Electronic supplementary information (ESI) for

BODIPY-based selenides as fluorescent probes for rapid, sensitive and mitochondria-specific detection of hypochlorous acid

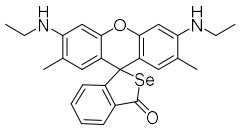
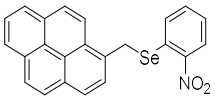
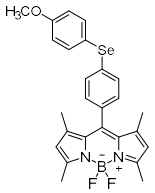
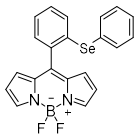
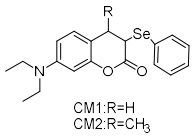
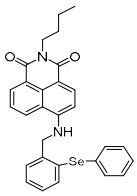
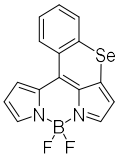
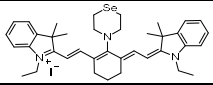
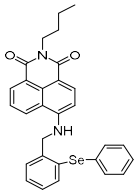
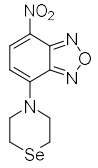
Xiang-Hong Xu, Chao Liu, Yuan Mei and Qin-Hua Song*

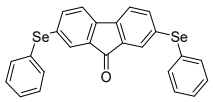
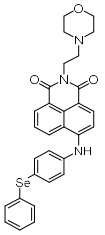
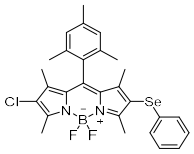
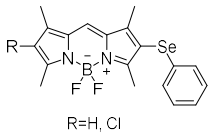
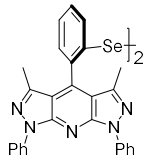
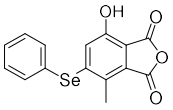
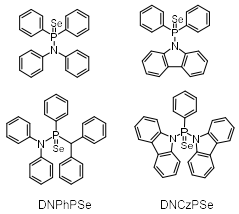
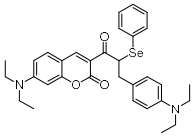
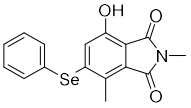
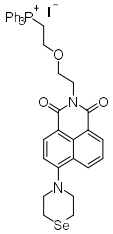
Department of Chemistry, University of Science and Technology of China, Hefei 230026, P. R. China

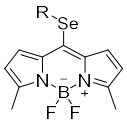
E-mail: qhsong@ustc.edu.cn

Contents

1. Table 1. Summary of organoselenium-based fluorescent probes for HClO

entry	Probes	Response time	LOD	λ^{abs}_{max} /λ^{em}_{max}	References
1		--[a]	--[a]	500 nm /545 nm	*Chem. Commun.* **2011**, *47*, 4373.
2		10 min	--[a]	--/392 nm	*Tetrahedron Lett.* **2012**, *53*, 3843.
3[b]		5 min	980 nM	500 nm /510 nm	*Chem. Commun.* **2013**, *49*, 1014.
4		5 min	7.98 nM	--[a] /526 nm	*Org. Lett.* **2013**, *15*, 878.
5		CM1: 20 s	CM1: 10 nM	CM1: -- /480 nm	*Org. Lett.* **2013**, *15*, 2002.
6		10 min	586 nM	430 nm /523 nm	*Chem. Commun.* **2013**, *49*, 2445.
7		--[a]	--[a]	524 nm /590 nm	*Org. Lett.* **2014**, *16*, 520.
8		100 s	310 nM	750 nm /786 nm	*Chem. Commun.* **2014**, *50*, 1018.
9		10 min	586 nM	430 nm /523 nm	*Phys. Chem. Chem. Phys.* **2014**, 16, 3749.
10[b]		< 10 s	3.3 nM	483 nm /544 nm	*RSC Adv.* **2015**, *5*, 79519.

11[b]		2 min	350 nM	415 nm /520 nm	*Chem. Commun.* **2015**, *51*, 10150.
12[b]		a few seconds	18.5 nM	452 nm /540 nm	*J. Photochem. Photobiol. A.* **2015**, *299*, 1.
13[a]		1-2 min	19.6 nM	520 nm /526 nm	*Chem.-Asian J.* **2016**, *11*, *24*, 3598.
14[b]	 R=H, Cl	< 1 s	R=H: 30.9 nM R=Cl: 4.5 nM	R=H: 512 nm /507 nm R=Cl: 526 nm /526 nm	*Chem. Eur. J.* **2016**, *22*, 9642.
15[b]		7 min	360	--[a] /436 nm	*RSC Adv.* **2016**, *6*, 32013.
16		< 1 s	--[a]	403 nm /502 nm	*Org. Lett.* **2018**, *20,* 3557
17	 DNPhPSe DNCzPSe	--[a]	--[a]	NCzPSe: 360 nm /345 nm DNCzPSe: 360 nm /360 nm	*Chem. Commun.* **2018**, *54*, 2926.
18		< 5 s	4.6 nM	450 nm /618 nm	*Chem. Commun.* **2018**, *54*, 11965.
19		< 1 s	90 nM	396 nm /523 nm	*ACS Omega* **2018**, *3*, 13474.
20		< 2 s	4.8 nM	410 nm /540 nm	*New J. Chem.* **2018**, *42*, 15105.

21	 BSe-1: R = Et BBSe: R = CH_2Ph BSe-2: R = Ph	BSe-Et: < 2 s BSe-Bz: < 5 s BSe-Ph: > 100 s	BSe-Et: 0.3 nM BSe-Bz: 0.8 nM BSe-Ph: 9.2 nM	BSe-Et: 520 nm /548 nm BSe-Bz: 532 nm /545 nm BSe-Ph: 532 nm /550 nm	This work

[a] No available.

[b] Work in a solvent mixture.

2. Determination of Fluorescence Quantum Yield (Φ_f)

The fluorescence quantum yields for three probes and their reaction systems with HClO were determined with 8-chloro-BODIPY (Φ_f = 0.72 in THF) as a reference. The probe was dissolved in phosphate buffer solutions (20 mM, pH 7.4, 0.1% CH_3CN) and 8-Cl-BODIPY was dissolved in THF to suitable concentration (A < 0.05 at 495 nm). Their absorbance and fluorescence spectra were determined at this concentration. Finally, the fluorescence quantum yields of the probe and the reaction system with HClO (molar ratio of probe/HClO, 1:1) were calculated by the equation of $\Phi_x = \Phi_s (F_x/F_s)(A_s/A_x)(n_x/n_s)^2$. Where, x & s indicate the unknown and standard solution respectively, Φ = quantum yield, F = area under the emission curve, A = absorbance at excitation wave length, n = index of refraction of the solvent.

