Supporting information

Reaction Based Fluorescent Probes for Hydrogen Sulfide

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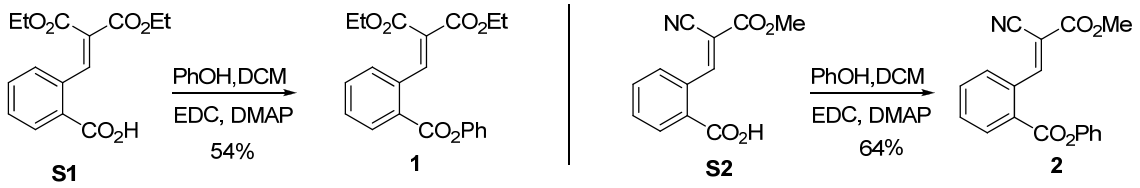
Materials and Methods: All solvents were reagent grade. Reactions were magnetically stirred and monitored by thin layer chromatography (TLC) with 0.25 mm pre-coated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.062mm). Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. Proton and carbon-13 NMR spectra were recorded on a 300 MHz spectrometer. Chemical shifts are reported relative to chloroform (δ 7.26) for ^{1}H NMR and chloroform (δ 77.0) for ^{13}C NMR. Absorption spectra were recorded on a Lambda 20 UV/VIS spectophotometer using 1 cm quartz cells. Fluorescence excitation and emission spectra were measured on Cary Eclipse fluorescence spectrophotometer.

Preparation of Compounds 1 and 2



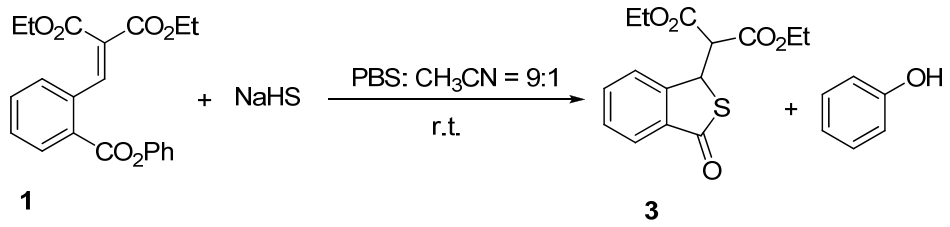
Scheme S1

Compounds **1** and **2** were prepared from **S1** and **S2**, which are known compounds.[1] The procedure was as following:

To a mixture of compounds **S1** (292 mg, 1.0 mmol), phenol (94 mg, 1.0 mmol), EDC (192 mg, 1.0 mmol) and DMAP(12.2 mg, 0.1 mmol) was added CH_2Cl_2 (5 mL) at room temperature. The mixture was stirred for 12 h. The solvent was then removed under reduced pressure and the resulted residue was purified by falsh column chromatography. Compound **1** was obtained as a light yellow solid (200 mg, 54 % yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.06 (t, *J* = 7.2 Hz, 3H), 1.31 (t, *J* = 7.2 Hz, 3H), 4.12 (q, *J* = 7.2 Hz, 2H), 4.28 (q, *J* = 7.2 Hz, 2H), 7.21-7.31 (m, 3 H), 7.41-7.70 (m, 5 H), 8.29 (m, 1 H), 8.43 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8，164.8，164.0, 150.9，144.6, 137.1, 135.2, 134.3, 133.3, 131.6, 130.4, 129.7, 128.8, 126.3, 124.0, 121.9, 93.5, 61.9, 61.6, 14.3, 14.0; MS (ESI^+) m/z 391.2 ($M+Na^+$); IR 3429,. 3006, 2161, 1725, 1252, 1193, 1055, 766; mp 60-61 ^{o}C.

Compound **2** was obatained via a silimliar procedure in 64 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.91 (s, 3H), 7.20-7.32 (m, 3 H), 7.45 (m, 2 H), 7.65-7.86 (m, 2 H), 7.86-7.89 (m, 1 H), 8.38 (dd, *J* = 7.5, 1.5 Hz, 1 H), 9.04 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.6, 162.4, 157.3, 150.7, 134.7, 134.0, 133.9, 131.9, 131.7, 130.1, 130.0, 129.9, 129.0, 126.6, 126.5, 121.8, 106.9, 53.6; MS (ESI^+) m/z 330.1 ($M+Na^+$); IR 3445, 3069, 2226, 1979, 1733, 1608, 1199, 751. mp 85-86 ^{o}C.

Reaction of Compounds 1 and 2 with H_2S

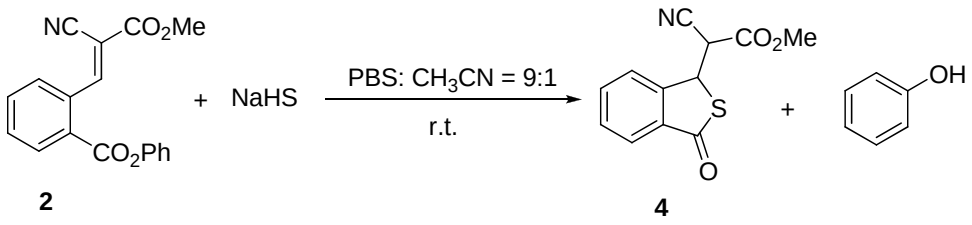


Scheme S2

To the solution of **1** (36.8 mg, 0.1 mmol) in CH_3CN (2.0 mL) and PBS buffer (18.0 mL,

[1] (a) V.M. Rodinov,E.I. Chukhina. *Zhurnal Obshchei Khimii.* **1956**, *26*, 143-146. (b) P. Kolsaker, J. Arukwe, J. Barcoczy, A. Wilberg, A.K. Fagerli. *Acta Chemica Scandinavica.* **1998**, *52*, 490-498.

20 mM, pH 7.4) was added NaHS (56 mg, 1.0 mmol). The mixture was stirred for 1 hour at rt and then diluted with ethyl acetate (50 mL). The organic layer was seperated and dried by $MgSO_4$, and concentrated. Purification by flash column chromatography afforded compound **3** (9.6 mg, 31% yield) and recovered unreacted **1** (>55%) as the major material. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (t, *J* = 7.2 Hz, 3H), 1.29 (t, *J* = 7.2 Hz, 3H), 3.99 (d, *J* = 7.2 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 4.29 (q, *J* = 7.2 Hz, 2H), 5.44 (d, *J* = 7.2 Hz, 1 H), 7.48-7.54 (m, 2 H),7.62(d, *J* = 7.6 Hz, 1 H), 7.82 (d, J = 7.3 Hz, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.6, 166.6, 147.7, 136.6, 133.63, 133.60, 129.1, 125.7, 124.3, 62.6, 62.4, 57.7, 48.4, 14.4, 14.2; MS (ESI^+) m/z 331.1 ($M+Na^+$). IR 3070, 2983, 2038, 1732, 1687, 1455, 1230, 775.

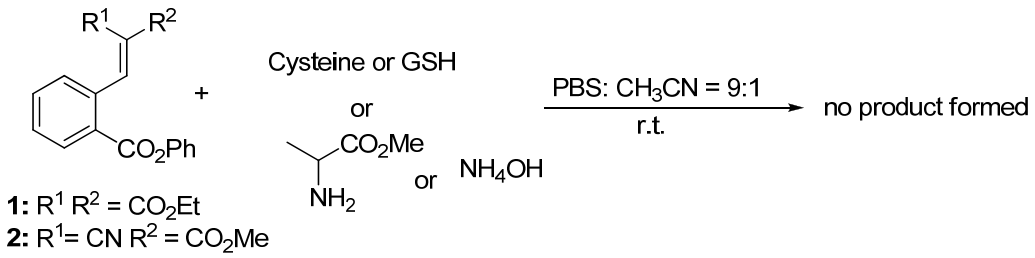


2 **4**

Scheme S3

The reaction between **2** and NaHS was carried using the same procedure as above. In this case, compound **4** was isolated as white solid in 91% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.86 (s, 3 H),), 4.42 (d, *J* = 5.2 Hz, 1H), 5.36 (d, *J* = 5.2 Hz, 1H), 7.47-7.51(m, 2H), 7.66 (m, 1H), 7.82 (m, 1H).^{13}C NMR (75 MHz, $CDCl_3$) δ 197.7, 164.6, 145.6, 136.5, 134.5, 130.1, 125.0, 124.9, 112.7, 54.6, 48.4, 44.6; MS (ESI^+) m/z 270.0 ($M+Na^+$); IR 3481, 3366, 2905, 1750, 1690,. 1453, 1262, 1215, 779; mp 112-113 ^{o}C.

