Supporting Information

"A time-resolved long-lived luminescence imaging method employing luminescent lanthanide probes with a new microscopy system"

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Synthesis of luminescent Eu^{3+} complexes (R = H, F, CH_3, CH_3O, NHAc, NH_2 at the 6-position of the quinoline). The basic synthetic scheme was as follows. Starting from 2-methylquinoline having various substituents (= R) at the 6-position, the 2-methylquinoline *N*-oxide was generated by treatment with 3-chloroperoxybenzoic acid at 40 ˚C in CH_2Cl_2, then chlorination of the *N*-oxide with p-toluenesulfonyl chloride at 100 ˚C in dichloroethane under Ar gave 2-chloromethylquinoline. The tetraazamacrocyclic chelators for Eu^{3+} with *t*-butyl ester protecting groups on three carboxylic acid moieties were obtained by treatment of the 2-chloromethylquinoline with 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane and anhydrous Na_2CO_3 in CH_3CN at reflux under Ar. The *t*-butyl ester protecting groups were removed with trifluoroacetic acid (TFA). The tetraazamacrocyclic chelators were purified by reversed-phase (ODS) HPLC. The Eu^{3+} complexes

were prepared by reacting tetraazamacrocyclic ligand and $EuCl_3$-$6H_2O$ salt (1.1 equiv.) at pH 7.0 and 70 ˚C in H_2O under Ar.

General procedure for the synthesis of quinoline *N*-oxides (4b-e). Compounds **4b-e** were synthesized according to the literature.[SR1] To a stirred solution of a 2-methylquinoline derivative (**3b-e**) (1.00 equiv.) in CH_2Cl_2 was added dropwise a solution of 70% 3-chloroperoxybenzoic acid (1.23 equiv.) in CH_2Cl_2. The mixture was stirred at 40 ˚C overnight. The resulting solution was washed with 10% aqueous Na_2SO_3, 10% aqueous K_2CO_3 and brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel or alumina to give the product.

6-Fluoro-2-methyl-1-oxyquinoline (4b). Reaction of 6-fluoro-2-methylquinoline (**3b**) (2.00 g, 12.4 mmol) in CH_2Cl_2 (15 ml) with 70% 3-chloroperoxybenzoic acid (3.79 g, 15.4 mmol) in CH_2Cl_2 (15 ml) was performed according to the general procedure. Purification of the product by column chromatography on silica gel (3 : 97 methanol / CH_2Cl_2 as eluent) gave **4b** (1.83 g, 10.3 mmol, 83% yield) as a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.80 (1H, dd, *J* = 5.1, 9.3 Hz, Ar*H*), 7.58 (1H, d, *J* = 8.6 Hz, Ar*H*), 7.44-7.53 (2H, m, Ar*H*), 7.34 (1H, d, *J* = 8.6 Hz, Ar*H*), 2.71 (3H, s, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.89, 159.57, 145.28, 138.54, 130.27, 130.14, 124.47, 124.40, 124.25, 122.53, 122.41, 120.11, 119.76, 111.64, 111.33, 18.57. MS (ESI^+): *m/z* 178 ($M + H^+$).

2,6-Dimethyl-1-oxyquinoline (4c). Reaction of 2,6-dimethylquinoline (**3c**) (2.13 g, 13.5 mmol) in CH_2Cl_2 (15 ml) with 70% 3-chloroperoxybenzoic acid (4.14 g, 16.8 mmol) in CH_2Cl_2 (15 ml) was performed according to the general procedure. Purification of the product by column chromatography on silica gel (5 : 95 methanol / CH_2Cl_2 as eluent) gave **4c** (2.11 g, 12.2 mmol, 90% yield) as a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.66 (1H, d, *J* = 9.3 Hz, Ar*H*), 7.55-7.59 (3H, m, Ar*H*), 7.27 (1H, d, *J* = 7.8 Hz, Ar*H*), 2.71 (3H, s, CH_3), 2.53 (3H, s, CH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 144.61, 139.86, 137.51, 132.13, 129.10, 126.73, 124.38, 122.73, 119.06, 21.08, 18.43. MS (ESI^+): *m/z* 174 ($M + H^+$).

6-Methoxy-2-methyl-1-oxyquinoline (4d). Reaction of 6-methoxy-2-methylquinoline (**3d**) (2.50 g, 14.4 mmol) in CH_2Cl_2 (15 ml) with 70% 3-chloroperoxybenzoic acid (4.41 g, 17.9 mmol) in CH_2Cl_2 (15 ml) was performed according to the general procedure. Purification of the product by column chromatography on silica gel (5 : 95 methanol / CH_2Cl_2 as eluent) gave **4d** (2.45 g, 12.9 mmol, 90% yield) as a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.69 (1H, d, *J* = 9.5 Hz, Ar*H*), 7.54 (1H, d, *J* = 8.8 Hz, Ar*H*), 7.37 (1H, dd, *J* = 2.6, 9.5 Hz, Ar*H*), 7.27 (1H, d, *J* = 8.8 Hz, Ar*H*), 7.08 (1H, d, *J* = 2.6 Hz, Ar*H*), 3.93 (3H, s, OCH_3), 2.69 (3H, s, ArCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 158.39, 143.27, 137.00, 130.23, 123.82, 123.18, 121.96, 120.88, 105.65, 55.36, 18.17. MS (ESI^+): *m/z* 190 ($M + H^+$).