3. Spectral response and limit of detections (LODs)

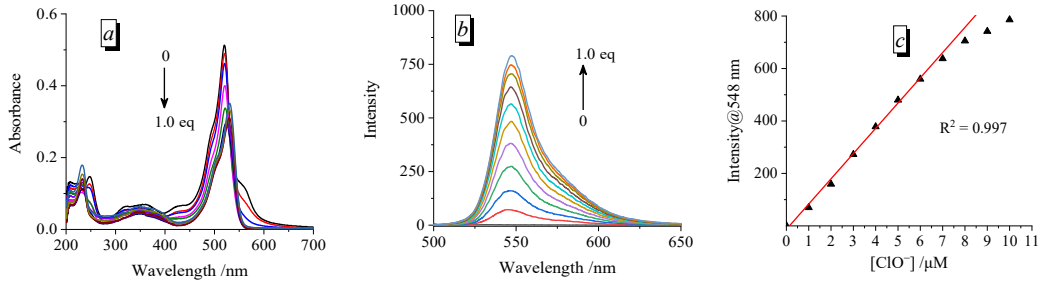


Figure S1. (a) UV/vis absorption and (b) fluorescence spectra of the probe BSe-Et (10 μM) in PBS buffer (pH=7.4, 20 mM, 0.1% CH_3CN) upon the addition of ClO^- (0-1.0 equiv.) recorded after 1 min, excitation at 495 nm. (c) The linear fitting diagram from (b).

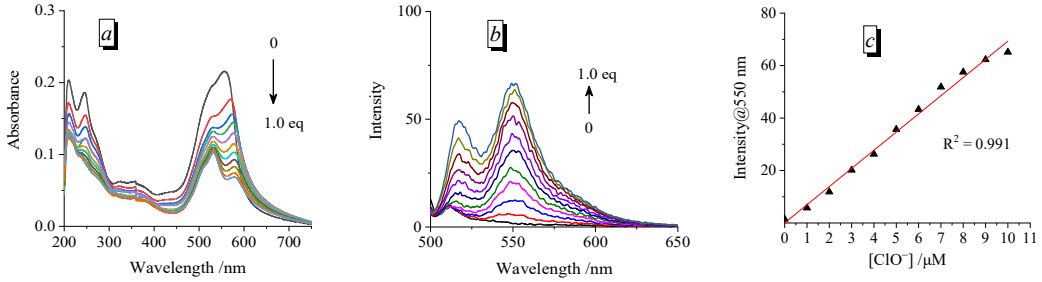


Figure S2. (a) UV/vis absorption and (b) fluorescence spectra of the probe BSe-Ph (10 μM) in PBS buffer (pH=7.4, 20 mM, 0.1% CH_3CN) upon the addition of ClO^- (0-1.0 equiv.) recorded after 5 min, excitation at 495 nm. (c) The linear fitting diagram from (b).

4. Photo- and thermostability of BSe-Bz, BSe-Et or BSe-Ph and their sensing HClO solutions

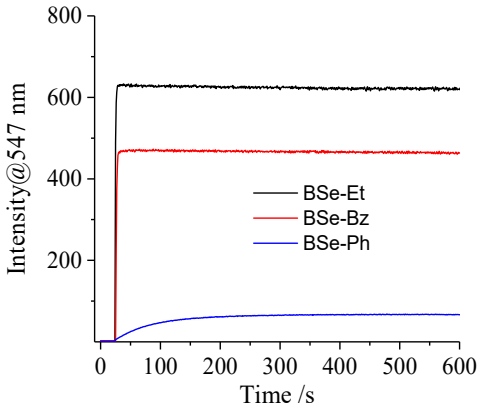


Figure S3. Time-dependent fluorescence intensity of three probes, BSe-Et, BSe-Bz, or BSe-Ph (10 μM) upon the addition of HClO (10 μM), excitation at 495 nm.

