Cytosine derivatized diethylenetriaminepentaacetic acid (dtpa) and Eu(III) complexes for selective recognition of 6-Thioguanine

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A B S T R A C T

Eu^{III}-dtpa-bis(cytosine) complex was designed and synthesized to serve as a fluorescent probe for the

determination of 6-Thioguanine (6-TG). With the addition of 6-TG, the fluorescence intensity of the Eu^{III} -dtpa-bis(cytosine) complex was enhanced at 575 nm, meanwhile, a new fluorescence emission peak emerged at 525 nm. The influence of pH value and the interference of 6-mercaptopurine, Guanine, Cytosine, Adenine, Hypoxanthine, Xanthine, Thymine, Uracil and Uric acid on the detection of 6-TG using the Eu^{III} -dtpa-bis(cytosine) complex as a fluorescence probe was examined. It was found that the sensitivity and specificity of the Eu^{III} -dtpa-bis(cytosine) complex in the 6-TG detection could be confirmed. The detection mechanism of 6-TG using the Eu^{III} -dtpa-bis(cytosine) complex in buffer solution was studied by ultraviolet-visible (UV-vis) and fluorescence spectroscopy. A linear response of fluorescence intensity of the Eu^{III} -dtpa-bis(cytosine) complex at 575 nm as a function of 6-TG concentration in the range of $0.00 - 7.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ was observed. The detection limit is about $5.11 \times 10^{-8} \text{ mol}\cdot\text{L}^{-1}$. The corresponding correlation coefficient of the linear equation was 0.989. Besides, we have also studied the detection of 6-TG in the actual urines. It was found that the fluorescent probe could be reliable and effective for the determination of 6-TG in real urines.

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1. Introduction

6-Thioguanine (6-TG), a close structural analogue of Guanine (Gu), was introduced into the clinic and first studied in patients in the early 1950s [1]. 6-TG has long been used as a popular drug for leukemia treatment, similar to the 6-mercaptopurine (6-MP), in the last two decades [2]. In recent years, it has been applied to treat breast cancer and influence immune response in the treatment of inflammatory diseases such as ulcerative colitis, dermatitis and rheumatoid arthritis [3-6]. However, the issue of its dosage is of crucial importance, that is, the underdosage will fail in the treatment of the diseases, while relatively adverse effects can be caused by the overdosage, with myelosuppression as the most common [7,8]. Because the safety margin between a therapeutic dose and a toxic dose is very narrow, it is necessary to come up with methods to identify and quantify the drug for forensic medicine and therapeutic drug monitoring.

Currently, various kinds of analytical methods including high-performance liquid chromatography,

electrochemical method, phosphorimetry, localized surface plasmon resonance, fluorimetry, and SERS [9-15] have been reported for the determination and quantification of 6-TG. In fact, these analytic methods were thought to be rapid and sensitive, but they also show their limitations and demerits such as high cost, complicate separation process, tedious pretreatment steps and sophisticated instrumentation. For instance, chromatography generally requires complicated and expensive equipment, electrochemical methods encounter the problem of laborintensive electrode procedures and the poor stability of electrode. Therefore, it is necessary to develop new method for the detection of 6-TG.

In this paper, the diethylenetriaminepentaacetic acid (dtpa) was modified with Cytosine (Cy), forming a new polydentate ligand, that is, dtpa-bis(cytosine). And then, the Eu^{3+} ion was used to react with this ligand to form a nine-coordinate complex, Eu^{III} -dtpa-bis(cytosine) [15-18]. It may be served as a fluorescent probe for the determination of 6-TG. In Eu^{III} -dtpa-bis(cytosine) complex, two Cy molecules locate at two ends (up and down) of the complex. When the Eu^{III} -dtpa-bis(cytosine) encounters the 6-TG, to some extent, whose chemical composition and molecular structure are similar to Cy. Two arms (Cy) can capture the 6-TG tightly through the hydrogen bond and π - π stacking, because of their similar flat structures and nitrogenous heterocyclic compositions. In addition, as the 6-TG draws closer to the Eu^{3+} ion in the Eu^{III} -dtpa-bis(cytosine) complex, it will replace the original coordinate water (H_2O), forming a new coordination bond with the Eu^{3+} ion. In consequence, the Eu^{III} -dtpa-bis(cytosine) can combine with 6-TG steadily and exclusively through the hydrogen bond, π - π stacking and coordination bond. The change of ligand field around the Eu^{3+} ion enhances the fluorescence of Eu^{III} -dtpa-bis(cytosine). And the formation of tripolymer of one 6-TG and two Cy molecules produces new fluorescence.

Subsequently, by means of fluorescence spectrum, it could be found that the fluorescence

emission peak around 575 nm was significantly enhanced when the 6-TG or Gu was introduced to the Eu^{III} -dtpa-bis(cytosine) solution. At the same time, a new strong fluorescence emission peak could be found around 525 nm only when the 6-TG was added to the Eu^{III} -dtpa-bis(cytosine) solution. Meanwhile, in this work, the interferences of some other similar base compounds on the fluorescence of Eu^{III} -dtpa-bis(cytosine) solution were also studied. It can be found that, when these base compounds, such as Guanine (Gu), 6-mercaptopurine (6-MP), Adenine (Ad), Hypoxanthine (Hy), Xanthine (Xa), Thymine (Th), Uracil (Ur) and Uric acid (Ua) were added to the Eu^{III} -dtpa-bis(cytosine) solution, respectively, the fluorescence intensity was slightly decreased or nearly invariable. Therefore, it can be confirmed that the Eu^{III} -dtpa-bis(cytosine) as a fluorescence probe can detect 6-TG and Gu with specificity and sensitivity and not be affected by other base compounds.

