SUPPORTING INFORMATION

Small molecule inhibitors of Ca^{2+}-S100B reveal two protein conformations

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Table S1. Calculated RMSD (Å) of Models from C$^{\alpha}$ of 1MHO		
^{Ca}S100B•**5a**	0.830 (0.307)	
^{Ca}S100B•**6b**	0.829 (0.328)	
^{Ca}S100B•**17**	0.215 (0.215)	0.360 (0.235)

The protein chains of the introduced models were aligned with the protein chain within 1MHO[46]. Numbers in parentheses are the calculated RMSD of only the globally conserved residues 1-84.

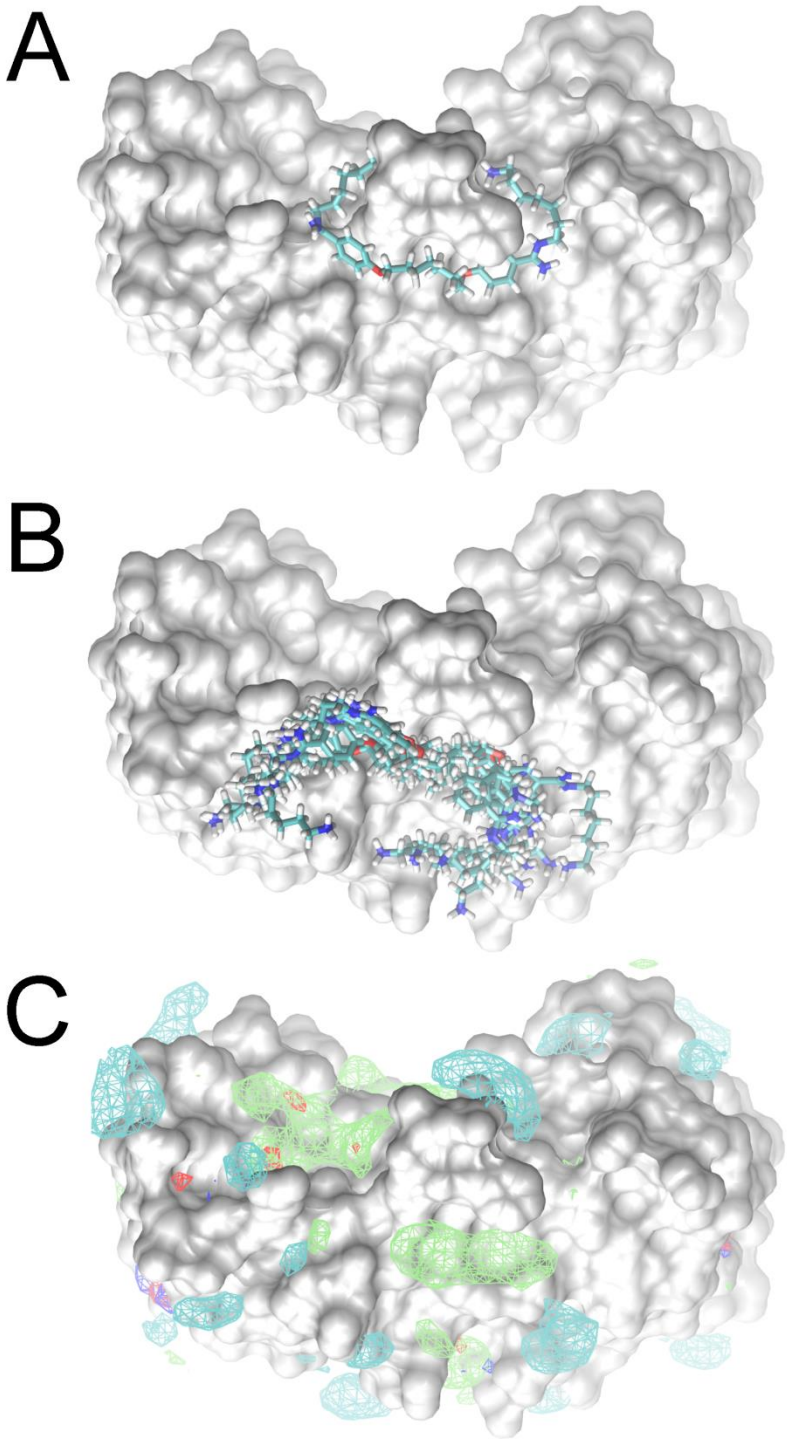
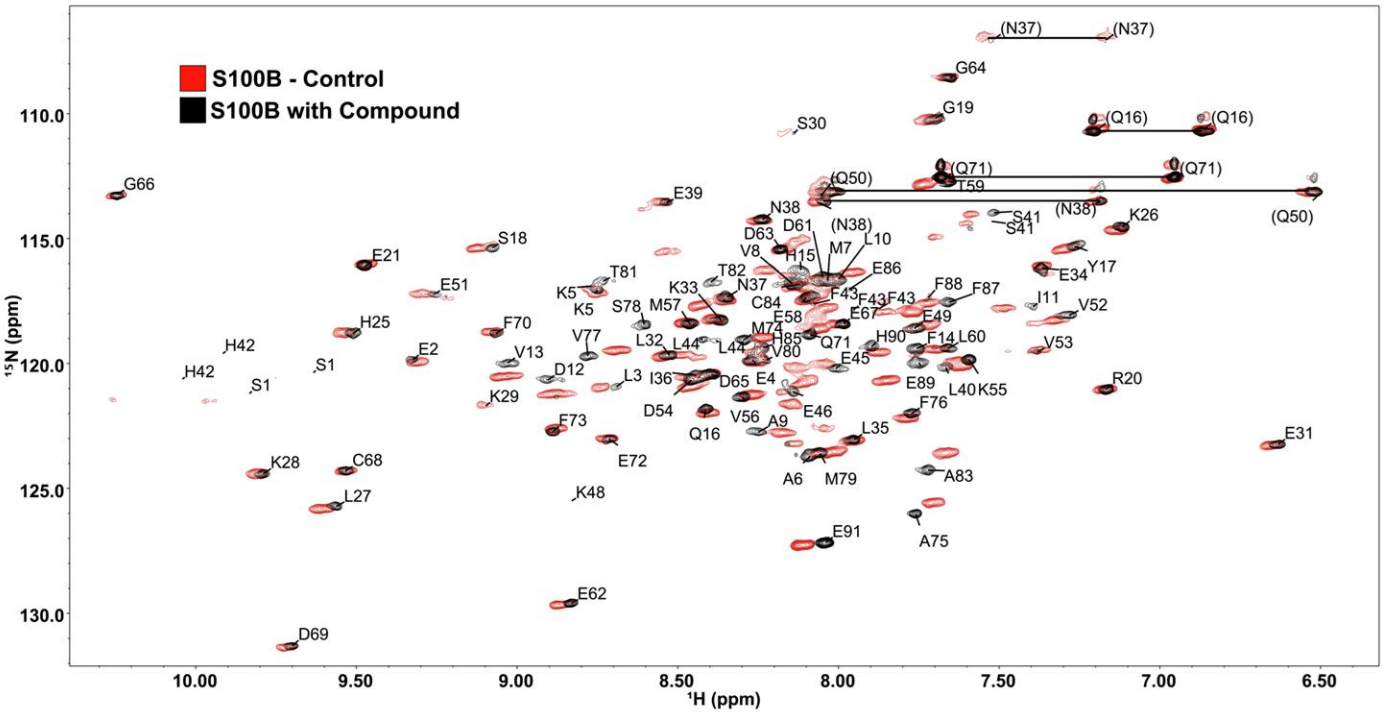
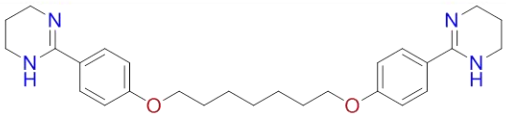


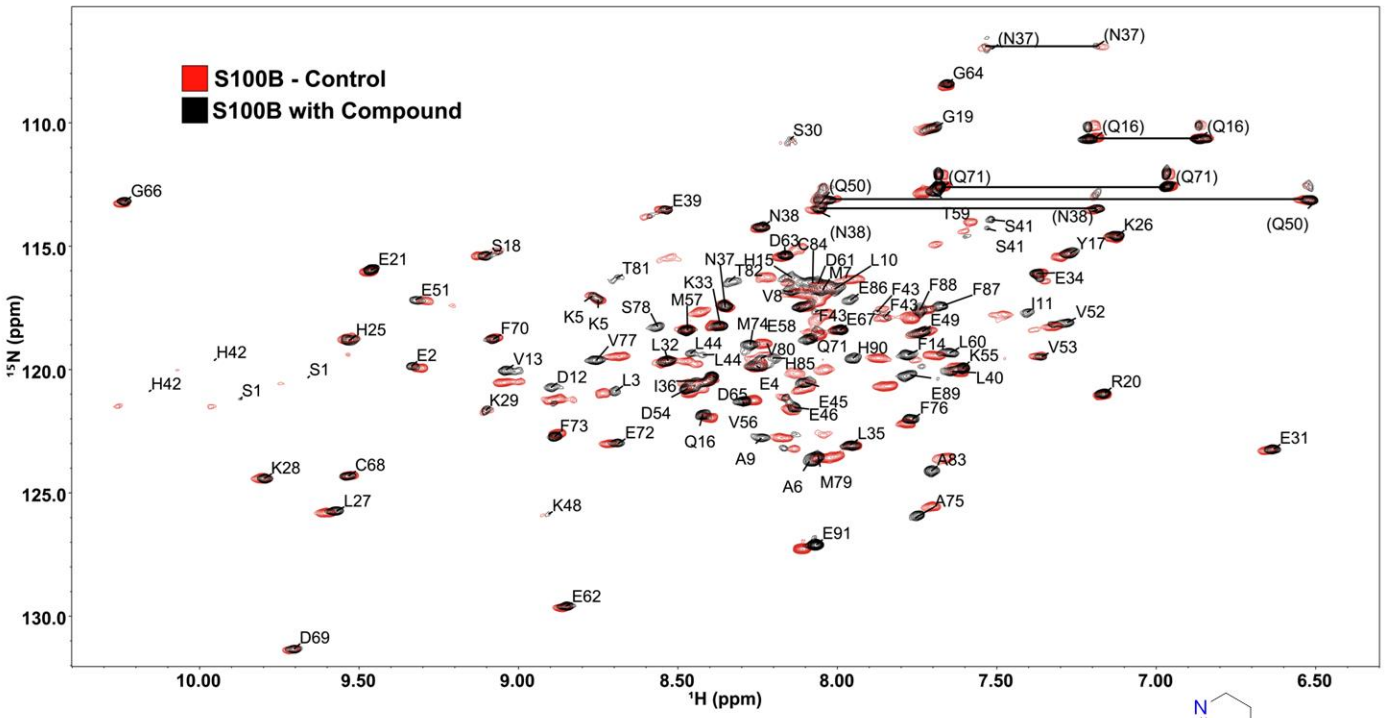
Figure S1. The crystal structure of S100B overlaid with compound 9a. (A) Top scoring conformation predicted by AutoDock, (B) Representative conformations calculated by MC-SILCS, (C) SILCS FragMaps are shown at a cutoff of -1.2 kcal/mol. Nonpolar maps are shown in green and positively charged group maps in cyan. The positively charged group maps drive the placement of the basic alkyl groups in the MC-SILCS docking.

Figures S2-5. Assigned 2D ^{1}H-^{15}N HSQC NMR Spectra. The binding of inhibitors to ^{Ca}S100B was assessed by monitoring perturbations of backbone ^{1}H–^{15}N HSQC NMR experiments.

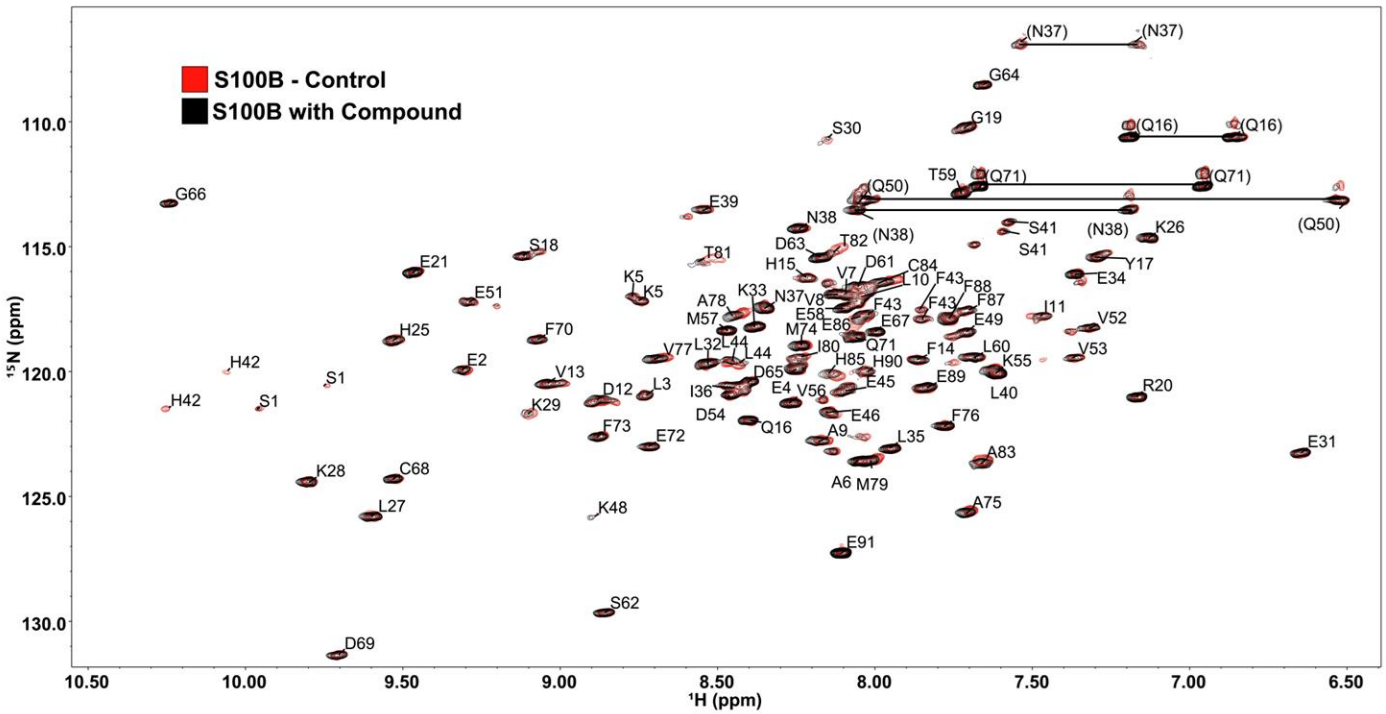


6a HSQC Overlay

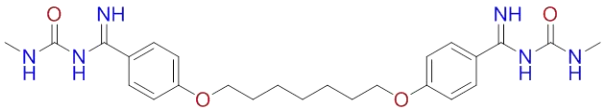


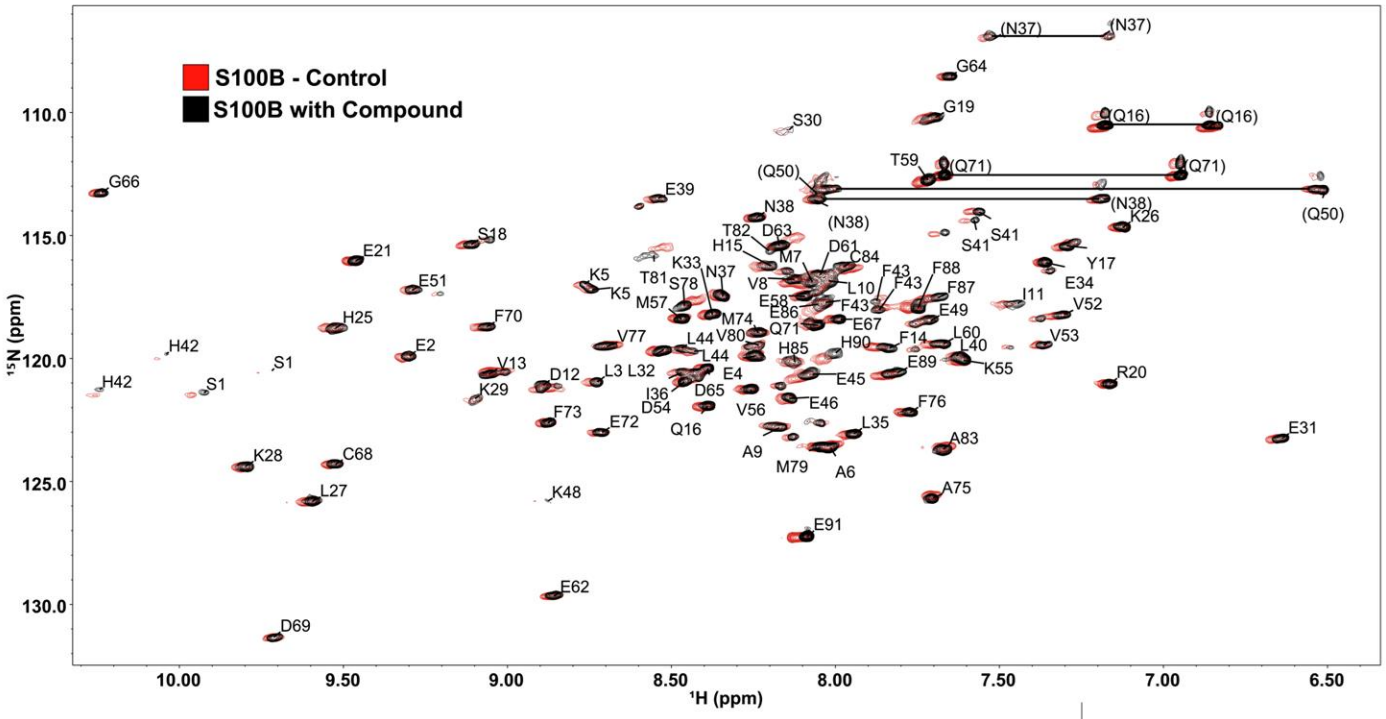


6c HSQC Overlay

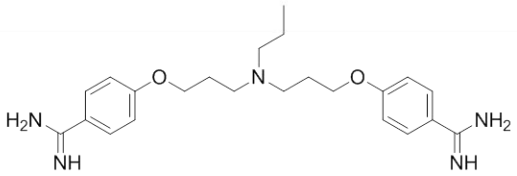


11 HSQC Overlay





16b HSQC Overlay



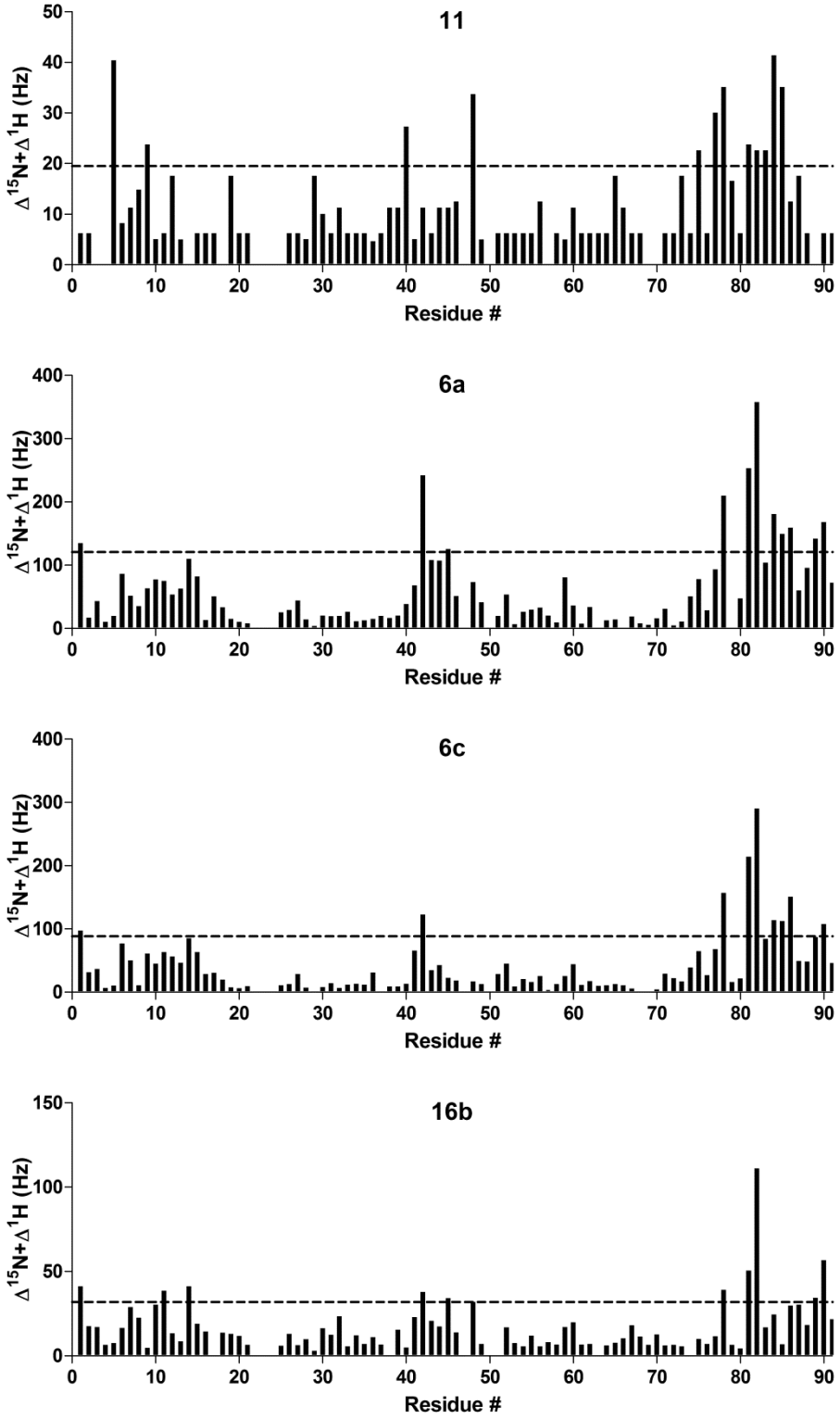
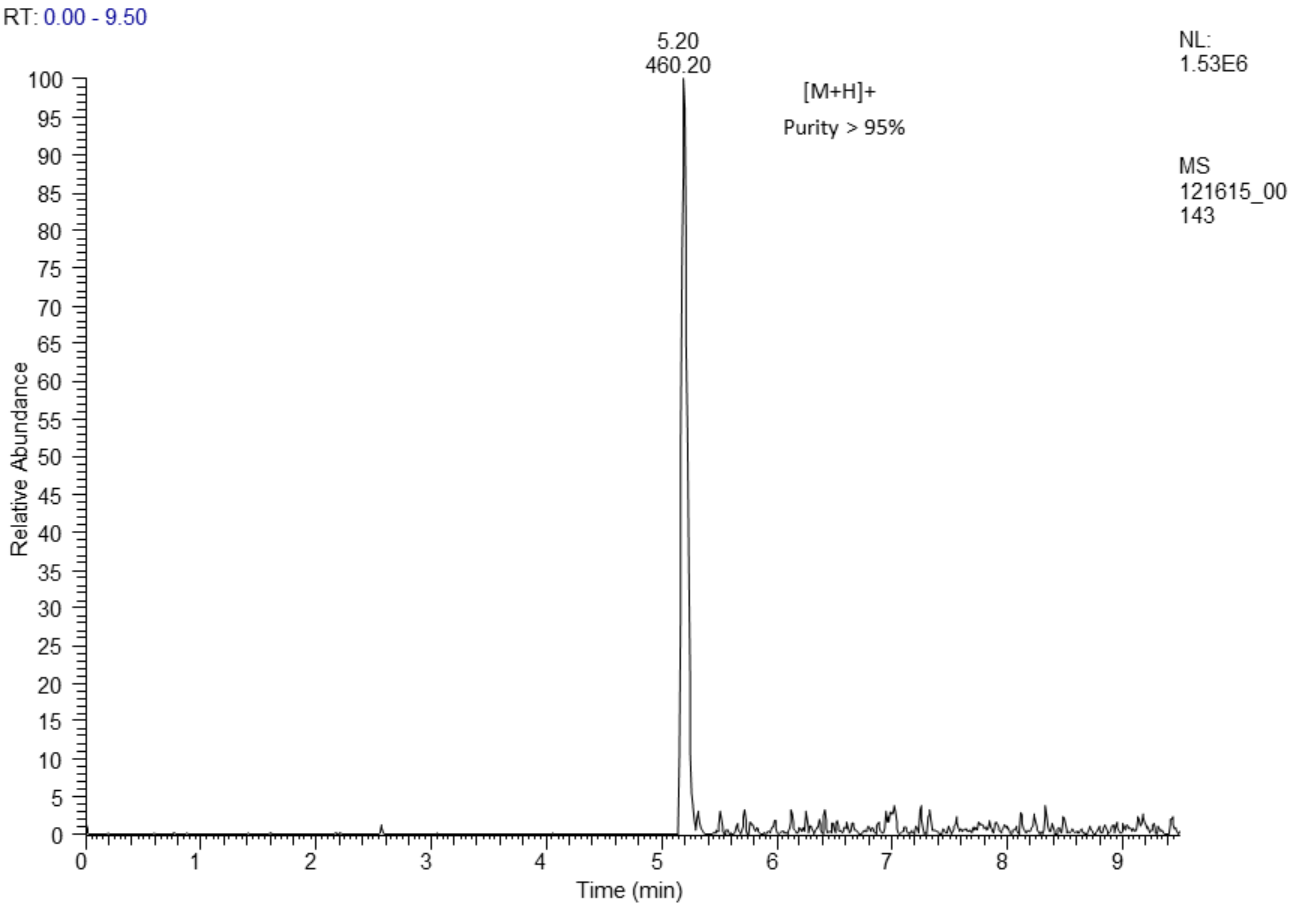


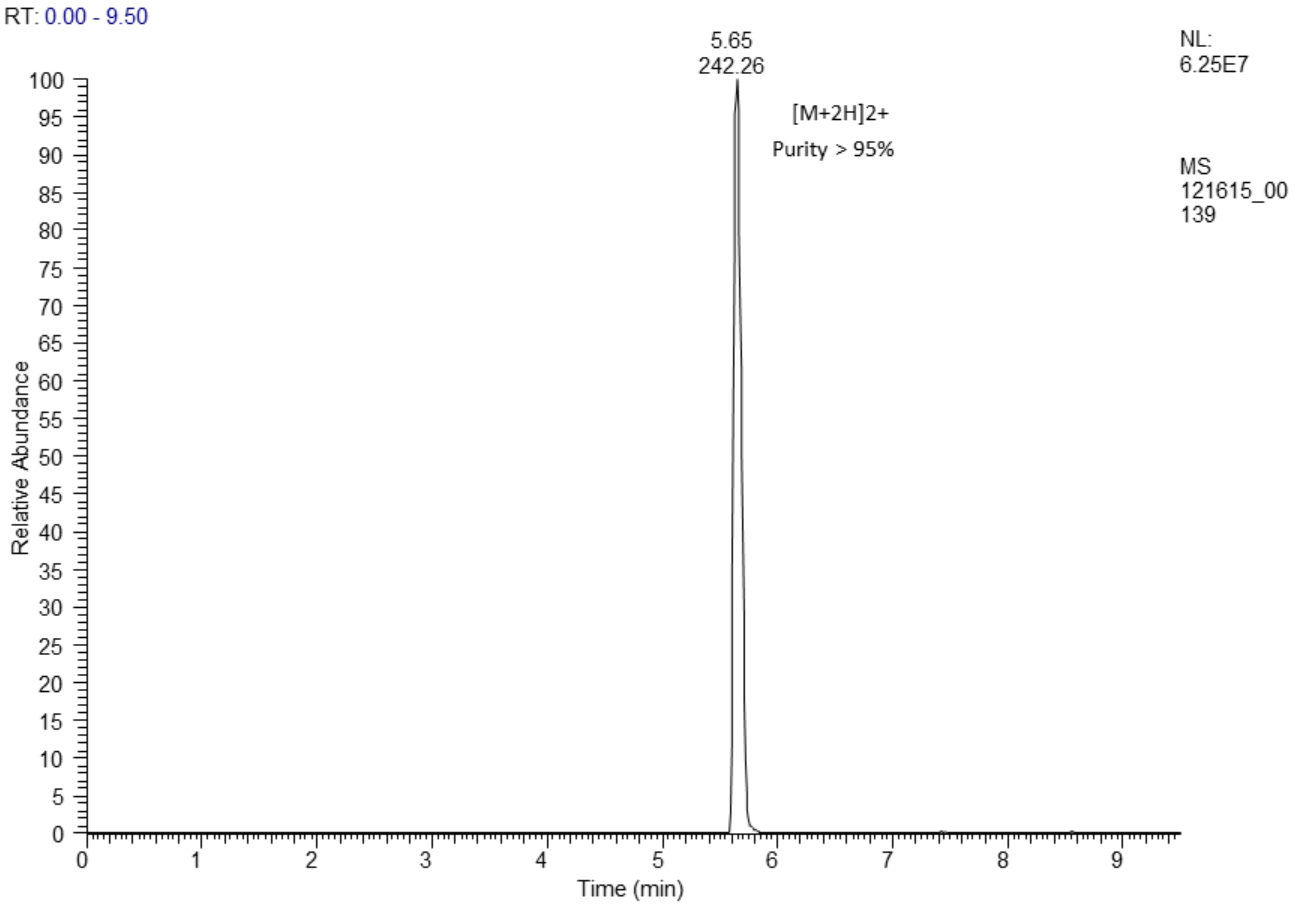
Figure S6. Measurements of Chemical Shift Perturbations from Assigned 2D ^{1}H-^{15}N HSQC NMR spectra. The solid horizontal line is plotted at the mean perturbation (Hz) plus one standard deviation for each dataset.

