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

A Potent and Selective C-11 Labeled PET Tracer for Imaging Sphingosine-1-phosphate Receptor 2 in the CNS Demonstrates Sexually Dimorphic Expression

Xuyi Yue[1], Hongjun Jin[1], Hui Liu[1], Adam J. Rosenberg[1], Robyn S. Klein[2,*] and Zhude Tu[1,*]

[1]Department of Radiology, Washington University School of Medicine, St. Louis, MO 63110, USA;

[2]Departments of Medicine, Anatomy & Neurobiology, Pathology & Immunology, Washington University School of Medicine, St. Louis, MO 63131, USA

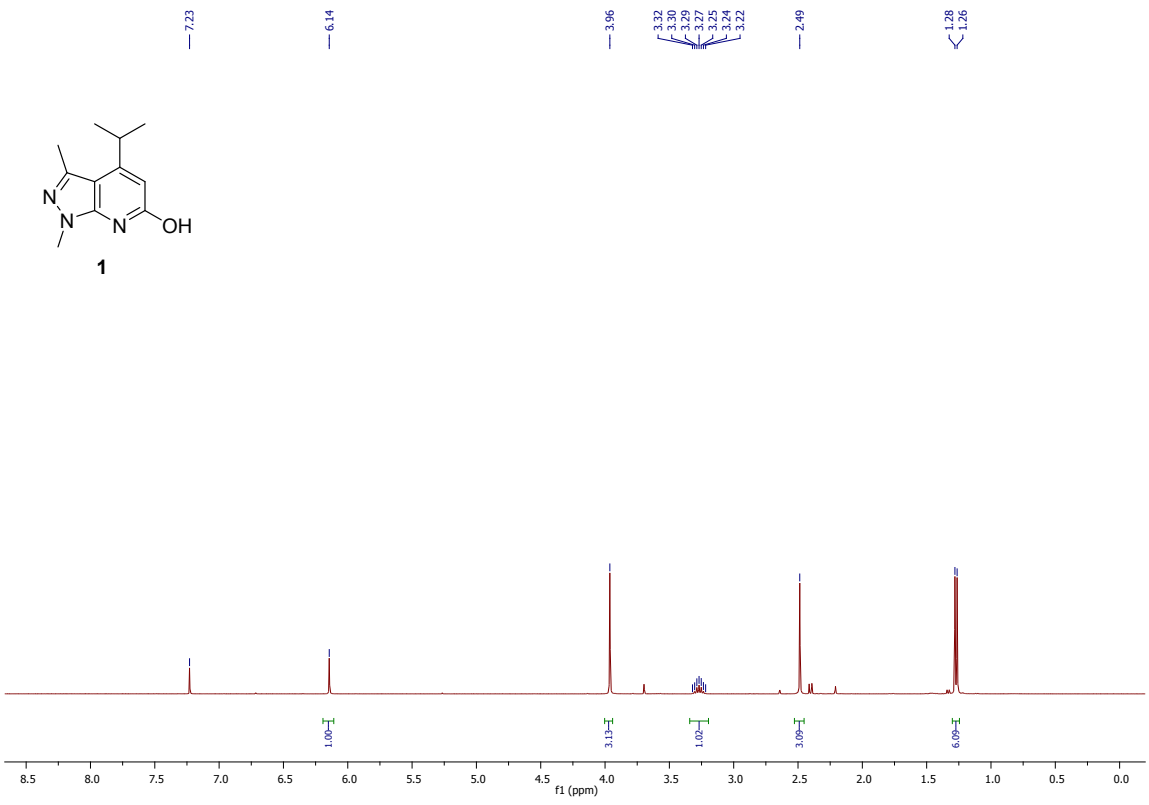
*Co-corresponding authors:

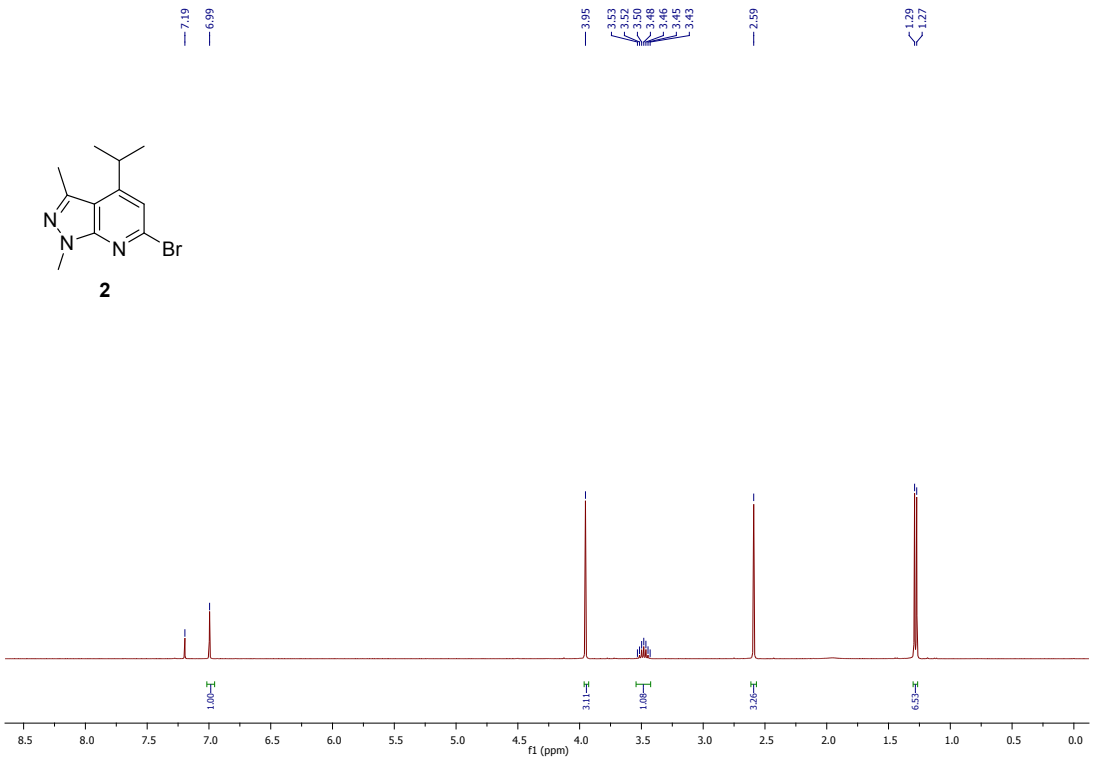
Tel.: +1-314-362-8487; Fax: +1-314-362-8555; E-mail: tuz@mir.wustl.edu

Tel.: +1-314-286-2140; Fax: +1-314-362-9320; E-mail: rklein@dom.wustl.edu

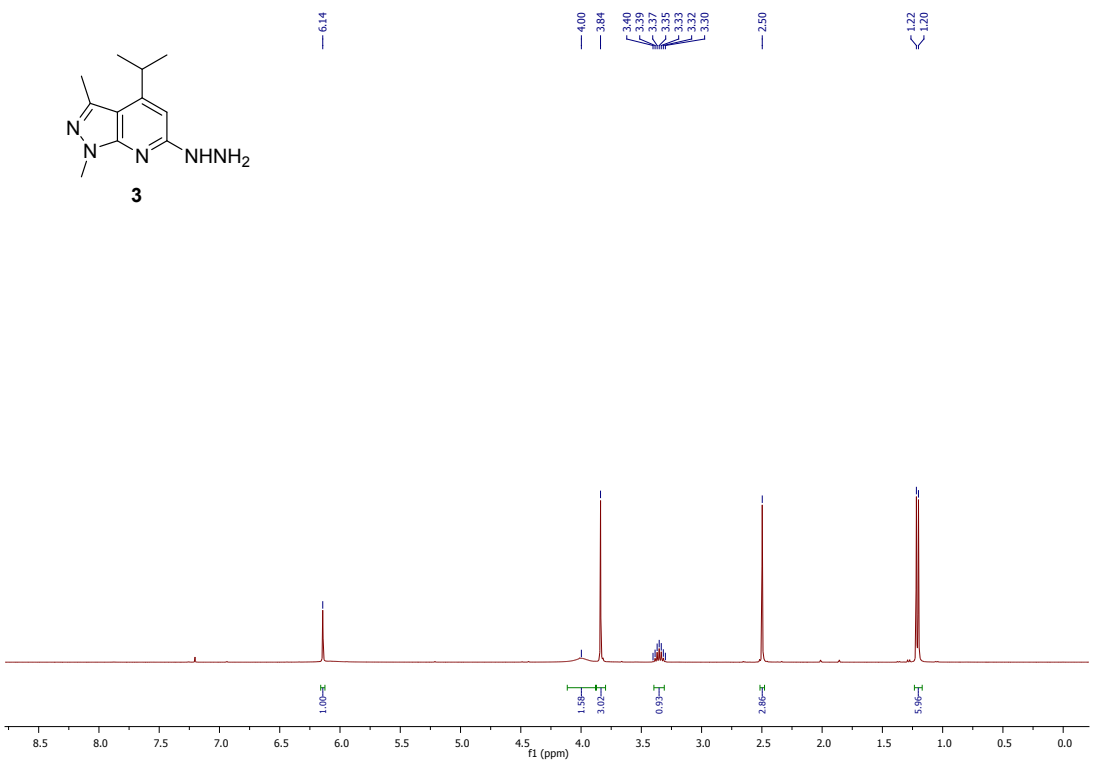
Supporting Information

Copies of ^{1}H NMR spectra for known compounds and ^{1}H NMR, ^{13}C NMR spectra for new compounds




7.19
6.99
3.95
3.53
3.52
3.50
3.48
3.46
3.45
3.43
2.59
1.29
1.27
N
N
N
Br
1.00
3.11
1.08
3.26
6.53
f1 (ppm)

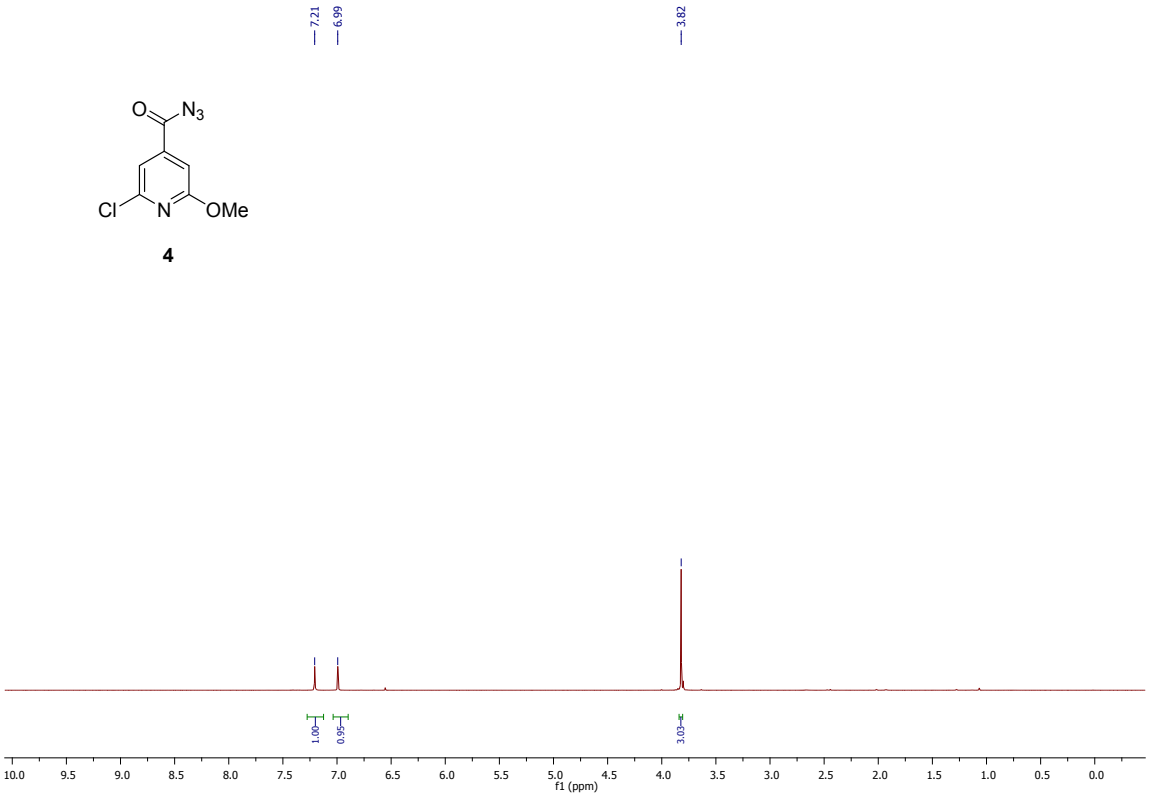
2

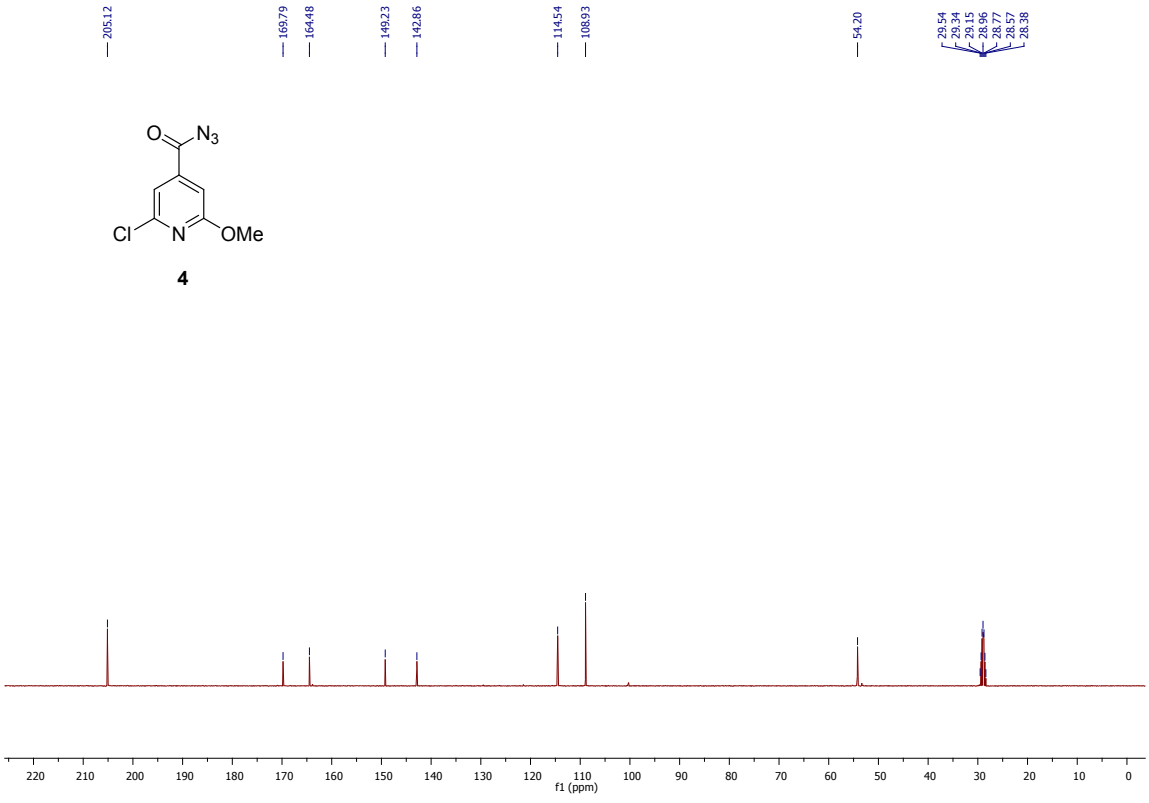

6.14
4.00
3.84
3.40
3.39
3.37
3.35
3.33
3.32
3.30
2.50
1.22
1.20
N
N
N
NHNH2
1.00
1.58
3.02
0.93
2.86
5.96
f1 (ppm)

3

O
N3
Cl
N
OMe

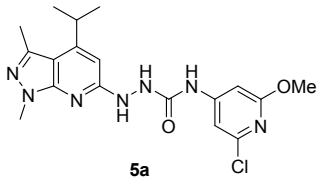
4


7.21
6.99
3.82
1.00
0.95
3.03
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
f1 (ppm)


205.12
169.79
164.48
149.23
142.86
114.54
108.93
54.20
29.54
29.34
29.15
28.96
28.77
28.57
28.38
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

