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A highly selective wavelength-ratiometric and colorimetric probe for cysteine

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ABSTRACT

Based on the nucleophilic aromatic substitution reaction mechanism, a new highly selective probe for cysteine (Cys), *N*-butyl-4-bromo-3-nitro-1,8-naphthalimide (**1**), was designed and synthesized. The probe displayed a remarkable (58 nm) red-shift in the absorption spectra and the color changes from colorless to yellow upon reaction with Cys. The probe could detect Cys quantitatively in the range of 0 −0.9 mM by both normal and ratiometric absorption spectrometry methods. Moreover, **1** could also serve as a "naked-eye" probe for Cys with a minimum detectable concentration of approximately 50 μM.

1. Introduction

Among the twenty amino acids used as building blocks for protein, the thiol-containing amino acids, such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy), play crucial roles in maintaining the biological redox homeostasis for their participation in the process of reversible redox reactions [1–6]. Their abnormal levels have been directly linked to some diseases and cancers. For example, Cys deficiency is involved in many syndromes, including slowed growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness [7–9]. Due to its important roles in biological systems, great attention has been paid to the detection of Cys. Several analytical techniques, such as high-performance liquid chromatography (HPLC) [10,11], capillary electrophoresis (CE) [12], electrochemical assay [13,14], UV/Vis spectroscopy [15,16], mass spectrometry [17,18], fluorescence spectroscopy [1–7,19–23], and colorimetric assay [6,8,24–36], have been employed to detect Cys. Among them, colorimetric indicators are widely developed because they have the capability to detect Cys by naked-eye, without the aid of any advanced instruments [37,38]. However, most of the reported examples still have some limitations, such as unsuitable working range and poor selective, *etc*. To the best of our knowledge, up to now, only Yang et al. [32] have described a colorimetric probe for Cys with suitable working range which can cover the physiological level of Cys in normal organisms. Additionally, ratiometric probes can overcome some problems produced by variabilities in sample environment and probe distribution in the quantitative measurement of analytes [39,40]. Therefore, the design of ratiometric and colorimetric cysteine probes has been the focus of numerous research efforts because of their remarkable importance in the qualitative and quantitative detection.

Recently, Li and Huang et al. [6] have demonstrated the first indicator for Cys/Hcy based on a mechanism of nucleophilic aromatic substitution, and the reaction with Cys/Hcy induces changes of absorption spectra. Inspired by this strategy, we designed and synthesized a new wavelength-ratiometric and colorimetric Cys probe, *N*-butyl-4-bromo-3-nitro-1,8-naphthalimide (Scheme 1, **1**), and **1** could detect Cys quantitatively by both normal and ratiometric absorption spectrometry methods.

1,8-naphthalimide and its derivatives are excellent chromogenic and fluorogenic dyes that are widely utilized as reporters in chemosensors [41–44]. We chose 1,8-naphthalimide as chromophore core for probe **1** due to its excellent photophysical properties. In addition, we added a nitryl group to 1,8-naphthalimide. The introduction of a nitryl group has two important functions. (i) It

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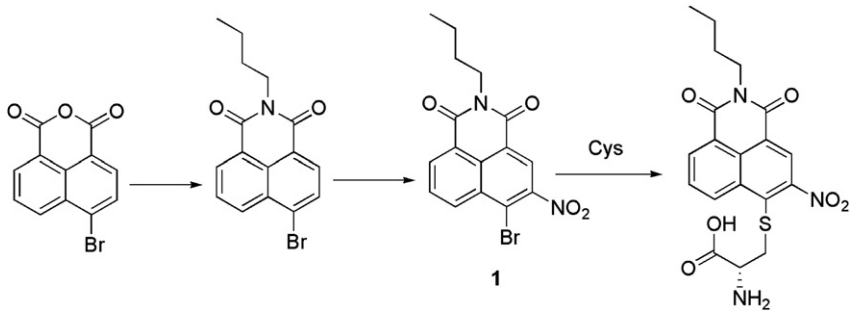