5. HRMS for the mixture of BSe-Bz or BSe-Et with HClO

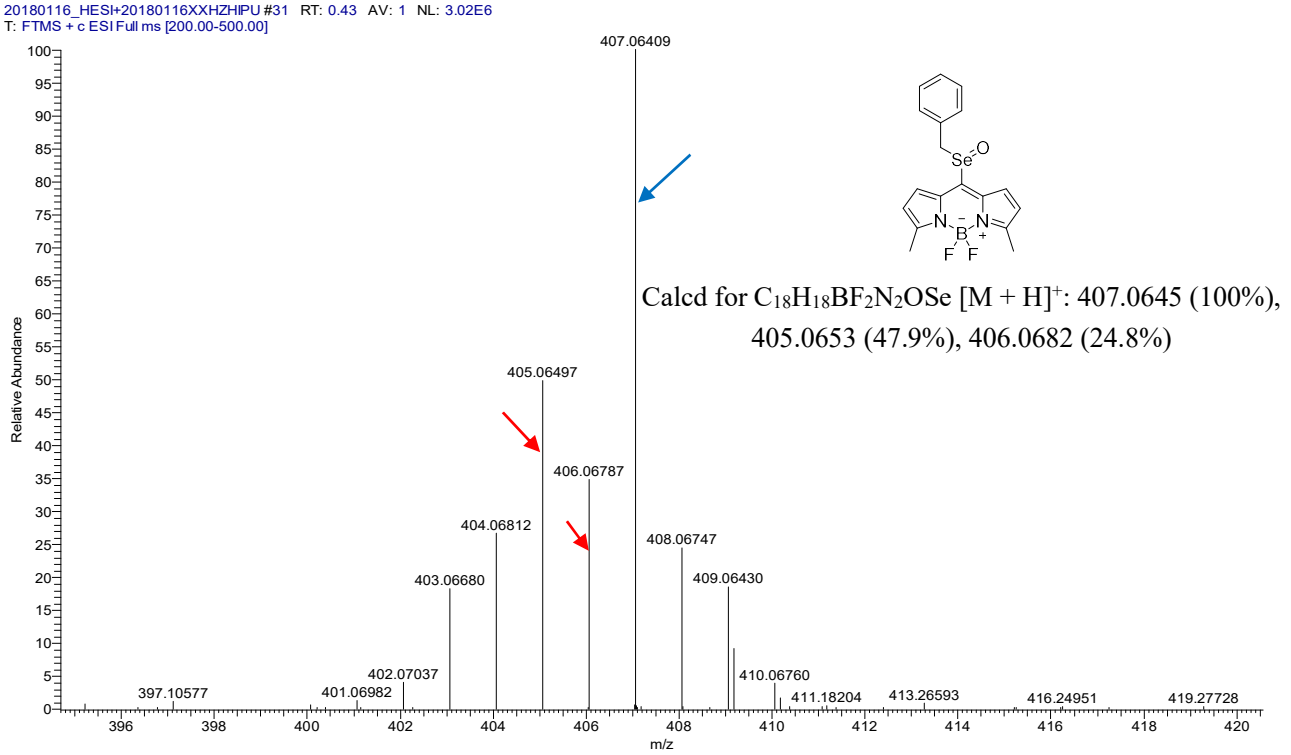


Figure S4. HRMS of the sensing mixture of BSe-Bz toward NaClO

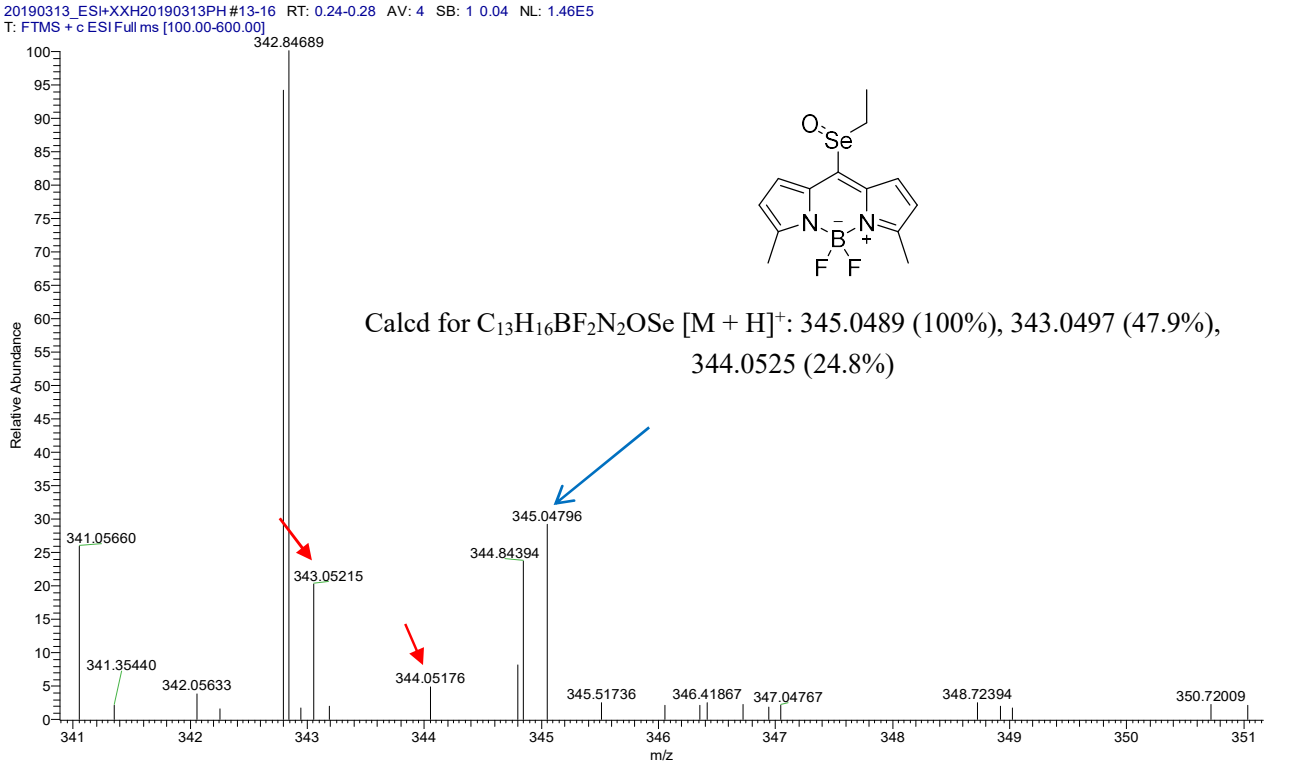


Figure S5. HRMS of the sensing mixture of BSe-Et toward NaClO

6. The selectivity of three probes for HClO

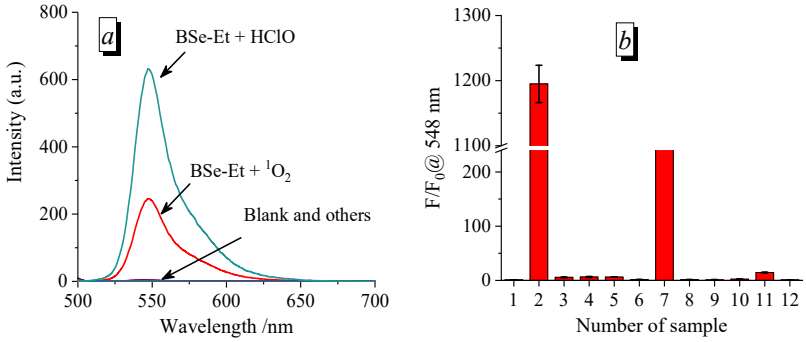


Figure S6a. (a) Fluorescence spectra and (b) intensity at 548 nm of BSe-Et (10 μM) upon the addition of 10 μM HClO and 100 μM other species (1: blank, 2: HClO, 3: H_2O_2, 4: $ONOO^-$, 5: $^{\bullet}OH$, 6: $O_2^{\bullet-}$, 7: $^1O_2$, 8: TBHP, 9: CH_3COO^-, 10: NO, 11: NO_2^-, 12: $TBO^{\bullet}$) in PBS buffer (pH=7.4, 20 mM, 0.1% CH_3CN) recorded after 30 min. Excitation at 495 nm.

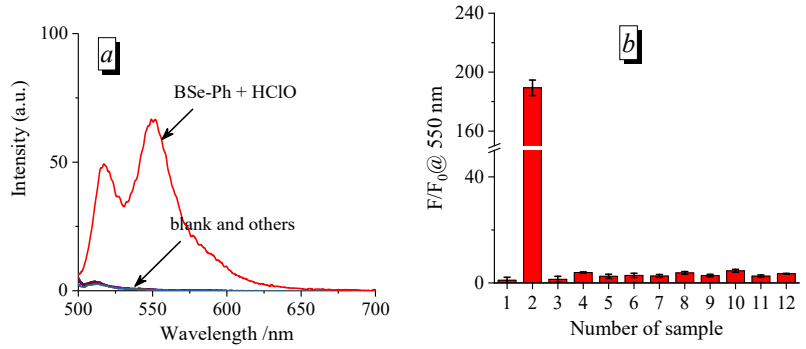


Figure S6b. (a) Fluorescence spectra and (b) intensity at 550 nm of BSe-Ph (10 μM) upon the addition of 10 μM HClO and 100 μM other species (1: blank, 2: HClO, 3: H_2O_2, 4: $ONOO^-$, 5: $^{\bullet}OH$, 6: $O_2^{\bullet-}$, 7: $^1O_2$, 8: TBHP, 9: CH_3COO^-, 10: NO, 11: NO_2^-, 12: $TBO^{\bullet}$) in PBS buffer (pH=7.4, 20 mM, 0.1% CH_3CN) recorded after 30 min. Excitation at 495 nm.

