

# A selective colorimetric chemosensor for lanthanide ions

Zhilian Liu,<sup>a,b</sup> Lin Jiang,<sup>a,b</sup> Zhi Liang<sup>a,b</sup> and Yunhua Gao<sup>a,\*</sup>

<sup>a</sup>Technical Institute of Physics and Chemistry, Chinese Academy of Sciences (CAS), Box 5131, Beijing 100101, People's Republic of China

<sup>b</sup>Graduate School of Chinese Academy of Sciences (GSCAS), Beijing 100039, People's Republic of China

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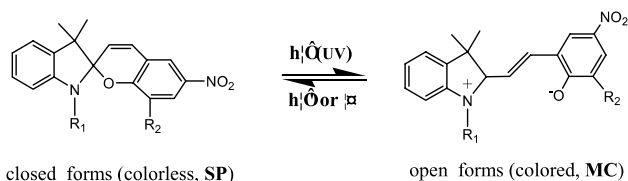
**Abstract**—The synthesis and complexing properties of a calix[4]arene derivate (**6**) carrying two spirobenzopyran moieties are described. The addition of lanthanide ions resulted in significant UV–vis spectral shifts (68–84 nm) in visible region. It indicates that the synthetic receptor can recognize lanthanide ions by naked eyes over other cations including Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup> and Zn<sup>2+</sup>. The mechanism of recognition was studied with <sup>1</sup>H NMR, UV–vis spectra and emission spectra. The receptor may be applied to sense lanthanide ions.

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## 1. Introduction

Lanthanide ions have been widely applied in many fields, for example, as probes and labels in chemical and biological applications.<sup>1</sup> In order to overcome the weak absorption coefficients of the lanthanide ions and sensitize their luminescence, many lanthanide (III) chelates have been designed containing ‘antenna effect’.<sup>2</sup> The macrocyclic chelating agents including cryptands,<sup>3</sup> calixarene,<sup>4</sup> cyclodextrins<sup>5</sup> and crown-ether derivatives,<sup>6</sup> are commonly used to sensitize luminescence of Eu (III) and Tb (III) ion.

Spirobenzopyran derivatives have been extensively investigated for their photochromism<sup>7</sup> and applications in photo-switchable molecular devices.<sup>8</sup> Under the irradiation of ultraviolet light (or in dark conditions), the colorless, neutral spiroopyran (SP) forms are isomerized to the colored, zwitterionic merocyanine forms (MC) while the s–p hybridization of a single C atom becomes p–conjugation over the whole molecule (Scheme 1). The zwitterionic merocyanine forms may have stronger affinity for lanthanide ions than the neutral host for their electrostatic interaction.<sup>9</sup>



**Scheme 1.** Photochromic reactions of spirobenzopyran derivatives.

**Keywords:** Calix[4]arene derivatives; Spirobenzopyrans; Recognition; Lanthanide ions; Sensors.

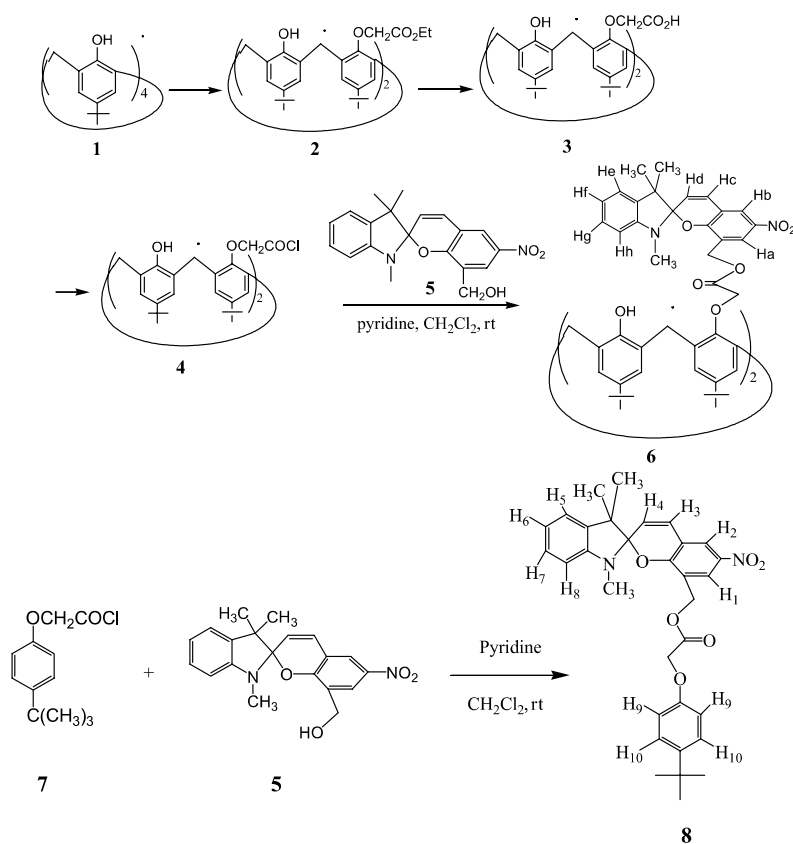
\* Corresponding author. Tel.: +86 10 64859580; fax: +86 10 64879375; e-mail: yhgao@mail.ipc.ac.cn

