Supplementary data

Synthesis of Novel FTY720 Analogs with Anticancer Activity through PP2A Activation

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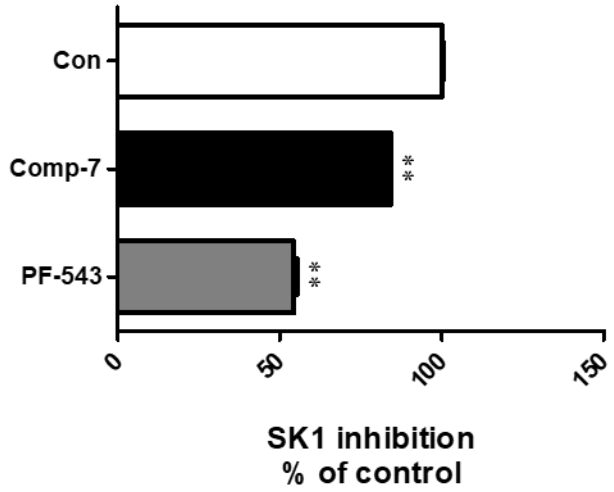


Figure S1. SK1 inhibition assay of compound **7.** SK1 activity was measured with 40 μM compound **7** (Comp-7) and PF-543 using Echelon's Sphingosine Kinase Activity Assay kit according to the manufacturer's protocol (100 μM sphingosine, 10 μM ATP and 0.5 ng/μL of recombinant sphingosine kinase 1).

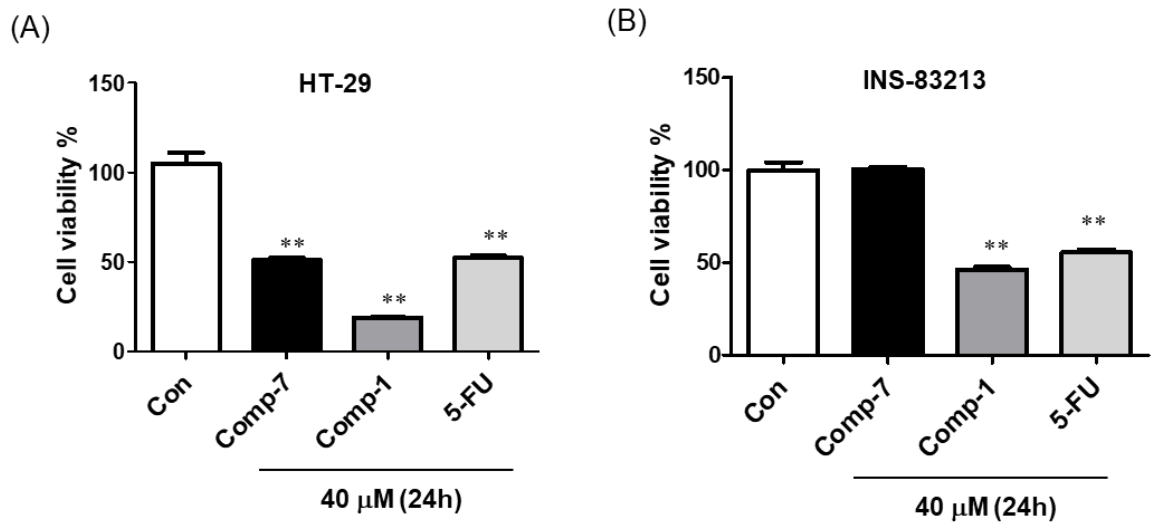
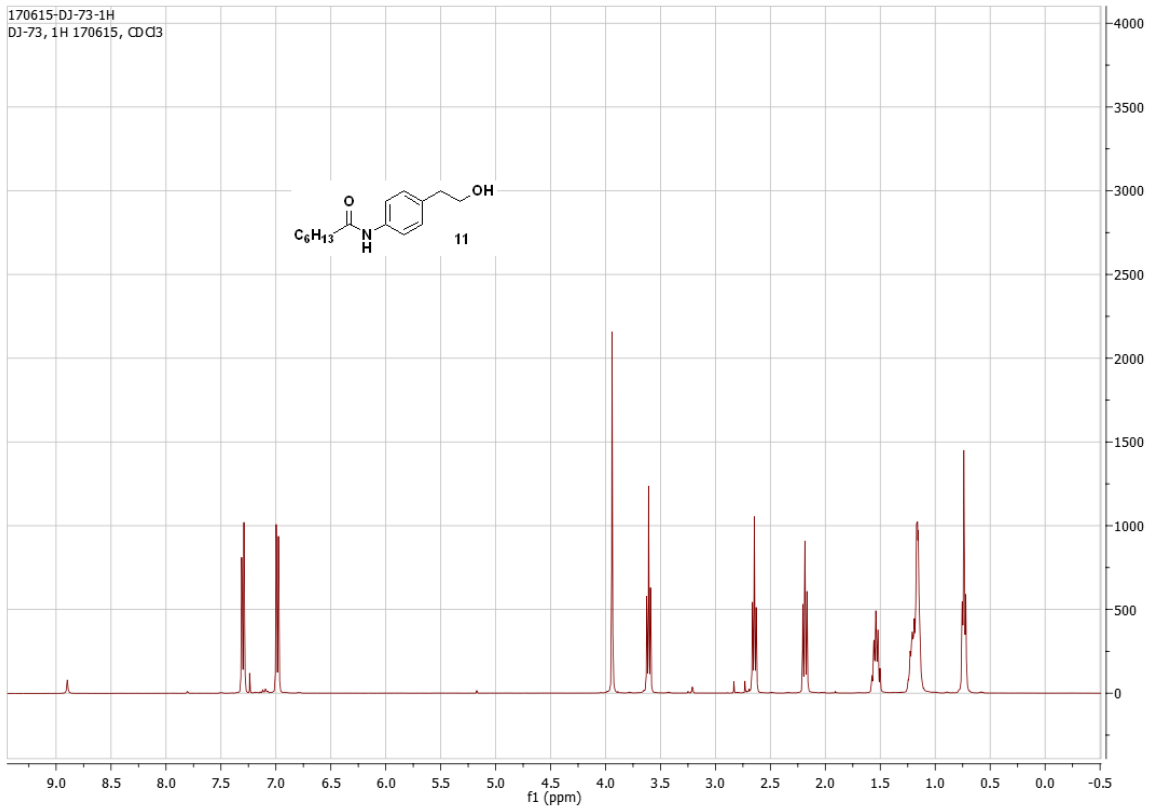
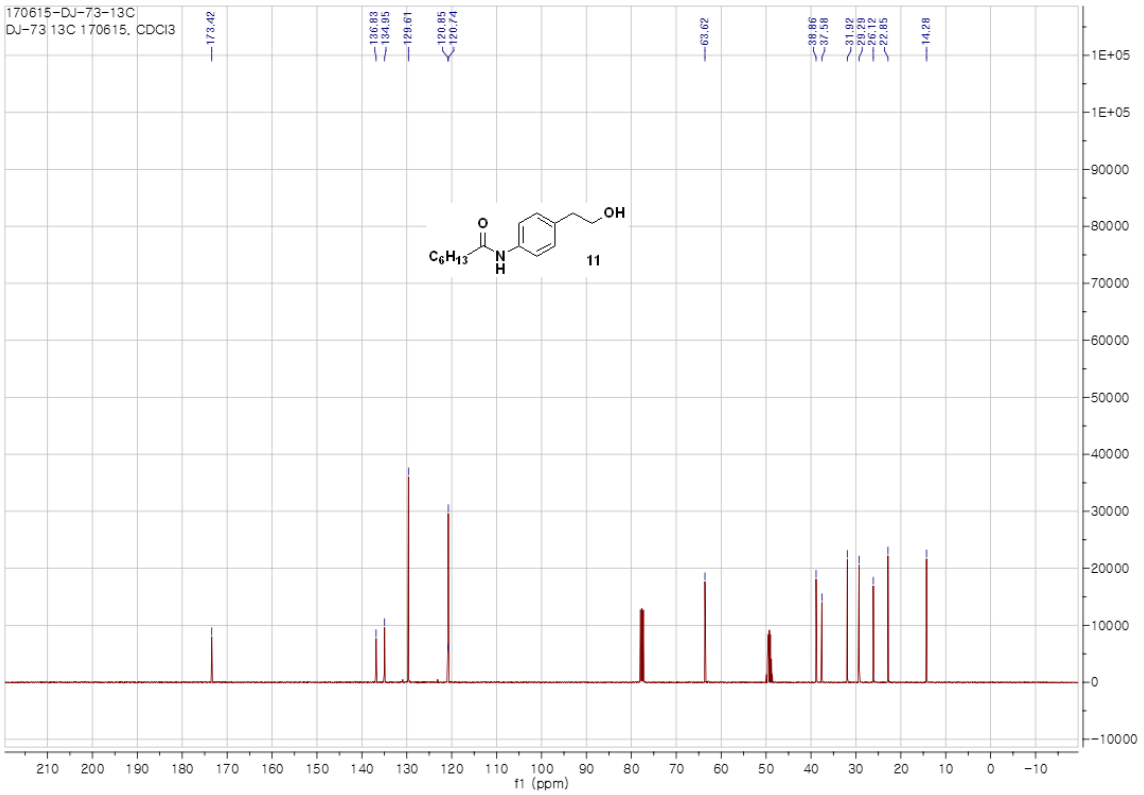
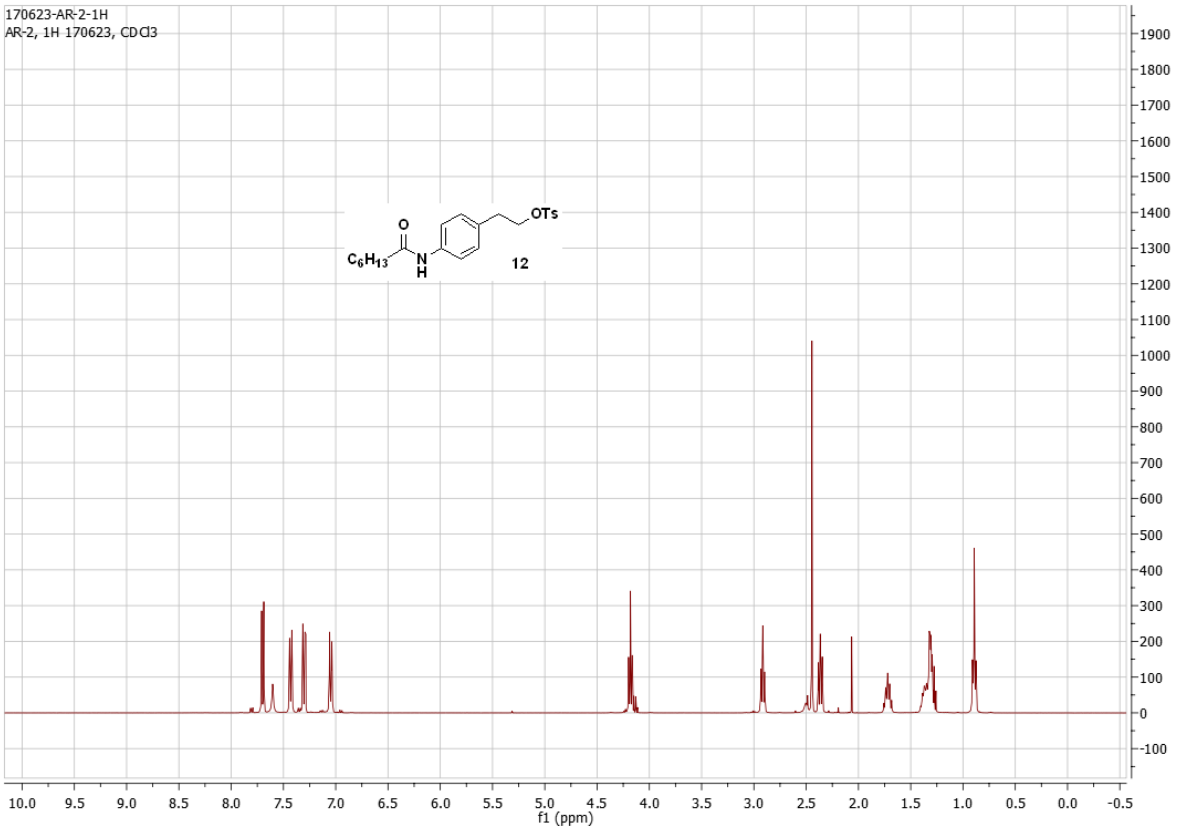