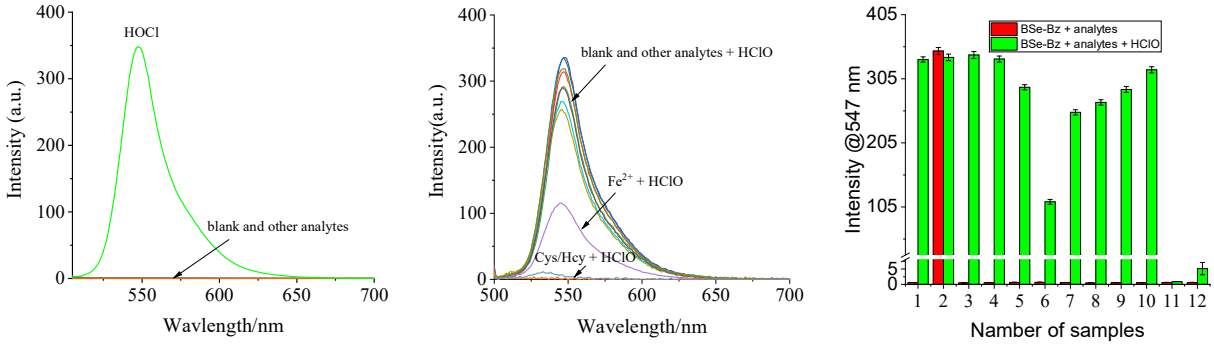


Figure S7. (a) Fluorescence intensity of BSe-Bz (10 μM) upon the addition of 10 μM NaClO and 100 μM other analytes (1: blank, 2: ClO^-, 3: Fe^{3+}, 4: Cu^{2+}, 5: Mg^{2+}, 6: Fe^{2+}, 7: Zn^{2+}, 8: Ca^{2+}, 9: K^+, 10: Na^+, 11: Cys, 12: Hcy) in PBS buffer (pH = 7.4, 20 mM, 0.1% CH_3CN) recorded after 30 min, (b) further addition of HClO into above solutions, excitation at 495 nm. (c) Intensities at 547 nm for all samples from (a) and (b).

7. pH effect on the probes BSe-Et, BSe-Bz and BSe-Ph and their sensing HClO

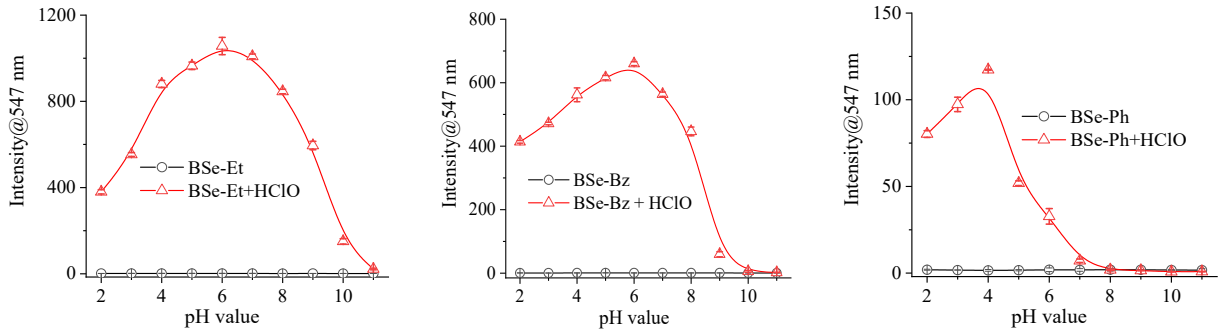


Figure S8. Fluorescence responses of three probes BSe-Et, BSe-Bz or BSe-Ph (10 μM) and their sensing NaClO (10 μM) at different pH solutions (pH 2-11).

8. Cell experiments

MTT assay

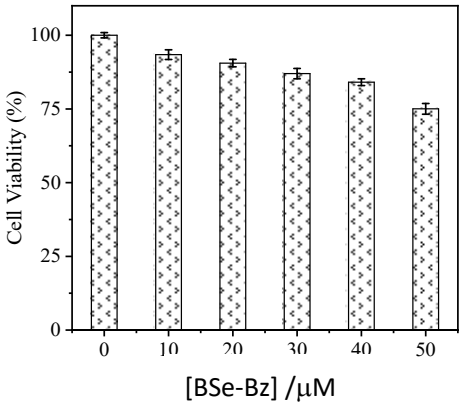
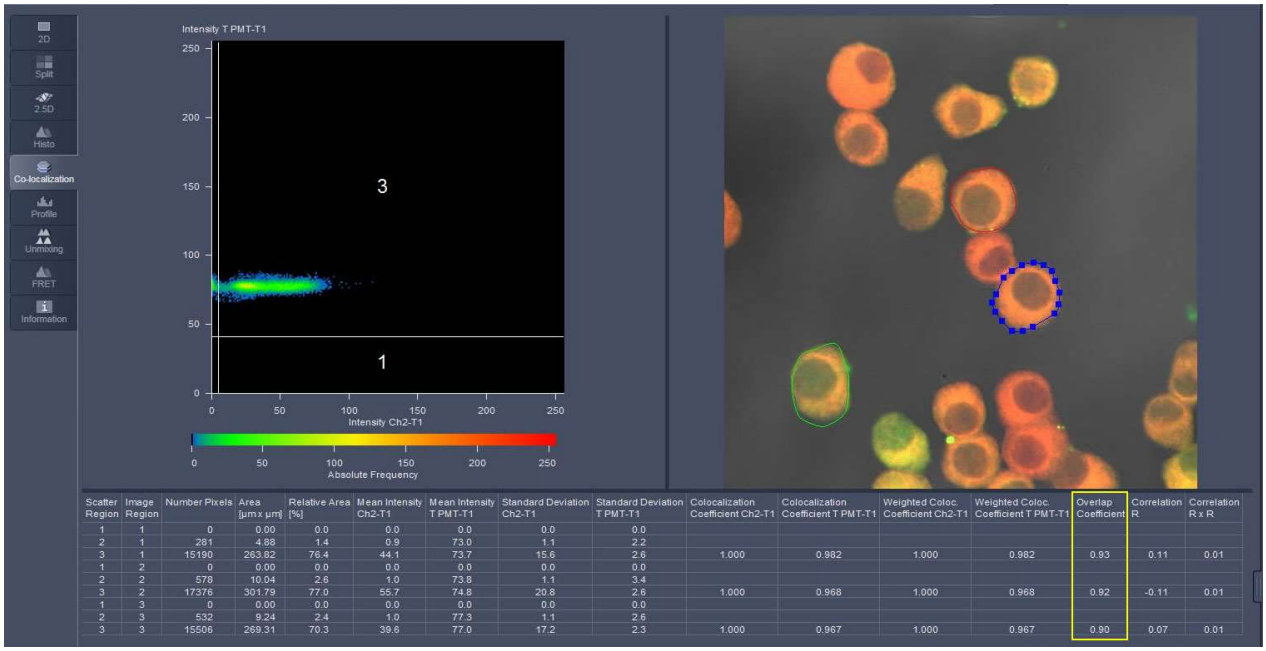


Figure S9. Viability of RAW264.7 cells incubated with probe BSe-Bz (0-50 μM) for 24 h. Data are mean ± SE (bars) (n = 3).

