Supporting Information

A Europium(III)-based PARACEST Agent that Senses Singlet Oxygen by MRI

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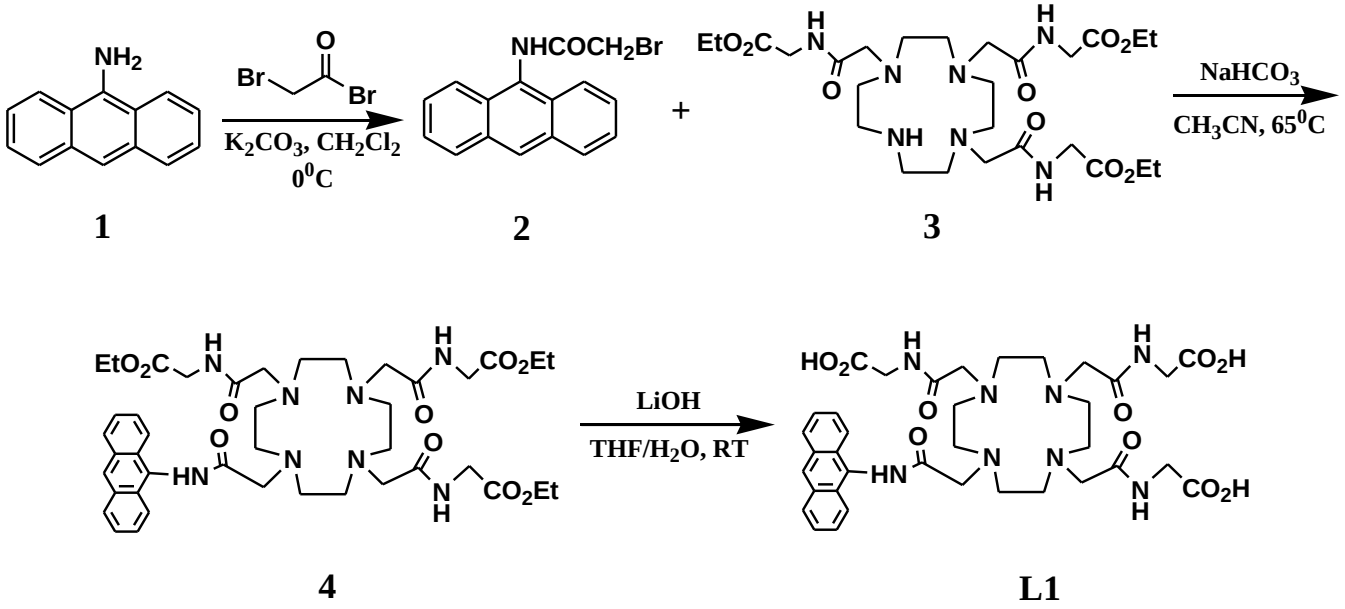
1. General remarks and synthesis of the ligand L

5,10,15,20-Tetrakis(1-methyl-4-pyridinio)porphyrin tetra(p-toluenesulfonate) (TMPyP) was purchased from Aldrich. Prior to use, H_2O_2 was diluted from a stabilized 30% solution, and then assayed by using its molar extinction coefficient of 43.6 $M^{-1}cm^{-1}$ at 240 nm.[1] Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification. ^{1}H, ^{13}C NMR, and CEST spectra were recorded using a Bruker AVANCE III 400 MHz NMR spectrometer. A pre-saturation pulse of 4 s duration and 9.4 μT power was used during the CEST acquisitions. CEST imaging was recorded on a Varian 9.4 T small animal imaging system. The CEST spectra of EuL and EP-EuL recorded at 298K or 310K were fitted to the Bloch equations with 3-pool model by use of a nonlinear fitting algorithm written in MATLAB 7 (Mathworks Inc., Natick, MA).[2] Ultraviolet absorbance spectra were recorded using a Varian Cary 300 Bio UV/Vis spectrophotometer. The fluorescence spectra were collected by a PTI QuantaMaster™ 30 Fluorescence Spectrophotometer (Birmingham, NJ).

2. Syntheses and Characterization

Scheme S1 shows the synthesis procedure of the new ligand **1-N-(9-anthryl)-4,7,10,-tris-(N-acetic acid)-1,4,7,10,-tetraazacyclododecane-1,4,7,10-tetraacetamide (L)**.



Scheme S1. Synthesis of **L**.

The details of the procedure are described as follows:

Anthracen-9-ylamine (1)[3] and **Tris--(N-ethylacetate)-1,4,7,10,-tetraazacyclododecane-1,4,7,10-tetraacetamide (3)** [4] were synthesized using established procedures.

