Supporting information for

A Nitroolefin Functionalized BODIPY Chemodosimeter for Biothiols Driven by an Unexpected Conjugated Addition Mechanism

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Contents:

1. Experimental Section

General: Reagents and solvents were used as received from commercial suppliers unless noted otherwise. All reactions were performed in oven-dried or flame-dried glassware unless otherwise stated, and were monitored by TLC using 0.25 mm silica gel plates with UV indicator (60F-254). ^{1}H and ^{13}C NMR are obtained on a 300 MHz NMR spectrometer at room temperature. Chemical shifts (δ) are given in ppm relative to $CDCl_3$ (7.26 ppm for ^{1}H and 77 ppm for ^{13}C) or to internal TMS (0 ppm for ^{1}H). High-resolution mass spectra were obtained using APCI-TOF in positive mode.

UV-visible absorption spectra and Fluorescence emission spectra were recorded on a commercial spectrophotometer (190-1100 nm scan range). Relative fluorescence quantum efficiencies of BODIPY derivatives were obtained by comparing the areas under the corrected emission spectrum of the test sample in various solvents with fluorescein (λ_{ex} = 480 nm) in 0.1 M NaOH aqueous solution (Φ = 0.90) as the standard or Cresy Violet perchlorate (λ_{ex} = 540 nm) in methanol as the standard (Φ = 0.54).[1] Non-degassed, spectroscopic grade solvents and a 10 mm quartz cuvette were used. Dilute solutions (0.01<A<0.05) were used to minimize the reabsorption effects. Quantum yields were determined using the following equation:[2]

$$\Phi_X = \Phi_S (I_X/I_S) (A_S/A_X) (\eta_X/\eta_S)^2$$

Where Φ_S stands for the reported quantum yield of the standard, I stands for the integrated emission spectra, A stands for the absorbance at the excitation wavelength and η stands for the refractive index of the solvent being used (η = 1 when the same solvent was used for both the test sample and the standard). X subscript stands for the test sample, and S subscript stands for the standard.

Crystals of BODIPY **4** suitable for X-ray analysis were obtained by slow evaporation of their dichloromethane solutions. The vial containing this solution was placed, loosely capped, to promote the crystallization. The structure was solved by the direct method using the SHELXS-974 program and refined by the least-squares method on F^2, SHELXL-97,[3] incorporated in SHELXTL V5.10.[4]

Procedures of thiols sensing: Deionized water was used throughout all experiments. A stock solution of **1** (1 mM) was prepared in CH_3CN. The stock solution of **1** was then diluted to the corresponding concentration with the solution of CH_3CN/HEPES (1:1, v/v, 0.1 M, pH 7.4). Spectra data were recorded in an indicated time after the addition of amino acids.

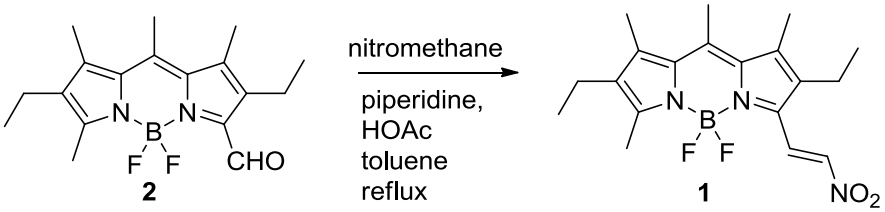
Fluorescence Imaging: Human gastric cancer SGC7901 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), streptomycin sulfate (100 μg/ml), and maintained at 37°C with

5% CO_2 in a humidified incubator. One day before imaging, cells were seeded in 6-well flat-bottomed plates in an atmosphere of 5% CO_2, 95% air at 37 ℃. Fluorescence imaging of intracellular cysteine was observed under OLYMPUS-IX71 inverted fluorescence microscope and imaged using FITC channel or TRITC channel. The microscope settings (brightness, contrast, and exposure time) were held constant before and after pretreatment of cells with N-ethylmaleimide to compare the relative intensity of intracellular cysteine fluorescence.

The cells were treated with 5 mM Cys in culture media for 30 min at 37 ℃ with 5% CO_2 in a humidified incubator. After washing with phosphate buffered saline (PBS) to remove the remaining NEM, the cells were further incubated with 10 μM of **1** in culture media for 30 min at 37 ℃ with 5% CO_2 in a humidified incubator. For the control experiment, the cells were treated with 20 mM N-ethylmaleimide (NEM) in culture media for 30 min at 37 ℃ with 5% CO_2 in a humidified incubator. After washing with PBS to remove the remaining NEM, the cells were further incubated with 10 μM of **1** in culture media for 30 min at 37 ℃ with 5% CO_2 in a humidified incubator. Fluorescence imaging was then carried out after washing the cells with the PBS.

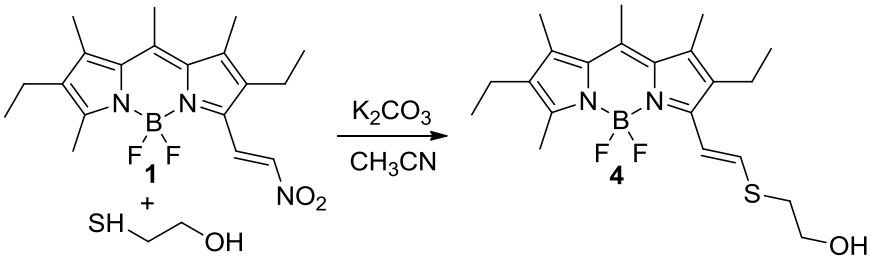
Synthesis of BODIPYs

3-formylBODIPY **2** was synthesized according to the literature.[5]



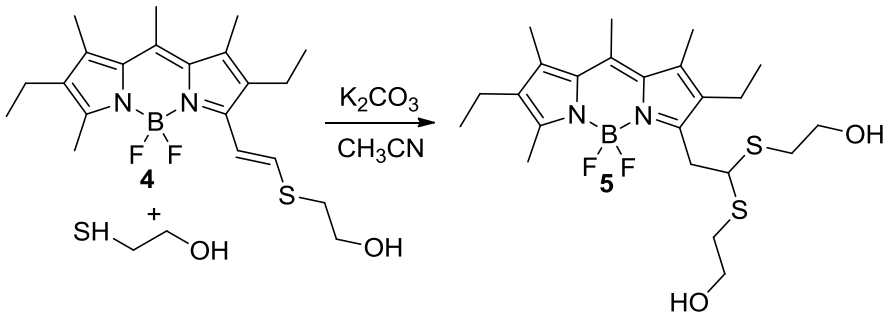
BODIPY 1: To a mixture of 3-formylBODIPY **2** (166 mg, 0.5 mmol) and nitromethane (122 mg 2.0 mmol) in toluene (40 mL) were added one drop of piperidine and a catalytic amount of glacial acetic acid. The mixture was heated to reflux for 2 h in a Sochlet's apparatas under argon, extracted with dichloromethane (3 × 50 mL). Organic layers were combined and washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. The crude product was purified using column chromatography on silica gel (hexane/dichloromethane = 2/1, v/v) to give **1** as a purple powder in 51% isolated yield (96 mg). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (d, J = 15.0 Hz, 1H), 7.78 (d, J = 15.0 Hz, 1H), 2.68 (s, 3H), 2.61 (m, 5H), 2.44 (m, 2H), 2.39 (s, 3H), 2.36 (s, 3H), 1.14 (t, J = 6.0 Hz, 3H), 1.08 (t, J = 9.0 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.2, 141.6, 140.5, 137.3, 136.4, 135.3, 134.7, 132.8, 127.9, 18.2, 17.7, 17.2, 14.9, 14.7, 14.4, 13.8, 13.4. HRMS(APCI) calcd. for $C_{19}H_{25}BF_2N_3O_2$ $[M+H]^+$: 376.2002, found 376.2003; HRMS(APCI) calcd. for $C_{19}H_{24}BFN_3O_2$ $[M-F]^+$: 356.194, found 356.1941.

BODIPY 4:



To a mixture of **1** (75 mg, 0.2 mmol) and β-Mercaptoethanol (32 mg 0.4 mmol) in acetonitrile (10 mL) was added a catalytic amount of K_2CO_3. The reaction mixture was stirred at room temperature for 3 min and then extracted with dichloromethane (3 × 30 mL). Organic layers were combined and washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. The crude product was purified using column chromatography on silica gel (hexane/ethylacetate = 3/2, v/v) to give **2a** as a purple powder in 98% isolated yield (80 mg). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (d, *J* = 18.0 Hz, 1H), 6.91 (d, *J* = 15.0 Hz, 1H), 3.92 (m, 2H), 3.10 (m, 2H), 2.62 (s, 3H), 2.54 (m, 2H), 2.50 (s, 3H), 2.39 (m, 2H), 2.34 (s, 6H), 1.60 (brs, 1H), 1.12 (t, *J* = 7.5 Hz, 3H), 1.05 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.3, 146.3, 139.1, 136.9, 136.4, 133.3, 132.7, 132.3, 131.9, 131.6, 117.9, 61.0, 35.2, 18.0, 17.2, 17.1, 14.8, 14.5(4),14.5, 14.1, 12.6. HRMS(APCI) calcd. for $C_{21}H_{30}BF_2N_2OS$ $[M+H]^+$: 407.2126, found 407.2135; HRMS(APCI) calcd. for $C_{21}H_{29}BFN_2OS$ $[M-F]^+$: 387.2072, found 387.2067.

BODIPY 5:



To a mixture of **4** (41 mg, 0.1 mmol) and β-Mercaptoethanol (32 mg 0.4 mmol) in acetonitrile (10 mL) was added a catalytic amount of K_2CO_3. The reaction mixture was stirred at room temperature for 3 hour and then extracted with dichloromethane (3 × 30 mL). Organic layers were combined and washed with water, dried over anhydrous Na_2SO_4, filtered and evaporated under vacuum. The crude product was purified using column chromatography on silica gel (Hexane/Ethylacetate = 1/1, v/v) to give **3** as an orange powder in 87% isolated yield (42 mg). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.71 (t, *J* = 6.0 Hz, 1H), 3.76-3.78 (m, 4H), 3.37 (d, *J* = 6.0 Hz, 2H), 2.88-2.95 (m, 4H), 2.64 (s, 3H), 2.49-2.54 (m, 5H), 2.35-2.42 (m, 8H), 1.62 (brs, 2H), 1.02-1.10 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.2, 148.2, 140.4, 137.7, 135.5, 133.4, 133.1, 60.8, 49.9, 34.9, 34.0, 17.3, 17.0, 16.8, 14.7, 14.5, 14.4, 14.2, 12.4.

