Wavelength-selective one- and two-photon uncaging of GABA.

Supporting Information

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(E)-di-tert-Butyl 2-(3-(4-(((4-((tert-butoxycarbonyl)amino)butanoyl) oxy)methyl)-7-(diethylamino)-2-oxo-2H-chromen-3-yl)acrylamido) succinate: A solution of (E)-di-tert-butyl 2-(3-(7-(diethylamino)-4-(hydroxymethyl)-2-oxo-2H-chromen-3-yl) acrylamido) succinate (**2**) (130 mg, 0.239 mmol, 1 eq) in dichloromethane (15 mL) was treated with Boc-L-aminobutyric acid (73 mg, 0.358 mmol, 1.5 eq), then 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (288 mg, 1.49 mmol, 6.25 eq) and cat. dimethylaminopyridine (15 mg). The reaction was stirred for 16 hours, then poured into water and extracted into ethyl acetate. The combined organics were dried over $MgSO_4$, filtered and concentrated to obtain the crude product. The crude material was then purified by column chromatography (silica, 1:1 Hexanes: Ethyl Acetate) to obtain the desired compound in 84% yield (147 mg, 0.201 mmol) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.78 (d, 1H, J = 15.0 Hz), 7.52 (d, 1H, J = 9.3 Hz), 7.31 (d, 1H, J = 15.0 Hz), 6.68 (d, 1H, J = 8.1 Hz), 6.64 (dd, 1H, J = 2.1 Hz, 9.0 Hz), 6.47 (d, 1H, J = 2.7 Hz), 5.39 (s, 2H), 4.99 (bs, 1H), 4.82 (m, 1H), 3.44 (q, 4H, J = 7.2 Hz), 3.14 (m, 1H), 2.85 (dq, 2H, J = 50.1 Hz, 17.1 Hz, 4.5 Hz), 2.38 (t , 2H, J = 7.2 Hz), 1.83 (t, 2H, J = 7.2 Hz), 1.46 (s, 27H), 1.23 (t, 6H, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.7, 170.4, 169.9, 166.2, 160.3, 155.8, 151.4, 146.9, 132.6, 131.1, 128.9, 126.8, 125.0, 115.0, 109.8, 108.2, 97.5, 82.6, 81.8, 68.5, 57.9, 49.7, 45.2, 40.1, 37.9, 31.6, 28.8, 28.5, 28.3, 25.6, 12.9 ; LCMS (ESI) m/z calc'd for $C_{38}H_{55}N_3O_{11}[M+H]^+$ 730.6137, found 730.6149.

(*E*)-di-*tert*-butyl 2-(3-(4-(((4-aminobutanoyl)oxy)methyl)-7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)acrylamido)succinate (1): (*E*)-di-*tert*-butyl 2-(3-(4-(((4-aminobutanoyl)oxy)methyl)-7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)acrylamido) succinate (110 mg, 0.151 mmol) was dissolved in dichloromethane (2 mL), and trifluoroacetic acid (6 mL) and stirred for 2.5 hours. The solvent was then removed and the crude material was redissolved in dichloromethane and concentrated to obtain the crude product. The crude product was purified by reverse phase HPLC (35% CH_3CN/ 0.1% TFA in H_2O, Alltimu C18 column) then

lyophilized to obtain (**1**) in 50% yield as a white powder. ^{1}H NMR (600 MHz, CD_3OD) δ: 7.71 (m, 2H), 7.31 (d, 1H, J = 12.5 Hz), 6.79 (m, 1H), 6.54 (d, 1H, J = 2.4 Hz), 5.49 (s, 2H), 3.52 (q, 4H, J = 7.2 Hz), 3.00-2.85 (m, 4H), 2.53 (t, 2H, J = 7.2 Hz), 1.95 (m, 2H), 1.22 (t, 6H, J = 7.2 Hz); ^{13}C NMR (75 MHz, CD_3OD) δ: 172.5, 171.9, 167.8, 160.6, 155.7, 151.8, 147.9, 132.5, 126.8, 123.8, 113.4, 109.8, 107.6, 96.4, 57.6, 44.4, 38.6, 30.1, 22.3, 11.3; LCMS (ESI) m/z calc'd for $C_{25}H_{31}N_3O_9$ $[M+H]^+$ 518.2133, found 518.2135.

(*E*)-*N*-(20-azido-3,6,9,12,15,18-hexaoxaicosyl)-3-(4-(((*tert*-butyldimethylsilyl)oxy) methyl)-7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)acrylamide (4): A solution of O-(Aminoethyl)-2-azidoethylpentaethyleneglycol (180 mg, 0.514 mmol, 2 eq) and (E)-3-(4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2H-chromen-3-yl)acrylic acid (**2**) (111 mg, 0.257 mmol, 1 eq) in acetonitrile (12 mL) was treated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (99 mg, 0.514 mmol, 2 eq). The reaction was stirred for 16 hours, and then concentrated to obtain the crude product. The crude material was then purified by column chromatography (silica, 9:1 Dichloromethane: Methanol) to obtain (**4**) in 89% yield (176 mg, 0.229 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.84 (d, 1H, J = 14.7 Hz), 7.69 (d, 1H, J = 9.3 Hz), 7.23 (d, 1H, J = 15.3 Hz), 6.61 (dd, 1H, J = 9.3 Hz, 2.4 Hz), 6.45 (d, 1H, J = 2.4 Hz), 6.37 (s, 1H), 4.96 (s, 2H), 3.66-3.36 (m, 36H), 1.22 (t, 6H, J = 7.0 Hz), 0.895 (s, 9H), 0.17 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.5, 160.6, 155.5, 151.3, 150.7, 131.7, 127.5, 124.8, 109.1, 108.3, 96.9, 70.6, 70.5, 70.4, 70.0, 69.9, 57.5, 50.7, 44.8, 39.5, 25.9, 18.3, 12.6, 12.5, -5.0; LCMS (ESI) m/z calc'd for $C_{37}H_{62}N_5O_{10}Si$ $[M+H]^+$ 764.4266, found 764.4274.

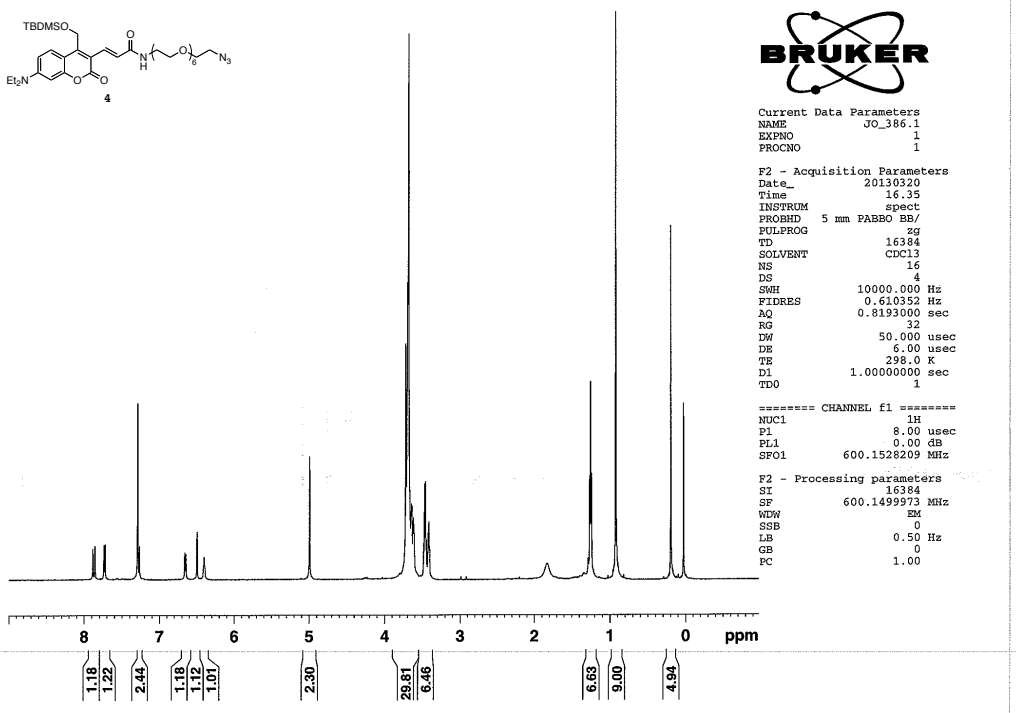
(*E*)-*N*-(20-azido-3,6,9,12,15,18-hexaoxaicosyl)-3-(7-(diethylamino)-4-(hydroxymethyl)-2-oxo-2*H*-chromen-3-yl)acrylamide (5): (*E*)-*N*-(20-azido-3,6,9,12, 15,18-hexaoxaicosyl)-3-(4-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)acrylamide (**4**) (0.166 g, 0.209 mmol, 1

