Accepted Manuscript

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PII:	S1386-1425(18)30545-6
DOI:	doi:10.1016/j.saa.2018.06.011
Reference:	SAA 16170
To appear in:	*Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*
Received date:	7 March 2018
Revised date:	21 May 2018
Accepted date:	2 June 2018

Please cite this article as: Fan Yang, Zhiyue Yu, Xinyi Li, Peipei Ren, Guanhong Liu, Youtao Song, Jun Wang , Design and synthesis of a novel lanthanide fluorescent probe (TbIII-dtpa-bis(2,6-diaminopurine)) and its application to the detection of uric acid in urine sample. Saa (2017), doi:10.1016/j.saa.2018.06.011

Spctrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 00 (2018) 0000-0000

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Received 00 June 2018; received in revised form 00 June 2018; accepted 00 June 2018; Available online 00 June 2018

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http://dx.doi.org/00.0000/j.saa.2018.00.000

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Design and synthesis of a novel lanthanide fluorescent probe (Tb^{III}-dtpa-bis(2,6-diaminopurine)) and its application to the detection of uric acid in urine sample

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A R T I C L E I N F

Article history:

Received 00 June 2018

Revised 00 June 2018

Accepted 00 June 2018

Available online 00 June 2018

Keywords:

Fluorescent probe

Detection of uric acid

2,6-Diaminopurine

Diethylenetriaminepentaacetic acid (DTPA)

Tb^{III}-dtpa-bis(2,6-diaminopurine) complex

A B S T R A C T

In this study, a novel fluorescent probe, Tb^{III}-dtpa-bis(2,6-diaminopurine) (Tb-dtpa-bdap), is designed based on

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http://dx.doi.org/00.0000/j.saa.2018.00.000

the principle of complementary base pairing and synthesized for uric acid detection. The synthesized fluorescent probe is characterized by ^{1}H-NMR, ^{13}C-NMR), infra-red (IR) spectrum and ultraviolet-visible (UV-vis) spectra. It is found that the fluorescence of Tb-dtpa-bdap solution can be quenched obviously in the presence of uric acid. The affecting factors, including solution acidity, uric acid concentration and interfering substances, on the detection of uric acid using this probe are examined. Under optimized conditions, the fluorescence intensities of Tb-dtpa-bdap solution towards different uric acid concentrations show a linear response in the range from 1.00×10^{-5} mol·L^{-1} to 5.00×10^{-5} mol·L^{-1} with a linear correlation coefficient (R^2) of 0.9877. And the obtained limit of detection (LOD) is about 5.80×10^{-6} mol·L^{-1}, which is lower than the level of uric acid in actual urine. The mechanism on the detection of uric acid by using Tb-dtpa-bdap is inferred from the experimental results. The facts demonstrate that the proposed fluorescent probe can be successfully applied for the determination of uric acid in human urine samples.

1. Introduction

Uric acid (2,6,8-trihydroxypurine) is the principal end product of the purine catabolism in human system [1]. Also, it is a very important marker molecular compound in body fluids, such as serum and urine, and can be used as an diagnosis indicator of some diseases [2]. Generally, the normal concentration of uric acid in human urine changes within the range of 1.49×10^{-3} mol·L^{-1} - 4.46×10^{-3} mol·L^{-1} (25 mg·dL^{-1} - 74 mg·dL^{-1}) [3], which is maintained through production and excretion from the body by various bio-processes [4]. Abnormal uric acid levels will cause various diseases, such as hypouricemia, leading frequently to multiple sclerosis [5]. In addition, it is worth noting that the lack of exercise, unhealthy diet and incorrect use of drugs easily result in the elevated uric acid level

(hyperuricemia) in vivo. The hyperuricemia can cause other some diseases, such as gout, high cholesterol, kidney disease, renal impairment, cardiovascular diseases and Lesch-Nyhan syndrome [6]. Worst of all, there is the evidence to indicate that the concentrations of uric acid in vivo is related to the preeclampsia, which could usually cause maternal death [7,8]. For these reasons, it is an urgent requirement to monitor the uric acid level anytime in human bodily fluids (such as blood and urine) for physiological investigations and disease diagnosis.

Now, multitudinous approaches have been developed for the detection of uric acid, including spectrophotometry [9,10], electrochemical methods [11,12], chromatography [13,14], high performance liquid chromatographic (HPLC) [15–17] and molecular fluorescent probe techniques [18,19] and so on. They can be summarized as the detection of uric acid usually through two types of methods, non-enzymatic and enzymatic analyses. Since the spectrophotometric and electrochemical methods are related with the use of uricase enzyme reagents and time-consuming nature and also easy to be interfered, these approaches were often limited in practical application. The fluorescent probe techniques have attracted much attention all through because of innate advantages over other methods, such as high sensitivity, high specificity, low cost and convenience. Therefore, the fluorescent probe has become an integral part of analytical and bioanalytical chemical fields due to its high selectivity, sensitivity, anti-interference and designability, and have found applications in a diverse range of areas, including medical, biological and environmental fields [20–23].

In the previous studies, some aminopolycarboxylic acids were used as ligands to combine with various rare earth metal ions forming probes to detect melamine and adenine [24,25]. In present study, the diethylenetriamine-*N*, *N*, *N'*, *N''*, *N''*-pentaacetic acid (dtpa) was chosen as precursor to synthesize a new fluorescent probe for uric acid detection. In general, most of rare earth metal ions form fairly stable nine-coordinate complexes with aminopolycarboxylic acid ligands [26,27]. As an eight-dentate

ligand, the dtpa only can provide eight coordination atoms to combine with various rare earth metal ions. Therefore, one water molecule as ninth ligand together with dtpa can coordinate with rare earth metal ions forming nine-coordinate complexes. Particularly, as we all known, the recognition ability of the fluorescent probe can be improved when a similar or complementary compound to target molecule in molecular structure and chemical composition is attached [28–30]. The longitudinal complementary base pairing in single-strand DNA is the main force for maintaining the stability of double-helix structure of DNA. So, based on the principle of complementary base pairing in single-stranded DNA and the coordinate structure of Tb^{III} complex with dtpa, two molecules of 2,6-diaminopurine (dap) were used to modify the Tb^{III}-dtpa complex, obtaining a novel fluorescent probe. Due to the complementary pairing in structure, the uric acid could be catched by the Tb-dtpa-bdap complex more easily. The presence of 2,6-diaminopurine (dap) changes the ligand field around the Tb^{III} ion, and then leads to the change of fluorescence. It provides better selectivity and sensitivity, especially strong targeting property and high recognition ability.

In this work, based on the principle of complementary base pairing, a novel fluorescent probe, Tb^{III}-dtpa-bis(2,6-diaminopurine) (Tb-dtpa-bdap), is designed and synthesized for uric acid detection. The synthesized fluorescent probe is characterized by ^{1}H-NMR, ^{13}C-NMR, infra-red (IR) and ultraviolet-visible (UV-vis) spectra. The affecting factors, including solution pH values, uric acid concentration and interfering substances, on the detection of uric acid using this probe are examined. The mechanism on the detection of uric acid by using Tb-dtpa-bdap is inferred. The facts demonstrate that the proposed fluorescent probe can be successfully applied for the determination of uric acid in human urine samples.

2. Experimental

2.1. Apparatus and reagents

Absorption spectra of the samples were measured with a UV-vis spectrophotometer (Cary 50, Varian Company, USA). Fluorescence measurements were carried out by a fluorophotometer (Cary 300, Varian Company, USA). The Fourier Transform Infrared (FT-IR) spectra were obtained on a FT-IR spectrophotometer (5700 FT-IR, Nicolet Company, USA). The Nuclear Magnetic Resonance (NMR) Spectrometer (Technologies Plus-400 MR, Agilent Company, USA.) was used to collect the NMR spectra of dtpa and dtpa-bis(2,6-diaminopurine), and the DMSO-*d*6 and tetramethysilane (TMS) were used as solvent and internal standard, respectively.

All reagents were of analytical reagent grade. The dtpa-bis(2,6-diaminopurine) was synthesized by use the diethylenetriaminepentaacetic acid (dtpa) from Beijing SHLHT Science & Trade Co., Ltd (China) and 2,6-diaminopurine from Shanghai Macklin Biochemical Co., Ltd. (China). Tyrosine, histidine, ascorbic acid (Sinopharm Chemical Reagent Co., Ltd., China), tryptophan, creatinine, hippuric acid and uric acid (Shanghai Macklin Biochemical Co., Ltd., China) were purchased and used as interference and detect substances. Anhydrous acetic anhydride and DMF (analytical purity, Shenyang Chemical Reagent Plant, China) were purchased and used as solvents. Pyridine and Triethylamine (analytical purity, Shenyang Chemical Reagent Plant, China) were obtained and used as acid binding agent. Tris-HCl buffer solution was prepared via Tris ((hydroxyl-methyl) aminomethane) and HCl (analytical purity, Shenyang Chemical Reagent Plant, China).

