*Supporting Information for*

# A Novel Fluorescent Probe with Si-O-Si Bond as Bridged for Reversible Detection of Hypochloric Acid and Biothiol Amino Acids in Live Cells and Zebrafishes

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## Content

## Materials and instruments

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. Solvents used were purified by standard methods prior to use. Twice-distilled water was used throughout all experiments; Mass spectrometric analyses were measured on a Finnigan MAT 95 XP spectrometer; High resolution mass spectrometric (HRMS) analyses were measured on an Agilent 1100 HPLC/MSD spectrometer; NMR spectra were recorded on an AVANCE III 400 MHz Digital NMR Spectrometer, using TMS as an internal standard; Electronic absorption spectra were obtained on a Shimadzu UV-2700 power spectrometer; Photoluminescent spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer with a 1 cm standard quartz cell; The fluorescence imaging of cells was performed with a Nikon A1MP confocal microscope; The pH measurements were carried out on a Mettler-Toledo Delta 320 pH meter; TLC analysis was performed on silica gel plates and column chromatography was conducted over silica gel (mesh 200–300), both of which were obtained from the Qingdao Ocean Chemicals. The imaging experiments used 40 × objective lens. Two-photon imaging was obtained by Nikon A1MP confocal microscopy. The Mai Tai DeepSee laser offers 2.5 W of average power and 350 nm (690-1040 nm) in useable tuning range. Living cells fresh were cultivated using fresh medium

## Determination of the detection limit

The detection limit was determined from the fluorescence titration data based on a

reported method. **BSi-1** (10.0 μM) was titrated with different concentrations of HClO, the linear relationship between the fluorescence intensity ($I_{500}$) and the concentration of HClO ($\lambda_{ex}$ = 405 nm) was fitted based on the fluorescence titration

$$\text{Detection limit} = 3\sigma/k$$

Where "σ" is the standard deviation of the blank sample and "k" is the slope of the linear regression equation.

## Preparation of the test solutions

The stock solution of the probe **BSi-1** was prepared at 1 mM in EtOH. The solutions of various testing solvents were prepared from EtOH, DCM, MeCN, THF, and DMF. The test solutions of the probe **BSi-1** (1 μM) in 2 mL different solvents were prepared. Unless otherwise noted, for the fluorescence spectra experiments, excitation and emission slit widths were 5 nm and 5 nm.

## Measurement of the fluorescence quantum yields

The fluorescence quantum yields (Φ) were calculated by the equation (1):

$$\Phi_s = \Phi_r \left( \frac{A_r}{A_s} \right) \left( \frac{\eta_s^2}{\eta_r^2} \right) \frac{I_s}{I_r} \qquad (1)$$

Where the subscripts s and r represent the sample and reference molecule, respectively. A stands for the absorbance. I represent the integrated emission intensity, n is the refractive index of the solvent. Φ is quantum yield. In this paper, fluorescence quantum yields were determined by using quinine sulfate (Φ=0.58 in 1 M $H_2SO_4$) as

fluorescence standard.

M).

## Culture and preparation of cell

The RAW 264.7 cell line and HeLa cell line were purchased from Procell Life Science&Technology Co,.Ltd. RAW 264.7 cells and HeLa cells were cultured in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10 % FBS (fetal bovine serum) in an atmosphere of 5 % $CO_2$ and 95 % air at 37°C. Before the experiments, seed the RAW 264.7 or HeLa cells in 35 mm glass-bottomed dishes at a density of 2×105 cells per dish in 2 mL of culture medium and incubate them inside an incubator containing 5% $CO_2$ and 95% air at 37°C. Incubating the cells for 24 h. Cells will attach to the glass surface during this time.

