

Highly Selective Luminescence Determination of Terbium at the Sub-ppb Level with Sulfonylcalix[4]arene-*p*-tetrasulfonate

Takayuki Horiuchi, Nobuhiko Iki,* Hiromi Oka, and Sotaro Miyano*

Department of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, Aramaki-Aoba 07, Sendai 980-8579

(Received June 4, 2002)

The energy-transfer luminescence of complexes of Tb^{3+} with calix- (CAS), thiacalix- (TCAS), and sulfonylcalix[4]arene-*p*-tetrasulfonates (SO_2CAS), in which four *p*-phenolsulfonates are jointed by $-\text{CH}_2-$, $-\text{S}-$, and $-\text{SO}_2-$, respectively, was applied to an ultratrace determination of the Tb^{3+} ion. Based on the complexation behavior of each calix ligand, a determination procedure was established. A time-resolved measurement was conveniently employed to separate the background fluorescence from the luminescence of the calix- Tb^{3+} complexes. An improved sensitivity was attained by sulfur-bridged calixes, TCAS and SO_2CAS , as compared to CAS, owing to the photophysical properties of the Tb^{3+} -complexes. The detection limits for Tb^{3+} ion by CAS, TCAS, and SO_2CAS ligands were estimated to be $8.2 \times 10^{-10} \text{ mol dm}^{-3}$ (131 ppt), $2.0 \times 10^{-10} \text{ mol dm}^{-3}$ (32 ppt), and $2.3 \times 10^{-10} \text{ mol dm}^{-3}$ (37 ppt) at $S/N = 3$, respectively. In terms of the selectivity, the effect of diverse coexisting ions on the luminescence intensity of the Tb^{3+} complexes was studied. The luminescence of the TCAS complex was interfered by the presence of a 5-fold amount of other lanthanide ions, while that of the SO_2CAS complex was more tolerant for those metal ions, allowing the presence of as much as 50 to 100-fold amounts. Also, 10 to 1500-fold amounts of other common ions were tolerated by using SO_2CAS . Thus, SO_2CAS was proved to be a practical reagent for determining the Tb^{3+} ion at the sub-ppb level.

The energy-transfer luminescence of lanthanide complexes has been widely applied to luminescent probes,^{1–3} owing to their characteristic properties such as long lifetime, narrow emission band, and large Stoke's shift. In particular, the detection capability of energy-transfer luminescence with high sensitivity and selectivity by simple operations at low running cost could make the luminescent spectrometry a more advantageous alternative of the atomic absorption and/or emission spectrometry for the determination of lanthanide ions. In order to further improve the limit of the luminescent-detection protocol, the molecular design of the ligand has generally relied on introducing either additional ligating groups to the metal center to expel the coordinated water responsible for quenching, or a chromophore, which efficiently transfers the absorbed energy to the lanthanide ion. In this context, calixarenes have been frequently used as a platform for building such ligands because of their ease in the functionalization with ligating groups and chromophores.⁴ In view of these general strategies for ligand design, a recent report by Yoshida and Shinkai is rather unexpected to reveal that calix[4]arene-*p*-tetrasulfonate (CAS, Fig. 1), without having such auxiliary functional groups, could serve as a good ligand to form a highly luminescent complex with Tb^{3+} .⁵ It has been suggested that the two phenoxide oxygens of CAS coordinate to Tb^{3+} and the phenol moiety serves as an inherent chromophore. By using the highly luminescent CAS- Tb^{3+} complex, they applied luminescence for the sensitive determination of Tb^{3+} at a detection limit (D.L.) of as low as $2 \times 10^{-9} \text{ M}$ ($\text{M} \equiv \text{mol dm}^{-3}$).⁶ On the other hand, our recent development of facile methods for preparing sulfur-bridged analogues of CAS, i.e. thiacalix- (TCAS)

