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Eu(III), Tb(III) complexes with novel ligands containing pyridine-2,6-dicarboxylic acid unit: Synthesis, characterization, fluorescence properties and application in biological imaging

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1. Introduction

The unique luminescence properties of Ln^{III} ions (sharp emission, large Stokes shift, insensitivity to oxygen) and particularly their long excited state lifetime ranging from microseconds (Yb, Nd) to milliseconds (Eu, Tb) triggered the development of timeresolved spectroscopy or microscopy for applications in biological, environmental, or clinical analysis [1,2]. These techniques consist of the introduction of a time delay before the detection of the lanthanide luminescence in order to eliminate parasitic scattering and short-lived luminescence resulting in an enhanced signal/noise ratio [1]. The absorption coefficients of the optical transitions for lanthanide ions are, however, very low which limits their practical application considerably. This drawback can be overcome through the use of highly absorbent chelating ligands, which serve as efficient sensitizers [3]. Pyridine-2,6-dicarboxylic (dipicolinic) acid and its derivatives are highly useful tridentate ligands, they can form nine-coordinate complexes with lanthanide ions [4-6]. In this paper, we designed and synthesized a series of novel chromophores containing dipicolinic acid unit, namely, trans-4-(4'-methoxystyryl) pyridine- 2.6-dicarboxylic acid, trans-4-(4'-(dimethylamino)styryl)pyridine-2,6- dicarboxylic acid, and trans-4-(4'-(diphenylamino)styryl)pyridine-2,6-dicarboxylic acid (Scheme 1), studied the fluorescence properties of their Eu(III), Tb(III) complexes. In addition, the applications in cell imaging of the europium and terbium complexes were investigated.

ABSTRACT

Three novel ligands containing pyridine-2,6-dicarboxylic acid unit, trans-4 -(4'-methoxystyryl) pyridine-2,6-dicarboxylic acid, trans-4-(4'-(dimethylamino)styryl)pyridine-2,6-dicarboxylic acid, and trans-4-(4'-(diphenylamino)styryl)pyridine-2,6-dicarboxylic acid were synthesized and their complexes with Eu(III), Tb(III) ions were successfully prepared. The ligands and the corresponding metal complexes were characterized by means of MS, elemental analysis, IR, ¹H NMR and TG–DTA. The luminescence spectra of Eu(III) and Tb(III) complexes in solid state were studied. The strong luminescence emitting peaks at 615 nm for Eu(III) and 545 nm for Tb(III) can be observed. The applications in cell imaging of the europium and terbium complexes were investigated.

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2. Experimental

2.1. Materials and apparatus

All reagents and solvents were commercial reagents of analytical grade and were used as received. Further purification and drying by standard methods were employed and they were distilled prior to use when necessary.

Trans-dimethyl-4-(4'-methoxystyryl)pyridine-2,6-

dicarboxylate, trans-dimethyl-4-(4'-(dimethylamino)styryl)pyridine-2,6-dicarboxylate, and trans-dimethyl-4-(4'-(diphenylamino)styryl)pyridine-2,6-dicarboxylate were prepared starting from 2,6-dimethylpyridine according to the previous method [4,7].

Melting points were determined with an XT4A apparatus and are uncorrected. Mass spectral studies were carried out using VG12-250 mass spectrometer. ¹H NMR and ¹³C NMR spectra were obtained on a Bruker DRX 400 MHz spectrometer. Chemical shifts were reported in ppm relative to a Me₄Si standard. IR spectra were obtained on a Nicolet Avatar 370 DTGS spectrometer. Elemental analyses were performed by Atlantic Microlab. Steady-state emission and excitation spectra were recorded on PerkinElmer LS55 instrument. Visible absorption spectra were determined on PerkinElmer Lambda 35 spectrophotometer.

