Optically Selective Two-photon Uncaging of Glutamate at 900 nm.

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Supporting Information.

Chemical synthesis of new compounds.

4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2H-chromen-2-one (1): To a solution of 7-(diethylamino)-4-(hydroxymethyl)-2*H*-chromen-2-one (0.495 g, 2.0 mmol, 1 eq) in dichloromethane (25 mL) was added tert-butyldimethylsilyl chloride (0.362 g, 2.4 mmol, 1.2 eq), followed by triethylamine (0.304 g, 3.0 mmol, 1.5 eq) and dimethylaminopyridine (73 mg, 0.6 mmol, 0.05 eq). The resulting solution was stirred for 3 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 Hexanes: Ethyl Acetate) to obtain **1** in 83% yield (0.602 g, 1.67 mmol) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.25 (d, 1H, J = 9.0 Hz), 6.52 (m, 2H), 6.28 (s, 1H), 4.81 (s, 2H), 3.40 (q, 4H, J = 7.0 Hz), 1.20 (t, 6H, J = 7.0 Hz), 0.96 (s, 9H), 0.14 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 162.4, 155.9, 154.5, 150.2, 123.8, 108.2, 106.2, 105.4, 97.8, 61.1, 44.7, 25.9, 18.5, 12.5, -5.2; LCMS ((Agilent Jet Stream) m/z calc'd for $C_{20}H_{31}NO_3Si$ $[M+H]^+$ 362.2146, found 362.2144.

3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2H-chromen-2-one (2): 4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2H-chromen-2-one (**1**) (2.28g, 6.31 mmol, 1 eq) was dissolved in acetonitrile (40 mL) and treated with N-Bromosuccinimide (1.24 g, 6.94 mmol, 1.1 eq). Ammonium acetate (49 mg, 0.631 mmol, 0.1 eq) was then added and the reaction

was stirred for 1 hour at which time the reaction was then poured into H_2O 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, 4:1 Hexanes: Ethyl Acetate) to obtain 2 in 65% yield (1.81 g, 4.10 mmol) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.75 (d, 1H, J = 9.0 Hz), 6.60 (dd, 1H, J = 9.0 Hz, 3.0 Hz), 6.47 (d, 1H, J = 3.0 Hz), 4.99 (s, 2H), 3.40 (q, 4H, J = 7.0 Hz), 1.20 (t, 6H, J = 7.0 Hz), 0.90 (s, 9H), 0.13 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 158.3, 154.8, 151.2, 150.4, 127.2, 108.8, 107.9, 104.6, 96.9, 63.2, 44.8, 25.9, 18.3, 12.5, -4.9; LCMS (Agilent Jet Stream) m/z calc'd for $C_{20}H_{30}BrNO_3Si$ $[M+H]^+$ 442.1233, found 442.1233.

(E)-tert-butyl 3-(4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2H-chromen-3-yl)acrylate (3): A solution of 3-bromo-4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2H-chromen-2-one (**2**) (1.81 g, 4.11 mmol, 1eq), and tert-Butyl acrylate (1.58 g, 12.33 mmol, 3eq) in dimethylformamide (40 mL) was degassed for 10 minutes, then treated with lithium chloride (0.296 g, 6.99 mmol, 1.7eq), sodium bicarbonate (1.04 g, 12.33 mmol, 3 eq), and tetrabutylammonium chloride (1.23 g, 4.52 mmol, 1.1 eq). The resulting suspension was degassed for 5 minutes, then treated with palladium acetate (46 mg, 0.206 mmol, 5 mol%) and heated to 110 °C for 30 min. The reaction was then cooled to room temperature poured into H_2O 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 Hexanes: Ethyl Acetate) to obtain **3** in 68% yield (1.35 g, 2.76 mmol) as a bright yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ:

7.75 (d, 1H, J = 16.0 Hz), 7.65 (d, 1H, J = 9.0 Hz), 7.00 (d,1H, J = 16.0 Hz), 6.61 (dd, 1H, J = 9.0 Hz, 3.0 Hz), 6.47 (d, 1H, J = 3.0 Hz), 4.90 (s, 2H), 3.42 (q, 4H, J = 7.0 Hz), 1.51 (s, 9H), 1.22 (t, 6H, J = 7.0 Hz), 0.90 (s, 9H), 0.16 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 166.7, 160.4, 155.6, 151.1, 150.8, 134.6, 127.3, 124.6, 113.5, 109.0, 108.2, 96.9, 79.9, 57.6, 44.8, 28.3, 25.8, 18.3, 12.6, -5.0; LCMS (Agilent Jet Stream) m/z calc'd for $C_{27}H_{41}NO_5Si$ $[M+H]^+$ 488.2827, found 488.2825.

(E)-3-(4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2H-chromen-3-yl)acrylic acid (4): (E)-tert-butyl 3-(4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2H-chromen-3-yl)acrylate (**3**) (0.550 g, 1.12 mmol) was dissolved in dichloromethane (5 mL), and trifluoroacetic acid (5 mL) and stirred for 30 min. The solvent was then removed and the crude material was redissolved in dichloromethane and concentrated to obtain (**4**) in 98% yield (0.471 g, 1.09 mmol) as an orange solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.00 (d, 1H, J = 15.3 Hz), 7.66 (d, 1H, J = 9.3 Hz), 7.17 (d,1H, J = 15.6 Hz), 6.64 (dd, 1H, J = 9.0, 2.1 Hz), 6.48 (d, 1H, J = 2.7 Hz), 4.93 (s, 2H), 3.44 (q, 4H, J = 7.0 Hz), 1.23 (t, 6H, J = 7.0 Hz), 0.91 (s, 9H), 0.17 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.2, 160.3, 156.0, 152.6, 151.2, 138.3, 127.5, 120.6, 112.8, 109.4, 108.3, 97.2, 57.5, 45.0, 25.8, 18.3, 12.6, -5.0; LCMS (Agilent Jet Stream) m/z calc'd for $C_{23}H_{33}NO_5Si$ $[M+H]^+$ 432.2201, found 432.2201.

(E)-di-tert-butyl 2-(3-(4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2H-chromen-3-yl)acrylamido)succinate (5): A solution of L-Aspartic acid di-tertbutylester (278 mg, 1.14 mmol, 2 eq) and (E)-3-(4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2H-chromen-3-yl)acrylic acid (**4**) (245 mg, 0.567 mmol, 1 eq) in dichloromethane (15 mL) and acetonitrile

(15 mL) was treated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (119 mg, 0.624 mmol, 1.1 eq). The reaction was stirred for 16 hours, then concentrated to obtain the crude product. The crude material was then purified by column chromatography (silica, 2:1 Hexanes: Ethyl Acetate) to obtain **5** in 55% yield (204 mg, 0.309 mmol) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.85 (d, 1H, J = 15.0 Hz), 7.69 (d, 1H, J = 9.3 Hz), 7.24 (d,1H, J = 9.3 Hz), 6.62 (m, 2H), 6.47 (d, 1H, J = 3.0 Hz), 4.95 (s, 2H), 4.85 (m, 1H), 3.44 (q, 4H, J = 7.0 Hz), 2.85 (dq, 2H, J = 50.1 Hz, 17.1 Hz, 4.5 Hz), 1.46 (s, 9H), 1.45 (s, 9H), 1.22 (t, 6H, J = 7.0 Hz), 0.89 (s, 9H), 0.16 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.1, 169.6, 165.9, 160.4, 155.6, 151.5, 150.8, 132.5, 127.5, 124.2, 112.9, 96.9, 82.1, 81.4, 57.5, 49.2, 44.8, 37.7, 28.1, 27.9, 25.9, 18.3, 12.6, -5.0 ; LCMS (Agilent Jet Stream) m/z calc'd for $C_{35}H_{54}N_2O_8Si$ $[M+H]^+$ 659.3722, found 659.3723.

(E)-di-tert-butyl 2-(3-(7-(diethylamino)-4-(hydroxymethyl)-2-oxo-2H-chromen-3-yl)acrylamido)succinate (6): To a solution of (E)-di-tert-butyl 2-(3-(4-(((tert-butyldimethylsilyl)oxy)methyl)-7-(diethylamino)-2-oxo-2H-chromen-3-yl)acrylamido)succinate (**5**) (83 mg, 0.126 mmol, 1 eq) in tetrahydrofuran (5 mL) was added tetrabutylammonium fluoride (1M in THF) (0.189 mL, 0.189 mmol, 1.5 eq). The reaction was stirred for 15 minutes, 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, 1:3 Hexanes: Ethyl Acetate) to obtain **6** in 83% yield (57 mg, 0.105 mmol) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.86 (d, 1H, J = 15.0 Hz), 7.72 (d, 1H, J = 9.3 Hz), 7.28 (d,1H, J = 14.7 Hz), 6.75 (d, 1H, J = 8.1 Hz), 6.62 (dd, 1H, J = 9.2 Hz, 2.4 Hz), 6.47 (d, 1H, J = 2. Hz),

4.97 (s, 2H), 4.83 (m, 1H), 3.44 (q, 4H, J = 7.0 Hz), 2.85 (dq, 2H, J = 50.1 Hz, 17.1 Hz, 4.5 Hz), 1.46 (s, 9H), 1.44 (s, 9H), 1.22 (t, 6H, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 170.0, 169.5, 166.5, 160.5, 155.7, 151.7, 150.9, 132.8, 127.3, 123.8, 113.1, 109.3, 108.2, 97.0, 82.2, 81.4, 56.4, 49.4, 44.9, 37.6, 29.7, 28.1, 27.9, 12.6 ; LCMS (Agilent Jet Stream) m/z calc'd for $C_{29}H_{40}N_2O_8$ $[M+H]^+$ 545.2857, found 545.2859.

(E)-1-tert-butyl 5-((3-(3-((1,4-di-tert-butoxy-1,4-dioxobutan-2-yl)amino)-3-oxoprop-1-en-1-yl)-7-(diethylamino)-2-oxo-2H-chromen-4-yl)methyl) 2-((tert-butoxycarbonyl)amino)pentanedioate: A solution of (E)-di-tert-butyl 2-(3-(7-(diethylamino)-4-(hydroxymethyl)-2-oxo-2H-chromen-3-yl) acrylamido) succinate (217 mg, 0.398 mmol, 1 eq) in dichloromethane (15 mL) was treated with Boc-L-glutamic acid 1-tert-butyl ester (181 mg, 0.598 mmol, 1.5 eq), then 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (479 mg, 2.49 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 (E)-1-tert-butyl 5-((3-(3-((1,4-di-tert-butoxy-1,4-dioxobutan-2-yl)amino)-3-oxoprop-1-en-1-yl)-7-(diethylamino)-2-oxo-2H-chromen-4-yl)methyl) 2-((tert-butoxycarbonyl)amino)pentanedioate in 69% yield (227 mg, 0.273 mmol) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.81 (d, 1H, J = 15.0 Hz), 7.52 (d, 1H, J = 9.3 Hz), 7.31 (d, 1H, J = 15.0 Hz), 6.68 (m, 2H), 6.47 (d, 1H, J = 2.4 Hz), 5.40 (s, 2H), 5.11 (d, 1H, J = 8.1 Hz), 4.82 (m, 1H), 4.15 (m, 1H), 3.44 (q, 4H, J = 7.2 Hz), 2.85 (dq, 2H, J = 50.1 Hz, 17.1 Hz, 4.5 Hz), 2.43 (m ,2H), 2.14 (m, 1H), 1.91 (m, 1H), 1.46 (s, 9H), 1.45 (s, 9H), 1.44 (s, 9H), 1.41 (s, 9H),

1.23 (t, 6H, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 171.9, 170.9, 169.9, 169.5, 165.7, 159.9, 155.3, 150.9, 146.3, 131.9, 126.6, 124.8, 114.6, 109.4, 107.7, 96.9, 82.0, 81.9, 81.3, 79.5, 57.6, 53.2, 49.2, 44.8, 37.6, 30.0, 28.2, 28.0, 27.9, 27.8, 12.5 ; LCMS (Agilent Jet Stream) m/z calc'd for $C_{43}H_{63}N_3O_{13}$ $[M+H]^+$ 830.4466, found 830.4469.

