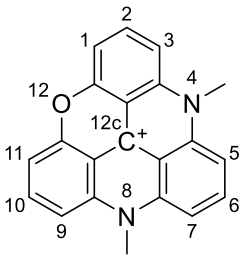
Diazaoxatriangulenium: synthesis of reactive derivatives and conjugation to bovine serum albumin

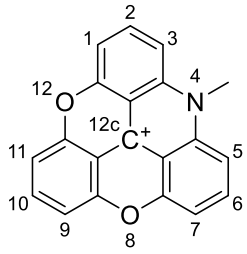
Ilkay Bora, Sidsel A. Bogh, Marco Santella, Martin Rosenberg, Thomas Just Sørensen* and Bo W. Laursen*

Contents

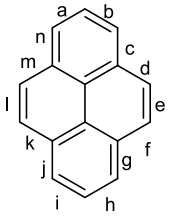
Nomenclature of triangulenium ions



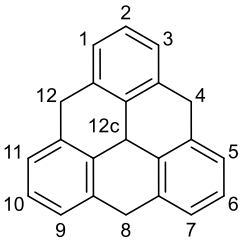
DAOTA



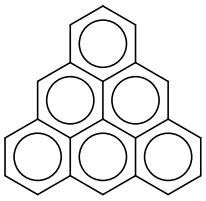
ADOTA



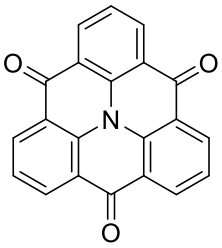
Pyrene



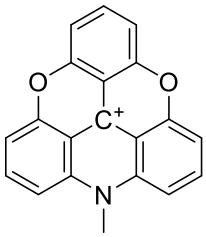
4,8,12,12c-tetrahydro-dibenzo[*cd*,*mn*]-pyrene



Triangulene
(all sp^2, non existing)



Heterotriangulenes
(all sp^2)



Triangulenium
(all sp^2)

ADOTA

The triangulenium dyes are after IUPAC nomenclature a functionalized pyrene derivative, but the fused ring system has the trivial name triangulene as named after Clar. The trivial name of the ionic versions is triangulenium. The heteroatom substitutions in the structure in the IUPAC nomenclature introduced as prefixes, thus the system with two oxygen bridges and a single nitrogen bridge becomes:

4-aza-8,12-dioxa-4,8,12,12c-tetrahydro-dibenzo[*cd*,*mn*]-pyrenylium

or

azadioxatriangulenium

Either name can lead to the abbreviation: ADOTA.

A *N*- substituent in the azadioxatriangulenium structure equals the 4-substituent, so e.g. the substituted azadioxatriangulenium dye, **4a** can be named either:

4-(3-Carboxypropyl)-4-aza-8,12-dioxa-4,8,12,12c-tetrahydro-dibenzo[*cd*,*mn*]-pyrenylium

or

N-(3-carboxypropyl)-azadioxatriangulenium

DAOTA

The heteroatom substitutions in the structure in the IUPAC nomenclature introduced as prefixes, thus the system with one oxygen bridge and two nitrogen bridges becomes:

4,8-diaza-12-dioxa-4,8,12,12c-tetrahydro-dibenzo[*cd*,*mn*]-pyrenylium

or

diazaoxatriangulenium

Either name can lead to the abbreviation: DAOTA.

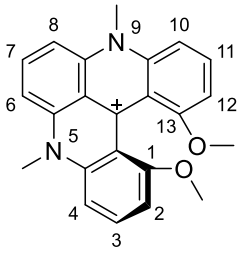
N, N'- substituents in the diazaoxatriangulenium structure equal the 4- and 8-subsitutents, so e.g. the substituted diazaoxatriangulenium dye, **5a** can be named either:

4-(3-Carboxypropyl)-8-methyl-4,8-diaza-12-oxa-4,8,12,12c-tetrahydro-dibenzo[*cd*,*mn*]-pyrenylium

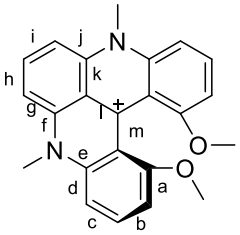
or

N-(3-Carboxypropyl)-*N*'-methyl-diazaoxatriangulenium

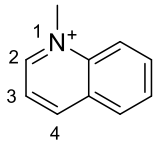
Systematic names for [4]helicenium ions



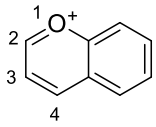
DMQA$^+$



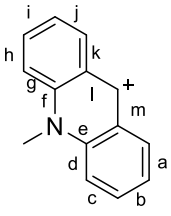
DMQA$^+$



Quinolinium



Chromenium



Acridinium

DMQA$^+$ 1,13-dimethoxy-5,9-dialkyl-quin[2,3,4-*kl*]acridinium or *N,N'*-dialkyl-1,13-dimethoxy-quin[2,3,4-*kl*]acridinium

Quantum Yield (QY)

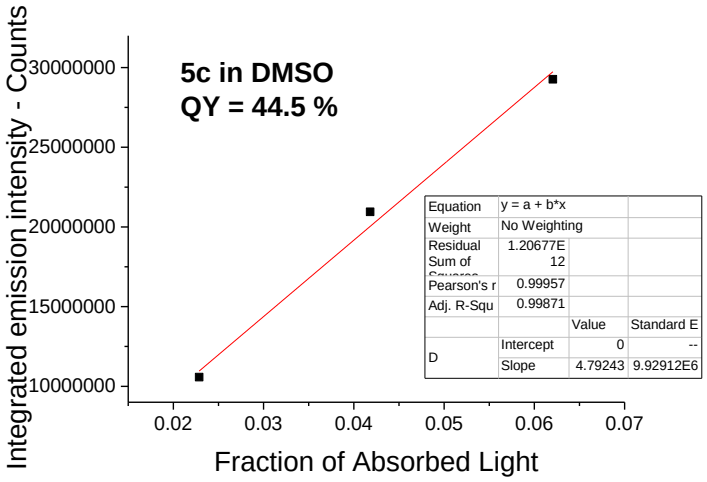
The QYs (ϕ_{fl}) were determined by the relative method using the formula:

$$\phi_{fl} = \phi_{fl,ref} \frac{\alpha \cdot n^2}{\alpha_{ref} \cdot n_{ref}^2}$$

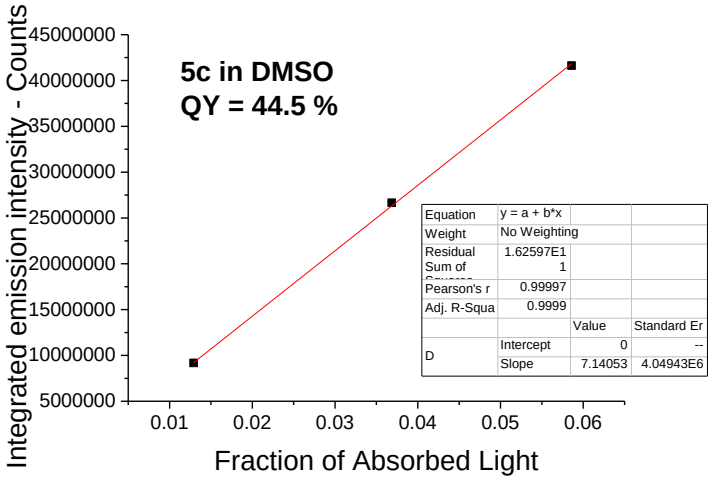
Where n is the refractive index of the solvent used and α is the slope determined from a linear fit for three or more points of I_{int} vs. $f_{a.}$ Here, f_a is the fraction of light absorbed (f_a = 1–10^{-A}, where A is the absorbance measured at the excitation wavelength) and I_{int} is the integrated emission intensity. The subscript "*ref*" refers to a fluorescence reference standard (here rhodamine 6G, $\phi_{fl,ref}$ = 0.95) measured using the instrumental same settings. The absorption spectra of the fluorophores were measured at three or more different concentrations (all between 1-12 μM) obtained by titration of a dye stock solution directly into the cuvette. Each absorption measurement was followed by an emission measurement with an excitation wavelength of 510 nm using a solid state laser excitation source. For all measurements the absorption was kept below 0.1 in the maximum of the longest wavelength absorption band. The settings for both the absorption and emission measurements were the same for every fluorescence quantum yield measurement to ensure comparability. The emission signal was measured with a PMT detector with a spectral range of 300-900 nm. The spectral bandwidth in the absorption spectrometer was 1 nm, whereas the laser used for excitation in the emission measurements has a spectral band width of 7 nm. To compensate for the difference in the spectral bandwidths of the excitation sources of the two instruments, the measured absorption spectra were multiplied by a Gaussian fit of the spectral band shape of the laser pulse before the fraction of absorbed light (f_a) was determined. Magic angle settings were used for the emission measurements and emission spectra were corrected for the wavelength dependent response of the detection system before use in the fluorescence quantum yield determinations. A worked example and all the plots used for quantum yield determination are shown below. Single-point QY measurements for **5c-BSA** with different degree of labeling (DOL) were calculated from the formula:

$$\phi_{fl} = \phi_{fl,ref} \frac{I_{int} \cdot f_{a,ref} \cdot n^2}{f_a \cdot I_{int,ref} \cdot n_{ref}^2}$$

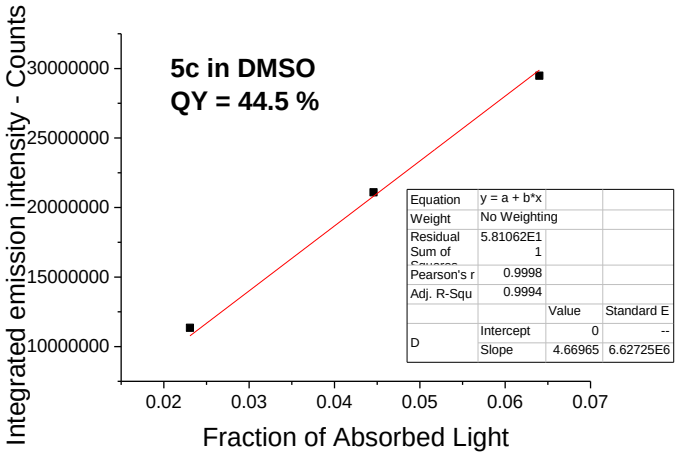
QY data for rhodamine 6G (Rh6G) in EtOH and 5c in DMSO, MeCN and PBS solutions

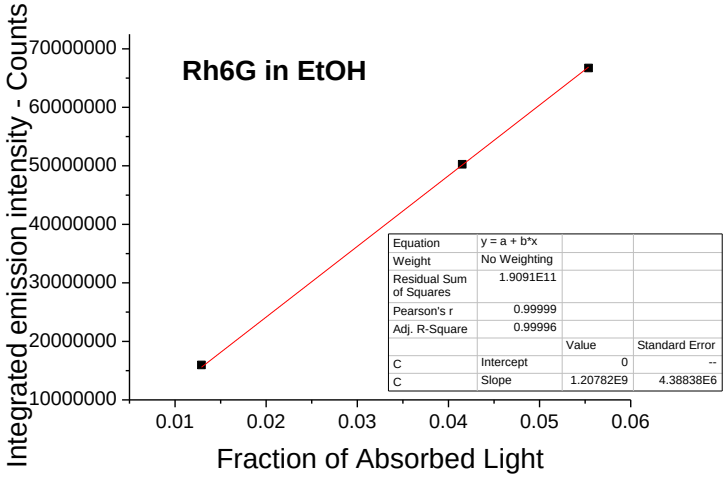


Equation	y = a + b*x		
Weight	No Weighting		
Residual Sum of	1.20677E12		
Pearson's r	0.99957		
Adj. R-Squ	0.99871		
		Value	Standard E
D	Intercept	0	--
	Slope	4.79243	9.92912E6



Equation	y = a + b*x		
Weight	No Weighting		
Residual Sum of	1.62597E11		
Pearson's r	0.99997		
Adj. R-Squa	0.9999		
		Value	Standard Er
D	Intercept	0	--
	Slope	7.14053	4.04943E6


5c in DMSO
QY = 44.5 %
Integrated emission intensity - Counts
30000000
25000000
20000000
15000000
10000000
0.02
0.03
0.04
0.05
0.06
0.07
Fraction of Absorbed Light
Equation y = a + b*x
Weight No Weighting
Residual Sum of 5.81062E11
Pearson's r 0.9998
Adj. R-Squ 0.9994
Value Standard E
D Intercept 0 --
Slope 4.66965 6.62725E6


Rh6G in EtOH
Integrated emission intensity - Counts
70000000
60000000
50000000
40000000
30000000
20000000
10000000
0.01
0.02
0.03
0.04
0.05
0.06
Fraction of Absorbed Light
Equation y = a + b*x
Weight No Weighting
Residual Sum of Squares 1.9091E11
Pearson's r 0.99999
Adj. R-Square 0.99996
Value Standard Error
C Intercept 0 --
C Slope 1.20782E9 4.38838E6

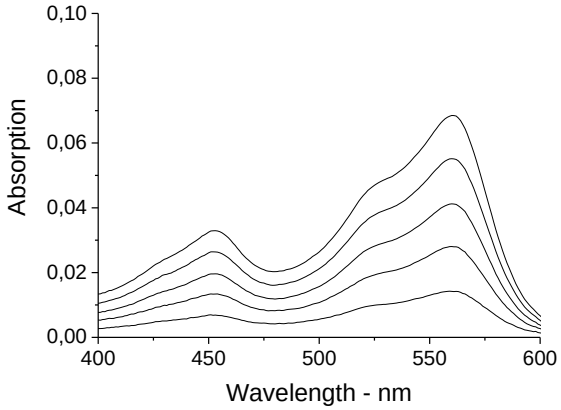
Labeling

For the labeling experiment the conjugation of **6c** and BSA was carried out in BBS buffer (pH 8.2). **5c** was dissolved in DMSO and 1.2 equivalents of TSTU and 3 equivalents of DIPEA was added to produce the activated NHS ester. After stirring at ambient temperatures for approximately 1 hour, 0.4 mL of the NHS ester solution in DMSO was added to 1.2 mL of BBS buffer while gently shaking. The solution was then slowly transferred to 2.4 mL of BSA (30 mg/mL) in BBS buffer. After 2 hours on a rocking table, 3 mL of each solution were transferred to dialysis cassettes with molecular weight cut-off at 10 kDa (Slide-A-Lyzer from Thermo Fisher Scientific) to remove unreacted ADOTA from the sample. The sample was dialyzed in 800 mL of PBS buffer with buffer change three times with at least 8 hours in between.

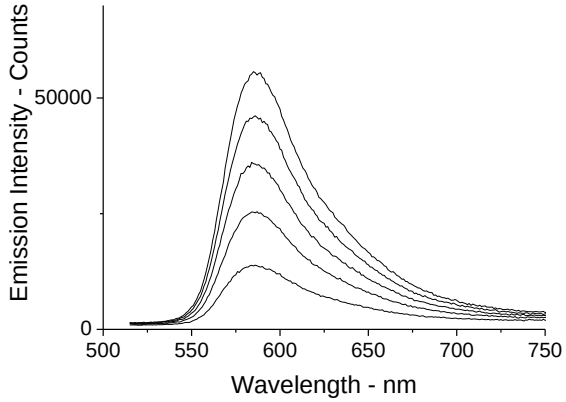
The degree of labeling (DOL) was determined using the absorbance at 280 nm (A_{280}) to determine the BSA concentration, correcting for the absorbance of the DAOTA chromophore in this wavelength region by use of the correction factor ($CF_{ADOTA}=A_{280}/A_{max}$), and from the longest wavelength absorption maximum for the DAOTA chromophore (A_{max}). The DOL is then $DOL=C_{DAOTA}/C_{BSA}$. The absorption spectra below were used to determine the DOL for the **5c-BSA** conjugates.

QY for 5c-BSA, DOL 0.9

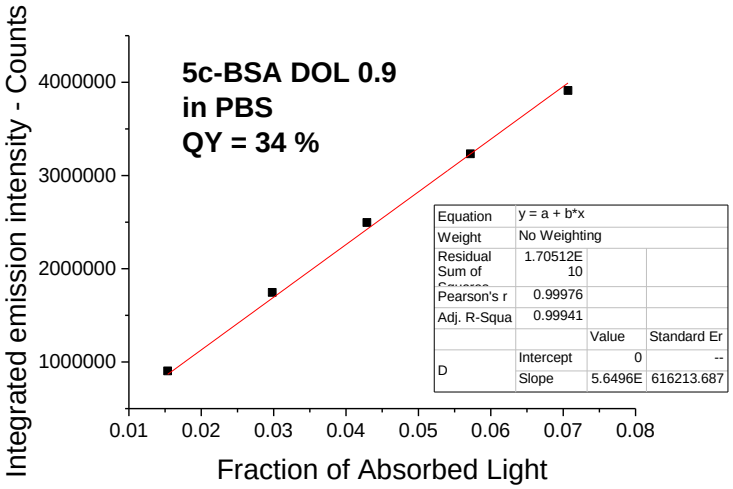
Experimental procedures as described above.



Absorption spectra of 5c-BSA with DOL 0.9 measured at different concentrations in PBS solution.

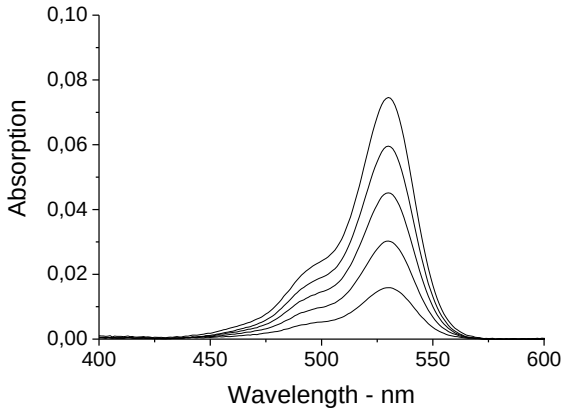


Emission spectra of 5c-BSA with DOL 0.9 measured at different concentrations in PBS solution.