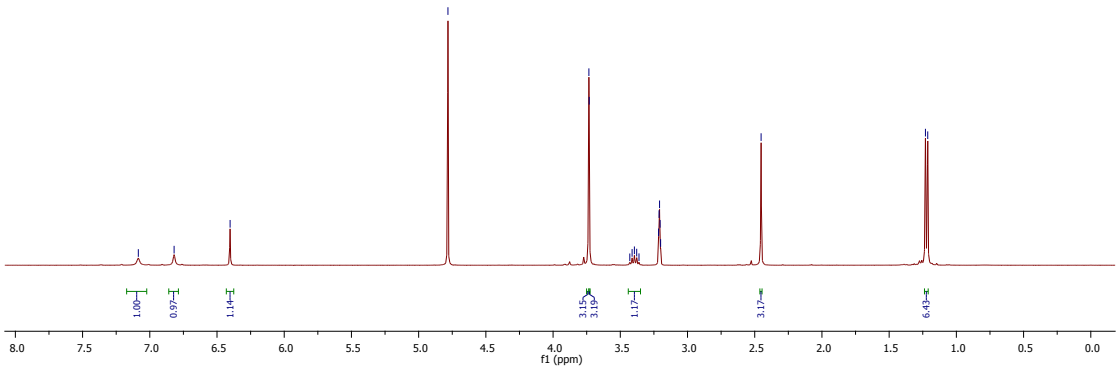
O
N3
Cl
N
OMe

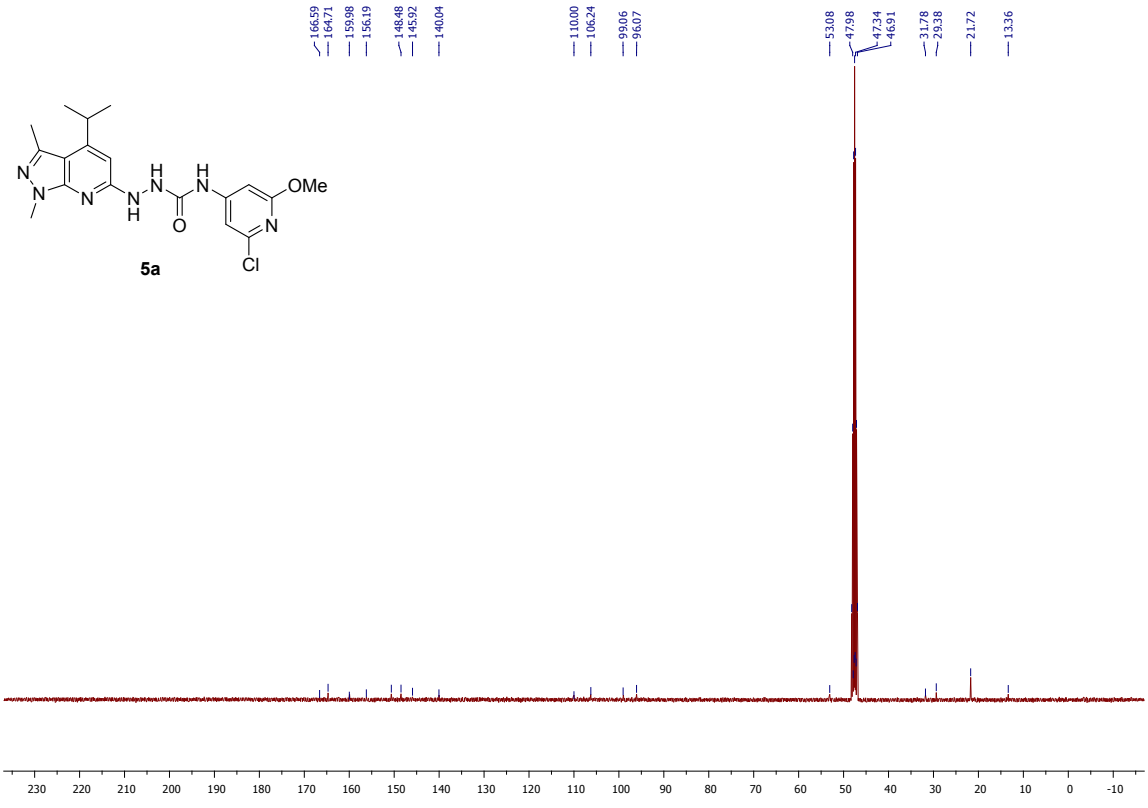
4


7.09
6.82
6.40
4.78
3.73
3.73
3.40
3.38
3.36
3.21
3.21
3.20
3.20
2.45
1.23
1.21

OMe
Cl

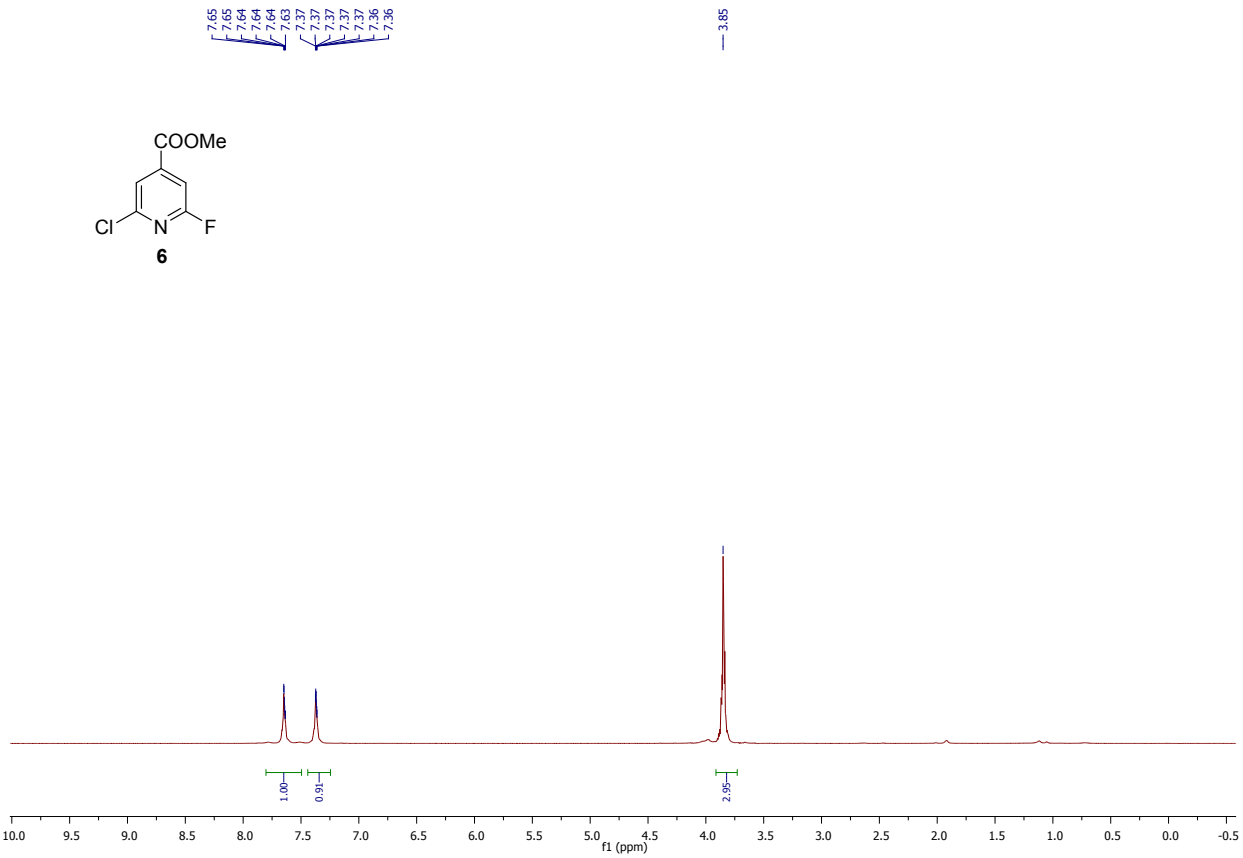
5a

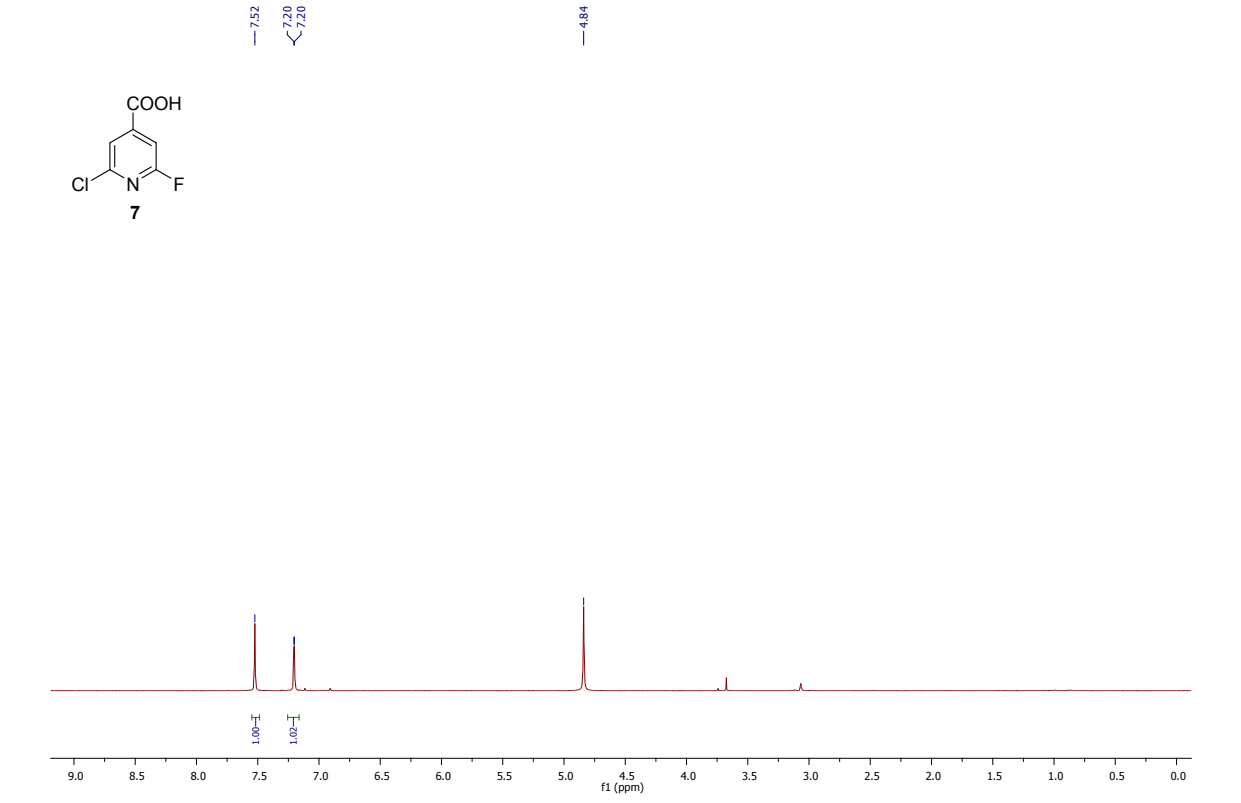

1.00
0.97
1.14
3.15
3.19
1.17
3.17
6.49
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

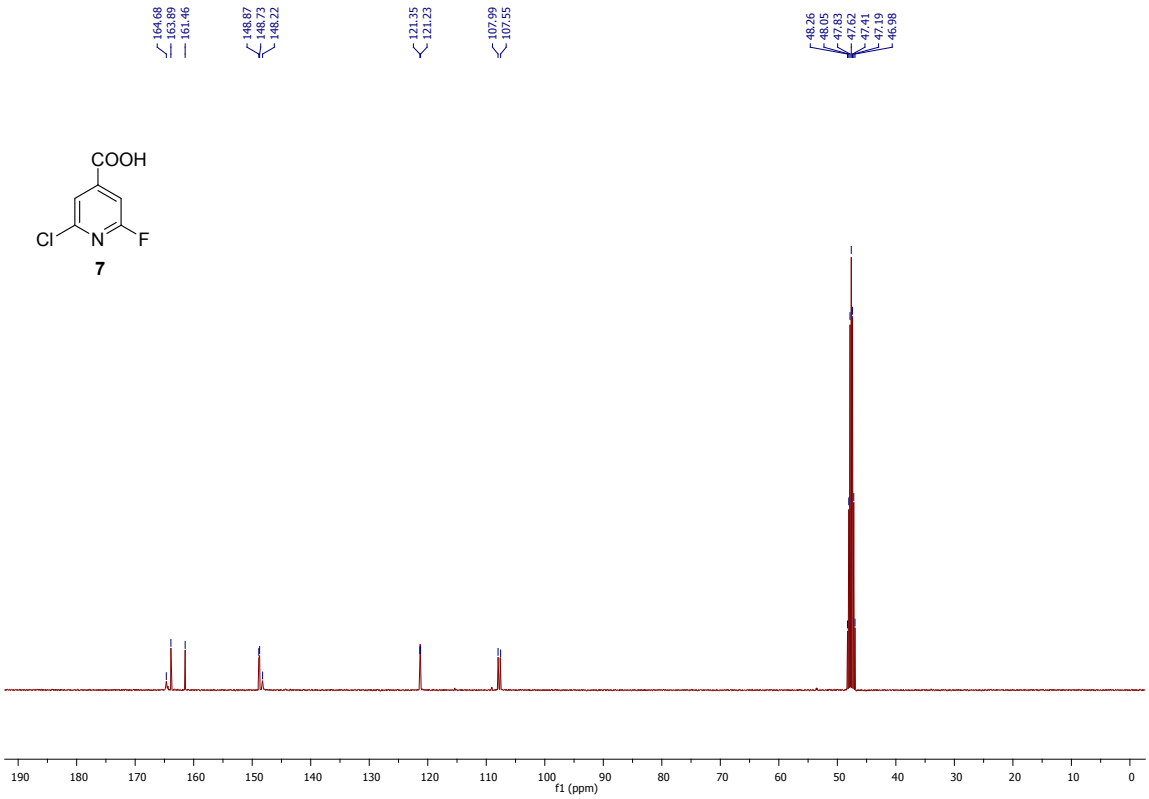

166.59
164.71
159.98
156.19
148.48
145.92
140.04
110.00
106.24
99.06
96.07
53.08
47.98
47.34
46.91
31.78
29.38
21.72
13.36
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10

