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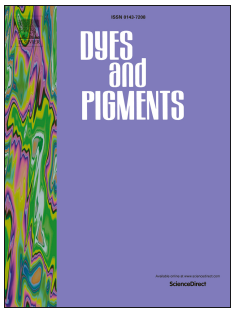

A triphenylamine as a fluorophore and maleimide as a bonding group selective turn-on fluorescent imaging probe for thiols

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Abstract Graphic

The Title:

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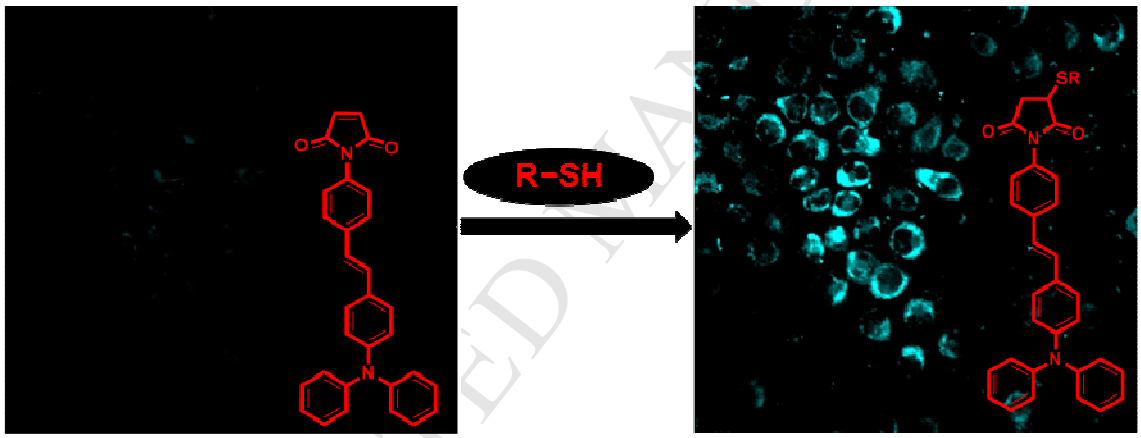
The statement:



Based on the Michael addition reaction of thiol with the C=C bond under mild conditions, we herein report a new fluorescent probe which features a rapid signal response time, a good linearity range and a low detection limit. The potential application of this new fluorescent probe was demonstrated by fluorescent imaging of thiol in living cells.

A triphenylamine as a fluorophore and maleimide as a bonding group selective turn-on fluorescent imaging probe for thiols

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Abstract: With the biological importance of biothiols, the development of probes for thiols has been an active research area in recent years. Here, we report a novel thiol-reactive fluorescent probe based on Michael addition reaction for selectively detecting thiols over other relevant biological species. The thiol adduct was characterized using NMR and mass spectroscopy and detection mechanism was further confirmed. This sensor with excellent selectivity for biothiols over other amino acids features a rapid signal response time, a good linearity range and a low detection limit. For the practical application of the sensor, it can be used to monitor thiol in live cells with turn-on fluorescence imaging.

Keywords: Thiols; Detection; Fluorescent probe; Bioimaging

1. Introduction

Biological thiols including cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) are components of many peptides, which play crucial roles in maintaining the biological redox homeostasis through the equilibrium of free thiols and oxidized disulfides in biological systems [1-3]. It is known that intracellular concentration of GSH is much higher than Cys (Cys: 30-200 μM; GSH: 1-10 mM) [4,5]. In contrast, in healthy human plasma, Cys concentration is typically 10 times that of GSH, 20-30 times that of Hcy, which normally presents below 12-15 μM [4]. However, the alterations in the level of thiols in biological fluids are implicated in a variety of diseases [6-8]. For example, Cys deficiency is involved in many syndromes such as slow growth in children, hair depigmentation, edema, lethargy, liver damage, loss of muscle and fat, skin lesions, and weakness [9]. At elevated levels in plasma, Hcy is a risk factor for Alzheimer's disease [10], folate and cobalamin (vitamin B12) deficiencies [11], and cardiovascular diseases [12]. GSH is the most abundant intracellular non-protein thiol [13], which serves many cellular functions, including maintenance of intracellular redox activities, xenobiotic metabolism, intracellular signal transduction, and gene regulation [14, 15]. Owing to their important roles, sensitive and selective detection of thiols has received growing attention in recent years.

In recent times, fluorescent molecular probes have emerged as an attractive tool for selective detection of various chemical and biological components [16–19], including thiols [20–27]. Compared to traditional techniques, chemical probes based on absorption or fluorescence changes are more feasible for detecting analyses owing to

their many appealing advantages such as low detection limit, high selectivity, its real-time monitoring and its potential for in vivo imaging of living cells. However, fluorescence quenching may be caused by a number of factors other than the target analyte, and thus the sensing behavior may be nonspecific [28]. Probes that rely on fluorescence quenching suffer from inherent drawbacks including low signal-to-noise ratio and non-specific quenching [29], so that “turn-on” type fluorescence probes are preferred [30-33].

Maleimide groups are known to react fairly selectively with thiols via addition reactions involving their C=C double bond. They are also known to quench fluorescence in their conjugated form, but not as their thiol adduct products [34]. These properties were demonstrated in the characterization of fluorophores bearing a maleimide group whose fluorescence increased dramatically upon reaction with thiols [35-37]. Based on these issues, we synthesized a new thiol-reactive fluorescent probe containing triphenylamine and maleimide (Scheme 1). When the concentration of probe is low, Hcy/GSH induced a significant enhancement in fluorescence intensity whereas Cys induced almost no change, while increasing amount of probe, fluorescence enhancement induced by Cys was enough to make clear the recognition. Furthermore, this probe was successfully applied in fluorescent imaging in living cells.

<Inserted Scheme 1>

2. Materials and Methods

2.1. Materials

4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from

Sigma-Aldrich (St. Louis, MO). Sodium hydroxide solution (0.1 mol/L) was added to aqueous HEPES (10 mmol/L) to adjust the pH to 7.4. Amino acids were purchased from Shanghai Experiment Reagent Co., Ltd (Shanghai, China). All other chemicals used were of analytical grade.

2.2. Instruments

A pH meter (Mettler Toledo, Switzerland) was used to determine the pH. Ultraviolet–visible (UV–vis) spectra were recorded on an Agilent 8453 UV-Visible spectrophotometer. Fluorescence spectra were measured on F-7000 FL Spectrophotometer. A PO-120 quartz cuvette (10 mm) was purchased from Shanghai Huamei Experiment Instrrument Plants, China. ^{1}H NMR, ^{13}C NMR spectra were recorded on a Bruker AVANCE-300 MHz (and 600MHz) and 75 MHz NMR spectrometer, respectively (Bruker, Billerica, MA). ESI was measured with an LTQ-MS (Thermo) instrument. LC-MS was measured with Bruker solari X FTMS. The ability of probe reacting to thiols in the living cells was also evaluated by laser confocal fluorescence imaging using an Olympus FV1000 laser scanning microscope.

2.3. Preparation and characterization of probes

2.3.1. Preparation and characterization of B

The synthesis route is summarized in Scheme 1. $POCl_3$ (11 mL) was added to a DMF solution (250 mL) with stirring for 2 h in ice-water bath. After the color change to nacarat, added triphenylamine (100 mmol) to the solution with stirring at 40℃. After the reaction was complete, the reaction mixture was poured into ice water (1000 mL) and adjusted pH to 9 with NaOH to separate faint yellow crystals out. The solid

obtained by filtered, washed with water and recrystallized in ethanol to give compound B in 86% as a faint yellow powder. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm): 9.77 (s, 1H), 7.72 (d, 2H, J = 8.7 Hz), 7.43 (t, 4H, J = 15.6 Hz), 7.23 (m, 6H), 6.88 (d, 2H, J = 8.7 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm): 117.7, 125.0, 126.0, 128.1, 129.6, 130.9, 145.1, 152.3, 190.1; Elemental analysis (calcd. %) for $C_{19}H_{15}NO$: C, 83.49, H, 5.53, N, 5.12, Found: C, 83.46, H, 5.54, N, 5.14; ESI–MS m/z: $[B + H]^+$ Calcd for $C_{19}H_{16}NO$ 274.12, Found 273.92 (Fig. S1).

2.3.2. Preparation and characterization of C

A mixture of C (triphenylphosphine, 0.16 mol) and 4-nitrobenzyl chlorine (0.15 mol) in paraxylene (200 mL) was stirred and refluxed at 150℃ for 2 h. The mixture was then cooled to 0℃ and filtered. The taupe solid thus obtained was dried under vacuum to give compound D in 92%. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm): 8.11 (d, 2H, J = 8.1 Hz), 7.93 (s, 3H), 7.75 (d, 12H, J = 9.9 Hz), 7.30 (d, 2H, J = 8.1 Hz), 5.60 (s, 1H), 5.54 (s, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm): 116.0, 117.1, 122.9, 129.3, 129.5, 131.4, 133.2, 133.4, 134.5, 135.4, 135.6, 146.5; Elemental analysis (calcd. %) for $C_{25}H_{21}ClNO_2P$: C, 69.21, H, 4.88, N, 3.23, Found: C, 69.20, H, 4.90; N, 3.20 (Fig. S2).

2.3.3. Preparation and characterization of D

In a 250 mL 3 mouth flask, added potassium tert-butoxide to a THF solution (100 mL) of D (12.5 mmol) in ice-water bath with stirring for 1h. Afterwards 8.5 mmol B was added to the solution with stirring and refluxing at 70℃. After the reaction was complete, parts of the solvents were then evaporated in vacuo and the residue was dissolved in dichloromethane. 30 mL of water was then added to the dichloromethane

solution, and the organic layer was purified by chromatography on a silica gel column to give a crimson product in 82% yield. A CH_3Cl solution containing the product was allowed to evaporate slowly at room temperature for several days, and the crystals that subsequently formed were suitable for X-ray crystallography. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm): 8.21 (d, 2H, J = 8.7 Hz), 7.81 (d, 2H, J = 8.7 Hz), 7.55 (t, 2H, J = 15.6 Hz), 7.30 (m, 6H), 7.09 (m, 6H), 6.95 (d, 2H, J = 8.4 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm): 121.7, 123.2, 124.1, 126.4, 127.9, 129.1, 129.6, 132.4, 144.0, 145.3, 146.2, 147.3; Elemental analysis (calcd. %) for $C_{26}H_{20}N_2O_2$: C, 79.57, H, 5.14, N, 7.14, Found: C, 79.59, H, 5.14, N, 7.12; ESI–MS m/z: $[E + H]^+$ Calcd for $C_{26}H_{21}N_2O_2$ 393.15, Found 393.08; Crystal data for $C_{26}H_{20}N_2O_2$: crystal size: 0.20 × 0.16 × 0.06, monoclinic, space group P 1 21/n 1. a = 8.4057(13) Å, b = 8.8548(13) Å, c = 27.015(4) Å, β = 96.654(3)°, V =1997.2(5) Å^3, Z = 4, T = 173.1500 K, max = 27.483°, 13867 reflections measured, 4562 unique (R_{int} = 0.0355). Final residual for 271 parameters and 4562 reflections with I > 2(I): R_1 = 0.0596, wR_2 = 0.1248 and GOF = 1.104 (Fig. S3).

*2.3.4. Preparation and characterization of **F***

A solution of E (10 mmol) dissolved in 150 mL ethanol was added into a round-bottom flask equipped with a magnetic stirrer and heated at 80℃. Then 0.3 g of Pd/C catalyst was added into the preceding reaction system and a solution of 4.9 mL of 85% hydrazine hydrate was added dropwise for about 0.5 h. The reaction was monitored by TLC. After the completion of the reaction, the reaction mixture was filtered immediately and the solution was poured into saturated sodium chloride solution to give a faint yellow solid. The solid obtained by filtered was purified by

chromatography on a silica gel column to give compound F in 65% yield. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm): 7.40 (d, 2H, J = 8.1 Hz), 7.27 (m, 6H), 6.85 (m, 10H), 6.53 (d, 2H, J = 8.1 Hz), 5.26 (s, 2H); ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm): 112.2, 112.7, 121.0, 121.7, 122.5, 12.7, 125.6, 126.2, 126.7, 128.3, 131.5, 14.4, 145.9, 147.3; Elemental analysis (calcd. %) for $C_{26}H_{22}N_2$: C, 86.15, H, 6.12, N, 7.73, Found: C, 86.14, H, 6.12, N, 7.74; ESI–MS m/z: $[F + H]^+$ Calcd for $C_{26}H_{23}N_2$ 363.18, Found 362.92 (Fig. S4).

