Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

A colorimetric and far-red fluorescent probe for the highly sensitive detection of silver (I)

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Materials and measurements

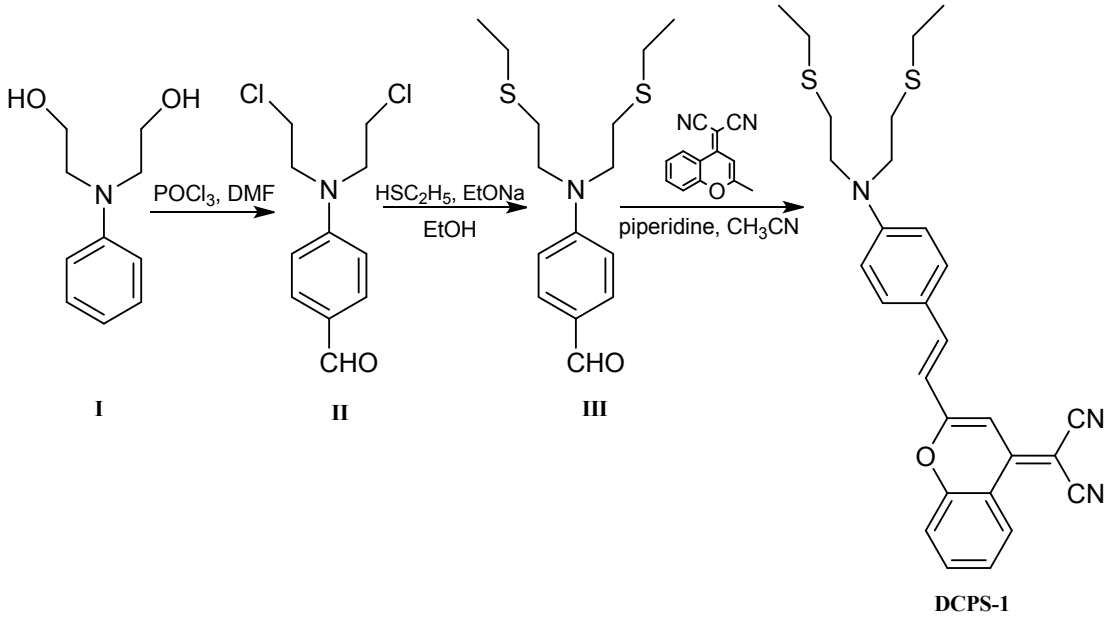
All spectroscopic measurements were performed in MeOH. For the spectrometer measurements, a quartz cuvette with 10 mm path length was used to hold each sample. The UV-vis spectra were measured using a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence spectra were acquired using an FL-4500 fluorescence spectrophotometer (Hitachi, Japan). Excitation wavelength was 460 nm and fluorescence was detected in a wavelength range between 480 nm and 800 nm. Unless otherwise noted, the spectra were measured in MeOH recorded at 30 min intervals at room temperature.

Reagents and instrumentation

All solvents and reagents (analytical grade) were obtained commercially and used as received unless otherwise mentioned. Column-layer chromatographic silica gel was purchased from Branch of Qingdao Haiyang Chemical Co., Ltd. HPLC **analyses were recorded on Agilent1200.** NMR spectra were collected on **AVANCE III HD 400 (Bruker, Germany)** with TMS as an internal standard. High-resolution mass spectral analyses were carried out using **Agilent1290/maXis impact (Bruker, Germany).** Absorption spectra were recorded on a UV-2450 spectrophotometer (Shimadzu, Japan). Fluorescence measurements were performed on an F-4500 fluorescence spectrophotometer (Hitachi, Japan) equipped with quartz cell of 10 mm path length. Confocal fluorescence imaging studies were performed with a Zeiss laser scanning microscope 710 with a 40× oil objective lens and Zen 2009 software (Carl Zeiss, **Germany**).

1. Synthesis of probe DCPS-1

The probe DCPS-1 was easily prepared and the synthetic route was divided into three steps (**Scheme** 1):



Scheme 1: The synthetic route of **DCPS-1**

The synthesis of (2-methyl-4H-1-benzopyran-4-ylidene)-propanedinitrile was according to the reported literature [1]

(1) Synthesis of 4-(bis(2-chloroethyl)amino)benzaldehyde (**II**)

A solution of compound 2,2'-(phenylazanediyl)diethanol (5.4 g, 29.8 mmol) in DMF (15 mL), was added dropwise to the DMF (25 mL) solution containing phosphorus oxychloride (11.5mL, 120mmol) under the ice bath. After the addition was completed, the reaction mixture was stirred at 90 ℃ for 2 h, then the solution was cooled to room temperatureand was poured into 300 mL of ice water. The mixture was neutralized with NaOH accompanied product from the precipitate in the solution. After vacuum filtration, the filter cake was recrystalized from 100 mL ethanol and get (**II)** as yellow crystal (6.4 g, yield 87.7%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.81 (s, 1H), 7.80 (d, *J* = 12.0 Hz, 2H), 6.77 (d, *J* = 8.0 Hz, 2H), 3.87 (t, *J* = 8.0 Hz, 4H), 3.71 (t, *J* = 8.0 Hz, 4H).

