CHEMBIOCHEM

Supporting Information

Rational Design, Synthesis and Biological Evaluation of Modular Fluorogenic Substrates with High Affinity and Selectivity for PTP1B

Silvano Sanchini,* Francesca Perruccio, and Grazia Piizzi[a]

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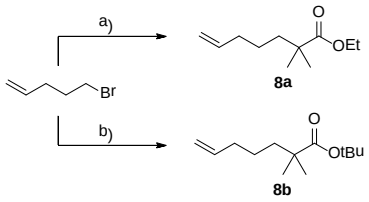
I. Synthetic procedures and compound characterization.

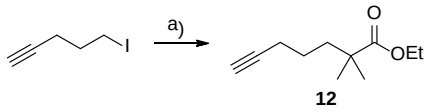


Scheme S1. Reagents and conditions: a) ethyl isobutyrate, LDA, THF, –78 °C to rt, overnight; b) *t*-butyl isobutyrate, LDA, THF, –78 °C to rt, overnight.

General procedure for the synthesis of compounds 8a[i] and 8b: to a stirred solution of freshly prepared LDA (1.1 equiv.) in THF (0.05 M), the opportune isobutyrate (1.0 equiv., ethyl isobutyrate for **8a**; *t*-butyl isobutyrate for **8b**) was added at -78 °C and under argon atmosphere. After 2 hours 5-bromo-pent-1-ene (1.0 equiv) was added, the mixture was allowed to warm to room temperature and stirred overnight. The reaction was then quenched with HCl 2N and extracted with Et_2O. Organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was filtered over a short pad o silica gel by eluting with heptane and used as is for the next step.

Ethyl 2,2-dimethylhept-6-enoate (8a): the spectroscopic data recorded are in agreement with those reported in the reference. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.76 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1H), 4.98 (dd, *J* = 17.1, 1.4 Hz, 1H), 4.93 (d, *J* = 10.2 Hz, 1H), 4.10 (qd, *J* = 7.2, 2.4 Hz, 2H), 2.05 – 1.97 (m, 2H), 1.53 – 1.48 (m, 2H), 1.37 – 1.25 (m, 2H), 1.23 (t, *J* = 7.1 Hz, 4H), 1.14 (s, 1H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.0, 138.7, 114.6, 60.3, 42.2, 40.3, 34.2, 25.3, 24.4, 14.4 ppm.

***tert*-Butyl 2,2-dimethylhept-6-enoate (8b):** ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.78 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 4.98 (dd, *J* = 17.1, 1.4 Hz, 1H), 4.93 (d, *J* = 10.2 Hz, 1H), 2.02 (q, *J* = 7.0 Hz, 2H), 1.51 – 1.44 (m, 2H), 1.42 (s, 9H), 1.37 – 1.27 (m, 2H), 1.10 (s, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.4, 138.8, 114.6, 79.7, 42.7, 40.3, 34.3, 28.1, 25.3, 24.4 ppm.



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Scheme S2. Reagents and conditions: a) ethyl isobutyrate, LDA, THF, -78 °C to rt, overnight.

Synthesis of ethyl 2,2-dimethylhept-6-ynoate (**12**): to a stirred solution of freshly prepared LDA (1.1 equiv.) in THF (0.05 M), ethyl isobutyrate (1.1 equiv.) was added at -78 °C and under argon atmosphere. After 2 hours 5-iodo-pent-1-yne (1.0 equiv) was added, the mixture was allowed to warm to room temperature and stirred overnight. The reaction was then quenched with HCl 2N and extracted with Et_2O. Organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was filtered over a short pad o silica gel by eluting with heptane and used as is for the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.12 (q, *J* = 7.1 Hz, 2H), 2.17 (td, *J* = 7.0, 2.6 Hz, 2H), 1.95 (t, *J* = 2.6 Hz, 1H), 1.66 – 1.59 (m, 2H), 1.52 – 1.42 (m, 2H), 1.25 (t, *J* = 7.2 Hz, 3H), 1.18 (s, 6H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.8, 84.3, 68.5, 60.4, 42.1, 39.9, 25.2, 24.2, 19.0, 14.3 ppm.

ABBREVIATIONS USED

TMSBr, bromotrimethylsilane; DCM, dicloromethane; SiO_2, silica gel; MOMCl, chloromethyl methyl ether; DIPEA, N,N-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DIBAL-H, diisobutylaluminium hydride; TEA, triethylamine; LDA, lithium diisopropylamide; LiTMP, lithium tetramethylpiperidine.

II. Stability Studies

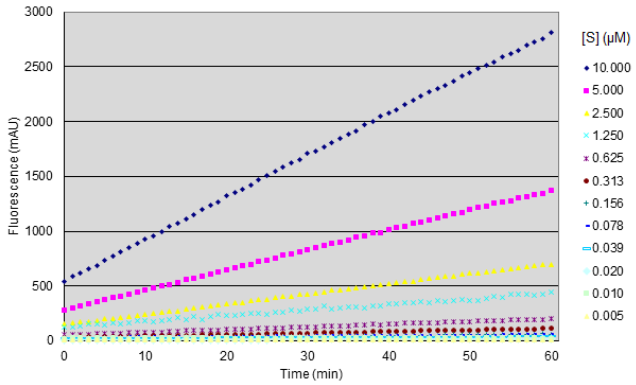


Figure S1. Spontaneous degradation of compound **4f**. Compound **4f** was serially diluted (10 µM, 5 µM, 2.5 µM, 1.25 µM, 0.625 µM, 0.313 µM, 0.156 µM, 0.078 µM, 0.039 µM, 0.020 µM, 0.010 µM and 0.005 µM) in cold reaction buffer (50 mM HEPES, pH 7.1, 50 mM KCl, 1mM EDTA, 3 mM DTT, 0.05% NP-40) for a total volume of 25 µl. Fluorescence production (mAU, y-axis) was monitored (λ_{ex} = 342 nm, λ_{em} = 441 nm) every minute for one hour. The assay was run in duplicate.

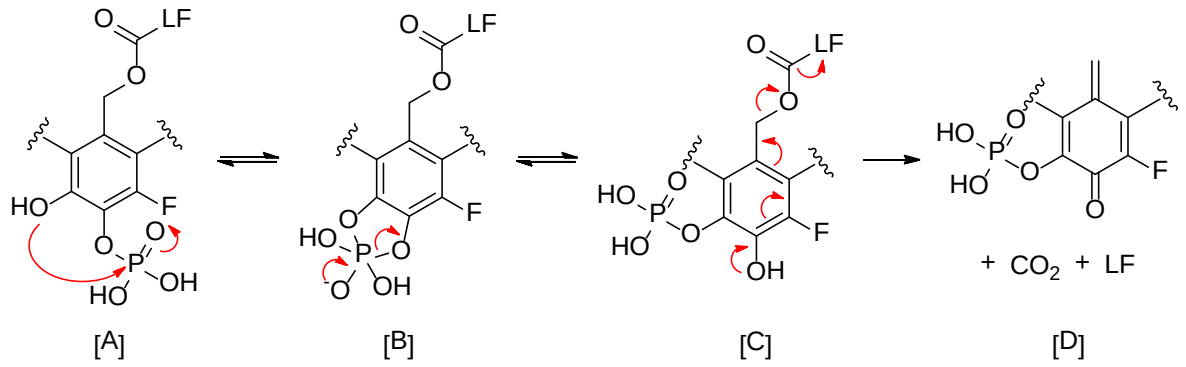


Figure S2. Proposed decomposition mechanism for compounds **4h** and **4k**. [A] When unmasked, the free phenol attacks the phosphate group to generate an unstable pentacyclic intermediate [B] which re-opens and triggers the 1,6-elimination [C] with subsequent decomposition of the scaffolds [D].

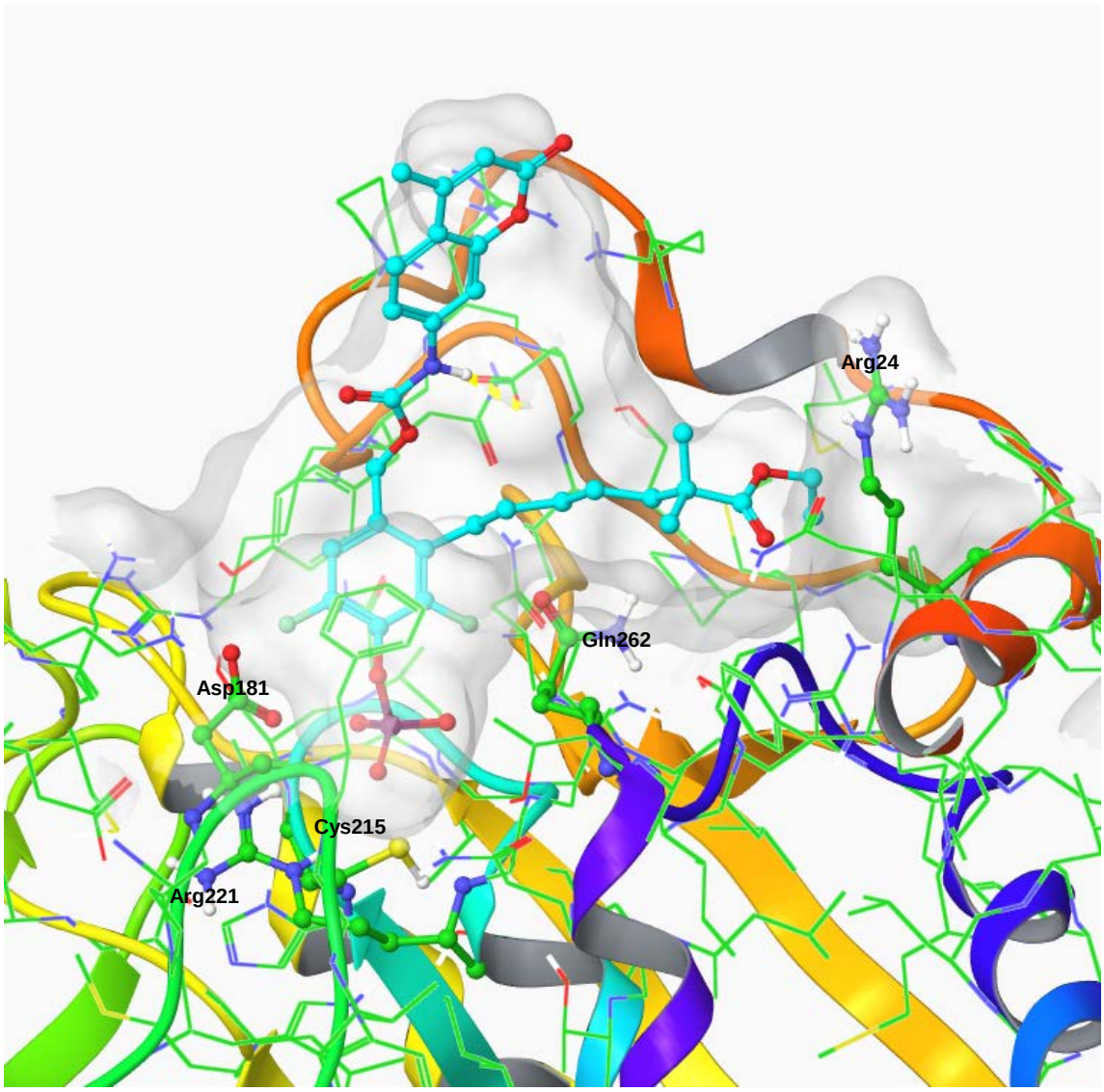
III. Computational Studies

Table S1 – Graphical summary of the BLAST output

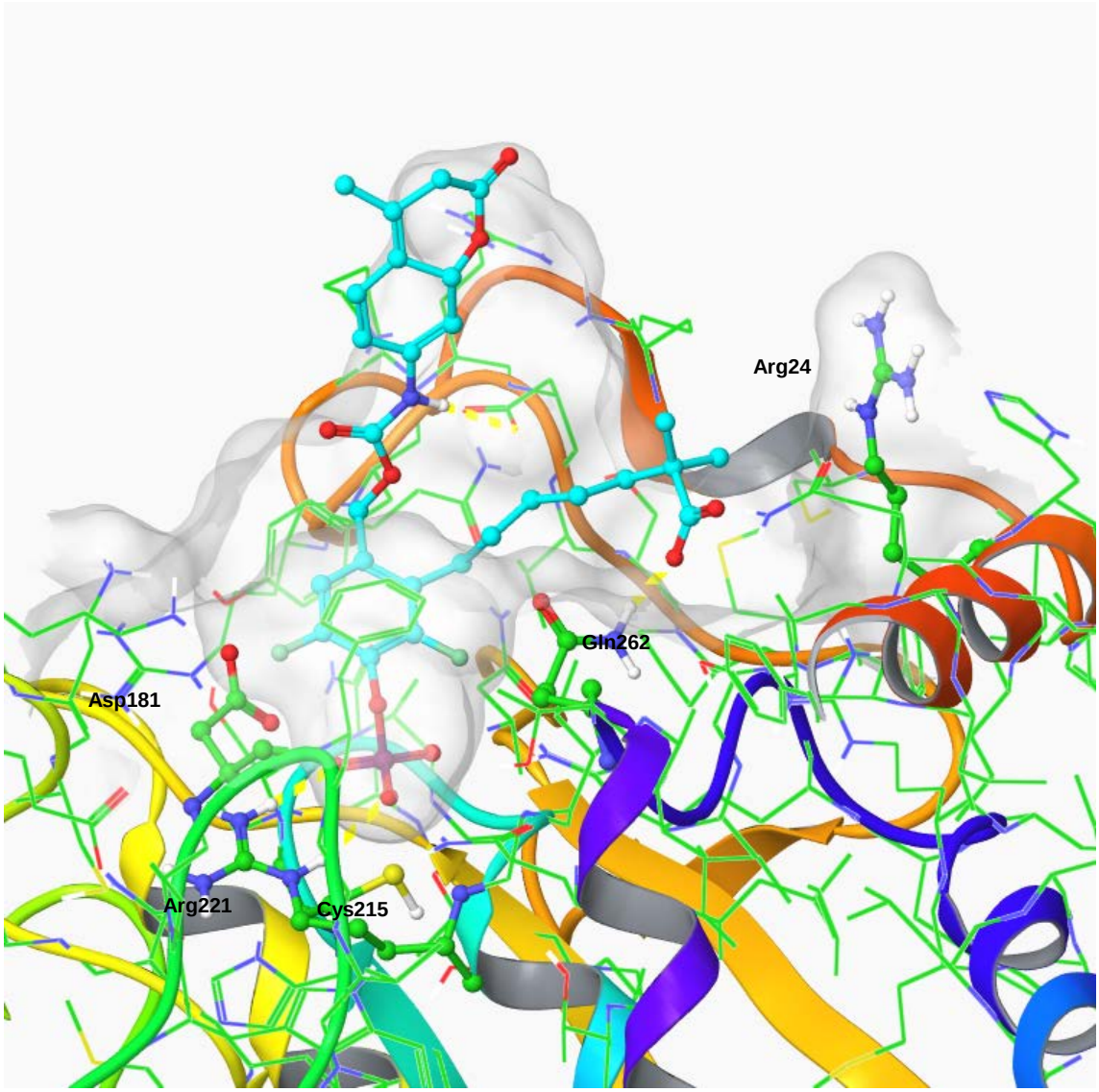
Protein	Pdb ID	Score[a]	Identities[b]	Positives[c]
TCPTP	1L8K	449.5	68 %	84 %
PTP-β	2I4E	197.2	39%	58%
SHP1	305X	186.2	38 %	59 %
LAR	1LAR	182.6	35 %	51 %
CD45	1YGR	115.2	25 %	45%
VHR*	ND	ND	ND	ND
PP2A*	ND	ND	ND	ND

a) numerical value describing the overall quality of an alignment: higher numbers correspond to a higher similarity; b) parameter indicating amino acid residues that are identical in the query (PTP1B) and in the hit when the two are optimally aligned; c) parameter indicating residues that are similar to each other.

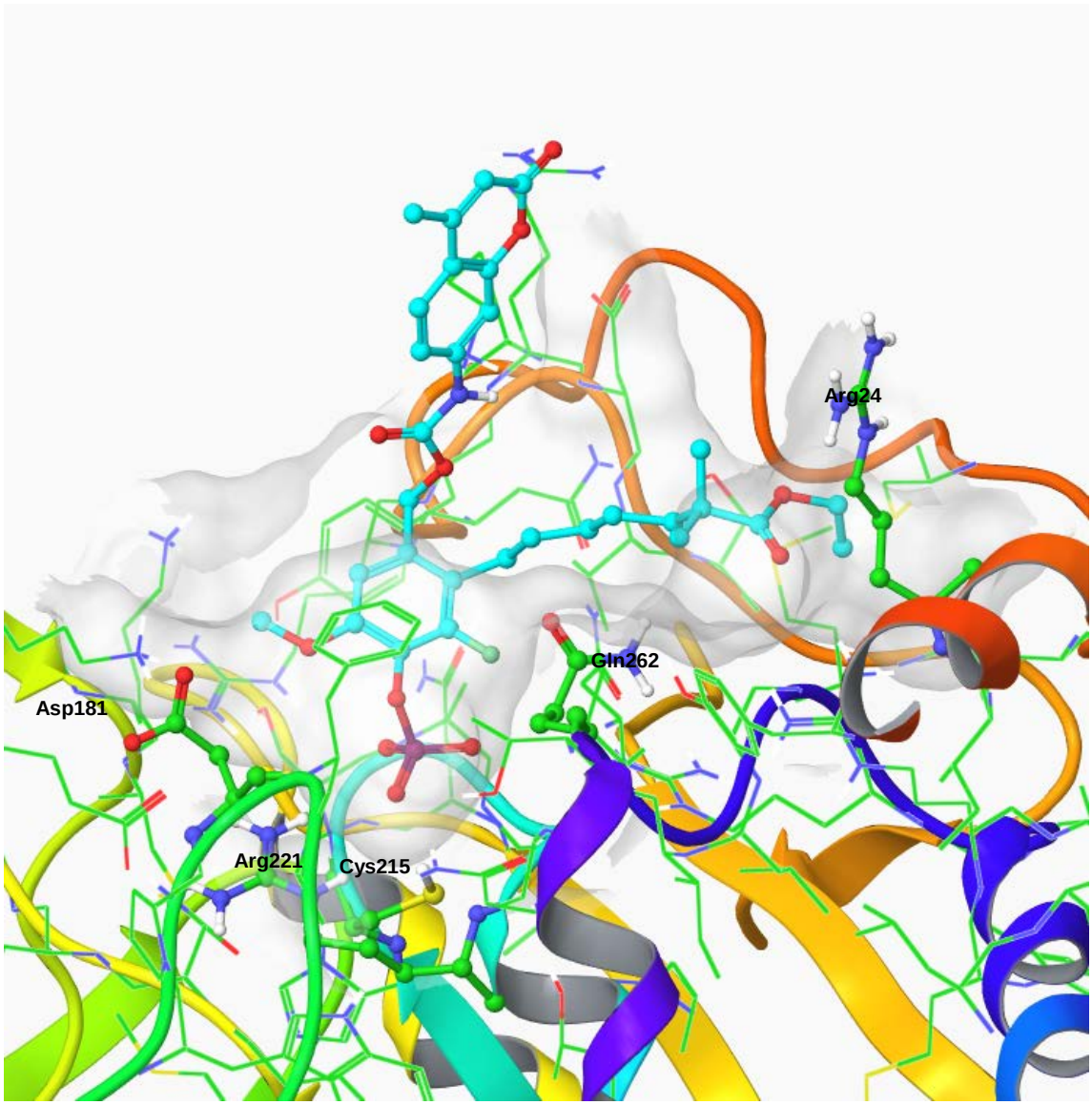
BLAST output highlights a very high degree of similarity between PPT1B and TCPTP. A minor degree of homology was found in PTP-β, SHP1, LAR and CD45 whereas PP2A and VHR scored below the threshold value. The obtained data was in complete agreement with the biological results on the potential secondary targets for the synthesized substrates.



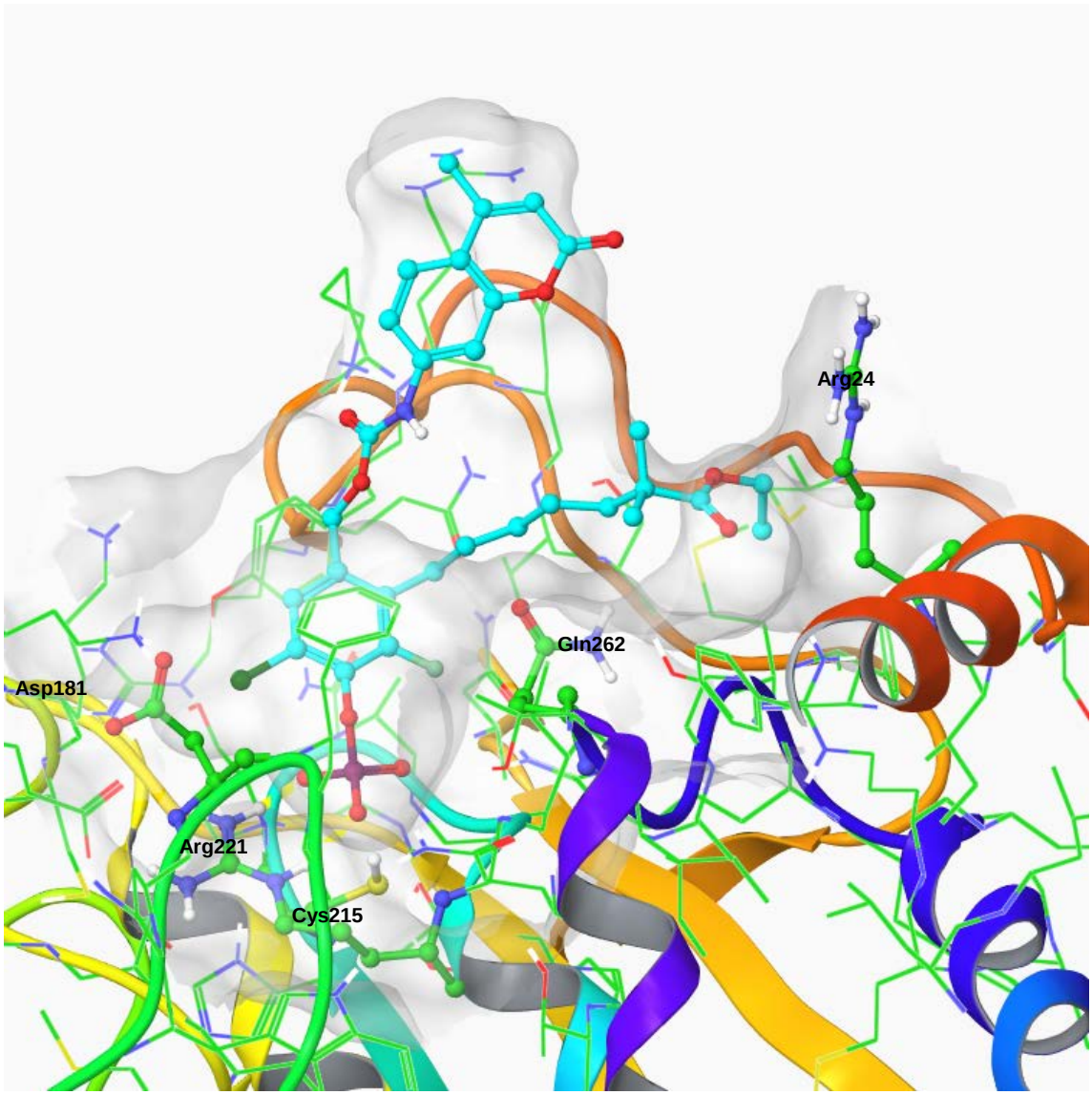
Compound **4d** docked into the active site of 2BGE



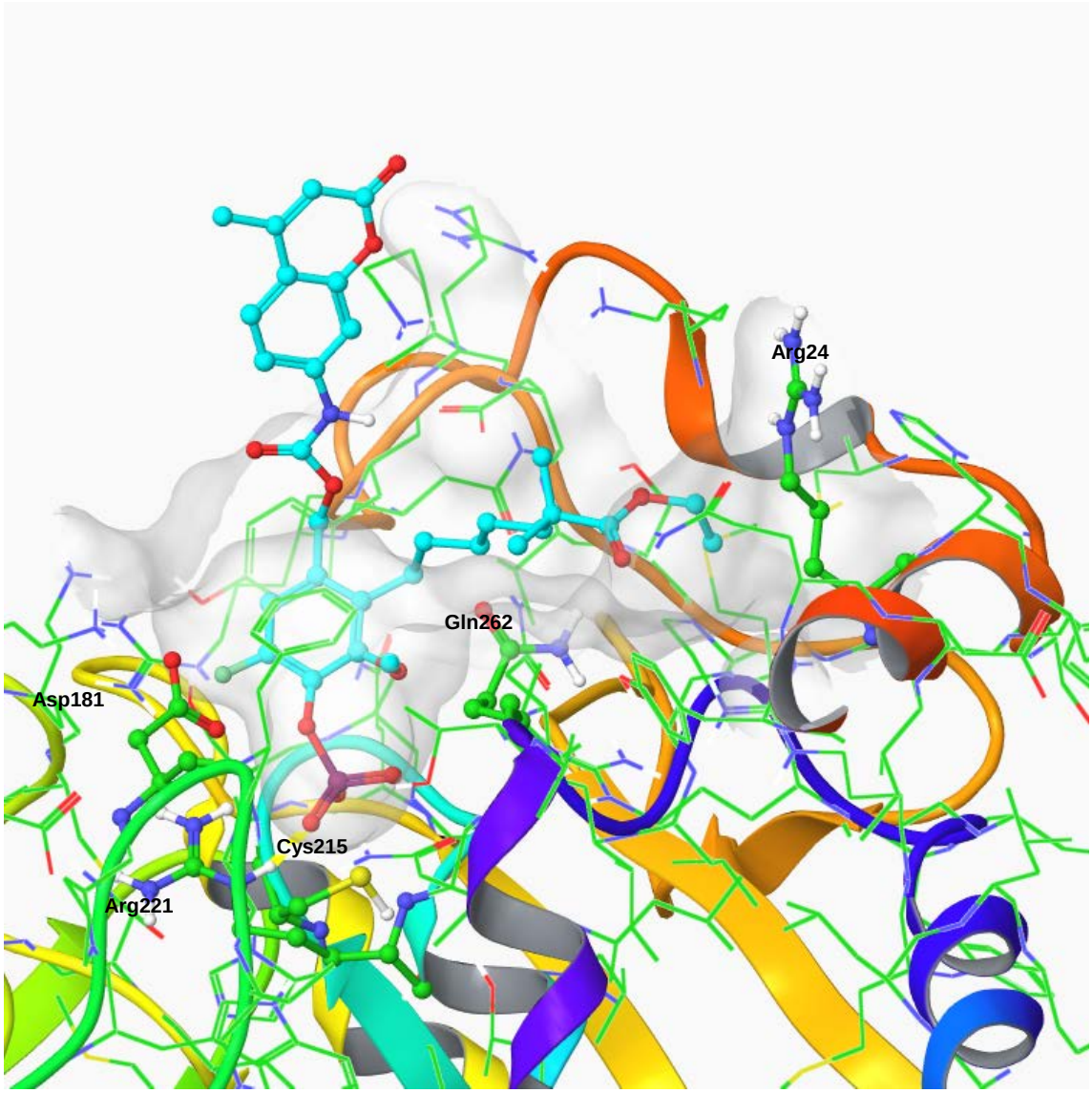
Compound **4e** docked into the active site of 2BGE



Compound **4g** docked into the active site of 2BGD

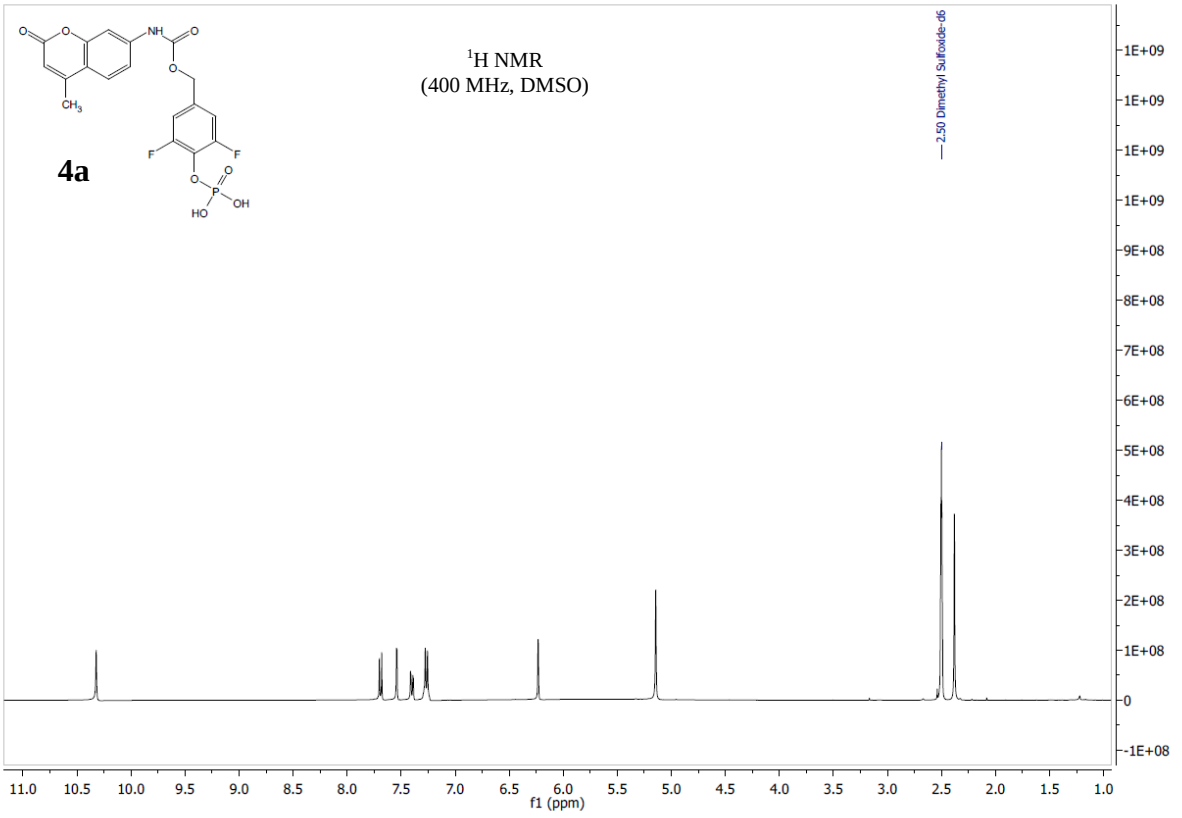


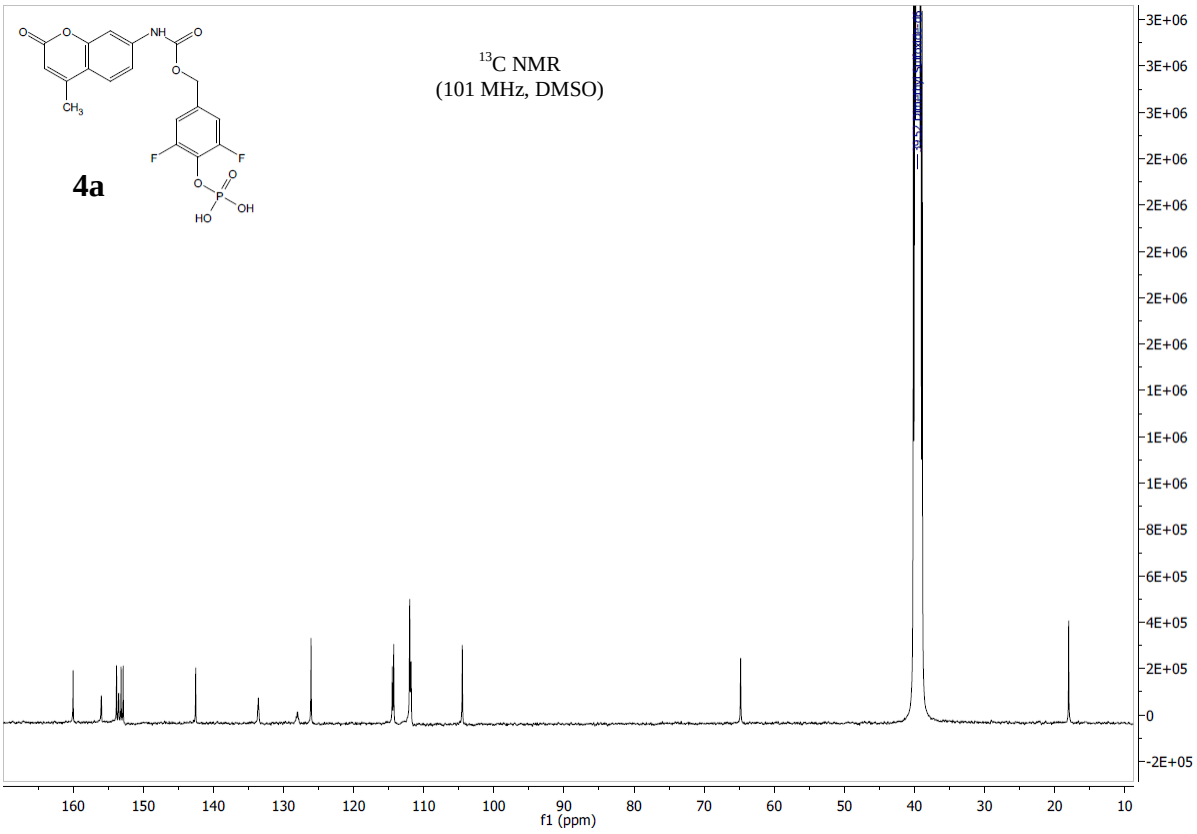
Compound **4i** docked into the active site of 2BGD