2. Experimental

2.1. Apparatus

Fourier Transform-Infrared (FT-IR) spectra were taken in KBr disks on a Nicolet 5700 FTIR spectrometer. NMR spectra were conducted with an Agilent Technologies Plus-400MR spectrometer with $\text{DMSO}-d_6$ as the solvent and tetramethylsilane (TMS) as internal standard. Fluorescence determination experiments were carried out by fluorophotometer (Cary 300, Varian Company, USA) and UV-vis absorption spectra were recorded with an UV-vis spectrophotometer (Cary 50, Varian Company, USA).

2.2. Materials

Diethylenetriaminepentaacetic acid (dtpa) and Cytosine (Cy) (A.R., Beijing SHLHT Science & Trade Co., Ltd, China) were purchased and used to synthesize the dtpa-bis(cytosine) ligand.

6-Thioguanine (6-TG), Guanine (Gu), 6-mercaptopurine (6-MP), Adenine (Ad), Hypoxanthine (Hy), Xanthine (Xa), Thymine (Th), Uracil (Ur) and Uric acid (Ua) (A.R., Beijing SHLHT Science & Trade Co., Ltd, China) were purchased and used to be analytic targets. Their molecular structures were given in Fig. 1. $\text{Eu}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (99.999 %, Yuelong Rare Earth Co., Ltd, China) was obtained to prepare the Eu^{III} -dtpa-bis(cytosine) complex as fluorescent probe. Anhydrous acetic anhydride and DMF (analytical purity, Shenyang Chemical Reagent Plant, China) were purchased and used as solvent. Pyridine and Triethylamine (analytical purity, Shenyang Chemical Reagent Plant, China) were obtained and used as acid-binding-agent. Tris (hydroxyl-methyl) aminomethane (Tris) and HCl (analytical purity, Shenyang Chemical Reagent Plant, China) were used to prepare the Tris-HCl ($\text{pH} = 7.4$ and $[\text{Tris-HCl}] = 50 \text{ mmol} \cdot \text{L}^{-1}$) buffer solution in order to maintain the ionic strength and adjust the solution acidity.

Fig. 1.

2.3. Synthesis of probe Eu^{III} -dtpa-bis(cytosine)

2.3.1. Synthesis of diethylenetriamine pentaacetic acid dianhydride (dtpaa)

The diethylenetriamine pentaacetic acid dianhydride (dtpaa) is demand for the start of all experiments and its synthesis procedure is described in Scheme 1 according to the reported method [19-21]. Diethylenetriamine pentaacetic acid (dtpa) (7.80 g, 0.02 mmol) was dissolved in acetic anhydride (8.00 mL, 0.08 mmol) and pyridine (10.00 mL, 0.12 mmol) as base agent under anhydrous condition. The mixed solution was stirred for 24 h under heat-refluxing at 65°C . And then, the reaction mixture was cooled down to room temperature, and the solvent was removed under reduced pressure. The residue was washed with acetic anhydride and anhydrous diethyl ether twice. Finally, drying it in vacuum (52 kpa) at 80°C 6.50 g white powder with yield of 83 % was obtained. FT-IR

(KBr, cm^{-1}): 2979, 2820, 2341, 1821, 1772 and 1642. ^1H NMR (500 MHz, DMSO): δ = 2.593 (t, 4H), 2.748 (t, 4H), 3.300 (s, 2H), 3.705 (s, 8H) and 11.013 (s, 1H).

Scheme 1.

2.3.2. Synthesis of dtpa-bis(cytosine)

Dtpa-bis(cytosine) ligand was synthesized by the aminolysis reaction between dtpaa and cytosine by a new improving method [22-26]. Its synthesis procedure is shown in Scheme 1. The cytosine (1.83 g, 165 mmol) was dissolved in 25 mL of distilled water, then drop slowly dtpaa (1.96 g, 55 mmol) which was dissolved in DMF (50 mL) into the aqueous solution, and Triethylamine as base under anhydrous condition, was stirred for 24 h under heat-refluxing at 100 °C. The mixture was then cooled down to room temperature. H_2O and DMF were removed under reduced pressure, and the residue was diluted with water and filtrated. Finally, the straw yellow solid was obtained. The straw yellow solid was evaporated to dryness under vacuum (52 kpa) at 50 °C to give 3.18 g straw yellow powdery solid with yield of 84 %. FT-IR (KBr, cm^{-1}): 3378, 3312, 1726, 1648, 1372, 1234 and 906. ^1H NMR (500 MHz, DMSO): δ = 2.620 (m, 4H), 3.263 (m, 4H), 3.303 (s, 2H), 4.108 (s, 4H), 4.506 (d, 2H), 6.018 (s, 2H), 7.305 (m, 2H), 8.010 (d, 2H) and 11.012 (s, 3H).

2.3.3. Synthesis of the Eu(III) complex (Eu^{III} -dtpa-bis(cytosine)) with dtpa-bis(cytosine) ligand

Dtpa-bis(cytosine) (0.073 g, 0.13 mmol) was dissolved in 50 mL Tris-HCl (pH = 7.4 and $[\text{Tris-HCl}] = 50 \text{ mmol}\cdot\text{L}^{-1}$) buffer solution. $\text{Eu}(\text{NO}_3)_3\cdot 6\text{H}_2\text{O}$ (0.056 g, 0.13 mmol) powder was dissolved in 50 mL Tris-HCl (pH = 7.4 and $[\text{Tris-HCl}] = 50 \text{ mmol}\cdot\text{L}^{-1}$) buffer solution. After that the two above buffer solution were mixed together, refluxed and stirred for one hour, constant volume in 250 mL volumetric flask and kept in refrigerator 4.0 °C as stock solution to be used.

2.4. Preparation of the solutions of various base compounds

6-TG (0.021 g, 0.13 mmol), Gu (0.019 g, 0.13 mmol), 6-MP (0.019 g, 0.13 mmol), Cy (0.014g, 0.13 mmol), Ad (0.017 g, 0.13 mmol), Hy (0.017 g, 0.13 mmol), Xa (0.019 g, 0.13 mmol), Th (0.016g, 0.13mmol), Ur (0.014g, 0.13mmol) and Ua (0.021 g, 0.13 mmol) were dissolved in 20 mL Tris-HCl (pH = 7.4 and [Tris-HCl] = 50 mmol·L⁻¹) buffer solution and constanted in 250 mL volumetric flask to obtain 5.00×10^{-4} mol·L⁻¹ concentration of solutions, respectively. The stock solutions were kept in refrigerator 4.0 °C. The stock solutions were diluted to required concentrations with Tris-HCl (pH = 7.4 and [Tris-HCl] = 50 mmol·L⁻¹) buffer solution when needed.