Figures S7-29. HPLC-MS. Purity of all compounds was determined to be >95% by HPLC.

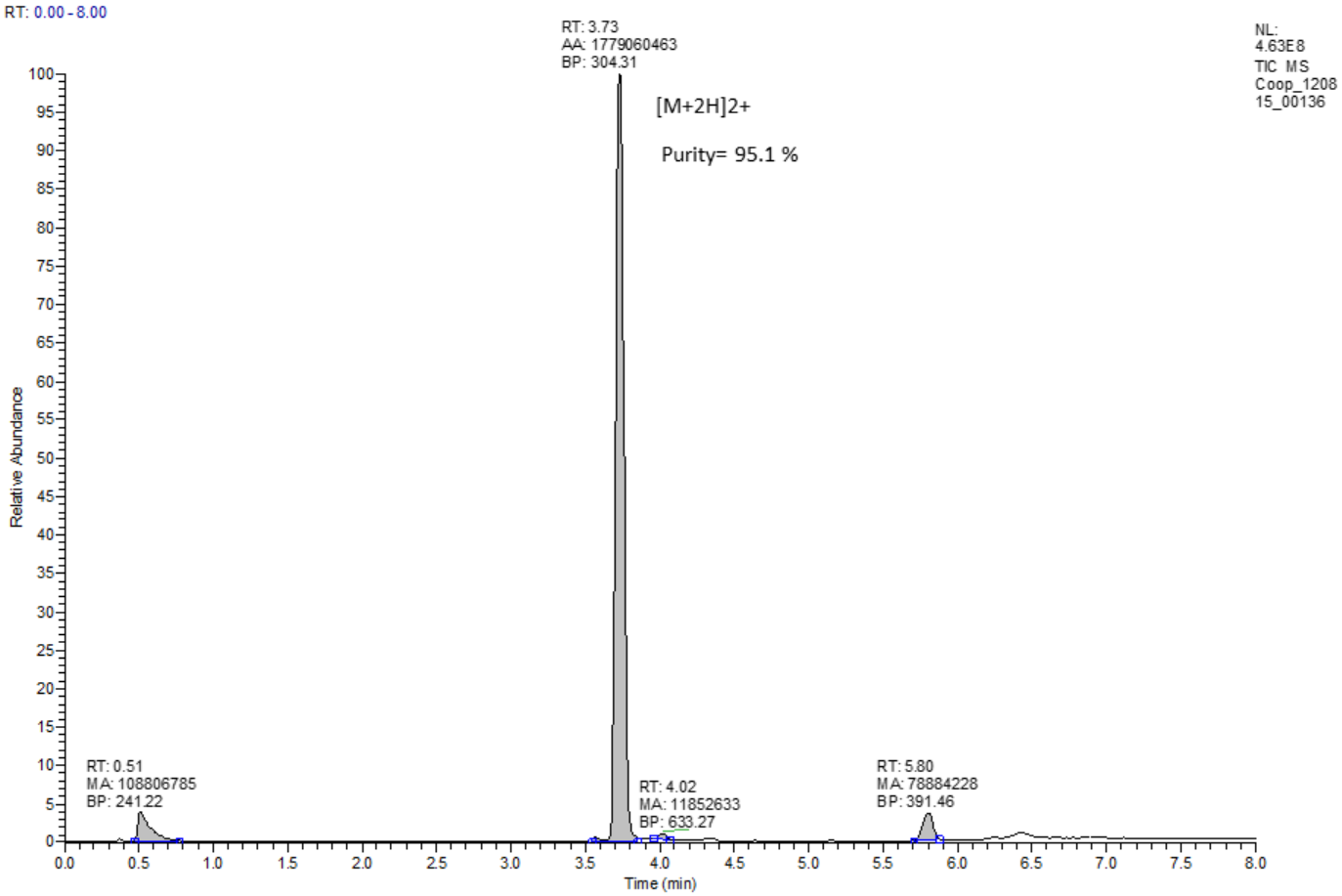
Compound 16a



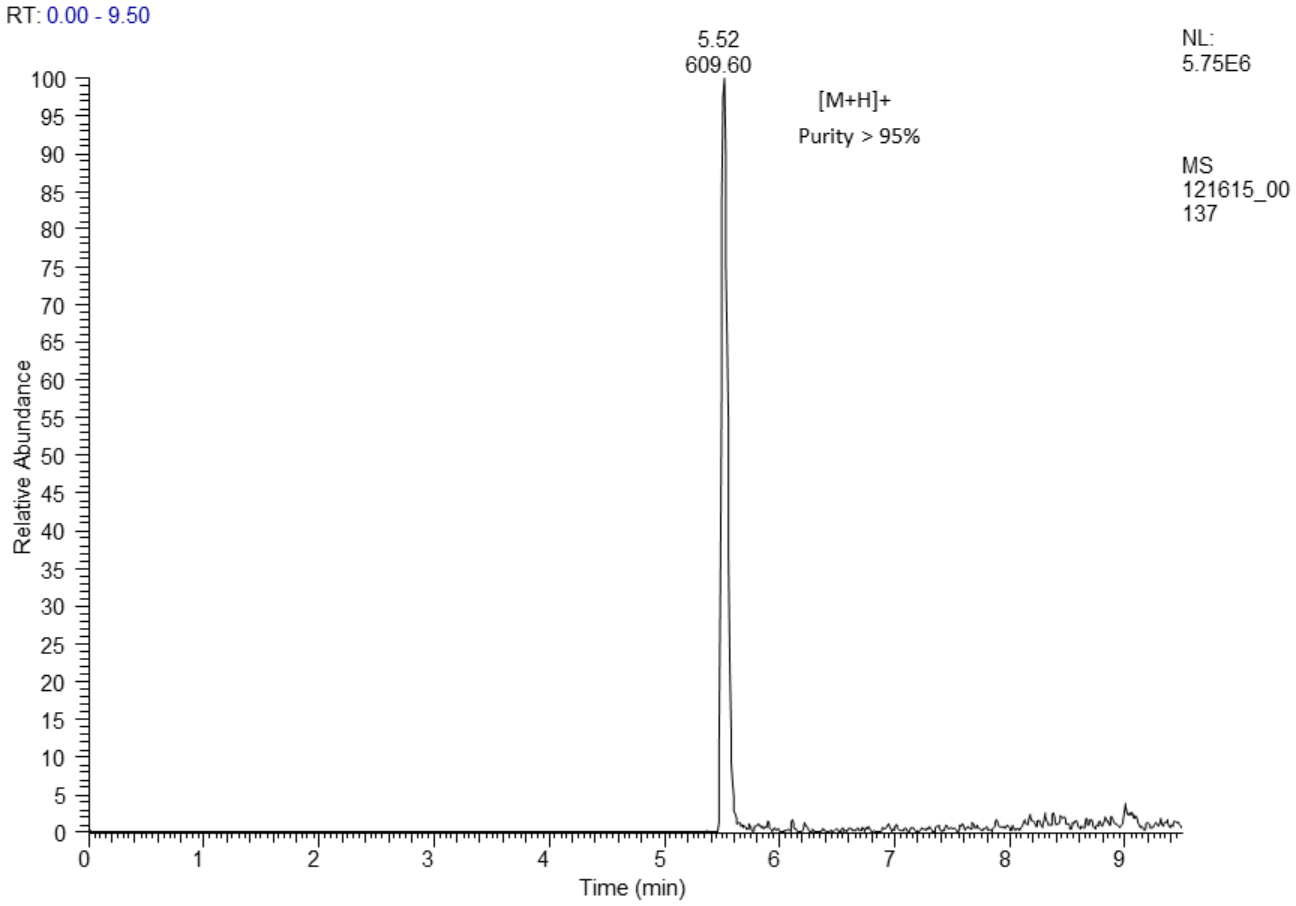
Compound 11


RT: 0.00 - 9.50
5.65
242.26
[M+2H]2+
Purity > 95%
NL:
6.25E7
MS
121615_00
139
Relative Abundance
Time (min)

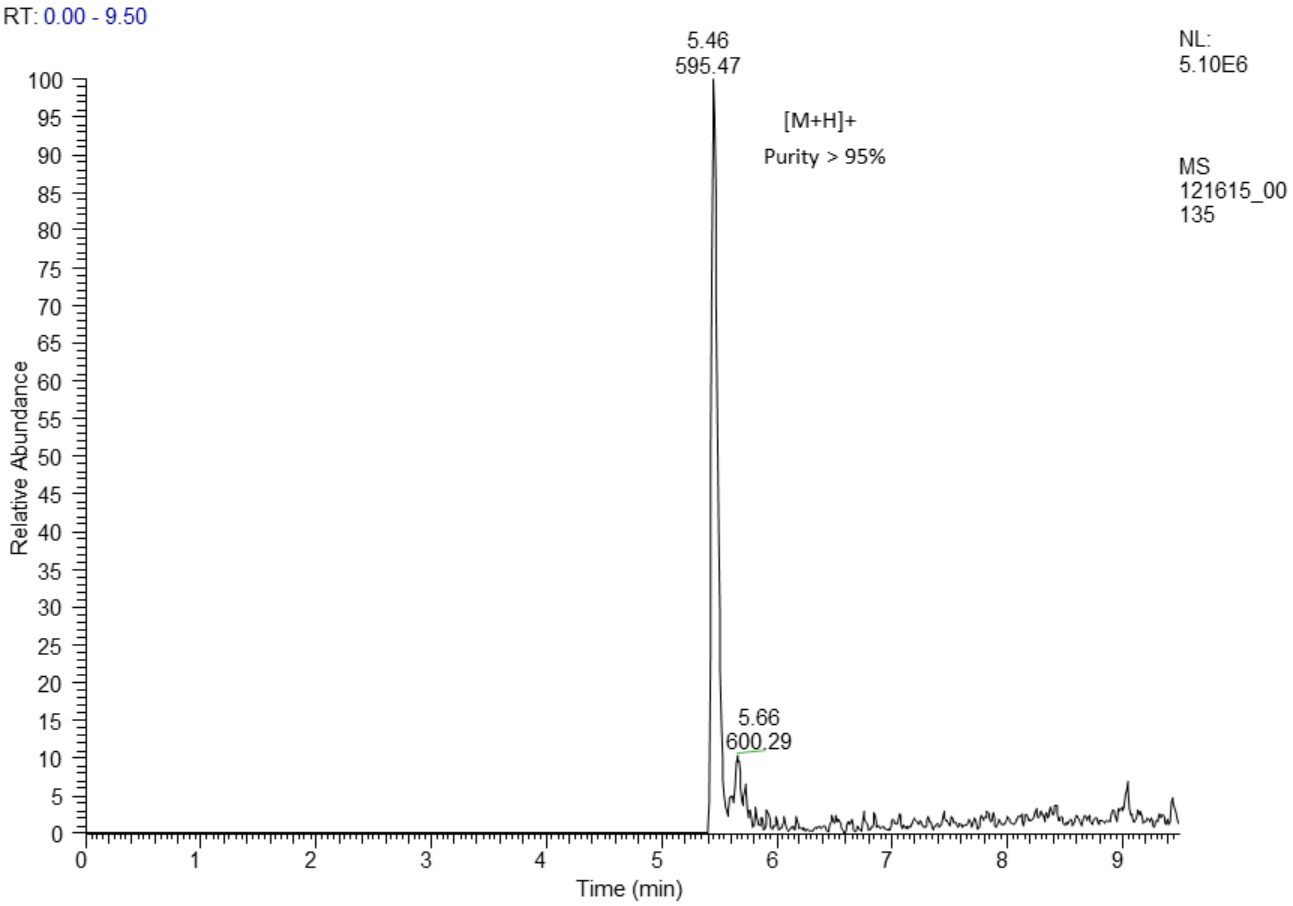
Compound 10


RT: 0.00 - 8.00
RT: 3.73
AA: 1779060463
BP: 304.31
[M+2H]2+
Purity= 95.1 %
NL:
4.63E8
TIC MS
Coop_1208
15_00136
Relative Abundance
Time (min)
RT: 0.51
MA: 108806785
BP: 241.22
RT: 4.02
MA: 11852633
BP: 633.27
RT: 5.80
MA: 78884228
BP: 391.46

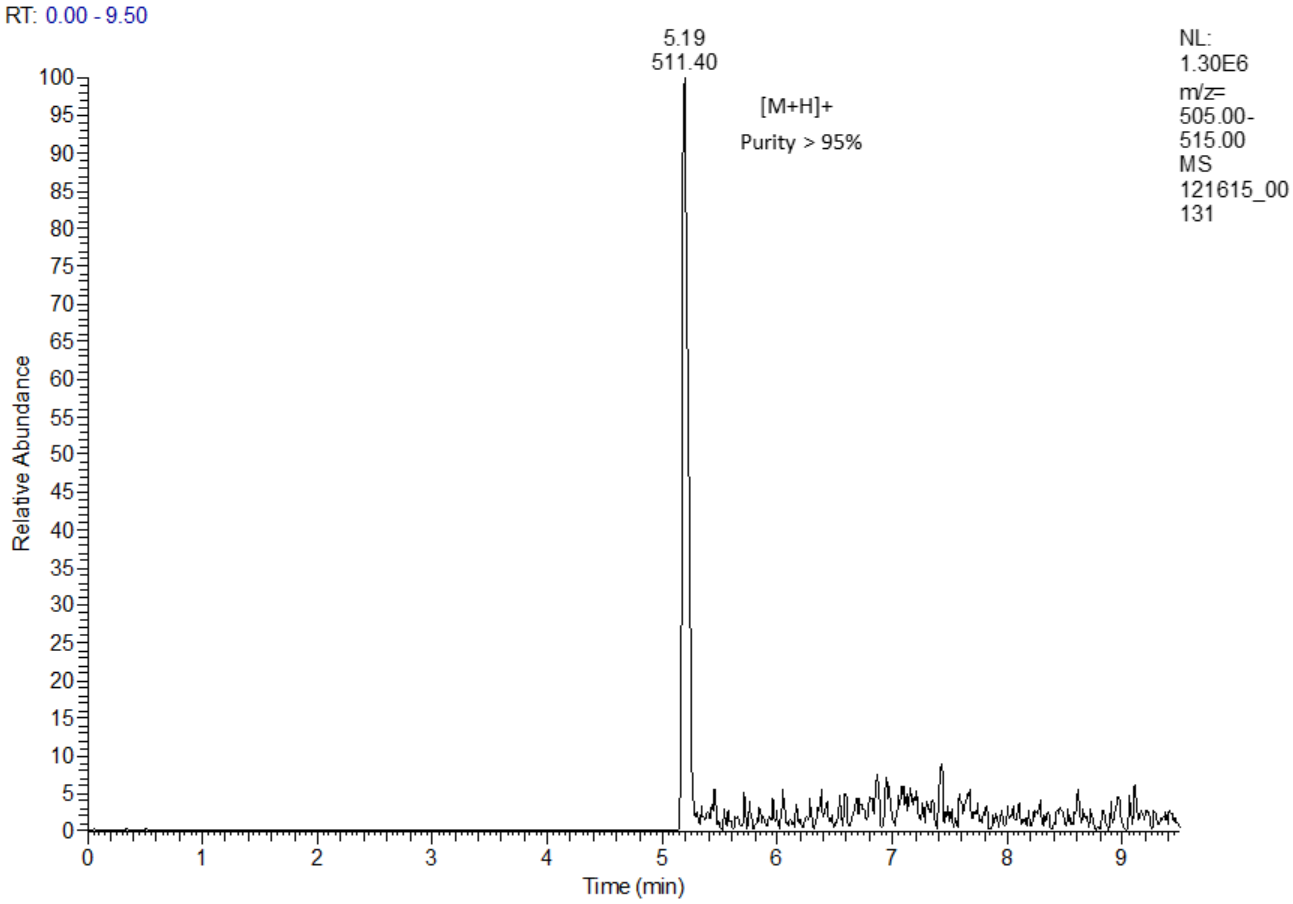
Compound 9b


RT: 0.00 - 9.50
5.52
609.60
[M+H]+
Purity > 95%
NL:
5.75E6
MS
121615_00
137
Relative Abundance
Time (min)

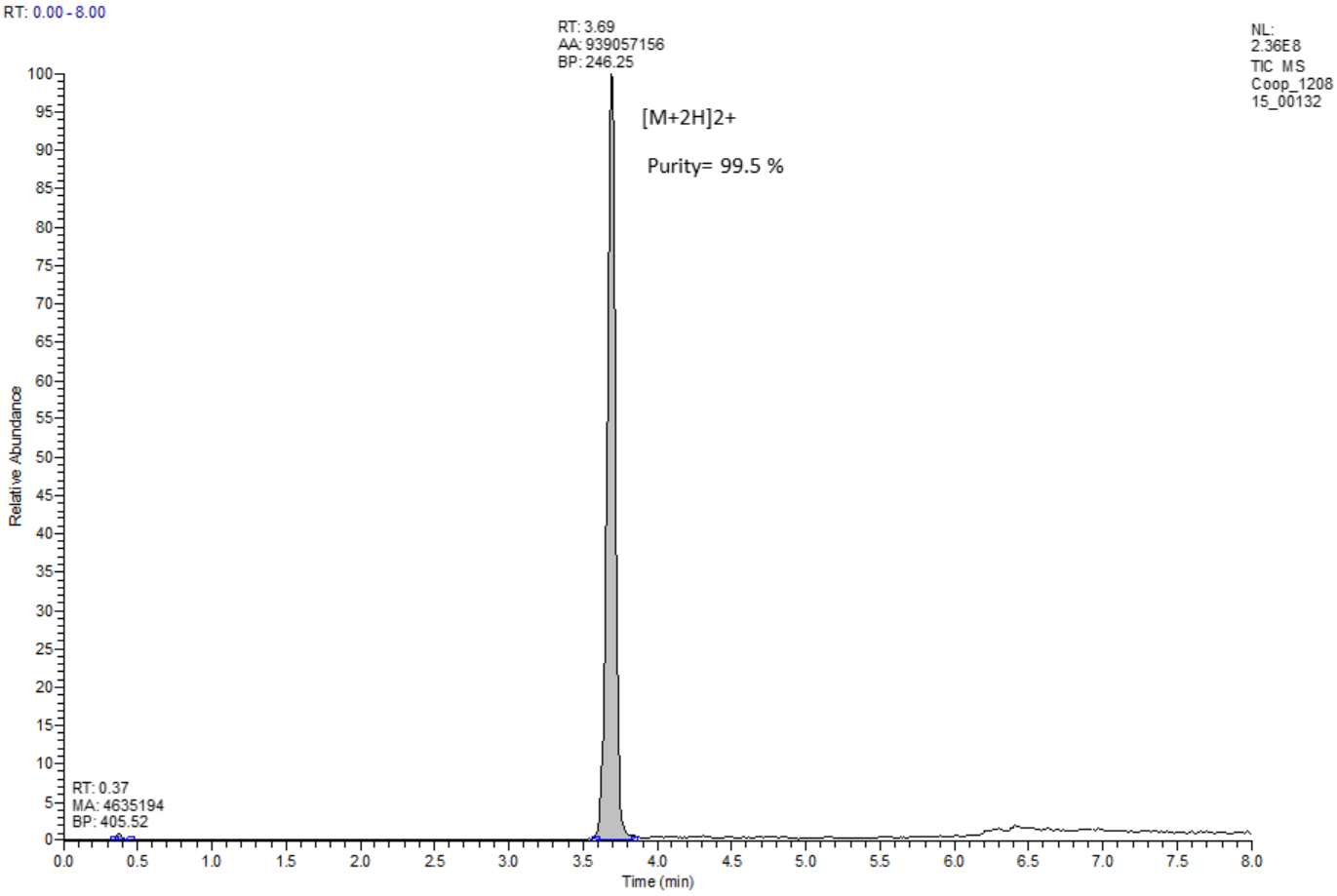
Compound 9a


RT: 0.00 - 9.50
5.46
595.47
[M+H]+
Purity > 95%
NL:
5.10E6
MS
121615_00
135
5.66
600.29
Relative Abundance
Time (min)

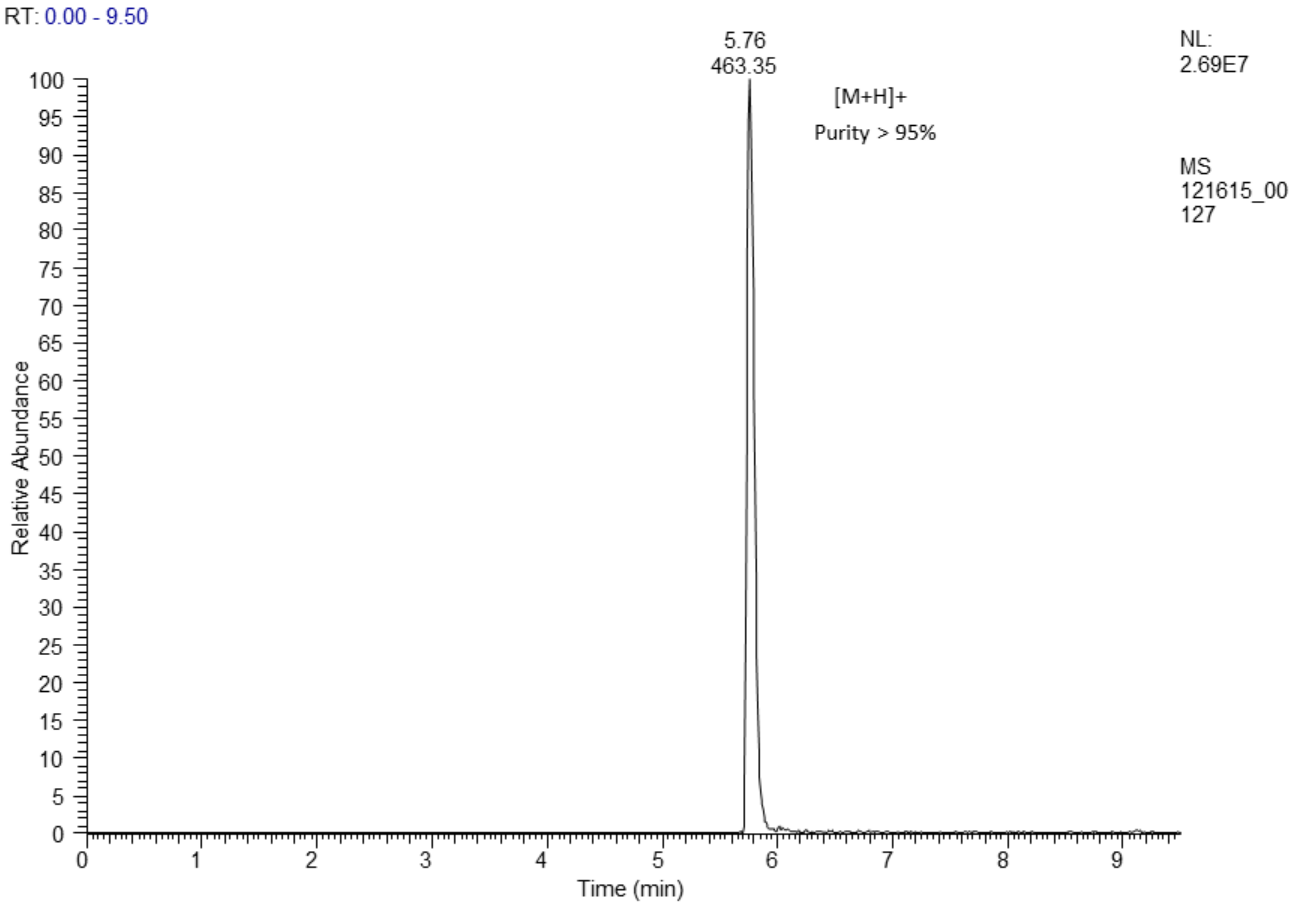
Compound 8


RT: 0.00 - 9.50
5.19
511.40
[M+H]+
Purity > 95%
NL:
1.30E6
m/z=
505.00-
515.00
MS
121615_00
131
Relative Abundance
Time (min)