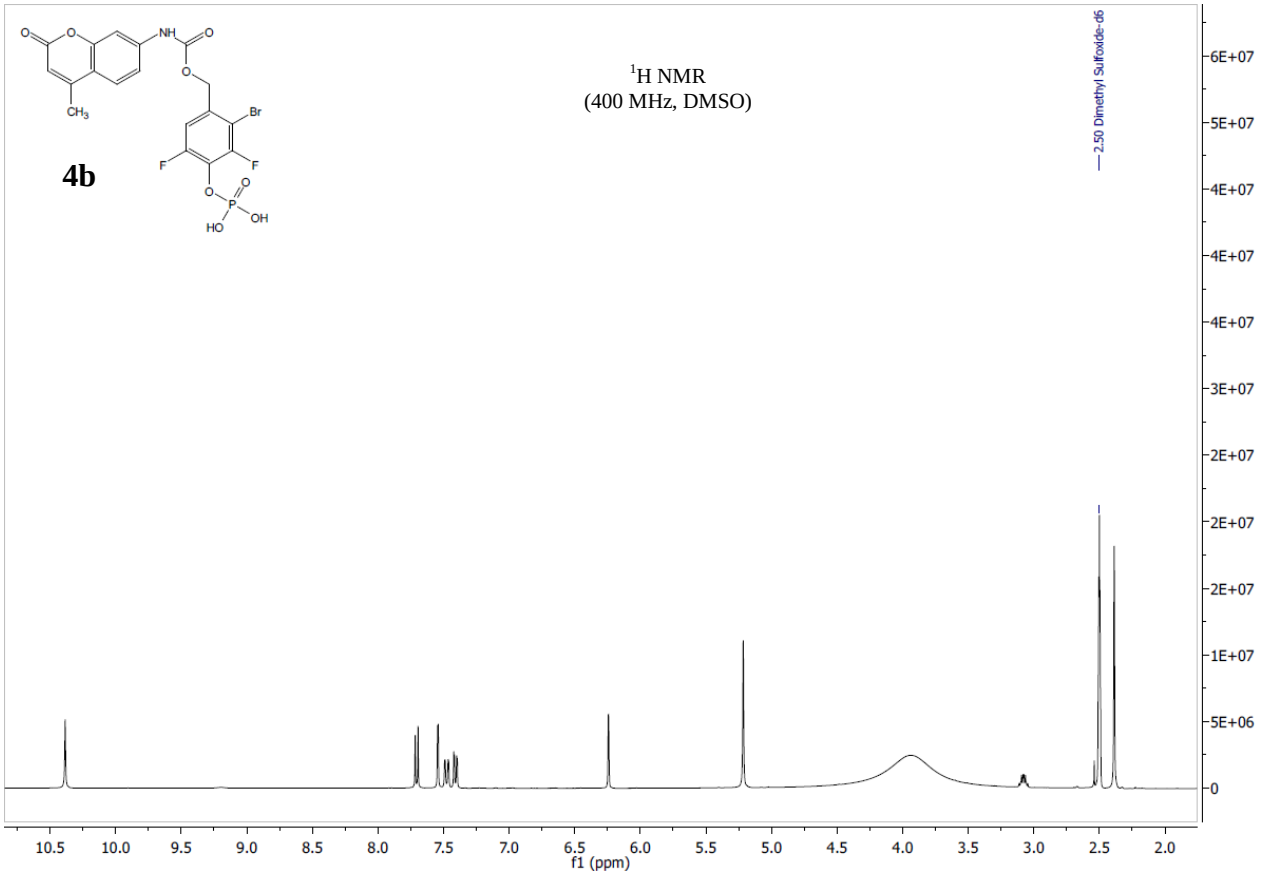


Compound **4j** docked into the active site of 2BGE

V. NMR spectra



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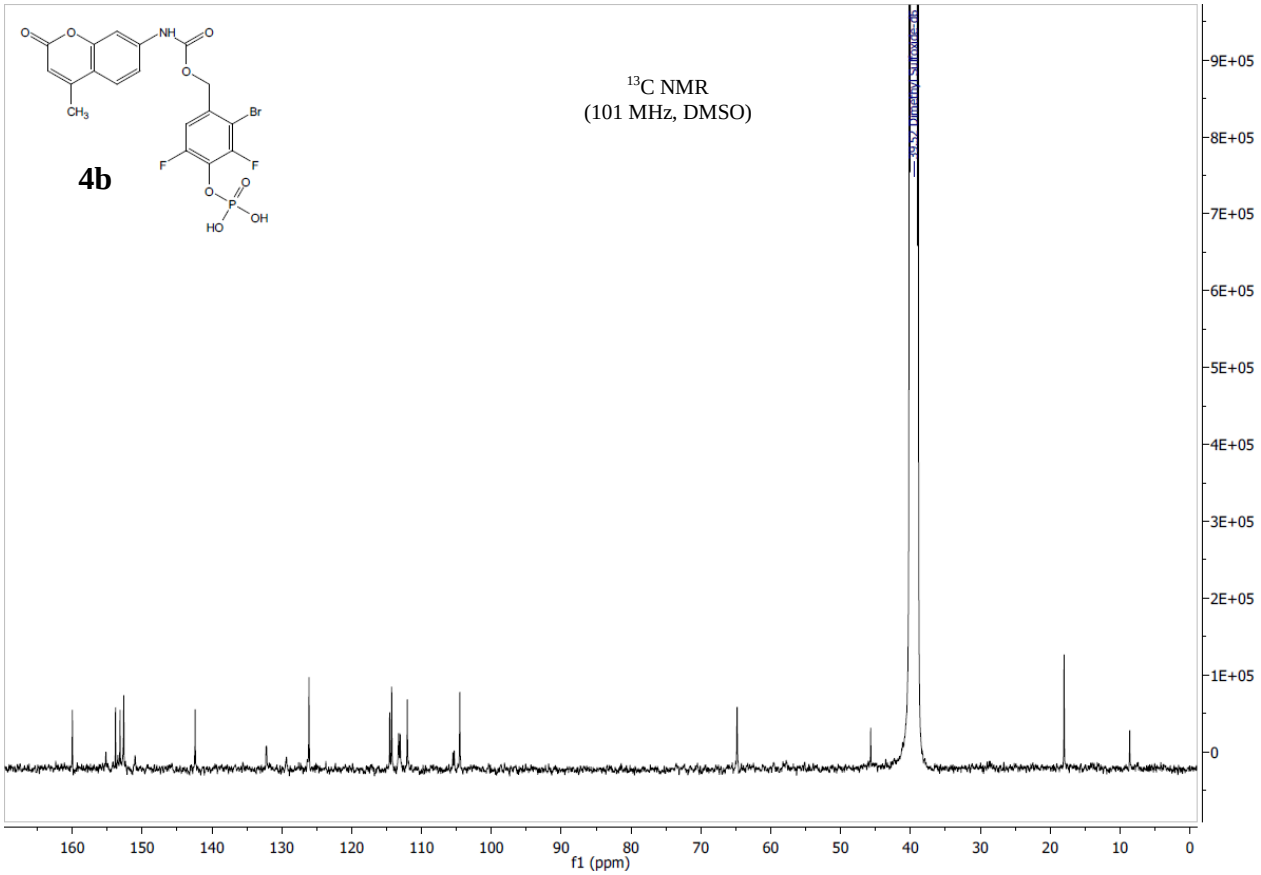
f1 (ppm)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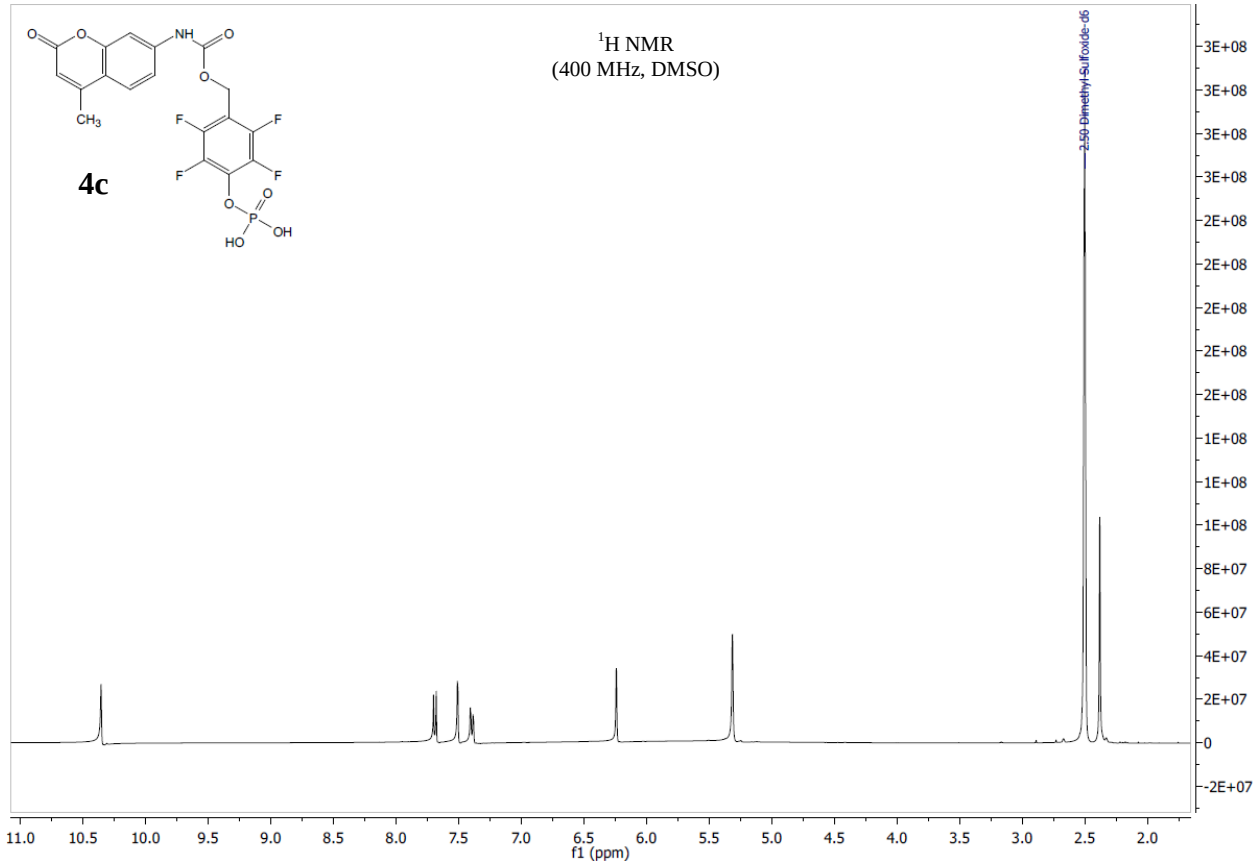
(101 MHz, DMSO)
f1 (ppm)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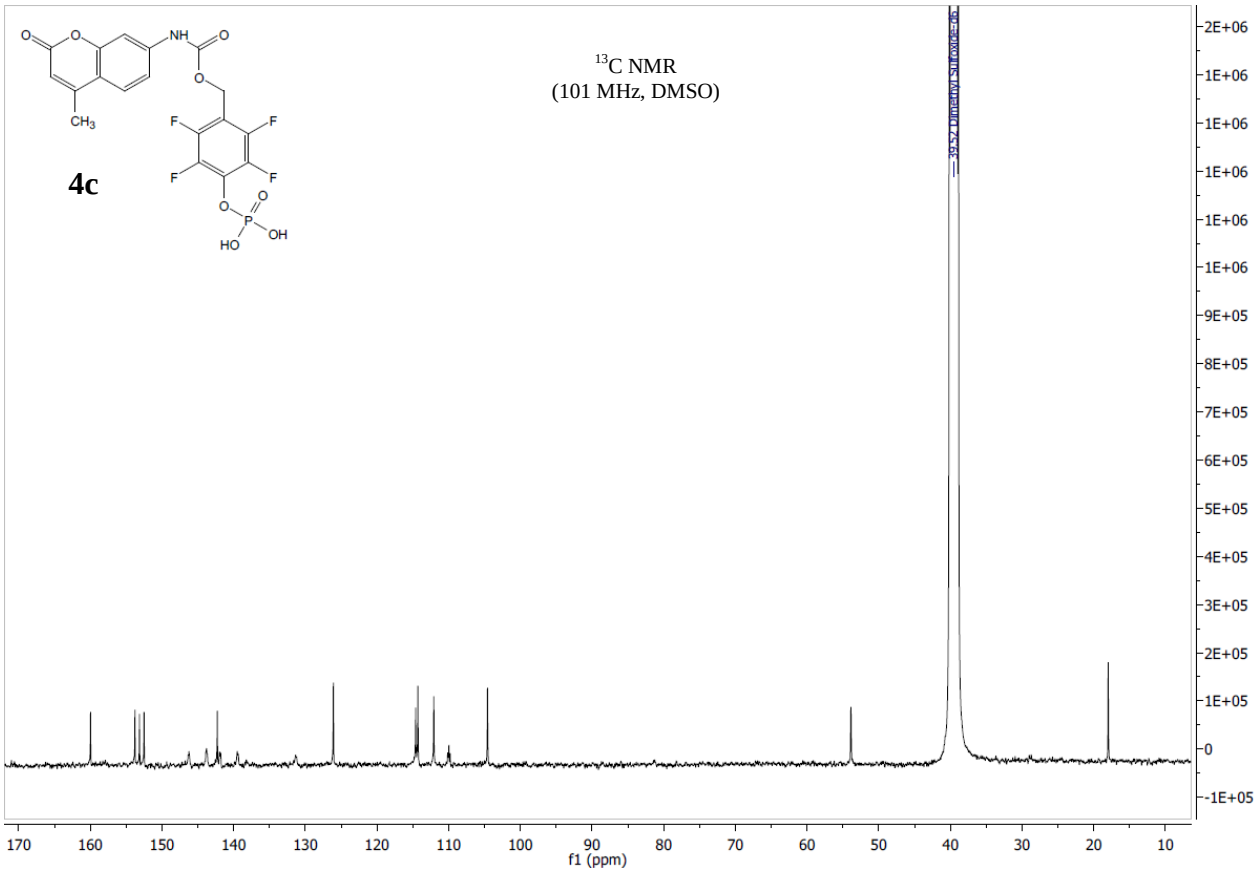
(400 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)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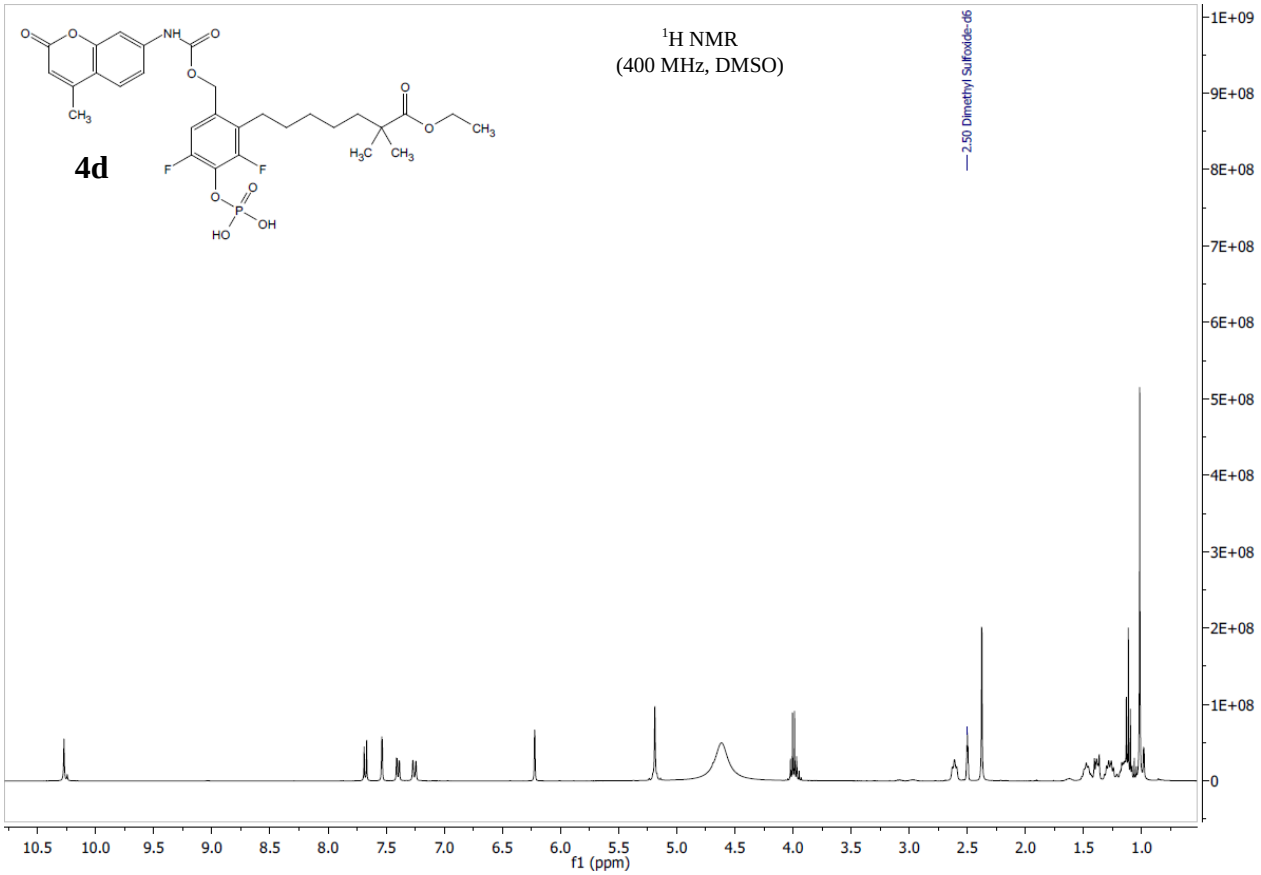
39.52 Dimethyl Sulfoxide-d6
f1 (ppm)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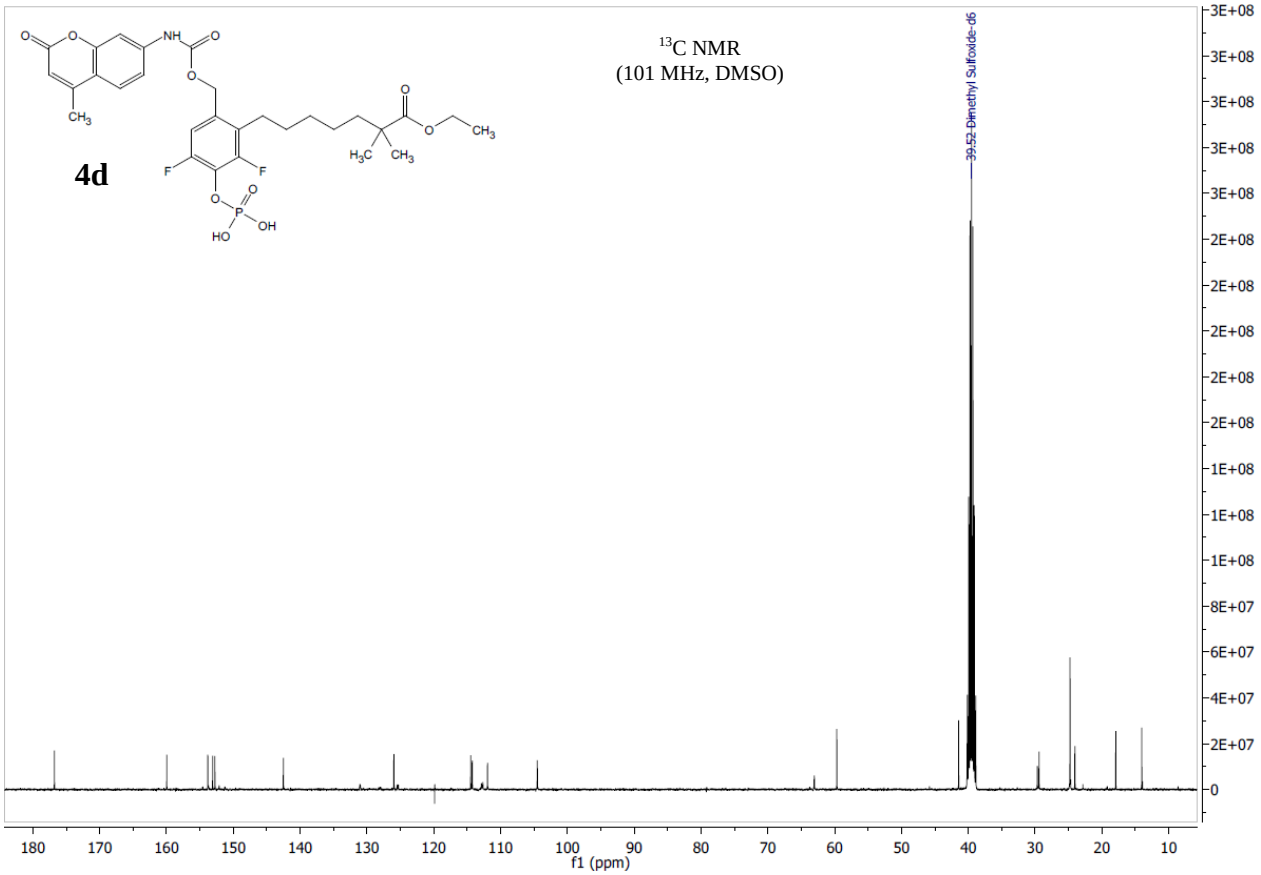
(400 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)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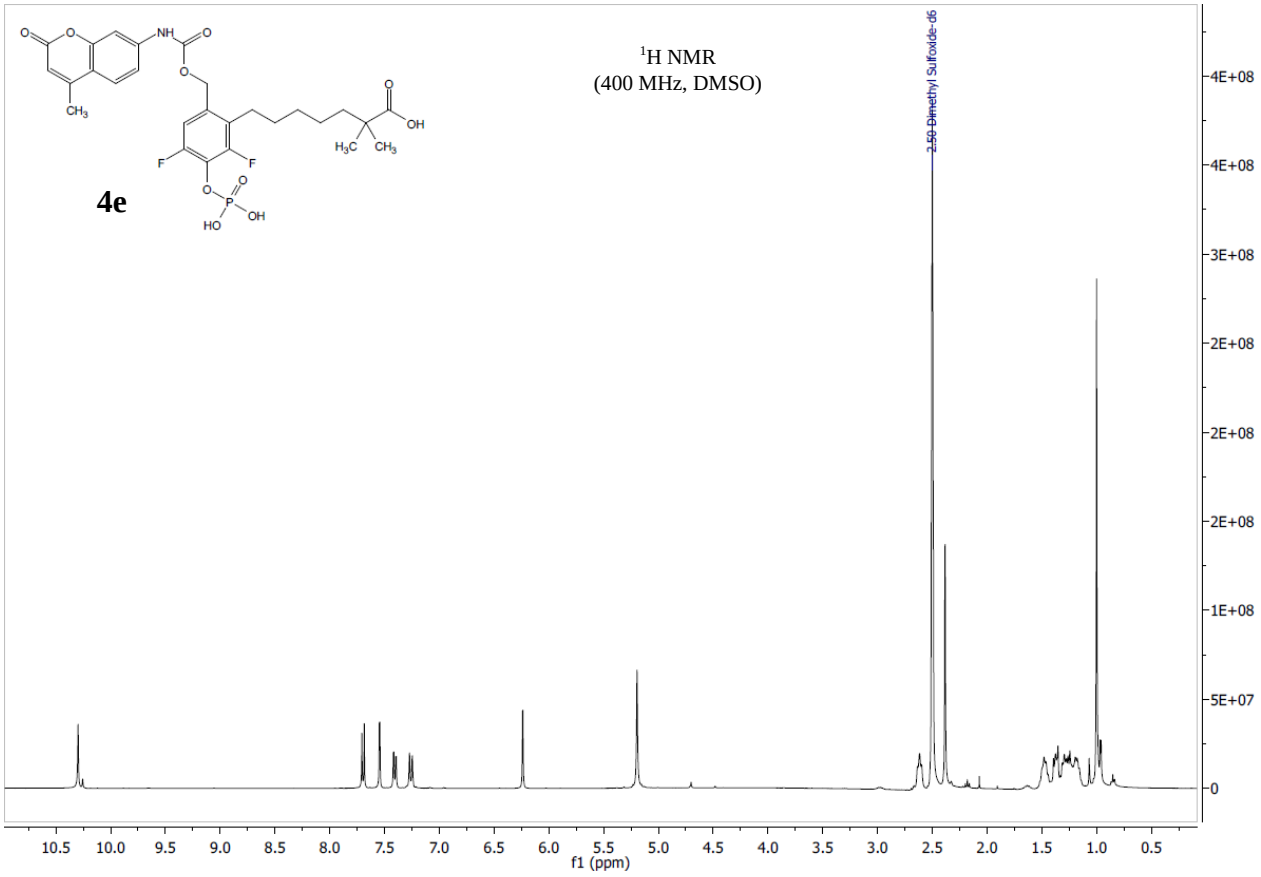
^{13}C NMR
(101 MHz, DMSO)
39.52 Dimethyl Sulfoxide-d6
f1 (ppm)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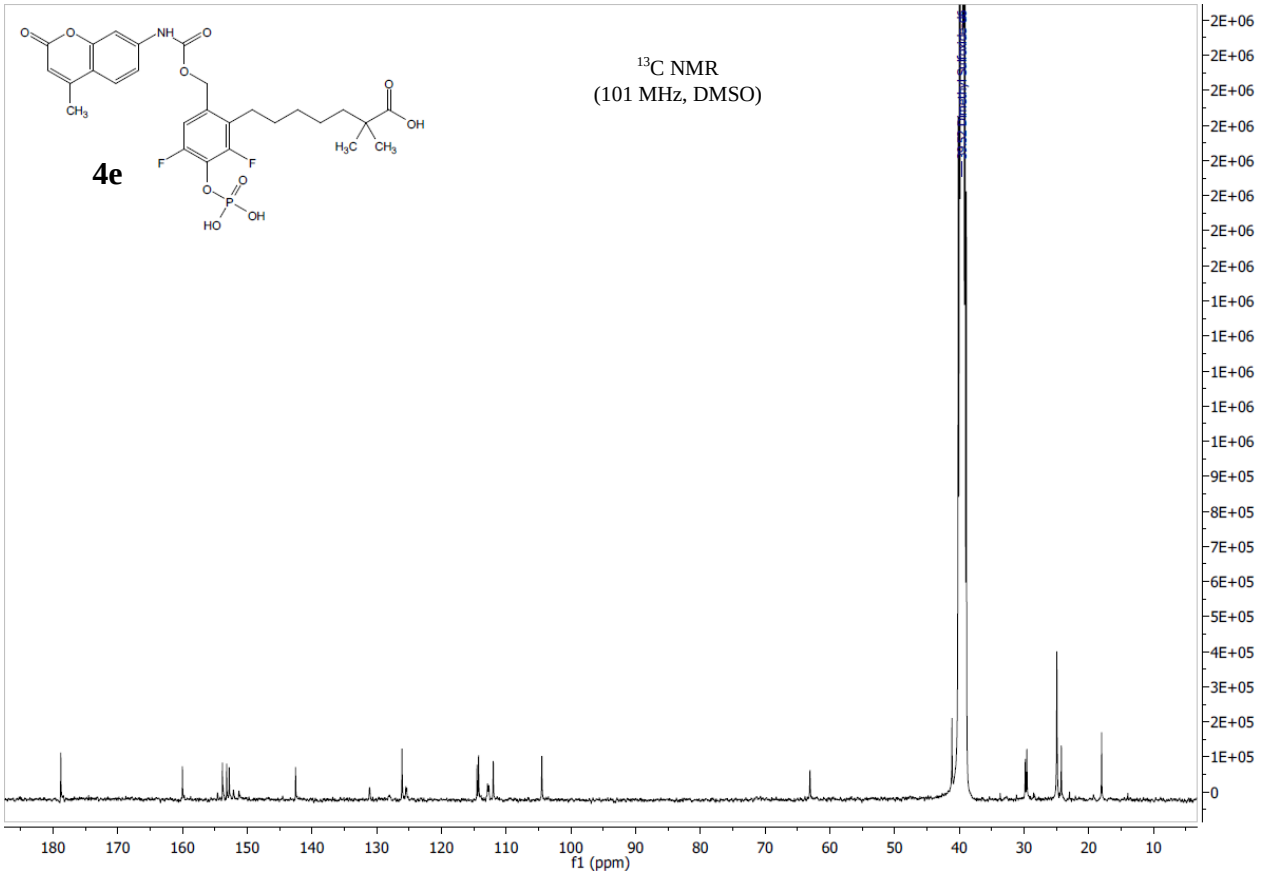
(400 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)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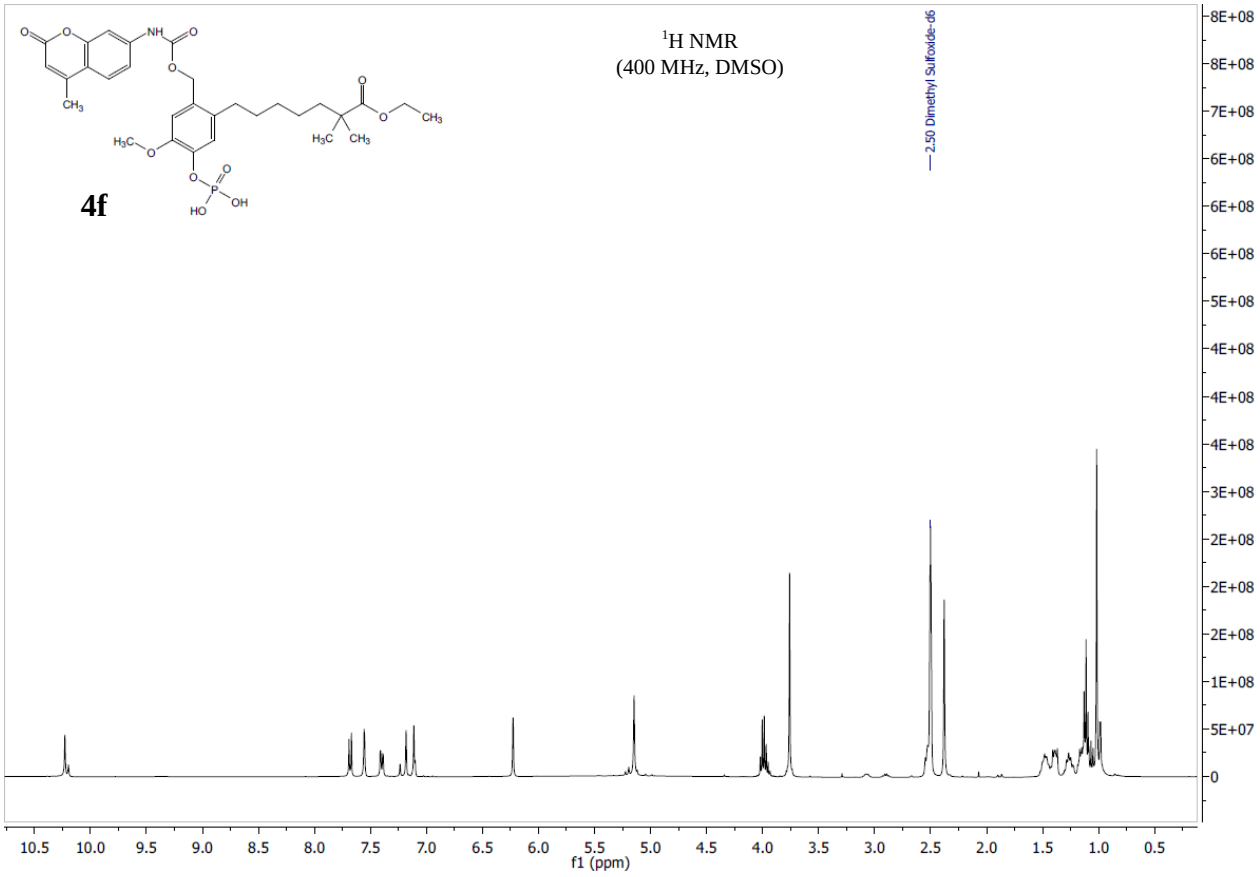
^{13}C NMR
(101 MHz, DMSO)
f1 (ppm)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(400 MHz, DMSO)
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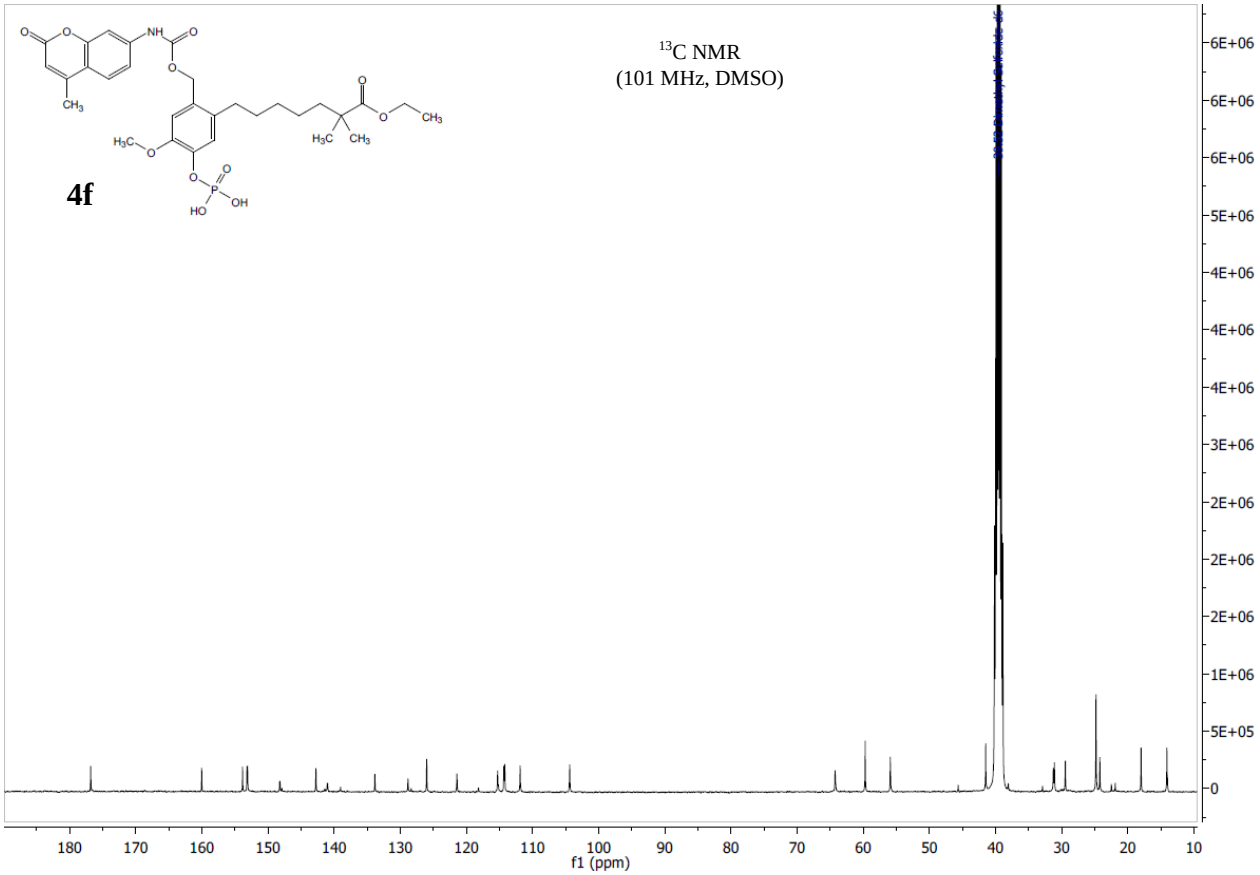
f1 (ppm)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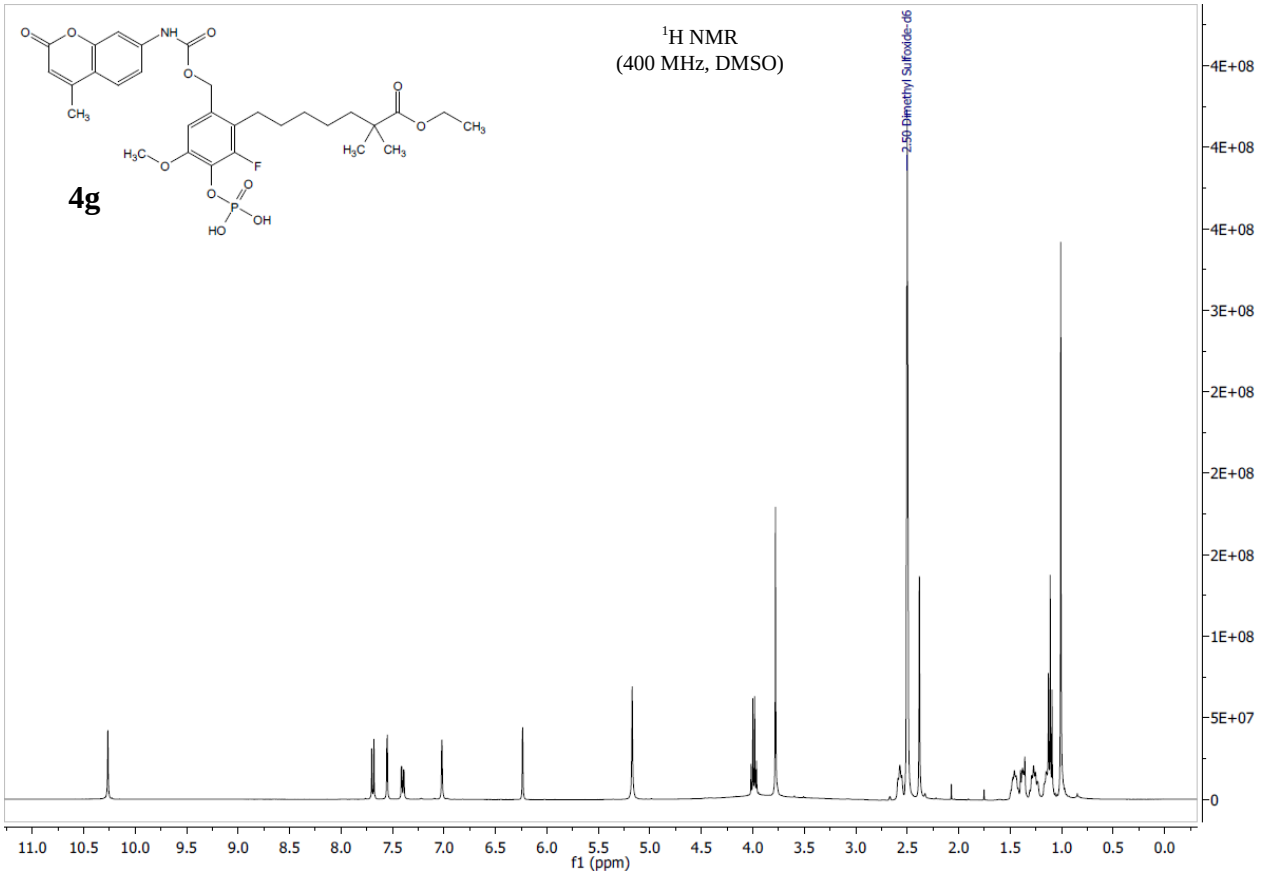
^{13}C NMR
(101 MHz, DMSO)
f1 (ppm)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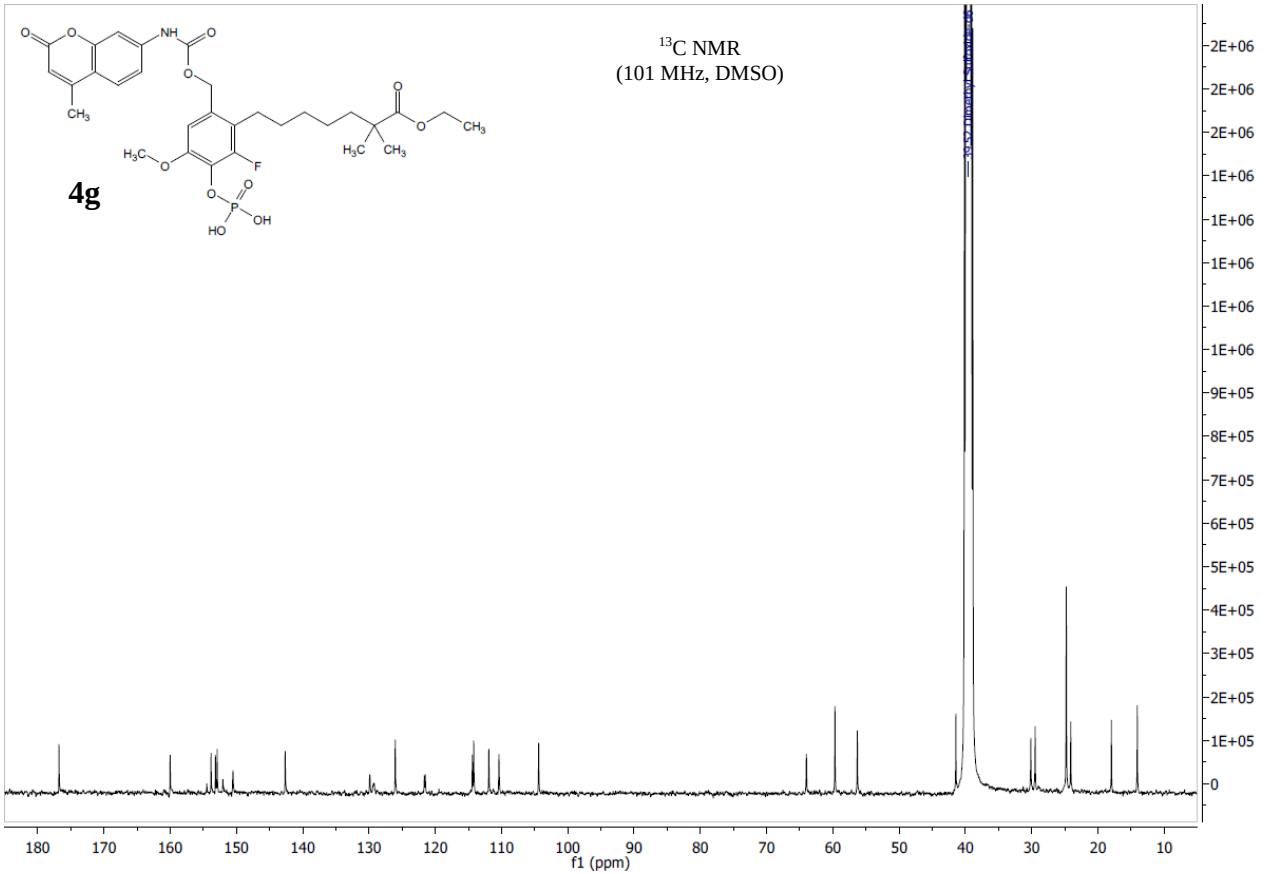
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(101 MHz, DMSO)
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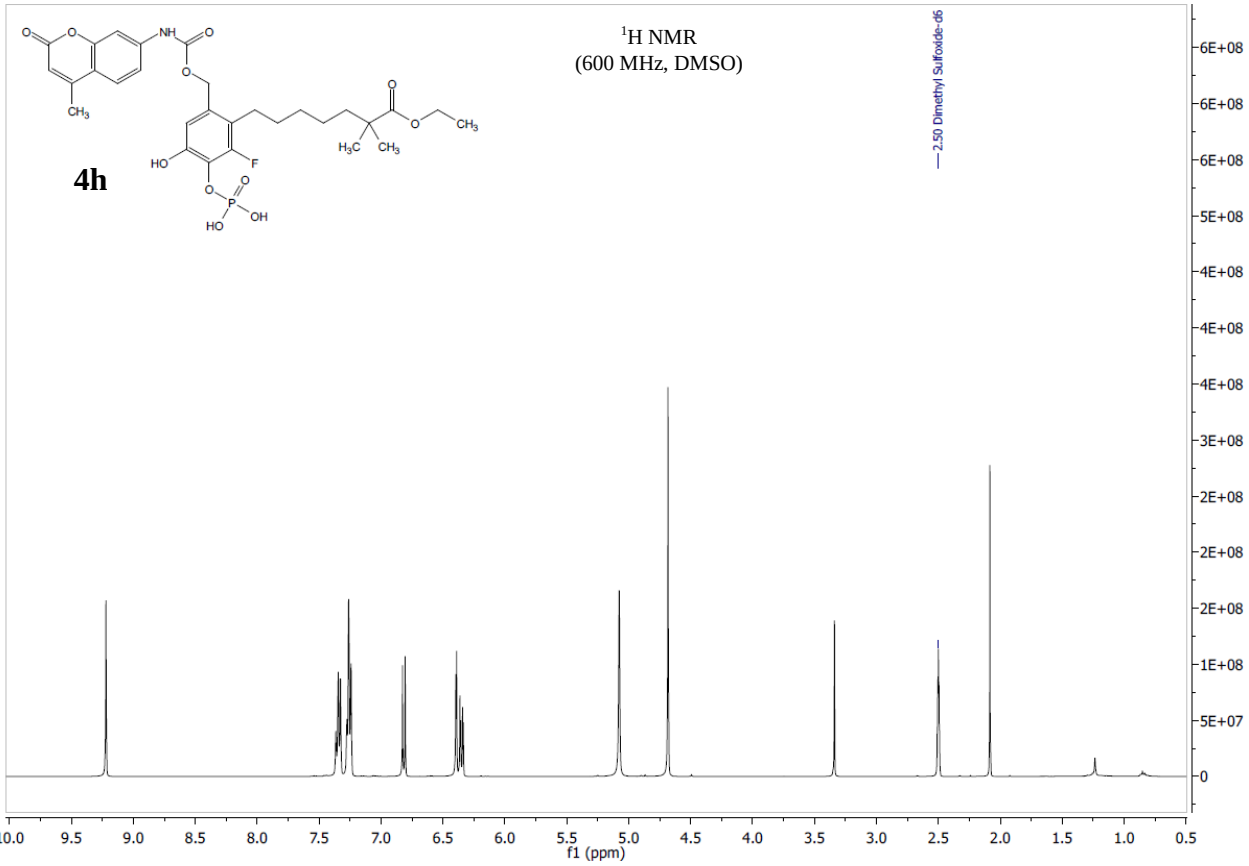
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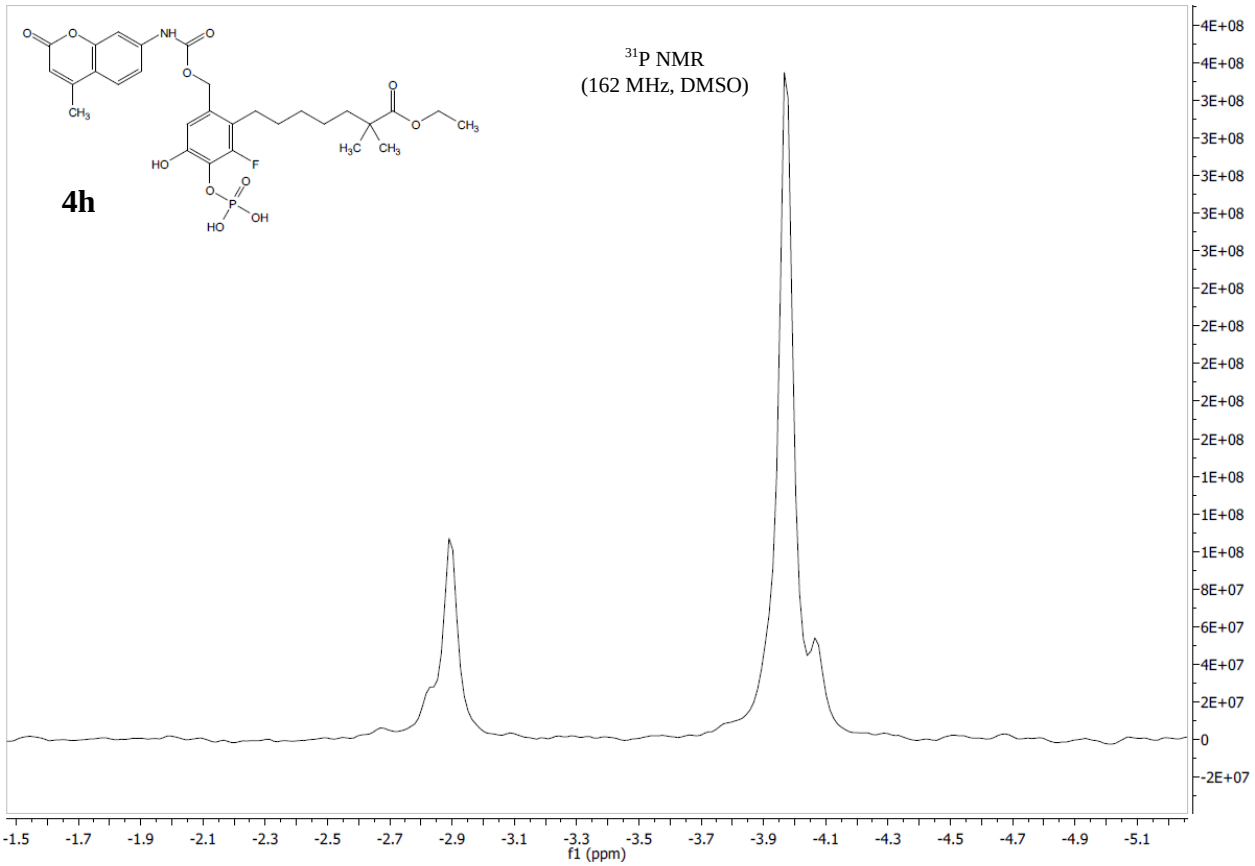
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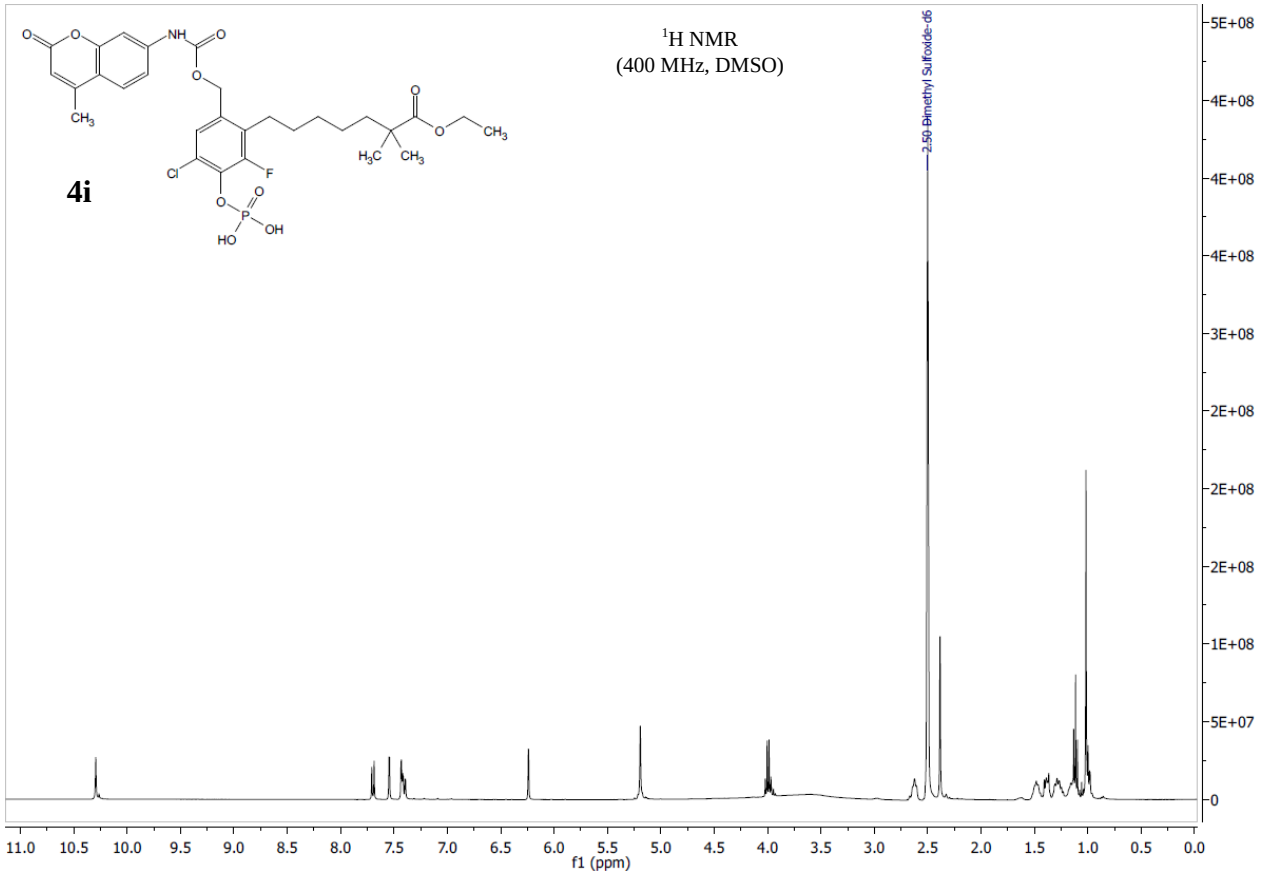
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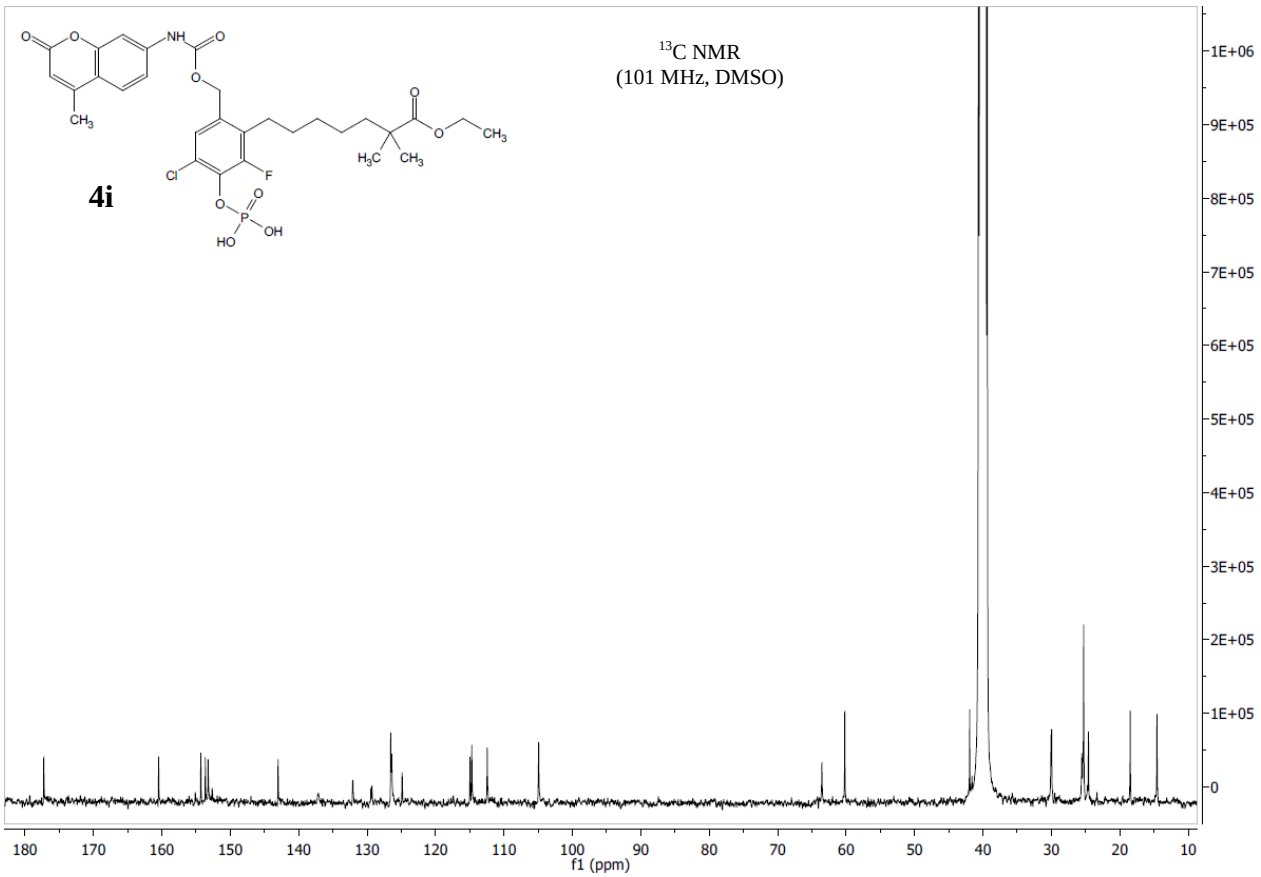
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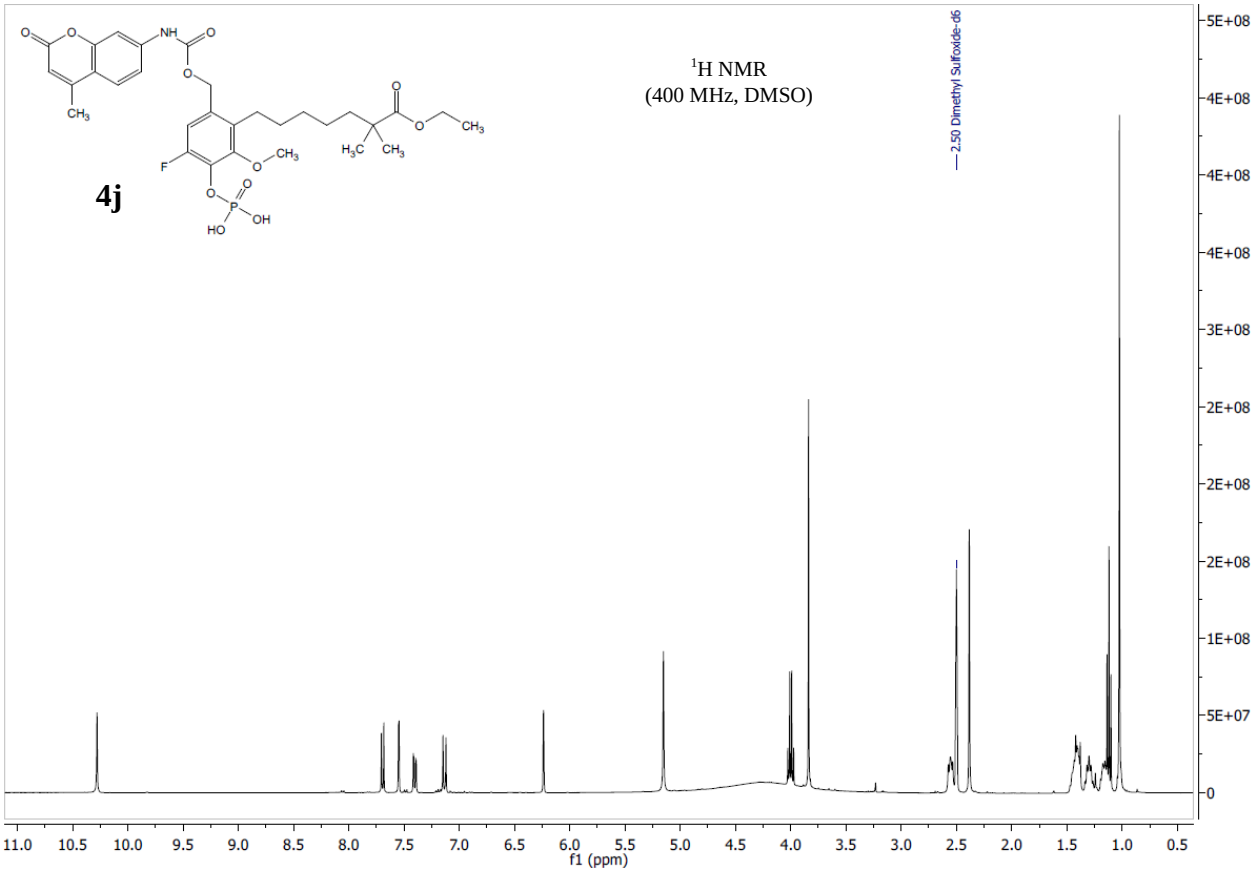
f1 (ppm)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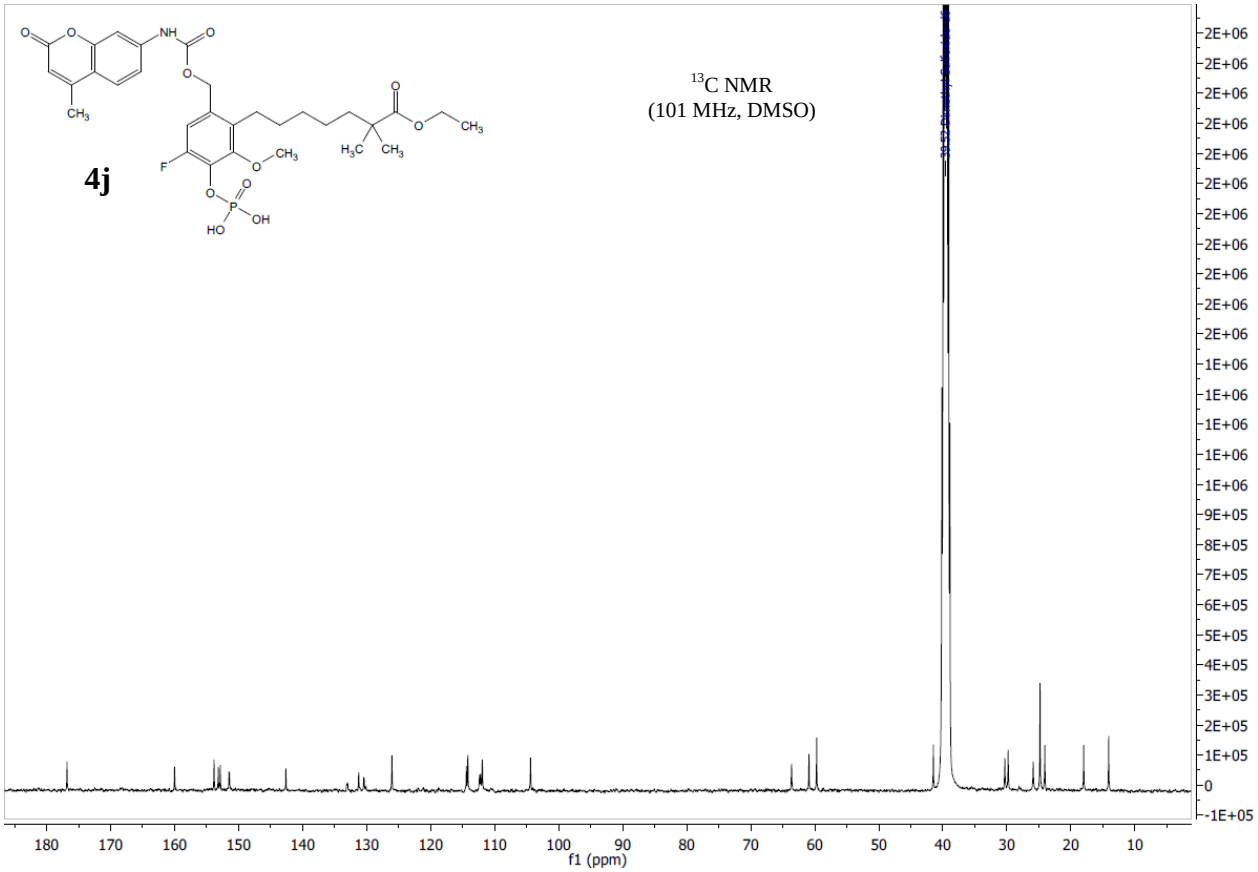
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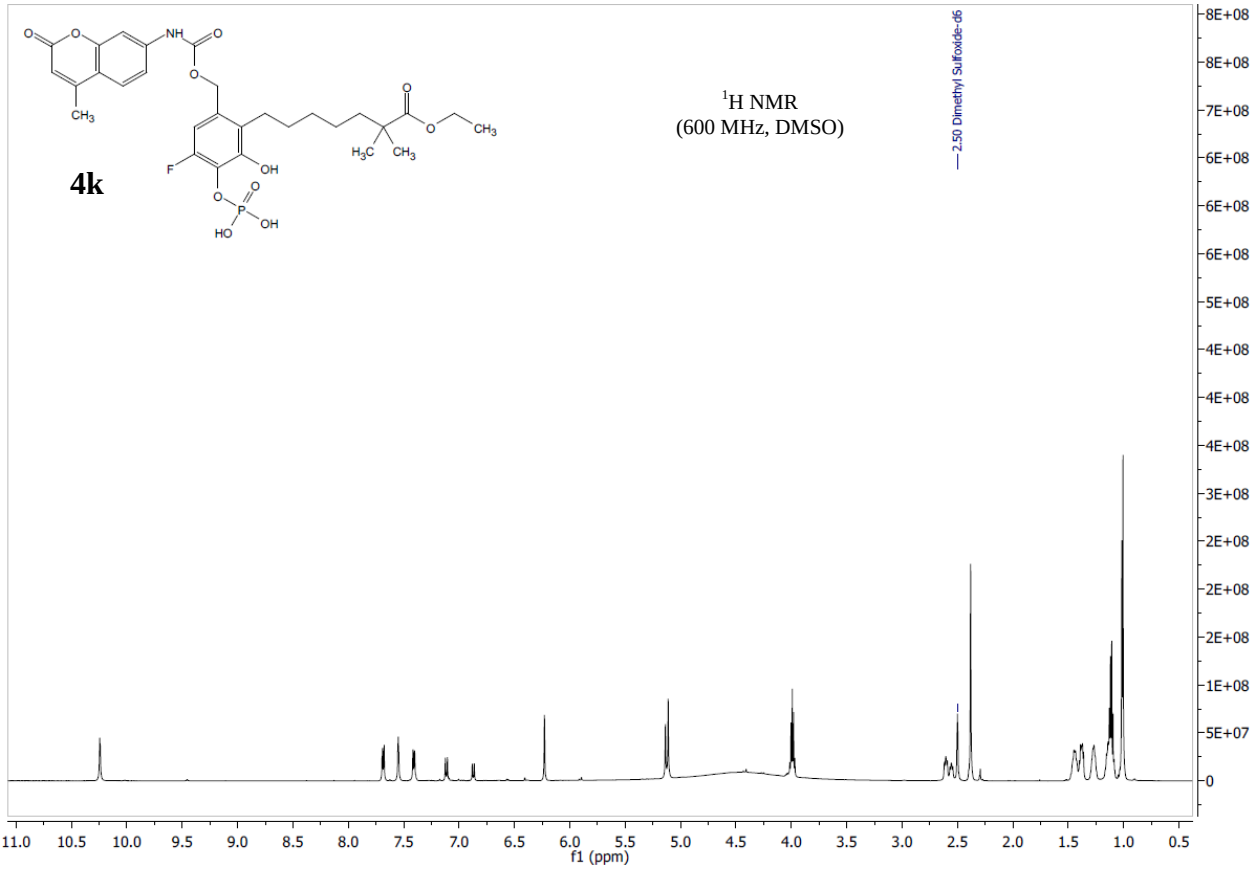
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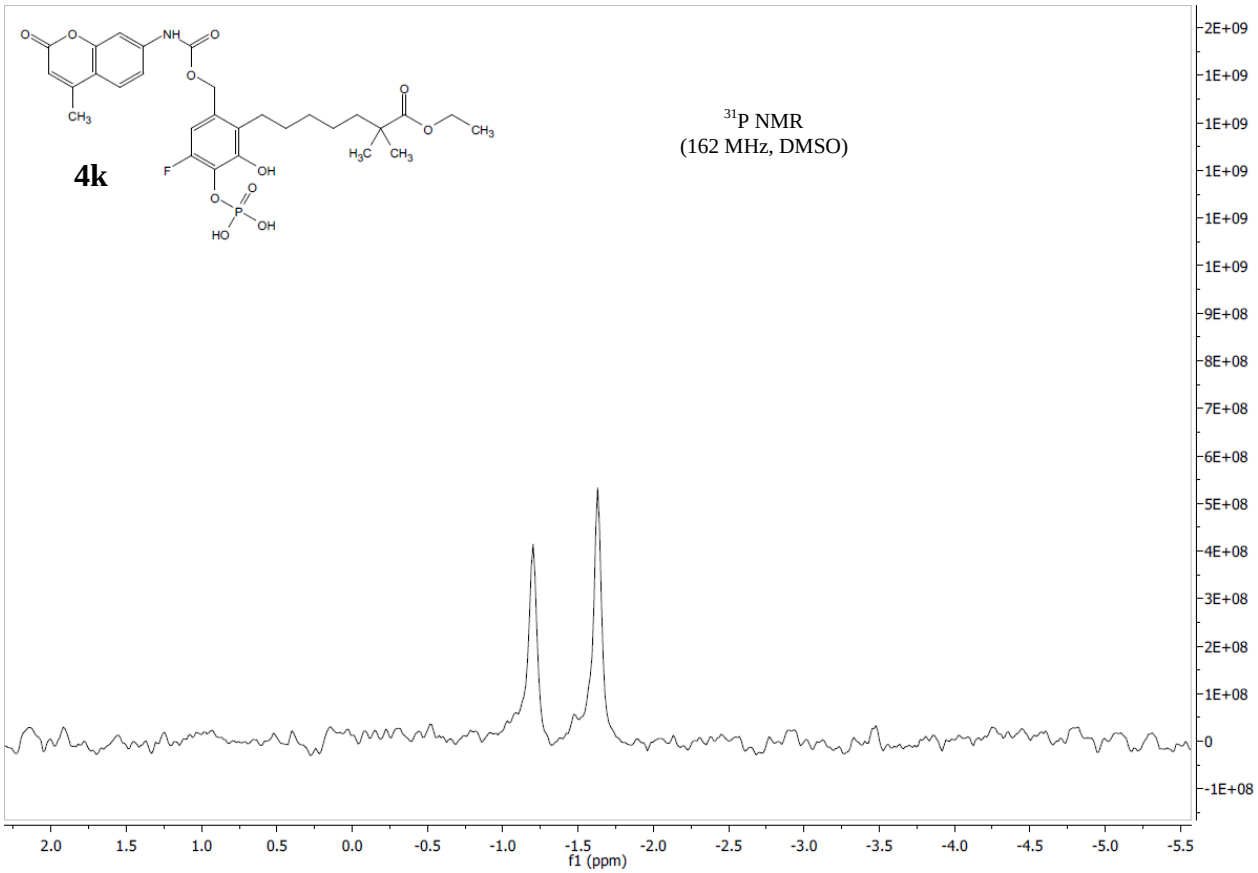
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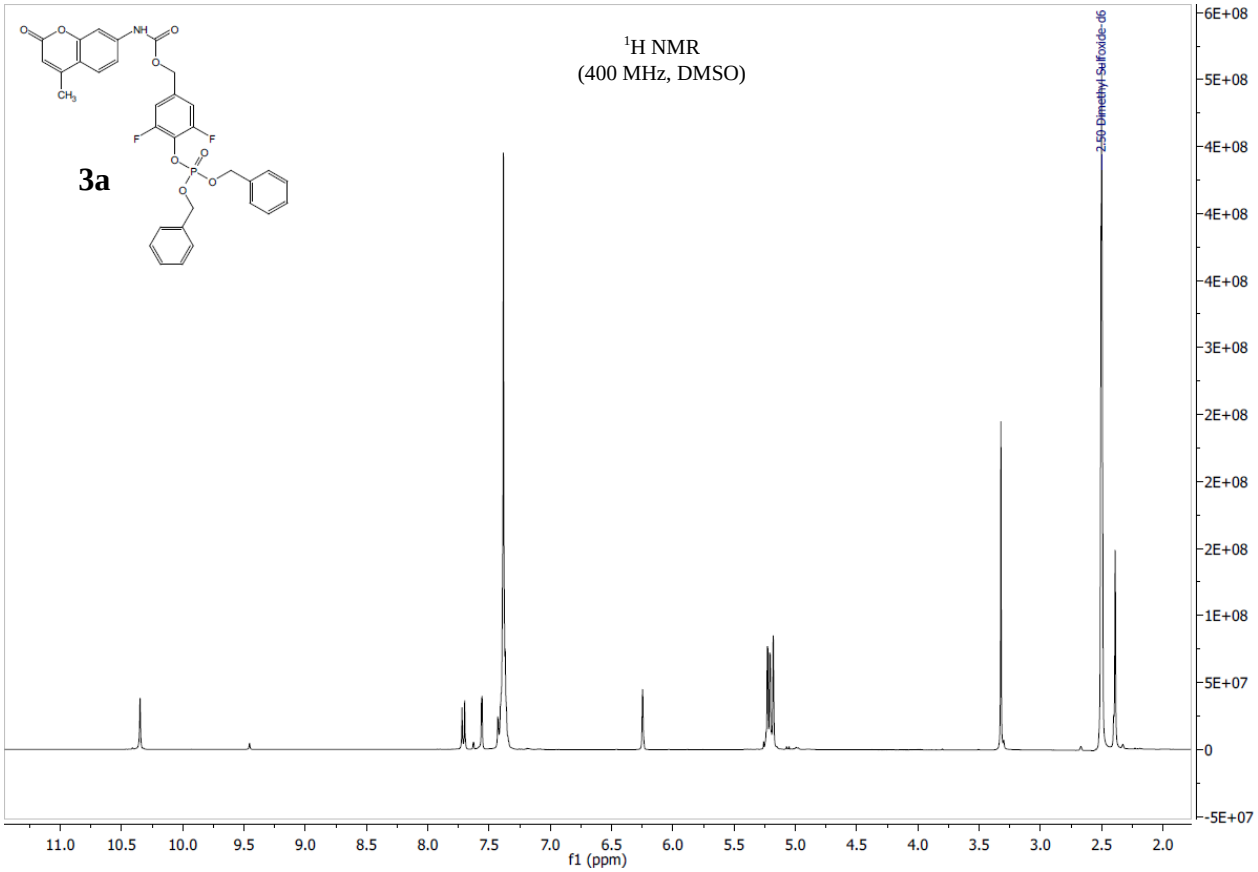
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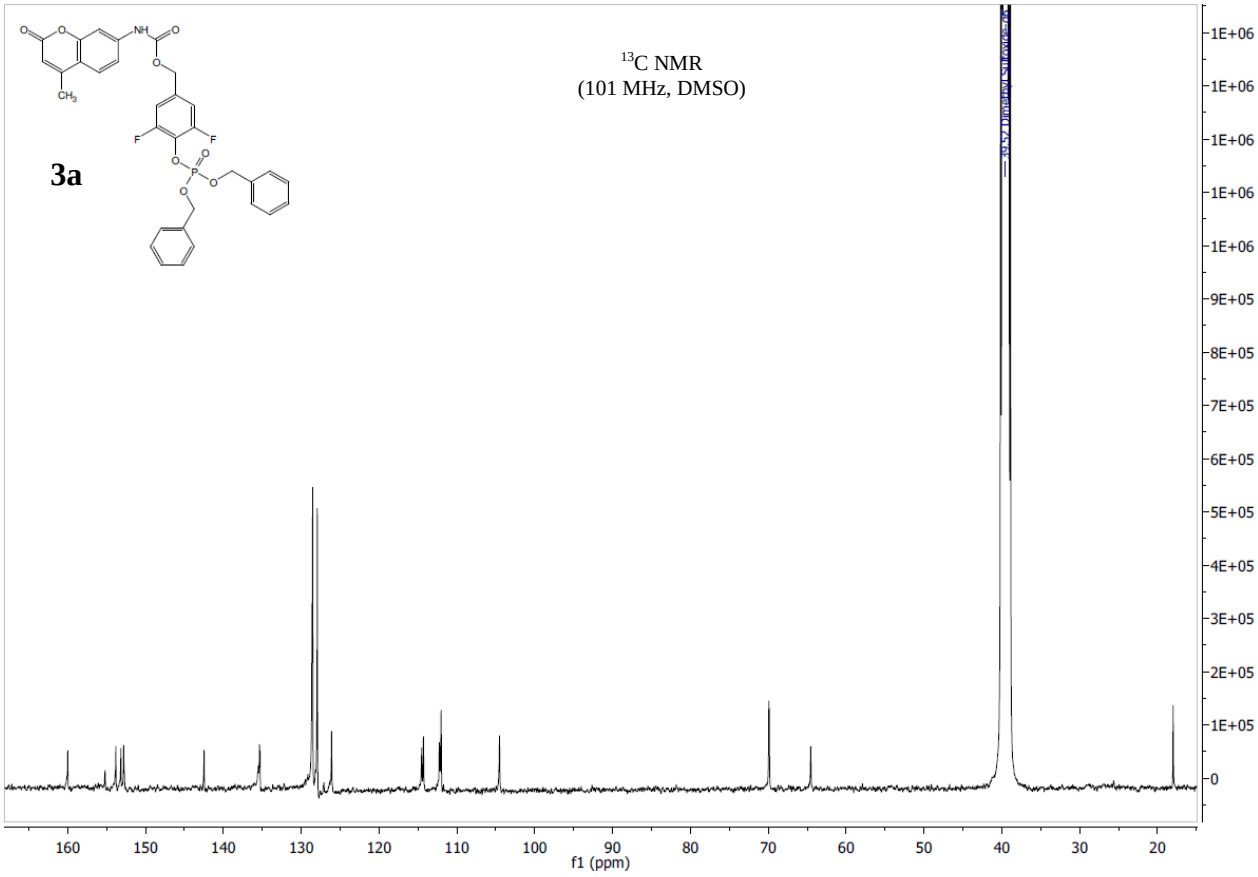
f1 (ppm)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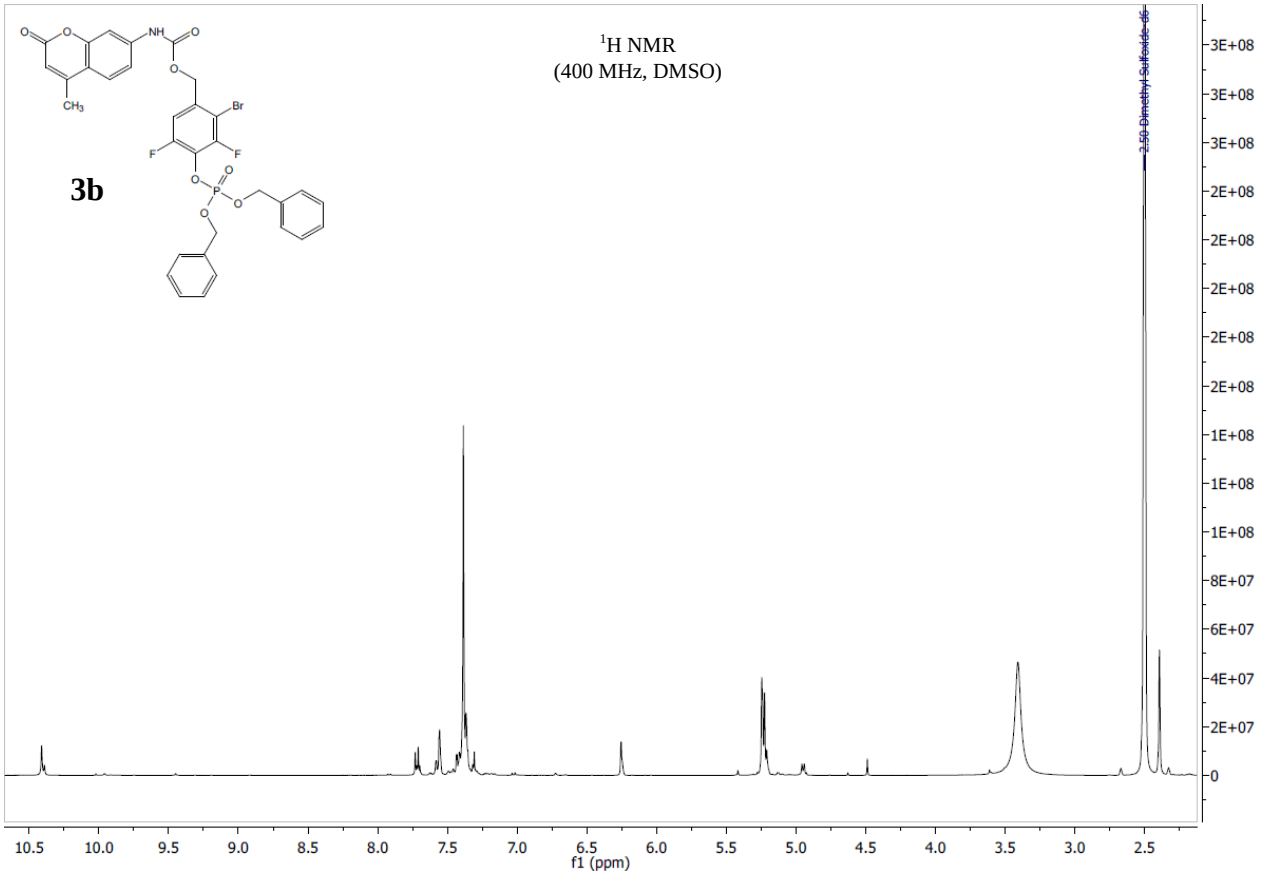
f1 (ppm)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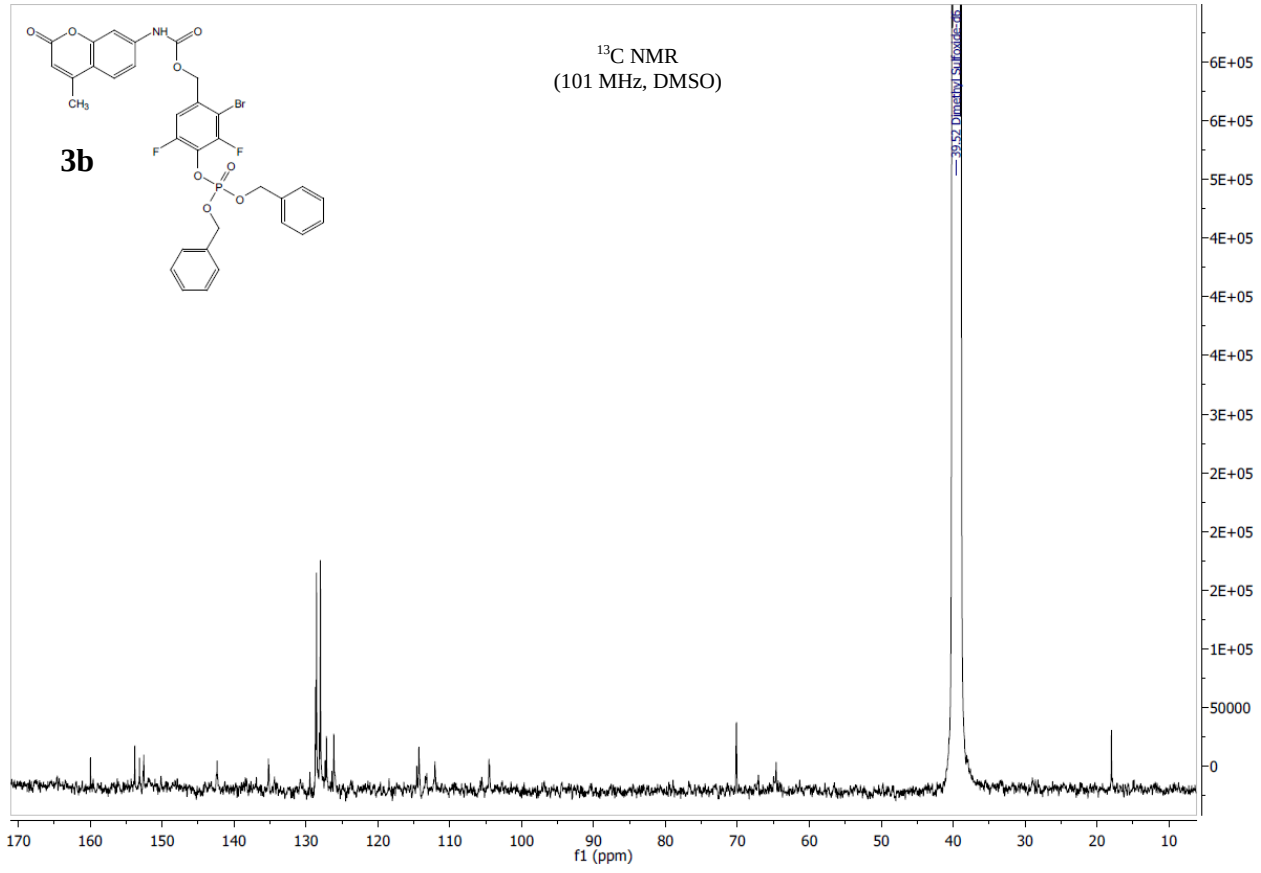
(400 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)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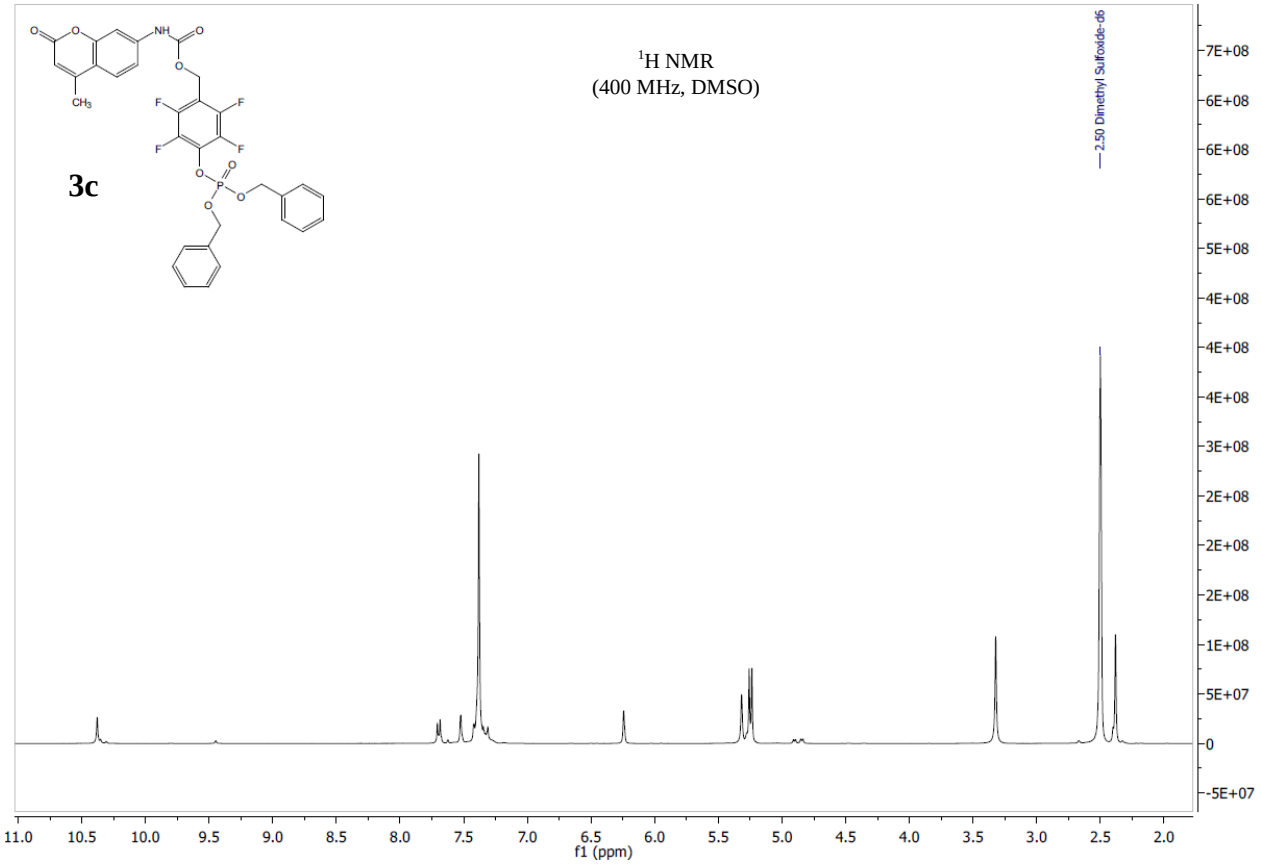
(101 MHz, DMSO)
39.52 Dimethyl Sulfoxide-d6
f1 (ppm)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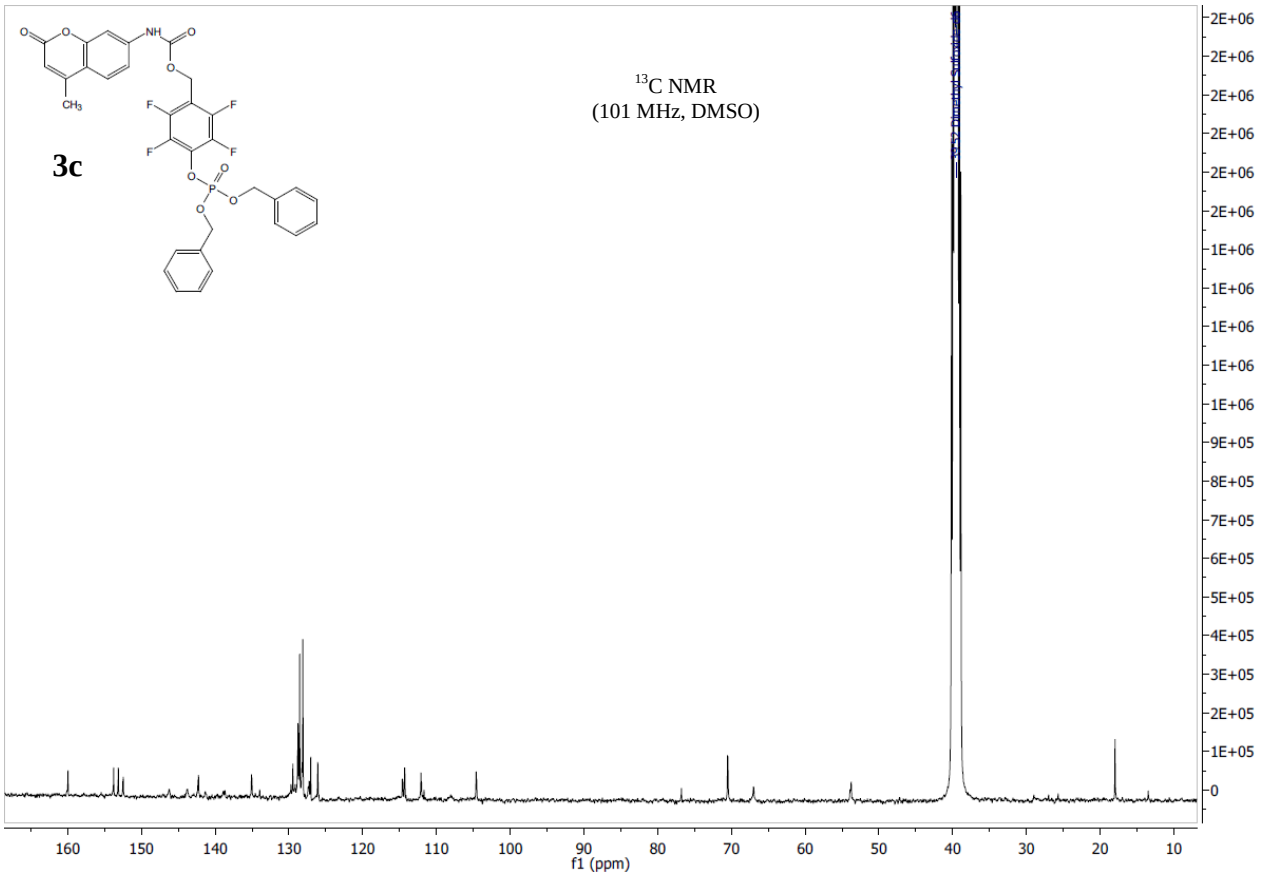
(400 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)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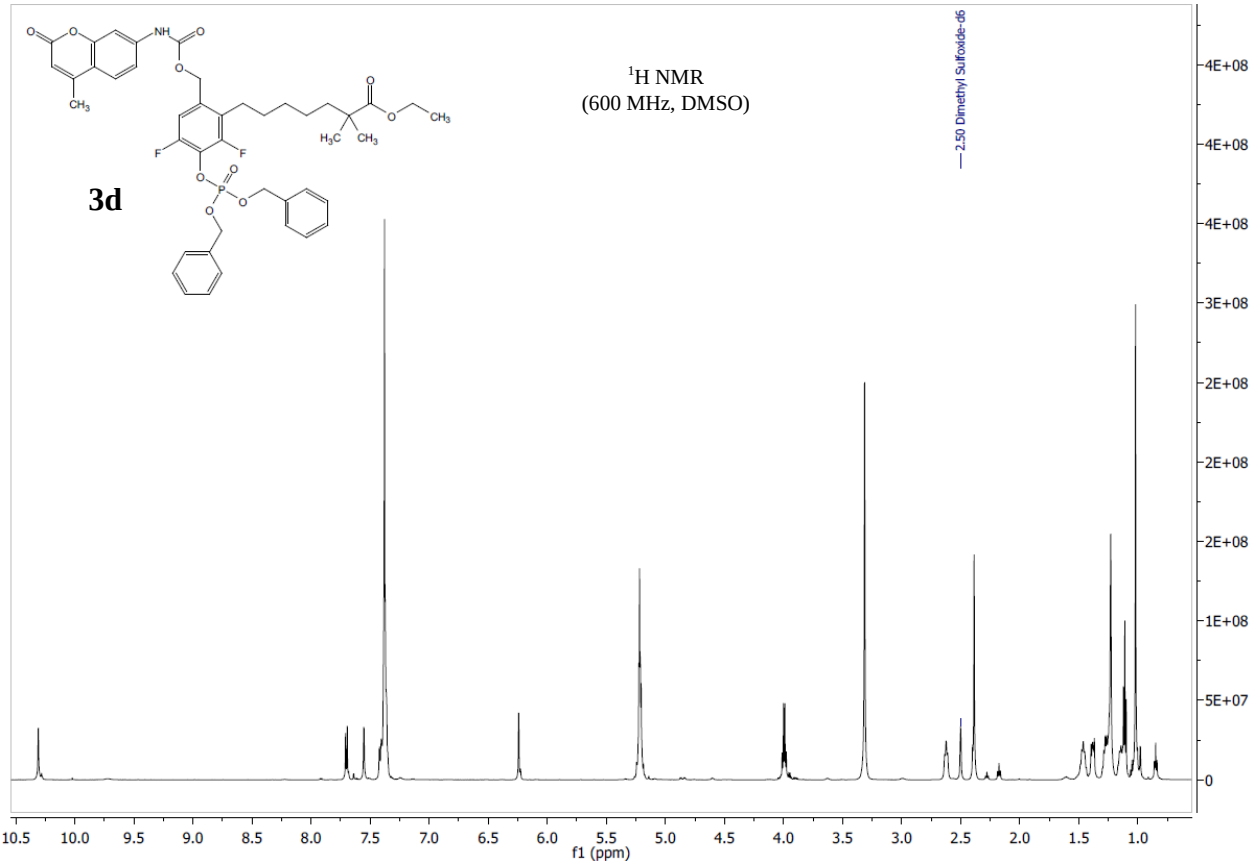
(101 MHz, DMSO)
f1 (ppm)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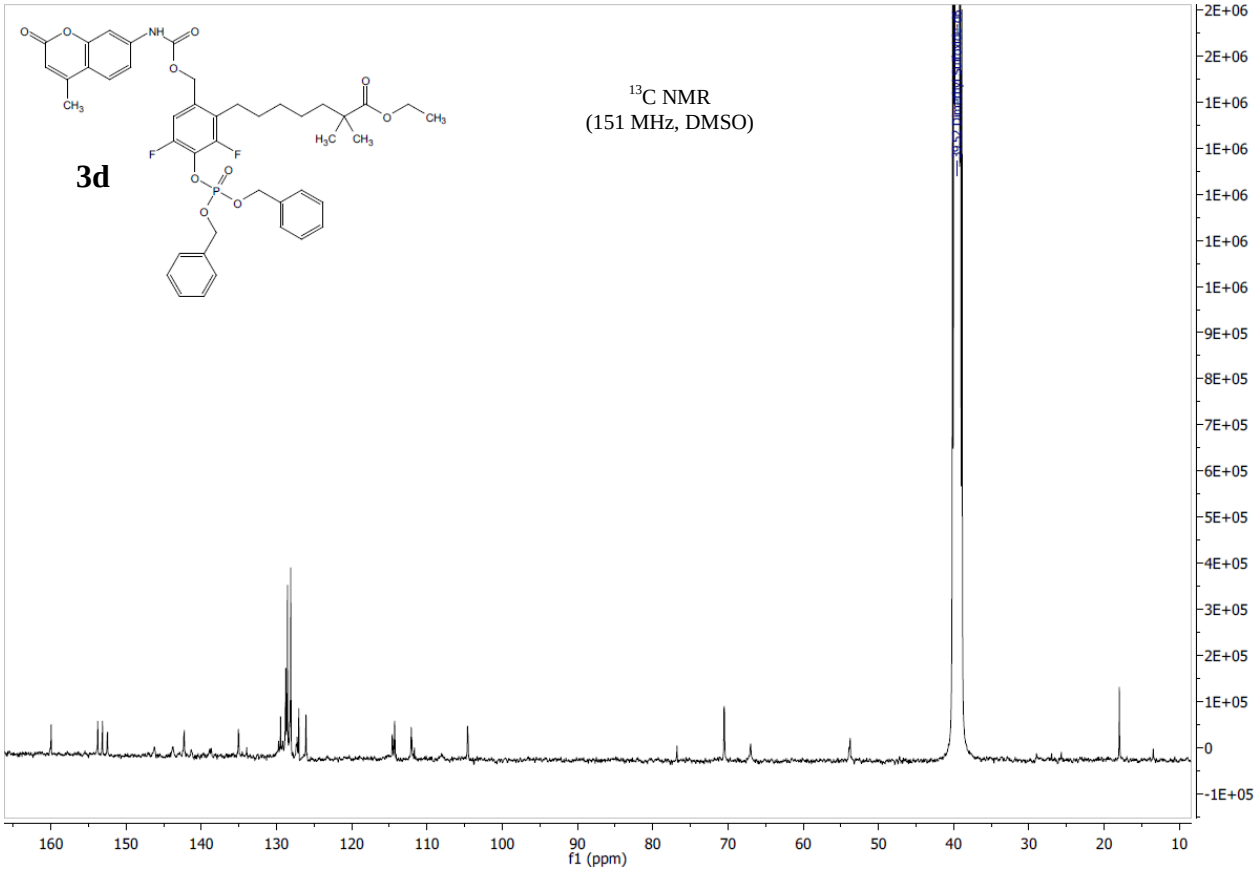
(600 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)