2.5. Determination of spectra

2.5.1. Determination of UV-vis spectra of Eu^{III}-dtpa-bis(cytosine) solution

The dilute solution of Eu^{III}-dtpa-bis(cytosine), dtpa-bis(cytosine) and Eu³⁺ ion (5.00×10^{-5} mol·L⁻¹) were prepared in Tris-HCl (pH = 7.4 and [Tris-HCl] = 50 mmol·L⁻¹) buffer solution. These above solutions were put in a quartz cell (10.00 mm width), and the absorption spectra were recorded at room temperature.

2.5.2. Determination of fluorescence spectra of Eu-dtpa-bis(cytosine) solution

The dilute solution of Eu^{III}-dtpa-bis(cytosine) (5.00×10^{-5} mol·L⁻¹) was prepared in Tris-HCl (pH = 7.4 and [Tris-HCl] = 50 mmol·L⁻¹) solution. Also, the mixed dilute solution of Eu^{III}-dtpa-bis(cytosine) (5.00×10^{-5} mol·L⁻¹) and 6-TG, Gu, 6-MP, Cy, Ad, Hy, Xa, Th, Ur and Ua (5.00×10^{-5} mol·L⁻¹) and the mixed dilute solution of Eu^{III}-dtpa-bis(cytosine) + 6-TG (5.00×10^{-5} mol·L⁻¹) and Gu, 6-MP, Cy, Ad, Hy, Xa, Th, Ur and Ua (5.00×10^{-5} mol·L⁻¹) were prepared in Tris-HCl (pH = 7.4 and [Tris-HCl] = 50 mmol·L⁻¹) solutions, respectively. These above solutions were placed in quartz cell (10.00 mm width), respectively, and the determination of fluorescence spectra was carried out at room temperature. In addition, the dilute solution of Eu^{III}-dtpa-bis(cytosine) (5.00×10^{-5} mol·L⁻¹) with various

concentrations of 6-TG ($0.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ - $7.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ at intervals of $0.50 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$) were prepared in Tris-HCl (pH = 7.4 and $[\text{Tris-HCl}] = 50 \text{ mmol}\cdot\text{L}^{-1}$) solution and the corresponding fluorescence spectra were also determined.

3. Results and discussion

3.1. FT-IR spectra of *Eu-dtpa-bis(guanine)*

The diethylenetriamine pentaacetic acid dianhydride (dtpaa) was synthesized by the decarboxylic reaction of diethylenetriamine pentaacetic acid (dtpa). And the dtpa-bis(cytosine) was synthesized by the acylation reaction between dtpaa and Cy. All obtained compounds were characterized by ^1H NMR (the corresponding data were given in the experimental part) and fourier transform infrared spectra (FT-IR). The comparison of FT-IR spectra among dtpa, Cy and dtpa-bis(cytosine) is shown in Fig. 2. It can be found that the $\nu(\text{C-N})$ of dtpa-bis(cytosine) appears at 906 cm^{-1} , which displays a red-shift by 76 cm^{-1} compared with 982 cm^{-1} of Cytosine (Cy). The $\nu_s(\text{COO})$ of dtpa-bis(cytosine) appears at 1372 cm^{-1} , which display a red-shift by 27 cm^{-1} compared with 1399 cm^{-1} of dtpa. Furthermore, the $\nu_{as}(\text{COO})$ of dtpa-bis(cytosine) appears at 1726 cm^{-1} , while the $\nu_{as}(\text{COO})$ of dtpa appears at 1734 cm^{-1} , so the dtpa-bis(cytosine) displays a red-shift by 8 cm^{-1} . And that the $\nu_{as}(\text{CONH})$ of dtpa-bis(cytosine) appears at 1648 cm^{-1} and the characteristic broad absorption peaks of hydroxy group appears at 3378 cm^{-1} . These shifts confirmed that the dtpa-bis(cytosine) has been synthesized by the acylation action between dtpaa and cytosine.

Fig. 2.

3.2. UV-vis spectra of *Eu-dtpa-bis(cytosine)* solution

The UV-vis absorption spectra of Eu^{III} -dtpa-bis(cytosine), dtpa-bis(cytosine) and Eu^{3+} ion in

Tris-HCl (pH = 7.4 and [Tris-HCl] = 50 mmol·L⁻¹) buffer solution (5.00×10^{-5} mol·L⁻¹) were determined and all shown in Fig. 3. It can be found that the Eu³⁺ ion solution gives a maximum absorption peak at 220 nm wavelength ($\lambda_{\text{max}} = 220$ nm). And that the dtpa-bis(cytosine) solution displays a very weak absorption peak at 205 nm. Upon the addition of dtpa-bis(cytosine), the formed Eu^{III}-dtpa-bis(cytosine) complex also gives a maximum absorption peak at 220 nm, but the absorption peak obviously becomes strong compared with that of the Eu³⁺ ion solution. In addition, a new absorption peak appears at 288 nm. It indicates that the dtpa-bis(cytosine) can form new ligand field and change the electronic structure of the Eu³⁺ ion, resulting in the potential acting as a fluorescence probe.

Fig. 3.