6-*N*-Acetylamino-2-methyl-1-oxyquinoline (4e). Reaction of 6-*N*-acetylamino-2-methylquinoline (**3e**) (1.20 g, 5.99 mmol) in CH_2Cl_2 (15 ml) with 70% 3-chloroperoxybenzoic acid (1.83 g, 7.42 mmol) in CH_2Cl_2 (15 ml) was performed according to the general procedure. The mixture was stirred at 40 ˚C overnight, and the precipitate was collected by filteration and washed with cold CH_2Cl_2. Purification of the product by column chromatography on silica gel (7 : 93 methanol / CH_2Cl_2 as eluent) gave **4e** (801 mg, 3.70 mmol, 62% yield) as a colorless solid. ^{1}H-NMR (300 MHz, CD_3OD): δ 8.56 (1H, d, *J* = 9.3 Hz, Ar*H*), 8.48 (1H, d, *J* = 2.4 Hz, Ar*H*), 7.95 (1H, d, *J* = 8.8 Hz, Ar*H*), 7.84 (1H, dd, *J* = 2.4, 9.3 Hz, Ar*H*), 7.53 (1H, d, *J* = 8.8 Hz, Ar*H*), 2.70 (3H, s, ArCH_3), 2.20 (3H, s, COCH_3). ^{13}C-NMR (75 MHz, CD_3OD): δ 172.09, 147.69, 139.88, 138.64, 131.36, 129.93, 125.78, 124.97, 120.35, 117.31, 23.99, 18.47. MS (ESI^+): *m/z* 239 ($M + Na^+$).

General procedure for the synthesis of 2-chloromethylquinolines (5b-e). Compounds **5b-e** were synthesized according to the literature.[SR1] A quinoline *N*-oxide (**5b-e**) (1 equiv.) was added to a stirred solution of *p*-toluenesulfonyl chloride (1.1 equiv.) in dichloroethane. The reaction mixture was stirred under Ar for 5 h at 100 ˚C and then cooled to room temperature. The reaction mixture was diluted with CH_2Cl_2 and washed with 10% aqueous K_2CO_3. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to afford a crude solid, which was purified by column chromatography on silica gel to give the product.

2-Chloromethyl-6-fluoroquinoline (5b). Reaction of 6-fluoro-2-methyl-1-oxyquinoline (**4b**) (1.60 g, 9.03 mmol) in dichloroethane (120 ml) with *p*-toluenesulfonyl chloride (1.89 g, 9.91 mmol) was performed according to the general procedure. Purification of the product by column chromatography on silica gel (1 : 9 ethyl acetate / n-hexane as eluent) gave **5b** (1.02 g, 5.21 mmol, 58% yield) as a pale brown solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.16 (1H, d, J = 8.6 Hz, ArH), 8.07 (1H, dd, *J* = 5.3, 9.3 Hz, Ar*H*), 7.63 (1H, d, *J* = 8.6 Hz, Ar*H*), 7.43-7.54 (2H, m, Ar*H*), 4.83 (2H, s, ClCH_2). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 162.55, 159.25, 156.31, 156.27, 144.63, 136.95, 136.89, 132.00, 131.89, 128.34, 128.21, 121.51, 120.58, 120.24, 111.02, 110.73, 47.43. MS (ESI^+): *m/z* 196, 198 ($M + H^+$).

2-Chloromethyl-6-methylquinoline (5c). Reaction of 2,6-dimethyl-1-oxyquinoline (**4c**) (1.90 g, 11.0 mmol) in dichloroethane (150 ml) with *p*-toluenesulfonyl chloride (2.30 g, 12.1 mmol) was performed according to the general procedure. The reaction mixture was stirred under Ar at 100 ˚C overnight. Purification of the product by column chromatography on silica gel (3 : 2 CH_2Cl_2 / n-hexane as eluent) gave **5c** (1.23 g, 6.42 mmol, 58% yield) as a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.12 (1H, d, *J* = 8.6 Hz, Ar*H*), 7.96 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.56-7.59 (3H, m, Ar*H*), 4.83 (2H, s, ClCH_2), 2.55 (3H, s, ArCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 155.61, 145.89, 136.79, 136.48, 132.10, 128.74, 127.34, 126.31, 120.34, 47.33, 21.46. MS (ESI^+): *m/z* 192, 194 ($M + H^+$).

2-Chloromethyl-6-methoxyquinoline (5d). Reaction of 6-methoxy-2-methyl-1-oxyquinoline (**4d**) (2.00 g, 10.6 mmol) in dichloroethane (150 ml) with *p*-toluenesulfonyl chloride (2.22 g, 11.6 mmol) was performed according to the general procedure. The reaction mixture was stirred under Ar at 100 ˚C overnight. Purification of the product by column chromatography on silica gel (3 : 2 CH_2Cl_2 / n-hexane as eluent) gave **5d** (449 mg, 2.16 mmol, 20% yield) as a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.09 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.97 (1H, d, *J* = 9.2 Hz, Ar*H*), 7.56 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.39 (1H, dd, *J* = 2.7, 9.2 Hz, Ar*H*), 7.08 (1H, d, *J* = 2.7 Hz, Ar*H*), 4.82 (2H, s, ClCH_2), 3.94 (3H, s, OCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 157.93, 153.91,

143.29, 135.81, 130.42, 128.33, 122.52, 120.65, 104.85, 55.37, 47.31. MS (ESI^+): *m/z* 208, 210 ($M + H^+$).