2.3.5. Preparation and characterization of probe **1**

The synthesis of probe **1** is that a mixture of F (2 mmol), maleic anhydride (2 mmol) in glacial acetic acid (10 mL) was heated under reflux with monitoring by TLC. The mixture solution was poured into saturated sodium carbonate solution to separate crystals out. The solid obtained by filtered was purified by chromatography on a silica gel column to give compound F in 58% yield. ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm): 7.67 (d, 2H, J = 8.5 Hz), 7.53 (d, 2H, J = 8.6 Hz), 7.35 – 7.32 (m, 4H), 7.31 (d, 2H, J = 2.0 Hz), 7.25 (d, 2H, J = 16.4 Hz), 7.19 (s, 2H), 7.15 (d, 2H, J = 16.4 Hz), 7.07 (t, 2H, J = 7.4 Hz), 7.05 (d, 4H, J = 7.6 Hz), 6.97 (d, 2H, J = 8.6 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz): δ (ppm): 122.5, 122.9, 123.8, 125.5, 126.1, 126.5, 127.4, 128.4, 129.2, 130.0, 130.7, 134.3, 136.4, 146.5, 169.5; Elemental analysis (calcd. %) for $C_{30}H_{22}N_2O_2$: C, 81.43, H, 5.01, N, 6.33, Found: C, 81.44, H, 5.02, N, 6.31. LC–MS m/z: [probe + CH_3OH] Calcd for $C_{31}H_{26}N_2O_3$ 474.19434, Found 474.19364 (Fig. S5).

2.4. General UV-Vis and fluorescence spectra measurements

Probe stock solutions were prepared in DMSO. Aqueous amino acid solutions

were also prepared using deionized water. Fluorescence measurements were carried out with a slit width of 5 nm (λ_{ex} = 370 nm). Fluorescence spectra were obtained in HEPES aqueous buffer (10 mmol/L, pH 7.4) solutions.

2.5. Detection range

Fluorescence spectra were measured from 385 nm to 620 nm with excitation at 370 nm. The detection threshold for Cys, Hcy and GSH was 10^{-8} to 10^{-7} mol/L, and at this level the fluorescence color change was very obvious.

3. Results and discussion

3.1. The Selectivity over thiols

The spectral responses of probe **1** towards various amino acids, including thiols with mercapto groups (GSH, Hcy and Cys), and those without mercapto groups (L-alanine (Ala), L-arginine (Arg), L-asparagine(Asp), L-glutamine (Gln), L-glutamic acid (Glu), L-glycine (gly), L-histidine (His), L-isoleucine (Ile), L-leucine (Leu), L-lysine (Lys), L-methionine (Met), L-phenylalanine (Phe), L-proline (Pro), L-serine (Ser), L-threonine (Thr), L-tryptophan (Trp), L-tyrosine (Tyr), L-valine (Val) and SH^-) were examined. The probe **1** is essentially non-fluorescent in the absence of thiol. Only in the presence of thiols there was a significant enhancement in fluorescence intensity observed for probe **1**. In the buffer containing 0.5 μmol/L probe, addition of Hcy/GSH will induce a significant fluorescence enhancement, whereas Cys induce a little (Fig.1). Interestingly, only using 5 μmol/L probe **1**, fluorescence enhancement induced by Cys was obvious (Fig. S6). The other amino acid samples exhibited no noticeable increase of the fluorescence signal. For ultraviolet spectrum, there is no significant change even

in the presence of 20 eq. of thiols (Fig. S7). For other thiols, such as ME (2-mercaptoethanol), **1** showed similar fluorescence responses (Fig. S6).

<Inserted Figure 1>

3.2. The fluorescence spectra for thiol

As a typical biological thiol, GSH was used to further examine the fluorescence response of probe **1**. The changes in the fluorescence spectra of probe **1** (0.5 μmol/L) in the absence or presence of GSH (0–3.5 μmol/L) in HEPES buffer are displayed in Fig. 2. The probe **1** is essentially non-fluorescent in the absence of GSH; however, the addition of GSH caused a dramatic change in the fluorescence spectra. A strong new emission peak at 480 nm appeared, and an enhancement of the fluorescence intensity by up to 24-fold was observed. Upon addition of Hcy (3.0 μmol/L) to probe, a similar phenomenon on fluorescence spectra could be observed (Fig. S6). Cys induced a negligible response for 0.5 μmol/L probe **1**. However, Cys (35 μmol/L) caused 12-fold fluorescence enhancement in the presence of 5 μmol/L probe **1**.

<Inserted Figure 2>

To investigate the detection limit of probe for GSH, probe **1** (0.5 μmol/L) was treated with various concentrations of GSH (0–3.5 μmol/L) and the relative emission intensity at 480 nm was plotted as a function of the GSH concentration (Fig. 3). The emission intensity of probe **1** was linearly proportional to GSH concentrations of 0–3.5 μmol/L. The detection limit, based on the definition by IUPAC (C_{DL} = 3 Sb/m) [38], was found to be 0.085 μmol/L. Similarly, the detection limits were estimated to be 0.12 μmol/L for Hcy and 0.13 μmol/L for Cys, respectively (Fig. S8). The probe shows a

high sensitivity towards thiols which is comparable to that of other reported thiol chemosensors (Table 1) [39-41].

<Inserted Figure 3>

<Inserted Table 1>

3.3. Time-dependence in the detection process of thiols

Time-dependent modulations in the fluorescence spectra of probe **1** were monitored in the presence of 10 eq. of thiol. The kinetic study (Fig. 4) showed that the reaction was complete within 75 s for Hcy and GSH, indicating that probe **1** reacts rapidly with Hcy/GSH under the experimental conditions. Slightly inferior to Hcy/GSH, the reaction time for Cys is 150 s which is also faster than that of reported probes [26,42].

<Inserted Figure 4>

3.4. pH dependence

To investigate the effect of pH on the fluorescence response of **1** to GSH, the fluorescence intensity changes of **1** induced by GSH were measured at various pHs (Fig. S9). When the solution pH was between 2.0 and 4.0, the probe has slight fluorescence. When pH value exceeds 4.0, the probe **1** almost has no fluorescence. However, when the solution pH exceeds 5.0, fluorescence increase induced by GSH is obvious with intensifying pH value. Therefore, physiological pH 7.4 was chosen for further studies

3.5. Michael addition mechanism

Maleimide groups are known to react fairly selectively with thiols via addition reactions involving their C=C double bond. Michael addition of thiols to the

electron-deficient alkene groups in probe **1** gave rise to emission enhancement (λ_{ex} = 370 nm, λ_{em} = 480 nm). Mass spectrometry analysis of a product obtained from the reaction of probe with 2-mercaptoethanol also supports the formation of probe-thiol derivatives. A peak at 521.16 corresponding to [probe-ME + H]$^+$ was clearly observed (Fig. S10). Further NMR spectroscopic analysis also provided the evidence for the 1,2-addition of the thiol to the maleimide ring in probe **1**. With addition of 2 eq. of 2-mercaptoethanol (ME) to probe in DMSO-d_6, the resonance of the original proton ($CH_{maleimide\ ring}$) at 7.19 ppm disappeared and new peaks at 4.90, 4.19, 3.62, 2.7–3.0 ppm appeared (Fig. S11). Thus, the sensing mechanism of probe **1** towards thiol, which is in keeping with reported literature [43], was based on the Michael addition as shown in Scheme 2.

<Inserted Scheme 2>

3.6. Cellular Imaging

In order to evaluate the cell permeability and capability of probe **1** to selectively detect intracellular thiols, live-cell imaging studies were carried out. As shown in Fig. 5a, HepG2 cells that were pre-treated with 10 μmol/L N-ethylmaleimide (NEM, a thiol blocking reagent) and then incubated with 0.5 μmol/L probe **1** for 30 min at 37 ℃ showed no fluorescence. In a further experiment it was found that HepG2 cells displayed cyan fluorescence when the cells were first incubated with 0.5 μmol/L of probe **1** for 30 min at 37℃ and then incubated with 3.5 μmol/L GSH (Fig. 5b). These cell experiments show the good cell-membrane permeability of probe, and it can thus be used to mark thiols within living cells.

<Inserted Figure 5>

4. Conclusions

In summary, here we report an easy-prepared probe based on triphenylamine for the detection of thiol-containing molecules with high selectivity and sensitivity. The observed fluorescence enhancement is ascribed to a selective thiol-induced addition reactions to C=C double bond of maleimide group. The results of the investigation show that thiol induce an enhancement of the fluorescence intensity of **1** in pH 7.4 solutions. Moreover, because of its good water solubility, cell-penetration ability and biocompatibility, **1** serves as a fluorescent sensor to visualize thiol in live cells at micromolar concentrations. However, one shortcoming is that the excitation and emission wavelengths are in the ultraviolet region, which might be avoided in the future directions.

Acknowledgments

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Figure captions

Scheme 1. The synthesis of the probe 1.

Fig. 1. (a) Fluorescence emission spectra of the probe 1 (0.5 μmol/L) in CH_3OH–HEPES buffer (10 mmol/L, pH=7.4, 1 : 1, v/v) in the presence of 50 μmol/L Ala, Arg, Asp, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Val and 3.5 μmol/L Cys, GSH, Hcy. (b) Optical density two-dimensional graph of the probe at 480 nm upon the addition of amino acids.

Fig. 2. Fluorescence spectra of the probe (0.5 μmol/L) in the presence of various concentrations of GSH (0–3.5 μmol/L) in CH_3OH–HEPES buffer (10 mmol/L, pH 7.4, 1 : 1, v/v) (λ_{ex} = 370 nm, slit: 5 nm/5 nm).

Fig. 3. The linearity of the relative fluorescence intensity versus GSH concentration.

Table 1. A comparison table of the detection limits for Hcy.

Fig. 4. Reaction time profiles of probe (0.5 μmol/L) with GSH, Hcy and probe (5 μmol/L) for Cys.

Scheme 2. Proposed detection mechanism of the probe to thiols.

Fig. 5. Confocal fluorescence images of HepG2 cells. (a) Fluorescence image of HepG2 cells pre-treated with 10 μmol/L NEM and then incubated with 0.5 μmol/L probe for 30 min at 37℃ and its bright field image (c); (b) fluorescence image of HepG2 cells first incubated with 0.5 μmol/L of probe for 30 min at 37℃ and then incubated with 3.5 μmol/L GSH for 30 min at 37℃ and its bright field image (d).

