

Supporting Information

A Phenylselenium-Substituted BODIPY Fluorescent Turn-off Probe for Fluorescence Imaging of Hydrogen Sulfide in Living Cells

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Table S1. Spectroscopic/photophysical data of **1** (10 μM) in addition of Na_2S .

(Spectrum of **1**+ Na_2S was recorded 20 min after Na_2S addition in Triton X-100/DMSO/HEPES buffer (0.01: 1: 9, v/v/v, 10 mM, pH 7.4) at 37 $^\circ\text{C}$.)

Compounds	Reaction time/min	$\lambda_{\text{abs}}(\text{max})/\text{nm}$	$\lambda_{\text{ems}}(\text{max})/\text{nm}$	ϕ
1	—	587	610	0.299
1 + Na_2S	20	516	602	0.008

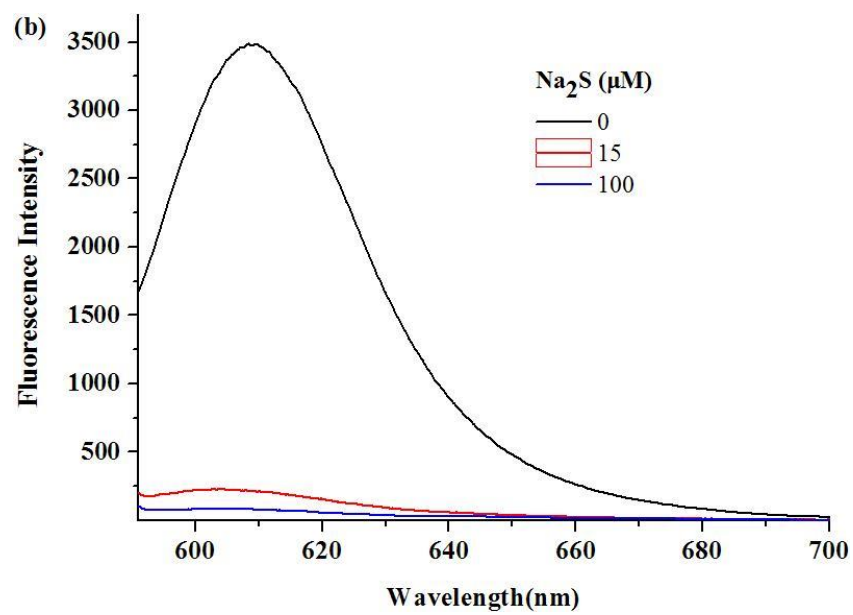
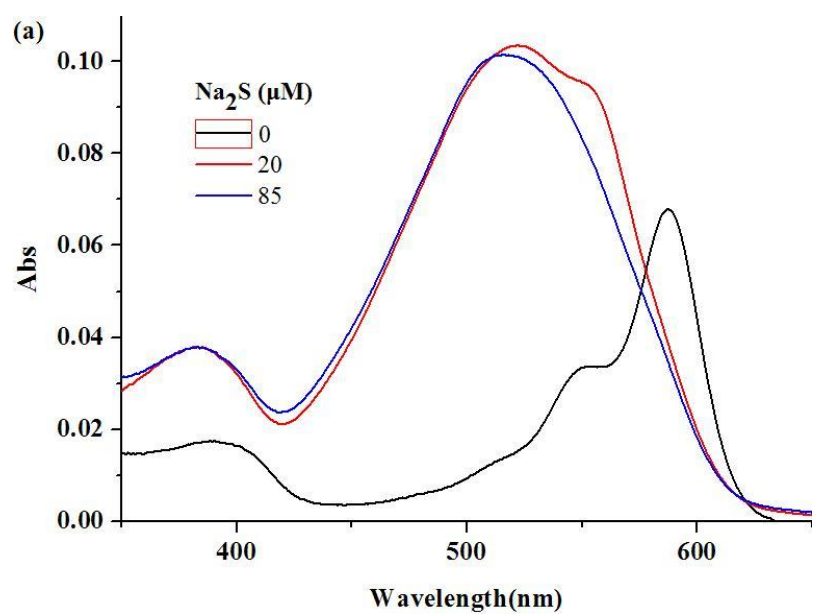
Table S2. Comparison of the proposed method with other methods for H_2S detection.

Linear range	Detection Limit	React time	Reference
0-100 μM	2.46 μM	40min	Anal.Chem., 2016, 88, 5476–5481
0-15 μM	0.05 μM	10min	Chem. Commun., 2012, 48, 10529–10531.
0-8 μM	0.007 μM	140 s	J. Am. Chem. Soc., 2015, 137, 8490–8498
0-10 μM	0.01 μM	15min	Chem. Commun., 2013, 49, 403–405.
0-300 μM	0.0682 μM	2min	Chem. Commun., 2015, 51, 16225-16228
0-125 μM	0.38 μM	30min	Chem. Commun., 2015, 51, 10463-10466.
0-16 μM	0.086 μM	90min	J. Am. Chem. Soc., 2015, 137, 10216–10223
1.0-30 μM	0.052 μM	60min	Anal. Chem., 2016, 88, 1434–1439.
0-30 μM	0.85 μM	20min	Anal. Chem., 2015, 87, 2678–2684
0-15 μM	0.0025 μM	20min	this work

Table S3. Photophysical Properties of **1** (10 μM) in the absence and in addition of Na_2S in Triton X-100/DMSO/HEPES buffer (0.01: 1: 9, v/v/v, 10 mM, pH 7.4) at 37

°C. Global analyses of decay times τ_1 , τ_2 and τ_3 , and the relative amplitude α_i (%), each spectrum was recorded 20 min after Na₂S addition at the same excitation wavelength (560 nm), but at different emission wavelength (590, 610, 630 and 650 nm).