2.2. Synthesis of Diethylenetriamine pentaacetic acid dianhydride (dtpaa)

The diethylenetriamine pentaacetic acid dianhydride (dtpaa) was prepared from diethylenetriaminepentaacetic acid (dtpa) according to a modified method proposed in our previous work [31–33], and its synthetic procedure is described in Scheme 1. In brief, 7.8020 g dtpa (0.02

mmol) was added to a three-necked flask, then, the 8.00 mL acetic anhydride (0.08 mmol) and 10.00 mL pyridine (0.12 mmol) were added to the flask. The reaction mixture was stirred and refluxed for 24 h. After the reaction was completed, the product was cooled down to room temperature, and the solvent was removed through vacuum distillation. The crude product was washed three times with acetic anhydride and anhydrous diethyl as the eluent, respectively. Finally, the product was vacuum dried (52 kpa) at 80 ºC and the dtpaa was obtained as white solid. The overall yield was above 83 %. FT-IR (KBr, cm-1) of dtpaa: 1642.41, 1772.10, 1821.08, 2341.42, 2820.47 and 2979.80. ^{1}H-NMR (ppm, 300 MHz, DMSO) of dtpa: δ = 2.45 (t, 8H), 3.30 (s, 10H) and 11.01 (s, 3H). ^{1}H-NMR (ppm, 300 MHz, DMSO) of dtpaa: δ = 2.59 (t, 4H), 2.74 (t, 4H), 3.30 (s, 2H), 3.70 (s, 8H) and 11.01 (s, 1H).

Scheme 1.

2.3. Synthesis of dtpa-bis(2,6-diaminopurine)

The reported methods were improved for synthesizing dtpa-bis(2,6-diaminopurine) (dtpa-bdap) [34,35]. The synthesis process is shown in Scheme 1. 1.9652 g dtpaa (55.00 mmol) was dissolved in a mixed solvent of 30 mL DMF and 8 mL triethylamine, and then the obtained solution was stirred to transparent liquid at 50 ºC. At the same time, 1.6515 g 2,6-diaminopurine (11.00 mmol) was dissolved in 20 mL DMF, and then stirred and heated under 50 ºC. Afterwards, above two solutions were mixed and stirred for 24 h at 100 ºC. Through reduced pressure distillation and filtration the DMF was removed. Finally, the obtained flavescens powder was evaporated under vacuum (52 kpa) at 50 ºC. The final product was 2.9806 g and the yield was about 82 %. FT-IR (KBr, cm^{-1}) of dtpa-bdap: 937, 1210, 1396, 1630 and 3108. ^{1}H-NMR (ppm, 300 MHz, DMSO, δ): 2.46 (t, 8H), 3.25 (t, 4H), 4.10 (s, 4H), 7.10 (d, 2H) and 8.01 (s, 2H). ^{13}C-NMR (ppm, 101 MHz, DMSO, δ): 173.16 (s), 159.71 (s),

155.81 (s), 152.75 (s), 137.03 (s), 122.03 (s), 56.01 (s), 51.84 (s), 50.35 (s), 40.54 (s), 40.33 (s), 40.12 (s), 39.91 (s), 39.71 (s), 39.50 (s) and 39.29 (s).

2.4. Synthesis of Tb^{III}-dtpa-bis(2,6-diaminopurine)

0.0667 g (0.125 mmol) dtpa-bis(2,6-diaminopurine) was added to 50 mL Tris-HCl ([TrisHCl] = 50 mmol·L^{-1} and pH = 7.40) buffer solution and then stirred and slowly heated. Then, 0.0566 g (0.125 mmol) $Tb(NO_3)_3 \cdot 6H_2O$ was mixed in above solution, following by stirring and heating circumfluence for 3.0 hours. And then, it was transfered into a constant 250 mL volumetric flask. The stock solution (5.00×10^{-4} mol·L^{-1}) was obtained and kept in refrigerator at 4.0 ºC as to be used.

2.5. Preparation of various tested solutions

0.0210 g uric acid (UA), 0.0226 g tyrosine (Tyr), 0.0141 g creatinine (Cre), 0.0220 g ascorbic acid (AA), 0.0224 g hippuric acid (Hipa), 0.0255 g tryptophan (Try) and 0.0194 g histidine (His) were dissolved in Tris-HCl ([Tris-HCl] = 50 mmol·L^{-1} and pH = 7.40) buffer solution and diluted with buffer solution to volume in 250 mL volumetric flask to obtaine 5.00×10^{-4} mol·L^{-1} solutions, respectively. The stock soultions were kept in 4.0 ºC. The stock solutions were diluted to required concentrations with Tris-HCl ([Tris-HCl] = 50 mmol·L^{-1} and pH = 7.40) buffer solution when needed in experiments. The molecular structures of uric acid, 2,6-diaminopurine (dap), tyrosine (Tyr), creatinine (Cre), ascorbic acid (AA), hippuric acid (Hipa), tryptophan (Try) and histidine (His) were shown in Fig. 1.

Fig. 1.

2.6. Spectral measurements

5.00 mL above stock solutions were taked out and tranfered to 50 mL volumetric flask,

respectively. 5.00 mL stock solutions of Tb-dtpa-bdap were added to above solutions. And then, the solutions were diluted with Tris-HCl ([Tris-HCl] = 50 mmol·L^{-1} and pH = 7.40) solutions to 50 mL (5.00×10^{-5} mol·L^{-1}). Tb-dtpa-bdap slutuion as blank without other interfering substances was also prepared by the same procedure. The fluorescence emission spectra were recorded in the wavelength range from 200 nm to 600 nm under excitation of wavelength light at 241 nm. Both excitation and emission slits were set at 5.0 nm. All experiments were carried out at room temperature (25.00 ± 0.01 ^{o}C).

2.7. Preparation of real samples

Here, the human urine samples were obtained from a healthy man and no pretreatment was necessary and significant for urine samples. The detection and analysis of uric acid were carried out according to the general procedure. 5.00 mL of urine and 5.00 mL Tb-dtpa-bdap stock solution were taken to a 50 mL volumetric flask, and then the solution was diluted with Tris-HCl ([Tris-HCl] = 50 mmol·L^{-1} and pH = 7.40) solution to the mark. In addition, 1.00 mL, 3.00 mL and 5.00 mL of different concentrations of uric acid solutions were sequentially added and the same operations were carried out. The fluorescence intensities of the solutions were measured at excitation wavelength of 241 nm with slit width of 5.00 nm for both excitation and emission.

3. Results and discussion

3.1. ^{1}H-NMR, ^{13}C-NMR and FT-IR spectra

In order to confirm the composition and structure of dtpa-bdap ligand, the ^{1}H-NMR and ^{13}C-NMR were determined and the results were given in Fig. 2. As shown in Fig. 2(a), a distinct (singlet) signal can be found at δ = 8.01 ppm, which corresponds to the –NH proton in dtpa-bdap.

Nevertheless, the signal for the –NH proton in the dtpa can not be found, demonstrating that the dtpa-bdap has been synthesized through the aminolysis reaction between dtpaa and 2,6-diaminopurine. Fig. 2(b) also shows that the synthesized ligand consists of the above two parts, that is, dtpa and 2,6-diaminopurine. In general, the signal peaks of carboxyl carbon atoms in dtpa appear at 172.43 ppm [36]. However, for synthesized ligand a new signal peak at 159.71 ppm can be found, which should belong to the amide carbon. At the same time, the signal peaks of carbon atoms in 2,6-diaminopurine typically appear at 152.10 ppm and 138.60 ppm [37], respectively. And that, the corresponding signal peaks in the synthesized ligand are found at 152.75 ppm and 122.03 ppm, respectively. The appearance of new carbon atom peak of amide bonds and the shift of carbon atom signal peaks of 2,6-diaminopurine proved that the dtpa-bdap ligand was successfully synthesized. Other relevant data were provided in 2.3 section.

Fig. 2.

Additionally, the FT-IR spectra of diethylenetriamine pentaacetic acid (dtpa) (a), 2,6-diaminopurine (dap) (b) and dtpa-bdap (c) were also investigated and the determined results were shown in Fig. 3. All the FT-IR spectra were determined in the region of 4000-500 cm^{-1}. From Fig. 3(c), it can be found that the ν_s(C-O) and ν_{as}(C=O) of dtpa-bdap ligand appear at 1396 cm^{-1} and 1630 cm^{-1}, respectively, both demonstrating a slight shift compared with that of dtpa in Fig. 3(a). The absorption at 3108 cm^{-1} is originated from the vibration band (-NH-) of acidamide groups in Fig. 3(c). In addition, there exists an absorption band around 1210 cm^{-1}, corresponding to the vibration absorption of C–N group [38], which has a 32 cm^{-1} shift compared with that of dtpa in Fig. 3(a). These observations confirm the formation of the dtpa-bdap via acylation action between dtpaa and dap. It is worth noting that the absorption of $–NH_2$ at 937 cm^{-1} of dtpa-bdap in Fig. 3(c) hardly changes

compared with that of dap in Fig. 3(b), which illustrates that the synthesized dtpa-bdap was just only take up one $–NH_2$ and the other $–NH_2$ of dap was retained.