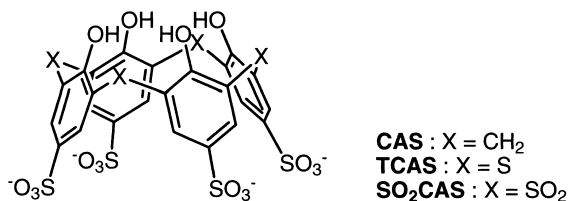


Fig. 1. Calix[4]arene-*p*-tetrasulfonate and the analogues with sulfur bridges at X.

and sulfonylcalix[4]arene-*p*-tetrasulfonates (SO_2CAS , see Fig. 1), prompted us to study their photophysical properties of the lanthanide complexes.⁷ Improved properties of their Tb^{3+} complexes were discovered, as compared with those of the CAS- Tb^{3+} complex, such as longer emission lifetimes and larger quantum yields by virtue of their higher coordination ability.⁸ The absorption maxima of Tb^{3+} complexes of TCAS and SO_2CAS complexes at longer wavelength than that of CAS- Tb^{3+} are other merits from the view point of choosing the excitation source. Encouraged by these findings, we herein further extended our study on the applicability of the sulfur-containing macrocycles to the determination of Tb^{3+} at the sub-ppb level.

Experimental

Equipment. Corrected luminescence spectra and emission decay curves were measured by a Hitachi F-4500 spectrofluorimeter with a $1 \times 1 \text{ cm}$ quartz cell. UV absorption spectra were measured using a Shimadzu UV-2500PC. The pHs of the solutions were measured by a Horiba D-14 with an S8720 electrode.

Materials. Calixarene ligands are obtained and purified as described previously.⁷ The stock solutions of lanthanide ions (0.01 M) were prepared by dissolving an appropriate amount of the nitrates (Kanto Chemical Co., Inc., Tokyo) in 0.01 M HNO₃. Before use, it was diluted to the desired concentration with a 0.01 M HNO₃ solution. The pH buffers piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) and *N*-cyclohexyl-2-aminoethanesulfonic acid (CHES) were purchased from Dojindo Laboratories, Kumamoto. Deionized water was used throughout this study.

Results and Discussion

Determination Protocol for Tb³⁺ Ion. Our previous study on the complexation of lanthanide ions with calix ligands revealed that TCAS and SO₂CAS form 1:1 (= metal:ligand) complexes with Tb³⁺ in the pH region above 8.5 and 5.5, respectively, via coordination of the bridging group (X) as well as of two adjacent phenoxides (Fig. 2).⁷ This contrasted well with CAS, which formed a 1:2 complex above pH 12 via the coordination of two phenoxide oxygens.⁵ The difference in the pH regions to form the complexes is ascribed to the coordination ability of the ligands toward the Tb³⁺ ion as well as to the acidity of the calixarene ligands, which is in the order SO₂CAS ≫ TCAS > CAS.⁹

All of the resulting complexes of Tb³⁺ exhibited characteristic emission spectra consisting of four bands at 488, 543, 583, and 618 nm corresponding to metal-centered *f*-*f* transitions (Fig. 3). As shown, the most intense band for each calix-Tb³⁺ was observed at 543 nm, which was set as the detection wavelength in the following experiments. In addition, a slight background fluorescence from the free ligand was observed in each case, suggesting that the time-resolved mode for the luminescence measurement is suitable for the determination of trace amounts of Tb³⁺ ion.

Depending on the optimal pH regions for the formation of Tb³⁺ complexes as well as the absorption⁷ and emission spectra, the determination procedure for Tb³⁺ ion was established as follows. To a volumetric flask (25 cm³) was pipetted a sample solution containing Tb³⁺, 0.02 cm³ of 5 mM calix[4]arene (CAS, TCAS, or SO₂CAS) solution, and deionized water (ca. 15 cm³). To the mixture, 0.25 cm³ of 0.5 M buffer solution or 0.3 cm³ of 1 M NaOH was added dropwise under mixing to adjust the pH (Table 1). After being made up with water, the mixture was shaken and allowed to stand for 1 h at room temperature to allow complexation. After nitrogen gas was bubbled for 1 min to purge dissolved oxygen, emission decay curves at 543 nm were collected and accumulated ten times by