## Cytotoxicity assay

In vitro cytotoxicity was measured using the colorimetric methyl thiazolyl tetrazolium (MTT) assay on HeLa cells. Cells were seeded into the 24-well tissue culture plate in the presence of 500 μL Dulbecco's modifed eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1 % penicillin/streptomycin at 37°C and 5% $CO_2$ atmosphere for overnight and then incubated for 24 h in the presence of CR-HA at different concentrations (0 μM, 5 μM, 10 μM, 20 μM, 30 μM, 50 μM). Then cells were washed with PBS buffer and 500 μL supplemented DMEM medium was added. Subsequently, 50 μL MTT (5 mg/ mL) was added to each well and incubated for 4 h. Violet formazan was dissolved in 500 μL sodium dodecyl

sulfate solution in the water-DMF mixture. Absorbance of the solution was measured at 570 nm using a microplate reader. The cell viability was determined by assuming 100 % cell viability for cells without **BSi-1**.

## Imaging of $OCl^-$ in living cells and zebrafishes

1. Fluorescent imaging of exogenous HClO in HeLa cells

Before the experiments, the HeLa cells were seeded on two 35-mm glass-bottomed dishes. and allowed to adhere for 24 h. The cells were washed with PBS (pH=7.4) buffer three times. Subsequently, the first group was incubating with probe **BSi-1**(10 μM) (containing 0.1 % EtOH as a cosolvent) for 30 min at 37 ℃. The HeLa cells were rinsed with PBS three times.

The second group was incubating with probe **BSi-1** (10 μM) (containing 0.1 % EtOH as a cosolvent) for 30 min at 37 ℃. The HeLa cells were rinsed with PBS three times and the cells were incubated with HClO (30 μM) for 30 min at 37 ℃. And then the probe was washed with PBS three times, and the fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera

2. Imaging of endogenous HClO in RAW 264.7 cells

Before the experiments, the RAW 264.7 cells were placed on 6-well plates and allowed to adhere for 24 h and then incubated with probe **BSi-1** (10 μM) for 30 min at 37℃, washed by PBS buffer and subsequently incubated with 2 μg/mL PMA (phorbol 12-myristate13 acetate) and 2 μg/mL LPS (lipopolysaccharides) for 2 h. For the

control experiments, the cells without treated with PMA/LPS were incubated with probe **BSi-1** (10 μM) for 2 hours under the same conditions. For negative control group, the RAW 264.7 cells incubated with probe **BSi-1** (10 μM) for 30 min at 37 C, washed by PBS buffer and subsequently incubated with 20 μg/mL PMA, 20 μg/mL LPS and 4-aminobenzoic acid hydrazide (ABH, 200 μM) for 2 h prior to imaging. The cells were washed with PBS (pH=7.4) buffer. The fluorescence images were acquired through a Nikon A1MP confocal microscopy inverted fluorescence microscopy equipped with a cooled CCD camera.

3. Imaging of HClO in zebrafishes

5-day-old zebra fishes were took into a 30 mm glass culture dishes using a disposable sterilized dropper in fluorescence imaging experiments. All animal procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Shandong University and approved by the Animal Ethics Committee of Shandong University.

## Synthesis

***Synthesis of compound Bis-Na***

4-Bromo-1, 8-naphthalic anhydride (2.77 g) and Sodium thiomethoxide (3 g) were added in ethanol (100 mL).The mixture was heated at room temperature for 48 h. Then adding the deionized water (100 mL) and the yellow precipitate appeared. Washing it with three times and drying to obtain the crude product. Purification by

column chromatography to obtain **Bis-Na** (yield: 92 %).

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 8.67-8.52 (q, 2H), 8.47-8.42 (d, 1H), 8.00-7.87 (q, 1H), 7.77-7.65 (d, 1H), 2.83-2.70 (d, 3H).

HRMS (EI) *m/z* calculated for $C_{13}H_8O_3S$: 244.0194 Found: 245.0267 (M+H).