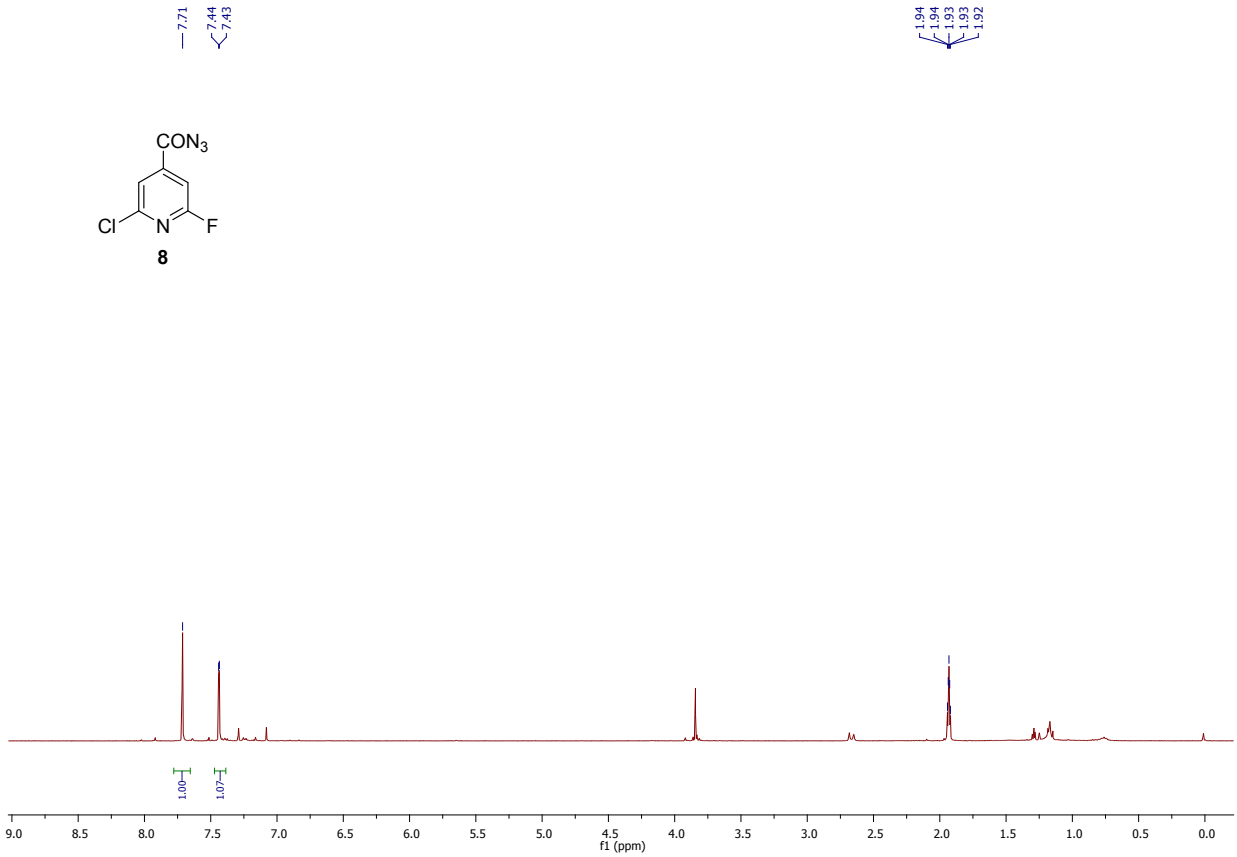
OMe
Cl

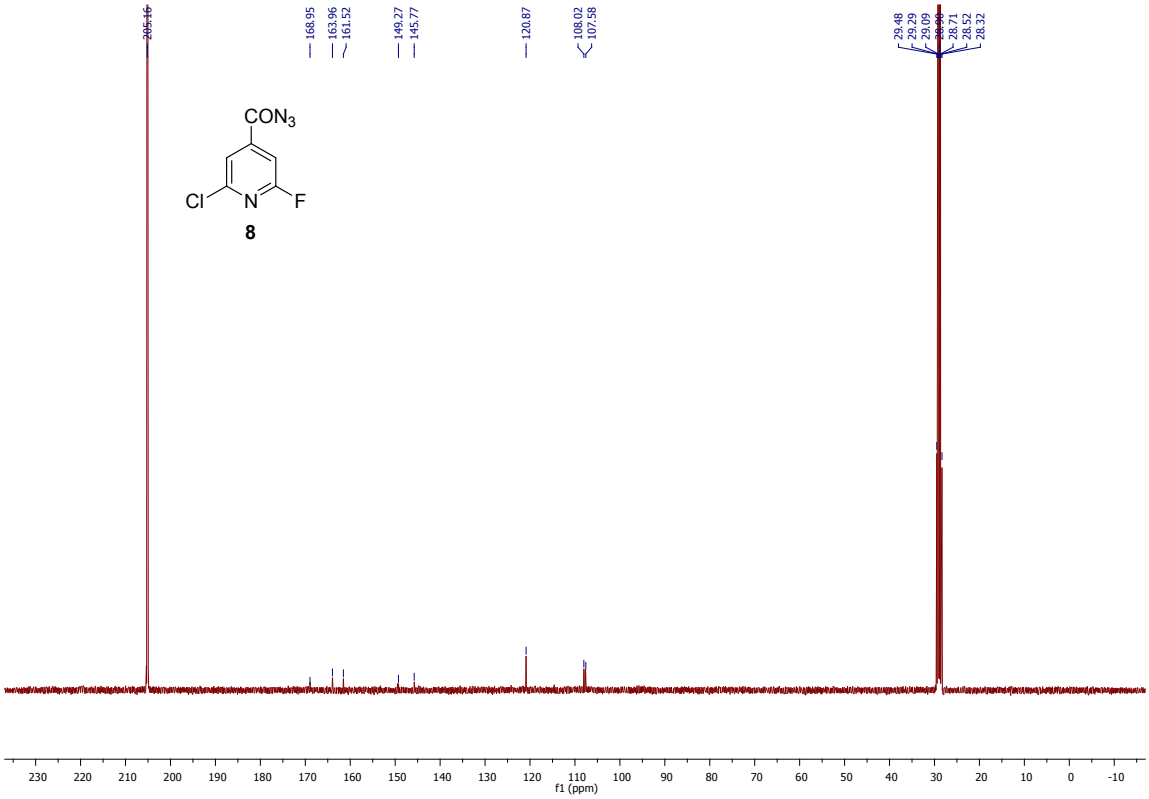
5a

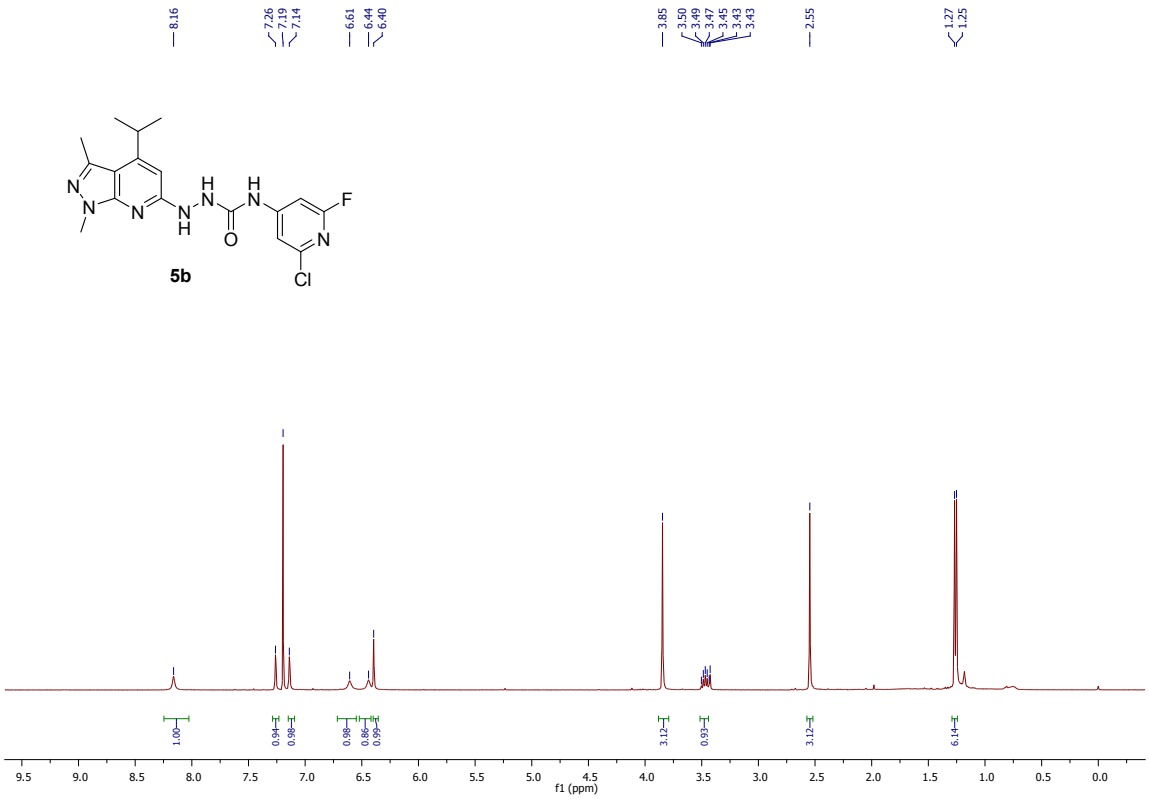

7.65
7.65
7.64
7.64
7.64
7.63
7.37
7.37
7.37
7.37
7.37
7.36
7.36
3.85
COOMe
Cl
N
F
6
1.00
0.91
2.95
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

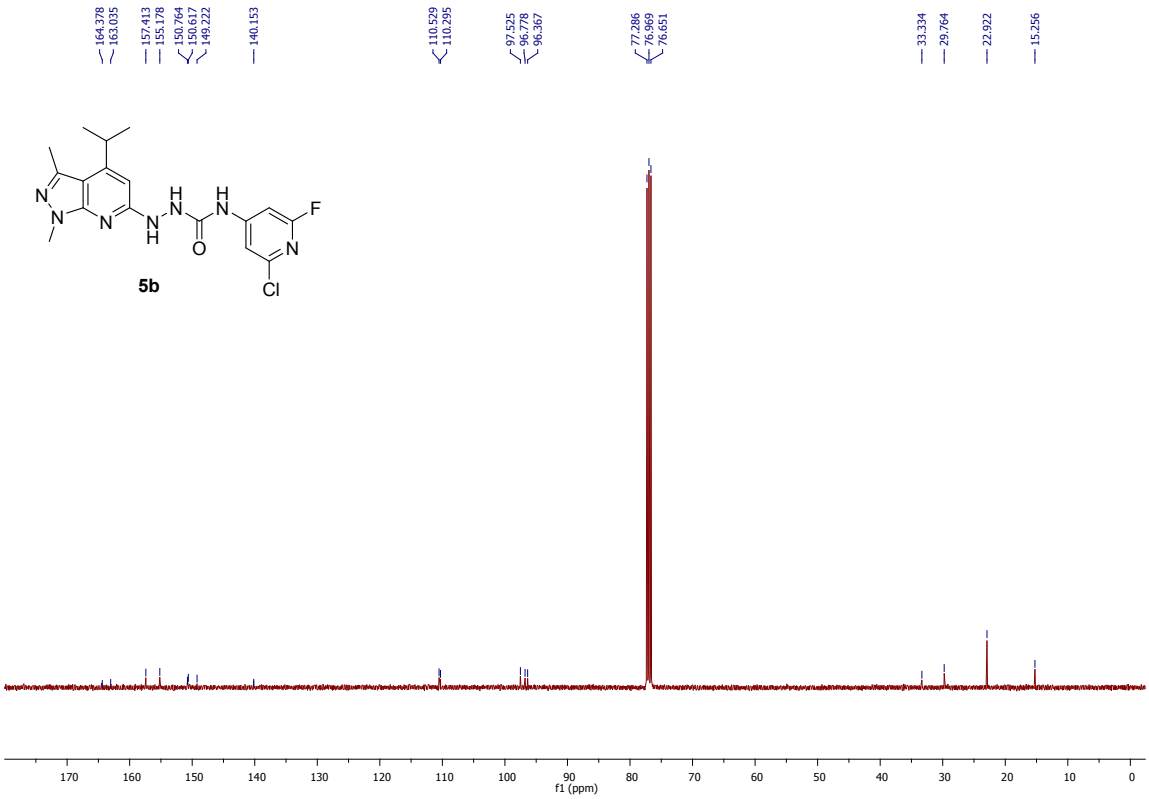

7.52
7.20
7.20
4.84
COOH
Cl
N
F
7
1.00
1.02
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

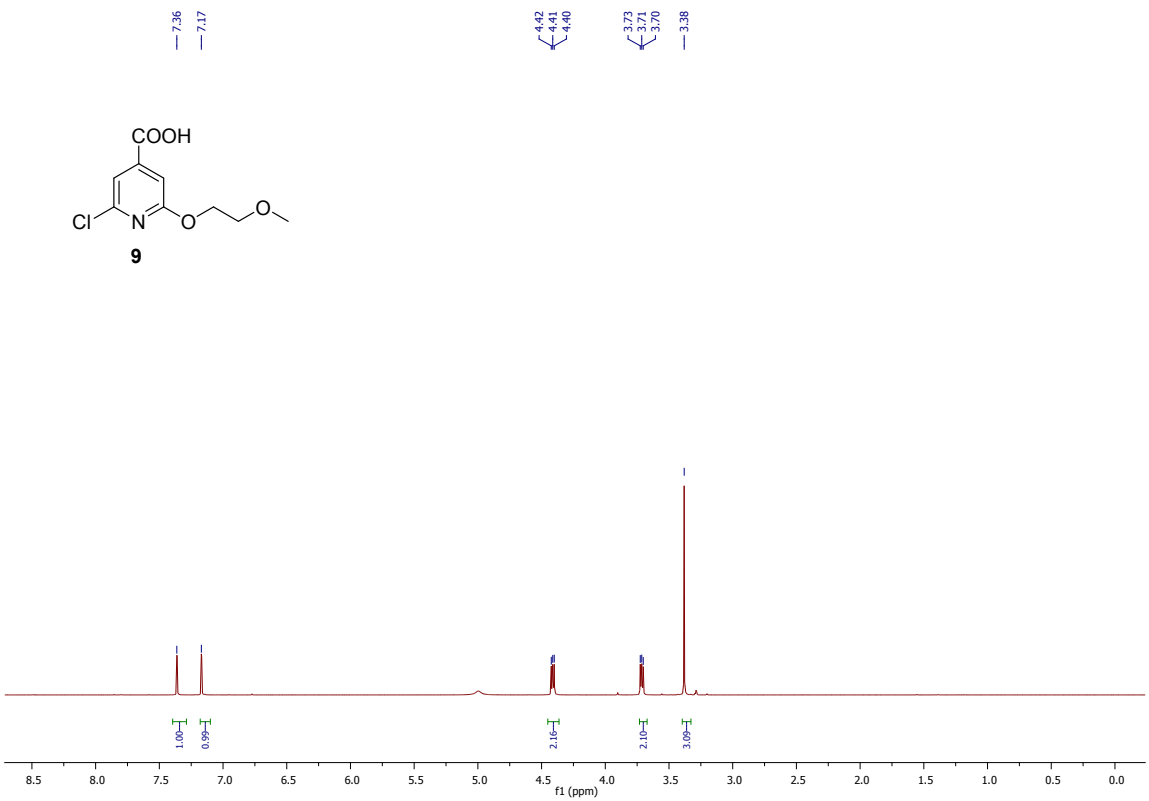

164.68
163.89
161.46
148.87
148.73
148.22
121.35
121.23
107.99
107.55
48.26
48.05
47.83
47.62
47.41
47.19
46.98
COOH
Cl
N
F
7
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

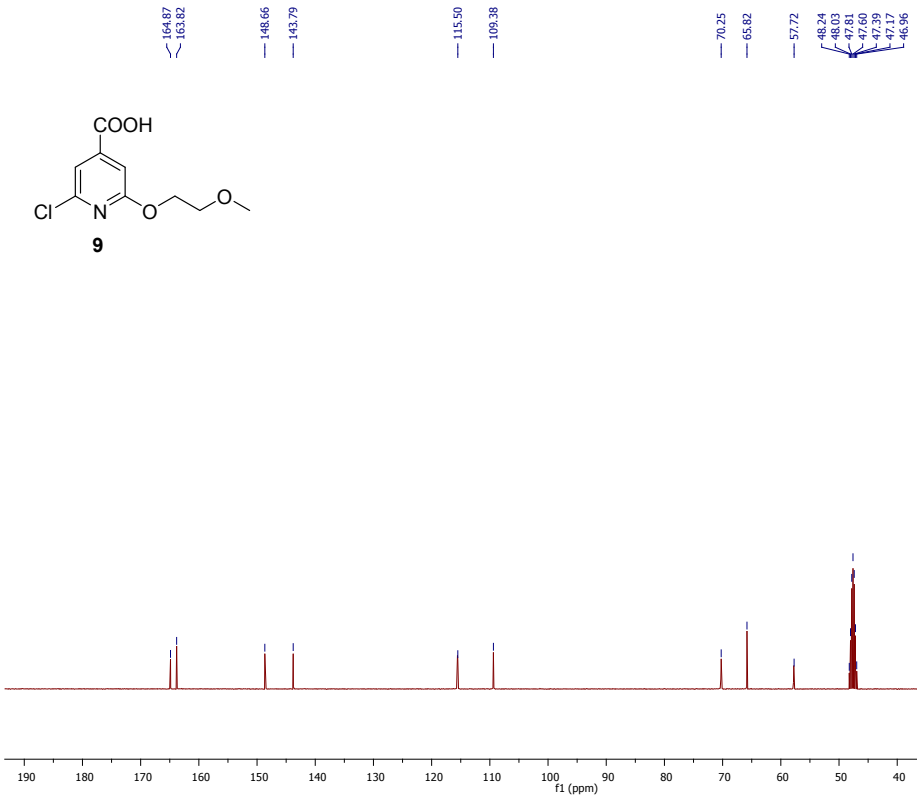

7.71
7.44
7.43
1.94
1.94
1.93
1.93
1.92
CON3
Cl
N
F
8
1.00
1.07
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

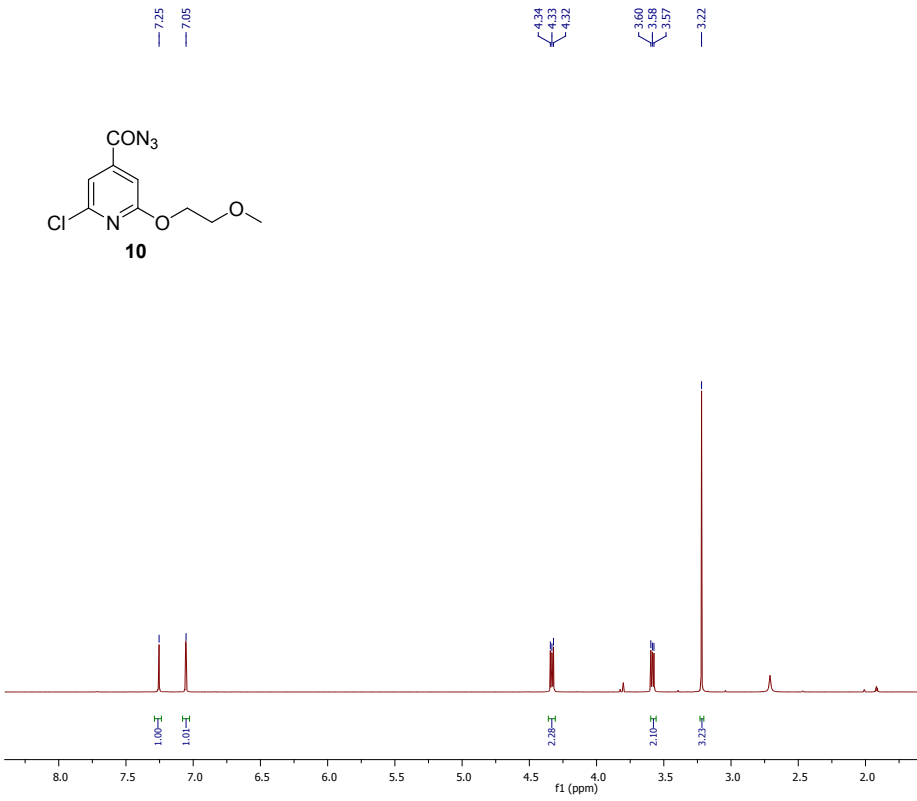

205.16
168.95
163.96
161.52
149.27
145.77
120.87
108.02
107.58
29.48
29.29
29.09
28.71
28.52
28.32
CON3
Cl
N
F
8
f1 (ppm)

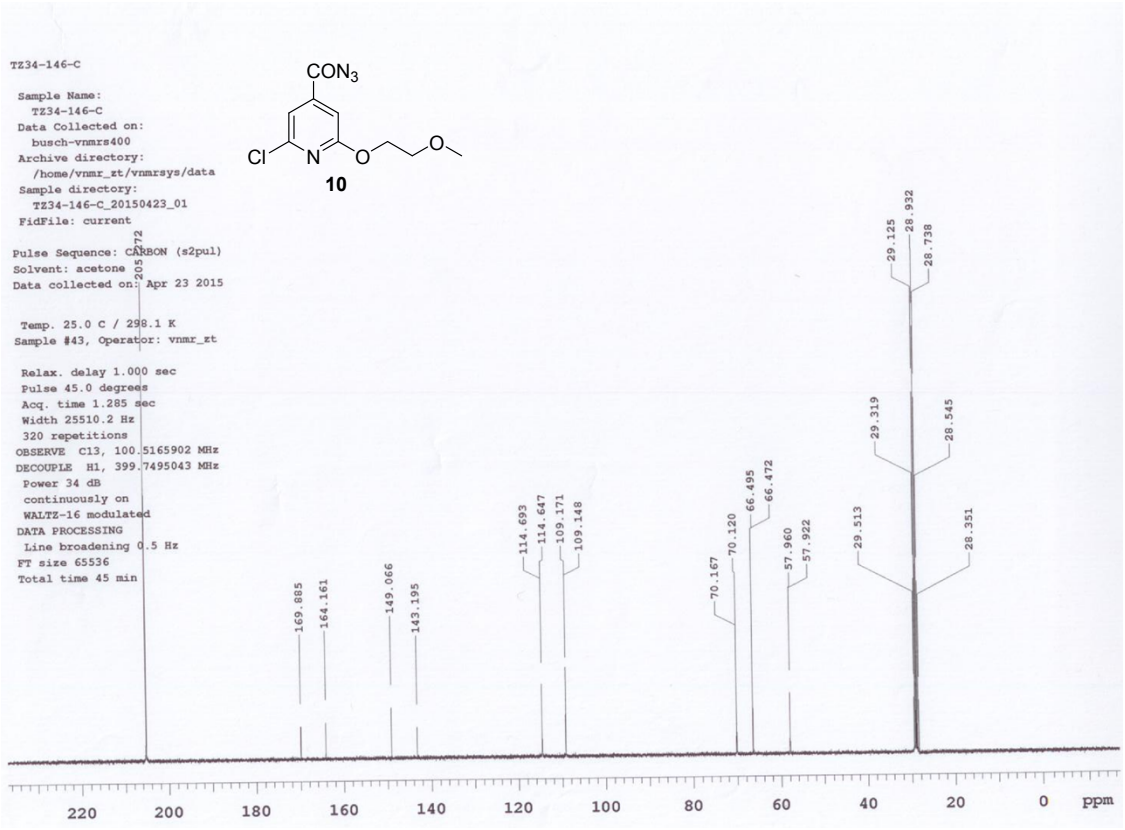

8.16
7.26
7.19
7.14
6.61
6.44
6.40
3.85
3.50
3.49
3.47
3.45
3.43
3.43
2.55
1.27
1.25
N
N
H
N
N
H
N
O
F
N
Cl
5b
1.00
0.94
0.98
0.98
0.88
0.99
3.12
0.93
3.12
6.14
f1 (ppm)

