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Highly selective fluorescent chemosensor with red shift for cysteine in buffer solution and its bioimage: symmetrical naphthalimide aldehyde

Liping Duan, Yufang Xu, Xuhong Qian *, Fang Wang, Jianwen Liu, Tanyu Cheng

Shanghai Key Laboratory of Chemical Biology, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China

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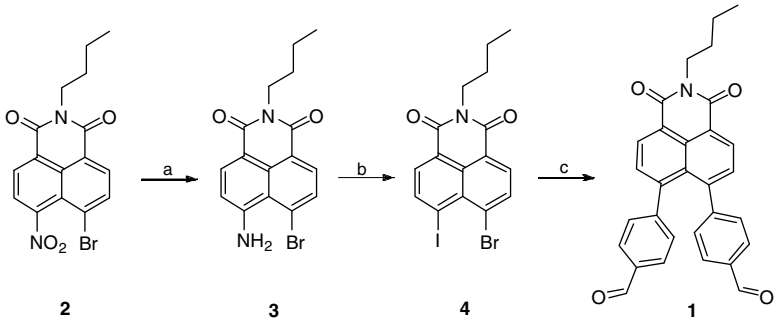
ABSTRACT

A new fluorescent probe **1**, *N*-butyl-4, 5-(*p*-aldehyde)phenyl-1,8-naphthalimide, was designed and synthesized for the determination of the cysteine (Cys). Upon addition of Cys, the emission of **1** was enhanced with about 25 nm red-shift in the emission maximum (from 455 to 480 nm), accompanied with the fluorescent color change from blue to cyan, which was attributed to the reaction of the aldehyde groups in **1** with cysteine to form very stable thiazolidines derivative. Compound **1** was highly selective for cysteine detection without the interference of other amino acids and can be used for bioimaging of Cys.

Cysteine deficiency causes many diseases such as slowed growth in childhood, depigmentation of hair, edema, lethargy, liver damage, and loss of muscle.[1–5] In light of the important roles of Cys in a variety of fundamental physicological processes in organisms, selective and sensitive detections for Cys have gained tremendous attentions. The determination of Cys is generally accomplished by high-performance liquid chromatography (HPLC) or high-performance capillary electrophoresis (HPCE).[6–12] There are also some examples of fluorescence quenching chemosensors and UV/vis spectroscopic response for Cys in organic solution.[13–16] However, there are a few chemosensors with fluorescence enhancement (FE) for Cys in organic solutions, and only two reports on fluorescence turn-on sensing and bioimaging of Cys in aqueous solutions.[17] Therefore, we stress to pay more attention to novel selective chemosensors with FE for Cys in aqueous solution.

Electron donor (D)–electron acceptor (A) interactions play a fundamental role in organic chemistry. Reactivities and physical properties of organic compounds largely depend on the strength of the D–A interactions. The guest-binding will induce changes in two-channel output signals (color change and fluorescence variation), which are convenient and sensitive for practical utilization, especially when the substituent in the D–π–A system was strongly electron-withdrawing or electron-acceptor. 4,5-Substituted-1,8-naphthalimide, a frequently used fluoroionophore, possesses desirable spectroscopic properties. In addition, the special reaction of aldehydes with N-terminal cysteines to form thiazolidines has



Scheme 1. Synthesis of **1**. Reagents and conditions: (a) $SnCl_2{\cdot}2H_2O$, AcOH, HCl (g), room temperature, yield: 90%; (b) $NaNO_2$, HCl, KI, −5 to 0 °C, yield: 48%; (c) *p*-aldehydephenylboric acid, $Pd(PPh_3)_4$, toluene, N_2, reflux, yield: 85%.

* Corresponding author.
E-mail address: xhqian@ecust.edu.cn (X. Qian).

