# Synthesis and Application of Salicylhydrazone Probes with High Selectivity for Rapid Detection of $Cu^{2+}$

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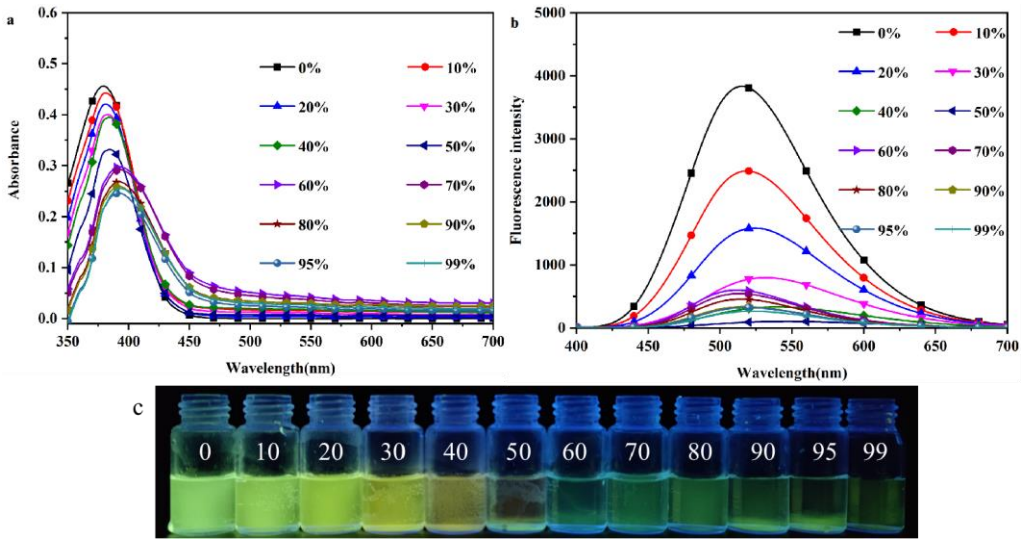



Figure S1 Ultraviolet absorption spectra (a) and Fluorescence emission spectra (b) of DHBYMH ($1\times10^{-5}$ M) in different ratios of $H_2O$/DMS; (c) Physical images of DHBYMH ($1\times10^{-5}$ M) in different ratios of $H_2O$/DMSO under UV lamp (365 nm) irradiation.

The Lippert-Mataga equation [1]

$$\Delta v = v_A - v_F = \frac{2\Delta\mu^2\Delta f}{hca_0^3} + \text{const} \quad \text{(S1)}$$

$$\Delta\mu = \mu_e - \mu_g \quad \text{(S2)}$$

$$\Delta f = \frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2+1} \quad \text{(S3)}$$

$$a_0 = \sqrt[3]{\frac{3M}{4N\pi d}} \quad \text{(S4)}$$

where Δν is the Stokes shift (in $cm^{-1}$) between the absorption and fluorescence emission peak, $h$ is the Planck constant, $c$ is the velocity of light in vacuum, $\Delta\mu$ is the transition dipole moments between the ground ($\mu_g$) and excited ($\mu_e$) states, $\Delta f$ is the orientation polarizability of the solvents expressed by the dielectric constant ($\varepsilon$) and refractive index ($n$) of the solvents and $a_0$ is the Onsager cavity radius, which can be derived from the Avogadro number $N$, molecular weight $M$, and density $d$ ($d$ = 1.0 $g/cm^3$). The Onsager cavity radius ($a_0$) of DHBYMH was calculated to be 5.83 nm according to the equation (S4).

Obviously, the experimental data do not obey the linear relationship predicted by the Lippert-Mataga equation well, in the whole range of solvent polarity, as shown in Figure S2. A transformation in the slope of the ftted line is observed between the toluene ($f$=0.014)and ethanol ($f$= 0.295) solvents. Through the analysis of the fitted line in high-polarity solvents, a slope value of ≈4095.08 ($R^2$=0.77) was obtained.