eq) was dissolved in tetrahydrofuran (15 mL) was added tetrabutylammonium fluoride (1M in THF) (0.313 mL, 0.313 mmol, 1.5 eq). The reaction was stirred for 2 hours, then quenched with sat. NH_4Cl and extracted into ethyl acetate. The combined organics were dried over $MgSO_4$, filtered and concentrated to obtain the crude product. The crude material was then purified by column chromatography (silica, 9:1 Dichloromethane: Methanol) to obtain (**5**) in 84% yield (114 mg, 0.176 mmol) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.81 (d, 1H, J = 15.3 Hz), 7.71 (d, 1H, J = 9.3 Hz), 7.22 (d, 1H, J = 15.3 Hz), 6.71 (s, 1H), 6.62 (dd, 1H, J = 9.3 Hz, 2.7 Hz), 6.45 (d, 1H, J = 2.7 Hz), 4.95 (s, 2H), 3.66-3.36 (m, 40H), 1.21 (t, 6H, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.0, 160.6, 155.6, 151.6, 150.8, 131.9, 127.4, 124.6, 113.2, 109.2, 108.3, 96.9, 70.4, 70.2, 69.9, 69.7, 56.3, 50.6, 44.8, 39.6, 29.7, 12.6; LCMS (ESI) m/z calc'd for $C_{31}H_{47}N_5O_{10}$ $[M+H]^+$ 649.3323, found 649.3324.

(*E*)-(3-(1-azido-22-oxo-3,6,9,12,15,18-hexaoxa-21-azatetracos-23-en-24-yl)-7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)methyl 4-((*tert*-butoxycarbonyl)amino) butanoate: A solution of (*E*)-*N*-(20-azido-3,6,9,12,15,18-hexaoxaicosyl)-3-(7-(diethylamino)-4-(hydroxymethyl)-2-oxo-2*H*-chromen-3-yl)acrylamide (**5**) (85 mg, 0.130 mmol, 1 eq) in dichloromethane (10 mL) was treated with Boc-L-aminobutyric acid (40 mg, 0.196 mmol, 1.5 eq), then 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (157 mg, 0.813 mmol, 6.25 eq) and cat. dimethylaminopyridine (2 mg). The reaction was stirred for 16 hours, then poured into water and extracted into ethyl acetate. The combined organics were dried over $MgSO_4$, filtered and concentrated to obtain the crude product. The crude material was then purified by column chromatography (silica, 1:1 Hexanes: Ethyl Acetate) to obtain the desired product in 94% yield (102 mg, 0.122 mmol) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.75 (d, 1H, J = 15.3 Hz), 7.52 (d, 1H, J = 9.3 Hz), 7.28 (d, 1H, J = 14.7 Hz), 6.64 (dd, 1H, J = 2.7 Hz, 9.3 Hz), 6.58 (s, 1H), 6.47 (d, 1H, J = 2.4 Hz), 5.39 (s, 2H), 5.02 (bs, 1H), 3.68-3.59 (m, 38H), 3.12 (m, 2H), 2.39 (t , 2H, J = 7.2 Hz), 1.81 (t, 2H, J = 7.2 Hz), 1.41 (s, 9H), 1.21 (t, 6H, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.7,

166.7, 160.4, 156.2, 155.7, 151.4, 146.7, 131.8, 126.8, 128.7, 115.2, 109.8, 108.2, 97.5, 70.9, 70.7, 70.3, 70.2, 57.9, 51.0, 45.2, 40.1, 39.9, 31.6, 28.8, 25.6, 12.9; LCMS (ESI) m/z calc'd for $C_{40}H_{62}N_6O_{13}$ $[M+H]^+$ 835.4453, found 835.4469.

