Supporting Information for

Design of a FRET-based fluorescent probe for reversible detection of SO_2 and formaldehyde in living cells and mice

Wenjie Gao, Yanyan Ma and Weiying Lin*

Institute of Fluorescent Probes for Biological Imaging, School of Chemistry and Chemical Engineering, School of Materials Science and Engineering, University of Jinan, Jinan, Shandong 250022, P.R. China.

E-mail: weiyinglin2013@163.com

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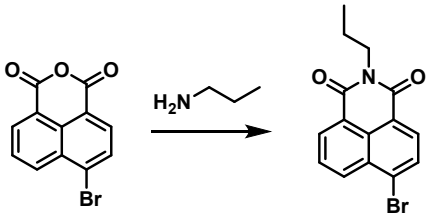
Materials and instruments

Unless otherwise stated, ultrapure water was used in all experiments and all reagents were obtained from commercial suppliers without further purification. Solvents were purified by standard methods prior to use. UV−vis absorption spectra were measured on a Shimadzu UV-2700 spectrophotometer and fluorescence spectra were recorded with a HITACHI F4600 fluorescence spectrophotometer. TLC analysis carried out on silica gel plates and column chromatography was conducted over silica gel (mesh 200−300), both of them were purchased from the Qingdao Ocean Chemicals. MTT was purchased from J&K Scientific Ltd. Fluorescence imaging experiments were performed with Nikon A1MP confocal microscopy. ^{1}H and ^{13}C NMR spectra were recorded on an AVANCE III 400 Nanobay (Bruker, 400 MHz for ^{1}H, 100 MHz for ^{13}C) at room temperature, using DMSO-d6 as solvent and tetramethylsilane (TMS) as internal reference. High-resolution mass spectrometric (HRMS) analyses were measured on a Bruker apex-Ultra mass spectrometer (Bruker Daltonics Corp., USA) in electrospray ionization (ESI) mode. HRMS for response mechanism was collected using Agilent 6510 Q-TOF LC/MS for studying response mechanism.

Synthesis of compound of NaP

Synthesis of compound 1

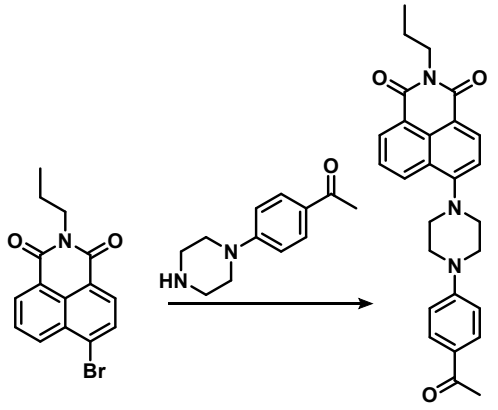


1

4-bromo-1,8-naphthalene dicarboxylic anhydride (5.0 g, 18 mmol) and aminopropane (1.64 g ,19 mmol) were put into a suitable reaction bottle, and then 10 mL ethanol was added and heat to reflux at 80℃ for 3 h under nitrogen protection. After cooling to room temperature, the compound **1** was obtained by vacuum

filtration.

