Table of Contents

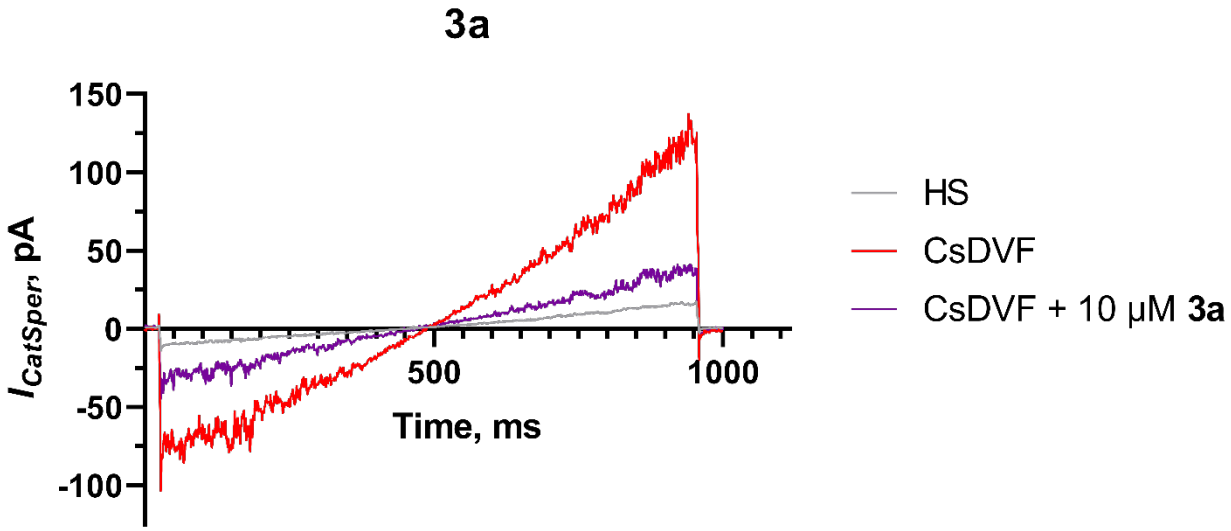


Figure S1. Representative $I_{CatSper}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound **3a**. Baseline signal in high saline (HS) buffer shown in grey. $I_{CatSper}$ currents recorded in cesium divalent free media (CsDVF) elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.

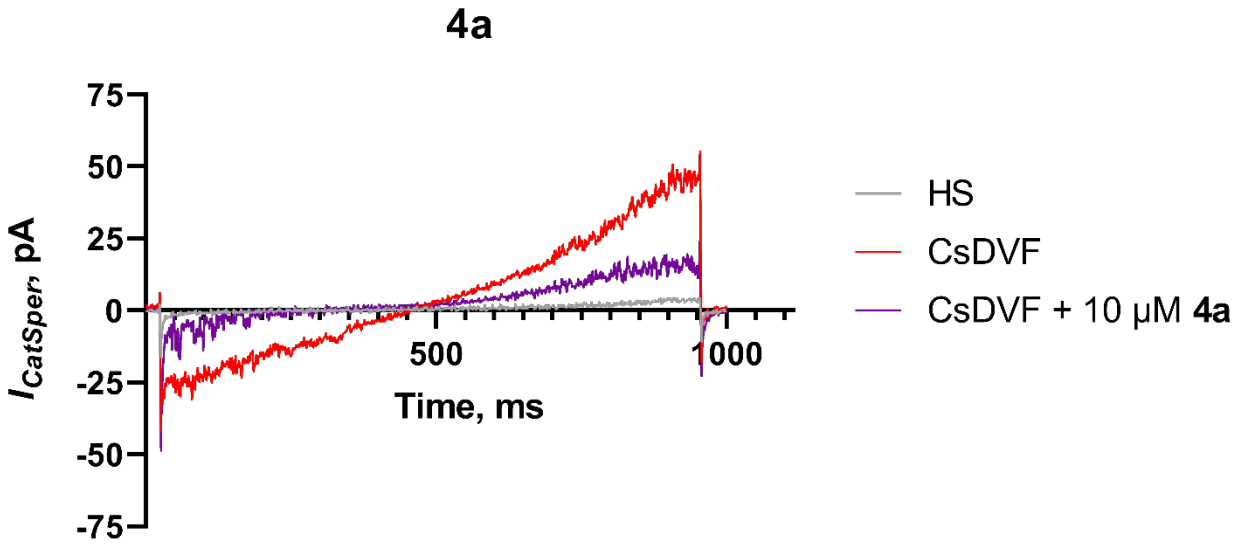


Figure S2. Representative $I_{CatSper}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound **4a**. Baseline signal in high saline (HS) buffer shown in grey. $I_{CatSper}$ currents recorded in CsDVF elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.

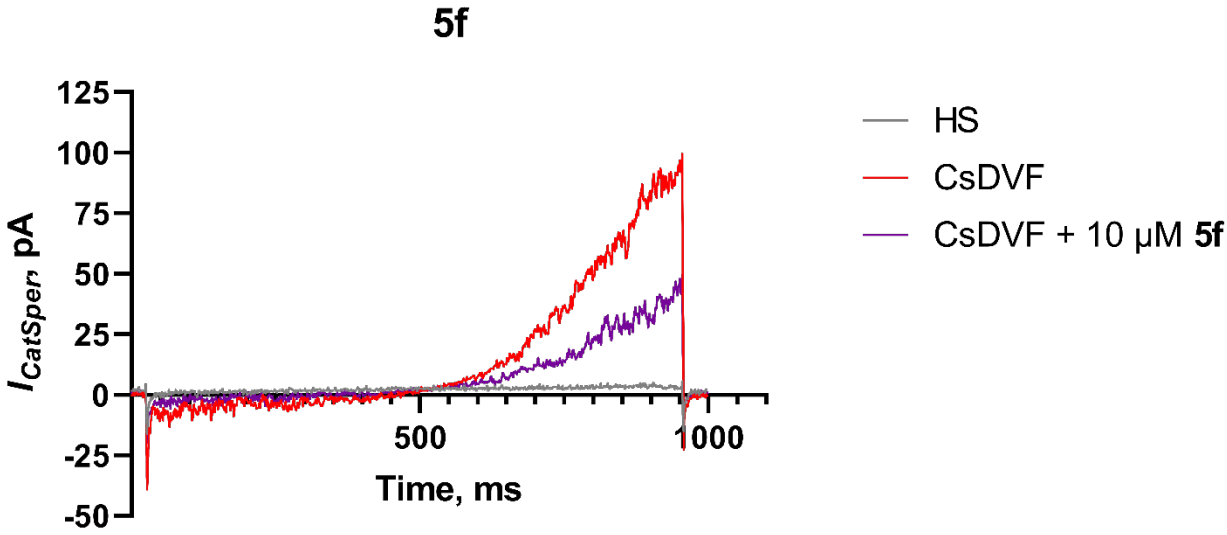


Figure S3. Representative $I_{CatSper}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound **5f**. Baseline signal in high HS buffer shown in grey. $I_{CatSper}$ currents recorded in CsDVF elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.

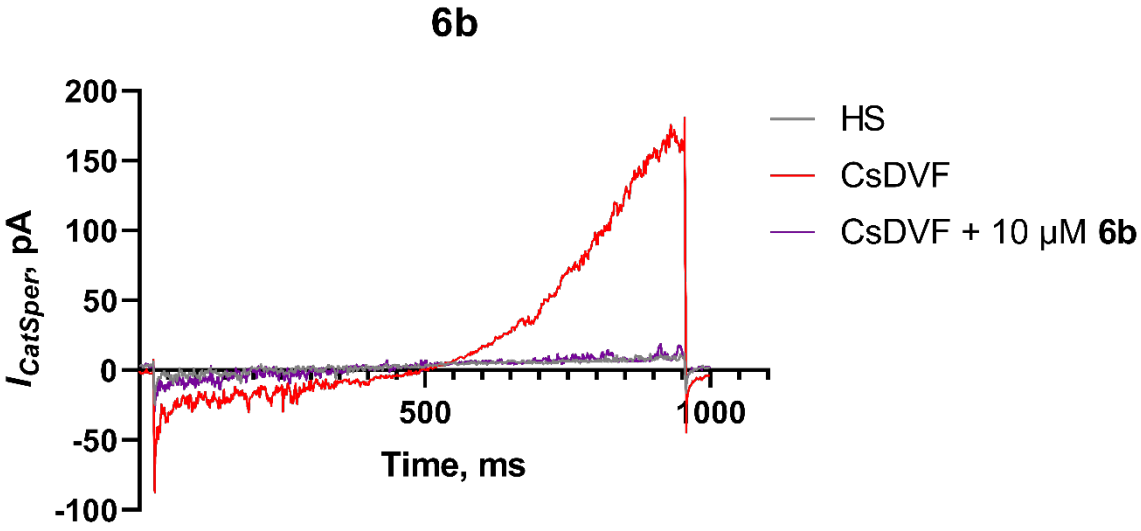


Figure S4. Representative $I_{CatSper}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound **6b**. Baseline signal in high saline (HS) buffer shown in grey. $I_{CatSper}$ currents recorded in CsDVF elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.

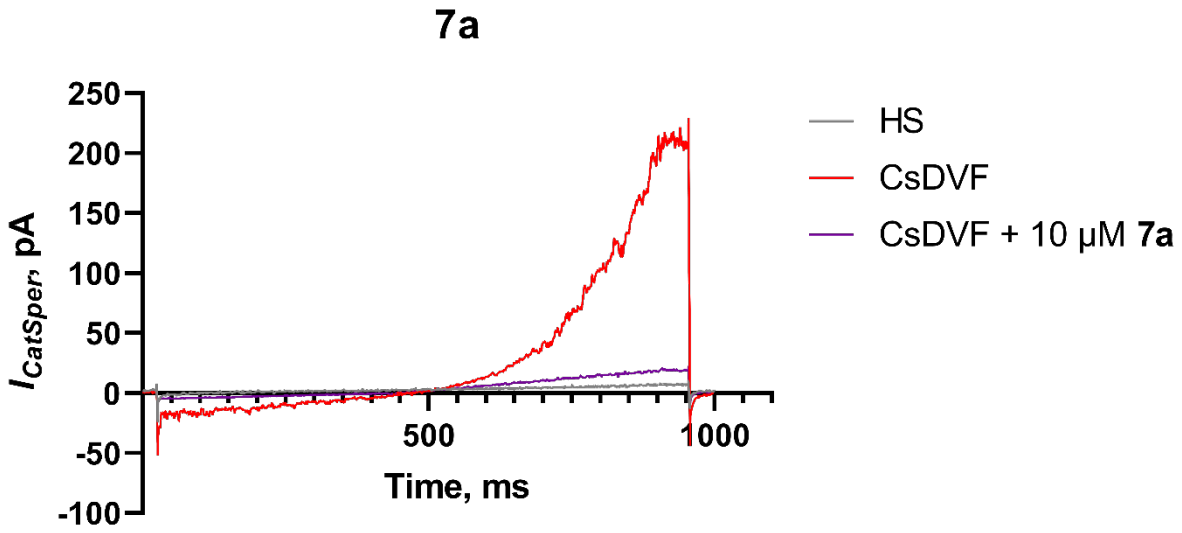
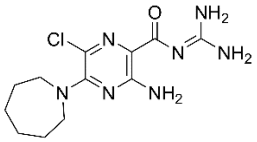
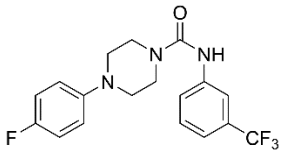
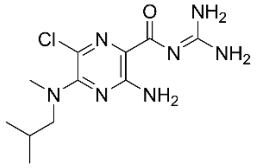
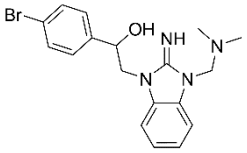
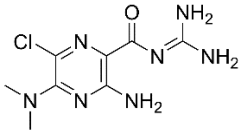
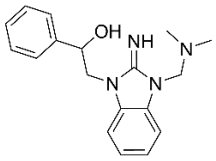
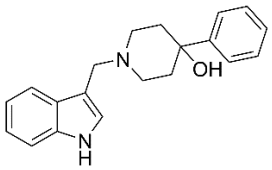
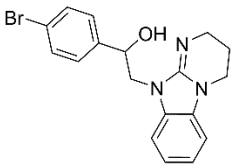
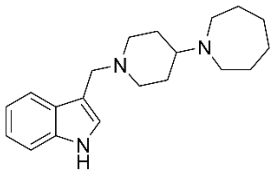
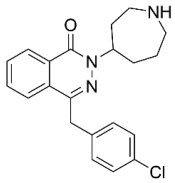
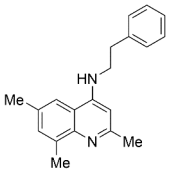
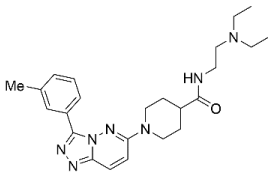
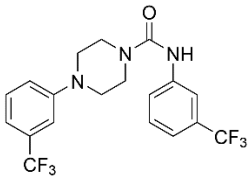


Figure S5. Representative $I_{CatSper}$ recordings from healthy human sperm in the absence (red) and presence (purple) of test compound **7a**. Baseline signal in HS buffer shown in grey. $I_{CatSper}$ currents recorded in CsDVF elicited by the -80 mV (negative scale) or +80 mV (positive scale) voltage ramp.

Table S1. Inhibition of potassium-activation of CatSper by compounds from each hit series

ID	Structure	IC_{50}, μM	ID	Structure	IC_{50}, μM
1a		9.6	**4b**		6.9
1b		12	**5a**		6.3
1c		59	**5d**		33
2a		12	**5k**		18
2d		12	**6b**		5.1
3a		7.8	**7a**		11
4a		9.2			

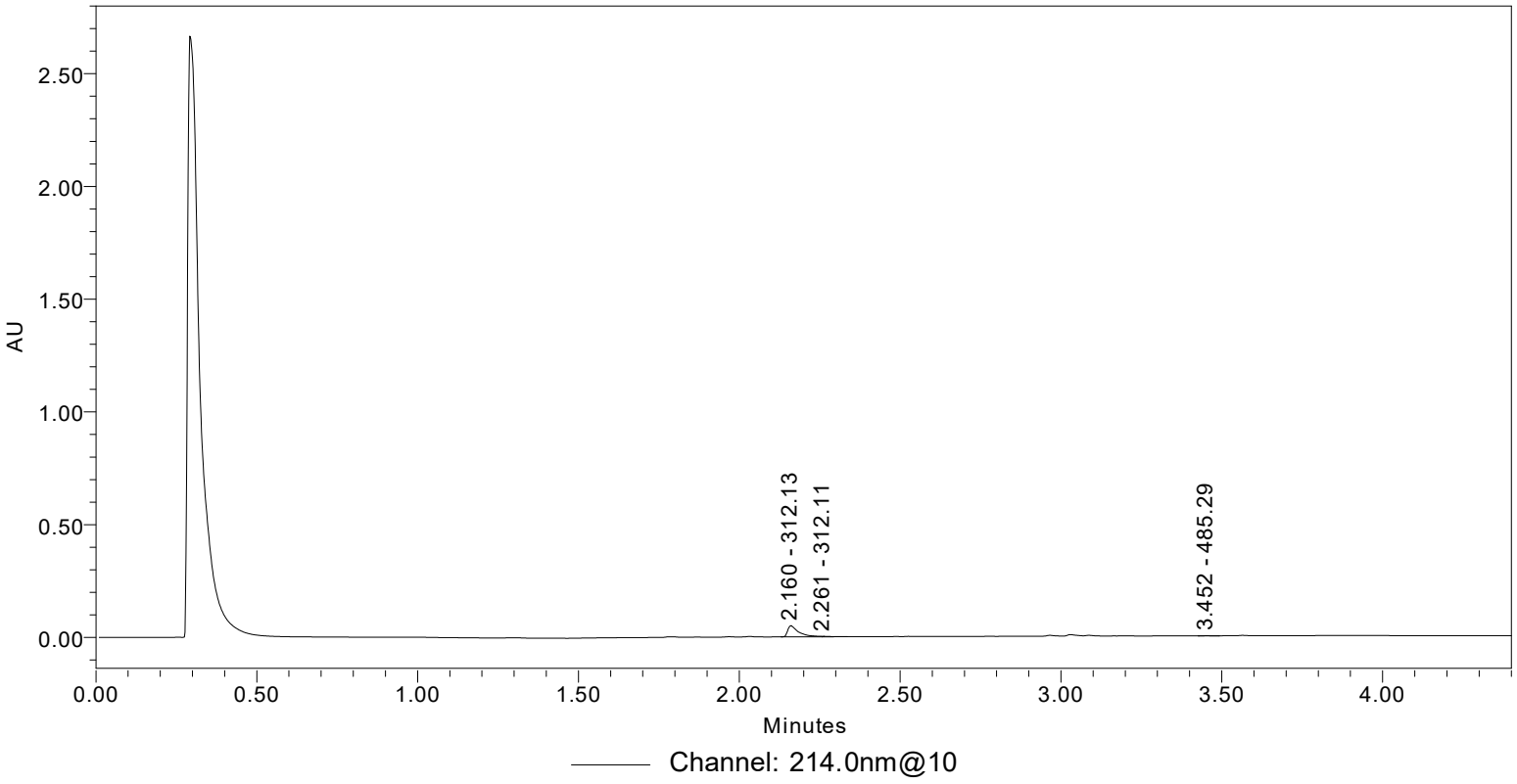
CatSper activated by high potassium/high pH buffer. IC_{50} values calculated from single experiments.

