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Selective Detecting of Zinc Ions with Novel Luminescent Lanthanide Probes**

Kenjiro Hanaoka, Kazuya Kikuchi, Hirotatsu Kojima, Yasuteru Urano, and Tetsuo Nagano*

Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Experimental protocols

All time-resolved fluorescence spectra and lifetimes were recorded on a Perkin Elmer LS-50B (Beaconsfield, Buckinghamshire, England). UV-visible spectra were obtained on a Shimadzu UV-1600.

Synthesis of luminescent lanthanide complexes

[Tb**L₁**], [Eu**L₁**]: [Ln^{III}**L₁**] complexes were prepared by mixing an equimolar quantity of $LnCl_3$ solution with ligand **L₁** (120 mg, 0.14 mmol) in Tris buffer (1 M; 5 ml) at pH 8.0.[S1] The mixture was stirred at room temperature overnight. These products were purified by reversed-phase HPLC on a C_{18} column using methanol-H_2O (3 : 2) as the eluent. [Tb^{III}**L₁**]·$5H_2O$ (yield 32%). MS (FAB^+ in *m*-nitrobenzyl alcohol as a matrix): m/z 998 [M^+ + H]. IR (KBr): ν [cm^{-1}] = 3397 (H_2O), 3235, 3079, 2944, 1624, 1435, 1400, 1327, 1155, 1098, 1001, 930, 770. Anal. Calcd. for $C_{42}H_{52}N_{11}O_8Tb·5H_2O$: C 46.37, H 5.74, N 14.16; found: C 46.53, H 5.72, N 14.08. [Eu^{III}**L₁**]·$5H_2O$ (yield 49%). MS (FAB^+ in *m*-nitrobenzyl alcohol as a matrix): m/z 992 [M^+ + H]. IR (KBr): ν [cm^{-1}] = 3403 (H_2O), 3245, 3084, 2934, 1624, 1435, 1399, 1327, 1155, 1098, 997, 928, 770. Anal. Calcd. for $C_{42}H_{52}N_{11}O_8Eu·5H_2O$: C 46.67, H 5.78, N 14.25; found: C 46.67, H 5.75, N 14.08.

(S1) K. Hanaoka, K. Kikuchi, Y. Urano, T. Nagano, *J. Chem. Soc., Perkin Trans. 2* **2001**, 1840-1843.

Preparation of Zn^{II} solution

We prepared 100 mM HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid) buffer (pH 7.4, I = 0.1 ($NaNO_3$)) including 10 mM NTA (nitrilotriacetic acid) and 0-9 mM $ZnSO_4$. The stability constant for the Zn^{II} complex of NTA was taken from ref. S2.

Thus, for NTA (20°C, 0.1 M KNO_3),

pK_{a1} = 9.73, pK_{a2} = 2.49, pK_{a3} = 1.89, log$K$(ZnL) = 10.66

Protonation constants must be corrected upward by 0.11 when working at 0.1 M ionic strength[S3]. Using these values, free Zn^{II} concentration was calculated using the method described in ref. S4. Thus,

$$[Zn^{II}] = [Zn^{II}]_{total} / \alpha'_{M}[L]_{free} \quad (1)$$

$$\alpha' = K(ZnL)/(\alpha_M \alpha_L)$$

$$\alpha_L = 1+10^{(pKa1-pH)}+10^{(pKa1+pKa2-2pH)}+10^{(pKa1+pKa2+pKa3-3pH)}$$

$$[L]_{free} \approx [L]_{total} - [Zn^{II}]_{total}$$

$[L]_{total}$ was set at 10 mM, and $[Zn^{II}]_{total}$ was varied from 0-9 mM.

The value of $[Zn^{II}]$ obtained from equation (1):

$[Zn^{II}]_{total}$ (mM)	0.16	0.40	0.95	2.1	4.0	6.2	8.1	8.7
$[Zn^{II}]$ (nM)	0.10	0.25	0.63	1.6	4.0	10	25	40

(S2) A. E. Martell, R. M. Smith, *NIST Critical Stability Constants of Metal Complexes. NIST Standard Reference Database 46, Version 5.0*, **1998**.