Co-localization assay

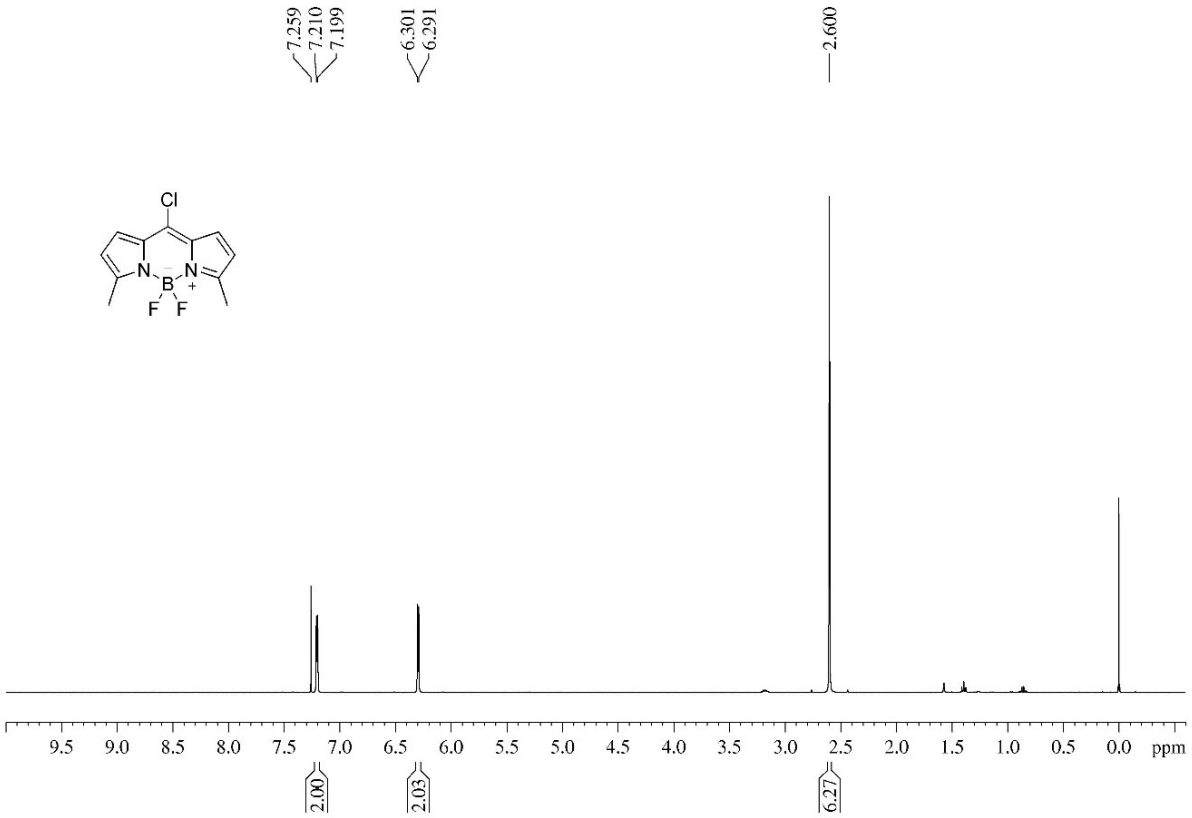


Scatter Region	Image Region	Number Pixels	Area [µm x µm]	Relative Area [%]	Mean Intensity Ch2-T1	Mean Intensity T PMT-T1	Standard Deviation Ch2-T1	Standard Deviation T PMT-T1	Colocalization Coefficient Ch2-T1	Colocalization Coefficient T PMT-T1	Weighted Coloc. Coefficient Ch2-T1	Weighted Coloc. Coefficient T PMT-T1	Overlap Coefficient	Correlation R	Correlation R x R
1	1	0	0.00	0.0	0.0	0.0	0.0	0.0							
2	1	281	4.88	1.4	0.9	73.0	1.1	2.2							
3	1	15190	263.82	76.4	44.1	73.7	15.6	2.6	1.000	0.982	1.000	0.982	0.93	0.11	0.01
1	2	0	0.00	0.0	0.0	0.0	0.0	0.0							
2	2	578	10.04	2.6	1.0	73.8	1.1	3.4							
3	2	17376	301.79	77.0	55.7	74.8	20.8	2.6	1.000	0.968	1.000	0.968	0.92	-0.11	0.01
1	3	0	0.00	0.0	0.0	0.0	0.0	0.0							
2	3	532	9.24	2.4	1.0	77.3	1.1	2.6							
3	3	15506	269.31	70.3	39.6	77.0	17.2	2.3	1.000	0.967	1.000	0.967	0.90	0.07	0.01