(E)-2-(3-(4-(((4-amino-4-carboxybutanoyl)oxy)methyl)-7-(diethylamino)-2-oxo-2H-chromen-3-yl)acrylamido)succinic acid (7): (E)-1-tert-butyl 5-((3-(3-((1,4-di-tert-butoxy-1,4-dioxobutan-2-yl)amino)-3-oxoprop-1-en-1-yl)-7-(diethylamino)-2-oxo-2H-chromen-4-yl)methyl) 2-((tert-butoxycarbonyl)amino)pentanedioate (50 mg, 0.602 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, Altima C18 column, then lyophilized to obtain **7** in 68% yield (23 mg, 0.041 mmol) as a yellow powder. ^{1}H NMR (600 MHz, CD_3OD) δ: 7.75 (d, 1H, J = 15.0 Hz), 7.70 (d, 1H, J = 9.6 Hz), 7.32 (d, 1H, J = 15.0 Hz), 6.79 (dd, 1H, J = 9.3 Hz, 2.4 Hz), 6.54 (d, 1H, J = 2.4 Hz), 5.49 (s, 2H), 4.02 (t, 1H, J = 7.2 Hz), 3.44 (q, 4H, J = 7.2 Hz), 2.85 (dq, 2H, J = 51.6 Hz, 16.8 Hz, 7.2 Hz), 2.60 (m ,2H), 2.26 (m, 1H), 2.17 (m, 1H), 1.25 (t, 6H, J = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.9, 171.8, 170.2, 167.8, 160.7, 155.8, 151.8, 147.8, 132.5, 126.9, 123.9, 113.5, 109.8, 107.6, 96.4, 57.7, 51.9, 49.2, 44.4, 35.6, 29.0, 25.2, 11.3; LCMS (Agilent Jet Stream) m/z calc'd for $C_{26}H_{31}N_3O_{11}$ $[M+H]^+$ 562.2031, found 562.2033. RP-HPLC; retention time= 14.38 min, 35% CH_3CN/ 0.1% TFA in H_2O, Altima C-18 column

Photochemical and physicochemical methods. The quantum yield of uncaging was measured by comparative photolysis as described previously for other caged compounds[22,23,29,30]. Concentrations of RuBi-Glu and DEAC450-Glu were set to give an OD = 0.2 at 473 nm in a 1 mm cuvette. A defocused 473-nm laser with a diameter of 3 cm was used for irradiation of 100μL solutions of the caged compounds in phosphate buffer (100 mM) at pH 7.4. Inosine (0.5 mM) was also included in the photolysis reaction mixture, as a photochemically inert standard. RuBi-Glu has a quantum yield of photolysis of 0.13. Analysis of the time course of the reaction by HPLC showed that DEAC450-Glu was photolyzed 3 times greater than RuBi-Glu, giving a quantum yield of photolysis of 0.39 (n=4).

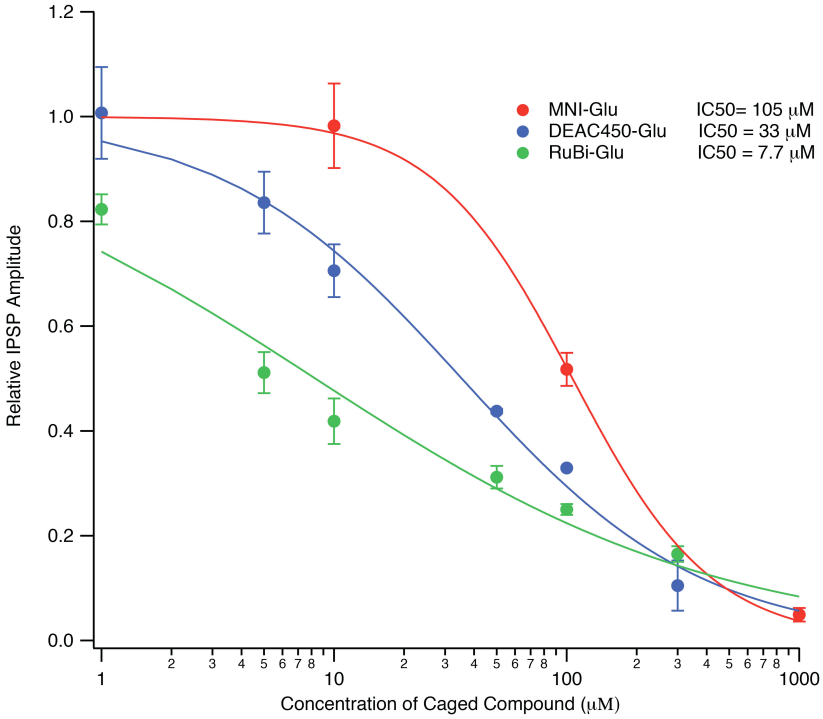
The solubility of DEAC450-Glu was determined by dissolving a known amount of compound in a defined volume of phosphate buffer at pH 7.4 to give a concentration of 7.5 mM. This solution was centrifuged at 13,500 rpm for 10 min. After removal of the supernatant, no pellet was visible.

The relative two-photon excitation efficiency of DEAC450 was determined by irradiation of a solution (0.02 mM) with mode-locked Ti:sapphire laser (Coherent Ultra II) through a 0.8 NA 40x lens on a Prairie Technologies Ultima two-photon microscope. Emitted fluorescence was detected after excitation at 720 nm and 900nm. Note, DEAC450 is non-fluorescent at 1000 nm.

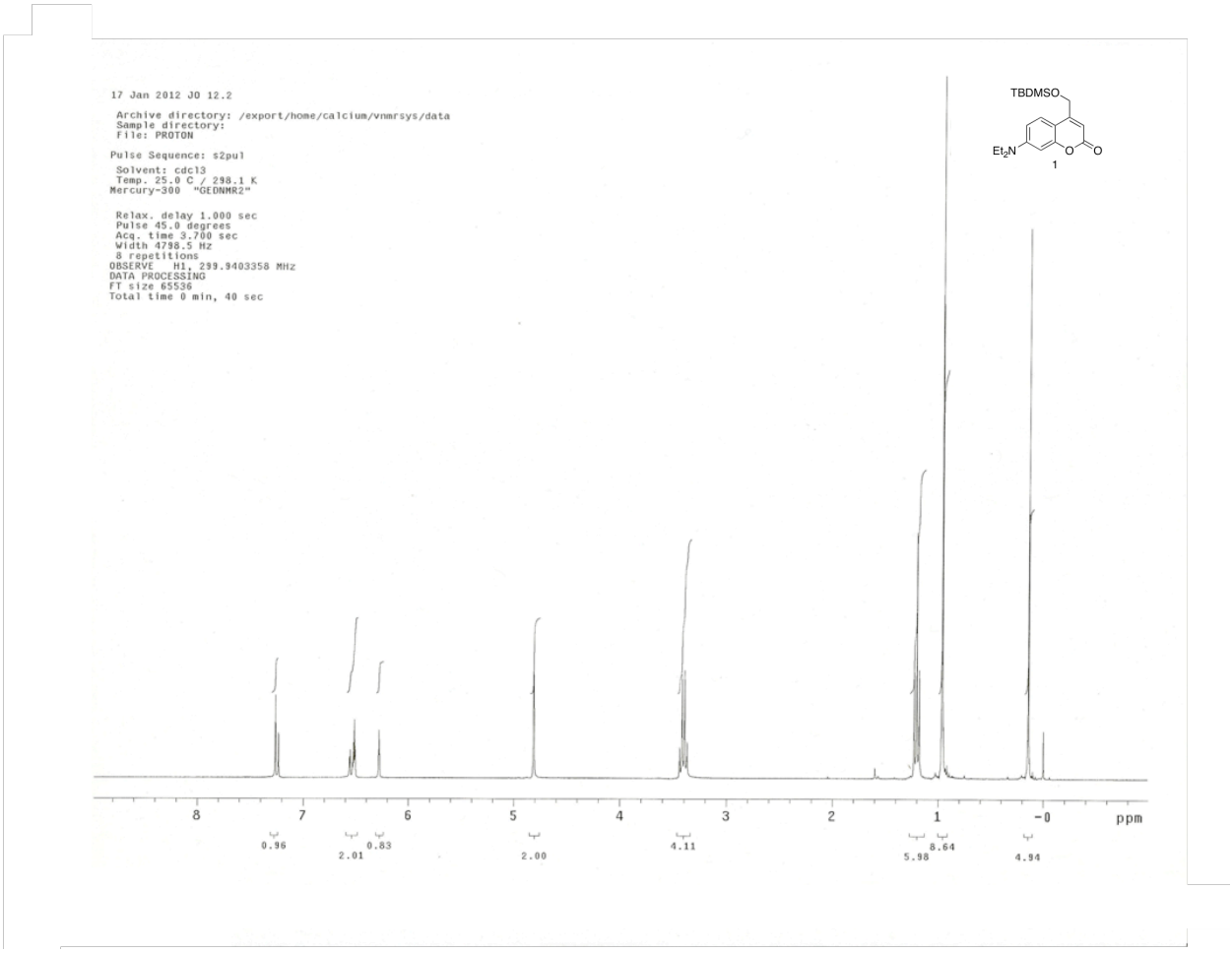
Physiology methods. All animal handling was performed in accordance with guidelines approved by the Harvard and Yale Institutional Animal Care and Use Committees and federal guidelines. Brain slices were acutely isolated from mice as described previously.[27,28] Two-photon uncaging at visually spine heads defined was performed using custom-built microscopes and software as

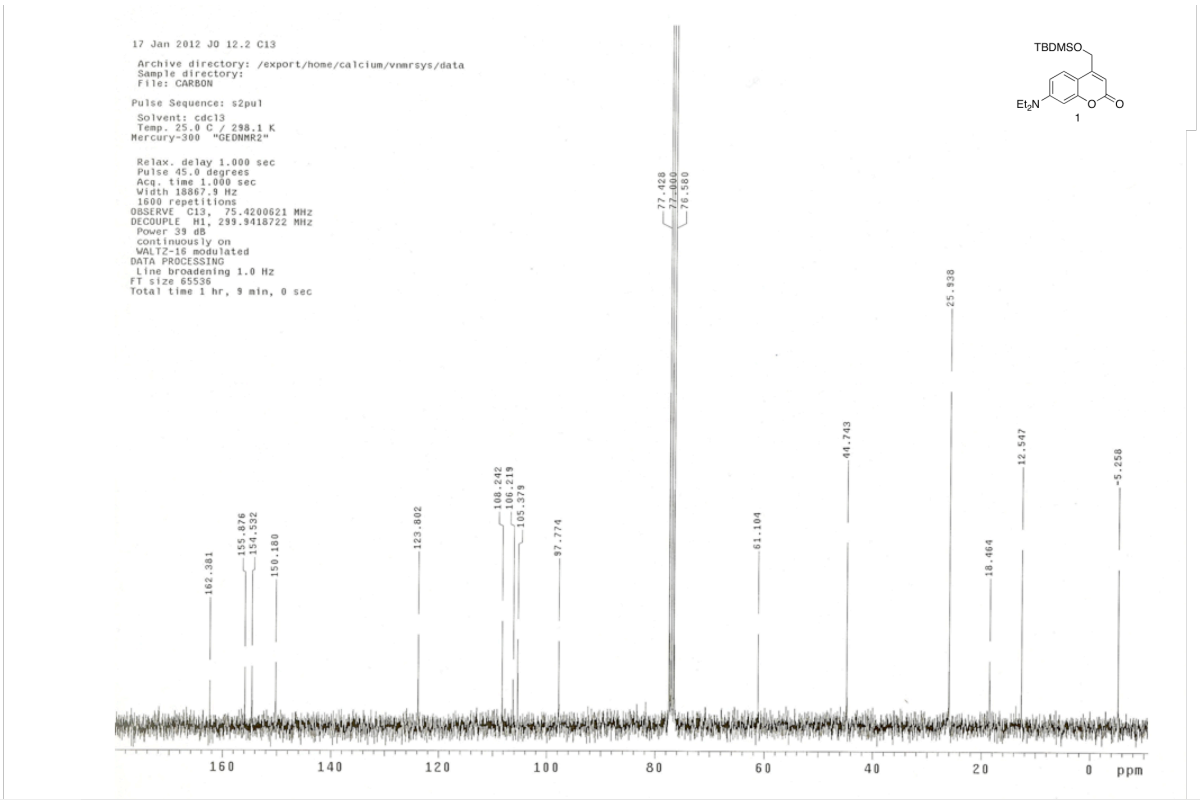
described previously[27,28]. For uncaging experiments, the caged glutamate probes were applied using a puffer pipette positioned just above the brain slice[26]. Dendrites filled with Alexa-594 were imaged at 1000 nm.

