# Electronic Supplementary Information

## A ratiometric fluorescence probe for detection of hydrogen sulfide in cells

Bin Wang, Na Jiang*, Wei Sun, Qiufen Wang, Gengxiu Zheng*

a School of Chemistry and Chemical Engineering, University of Jinan, 336 Nanxinzhuang West Road, 250022, Jinan (China)

* E-mail: chm_jiangn@ujn.edu.cn; chm_zhenggx@ujn.edu.cn

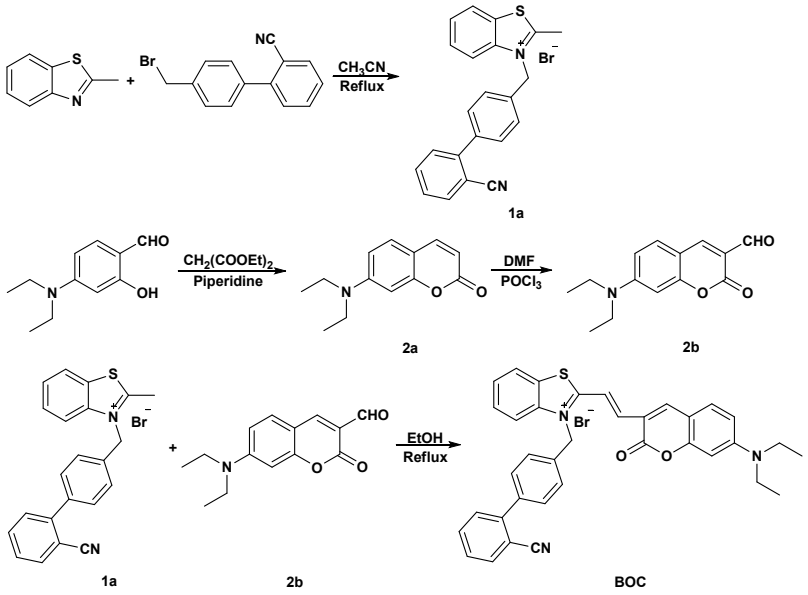
All chemicals and solvents were purchased from commercial suppliers and used without further purification. Distilled water was used after passing through a water ultrapurification system. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on a Varian Mercury 400 spectrometer. HR-MS data were obtained with a LC-MS spectrometer. UV-Vis and fluorescence spectra were recorded on a UV-Vis spectrophotometer and a fluorescence spectrophotometer with a temperature controller, respectively. Standard quartz cuvettes with a 1.0 cm lightpath were used for all optical spectra measurements.

**Preparation of Solutions of BOC and Analytes.** Stock solution of **BOC** (1.0 mM) was prepared in HPLC grade $CH_3CN$. Analytes cysteine (Cys), homocysteine (Hcy), glutathione (GSH), $Na^+$, $K^+$, $Ca^{2+}$, $NH_4^+$, $Ba^{2+}$, $Cu^+$, $F^-$, $Br^-$, $NO_2^-$, $ClO_4^-$ $SO_4^{2-}$, $S_2O_8^{2-}$, $S_2O_3^{2-}$, $SCN^-$ were dissolved in distilled water (10 mL) to afford 1 mM (for Cys, Hcy, GSH) or 5 mM (for others) aqueous solution.

**Measurements of ratios of fluorescence intensity of $I_{492}/I_{652}$ of BOC upon addition of various analytes.** A solution of **BOC** (10 μM) was prepared in $CH_3CN$-PBS solution (7:3, v/v, 10 mM PBS buffer). Then 3.0 mL of **BOC** solution was placed in a quartz cell until the temperature reached at 37 °C over a few minutes. The fluorescence spectra were then recorded upon addition of various analytes.

**Cell incubation and imaging.** HeLa and MCF-7 cells were cultured in DEME medium supplemented with 10% fetal bovine serum at 37 ºC in an atmosphere containing 5% $CO_2$. For live cell imaging, BOC were added to cells grown in a confocal microscope dish for 15 min and washed with PBS (phosphate-buffered saline) three times. After replacement of the medium, cells were imaged using a Olympus (FV1000) confocal laser scanning microscope with a 200×objective lens. HeLa and MCF-7 cells were first stained with 5.0 μM of **BOC** at 37 ºC in an atmosphere of 5% $CO_2$ for 30 min, and then washed with PBS three times. Cells were then incubated with $HS^-$ (200 μM ) at 37 ºC in an atmosphere of 5% $CO_2$ for another 30 minutes, and then washed with PBS three times. After replacement of medium, cells were imaged using OLYMPUS FV1000 confocal fluorescence microscope with a 200×objective lens.