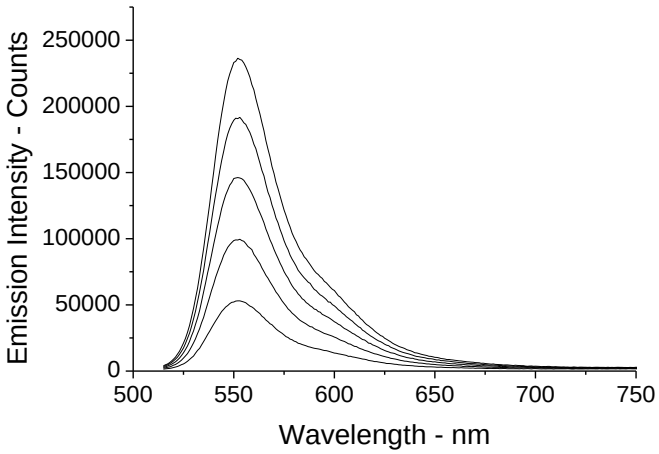


Plot of integrated emission intensity (I_{int}) vs. fraction of absorbed light (f_a) for 5c-BSA with DOL 0.9.

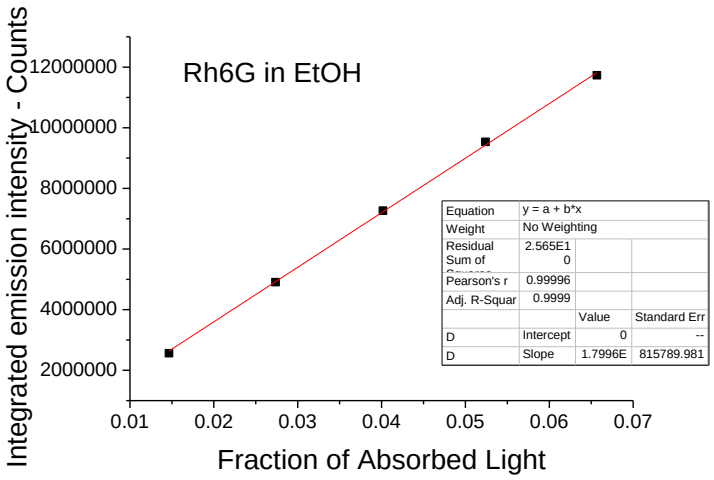
Rh6G reference data for 5c-BSA



Absorption spectra of Rh6G measured in EtOH solution at different concentrations.



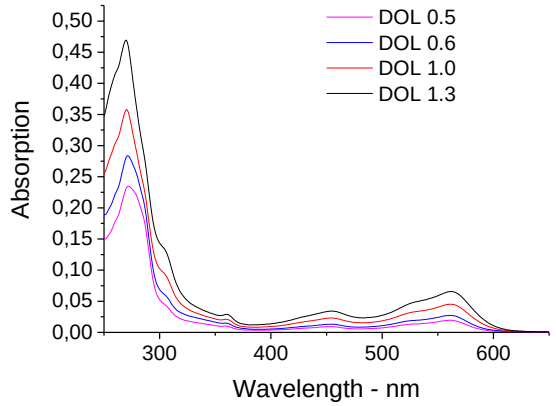
Emission spectra of Rh6G measured in EtOH solution at different concentrations.



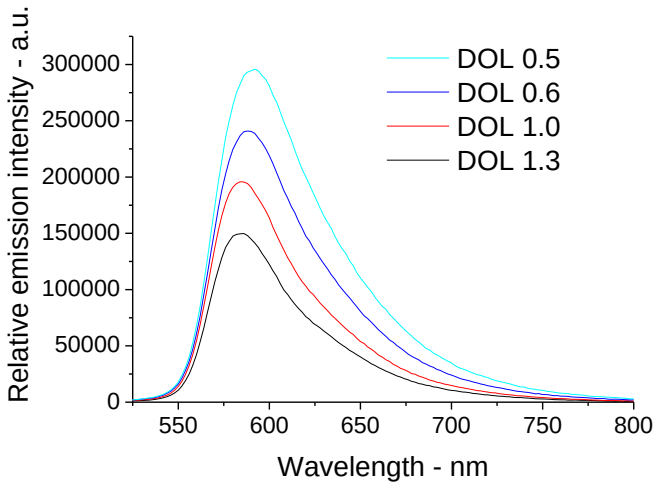
Plot of the integrated emission intensity (I_{int}) vs. fraction of absorbed light (f_a) for the Rh6G reference .

QY data 5c-BSA with different DOL

The degree of labelling (DOL) was varied by using different **5c-BSA** ratios. The relative quantum yields of different DOL were estimated using single point measurements:



Absorption spectra of 5c-BSA with varying DOL measured in PBS solution.



Emission spectra of 5c-BSA with varying DOL measured in PBS solution.

Time-dependent photophysical properties

Photophysical properties of 5c-BSA and 5c in PBS solution

Data from QY determinations and fitting the time-resolved emission decay profiles of non-bound **5c** and **5c-BSA**, are given in figure 3 in the main text. **5c** has a mono-exponential time-resolved emission decay profile, while the **5c-BSA** conjugate exhibits an emission decay profile that requires a multi-exponential fit. Thus, the non-bound dyes are present in one emitting population, while the dye when conjugated to BSA can be found in at least three different environments. Despite having two quenched populations the resulting intensity averaged lifetime $<\tau_{Int}>$ of **5c-BSA** is 19.4 ns.

Photophysical properties of 5c-BSA and 5c measured in PBS solution. Quantum yields (ϕ_{fl}) are measured with Rh6G as reference (ϕ_{fl} = 0.95). Fluorescence lifetime τ_n (n = 1-3) components are given in ns, and the corresponding fractional contributions A_n (n = 1-3) are given in percent. The intensity averaged fluorescence lifetimes $<\tau_{int}>$ are given in ns.

	ϕ_{fl}	τ_1 / ns	A_1 / %	τ_2 / ns	A_2 / %	τ_3 / ns	A_3 / %	$<\tau_{int}>$ / ns	χ^2
5c	0.35	14.2	100	-	-	-	-	14.2	1.166
5c-BSA, DOL 0.9	0.34	21.2	63.7	8.74	23.2	1.37	13.0	19.4	1.001

Rotational correlation times (θ) measured for 5c-BSA

The parameters needed to fit the time-resolved anisotropy decay profiles for **5c-BSA**, are given in figure 3 in the main text. Two components are required to fit the anisotropy decay, a minor contributing short rotational correlation time component (θ_1) is attributed to the fast local motion of the dye, and a dominating long correlation time component (θ_2), which is attributed to the overall motion of the **5c-BSA** conjugate.

The intensity averaged fluorescence lifetime $<\tau_{int}>$ is given in ns. Rotational correlation times (θ_n, n = 1-2) measured for 5c-BSA in PBS solution, given in ns. The corresponding fractional´anisotropy contributions r_n (n = 1-2).

	$<\tau_{int}>$ / ns	θ_1 / ns	r_1	θ_2 / ns	r_2	χ^2
5c-BSA, DOL 0.9	19.4	0.3	0.06	40	0.24	1.388

Quenching by tryptophan

To form the **5c**-tryptophan (**5c-Trp**) conjugate shown below **6c** was reacted with tryptophan. This was done in order to investigate the extent of quenching of the DAOTA chromophore by tryptophan by comparing the relative quantum yield of **5c-Trp** to that **5c**. The geometry of **5c-Trp** corresponds both to a very high local concentration of tryptophan for a quenching experiment, and to an exaggerated close packing of **5c** and tryptophan residues in **5c-BSA** conjugates. The very low degree of quenching in **5c-Trp** demonstrates that **5c** should experience a very limited amount of quenching by tryptophan, when used in bioconjugation experiments.

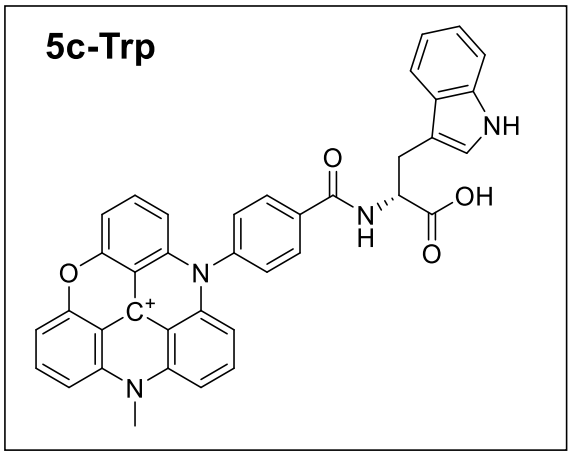
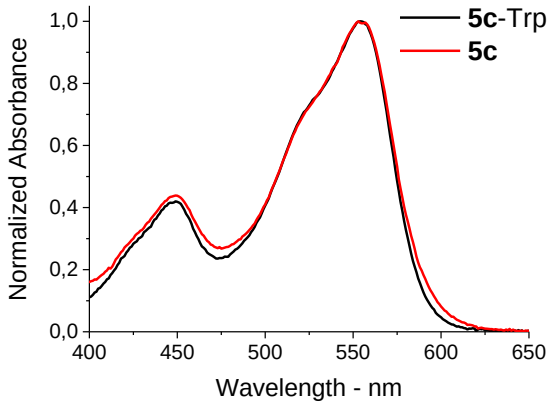
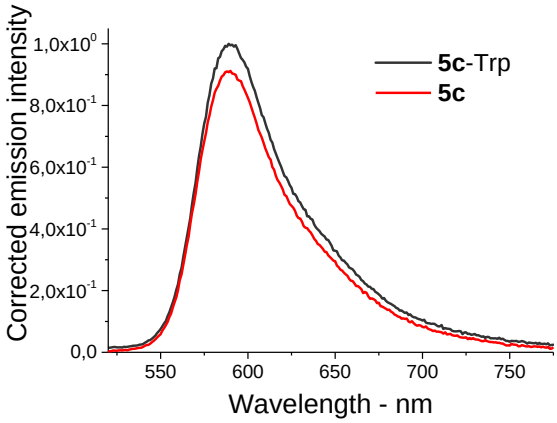


Table showing the redox potential of tryptophan, ADOTA and DAOTA, the former in water pH = 7, while the altter is in acetonitrile.

	E_{red} vs. SCE	E_{ox} vs. SCE
Tryptophan[1]	-	0.77
ADOTA[2, 3]	-0.50	-
DAOTA[2, 3]	-0.85	1.40



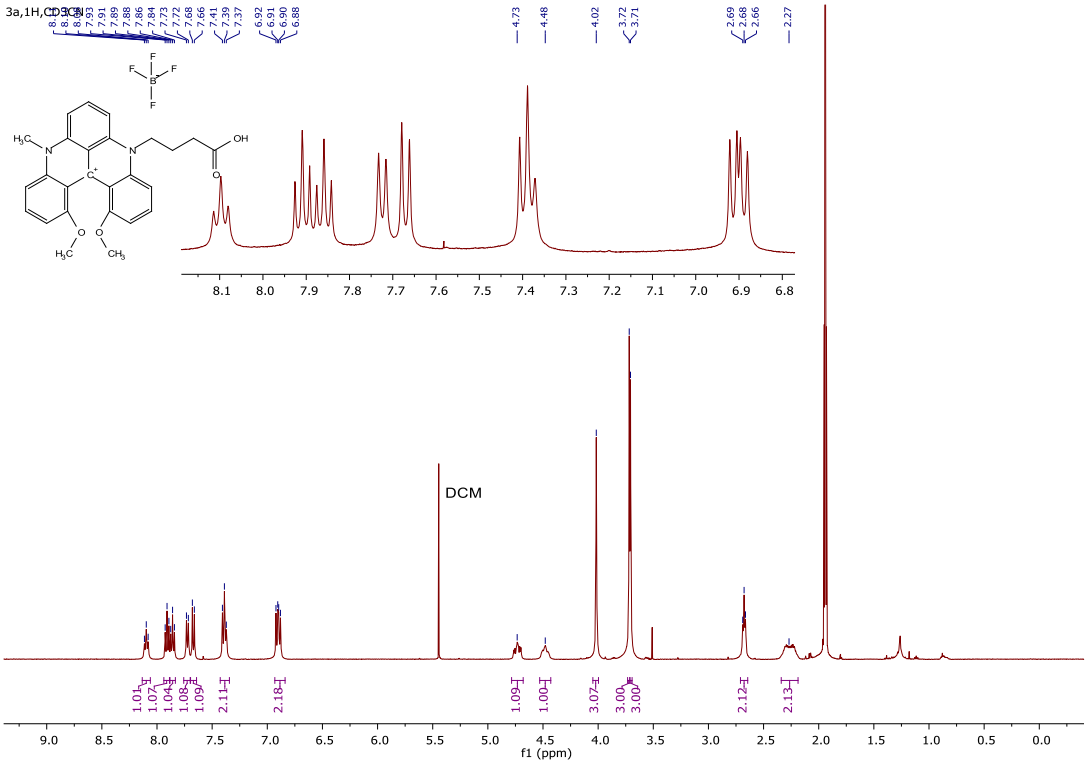
Normalized absorption spectra of 5c and 5c-Trp measured in PBS solution.

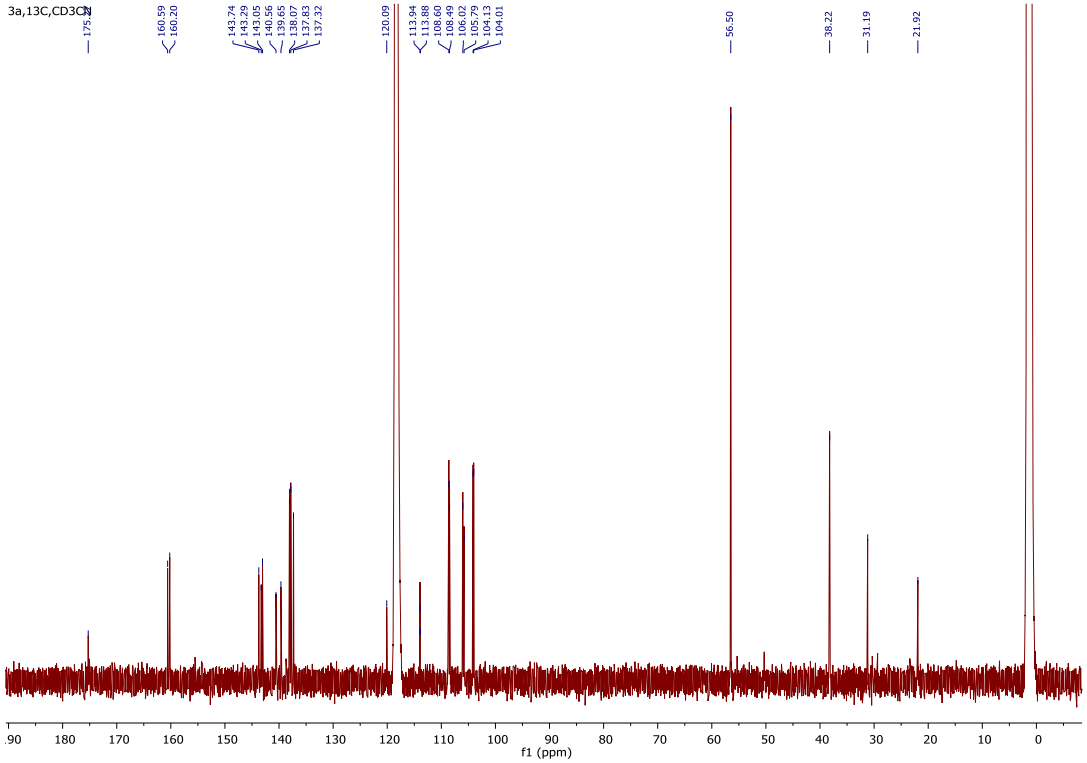


Emission spectra corrected for their absorbance at the excitation wavelength of 5c and 5c-Trp, respectively.

