

Supporting Information

Carboxy Bodipy-based Fast Trigger Fluorescent Probe for Imaging Endogenous Hypochlorous Acid

Hao Zhang¹, Qincong Feng¹, Ji-Ting Hou², Zhipeng Li^{2,*} and Jianliang Shen^{1,2,3,*}

¹ School of Basic Medical Sciences, Youjiang Medical University for Nationalities, Baise 533000, China; zhangxiaohao0209@163.com (H.Z.); 20211001060@stu.ymun.edu.cn (Q.F.)

² School of Ophthalmology & Optometry, School of Biomedical Engineering, Wenzhou Medical University, Wenzhou 325000, China; houjiting2206@163.com

³ Wenzhou Institute, University of Chinese Academy of Sciences, Wenzhou, 325000, China

* Correspondence: lizhipeng1991520@gmail.com (Z.L.); shenjl@wiucas.ac.cn (J.S.).

Table of Contents

	Content	Page
1	Synthesis Methods	S2-S3
2	Supporting Scheme	S3
3	Supporting Figures	S4-10
4	Supporting Tables	S11-12

Abbreviations. **ACN**- Acetonitrile, **Ar**- Argon, **CLSM**- Confocal Laser Scanning Microscope, **DCM**- Dichloromethane, **DMF**- N, N-Dimethylformamide, **EA**- Ethylacetate, **NaOH**- Sodium hydroxide, **HPLC**- High Performance Liquid Chromatography.

1. Synthesis Methods

The synthetic protocols and the details of all compound characterization are provided below.

Compound 2

To a solution of sodium hydroxide (240 mg, 6.0 mmol) in water (5 mL) at room temperature was added p-Hydroxybenzaldehyde (610 mg, 5.0 mmol). The solution was stirred at rt for 20 min and concentrated to give a white solid. The resulting solid was dispersed into 5 mL acetonitrile and then the N, N-Dimethylcarbamothioic chloride (677 mg, 5.5 mmol) was added slowly dropwise. Then stir at room temperature for 3 h. After the end of the reaction, the reaction solution was diluted with ethyl acetate, washed, anhydrous magnesium sulfate dried, and concentrated to give 780 mg crude compound **1**. The resulting crude product was dissolved into 20 mL ethanol, and then slowly added sodium borohydride, and continued to stir for 1 h, and then the reaction solution was diluted with ethyl acetate, washed with water, dried with anhydrous magnesium sulfate, and concentrated to give 700 mg crude compound **2** (66%). HRMS (ESI) calculated for $C_{10}H_{14}NO_2S^+$, $[M+H]^+$, 212.2881, found, 212.2887. (Figure. S1)

Bp-COOH synthesis according to our previous report paper (Chemistry 21, 9645–9649 (2015)).

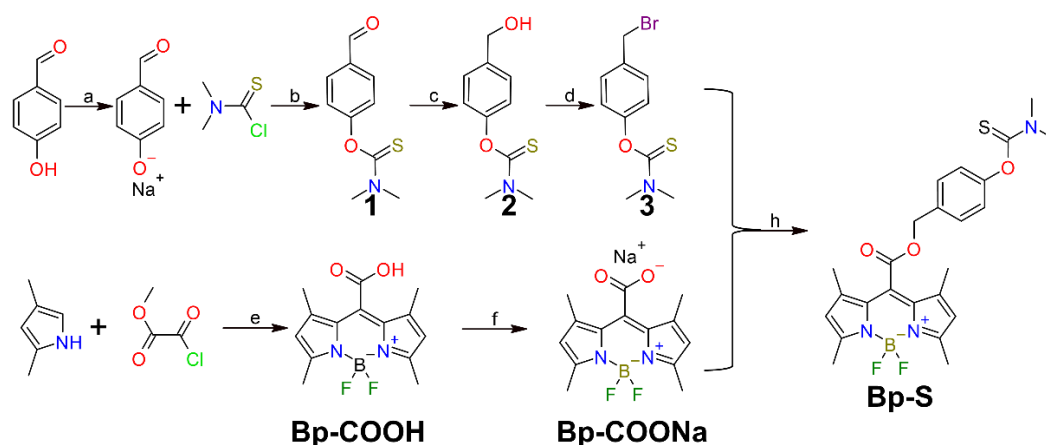
Compound 3

To a solution of compound **2** (316 mg, 1.5 mmol) in dry DCM (5 mL) at room temperature was added PBr_3 (610 mg, 5.0 mmol). The solution was stirred at rt for 60 min and then the reaction solution was diluted with DCM, wash with saturated sodium bicarbonate, dried with anhydrous magnesium sulfate, and concentrated to give crude compound **3**. Compound **3** was directly used in the next reaction without purification.