## Synthesis of intermediates and BOC




**Scheme S1.** Synthesis of intermediates and **BOC**

**Synthesis of compound 1a.** 4-Bromomethyl-2-cyanobiphenyl (1.3 g, 4.8 mmol), 2-methyl benzothiazole (0.5 g, 3.4 mmol) was added into 20 ml acetonitrile under nitrogen to get a mixed solution. Then stirred for 24 hours under a refluxing condition (Scheme S1). The mixture was then cooled down, the precipitate filtered off and washed with diethyl ether to give compound 1a as crude product (1.1 g, yield 80 %).

$^1$H NMR (400 MHz, DMSO) $\delta$ 8.55 (1 H, d, *J* 7.9), 8.27 (1 H, d, *J* 8.3), 7.96 (1 H, d, *J* 7.6), 7.88 (1 H, t, *J* 7.6), 7.81 (2 H, dd, *J* 15.8, 7.9), 7.61 (4 H, q, *J* 7.5), 7.51 (2 H, d, *J* 7.9), 6.21 (2 H, s), 3.33 (3 H, s).

**Compound 2b** was prepared according to the reported procedure.[S1]

**Synthesis of BOC. BOC** was conveniently synthesized via the condensation of compound 1a with 2b (Scheme S1). Compound 1a (1.03 g, 2.5 mmol), compound 2b (0.5 g, 2 mmol) were mixed in an absolute ethanol solution (20 ml). The solution was refluxed under nitrogen for 24 h, and then cooled down. The precipitate was collected, washed with diethyl ether, then dried, giving a violet solid (1.13 g, 85.7%).

$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 9.41 (1 H, s), 8.54 (2 H, dd, *J* 32.2, 15.0), 8.19 (1 H, d, *J* 8.0), 7.88 (1 H, d, *J* 8.3), 7.79 – 7.71 (2 H, m), 7.71 – 7.57 (3 H, m), 7.55 (2 H, d, *J* 8.1), 7.45 (4 H, dd,

*J* 13.8, 7.8), 6.64 (1 H, d, *J* 10.5), 6.42 (1 H, s), 6.33 (2 H, s), 3.49 (4 H, q, *J* 6.9), 1.84 (4 H, s), 1.26 (8 H, t, *J* 6.9).

$^{13}$C NMR (100 MHz, $CDCl_3$) 172.82, 158.06, 153.88, 150.08, 147.98, 144.23, 141.26, 138.77, 133.79, 133.35, 133.03, 130.11, 129.82, 129.71, 128.22, 127.95, 127.85, 127.37, 124.20, 118.53, 115.72, 112.72, 110.97, 110.59, 110.29, 96.70, 77.36, 77.04, 76.73, 51.98, 45.46, 29.70, 12.58.

HR-MS (ESI): *m*/*z*, calcd for $C_{36}H_{30}N_3O_2S^+$ 568.2059 (M + $H^+$), found 568.2055.

**Table S1.** Spectral properties of **BOC** in various solvents.

| Solvents | $\lambda_{abs}$[a] | $\lambda_{em}$[b] | $\Delta\lambda$[c] | $\Phi_f$[d] | $\varepsilon$[e] |
|---|---|---|---|---|---|
| PBS | 357, 568 | 477, 647 | 120, 79 | 0.01 | 8100, 30200 |
| $CH_2Cl_2$ | 363, 593 | 479, 646 | 116, 53 | 0.258 | 8800, 75500 |
| $CH_3CH_2OH$ | 355, 565 | 482, 649 | 127, 84 | 0.172 | 8600, 81900 |
| $CH_3CN$ | 354, 565 | 482, 653 | 128, 88 | 0.08 | 7700, 69600 |
| DMSO | 357, 568 | 489, 667 | 132, 99 | 0.186 | 7100, 56200 |

[a] The two absorption peaks of **BOC** (nm). [b] The two emission peaks of **BOC** (nm), excited at 430 nm. [c] Stokes shift of **BOC** (nm). [d] Fluorescence quantum yields of **BOC**. [e] $\times 10^4$ $mol^{-1}$ $cm^{-1}$ L.