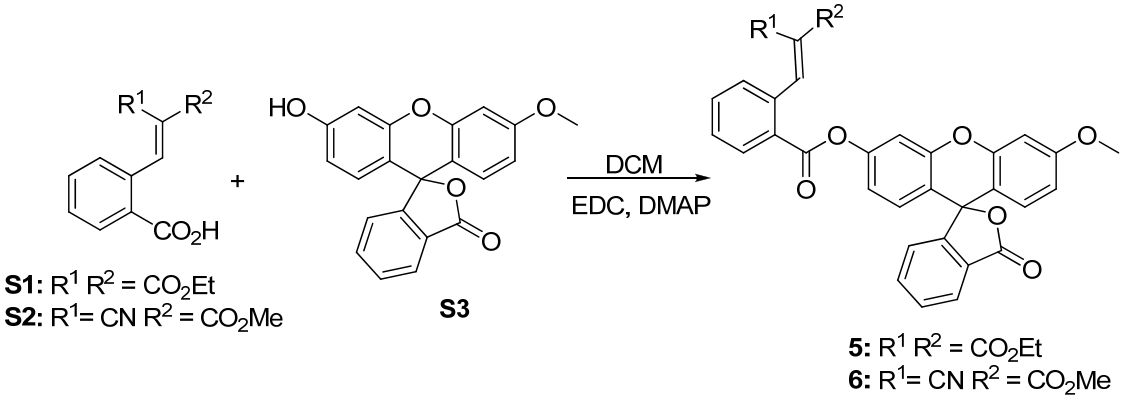
Reactions between compound 1 (or 2) and thiols/amines



Scheme S4

General proceudre of control experiment: To the solution of **1** or **2** (0.1 mmol) in CH_3CN (2 mL) and PBS buffer (18 mL, 20 mM, pH = 7.4) was added cysteine, glutathione, alanine, or ammonia seperately (1.0 mmol each). The mixture was stirred for 1 hour at rt. No product was observed on TLC. Then, ethyl acetate (50 mL) was added into the solution to extract the reaction mixture. The organic layer was seperated, dried by $MgSO_4$, and concentrated. Starting materials **1** or **2** were recovered in 89-92 % yields by column chromatography.

Synthesis of probe 5 and 6.



Scheme S5

Compounds **5** and **6** were prepared using the same procedure for compounds **1** and **2**.

Compound 5: 44 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (t, *J* = 7.2 Hz, 3 H), 1.31 (t, *J* = 7.2 Hz, 3 H), 3.85 (s, 3 H), 4.12 (q, *J* = 7.2 Hz, 2 H), 4.29 (q, *J* = 7.2 Hz, 2 H), 6.61-6.94 (m, 5 H), 7.17-7.28 (m, 2 H), 7.42-7.71 (m, 5 H), 8.04 (m, 1 H), 8.27 (m, 1 H), 8.40 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.5, 165.7, 164.3, 163.9, 161.7, 153.2, 152.5, 152.2, 152.1, 144.3, 137.3, 135.4, 133.6, 131.6, 130.1, 129.6, 129.5, 129.43, 129.40, 129.3, 128.4, 127.8, 126.7, 125.3, 124.2, 117.7, 117.3, 112.2, 111.0, 101.1, 82.6, 61.9, 61.6, 55.8, 14.3, 14.0; MS (ESI$^+$) m/z 643.3 (M+Na$^+$); IR 3070, 2979, 2180, 1766, 1731, 1606, 1246, 761; mp 90-91 ^{o}C.

Compound 6: 42 % yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.85 (s, 3 H), 3.92 (s, 3 H), 6.63-6.94 (m, 5 H), 7.17-7.22 (m, 2 H), 7.62-7.90 (m, 5 H), 8.04 (m, 1 H), 8.38 (m, 1 H), 9.02 (s, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.4, 169.5, 164.2, 162.3, 161.7, 157.1, 153.2, 152.4, 152.2, 151.8, 135.4, 134.9, 134.3, 132.0, 131.8, 130.18, 130.15, 129.6,

129.3, 128.4, 126.7, 125.4, 124.2, 117.6, 114.7, 112.3, 111.0, 110.7, 107.1, 101.1, 82.5, 55.8, 53.7; MS (ESI^+) m/z 560.1 ($M+H^+$); IR 3071, 2953, 2227, 2019, 1765, 1731, 1244, 1196, 759; mp 115-116 ^oC.

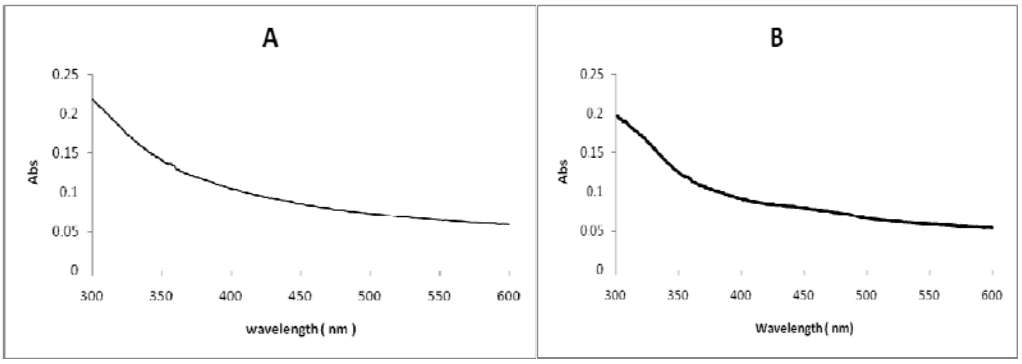


Figure S1. Absorbance spectra of a) 10 μM of compound **5** and b) 10 μM of compound **6** in PBS buffer (pH 7.4).

Quantum Yields.

Quantum yields were determined using fluorescein as a standard according to a published method.[2] The quantum yield was calculated according to the equation: ($\Phi_{sample} = \Phi_{standard} * (I_{sample}/ I_{standard}) * (A_{sample}/ A_{standard})$); where Φ is the quantum yield, $\Phi_{standard} = 0.95$ in 0.1 M NaOH; I_{sample} and $I_{standard}$ are the integrated fluorescence intensities of the sample and the standard, A_{sample} and $A_{standard}$ are the optical densities, at the excitation wavelength, of the sample and the standard, respectively.

Quantum yield of compound **5**: Φ = 0.0002; Quantum yield of compound **6**: Φ= 0.0008

Preparation of stock solutions

Compound **5** stock solution in CH_3CN: 62 mg of **5** was dissolved in 100 mL CH_3CN ([Compd. **5**]=1.0 mM).

Compound **6** stock solution in CH_3CN: 56 mg of **6** was dissolved into 100 mL CH_3CN ([Compd. **6**]=1.0 mM).

[2] a) A. A. Musse, J. Wang, G. P. deLeon, G. A. Prentice, E. London, A. R. Merrill, *J Biol. Chem,* **2006**, *281*, 885. b) J. J. Jankowski, D. J. Kieber, K. Mopper, P. J. Neale, *Photochem. Photobiol.* **2000**, *71*, 431.

NaHS stock solution in PBS buffer: 84 mg of $NaHS.H_2O$ (from Acros) was dissolved into 1000 mL of PBS buffer (pH 7.4, 10.0 mM) to get the desired stock solution ([NaHS] = 1.0 mM). This solution was freshly prepared each time before use.

Cysteine stock solution in PBS buffer: 122 mg of cysteine was dissolved in 100 mL of PBS buffer (pH 7.4, 10.0 mM) ([cysteine]=10.0 mM).

Glutathione (GSH) stock solution in PBS buffer: 307 mg of GSH was dissolved in 100 mL of PBS buffer (pH 7.4, 10.0 mM) ([GSH] = 10.0 mM).

Figure 1--Fluorescent images of probes

Top row:

a): 20 μL of compound **5** stock solution was diluted into 4.0 mL PBS buffer.

b): 20 μL of compound **5** stock solution was diluted with 3.6 mL PBS buffer. Then 0.4 mL of NaHS stock solution (1.0 mM) was added into the mixture.

c): 20 μL of compound **5** stock solution was diluted with 3.6 mL PBS buffer. Then 0.4 mL of cysteine stock solution (10.0 mM) was added into the mixture.

d): 20 μL of compound **5** stock solution was diluted with 3.6 mL PBS buffer. Then 0.4 mL of GSH stock solution (10.0 mM) was added into the mixture.

e): To a mixture of 0.4 mL of cysteine stock solution and 0.4 mL of NaHS stock solution was added 3.2 mL PBS buffer. Then 20 μL of compound **5** stock solution was added into the mixture.

f): To a mixture of 0.4 mL of GSH stock solution and 0.4 mL of NaHS stock solution was added 3.2 mL PBS buffer. Then 20 μL of compound **5** stock solution was added into the mixture.

The images were taken after 30 min.

Bottom row:

The images of probe **6** were taken using the same procedures described above.