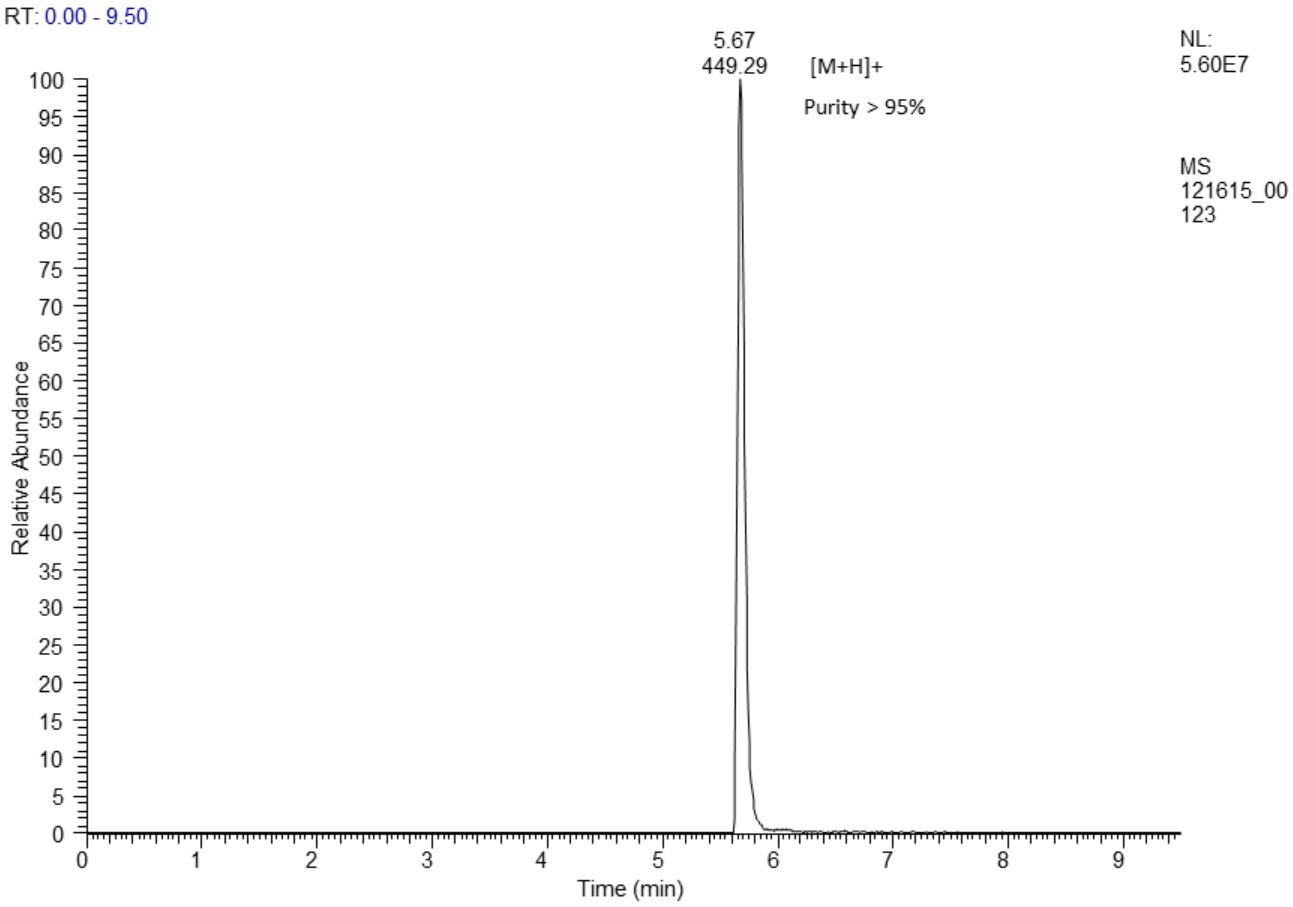
Compound 7d


RT: 0.00 - 8.00
RT: 3.69
AA: 939057156
BP: 246.25
NL:
2.36E8
TIC MS
Coop_1208
15_00132
[M+2H]2+
Purity= 99.5 %
RT: 0.37
MA: 4635194
BP: 405.52
Relative Abundance
Time (min)

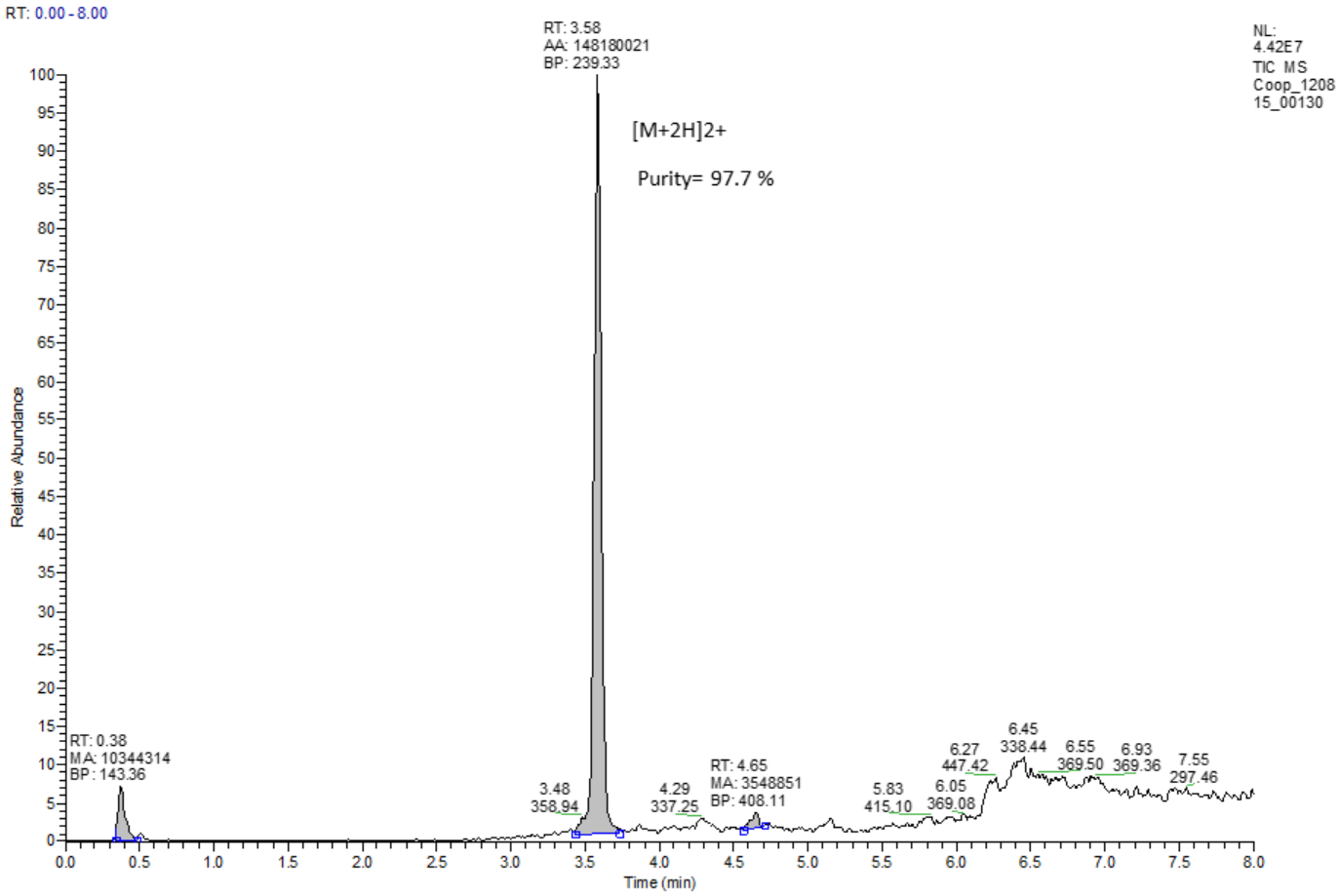
Compound 7c


RT: 0.00 - 9.50
5.76
463.35
[M+H]+
Purity > 95%
NL:
2.69E7
MS
121615_00
127
Relative Abundance
Time (min)

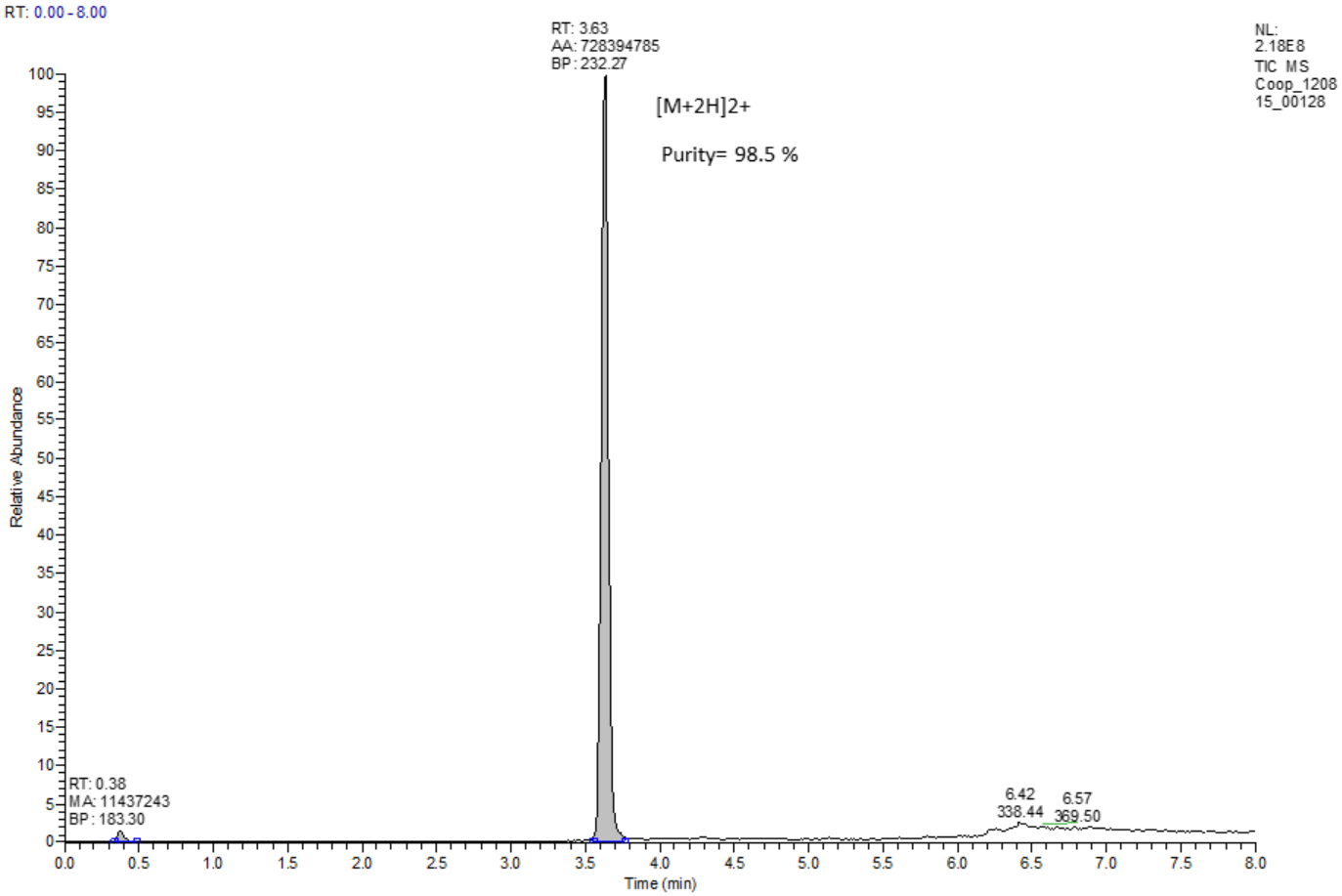
Compound 7b


RT: 0.00 - 9.50
5.67
449.29
[M+H]+
Purity > 95%
NL:
5.60E7
MS
121615_00
123
Relative Abundance
Time (min)

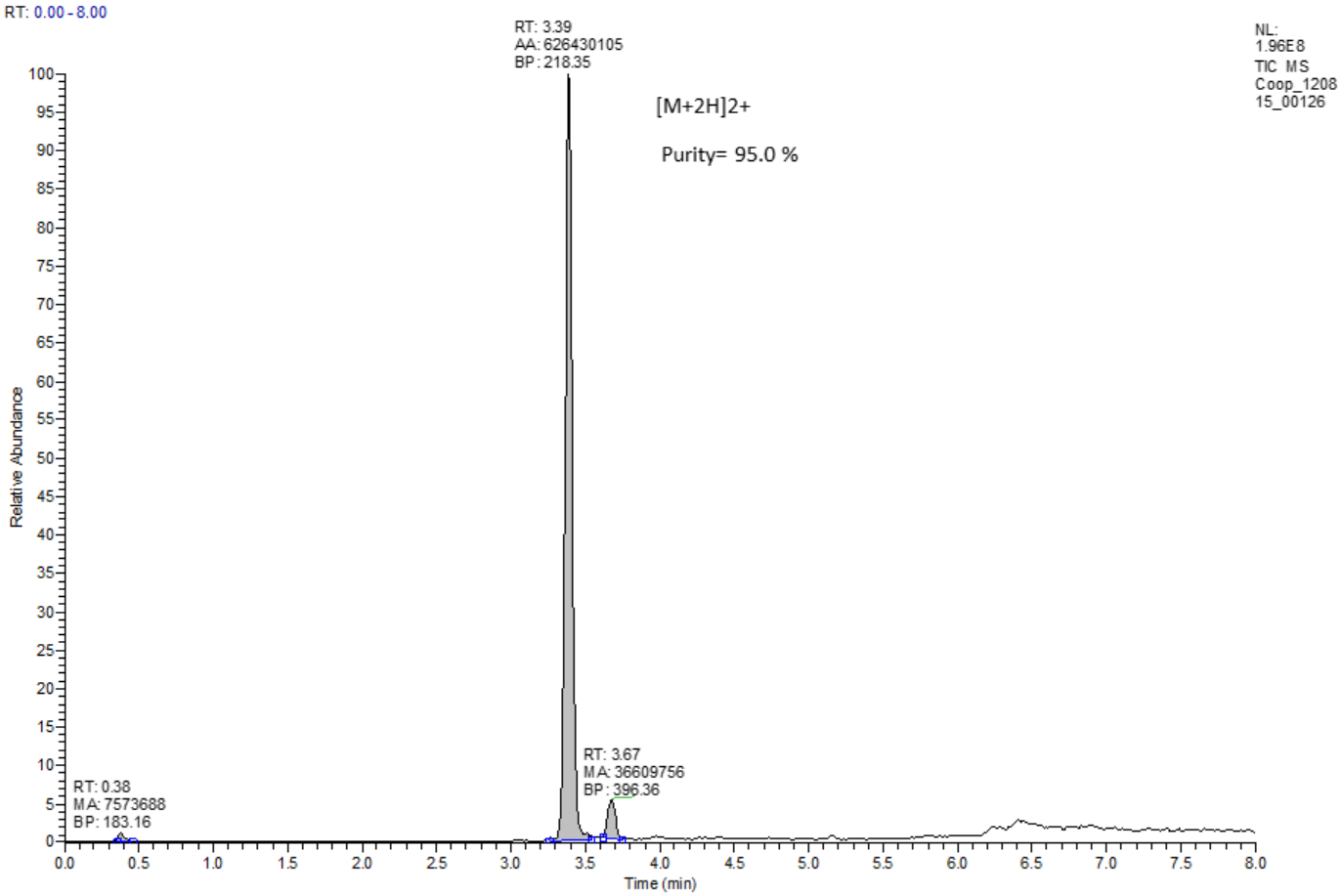
Compound 7a


RT: 0.00 - 8.00
RT: 3.58
AA: 148180021
BP: 239.33
NL:
4.42E7
TIC MS
Coop_1208
15_00130
[M+2H]2+
Purity= 97.7 %
Relative Abundance
Time (min)
RT: 0.38
MA: 10344314
BP: 143.36
3.48
358.94
4.29
337.25
RT: 4.65
MA: 3548851
BP: 408.11
5.83
415.10
6.05
369.08
6.27
447.42
6.45
338.44
6.55
369.50
6.93
369.36
7.55
297.46

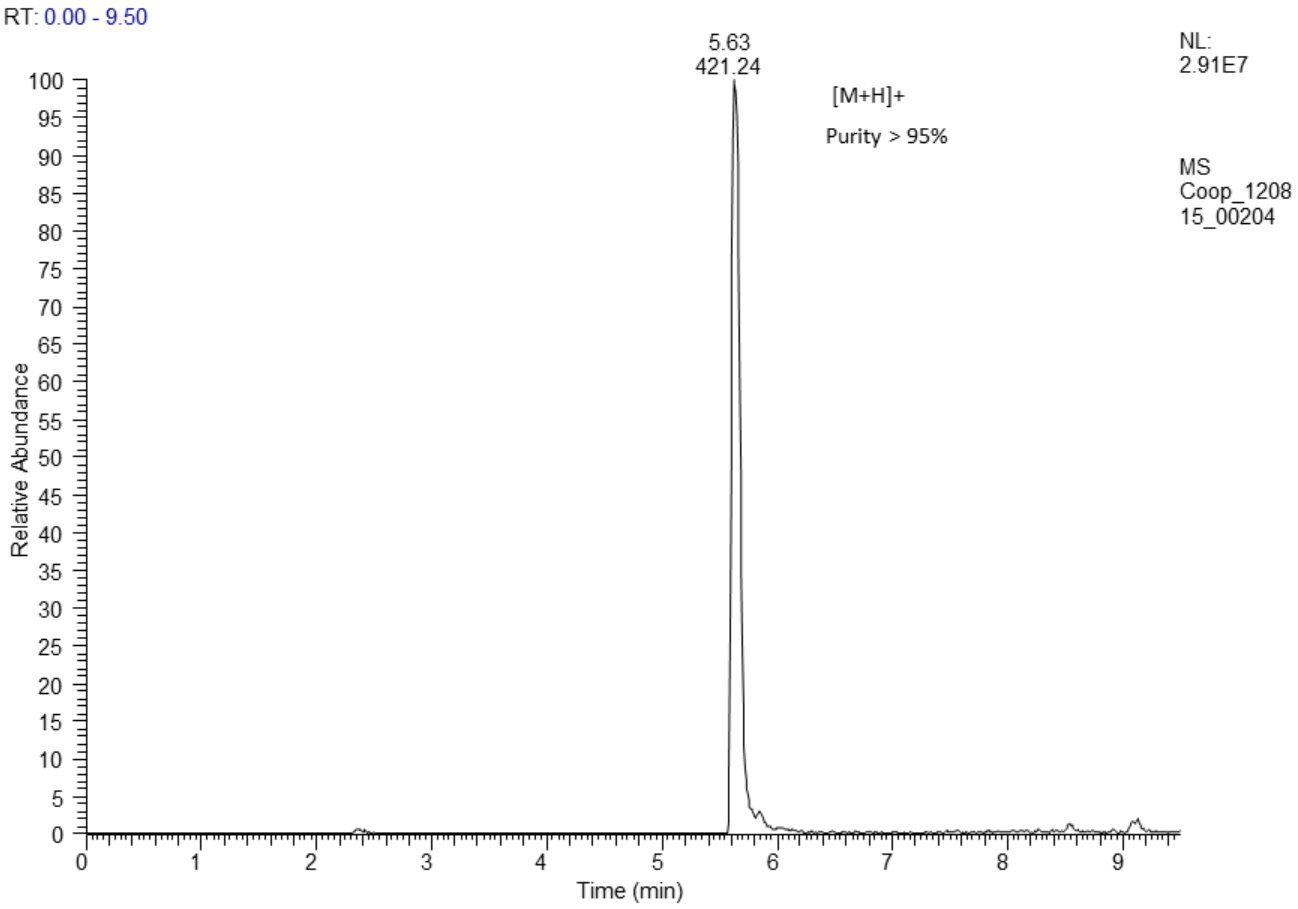
Compound 6d


RT: 0.00 - 8.00
RT: 3.63
AA: 728394785
BP: 232.27
[M+2H]2+
Purity= 98.5 %
NL:
2.18E8
TIC MS
Coop_1208
15_00128
Relative Abundance
RT: 0.38
MA: 11437243
BP: 183.30
6.42
338.44
6.57
369.50
Time (min)

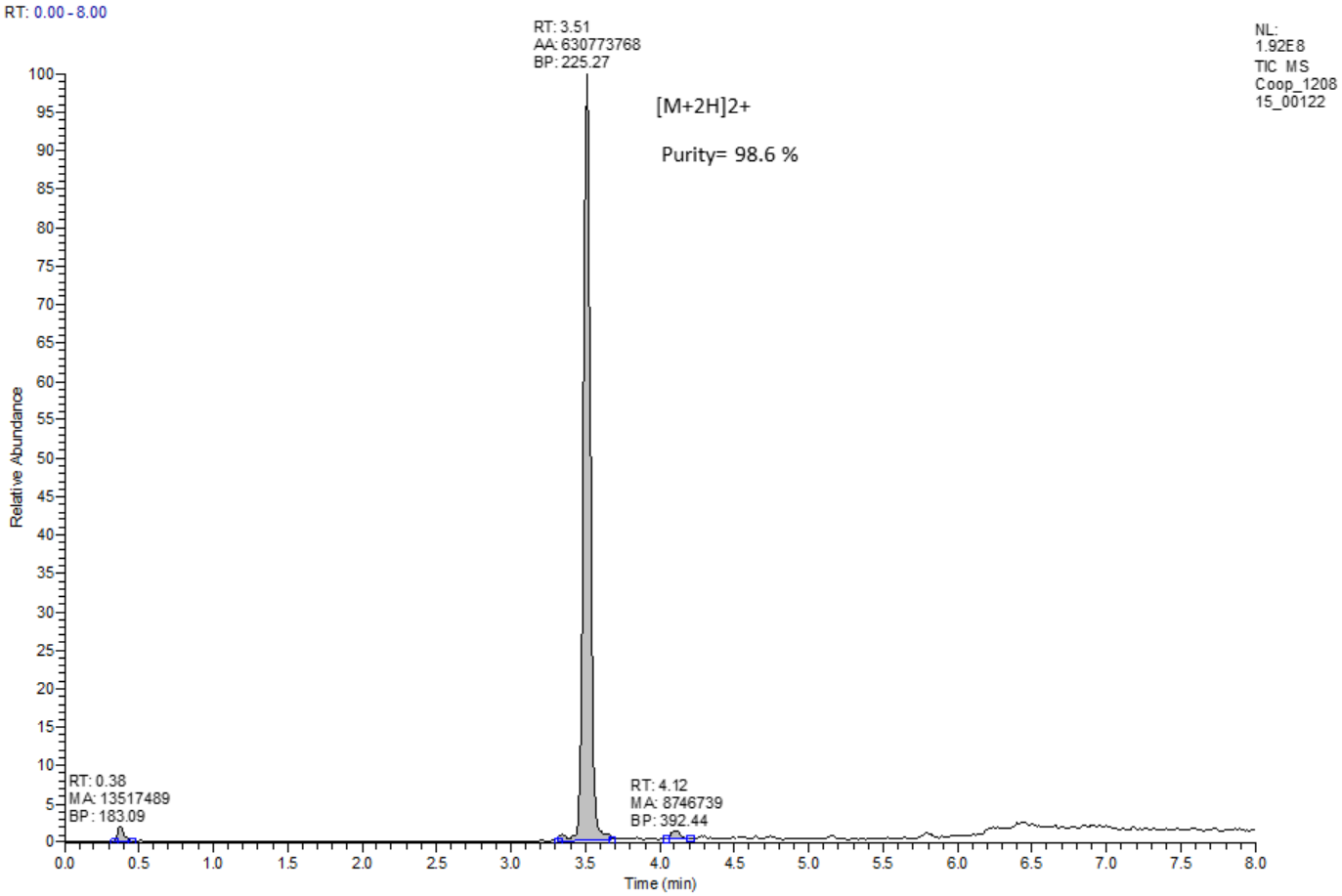
Compound 6c


RT: 0.00 - 8.00
RT: 3.39
AA: 626430105
BP: 218.35
[M+2H]2+
Purity= 95.0 %
NL:
1.96E8
TIC MS
Coop_1208
15_00126
RT: 3.67
MA: 36609756
BP: 396.36
RT: 0.38
MA: 7573688
BP: 183.16
Relative Abundance
Time (min)