HRMS(APCI) calcd. for $C_{23}H_{36}BF_2N_2O_2S_2$ $[M+H]^+$: 485.2274, found 485.2273; HRMS(APCI) calcd. for $C_{23}H_{35}BFN_2O_2S_2$ $[M-F]^+$: 465.2212, found 465.2208.

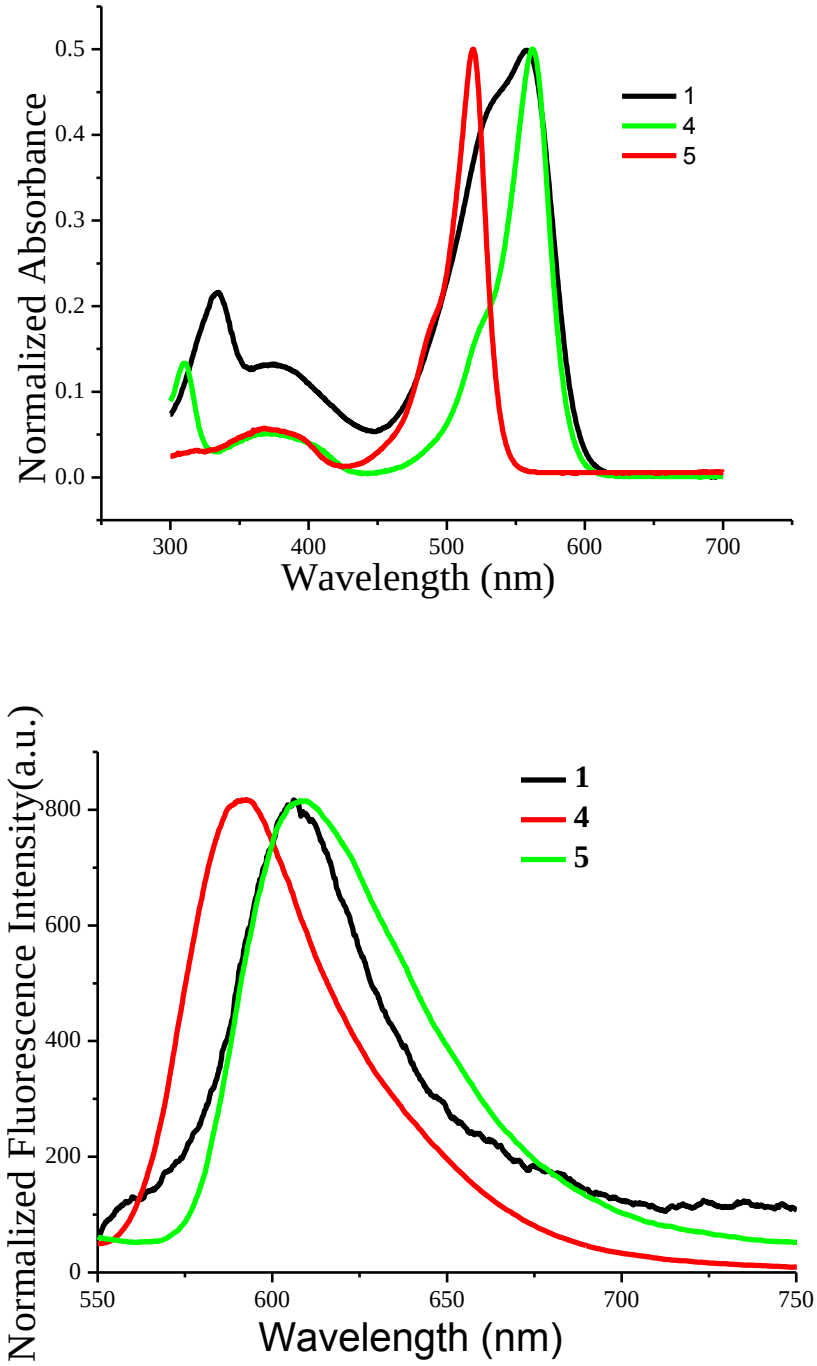


Figure S1: Normalized absorption (top) and emission spectra (botton) of BODIPY **1**, **4** and **5** recorded in CH_3CN.

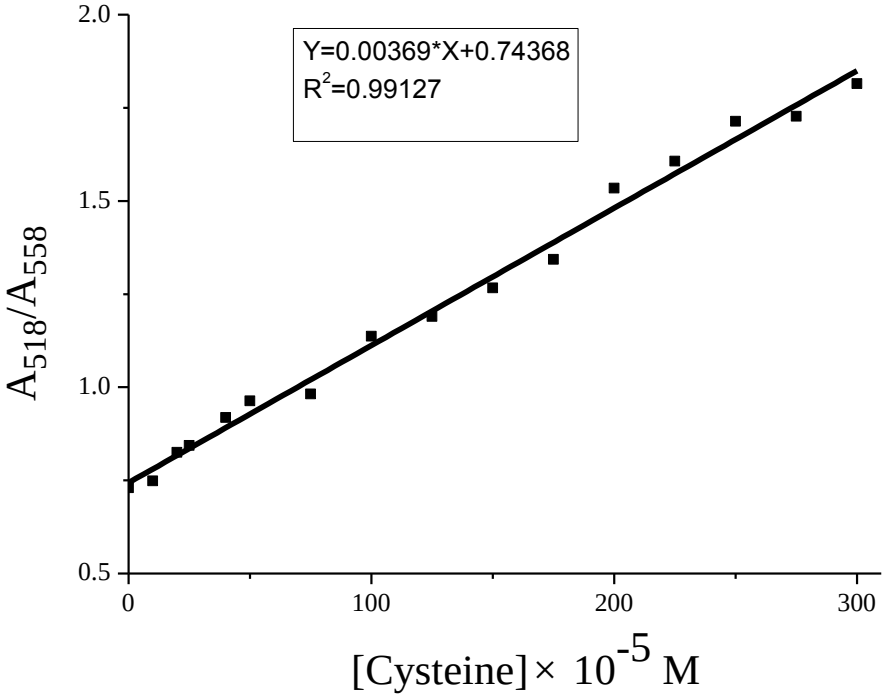


Figure S2: Plot of the absorption intensity ratios at 518 nm and 558 nm of **1** (10 μM) upon addition of Cys (0-3000 μM).

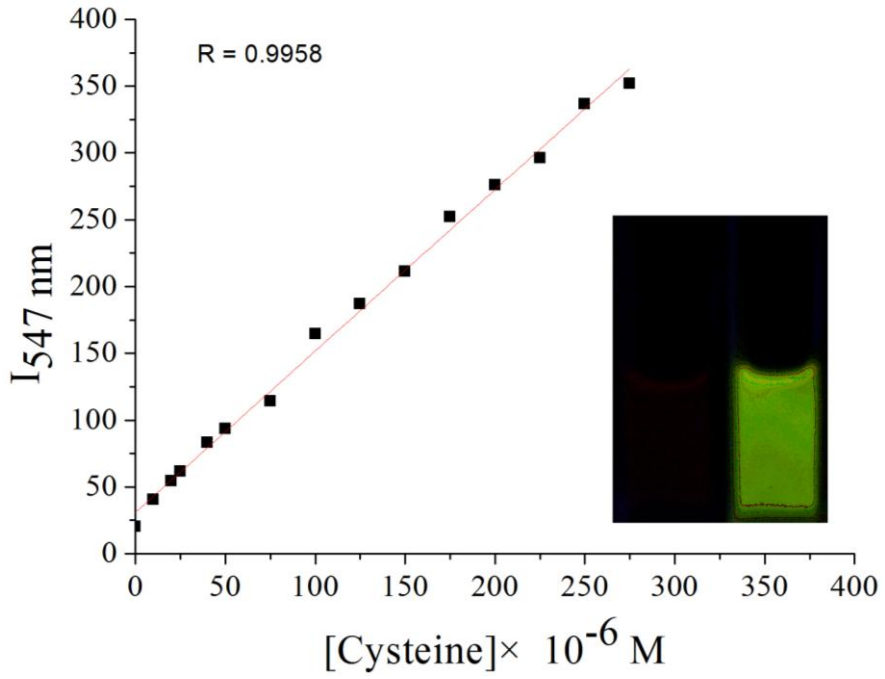


Figure S3: Plot of the fluorescence intensity at 547 nm of **1** (1 μM) upon addition of Cys (0-300 μM). Inset: Color change of **1** with the addition of 200 equiv of Cys (right) under UV-irradiation condition (365 nm).

Detection limit: The detection limit was calculated based on the fluorescence titration. **1** was employed at 1 μM and the slits were adjusted to 5.0 nm. To determine the *S/N* ratio, the emission intensity of **1** without Cys was measured by 10 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the Cys concentration could be obtained in the 0-250 μM (R = 0.9981), as shown in Figure S4. The detection limit is then calculated with the equation: detection limit = $3\sigma_{bi}/m$, where σ_{bi} is the standard deviation of blank measurements, m is the slope between intensity versus sample concentration. The detection limit was measured to be 8.77×10^{-7} M at S/N = 3 (signal-to-noise ratio of 3:1).

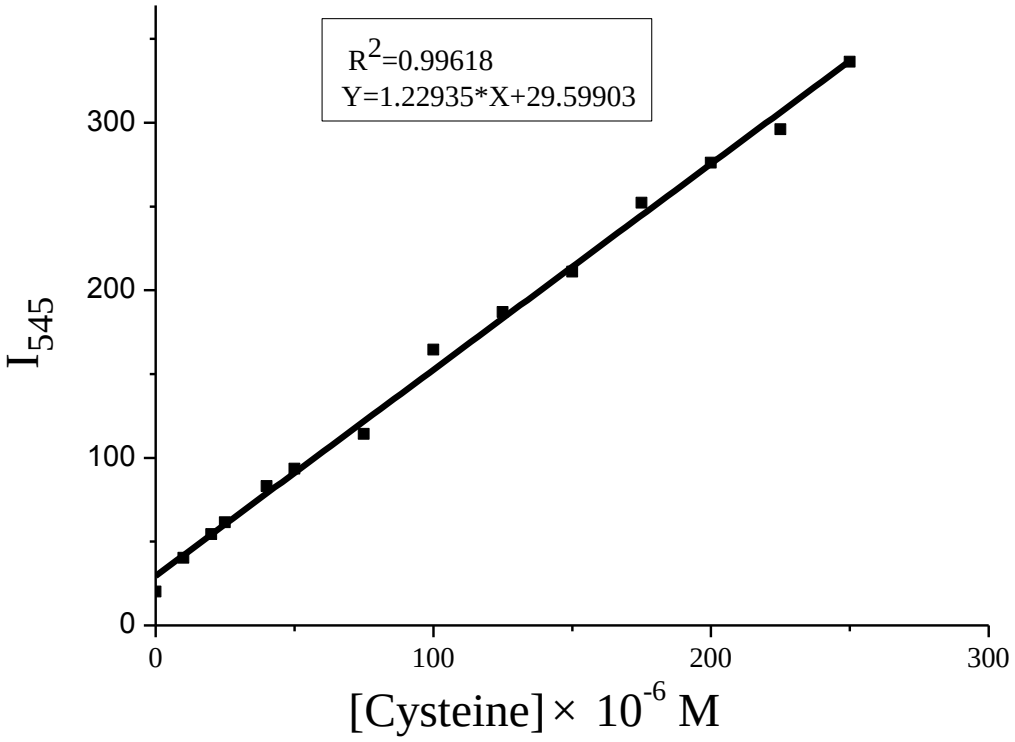


Figure S4: Fluorescence intensity at 545 nm of **1** (1 μM) upon addition of Cys (0-250 μM).