Addition of Na ₂ S / μ M	Monitored wavelength/ nm	τ_1 /ns	τ_2 /ns	τ_3 /ns	α_1 (%)	α_2 (%)	α_3 (%)
0	590				1%	99%	-
	610	2.73 \pm 0.01	1.04 \pm 0.01	-	4%	96%	-
	630				5%	95%	-
	650				5%	95%	-
10	590				22%	45%	33%
	610	3.26 \pm 0.01	1.31 \pm 0.01	0.49 \pm 0.03	26%	54%	20%
	630				31%	51%	18%
	650				35%	44%	21%
25	590				27%	40%	33%
	610	3.34 \pm 0.01	1.47 \pm 0.01	0.48 \pm 0.02	28%	47%	25%
	630				30%	47%	23%
	650				31%	45%	24%
100	590				42%	42%	16%
	610	2.99 \pm 0.01	1.28 \pm 0.01	0.44 \pm 0.06	40%	43%	17%
	630				42%	43%	15%
	650				42%	47%	11%



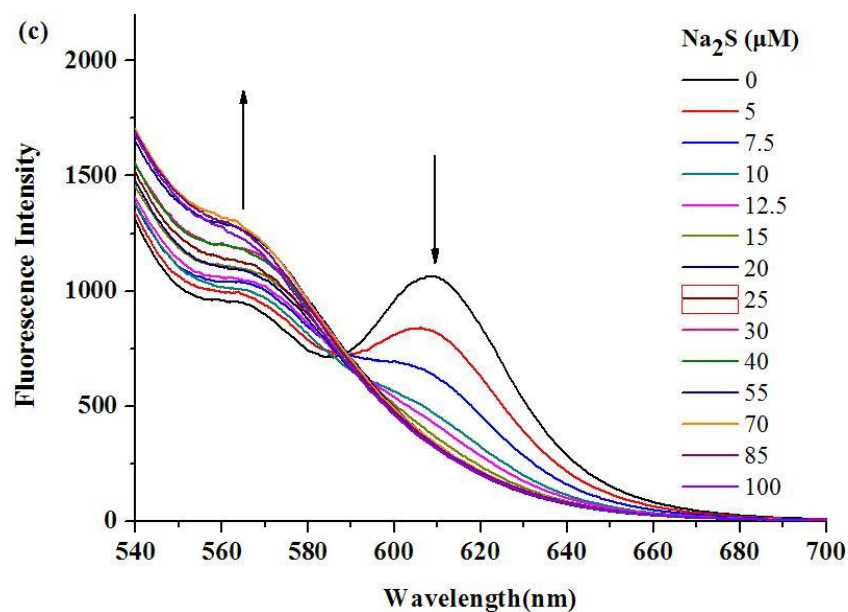


Figure S1. (a) Absorption and (b) fluorescent spectra of **1** (10 μM) in the absence and presence of different concentration of Na_2S . Each data point was acquired 20 min after addition in Triton X-100/DMSO/HEPES buffer (0.01: 1: 9, v/v/v, 10 mM, pH 7.4) at 37 $^{\circ}\text{C}$. $\lambda_{\text{ex}} = 582$ nm. (c) fluorescence spectral changes of **1** (10 μM) upon addition of Na_2S . Each spectrum was recorded 20 min after Na_2S addition. $\lambda_{\text{ex}} = 500$ nm.

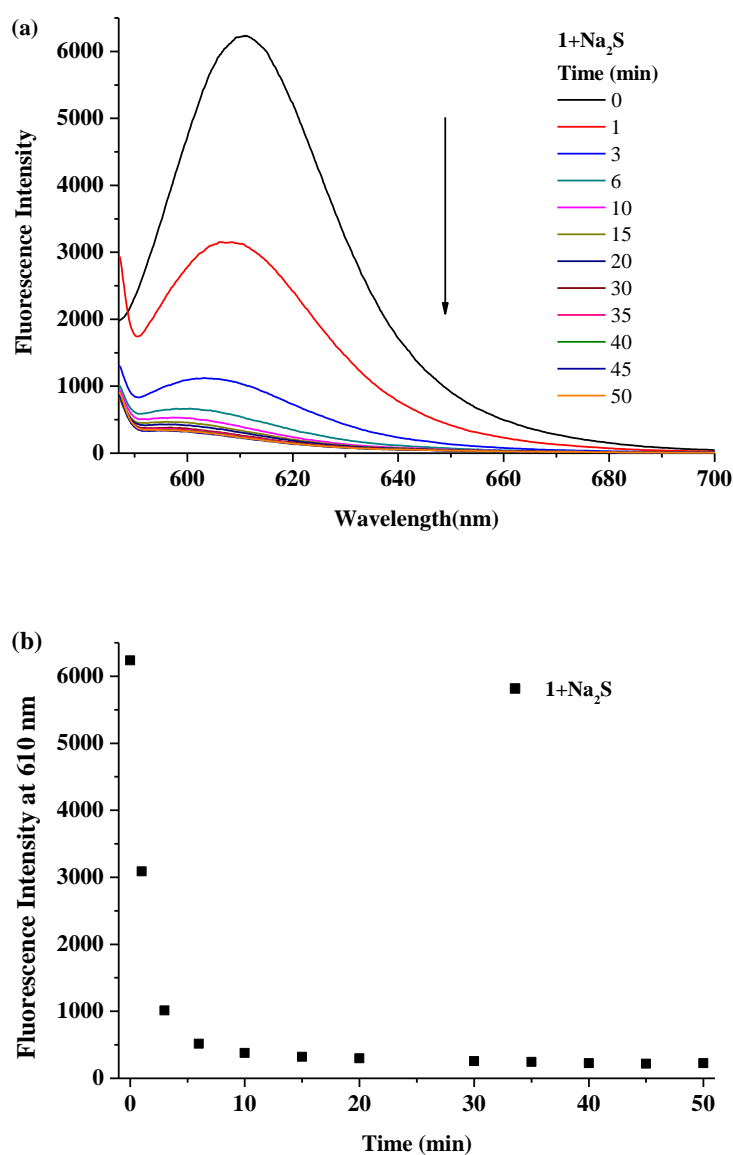
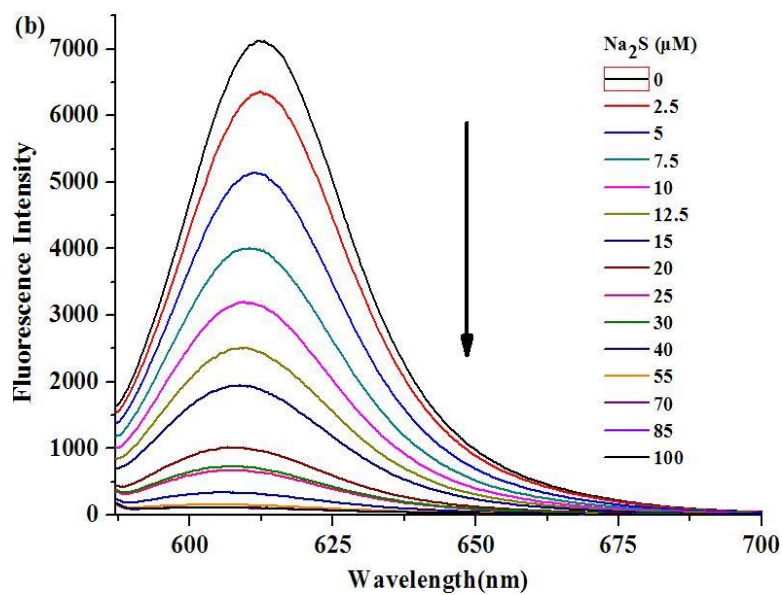
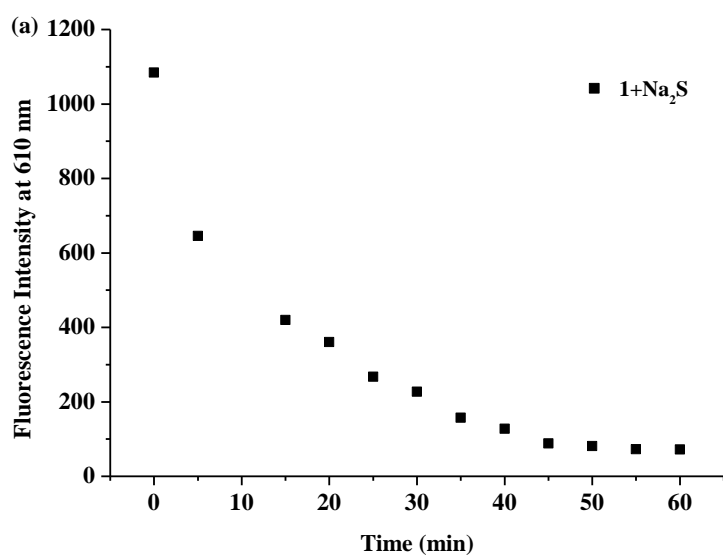


