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ARTICLE TYPE

Glucosamine Modified Near-infrared Cyanine as a Sensitive Colorimetric Fluorescent Chemosensor for Aspartic and Glutamic Acid and its Application

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Abstract: A glucosamine modified near-infrared cyanine fluorescent probe has been synthesized and developed as an efficient and visual sensor for the trace level determination of glutamtic acid (Glu) and aspartic acid (Asp) with no interference by other amino acids. A prominent fluorescence enhancement at 805 nm and a remarkable color change from pale red to colorless were observed in the presence of Glu and Asp. Moreover, this probe may also serve as a highly sensitive acidic pH probe with a pKa value of 5.82. Furthermore, the chemosensor has been successfully applied for the imaging of Glu and Asp in living cells, and the cytotoxicity assay exhibited that it is of low toxicity under the experimental conditions.

Introduction

Aspartic acid (Asp) and glutamtic acid (Glu), major excitatory amino acids (EAAS), can activate N-methyl-D-aspartate (NMDA) reporters in the mammalian central nervous system (CNS). EAAS exist in more than half of CNS synapses and play crucial roles in memory, learning, movement disorders and drug additions as well as many other normal or abnormal physiological processes and behaviors [1]. The balance of Asp and Glu has an effect on brain function, and any alterations in such a balance may cause several neurological or psychiatric disorders [2,3]. The altered levels of EAAS in humans are also closely related to some pathologies, such as epilepsy [4], Parkinson's disease [5, 6], ischemic brain injuries [7, 8], and so on.

Thus, development of efficient methods for the selective detection and quantification of Asp and Glu in physiological media has been of intense interest. Up to date, many methodologies have been designed to detect them [9-11], such as liquid or gas chromatography, capillary electrophoresis, electrochemical or optical assays [1, 12, 13]. Among them, optical assays based on synthetic colorimetric and fluorescent probes have received increasing attention owing to their simplicity, high selectivity and sensitivity. A Co(II) complex with 2-(2-pyridyl) benzimidazole [1] and a polythiophene-gold nanoparticles composite [13] were discovered as probes for EAAS. Though considerable efforts have been made to develop fluorescent probes for the detection of EAAS, it remains a challenge to acquire new simple molecular-based EAAS sensors, which have properties of high sensitivity, good aqueous solubility, biocompatibility and quick response for real-time detection.

Near-infrared (NIR) fluorescent probes with long wavelength emission (650–900 nm) are preferred for biological imaging applications, because they can reduce background absorption and tissue autofluorescence, reduce photodamage to living cells and penetrate deeply with low light scattering to achieve a distinct image *in vivo* assay [14-16]. Herein, we designed and synthesized a NIR cyanine fluorescent probe as a chemosensor for EAAS, which bearing a non-N-alkylated indolium moiety as a recognition site of Glu and Asp. To improve the probe's solubility in aqueous media and reduce its aggregation in solution, glucosamine functional group was introduced [17]. In the absence of Glu and Asp, this probe (10^{-5} M) showed a very weak fluorescence, upon treatment with Glu or Asp, a prominent enhancement of fluorescence intensity accompanying with a remarkable color change from pale red to colorless were observed due to the protonation of indole nitrogen atoms. It was also found that the probe was sensitive to pH value. Moreover, the probe has been successfully applied for the detection of Glu and Asp in living cells. Cytotoxicity assay has confirmed that this probe is of low toxicity under the experimental conditions. Hence, this probe has potential application for detection of Glu and Asp or pH variations in living cells with a fluorescence turn-on signal.

Results and discussion

We have designed and synthesized a NIR fluorescent chemosensor for detection of Glu and Asp. To improved the probe's solubility and reduce its aggregation in solution, glucosamine functional group was introduced. The synthetic procedures of the probe were summarized in scheme1. Ethyl-3-(3, 3-dimethyl-2-methyleneindolinl-yl)-propionate (compound 2) was obtained by a multistep synthesis starting from phenyl hydrazine and 3-methyl-2-butanone and then modification by nucleophilic substitution reaction with ethyl-3-bromo propionate. 2-Chloro-1-formyl-3-(hydroxymethylene)-cyclohex-1-ene

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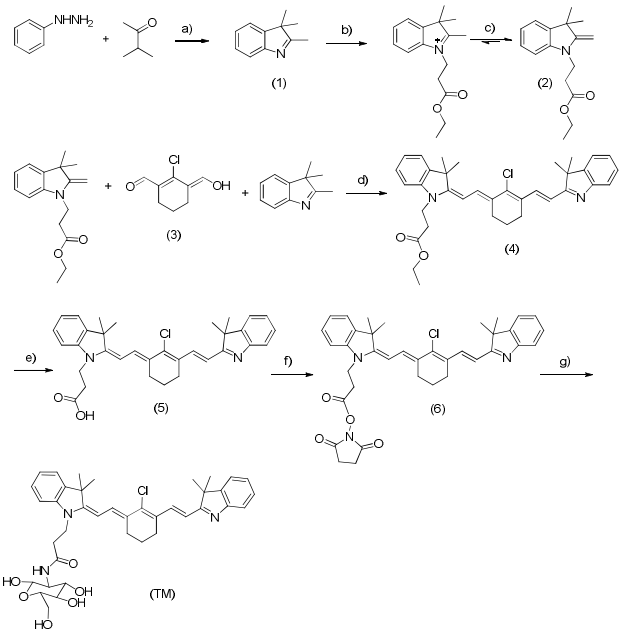
(compound 3) was prepared according to literature method [18]. Then a reaction of compound 2 and compound 3 in ethanol provided the compound 4. TM (the target compound) was prepared smoothly from compound 5 (the hydrolyzate of compound 4) and D-glucosamine hydrochloride through acylation reaction in a satisfactory yield. The structures of TM and intermediates were characterized by ^{1}H NMR, ^{13}C NMR and ESI-MS analysis.



Scheme 1. Synthetic scheme for the probe

Reagents and conditions: a) 100 ℃, 2 h, then acetic acid, reflux, 5 h; b) ethyl 3-bromo propionate, 100 ℃, 5 h; c) 1 M NaOH, r.t., 2 h; d) ethanol, reflux, 3 h; e) 2 M NaOH in ethanol, 50 ℃, 3 , N_2 atmosphere; f) EDC·HCl, NHS, DMF, ice bath, then r.t., 12 h, N_2 atmosphere; g) D-glucosamine hydrochloride, DIPEA, DMF, 5 h, N_2 atmosphere.

To investigate the sensitivity of probe to Glu and Asp, UV-Vis titration of probe (10^{-5} M) toward Glu and Asp (0 to 25 equiv) were measured firstly. As shown in Fig.1 (A and B), upon addition of EAAS, a new absorption peak at 780 nm progressively increased, concomitantly the absorption peak of probe at 520 nm gradually decreased. The absorbance reached maximum when the concentration of Asp and Glu is 5 equiv and 12 equiv, respectively. Meanwhile, the color of the solution changed from pale red to colorless, which indicated that this probe could serve as a visual indicator for Glu and Asp. Moreover, a further observation is the presence of an isosbestic point at 599 nm which indicated that only one species was produced upon the reaction of probe with EAAS.