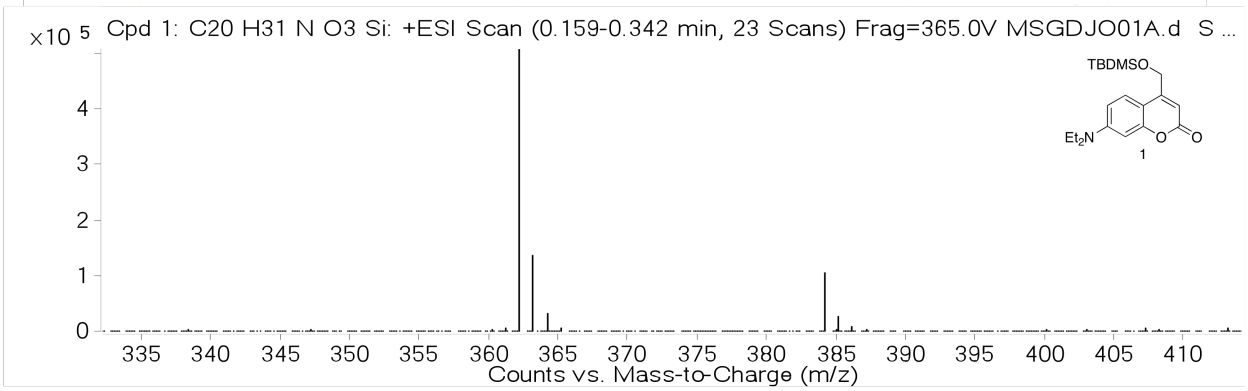
For determination of GABA-A receptor block, inhibitory postsynaptic currents were evoked using local electrical stimulation and compounds were bath applied at a range of concentrations (0.01-1.0 mM).



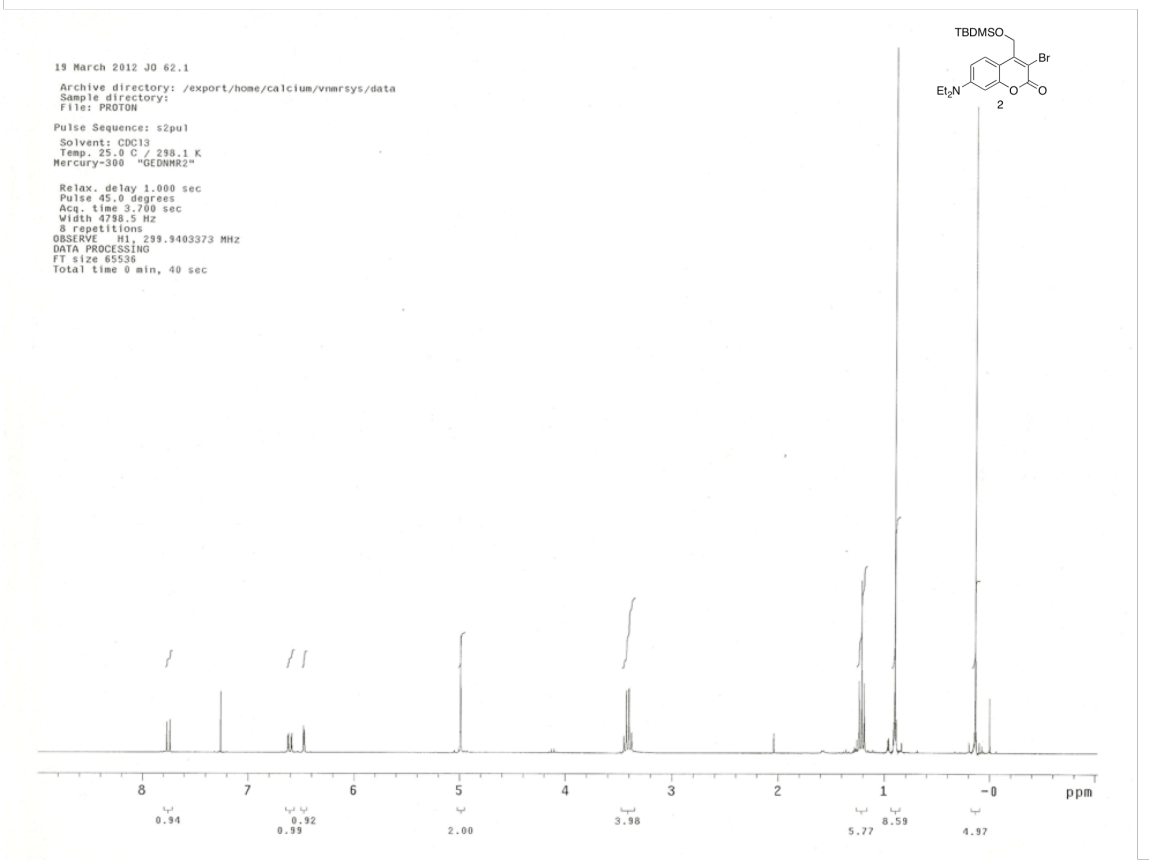
Spectral data (scans of NMR and HR-MS).







m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
362.2144	362.2146	0.41	1	508095.28	C20H31NO3Si	(M+H)+
363.2172	363.2172	0.02	1	134908.19	C20H31NO3Si	(M+H)+
364.2165	364.2161	-1.07	1	30690.04	C20H31NO3Si	(M+H)+
365.2151	365.2176	6.78	1	4812.25	C20H31NO3Si	(M+H)+
366.2221	366.2196	-6.74	1	662.06	C20H31NO3Si	(M+H)+
384.1964	384.1965	0.4	1	103681.56	C20H31NO3Si	(M+Na)+
385.1998	385.1992	-1.55	1	26934.23	C20H31NO3Si	(M+Na)+
386.1997	386.198	-4.22	1	6631.77	C20H31NO3Si	(M+Na)+
387.1972	387.1996	6.1	1	1503.93	C20H31NO3Si	(M+Na)+
388.2047	388.2016	-7.98	1	379.76	C20H31NO3Si	(M+Na)+


19 March 2012 JO 62.1
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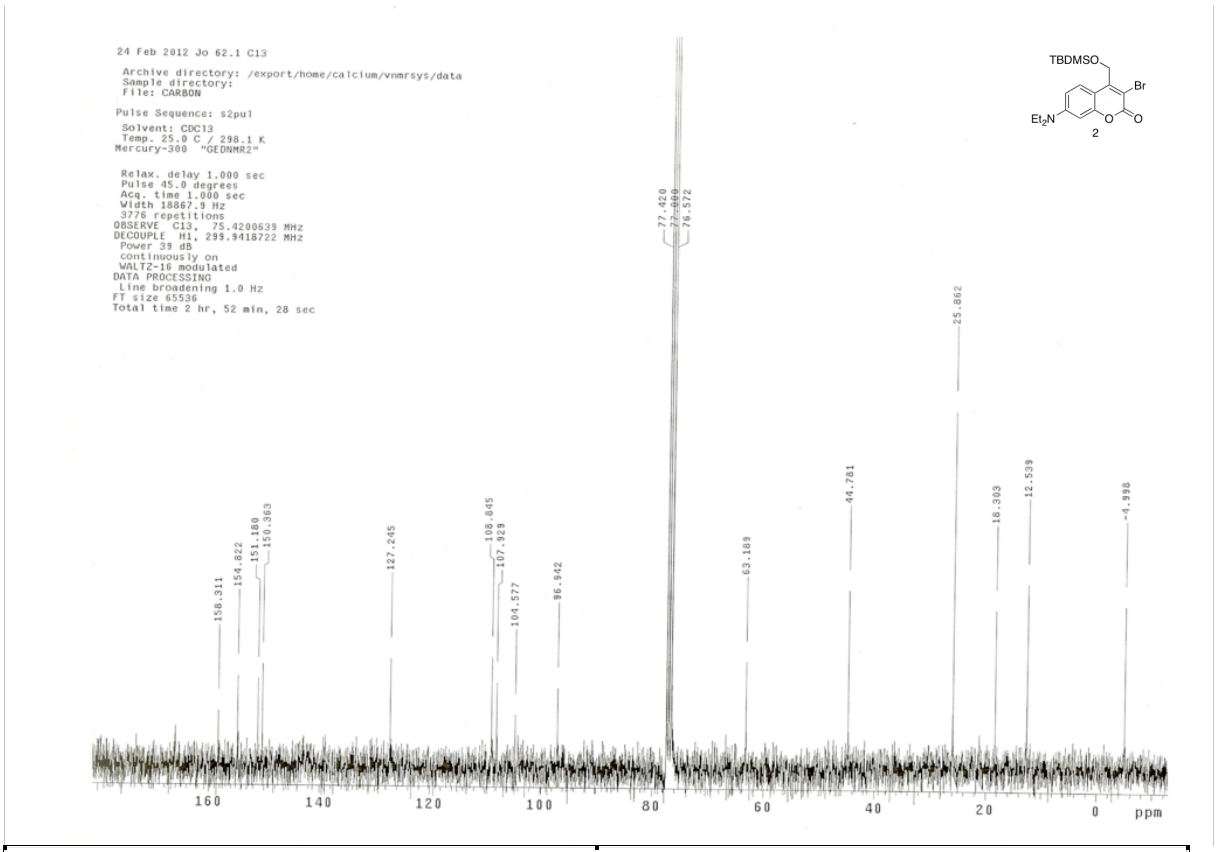
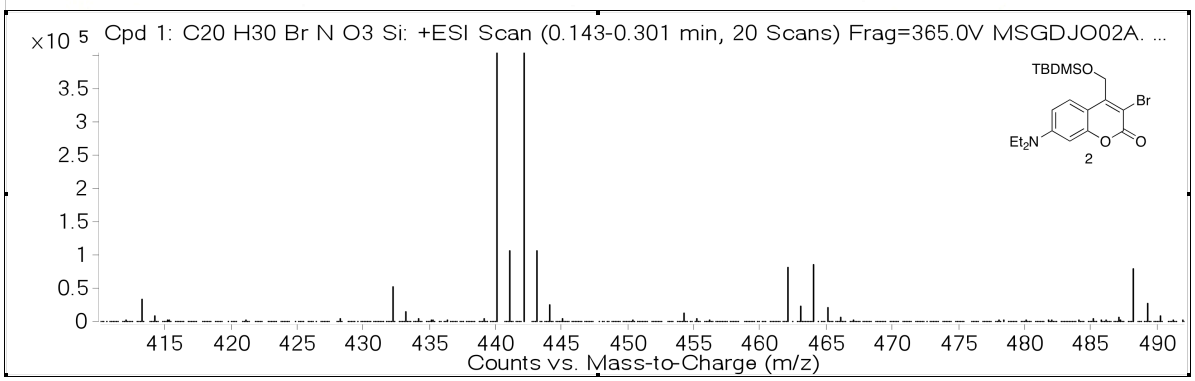
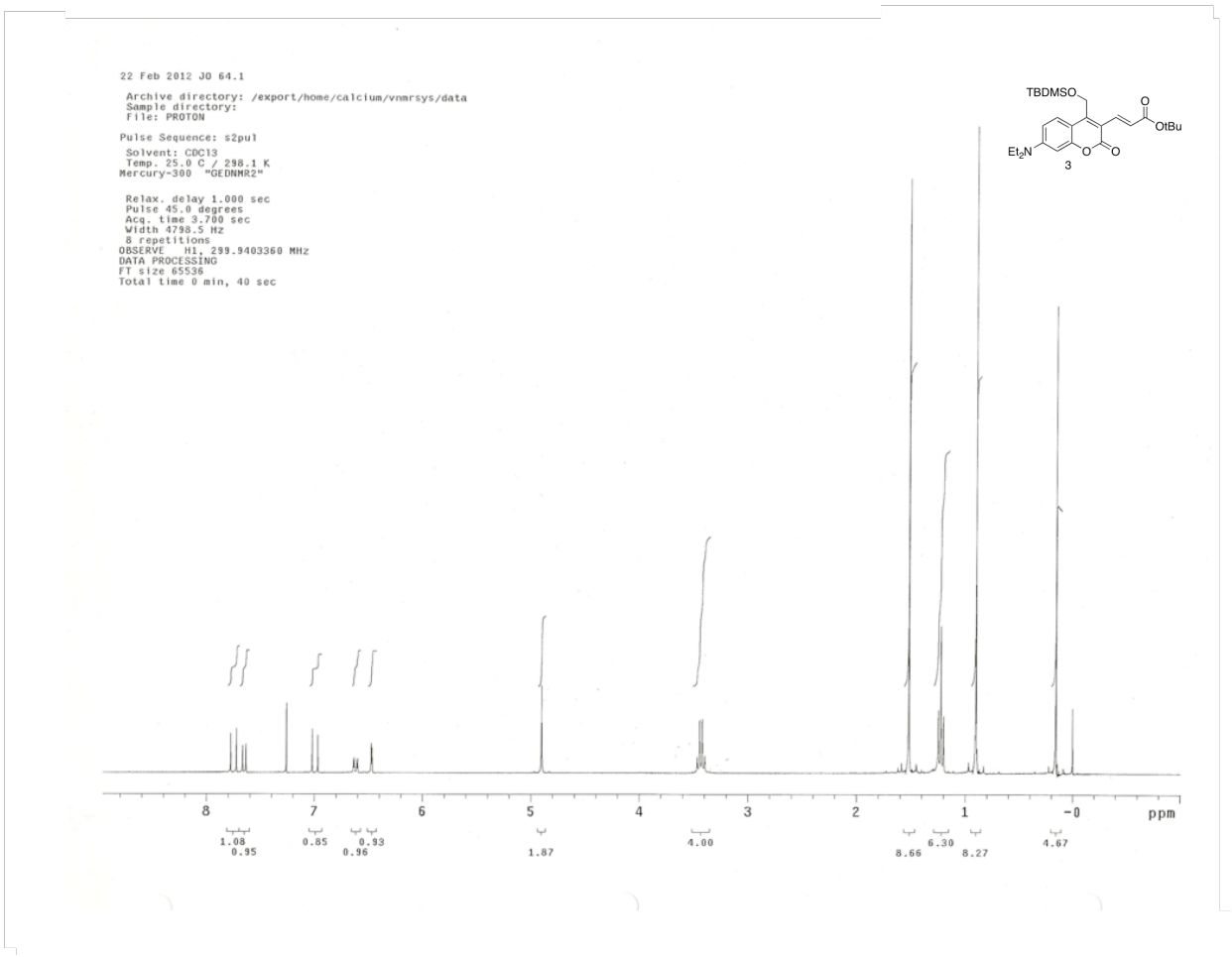




