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Design and synthesis of novel adenine fluorescence probe based on Eu(III) complexes with dtpa-bis(guanine) ligand

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ABSTRACT

A novel adenine (Ad) fluorescence probe (Eu^{III}-dtpa-bis(guanine)) was designed and synthesized by improving experimental method based on the Eu(III) complex and dtpa-bis(guanine) ligand. The dtpa-bis(guanine) ligand was first synthesized by the acylation action between dtpaa and guanine (Gu), and the corresponding Eu(III) complex was successfully prepared through heat-refluxing method with dtpa-bis(guanine) ligand. As a novel fluorescence probe, the Eu^{III}-dtpa-bis(guanine) complex can detect adenine (Ad) with characteristics of strong targeting, high specificity and high recognition ability. The detection mechanism of the adenine (Ad) using this probe in buffer solution was studied by ultraviolet-visible (UV–vis) and fluorescence spectroscopy. When the Eu^{III}-dtpa-bis(guanine) was introduced to the adenine (Ad) solution, the fluorescence emission intensity was significantly enhanced. However, adding other bases such as guanine (Gu), xanthine (Xa), hypoxanthine (Hy) and uric acid (Ur) with similar composition and structure to that of adenine (Ad) to the Eu^{III}-dtpa-bis(guanine) solution, the fluorescence emission intensities are nearly invariable. Meanwhile, the interference of guanine (Gu), xanthine (Xa), hypoxanthine (Hy) and uric acid (Ur) on the detection of the adenine using Eu^{III}-dtpa-bis(guanine) probe was also studied. It was found that presence of these bases does not affect the detection of adenine (Ad). A linear response of fluorescence emission intensities of Eu^{III}-dtpa-bis(guanine) at 570 nm as a function of adenine (Ad) concentration in the range of 0.00–5.00 $\times 10^{-5}$ mol L^{-1} was observed. The detection limit is about 4.70 $\times 10^{-7}$ mol L^{-1}.

1. Introduction

Adenine (Ad) is an important component of nucleic acid which is not only participate in the synthesis of genetic material playing a crucial role in life process and fulfilling a variety of functions in the metabolism of the cell, but also has widespread influences on coronary and cerebral circulation [1–3]. It can also control of blood flow, prevention of cardiac arrhythmias, inhibition of neurotransmitter release and modulation of adenylate cyclase activity [4]. In addition, adenine (Ad) can be used as drugs for leukopenia caused by tumor chemotherapy disease, also used in acute granulocytopenia. The abnormal change of adenine (Ad) in organism may indicate the mutation and deficiency of the immune system leading to the manifestation of various diseases including epilepsy, cancer, tumorigenesis, mental retardation, HIV infection, Parkinson's disease, carcinoma and liver diseases [5–10]. Therefore, the determination of concentration level of adenine (Ad) is considered to be a challenging and important task in the field of physiology and clinical pathology [11].

In recent years, a variety of methods including high-performance liquid chromatography, ion-pairing liquid chromatography, micellar electrokinetic chromatography, electrochemistry, capillary electrophoresis, chemiluminescence, spectrophotometry, surface enhanced Raman scattering and mass spectrometry have been developed for the determination and quantification of adenine (Ad) in biological fluids [12–26]. In fact, these analytic methods were thought to be rapid and sensitive, but they also have several shortcomings such as high cost, high time consumption, tedious pretreatment steps and poor capture ability to substrates. Therefore, it is necessary to develop new method to detect adenine (Ad). Compared to these methods, the fluorescence probe methods not only have characteristics of high sensitivity, high accuracy and low detection concentration, but also have features of high specificity and strong targeting property [27–30]. It is well known that some Eu(III) complexes are successfully applied in materials science field as luminescent probe with their high and sharply spiked fluorescence emission efficiency, long lifetime and large Stokes shift [31–33]. Aminopolycarboxylic acid such as dtpa as a good chelating agent can form extraordinarily stable complexes with many rare earth metal ions and emit a specific fluorescence under excitation of light of appropriate wavelength [34]. However, as a good fluorescent probe, in addition to possessing obvious photo-responsive property, it should have

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good specificity, high recognition ability and strong choice ability. The Eu(III) complexes with some aminopolycarboxylic acid ligands can be used as fundamental fluorescence probes due to the obvious photo-responsive property. In order to make these fundamental fluorescence probes have better recognition ability and strong choice ability, it is necessary to pointedly modify the dtpa ligand by using some targeting compounds to detected object.

