Synthesis, Luminescence Properties of Eu (III) and Tb (III) Complexes with a Polyamine Polycarboxylic Ligand as well as Their Binding Characteristics with Bovine Serum Albumin (BSA)

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A novel polyamine polycarboxylic ligand (L) with imidazole and pyridyl moieties and its corresponding complexes 

\[ \text{Na}_2\text{Eu(LCl)}_3 \cdot \text{H}_2\text{O} \text{ and Na}_2\text{TM(LCl)}_3 \cdot \text{H}_2\text{O} \text{, on to which Eu(III) and Tb(III) were bound via coordination, were prepared and characterized by FT-IR, } ^1\text{HNMRI, TG-DTA and CHN elemental analysis. The thermogravimetric analysis shows the Eu(III) and Tb(III) complexes have good thermal stabilities with decomposition temperatures 380 and 398 °C, respectively. The luminescence properties of the complexes show the ligand has excellent antenna effect to sensitize the lanthanide ions while using ethyl p-dimethylaminobenzoate in ethanol as reference sample, the quantum efficiency of the Eu(III) and Tb(III) complexes are 0.264 and 0.280 in the DMSO at room temperature, respectively. The interactions of the complexes with bovine serum albumin (BSA) were analyzed by fluorescence measurements under physiological conditions. The UV–vis absorbance and the Stern-Volmer analysis indicate that the main quenching mechanism of BSA by the complexes is a static quenching procedure. The binding constants, binding site number at different temperatures were calculated by van’t Hoff equation, which confirms that the Van der Waals and hydrogen bond interactions are mainly impulsion to the formation of complexes BSA-L coordination compound. Furthermore, the effect of complex on the conformation of BSA was analyzed according to synchronous fluorescence and circular dichroism (CD) spectra.

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Manuscript submitted March 9, 2015; revised manuscript received April 6, 2015. Published April 18, 2015.

Within recent years, the chemistry of organic lanthanide complexes has developed rapidly and extensively due to their enormous potential applications in fluorescent displays, phosphorescence devices, lasers, electroluminescence optical devices, probes for chemical and biological molecules and for the study of drugs with regard to activities, reactions and dynamics. However, as is well known, the absorption and emission spectra intensity of the lanthanide ion are weak because of their low quantum yields and poor molar absorptivities. Organic ligand sensitized fluorescence with high fluorescent efficiency, narrow band f-f emissions and excellent monochromatocity, which acts as “antenna” is employed to overcome these drawbacks. Based on the different chromophoric antennae and the central Re(III), many novel ligands and complexes have been designed and synthesized. Among them, the chromophore of containing pyridine and pyrazole rings is one of the promising ligands, as it can show a new ployfunctional molecule featuring large π-conjugated system

\[ \text{L} \], which with pyridine and pyrazole rings is one of the promising ligands, as it can show a great conjugate structure that can sensitize the emissive states of lanthanide ions through highly efficient intramolecular energy transfer. The ionic molecules of these lanthanide complexes, we were prompted to construct a new ployfunctional molecule featuring large π-conjugated system named (4, 4′-t(3, 3′-pyridine-2, 6-diy)bis(5-phenyl-1H-pyrazole-3, 1-diy)bis(methylene)bis (pyridine-2, 6-dicarboxylic acid L), which has multiple N and O donor atoms as coordination sites, providing a nine-coordinating environment for the Eu(III) and Tb(III) ions and reducing the possibility of coordinated water molecules involved. When the complexes are formed, the shielding effect is expected to reduce nonradiative deactivation processes and protects metal ions from environmental interference. Furthermore, the structure of L contains pyridine and pyrazole rings, which are indispensable in many drug molecules. On the other hand, serum albumins are the most abundant proteins in circulatory system, serving as transporters for a variety of ligands such as fatty acids, hormones, and drugs. The luminescence properties of the complexes were studied and they were proved to be efficient sensitizers. Moreover, the binding interaction mechanism between the complexes and BSA were investigated based on fluorescence quenching. UV-vis absorbance spectra, synchronous fluorescence and circular dichroism (CD) spectra, which was of great importance to provide a theoretical basis for their potential medical and chiroptical systems, showed and a model of interaction for drug design as well. The synthetic route of the ligand is outlined in Scheme 1.