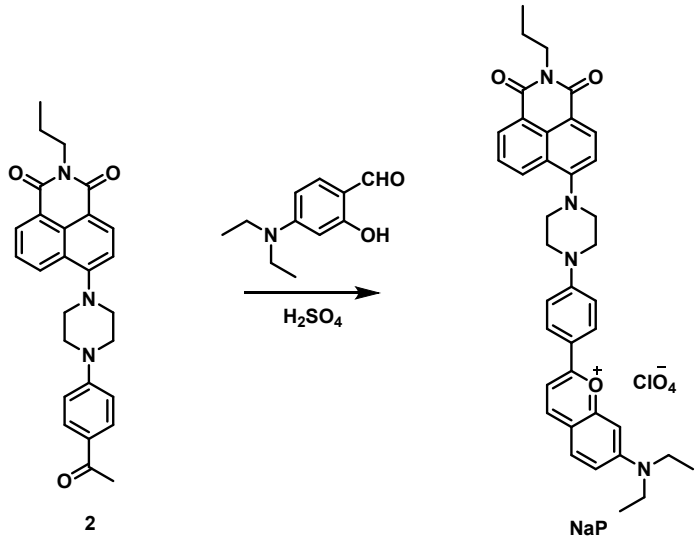
Synthesis of compound 2



1 2

Compound **1** (1.5 g, 5 mmol) was added to a flask containing 10 ml of ethylene glycol monomethyl ether. Then 1-(4-piperazin-1-yl-phenyl)e thanone (1.3 g, 6 mmol) was added to the above soultion and heated to reflux at 130°C for 5h. Then, the solvent was evaporated and purified by silica gel column chromatography (CH_2Cl_2: MeOH=30:1) to obtain compound **2**.

Synthesis of compound NaP



2 NaP

Preparation for the spectral measurement

Detection limit

The detection limit was based on a reported method. [S1] According to the result of titrating experiment, the fluorescence ratio intensities (I_{540}/I_{640}) of **NaP** treated with different $NaHSO_3$ were normalized between the minimum intensity and the maximum intensity. A linear regression curve was then fitted to the normalized fluorescent intensity data and the point at which this line crossed the axis was considered as the detection limit.

Cell culture and cytotoxicity assays

HeLa cells were cultured in Dulbecco's Modified Eagle Medium media (DMEM, Hyclone) supplemented with 10 % fetal bovine serum (FBS, Sijiqing), penicillin (100 U/ml, Hyclone) and streptomycin sulfate (100 U/ml, Hyclone) under an atmosphere of 5% CO_2 and 95% air at 37 °C.

The cytotoxicity of **NaP** to living cells was performed by standard MTT assays. 2×10^4 cells/mL living cells were seeded in 96-well plates and then incubated with different concentrations of **NaP** (0-20 μM) for 24 h. Subsequently, HeLa cells were incubated with 5 mg/mL MTT (10 μL per well) and treated for 4 h. After that the supernatants were aspirated and 100 μL DMSO was added to per well. The absorbance of the solution at 570 nm was recorded using microplate reader. The cell viability (%) = $(OD_{sample}-OD_{blank}) / (OD_{control}-OD_{blank}) \times 100$ %.

OD_{sample} denotes cells treated with various concentrations of **NaP**; OD_{blank} denotes the plates with DMEM; $OD_{control}$ denotes cells without treated with **NaP**. Each concentration was conducted with three parallel samples, and the results were expressed as mean ± standard deviation (SD).

Ethical statement of living mice

The 4-week old babl/c mice were obtained from School of Pharmaceutical Sciences, Shandong University. All animal experiments were conducted in accordance with the Animal Management Regulations of the People's Republic of China (No. 55 of 2001) and the Guidelines for the Care and Use of the Animal Ethics Experimental Committee of Shandong University. The procedures for the use of animals used in this study have been approved by the ethics committee of the institutional animal care and use Committee (IACCC), and have been approved by all applicable agencies and government regulations concerning the ethical use of animals.