***Synthesis of compound BSi-1***

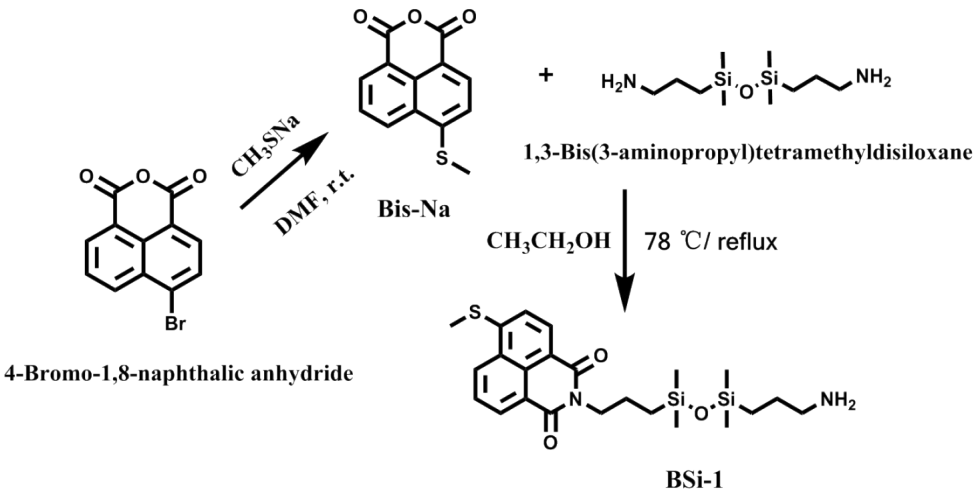
**BSi-1** was synthesized by the **Bis-Na** and 1, 3-Bis (3-aminopropyl) tetramethyldisiloxane (1: 1) in ethanol solvent under reflux in 78 ℃. **BSi-1** was obtained as pure green liquid (yield: 70%) through chromatographic purification on silica gel columns (Scheme 1). They were characterized by $^1$H NMR, $^{13}$C NMR and HRMS.

$^1$H NMR (400 MHz, $CDCl_3$) δ 8.61-8.55 (-Ph, 1H), 8.46-8.39 (-Ph, 2H), 7.72-7.66 (-Ph, 1H), 7.43-7.36 (-Ph, 1H), 4.17-4.05 (γ-Si, 4H), 3.01-2.82 (-$NH_2$, 2H), 2.72-2.66 (β-Si, 4H), 0.68-0.59 (α-Si, 4H), 0.08-0.03 (Si-$CH_3$, 12H).

$^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.56, 146.17, 131.36, 130.79, 129.54, 128.99, 126.48, 123.21, 121.04, 118.87, 113.54, 64.47, 43.09, 24.59, 22.06, 11.53, 10.30, 5.33.

HRMS (EI) *m/z* calculated for $C_{23}H_{34}N_2O_3SSi_2$: 474.19 Found: 475.1904 (M+H).

**Scheme S1**. Design and synthesis of **Bis-Na** and **BSi-1**.



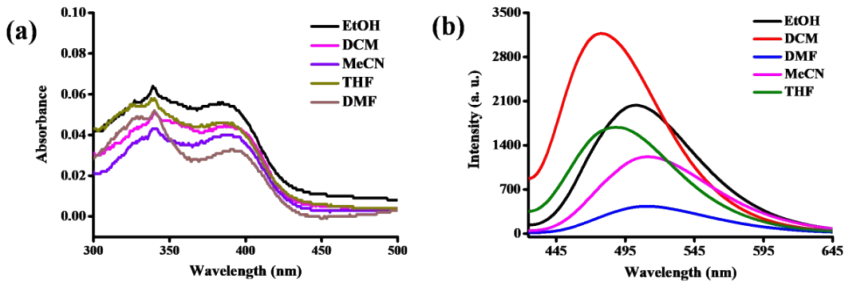




**Figure S1.** (a) Absorption spectra of **BSi-1** in various solvents; (b) fluorescence spectra of **BSi-1** in various solvents. Excitation wavelength was 405 nm.