3.3. Fluorescence spectra of Eu-dtpa-bis(cytosine) solution in the presence of some base compounds

The fluorescence spectra of pure Eu^{III}-dtpa-bis(cytosine) solution and the mixed solutions with a series of base compounds, such as 6-TG, Gu, 6-MP, Cy, Ad, Hy, Xa, Th, Ur and Ua, are depicted in Fig. 4(a). Under the excitation of 288 nm wavelength light, it can be found that the Eu^{III}-dtpa-bis(cytosine) in aqueous solution emits a moderately strong green fluorescence at 575 nm owing to the presence of Eu³⁺ ion [27]. When the 6-TG was introduced to the Eu^{III}-dtpa-bis(cytosine) solution, the fluorescence emission intensity of Eu^{III}-dtpa-bis(cytosine) solution at 575 nm was significantly enhanced about three times, as shown in Fig. 4(b-1). In addition, it can also be found that a new strong fluorescence emission peak emerged at 525 nm at the same time after adding 6-TG. Nevertheless, with the addition of other base compounds, like Cy, Ad, Hy, Xa, Th, Ur and Ua to the Eu^{III}-dtpa-bis(cytosine) solution, the fluorescence intensity of Eu^{III}-dtpa-bis(cytosine) solution was slightly decreased or unchanged at

575 nm as shown in **Fig. 4(b-2)**. For fluorescence emission peak at 525 nm, in other words, these base compounds including 6-MP, Cy, Ad, Hy, Xa, Th, Ur and Ua can only slightly quench the fluorescence of Eu^{III} -dtpa-bis(cytosine) solution or hardly change it. It is well known that the photoluminescence signals can be significantly quenched by highly conjugated organic compounds through close interaction [28]. The 6-TG and these base compounds including 6-MP, Gu, Cy, Ad, Hy, Xa, Th, Ur and Ua have relatively high π - π conjugation level and several molecules can form stacked structures. Thus, added these base compounds including 6-MP, Cy, Ad, Hy, Xa, Th, Ur and Ua into the Eu^{III} -dtpa-bis(cytosine) solution, respectively, the fluorescence of Eu^{III} -dtpa-bis(cytosine) solution was slightly quenched. It is worth noting that when adding Gu, whose chemical composition and molecular structure are similar to that of 6-TG, to the Eu^{III} -dtpa-bis(cytosine) solution, the fluorescence intensity at 575 nm was also enhanced dramatically. However, there was no new fluorescence-emission peak formed at 525 nm. Although the 6-TG and Gu have similar chemical composition and molecular structure, it can be confirmed that the Eu^{III} -dtpa-bis(cytosine) as fluorescence probe can sensitively recognize 6-TG and Gu by means of fluorescence spectra. In a word, due to the obvious increase of fluorescence intensity at 575 nm the 6-TG and Gu can be detected by the fluorescence probe at the same time. And that, because of the new fluorescence emission peak appeared at the 525 nm only caused by 6-TG, the Eu^{III} -dtpa-bis(cytosine) can be used to recognize and differentiate the 6-TG and Gu.

Fig. 4.

3.4. Effects of other base compounds on fluorescence spectrum of Eu^{III} -dtpa-bis(cytosine) + 6-TG solution

The effects of other base compounds including Gu, Ad, 6-MP, Cy, Hy, Xa, Th, Ur and Ua on the

fluorescence of Eu^{III} -dtpa-bis(cytosine) and 6-TG mixed solution were researched. As shown in **Fig. 5(a)** and **Fig. 5(b-1)**, when adding these base compounds into the Eu^{III} -dtpa-bis(cytosine) and 6-TG mixed solution, respectively, the fluorescence-emission peak position did not move and the intensity at 575 nm was hardly change either. However, it can be found that the fluorescence intensity at 525 nm is decreased by half after adding these base compounds as shown in **Fig. 5(b-2)**. It can be predicted that these base compounds will not disturb the detection and analysis of 6-TG by using the Eu^{III} -dtpa-bis(cytosine) complex as a probe, though the fluorescence intensity at 525 nm reduces to a certain extent. In fact, there is not new fluorescence-emission peak formed at 525 nm for Eu^{III} -dtpa-bis(cytosine) solution except the 6-TG. It indicates that the Eu^{III} -dtpa-bis(cytosine) as fluorescence probe to detect 6-TG exhibits high sensitivity and recognizability as well as good specific binding ability. Moreover, it can be confirmed that the Eu^{III} -dtpa-bis(cytosine) brings the sensitive recognition to 6-TG through hydrogen bond, π - π stacking and coordination bond due to the similar chemical composition and molecular structure between 6-TG and Cy as two arms.

Fig. 5.

3.5. Fluorescence spectra of Eu^{III} -dtpa-bis(cytosine) solution along with 6-TG concentration

Fluorescence spectra of Eu^{III} -dtpa-bis(cytosine) solution along with 6-TG concentration were given in **Fig. 6(a and b)** for two concentration ranges ($0.00 - 7.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ (a) and $4.50 - 7.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ (b)). And the corresponding fluorescence intensities at 575 nm and 525 nm were given in **Fig. 6(c-1 and c-2)**, respectively. It can be seen from **Fig. 6(a)** that the fluorescence intensity around 575 nm generally increases along with the increase of 6-TG concentration in the range of $0.00 - 5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$. When the concentration of 6-TG reached $5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, the fluorescence intensity of Eu^{III} -dtpa-bis(cytosine) solution becamen the maximum. Along with the further increase of the

6-TG concentration ranged from 5.00 to $7.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, the fluorescence intensity increases only slightly and then until it dose not change. It indicates that a growing number of 6-TG react with the Eu^{III} -dtpa-bis(cytosine) to form the Eu^{III} -dtpa-bis(cytosine)-6-TG complexes. However, the fluorescence emission peak around 525 nm does not appear until $4.50 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ concentration of 6-TG. Sequentially, from Fig. 6(b), it can be found that the fluorescence emission peak around 525 nm increases along with the increase of 6-TG concentration from $4.50 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ to $6.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ and then decreases after $6.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$. As shown in Fig. 6(c-2), the strongest fluorescence intensity at 525 nm appears at $6.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ of 6-TG. It demonstrates that the fluorescence peak around 525 nm is sensitive to the concentration of 6-TG. Taking into consideration of the changes of these two fluorescence emission peaks, the Eu^{III} -dtpa-bis(cytosine) may be used for the detection and analysis of 6-TG.