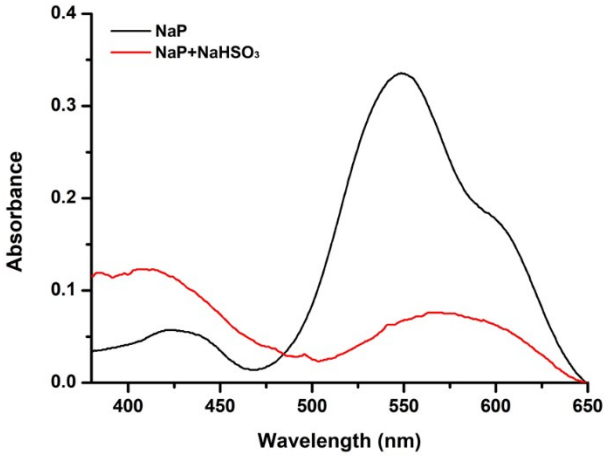
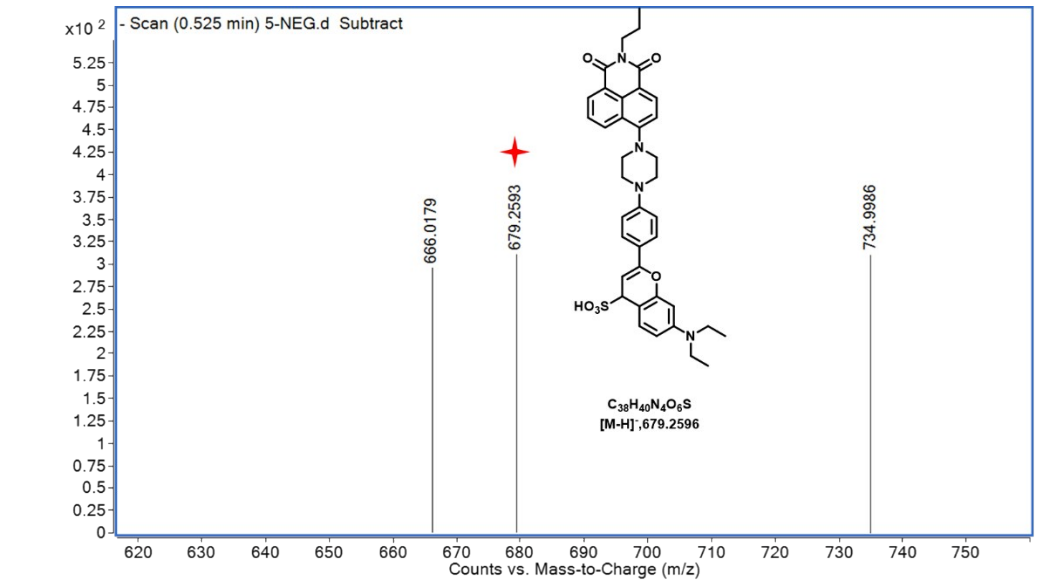
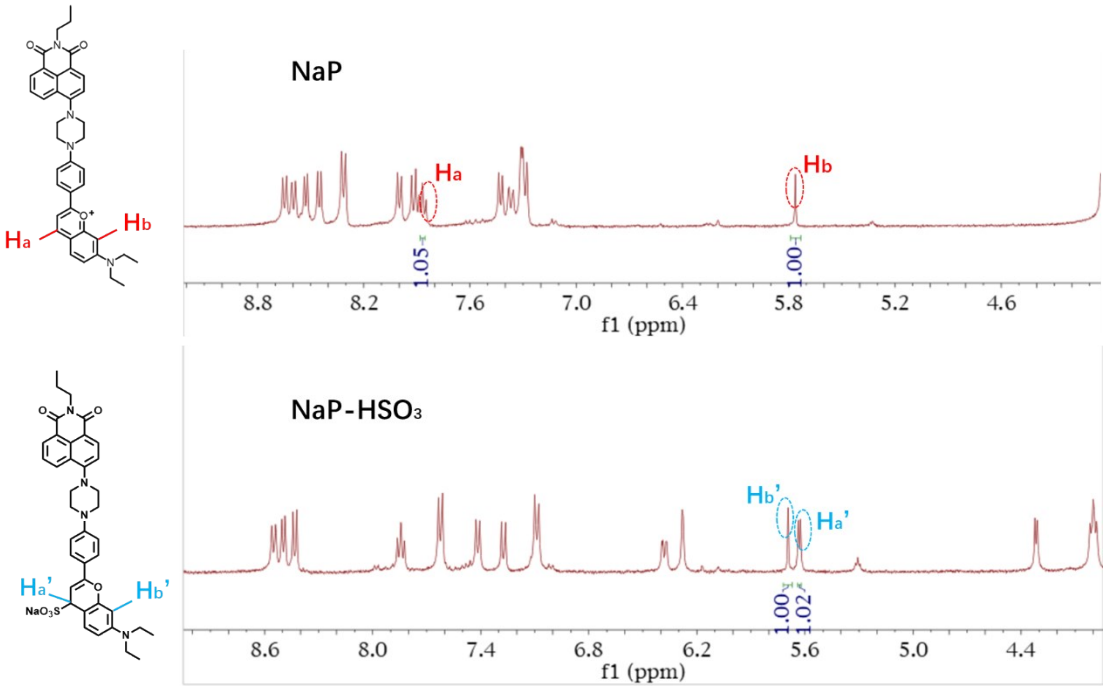


Fig. S1. Absorbance spectra of the probe **NaP** (10 μM) upon treating with 70 μM $NaHSO_3$.