Fig. 3.

3.2. Absorption and fluorescence spectra

Fig. 4 shows the ultraviolet absorption spectra of Tb^{3+} ion, dtpa-bdap and Tb-dtpa-bdap solutions. It can be seen that the Tb^{3+} ion solution (5.00×10^{-5} mol·L^{-1}) gives a weak absorption peak at 226 nm. And that, for dtpa-bdap solution (5.00×10^{-5} mol·L^{-1}) three strong absorption peaks can be found at 222 nm, 246 nm and 280 nm, respectively. After adding Tb^{3+} ion into dtpa-bdap solution, the absorption peaks at 222 nm, 246 nm and 280 nm were all enhanced. Particularly, the absorption peak at 222 nm was obviously strengthened. It indicates that the dtpa-bdap can react with Tb^{3+} ion in aqueous solution, forming Tb-dtpa-bdap complex. In addition, the strong absorption peaks lay the foundation for the further fluorescence determination.

Fig. 4.

Sequentially, the fluorescence properties of uric acid, Tb-dtpa-bdap and Tb-dtpa-bdap-UA solutions were investigated and the results were shown in Fig. 5. Referring to the ultraviolet absorption spectrum, the excitation wavelength of 241 nm was chosen to excite uric acid, Tb-dtpa-bdap and Tb-dtpa-bdap-UA solutions. It can be found that the uric acid solution hardly gives any emission peak between 250 nm and 450 nm. Under the same conditions, the Tb-dtpa-bdap solution displays a very strong characteristic peak at 350 nm. Hopefully, the fluorescence intensity of Tb-dtpa-bdap solution was obviously quenched a half when the uric acid was added. The preliminary experimental results prove that the Tb-dtpa-bdap complex may have a potential capacity as fluorescent

probe to detect uric acid.

Fig. 5.

3.3. Effect of solution pH value

The effect of different solution acidities, such as pH = 4.40, 5.40, 6.40, 7.40, 8.40, 9.40 and 10.40, on the fluorescence intensity of Tb-dtpa-bdap+UA solution was tested and the obtained results were shown in Fig. 6(a). Previous reports suggested that the pH of various buffer solutions could generally influence the interaction between probe and detected molecules [39,40]. It forces us to optimize the experimental conditions including pH values. However, in Fig. 6(b) the intuitive observation demonstrates that the solution pH has hardly any effect on the fluorescence intensity of Tb-dtpa-bdap+UA solution. It indicates that the uric acid can be detected by using Tb-dtpa-bdap in a wide pH range (pH = 4.40 - 10.40). In general, the pH of actual urine is in the range of 4.50-8.00 for a health people [41]. Even for a patient suffering from some diseases, the pH of urine only shows a slight increase or decrease compared with that of normal people. Apparently, the use of Tb-dtpa-bdap gives a great convenience for the detection of real urine samples, which need not to consider the influence of solution pH for the detection of uric acid.

Fig. 6.

3.4. Selectivity of the proposed Tb-dtpa-bdap as probe for uric acid detection

As everyone knows, there also are other several coexisting substances besides uric acid in the urine, for example, tyrosine (Tyr), creatinine (Cre), ascorbic acid (AA), hippuric acid (Hipa), tryptophan (Try) and histidine (His). Hence, these substances were selected to examine the selectivity of Tb-dtpa-bdap as probe for uric acid detection. In order to examine the effect of these coexisting

substances on the detection of uric acid, the fluorescence determination of the above substances were carried out in the absence of Tb-dtpa-bdap. In Fig. 7(a), it can be seen that, in addition to Try, other substances (Tyr, Cre, AA, Hipa and His) hardly have fluorescent emission around 350 nm under the excitation of 241 nm wavelength light. As a strongly fluorescent compound, the Try solution gives an obvious emission peak at 350 nm. Fortunately, the concentration of Try in actual urine is very low, so the detection of uric acid will not be disturbed by using Tb-dtpa-bdap as probe.

When the Tb-dtpa-bdap was mixed with uric acid in aqueous solution, as shown in Fig. 7(b) the Tb-dtpa-bdap displays a notable fluorescence quenching. On the contrary, when the Tb-dtpa-bdap was mixed with some coexisting substance (except Try) in aqueous solutions, respectively, the fluorescence of Tb-dtpa-bdap was only slightly decreased, which indicates that these coexisting substance (except Try) will hardly interfere the detection of uric acid. And that, Try and Tb-dtpa-bdap were mixed with the same concentration, the fluorescence intensity of Tb-dtpa-bdap solution was enhanced due to the strong fluorescence of Try itself. The detailed comparison can be visually seen in Fig.7(c). High concentration of Try may have interference seemingly for uric acid detection by using Tb-dtpa-bdap as probe. In fact, the amount of Try in urine of a normal person is 10^{-5} mol·L^{-1} - 10^{-6} mol·L^{-1} [48], which is far less than that of uric acid. Apparently, it will not have any affect on the Tb-dtpa-bdap as probe in process of uric acid detection. The results indicated that the proposed Tb-dtpa-bdap as probe exhibited high selectivity to uric acid.

Fig. 7.

3.5. Effects of other substances on fluorescence spectra of Tb-dtpa-bdap and uric acid mixed solution

In order to evaluate the specificity of Tb-dtpa-bdap as fluorescent probe for uric acid detection,

the interference experiments were implemented. As shown in Fig. 8(a), the addition of uric acid can markedly quench the fluorescence of Tb-dtpa-bdap solution, indicating the effective interaction between Tb-dtpa-bdap and uric acid in aqueous solution. When these used interfering (coexisting) substances (Tyr, Cre, AA, Hipa and His) were added into Tb-dtpa-bdap+UA mixed solution, respectively, the fluorescence intensities hardly changed. It indicates that the presence of Tyr, Cre, AA, Hipa and His as the major ingredients of human urine do not affect the detection of uric acid by using Tb-dtpa-bdap as fluorescent probe. As a special case, the addition of Try with the same concentration (5.00×10^{-5} mol·L^{-1}) as that of Tb-dtpa-bdap+UA mixed solution increases the fluorescence intensity to some extent, but it is much lower than that of Tb-dtpa-bdap solution. In addition, the actual concentration of Try in human urine is very low. Therefore, it is difficult to find the fluorescence of human urine, particularly, by using 241 nm as excited wavelength. The detailed comparison can be visually seen in Fig.8(b). Apparently, the Tb-dtpa-bdap has good anti-interference as fluorescent probe to detect uric acid in human urine.

Fig. 8.

The effect of tryptophan in detecting uric acid using Tb-dtpa-bdap as probe was further investigated. As we all known, the concentration of uric acid is 1.49 - 4.46×10^{-3} mol·L^{-1} in actual urine of healthy people, and the concentration of tryptophan is 2.10 - 2.30×10^{-5} mol·L^{-1}. It is easy to see that the concentration of uric acid is 100 times higher than that of tryptophan in the actual urine sample. However, the tested concentration in above experiment is 5.00×10^{-5} mol·L^{-1} for both uric acid and tryptophan. Therefore, the existence of tryptophan seems to affect the detection of uric acid. However, uric acid concentrations are still within the range that can be detected after the real urine sample was diluted 100-fold. And that, the concentration of tryptophan was diluted to 2.50×10^{-7}

mol·L^{-1} in the next experiment. In Fig. 9(a), it can be seen that the fluorescence intensity of Tb-dtpa-bdap solution was obviously quenched when uric acid was added into the Tb-dtpa-bdap solution, at this point, the concentrations of uric acid and Tb-dtpa-bdap are both 5.00×10^{-5} mol·L^{-1}. The tryptophan solution with 2.30×10^{-7} mol·L^{-1} has hardly any fluorescence at 350 nm. When the tryptophan solution (2.50×10^{-6} mol·L^{-1}) was added to the Tb-dtpa-bdap solution with 5.00×10^{-4} mol·L^{-1}, the fluorescence intensity of Tb-dtpa-bdap solution hardly changed. In this mixed solution, the concentration of tryptophan is 2.50×10^{-7} mol·L^{-1} and the uric acid was 5.00×10^{-5} mol·L^{-1}. Similarly, there is no change in fluorescence intensity of Tb-dtpa-bdap solution when the solutions of uric acid (5.00×10^{-4} mol·L^{-1}) and tryptophan (2.50×10^{-6} mol·L^{-1}) were added into Tb-dtpa-bdap solution at the same time. The detailed comparison diagram is given in Fig. 9(b). This result indicates that the tryptophan will not affect the detection of uric acid using Tb-dtpa-bdap as fluorescent probe in the analysis of actual urine sample.

Fig. 9.