3d
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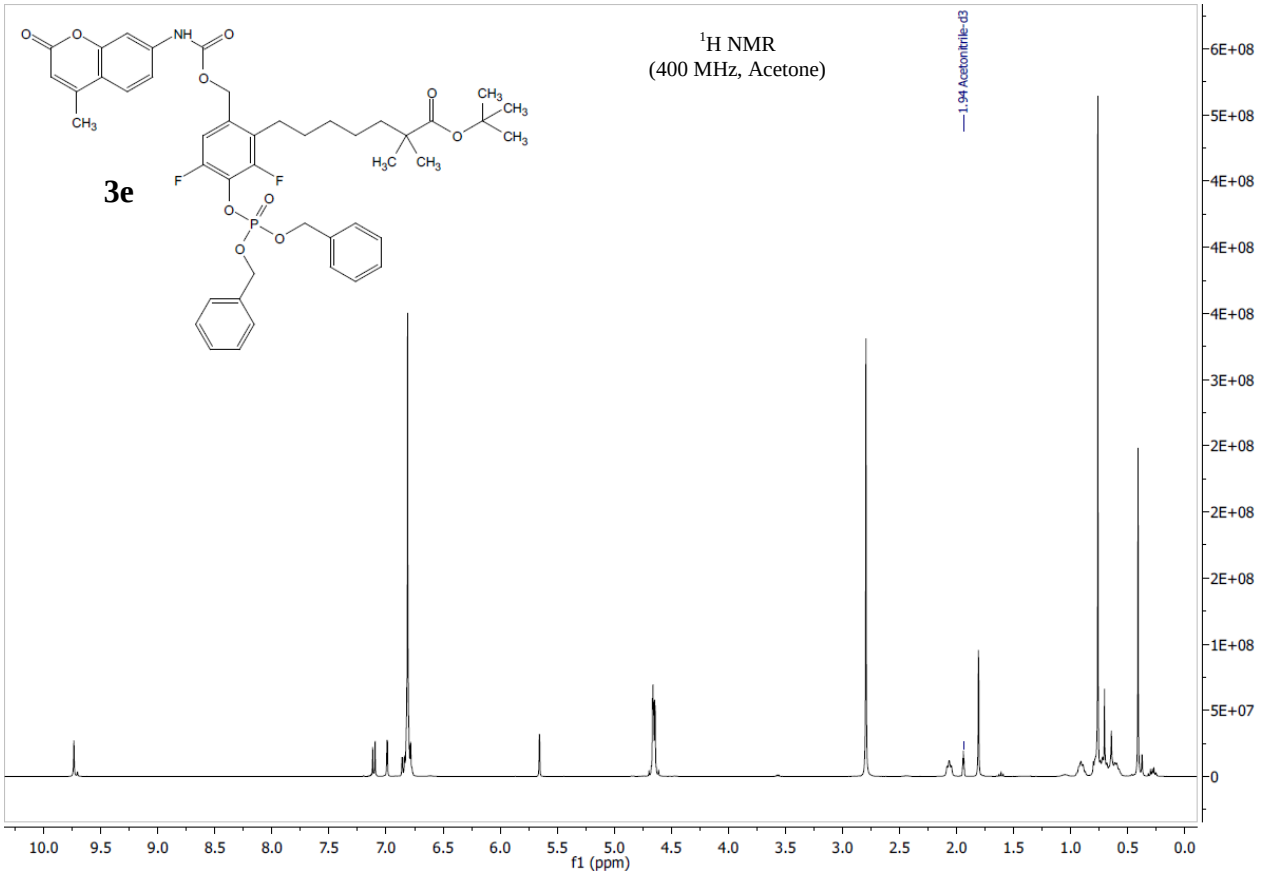
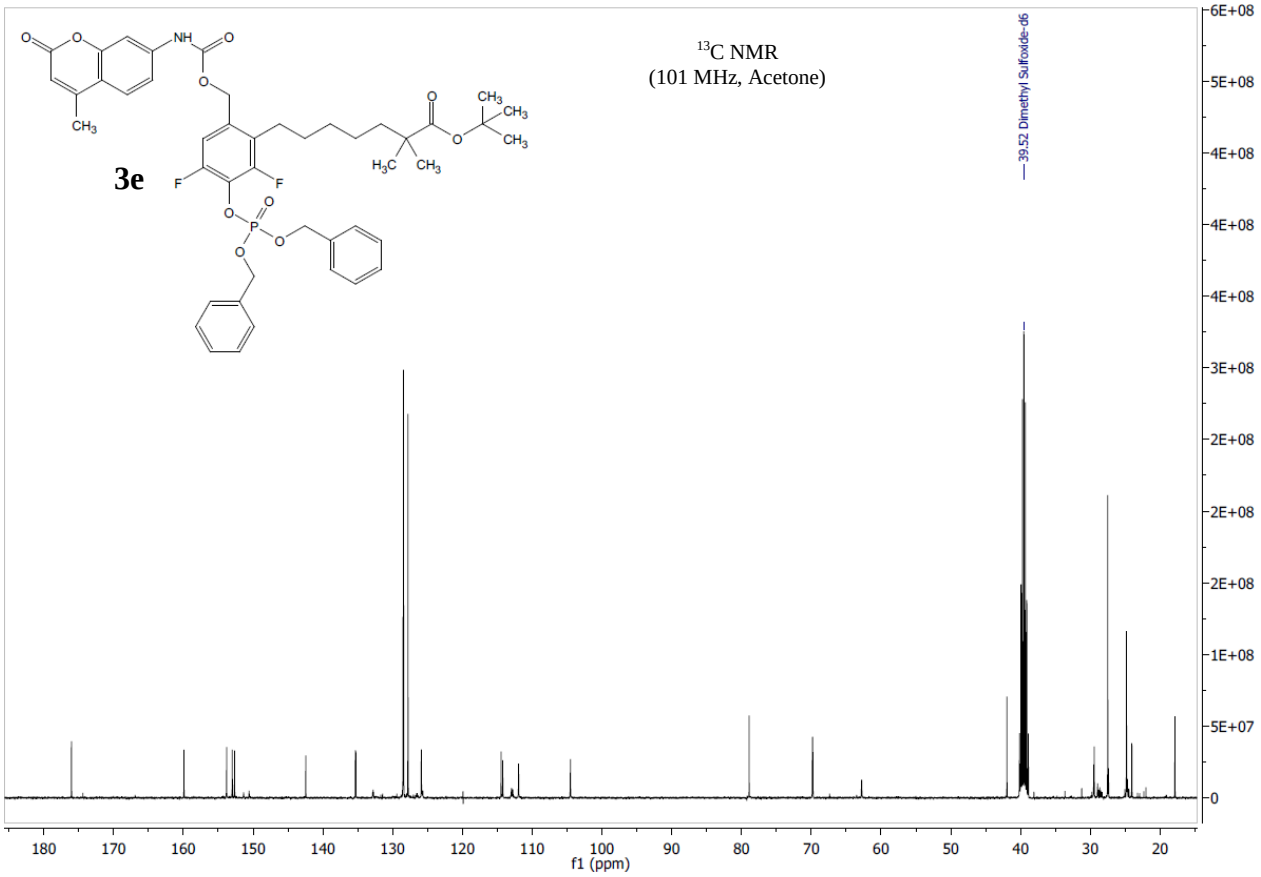
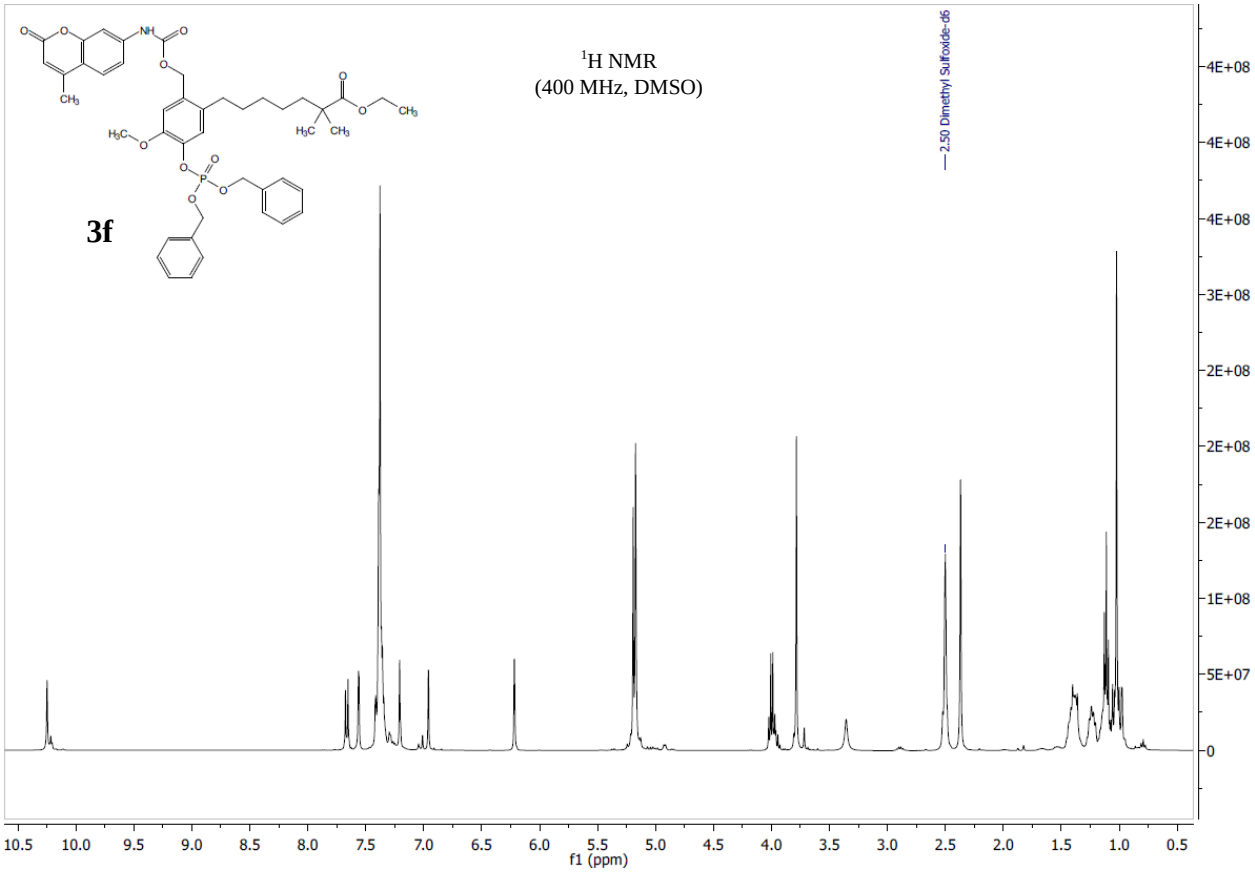
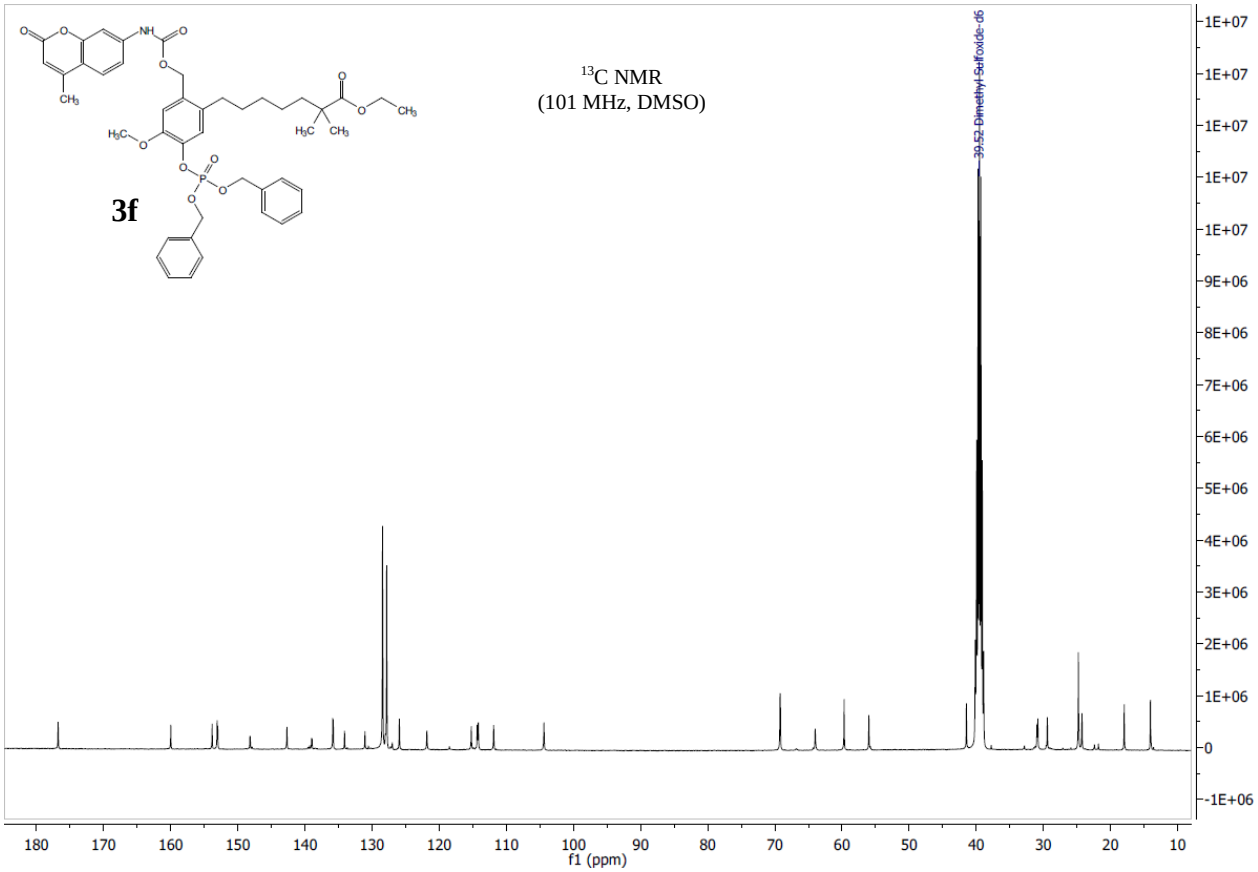
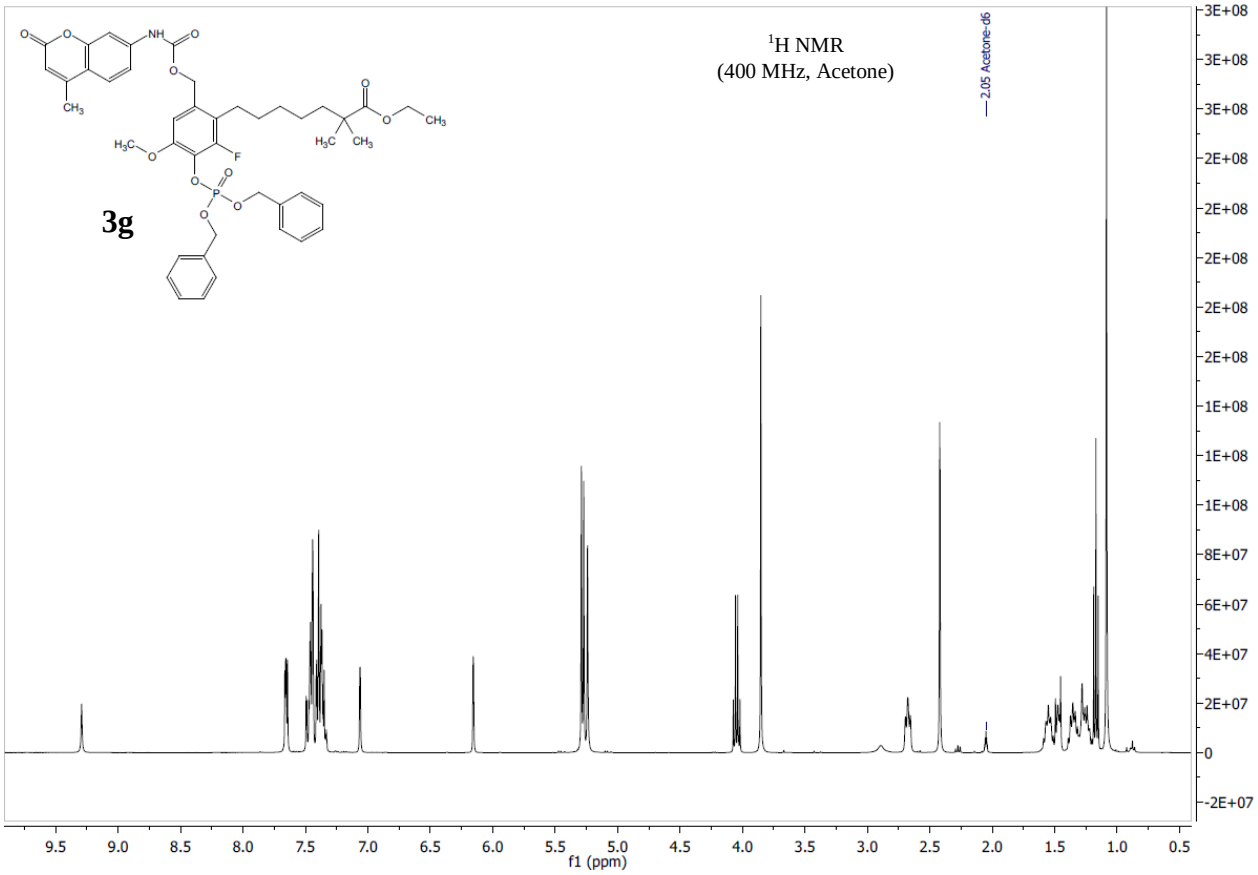
(151 MHz, DMSO)
f1 (ppm)

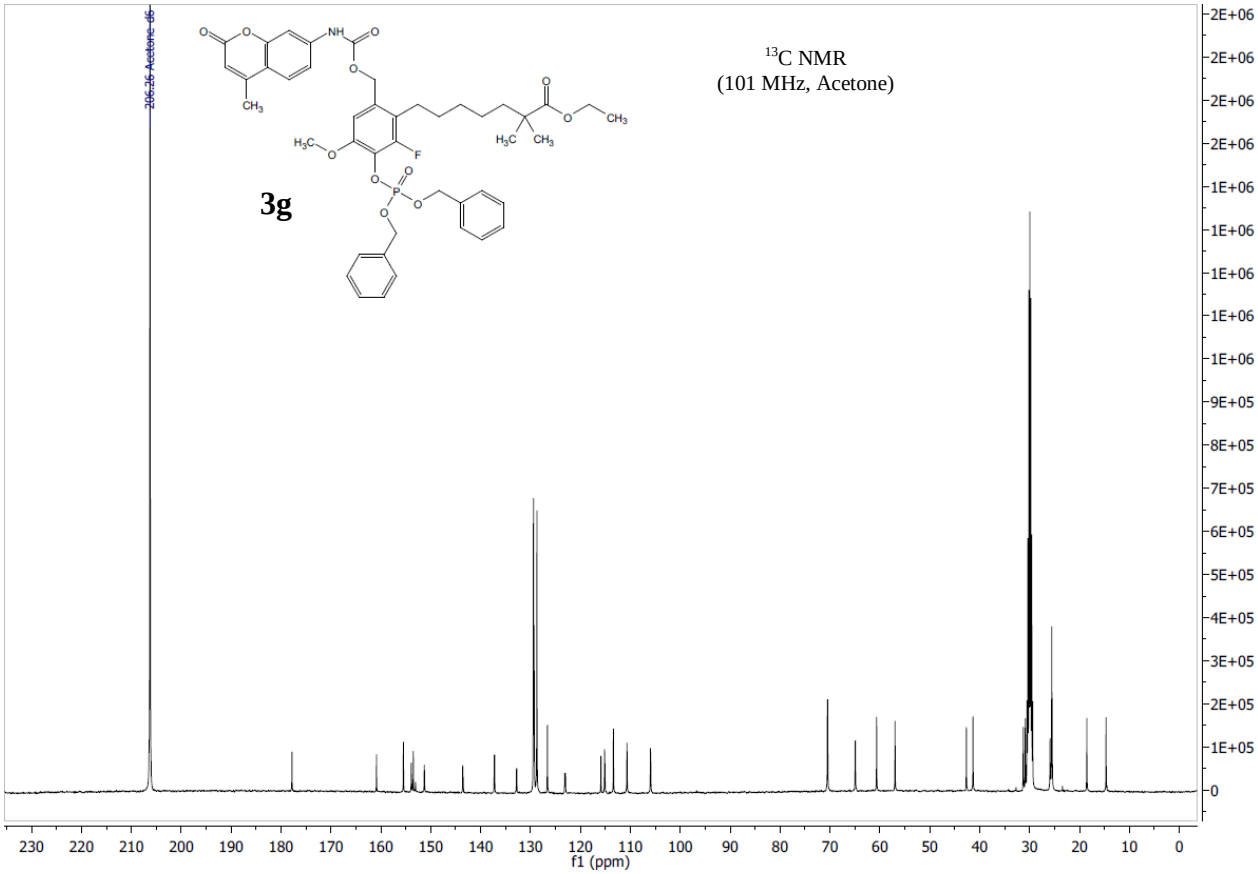

3e
^{1}H NMR
(400 MHz, Acetone)
1.94 Acetonitrile-d3
f1 (ppm)

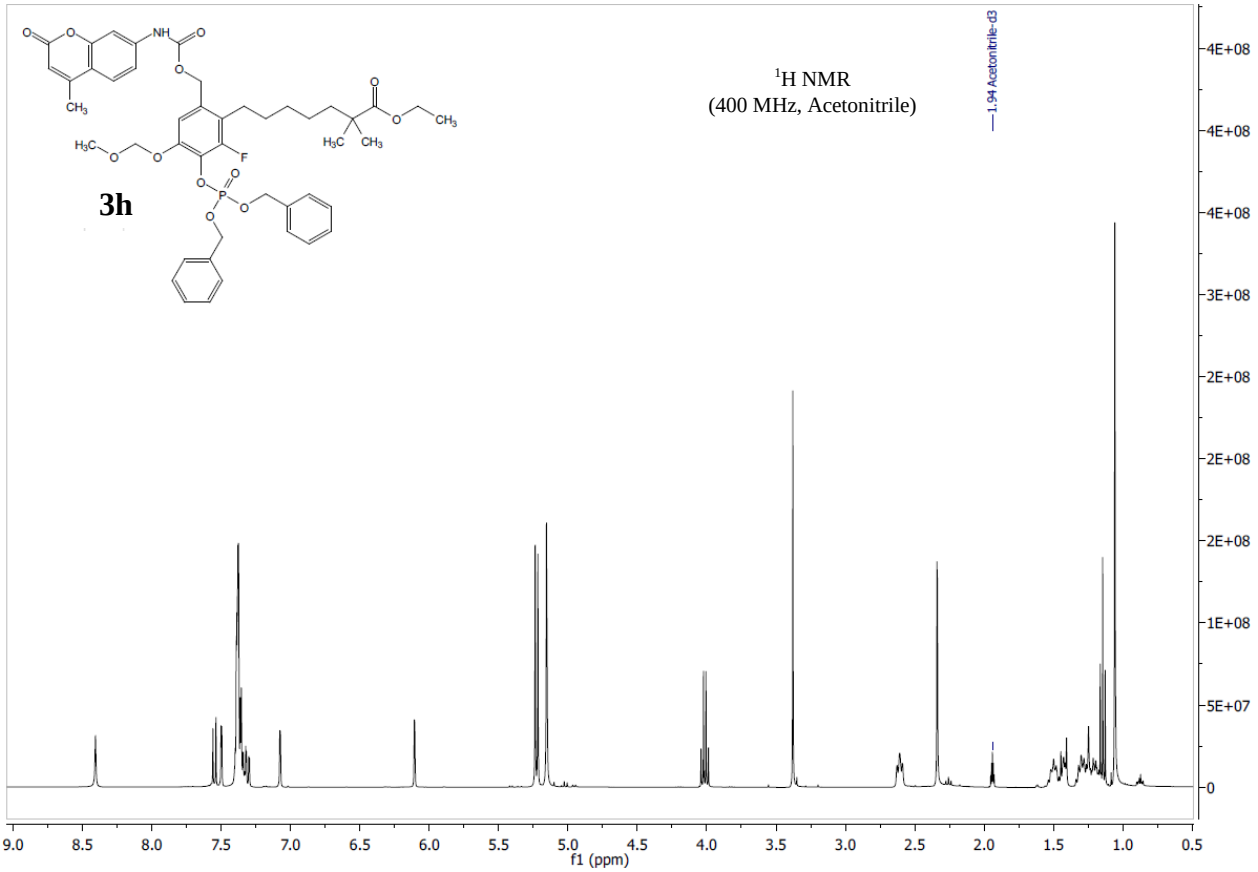

3e
^{13}C NMR
(101 MHz, Acetone)
39.52 Dimethyl Sulfoxide-d6
f1 (ppm)

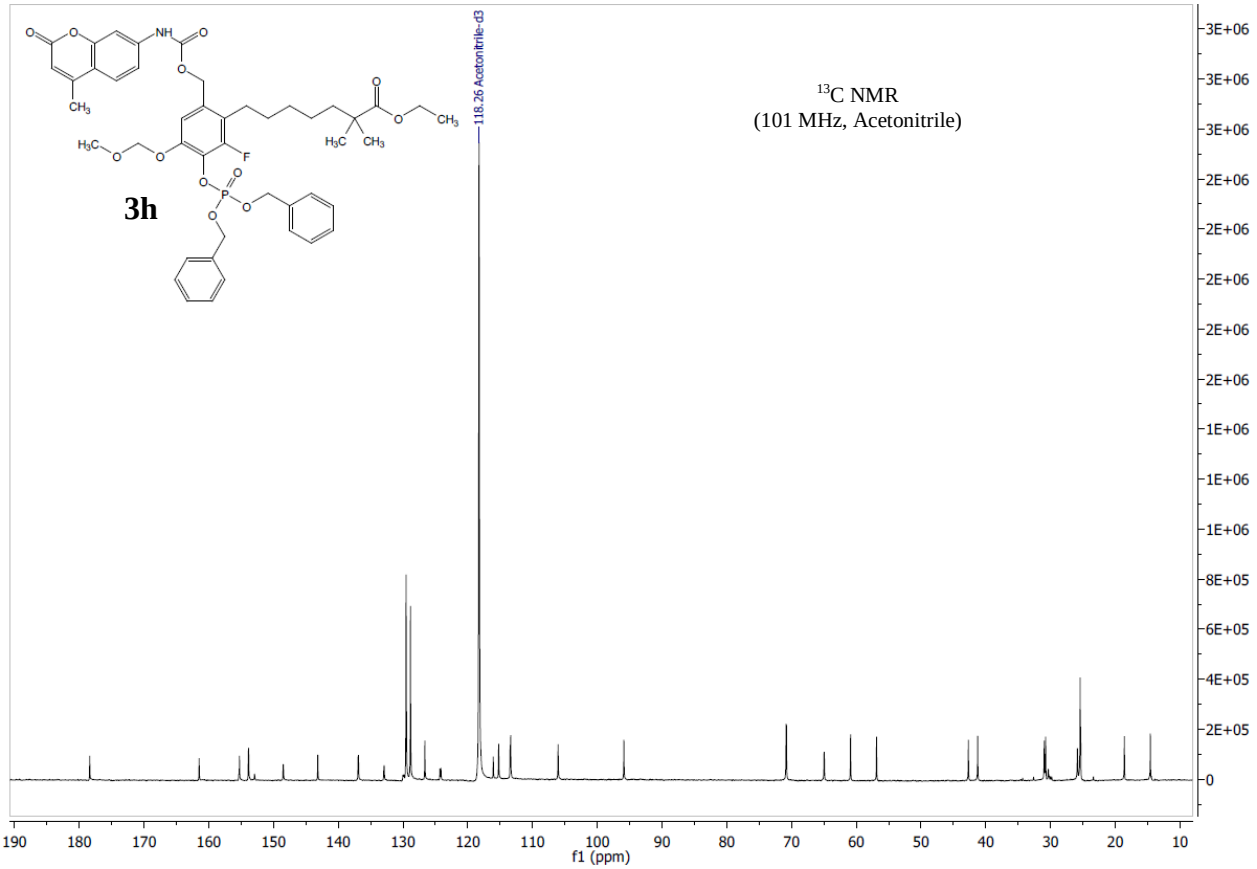

3f
^{1}H NMR
(400 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)

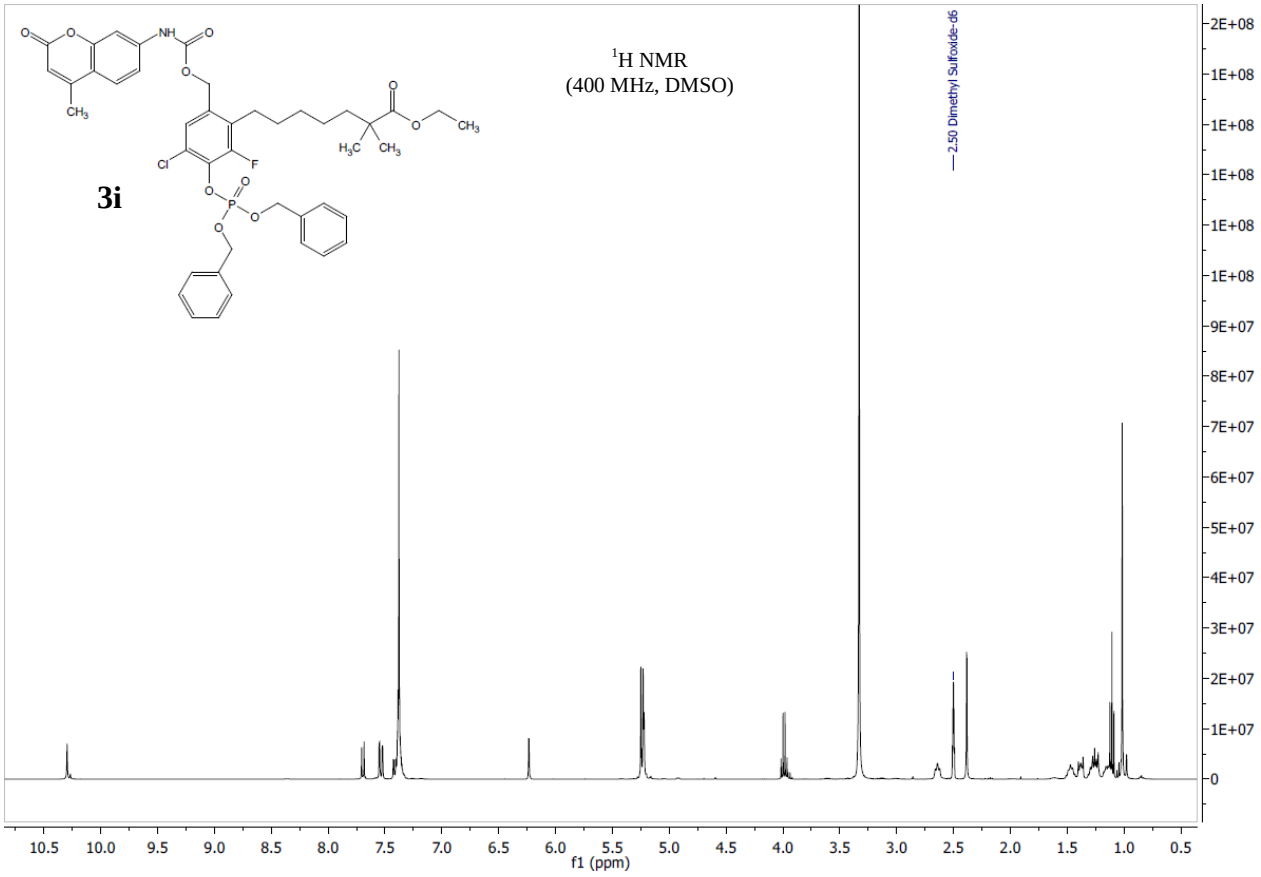

3f
^{13}C NMR
(101 MHz, DMSO)
39.52 Dimethyl Sulfoxide-d6
f1 (ppm)

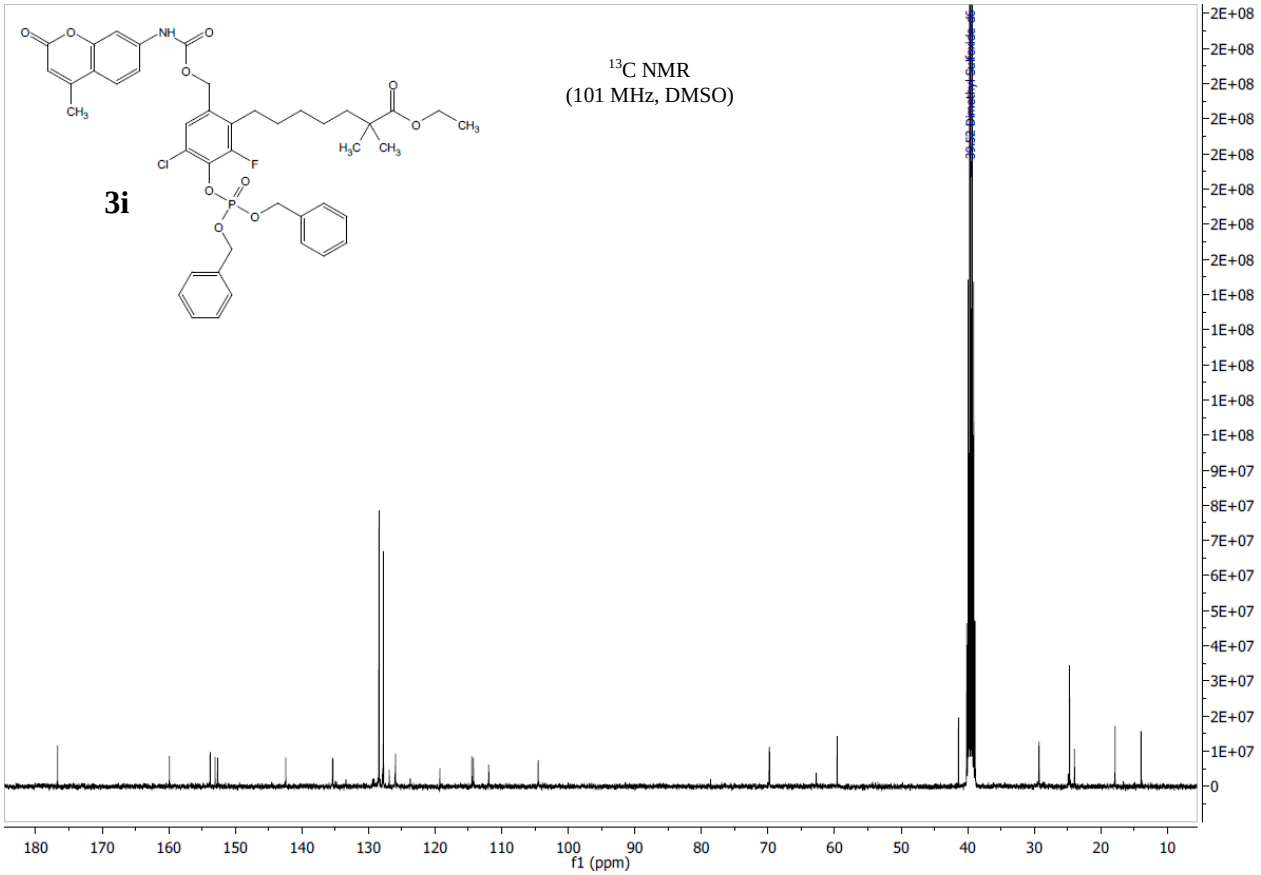

3g
^{1}H NMR
(400 MHz, Acetone)
2.05 Acetone-d6
f1 (ppm)

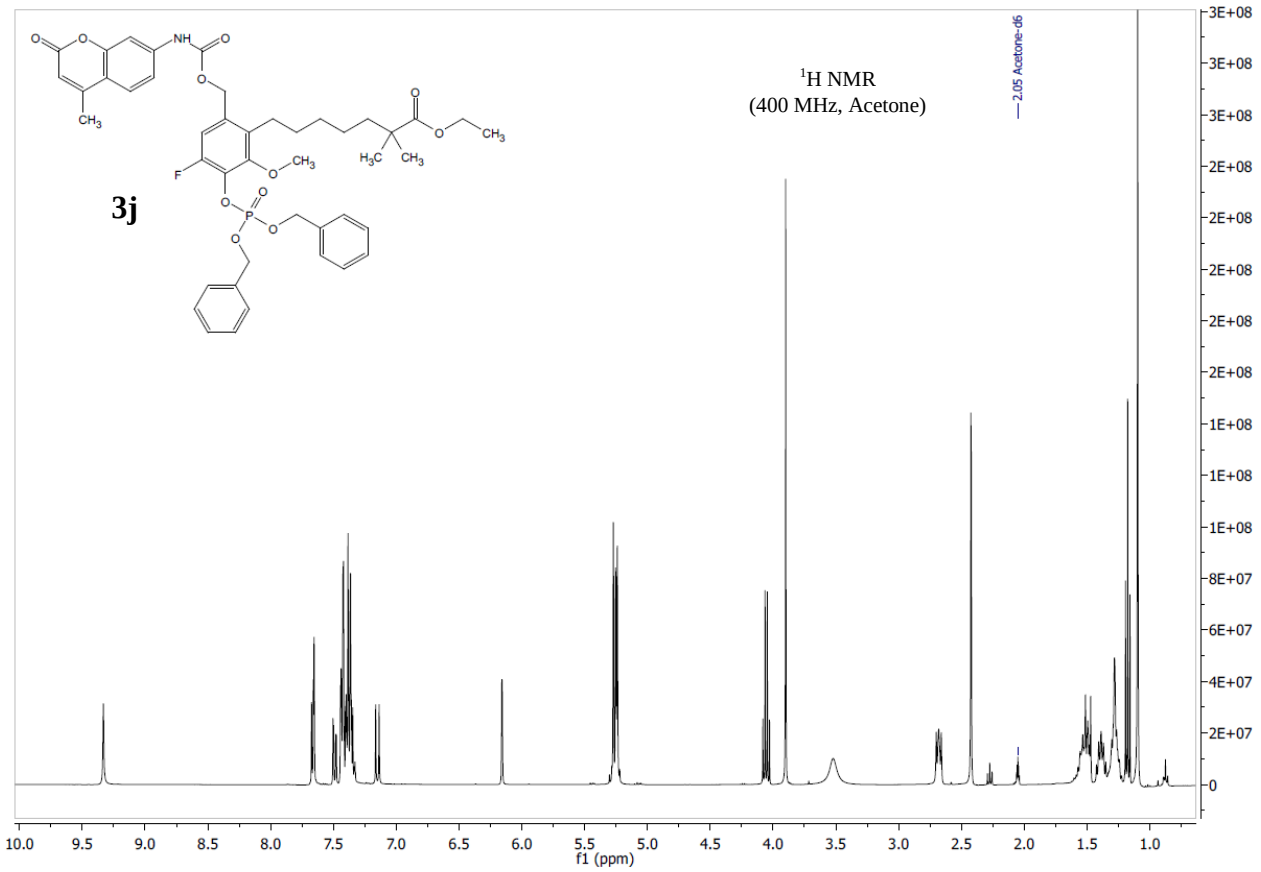

3g
^{13}C NMR
(101 MHz, Acetone)
206.26 Acetone-d6
f1 (ppm)

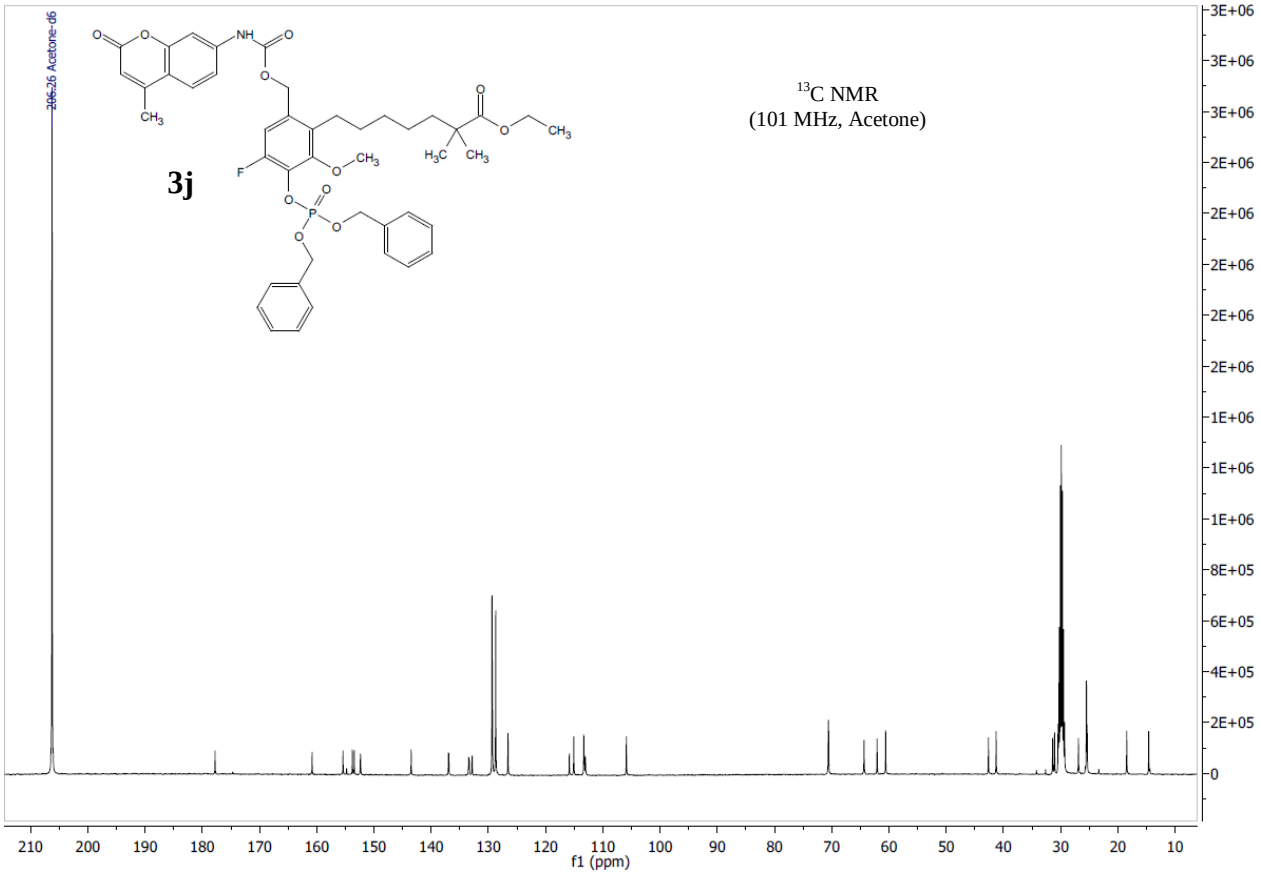

3h
^{1}H NMR
(400 MHz, Acetonitrile)
1.94 Acetonitrile-d3
f1 (ppm)

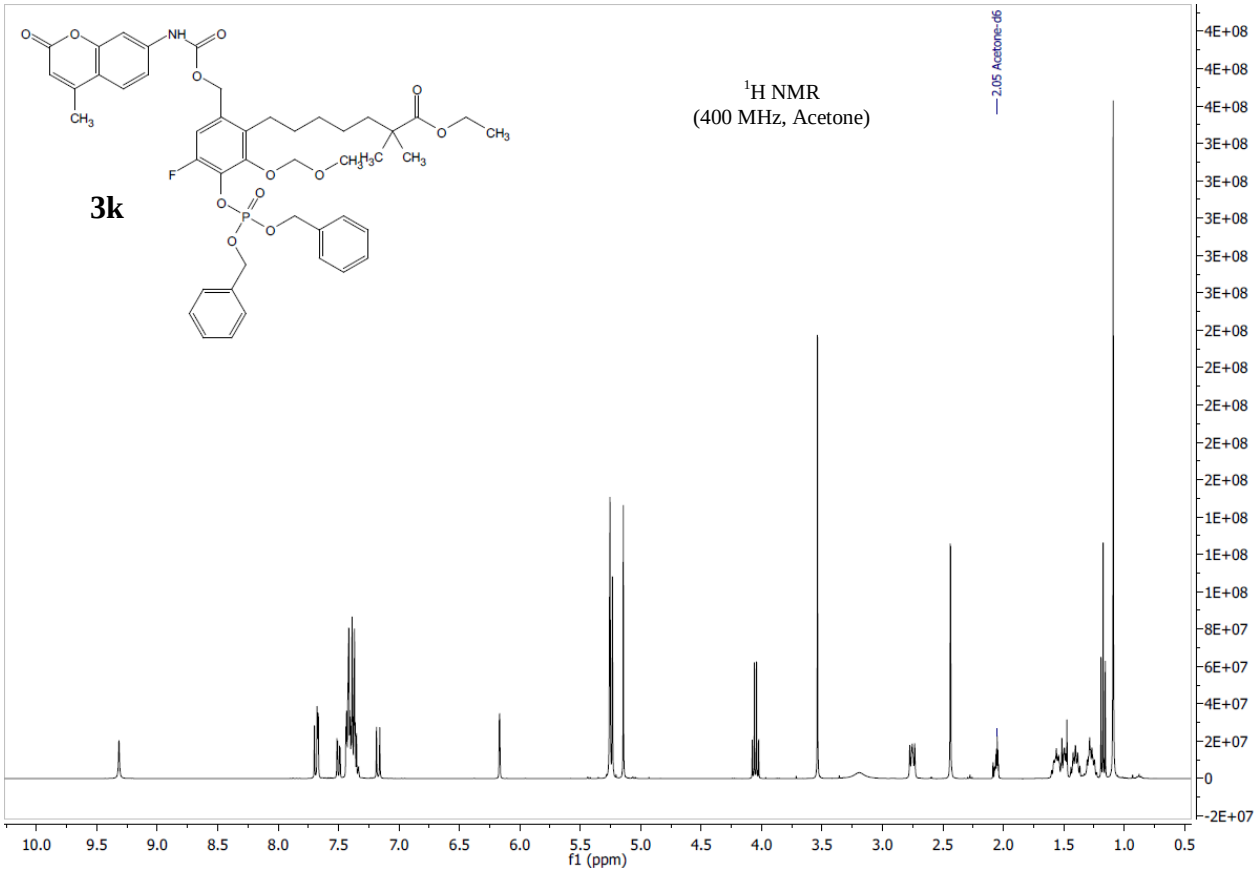

3h
^{13}C NMR
(101 MHz, Acetonitrile)
118.26 Acetonitrile-d3
f1 (ppm)

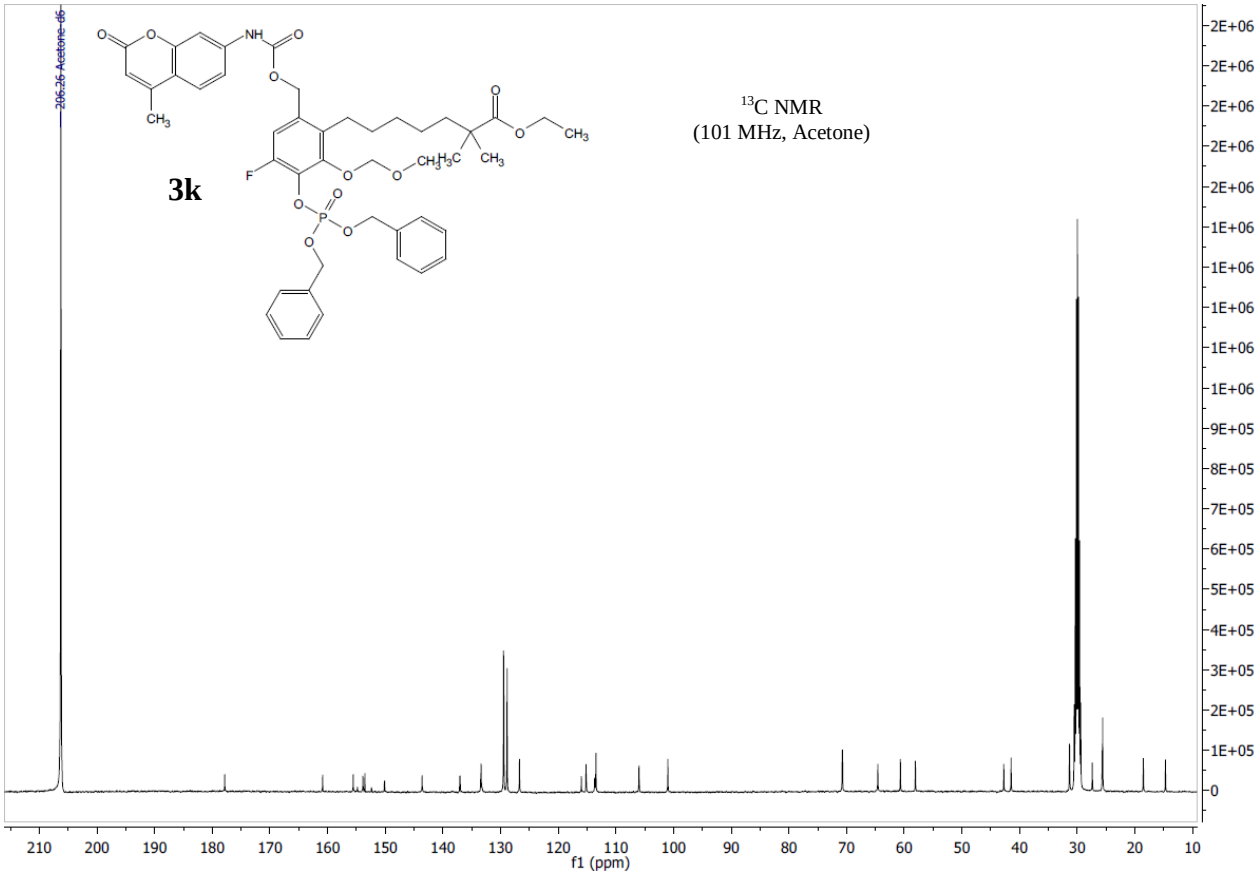

3i
^{1}H NMR
(400 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)

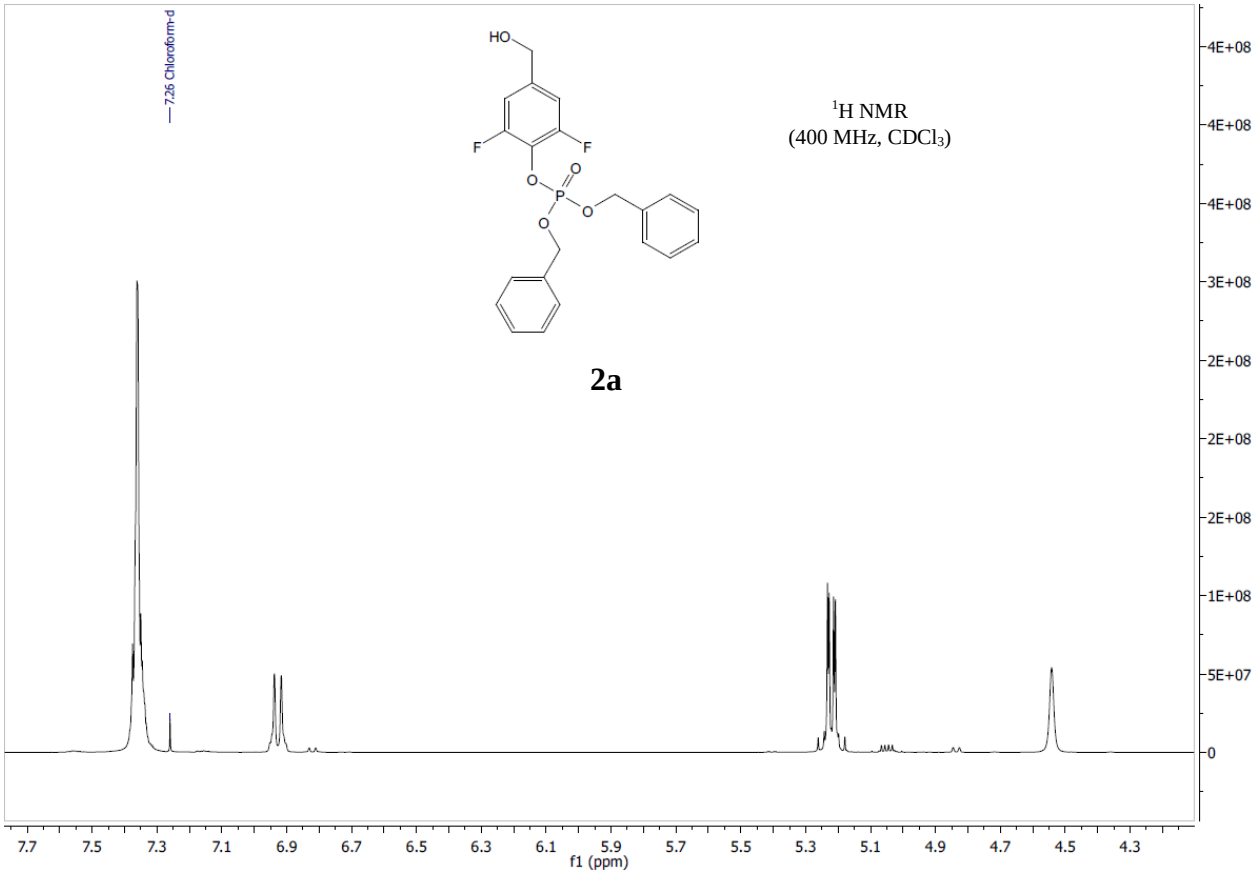

3i
^{13}C NMR
(101 MHz, DMSO)
39.52 Dimethyl Sulfoxide-d6
f1 (ppm)