Bp-S

To a solution of Bp-COOH (292 mg, 1.0 mmol) in EtOH (2 mL) at room temperature was added NaOH solution (40 mg, 1.0 mmol, in 5 mL water) slowly. After dropwise addition is completed, the solvent is removed by concentration. The residual solid was dissolved into 10 mL DMF, crude compound 3 was added, and stirred at room temperature overnight. The reaction solution was diluted with ethyl acetate, washed sequentially with water, sodium chloride solution, and finally dried with anhydrous magnesium sulfate. The crude material was purified by flash column (SiO₂) to give 252 mg (52% yield) of the title compound as a red solid. HRMS (ESI) calculated for C₂₄H₂₆BFN₃O₃S⁺, [M-F]⁺, 466.3554, found, 466.3554. (Fig. S2)

2. Supporting Schemes



Scheme S1: Synthetic procedures of **Bp-S**. Reaction conditions: a. NaOH, water; b. ACN, rt, 3h; c. NaBH₄, EtOH, rt, 1h. d. PBr₃, DCM, rt, 1h. e. 1: BF₃·(Et)₂O, TEA, DCM. 2: LiI, EA, reflux, overnight. f. EtOH, water, NaOH, rt, 30 min. h. DMF, rt, overnight.

3. Supporting Figures

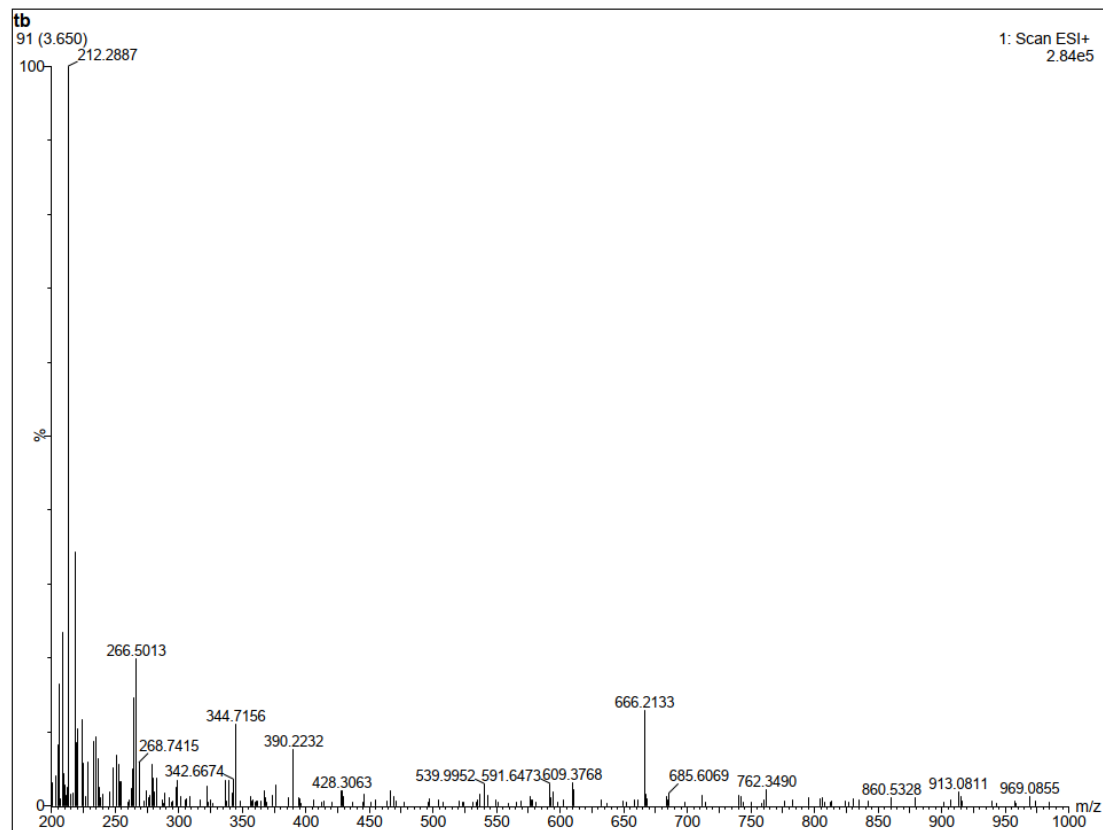


Figure S1: ESI-HRMS of Compound 2

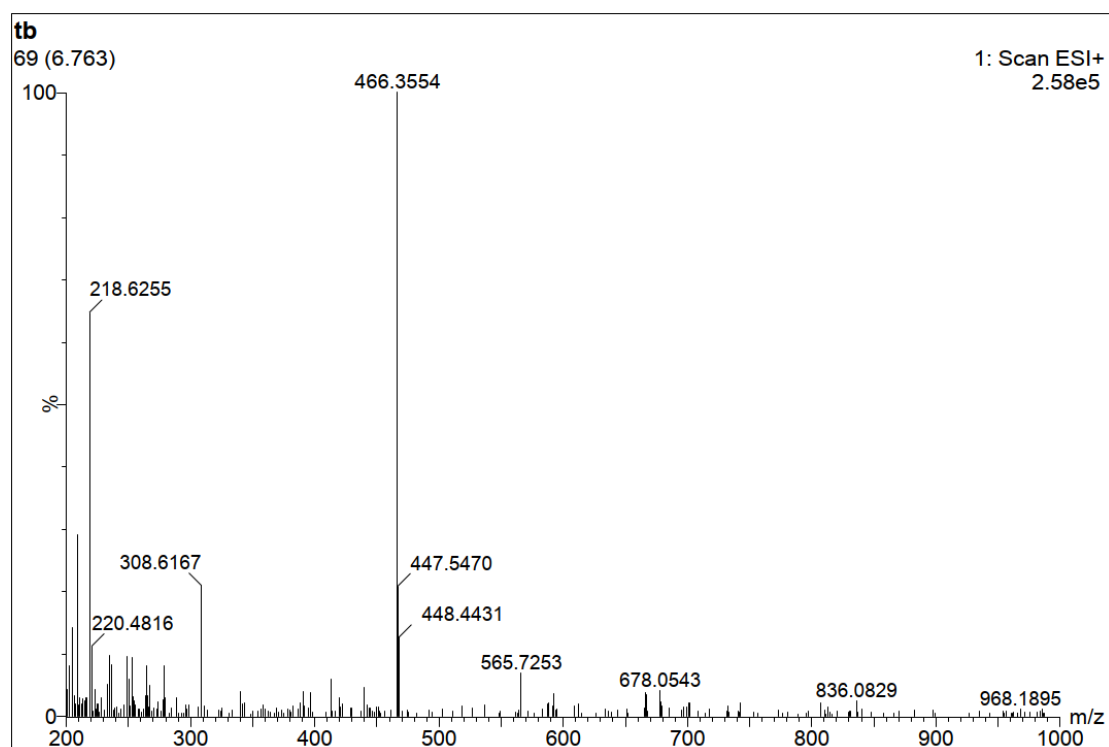


Figure S2: ESI-MS of Bp-S.

