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A colorimetric and fluorescent chemodosimeter for discriminative and simultaneous quantification of cysteine and homocysteine

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ABSTRACT

A highly selective, visible-light-excited and OFF–ON fluorescent chemodosimeter **1** employed conjugate addition/cyclization sequence mechanism, was designed and synthesized to discriminatively detect cysteine (Cys) and homocysteine (Hcy). The addition of Cys and Hcy resulted in the color of the solution of **1** changing from colorless to green under the simulation of physiological condition, and **1** could serve as a "naked-eye" indicator. Our chemodosimeter can detect Cys and Hcy quantitatively by fluorescence spectrometry method with a detection limit of 0.5 μM (for Cys) and 0.8 μM (for Hcy). To the best of our knowledge, **1** is the first visual and visible-light-excited fluorescent indicator for discriminative and simultaneous detection of Cys and Hcy. Furthermore, the mechanism of the reaction between **1** and Cys was confirmed using ESI-MS and fluorescence spectra.

1. Introduction

Amino acids are of significant importance to the physiological processes [1]. Among them, the thiol-containing amino acids, such as cysteine (Cys) and homocysteine (Hcy), play crucial roles in maintaining the biological redox homeostasis for their participation in the process of reversible redox reactions, and their levels have been directly linked to some diseases and cancers [2–6]. For example, a depressed level of Cys is associated with slowed growth, hair depigmentation, edema, lethargy, liver damage, muscle and fat loss, skin lesions, and weakness [7–9], and an elevated level of Hcy is a risk factor for Alzheimer's and cardiovascular diseases [10–12]. Thus, the selective and quantitative detection of Cys and Hcy is very important.

Among the various reported analytical techniques for the detection of Cys and Hcy, fluorescent chemodosimeters are widely developed due to operational simplicity and high sensitivity [13–23]. However, up to now, few reports on the highly selective fluorescent chemodosimeters for Cys and/or Hcy have been published [24–27]. Very recently, based on the kinetic differences in the intramolecular cyclization reactions, Yang and Strongin reported the first conjugate addition/cyclization sequence chemodosimeter for the selective and simultaneous quantitative detection of Cys and Hcy [11]. The use of the long excitation and emission wavelength may eliminate or decrease background interference from biological samples and its damage to living cells. In addition, colorimetric chemodosimeters are widely developed because they have the capability to detect analytes by naked-eye, without the aid of any advanced instruments [28–34]. Therefore, colorimetric and long wavelength fluorescent chemodosimeters for the selective and simultaneous determination of Cys and Hcy become our target.

Herein, we present the design, synthesis and properties of a highly selective colorimetric and visible-light-excited fluorescent chemodosimeter for simultaneous determination of Cys and Hcy. Chemodosimeter **1** is composed of a latent fluorescein fluorophore and a receptor of acrylate (Scheme 1), and it becomes highly fluorescent upon the spirolactone-opening reaction by Cys and Hcy, accompanied with the color changing from colorless to green.

2. Material and methods

2.1. General

All the chemicals used in this paper were commercial products of analytic grade. ^{1}H NMR and ^{13}C NMR spectra were taken on

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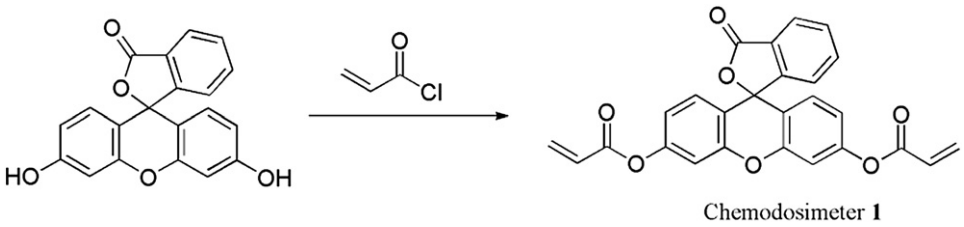


Scheme 1. Synthesis of chemodosimeter **1**.

a Bruker AMX400 spectrometer. Chemical shifts (δ) were reported in ppm relative to a Me_4Si standard in $CDCl_3$. Electrospray ionization (ESI) mass spectra were measured with an LC-MS 2010A (Shimadzu) instrument. Absorption spectra were recorded on UV-3101PC spectrophotometer. Fluorescence emission spectra were measured on Perkin–Elmer Model LS-55. All pH measurements were made with a Sartorius basic pH-meter PB-10.