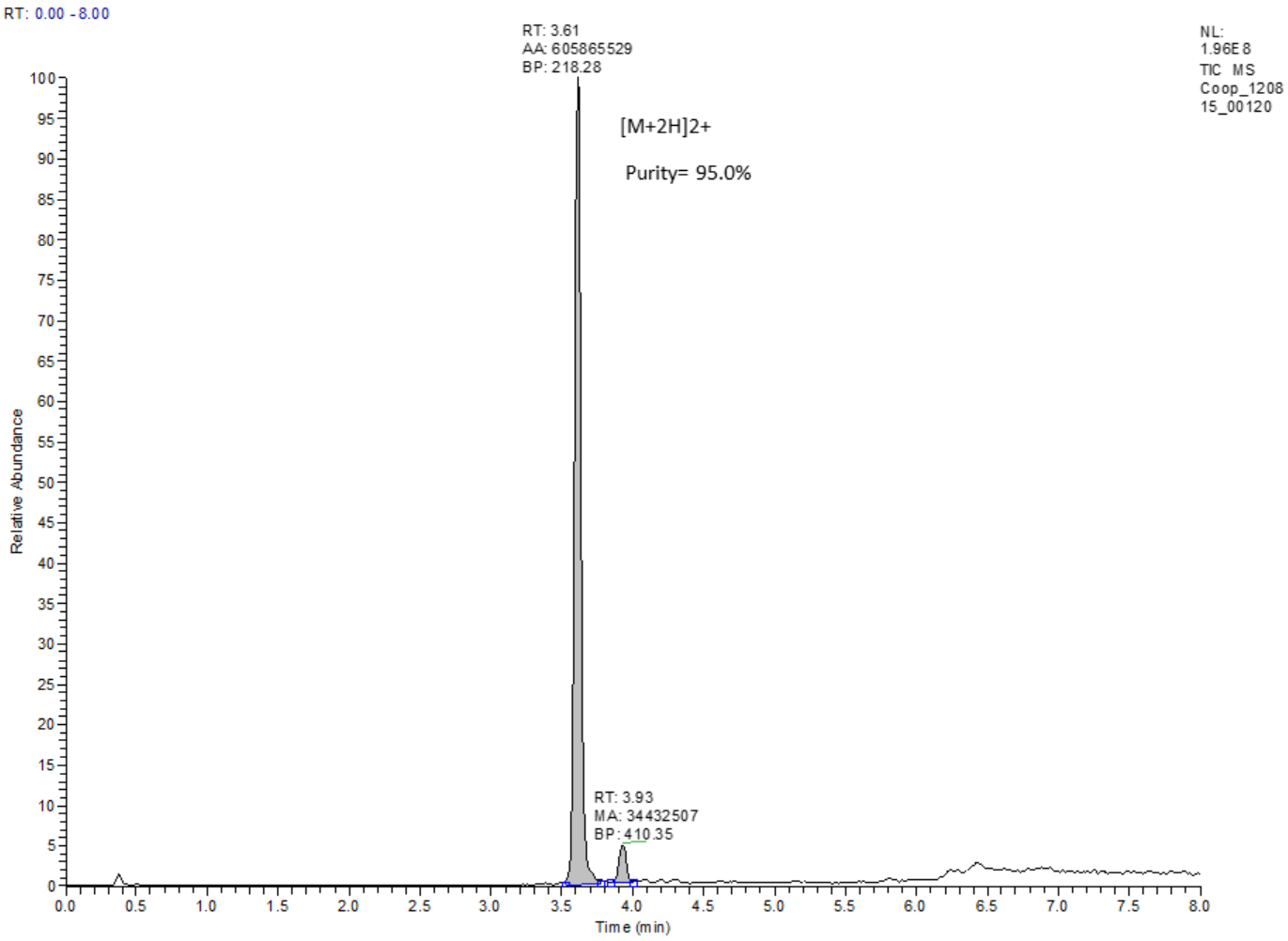
Compound 6b


RT: 0.00 - 9.50
5.63
421.24
[M+H]+
Purity > 95%
NL:
2.91E7
MS
Coop_1208
15_00204
Relative Abundance
Time (min)

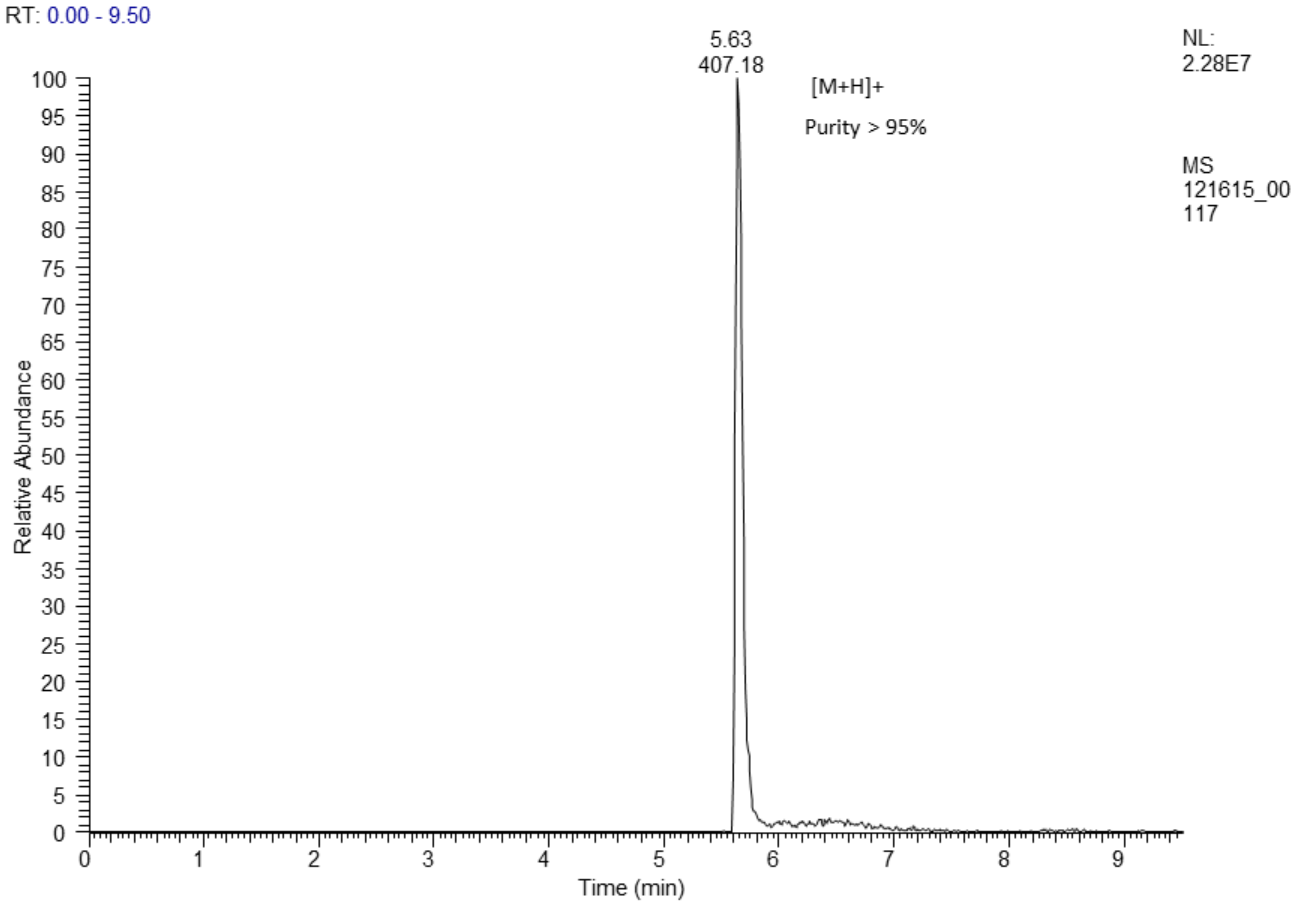
Compound 6a


RT: 0.00 - 8.00
RT: 3.51
AA: 630773768
BP: 225.27
[M+2H]2+
Purity= 98.6 %
NL:
1.92E8
TIC MS
Coop_1208
15_00122
Relative Abundance
RT: 0.38
MA: 13517489
BP: 183.09
RT: 4.12
MA: 8746739
BP: 392.44
Time (min)

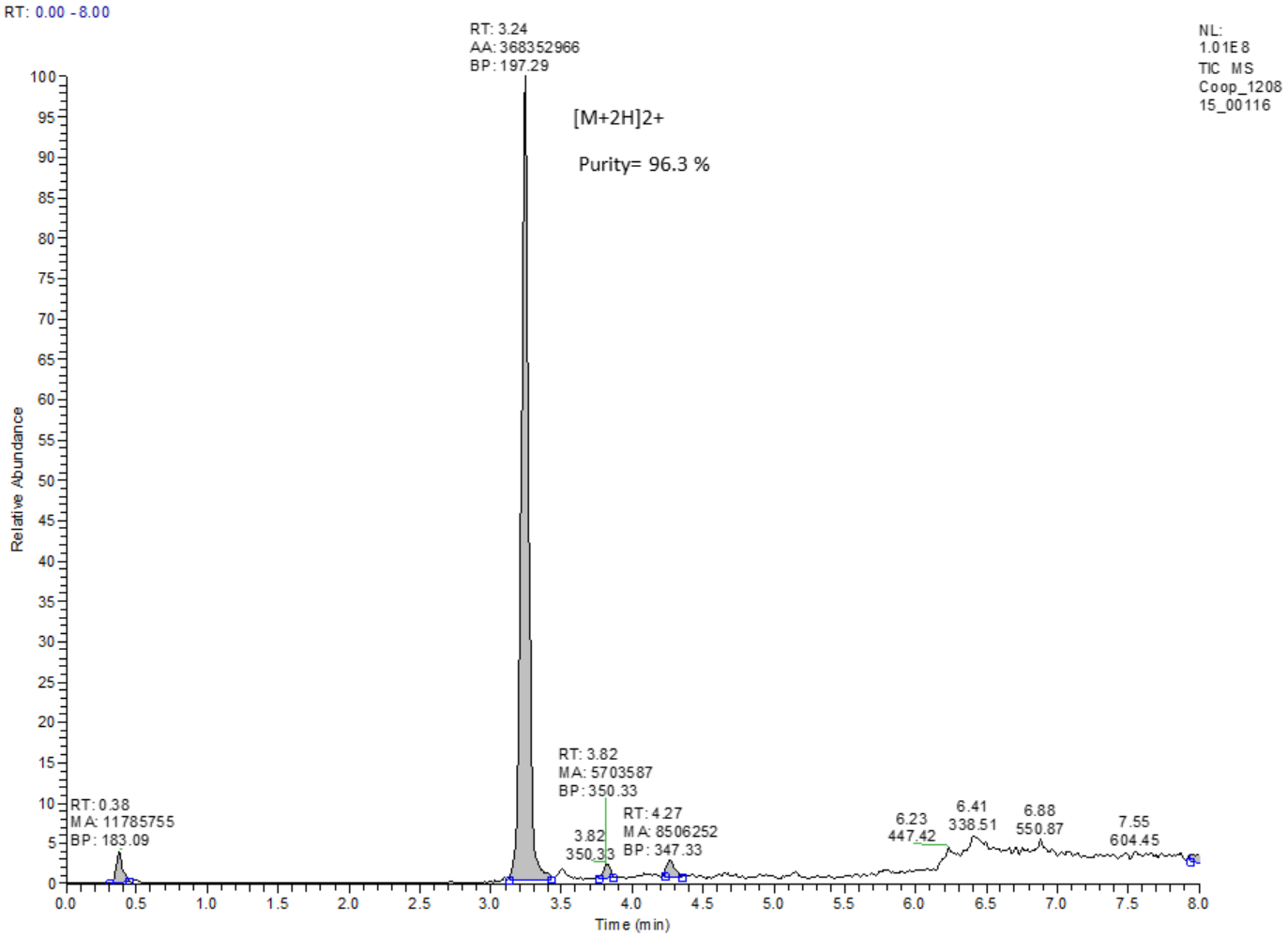
Compound 5d


RT: 0.00 - 8.00
RT: 3.61
AA: 605865529
BP: 218.28
NL:
1.96E8
TIC MS
Coop_1208
15_00120
[M+2H]2+
Purity= 95.0%
RT: 3.93
MA: 34432507
BP: 410.35
Relative Abundance
Time (min)

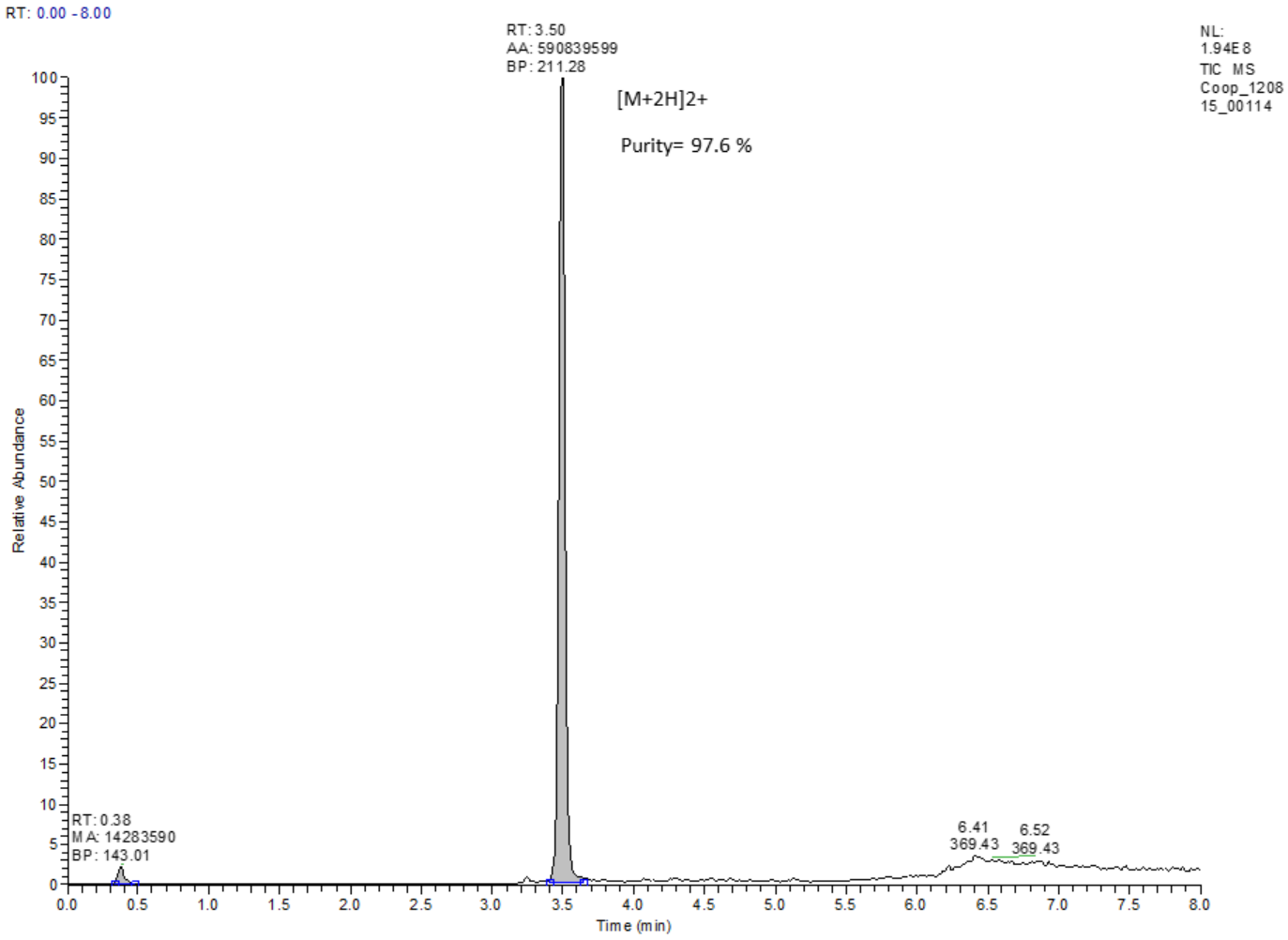
Compound 5c


RT: 0.00 - 9.50
5.63
407.18
[M+H]+
Purity > 95%
NL:
2.28E7
MS
121615_00
117
Relative Abundance
Time (min)

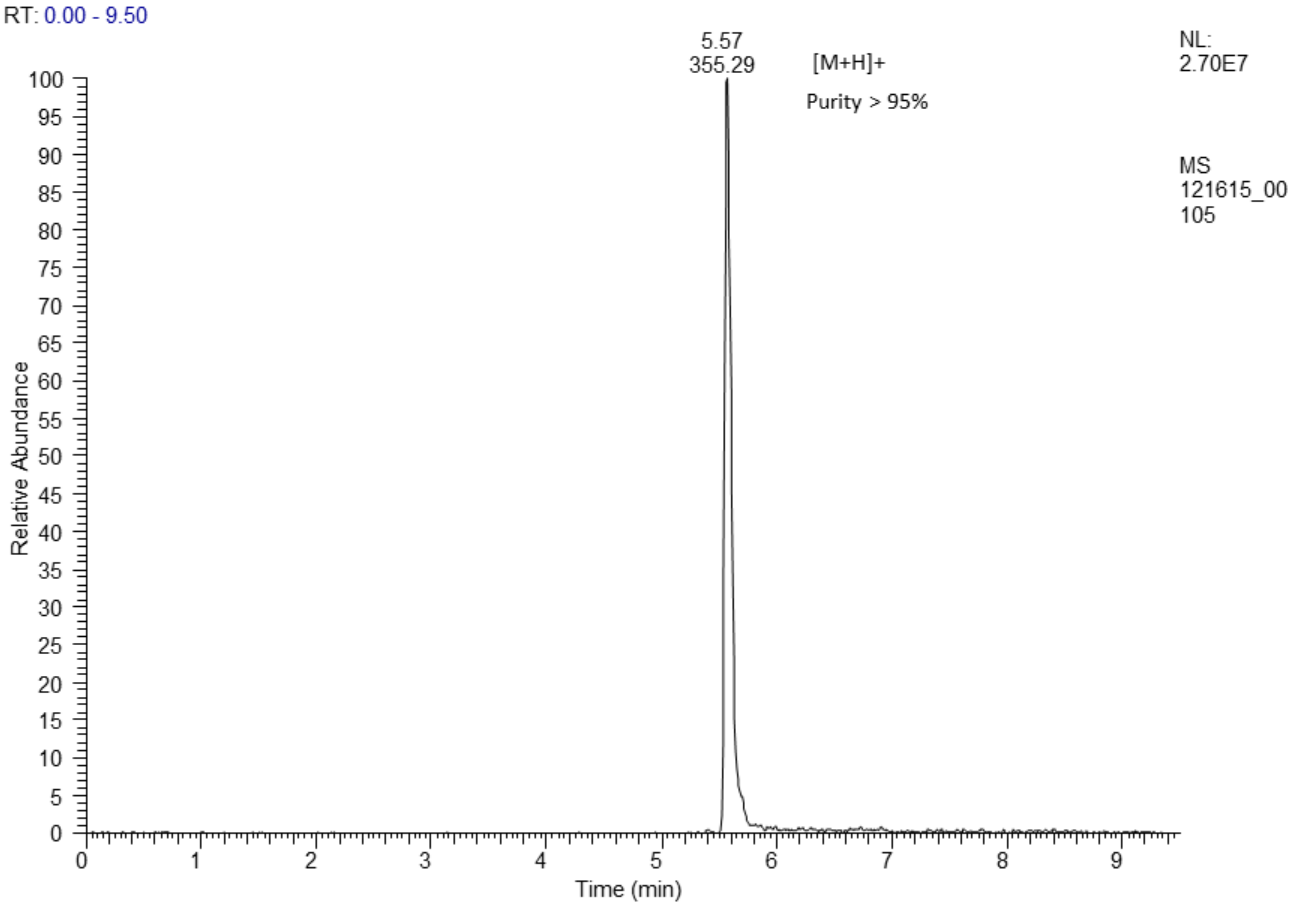
Compound 5b


RT: 0.00 - 8.00
RT: 3.24
AA: 368352966
BP: 197.29
NL:
1.01E8
TIC MS
Coop_1208
15_00116
[M+2H]2+
Purity= 96.3 %
Relative Abundance
Time (min)
RT: 0.38
MA: 11785755
BP: 183.09
RT: 3.82
MA: 5703587
BP: 350.33
RT: 4.27
MA: 8506252
BP: 347.33
3.82
350.33
6.23
447.42
6.41
338.51
6.88
550.87
7.55
604.45

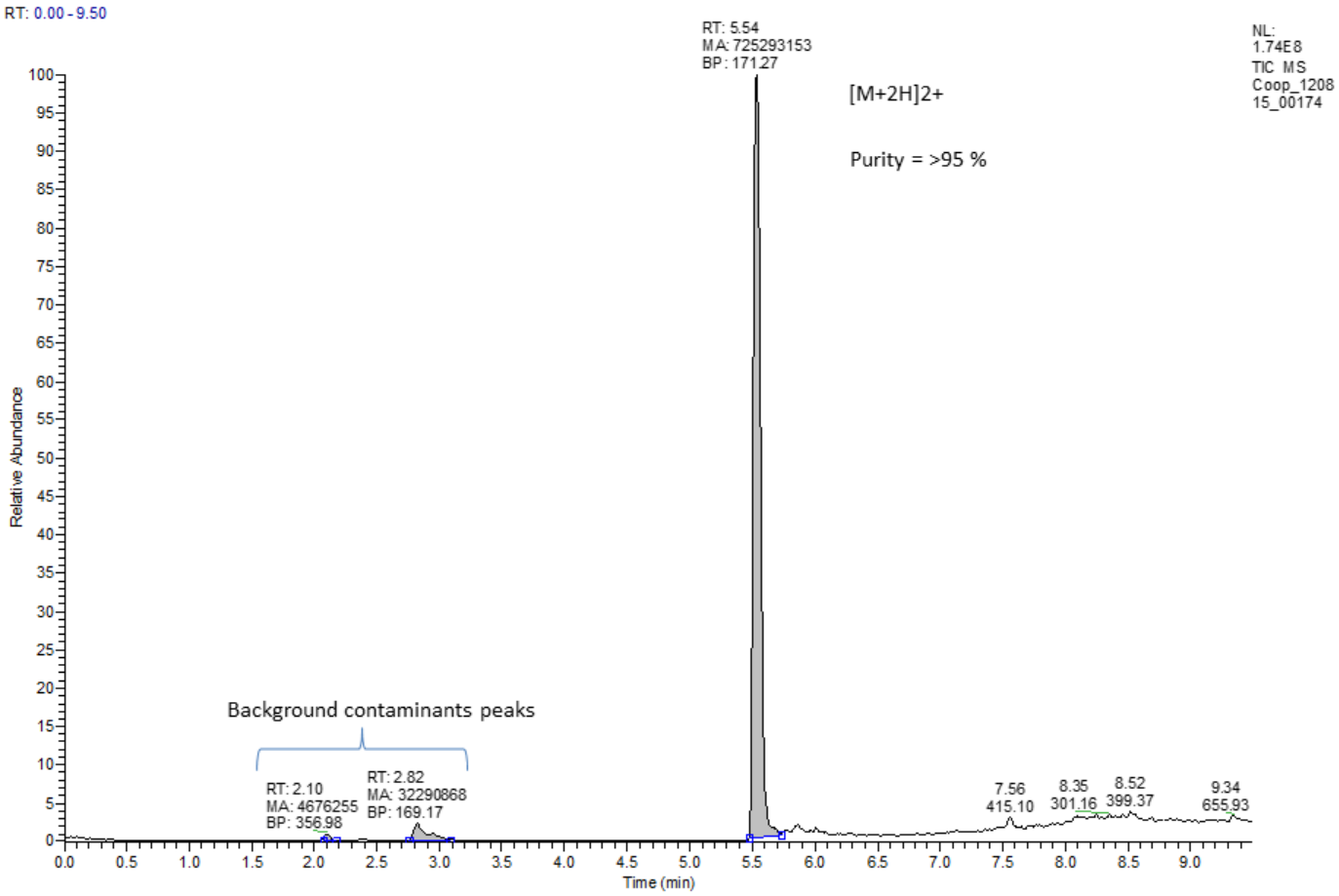
Compound 5a


RT: 0.00 - 8.00
RT: 3.50
AA: 590839599
BP: 211.28
[M+2H]2+
Purity= 97.6 %
NL:
1.94E8
TIC MS
Coop_1208
15_00114
Relative Abundance
RT: 0.38
MA: 14283590
BP: 143.01
6.41
369.43
6.52
369.43
Time (min)

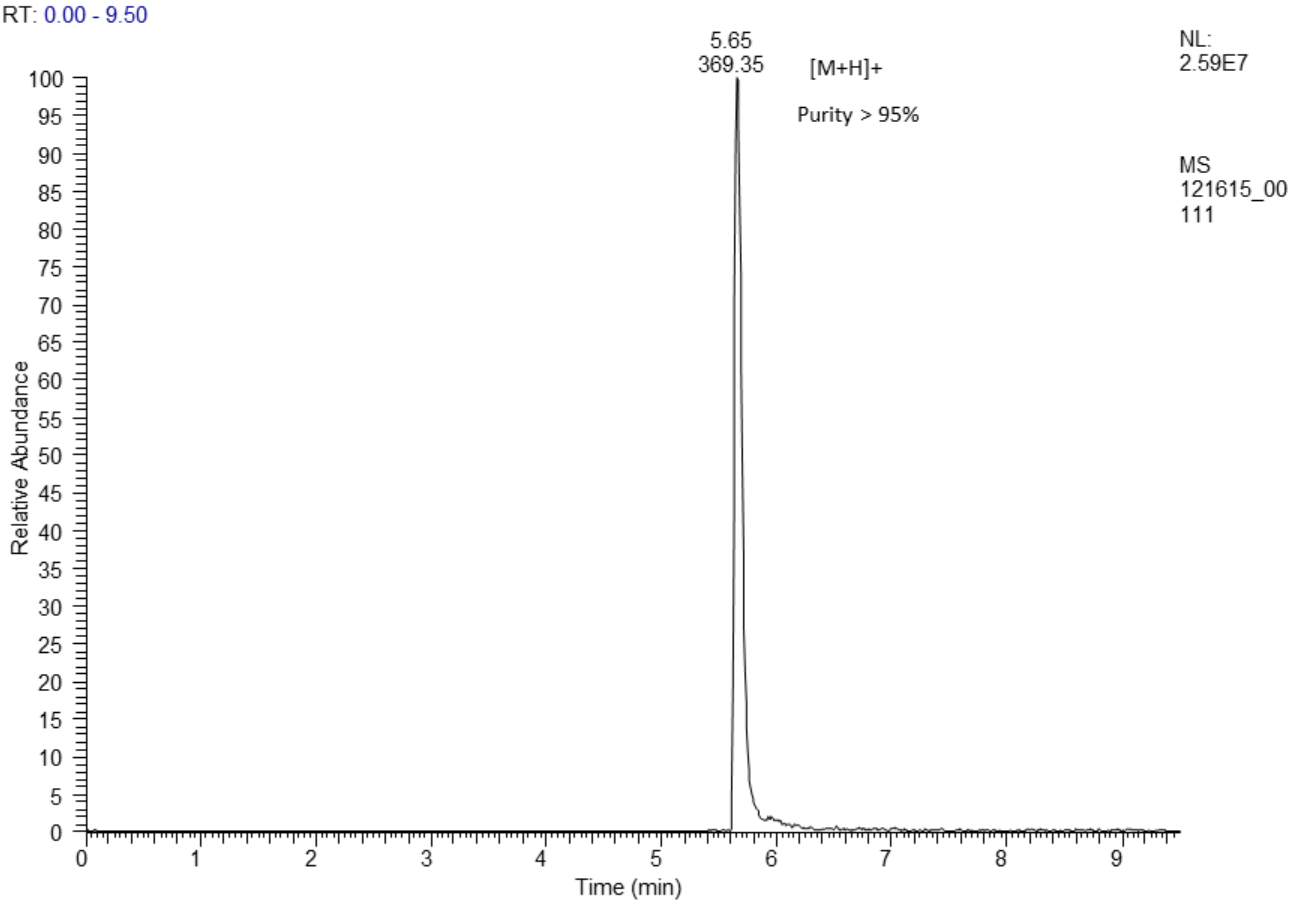
Compound 4c


RT: 0.00 - 9.50
5.57
355.29
[M+H]+
Purity > 95%
NL:
2.70E7
MS
121615_00
105
Relative Abundance
Time (min)