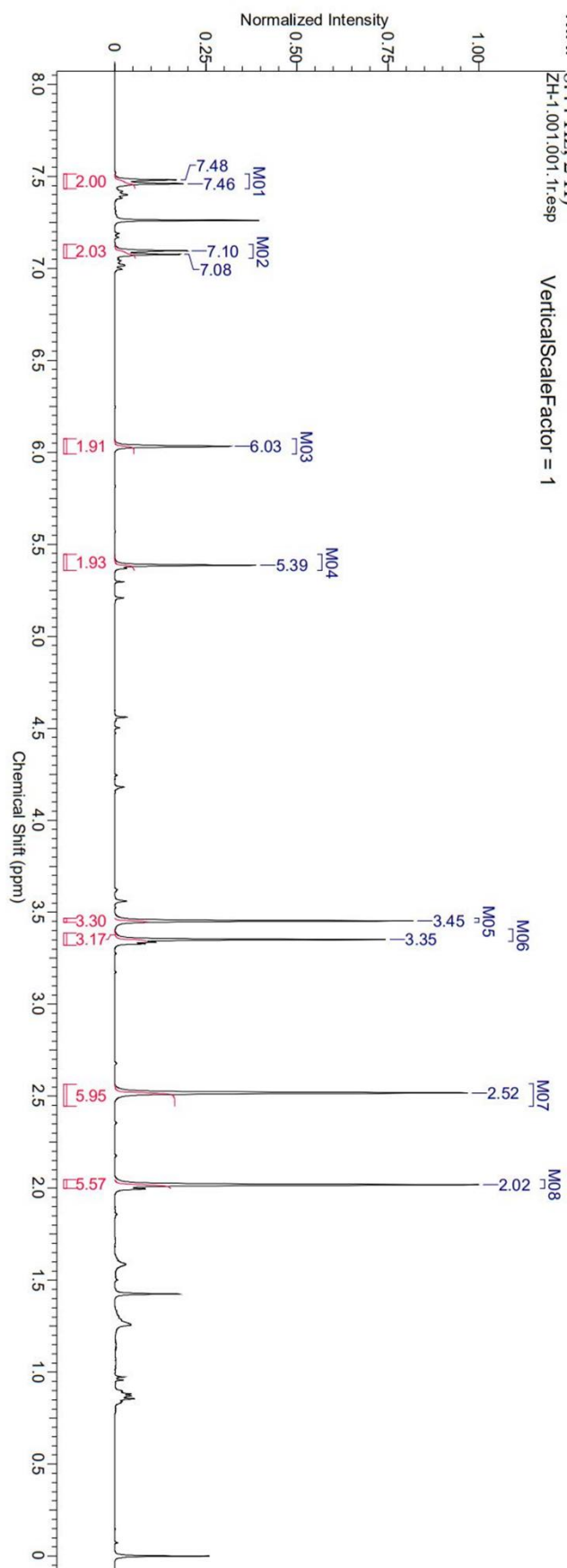


Figure S3: ^1H NMR of Compound **Bp-S**.