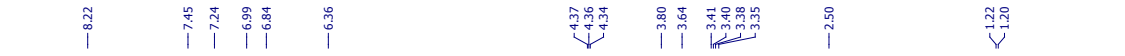

164.378
163.035
157.413
155.178
150.764
150.617
149.222
140.153
110.529
110.295
97.525
96.778
96.367
77.286
76.969
76.651
33.334
29.764
22.922
15.256
5b
f1 (ppm)

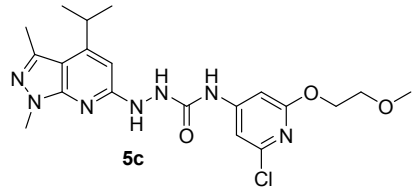

7.36
7.17
4.42
4.41
4.40
3.73
3.71
3.70
3.38
9
1.00
0.99
2.16
2.10
3.09
f1 (ppm)

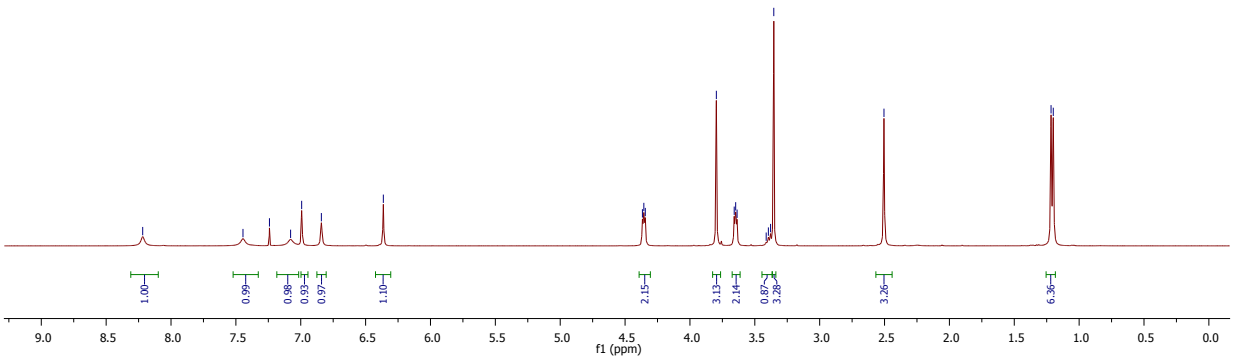

164.87
163.82
148.66
143.79
115.50
109.38
70.25
65.82
57.72
48.24
48.03
47.81
47.60
47.39
47.17
46.96
COOH
Cl
N
O
O
9
f1 (ppm)

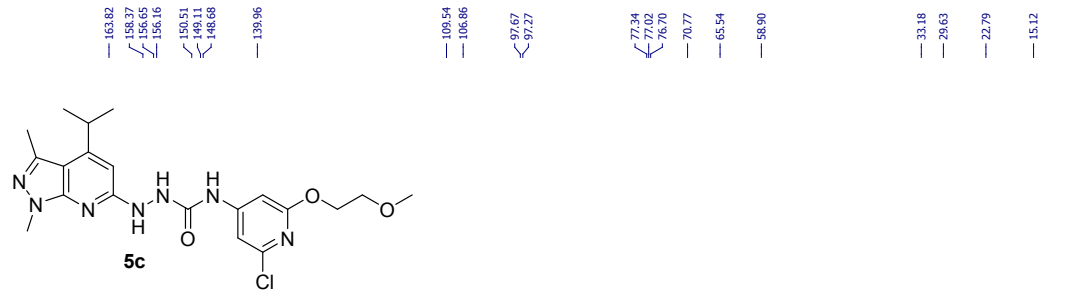

7.25
7.05
4.34
4.33
4.32
3.60
3.58
3.57
3.22
CON3
Cl
N
O
O
10
1.00
1.01
2.28
2.10
3.23
f1 (ppm)


TZ34-146-C
Sample Name:
TZ34-146-C
Data Collected on:
busch-vnmrs400
Archive directory:
/home/vnmr_zt/vnmrsys/data
Sample directory:
TZ34-146-C_20150423_01
FidFile: current
Pulse Sequence: CARBON (s2pul)
Solvent: acetone
Data collected on: Apr 23 2015
Temp. 25.0 C / 298.1 K
Sample #43, Operator: vnmr_zt
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.285 sec
Width 25510.2 Hz
320 repetitions
OBSERVE C13, 100.5165902 MHz
DECOUPLE H1, 399.7495043 MHz
Power 34 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 45 min
CON3
Cl
N
O
O
10
205.772
169.885
164.161
149.066
143.195
114.693
114.647
109.171
109.148
70.167
70.120
66.495
66.472
57.960
57.922
29.513
29.319
29.125
28.932
28.738
28.545
28.351
220
200
180
160
140
120
100
80
60
40
20
0
ppm

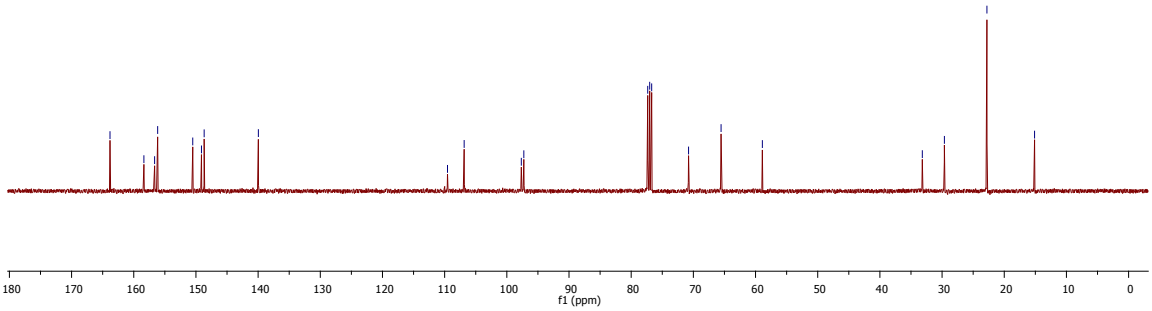

8.22
7.45
7.24
6.99
6.84
6.36
4.37
4.36
4.34
3.80
3.64
3.41
3.40
3.38
3.35
2.50
1.22
1.20


N
N
N
H
N
N
H
H
N
O
O
O
N
Cl
5c


1.00
0.99
0.98
0.93
0.97
1.10
2.15
3.13
2.14
0.87
3.28
3.26
6.36
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
f1 (ppm)

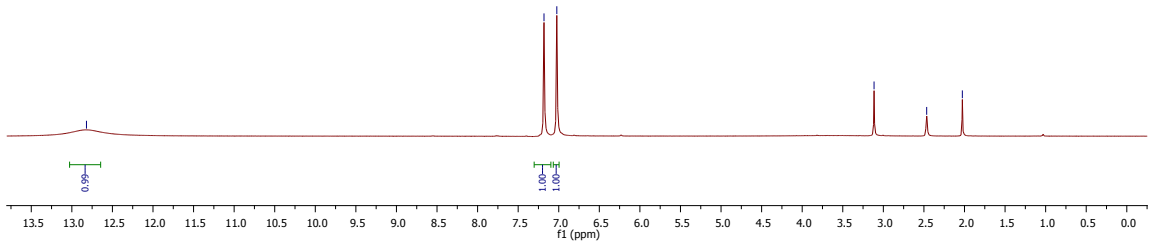

163.82
158.37
156.65
156.16
150.51
149.11
148.68
139.96
109.54
106.86
97.67
97.27
77.34
77.02
76.70
70.77
65.54
58.90
33.18
29.63
22.79
15.12

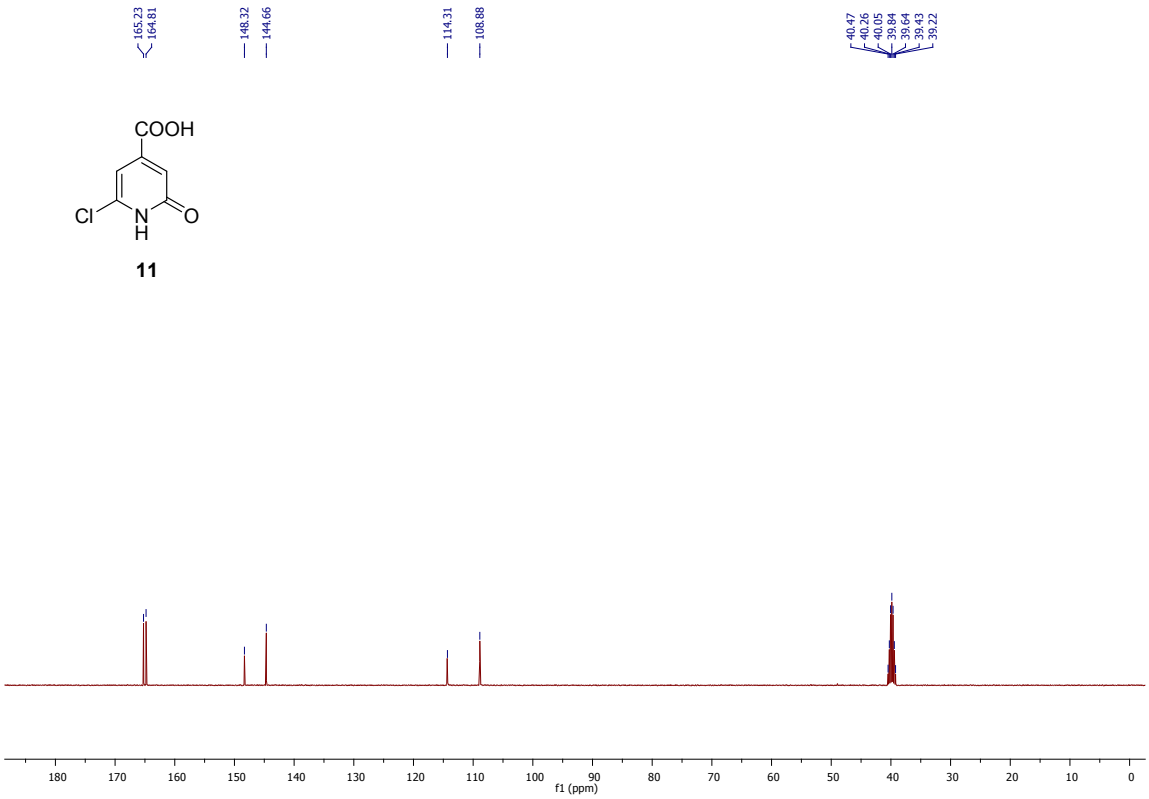
5c


f1 (ppm)

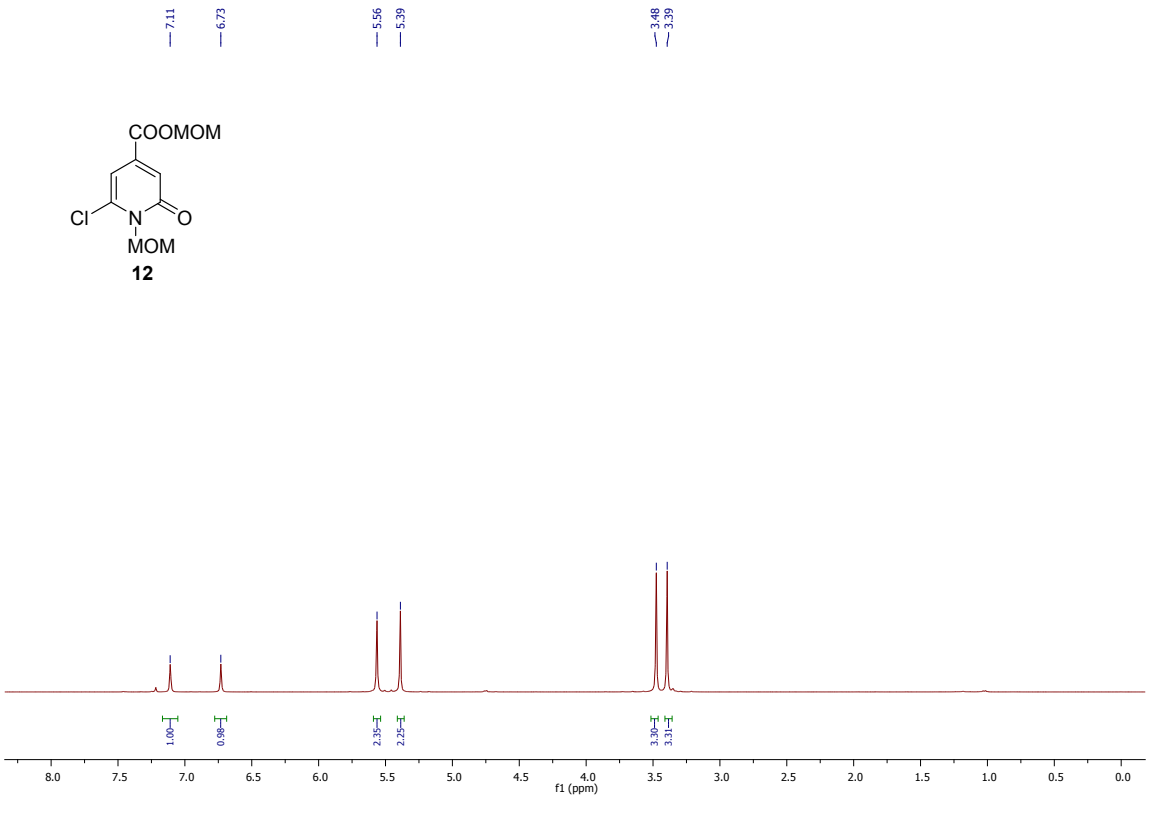

12.82
7.18
7.02
3.11
2.47
2.03

11

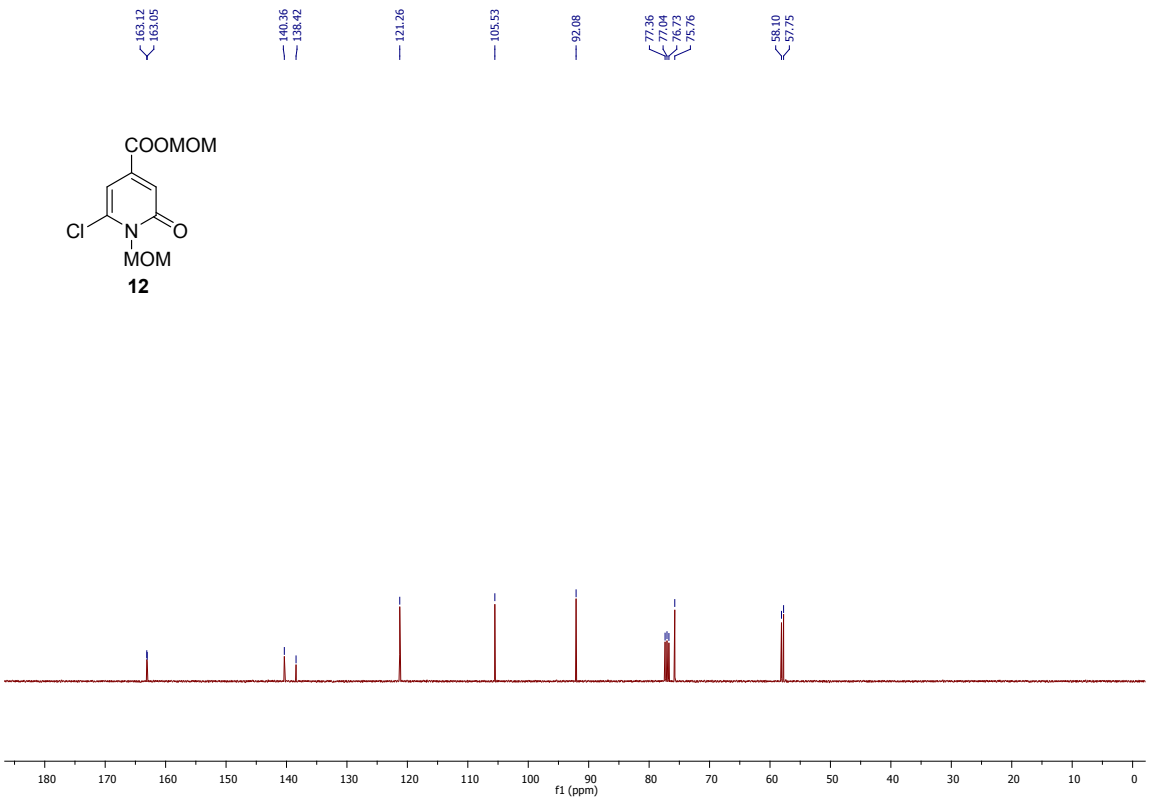

0.99
1.00
1.00
f1 (ppm)


165.23
164.81
148.32
144.66
114.31
108.88
40.47
40.26
40.05
39.84
39.64
39.43
39.22
COOH
Cl
N
H
O
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