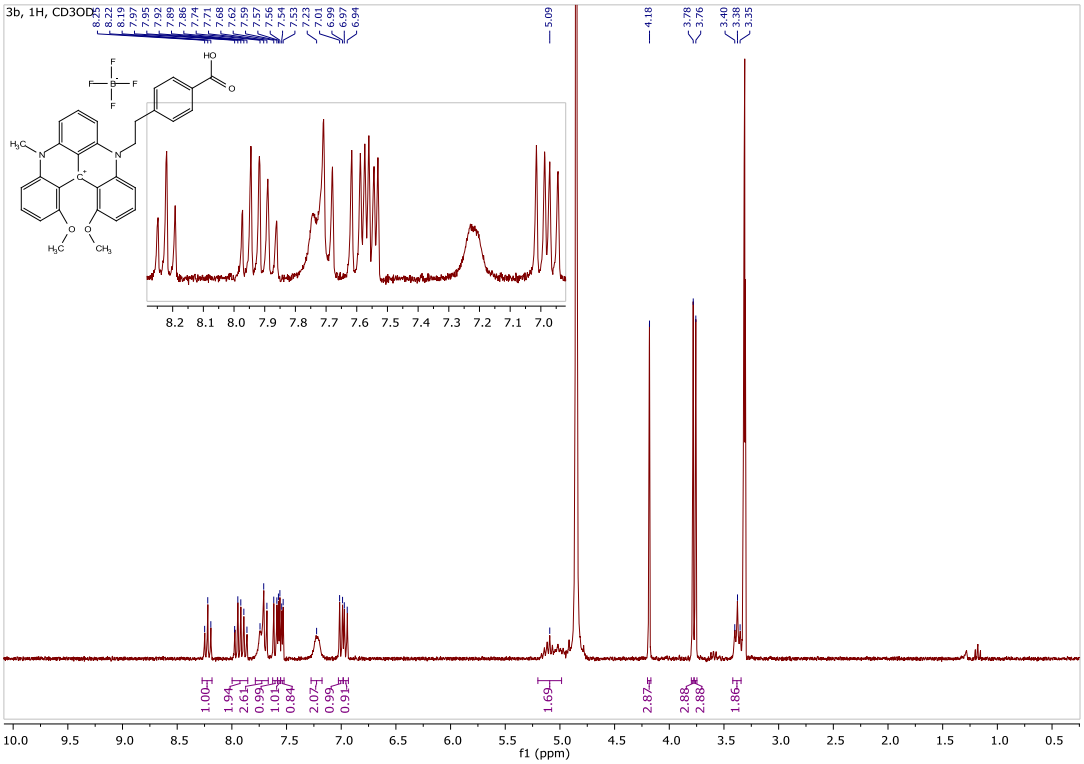
NMR data

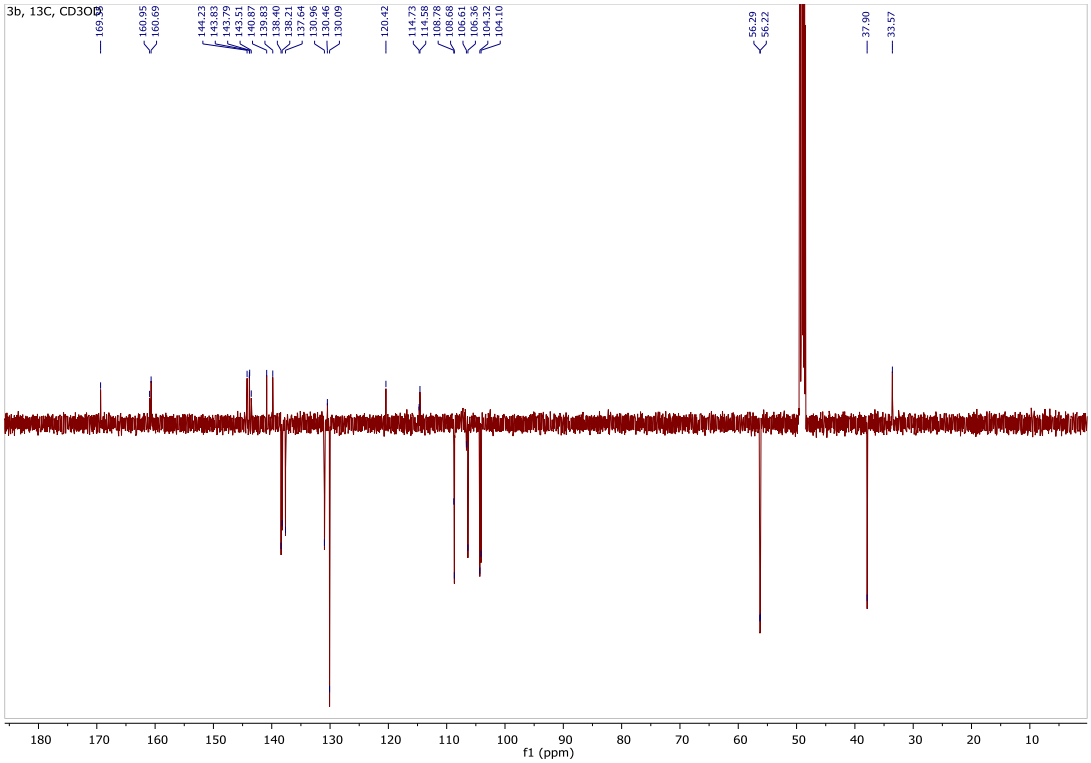
3a

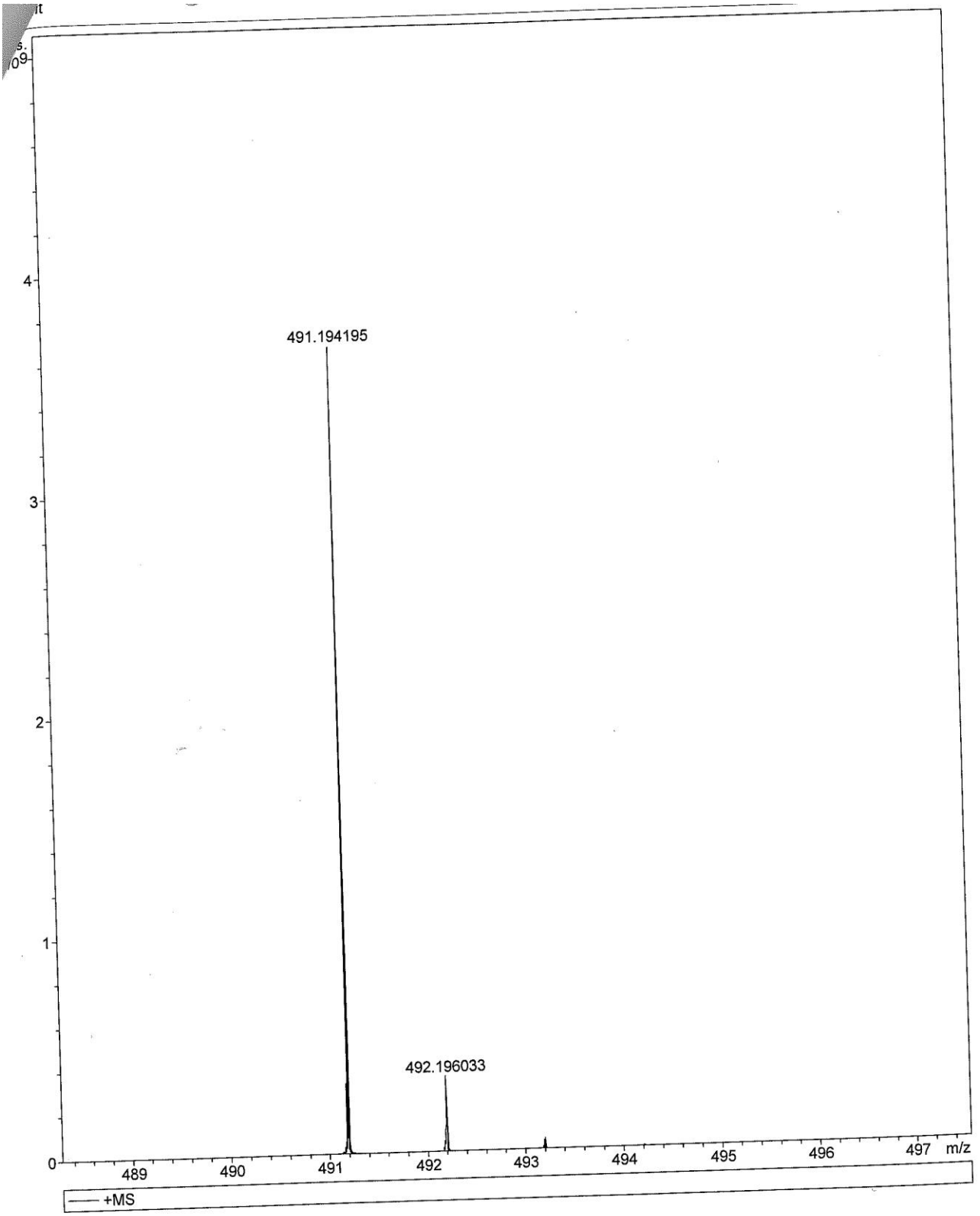




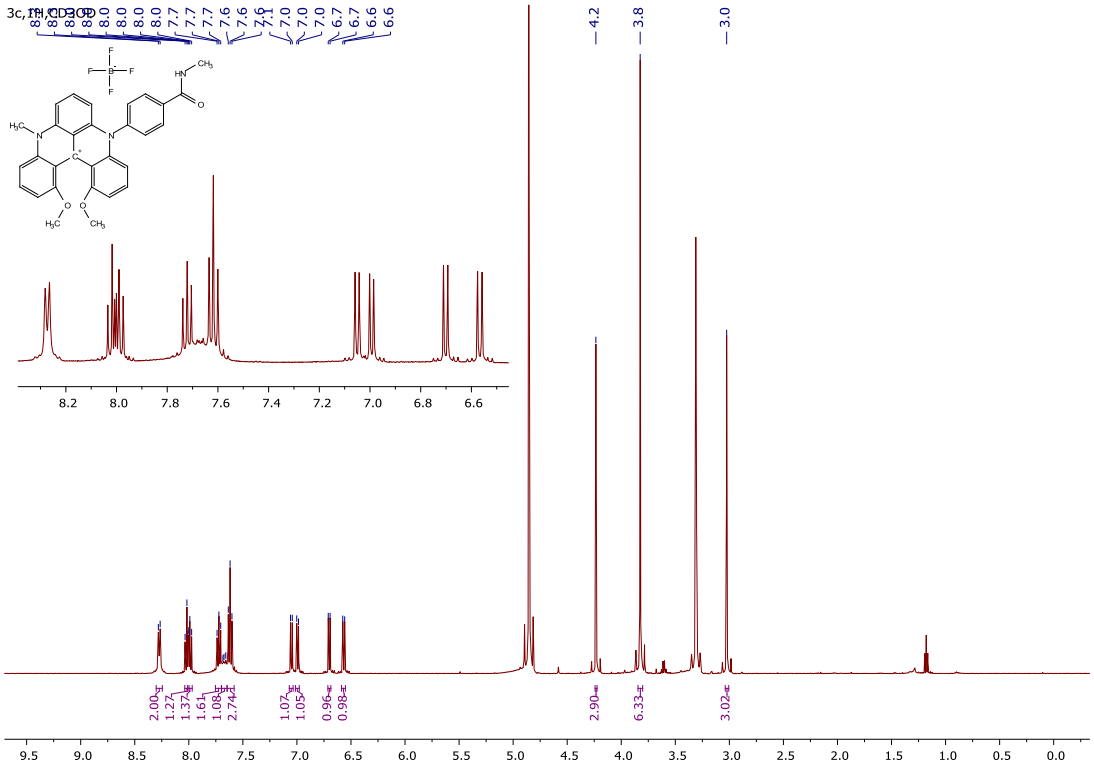
3b

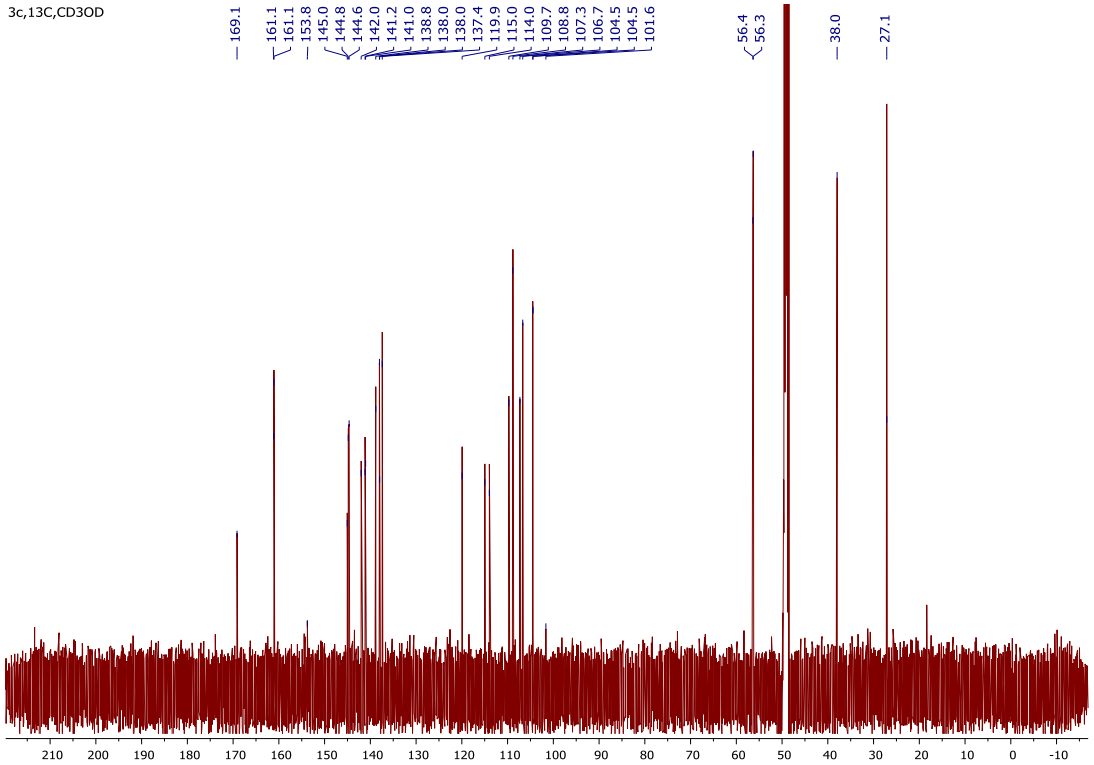


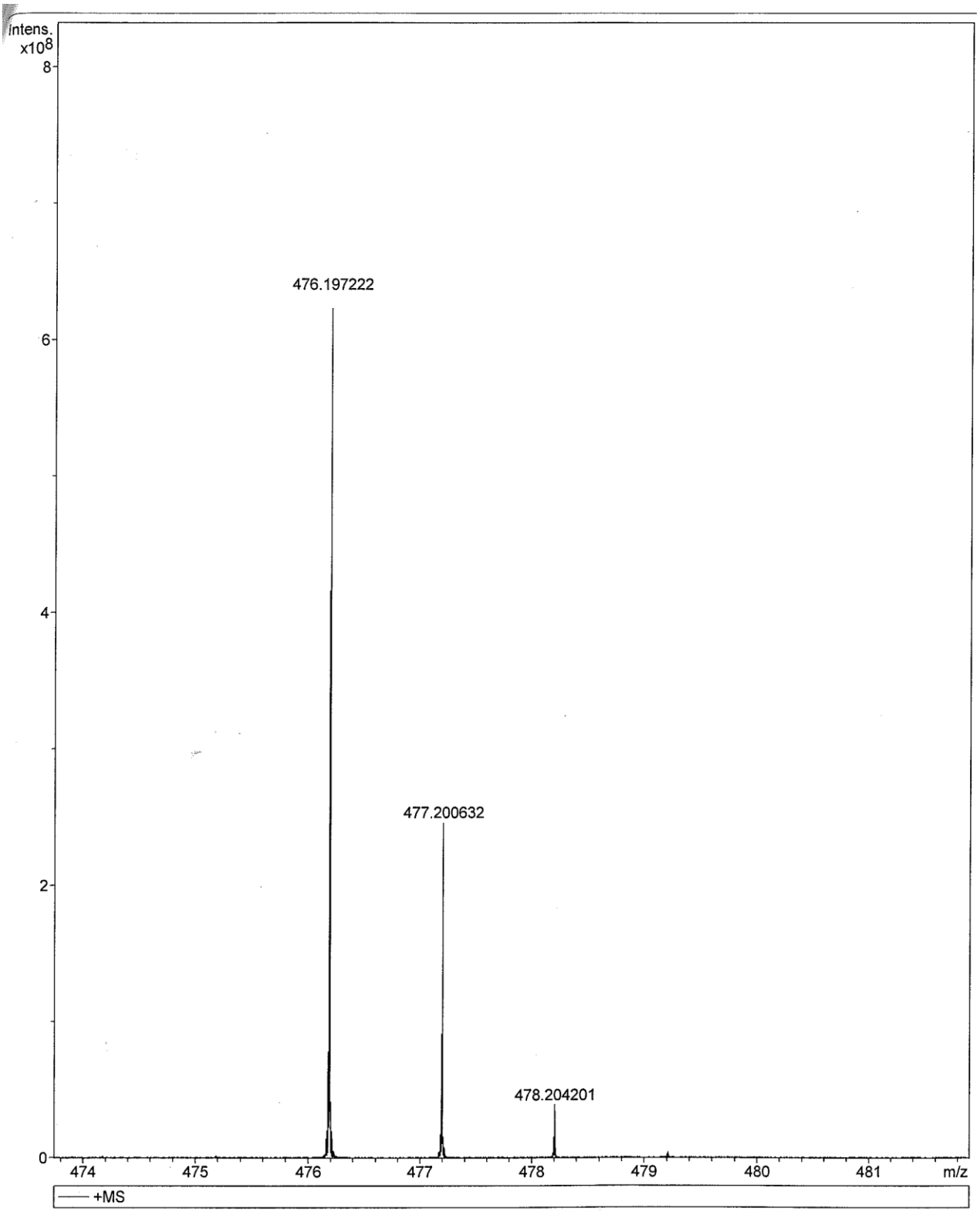



491.194195
492.196033
4
3
2
1
0
489
490
491
492
493
494
495
496
497
m/z
+MS

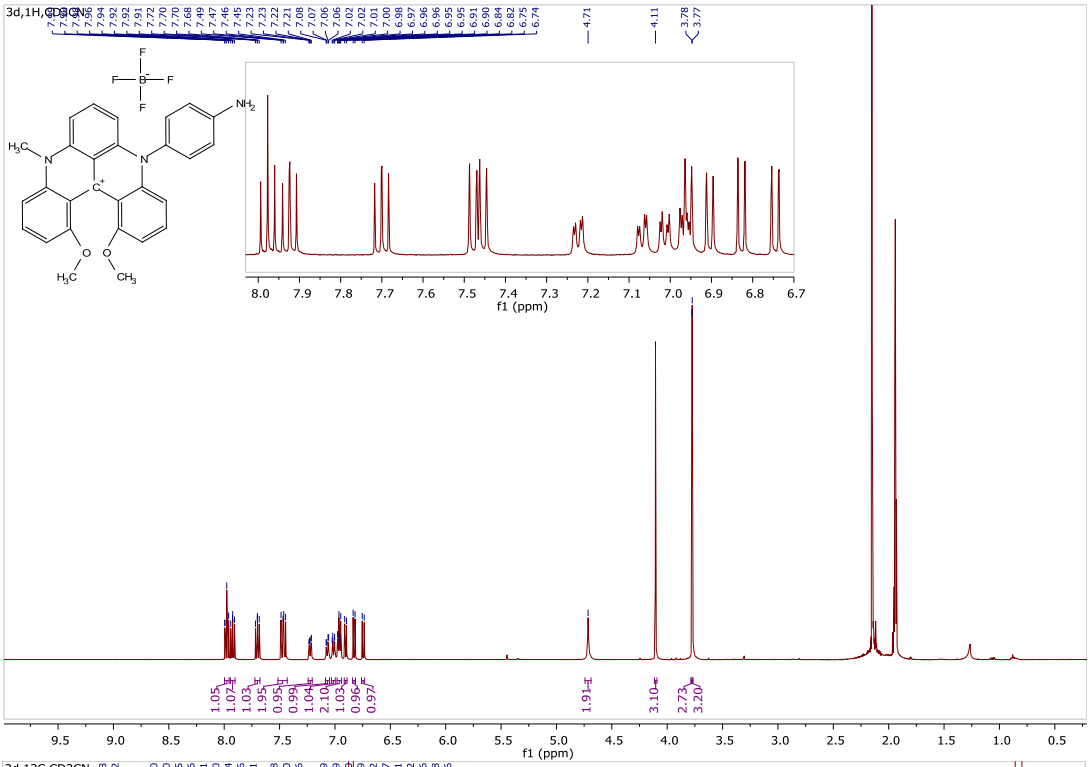
3c’