**Table S1** The photophysical properties of **BSi-1** in various solvents.

| Sample | **BSi-1** | | |
|---|---|---|---|
| | $\lambda_a/\lambda_b$(nm ) | SSc(nm) | $\Phi_d$/% |
| DCM | 405/478 | 73 | 37.2 |
| DMF | 405/509 | 104 | 15.8 |
| THF | 405/488 | 83 | 28.3 |
| MeCN | 405/510 | 105 | 21.1 |

| | | | |
|---|---|---|---|
| EtOH | 405/502 | 97 | 31.0 |

[a]Maximum absorption wavelength (nm). [b]Maximum emission wavelength(nm). [c]Stokes shift (SS); $\Phi_d$ fluorescence quantum yield (error limit: 8%) determined by using fluorescein ($\Phi$= 0.58) in aqueous $H_2SO_4$ (1 M) as the standard.

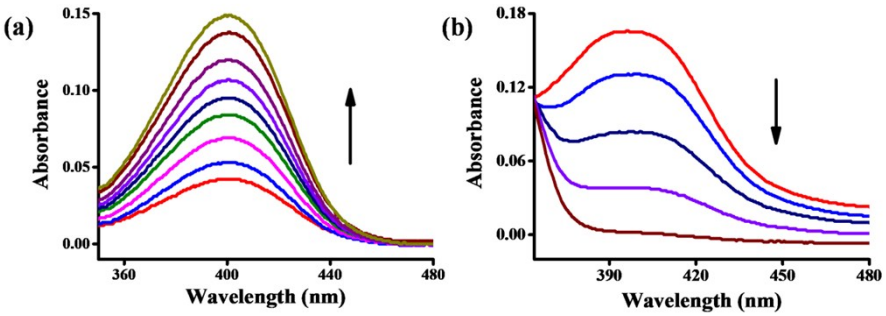



**Figure S2.** (a) The UV–Vis absorption spectra of with increasing concentrations of **BSi-1**; (b) The UV–Vis absorption spectra of **BSi-1** was decreasingly with increasing $OCl^-$ concentrations.

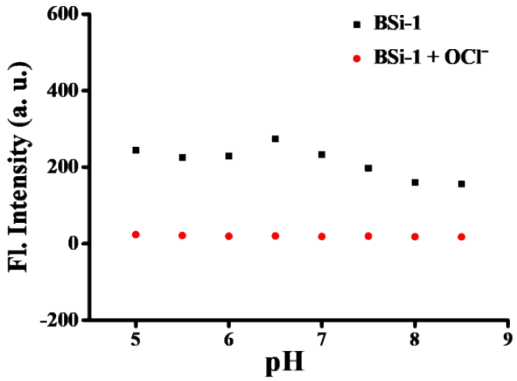



**Figure S3.** The fluorescent emission intensities at 498 nm of **BSi-1** (10 μm) in the absence (●) or presence (■) of $ClO^-$ (20 equiv) at various pH values of PBS buffer. Excitation wavelength was 405 nm.