Table S2. Reduction of $I_{CatSper}$ current density in human sperm by compounds from series **2** through **7**.		
Compound	**Current Density (pA/pF)**	
	Inward (–80 mV)	**Outward (+80 mV)**
CsDVF	-20 ± 3	86 ± 12
2a	-5.0 ± 0.5	23 ± 4
3a	-4.0 ± 0.3	3.3 ± 2.9
4a	-3.4 ± 0.2	15 ± 2.4
5f	-15 ± 3	35 ± 4
6b	-2.5 ± 1.1	0.1 ± 1.4
7a	-1.2 ± 0.4	5.9 ± 1.4
CsDVF = Cesium divalent free media. Values are the mean ± SEM of at least 3 independent experiments.		

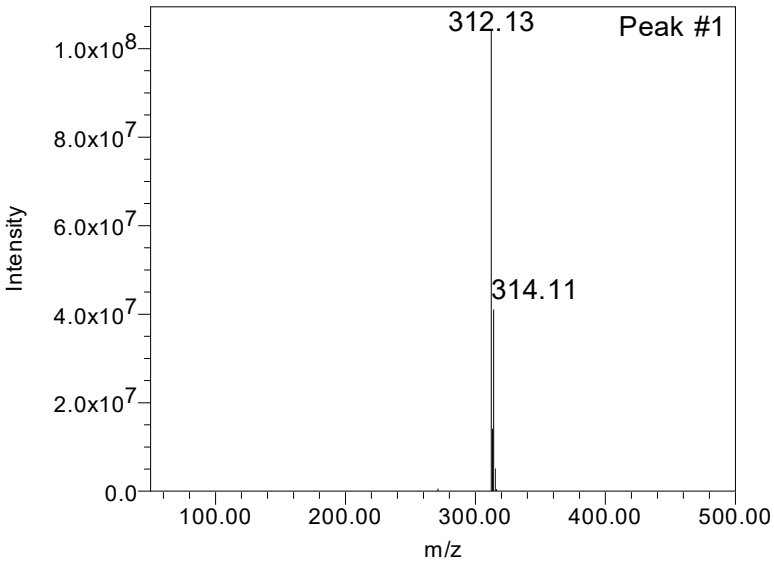
Mass Analysis Report

SAMPLE INFORMATION

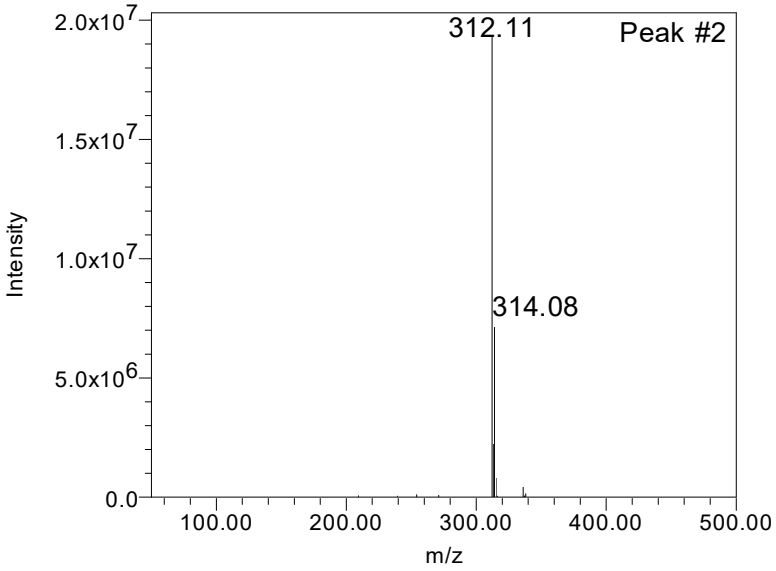
Sample Name: 1a
Acq Method Set: Col2_50to500_PosOnly
Acquired: 1/28/2022 8:12:07 PM CST
InjVol: 3.00 uL



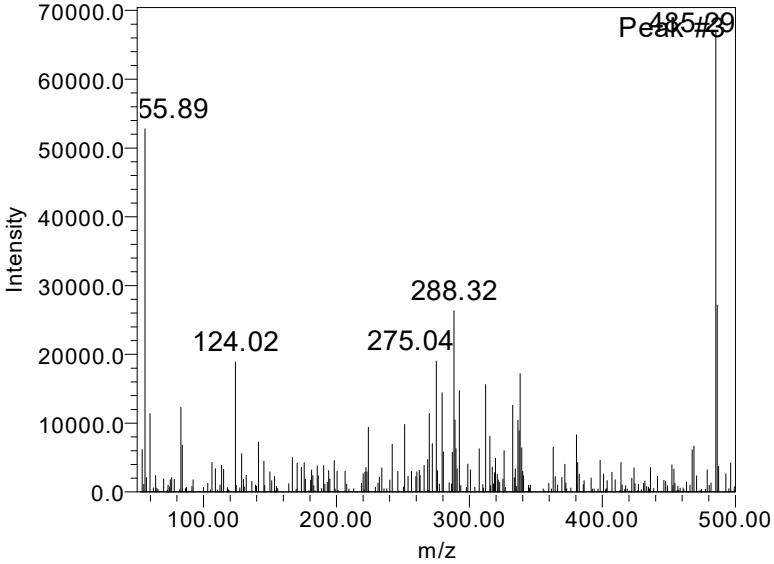
	RT	Area	% Area	Height	Base Peak (m/z)
1	2.160	116430	96.48	48975	312.13
2	2.261	3612	2.99	1843	312.11
3	3.452	636	0.53	389	485.29



RT: 2.160



RT: 2.261

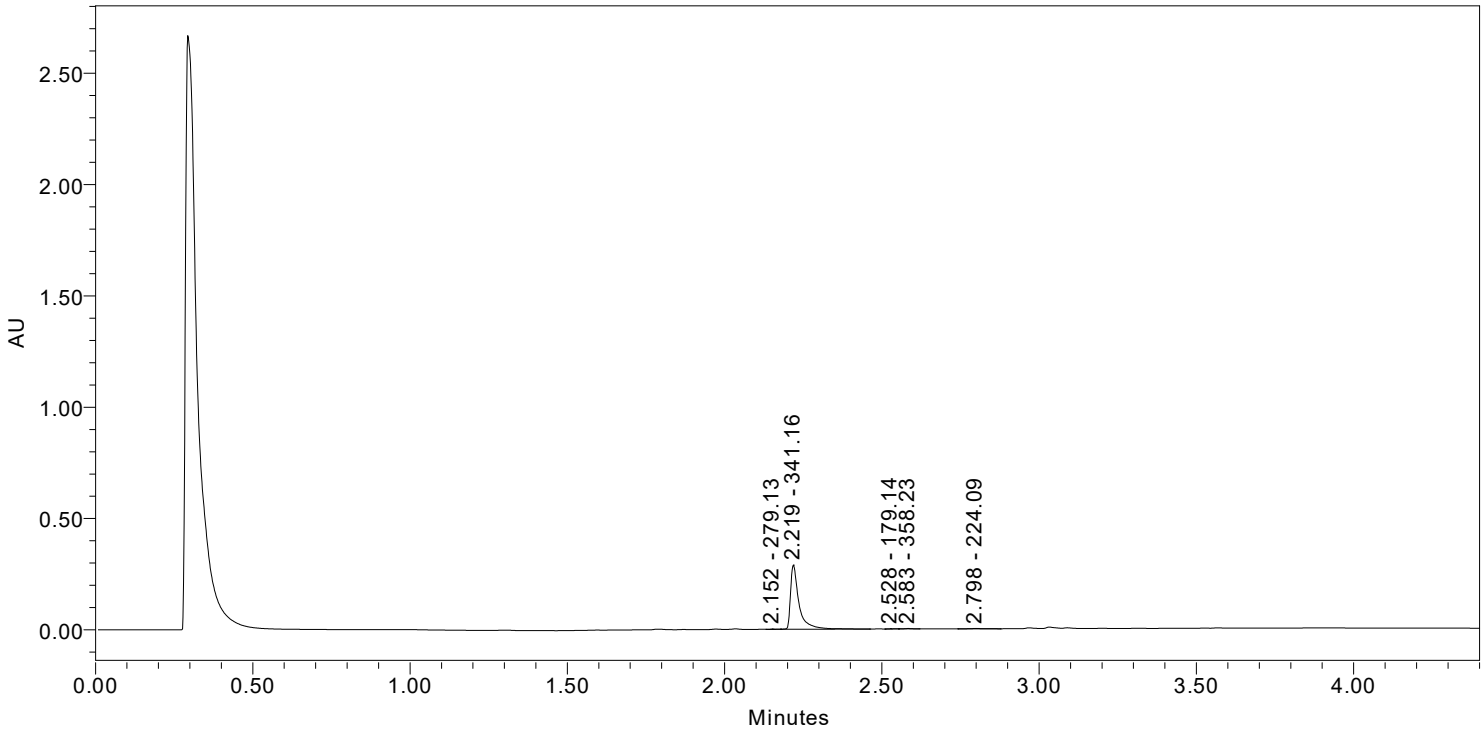


RT: 3.452

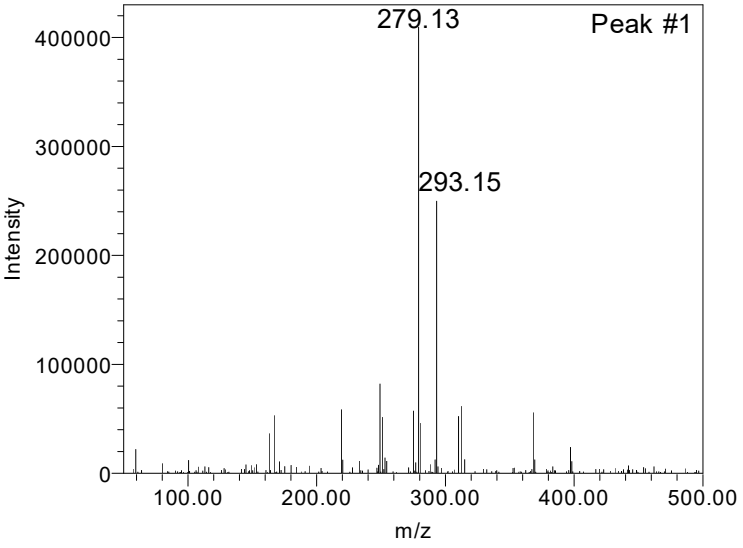
Mass Analysis Report

SAMPLE INFORMATION

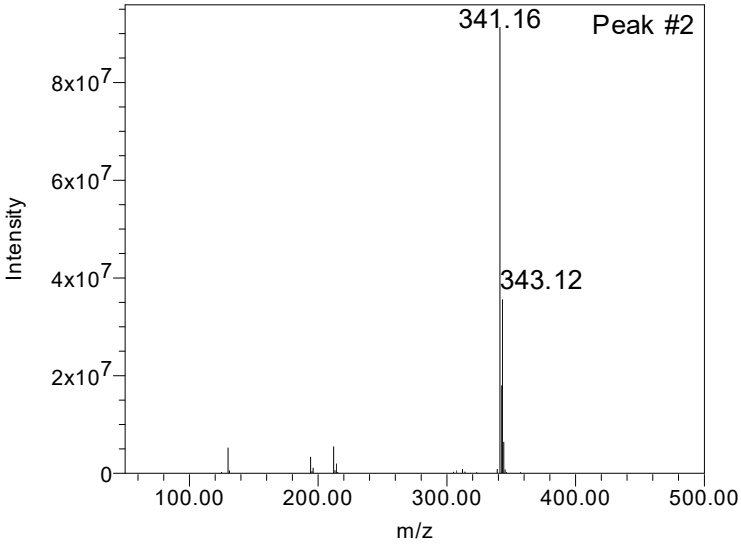
Sample Name: 2a
Acq Method Set: Col2_50to500_PosOnly
Acquired: 1/28/2022 8:19:07 PM CST
InjVol: 3.00 uL



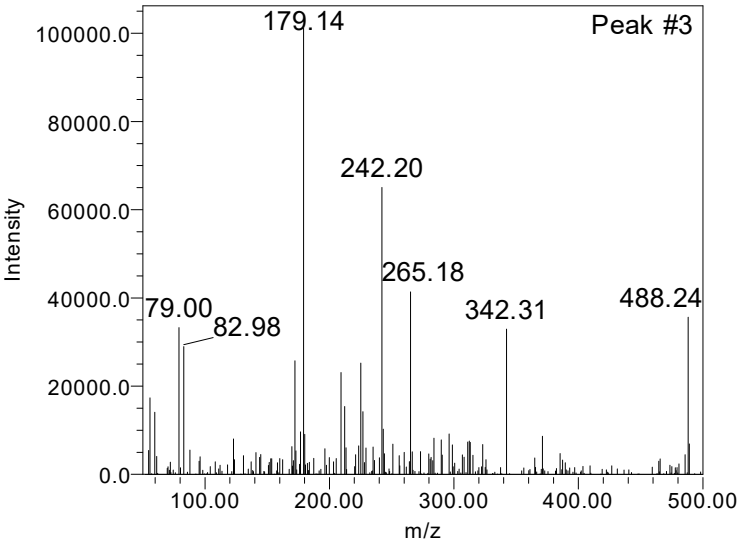
	RT	Area	% Area	Height	Base Peak (m/z)
1	2.152	2054	0.37	1309	279.13
2	2.219	541358	98.20	288613	341.16
3	2.528	1156	0.21	944	179.14
4	2.583	2494	0.45	1661	358.23
5	2.798	4191	0.76	1555	224.09