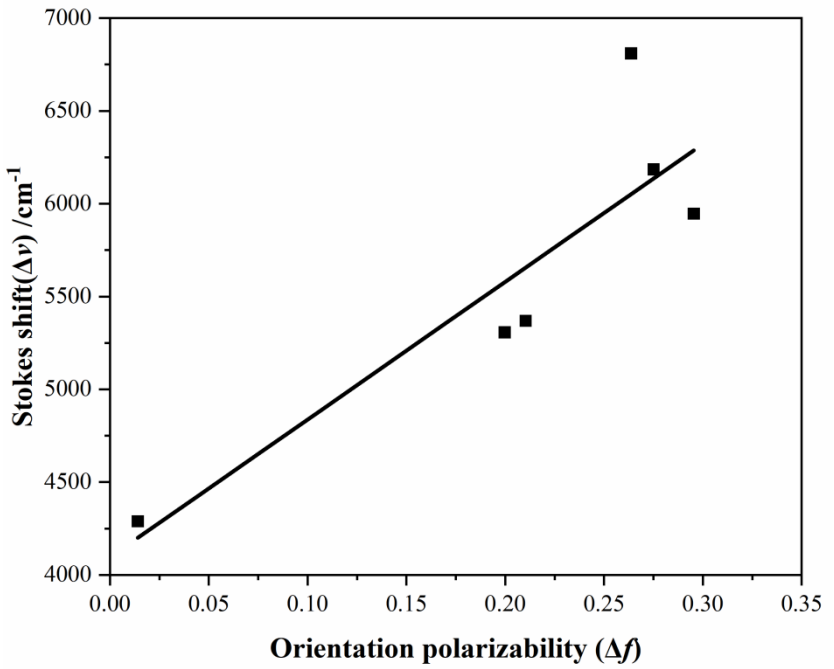



Figure S2 Lippert−Mataga plot for DHBYMH in various solvents.

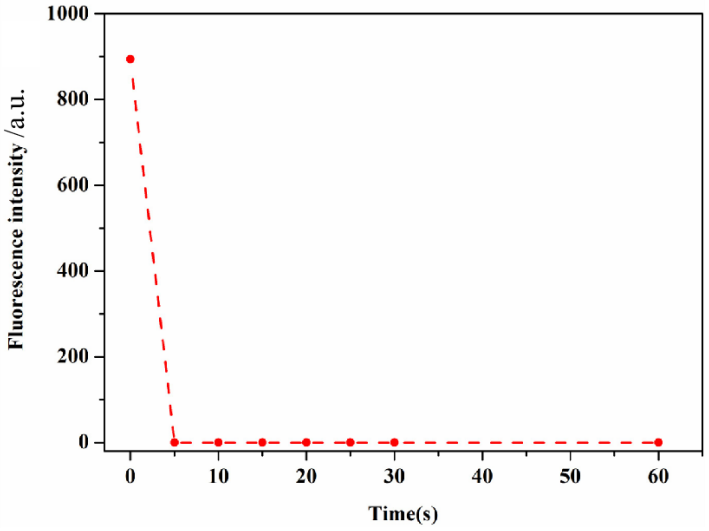



Figure S3 DHBYMH($1\times10^{-5}$ M, DMSO/$H_2O$, v/v=7/3, pH=7.0, Cys: $1\times10^{-4}$ M) recognition $Cu^{2+}$ ion response time.

Optical study

The maximum UV absorption wavelength of DHBYMH in in different polar organic solvents (toluene, ethyl acetate, THF, ethanol, DMF, and DMSO) was used as the excitation wavelength for measuring the fluorescence wavelength. UV−vis spectra were recorded using UV-2450 type ultraviolet-visible spectrophotometer. Photoluminescence (PL) spectra were obtained using RF-5301PC fluorescence spectrophotometer. The

photoluminescence quantum yields (QY) in solutions were determined by comparing to quinine sulfate in 0.10 M $H_2SO_4$ at room temperature as a standard. The QY in solid states were obtained on a HORIBA Fluorolog-3 spectrophotometer equipped with an integrating sphere.