Synthesis of 9-anthryl bromoacetamide (2). To a solution of Anthracen-9-ylamine (4.7g, 24.4mmol) in dichloromethane (70 ml) was added potassium carbonate (4.04g, 29.2mmol). The reaction mixture was stirred vigorously and cooled to 0 °C in an ice bath. Bromoacetyl bromide (5.9g, 29.2mmol) in dichloromethane (30 ml) was then added drop-wise over a period of 30 min. The reaction mixture was then allowed to warm to room temperature and stirred overnight. The precipitate was filtered and washed with water and CH_3CN, and dried. Compound **2** was obtained as a pale yellow powder (5.86 g, 76.5% yield). ^{1}H NMR (400 MHz, DMSO, 25 °C): δ□= 4.35 (s, 2H, CH_2), 7.55-7.60 (m, 4H, Ar), 8.11-8.15 (m, 4H, Ar), 8.63 (s, , 1H, Ar), 10.69 (s, 1H, NH), ^{13}C NMR (400 MHz, DMSO, 25 °C): δ= 30.09 (CH_2), 123.90 (Ar), 126.06 (Ar), 126.71 (Ar), 128.41 (Ar), 128.90 (Ar), 131.68 (Ar), 167.03(C=O).

Synthesis of 1-N-(9-anthryl)-4,7,10,-tris-(N-ethylacetate)-1,4,7,10,-tetraazacyclododecane-1,4,7,10-tetraacetamide (4). Sodium bicarbonate (1.01 g, 12.0 mmol) was added to a solution of

compound **3** (1.83 g, 3.0 mmol) in anhydrous acetonitrile (150 ml) and mixture was stirred for 15 min. The compound **2** (1.05 g, 3.3 mmol) was added and the reaction was heated to 65 °C under N_2 for 18 hours with stirring. The reaction was cooled to room temperature and filtered to remove the inorganic salts. After removal of solvent under reduced pressure, the residue was purified by chromatography on neutral alumina, eluting with an increasing gradient of methanol in dichloromethane (0-5%). The crude product was then crystallized from ethyl acetate to afford a pale yellow powder (1.67 g, 66.5% yield). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.15-1.21 (m, 9H, CH_3), 2.76-2.80 (d br, 8H, ring CH_2N), 3.00 (s br, 4H, ring CH_2N), 3.10-3.13 (d br, 6H, ring CH_2N and $NCH_2C{=}O$), 3.22 (s, 4H, $NCH_2C{=}O$), 3.60 (s, 2H, $NCH_2C{=}O$), 3.67 (s, 6H, $NHCH_2C{=}O$), 3.96-4.04 (m, 6H, OCH_2CH_3), 7.44-7.51 (m, 8H, Ar and $CH_2NHC{=}O$), 7.97 (d, ^3J(H,H) = 8.0 Hz, 2H, Ar), 8.05 (d, ^3J(H,H) = 8.0 Hz, 2H, Ar), 8.48 (s, 1H, Ar), 9.27 (s, 1H, ArNHC=O). ^{13}C NMR (400 MHz, $CDCl_3$, 25 °C): δ□= 14.01 (CH_3), 14.05 (CH_3), 40.72 ($NHCH_2C{=}O$), 53.48 (ring CH_2N), 53.72 (ring CH_2N), 54.01 (ring CH_2N), 58.76 ($NCH_2C{=}O$), 59.47 ($NCH_2C{=}O$), 60.04 ($NCH_2C{=}O$), 61.23 (OCH_2CH_3), 61.28 (OCH_2CH_3), 122.94 (Ar), 125.48 (Ar), 126.48 (Ar), 126.93 (Ar), 127.93 (Ar), 128.30 (Ar), 128.76 (Ar), 131.69 (Ar), 170.03 (C=O), 170.11 (C=O), 170.93 (C=O), 171.13 (C=O), 171.53 (C=O). ESI: *m/z*: 835 (100%, $[M + H]^+$) and 857 (30% . $[M + Na]^+$).

Synthesis of 1-N-(9-anthryl)-4,7,10,-tris-(N-acetic acid)-1,4,7,10,-tetraazacyclododecane-1,4,7,10-tetraacetamide (L). Compound **4** (1.25g, 1.5 mmol) was added into a mixture of aq. LiOH (0.25 M, 35 mL) and THF (35 mL). The reaction mixture was stirred at 0 °C for 2 hours and then at room temperature for 2 hours. After THF was evaporated under reduced pressure, the resulting solution was adjusted to pH 2.0 by addition of HCl and lyophilized to dryness giving the title compound as pale yellow solid. The crude product was thoroughly washed with acetone (10×20ml) and dried in vacuo over P_2O_5. **L** was obtained as a white powder (1.27 g, 92.6% yield). ^{1}H NMR (400 MHz, D_2O, 25 °C pD=10.0): δ = 2.39 (br, 16H, ring CH_2N), 2.95 (s br, 8H, $NCH_2C{=}O$), 3.37-3.61(m, 6H, $NHCH_2C{=}O$), 7.47-7.51 (m, 4H, Ar), 7.89 (d, ^3J(H,H) = 4.0 Hz, 2H, Ar), 7.97(d, ^3J(H,H) = 8.0 Hz, 2H, Ar), 8.42 (s,