Figure S2. Cell cytotoxic effect of compound **7** in cancer and normal cell line. (A) HT-29 and (B) INS-83213 cells were plated in 96 well and treated with compound **7**, compound **1** (FTY720) and 5-fluorouracil (5-FU) 40 μM for 24 h, and cell viability was measured by EZ-CYTOTOX kit. Data are means ± SD from three independent experiments. (n=8) **p<0.01 compared with control cells.

^{1}H and ^{13}C NMR Spectra



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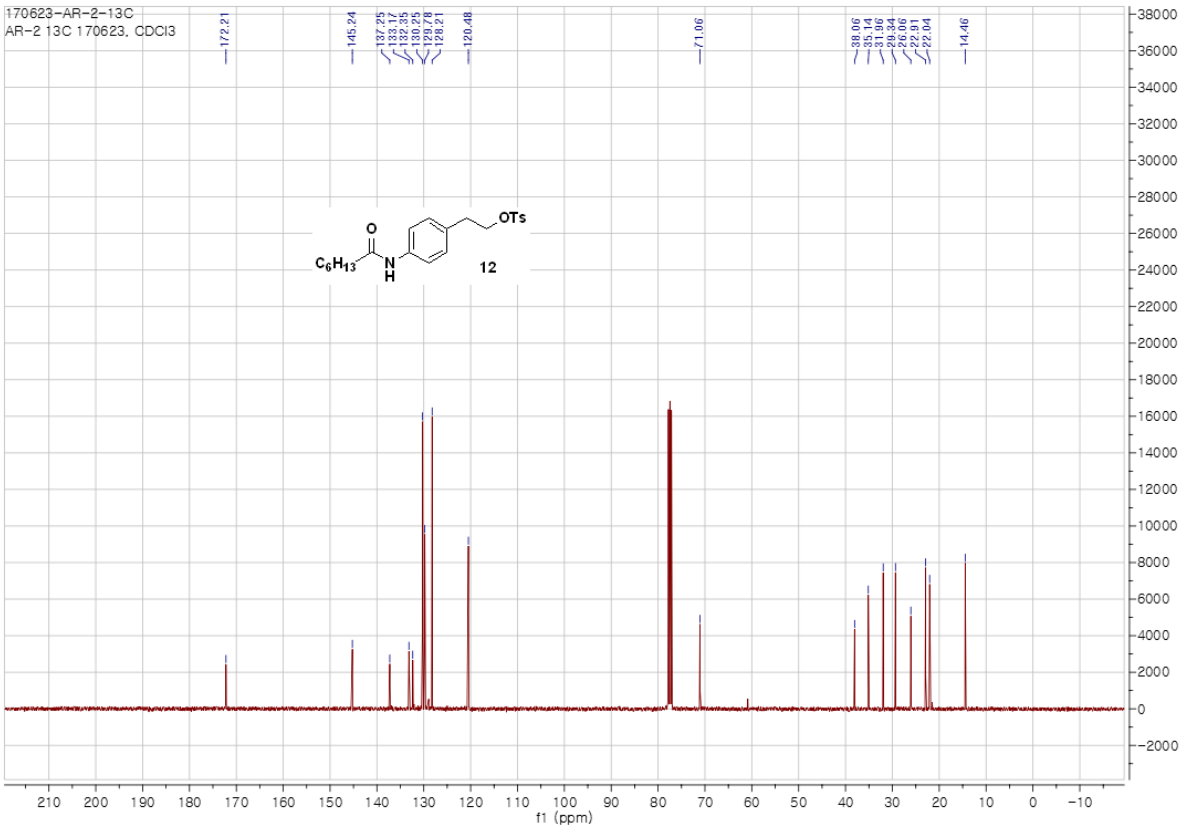
AR-2, 1H 170623, CDCl3
OTs
O
N
H
C_6H_{13}
12
f1 (ppm)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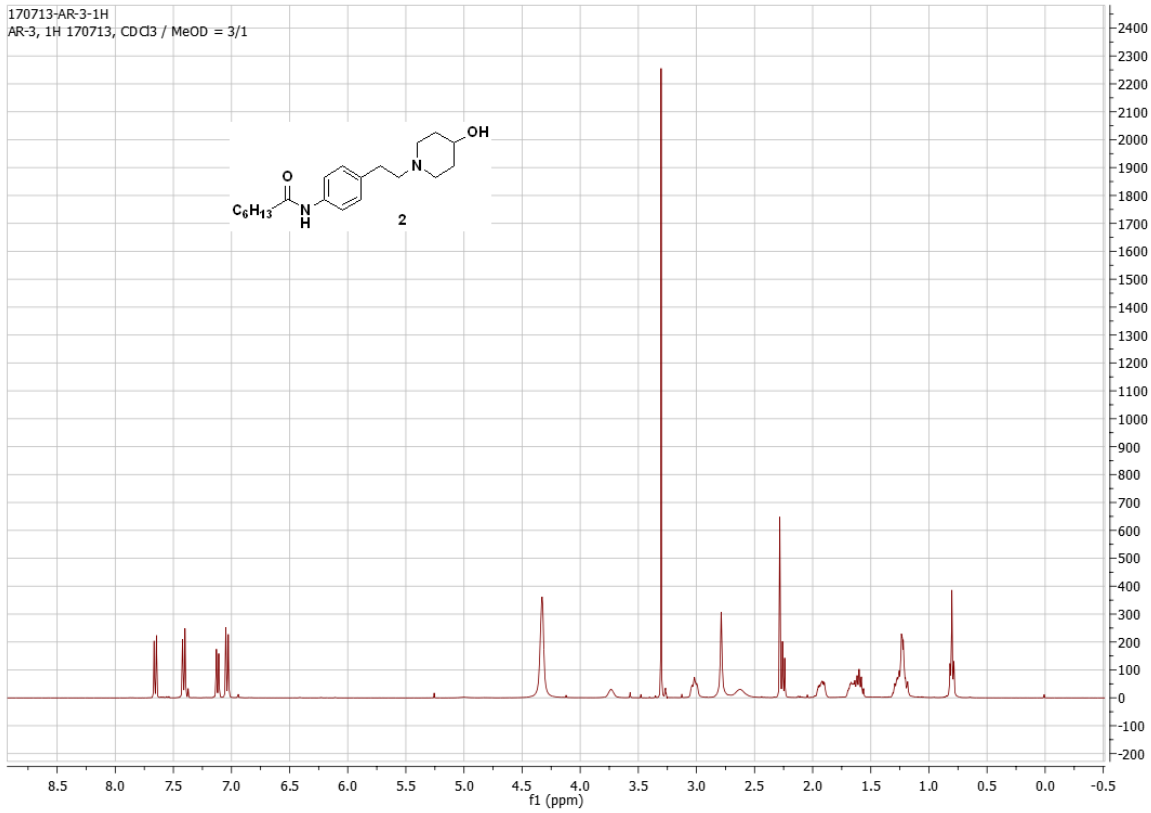
AR-2 13C 170623, CDCl3
172.21
