Supplementary Information

# A Practical Strategy to Create Near-Infrared Luminescent Probes: Conversion from Fluorescein-Based Sensors

*Takuya Terai,[a] Yasuteru Urano,[b] Saki Izumi,[a] Hirotatsu Kojima,[c] and Tetsuo Nagano*[a]*

[a]*Graduate School of Pharmaceutical Sciences, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

[b]*Graduate School of Medicine, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

[c]*Open Innovation Center for Drug Discovery, The University of Tokyo 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033 Japan.*

Correspondence should be addressed to T.N. (tlong@mol.f.u-tokyo.ac.jp)
Tel: +81-3-5841-4850, Fax: +81-3-5841-4855

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# 1. Materials and methods

## Materials

General chemicals were of the best grade available, supplied by Aldrich Chemical Co., Ltd, Tokyo Chemical Industries, and Wako Pure Chemical Industries and were used without further purification. Dimethyl sulfoxide and *N*,*N*-dimethylformamide (from Dojindo) used in spectroscopic analysis were of fluorometric grade. NOC5 was purchased from Dojindo Molecular Technologies, Inc., Japan. EP-1 was synthesized as described in the literature.[S1] $[Yb(tropolonate)_4]^-$ was synthesized as described in the literature.[7]

## Instruments

NMR spectra were recorded on a JEOL JNM-LA400 instrument at 400 MHz for $^1H$ NMR and 100 MHz for $^{13}C$ NMR. δ values are given in ppm relative to tetramethylsilane. Mass spectra were measured with a JEOL JMS T100LC AccuTOF mass spectrometer for ESI+ and ESI-. UV-Visible spectra were obtained on a Shimadzu UV-1800 spectrophotometer. Steady-state fluorescence studies were performed on a Horiba Jobin Yvon Fluorolog-3 spectrofluorimeter equipped with an InGaAs detector (DSS-IGA020L, Electro-Optical Systems, Inc.) for infrared emission. For lifetime measurements of $Yb^{3+}$, a time-resolved near-infrared detector system C8232TR3-45 with a Nd-YAG laser (532 nm) (Hamamatsu Photonics, Japan) was used. HPLC analyses were performed on an Inertsil ODS-3 (4.6 × 250 mm) column (GL Sciences Inc., Japan) using an HPLC system composed of a pump (PU-2080, JASCO, Japan) and a detector (MD-2015, JASCO, Japan). Preparative HPLC was performed with an Inertsil ODS-3 (10.0 mm × 250 mm) reverse-phase column (GL Science Inc., Japan) fitted on a JASCO PU-1587S2 system, using eluent A (0.1% trifluoroacetic acid) and eluent B ($CH_3CN$ with 20% $H_2O$ containg 0.1% trifluoroacetic acid). Silica gel column chromatography was performed with silica gel 60N (Kanto Chemical Co. Inc., Japan).

### Spectroscopic measurements

Measurements were performed at 298K unless otherwise indicated. Buffers were pretreated with Chelex 100 resin (Bio-Rad) to remove residual heavy metals, before sample preparation. Samples were prepared in a quartz cuvette ($l$ = 1 cm). Throughout the work, $Yb^{3+}$ complexes were formed by mixing equimolar amounts of the ligand and $YbCl_3$ in DMF or DMSO, and the mixture was diluted with an appropriate aqueous buffer to the indicated concentration. Each solution contained up to 0.2% (v/v) DMSO or DMF as a cosolvent. Luminescence spectra were corrected according to the manufacturer's instructions.