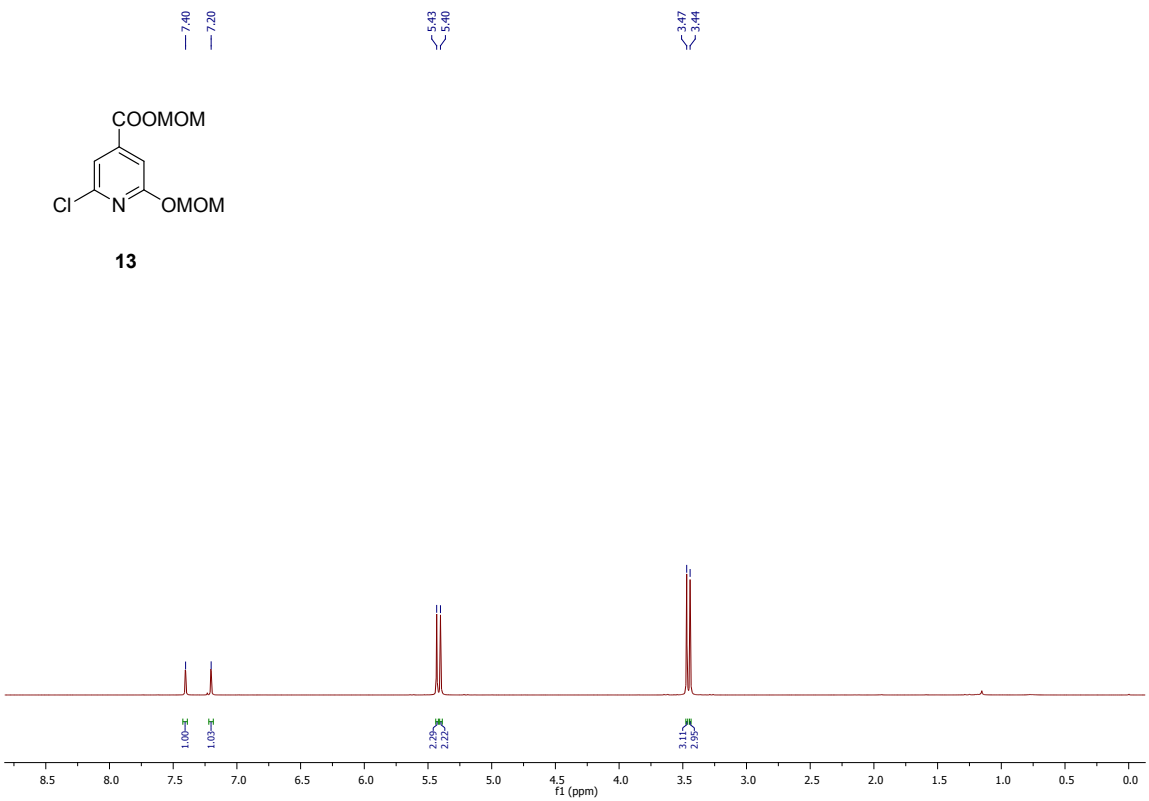
11


7.11
6.73
5.56
5.39
3.48
3.39
COOMOM
Cl
N
O
MOM
1.00
0.98
2.35
2.25
3.30
3.31
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
f1 (ppm)

12


163.12
163.05
140.36
138.42
121.26
105.53
92.08
77.36
77.04
76.73
75.76
58.10
57.75
COOMOM
Cl
N
O
MOM
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

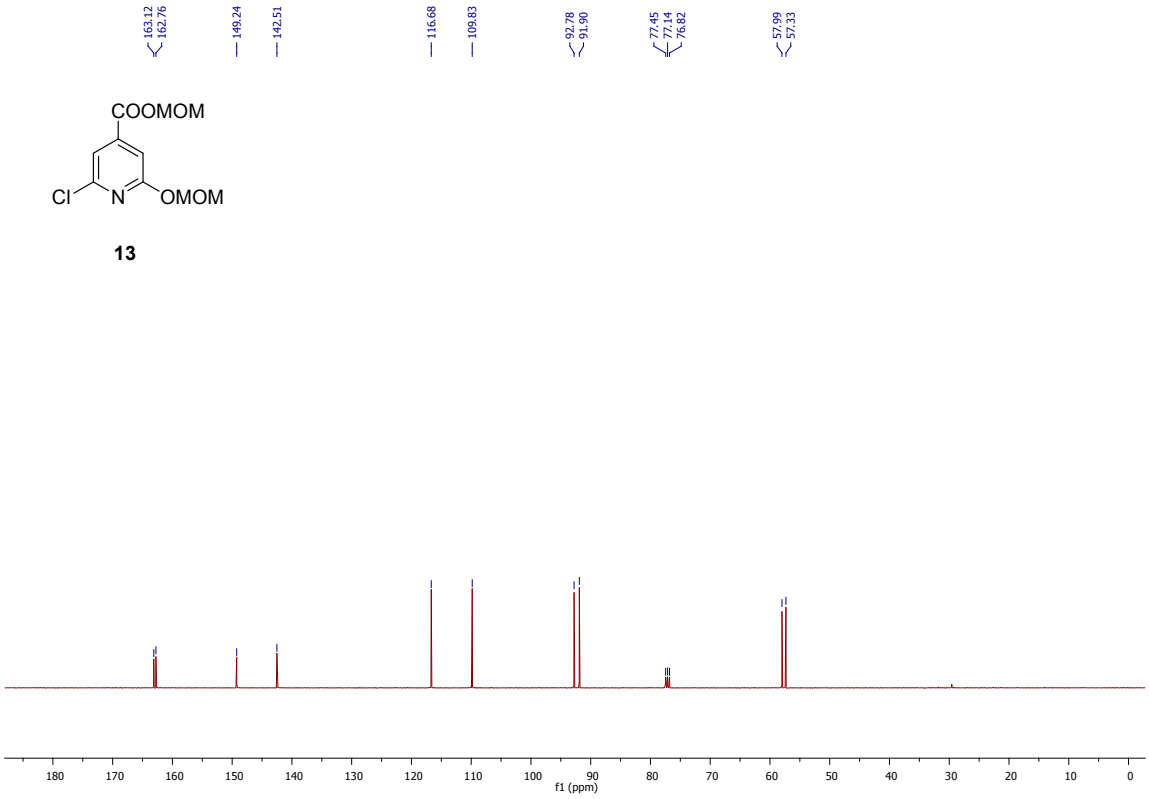
12

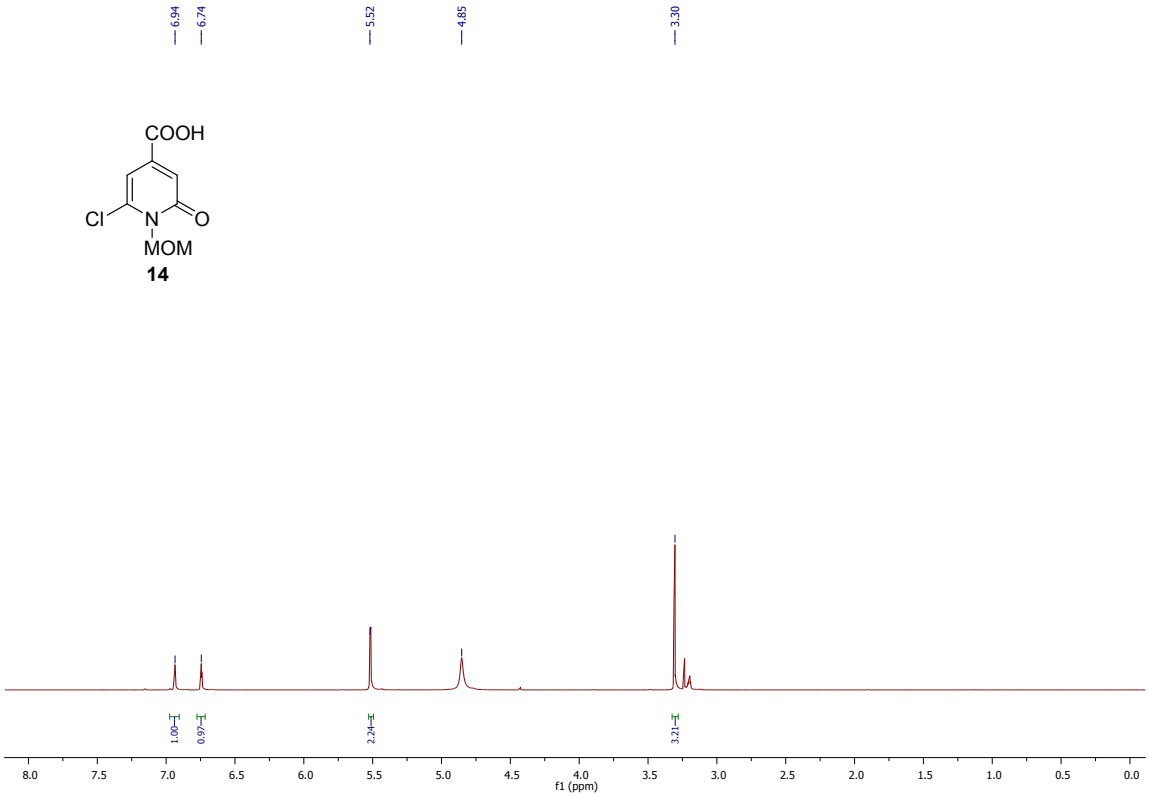

7.40
7.20
5.43
5.40
3.47
3.44
COOMOM
Cl
N
OMOM
1.00
1.03
2.29
2.22
3.11
2.95
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

13

COOMOM
Cl
N
OMOM

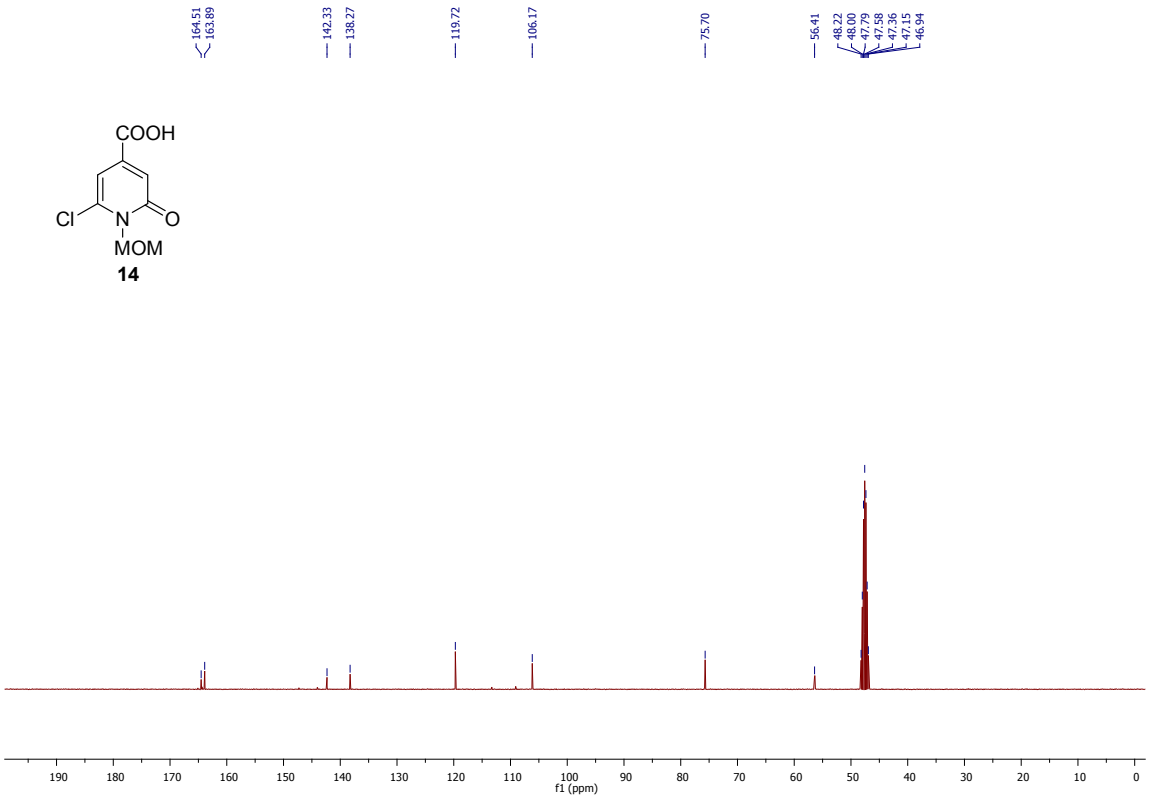
13

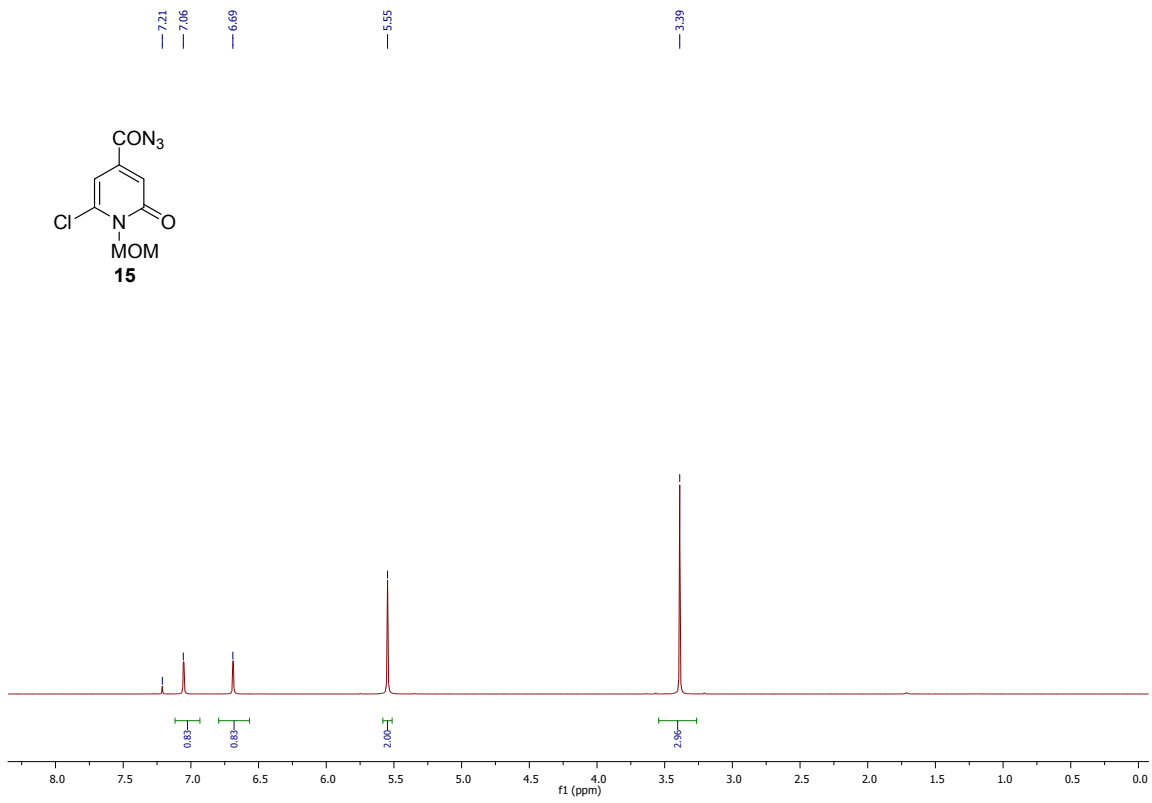

163.12
162.76
149.24
142.51
116.68
109.83
92.78
91.90
77.45
77.14
76.82
57.99
57.33
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

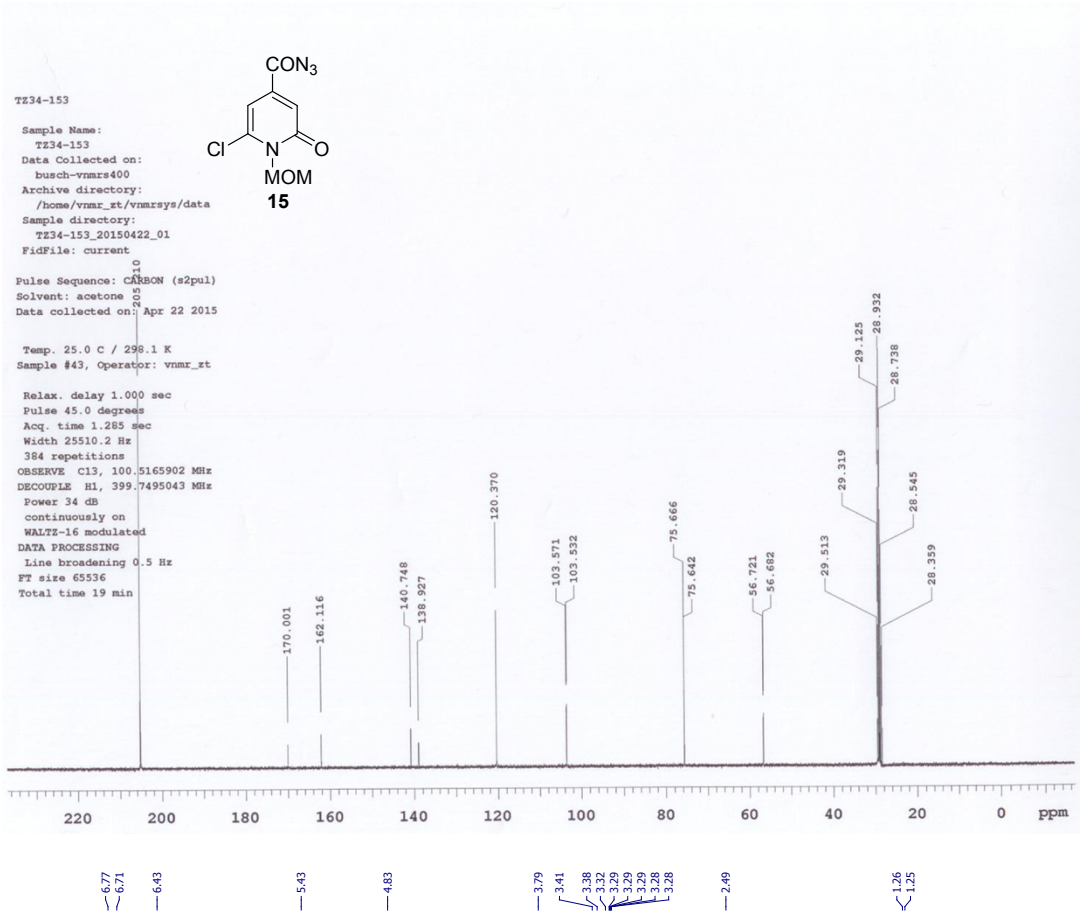

6.94
6.74
5.52
4.85
3.30
1.00
0.97
2.24
3.21
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

COOH
Cl
N
O
MOM

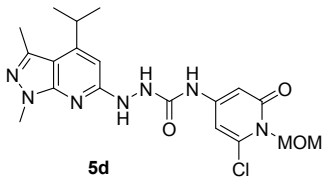
14

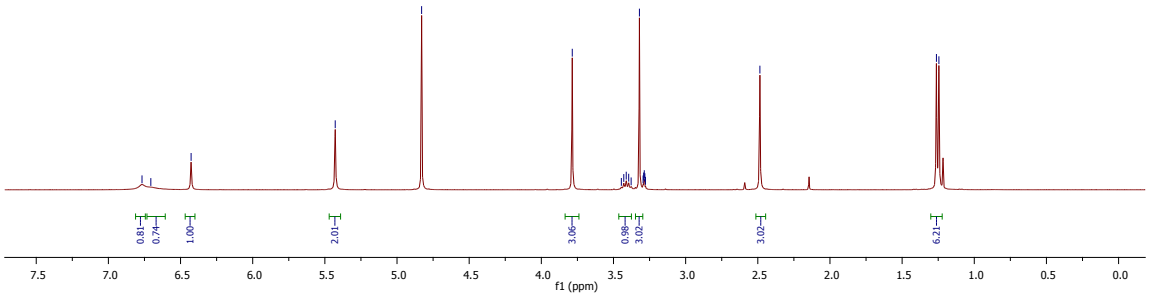

164.51
163.89
142.33
138.27
119.72
106.17
75.70
56.41
48.22
48.00
47.79
47.58
47.36
47.15
46.94
COOH
Cl
N
O
MOM
14
f1 (ppm)