Scheme 1. The synthesis of the probe **1** and the proposed reaction mechanism of **1** with Cys.

could activate the 4-position of 1,8-naphthalimide, which provides a precondition for the interaction with Cys. (ii) It enhances and extends intramolecular charge transfer (ICT), which in turn exhibits enhanced sensitivity and color changes toward Cys. We hypothesize that the nucleophilic aromatic substitution reaction of Cys with **1** would result in the formation of a new stronger ICT because sulfur atom is better electron donor than bromine [45]. Thus, the red-shift absorption spectra and color changes could be expected upon addition of Cys.

2. Experiments

2.1. Materials and general methods

All chemicals used in this paper were commercial products of analytic grade. ^{1}H-NMR and ^{13}C-NMR spectra were taken on a Bruker AMX400 spectrometer. Chemical shifts (δ) were reported in ppm relative to a Me_4Si standard in $CDCl_3$. High-resolution mass data were measured with fourier transform ion cyclotron resonance mass spectrometer (APEX IV). Absorption spectra were recorded on TU-1901 UV–vis spectrophotometer. All pH measurements were made with a Sartorius basic pH-meter PB-10.

2.2. Synthesis of N-butyl-4-bromo-1,8-naphthalimide

The condensation of 4-bromo-1,8-naphthalic anhydride (10 g, 36 mmol) and *n*-butylamine (2.9 g, 40 mmol) was carried out in ethanol (200 mL) at refluxing under nitrogen atmosphere according to a reported literature [42]. After removal of ethanol, the residues were purified by silica gel column chromatography using dichloromethane as eluent to afford 10.3 g (86%) of pure products. Mp: 103.0–105.6 °C.

2.3. Synthesis of N-butyl-4-bromo-3-nitro-1,8-naphthalimide (1)

N-butyl-4-bromo-1,8-naphthalimide (1.577 g, 5.49 mmol) was dissolved in concentrated vitriol (50 mL), and the mixture was cooled to −5°. Sodium nitrate (467 mg, 5.49 mmol) was added to the mixture gradually with stirring, and the reaction mixture was stirred at room temperature for an additional 3 h. The solution was transferred to the mixture of water and ice. The product is collected by filtration and washed with 5 × 3 mL cooled water to give 1.641 g (90%) of pure product. ^{1}H-NMR (400 MHz, $CDCl_3$, δ ppm): 0.98(t, J = 7.3 Hz, 3H), 1.40–1.49(m, 2H), 1.67–1.75(m, 2H), 4.18(t, J = 7.5 Hz, 2H), 8.01(t, J = 8.2 Hz, 1H), 8.75–8.78(m, 3H). ^{13}C-NMR (100 MHz, $CDCl_3$, δ ppm): 14.0, 20.5, 30.2, 40.8, 121.9, 123.5, 123.7, 125.3, 129.1, 130.2, 131.9, 134.1, 134.9, 149.5, 162.1, 162.9. HRMS (ESI positive) calcd for $C_{16}H_{14}BrN_2O_4$ $[M + H]^+$ 377.01315, found 377.01287. Mp: 147.8–150.0 °C.

3. Results and discussion

3.1. Characteristic spectrum

Probe **1** was easily synthesized through the nitration of *N*-butyl-4-bromo-1,8-naphthalimide, which was prepared from 4-bromo-1,8-naphthalic anhydride and *N*-butylamine.

The spectral responses of **1** (10 μM) toward Cys were investigated in a mixture of ethanol and HEPES (7:3, v/v) solution at pH 7.3. The subsequent addition of Cys to the solution of **1** elicited a decrease of the absorption peak at 342 nm and an increase of a new absorption band centered at around 400 nm, accompanied the color of the solution of **1** changing from colorless to yellow (Fig. 1). Two well-defined isosbestic points were noted at 311 nm and 365 nm, which might indicate the formation of a new species because of the reaction of **1** with Cys. Additionally, a remarkable red-shift (58 nm) in the absorption spectra suggests the formation of the new ICT structure in this system. This result is in good agreement with the conclusion reported by Li and Huang et al. [6].