**Quantum yields of luminescence**

For determination of the quantum efficiency of luminescence ($\Phi_{lum}$), $[Yb(tropolonate)_4]^-$ in non-deuterated MeOH ($\Phi_{lum} = 1.3 \times 10^{-3}$) was used as a standard.[7] Excitation wavelength was 330 nm. Efficiency was calculated with the following equation ($L$ denotes the area under the luminescence band ($L = \Sigma I_{lum}(\lambda)$, where $I_{lum}(\lambda)$ is the luminescence intensity at each emission wavelength), Abs denotes the absorbance at the excitation wavelength, and $n$ denotes the refractive index of the solvent).

$$\Phi_{lum}^{sample} = \Phi_{lum}^{standard} \times (L^{sample} / L^{standard}) \times (n^{sample} / n^{standard})^2 \times (Abs^{standard} / Abs^{sample})$$

**Estimation of water coordination number ($q$)**

Time-dependent luminescence profiles of the $Yb^{3+}$ complexes in $H_2O$ and $D_2O$ containing 10 mM HEPES (pH or pD 7.4) were obtained with a C8232TR3-45 spectrofluorimeter (Hamamatsu Photonics, Japan), and the data were fitted to single exponential decay curves to calculate luminescence lifetimes ($\tau$). The coordination number of water molecules ($q$) was estimated from $\tau$ ($\mu$s) in $H_2O$ and $D_2O$ by use of the following equation, which was proposed by Parker and coworkers.[23]

$$q_{Yb} = 1.0 \times \left( \frac{1}{\tau_{H_2O}} - \frac{1}{\tau_{D_2O}} - 0.2 \right)$$

**Production of ROSs**

(a) $H_2O_2$

$H_2O_2$ (35% aqueous solution) was diluted appropriately in water to make a $H_2O_2$ stock solution. To a solution of a complex in 50 mM HEPES buffer at pH 7.5 containing 0.2% DMF as a cosolvent, the $H_2O_2$ stock solution (final 1 mM) was added at 37 °C, and the spectra were measured 30 min later.

(b) Generation of •OH

$Fe(ClO_4)_2$ was dissolved in water. To a solution of a complex and $H_2O_2$ in 50 mM HEPES buffer at pH 7.5 containing 0.2% DMF as a cosolvent, the $Fe(ClO_4)_2$ solution was added at 25 °C. Then, •OH was generated from $Fe^{2+}$ and $H_2O_2$ (Fenton reaction). The final concentration of $H_2O_2$ was 1 mM and that of $Fe(ClO_4)_2$ was 50 μM. Spectra were measured 5 min later.

(c) $ONOO^-$

NaONOO solution was diluted appropriately in 0.1 M NaOH aq. to obtain a $ONOO^-$ stock solution. To a solution of a complex in 50 mM HEPES buffer at pH 7.5 containing 0.2% DMF as a cosolvent, the $ONOO^-$ stock solution was added at 25 °C. The final concentration of $ONOO^-$ was 10 μM. Spectra were measured 5 min later.

(d) $^{-}$OCl

NaOCl solution (1.6 M) was diluted appropriately in 0.1 M NaOH aq. to obtain a $^{-}$OCl stock solution. To a solution of a complex in 50 mM HEPES buffer at pH 7.5 containing 0.2% DMF as a cosolvent, the $^{-}$OCl stock solution was added at 25 °C. The final concentration of $^{-}$OCl was 10 μM. Spectra were measured 5 min later.

(e) Generation of $O2^{\bullet-}$

$KO_2$ was suspended in DMF to make a stock solution (100 mM). To a solution of a complex in 50 mM HEPES buffer at pH 7.5 containing 0.2% DMF as a cosolvent, this solution was added at 25 °C. The final concentration of $O2^{\bullet-}$ was 1 mM. Spectra were measured 5 min later.

(f) $^1O_2$

EP-1 was dissolved in DMF to obtain a stock solution (10 mM). To a solution of a complex in 50 mM HEPES buffer at pH 7.5 containing 0.2% DMF as a cosolvent, EP-1 solution (final 0.1 mM) was added at 37 °C. Spectra were obtained at 30 min after the addition of EP-1.

(g) NO

NOC5 was dissolved in 0.1 M NaOH aq. to obtain a stock solution (10 mM). To a solution of a complex in 50 mM HEPES buffer at pH 7.5 containing 0.2% DMF as a cosolvent, the NOC5 solution was added at 37 °C. Spectra were obtained at 60 min after the addition of NOC5. The final concentration of NO was as indicated in each figure.