In this paper, the structure of dtpa was modified by guanine and formed a new ligand dtpa-bis(guanine). The Eu^{3+} ion can form a nine-coordinate Eu^{III}-dtpa-bis(guanine) complex with the new ligand dtpa-bis(guanine) [35–37]. In the Eu^{III}-dtpa-bis(guanine) complex as fluorescence people, two guanines at the two ends (up and down), like the two arms. When the Eu^{III}-dtpa-bis(guanine) encounters the adenine, whose molecular structure and chemical composition are similar to that of guanine, these two arms (guanines) can capture the adenine tightly. Because of the change of ligands from water to adenine forming the new coordination bond, the fluorescence intensity of Eu^{III}-dtpa-bis(guanine) was changed obviously. Due to the highly chemical similarity, the Eu^{III}-dtpa-bis(guanine) complex can combine with adenine exclusively. Thus, as adenine fluorescence probe the Eu^{III}-dtpa-bis(guanine) complex not only have the characteristics of high sensitivity, high accuracy and low detection concentration, but also the advantages of strong targeting, high recognition ability and choice ability. Subsequently, by means of fluorescence spectrum, it was found that the fluorescence emission intensity was significantly enhanced, when the adenine was introduced to the Eu^{III}-dtpa-bis(guanine) solution. While the other base compounds, such as guanine, xanthine, hypoxanthine and uric acid, were added to the Eu^{III}-dtpa-bis(guanine) solution, the fluorescence emission intensity was hardly changed. Meanwhile, in this work, the effects and interferences of guanine, hypoxanthine, xanthine and uric acid were also studied. It was found that the Eu^{III}-dtpa-bis(guanine) complex as fluorescence probe can detect adenine with specificity and not be affected by other base compounds. Therefore, the sensitivity and specificity of the Eu^{III}-dtpa-bis(guanine) complex in the adenine detection could be confirmed.

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 DMSO-d_6, D_2O and NaOH-*d1* as the solvent and tetramethysilane (TMS) as internal standard. Fluorescence determination experiments were carried out by fluorophotometer (Cary 300, Varian Company, USA) and the UV–vis absorption spectra were recorded with an UV–Vis spectrophotometer (Cary 50, Varian Company, USA).

2.2. Material

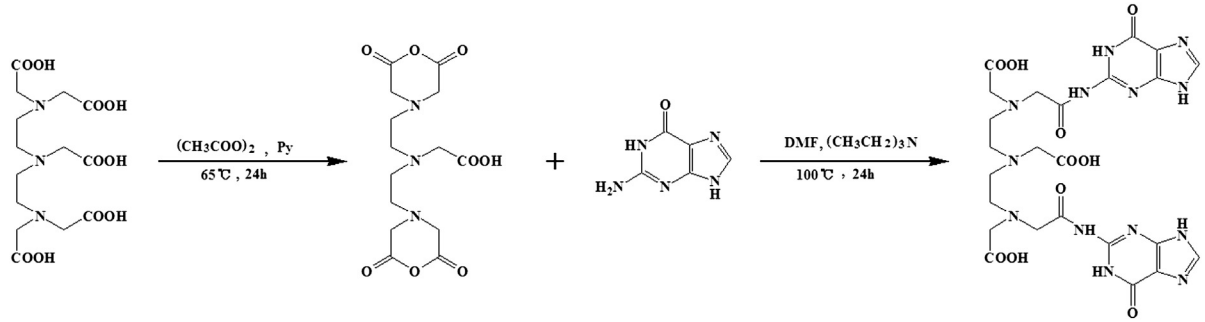
Diethylenetriamine pentaacetic acid (dtpa) and guanine (Gu) (A.R., Beijing SHLHT Science & Trade Co., Ltd., China) were purchased and used to synthesize the dtpa-bis(guanine) ligand. Adenine (Ad), hypoxanthine (Hy), xanthine (Xa) and uric acid (Ur) (A.R., Beijing SHLHT Science & Trade Co., Ltd., China) were purchased and used to be target client. $Eu(NO_3)_3 \cdot 6H_2O$ (99.999%, Yuelong Rare Earth Co., Ltd., China) was obtained to prepare the Eu^{III}-dtpa-bis(guanine) 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 (pH = 7.4 and [Tris-HCl] = 50 mmol L^{-1}) buffer solution in order to maintain the ionic strength and adjust the solution acidity.

2.3. Synthesis of Diethylenetriamine Pentaacetic Acid Dianhydride (dtpaa)

It must be pointed out that the diethylenetriamine pentaacetic acid dianhydride (dtpaa) is demand for the start of all experiments and its synthesis procedure is described in Scheme 1. 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 acid-binding agent under anhydrous condition. The mixed solution was stirred for one day under heat-refluxing at 65 °C. Afterwards, the reaction mixture was cooled down to room temperature, and the solvent was removed by reduced pressure filter. The residue washed twice by acetic anhydride and anhydrous diethyl ether. Finally, the residue was dried to give 6.50 g white powder under vacuum (52 kpa) at 80 °C with yield of 83%. FT-IR (KBr, cm^{-1}): 1642.41, 1772.10, 1821.08, 2341.42, 2820.47 and 2979.80. ^1H NMR (500 MHz, DMSO): d = 2.593 (t, 4H), 2.748 (t, 4H), 3.300 (s, 2H), 3.705 (s, 8H) and 11.013 (s, 1H).