m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
440.125	440.1251	0.15	1	402777.25	C20H30BrNO3Si	(M+H)+
441.1275	441.1277	0.42	1	105154.92	C20H30BrNO3Si	(M+H)+
442.1233	442.1233	0.14	1	403264.06	C20H30BrNO3Si	(M+H)+
443.126	443.1258	-0.43	1	105643.72	C20H30BrNO3Si	(M+H)+
444.1253	444.1247	-1.48	1	24863.38	C20H30BrNO3Si	(M+H)+
462.1068	462.1071	0.53	1	81940.59	C20H30BrNO3Si	(M+Na)+
463.1095	463.1097	0.28	1	21944.5	C20H30BrNO3Si	(M+Na)+
464.1051	464.1053	0.37	1	85689.31	C20H30BrNO3Si	(M+Na)+
465.1076	465.1077	0.24	1	21001.67	C20H30BrNO3Si	(M+Na)+
466.1072	466.1066	-1.33	1	5259.36	C20H30BrNO3Si	(M+Na)+


22 Feb 2012 JO 64.1
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Total time 0 min, 40 sec
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OtBu
Et2N
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8
7
6
5
4
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2
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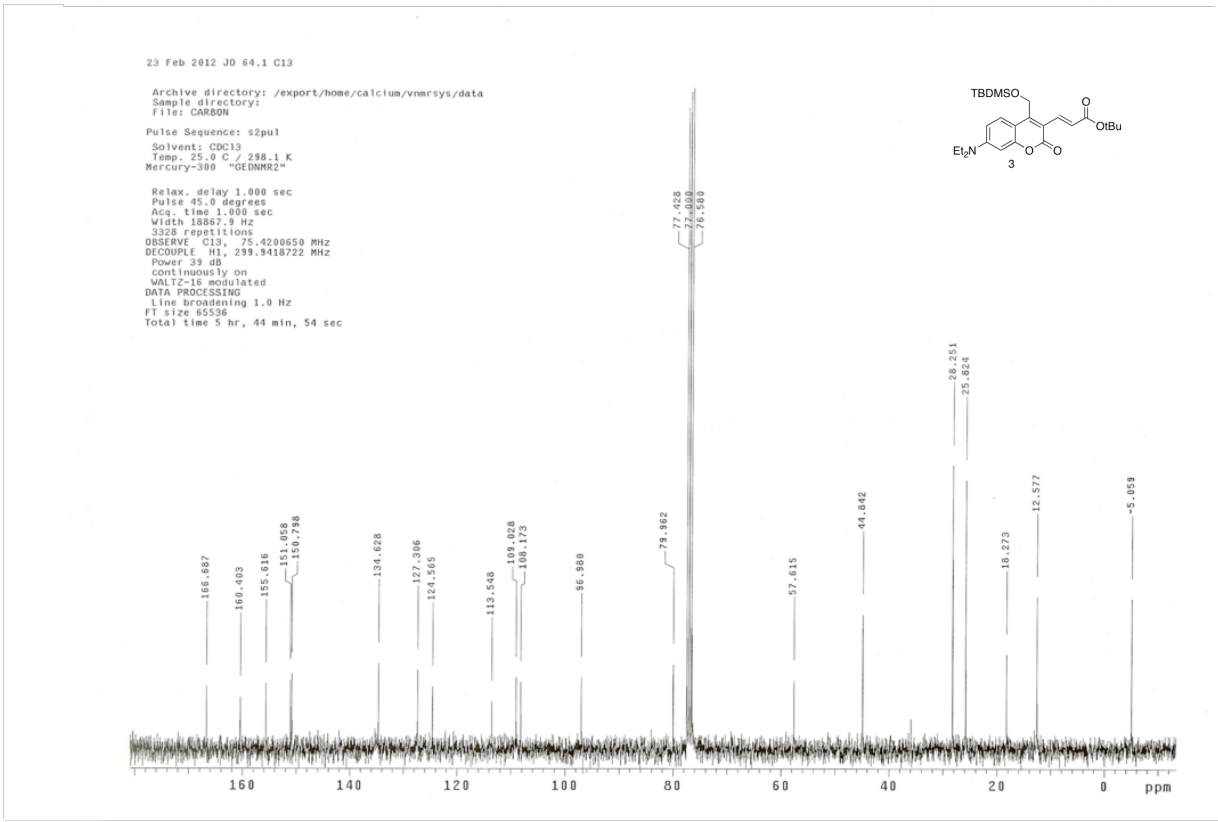
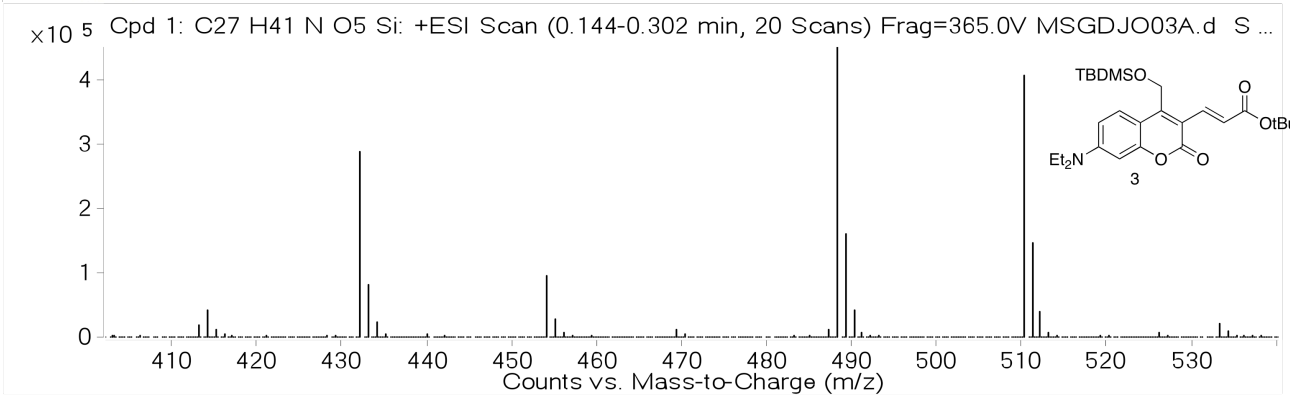
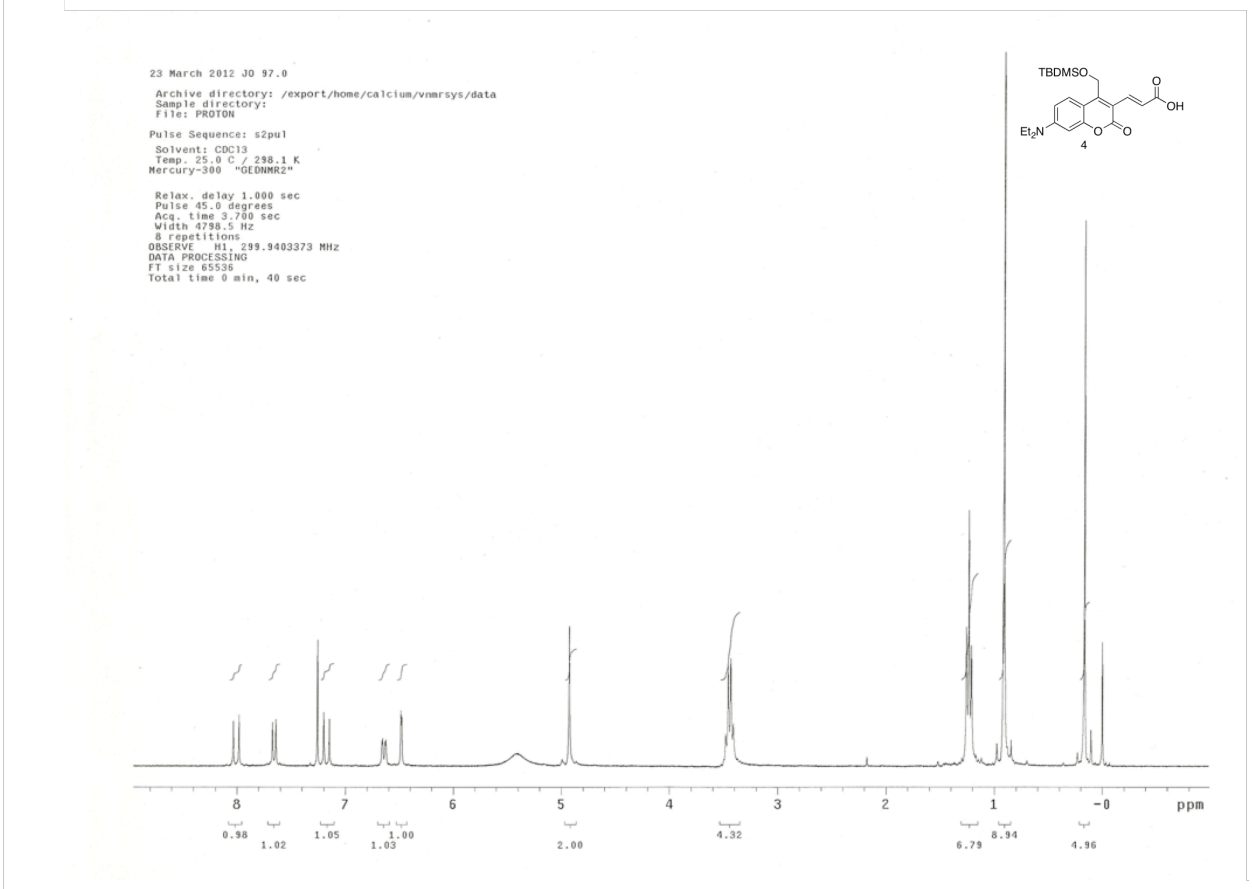
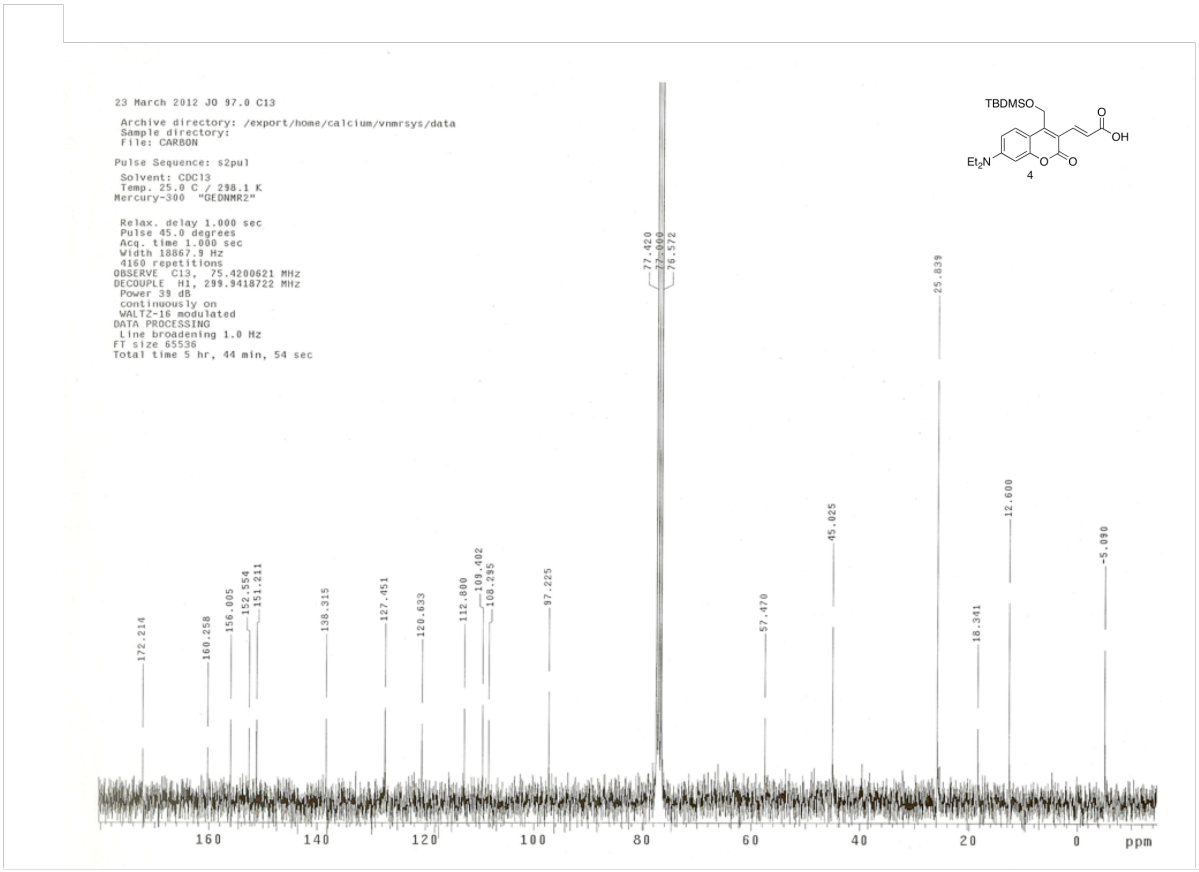
0.95
0.85
0.93
0.96
1.87
4.00
8.66
6.30
8.27
4.67