## 2. Supplementary figures

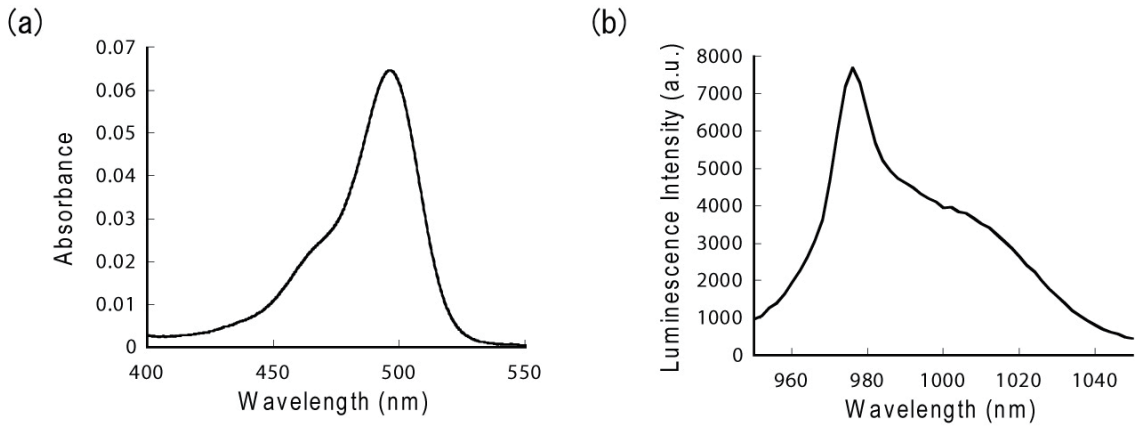



**Fig. S1** Absorption (a) and luminescence (b) spectra of the complex **1** (1 μM) in 0.1 M HEPES buffer (pH 7.4). Ex. 490 nm.

