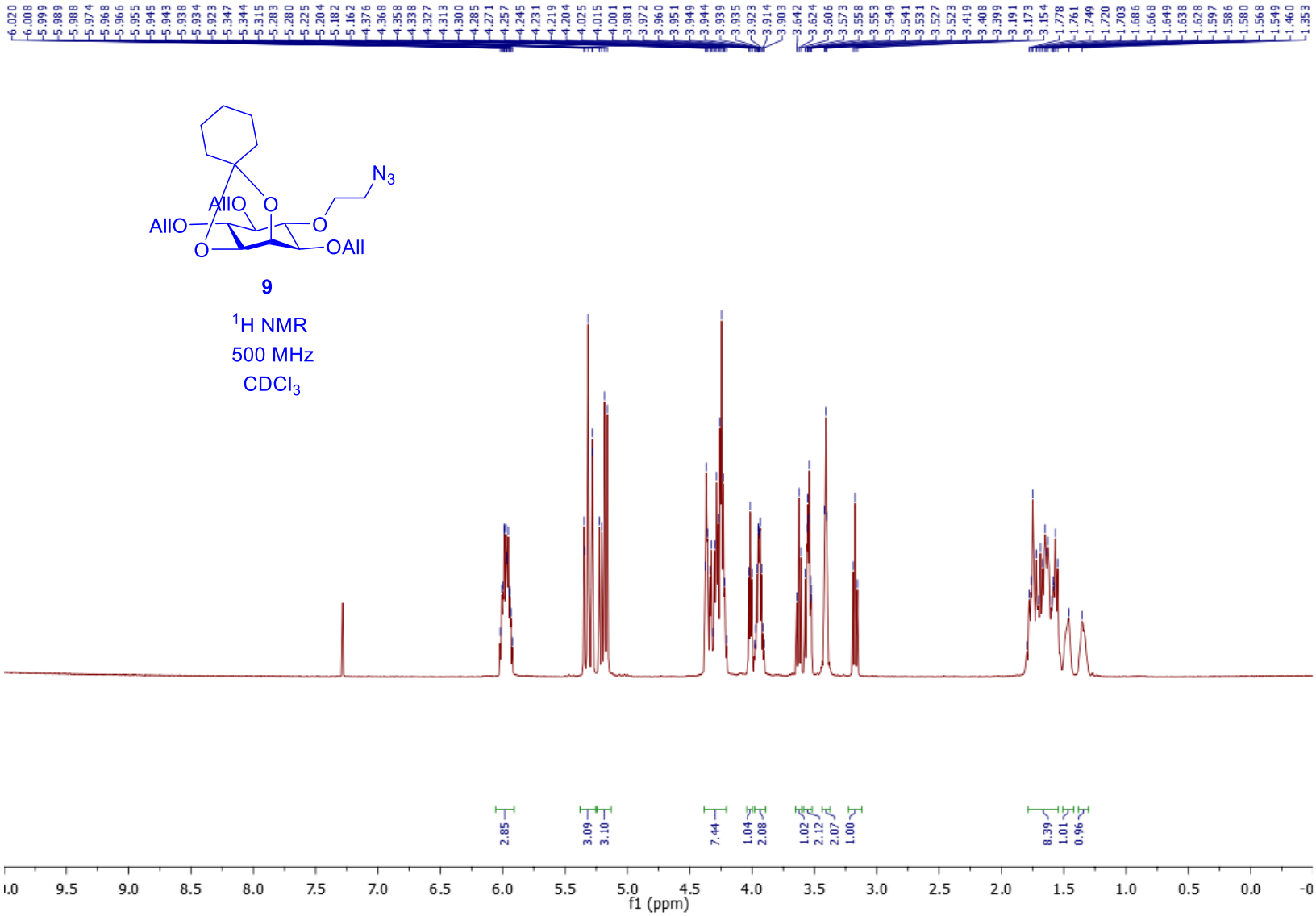
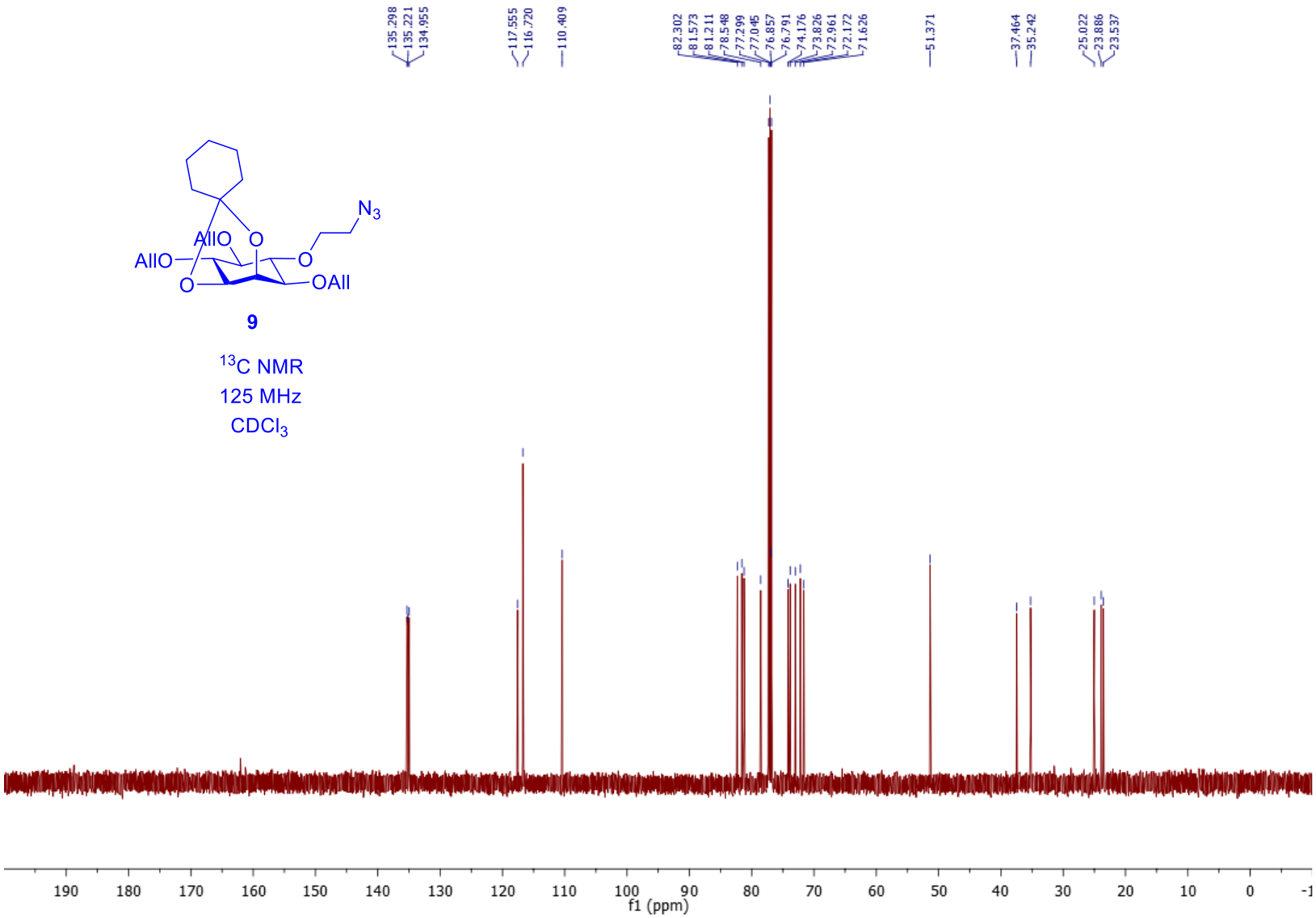


Figure S4: Flow cytometry results of HepG2 cell treated with PBS (control) or with 200 μM of **2** or **4** for 48 h and then with DBCO-Cy5 (50 μM, 37 °C, 1 h). Data are presented as mean ± standard deviation of two parallel experiments and analyzed by two-tailed student's *t* test: **statistically very different ($P \leq 0.01$) from the PBS group; NS, no significant difference ($P > 0.05$).

II. NMR and MS Spectra of Compounds

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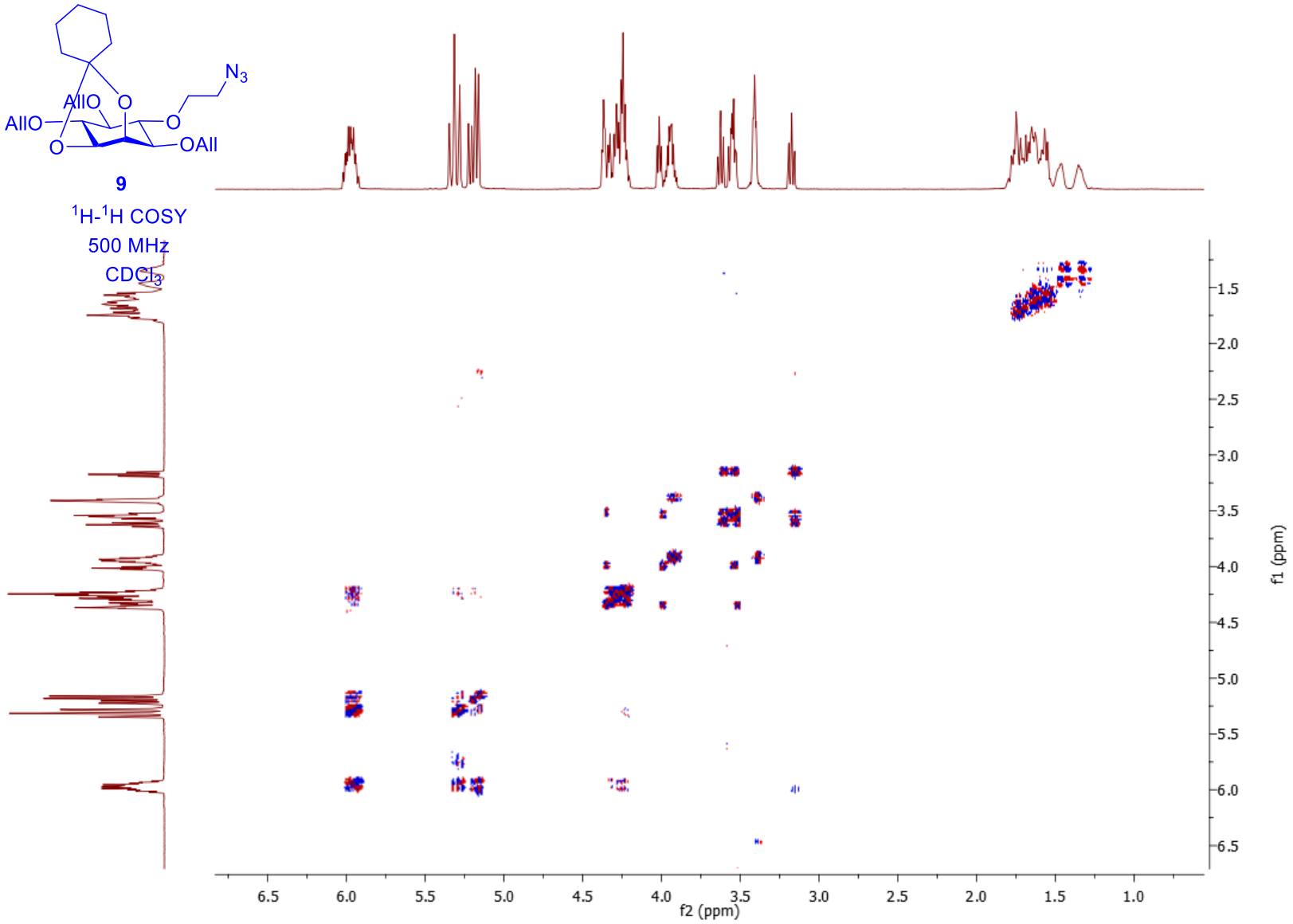
134.955
117.555
116.720
110.409
82.302
81.573
81.211
78.548
77.299
77.045
76.857
76.791
74.176
73.826
72.961
72.172
71.626
51.371
37.464
35.242
25.022
23.886
23.537
AllO
O
O
AllO
O
OAll
N3
9
13C NMR
125 MHz
CDCl3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)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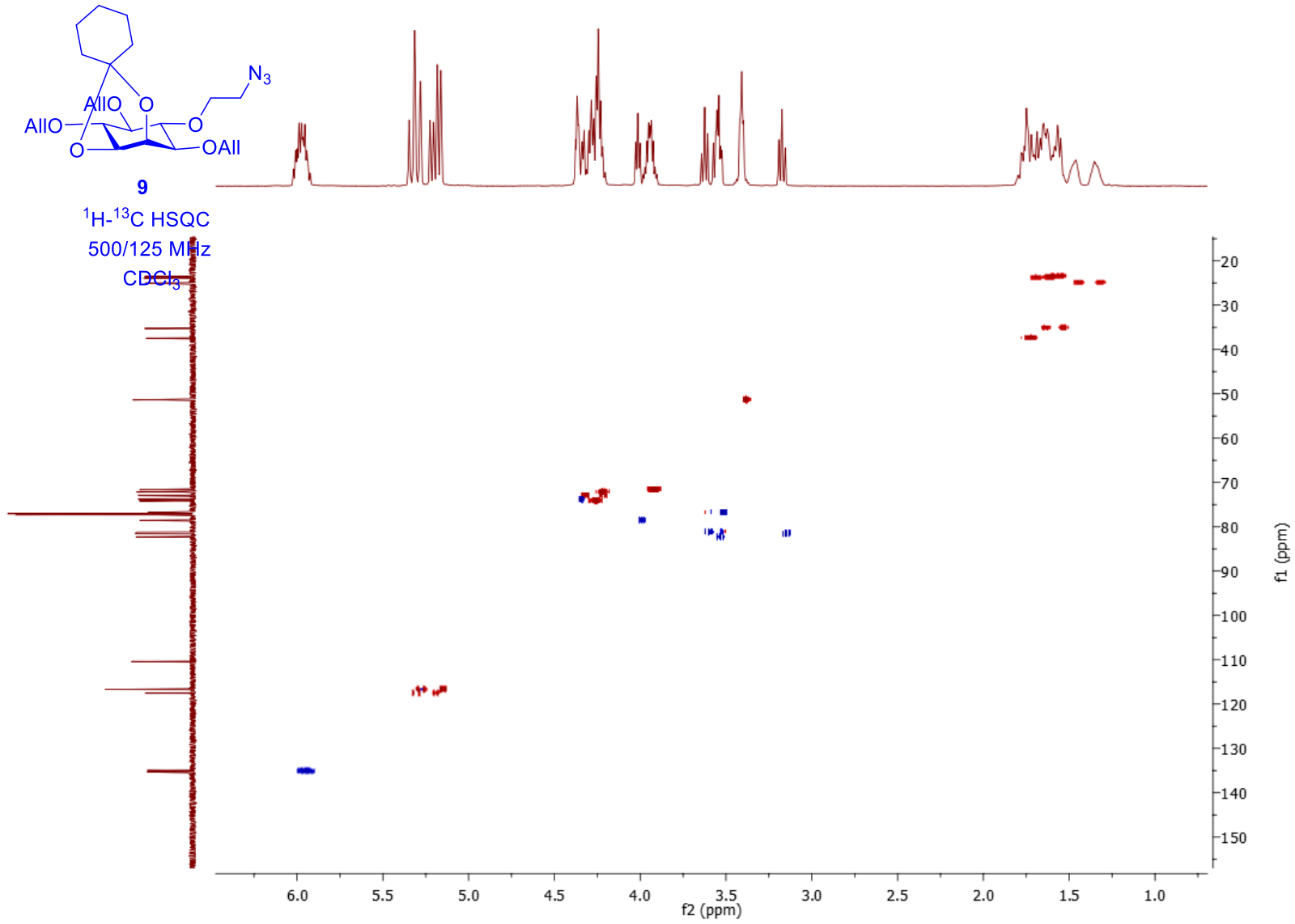
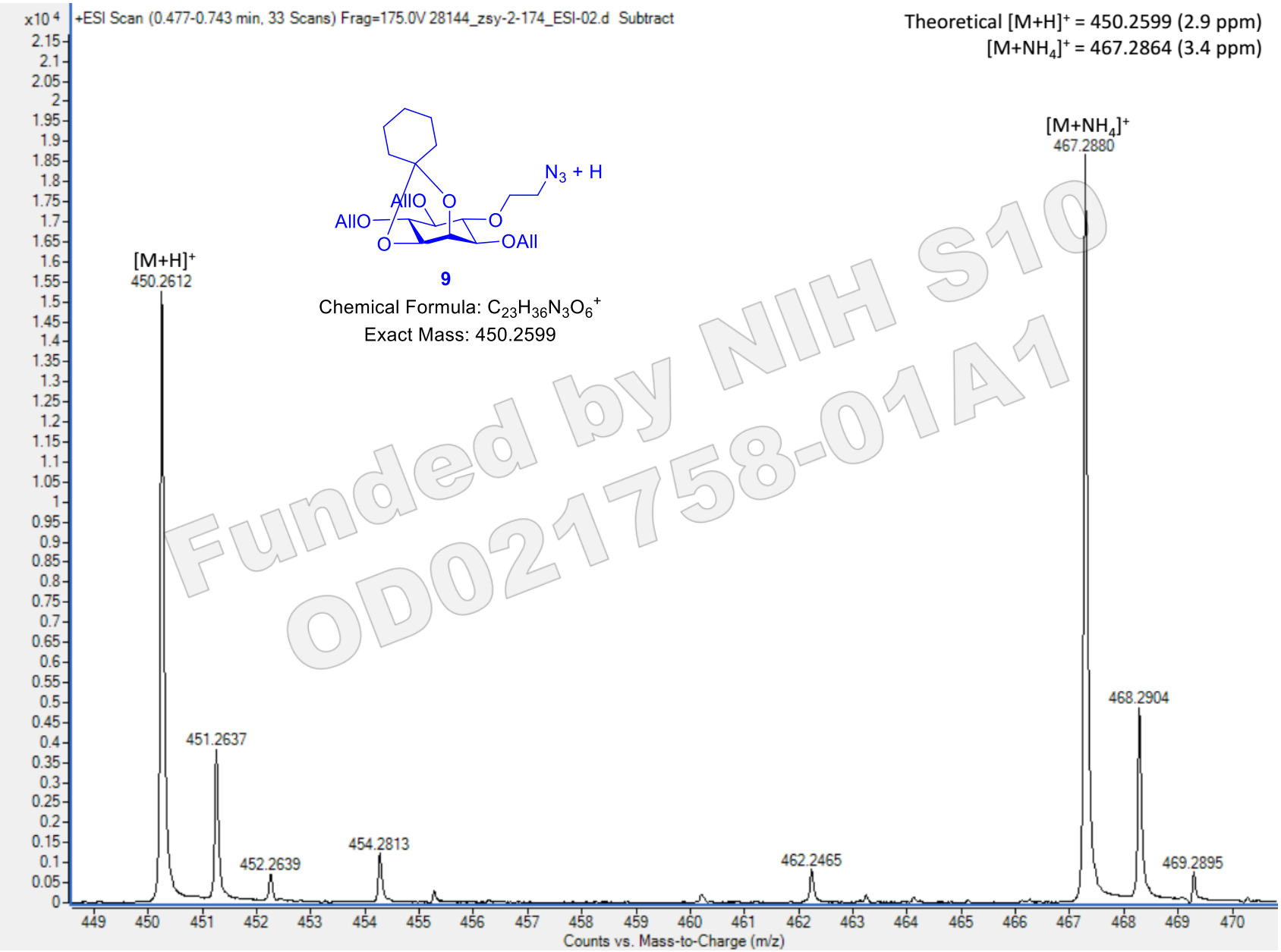
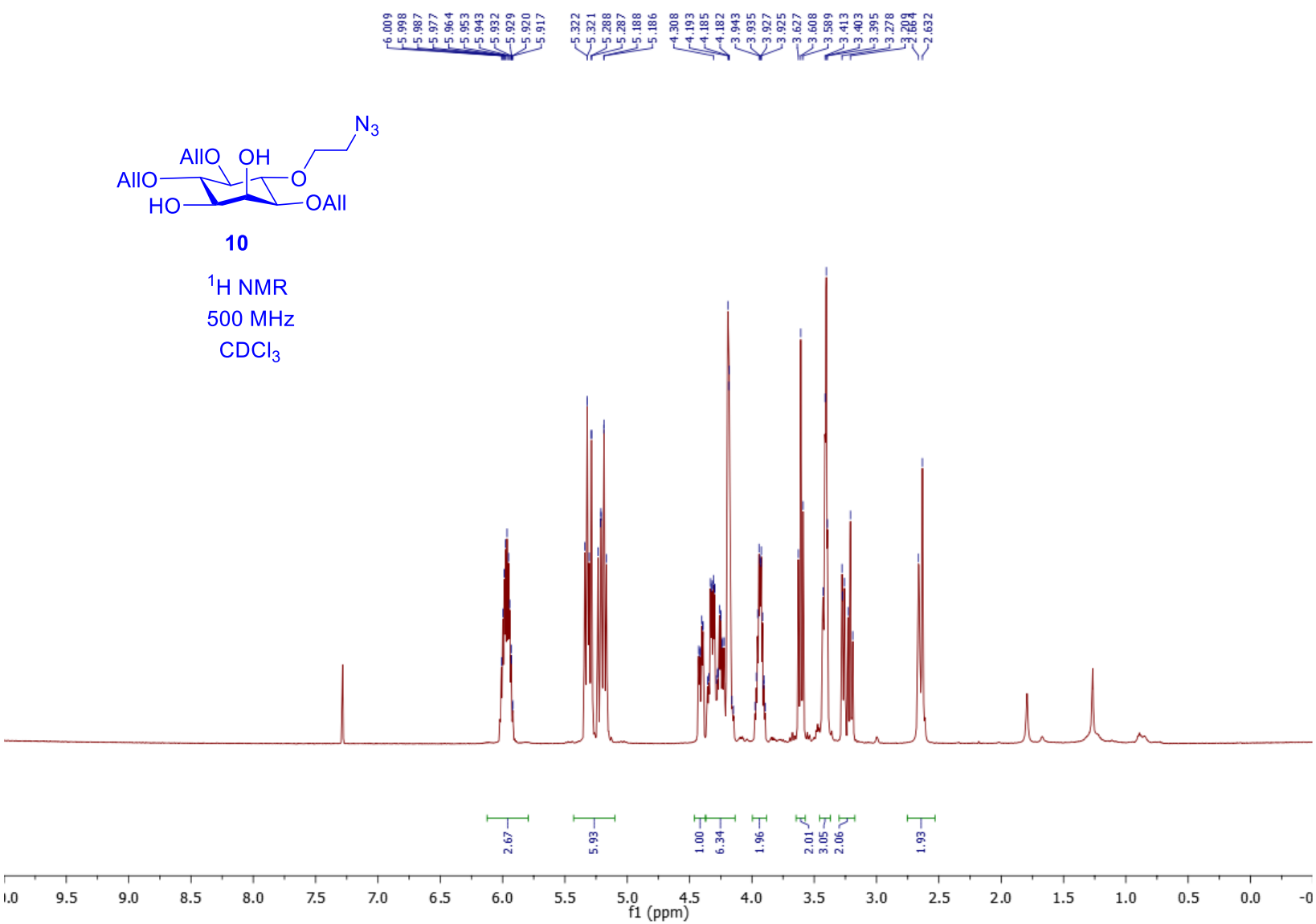
AllO
O
O
O
OAll
N3
9
1H-1H COSY
500 MHz
CDCl3
f2 (ppm)
f1 (ppm)


AllO
AllO
O
O
O
OAll
N3
9
1H-13C HSQC
500/125 MHz
CDCl3
f2 (ppm)
f1 (ppm)

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5.987
5.977
5.964
5.953
5.943
5.932
5.929
5.920
5.917
5.322
5.321
5.288
5.287
5.188
5.186
4.308
4.193
4.185
4.182
3.943
3.935
3.927
3.925
3.627
3.608
3.589
3.413
3.403
3.395
3.278
3.209
2.664
2.632
N3
AllO
OH
AllO
O
HO
OAll
10
1H NMR
500 MHz
CDCl3
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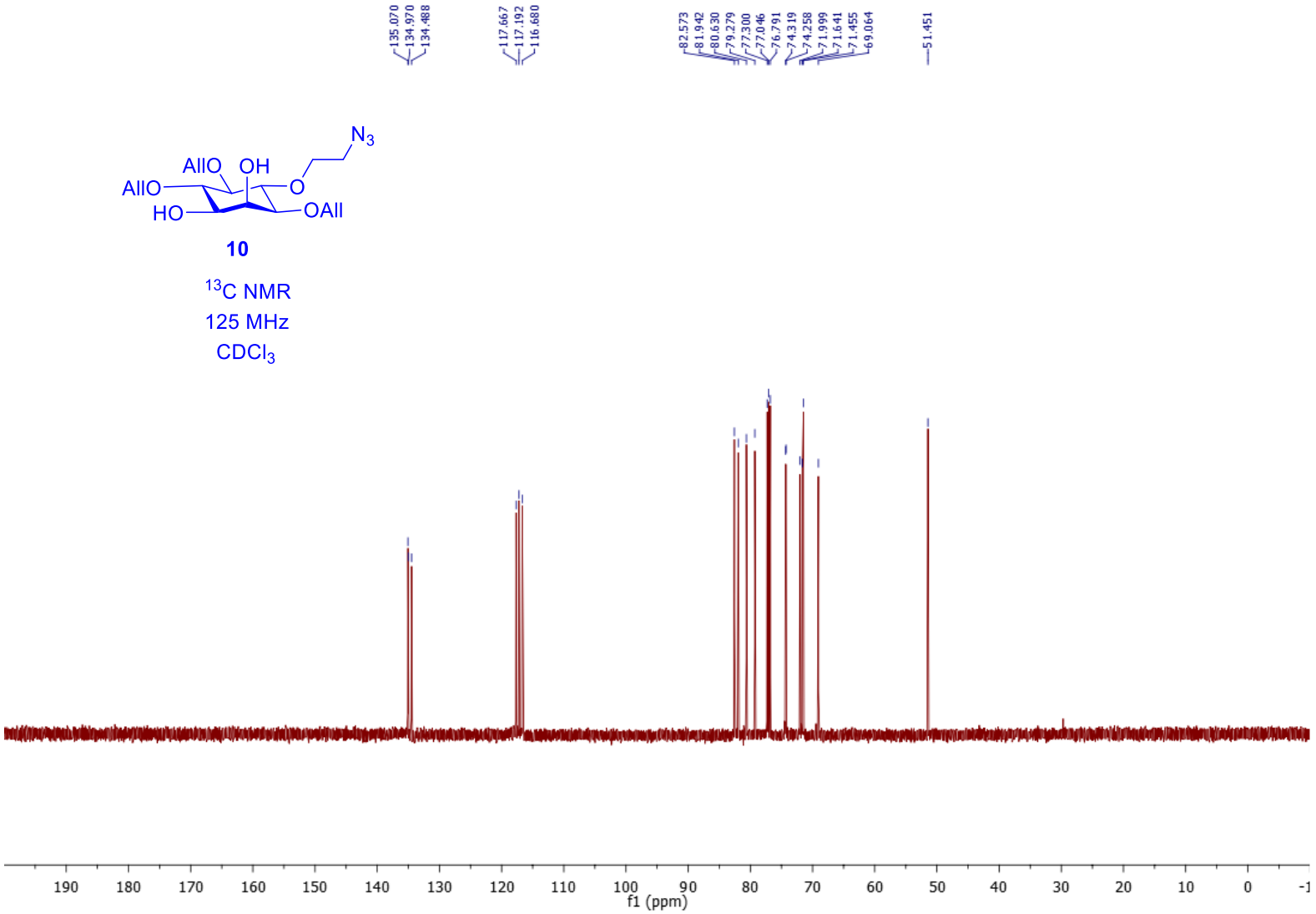
5.93
1.00
6.34
1.96
2.01
3.05
2.06
1.93
f1 (ppm)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134.970
134.488
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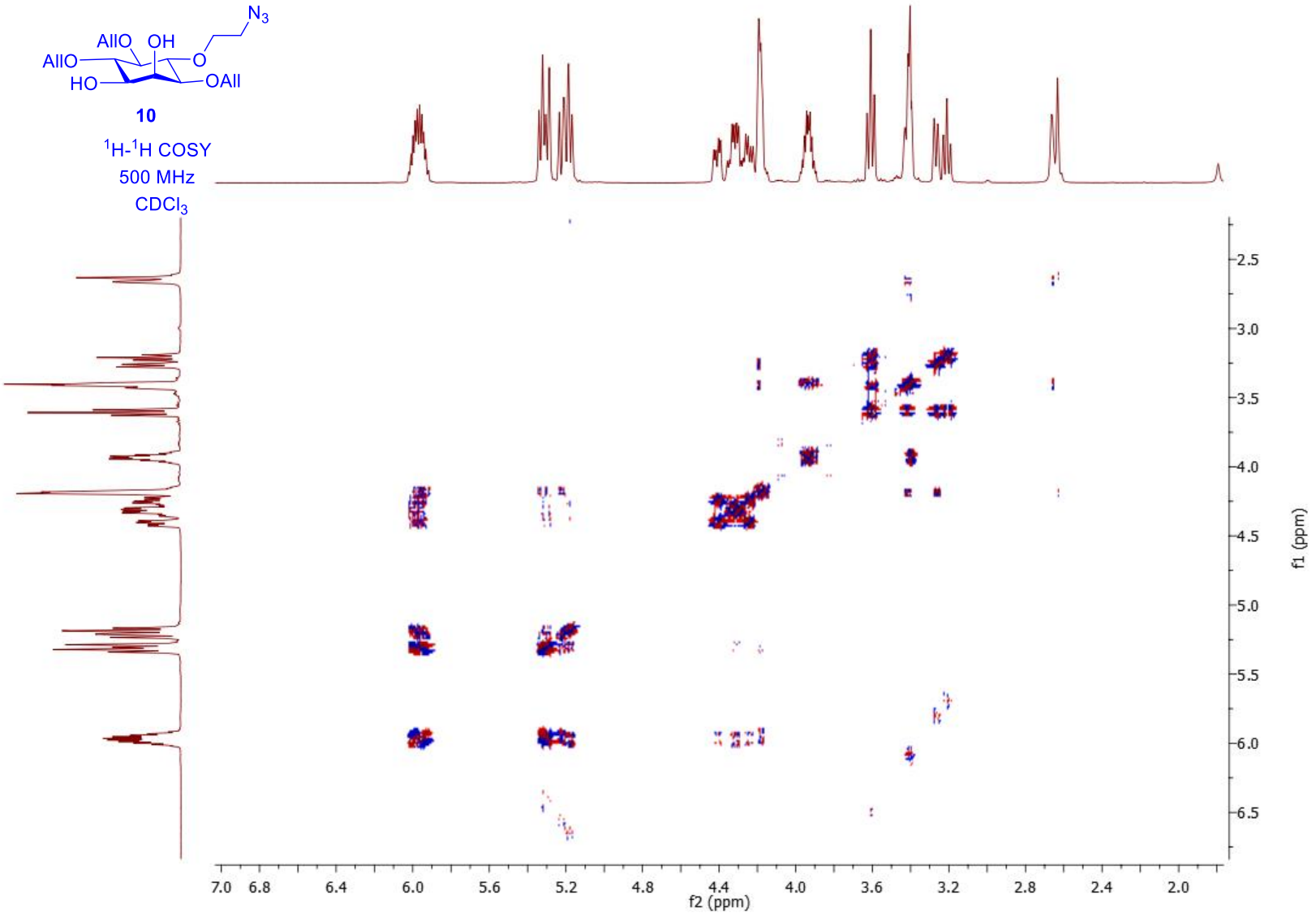
117.192
116.680
82.573
81.942
80.630
79.279
77.300
77.046
76.791
74.319
74.258
71.999
71.641
71.455
69.064
51.451
N3
AllO
OH
AllO
O
HO
OAll
10
13C NMR
125 MHz
CDCl3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)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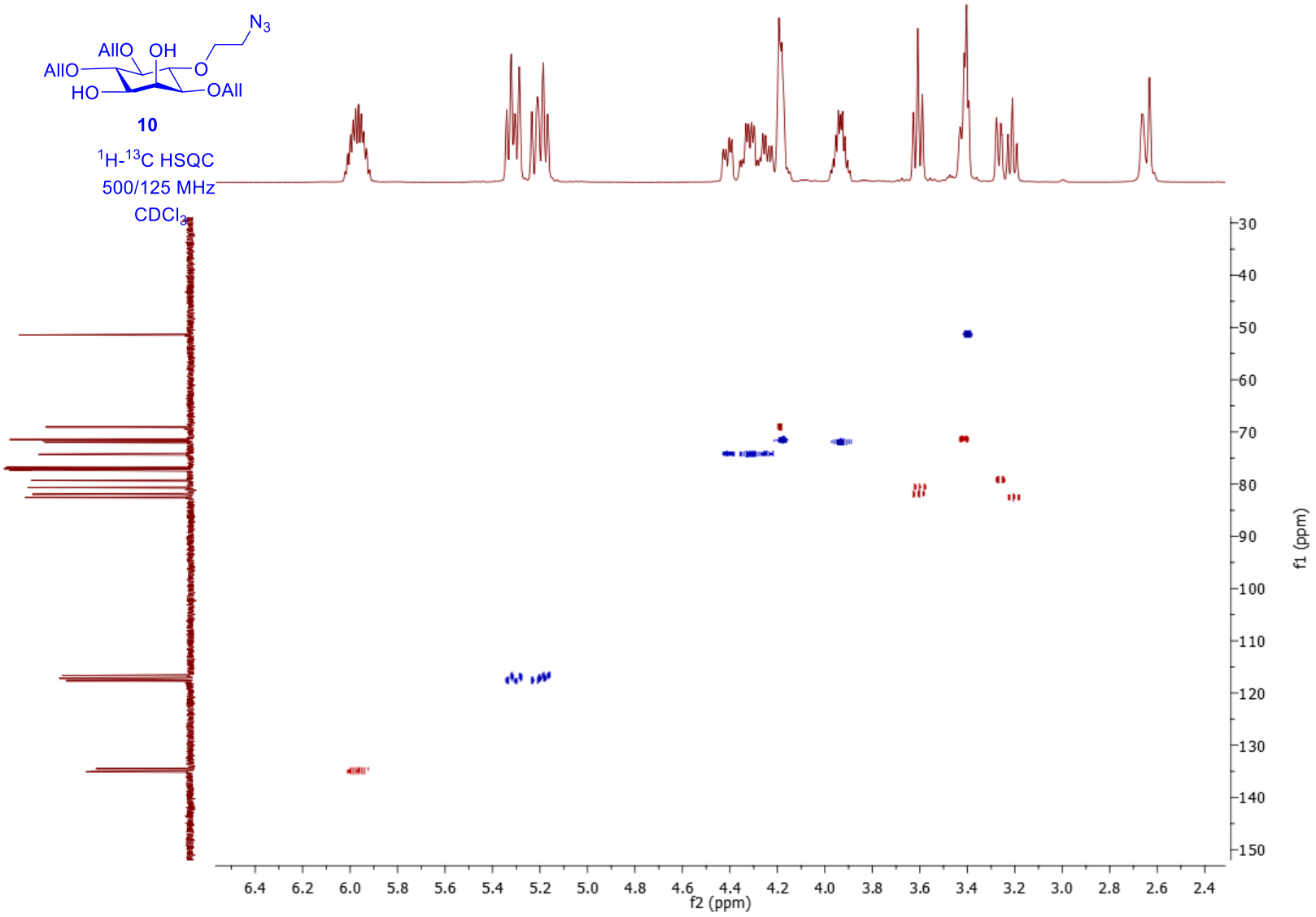
AllO
O
HO
OAll
N3
10
1H-1H COSY
500 MHz
CDCl3
f2 (ppm)
f1 (ppm)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HO
OAll
N3
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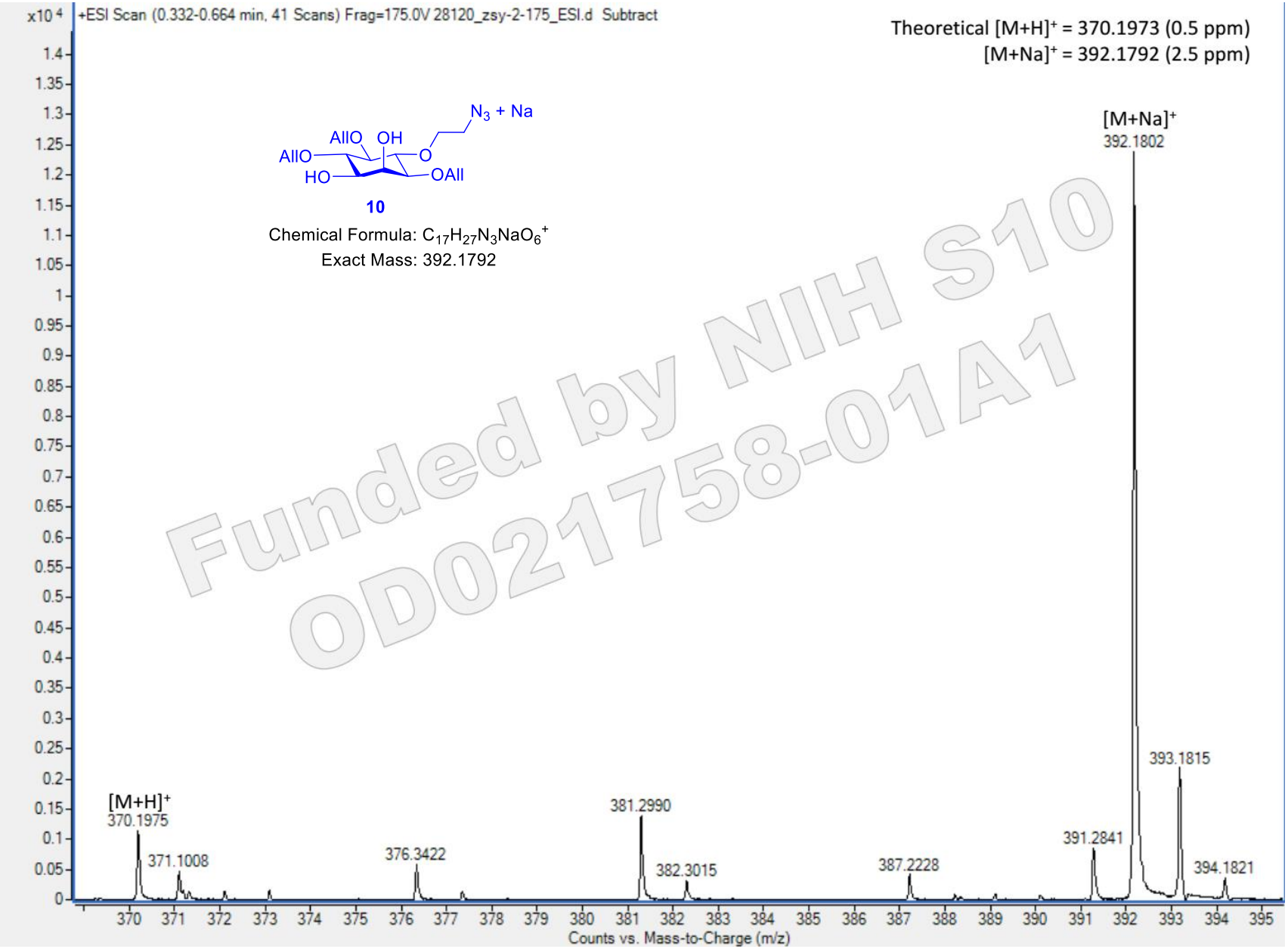
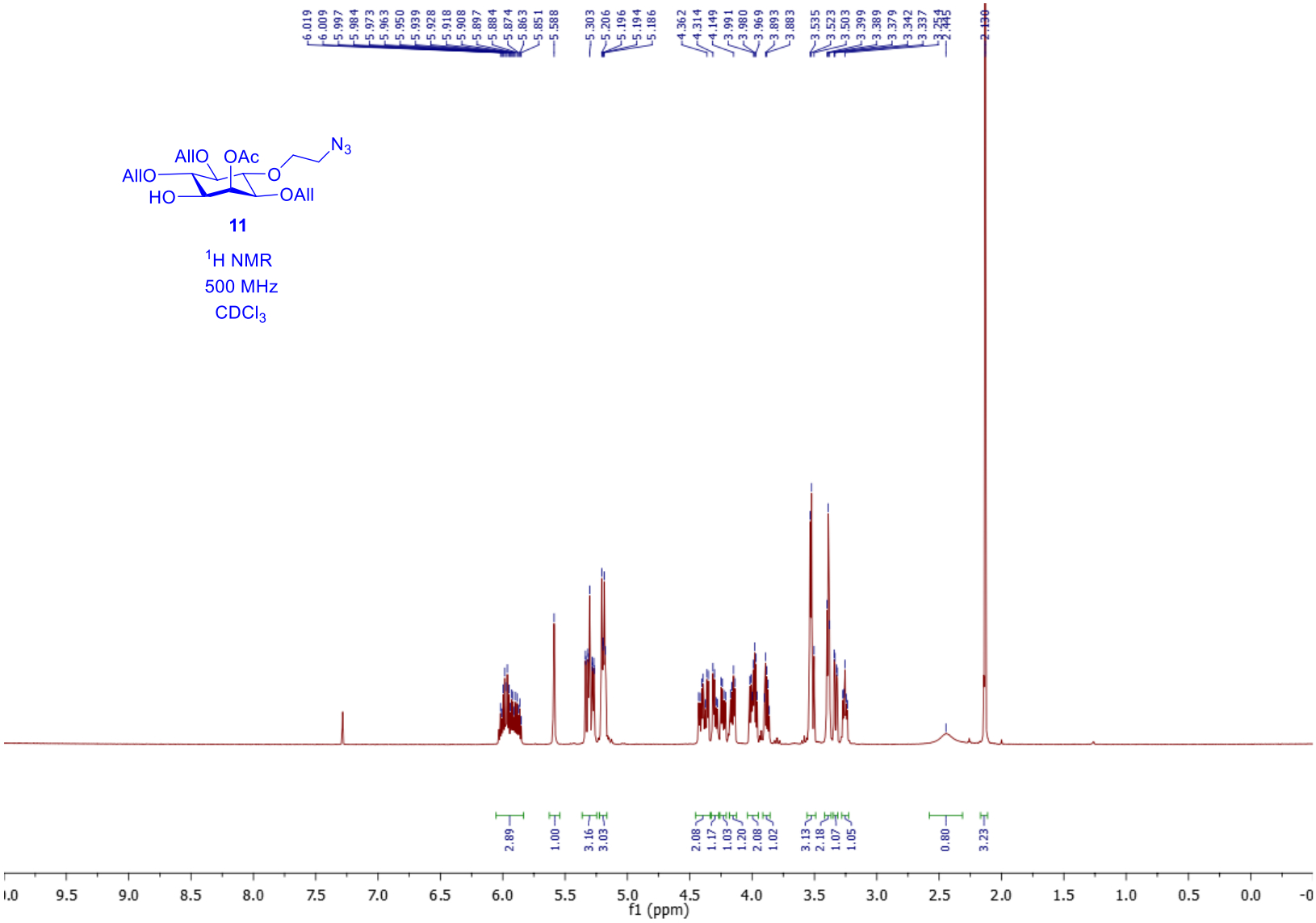
1H-13C HSQC
500/125 MHz
CDCl3
f2 (ppm)
f1 (ppm)