Experimental

Materials and methods.—The stock solution of BSA (purity ≥ 99%, purchased from Sino-Biotechnology Company, Shanghai, China) was prepared to be the concentration of 1.0 × 10^{-6} mol · L^{-1} and kept in the dark at 0–4 °C. After TbCl₃·2H₂O was dissolved in water, and diluted to the desired concentration, the working solutions of TbCl₃·2H₂O was obtained. The buffer Tris was purchased from Acros (Geel, Belgium). Still, Tris-HCl buffer solution (pH = 7.4) was given via the dropwise addition of HCl (0.1 mol · L^{-1}) to Tris solution (0.1 mol · L^{-1}). 0.9% NaCl solution was used to maintain the ionic strength. Dimethylpyridine-2, 6-dicarboxylate 2 was prepared by literature. EuCl₃, TbCl₃ and 4-Hydroxymethyl-pyridine-2, 6-dicarboxylate 3 was prepared as described in the literature. Other chemicals were of A. R. grade and used without further purification. Water used in this study was deionized. Melting points were determined on a XRD-4 apparatus (thermometer uncorrected). Elemental analysis was carried out by a PerkinElmer2400 elemental analyzer. Infrared spectra (4000–400 cm⁻¹) were recorded with samples as KBr discs using a Nicolet NEXUS 670 FT-IR spectrophotometer. ¹HNMRI was measured with a Bruker-400 MHz nuclear magnetic resonance spectrometer with CDCl₃, DMSO or D₂O as solvents and TMS as internal reference. The UV–vis spectra were recorded on an UV-2450 spectrophotometer. The luminescence measurements were made on a Hitachi F-4600 spectrophotometer, the widths of both the excitation and emission slit were set to 5 nm with the photomultiplier tube voltage at 700 V. CD measurements were carried out on a J-810 Spectrophotometer.

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spectropolarimeter (Jasco, Tokyo, Japan) in a cell of path length 1.0 mm at room temperature.

Synthesis of the ligand L.—Synthesis of 2, 6-dimethoxycarbonyl-4-bromomethylpyridine 4.—Phosphorous bromide (0.9 g, 3.32 mmol) was added dropwise to a solution of 4-hydroxymethyl-pyridine-2, 6-dicarboxylic acid dimethyl ester 3 (0.5 g, 2.22 mmol) in tetrahydrofuran (15 ml) and stirred at room temperature for 12 h. The reaction mixture was treated with the solution of NaHCO₃ to pH 7, and extracted with dichloromethane (3 × 30 ml). The organic phase was dried over sodium sulfate, filtered, and concentrated by vacuum and the residues were recrystallized using dichloromethane and petroleum ether mixture (1/4, v/v) to give the white compound 4 (0.5 g, 78.3%). mp: 105–107 ◦C. IR (KBr); ν max(cm⁻¹): 3077 (CH₂), 2949 (CH₃), 1720 (C=O), 1604 (C–O), 1443, 1420, 1356 (skeleton of Py); ¹HNMR (CDCl₃, ppm): δ 8.73 (s, 2H, Py–H), 4.50 (s, 2H, CH₂Br), 4.03 (s, 6H, CO₂CH₃).

Synthesis of 1-[6-(3-oxo-3-phenyl-propionyl)-pyridin-2-yl]-3-phenylpropane-1, 3-dione 5.—Dimethyl-2, 6-pyridinedicarboxylate 2 (1.95 g, 10 mmol) was added to a solution of metal sodium (0.92 g, 40 mmol) in dry toluene (40 ml), and then a solution of acetophenone (4.8 g, 40 mmol) in 10 ml dry toluene was added dropwise under stirring at 60 ◦C. Then, the mixture was heated to 110 ◦C for about 24 h. The yellow precipitate was collected by filtration, washed thoroughly with petroleum ether. The residue was dissolved in 10 ml water, treated to pH 3–4 with 2 M HCl, and the crude products were recrystallized from ethyl acetate to obtain the yellow powder compound 5 (0.74 g, 85.0%). mp: 176–177 ◦C; IR (KBr): ν max(cm⁻¹): 3422 (C=O–OH), 1620 (C=O–OH), 1582, 1529, 1466, 1315 (skeleton of Ph, Py and Pyraz); ¹HNMR (DMSO-d₆, ppm): δ 16.5 (s, 2H, OH), 8.04–8.32 (m, 3H, J= 8 Hz, Py–CH=), 7.25–7.75 (m, 10H, J= 8 Hz, Ar–H), 6.9 (s, 2H, CH).

Synthesis of 2, 6-bis-(5-phenyl-1H-pyrazol-3-yl)-pyridine 6.—Hydrazine hydrate (8.0 ml, 85.0%) was added dropwise to a solution of 5 (0.74 g, 2 mmol) in acetic acid (30 ml) and then the mixture was refluxed for 24 h with stirring. The mixture was filtered and washed with water. Recrystallization from ethyl acetate, white powder compound 6 was obtained (0.58 g, 79.9%). mp: 232–234 ◦C. IR (KBr): ν max(cm⁻¹): 3201 (N–H), 1675, 1582, 1529, 1466, 1315 (skeleton of Ph, Py and Pyraz); ¹HNMR (DMSO-d₆, ppm): δ 13.57–13.62 (d, 2H, J= 2 Hz, NH), 7.94–8.05 (m, 3H, J= 8 Hz, Py–CH=), 7.22–7.83 (m, 10H, J= 8 Hz, Ar–H), 7.35–7.37 (d, 2H, J= 8 Hz, Pyraz).