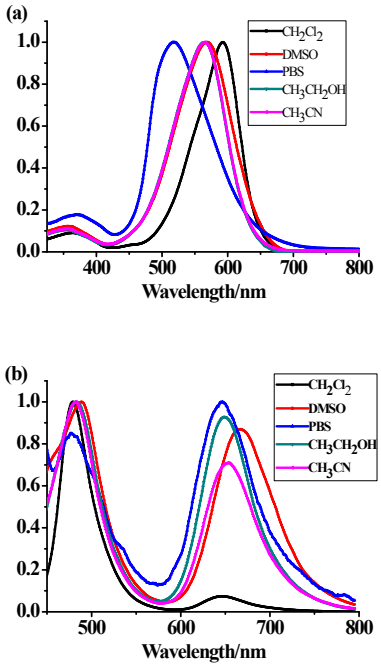
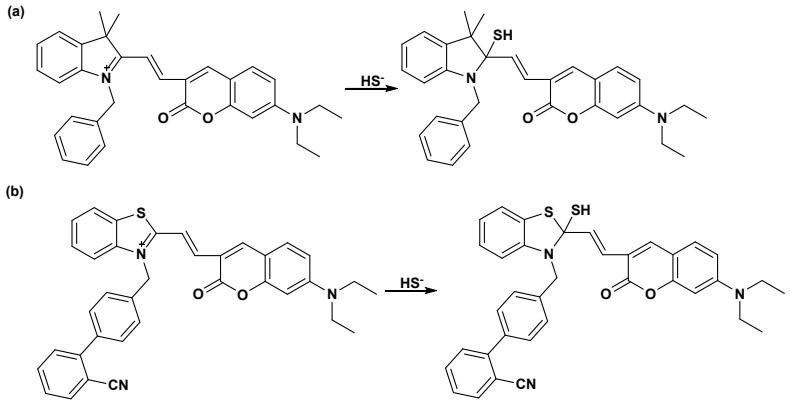



**Figure S1.** (a) Normalized UV–vis spectra of **BOC** (10 μM) in various solvents; (b) Normalized fluorescence

spectra of **BOC** (10 μM) in various solvents. $\lambda_{ex}$ = 430 nm.

## Data for investigation of the sensing mechanism




**Scheme S2.** (a) $H_2S$ sensing mechanism mentioned in the literature.[S2] (b) Proposed $H_2S$ sensing mechanism of **BOC**.

To examine the sensing mechanism of **BOC** to $H_2S$, the following $^1H$ NMR titration experiment was performed. **BOC** was dissolved in $CD_3CN$ to get a mixed solution of 10 mM. NaHS was dissolved in $D_2O$, then added into the above solution in a gradient of 4 mM. From the following comparison chart we can see that the conjugated hydrogen in the minimum field is decreased continuously with the increasing concentration of NaHS. The change of absorption is saturated when the molar ratio of NaHS to **BOC** is 1:1, which indicated the sensing mechanism of **BOC** (Fig. S2).

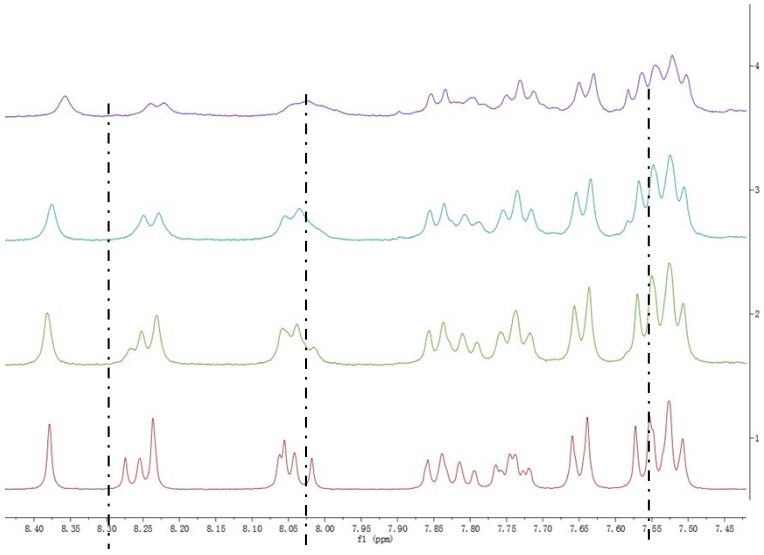



**Figure S2.** The sensing mechanism of **BOC** for $HS^-$. $^1H$ NMR spectral change of **BOC** (10 mM) in the absence and presence (2, 3, 4) of $HS^-$, the gradient of $HS^-$ is 4 mM.