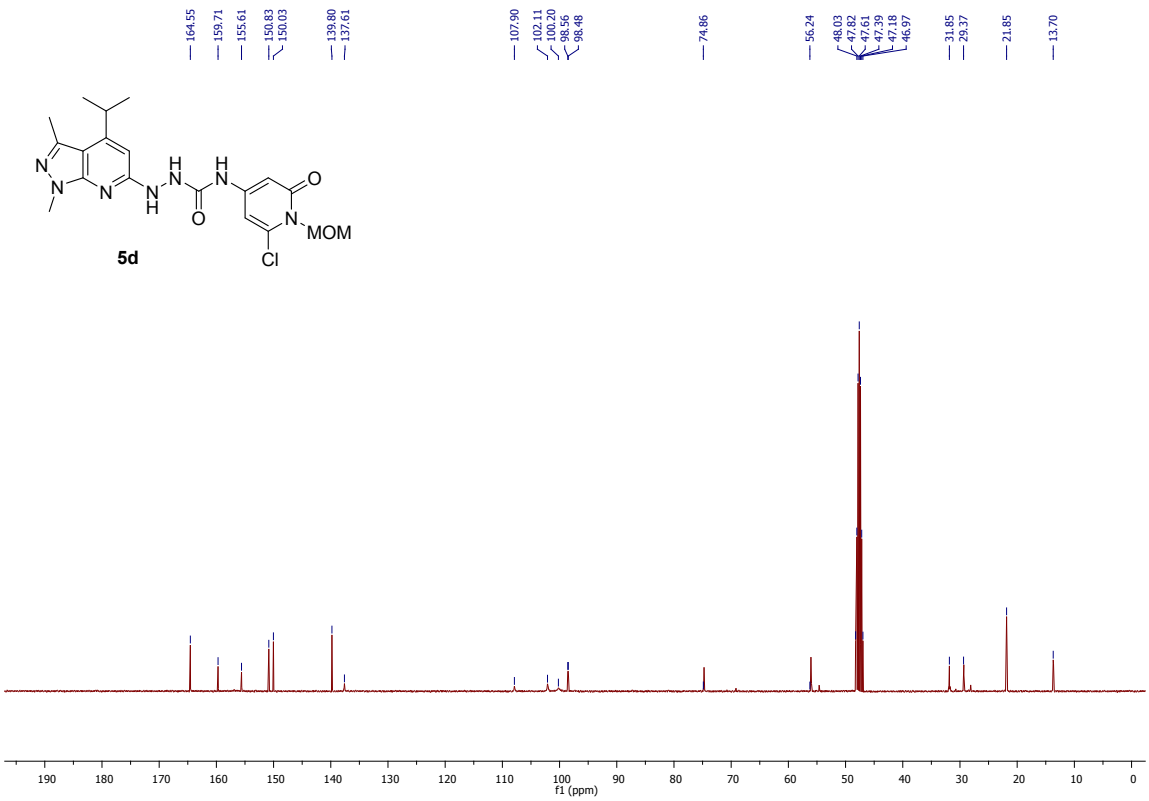

7.21
7.06
6.69
5.55
3.39
CON3
Cl
N
O
MOM
15
0.83
0.83
2.00
2.96
f1 (ppm)


CON3
Cl
N
O
MOM
15
TZ34-153
Sample Name:
TZ34-153
Data Collected on:
busch-vnmrs400
Archive directory:
/home/vnmr_zt/vnmrsys/data
Sample directory:
TZ34-153_20150422_01
FidFile: current
Pulse Sequence: CARBON (s2pul)
Solvent: acetone
Data collected on: Apr 22 2015
Temp. 25.0 C / 298.1 K
Sample #43, Operator: vnmr_zt
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.285 sec
Width 25510.2 Hz
384 repetitions
OBSERVE C13, 100.5165902 MHz
DECOUPLE H1, 399.7495043 MHz
Power 34 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 19 min
205.210
170.001
162.116
140.748
138.927
120.370
103.571
103.532
75.666
75.642
56.721
56.682
29.513
29.319
29.125
28.932
28.738
28.545
28.359
220
200
180
160
140
120
100
80
60
40
20
0
ppm

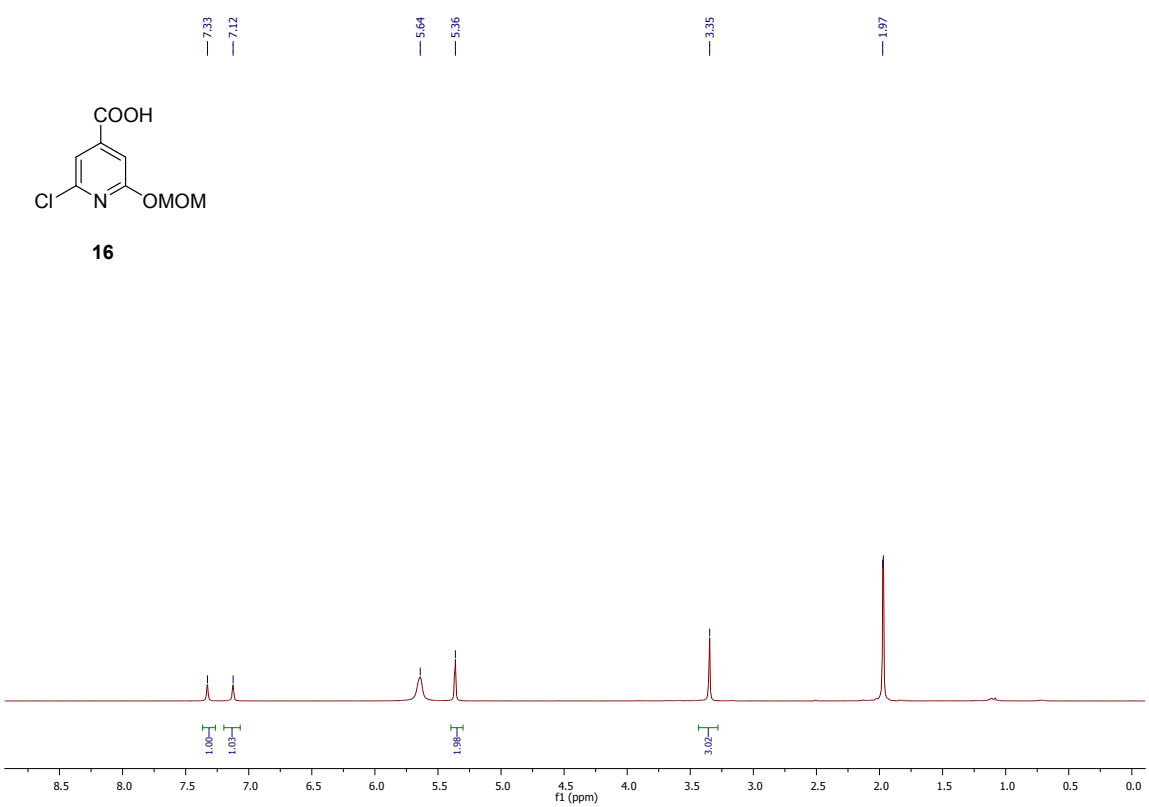
6.77
6.71
6.43
5.43
4.83
3.79
3.41
3.38
3.32
3.29
3.29
3.29
3.28
3.28
2.49
1.26
1.25


N
N
N
H
N
N
H
H
N
O
O
N
MOM
Cl
5d

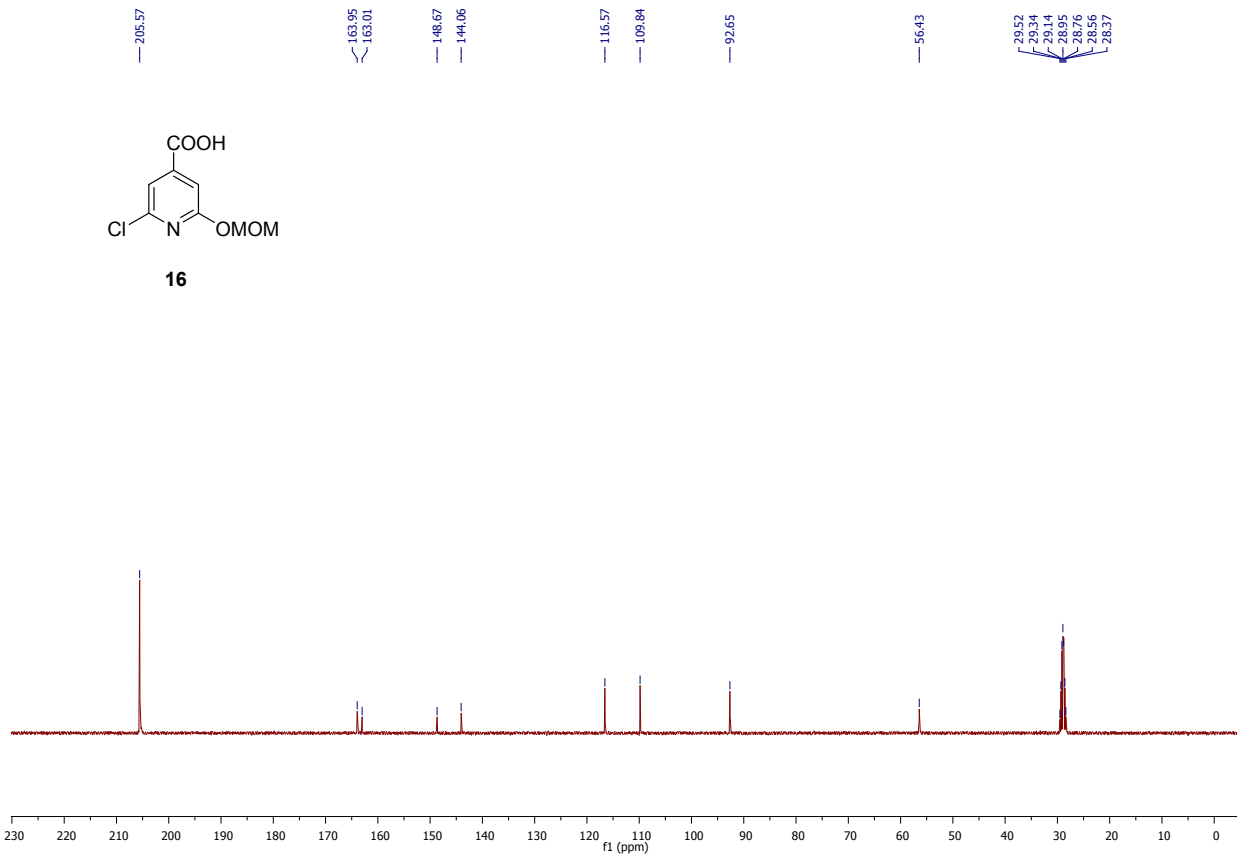

0.81
0.74
1.00
2.01
3.06
0.98
3.02
3.02
6.21
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

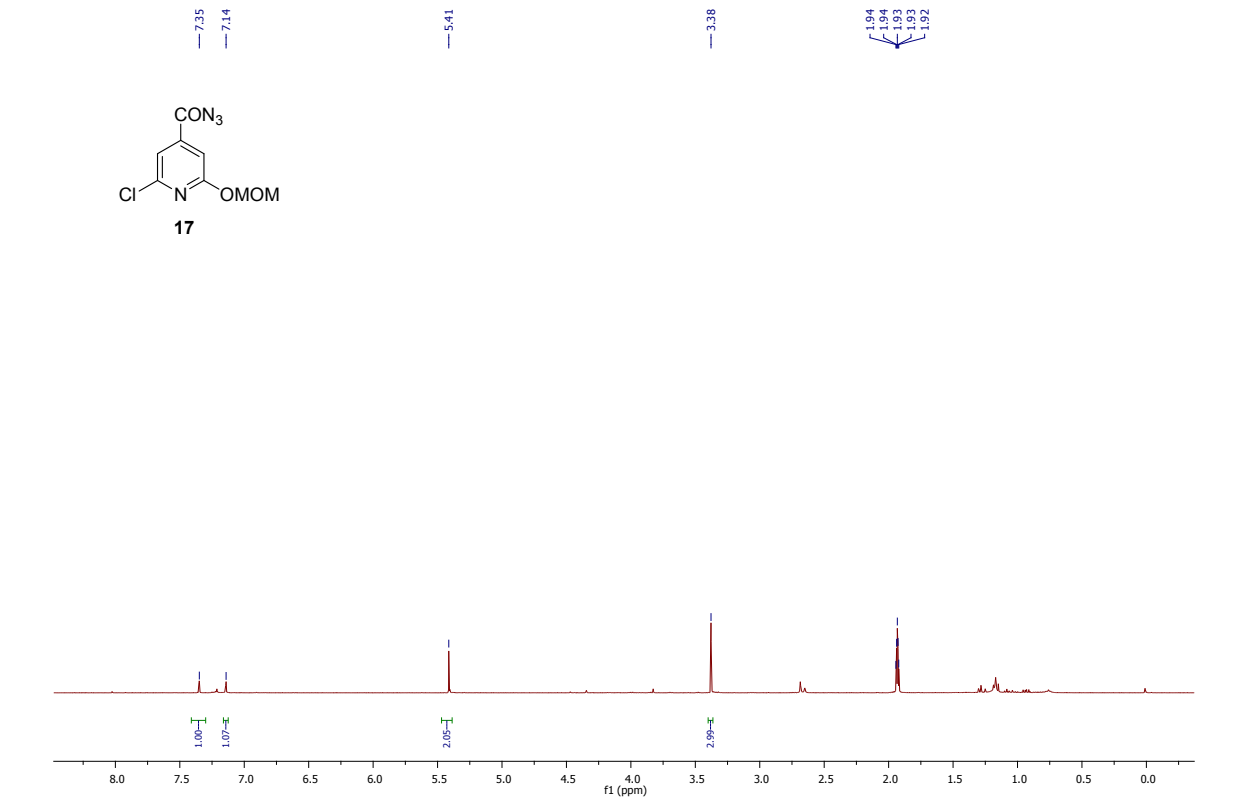

164.55
159.71
155.61
150.83
150.03
139.80
137.61
107.90
102.11
100.20
98.56
98.48
74.86
56.24
48.03
47.82
47.61
47.39
47.18
46.97
31.85
29.37
21.85
13.70
MOM
Cl
f1 (ppm)

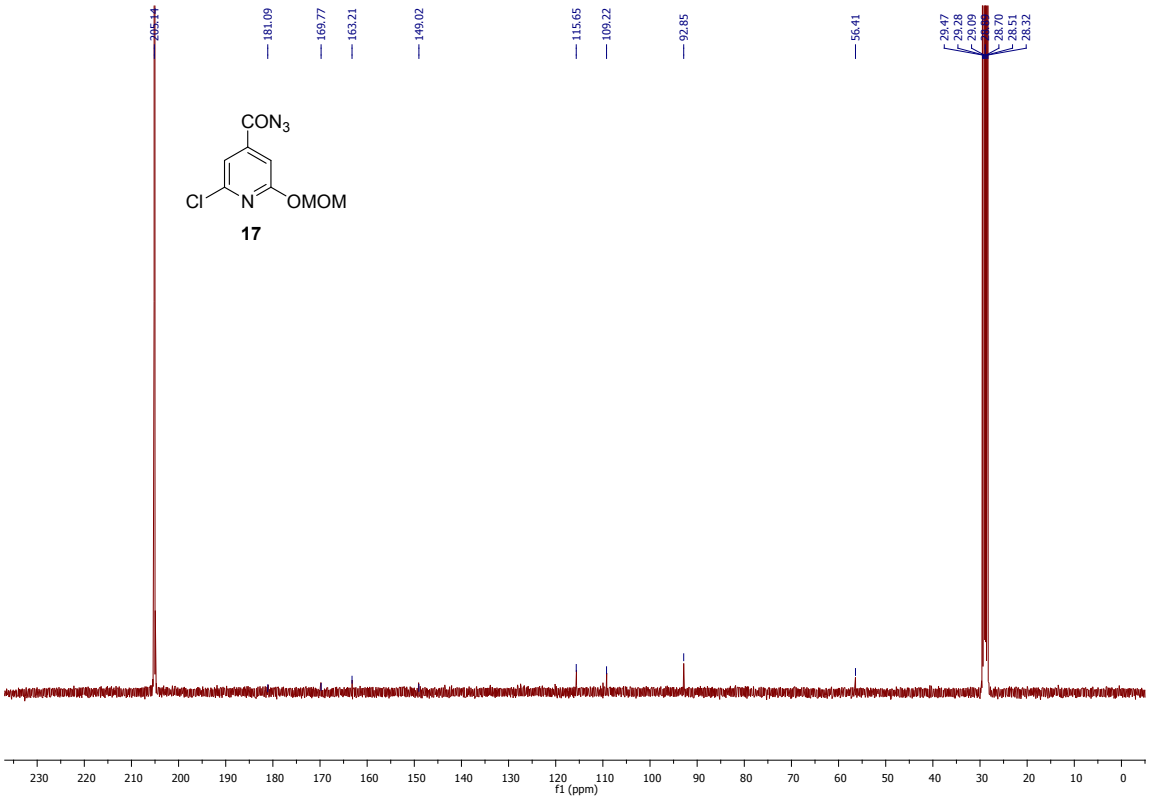
5d

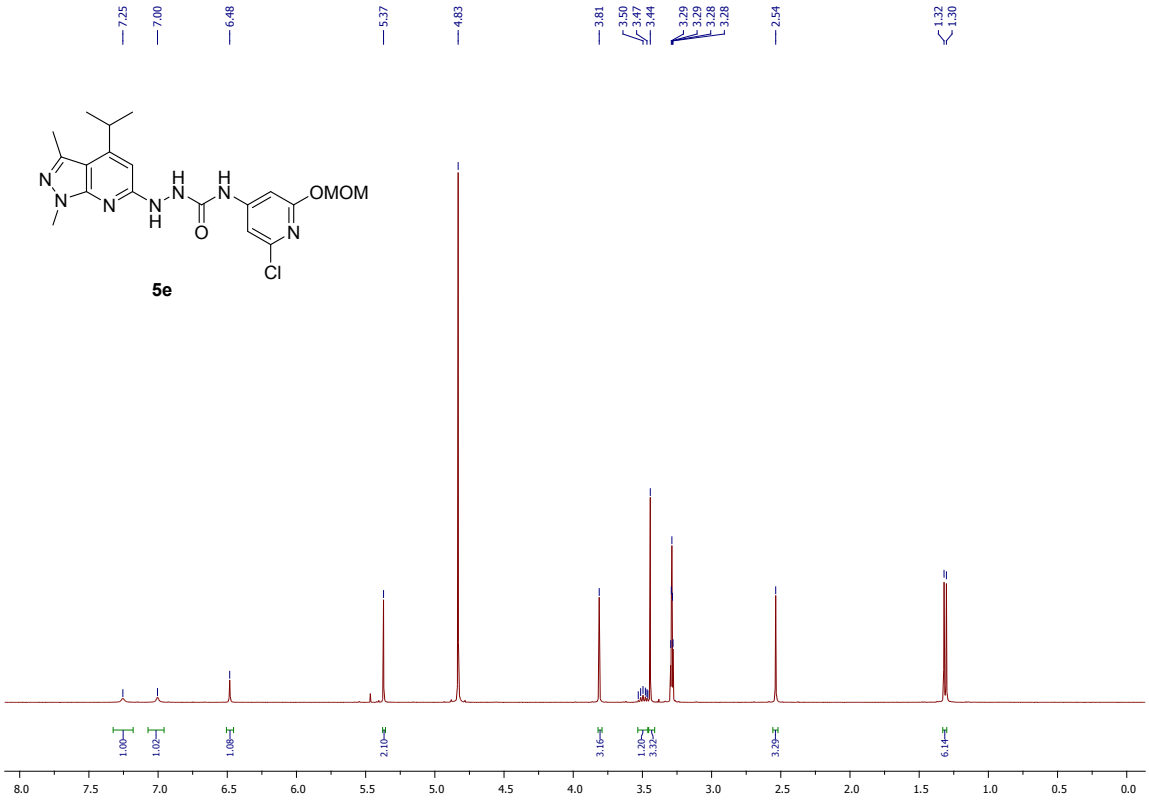

7.33
7.12
5.64
5.36
3.35
1.97
COOH
Cl
N
OMOM
1.00
1.03
1.98
3.02
f1 (ppm)