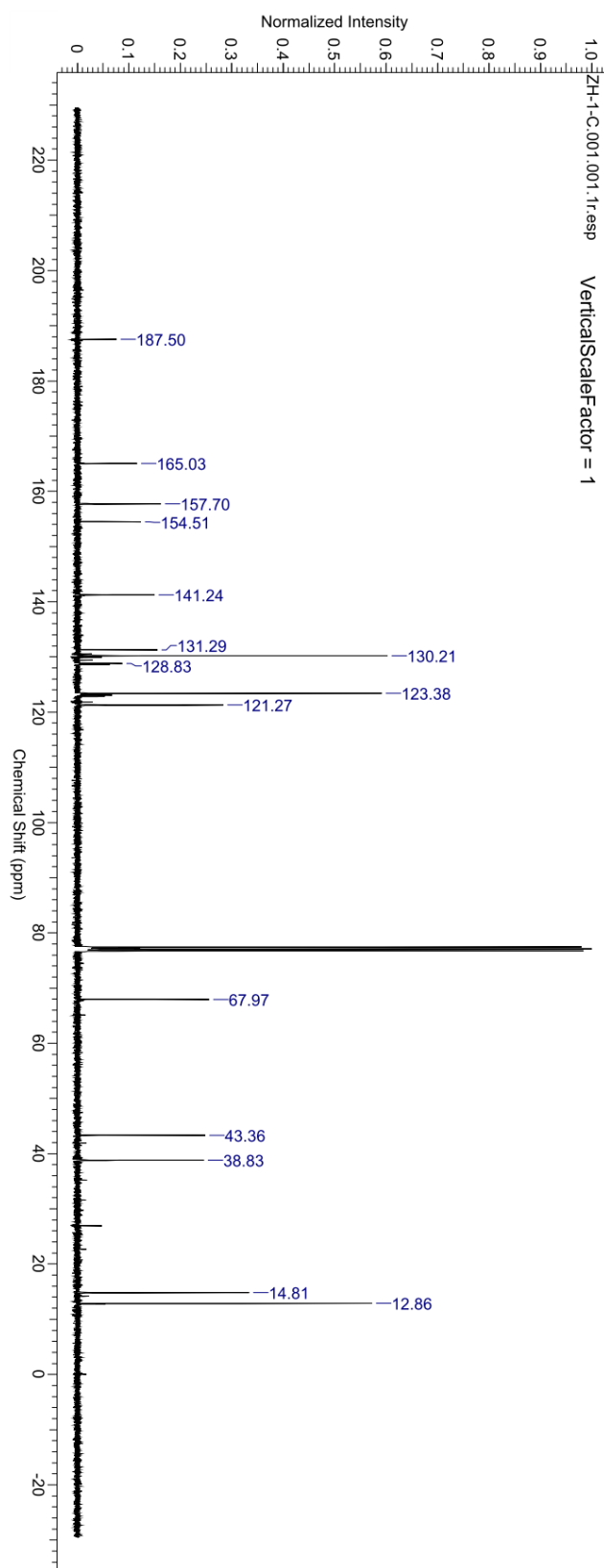


Figure S4: ^{13}C NMR of **Bp-S**.

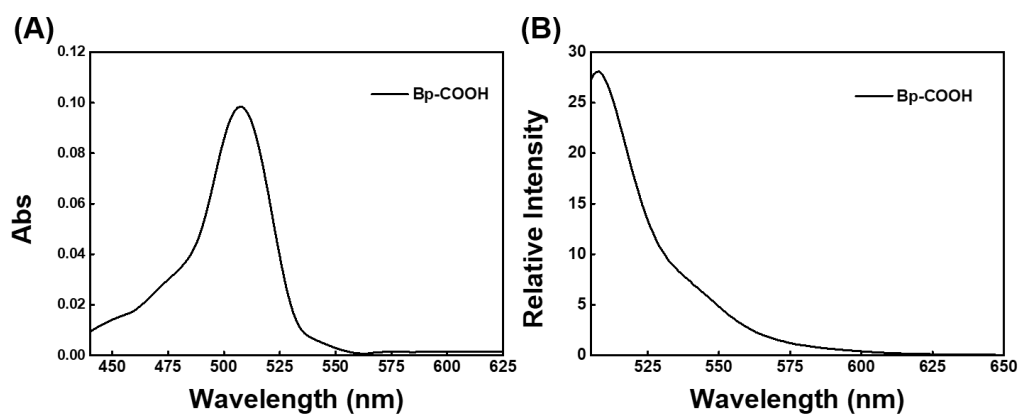


Figure S5. (A) Absorption and (B) fluorescence emission spectra of **Bp-COOH** (1 μ M) in pH7.4 PBS. λ_{ex} = 495 nm, slit width: 1.5 mm/1.5 mm.