(2) Synthesis of 4-[bis [2-(ethylthio) ethyl] amino]-benzaldehyde (**III**)

A solution of ethyl mercaptan (5.5 mL, 76.3 mmol) and sodium metal (1.0 g, 43.5 mmol) in anhydrous ethanol (75 mL) was stirred for 1 h at room temperature. Then a solution of compound 4-(bis(2-chloroethyl)amino)benzaldehyde (6.4 g, 26.0 mmol) in anhydrous ethanol (25 mL) was added to the mixture. After the addition was completed, the reaction mixture was stirred at 80 ℃ overnight. The solution was cooled to room temperature and concentrated under vacuum, the residue was dissolveed in DCM (250 mL), which was washed with water (2 × 250 mL), dried ($MgSO_4$), and concentrated under cacuum to give (**III**) (7.0 g, yield 90.5%) as red oils, which was subjected to the next step without any purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.73 (s, 1H), 7.74 (d, *J* = 8.0 Hz, 2H), 6.69 (d, *J* = 12.0 Hz, 2H), 3.63 (t, *J* = 8.0Hz, 4H), 2.76 (t, *J* = 8.0 Hz, 4H), 2.61 (q, *J* = 8.0 Hz, 4H), 1.29 (t, *J* = 8.0 Hz, 6H).

(3) Synthesis of 2 - (2 - (4 - (bis (2 - (ethylthio) ethyl) amino) styryl) - 4H - chromen - 4 - ylidene) malononitrile (**DCPS-1**)

A solution of (2-methyl-4H-1-benzopyran-4-ylidene)-propanedinitrile (0.2 g, 1.0 mmol), 4-[bis [2-(ethylthio) ethyl] amino]-benzaldehyde (0.3 g, 1.0 mmol) and piperidine (0.2 mL, 2.0 mmol) in acetonitrile (15 mL), was stirred overnight at 80 ℃. The mixture was cooled to room temperature and concentrated under vacuum, the residual was recrystalized from 20 mL ethanol as red solid (236 mg, yield 48.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.88 (d, *J* = 8.0 Hz, 1H), 7.70 (t, *J* = 4.0 Hz, 1H), 7.55-7.51 (m, 2H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.41 (t, *J* = 4.0 Hz, 1H), 6.75 (s, 1H), 6.68 (d, *J* = 4.0 Hz, 2H), 6.55 (d, *J* = 8.0 Hz, 1H), 3.62 (t, *J* = 4.0 Hz, 4H), 2.77 (t, *J* = 4.0 Hz, 4H), 2.63 (q, *J* = 4.0 Hz, 4H), 1.30 (t, *J* = 8.0 Hz, 6H). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ (ppm): 158.72, 152.78, 152.40, 148.80, 139.36, 134.24, 130.26, 125.78, 125.66, 123.16, 118.46, 118.01, 117.35, 116.28, 113.54, 111.91, 105.49, 60.66, 51.51, 28.93, 26.41, 14.85. ESI-HRMS: m/z, $C_{13}H_{14}N_4O_5$: caculate 488.1825 ($[M+H]^+$); found 488.1822.

General spectral measurements

The stock solutions of **DCPS-1**(10 mM) and $AgNO_3$ were prepared by dissolving the required

amount in DMSO. The stock solutions (50 mM) of relevant analytes (Fe^{2+}, K^{+}, Na^{+},Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, Pb^{2+}, Ni^{2+}, Co^{2+}, Ba^{2+}, Mn^{2+}, Cu^{+}) were prepared in DMSO. Absorption and fluorescence titrations were performed by adding small aliquots of **DCPS-1** into working solutions in a quartz cell (10.0 mm width). The fluorescence intensities were measured at the excitation wavelength of 460 nm with the emission approximately 620 nm.

HPLC analyses:

Reversed-phase HPLC analysis was performed with column (C18 5μ 250×4.6 mm). HPLC separation was carried out with 57% acetonitrile, 30% methanol and 13% water. The flow velocity is 1 mL/min. Column temperature is 40 ℃. The detection wavelength is 210 nm.