(*E*)-(3-(1-azido-22-oxo-3,6,9,12,15,18-hexaoxa-21-azatetracos-23-en-24-yl)-7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)methyl 4-aminobutanoate (6): (*E*)-(3-(1-azido-22-oxo-3,6,9,12,15,18-hexaoxa-21-azatetracos-23-en-24-yl)-7-(diethylamino)-2-oxo-2*H*-chromen-4-yl)methyl 4-((*tert*-butoxycarbonyl)amino) butanoate (95 mg, 0.114 mmol) was dissolved in dichloromethane (4 mL), and trifluoroacetic acid (4 mL) and stirred for 2.5 hours. The solvent was then removed and the crude material was redissolved in dichloromethane and concentrated to obtain the crude product. The crude product was purified by reverse phase HPLC (50% CH_3CN/ 0.1% TFA in H_2O, Alltimu C18 column) then lyophilized to obtain (**6**) in 73% yield as a red oil. ^1H NMR (300 MHz, CD_3OD) δ: 7.73 (s, 1H), 7.69 (d, 1H, J = 6.0 Hz), 7.29 (d, 1H, J = 15.3 Hz), 6.80 (dd, 1H, J = 2.4 Hz, 9.6 Hz), 6.55 (d, 1H, J = 2.7 Hz), 5.50 (s, 2H), 3.66-3.53 (m, 30H), 3.34 (m, 2H), 3.01 (t, 2H, J = 7.8 Hz), 2.53 (t, 2H, J = 6.9 Hz), 1.95 (m, 2H), 1.23 (t, 6H, J = 6.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.0, 168.1, 160.6, 155.7, 151.8, 147.8, 132.1, 127.0, 124.5, 113.7, 110.0, 107.8, 96.7, 70.3, 70.2, 70.1, 69.9, 69.7, 57.9, 50.7, 44.8, 39.5, 39.0, 30.5, 22.8, 11.8; LCMS (ESI) m/z calc'd for $C_{35}H_{54}N_6O_{11}$ $[M+H]^+$ 734.3851, found 734.3856.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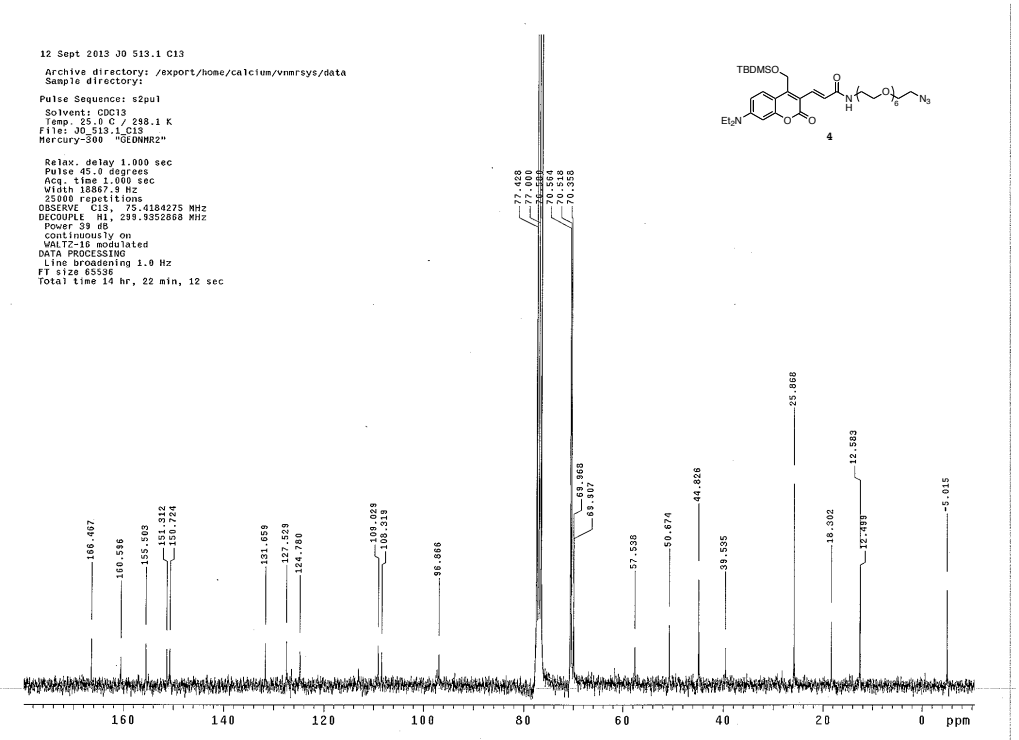
12 Sept 2013 JO 513.1 C13
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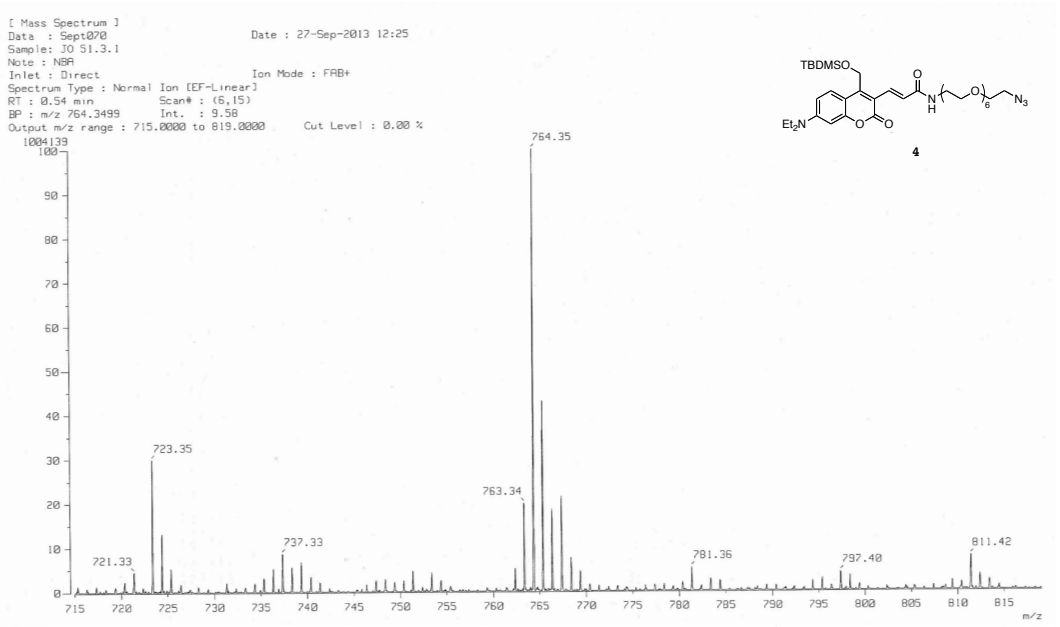

Observed m/z Int% Err[ppm / mmu] U.S. Composition
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[Theoretical Ion Distribution]
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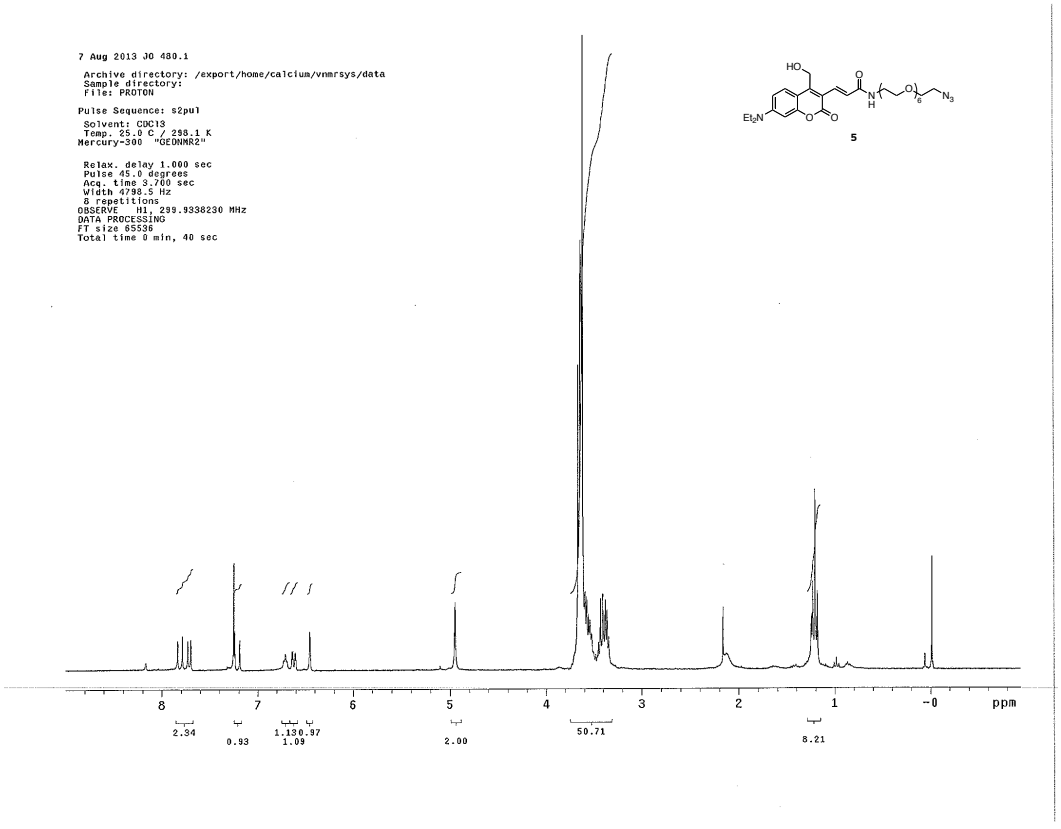