Synthesis of tetramethyl-4, 4′-[((3, 3′-(pyridine-2, 6-diyl)bis(5-phenyl-1H-pyrazole-3, 1-diyl)) bis(methylene))bis(pyridine-2, 6-dicarboxylate) 7.—To a solution of 15 ml of dry CH₃CN containing 0.3 g of 6 (1.04 mmol), 0.18 g of 4 (0.5 mmol) and 0.3 g of K₂CO₃ were added. The mixture was refluxed for about 24 h with stirring. The solution was filtered to remove precipitate and the solvent was evaporated. The crude product was obtained and purified by silica gel column chromatography using methanol and dichloromethane mixture (1/19, v/v) as eluent to yield the white powder compound 7 (0.28 g, 71.3%). mp: (>300 ◦C). IR (KBr): ν max(cm⁻¹): 3422 (3064 (CH)), 1620 (C=O), 1585 (C=N); ¹HNMR (CDCl₃, ppm): δ 16.5 (s, 2H, OH), 8.04–8.32 (m, 3H, J= 8 Hz, Py–CH=), 7.25–7.75 (m, 10H, J= 8 Hz, Ar–H), 6.9 (s, 2H, CH).
NaOH (0.01 M) was added dropwise to pH 6.0. Then the mixture of the following equation described by Greenfield and Fasman:

\[
\alpha_{\text{helix}} = \left[1 - \frac{ME_{208}}{30000 - 4000}\right] \times 100
\]

The elemental analytical data for the complexes were presented in Table I. The results of elemental analyses of C, H, N in complexes are in good agreement with structural for-

| Complexes | C (%) | H (%) | N (%) |
|-----------|-------|-------|-------|
| Na3EuLCl3·6H2O | 33.16 (34.24) | 2.76 (2.75) | 10.03 (10.01) |
| Na3TbLCl3·6H2O | 32.99 (33.08) | 2.74 (2.71) | 9.96 (9.94) |

The complexes are yellowish powder, stable under atmospheric condition and slightly soluble in acetone, DMF and DMSO.

IR spectra of the complexes.—The FT-IR spectra of the two complexes are similar, indicating that they are structurally homologous (Figure 1). The FT-IR spectrum of the free ligand shows bands at 3448, 1722 and 1597 cm⁻¹, which can be assigned to v(OH), v(C=O) and v(C≡N) of the ligand, respectively. In comparison with the ligand, the FT-IR spectra of the two complexes are changed greatly, the band v(C=O) at 1722 cm⁻¹ in free ligand disappears and the new bands appear at 1611–1604 cm⁻¹ and 1411–1405 cm⁻¹ assignable to \[\nu_{\text{as}}(\text{COO}^-) + \nu_{\text{s}}(\text{COO}^-)\], stretching vibration modes of the carboxylate group, respectively. The absorption band at 409 cm⁻¹ and 420 cm⁻¹ are assigned to v(Tb-O) and v(Eu-O). These indicate that the carboxylic acid groups are converted into carboxylate anions due to the formation of the stable Ln³⁺-cored complexes. The high intensity bands appearing around 1597 cm⁻¹ in ligand, due to C=O group in pyridine ring downshifts to about 1448–1453 cm⁻¹ in the Ln(III) complexes. The obvious shifts indicate that the C=O groups of the ligands coordinate to the Ln(III) ions through nitrogen atoms.

Thermogravimetric analysis.—The results of TGA of the two complexes Na3TbLCl3·6H2O and Na3EuLCl3·6H2O are presented in Figures 2–3. There are two main successive mass loss stages in the TG-DTA curves. For Tb(III) complex, the first stage loss in the range 30–180 °C owing to the loss of the solvated water content (weight loss: 9.54%), and the second loss step ranging from 280 to 700 °C corresponding to the loss of the ligand L (weight loss: 50.36%). While for Eu(III) complex, the first stage decomposition appears within 30–160 °C accounts for the loss of solvated water (weight loss: 10.23%), and the second loss stage occurs in the temperature range 270–700 °C is attributed to the loss of L content (weight loss: 53.17%), which is