2.2. Synthesis of the ligand (L)

2.2.1. Trans-4 -(4'-methoxystyryl) pyridine-2,6-dicarboxylic acid (H_2L_1)

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X=OCH₃, N(CH₃)₂, N(Ph)₂

Scheme 1. Synthesis of the ligands.

for 12 h. Then the solvent was completely removed under reduced pressure. The residue was acidified to pH \approx 2 by dropwise addition of 1 N HCl solution, stirred at room temperature for 2 h and gave 0.275 g orange solid in 92% yield. m.p. > 250 °C. EI–MS:*m*/*z* 299(M⁺).¹H NMR (400 MHz,DMSO-d₆, ppm) δ : 13.36 (s,2H), 8.36 (s, 2H), 7.72–7.76 (d, 1H, *J*=16Hz), 7.69–7.67 (d, 2H, *J*=8Hz), 7.35–7.31(d, 1H, *J*=16.4Hz), 7.01–6.99 (d, 2H, *J*=8.4Hz), 3.79 (s, 3H). ¹³C NMR (DMSO-d₆, δ ppm) δ :166.16, 160.90, 149.21, 148.93, 135.67, 129.72, 129.27,124.37, 122.99, 115.09, 56.02.

2.2.2.

Trans-4-(4'-(dimethylamino)styryl)pyridine-2,6-dicarboxylic acid (H₂L₂)

Compound H_2L_2 was obtained following the same procedure described as H_2L_1 . Yellow solid in 93% yield: ¹H NMR(400 MHz, DMSO-d₆, ppm) δ : 9.41(s,2H), 8.33(s,2H), 8.09 (d,2H, *J*=4.8 Hz), 7.71–7.68 (d,1H, *J*=13.2 Hz), 7.29–7.26 (d,1H, *J*=13.6 Hz), 6.98 (d,2H, *J*=4.4 Hz), 3.00 (s, 6H). ¹³C NMR (DMSO-d₆, ppm) δ :166.22, 151.38, 148.49, 133.88, 132.76, 132.22, 132.13, 129.52,129.40, 128.81, 21.14.

2.2.3.

Trans-4-(4'-(diphenylamino)styryl)pyridine-2,6-dicarboxylic acid (H₂L₃)

Compound H_2L_3 was obtained following the same procedure described as H_2L_1 . Dark red solid in 92% yield. ¹H NMR(300 MHz, DMSO-d₆, ppm) δ : 12.94 (s, 2H), 8.36 (s, 2H), 7.75–7.70 (d, 1H, *J*=15.6 Hz), 7.63–7.61(d, 2H, J=8.4 Hz), 7.37–7.28 (m, 5H), 7.13–7.06 (m, 6H), 6.97–6.94 (d, 2H, J=8.4 Hz). ¹³C NMR(DMSO-d₆, ppm) δ 191.04, 153.30, 146.07, 133.78, 132.76, 132.52, 132.03, 131.93, 130.52, 129.30, 129.18, 126.90, 125.97, 118.70.

2.3. Synthesis of the complexes

To a stirring solution of the ligand (0.160 mmol) in methanol, the methanol solution of $EuCl_3 \cdot 6H_2O$ (19.5 mg, 0.053 mmol) or $Tb(NO_3)_3 \cdot 6H_2O$ (24.5 mg, 0.053 mmol) was added and refluxed for 24 h under nitrogen atmosphere. The precipitate was filtered, washed with methanol and air-dried to give the desired complex (typically about 54% yield).
 Table 1

 Elemental analytical data for the complexes.

Compounds	C (%) found	H (%) found	N (%) found
	(calc.)	(calc.)	(calc.)
$\begin{array}{l} H_6 Eu(L_1)_3 Cl_3 {\cdot} 16H_2 O \\ H_6 Eu(L_2)_3 Cl_3 {\cdot} 6H_2 O \\ H_6 Eu(L_3)_3 Cl_3 {\cdot} 16H_2 O \\ H_6 Tb(L_2)_3 (NO_3)_3 {\cdot} 9H_2 O \\ H_6 Tb(L_3)_3 (NO_3)_3 {\cdot} 16H_2 O \end{array}$	39.68(39.91)	4.89(4.95)	2.94(2.91)
	46.73(47.00)	4.59(4.64)	6.51(6.45)
	52.12(52.42)	4.95(5.00)	4.58(4.53)
	42.20(42.42)	4.56(4.61)	8.82(8.73)
	49.79(50.08)	4.72(4.77)	6.55(6.49)