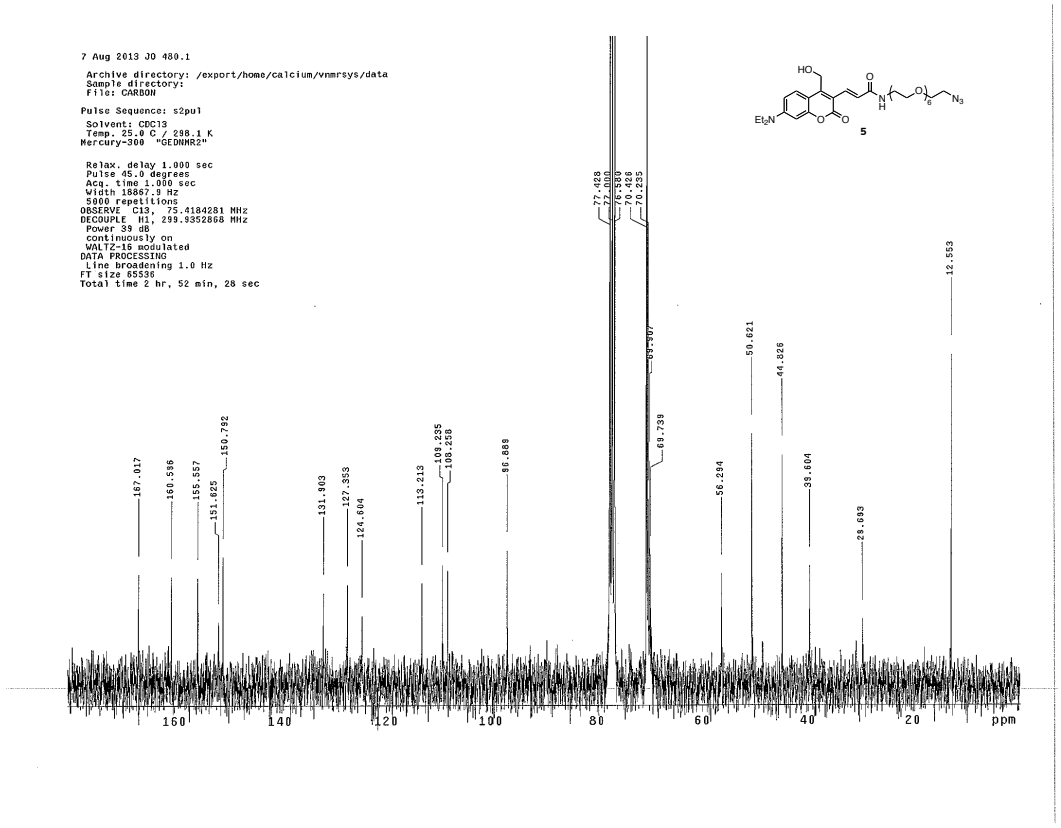

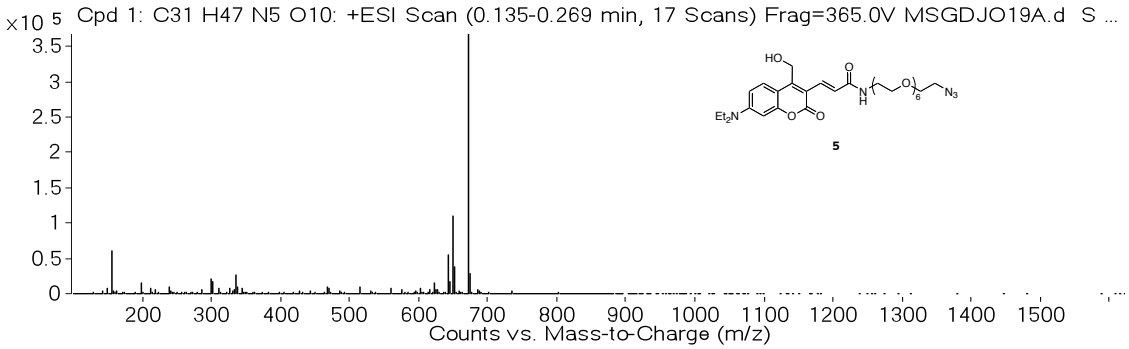

MS Spectrum Peak List

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652.3447	652.3453	0.83	1	9186.03	C31H47N5O10	(M+H)+
653.3462	653.3478	2.47	1	1812.08	C31H47N5O10	(M+H)+
654.3426	654.3504	11.81	1	396.65	C31H47N5O10	(M+H)+
672.3218	672.3215	-0.49	1	367703.44	C31H47N5O10	(M+Na)+
673.3248	673.3246	-0.25	1	129113.1	C31H47N5O10	(M+Na)+
674.3269	674.3272	0.52	1	28219.11	C31H47N5O10	(M+Na)+
675.3286	675.3298	1.73	1	5164.59	C31H47N5O10	(M+Na)+

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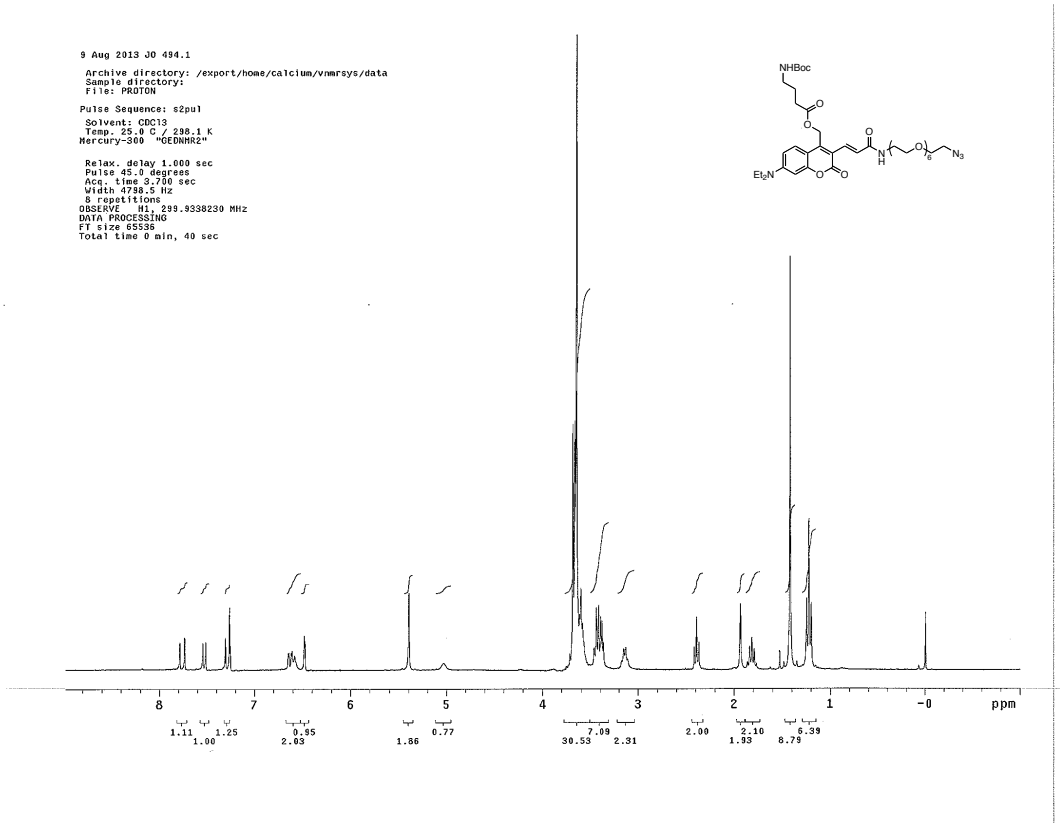