interactions with BSA.—Fluorescence spectroscopy.—To 1 ml of BSA stock solution in a 3 ml of quartz cell, the complex working solution (4×10⁻⁵ mol/L) was gradually added in turn and the concentration of it varied from 0 to 4×10⁻⁵ mol/L. The solution was excited at 298 K in the region of 260–300 nm. The 1 ml of BSA stock solution in a 3 ml of quartz cell, the complex working solution (4×10⁻⁵ mol/L) was gradually added in turn and the concentration of it varied from 0 to 4×10⁻⁵ mol/L. The solution was excited at 298 K in the region of 260–300 nm. The 1 ml of BSA stock solution in a 3 ml of quartz cell, the complex working solution (4×10⁻⁵ mol/L) was gradually added in turn and the concentration of it varied from 0 to 4×10⁻⁵ mol/L. The solution was excited at 298 K in the region of 260–300 nm. The 1 ml of BSA stock solution in a 3 ml of quartz cell, the complex working solution (4×10⁻⁵ mol/L) was gradually added in turn and the concentration of it varied from 0 to 4×10⁻⁵ mol/L. The solution was excited at 298 K in the region of 260–300 nm. The 1 ml of BSA stock solution in a 3 ml of quartz cell, the complex working solution (4×10⁻⁵ mol/L) was gradually added in turn and the concentration of it varied from 0 to 4×10⁻⁵ mol/L. The solution was excited at 298 K in the region of 260–300 nm. The 1 ml of BSA stock solution in a 3 ml of quartz cell, the complex working solution (4×10⁻⁵ mol/L) was gradually added in turn and the concentration of it varied from 0 to 4×10⁻⁵ mol/L. The solution was excited at 298 K in the region of 260–300 nm. The 1 ml of BSA stock solution in a 3 ml of quartz cell, the complex working solution (4×10⁻⁵ mol/L) was gradually added in turn and the concentration of it varied from 0 to 4×10⁻⁵ mol/L. The solution was excited at 298 K in the region of 260–300 nm.
confirmed by comparing the observed and the calculated mass of the complexes. The detailed data are listed in Table II. The above results prove that the complexes have relatively high thermal stability. And further proof that the water in the complexes is solvated water rather than participated in coordination, since for the coordinated water of analogous complexes releases usually above 200 °C.22,23

Thus, the results of EA, TGA and FT-IR spectroscopy indicate that the ligand L have four carboxylic acid groups and five nitrogen atom which involved with the coordination, the chemical structure of complexes can be suspected as shown in Scheme 2.

**Fluorescence studies of complexes.**—Photoluminescence properties of complexes.—The excitation and emission spectra for the two complexes were measured in DMSO solution at room temperature (Figure 4). The luminescence data for the complexes are listed in Table III. The maximum excitation wavelengths of the Tb (III) and Eu (III) complexes could be observed at 306 nm and 305 nm, respectively, due to the π-π* transition centering at the ligand. For Tb (III) complex, the emission band at 542 nm, 581 nm and 618 nm, which root in deactivation of the 5D4 excited state to the corresponding ground state 7Fj, (J = 5–3) of the Tb3+ ion and the most intense emission is located at 542 nm, and corresponded to 5D4-7F5 (induced electric dipole transition) owing to a highly polarizable chemical environment around the central ion. The emission spectrum of the Eu(III) complex consists of four main bands at approximate 584 nm (5D0-7F1), 618 nm (5D0-7F2), 655 nm (5D0-7F3) and 699 nm (5D0-7F4), and the band at 618 nm is the strongest. Still, each emission band is very narrow and the width of half band is about several nanometers, indicating that the complex has high color purity and the ligand is a comparative good organic chelator to sensitize fluorescence of Tb (III) and Eu (III) ions.

Compared with the similar complexes synthesized by Xiao,24 we find that the complexes prepared by us possess much stronger luminescence intensity. The structures of the ligands are very similar and the primary difference between them is that the novel ligand has more coordination sites. It may be attributed to that the ligand L has two pyrazole rings as well as three pyridine rings, while the former ligand has only a pyridine ring and two pyrazole rings. Although the five rings are not fully conjugated, they all participate in coordination that the increase of the number of the coordination atoms can shield the

| Complexes | Stage | Temperature (°C) | Mass loss (%) found (calc.) | Probable lost molecules |
|-----------|-------|-----------------|-----------------------------|-------------------------|
| Na3EuLCl3 · 6H2O | I | 30–160 | 10.23 (9.34) | 6H2O |
| | II | 270–700 | 53.17 (56.20) | L |
| Na3TbLCl3 · 6H2O | I | 30–180 | 9.54 (9.28) | 6H2O |
| | II | 280–700 | 50.36 (52.37) | L |
Where, the chromophore of BSA is decreased.\textsuperscript{25,26} In given conditions (such as fixed pH, temperature and ionic strength), fluorescence quenching mechanisms usually result from dynamic quenching and static quenching.

The quenching effect of Na\textsubscript{3}TbCl\textsubscript{3} · 6H\textsubscript{2}O on fluorescence intensity of BSA at 280 K; \( \lambda_{\text{ex}} = 280 \text{ nm} \); a-h, c(BSA) = 5 × 10\textsuperscript{-6} mol. L\textsuperscript{-1}; c(L)(10\textsuperscript{-6} mol. L\textsuperscript{-1}): 0, 2.5, 5, 7.5, 10, 15, 20 and 40, respectively.