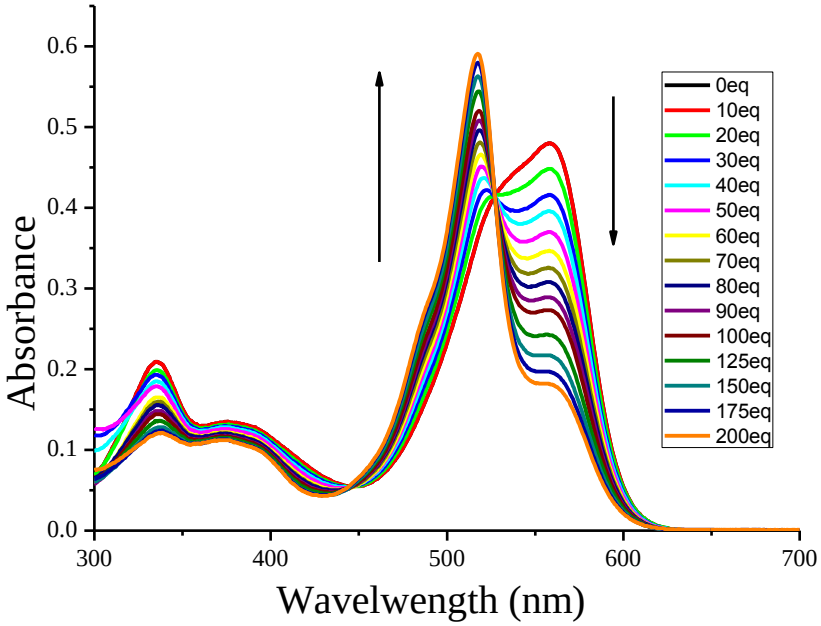


Figure S5: Absorption spectral changes of **1** (10 μM) in CH_3CN-HEPES buffer (0.1 M, 1:1, v/v, pH = 7.4) upon addition of HCy (0–200 eq). Each spectrum was acquired 30 min after HCy addition.

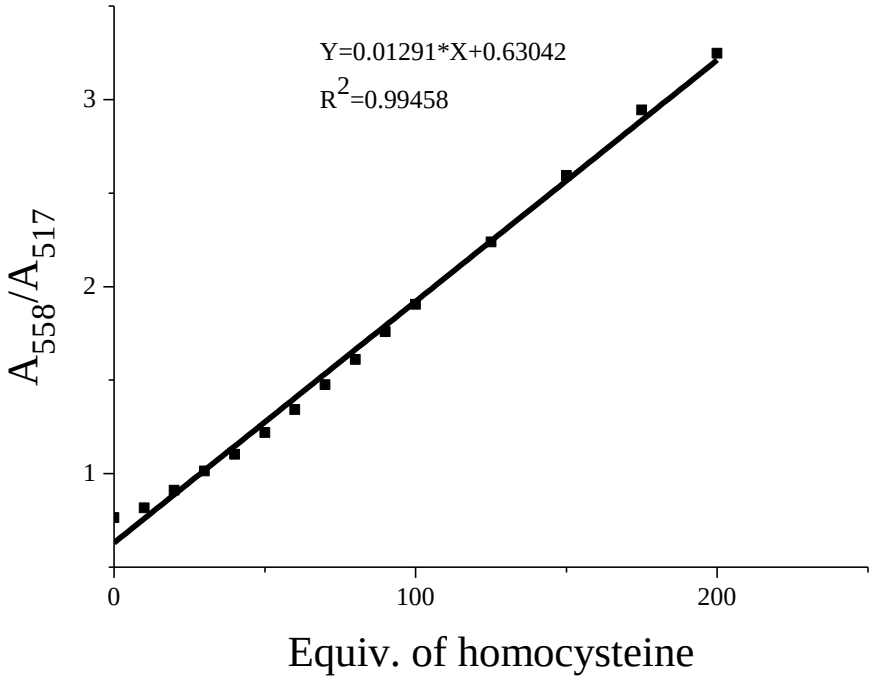


Figure S6: Plot of the absorption intensity ratios at 517 nm and 558 nm of **1** (10 μM) upon addition of Hcy (0-2000 μM).

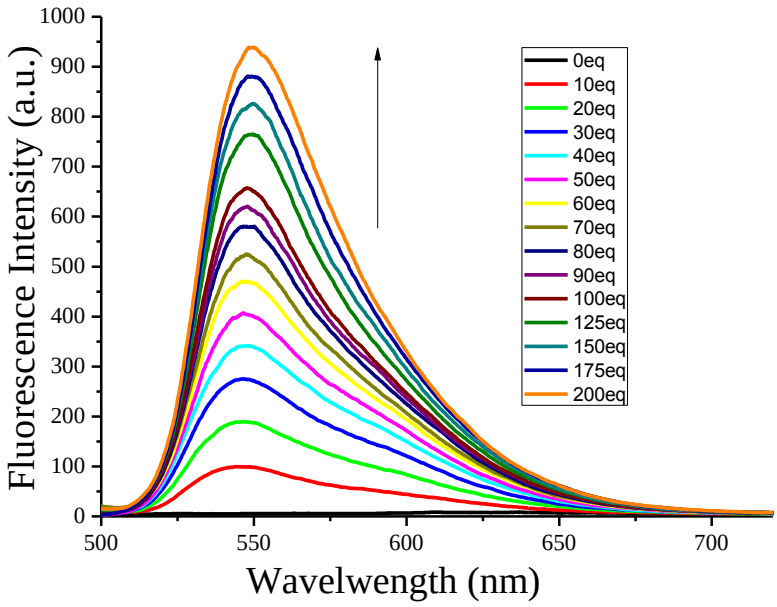


Figure S7： Fluorescence spectral change of 1 (1 μM) in CH_3CN-HEPES buffer (0.1 M, 1:1, v/v, pH = 7.4) upon addition of Hcy(0–200eq). Each spectrum is acquired 30 min after Hcy addition.

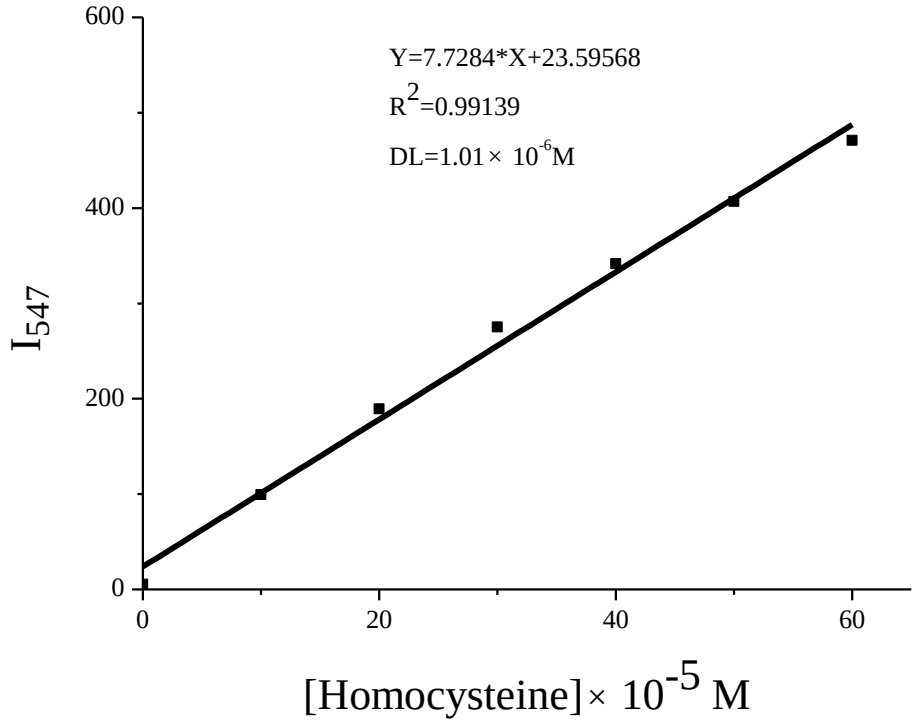


Figure S8: Plot of the fluorescence intensity at 547 nm of **1** (1 μM) in CH_3CN-HEPES buffer (0.1 M, 1:1, v/v, pH = 7.4) upon addition of Hcy (0-60 μM).