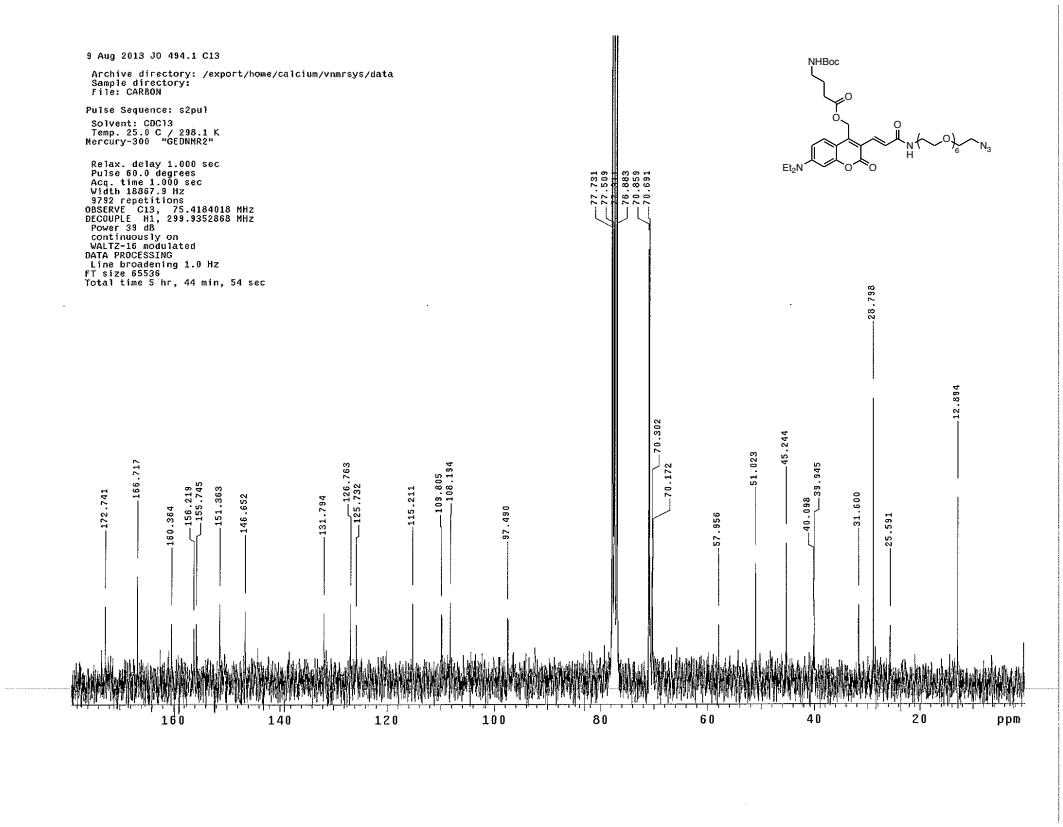

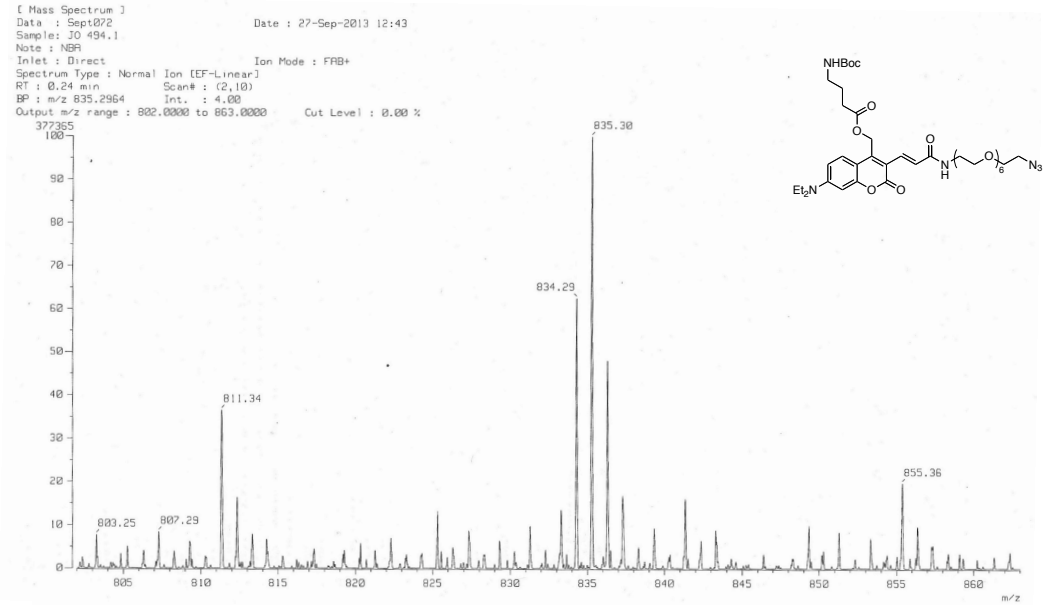

Observed m/z Int% Err[ppm / mmu] U.S. Composition
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[Theoretical Ion Distribution]
Molecular Formula : C40 H63 O13 N6
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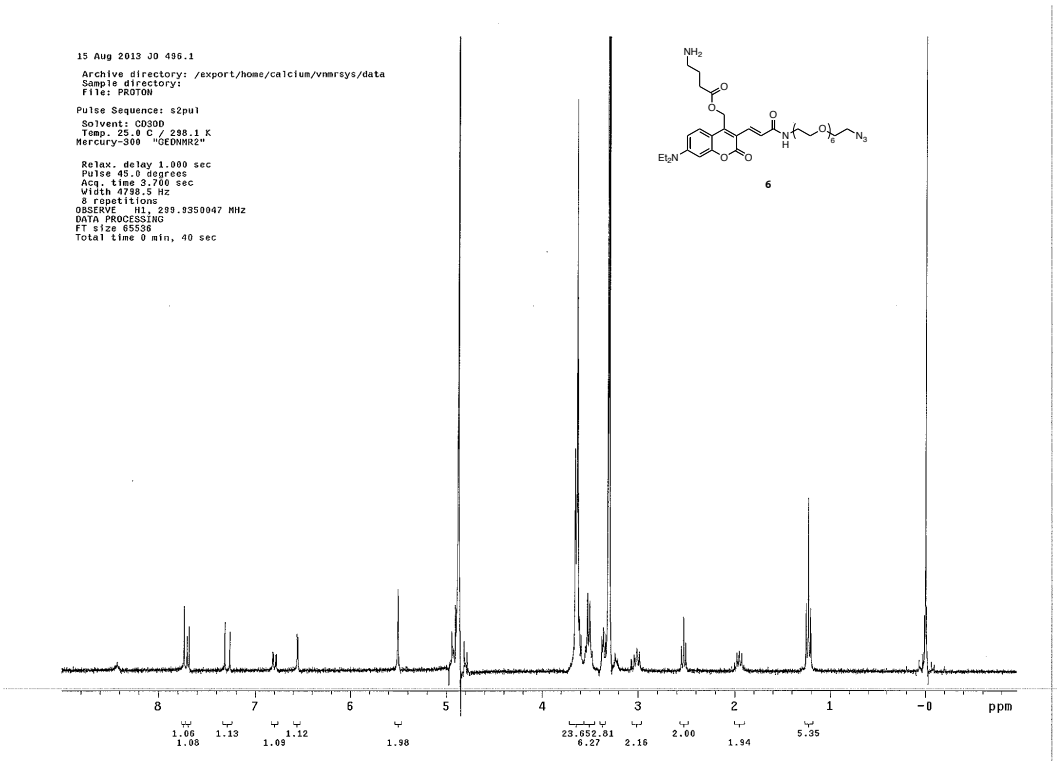
Base Peak : 835.4453, Averaged MW : 835.9753(a), 835.9760(w)