6-*N*-Acetylamino-2-chloromethylquinoline (5e). Reaction of 6-*N*-acetylamino-2-methyl-1-oxyquinoline (**4e**) (0.75 g, 3.47 mmol) in dichloroethane (50 ml) with *p*-toluenesulfonyl chloride (727 mg, 3.81 mmol) was performed according to the general procedure. The reaction mixture was stirred under Ar at 100 ˚C overnight. Purification of the product by column chromatography on silica gel (4 : 1 ethyl acetate / n-hexane as eluent) gave **5e** (450 mg, 1.92 mmol, 55% yield) as a pale yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.36 (1H, d, *J* = 2.4 Hz, Ar*H*), 8.17 (1H, d, *J* = 8.4 Hz, Ar*H*), 8.01 (1H, d, *J* = 9.3 Hz, Ar*H*), 7.59 (1H, d, *J* = 8.4, Ar*H*), 7.54 (1H, dd, *J* = 2.4, 9.3 Hz, Ar*H*), 7.35 (1H, br s, N*H*), 4.82 (2H, s, ClCH_2), 2.26 (3H, s, CH_3). ^{13}C-NMR (75 MHz, CD_3OD): δ 172.00, 157.28, 145.34, 138.87, 138.80, 129.73, 129.49, 125.34, 122.61, 116.95, 47.45, 23.96. MS (ESI^+): *m/z* 235, 237 ($M + H^+$).

General procedure for the synthesis of 2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinolines (6a-e). Hydrobromide salt of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**9**) was prepared according to the literature.[SR2] To a solution of a 2-chloromethylquinoline (**5b-e** or 2-chloromethylquinoline hydrochloride) (1 equiv.) and hydrobromide salt of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**9**) (1 equiv.) in acetonitrile was added anhydrous Na_2CO_3 (4 equiv.). The resulting suspension was heated under reflux under Ar for 48 h, cooled to room temperature, filtered and evaporated to dryness. The residue was dissolved in CH_2Cl_2, then the solution was washed with H_2O and brine, dried over anhydrous K_2CO_3 and evaporated to dryness. The residue was purified by silica gel column chromatography to give the product.

2-[4,7,10-Tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (6a). Reaction of 2-chloromethylquinoline hydrochloride (158 mg, 0.738 mmol, 1.1 equiv.), hydrobromide salt of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**9**) (399 mg, 0.670 mmol) and Na_2CO_3

(284 mg, 2.68 mmol) in acetonitrile (50 ml) was performed according to the general procedure. The resulting suspension was heated under reflux under Ar for 15 h. Purification of the product by column chromatography on silica gel (5 : 95 methanol / CH_2Cl_2 as eluent) gave **6a** (191 mg, 0.291 mmol, 43% yield) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.14 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.98-8.02 (1H, m, Ar*H*), 7.79-7.82 (1H, m, Ar*H*), 7.44-7.50 (2H, m, Ar*H*), 7.32 (1H, d, *J* = 8.4 Hz, Ar*H*), 3.92 (2H, br s, ArCH_2N), 2.37-3.13 (22H, m, ring and CH_2CO), 1.57 (9H, s, ^{t}*Bu*), 1.24 (18H, s, ^{t}*Bu*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.09, 171.93, 158.99, 147.83, 137.02, 129.47, 129.06, 127.61, 127.28, 126.13, 121.33, 82.25, 81.97, 60.48, 56.64, 55.81, 50.87 (broad), 28.02, 27.89. MS (ESI^+): *m/z* 656 ($M + H^+$).

6-Fluoro-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (6b). Reaction of 2-chloromethyl-6-fluoroquinoline (**5b**) (0.23 g, 1.18 mmol), hydrobromide salt of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**9**) (0.7 g, 1.18 mmol) and Na_2CO_3 (0.5 g, 4.72 mmol) in acetonitrile (90 ml) was performed according to the general procedure. The resulting suspension was heated under reflux under Ar for 24 h. Purification of the product by column chromatography on silica gel (7 : 93 methanol / CH_2Cl_2 as eluent) gave **6b** (596 mg, 0.884 mmol, 75% yield) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.10 (1H, d, *J* = 8.6 Hz, Ar*H*), 8.02 (1H, dd, *J* = 5.5, 9.3 Hz, Ar*H*), 7.42 (1H, dd, *J* = 2.7, 8.8 Hz, Ar*H*), 7.36 (1H, d, *J* = 8.6 Hz, Ar*H*), 7.19-7.25 (1H, m, Ar*H*), 3.91 (2H, br s, ArCH_2N), 2.34-3.11 (22H, m, ring and CH_2CO), 1.57 (9H, s, ^{t}*Bu*), 1.25 (18H, s, ^{t}*Bu*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.12, 172.02, 161.61, 158.53, 158.49, 158.33, 144.79, 136.50, 136.43, 131.50, 131.38, 128.00, 127.86, 122.29, 119.45, 119.11, 110.84, 110.55, 82.22, 81.95, 60.25, 56.62, 55.76, 50.77 (broad), 27.98, 27.85. MS (ESI^+): *m/z* 674 ($M + H^+$).