Quantum yields

Quantum yields were determined according to the literature using cresyl violet acetate as the standard.[2] The quantum yield was calculated according to the equation[3]:

$$\emptyset_{sample} = \emptyset_{standard} \times \frac{OD_{standard} \times A_{sample} \times \eta_{sample}^{2}}{OD_{sample} \times A_{standard} \times \eta_{standard}^{2}}$$

where Φ is quantum yield; $\Phi_{standard}$ = 0.54 in methanol; *OD* is optical density of the compound at the excitation wavelength; *A* is the area under the fluorescence spectral curve and the η is the refractive index of the solvent.

General procedure of MTT assay

The MTT assay was used to measure the cytotoxicity of **DCPS-1** to HeLa cells. Cells were seeded into a 96-well cell-culture plate. Various concentrations of DCPS-1 were added to the wells. The cells were incubated at 37 °C under 5% CO2 for 48 h. 10 μL MTT (5 mg mL-1) was added to each well and incubated at 37 °C under 5% CO2 for 4 h. Remove the MTT solution and yellow precipitates (formazan) observed in plates were dissolved in 100 μL DMSO. Microplate

reader was used to measure the absorbance at 570 nm for each well. The viability of cells was calculated according to the following equation: Cell viability = A570(sample)/A570(control)

General procedure of cell imaging

HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/mL penicillin, 100 mg/mL streptomycin and 10% heat inactivated fetal bovin serum. The cells were seeded on a Ø 30mm glass-bottomed dish at the density of 1×105 cells in a culture medium and incubated overnight for live-cell imaging by confocal laser scanning microscopy (CLSM). The HeLa cells were treated with 10 μM of Hochest 33342 and **DCPS-1**, and incubated for 30 min at 37 °C and washed with three times with PBS before imaging by CLSM. And the cells were subsequently incubated with Ag^{+} (30 μM) for 30 min at 37 °C and washed three times with PBS before imaging by CLSM. The cells were imaged with a 40× objective lens. The excitation wavelengths were 405 nm for Hochest 33342 and 488 nm for **DCPS-1**, respectively

Table 1. Detection of Ag+ in water samples (n=3)

Sample	Added (μM)	Found (μM)	Recovery
Tap water	5.0	4.66 ± 0.06	93.2%
Pearl river water	5.0	5.19 ± 0.01	103.8%
Waste water	5.0	4.82 ± 0.06	96.4%