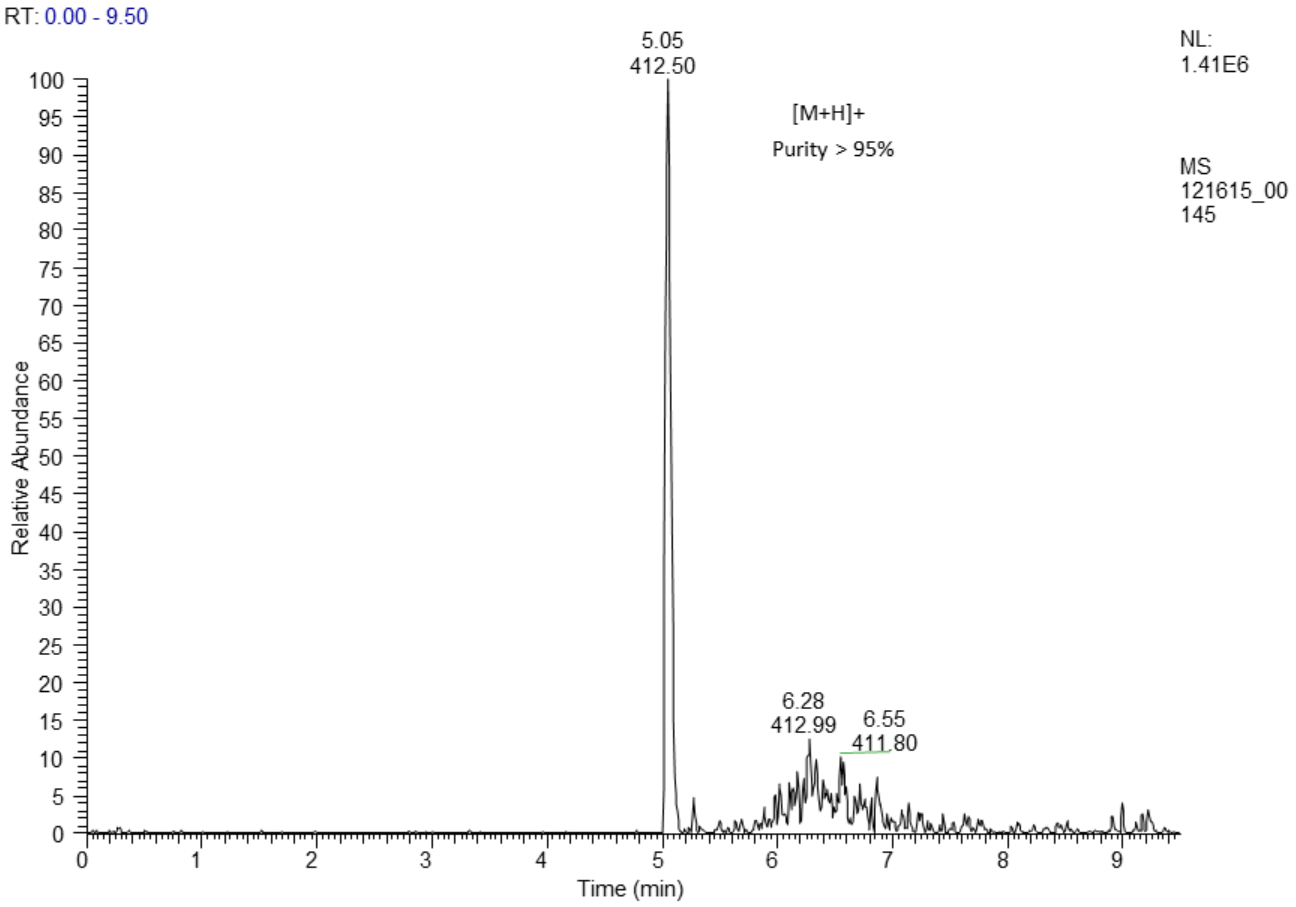
Compound 4b

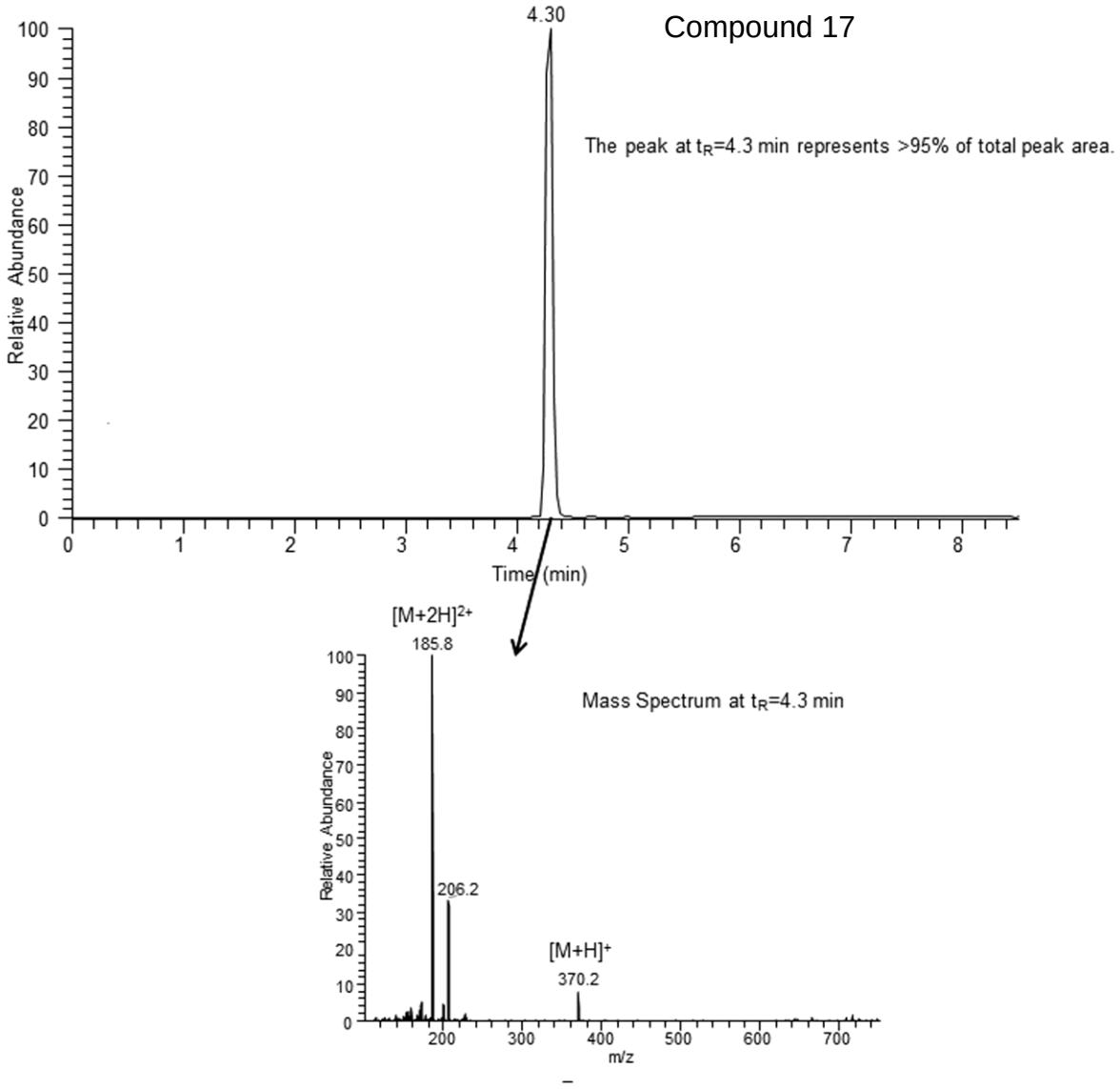

RT: 0.00 - 9.50
RT: 5.54
MA: 725293153
BP: 171.27
[M+2H]2+
Purity = >95 %
NL:
1.74E8
TIC MS
Coop_1208
15_00174
Relative Abundance
Time (min)
Background contaminants peaks
RT: 2.10
MA: 4676255
BP: 356.98
RT: 2.82
MA: 32290868
BP: 169.17
7.56
415.10
8.35
301.16
8.52
399.37
9.34
655.93

Compound 4a

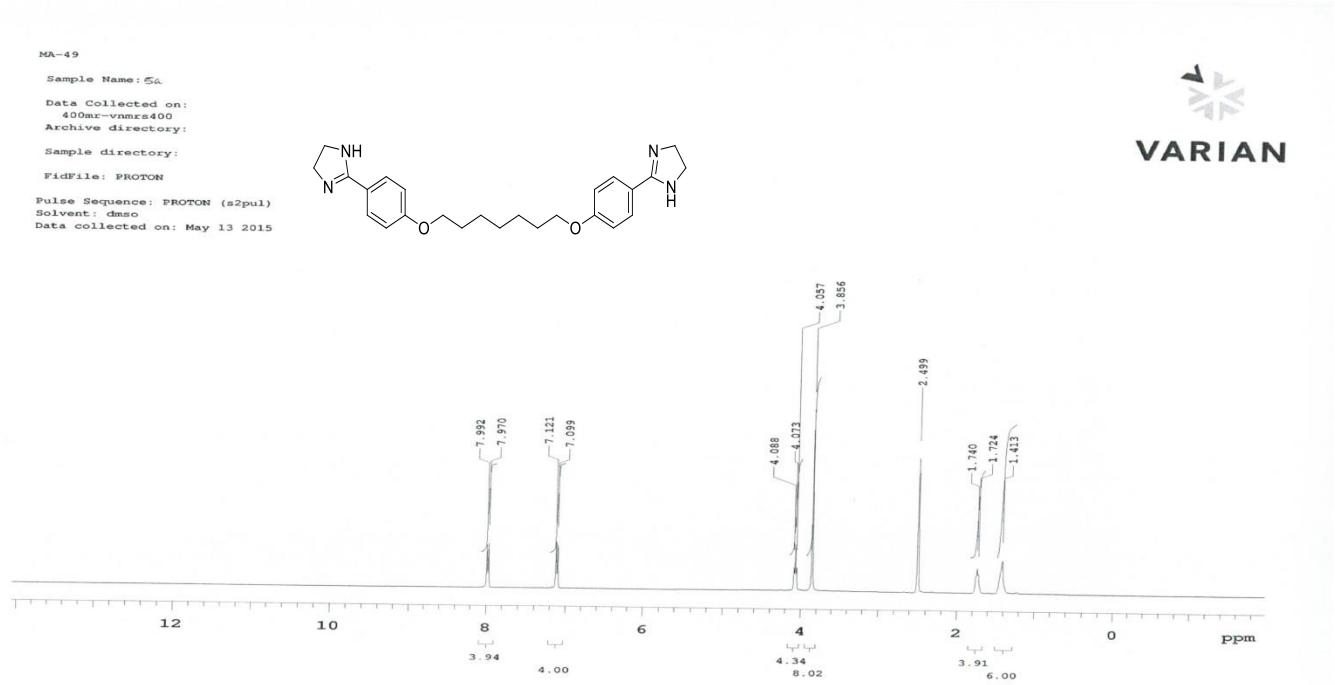

RT: 0.00 - 9.50
5.65
369.35
[M+H]+
Purity > 95%
NL:
2.59E7
MS
121615_00
111
Relative Abundance
Time (min)

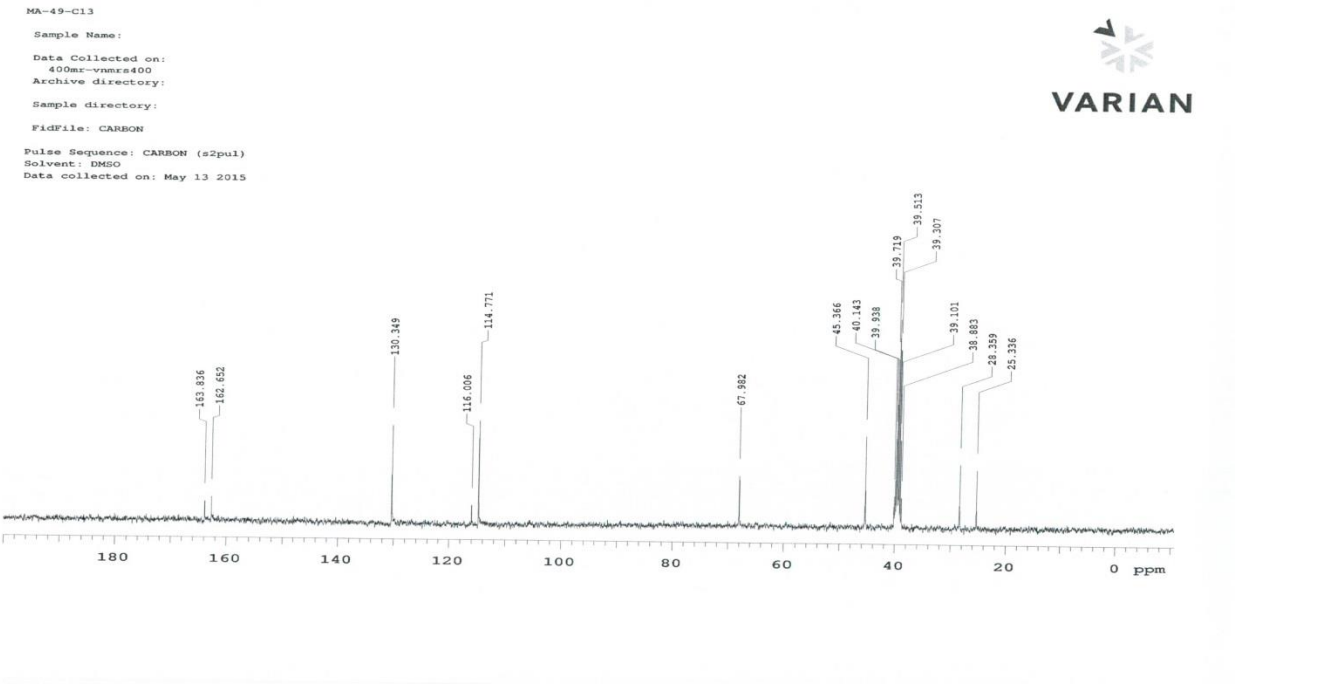
Compound 16b



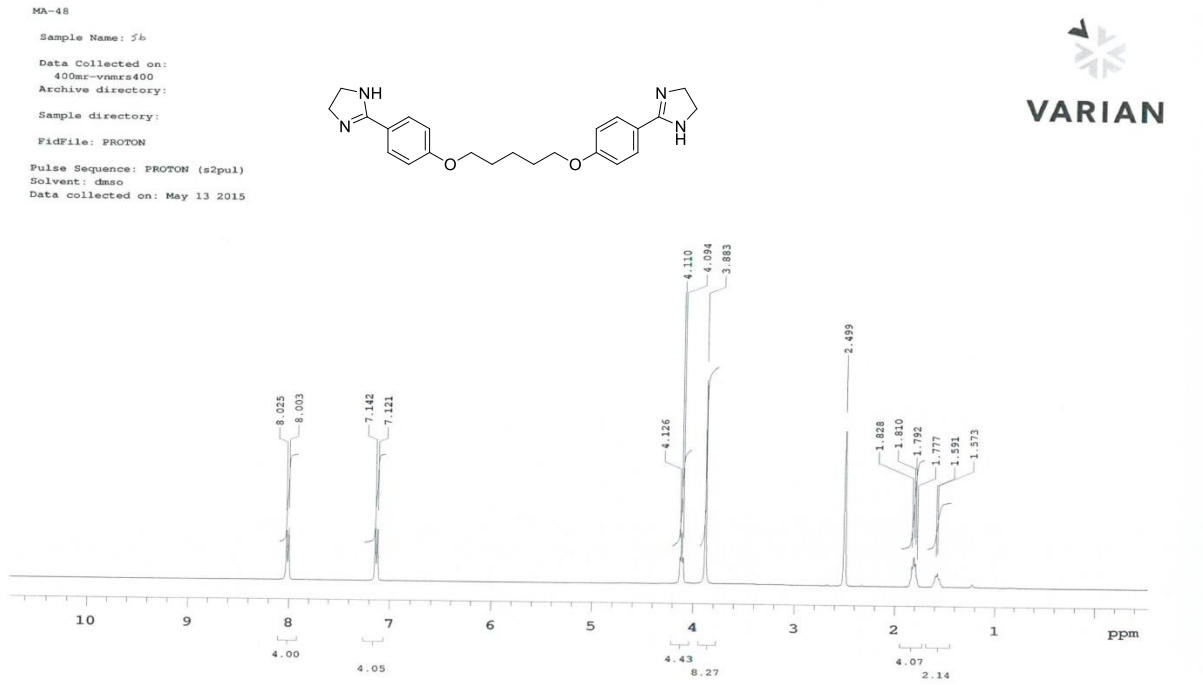

Compound 17
4.30
The peak at t_R=4.3 min represents >95% of total peak area.
Relative Abundance
Time (min)
[M+2H]2+
185.8
Mass Spectrum at t_R=4.3 min
206.2
[M+H]+
370.2
m/z

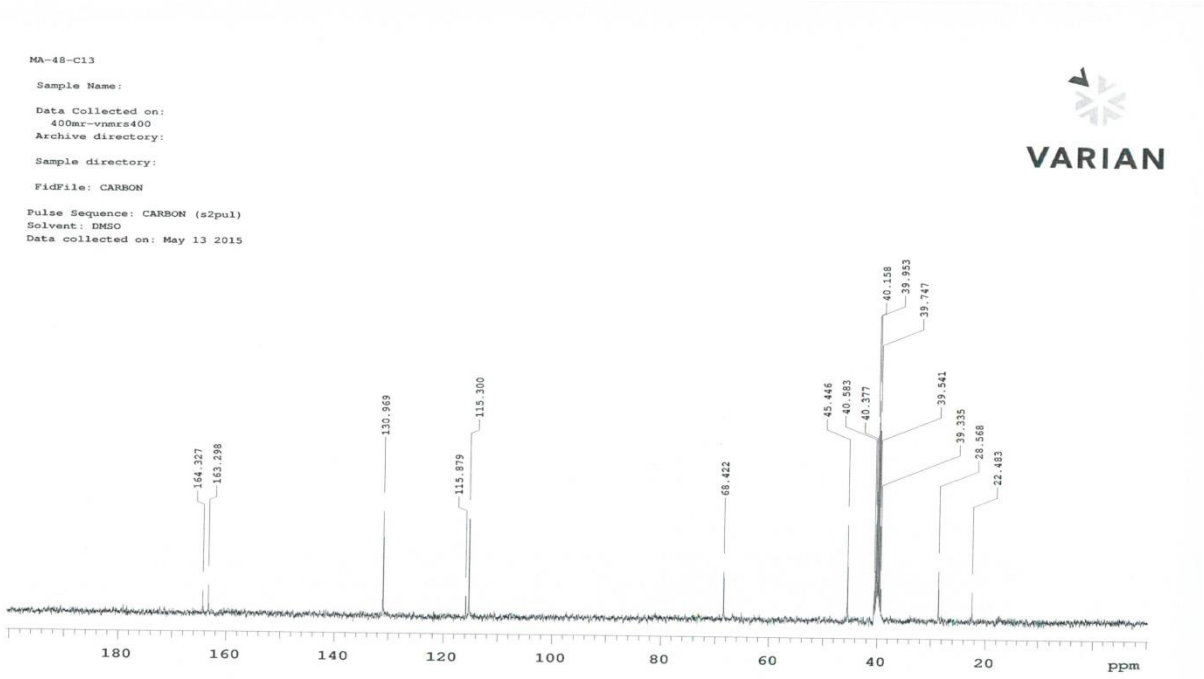
Figures S30-48(below). ^{1}H and ^{13}C NMR spectra for synthesized compounds obtained using a 500 MHz Varian NMR Spectrometer.