6-Methyl-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (6c). Reaction of 2-chloromethyl-6-methylquinoline (**5c**) (0.225 g, 1.17 mmol), hydrobromide salt of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**9**) (0.7 g, 1.18 mmol) and Na_2CO_3 (0.5 g, 4.72 mmol) in acetonitrile (90 ml) was performed according to the general procedure. The resulting suspension was heated under reflux under Ar for 48 h. Purification of the product by column chromatography on silica gel

(7 : 93 methanol / CH_2Cl_2 as eluent) gave **6c** (737 mg, 1.10 mmol, 94% yield) as an orange oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.04 (1H, d, *J* = 8.3 Hz, Ar*H*), 7.89 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.55 (1H, s, Ar*H*), 7.24-7.31 (2H, m, Ar*H*), 3.88 (2H, br s, ArCH_2N), 2.37-3.12 (22H, m, ring and CH_2CO), 1.58 (9H, s, ^{t}*Bu*), 1.26 (18H, s, ^{t}*Bu*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.65, 171.47, 157.51, 146.03, 135.96, 135.55, 131.29, 128.39, 126.88, 126.01, 120.98, 81.85, 81.54, 59.95, 56.18, 55.43, 50.49 (broad), 27.63, 27.49, 21.05. MS (ESI$^+$): *m/z* 670 (M + H$^+$).

6-Methoxy-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (6d). Reaction of 2-chloromethyl-6-methoxyquinoline (**5d**) (0.244 g, 1.17 mmol), hydrobromide salt of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**9**) (0.7 g, 1.18 mmol) and Na_2CO_3 (0.5 g, 4.72 mmol) in acetonitrile (90 ml) was performed according to the general procedure. The resulting suspension was heated under reflux under Ar for 72 h. Purification of the product by column chromatography on silica gel (7 : 93 methanol / CH_2Cl_2 as eluent) gave **6d** (641 mg, 0.935 mmol, 80% yield) as a yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.05 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.89 (1H, d, *J* = 9.2 Hz, Ar*H*), 7.28 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.11 (1H, dd, *J* = 2.7, 9.2 Hz, Ar*H*), 7.07 (1H, d, *J* = 2.7 Hz, Ar*H*), 3.92 (3H, s, OCH_3), 2.36-3.12 (22H, m, ring and CH_2CO), 1.57 (9H, s, ^{t}*Bu*), 1.26 (18H, s, ^{t}*Bu*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.04, 171.86, 157.37, 156.17, 143.90, 135.90, 130.42, 128.30, 122.18, 121.66, 104.98, 82.22, 81.94, 60.17, 56.58, 55.81, 55.65, 50.82 (broad), 28.00, 27.88. MS (ESI$^+$): *m/z* 686 (M + H$^+$).

6-*N*-Acetylamino-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (6e). Reaction of 6-*N*-acetylamino-2-chloromethylquinoline (**5e**) (0.276 g, 1.18 mmol), hydrobromide salt of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**9**) (0.7 g, 1.18 mmol) and Na_2CO_3 (0.5 g, 4.72 mmol) in acetonitrile (90 ml) was performed according to the general procedure. The resulting suspension was heated under reflux under Ar for 48 h. Purification of the product by column chromatography on silica gel (7 : 93 methanol / CH_2Cl_2 as eluent) gave **6e** (463 mg, 0.649 mmol, 55% yield) as a pale yellow solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 11.25 (1H, s, CON*H*), 8.50 (1H, d, *J* = 2.4 Hz, Ar*H*), 8.24 (1H, dd, *J* = 2.4, 9.2 Hz, Ar*H*), 8.06 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.81 (1H, d, *J* = 9.2 Hz, Ar*H*), 7.13 (1H, d, *J* = 8.4 Hz, Ar*H*),

3.82 (2H, br s, ArCH_2N), 2.38-3.09 (25H, m, ring and CH_2COO and CH_3CON), 1.58 (9H, s, ^{t}*Bu*), 1.28 (18H, s, ^{t}*Bu*). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 172.74, 171.70, 170.81, 156.20, 144.82, 138.04, 136.70, 128.75, 127.63, 125.09, 120.91, 115.37, 82.69, 82.40, 60.31, 56.49, 55.77, 50.68, 28.03, 27.93, 24.68. MS (ESI$^+$): *m/z* 713 (M + H$^+$).

General procedure for the synthesis of 2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinolines (7a-e). Trifluoroacetic acid (TFA) (50 ml) was added dropwise to a 2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**6a-e**) at 0 ˚C. The mixture was stirred at room temperature overnight and evaporated to dryness. The residue was dissolved in distilled water (20 ml) and evaporated to dryness three times. The residue was purified by reversed-phase HPLC.

2-[4,7,10-Tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (7a). Reaction of 2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**6a**) (0.191 g, 0.291 mmol) with TFA was performed according to the general procedure. Purification of the product by reversed-phase HPLC (a 120-min linear gradient, from 0% to 80% solvent B (solvent A, H_2O, 0.1% TFA; solvent B, acetonitrile-H_2O (80 : 20), 0.1% TFA)) gave **7a** (72 mg, 0.148 mmol, 51% yield) as an orange oil. ^{1}H-NMR (300 MHz, D_2O): δ 8.91 (1H, d, *J* = 8.6 Hz, Ar*H*), 8.20 (1H, d, *J* = 8.6 Hz, Ar*H*), 8.13 (1H, d, *J* = 8.6 Hz, Ar*H*), 8.04 (1H, dd, *J* = 7.7, 8.6 Hz, Ar*H*), 7.97 (1H, d, *J* = 8.6 Hz, Ar*H*), 7.82 (1H, dd, *J* = 7.7, 8.6 Hz, Ar*H*), 4.19 (2H, s, ArCH_2N), 2.83-3.62 (22H, m, ring and CH_2COO). ^{13}C-NMR (75 MHz, D_2O): δ 175.65, 169.49, 154.26, 149.09, 139.17, 136.60, 131.18, 129.80, 129.19, 123.12, 121.36, 55.84, 55.24, 53.85, 52.87, 51.31, 49.14, 48.72. HRMS (ESI$^+$): Calcd for $C_{24}H_{34}N_5O_6^+$: 488.2509 (M + H$^+$), found: 488.2472 (M + H$^+$).