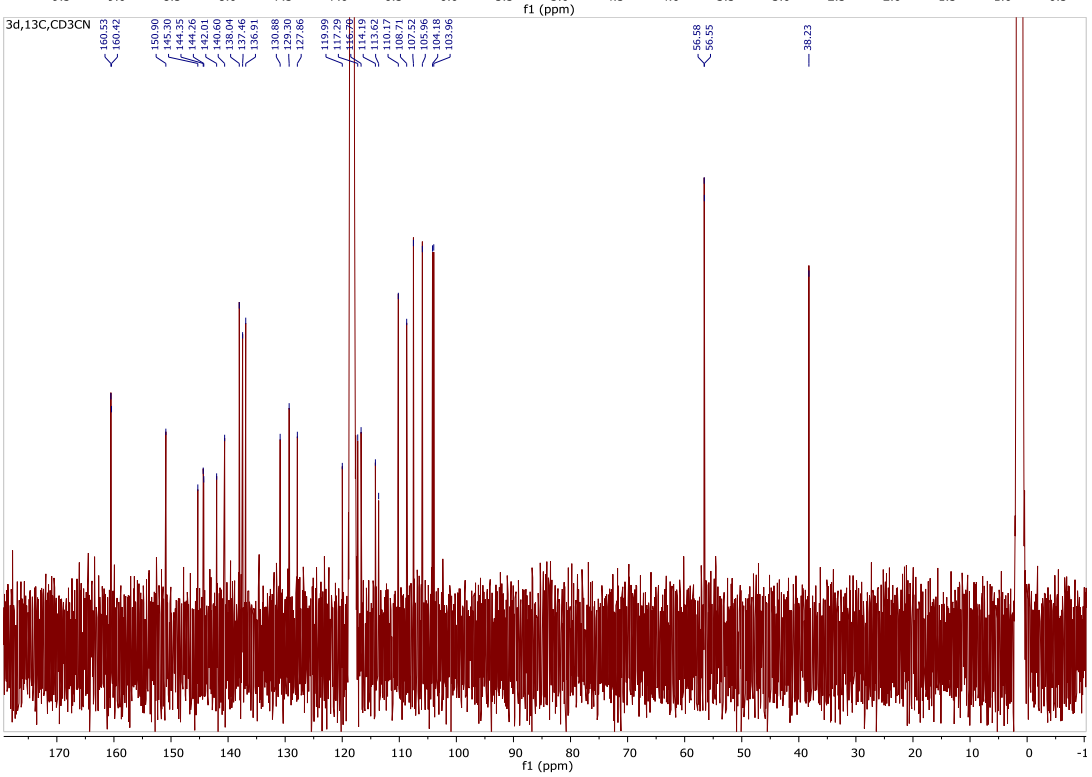

3c,1H,CD3OD
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
4.2
3.8
3.0
2.00
1.27
1.37
1.61
1.08
2.74
1.07
1.05
0.96
0.98
2.90
6.33
3.02
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

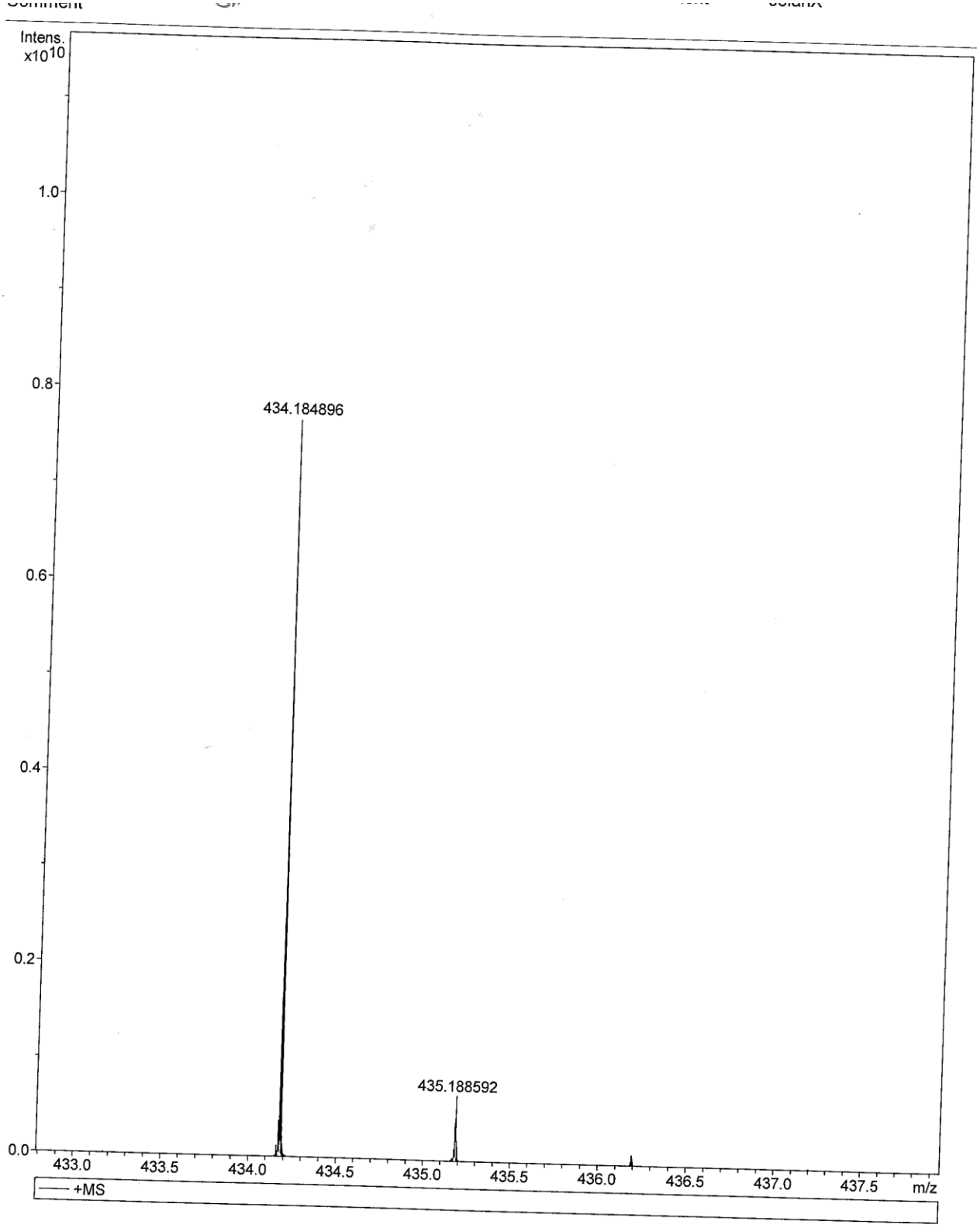

3c,13C,CD3OD
169.1
161.1
161.1
153.8
145.0
144.8
144.6
142.0
141.2
141.0
138.8
138.0
138.0
137.4
119.9
115.0
114.0
109.7
108.8
107.3
106.7
104.5
104.5
101.6
56.4
56.3
38.0
27.1
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10


Intens.
x10^8
8
6
4
2
0
476.197222
477.200632
478.204201
474
475
476
477
478
479
480
481
m/z
+MS

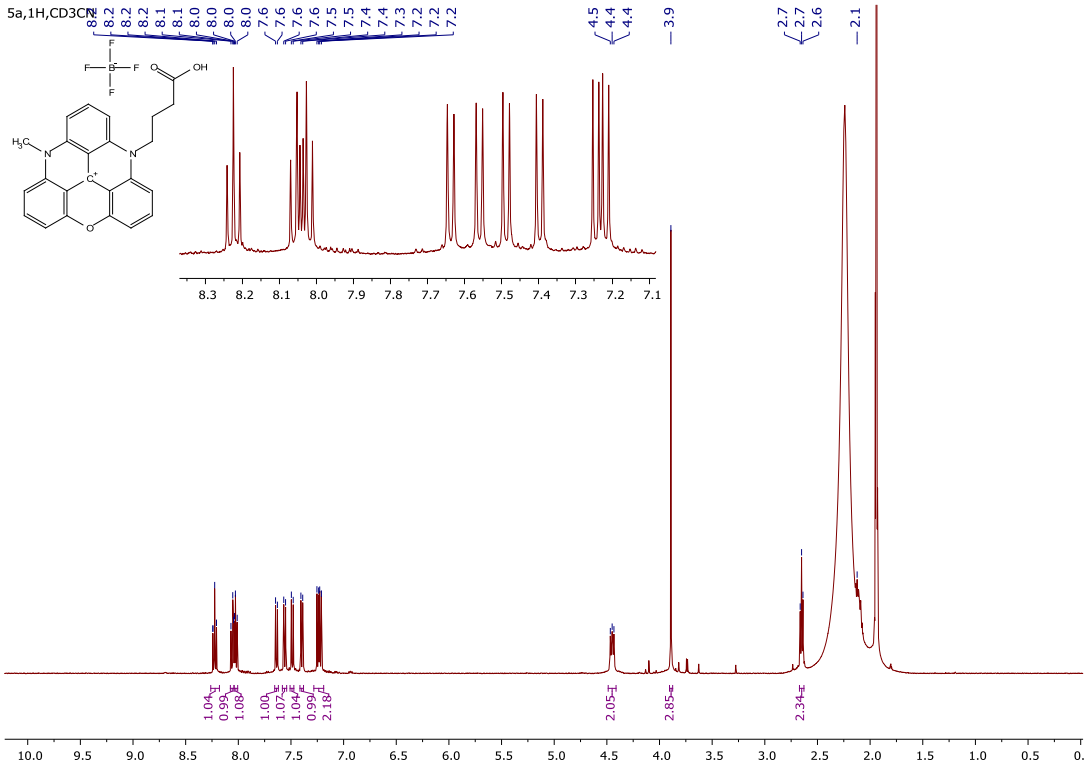
3d

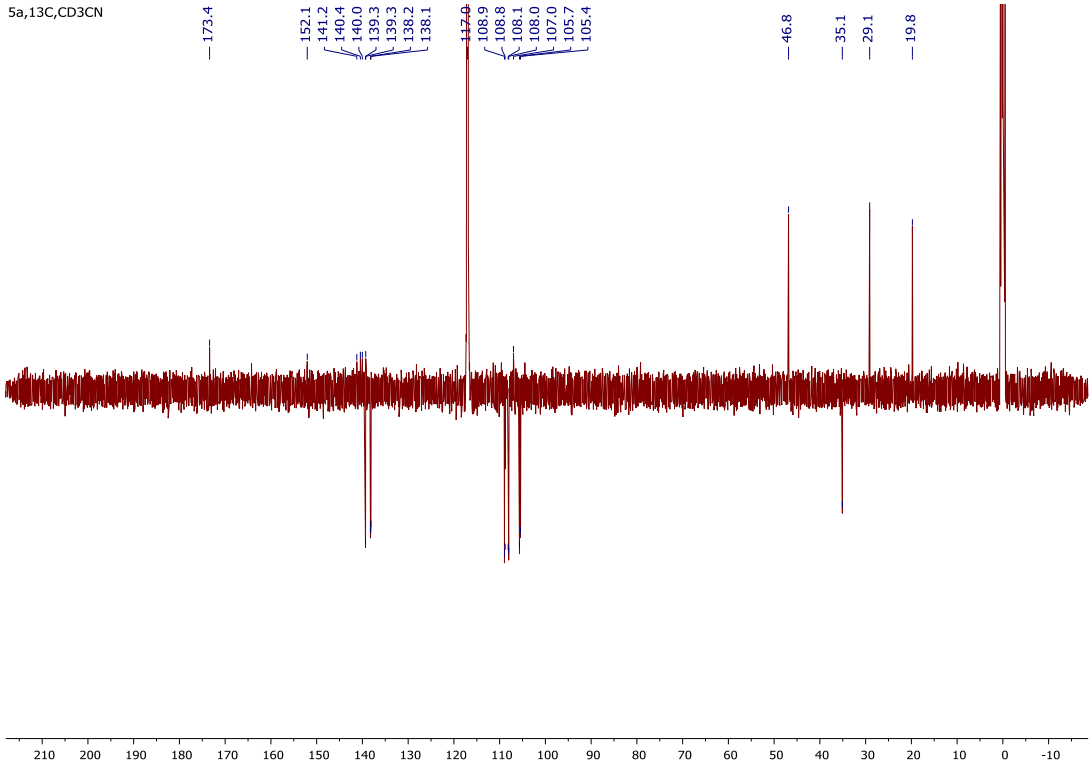





Intens.
x10^10
1.0
0.8
0.6
0.4
0.2
0.0
434.184896
435.188592
433.0
433.5
434.0
434.5
435.0
435.5
436.0
436.5
437.0
437.5
m/z
+MS
Bruker Compass DataAnalysis 4.0
printed: 6/26/2014 8:18:18 AM
Page 1 of 1

5a

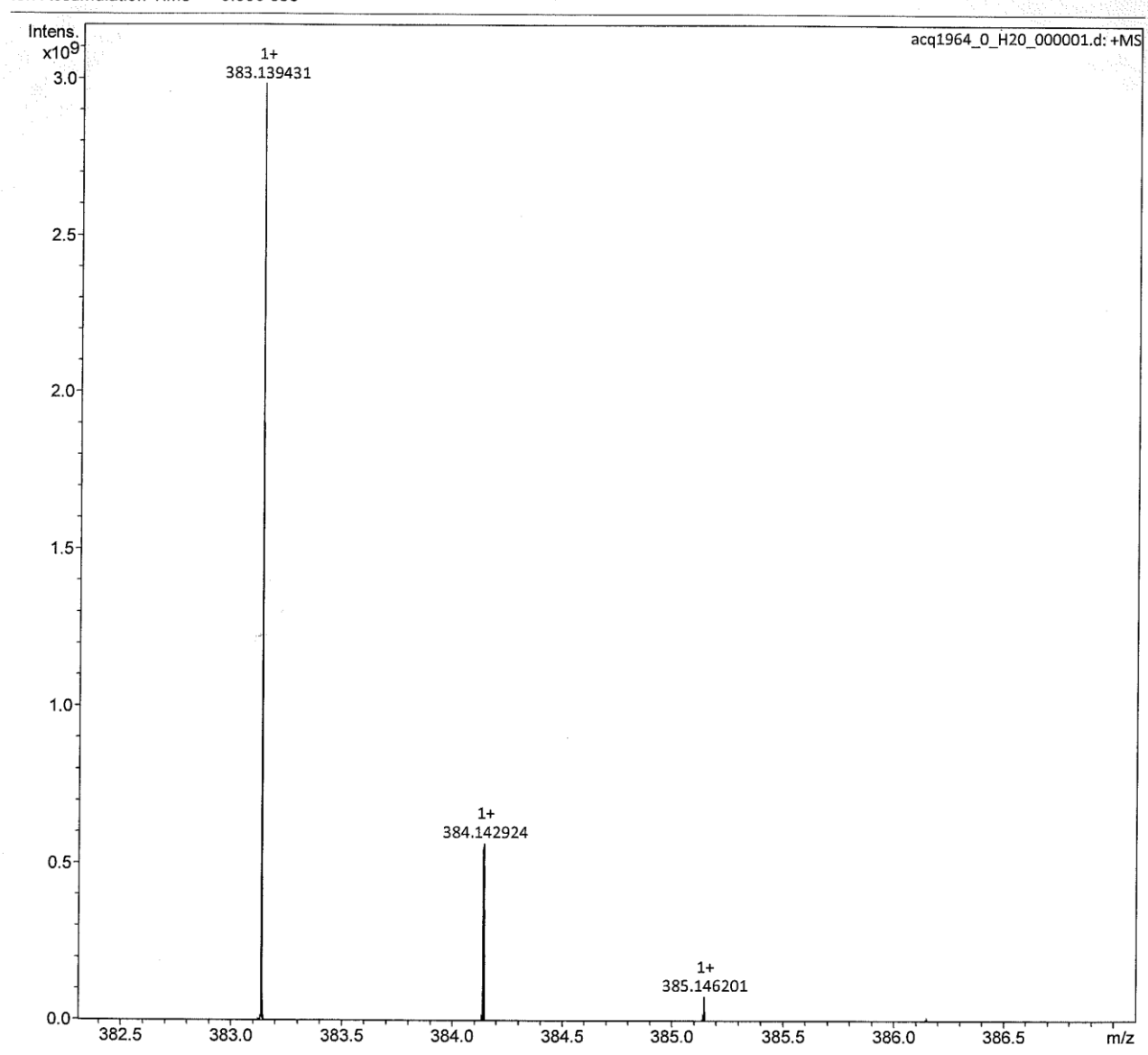

5a,1H,CD3CN
8.2 8.2 8.2 8.2 8.1 8.1 8.0 8.0 8.0 8.0 7.6 7.6 7.6 7.6 7.5 7.5 7.4 7.4 7.3 7.2 7.2 7.2
4.5 4.4 4.4
3.9
2.7 2.7 2.6
2.1
1.04 0.99 1.08 1.00 1.07 1.04 0.99 2.18 2.05 2.85 2.34
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5