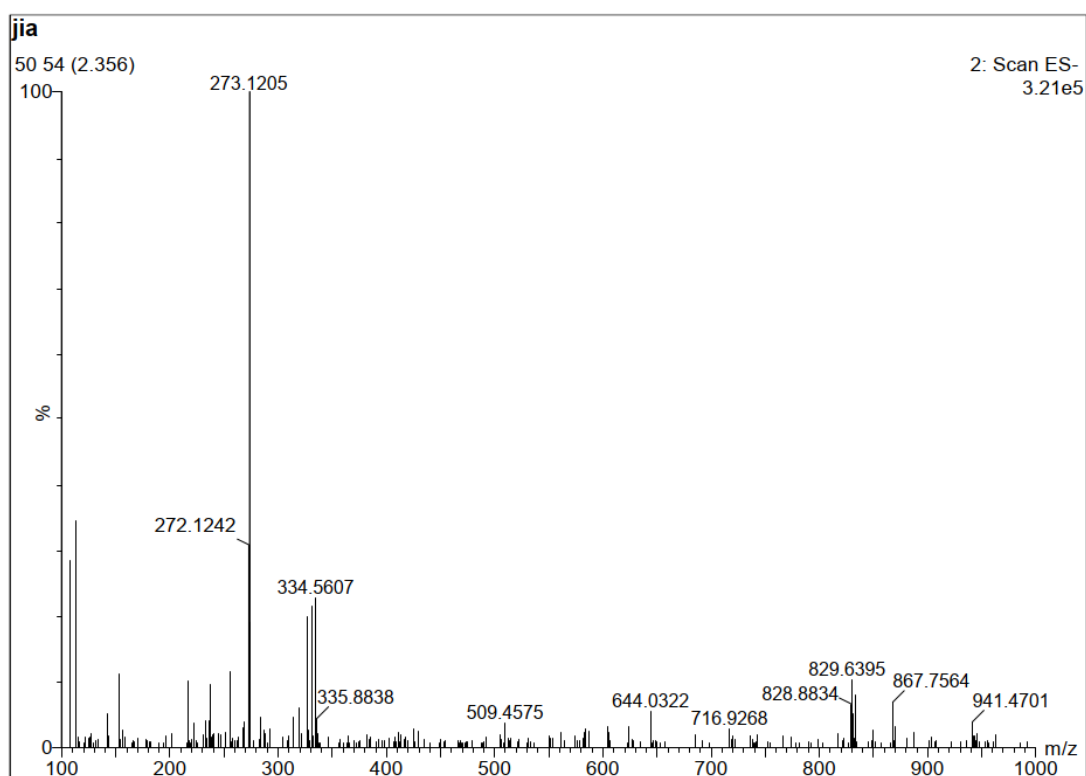


Figure S6. ESI-MS of the reaction solution of **Bp-S** (2 μ M) with NaClO (40 μ M).

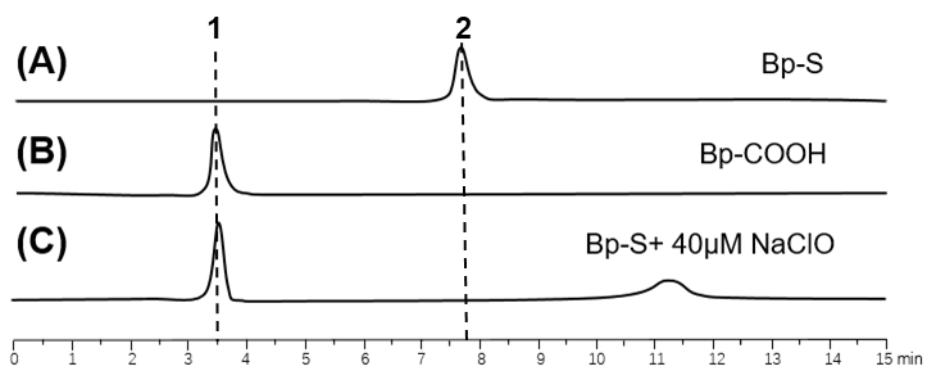


Figure S7. Chromatograms of different reaction systems. (A) **Bp-S** (2 μM); (B) **Bp-COOH** (2 μM); (C) the reaction solution of 2 μM **Bp-S** with 100 μM NaClO for 5 min. The assignments of the peaks: (1) 3.51 min, **Bp-COOH**; (2) 5.78 min, **Bp-S**.