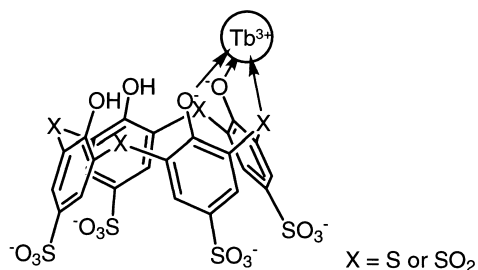


Fig. 2. A possible structure of Tb³⁺ complex with TCAS and SO₂CAS.

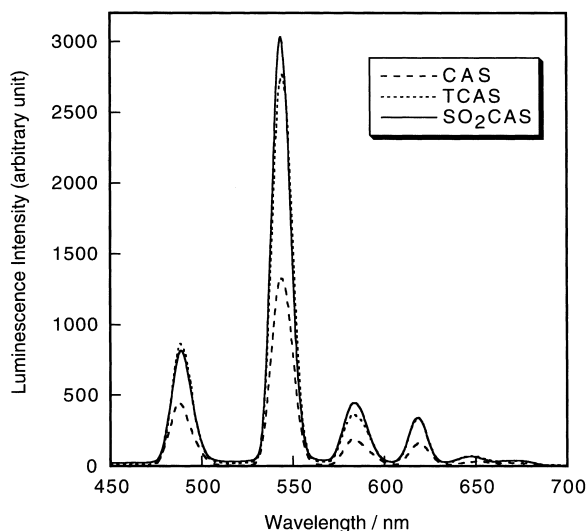


Fig. 3. Corrected emission spectra of Tb³⁺ ion complexed with CAS, TCAS, and SO₂CAS. [Calixarene ligand]_T = 4.0 × 10⁻⁶ M, [Tb³⁺]_T = 2.0 × 10⁻⁷ M, [buffer] = 1 mM. λ_{ex} = 263 (Tb³⁺-CAS), 261 (Tb³⁺-TCAS), and 330 nm (Tb³⁺-SO₂CAS). The pHs are 12.0 (Tb³⁺-CAS), 9.7 (Tb³⁺-TCAS), and 6.5 (Tb³⁺-SO₂CAS). Excitation and emission slit width were 5 and 10 nm, respectively. cf. The conditions such as λ_{ex} and slit width are different from that for the previously reported emission spectra in Ref. 7.

Table 1. Conditions for Luminescence Determination of Tb³⁺ ion by CAS, TCAS and SO₂CAS Systems

Ligand	Buffer	pH	λ _{ex} /nm
CAS	1.0 M NaOH	12.1	263
TCAS	0.5 M CHES-NaOH	9.5	261
SO ₂ CAS	0.5 M PIPES-NaOH	6.6	330

pulse excitation (Table 1), followed by the integration from 0.04 to 3 ms to estimate the luminescence intensity.

Sensitivity. Calibration curves of Tb³⁺ ion in the nM range obtained by the above-mentioned procedure with each calixarene are shown in Fig. 4. Obviously, the sensitivity is in the order TCAS > SO₂CAS > CAS, which may be mainly determined by the photophysical properties of the complexes, such as the molar absorptivity (ε) and Φ (Table 2). The detection limits (D.L.) based on 3σ of a blank signal were estimated (Table 2). Owing to the time-resolved measurement, the D.L. for CAS system was substantially improved from the reported value of 2 × 10⁻⁹ M by Yoshida to 8.2 × 10⁻¹⁰ M. However, the D.L. was the highest among the calixarenes examined, due to the high blank value and the deviation in addition to the lowest sensitivity. On the other hand, quite similar D.L. values were obtained by both of the sulfur-bridged calixarenes (Table 2). Although TCAS-Tb³⁺ has a much improved sensitivity, as compared to the CAS complex, it still suffers from a rather high blank signal. These D.L. attained by TCAS and SO₂CAS systems are not so low as the one (D.L. 2.1 × 10⁻¹¹ M) attained by a different type of phenol-based ligand, *N,N'*-bis(2-hydroxybenzyl)ethylenediamine-*N,N'*-diacetic acid (HBED).¹⁰