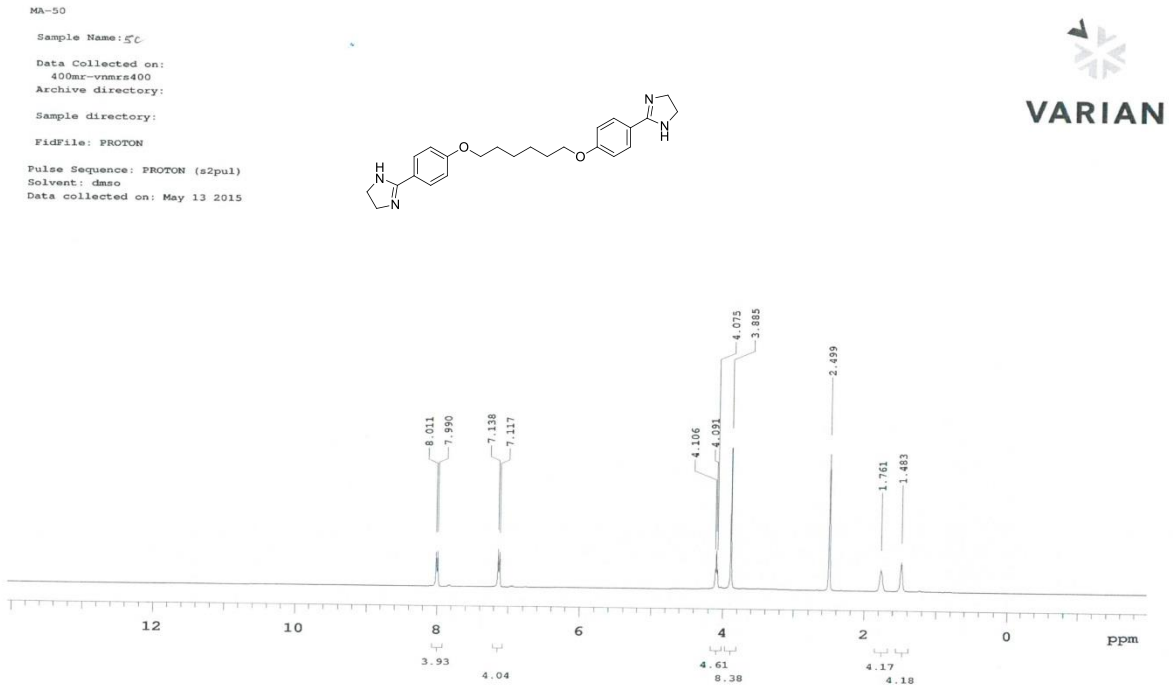


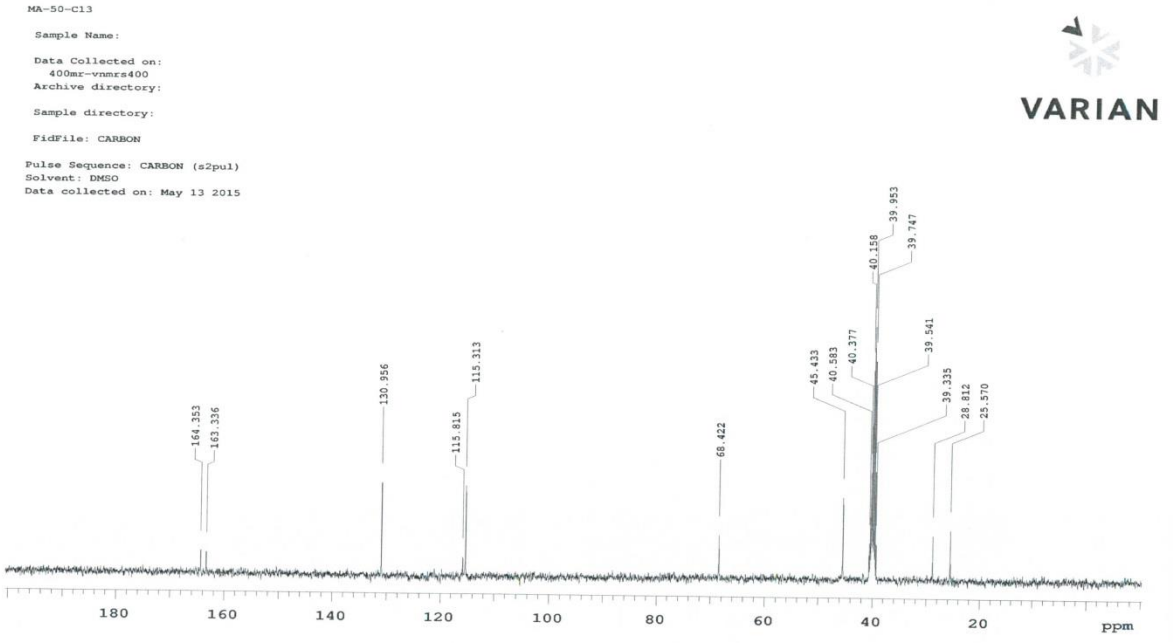
^{1}H and ^{13}C NMR of 5a



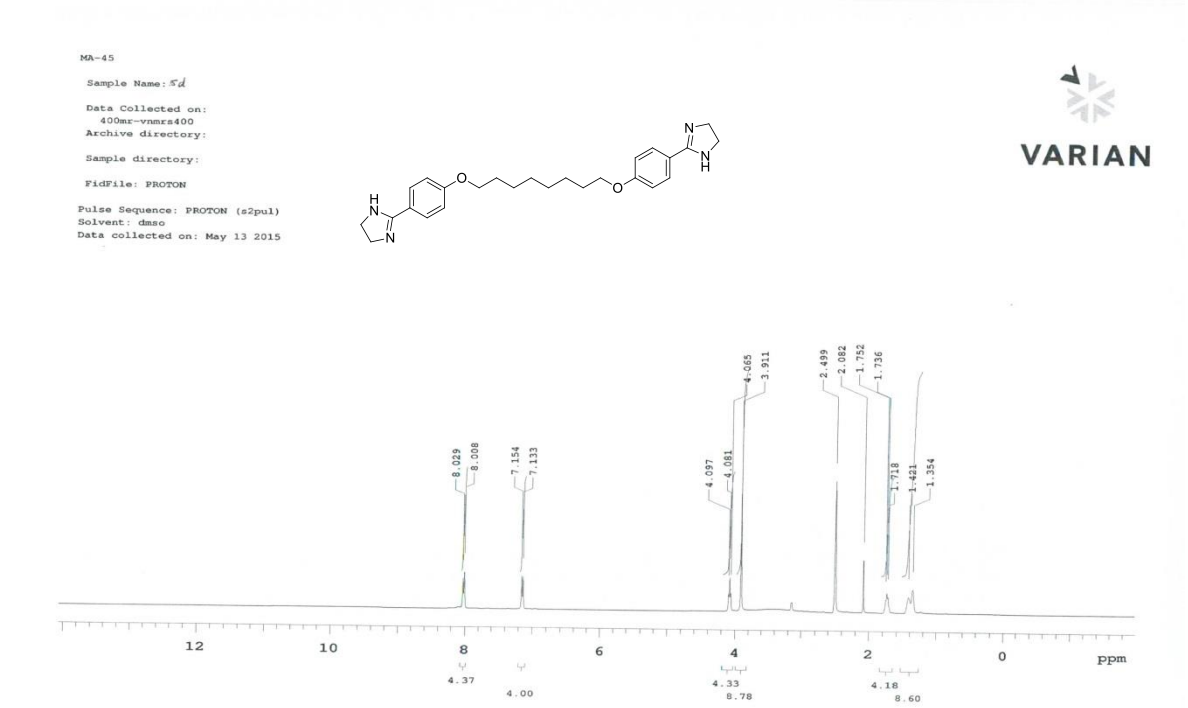


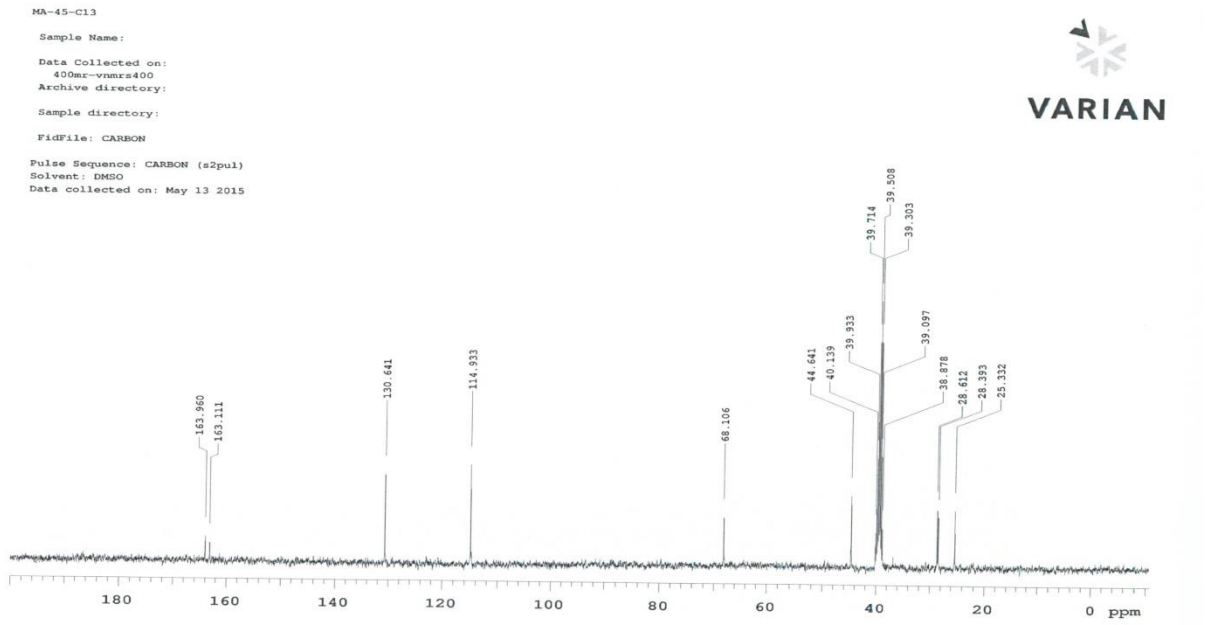
^{1}H and ^{13}C NMR of 5b



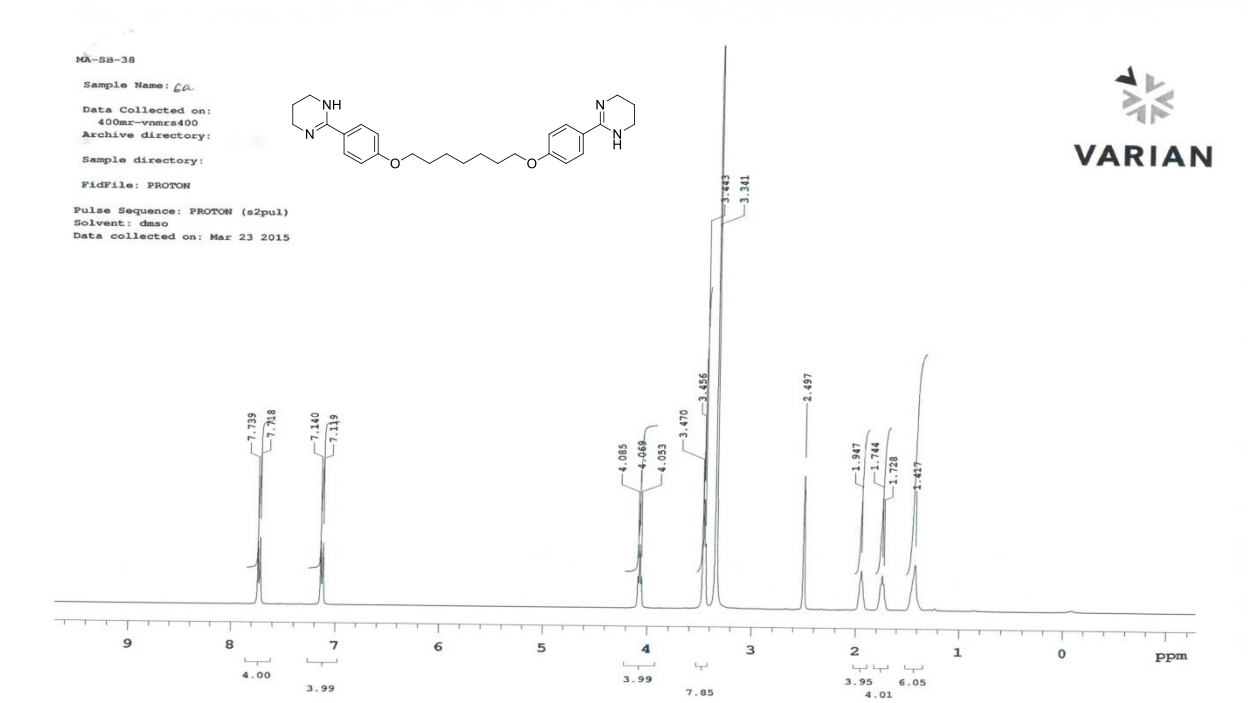


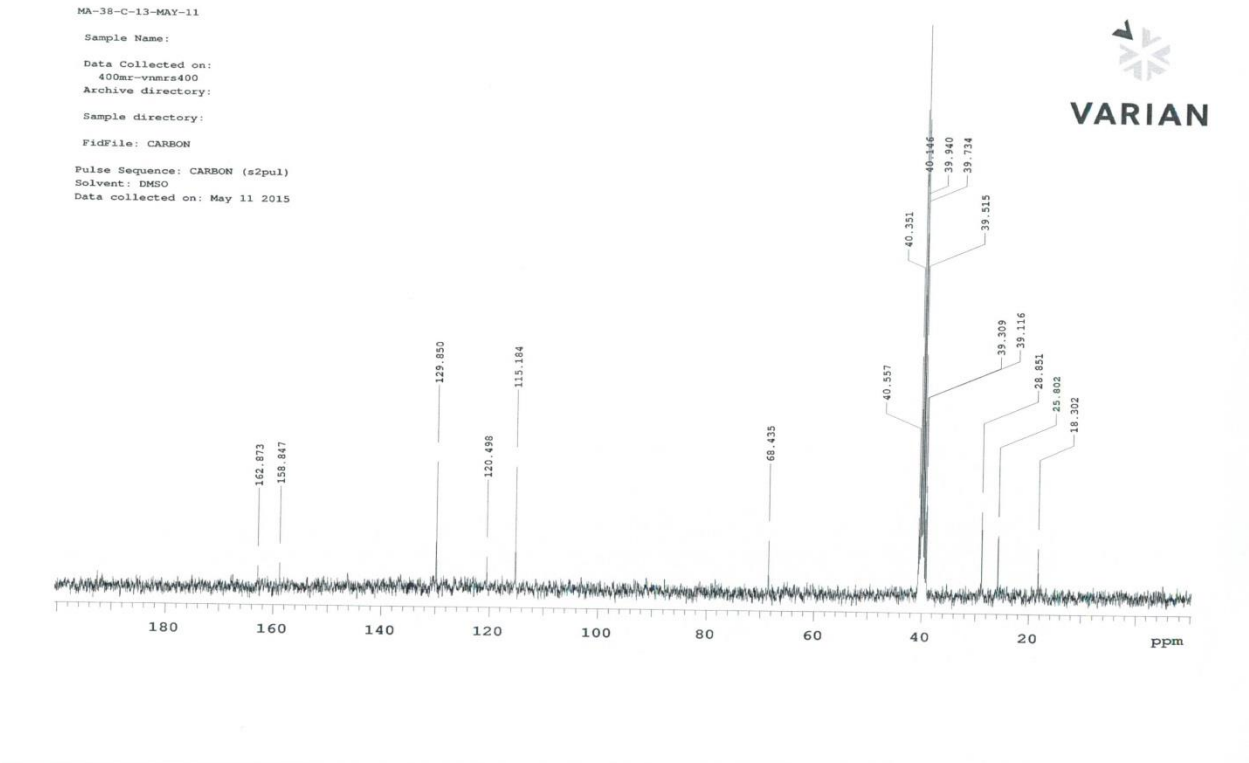
^{1}H and ^{13}C NMR of 5c



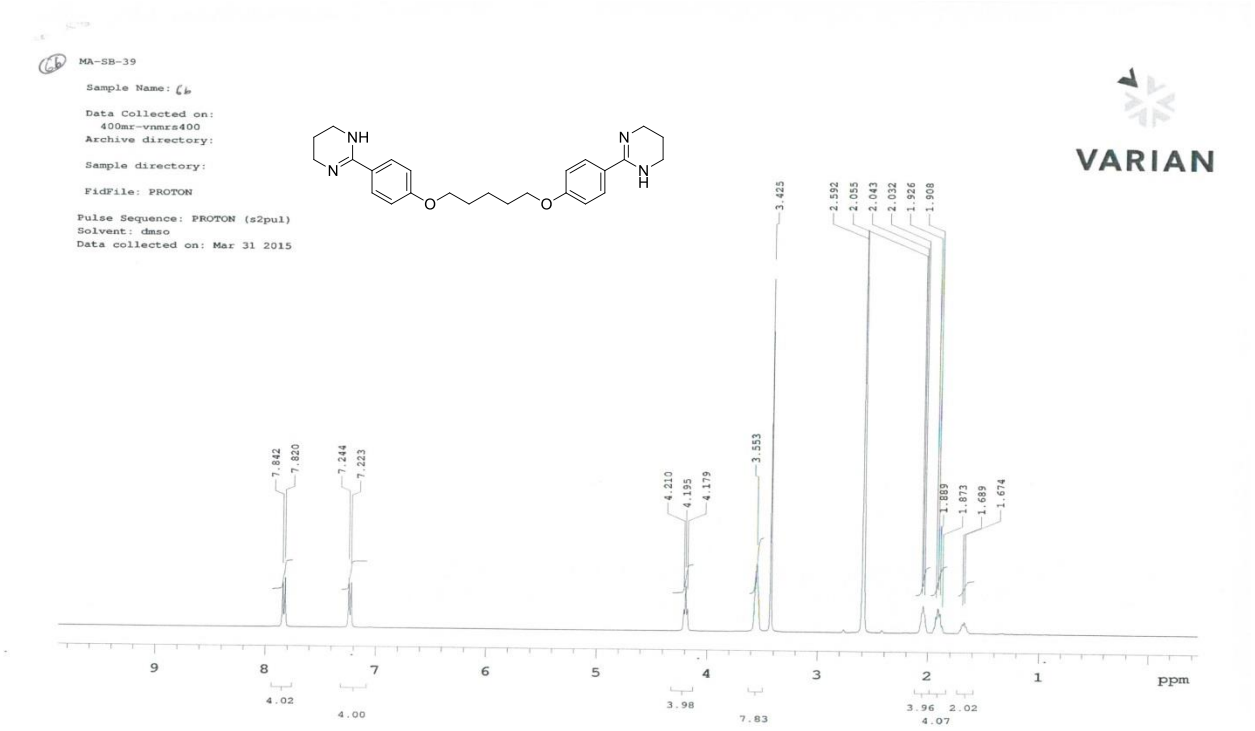


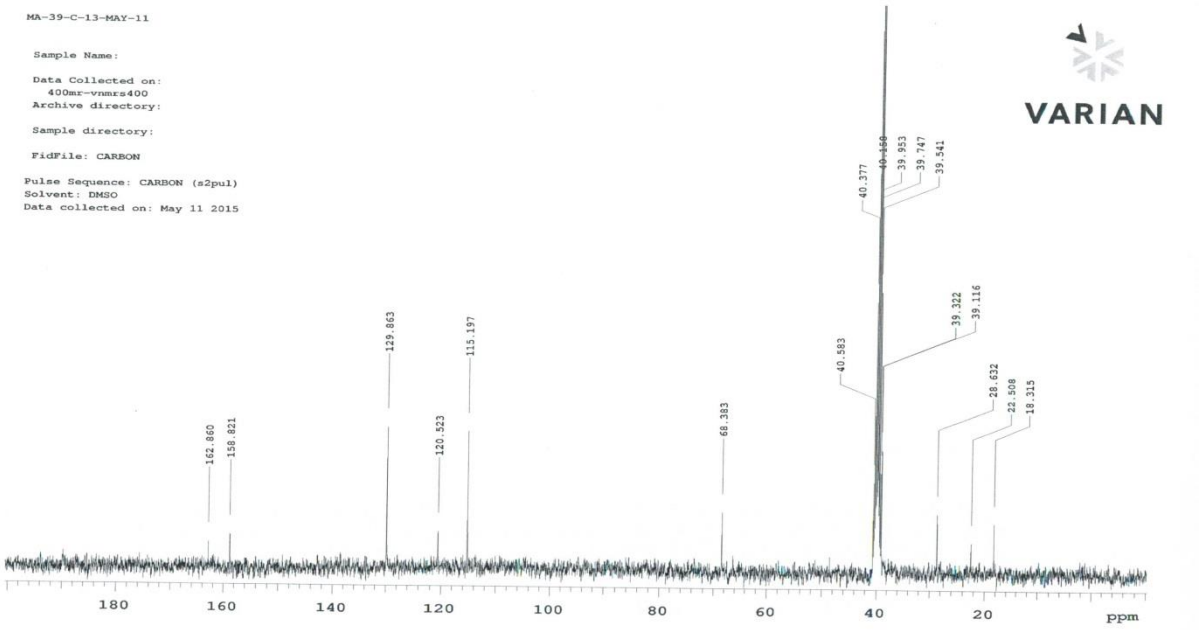
^{1}H and ^{13}C NMR of 5d



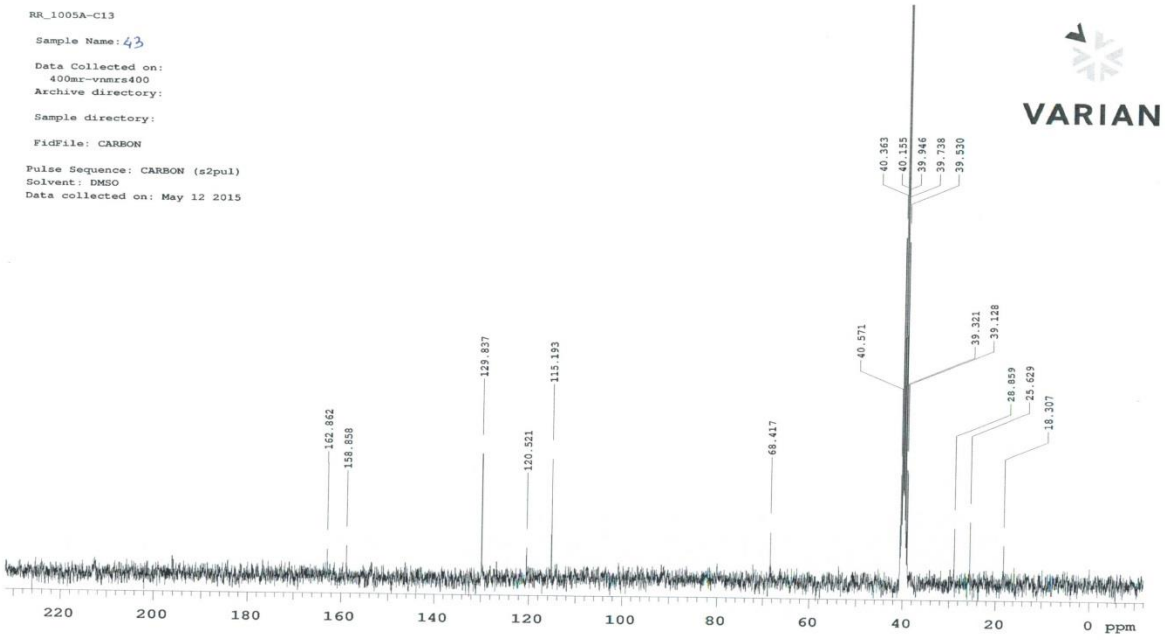


^{1}H and ^{13}C NMR of 6a

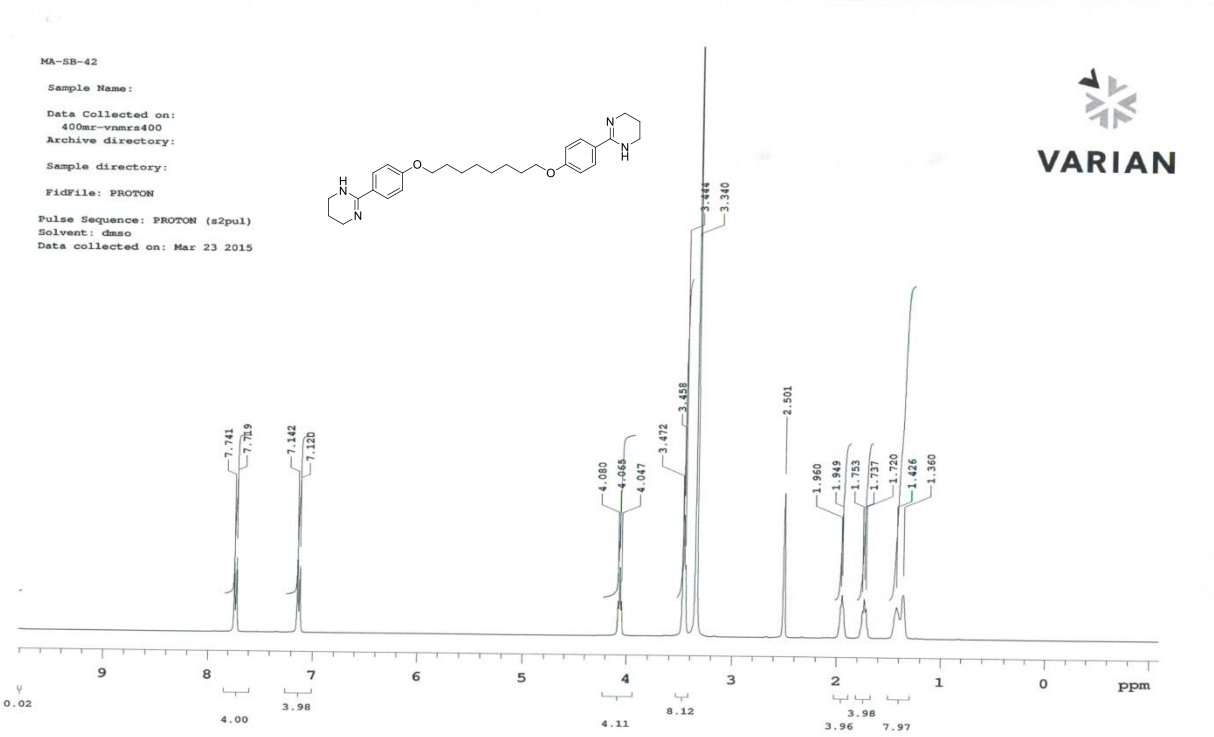


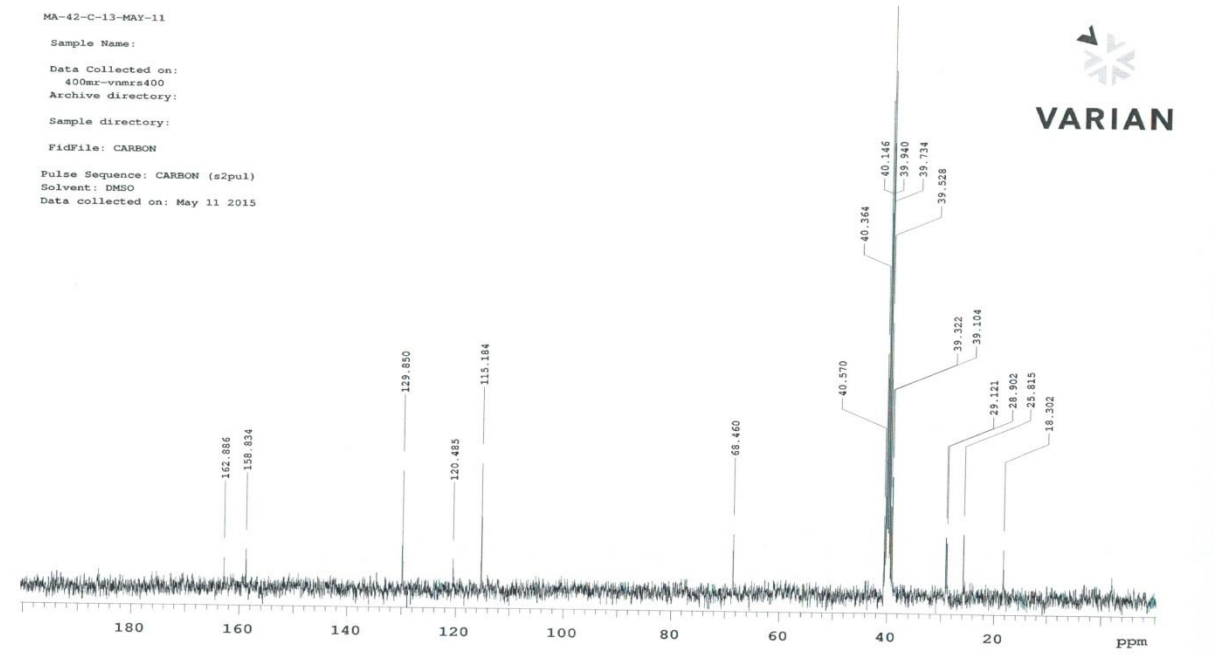


^{1}H and ^{13}C NMR of 6b

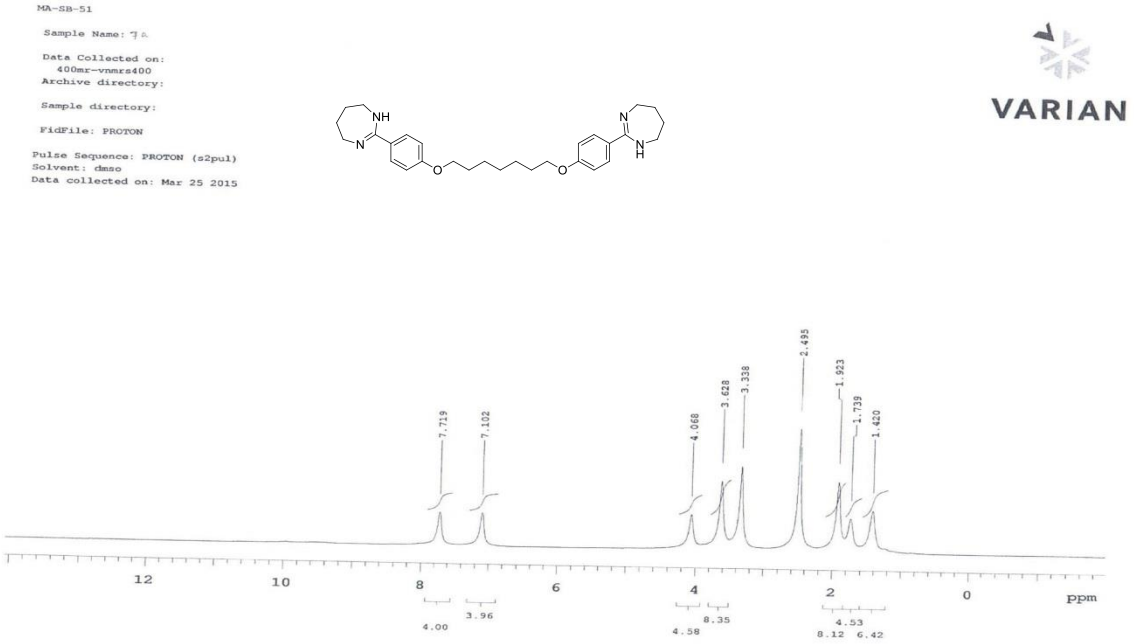


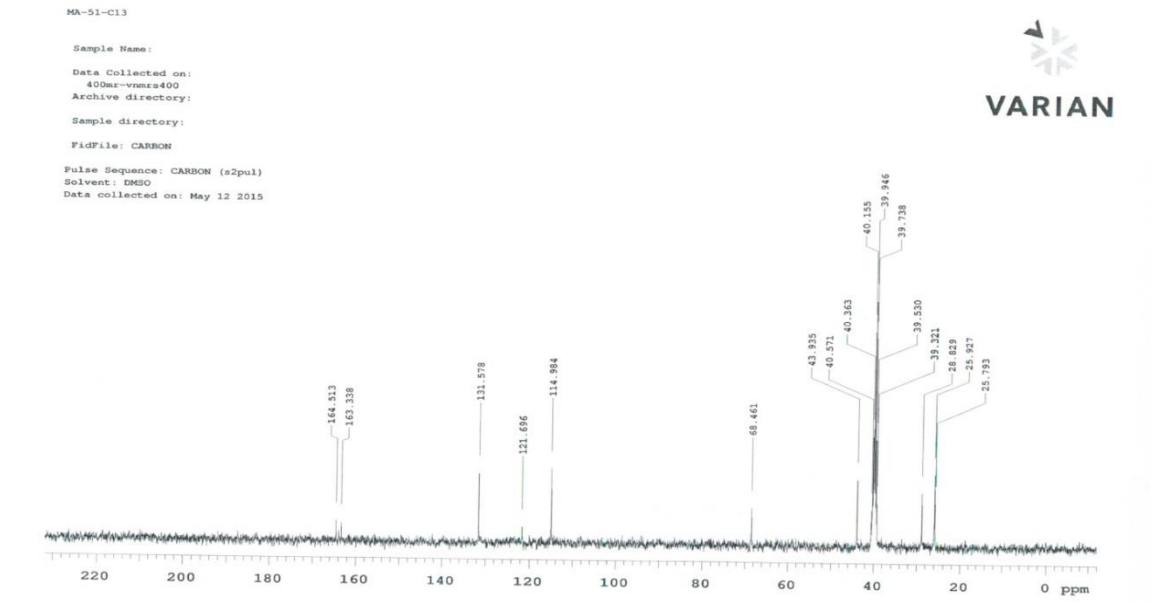
^{1}H and ^{13}C NMR of 6c



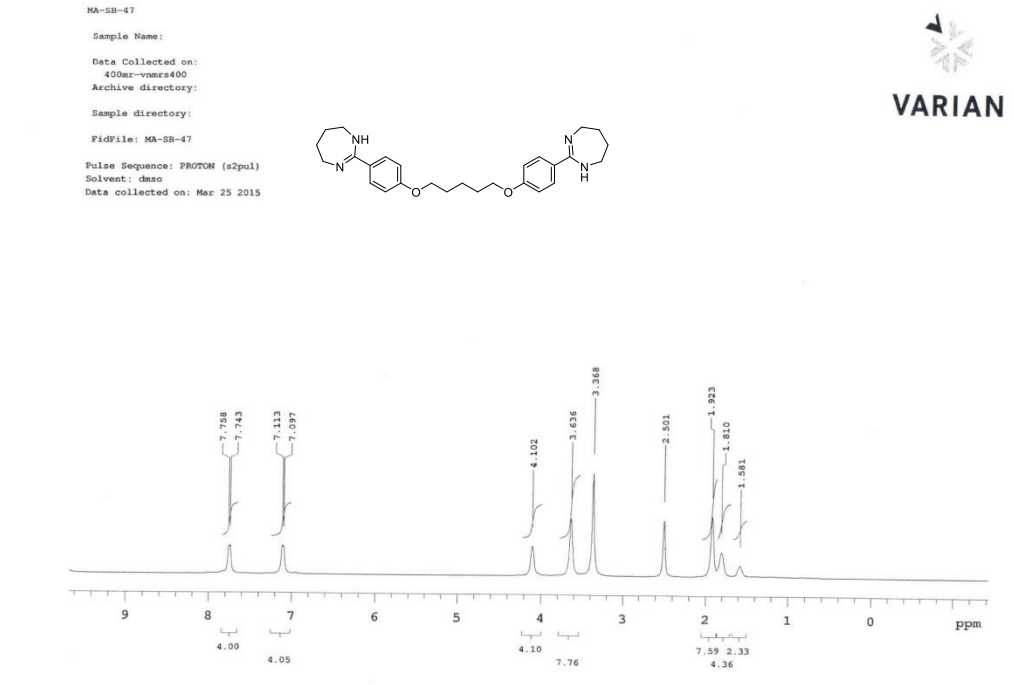


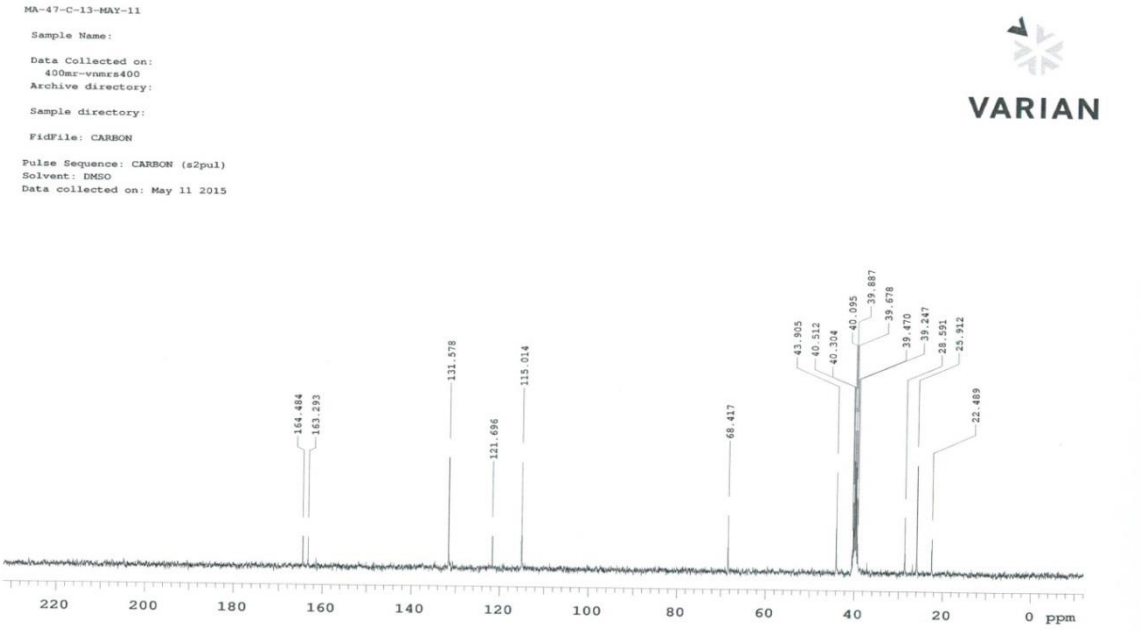