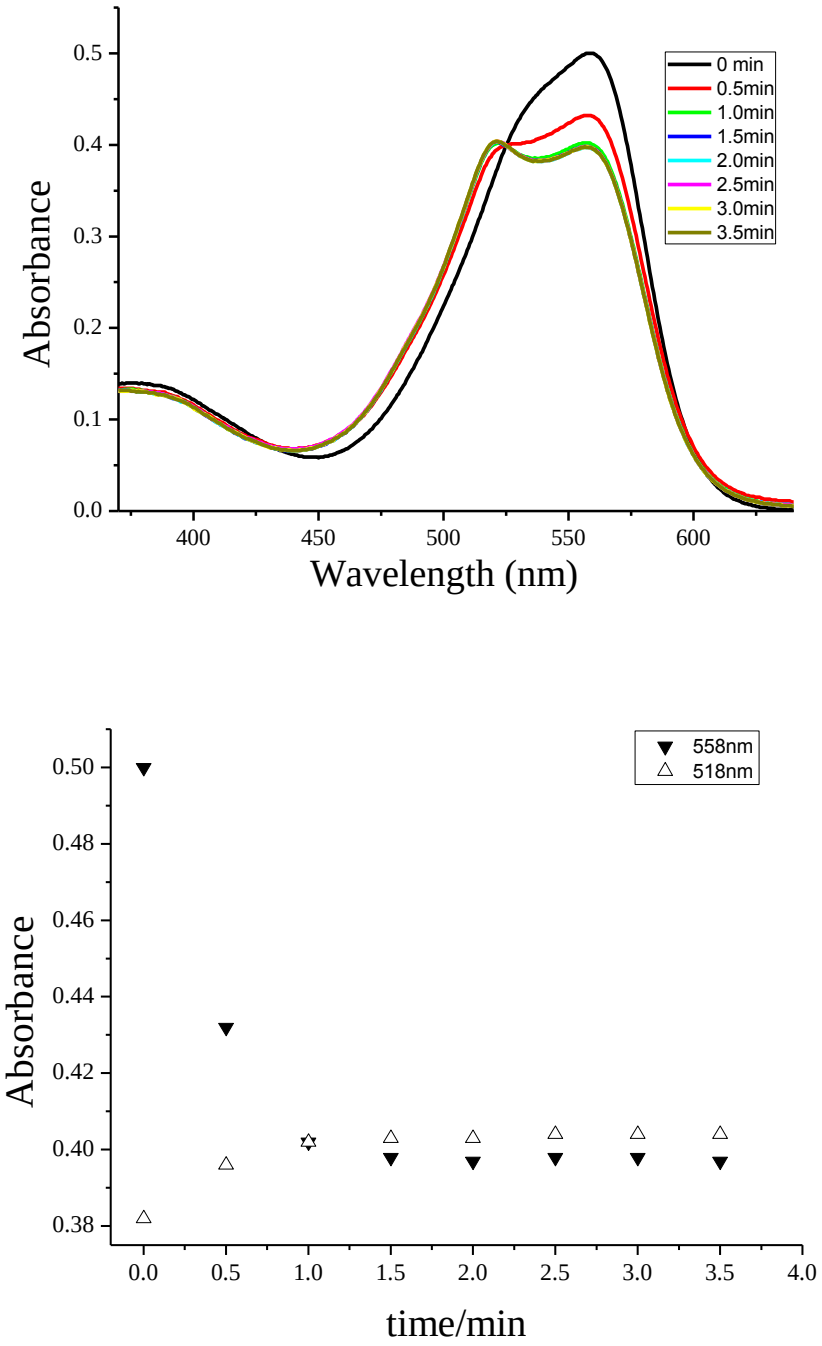


Figure S9: Time-dependent of absorption spectral changes (top) and time-dependent of absorption intensity changes at 518 nm and 558 nm (bottom) of probe **1** (10 μM) in the absence or presence of Cys (100 μM) in CH_3CN-HEPES buffer (0.1 M, 1:1, v/v, pH = 7.4).

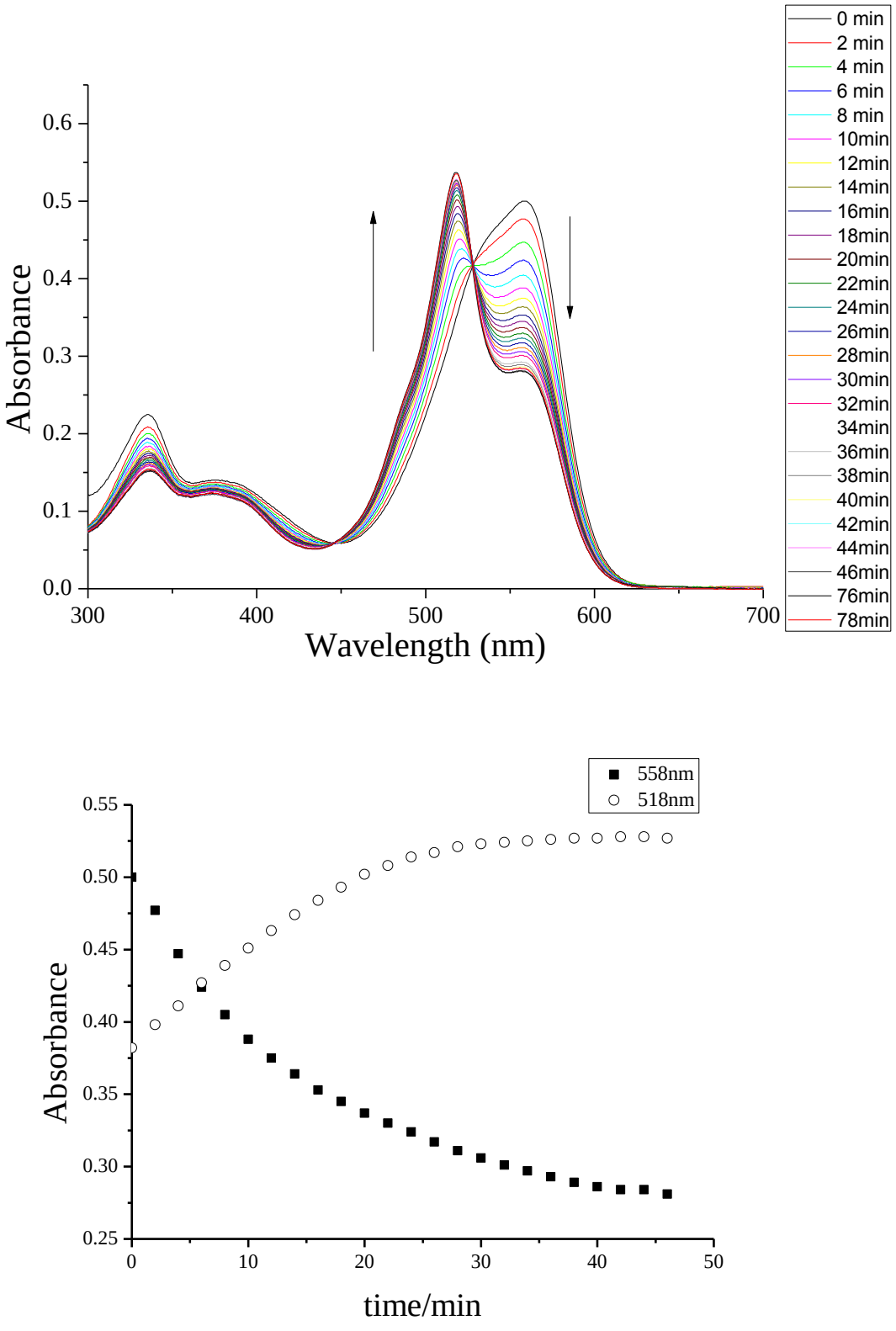


Figure S10: Time-dependent of absorption spectral changes (top) and time-dependent of absorption intensity changes at 518 nm and 558 nm (bottom) of probe **1** (10 μM) in the absence or presence of Hcy (100 μM) in CH_3CN-HEPES buffer (0.1 M, 1:1, v/v, pH = 7.4).

Table S1. Photophysical properties of BODIPYs **1** in different solvents.

solvents	λ_{abs} (nm), log ε	λ_{em} (nm)	Φ^a	stokes-shift (cm^{-1})
n-hexane	558, 4.36	587	0.54	885
cyclohexane	561, 4.47	591	0.44	937
toluene	566, 4.67	598	0.37	945
tetrahydrofuran	562, 4.64	598	0.26	1072
ethyl acetate	558, 5.67	593	0.25	987
dichloromethane	565, 4.65	609	0.17	1279
acetonitrile	558, 4.69	606	0.03	1419
methanol	559, 4.61	616	0.01	1655

[a]the fluorescence quantum yields Φ were calculated using cresyl violet perchlorate (λ_{ex} = 540 nm) in methanol as the standard (Φ = 0.54).

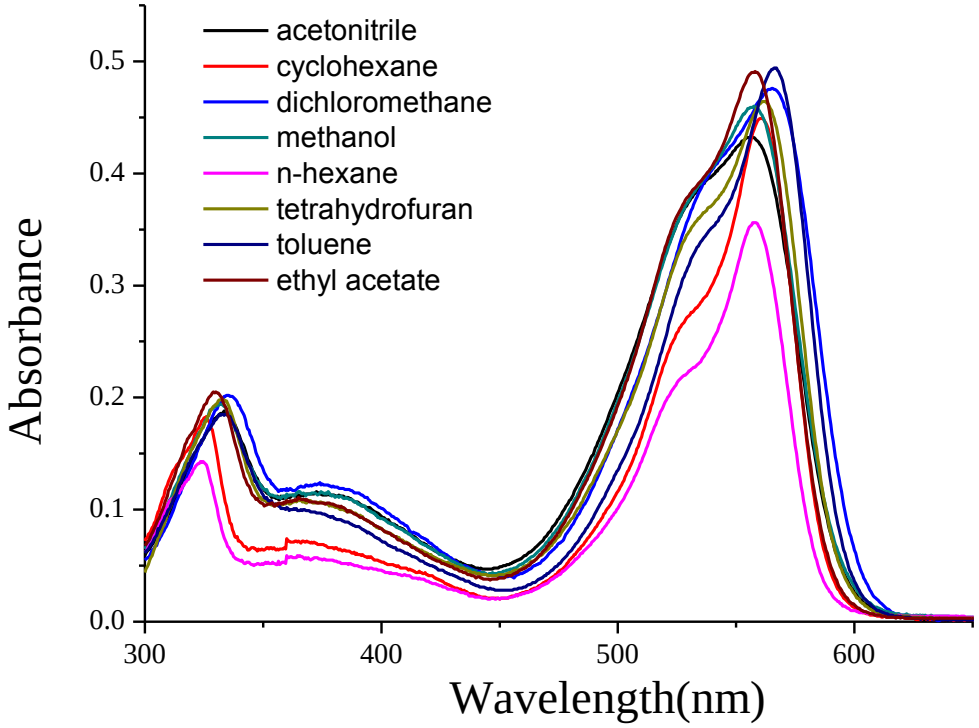


Figure S11: Absorption absorptions of probe **1** in different solvents.