1H, Ar). ^{13}C NMR (400 MHz, D_2O, 25 °C pD=10.0): 42.97 (NH<u>C</u>H_2C=O), 50.34 (ring CH_2N), 56.53 (N<u>C</u>H_2C=O), 57.73 (N<u>C</u>H_2C=O), 122.41 (Ar), 126.00 (Ar), 127.32 (Ar), 127.93 (Ar), 128.78 (Ar), 131.32 (Ar), 172.88 (C=O), 175.99 (C=O). ESI: *m/z*: 751 (100%, $[M + H]^+$) and 757 (30% . $[M + Li]^+$). Elemental analysis calcd for $C_{36}H_{46}N_8O_{10}$·0.5LiCi·2.5HCl·3.5H_2O: C 46.68%, H 6.04%, N 12.01%, found: C 46.72%, H 6.27%, N 11.86%.

Synthesis of Europium(III) 1-N-(9-anthryl)-4,7,10,-tris-(N-acetic acid)-1,4,7,10,-tetraazacyclododecane-1,4,7,10-tetraacetamide triflate salt (Eu(III)-L). 92.2 mg **L** (0.1 mmol) and 59.9 mg $Eu(OTf)_3$ (0.1 mmol) were dissolved in 4 ml water. The reaction mixture was adjusted and maintained to a pH around 6.0 by careful addition of aq. KOH. The solution was stirred at room temperature for 12 hours. The presence of excess of free Eu^{3+} was then checked with xylenol orange. If there is excess of free Eu^{3+} in solution, more free ligand **L** (1 mg) was added, the pH of solution was adjusted to 6.0, and the mixture was stirred for an additional 12 hours, before checking for excess free Eu^{3+} again. When no free Eu^{3+} was detected, the aqueous solution was lyophilized to give the complex as pale yellow powder. The EuL complex was used without further purification. ^{1}H NMR (400 MHz, D_2O,): δ = -13.87 ─ -11.70(4H), -10.74 ─ -8.65 (6H), -7.13 ─ - 5.01 (4H), -3.78 ─ -1.27 (5H), 0.75 ─ 5.07 (7H), 6.66 ─ 10.54 (9H, Ar), 23.80 ─ 26.44 (t, 4H, br, ring *ax*[s]). ESI: *m/z*: 901 (40%, $[M + H]^+$) and 939 (100% . $[M + K]^+$). HPLC analysis: retention time, 5.43 min (purity, 97.1% integrated intensity), RESTEK Ultra IBD 3μm 100 mm × 4.6 mm C_{18} reverse-phase column, eluent, 10 min increasing linear gradient acetonitrile/H_2O from 5% to 95%, flow rate, 1 ml/min. The elution was monitored at 215 nm.

Synthesis and characterization of endoperoxide of EuL (EP-EuL)

12.1 mg Na_2MoO_4·2H_2O (0.05 mmol) was added to a 1 ml solution of EuL(5mM) in 0.1 M Na_2CO_3 buffer of pH 10.5, and 10 μl 30% H_2O_2 were added, and the solution was stirred for 30 min. The reaction was monitored by HPLC to check the complete conversion of EuL to EP-EuL. The aqueous solution was neutralized and lyophilized to give the complex as pale yellow powder. The EP-EuL

complex was used without further purification. ^{1}H NMR (400 MHz, D_2O,): δ = -15.76 ─ -10.81 (8H), -9.17 ─ -6.58 (5H), -4.08 ─ -0.36 (7H), 1.16 ─ 4.37 (8H), 5.30 ─ 14.10 (s, 7H, Ar), 26.64 ─ 29.30 (m, br, 4H, ring *ax*^{s}). MALDI-TOF: *m/z*: 724 (82%, $[M+H-O_2-C_{14}H_9]^+$) and 746 (84%, $[M+Na-O_2-C_{14}H_9]^+$). HPLC analysis: retention time, 3.95 min (purity, 95.6% integrated intensity), RESTEK Ultra IBD 3µm 100 mm × 4.6 mm C_{18} reverse-phase column, eluent, 10 min increasing linear gradient acetonitrile/H_2O from 5% to 95%, flow rate, 1 ml/min. The elution was monitored at 215 nm.

