Electronic Supporting Information

A colorimetric and fluorometric probe for phenylhydrazine and its application in real samples

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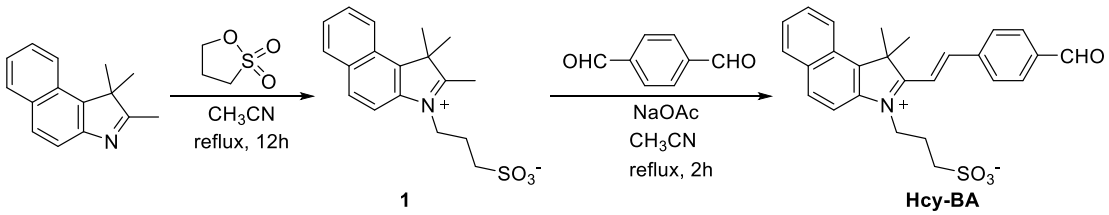
CONTENT

1. Materials and instruments

All chemical reagents and solvents were obtained from commercial suppliers (Adamas Reagents or Cologne Reagents) without further purification unless mentioned. Ultrapure water was purified from Millipore water purification system. Silica gel (Qingdao, mesh 100-200) was used for TLC analysis and column chromatography. NaCl, Na_2CO_3, Na_2SO_4, $NaNO_2$, $MgCl_2$, $CaCl_2$, $CuCl_2$, NH_4Cl, *L*-histidine, *L*-proline, cyclohexylamine, aniline, hydrazine, hydroxylamine hydrochloride, acethydrazide, isopropyl hydrazine, hydrazinoethnol, benzyl hydrazine and phenylhydrazine were analytical grade products and their respective PBS solutions were used for spectral measurements.

^1H NMR and ^{13}C NMR spectra were measured on a Bruker AVANCE NMR instrument (400 or 600 M) and chemical shifts were given in ppm using the peak of residual proton signals of DMSO as the internal standard. Mass spectra were carried out on a Finnigan LCQ^{DECA} spectrometer with ESI mode. UV−vis and fluorescence spectra were performed on a SHIMADZU UV-2450 spectrophotometer and a VARIAN Cary Eclipse FL1003 M013 spectrometer. A digital pH meter (pHS-25, Century Ark, China) was used to measure the pH value. The fluorescence of test strip was recorded under 365 nm UV light irradiation (LUV-270A, LuYang, Shanghai).

2. Synthesis and characterizations of Hcy-BA



Scheme S1 Synthetic route of probe **Hcy-BA**.

Synthesis of intermediate 1

To the solution of 2,3,3-trimethyl-3*H*-benzo[*g*] indole (2.09 g，10.00 mmol) in 50

mL acetonitrile was added 1,3-propane sultone (2.44 g， 20.00 mmol), then, the mixture was refluxed for 12 h. After cooling to room temperature, the reaction mixture was filtered and the solid collected was washed three times with acetonitrile and dried under vacuum. Then, intermediate **1** was obtained as a pale-white solid (2.97 g, 89.7%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.36 (d, J = 8.4 Hz, 1H), 8.29 (d, J = 8.9 Hz, 1H), 8.26 - 8.19 (m, 2H), 7.78 (t, J = 7.6 Hz, 1H), 7.72 (t, J = 7.5 Hz, 1H), 4.78 (t, J = 8.0 Hz, 2H), 2.94 (s, 3H), 2.67 (t, J = 6.5 Hz, 2H), 2.22 (dt, J = 14.5, 6.8 Hz, 2H), 1.76 (s, 6H). The spectral data were in accordance with those reported in the literature. [1]

Synthesis of probe Hcy-BA

Intermediate **1** (0.33 g, 1.00 mmol) was dissolved in 25 mL acetonitrile at room temperature and terephthalaldehyde (0.20 g, 1.50 mmol) was added. After the addition of sodium acetate (3 mg), the mixture was refluxed for 2 h. After cooling to room temperature, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. Then, the residue was purified by column chromatography on silica gel (dichloromethane: methanol = 20:1, v/v) to afford probe **Hcy-BA** as a dark red solid (0.28 g, 63.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.13 (s, 1H), 8.62 (d, J = 16.5 Hz, 1H), 8.55 (d, J = 8.0 Hz, 2H), 8.46 (d, J = 8.5 Hz, 1H), 8.31 (d, J = 9.1 Hz, 2H), 8.25 (d, J = 8.2 Hz, 1H), 8.11 (d, J = 15.9 Hz, 2H), 8.07 (s, 1H), 7.82 (d, J = 7.0 Hz, 1H), 7.75 (d, J = 7.0 Hz, 1H), 5.07 (t, J = 8.1 Hz, 2H), 2.74 (t, J = 6.2 Hz, 2H), 2.31 - 2.24 (m, 2H), 2.06 (s, 6H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 193.28, 182.84, 151.05, 140.25, 139.67, 138.93, 138.62, 133.87, 131.65, 131.48, 130.50, 130.21, 128.94, 127.96, 127.13, 123.76, 115.67, 113.94, 54.62, 47.61, 46.81, 25.56, 25.51. HRMS (ESI, m/z): calculated for $[C_{26}H_{26}NO_4S]^+$, 448.1577, found 448.1578.