5a,13C,CD3CN
173.4
152.1 141.2 140.4 140.0 139.3 139.3 138.2 138.1
117.0 108.9 108.8 108.1 108.0 107.0 105.7 105.4
46.8
35.1
29.1
19.8
210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10

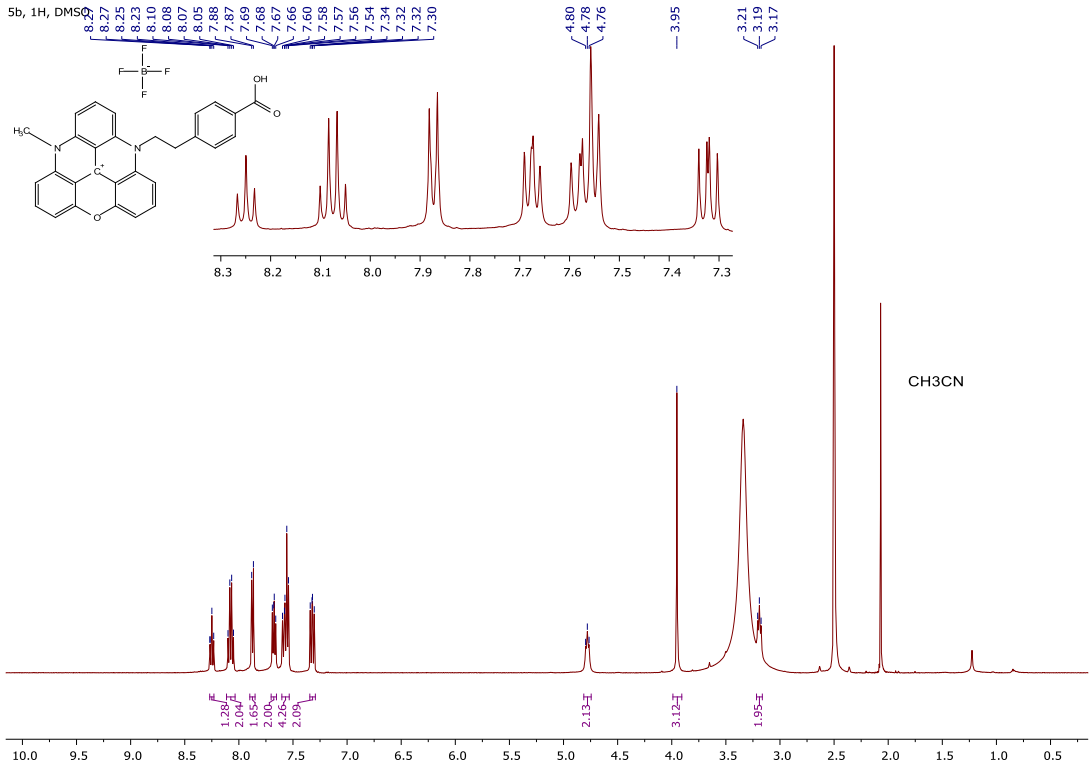
Matrix: Dithranol in DCM

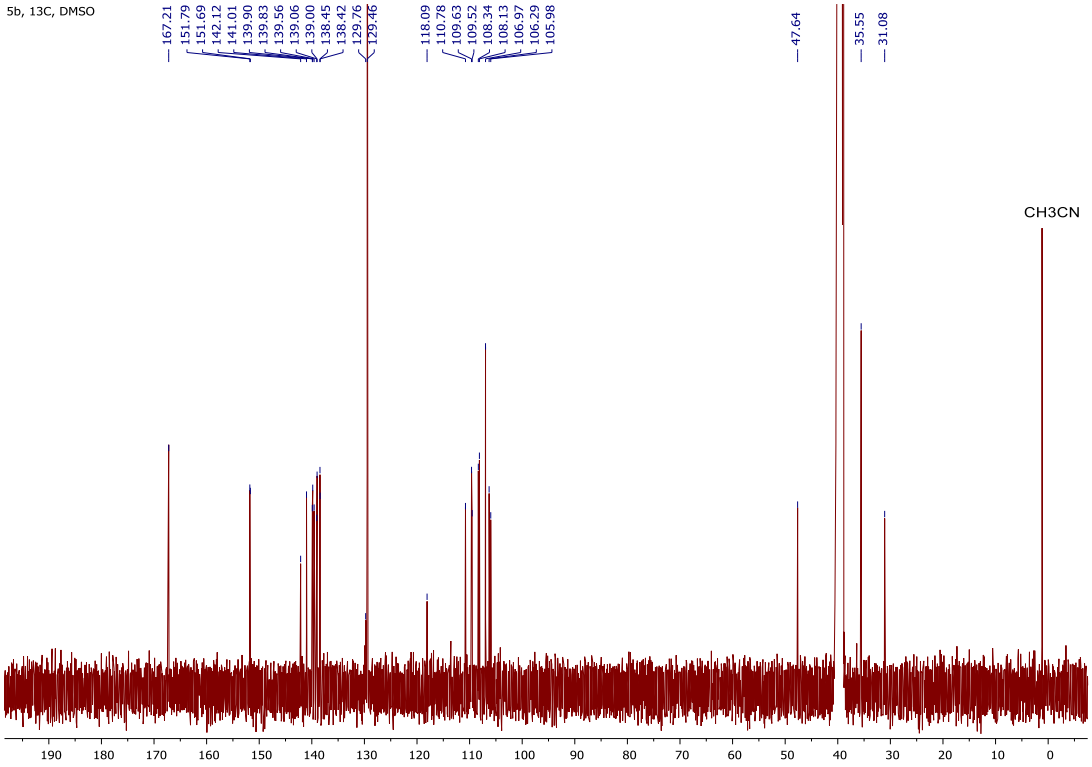
Acquisition Parameter

Acquisition Mode	Single MS	Acquired Scans	4	Calibration Date	Tue Jan 27 05:35:41 2015
Polarity	Positive	No. of Cell Fills	1	Data Acquisition Size	4194304
Broadband Low Mass	150.5 m/z	No. of Laser Shots	20	Data Processing Size	8388608
Broadband High Mass	4000.0 m/z	Laser Power	20.0 lp	Apodization	Full-Sine
Source Accumulation	0.010 sec	Laser Shot Frequency	0.002 sec		
Ion Accumulation Time	0.050 sec				



5b

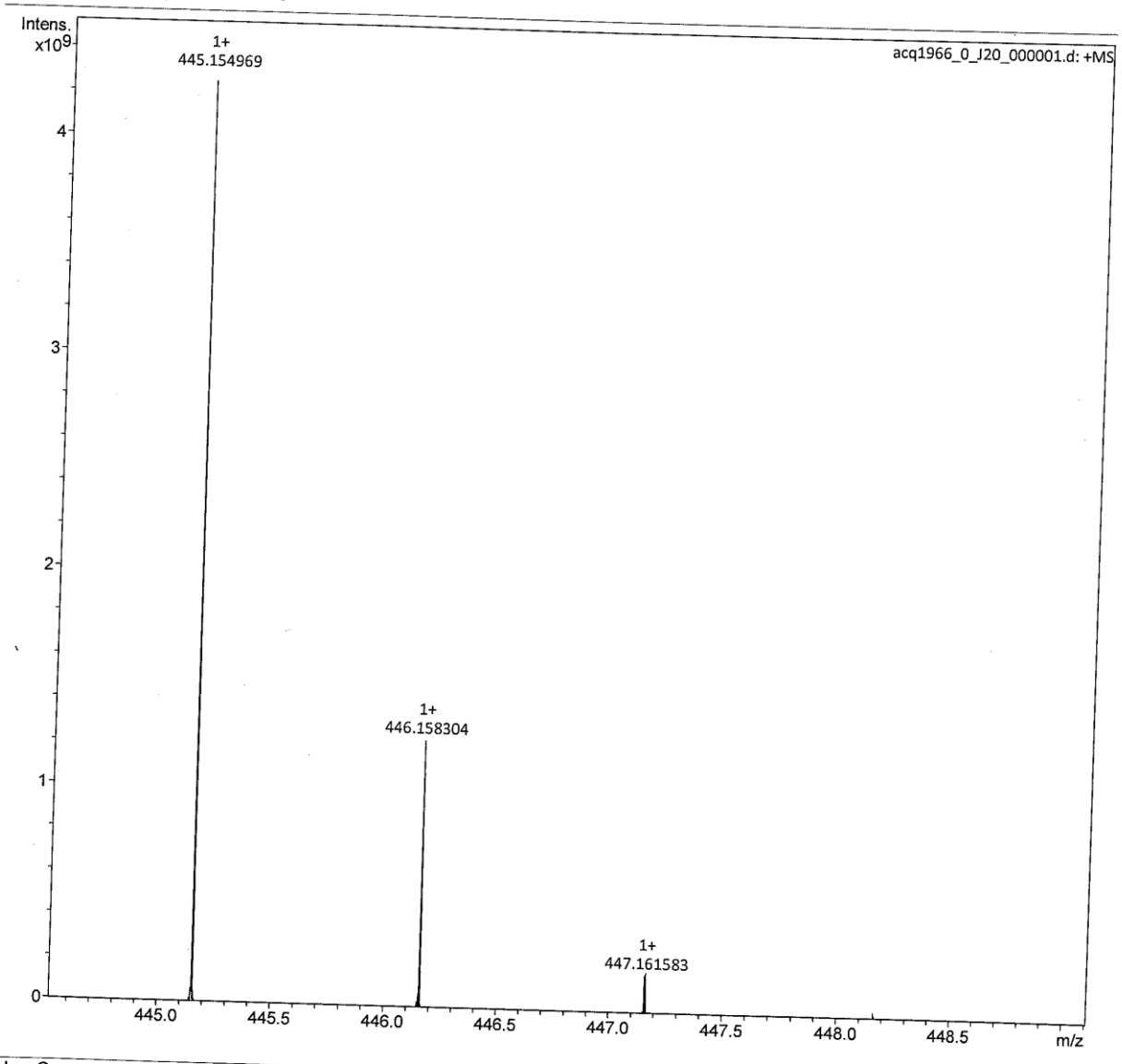

5b, 1H, DMSO
8.27
8.25
8.23
8.10
8.08
8.07
8.05
7.88
7.87
7.69
7.68
7.67
7.66
7.60
7.58
7.57
7.56
7.54
7.34
7.32
7.30
4.80
4.78
4.76
3.95
3.21
3.19
3.17
CH3CN
1.28
2.04
1.65
2.00
4.26
2.09
2.13
3.12
1.95


5b, 13C, DMSO
167.21
151.79
151.69
142.12
141.01
139.90
139.83
139.56
139.06
139.00
138.45
138.42
129.76
129.46
118.09
110.78
109.63
109.52
108.34
108.13
106.97
106.29
105.98
47.64
35.55
31.08
CH3CN

Matrix: Dithranol in DCM

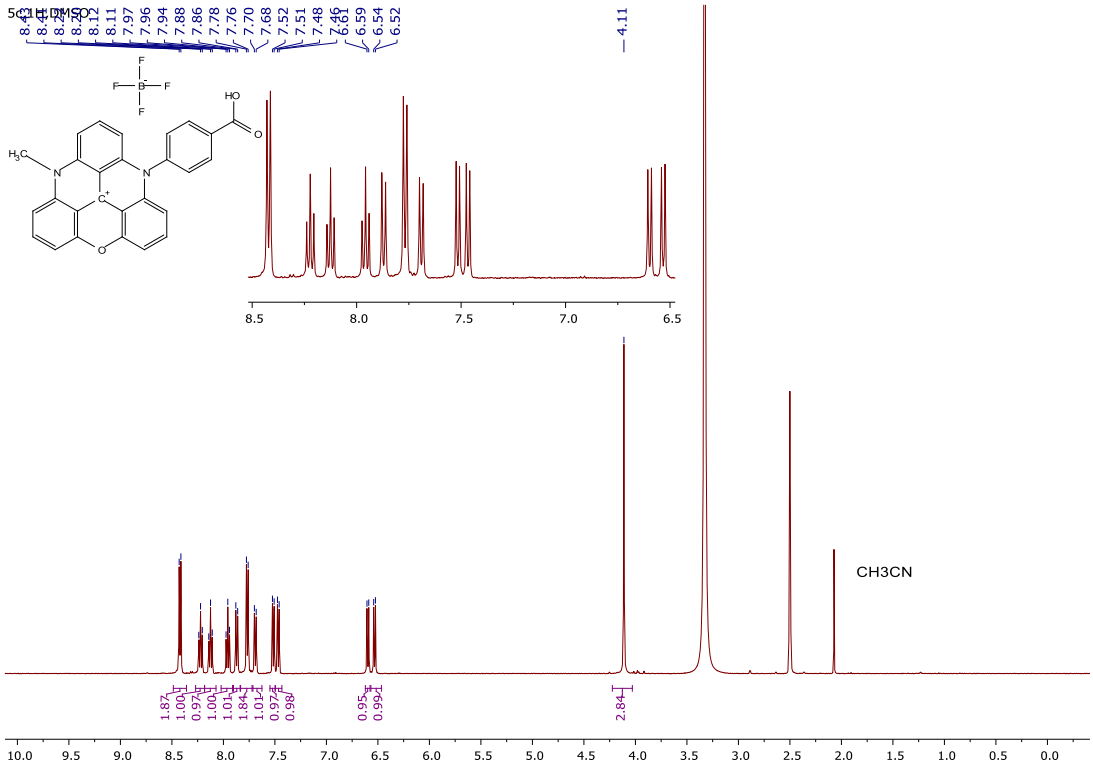
Acquisition Parameter

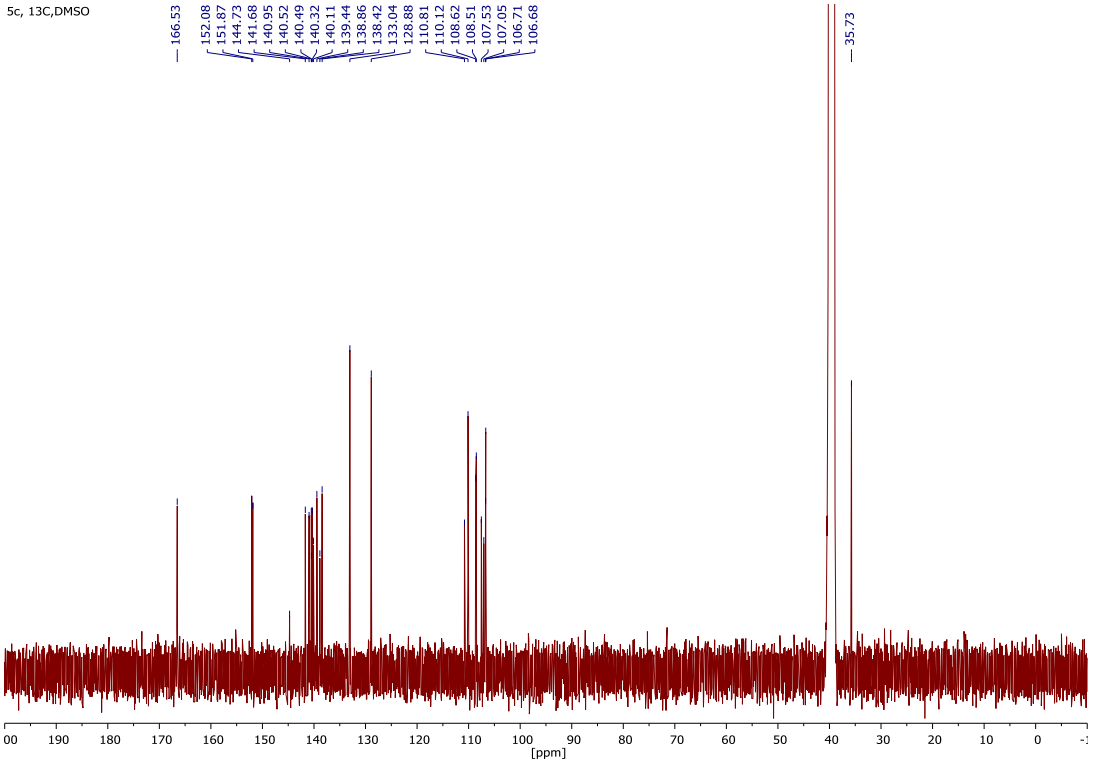
Acquisition Mode	Single MS	Acquired Scans	4	Calibration Date	Tue Jan 27 05:35:41 2015
Polarity	Positive	No. of Cell Fills	1	Data Acquisition Size	4194304
Broadband Low Mass	150.5 m/z	No. of Laser Shots	20	Data Processing Size	8388608
Broadband High Mass	4000.0 m/z	Laser Power	20.0 lp	Apodization	Full-Sine
Source Accumulation	0.010 sec	Laser Shot Frequency	0.002 sec		
Ion Accumulation Time	0.050 sec				