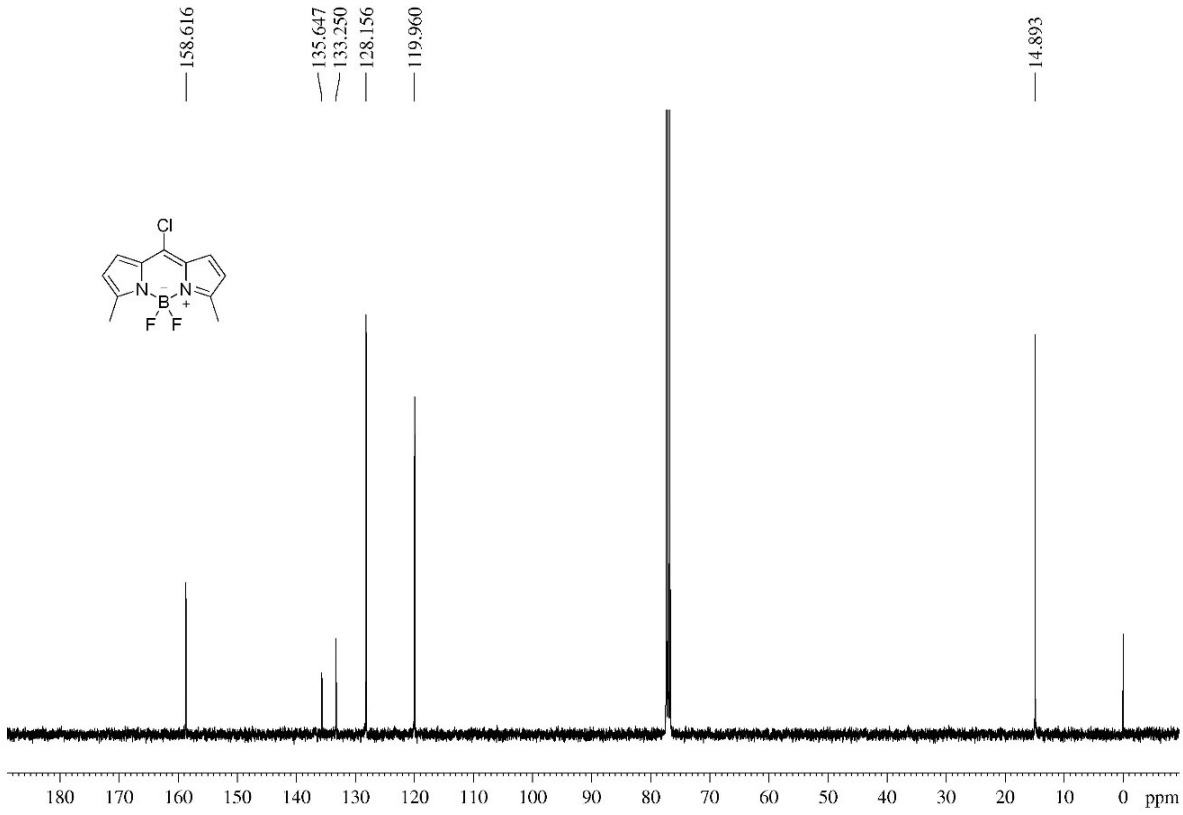
Figure S10. Co-localization assay for fluorescence images of RAW 264.7 cells incubated with BSe-Bz (10 µM) for 0.5 h, further incubated with HClO (10 µM) for 0.5 h, and a mitochondrial dye (MitoTracker@ Deep Red FM) (0.5 µM) for 0.5 h. Merged image of (a) fluorescence image from a green-channel (495-598 nm), λ_{ex} = 488 nm and (b) fluorescence image from a red channel (640-728 nm), λ_{ex} = 633 nm; and overlap coefficients of two-channel images in three interesting area are 0.93, 0.92 and 0.90.

9. Copies of NMR spectra of related compounds

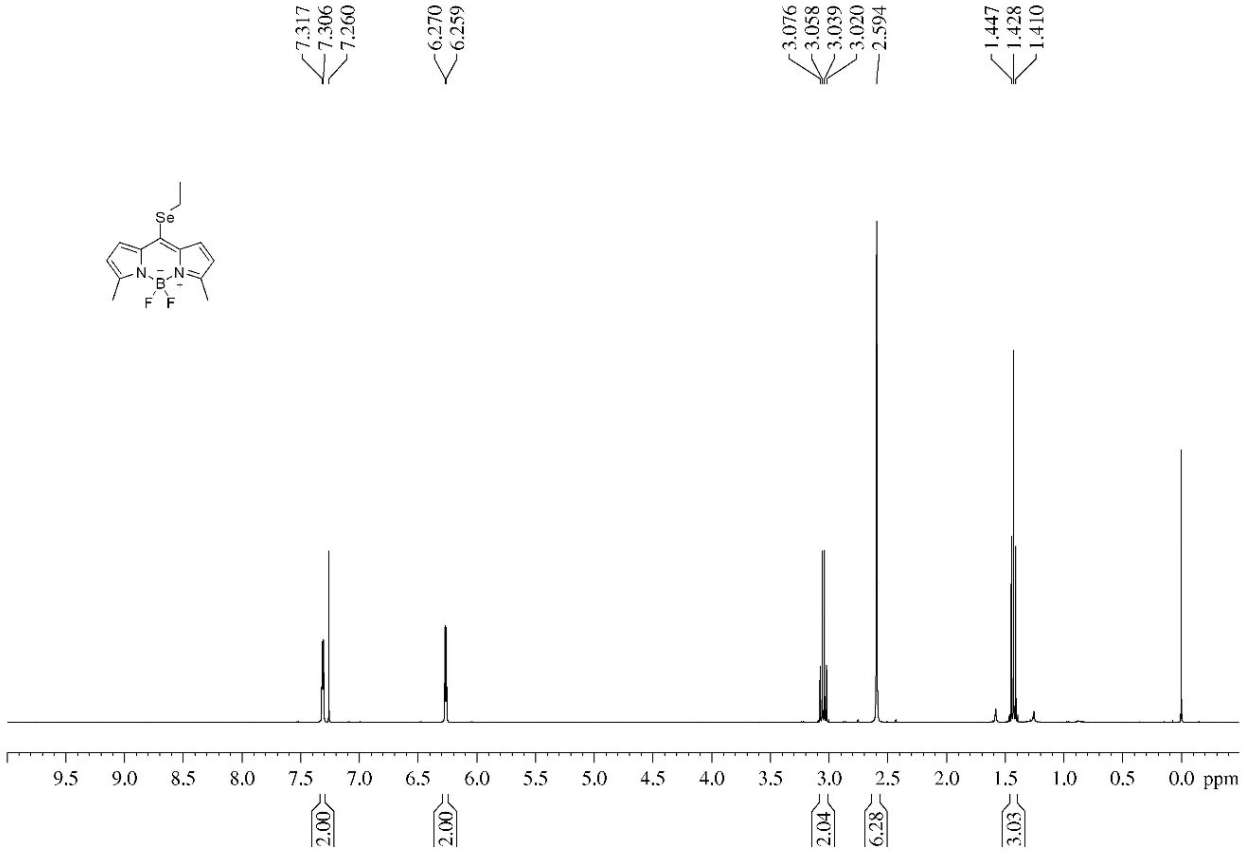
^{1}H NMR of 8-chloro-3,5-dimethyl BODIPY in $CDCl_3$, 400 MHz