2.2. Synthesis of chemodosimeter *1*

To a solution of fluorescein (332.3 mg, 1 mmol) and triethylamine (303.5 mg, 3 mmol) in anhydrous CH_2Cl_2 (20 mL), acryloyl chloride (200.2 mg, 2.2 mmol) mixed with 3 mL anhydrous CH_2Cl_2 were added dropwise at −10 °C. And then, the resulting mixture was allowed to stir at room temperature for 8 h. After removal of solvent, the residues were purified by silica gel column chromatography using dichloromethane as eluent to afford pure product. ^{1}H NMR (400 MHz, $CDCl_3$) δ (*10^{-6}): 6.06(d, J = 10.4 Hz, 2H), 6.28–6.35(m, 2H), 6.63(d, J = 17.2 Hz, 2H), 6.86(t, J = 9.8 Hz, 4H), 7.15(s, 2H), 7.21(d, J = 7.6 Hz, 1H), 7.63–7.72(m, 2H), 8.04(d, J = 7.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (*10^{-6}): 81.66, 110.38, 116.55, 117.72, 124.08, 125.24, 126.13, 127.50, 128.97, 130.07, 133.31, 133.37, 135.30, 151.60, 152.00, 152.93, 163.88, 163.91, 169.11, 169.14. ESI-MS calcd for $C_{26}H_{17}O_7$ $[M + H]^+$ 441.1, found 441.1.

3. Results and discussion

3.1. Characteristic spectrum

In this paper, the spectral responses of chemodosimeter **1** (2 μM) toward Cys and Hcy were investigated in a mixture of ethanol and water (2:3, v/v) solution buffered at pH 7.4 (phosphate buffer, 20 mM).

As shown in Fig. 1, the obvious absorption and fluorescence band of the solution of free **1** were not observed. Addition of Cys (16 μM) and Hcy (20 μM) to the solution of **1** resulted in a new absorption band centered at around 495 nm, accompanied with the color of the solution of **1** changing from colorless to green (Fig. 1a). Additionally, in the fluorescence spectra (Fig. 1b), a remarkable fluorescent enhancement at around 520 nm was observed in the presence of Cys or Hcy. The results imply that spirolactone-opening reaction was generated by Cys and Hcy (Scheme 2).

3.2. Effects of reaction time on sensing Cys and Hcy

Reaction time is an important factor for chemodosimeters and the time required for reaction of **1** with Cys and Hcy at 25 °C were investigated. As shown in Fig. 2, the fluorescence intensity increases with reaction time and then almost levels off at reaction time greater than about 50 min and 400 min for Cys and Hcy, respectively. So, assay time of 60 min and 420 min were selected for the quantification of Cys and Hcy. Furthermore, we have investigated the kinetics of fluorescence enhancement for Cys and Hcy at different concentrations (5, 10, 15, 20, 25 μM). The result showed the second-order rate constant for Cys is 25-fold faster than that for Hcy. The results are in good agreement with the conclusion reported by Yang and Strongin [11], and these allowed the discrimination of Cys and Hcy based on their different relative rates of intramolecular cyclization.

3.3. Quantification of Cys and Hcy

The subsequent addition of Cys to the solution of **1** elicited a progressive increase of fluorescence band centered at around 520 nm. Moreover, there was a good linearity between the