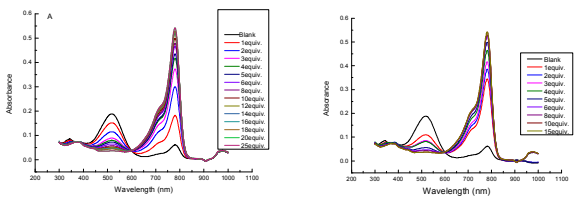


Fig.1 (A and B). Effect of Glu and Asp on UV-Vis absorption. (A) shows the UV-Vis absorption changes of probe upon the addition of Glu in ethanol-water (20/80, V/V) solution. [probe]=10^{-5} M, [Glu]=0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25 equiv, respectively. (B) shows the UV-Vis absorption changes of probe upon the addition of Asp at the similar condition.

Then, fluorescence emission spectra of probe in the presence of Glu and Asp at various concentrations were assessed (shown in Fig.2). As concentration of Glu or Asp increases, the fluorescence intensity at 805 nm enhanced prominently, and the color of solution changed from pale red to colorless. In order to understand how amino acid concentration affect on fluorescence intensity clearly, the fluorescence intensity at 805 nm were plotted as a function of the Asp and Glu concentration (Fig.3 A and B). The results exhibited that about 5 equiv of Asp and 12 equiv of Glu are required for the saturation of the fluorescence intensity under the titration conditions, respectively. This result was in good agreement with UV-Vis spectra's. Moreover, the probe with 805 nm (NIR) emission is of great potential for *in vivo* imaging due to the increased optical penetrability and minimal tissue autfluorescence in this region.

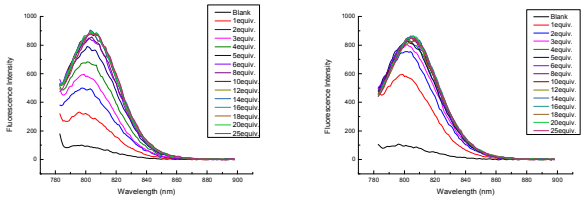


Fig.2 Fluorescence spectra (λ_{ex}=780 nm, λ_{em}=805 nm) of probe (10^{-5} M) in ethanol-water (20/80, V/V) solution. (a) In the presence of Glu (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25 equiv, respectively). (b) In the presence of Asp (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25 equiv, respectively).

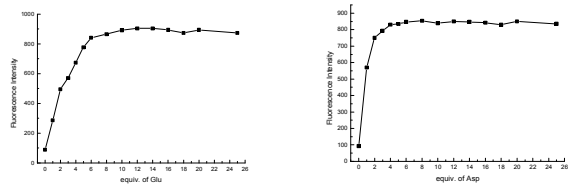


Fig.3 Fluorescence intensity at 805 nm vs. Asp and Glu concentration

To investigate the detection limit of probe for Glu and Asp, a typical fluorescence titration of Glu (10^{-6}-10^{-5} M) and Asp (10^{-7}-10^{-5} M) were supplemented using a 10^{-5} M solution of probe in ethanol-water (20/80, V/V) (Fig.4, A and B). The fluorescence intensity at 805 nm was plotted as a function of the Asp and Glu concentration (Fig.5). A noticeable change of fluorescence intensity still appeared though Glu and Asp are at concentration of 1×10^{-6} M and 1×10^{-7} M, rescepctively. This results strongly indicated that this probe could serve as a trace level determination method of Glu and Asp.

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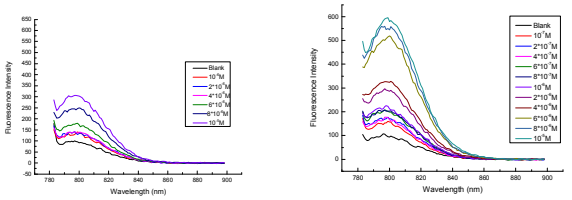


Fig.4 The fluorescence spectral of probe toward Glu (A) and Asp (B).

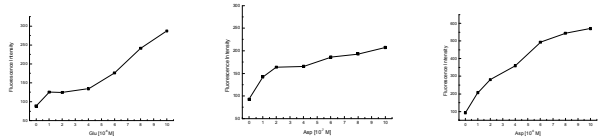


Fig.5 The fluorescence intensity at 805 nm vs. the concentration of Glu or Asp.

The selectivity of probe has been investigated with other natural amino acids over the concentration of 2×10^{-4} M (20 equiv). The UV-Vis and fluorescence spectra upon exposing to various amino acids are shown in Fig. 6 (A and B). The fluorescent response of probe to Pro, Leu, Trp, Ty, Gly, Asn, Val, Ala, Met, Gln, Ser, Phe, Thr, Ile and His exhibited no change, the fluorescence intensity of probe showed a slight enhancement with Cys, a little decrease with Arg, Lys. Interestingly, only when Glu or Asp was added to probe, a remarkable enhancement of fluorescent intensity at 805 nm and a prominent change of color were observed promptly. The fluorescent intensity variation at 805 nm and the color change were shown in Fig.7. These results suggest that the probe exhibits quick response and high selectivity toward Glu and Asp and can be used as a "naked eye" chemosensor for them without interference from other natural amino acids. To further explore the tolerance of probe to Glu and Asp over other natural amino acids, competitive experiments were performed in the presence of Glu and Asp (20 equiv) with other amino acids (10 equiv) respectively. The results indicated that the amplitude of Glu and Asp induced fluorescence enhancement was not interfered by other natural amino acids such as Pro, Leu, , Trp, Ty, Gly, Asn, Val, Ala, Met, Gln, Ser, Phe, Thr, Ile and His.

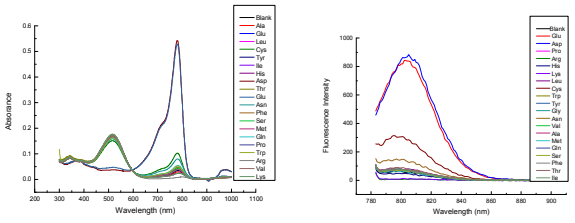
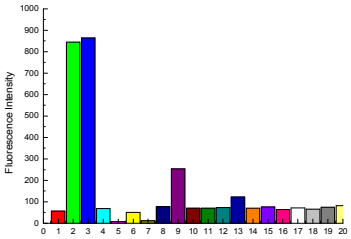


Fig.6 Effects of amino acids (20 equiv) on the UV-Vis spectra (A) and fluorescence spectra (λ_{ex}=780 nm, λ_{em}=805 nm) (B) of probe (10^{-5} M) in ethanol-water (20/80, V/V) solution.