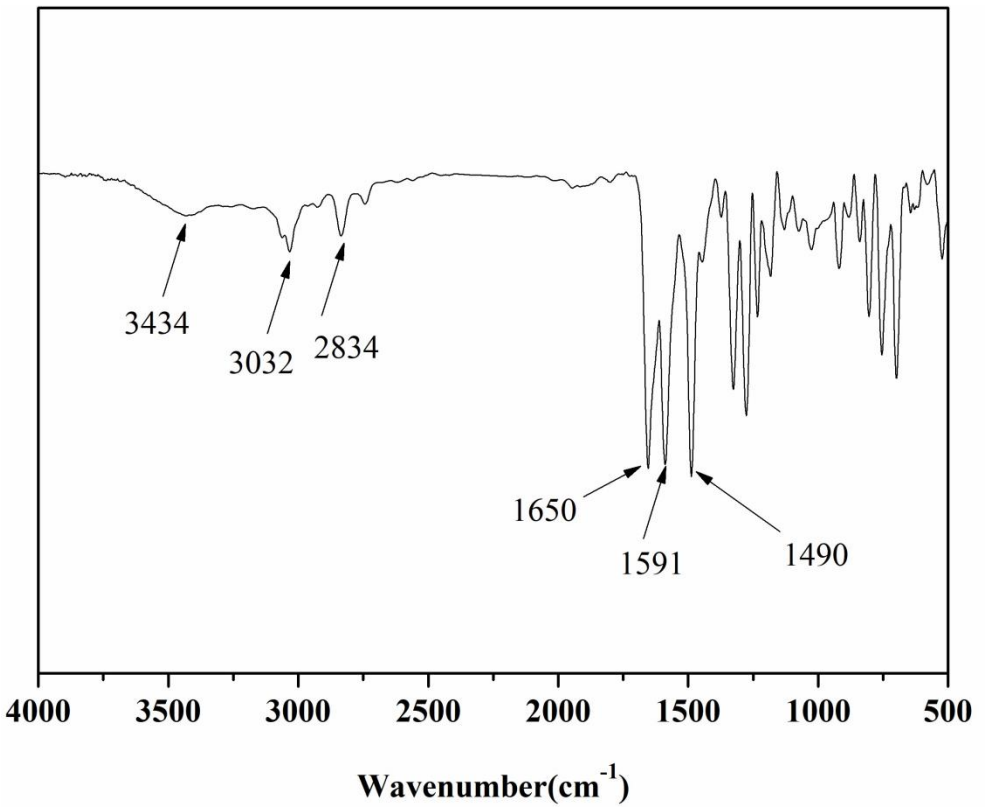



Figure S4 FT-IR of DHB

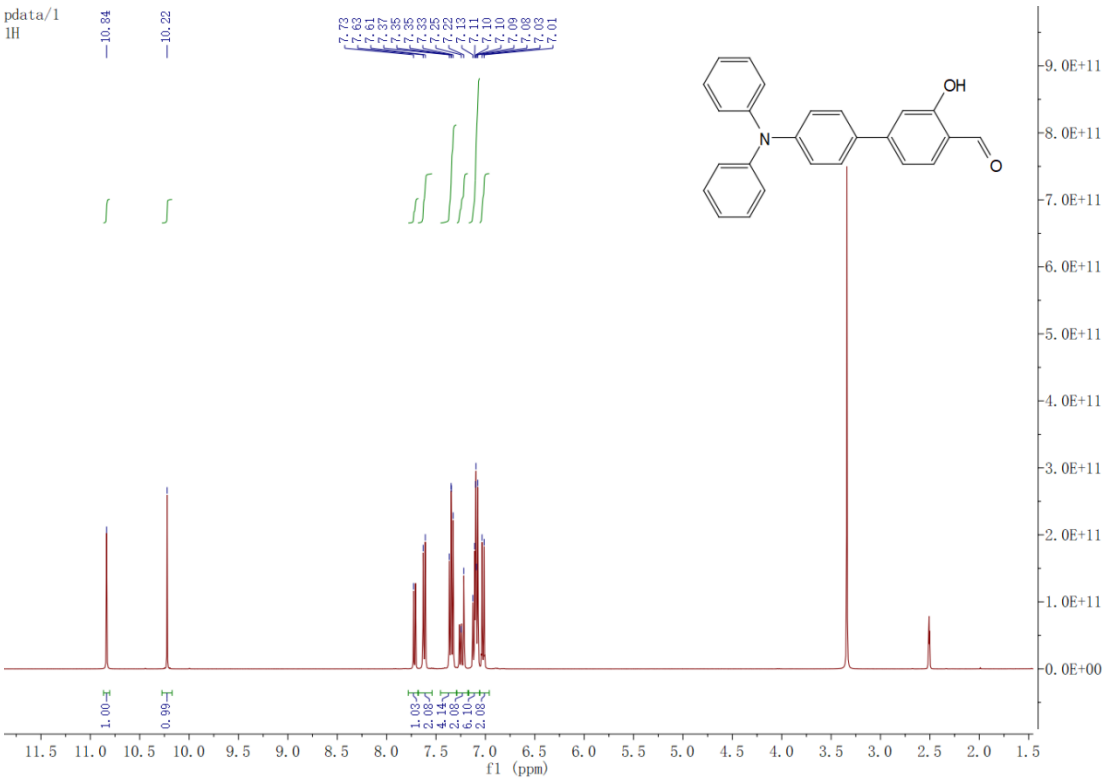



Figure S5 $^{1}$H NMR (400 MHz, DMSO-$d_6$) spectrum of DHB