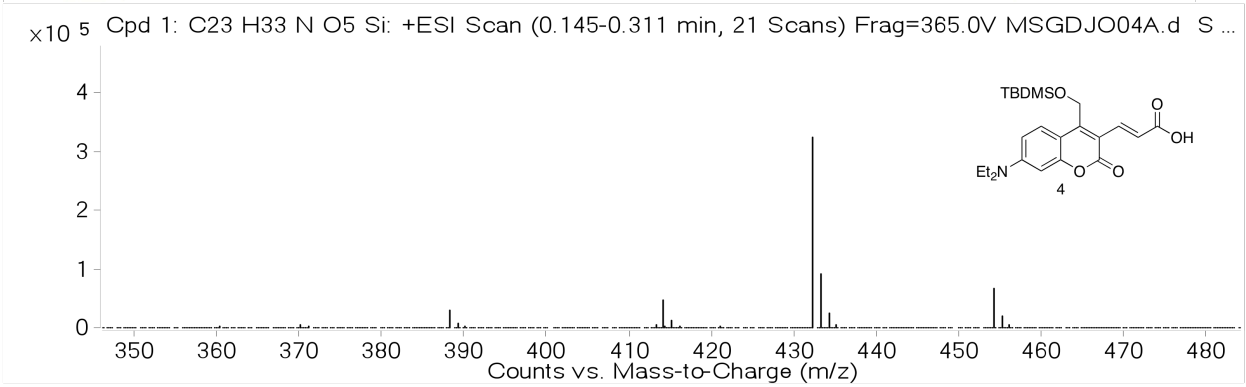




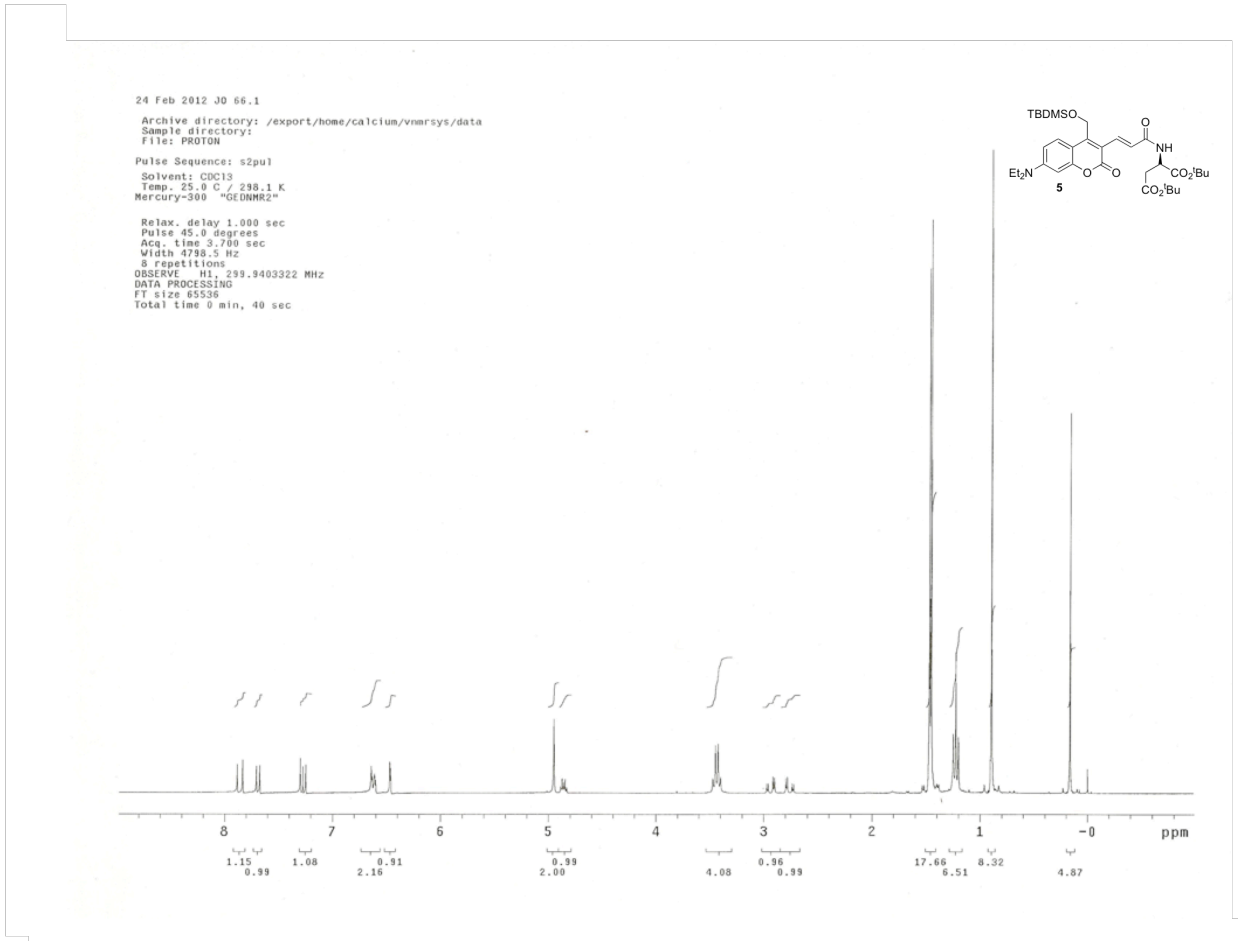
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
432.2203	432.2201	-0.58	1	287455.59	C27H41NO5Si	(M+H)+[-C4H8]
433.2228	433.2228	-0.04	1	82514.23	C27H41NO5Si	(M+H)+[-C4H8]
449.2453	449.2466	2.92	1	124.81	C27H41NO5Si	(M+NH4)+[-C4H8]
454.2019	454.202	0.22	1	95533.08	C27H41NO5Si	(M+Na)+[-C4H8]
488.2825	488.2827	0.31	1	451490.63	C27H41NO5Si	(M+H)+
489.2857	489.2855	-0.5	1	161447.64	C27H41NO5Si	(M+H)+
490.2862	490.2854	-1.75	1	42248.73	C27H41NO5Si	(M+H)+
510.2645	510.2646	0.17	1	408036.19	C27H41NO5Si	(M+Na)+
511.2677	511.2674	-0.53	1	146797.28	C27H41NO5Si	(M+Na)+
512.2679	512.2673	-1.26	1	38817.13	C27H41NO5Si	(M+Na)+