3.6. Fluorescence spectra of Tb-dtpa-bdap solution with uric acid concentration

Under optimal conditions, the performance of the Tb-dtpa-bdap as fluorescent probe was evaluated and the fluorescent responses to uric acid with different concentrations were recorded as shown in Fig. 10(a). Under the excitation of 241 nm wavelength light, along with the increasing of uric acid concentrations in the range from 0.00 mol·L^{-1} to 10.00 mol·L^{-1} at 0.50 mol·L^{-1} intervals the fluorescence intensity (at 350 nm) of Tb-dtpa-bdap solution decreases gradually. However, from Fig. 10(b) it can be noticed that the decreasing tendency of fluorescence intensity begins to slow down after 5.00×10^{-5} mol·L^{-1} uric acid concentration. It can be inferred that the combination ratio between Tb-dtpa-bdap and uric acid is 1:1 in stoichiometry ratio.

Fig. 10.

In addition, as shown in Fig. 10(c) the relative fluorescence intensity, F_0/F, exhibits a linear relationship with the concentration of uric acid in the range from 1.00×10^{-5} mol·L^{-1} to 5.00×10^{-5} mol·L^{-1}, which is described by the equation: $F_0/F = 0.9353 + 0.2217$ [UA] ($R^2 = 0.9877$), where F_0 and F are the fluorescence intensities of the Tb-dtpa-bdap solution in the absence and presence of uric acid, respectively, [UA] is the concentration of uric acid (mol·L^{-1}) and the correlation coefficient is 0.9877. The limit of detection (LOD) calculated according to IUPAC definitions is about 5.80×10^{-6} mol·L^{-1}. The results of some other methods were shown in Table 1. It can be seen that the fluorescent probe mentioned in this paper has a wide detection range and a low limit of detection (LOD). Therefore, compared with other methods, the detection based on Tb-dtpa-bdap as fluorescent probe can be used as a rapid and simple method for uric acid analysis.

Table 1

3.7. Mechanism of Tb-dtpa-bdap as fluorescent probe for uric acid detection

It is well known that the Tb^{3+} ion can form nine-coordinate complexes with various aminopolycarboxylic acid ligands [48]. Therefore, in Tb-dtpa-bdap, the diethylenetriamine pentaacetic acid (dtpa) provides eight coordination atoms and a molecule of water should also be coordinate with Tb^{3+} ion as ninth coordination atom.

It is worth mentioning that adenines are pair with cytosines through π-π stacking interaction in single-stranded DNA. The same situation also appears on guanines and thymines. The principle of complementary base pairing has a very important guiding significance for the choice of modified substance in our experiment. In this study, 2,6-diaminopurine (dap) was used to modify the dtpa forming dtpa-bdap ligand. It can be found that 2,6-diaminopurine and uric acid are also

complementary in charge besides similar structure, because the uric acid is electropositivity, and that the 2,6-diaminopurine is electronegativity. Therefore, it can be inferred that the Tb-dtpa-bdap as fluorescent probe to specifically detect uric acid is related with their similar structure and complementary electrical property.

As showed in Fig. 11, when the Tb^{3+} ion complex is photoexcited, the organic ligand part, dtpa-bdap, absorbs the energy of excited light and their electrons are jumped from the singlet ground state (S_0) to the first singlet excited state (S_1). And then, the energy of ligand is transferred to the triplet excited state (T_1) of ligand by intersystem crossing (non-radiative) process. When the T_1 energy level is higher than that of Tb^{3+} ion, the energy could be transmitted to the Tb^{3+} ion, and then the characteristic fluorescence of Tb^{3+} ion is emitted. Upon addition of the uric acid, due to the similar molecular structure, the π-π stacking binding will be formed between uric acid and 2,6-diaminopurine (dap) in Tb-dtpa-bdap. In addition, the hydrogen bonds are also formed between uric acid and 2,6-diaminopurine (dap). At the same time, the original coordinate water molecule will be replaced by the closer uric acid, which leads to the change in the fluorescence spectrum.

Thus, through the above analyses it is confirmed that the Tb-dtpa-bdap as fluorescent probe can be used to detect uric acid sensitively and specifically.

Fig. 11.

3.8. Analysis of uric acid in real samples

To demonstrate the applicability of the Tb-dtpa-bdap as fluorescent probe for real sample, the standard addition experiments and recovery tests were used to evaluate the accuracy of the proposed probe. Considering the interference of original uric acid in urine, 100-fold diluted urine samples with buffer solution were used for all samples. The obtained results were shown in Fig. 12. From Fig. 12(a)

it can be seen that the diluted urine sample has no obvious fluorescence under excitation of 241 nm wavelength light, and the Tb-dtpa-bdap solution (5.00×10^{-5} mol·L^{-1}) gives a strong fluorescence peak at 350 nm. However, when the urine sample was added the fluorescence intensity of Tb-dtpa-bdap solution (5.00×10^{-5} mol·L^{-1}) was quenched obviously about half. Moreover, three different volumes (1.00 mL, 3.00 mL and 5.00 mL) of uric acid solutions (5.00×10^{-4} mol·L^{-1}) were added to the mixed solutions of Tb-dtpa-bdap and urine samples and the concentrations were 1.00×10^{-5} mol·L^{-1}, 3.00×10^{-5} mol·L^{-1} and 5.00×10^{-5} mol·L^{-1}, respectively. It can be found that the fluorescence intensity of Tb-dtpa-bdap solution (5.00×10^{-5} mol·L^{-1}) was further decreased gradually along with the increase of uric acid concentration. It indicates that there is a certain amount of uric acid in tested urine samples and the fluorescence of Tb-dtpa-bdap solution (5.00×10^{-5} mol·L^{-1}) can be further quenched by added uric acid due to their specific combination.

Fig. 12.

In addition, as shown in Fig. 12(b) the decrease of the fluorescence intensity of Tb-dtpa-bdap solution apparently demonstrates a reasonable linear response with the increase of uric acid concentration. The results obtained using the standard addition method were listed in Table 2, and the excellent recoveries in the range from 95 % to 103 % were obtained for human urine sample, demonstrating this is an ideal detection method of uric acid in actual urine sample.

Table 2

4. Conclusion

In summary, a novel and convenient fluorescent probe was developed for uric acid determination based on the fluorescence quenching of Tb-dtpa-bdap. The common coexisting interferents in real

urine samples do not disturb the fluorescent response. The solution pH has not impact on the detection of uric acid by using this fluorescent probe. The practical application of this fluorescent probe in the detection of uric acid in urine samples was proved by recovery experiments. Under optimum conditions, the method displays a good linear relationship between fluorescence intensity and uric acid concentration in the concentration range from 1.00×10^{-5} mol·L^{-1} to 5.00×10^{-5} mol·L^{-1}. A low limit of detection (LOD) of 5.80×10^{-6} mol·L^{-1} was obtained. It is thought that the mentioned Tb-dtpa-bdap may be an economical, sensitive and selective fluorescent probe and this method may be successfully applied to assess uric acid in urine samples.

Acknowledgments

The authors greatly acknowledge the National Science Foundation of China (21371084 and 31570154), Key Laboratory Basic Research Foundation of Liaoning Provincial Education Department (L2015043), Liaoning Provincial Department of Education Innovation Team Projects (LT2015012) and Undergraduate Teaching Reform Projects of Liaoning University (JG2016YB0034) for financial support. The authors also thank our colleagues and other students for their participating in this work.

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Content of Scheme, Figures and Table:

Scheme:

Scheme. 1. The synthesis route of Tb^{3+}-dtpa-bdap complex.

Figures:

Fig. 1. The molecular structures of uric acid, 2,6-diaminopurine and analogues as possibly co-existing compounds in urine.

Fig. 2. ^{1}H-NMR (a) spectra of dtpa-bdap and dtpa and ^{13}C-NMR (b) spectrum of dtpa-bdap in DMSO-d_6.

Fig. 3. Infrared spectra of (a) diethylenetrinmine pentaacetic acid (dtpa), (b) 2,6-diaminopurine (dap) and (c) dtpa-bis(2,6-diaminopurine) (dtpa-bdap).

Fig. 4. Ultraviolet absorption spectra of Tb^{3+} ion, dtpa-bdap and Tb^{3+}-dtpa-bdap in Tris-HCl buffer solutions. ([Tb^{3+}] = [dtpa-bdap] = [Tb-dtpa-bdap] = 5.00×10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

Fig. 5. Fluorescence spectra (λ_{ex} = 241 nm) of Tb^{3+}-dtpa-bdap in the absence and presence of uric acid (UA) in Tris-HCl buffer solutions. ([Tb^{3+}-dtpa-bdap] = [UA] = 5.00×10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

Fig. 6. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap in the presence of uric acid (UA) at different pH values (4.40 - 10.40) and corresponding intensities (λ_{em} = 350 nm) (b). ([Tb-dtpa-bdap] = [UA] = 5.00×10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1} and T_{solu} = 25.00 ± 0.02 °C.)