3. Results and discussion

3.1. Properities of the complexes

The result of elemental analysis (Table 1) indicated that the composition of the complexes conforms to $H_6Eu(L_1)_3Cl_3\cdot 16H_2O$ (5), $H_6Eu(L_2)_3Cl_3\cdot 6H_2O$ (1), $H_6Eu(L_3)_3Cl_3\cdot 16H_2O$ (3), $H_6Tb(L_2)_3(NO_3)_3\cdot 9H_2O$ (2), $H_6Tb(L_3)_3(NO_3)_3\cdot 16H_2O$ (4), respectively. The complexes are soluble in DMSO, DMF, slightly in ethanol and acetone, and insoluble in diethylether and tetrhydrofuran. Because of the insolubility of complexes in suitable solvents we were unsuccessful in growing crystals for single crystal X-ray structural studies.

3.2. IR spectra

The bands useful for suggesting the mode of coordination of ligands are listed in Table 2. The uncoordinated ligands show the bands of middle intensity at $3059 \text{ cm}^{-1}(\text{H}_2\text{L}_1)$, $3016 \text{ cm}^{-1}(\text{H}_2\text{L}_2)$, $3055 \text{ cm}^{-1}(\text{H}_2\text{L}_3)$ attributed to free $v_{(OH)}$ of COOH, and strong and sharp bands at $1725 \text{ cm}^{-1}(\text{H}_2\text{L}_1)$, $1703 \text{ cm}^{-1}(\text{H}_2\text{L}_2)$, $1728 \text{ cm}^{-1}(\text{H}_2\text{L}_3)$ assignable to $\upsilon_{(C=0)}$. The bands at $1598 \text{ cm}^{-1}(\text{H}_2\text{L}_1)$, $1583 \text{ cm}^{-1}(\text{H}_2\text{L}_2)$, $1586 \text{ cm}^{-1}(\text{H}_2\text{L}_3)$ were assigned to pyridine ring stretching $[v_{(C=N)}]$. In the complexes, the bands for $v_{(C=N)}$ of pyridine ring shows a shift to a lower frequency about 89-154 cm⁻¹ as a result of the coordination through metal-nitrogen bond [8]. The bands for $v_{(C=0)}$ of carboxylic groups are disappeared completely, thus indicates that all the carboxylic groups take part in coordination. Two strong absorption bands lying at $1636-1627 \text{ cm}^{-1}$ and $1400-1381 \text{ cm}^{-1}$ were observed in complexes, which were attributed to the asymmetric vibration absorption $\upsilon_{\rm as(COO^-)}$ and symmetric vibration absorption $\upsilon_{\rm s(COO^-)}$

Table 2	
Characteristic IR bands (cm ⁻¹) of the ligands and their complexes.	

Compounds	υ(C=0)	υ (OH)	Py(C=N)	$\delta (=C-H)$	υ_{as} (COO ⁻)	υ_{s} (COO ⁻)	Δv_{as-s}
H ₂ L ₁	1727	3059	1598	966			
H_2L_2	1729	3016	1583	968			
H ₂ L ₃	1728	3055	1586	963			
Na ₂ L ₁			1552	1027	1634	1385	249
Na ₂ L ₂			1599	1024	1625	1384	241
Na ₂ L ₃			1535	1020	1628	1406	222
$H_6Eu(L_1)_3Cl_3 \cdot 16H_2O(5)$			1436	1023	1629	1384	245
$H_6Eu(L_2)_3Cl_3 \cdot 6H_2O(1)$			1448	1023	1627	1384	243
$H_6Eu(L_3)_3Cl_3 \cdot 16H_2O(3)$			1446	1000	1636	1400	236
$H_6Tb(L_2)_3(NO_3)_3 \cdot 9H_2O(2)$			1445	1023	1633	1381	252
$H_6Tb(L_3)_3(NO_3)_3 \cdot 16H_2O(4)$			1445	1001	1633	1400	233