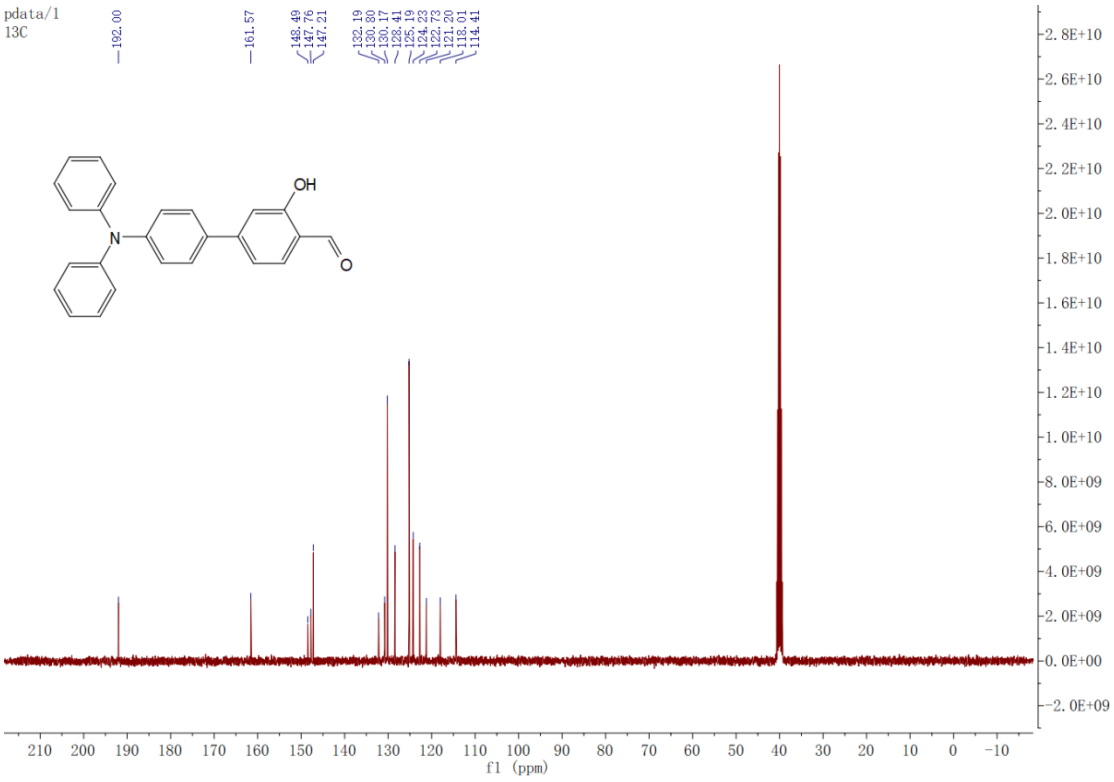



Figure S6 $^{13}$C NMR (101 MHz, DMSO-$d_6$) spectrum of DHB

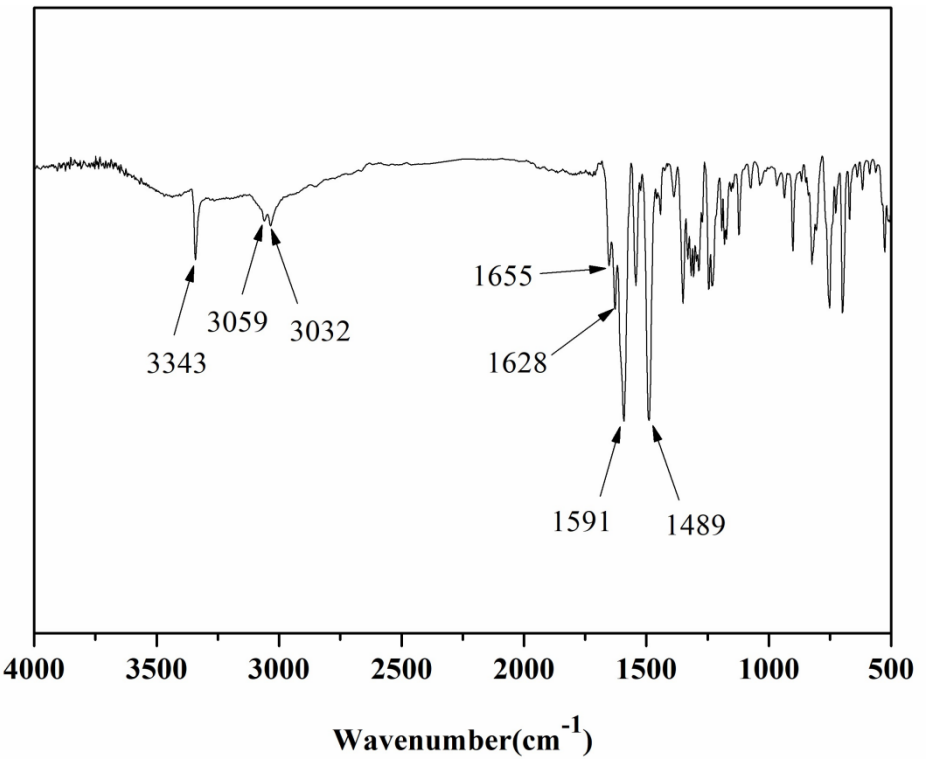