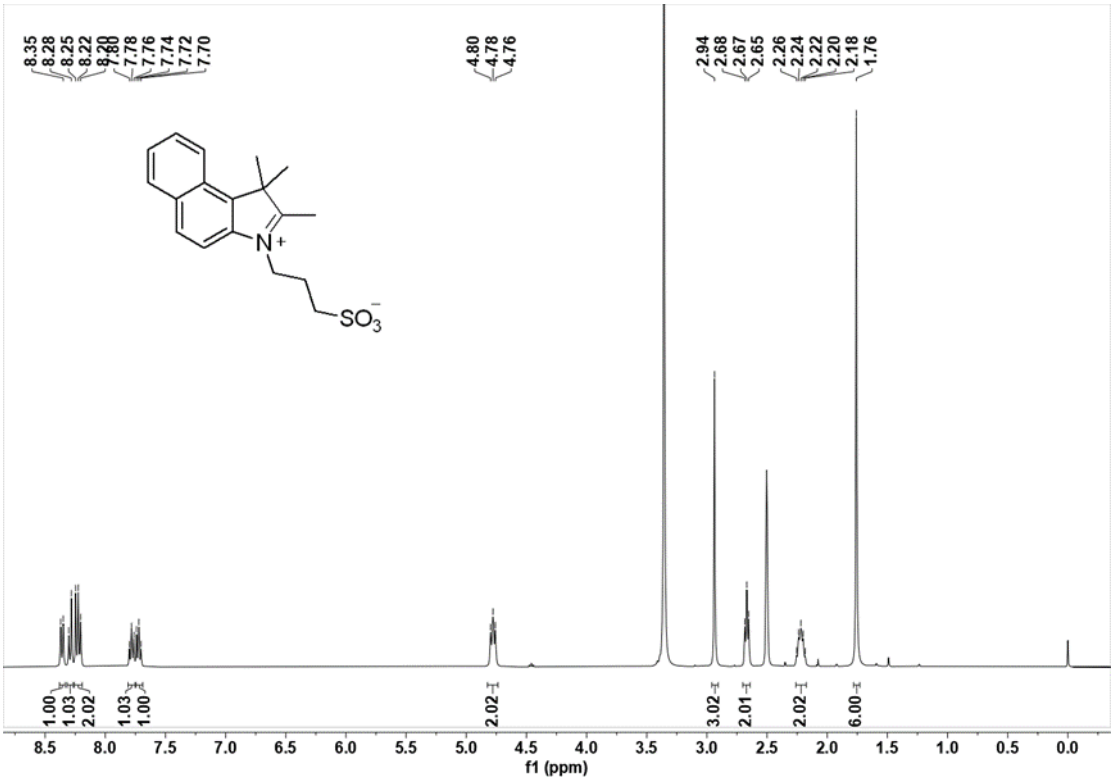


Fig. S1 [1]H-NMR spectra of intermediate **1** in DMSO-d_6.