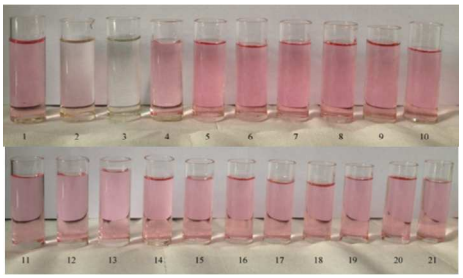


Fig.7 The fluorescent intensity variation and color change of probe in enthanol-water (20/80, V/V) solution induced by the addition of different amino acid. 1. probe; 2.probe-Glu; 3.probe-Asp; 4. probe-Pro; 5. probe-Arg; 6. probe-His; 7. probe-Lys; 8. probe-Leu; 9. probe-Cys; 10. probe-Trp; 11. probe-Tyr; 12. probe-Gly; 13. probe-Asn; 14. probe-Val; 15. probe-Ala; 16. probe-Met; 17. probe-Gln; 18. probe-Ser; 19. probe-Phe; 20. probe-Thr; 21. probe-Ile.[Probe]=10^{-5} M, [amino acid]=20 equiv.

For practical applications, the cytotoxicity of probe was measured using a CCK8 assay in MCF-7 cells with concentrations of probe from 10^{-3} to 10^{-8} M. The results showed IC_{50}=1046 uM, which clearly confirmed that the probe was of low toxicity to cultured cell lines at the concentrations of 10^{-5} M.

To further demonstrate the practical ability of probe, the imaging of probe in living cells (MCF-7) were also evaluated (Fig. 8). When MCF-7cells were pretreated with Glu or Asp and then incubated with probe, strong fluorescence was observed. By contrast, addition of probe (TM) or these amino respective, leading to almost no fluorescence. Addition of the contrast, a always on probe to the cell culture prior to the induction of EAAS led to strong fluorescence also. The results indicated that this probe is cell-permeable and can make turn on detection for these amino in living cells.

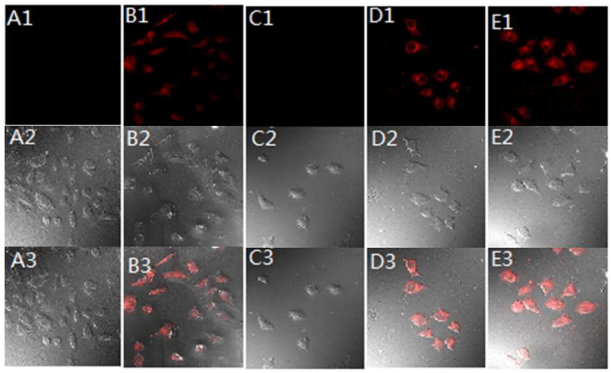


Fig.8 Confocal fluorescence and bright-field images of living MCF-7cells: A1-3: MCF-7 cells, B1-3: MCF-7 cells were incubated with reference probe (always on, IR783, 1×10^{-5} M) for 20 min, C1-3: MCF-7 cells were incubated with probe (TM, 1×10^{-5} M) for 20 min, D1-3: MCF-7 cells were pre-incubated with 2×10^{-4} M Glu for 24 h, and then treated with probe (TM, 1×10^{-5} M) for 20 min, E1-3: MCF-7 cells were pre-incubated with 2×10^{-4} M Asp for 24 h, and then treated with probe (TM, 1×10^{-5} M)

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for 20 min.. A1,B1, C1, D1, E1: fluorescence images, A2,B2,C2,D2, E2: bright-field images, A3,B3,C3,D3, E3: merged images.

In conclusion, the near-infrared fluorescent probe based on glucosamine modified cyanine is useful for detection of Glu and Asp whereby a fluorescence enhancement at 805 nm and a color change from pale red to colorless can be visibly observed. The low cytotoxicity and the results of living cells imaging indicated that this probe has potential application for biological applications.

As predicating by previous studies, one or both of the indole nitrogen atoms nonalkylated in cyanine, are pH-sensitive. In acidic environments, protonation of the indole nitrogen atoms gives rise to long wavelength absorption and strong fluorescence. Deprotonation of the nitrogen atoms results in a blue-shifted absorption band and little or no observable fluorescence emission [18]. Interestingly, the spectral responses of probe toward Glu and Asp presented similar results. Therefore, we predict that the pH value changes depending on Glu and Asp may serve as the reason for remarkable enhancement of fluorescence emission.

In order to gain insight into the sensing mechanism of probe towards Glu and Asp, the pH value change of the solution depending on concentration of Glu and Asp (Fig.9), the UV-Vis and fluorescence spectra of probe in ethanol-water (20/80, V/V) solution over a pH range of 2 to 11 (Fig.9) were measured. The result exhibited that the concentration of Glu and Asp would significantly alter the pH value of the solution. From the fluorescence spectra, it can be observed that the fluorescent intensity of the probe greatly increased as the solution become more acidity. This is due to increased protonation of the cyanine dye and leads to the change of the existing form of probe. Further proof of this acid–base equilibrium can be achieved by observation of the UV-Vis spectra of probe at different pH (Fig.10 A). It can be seen that as the solution become more acidity, the absorbance at 520 nm decreased and that at wavelength around 780 nm increased with an isosbestic point appearing at 599 nm, much like that described in the UV-Vis titration of Glu and Asp. This phenomenon can further support our hypothesis.

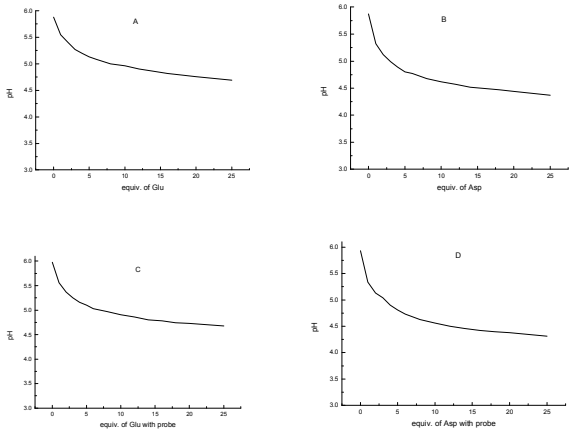

Fig.9 The pH value change depending on Glu and Asp (or with probe) in ethanol-water (20/80, V/V) solution. A and B show the pH value change depending on Glu and Asp (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25equiv, respectively); C and D show the pH value change depending on Glu and Asp (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25equiv, respectively) with probe.

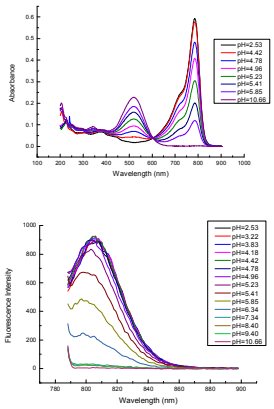

Fig.10 UV-Vis spectra (a) and Fluorescence spectra (b) of probe (10^{-5} M) at different pH value

Fig.11 illustrated the pH response of probe as a function of I / I_{max} vs. pH, where I is the fluorescent intensity at different pH, and I_{max} is the maximum output of the probe. It can be seen that a regular sigmoidal response is observed for this probe in response to pH. The pKa of probe can be estimated where I / I_{max} is 0.5 [19]. This provides a pKa value of 5. 82 for this probe.