Bruker Compass DataAnalysis 4.2 printed: 6/25/2015 11:14:04 AM by: Admin Page 1 of 1

5c

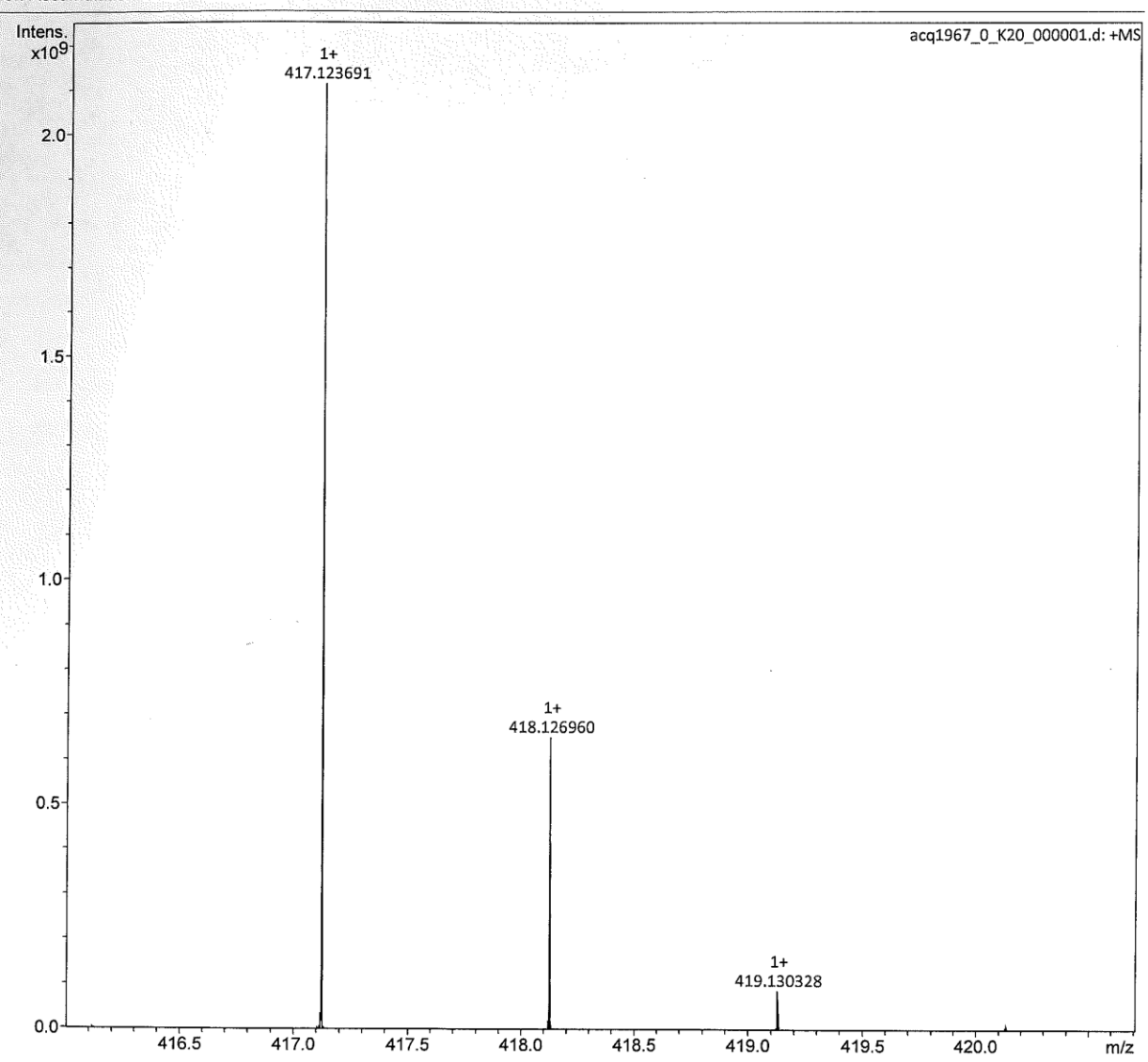

5c, 1H,DMSO
8.43
8.42
8.29
8.28
8.12
8.11
7.97
7.96
7.94
7.88
7.86
7.78
7.76
7.70
7.68
7.52
7.51
7.48
7.46
7.41
6.59
6.54
6.52
4.11
CH3CN
1.87
1.00
0.97
1.00
1.01
1.84
1.01
0.97
0.98
0.95
0.99
2.84


5c, 13C,DMSO
166.53
152.08
151.67
144.73
141.68
140.95
140.52
140.49
140.32
140.11
139.44
138.86
138.42
133.04
128.88
110.81
110.12
108.62
108.51
107.53
107.05
106.71
106.68
35.73
[ppm]

Matrix: Dithranol in DCM

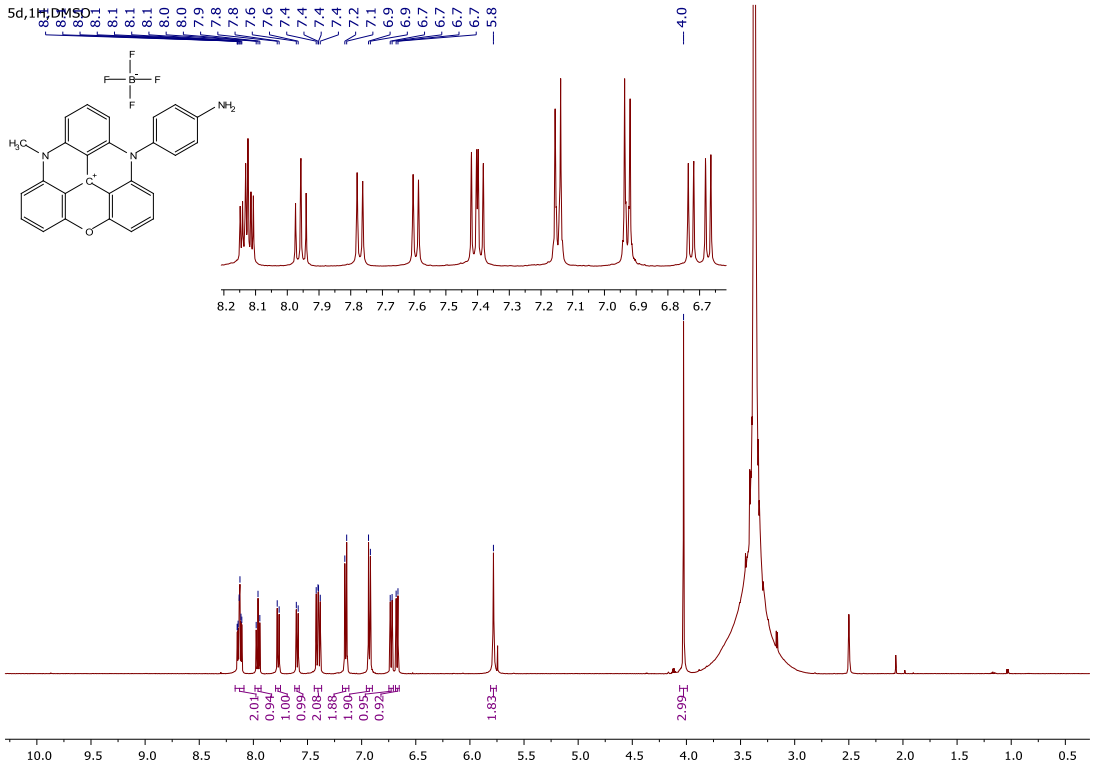
Acquisition Parameter

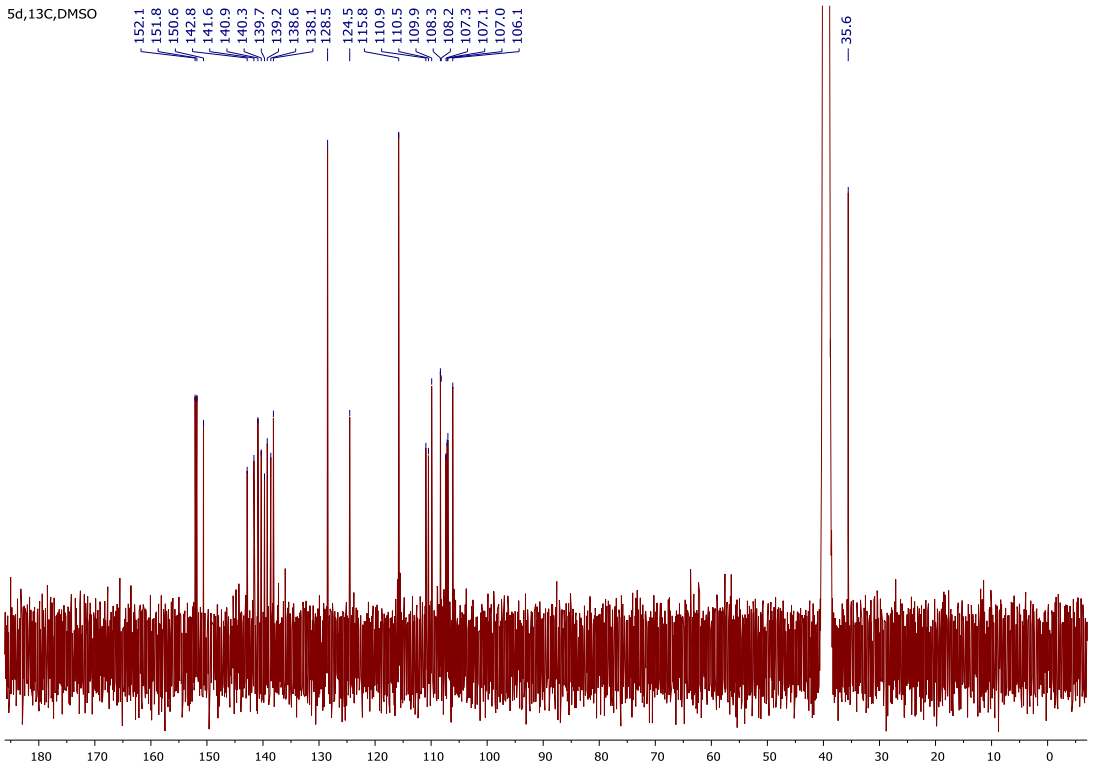
Acquisition Mode	Single MS	Acquired Scans	4	Calibration Date	Tue Jan 27 05:35:41 2015
Polarity	Positive	No. of Cell Fills	1	Data Acquisition Size	4194304
Broadband Low Mass	150.5 m/z	No. of Laser Shots	20	Data Processing Size	8388608
Broadband High Mass	4000.0 m/z	Laser Power	20.0 lp	Apodization	Full-Sine
Source Accumulation	0.010 sec	Laser Shot Frequency	0.002 sec		
Ion Accumulation Time	0.050 sec				



Bruker Compass DataAnalysis 4.2 printed: 6/25/2015 11:16:32 AM by: Admin Page 1 of 1

5d

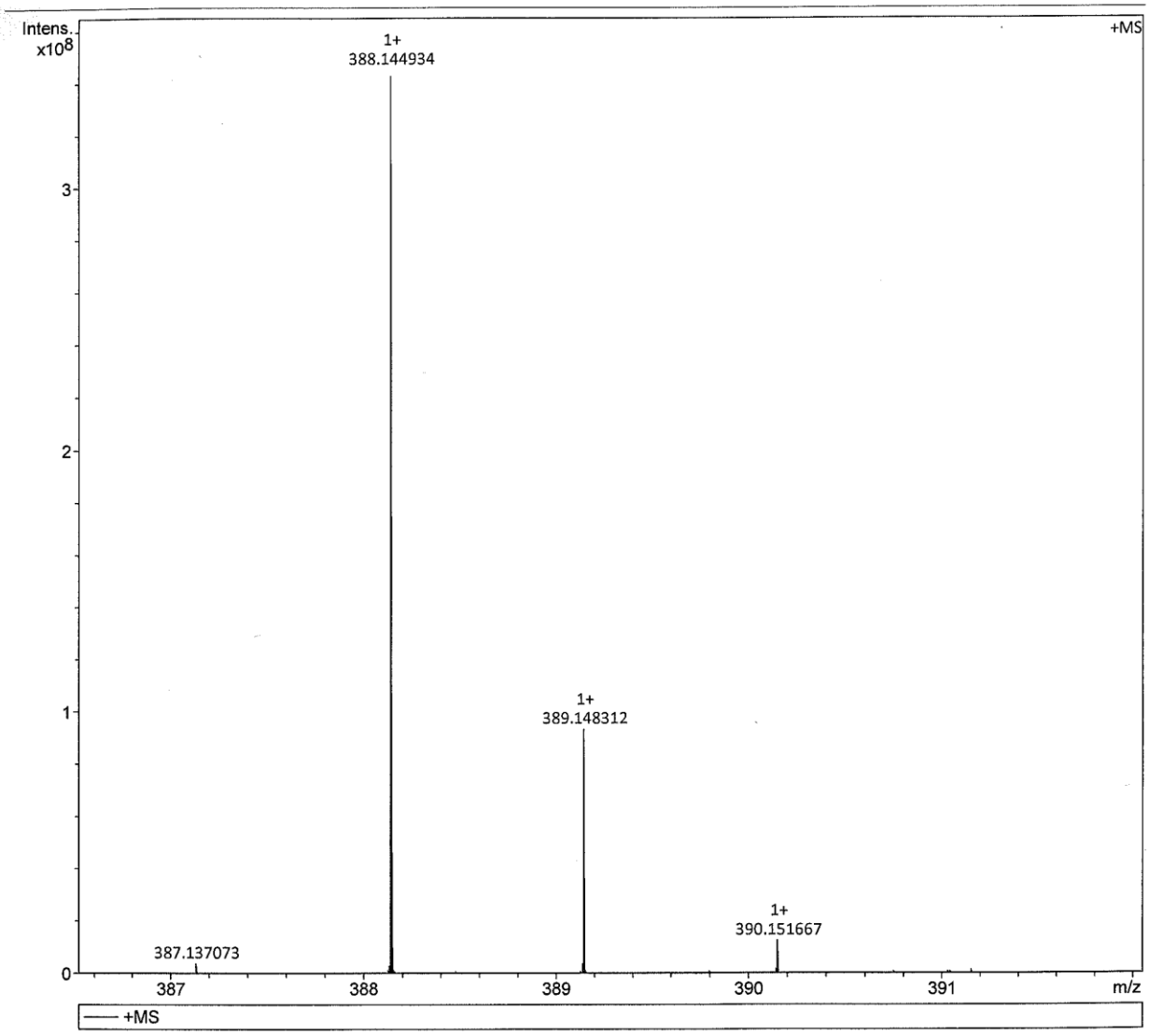

5d,1H,DMSO
8.2 8.2 8.0 8.1 8.1 8.1 8.1 8.0 8.0 7.9 7.8 7.8 7.6 7.6 7.4 7.4 7.4 7.4 7.2 7.1 6.9 6.9 6.7 6.7 6.7 6.7
5.8
4.0
2.01 0.94 1.00 0.99 2.08 1.88 1.90 0.95 0.92 1.83 2.99


5d,13C,DMSO
152.1 151.8 150.6 142.8 141.6 140.9 140.3 139.7 139.2 138.6 138.1 128.5 124.5 115.8 110.9 110.5 109.9 108.3 108.2 107.3 107.1 107.0 106.1
35.6