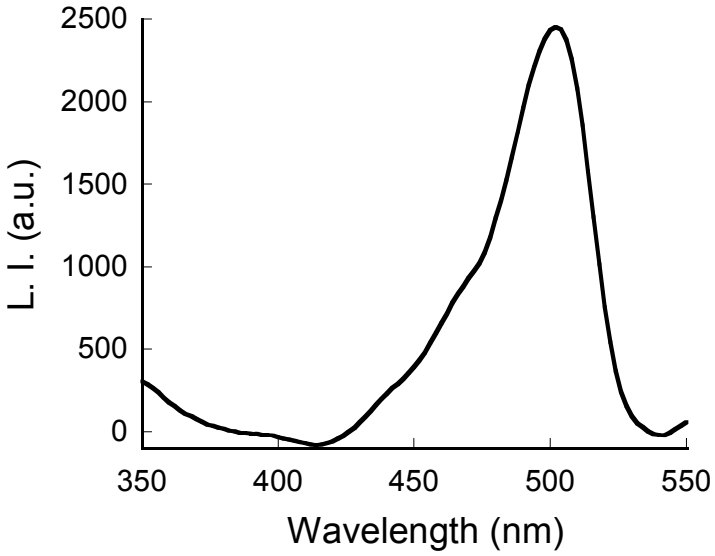



**Fig. S2** Excitation spectrum of the complex **1** (4 μM) in 0.1 M HEPES buffer (pH 7.4). Em. 980 nm.

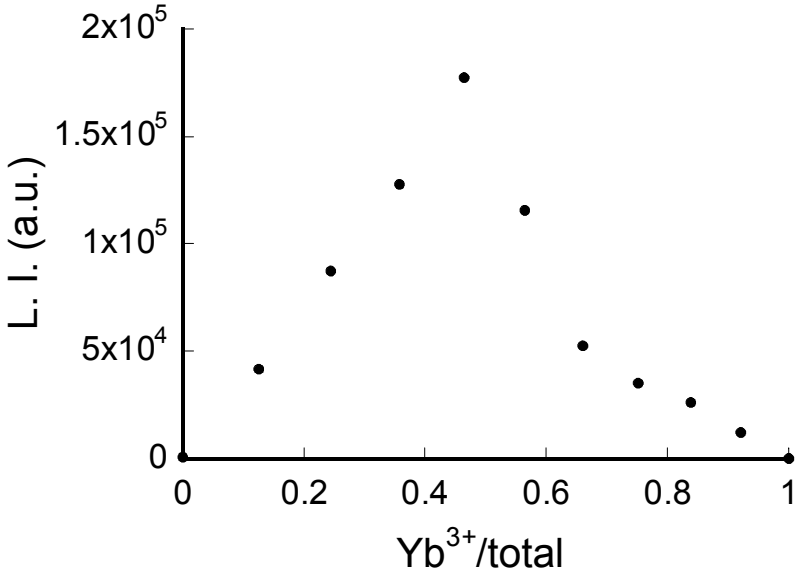



**Fig. S3** Job's plot of the complex **1**. Total concentration of the ligand and $Yb^{3+}$ was maintained at 10 μM. Measurement was performed in 10 mM Tris-HCl buffer (pH 7.4). Ex. 500 nm. Em. 976 nm. The peak was observed at $Yb^{3+}$/total = 0.5, indicating a 1:1 binding ratio.

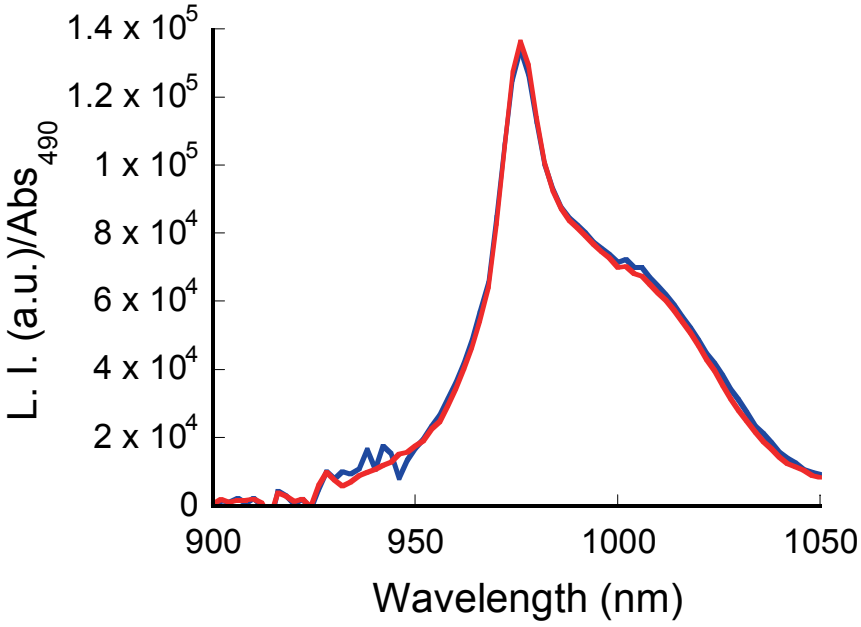



**Fig. S4** Comparison of the antenna efficiency of calcein and 2,7-dichlorocalcein. Luminescence spectra of the two complexes ($[Yb(calcein)]^{10}$: blue line, complex **1**: red line) were measured and normalized using the absorbance at the excitation wavelength (490 nm).