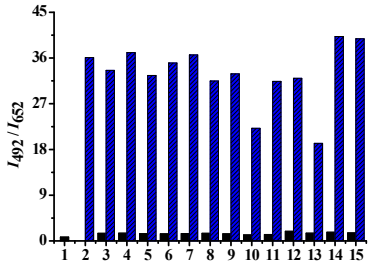



**Figure S3**. Fluorescence ratio responses of **BOC** (10 μM) to $HS^-$ (667 μM), various ions and biologically relevant species at 492 nm and 652 nm in PBS (10 mM, pH 7.40, 70% $CH_3CN$, v/v). Black bars represent the addition of various ions or biomacromolecules. Blue bars represent the addition of $HS^-$ to the above solution, respectively. 1. Probe alone, 2. $HS^-$, 3. $Na^+$, 4. $K^+$, 5. $F^-$, 6. $Br^-$, 7. $NO_2^-$, 8. $ClO_4^-$, 9. $SO_4^{2-}$, 10. $S_2O_8^{2-}$, 11. $S_2O_3^{2-}$, 12. $SCN^-$, 13. Cys, 14. GSH, 15. Hcy, (3 – 12: 5 mM and 13 – 15: 1 mM). $\lambda_{ex}$ = 430 nm.

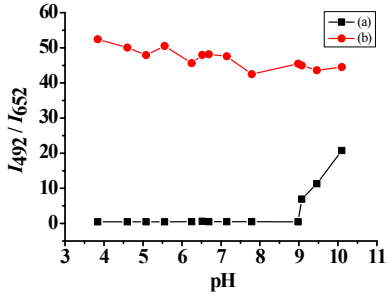



**Figure S4.** (a) fluorescence spectra of **BOC** (10 μM) under different pH conditions. (b) fluorescence spectra of **BOC** (10 μM) + $HS^-$ (833 μM) under different pH conditions.

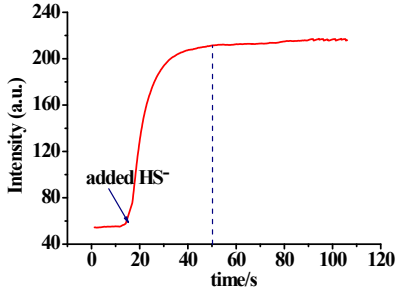



**Figure S5.** Time-dependent fluorescence changes of **BOC** (10 μM) with $HS^-$ (666.7 μM). $\lambda_{ex}$ = 430 nm, $\lambda_{em}$ = 495 nm.

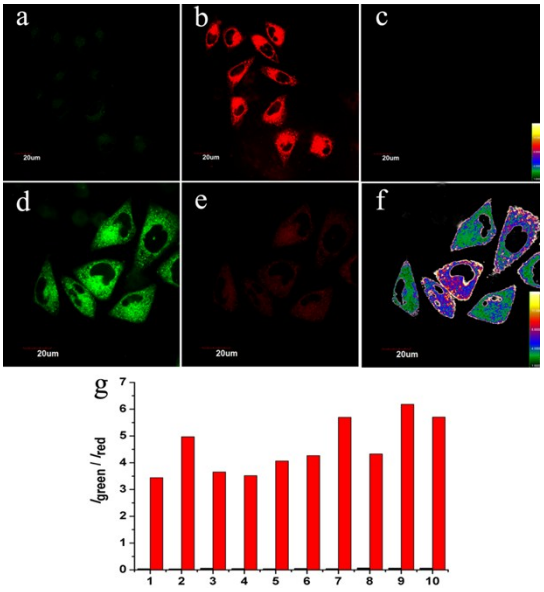
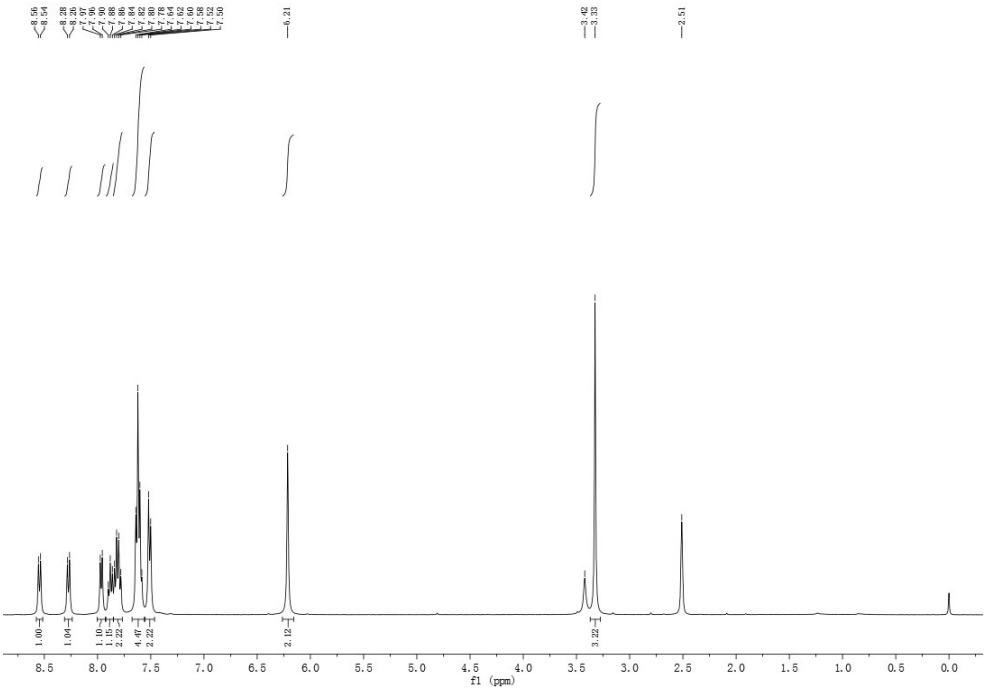