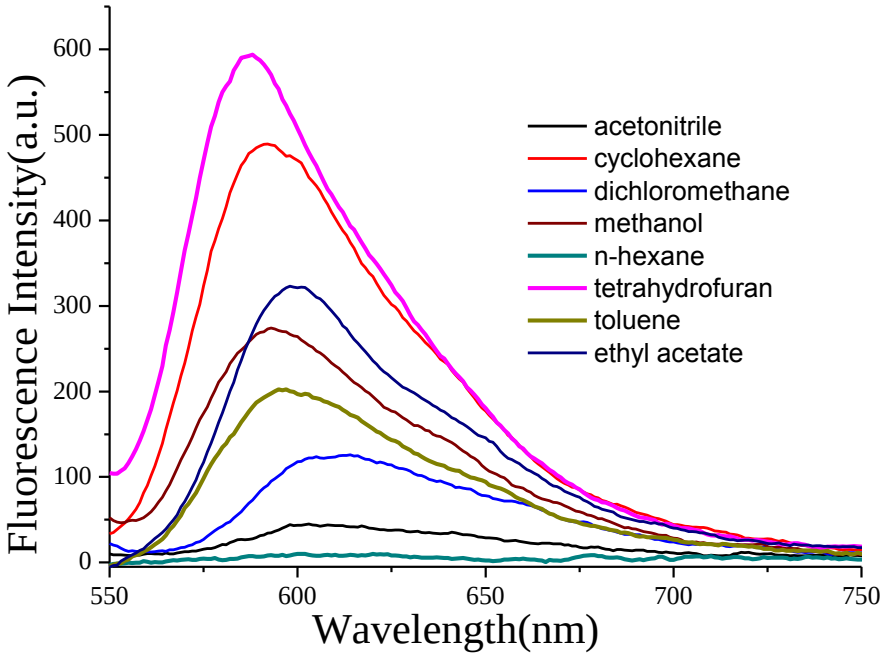


Figure S12: Fluorescence emissions of probe **1** in different solvents (λ_{ex} = 540 nm).

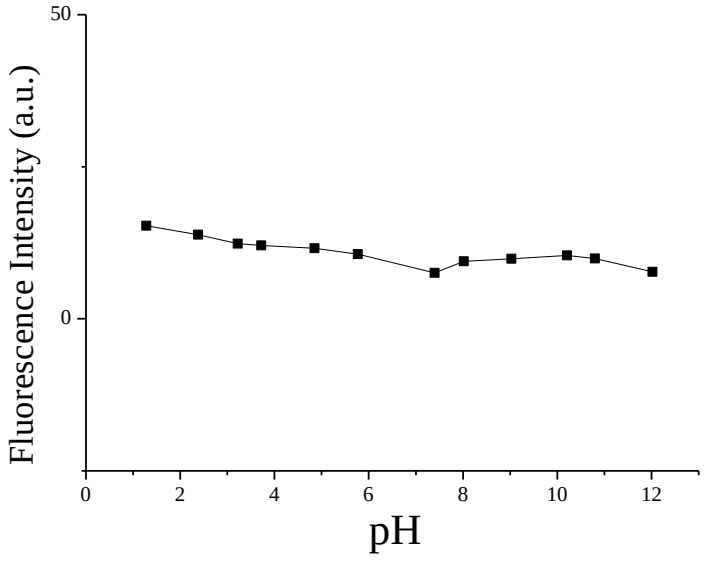


Figure S13: Influence of pH on fluorescence intensity for probe **1** (10 μM) at 606 nm in CH_3CN-HEPES buffer (1:1, v/v, pH = 7.4). (λ_{ex} = 490 nm).

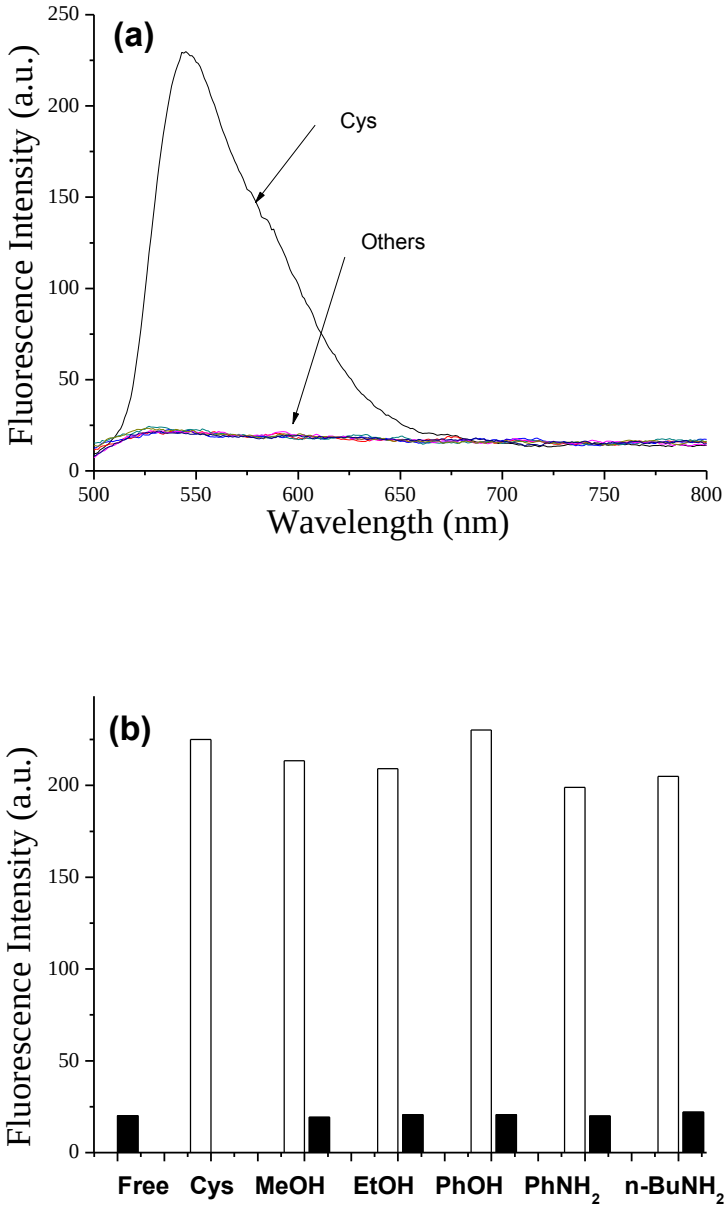
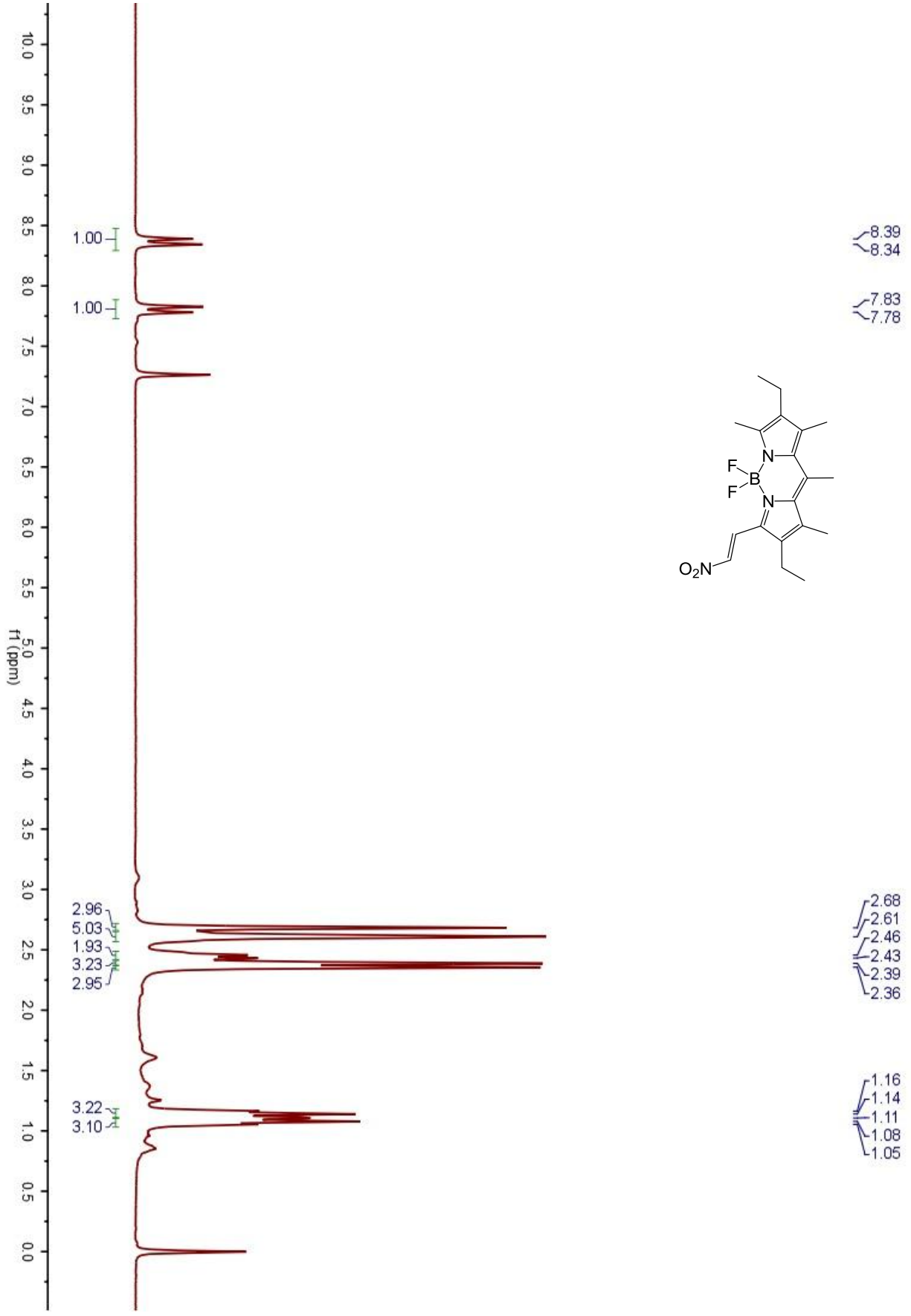
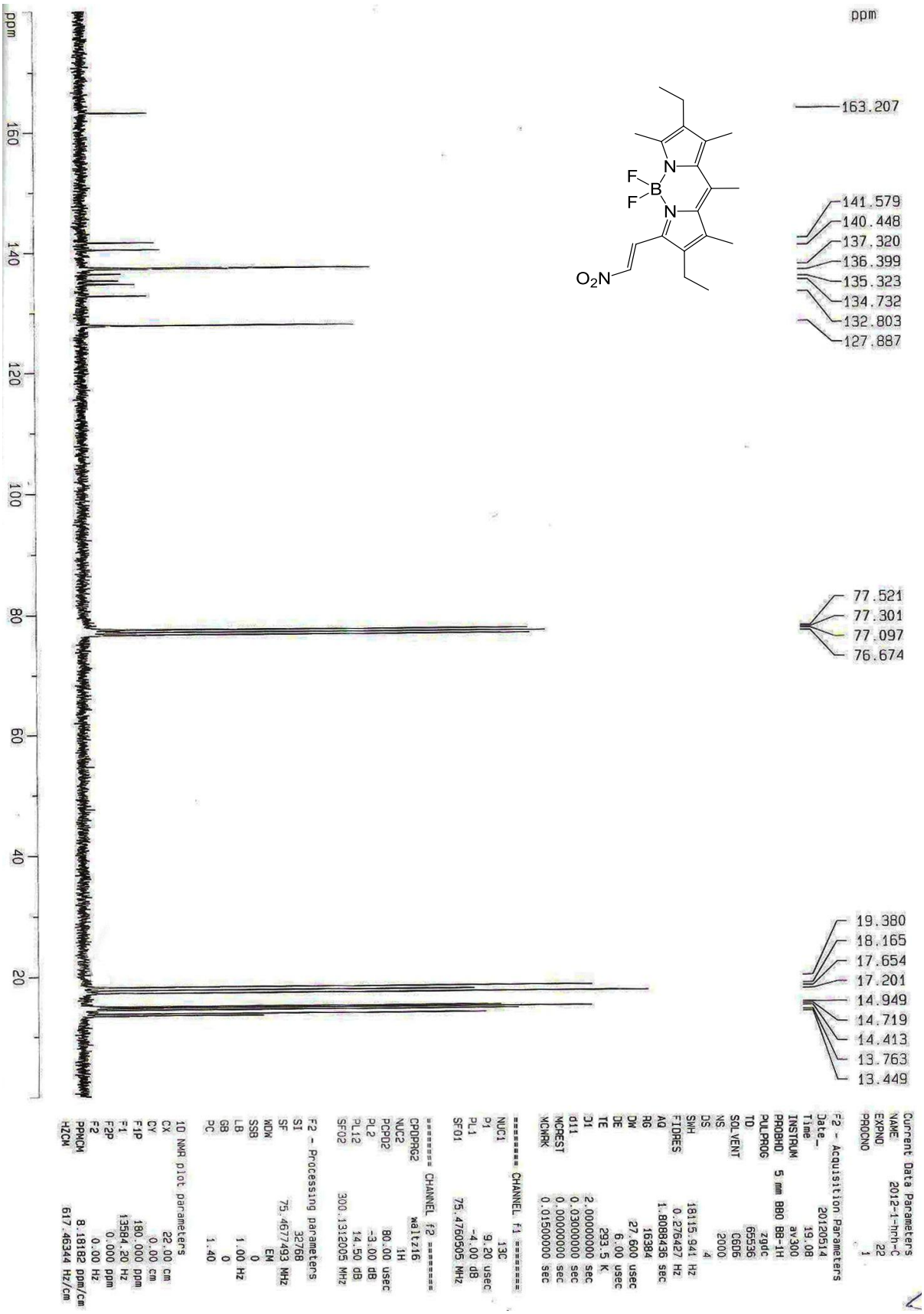