6-Fluoro-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (7b). Reaction of

6-fluoro-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**6b**) (0.596 g, 0.884 mmol) with TFA was performed according to the general procedure. Purification of the product by reversed-phase HPLC (a 40-min linear gradient, from 0% to 80% solvent B (solvent A, H_2O, 0.1% TFA; solvent B, acetonitrile-H_2O (80 : 20), 0.1% TFA)) gave **7b** (401 mg, 0.793 mmol, 90% yield) as a colorless solid. ^{1}H-NMR (300 MHz, D_2O): δ 8.86 (1H, d, *J* = 8.6 Hz, Ar*H*), 8.26 (1H, dd, *J* = 4.6, 9.7 Hz, Ar*H*), 7.99 (1H, d, *J* = 8.6 Hz, Ar*H*), 7.81-7.89 (2H, m, Ar*H*), 4.20 (2H, s, ArCH_2N), 2.84-3.63 (22H, m, ring and CH_2COO). ^{13}C-NMR (75 MHz, D_2O, 50 ˚C): δ 175.05, 170.03, 164.37, 161.03, 153.79, 147.56, 137.66, 130.70, 130.54, 126.61, 126.27, 125.76, 124.23, 113.44, 113.14, 56.19, 55.84, 54.31, 52.67, 51.44, 49.71, 49.36. HRMS (ESI$^+$): Calcd for $C_{24}H_{33}FN_5O_6^+$: 506.2415 (M + H^+), found: 506.2387 (M + H^+).

6-Methyl-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (7c).

Reaction of 6-methyl-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**6c**) (0.737 g, 1.10 mmol) with TFA was performed according to the general procedure. Purification of the product by reversed-phase HPLC (a 40-min linear gradient, from 0% to 80% solvent B (solvent A, H_2O, 0.1% TFA; solvent B, acetonitrile-H_2O (80 : 20), 0.1% TFA)) gave **7c** (491 mg, 0.979 mmol, 89% yield) as a colorless solid. ^{1}H-NMR (300 MHz, D_2O): δ 8.79 (1H, d, *J* = 8.4 Hz, Ar*H*), 8.08 (1H, d, *J* = 9.2 Hz, Ar*H*), 7.88-7.92 (3H, m, Ar*H*), 4.16 (2H, s, ArCH_2N), 2.82-3.64 (22H, m, ring and CH_2COO), 2.48 (3H, s, CH_3). ^{13}C-NMR (75 MHz, D_2O): δ 175.51, 169.45, 152.72, 147.97, 142.46, 138.83, 137.60, 129.26, 128.10, 123.02, 120.82, 55.93, 55.01, 53.84, 52.78, 51.28, 49.07, 48.70, 21.59. HRMS (ESI$^+$): Calcd for $C_{25}H_{36}N_5O_6^+$: 502.2666 (M + H^+), found: 502.2656 (M + H^+).

6-Methoxy-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (7d).

Reaction of 6-methoxy-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**6d**) (0.641 g, 0.935 mmol) with TFA was performed according to the general procedure. Purification of the product

by reversed-phase HPLC (a 40-min linear gradient, from 0% to 80% solvent B (solvent A, H_2O, 0.1% TFA; solvent B, acetonitrile-H_2O (80 : 20), 0.1% TFA)) gave **7d** (403 mg, 0.779 mmol, 83% yield) as a colorless solid. ^{1}H-NMR (300 MHz, D_2O): δ 8.74 (1H, d, *J* = 8.6 Hz, Ar*H*), 8.08 (1H, d, *J* = 9.4 Hz, Ar*H*), 7.89 (1H, d, *J* = 8.6 Hz, Ar*H*), 7.65 (1H, dd, *J* = 2.8, 9.4 Hz, Ar*H*), 7.46 (1H, d, *J* = 2.8 Hz, Ar*H*), 4.14 (2H, s, ArCH_2N), 3.89 (3H, s, CH_3O), 2.84-3.69 (22H, m, ring and CH_2COO). ^{13}C-NMR (75 MHz, D_2O): δ 175.62, 169.41, 160.66, 150.72, 146.88, 135.14, 131.06, 129.29, 123.44, 122.84, 107.12, 56.86, 55.67, 54.90, 53.81, 52.85, 51.33, 49.03, 48.71. HRMS (ESI$^+$): Calcd for $C_{25}H_{36}N_5O_7^+$: 518.2615 (M + H^+), found: 518.2637 (M + H^+).