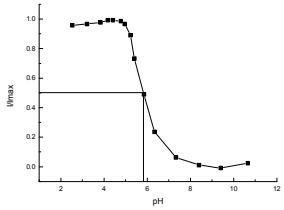

Fig.10 The pH response of probe as a function of I/I_{max} vs. pH.

The reversibility of the sensor is a very important aspect when considering some practical applications. The interaction between probe and H^+ was reversible, which can be verified by fluorescence titration experiment of probe in ethanol-water (20/80, V/V) with NaOH solution and HCl solution. The experiment showed that the introduction of H^+ could lead to the increase of fluorescence intensity and the disappearance of the color of the system. When OH^- was added to the system again, the fluorescence intensity was decreased and the color of system was recovered. Further switching on and off experiment was accomplished by the alternating addition of HCl or NaOH. This can be reversibly performed for at least five cycles.

Through the above research, we can come to the conclusion that the near-infrared colormetric fluorescent chemosensor based on glucosamine modified cyanine can be used conveniently as a visual fluorescence sensor for EAAS and pH value.

Conclusion

A novel colormetric NIR fluorescent chemosensor based on glucosamine modified cyanine has been designed and synthesized. This chemosensor exhibits excellent properties for detecting of Glu and Asp. A remarkable enhancement of fluorescence intensity at 805 nm and a visible change of color were observed in the presence of Glu and Asp. Moreover, this chemosensor may also sever as a highly sensitive acidic pH probe

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and the fluorescent switch can be reversible turned off and on by alternating pH value. Furthermore, the chemosensor has been successfully applied for the imaging of Glu and Asp in living cells. The cytotoxicity assay exhibits it is of low toxicity at the concentration of measurement. Hence, this probe is suitable for detecting Glu and Asp or acidic pH variations in living cells with a fluorescence turn-on signal.

Experimental

Materials and equipments

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxy succinimide (NHS) and ethyl 3-bromo propionate were purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). CCK8 was purchased from Sigma (Poole, Dorset, England). Other reagents and solvents such as sodium carbonate (anhydrous) and DMSO (analytical reagent) were purchased from Tianjin chemicals Co. (Tianjin, China) and used without further purification unless otherwise noted. DMSO was purified by refluxing with calcium hydride for 4 h and then distilled [20]. Deionized water was used throughout this work. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Avance 600 spectrometer, using TMS as an internal standard. Mass spectrometry (MS) was obtained using a Thermo Fisher Scientific LCQ Advantage Max. High-resolution mass spectra were measured with a Bruker Daltonics micrOTOF-Q II instrument (ESI). All pH measurements were made with a Sartorius basic pH-meter PB-10. UV-Vis spectra were obtained on an Evolution 300 UV-Visible spectrophotometer. Fluorescence spectra were recorded on a VARINA Eclipse fluorescence spectrophotometer. All measurements were performed under ambient atmosphere at room temperature.

General procedure for spectroscopic measurements

Working solutions of amino acids and probe were prepared by serial dilution of the stock solutions (1×10^{-2} M of amino acids in deionized water and 1×10^{-3} M of probe in ethanol). Solutions of amino acids were mixed separately with the solution of probe in different equiv and their UV-Vis and fluorescence studies were performed. For all measurements, the excitation and emission silt widths were both 5 nm, excitation wavelength was 780 nm.

Cytotoxicity assay andflorescence imaging

In vitro cytotoxicity of the probe was measured using CCK8 assay in MCF-7 cells according to the standard method of MTT assay [21, 22]. The CCK8 assay is similar to the MTT assay, except it uses a water-soluble tetrazoloum salt that produces a water-soluble formazan dye upon reduction by cellular dehydrogenases, and therefore doesn't require a solubilization step. Cells were seeded into 96-well cell culture plate at 200 μL/well, cultured for 24 h at 37 ℃ under 5 % CO_2, and then different concentrations of probe (0, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} M) were respectively added to the wells. The cells were then incubated for 24 h at 37 ℃ under 5 % CO_2. Subsequently, 20 μL CCk8 was added to each well and incubated for additional 4 h at 37 ℃ under 5 % CO_2. The amount of CCK8 formazan was quantified by determining the absorbance at 450 nm with a reference wavelength at 650 nm using a microplate reader (Tecan, Austria). For each concentration, each treatment was done in triplicate. IC_{50} value was calculated according to Huber and Koella [23].

MCF-7 cells were seeded in a 6-well plate at a density of 5×10^5 cells per well in culture media. The cells were incubated with 2×10^4 M Glu or Asp in culture media for 24 h at 37 ℃ in a humidified incubator. After washing three times with PBS, the cells were further incubated with 1×10^5 M of probe in culture media for 20 min. For the control experiments, the cells were treated with an always on probe (IR783), or treated with probe (TM) or EAAS respectively. Confocal fluorescence imaging was acquired on Olympus FV1000 confocal laser-scanning microscope with a 40×oil-immersion object lens. Fluorescence was excited at 635 nm and emission was collected by a 700-800 nm band pass filter.

Synthesis

2, 3, 3-Trimethyl-3*H*-indoleine (1): The mixture of phenyl hydrazine (5.40 g, 50 mmol) and 3-methyl-2-butanone (4.40 g, 50 mmol) was stirred at 100 ℃ for 2 h. After cooling to room temperature, the red liquid was extracted with CH_2Cl_2 (2×20 mL), the combined organic extracts were dried over $MgSO_4$, filtered and concentrated. The residue was diluted with acetic acid (20 mL) and stirred under reflux for 5 h, and then acetic acid was evaporated. The resulting residue was diluted with CH_2Cl_2 (20 mL) and washed with saturated aqueous Na_2CO_3 (3×30 mL), brine, then dried over $MgSO_4$, filtered and concentrated to give red oil. The crude product was purified by flash chromatography with gradient elution (petroleum ether/ethyl acetate of 20:1 to 3:1) to give pale yellow oil (6.41g, 81.1 %).

^{1}H NMR (600 MHz, $CDCl_3$) δ(ppm): 7.53 (1H, d, *J=7.8 Hz*, ArH), 7.31-7.27 (2H, m, ArH), 7.19 (1H, t, *J=7.2 Hz*, ArH), 2.28 (3H, s, CH_3), 1.30 (6H, s, CH_3).