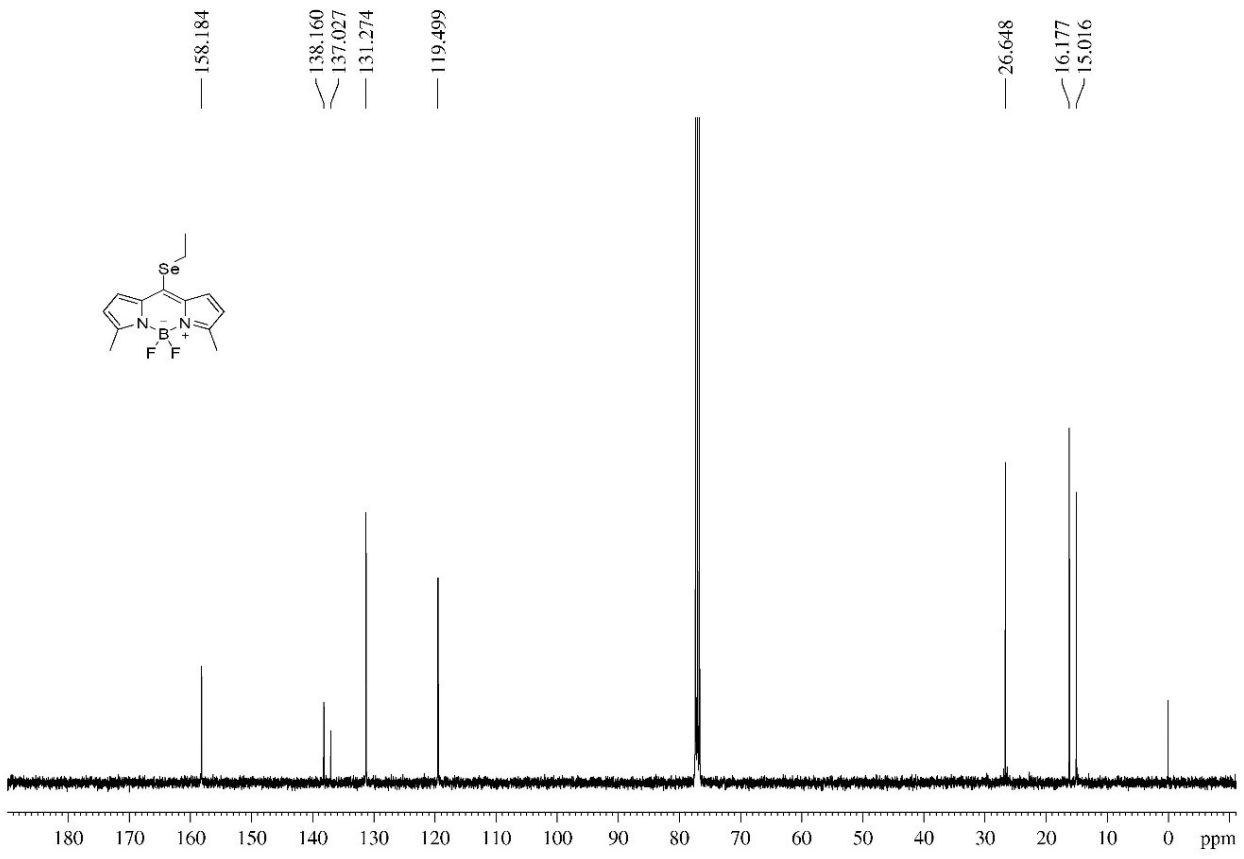
^{13}C NMR of 8-chloro-3,5-dimethyl BODIPY in $CDCl_3$, 100 MHz



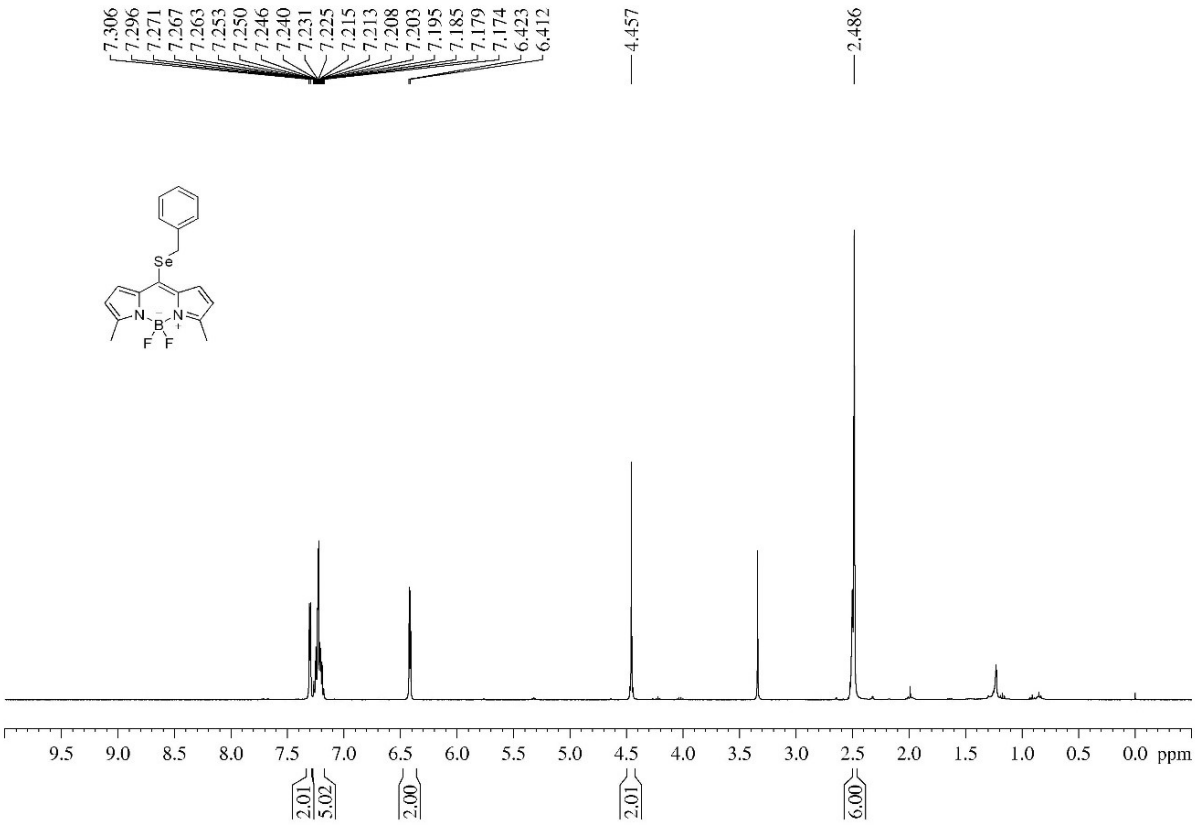
^{1}H NMR of BSe-Et in $CDCl_3$, 400 MHz