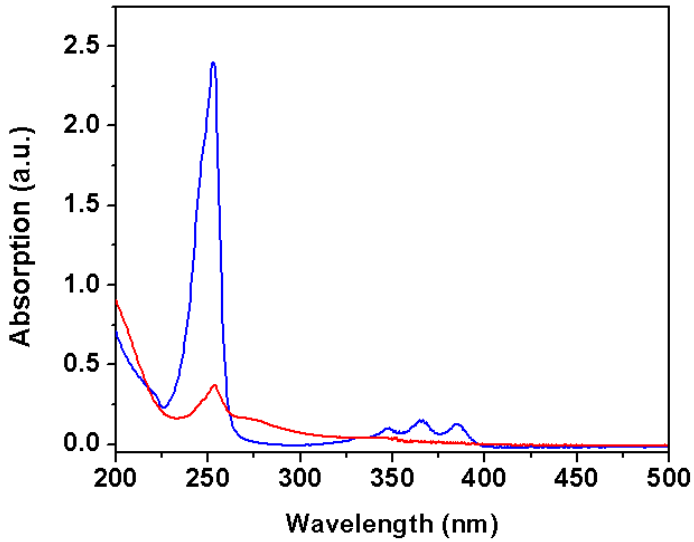


Figure S1. UV-VIS spectra of EuL (25 µM, blue line) and EP-EuL (25 µM, red line) in 0.1 M HEPES buffer, pH 7.0.

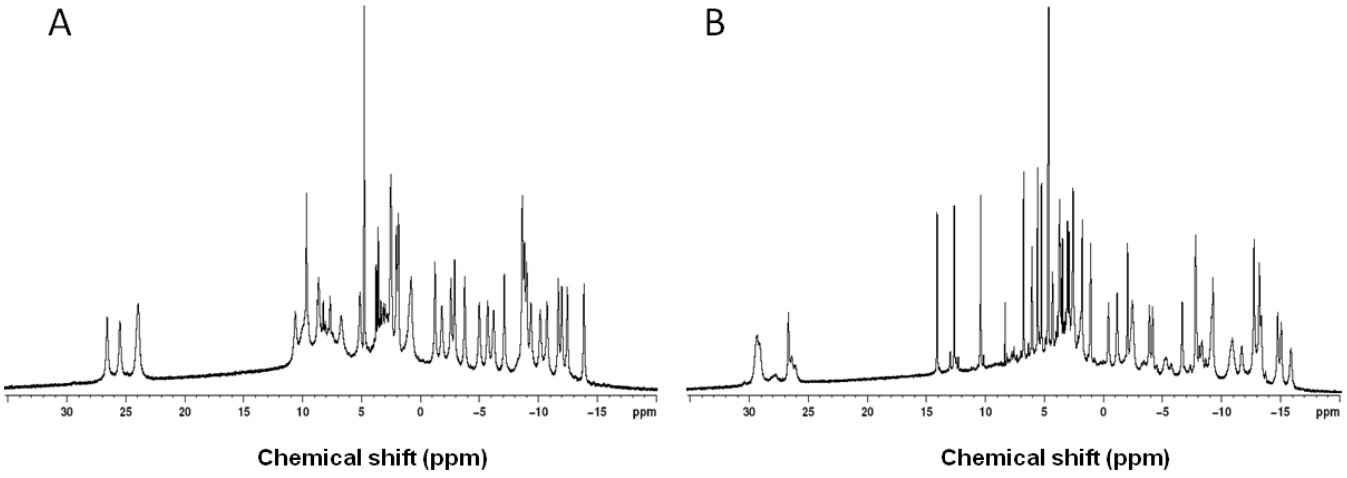


Figure S2. ^{1}H NMR spectra of EuL (A) and EP-EuL (B) in D_2O.

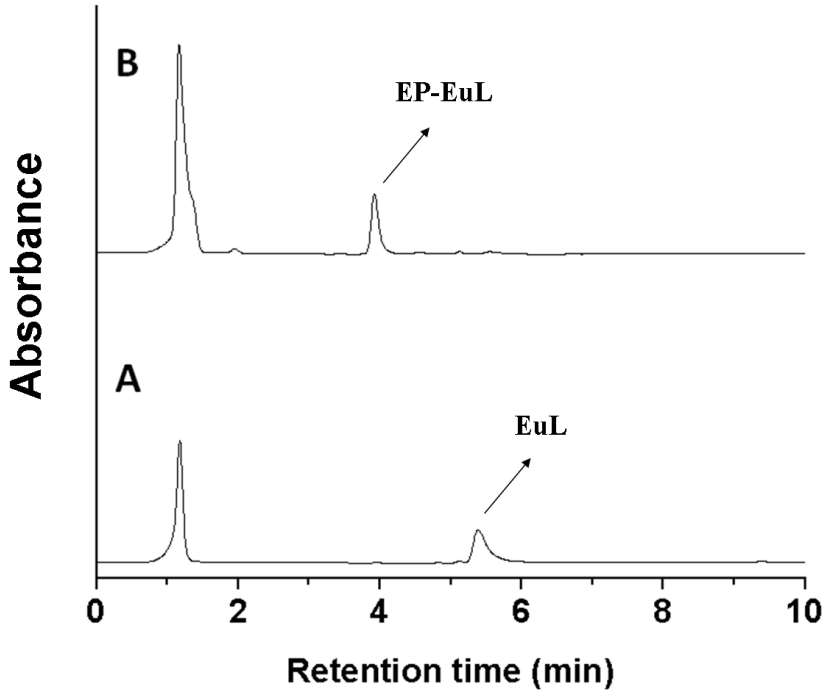


Figure S3. HPLC chromatograms of EuL (A) and EP-EuL (B).