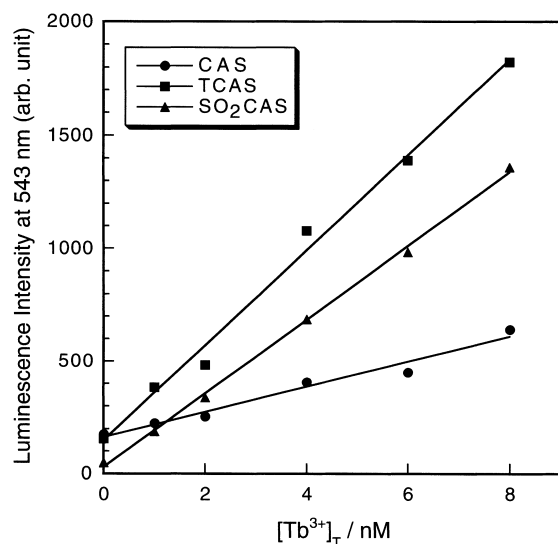


Fig. 4. Calibration curves for Tb^{3+} ion obtained with CAS, TCAS, and SO_2CAS . [calixarene ligand] = 4.0×10^{-6} M, $[\text{Tb}^{3+}] = 0\text{--}8.0 \times 10^{-9}$ M, [buffer] = 5.0×10^{-3} M. Excitation and emission slit width: 10 and 20 nm, respectively. PMT voltage: 700 V, response: 0.004 s. For other conditions, see Table 1.

This suggests that the tridentate nature of TCAS and SO_2CAS is an essential weakness to allow the center Tb^{3+} ion to have a higher number of coordinated water molecules ($q = 4.2\text{--}4.5$), rendering the quantum yield lower. In contrast, HBED is hexadentate to exclude water molecules from the first coordination shell of Tb^{3+} ($q = 1.8$) to have a higher quantum yield ($\Phi = 0.22$). Thus, there may be a possibility to improve the sensitivity of the TCAS and SO_2CAS systems via removal of the coordinated water by introducing suitable ligating groups, or the addition of an auxiliary ligand to form a ternary complex with a sulfur-bridged calixarene- Tb^{3+} complex. None the less, TCAS and SO_2CAS systems have lower detection limits than atomic spectrometric methods, such as inductively coupled plasma atomic emission spectrometry (ICP-AES) (0.8 ppb), flame atomic absorption spectrophotometry (FAAS) (600 ppb), and atomic fluorometry (500 ppb),¹¹ strongly suggesting that these sulfur-bridged calixarenes are hopeful candidates for a ligand to be applied to the ultratrace determination of the Tb^{3+} ion.

Selectivity. In principle, the selectivity of the luminescence signal from the lanthanide ion is good, because of the narrow emission band and the long lifetime. The former al-

lows signal separation in the wavelength domain, while the latter allows a time-resolved measurement to effectively remove any background fluorescence signal having a much shorter lifetime. In fact, TCAS and SO_2CAS form luminescent complexes with a few kinds of lanthanide ions, such as Sm^{3+} , Eu^{3+} , and Dy^{3+} , having only weak emission bands, which are well separable on the emission spectra.⁷ Also, the Tb^{3+} luminescence signal could be easily distinguished from the fluorescence of calixarene ligands by a time-resolved measurement (vide supra). To clarify other factors, if any, which might interfere in the luminescence determination, the effect of diverse ions on the luminescence intensity was studied.