Figure S2. (a) Time-dependent fluorescence spectra of **1** (10 μM) with 10 equiv. of Na_2S in Triton X-100/DMSO/HEPES buffer (0.01: 1: 9, v/v/v, 10 mM, pH 7.4) at 37 $^\circ\text{C}$. (b) is time course of the response at 610 nm of (a), $\lambda_{\text{ex}} = 582 \text{ nm}$.



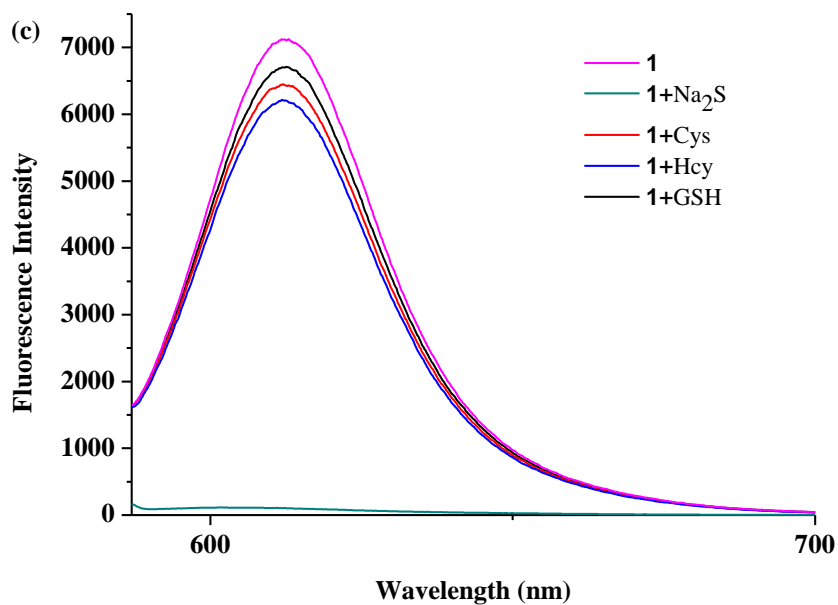


Figure S3. (a) Time course of the response at 610 nm of time-dependent fluorescence spectra of **1** (10 μM) with 10 equiv. of Na_2S . (b) Fluorescence spectral changes of **1** (10 μM) upon addition of Na_2S . (c) Emission response of **1** (10 μM) upon addition of 10 equiv. of biothiols and Na_2S . Each spectrum was recorded 50 min after Na_2S addition in DMSO/HEPES buffer (1: 1, v/v, 10 mM, pH 7.4) at 37 $^\circ\text{C}$, $\lambda_{\text{ex}} = 582 \text{ nm}$.)