**Figure S6.** Confocal fluorescence images (a) and (b) of MCF-7 cells incubated with 5 μM **BOC**. Images (d) and (e) of the aboved MCF-7 cells after added 200 μM $HS^-$. Ratio images (c) and (f) of green channel compared to the red channel. (g) Ratio of 10 points that drawed from (c) and (f), respectively, black bars represent **BOC** and red bars represent **BOC** + **$HS^-$**.

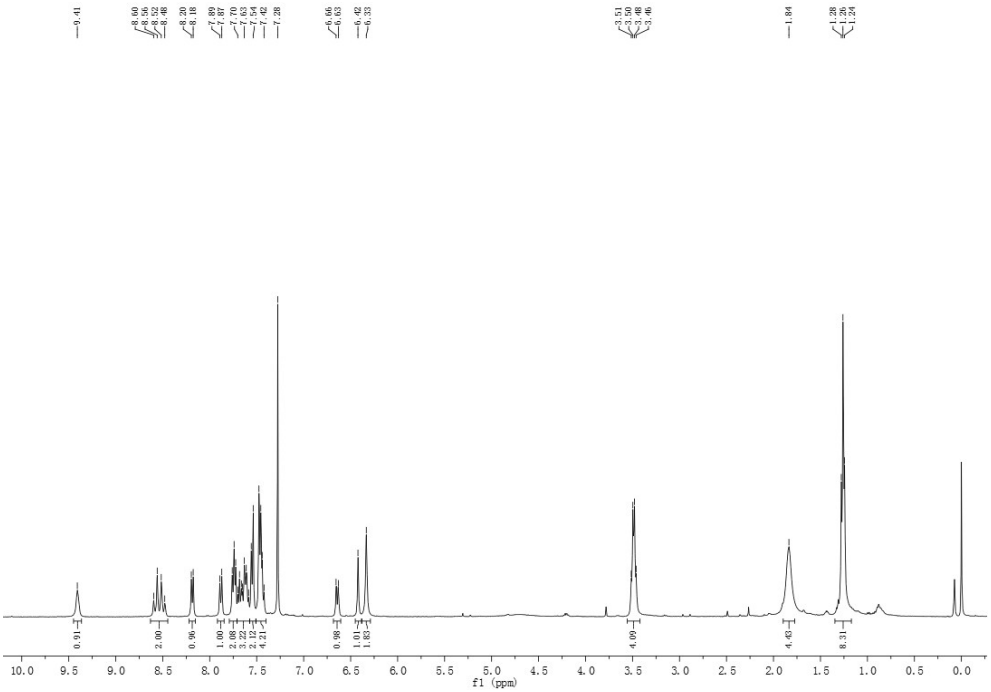
## References

(S1) (a) Wu. J. S, Liu. W. M, Zhuang. X. Q, Wang. F, Wang. P. F, Tao. S. L, Zhang. X. H, Wu. S. K and Lee. S. T, *Org. Lett*., 2007, **9**, 33-36. (b) Oushiki. D, Kojima. H, Terai. T, Arita. M, Hanaoka. K, Urano. Y and Nagano. T, *J. Am. Chem. Soc*., 2010, **132**, 2795-2801.