Because the chemical properties of lanthanoid (Ln^{3+}) ions resemble each other, we first studied the effect of the addition of Ln^{3+} on the luminescence intensity of Tb^{3+} complexes with TCAS and SO_2CAS . In the TCAS system (Fig. 5a), the addition of an equimolar amount to a few-fold excess of La^{3+} , Dy^{3+} , Pr^{3+} , Sm^{3+} , and Eu^{3+} slightly increased the luminescence intensity, while addition of more than a several-fold excess of Ln^{3+} caused a significant decrease in the signal. In the low $[\text{Ln}^{3+}]_{\text{T}}/[\text{Tb}^{3+}]_{\text{T}}$ region, the effect is too subtle to estimate the cause, while in the region $[\text{Ln}^{3+}]_{\text{T}}/[\text{Tb}^{3+}]_{\text{T}} > 5$ the competition between Tb^{3+} and Ln^{3+} to bind to TCAS may be responsible for the decrement of the signal. The use of a selective masking agent to bind to Ln^{3+} , except for Tb^{3+} , should be the most appropriate resort to avoid interference. However, the molecular design of such an ideal agent has been unsuccessful so far. In contrast to the TCAS system, SO_2CAS tolerated as much as 50 to 100-fold of all other kinds of Ln^{3+} ions (Fig. 5b), which would be due to the higher coordination ability of SO_2CAS than that of TCAS. Thus, SO_2CAS is a highly promising agent in terms of selectivity, which tempted us to further survey the tolerance limits of SO_2CAS for other ions.

Table 3 lists the tolerance limits of the luminescence signal of the $\text{SO}_2\text{CAS-Tb}^{3+}$ system for common cations and anions. Obviously, the present system can tolerate large amounts of main-group metal ions and anions. On the other hand, a 10-fold amount of Cr^{3+} decreased the luminescence signal, which may be caused by the paramagnetic nature to markedly quench the $\text{SO}_2\text{CAS-Tb}^{3+}$ complex in the excited states. While Ni^{2+} gave a positive error at the 10 to 30-fold amount to Tb^{3+} and a negative error above a 50-fold amount, the reason for which is not clear. Other transition-metal ions showed a smaller quenching effect. Although we have not studied the tolerance limits for the CAS- Tb^{3+} system, the reported values⁶ clearly suggest that the $\text{SO}_2\text{CAS-Tb}^{3+}$ system is more tolerant than the CAS- Tb^{3+} system. This may again be due to the higher coordinating ability of SO_2CAS than CAS toward Tb^{3+} in

Table 2. Photophysical Properties of the Tb^{3+} Complexes and Sensitivity and Detection Limits of Tb^{3+} by CAS, TCAS and SO_2CAS Systems

Ligand	$\epsilon/\text{mol}^{-1} \text{dm}^3 \text{cm}^{-1}$ (at λ_{Ex})	$\Phi^{\text{a)}$	Sensitivity (slope)	Blank average	σ_{Blank}	Detection limit/M
CAS	2.70×10^4	0.12	55.9	67.1	11.0	8.22×10^{-10} (131 ppt)
TCAS	4.33×10^4	0.15	211	59.7	12.0	2.02×10^{-10} (32 ppt)
SO_2CAS	3.28×10^4	0.13	164	23.2	5.47	2.33×10^{-10} (37 ppt)

a) See Ref. 7.