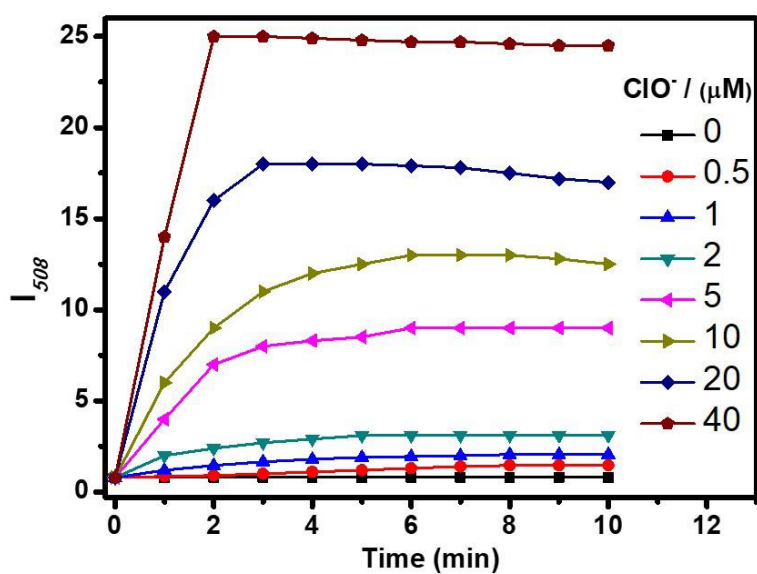


Figure S8. Fluorescence emission of **Bp-S** (2 μM) for different concentrations of ClO^- with time.

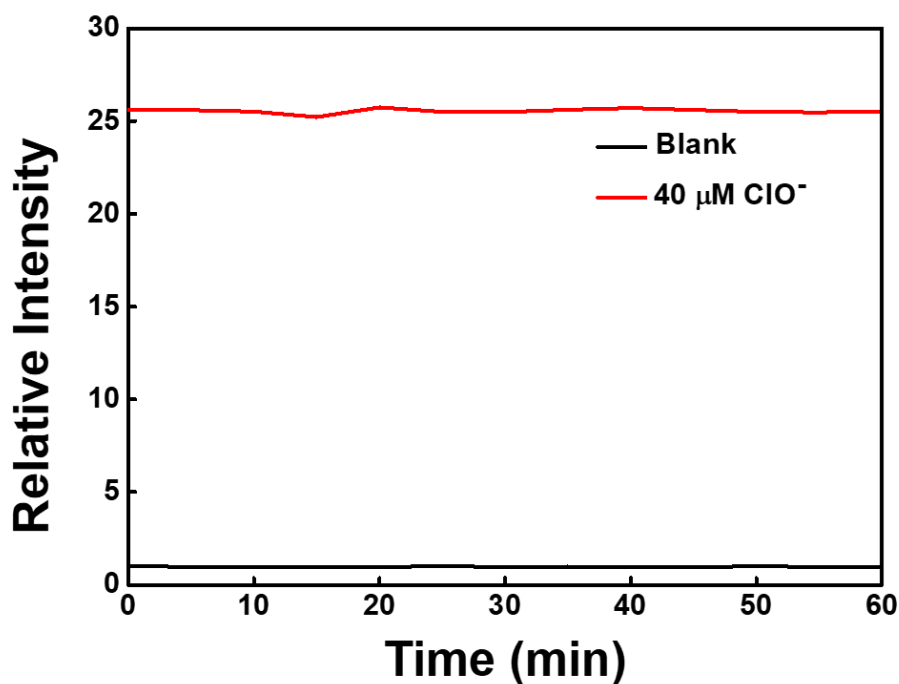


Figure S9. Dynamic emission intensity of **Bp-S** (2 μM) with or without ClO[−] (40 μM). λ_{ex} = 495 nm, slit width: 1.5 mm/1.5 mm.

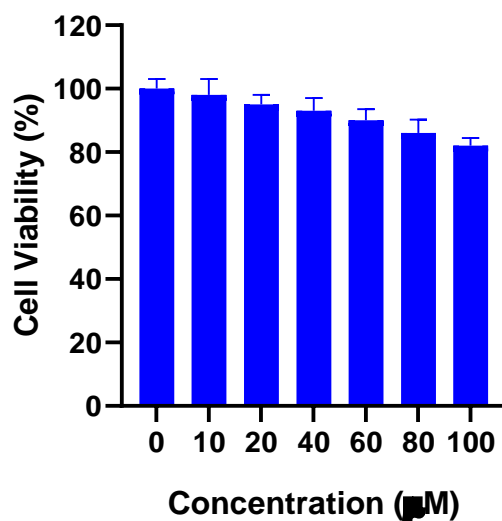


Figure S10. Cytotoxic effect of **Bp-S**. RAW 246.7 cells was incubated with different concentration (of probes for 24 h. Cell viability was assayed with CCK-8 test. Results are expressed as mean \pm standard deviation of three independent experiments.