(a)



(b)

Fig. S2. (a) HR-MS spectrum of the reaction of **NaP** (10 μM) with 70 μM $NaHSO_3$. (b) Partial ^1H NMR spectra of **NaP** in the presence or absence of $NaHSO_3$ in DMSO-d6/D2O (v/v, 10:1) solution.

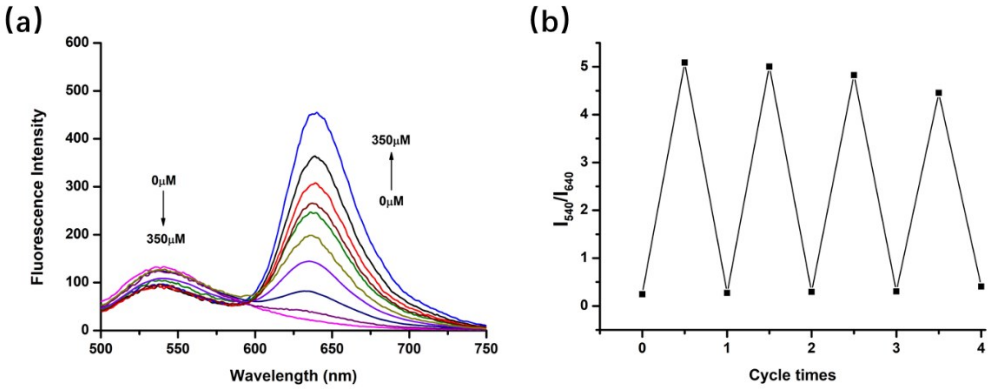


Fig. S3. (a) Fluorescence spectra of increasing FA (0-350 μM) concentration were added to the **NaP** (10 μM) solution containing $NaHSO_3$ (70 μM). (b) Reversible cycle of **NaP** upon addition of $NaHSO_3$ and FA alternately. λ_{ex} = 425 nm.

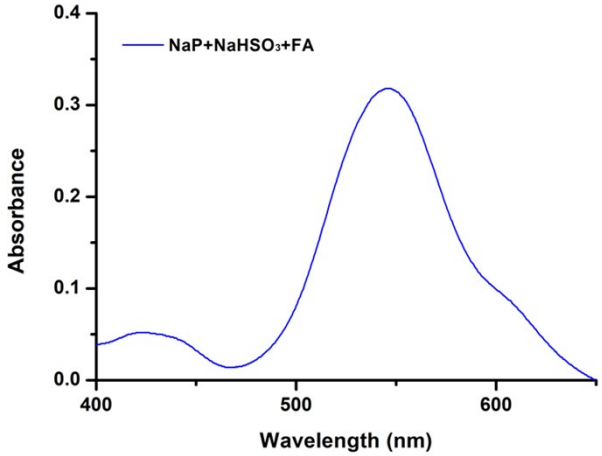


Fig. S4. Absorbance spectra of the probe **NaP** (10 μM) upon treated with 70 μM $NaHSO_3$ and FA (350 μM).