15 Aug 2013 JO 496.1
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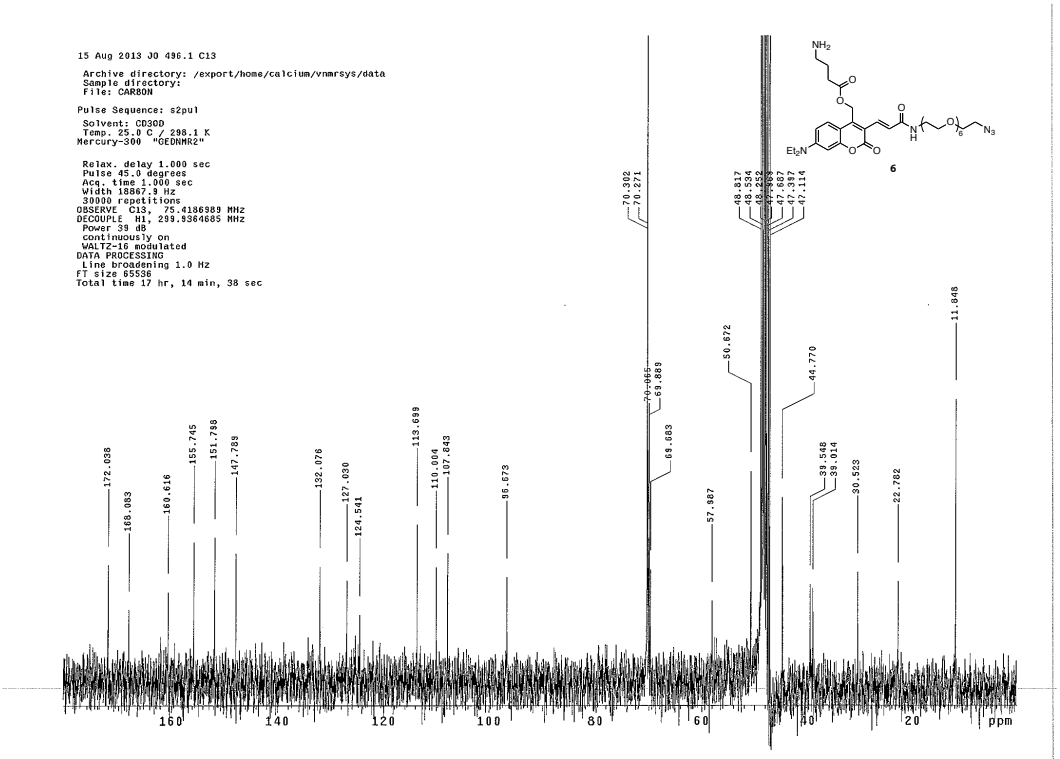
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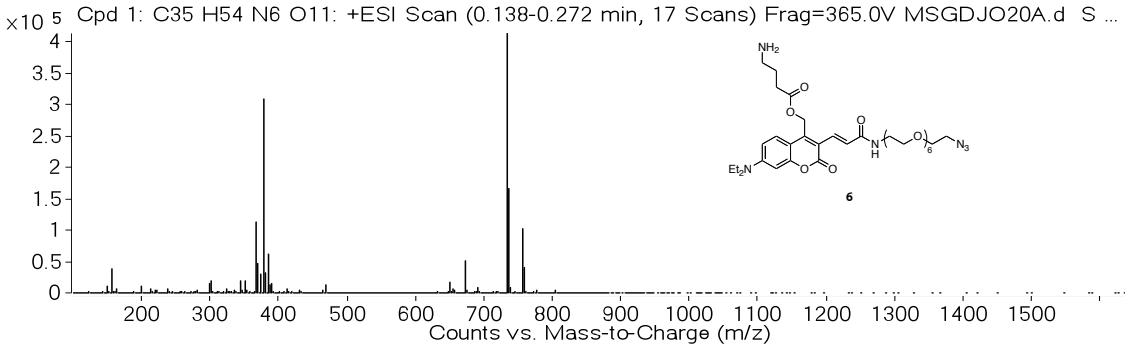