Table 3
The fluorescence spectra of the europium and terbium complexes

Compounds	λ_{ex} (nm)	λ_{em} (nm)	Assignment
$H_6Eu(L_1)_3Cl_3 \cdot 16H_2O(5)$	277	591	${}^{5}D_{0} {}^{7}F_{1}$
0 (1)5 5 2 (1)		615	${}^{5}D_{0} {}^{7}F_{2}$
		698	⁵ D ₀ ⁷ F ₄
$H_6Eu(L_2)_3Cl_3.6H_2O(1)$	275	590	⁵ D ₀ ⁷ F ₁
		615	⁵ D ₀ ⁷ F ₂
		699	⁵ D ₀ ⁷ F ₄
$H_6Eu(L_3)_3Cl_3 \cdot 16H_2O(3)$	275	591	⁵ D ₀ ⁷ F ₁
		615	⁵ D ₀ ⁷ F ₂
		691	⁵ D ₀ ⁷ F ₄
$H_6Tb(L_2)_3(NO_3)_3 \cdot 9H_2O(2)$	276	489	⁵ D ₄ ⁷ F ₆
		545	⁵ D ₄ ⁷ F ₅
		584	⁵ D ₄ ⁷ F ₄
		620	${}^{5}D_{4} {}^{7}F_{3}$
$H_6Tb(L_3)_3(NO_3)_3 \cdot 12H_2O(4)$	272	488	⁵ D ₄ ⁷ F ₆
		545	⁵ D ₄ ⁷ F ₅
		584	⁵ D ₄ ⁷ F ₄
		621	⁵ D ₄ ⁷ F ₃

of the carboxylic groups. For ligands L₂ and L₃, the asymmetric stetching vibration frequencies $v_{as(COO^-)}$ in the complexes show a shift to a higher frequency, meanwhile the symmetric stretching vibration frequencies $v_{s(COO^-)}$ show a shift to a lower frequency. The determined Δv_{as-s} for the Ln(III) complexes is larger than that of Na₂L. This state clearly that the carboxlic group acting as a single dentate chelate coordinated to the lanthanide ions in the complexes[9,10]. The $v_{as(COO^-)}$ of the L₁ ligand in coordination compound was shifted to a lower frequency region as compared with free ligand, therefore the Δv_{as-s} was decreased and the carboxylic group was coordinated with a Eu(III) ion as a form of a symmetrical chelating bi-dentate [11]. The broad bands at about 3430 cm⁻¹ in all complexes indicate the presence of crystal water molecules [12,13]. The IR spectra and elemental analytical data suggest that the coordination numbers of Eu(III) and Tb(III) ions is nine.

3.3. Fluorescence properties of complexes

Fluorescence properties of complexes in solid were shown in Table 3, Figs. 1 and 2. The europium and terbium complexes exhibited strong characteristic luminescence of europium and terbium ions when excited at about 276 nm. The most intense transition of the Tb(III) complexes is observed at 545 nm due to the ${}^{5}D_{4} \rightarrow {}^{7}F_{5}$



Fig. 1. Emission spectra of complexes with Eu(III) ions.



Fig. 2. Emission spectra of complexes with Tb(III) ions.

(Fig. 2). The components at 489 nm, 580 nm and 620 nm are assigned to the ${}^{5}D_{4} \rightarrow {}^{7}F_{6}$, ${}^{5}D_{4} \rightarrow {}^{7}F_{4}$, ${}^{5}D_{4} \rightarrow {}^{7}F_{3}$ transition, respectively. And the intensity of ${}^{5}D_{4} \rightarrow {}^{7}F_{5}$ transition is far stronger than that of ${}^{5}D_{4} \rightarrow {}^{7}F_{6}$, the intensity ratio value (${}^{5}D_{4} \rightarrow {}^{7}F_{5}/{}^{5}D_{4} \rightarrow {}^{7}F_{6}$) is 2.0, indicating a low symmetry for the electrostatic field



Fig. 4. DTA analysis of complexes.