Figure 5. The quenching effect of Na\textsubscript{3}TbCl\textsubscript{3} · 6H\textsubscript{2}O on fluorescence intensity of BSA at 280 K; \( \lambda_{\text{ex}} = 280 \text{ nm} \); a-h, c(BSA) = 5 × 10\textsuperscript{-6} mol. L\textsuperscript{-1}; c(L)(10\textsuperscript{-6} mol. L\textsuperscript{-1}): 0, 2.5, 5, 7.5, 10, 15, 20 and 40, respectively.

Interaction of L with BSA.—The main quenching mechanism of BSA by the ligand L.— BSA shows a fluorescence band at 340 nm when excited at 280 nm, whereas the complex shows almost no fluorescence band at this excitation wavelength. The fluorescence intensity of BSA at 340 nm is observed to quench gradually with gradual addition of the complex (Figure 5) in buffer solution. At the same time, a slight redshift of the emission maximum wavelength is observed. Results above indicate that the strong interactions between BSA and the complexes exist, and the polarity of microenvironment around the fluorophore of BSA is decreased.\textsuperscript{25,26} In given conditions (such as fixed pH, temperature and ionic strength), fluorescence quenching mechanisms usually result from dynamic quenching and static quenching.

To estimate the difference between the static and dynamic quenching, we have studied the fluorescence at different temperatures. The fluorescence quenching studies are analyzed by the Stern–Volmer equation (Eq. 3), which allows for calculating of quenching constants:\textsuperscript{27}

\[
F_0/F = 1 + K_{\text{sv}}[Q] = 1 + \tau_0 k_0[Q]
\]

Where, \( F_0 \) and \( F \) are the fluorescence intensities of BSA in the absence and presence of quencher, respectively. \( K_{\text{sv}} \) is the Stern–Volmer quenching rate constant (M\textsuperscript{-1} · s\textsuperscript{-1}), \([Q]\) is the concentration of the quencher (M) and \( \tau_0 \) is the average fluorescence lifetime of biomolecule at about 10\textsuperscript{-8} s.\textsuperscript{28} The S–V plots at different temperatures in both the media are shown in Figure 6. It is seen that the \( K_{\text{sv}} \) and \( K_{\text{q}} \) values for BSA increase with the increasing temperature (Table IV), which indicate that the probable quenching mechanism of BSA–complex interaction is initiated by complex formation rather than by dynamic collision. The rate constants \( (K_{\text{q}}) \) for the quenching of BSA by complexes are larger than that of the maximum scattering collision quenching constant \( (2.0 \times 10\textsuperscript{10} \text{ L mol}^{-1} \text{ s}^{-1}) \) and the slope of the Stern–Volmer plot are inversely correlated with the temperature. This confirms that the fluorescence quenching is not the result of dynamic collision quenching, rather a consequence of static quenching.

To further confirm the quenching mechanism induced by the complex, the difference of UV–vis absorption spectra of BSA in the absence and presence of the complex are shown in Figure 7. We can clearly see that with the addition of the complex, the UV–vis absorption spectra of BSA are raised regularly around 280 nm. All we know that the dynamic quenching only affects the excited state of fluorophore and does not change the absorption spectrum. However, the static quenching refers to the formation of fluorophore-quencher complex, which can induce the change of absorption spectrum of fluorophore.\textsuperscript{29} It is clear that the mechanism of fluorescence quenching is a static quenching procedure and indicates BSA molecules binding complexes to form a BSA-L complex.

Binding constants and binding sites.—The above studies clearly confirm that complex forms a complex-BSA and experiences a static quenching process of BSA. The apparent binding constants and the number of binding sites can be determined according to the following equation (Eq. 4):\textsuperscript{30}

\[
\log(F/(F_0 - F)) = \log 1/k_a + n \log[1/[Q]]
\]

where \( K_a \) is the binding constant, and \( n \) is the number of binding sites per BSA.\textsuperscript{31} The plot of \( F/(F_0 - F) \) versus \( \log[1/[Q]] \) gives a linear plot (Figure 8). The value of ‘\( n \)’ is obtained from the slope of the linear plot which is close to 1, indicating the interaction of the complex with BSA has only a single binding site. All the values of \( K_a \) for the BSA–complex system at different temperatures are obtained from the intercept (Table IV). It is noticed that the binding constant values are equal to 10\textsuperscript{5} and decreased with increasing in temperature, indicating the denaturation of BSA with complexes are not very strong, and the unstable compound will be partly decomposed with the rising temperature.