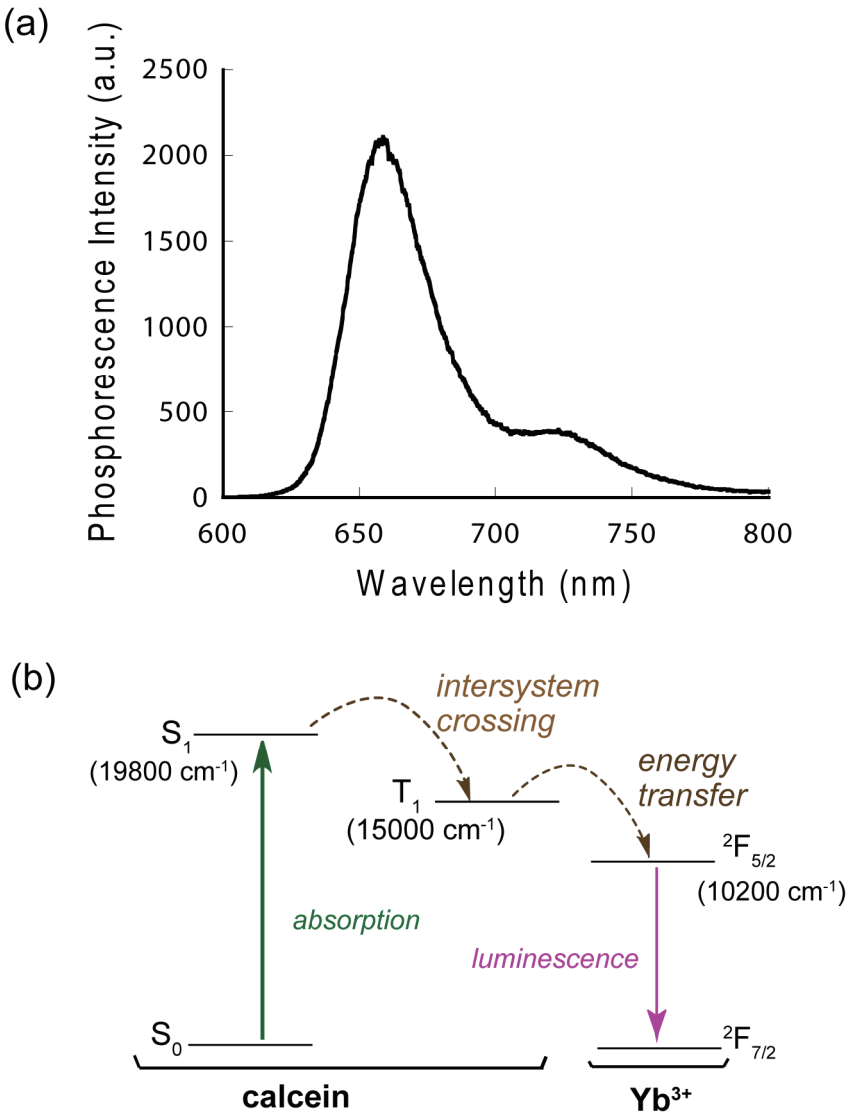



**Fig. S5** (a) Phosphorescence spectrum of [Gd(2,7-dichlorocalcein)] (5 μM) in EtOH/MeOH = 1/1 at 77K. Ex. 500 nm. Emission maximum was observed at 660 nm (15000 $cm^{-1}$). (b) Energy diagram of the complex **1**.

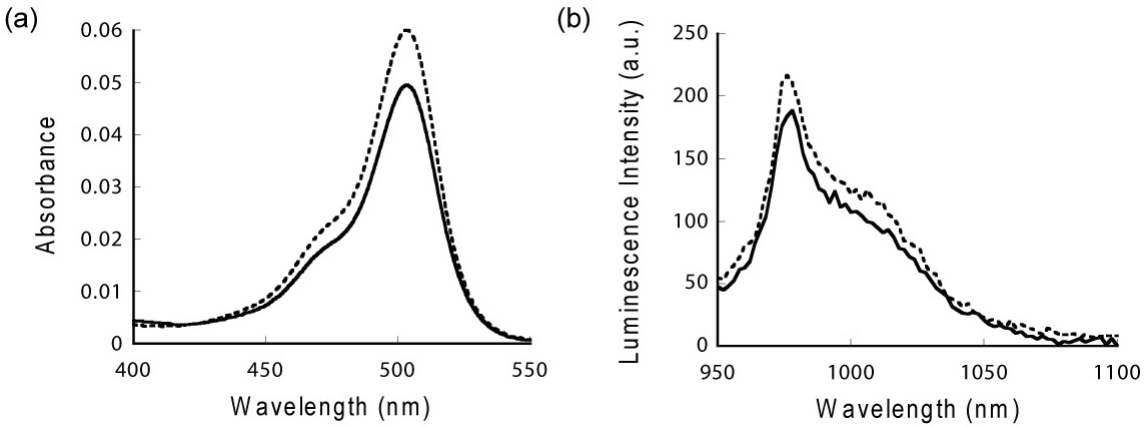



**Fig. S6** Absorption (a) and luminescence (b) spectra of the complex **1** in the presence (solid line) and absence (dashed line) of NO (50 μM). Measurement was performed in 50 mM HEPES buffer (pH 7.2). Ex. 500 nm.