Scheme 1

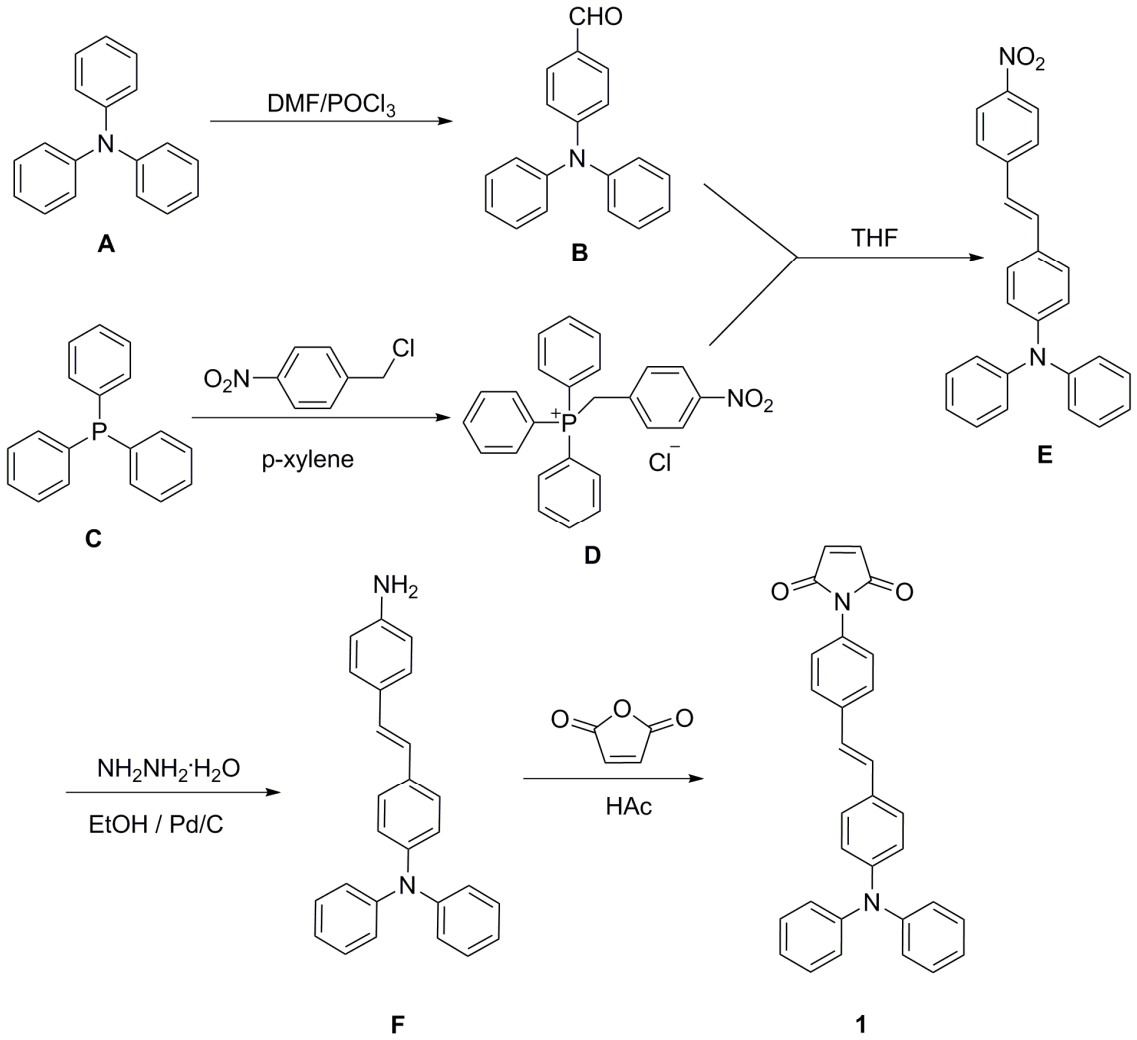
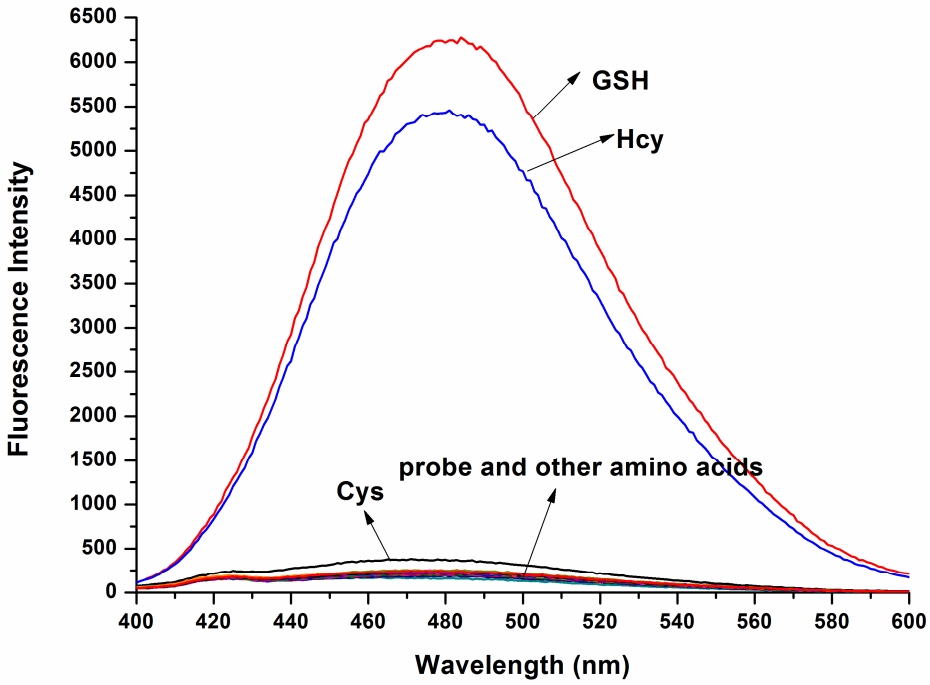


Figure 1(a)



(a)

Figure 1 (b)

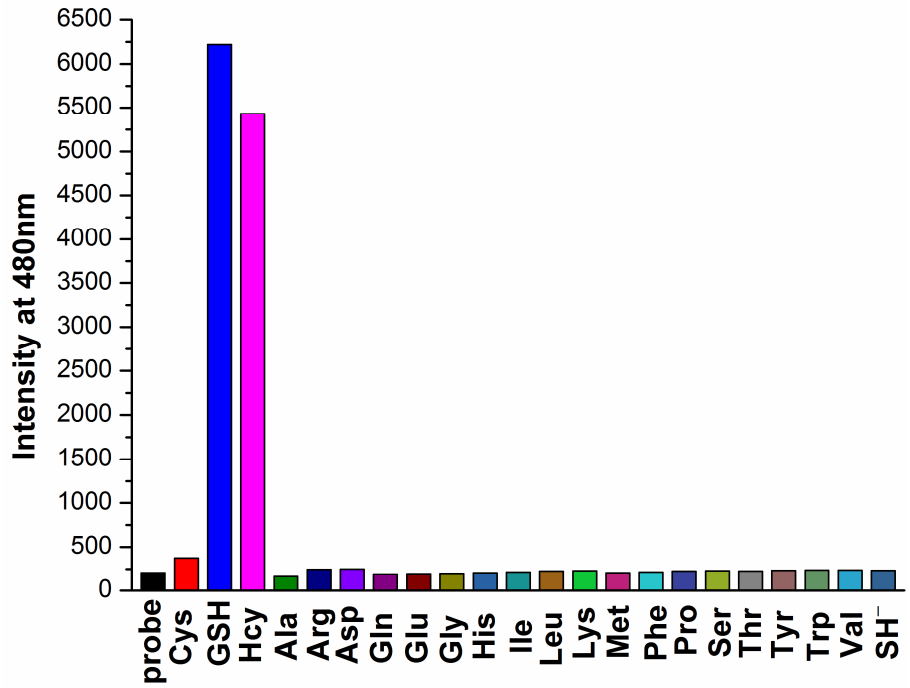


Figure 2

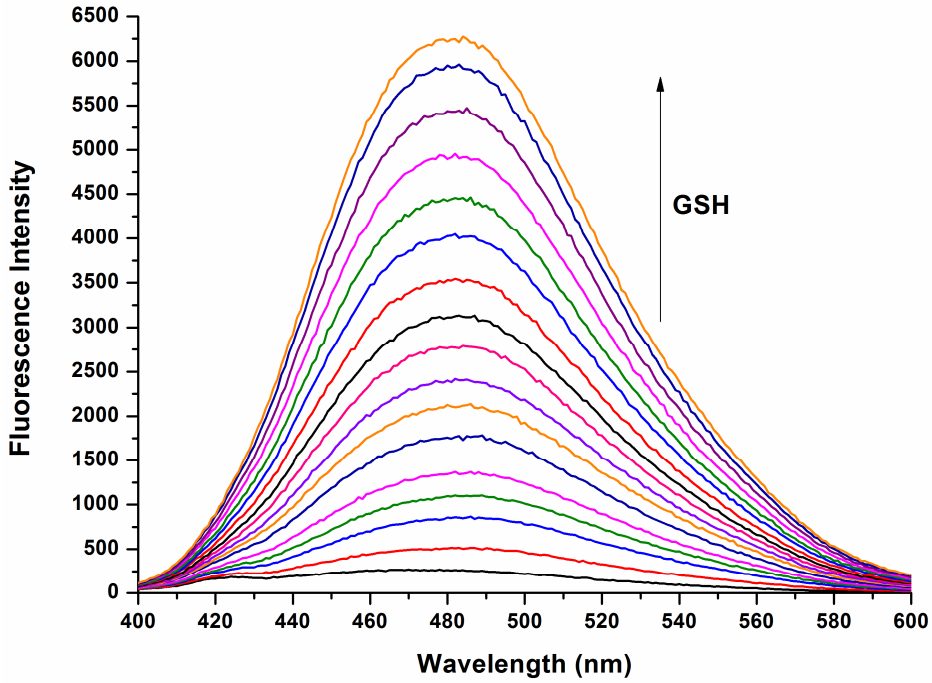


Figure 3

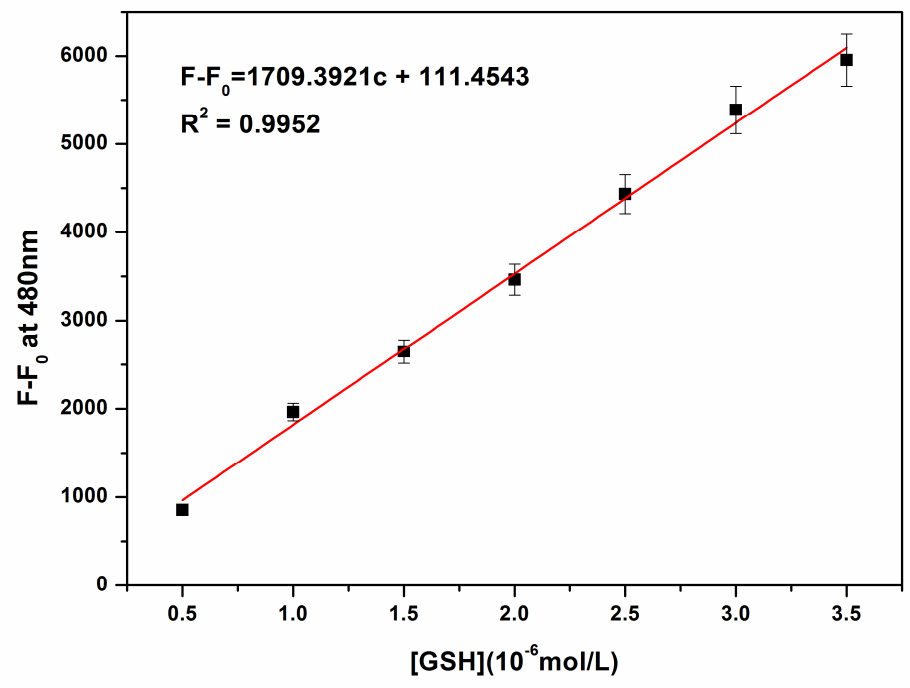
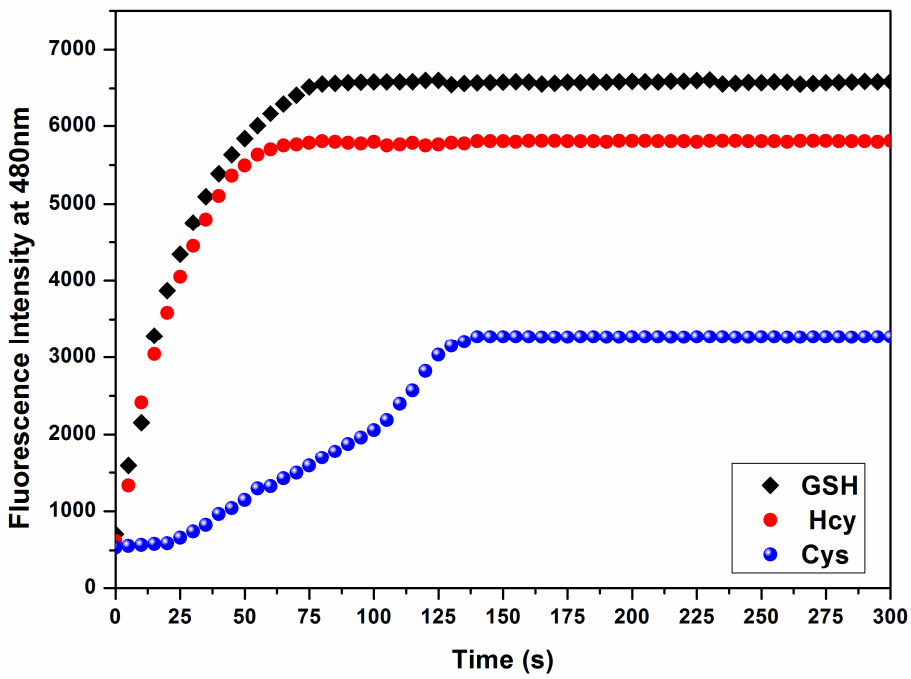


Table 1

Method	Analyte	Signal output	Solvent	Detection limit
Ref.[39]	Hcy	Fluorescence	DMSO/H_2O	1.19 μM
Ref.[40]	Hcy	Fluorescence	DMSO/H_2O	1.96μM
Ref.[41]	Hcy	Fluorescence	CH_3CN/H_2O	0.13μM
This work	Hcy	Fluorescence	DMSO/H_2O	0.12μM