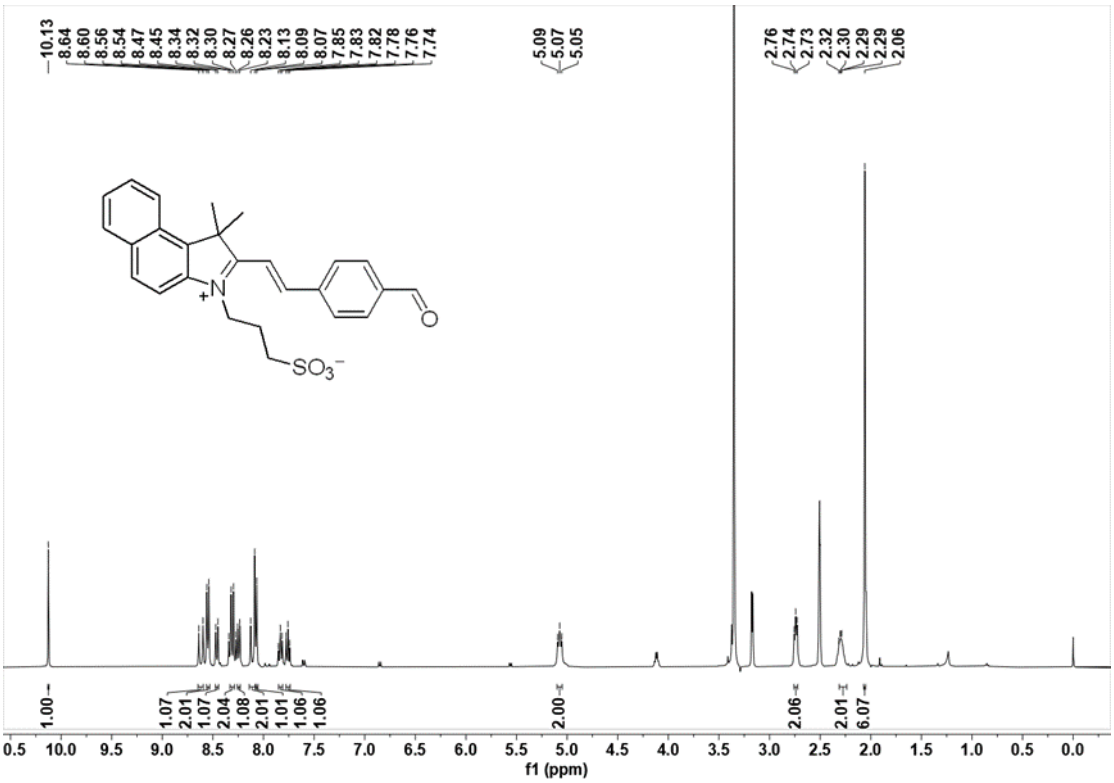


Fig. S2 [1]H-NMR spectra of probe **Hcy-BA** in DMSO-d_6.

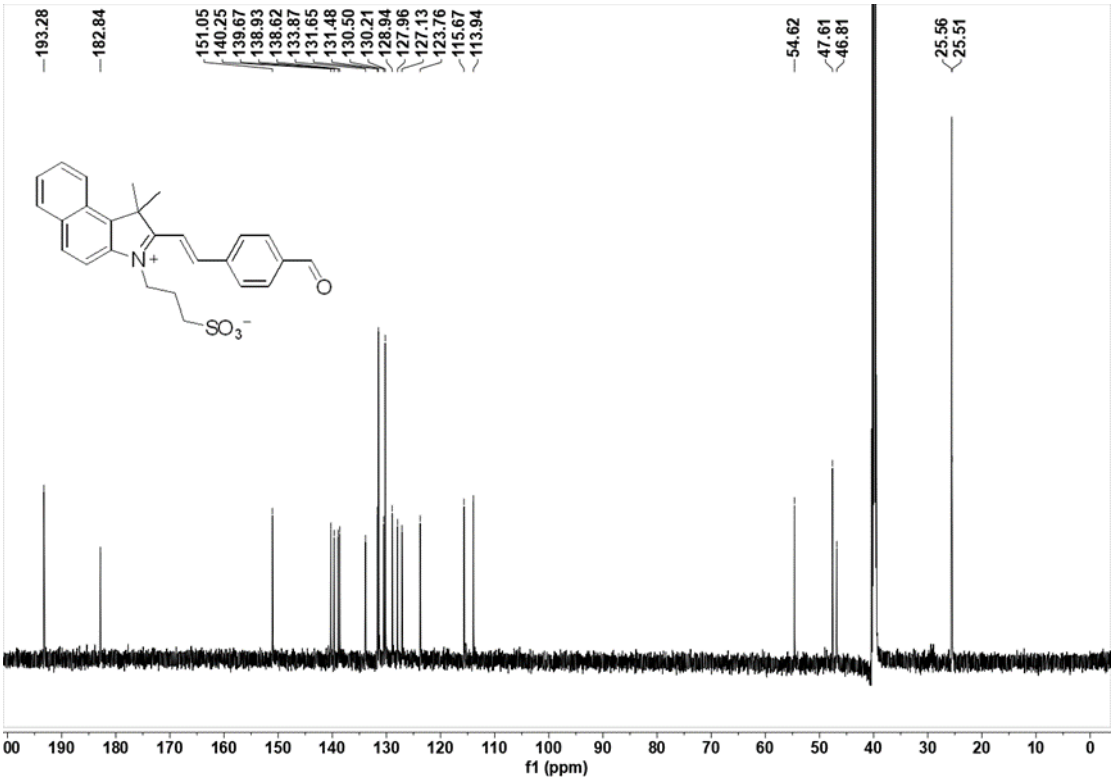


Fig. S3 ^{13}C-NMR spectra of probe **Hcy-BA** in DMSO-d_6.