Figure 2: Fluorescence responses of the probes toward H_2S and other thiols

A

a): 20 μL of compound **5** stock solution was diluted into 4.0 mL PBS buffer.

b): 20 μL of compound **5** stock solution was diluted with 3.6 mL PBS buffer. Then 0.4 mL of NaHS stock solution (1.0 mM) was added into the mixture.

c): 20 μL of compound **5** stock solution was diluted with 3.6 mL PBS buffer. Then 0.4 mL of cysteine stock solution (10.0 mM) was added into the mixture.

d): 20 μL of compound **5** stock solution was diluted with 3.6 mL PBS buffer. Then 0.4 mL of GSH stock solution (10.0 mM) was added into the mixture.

e): To a mixture of 0.4 mL of cysteine stock solution and 0.4 mL of NaHS stock solution was added 3.2 mL PBS buffer. Then 20 μL of compound **5** stock solution was added into the mixture.

f): To a mixture of 0.4 mL of GSH stock solution and 0.4 mL of NaHS stock solution was added 3.2 mL PBS buffer. Then 20 μL of compound **5** stock solution was added into the mixture.

The fluorescent intensity was measured after mixing at rt for 30 min (λ_{ex} = 476 nm, λ_{em} = 513 nm).

Table S1. Fluorescence emission intensities a)-f)

Vial #	a	b	c	d	e	f
Intensity (a.u.)	31±1.5	328±16.4	32±1.7	72±3.6	324±16.0	284±14.2

B

The intensities of probe **6** were taken using the same procedures described above.

Table S2. Fluorescence emission intensities a)-f)

Vial #	a	b	c	d	e	f
Intensity (a.u.)	5±0.2	854±41.5	15±0.6	14±0.5	789±39.5	778±33.8

Figure S2: Linear correlation of fluorescent emission intensity and the concentrations of NaHS

A: 20 μL compound **5** stock solution was diluted to 4 mL PBS buffer. Then a series of NaHS stock solutions (0, 40, 80, 120, 280, 360 μL) were added into the mixture respectively. The fluorescence intensity was measured after the mixutre was kept at rt for 30 min (λ_{ex} = 476 nm, λ_{em} = 513 nm).

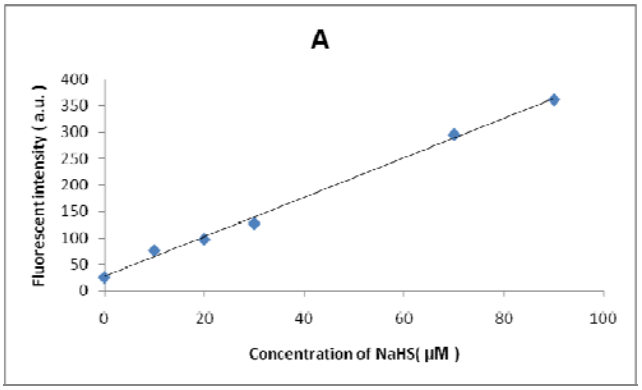


Figure S2-A. Linear correlation of fluorescent intensity toward NaHS concentration with probe **5**.

B: 20 μL compound **6** stock solution was diluted to 4 mL PBS buffer. Then a series of NaHS stock solutions (0, 20, 40, 120, 160, 200 μL) were added into the mixture respectively. The fluorescence intensity was measured after the mixutre was kept at rt for 30 min (λ_{ex} = 476 nm, λ_{em} = 513 nm).

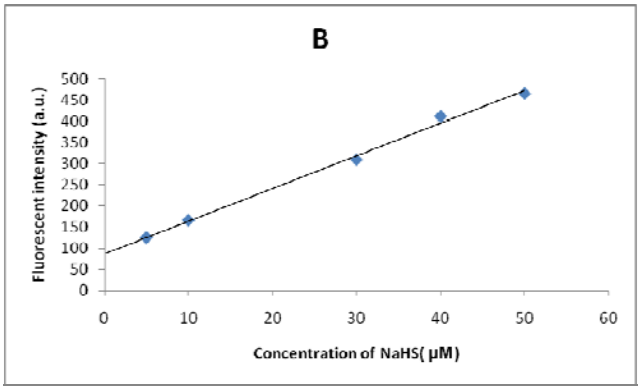


Figure S2-B. Linear correlation of fluorescent intensity toward NaHS concentration with probe **6**.

Figure 4 Test the stability of probes 5 and 6 towards esterase

Esterase activity assay

The protocol provided by Sigma-Aldrich was followed (http://www.sigmaaldrich.com/technical-documents/protocols/biology/enzymatic-assay-of-esterase.html). Briefly, a 0.62 mg/mL solution of boric acid in purified water was prepared. pH was ajusted to 8.0 with 1.0 N NaOH to result in 10 mM borate buffer. Then 10 mg esterase (E-0887 from Sigma) was dissolved in 10 mL borate buffer (10 mM , pH 8.0) (Con. = 1 mg/mL). To 25 mL of borate buffer was added 0.1 mL of ethyl butyrate. Then several drops of 0.01 N NaOH was added into the solution till pH reached 8.17. Next 0.1 mL of esterase solution was added in and after 2 min, pH was changed to 8.0. After that, 0.1 mL of 0.01 N NaOH was added in and pH was changed to 8.02 and then

decreased to 8.0 by esterase hydrolysis. These steps were repeated for 11 times in 5 min which demonstrated the activity of the enzyme.

*Stability assay of **5** and **6***

20 μL of compound **5** or **6** stock solution was mixed with 0.1 mL of esterase stock solution. The resultant mixture was diluted with 3.9 mL phosphate buffer (pH 7.4, 10.0 mM). The fluorescence intensity was measured after the mixutre was kept at room temperature for 0, 0.5, 1.0, 2.0 hour. After 2 h, to the mixture was added 40 μL of NaHS stock solution (for probe **5**) or 28 μL of NaHS stock solution (for probe **6**). The fluorescence intensity was measured after 0.5 h (λ_{ex} = 476 nm, λ_{em} = 513 nm).

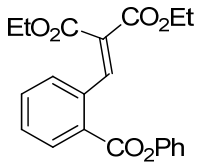
Cell Culture and Treatment

COS7 cells were obtained from ATCC and maintained in Dulbecco's modified Eagle's medium with high glucose supplemented with 10% fetal bovine serum. For the experiments, cells were passaged and allowed to grow on glass coverslips for two days. Stock solution of **6** (0.2 mM) was prepared in DMSO at the same day of the experiments and was diluted into the cell culture media at 100 μM. To start the experiment, living cells were preloaded with 100 μM probe **6** for 10 minutes at 37 ^{o}C in 5% CO_2 incubator, and then washed twice with PBS buffer to remove the extracellular probe. Cells were then treated with or without 200 μM sodium sulfide as indicated in the culture media. After 10 minutes of treatment, cells were rinsed twice with PBS buffer, and mounted by ProLong Gold Antifade reagent (Invitrogen) for imaging.