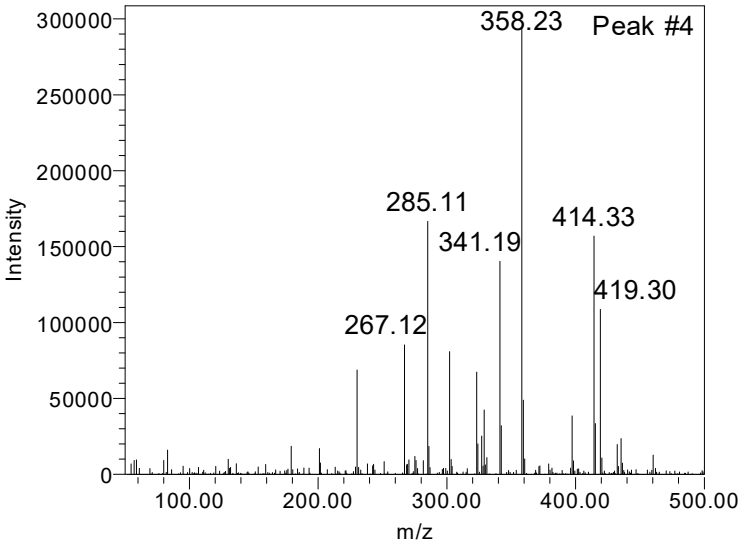
RT: 2.152



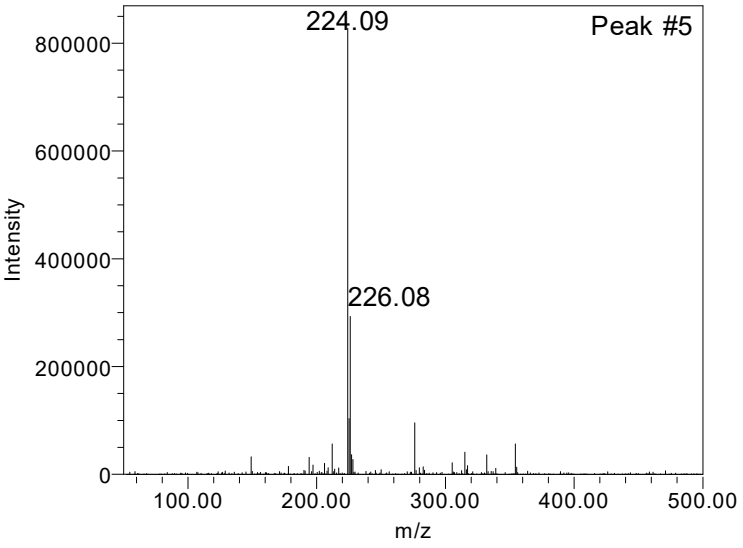
RT: 2.219



RT: 2.528



RT: 2.583

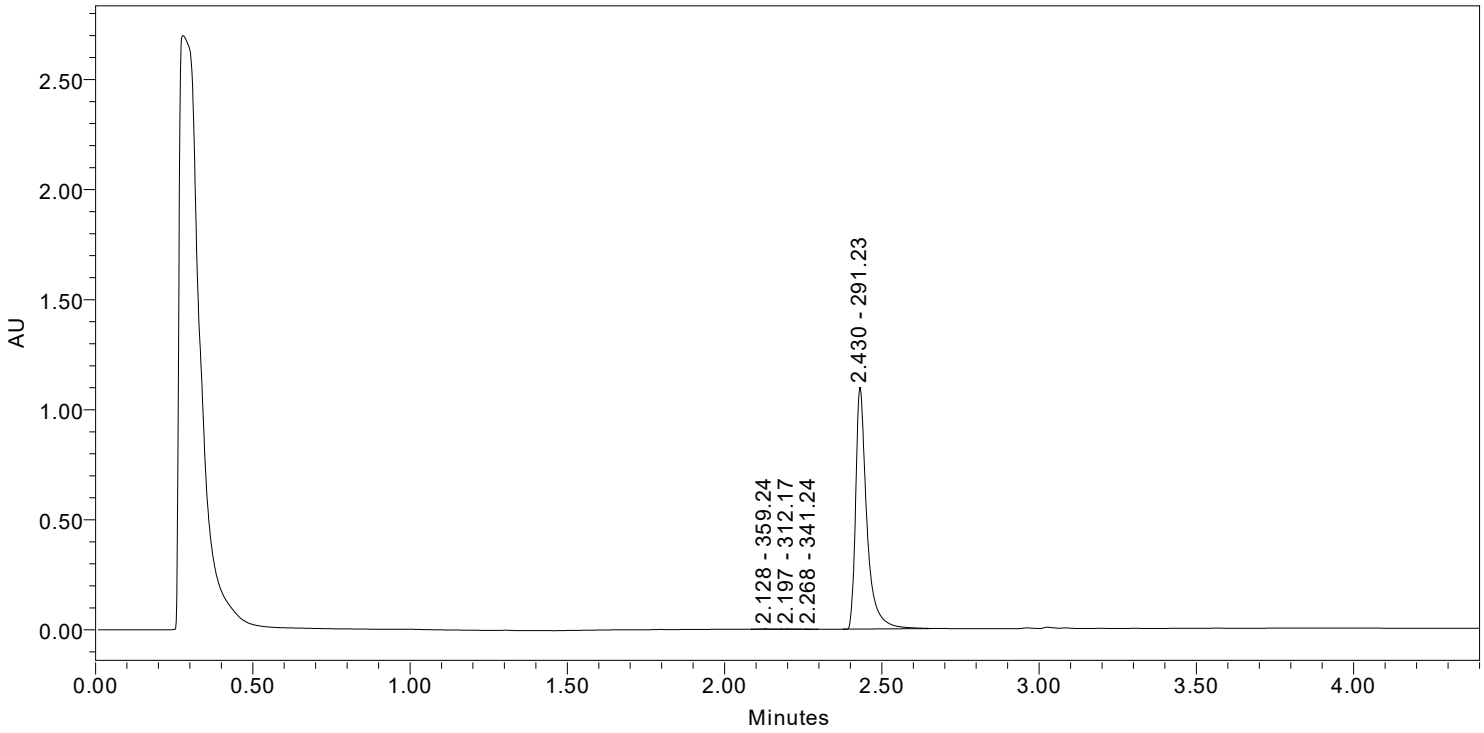


RT: 2.798

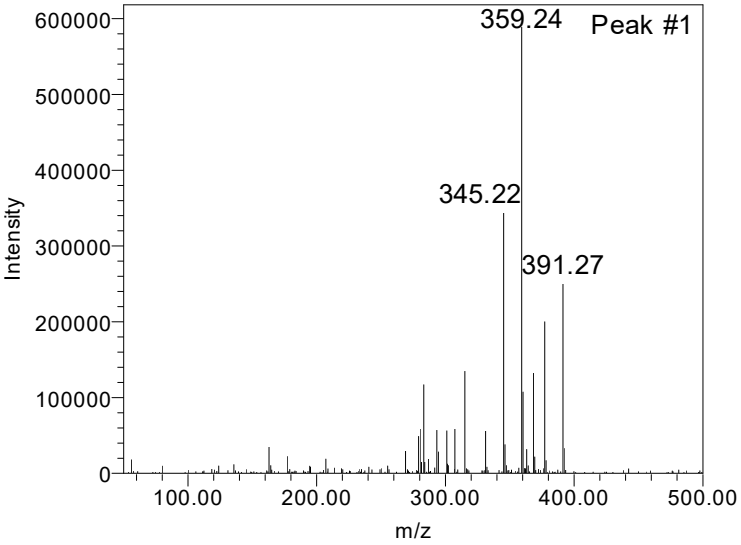
Mass Analysis Report

SAMPLE INFORMATION

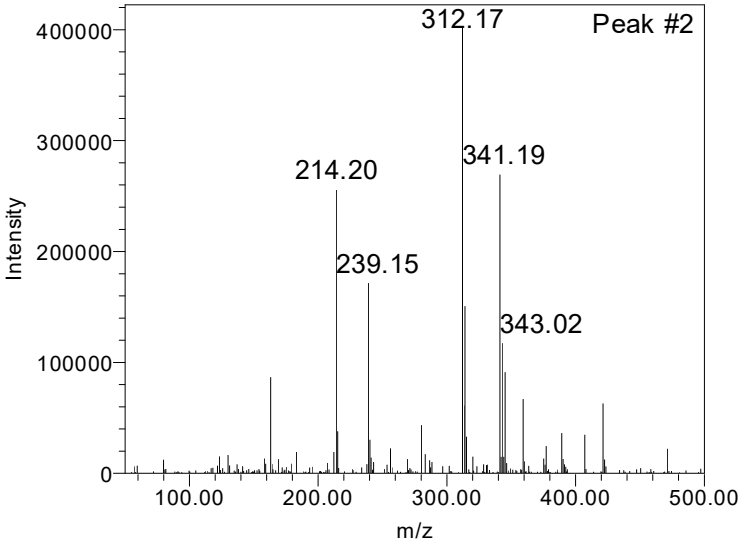
Sample Name: 3a
Acq Method Set: Col2_50to500_PosOnly
Acquired: 1/28/2022 10:27:28 PM CST
InjVol: 7.50 uL



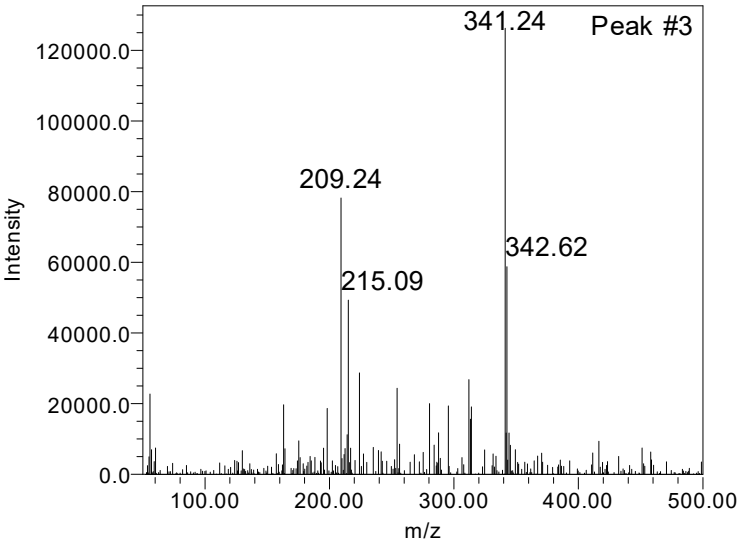
	RT	Area	% Area	Height	Base Peak (m/z)
1	2.128	4919	0.18	1590	359.24
2	2.197	3898	0.14	1234	312.17
3	2.268	364	0.01	288	341.24
4	2.430	2714937	99.66	1098416	291.23