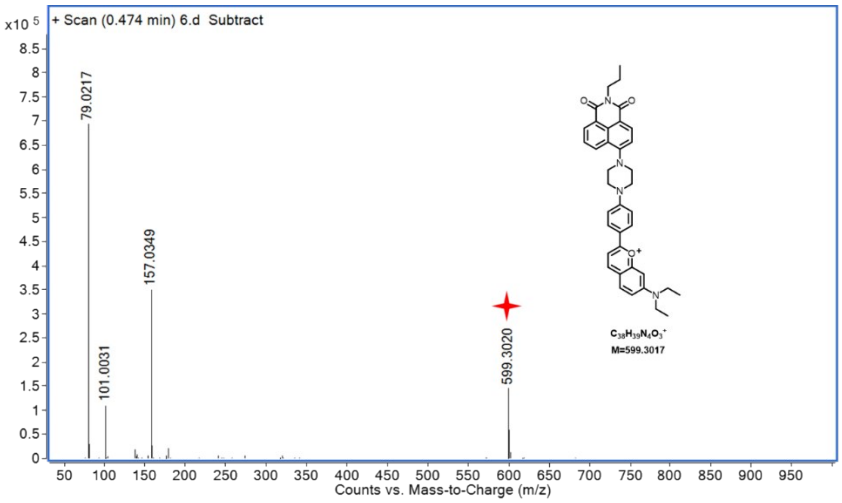


Fig. S5. HR-MS spectrum of the mixture of 10 μM **NaP** with 70 μM $NaHSO_3$ upon addition of

350 μM FA.

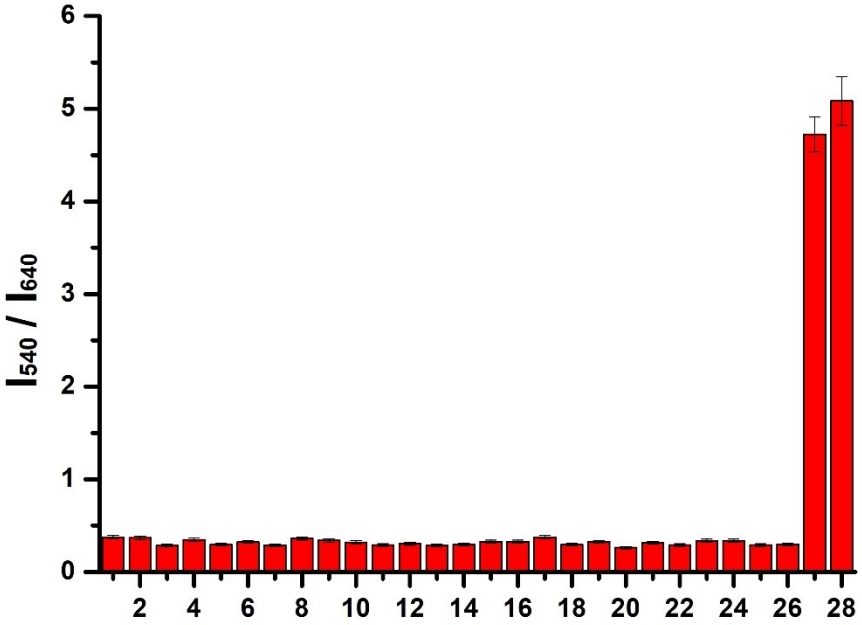


Fig. S6. Fluorescent intensity ratio (I_{540}/I_{640}) of the probe **NaP** (10 μM) to various relevant analytes in PBS/CH_3CN (V/V, 4/1). 1.**NaP** (10 μM), 2. Hcy (500 μM), 3. Cys (500 μM), 4. GSH (500 μM), 5. Na_2S (50 μM), 6. NaClO (100 μM), 7. H_2O_2 (100 μM), 8. tert-butyl peroxide (TBHP) (100 μM), 9. KF (2.5 mM), 10. NaF (2.5 mM), 11. KI (2.5 mM), 12. $FeSO_4$ (2.5 mM), 13. KSCN (2.5 mM), 14. $MgCl_2$ (2.5 mM), 15. NaBr (2.5 mM), 16. $NaHCO_3$ (2.5 mM), 17. $NaNO_2$ (2.5 mM), 18. $NaNO_3$ (2.5 mM), 19. $BaCl_2$ (2.5 mM), 20. Na_2HPO_4 (2.5 mM), 21. $AgNO_3$ (2.5 mM), 22. $ZnCl_2$ (2.5 mM), 23. NaOAc (2.5 mM), 24. $Na_2S_2O_3$ (2.5 mM), 25. $CaCl_2$ (2.5 mM), 26. $CuCl_2$ (2.5 mM) 27. Na_2SO_3 (70 μM), 28. $NaHSO_3$ (70 μM). λ_{ex} = 425nm. Error bars represent mean values ± SD. (n = 3)