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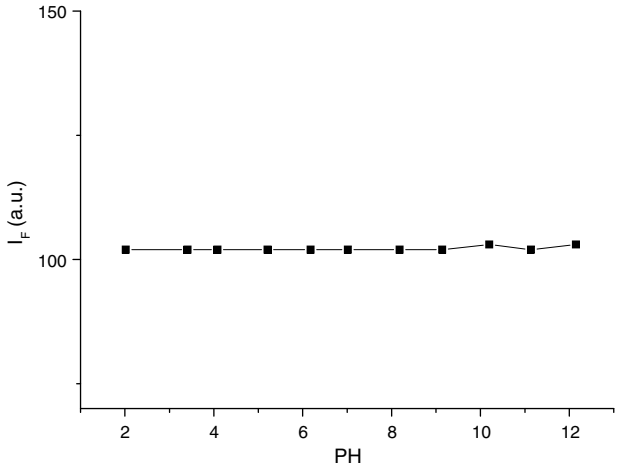


Figure 1. Influence of pH on the fluorescence of **1** in the ethanol–water (60:40, v/v) solution, IF = fluorescence intensity, excitation wavelength = 365 nm, emission wavelength = 455 nm, [**1**] = 10 μm.

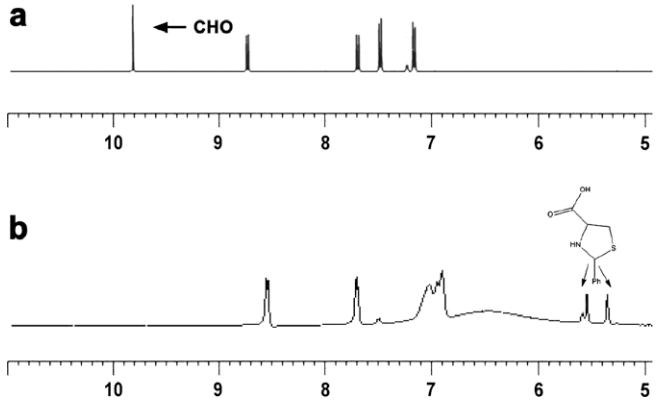


Figure 3. ^{1}H NMR spectra of **1** in DMSO-d_6 in the absence (a) and the presence of 40 equiv of Cys at 25 °C (b).

been used to label and immobilize peptides and proteins.[18] So we designed a simple and aqueous-soluble chemosensor **1**, which is composed of the naphthalimide fluorophore and the two aldehydes as Cys receptors.

From the starting material *N*-butyl-4-bromo-5-nitro-1, 8-naphthalimide,[19] through the intermediate *N*-butyl-4-bromo-5-amino-1.8-naphthalimide **3**[20] and *N*-butyl-4-bromo-5-iodo-1.8-naphthalimide **4**,[21] the target compound *N*-Butyl-4, 5-(p-aldehyde)phenyl-1,8-naphthalimide was obtained (Scheme 1);[22] its fluorescence quantum yield is 0.30 in absolute ethanol, which was determined by using quinine sulphate as standard.

The fluorescence of sensors is usually disturbed by proton in the detection of metal ions, so their low sensitivity to pH is desired. The effect of pH on the fluorescence of **1** was determined in ethanol–water (60:40, v/v) solution. The emission spectra of **1** have no