In the present work, we report the synthesis of receptor **6** by incorporating two 1',3',3'-trimethyl-6-nitro-8-hydro-methyl-spiro-(2-*H*-1-benzopyran-2,2'-indoline) (**5**) groups to a 5,11,17,23-tetra-*tert*-butyl-25,27-bis(chloroformyl-methoxy)-26,28-dihydroxycalix[4]arene (**4**) (Scheme 2). The structure of compound **6** was identified by IR, <sup>1</sup>H, <sup>13</sup>C NMR, elemental analysis and MALDI-TOF MS. Two doublets of the CH<sub>2</sub> bridging groups (at 3.26 and 4.35 ppm) in the <sup>1</sup>H NMR data revealed that **6** is in the cone conformation. The host compound **6** can selectively recognize lanthanide ions with significant hypsochromic shifts and enhancement of maximum absorption intensity in visible region. Meanwhile, the color of the solution changed from purple to yellow. Whereas, addition of alkali metal cations (such as Na<sup>+</sup>, K<sup>+</sup>), alkali earth metal cations (such as Mg<sup>2+</sup>, Ca<sup>2+</sup>) or transition metal cations (such as Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>) resulted in no visible changes in the UV–vis absorption spectra. Thus, compound **6** may be applied as a lanthanide sensor with naked eye. The model compound **8** (Scheme 2) without calix[4]arene cavity had poorer selective ability for lanthanide ions than compound **6**. Compound **5** (Scheme 2) is of the least selectivity for the investigated ions among these three receptors. In this paper, we studied the mechanism of recognition by means of <sup>1</sup>H NMR, UV–vis spectra and emission spectra of the **6**-Eu<sup>3+</sup> complexes.

## 2. Results and discussion

### 2.1. Metal cations recognition

We investigated the recognition ability of receptor **6** for metal ions such as alkali metal cations (Na<sup>+</sup>, K<sup>+</sup>), alkali earth metal cations (Mg<sup>2+</sup>, Ca<sup>2+</sup>), transition metal cations (Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>) and lanthanide cations (La<sup>3+</sup>, Pr<sup>3+</sup>, Eu<sup>3+</sup>, Gd<sup>3+</sup>,



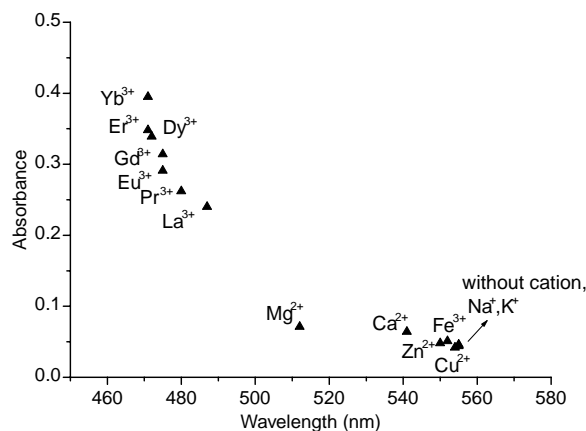
**Scheme 2.** Synthesis of receptor **6** and model compound **8**.

$\text{Dy}^{3+}$ ,  $\text{Er}^{3+}$ ,  $\text{Yb}^{3+}$ ). When the colorless lanthanide solutions in acetonitrile were added to the purple solution of receptor **6** in acetonitrile, the color changed to yellow immediately. However, there was no remarkable color change upon the addition of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ . As shown in Figure 1, the color change of **6** upon the addition of lanthanide ions is visible to the naked eye. Figure 2 shows the absorbance at the maximum absorption wavelength of the mixed solution of receptor **6** with equally molar metal cations, which were allowed to stand for 48 h under dark conditions for complete complexation. The addition of lanthanide ions caused significant hypsochromic shifts (68–84 nm) and enhancement of maximum absorption intensity (5.5–9.0 folds, Table 1) in visible region. The hypsochromic shifts can be explained by the location of electron cloud of the open merocyanine forms (**Calix-2MC**) attributed to electrostatic interaction between the phenolate anions of merocyanine groups (**MC**) and trivalent lanthanide ions under dark conditions. These changes suggest **6** coordinated with lanthanide ions. The complexing process was described in Scheme 3 (**Calix-2SP** and **Calix-2MC** denote the closed forms and the open forms of receptor **6**, respectively. **Calix-**



**Figure 1.** Color change of compound **6** (50  $\mu\text{M}$ ) in acetonitrile induced by addition of 2 equiv of metal nitrate (from left to right: compound **6** without metal ion; addition of  $\text{Na}^+$ ;  $\text{Ca}^{2+}$ ;  $\text{Zn}^{2+}$ ;  $\text{La}^{3+}$ ;  $\text{Pr}^{3+}$ ;  $\text{Eu}^{3+}$ ;  $\text{Gd}^{3+}$ ;  $\text{Er}^{3+}$ ).

**2MC**· $\text{Ln}^{3+}$  means the complexes of the open **6** and lanthanide ions). According to hard soft acid base principle, lanthanide ions belong to hard acid, which have strong affinity for hard alkali (such as oxygen). In the lower rim of the receptor **6**, there are 10 oxygen atoms, which are not only able to coordinate with lanthanide ions but also meet their large coordination number. What is interesting is that the trend of spectral changes is  $\text{Yb}^{3+} > \text{Er}^{3+} > \text{Gd}^{3+} > \text{Dy}^{3+} > \text{Eu}^{3+} > \text{Pr}^{3+} > \text{La}^{3+}$  opposite to that of the lanthanide ions' radii. This implies that the recognition process is relative to the size-fit effect. Hence upon the addition of lanthanide ions, the receptor changes color due to strong electrostatic interaction, hard acid–hard alkali interaction and size-fit effect between **Calix-2MC** and lanthanide ions.