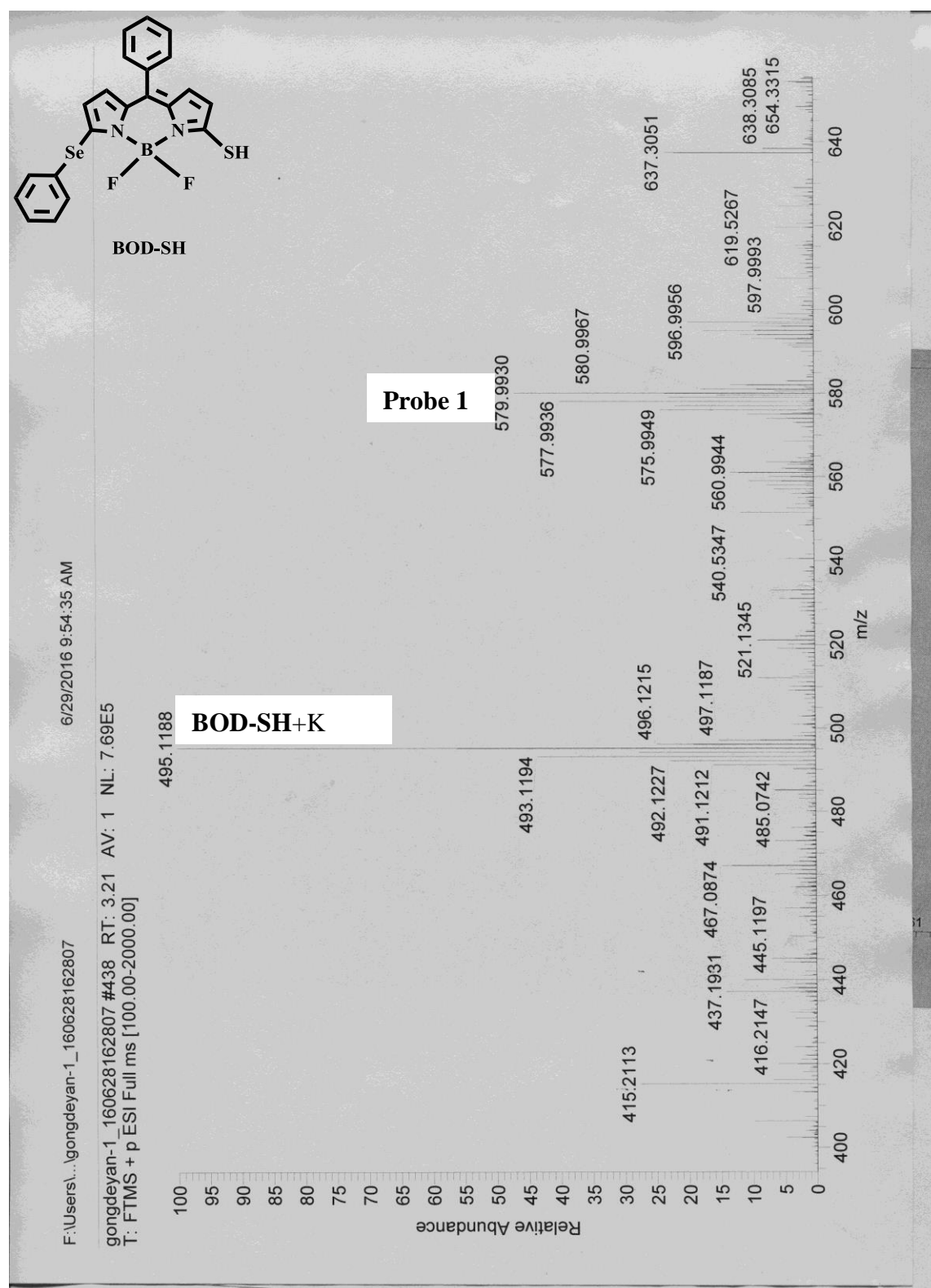


Figure S4. MS Spectrum of **1** in EtOH with Na₂S (1 equiv.) at 2 h reaction time at 25 °C. The predicted product is **BOD-SH** (the molecular weight of [**BOD-SH**] (C₂₁H₁₅BF₂N₂SSe) is 456.0). The peaks at m/z 495.1 (calcd = 495.0) corresponding to [**BOD-SH**+K].

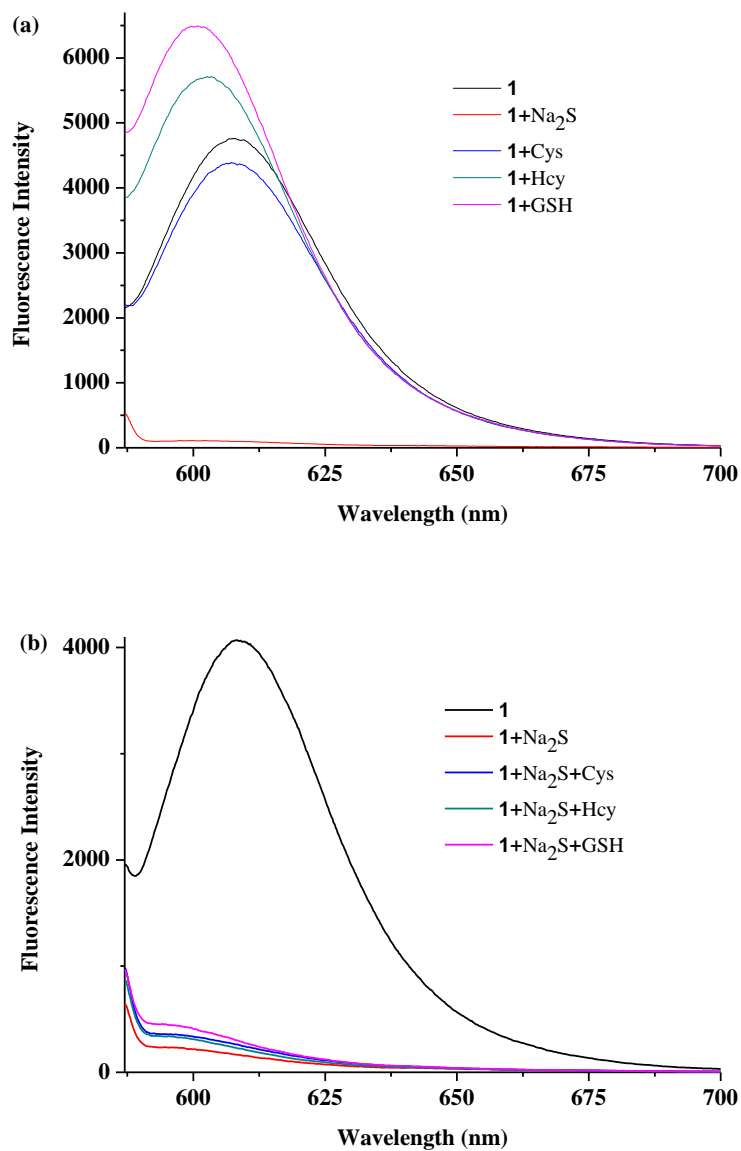


Figure S5. (a) Emission response of **1** (10 μ M) upon addition of 10 equiv. of biothiols and Na₂S. (b) the competition graph of **1** (10 μ M) in the presence of 10 equiv. of biothiols with Na₂S. Each data point was acquired 50 min after addition at 37 °C. λ_{ex} = 582 nm.

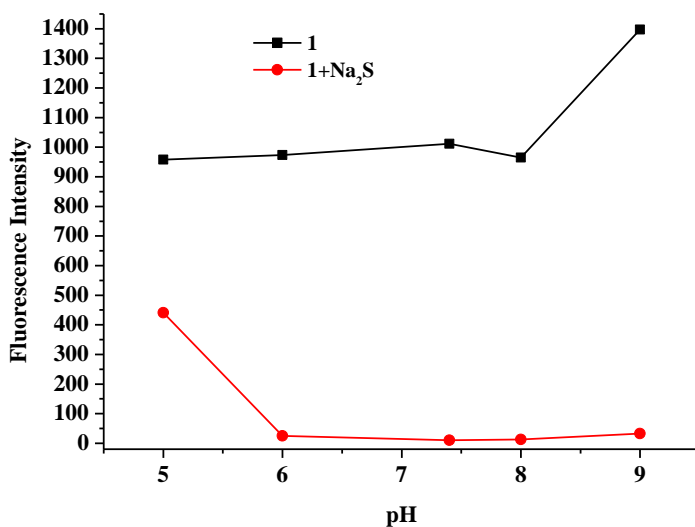


Figure S6. Fluorescence intensity of **1** at 610 nm in the absence and presence of 10 equiv. of Na₂S as a function of pH. **1** = 10 μ M, λ_{ex} = 582 nm. Each data point was acquired 50 min after addition of Na₂S in Triton X-100/DMSO/HEPES buffer (0.01: 1: 9, v/v/v, 10 mM, pH 7.4) at 37 °C.