3.2. Effect of pH value

The pH value of system is often considered as a significant influence factor on interactions, and the effect of pH was investigated in the paper. The absorbance intensity of 400 nm was recorded (Fig. 2). As it can be seen, at the range of 3–11, the absorption spectra of probe **1** have no significant changes. The absorption intensity was low at the pH range of 3–5. With the

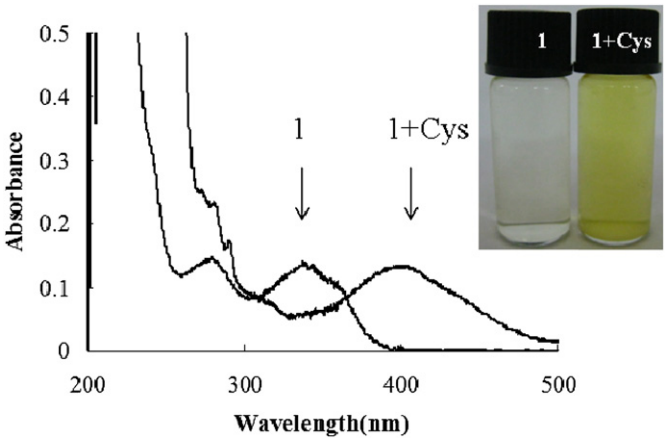

Fig. 1. Absorption spectra of **1** (10 μM) and Cys (5 mM) in a mixture of ethanol and HEPES (7: 3, v/v, pH 7.3). Insert photo is the photographs of the solution of **1** (50 μM) in the absence (left) and presence (right) of Cys (1 mM). Each spectrum was acquired 2 h after various analyses addition at 25 °C.

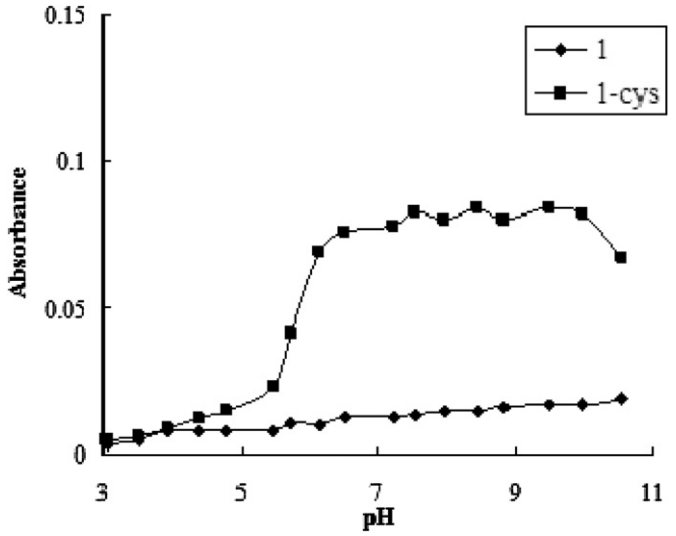

Fig. 2. The effect of pH on **1** (10 μM)-Cys (1 mM) system at 400 nm in a mixture of ethanol and water (7:3, v/v).