2.4. Synthesis of dtpa-bis(guanine)

Dtpa-bis(guanine) ligand was synthesized by the aminolysis reaction between dtpaa and guanine and the synthesis procedure is shown in Scheme 1. Dtpaa (1.96 g, 55 mmol) was dissolved in DMF (50 mL) and Trithylamine as base under anhydrous condition. Subsequently, the guanine was added to the mixed solution slowly. The mixed solution was stirred 24 h under heat-refluxing at 100 °C. The mixture was then cooled down to room temperature. After the solvent was removed by vacuum filter, the white solid was obtained. The white solid was evaporated to dryness under vacuum (52 kpa) at 50 °C to give 2.30 g white powdery solid with yield of 92%. FT-IR (KBr, cm^{-1}): 1668, 1710, 2691, 2854, 2908, 3119 and 3321. ^1H NMR (500 MHz, DMSO): d = 2.010 (m, 2H), 2.717 (s, 2H), 2.807 (d, 2H), 2.460 (t, 8H), 3.254 (s, 4H), 3.301 (s, 6H), 7.503 (d, 2H), 7.973 (s, 2H) 8.010 (s, 2H) and 11.012 (s, 3H).



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

2.5. Synthesis of the Eu(III) Complex With dtpa-bis(guanine) Ligand

0.0870 g (0.13 mmol) dtpa-bis(guanine) was added to 50 mL Tris-HCl buffer solution. 0.0570 g (0.13 mmol) $Eu(NO_3)_3 \cdot 6H_2O$ powder was added to 50 mL Tris-HCl buffer solution. After that the two above buffer solution were mixed together and refluxed and stirred for one hour until solution became transparent, constanted volume in 250 mL volumetric flask and kept in refrigerator 4.0 °C as stock solution to be used.

2.6. Preparation of the Solution of Various Bases

0.017 g (0.13 mmol) adenine, 0.019 g (0.13 mmol) guanine, 0.019 g (0.13 mmol) xanthine, 0.017 g (0.13 mmol) hypoxanthine and 0.021 g (0.13 mmol) uric acid were dissolved in 20 mL Tris-HCl buffer solution and constanted in 250 mL volumetric flask to obtain 5.00×10^{-4} mol L^{-1} solution, respectively. The stock solutions were kept in refrigerator 4.0 °C. The stock solutions were diluted to required concentrations with Tris-HCl buffer solution when needed.

2.7. Determination of UV–Vis Spectra of Eu-dtpa-bis(guanine) solution

The dilute solution of Eu^{III}-dtpa-bis(guanine) and dtpa-bis(guanine) (5.00×10^{-5} mol L^{-1}) were prepared in Tris-HCl buffer solution. The solution of Eu^{3+} (5.00×10^{-5} mol L^{-1}) was also prepared in Tris-HCl buffer solution. These above solutions were put in a quartz cell (10.0 mm width), and the absorption spectra were recorded at room temperature.

2.8. Determination of Fluorescence Spectra of Eu-dtpa-bis(guanine) Solution With Adenine

The dilute solution of Eu^{III}-dtpa-bis(guanine), adenine, guanine, hypoxanthine, xanthine and uric acid (5.00×10^{-5} mol L^{-1}) were prepared in Tris-HCl solution, respectively. These above solutions were placed in quartz cell (10.0 mm width) and all fluorescence spectra were carried out at room temperature.

3. Results and Discussion

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

Dtpaa was synthesized by the decarboxylic reaction of dtpa. And the dtpa-bis(guanine) was synthesized by the acylation reaction between dtpaa and guanine. All obtained compounds were characterized by ^1H NMR (the corresponding data were given in the experimental part) and Fourier transform infrared spectra. The comparison of FT-IR spectra among dtpa, guanine and dtpa-bis(guanine) is shown in Fig. 1. It can be found that the ν(C—N) of dtpa-bis(guanine) appears at 940 cm^{-1}, which displays a red-shift by 10 cm^{-1} compared with the 950 cm^{-1} of guanine. The ν_s(COO) of dtpa-bis(guanine) appears at 1418 cm^{-1}, which displays a red-shift by 22 cm^{-1} compared with that 1397 cm^{-1} of dtpa. Furthermore, the ν_{as}(COO) of dtpa-bis(guanine) appears at 1710 cm^{-1}, while the ν_{as}(COO) of dtpa appears at 1732 cm^{-1}, so the dtpa-bis(guanine) displays a red-shift by 22 cm^{-1}. And that the ν_{as}(CONH) of dtpa-bis(guanine) appears at 1668 cm^{-1} and the characteristic broad absorption peaks of hydroxy group appears at 3446 cm^{-1}. These shifts confirmed that the dtpa-bis(guanine) was synthesized by the acylation action between dtpaa and guanine.