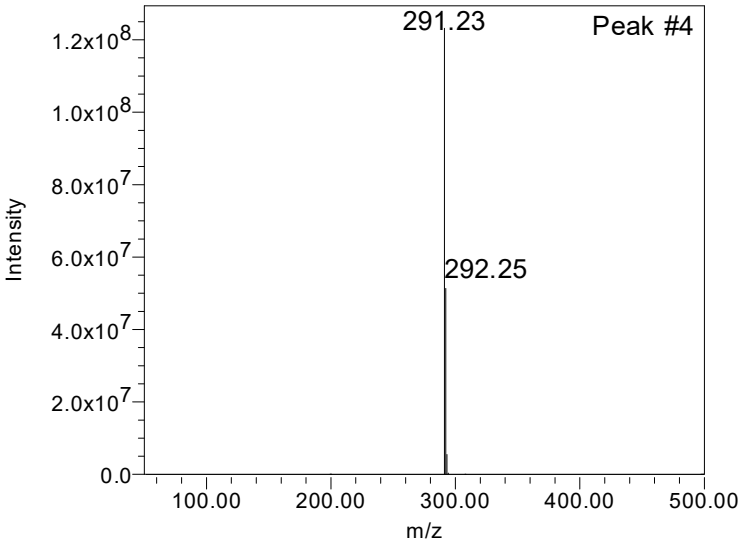
RT: 2.128



RT: 2.197



RT: 2.268

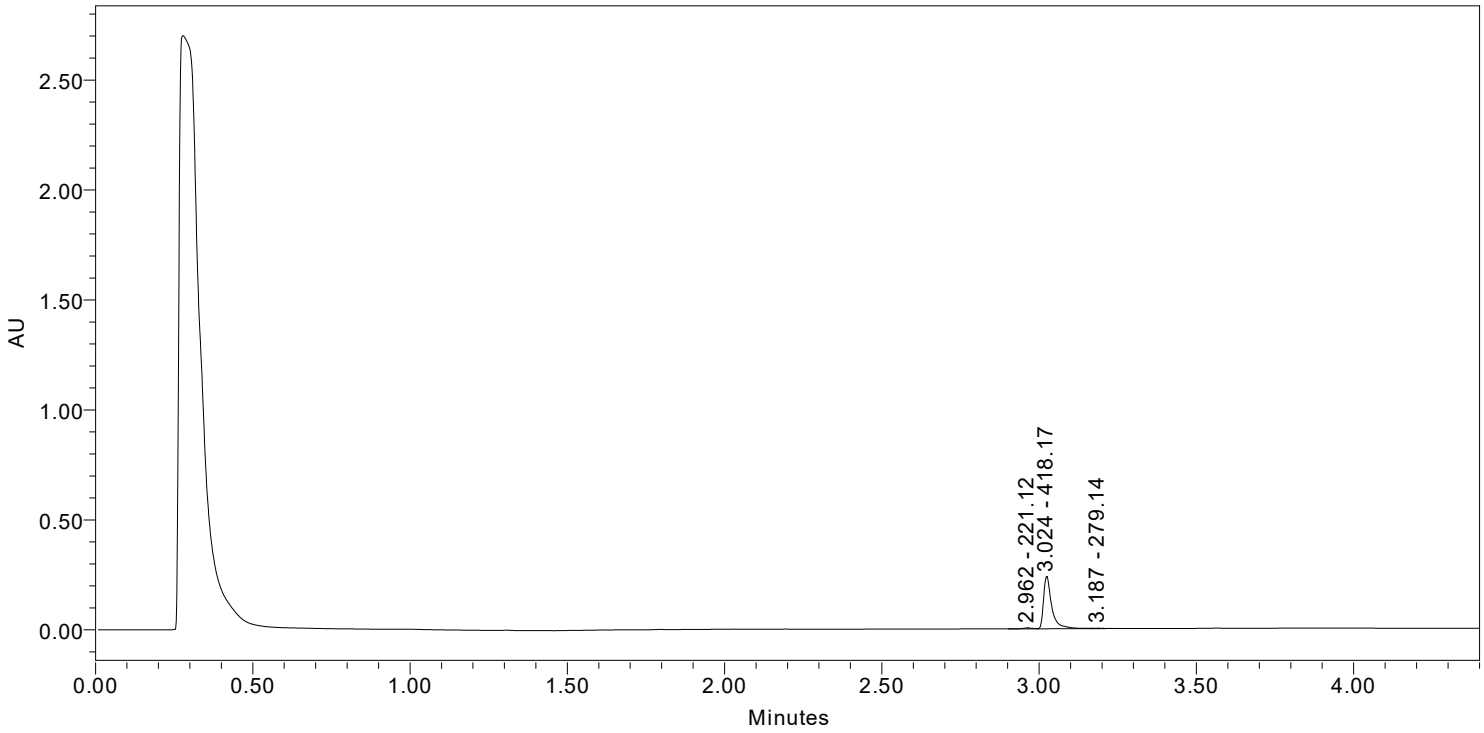


RT: 2.430

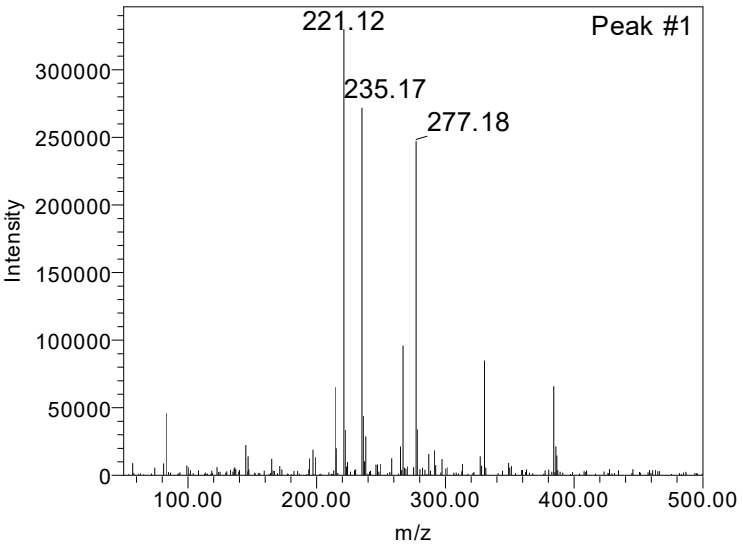
Mass Analysis Report

SAMPLE INFORMATION

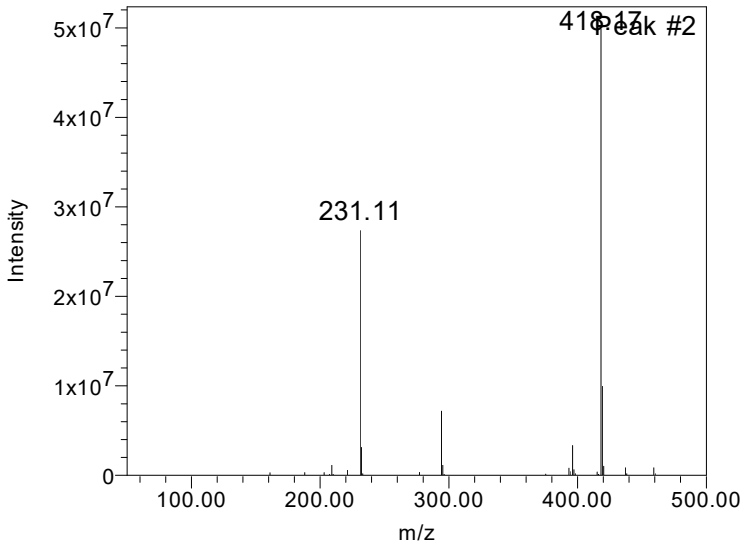
Sample Name: 4a
Acq Method Set: Col2_50to500_PosOnly
Acquired: 1/28/2022 10:34:35 PM CST
InjVol: 7.50 uL



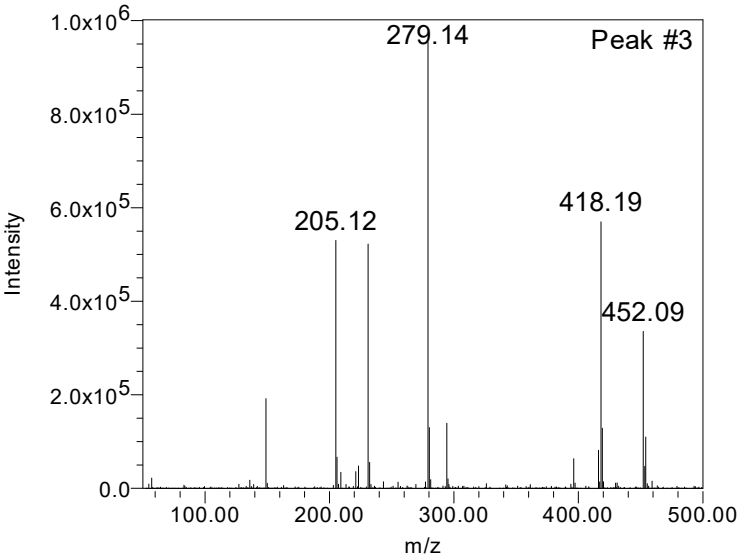
	RT	Area	% Area	Height	Base Peak (m/z)
1	2.962	8491	1.91	3932	221.12
2	3.024	432574	97.18	238038	418.17
3	3.187	4053	0.91	1755	279.14



RT: 2.962



RT: 3.024



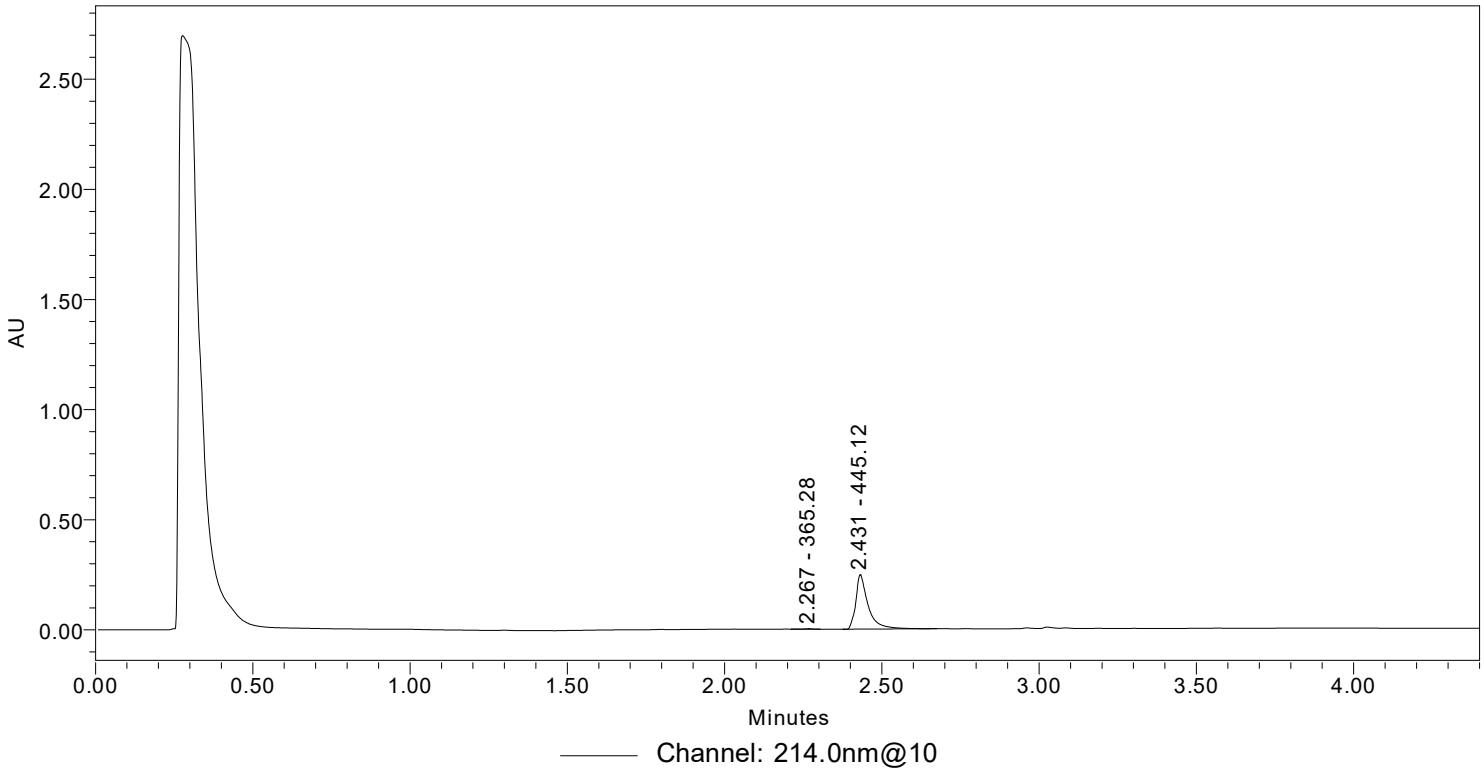
RT: 3.187

Mass Analysis Report

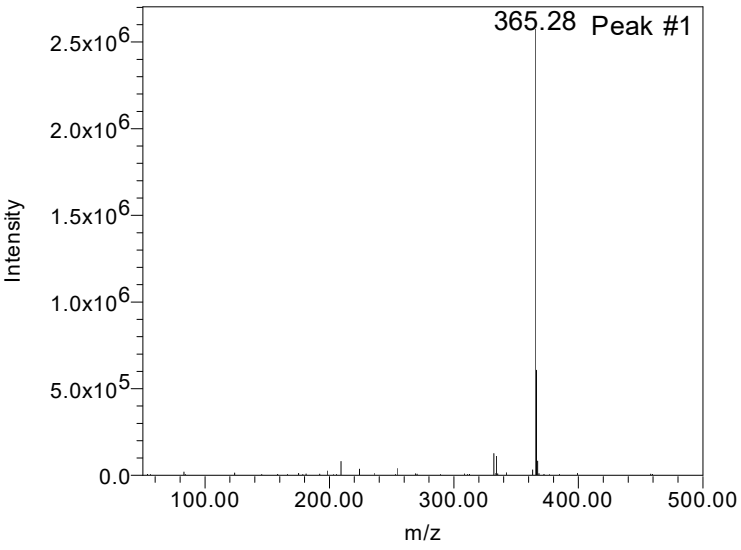
SAMPLE INFORMATION

Sample Name: 5f
Acq Method Set: Col2_50to500_PosOnly

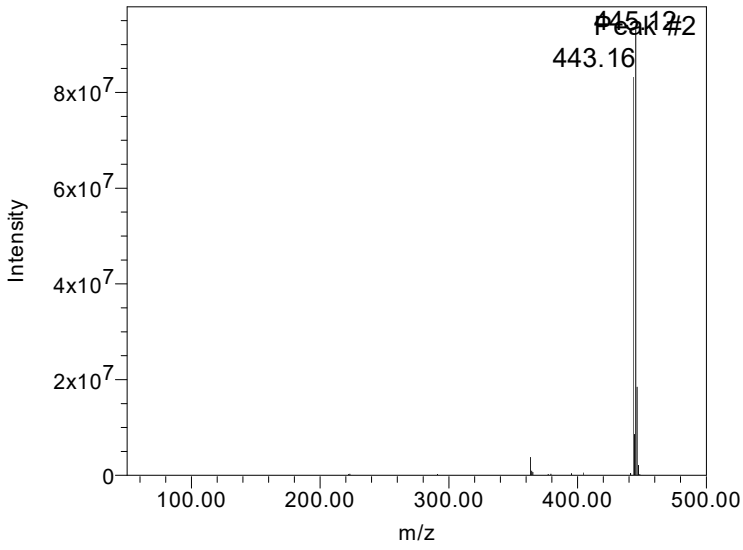
Acquired: 1/28/2022 10:41:39 PM CST
InjVol: 7.50 uL



	RT	Area	% Area	Height	Base Peak (m/z)
1	2.267	4324	0.64	1859	365.28
2	2.431	674377	99.36	246673	445.12



RT: 2.267

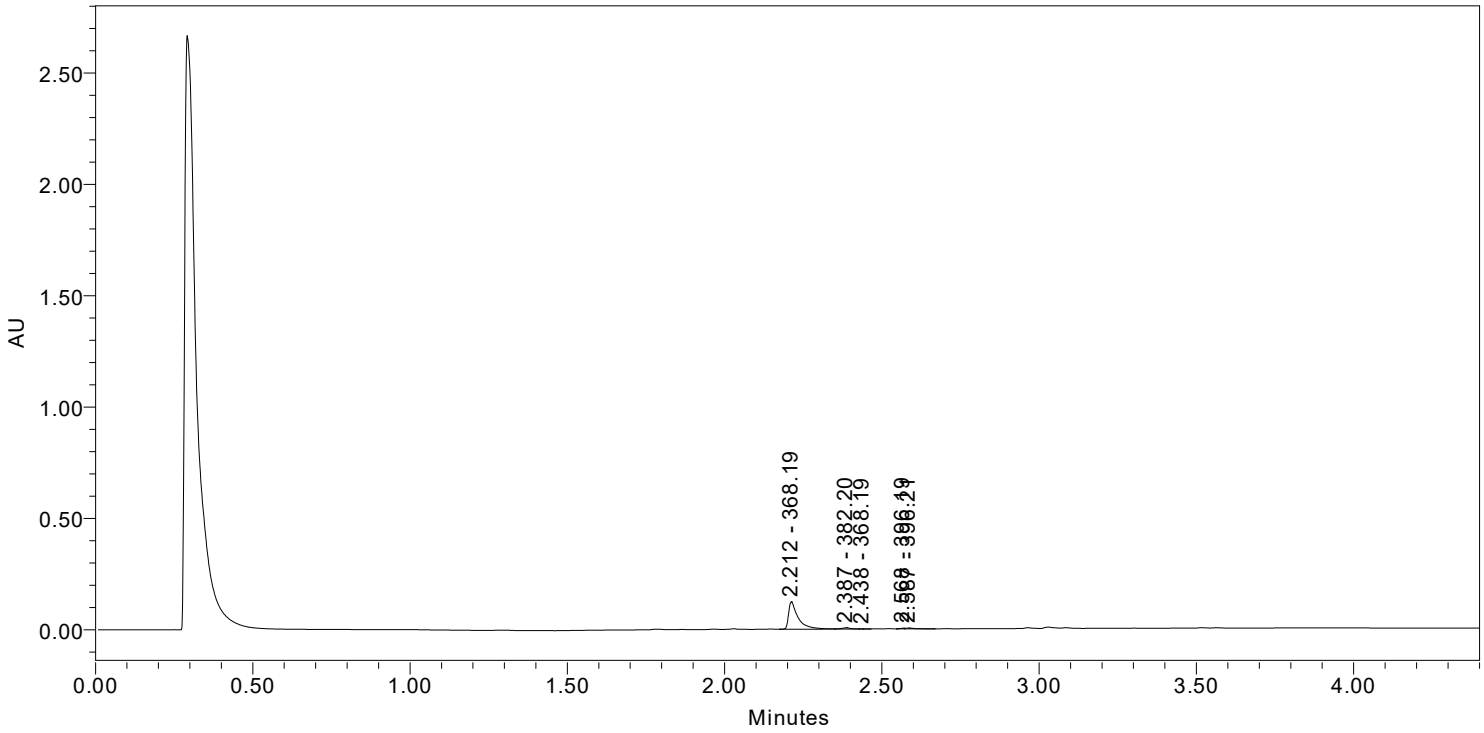


RT: 2.431

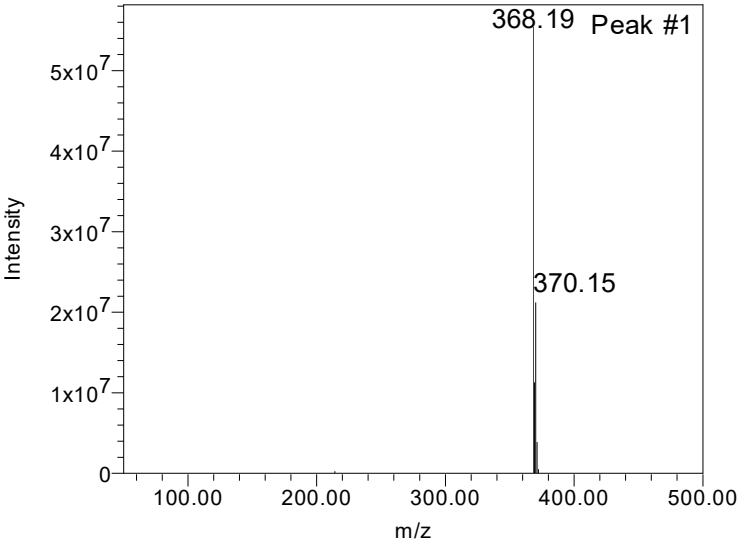
Mass Analysis Report

SAMPLE INFORMATION

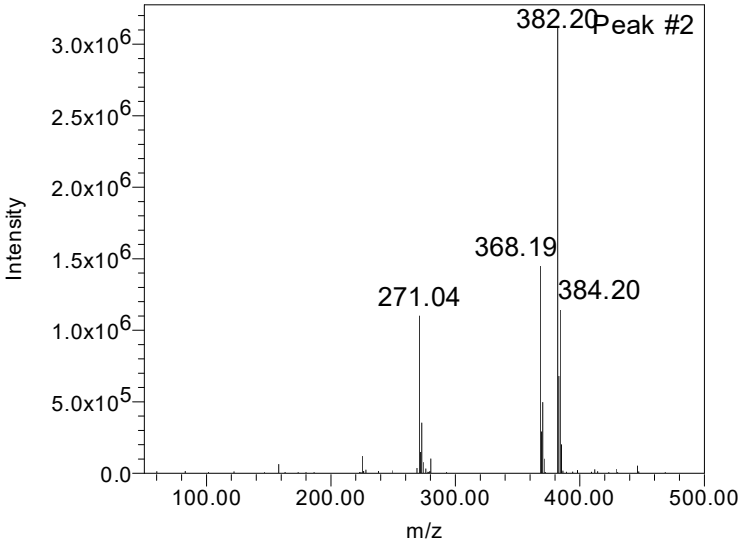
Sample Name: 6b
Acq Method Set: Col2_50to500_PosOnly
Acquired: 1/28/2022 8:47:06 PM CST
InjVol: 3.00 uL