Figure S7FT-IR of DHBYMH

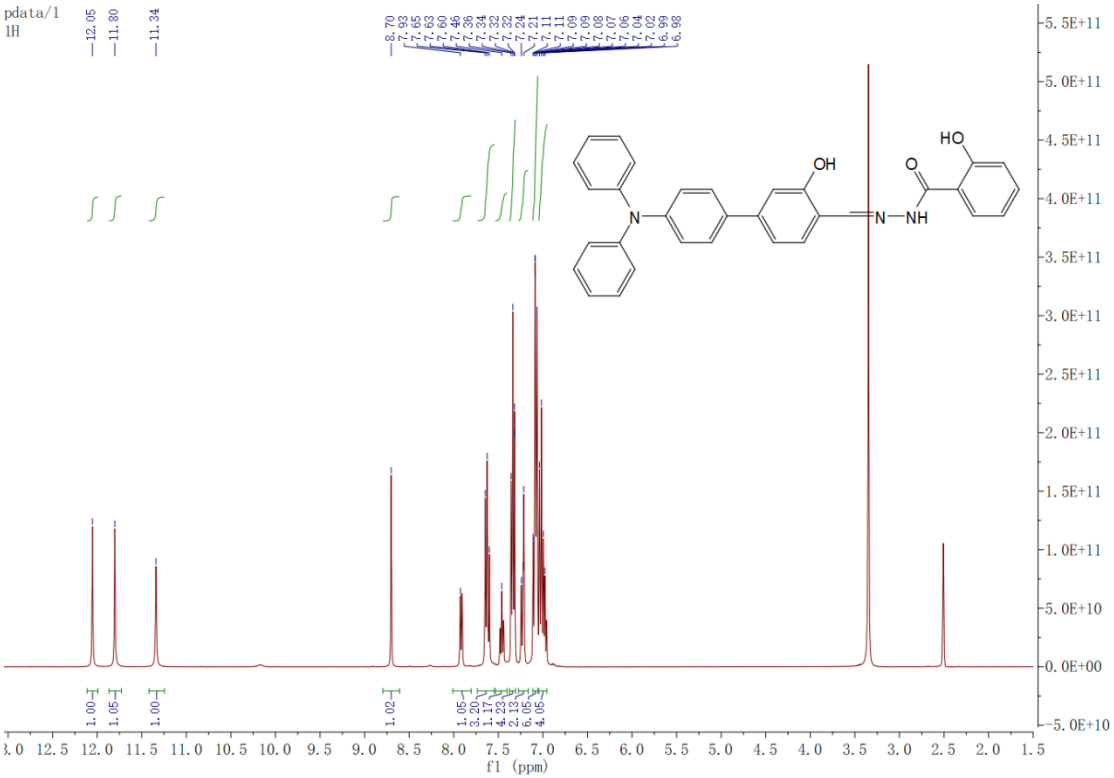



Figure S8 $^{1}$H NMR (400 MHz, DMSO-$d_6$) spectrum of DHBYMH

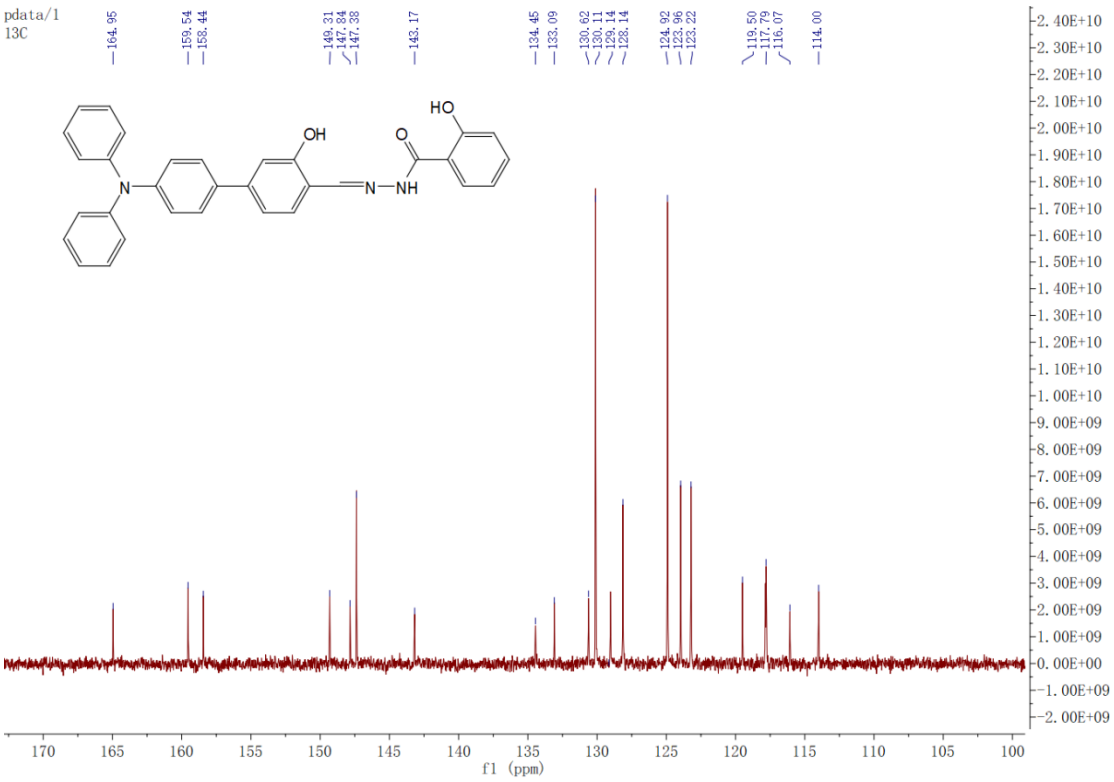