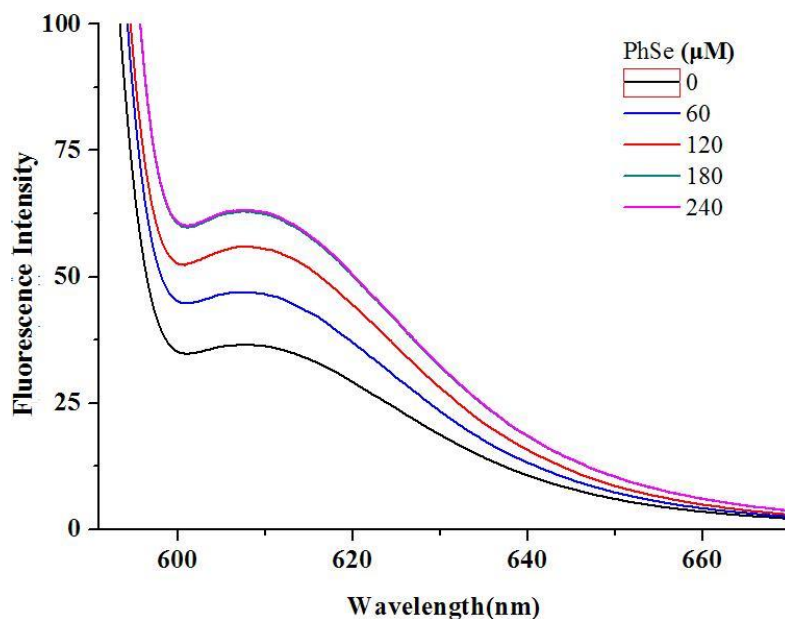
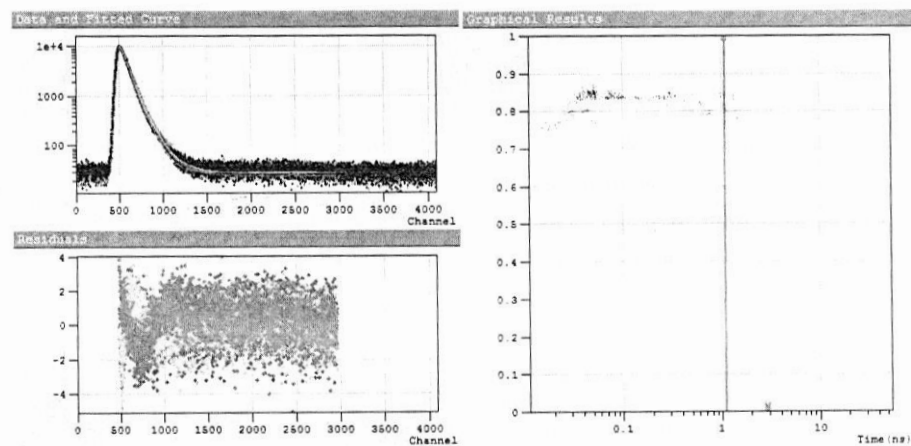


Figure S7. The fluorescence spectral changes of the **BOD-SH** mixture solution upon addition of different concentration of benzeneselenol. Each data point was acquired 20 min after addition of benzeneselenol in Triton X-100/DMSO/HEPES buffer (0.01: 1: 9, v/v/v, 10 mM, pH 7.4) at 37 °C, λ_{ex} = 582 nm.

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- ◆ 2PHSE-EX560EM630-DMSO-add irf.FL
- ◆ 2PHSE-EX560EM650-DMSO-add irf.FL
- ◆ 2PHSE-EX560EM590-DMSO-add irf.FL



File: 2PHSE-EX560EM610-DMSO-add irf.FL

❖ Global Analysis (Reconvolution)

Fitting range : [475; 2950] channels
Global χ^2 : 1.314
 χ^2 : 1.100

	B_i	ΔB_i	f_i (%)	Δf_i (%)	τ_i (ns)	$\Delta \tau_i$ (ns)
1	0.0282	4.2e-5	96.28	0.2803	1.037 linked	0.0013
2	0.0004	2.7e-5	3.7242	0.2645	2.733 linked	0.0072

Shift : 0.0026 ns (\pm 0 ns)
Decay : 27.27 (\pm 0)
Background : 12.40
IRF Background : 12.40

File: 2PHSE-EX560EM630-DMSO-add irf.FL

❖ Global Analysis (Reconvolution)

Fitting range : [475; 2950] channels
Global χ^2 : 1.314
 χ^2 : 1.454

	B_i	ΔB_i	f_i (%)	Δf_i (%)	τ_i (ns)	$\Delta \tau_i$ (ns)
1	0.0281	5.5e-5	94.85	0.3920	1.037 linked	0.0015
2	0.0006	3.9e-5	5.1468	0.4331	2.733 linked	0.0082

Shift : 0.0074 ns (\pm 0 ns)
Decay : 23.17 (\pm 0)
Background : 12.40
IRF Background : 12.40

File: 2PHSE-EX560EM650-DMSO-add irf.FL

❖ Global Analysis (Reconvolution)

Fitting range : [475; 2950] channels

Global χ^2 : 1.314

χ^2 : 1.243

	B_i	ΔB_i	f_i (%)	Δf_i (%)	τ_i (ns)	$\Delta \tau_i$ (ns)
1	0.0282	5.3e-5	94.56	0.3410	1.037 linked	0.0014
2	0.0006	3.8e-5	5.4400	0.3890	2.733 linked	0.0076

Shift : 0.0082 ns (\pm 0 ns)

Decay : 26.88 (\pm 0)

Background : 12.40

File: 2PHSE-EX560EM590-DMSO-add irf.FL

❖ Global Analysis (Reconvolution)

Fitting range : [475; 2950] channels

Global χ^2 : 1.314

χ^2 : 1.458

	B_i	ΔB_i	f_i (%)	Δf_i (%)	τ_i (ns)	$\Delta \tau_i$ (ns)
1	0.0280	4.6e-5	98.88	0.3729	1.037 linked	0.0015
2	0.0001	1.8e-5	1.1161	0.2079	2.733 linked	0.0082