(S3) C. J. Fahrni, T. V. O'Halloran, *J. Am. Chem. Soc.* **1999**, *121*, 11448-11458.

(S4) D. D. Perrin, B. Dempsy, *Buffers for pH and Metal Ion Control*, John Wiley & Sons: Chapman and Hall, New York, London, **1974**.

Determination of apparent dissociation constant with Zn^{II}

Stock solutions of [Tb**L_1**] (10 mM) in 100 mM HEPES buffer (pH 7.4, I = 0.1 ($NaNO_3$)) were diluted to a final concentration of 100 μM with 100 mM HEPES buffer (pH 7.4, I = 0.1 (KNO_3)) including 10 mM NTA and 0-9 mM $ZnSO_4$. The luminescence intensity (excitation: 260 nm, emission: 545 nm) of each solution was measured, and was fitted to the following equation:

$$(F-F_0)/(F_{max}-F_0) = [Zn^{II}]/(K_d+[Zn^{II}])$$

where F_0 is the luminescence intensity with no addition of Zn^{II}, F_{max} is the maximum luminescence intensity and $[Zn^{II}]$ is the free Zn^{II} concentration calculated from equation (1). The value of apparent dissociation constant with Zn^{II} were 2.57 nM. This was determined from the fittings showed in Figure S3.

Determination of the detection limit (DL)

At the excitation wavelength of 260 nm with the emission wavelength of 545 nm, the luminescence increment of [Tb**L_1**] (100 μM) in 100 mM HEPES buffer at pH 7.4 shows a linear relationship with the concentration of Zn^{II} in the range from 0 to 1.0×10^{-4} M (r^2=0.996).

In signals affected by Gaussian noise the usual criterion for critical threshold is that the signal be equal to or greater than three times the standard deviation (σ) of the noise level.[S6] This criterion results in the following condition:

$$1 \geq 3\sigma/N \qquad (3)$$

where N is the magnitude of the signal in the region of interest of the spectrum.

In our experiment, the magnitude N of the luminescence intensity is proportional to the concentration C_{Zn} of Zn^{II}, therefore, the detection limit can be expressed as a function of concentration C_{Zn}:

$$DL \geq C_{Zn}(3\sigma)/(N_{Zn}-N_0) \qquad (4)$$

where C_{Zn} (M) is the concentration of Zn^{II} added to the solution, N_{Zn} is the luminescence intensity with addition of

C_{Zn} molar of Zn^{II}, N_0 is the luminescence intensity with no addition of Zn^{II}.[S6]

The value of the detection limit was 4.8×10^{-6} M (3σ). This was determined from above equation.

(S5) a) L. Bennun, E. D. Greaves, J. J. Blostein, *X-Ray Spectrom.* **2002**, *31*, 289-295; b) S. C. Liang, H. Wang, Z. M. Zhang, X. Zhang, H. S. Zhang, *Spectrochim. Acta, Part A* **2002**, *58*, 2605-2611; c) X. F. Yang, X. Q. Guo, Y. B. Zhao, *Talanta* **2002**, *57*, 883-890.

Scheme S1. Synthetic scheme of luminescent lanthanide complexes [Tb$\mathbf{L_1}$] and [Eu$\mathbf{L_1}$].[S1]