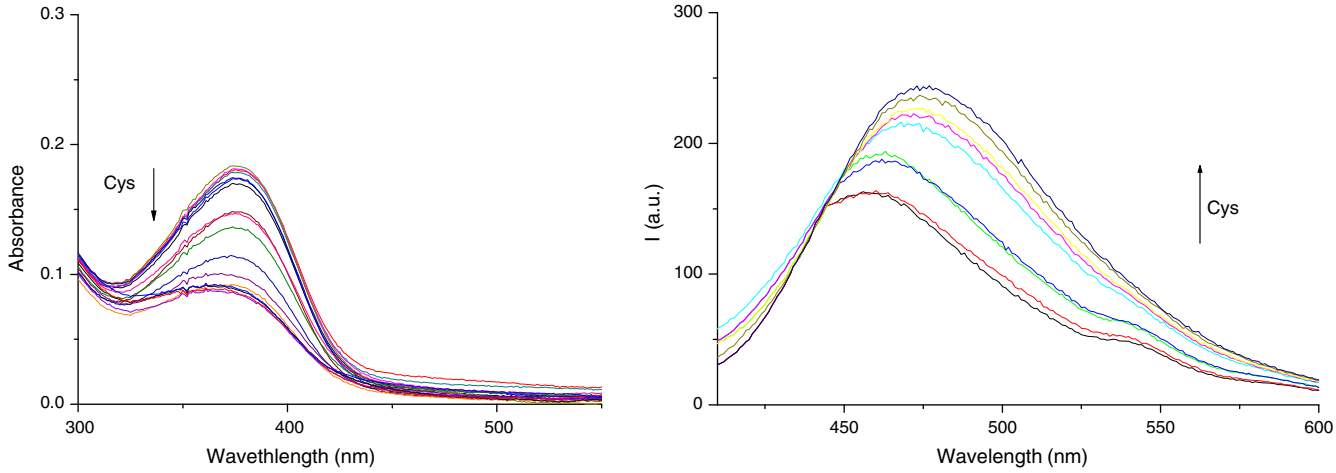
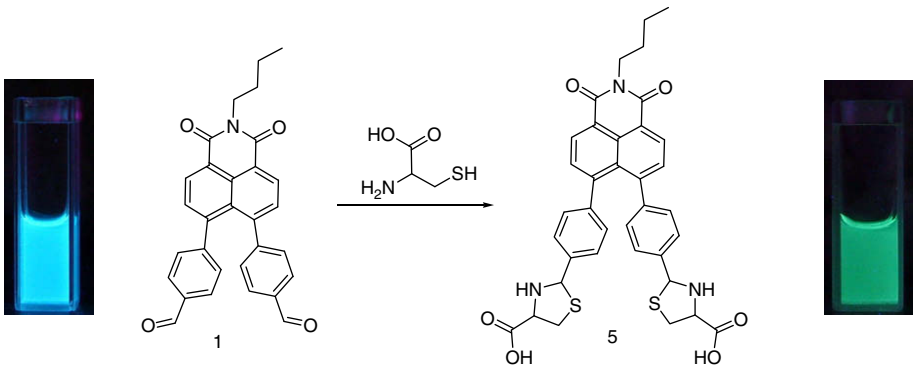


Figure 2. UV–vis and emission spectra of **1** (10 μm) with the addition of Cys (0–400 μm) in a mixed solution of ethanol and water (60:40, v/v) at pH 7.2 maintained with HEPES buffer (50 mM) at 25 °C. λ_{EX} = 375 nm.



Scheme 2. The change in fluorescent color and possible reaction.

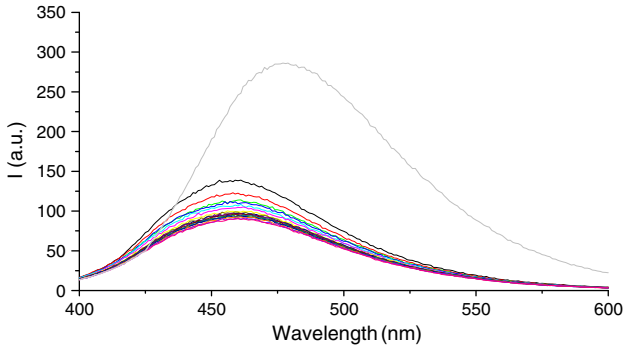


Figure 4. Fluorescence spectra of **1** (10 μm) in a mixed solution of ethanol and water (60:40, v/v) at pH 7.2 maintained with HEPES buffer (50 mM) in the presence of different amino acids (40 equiv) at 25 °C, and nearly no response to other amino acids detected (alanine, arginine, asparagine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, proline, serine, threonine, tyrosine, and valine).

obvious change between pH 12.0 and 2.00 (Fig. 1). So the detections of metal cations were operated in the ethanol–water (60:40, v/v) solution at pH 7.2 maintained with HEPES buffer (50 mM).

The resulting absorption and fluorescence spectra are shown in Figure 2. Upon the addition of Cys to the probe solution, the absorbance at 375 nm decreased and the maximum of the absorption peak shifted to the shorter wavelength (to 370 nm). At the same time, its emission spectra also displayed a significant change (Fig. 2): the fluorescence intensity increased with about 25 nm red-shift in emission band (from 455 to 480 nm), accompanied with a fluorescent color change from blue to cyan. The fluorescence quantum yield of **1** increased from 0.25 to 0.40.