**Figure 2.** UV-vis spectra of compound **6** (20  $\mu\text{M}$ ) in the presence of and without metal ions (20  $\mu\text{M}$ ) in acetonitrile under dark conditions for 48 h.

**Table 1.** Spectral changes of compound **6**, compound **8** and compound **5** on addition of metal ions<sup>a</sup>

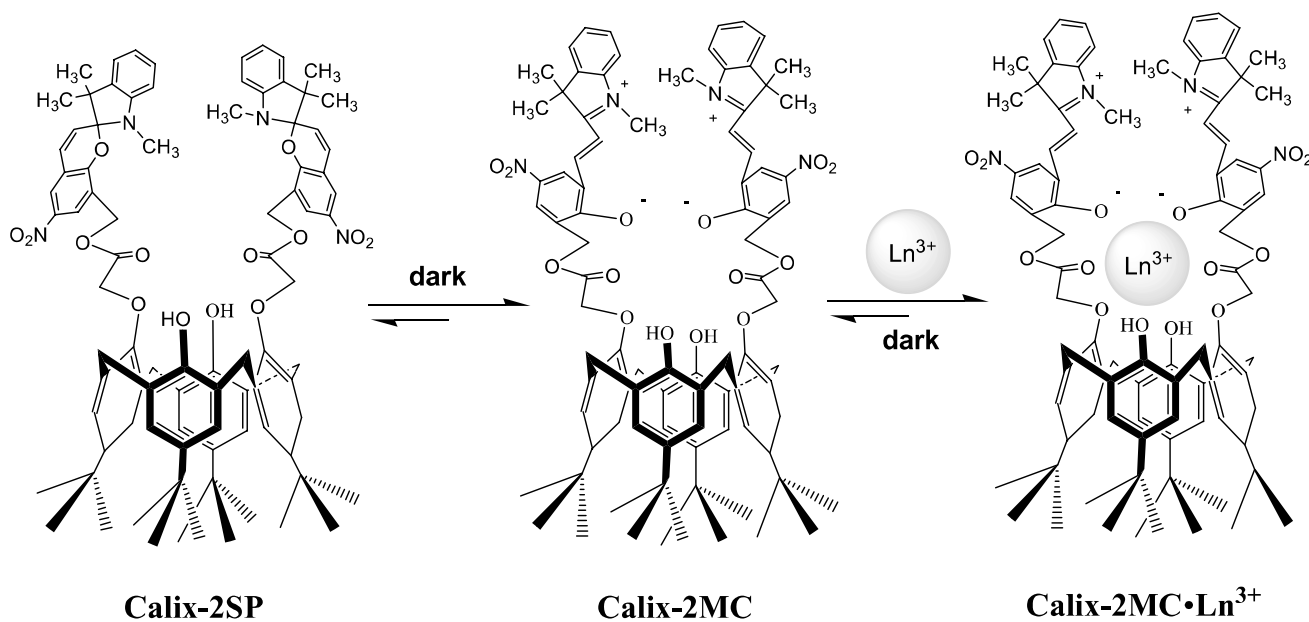
Metal ion	<i>r</i> (nm) <sup>b</sup>	Compound <b>6</b>		Compound <b>8</b>		Compound <b>5</b>	
		$\lambda_{\max}$ (nm) ( $\Delta\lambda$ ) <sup>c</sup>	<i>A</i> ( $\Delta A$ ) <sup>d</sup>	$\lambda_{\max}$ (nm) ( $\Delta\lambda$ ) <sup>c</sup>	<i>A</i> ( $\Delta A$ ) <sup>d</sup>	$\lambda_{\max}$ (nm) ( $\Delta\lambda$ ) <sup>c</sup>	<i>A</i> ( $\Delta A$ ) <sup>d</sup>
None		555	0.044 (1)	550	0.054 (1.0)	550	0.030 (1.0)
Na <sup>+</sup>	0.095	555 (0)	0.046 (1)	550 (0)	0.052 (1.0)	547 (−3)	0.025 (0.8)
K <sup>+</sup>	0.133	555 (0)	0.045 (1)	550 (0)	0.053 (1.0)	547 (−3)	0.026 (0.8)
Mg <sup>2+</sup>	0.065	512 (−43)	0.071 (1.6)	550 (0)	0.053 (1.0)	485 (−65)	0.119 (3.9)
Ca <sup>2+</sup>	0.099	541 (−14)	0.064 (1.5)	550 (0)	0.051 (0.9)	538 (−12)	0.034 (1.1)
Fe <sup>3+</sup>	0.064	552 (−3)	0.051 (1.2)	550 (0)	0.052 (1.0)	469 (−81)	0.048 (1.6)
Cu <sup>2+</sup>	0.069	554 (−1)	0.042 (0.9)	549 (−1)	0.051 (0.9)	540 (−10)	0.030 (1.0)
Zn <sup>2+</sup>	0.074	550 (−5)	0.048 (1.1)	550 (0)	0.052 (1.0)	470 (−80)	0.056 (1.9)
La <sup>3+</sup>	0.120	487 (−68)	0.240 (5.5)	548 (−2)	0.048 (0.9)	478 (−72)	0.262 (8.7)
Pr <sup>3+</sup>	0.115	480 (−75)	0.262 (6.0)	499 (−51)	0.051 (0.9)	477 (−73)	0.410 (13.7)
Eu <sup>3+</sup>	0.109	475 (−80)	0.291 (6.6)	478 (−72)	0.063 (1.2)	471 (−79)	0.392 (13.1)
Gd <sup>3+</sup>	0.108	475 (−80)	0.314 (7.1)	477 (−73)	0.047 (0.9)	469 (−81)	0.328 (10.9)
Dy <sup>3+</sup>	0.105	472 (−83)	0.339 (7.7)	467 (−83)	0.124 (2.3)	467 (−83)	0.390 (13)
Er <sup>3+</sup>	0.102	471 (−84)	0.348 (7.9)	468 (−82)	0.114 (2.1)	466 (−84)	0.402 (13.4)
Yb <sup>3+</sup>	0.100	471 (−84)	0.395 (9.0)	468 (−82)	0.092 (1.7)	465 (−85)	0.374 (12.5)