Figure S9 $^{13}$C NMR (101 MHz, DMSO-$d_6$) spectrum of DHBYMH

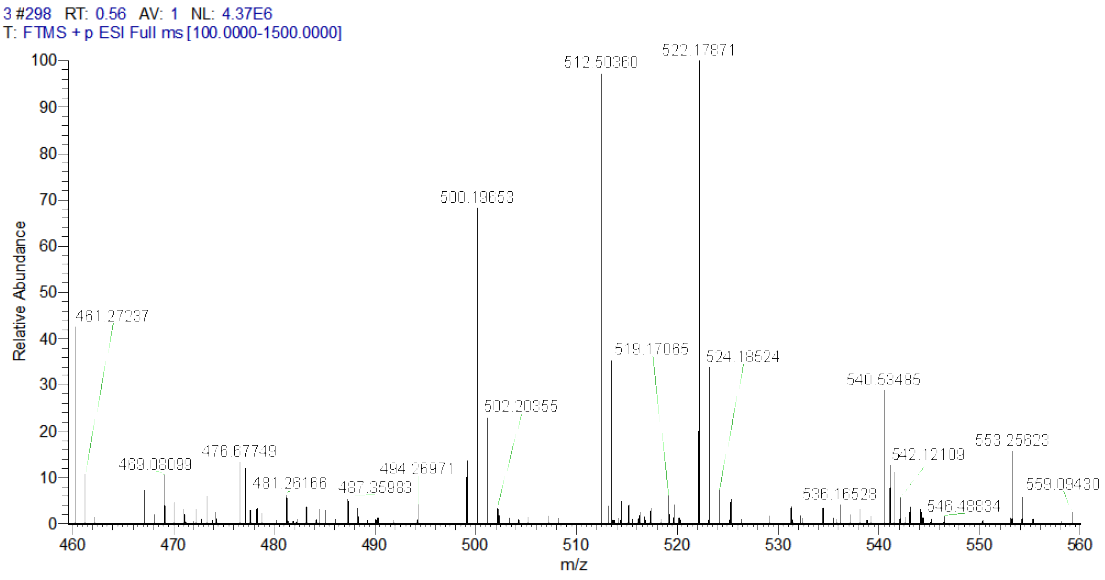
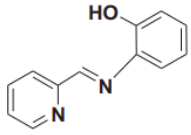
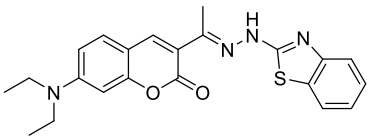
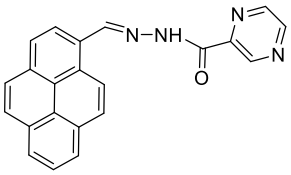
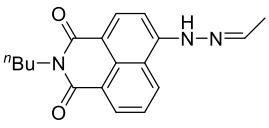
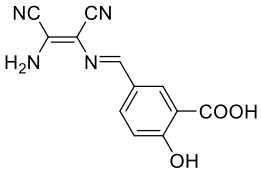