In addition, for the fluorescence-emission peak around 575 nm a linear response of fluorescence intensity of Eu^{III} -dtpa-bis(cytosine) as a function of 6-TG concentration was also observed in Fig. 6(c-1) in the range of $0.00 - 7.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$. The corresponding linear equation is $y = 29.35x + 60.64$ ($R^2 = 0.9892$), where y is the fluorescence intensity at 575 nm measured at a given 6-TG concentration and x represents the concentration of added 6-TG. Apparently, it can be found that when the concentration of 6-TG reaches $5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, the fluorescence intensity attains maximum, which indicates the complexation ratio of Eu^{III} -dtpa-bis(cytosine) and 6-Thioguanine (6-TG) is 1:1. And then, with the increasing of the 6-TG concentration ranged from $5.00 - 7.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, the fluorescene intensity was almost invariable. Thus, the stability constant (k) was 1.21×10^7 , calculated from the formula: $k = \frac{1-\alpha}{[\text{Complex}] \cdot \alpha^2}$, $\alpha = \frac{A'-A}{A'}$, A' represents the fluorescence intensity value corresponding the point of the tangents intersection and A represents the fluorescence intensity value

corresponding curve intersection, as shown in **Fig. 6(c-1)**. It can be found that the complex of Eu^{III} -dtpa-bis(cytosine) and 6-TG was extremely stable, because of the larger value of k . According to the formula of LOD (limit of detection): $\text{LOD} = 3\sigma/s$, the calculated LOD is about $5.11 \times 10^{-8} \text{ mol}\cdot\text{L}^{-1}$. It can be seen from **Table 1** that the method proposed in this work shows a relatively wide detection range and low detection of limit compared with those reported in literatures [10-12,15-17]. Particularly, due to the high sensitivity and specificity to 6-TG and outstanding anti-interference to other base compounds, the Eu^{III} -dtpa-bis(cytosine) can be used as a excellent fluorescence probe to detect 6-TG.

Finally, as can be seen from **Fig. 6(a and b)**, when the concentration of 6-TG reaches $5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, the fluorescence peak of the Eu^{III} -dtpa-bis(cytosine) solution begins to appear around 525 nm. It indicates that Eu^{III} -dtpa-bis(cytosine) and 6-TG may form a new type of complex with 1:2 molar ratio. Along with increasing concentration of 6-TG, the fluorescence peak was increased at first, and then decreased. That is, when the concentration of 6-TG reaches $6.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, the fluorescence intensity attains the maximum. Apparently, only when the concentration of 6-TG reaches $6.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, except for 1:2 molar ratio complexes the Eu^{III} -dtpa-bis(cytosine) can form the greatest amount of 1:2 molar ratio complexes with 6-TG. Therefore, the fluorescence intensity at 525 nm reaches the maximum. However, when the concentration of 6-TG is larger than $6.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, a certain amount of 1:3 molar ratio complexes, which does not generate the fluorescence-emission peak around 525 nm, may form in aqueous solution. As a result, the whole fluorescence intensity is decreased. Thus, it can draw a conclusion that the Eu^{III} -dtpa-bis(cytosine) can combine with more 6-TG molecules. That is, when the concentration of 6-TG is lower than $5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, the Eu^{III} -dtpa-bis(cytosine) can mainly form 1:1 molar ratio complexes with 6-TG, which hardly give fluorescence emission. When the concentration of 6-TG is $6.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, some 1:2 molar ratio complexes can be produced and these 1:2 molar ratio complexes can give strong fluorescence

emission. And that, when the concentration of 6-TG is larger than $6.00 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$, 1:3 molar ratio complexes may be formed, which also do not generate fluorescence.

Fig. 6.

Table 1.

3.6. Effect of pH value on fluorescence spectrum of Eu^{III} -dtpa-bis(cytosine) + 6-TG solution

In this section, the effect of pH value on the fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) of Eu^{III} -dtpa-bis(cytosine) and 6-TG mixed solution were investigated and the corresponding results were given in Fig. 7. The pH value is adjusted using Tris-HCl ($[\text{Tris-HCl}] = 50 \text{ mmol} \cdot \text{L}^{-1}$ and $\text{pH} = 7.40$) buffer solution ranged from $\text{pH} = 2.60$ to $\text{pH} = 8.90$. From Fig. 7(a), it can be observed that the fluorescence intensity ($\lambda_{\text{em}} = 580 \text{ nm}$) slowly increases along with increasing pH value, and then grows rapidly until close to neutral solution. Afterwards, the fluorescence intensity ($\lambda_{\text{em}} = 575 \text{ nm}$) drops rapidly with further increasing pH value to weakly alkaline solution. Specifically, as shown in Fig. 7(b), the strongest fluorescence-emission peak ($\lambda_{\text{em}} = 575 \text{ nm}$) appears at $\text{pH} = 7.10$. It indicates that the fluorescence spectrum ($\lambda_{\text{ex}} = 288 \text{ nm}$) of Eu^{III} -dtpa-bis(cytosine) and 6-TG mixed solution is sensitive to acidity, since the 6-TG proposes a scheme of the acid-base tautomeric equilibrium in the aqueous solution due to the presence of amino group [29]. Therefore, the solution pH value plays an important role in the experiments and the optimum pH value for detection of 6-TG is around $\text{pH} = 7.10$.

Fig. 7.