145.24
137.25
133.17
130.25
129.78
128.21
120.48
71.06
38.06
35.14
31.66
29.34
26.06
22.91
22.04
14.46
OTs
O
N
H
C_6H_{13}
12
f1 (ppm)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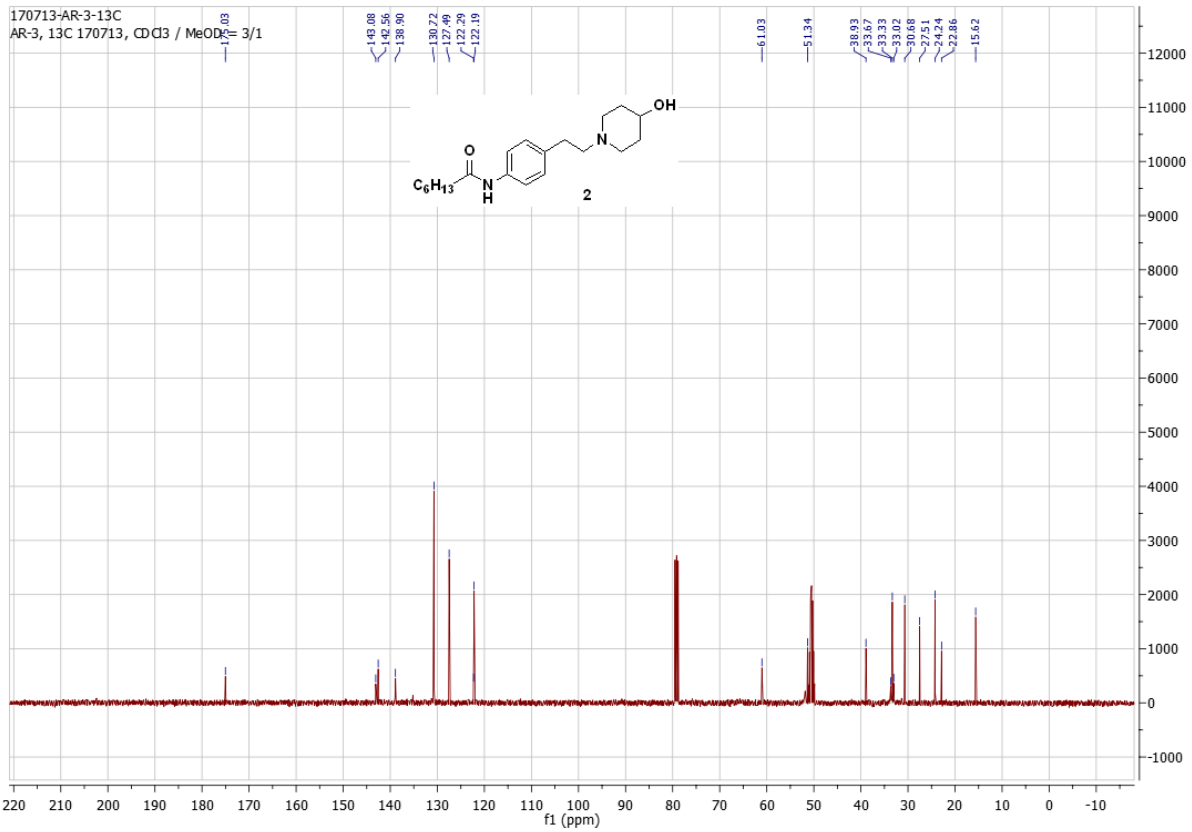
OH
N
O
N
H
C6H13
2
f1 (ppm)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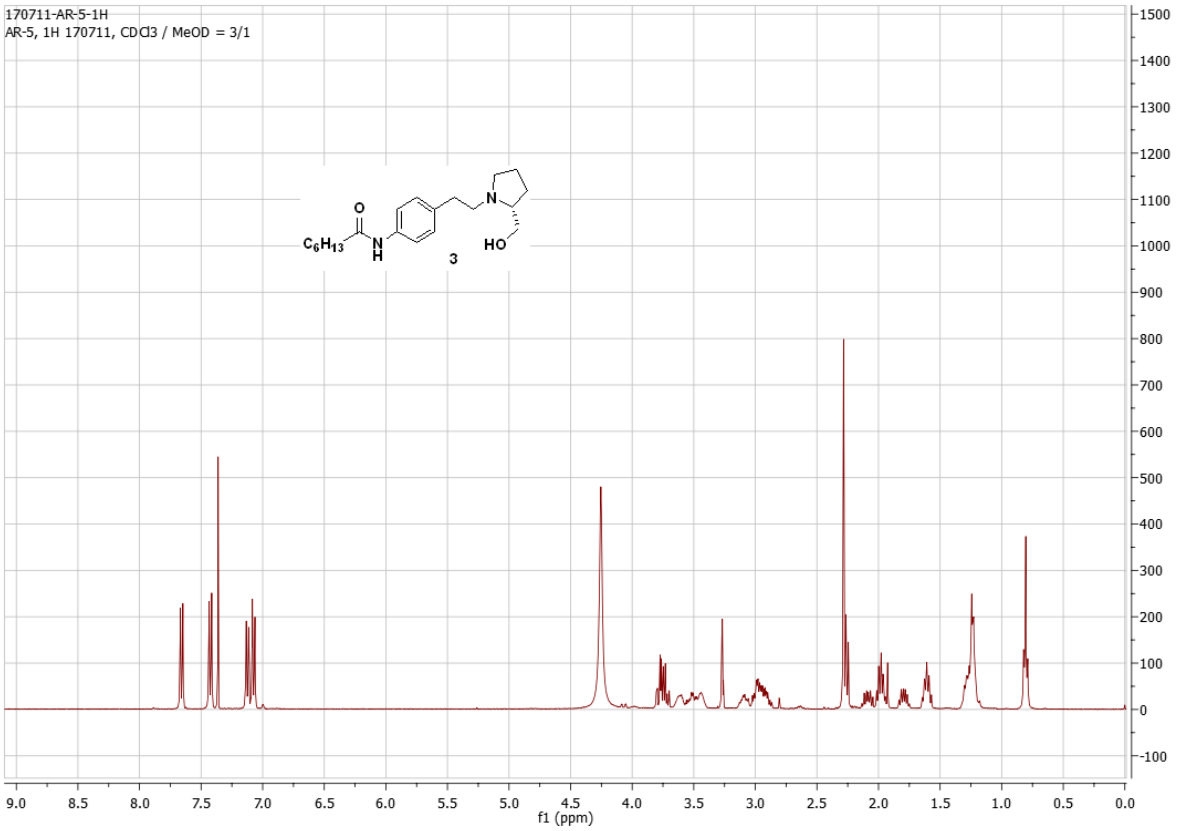
143.08
142.56
138.90
130.72
127.49
122.29
122.19
61.03
51.34
38.93
33.67
33.33
33.02
30.68
27.51
24.24
22.86
15.62
OH
N
O
N
H
C6H13
2
f1 (ppm)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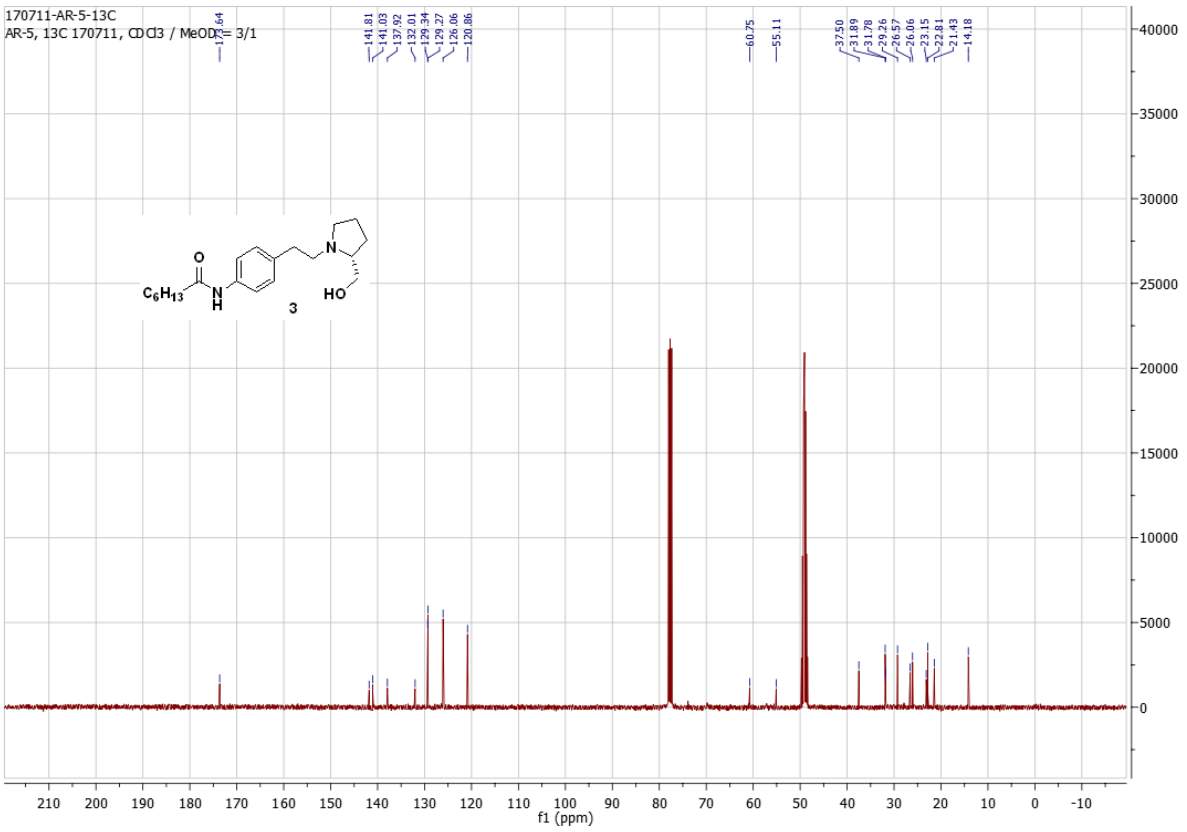
3
f1 (ppm)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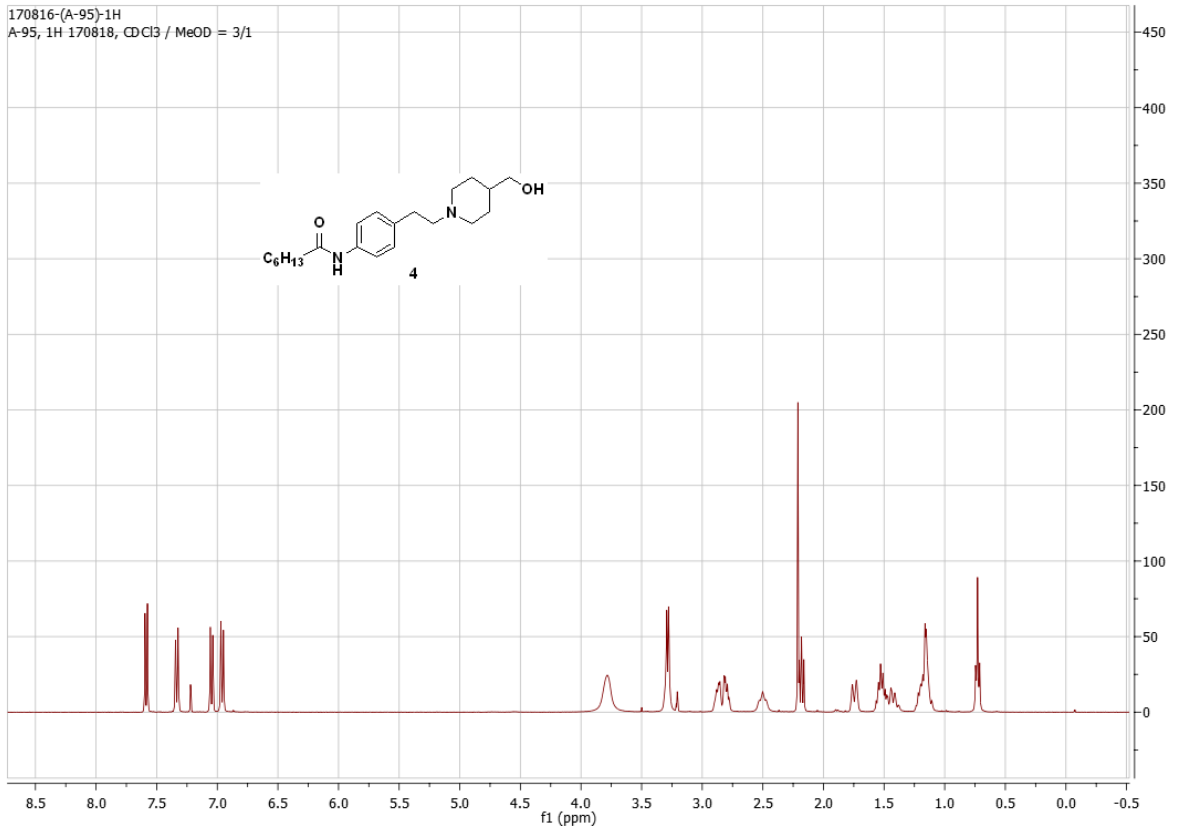