3. Bound water lifetime (τ_M) of EuL and EP-EuL

The bound water lifetimes (τ_M) of EuL and EP-EuL were determined by fitting the experimental CEST spectra to the Bloch equations modified for exchange.

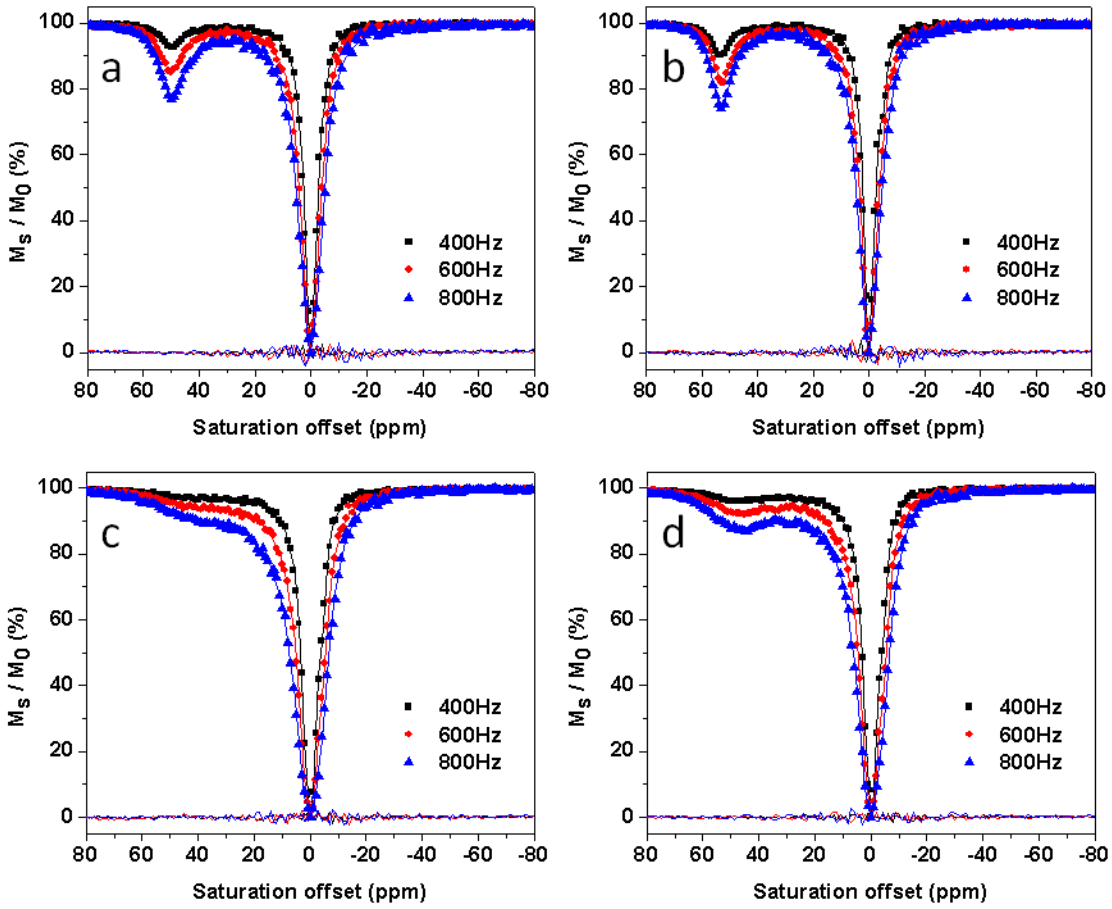


Figure S4. CEST Spectra of (a) 5 mM EuL recorded at 298K, (b) 5 mM EP-EuL recorded at 298K, (c) 5 mM EuL recorded at 310K and (d) 5 mM EP-EuL recorded at 310K. Water exchange rates were calculated by fitting the experimental data to a three pool model using the Bloch equations modified for exchange written in MATLAB. The points represent the experimental data and the lines show the data from fitting.

4. Detection of $^1O_2$ in aqueous media

The reactions of EuL with $^1O_2$ generated from a MoO_4^{2-}-H_2O_2 system and a photosensitization system were investigated. The detailed experimental procedures are described as follows:

(i) The reaction of EuL with $^1O_2$ generated from a MoO_4^{2-}-H_2O_2 system was performed in 0.1 M carbonate buffer of pH 10.5. Various concentrations of H_2O_2 solutions were added to the buffer solutions containing 5 mM of EuL and 50 mM of Na_2MoO_4. After 20 min incubation at 37 ^{0}C, the solutions were adjusted to pH 7.0 and the CEST spectra were obtained using a Bruker AVANCE III 400 MHz NMR spectrometer at 298K or 310K.