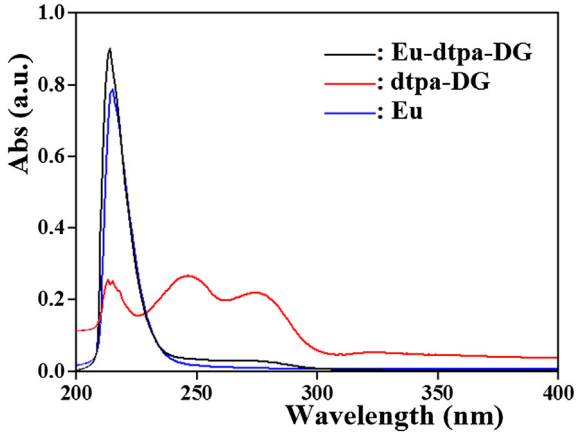


Fig. 2. UV–vis spectra of Eu(III) complexes (5.00×10^{-5} mol L^{-1}) and Eu-dtpa-bis(guanine) complex (5.00×10^{-5} mol L^{-1}) and dtpa-bis(guanine) (5.00×10^{-5} mol L^{-1}) solution. (Eu-dtpa-DG: Eu-dtpa-bis(guanine)).

3.2. UV–Vis Spectra of Eu-dtpa-bis(guanine) Solution

The UV–vis absorption spectra of dtpa-bis(guanine), Eu^{III}-dtpa-bis(guanine) and Eu(III) ion in Tris-HCl (pH = 7.4 and [Tris-HCl] = [NaCl] = 50 mmol L^{-1}) buffer solution solutions (5.00×10^{-5} mol L^{-1}) were all shown in Fig. 2. It can be found that the Eu(III) solution gives a maximum absorption peak at about 220 nm wavelength (λ_{max}). And that, for dtpa-bis(guanine) its solution displays three absorption peaks at 214 nm, 242 nm and 274 nm, respectively. Upon the addition of dtpa-bis(guanine), the formed Eu^{III}-dtpa-bis(guanine) complex also gives a maximum absorption peak at 220 nm, but slightly becomes stronger compared with that of Eu^{3+} ion solution. The other absorption peaks obviously become weaker compared with those of dtpa-bis(guanine) solution. It indicates that the Eu^{3+} ion and dtpa-bis(guanine) can change their electronic structure each other resulting in the potential acting as fluorescence probe.

3.3. Fluorescence Spectra of Eu-dtpa-bis(guanine) Solution With Adenine

The fluorescence spectra of Eu^{III}-dtpa-bis(guanine) complex and corresponding mixed solutions with a series of base compounds, such as adenine (Ad), guanine (Gu), hypoxanthine (Hy), xanthine (Xa) and

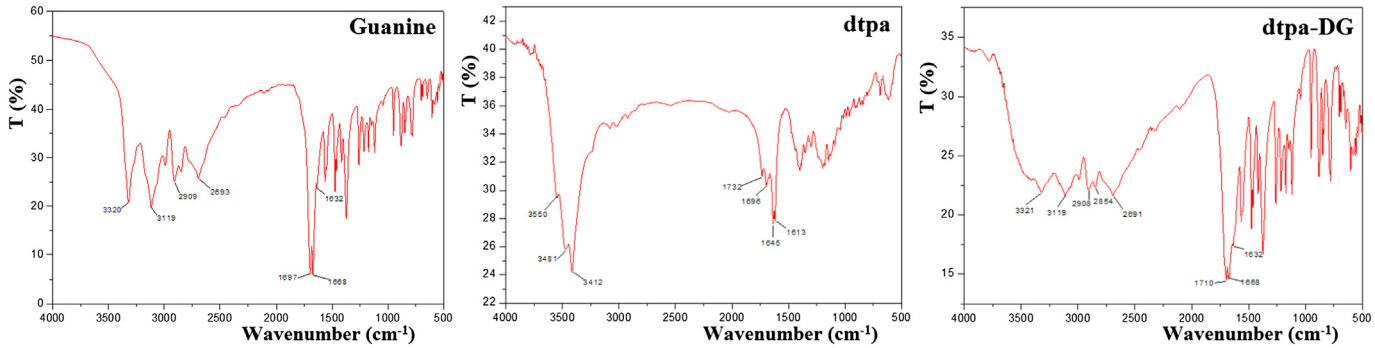


Fig. 1. Infrared spectra of guanine (Gu), diethylenetriamine pentaacetic acid (dtpa) and dtpa-bis(guanine). (Eu-dtpa-DG: Eu-dtpa-bis(guanine)).