<sup>a</sup> [Compound **6**] = [metal ions] = 20  $\mu$ M, [compound **8**] = [compound **5**] = 40  $\mu$ M in acetonitrile.

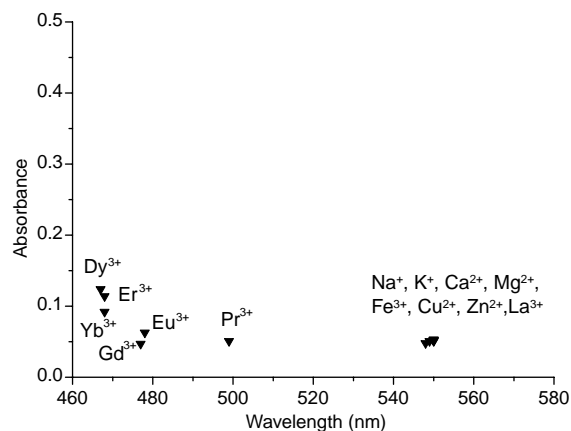
<sup>b</sup> Pauling radius of the investigated ions.

<sup>c</sup> Shifts of the  $\lambda_{\max}$  (minus denotes hypsochromic shift).

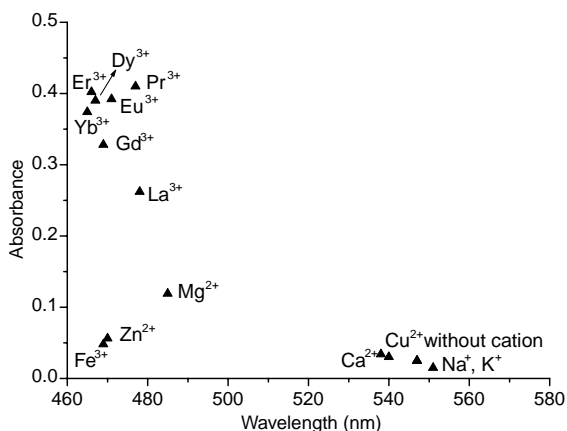
<sup>d</sup> The increased folds of the absorbance of compound **6**, **8** and **5**.

**Scheme 3.** Sketches of equilibrium reactions of compound **6** in the presence of and without lanthanide ions.

In order to investigate the contribution of the calix[4]arene cavity to the selectivity, we designed a model compound **8** without cavity (Scheme 2). As shown in Figure 3, the compound **8** have selectivity for Pr<sup>3+</sup>, Eu<sup>3+</sup>, Gd<sup>3+</sup>, Dy<sup>3+</sup>, Er<sup>3+</sup>, Yb<sup>3+</sup> with significant blue shifts. In these experiments, the concentration of compound **8** and **5** (40  $\mu$ M) is double of that of compound **6** (20  $\mu$ M) so that the concentration of spirobenzopyran groups is consistent. However, the addition of these ions to a solution of compound **8** caused less visible color changes than those of compound **6**. It may be explained that the concentration of the complexes in the mixture of the compound **6** and Ln<sup>3+</sup> is higher than that of the compound **8** and lanthanide ions systems. Figure 4 shows the recognition ability of the compound **5** to the investigated ions. It has poor selectivity for lanthanide ions for the interference of Fe<sup>3+</sup>, Zn<sup>2+</sup> and Mg<sup>2+</sup>. Data of spectral changes are listed in Table 1. These data shows compound **6** and **8** have better selectivity for

**Figure 3.** UV-vis spectra of compound **8** (40  $\mu$ M) in the presence of and without metal ions (20  $\mu$ M) in acetonitrile under dark conditions for 48 h.

lanthanide ions than compound **5**. Nevertheless, the addition of lanthanide ions caused larger enhancement of absorbance for compound **6** and **5** than that of compound **8**, which indicates compound **6** and **5** have higher sensitivity for lanthanide ions than compound **8**. Hence, compound **6** has high sensitivity and high selectivity for lanthanide ions. Compared with compound **5**, compound **8** bearing a *p*-tert-butylphenoxyacetyl substitute have an increasing spatial hindrance for the formation of complexes it has lower affinity but higher selectivity for the investigated ions than compound **5**. Especially in the presence of  $\text{Fe}^{3+}$  and  $\text{Zn}^{2+}$ , the absorption spectral differences between compound **5** and **8** is a good example for different binding ability caused by different structure. The introduction of calix[4]arene blocks increases selectivity of compound **6** likewise. However, it enhances coordinative ability simultaneously for the calix[4]arene cavity playing an important role for the stability of the complexes. We studied the  $^1\text{H}$  NMR spectra, UV-vis spectra and luminescence spectra of the receptor **6** with and without  $\text{Eu}^{3+}$  for illustrating the coordinating process.