Figure S14: (a) Fluorescence spectral changes of **1** (1 μM) with or without 200 equiv of Cys or 400 equiv of various other compounds (MeOH, EtOH, PhOH, $PhNH_2$ and n-$BuNH_2$) in CH_3CN-HEPES buffer (0.1 M, 1:1, v/v, pH = 7.4). (B) Fluorescence responses of **1** (1 μM) to various amino acids and their competition graph with Cys at 547 nm. Black bar represent the addition of 200 equiv of Cys or 400 equiv of various other compounds. White bars represent the addition of 200 equiv of Cys to the above solutions. Each spectrum was recorded after 2 min after addition.

Copies of ^{1}H NMR, ^{13}C NMR and HRMS for all new compounds

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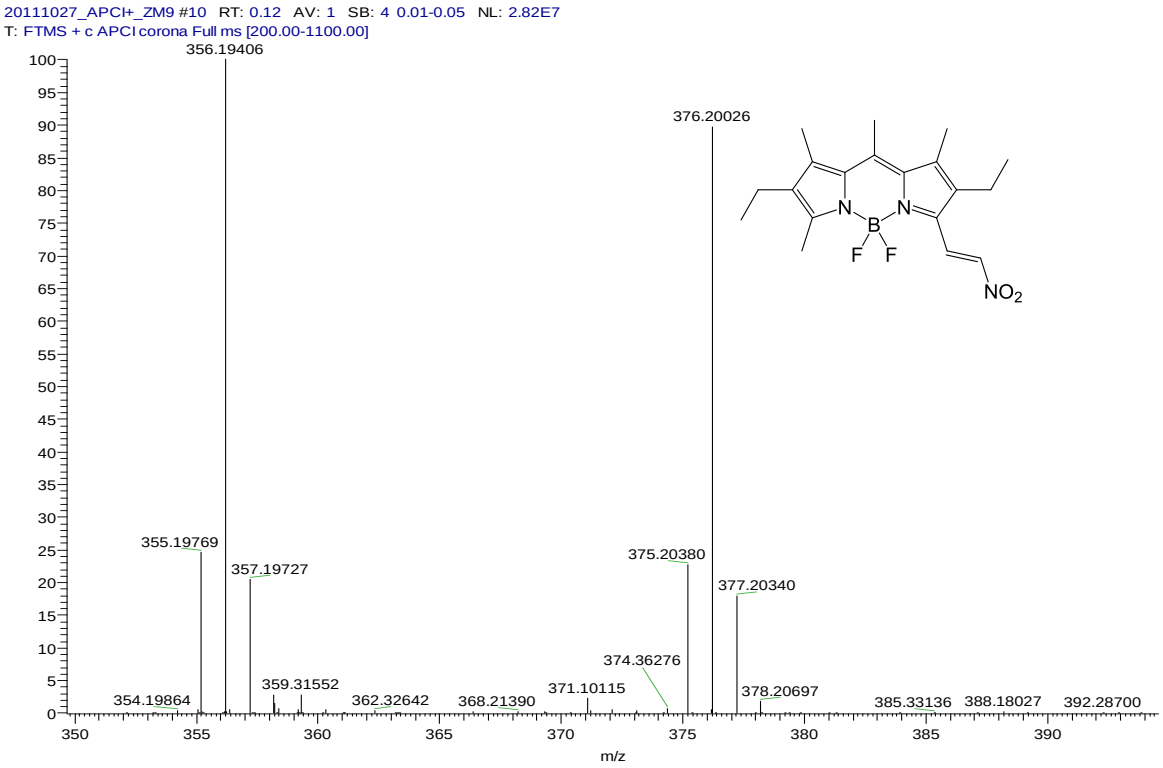
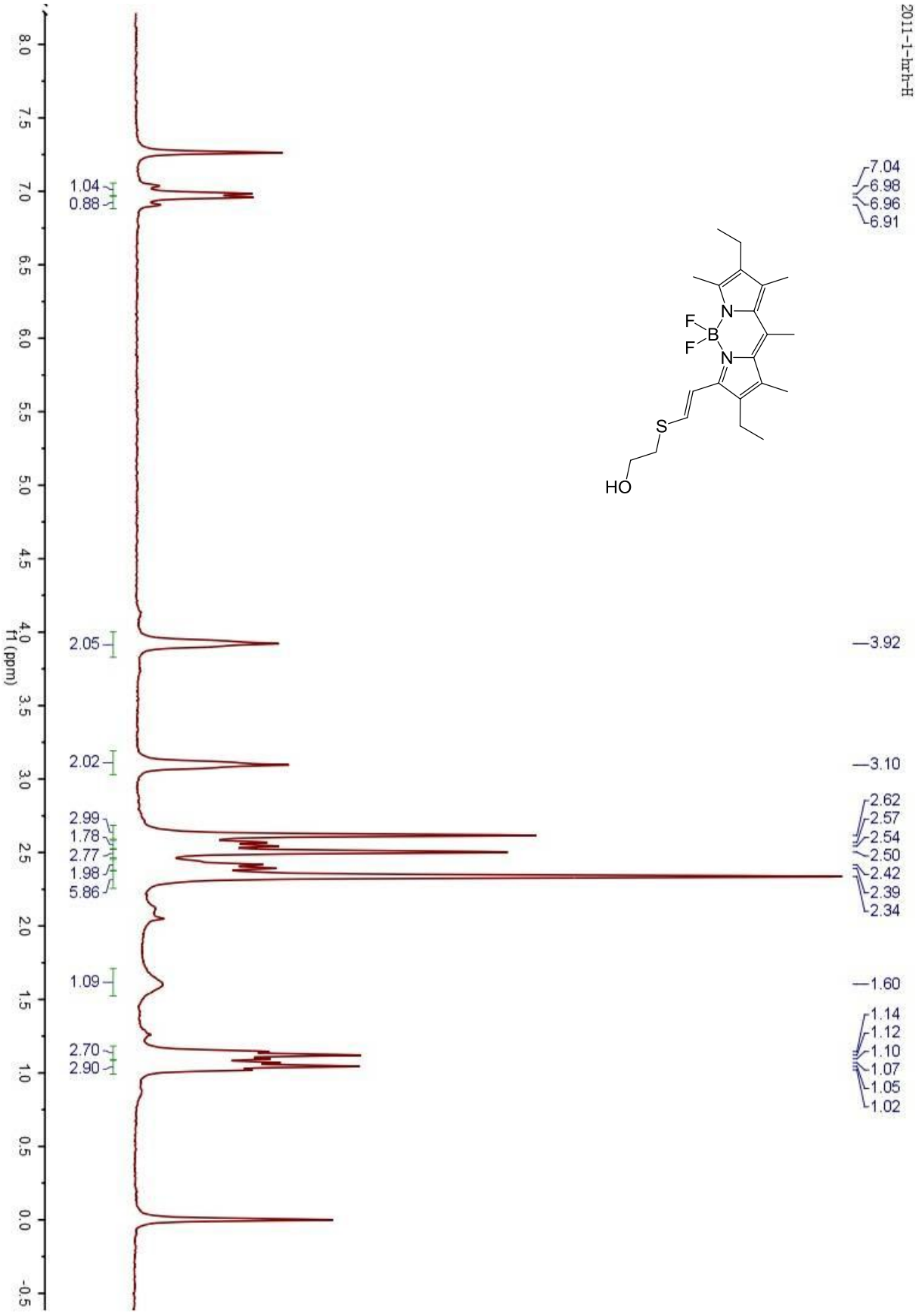