Fig. 7. Fluorescence spectra (λ_{ex} = 241 nm) of Tb^{3+}-dtpa-bdap in Tris-HCl buffer solution in the absence (a) and presence (b) of tryptophan (Try), creatinine (Cre), ascorbic acid (AA), tyrosine (Tyr), hippuric acid (Hipa), uric acid (UA) and histidine (His) and the corresponding fluorescence intensities (λ_{em} = 350 nm) (c). ([Tb^{3+}-dtpa-bdap] = [UA] = [Try] = [Cre] = [AA] = [Tyr] = [Hipa] = [His] = 5.00×10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

Fig. 8. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap, Tb^{3+}-dtpa-bdap+UA and Tb^{3+}-dtpa-bdap+UA upon the addition of tryptophan (Try), creatinine (Cre), ascorbic acid (AA), tyrosine (Tyr), hippuric acid (Hipa) and histidine (His) in Tris-HCl buffer solutions and the corresponding fluorescence intensities (λ_{em} = 350 nm) (b). ([Tb^{3+}-dtpa-bdap] = [UA] = [Try] = [Cre] = [AA] = [Tyr] = [Hipa] = [His] = 5.00×10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

Fig. 9. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap, Tb^{3+}-dtpa-bdap+Try, Tb^{3+}-dtpa-bdap+UA Tb^{3+}-dtpa-bdap+UA+Try and Try in Tris-HCl buffer solutions and the corresponding fluorescence intensities (λ_{em} = 350 nm) (b). ([Tb^{3+}-dtpa-bdap] = [UA] = 5.00 × 10^{-5} mol·L^{-1}, [Try] = 2.50 × 10^{-7} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and Tsolu = 25.00 ± 0.02 °C.)

Fig. 10. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap in Tris-HCl buffer solutions with different concentrations of uric acid (UA) from 0.00 × 10^{-5} mol·L^{-1} to 10.00 × 10^{-5} mol·L^{-1}, corresponding fluorescence intensities (λ_{em} = 350 nm) (b) and linear responses (c) of Tb^{3+}-dtpa-bdap as a function of uric acid (UA) concentration (1.00 × 10^{-5} - 5.00 × 10^{-5} mol·L^{-1}). ([Tb^{3+}-dtpa-bdap] = 5.00 × 10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

Fig. 11. The reaction mechanism of Tb^{3+}-dtpa-bdap with uric acid.

Fig. 12. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap and Tb^{3+}-dtpa-bdap with different concentrations of uric acid (UA) in diluted urine samples with 100-folds as well as blank urine and corresponding fluorescence intensities (λ_{em} = 350 nm) (b). ([Tb^{3+}-dtpa-bdap] = 5.00 × 10^{-5} mol·L^{-1}, [UA] = 1.00 × 10^{-5}, 3.00 × 10^{-4} and 5.00 × 10^{-5} mol·L^{-1}. pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

Table:

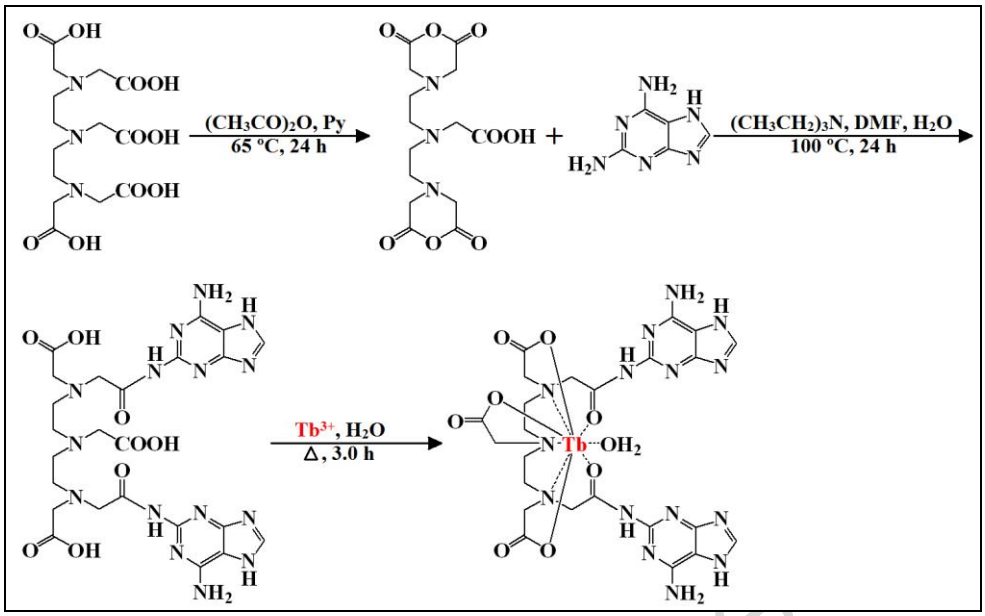
Table 1

Comprasion of the proposed method with others.

Table 2

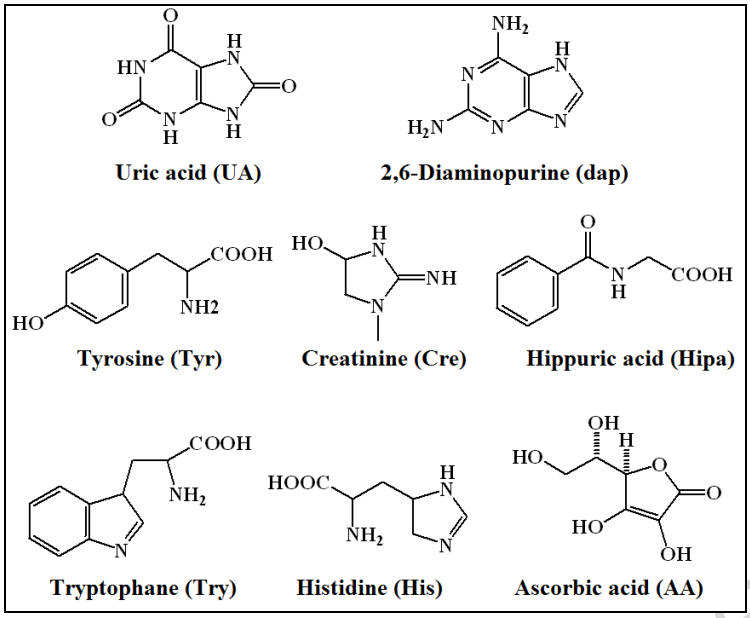
Determination results of uric acid (UA) content in diluted urine samples with 100-folds.

Scheme:



Scheme. 1. The synthesis route of Tb^{3+}-dtpa-bdap complex.

Figures:



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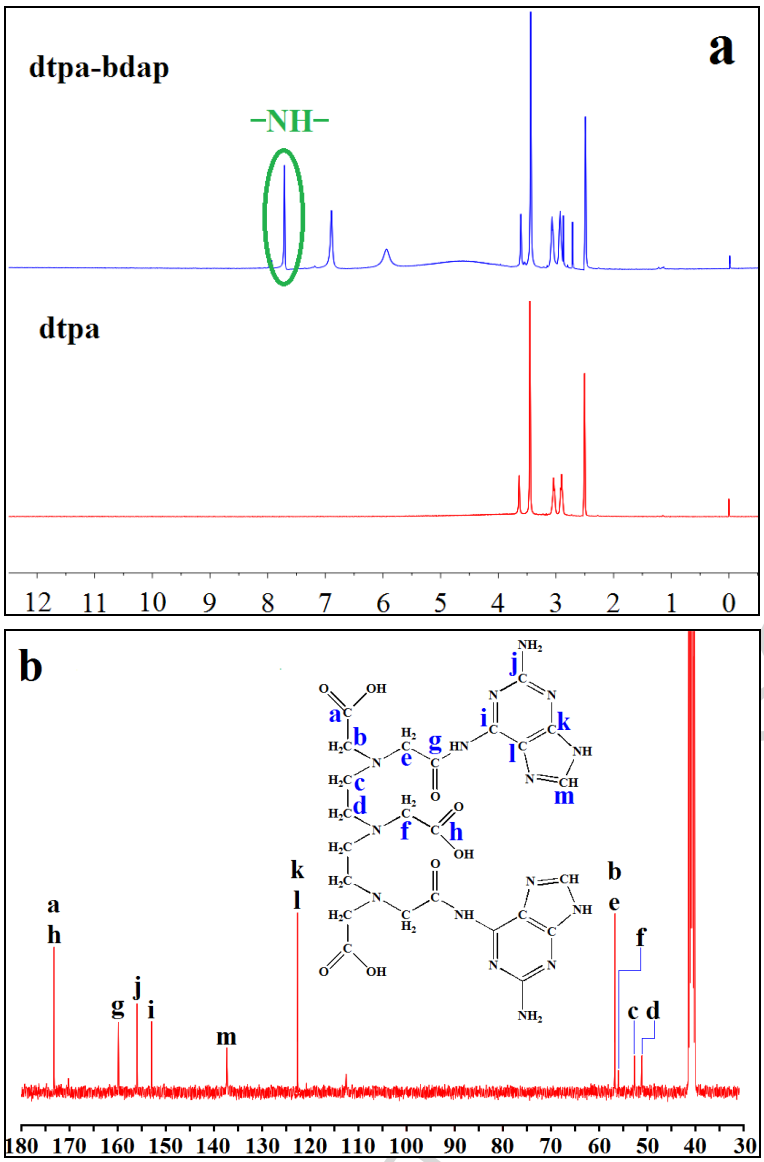