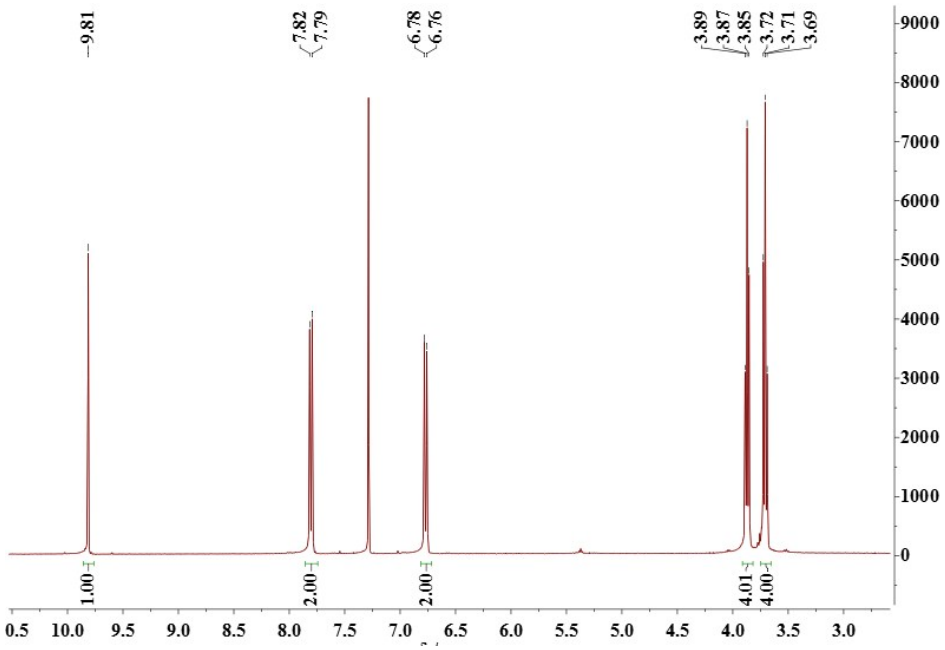


Fig. S1. ^{1}H NMR spectra of **II.**

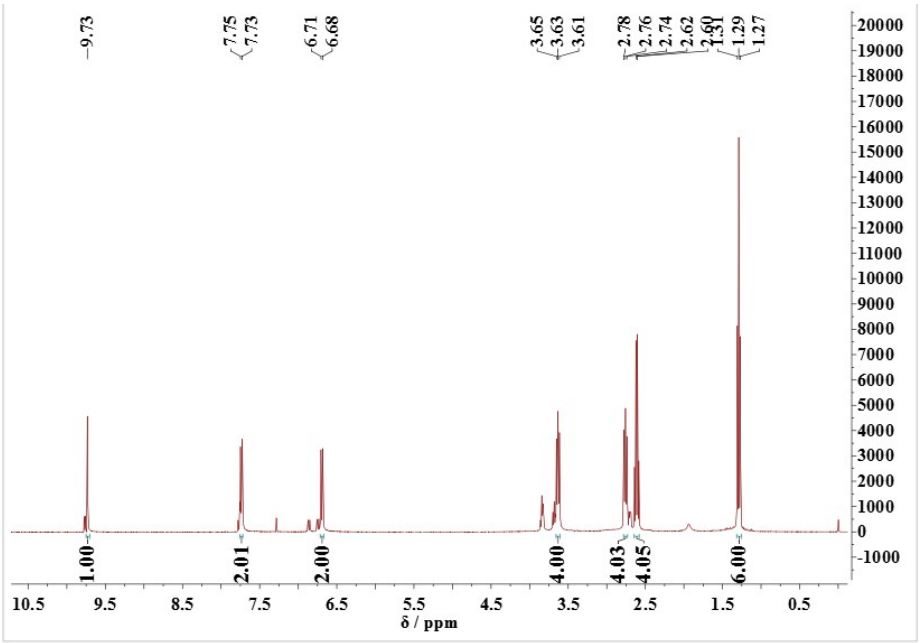


Fig. S2. ^{1}H NMR spectra of **III.**

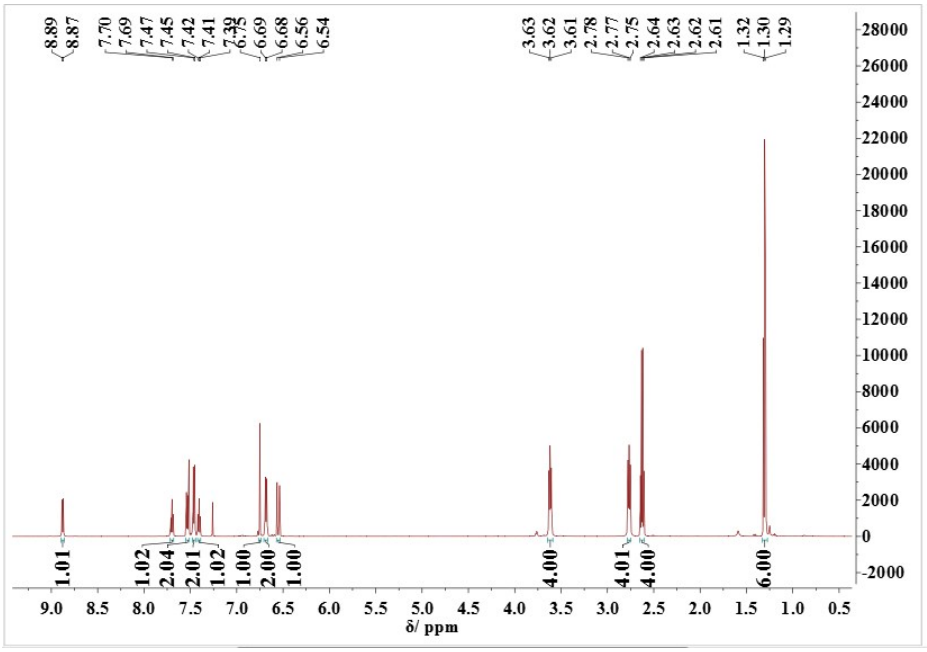


Fig. S3. ^{1}H NMR spectra of **DCPS-1.**

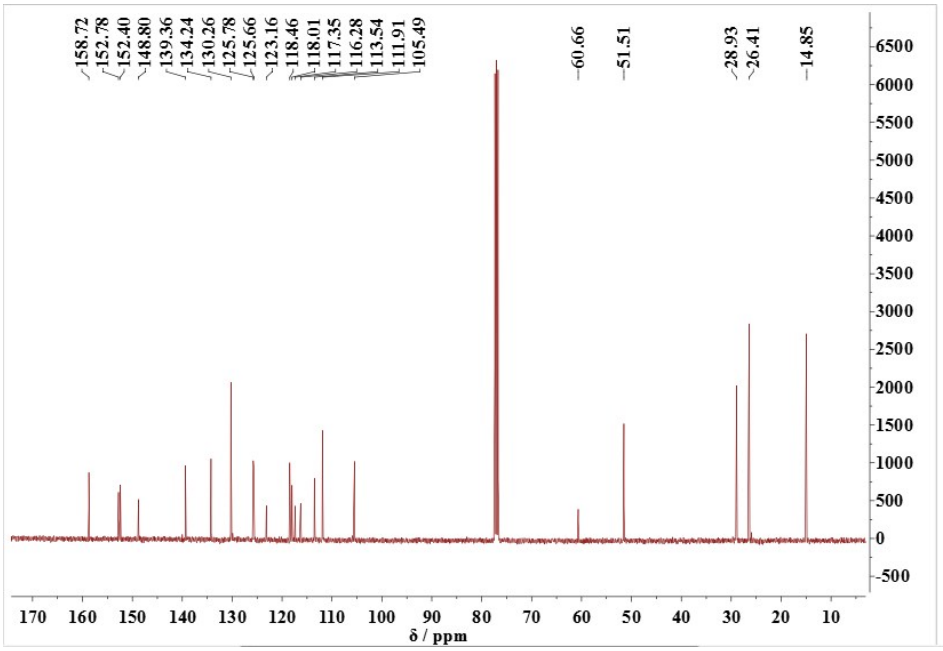
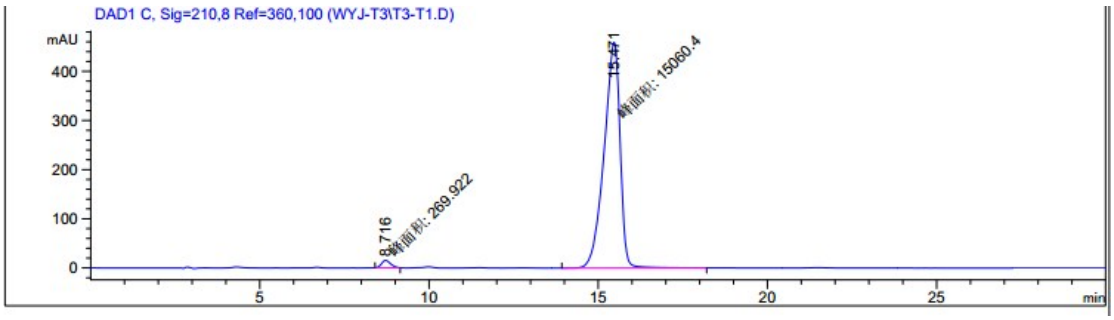