Thermodynamic parameters and binding mode.—In general, the intermolecular interaction between complex and protein mainly include many forces like hydrophobic forces, hydrogen bonds, electrostatic forces in the interaction with H\textsubscript{2}O molecules, which would otherwise cause nonradiative deactivation of the excited state. Thus, it can be explained that the number of the coordination atoms of ligand can be a prominent factor in the efficiency of ligand-to-metal energy transfer, and it is also in agreement with our former study.\textsuperscript{3}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{Complexes} & \textbf{\( \lambda_{\text{ex}} \) (nm)} & \textbf{\( \lambda_{\text{em}} \) (nm)} & \textbf{L} & \textbf{Assignment} \\
\hline
Na\textsubscript{3}TbCl\textsubscript{3} · 6H\textsubscript{2}O & 306 & 542 & 6718 & \( \text{^5}D_4-\text{^7}F_2 \) \\
Na\textsubscript{3}EuCl\textsubscript{3} · 6H\textsubscript{2}O & 305 & 584 & 2603 & \( \text{^5}D_0-\text{^7}F_2 \) \\
\hline
\end{tabular}
\caption{Luminescence data for the complexes.}
\end{table}
Table IV. Fluorescence quenching constants of L-BSA system at different temperatures and different concentrations of BSA.

| Complex      | T (K) | n   | $K_{sv}$ (L·mol$^{-1}$) | $k_{a}$ (L·mol$^{-1}$·s$^{-1}$) | $K_a$ (L·mol$^{-1}$) | $\Delta H$ (kJ·mol$^{-1}$) | $\Delta S$ (J·mol$^{-1}$·K$^{-1}$) | $\Delta G$ (kJ·mol$^{-1}$) | $R^2$  |
|--------------|-------|-----|-------------------------|---------------------------------|----------------------|--------------------------|-------------------------------|----------------------------|-------|
| Na$_3$EuLCl$_3$·6H$_2$O | 293   | 1.12| $9.23 \times 10^4$     | $9.23 \times 10^{12}$          | 1.92 $\times 10^9$  | $-58.36$                | $-98.06$                      | $-29.63$                   | 0.99668 |
|               | 300   | 1.06| $7.47 \times 10^4$     | $7.47 \times 10^{12}$          | 1.08 $\times 10^9$  | $-20.57$                |                                | $-20.57$                   | 0.99223 |
|               | 310   | 0.96| $1.15 \times 10^4$     | $1.15 \times 10^{12}$          | 5.56 $\times 10^9$  | $-20.43$                |                                | $-20.43$                   | 0.99723 |

$R^2$ is the correlation coefficient of Stern–Volmer equation.

Where $K_a$ is analogous to the associative binding constant at the corresponding temperature and $R$ is the gas constant. The linear plot of log $K_a$ versus $1/T$ (Figure 9) shows that the enthalpy $\Delta H$ remains constant for the range of temperature discussed. The data of thermodynamic parameters are presented in Table IV, shows the values of $\Delta H$ and $\Delta S$ obtained for the binding site from the slopes and in accordance with the reported literatures. The negative free energy change $\Delta H$ and $\Delta S$ of the interaction drive the formation of BSA-L complex reveal that van der Waals interactions and hydrogen bonds played major roles in the binding reaction. $\Delta G$ is obtained from Eq. 6

$$\Delta G = \Delta H - T \Delta S$$

We obtain $\Delta G < 0$ and $\Delta H < 0$, which implies that the above quenching process is spontaneous and exothermic.

Quantum efficiency.—The fluorescence quantum yield is defined as the ratio of the number of photons emitted to the number of photons absorbed, which reflects the efficiency of the fluorescence process. The overall quantum yields ($\phi_{\text{overall}}$) of the sensitized lanthanide ions emissions of the complexes were measured in the DMSO solution at room temperature and were cited relative to a reference solution of ethyl p-dimethylaminobenzoate in ethanol ($\phi_{\text{ref}} = 29\%$). The overall quantum yields of the complexes were calculated according to the well-known equation

$$\phi_{\text{overall}} = \phi_{\text{ref}} \left( \frac{n^2 A_{\text{ref}} I}{n^2_{\text{ref}} A_{I_{\text{ref}}}} \right)$$

Here, $n$, $A$ and $I$ denote the refractive index of solvent, the area of the emission absorbance spectrum, and the emission at the excitation wavelength, respectively. Subscript ref denotes the refractive, and the absence of a subscript denotes the unknown sample, and the $\phi_{\text{overall}}$ is the quantum yield of the sample, $\phi_{\text{ref}}$ is the quantum yield of the ethyl p-dimethylaminobenzoate in ethanol. The refractive index is...
assumed to be equivalent to that of the pure solvent: 1.096 for DMSO and 1.4783 for ethanol at room temperature. The estimated error for the quantum yields is (±10%).