MS Spectrum Peak List

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369.2035	369.2027	-2.27	2	12572.82	C35H54N6O11	(M+2H)+2
390.1822	390.1817	-1.23	2	15188.57	C35H54N6O11	(M+2Na)+2
735.3927	735.3923	-0.56	1	413248.34	C35H54N6O11	(M+H)+
736.3961	736.3954	-0.93	1	165560.97	C35H54N6O11	(M+H)+
737.3981	737.3981	-0.08	1	39580.2	C35H54N6O11	(M+H)+
757.3746	757.3743	-0.39	1	101382.55	C35H54N6O11	(M+Na)+
758.3774	758.3773	-0.02	1	39481.48	C35H54N6O11	(M+Na)+
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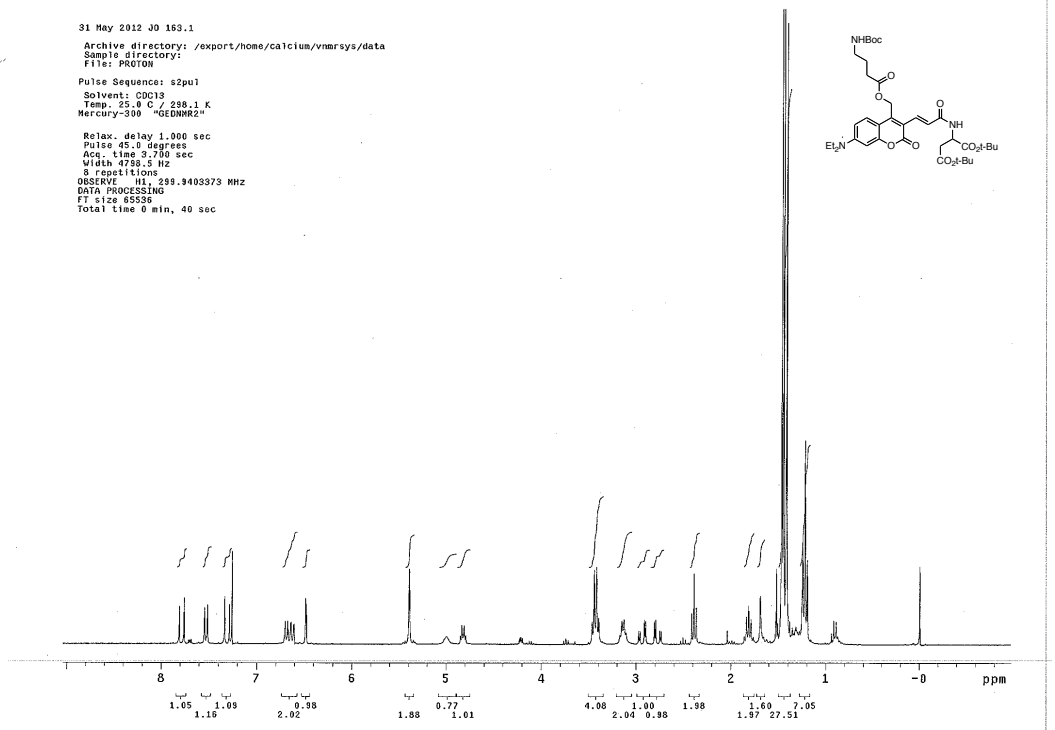
31 May 2012 JO 163.1
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Solvent: CDCl3
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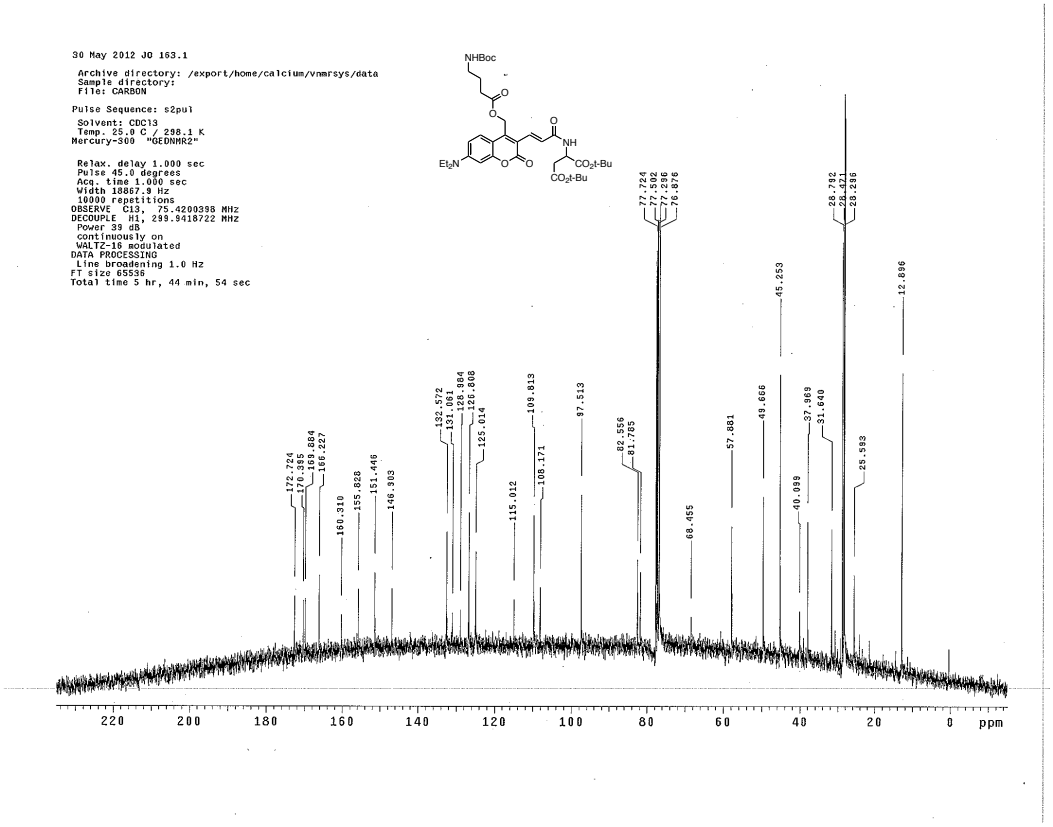
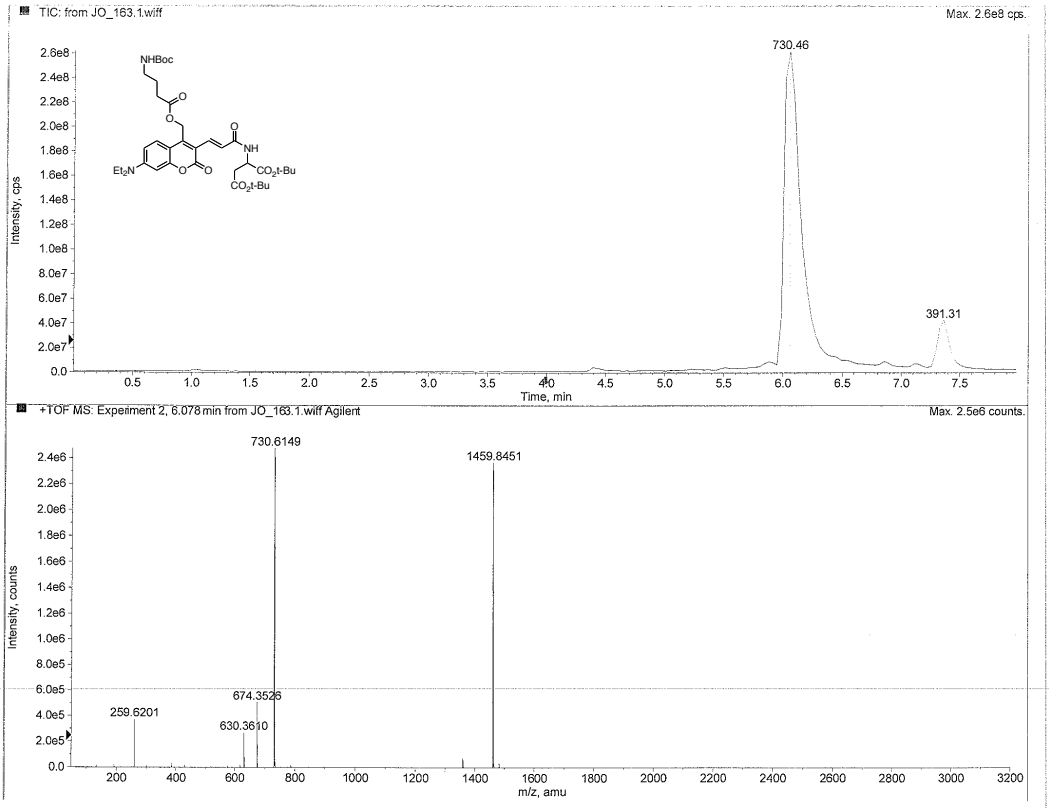
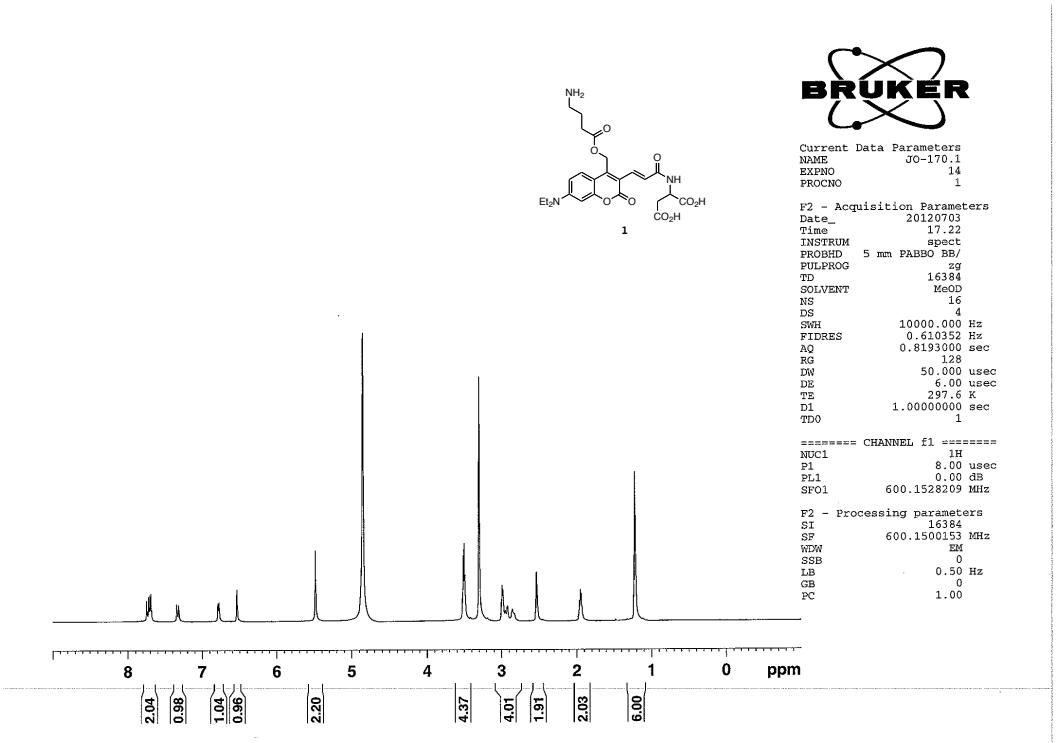
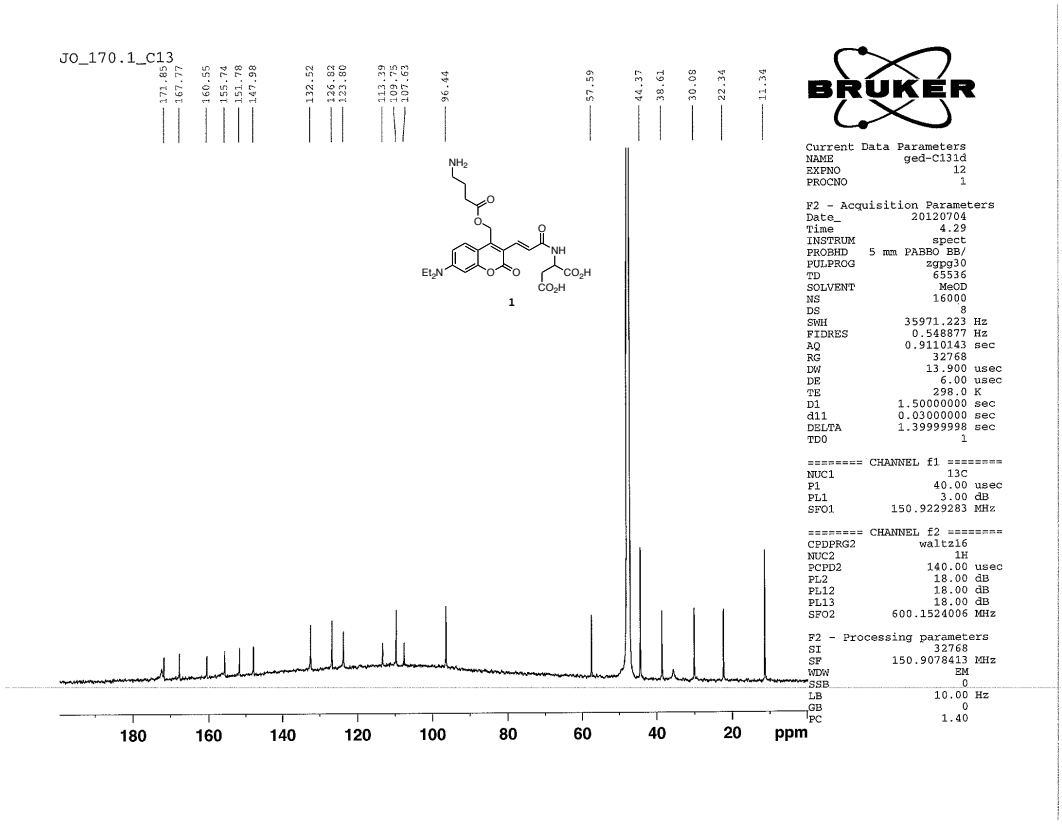
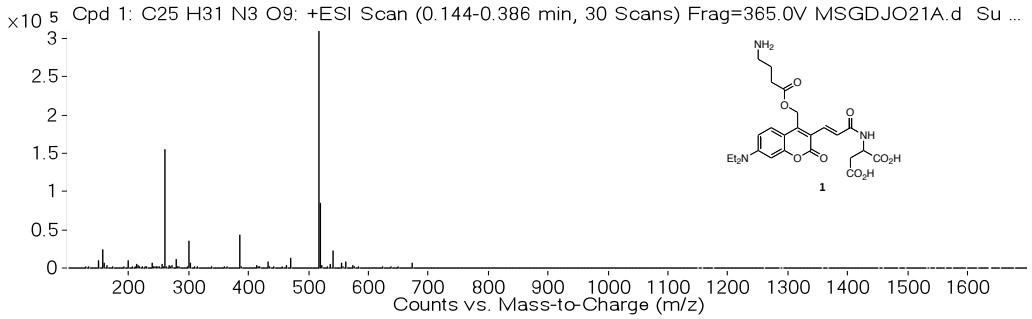
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.700 sec
Width 4798.5 Hz
8 repetitions
OBSERVE H1, 299.9403373 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 40 sec
NHBoc
Et2N
CO2t-Bu
CO2t-Bu
8
7
6
5
4
3
2
1
-0
ppm
1.05
1.16
1.09
0.98
2.02
1.88
0.77
1.01
4.08
2.04
1.00
0.98
1.98
1.60
1.97
7.05
27.51