f1 (ppm)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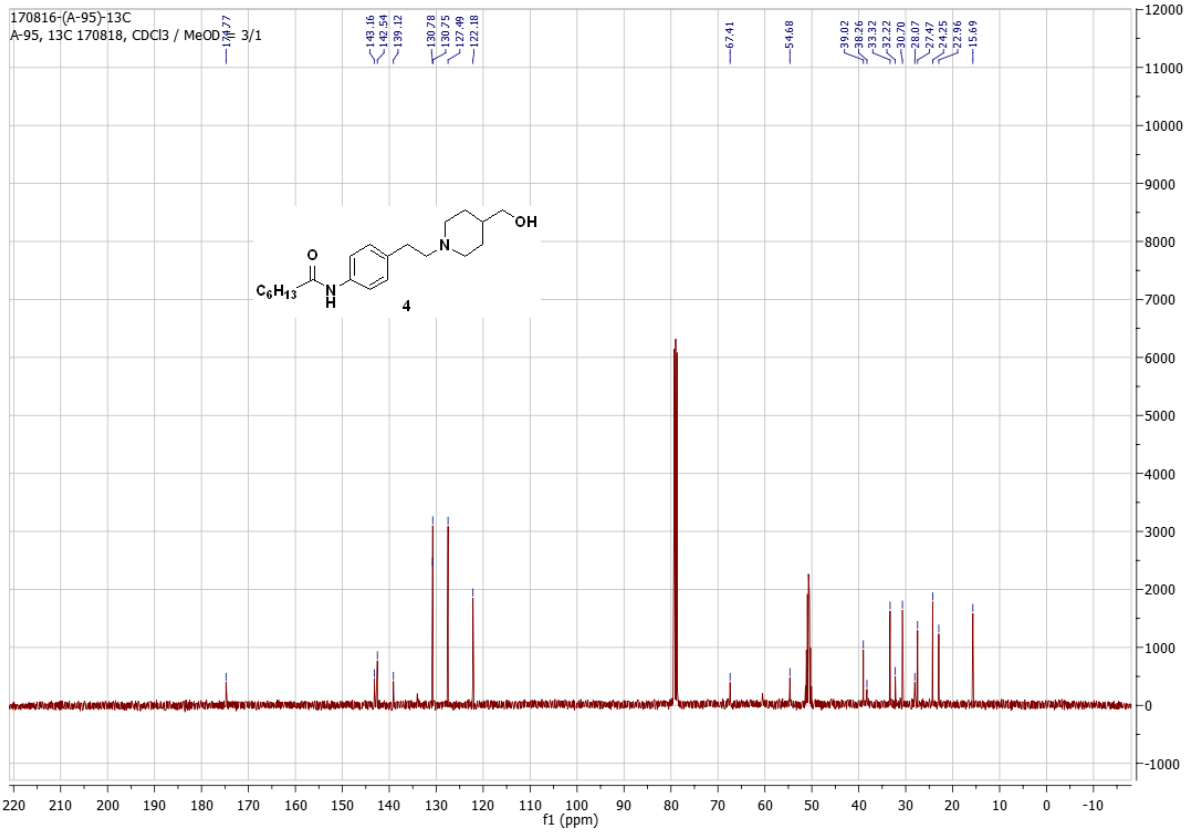
OH
N
O
N
H
C_6H_{13}
4
f1 (ppm)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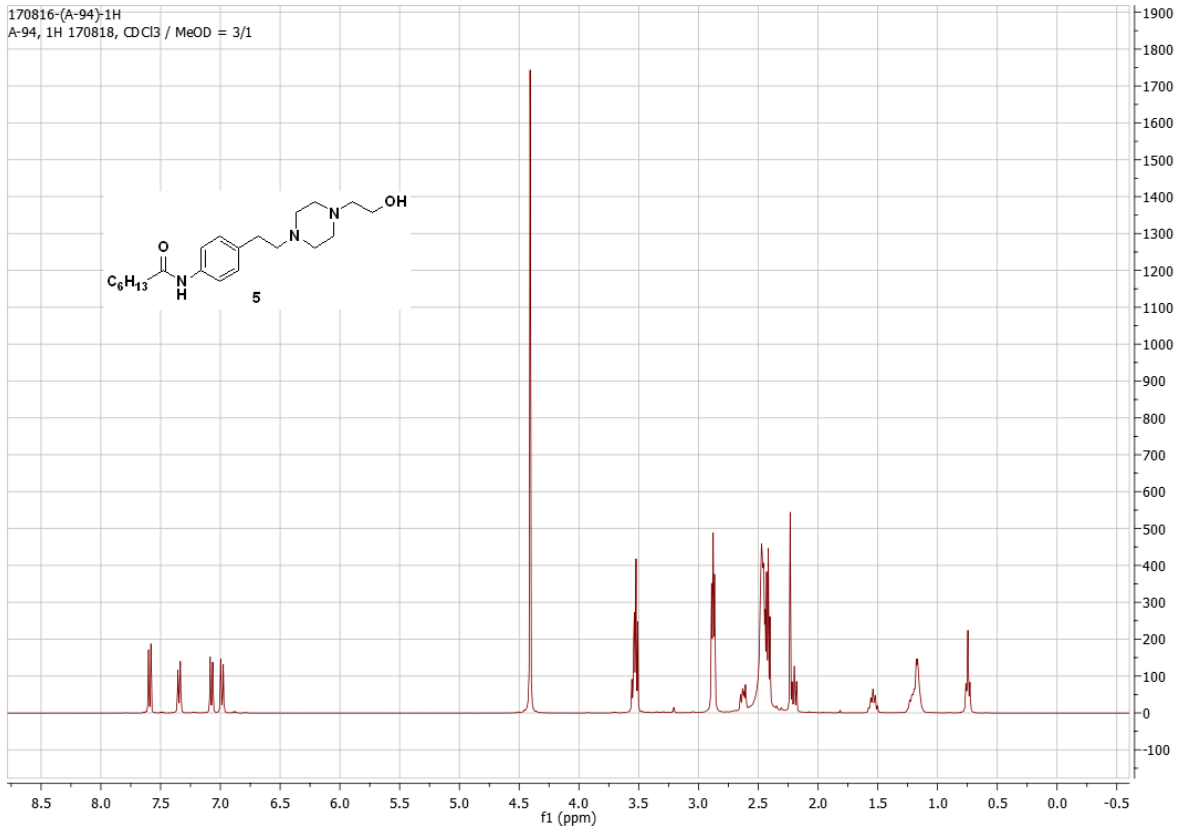
A-95, 13C 170818, CDCl3 / MeOD = 3/1
143.16
142.54
139.12
130.78
130.75
127.49
122.18
67.41
54.68
39.02
38.26
33.32
32.22
30.70
28.07
27.47
24.25
22.96
15.69
OH
N
O
N
H
C_6H_{13}
4
f1 (ppm)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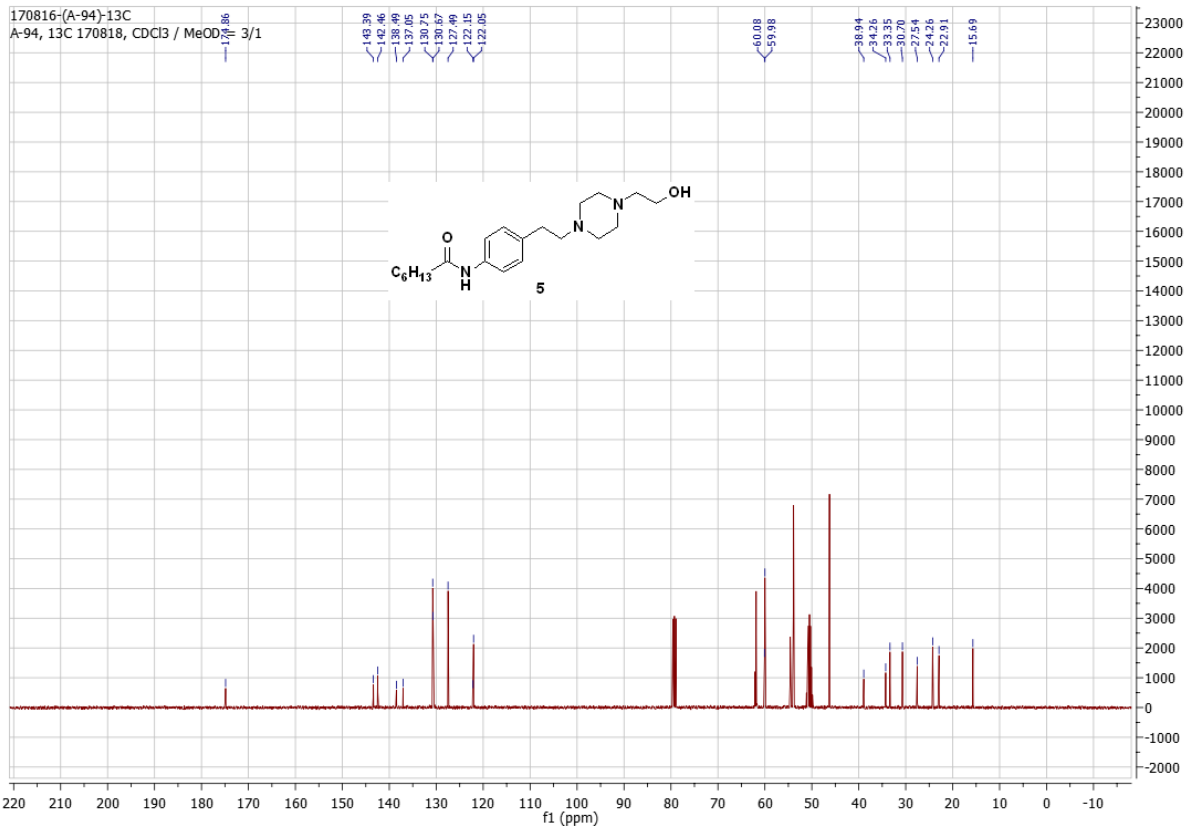
A-94, 1H 170818, CDCl3 / MeOD = 3/1
OH
N
N
O
N
H
C6H13
5
f1 (ppm)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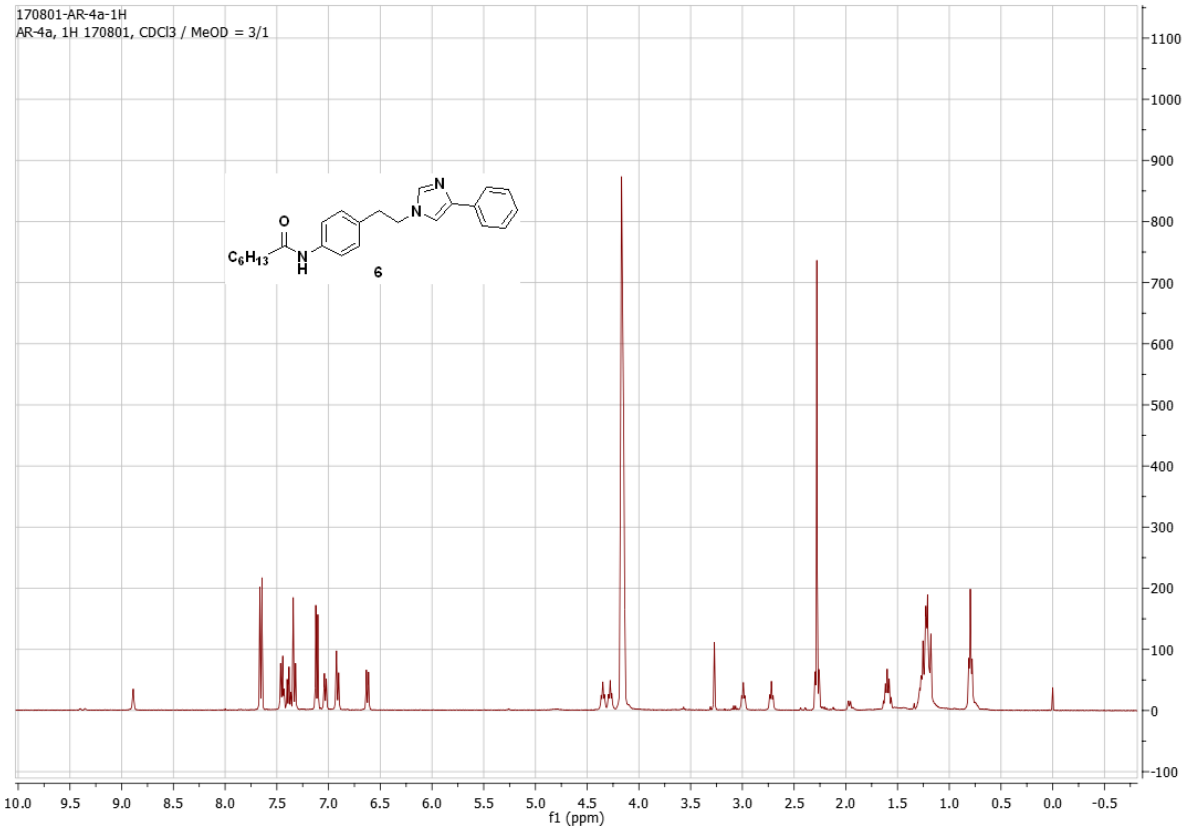
N
N
O
N
H
C6H13
5
f1 (ppm)