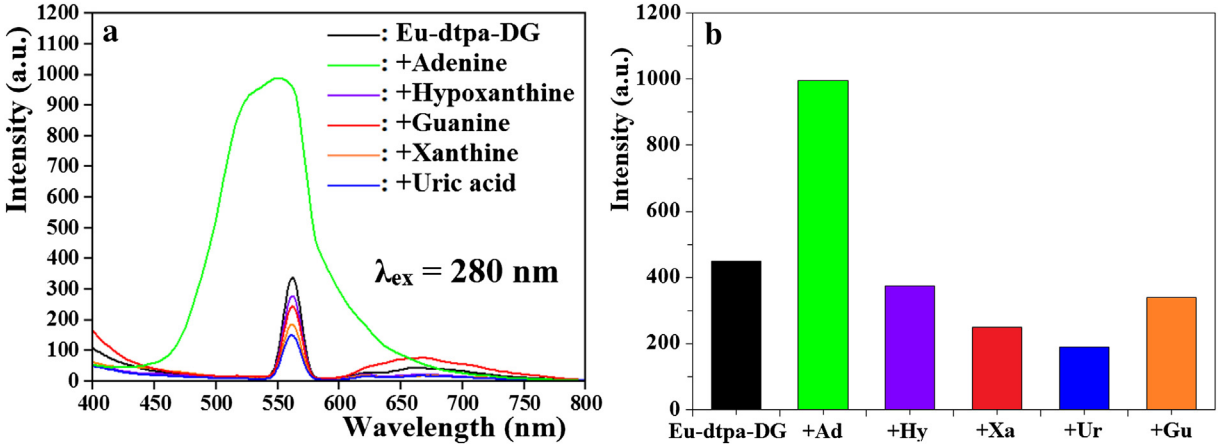


Fig. 3. Fluorescence spectra (λ_{ex} = 280 nm) (a) of Eu-dtpa-bis(guanine) in Tris-HCl buffer solution upon the addition of adenine (Ad), hypoxanthine (Hy), xanthine (Xa), uric acid (Ur) and guanine (Gu) and the corresponding fluorescence intensities (b). ([Eu-dtpa-bis(guanine)] = [Ad] = [Hy] = [Xa] = [Ur] = [Gu] = 5.00×10^{-5} mol L^{-1}, [Tris-HCl] = 50 mmol L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C. Eu-dtpa-DG: Eu-dtpa-bis(guanine)).

uric acid (Ur), are depicted in Fig. 3. It can be found that, under the excitation of ultraviolet light at 280 nm, the Eu^{III}-dtpa-bis(guanine) complex in aqueous solution emita moderately strong green fluorescence at maximum wavelength at 560 nm owing to the presence of Eu^{3+} ion. When the adenine (Ad) was introduced to the Eu^{III}-dtpa-bis(guanine) solution, the fluorescence emission intensity was significantly enhanced about 2.5 times. While with the addition of guanine (Gu), hypoxanthine (Hy), xanthine (Xa) and uric acid (Ur) to the Eu^{III}-dtpa-bis(guanine) solution, the fluorescence emission intensity was obviously decreased. That is, the guanine (Gu), hypoxanthine (Hy), xanthine (Xa) and uric acid (Ur) can quench the fluorescence of Eu^{III}-dtpa-bis(guanine) solution more or less and the quenched order is as uric acid (Ur) > xanthine (Xa) > guanine (Gu) > hypoxanthine (Hy). It could be confirmed that a sensitive response of Eu^{III}-dtpa-bis(guanine) solution was observed when only adenine (Ad) was added, though these base compounds have similar molecular structure and chemical composition. In addition, it can be estimated that the adenine (Ad) adopts a different interaction mode with Eu^{III}-dtpa-bis(guanine) is from other base compounds (guanine (Gu), hypoxanthine (Hy), xanthine (Xa) and uric acid (Ur). It suggested that the Eu^{III}-dtpa-bis(guanine) complex can be used as a selective fluorescence probe to detect adenine (Ad).