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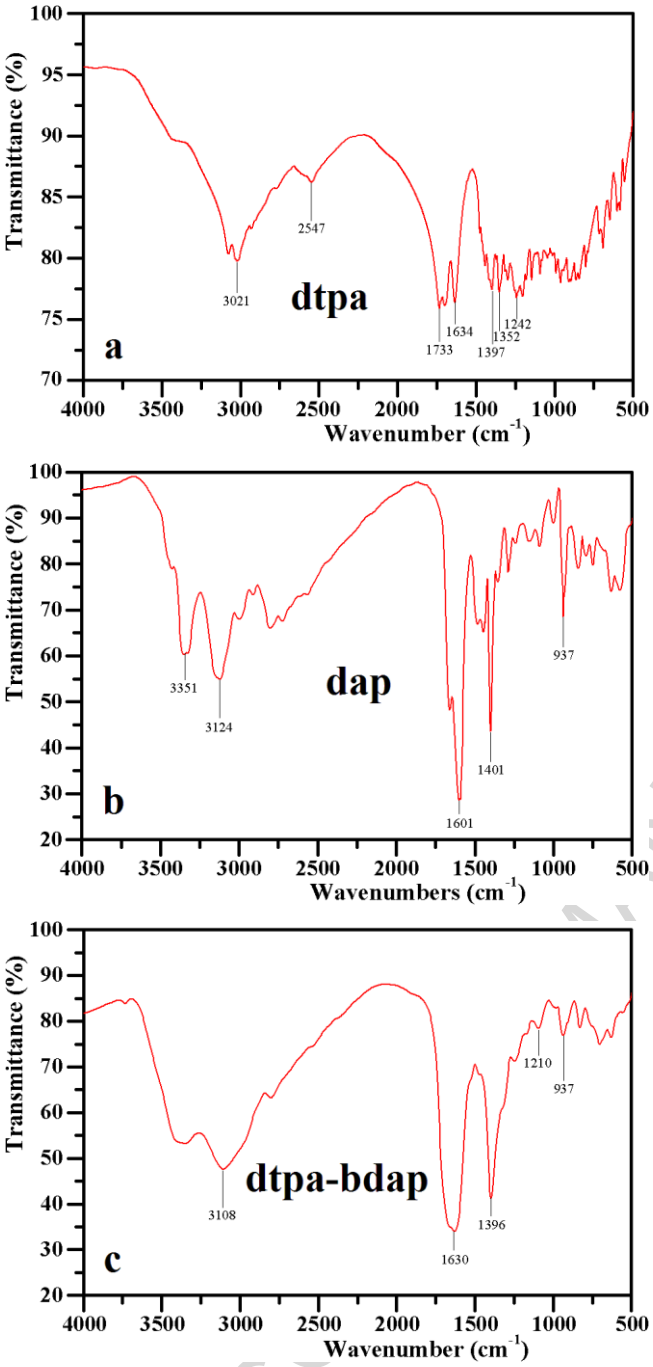

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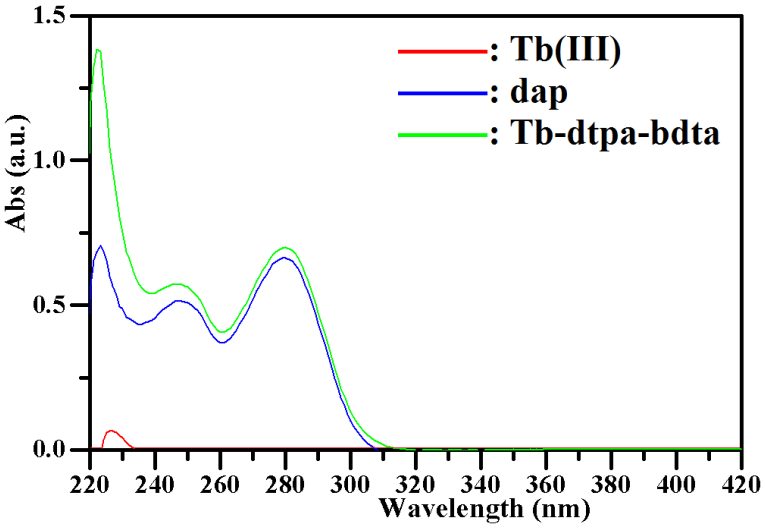

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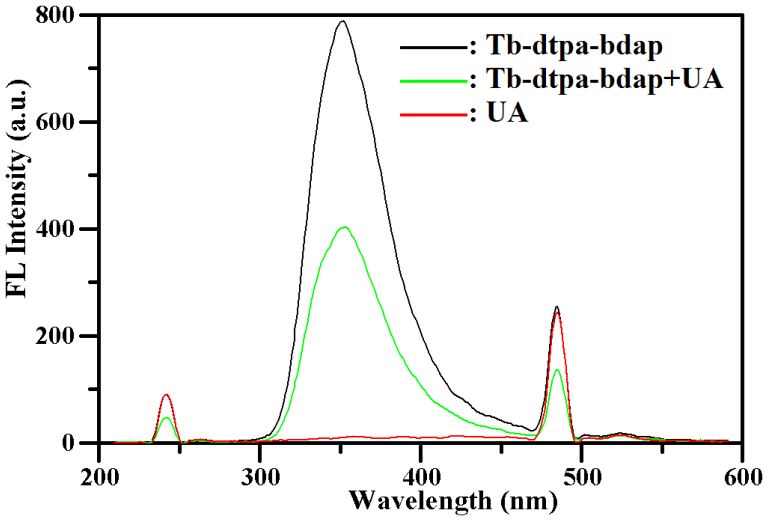

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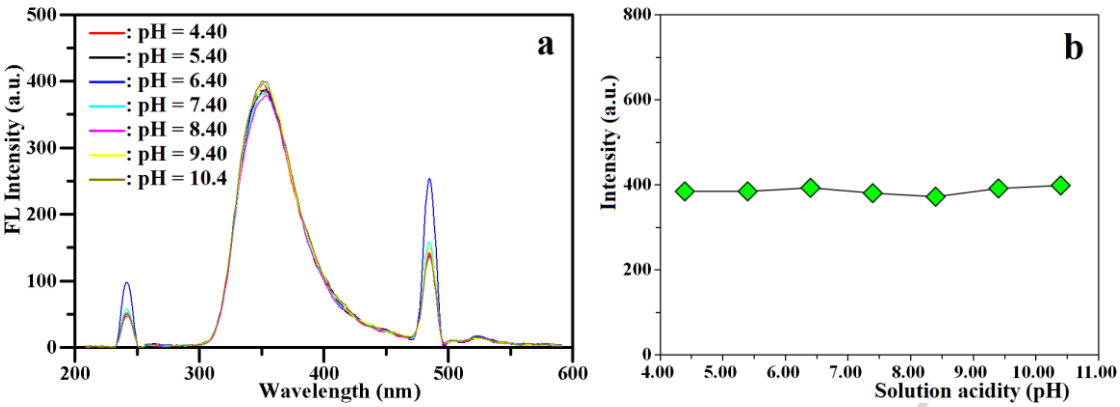


Fig. 6. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap in the presence of uric acid (UA) at different pH values (4.40 - 10.40) and corresponding intensities (λ_{em} = 350 nm) (b). ([Tb-dtpa-bdap] = [UA] = 5.00×10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1} and T_{solu} = 25.00 ± 0.02 °C.)