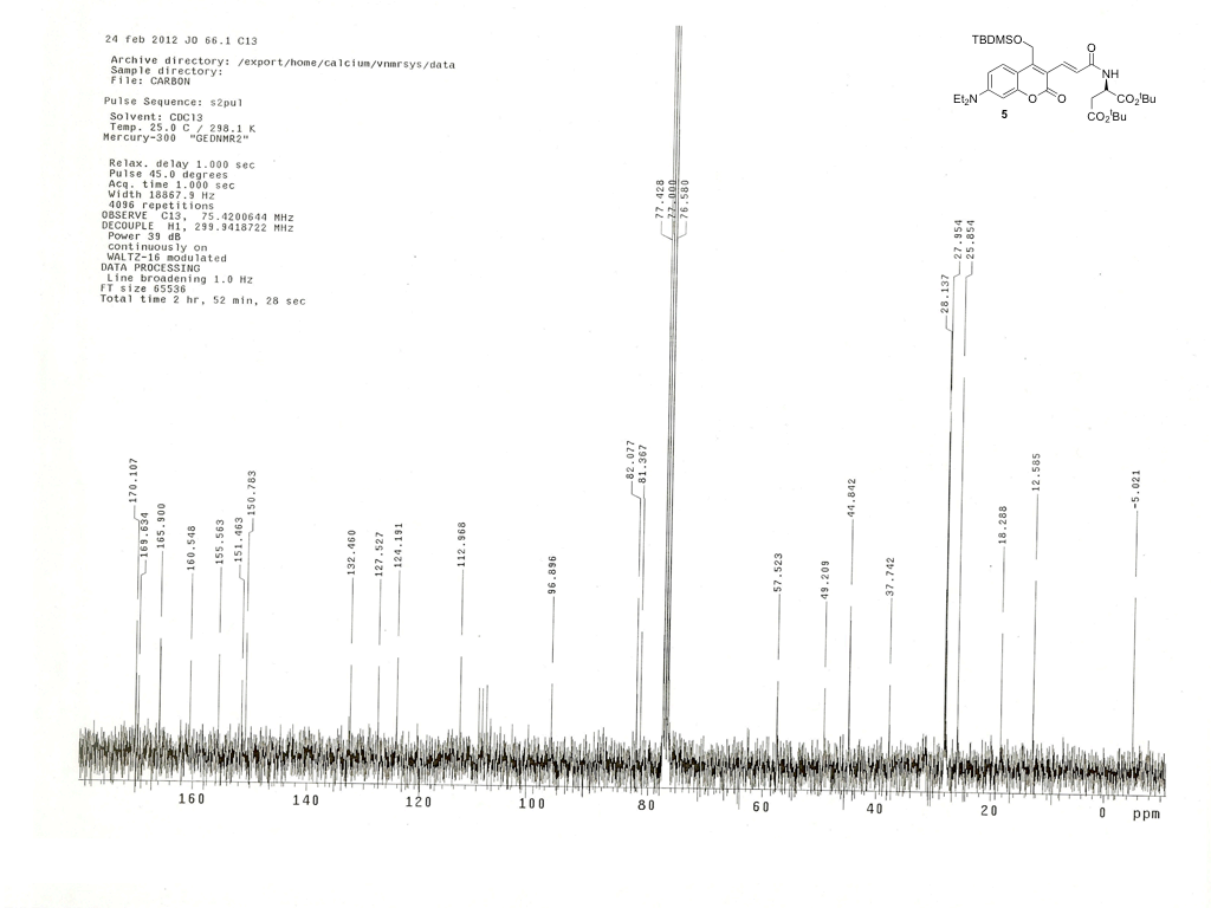

23 March 2012 JO 97.0
Archive directory: /export/home/calcium/vnmrsys/data
Sample directory:
File: PROTON
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Mercury-300 "GEDNMR2"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.700 sec
Width 4798.5 Hz
8 repetitions
OBSERVE H1, 299.9403373 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 40 sec
TBDMSO
OH
Et_2N
4
8
7
6
5
4
3
2
1
-0
ppm
0.98
1.02
1.05
1.00
1.03
2.00
4.32
6.79
8.94
4.96

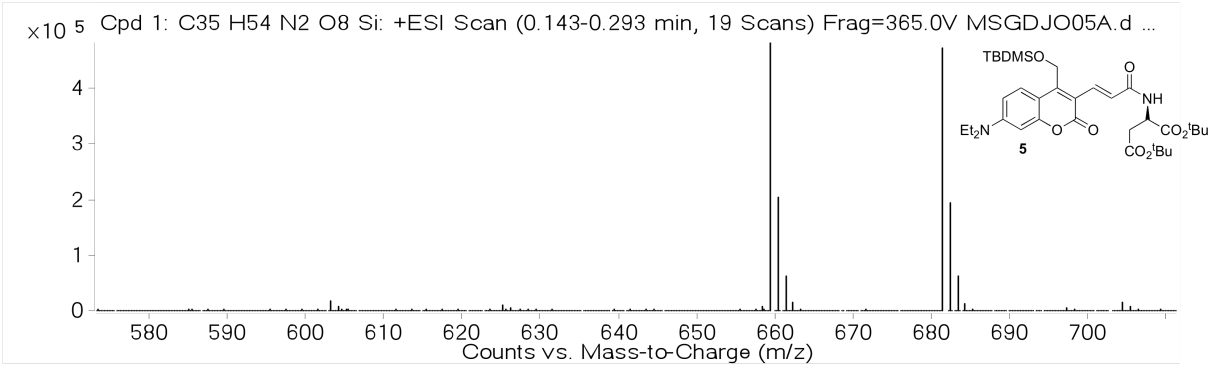




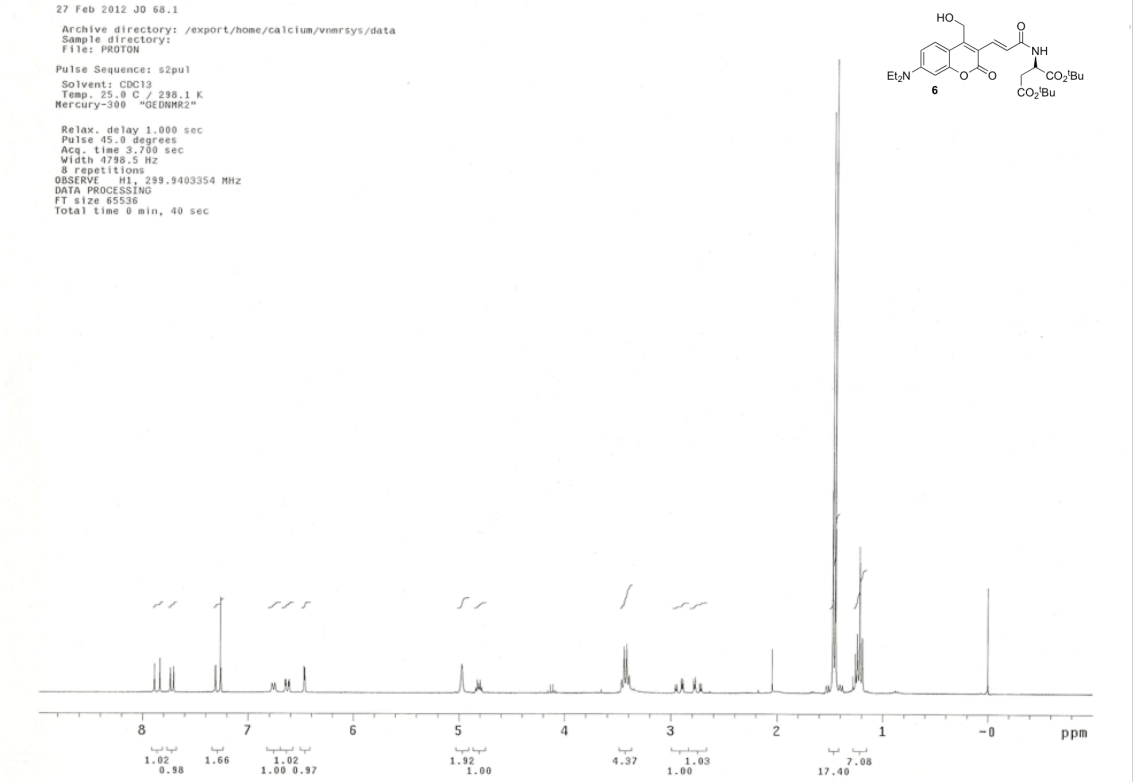
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
376.1547	376.1575	7.5	1	183.01	C23H33NO5Si	(M+H)+[-C4H8]
414.2092	414.2095	0.63	1	45997.63	C23H33NO5Si	(M+H)+[-H2O]
415.2121	415.2122	0.3	1	12875.92	C23H33NO5Si	(M+H)+[-H2O]
432.2201	432.2201	-0.07	1	323857.03	C23H33NO5Si	(M+H)+
433.2229	433.2228	-0.31	1	92371.99	C23H33NO5Si	(M+H)+
434.223	434.2222	-1.83	1	24095.42	C23H33NO5Si	(M+H)+
454.2018	454.202	0.42	1	67524.16	C23H33NO5Si	(M+Na)+
455.2047	455.2047	0.15	1	19342.42	C23H33NO5Si	(M+Na)+
456.2054	456.2041	-2.75	1	5068.97	C23H33NO5Si	(M+Na)+

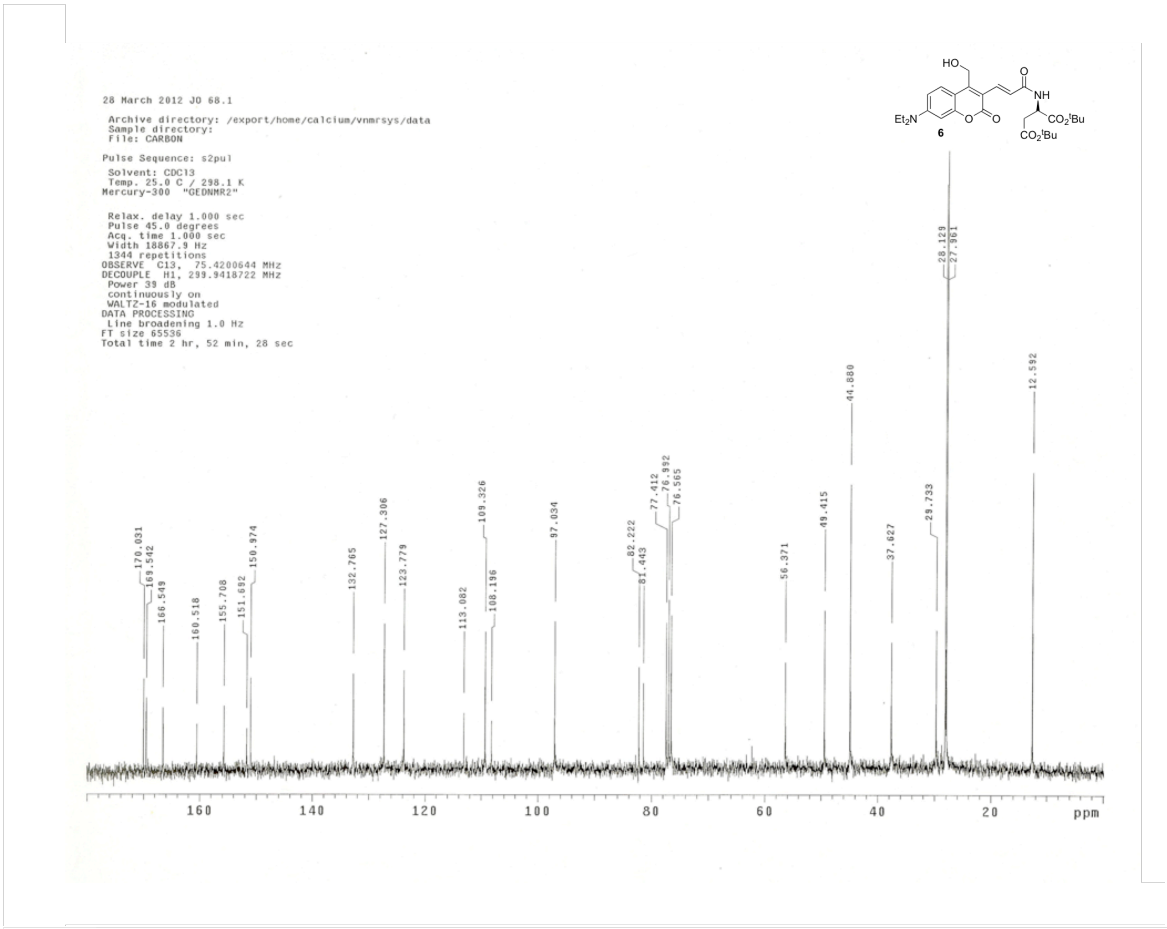

24 Feb 2012 JO 66.1
Archive directory: /export/home/calcium/vnmrsys/data
Sample directory:
File: PROTON
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Mercury-300 "GEONMR2"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.700 sec
Width 4798.5 Hz
8 repetitions
OBSERVE H1, 299.9403322 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 40 sec
TBDMSO
O
NH
Et2N
O
O
CO2tBu
CO2tBu
5
8
7
6
5
4
3
2
1
-0
ppm
1.15
0.99
1.08
0.91
2.16
0.99
2.00
4.08
0.96
0.99
17.66
6.51
8.32
4.87