Ethyl-3-(3,3-dimethyl-2-methyleneindolinl-yl)-propionate (2): The reaction mixture of 2,3,3-trimethyl-3*H*-indole (6.32 g, 40 mmol) and ethyl-3-bromo propionate (7.20 g, 5.1 mL, 40 mmol) was stirred at 100 ℃ for 5 h. After cooling to room temperature, the red precipitate was collected and washed with petroleum ether (3×30 mL), and then it was dissolved in 1 M NaOH (30 mL) and stirred for 2 h at room temperature. The solution was extracted with CH_2Cl_2 (3×30 mL) and the combined organic phases were washed with brine, dried over $MgSO_4$, filtered and concentrated to give red oil. The crude product was purified by flash chromatography with gradient elution (petroleum ether to petroleum ether/ethyl acetate of 40:1) to obtain the title compound as pale yellow oil (7.27 g, 70.2 %).

^{1}H NMR (600 MHz, $CDCl_3$) δ(ppm): 7.11 (1H, t, *J=7.2 Hz*, ArH), 7.07 (1H, d, *J=7.2 Hz*, ArH), 6.76 (1H, t, *J=7.8 Hz*, ArH), 6.60 (1H, d, *J=7.8 Hz*, ArH), 4.09 (2H, dd, J_1*=7.2 Hz*, J_2*=14.4 Hz*, CH_2), 3.89 (2H, d, *J=15.6 Hz*, CH_2), 3.84 (2H, t, *J=7.2 Hz*, CH_2), 2.61 (2H, t, *J=7.2 Hz*, CH_2), 1.32 (6H, s, CH_3), 1.19 (3H, t, *J=7.2 Hz*, CH_3).

^{13}C NMR (150 MHz, $CDCl_3$) δ(ppm): 171.97, 171.92, 160.77, 145.27, 137.48, 127.64, 121.98, 118.61, 105.38, 74.01, 60.73, 44.19, 38.6, 30.84, 30.06, 14.19.

MS: m/z 260.3 $[M+H]^+$.

2-Chloro-1-formyl-3-(hydroxymethylene)-cyclohex-1-ene (3): Title compound was prepared according to literature method

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with some modifications [18]. To a chilled solution of DMF (21.93 g, 23.1 mL, 300 mmol) in 10 mL CH_2Cl_2, $POCl_3$ (23 g, 13.8 mL, 150 mmol) in 10 mL CH_2Cl_2 were added dropwise under an ice bath. After 30 min, cyclohexanone (4.91 g, 5.2 mL, 50 mmol) in 5 mL CH_2Cl_2 was added, and the resulting mixture was stirred at 80 ℃ for 4 h, then it was cooled to room temperature and poured into ice water, and kept it overnight to obtain compound **3** as yellow solid (7.56 g, 87.9%).

Compound 4: A round bottom flask was charged with compounds **2** (7.79 g, 30 mmol) and **3** (5.16 g, 30 mmol) in ethanol solution (30 mL) and the resulting mixture was stirred at 50 ℃ for 0.5 h under N_2 atmosphere. After cooling to room temperature, compound **1** (5.53 g, 35 mmol) was added to the mixture, then it was stirred under reflux for an additional 3 h to afford a dark red solution. After cooling to room temperature, the solvent was removed. The crude red solid was purified by flash chromatography with gradient elution (petroleum ether/ethyl acetate of 15:1 to 3:1) affording the compound **4** as red solid (5.56 g, 33.5 %).

^{1}H NMR (600 MHz, $CDCl_3$) δ(ppm): 8.10 (1H, d, *J=16.2 Hz*, -CH=CH-), 7.61 (1H, d, *J=7.2 Hz*, ArH), 7.52 (1H, d, *J=12.6 Hz*, -CH=CH-), 7.32 (2H, dd, J_1*=8.4 Hz*, J_2*=14.4 Hz*, ArH), 7.21 (1H, t, *J=7.2 Hz*, ArH), 7.16-7.19 (2H, m, ArH), 6.89 (1H, d, *J=7.2 Hz*, ArH), 6.70 (1H, d, *J=7.8 Hz*, ArH), 6.65 (1H, d, *J=16.2 Hz*, -CH=CH-), 5.51 (1H, d, *J=12.6 Hz*, -CH=CH-), 4.12 (2H, dd, J_1*=7.2 Hz*, J_2*=14.4 Hz*, CH_2), 4.00 (2H, t, *J=7.2 Hz*, CH_2), 2.67 (2H, t, *J=7.2 Hz*, CH_2), 2.60 (4H, t, *J=6 Hz*, CH_2), 1.87-1.91 (2H, m, CH_2), 1.65 (6H, s, CH_3), 1.50 (6H, s, CH_3), 1.21 (3H, t, *J=7.2Hz*, CH_3).

^{13}C NMR (150 MHz, $CDCl_3$) δ(ppm)**:** 183.92, 171.60, 157.84, 147.19, 143.83, 138.96, 137.45, 136.85, 128.68, 127.79, 126.62, 125.40, 122.54, 121.85, 121.00, 120.50, 120.35, 120.29, 108.26, 106.44, 93.42, 61.03, 52.46, 45.99, 38.22, 31.20, 28.21, 26,89, 26.40, 24.78, 21.50, 14.15

HRMS (ESI Positive) calc. for $C_{35}H_{39}ClN_2O_2$, $[M+H]^+$ 555.2778, found 555.2774.

Compound 5: To an ethanol solution (20 mL) of intermediate **4** (5.54 g, 10 mmol), 2 M NaOH solution (10 mL) was added, and the resulting mixture was stirred at 50 ℃ for 3 h under N_2 atmosphere. After cooling to room temperature, the solvent was removed, then the residue was dissolved in water (30 mL) and acidified to pH=3 with 1 M HCl solution. The precipitation was collected by filtration and washed with water, then purified by flash chromatography with gradient elution (petroleum ether/ethyl acetate of 3:1 to methanol) to afford compound **5** as blue solid (4.84 g, 92.1 %).

^{1}H NMR (600MHz, MeOD) δ(ppm): 8.11 (1H, d, *J=24 Hz*, -CH=CH-), 7.70 (1H, d, *J=6.6 Hz*, ArH), 7.60 (1H, d, *J=19.2 Hz*, -CH=CH-), 7.36 (2H, t, *J =11.4 Hz*, ArH), 7.25-7.19 (3H, m, ArH), 6.93 (1H, t, *J=10.8 Hz*, ArH), 6.82 (1H, d, *J=24 Hz*, -CH=CH-), 6.79 (1H, d, *J=12.6 Hz*, ArH), 5.65 (1H, d, *J=18.6 Hz,* -CH=CH-), 4.08 (2H, s, CH_2), 2.75 (2H, s, CH_2), 2.66-2.62 (4H, m, CH_2), 1.90 (2H, s, CH_2), 1.67 (6H, s, CH_3), 1.56 (6H, s, CH_3).

HRMS (ESI Positive) calc. for $C_{33}H_{35}ClN_2O_2$, $[M+H]^+$ 527.2465 and [M-Cl] 491.2699, found 527.2464 and 491.2704.