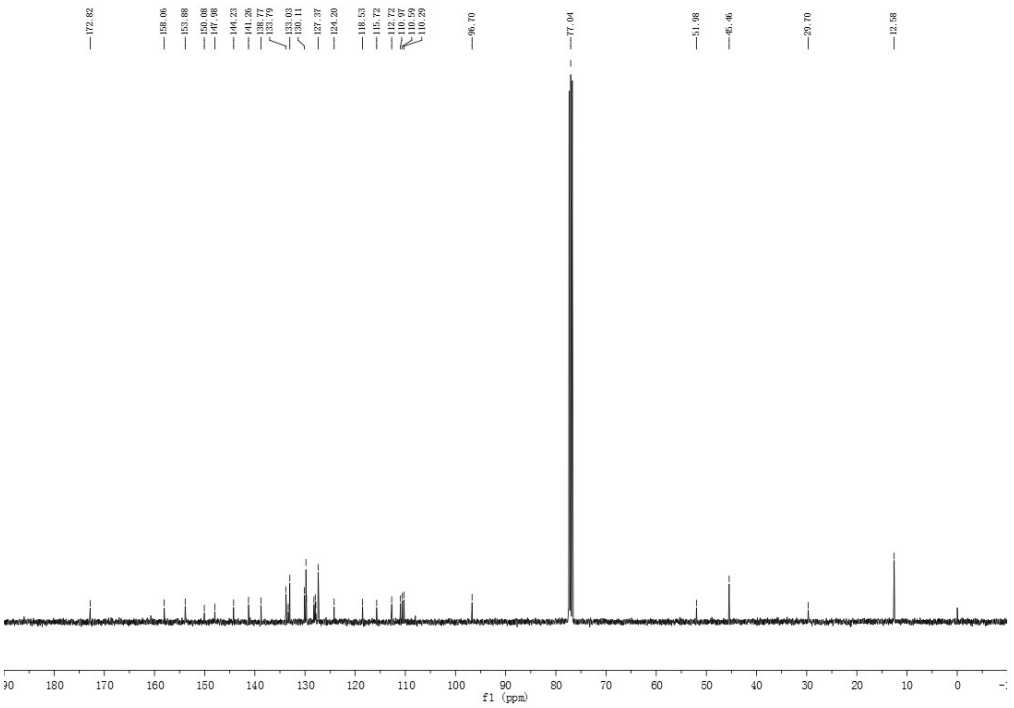
(S2) Chen. Y. C, Zhu. C. C, Yang. Z. H, Chen. J. J, He. Y. F, Jiao. Y, He. W. J, Qiu. L, Cen. J. J and Guo. Z. J, *Angew. Chem. Int. Edit*., 2013, **125**, 1732-1735.



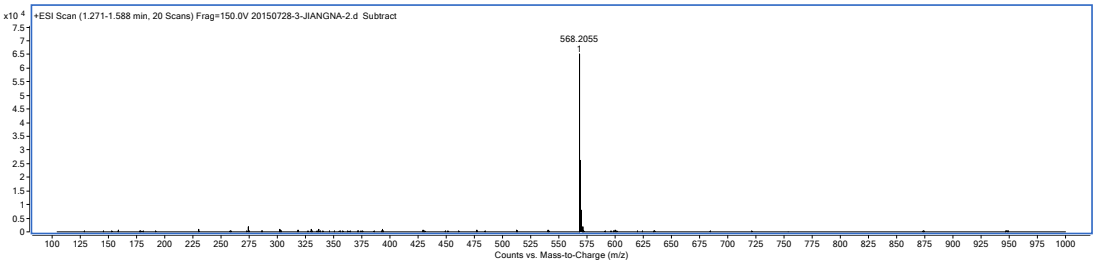
$^1$H NMR (400 MHz) spectra of compound **1a** in DMSO



$^1$H NMR (400 MHz) spectra of **BOC** in $CDCl_3$




$^{13}C$ NMR (400 MHz) spectra of **BOC** in $CDCl_3$




ESI-MS spectra of **BOC** in $CDCl_3$