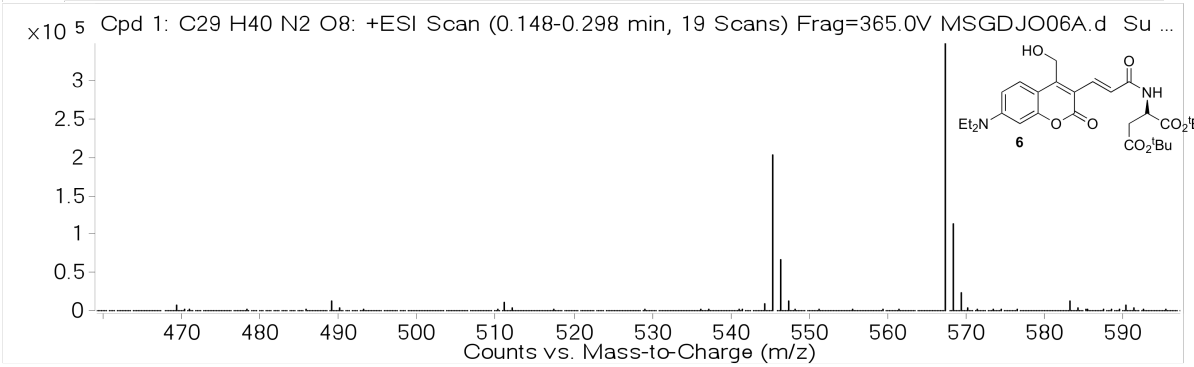




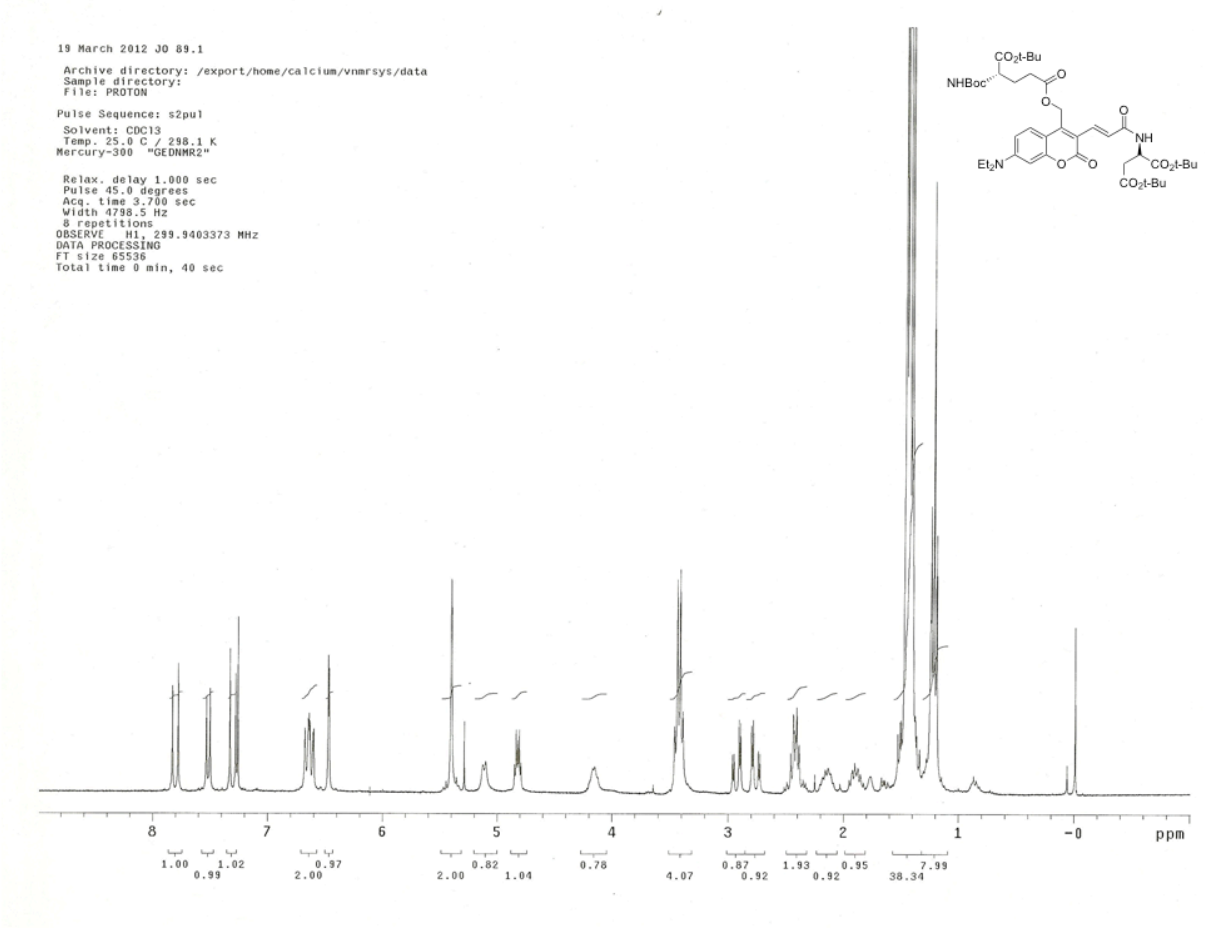
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
603.3094	603.3096	0.42	1	16432.81	C35H54N2O8Si	(M+H)+[-C4H8]
625.2915	625.2916	0.05	1	9437.64	C35H54N2O8Si	(M+Na)+[-C4H8]
659.3723	659.3722	-0.14	1	482210.13	C35H54N2O8Si	(M+H)+
660.3756	660.3751	-0.82	1	203887.36	C35H54N2O8Si	(M+H)+
661.3763	661.3758	-0.74	1	62016.17	C35H54N2O8Si	(M+H)+
662.3775	662.3773	-0.35	1	13959.85	C35H54N2O8Si	(M+H)+
681.3543	681.3542	-0.17	1	473281.03	C35H54N2O8Si	(M+Na)+
682.3576	682.357	-0.87	1	194359.84	C35H54N2O8Si	(M+Na)+
683.3586	683.3577	-1.31	1	60960.01	C35H54N2O8Si	(M+Na)+
684.3597	684.3592	-0.66	1	13206.08	C35H54N2O8Si	(M+Na)+

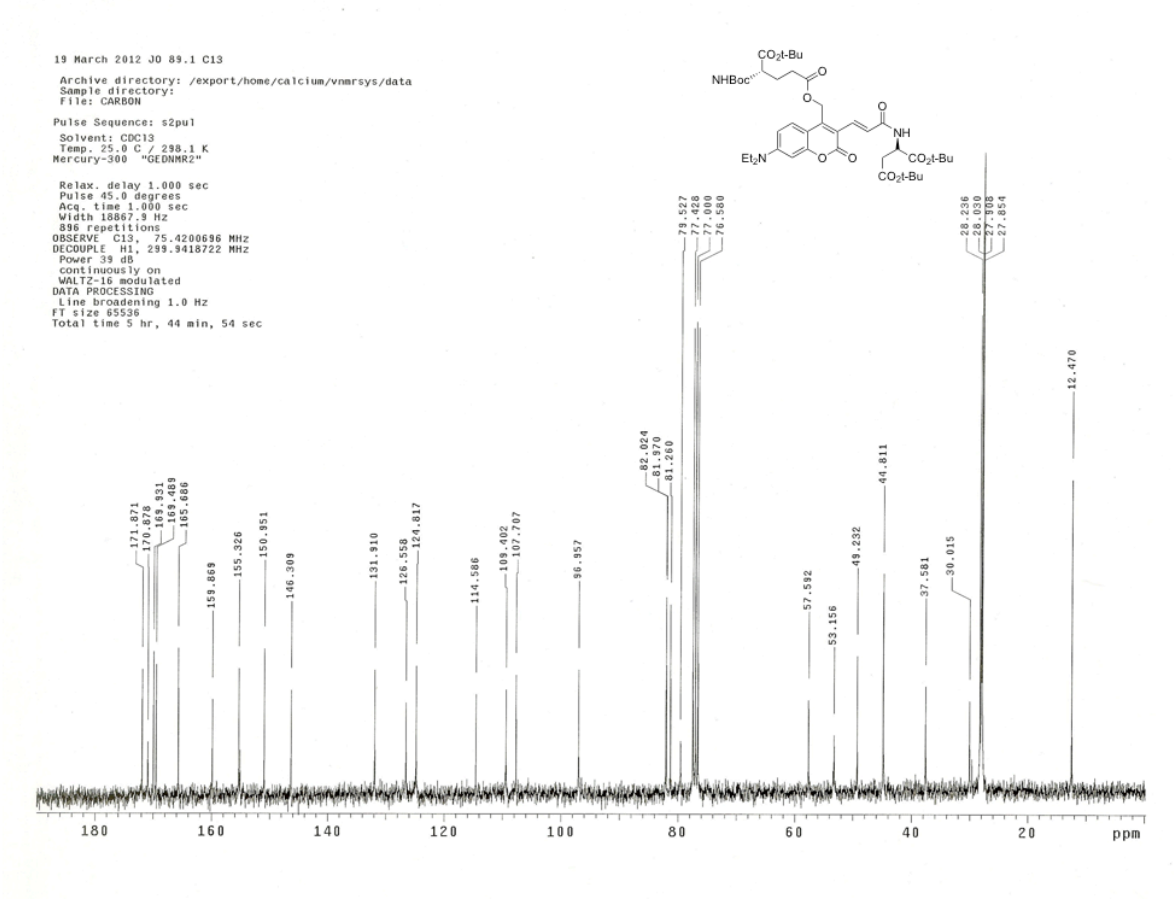

27 Feb 2012 JO 68.1
Archive directory: /export/home/calcium/vnmrsys/data
Sample directory:
File: PROTON
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Mercury-300 "GEDNMR2"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.700 sec
Width 4798.5 Hz
8 repetitions
OBSERVE H1, 299.9403354 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 40 sec
HO
O
NH
Et2N
O
O
CO2tBu
CO2tBu
6
8
7
6
5
4
3
2
1
-0
ppm
1.02
0.98
1.66
1.02
1.00 0.97
1.92
1.00
4.37
1.03
1.00
7.08
17.40