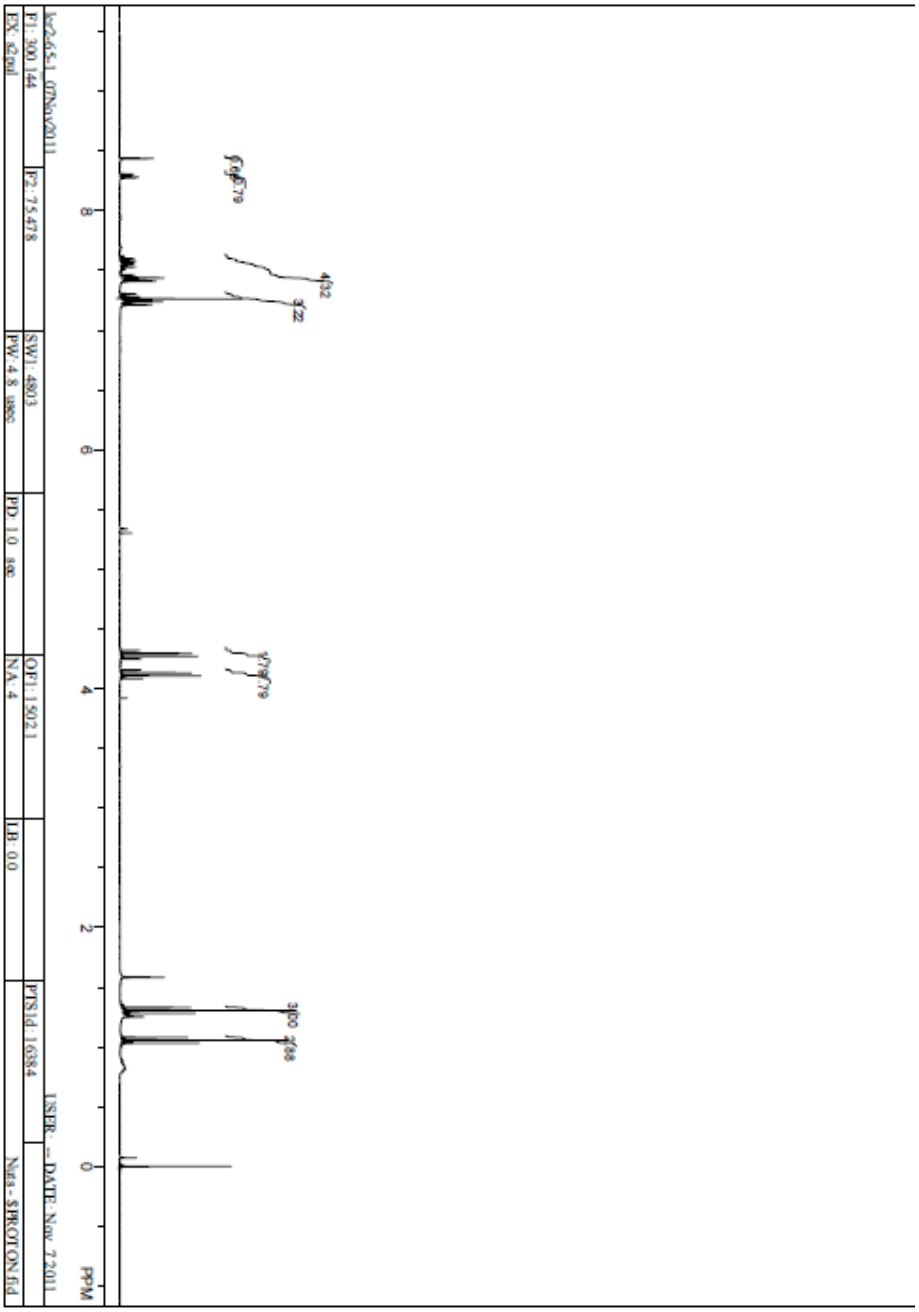
Fluorescence Microscopy

Images were acquired using a Zeiss Axio Imager microscope (Carl Zeiss) with a 20X/NA0.50 objective lens. For probe labeling: ex 470 +/- 20 nm, em 525 +/- 25 nm. The imaging process was controlled by the MetaMorph 7.7 software.

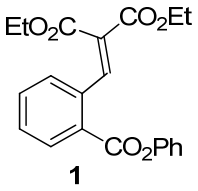
^{1}H NMR of **1** (300 MHz, in $CDCl_3$)



1



^{13}C NMR of **1** (75 MHz, in $CDCl_3$)



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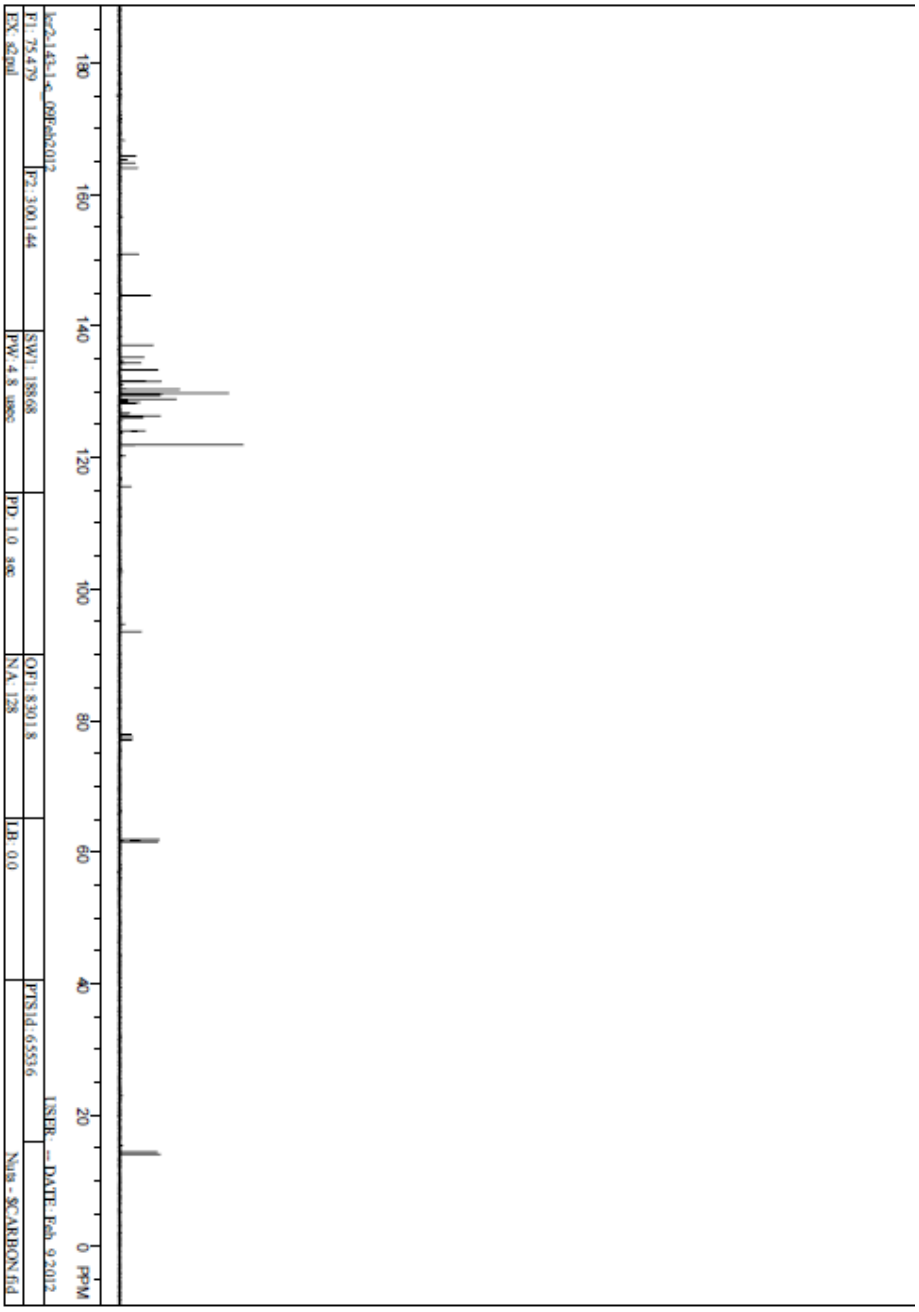
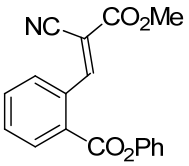


^{1}H NMR of **2** (300 MHz, in $CDCl_3$)



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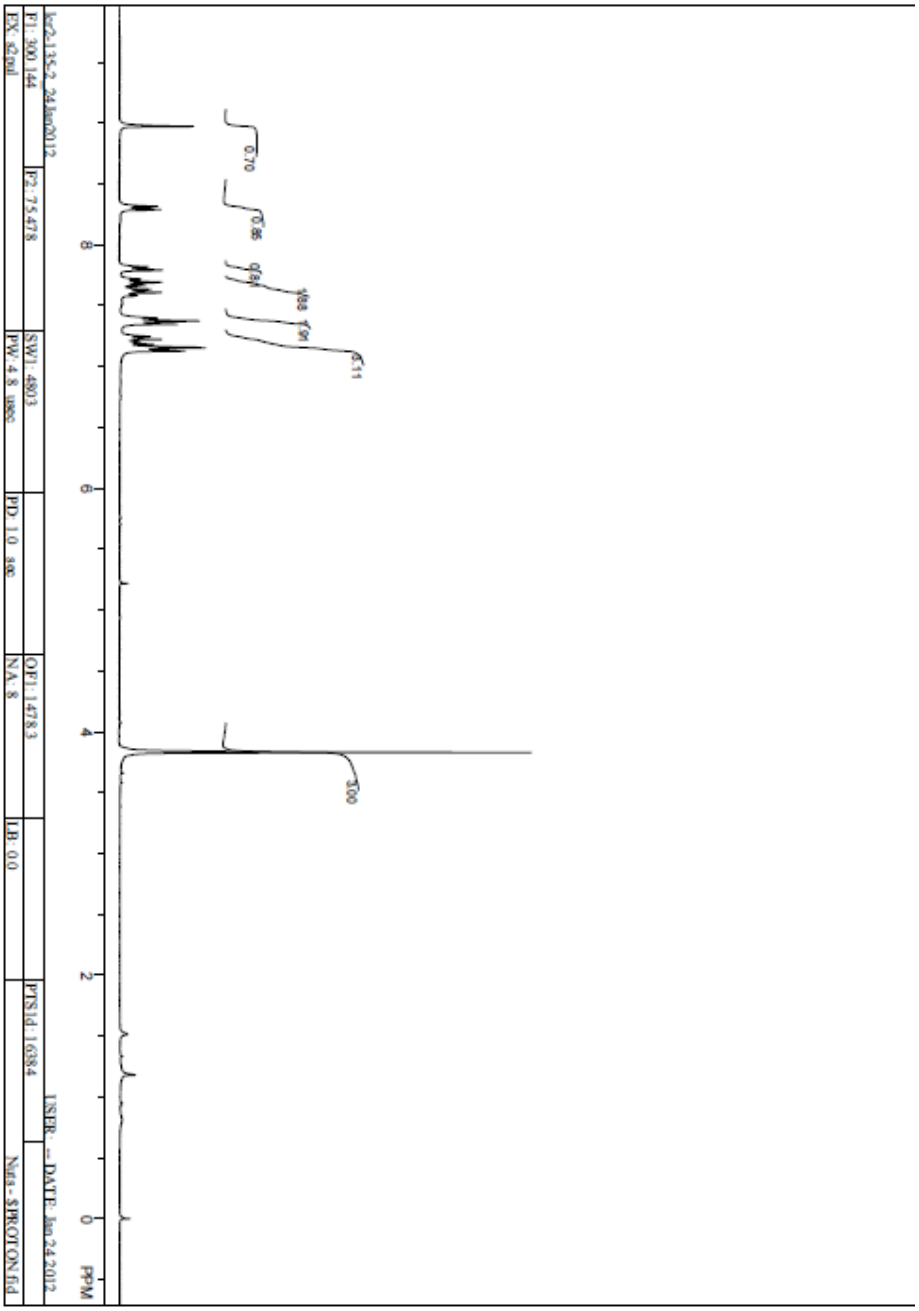
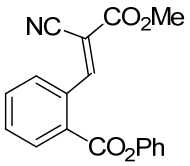