Figure 4



Scheme 2

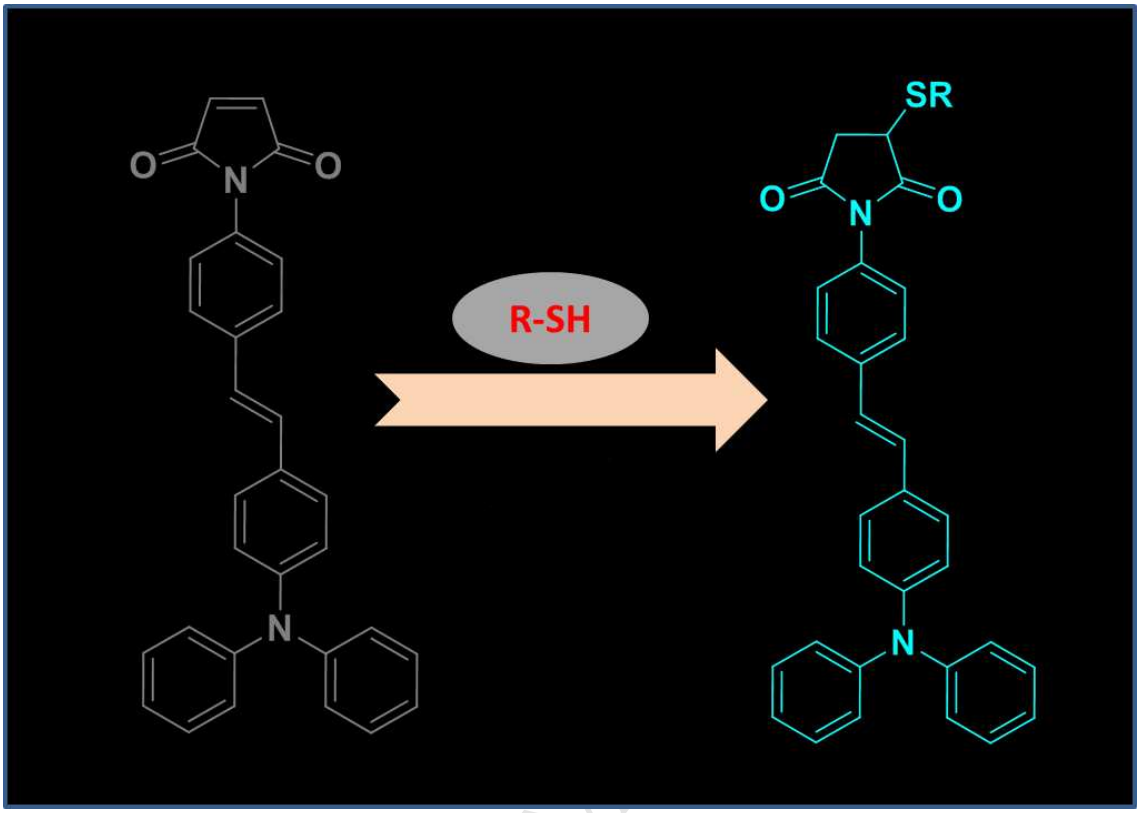
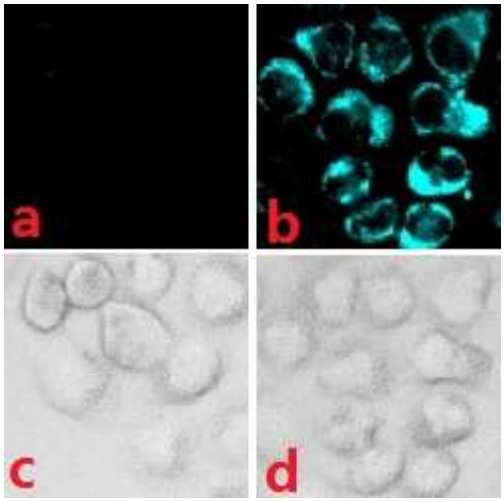


Figure 5



Supporting Information for

A triphenylamine as a fluorophore and maleimide as a bonding group selective turn-on fluorescent imaging probe for thiols

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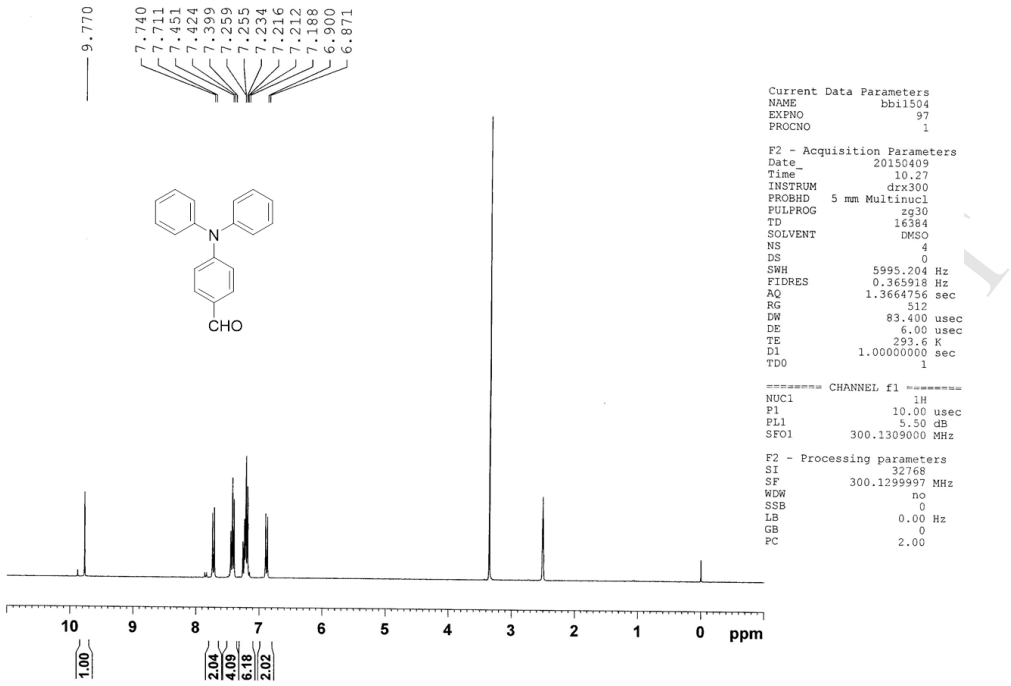
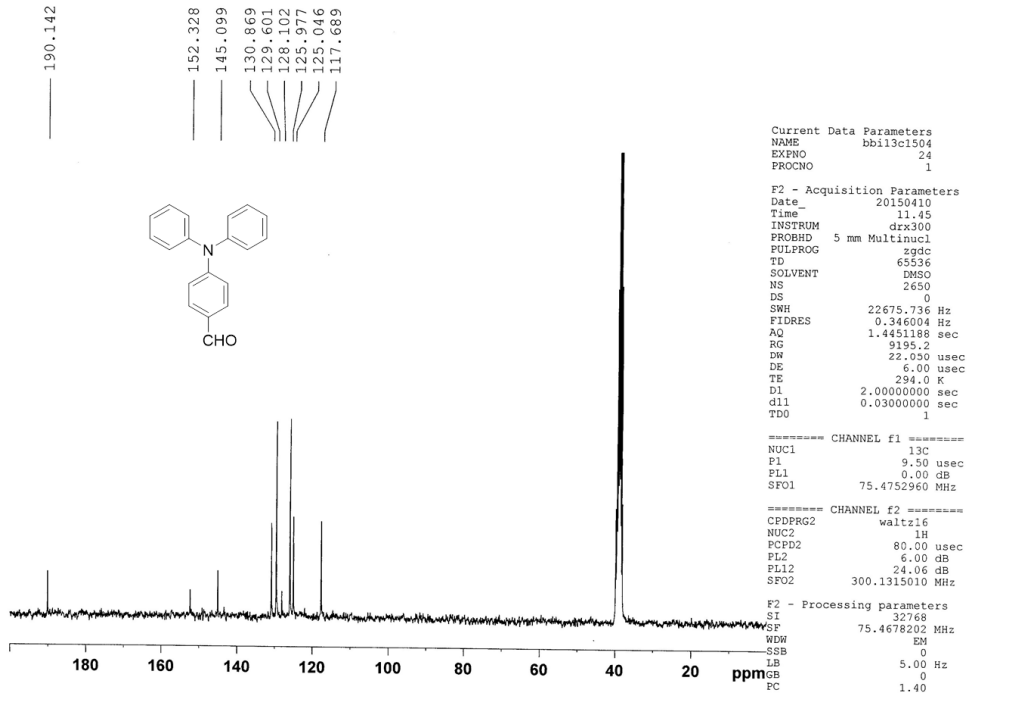
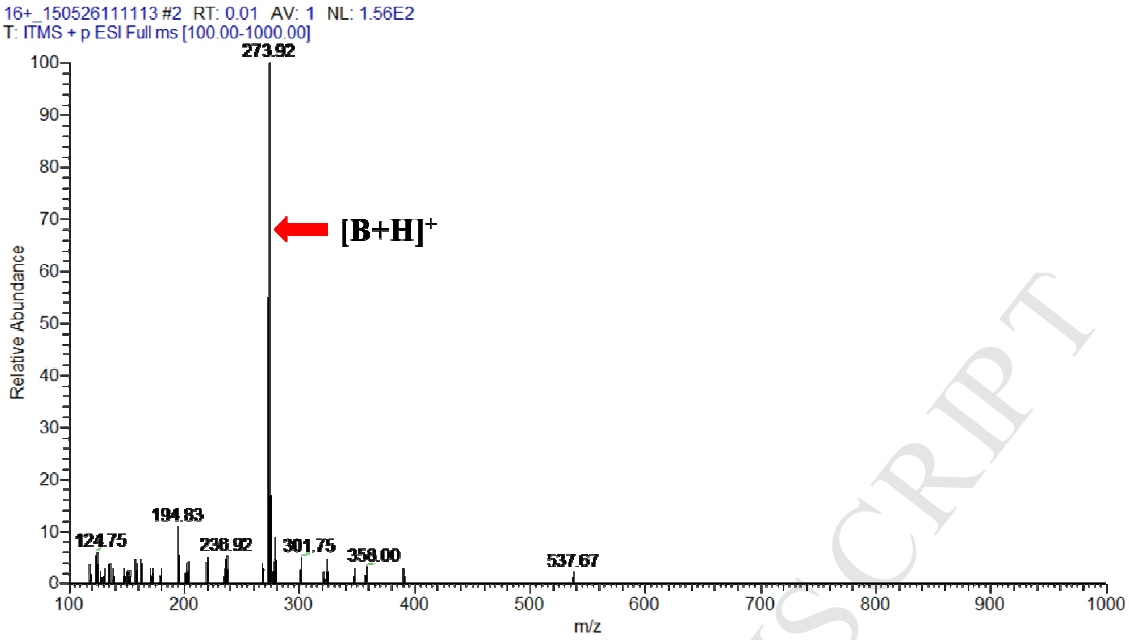
Figure S1: ^{1}H NMR, ^{13}C NMR, ESI-MS of the compound **B**



The ^{1}H NMR (300MHz) spectra of the B in DMSO-d_6.

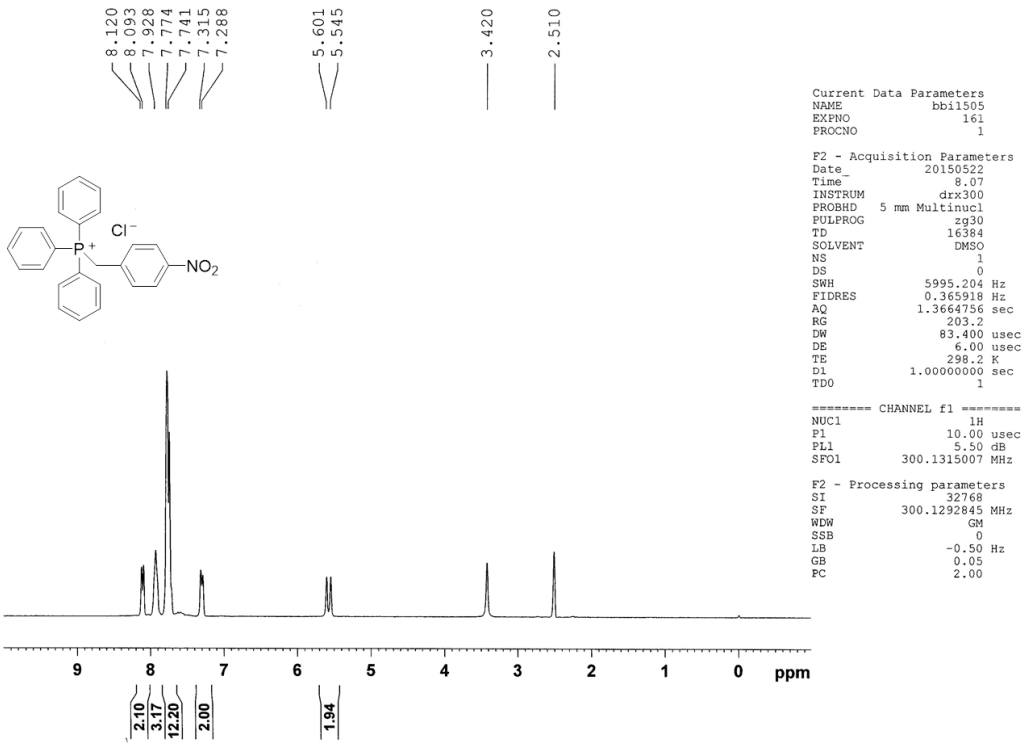


The ^{13}C NMR (75 MHz) spectra of B in DMSO-d_6.