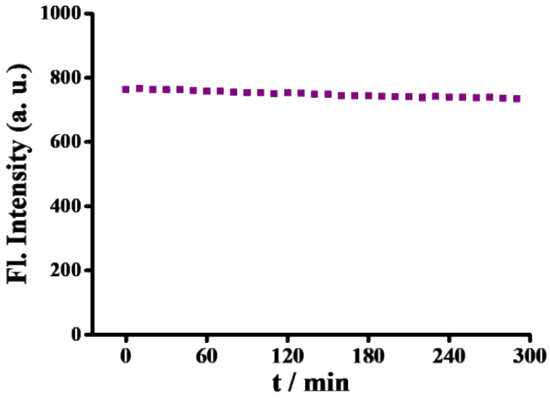



**Figure S4.** The photostability of **BSi-1**. Excitation wavelength was 405 nm.

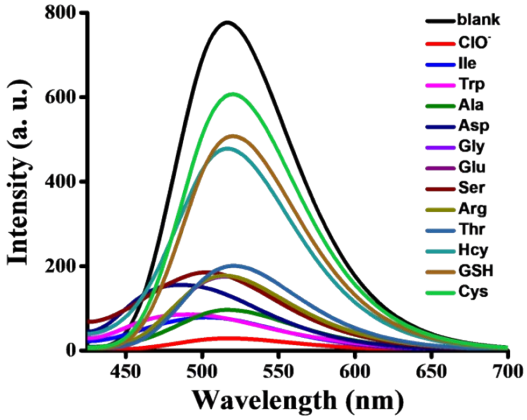



**Figure S5.** Fluorescence intensity recovery of **BSi-1**+$OCl^-$ in various α-amino acids aqueous solution (100 mM PBS buffer, pH=7.4) ($\lambda_{ex}$ = 405 nm).

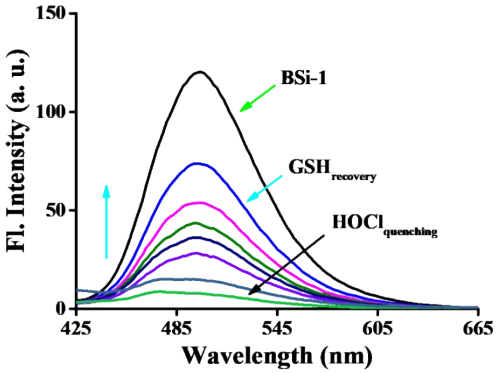



**Figure S6.** Fluorescence intensity recovery of **BSi-1** in GSH aqueous solution with time (100 mM PBS buffer, pH=7.4) ($\lambda_{ex}$ = 405 nm).