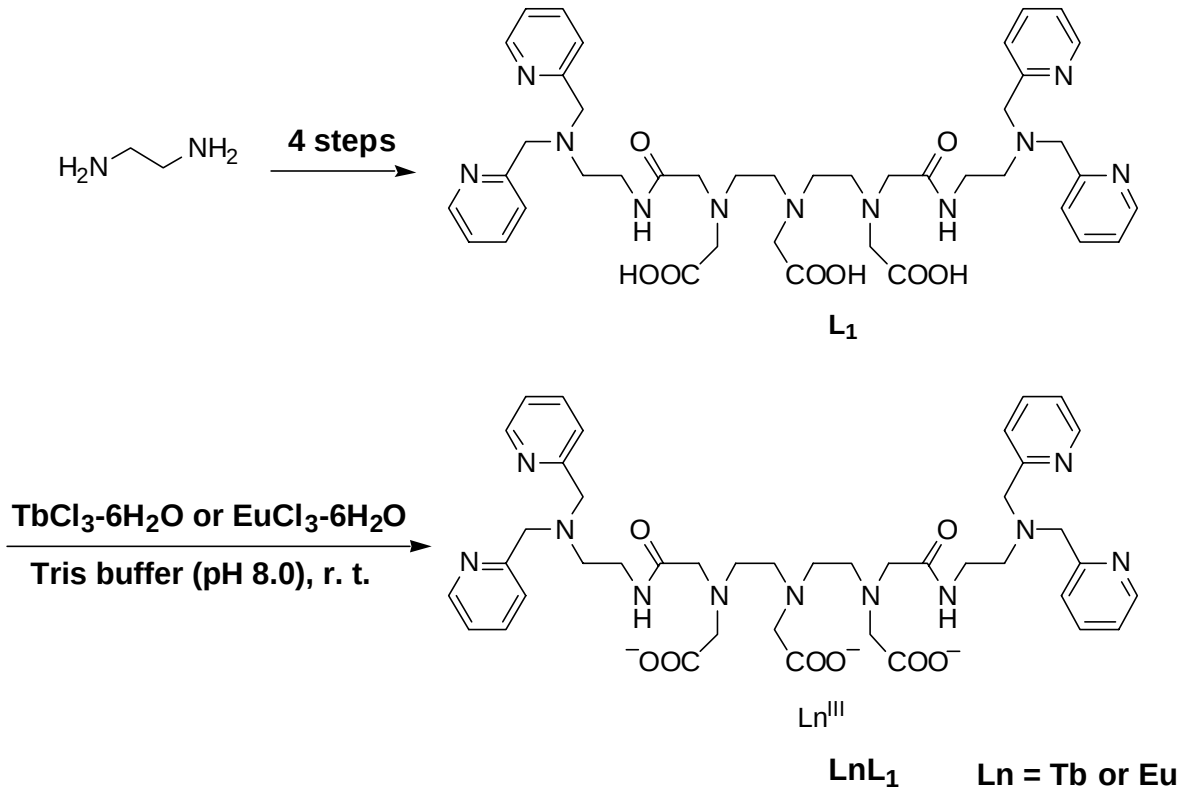

Figure S1. Emission spectra in D_2O solution (100 mM HEPES buffer; pD 7.4) of [Tb**L**$_1$] (100 μM) in the presence of various concentrations of Zn^{II}: 0, 0.33, 0.67 and 1.0 equiv. Zn^{II} to [Tb**L**$_1$]. (excitation at 260 nm, 22 °C). *I*=Intensity (arbitrary units).