TM: The mixture of EDC·HCl (1.53 g, 8 mmol) and NHS (1.15 g, 10 mmol) was added to a solution of compound **5** (2.63 g, 5 mmol) in anhydrous DMSO (15 mL) on an ice bath under N_2 atmosphere. After stirring for 1 h, the mixture was allowed to warm up to room temperature and stirred for an additional 12 h in the dark, then water was slowly added to the mixture until no precipitation produced and then filtered. This material was used immediately for the next step without further purification.

The mixture of succinimidyl ester of compound **5**, *N,N*-diisopropylethylamine (DIPEA) (1.94 g, 1.3 mL, 15 mmol) and D-glucosamine hydrochloride (1.73 g, 8 mmol) were added in anhydrous DMSO (15 mL). The mixture was stirred at room temperature for 5 h under N_2 atmosphere, and then poured into ether (50 mL). The precipitation was collected by filtration and purified by flash chromatography (CH_2Cl_2/Methanol of 10:1) to obtain the title compound as red solid (2.40 g, 69.7 %).

^{1}H NMR (600MHz, DMSO-d_6) δ(ppm): 8.12 (1H, d, *J=24 Hz*, -CH=CH-), 7.83 (1H, d, *J=12.6 Hz*, ArH), 7.52 (1H, d, *J=11.4 Hz*, ArH), 7.45 (2H, dd, J_1*=9.6 Hz*, J_2*=11.4 Hz*, ArH), 7.32-7.27 (2H, m, ArH), 7.21-7.16 (2H, m, ArH), 6.88 (1H, d, *J=11.4 Hz*, ArH), 6.63 (1H, d, *J=17.4 Hz*, -CH=CH-), 5.65 (1H, d, *J=17.4 Hz*, -CH=CH-), 4.95-4.81 (3H, m), 4.61-4.40 (3H, m), 3.94 (2H, t, *J =10.8 Hz*, CH_2), 3.61 (2H, t, *J=10.8 Hz*, CH_2), 2.61-2.60 (4H, m, CH_2), 1.87-1.76 (2H, m, CH_2), 1.57 (6H, s, CH_3), 1.36 (6H, s, CH_3).

HRMS (ESI Positive) calc. for $C_{39}H_{46}ClN_3O_6$, $[M+H]^+$ 688.3153, found 688.3146.

Acknowledgements

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Notes and references

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† Electronic Supplementary Information (ESI) available: [^{1}H NMR spectrum, ^{13}C NMR spectrum and ESI-MS spectrum]. See DOI: 10.1039/b000000x/

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Figure Captions

Fig.1 Effect of Glu and Asp on UV-Vis absorption.

Fig.2 Fluorescence spectra (λ_{ex}=780 nm, λ_{em}=805 nm) of probe (10^{-5} M) in ethanol-water (20/80, V/V) solution.

Fig.3 Fluorescence intensity at 805 nm vs. Asp and Glu concentration

Fig.4 The fluorescence spectral of probe toward Glu (A) and Asp (B).

Fig.5 The fluorescence intensity at 805 nm vs. the concentration of Glu or Asp.

Fig.6 Effects of amino acids (20 equiv) on the UV-Vis spectra (A) and fluorescence spectra (λ_{ex}=780 nm, λ_{em}=805 nm) (B) of probe (10^{-5} M) in ethanol-water (20/80, V/V) solution.

Fig.7 The fluorescent intensity variation and color change of the solution of probe in the presence of different amino acid.

Fig.8 Confocal fluorescence and bright-field images of living MCF-7cells.

Fig.9 The pH value change depending on Glu and Asp (or with probe) in ethanol-water (20/80, V/V) solution.

Fig.10 UV-Vis spectra (a) and Fluorescence spectra (b) of probe (10^{-5} M) at different pH value

Fig.11 The pH response of probe as a function of I/I_{max} vs. pH.

Scheme 1. Synthetic scheme for the probe

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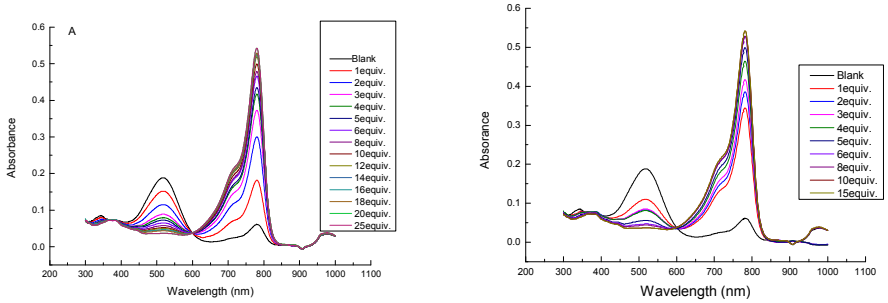

Fig.1 (A and B). Effect of Glu and Asp on UV-Vis absorption. (A) shows the UV-Vis absorption changes of probe upon the addition of Glu in ethanol-water (20/80, V/V) solution. [probe]=10^{-5} M, [Glu]=0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25 equiv, respectively. (B) shows the UV-Vis absorption changes of probe upon the addition of Asp at the similar condition.

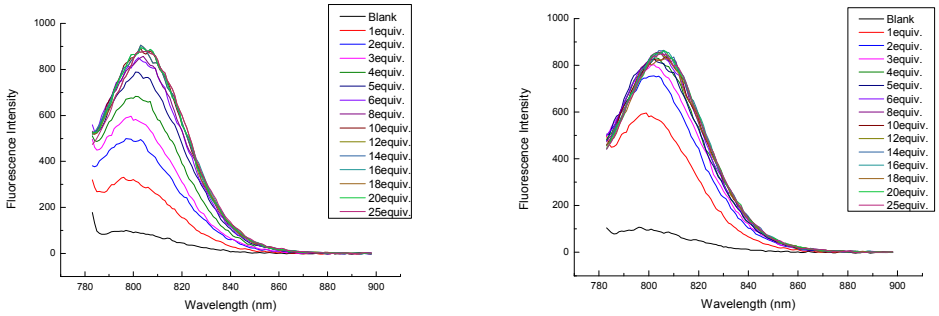

Fig.2 Fluorescence spectra (λ_{ex}=780 nm, λ_{em}=805 nm) of probe (10^{-5} M) in ethanol-water (20/80, V/V) solution. (a) In the presence of Glu (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25 equiv, respectively). (b) In the presence of Asp (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25 equiv, respectively).