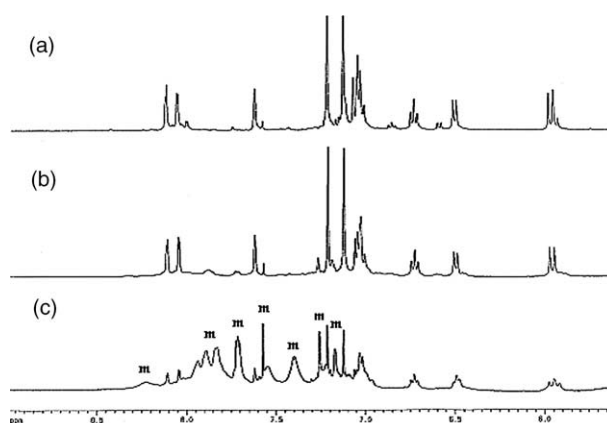


**Figure 4.** UV-vis spectra of compound **5** (40  $\mu\text{M}$ ) in the presence of and without metal ions (20  $\mu\text{M}$ ) in acetonitrile under dark conditions for 48 h.

## 2.2. $^1\text{H}$ NMR spectra studies

The spectrum **a** (Fig. 5) is a partial  $^1\text{H}$  NMR spectra of compound **6** in  $\text{CD}_3\text{CN}$ . Two equivalents of europium (III) nitrate were added to the solution of **6** and then the mixture was kept in dark. Two hours later, the  $^1\text{H}$  NMR spectrum was measured (spectrum **b** in Fig. 5). There are no remarkable differences between spectrum **a** and **b** except two small peaks at 7.72 and 7.94 ppm in spectrum **b**, which was assigned to signals of receptor **6**'s merocyanine structure (**Calix-2MC**). It means that the isomerization of the closed forms to the open forms occurred in the presence of  $\text{Eu}^{3+}$ . Significant spectral changes were observed after the mixture had been kept under dark conditions for 18 h. Spectrum **c** (Fig. 5) shows the concentration of the spiropyran forms (**Calix-2SP**) had decreased, meanwhile that of the merocyanine forms (**Calix-2MC**) increased. It suggests that the equilibrium of the first reaction in Scheme 3 was broken by addition of  $\text{Eu}^{3+}$ .

The changes of  $^1\text{H}$  NMR spectra disclosed the complexing process was that the formation of the **Calix-2MC**· $\text{Eu}^{3+}$

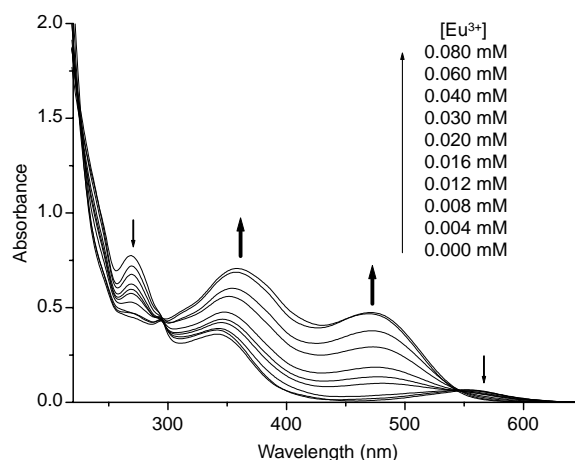


**Figure 5.**  $^1\text{H}$  NMR spectra (400 MHz) of compound **6** in  $\text{CD}_3\text{CN}$  (a) before the addition of  $\text{Eu}^{3+}$ , (b) 2 h after the addition in dark, and (c) 18 h after the addition in dark. The signals marked with 'm' are for the merocyanine structure **Calix-2MC**.

caused the concentration of **Calix-2MC** to decrease and the equilibrium between **Calix-2SP** and **Calix-2MC** was broke and then **Calix-2SP** was isomerized to **Calix-2MC** gradually. Subsequently, **Calix-2MC** produced newly coordinated with  $\text{Eu}^{3+}$  again (Scheme 3). The process was repeated until a new equilibrium was established.

## 2.3. UV-vis spectra

The receptor **6** showed absorption band centered at 555 nm in visible region (shown in Fig. 6). We added different equivalents of  $\text{Eu}^{3+}$  (0, 0.2, 0.4, 0.6, 0.8, 1, 1.5, 2, 3, 4 equiv) to the solution of receptor **6** (20  $\mu\text{M}$ ) in acetonitrile. Then these mixtures had been kept in dark for 48 h for a complete complexation. On the concentration of the  $\text{Eu}^{3+}$  increasing, the absorbance at 555 and 269 nm decreased and meanwhile that at 475 nm (a new ligand-centred absorption band) and 358 nm increased. Three isosbestic points were observed at 543, 293 and 227 nm. These results confirmed the formation of the complexes.



**Figure 6.** Changes in the UV-vis spectra of **6** (20  $\mu\text{M}$ ) upon titration by  $\text{Eu}(\text{NO}_3)_3$  in a acetonitrile solution, where the concentration of the  $\text{Eu}(\text{NO}_3)_3$  varies from 0 to 80  $\mu\text{M}$ .

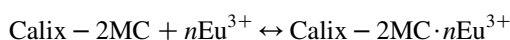
## 2.4. Fluorescence spectra

It has been reported that spirobenzopyran derivatives can emit red fluorescence.<sup>10</sup> The formation of complexes gave rise to the changes of emission spectra.<sup>11</sup> Therefore, the changes of luminescence spectra can be a witness of the interaction between hosts and guests. We studied the fluorescence spectra of the receptor **6** with and without the presence of the  $\text{Eu}^{3+}$  ( $[\mathbf{6}] = 20 \mu\text{M}$ ,  $[\text{Eu}^{3+}]/[\mathbf{6}] = 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 3.0, 4.0$ ). As shown in Figure 8, the relative fluorescence intensity of these mixed solutions was enhanced with the increasing concentration of  $\text{Eu}^{3+}$  when they were excited at 475 nm. Compared with the fluorescence spectrum of the compound **6**, that of the mixed solutions showed a blue shift of 42 nm (from 637 to 595 nm).