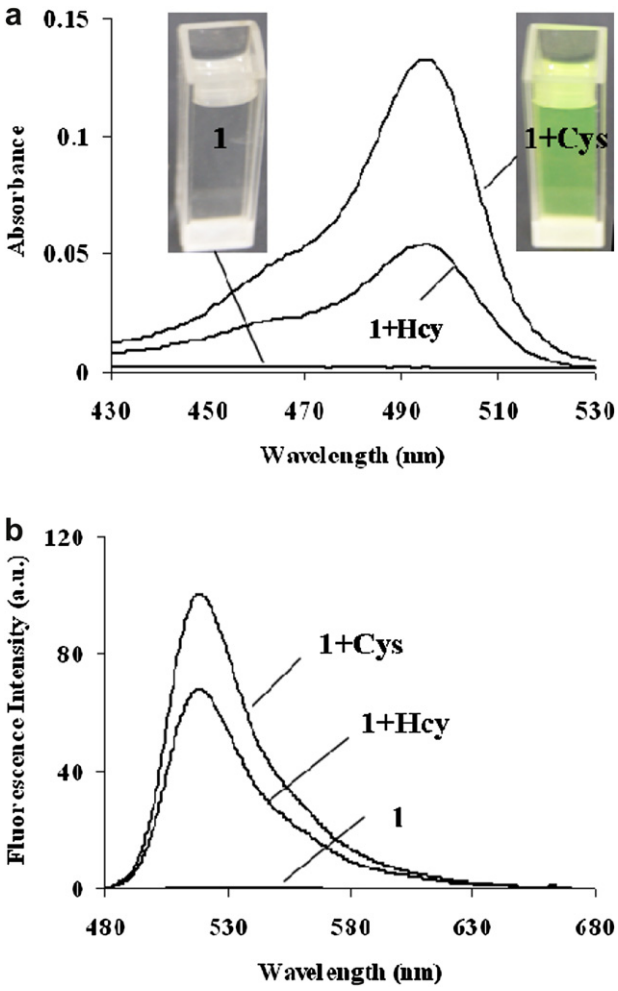
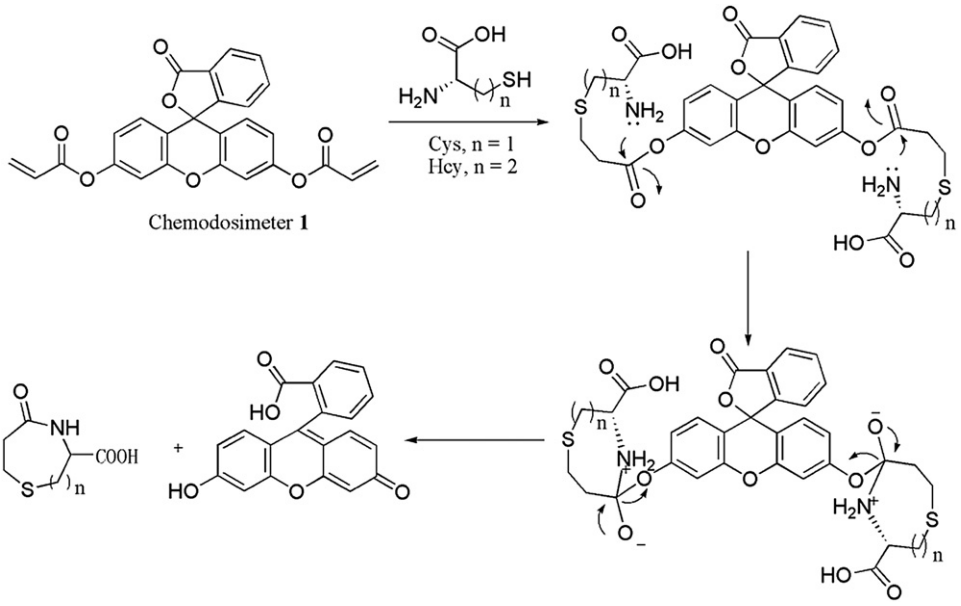


Fig. 1. Absorbance (a) and fluorescence (b) spectra of **1** (2 μM) upon reaction with Cys (16 μM) and Hcy (20 μM) in a mixture of ethanol and water (2:3, v/v) solution buffered at pH 7.4 (phosphate buffer, 20 mM). Inset photos are the photographs of the solution of **1** in the absence (left) and presence (right) of Cys. For Cys, its spectra were acquired 60 min after Cys addition at 25 °C. For Hcy, its spectra were acquired 420 min after Hcy addition at 25 °C.



Scheme 2. A proposed reaction mechanism of chemodosimeter **1** with Cys and Hcy.