3j
^{1}H NMR
(400 MHz, Acetone)
2.05 Acetone-d6
f1 (ppm)


3j
^{13}C NMR
(101 MHz, Acetone)
206.26 Acetone-d6
f1 (ppm)

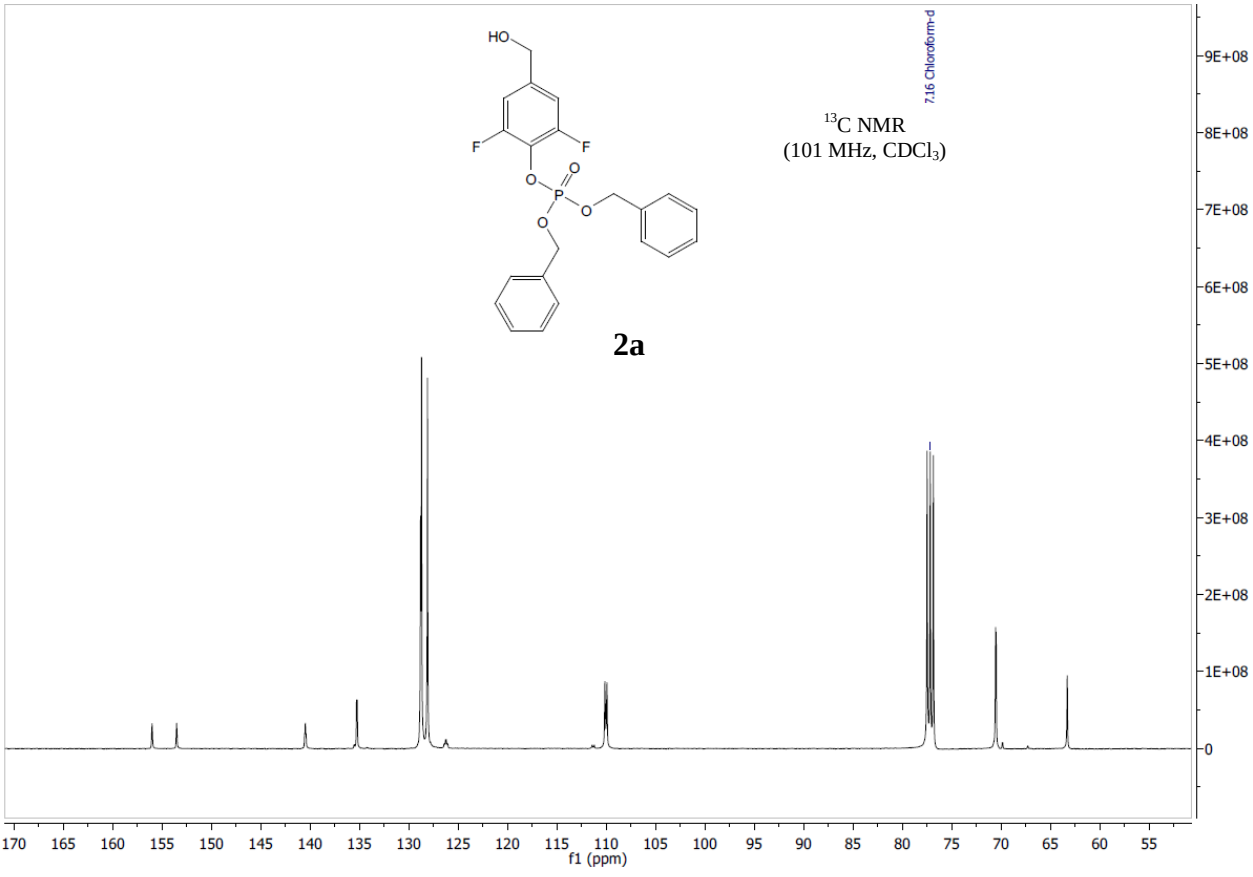

3k
^{1}H NMR
(400 MHz, Acetone)
2.05 Acetone-d6
f1 (ppm)


3k
^{13}C NMR
(101 MHz, Acetone)
206.26 Acetone-d6
f1 (ppm)



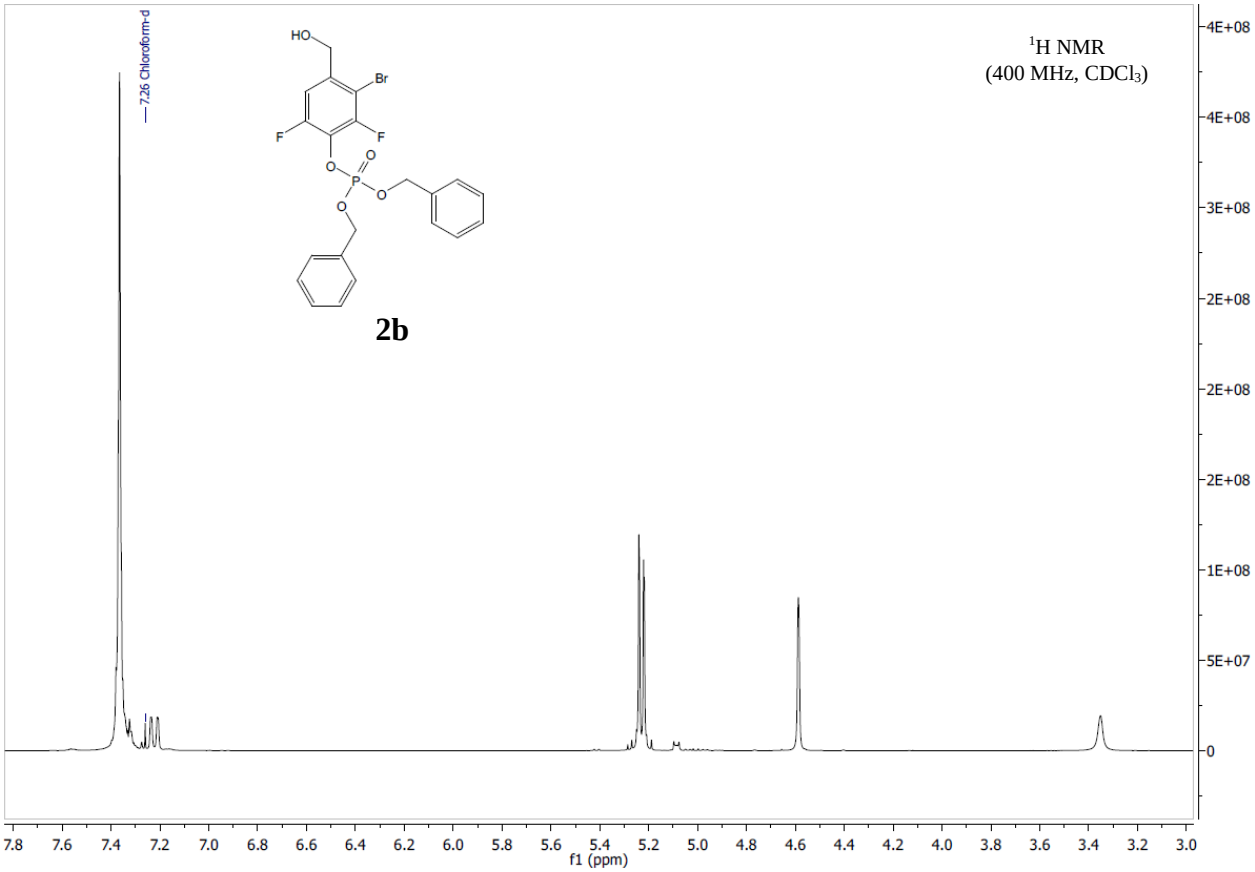
^{1}H NMR
(400 MHz, $CDCl_3$)

2a

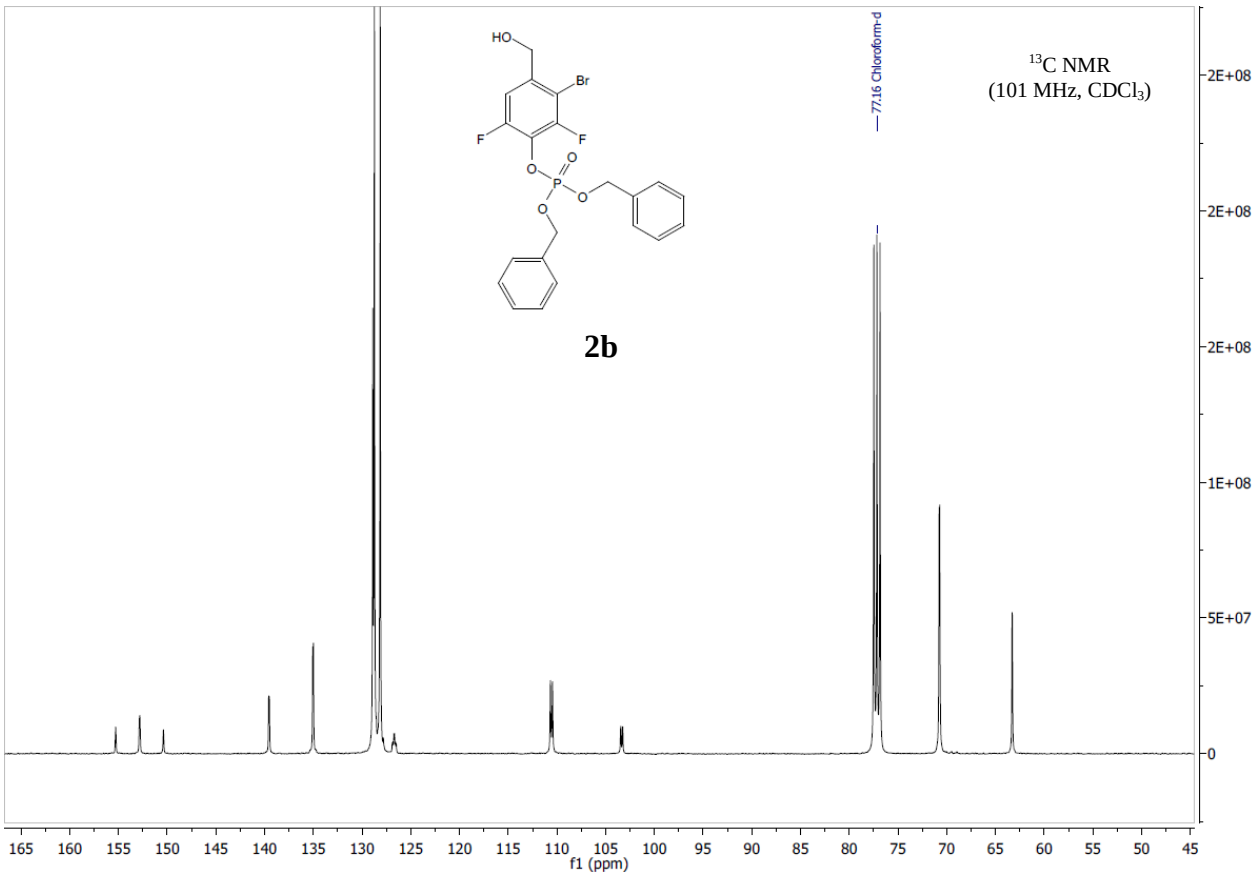


^{13}C NMR
(101 MHz, $CDCl_3$)

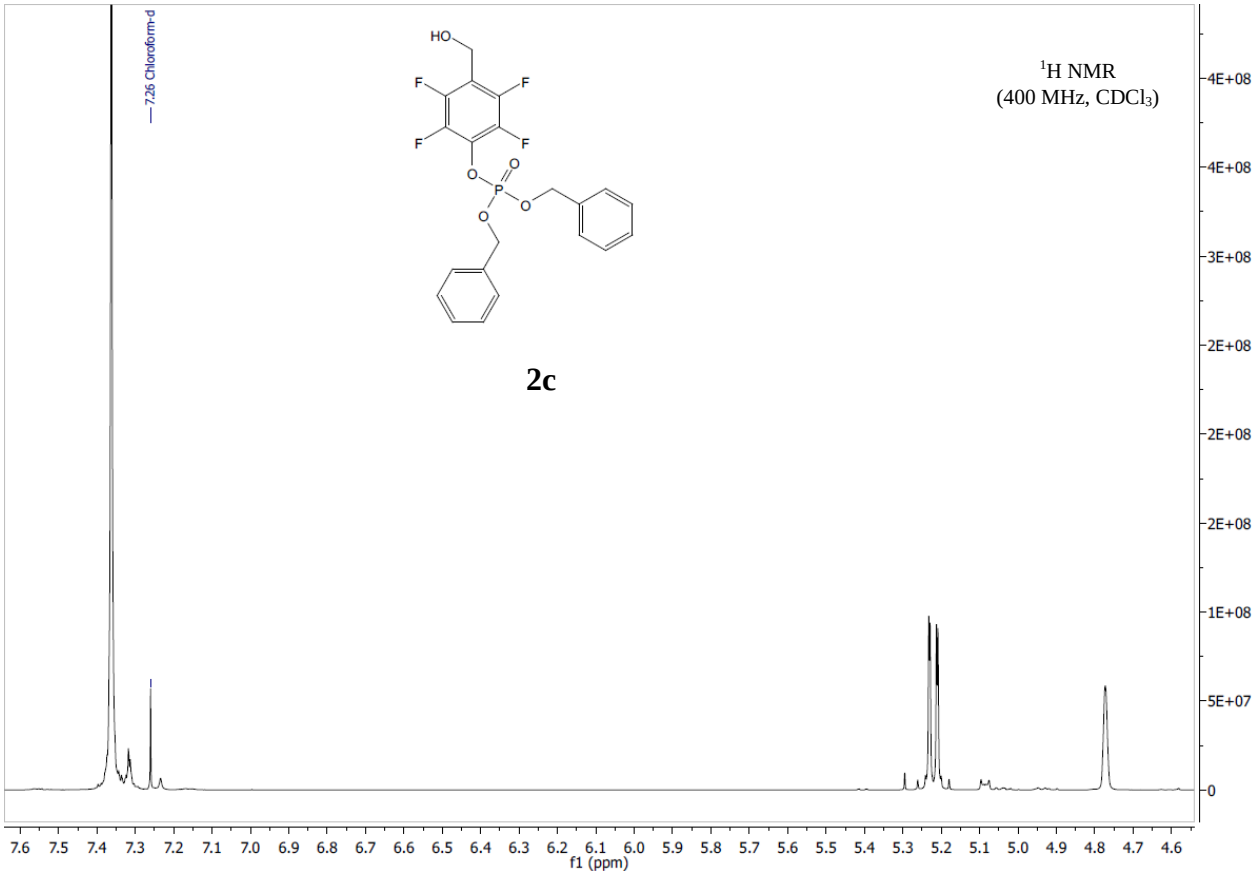
2a


7.26 Chloroform-d
HO
Br
F
F
O
P
O
O
O
^{1}H NMR
(400 MHz, $CDCl_3$)
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0
f1 (ppm)

2b

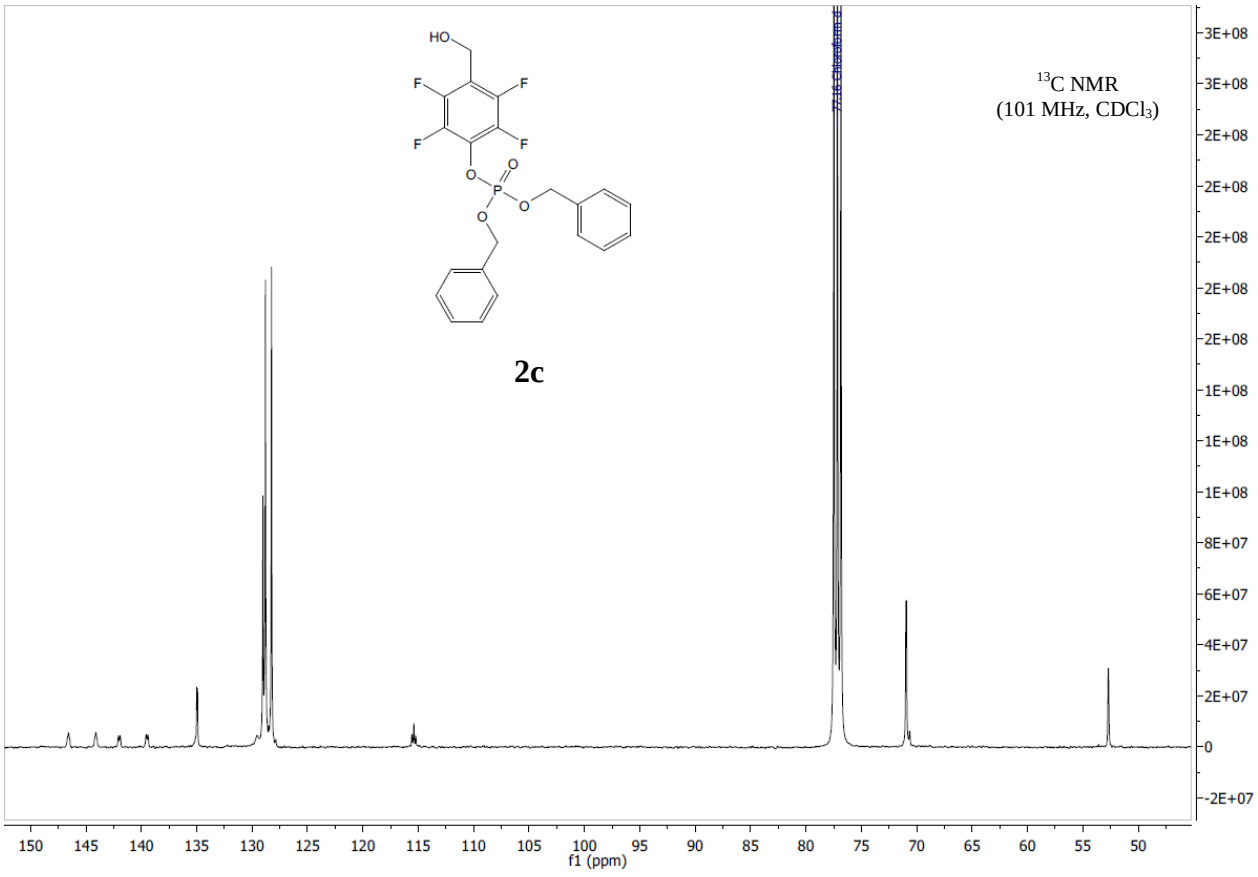

77.16 Chloroform-d
HO
Br
F
F
O
P
O
O
O
^{13}C NMR
(101 MHz, $CDCl_3$)
2E+08
2E+08
2E+08
1E+08
5E+07
0
165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45
f1 (ppm)

2b



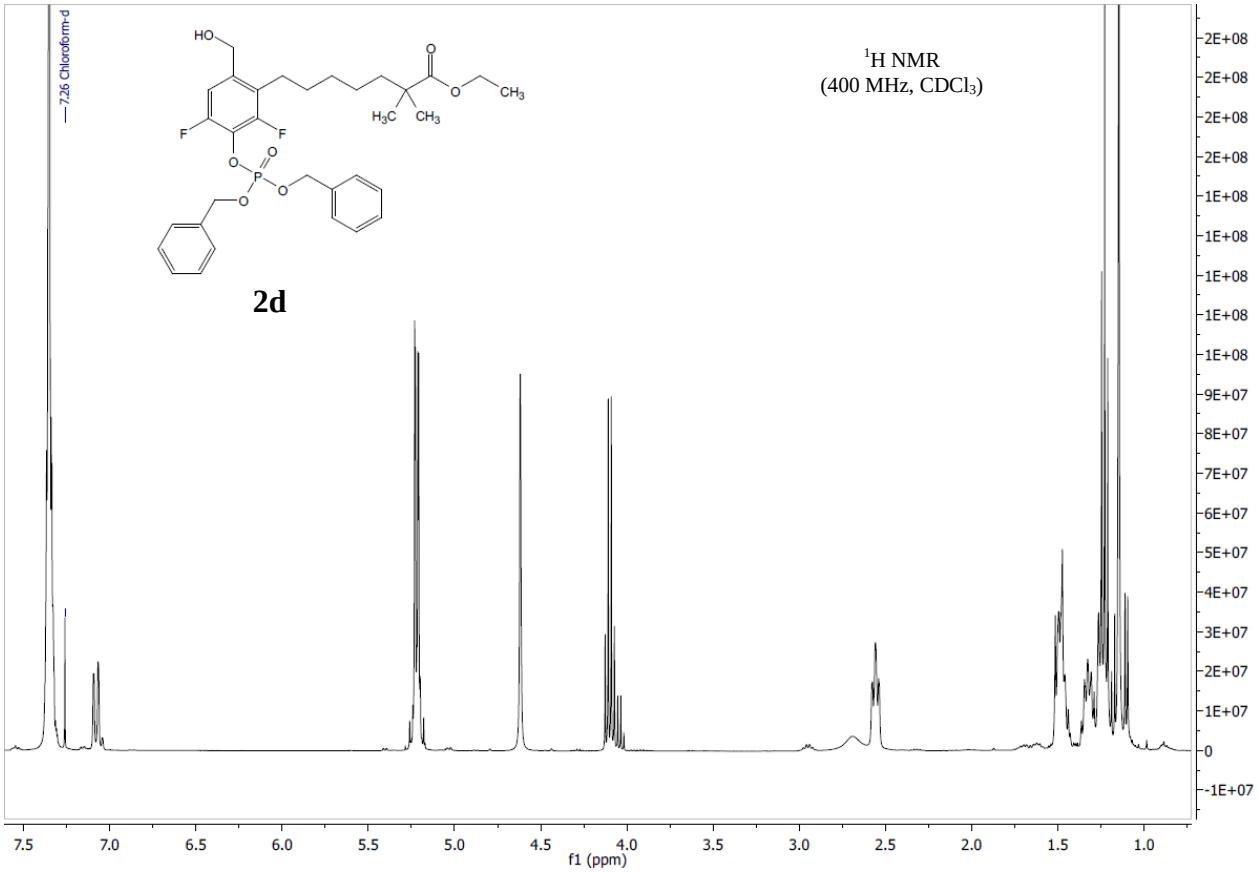
^{1}H NMR
(400 MHz, $CDCl_3$)

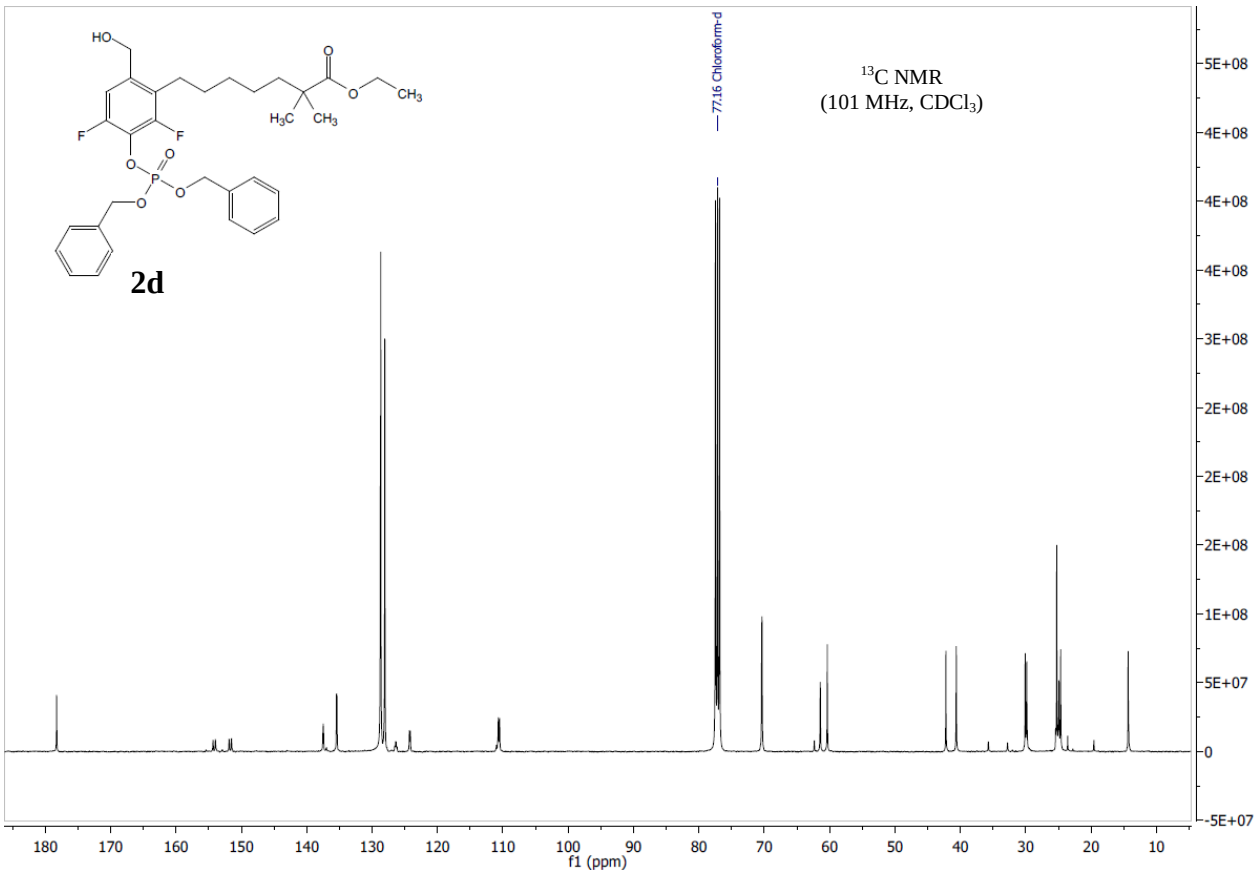
2c

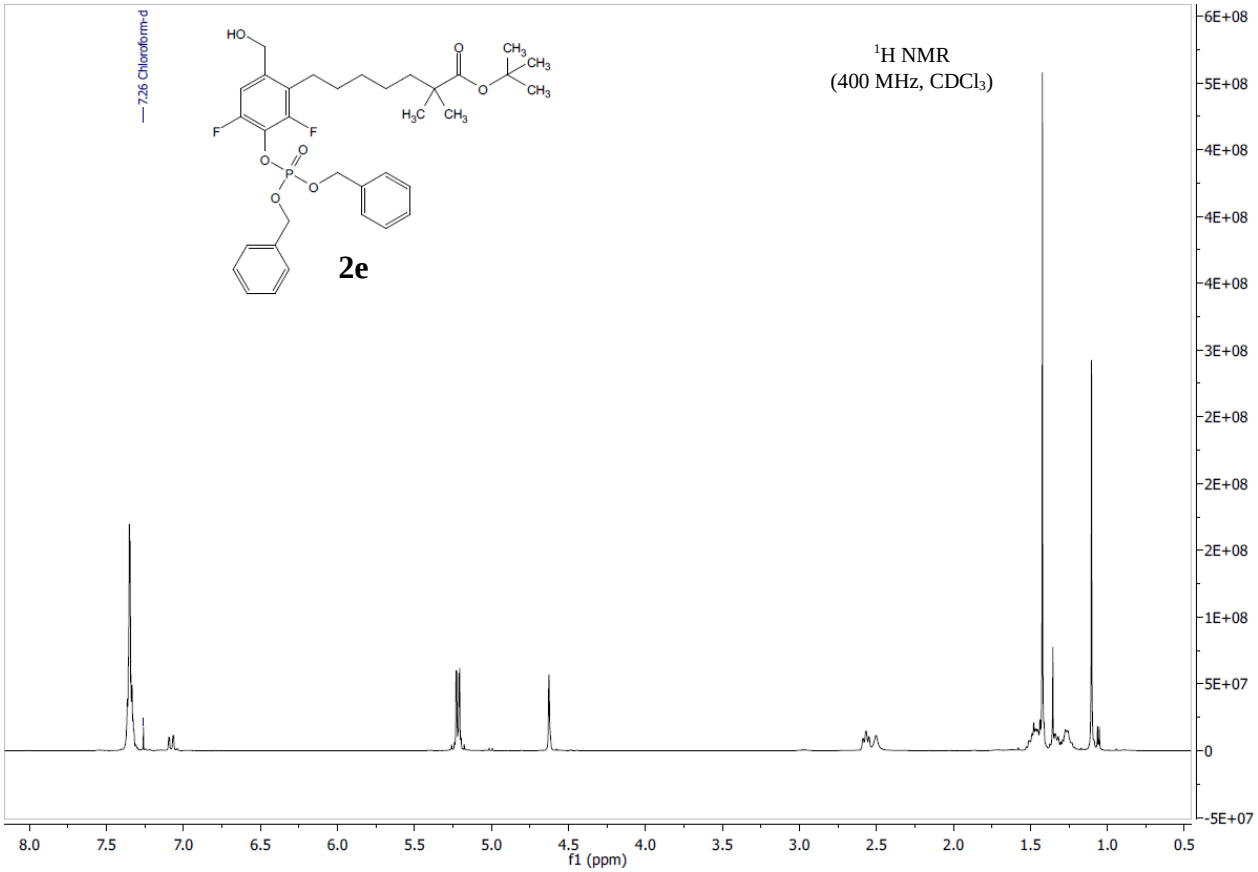


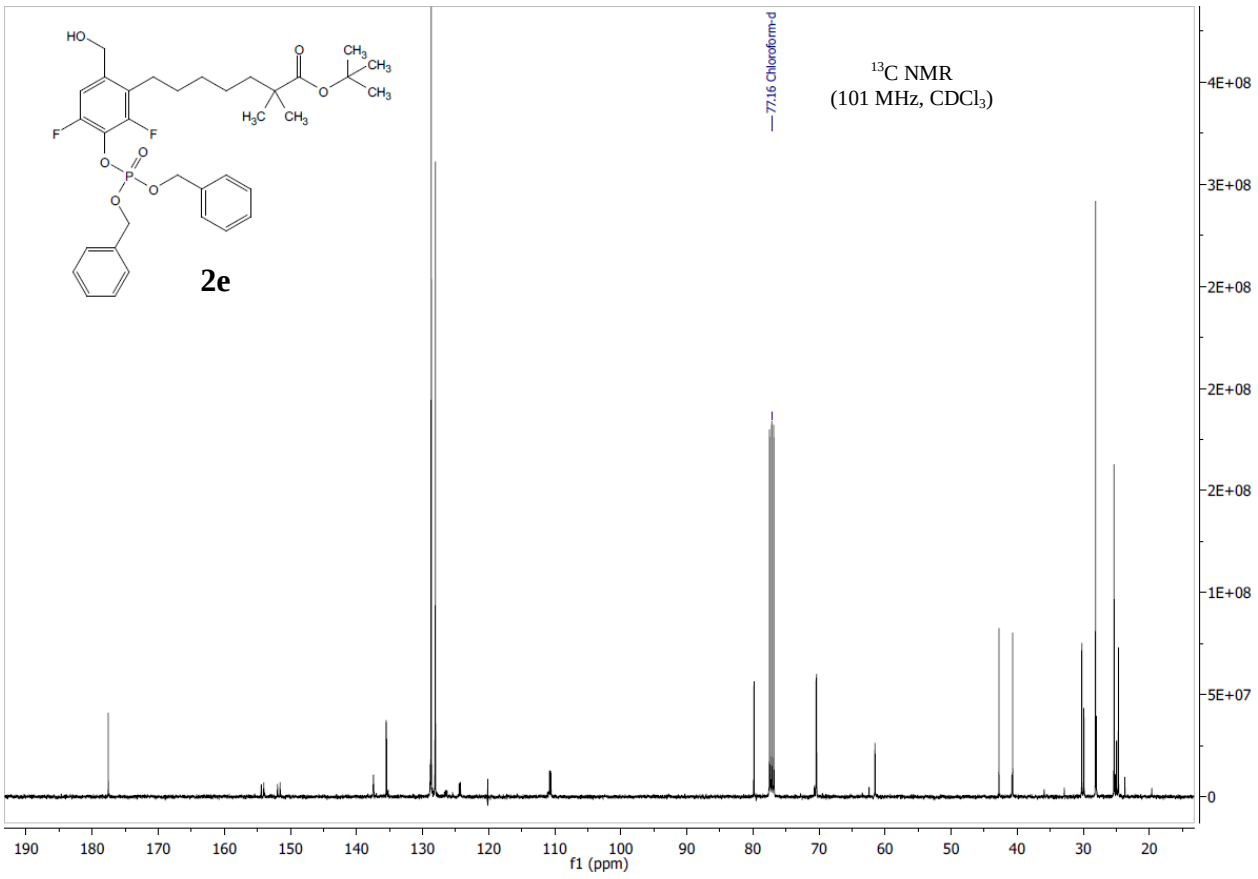
^{13}C NMR
(101 MHz, $CDCl_3$)

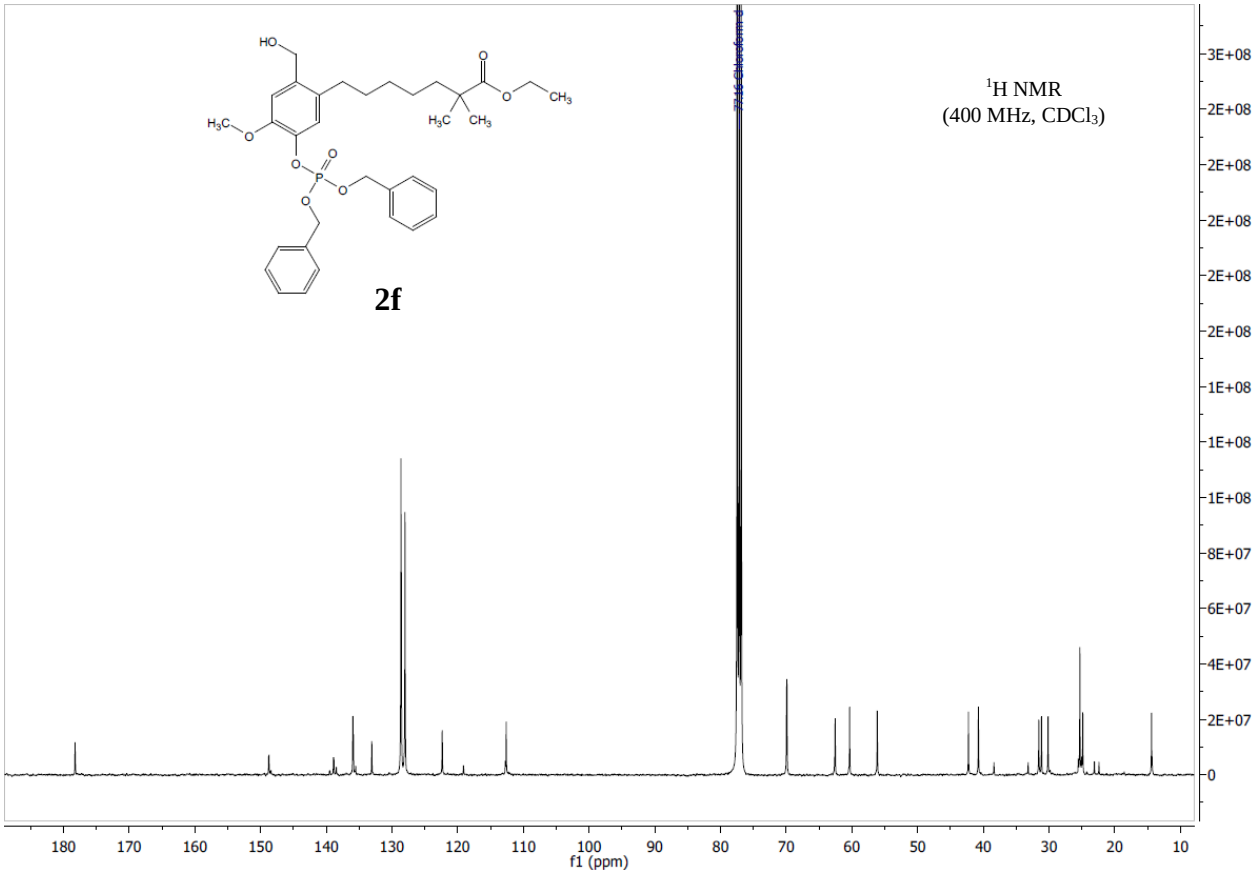
2c


7.26 Chloroform-d
HO
F
F
O
P
O
O
O
O
O
H3C
CH3
CH3
2d
1H NMR
(400 MHz, CDCl3)
2E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
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
f1 (ppm)

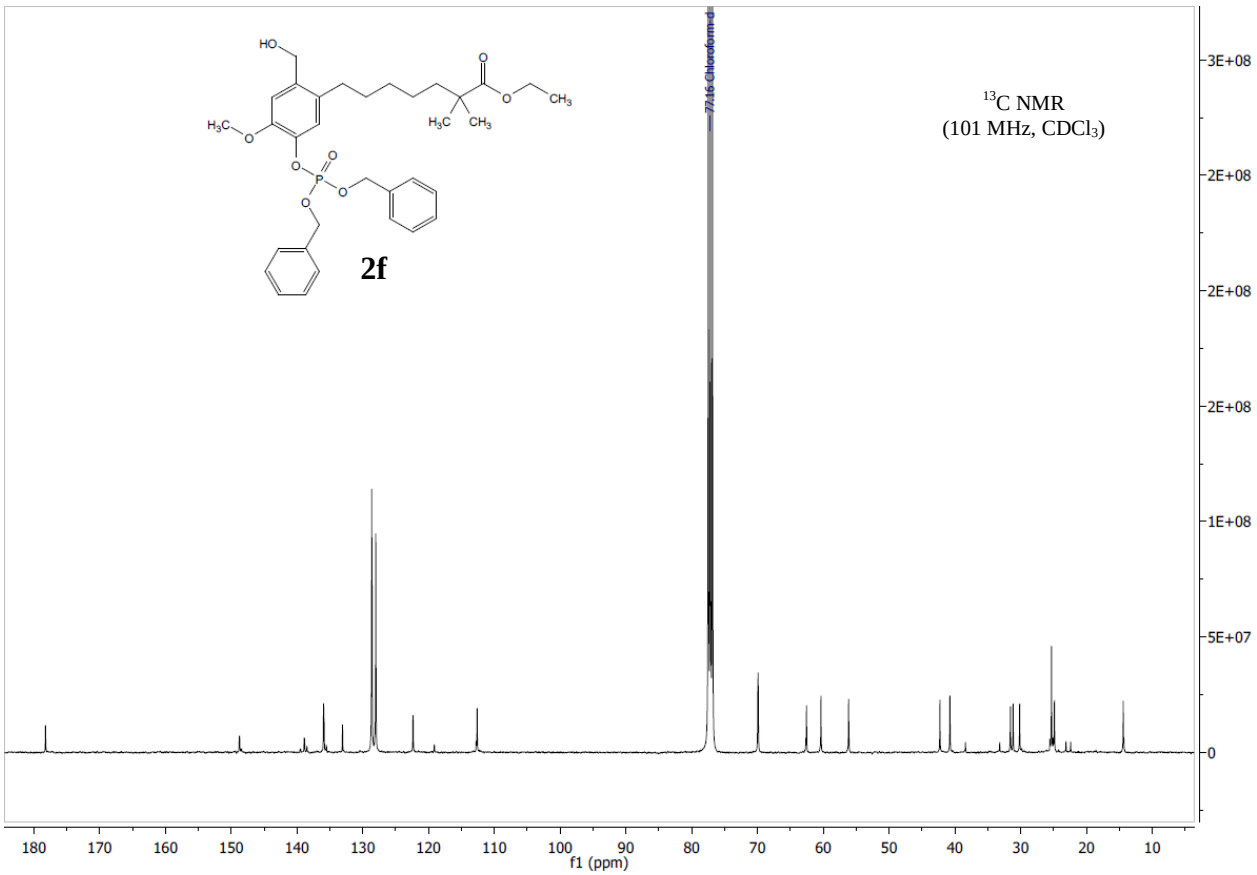

77.16 Chloroform-d
HO
F
F
O
P
O
O
O
O
O
H3C
CH3
CH3
2d
13C NMR
(101 MHz, CDCl3)
5E+08
4E+08
3E+08
2E+08
1E+08
0
-5E+07
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


7.26 Chloroform-d
HO
F
F
O
P
O
O
O
O
O
CH3
CH3
CH3
H3C
CH3
2e
1H NMR
(400 MHz, CDCl3)
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
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
f1 (ppm)

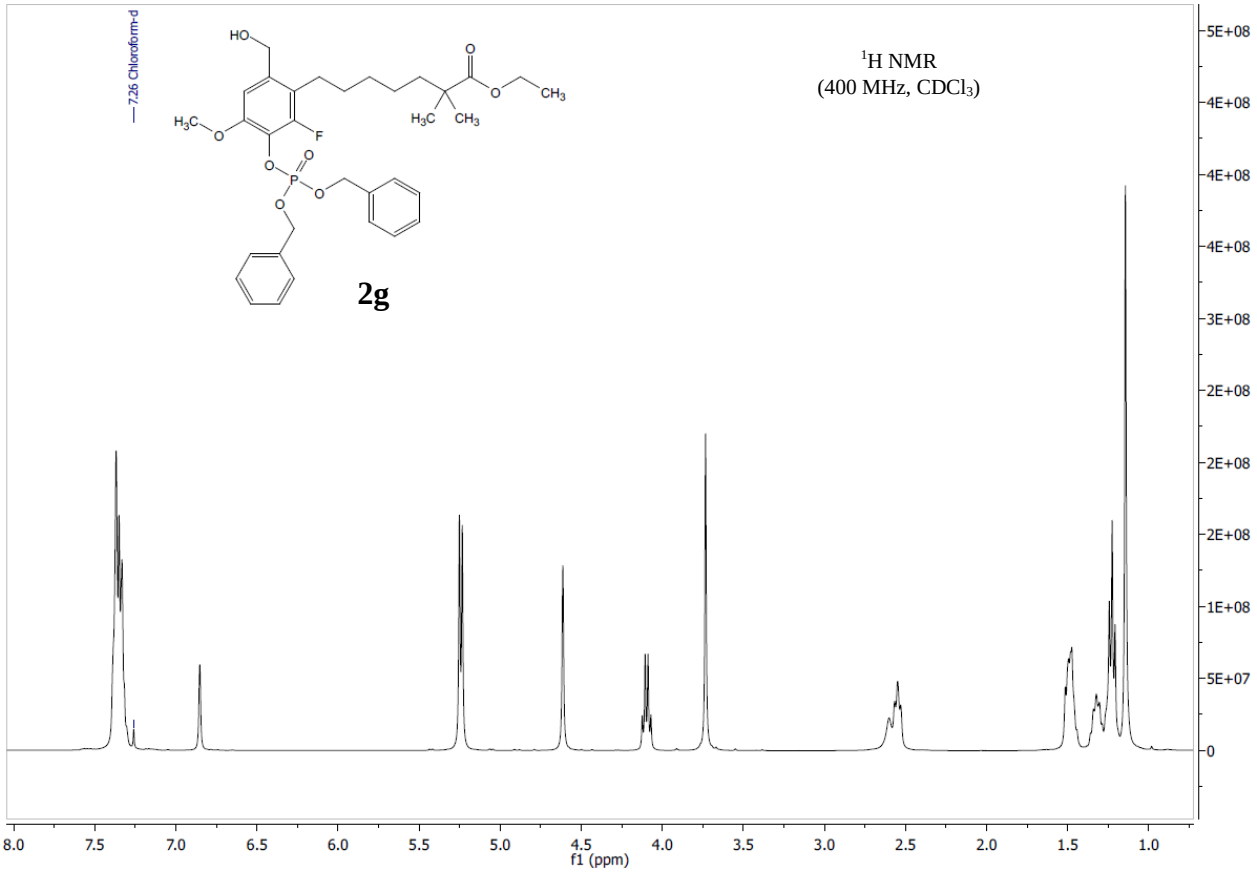

HO
F
F
O
P
O
O
O
O
O
CH3
CH3
CH3
H3C
CH3
2e
77.16 Chloroform-d
13C NMR
(101 MHz, CDCl3)
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

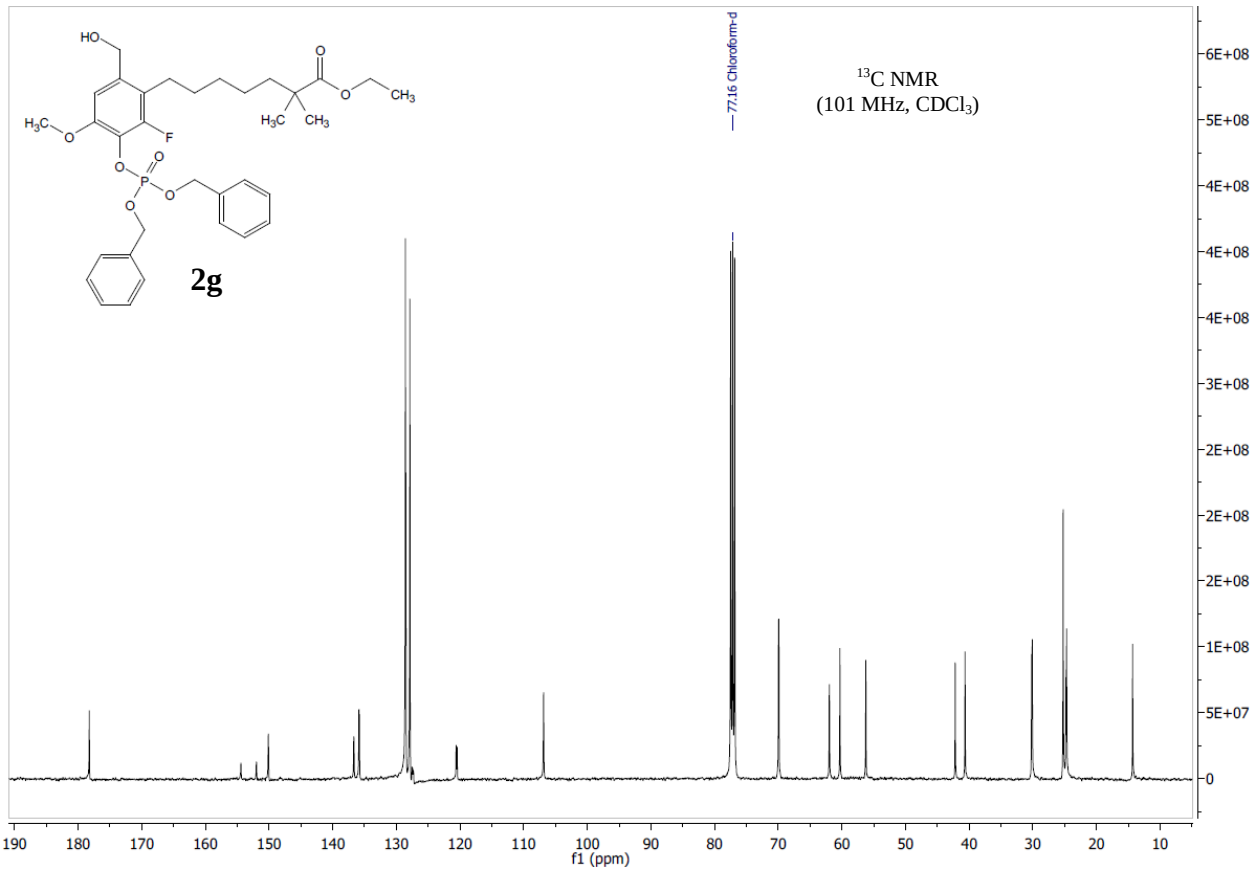


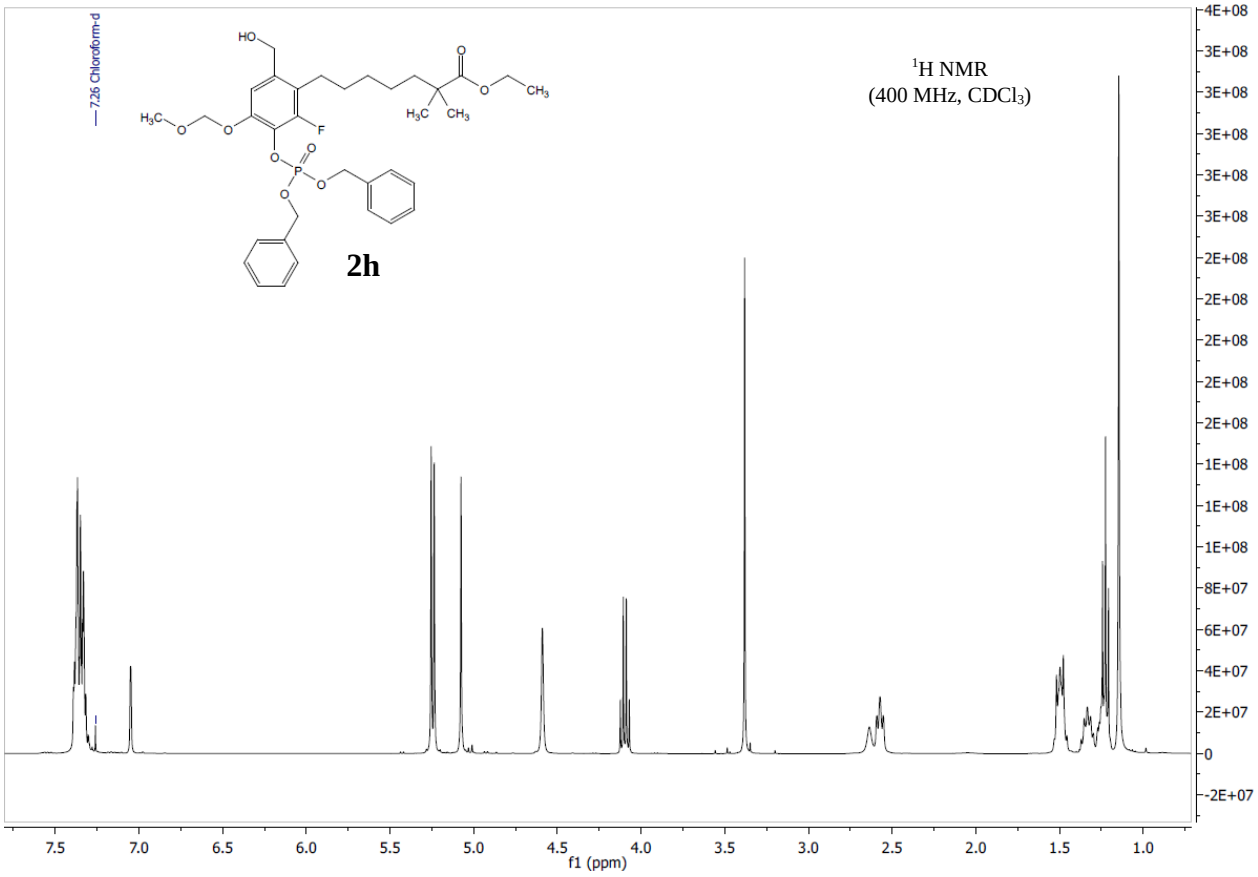
^{1}H NMR
(400 MHz, $CDCl_3$)

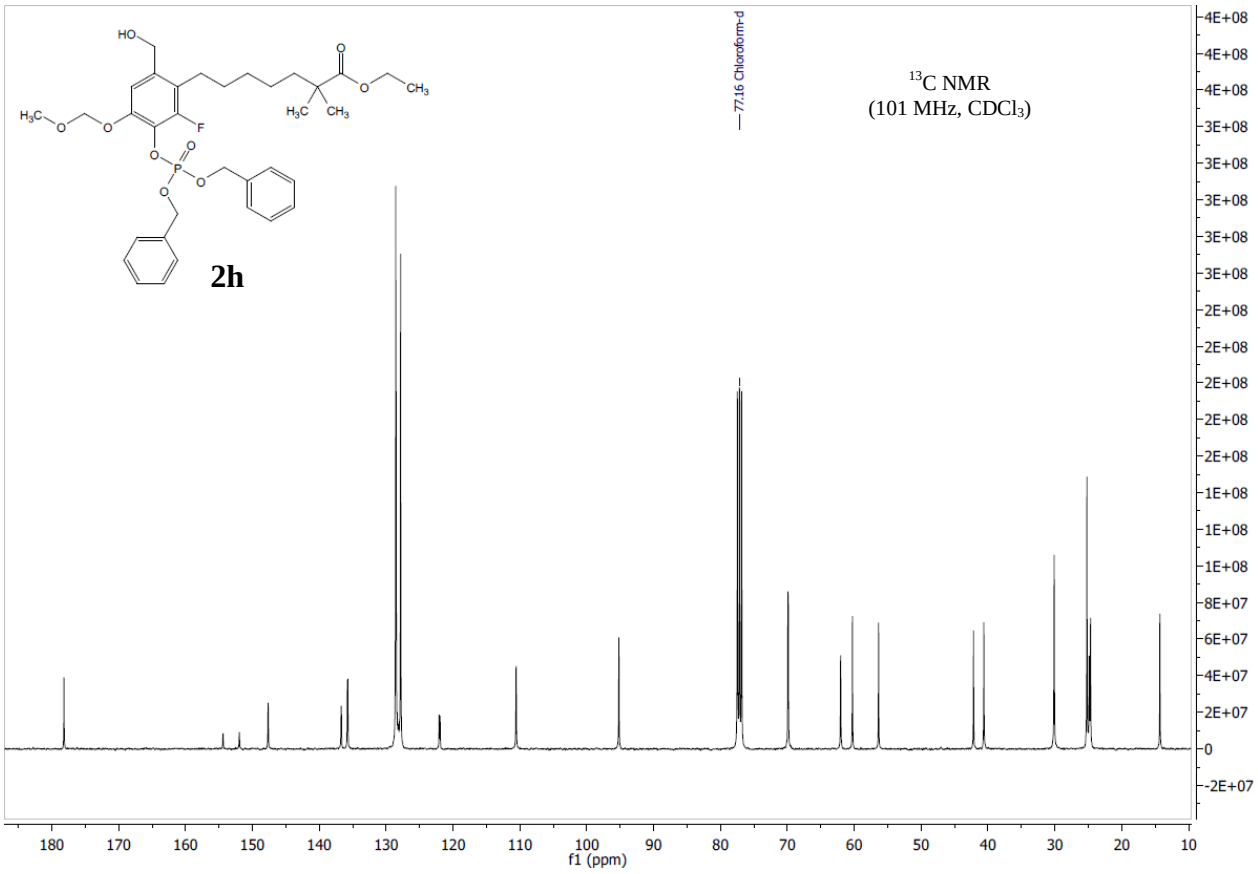


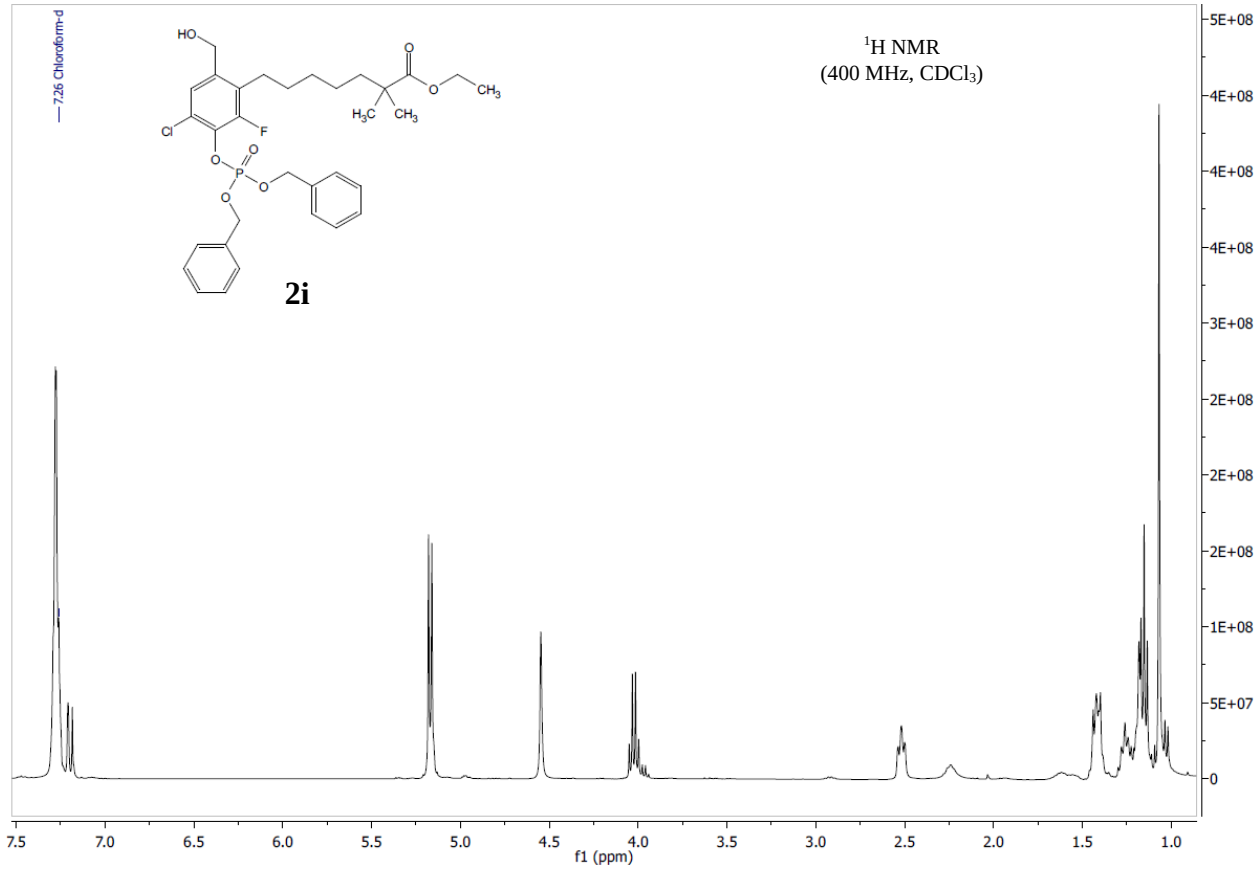
^{13}C NMR
(101 MHz, $CDCl_3$)

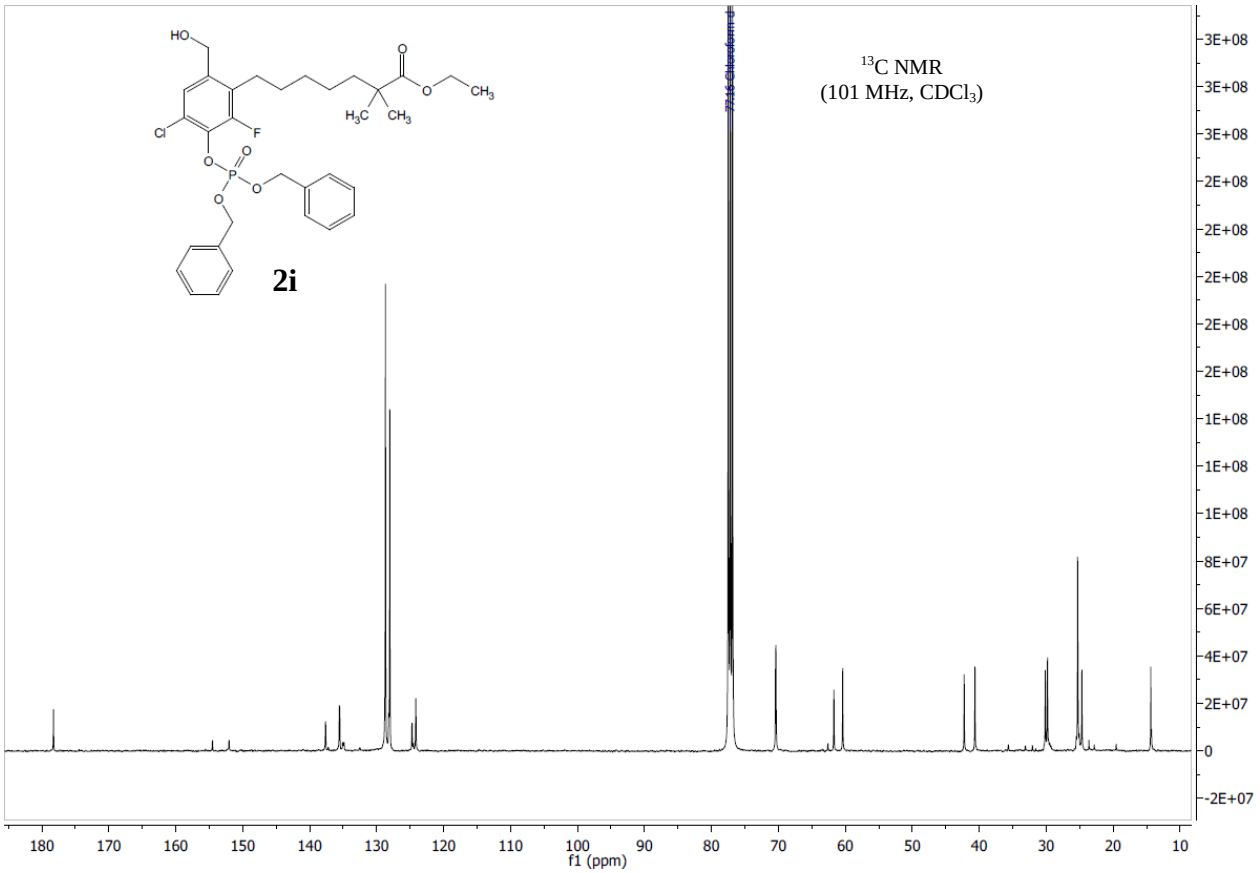

7.26 Chloroform-d
HO
O
O
CH3
H3C
CH3
H3C
O
F
O
P
O
O
O
2g
1H NMR
(400 MHz, CDCl3)
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