Compound **1** is a A–F–A′ π–π conjugation system (A—electron accepting group, imide; F—aromatic fluorophore; A′—electron accepting group, phenyl formaldehyde) due to the electron pull effects of imide group and phenyl formaldehyde. When **1** reacts with Cys to form compound **5**, the π–π conjugation will transform to A–F–D π–π conjugation system (A—electron accepting group, imide; F—aromatic fluorophore; D—electron donating group, alkyl substituent with sulfur and amino group) because of the slight electron push effect of alkyl group. Therefore, the push–pull effect is more efficient for compound **5**, which leads to higher fluorescence yield accompanied with red-shift in emission spectrum.

Similar results were obtained when Cys was added to 0.1 M carbonate buffer solution of **1**, which revealed that the Cys detection could be operated in aqueous solution.

The ^{1}H NMR experiments were carried out to confirm the formation of thiazolidine derivative **1**-Cys (Fig. 3). In neutral condition, when excess Cys (in DMSO) was added into solution of **1**, the aldehyde resonance peak (9.84 ppm) of **1** disappeared after 5 min and two new equal peaks centered at 5.63 and 5.35 ppm appeared, which could be assigned to the methine protons of the thiazolidine diastereomer.[15a] These results indicated that thiazolidine was formed by the interaction of aldehyde with Cys.

A possible mechanism was proposed as shown in Scheme 2. When Cys was added, the CHO group reacted with Cys to form thiazolide (compound **5**).

Selectivity is an important issue in the probe application of amino acids detection. The effects of some relative amino acids on the fluorescence spectra of **1** were determined. As shown in Figure 4, the emission spectra of **1** in a mixed solution of ethanol and water (60:40, v/v) at pH 7.2 maintained with HEPES buffer (50 mM) were also investigated with the addition of other amino acids. The results showed that upon addition of other amino acids, slight fluorescence quenching was observed without any color change, which indicated that the CHO group only reacted with Cys, which was the key for the selective recognition of Cys. Thus, we confirmed that this new probe offers good selectivity.

We also investigated the living cell bioimaging of Cys by using DMSO–PBS(1:80, v/v, pH 7) as a staining medium. V79 cells were used for the evaluation of **1** as potential cell imaging agent for Cys, which were maintained as exponentially growing suspension cultures in Eagle's minimal essential medium with Earle's salts, modified for suspension cultures with 7.5% fetal calf serum. After incubation with **1** for 2 h, it showed an intense intracellular fluorescence (**1** was added to cell suspension to give the appropriate concentration at 10 μM) (Fig. 5).

In summary, an aqueous soluble fluorescent chemosensor **1** for recognition of Cys was designed and synthesized. It showed high selectivity for Cys by fluorometric enhancement with red-shift in aqueous solution. Moreover, fluorescence images indicate that **1** can be used for bioimaging of Cys in living cell.

Acknowledgments

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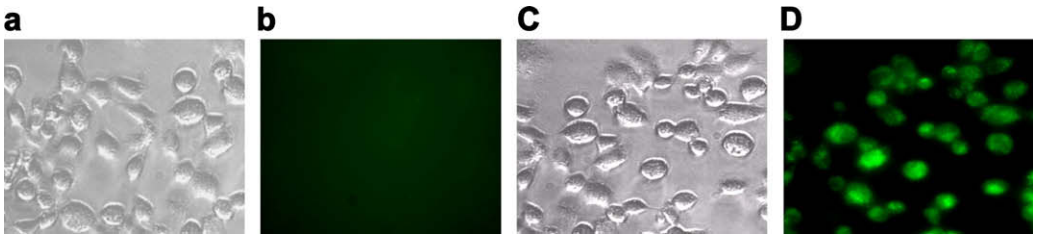

Figure 5. Fluorescence images of V79 cells incubated with(c, d) or without (a, b) 10 μM compound **1** at 37 °C After 2 h incubation, scanning was taken. Magnification was 1000×. (a) Brightfield image of cells; and (b) excited in 365 nm, no obvious fluorescence was observed; (c) scanning was taken on brightfield; (d) excited at 365 nm.