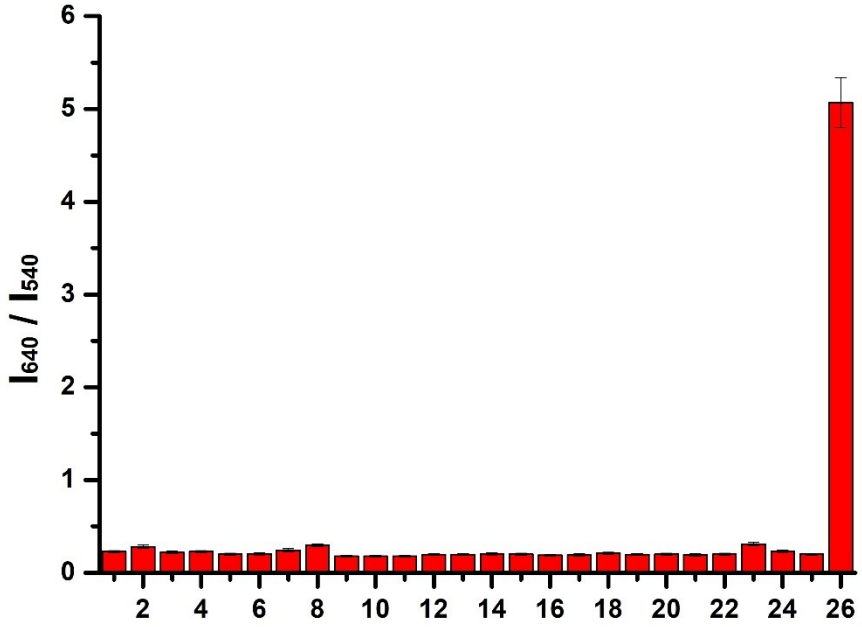


Fig. S7. The fluorescence intensity ratio (I_{640} / I_{540}) of the relevant analytes added to the solution of **NaP** (10μM) containing $NaHSO_3$ (70μM). 1. $NaHSO_3$ (70 μM), 2. NaClO (100 μM), 3. Na_2S (50 μM), 4. H_2O_2 (100 μM), 5. tert-butyl peroxide (TBHP) (100 μM), 6. Hcy (500 μM), 7. Cys (500 μM), 8. GSH (500 μM), 9. KF (2.5 mM), 10. NaF (2.5 mM), 11. KI (2.5 mM), 12. $FeSO_4$ (2.5 mM), 13. KSCN (2.5 mM), 14. $MgCl_2$ (2.5 mM), 15. NaBr (2.5 mM), 16. $NaHCO_3$ (2.5 mM), 17. $NaNO_2$ (2.5 mM), 18. $NaNO_3$ (2.5 mM), 19. $BaCl_2$ (2.5 mM), 20. Na_2HPO_4 (2.5 mM), 21. $AgNO_3$ (2.5 mM), 22. NaOAc (2.5 mM), 23. $Na_2S_2O_3$ (2.5 mM), 24. $CaCl_2$ (2.5 mM), 25. $CuCl_2$ (2.5 mM), 26. FA (350μM). Error bars represent mean values ± SD. (n = 3)