m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
355.07	146842.1	0.52	355.0685	1.48	C20 H9 O3 N2 B F
355.1977	6941392	24.59	355.1988	-1.07	C20 H25 O2 N2 B F
356.1866	212192.8	0.75	356.1866	-0.05	C20 H23 O N2 B F2
356.1941	28229312	100	356.194	0.05	C19 H24 O2 N3 B F
357.1902	171100.5	0.61	357.1906	-0.39	C20 H25 O3 N B F
357.1973	5779760	20.47	357.1984	-1.16	C18 H27 O3 N2 F2
358.1896	795770.8	2.82	358.1897	-0.07	C19 H23 O N3 B F2
358.2009	426433.1	1.51	358.2023	-1.37	C20 H25 O N2 B F2
359.1938	150239.1	0.53	359.1937	0.08	C19 H25 O3 N2 B F
359.3155	800522.6	2.84	359.3166	-1.03	C20 H40 O N B F2
360.3188	151830.3	0.54	360.3185	0.34	C20 H40 N3 F2
371.1012	653812.9	2.32	371.1076	-6.45	C19 H15 O3 N3 F2
372.101	167429.1	0.59	372.095	5.93	C20 H12 O3 N3 B F
374.3628	213761.2	0.76	374.3549	7.91	C19 H45 O3 N3 B
375.2038	6423093	22.75	375.205	-1.2	C20 H26 O2 N2 B F2
376.2003	25316014	89.68	376.2002	0.02	C19 H25 O2 N3 B F2
376.2115	157483.6	0.56	376.2128	-1.36	C20 H27 O2 N2 B F2
377.2034	5048377	17.88	377.2081	-4.67	C19 H26 O2 N3 B F2
378.207	497592.2	1.76	378.2047	2.31	C20 H27 O3 N B F2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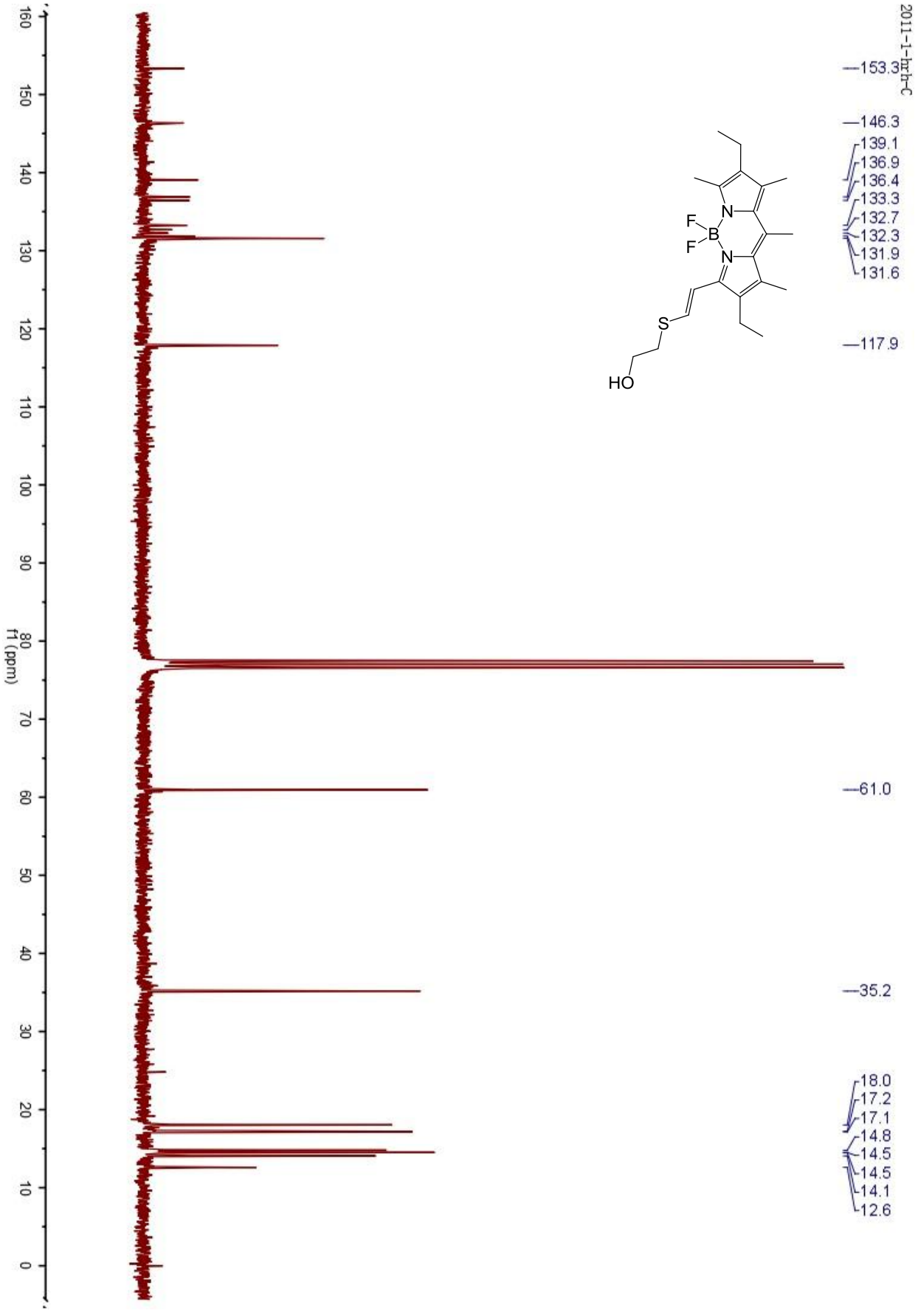
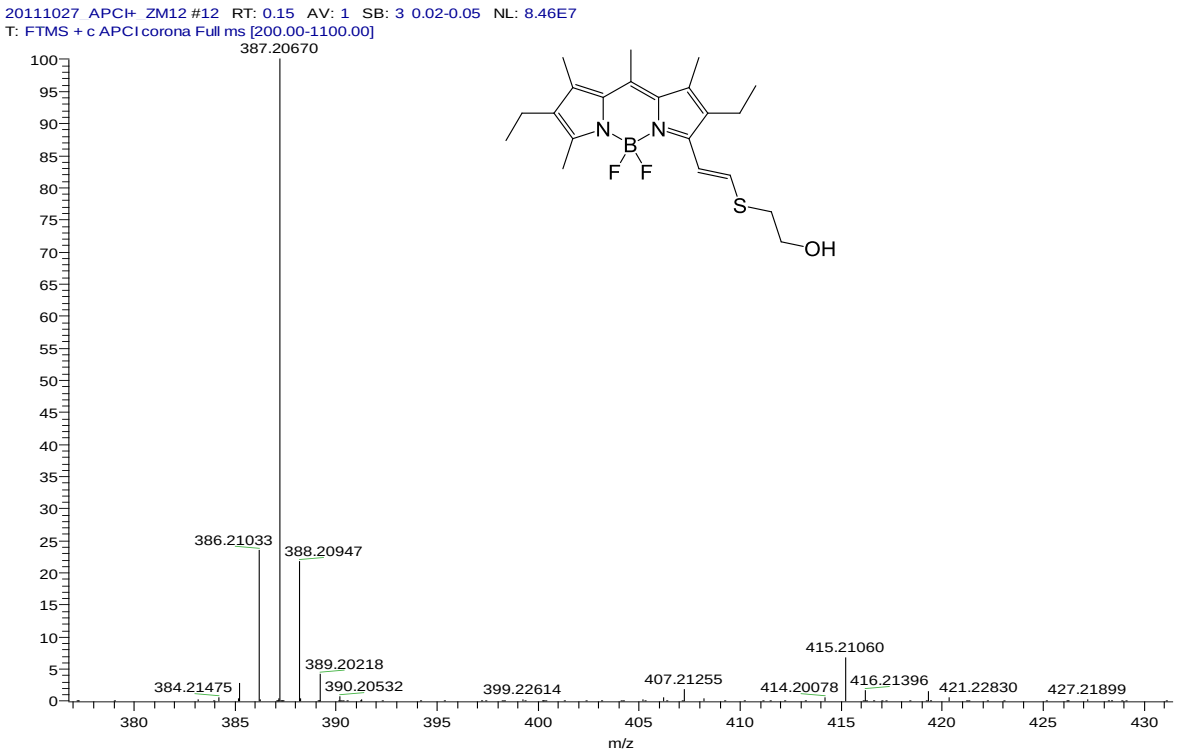
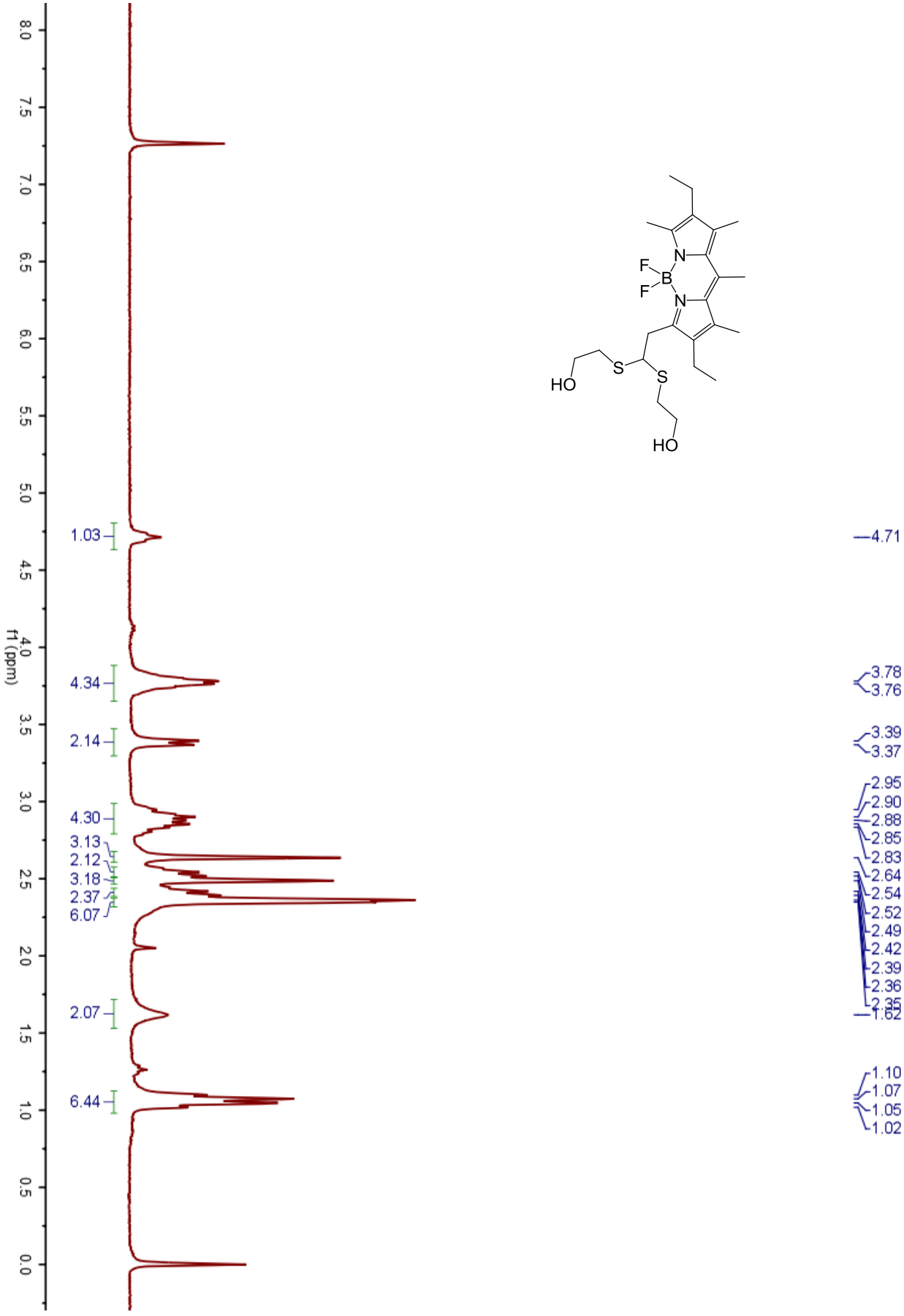
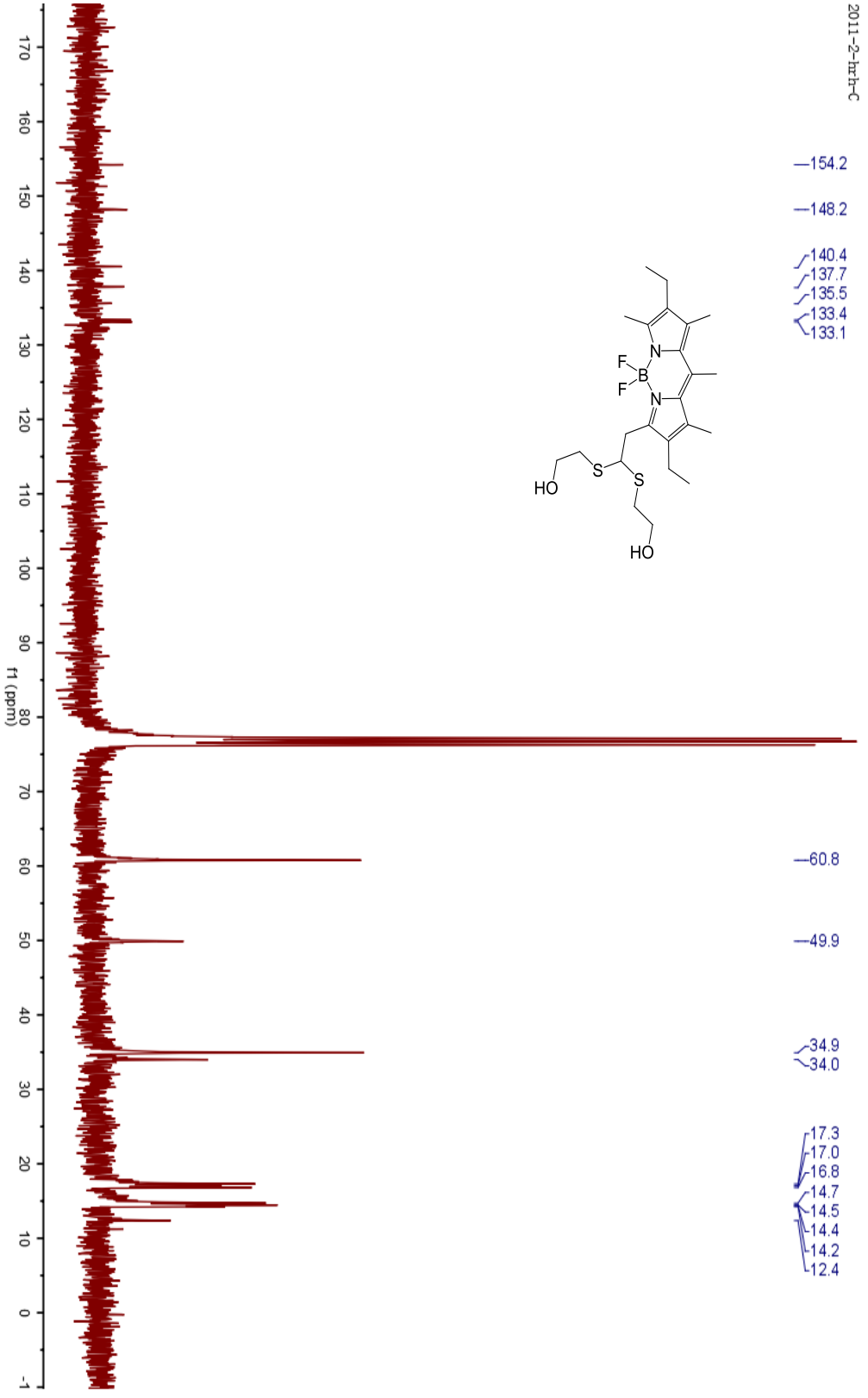
2.62
2.57
2.54
2.50
2.42
2.39
2.34
1.60
1.14
1.12
1.10
1.07
1.05
1.02
F
B
F
N
N
S
HO