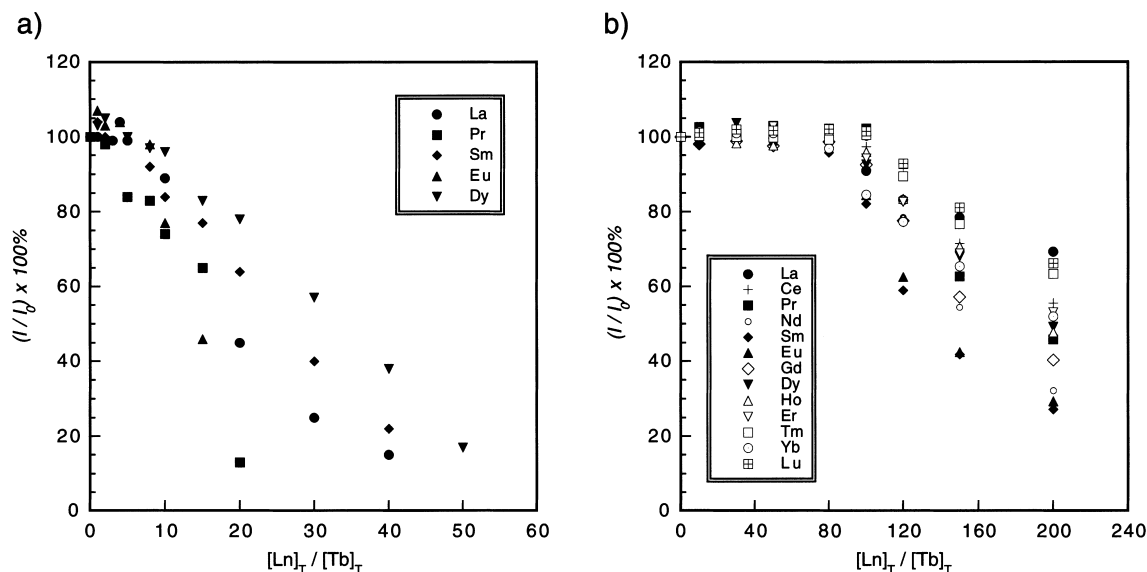


Fig. 5. Effect of foreign lanthanoid ions on the luminescence intensity of Tb^{3+} complexes with (a) TCAS and (b) SO_2CAS . I_0, I : luminescence intensity at 543 nm in the absence and presence of a foreign lanthanoid ion, respectively. $[\text{Tb}^{3+}]_{\text{T}} = 4.0 \times 10^{-8}$ M, $[\text{Foreign lanthanoid ion}]_{\text{T}} = 0\text{--}8.0 \times 10^{-6}$ M, $[\text{Calixarene}] = 4.0 \times 10^{-6}$ M, pH = (a) 9.5, (b) 6.5. Excitation and emission slit width were 5 and 10 nm, respectively. PMT voltage: 700 V, response: auto.

Table 3. Tolerance Limits of Foreign Ions for Determination of Tb^{3+} Ion^{a)}

$[\text{ion}]/[\text{Tb}^{3+}]$	Foreign ions
10	$\text{Cr}^{3+}, \text{Ni}^{2+}$
20	Co^{2+}
30	$\text{Ti}^{4+}, \text{Cu}^{2+}, \text{Zn}^{2+}$
50	$\text{Y}^{3+}, \text{Sm}^{3+}, \text{Dy}^{3+}, \text{Yb}^{3+}, \text{Tl}^{+}$
80	$\text{La}^{3+}, \text{Ce}^{3+}, \text{Eu}^{3+}, \text{Gd}^{3+}, \text{Ho}^{3+}, \text{Er}^{3+}$
100	$\text{Mg}^{2+}, \text{Al}^{3+}, \text{Mn}^{2+}, \text{Sr}^{2+}, \text{Ag}^{+}, \text{Pr}^{3+}, \text{Nd}^{3+}, \text{Tm}^{3+}, \text{Lu}^{3+}$
150	Ba^{2+}
200	$\text{Fe}^{3+}, \text{Cd}^{2+}, \text{Hf}^{4+}, \text{B}_4\text{O}_7^{2-}$
500	$\text{Zr}^{4+}, \text{CH}_3\text{COO}^-, \text{NO}_2^-, \text{P}_2\text{O}_4^{2-}$
1,000	$\text{Ca}^{2+}, \text{CO}_3^{2-}, \text{MoO}_4^{2-}, \text{NO}_3^-, \text{SO}_4^{2-}$
1,500	$\text{Ga}^{3+}, \text{ClO}_4^-, \text{SCN}^-$

a) $[\text{Tb}^{3+}] = 4.0 \times 10^{-8}$ M, $[\text{SO}_2\text{CAS}] = 4.0 \times 10^{-6}$ M, $[\text{Buffer}] = 5.0 \times 10^{-3}$ M, $\lambda_{\text{Ex}} = 330$ nm, $\lambda_{\text{Em}} = 543$ nm, R.S.D. = 2.32% ($n = 15$).