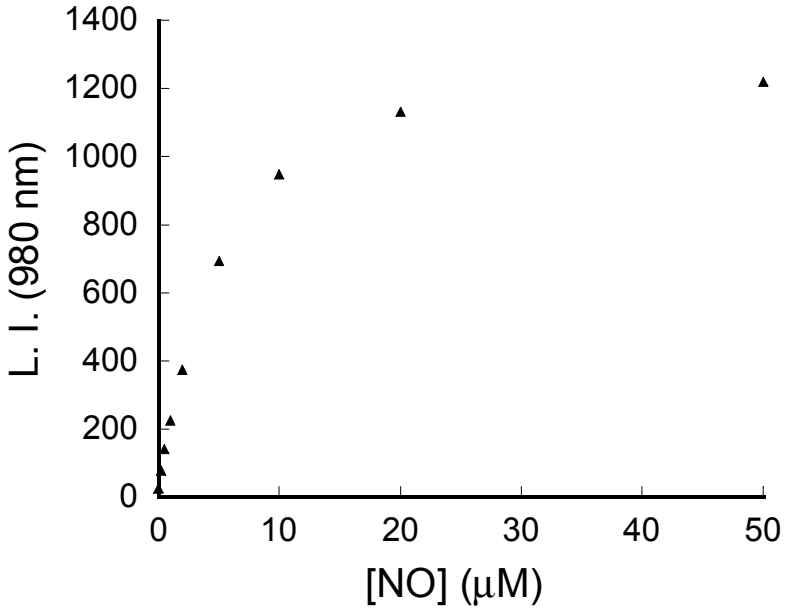



**Fig. S7** Luminescence intensity of the complex **2** (1.5 μM) as a function of added NO. Measurement was performed in 50 mM HEPES buffer (pH 7.4). Ex. 500 nm. Em. 980 nm. NO was produced in situ from NOC5, and the luminescence was measured after 90 min incubation at 37 °C.