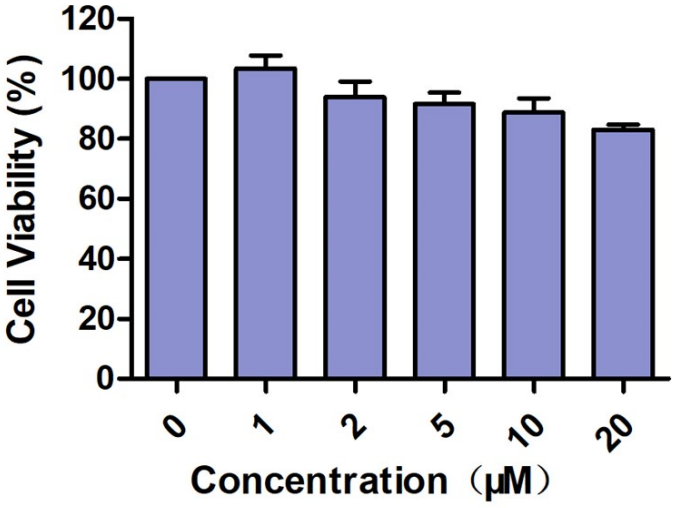


Fig. S8. Viability of HeLa cells treated with various concentrations (0 - 20 μM) of **NaP** for 24 h.

Error bars represent mean values ± SD. (n = 3)

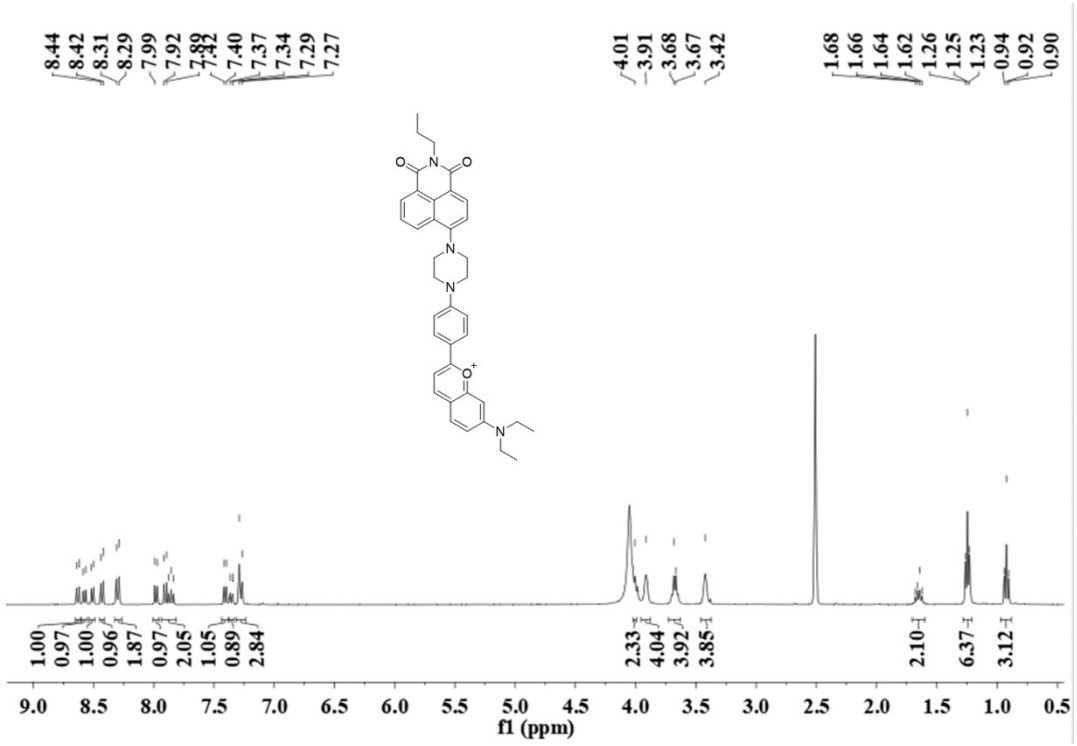


Fig. S9. ^{1}H NMR spectrum of Compound **NaP** in d_6-DMSO.