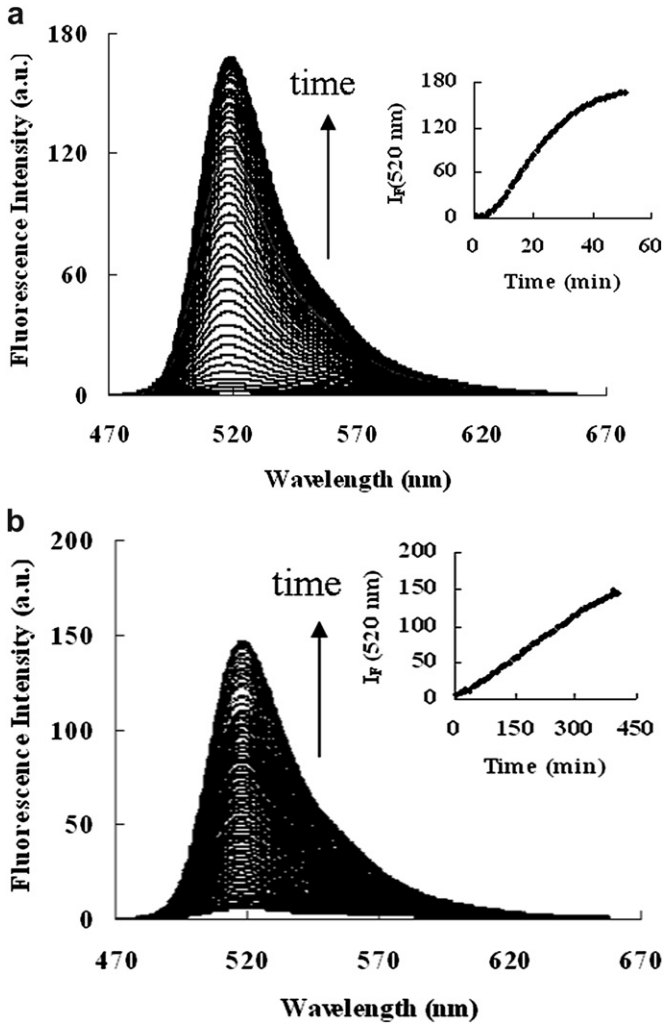


Fig. 2. The fluorescence spectra of **1** (2 μM) after the addition of Cys (25 μM) (a) and Hcy (25 μM) (b) during different time in a mixture of ethanol and water (2:3, v/v) solution buffered at pH 7.4 (phosphate buffer, 20 mM), $\lambda_{ex} = 450$ nm.

fluorescence intensity and the concentrations of Cys in the range of 2–25 μM with a detection limit of 0.5 μM (Fig. 3a). Similarly, the observed fluorescence intensity is proportion to the concentrations of Hcy in the range of 0–20 μM with a detection limit of 0.8 μM (Fig. 3b). These results implied that chemodosimeter **1** might detect Cys and Hcy qualitatively and quantitatively by the fluorescence spectrometry method.

To discriminate Cys and Hcy, the fluorescence spectra were determined after the addition of Cys and Hcy for 60 min and 420 min, respectively. The results show that chemodosimeter **1** could allow the discrimination and quantification of Cys and Hcy (Fig. 3c).

3.4. Selectivity to Cys and Hcy

The effects of some relative amino acids, such as Cys, Hcy, glutathione (GSH), glycine (Gly), leucine (Leu), alanine (Ala), glutamine (Glu), lysine (Lys), threonine (Thr), valine (Val), and praline (Pro), on fluorescence spectra of **1** were investigated. Remarkable changes were observed for Cys and Hcy (Fig. 4). Also, the effects of interference of other relative amino acids on monitoring Cys and Hcy were studied (Fig. 4). GSH obviously disturb the detection of Cys and Hcy for its addition reaction with carbon–carbon double bond of chemodosimeter **1**. Further, the effects of GSH on monitoring Cys and Hcy in the presence of excess chemodosimeter **1** were investigated. The primary experimental results showed that the interference of GSH may be ignored. Therefore, chemodosimeter **1** possesses high selectivity toward Cys and Hcy in the presence of other amino acids.

3.5. Mechanism of *1* in sensing Cys and Hcy

To confirm the reaction of **1** with Cys and Hcy by conjugate addition/cyclization sequence, a mixture of *N*-ethylmaleimide (NEM, a known thiol-blocking agent) and Cys was added to the solution of **1**, no obvious change in the fluorescence spectra was observed (Fig. 5), implying the addition reaction of **1** with thiol of Cys. Next, to demonstrate the reaction of **1** with Cys by the subsequent cyclization reaction, addition of thioglycolic acid (TA) did not elicit a remarkable fluorescence change. However, addition