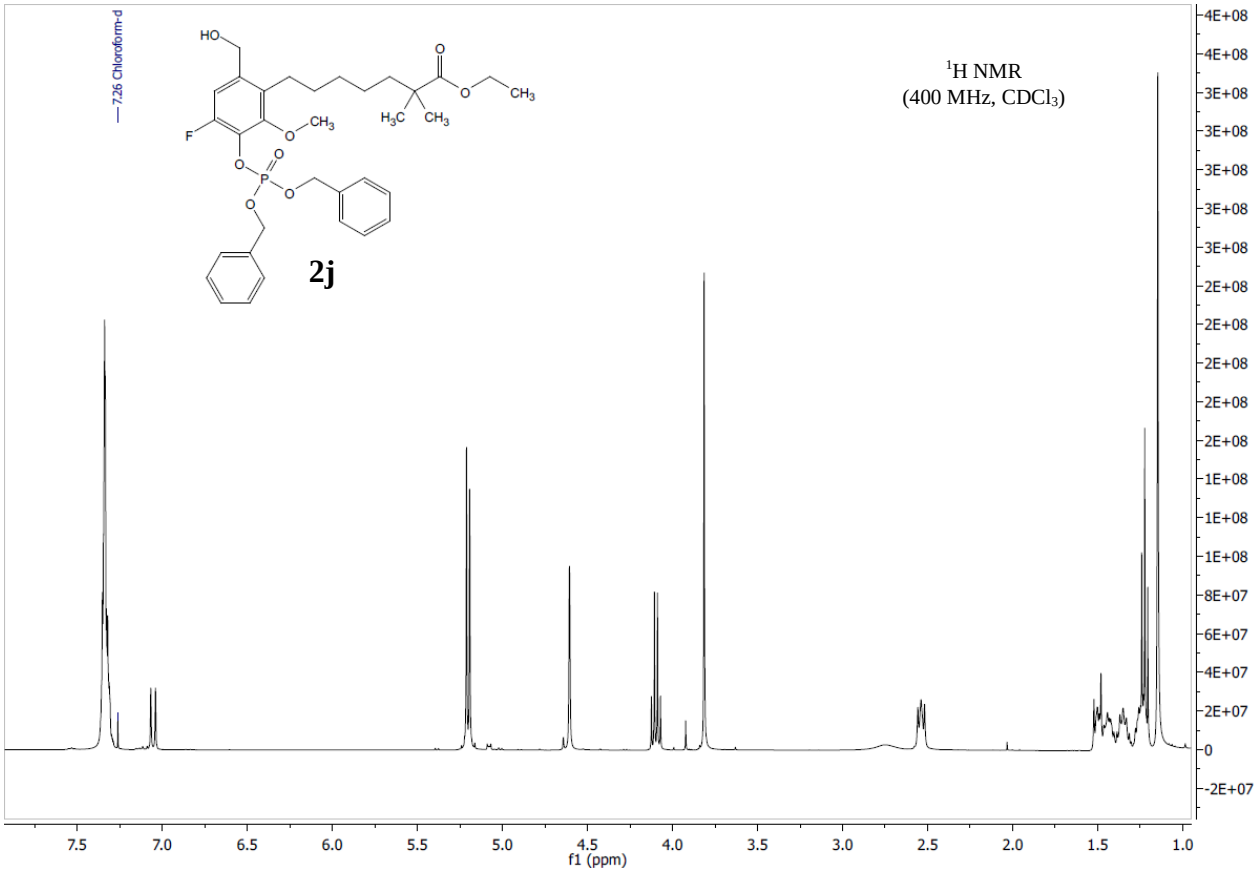

HO
O
O
CH3
H3C
CH3
H3C
O
F
O
P
O
O
O
2g
77.16 Chloroform-d
13C NMR
(101 MHz, CDCl3)
6E+08
5E+08
4E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

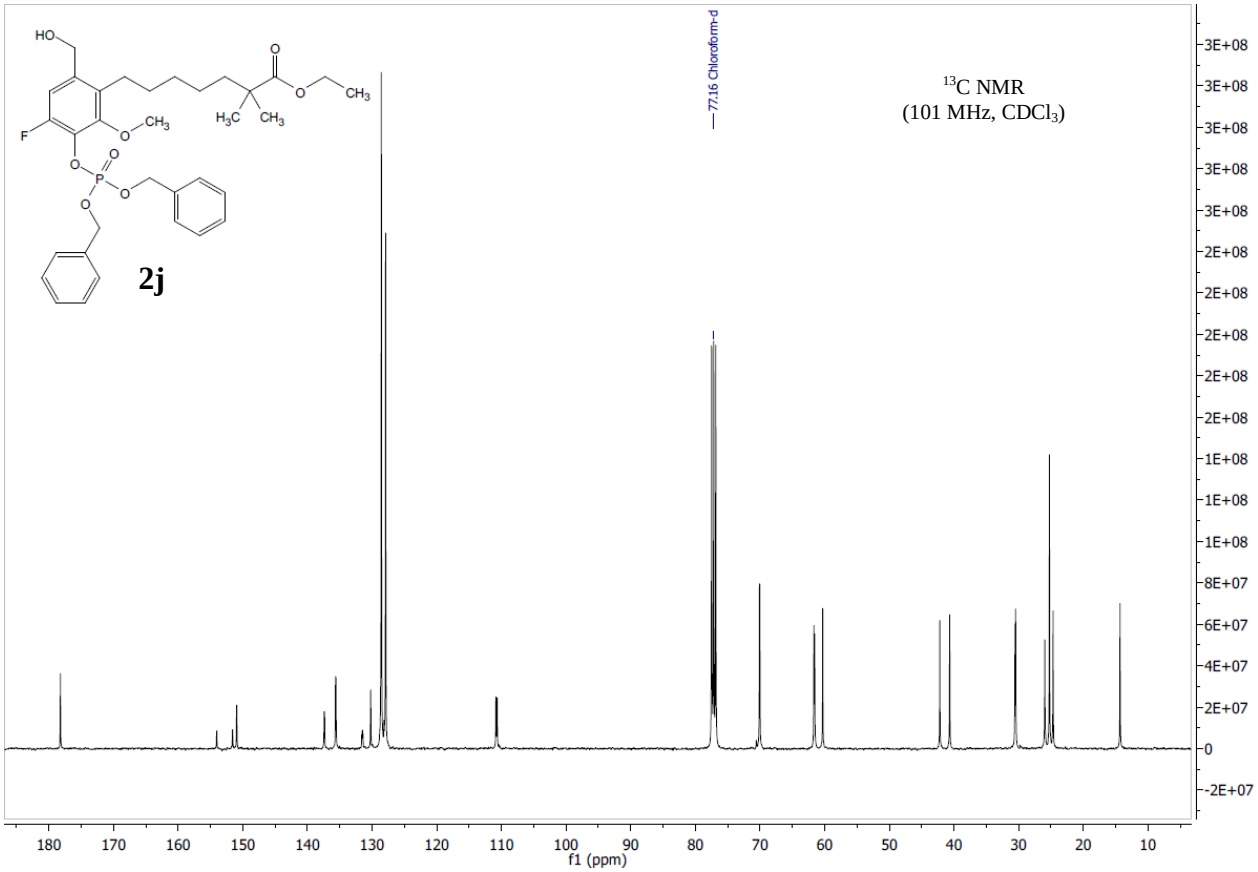

7.26 Chloroform-d
HO
O
CH3
H3C
CH3
H3C
O
O
F
O
P
O
O
2h
1H NMR
(400 MHz, CDCl3)
4E+08
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

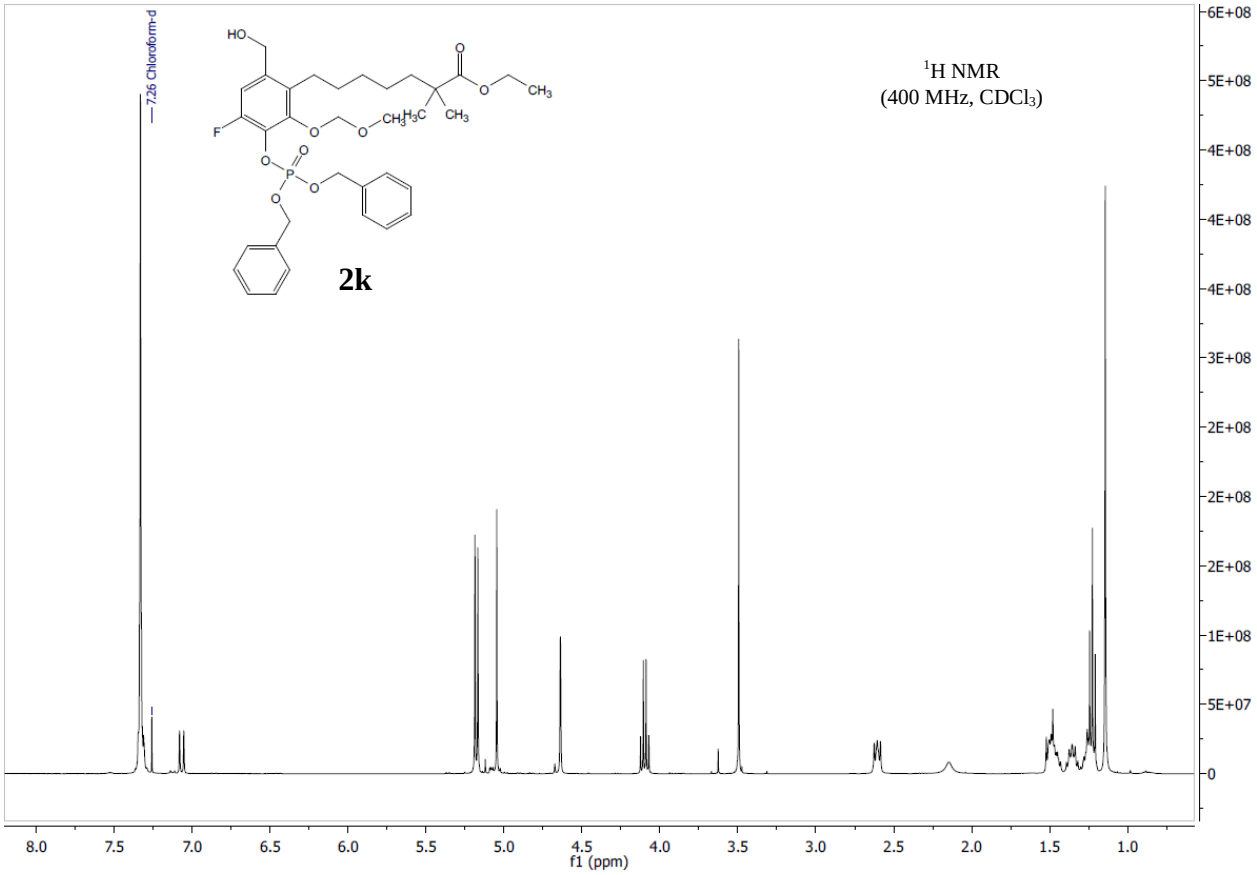

HO
O
CH3
H3C
CH3
H3C
O
O
F
O
P
O
O
2h
77.16 Chloroform-d
13C NMR
(101 MHz, CDCl3)
4E+08
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

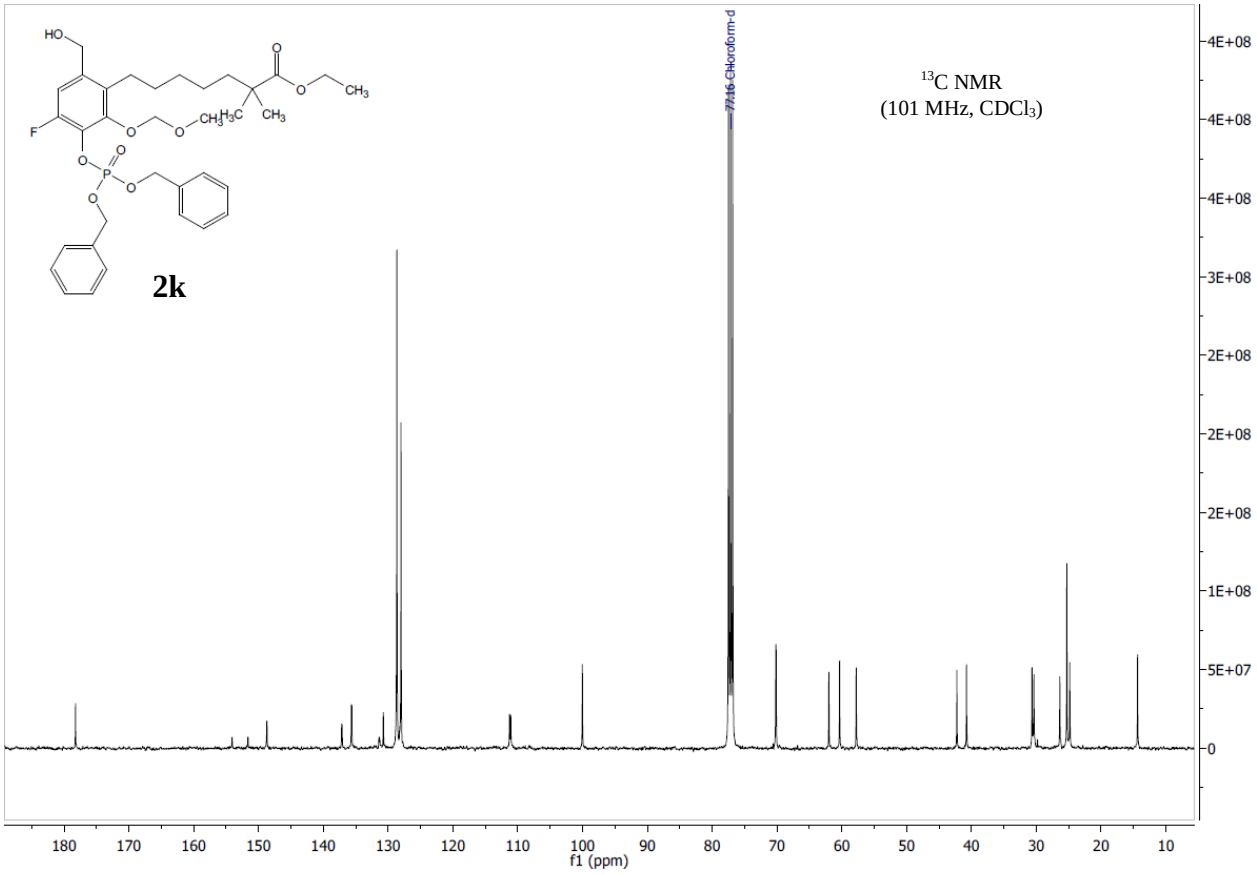

7.26 Chloroform-d
HO
O
O
CH3
H3C
CH3
Cl
F
O
P
O
O
O
2i
1H NMR
(400 MHz, CDCl3)
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

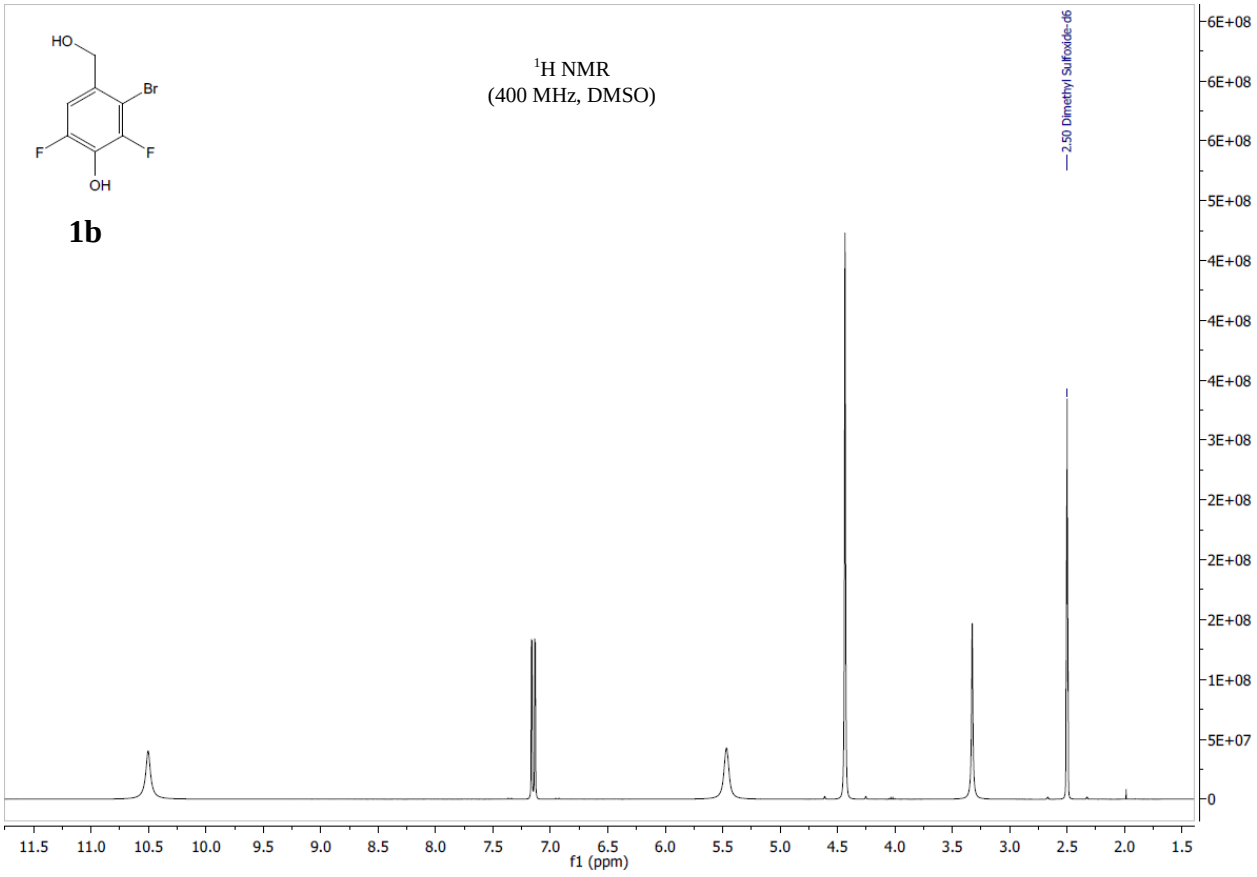

HO
O
O
CH3
H3C
CH3
Cl
F
O
P
O
O
O
2i
13C NMR
(101 MHz, CDCl3)
3E+08
3E+08
3E+08
2E+08
2E+08
2E+08
2E+08
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

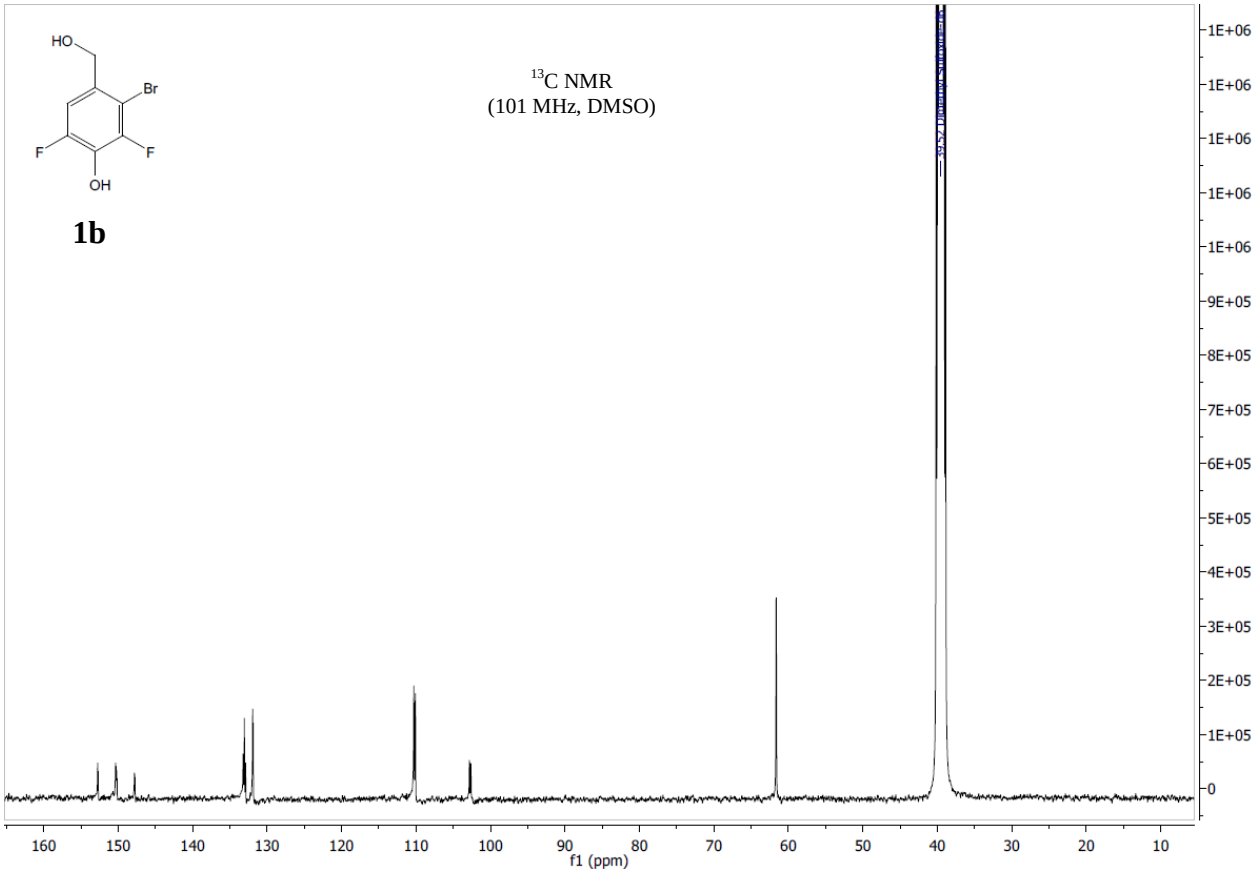

7.26 Chloroform-d
HO
F
O
CH3
H3C
CH3
O
O
P
O
O
O
CH3
2j
1H NMR
(400 MHz, CDCl3)
4E+08
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

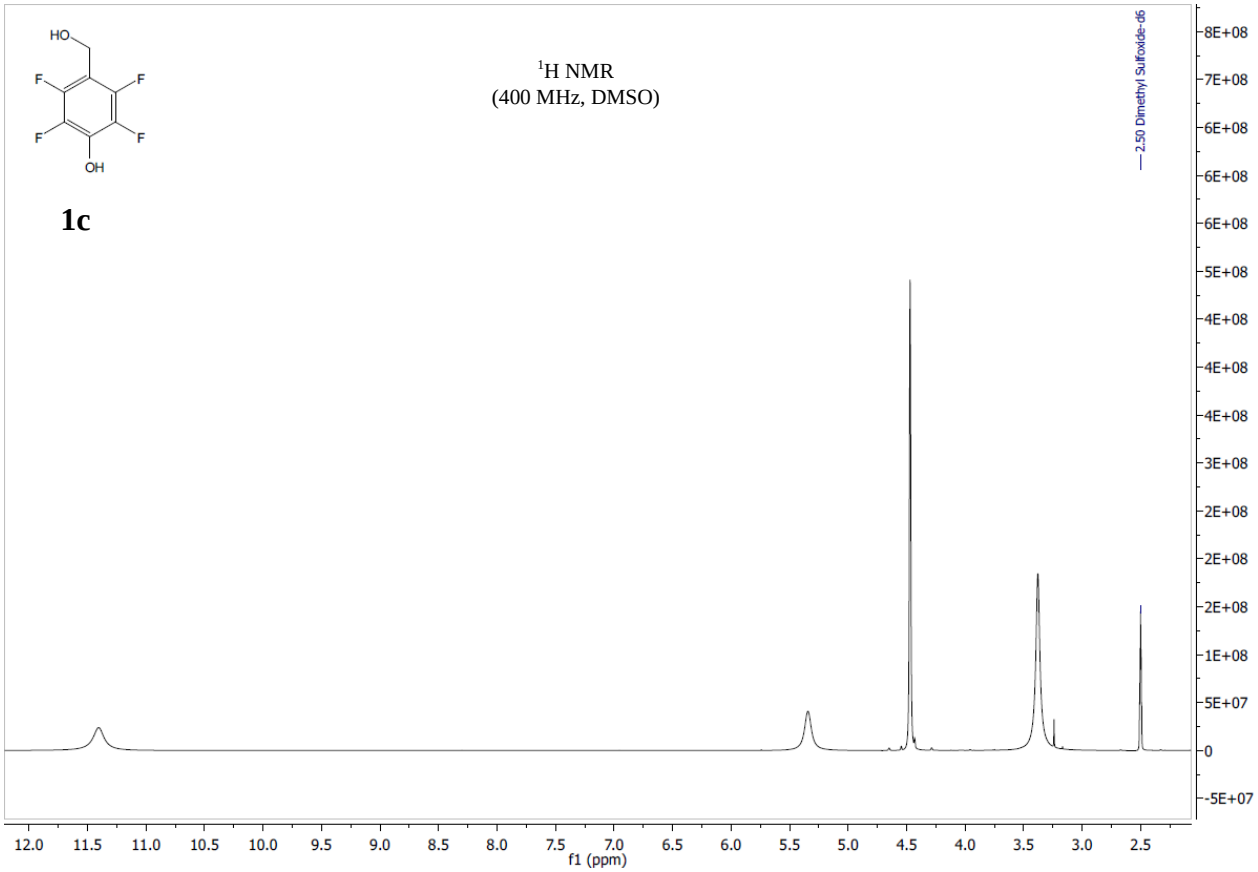

77.16 Chloroform-d
HO
F
O
CH3
H3C
CH3
O
O
P
O
O
O
CH3
2j
13C NMR
(101 MHz, CDCl3)
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

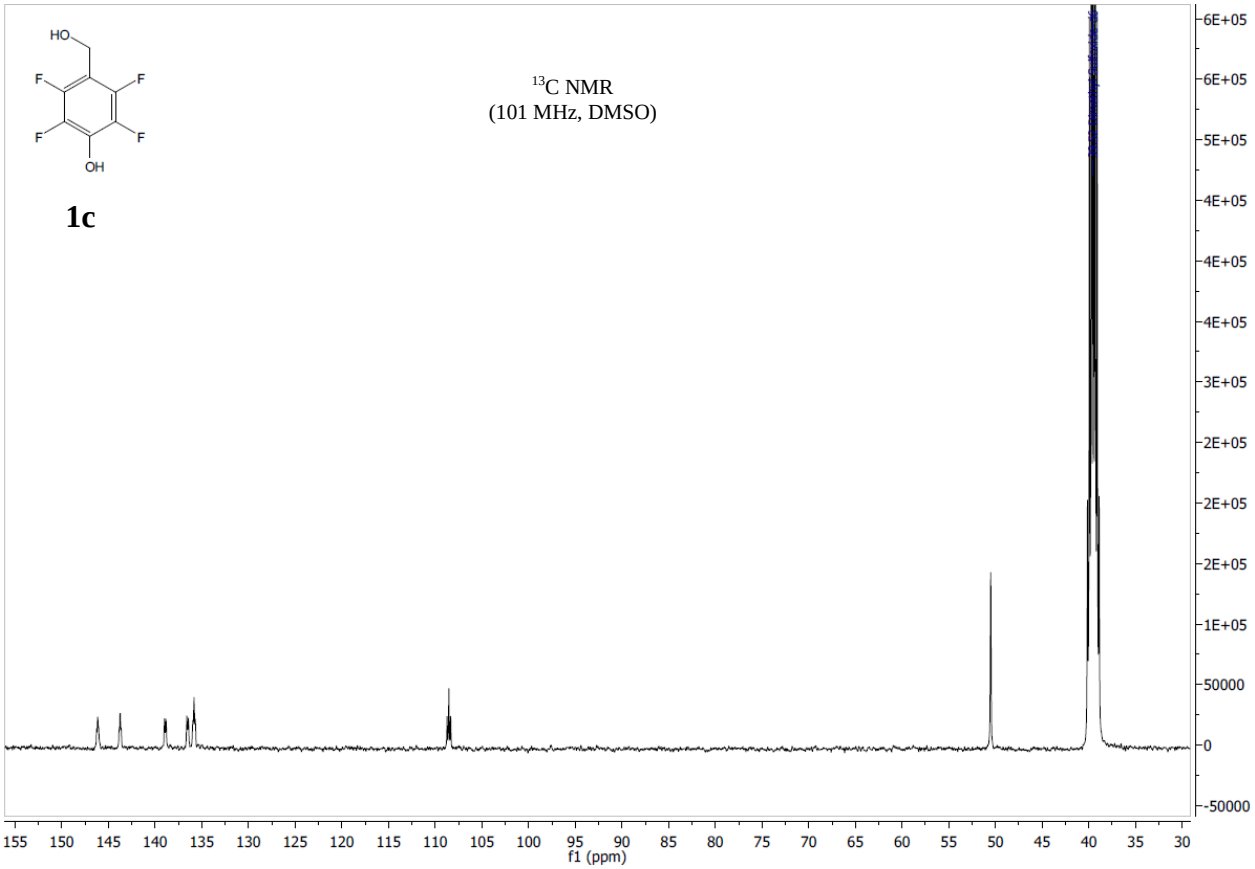

7.26 Chloroform-d
HO
F
O
CH3
H3C
CH3
2k
1H NMR
(400 MHz, CDCl3)
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

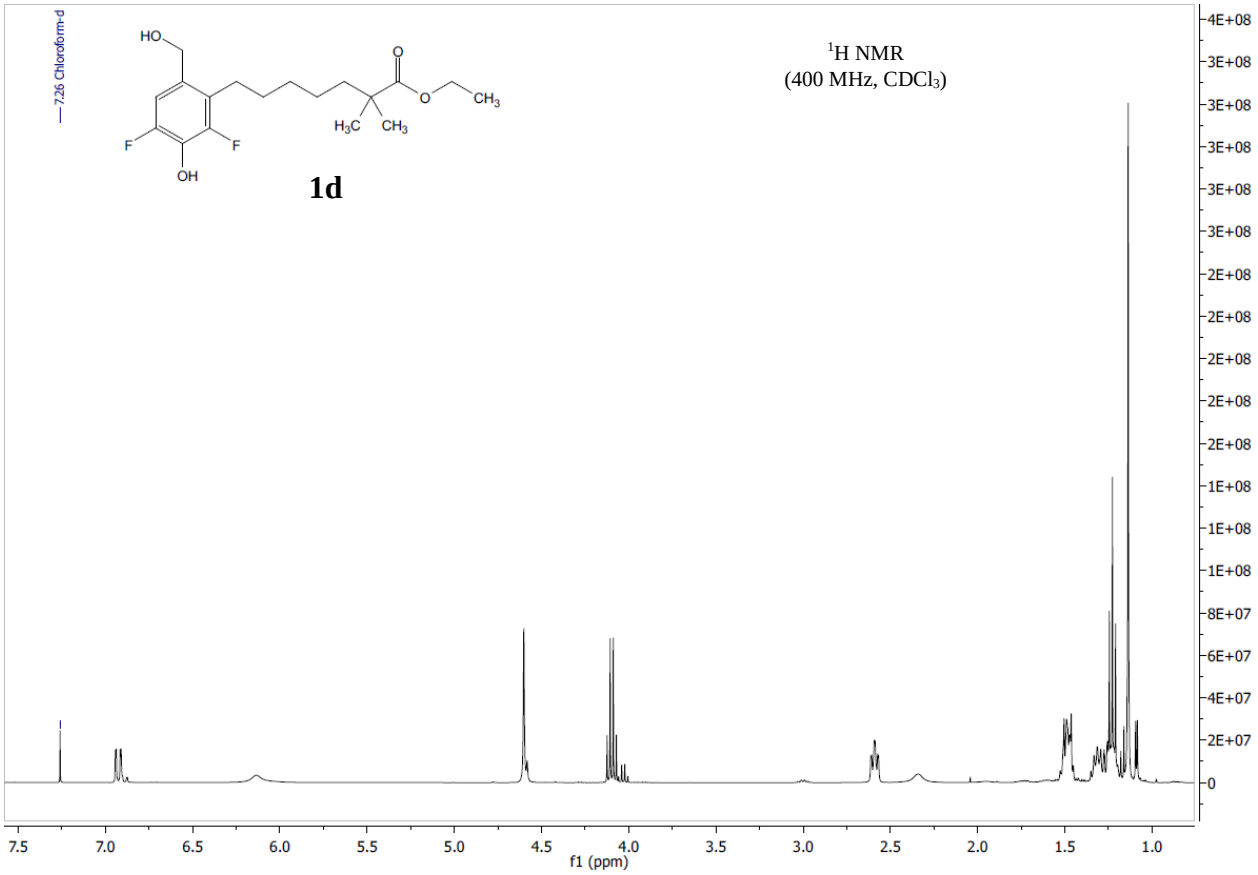

HO
F
O
CH3
H3C
CH3
2k
77.16 Chloroform-d
13C NMR
(101 MHz, CDCl3)
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

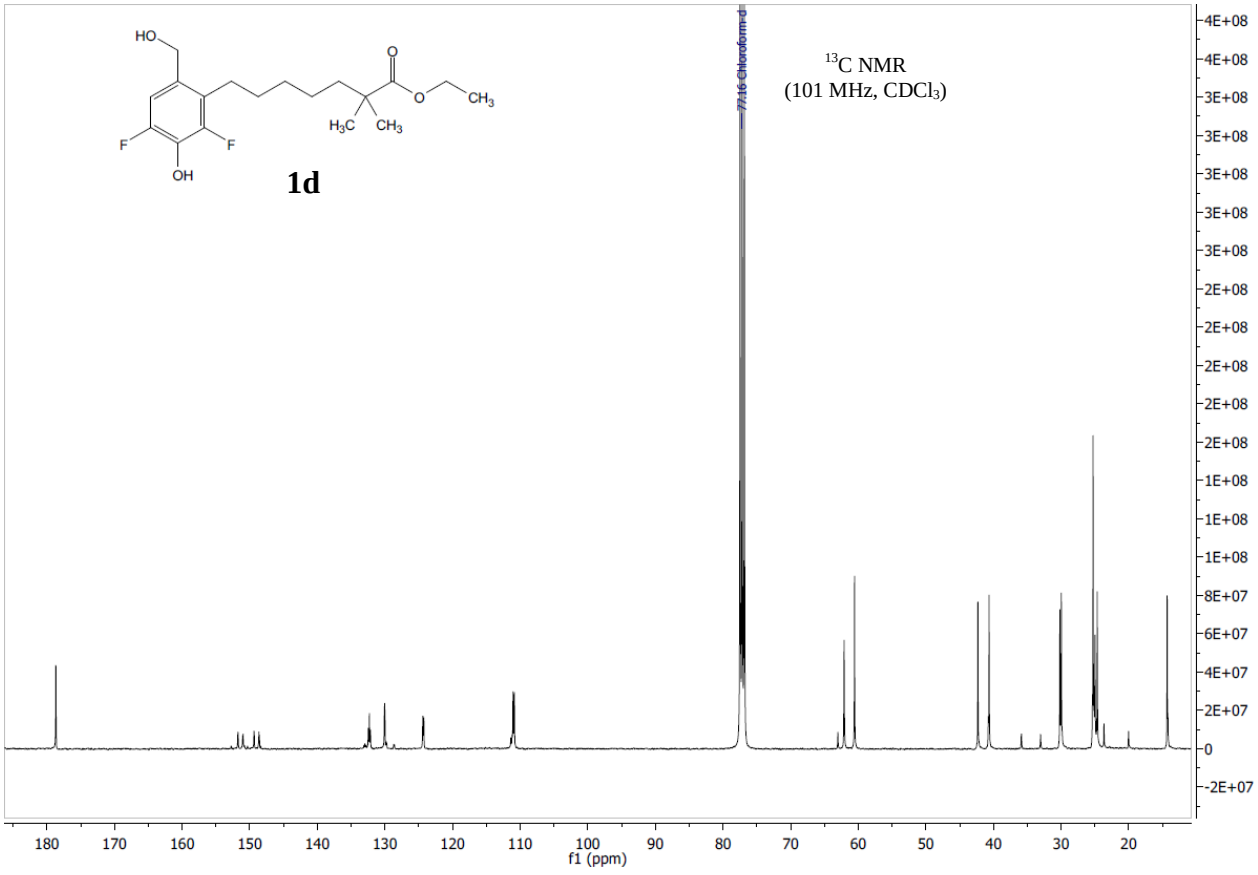

HO
Br
F
F
OH
1b
^{1}H NMR
(400 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)

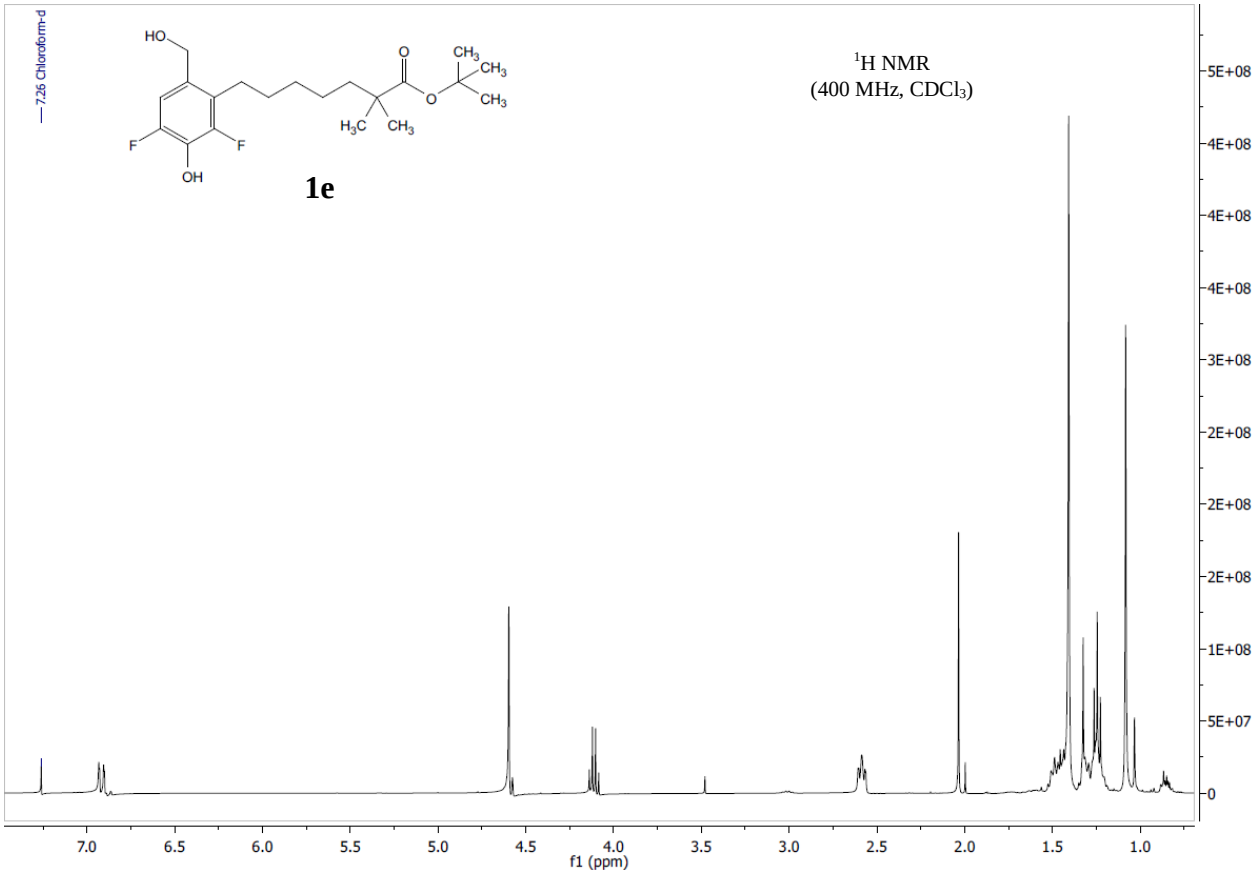

HO
Br
F
F
OH
1b
^{13}C NMR
(101 MHz, DMSO)
39.52 Dimethyl Sulfoxide-d6
f1 (ppm)

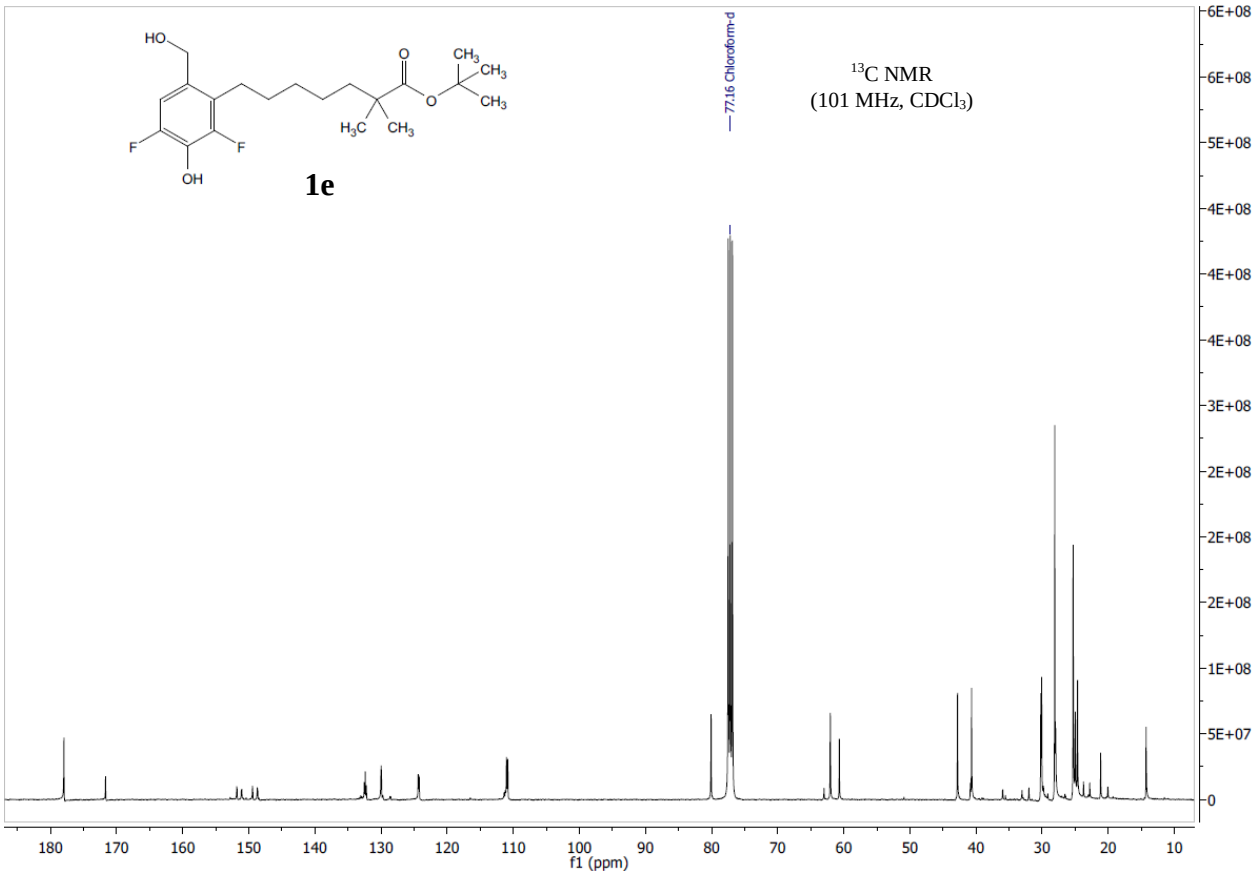

HO
F
F
F
F
OH
1c
^{1}H NMR
(400 MHz, DMSO)
2.50 Dimethyl Sulfoxide-d6
8E+08
7E+08
6E+08
6E+08
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5
f1 (ppm)

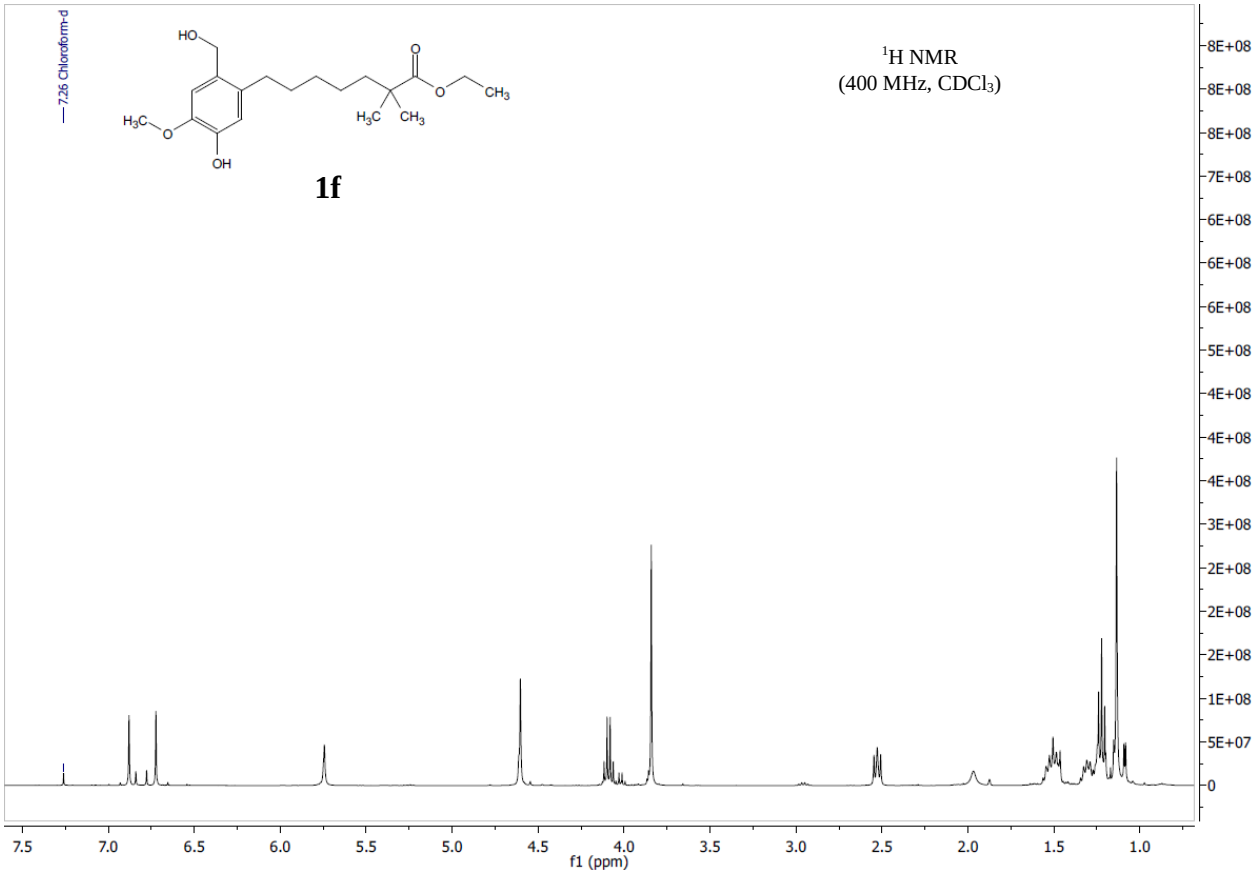

HO
F
F
F
F
OH
1c
^{13}C NMR
(101 MHz, DMSO)
6E+05
6E+05
5E+05
4E+05
4E+05
4E+05
3E+05
2E+05
2E+05
2E+05
1E+05
50000
0
-50000
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30
f1 (ppm)


7.26 Chloroform-d
HO
O
O
CH3
H3C
CH3
F
F
OH
1d
1H NMR
(400 MHz, CDCl3)
4E+08
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
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
f1 (ppm)


77.16 Chloroform-d
HO
O
O
CH3
H3C
CH3
F
F
OH
1d
13C NMR
(101 MHz, CDCl3)
4E+08
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

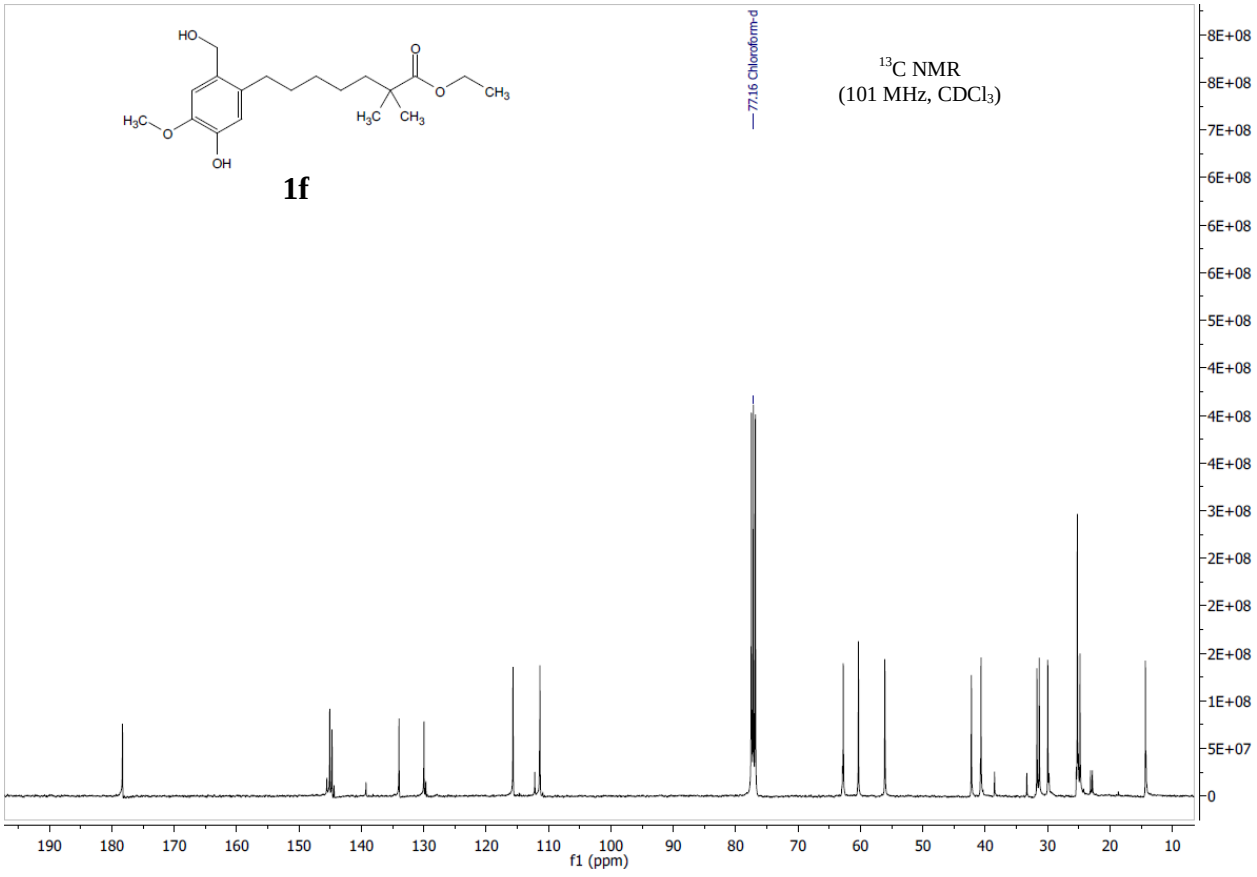

7.26 Chloroform-d
HO
F
F
OH
O
O
CH3
CH3
CH3
H3C
CH3
1e
1H NMR
(400 MHz, CDCl3)
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


77.16 Chloroform-d
HO
F
F
OH
O
O
CH3
CH3
CH3
H3C
CH3
1e
13C NMR
(101 MHz, CDCl3)
6E+08
6E+08
5E+08
4E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)



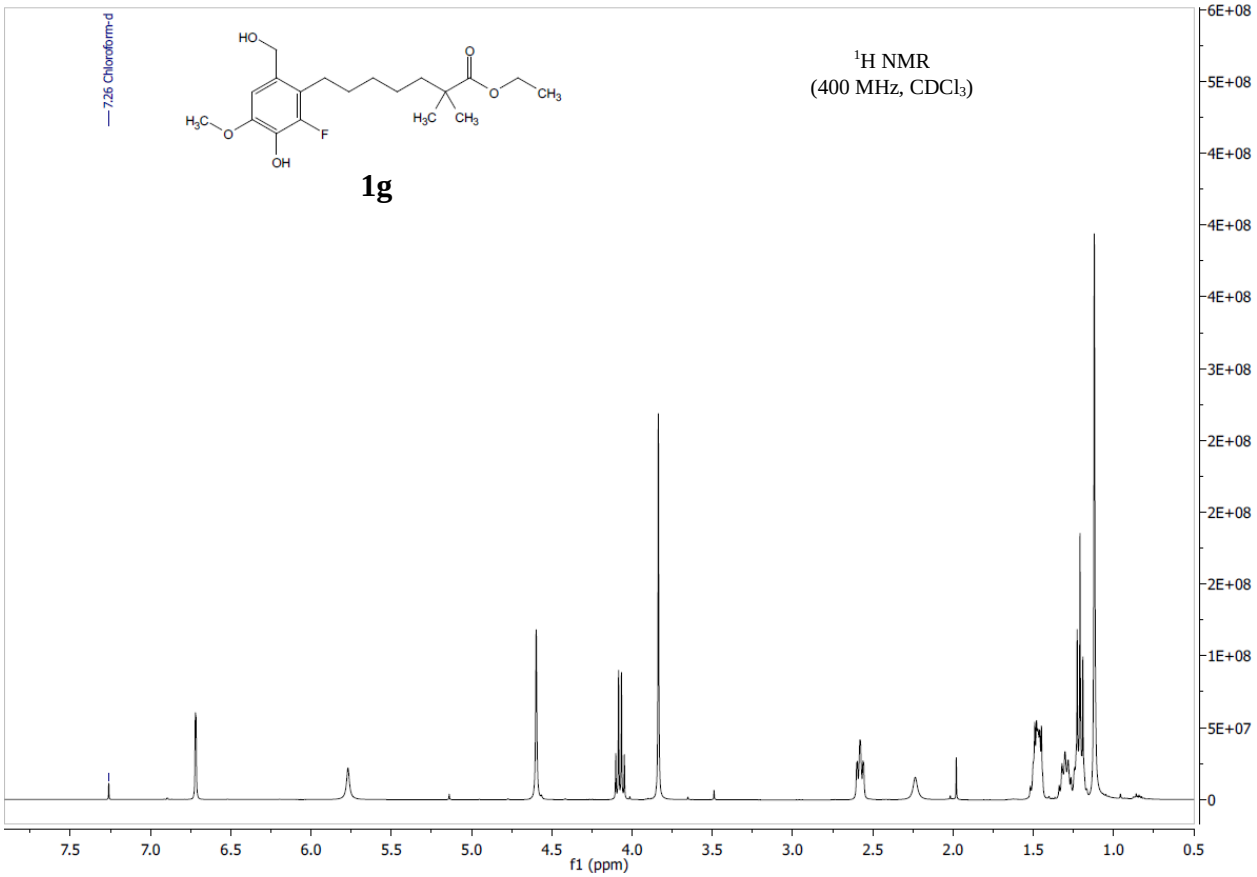
1f

^{1}H NMR
(400 MHz, $CDCl_3$)



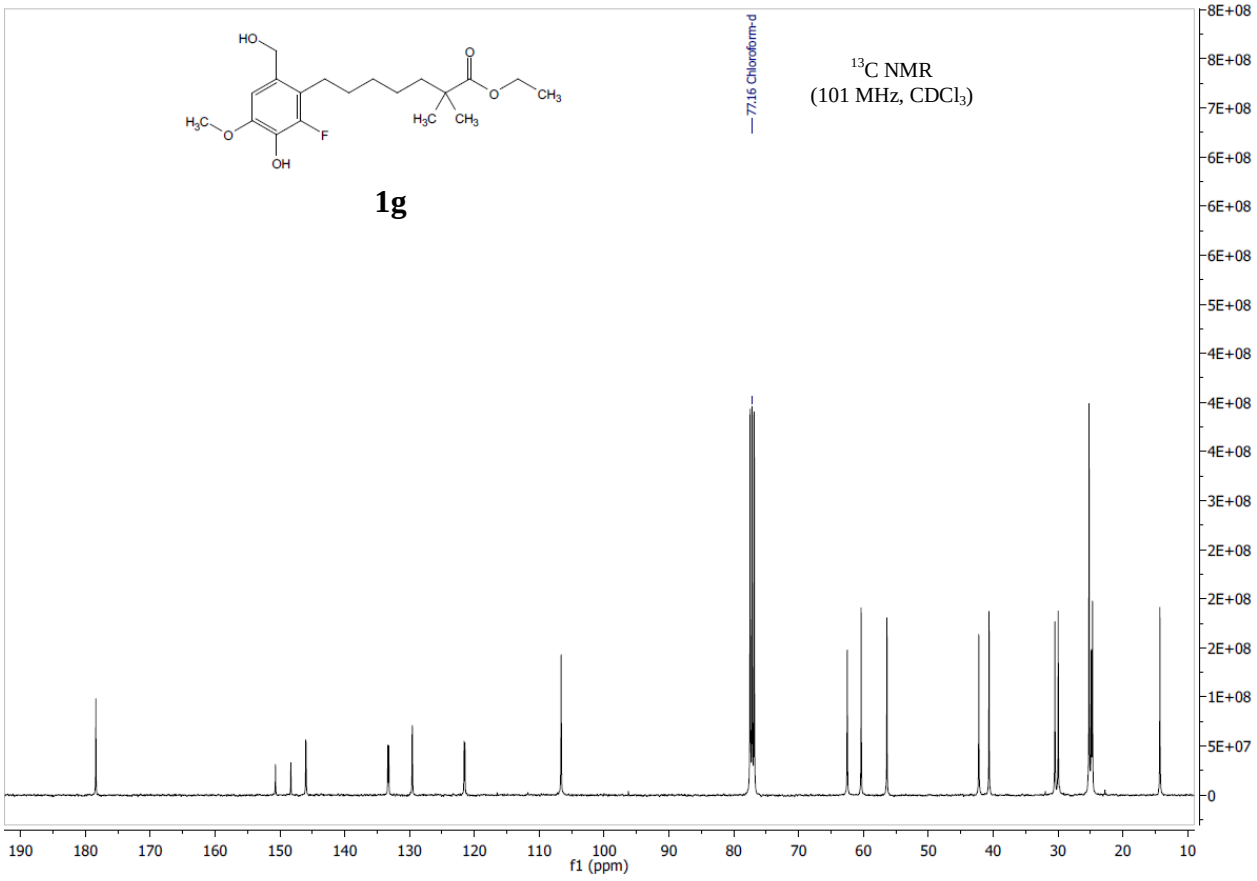
1f

^{13}C NMR
(101 MHz, $CDCl_3$)



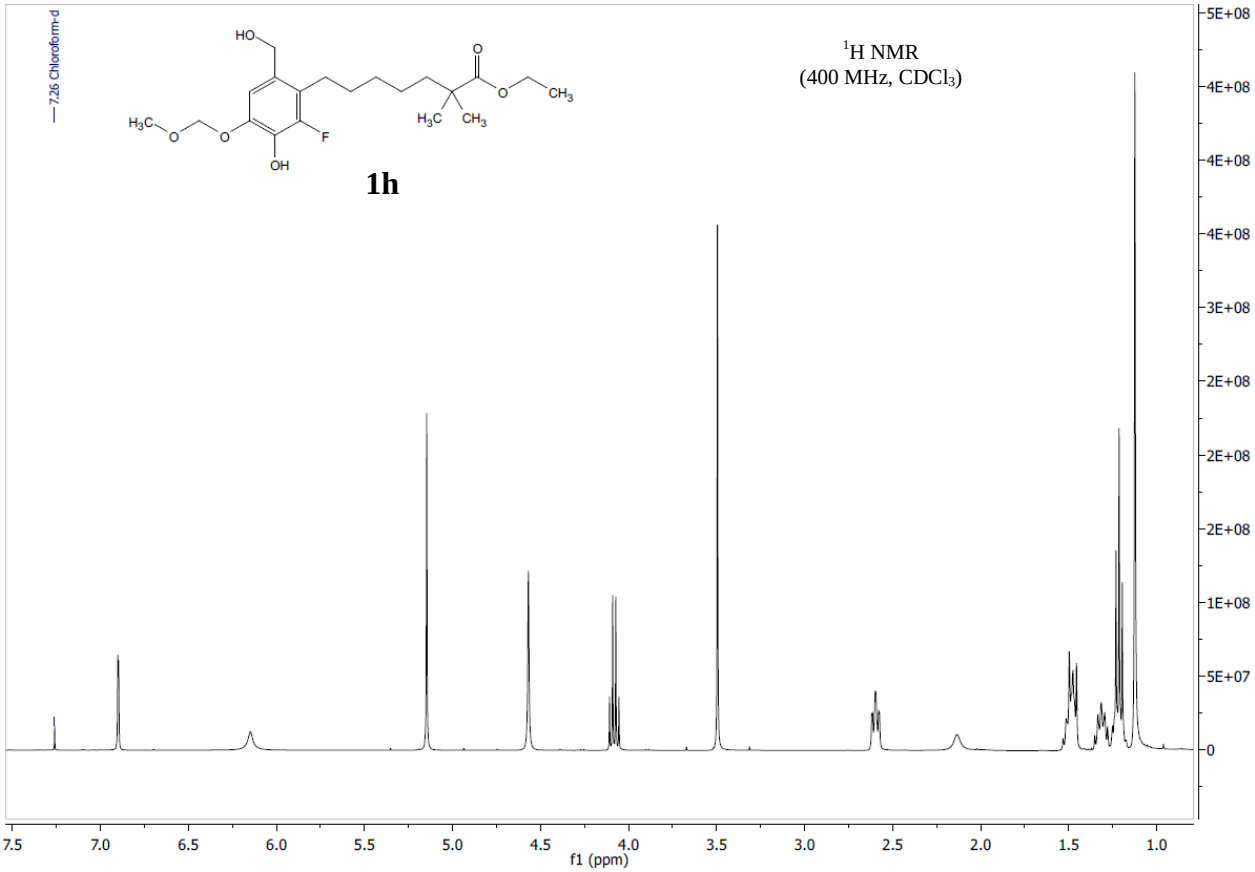
1g

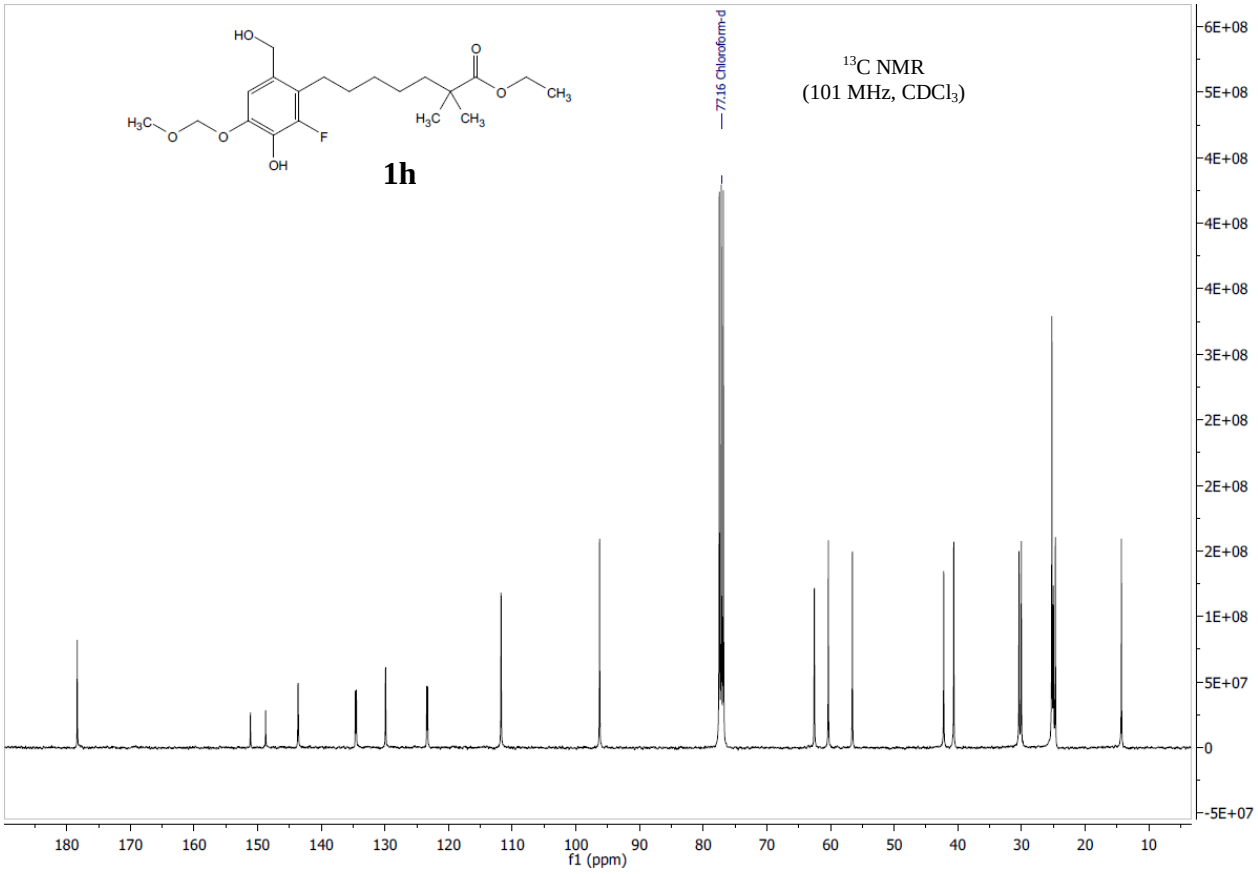
^{1}H NMR
(400 MHz, $CDCl_3$)