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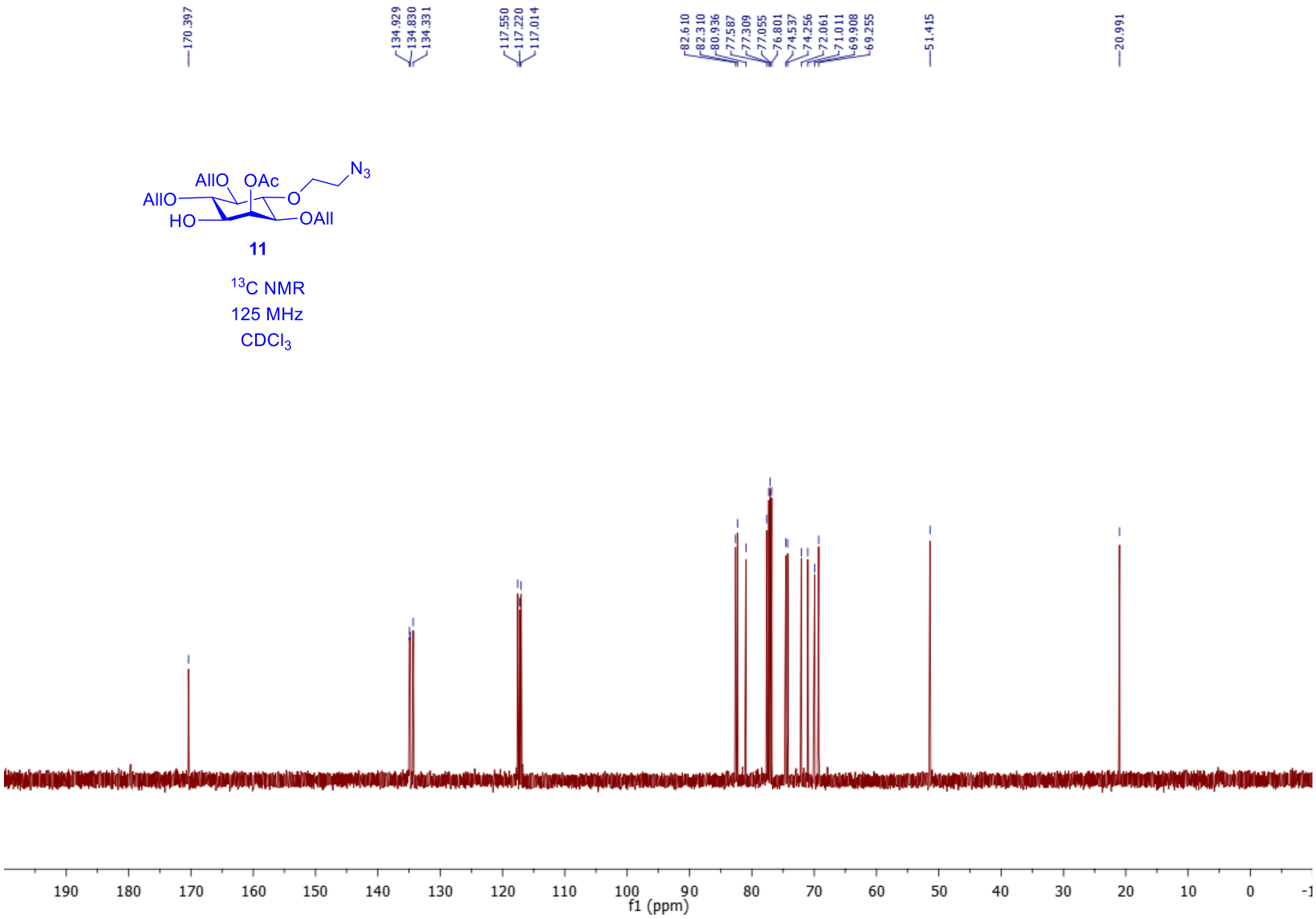
OAc
N3
AllO
O
HO
OAll
11
1H NMR
500 MHz
CDCl3
f1 (ppm)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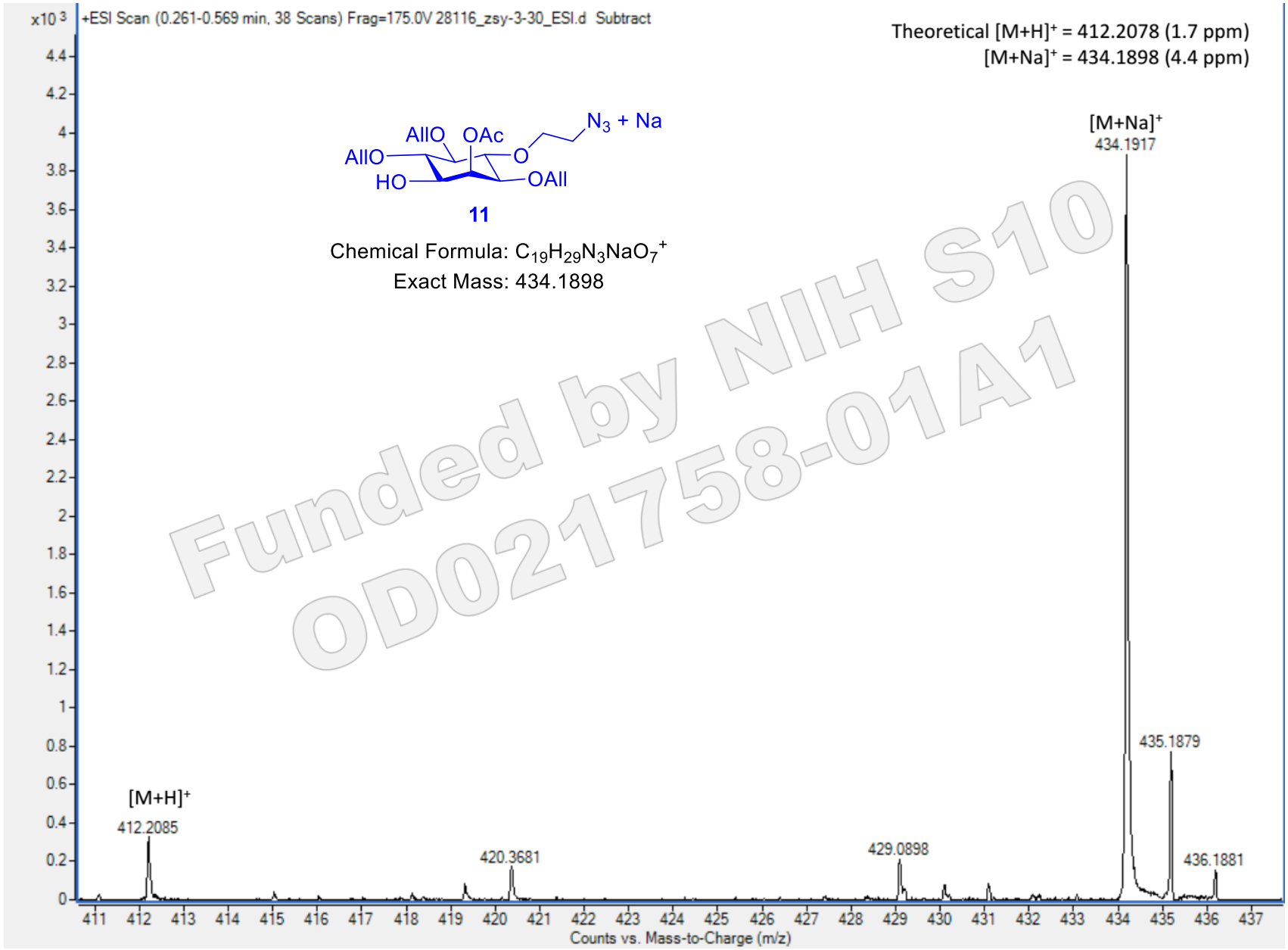
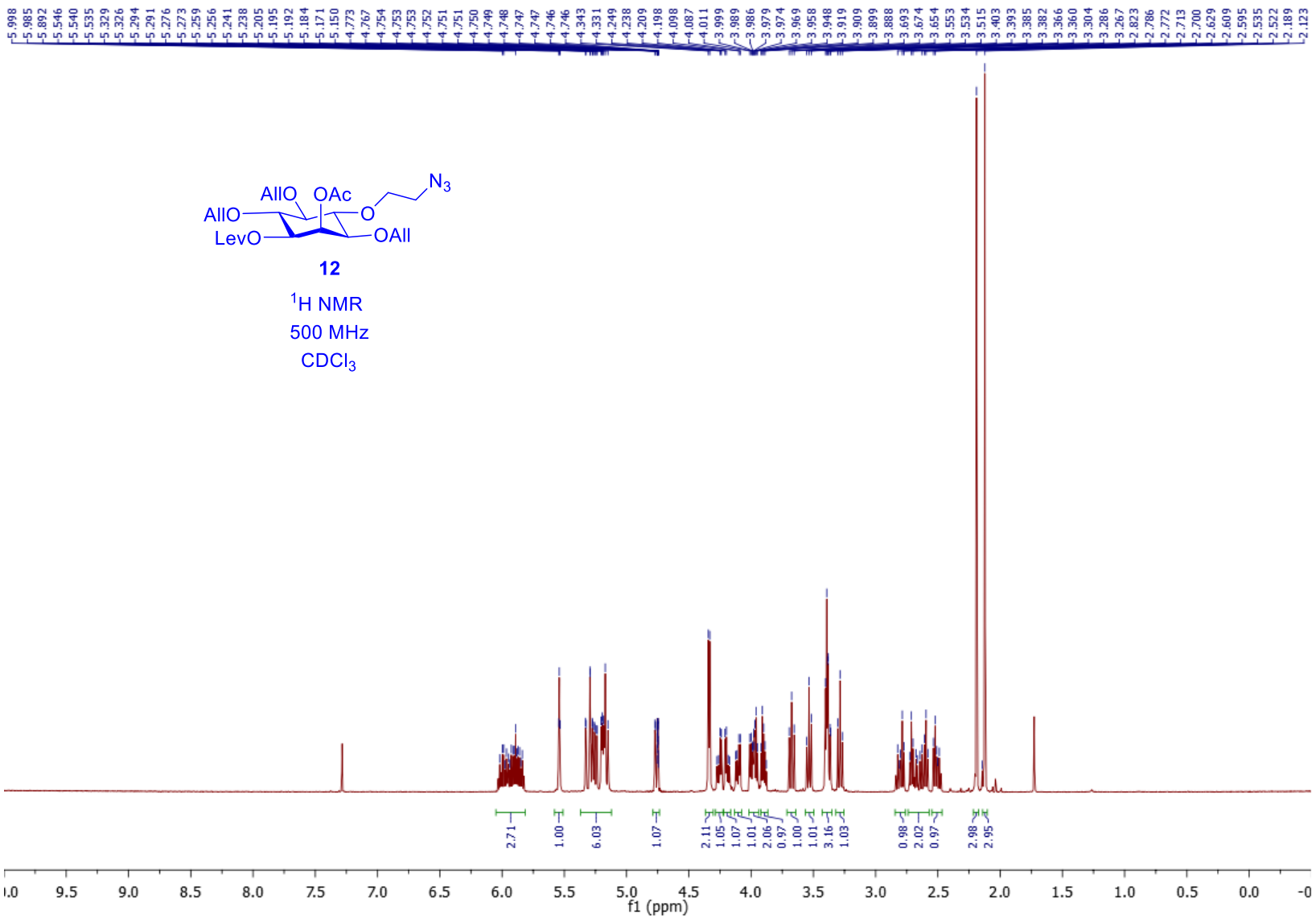
134.830
134.331
117.550
117.220
117.014
82.610
82.310
80.936
77.587
77.309
77.055
76.801
74.537
74.256
72.061
71.011
69.908
69.255
51.415
20.991
AllO
OAc
AllO
O
N3
HO
OAll
11
13C NMR
125 MHz
CDCl3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1
f1 (ppm)

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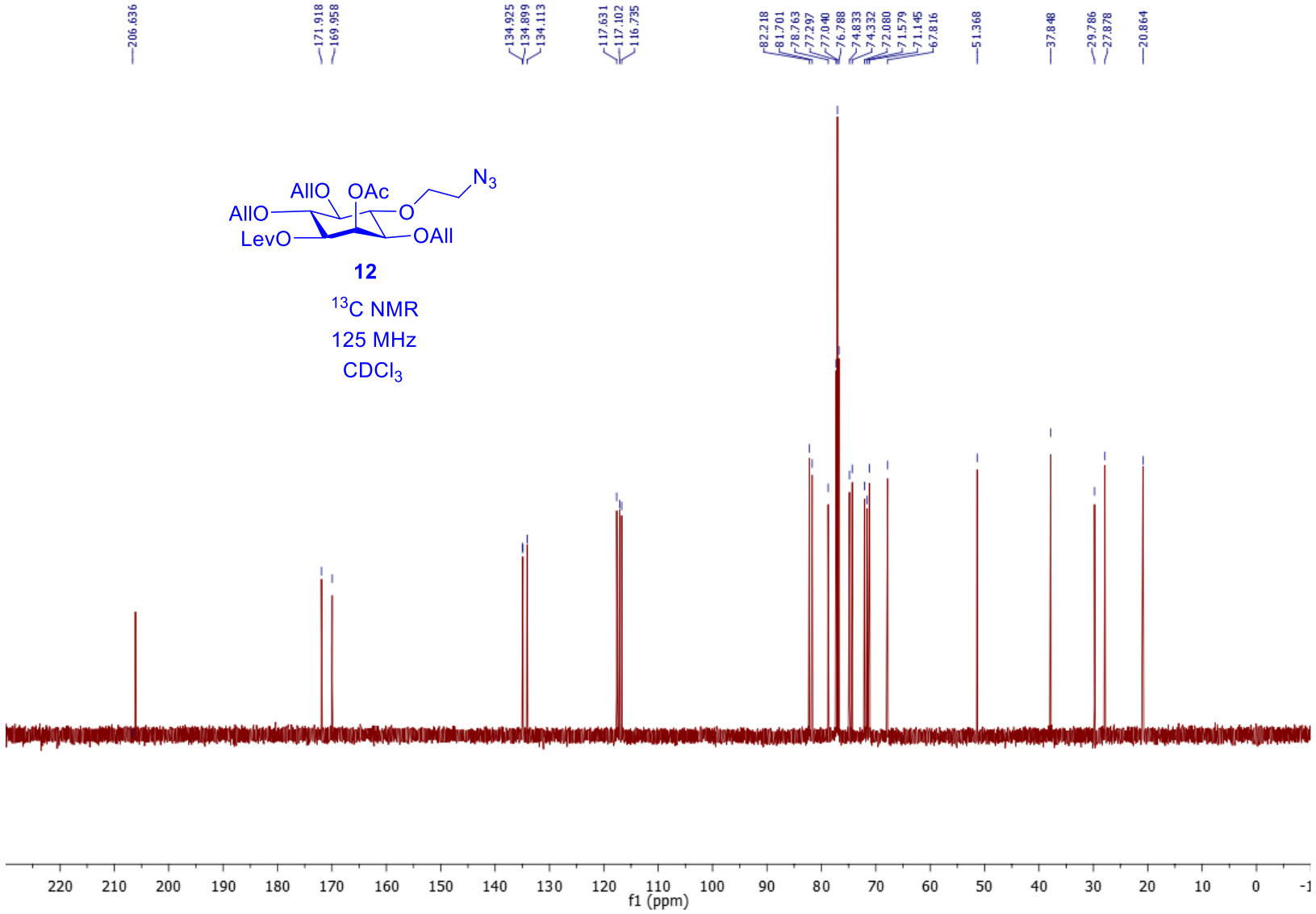
N3
AllO
O
LevO
OAll
12
1H NMR
500 MHz
CDCl3
f1 (ppm)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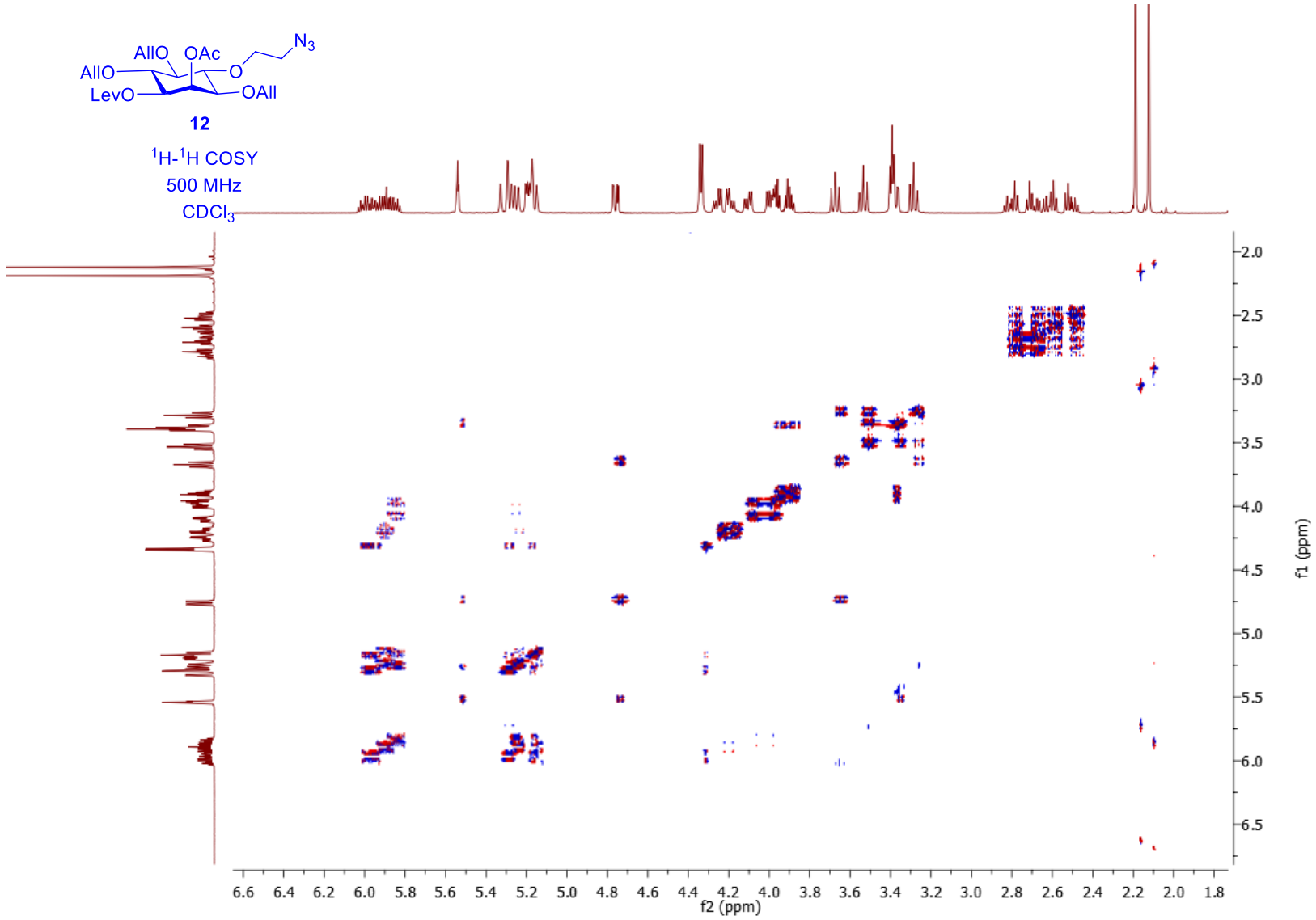
134.925
134.899
134.113
117.631
117.102
116.735
82.218
81.701
78.763
77.297
77.040
76.788
74.833
74.332
72.080
71.579
71.145
67.816
51.368
37.848
29.786
27.878
20.864
AllO
OAc
N3
AllO
O
LevO
OAll
12
13C NMR
125 MHz
CDCl3
f1 (ppm)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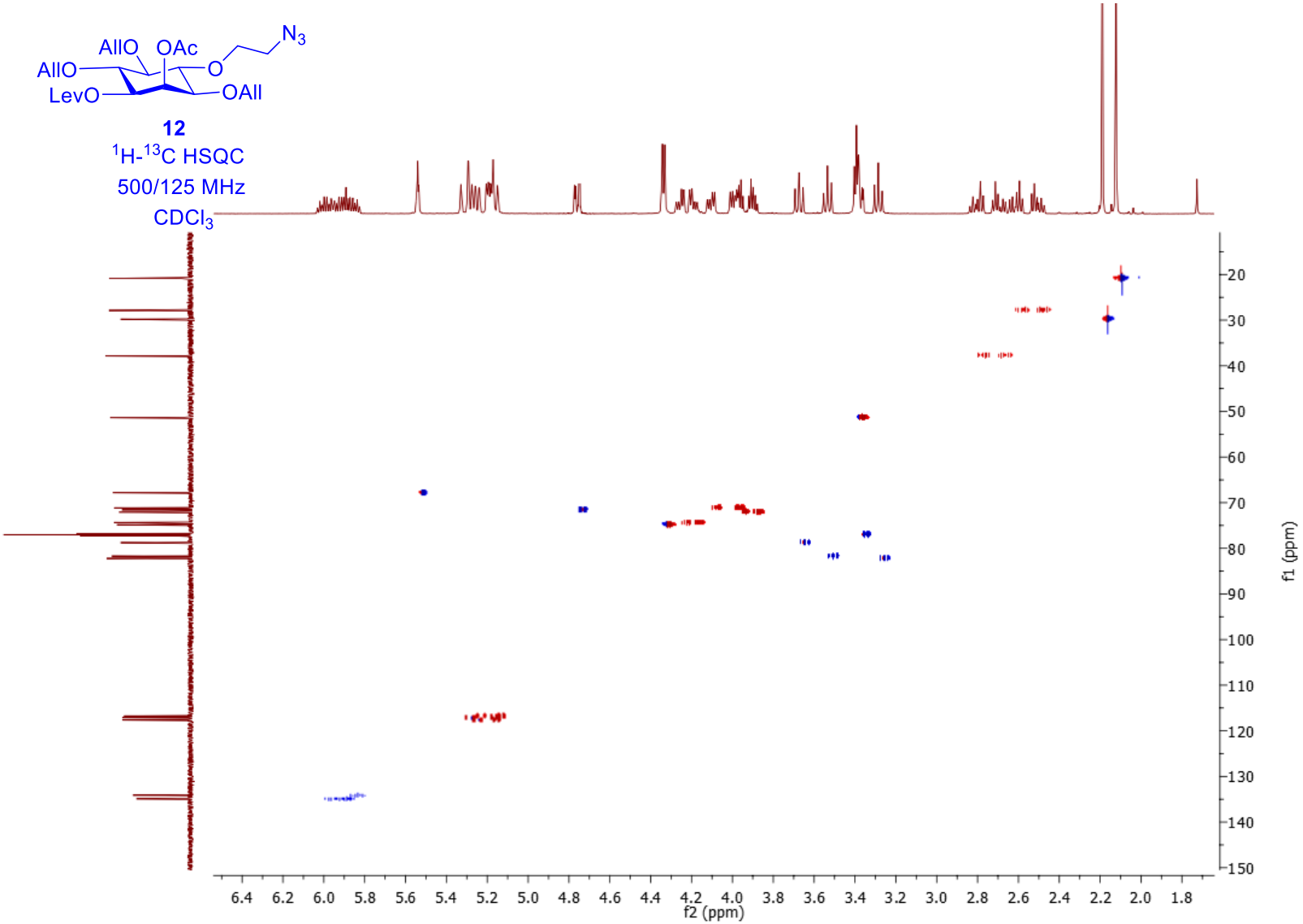
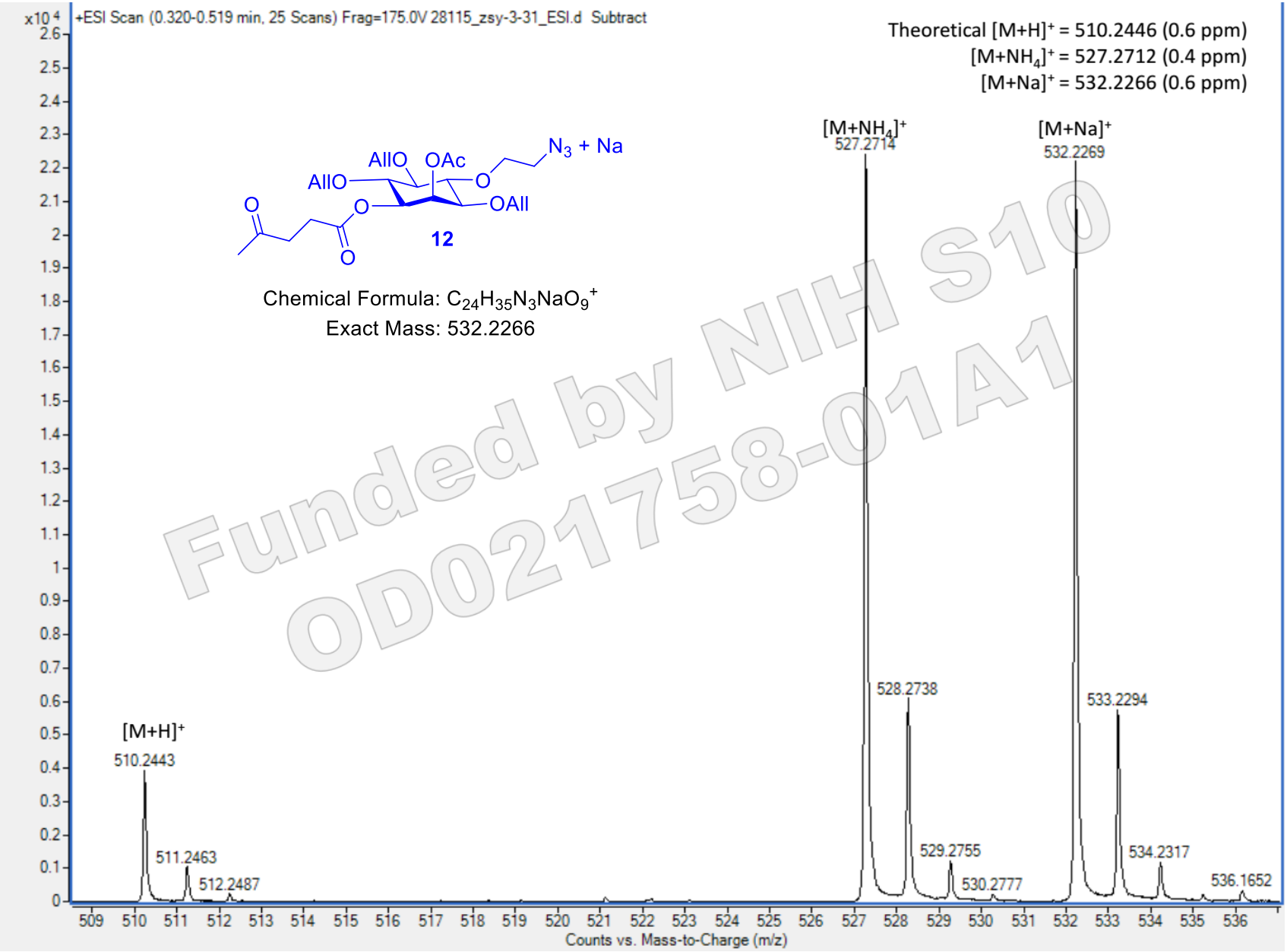
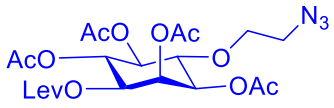
OAc
N3
AllO
O
LevO
OAll
12
1H-1H COSY
500 MHz
CDCl3
f2 (ppm)
f1 (ppm)