Energy transfer from BSA to complexes.—The overlap of the UV–vis absorption spectrum of complexes with the fluorescence emission spectrum of BSA is shown in Figure 10. The importance of the energy transfer in biochemistry is that the efficiency of transfer can be used to evaluate the distance between the ligand and the tryptophan residues in the protein. The energy transfer efficiency is related not only to the distance r between the acceptor and the donor but also to the critical energy transfer distance $R_0$:

$$R_0 = 8.79 \times 10^{-23} K^2 n^{-4} \phi J$$

where $K^2$ is random orientation, usually assumed to be equal to 2/3, n is the average refractive index of medium in the wavelength range where spectral overlaps are significant, $\phi$ is the quantum yield of the donor in the absence of acceptor and J(λ) is the overlap integral of the fluorescence emission spectrum of the donor with the absorption spectrum of the acceptor, which can be obtained from the following expression:

$$J = \frac{\int_\lambda \varepsilon(\lambda) \cdot \lambda^4 d\lambda}{\int_\lambda F(\lambda) d\lambda}$$

where $F(\lambda)$ is the fluorescence intensity of the BSA at wavelength, $\varepsilon(\lambda)$ is the molar absorption coefficient of the flavonoid at the wavelength. The energy transfer efficiency $E$ is given by

$$E = 1 - \frac{F}{F_0} = R_0^6 / (R_0^6 + r^6)$$

In the present case, $n = 1.366$ and $\phi = 0.15$. From the available data, it resulted that $J = 1.95 \times 10^{-4}$ cm$^3$ L$^{-1}$ mol$^{-1}$, $E = 0.207$, $R_0 = 2.22$ nm and $r = 2.78$ nm were calculated. The binding distance $r = 2.78$ nm is less than 8 nm, and $0.5R_0 < r < 1.5R_0$, which indicates that the energy transfer from BSA to complexes occurs with high probability.

Conformation investigations.—Synchronous fluorescence spectra.—The synchronous fluorescence spectra can provide much valuable information about the microenvironment in the vicinity of the chromophore molecules. In the synchronous fluorescence of BSA, the shift in the position of the maximum emission wavelength corresponds to the changes of the polarity around the fluorophore of amino acid residues. When $\Delta\lambda$ values are stabilized at 15 or 60 nm, the synchronous fluorescence of BSA is characteristic of tryptophan residues (Trp) and tyrosine residues (Tyr), respectively. The effect of Na$_3$TbLCl$_3$·6H$_2$O on BSA synchronous fluorescence spectroscopy is shown in Figure 11. We can observe that the fluorescence intensities of Trp is stronger than those of Tyr. The maximum emission wavelength of Tyr keep unchanged at $\Delta\lambda = 15$ nm, whereas a notable redshift of the maximum emission wavelength of Trp are observed upon addition of Na$_3$TbLCl$_3$·6H$_2$O when $\Delta\lambda$ is 60 nm. This reflects that the conformation of BSA is changed in that the polarity around the Trp microenvironment is significantly increased by the combination of Na$_3$TbLCl$_3$·6H$_2$O and BSA.

Circular Dichroism (CD) Spectroscopy.—CD spectra is a sensitive technique for monitoring the conformational changes of protein upon interaction with the ligand. The CD spectra of BSA (shown in Figure 12) exhibit two negative bands at 208 and 222 nm, which are characteristic features of the $\alpha$-helix structure of proteins and any variation in these bands will indicate conformational change in the native structure. The negative ellipticity values of BSA at these wavelengths are decreased slightly in the presence of complex, indicating minor conformational changes in $\alpha$-helical structure. Using the Eq. 1 and 2, the calculated results exhibit a reduction of $\Delta\alpha$ from 60.35% to 57.12% at BSA molar ratio of 1:1. From the above results, it is apparent that the effect of on BSA causes a conformational change of the protein, with the loss of $\alpha$-helical stability.
Conclusions

In summary, we have designed, synthesized, and characterized a new polyamine polycarboxylic ligand with imidazole and pyridyl moieties. The coordination of the metal ions to the ligand occurred at the nitrogen atoms and the oxygen atoms and that water molecules did not participate in coordination. The good quantum yields and the high fluorescent spectra and the CD spectra indicated that the interaction of complex with BSA caused conformational change of BSA. These results are expected to give important insight into interactions of the rare earth complexes and BSA, which show great reference value for a model of application for drug design.

Acknowledgment

This work was supported by the National Natural Science Foundation of China (No 21071152).