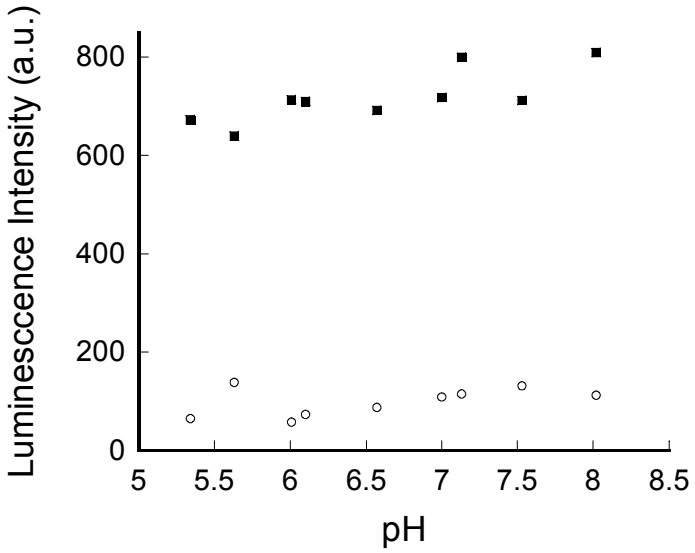
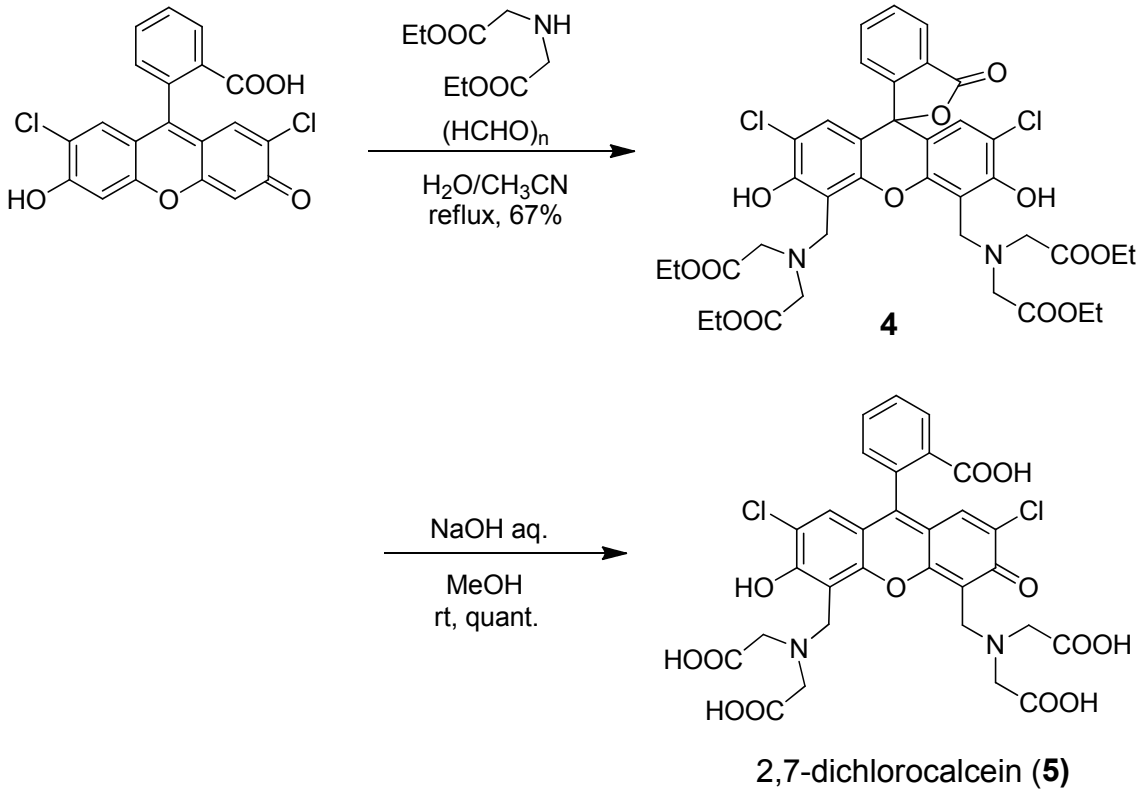



**Fig. S8** Luminescence intensity of the complexes **2** (white circle) and **3** (black square) (each 2 μM) as a function of pH. Measurement was performed in 0.1 M MES buffer (pH 5.34, 5.63, 6.01), PIPES buffer (pH 6.10, 6.57, 7.00), and HEPES buffer (pH 7.13, 7.53, 8.02). Ex. 500 nm. Em. 980 nm.

## 3. Synthetic procedures

Ligands of the complexes **2** and **3** were synthesized as described in our previous work.[18] 2,7-Dichlorocalcein was synthesized according to the reported procedures, with slight modifications as shown below (Scheme S1).[S2]