AllO
OAc
N3
AllO
O
LevO
OAll
12
1H-13C HSQC
500/125 MHz
CDCl3
f2 (ppm)
f1 (ppm)



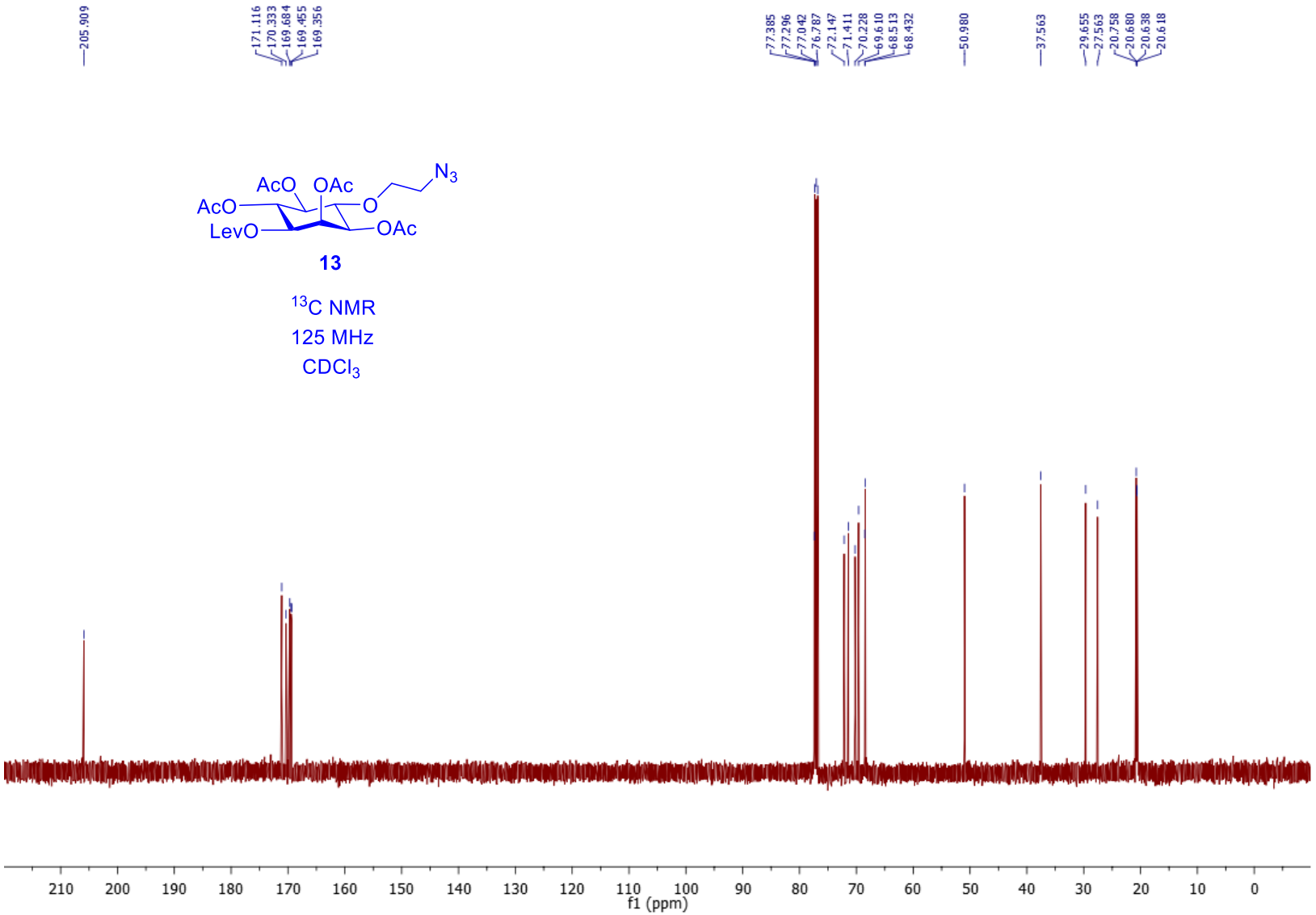


13

^{1}H NMR

500 MHz

$CDCl_3$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169.455
169.356
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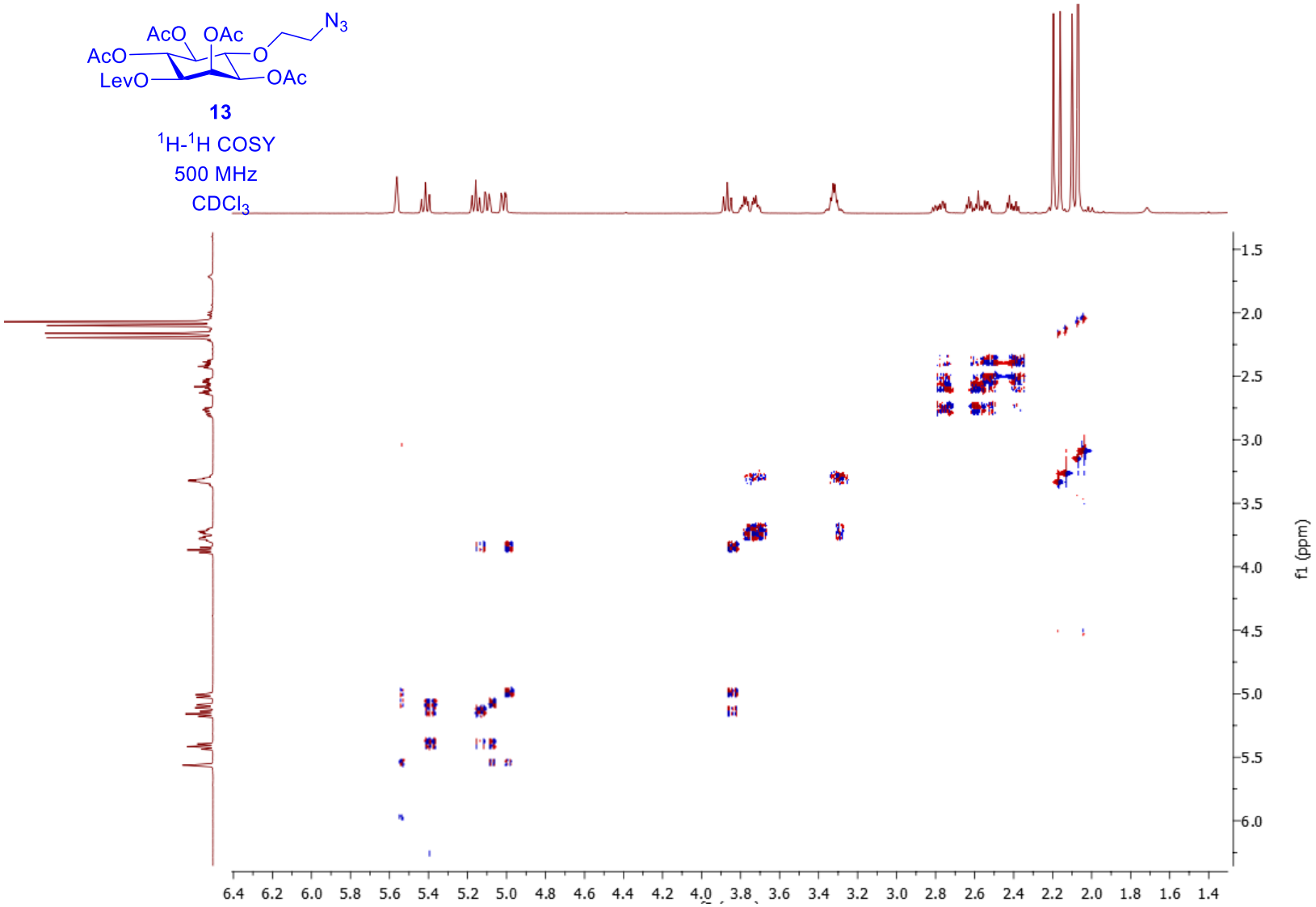
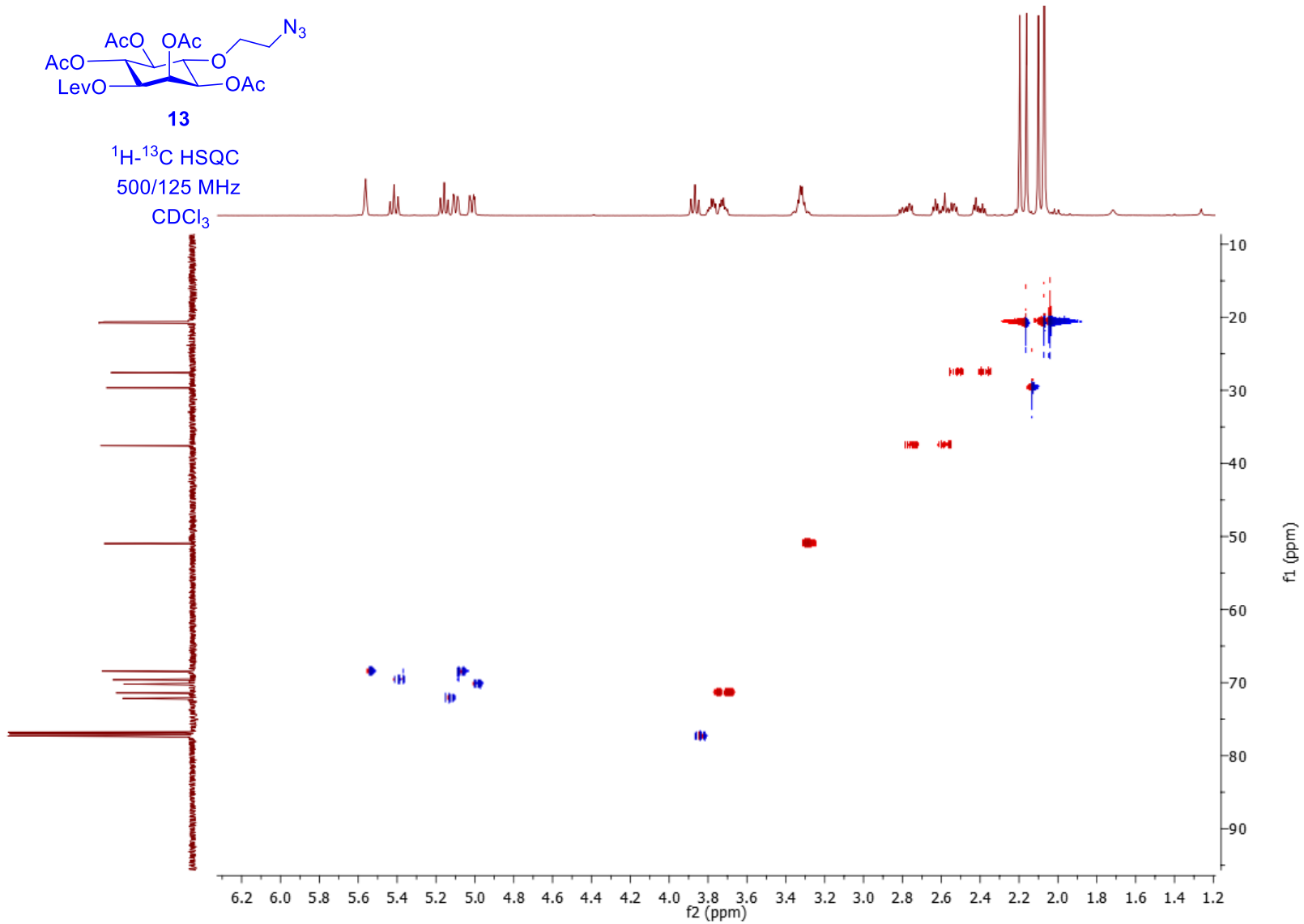
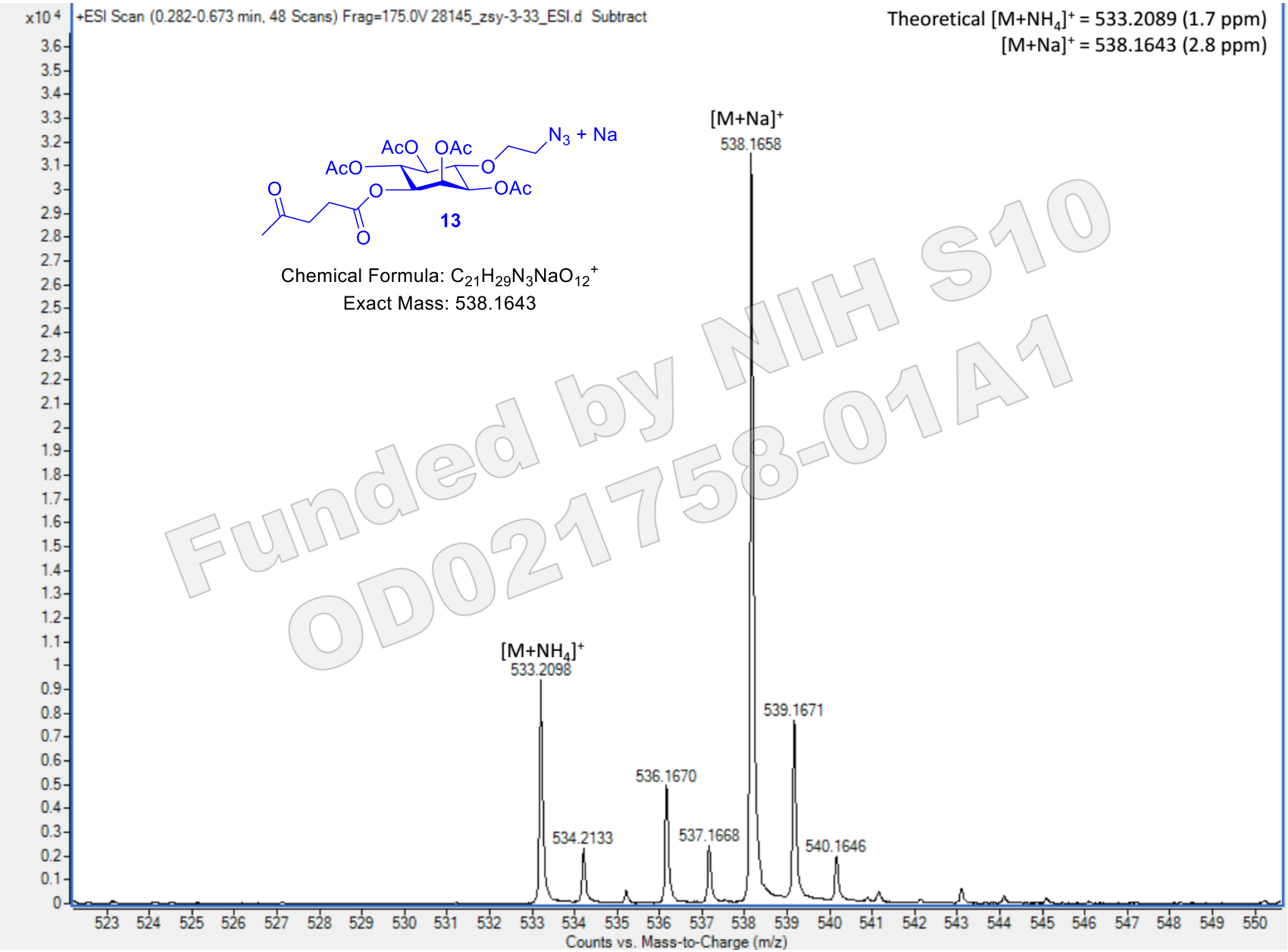
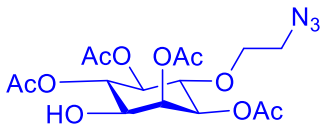
77.296
77.042
76.787
72.147
71.411
70.228
69.610
68.513
68.432
50.980
37.563
29.655
27.563
20.758
20.680
20.638
20.618
AcO
OAc
AcO
O
N3
LevO
OAc
13
13C NMR
125 MHz
CDCl3
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


AcO
OAc
AcO
O
N3
LevO
OAc
13
1H-1H COSY
500 MHz
CDCl3
f1 (ppm)
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.4
6.2
6.0
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


AcO
OAc
N3
AcO
O
LevO
OAc
13
1H-13C HSQC
500/125 MHz
CDCl3
f2 (ppm)
f1 (ppm)



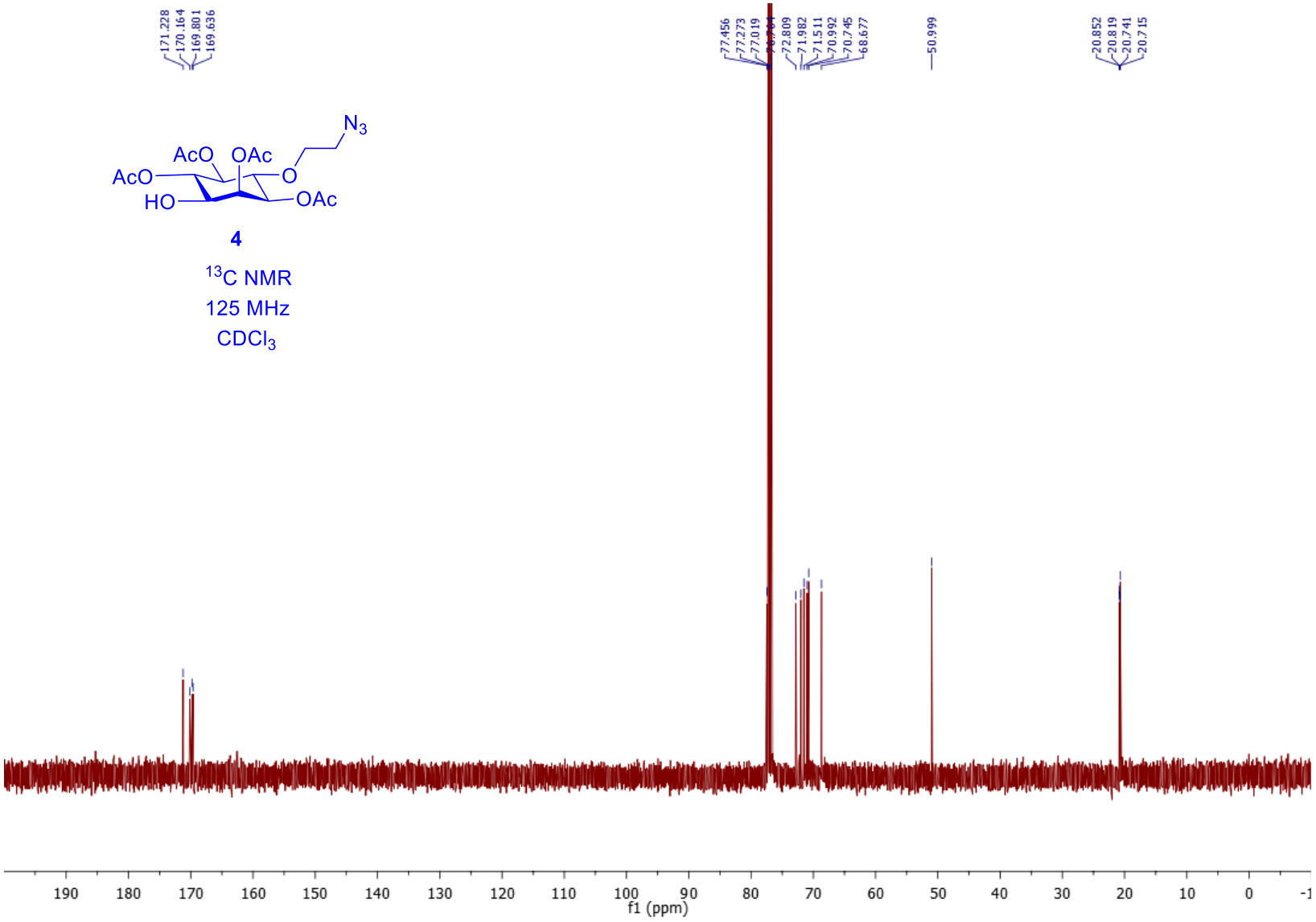


4

^{1}H NMR

500 MHz

$CDCl_3$


171.228
170.164
169.801
169.636
77.456
77.273
77.019
72.809
71.982
71.511
70.992
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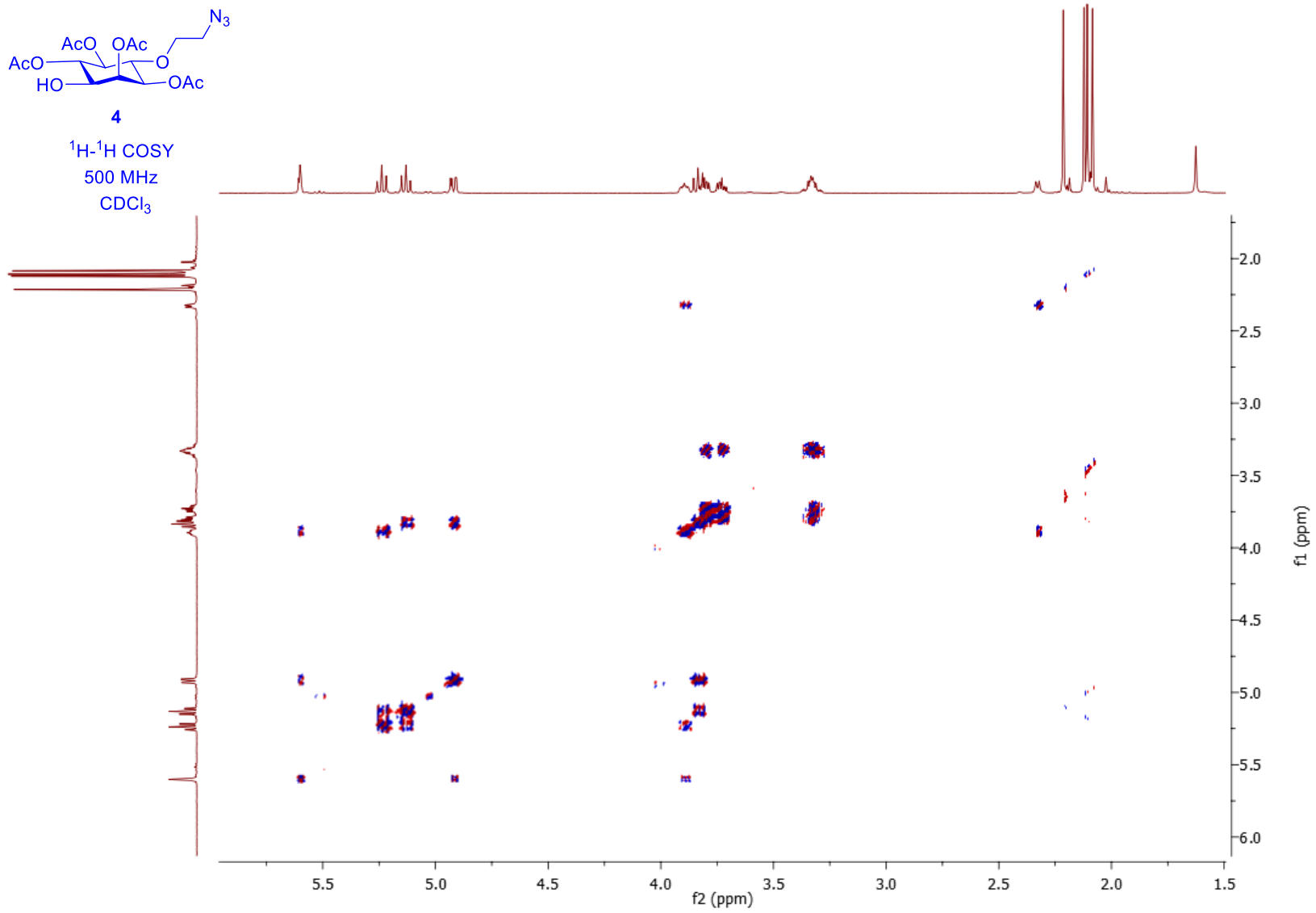
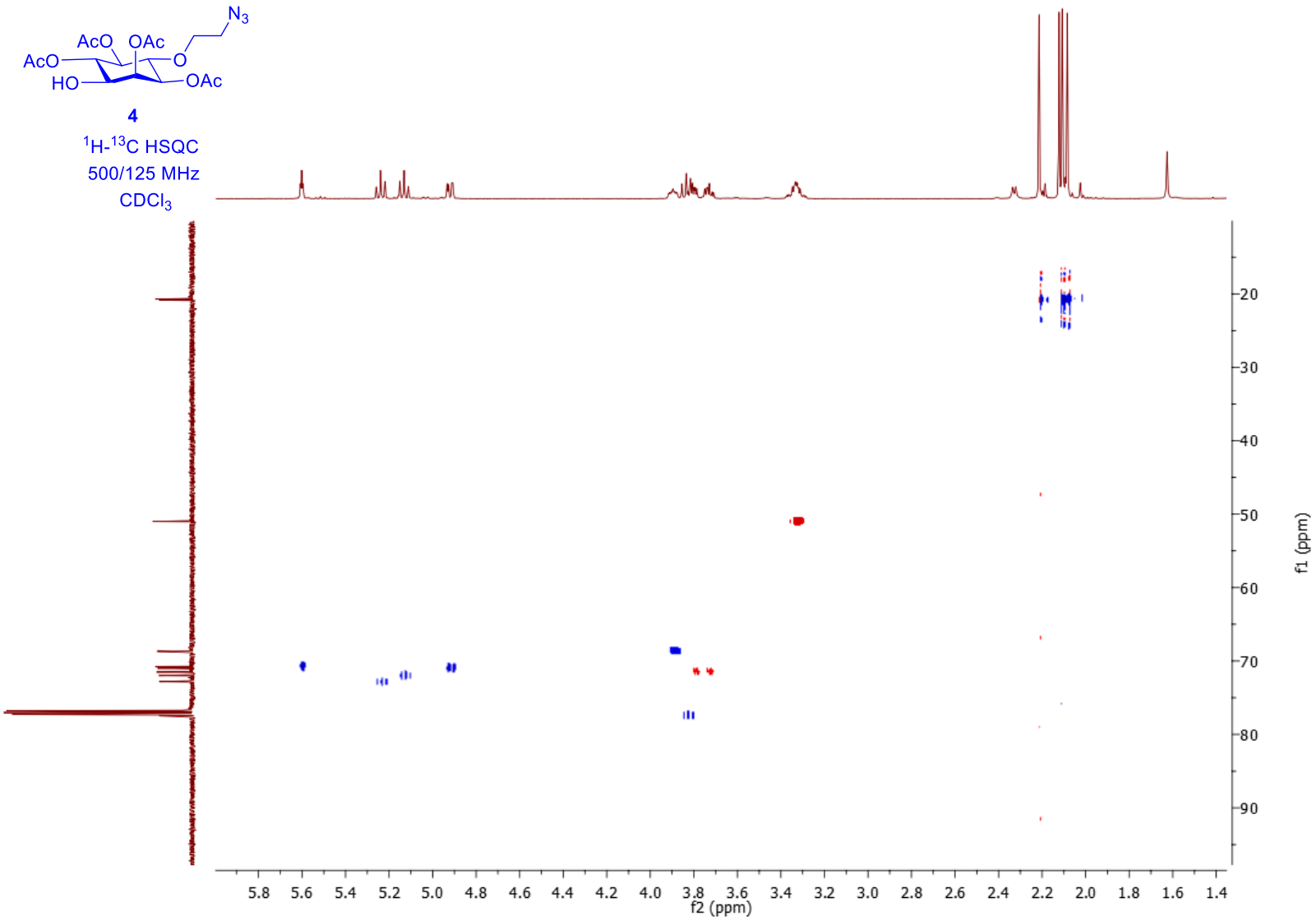
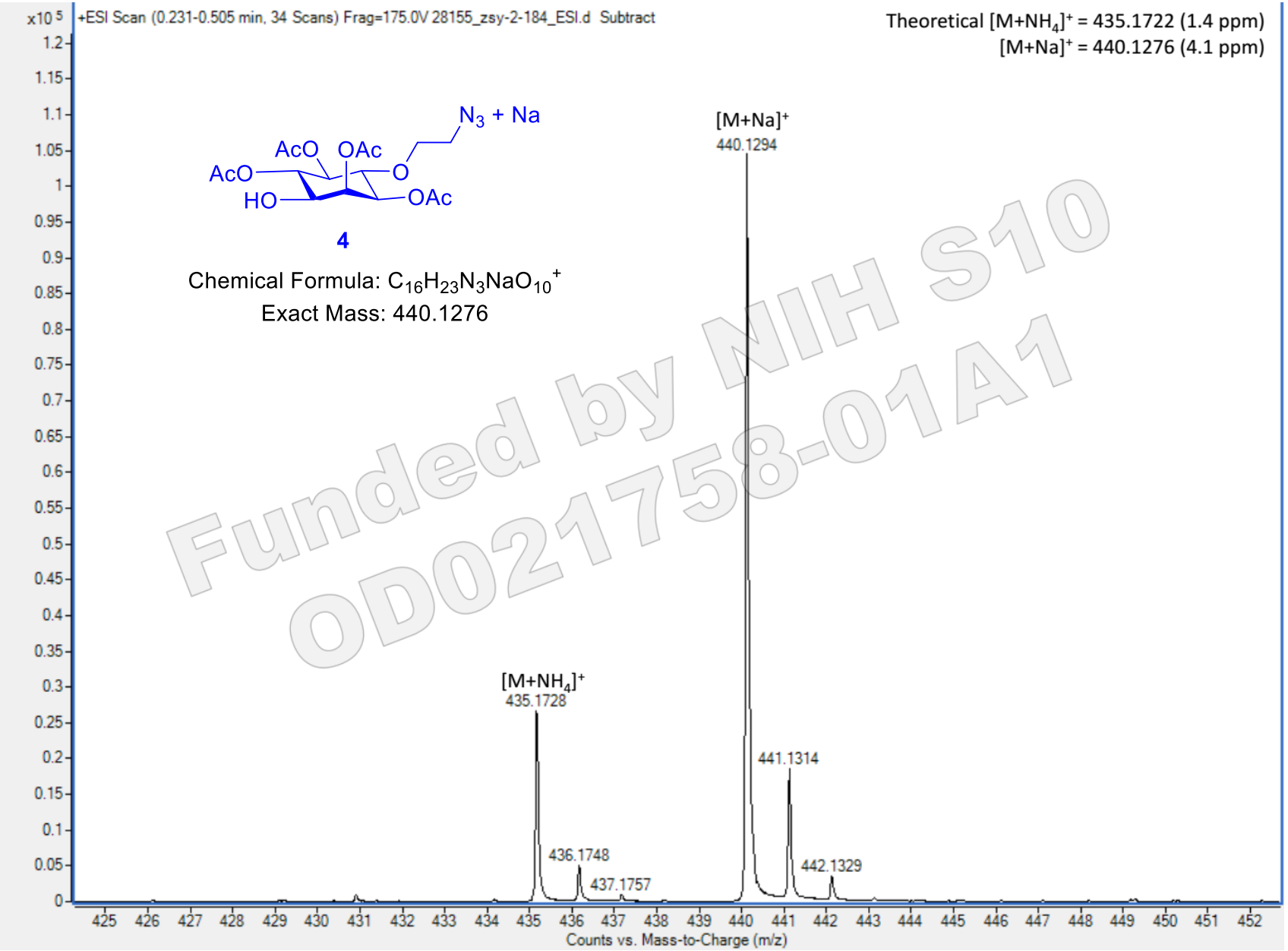
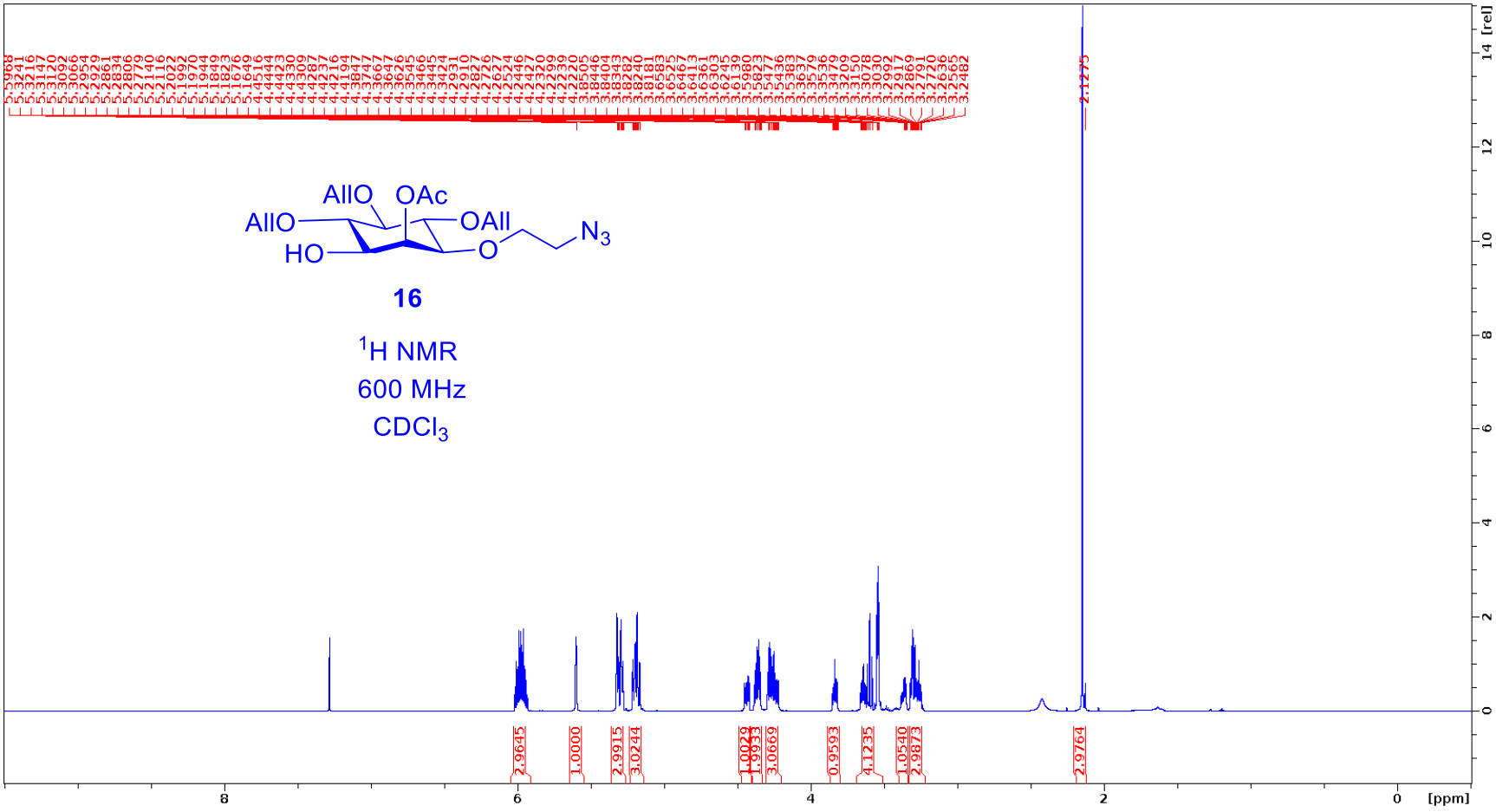
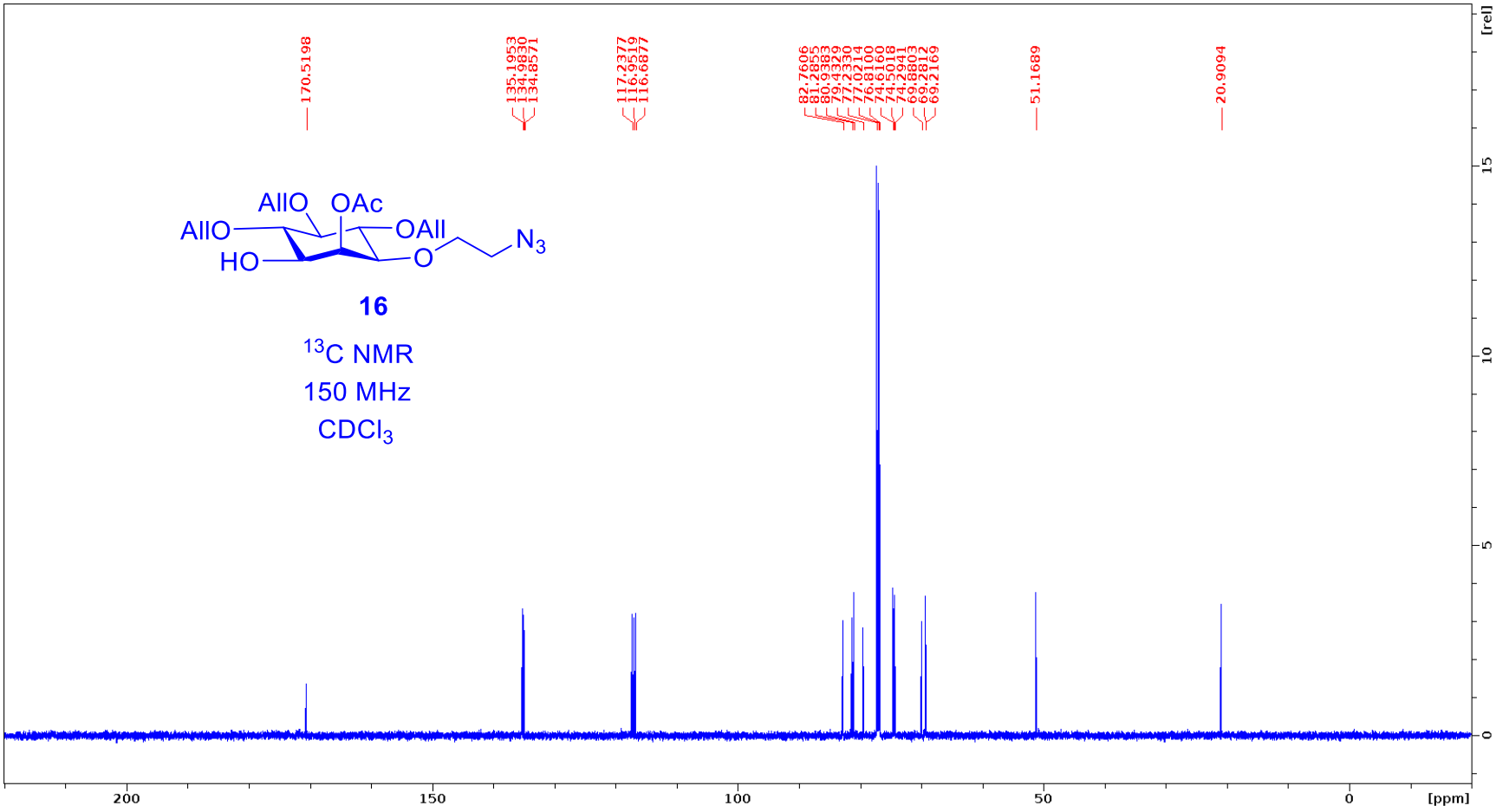
68.677
50.999
20.852
20.819
20.741
20.715
N3
AcO
OAc
AcO
O
HO
OAc
4
13C NMR
125 MHz
CDCl3
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