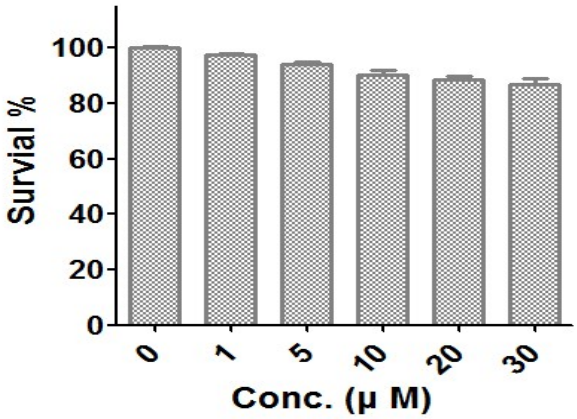



**Figure S7.** Cytotoxicity assays of **Bsi-1** at different concentrations for HeLa cells.

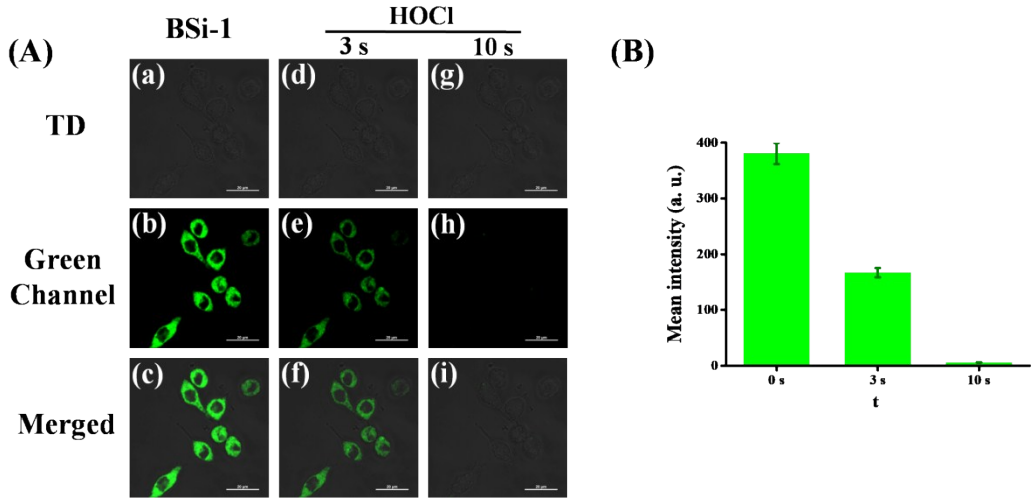



**Figure S8.** (A) (a-c) The fluorescence images of **BSi-1** in HeLa cells, (d-f) **BSi-1** was treated with NaOCl within 3 seconds, (g-i) the fluorescence imaging of **BSi-1** with NaOCl within 10 seconds; (B) the mean fluorescent intensity of **BSi-1** in HeLa cells. Green channel: $\lambda_{ex}$ = 405 nm, $\lambda_{em}$=425-475 nm. Scale bar = 20 μm.

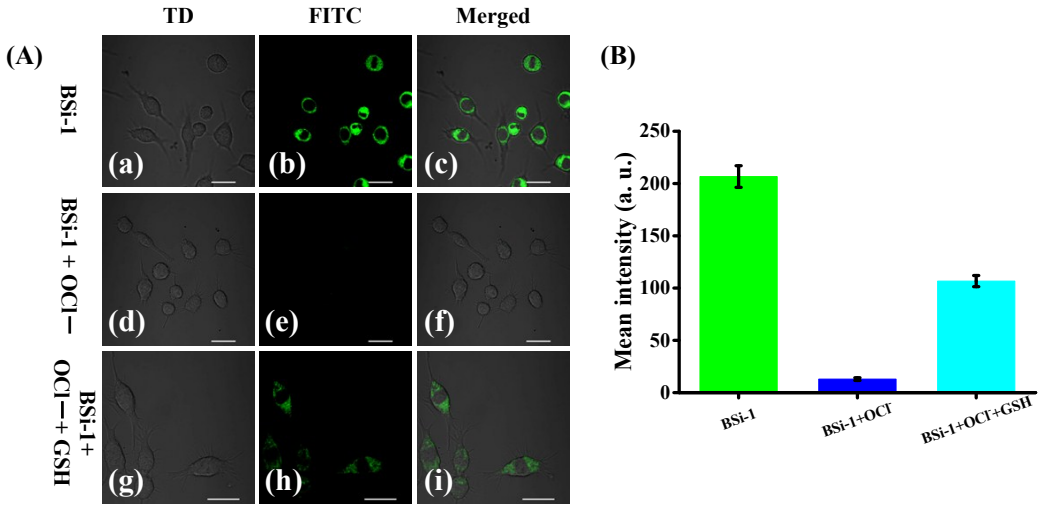



**Figure S9.** (A) (a-c) The fluorescence images of **BSi-1** in RAW 264.7 macrophages cells, (d-f) **BSi-1** was treated with NaOCl, (g-i) the fluorescent recovery of **BSi-1** with GSH; (B) the mean fluorescent intensity of **BSi-1** in RAW 264.7 macrophages cells. Green channel: $\lambda_{ex}$ = 405 nm, $\lambda_{em}$= 425-475 nm. Scale bar = 20 μm.