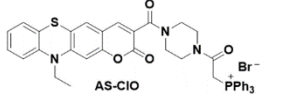
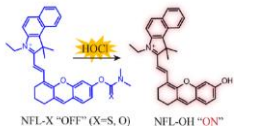
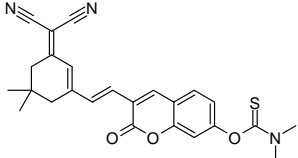
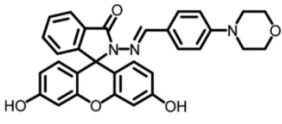
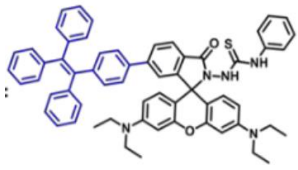
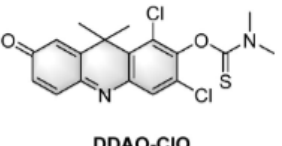
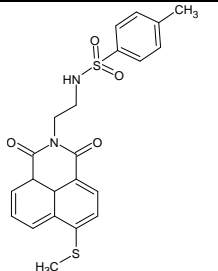
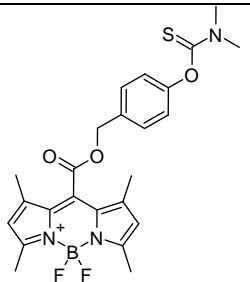
Table S1. Photophysical Properties of Bp-COOH and Bp-S.

Compound	$\lambda_{\text{abs}}(\text{nm})$	$\epsilon (\text{M}^{-1}\text{cm}^{-1})$	$\lambda_{\text{em}}(\text{nm})$	$\Phi_{\text{fl}}(\%)$
Bp-COOH	492	112800	506	52 ^a
Bp-COOH	495	146000	506	59 ^b
Bp-S	513	52000	508	0.3 ^b

^a Data from literature ([jacs, 2017 139,10157](#)).

^b Data were collected in 10 mM PBS (pH 7.4; 20% v/v EtOH) at 25 °C.

Table S2. Some recently published ClO⁻ fluorescent probes

Structure	properties	Reference
 <p>AS-CIO</p>	$\lambda_{\text{abs}} = 383 \text{ nm}$ $\lambda_{\text{em}} = 520 \text{ nm}$ LOD = 12.53 nM Response < 5 s	Sensors and Actuators B: Chemical 348 , 130695 (2021)
 <p>NFL-X "OFF" (X=S, O) NFL-OH "ON"</p>	$\lambda_{\text{abs}} = 607 \text{ nm}$ $\lambda_{\text{em}} = 700 \text{ nm}$ LOD = 35 nM Response < 30 s	Dyes and Pigments 188 , 109218 (2021)
	$\lambda_{\text{abs}} = 510 \text{ nm}$ $\lambda_{\text{em}} = 700 \text{ nm}$ LOD = no data Response < 2 s	Talanta 223 , 121768 (2021)
 <p>FCMB</p>	$\lambda_{\text{abs}} = 505 \text{ nm}$ $\lambda_{\text{em}} = 530 \text{ nm}$ LOD = 57.8 nM Response < 20 s	Sensors and Actuators B: Chemical 327 , 128848 (2021)
	$\lambda_{\text{abs}} = 480 \text{ nm}$ $\lambda_{\text{em}} = 590 \text{ nm}$ LOD = no data Response < 3 min	Chem Commun (Camb) 56 , 14613–14616 (2020)
 <p>DDAO-CIO</p>	$\lambda_{\text{abs}} = 645 \text{ nm}$ $\lambda_{\text{em}} = 658 \text{ nm}$ LOD = 7.3 nM Response < 3 s	Talanta 215 , 120901 (2020)
	$\lambda_{\text{abs}} = 402 \text{ nm}$ $\lambda_{\text{em}} = 509 \text{ nm}$ LOD = 120 nM Response < 3 min	Spectrochim Acta A Mol Biomol Spectrosc 229 , 117992 (2020)
	$\lambda_{\text{abs}} = 495 \text{ nm}$ $\lambda_{\text{em}} = 508 \text{ nm}$ LOD = 6.3 nM Response < 5 min	This work