AcO
OAc
AcO
O
HO
OAc
N3
4
1H-1H COSY
500 MHz
CDCl3
f2 (ppm)
f1 (ppm)


AcO
OAc
AcO
O
HO
OAc
N3
4
1H-13C HSQC
500/125 MHz
CDCl3
f1 (ppm)
f2 (ppm)




AllO
OAc
AllO
OAll
HO
O
N_3
16
^{1}H NMR
600 MHz
$CDCl_3$
2.1275
2.9645
1.0000
2.9915
3.0244
1.0029
1.0033
3.0669
0.9593
4.1235
1.0540
2.9873
2.9764
8
6
4
2
0
[ppm]
14 [rel]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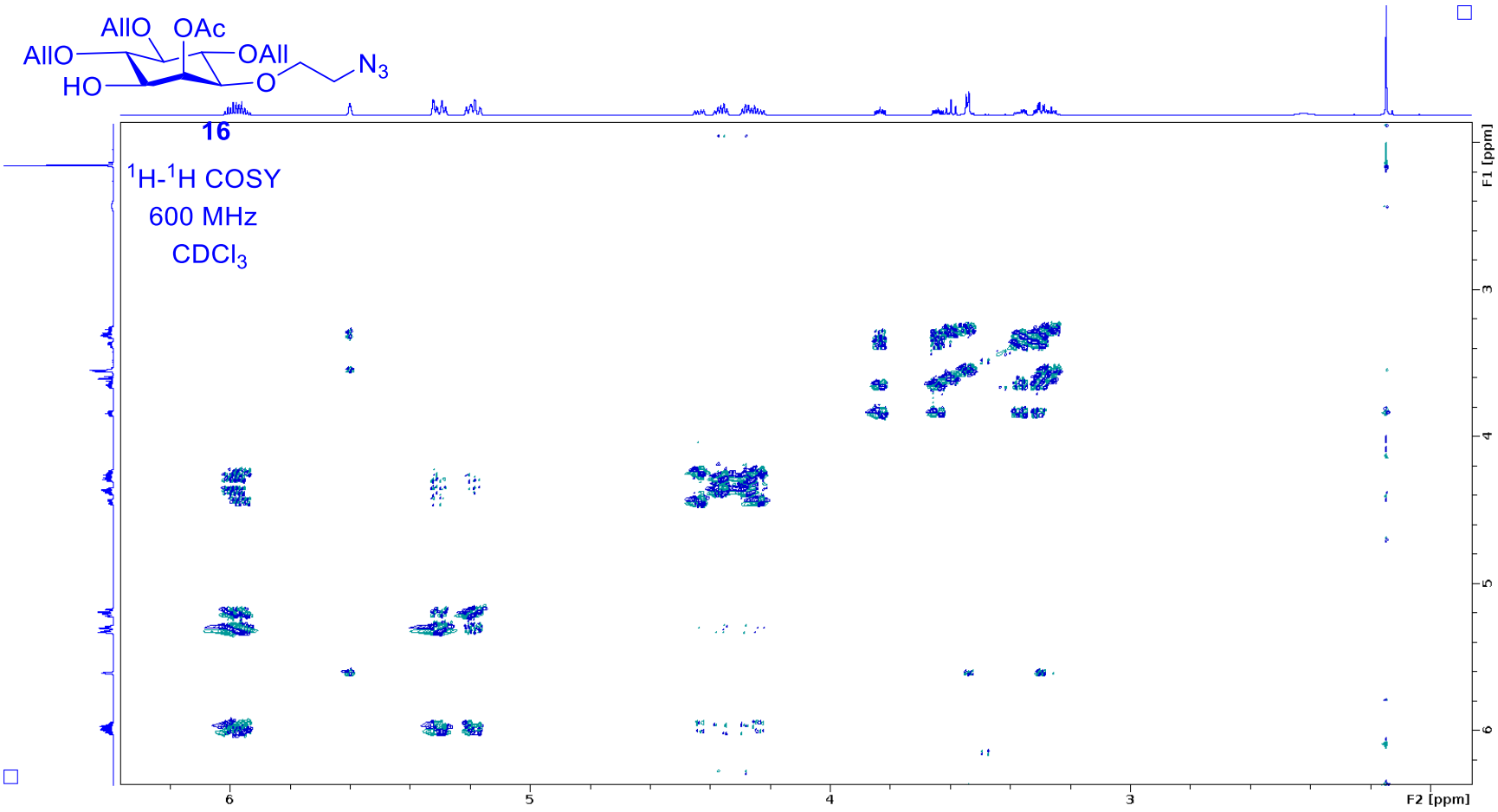
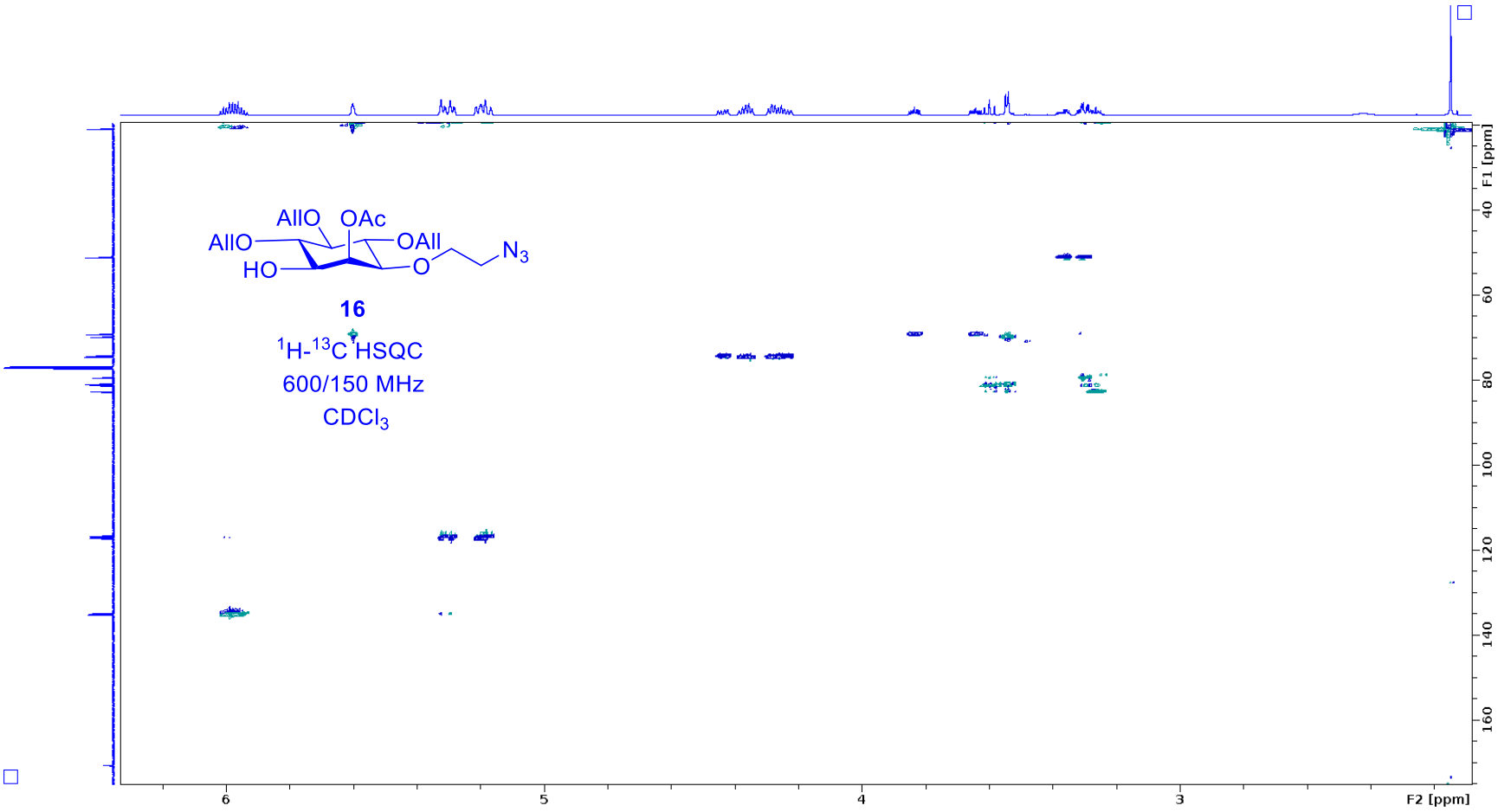
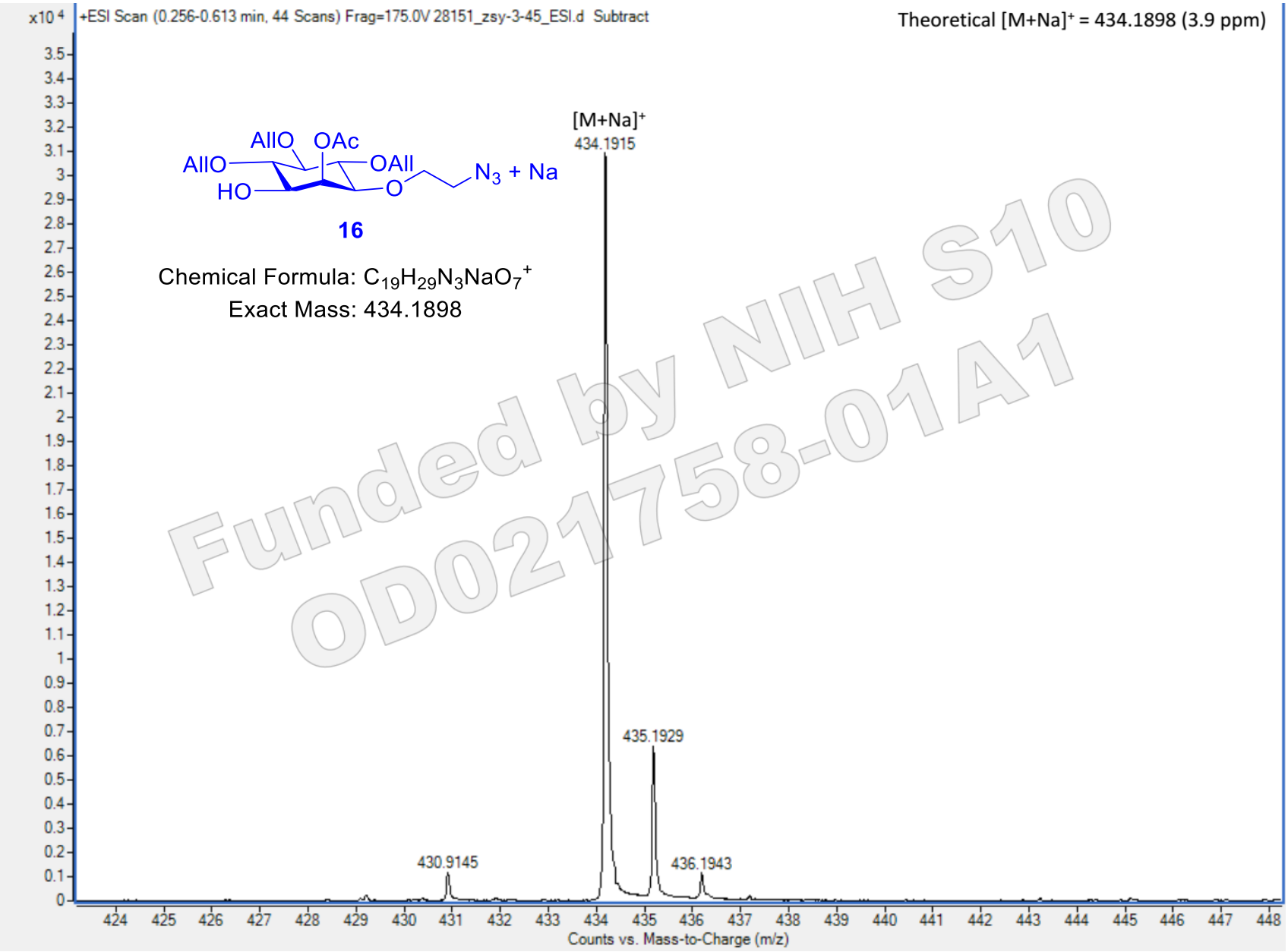
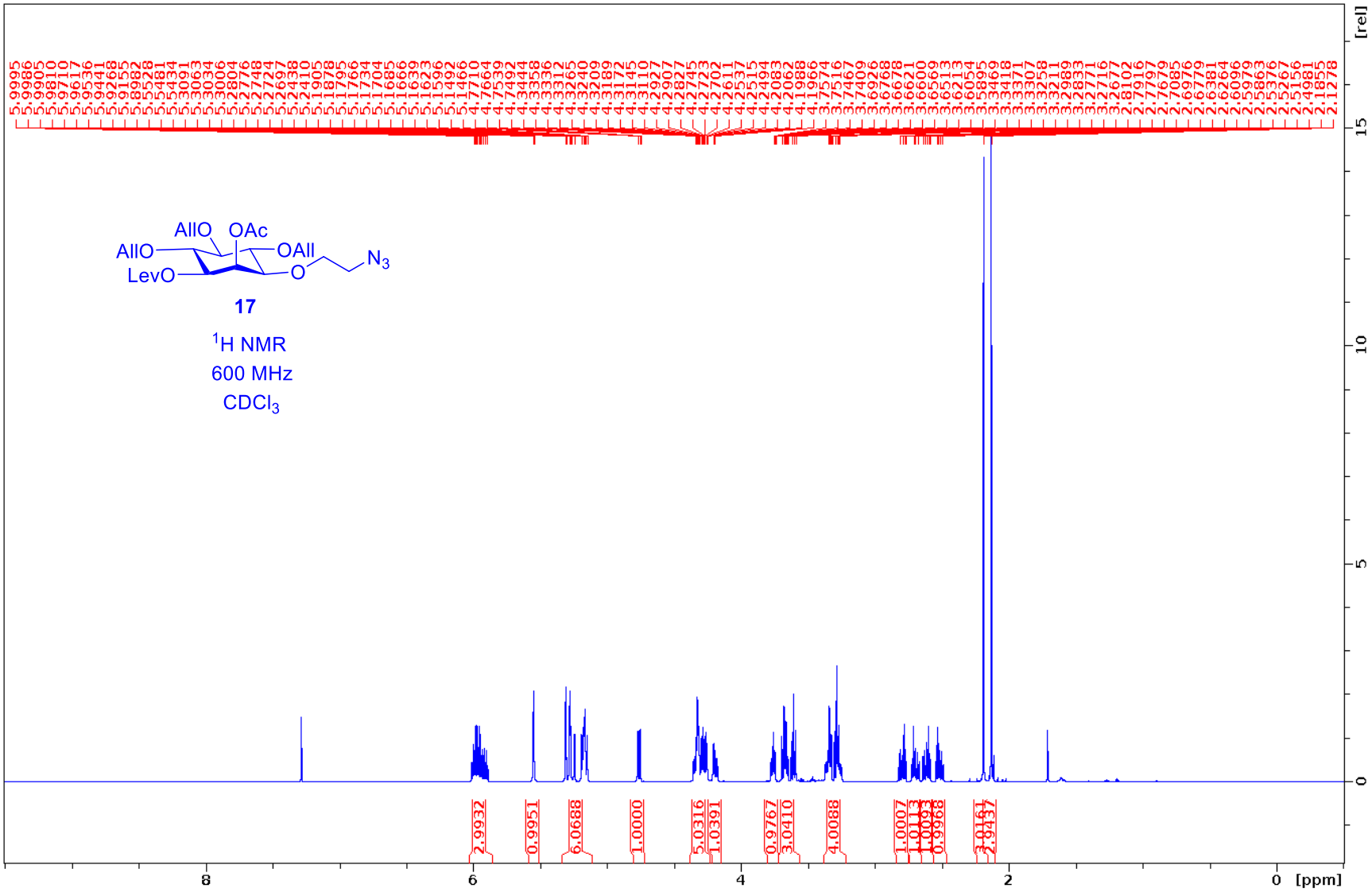
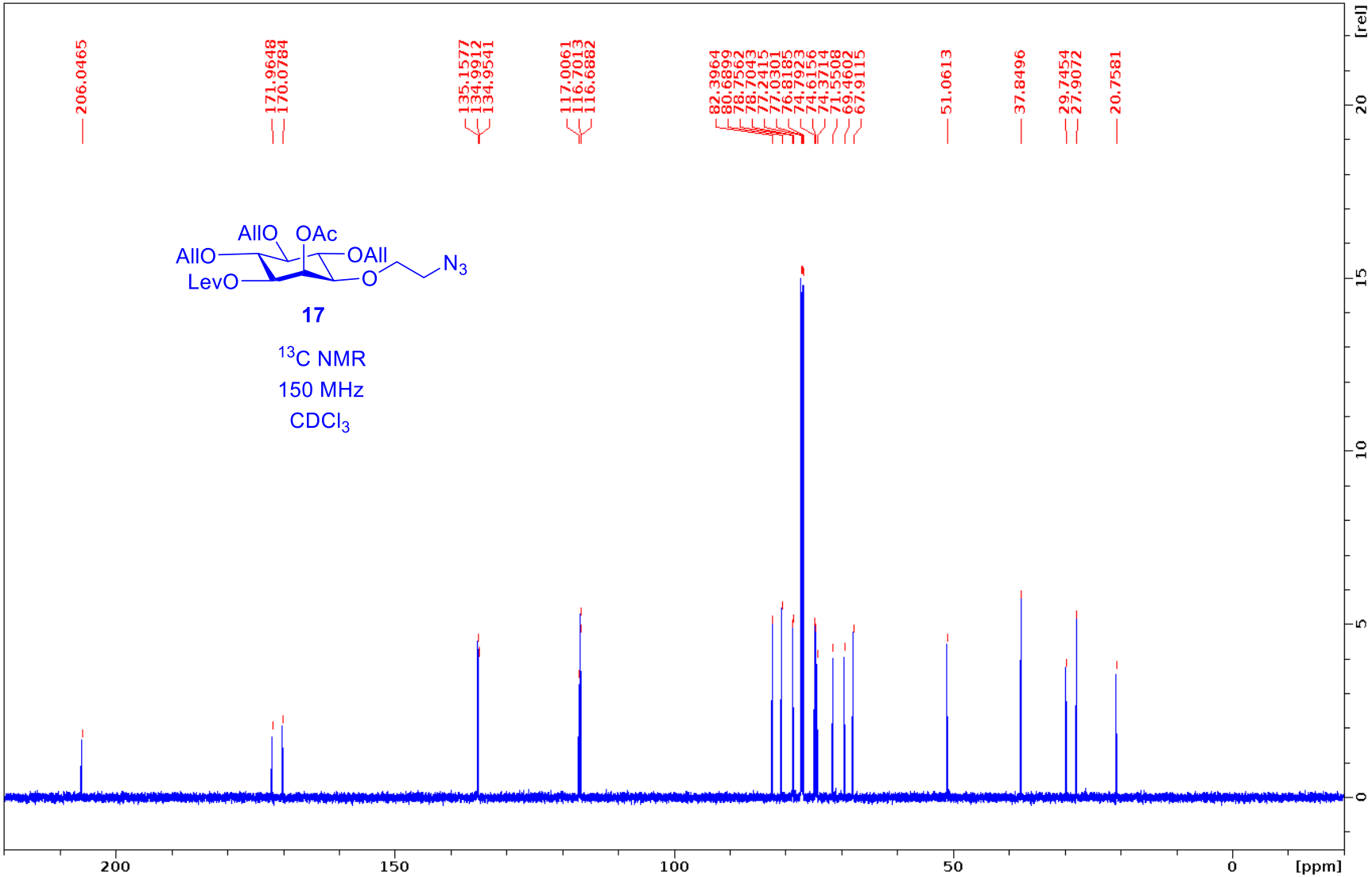
135.1953
134.9830
134.8571
117.2377
116.9519
116.6877
82.7606
81.2855
80.9383
79.4329
77.2330
77.0214
76.8100
74.6160
74.2941
69.8803
69.2812
69.2169
51.1689
20.9094
AllO
OAc
AllO
OAll
HO
O
N3
16
13C NMR
150 MHz
CDCl3
[rel]
15
10
5
0
200
150
100
50
0
[ppm]


AllO
OAc
AllO
OAll
HO
O
N_3
16
^{1}H-^{1}H COSY
600 MHz
$CDCl_3$
F1 [ppm]
F2 [ppm]
3
4
5
6
6
5
4
3


AllO
OAc
AllO
OAll
HO
O
N3
16
1H-13C HSQC
600/150 MHz
CDCl3
F1 [ppm]
40
60
80
100
120
140
160
6
5
4
3
F2 [ppm]