3.4. Effects of Adding Other Bases on Fluorescence Spectrum of Eu^{III}-dtpa-bis(guanine) and Adenine (Ad) Solution

The effects of other bases including guanine (Gu), hypoxanthine (Hy), xanthine (Xa) and uric acid (Ur) on the fluorescence of Eu^{III}-dtpa-bis(guanine) and adenine (Ad) solution were researched. As shown in Fig. 4, when adding guanine (Gu), hypoxanthine (Hy), xanthine (Xa) and uric acid (Ur) into the Eu^{III}-dtpa-bis(guanine) and adenine (Ad) solution, respectively, the fluorescence emission intensity and peak position are hardly changed. It can be predicted that the presence of these base compounds will not disturb the detection and analysis of adenine (Ad) due to their different interaction mode with Eu^{III}-dtpa-bis(guanine) complex. It indicates that the Eu^{III}-dtpa-bis(guanine) complex as fluorescence probe to detect adenine (Ad) exhibits high sensitivity and specificity as well as good recognition ability. Moreover, it can be confirmed that the use of guanine (Gu) combined with the Eu^{III}-dtpa forming Eu^{III}-dtpa-bis(guanine) complex brings the specific

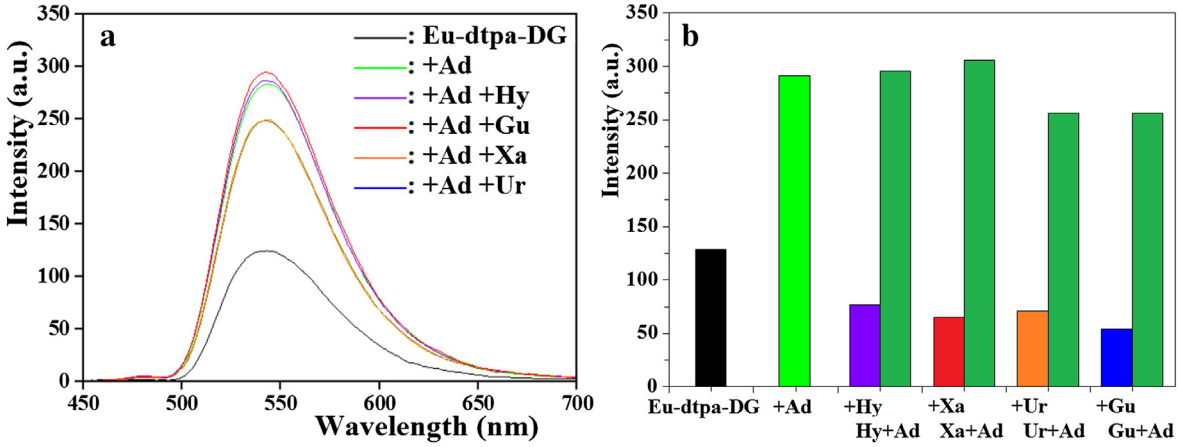


Fig. 4. Fluorescence spectra (λ_{ex} = 280 nm) (a) of Eu-dtpa-bis(guanine) and Eu-dtpa-bis(guanine) + A (adenine) in Tris-HCl buffer solution upon the addition of hypoxanthine (Hy), xanthine (Xa), uric acid (Ur) and guanine (Gu) and the corresponding fluorescence intensities (b) at λ_{em} = 560 nm. ([Eu-dtpa-bis(guanine)] = [Ad] = [Hy] = [Xa] = [Ur] = [Gu] = 5.0×10^{-5} mol L^{-1}, [Tris-HCl] = 50 mmol L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C. Eu-dtpa-DG: Eu-dtpa-bis(guanine)).