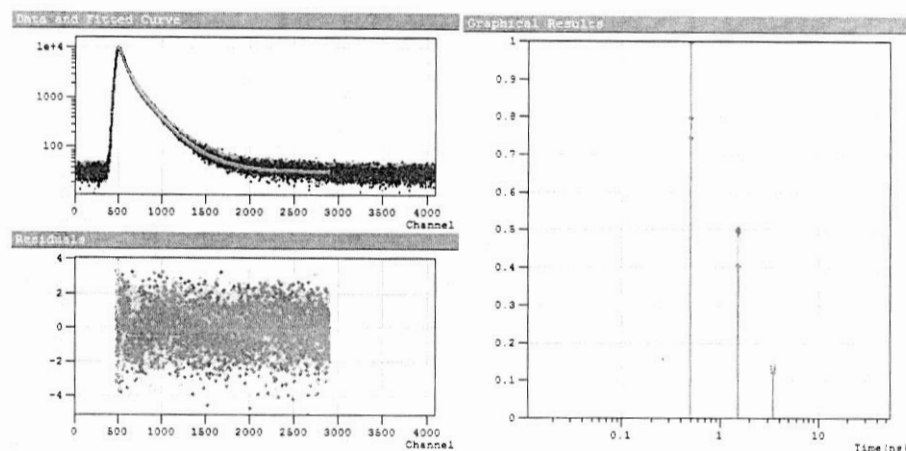
Shift : -0.0131 ns (\pm 0 ns)

Decay : 27.72 (\pm 0)

Background : 12.40

Figure S8. Global analysis of decay times result of **1** (10 μ M) in Triton X-100/DMSO/HEPES buffer (0.01: 1: 9, v/v/v, 10 mM, pH 7.4) at 37 °C, each spectrum was recorded at the same excitation wavelength (560 nm), but at different emission wavelength (590, 610, 630 and 650 nm).

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- ◆ 2PHSE-EX560EM630-DMSO-13-25-add irf.FL
- ◆ 2PHSE-EX560EM650-DMSO-13-25-add irf.FL
- ◆ 2PHSE-EX560EM590-DMSO-13-25-add irf.FL



File: 2PHSE-EX560EM610-DMSO-13-25-add irf.FL

◆ Global Analysis (Reconvolution)

Fitting range : [475; 2890] channels
Global χ^2 : 1.109
 χ^2 : 1.057

	B_i	ΔB_i	f_i (%)	Δf_i (%)	τ_i (ns)	$\Delta \tau_i$ (ns)
1	0.0177	0.0002	24.90	1.6047	0.483 linked	0.0246
2	0.0109	0.0001	47.00	0.9672	1.473 linked	0.0098
3	0.0029	10.0e-5	28.10	1.0211	3.339 linked	0.0025

Shift : -0.1332 ns (± 0 ns)
Decay : 29.74 (± 0)
Background : 12.40
IRF Background : 12.40

File: 2PHSE-EX560EM630-DMSO-13-25-add irf.FL

◆ Global Analysis (Reconvolution)

Fitting range : [475; 2890] channels
Global χ^2 : 1.109
 χ^2 : 1.087

	B_i	ΔB_i	f_i (%)	Δf_i (%)	τ_i (ns)	$\Delta \tau_i$ (ns)
1	0.0165	0.0002	23.07	1.5532	0.483 linked	0.0250
2	0.0111	0.0001	47.45	0.9732	1.473 linked	0.0099
3	0.0031	0.0001	29.48	1.0904	3.339 linked	0.0026

Shift : -0.1182 ns (± 0 ns)
Decay : 29.74 (± 0)

Background : 31.40 (± 0)
 IRF Background : 12.40

File: 2PHSE-EX560EM650-DMSO-13-25-add irf.FL

❖ Global Analysis (Reconvolution)

Fitting range : [475; 2890] channels
 Global χ^2 : 1.109
 χ^2 : 1.150

	B_i	ΔB_i	f_i (%)	Δf_i (%)	τ_i (ns)	$\Delta \tau_i$ (ns)
1	0.0173	0.0002	24.32	1.6864	0.483 linked	0.0257
2	0.0105	0.0001	45.15	0.9962	1.473 linked	0.0102
3	0.0031	0.0001	30.54	1.1556	3.339 linked	0.0027

Shift : -0.1041 ns (± 0 ns)
 Decay : 32.28 (± 0)
 Background : 32.28 (± 0)
 IRF Background : 12.40

File: 2PHSE-EX560EM590-DMSO-13-25-add irf.FL

❖ Global Analysis (Reconvolution)

Fitting range : [475; 2890] channels
 Global χ^2 : 1.109
 χ^2 : 1.143

	B_i	ΔB_i	f_i (%)	Δf_i (%)	τ_i (ns)	$\Delta \tau_i$ (ns)
1	0.0222	0.0002	32.78	2.1117	0.483 linked	0.0256
2	0.0090	0.0002	40.38	1.0272	1.473 linked	0.0102
3	0.0026	8.9e-5	26.85	0.9948	3.339 linked	0.0026

Shift : -0.0598 ns (± 0 ns)
 Decay : 29.11 (± 0)
 Background : 29.11 (± 0)
 IRF Background : 12.40

Figure S9. Global analysis of decay times result of **1** (10 μ M) in addition of 25 μ M Na₂S in Triton X-100/DMSO/HEPES buffer (0.01: 1: 9, v/v/v, 10 mM, pH 7.4) at 37 °C, each spectrum was recorded 20 min after Na₂S addition at the same excitation wavelength (560 nm), but at different emission wavelength (590, 610, 630 and 650 nm).