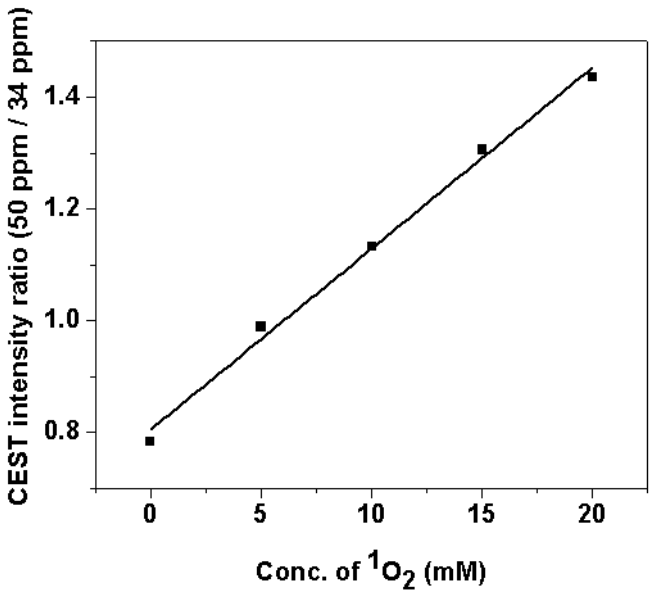


Figure S5. Calibration curve for $^1O_2$ detection derived the EuL CEST intensity ratio at 50 ppm versus 34 ppm recorded at 9.4 T and 310 K.

(ii) The photosensitization reaction was carried out in an aqueous buffer of pH 7.0. The solution containing 1 mM TMPyP and 5 mM of EuL in a glass vial was irradiated from a distance of 5 cm by a 150-W tungsten lamp. After the reaction, the solution was subjected to the CEST measurement.

5. Reactions of EuL with reactive oxygen species (ROS)

The reaction of EuL with $^1O_2$ was performed as the described procedure. All the other reactions were carried out in 0.1 M HEPES buffer of pH 7.0 with the same EuL concentration (5 mM) for 0.5 h at room temperature. Peroxynitrite was synthesized from sodium nitrite (0.6 M) and H_2O_2 (0.65 M) in a quenched-flow reactor (excess H_2O_2 was used to minimize nitrite contamination). After the reaction, the solution was treated with MnO_2 to eliminate the excess H_2O_2. The concentration of the $ONOO^-$ stock solution was determined by measuring the absorbance at 302 nm with a molar extinction coefficient of 1670 $M^{-1}cm^{-1}$.[5] Superoxide solution was prepared by adding KO_2 to dry dimethylsulfoxide and stirring vigorously for 10 min.[6] Hydroxyl radical ($^{\cdot}OH$) was generated in the Fenton system from ferrous chloride and hydrogen peroxide.[7] Singlet oxygen was chemically generated from the MoO_4^{2-}-H_2O_2 system in alkaline media.[8]

6. Reaction rate constant of EuL with $^1O_2$

The reaction rate constant of EuL with $^1O_2$ was determined by a HPLC method using the competitive reactions between the EuL and sodium azide with $^1O_2$ produced by MoO_4^{2-}-H_2O_2 system.

Different concentrations of NaN_3 were used for the competitive reactions between the EuL (1 mM) and NaN_3 with $^1O_2$ in 0.1 M Na_2CO_3 buffer of pH 10.5 containing 10 mM Na_2MoO_4 and 6 mM H_2O_2. In all the experiments, the solutions were stirred for 30 min at room temperature and the resulted EP-EuL was evaluated by HPLC. The reaction rate constant of EuL was calculated according to the eq (1),[9] where I_0/I is the ratio of the HPLC signal intensity of EP-EuL in the absence and the presence of NaN_3, k_T is the rate constant for the EuL-$^1O_2$ reaction, k_d is the decay rate of $^1O_2$ in an aqueous solution ($k_d = 2.38 \times 10^5$ s^{-1}),[10] kq is the rate constant for NaN_3-$^1O_2$ reaction (kq $= 4.5 \times 10^8$ M^{-1} s^{-1}),[11] [T] is the concentration of the EuL probe, and [Q] is the concentration of NaN_3.