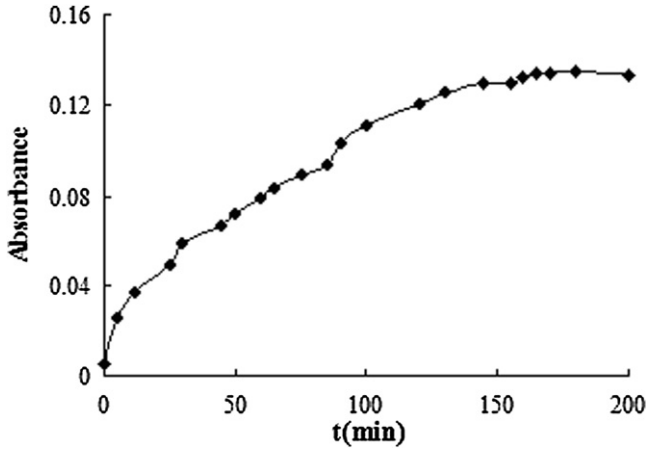

Fig. 3. The absorption intensity of the monitoring of the **1** (10 μM)-Cys (5 mM) at 400 nm in real time in a mixture of ethanol and HEPES (7:3, v/v, pH 7.3).

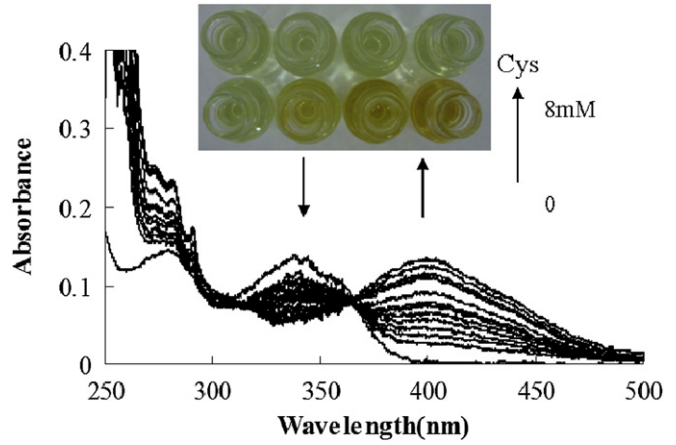

Fig. 4. The absorption responses of **1** (10 μM) toward different concentrations of Cys (final concentration: 0, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 3, 5, 8 mM) in a mixture of ethanol and HEPES (7:3, v/v, pH 7.3). Insert photo is the photographs of the solution of **1**(10 μM) in the presence of Cys (final concentration: 0, 0.005, 0.01, 0.02, 0.03, 0.05, 3, 8 mM). Each spectrum was acquired 2 h after various analyses addition at 25 °C.

addition of Cys, for the sulfhydryl group of Cys is difficult to take place a deprotonation process in this pH range. The absorption intensity increased gradually with pH and get stable from 5 to 10. As pH 7.3 is considered as a normal physiological pH value, we choose 7.3 as the optimal value for Cys detection.

3.3. The real time monitoring of 1-Cys system

To investigate how the absorption spectra of **1**-Cys system changed along with time, the absorption spectra of this system were recorded over time. Also, the absorbance intensity of 400 nm