AllO
OAc
AllO
OAll
LevO
O
N3
17
1H NMR
600 MHz
CDCl3
[rel]
[ppm]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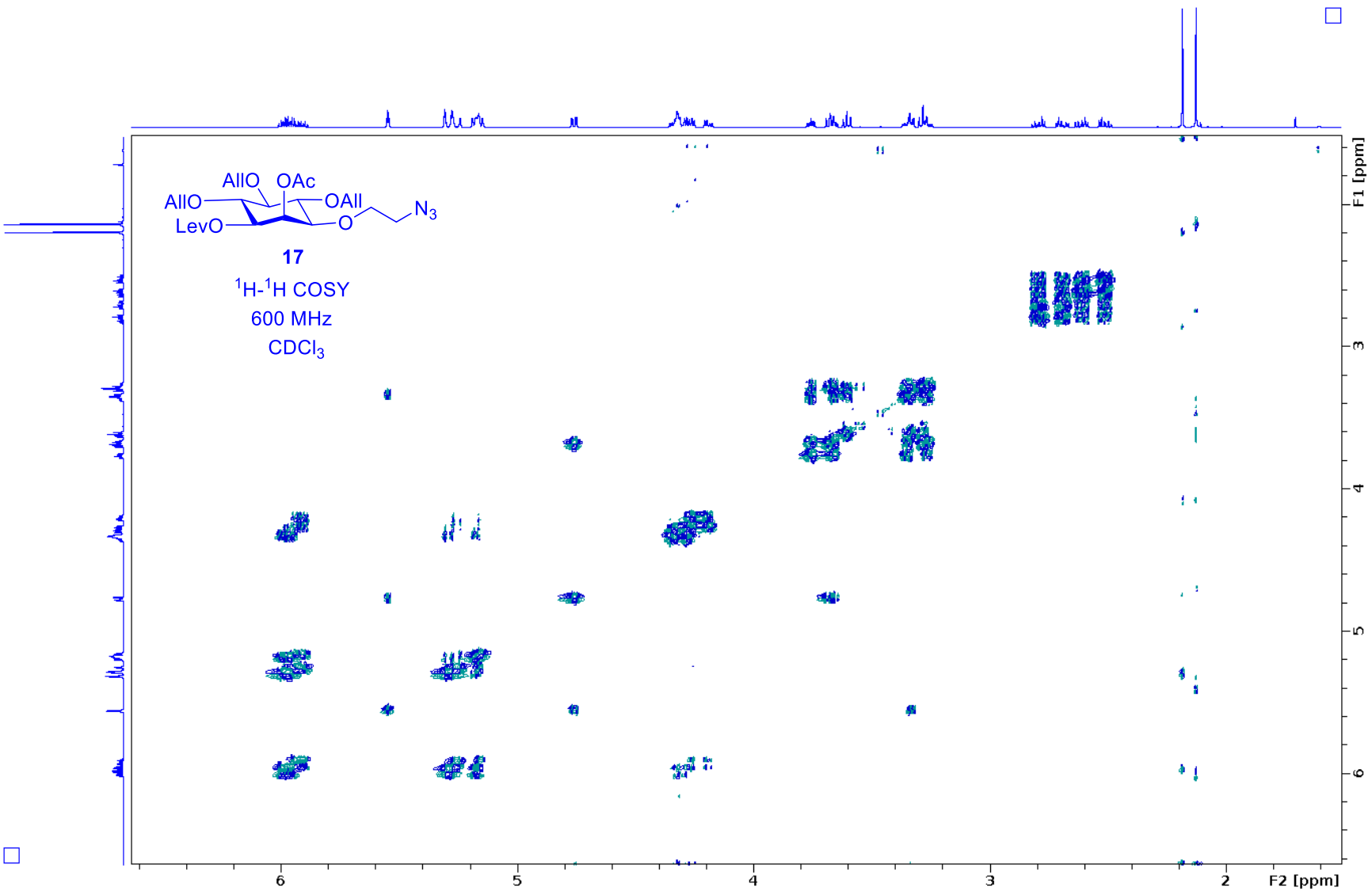
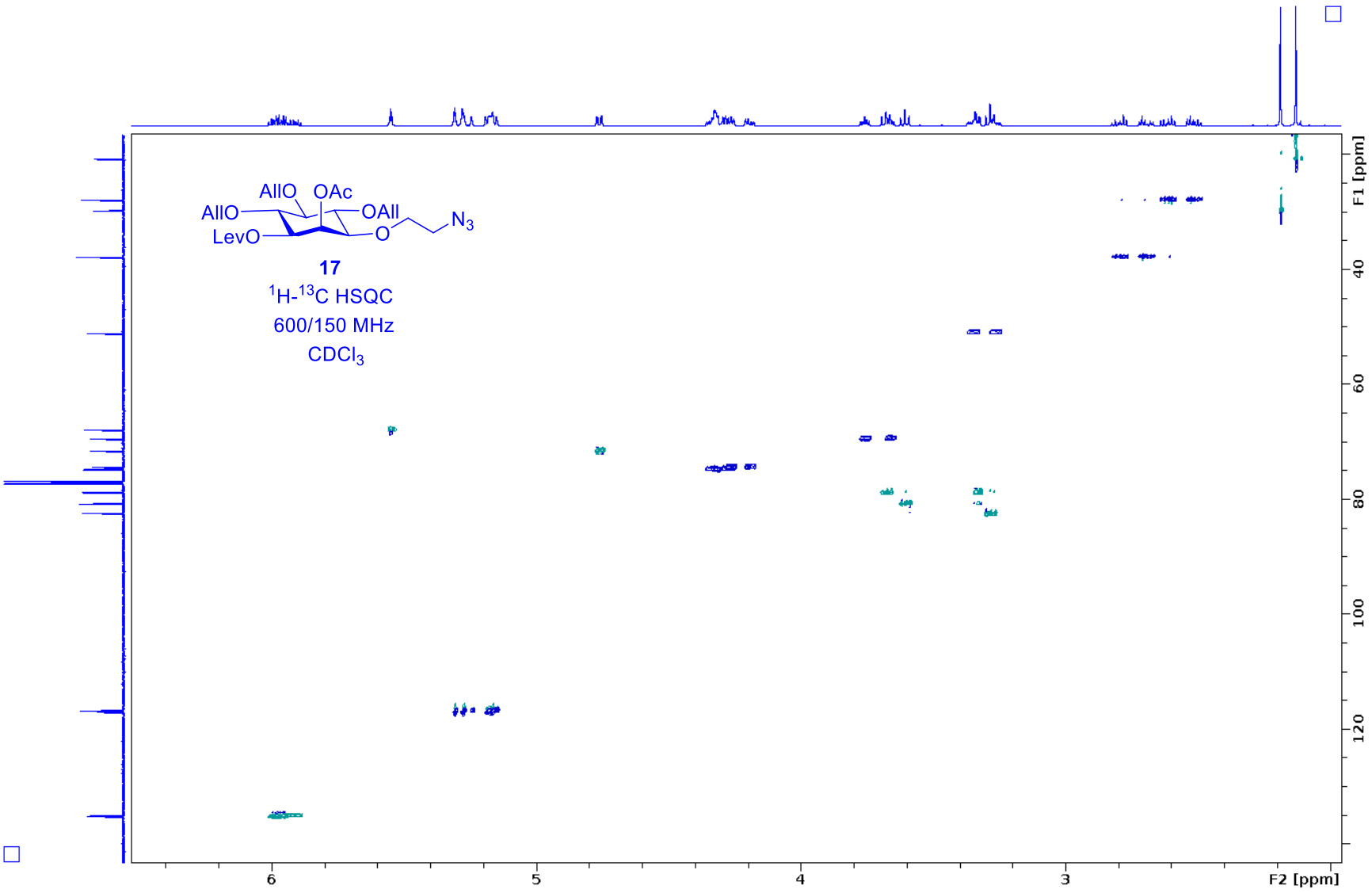
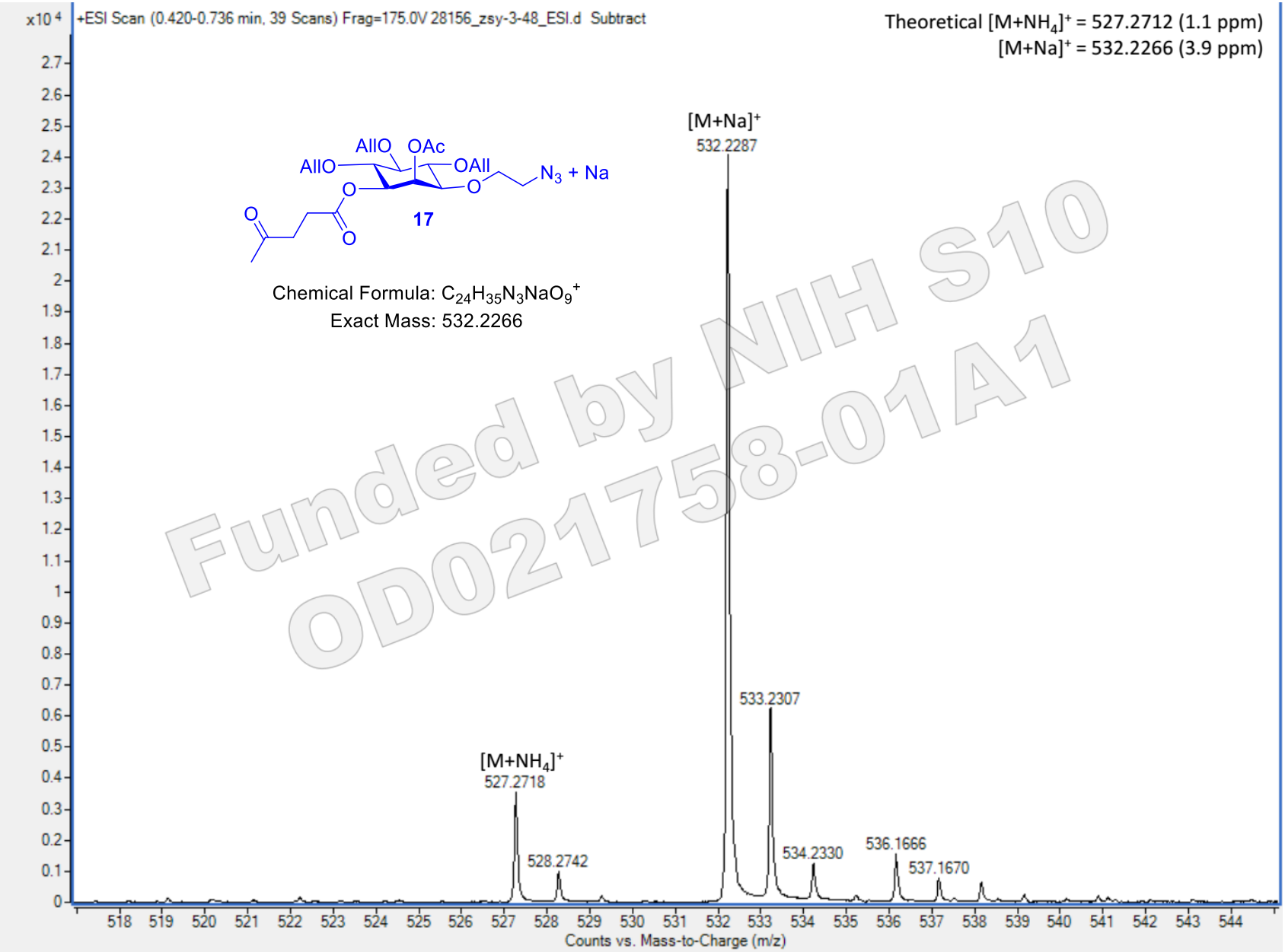
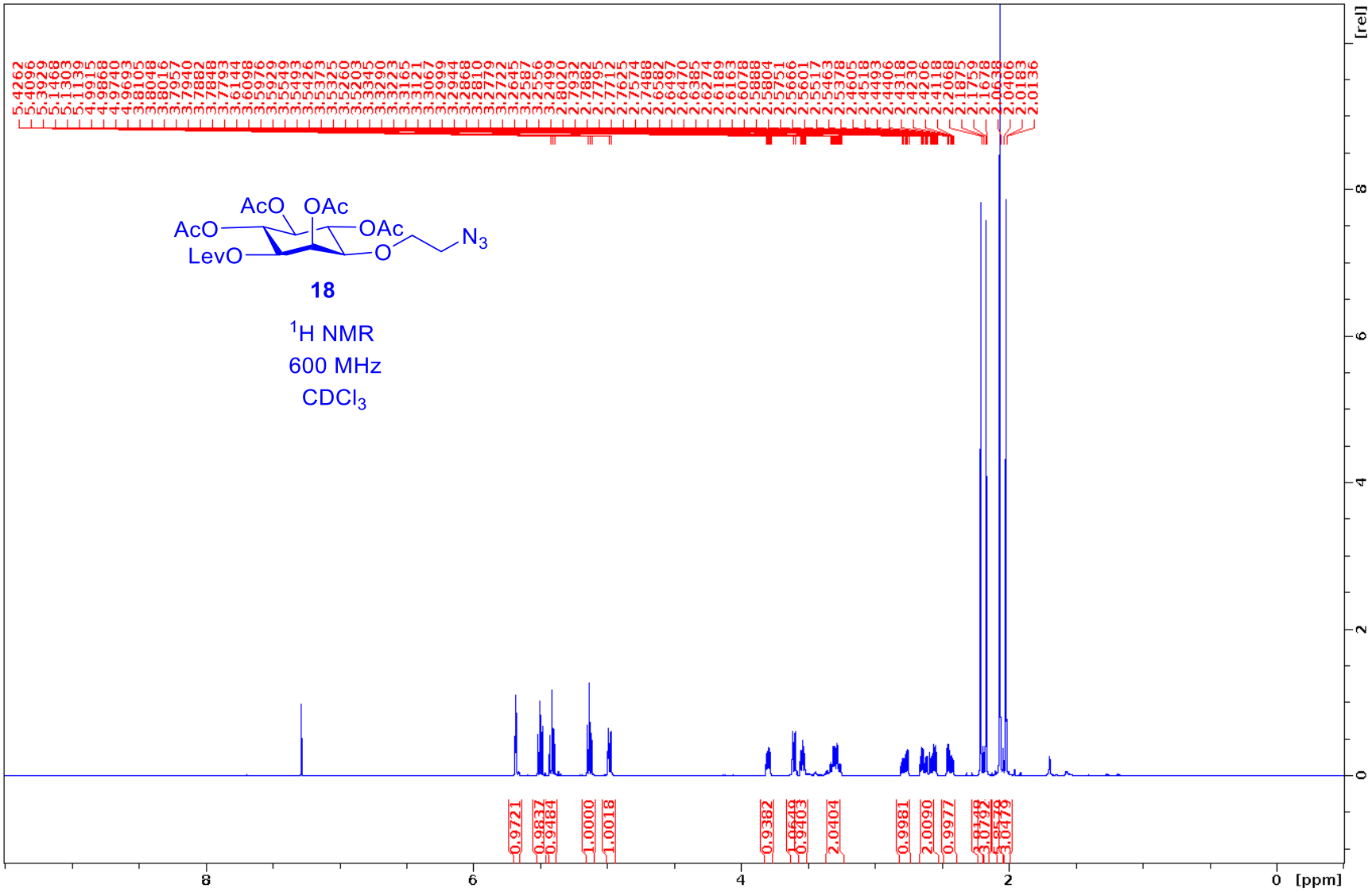
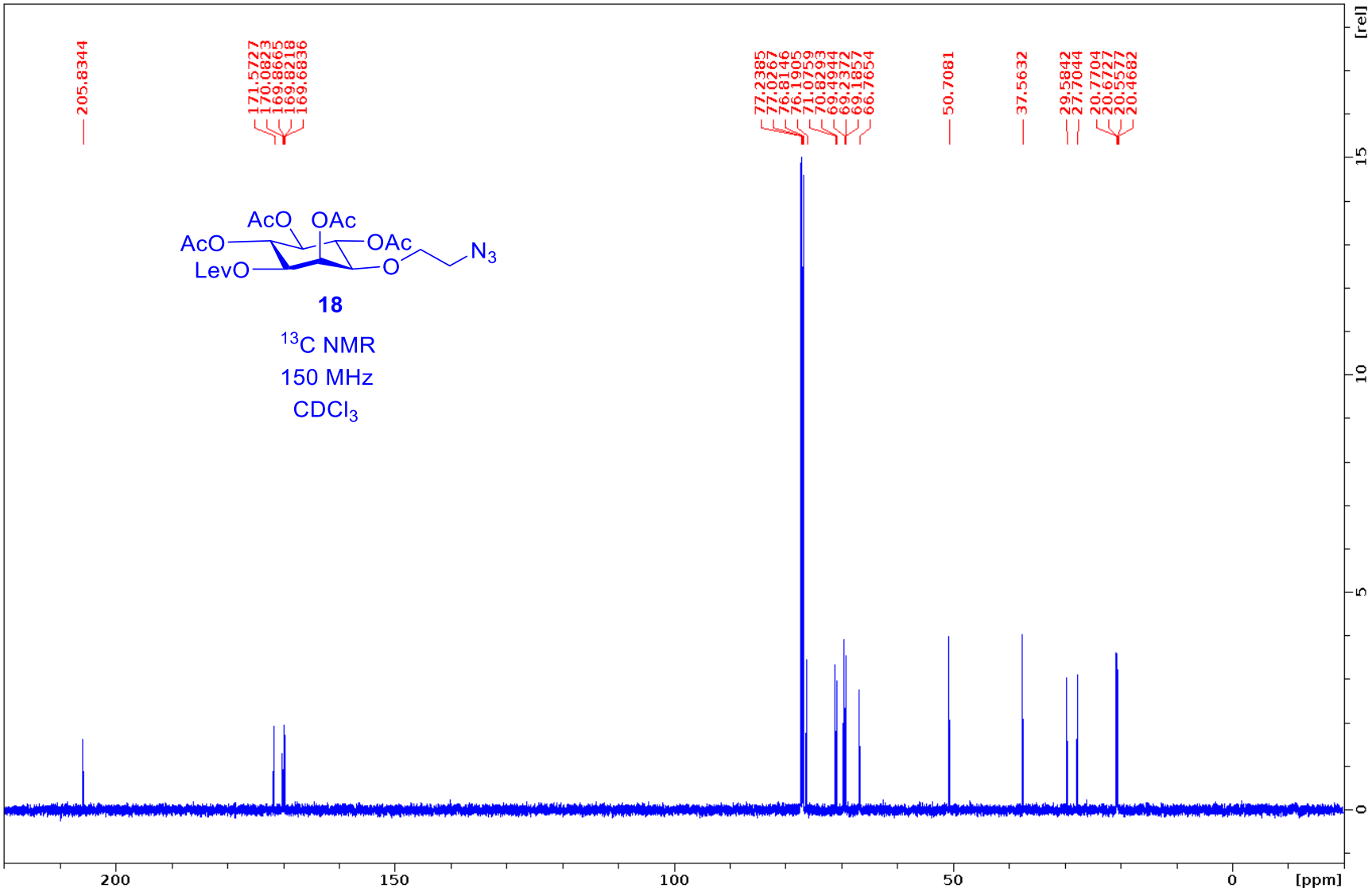
171.9648
170.0784
135.1577
134.9912
134.9541
117.0061
116.7013
116.6882
82.3964
80.6899
78.7562
78.7043
77.2415
77.0301
76.8185
74.7923
74.6156
74.3714
71.5508
69.4602
67.9115
51.0613
37.8496
29.7454
27.9072
20.7581
AllO
OAc
AllO
OAll
LevO
O
N3
17
13C NMR
150 MHz
CDCl3
[rel]
[ppm]


AllO
OAc
AllO
OAll
LevO
O
N3
17
1H-1H COSY
600 MHz
CDCl3
F1 [ppm]
F2 [ppm]
2
3
4
5
6


AllO
OAc
AllO
OAll
LevO
O
N3
17
1H-13C HSQC
600/150 MHz
CDCl3
F1 [ppm]
40
60
80
100
120
6
5
4
3
F2 [ppm]




AcO
OAc
AcO
OAc
LevO
O
N3
18
1H NMR
600 MHz
CDCl3
[rel]
[ppm]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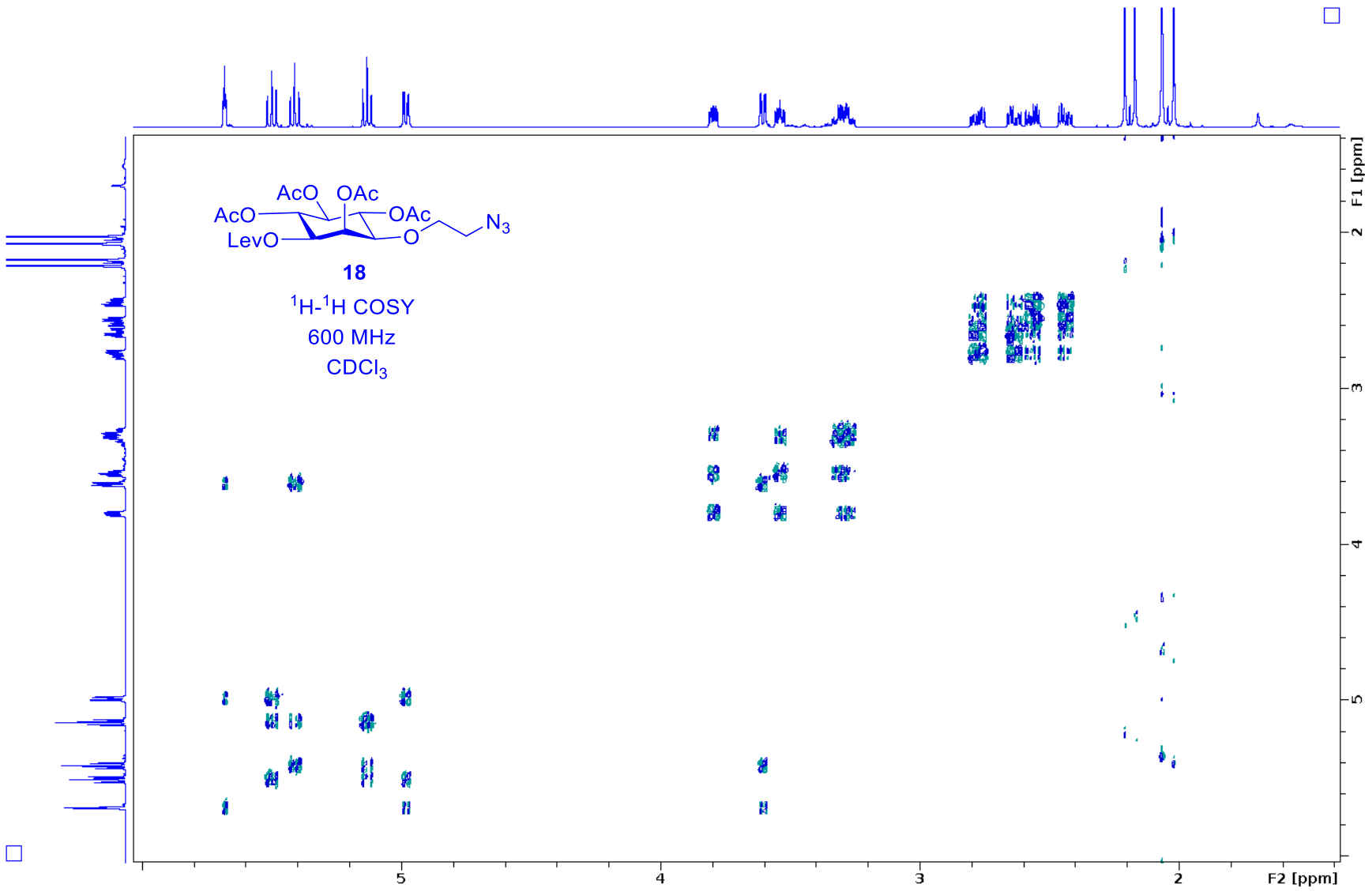
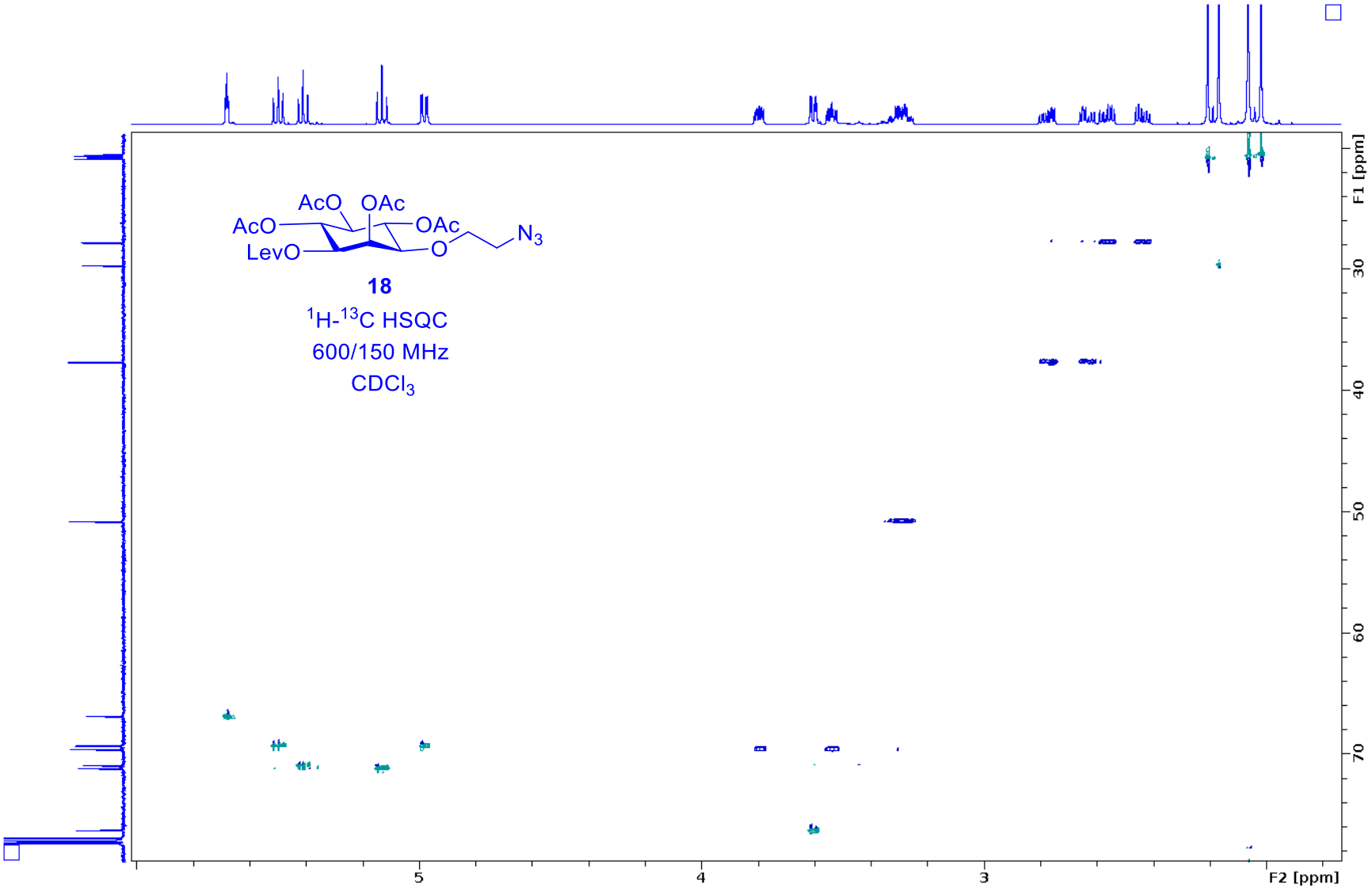
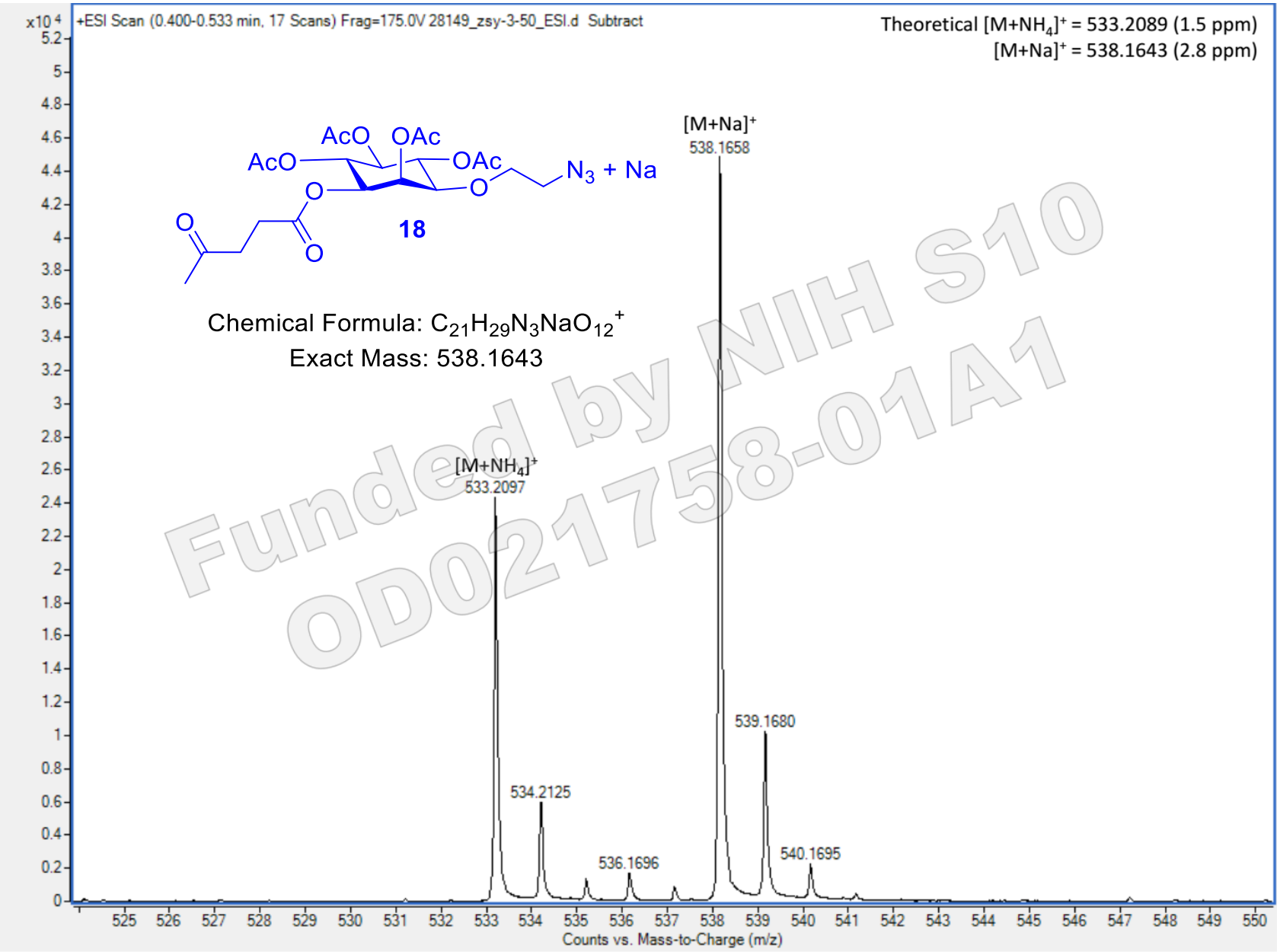
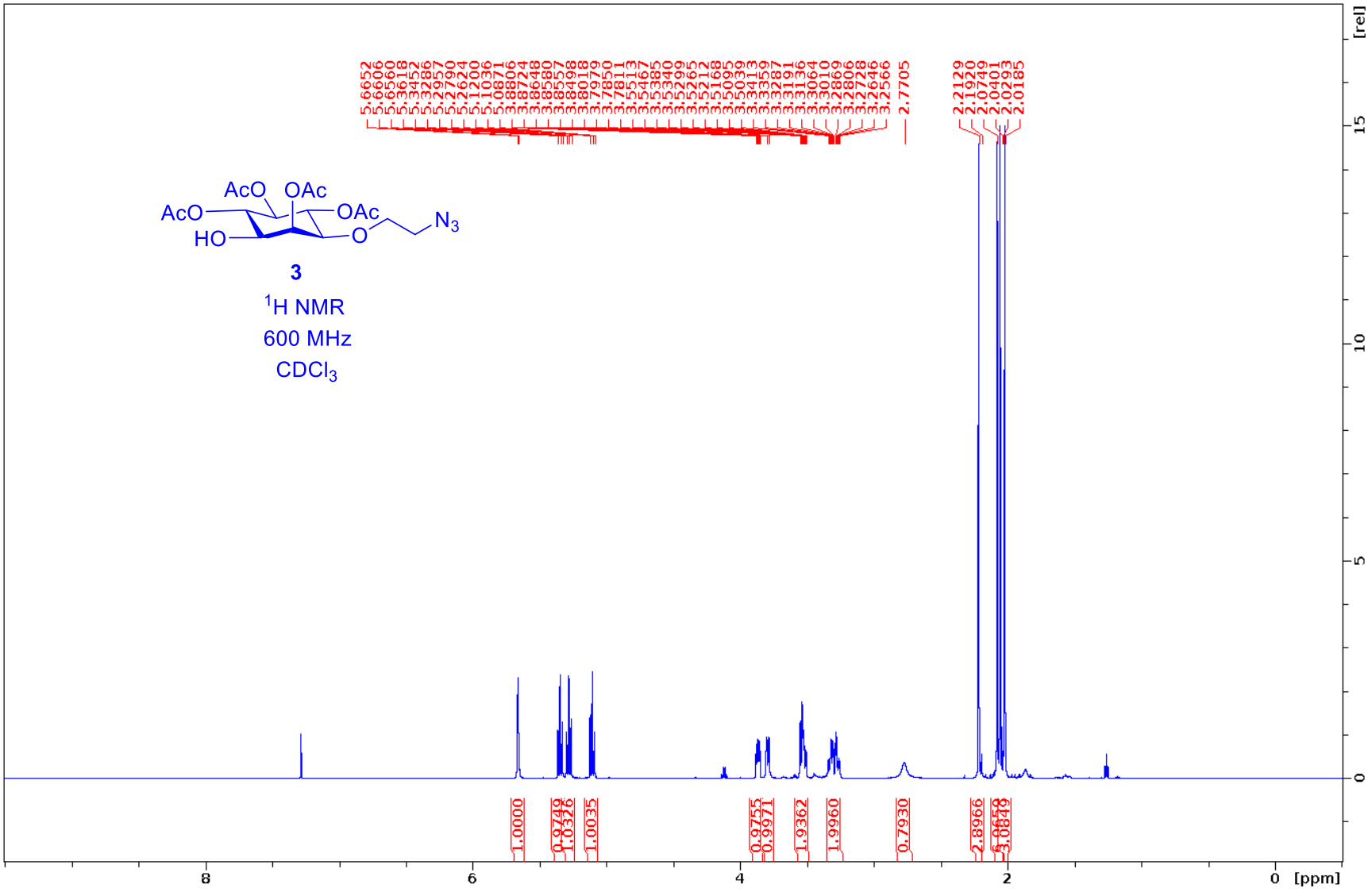
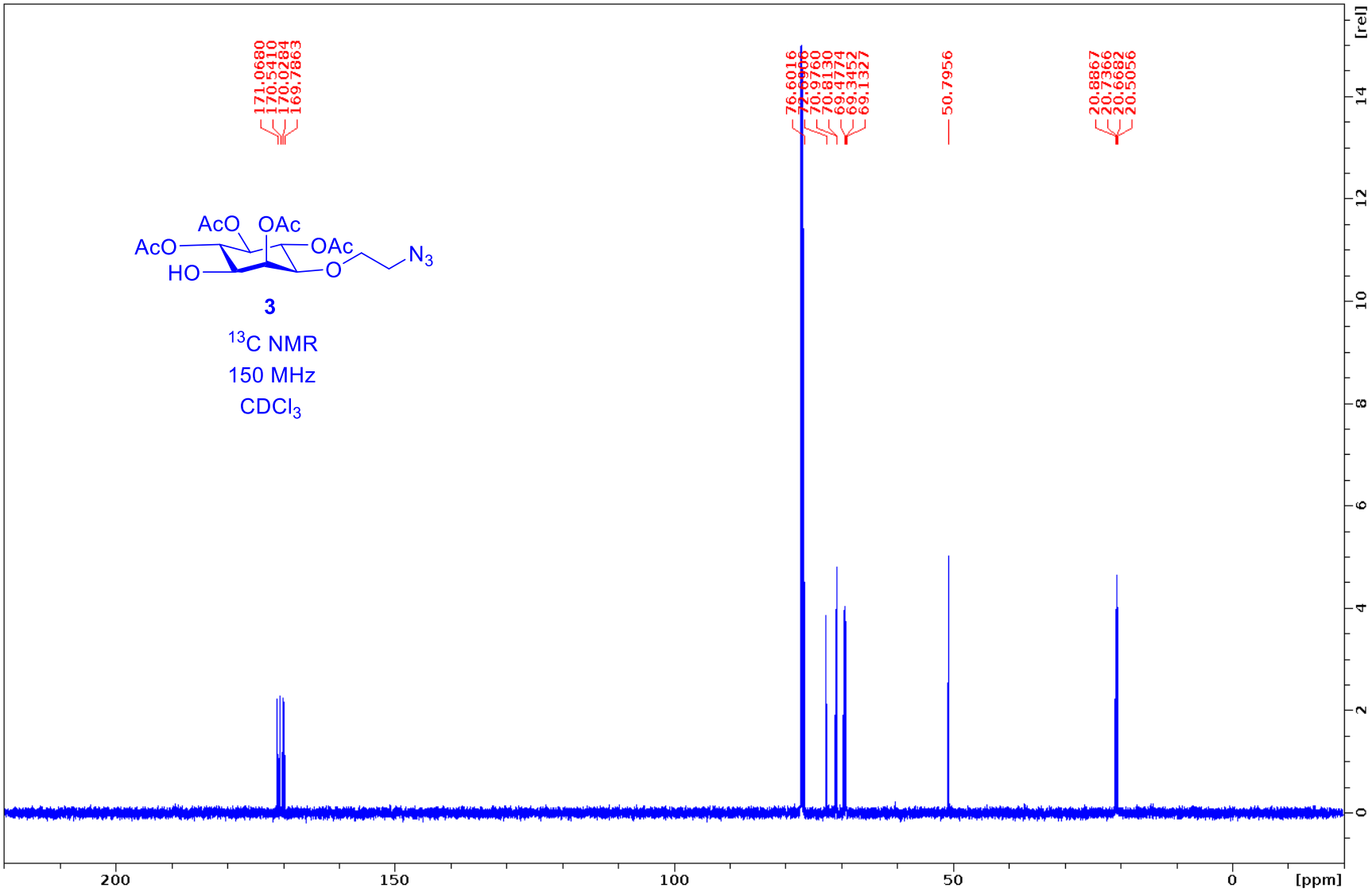
171.5727
170.0823
169.8665
169.8218
169.6836
77.2385
77.0267
76.8146
76.1905
71.0759
70.8293
69.4944
69.2372
69.1857
66.7654
50.7081
37.5632
29.5842
27.7044
20.7704
20.6727
20.5577
20.4682
AcO
OAc
AcO
OAc
LevO
O
N3
18
13C NMR
150 MHz
CDCl3
[rel]
15
10
5
0
200
150
100
50
0
[ppm]