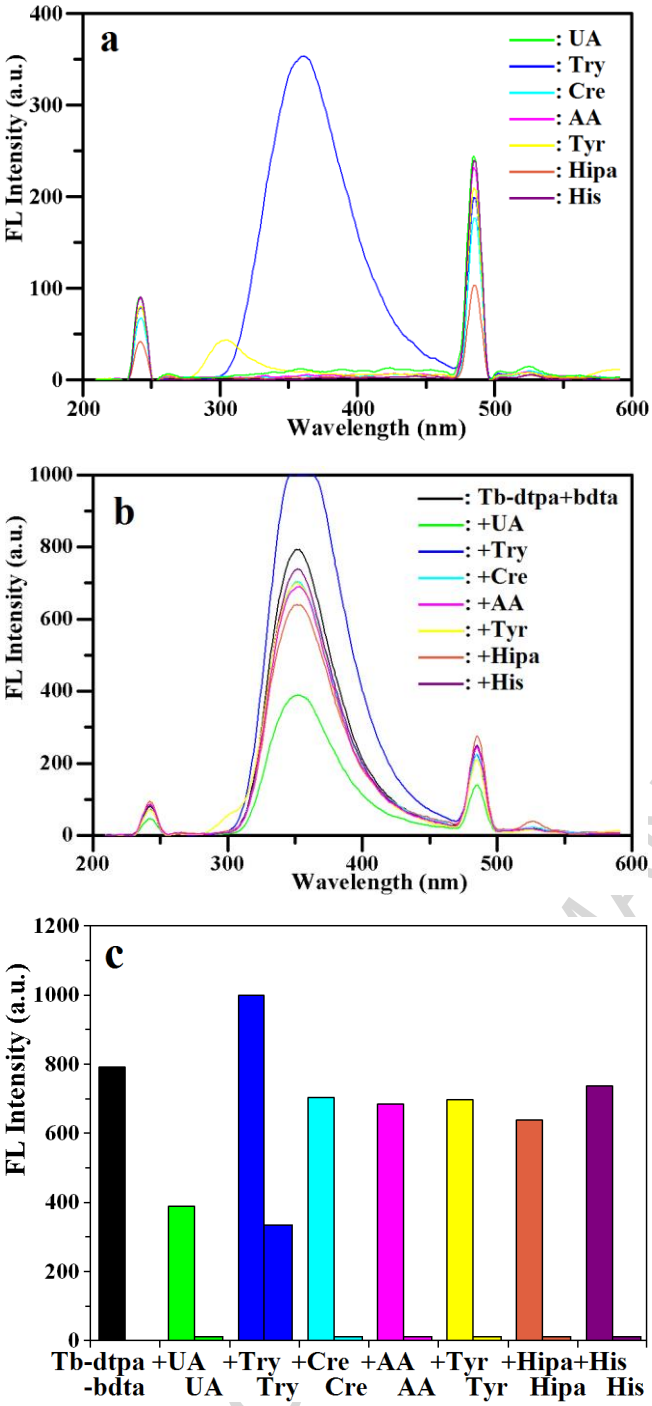


Fig. 7. Fluorescence spectra (λ_{ex} = 241 nm) of Tb^{3+}-dtpa-bdap in Tris-HCl buffer solution in the absence (a) and presence (b) of tryptophan (Try), creatinine (Cre), ascorbic acid (AA), tyrosine (Tyr), hippuric acid (Hipa), uric acid (UA) and histidine (His) and the corresponding fluorescence intensities (λ_{em} = 350 nm) (c). ([Tb^{3+}-dtpa-bdap] = [UA] = [Try] = [Cre] = [AA] = [Tyr] = [Hipa] = [His] = 5.00×10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

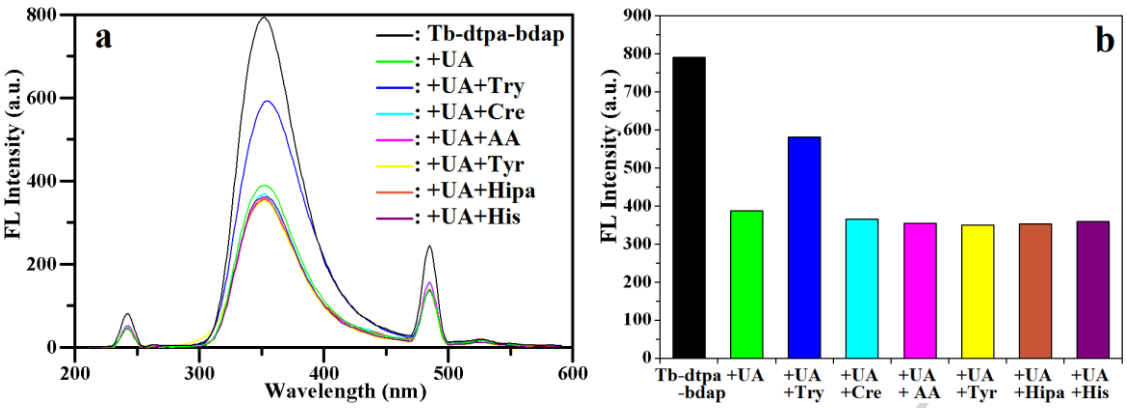


Fig. 8. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap, Tb^{3+}-dtpa-bdap+UA and Tb^{3+}-dtpa-bdap+UA upon the addition of tryptophan (Try), creatinine (Cre), ascorbic acid (AA), tyrosine (Tyr), hippuric acid (Hipa) and histidine (His) in Tris-HCl buffer solutions and the corresponding fluorescence intensities (λ_{em} = 350 nm) (b). ([Tb^{3+}-dtpa-bdap] = [UA] = [Try] = [Cre] = [AA] = [Tyr] = [Hipa] = [His] = 5.00×10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

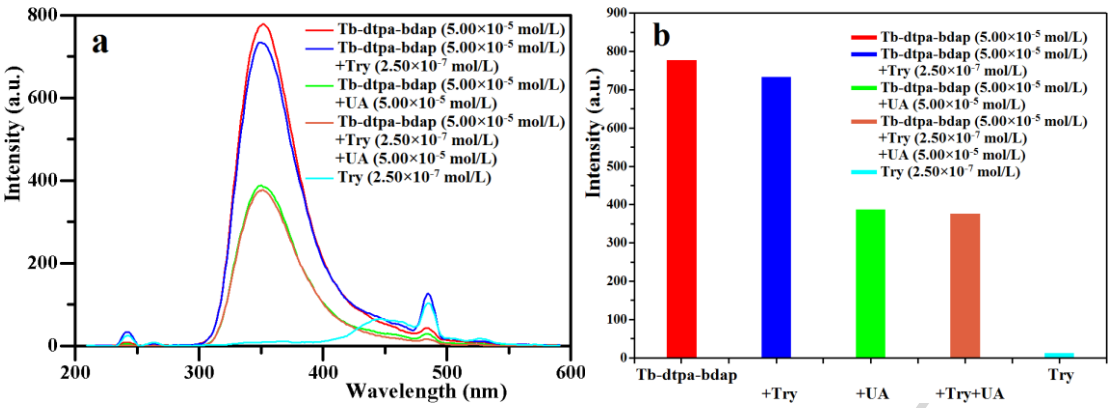


Fig. 9. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap, Tb^{3+}-dtpa-bdap+Try, Tb^{3+}-dtpa-bdap+UA Tb^{3+}-dtpa-bdap+UA+Try and Try in Tris-HCl buffer solutions and the corresponding fluorescence intensities (λ_{em} = 350 nm) (b). ([Tb^{3+}-dtpa-bdap] = [UA] = 5.00 × 10^{-5} mol·L^{-1}, [Try] = 2.50 × 10^{-7} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and Tsolu = 25.00 ± 0.02 °C.)