The broad-band emission is neither similar to that of  $\text{Eu}^{3+}$  whose emission is narrow and line-like nor to that of  $\text{Eu}^{2+}$  (a broad emission at about 430 nm). Therefore, the emission can be assigned to the  $\pi$ - $\pi^*$  emission of the conjugated system of the open forms of compound **6** (Calix-2MC). The blue shifts were caused by **6**'s coordination with  $\text{Eu}^{3+}$ , which is in accordance with the blue shifts in the absorption spectra. These results further favor the fore conclusion of the formation of the Calix-2MC· $\text{Eu}^{3+}$ .

## 2.5. Determination of stoichiometry and stability constant of the complexes

The equilibrium of complexation in the mixture of compound **6** and  $\text{Eu}(\text{NO}_3)_3$  is



The stability constant of the complex is defined as

$$K_n = \frac{[\text{M}_n\text{L}]}{[\text{L}][\text{M}]^n} \quad (1)$$

where  $\text{M}_n\text{L}$ , L and M stand for Calix-2MC· $n\text{Eu}^{3+}$ , Calix-2MC and  $\text{Eu}^{3+}$ . Calix-2SP and  $\text{Eu}^{3+}$  have no absorption above 400 nm, so the absorbance at visible region is the absorbance sum of Calix-2MC and Calix-2MC· $n\text{Eu}^{3+}$ . We select 440 and 555 nm as investigated wavelength because the ligand has minimum and maximum absorbance at these two wavelength. Thus, we have

$$A_{440} = \varepsilon_{\text{M}_n\text{L} 440}[\text{M}_n\text{L}] + \varepsilon_{\text{L} 440}[\text{L}] \quad (2)$$

$$A_{555} = \varepsilon_{\text{M}_n\text{L} 555}[\text{M}_n\text{L}] + \varepsilon_{\text{L} 555}[\text{L}] \quad (3)$$

In Eqs. 2 and 3,  $\varepsilon_{\text{M}_n\text{L} 440}$  and  $\varepsilon_{\text{M}_n\text{L} 555}$  is the molar extinction coefficient of Calix-2MC· $n\text{Eu}^{3+}$  at 440 and 555 nm, respectively.  $\varepsilon_{\text{L} 440}$  and  $\varepsilon_{\text{L} 555}$  is the molar extinction coefficient of Calix-2MC at 440 and 555 nm, respectively. And  $l$  is the optical path length (cm). The Eqs. 1–3 lead to

$$\frac{A_{440}}{A_{555}} = \frac{(K_n \varepsilon_{\text{M}_n\text{L} 440} [\text{M}]^n + \varepsilon_{\text{L} 440})}{(K_n \varepsilon_{\text{M}_n\text{L} 555} [\text{M}]^n + \varepsilon_{\text{L} 555})} \quad (4)$$

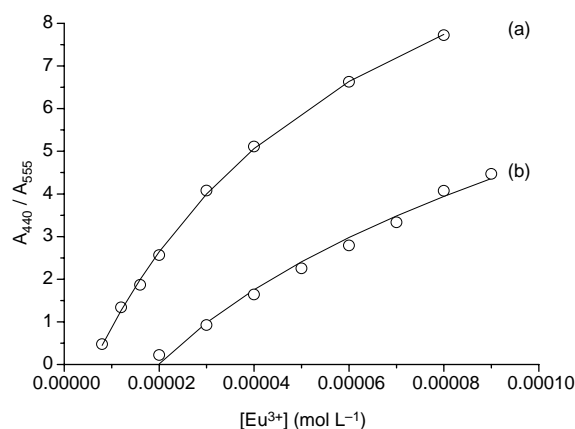
The overall concentration in cation is

$$C_{\text{M}} = [\text{M}] + n[\text{M}_n\text{L}] \quad (5)$$

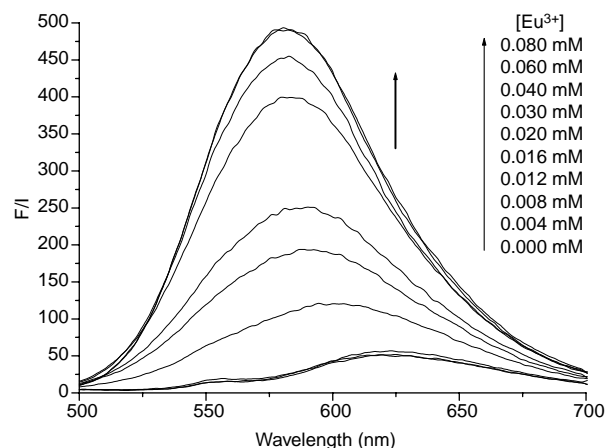
Thus,

$$[\text{M}] = C_{\text{M}} - n[\text{M}_n\text{L}] = \frac{C_{\text{M}} - nA_{440}}{\varepsilon_{\text{M}_n\text{L} 440} l} \quad (6)$$

The absorbance data were analyzed by using a nonlinear least-squares method<sup>14</sup> for  $n$  and  $K_n$ . The analysis provided the stoichiometry of the complex formed from compound **6** and  $\text{Eu}(\text{NO}_3)_3$  (Fig. 7a) was 1:1 (**6**:  $\text{Eu}^{3+}$ ) and the association constant  $\log K = 5.14 \pm 0.02$  with the correlation coefficient 0.9996. For the complex of compound **5** and  $\text{Eu}(\text{NO}_3)_3$  (Fig. 7b), the stoichiometry was 2:1 (**5**:  $\text{Eu}^{3+}$ ) and the association constant  $\log K = 4.31 \pm 0.05$  with the correlation coefficient 0.9981. These results of compound **5** and **6** are consistent and therefore verify the proposed coordinating process (as shown in Scheme 3) further (Fig. 8).