6-*N*-Acetylamino-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (7e). Reaction of 6-*N*-acetylamino-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**6e**) (0.463 g, 0.649 mmol) with TFA was performed according to the general procedure. Purification of the product by reversed-phase HPLC (a 40-min linear gradient, from 0% to 80% solvent B (solvent A, H_2O, 0.1% TFA; solvent B, acetonitrile-H_2O (80 : 20), 0.1% TFA)) gave **7e** (339 mg, 0.622 mmol, 96% yield) as a pale yellow solid. ^{1}H-NMR (300 MHz, D_2O): δ 8.82 (1H, d, *J* = 8.6 Hz, Ar*H*), 8.33 (1H, d, *J* = 2.2 Hz, Ar*H*), 8.19 (1H, d, *J* = 9.2 Hz, Ar*H*), 8.00 (1H, dd, *J* = 2.2, 9.2 Hz, Ar*H*), 7.94 (1H, d, *J* = 8.6, Ar*H*), 4.17 (2H, s, ArCH_2N), 2.79-3.69 (22H, m, ring and CH_2COO), 2.14 (3H, s, COCH_3). ^{13}C-NMR (75 MHz, D_2O): δ 175.48, 173.80, 169.58, 152.75, 147.83, 139.51, 136.27, 130.17, 129.82, 123.54, 122.40, 117.10, 55.99, 55.10, 53.87, 52.74, 51.28, 49.13, 48.74, 24.06. HRMS (ESI$^+$): Calcd for $C_{26}H_{37}N_6O_7^+$: 545.2724 (M + H^+), found: 545.2707 (M + H^+).

General procedure for the synthesis of europium(III) salt of 2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinolines (8a-e). The complexation and purificaton procedures followed the reported protocol with some modifications.[SR3-6] Briefly, a 2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**7a-e**) (1 equiv.) was dissolved in 27 ml of distilled water. To this solution, 0.1 M aqueous NaOH was added until pH 7 was reached.

An aqueous solution (3.0 ml) of $EuCl_3$-$6H_2O$ (1.1 equiv.) was then added slowly. The mixture was heated at 70 ˚C for 1 h under Ar, and cooled to room temperature. To this solution, 0.1 M aqueous NaOH was added until pH 7 was reached. The mixture was evaporated to dryness. The residue was dissolved in distilled water (2 ml), and the product was precipitated by the addition of tetrahydrofuran (THF), washed with THF and dried under reduced pressure. The residue was further purified by reversed-phase chromatography (Chromatorex-ODS, Fuji Silysia Chemical Ltd.) (from H_2O to 1 : 1 methanol / H_2O as eluent) to give **(8a-e)**.

Europium(III) salt of 2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (8a). Reaction of 2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**7a**) (50.0 mg, 0.103 mmol) with $EuCl_3$-$6H_2O$ (41.4 mg, 0.113 mmol) was performed according to the general procedure. Yield: 27.0 mg, 0.0424 mmol as a colorless solid (41%). Mp > 327 ˚C (decomp.). IR (KBr): 3409 (broad), 2984, 2866, 1603, 1391, 1321, 1084, 937, 835, 714 cm^{-1}. HPLC analysis: retention time, 10.2 min (purity, 98.7% integrated intensity); Inertsil ODS-3 4.6 mm × 250 mm (Gl Sciences); eluent, a 20-min linear gradient, from 0% to 80% solvent B (solvent A, 0.1 M triethylammonium acetate (pH 6.5); solvent B, acetonitrile / H_2O = 4 : 1); flow rate, 1.0 ml/min; UV, 300 nm. HRMS (ESI^+): Calcd for $C_{24}H_{31}N_5O_6Eu^+$: 638.1487 ($M + H^+$), found: 638.1533 ($M + H^+$).

Europium(III) salt of 6-Fluoro-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (8b). Reaction of 6-fluoro-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**7b**) (120 mg, 0.237 mmol) with $EuCl_3$-$6H_2O$ (95.7 mg, 0.261 mmol) was performed according to the general procedure. Yield: 114 mg, 0.174 mmol as a colorless solid (73%). Mp > 332 ˚C (decomp.). IR (KBr): 3412 (broad), 2986, 2868, 1607, 1514, 1387, 1321, 1227, 1084, 936, 835, 718 cm^{-1}. HPLC analysis: retention time, 10.5 min (purity, 99.3% integrated intensity); Inertsil ODS-3 4.6 mm × 250 mm (Gl Sciences); eluent, a 20-min linear gradient, from 0% to 80% solvent B (solvent A, 0.1 M triethylammonium acetate (pH 6.5); solvent B, acetonitrile / H_2O = 4 : 1); flow rate, 1.0 ml/min; UV, 300 nm. HRMS (ESI^+): Calcd for $C_{24}H_{30}FN_5O_6Eu^+$: 656.1392 ($M + H^+$), found: 656.1441 ($M + H^+$).

Europium(III) salt of

6-Methyl-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (8c).

Reaction of 6-methyl-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**7c**) (140 mg, 0.279 mmol) with $EuCl_3$-$6H_2O$ (112.5 mg, 0.307 mmol) was performed according to the general procedure. Yield: 121 mg, 0.186 mmol as a colorless solid (67%). Mp > 332 ˚C (decomp.). IR (KBr): 3405 (broad), 2986, 2866, 1605, 1385, 1321, 1084, 936, 839, 718 cm^{-1}. HPLC analysis: retention time, 9.0 min (purity, 99.6% integrated intensity); Inertsil ODS-3 4.6 mm × 250 mm (Gl Sciences); eluent, a 20-min linear gradient, from 0% to 80% solvent B (solvent A, 0.1 M triethylammonium acetate (pH 6.5); solvent B, acetonitrile / H_2O = 4 : 1); flow rate, 1.0 ml/min; UV, 300 nm. HRMS (ESI^+): Calcd for $C_{25}H_{33}N_5O_6Eu^+$: 652.1643 ($M + H^+$), found: 652.1607 ($M + H^+$).

Europium(III) salt of

6-Methoxy-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (8d).