**Scheme S1** Synthesis of 2,7-dichlorocalcein.

**Synthesis of 2,7-dichlorocalcein ethyl ester (4)**

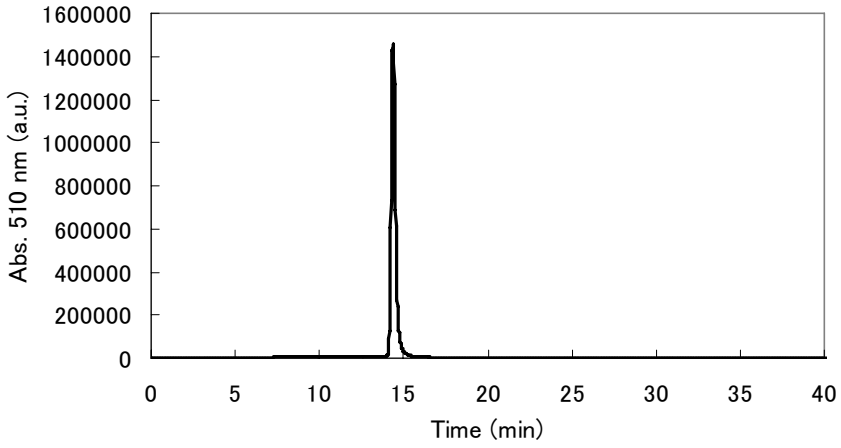
2,7-Dichlorofluorescein (210 mg, 0.52 mmol), diethyl iminodiacetate (0.54 mL, 3 mmol), and paraformaldehyde (180 mg, 6 mmol) were dissolved in a mixed solvent of $H_2O/CH_3CN$ (15/8 mL). The mixture was refluxed for 36 hours in a sealed flask, and the solvent was evaporated. The residue was roughly chromatographed ($SiO_2$, 10% MeOH in $CH_2Cl_2$ as the eluent), and the product was recrystallized from MeOH to obtain **4** as colorless needles (280 mg, yield 67%). $^1$H-NMR ($CDCl_3$): δ 1.28 (t, 12H, $J$ = 7.1 Hz), 3.61 (s, 8H), 4.21 (q, 8H, $J$ = 7.1 Hz), 4.25 (d, 2H, $J$ = 14.2 Hz), 4.45 (d, 2H, $J$ = 14.2 Hz), 6.65 (s, 2H), 7.20 (d, 1H, $J$ = 7.8 Hz), 7.67-7.71 (m, 1H), 7.72-7.76 (m, 1H), 8.05 (d, 1H $J$ = 7.3 Hz), 10.97 (br, 2H). $^{13}$C-NMR ($CDCl_3$): δ 14.2, 48.8, 54.1, 61.3, 82.7, 109.7, 110.6, 117.6, 124.0, 125.5, 127.0, 128.0, 130.3, 135.4, 148.3, 151.3, 155.6, 168.7, 170.4. HRMS (ESI$^+$) *m/z* calcd for $[M + H]^+$: 803.1986, found: 803.1948.

**Synthesis of 2,7-dichlorocalcein (5)**

Compound **4** (78 mg, 0.097 mmol) was dissolved in 2 N NaOH aq./MeOH = 5/5 mL, and the mixture was stirred at room temperature for 6 hours. Completion of the reaction was confirmed by RP-HPLC, then the mixture was neutralized with Amberlite IR-120(H) (Sigma-Aldrich). The resin was filtered off, and the filtrate was lyophilized to give pure **5** as a red powder (69 mg, yield quant.). $^{1}$H-NMR ($D_2O$ containing NaOD): δ 2.94-3.10 (m, 8H), 3.72-3.92 (m, 4H), 7.11-7.16 (m, 1H), 7.13 (s, 2H), 7.40-7.49 (m, 2H), 7.63 (d, 1H, $J$ = 7.8 Hz). $^{13}$C-NMR ($D_2O$ containing NaOD): δ 49.3, 60.3, 111.9, 113.0, 127.9, 128.3, 129.0, 130.2, 130.3, 130.9, 132.0, 140.1, 156.4, 156.6, 175.4, 175.9, 180.3. HRMS (ESI$^-$) *m/z* calcd for [M − COOH]$^-$: 645.0679, found: 645.0690.*

**m/z* 733 [M − 3H + 2Na]$^-$ and other peaks associated with **5** were detected, which indicated that decarboxylation occurred inside the mass spectrometer.

HPLC (A:B = 70:30 to 30:70 over 40 minutes) analysis confirmed the purity of the compound. The chromatogram below was obtained with detection in terms of absorbance at 510 nm. Rt 14.3 min.



## 4. Supplementary references

S1. M. Nakano, Y. Kambayashi, H. Tatsuzawa, T. Komiyama, and K. Fujimori, *FEBS Lett.,* 1998, **432,** 9.

S2. E. J. Jun, J.-A. Kim, K. M. K. Swamy, S. Park, and J. Yoon, *Tetrahedron Lett.,* 2006, **47,** 1051.