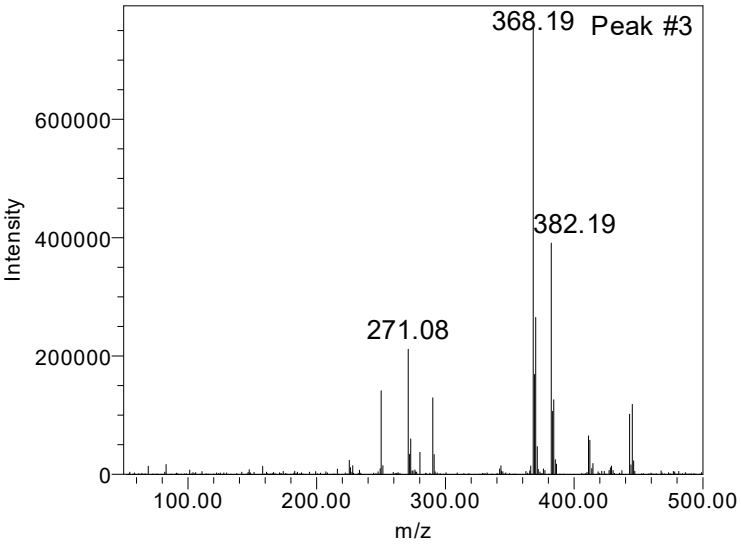
	RT	Area	% Area	Height	Base Peak (m/z)
1	2.212	261797	91.79	123446	368.19
2	2.387	12417	4.35	5882	382.20
3	2.438	1349	0.47	759	368.19
4	2.568	2820	0.99	2823	396.19
5	2.587	6844	2.40	3792	396.21



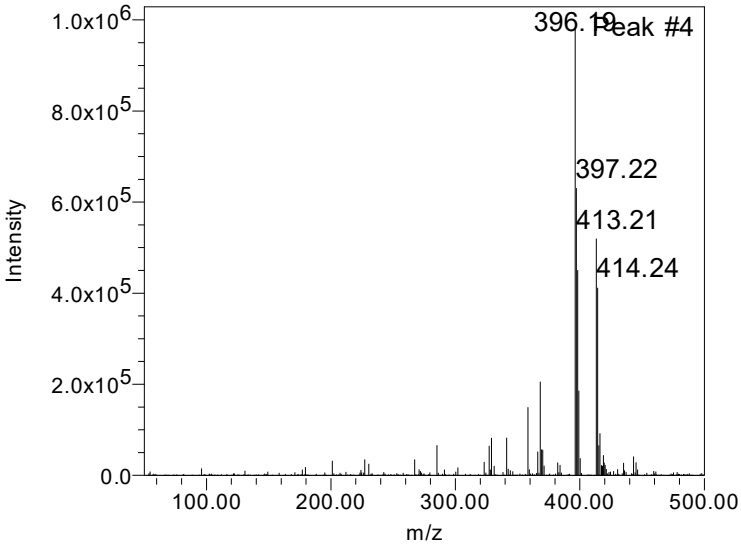
RT: 2.212



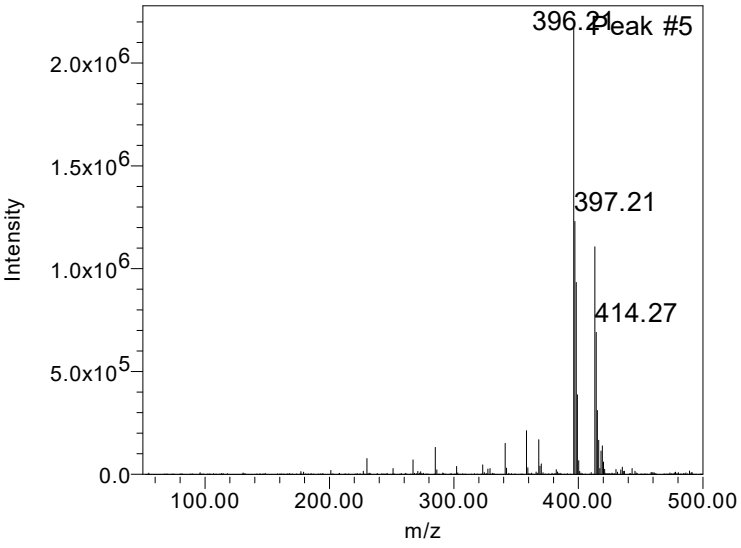
RT: 2.387



RT: 2.438



RT: 2.568



RT: 2.587

LCMS Analysis Report

Acquired by	: System Administrator
Sample Name	: YTL-12-6-2-1
Injection Volume	: 1
Data File	: YTL-12-6-2-1.lcd
Method File	: ACN-Water-0.05%TFA-5%B-1.5-3.0MIN(90-900).lcm
Date Acquired	: 2022/2/22 17:24:34
Comment	: Mobile phaseA:water/0.05%TFA Mobile phaseB:ACN/0.05%TFA

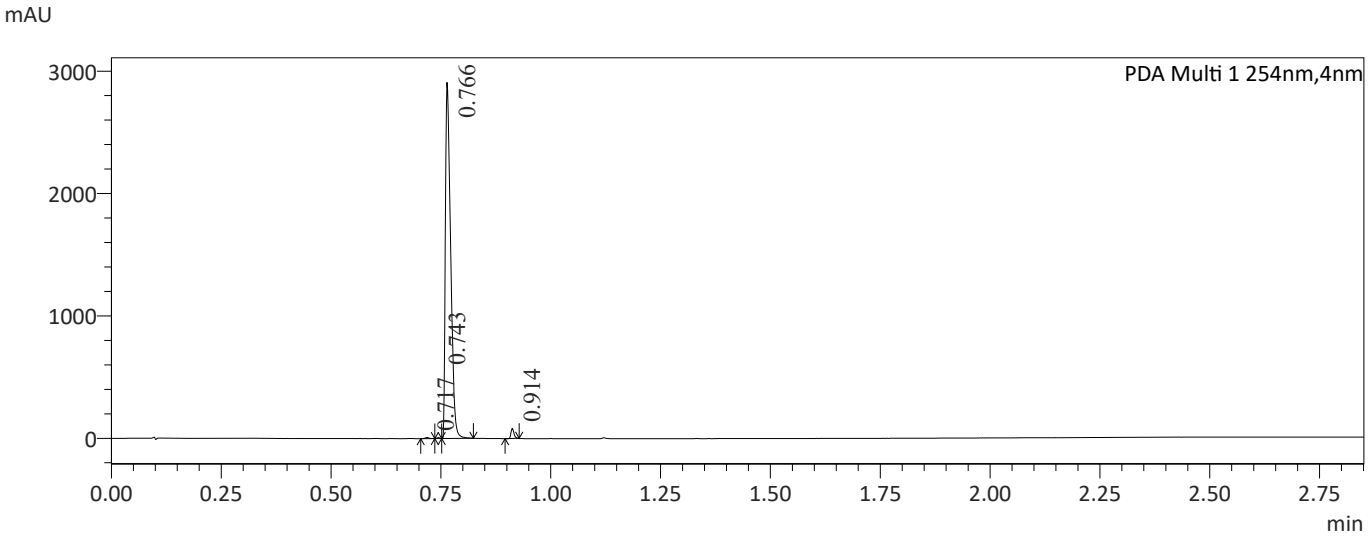
Instrument Name	: Shimadzu LCMS-2020
<<Pump>>	
Mode	: Binary gradient
Pump A	: LC-20ADXR
Pump B	: LC-20ADXR
Total Flow	: 1.5000 mL/min
B Conc.	: 5.0 %
<<Oven>>	
Oven Temperature	: 40 C
<<PDA>>	
PDA Model	: SPD-M20A
Lamp	: D2
Start Wavelength	: 190 nm
End Wavelength	: 400 nm
<<Column>>	
Column Name	: HALO C18
Length	: 30 mm
Internal Diameter	: 3.0 mm

<<Interface>>	
Interface	:ESI
DL Temperature	:250 C
Nebulizing Gas Flow	:1.50 L/min
Heat Block	:250 C
Drying Gas	:On
	15.00 L/min
<<MS Parameter>>	
Initial Valve Position	:-
--Segment 1 Event 1--	
Start Time	:0.00 min
End Time	:2.85 min
Acquisition Mode	:Scan
Polarity	:Positive
Event Time	:0.30 sec
Detector Voltage	:+0.90 kV
Threshold	:0
Start m/z	:90.00
End m/z	:900.00
Scan Speed	:3000 u/sec
Interface Volt.	:Use the Data in the Tuning File
DL Volt.	:Use the Data in the Tuning File
Qarray DC Voltage	:Use the Data in the Tuning File
Qarray DC Voltage	:Use the Data in the Tuning File

<<LC Time Program>>

Time	Module	Command	Value
0.01	Pumps	B.Conc	5
2.00	Pumps	B.Conc	100
2.70	Pumps	B.Conc	100
2.75	Pumps	B.Conc	5
3.00	Controller	Stop	

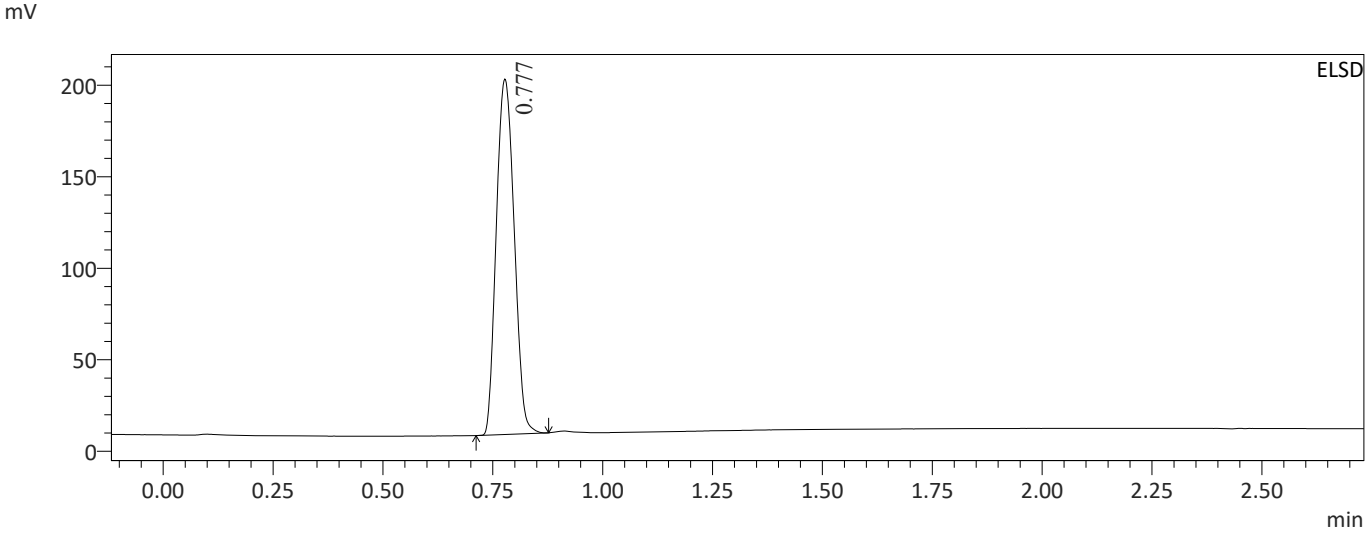
Chromatogram



Peak Table

PDA Ch1 254nm

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.717	6809	0.254	4945	0.201
2	0.743	3550	0.132	3076	0.125
3	0.766	2633161	98.096	2417257	98.173
4	0.914	40753	1.518	36961	1.501
Total		2684272	100.000	2462239	100.000

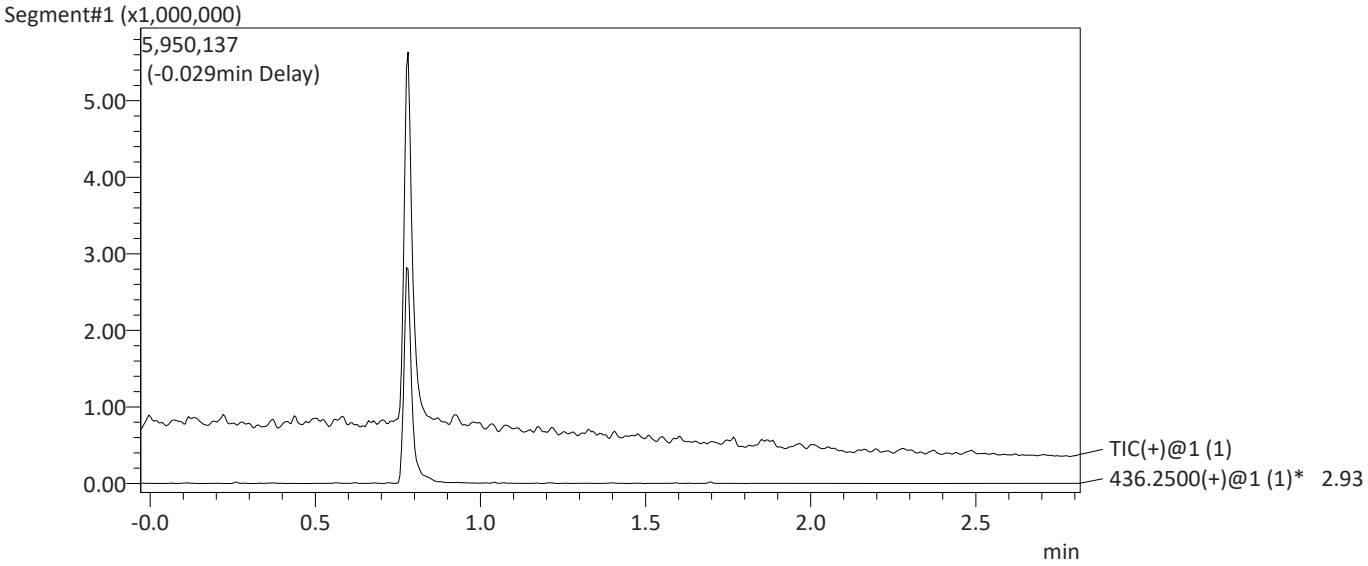


Peak Table

ELSD

Peak#	Ret. Time	Height	Height%	Area	Area%
1	0.777	192746	100.000	551055	100.000
Total		192746	100.000	551055	100.000