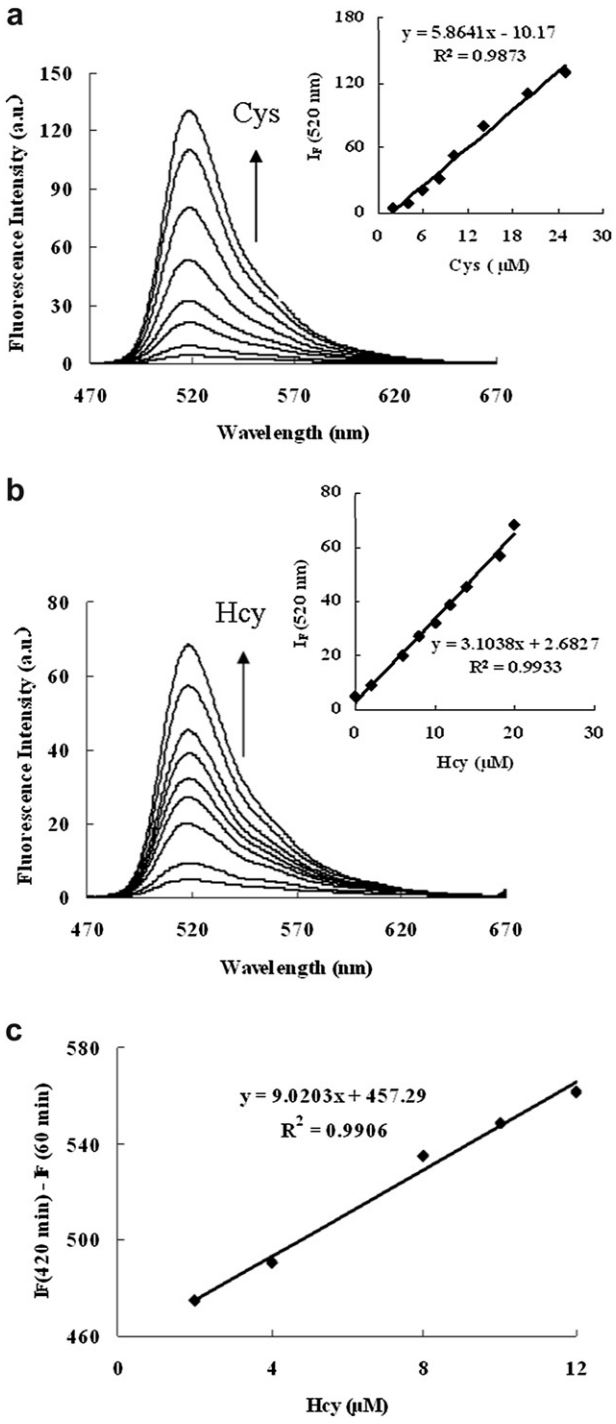


Fig. 3. (a) The fluorescence responses of **1** (2 μM) toward different concentrations of Cys (final concentration: 2, 4, 6, 8, 10, 14, 20, 25 μM) in a mixture of ethanol and water (2:3, v/v) solution buffered at pH 7.4 (phosphate buffer, 20 mM), λ_{ex} = 450 nm. Inset is the plot of fluorescence intensity at 520 nm *vs* concentration of Cys. Each spectrum was acquired 60 min after Cys addition at 25 °C. (b) The fluorescence responses of **1** (2 μM) toward different concentrations of Hcy (final concentration: 0, 2, 6, 8, 10, 12, 14, 18, 20 μM) in a mixture of ethanol and water (2:3, v/v) solution buffered at pH 7.4 (phosphate buffer, 20 mM), λ_{ex} = 450 nm. Inset is the plot of fluorescence intensity at 520 nm *vs* concentration of Hcy. Each spectrum was acquired 420 min after Hcy addition at 25 °C. (c) The fluorescence responses of **1** (40 μM) in the presence of 25 μM Cys toward different concentrations of Hcy (final concentration: 2, 4, 8, 10, 12 μM) in a mixture of ethanol and water (2:3, v/v) solution buffered at pH 7.4 (phosphate buffer, 20 mM), λ_{ex} = 450 nm. The plot of the changes of fluorescence intensity at 520 nm from 60 min to 420 min *vs* concentration of Hcy.