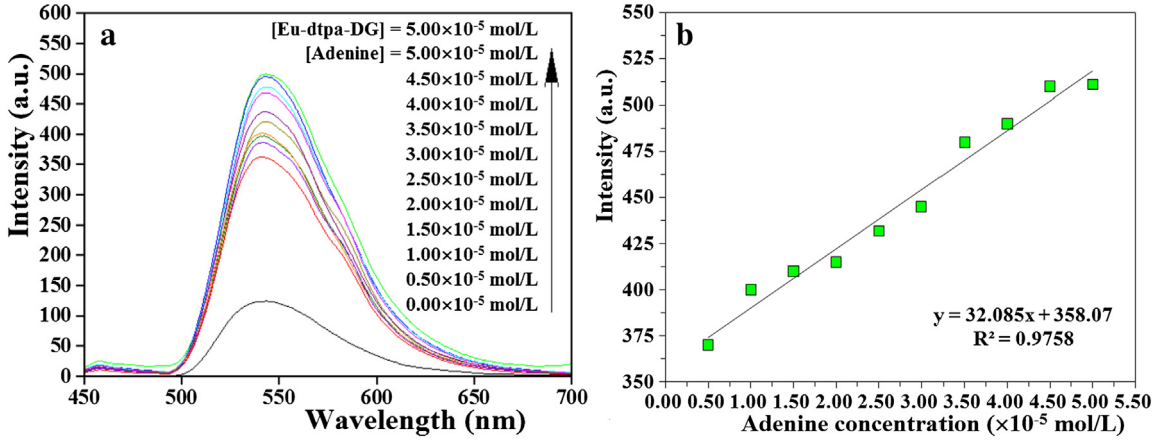


Fig. 5. Fluorescence spectra ($\lambda_{ex} = 280$ nm) (a) of Eu-dtpa-bis(guanine) in Tris-HCl buffer solution upon the addition of adenine (Ad) with different concentrations and linear responses (b) of Eu-dtpa-bis(guanine) as a function of adenine (Ad) concentrations (0.00–5.00 × 10^{-5} mol L^{-1}). ([Eu-dtpa-bis(guanine)] = 5.00 × 10^{-5} mol L^{-1}, [Tris-HCl] = 50 mmol L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C. Eu-dtpa-DG: Eu-dtpa-bis(guanine)).

targeting to adenine (Ad) due to their similar molecular structure and chemical composition.

3.5. Fluorescence Spectra of Eu^{III}-dtpa-bis(guanine) Solution With Adenine (Ad) Concentration

It can be seen from Fig. 5(a) that the fluorescence emission intensity is obviously enhanced after adding adenine (Ad), and then further increases gradually with increasing adenine (Ad) concentration. It indicates that this fluorescence change of Eu^{III}-dtpa-bis(guanine) complex may be used for the detection and analysis of adenine (Ad). In addition, a linear response of fluorescence intensities of Eu^{III}-dtpa-bis(guanine) solution as a function of adenine (Ad) concentration at 570 nm was also observed in Fig. 5(b) in the range of 0.00–5.00 × 10^{-5} mol L^{-1}. The corresponding linear equation is $y = 32.085x + 358.07$ ($R = 0.9758$), where y is the fluorescence intensity at 570 nm measured at a given adenine (Ad) concentration and x represents the concentration of added adenine (Ad). According to the formula of LOD (limit of detection): LOD = 3σ/s, the calculated LOD is about 4.70 × 10^{-7} mol L^{-1}.

It can be seen from Table 1 that the method proposed in this work showed a relatively wide detection range and low detection limit compared with those reported in literatures. Particularly, due to the high sensitivity and specificity to adenine (Ad) and outstanding anti-interference to other base compounds with similar molecular structures and chemical compositions to adenine (Ad), the Eu^{III}-dtpa-bis(guanine) complex can be used as an excellent fluorescence probe to detect adenine (Ad).

3.6. Reaction Mechanism and Process of Eu^{III}-dtpa-bis(guanine) With Adenine (Ad)

As shown in Fig. 6, the binding mechanism and process of Eu^{III}-dtpa-bis(guanine) as fluorescence probe with adenine (Ad) was proposed. It is well known that the Eu^{3+} ion can form nine-coordinate complexes with various aminopolycarboxylic acid ligands [35–37]. For this newly synthesized dtpa-bis(guanine) ligand, which is modified dtpa by two guanines at its two ends (up and down), respectively, it also is an eight-dentate ligand. Therefore, in the Eu^{III}-dtpa-bis(guanine) complex, as ninth coordination ligand one water molecule (H_2O) should be coordinated with Eu^{III} ion. It was inferred that the Eu^{3+} is coordinated with the three nitrogen atoms and five oxygen atoms from dtpa-bis(guanine) ligand and one oxygen atom from H_2O molecule in Eu^{III}-dtpa-bis(guanine) complex. Upon addition of the adenine (Ad), Because the adenine (Ad) and guanine (Gu) both have flat molecular structure, the π-π stacking binding will be formed between adenine (Ad) and guanine (Gu) in Eu^{III}-dtpa-bis(guanine) due to their similar molecular structures and chemical compositions.

Figuratively, like two arms the guanines (Gu) can capture adenine (Ad) tightly and then two guanines (Gu) and one adenine (Ad) form a tripolymer layer structure. With the help of π-π stacking bonding, the adenine (Ad) can be near to Eu^{3+} ion in Eu^{III}-dtpa-bis(guanine) complex and form coordination bond. Moreover, the formation of π-π stacking binding makes the nitrogen atom (or amino group) of adenine (Ad) further be closed to the Eu^{3+} ion until forming a stable and complete coordination bond. At the same time, the original coordinate water molecule will be replaced by the closer adenine (Ad). Under normal conditions, the nitrogen atom is difficult to replace the oxygen atom. Nevertheless, due to the presence of π-π stacking bonding the adenine (Ad) is forced to be closed to the Eu^{3+} ion in Eu^{III}-dtpa-bis(guanine) complex forming a new coordination bond. Because of the change of ligand field around the Eu^{3+} ion, the fluorescence intensity of Eu^{III}-dtpa-bis(guanine) solution is also changed obviously. That is, the fluorescence intensity was distinctly enhanced after adding the adenine (Ad). In addition, only in the presence of adenine (Ad) the Eu^{III}-dtpa-bis(guanine) solution gives greater fluorescence intensity. In contrast,

Table 1
Comparison of the proposed method with others.