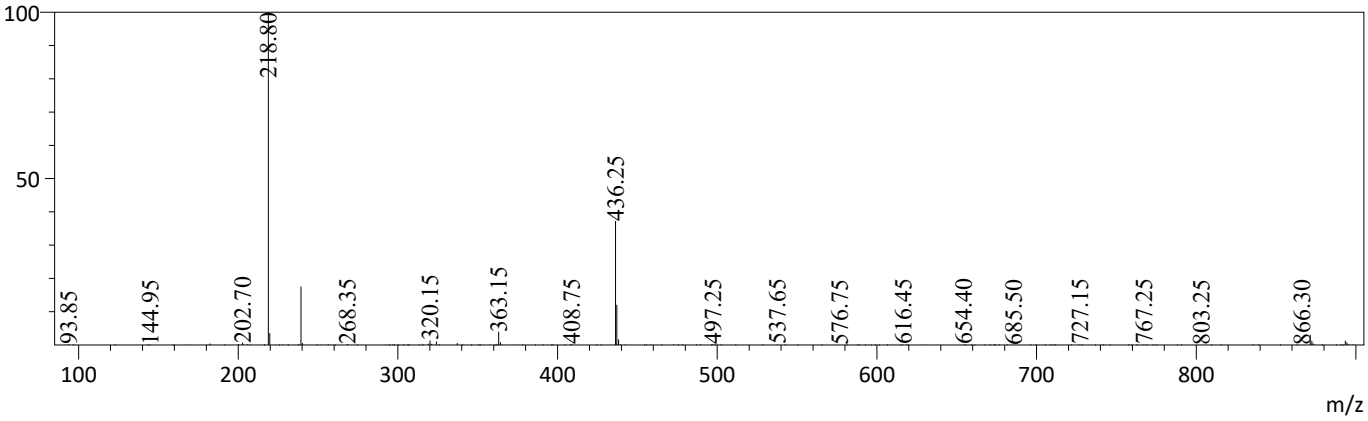
MS Chromatogram

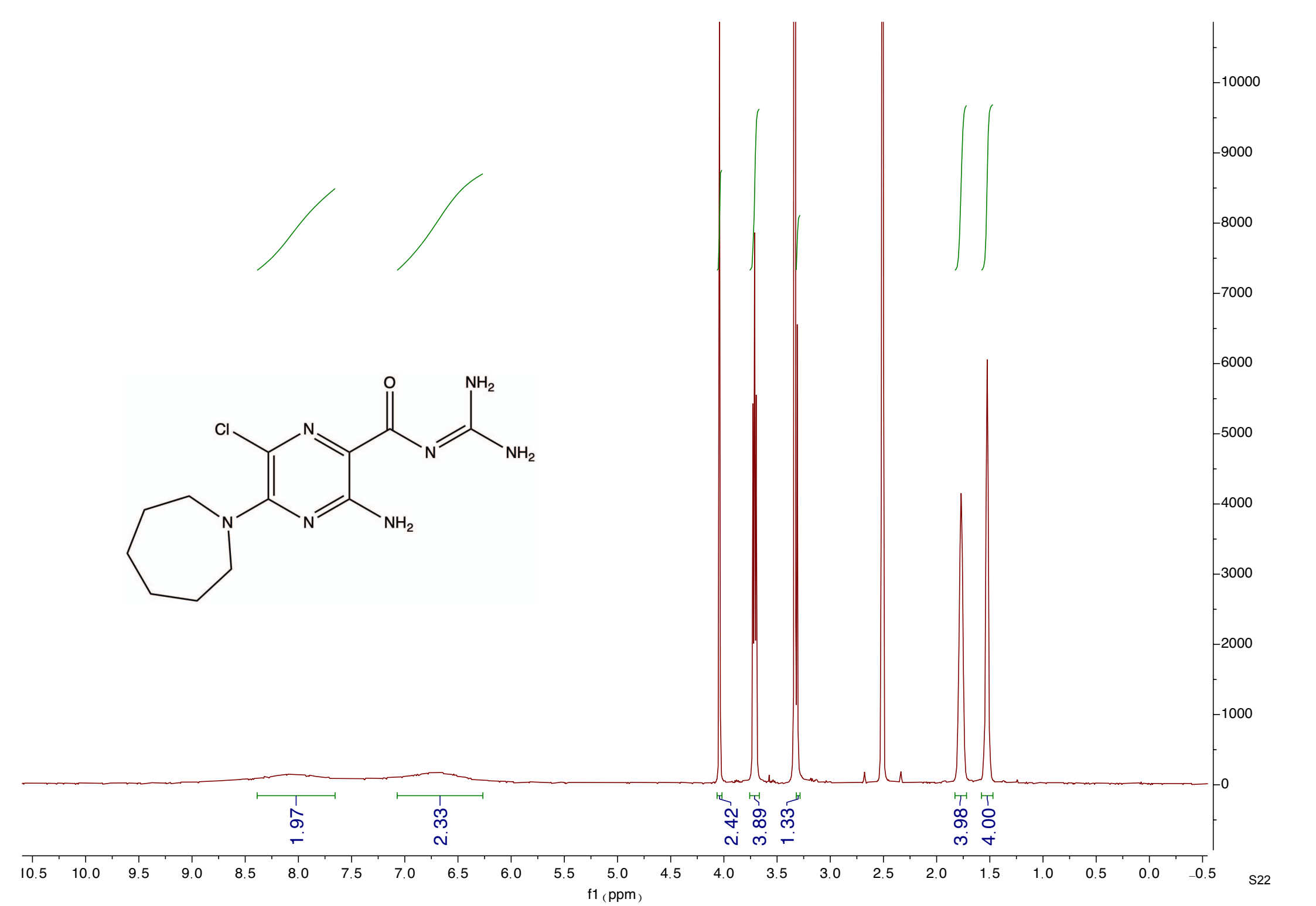


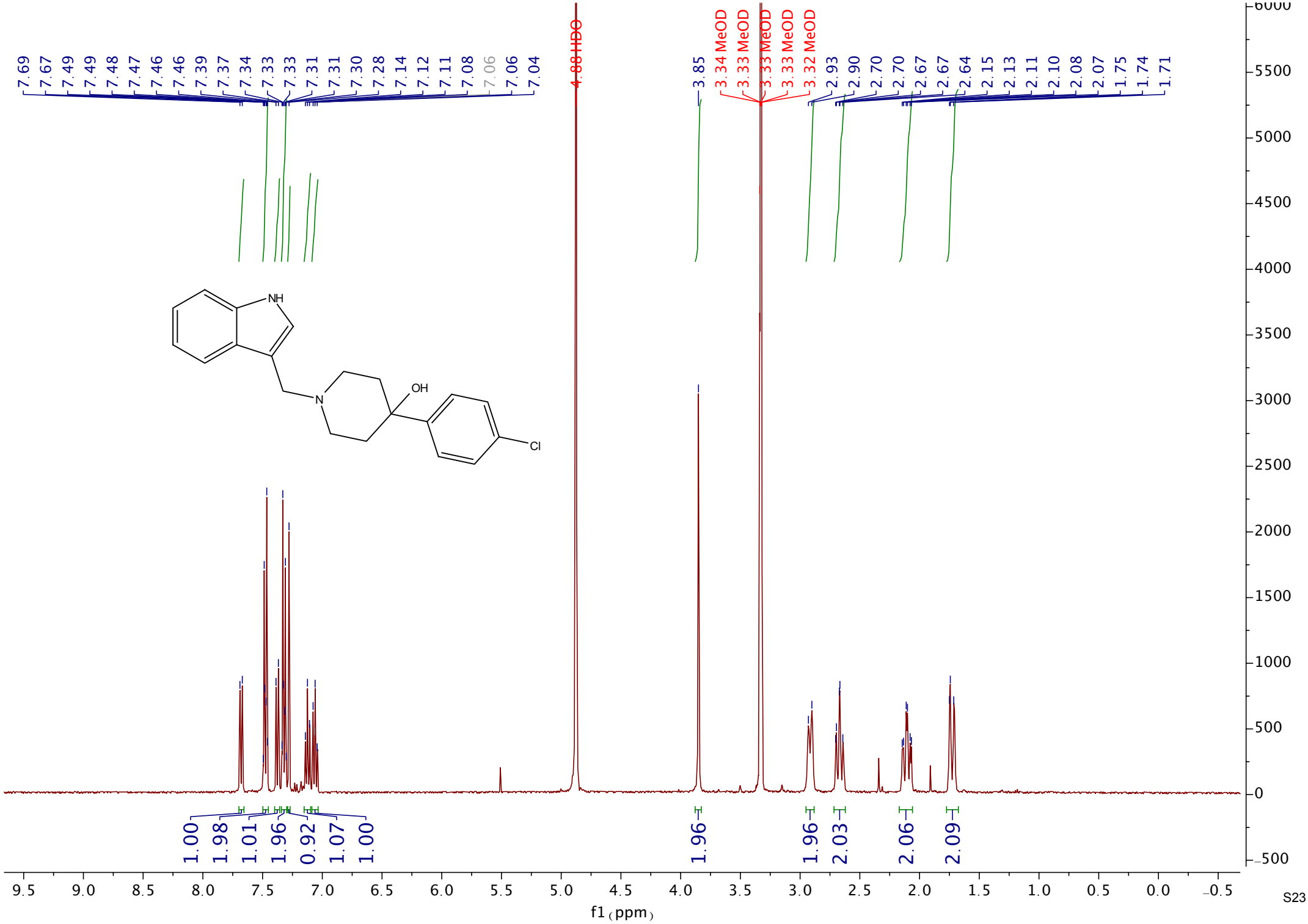
Mass Spectrum

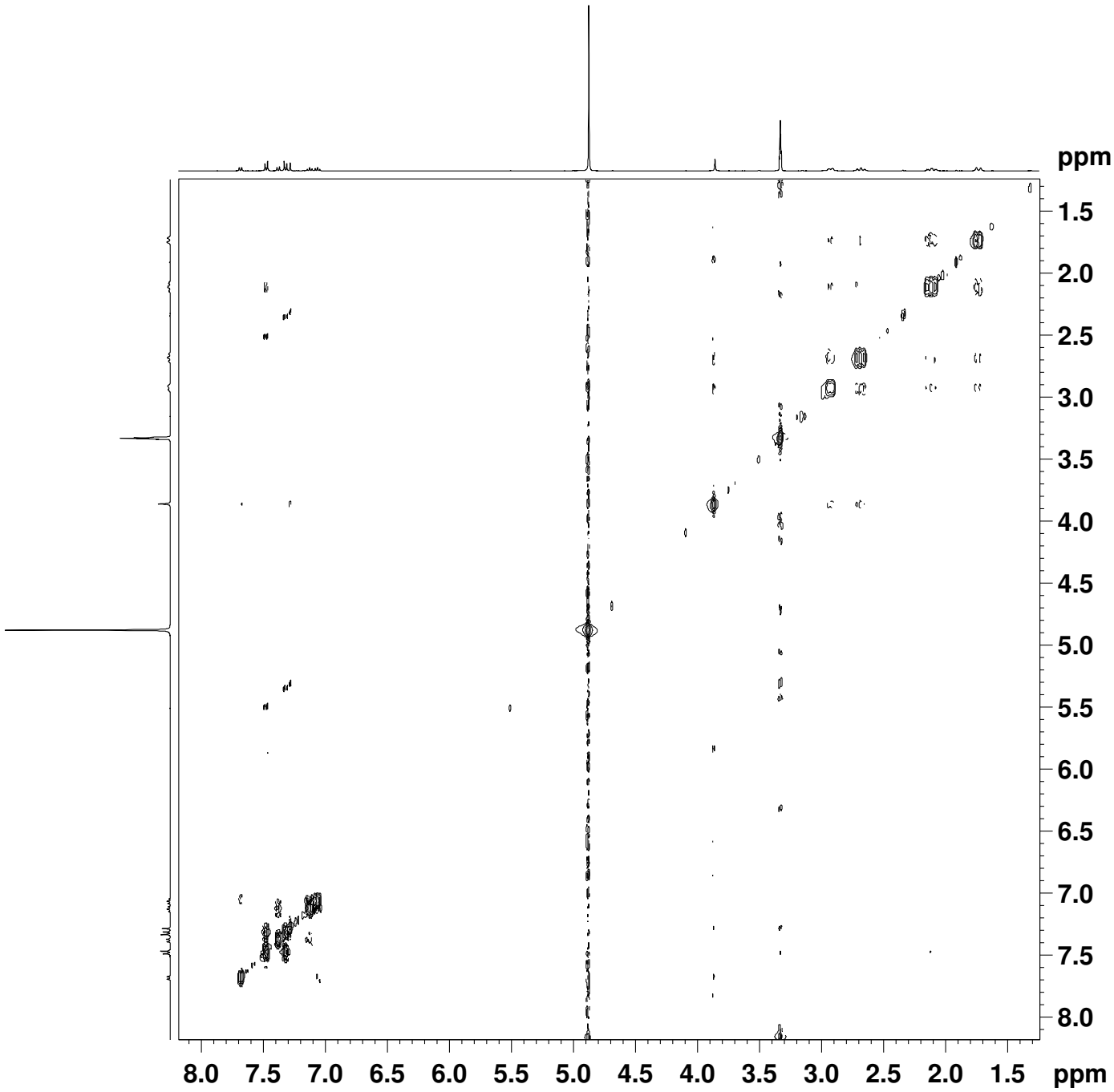
Retention time: 0.781

Spectrum Mode:Single 0.781(163) Base Peak:218.80(2647371)
BG Mode:Averaged 0.431-1.301(93-267) Segment 1 - Event 1