Matrix: Dithranol in MeCN

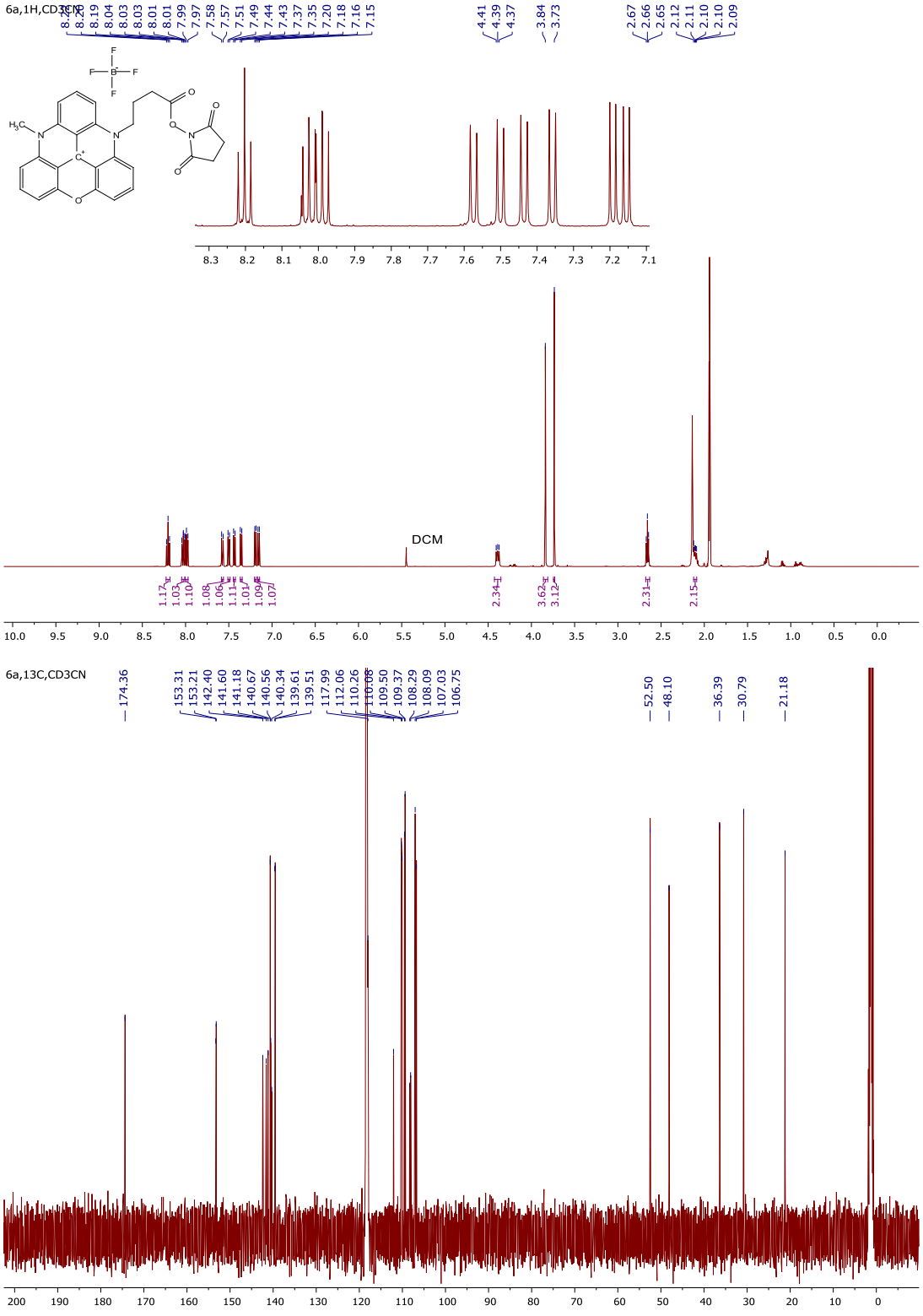
Acquisition Parameter

Acquisition Mode	Single MS	Acquired Scans	6	Calibration Date	Tue Jan 27 05:35:41 2015
Polarity	Positive	No. of Cell Fills	1	Data Acquisition Size	4194304
Broadband Low Mass	150.5 m/z	No. of Laser Shots	20	Data Processing Size	8388608
Broadband High Mass	4000.0 m/z	Laser Power	20.0 lp	Apodization	Full-Sine
Source Accumulation	0.010 sec	Laser Shot Frequency	0.002 sec		
Ion Accumulation Time	0.050 sec				



Bruker Compass DataAnalysis 4.2 printed: 10/19/2015 2:43:25 PM by: Admin Page 1 of 1

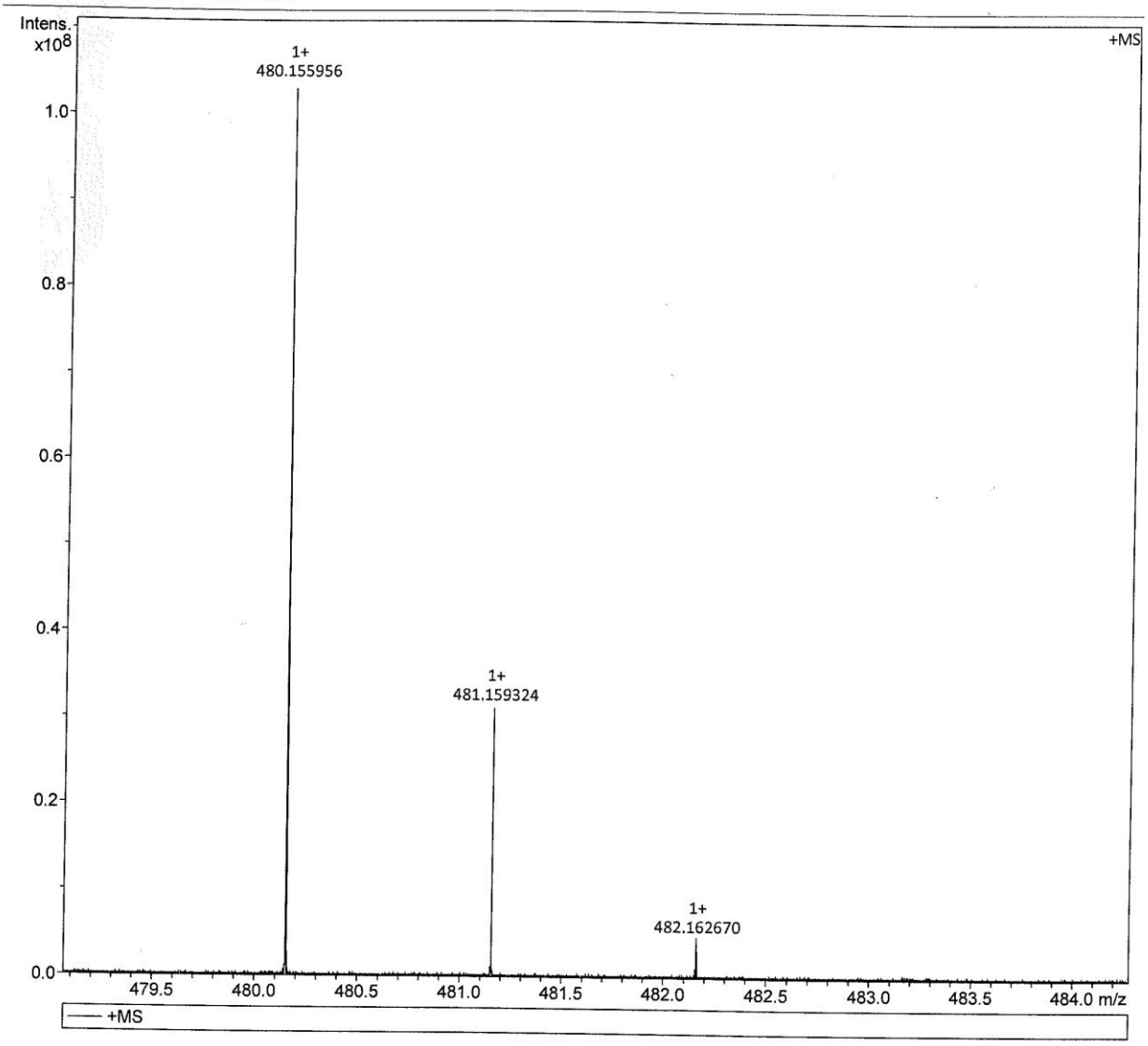
6a



Matrix: Dithranol in MeCN

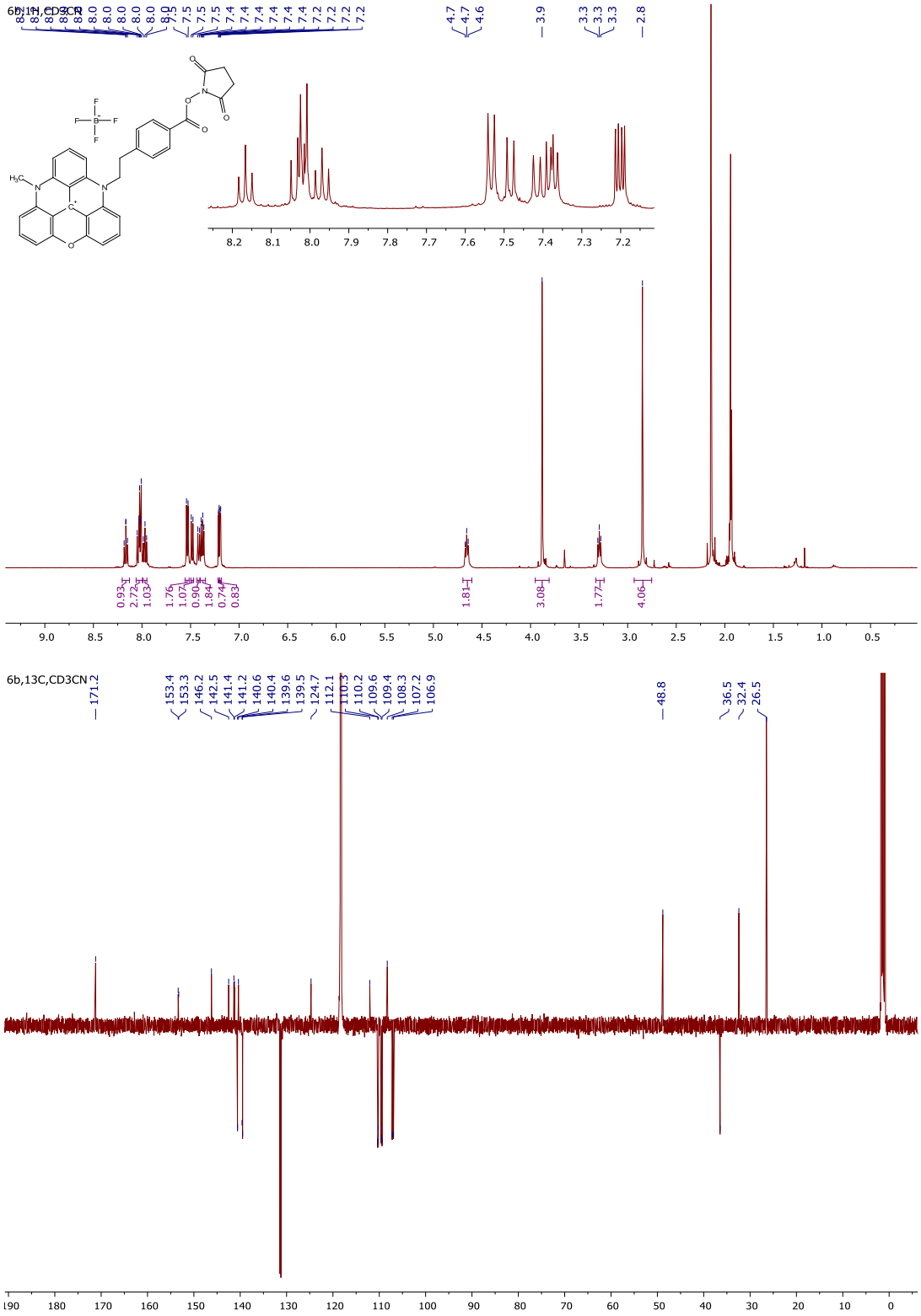
Acquisition Parameter

Acquisition Mode	Single MS	Acquired Scans	6	Calibration Date	Tue Jan 27 05:35:41 2015
Polarity	Positive	No. of Cell Fills	1	Data Acquisition Size	4194304
Broadband Low Mass	150.5 m/z	No. of Laser Shots	20	Data Processing Size	8388608
Broadband High Mass	4000.0 m/z	Laser Power	20.0 lp	Apodization	Full-Sine
Source Accumulation	0.010 sec	Laser Shot Frequency	0.002 sec		
Ion Accumulation Time	0.050 sec				



Bruker Compass DataAnalysis 4.2 printed: 10/19/2015 2:53:58 PM by: Admin Page 1 of 1