^{1}H and ^{13}C NMR of 6d



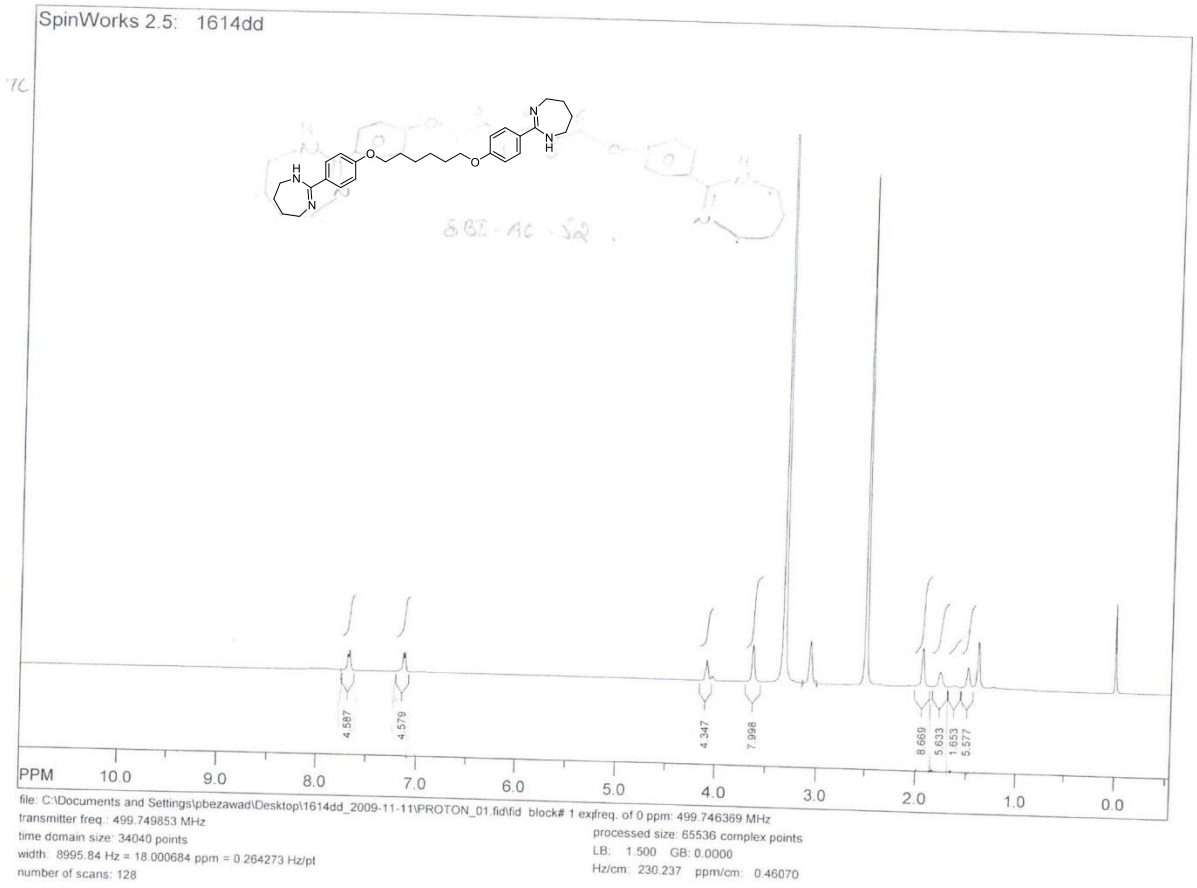


^{1}H and ^{13}C NMR of 7a





^{1}H and ^{13}C NMR of 7b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7C
SBI-AC-52
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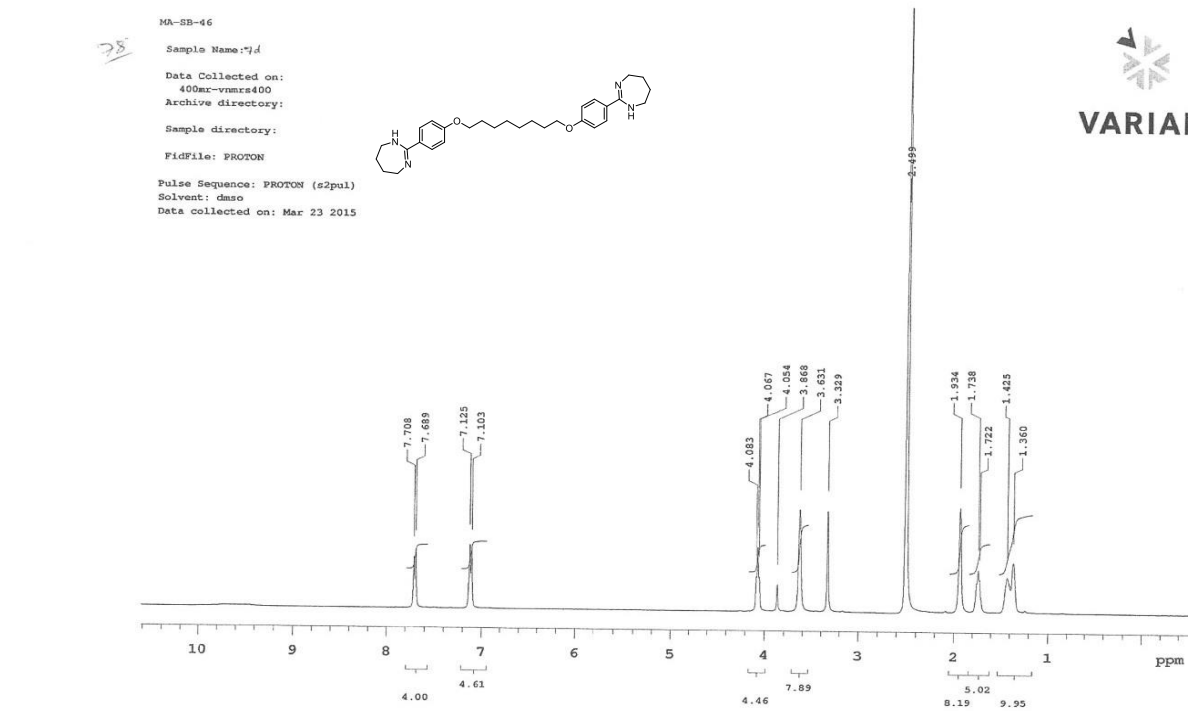
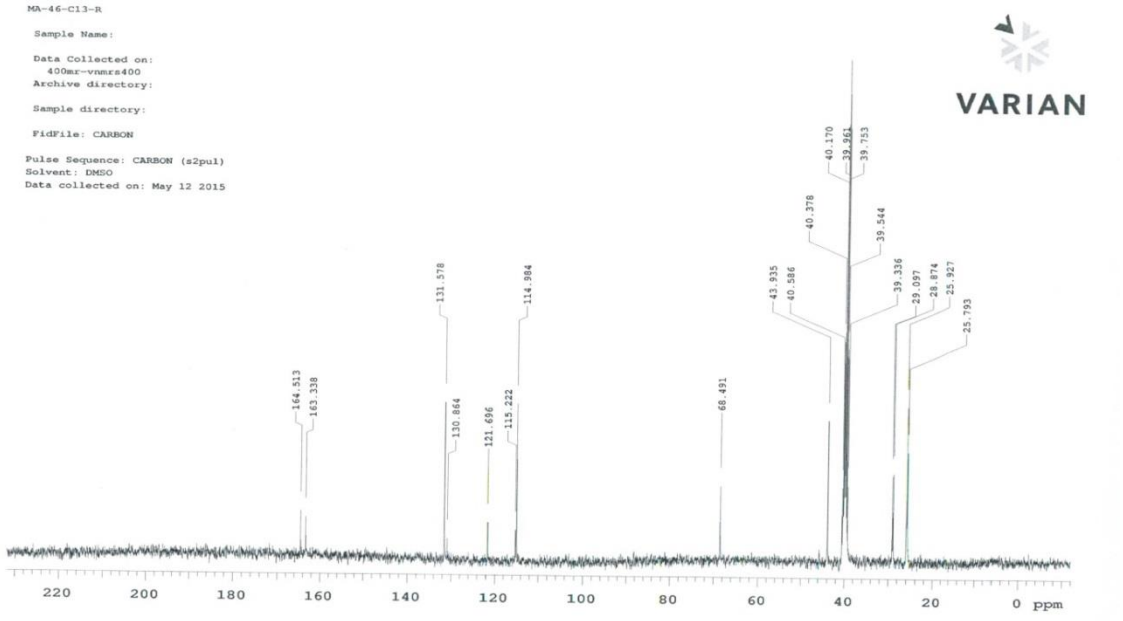
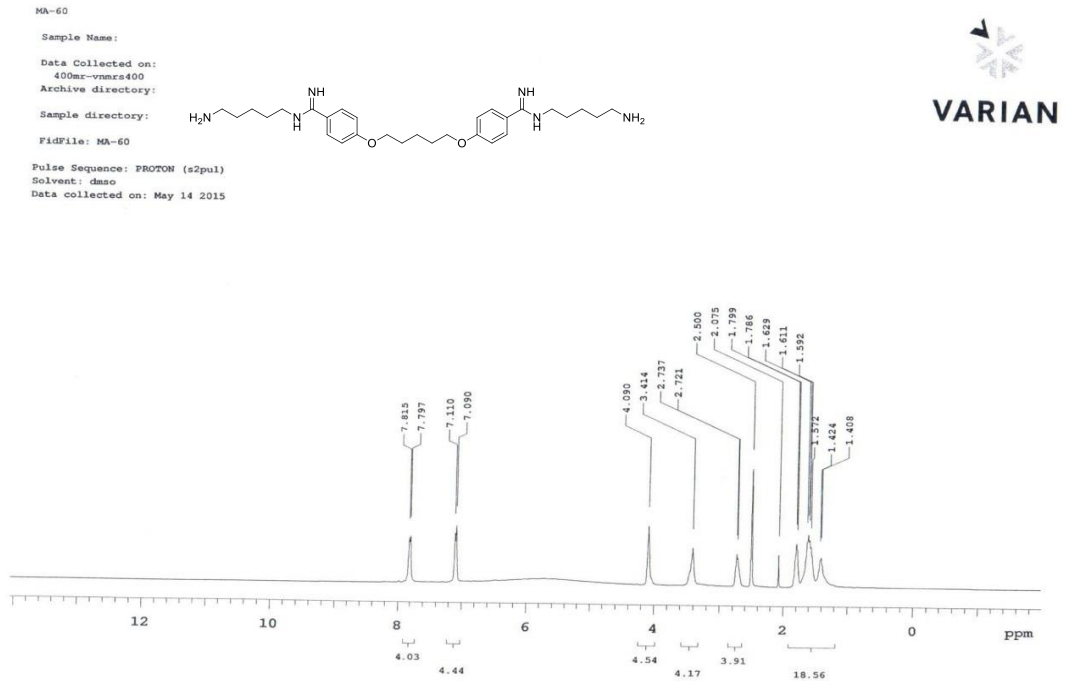
4.579
4.347
7.998
8.669
5.633
1.653
5.577
PPM
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
file: C:\Documents and Settings\pbezawad\Desktop\1614dd_2009-11-11\PROTON_01.fid\fid block# 1 exp
transmitter freq.: 499.749853 MHz
time domain size: 34040 points
width: 8995.84 Hz = 18.000684 ppm = 0.264273 Hz/pt
number of scans: 128
freq. of 0 ppm: 499.746369 MHz
processed size: 65536 complex points
LB: 1.500 GB: 0.0000
Hz/cm: 230.237 ppm/cm: 0.46070