Fig. S4. ^{13}C NMR spectra of **DCPS-1**



信号 2: DAD1 C, Sig=210,8 Ref=360,100

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1	8.716	MM	0.3039	269.92172	14.80533	1.7607
2	15.471	MM	0.5466	1.50604e4	459.19214	98.2393
总量：				1.53303e4	473.99747	

Fig. S5. HPLC spectrum of **DCPS-1.**

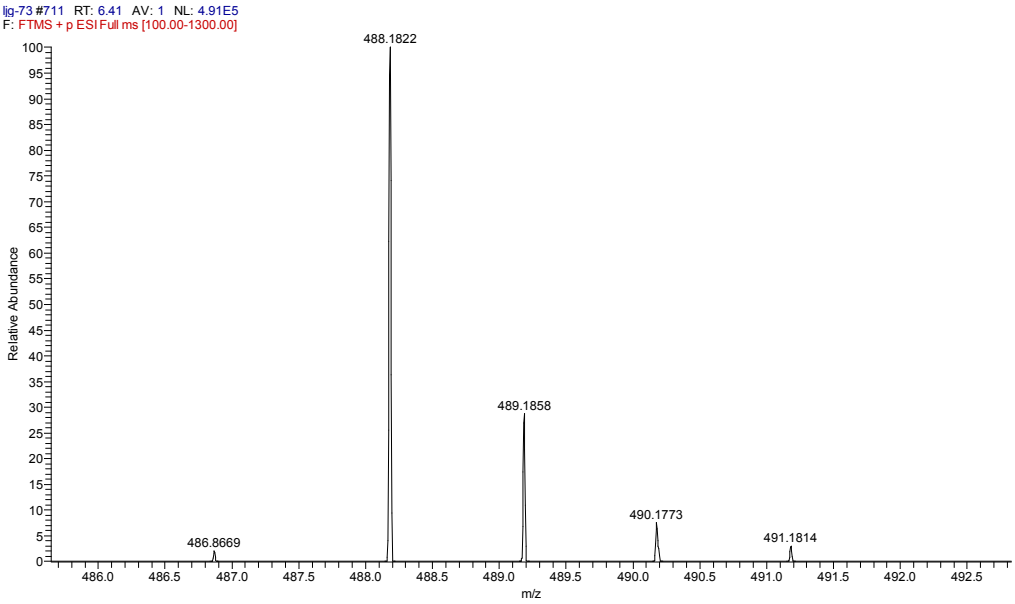
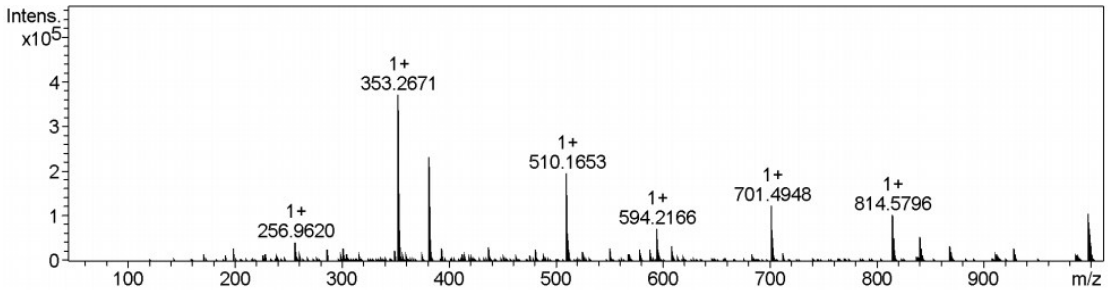