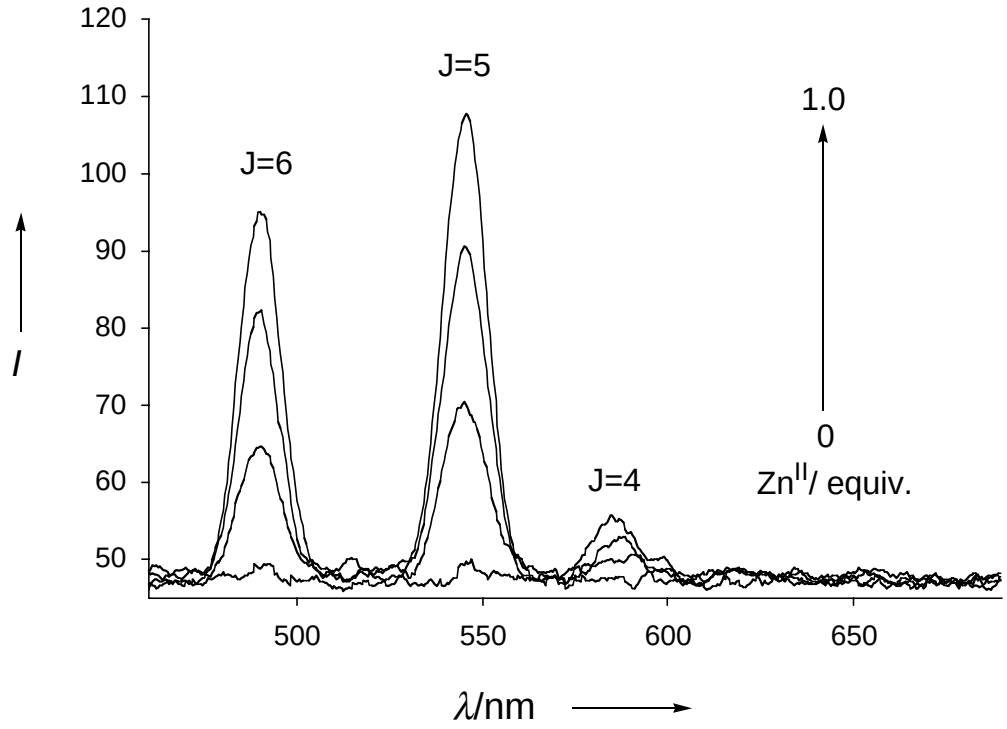

Figure S2. Decay of luminescence emission (at 545 nm) on [Tb$\mathbf{L_1}$] (100 μM) following the addition of 1.0 equiv. Zn^{II}. The data were collected in H_2O (100 mM HEPES buffer; pH 7.4) with 10 μs resolution (excitation at 260 nm, 22 °C), and fitted to a single exponential curve, which showed no residual structure, with r^2=0.982. Each point is the mean value of five measurements. The lifetimes of [Tb$\mathbf{L_1}$] are 1.45 ms. *I*=Intensity (arbitrary units).