170801-AR-4a-1H
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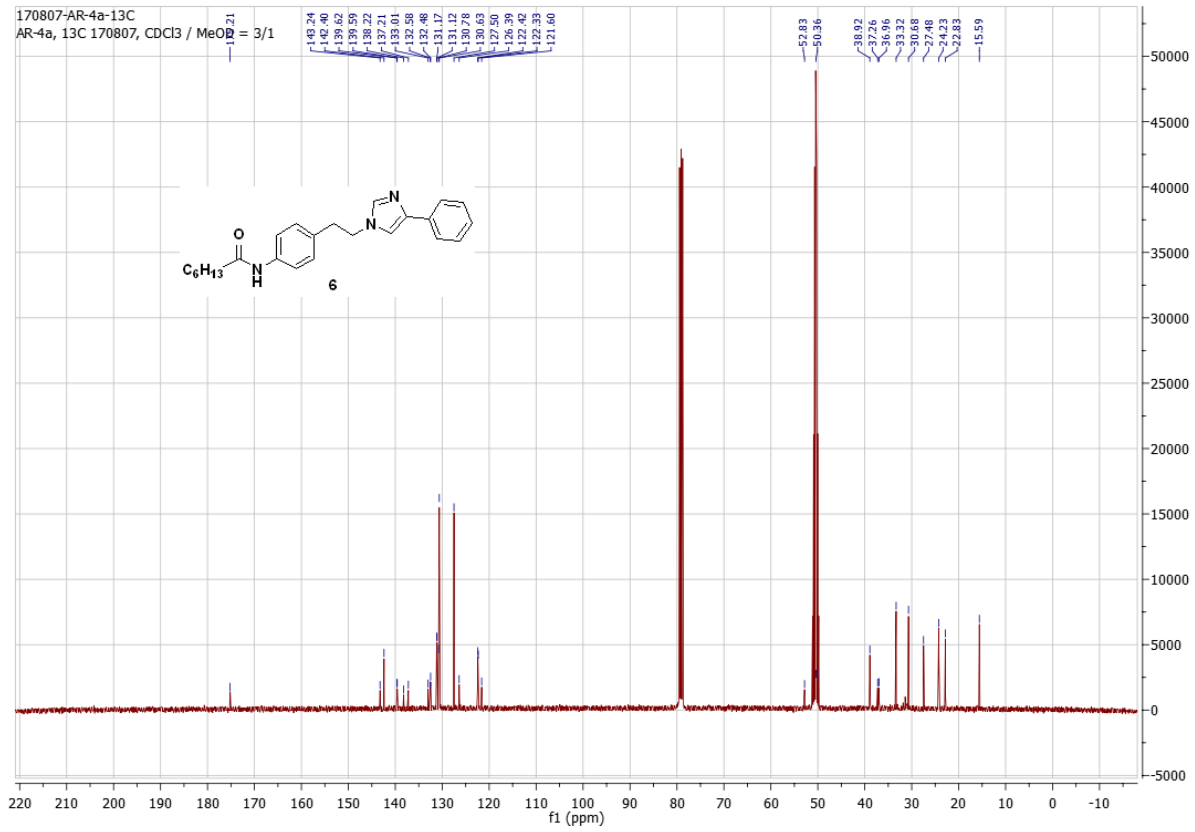
O
N
H
C_6H_{13}
N
N
6
f1 (ppm)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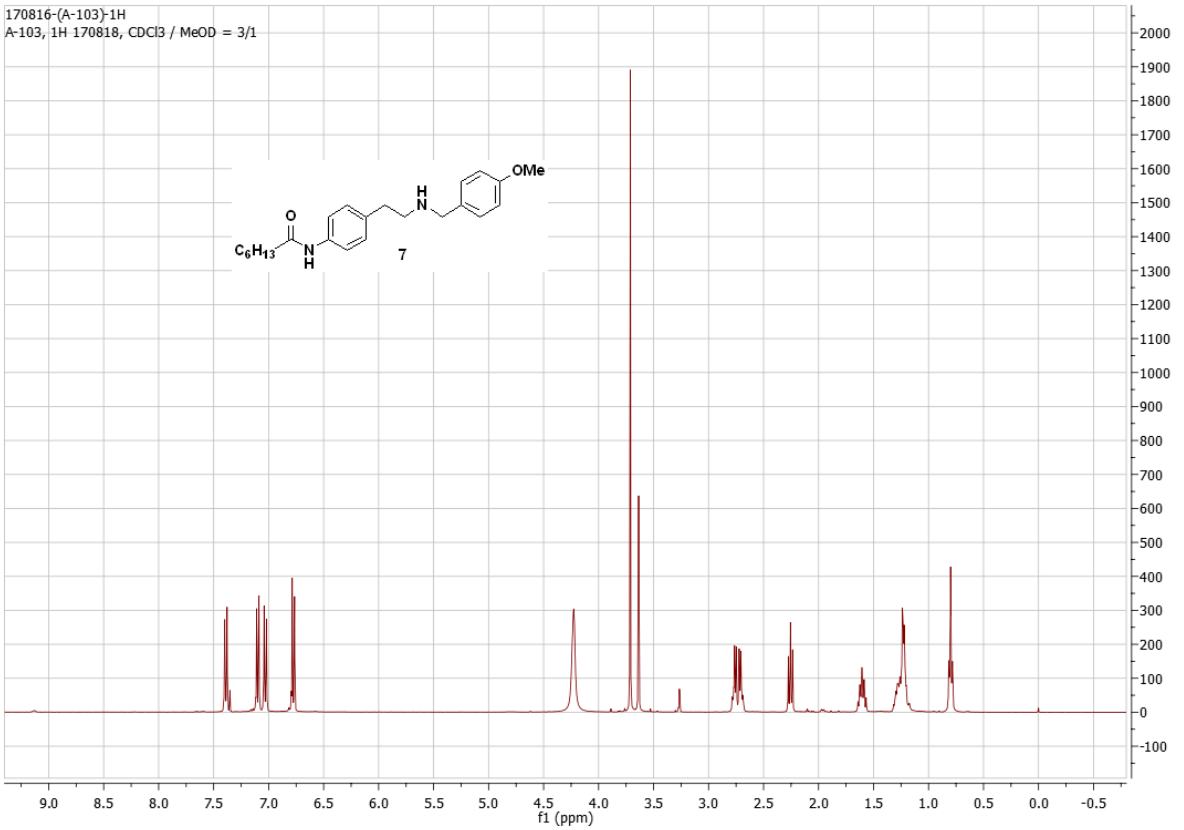
AR-4a, 13C 170807, CDCl3 / MeOD = 3/1
173.21
143.24
142.40
139.62
139.59
138.22
137.21
133.01
132.58
132.48
131.17
131.12
130.78
130.63
127.50
126.39
122.42
122.33
121.60
52.83
50.36
38.92
37.26
36.96
33.32
30.68
27.48
24.23
22.83
15.59
O
N
H
C_6H_{13}
N
N
6
f1 (ppm)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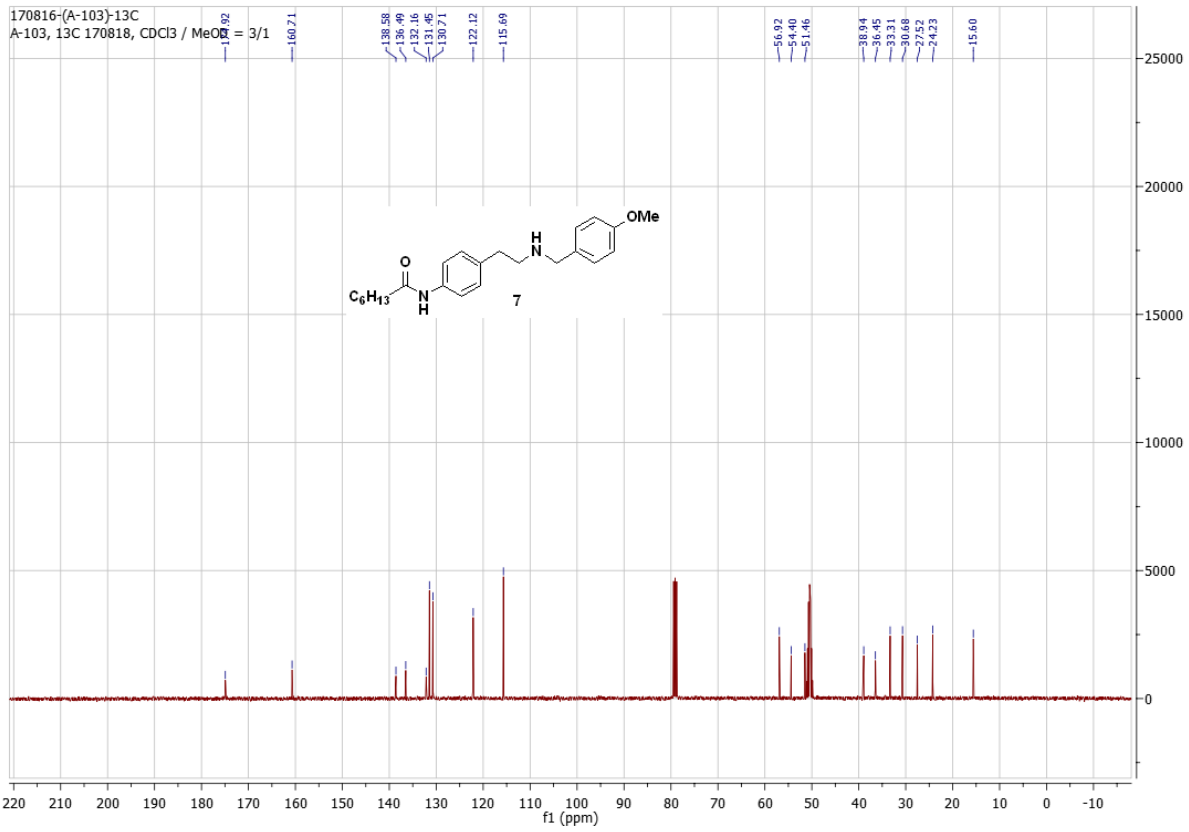
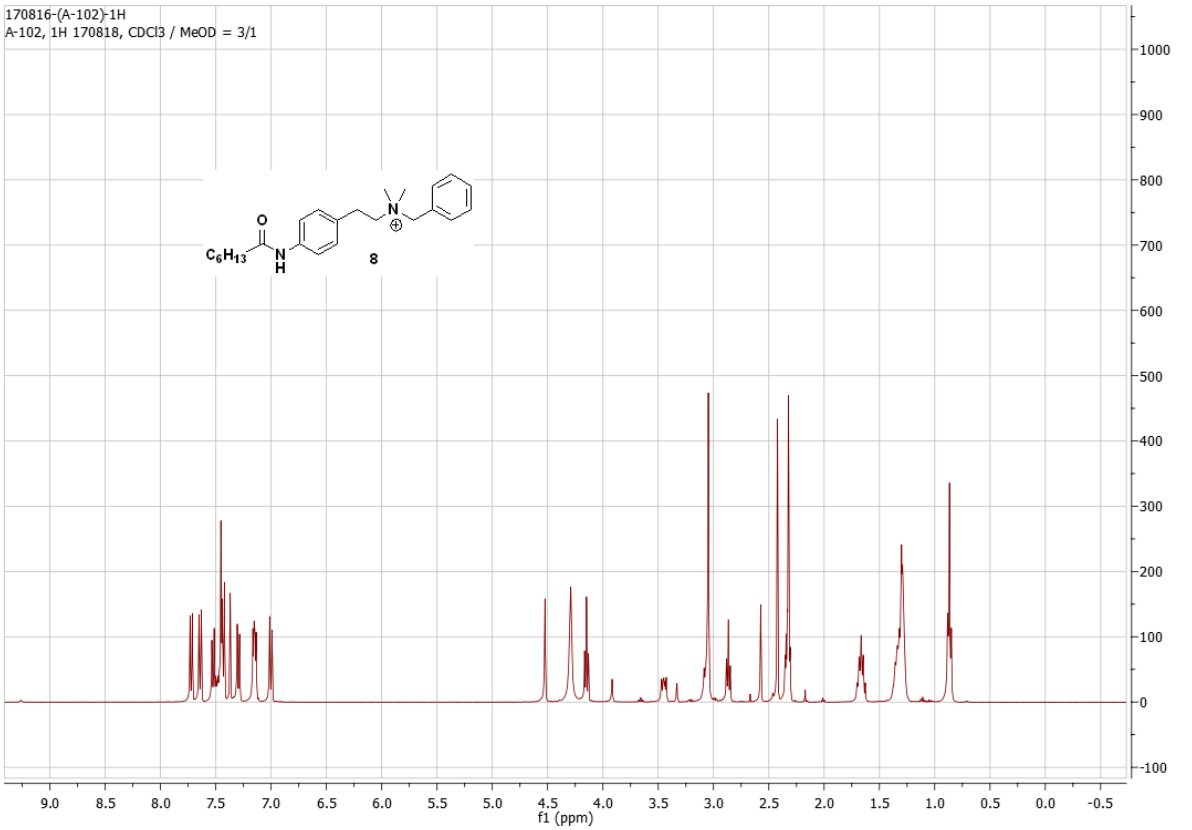
A-103, 1H 170818, CDCl3 / MeOD = 3/1
OMe
H
N
O
N
H
C_6H_{13}
7
f1 (ppm)