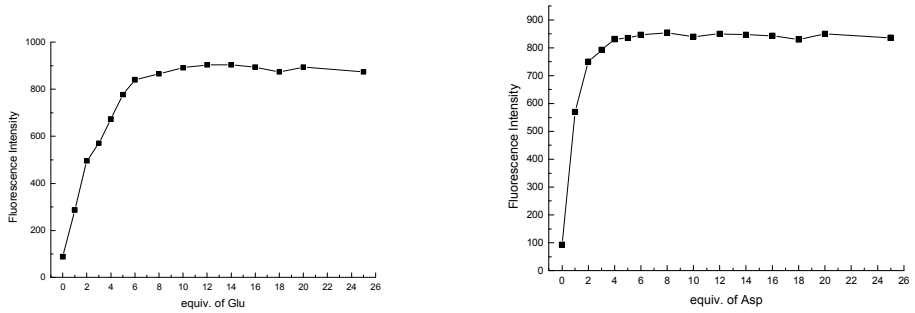

Fig.3 Fluorescence intensity at 805 nm vs. Asp and Glu concentration

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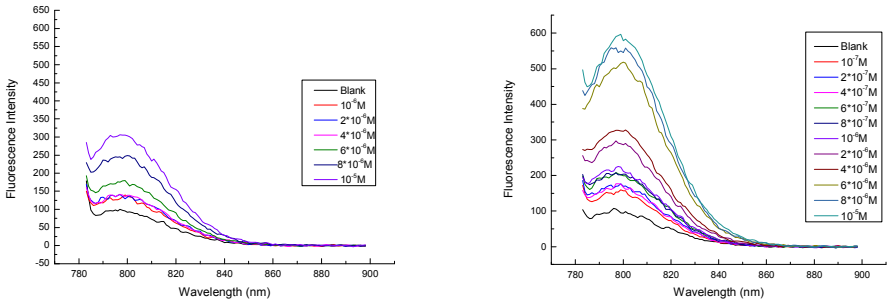

Fig.4 The fluorescence spectral of probe toward Glu (A) and Asp (B).

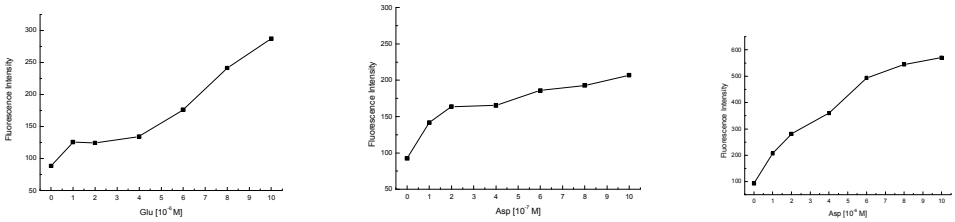

Fig.5 The fluorescence intensity at 805 nm vs. the concentration of Glu or Asp.

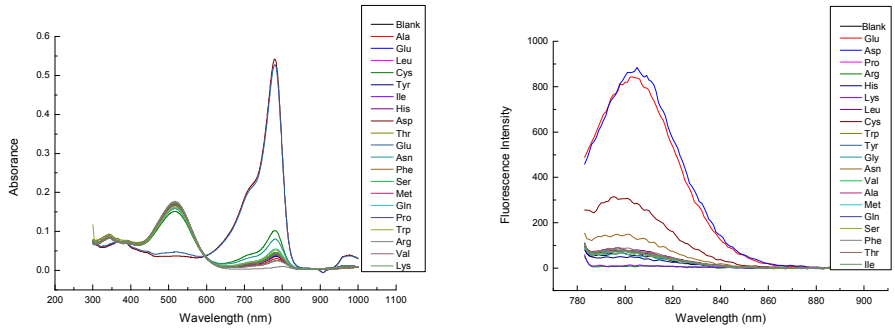

Fig.6 Effects of amino acids (20 equiv) on the UV-Vis spectra (A) and fluorescence spectra (λ_{ex}=780 nm, λ_{em}=805 nm) (B) of probe (10^{-5} M) in ethanol-water (20/80, V/V) solution.

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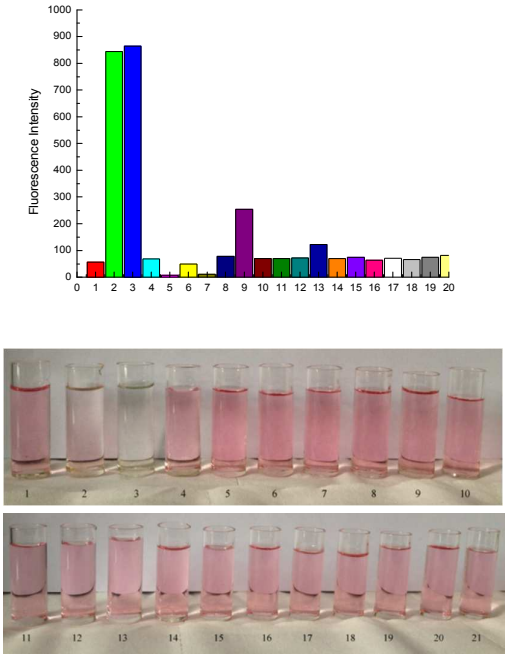

Fig.7 The fluorescent intensity variation and color change of probe in enthanol-water (20/80, V/V) solution induced by the addition of different amino acid. 1. probe; 2.probe-Glu; 3.probe-Asp; 4. probe-Pro; 5. probe-Arg; 6. probe-His; 7. probe-Lys; 8. probe-Leu; 9. probe-Cys; 10. probe-Trp; 11. probe-Tyr; 12. probe-Gly; 13. probe-Asn; 14. probe-Val; 15. probe-Ala; 16. probe-Met; 17. probe-Gln; 18. probe-Ser; 19. probe-Phe; 20. probe-Thr; 21. probe-Ile.
[Probe]=10^{-5} M, [amino acid]=20 equiv.

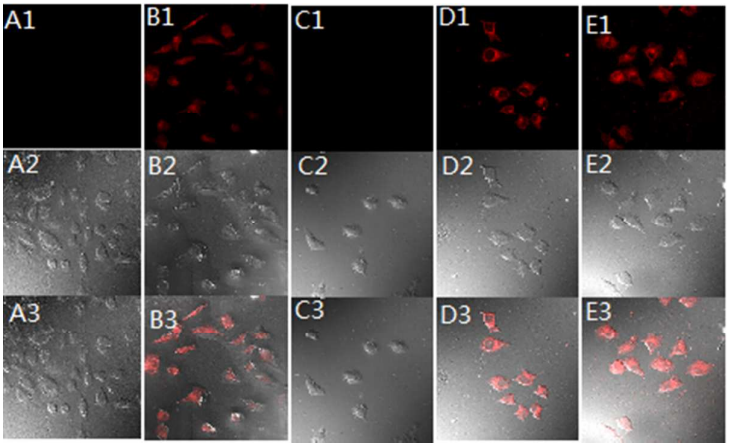

Fig.8 Confocal fluorescence and bright-field images of living MCF-7cells: A1-3: MCF-7 cells, B1-3: MCF-7 cells were incubated with reference probe (always on, IR783, 1×10^{-5} M) for 20 min, C1-3: MCF-7 cells were incubated with probe (TM, 1×10^{-5} M) for 20 min, D1-3: MCF-7 cells were pre-incubated with 2×10^{-4} M Glu for 24 h, and then treated with probe (TM, 1×10^{-5} M) for 20 min, E1-3: MCF-7 cells were pre-incubated with 2×10^{-4} M Asp for 24 h, and then treated with probe (TM, 1×10^{-5} M) for 20 min.. A1,B1, C1, D1, E1: fluorescence images, A2,B2,C2,D2, E2: bright-field images, A3,B3,C3,D3, E3: merged images.