^{13}C NMR of **2** (75 MHz, in $CDCl_3$)



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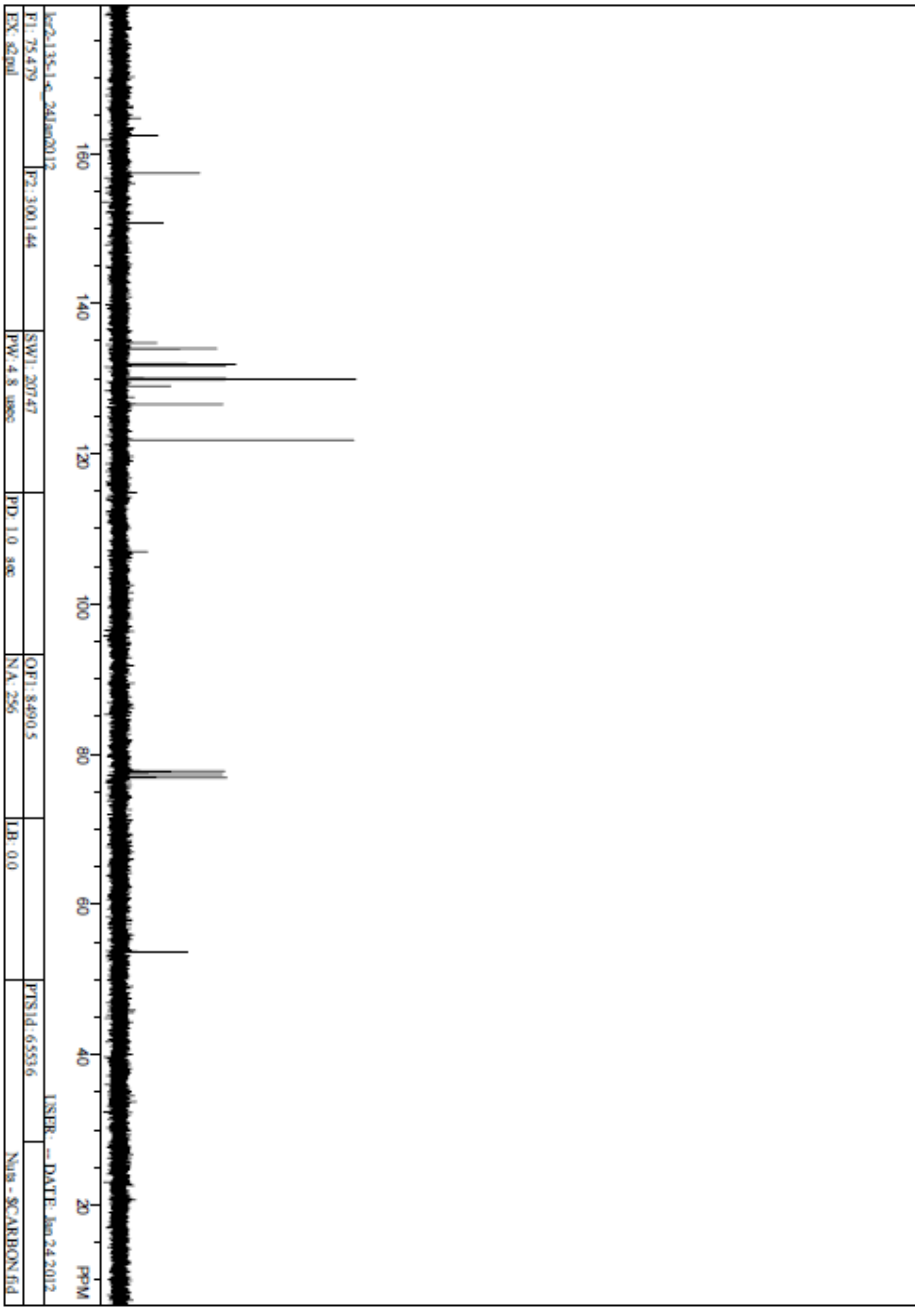
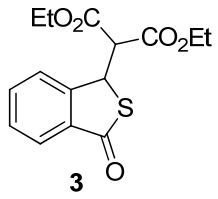


^{1}H NMR of **3** (300 MHz, in $CDCl_3$)



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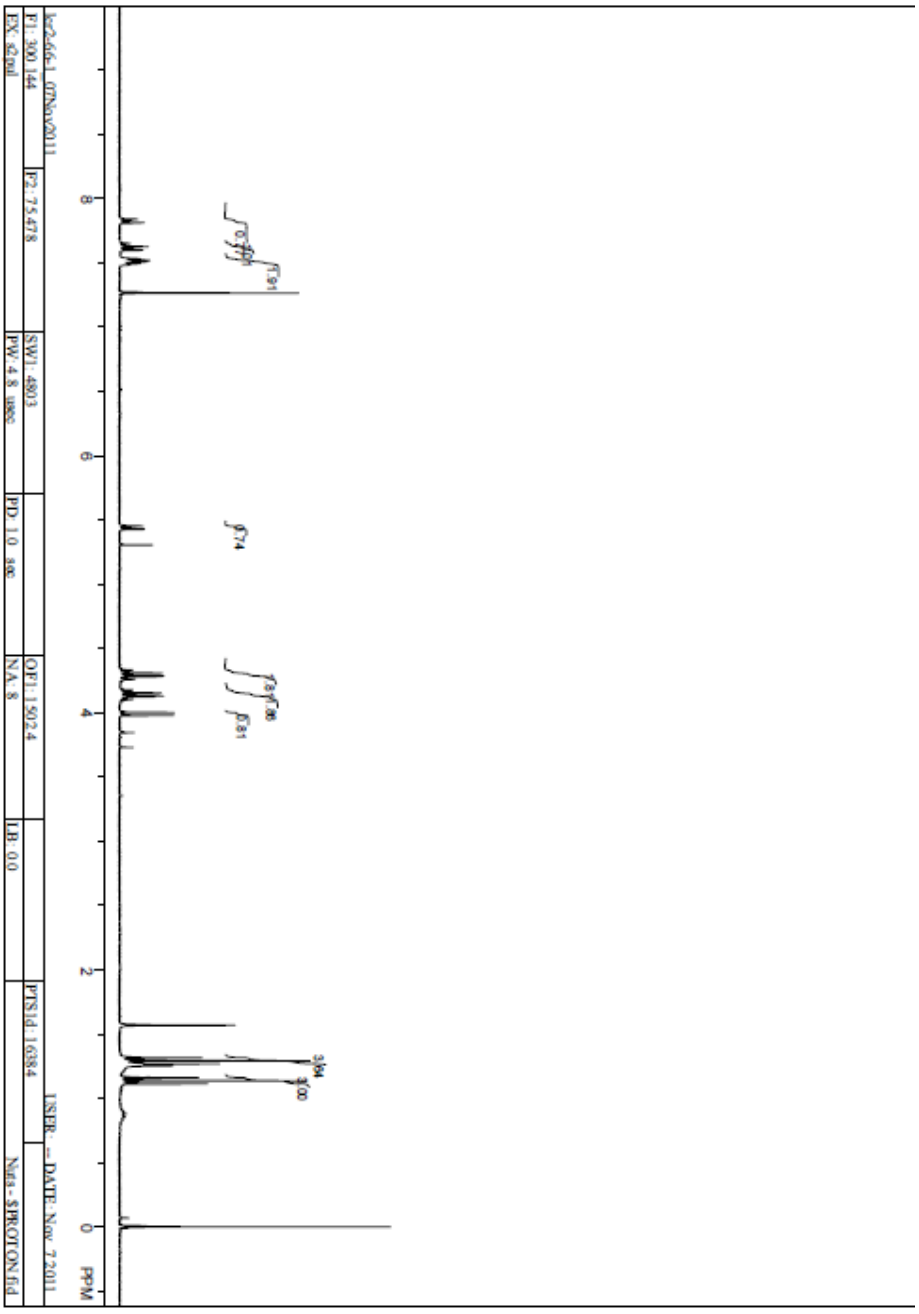
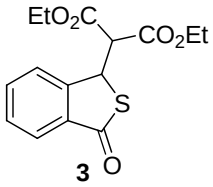