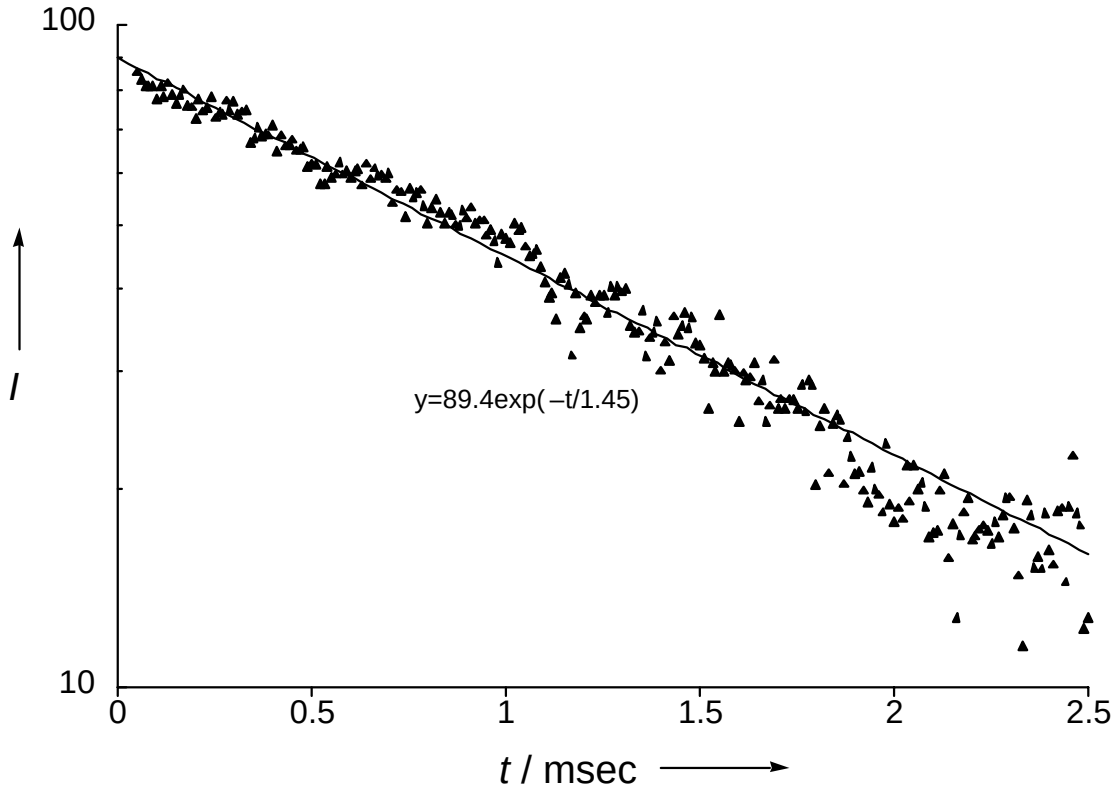

Figure S3. Apparent dissociation constant measurement of [Tb$\mathbf{L_1}$] with Zn^{II}. The luminescence intensity (excitation: 260 nm, emission: 545 nm) of each solution was measured. The fitted curve corresponds to the apparent dissociation constant = 2.57 nM. [Zn^{II}] was controlled with the Zn^{II}/NTA system. The buffers contained 100 mM HEPES, I = 0.1 ($NaNO_3$), pH 7.4, 10 mM NTA. I = Intensity (arbitrary units).

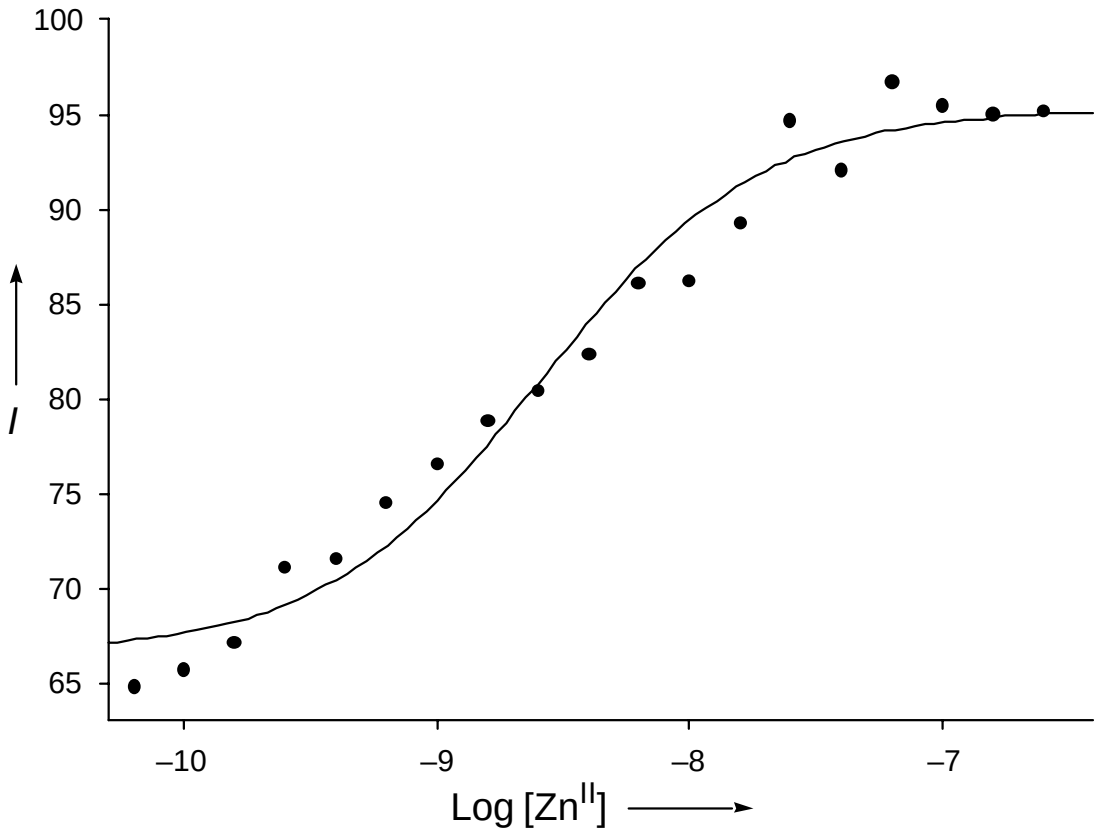

Figure S4. Effect of pH on the luminescence intensity at 545 nm of [Tb**L**$_1$]. [Tb**L**$_1$] (100 μM) was dissolved in distilled water and titrated with NaOH or HCl solution. *I* = Intensity (arbitrary units).