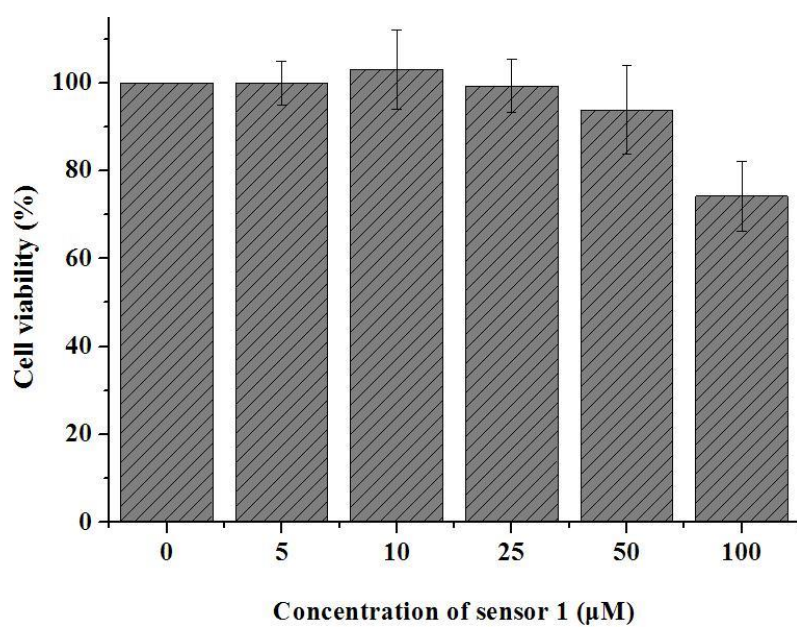
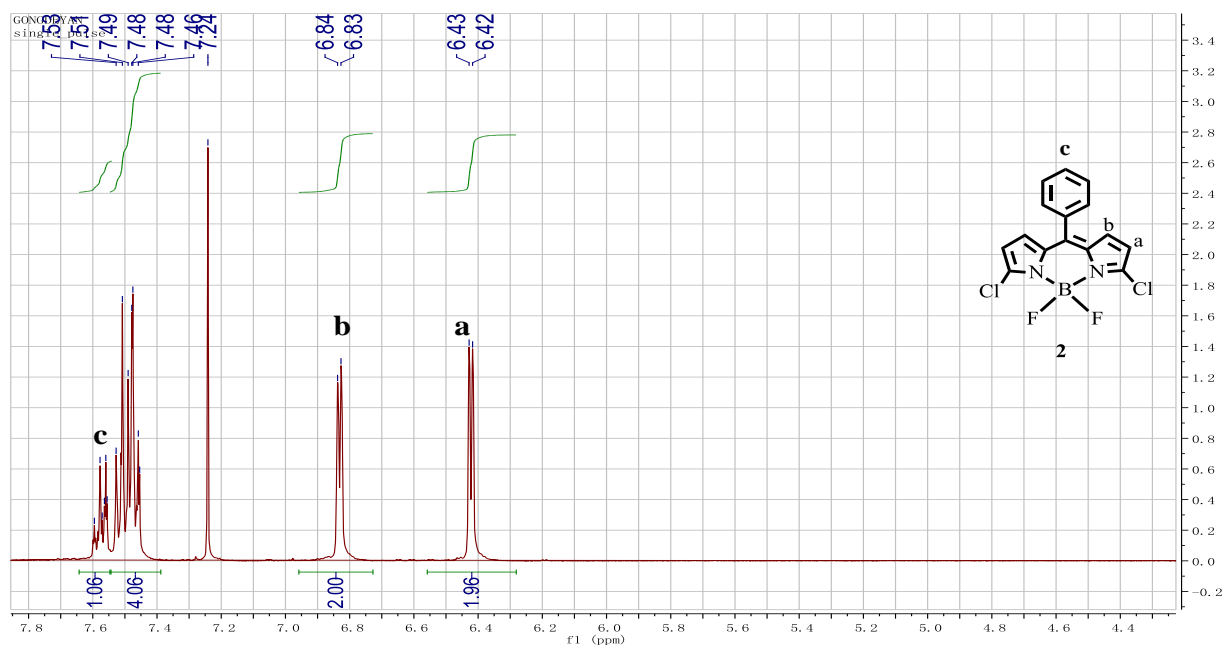
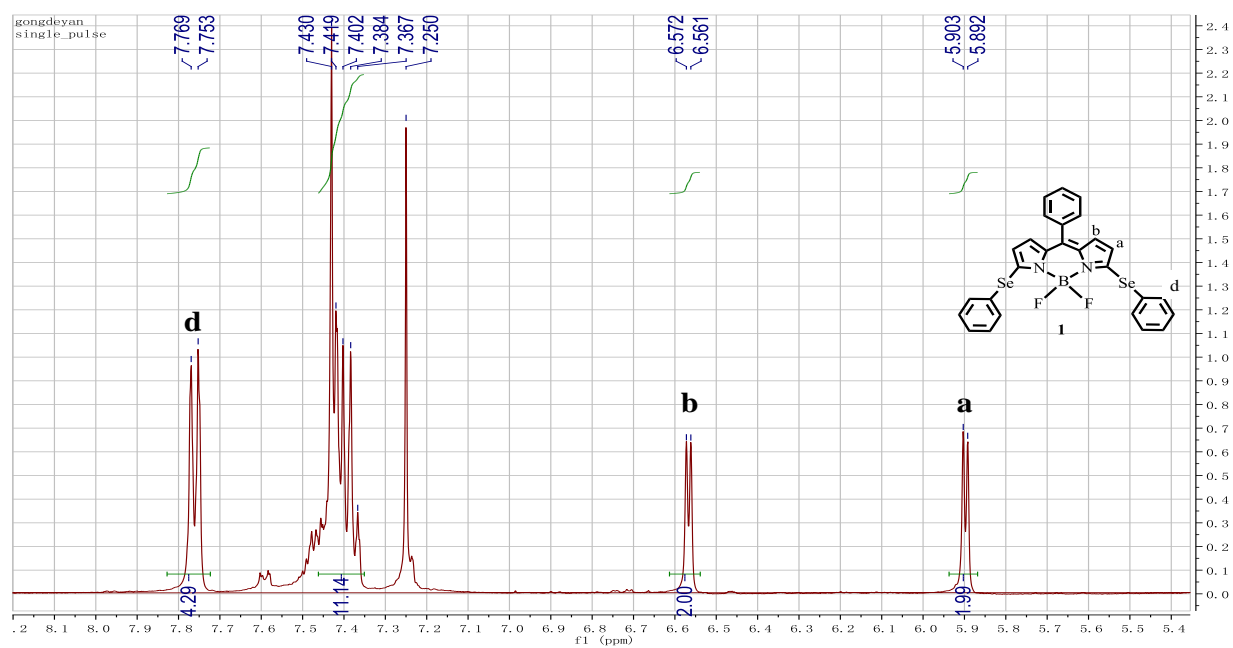


Figure S10. Effects of **1** at varied concentrations on the viability of BHK cells. The cell viability data were checked three times.