**Figure 7.** Variations in absorbance of  $A_{440}/A_{555}$  (O) of a solution of compounds **6** (a) and **5** (b) in acetonitrile ( $2 \times 10^{-3}$  M) as a function of the concentration of europium nitrate. The solid line represents the best fit with Eqs. 4 and 6.



**Figure 8.** Changes in the emission spectra of **6** ( $20 \mu\text{M}$ ) upon titration by  $\text{Eu}(\text{NO}_3)_3$  in a acetonitrile solution, where the concentration of the  $\text{Eu}(\text{NO}_3)_3$  varies from 0 to  $80 \mu\text{M}$ .

## 3. Conclusion

We synthesized a novel receptor **6** containing two photochromic spirobenzopyran groups, which can be used to recognize lanthanide ions with naked eye. The addition of lanthanide



ions to the solution of receptor **6** resulted in remarkable shifts of the UV–vis spectra (68–84 nm) and the emission spectra (42 nm for  $\text{Eu}^{3+}$ ), which suggested the formation of complexes. The  $^1\text{H}$  NMR explained the microcosmic changes during the coordination of the receptor **6** with  $\text{Eu}^{3+}$ . When  $\text{Eu}^{3+}$  were added to the solution of the receptor **6**, some of the  $\text{Eu}^{3+}$  coordinated with **Calix-2MC** at first, which resulted in the decrease of concentration of **Calix-2MC** and the breakage of original equilibrium of **Calix-2SP** and **Calix-2MC**. The system then tended to establish a new equilibrium among **Calix-2SP**, **Calix-2MC** and **Calix-2MC**· $\text{Eu}^{3+}$ . The calix[4]-arene cavity contributed to the stability of the complex **Calix-2MC**· $\text{Ln}^{3+}$ . The selectivity of **6** for lanthanide ions is relative to the strong electrostatic interaction, hard acid–hard alkali interaction and size–fit effect. These spectroscopic properties might be applied to sense lanthanide ions by light.

## 4. Experimental

### 4.1. General remarks

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded with a Bruker DPX 400 ( $\text{CD}_3\text{CN}$  and  $\text{CDCl}_3$ , TMS as internal standard, chemical shifts in ppm). Mass spectra (MALDI-TOF MS) were recorded with BIFLEXIII MALDI-TOF. UV–vis spectra were recorded with Hitachi U3010. Emission spectra were performed with PERKIN ELMER Luminescence Spectrometer LS 50 B. Elemental analyses were performed with FLASH EA1112 Elemental Analysis Apparatus.

### 4.2. Synthesis

Compound **1**, **2**, **3**, **4** and **5** were synthesized according to literatures.

**4.2.1. 5,11,17,23-Tetra-tert-butyl-25,27-bis(1',3',3'-trimethyl-6-nitrospiro[2-*H*-1-benzopyran-2,2'-indoline]-8-methoxycarbonylmethoxy)-26,28-dihydroxycalix[4]-arene, **6**.** To a solution of **4** (1.36 g, 1.7 mmol) in  $\text{CH}_2\text{Cl}_2$  (15 mL) was added dropwise a solution of 1,3,3-trimethyl-6'-nitro-8'-hydromethyl-spiro-(2'-*H*,1'-benzopyran-2,2'-indoline) (**5**, 1.232 g, 3.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (15 mL) with stirring. The color of the mixed solution changed from colorless to dark brown. Then, the  $\text{CH}_2\text{Cl}_2$  solution (5 mL) of pyridine (0.26 g, 3.3 mmol) was added dropwise to the mixture. The color changed to purple quickly. The mixture was stirred for 36 h at room temperature. Then the solvent was evaporated to dryness. The residue was dissolved in chloroform and washed with water 3 times. After chloroform was evaporated, the purple residue was purified by column chromatography with petroleum ether and ethyl acetate as an eluent and gave compound **6** as a purple powder in 15% yield. Mp 143–144 °C;  $^1\text{H}$  NMR (400 MHz,  $[\text{D}_1]$ chloroform, 25 °C):  $\delta$  0.93 (s, 18H;  $\text{Bu}^t$ ), 1.19 (s, 6H; SP- $\text{CH}_3$ ), 1.28 (s, 18H;  $\text{Bu}^t$ ), 1.31 (s, 6H; SP- $\text{CH}_3$ ), 2.68 (s, 6H; SP-N $\text{CH}_3$ ), 3.26 (d,  $J=13.2$  Hz, 4H; Ar $\text{CH}_2\text{Ar}$ ), 4.35 (d,  $J=13.2$  Hz, 4H; Ar $\text{CH}_2\text{Ar}$ ), 4.56 (s, 4H; ArO $\text{CH}_2\text{CO}_2$ –), 4.96 (d,  $J=12.9$  Hz, 2H; Ar $\text{CH}_2\text{O}$ –), 5.05 (d,  $J=12.9$  Hz, 2H; Ar $\text{CH}_2\text{O}$ –), 5.89 (d,  $J=10.3$  Hz, 2H; SP- $H_d$ ), 6.49 (d,  $J=7.5$  Hz, 2H; SP- $H_b$ ), 6.72 (s, 2H; Ar-OH), 6.75 (s, 4H; Ar-*H*), 6.80 (t,  $J=7.5$  Hz, 2H; SP- $H_f$ ), 6.92 (d,  $J=10.3$  Hz, 2H; SP- $H_c$ ), 7.01 (s, 4H; Ar-*H*), 7.02 (d,  $J=7.5$  Hz, 2H; SP-