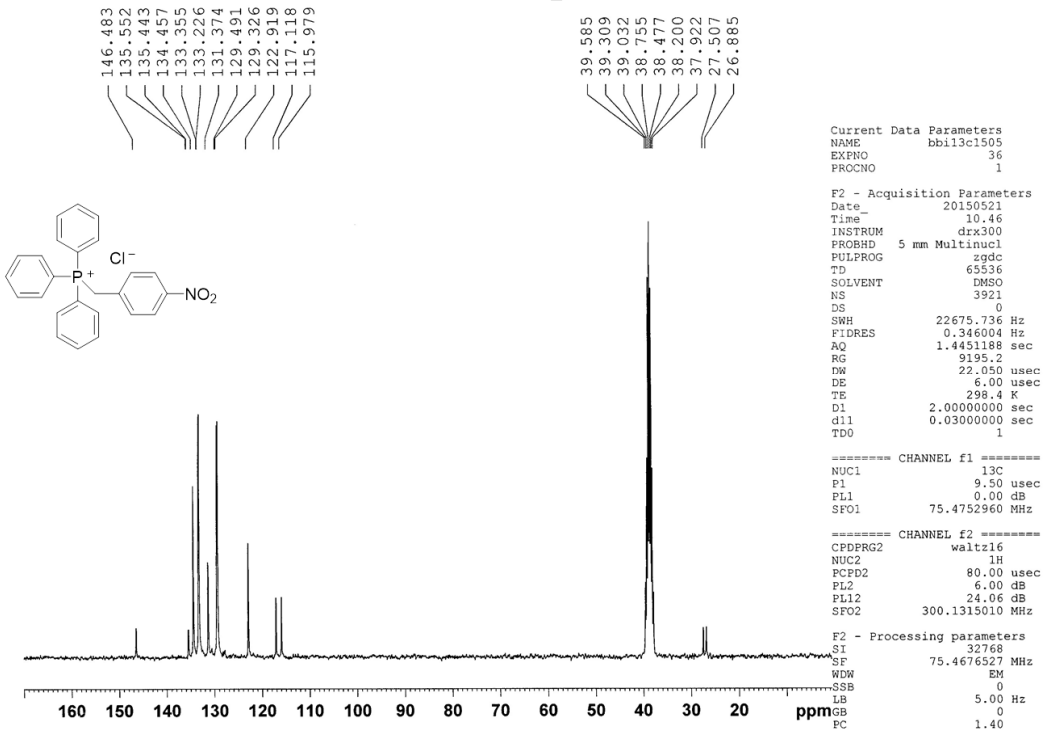


ESI-MS of the probe**:** m/z: $[B + H]^+$ Calcd for $C_{19}H_{16}NO$ 274.12, Found 273.92

Figure S2: ^{1}H NMR, ^{13}C NMR, ESI-MS of the compound D

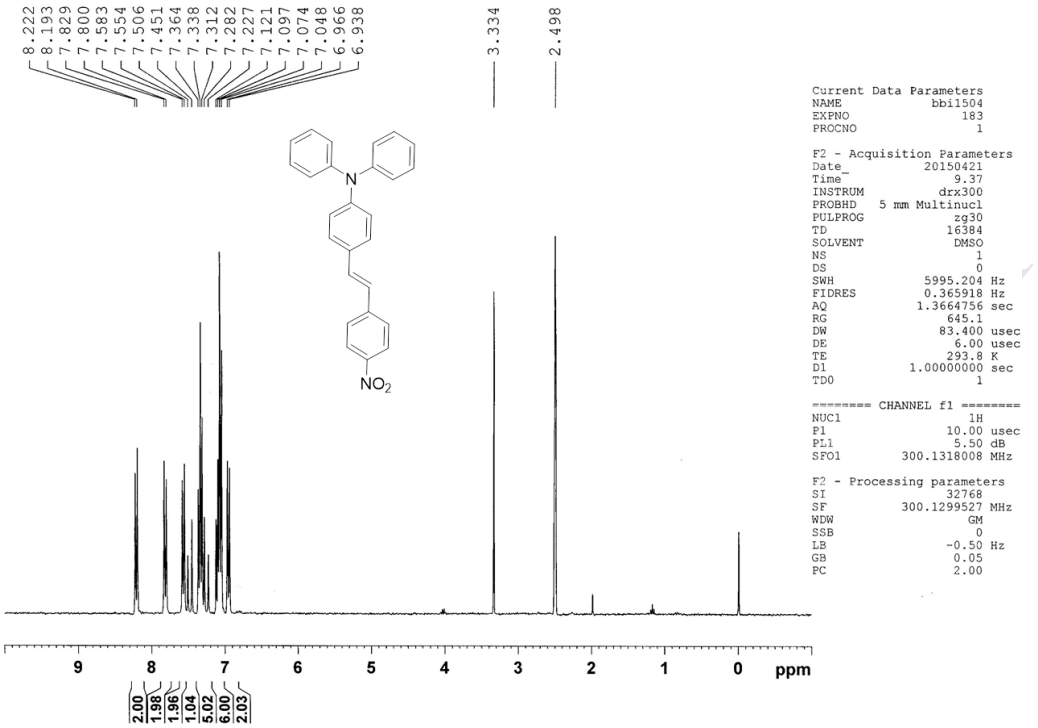


The ^{1}H NMR (300MHz) spectra of the **D** in DMSO

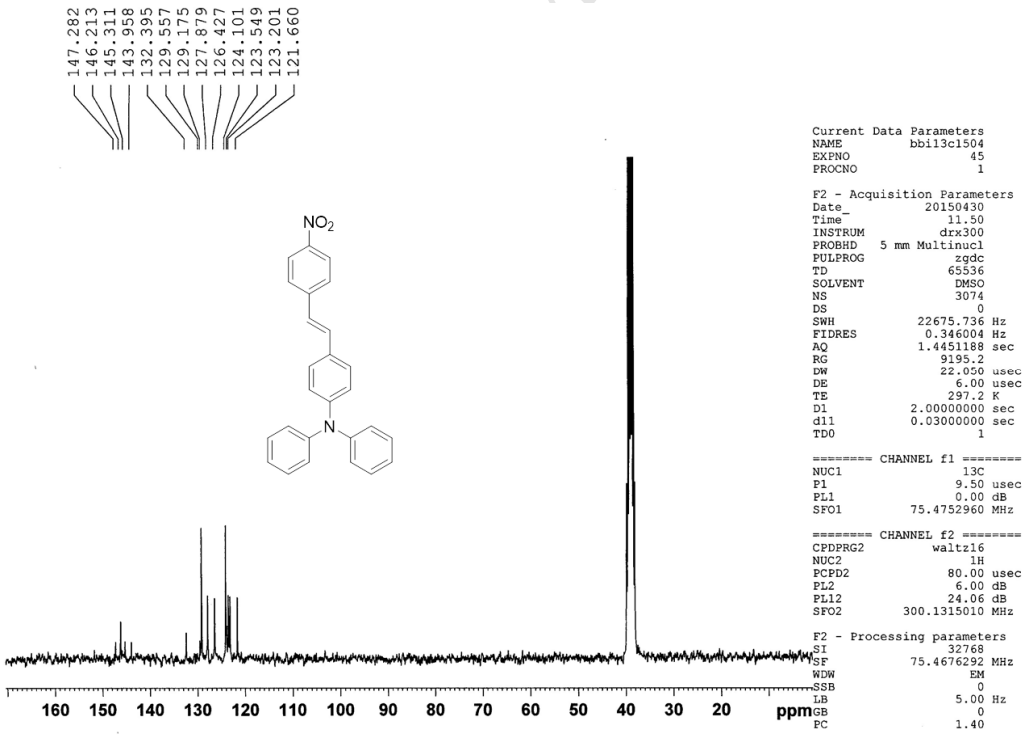


The ^{13}C NMR (75 MHz) spectra of **D** in DMSO

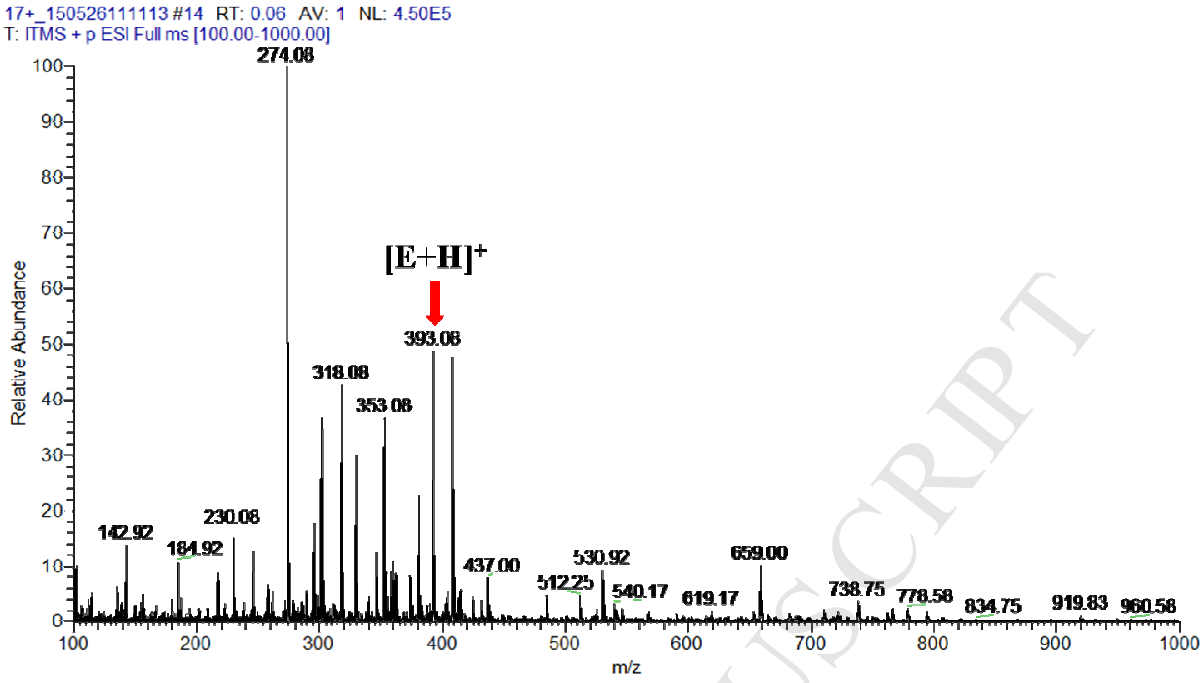
Figure S3: ^{1}H NMR, ^{13}C NMR, ESI-MS of the compound E



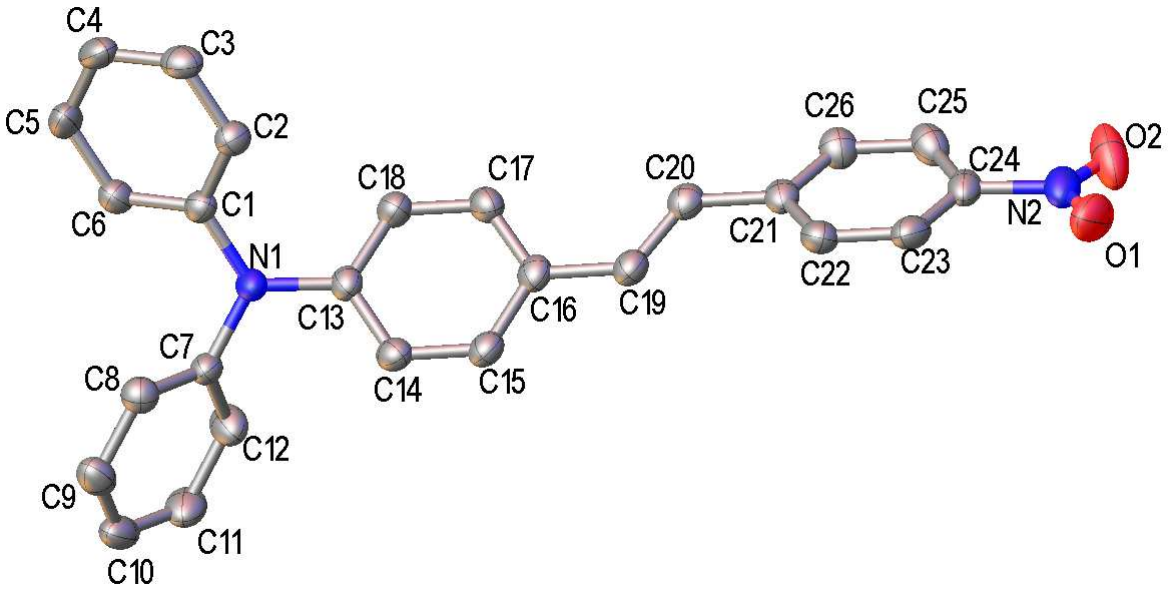
The ^{1}H NMR (300MHz) spectra of the **E** in DMSO