Fig. 5. Fluorescence (left) and phase contrast (right) images of sertoli cell fixed in paraformaldehyde and loaded with 4.

surrounding Tb(III) [14]. From Fig. 1, visible bright emission peaks, which centered at 615 nm were observed in europium complexes and assigned to ${}^{5}D_{0} \rightarrow {}^{7}F_{2}$. In all europium and terbium complexes, the ligand-luminescence is completely suppressed, indicating that the ligands are comparative good organic chelators to absorb energy and transfer them to Ln(III) ions (Tb(III) ion or Eu(III) ion).

3.4. TG-DTA analysis

Thermal decomposition of all of the studied complexes was carried out using TG and DTA techniques. Samples of about 10 mg were placed in a crucible, and heated up to 800 °C at the rate of 10 °C/min in an air atmosphere at ambient pressure, using α -Al₂O₃ as reference material.

TG and DTA curves of the complexes are presented in Figs. 3 and 4, respectively. The mass loss observed in the first step of decomposition of all complexes corresponds to the loss of water of crystallization (50-150°C) and coordination water (150–200 °C) [15,16]. After a complete dehydration stage, the subsequent mass loss stages were attributed to decomposition and/or elimination of ligands. The mass loss starts again at first smoothly and then steeply from 450 °C to 560 °C and then smoothly to end up at 800 °C in the case of $H_6Tb(L_2)_3(NO_3)_3.9H_2O$ or H₆Eu(L₂)₃Cl₃·6H₂O, respectively. Concerning H₆Eu(L₃)₃Cl₃·16H₂O and $H_6Tb(L_3)_3(NO_3)_3 \cdot 16H_2O$, the second stage begin first steeply from 230 °C to 330 °C and then gradually up to 800 °C. The decomposition process of $H_6Eu(L_1)_3Cl_3 \cdot 16H_2O$ is similar to that of $H_6Eu(L_3)_3Cl_3 \cdot 16H_2O$ or $H_6Tb(L_3)_3(NO_3)_3 \cdot 16H_2O$, albeit steeper decomposition pathways were observed for $H_6Eu(L_1)_3Cl_3 \cdot 16H_2O$. The TG curves of free ligands show that the residues obtained are close to 0% at 800 °C. The TG results were confirmed by the endothermic peaks observed in DTA curves. TG-DTA curves indicated that L₂-based complexes exhibit better thermal stability.

3.5. Application in cell fluorescence imaging

To test the feasibility of using the complexes for measurements in living cells, the complexes incorporation into cells was investigated [17]. For imaging experiments, sertoli cells were seeded at low density on Lab-Tek Chambered Coverglass grown for 48 h before being fixed for 10 min with paraformaldehyde 4%, washed three times with PBS and incubated with complexe at room temperature for 1 h and were then washed with PBS water solution to remove the excess of unbound complexes. The slides were mounted and imaged by confocal microscopy. The one-photon fluorescence image of complex 4 was presented in Fig. 5. Comparison with a phase contrast image clearly indicates that the complex is mainly localized in a perinuclear region. Bright spots are seldom observed in the nucleus, indicating that the complex difficultly targeted small organelles called nucleoli. When a comparison is made between the fluorescence images, we can find that the fluorescence intensity of the complexes in cells increases with the ligands in the order $L_3 > L_2 > L_1$. The promising results suggest their potential in cell imaging techniques.

4. Conclusions

The preparation, characterication, fluorescence properties and cell fluorescence imagings of the europium(III) and terbium(III) complexes of three novel ligands containing dipicolinic acid unit (L₁, L₂ and L₃) are reported. Difference in the IR spectra of the free ligands and the metal complexes indicated that coordination of the Ln(III) ions to the ligands were in the form of a tridentate ligand. The Eu(III) and Tb(III) complexes exhibited characteristic luminescence of Eu(III) and Tb(III) ions. TG–DTA curves indicated that all complexes are thermally stable and L₂-based complexes exhibit better thermal stability. The results of cell fluorescence imaging show that the fluorescence intensity of the complexes in cells increases with the ligands in the order L₃ > L₂ > L₁. These complexes would be potential candidate materials for application in bioimaging.

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