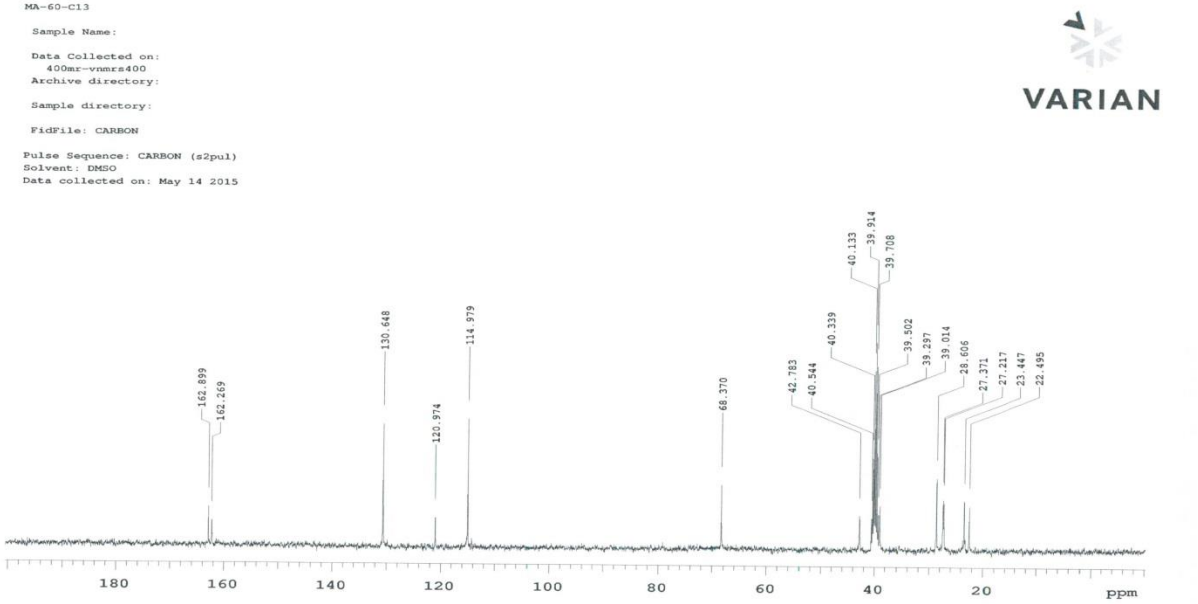
^{1}H NMR of 7c



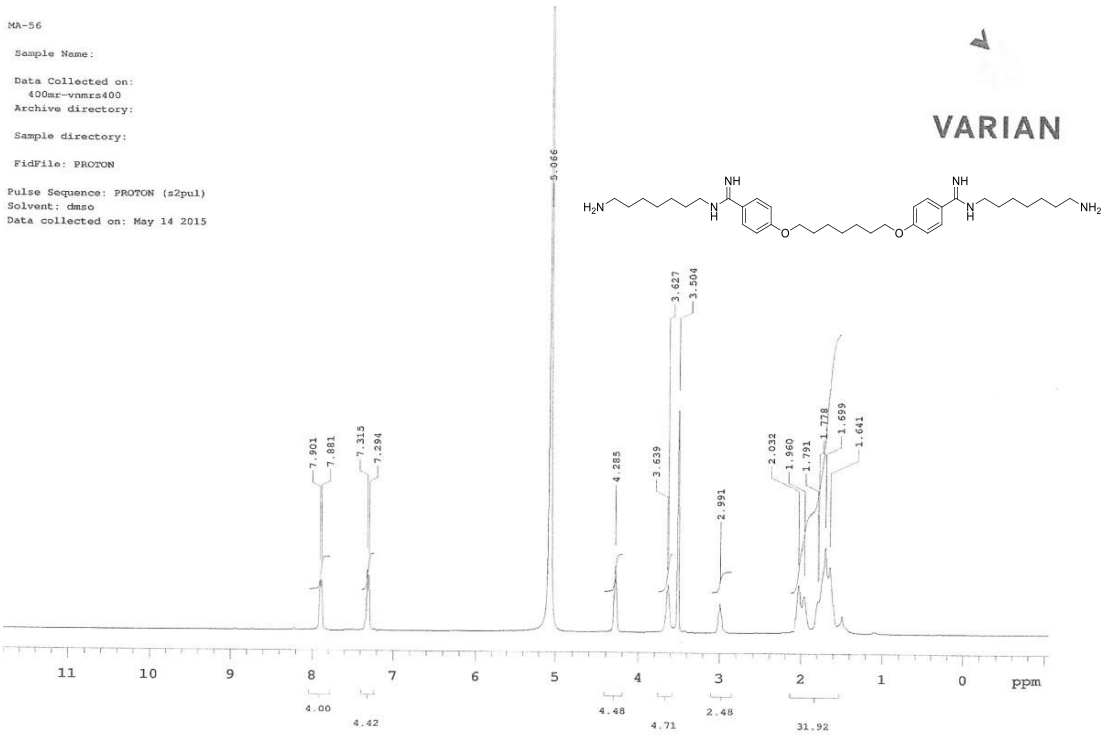


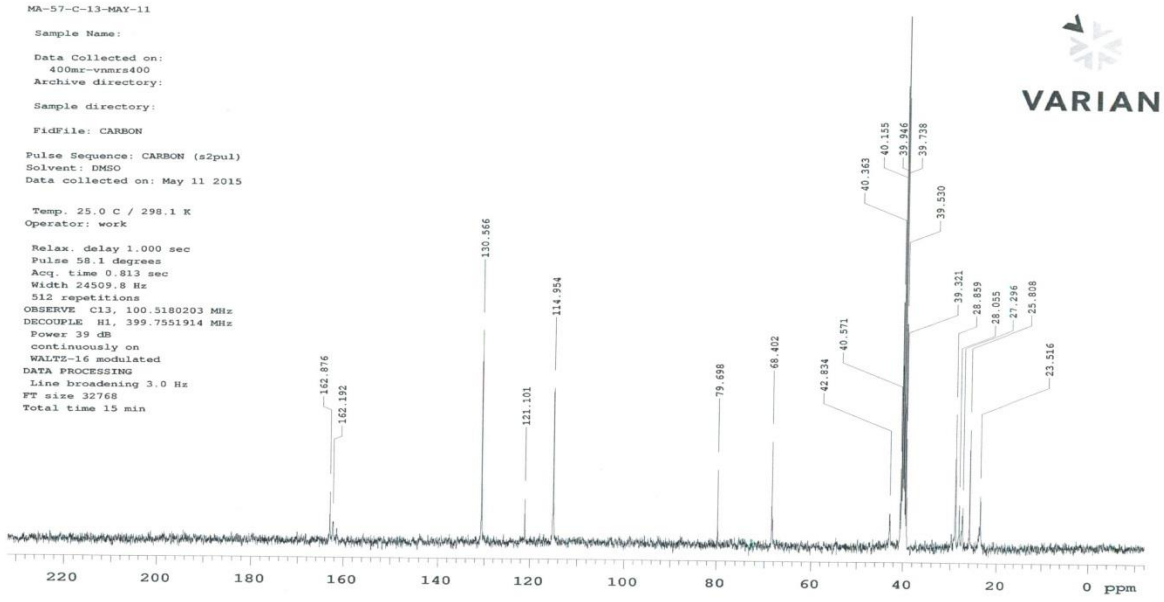
^{1}H and ^{13}C NMR of 7d



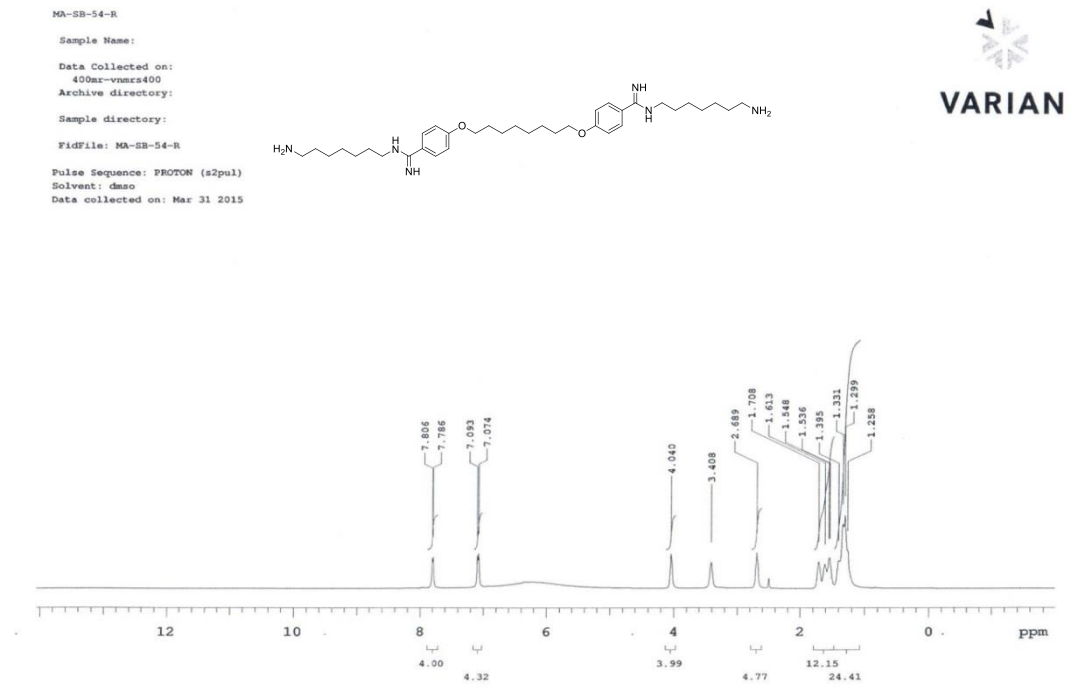


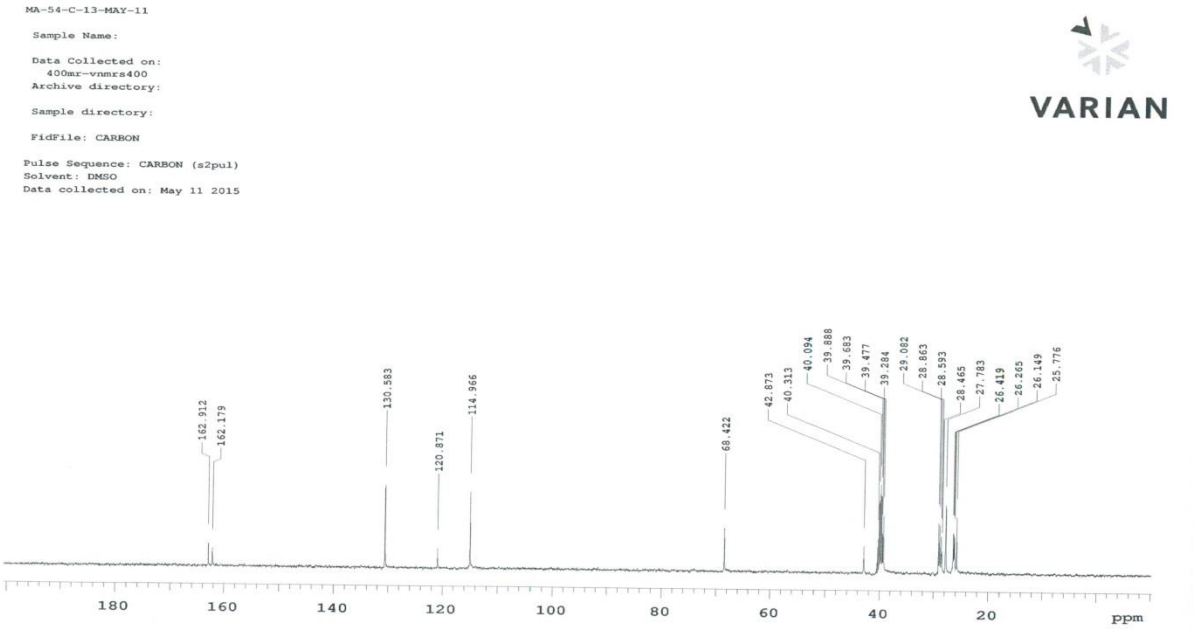
^{1}H and ^{13}C NMR of 8



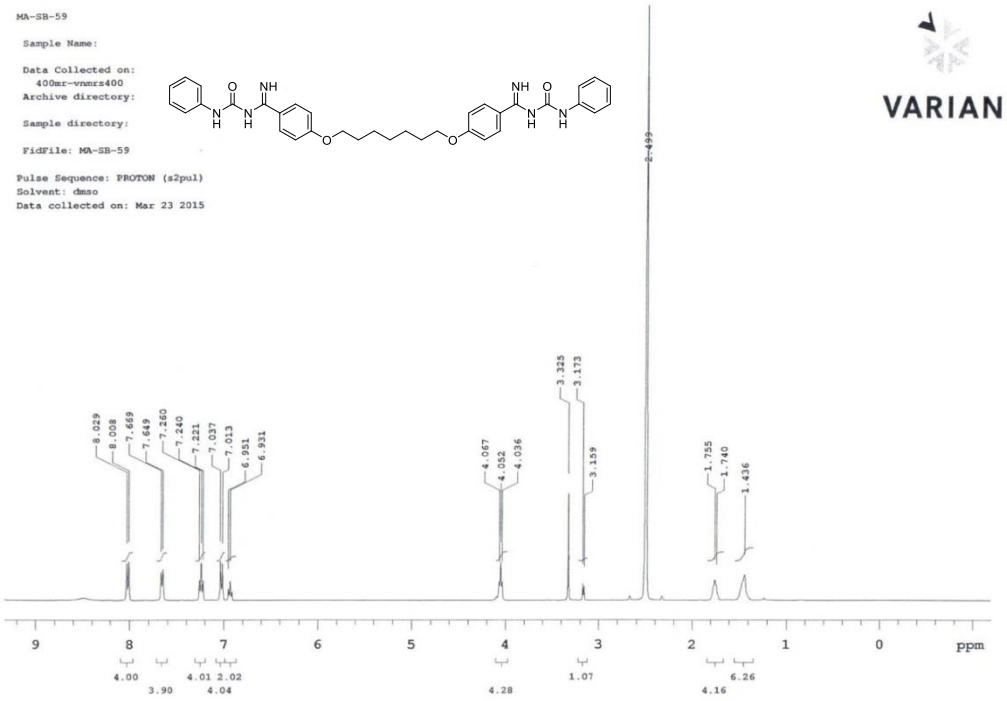


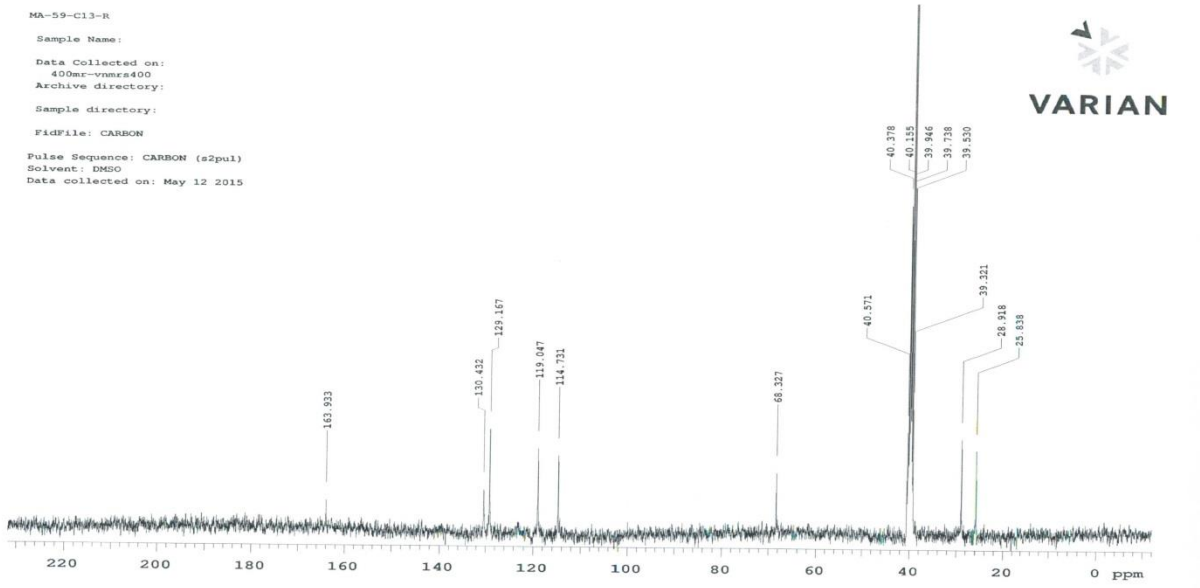
^{1}H and ^{13}C NMR of 9a



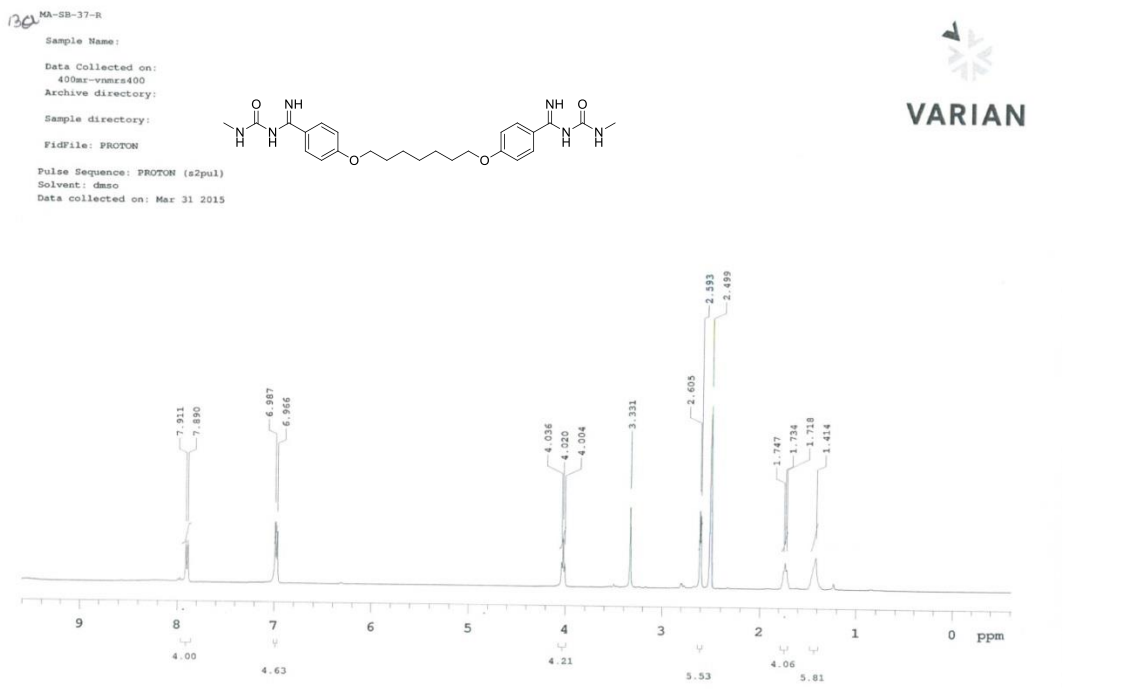


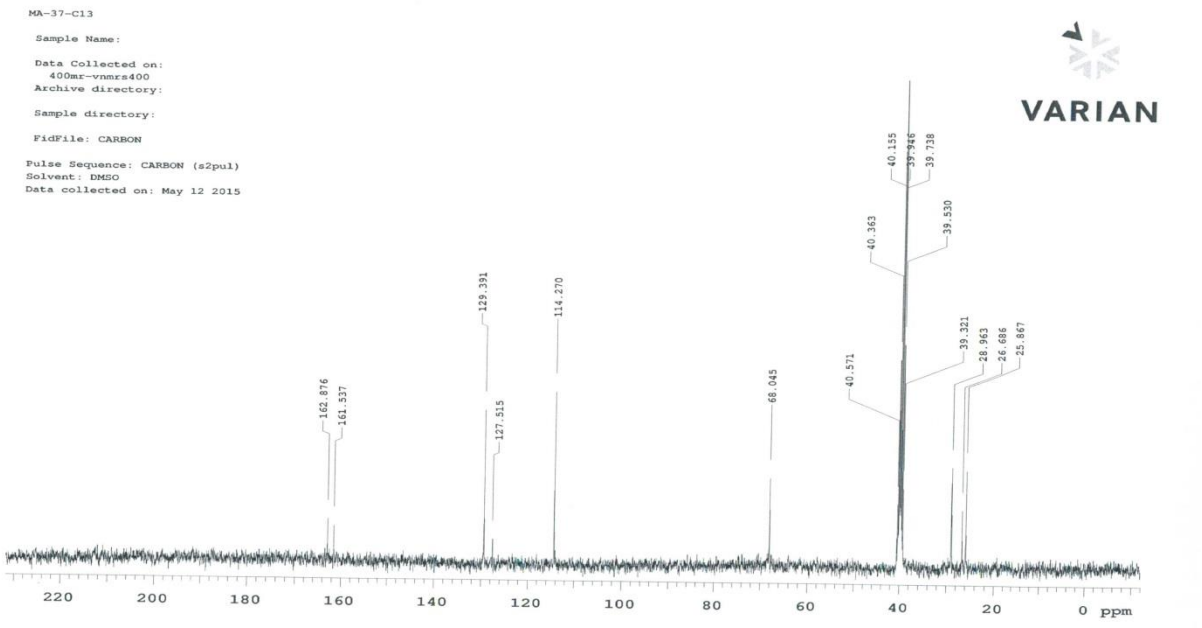
^{1}H and ^{13}C NMR of 9b



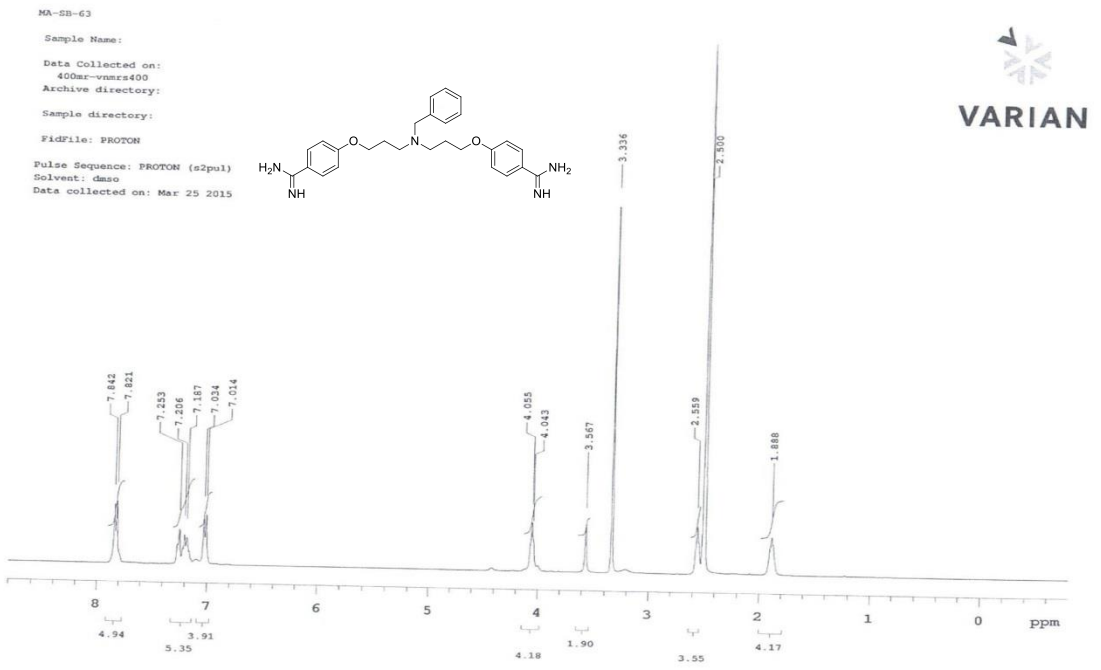


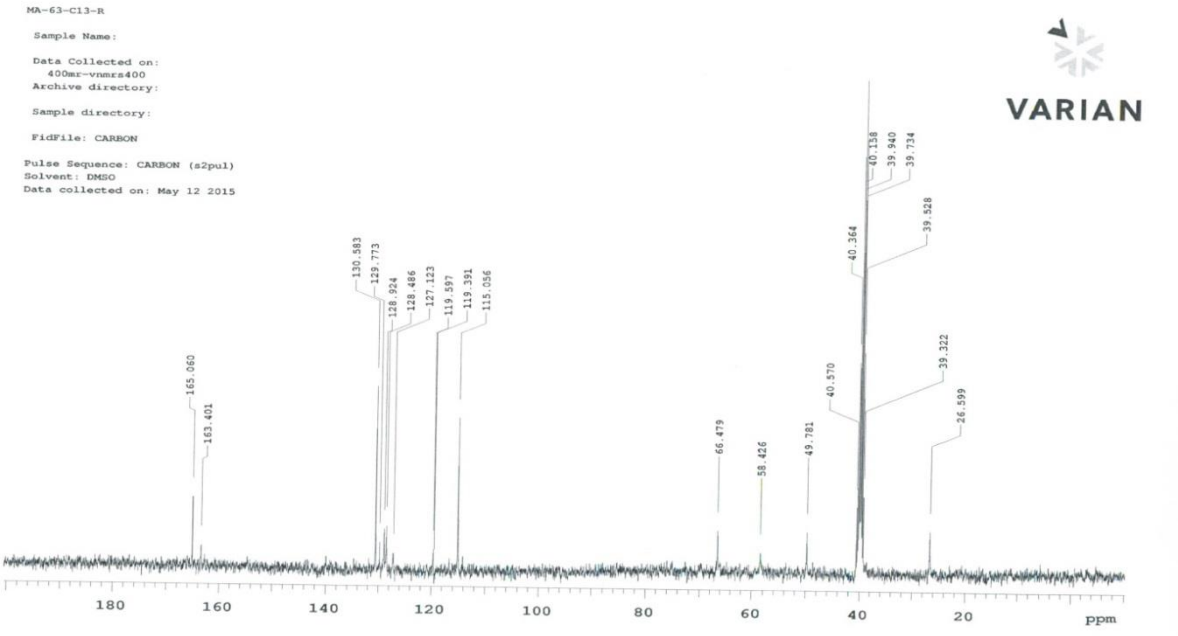
^{1}H and ^{13}C NMR of 10



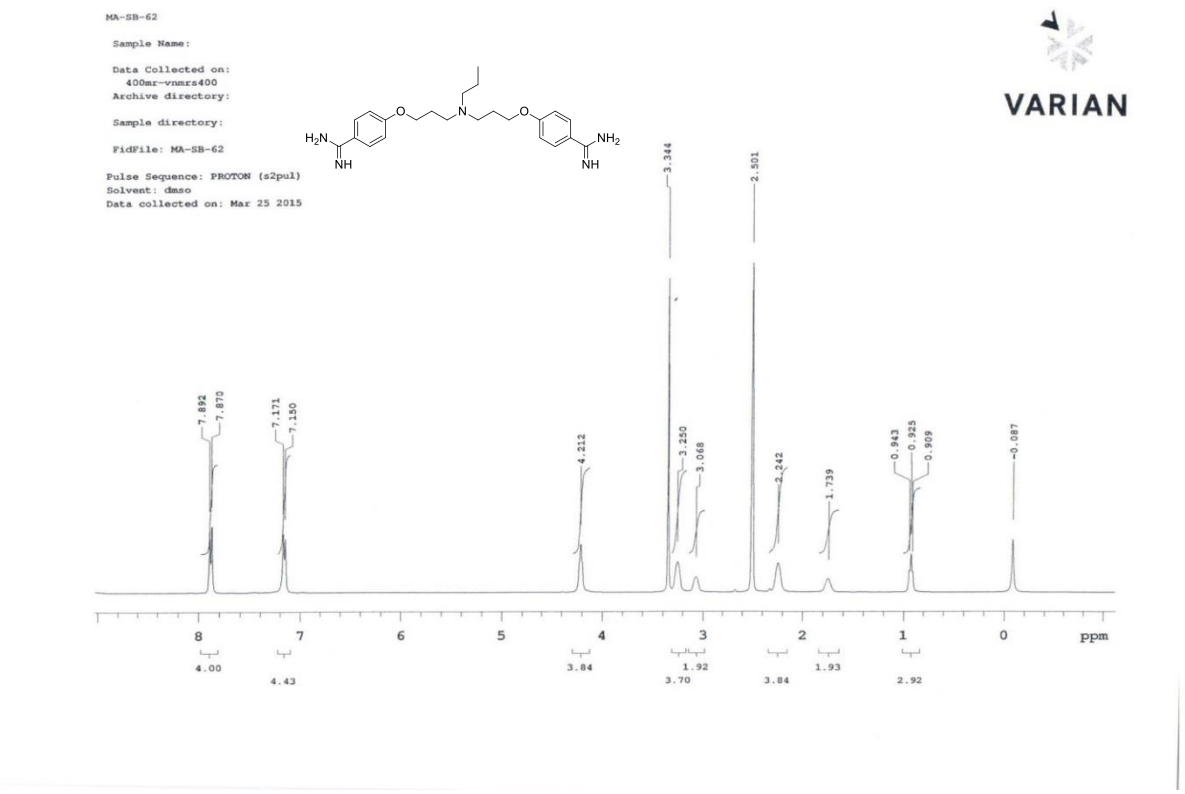


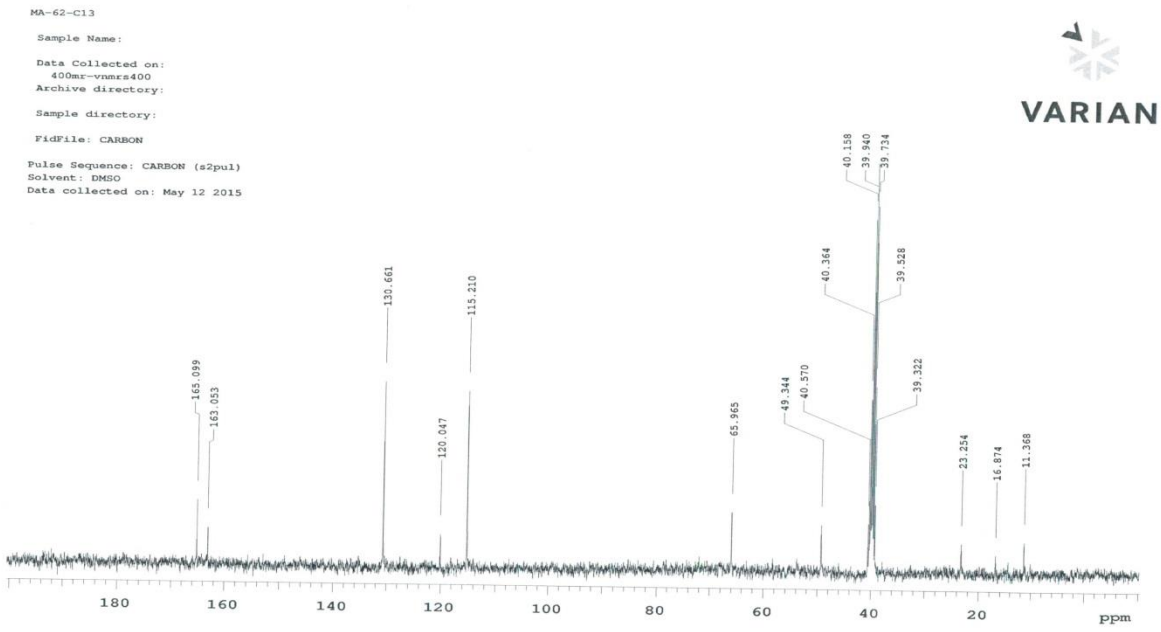
^{1}H and ^{13}C NMR of 11





^{1}H and ^{13}C NMR of 16a





^{1}H and ^{13}C NMR of 16b

Table S2. SMILES Documentation

Prep. I.D.	Internal I.D. SBiX	SMILE
4a	4211	N=C(N)C(C=C1)=CC=C1OCCCCCCCCOC2=CC=C(C(N)=N)C=C2
4b	1	N=C(C1=CC=C(OCCCCCOC2=CC=C(C(N)=N)C=C2)C=C1)N
4c	4210	N=C(N)C(C=C1)=CC=C1OCCCCCCCOC2=CC=C(C(N)=N)C=C2
5a	4225	C1(C2=NCCN2)=CC=C(OCCCCCCCCOC3=CC=C(C4=NCCN4)C=C3)C=C1
5b	4224	C1(C2=NCCN2)=CC=C(OCCCCCOC3=CC=C(C4=NCCN4)C=C3)C=C1
5c	4226	C1(C2=CC=C(OCCCCCCOC3=CC=C(C4=NCCN4)C=C3)C=C2)=NCCN1
5d	4221	C1(C2=CC=C(OCCCCCCCCOC3=CC=C(C4=NCCN4)C=C3)C=C2)=NCCN1
6a	4213	C1(C2=NCCCN2)=CC=C(OCCCCCCCCOC3=CC=C(C4=NCCCN4)C=C3)C=C1
6b	4214	C1(C2=NCCCN2)=CC=C(OCCCCCOC3=CC=C(C4=NCCCN4)C=C3)C=C1
6c	4218	C1(C2=NCCCN2)=CC=C(OCCCCCCOC3=CC=C(C4=NCCCN4)C=C3)C=C1
6d	4217	C1(C2=NCCCN2)=CC=C(OCCCCCCCCOC3=CC=C(C4=NCCCN4)C=C3)C=C1
7a	4227	C1(C2=CC=C(OCCCCCCCCOC3=CC=C(C4=NCCCCN4)C=C3)C=C2)=NCCCCN1
7b	4223	C1(C2=CC=C(OCCCCCOC3=CC=C(C4=NCCCCN4)C=C3)C=C2)=NCCCCN1
7c	4228	C1(C2=CC=C(OCCCCCCOC3=CC=C(C4=NCCCCN4)C=C3)C=C2)=NCCCCN1
7d	4222	C1(C2=CC=C(OCCCCCCCCOC3=CC=C(C4=NCCCCN4)C=C3)C=C2)=NCCCCN1
8	4236	N=C(NCCCCCN)C(C=C1)=CC=C1OCCCCCOC2=CC=C(C(NCCCCCN)=N)C=C2
9a	4232	N=C(NCCCCCCCN)C(C=C1)=CC=C1OCCCCCCCOC2=CC=C(C(NCCCCCCCN)=N)C=C2
9b	4230	N=C(NCCCCCCCN)C1=CC=C(OCCCCCCCCOC2=CC=C(C(NCCCCCCCN)=N)C=C2)C=C1
10	4235	N=C(NC(NC1=CC=CC=C1)=O)C(C=C2)=CC=C2OCCCCCCCOC3=CC=C(C(NC(NC4=CC=CC=C4)=O)=N)C=C3
11	4212	CNC(NC(C1=CC=C(OCCCCCCCOC2=CC=C(C(NC(NC)=O)=N)C=C2)C=C1)=N)=O
16a	4239	NC(C(C=C1)=CC=C1OCCCN(CC2=CC=CC=C2)CCCOC3=CC=C(C(N)=N)C=C3)=N
16b	4238	NC(C(C=C1)=CC=C1OCCCN(CCC)CCCOC2=CC=C(C(N)=N)C=C2)=N
17	29	N=C(C1=CC=C(OC2=CC=C(C3=CC(C=CC(C(N)=N)=C4)=C4N3)C=C2)C=C1)N