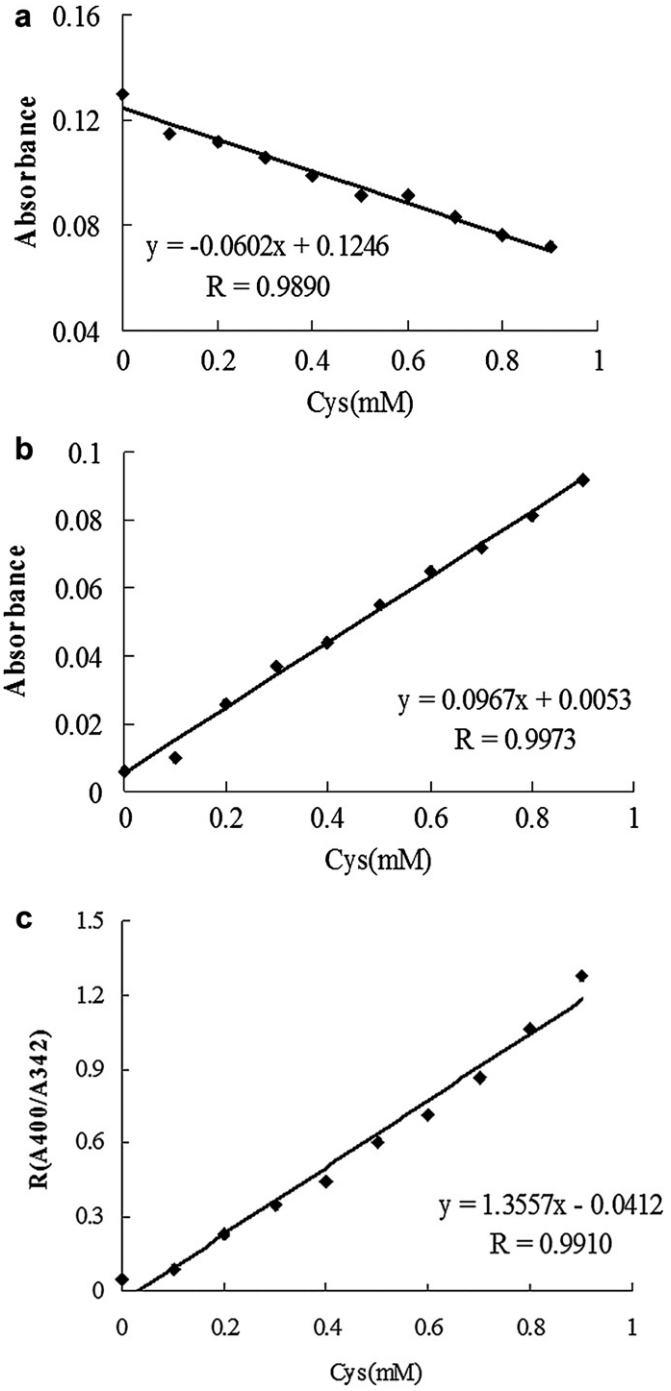

Fig. 5. The plot of absorbance at 342 nm(a), 400 nm(b) and R (A_{400}/A_{342})(c) *vs* concentration of Cys (final concentration: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 mM).

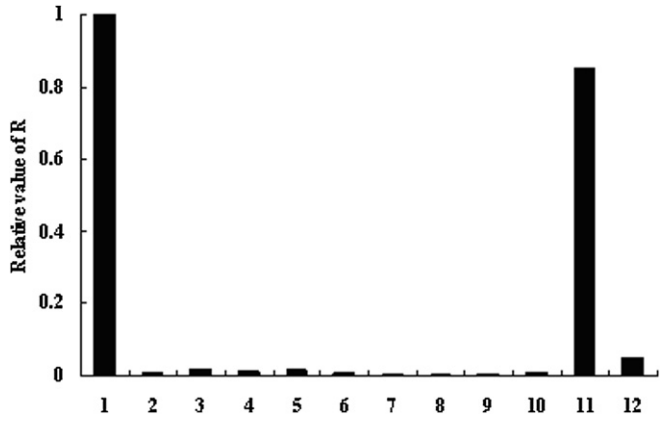

Fig. 6. The relative value of *R* of **1** in the presence of Cys and other amino acids (360 μM) in a mixture of ethanol and HEPES (7:3, v/v, pH 7.3). The relative value of *R* is normalized to Cys (1). Cys (1), Gly (2), Leu (3), Ala (4), Glu (5), Lys (6), Thr (7), Val (8), Pro (9), GSH (10) Hcy (11) and Hcy (12)(final concentration: 24 μM). Each spectrum was acquired 2 h after various analyses addition at 25 °C.

was especially recorded and the results were showed in Fig. 3. The experimental results showed that probe **1** requires at least 2 h reaching the stable absorption. All samples were equilibrated for at least 2 h before taking any measurement.