The ^{13}C NMR (75 MHz) spectra of **E** in DMSO

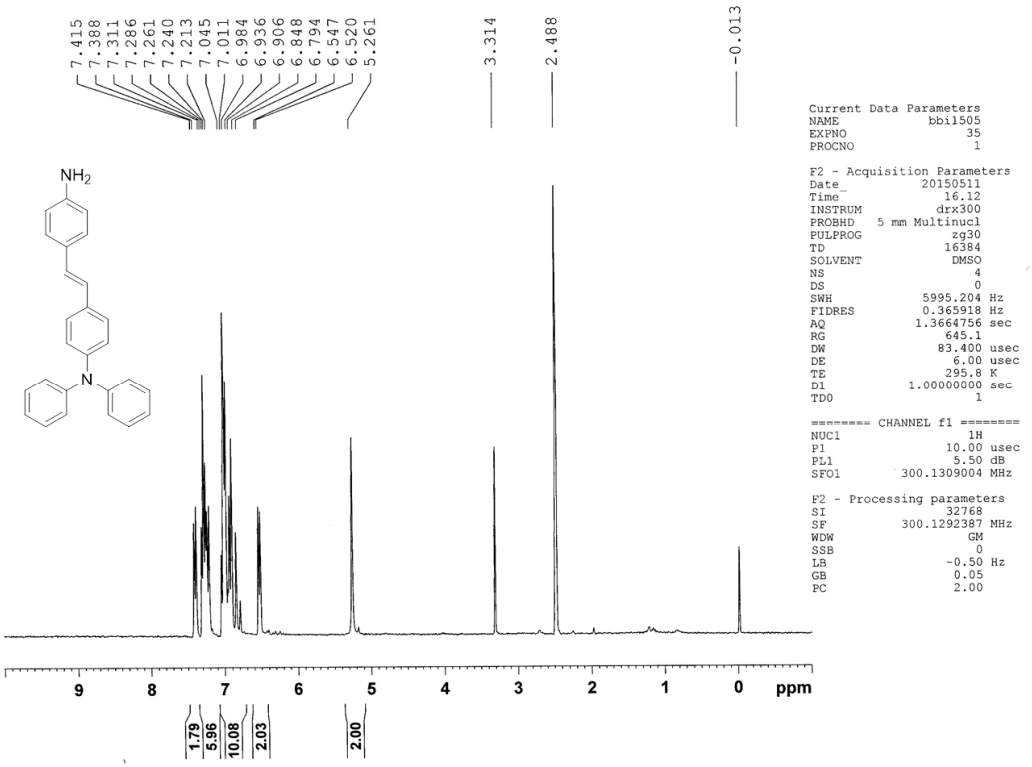


ESI-MS of the E**:** HRMS (ESI-TOF) m/z: $[E + H]^+$ Calcd for $C_{26}H_{21}N_2O_2$ 393.15, Found 393.08

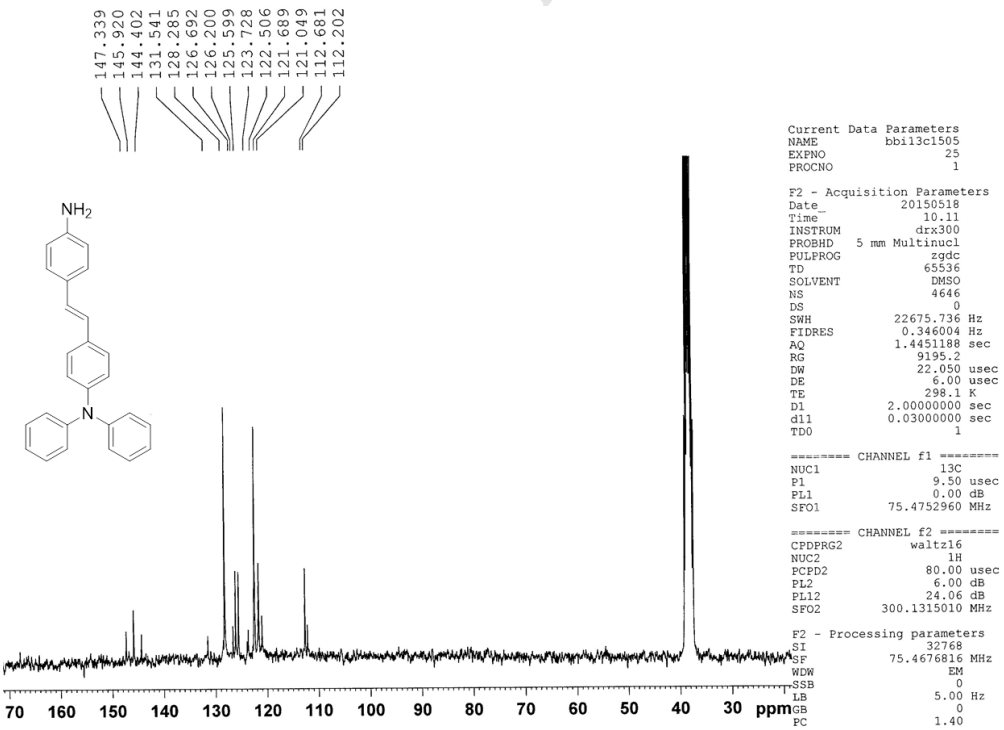


Thermal ellipsoids of compound **E** are drawn at the 50% probability level.

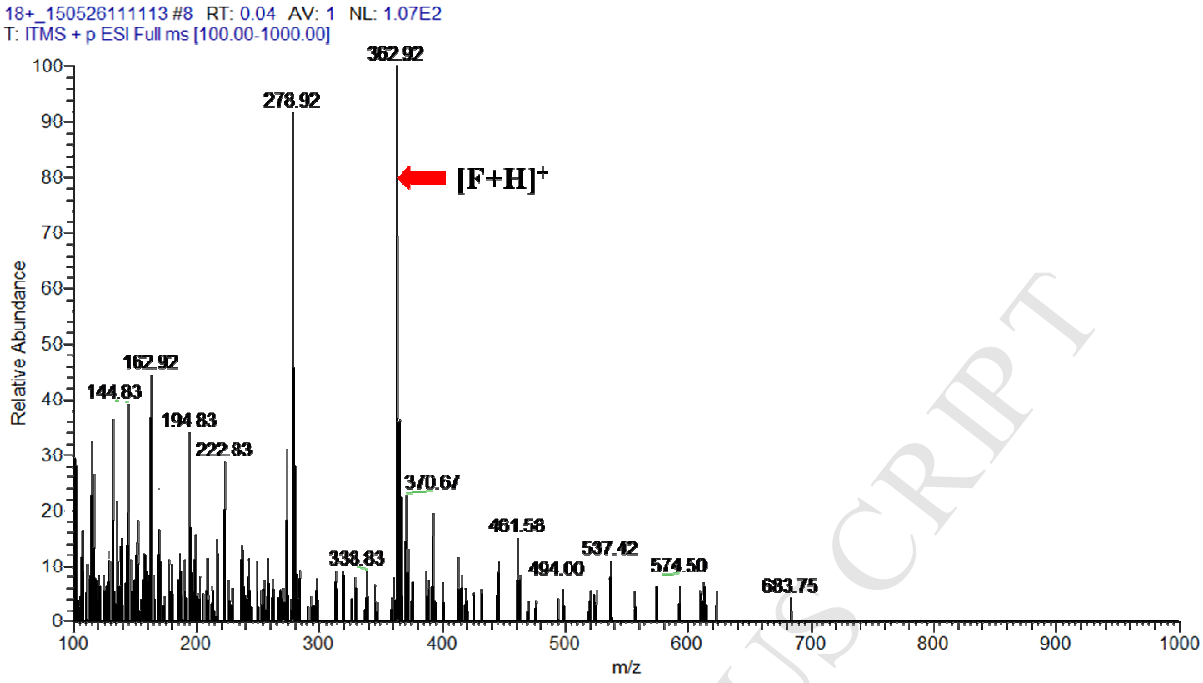
Figure S4: ^{1}H NMR, ^{13}C NMR, ESI-MS of the compound **F**



The ^{1}H NMR (300MHz) spectra of the **F** in DMSO

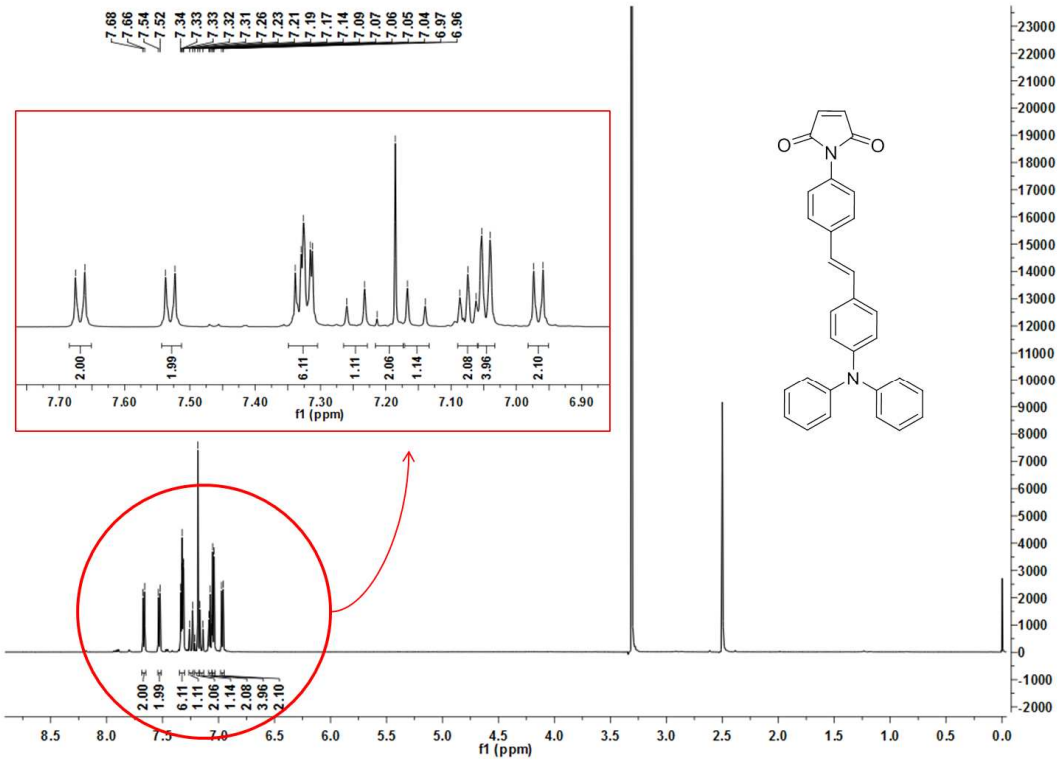


The ^{13}C NMR (75 MHz) spectra of **F** in DMSO

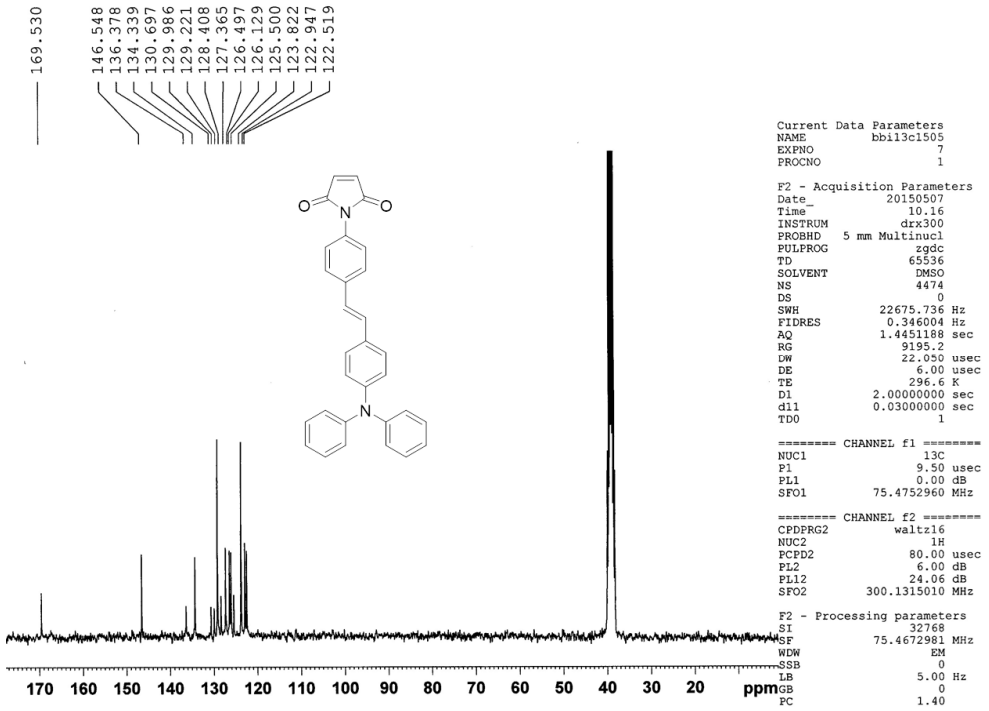


ESI-MS of the E**:** HRMS (ESI-TOF) m/z: $[F + H]^+$ Calcd for $C_{26}H_{23}N_2$ 363.18, Found 362.92