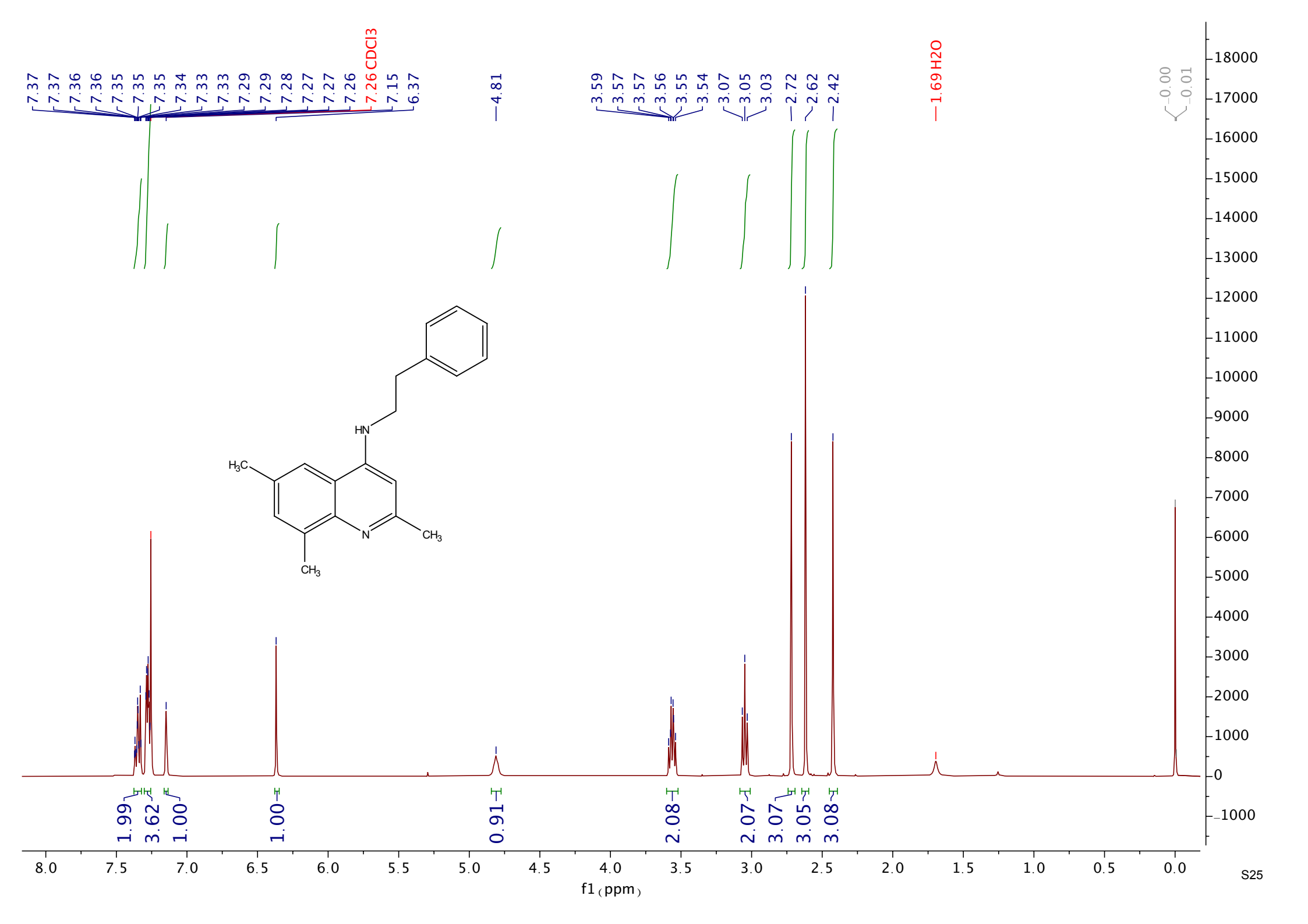


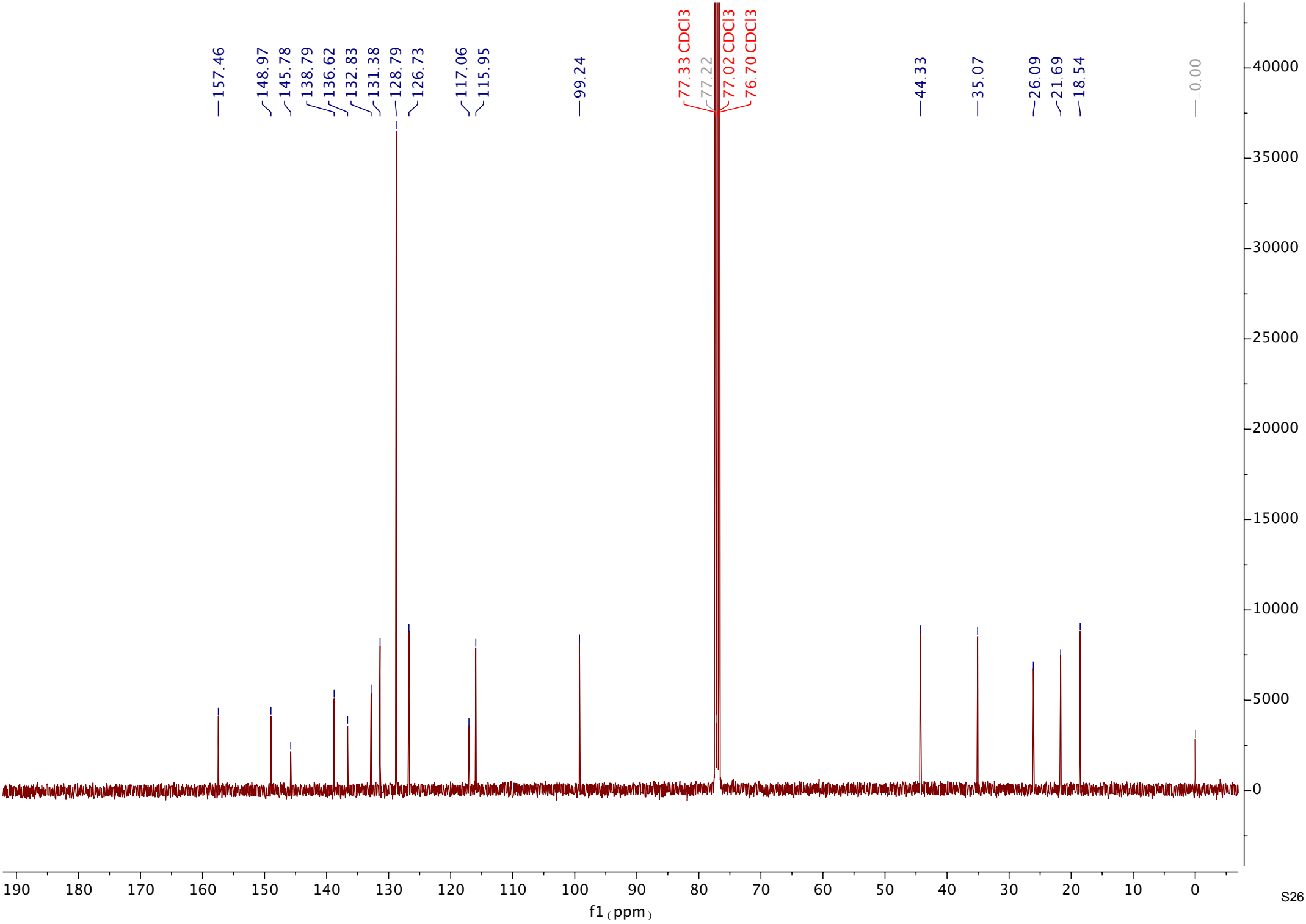

Cl
N
O
NH2
N
NH2
N
N
NH2
1.97
2.33
2.42
3.89
1.33
3.98
4.00
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

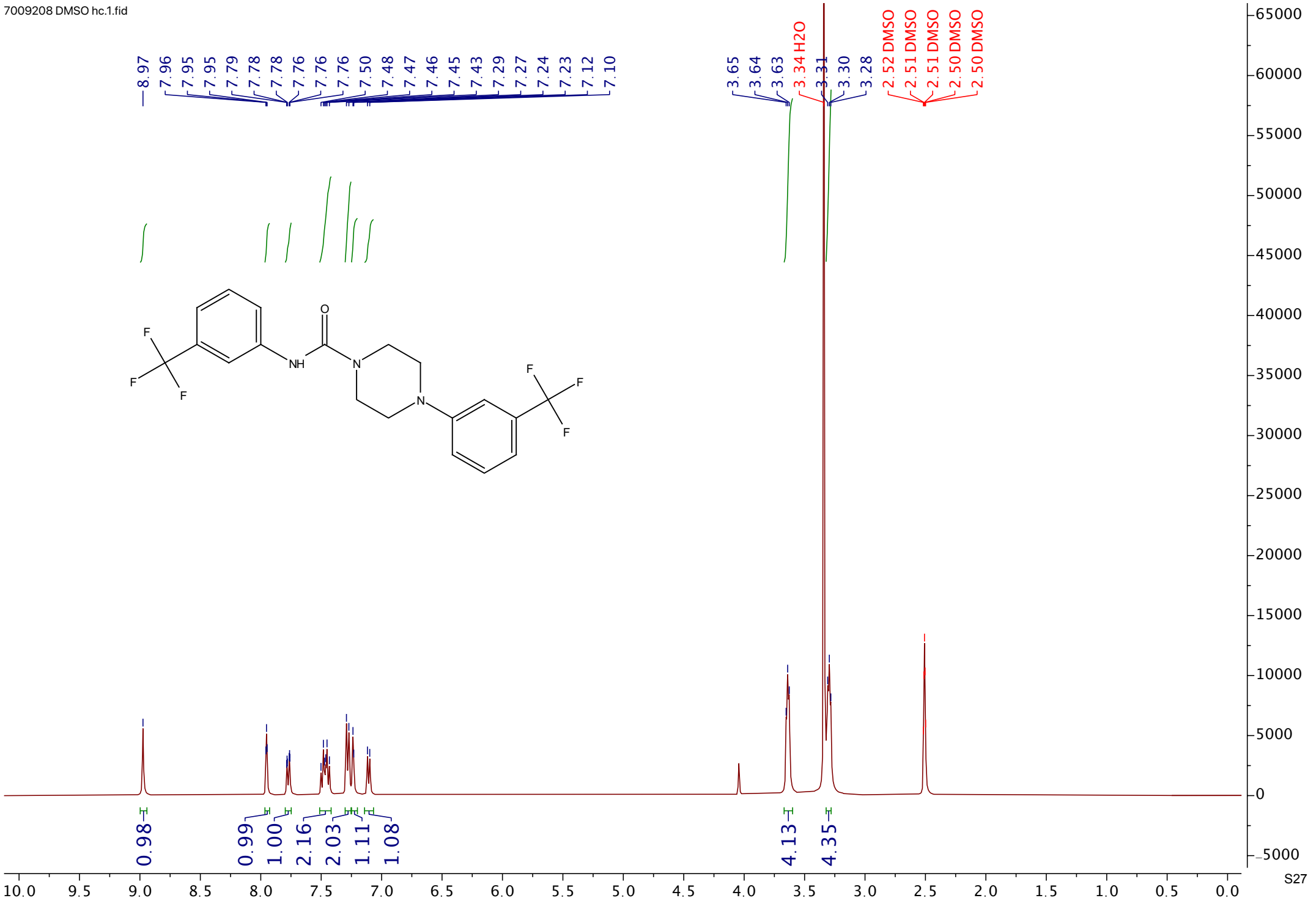

7.69
7.67
7.49
7.49
7.48
7.47
7.46
7.46
7.39
7.37
7.34
7.33
7.33
7.31
7.31
7.30
7.28
7.14
7.12
7.11
7.08
7.06
7.06
7.04
4.88 HDO
3.85
3.34 MeOD
3.33 MeOD
3.33 MeOD
3.33 MeOD
3.32 MeOD
2.93
2.90
2.70
2.70
2.67
2.67
2.64
2.15
2.13
2.11
2.10
2.08
2.07
1.75
1.74
1.71
NH
N
OH
Cl
1.00
1.98
1.01
1.96
0.92
1.07
1.00
1.96
1.96
2.03
2.06
2.09
f1 (ppm)
S23