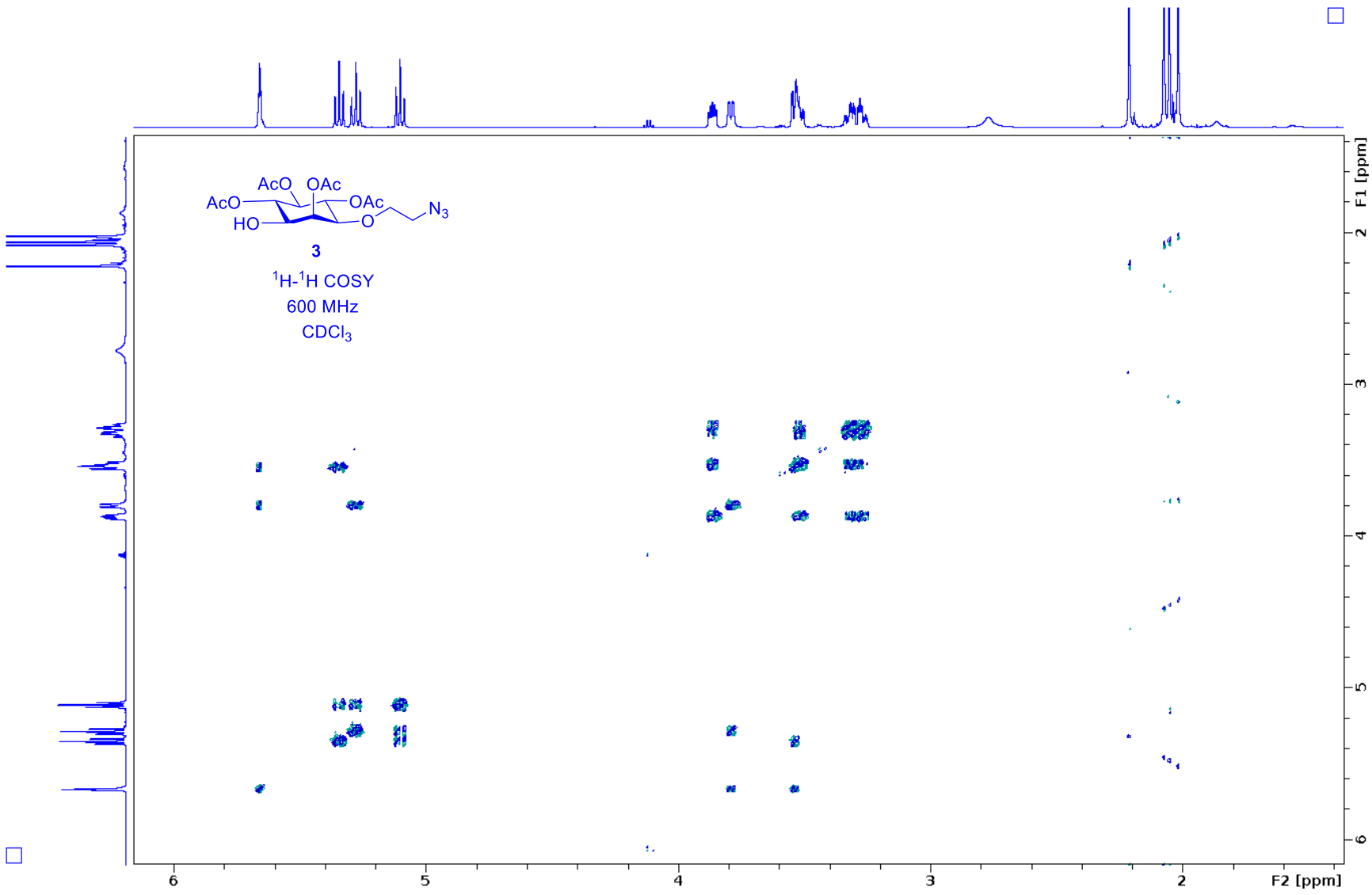

AcO
OAc
AcO
OAc
LevO
O
N3
18
1H-1H COSY
600 MHz
CDCl3
F1 [ppm]
F2 [ppm]
2
3
4
5

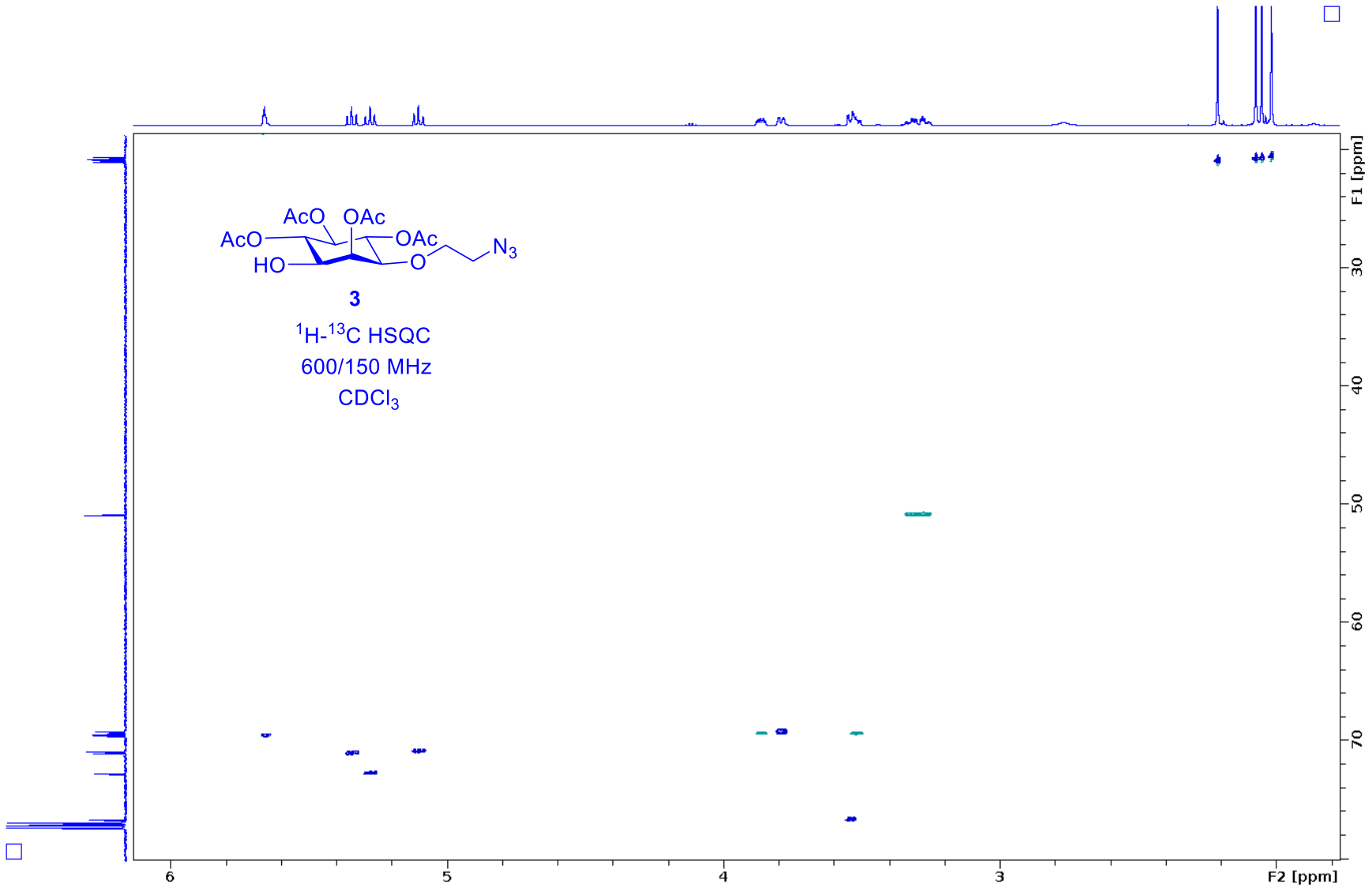

AcO
OAc
AcO
OAc
LevO
O
N3
18
1H-13C HSQC
600/150 MHz
CDCl3
F1 [ppm]
30
40
50
60
70
5
4
3
F2 [ppm]

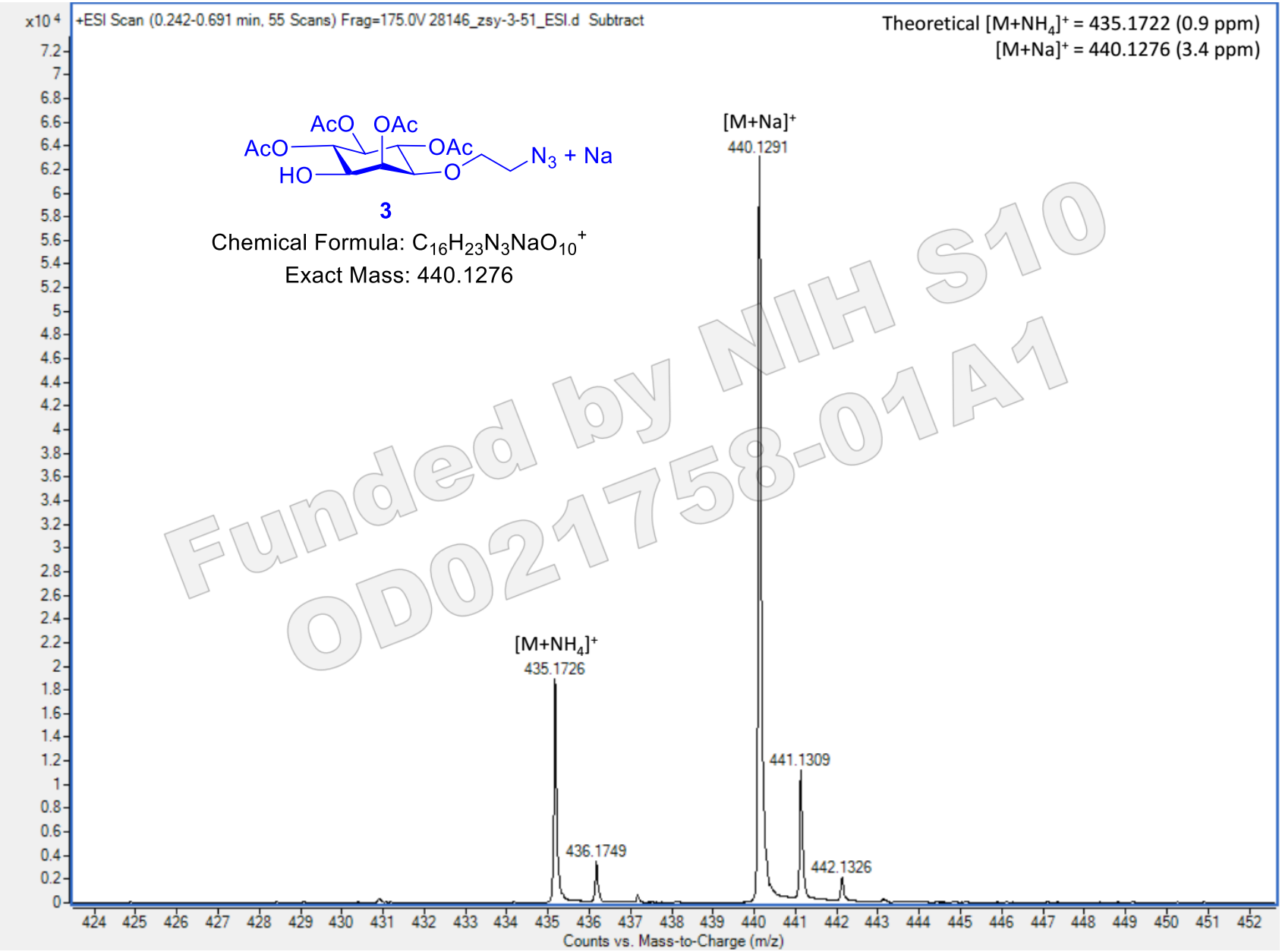


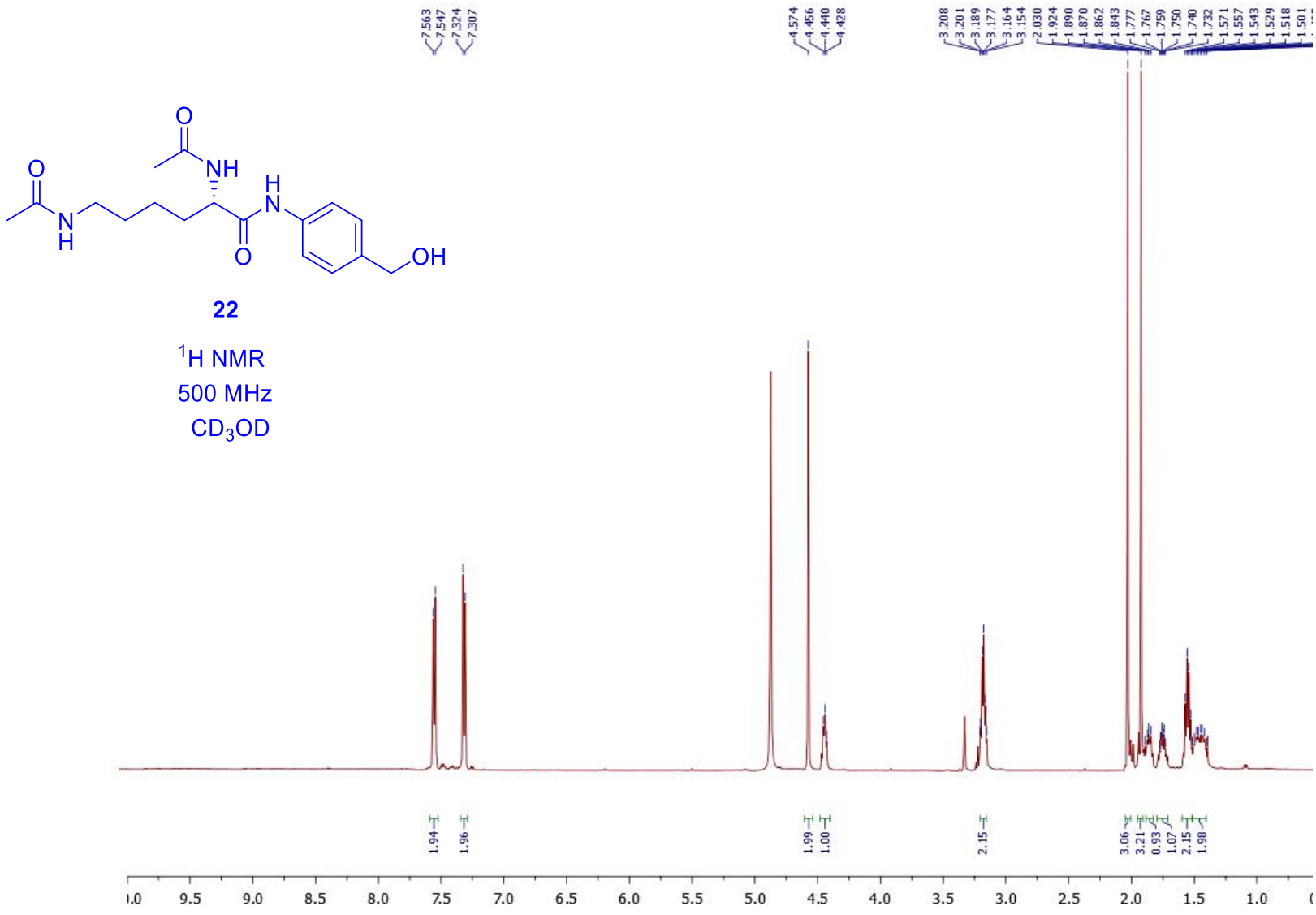

AcO
OAc
AcO
OAc
HO
O
N3
3
1H NMR
600 MHz
CDCl3
5.6652
5.6606
5.6560
5.3618
5.3452
5.3286
5.2957
5.2790
5.2624
5.1200
5.1036
5.0871
3.8806
3.8724
3.8648
3.8580
3.8557
3.8498
3.8018
3.7979
3.7850
3.7811
3.5513
3.5467
3.5385
3.5340
3.5299
3.5265
3.5212
3.5168
3.5095
3.5039
3.3413
3.3359
3.3287
3.3191
3.3136
3.3064
3.3010
3.2869
3.2806
3.2728
3.2646
3.2566
2.7705
2.2129
2.1920
2.0749
2.0401
2.0293
2.0185
1.0000
0.9749
1.0326
1.0035
0.9755
0.9971
1.9362
1.9960
0.7930
2.8966
1.0659
3.0849
8
6
4
2
0
[ppm]
[rel]
15
10
5
0

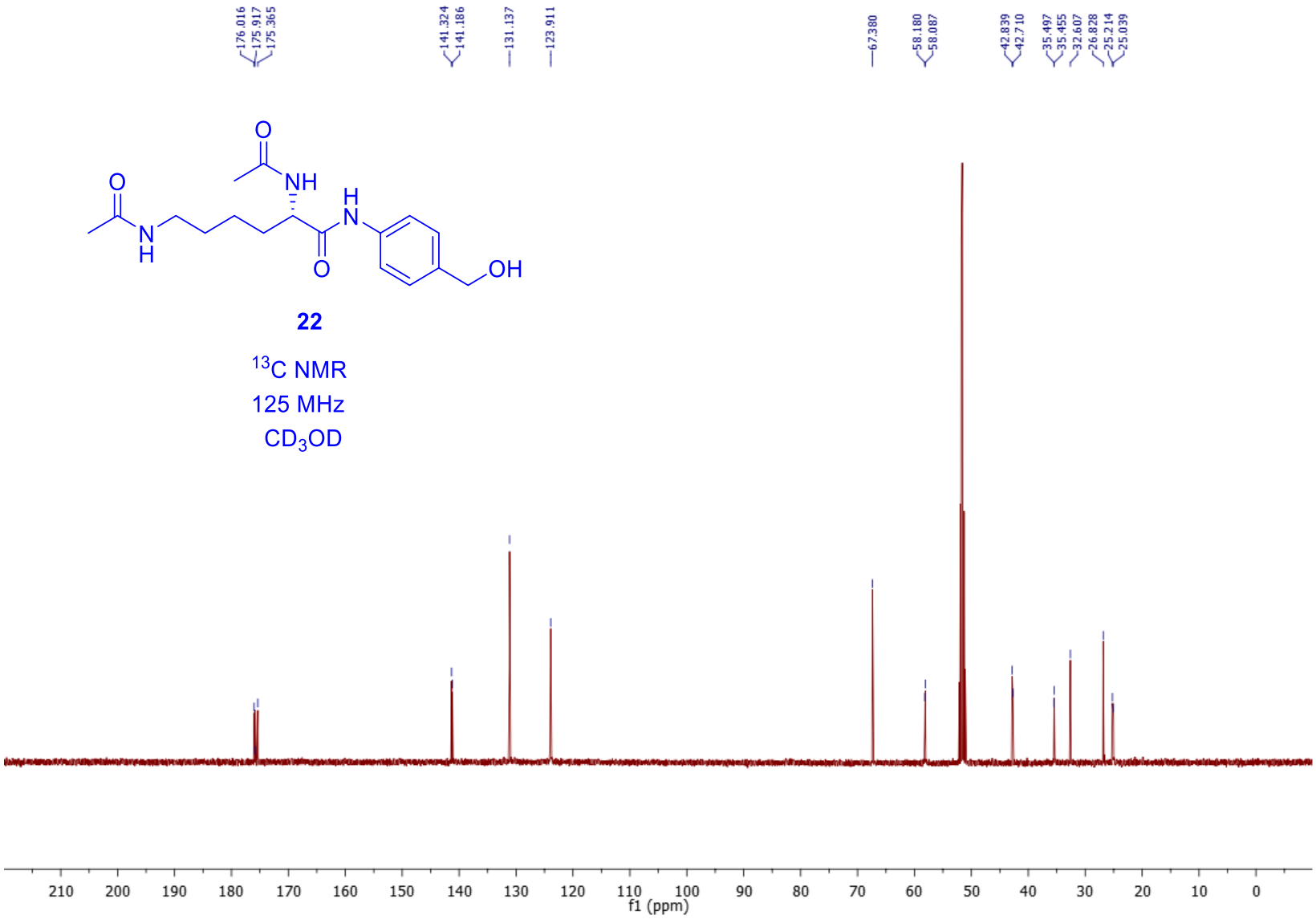

171.0680
170.5410
170.0284
169.7863
76.6016
77.0906
76.9760
70.8130
69.4774
69.3452
69.1327
50.7956
20.8867
20.7366
20.6682
20.5056
AcO
OAc
AcO
OAc
HO
O
N3
3
13C NMR
150 MHz
CDCl3
[rel]
[ppm]
200
150
100
50
0

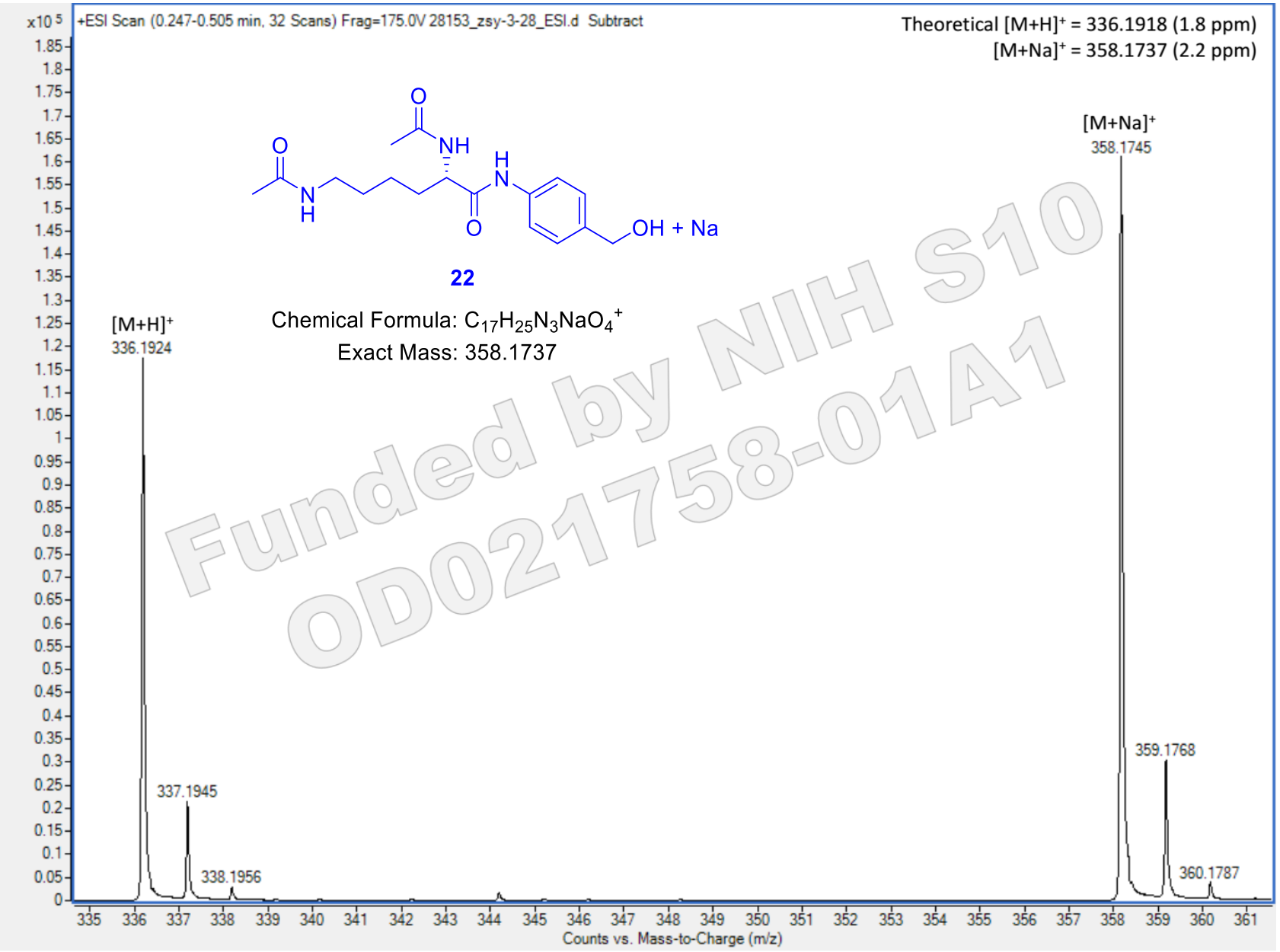

AcO
OAc
AcO
OAc
HO
O
N3
3
1H-1H COSY
600 MHz
CDCl3
F1 [ppm]
F2 [ppm]
2
3
4
5
6

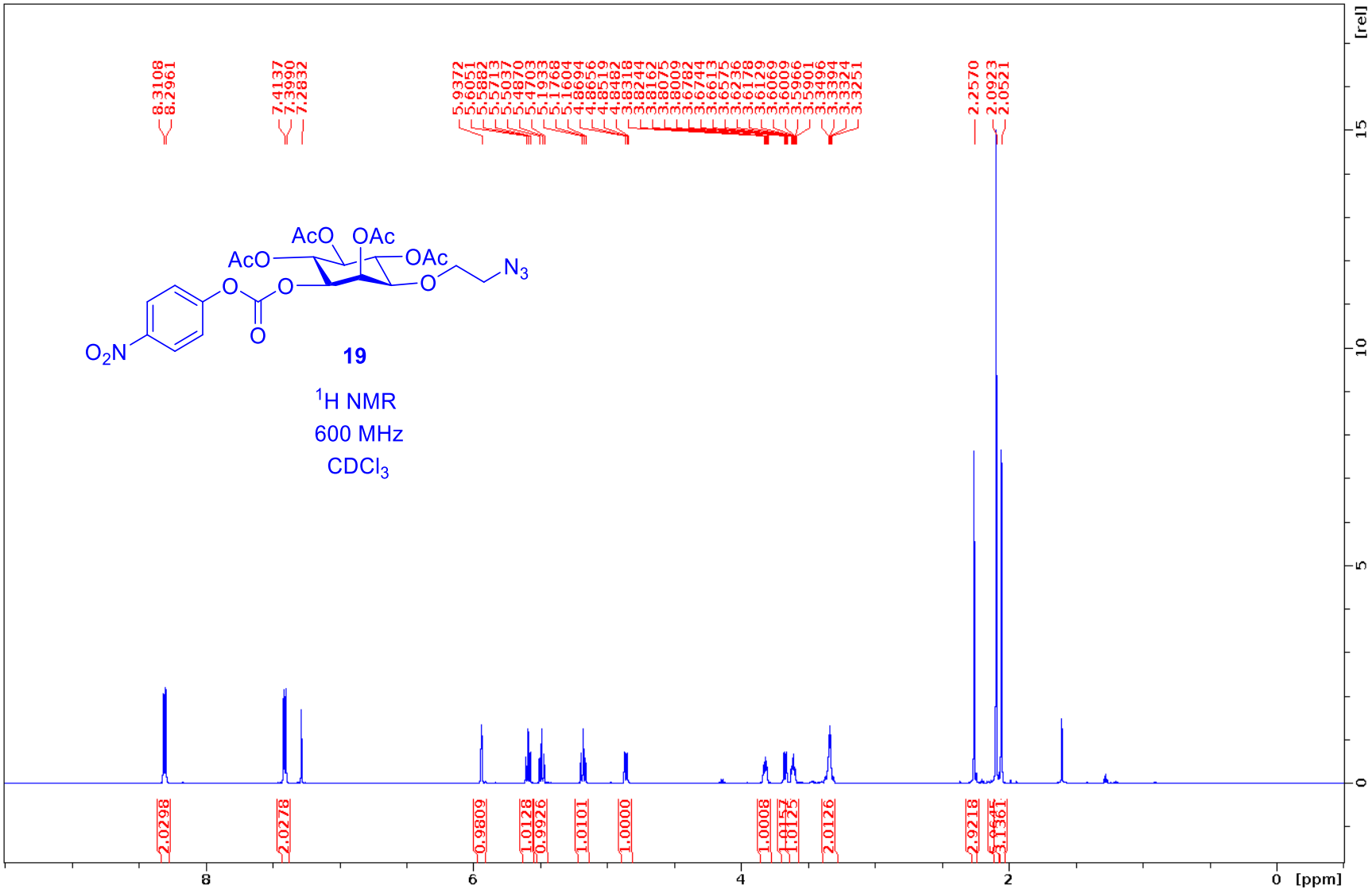

AcO
OAc
AcO
OAc
HO
O
N3
3
1H-13C HSQC
600/150 MHz
CDCl3
F1 [ppm]
30
40
50
60
70
6
5
4
3
F2 [ppm]