$$\frac{I_0}{I} = 1 + \frac{k_q}{k_T[T] + k_d}[Q] \qquad (1)$$

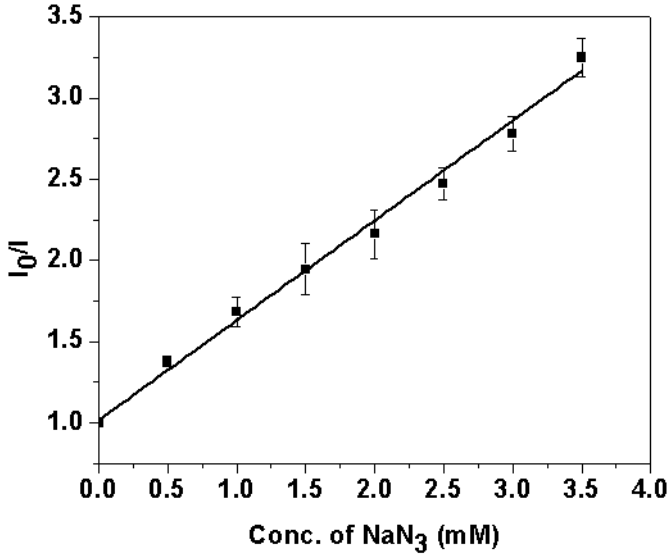


Figure S6. Stern-Volmer plots of The HPLC signal intensities of EP-EuL against the concentrations of NaN_3.

7. pH effects on the CEST signal of EuL

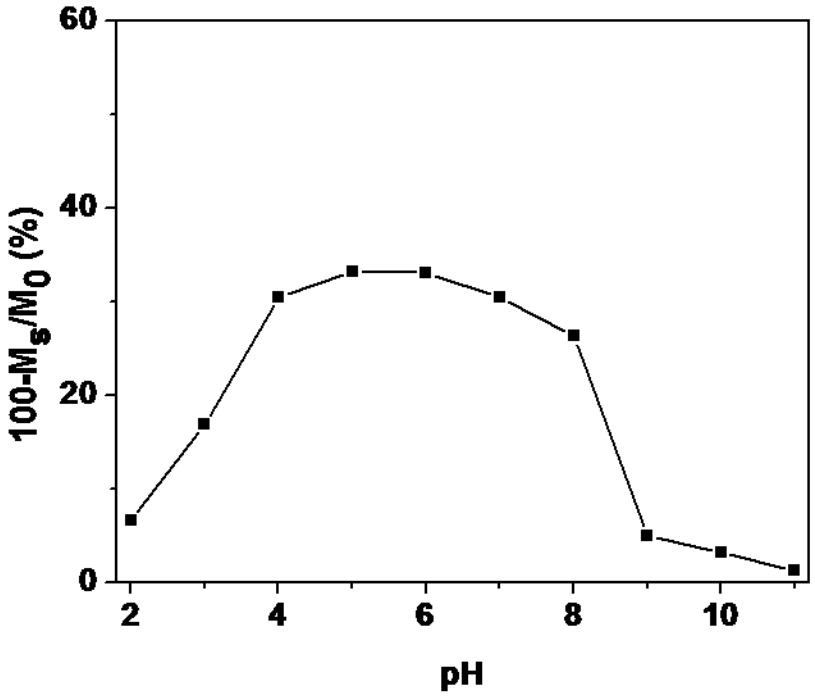


Figure S7. pH dependence of the metal-bound water CEST signal of EuL (20 mM) at 9.4T, 298K and B1 = 9.4 μT with an irradiation time of 4 s.

8. Preparation of HeLa cells

The cell line HeLa was purchased from ATCC (USA) and cultured in 75 cm^2 culture flasks using minimum essential medium (MEM) with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin (PS). Cultures were maintained at 37^0C under 5% CO2 and 95% air atmosphere. The growth medium was changed every other day until the time of using the cells.

For preparing EuL-deposited HeLa cells, HeLa cells cultured in a 75 cm^2 culture flask were washed three times with an isotonic saline solution consisting of 140 mM NaCl, 10 mM glucose, and 3.5 mM KCl, and then incubated with the isotonic saline solution containing 15 mM EuL for 1 h at 37 ^{0}C and 5% CO2.[12] The labeled HeLa cells were washed 7 times with the saline and collected to an Eppendorf tube by scraping. The cells suspension were sonicated to make it homogenous, and used for recording CEST spectrum and CEST image of phantom.

For fluorescence imaging, HeLa cells were seeded on cell culture dishes with 14 mm glass cover slips and allowed to adhere for 48 h. The cultured HeLa cells incubated with 5 mM EuL in MEM for 1 h at 37 ^{0}C in a 5% CO2/95% air incubator. The cells were then washed five times with phosphate buffered

saline (PBS) and used for fluorescence imaging measurement in PBS. Fluorescence and bright field live cell images were obtained by an IX-71 inverted fluorescence microscope (Olympus) with a 1.3NA 100X oil-immersion objective under Hg-lamp excitation (for fluorescence imaging, Ex: 325-375 nm; Em: 435-485 nm; 30 W/cm2; 0.5 s exposure time) and a Photon Max 512 CCD camera (Princeton Instrument).