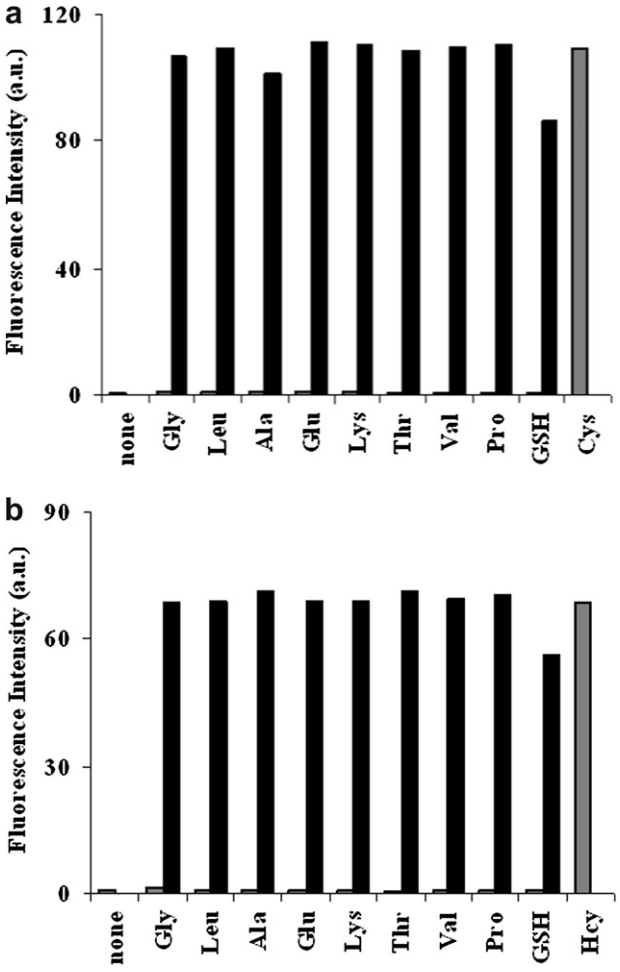


Fig. 4. The fluorescence responses of **1** (2 μM) toward Cys (a) and Hcy (25 μM) (b) in the absence and presence of other amino acids (final concentration: 25 μM, GSH: 5 μM) in a mixture of ethanol and water (2:3, v/v) solution buffered at pH 7.4 (phosphate buffer, 20 mM), λ_{ex} = 450 nm.

of 2-aminoethanethiol (TEA), as expected, resulted in a clear fluorescence enhancement. Furthermore, the reaction products of **1** with Cys were subjected to electrospray ionization mass spectral analyses. The peak at *m*/*z* 331 corresponding to the fluorescein was observed. Therefore, a proposed mechanism was proposed as shown in Scheme 2.

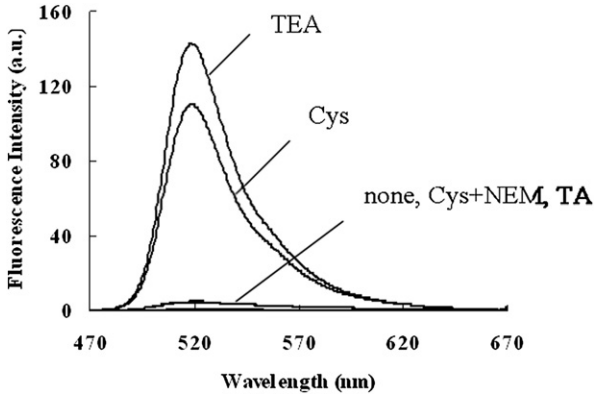


Fig. 5. The fluorescence spectra of **1** (2 μM) in the absence and presence of Cys (25 μM) thioglycolic acid (TA) (25 μM), 2-aminoethanethiol (TEA) (25 μM), and a pretreated solution of Cys (25 μM) with NEM (1 mM) in a mixture of ethanol and water (2:3, v/v) solution buffered at pH 7.4 (phosphate buffer, 20 mM), λ_{ex} = 450 nm.

4. Conclusions

In summary, we have presented the synthesis and properties of a highly selective visible-light-excited fluorescent chemodosimeter **1** for discrimination and simultaneous quantification of Cys and Hcy. The color of the solution of **1** changing from colorless to green and remarkably enhanced fluorescence upon reaction of Cys and Hcy were observed, and **1** could serve as a "naked-eye" indicator for Cys and Hcy. Furthermore, **1** can detect Cys and Hcy quantitatively in the range of 2–25 μM (for Cys) and in the range of 0–20 μM (for Hcy). Finally, the mechanism of **1** on sensing Cys and Hcy was confirmed using ESI-MS and fluorescence spectra.

Acknowledgments

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