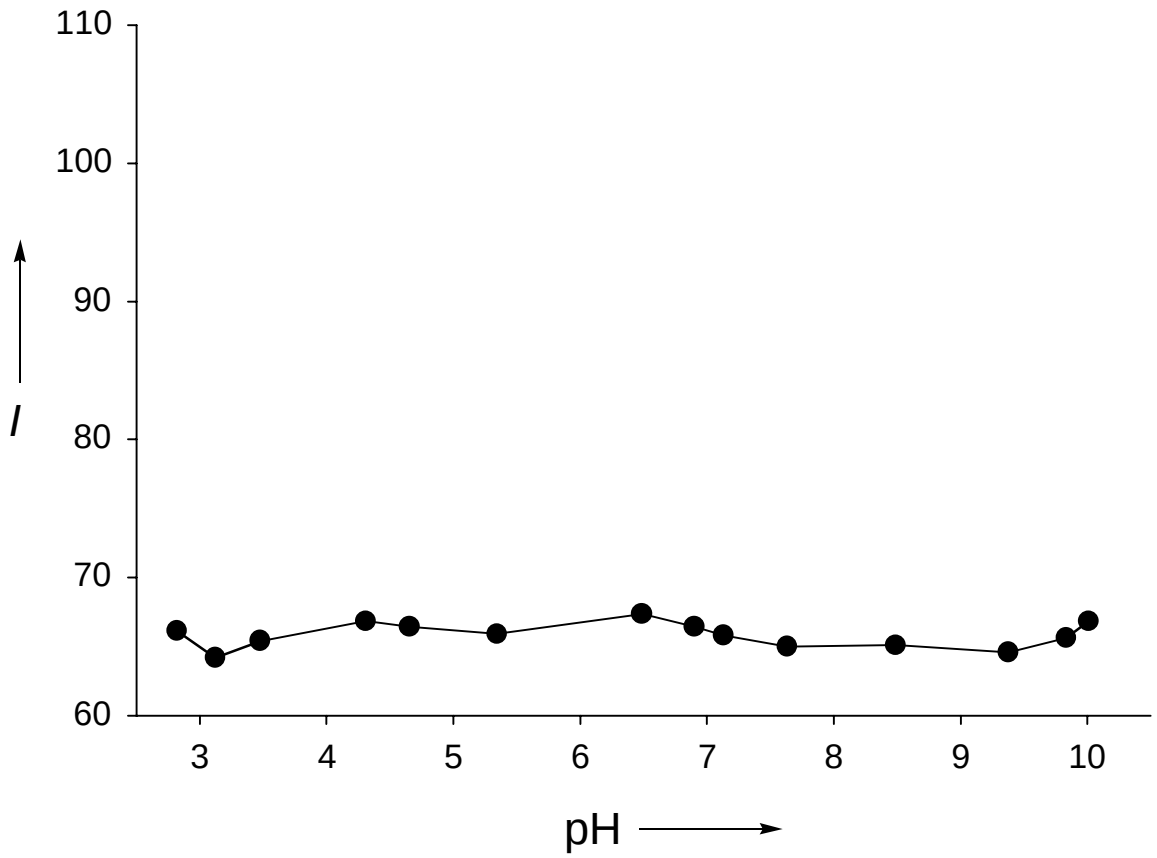

Figure S5. Job plot of [[Tb$\mathbf{L_1}$]-Zn^{II} complex] versus the mole fraction [Tb$\mathbf{L_1}$]/([Tb$\mathbf{L_1}$] + [Zn^{II}]) at constant [Tb$\mathbf{L_1}$] + [Zn^{II}] (20 μM). The luminescence intensity (excitation: 260 nm, emission: 545 nm) of each solution was measured. [Tb$\mathbf{L_1}$] and Zn^{II} were dissolved in 100 mM HEPES buffer at pH 7.4. *I* = Intensity (arbitrary units).