Detection method	Linear range (×10^{-6} mol/L)	LOD (×10^{-6} mol/L)	R	Reference
Electrochemical luminescence	5.0–100	1.40	0.9980	[4]
Electrochemistry	2.5–150	0.75	0.9993	[10]
Electrochemistry	1–132	0.70	–	[14]
Electrochemistry	2–200	0.58	0.9956	[15]
Electrochemistry	25–200	0.76	0.9980	[16]
Electrochemistry	1.0–46	0.33	0.9889	[17]
Fluorometry	0.6–90	0.08	0.9993	[21]
Fluorometry	1.3–66	0.53	0.9926	[22]
Fluorometry	4.96 × 10^{-2}–1.09	0.019	0.9991	[23]
Fluorometry	5.0–50	0.47	0.9758	This work

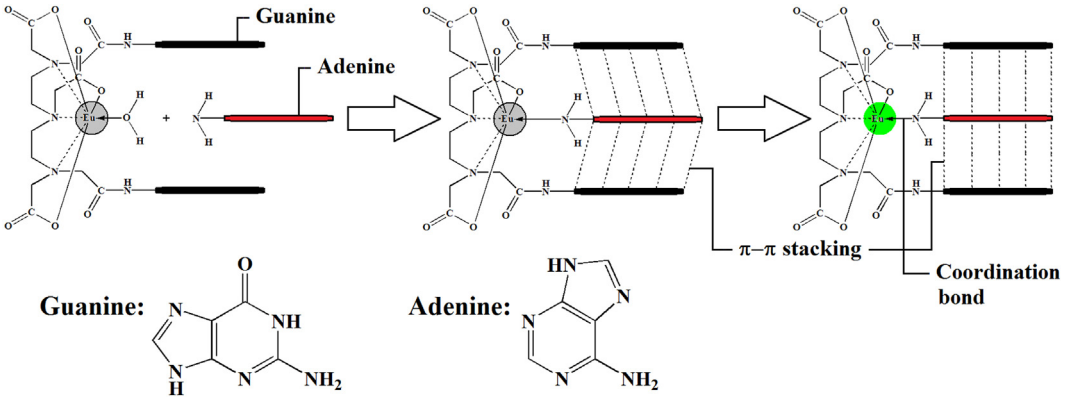


Fig. 6. The structure of Eu-dtpa-bis(guanine) + Ad (adenine) and reaction process of Eu-dtpa-bis(guanine) and adenine (Ad).

upon the addition of other base compounds including guanine (Gu), hypoxanthine (Hy), xanthine (Xa) and uric acid (Ur) to the Eu^{III}-dtpa-bis(guanine) solution, the fluorescence emission intensity was slightly quenched. Thus, it was confirmed that the sensitivity and specificity of Eu^{III}-dtpa-bis(guanine) complex as a fluorescence probe to detect adenine (Ad).

4. Conclusion

In this presented work, the adenine fluorescence probe (Eu^{III}-dtpa-bis(guanine)) was successfully designed and synthesized based on Eu(III) complex with new dtpa-bis(guanine) ligand. In the Eu^{III}-dtpa-bis(guanine) complex, two guanine molecules whose composition and structure are similar to that of adenine at the two ends (up and down), can combine with adenine exclusively with the help of π-π stacking interaction between adenine and guanine. Due to the formation of coordination bond of adenine with Eu(III) ion in the Eu^{III}-dtpa-bis(guanine) complex, the fluorescence intensity was enhanced clearly. The addition of other bases such as guanine, xanthine, hypoxanthine and uric acid to the Eu^{III}-dtpa-bis(guanine) solution does not obviously change the fluorescence emission intensity. Therefore, it was confirmed that the Eu^{III}-dtpa-bis(guanine) as a fluorescence probe has sensitivity and specificity in the detection of adenine. Furthermore, a linear response of fluorescence emission intensity of Eu^{III}-dtpa-bis(guanine) at 570 nm as a function of adenine concentration was observed in the range of 0.00–5.00 $\times 10^{-5}$ mol L^{-1}. According to the formula of LOD (limit of detection), the calculated limit of detection is about 4.70 $\times 10^{-7}$ mol L^{-1}.

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