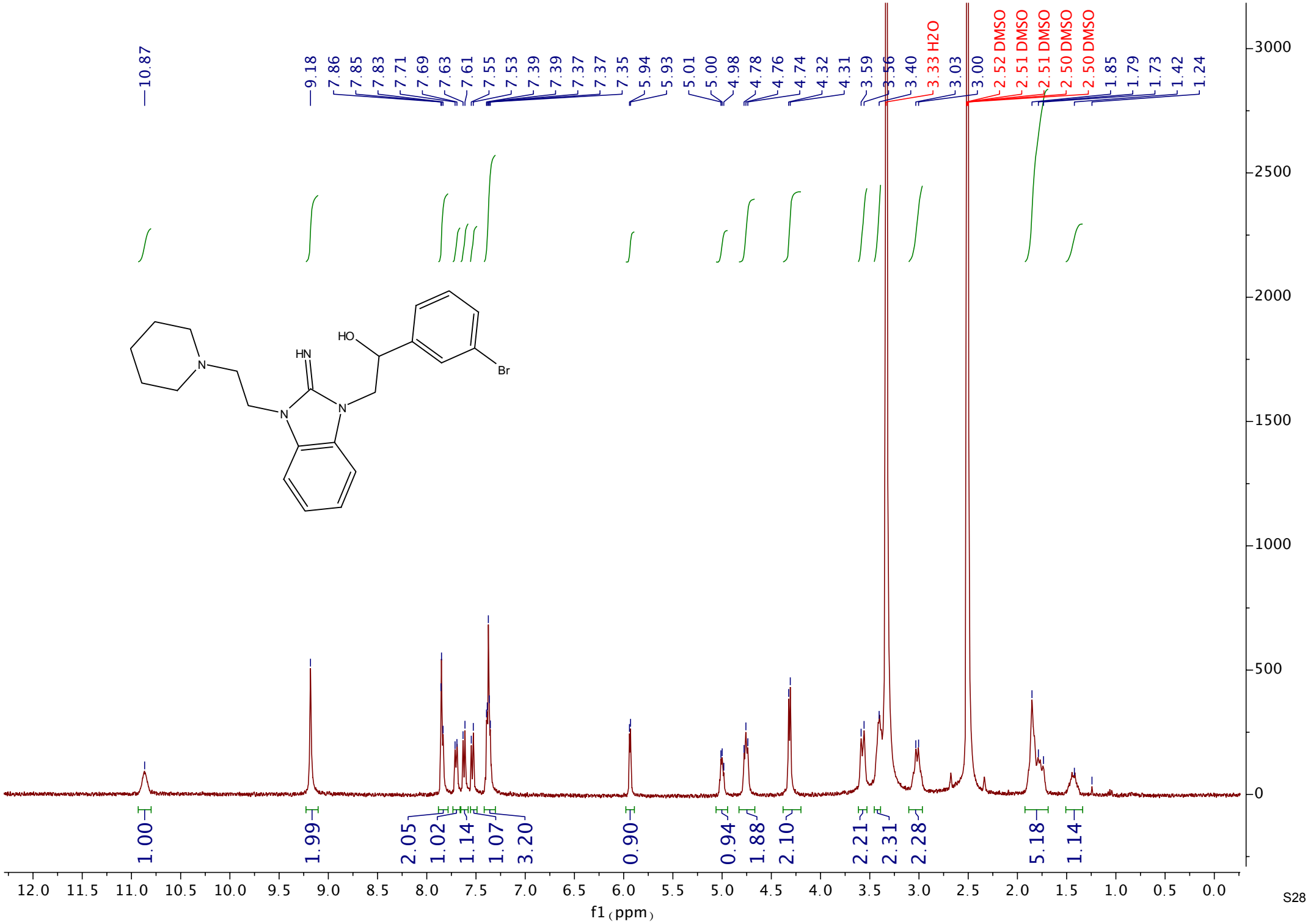


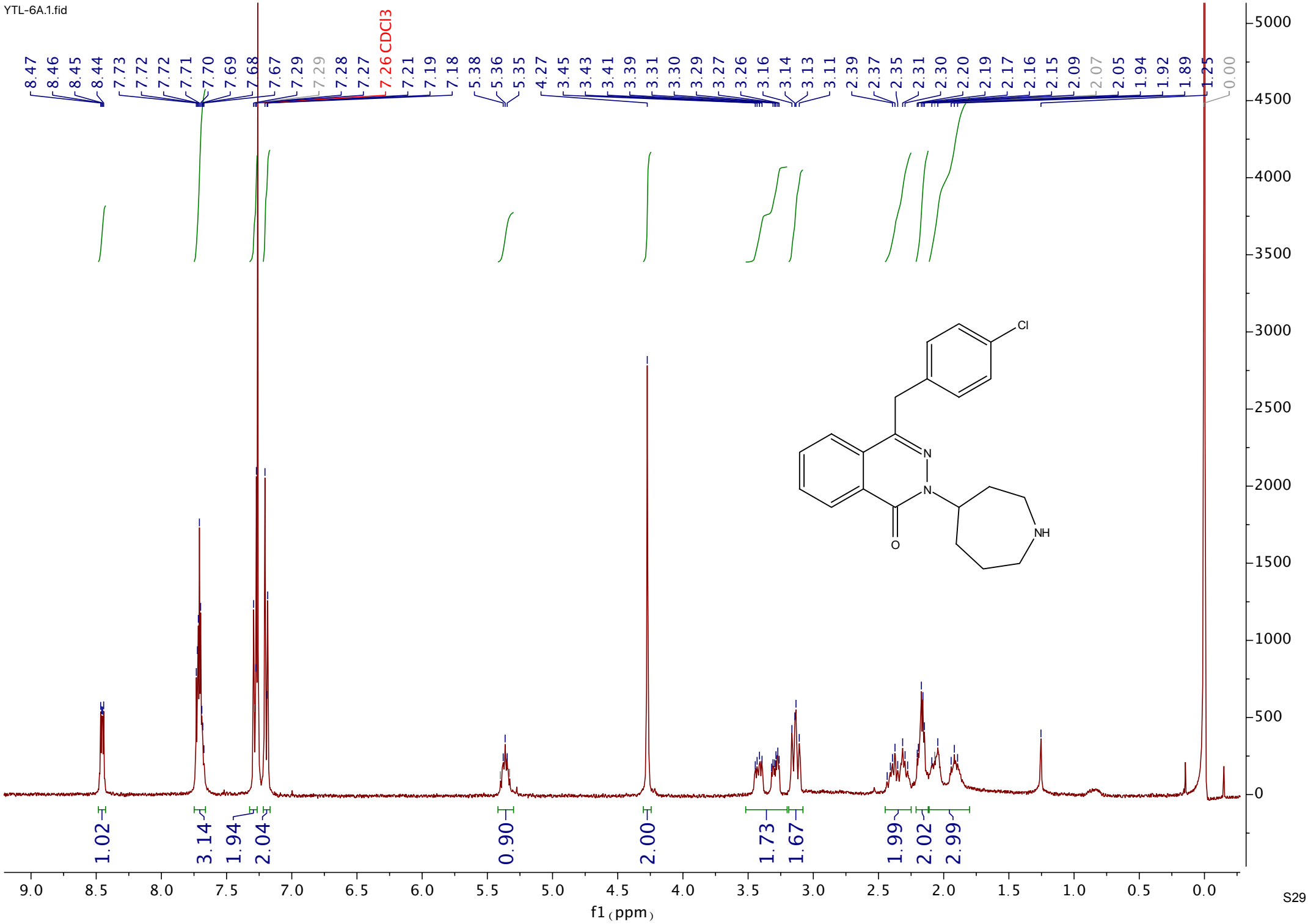
NAME	2NOESY	
EXPNO	2	
PROCNO	1	
Date_	20220422	
Time	22.06	
INSTRUM	spect	
PROBHD	5 mm PABBO BB-	
PULPROG	noesyph	
TD	2048	
SOLVENT	MeOD	
NS	16	
DS	4	
SWH	2777.778	Hz
FIDRES	1.356337	Hz
AQ	0.3686900	sec
RG	203	
DW	180.000	usec
DE	6.50	usec
TE	298.0	K
D0	0.00015976	sec
D1	1.88203502	sec
D8	0.30000001	sec
IN0	0.00036000	sec
======== CHANNEL f1 ========		
NUC1	1H	
P1	15.90	usec
PL1	-3.00	dB
PL1W	18.64416504	W
SFO1	400.1318852	MHz
ND0	1	
TD	256	
SFO1	400.1319	MHz
FIDRES	10.850695	Hz
SW	6.942	ppm
FnMODE	States-TPPI	
SI	1024	
SF	400.1300000	MHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	
PC	1.00	
SI	1024	
MC2	States-TPPI	
SF	400.1300000	MHz
WDW	QSINE	
SSB	2	
LB	0.00	Hz
GB	0	

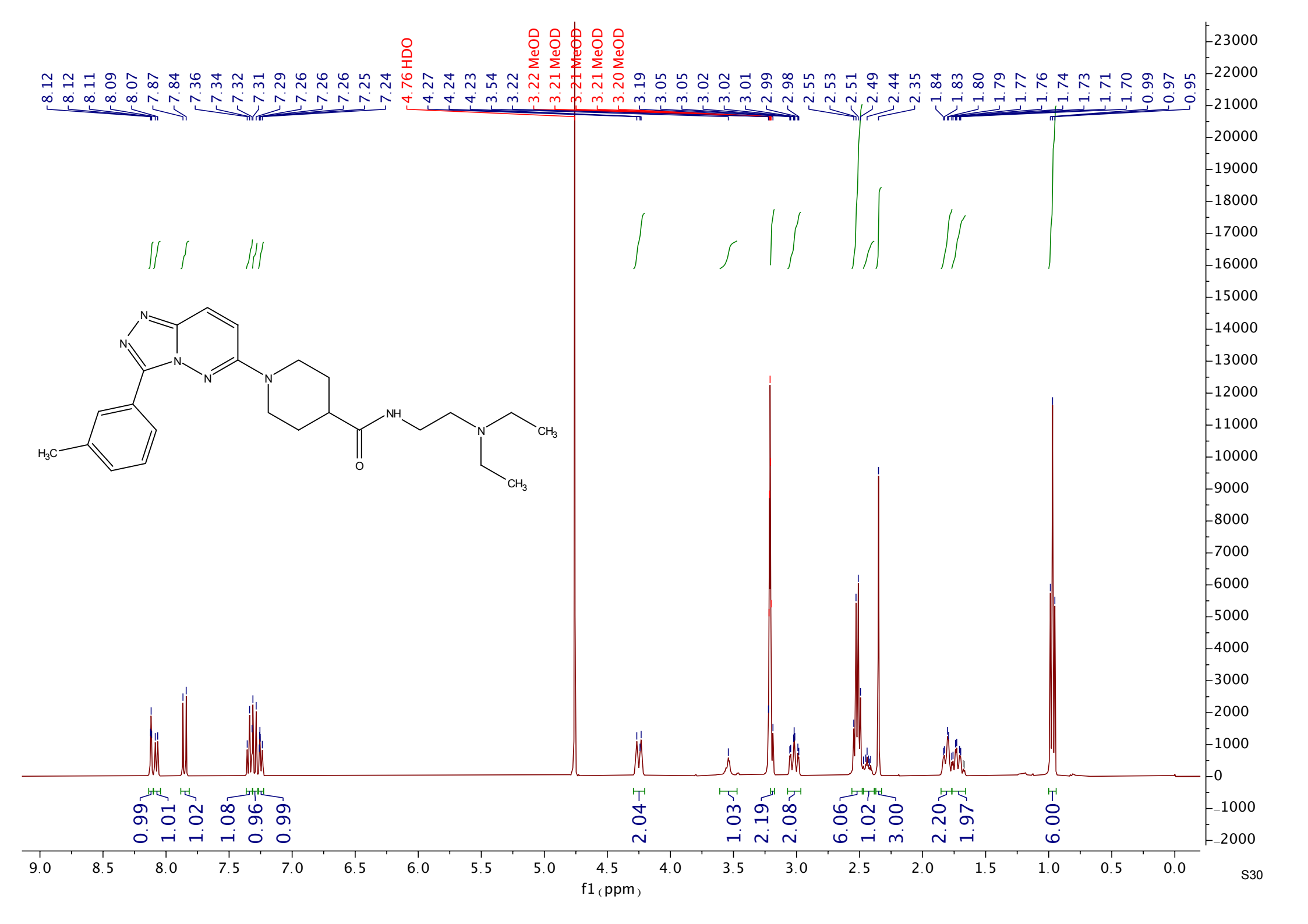

7.37
7.37
7.36
7.36
7.35
7.35
7.35
7.34
7.33
7.33
7.29
7.29
7.28
7.27
7.27
7.26
7.26 CDCl3
7.15
6.37
4.81
3.59
3.57
3.57
3.56
3.55
3.54
3.07
3.05
3.03
2.72
2.62
2.42
1.69 H2O
0.00
0.01
HN
H3C
N
CH3
CH3
1.99
3.62
1.00
1.00
0.91
2.08
2.07
3.07
3.05
3.08
f1 (ppm)
S25

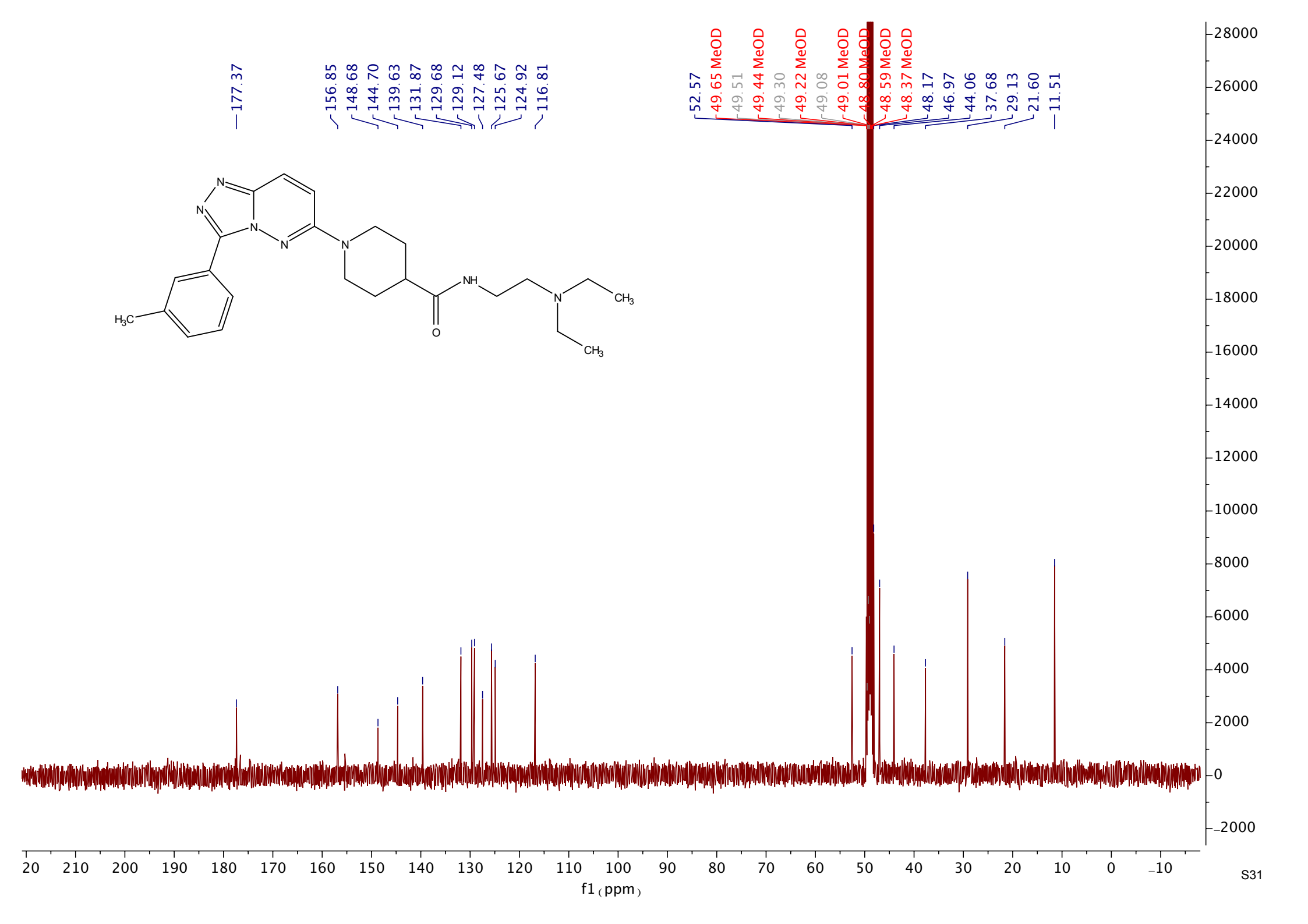

157.46
148.97
145.78
138.79
136.62
132.83
131.38
128.79
126.73
117.06
115.95
99.24
77.33 CDCl3
77.22
77.02 CDCl3
76.70 CDCl3
44.33
35.07
26.09
21.69
18.54
0.00
40000
35000
30000
25000
20000
15000
10000
5000
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7009208 DMSO hc.1.fid
8.97
7.96
7.95
7.95
7.79
7.78
7.78
7.76
7.76
7.76
7.50
7.48
7.47
7.46
7.45
7.43
7.29
7.27
7.24
7.23
7.12
7.10
3.65
3.64
3.63
3.34 H2O
3.31
3.30
3.28
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
O
NH
N
N
F
F
F
F
F
F
0.98
0.99
1.00
2.16
2.03
1.11
1.08
4.13
4.35
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
-5000
10.0
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


10.87
9.18
7.86
7.85
7.83
7.71
7.69
7.63
7.61
7.55
7.53
7.39
7.39
7.37
7.37
7.35
5.94
5.93
5.01
5.00
4.98
4.78
4.76
4.74
4.32
4.31
3.59
3.56
3.40
3.33 H2O
3.03
3.00
2.52 DMSO
2.51 DMSO
2.51 DMSO
2.50 DMSO
2.50 DMSO
1.85
1.79
1.73
1.42
1.24
HO
HN
N
N
N
Br
1.00
1.99
2.05
1.02
1.14
1.07
3.20
0.90
0.94
1.88
2.10
2.21
2.31
2.28
5.18
1.14
f1 (ppm)


YTL-6A.1.fid
8.47 8.46 8.45 8.44 7.73 7.72 7.72 7.71 7.70 7.69 7.68 7.67 7.29 7.29 7.28 7.27 7.26 CDCl3 7.21 7.19 7.18 5.38 5.36 5.35 4.27 3.45 3.43 3.41 3.39 3.31 3.30 3.29 3.27 3.26 3.16 3.14 3.13 3.11 2.39 2.37 2.35 2.31 2.30 2.20 2.19 2.17 2.16 2.15 2.09 2.07 2.05 1.94 1.92 1.89 1.25 0.00
Cl
N
N
NH
O
1.02 3.14 1.94 2.04 0.90 2.00 1.73 1.67 1.99 2.02 2.99
f1 (ppm)


8.12 8.12 8.11 8.09 8.07 7.87 7.84 7.36 7.34 7.32 7.31 7.29 7.26 7.26 7.26 7.25 7.24
4.76 HDO
4.27 4.24 4.23 3.54 3.22
3.22 MeOD 3.21 MeOD 3.21 MeOD 3.21 MeOD 3.20 MeOD
3.19 3.05 3.05 3.02 3.02 3.01 2.99 2.98 2.55 2.53 2.51 2.49 2.44 2.35 1.84 1.83 1.80 1.79 1.77 1.76 1.74 1.73 1.71 1.70 0.99 0.97 0.95
N N N N N N NH N CH3 CH3 H3C O
0.99 1.01 1.02 1.08 0.96 0.99 2.04 1.03 2.19 2.08 6.06 1.02 3.00 2.20 1.97 6.00
f1 (ppm)
S30


177.37
156.85
148.68
144.70
139.63
131.87
129.68
129.12
127.48
125.67
124.92
116.81
52.57
49.65 MeOD
49.51
49.44 MeOD
49.30
49.22 MeOD
49.08
49.01 MeOD
48.80 MeOD
48.59 MeOD
48.37 MeOD
48.17
46.97
44.06
37.68
29.13
21.60
11.51
N
N
N
N
N
NH
O
N
CH3
CH3
H3C
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
−2000
20
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
−10
f1 (ppm)
S31