Reaction of 6-methoxy-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**7d**) (140 mg, 0.270 mmol) with $EuCl_3$-$6H_2O$ (119 mg, 0.297 mmol) was performed according to the general procedure. Yield: 130 mg, 0.195 mmol as a colorless solid (72%). Mp > 337 ˚C (decomp.). IR (KBr): 3407 (broad), 2982, 2863, 1607, 1383, 1319, 1235, 1084, 937, 837, 716 cm^{-1}. HPLC analysis: retention time, 11.9 min (purity, 98.6% integrated intensity); Inertsil ODS-3 4.6 mm × 250 mm (Gl Sciences); eluent, a 20-min linear gradient, from 0% to 80% solvent B (solvent A, 0.1 M triethylammonium acetate (pH 6.5); solvent B, acetonitrile / H_2O = 4 : 1); flow rate, 1.0 ml/min; UV, 300 nm. HRMS (ESI^+): Calcd for $C_{25}H_{33}N_5O_7Eu^+$: 668.1592 ($M + H^+$), found: 668.1608 ($M + H^+$).

Europium(III) salt of

6-*N*-Acetylamino-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (8e).

Reaction of 6-*N*-acetylamino-2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**7e**) (140 mg, 0.257 mmol) with $EuCl_3$-$6H_2O$ (103.6 mg, 0.283 mmol) was performed according to the general

procedure. Yield: 128 mg, 0.185 mmol as a pale yellow solid (72%). Mp > 337 ˚C (decomp.). IR (KBr): 3420 (broad), 2986, 2865, 1611, 1383, 1318, 1084, 937, 837, 720 cm^{-1}. HPLC analysis: retention time, 9.6 min (purity, 98.7% integrated intensity); Inertsil ODS-3 4.6 mm × 250 mm (GI Sciences); eluent, a 20-min linear gradient, from 0% to 80% solvent B (solvent A, 0.1 M triethylammonium acetate (pH 6.5); solvent B, acetonitrile / H_2O = 4 : 1); flow rate, 1.0 ml/min; UV, 300 nm. HRMS (ESI^+): Calcd for $C_{26}H_{33}N_6NaO_7Eu^+$: 717.1521 ($M + Na^+$), found: 717.1481 ($M + Na^+$).

6-*N*-Acetylamino-2-methylquinoline (2). To a solution of 6-amino-2-methylquinoline (**1**) (2.11 g, 13.3 mmol) in pyridine (50 ml) was added acetic anhydride (1.4 ml, 14.8 mmol). The resulting mixture was stirred for 1 h at room temperature and evaporated to dryness. The residue was purified by column chromatography on silica gel (ethyl acetate as eluent) to give **2** (2.60 g, 13.0 mmol, 98% yield) as a colorless solid. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.29 (1H, d, *J* = 2.4 Hz, Ar*H*), 8.01 (1H, d, *J* = 8.3 Hz, Ar*H*), 7.95 (1H, d, *J* = 9.0 Hz, Ar*H*), 7.49 (1H, dd, *J* = 2.4, 9.0 Hz, Ar*H*), 7.35 (1H, br s, CON*H*), 7.27 (1H, d, *J* = 8.3 Hz, Ar*H*), 2.72 (3H, s, ArCH_3), 2.25 (3H, s, NCOCH_3). ^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.53, 157.84, 144.79, 136.18, 135.88, 128.60, 126.96, 123.43, 122.55, 116.36, 24.89, 24.36. MS (ESI^+): *m/z* 201 ($M + H^+$).

***N*-[2-[4,7,10-Tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinolin-6-yl] carbamic acid benzyl ester (11).** *N*-(2-Chloromethylquinolin-6-yl)carbamic acid benzyl ester (**10**) was prepared according to the literature.[SR7] To a solution of *N*-(2-chloromethylquinolin-6-yl)carbamic acid benzyl ester (**10**) (0.50 g, 1.53 mmol) and hydrobromide salt of 1,4,7-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**9**) (911 mg, 1.53 mmol) in acetonitrile (110 ml) was added anhydrous Na_2CO_3 (0.649 g, 6.12 mmol). The resulting suspension was heated under reflux under Ar for 24 h, cooled to room temperature, filtered and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (100 ml), washed with H_2O (100 ml × 2) and brine (100 ml), dried over anhydrous K_2CO_3 and evaporated to dryness. The residue was purified by silica gel column chromatography (7 : 93 methanol / CH_2Cl_2 as eluent) to give **11** (824 mg, 1.02 mmol, 67% yield) as a colorless solid. ^{1}H NMR (300 MHz,

$CDCl_3$): δ 8.57 (1H, br s, N*H*), 8.15 (1H, s, Ar*H*-quinolyl), 8.06 (1H, d, *J* = 8.4 Hz, Ar*H*-quinolyl), 7.87 (1H, d, *J* = 9.0 Hz, Ar*H*-quinolyl), 7.78 (1H, d, *J* = 9.0 Hz, Ar*H*-quinolyl), 7.47 (2H, m, Ar*H*-benzyl), 7.30-7.39 (3H, m, Ar*H*-benzyl), 7.21 (1H, d, *J* = 8.4 Hz, Ar*H*-benzyl), 5.25 (2H, s, ArCH_2-benzyl), 2.35-3.09 (22H, m, ring and CH_2CO), 1.57 (9H, s, ^{t}*Bu*), 1.26 (18H, s, ^{t}*Bu*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.43, 171.39, 156.03, 153.99, 144.16, 137.06, 136.41, 136.06, 128.70, 127.90, 127.30, 127.26, 127.19, 123.45, 120.82, 113.78, 82.06, 81.77, 65.77, 59.92, 56.11, 53.21, 50.30 (broad), 27.63, 27.53. MS (ESI^+): *m/z* 805 ($M + H^+$).