aqueous solutions. Thus, it is concluded that the present $\text{SO}_2\text{CAS-Tb}^{3+}$ system has high selectivity to be applied to determination of Tb^{3+} in practical samples.

Conclusion

Herein, a series of calix[4]arenes with bridges CH_2 , S and SO_2 were compared in terms of the sensitivity and selectivity toward Tb^{3+} ion. Having sufficient sensitivity and the highest selectivity among them, the SO_2CAS system provides a hopeful method for the practical determination of the Tb^{3+} ion at the sub-ppb level by ligand-sensitized luminescence, which may be applicable to biological¹² and environmental¹³ samples where the role of lanthanide is of great interest.

This work was supported by a Grant-in-Aid for Scientific Research (No. 12750710) from the Ministry of Education, Science, Sports and Culture, and also by the Proposal-Based

New Industry Creative Type Technology R&D Promotion Program from the New Energy and Industrial Technology Development Organization (NEDO).

References

- 1 M. P. O. Wolbers, F. C. J. M. van Veggel, B. H. M. Snellink-Ruel, J. W. Hofstraat, F. A. J. Geurts, and D. N. Reinhoudt, *J. Chem. Soc., Perkin Trans. 2*, **1998**, 2141.
- 2 J.-C. G. Bünzli and G. R. Choppin, "Lanthanide Probes in Life, Chemical and Earth Sciences," Elsevier, Amsterdam (1989).
- 3 D. Parker and J. A. G. Williams, *J. Chem. Soc., Dalton Trans.*, **1996**, 3613.
- 4 N. Sabbatini, M. Guardigli, I. Manet, and R. Ziessel, "Luminescent Probes," in "Calixarenes 2001," ed by Z. Asfari, V. Böhrer, J. M. Harrowfield, and J. Vicens, Kluwer Academic Publishers, Dordrecht (2001), Chapter 31.

5 N. Sato, I. Yoshida, and S. Shinkai, *Chem. Lett.*, **1993**, 1261.

6 I. Yoshida, K. Koyabu, M. Nishida, F. Sagara, D. Ishii, and S. Shinkai, *Anal. Sci.*, **10**, 353 (1944).

7 N. Iki, T. Horiuchi, H. Oka, K. Koyama, N. Morohashi, C. Kabuto, and S. Miyano, *J. Chem. Soc., Perkin Trans. 2*, **2001**, 2219.

8 Since luminescence of Tb^{3+} center in a complex is sensitized by energy-transfer from the ligand in the T_1 state, the introduction of "heavy" sulfur atom into the ligand may enhance the luminescence. However, the contribution of such luminescence enhancement is still ambiguous in the cases of TCAS and

SO_2 CAS complexes.

9 H. Matsumiya, Y. Terazono, N. Iki, and S. Miyano, *J. Chem. Soc., Perkin Trans. 2*, **2002**, 1166.

10 S. Saito, H. Hoshino, and T. Yotsuyanagi, *Bull. Chem. Soc. Jpn.*, **73**, 1817 (2000).

11 "Bunsekikagaku Binran," 5th ed, ed by Japan Society for Analytical Chemistry, Maruzen, Tokyo (2001).

12 A. Shinohara, in "Kidorui no Kagaku (Science of Rare Earth Elements)," ed by G. Adachi, Kagakudojin, Kyoto (1999), Chapter 12, p. 209.

13 W. Loveland, "Chapter 11, Environmental Sciences," in Ref. 2, p. 391.