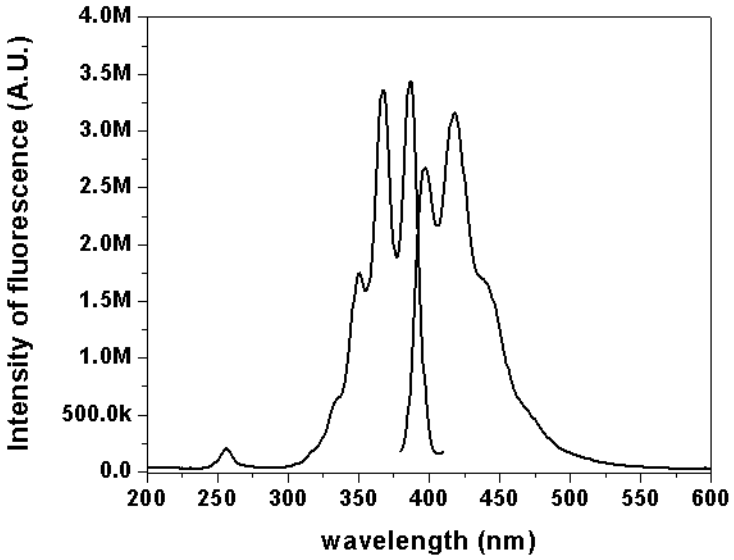


Figure S8. Fluorescence excitation and emission spectra of EuL (40 μM) in 0.1 M HEPES buffer of pH 7.0.

For quantifying the intracellular EuL, HeLa cells cultured in a 75 cm^2 culture flask were washed three times with an isotonic saline solution consisting of 140 mM NaCl, 10 mM glucose, and 3.5 mM KCl, and then incubated with the isotonic saline solution containing 15 mM EuL for 1 h at 37 ^{0}C and 5% CO2. The cells were washed with PBS five times and harvested by trypsin treatment. The cell density and viability, defined as the ratio of the number of viable cells over the total number of cells, of the cultures were determined by trypan blue staining with a Neubauer improved hemacytometer. The numbered EuL-deposited cells were suspended in 1 M HCl and subjected to the inductively coupled plasma - optical emission spectrometer (ICP-EOS) measurement.

For detection of intracellular $^1O_2$, HeLa cells cultured in a 75 cm^2 culture flask were washed three times with an isotonic saline solution consisting of 140 mM NaCl, 10 mM glucose, and 3.5 mM KCl, and then incubated with the isotonic saline solution containing 15 mM EuL for 1 h at 37 ^{0}C and 5% CO_2. After 2 mM TMPyP was added to culture medium in the culture flask, the flask was further incubated at 37 ^{0}C and 5% CO_2 for 30 min. The labeled HeLa cells were washed 7 times with the saline

and exposed to a 150-W tungsten light from a distance of 10 cm for 30 min. The cells were collected to an Eppendorf tube by scraping. The cells suspension were sonicated to make it homogenous, and used for recording CEST spectrum.

References

(1) Lei, B. P.; Adachi, N.; Arai, T. *Brain Res. Protoc.* **1998**, *3*, 33-36.

(2) Woessner, D. E.; Zhang, S. R.; Merritt, M. E.; Sherry, A. D. *Magn. Reson. Med.* **2005**, *53*, 790-799.

(3) Hirano, K.; Urban, S.; Wang, C.; Glorius, F. *Org. Lett.* **2009**, *11*, 1019-1022.

(4) Ratnakar, S. J.; Woods, M.; Lubag, A. J. M.; Kovacst, Z.; Sherry, A. D. *J. Am. Chem. Soc.* **2008**, *130*, 6-7.

(5) Miyamoto, S.; Martinez, G. R.; Martins, A. P. B.; Medeiros, M. H. G.; Di Mascio, P. *J. Am. Chem. Soc.* **2003**, *125*, 4510-4517.

(6) Zhao, H. T.; Kalivendi, S.; Zhang, H.; Joseph, J.; Nithipatikom, K.; Vasquez-Vivar, J.; Kalyanaraman, B. *Free Radic. Biol. Med.* **2003**, *34*, 1359-1368.

(7) Setsukinai, K.; Urano, Y.; Kakinuma, K.; Majima, H. J.; Nagano, T. *J. Biol. Chem.* **2003**, *278*, 3170-3175.

(8) Aubry, J. M.; Cazin, B. *Inorg. Chem.* **1988**, *27*, 2013-2014.

(9) Weng, M.; Zhang, M. H.; Shen, T. *Dyes Pigment.* **1998**, *36*, 93-102.

(10) Schmidt, R. *J. Am. Chem. Soc.* **1989**, *111*, 6983-6987.

(11) Miskoski, S.; Garcia, N. A. *Photochem. Photobiol.* **1993**, *57*, 447-452.

(12) Song, B.; Wang, G. L.; Tan, M. Q.; Yuan, J. L. *J. Am. Chem. Soc.* **2006**, *128*, 13442-13450.