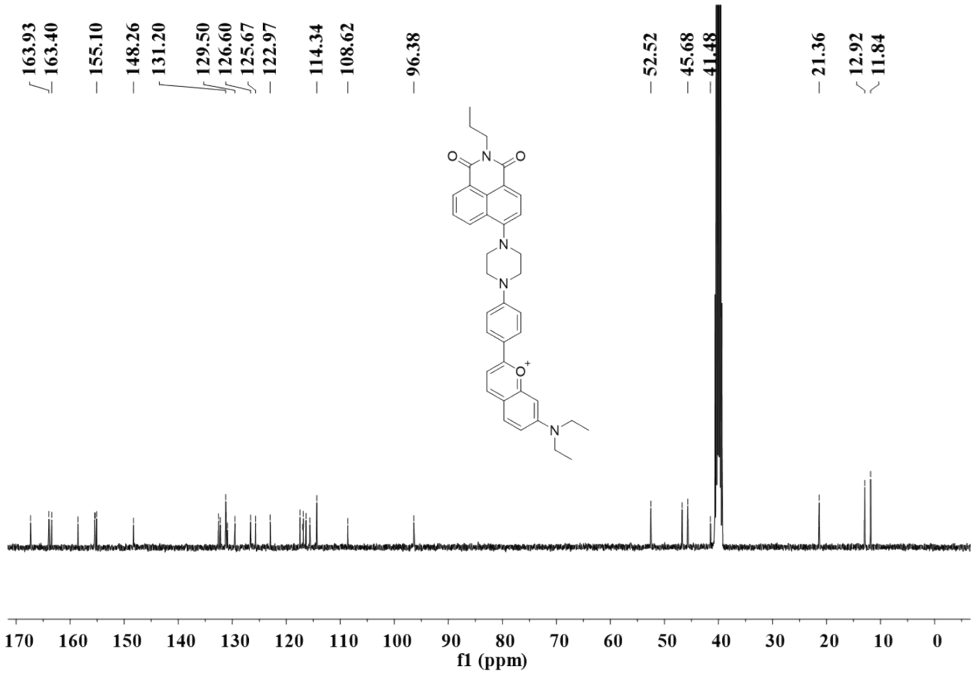


Fig. S10. ^{13}C NMR spectrum of **NaP** in d_6-DMSO.

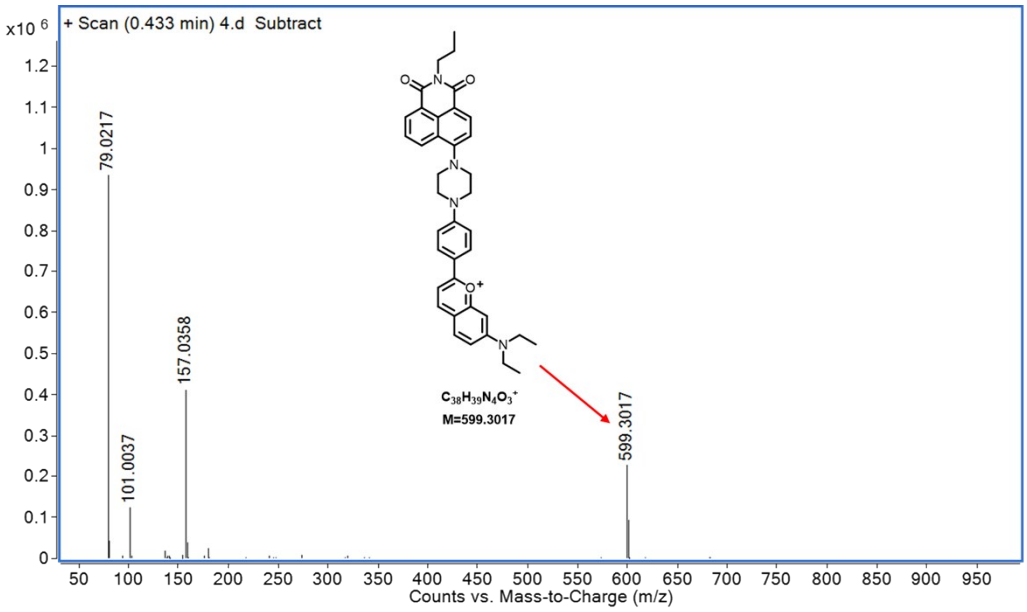


Fig. S11. HR-MS spectrum of **NaP**.

REFERENCES

[S1] Shortreed M.; Kopelman R.; Kuhn M.; Hoyland B.; *Anal. Chem.*, **1996**, *68*, 1414-1418.