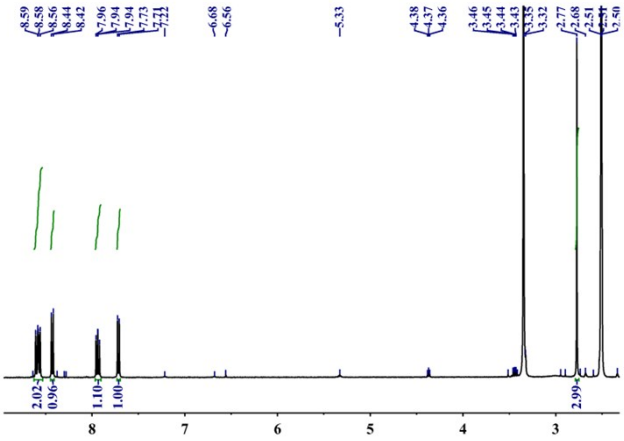


**Figure S10.** $^{1}$H-NMR (DMSO-*d*) spectrum of **Bis-Na**.

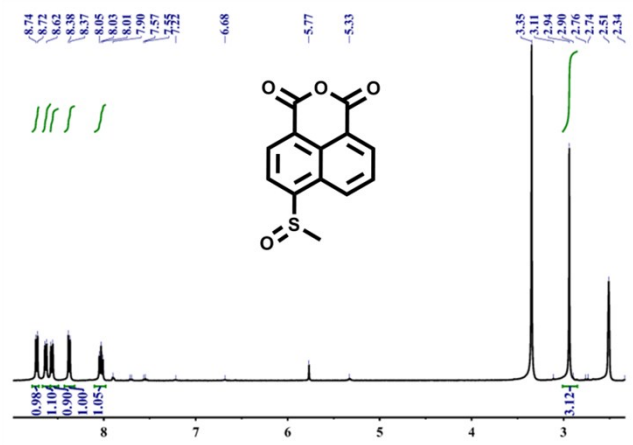


**Figure S11.** $^{1}$H-NMR (DMSO-$d_6$) spectrum of the oxides of **Bis-Na**.

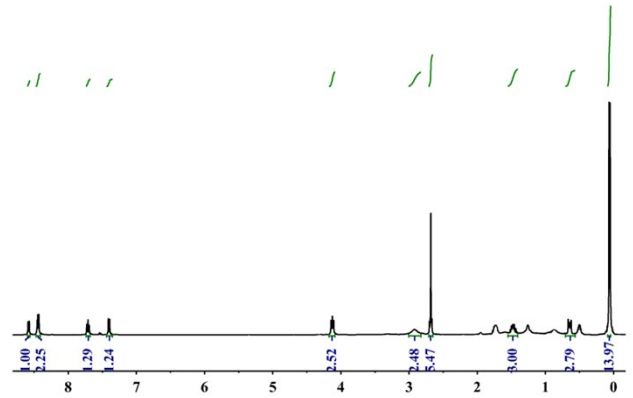


**Figure S12.** $^{1}$H-NMR ($CHCl_3$-$d_6$) spectrum of **BSi-1**.

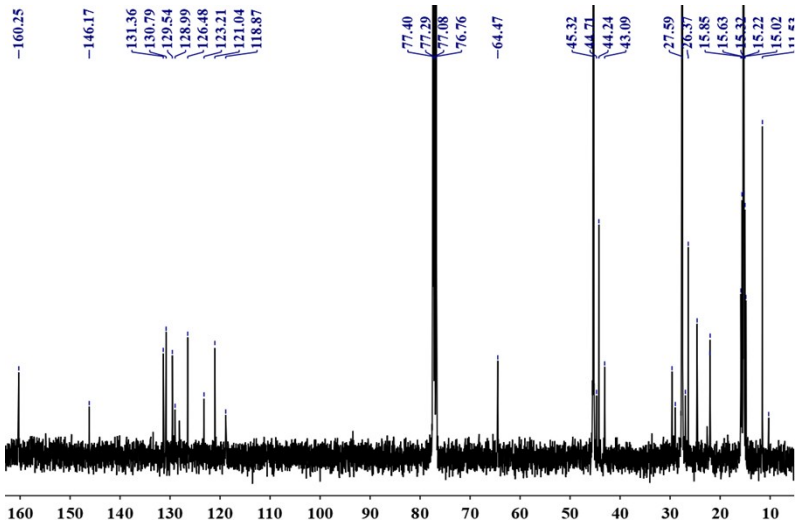