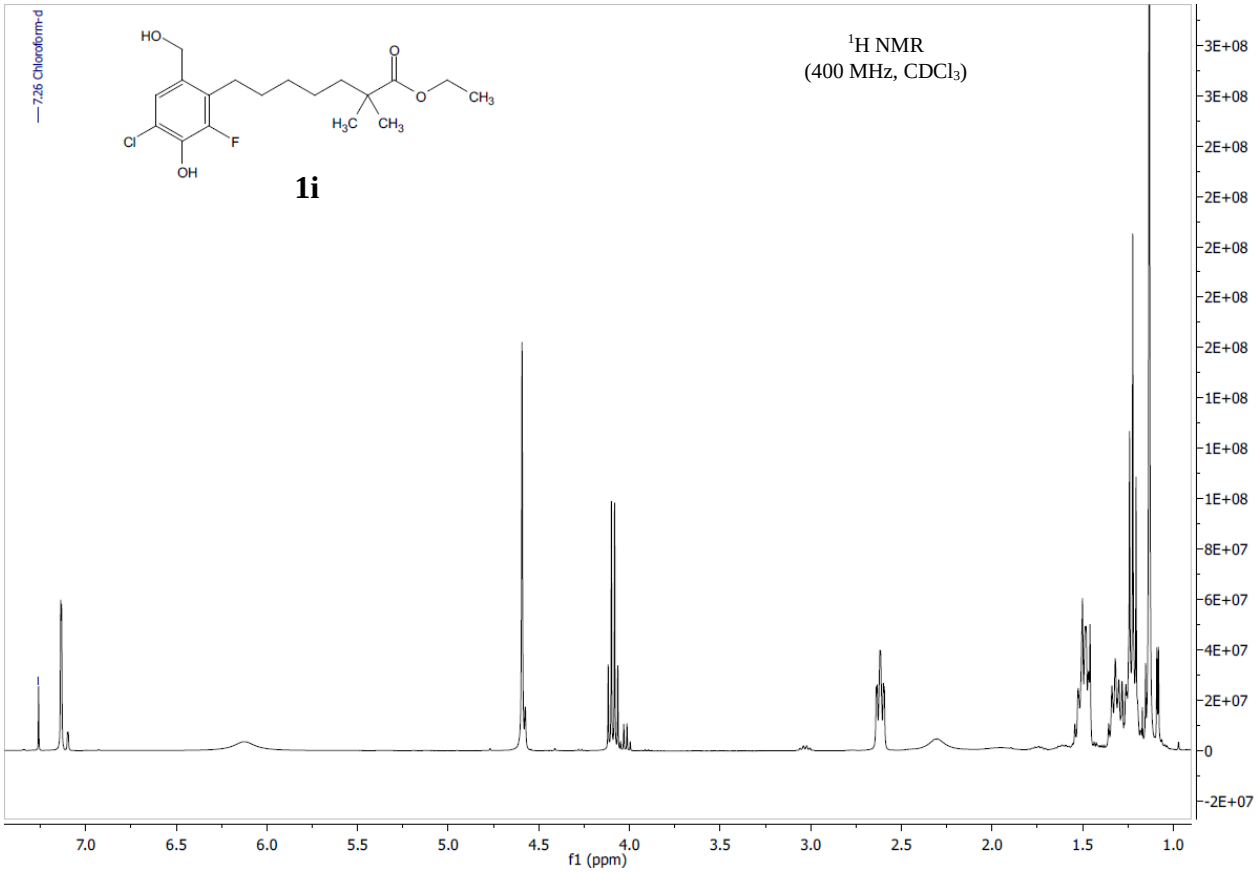


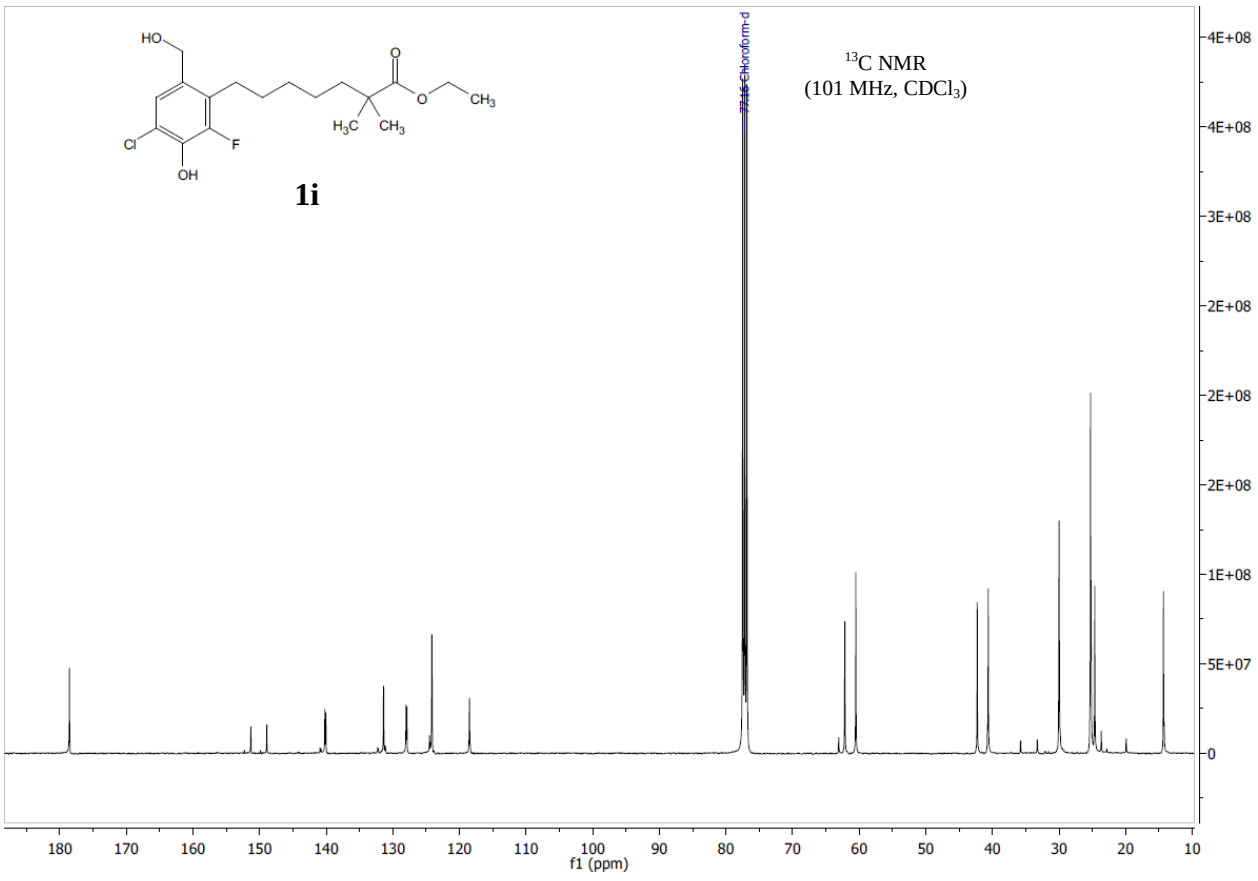
1g

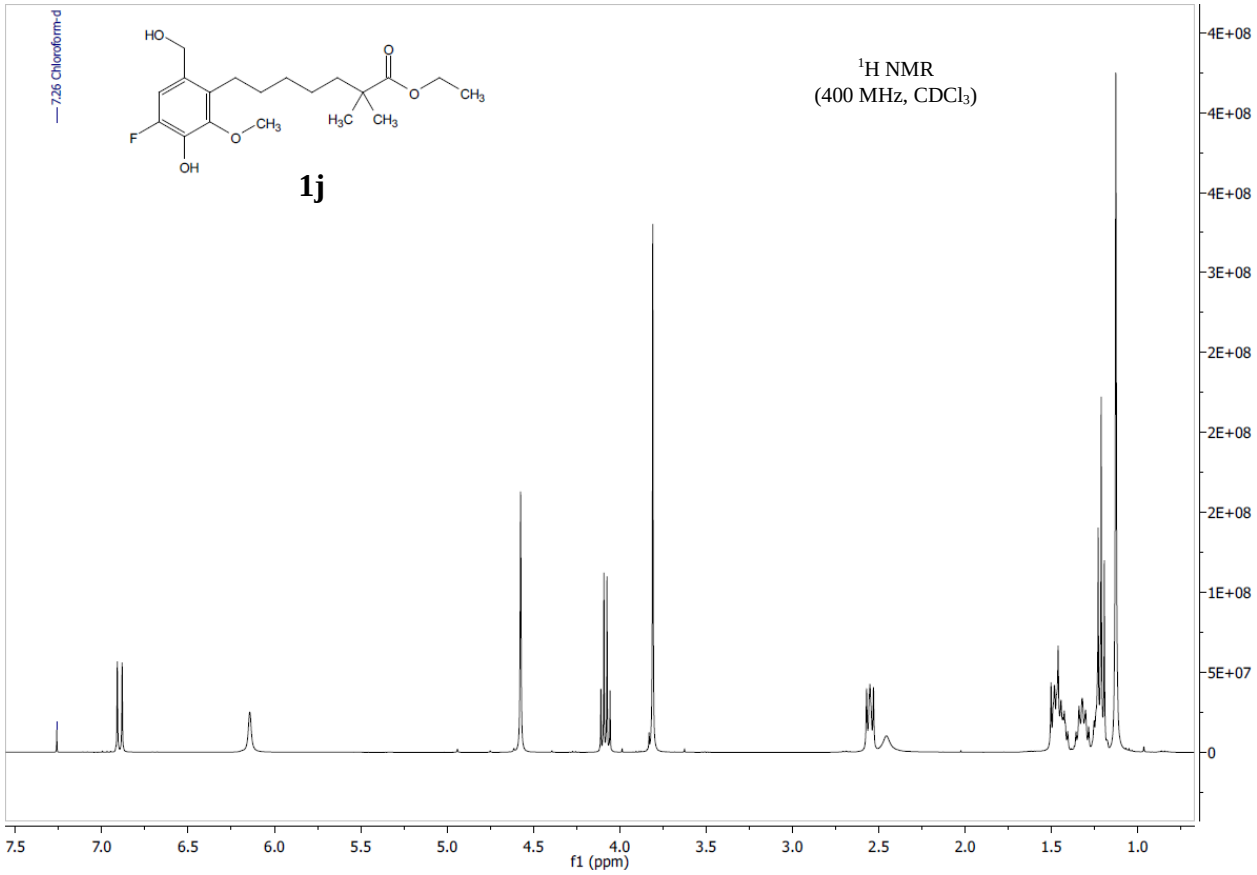
^{13}C NMR
(101 MHz, $CDCl_3$)

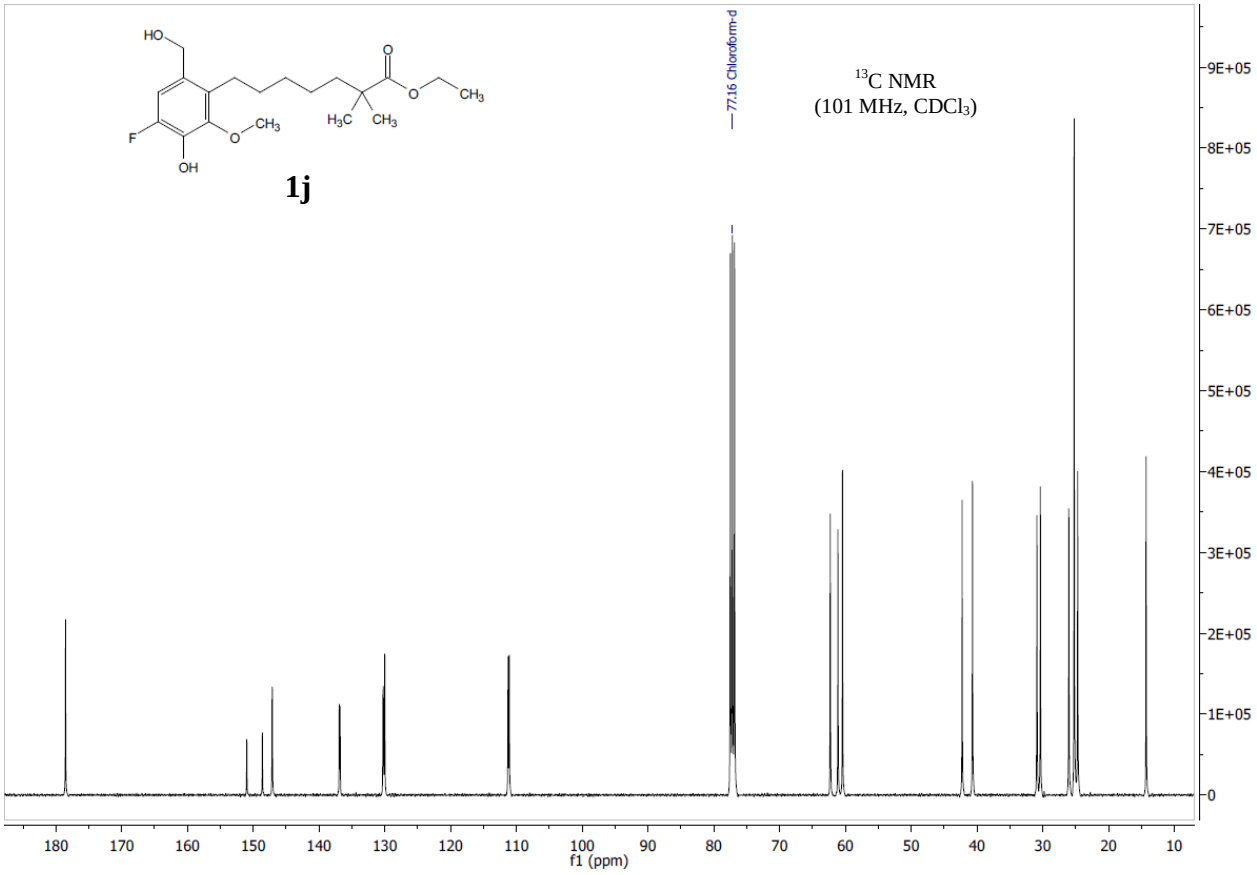

7.26 Chloroform-d
HO
O
O
CH3
H3C
O
O
H3C
CH3
F
OH
1h
1H NMR
(400 MHz, CDCl3)
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

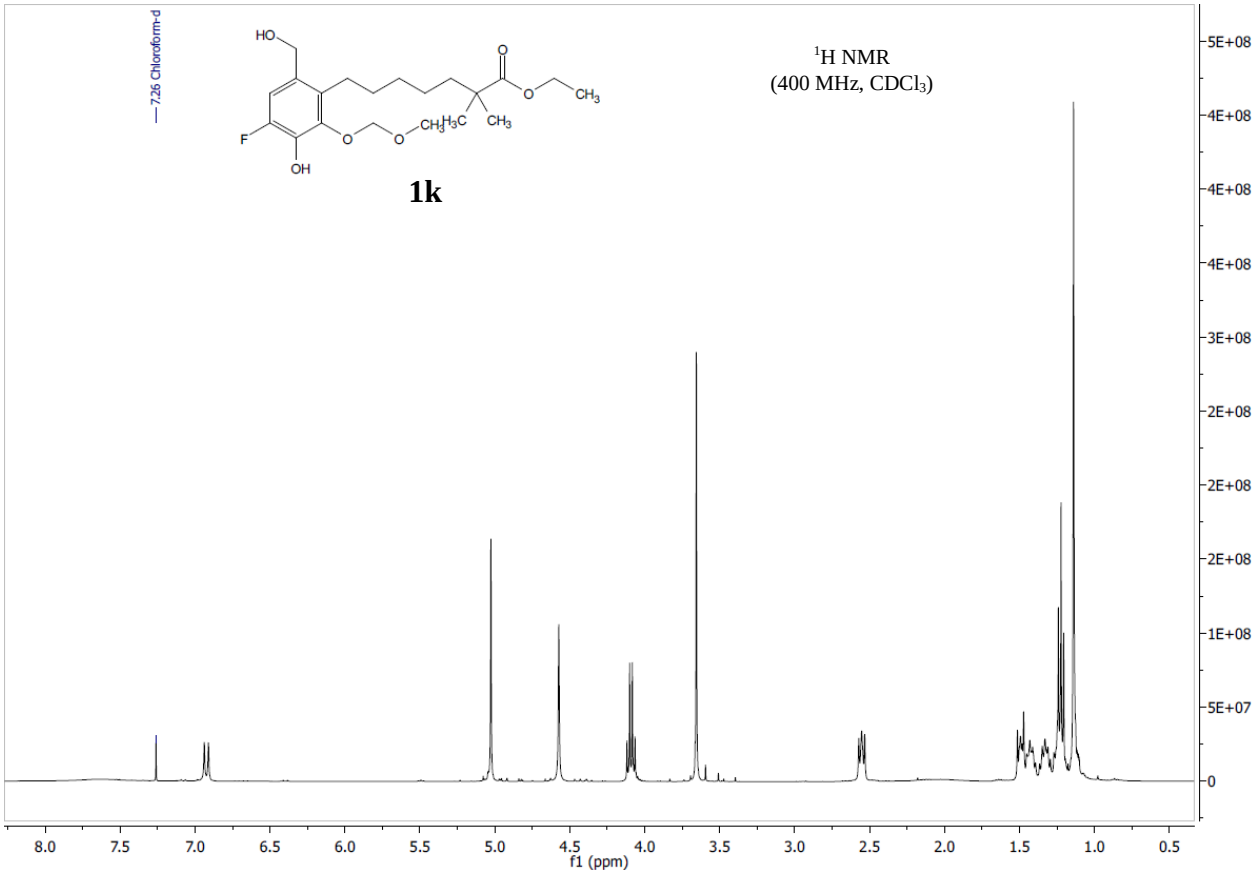

HO
O
O
CH3
H3C
O
O
H3C
CH3
F
OH
1h
77.16 Chloroform-d
13C NMR
(101 MHz, CDCl3)
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


7.26 Chloroform-d
HO
O
O
CH3
H3C
CH3
Cl
F
OH
1i
1H NMR
(400 MHz, CDCl3)
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
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
f1 (ppm)


HO
O
O
CH3
H3C
CH3
Cl
F
OH
1i
13C NMR
(101 MHz, CDCl3)
77.16 Chloroform-d
4E+08
3E+08
2E+08
1E+08
5E+07
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

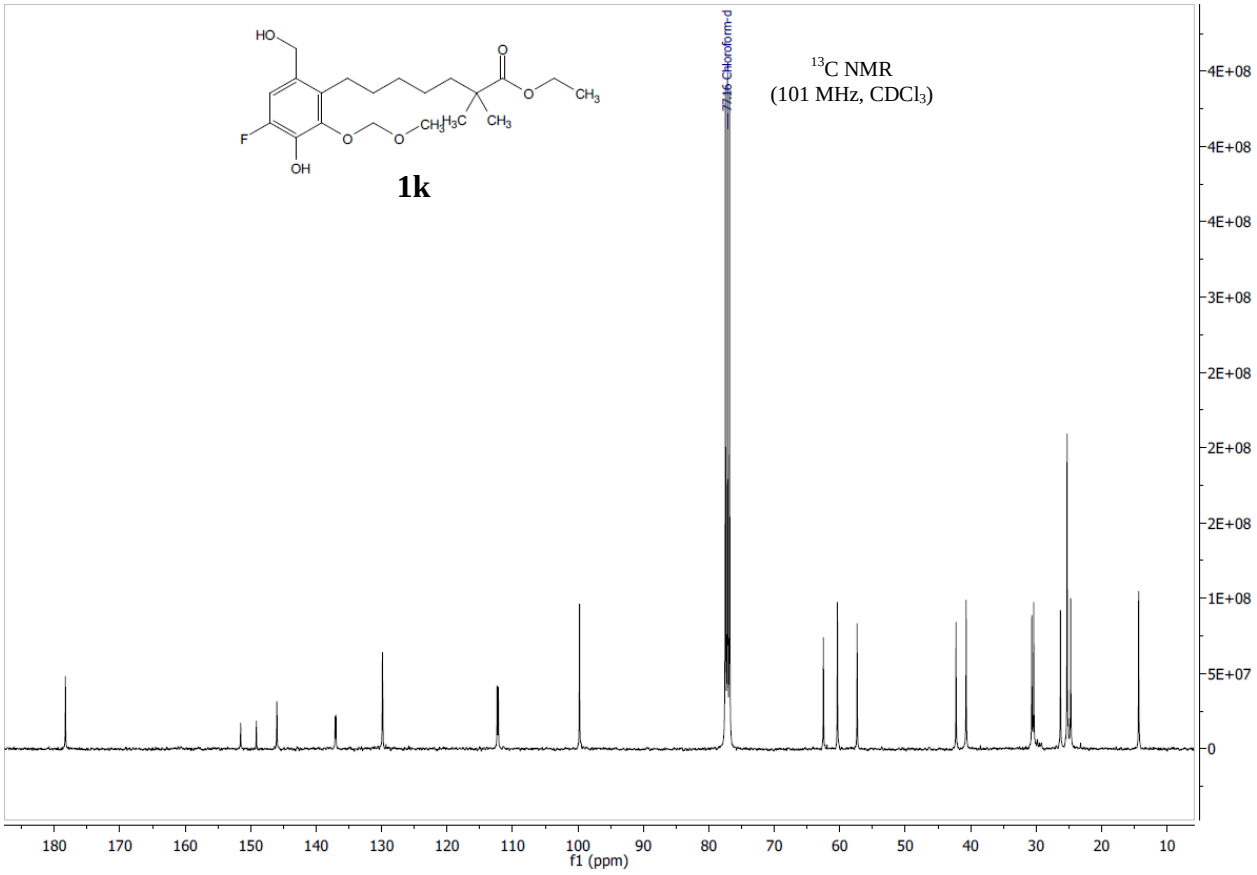

7.26 Chloroform-d
HO
F
OH
O
CH3
H3C
CH3
O
O
CH3
1j
1H NMR
(400 MHz, CDCl3)
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


77.16 Chloroform-d
HO
F
OH
O
CH3
H3C
CH3
O
O
CH3
1j
13C NMR
(101 MHz, CDCl3)
9E+05
8E+05
7E+05
6E+05
5E+05
4E+05
3E+05
2E+05
1E+05
0
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)



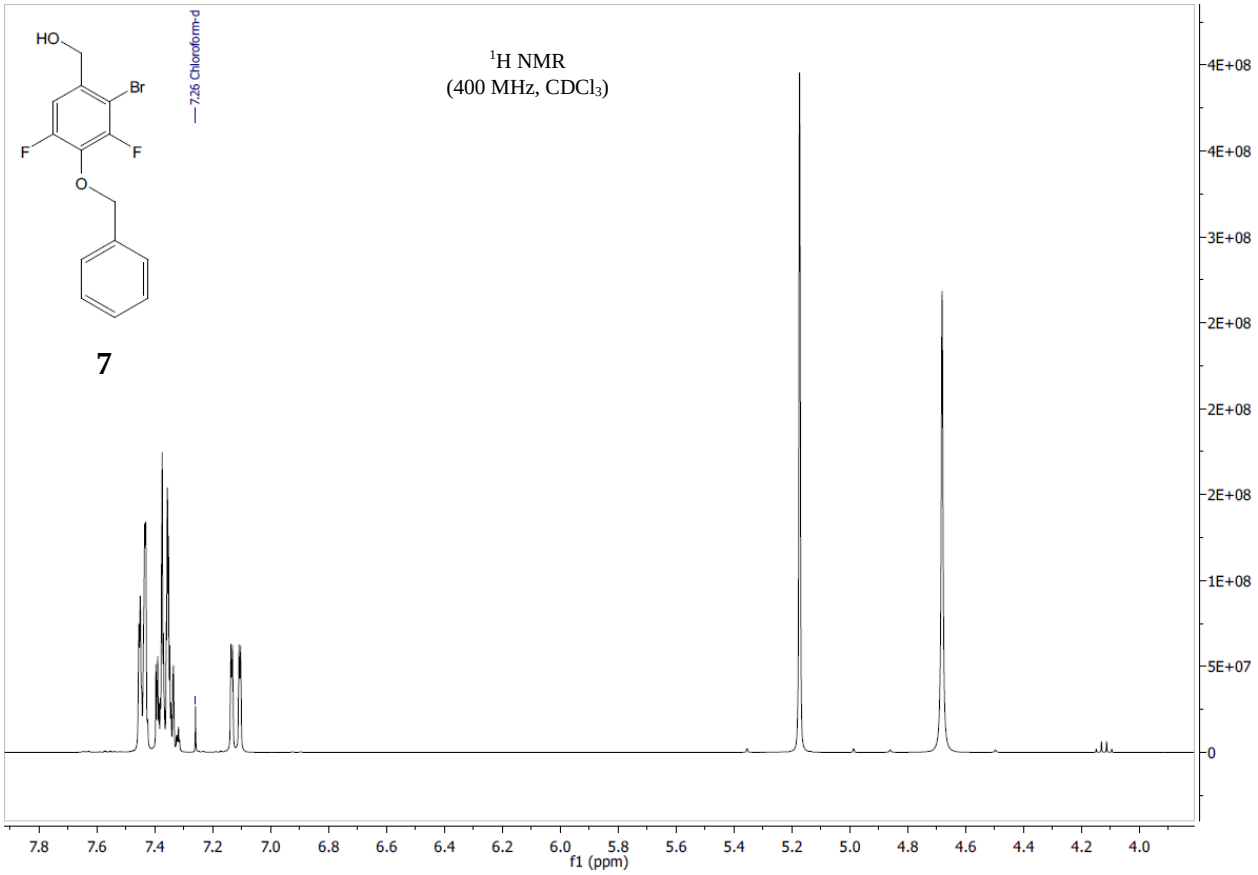
1k

^{1}H NMR
(400 MHz, $CDCl_3$)

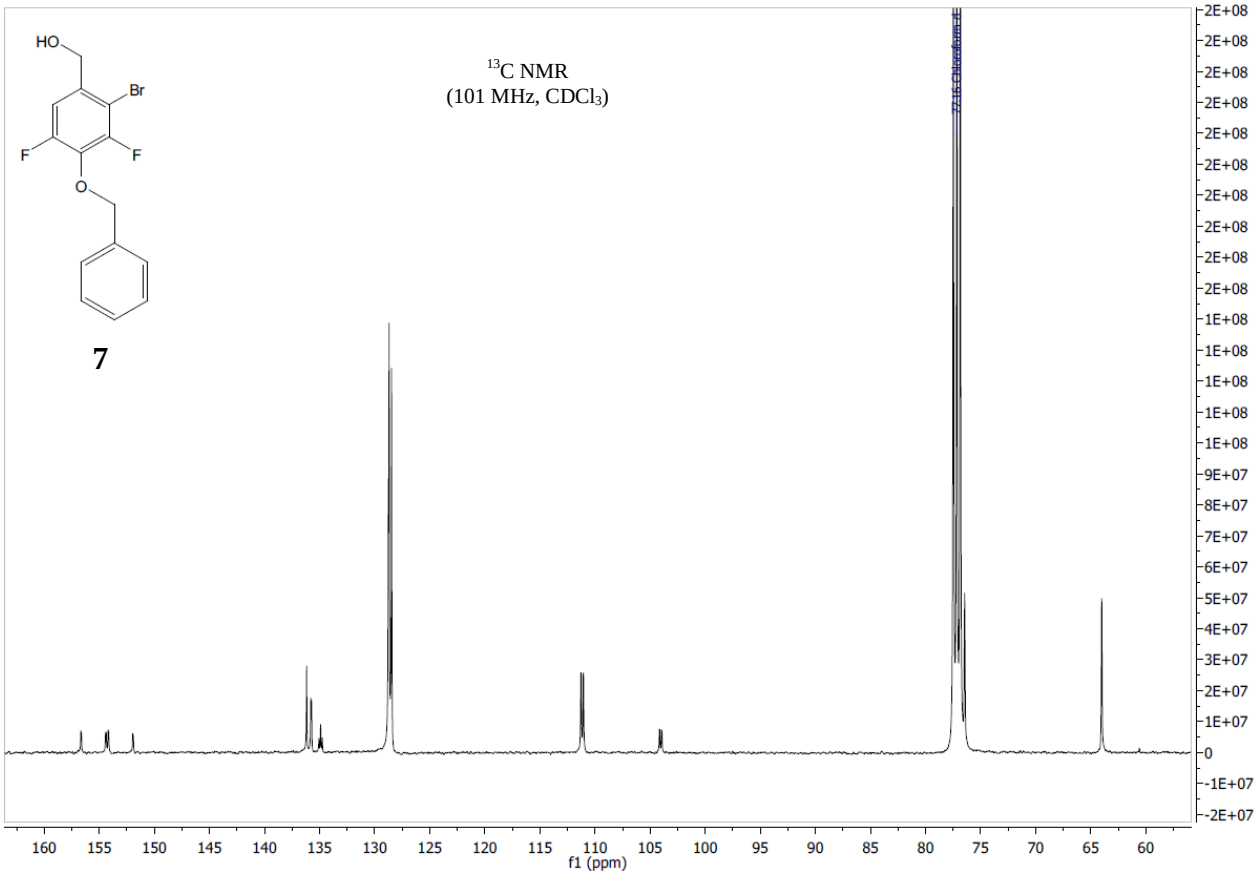


1k

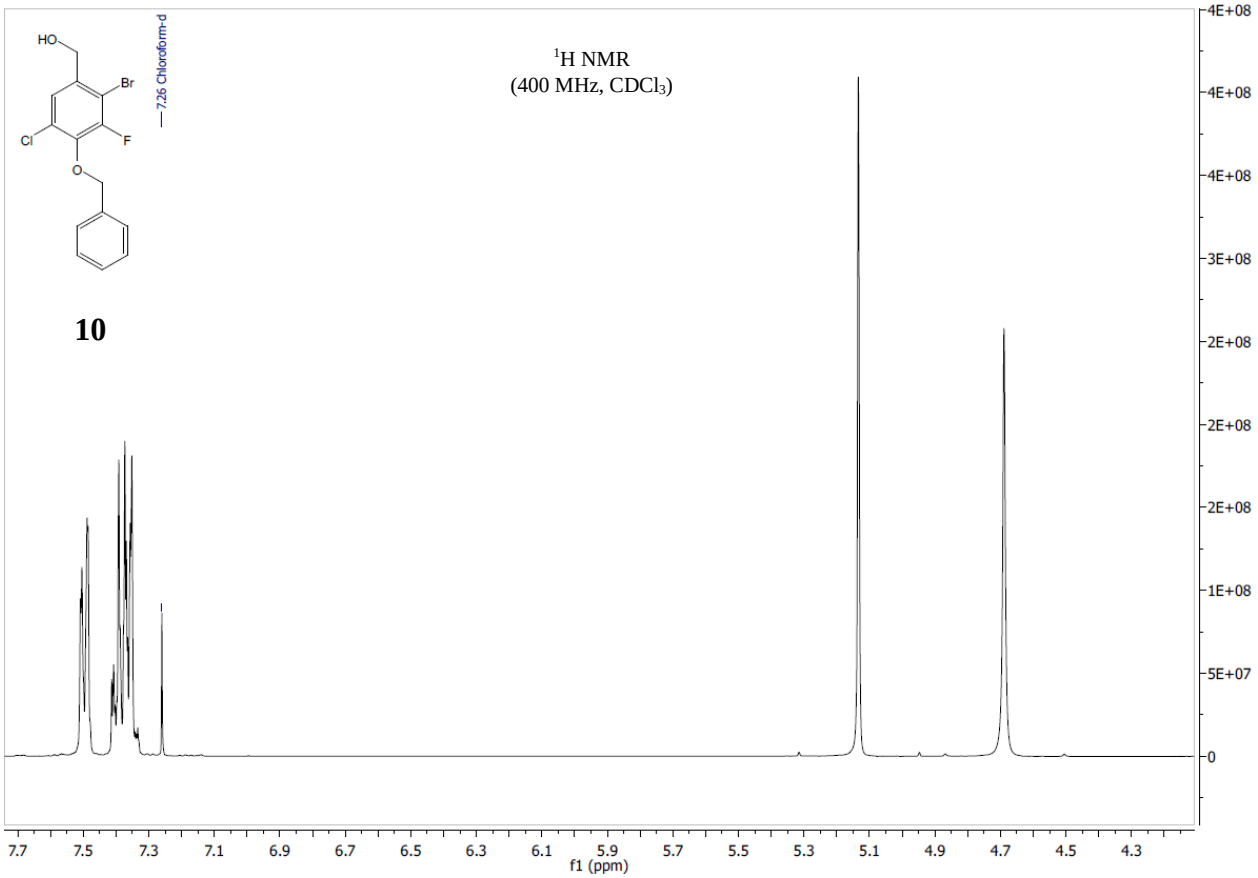
^{13}C NMR
(101 MHz, $CDCl_3$)

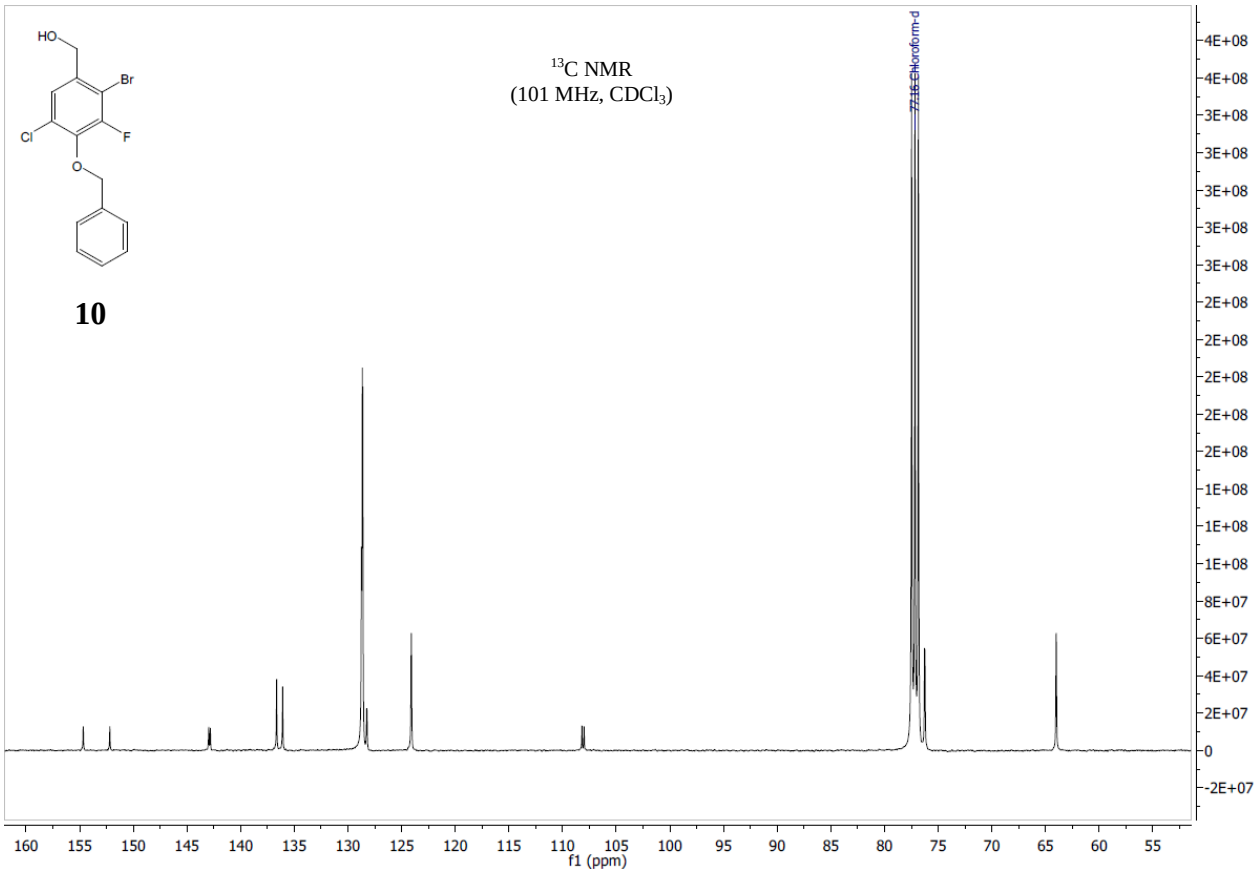

HO
Br
F
F
O
7.26 Chloroform-d
^{1}H NMR
(400 MHz, $CDCl_3$)
4E+08
3E+08
2E+08
1E+08
5E+07
0
7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0
f1 (ppm)

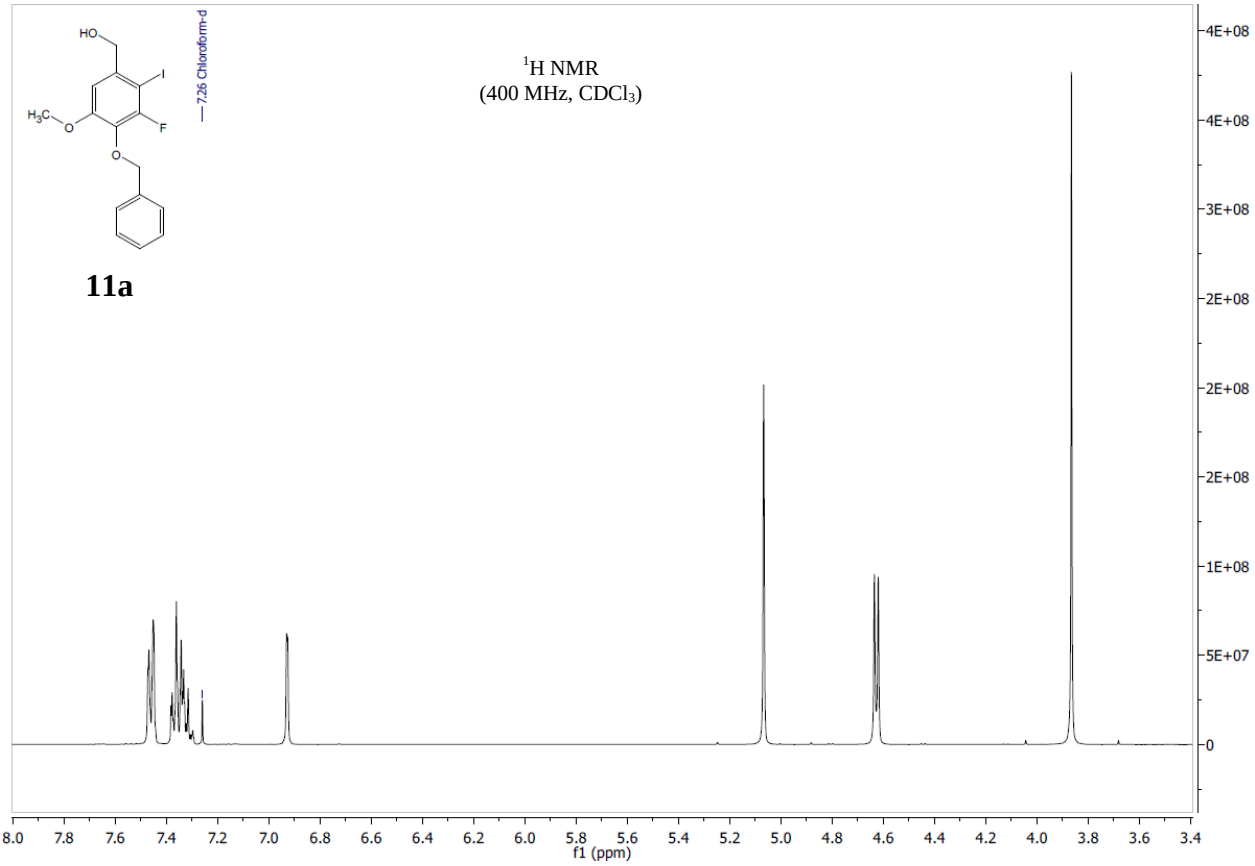
7


HO
Br
F
F
O
^{13}C NMR
(101 MHz, $CDCl_3$)
2E+08
1E+08
9E+07
8E+07
7E+07
6E+07
5E+07
4E+07
3E+07
2E+07
1E+07
0
-1E+07
-2E+07
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60
f1 (ppm)

7

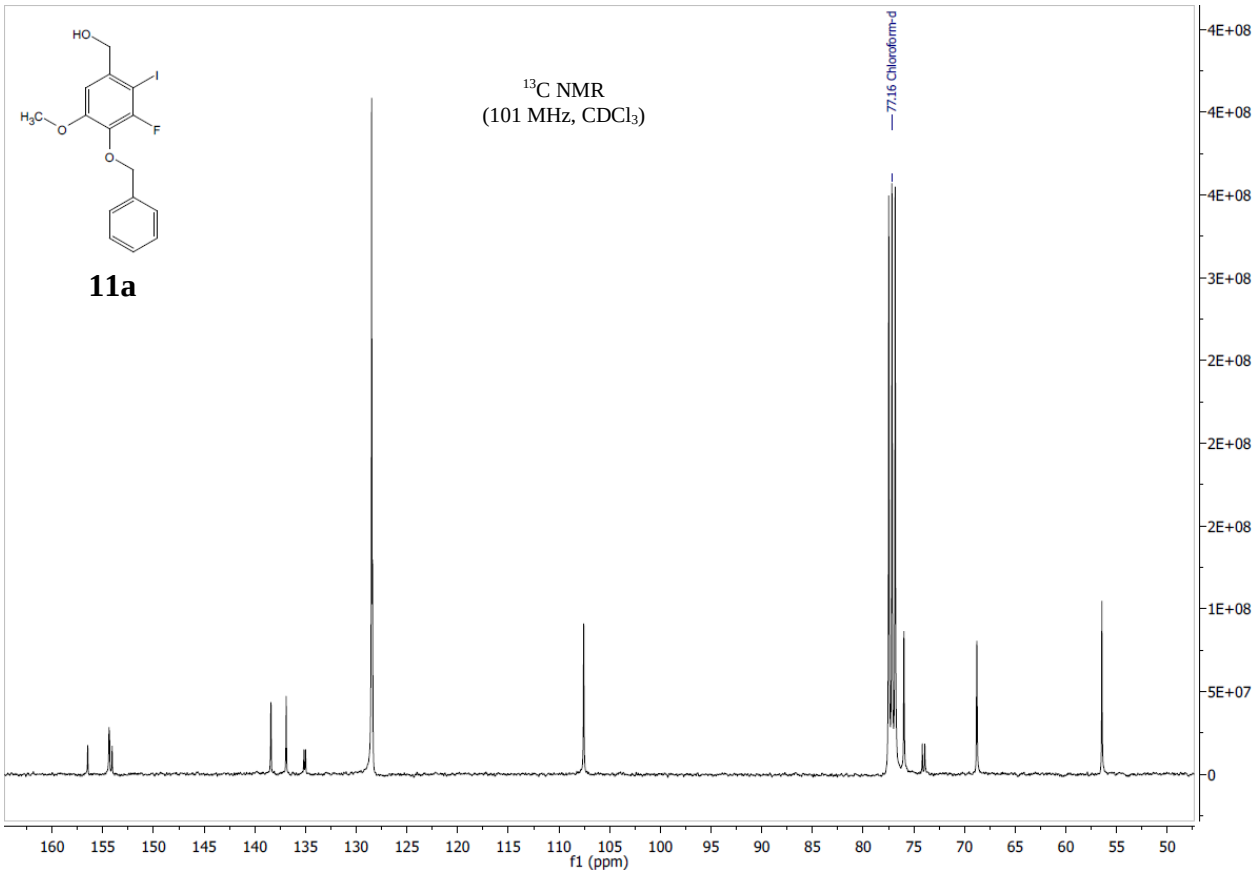

HO
Br
Cl
F
O
7.26 Chloroform-d
^{1}H NMR
(400 MHz, $CDCl_3$)
10
4E+08
3E+08
2E+08
1E+08
5E+07
0
7.7 7.5 7.3 7.1 6.9 6.7 6.5 6.3 6.1 5.9 5.7 5.5 5.3 5.1 4.9 4.7 4.5 4.3
f1 (ppm)


HO
Br
Cl
F
O
77.16 Chloroform-d
^{13}C NMR
(101 MHz, $CDCl_3$)
10
4E+08
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55
f1 (ppm)



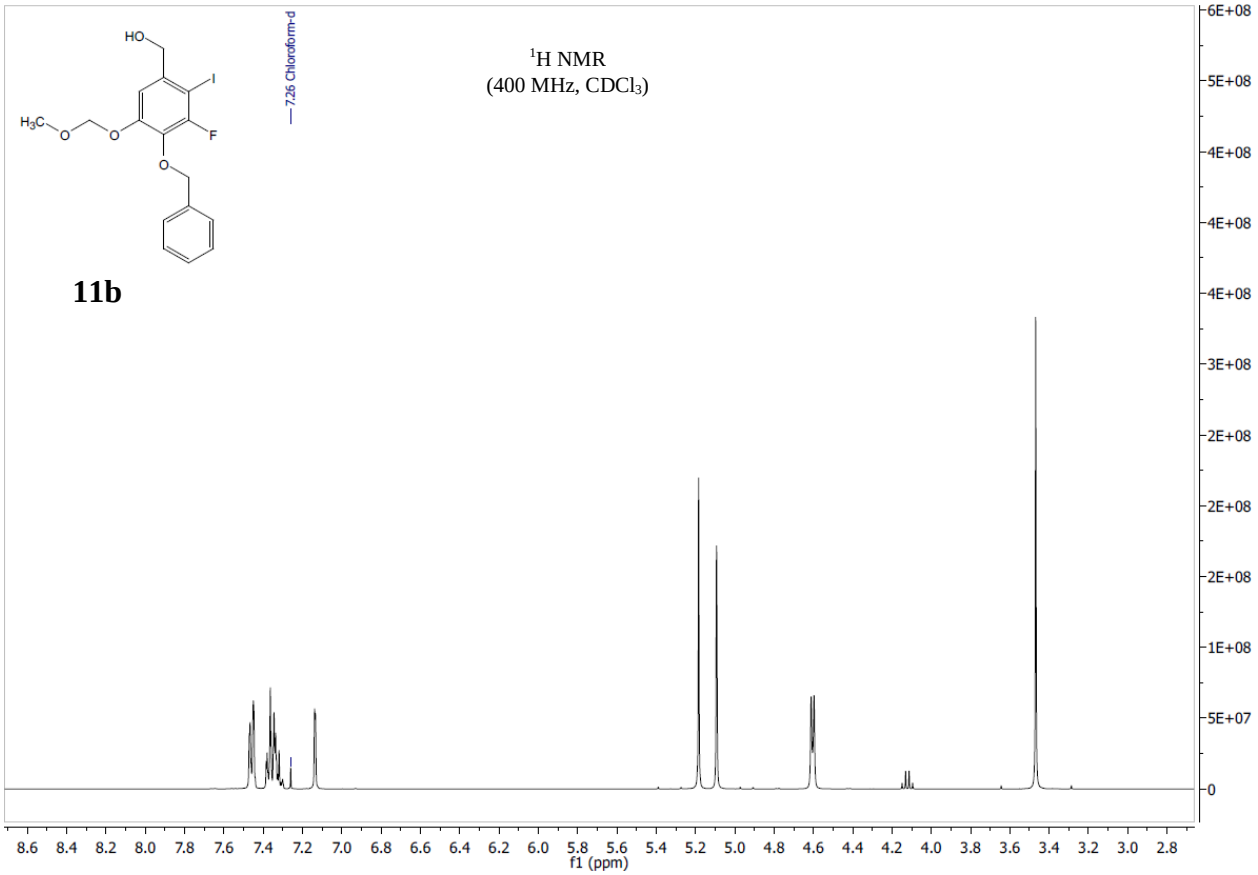
11a

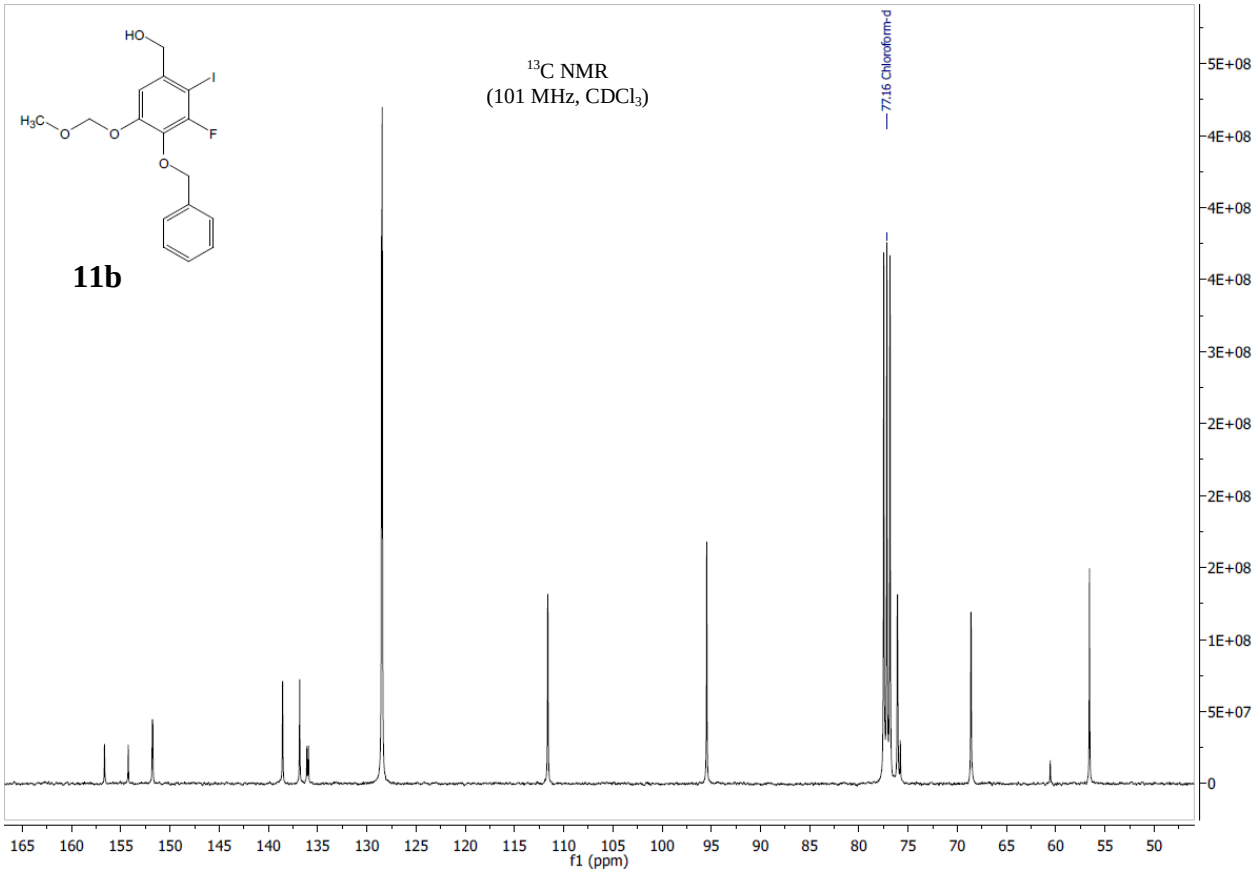
^{1}H NMR
(400 MHz, $CDCl_3$)

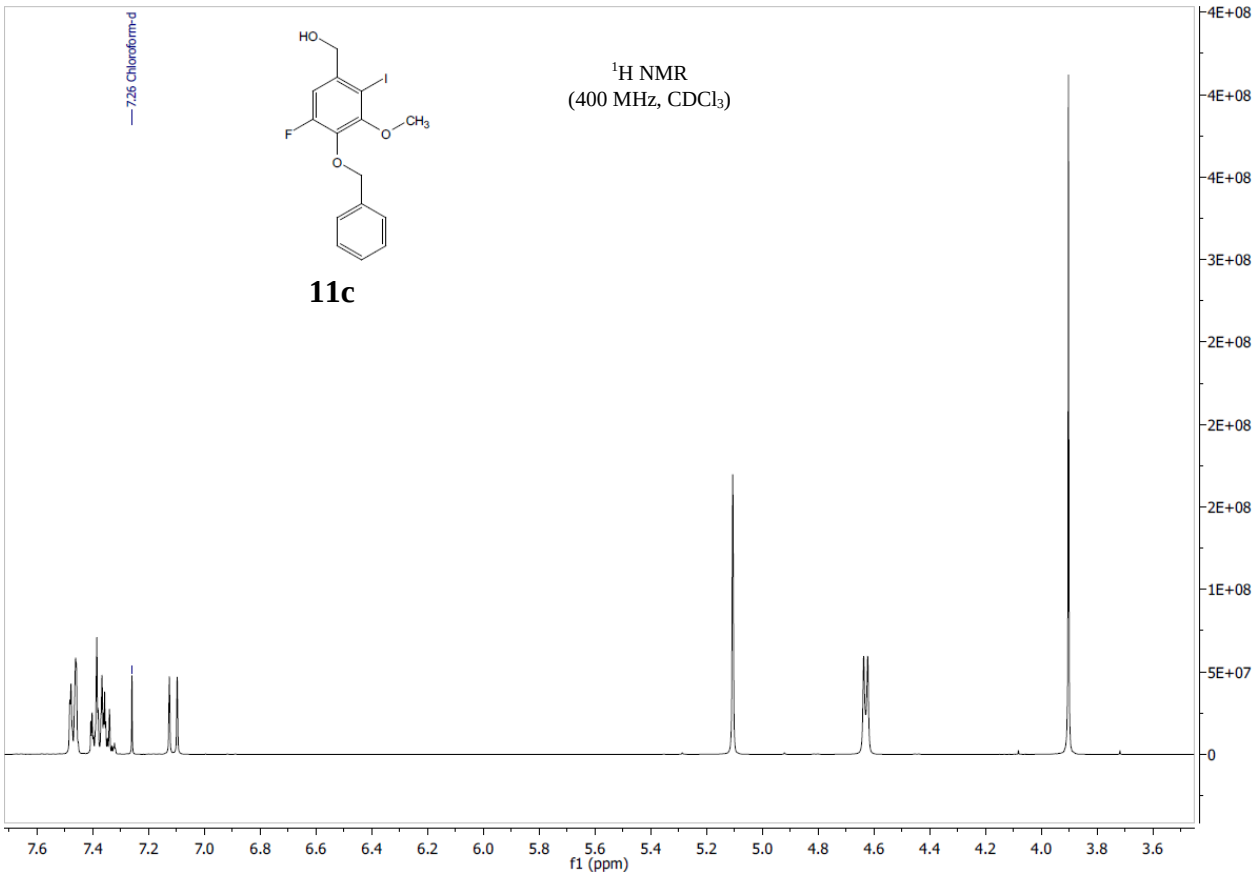


11a

^{13}C NMR
(101 MHz, $CDCl_3$)

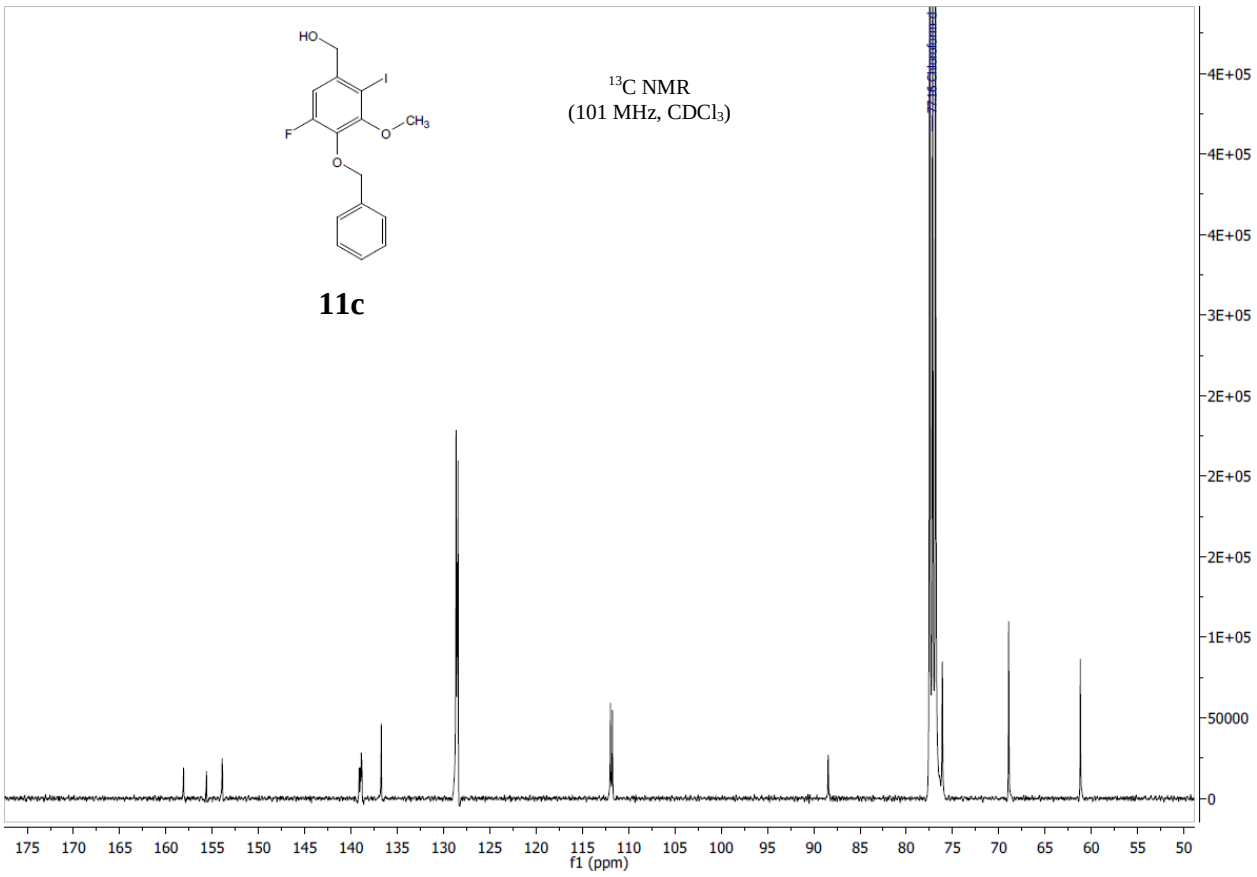

HO
I
H3C
O
O
F
O
7.26 Chloroform-d
1H NMR
(400 MHz, CDCl3)
11b
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8
f1 (ppm)


HO
I
H3C
O
O
F
O
77.16 Chloroform-d
13C NMR
(101 MHz, CDCl3)
11b
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50
f1 (ppm)