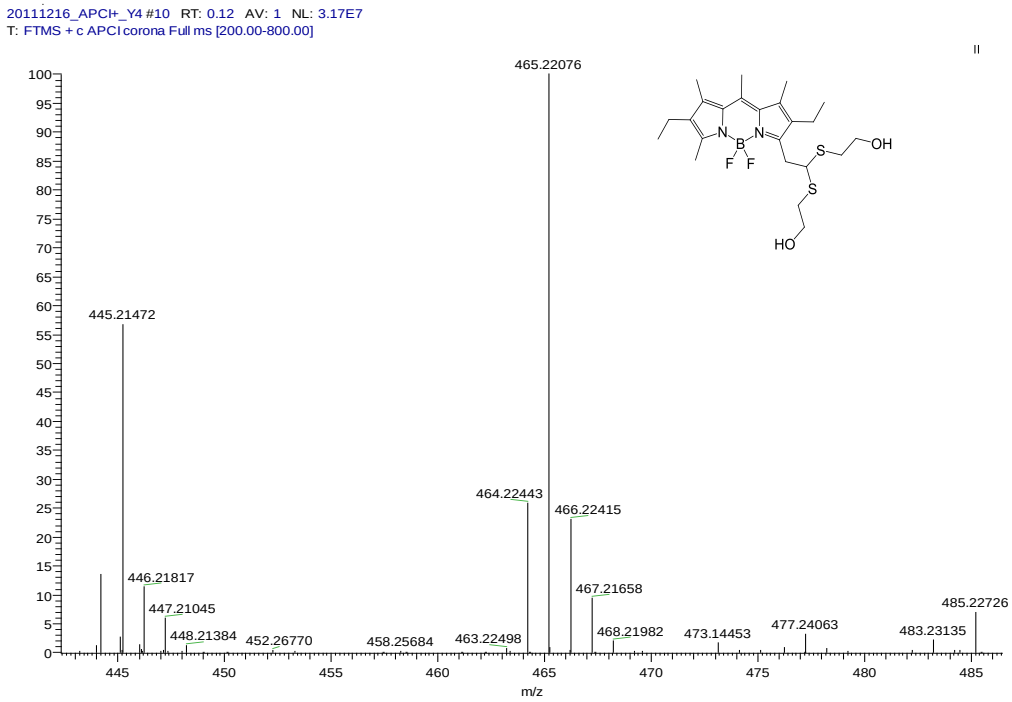

2011-1-hrh-C
153.3
146.3
139.1
136.9
136.4
133.3
132.7
132.3
131.9
131.6
117.9
61.0
35.2
18.0
17.2
17.1
14.8
14.5
14.5
14.1
12.6
f1 (ppm)
N
F
B
F
N
S
HO



m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
385.211	2388371	2.82	385.2108	0.2	C23 H30 N2 F S
386.2103	19887468	23.5	386.212	-1.64	C22 H30 O N B F S
387.2067	84616064	100	387.2072	-0.52	C21 H29 O N2 B F S
388.2095	18452256	21.81	388.2105	-1.02	C23 H31 O N F S
389.2022	3636145	4.3	389.2029	-0.7	C21 H28 N2 B F2 S
389.2129	1706031	2.02	389.2155	-2.58	C22 H30 N B F2 S
407.2126	1602596	1.89	407.2135	-0.89	C21 H30 O N2 B F2 S
415.2106	5725866	6.77	415.2185	-7.93	C23 H30 N2 B F2 S
416.214	1458169	1.72	416.2026	11.41	C23 H29 O N B F2 S
419.3144	1244540	1.47	419.3199	-5.51	C22 H44 O N B F2 S


f1 (ppm)
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
1.03
4.34
2.14
4.30
3.13
2.12
3.18
2.37
6.07
2.07
6.44
F
B
F
N
N
HO
S
S
HO
4.71
3.78
3.76
3.39
3.37
2.95
2.90
2.88
2.85
2.83
2.64
2.54
2.52
2.49
2.42
2.39
2.36
2.35
1.62
1.10
1.07
1.05
1.02


2011-2-hrh-C
154.2
148.2
140.4
137.7
135.5
133.4
133.1
60.8
49.9
34.9
34.0
17.3
17.0
16.8
14.7
14.5
14.4
14.2
12.4
f1 (ppm)
N
F
B
F
N
S
S
HO
HO



m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
444.2182	4290896	13.53	444.2201	-1.86	C23 H36 O N F2 S2
445.2147	17975982	56.7	445.2149	-0.21	C23 H34 O2 N2 B S2
446.0089	428059.9	1.35	446.0035	5.35	C21 H7 O2 N3 B F2 S2
446.2182	3624332	11.43	446.2165	1.69	C21 H35 O2 N B F2 S2
447.2105	1880033	5.93	447.2106	-0.14	C23 H33 O N2 B F S2
448.2138	395913	1.25	448.215	-1.17	C22 H36 O2 N F2 S2
464.2244	8178353	25.8	464.2212	3.27	C21 H36 O2 N3 F2 S2
465.2208	31703442	100	465.2212	-0.39	C23 H35 O2 N2 B F S2
466.2242	7339161	23.15	466.229	-4.83	C23 H36 O2 N2 B F S2
473.1445	541269.6	1.71	473.1335	11.05	C23 H24 O2 N2 B F2 S2
477.2406	1039186	3.28	477.2086	32.05	C23 H33 O2 N3 B F S2
483.2314	718984.8	2.27	483.2355	-4.19	C23 H36 O N3 B F2 S2
485.2273	44107	0.14	485.2274	-0.12	C23 H36 O2 N2 B F2 S2

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