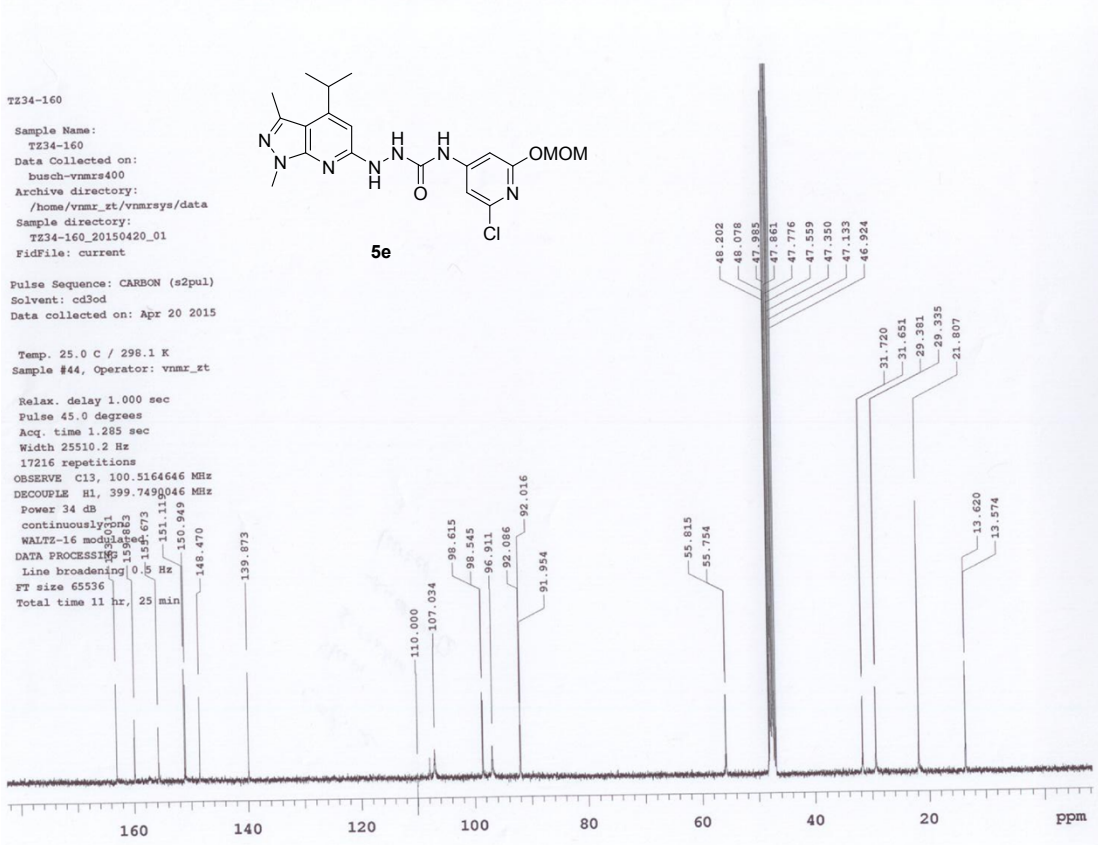
16

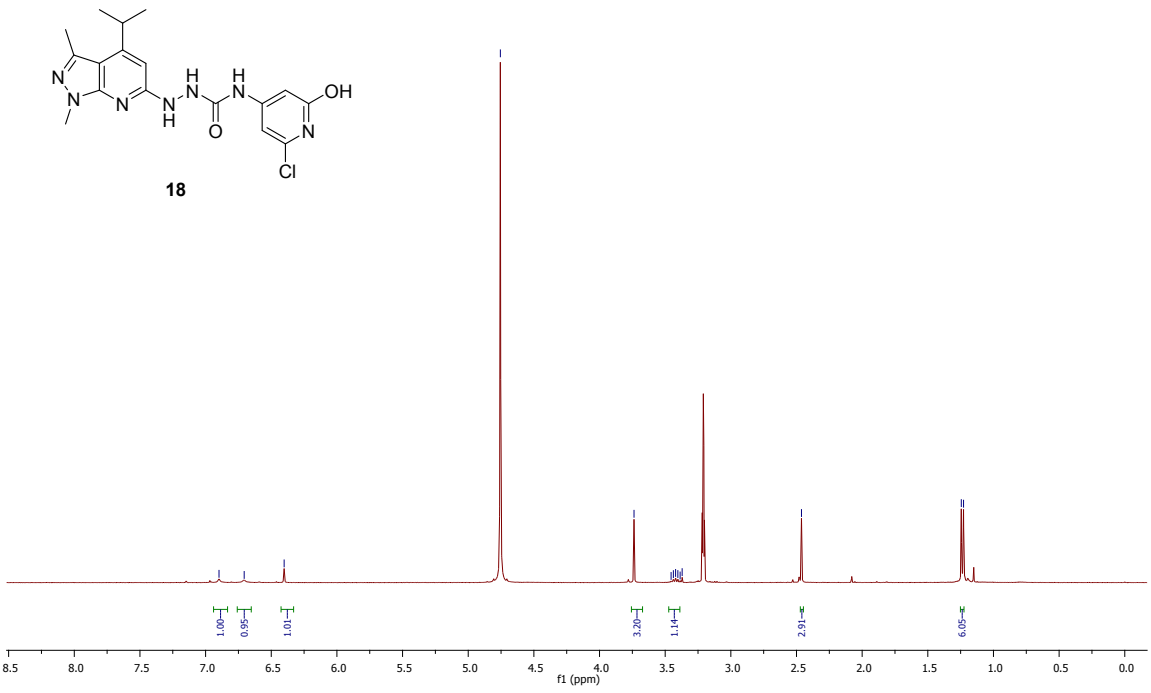

205.57
163.95
163.01
148.67
144.06
116.57
109.84
92.65
56.43
29.52
29.34
29.14
28.95
28.76
28.56
28.37
COOH
Cl
N
OMOM
16
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

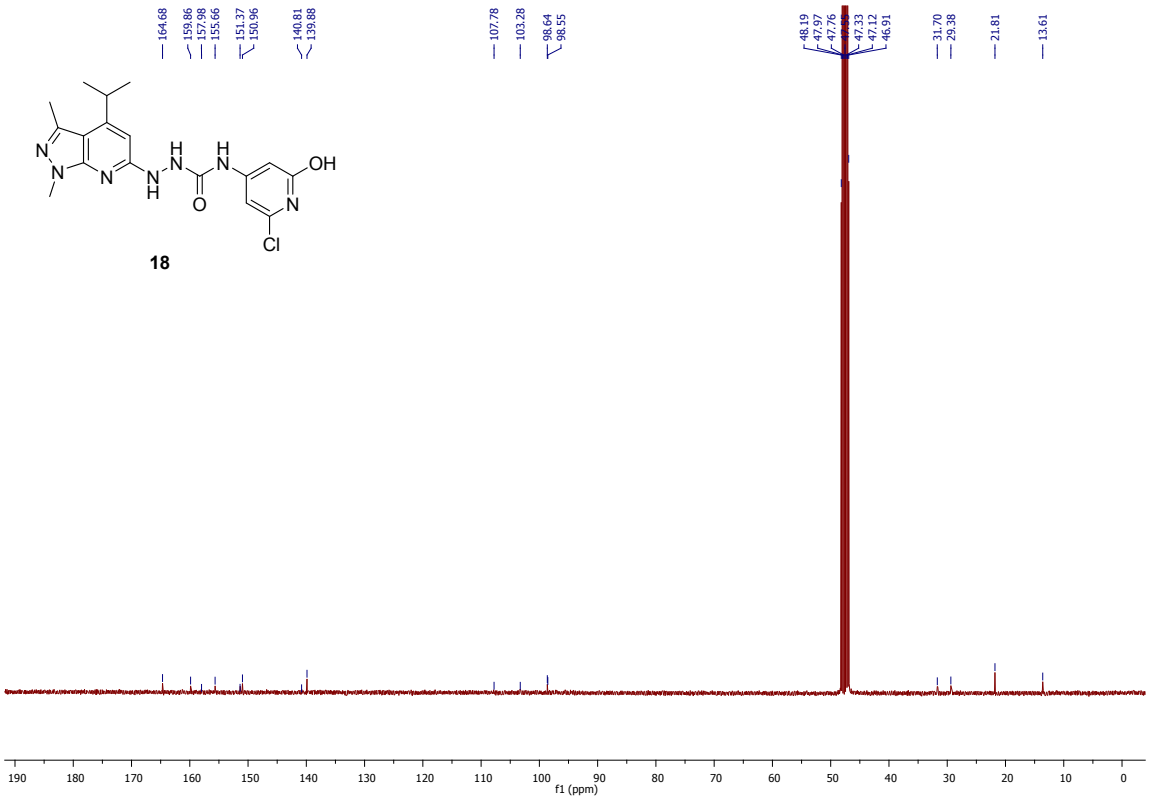

7.35
7.14
5.41
3.38
1.94
1.94
1.93
1.93
1.92
CON3
Cl
N
OMOM
17
1.00
1.07
2.05
2.99
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)


205.14
181.09
169.77
163.21
149.02
115.65
109.22
92.85
56.41
29.47
29.28
29.09
28.70
28.51
28.32
CON3
Cl
N
OMOM
17
f1 (ppm)

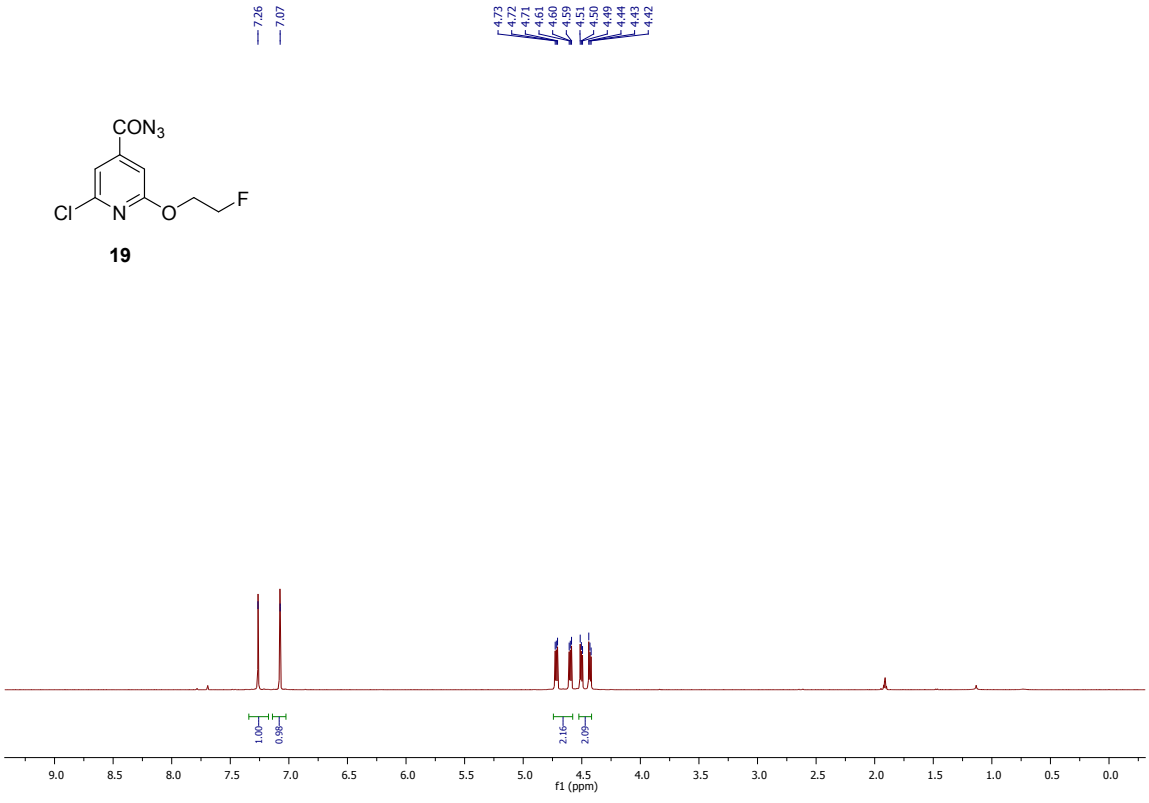

7.25
7.00
6.48
5.37
4.83
3.81
3.50
3.47
3.44
3.29
3.29
3.28
3.28
2.54
1.32
1.30
N
N
N
H
N
H
N
H
O
OMOM
N
Cl
5e
1.00
1.02
1.08
2.10
3.16
1.20
3.32
3.29
6.14


TZ34-160
Sample Name:
TZ34-160
Data Collected on:
busch-vnmrs400
Archive directory:
/home/vnmr_zt/vnmrsys/data
Sample directory:
TZ34-160_20150420_01
FidFile: current
Pulse Sequence: CARBON (s2pul)
Solvent: cd3od
Data collected on: Apr 20 2015
Temp. 25.0 C / 298.1 K
Sample #44, Operator: vnmr_zt
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.285 sec
Width 25510.2 Hz
17216 repetitions
OBSERVE C13, 100.5164646 MHz
DECOUPLE H1, 399.7490046 MHz
Power 34 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 11 hr, 25 min
OMOM
Cl
5e
48.202
48.078
47.985
47.861
47.776
47.559
47.350
47.133
46.924
31.720
31.651
29.381
29.335
21.807
13.620
13.574
55.815
55.754
98.615
98.545
96.911
92.086
92.016
91.954
110.000
107.034
ppm

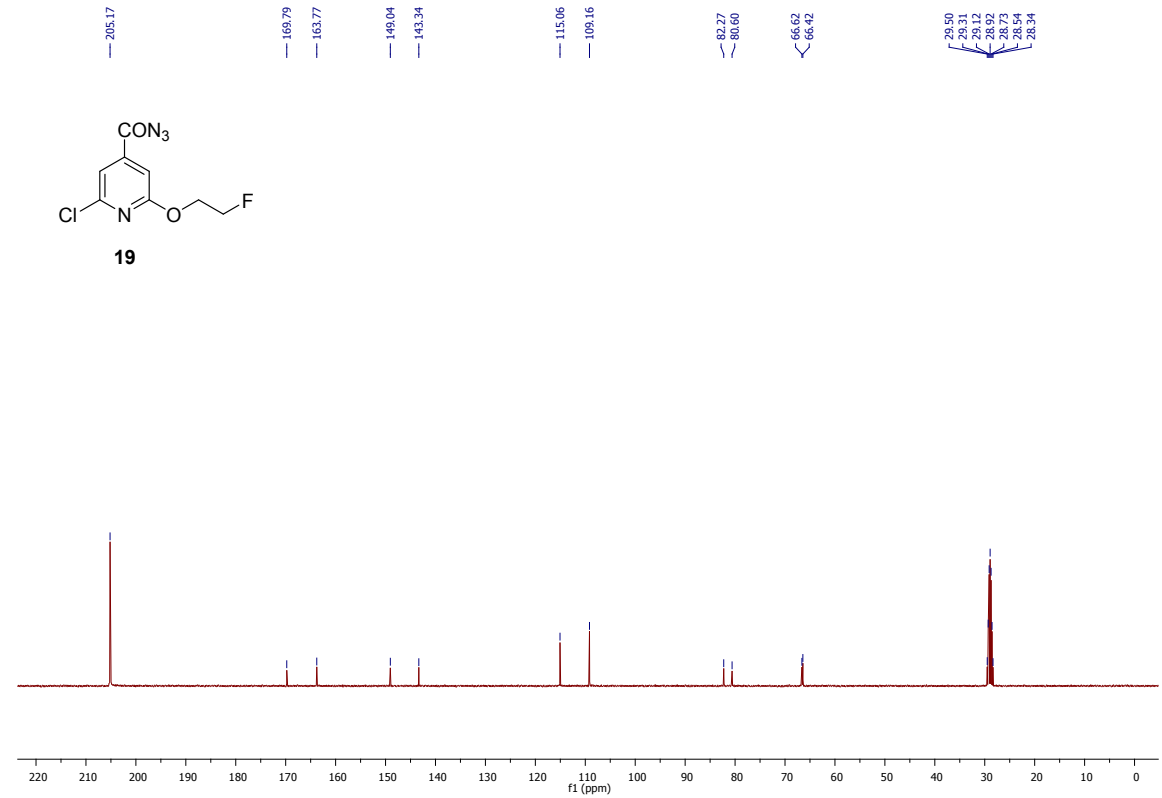

OH
Cl
18
6.90
6.71
6.40
4.76
3.74
3.46
3.44
3.42
3.40
3.39
3.37
2.46
1.24
1.23
1.00
0.95
1.01
3.20
1.14
2.91
6.05
f1 (ppm)