References

1. J. Wang, R. Wang, J. Yang, Z. Zheng, M. D. Carducci, and T. Cayou, J. Am. Chem. Soc., 123, 6179 (2001).
2. T. Gunnlaugsson, J. P. Leonard, K. Sn. Chal, and A. J. Harte, J. Am. Chem. Soc., 125, 12062 (2003).
3. G. Stein and E. Wurzburg, J. Chem. Phys., 62, 208 (1975).
4. J. H. Yang, G. Y. Zhu, and B. Wu, Anal. Chem. Acta, 198, 287 (1987).
5. S. Lis, M. Elbanowski, B. M. akowska, and Z. Hnatejko, J. Photochem. Photobiol. A, 150, 233 (2002).
6. Z. Bettencourt-Dias, Adv. Curr. Org. Chem., 11, 1460 (2007).
7. R. R. Tang, C. H. Tang, and C. Q. Tang, J. Organomet. Chem., 696, 2040 (2011).
8. E. Brunet, O. Juanes, M. A. Rodríguez-Blasco, S. P. Vila-Nueva, D. Garayalde, and J. C. Rodríguez-Ubis, Tetrahedron Lett., 46, 7801 (2005).
9. C. Picard, N. Geim, I. Nasso, B. Mestre, P. Tisnes, and S. Laurent et al., Bioorg Med Chem Lett, 16, 5309 (2006).
10. R. Shyni, S. Biju, M. L. P. Reddy, A. H. Cowley, and M. Findlater, Inorg. Chem., 46, 11025 (2007).
11. I. Nasso, S. Bedel, and C. Galup, Picard C. Eur. J. Inorg. Chem., 2008, 2064 (2008).
12. S. Raphael, M. L. P. Reddy, A. H. Cowley, and M. Findlater, Eur. J. Inorg. Chem., 2008, 4387 (2008).
13. Q. R. Wu, J. J. Wang, H. M. Hu, Y. Q. Shangguan, F. Fu, and M. L. Yang et al., Inorg. Chem. Commun., 14, 484 (2011).
14. G. Zolese, G. Falcioni, E. Bertoli, R. Galeazzi, M. Wozniak, and Z. Wypych et al., PROTEINS: Structure, Function, and Genetics, 40, 39 (2000).
15. C. P. Montgomery, E. J. New, D. Parker, and R. D. Peacock, Chem. Commun., 2008, 4261 (2008).
16. P. Caravan, N. J. Clostrier, M. T. Greenfield, S. A. McDermid, S. U. Dunham, and J. W. M. Bulte et al., J. Am. Chem. Soc., 124, 3152 (2002).
17. U. Katarhalli, S. Jaldappagari, and S. S. Kalanur, J. Lumin, 130, 211 (2010).
18. C. Schmuck and U. Machon, Chem. Eur. J., 11, 1109 (2005).
19. G. L. Gu, R. R. Tang, Y. H. Zheng, and X. M. Shi, Spectrochim. Acta A: Mol Biomol Spectrosc, 71, 209 (2008).
20. S. G. Roh, M. K. Nah, J. B. Oh, N. S. Baek, K.-M. Park, and H. K. Kim, Polyhedron, 24, 137 (2005).
21. X. M. Shi, R. R. Tang, G. L. Gu, and K. L. Huang, Spectrochim. Acta A: Mol Biomol Spectrosc, 72, 198 (2009).
22. K. S. Ghosh, S. Sen, B. K. Sahoo, and S. Dasgupta, Biopolymers, 91, 737 (2009).
23. Y. M. Luo, J. Li, L. X. Xiao, R. R. Tang, and X. C. Tang, Spectrochim. Acta A: Mol Biomol Spectrosc, 72, 703 (2009).
24. L. X. Xiao, Y. M. Luo, Z. Chen, J. Li, and R. R. Tang, Spectrochim. Acta A: Mol Biomol Spectrosc, 71, 321 (2008).
25. X. L. Lu, J. J. Fan, Y. Liu, and A. X. Hou, J. Mol. Struct., 934, 1 (2009).
26. T. Wang, Z. Zhao, L. Zhang, and L. Ji, J. Mol. Struct., 937, 65 (2009).
27. Y. C. Liu and Z. Y. Yang, J. Organomet. Chem., 694, 3691 (2009).
28. J. R. Lakowicz and G. Weber, Biochem., 12, 4161 (1973).
29. H. Y. Liu, Z. H. Xu, H. H. Liu, P. X. Xi, and Z. Z. Zeng, J. Am. Chem. Soc., 125, 1237 (2009).
30. J. Kang, Y. Liu, M. X. Xie, S. Li, M. Jiang, and Y. D. Wang, Biochim. Biophys. Acta, 1674, 205 (2004).
31. W. Brzyksa and W. Oiga, Thermochim. Acta, 247, 329 (1994).
32. M. Shi, F. Y. Li, T. Yi, D. Q. Zhang, H. M. Hu, and C. H. Huang, Inorg. Chem., 44, 8929 (2005).
33. S. V. Eliseeva, O. V. Kotova, Fr. Gumy, S. N. Semenov, V. G. Kessler, and L. S. Lepnev et al., J. Photochem. Photobio. A, 112, 3614 (2008).
34. B. P. Kandagul, A. Shashka, J. Seetharamappa, S. M. Shaikh, Y. Jadegoud, and O. B. Ijare, Spectrochim. Acta A, 696, 2040 (2011).
35. B. Klajpert and M. Bryszewska, Biochem., 55, 33 (2002).
36. H. Gao, L. Lei, J. Liu, Q. Kong, X. Chen, and Z. Hu, J. Photochem. Photobio. A, 167, 213 (2004).
37. N. Wang, C. R. Suri, and G. Shekhawat, Appl. Phys. Lett., 92, 133104 (2008).