$H_e$ ), 7.09 (t,  $J=7.5$  Hz, 2H; SP- $H_g$ ), 7.97 (s, 2H; SP- $H_b$ ), 8.07 (s, 2H; SP- $H_a$ );  $^{13}\text{C}$  NMR (100 MHz,  $[\text{D}_1]$ chloroform, 25 °C):  $\delta$  168.5, 157.3, 150.6, 150.3, 147.3, 147.1, 141.5, 140.5, 135.8, 132.2, 128.1, 127.9, 126.0, 125.7, 125.6, 125.3, 125.1, 122.6, 122.5, 121.6, 121.4, 120.1, 118.8, 107.1, 107.0, 71.9, 60.8, 52.0, 33.9, 33.8, 32.8, 32.1, 31.7, 31.5, 31.3, 31.0, 28.8, 26.0, 20.0; IR (Nujol):  $\nu=3430$   $\text{cm}^{-1}$  (C=O), 1762  $\text{cm}^{-1}$  (O–H); MALDI-TOF MS:  $m/z$  1431.8  $[\text{M}]^+$ , 1454.8  $[\text{M}+\text{Na}]^+$ , 1470.9  $[\text{M}+\text{K}]^+$ ; elemental analysis calcd (%) for  $\text{C}_{88}\text{H}_{96}\text{N}_4\text{O}_{14}$  (1433.72): C, 73.45; H, 7.02; N, 3.89; found: C, 73.18; H, 7.20; N, 3.64.

**4.2.2. 1',3',3'-Trimethyl-6-nitro-8-(4-tert-butylphenoxy-acetylmethyl)spiro[2-*H*-1-benzopyran-2,2'-indoline], **8**.** To a solution of **7** (Alfa Aesar, 0.45 g, 2 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) was added dropwise a solution of **5** (0.704 g, 2 mmol) in  $\text{CH}_2\text{Cl}_2$  (10 mL) with stirring. The color of the mixed solution changed from colorless to dark brown. Then, the  $\text{CH}_2\text{Cl}_2$  solution (5 mL) of pyridine (0.16 g, 2 mmol) was added dropwise to the mixture. The color changed to purple quickly. The mixture was stirred for 10 h at room temperature. Then the solvent was evaporated to dryness. The purple residue was purified by column chromatography with petrol ether and acetic ester as an eluent and gave compound **8** in 20% yield. Mp 59–61 °C;  $^1\text{H}$  NMR (400 MHz,  $[\text{D}_1]$ chloroform, 25 °C):  $\delta$  1.20 (s, 3H; SP- $\text{CH}_3$ ), 1.29 (s, 12H;  $\text{Bu}^t$  and SP- $\text{CH}_3$ ), 2.71 (s, 3H; SP-N $\text{CH}_3$ ), 4.49 (s, 2H; ArO $\text{CH}_2\text{CO}_2$ –), 4.98 (d,  $J=11.8$  Hz, 1H; Ar $\text{CH}_2\text{O}$ –), 5.05 (d,  $J=11.8$  Hz, 1H; Ar $\text{CH}_2\text{O}$ –), 5.91 (d,  $J=10.4$  Hz, 1H; SP- $H_4$ ), 6.53 (d,  $J=7.3$  Hz, 1H; SP- $H_8$ ), 6.78 (d,  $J=8.8$  Hz, 2H;  $H_9$ ), 6.87 (t,  $J=7.3$  Hz, 1H; SP- $H_6$ ), 6.96 (d,  $J=10.4$  Hz, 1H; SP- $H_3$ ), 7.06 (d,  $J=7.3$  Hz, 1H; SP- $H_5$ ), 7.17 (t,  $J=7.3$  Hz, 1H; SP- $H_7$ ), 7.29 (d,  $J=8.8$  Hz, 2H;  $H_{10}$ ), 8.01 (s, 1H; SP- $H_2$ ), 8.07 (s, 1H; SP- $H_1$ );  $^{13}\text{C}$  NMR (100 MHz,  $[\text{D}_1]$ chloroform, 25 °C):  $\delta$  168.6, 157.3, 155.4, 147.4, 144.5, 140.5, 135.9, 128.1, 127.9, 126.4, 125.3, 122.7, 122.4, 121.7, 121.5, 120.1, 118.8, 114.1, 107.1, 65.2, 60.9, 52.1, 34.1, 31.5, 28.8, 26.0, 20.0; MS (70 eV, EI):  $m/z$  (%) 542 (28)  $[\text{M}]^+$ , 335 (100)  $[\text{C}_{20}\text{H}_{19}\text{N}_2\text{O}_3]^+$ ; elemental analysis calcd (%) for  $\text{C}_{32}\text{H}_{34}\text{N}_2\text{O}_6$  (542.62): C, 70.83; H, 6.32; N, 5.16; found: C, 70.91; H, 6.39; N, 5.29.

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