170816-(A-103)-13C
A-103, 13C 170818, CDCl3 / MeOD = 3/1
173.92
160.71
138.58
136.49
132.16
131.45
130.71
122.12
115.69
56.92
54.40
51.46
38.94
36.45
33.31
30.68
27.52
24.23
15.60
OMe
H
N
O
N
H
C_6H_{13}
7
f1 (ppm)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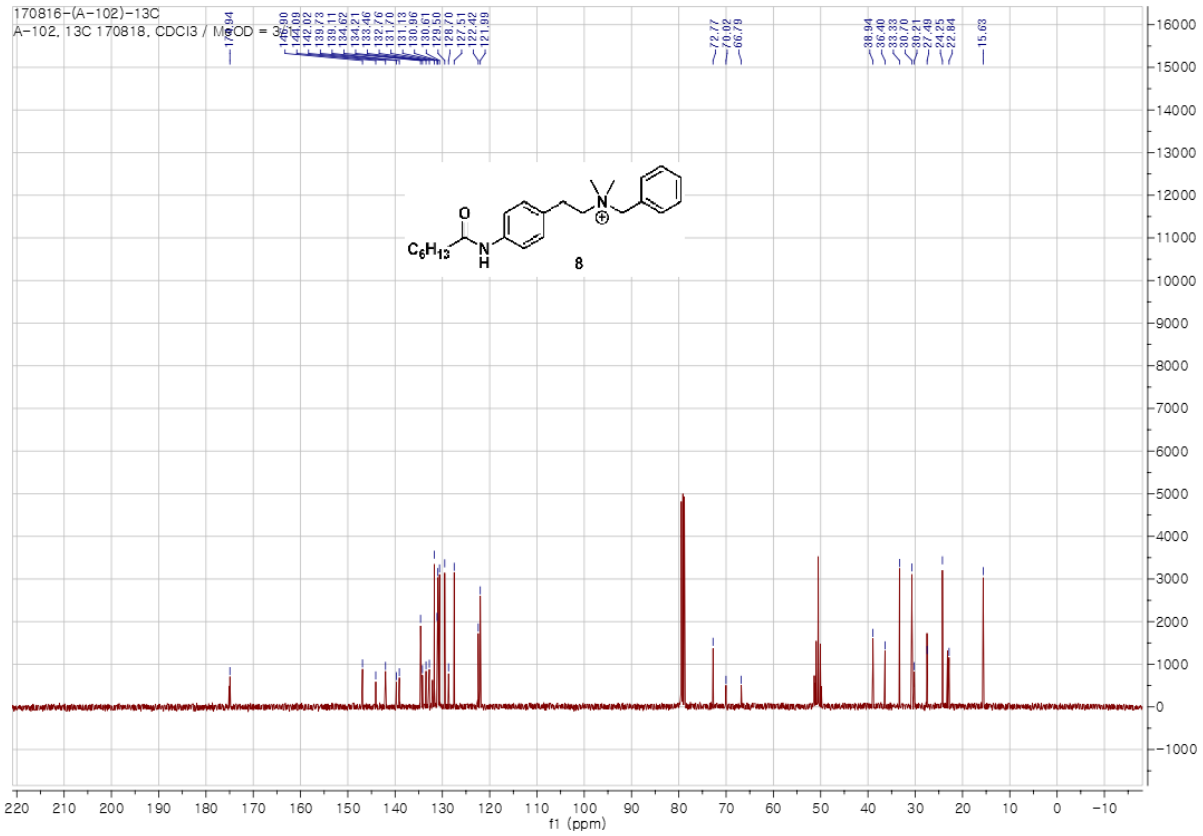
A-102, 1H 170818, CDCl3 / MeOD = 3/1
8
f1 (ppm)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A-102, 13C 170818, CDCl3 / MeOD = 3/1
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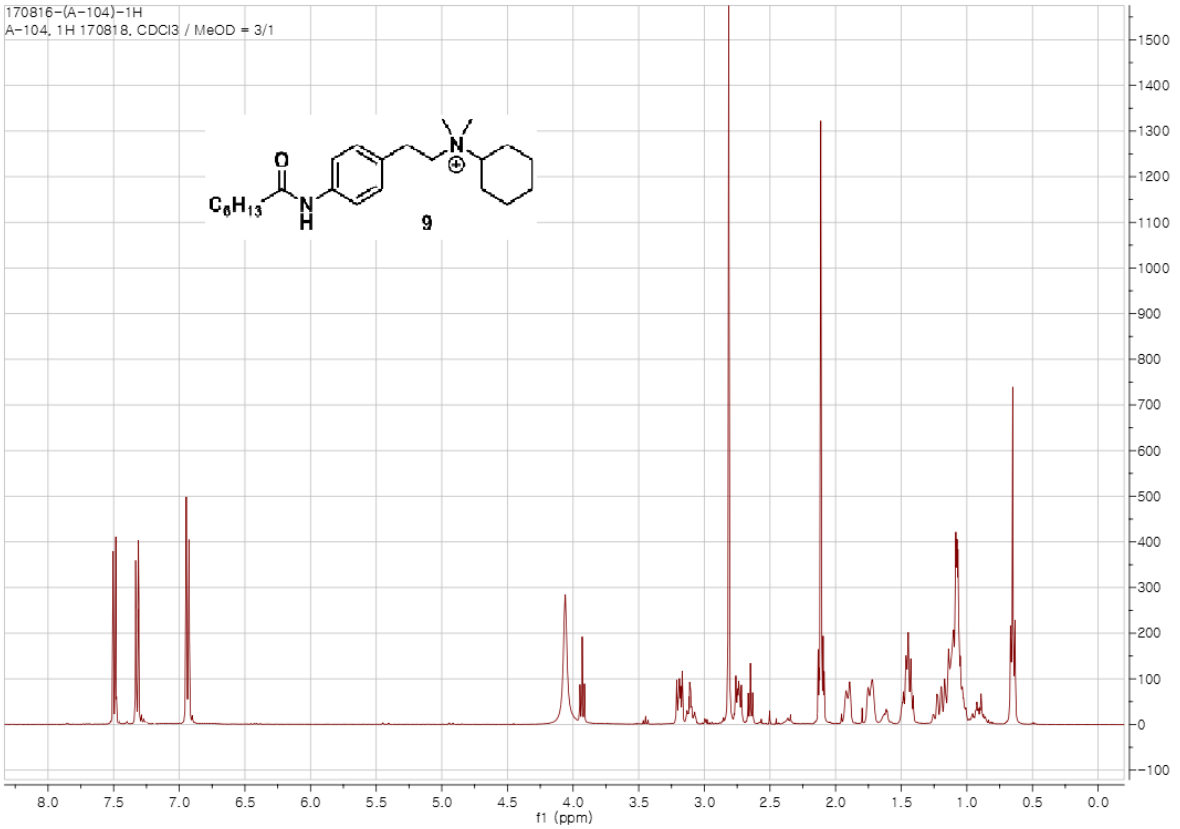
f1 (ppm)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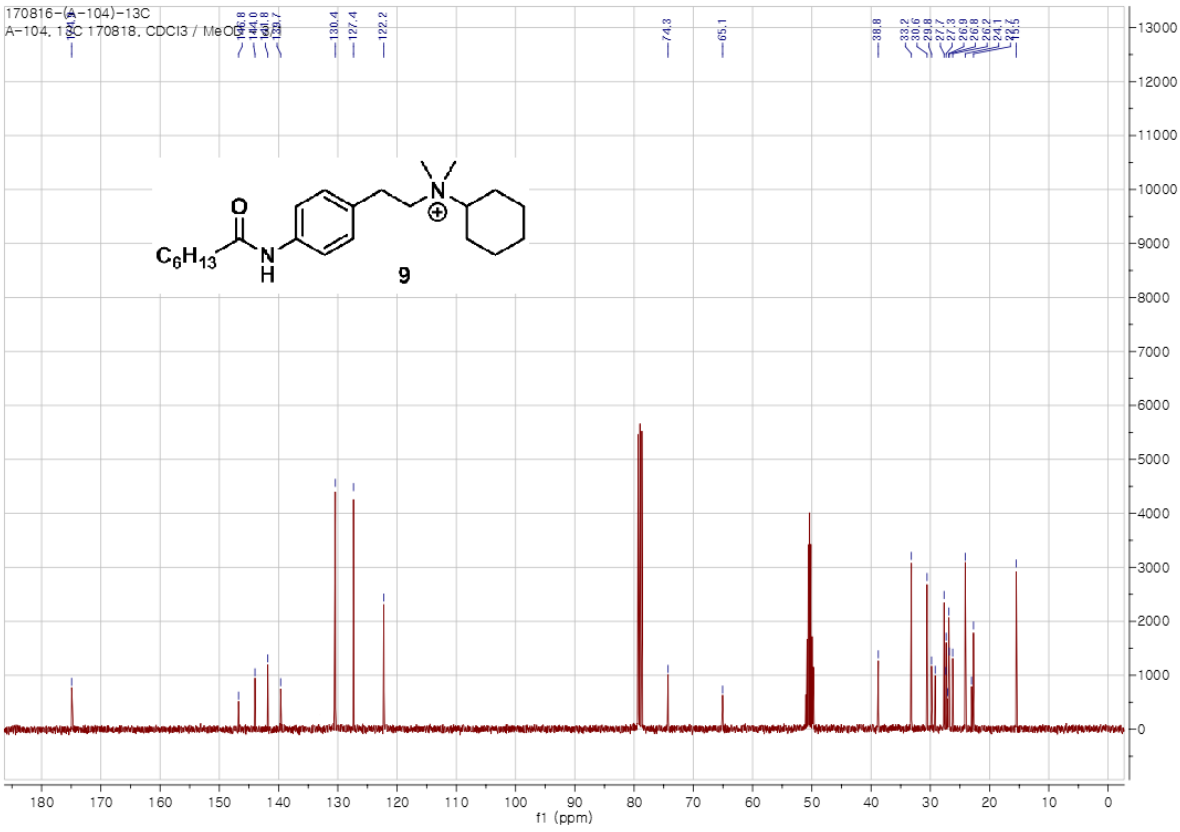
O
N
H
C_6H_{13}
N
9
f1 (ppm)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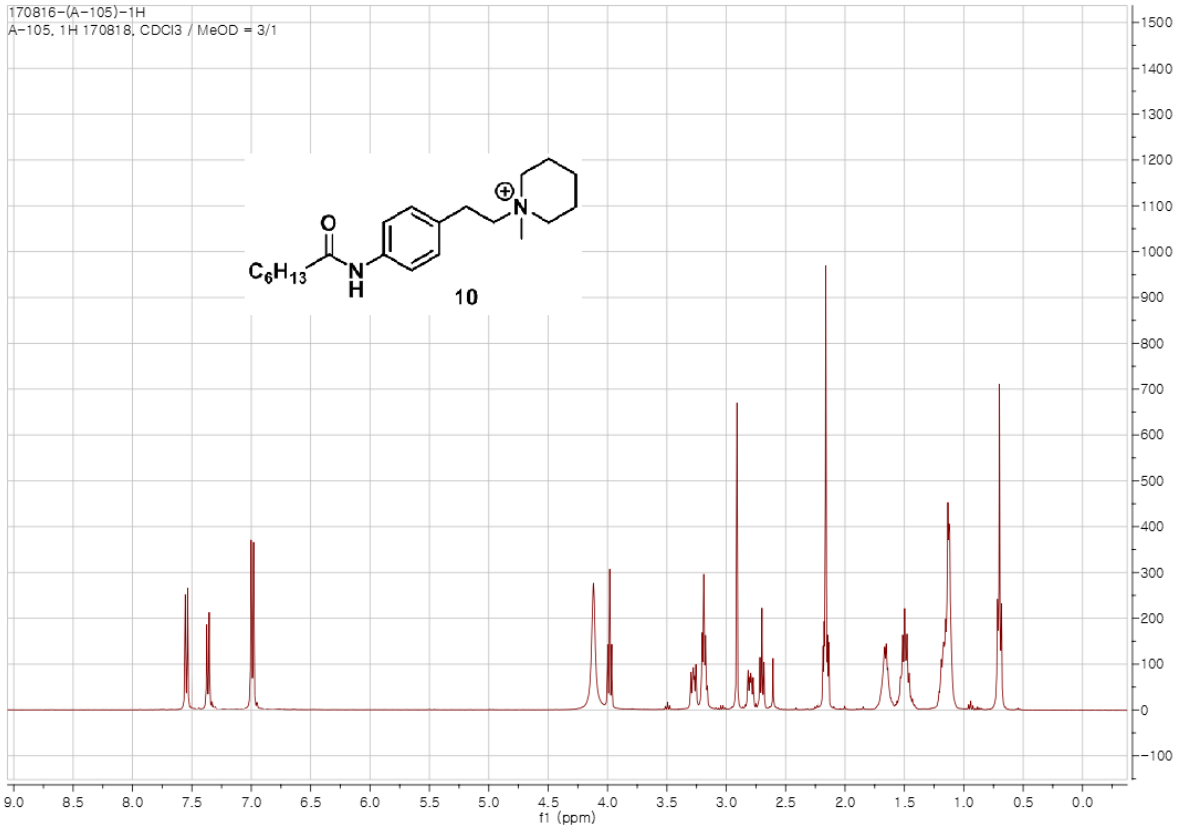
A-104, 13C 170818, CDCl3 / MeOD
130.4
127.4
122.2
74.3
65.1
38.8
33.2
30.6
29.8
O
N
H
C_6H_{13}
N
9
f1 (ppm)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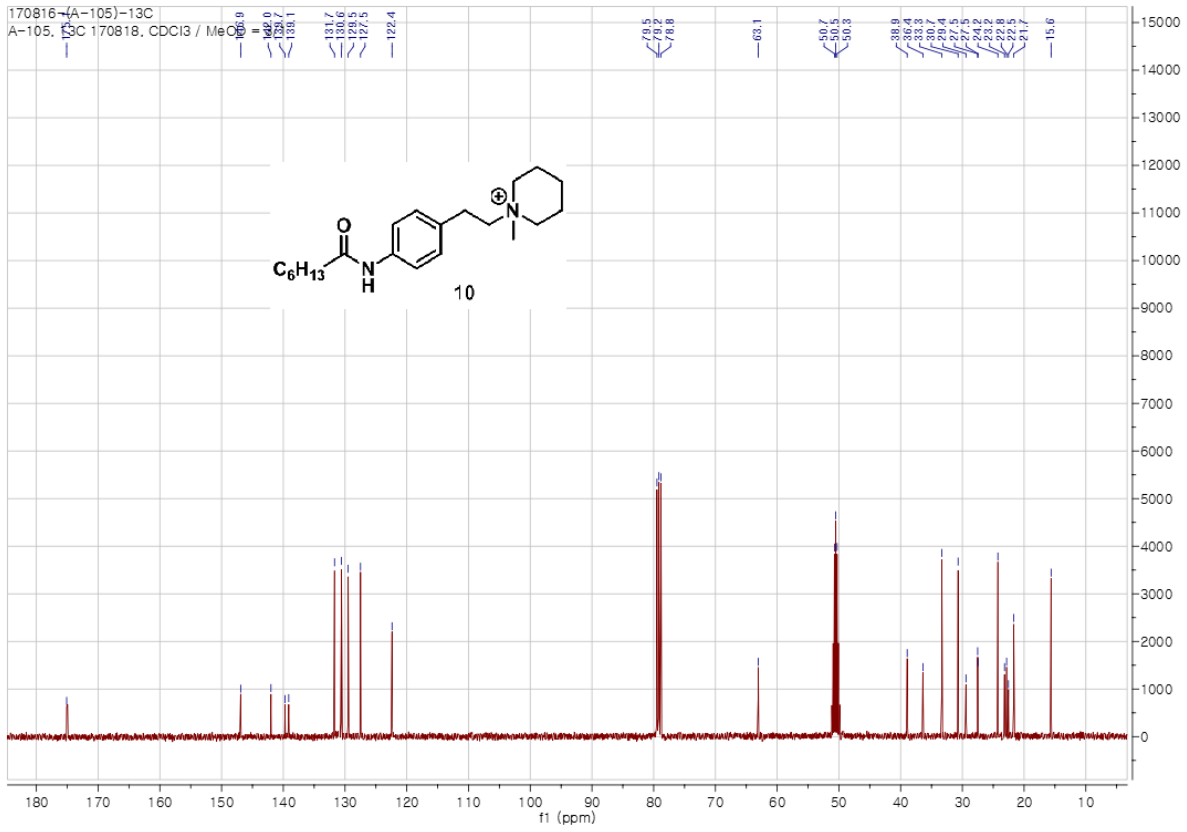
10
f1 (ppm)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A-105, 13C 170818, CDCl3 / MeOD = 3/1
10
f1 (ppm)