Fig. S6. HRMS spectrum of **DCPS-1.**



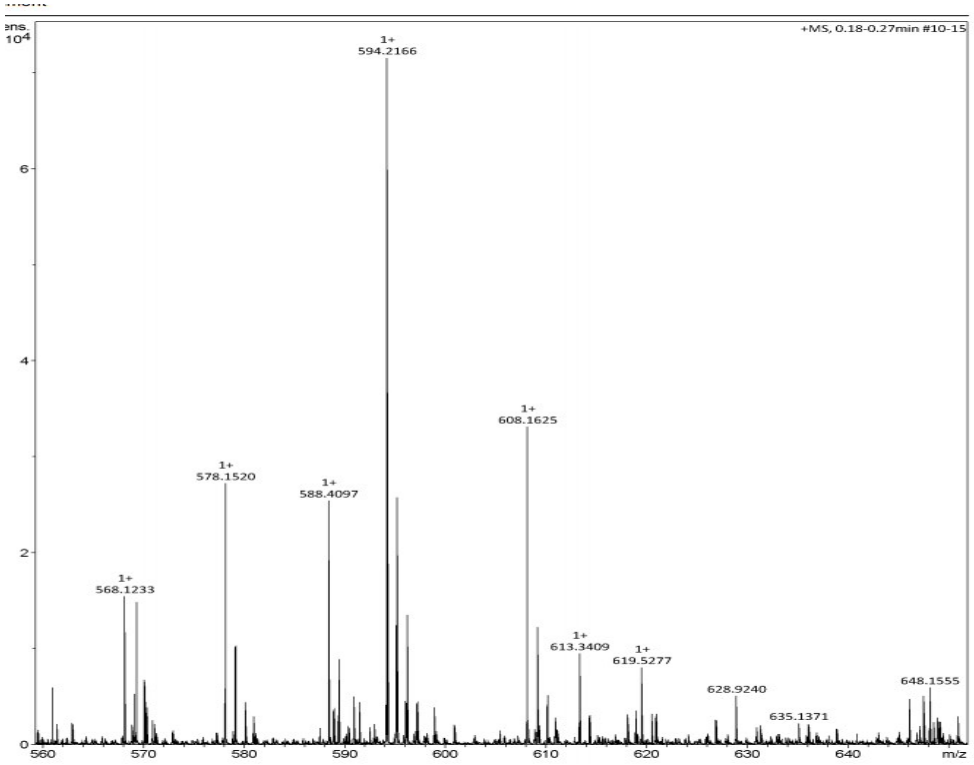


Fig. S7. HRMS spectrum of DCPS-1 form complexe with Ag^{+}.