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20. *Preparation of N-butyl-4-bromo-5-amino-1.8-naphthalimide* (**3**): Compound **2** (3.76 g, 0.01 mol) was added to a previously prepared solution of glacial acetic acid (40 ml) containing 15.3 g of $SnCl_2$ (0.08 mmol) and aerated with hydrogen chloride. The reaction mixture was stirred for 1 h. The precipitated solid was collected by filtration and washed with water. Crystallization from acetic acid gave **4** as yellow needles: 3.42 g (98.8%), mp 210.5–211.2 °C, IR (KBr) *v*: 3460, 3370, 1700, 1650, 1630, 1560, 1420, 1280, 1150, 1030. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.975 (t, J = 7.2 Hz, 3H), 1.43–1.45 (m, 2H), 1.70–1.75 (m, 2H), 4.14 (t, J = 7.2 Hz, 2H), 6.20–6.40 (br s, 2H), 6.85 (d, J = 8.0 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H), 8.34 (d, J = 8 Hz, 1H), 8.42 (d, J = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.83, 20.38, 30.15, 40.12, 112.12, 112.16, 117.52, 122.74, 125.46, 131.56, 131.79, 132.49, 134.23, 150.52, 163.61, 164.01, MS (ESI$^+$) $C_{16}H_{15}BrN_2O_2$ [M+H$^+$]: 374.0.
21. *Preparation of N-butyl-4-bromo-5-iodo-1.8-naphthalimide* (**4**): 30 mL of $NaNO_2$ (1.6 g, 0.023 mmol) aqueous solution was added dropwise to the HCl (22%, 25 mL) aqueous solution of **3** (3.5 g, 0.01 mol) at 0 °C. The reaction mixture was stirred for 2 h at this temperature. The resulting precipitate was added to 40 mL of KI (8.85 g, 0.05 mol) aqueous solution and the mixture reacted for 1 h, after which $NaHSO_3$ was added to destroy excessive I_2. The resulted solid was collected by filtration and then purified by column chromatography (SiO_2, $CHCl_3$) to give (**5**) as a white solid in 60% yield (2.7 g), mp 165.1–166.4 °C, IR (KBr) *v*: 2950, 1695, 1647, 1630, 1560, 1430, 1280, 1150, 1035 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.985 (t, J = 7.2 Hz, 3H), 1.42–1.47 (m, 2H), 1.69–1.75 (m, 2H), 4.16 (t, J = 7.2 Hz, 2H), 8.18 (d, J = 8.0 Hz, 1H), 8.25 (d, J = 8.0 Hz, 1H), 8.41 (d, J = 8 Hz, 1H), 8.72 (d, J = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.83, 20.29, 30.20, 40.36, 107.12, 125.87, 126.67, 128.99, 135.28, 136.66, 136.87, 139.15, 139.87, 141.65, 161.56, 163.86. MS (ESI) $C_{16}H_{13}BrINO_2$ [M+H$^+$]: 457.9.
22. *The preparation of N-butyl-4,5-(p-aldehyde)phenyl-1,8-naphthalimide* (**1**): To a solution of 46 mg (0.1 mmol) *N*-butyl-4-bromo-5-iodo-1,8-naphthalimide in 10 mL toluene under dry N_2, 45 mg (0.3 mmol) *p*-aldehydephenylboric acid and catalytic amounts of $Pd(PPh_3)_4$ were added. The mixture was then heated at reflux for 6 h at 110 °C and monitored by TLC. After the reaction was completed, the solvent was removed under reduced pressure. The crude product was then purified by column chromatography (SiO_2, $CHCl_3$) to give **1** as a white solid in 90% yield (40 mg). Mp 174–175 °C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.04 (t, J = 7.2 Hz, 3H), 1.47–1.55 (m, 2H), 1.75–1.82 (m, 2H), 4.26 (t, J = 7.2 Hz, 2H), 7.20 (d, J = 8.0 Hz, 4H), 7.52 (d, J = 8.0 Hz, 4H), 7.73 (d, J = 7.8 Hz, 2H), 8.76 (d, J = 7.8 Hz, 2H), 9.83 (s, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.83, 20.39, 30.20, 40.46, 123.12, 127.57, 128.99, 130.20, 130.36, 130.77, 131.65, 134.87, 145.15, 147.24, 163.86, 191.13. HRMS (ESI) calcd for $C_{30}H_{23}NO_4$ [M+Na$^+$]: 484.1511, found: 484.1511.