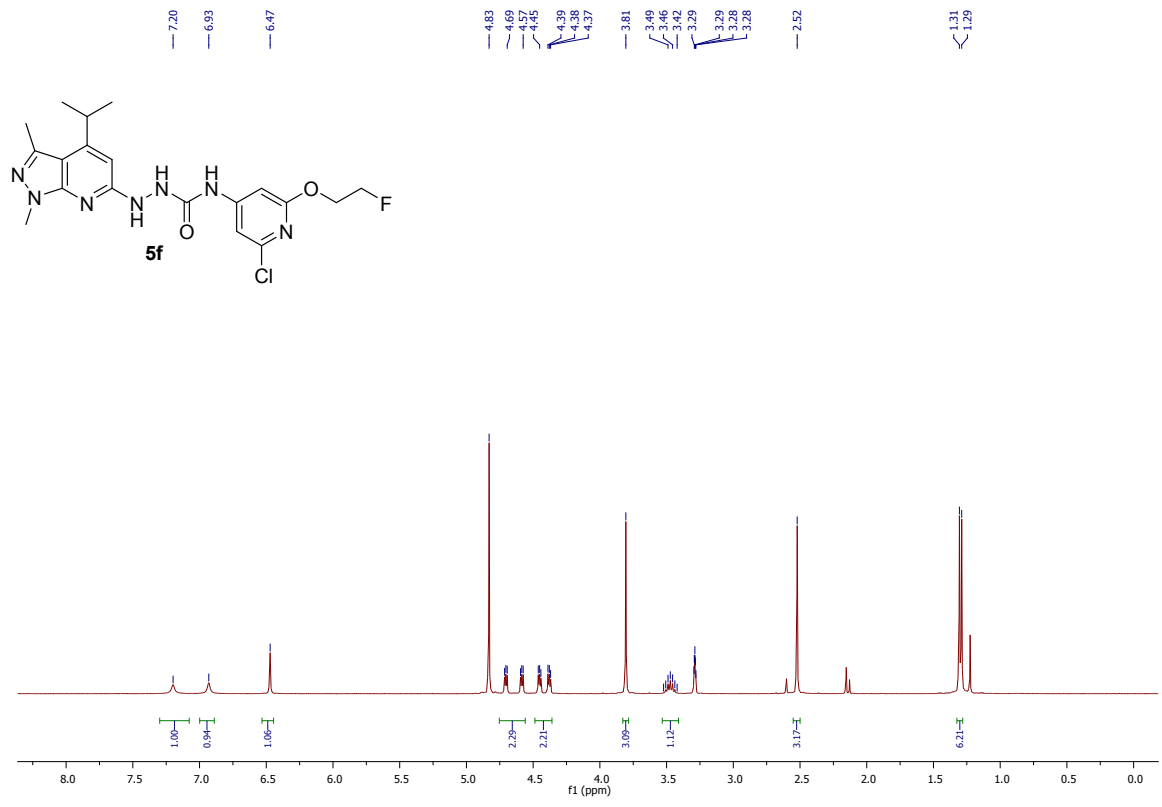

164.68
159.86
157.98
155.66
151.37
150.96
140.81
139.88
107.78
103.28
98.64
98.55
48.19
47.97
47.76
47.33
47.12
46.91
31.70
29.38
21.81
13.61
OH
Cl
f1 (ppm)

18


7.26
7.07
4.73
4.72
4.71
4.61
4.60
4.59
4.51
4.50
4.49
4.44
4.43
4.42
CON3
Cl
F
1.00
0.98
2.16
2.09
f1 (ppm)

19


205.17
169.79
163.77
149.04
143.34
115.06
109.16
82.27
80.60
66.62
66.42
29.50
29.31
29.12
28.92
28.73
28.54
28.34
CON3
Cl
N
O
F
19
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


7.20
6.93
6.47
4.83
4.69
4.57
4.45
4.39
4.38
4.37
3.81
3.49
3.46
3.42
3.29
3.29
3.28
3.28
2.52
1.31
1.29
N
N
H
N
H
N
H
O
O
N
F
Cl
5f
1.00
0.94
1.06
2.29
2.21
3.09
1.12
3.17
6.21
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
f1 (ppm)

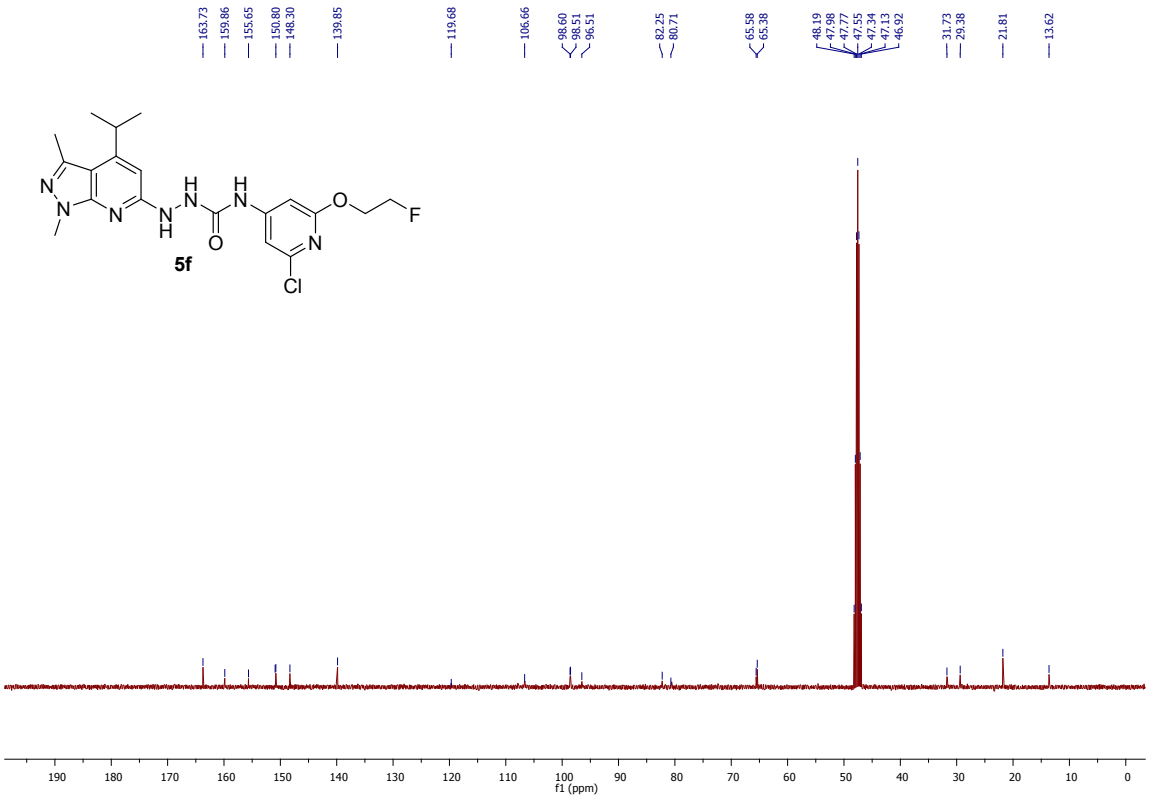


Table S1. Conditions screening for the radiolabeling of compound **18**.

Entry	Reaction conditions	HPLC conditions	Product Collected (mCi)	Radiochemical purity
1	[^{11}C]MeOTf, 5 $M_{(aq.)}$ KOH 3 μL, DMF 200 μL, 85 ^{o}C, 5 min	45% acetonitrile in 0.1 M ammonium formate, pH 4.5	14.7	> 99%
2	[^{11}C]MeOTf, 5 $M_{(aq.)}$ KOH 3 μL, DMF 200 μL, 60 ^{o}C, 5 min	45% acetonitrile in 0.1 M ammonium formate, pH 4.5	24.5	79%
3	[^{11}C]MeI, solid Cs_2CO_3, DMF 200 μL, 85 ^{o}C, 5 min	45% acetonitrile in 0.1 M ammonium formate, pH 4.5	2.1	> 99%
4	[^{11}C]MeI, 5 $M_{(aq.)}$ KOH 3 μL, DMF 200 μL, 85 ^{o}C, 5 min	45% acetonitrile in 0.1 M ammonium formate, pH 4.5	26.9	71%
5	[^{11}C]MeI, 5 $M_{(aq.)}$ CsOH 3 μL, DMF 200 μL, 85 ^{o}C, 5 min	45% acetonitrile in 0.1 M ammonium formate, pH 6.5	15.8	> 99%

6	**[^{11}C]MeI, 5 M$_{(aq.)}$ KOH, DMF 200 μL, 85 ^{o}C, 5 min**	**45% acetonitrile in 0.1 M ammonium formate, pH 6.5**	**31.1**	**> 99%**

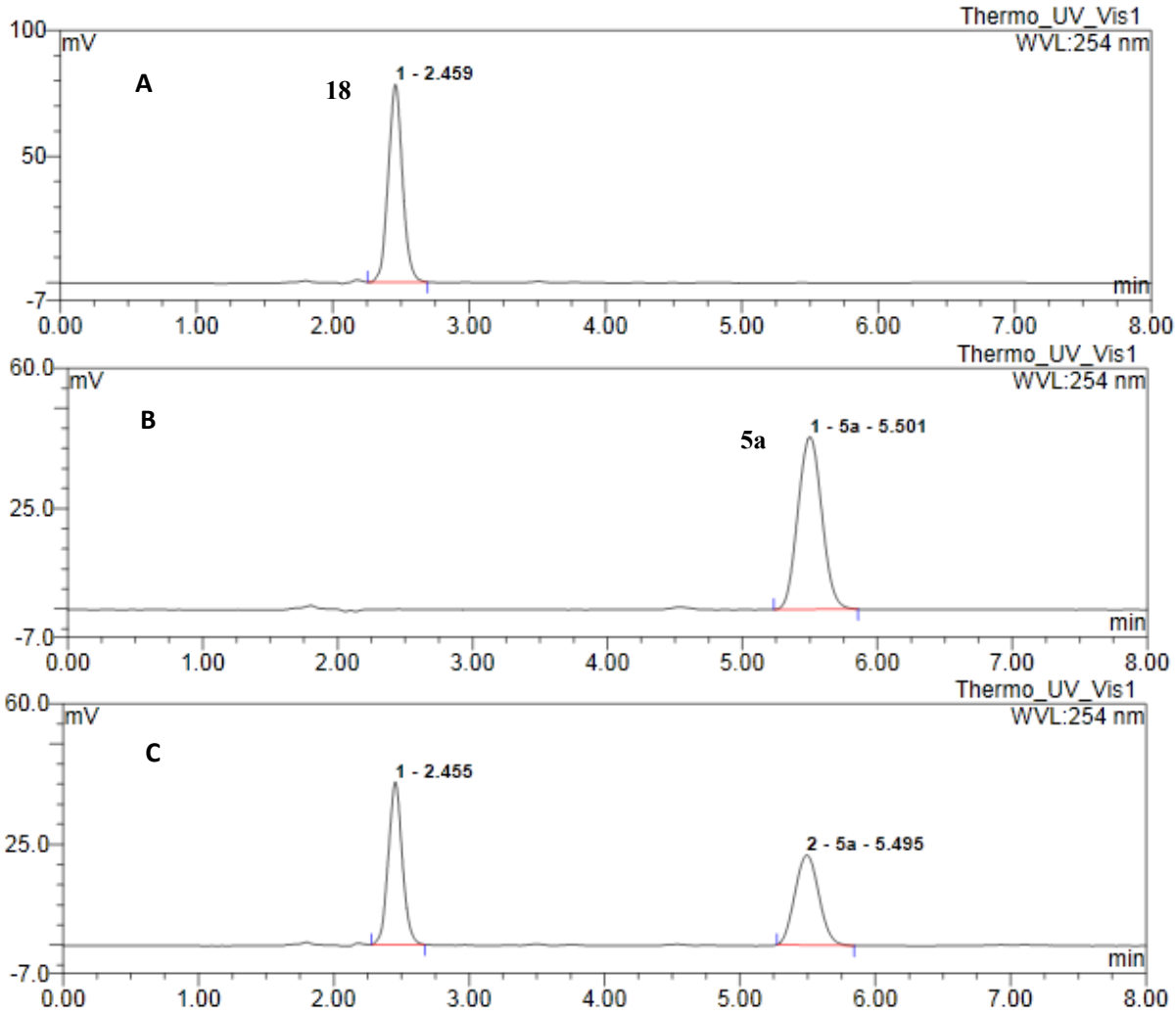


Figure S1. Analytical HPLC chromatograms of [^{11}C]**5a** precursor **18** (panel **A**), nonradiolabeled standard **5a** (panel **B**), and co-injection of **18** and **5a** (panel **C**). Analytical HPLC conditions: Agilent Zorbax SB-C18 column, 250 × 4.6 mm, mobile phase 55% acetonitrile in 0.1 M ammonium formate, pH 4.5, flow rate 1.2 mL/min, detection wavelength 254 nm.

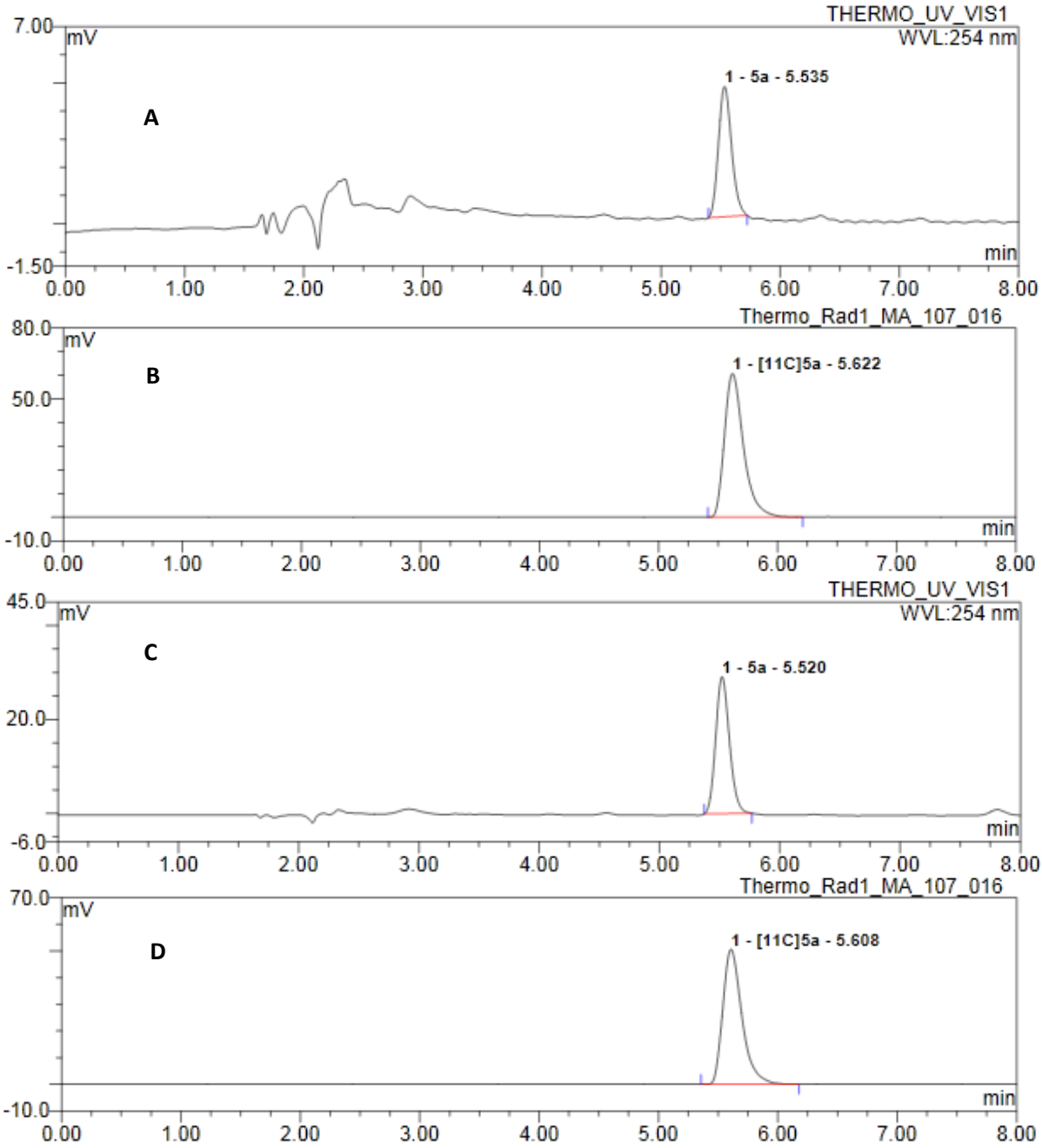


Figure S2. Typical analytical HPLC trace of formulated [^{18}F]**5a**. Panel **A** shows UV trace for [^{11}C]**5a** with 10% EtOH in saline; panel **B**: [^{11}C]**5a** radiochemical trace; panel **C**: UV trace for co-injection of [^{11}C]**5a** and **5a**; panel **D**: radiochemical trace for co-injection of [^{11}C]**5a** and **5a**. Analytical HPLC conditions: Agilent Zorbax SB-C18 column, 250 × 4.6 mm, mobile phase 55% acetonitrile in 0.1 M ammonium formate, pH 4.5, flow rate 1.2 mL/min, detection wavelength 254 nm.