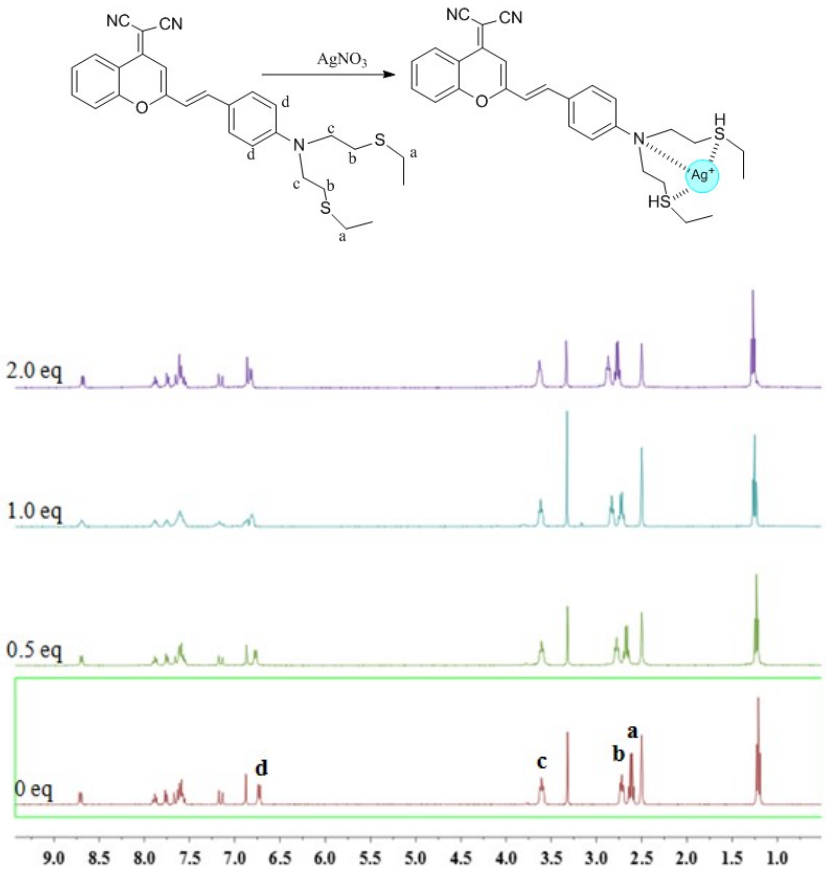


Fig. S8. ^{1}H-NMR spectra of DCPS-1 in DMSO-d_6 with Ag^{+} cation.

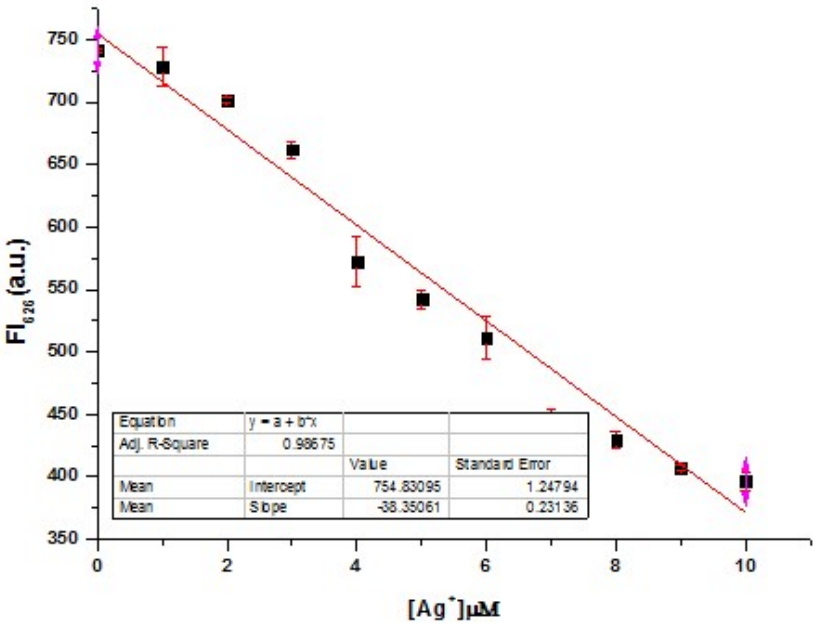


Fig. S9. Emission intensity at 620 nm of DCPS-1(5 μM) as a function of the concentration of Ag^{+} in MeOH.