^{13}C NMR of **3** (75 MHz, in $CDCl_3$)



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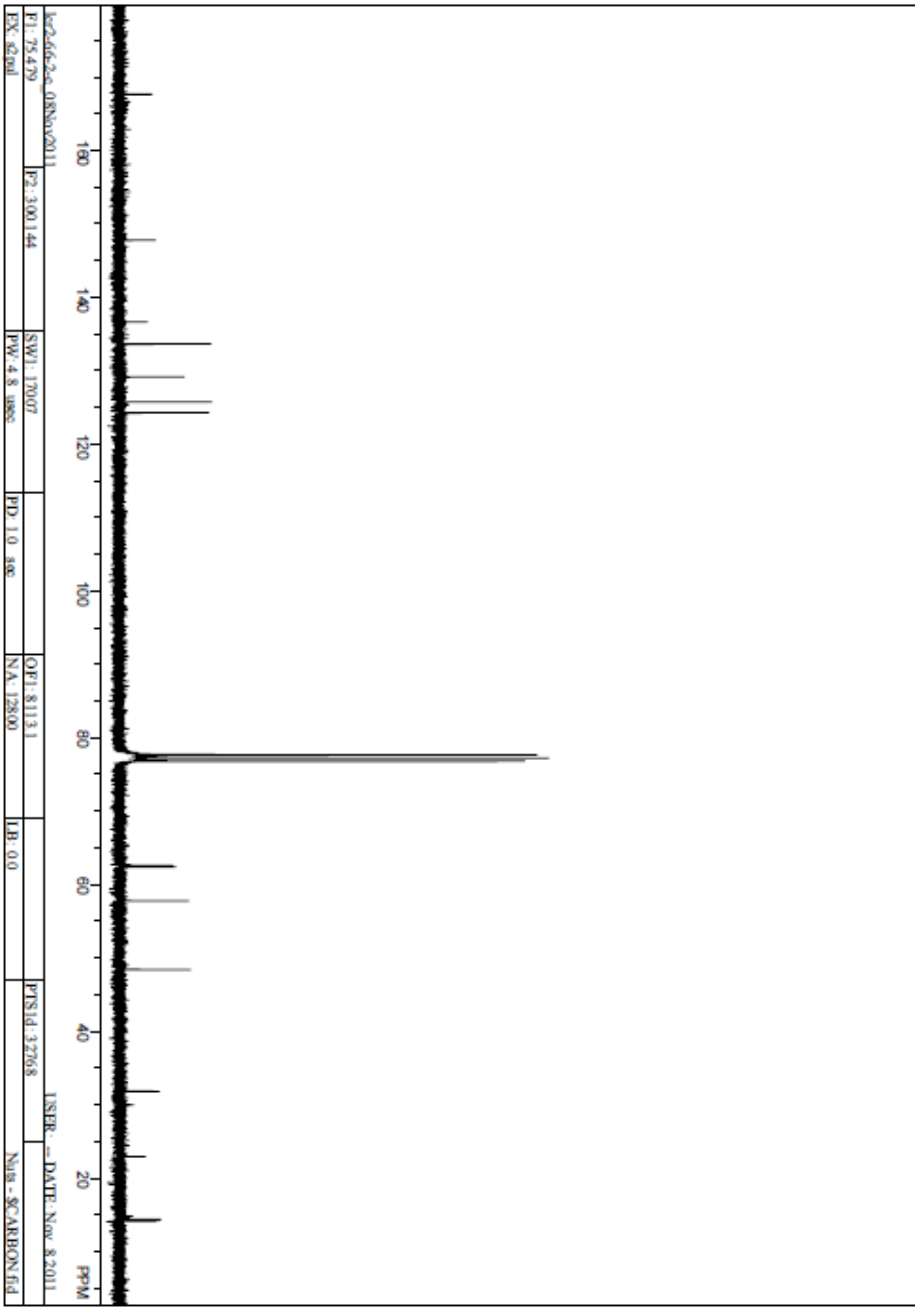
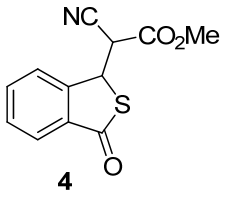


^{1}H NMR of **4** (300 MHz, in $CDCl_3$)