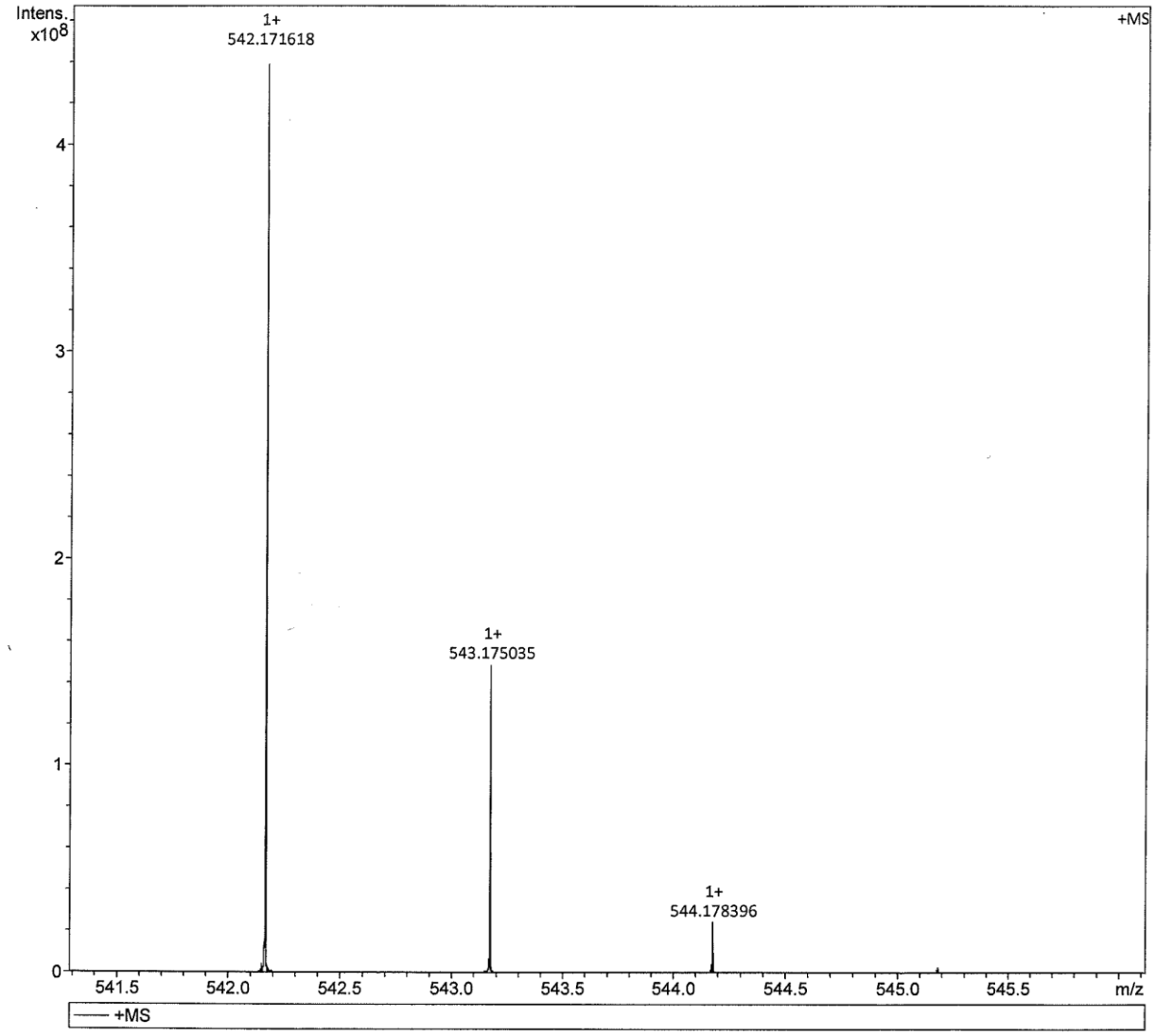
6b



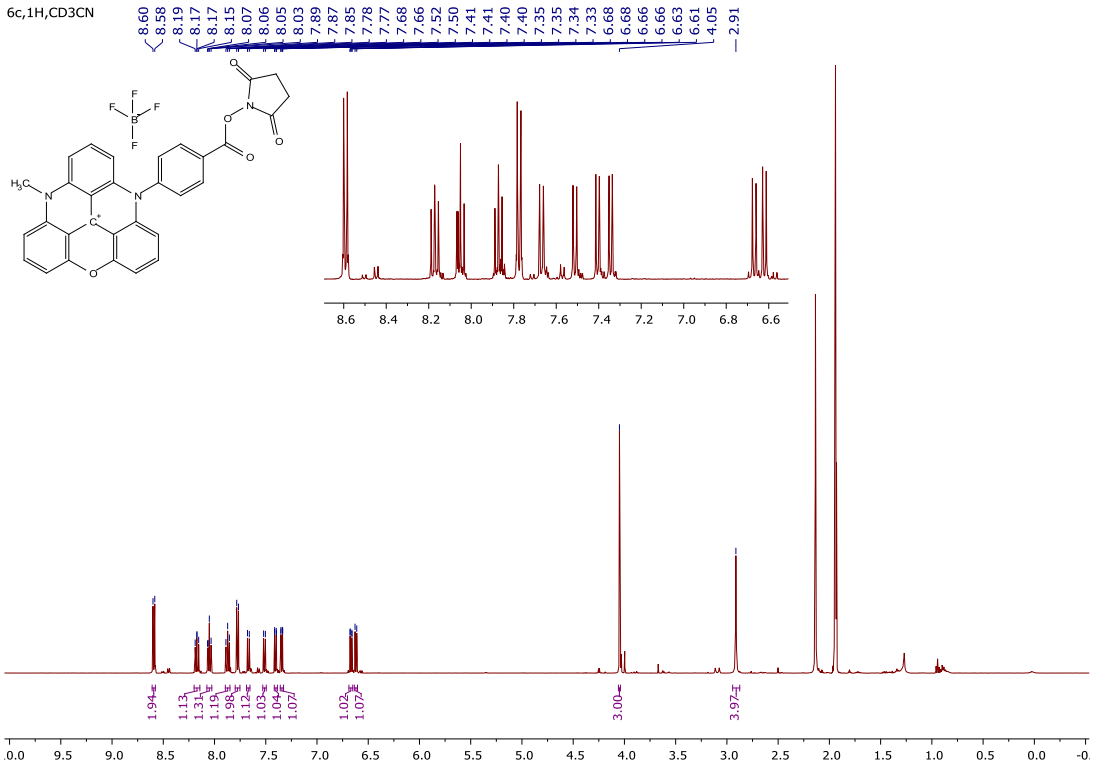
Matrix: Dithranol in MeCN

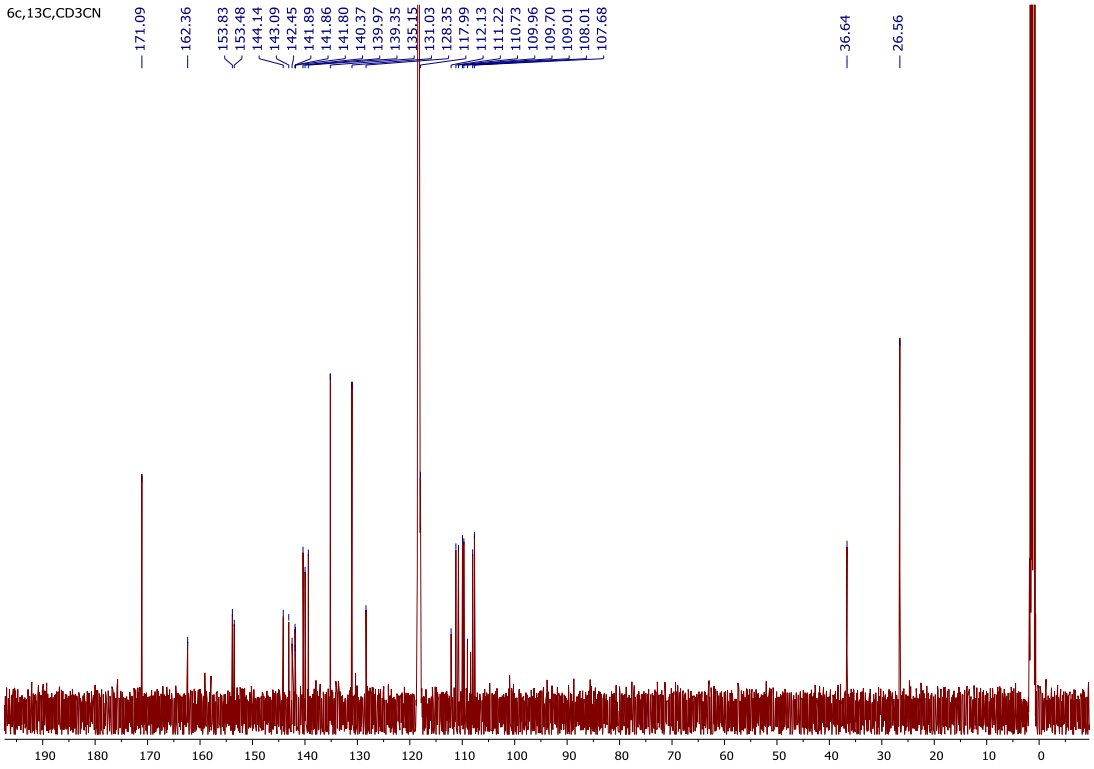
Acquisition Parameter

Acquisition Mode	Single MS	Acquired Scans	7	Calibration Date	Tue Jan 27 05:35:41 2015
Polarity	Positive	No. of Cell Fills	1	Data Acquisition Size	4194304
Broadband Low Mass	150.5 m/z	No. of Laser Shots	20	Data Processing Size	8388608
Broadband High Mass	4000.0 m/z	Laser Power	20.0 lp	Apodization	Full-Sine
Source Accumulation	0.010 sec	Laser Shot Frequency	0.002 sec		
Ion Accumulation Time	0.050 sec				



6c

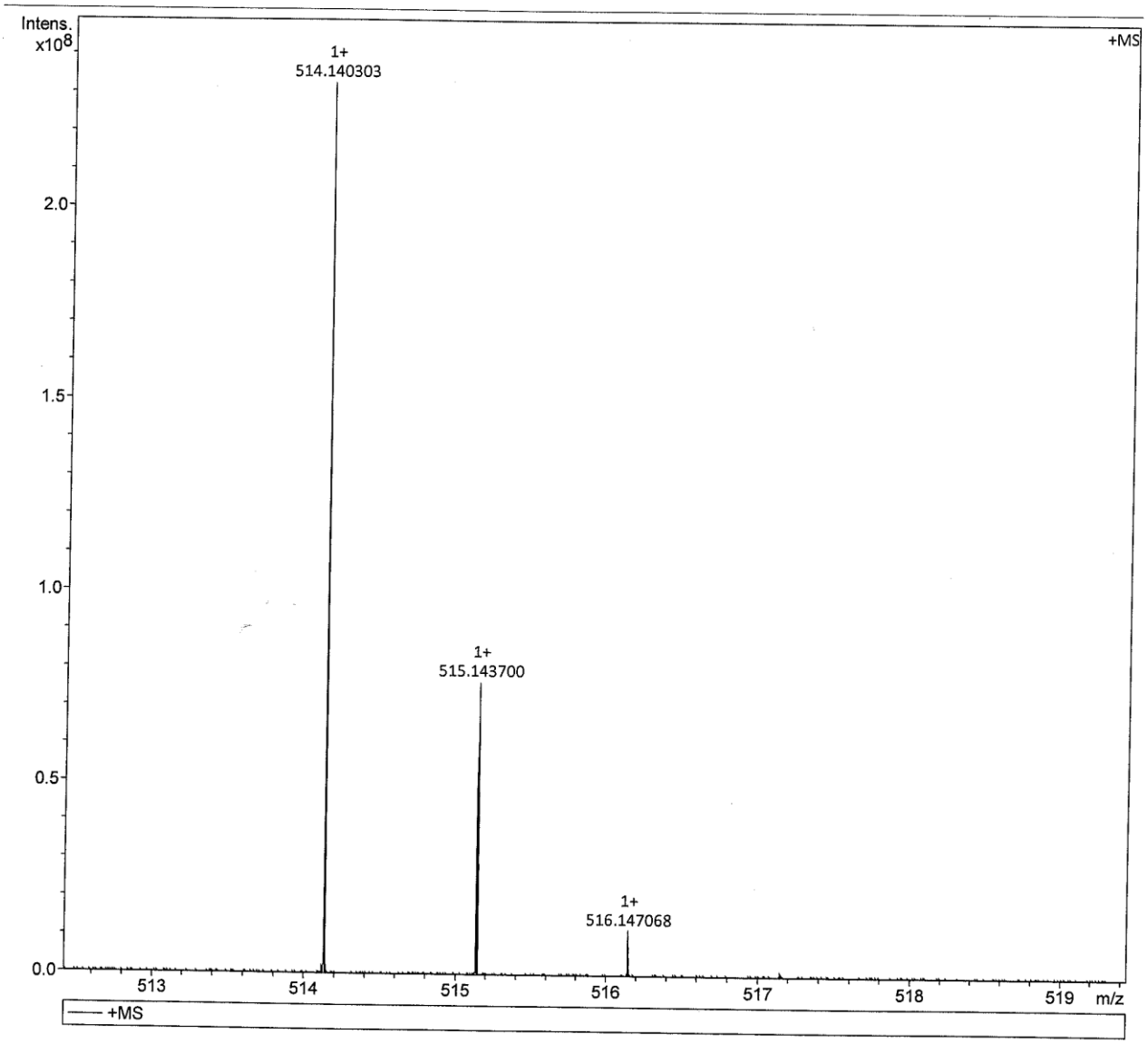

6c,1H,CD3CN
8.60
8.58
8.19
8.17
8.17
8.15
8.07
8.06
8.05
8.03
7.89
7.87
7.85
7.78
7.77
7.68
7.66
7.52
7.50
7.41
7.41
7.40
7.40
7.35
7.35
7.34
7.33
6.68
6.68
6.66
6.66
6.63
6.61
4.05
2.91
1.94
1.13
1.31
1.19
1.98
1.12
1.03
1.04
1.07
1.02
1.07
3.00
3.97


6c,13C,CD3CN
171.09
162.36
153.83
153.48
144.14
143.09
142.45
141.89
141.86
141.80
140.37
139.97
139.35
135.15
131.03
128.35
117.99
112.13
111.22
110.73
109.96
109.70
109.01
108.01
107.68
36.64
26.56

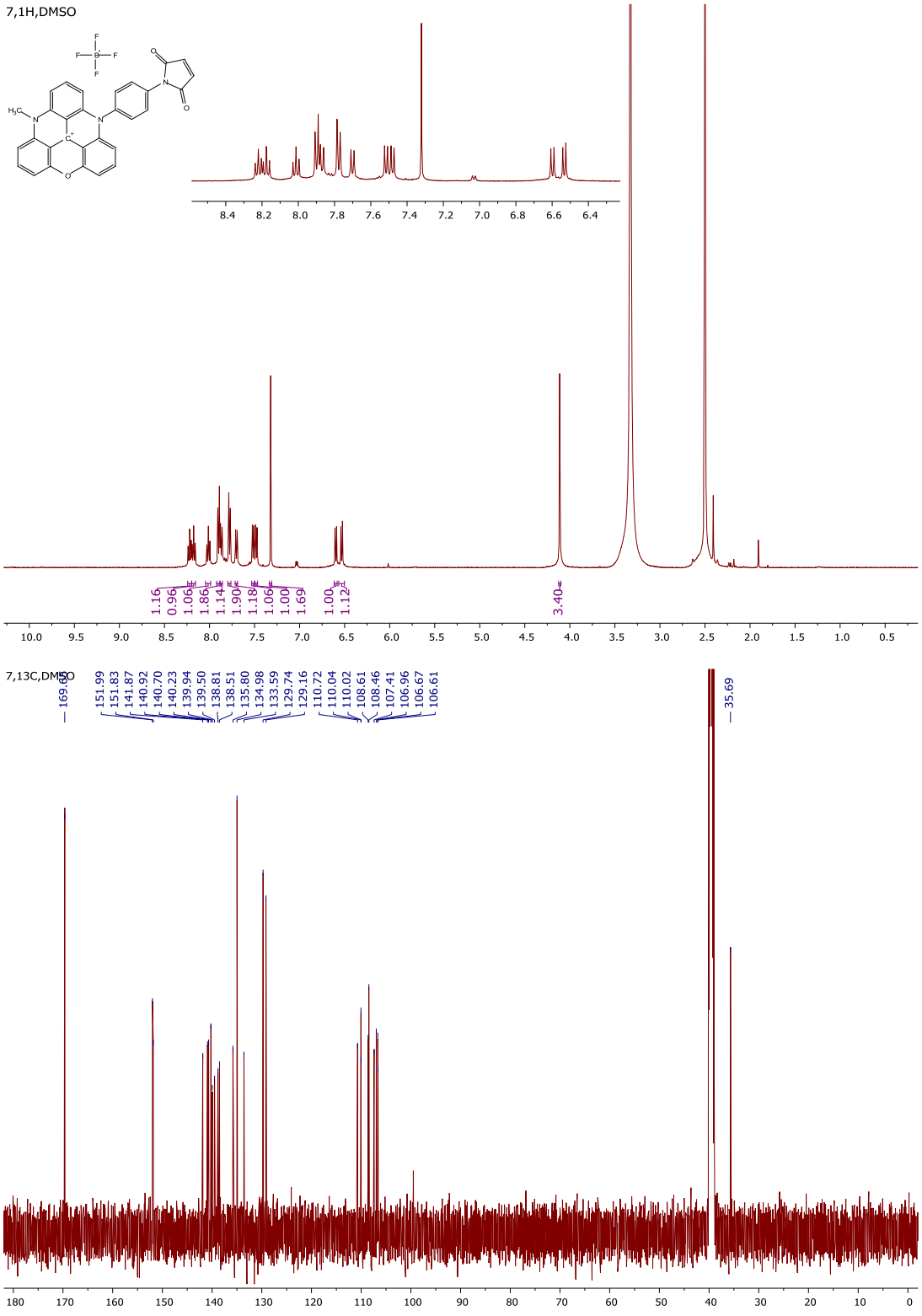
Matrix: Dithranol in MeCN

Acquisition Parameter

Acquisition Mode	Single MS	Acquired Scans	6	Calibration Date	Tue Jan 27 05:35:41 2015
Polarity	Positive	No. of Cell Fills	1	Data Acquisition Size	4194304
Broadband Low Mass	150.5 m/z	No. of Laser Shots	20	Data Processing Size	8388608
Broadband High Mass	4000.0 m/z	Laser Power	20.0 lp	Apodization	Full-Sine
Source Accumulation	0.010 sec	Laser Shot Frequency	0.002 sec		
Ion Accumulation Time	0.050 sec				



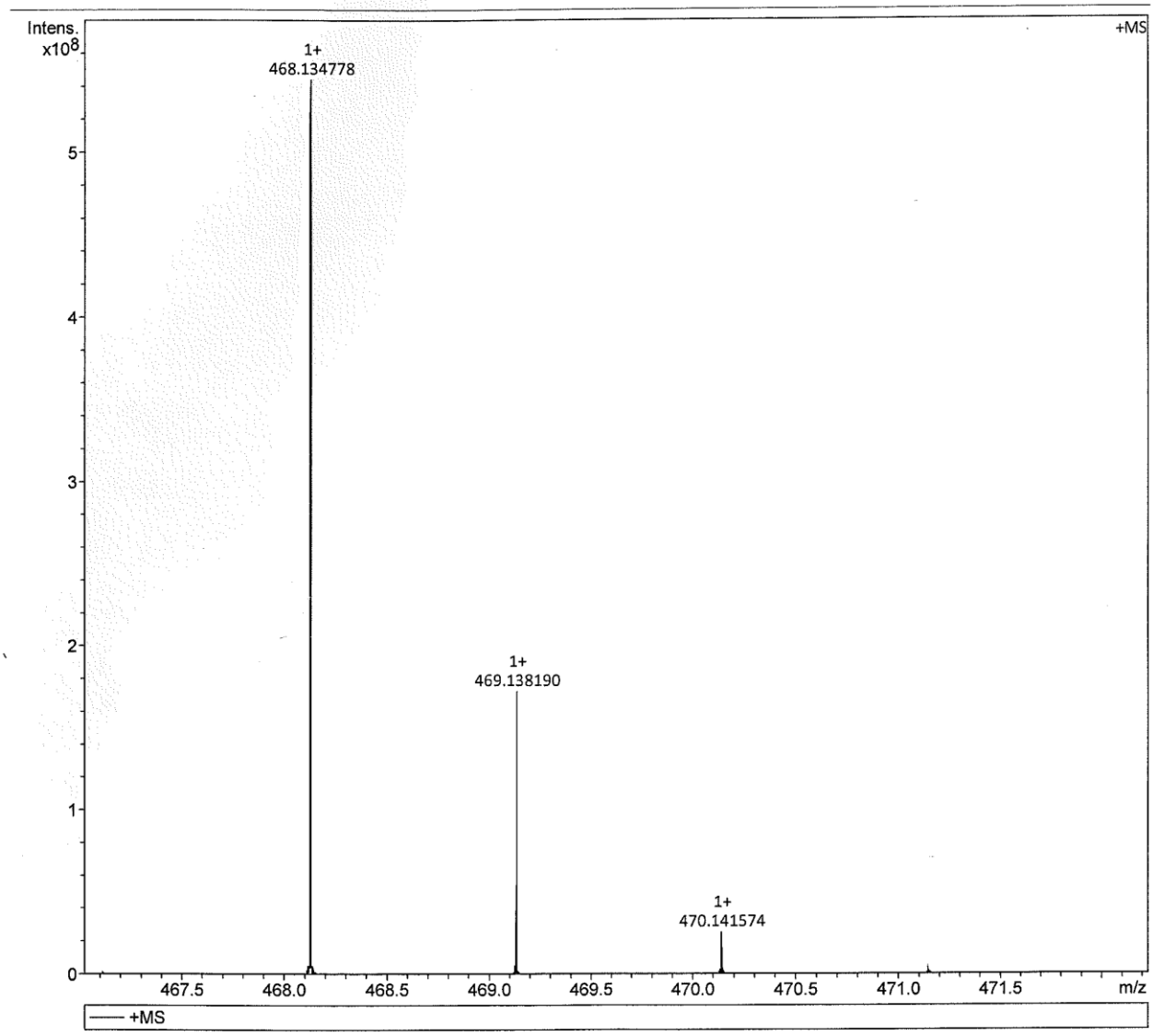
7


7,1H,DMSO
1.16
0.96
1.06
1.86
1.14
1.90
1.18
1.06
1.00
1.69
1.00
1.12
3.40
7,13C,DMSO
169.60
151.99
151.83
141.87
140.92
140.70
140.23
139.94
139.50
138.81
138.51
135.80
134.98
133.59
129.74
129.16
110.72
110.04
110.02
108.61
108.46
107.41
106.96
106.67
106.61
35.69

Matrix: Dithranol in MeCN

Acquisition Parameter

Acquisition Mode	Single MS	Acquired Scans	6	Calibration Date	Tue Jan 27 05:35:41 2015
Polarity	Positive	No. of Cell Fills	1	Data Acquisition Size	4194304
Broadband Low Mass	150.5 m/z	No. of Laser Shots	20	Data Processing Size	8388608
Broadband High Mass	4000.0 m/z	Laser Power	20.0 lp	Apodization	Full-Sine
Source Accumulation	0.010 sec	Laser Shot Frequency	0.002 sec		
Ion Accumulation Time	0.050 sec				



References

1. A. Harriman, *The Journal of Physical Chemistry*, 1987, **91**, 6102-6104.
2. J. Bosson, J. Gouin and J. Lacour, *Chem Soc Rev*, 2014, **43**, 2824-2840.
3. S. Dileesh and K. R. Gopidas, *J. Photochem. Photophys. A-Chem.*, 2004, **162**, 115-120.