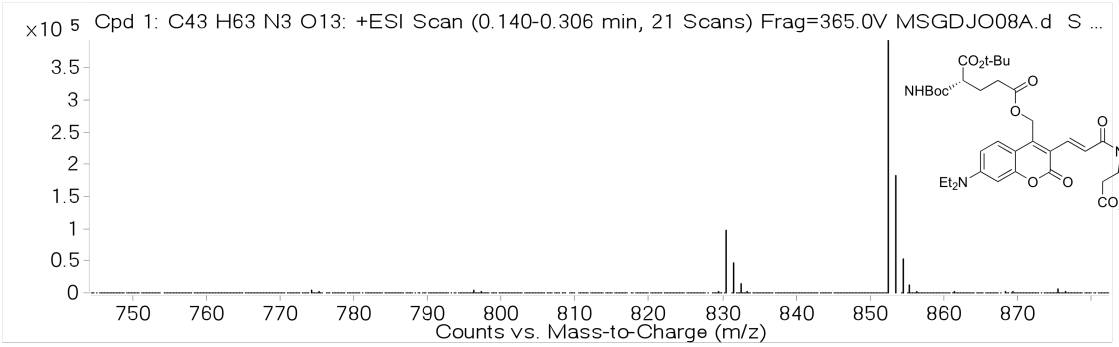




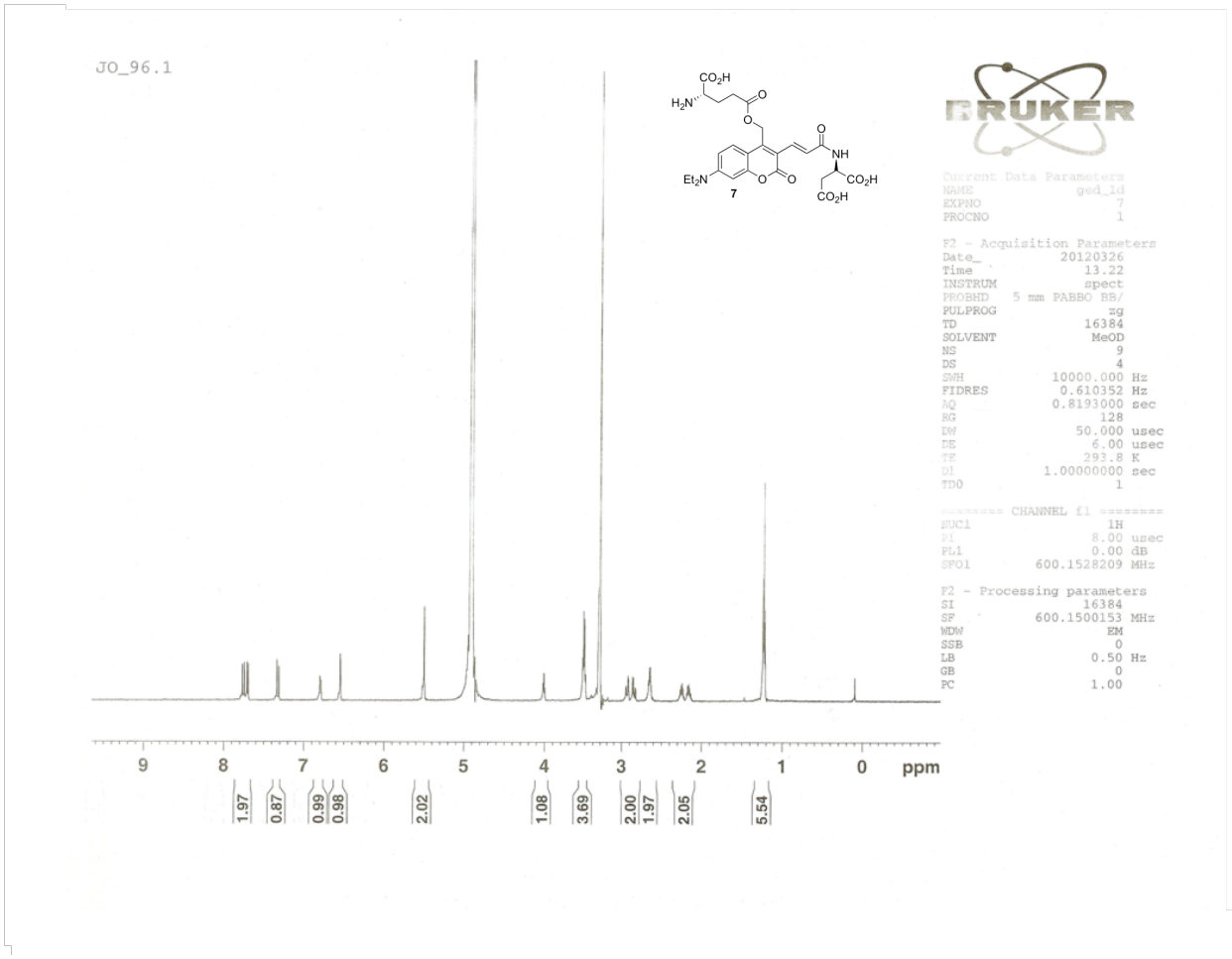
m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
489.223	489.2231	0.32	1	12406.19	C29H40N2O8	(M+H)+[-C4H8]
511.2051	511.2051	0.03	1	11571.46	C29H40N2O8	(M+Na)+[-C4H8]
545.2859	545.2857	-0.32	1	203309.16	C29H40N2O8	(M+H)+
546.2887	546.289	0.63	1	66083.05	C29H40N2O8	(M+H)+
547.2913	547.2917	0.68	1	12965.54	C29H40N2O8	(M+H)+
549.2608	549.2571	-6.78	1	58.1	C29H40N2O8	(M+Na)+[-H2O]
567.2679	567.2677	-0.44	1	349545.22	C29H40N2O8	(M+Na)+
568.2712	568.271	-0.4	1	113176.71	C29H40N2O8	(M+Na)+
569.2734	569.2737	0.4	1	23671.89	C29H40N2O8	(M+Na)+
570.2772	570.2763	-1.45	1	3716.38	C29H40N2O8	(M+Na)+

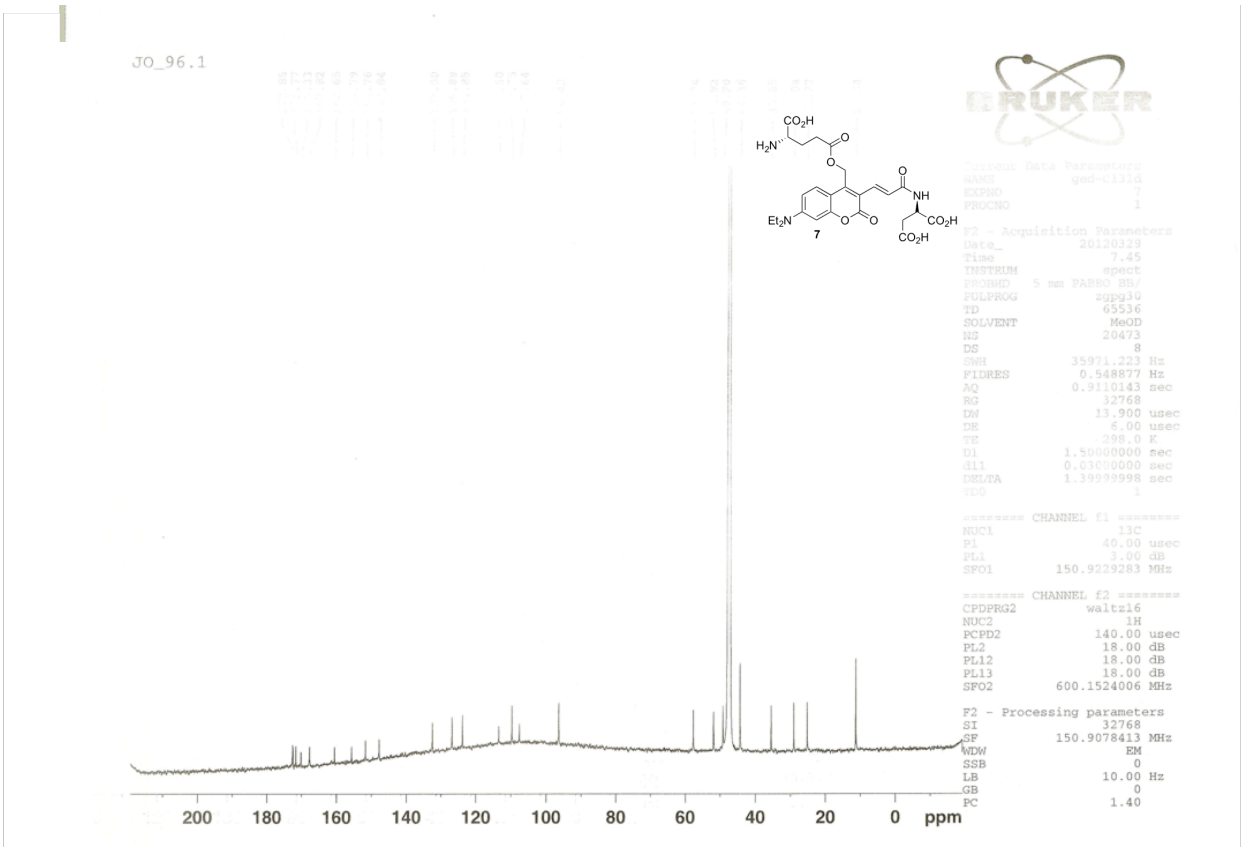

19 March 2012 JO 89.1
Archive directory: /export/home/calcium/vnmrsys/data
Sample directory:
File: PROTON
Pulse Sequence: s2pul
Solvent: CDCl3
Temp. 25.0 C / 298.1 K
Mercury-300 "GEDNMR2"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.700 sec
Width 4798.5 Hz
8 repetitions
OBSERVE H1, 299.9403373 MHz
DATA PROCESSING
FT size 65536
Total time 0 min, 40 sec
CO2t-Bu
NHBoc
O
O
O
NH
Et2N
O
O
CO2t-Bu
CO2t-Bu
8
7
6
5
4
3
2
1
-0
ppm
1.00
0.99
1.02
0.97
2.00
2.00
0.82
1.04
0.78
4.07
0.87
0.92
1.93
0.92
0.95
7.99
38.34

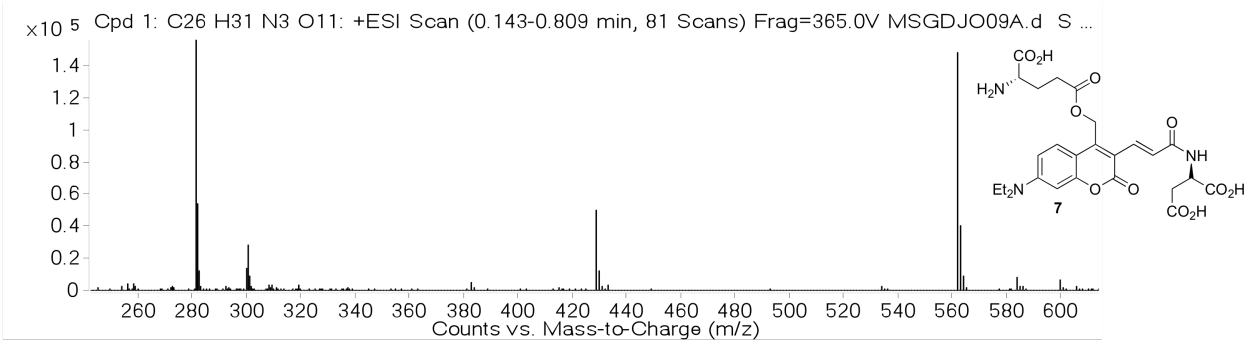




m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
774.3801	774.3808	0.81	1	3612.93	C43H63N3O13	(M+H)+[-C4H8]
791.4072	791.4073	0.12	1	54.12	C43H63N3O13	(M+NH4)+[-C4H8]
796.3621	796.3627	0.81	1	4861.2	C43H63N3O13	(M+Na)+[-C4H8]
830.4437	830.4434	-0.43	1	97661.36	C43H63N3O13	(M+H)+
831.4469	831.4466	-0.27	1	46513.77	C43H63N3O13	(M+H)+
832.4492	832.4495	0.29	1	13576.81	C43H63N3O13	(M+H)+
852.4259	852.4253	-0.67	1	392735.69	C43H63N3O13	(M+Na)+
853.4296	853.4286	-1.17	1	182556.56	C43H63N3O13	(M+Na)+
854.4315	854.4314	-0.05	1	53162.79	C43H63N3O13	(M+Na)+
855.4336	855.4341	0.59	1	11232.95	C43H63N3O13	(M+Na)+


JO_96.1
7
9
8
7
6
5
4
3
2
1
0
ppm
1.97
0.87
0.99
0.98
2.02
1.08
3.69
2.00
1.97
2.05
5.54
BRUKER
Current Data Parameters
NAME ged_id
EXPNO 7
PROCNO 1
F2 - Acquisition Parameters
Date_ 20120326
Time 13.22
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg
TD 16384
SOLVENT MeOD
NS 9
DS 4
SWH 10000.000 Hz
FIDRES 0.610352 Hz
AQ 0.8193000 sec
RG 128
DW 50.000 usec
DE 6.00 usec
TE 293.8 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





m/z	Calc m/z	Diff(ppm)	z	Abund	Formula	Ion
272.6001	272.5999	-0.72	2	2127.98	C26H31N3O11	(M+2H)+2[-H2O]
281.6059	281.6052	-2.34	2	156107.64	C26H31N3O11	(M+2H)+2
282.1076	282.1068	-2.69	2	53732.11	C26H31N3O11	(M+2H)+2
282.6085	282.608	-1.84	2	11992.53	C26H31N3O11	(M+2H)+2
303.5855	303.5872	5.55	2	191.8	C26H31N3O11	(M+2Na)+2
544.1919	544.1926	1.25	1	281.18	C26H31N3O11	(M+H)+[-H2O]
562.2033	562.2031	-0.31	1	148008.63	C26H31N3O11	(M+H)+
563.2063	563.2063	0.06	1	40599.27	C26H31N3O11	(M+H)+
564.2087	564.2088	0.16	1	9189.3	C26H31N3O11	(M+H)+
584.1842	584.1851	1.57	1	7657.34	C26H31N3O11	(M+Na)+