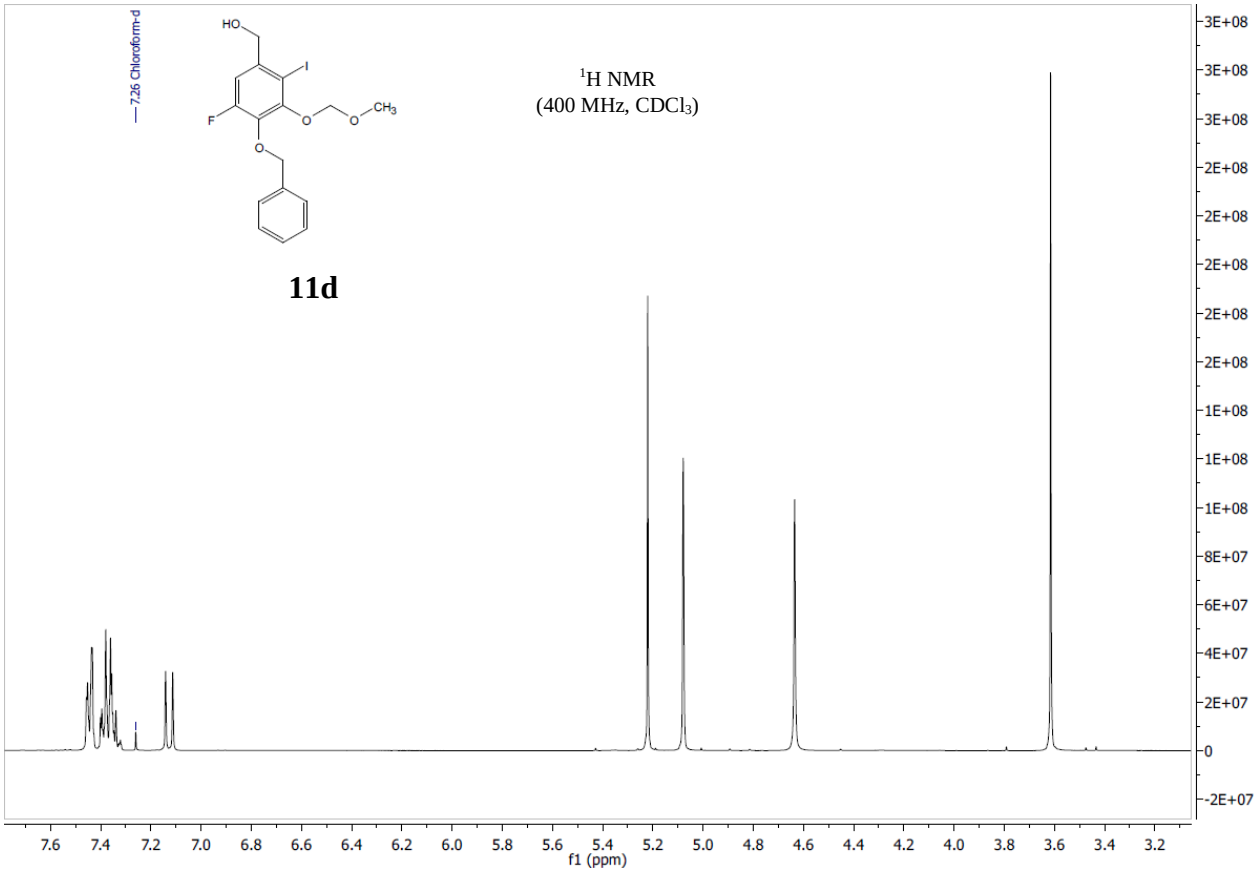
^{1}H NMR
(400 MHz, $CDCl_3$)

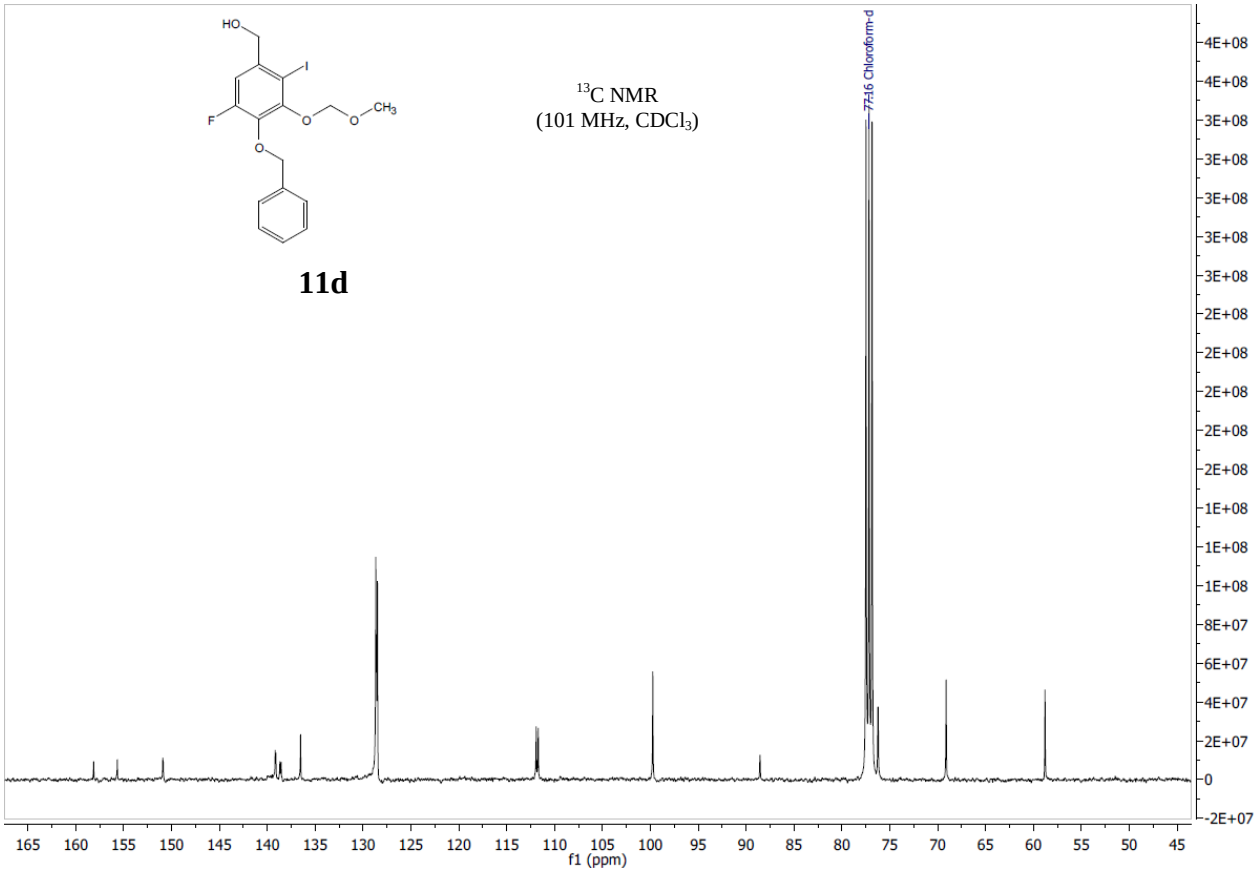
11c

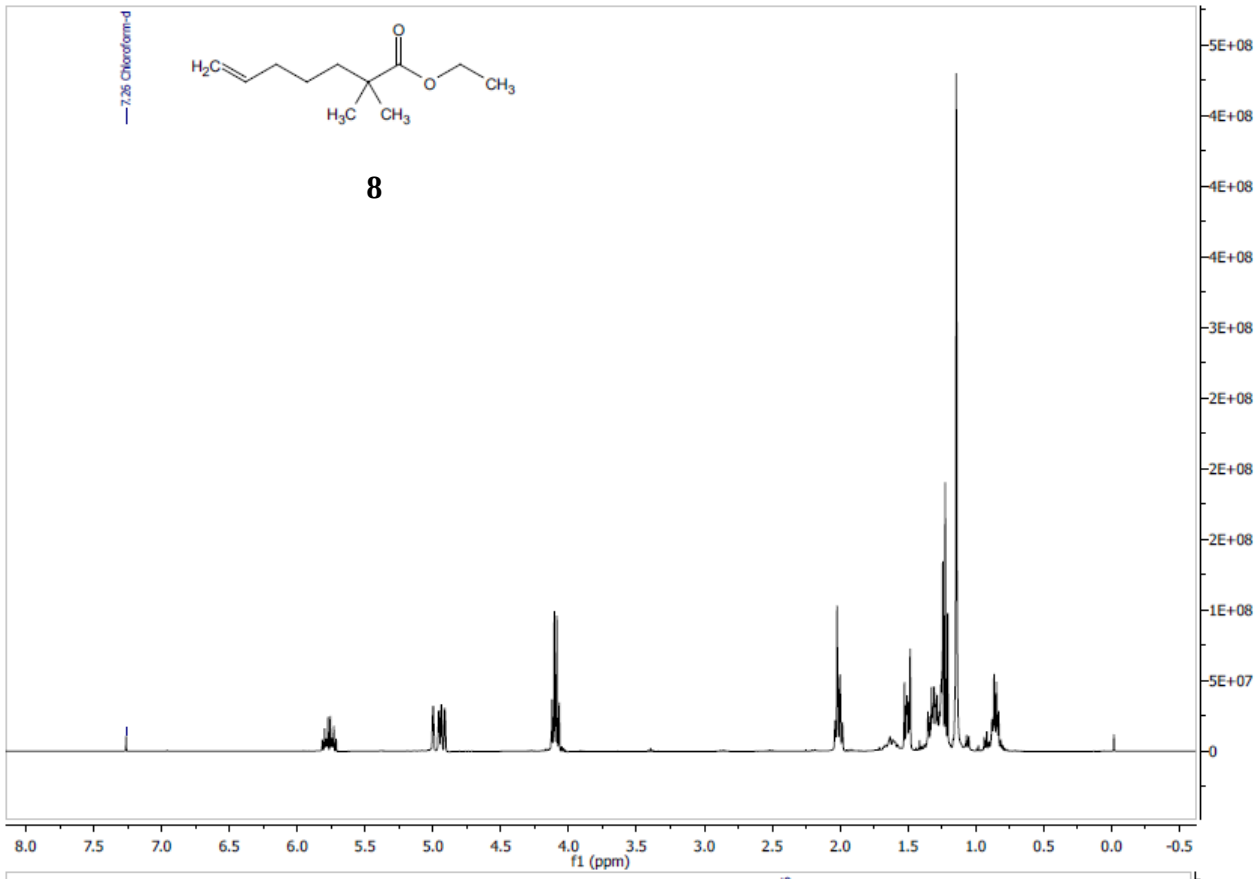


^{13}C NMR
(101 MHz, $CDCl_3$)

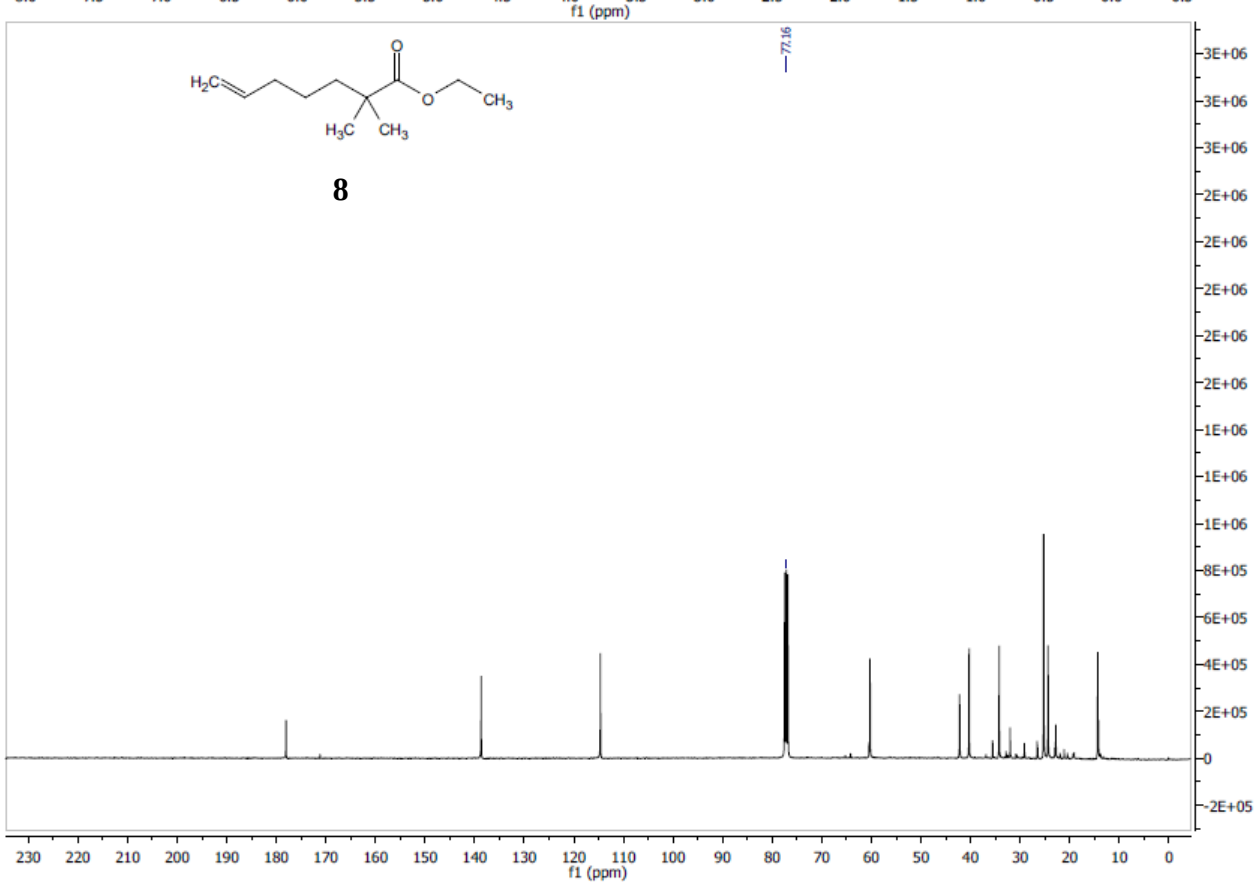
11c


7.26 Chloroform-d
HO
I
F
O
O
CH3
O
11d
1H NMR
(400 MHz, CDCl3)
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2
f1 (ppm)

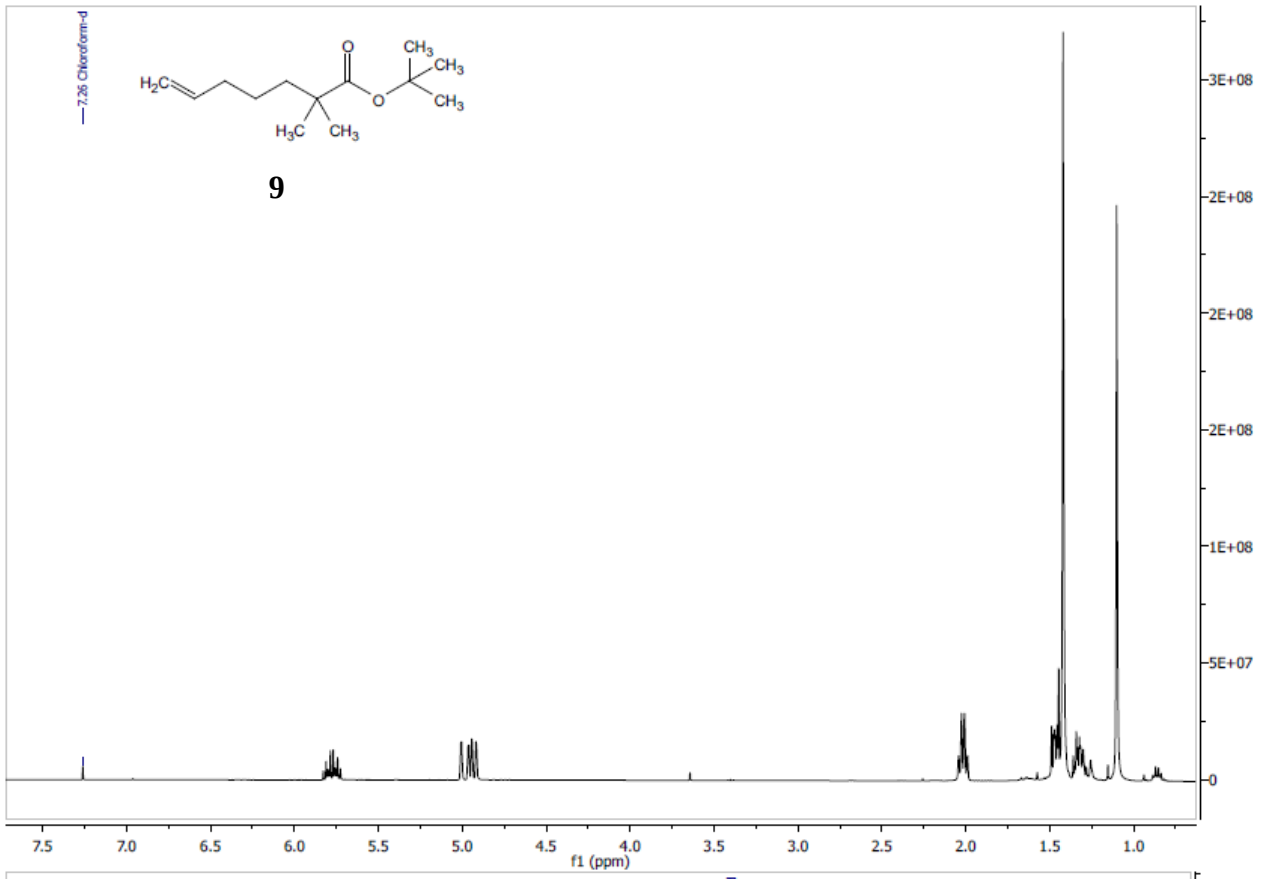

HO
I
F
O
O
CH3
O
11d
13C NMR
(101 MHz, CDCl3)
77.16 Chloroform-d
4E+08
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45
f1 (ppm)


7.26 Chloroform-d
H2C
O
O
CH3
H3C
CH3
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
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)

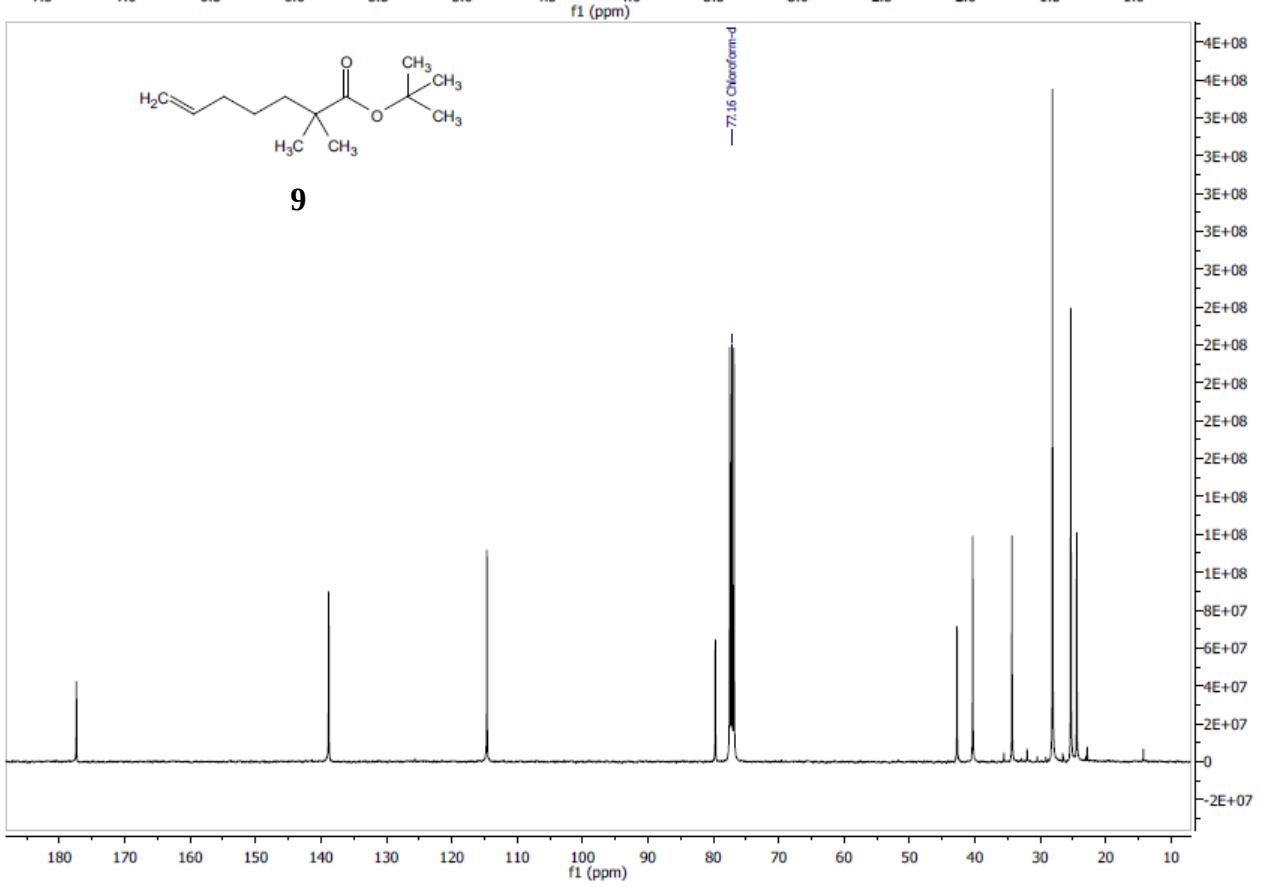
8


77.16
H2C
O
O
CH3
H3C
CH3
3E+06
3E+06
3E+06
2E+06
2E+06
2E+06
2E+06
2E+06
2E+06
1E+06
1E+06
1E+06
8E+05
6E+05
4E+05
2E+05
0
-2E+05
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)

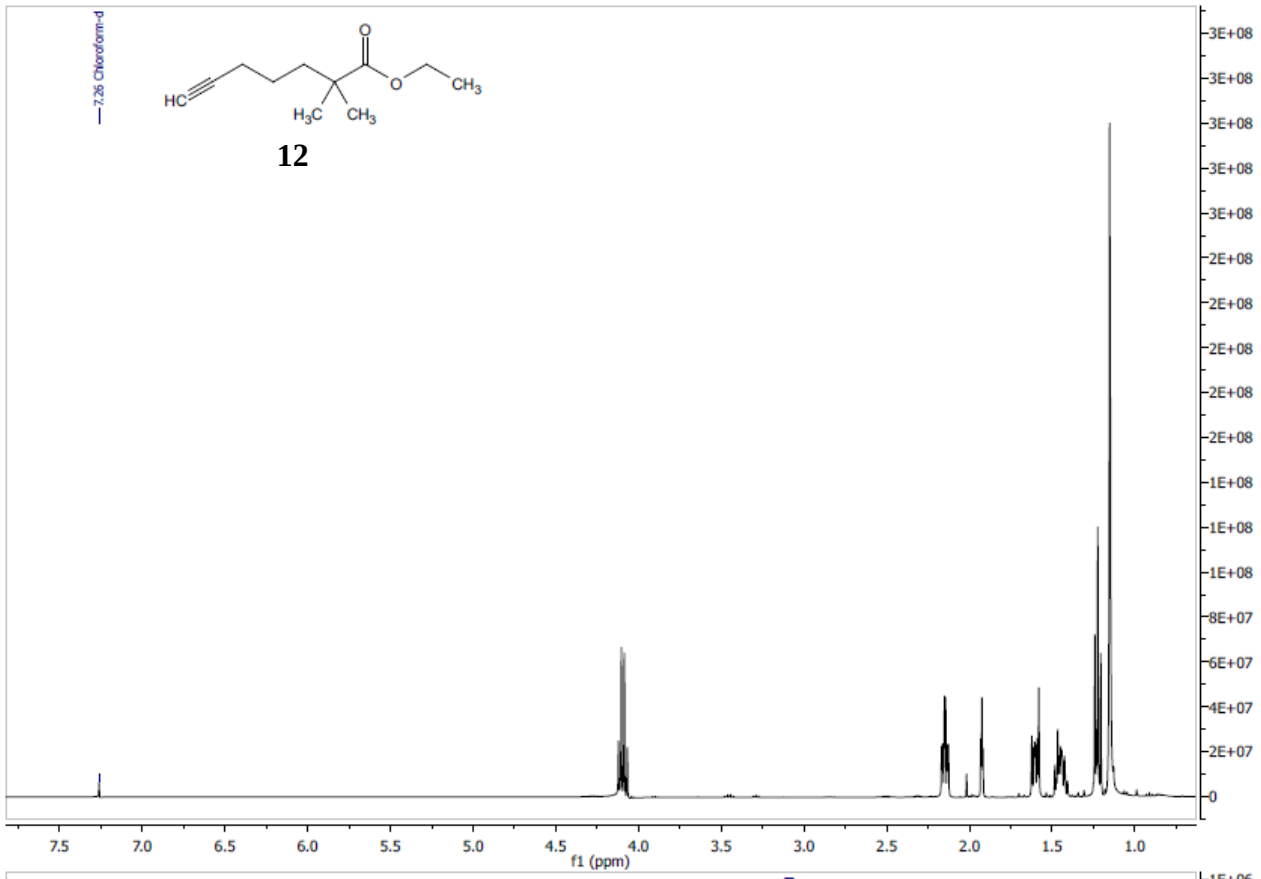
8


7.26 Chloroform-d
O
CH3
CH3
CH3
H2C
O
H3C
CH3
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
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
f1 (ppm)

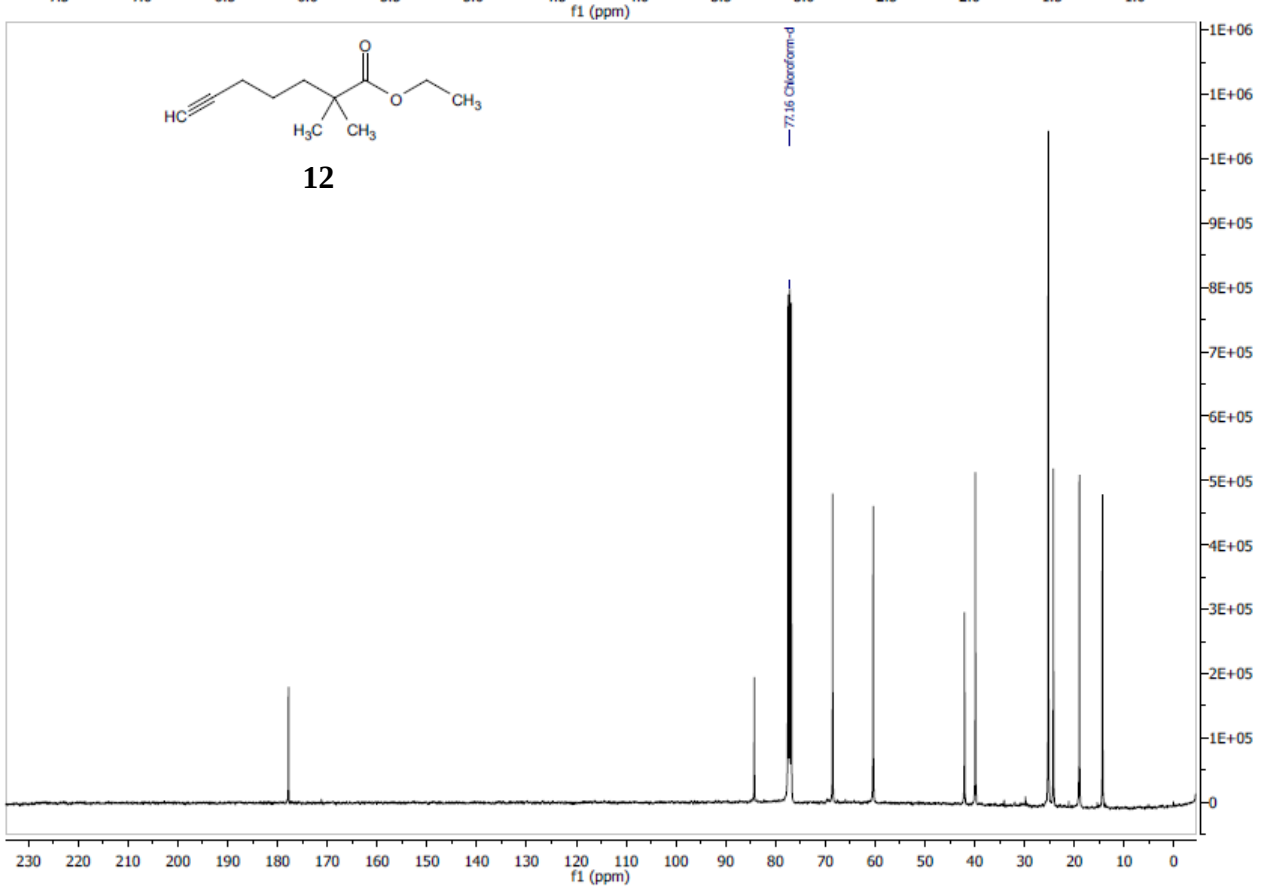
9


77.16 Chloroform-d
O
CH3
CH3
CH3
H2C
O
H3C
CH3
4E+08
4E+08
3E+08
3E+08
3E+08
3E+08
3E+08
3E+08
2E+08
2E+08
2E+08
2E+08
2E+08
2E+08
1E+08
1E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
-2E+07
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

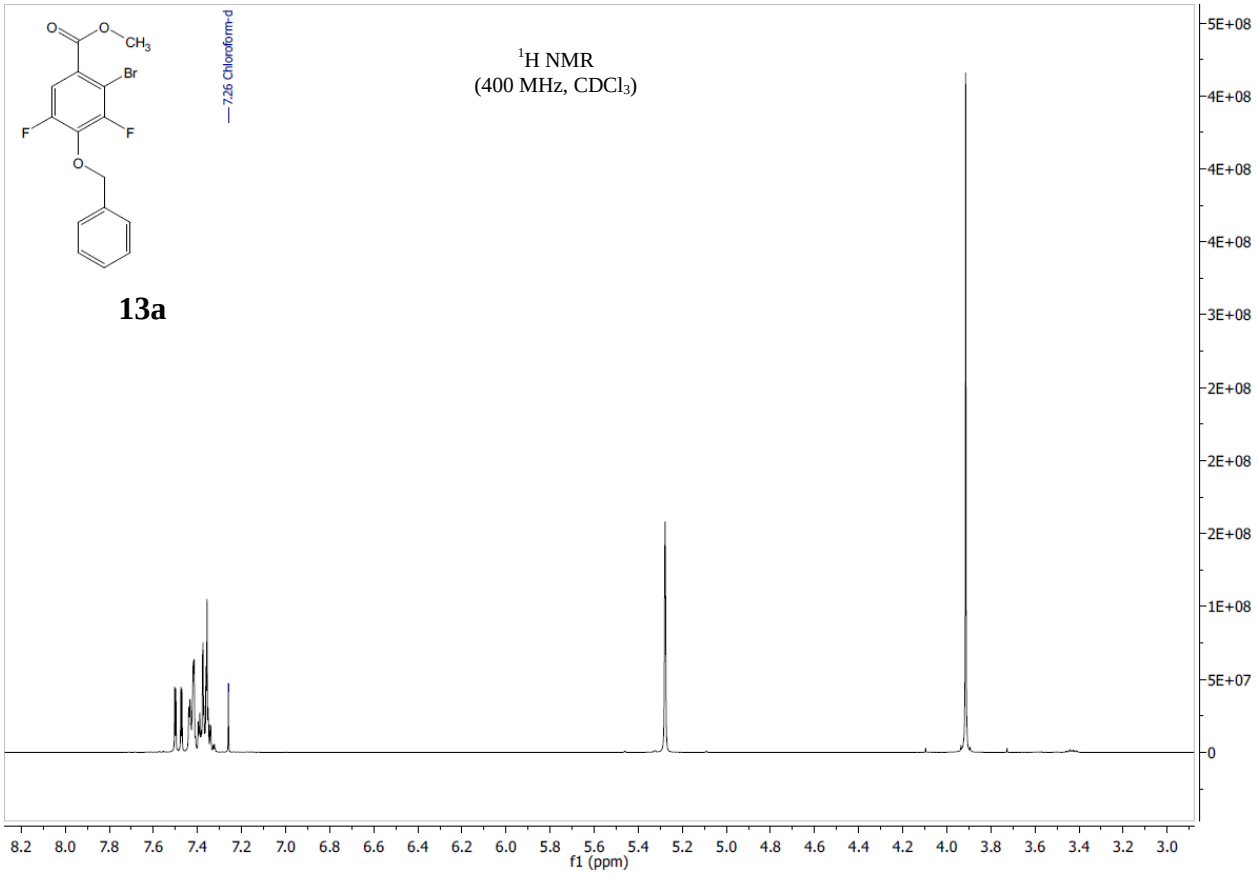
9

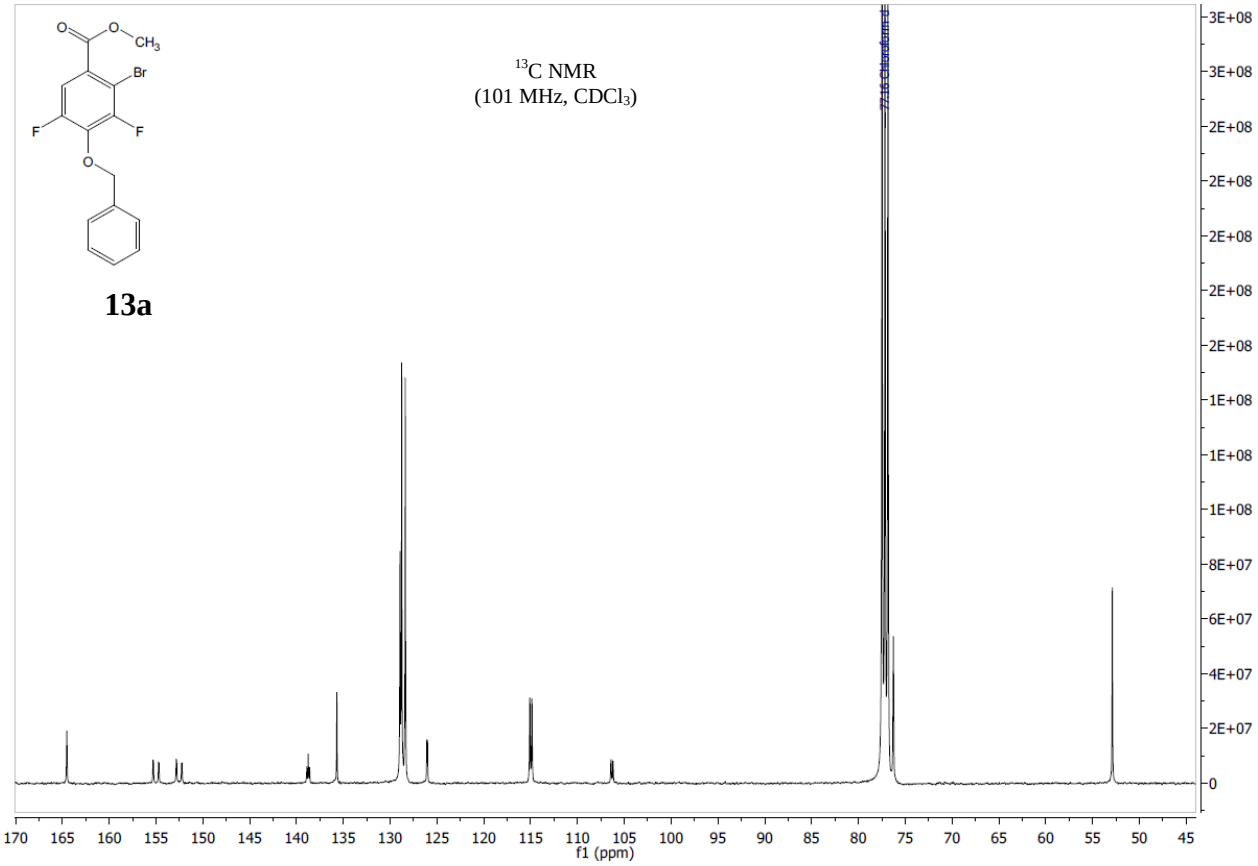

7.26 Chloroform-d
HC
O
O
CH3
H3C
CH3
3E+08
2E+08
1E+08
8E+07
6E+07
4E+07
2E+07
0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

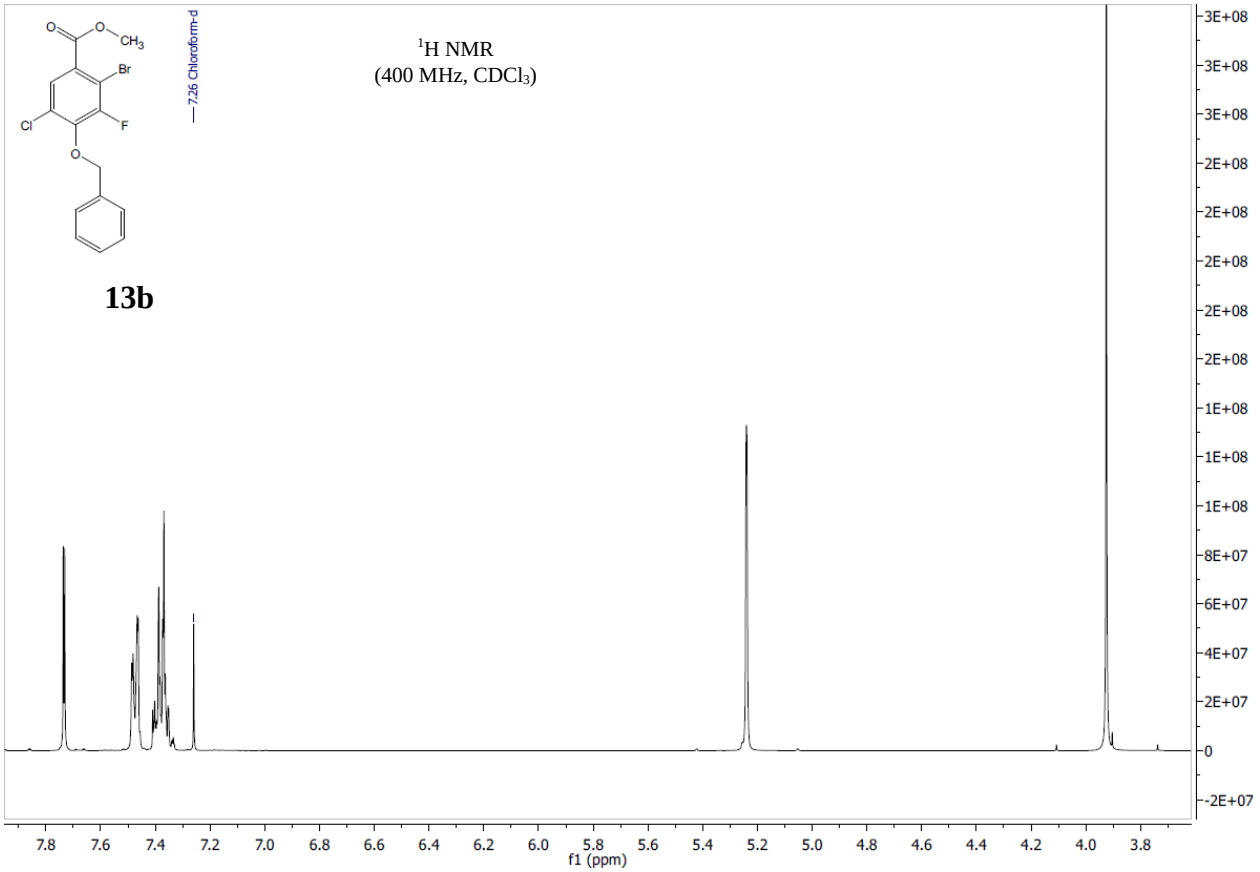
12


77.16 Chloroform-d
HC
O
O
CH3
H3C
CH3
1E+06
9E+05
8E+05
7E+05
6E+05
5E+05
4E+05
3E+05
2E+05
1E+05
0
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)

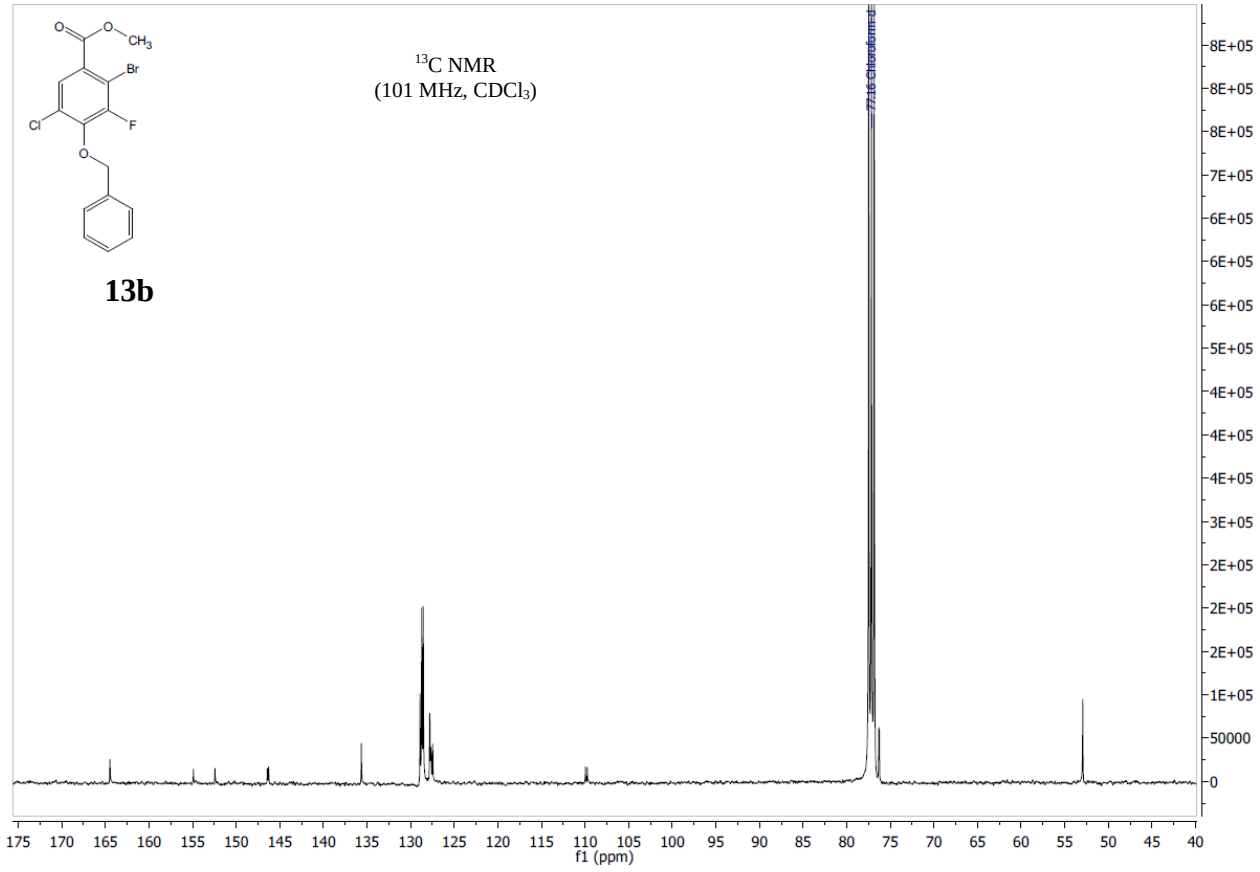
12


^{1}H NMR
(400 MHz, $CDCl_3$)
13a
7.26 Chloroform-d
f1 (ppm)

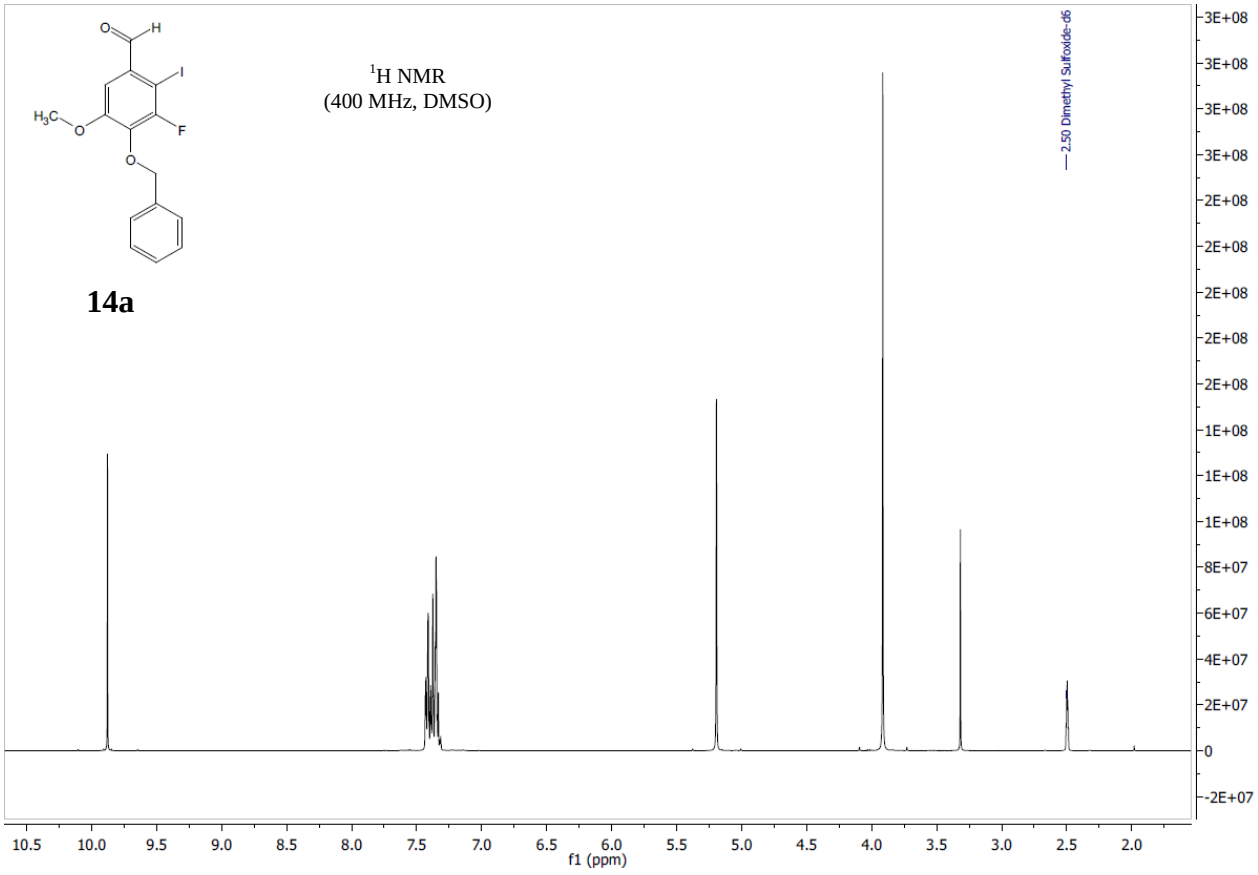

^{13}C NMR
(101 MHz, $CDCl_3$)
13a
77.16 Chloroform-d
f1 (ppm)

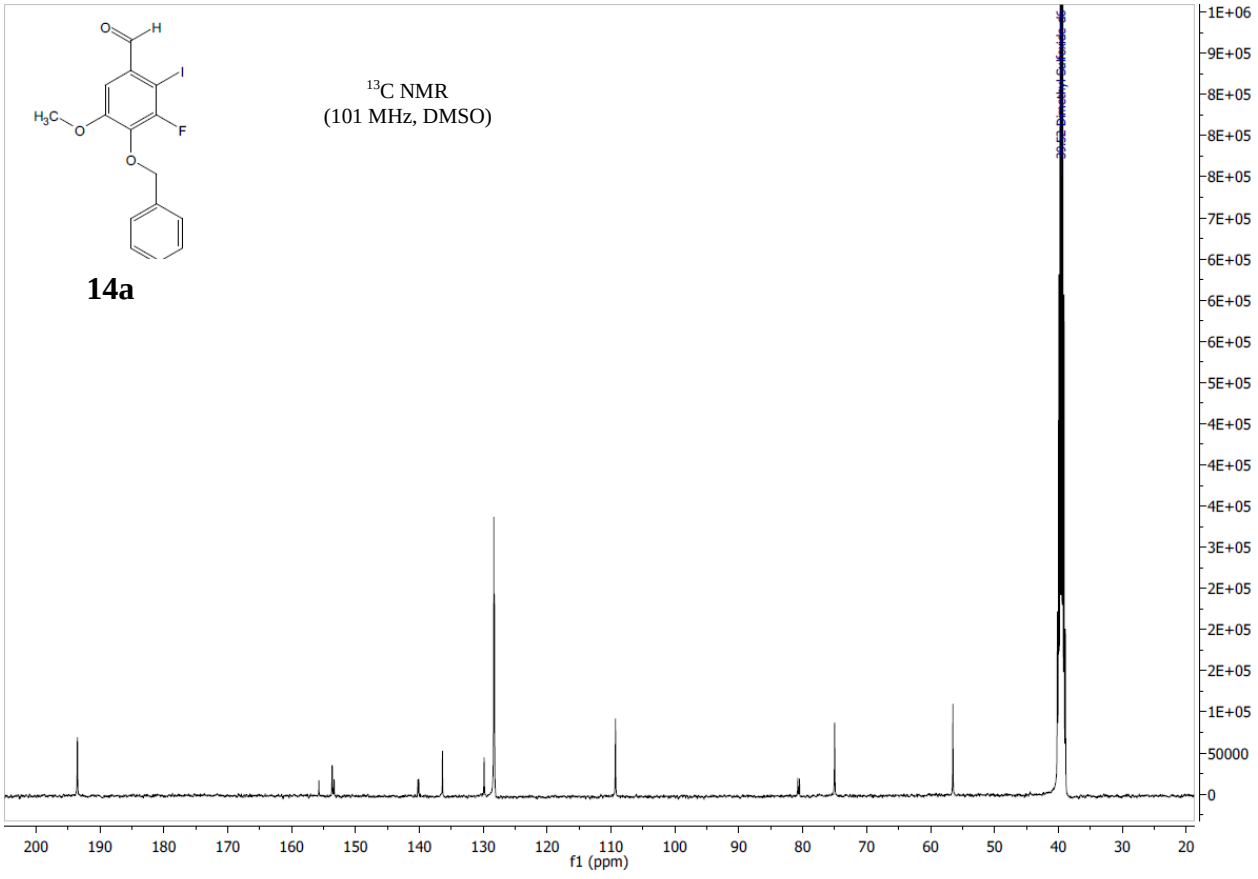

7.26 Chloroform-d
Br
Cl
F
O
CH3
1H NMR
(400 MHz, CDCl3)
f1 (ppm)

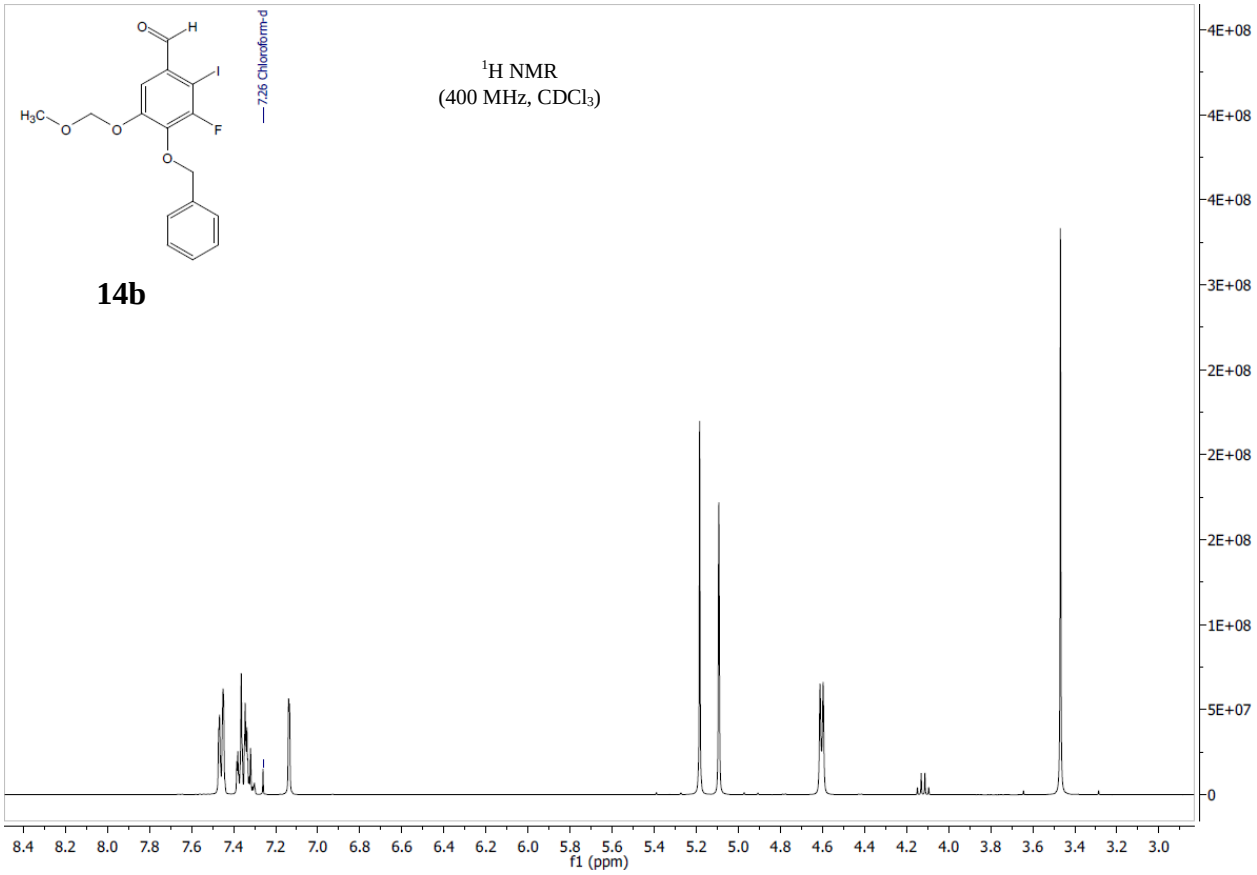
13b

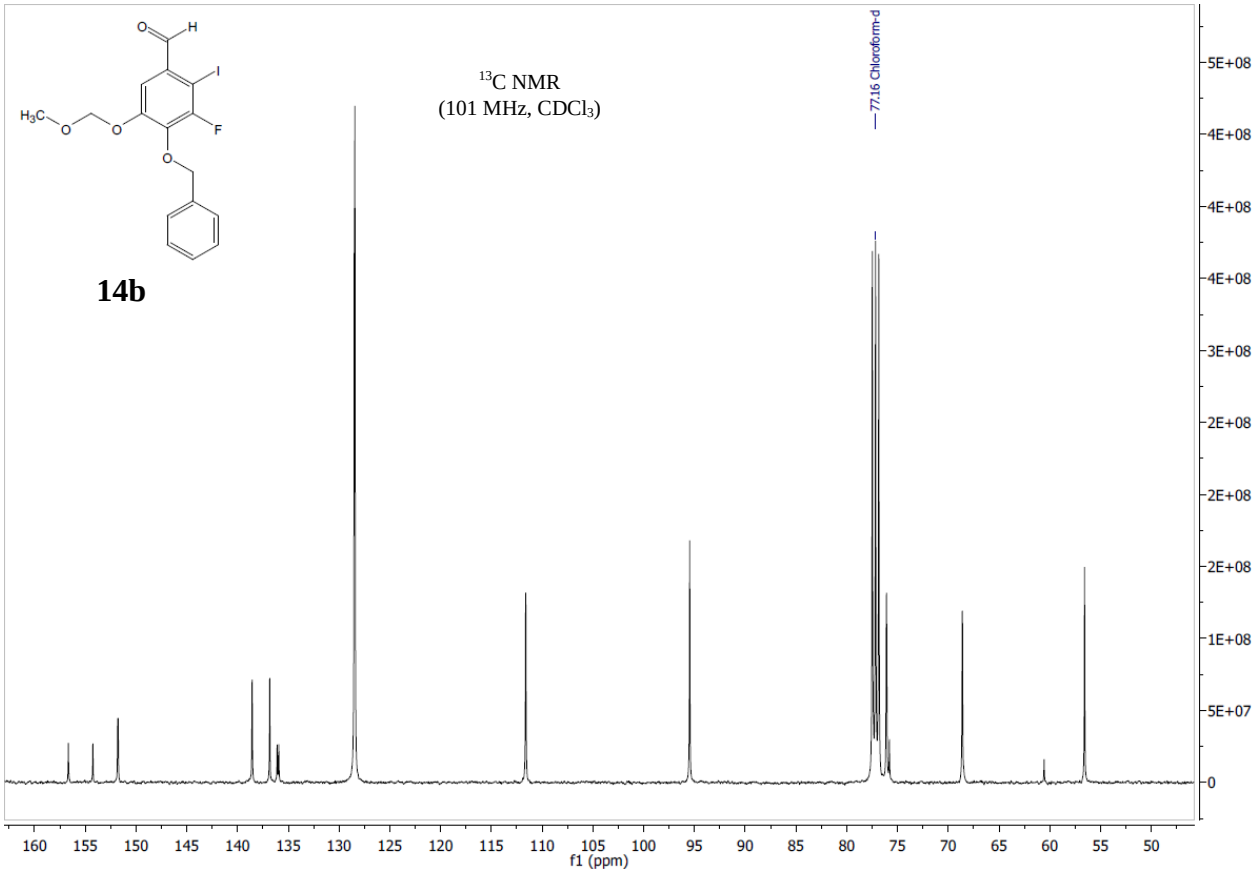

77.16 Chloroform-d
Br
Cl
F
O
CH3
13C NMR
(101 MHz, CDCl3)
f1 (ppm)

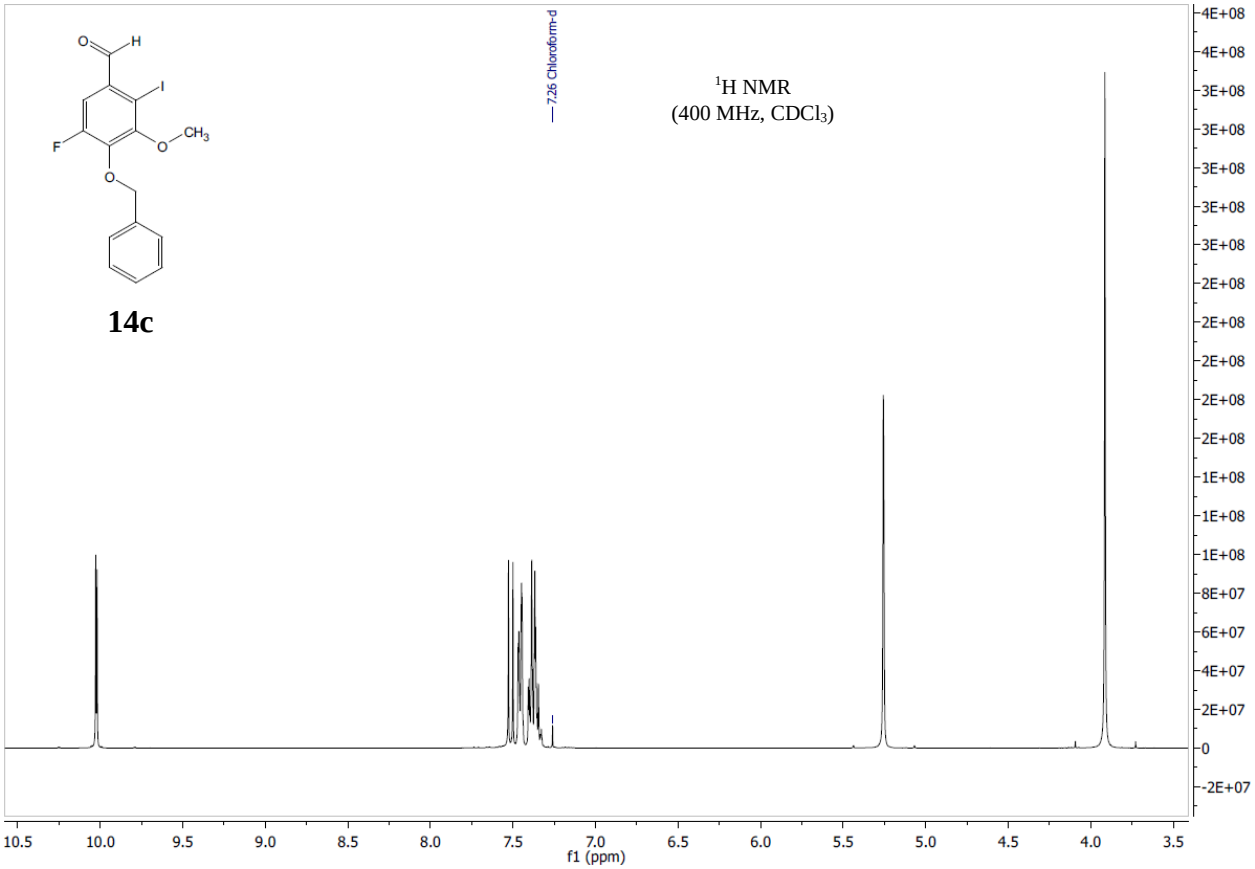
13b

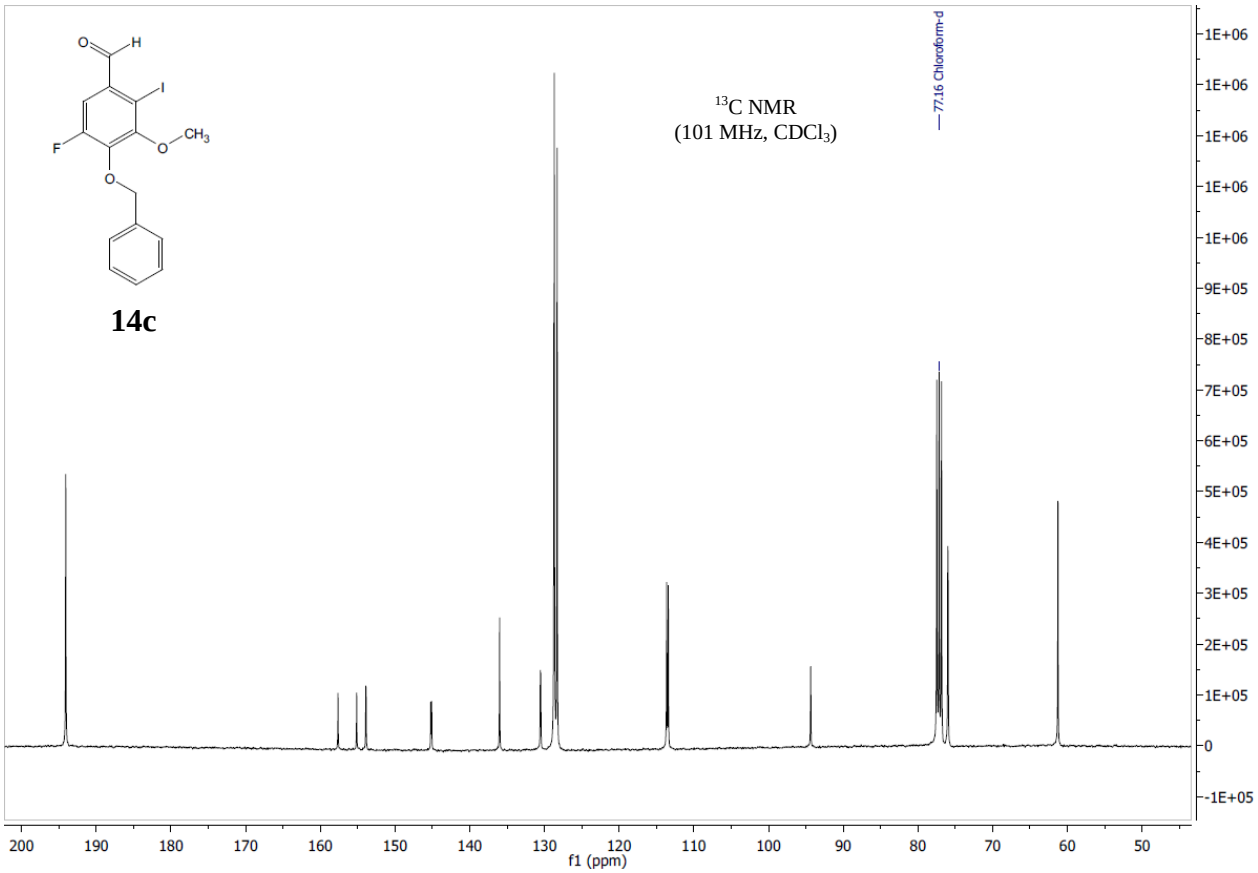

^{1}H NMR
(400 MHz, DMSO)
14a
2.50 Dimethyl Sulfoxide-d6
f1 (ppm)