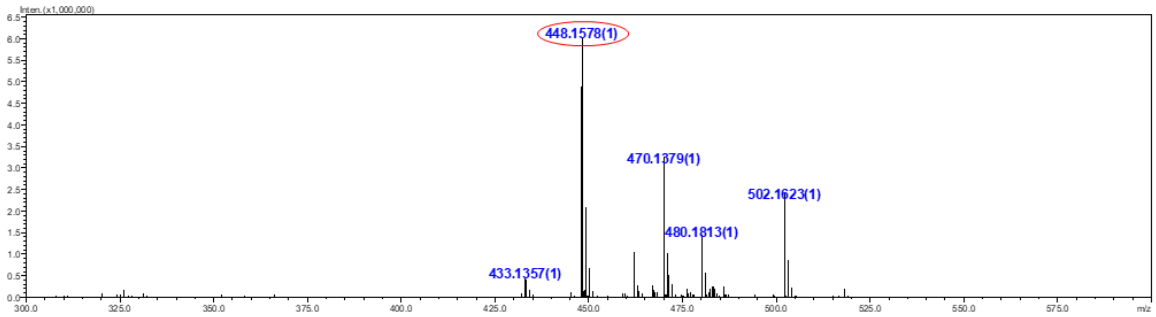


Fig. S4 HR-MS of probe **Hcy-BA**.

3. Experimental procedures for spectroscopic analysis

The stock solution of probe **Hcy-BA** was prepared in DMSO (1.0 mM) and the final concentration of **Hcy-BA** in all the spectroscopic experiments was 10 μM in DMSO/PBS buffer (5/95 v/v, pH = 5.0, 10 mM) unless otherwise stated. Stock solutions of phenylhydrazine and interfering substances were freshly prepared in PBS buffer (pH = 5.0, 10 mM). The absorption and fluorescence spectra (λ_{ex} = 370 nm, λ_{em} = 630 nm, slit width: 20 nm / 20 nm) measurements were carried out in 10-mm quartz cell at room temperature. The samples were mixed well and incubated for 16 min before the spectra were recorded.

In the preliminary experiment, **Hcy-BA** (10 μM) and phenylhydrazine (500 μM) were mixed in 5% DMSO-PBS (10 mM, v/v, pH=3.0, 4.0,5.0, 6.0, 7.0) and fluorescence spectra were taken every two minutes (λ_{ex} = 370 nm, λ_{em} = 630 nm, slit width: 20 nm / 20 nm). The changes of fluorescence intensity at 630 nm were then demonstrated in Fig. S5.

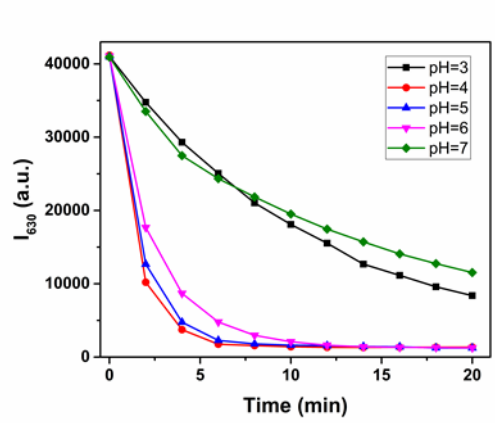


Fig. S5 The fluorescence intensity at 630 nm of probe **Hcy-BA** (10 μM) after the addition of phenylhydrazine (500 μM) in DMSO / PBS buffer (5:95 v/v) buffer at pH=3.0, 4.0, 5.0, 6.0 and 7.0, λ_{em} = 370 nm.