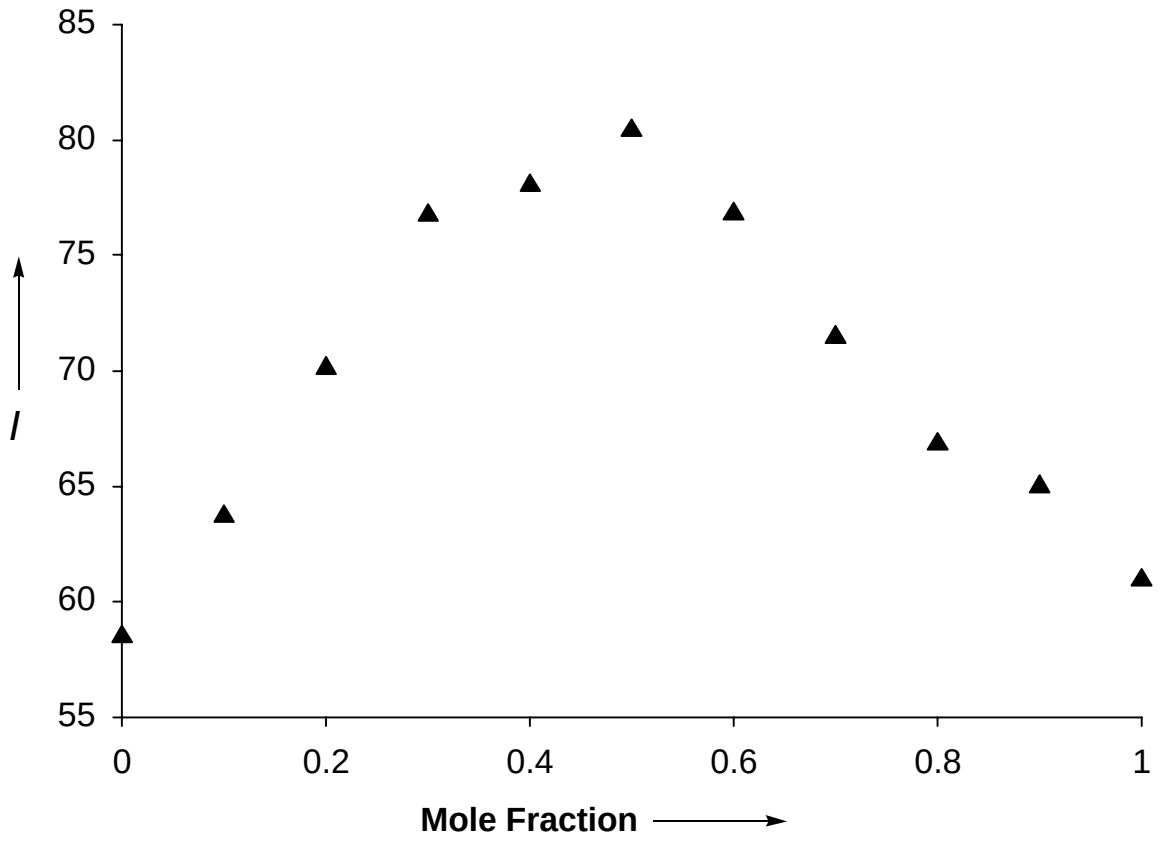

Figure S6. The luminescence intensity of [Tb$\mathbf{L_1}$] (100 μM) at 545 nm upon addition of various heavy metals (Fe^{II}, Fe^{III}, Cu^{II}, Ni^{II}, Co^{II}, Mn^{II}) and anions (Cl^-, carbonate and phosphate) (excitation at 260 nm) in 100 mM HEPES buffer (pH 7.4) at 22 °C. Heavy metal ions (1 equiv. to [Tb$\mathbf{L_1}$]) were added as $ZnSO_4$, $FeSO_4$, $Fe_2(SO_4)_3$, $CuSO_4$, $NiSO_4$, $CoSO_4$, $MnSO_4$. Anions (Cl^- (100mM), carbonate (10 mM), phosphate (10 mM)) were added as NaCl, $NaHCO_3$ and NaH_2PO_4. After addition of anions, the pH was adjusted to 7.4. *I*=Intensity (arbitrary units).