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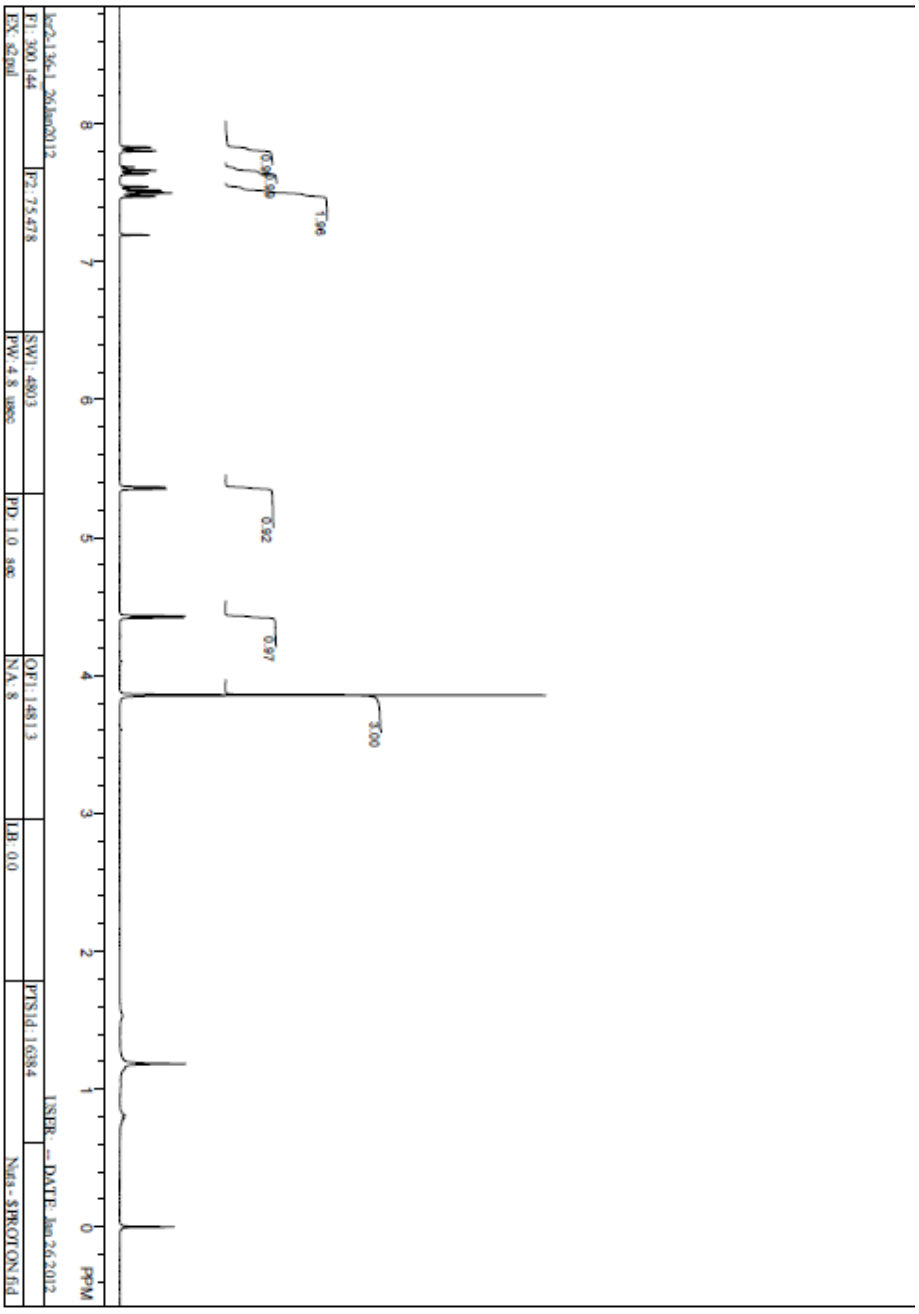
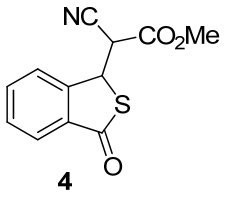
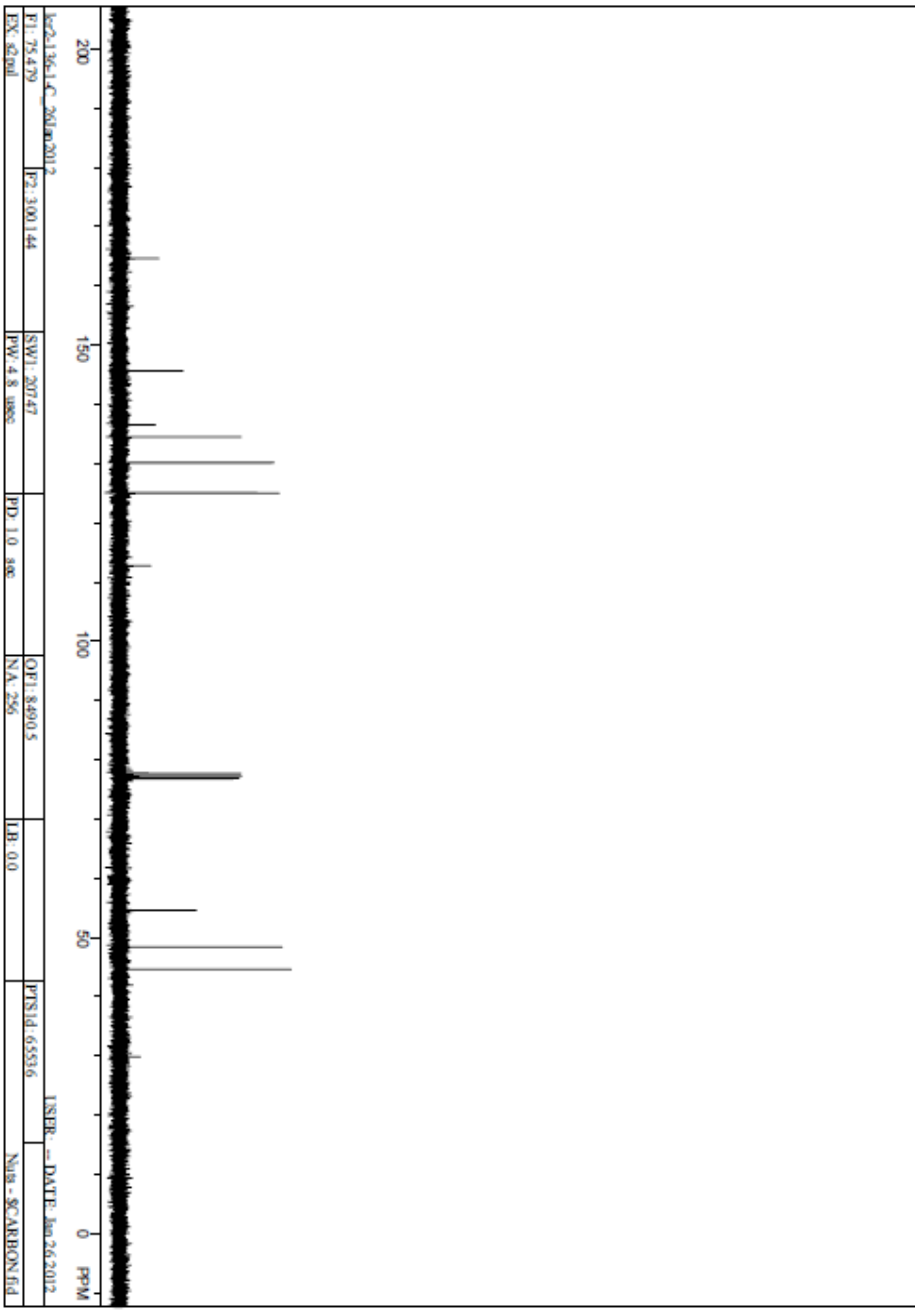


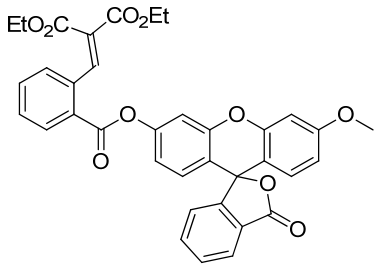
^{13}C NMR of **4** (75 MHz, in $CDCl_3$)



4



^{1}H NMR of **5** (300 MHz, in $CDCl_3$)



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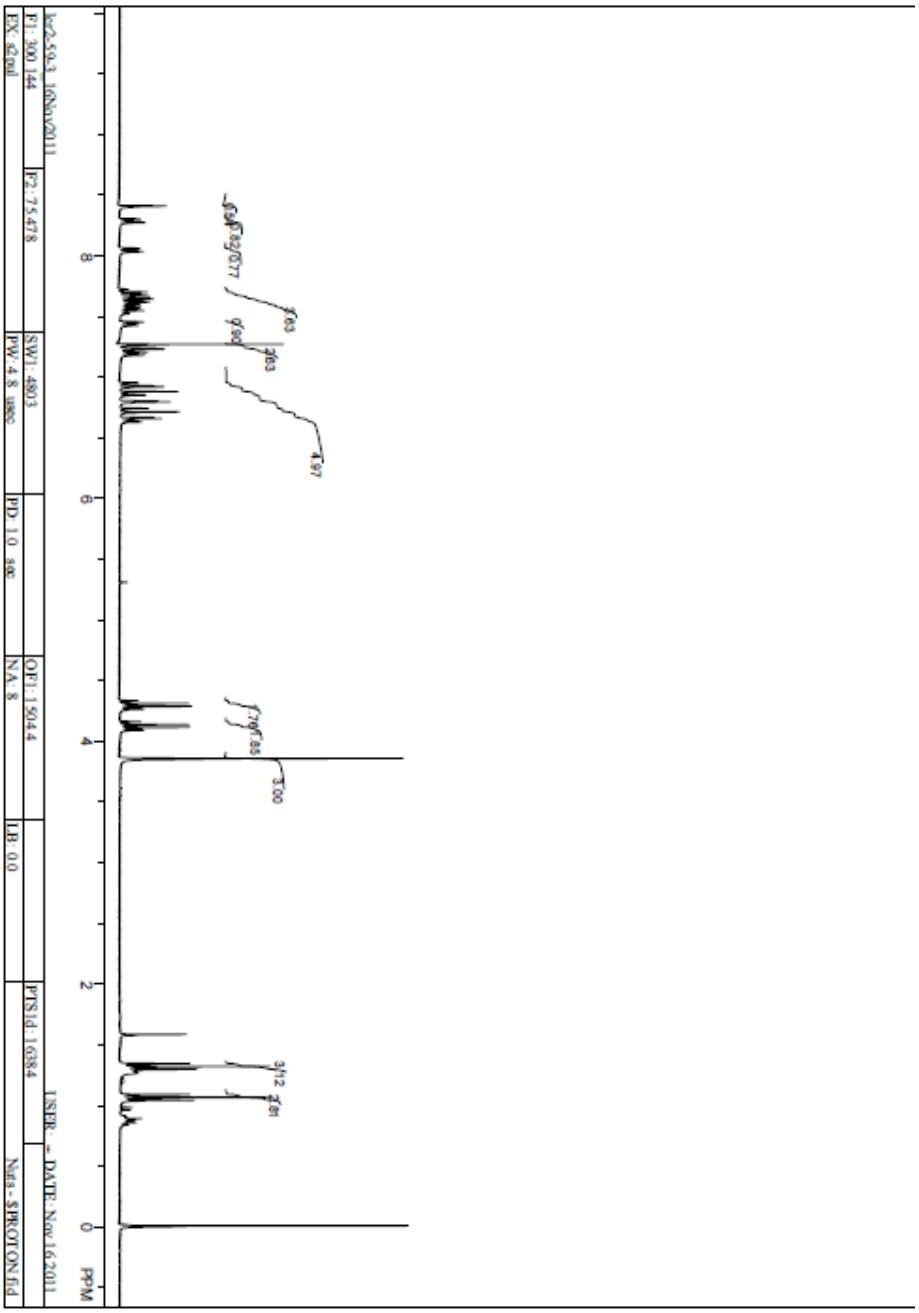
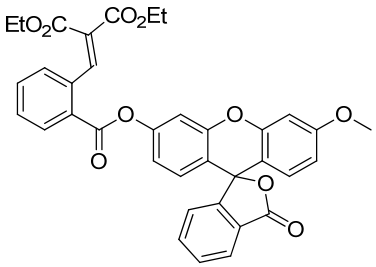