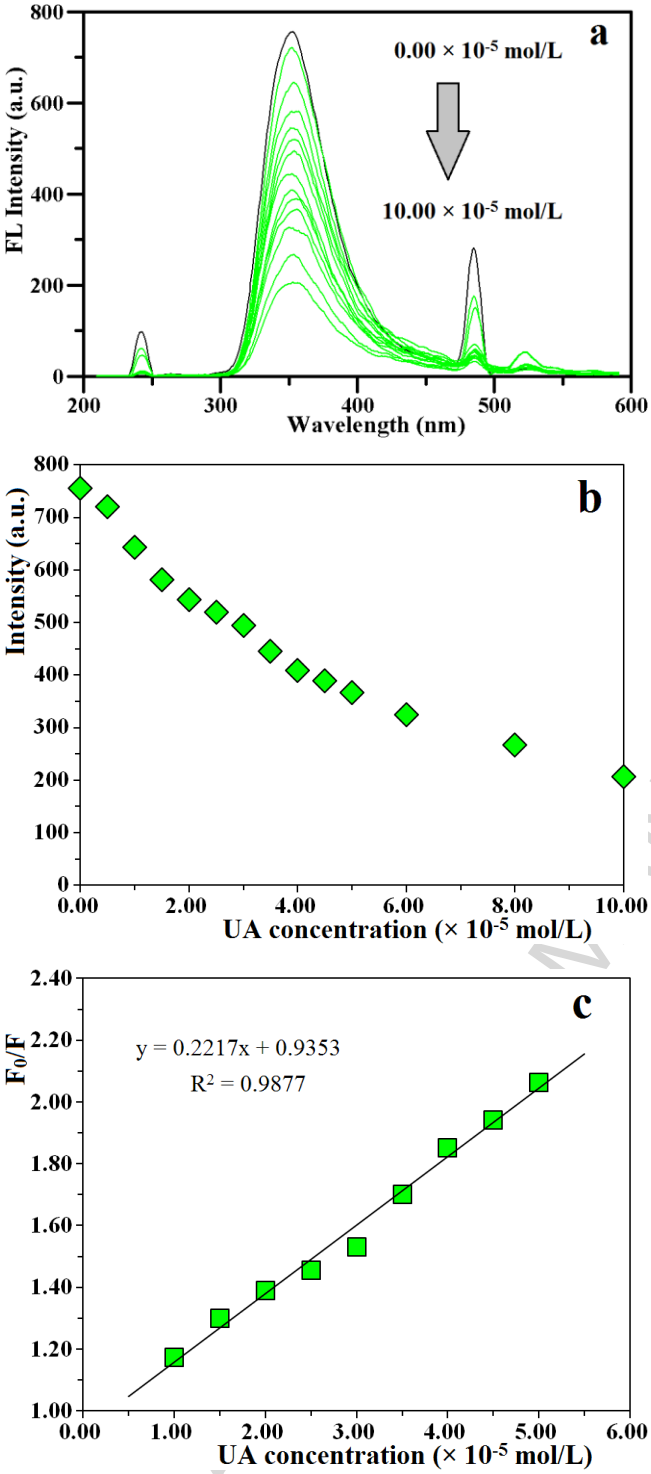


Fig. 10. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap in Tris-HCl buffer solutions with different concentrations of uric acid (UA) from 0.00 × 10^{-5} mol·L^{-1} to 10.00 × 10^{-5} mol·L^{-1}, corresponding fluorescence intensities (λ_{em} = 350 nm) (b) and linear responses (c) of Tb^{3+}-dtpa-bdap as a function of uric acid (UA) concentration (1.00 × 10^{-5} - 5.00 × 10^{-5} mol·L^{-1}). ([Tb^{3+}-dtpa-bdap] = 5.00 × 10^{-5} mol·L^{-1}, [Tris-HCl] = 50 mmol·L^{-1}, pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

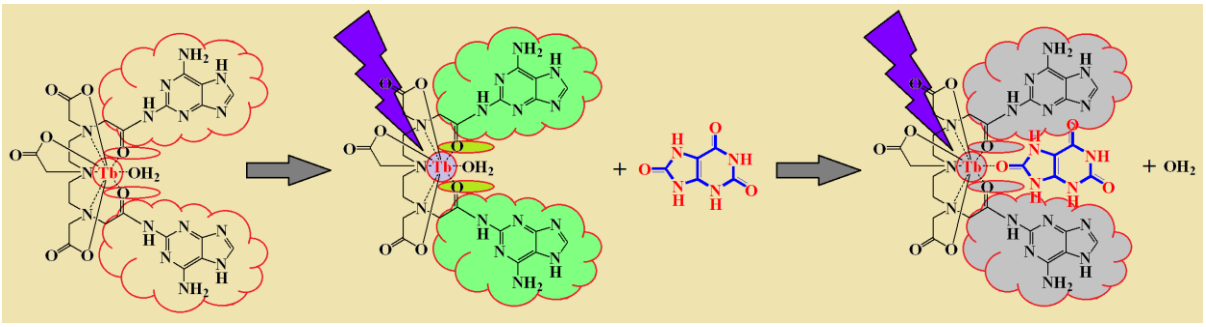


Fig. 11. The reaction mechanism of Tb^{3+}-dtpa-bdap with uric acid.

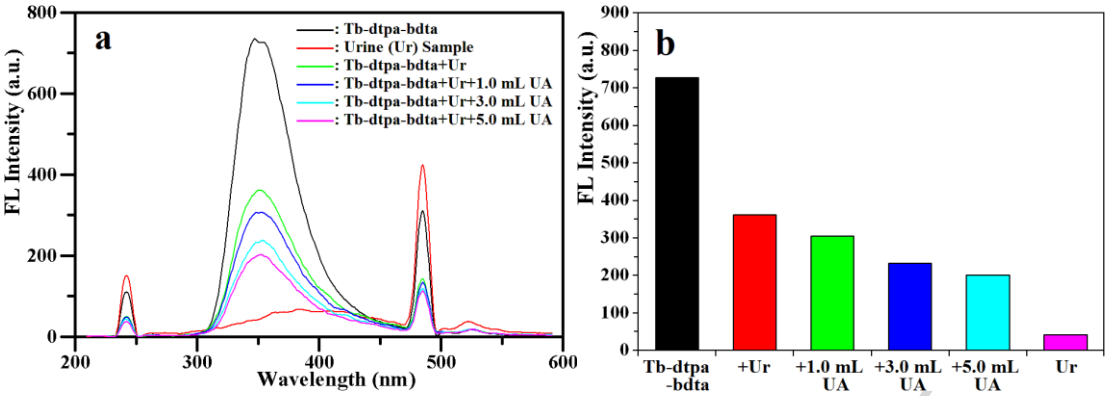


Fig. 12. Fluorescence spectra (λ_{ex} = 241 nm) (a) of Tb^{3+}-dtpa-bdap and Tb^{3+}-dtpa-bdap with different concentrations of uric acid (UA) in diluted urine samples with 100-folds as well as blank urine and corresponding fluorescence intensities (λ_{em} = 350 nm) (b). ([Tb^{3+}-dtpa-bdap] = 5.00×10^{-5} mol·L^{-1}, [UA] = 1.00×10^{-5}, 3.00×10^{-4} and 5.00×10^{-5} mol·L^{-1}. pH = 7.40 and T_{solu} = 25.00 ± 0.02 °C.)

Tables:

Table 1

Comprasion of the proposed method with others.

Analytical method	LOD/mol·L^{-1}	Linear range/mol·L^{-1}	Reference
Electrochemical (OMC-Fc)	1.80×10^{-6}	6.00×10^{-5} to 3.90×10^{-4}	[43]
Nanoparticle based-colorimetry	4.80×10^{-5}	Not satated	[44]
Enzymatic-fluorescent method	1.00×10^{-5}	2.00×10^{-5} to 6.00×10^{-6}	[45]
HPLC-UV	1.42×10^{-6}	1.70×10^{-6} to 4.00×10^{-6}	[46]
Fluorometric	1.00×10^{-6}	4.00×10^{-5} to 3.60×10^{-6}	[47]
Fluorescent probe	5.80×10^{-6}	1.00×10^{-5} to 5.00×10^{-5}	This work

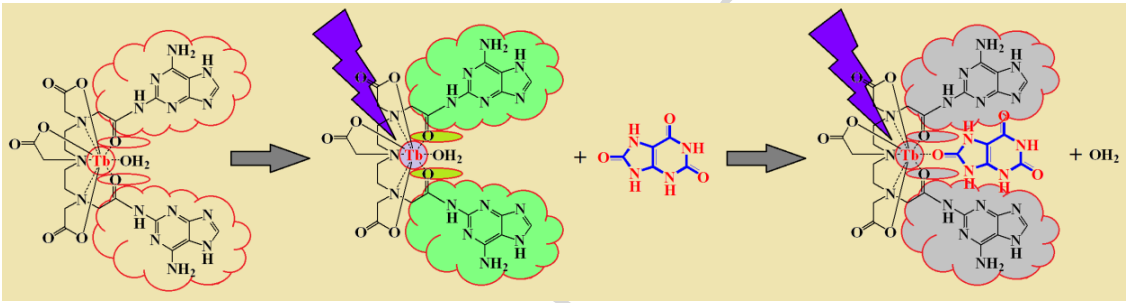
Table 2

Determination results of uric acid (UA) content in diluted urine samples with 100-folds.

Simple	Add ($mol \cdot L^{-1}$)	Found ($mol \cdot L^{-1}$)	Recovery (%)
1	0	4.84×10^{-5}	—
2	1.00×10^{-5}	5.87×10^{-5}	103
3	3.00×10^{-5}	7.69×10^{-5}	95
4	5.00×10^{-5}	9.89×10^{-5}	101

Graphical Abstract

A novel fluorescent probe, Tb-dtpa-bdap, is designed based on the principle of complementary base pairing and synthesized for uric acid detection. The affecting factors, including solution pH, uric acid concentration and interfering substances, on the detection of uric acid using this prepared probe are examined. Under the optimized conditions, the fluorescence intensities of Tb-dtpa-bdap solution towards different uric acid concentrations show a linear response in the range from 1.00×10^{-5} mol·L to 5.00×10^{-5} mol·L with the correlation coefficient (R^2) of 0.9877. And the obtained limit of detection (LOD) is about 5.80×10^{-6} mol·L^{-1}, which is much lower than the level of uric acid in actual urine. The mechanism on the detection of uric acid by using Tb-dtpa-bdap is inferred according to the above mentioned experimental results. The facts demonstrate that the proposed fluorescent probe may be successfully applied for the determination of uric acid in human urine samples.



Research Highlights

1. Tb-dtpa-bdap is designed and synthesized as fluorescent probe to detect uric acid.

2. 2,6-diaminopurine is used to improve recognition ability of Tb-dtpa to uric acid.

3. Synthesized Tb-dtpa-bdap as fluorescent probe is used to detect uric acid in urine.

4. Complementary base pairing principle is ued to design probe for detecting uric acid.