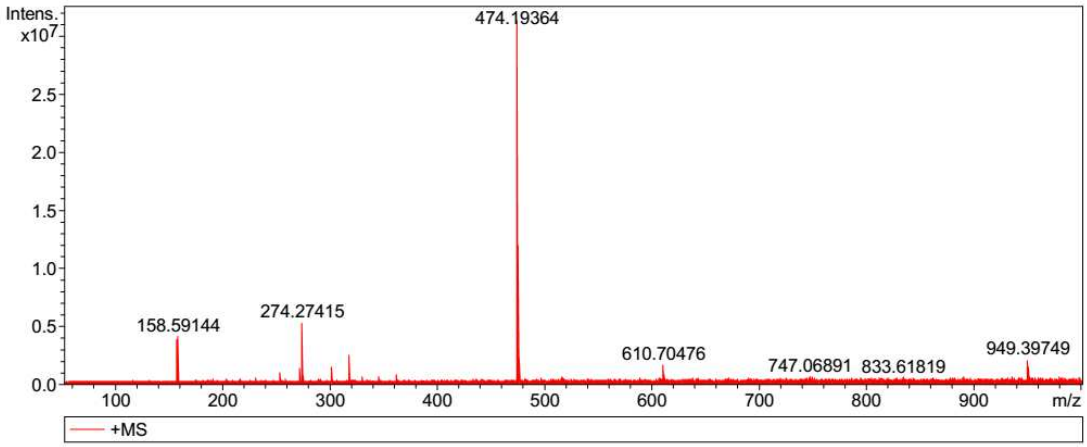
Figure S5: ^{1}H NMR, ^{13}C NMR, ESI-MS of the probe **1**



The ^{1}H NMR (600MHz) spectra of the probe **1** in DMSO

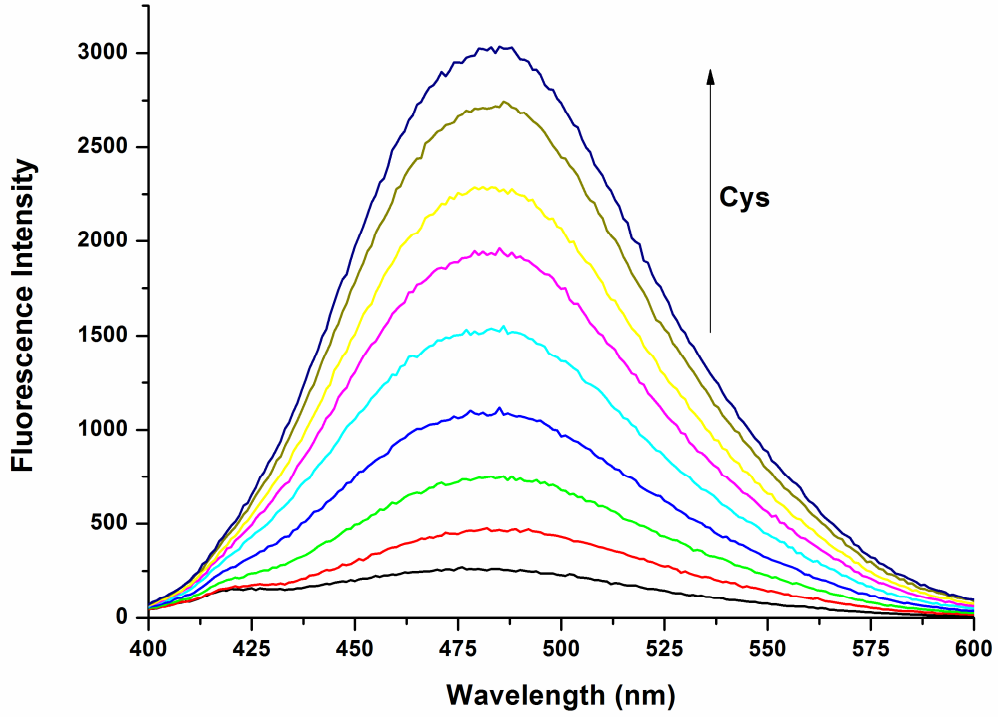


The ^{13}C NMR (75 MHz) spectra of probe in DMSO

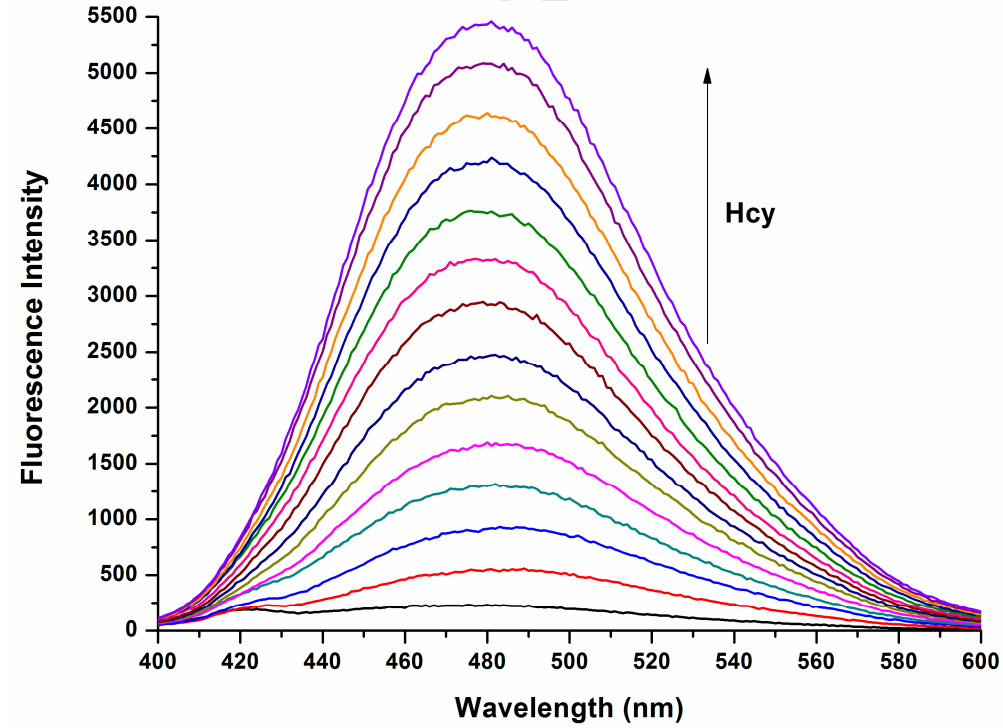


LC-MS of probe 1**:** HRMS (FTMS) m/z: [probe + CH_3OH] Calcd for $C_{31}H_{26}N_2O_3$ 474.19434, Found 474.19364

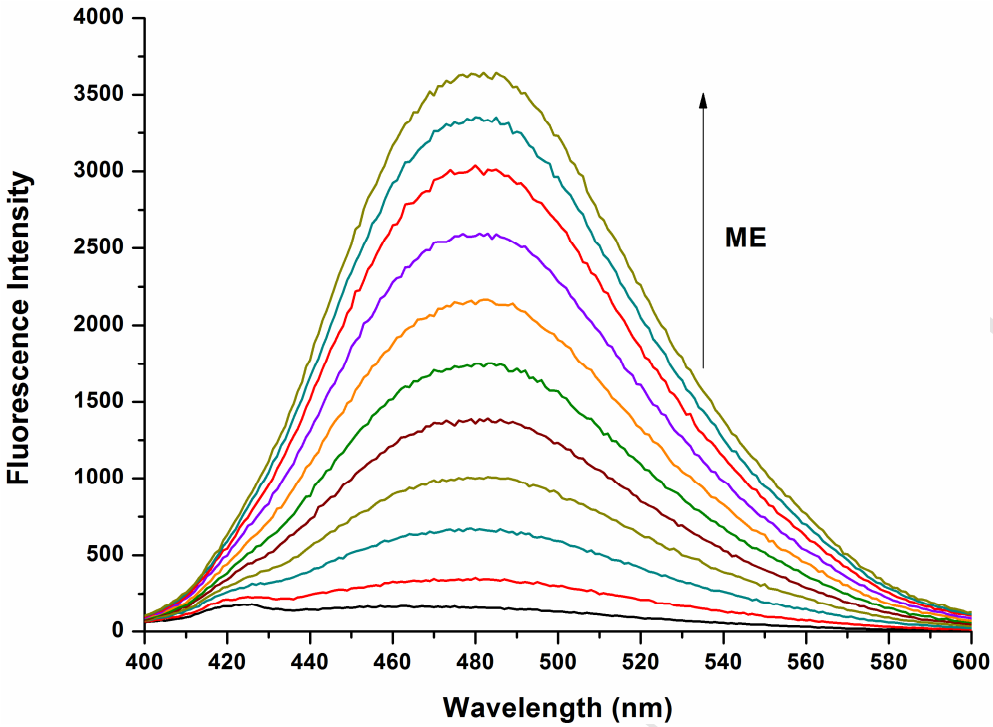
Figure S6: The fluorescence titration spectra of Cys, Hcy and ME



(a)



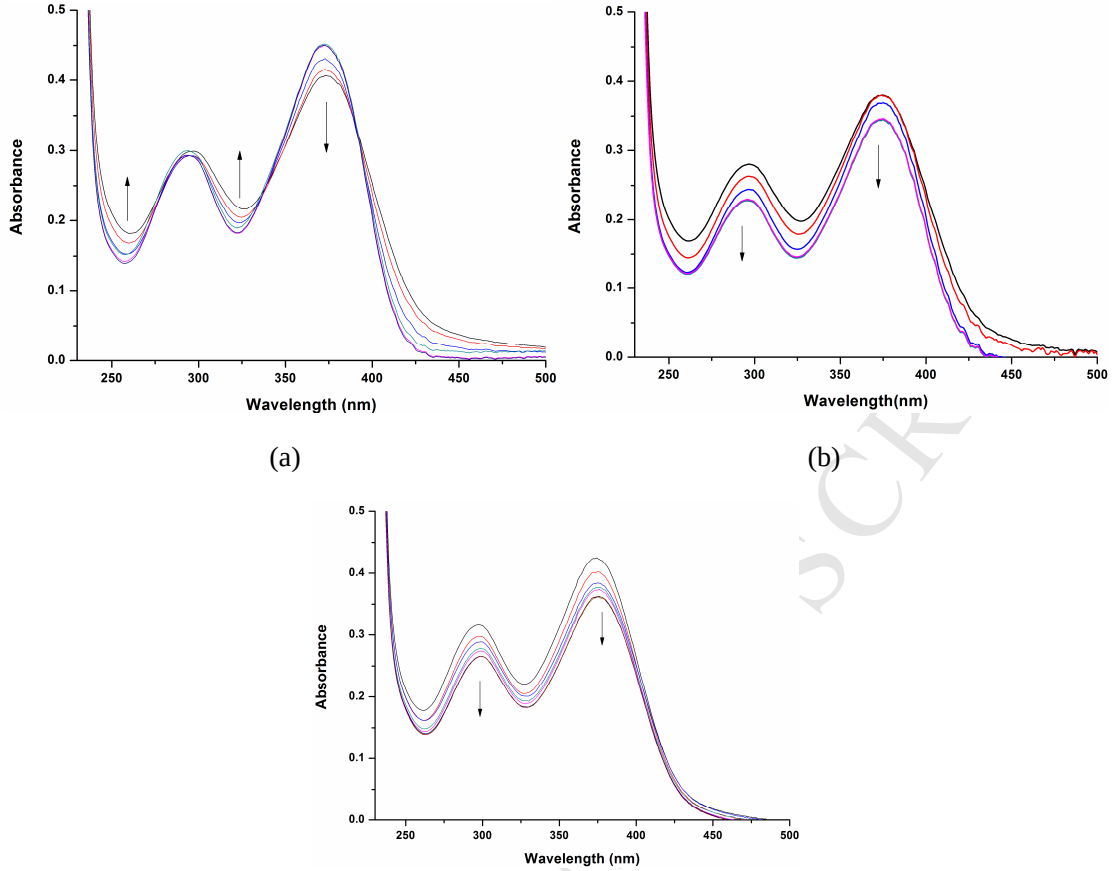
(b)



(c)

Figure S6: (a) Fluorescence spectra of the probe (5 μmol/L) in the presence of various concentrations of Cys (0–35 μmol/L) in CH_3OH–HEPES buffer (10 mmol/L, pH=7.4, 1 : 1, v/v). (b) and (c) Fluorescent titrations of probe (0.5 μmol/L) in response to the addition 0～3μmol/L Hcy and ME (2-mercaptoethanol) in HEPES buffer (10 mmol/L, pH 7.4) (λ_{ex} = 370 nm, slit: 5.0 nm/5.0 nm).