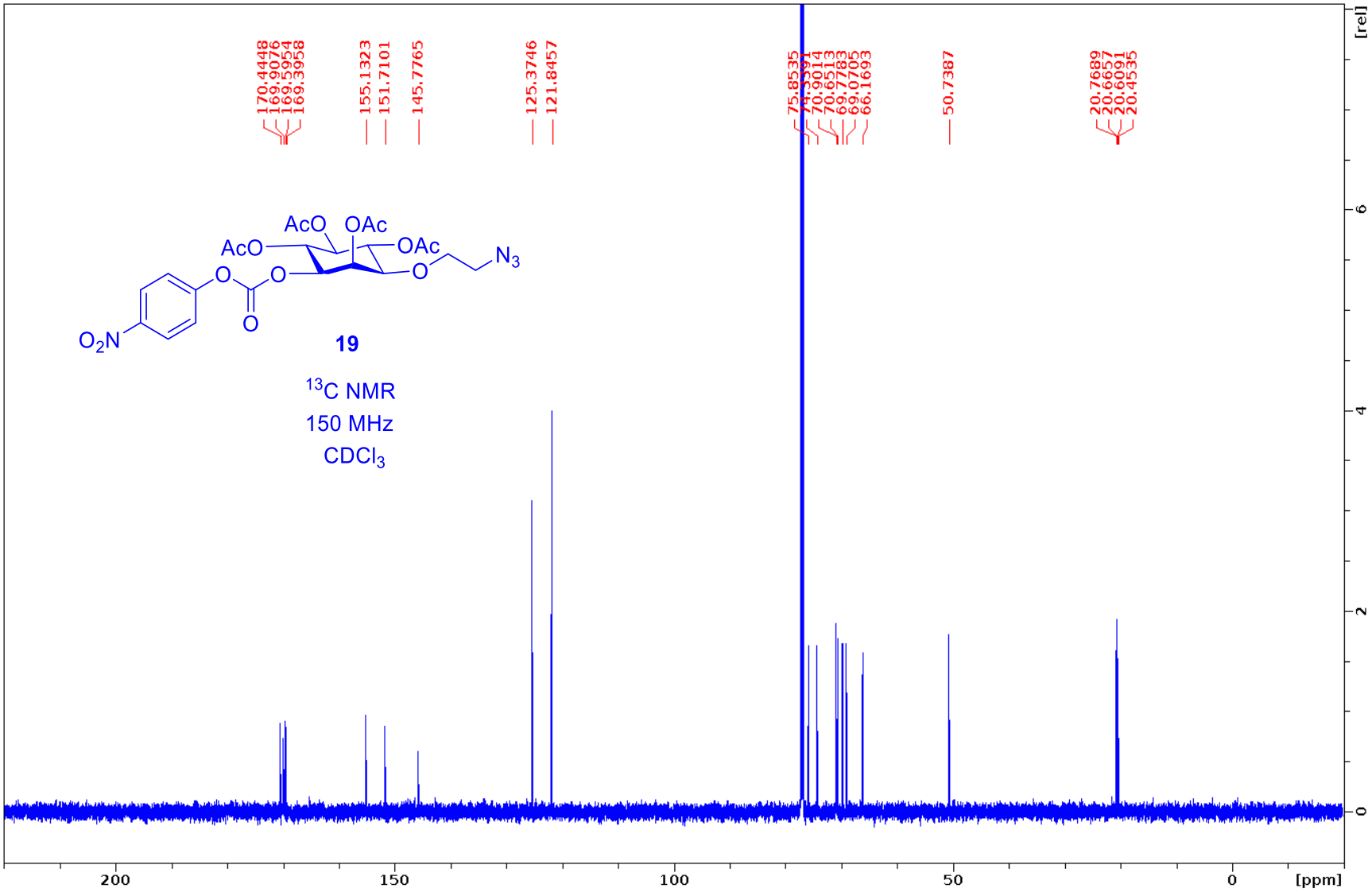


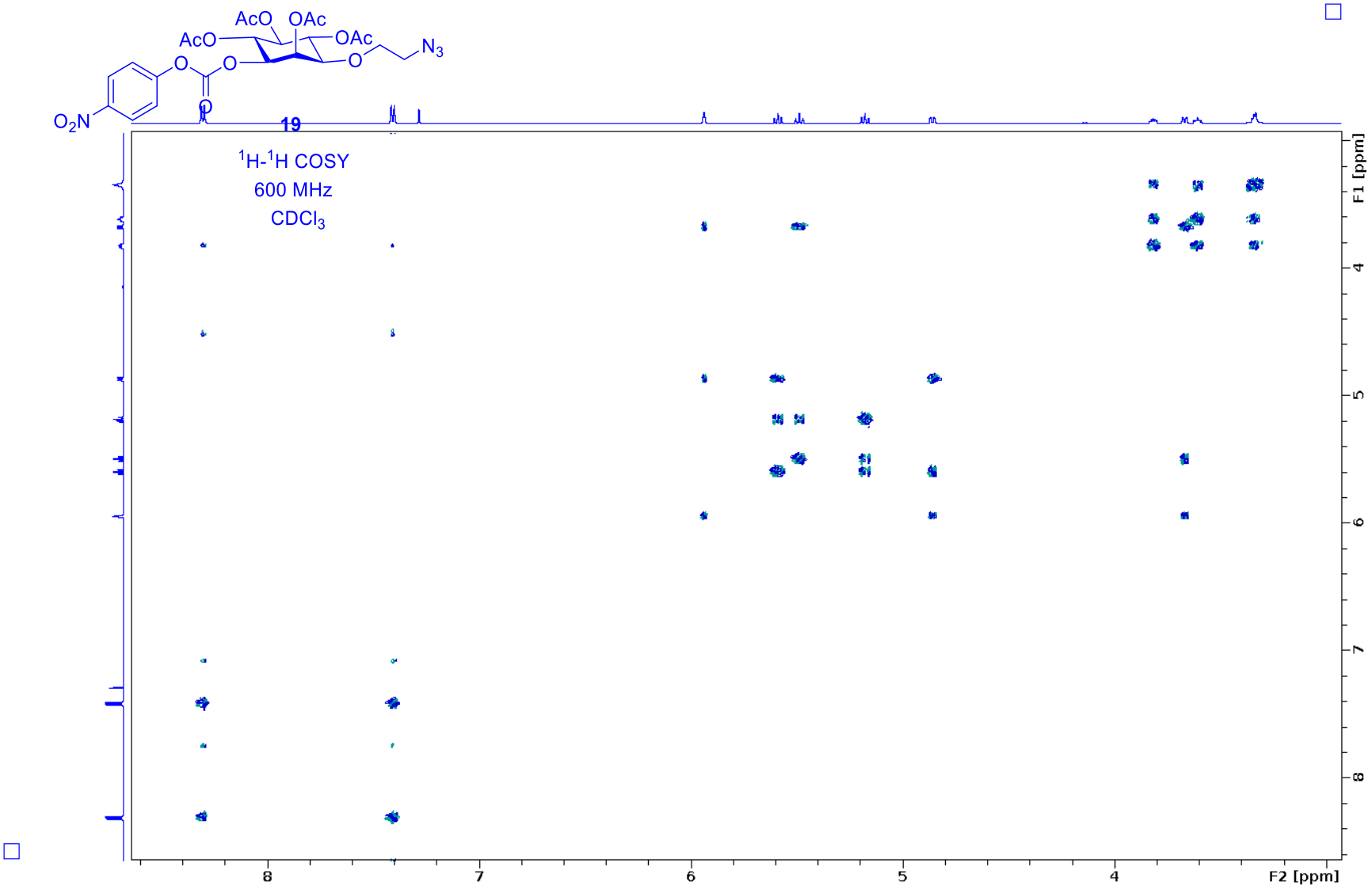

22
1H NMR
500 MHz
CD3OD

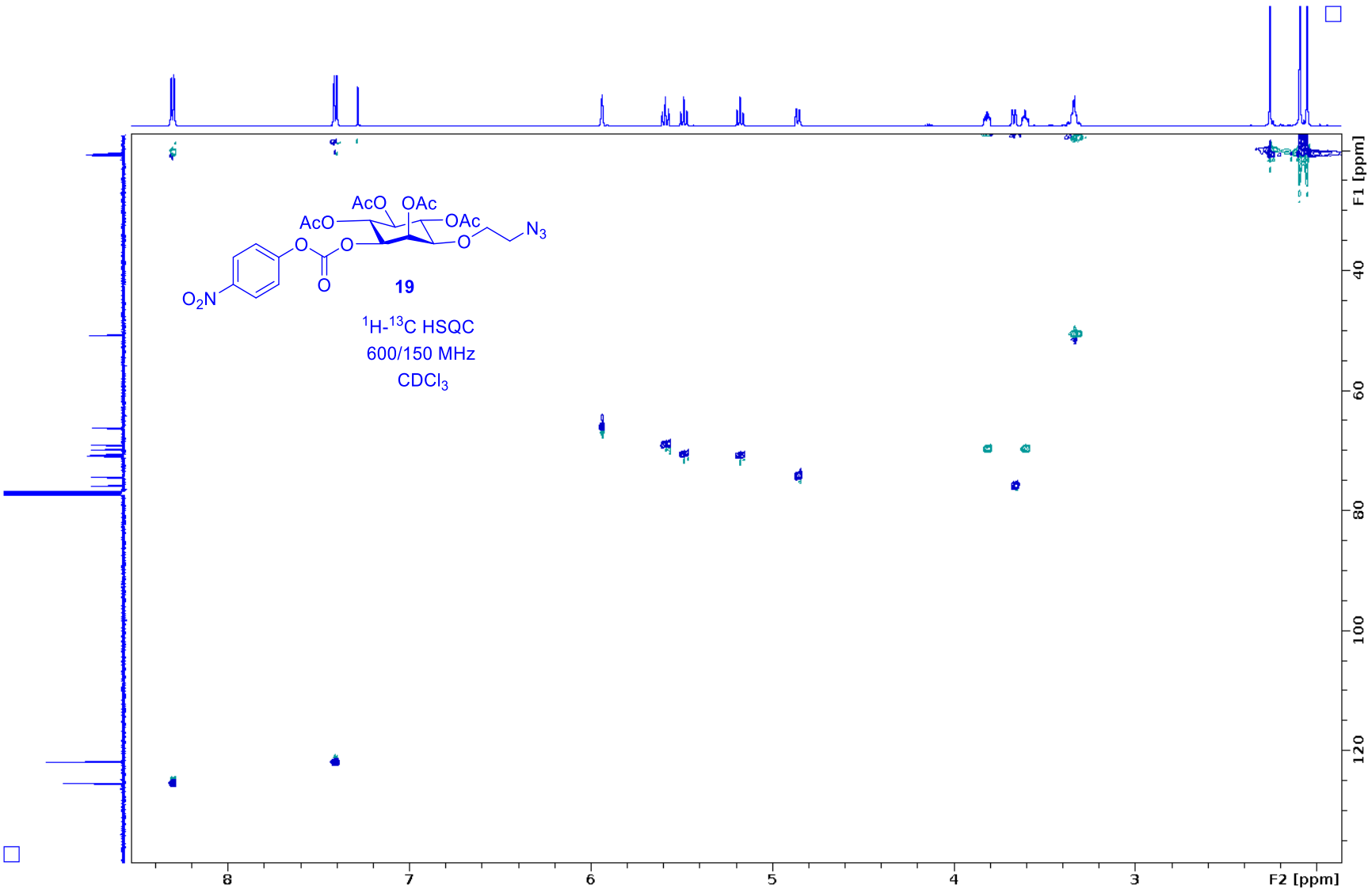

176.016
175.917
175.365
141.324
141.186
131.137
123.911
67.380
58.180
58.087
42.839
42.710
35.497
35.455
32.607
26.828
25.214
25.039
O
NH
H
N
O
N
H
O
OH
22
13C NMR
125 MHz
CD3OD
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

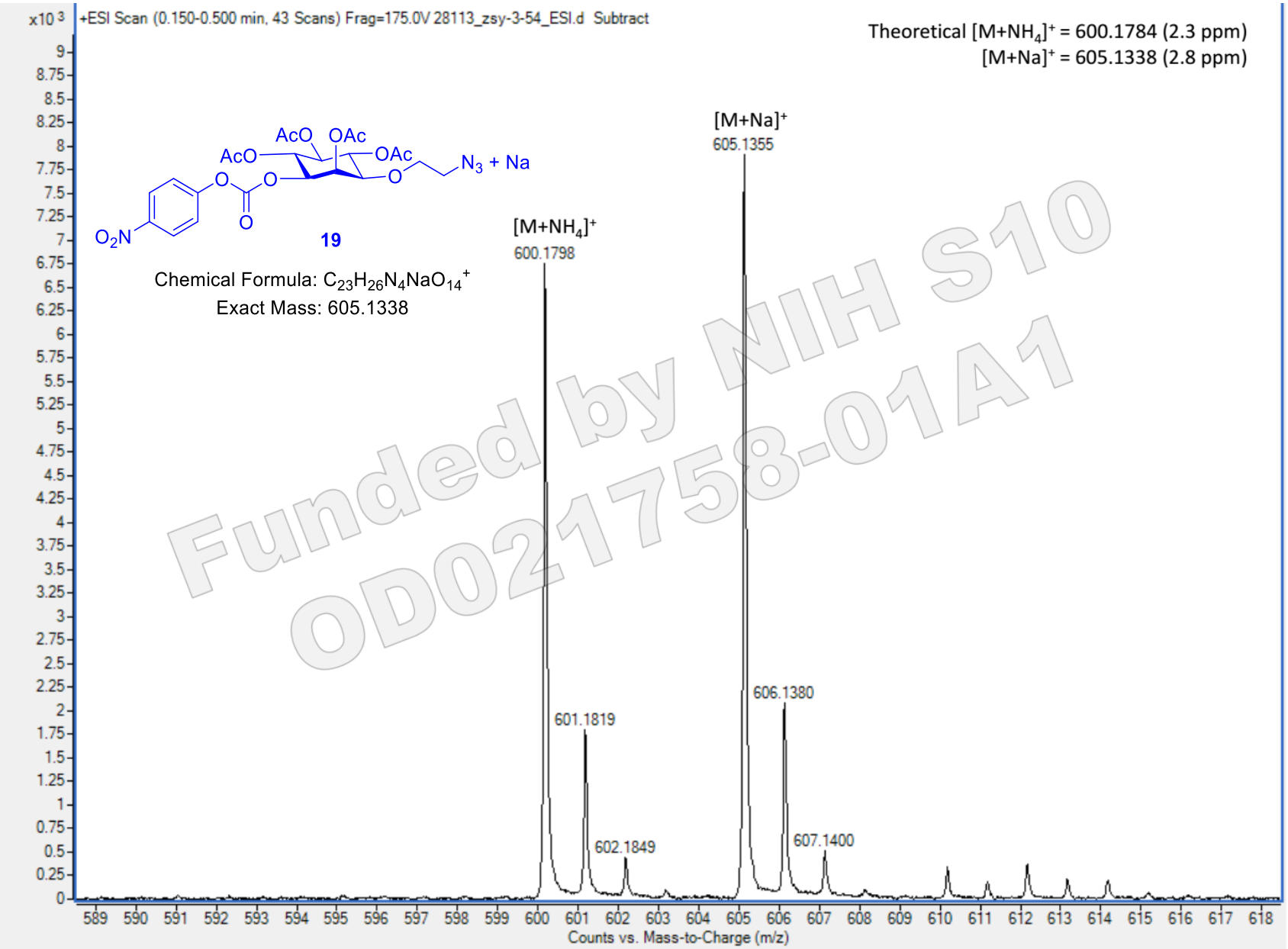


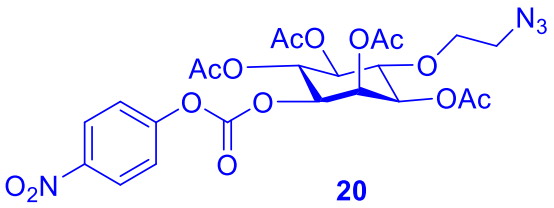

8.3108
8.2961
7.4137
7.3990
7.2832
5.9372
5.6051
5.5882
5.5713
5.5037
5.4870
5.4703
5.1933
5.1768
5.1604
4.8694
4.8656
4.8519
4.8482
3.8318
3.8244
3.8162
3.8075
3.8009
3.6782
3.6744
3.6613
3.6575
3.6236
3.6178
3.6129
3.6069
3.6009
3.5966
3.5901
3.3496
3.3394
3.3324
3.3251
2.2570
2.0923
2.0521
AcO
OAc
AcO
OAc
O
O
O
N3
O2N
O
19
1H NMR
600 MHz
CDCl3
2.0298
2.0278
0.9809
1.0128
0.9926
1.0101
1.0000
1.0008
1.0157
1.0125
2.0126
2.9218
5.0645
3.1361
8
6
4
2
0
[ppm]
[rel]
15
10
5
0


170.4448
169.9076
169.5954
169.3958
155.1323
151.7101
145.7765
125.3746
121.8457
75.8535
74.9391
70.9014
70.6513
69.7783
69.0705
66.1693
50.7387
20.7689
20.6657
20.6091
20.4535
AcO
OAc
AcO
OAc
O
O
O
O
N3
O2N
19
13C NMR
150 MHz
CDCl3
[rel]
0
2
4
6
200
150
100
50
0
[ppm]


AcO
OAc
AcO
OAc
O
O
O
N3
O2N
19
1H-1H COSY
600 MHz
CDCl3
F1 [ppm]
F2 [ppm]
8
7
6
5
4


AcO
OAc
AcO
OAc
N3
O2N
19
1H-13C HSQC
600/150 MHz
CDCl3
F1 [ppm]
40
60
80
100
120
8
7
6
5
4
3
F2 [ppm]


+ESI Scan (0.150-0.500 min, 43 Scans) Frag=175.0V 28113_zsy-3-54_ESI.d Subtract
x10 3
Theoretical [M+NH4]+ = 600.1784 (2.3 ppm)
[M+Na]+ = 605.1338 (2.8 ppm)
AcO
OAc
AcO
OAc
N3 + Na
O2N
19
Chemical Formula: C23H26N4NaO14+
Exact Mass: 605.1338
[M+Na]+
605.1355
[M+NH4]+
600.1798
601.1819
602.1849
606.1380
607.1400
Funded by NIH S10
OD021758-01A1
Counts vs. Mass-to-Charge (m/z)