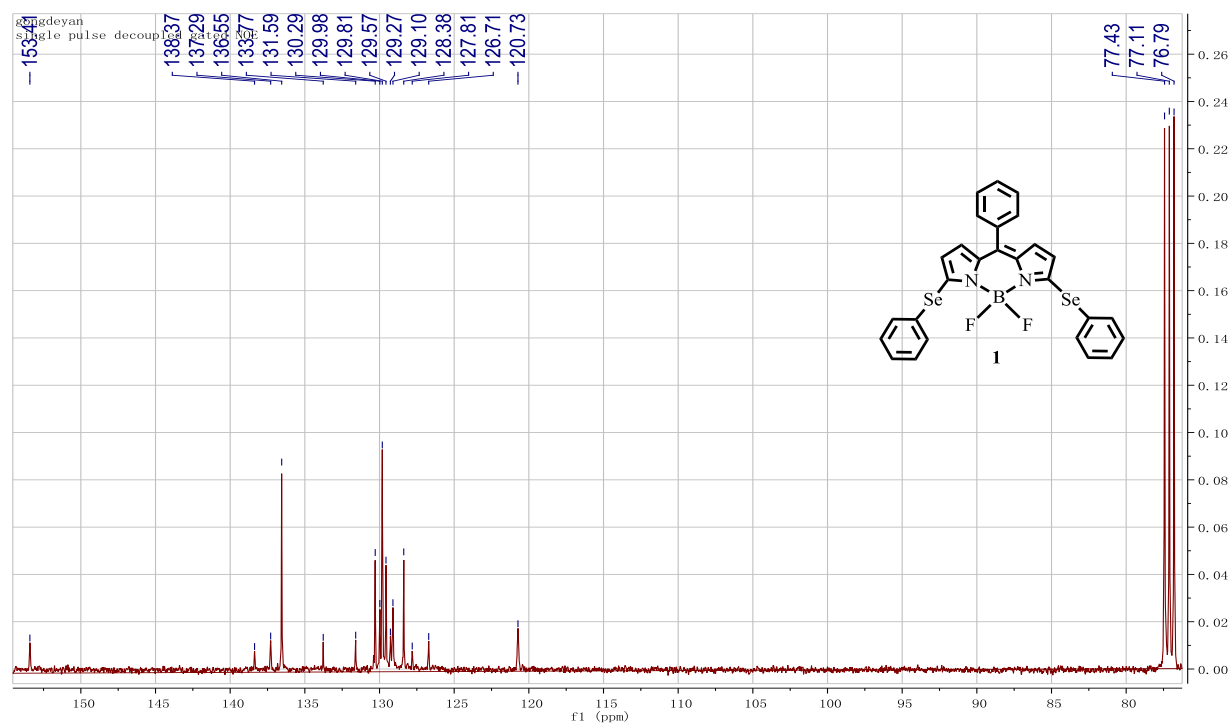
^1H NMR (CDCl_3 , 400 MHz) spectrum of 2



^1H NMR (CDCl_3 , 400 MHz) spectrum of 1



^{13}C NMR (CDCl₃, 400/4 MHz) spectrum of **1**



MS Spectrum of **2** (C₁₅H₉BCl₂F₂N₂, the molecular weight of **2** is 336.9).

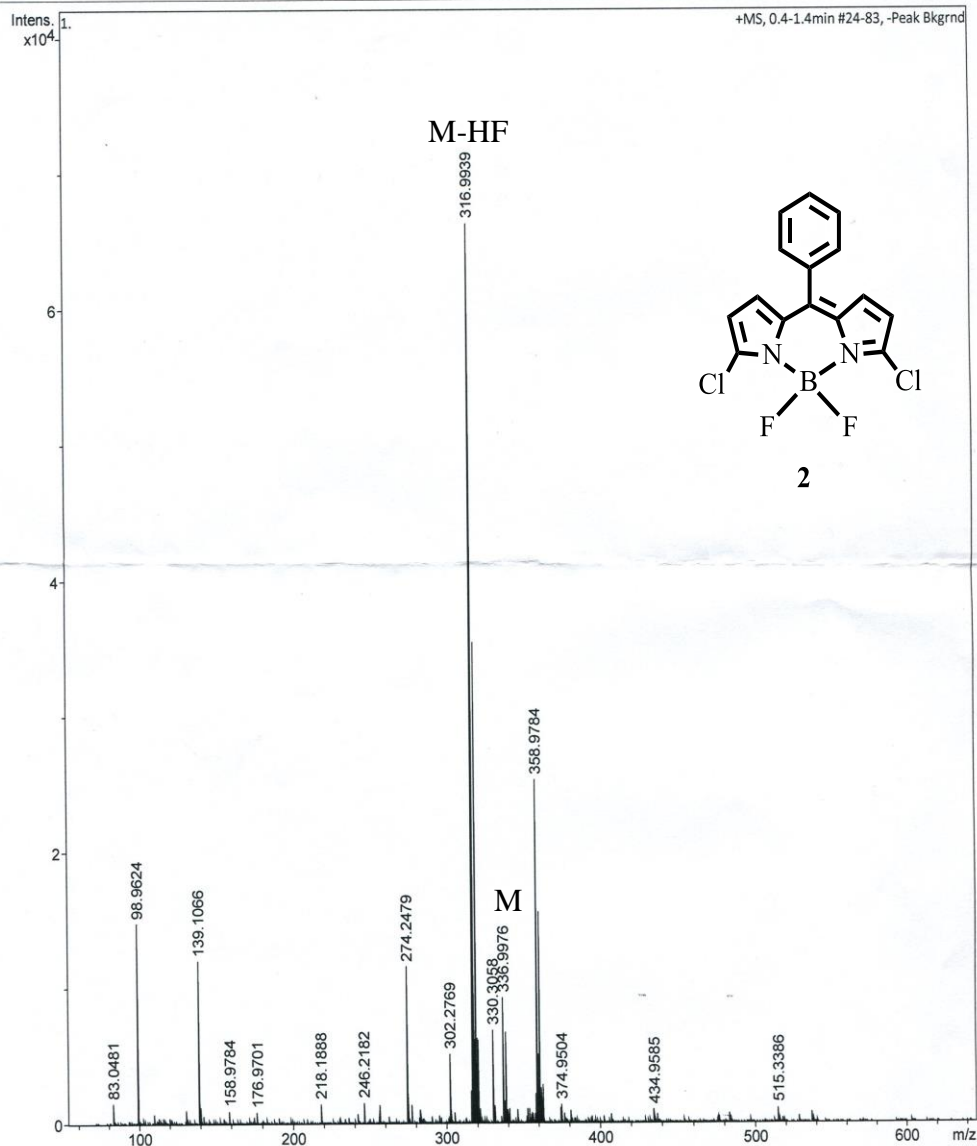
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Analysis Info

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Comment

Acquisition Date 3/5/2015 10:49:18 AM

Operator LZU
Instrument micrOTOF



printed: 3/5/2015 11:05:41 AM

by: LZU

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HRMS Spectrum of **1** (the molecular weight of [**1**] ($C_{17}H_{13}BClF_2N_4O$) is 579.9940).