30 May 2012 JO 163.1
Archive directory: /export/home/calcium/vnmrsys/data
Sample directory:
File: CARBON
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Mercury-300 "GEDNMR2"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 1.000 sec
Width 18867.9 Hz
10000 repetitions
OBSERVE C13, 75.4200398 MHz
DECOUPLE H1, 299.9418722 MHz
Power 39 dB
continuously on
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
FT size 65536
Total time 5 hr, 44 min, 54 sec
NHBoc
Et2N
CO2t-Bu
CO2t-Bu
172.724
170.385
158.884
156.227
160.310
155.828
151.446
148.303
132.572
131.081
128.984
126.806
125.014
115.012
109.813
108.171
97.513
82.556
81.785
77.724
77.502
77.296
76.876
68.455
57.681
49.666
45.253
40.099
37.969
31.640
28.732
28.421
28.296
25.583
12.896
220
200
180
160
140
120
100
80
60
40
20
0
ppm


TIC: from JO_163.1.wiff
Max. 2.6e8 cps.
730.46
391.31
Intensity, cps
Time, min
+TOF MS: Experiment 2, 6.078 min from JO_163.1.wiff Agilent
Max. 2.5e6 counts.
730.6149
1459.8451
674.3526
259.6201
630.3610
Intensity, counts
m/z, amu


1
BRUKER
Current Data Parameters
NAME JO-170.1
EXPNO 14
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120703
Time 17.22
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg
TD 16384
SOLVENT MeOD
NS 16
DS 4
SWH 10000.000 Hz
FIDRES 0.610352 Hz
AQ 0.8193300 sec
RG 128
DW 50.000 usec
DE 6.00 usec
TE 297.6 K
D1 1.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 8.00 usec
PL1 0.00 dB
SFO1 600.1528209 MHz
F2 - Processing parameters
SI 16384
SF 600.1500153 MHz
WDW EM
SSB 0
LB 0.50 Hz
GB 0
PC 1.00
ppm
2.04
0.98
1.04
0.96
2.20
4.37
4.01
1.91
2.03
6.00





MS Spectrum Peak List

m/z	*Calc m/z*	Diff(ppm)	z	Abund	Formula	Ion
259.6112	259.6103	-3.34	2	155220.63	C25H31N3O9	(M+2H)+2
260.1125	260.1119	-2.41	2	45514.71	C25H31N3O9	(M+2H)+2
260.6136	260.6131	-1.96	2	9676.08	C25H31N3O9	(M+2H)+2
281.592	281.5922	0.94	2	235.8	C25H31N3O9	(M+2Na)+2
518.2135	518.2133	-0.43	1	309856.53	C25H31N3O9	(M+H)+
519.2166	519.2165	-0.31	1	85228.64	C25H31N3O9	(M+H)+
520.2189	520.219	0.22	1	16953.01	C25H31N3O9	(M+H)+
535.2471	535.2399	-13.61	1	5511.28	C25H31N3O9	(M+NH4)+
540.1948	540.1953	0.83	1	22708.83	C25H31N3O9	(M+Na)+
541.1975	541.1984	1.59	1	6797.02	C25H31N3O9	(M+Na)+