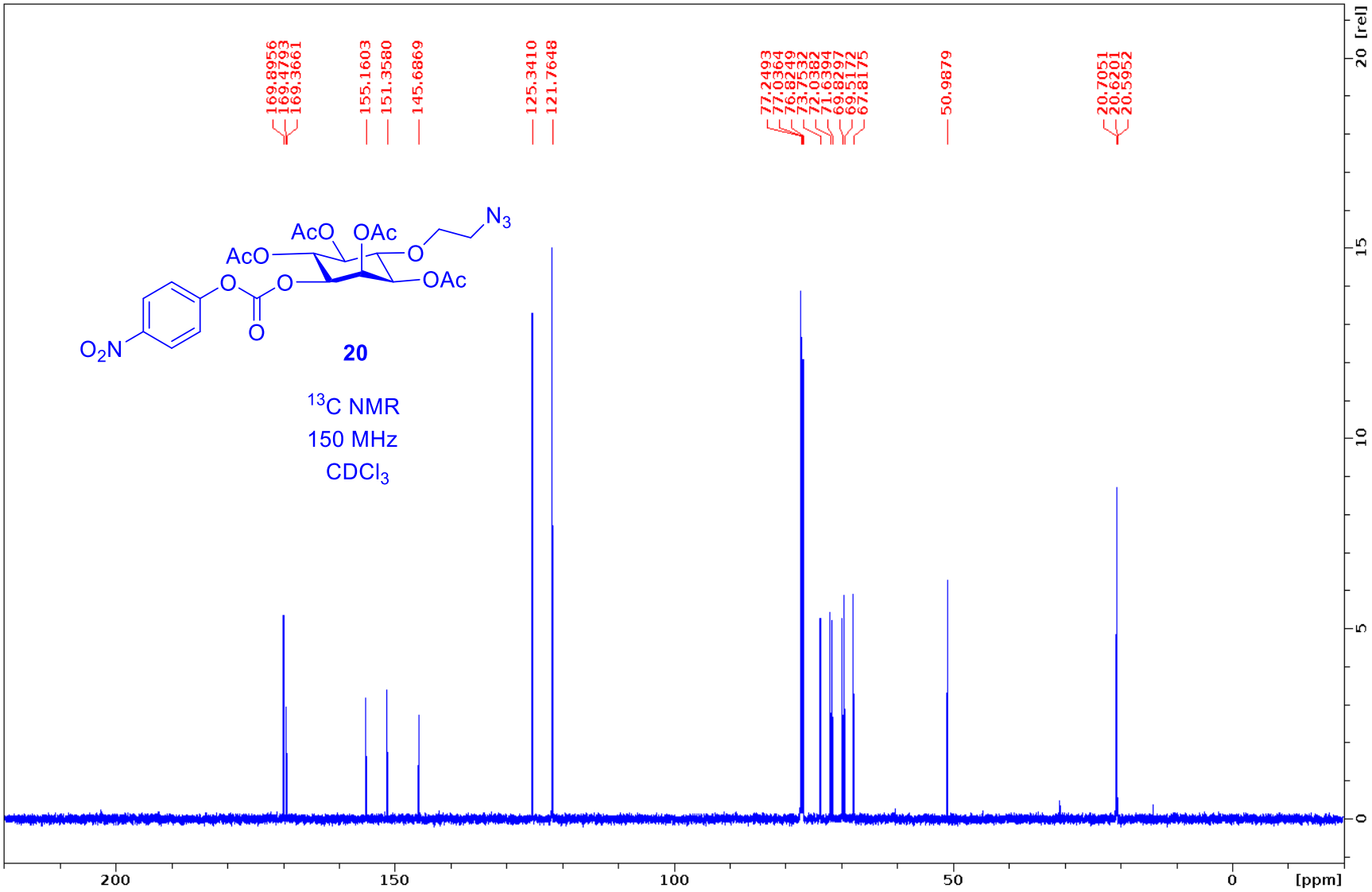
20

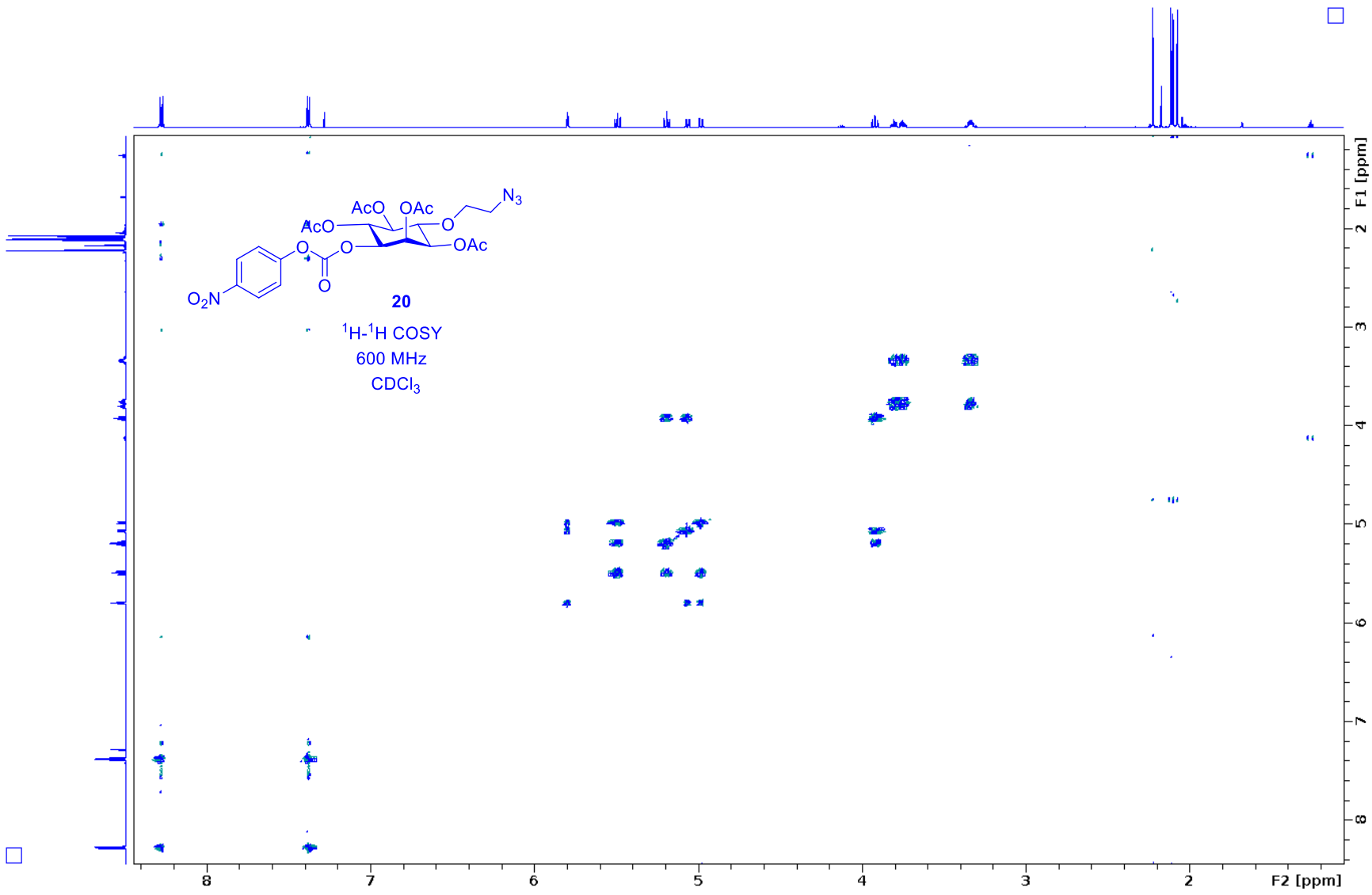
^{1}H NMR

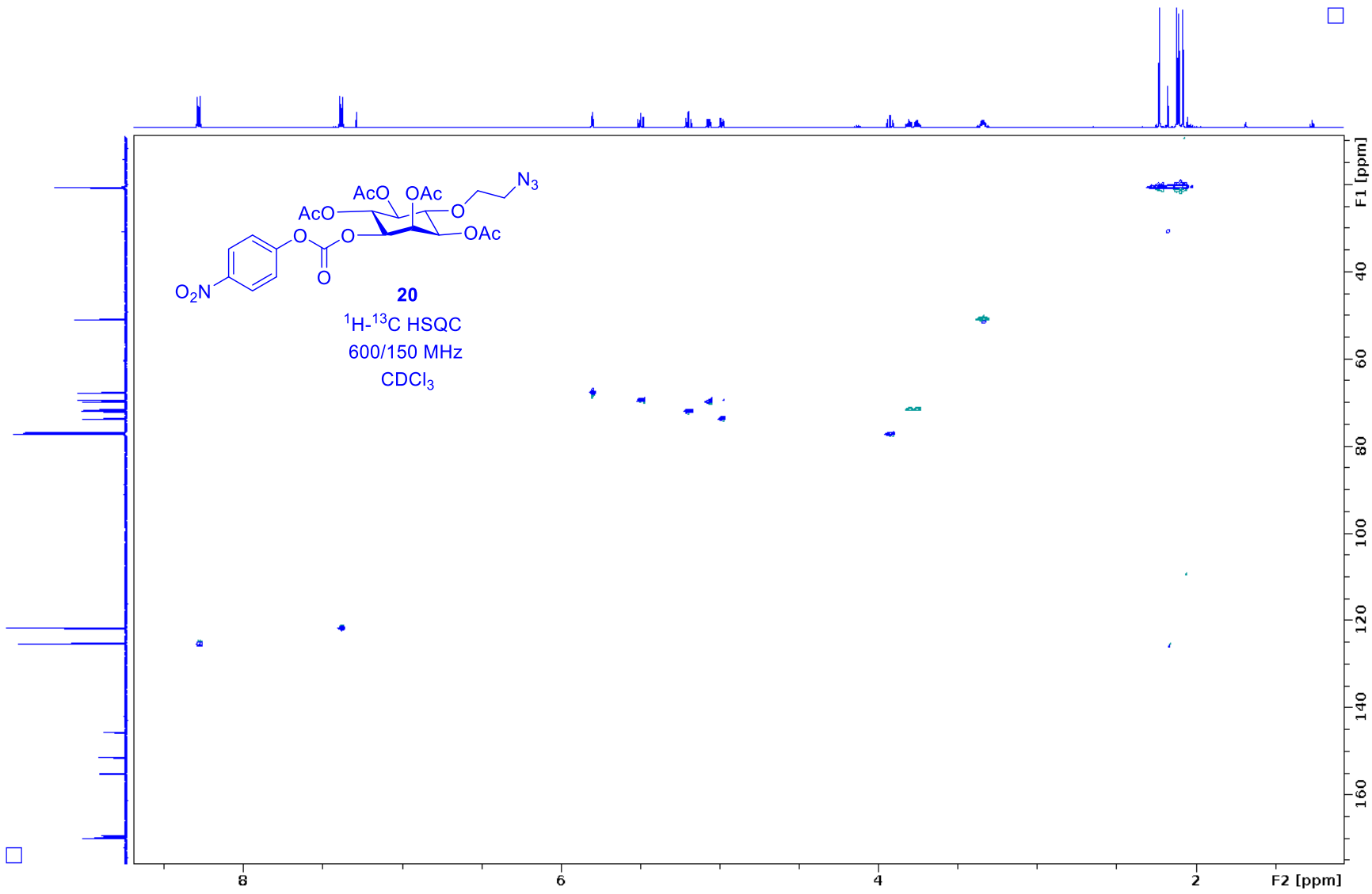
600 MHz

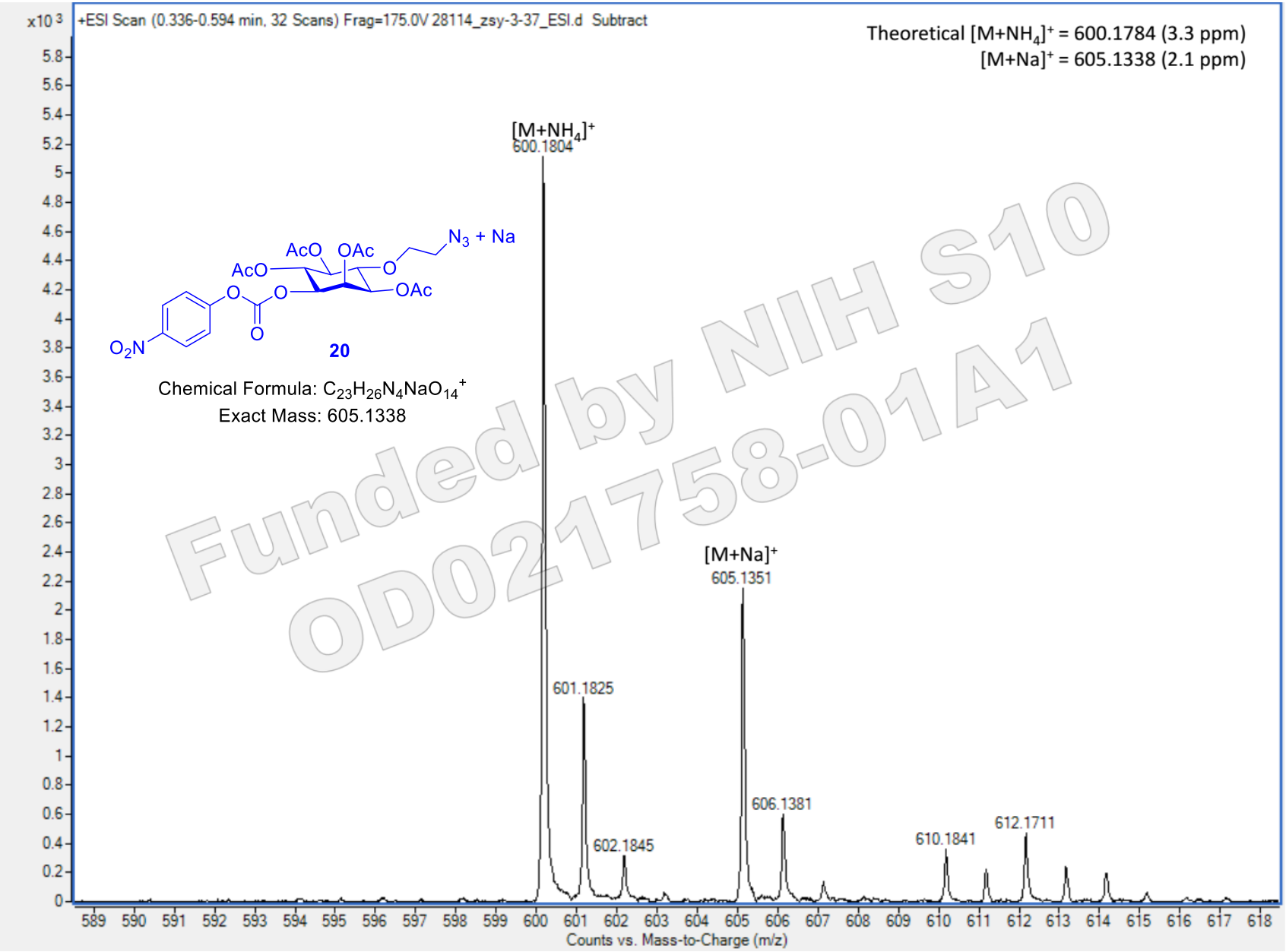
$CDCl_3$

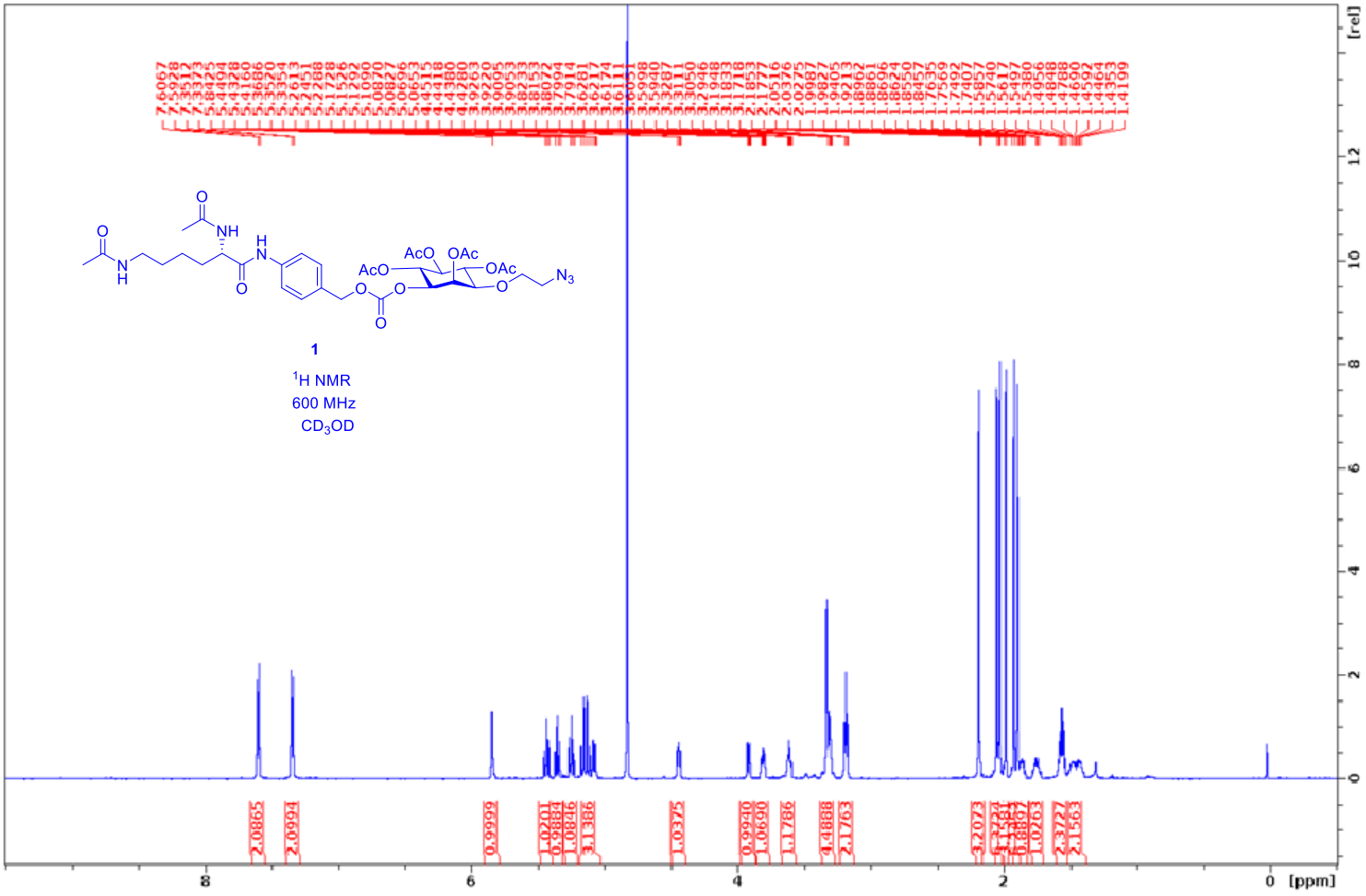

```
Current Data Parameters
NAME            zsy-3-37
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20180413
Time              16.09 h
INSTRUM            spect
PROBHD   Z847801_0073 (
PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                     8
DS                     0
SWH             6009.615 Hz
FIDRES          0.183399 Hz
AQ              5.4525952 sec
RG                   114
DW                83.200 usec
DE                 6.50 usec
TE                302.0 K
D1           1.00000000 sec
TD0                    1
SFO1         600.1627007 MHz
NUC1                  1H
P1                14.00 usec
PLW1         15.77099991 W

F2 - Processing parameters
SI                 65536
SF           600.1600000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

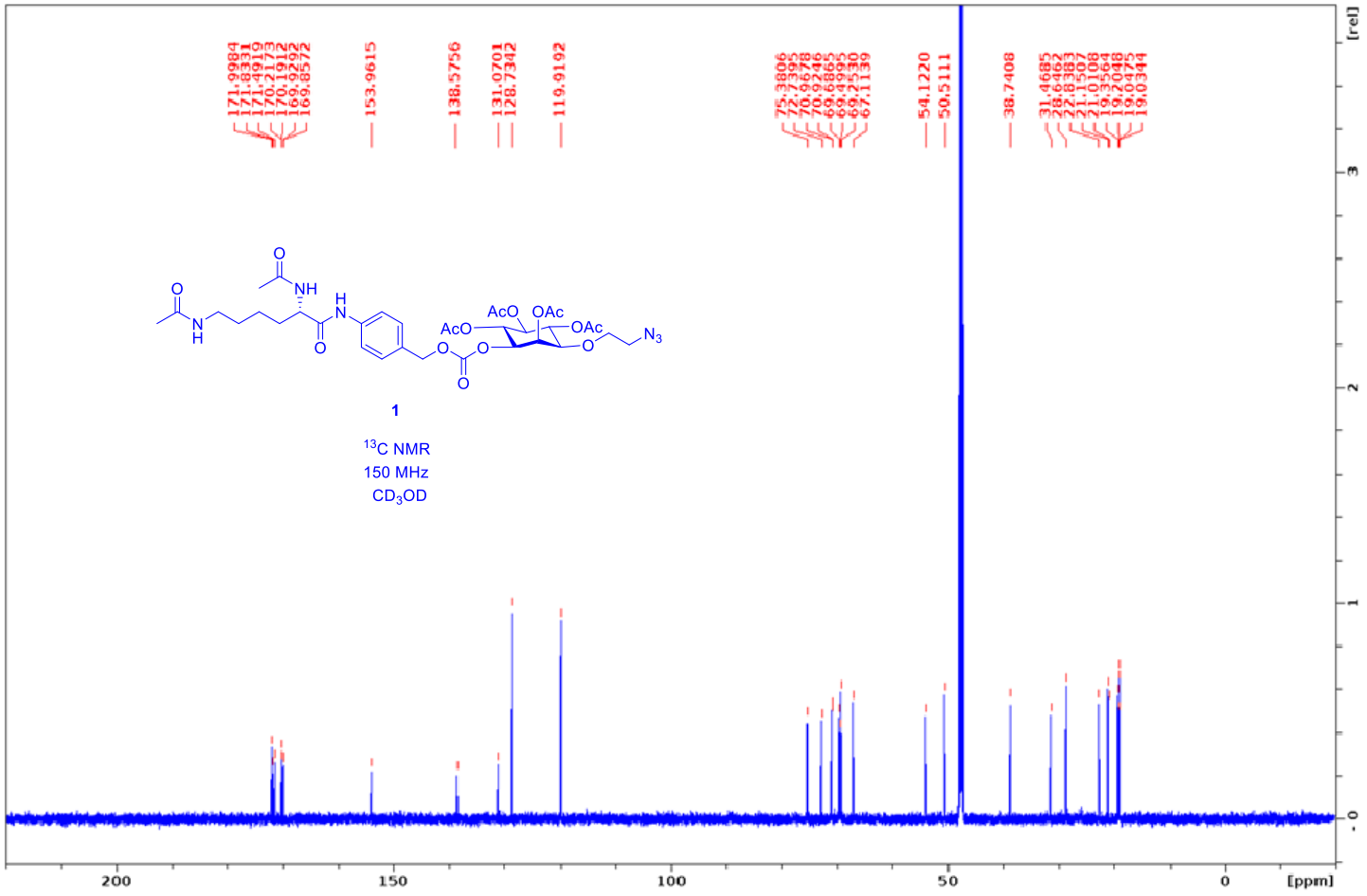

169.8956
169.4793
169.3661
155.1603
151.3580
145.6869
125.3410
121.7648
77.2493
77.0364
76.8249
73.7532
72.0382
71.6394
69.8297
69.5172
67.8175
50.9879
20.7051
20.6201
20.5952
AcO
OAc
AcO
OAc
O
O
O
O
N_3
O_2N
20
^{13}C NMR
150 MHz
$CDCl_3$
200
150
100
50
0
[ppm]
20 [rel]
15
10
5
0


AcO
OAc
AcO
O
OAc
O
O
O
O
N3
O2N
20
1H-1H COSY
600 MHz
CDCl3
F1 [ppm]
F2 [ppm]
2
3
4
5
6
7
8


AcO
OAc
AcO
OAc
N3
O2N
20
1H-13C HSQC
600/150 MHz
CDCl3
F1 [ppm]
F2 [ppm]
8
6
4
2
40
60
80
100
120
140
160




1
1H NMR
600 MHz
CD3OD
[rel]
[ppm]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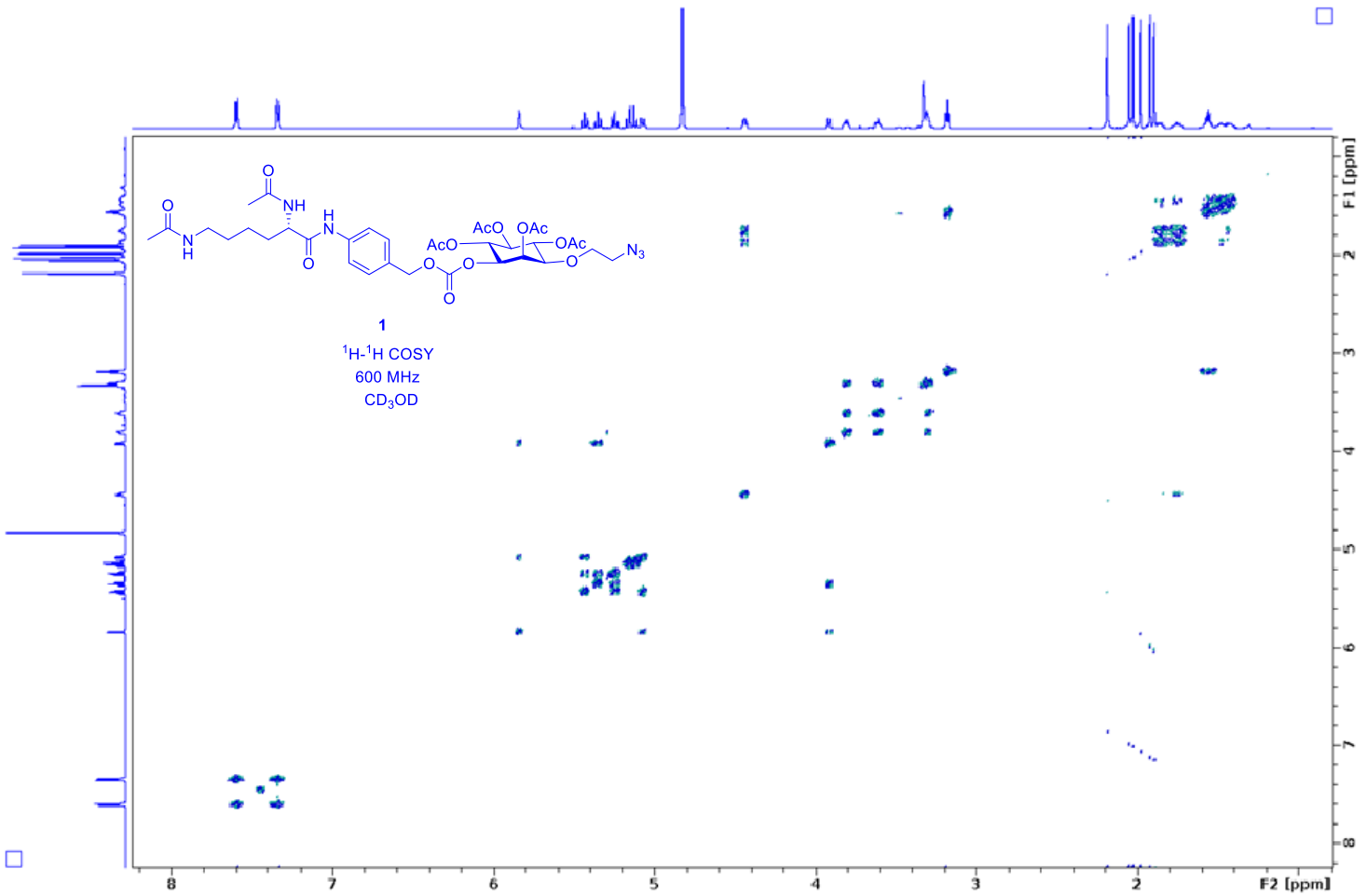
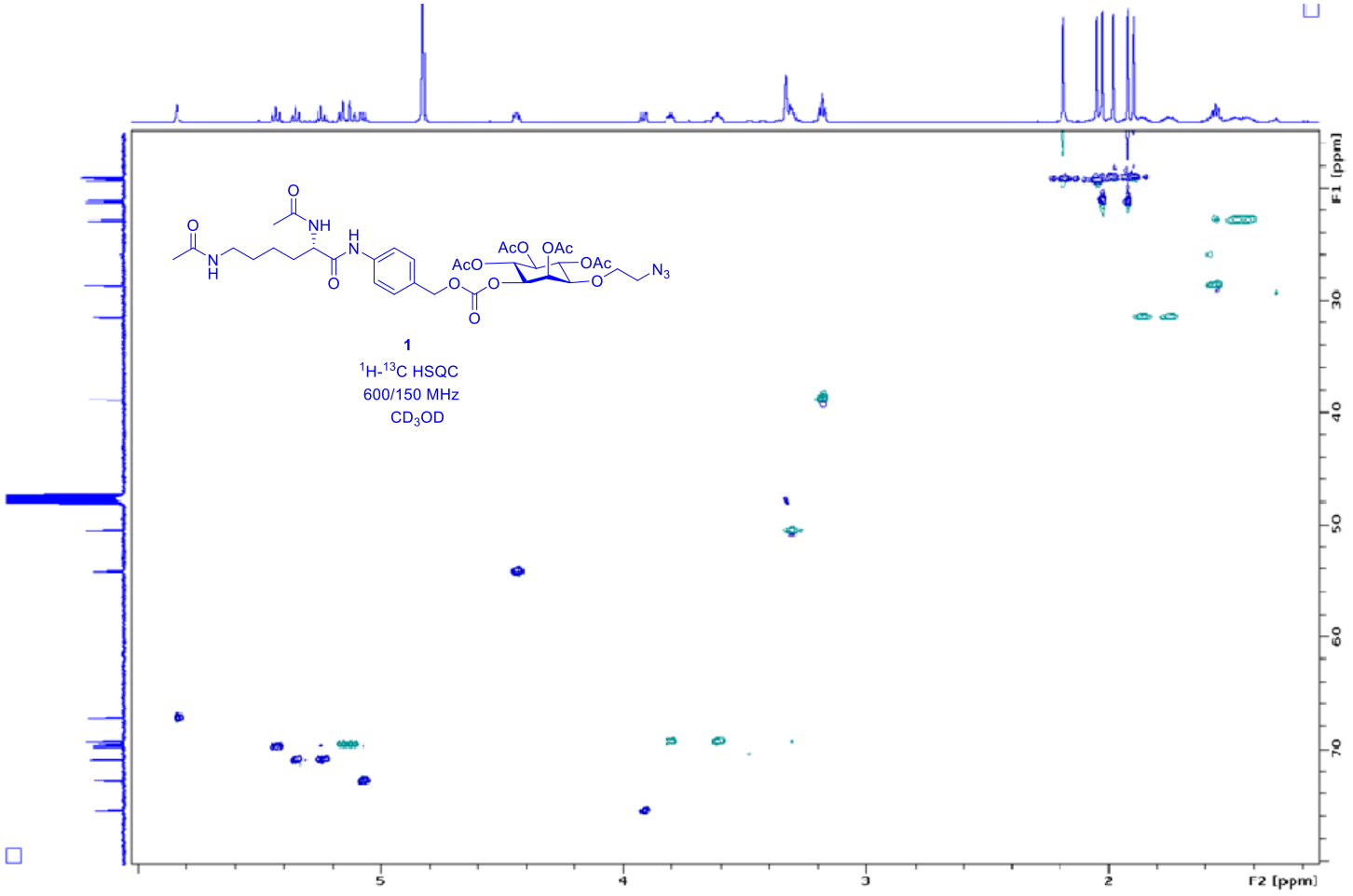
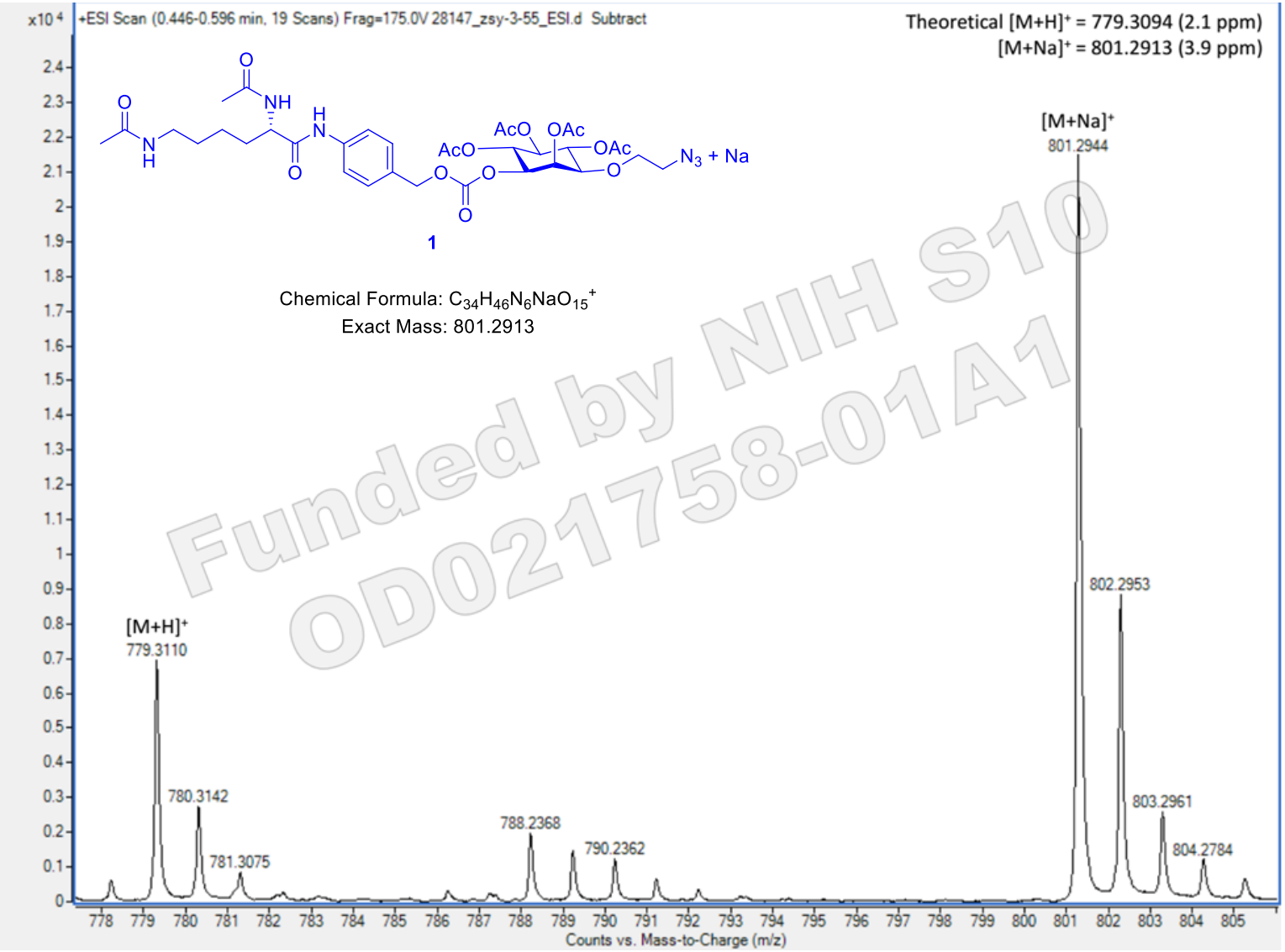
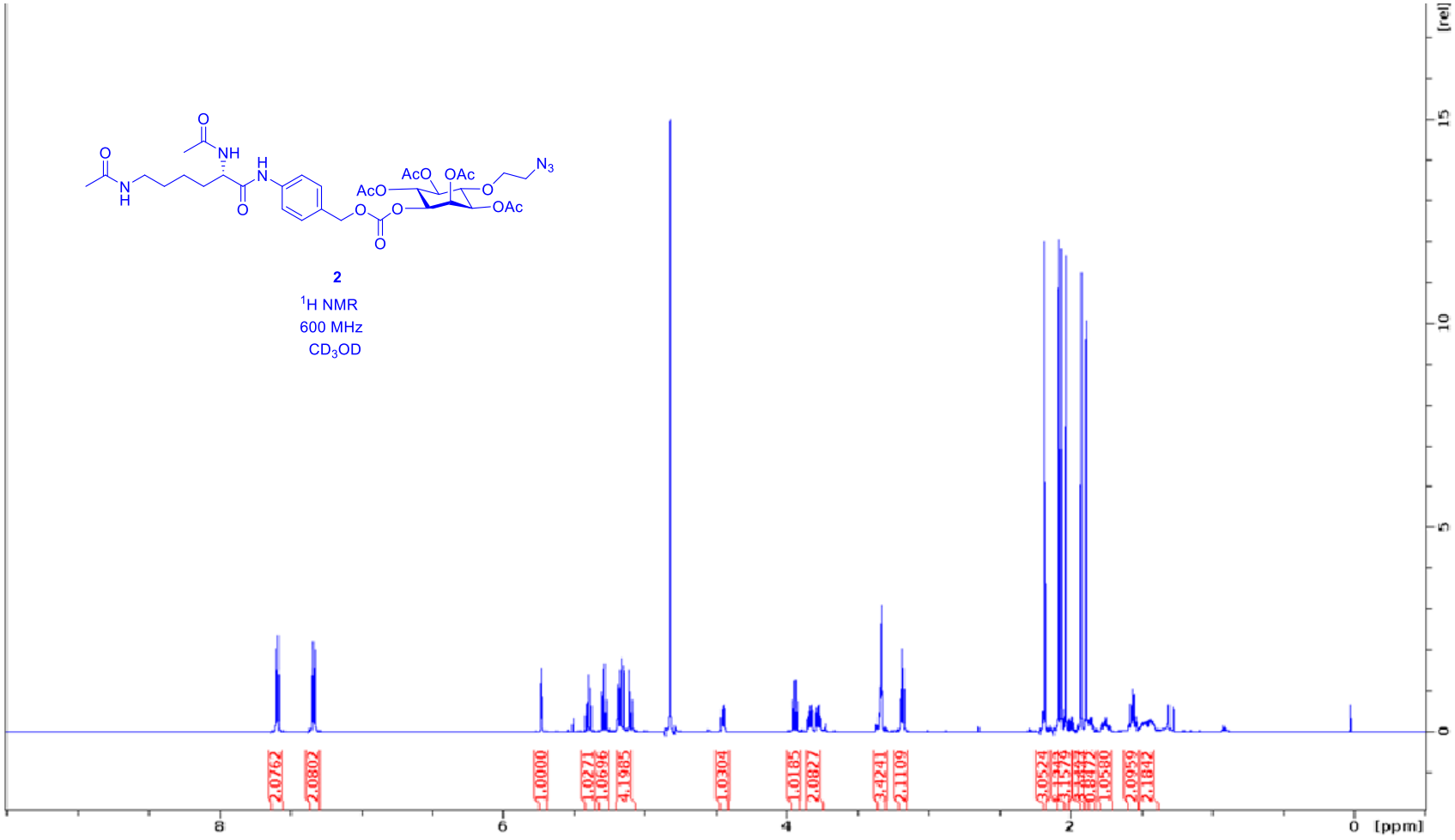
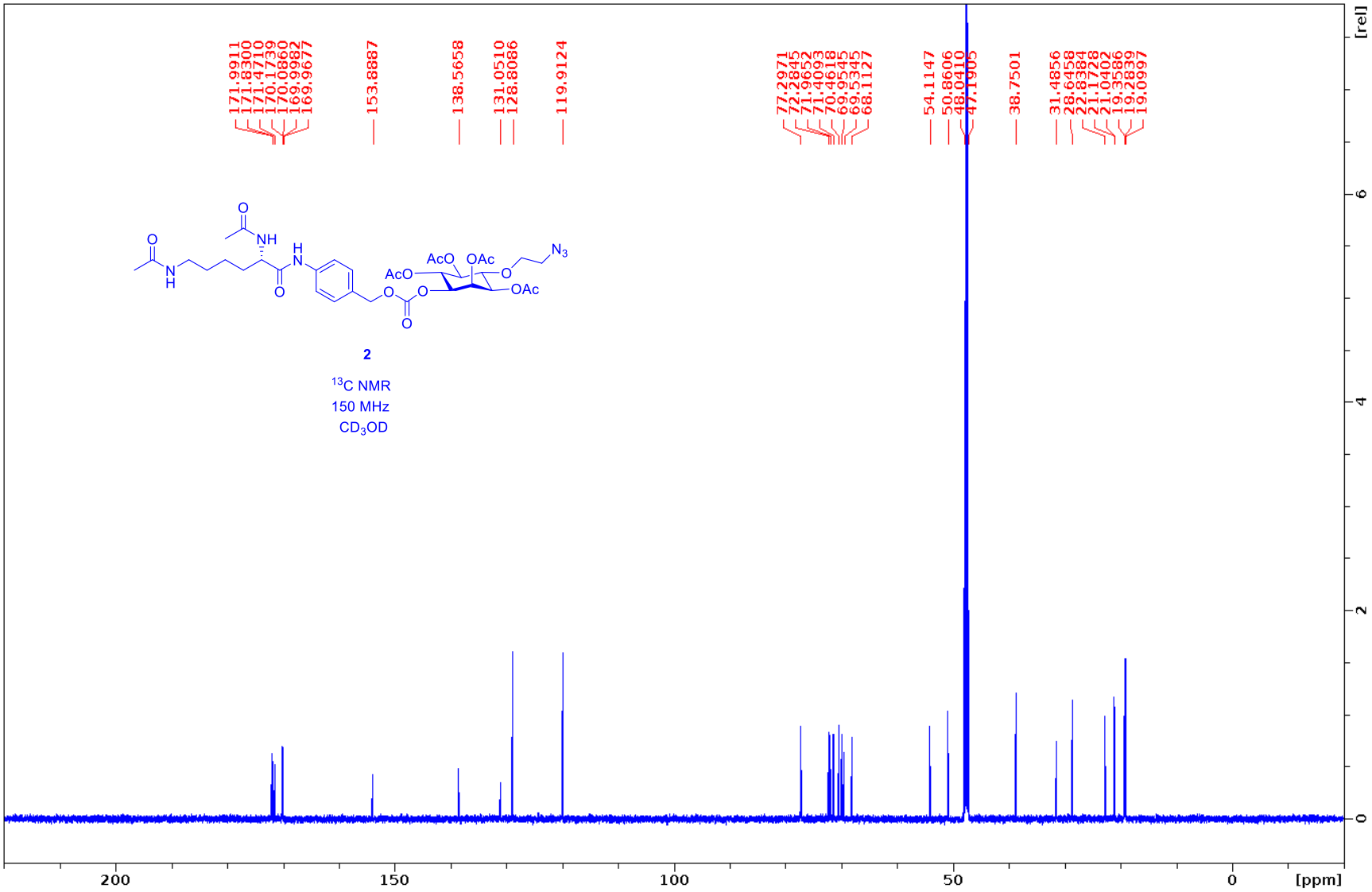
171.8331
171.4919
170.2173
170.1162
169.9262
169.8572
153.9615
138.5756
131.0701
128.7342
119.9192
75.3806
72.7395
70.9678
70.9246
69.6865
69.4995
69.2530
67.1139
54.1220
50.5111
38.7408
31.4685
28.6462
22.8383
21.1507
21.0108
19.3564
19.2046
19.0344
AcO
OAc
AcO
OAc
N3
1
13C NMR
150 MHz
CD3OD
[rel]
200
150
100
50
0
[ppm]

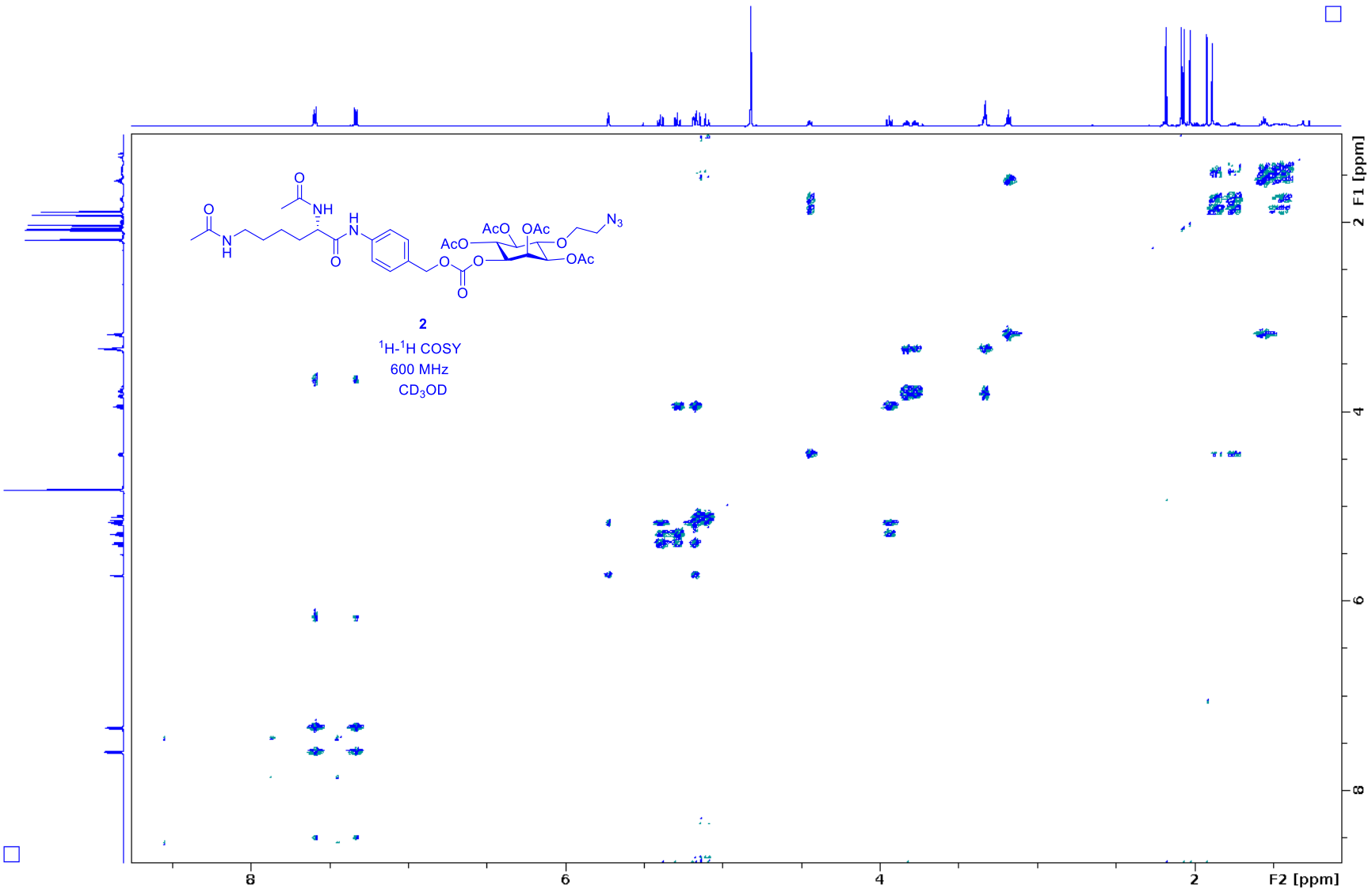

1
^{1}H-^{1}H COSY
600 MHz
CD_3OD
F1 [ppm]
F2 [ppm]

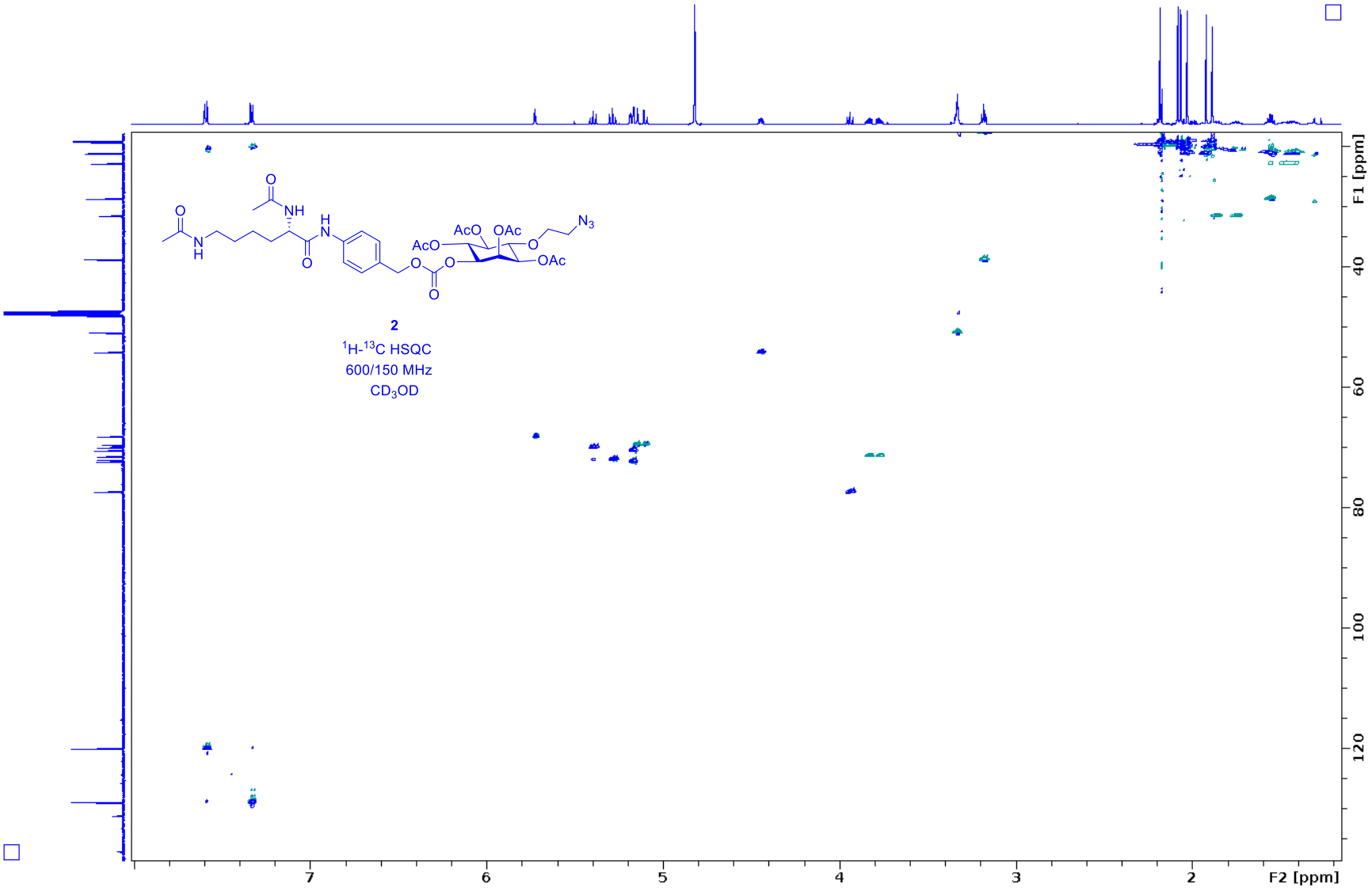

1
1H-13C HSQC
600/150 MHz
CD3OD
F1 [ppm]
F2 [ppm]




2
1H NMR
600 MHz
CD3OD
[rel]
15
10
5
0
8
6
4
2
0 [ppm]
2.0762
2.0802
1.0000
1.0271
1.0696
4.1985
1.0304
1.0185
2.0827
3.4241
2.1109
3.0524
3.1576
1.0580
2.0959
2.1842


171.9911
171.8300
171.4710
170.1739
170.0860
169.9982
169.9677
153.8887
138.5658
131.0510
128.8086
119.9124
77.2971
72.2845
71.9652
71.4093
70.4618
69.9545
69.5345
68.1127
54.1147
50.8606
48.0410
47.1905
38.7501
31.4856
28.6458
22.8384
21.1728
21.0462
19.3586
19.2839
19.0997
AcO
AcO
OAc
OAc
N3
2
13C NMR
150 MHz
CD3OD
[rel]
0
2
4
6
200
150
100
50
0
[ppm]


2
^{1}H-^{1}H COSY
600 MHz
CD_3OD
F1 [ppm]
F2 [ppm]
2
4
6
8


2
^{1}H-^{13}C HSQC
600/150 MHz
CD_3OD
F1 [ppm]
F2 [ppm]
7
6
5
4
3
2
40
60
80
100
120