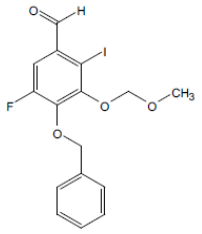

^{13}C NMR
(101 MHz, DMSO)
14a
39.52 Dimethyl Sulfoxide-d6
f1 (ppm)


7.26 Chloroform-d
^{1}H NMR
(400 MHz, $CDCl_3$)
14b
4E+08
3E+08
2E+08
1E+08
5E+07
0
8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0
f1 (ppm)


77.16 Chloroform-d
^{13}C NMR
(101 MHz, $CDCl_3$)
14b
5E+08
4E+08
3E+08
2E+08
1E+08
5E+07
0
160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50
f1 (ppm)

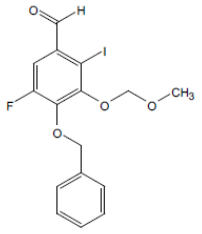

7.26 Chloroform-d
^{1}H NMR
(400 MHz, CDCl$_3$)
14c
f1 (ppm)


77.16 Chloroform-d
^{13}C NMR
(101 MHz, CDCl$_3$)
14c
f1 (ppm)



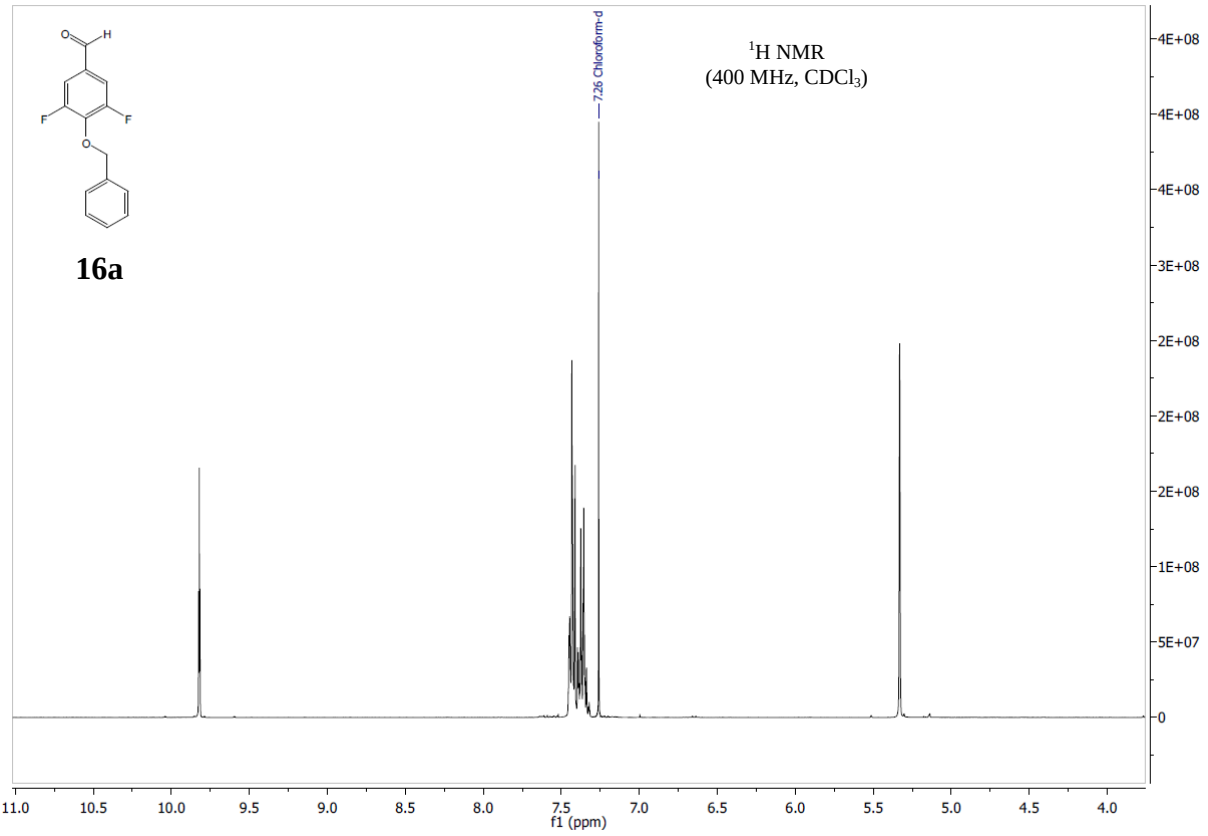
14d

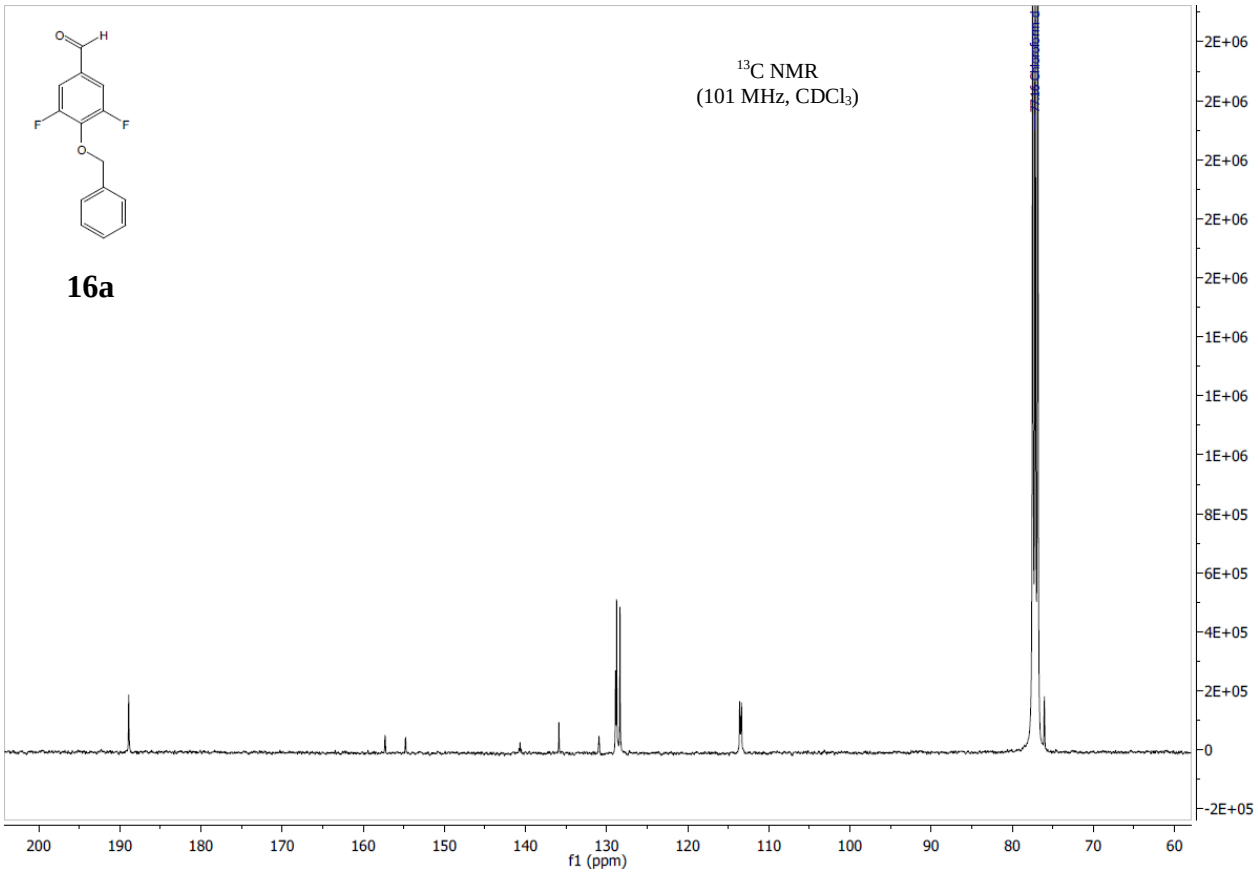
^{1}H NMR
(400 MHz, $CDCl_3$)

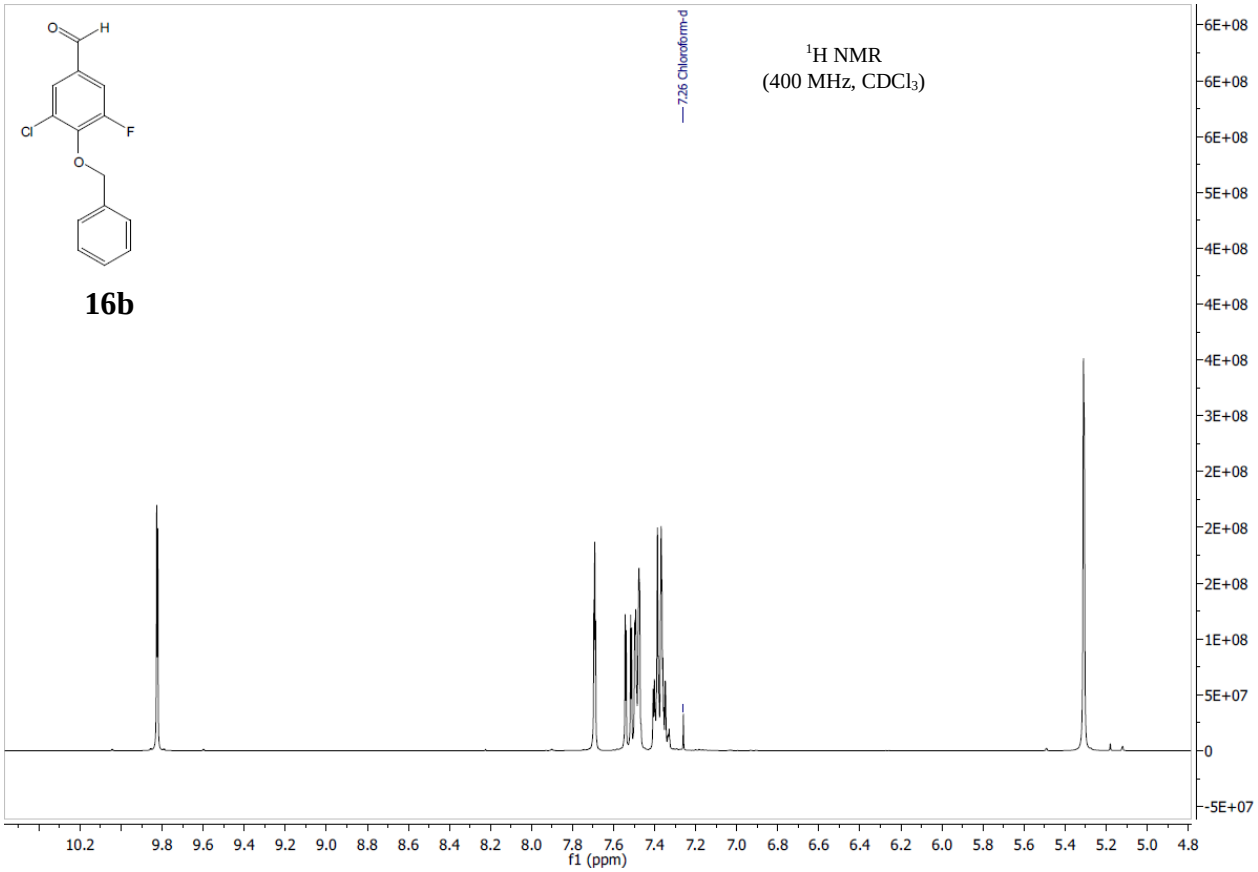


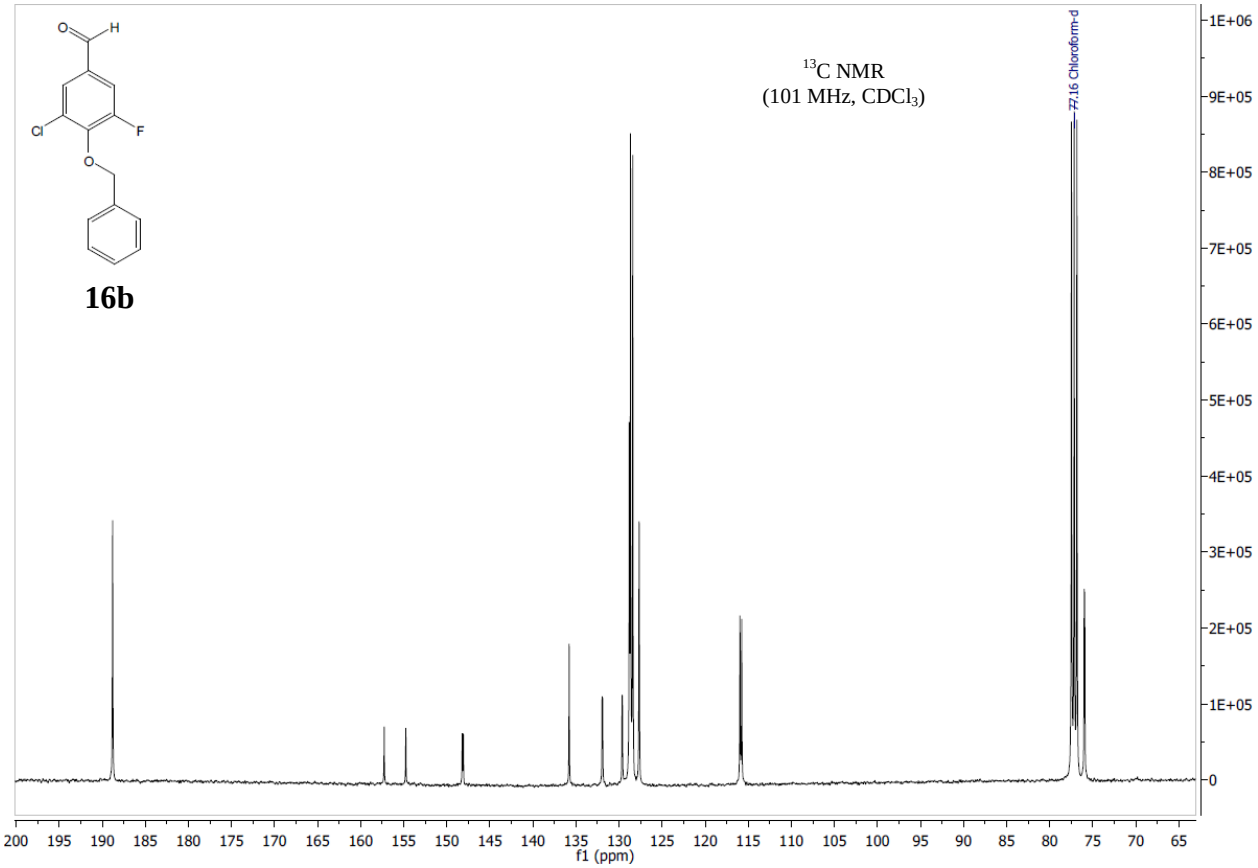
14d

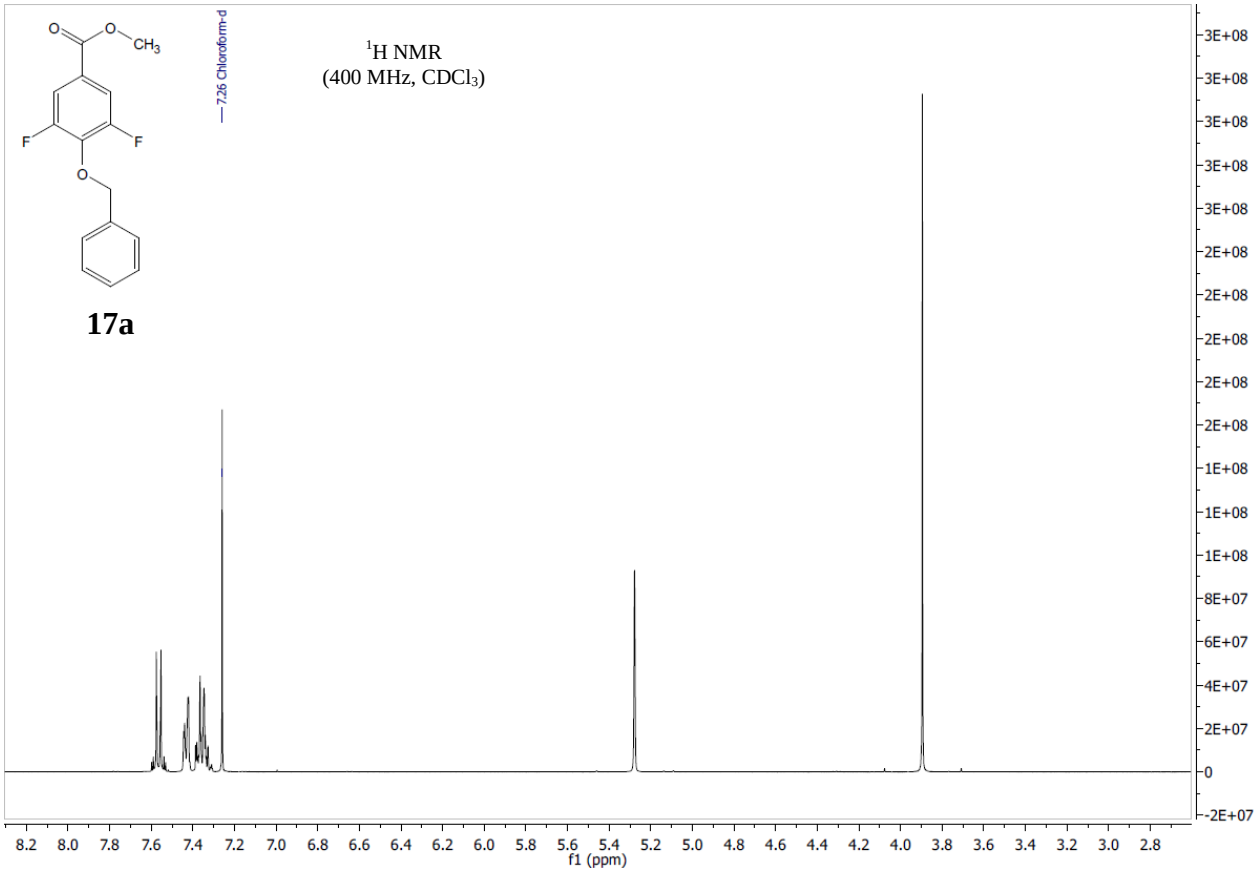
^{13}C NMR
(101 MHz, $CDCl_3$)


^{1}H NMR
(400 MHz, $CDCl_3$)
16a
7.26 Chloroform-d
f1 (ppm)

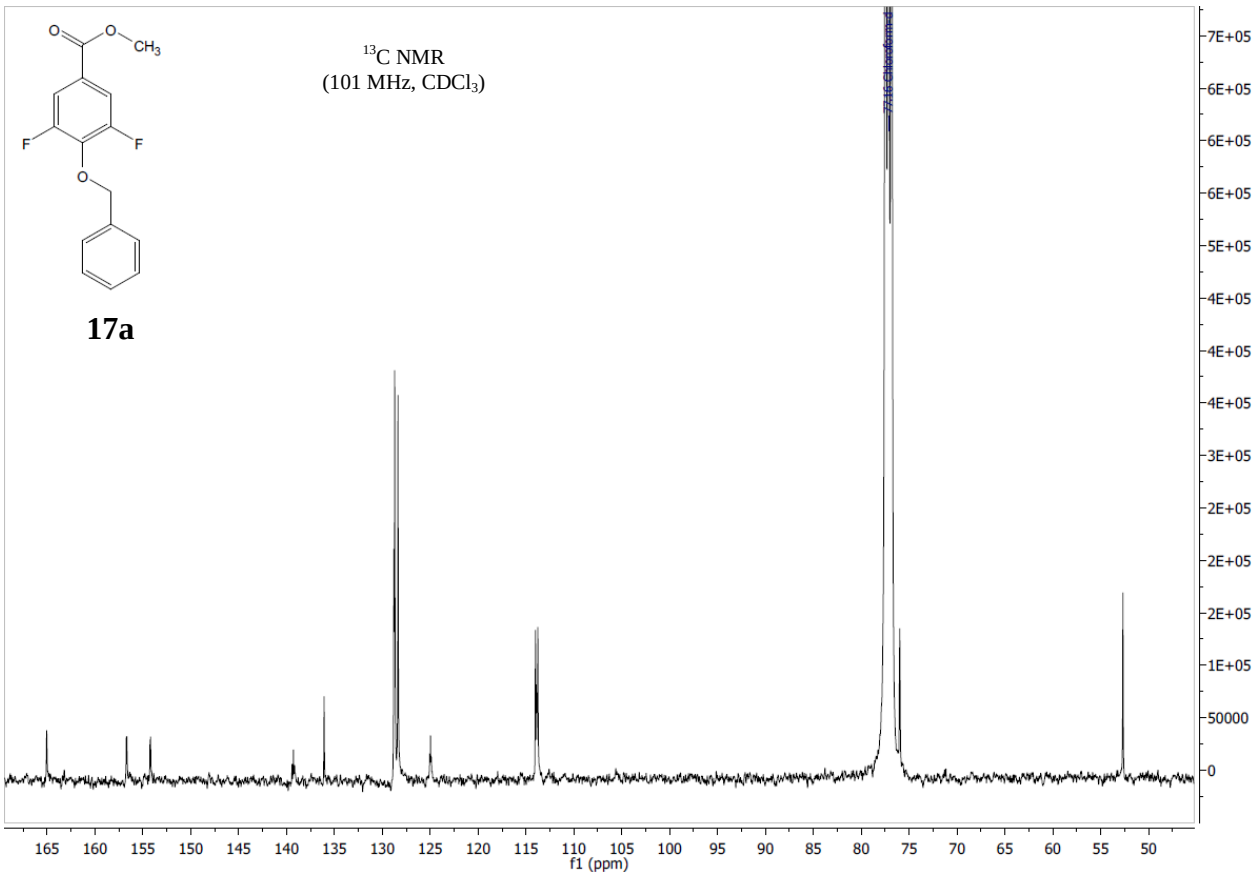

^{13}C NMR
(101 MHz, $CDCl_3$)
16a
77.16 Chloroform-d
f1 (ppm)


7.26 Chloroform-d
^{1}H NMR
(400 MHz, $CDCl_3$)
16b
f1 (ppm)

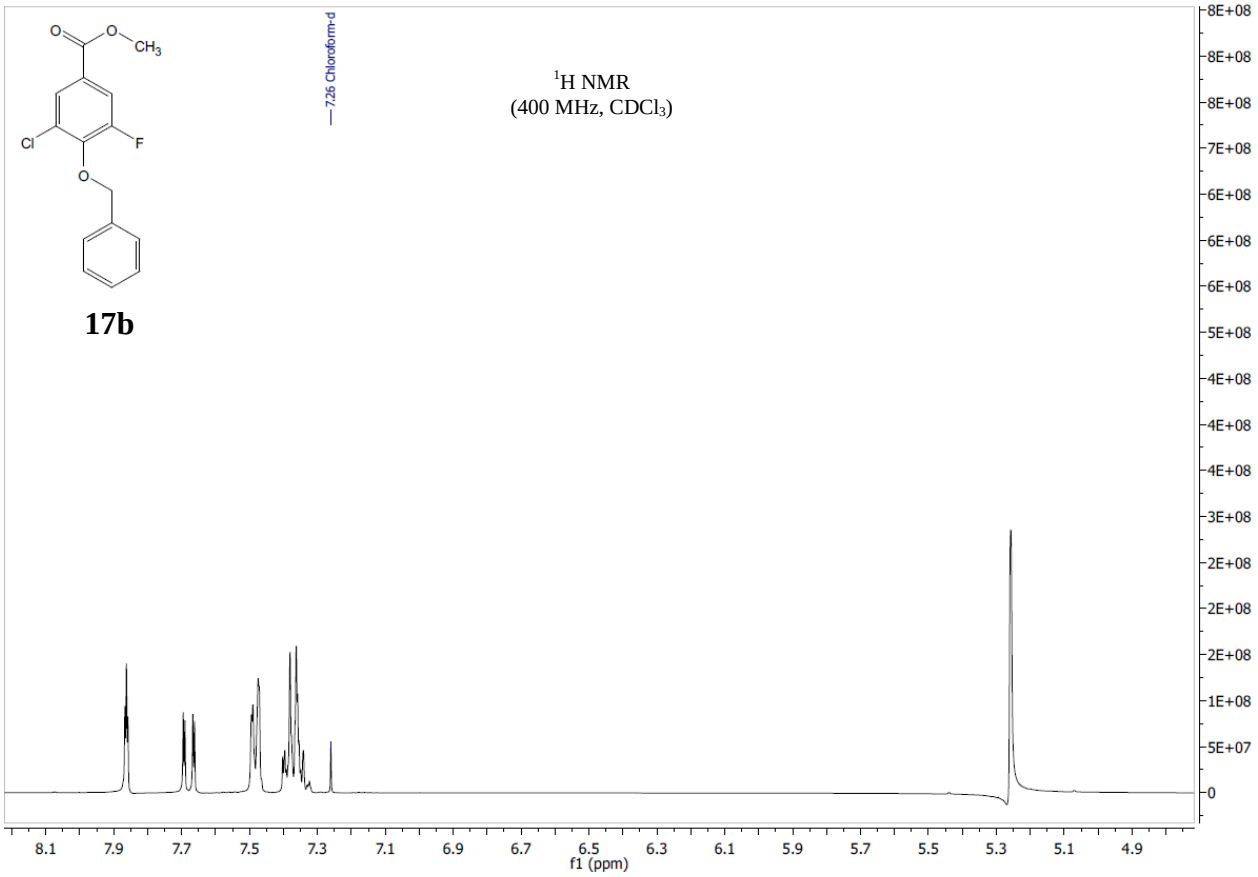

77.16 Chloroform-d
^{13}C NMR
(101 MHz, $CDCl_3$)
16b
f1 (ppm)


^{1}H NMR
(400 MHz, $CDCl_3$)
7.26 Chloroform-d
F
F
O
CH_3
f1 (ppm)

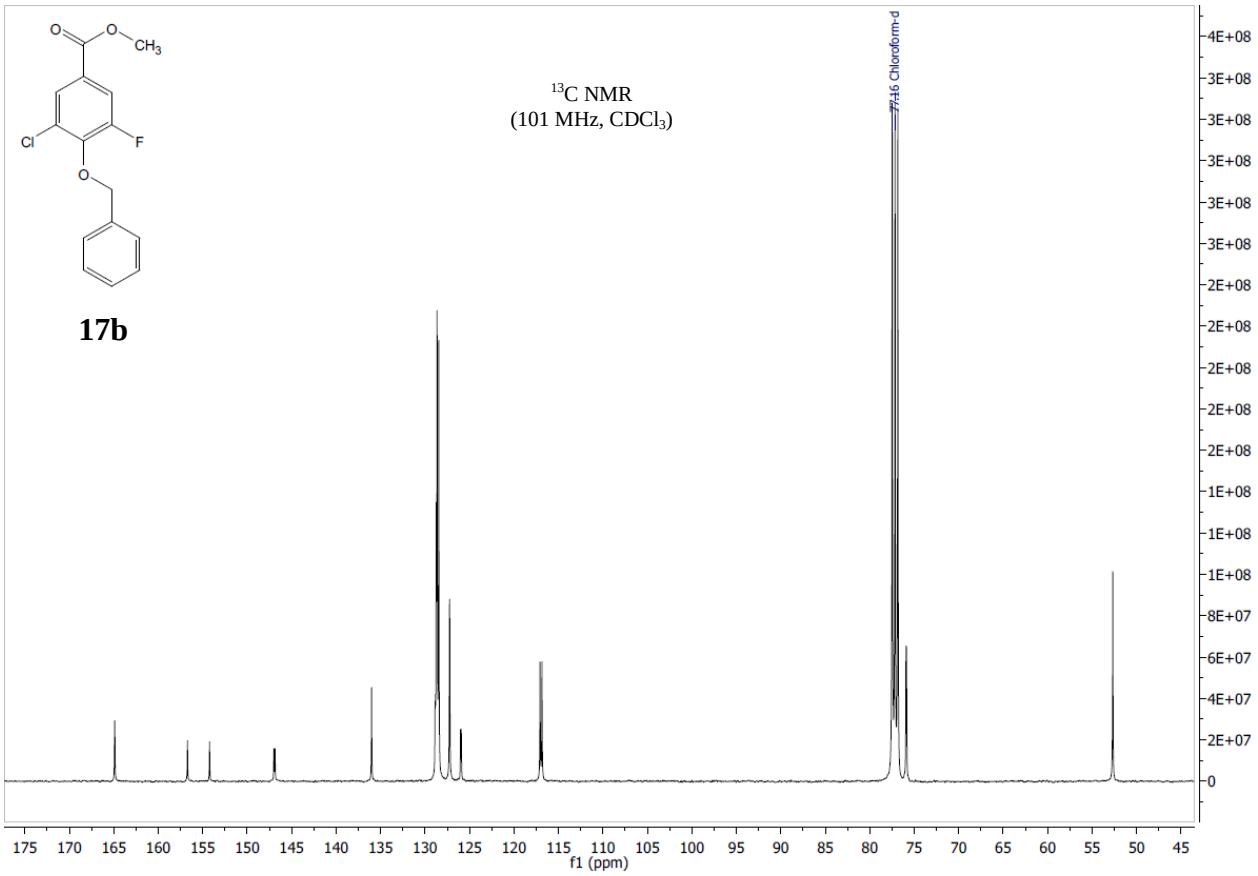
17a


^{13}C NMR
(101 MHz, $CDCl_3$)
77.16 Chloroform-d
F
F
O
CH_3
f1 (ppm)

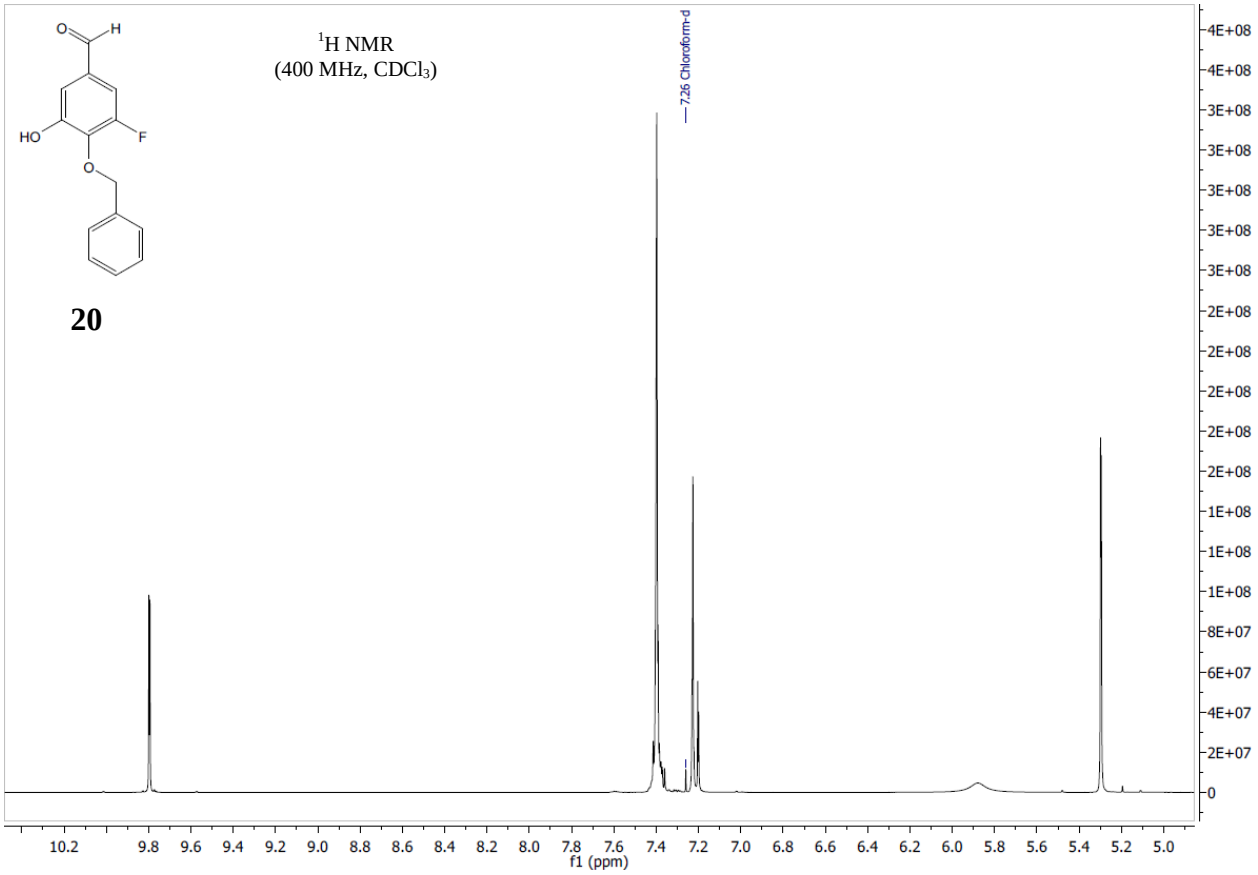
17a

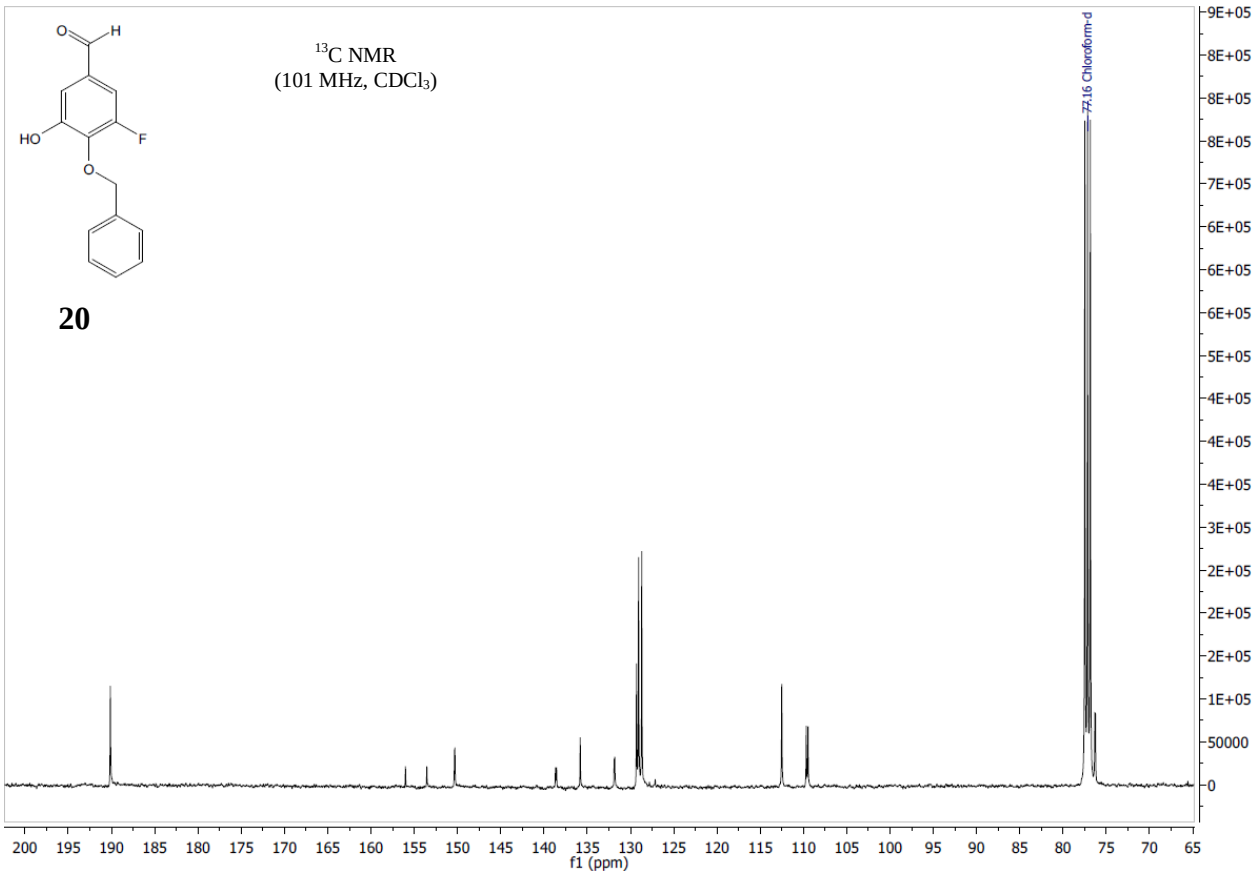

7.26 Chloroform-d
^{1}H NMR
(400 MHz, $CDCl_3$)
f1 (ppm)

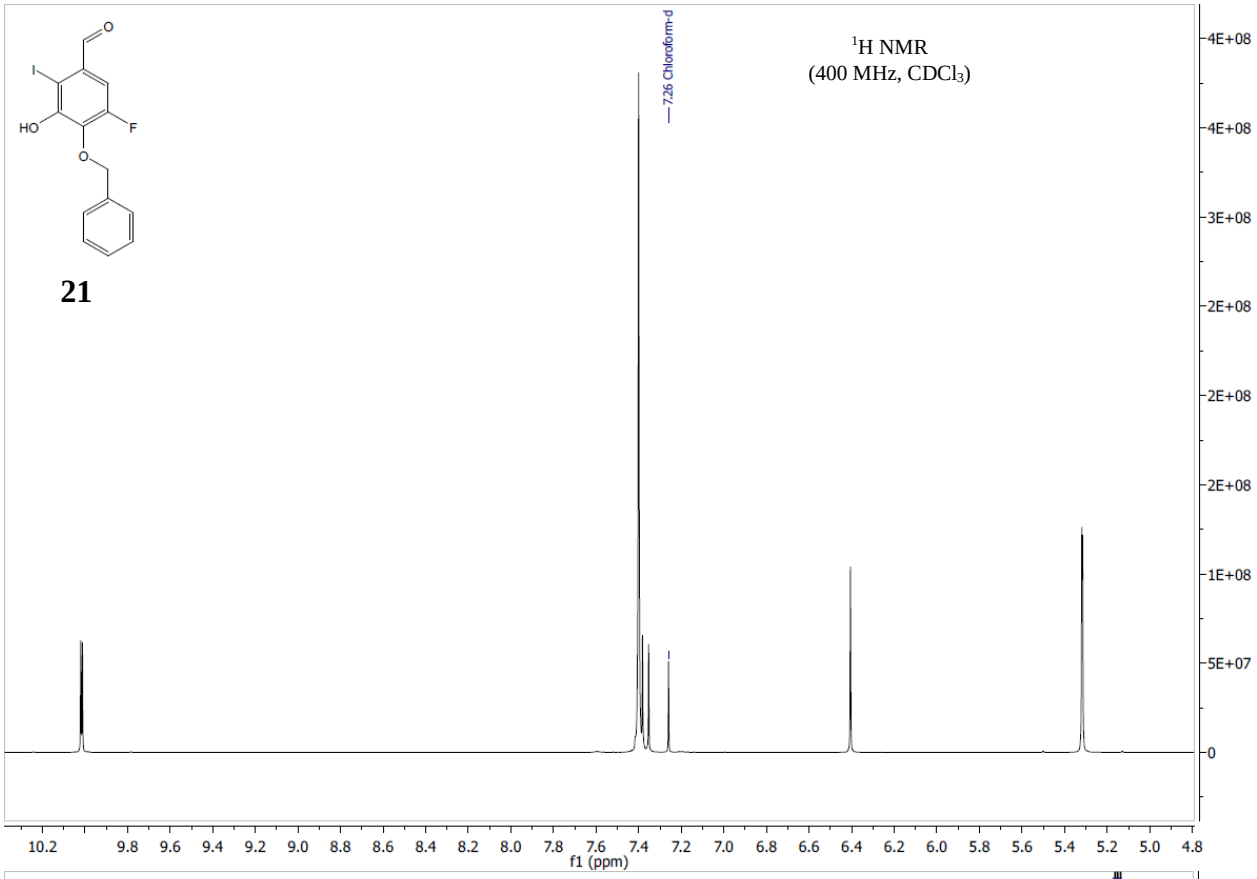
17b

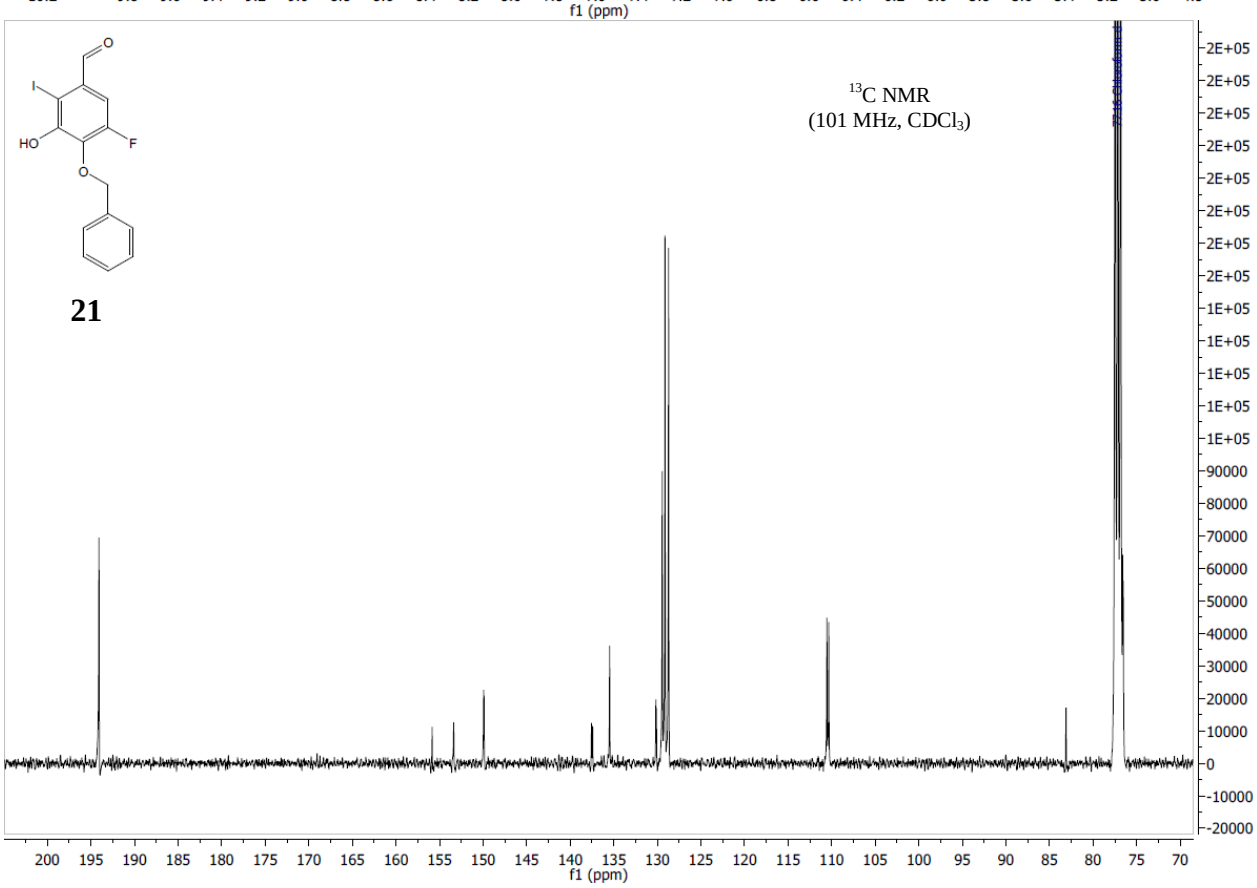

77.16 Chloroform-d
^{13}C NMR
(101 MHz, $CDCl_3$)
f1 (ppm)

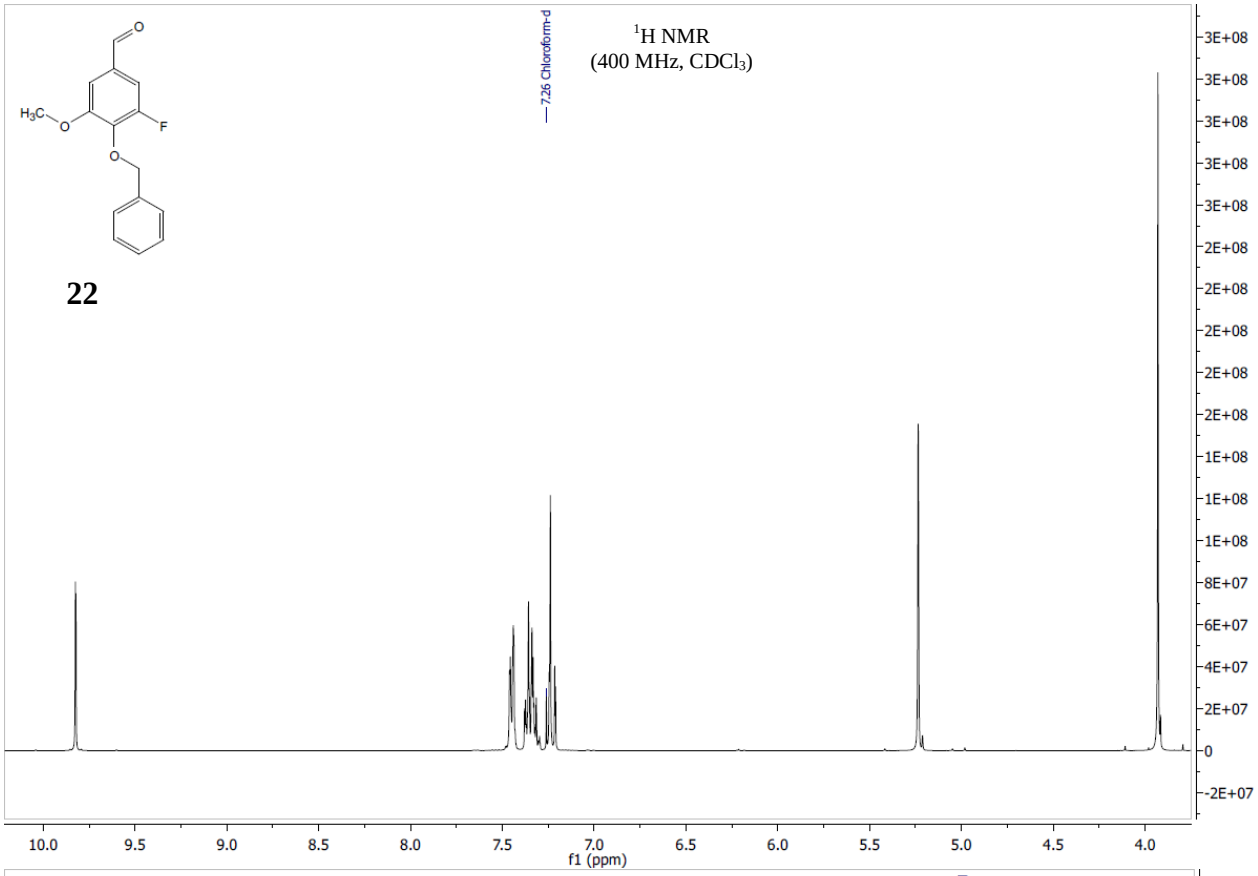
17b

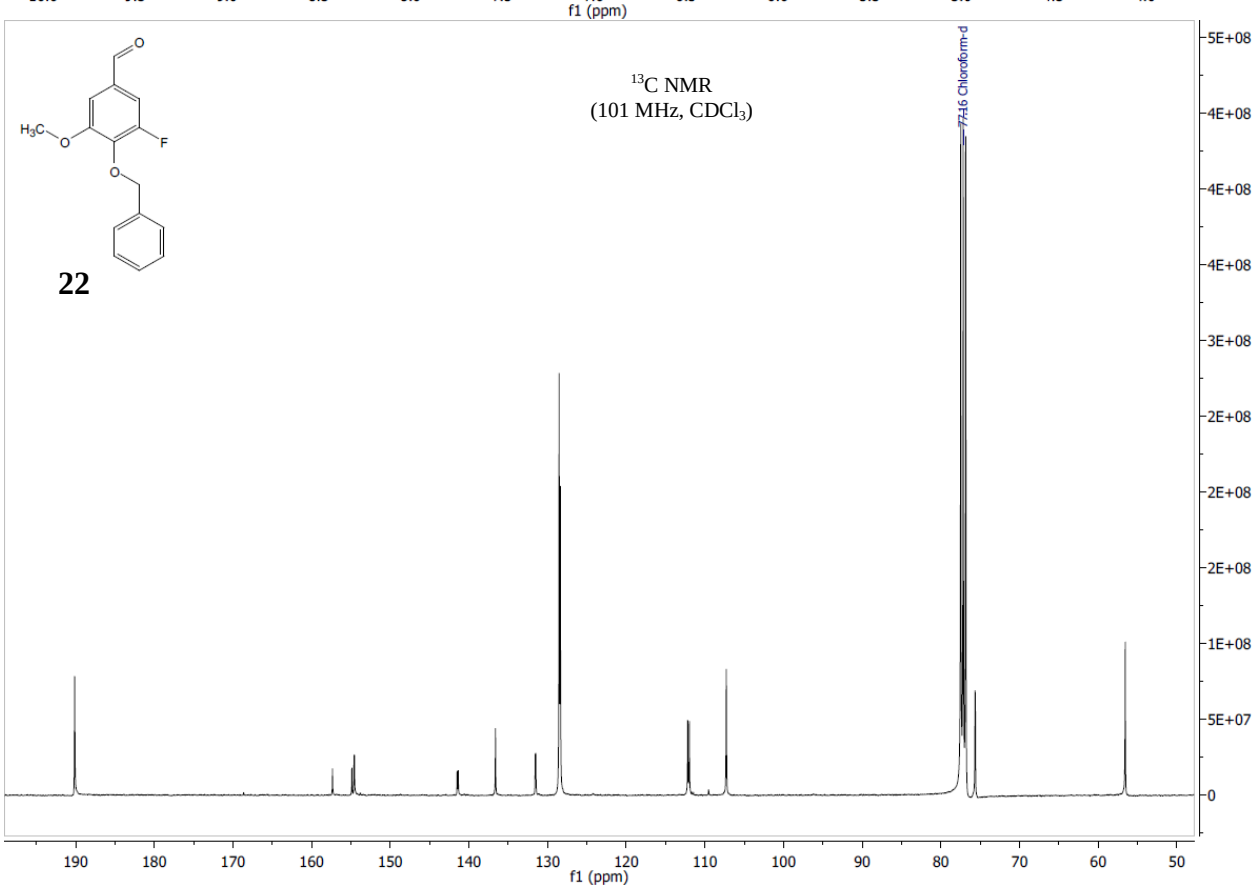

^{1}H NMR
(400 MHz, $CDCl_3$)
HO
F
O
H
O
20
7.26 Chloroform-d
f1 (ppm)

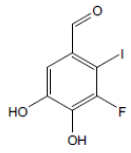

^{13}C NMR
(101 MHz, $CDCl_3$)
HO
F
O
H
O
20
77.16 Chloroform-d
f1 (ppm)


O
I
HO
F
O
21
7.26 Chloroform-d
^{1}H NMR
(400 MHz, $CDCl_3$)
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
10.2 9.8 9.6 9.4 9.2 9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8
f1 (ppm)


O
I
HO
F
O
21
^{13}C NMR
(101 MHz, $CDCl_3$)
77.16 Chloroform-d
2E+05
2E+05
2E+05
2E+05
2E+05
2E+05
2E+05
2E+05
1E+05
1E+05
1E+05
1E+05
1E+05
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
-20000
200 195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70
f1 (ppm)

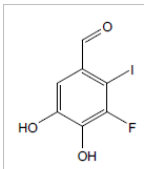

7.26 Chloroform-d
^{1}H NMR
(400 MHz, $CDCl_3$)
22
f1 (ppm)


77.16 Chloroform-d
^{13}C NMR
(101 MHz, $CDCl_3$)
22
f1 (ppm)



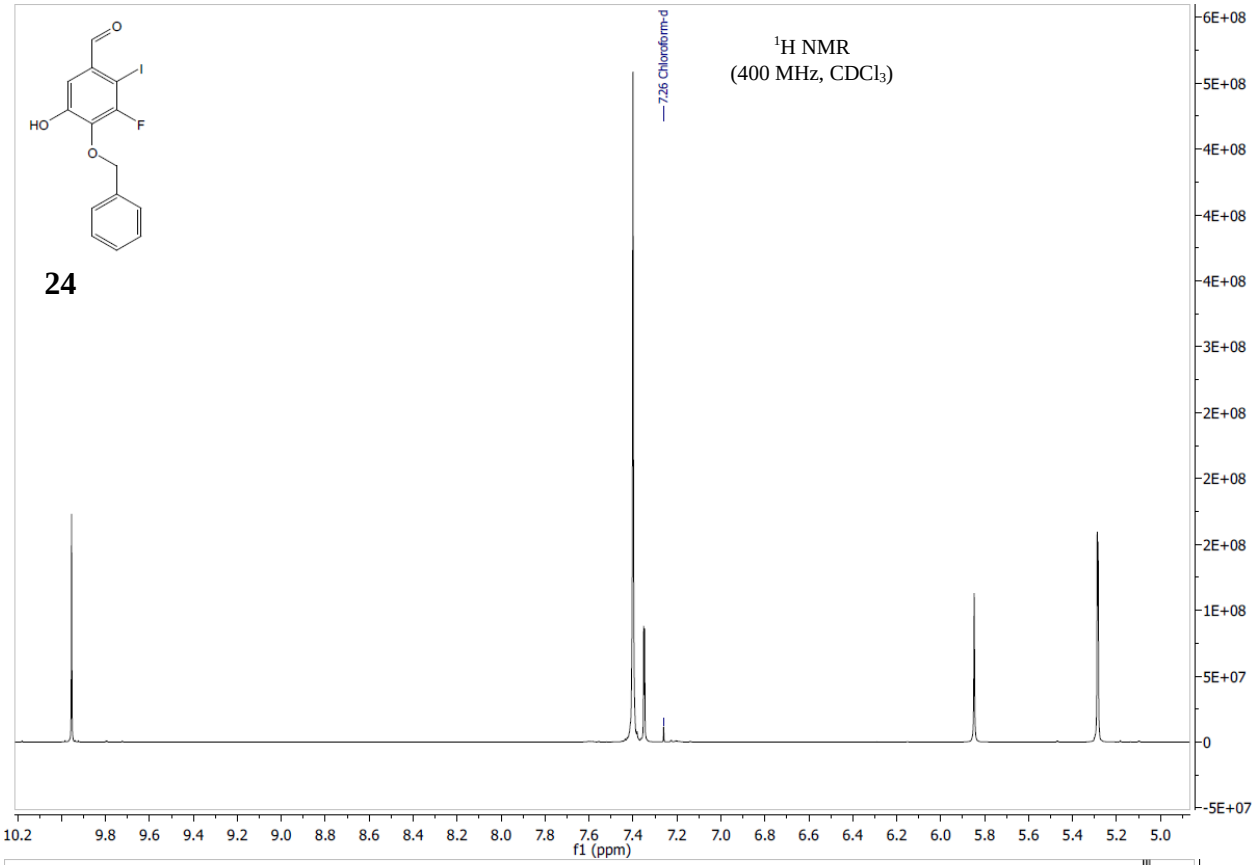
23

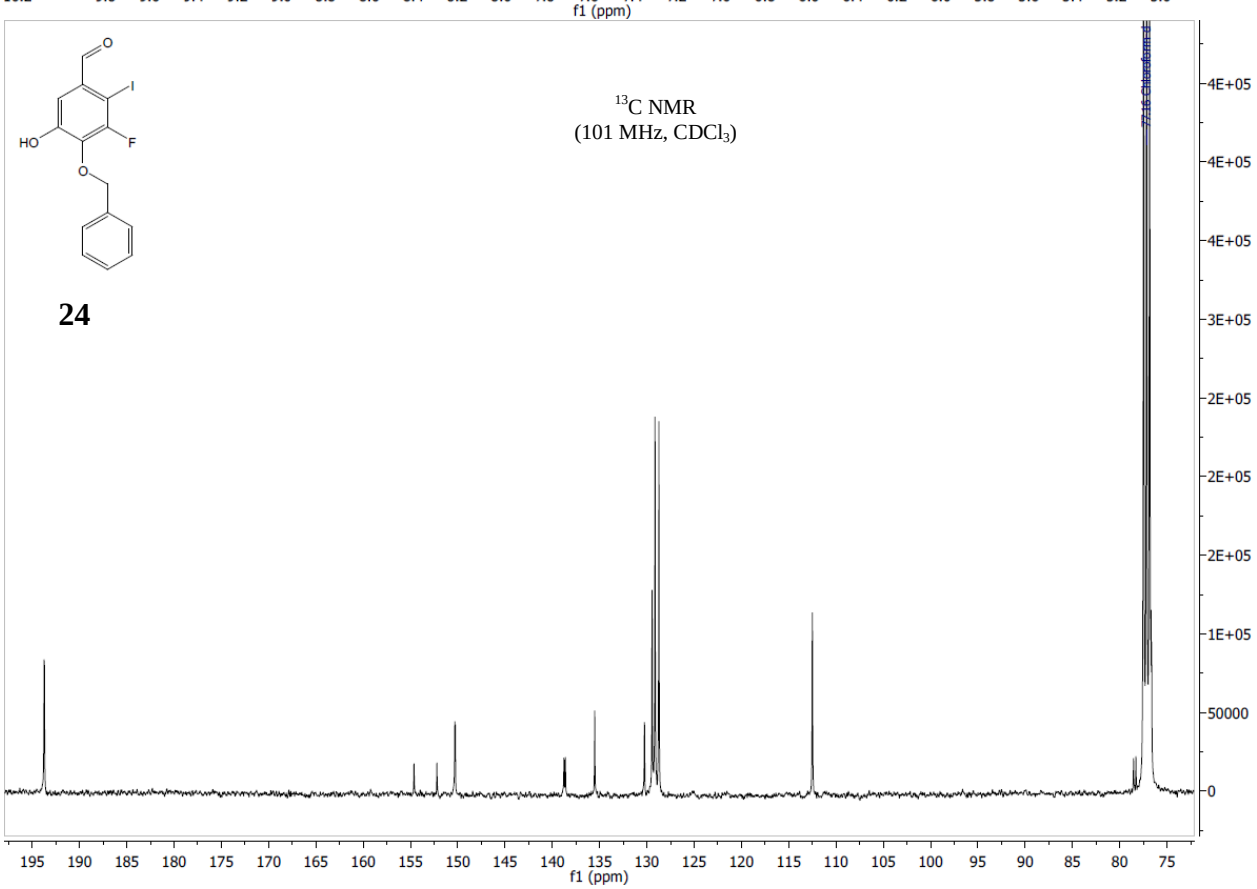
^{1}H NMR
(400 MHz, $CDCl_3$)



23

^{13}C NMR
(101 MHz, $CDCl_3$)


O
I
HO
F
O
24
7.26 Chloroform-d
^{1}H NMR
(400 MHz, $CDCl_3$)
6E+08
5E+08
4E+08
4E+08
4E+08
3E+08
2E+08
2E+08
2E+08
1E+08
5E+07
0
-5E+07
10.2 9.8 9.6 9.4 9.2 9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0
f1 (ppm)


O
I
HO
F
O
24
^{13}C NMR
(101 MHz, $CDCl_3$)
4E+05
4E+05
4E+05
3E+05
2E+05
2E+05
2E+05
1E+05
50000
0
195 190 185 180 175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75
f1 (ppm)

VI. References

[i]. H. M. Walborsky, M. Topolski, C. Hamdouchi, J. Pankowski, *J. Org. Chem.* **1992** *57*, 6188–6191.