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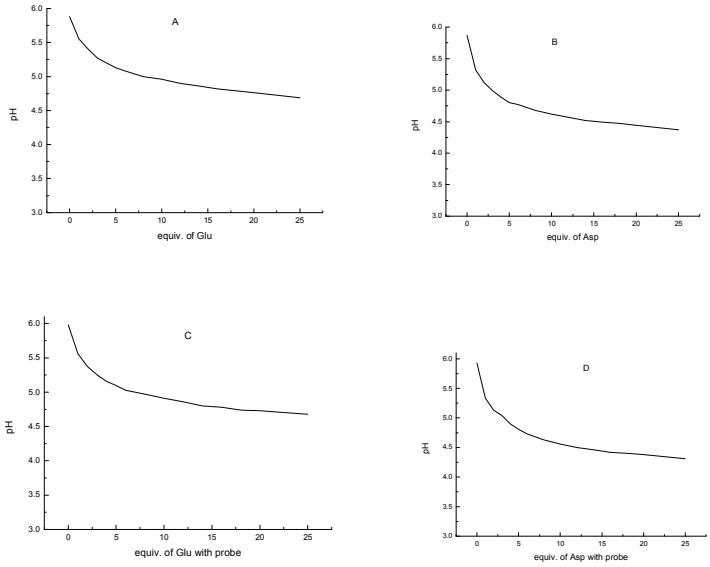

Fig.9 The pH value change depending on Glu and Asp (or with probe) in ethanol-water (20/80, V/V) solution. A and B show the pH value change depending on Glu and Asp (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25equiv, respectively); C and D show the pH value change depending on Glu and Asp (0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25equiv, respectively) with probe.

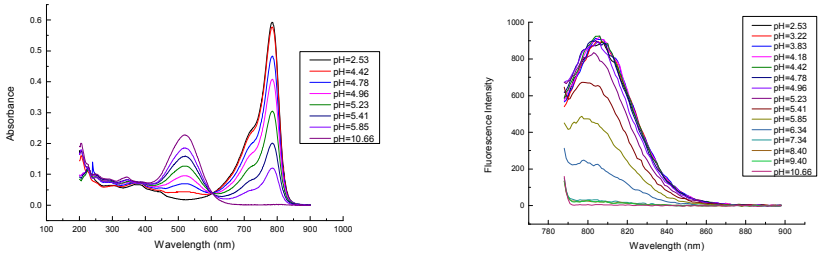

Fig.10 UV-Vis spectra (a) and Fluorescence spectra (b) of probe (10^{-5} M) at different pH value

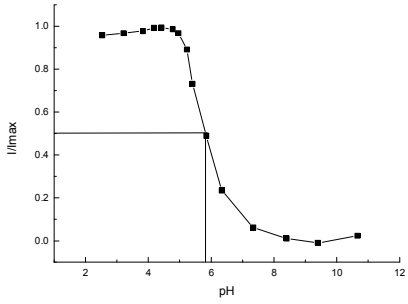

Fig.11 The pH response of probe as a function of I/I_{max} vs. pH.

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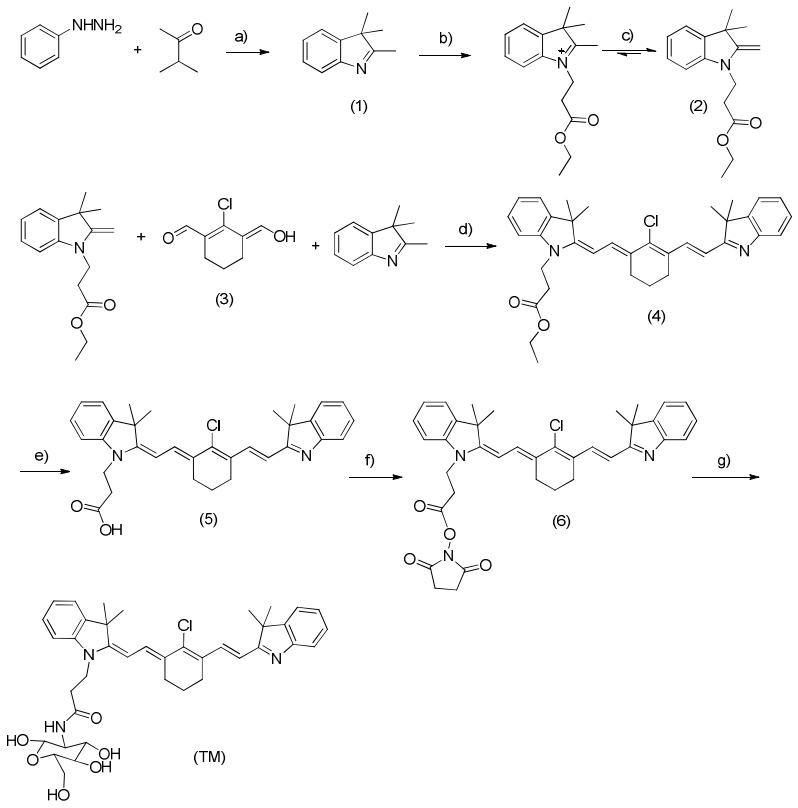

Scheme 1. Synthetic scheme for the probe

Reagents and conditions: a) 100 ℃, 2 h, then acetic acid, reflux, 5 h; b) ethyl 3-bromo propionate, 100 ℃, 5 h; c) 1 M NaOH, r.t., 2 h; d) ethanol, reflux, 3 h; e) 2 M NaOH in ethanol, 50 ℃, 3 , N_2 atmosphere; f) EDC·HCl, NHS, DMF, ice bath, then r.t., 12 h, N_2 atmosphere; g) D-glucosamine hydrochloride, DIPEA, DMF, 5 h, N_2 atmosphere.

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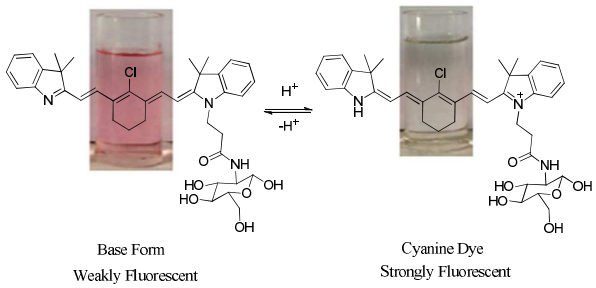
Table of contents entry

A near-infrared colorimetric fluorescent chemosensor has been developed and successfully applied for Glu and Asp imaging in living livings.



Base Form
Weakly Fluorescent

Cyanine Dye
Strongly Fluorescent

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