3.7. Reaction mechanism and process of Eu^{III} -dtpa-bis(cytosine) and 6-TG

As shown in Fig. 8, the binding mechanism and process of Eu^{III} -dtpa-bis(cytosine) as fluorescent

probe to detect 6-TG were proposed. In addition, the binding mechanism with Gu was also given in **Fig. 8**. It is well known that the Eu^{3+} ion can form nine-coordinate complexes with various aminopolycarboxylic acid ligands [30-32]. The dtpa was modified by two Cy at its two ends (up and down), respectively, forming a new octadentate dtpa-bis(cytosine) ligand. Therefore, in the Eu^{III} -dtpa-bis(cytosine) complex, one water molecule (H_2O), as ninth coordination ligand, should be coordinated with Eu^{3+} ion. It could be inferred that the Eu^{3+} is coordinated with the three nitrogen atoms and five oxygen atoms from a dtpa-bis(cytosine) ligand and one oxygen atom from H_2O in aqueous solution. 6-TG and Cy are similar in chemical composition and molecular structure to some extent, both of them containing a flat structure of nitrogen heterocyclic. When the Eu^{III} -dtpa-bis(cytosine) encounters the 6-TG, according to the base complementation pairing rule, the 6-TG can be pairing to the Cy with the help of the hydrogen bond and π - π stacking [1]. Therefore, the two arms (Cy) can capture the 6-TG tightly through the hydrogen bonds and π - π stackings among them. Besides, the ligand changes water into 6-TG, forming a new coordination bond, that is, the Eu^{III} -dtpa-bis(cytosine) can combine with 6-TG exclusively with the hydrogen bonds, π - π stackings and coordination bond [33]. So, the Eu^{III} -dtpa-bis(cytosine) in aqueous solution emits a moderately strong green fluorescence at 575 nm and a strong orange fluorescence at 525 nm owing to the presence of 6-TG [34-37]. Furthermore, we found that the binding sites or bonding styles between Eu^{III} -dtpa-bis(cytosine) and 6-TG could be changed with the increasing concentration of 6-TG, which causes the fluorescence emission at wavelength of 525 nm to decrease until disappear. When the concentration of 6-TG ranges in $4.50 - 6.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, most of 6-TG molecules locate at the middle position of the two arms (Cy) molecules with the hydrogen bond and π - π stackings as well as the coordination bond with Eu^{III} ions. And that, when the concentration of 6-TG is bigger than $6.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, overmany 6-TG are on the two external sides of the two arms with the hydrogen bond

and π - π stacking. Thus, the fluorescence intensity at 525 nm instead begins to decrease.

It is well known that 6-TG has a similar chemical composition and molecular structure with that of Gu, except one sulfur atom. Thus, when Gu was introduced to the Eu^{III} -dtpa-bis(cytosine) solution, it can also bind with the Eu^{III} -dtpa-bis(cytosine) also forming a new Eu^{III} -dtpa-bis(cytosine)-Gu complex through the hydrogen bonds, π - π stackings and coordination bond. Due to the nitrogen atom coordination to Eu^{III} ion replacing the water (H_2O) molecule, the fluorescence of Eu^{III} -dtpa-bis(cytosine) around 575 nm was also enhanced, but there was not new fluorescence-emission peak formed around 525 nm. Because of the different coordination atoms, as shown in **Fig. 8**, Eu^{III} -dtpa-bis(cytosine)-6-TG and Eu^{III} -dtpa-bis(cytosine)-Gu give two different fluorescence spectra. Therefore it can be confirmed that the Eu^{III} -dtpa-bis(cytosine) as a fluorescence probe not only detects sensitively the 6-TG through two fluorescence-emission peaks at 525 nm and 575 nm but also does the Gu through one fluorescence-emission peak at 575 nm.

Fig. 8.

3.8. Detection of 6-TG by using Eu^{III} -dtpa-bis(cytosine) in actual urine

The analysis and detection of 6-TG is considered to be an important task in the field of clinical pathology, which is helpful to not only improve the therapeutic effect of 6-TG, but also know the adverse effects caused by the overdosage in time. Based on the above researches and previous reports [1,7], it could be considered that the Eu^{III} -dtpa-bis(cytosine) complex as a fluorescence probe might be applied to the detection of 6-TG in actual urine. In this experiment, the samples were prepared through adding a certain amount of 6-TG standard solutions and the corresponding fluorescence spectra were determined in **Fig. 9**. From **Fig. 9(a)** it has been found that the pure urine and the 6-TG in urine both only show weak fluorescence, and the Eu^{III} -dtpa-bis(cytosine) in urine displays a relatively strong

fluorescence emission. However, the fluorescence intensity of Eu^{III} -dtpa-bis(cytosine) in urine could be increased significantly when the 6-TG was added. According to this phenomenon, it can be confirmed that the Eu^{III} -dtpa-bis(cytosine) complex as a fluorescence probe can be used to detect the 6-TG in real urine samples. Also, it can be seen from **Fig. 9(b)** that the position of the fluorescence-emission peak of Eu^{III} -dtpa-bis(cytosine) in urine gradually moves towards long wavelength along with the increase of 6-TG concentration in the range of $5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ - $5.00 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$. It is more helpful to detect the 6-TG in real urine samples. Therefore, the developed fluorescent probe (Eu^{III} -dtpa-bis(cytosine)) could be reliable and effective for the determination of 6-TG in real urine samples.

4. Conclusion

In this work, we have designed and synthesized a novel fluorescent probe (Eu^{III} -dtpa-bis(cytosine)) consisted of Eu^{3+} ion and dtpa-bis(cytosine) ligand, for potentially sensing the 6-TG in biological and pharmaceutical samples. The effects of pH value and interference of 6-MP, Gu, Cy, Ad, Hy, Xa, Th, Ur and Ua on the detection of 6-TG using Eu^{III} -dtpa-bis(cytosine) as fluorescent probe were also studied. In the neutral condition the mixed solution of Eu^{III} -dtpa-bis(cytosine) and 6-TG displays a strongest fluorescence intensity at 575 nm. The presence of some other similar base compounds does not affect the detection of 6-TG. A linear response of fluorescence intensity of Eu^{III} -dtpa-bis(cytosine) at 575 nm as a function of 6-TG concentration is in the range of $0.00 - 7.50 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$. The detection limit is about $5.11 \times 10^{-8} \text{ mol}\cdot\text{L}^{-1}$. In addition, we have also studied the detection of 6-TG in the actual urines. The experimental results indicates that the Eu^{III} -dtpa-bis(cytosine) as fluorescence probe to detect 6-TG exhibits high sensitivity and recognizability as well as good specific binding ability.