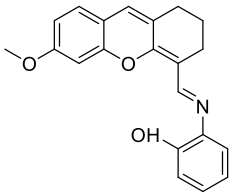
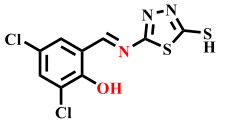
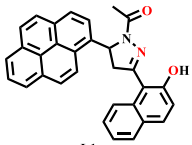
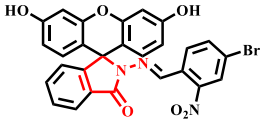
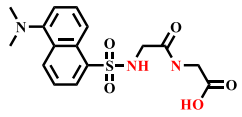
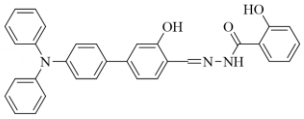

Figure S10 HR-MS spectra of DHBYMH

Table S1. The Stokes shift of DHBYMH in various solvents with a range of $\Delta f$ (orientation polarizability) values.

| DHBYMH | $\lambda_{abs}$(nm) | $\lambda_{em}$(nm) | $n$ | Stokes shift($cm^{-1}$) | $\varepsilon$ | $f$ |
|---|---|---|---|---|---|---|
| Toluene | 380 | 454 | 1.494 | 4289 | 2.38 | 0.014 |
| Ethyl acetate | 380 | 476 | 1.372 | 5307 | 6.02 | 0.199 |
| THF | 381 | 479 | 1.405 | 5369 | 7.58 | 0.210 |
| Ethanol | 386 | 501 | 1.354 | 5946 | 28.4 | 0.295 |
| DMF | 390 | 514 | 1.42817 | 6185 | 36.7 | 0.275 |
| DMSO | 384 | 520 | 1.4795 | 6810 | 48.9 | 0.264 |

**Table S2** Comparison of probe DHBYMH with other $Cu^{2+}$ probes reported.

| Probe | Solvent ($V$:$V$) | LOD/(mol•$L^{-1}$) | pH | Time | Cell | Ref. |
|---|---|---|---|---|---|---|
|  | $H_2O$/methanol (9:1 v/v) | $2.00\times10^{-5}$ | 7 | 60 s | — | [2] |
|  | $CH_3CN/H_2O$ (1:1) | $5.80\times10^{-8}$ | 7.2 | 1 h | MCF-7 cells | [3] |
|  | ACN/PBS (4:6) | $1.57\times10^{-7}$ | 7.4 | — | Hela cells | [4] |
|  | $CH_3CN$/HEPES (4:1) | $3.20\times10^{-7}$ | 7.4 | 20 min | 293 T cells | [5] |
|  | $CH_3CN$/HEPES (1:9) | $2.19\times10^{-7}$ | 7.0 | 11 min | HepG2 cells | [6] |

| | | | | | | |
|---|---|---|---|---|---|---|
|  | $CH_3CN$/HEPES (4:1) | $3.30\times10^{-5}$ | 7.3 | — | HepG2 cells | [7] |
|  SB-1 | $H_2O$ | $4.48\times10^{-6}$ | 4.2-10.5 | 150 s | Caco-2 cells | [8] |
|  L1 | EtOH–$H_2O$ =1:1 | $1.62\times10^{-6}$ | — | — | — | [9] |
|  A5 | PBS buffer/ EtOH (1:1) | 0.11 μM | 7.4 | 120s | — | [10] |
|  GG-Dns | $CH_3CN$/$H_2O$ (1/1) | $2.9\times10^{-7}$ | 1.0-3.5 | — | — | [11] |
|  | DMSO/$H_2O$ (7/3） | $1.62\times10^{-7}$ M | 7.0 | 5s | | **This study** |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9. Yang, Y.-S.; Cao, J.-Q.; Ma, C.-M.; Zhang, Y.-P.; Guo, H.-C.; Xue, J.-J. A novel pyrazoline-based fluorescence probe armed by pyrene and naphthol system for the selective detection of Cu2+ and its biological application. *Journal of the Iranian Chemical Society* **2022**, *19*, 3451-3461, doi:10.1007/s13738-022-02536-5.
10. Leng, X.; Wang, D.; Mi, Z.; Zhang, Y.; Yang, B.; Chen, F. Novel Fluorescence Probe toward Cu2+ Based on Fluorescein Derivatives and Its Bioimaging in Cells. *Biosensors* **2022**, *12*, doi:10.3390/bios12090732.
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