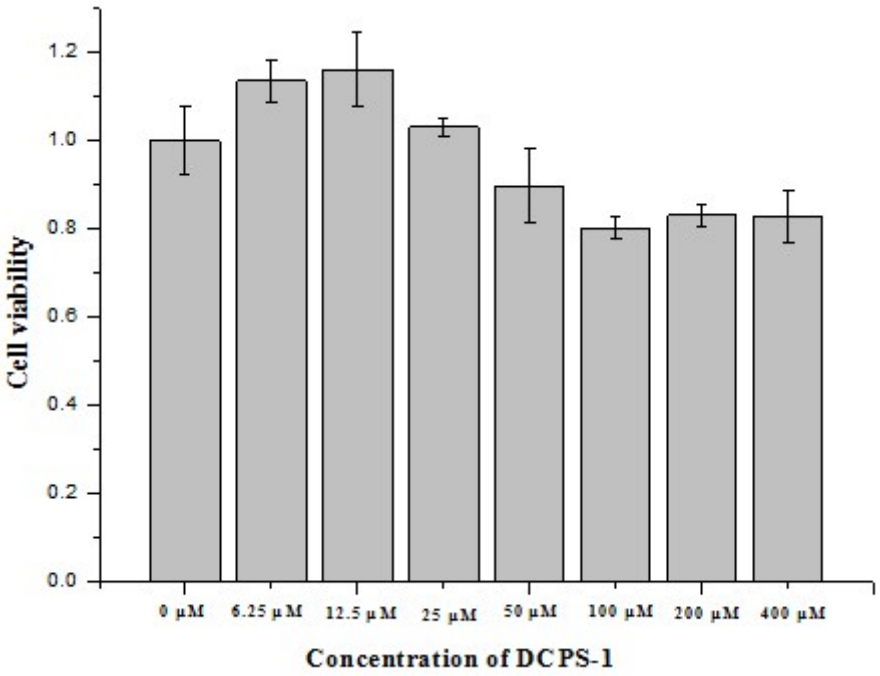


Fig. S10. MTT assay of Hela cells incubated with **DCPS-1** (0-400 μM) for 48 h.

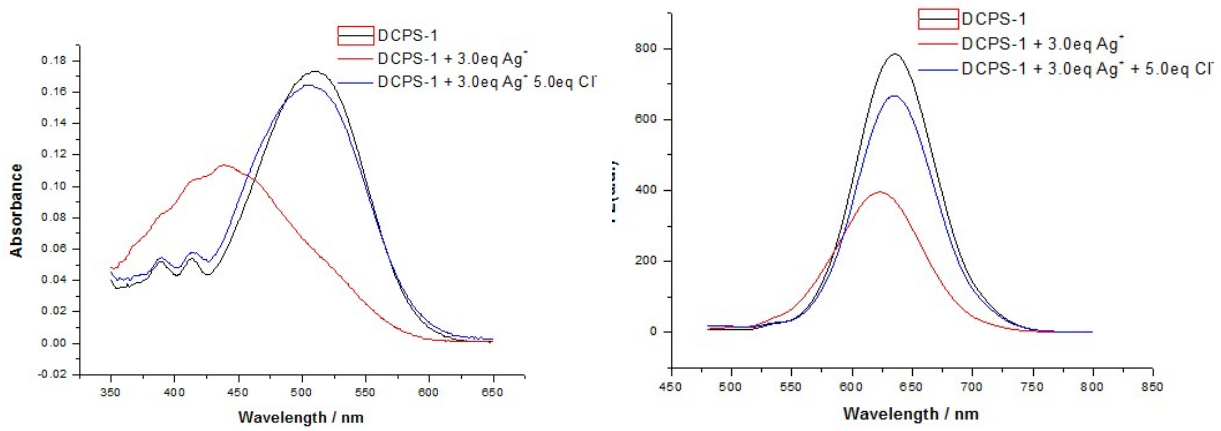


Fig. S11. The reversiblity of **DCPS-1** binding to Ag^{+}

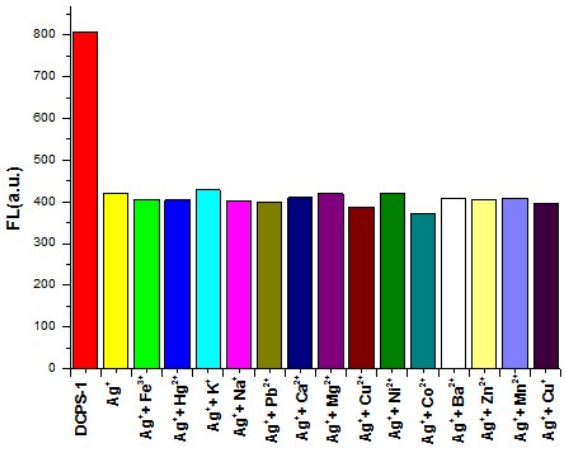


Fig. S12. The fluorescence of **DCPS-1**(5 μM) in the presence of Ag^{+} (5 equiv) and various of other mental ions (10 equiv) in MeOH solution at room temperature, λ_{ex} = 460 nm.

Reference

[1] Y. Zheng, M. Zhao, Q. Qiao, H. Liu, H. Lang and Z. Xu, *Dyes. Pigments.*, 2013, ***98*,** 367-371.

[2] D. Magde and J. H. Brannon, *J. Phys. Chem*., 1979, **83**, 696.

[3] E. Austin and M. Gouterman, *Bioinorg. Chem.*, 1978, **9**, 281.