Acknowledgments

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Content of Scheme, Figures and Table:

Scheme 1. The structure and synthetic route of the dtpa-bis(cytosine) ligands.

Fig. 1. Chemical structure of 6-Thioguanine (6-TG), 6-Mercaptopurine (6-MP), Guanine (Gu), Cytosine (Cy), Adenine (Ad), Hypoxanthine (Hy), Xanthine (Xa), Thymine (Th), Uracil (Ur) and Uric acid (Ua).

Fig. 2. Infrared spectra of Cytosine (Cy), diethylenetriamine pentaacetic acid (dtpa) and dtpa-bis(cytosine). (dtpa-DC: dtpa-bis(cytosine).)

Fig. 3. UV-vis spectra of Eu(III), dtpa-bis(cytosine) and Eu-dtpa-bis(cytosine) in Tris-HCl buffer solution. ([Eu(III)] = [dtpa-bis(cytosine)] = [Eu-dtpa-bis(cytosine)] = $5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, [Tris-HCl] = $50 \text{ mmol}\cdot\text{L}^{-1}$, pH = 7.40 and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$. dtpa-DC: dtpa-bis(cytosine), Eu^{3+} -dtpa-DC: Eu-dtpa-bis(cytosine).)

Fig. 4. Fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) (a) of Eu-dtpa-bis(cytosine) in Tris-HCl buffer solution upon the addition of 6-TG, Gu, Cy, Hy, Xa, Ur, Ad, Ua, Th and MP and the corresponding fluorescence intensities at 525 nm (b-1) and at 575 nm (b-2). ([Eu-dtpa-bis(cytosine)] = [6-TG] = [Gu] = [Cy] = [Hy] = [Xa] = [Ur] = [Ad] = [Ua] = [Th] = [MP] = $5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, [Tris-HCl] = $50 \text{ mmol}\cdot\text{L}^{-1}$, pH = 7.40 and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$. Eu-dtpa-DC: Eu-dtpa-bis(cytosine).)

Fig. 5. Fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) (a) of Eu-dtpa-bis(cytosine) and Eu-dtpa-bis(cytosine)+6-TG in Tris-HCl buffer solution upon the addition of Gu, Cy, Hy, Xa, Ur, Ad, Ua, Th and MP and the corresponding fluorescence intensities at $\lambda_{\text{em}} = 525 \text{ nm}$ (b-1) and at $\lambda_{\text{em}} = 575 \text{ nm}$ (b-2). ([Eu-dtpa-bis(cytosine)] = [6-TG] = [Gu] = [Cy] = [Hy] = [Xa] = [Ur] = [Ad] = [Ua] = [Th] = [MP] = $5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, [Tris-HCl] = $50 \text{ mmol}\cdot\text{L}^{-1}$, pH = 7.40 and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$. Eu-dtpa-DC: Eu-dtpa-bis(cytosine).)

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Fig. 8. The reaction mechanism and process of Eu-dtpa-bis(cytosine) with 6-TG and Gu.

Fig. 9. Fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) (a) of Eu-dtpa-bis(cytosine), 6-TG and Eu-dtpa-bis(cytosine)+6-TG in urine as well as blank urine and change (b) of fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) of Eu-dtpa-bis(cytosine) in urine with different 6-TG concentrations. ($[\text{Eu-dtpa-bis(cytosine)}] = 5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, $[\text{6-TG}] = 5.00 \times 10^{-5} - 5.00 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$, $\text{pH} = 7.40$ and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$. Eu-dtpa-DC: Eu-dtpa-bis(cytosine).)

Table 1. Comprasion of the proposed method with others.

Table:

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Detection method	Linear range ($\times 10^{-6}$ mol/L)	LOD ($\mu\text{mol/L}$)	Reference
SERS	0.1-15.0	0.065	[17]
HPLC	0.15-6.0	0.12	[10]
RP-HPLC	0.6-24	0.72	[11]
Electrochemisty	0.1-11	0.2	[12]
Fluorometry	0.015-0.75	0.01	[16]
LSPR	0.1-10.02	0.01	[15]
Fluorometry	5.0-50	0.051	This work

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$5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$.)

Fig. 8. The reaction mechanism and process of Eu-dtpa-bis(cytosine) with 6-TG and Gu.

Fig. 9. Fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) (a) of Eu-dtpa-bis(cytosine), 6-TG and Eu-dtpa-bis(cytosine)+6-TG in urine as well as blank urine and change (b) of fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) of Eu-dtpa-bis(cytosine) in urine with different 6-TG concentrations. ($[\text{Eu-dtpa-bis(cytosine)}] = 5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, $[\text{6-TG}] = 5.00 \times 10^{-5} - 5.00 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$, $\text{pH} = 7.40$ and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$. Eu-dtpa-DC: Eu-dtpa-bis(cytosine).)

Table 1. Comprasion of the proposed method with others.

Scheme:

Scheme 1. The structure and synthetic route of the dtpa-bis(cytosine) ligands.

Figures:

Fig. 1. Chemical structure of 6-Thioguanine (6-TG), 6-Mercaptopurine (6-MP), Guanine (Gu), Cytosine (Cy), Adenine (Ad), Hypoxanthine (Hy), Xanthine (Xa), Thymine (Th), Uracil (Ur) and Uric acid (Ua).

Fig. 2. Infrared spectra of Cytosine (Cy), diethylenetrinitrime pentaacetic acid (dtpa) and dtpa-bis(cytosine). (dtpa-DC: dtpa-bis(cytosine).)

Fig. 3. UV-vis spectra of Eu(III), dtpa-bis(cytosine) and Eu-dtpa-bis(cytosine) in Tris-HCl buffer solution. ([Eu(III)] = [dtpa-bis(cytosine)] = [Eu-dtpa-bis(cytosine)] = $5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, [Tris-HCl] = $50 \text{ mmol}\cdot\text{L}^{-1}$, pH = 7.40 and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$. dtpa-DC: dtpa-bis(cytosine), Eu^{3+} -dtpa-DC: Eu-dtpa-bis(cytosine).)