Figure S7: The absorption titration spectra of thiols.



(a) (b)

(c)

Figure S7: absorption spectra of probe (10 μM) with 200μM GSH (a), Hcy (b), Cys (c) in HEPES buffer (10 mM, pH 7.4,).

Figure S8: The detection limits of Hcy and Cys

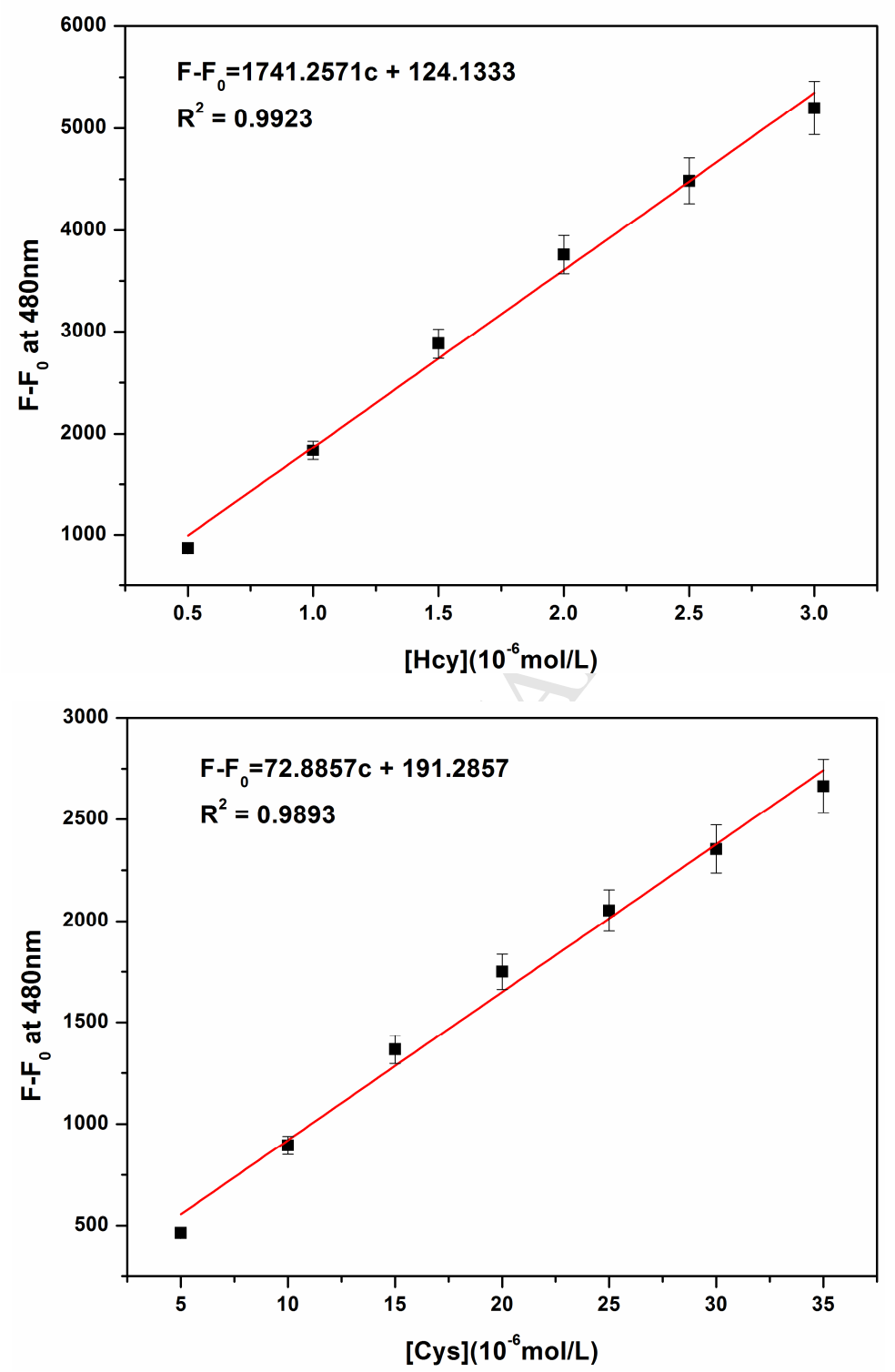


Figure S9: Choice of pH range for the measurements

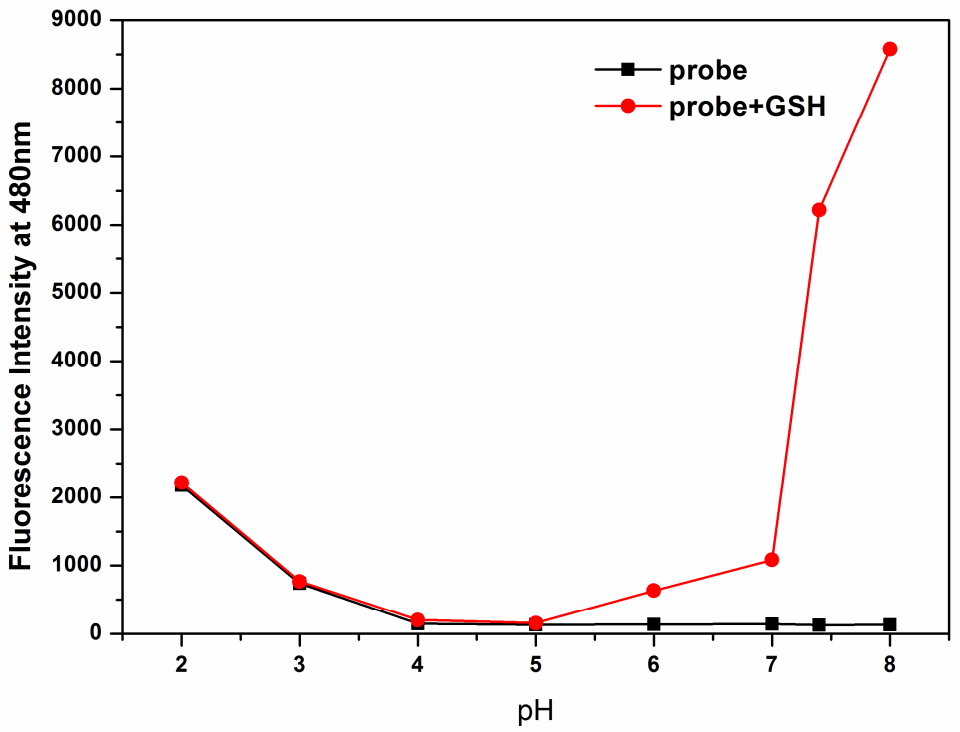


Figure S9: The fluorescence intensity of probe at 480 nm in the absence and presence of GSH under different pH (0.5 μmol/L probe in H_2O system; λ_{ex} = 370 nm; Slit: 5nm/5 nm).

Figure S10: LC-MS spectra of the probe-ME adduct

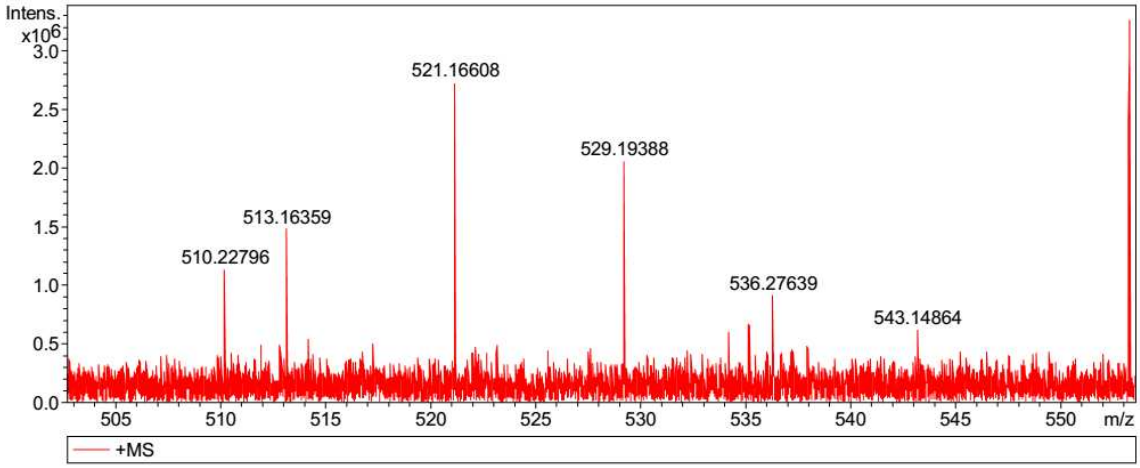
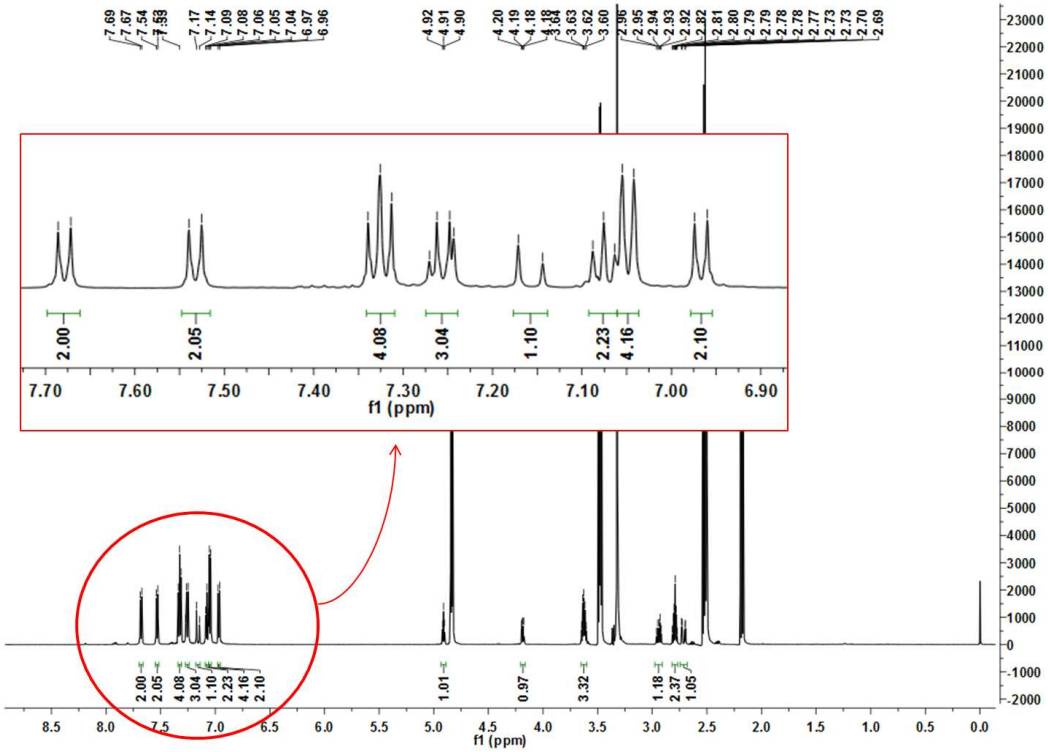


Figure S10: The LC-MS of product obtained by probe react with 2-mercaptoethanol: HRMS (FTMS) m/z: [probe-ME + H]$^+$ Calcd 521.18989, Found 521.16608

Figure S11: NMR spectra of probe and probe-ME



Highlight

1. A green turn on fluorescent probe for biothiols in solution was developed.
2. The detection limit is as low as 10^{-8}M.
3. The probe can be applied in bioimaging.