^{13}C NMR of **5** (75 MHz, in $CDCl_3$)



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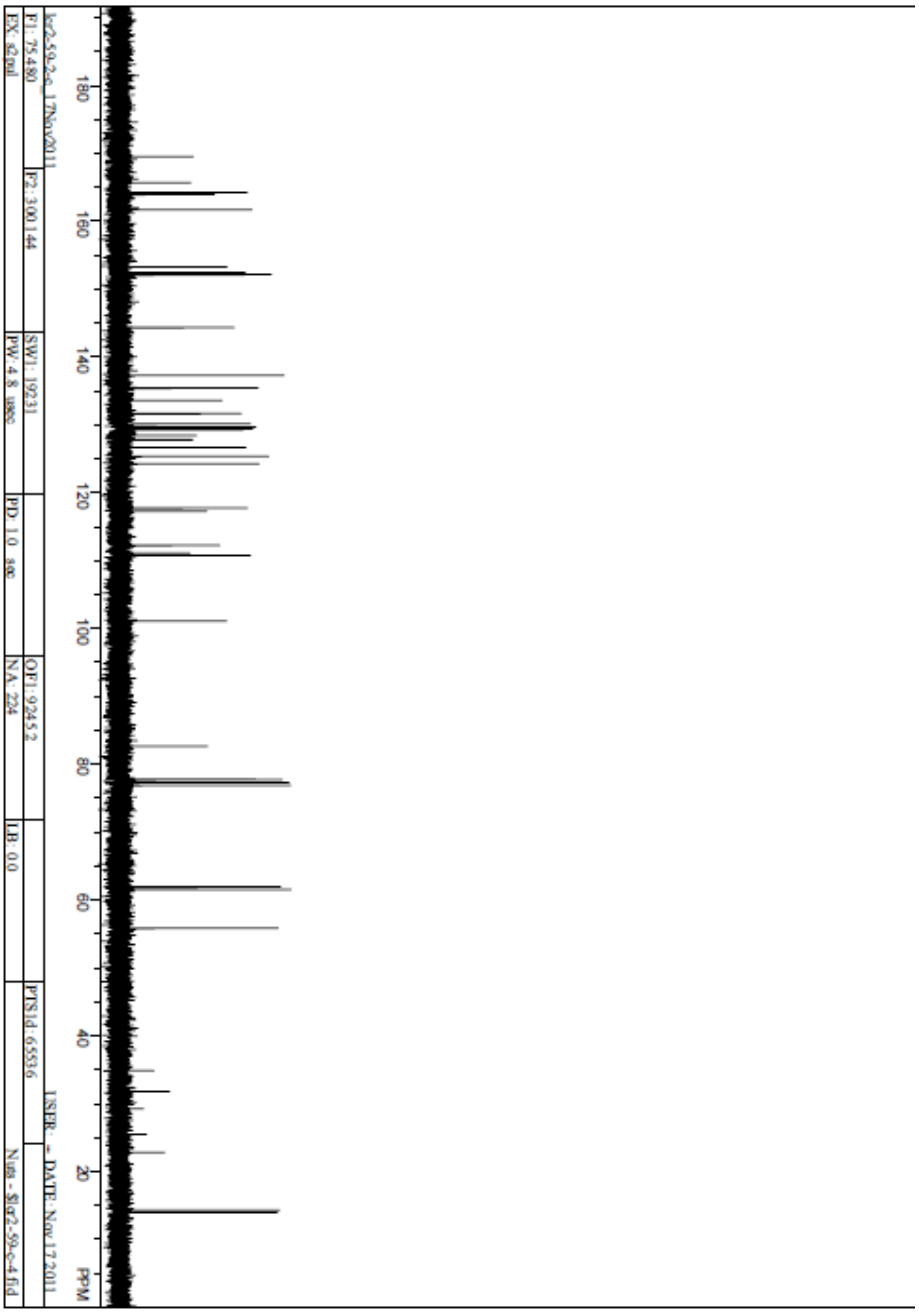
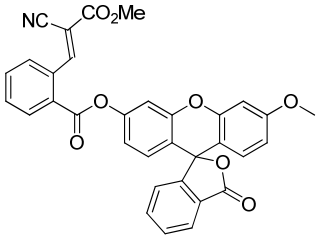


^{1}H NMR of **6** (300 MHz, in $CDCl_3$)



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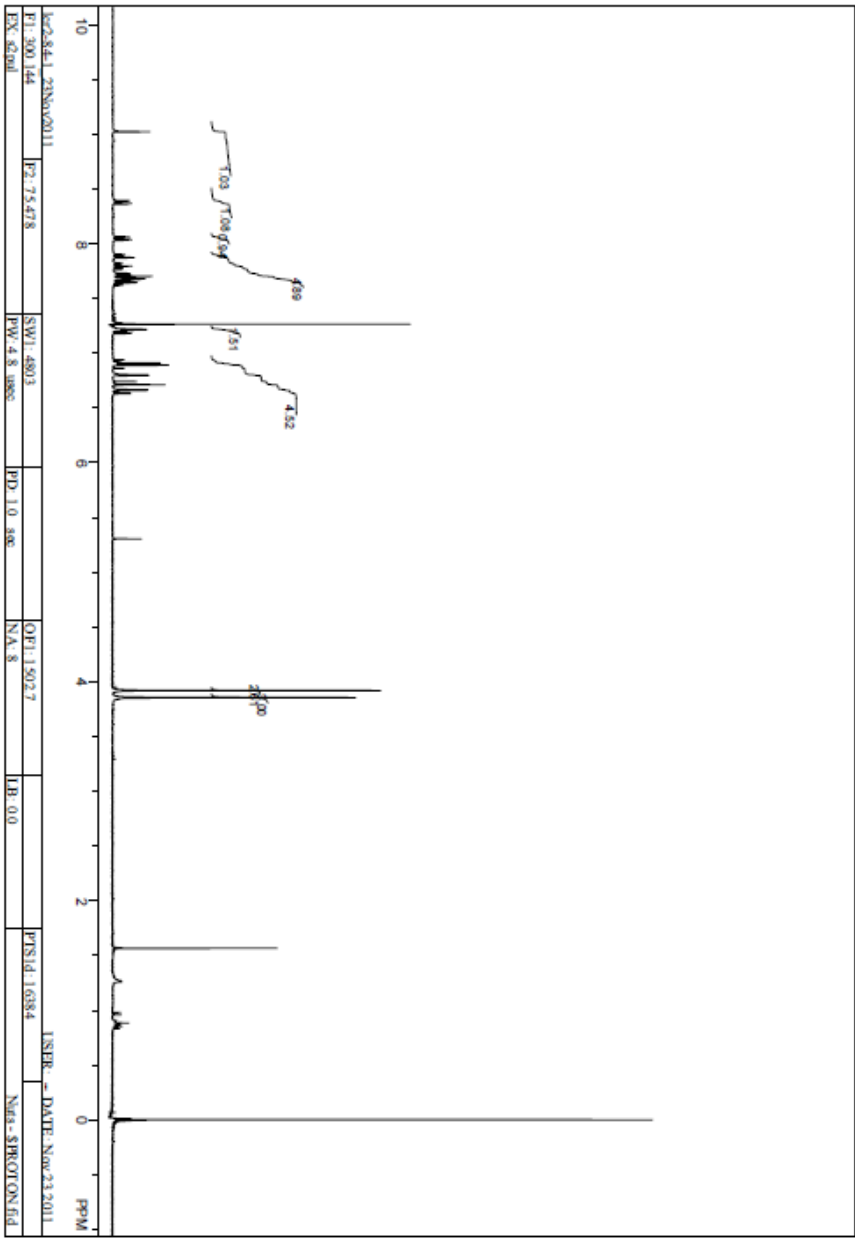
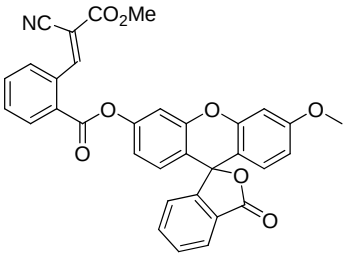


^{13}C NMR of **6** (75 MHz, in $CDCl_3$)



6