**Figure S13.** $^{13}$C-NMR ($CHCl_3$-$d_6$) spectrum of **BSi-1**.

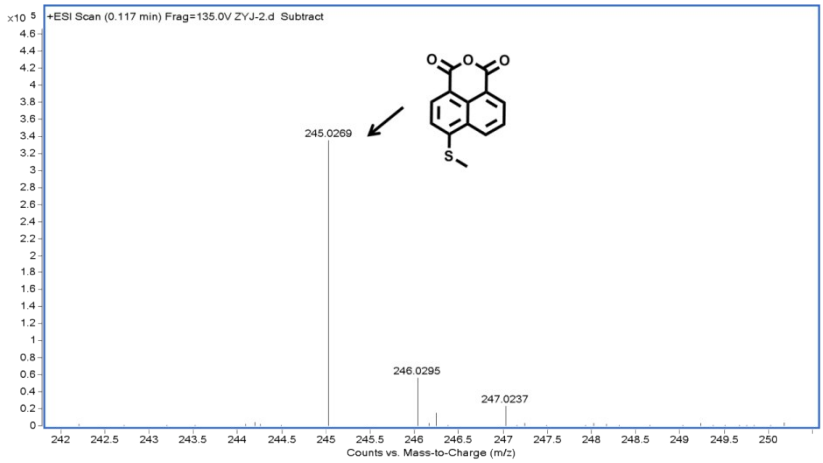



**Figure S14.** HRMS (ESI) spectrum of Bis-Na.

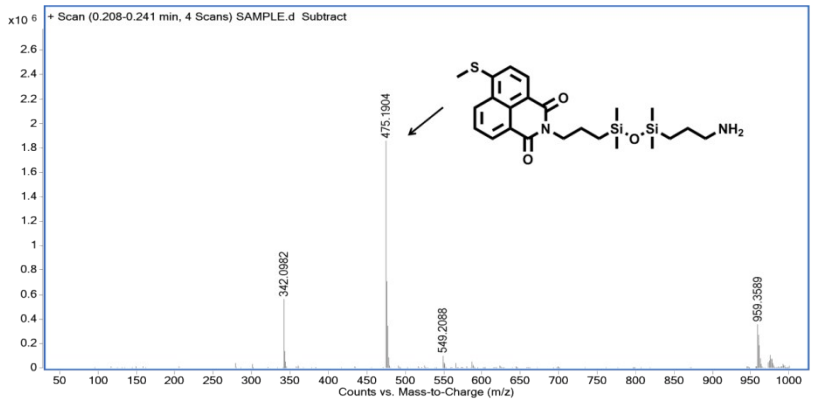



**Figure S15.** HRMS (ESI) spectrum of **BSi-1.**

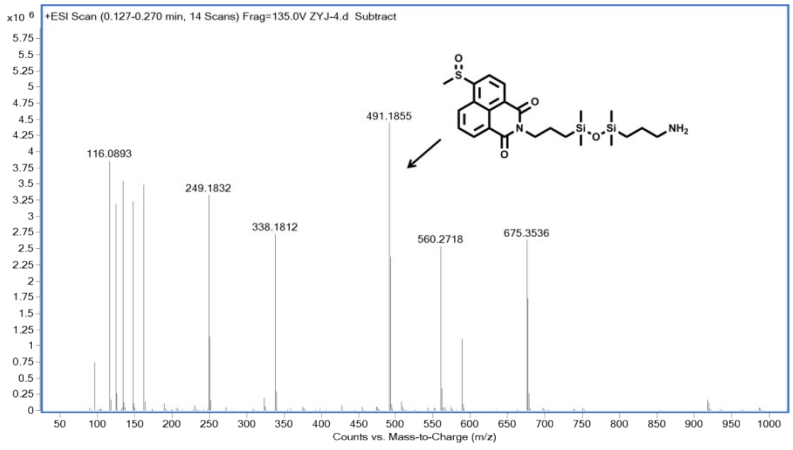



**Figure S16.** HRMS (ESI) spectrum of the oxides of **BSi-1**.