Fig. 4. Fluorescence spectra ($\lambda_{ex} = 288 \text{ nm}$) (a) of Eu-dtpa-bis(cytosine) in Tris-HCl buffer solution upon the addition of 6-TG, Gu, Cy, Hy, Xa, Ur, Ad, Ua, Th and MP and the corresponding fluorescence intensities at 525 nm (b-1) and at 575 nm (b-2). ($[\text{Eu-dtpa-bis(cytosine)}] = [\text{6-TG}] = [\text{Gu}] = [\text{Cy}] = [\text{Hy}] = [\text{Xa}] = [\text{Ur}] = [\text{Ad}] = [\text{Ua}] = [\text{Th}] = [\text{MP}] = 5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, $[\text{Tris-HCl}] = 50 \text{ mmol}\cdot\text{L}^{-1}$, $\text{pH} = 7.40$ and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$. Eu-dtpa-DC: Eu-dtpa-bis(cytosine).)

Fig. 5. Fluorescence spectra ($\lambda_{ex} = 288 \text{ nm}$) (a) of Eu-dpa-bis(cytosine) and Eu-dtpa-bis(cytosine)+6-TG in Tris-HCl buffer solution upon the addition of Gu, Cy, Hy, Xa, Ur, Ad, Ua, Th and MP and the corresponding fluorescence intensities at $\lambda_{em} = 525 \text{ nm}$ (b-1) and at $\lambda_{em} = 575 \text{ nm}$ (b-2). ($[\text{Eu-dtpa-bis(cytosine)}] = [6\text{-TG}] = [\text{Gu}] = [\text{Cy}] = [\text{Hy}] = [\text{Xa}] = [\text{Ur}] = [\text{Ad}] = [\text{Ua}] = [\text{Th}] = [\text{MP}] = 5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$, $[\text{Tris-HCl}] = 50 \text{ mmol}\cdot\text{L}^{-1}$, $\text{pH} = 7.40$ and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$. Eu-dtpa-DC: Eu-dtpa-bis(cytosine).)

Fig. 6. Fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) of Eu-dtpa-bis(cytosine) in Tris-HCl buffer solution upon the addition of 6-TG with different concentrations of $0.00 \text{ mol} \cdot \text{L}^{-1} - 5.00 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ (a) and $4.50 \text{ mol} \cdot \text{L}^{-1} - 7.50 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$ (b) and the corresponding intensities at $\lambda_{\text{em}} = 525 \text{ nm}$ (c-1) and $\lambda_{\text{em}} = 575 \text{ nm}$ (c-2). ($[\text{Eu-dtpa-bis(cytosine)}] = 5.00 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$, $[\text{Tris-HCl}] = 50 \text{ mmol} \cdot \text{L}^{-1}$, $\text{pH} = 7.40$ and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ } ^\circ\text{C}$. Eu-dtpa-DC: Eu-dtpa-bis(cytosine).)

Fig. 7. Fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) (a) of Eu-dtpa-bis(cytosine) upon the addition of 6-TG at different pH values (1.50 - 10.50) and corresponding intensities ($\lambda_{\text{em}} = 580 \text{ nm}$) (b). ($[\text{Eu-dtpa-bis(cytosine)}] = [\text{6-TG}] = 5.00 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$.)

Fig. 8. The reaction mechanism and process of Eu-dtpa-bis(cytosine) with 6-TG and Gu.

Fig. 9. Fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) (a) of Eu-dtpa-bis(cytosine), 6-TG and Eu-dtpa-bis(cytosine)+6-TG in urine as well as blank urine and change (b) of fluorescence spectra ($\lambda_{\text{ex}} = 288 \text{ nm}$) of Eu-dtpa-bis(cytosine) in urine with different 6-TG concentrations. ($[\text{Eu-dtpa-bis(cytosine)}] = 5.00 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$, $[\text{6-TG}] = 5.00 \times 10^{-5} - 5.00 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1}$, $\text{pH} = 7.40$ and $T_{\text{solu}} = 25.00 \pm 0.02 \text{ }^{\circ}\text{C}$. Eu-dtpa-DC: Eu-dtpa-bis(cytosine).)

Graphical Abstract

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Articles

Cytosine derivatized diethylenetriaminepentaacetic acid (dtpa) and Eu(III) complexes for selective recognition of 6-Thioguanine

Fengyun Tian^a, Fan Yang^a, Xiaoqing Jiang^a, Xuekai Dou^b, Guanhong, Liu^b, Peipei, Ren^b, Jun Wang^{a,*}, Youtao Song^{b,*}, Zhiqiang Xing^a, Guangxi Han^a

A novel Eu^{III}-dtpa-bis(cytosine) complex was designed and synthesized to serve as a fluorescent probe for the determination of 6-Thioguanine (6-TG). The detection mechanism of 6-TG using this fluorescent probe was studied by ultraviolet-visible (UV-vis) and fluorescence spectroscopy. Meanwhile, the interference of other some similar base compounds on the detection of 6-TG using Eu^{III}-dtpa-bis(cytosine) probe was also studied. It was found that the sensitivity and specificity of the Eu^{III}-dtpa-bis(cytosine) in the 6-TG detection could be confirmed. A linear response of fluorescence emission intensity of Eu^{III}-dtpa-bis(guanine) at 575 nm as a function of 6-TG concentration in the range of 0.00 - 7.50 × 10⁻⁵ mol·L⁻¹ was observed. The detection limit is about 5.11 × 10⁻⁷ mol·L⁻¹. The corresponding correlation coefficient of the linear equation was 0.989. In addition, we have also studied the detection of 6-TG in the actual urines. It was found that the developed fluorescent probe could be reliable and effective for the determination of 6-TG in the actual urines.