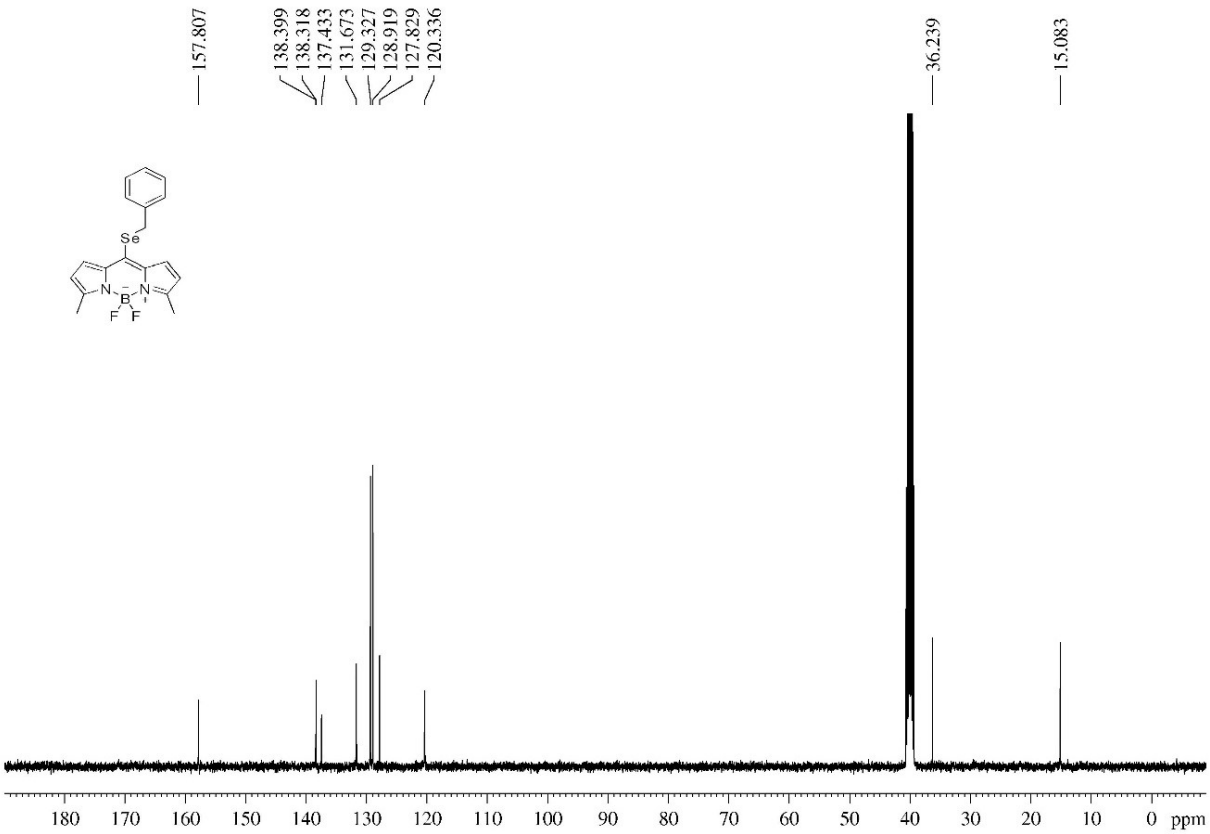
^{13}C NMR of BSe-Et in $CDCl_3$, 100 MHz



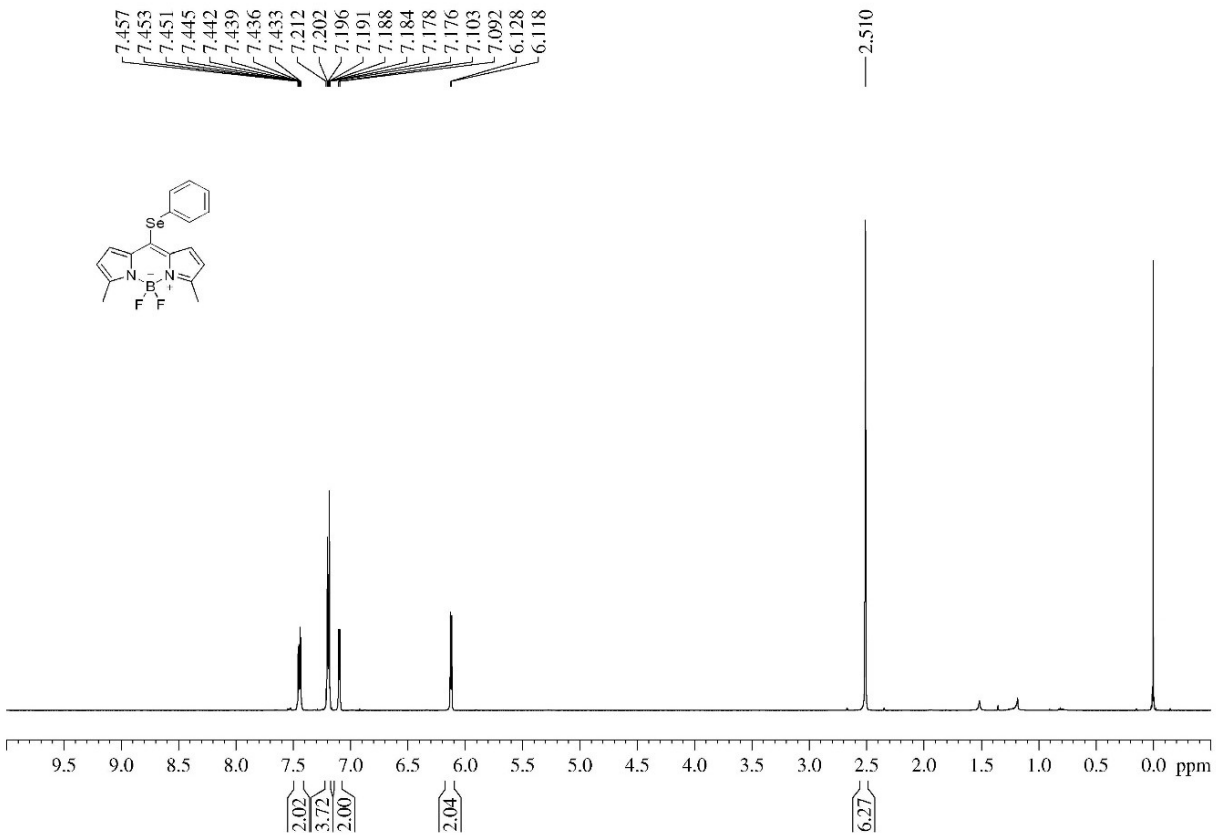
^{1}H NMR of BSe-Bz in DMSO-d_6, 400 MHz



^{13}C NMR of BSe-Bz in DMSO-d_6, 100 MHz



^{1}H NMR of BSe-Ph in $CDCl_3$, 400 MHz



^{13}C NMR of BSe-Ph in $CDCl_3$, 100 MHz