4. NMR and MS verification of sensing mechanism

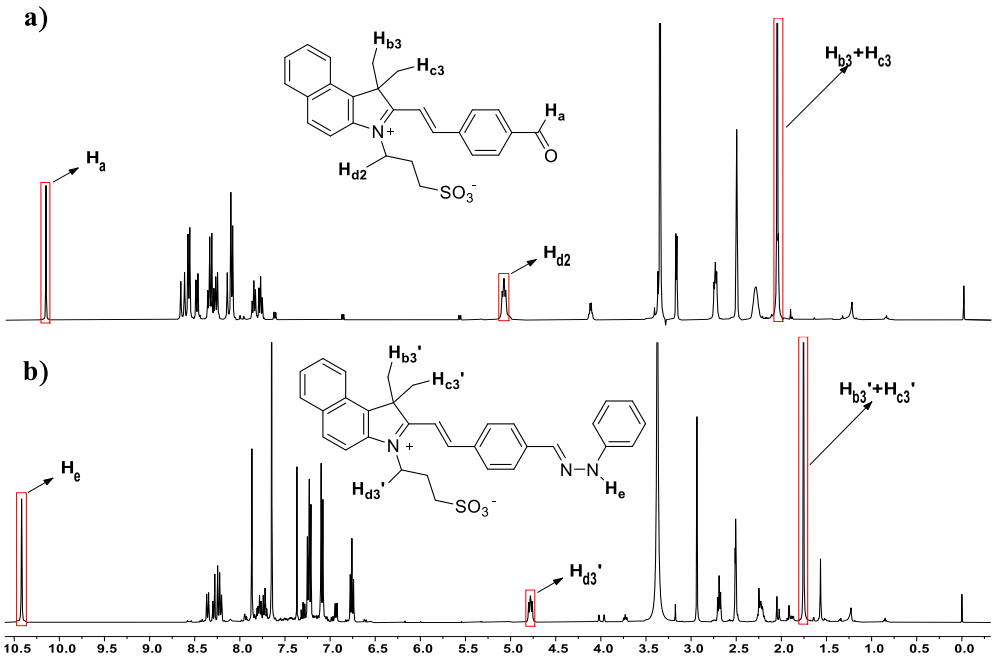


Fig. S6 ^{1}H NMR (400MHz, DMSO-d_6) spectra of (a) **Hcy-BA** (10 mM) and (b) **Hcy-BA** (10 mM) with the addition of 4 equiv of phenylhydrazine.

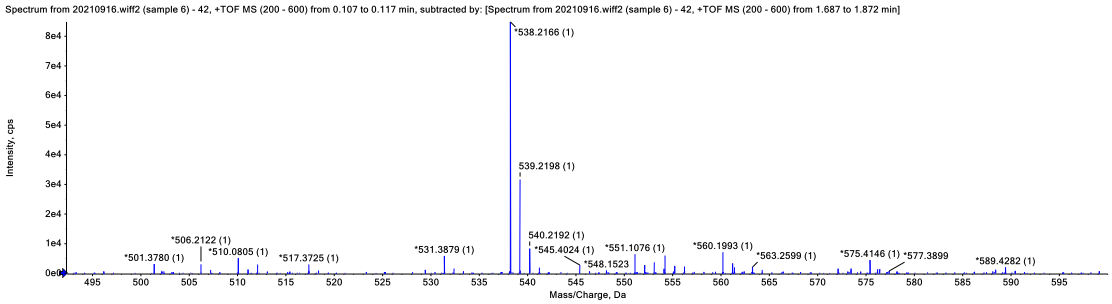


Fig. S7 HR-MS of **Hcy-BA** after the addition of phenylhydrazine.

5. Standard recovery experiment in real samples

The water samples collected from the urban water distribution network and Funan River of Chengdu were filtered with a microporous membrane (0.45 μm) and the pH values were 7.7 and 8.1, respectively. Then, 1.5 g and 1.8 g potassium dihydrogen phosphate were added to 100 mL of tap water and river water to adjust the pH values to 5.0. Subsequently, the pretreated water samples were used to prepare solutions of **Hcy-BA** (10 μM) and phenylhydrazine (0 μM, 5 μM, 10 μM, 20 μM, 50 μM, 100 μM, 200 μM). Each of the mixture was subjected to fluorescence spectra measurement three times and the fluorescence intensities at 630 nm were determined to calculate the corresponding concentrations of phenylhydrazine, as listed in Table 1.

6. Detection of PHZ with probe-loaded paper strips

Ethanol was selected as the solvent to dissolve **Hcy-BA** because of its good volatility and solubility. Paper strips were immersed in ethanol solution of **Hcy-BA** (1 mM) and dried in the air three times to obtain probe-loaded paper strips. Stock solution of phenylhydrazine (100 mM) was prepared in DMSO/PBS buffer (5/95 v/v, pH = 5.0, 10 mM) and samples (0.02 mM, 0.1 mM, 0.5 mM, 1mM, 5mM, 10mM) for experiment were prepared by dilution. Then, the probe-loaded paper strips were dipped into phenylhydrazine solutions with increasing concentration and dried in the air. The colors of paper strips under natural light and UV lamp were recorded with a camera.

References

1 L. Strekowski, C. J. Mason, H. Lee, R. Gupta, J. Sowell and G. Patonay, Synthesis of water-soluble near-infrared cyanine dyes functionalized with [(succinimido)oxy] carbonyl group, *J. Heterocyclic Chem.*, 2003, **40**(5):913-916.