HRMS sample name: A-73 (compound 11), AR-2 (compound 12), AR-3 (compound 2), AR-5 (compound 3), A-95 (compound 4), A-94 (compound 5), AR-4 (compound 6), A-103 (compound 7), A-102 (compound 8), A-104 (compound 9), A-105 (compound 10)

Data File	20180410A	**Sample Name**	A-73
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IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=249.3540 M=HC ESI Pos Small Molecule LT No HPLC.m		

Sample Group **Info.**
Molecular Formula C15H23NO2

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C15H23NO2	0.25	250.1807	42513	C15H23NO2

Compound Label	*m/z*	RT	Algorithm	Mass
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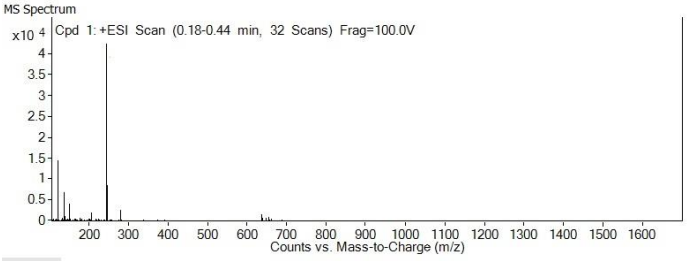
MS Spectrum



Data File	20180418F	**Sample Name**	AR-2
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IRM Calibration Status	Success	**DA Method**	Data 1.m
Comment	EM=403.5370 M=No HPLC MeOH 1.m		