3.4. The effect of Cys on 1

Moreover, the effect of Cys on **1** was studied. The absorbance at 342 nm, 400 nm and the ratio of the absorbance at 400 nm and 342 nm, *R* (A_{400}/A_{342}) linearly changed upon the gradual addition of Cys (Fig. 4). These linear relationships allowed the detection of Cys in the range of 0–0.9 mM by both normal and ratiometric absorption spectrometry methods (Fig. 5). More importantly, this work range covers the physiological level of Cys (240–360 μM) in normal organisms [21]. Additionally, the minimum detectable concentration of Cys based on the color change by naked-eye was evaluated, and this concentration is approximately 50 μM (Fig. 4). Therefore, **1** can be potentially used for the detection of Cys by naked-eye.

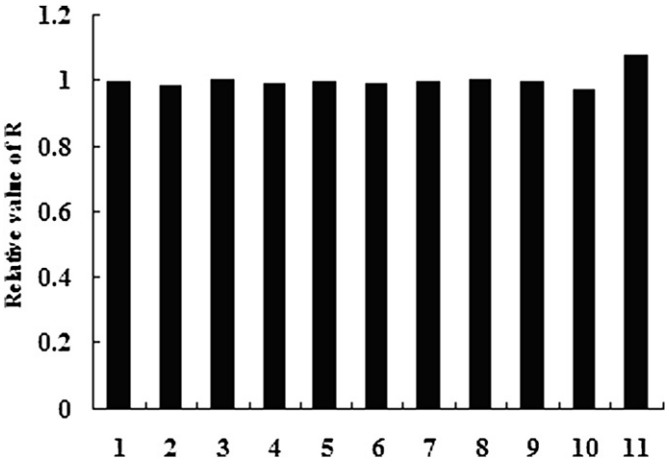

Fig. 7. The relative value of *R* (A_{400}/A_{342}) of **1** (10 μM) toward Cys (360 μM) in the absence (1) and presence of other amino acids (final concentration: 360 μM) in a mixture of ethanol and HEPES (7:3, v/v) solution at pH 7.3. The relative value of *R* is normalized to Cys (1), Gly (2), Leu (3), Ala (4), Glu (5), Lys (6), Thr (7), Val (8), Pro (9), GSH (final concentration: 0.5 mM) (10) and Hcy (final concentration: 24 μM) (11). Each spectrum was acquired 2 h after various analyses addition at 25 °C.

Table 1
The results for determination of Cys (300 μM) in synthetic samples. Each amino acid (300 μM) was added in a mixture of ethanol and HEPES (7:3, v/v) solution at pH 7.3, especially, the concentration of homocysteine is 12 μM and glutathione is 1 mM.

Cys in sample (μM)	Foreign substances	Found (μM)	Recovery (%)	RSD ($n = 3$) (%)
300.00	Glycine, Leucine, Alanine, Glutamic acid	290.67	96.89	1.68
300.00	Lysine, Threonine, Proline, Valine	293.73	97.91	1.73
300.00	Glycine, Leucine, Alanine, Homocysteine	293.85	97.75	1.54
300.00	Lysine, Threonine, proline, Glutathione	270.24	90.08	1.62

3.5. The specifity of the Cys probe

Next, the specificity of the probe **1** toward Cys was investigated. As shown in Fig. 6, remarkable changes of *R* (A_{400}/A_{342}) were observed for Cys and Hcy, and insignificant change was also observed for GSH. The results consist with the conclusion reported by Li and Huang et al. [6]. However, addition of 24 μM Hcy (healthy plasma total Hcy concentrations [46]) did not induce obvious change of *R* (A_{400}/A_{342}).

The effect of interference of other relative amino acids on monitoring Cys was also studied (Fig. 7). All results showed that **1** possesses high selectivity toward Cys in the presence of other amino acids. The results showed that this probe can be a good probe for Cys.

3.6. Analysis of synthetic samples

The Cys in synthetic samples containing some foreign substances was determined according to the above assay procedure and the experimental results are listed in Table 1. The recoveries of Cys in synthetic samples are 90.08%–97.75%, and the results are satisfied.