Sample Group **Info.**
Molecular Formula C22H29NO4S **Acquisition SW Version** 6200 series TOF/6500 series Q-TOF B.05.01 (B5125.1)

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C22H29NO4S	0.182	404.1896	192637	C22H29NO4S

Compound Label	*m/z*	RT	Algorithm	Mass
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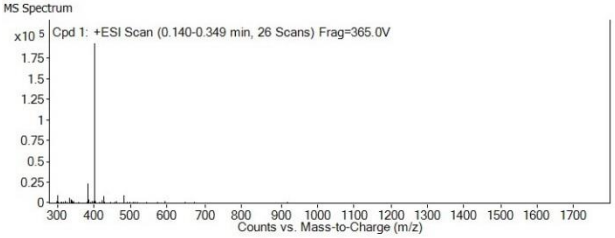
MS Spectrum



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Instrument Name	Instrument 1	**User Name**	
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IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=332.4880 M=HC ESI Pos Small Molecule LT No HPLC.m		

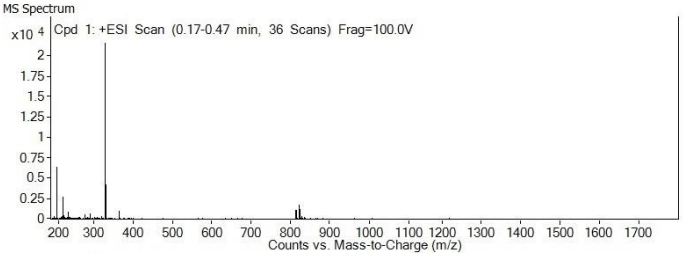
Sample Group **Info.**
Molecular Formula C20H32N2O2

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C20H32N2O2	0.23	333.2542	21580	C20H32N2O2

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C20H32N2O2	333.2575	0.23	Find By Formula	333.2542

MS Spectrum



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IRM Calibration Status	Success	**DA Method**	Data 1.m
Comment	EM=332.4880 M=No HPLC MeOH 1.m		

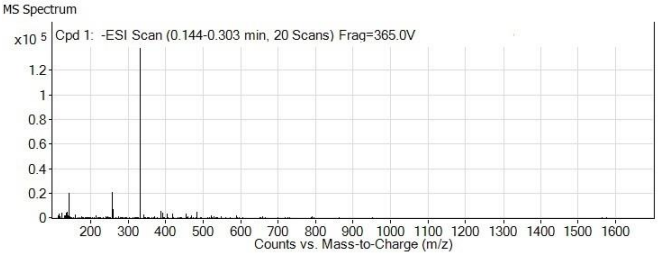
Sample Group **Info.**
Molecular Formula C20H32N2O2 **Acquisition SW Version** 6200 series TOF/6500 series Q-TOF B.05.01 (B5125)

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C20H32N2O2	0.186	333.2542	138368	C20H32N2O2

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C20H32N2O2	333.2517	0.186	Find By Formula	333.2542

MS Spectrum



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IRM Calibration Status	Success	**DA Method**	Data 1.m
Comment	EM=475.5160 M=No HPLC MeOH 1.m		

Sample Group		**Info.**	
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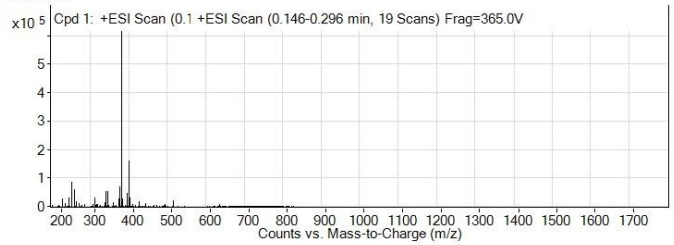
Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C24H29N3O	0.179	376.2389	614304	C24H29N3O

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C24H29N3O	376.2391	0.179	Find By Formula	376.2389

MS Spectrum



Data File	20180509A	**Sample Name**	A-94
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IRM Calibration Status	Success	**DA Method**	Data 1.m
Comment	EM=361.5300 M=No HPLC MeOH 1.m		

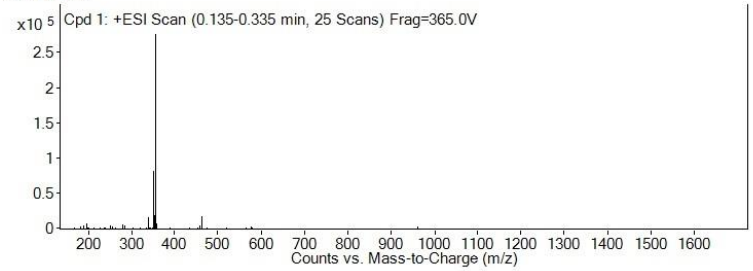
Sample Group		**Info.**	
Molecular Formula	C21H35N3O2	**Acquisition SW Version**	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.1)

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C21H35N3O2	0.185	362.2808	276800	C21H35N3O2

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C21H35N3O2	362.2833	0.185	Find By Formula	362.2808

MS Spectrum



Data File	20180509B	**Sample Name**	A-95
Sample Type	Sample	**Position**	
Instrument Name	Instrument 1	**User Name**	
Acq Method	No HPLC MeOH 1.m	**Acquired Time**	5/9/2019 9:44:12 AM
IRM Calibration Status	Success	**DA Method**	Data 1.m
Comment	EM=346.5150 M=No HPLC MeOH 1.m		

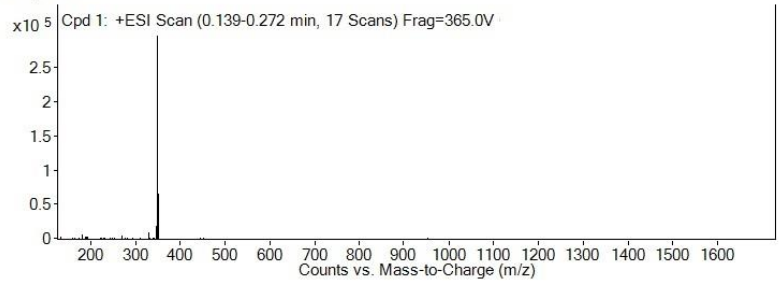
Sample Group		**Info.**	
Molecular Formula	C21H34N2O2	**Acquisition SW Version**	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.1)

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C21H34N2O2	0.181	347.2699	297920	C21H34N2O2

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C21H34N2O2	347.2629	0.181	Find By Formula	347.2699

MS Spectrum



Data File	20180509C	**Sample Name**	A-102
Sample Type	Sample	**Position**	
Instrument Name	Instrument 1	**User Name**	
Acq Method	No HPLC MeOH 1.m	**Acquired Time**	5/9/2018 10:11:49 AM
IRM Calibration Status	Success	**DA Method**	Data 1.m
Comment	EM=367.5565 M=No HPLC MeOH 1.m		

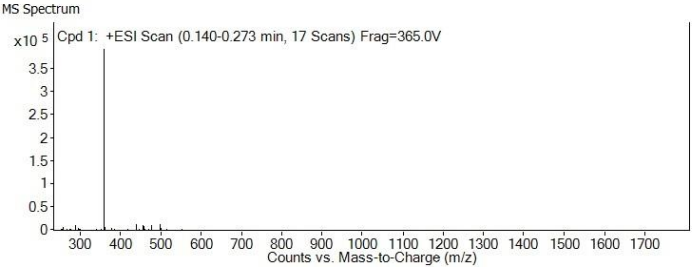
Sample Group		**Info.**	
Molecular Formula	C24H35N2O	**Acquisition SW Version**	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.1)

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C24H35N2O	0.181	368.2822	391837	C24H35N2O

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C24H35N2O	368.2809	0.181	Find By Formula	368.2822

MS Spectrum



Data File	20180509D	**Sample Name**	A-103
Sample Type	Sample	**Position**	
Instrument Name	Instrument 1	**User Name**	
Acq Method	No HPLC MeOH 1.m	**Acquired Time**	5/9/2018 10:38:25 AM
IRM Calibration Status	Success	**DA Method**	Data 1.m
Comment	EM=368.5210 M=No HPLC MeOH 1.m		

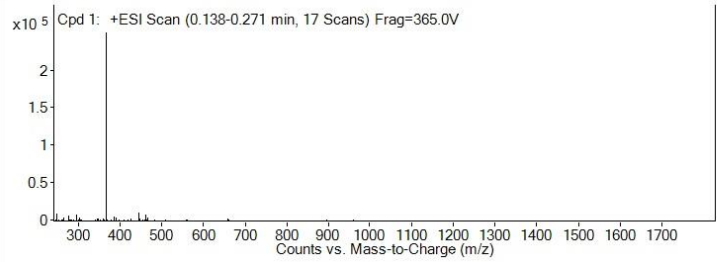
Sample Group		**Info.**	
Molecular Formula	C23H32N2O2	**Acquisition SW Version**	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.1)

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C23H32N2O2	0.18	369.2542	249707	C23H32N2O2

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C23H32N2O2	369.2538	0.18	Find By Formula	369.2542

MS Spectrum



Data File	20180509E	**Sample Name**	A-104
Sample Type	Sample	**Position**	
Instrument Name	Instrument 1	**User Name**	
Acq Method	No HPLC MeOH 1.m	**Acquired Time**	5/9/2018 11:01:15 AM
IRM Calibration Status	Success	**DA Method**	Data 1.m
Comment	EM=359.5775 M=No HPLC MeOH 1.m		

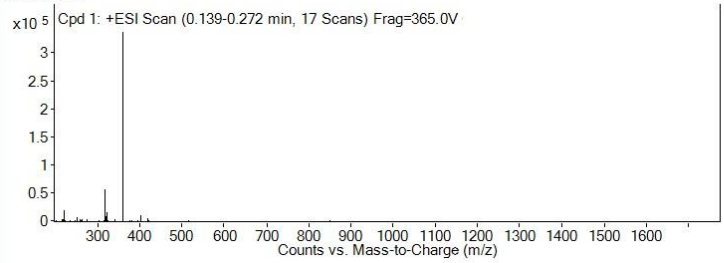
Sample Group		**Info.**	
Molecular Formula	C23H39N2O	**Acquisition SW Version**	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.1)

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C23H39N2O	0.181	360.3135	337012	C23H39N2O

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C23H39N2O	360.3123	0.181	Find By Formula	360.3135

MS Spectrum



Data File	20180509F	**Sample Name**	A105
Sample Type	Sample	**Position**	
Instrument Name	Instrument 1	**User Name**	
Acq Method	No HPLC MeOH 1.m	**Acquired Time**	5/9/2018 11:27:40 AM
IRM Calibration Status	Success	**DA Method**	Data 1.m
Comment	EM=331.5235 M=No HPLC MeOH 1.m		

Sample Group		**Info.**	
Molecular Formula	C21H35N2O	**Acquisition SW Version**	6200 series TOF/6500 series Q-TOF B.05.01 (B5125.1)

Compound Table

Compound Label	RT	Mass	Abund	Formula
Cpd 1: C21H35N2O	0.176	332.2822	260882	C21H35N2O

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C21H35N2O	332.2871	0.171	Find By Formula	332.2822

MS Spectrum