3.7. The reaction mechanism

To understand the reaction mechanism of **1** in sensing Cys, a mixture of *N*-ethylmaleimide (NEM, a known thiol-blocking

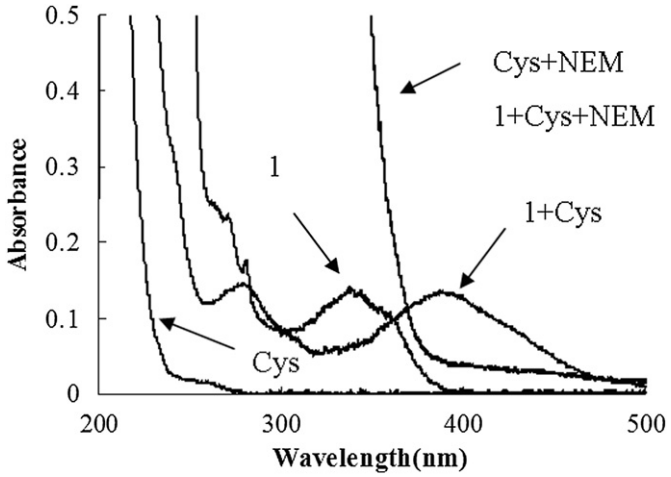

Fig. 8. The absorption spectra of **1** (10 μM) in the absence and presence of Cys (5 mM) and NEM (10 mM) in a mixture of ethanol and HEPES (7:3, v/v, pH 7.3) solution. Cys solution was pretreated with NEM for 2 h, and then 1 was added. Each spectrum was acquired 2 h after various analyses addition at 25 °C.

agent) and Cys was added to the solution of **1**, as expected, no obvious changes in the absorption spectra was observed (Fig. 8), implying the reaction of **1** to thiol of Cys. Next, to further confirm the reaction of **1** with Cys by the S_NAr^{Br}, the product of S_NAr^{Br} between 2-aminoethanethiol (to facilitate the purification of thiol adduct, 2-aminoethanethiol substituted for Cys.) and **1** was isolated and characterized by ^{1}H-NMR, ^{13}C-NMR, and HRMS [47]. Thus, combined with the previous study [45], a possible mechanism was proposed as shown in Scheme 1.

4. Conclusions

In summary, we have described a new probe molecule which exhibits excellent Cys-selectivity over GSH and other amino acids, and a 58 nm red-shift of absorption spectrum accompanied with the color change from colorless to yellow upon reaction with Cys. The probe could be used for the quantification of Cys by both normal and ratiometric absorption spectrometry methods with the working range covering the physiological level of Cys in normal organisms, and also by "naked-eye" with a minimum detectable concentration of approximately 50 μM. These present results may provide a useful approach for the development of colorimetric probe for Cys and other thiol-containing amino acids, especially for their ratiometric detection.

Acknowledgments

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[47] ^{1}H-NMR (500 MHz, $CDCl_3$) δ ($*10^{-6}$): 0.97(t, $J = 7.0$ Hz, 3H), 1.42-1.44(m, 2H), 1.61-1.68(m, 2H), 3.03(s, 2H), 4.13(s, 2H), 4.27(s, 2H), 7.74(t, $J = 8.0$ Hz, 1H), 8.55(t, $J = 8.8$ Hz, 1H), 8.67(d, $J = 6.5$ Hz, 1H), 9.19(s, 1H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ ($*10^{-6}$): 13.81, 20.33, 30.10, 38.74, 40.32, 48.60, 112.47, 123.48, 125.89, 129.04, 131.12, 132.33, 132.85, 133.96, 150.26, 162.43, 163.48. HRMS (ESI positive) calcd for $C_{18}H_{20}N_3O_4S$ $[M + H]^+$ 374.11690, found 374.11712; $C_{18}H_{19}N_3NaO_4S$ $[M + Na]^+$ 396.09885, found 396.09903.