6-Amino-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (12). To a solution of *N*-[2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinolin-6-yl] carbamic acid benzyl ester (**11**) (1.58 g, 1.96 mmol) in ethanol (120 ml) was added 10% palladium on activated carbon (88 mg). The reaction mixture was stirred under H_2 for 2 h at room temperature. The resulting mixture was filtered to remove the catalyst, and the filtrate was concentrated. Purification of the residue by column chromatography on silica gel (1 : 9 methanol / CH_2Cl_2 as eluent) gave **12** (1.06 g, 1.58 mmol, 81% yield) as a pale red solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (1H, d, *J* = 8.4 Hz, Ar*H*), 7.76 (1H, d, *J* = 9.0 Hz, Ar*H*), 7.14 (1H, d, *J* = 8.4 Hz, Ar*H*), 6.98 (1H, dd, *J* = 2.6, 9.0 Hz, Ar*H*), 6.92 (1H, d, *J* = 2.6 Hz, Ar*H*), 3.76 (2H, br s, NH_2), 2.37-3.11 (22H, m, ring and CH_2CO), 1.57 (9H, s, ^{t}*Bu*), 1.28 (18H, s, ^{t}*Bu*). ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.90, 171.77, 153.99, 145.26, 142.79, 134.73, 129.87, 128.87, 121.93, 121.32, 107.08, 82.36, 82.08, 60.18, 56.53, 55.87, 50.72, 28.04, 27.93. MS (ESI^+): *m/z* 671 ($M + H^+$).

6-Amino-2-[4,7,10-tris(carbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (13). Trifluoroacetic acid (TFA) (150 ml) was added dropwise to 6-amino-2-[4,7,10-tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**12**) (774 mg, 1.15 mmol) at 0 ˚C. The mixture was stirred at room temperature overnight and evaporated to dryness. The residue was dissolved in distilled water (50 ml) and evaporated to dryness three times. The residue was purified by reversed-phase HPLC. A 120-min linear gradient, from 0% to 80% solvent B (solvent

A, H_2O, 0.1% TFA; solvent B, acetonitrile-H_2O (80 : 20), 0.1% TFA) was used. This HPLC purification gave **13** (280 mg, 0.557 mmol, 48% yield) as a yellow solid. ^{1}H NMR (300 MHz, D_2O): δ 8.63 (1H, d, *J* = 9.3 Hz, Ar*H*), 8.08 (1H, d, *J* = 9.2 Hz, Ar*H*), 7.84 (1H, d, *J* = 9.2 Hz, Ar*H*), 7.64 (1H, d, *J* = 9.3 Hz, Ar*H*), 7.43 (1H, s, Ar*H*), 4.13 (2H, br s, ArC*H*$_2$N), 2.85-3.65 (22H, m, ring and C*H*$_2$CO). ^{13}C NMR (75 MHz, D_2O): δ 175.20, 169.83, 152.59, 146.58, 139.64, 137.01, 130.07, 129.99, 129.45, 123.95, 116.99, 55.86, 55.35, 53.99, 52.48, 51.13, 49.29, 48.94. HRMS (ESI$^+$): Calcd for $C_{24}H_{35}N_6O_6^+$: 503.2618 (M + H$^+$), found: 503.2653 (M + H$^+$).

Europium(III) salt of

6-amino-2-[4,7,10-tris(carbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (14). The complexation and purification procedures followed the reported protocol with some modifications.[SR3-6] Briefly, 6-amino-2-[4,7,10-tris(carbonylmethyl)-1,4,7,10-tetraazacyclododecan-1-yl]methylquinoline (**13**) (140 mg, 0.279 mmol) was dissolved in 27 ml of distilled water. To this solution, 0.1 M aqueous NaOH was added until pH 7 was reached. An aqueous solution (3.0 ml) of $EuCl_3$-6H_2O (112.2 mg, 0.306 mmol) was then added slowly. The mixture was heated at 70 ˚C for 1 h under Ar, and cooled to room temperature. To this solution, 0.1 M aqueous NaOH was added until pH 7 was reached. The mixture was evaporated to dryness. The residue was dissolved in distilled water (2 ml), and the product was precipitated by the addition of tetrahydrofuran (THF), washed with THF and dried under reduced pressure. The residue was further purified by reversed-phase chromatography (Chromatorex-ODS, Fuji Silysia Chemical Ltd.) (H_2O to 1 : 1 methanol / H_2O as eluent) to give **14** (121 mg, 0.186 mmol, 67% yield) as a pale yellow solid. Mp > 337 ˚C (decomp.). IR (KBr): 3418 (broad), 3229, 2988, 2861, 1607, 1508, 1387, 1321, 1084, 936, 839, 716 cm^{-1}. HPLC analysis: retention time, 9.7 min (purity, 98.8% integrated intensity); Inertsil ODS-3 4.6 mm × 250 mm (Gl Sciences); eluent, a 20-min linear gradient, from 0% to 80% solvent B (solvent A, 0.1 M triethylammonium acetate (pH 6.5); solvent B, acetonitrile / H_2O = 4 : 1); flow rate, 1.0 ml/min; UV, 350 nm. HRMS (ESI$^+$): Calcd for $C_{24}H_{32}N_6O_6Eu^+$: 653.1596 (M + H$^+$), found: 653.1624 (M + H$^+$).

Terbium (III) sodium salt of DTPA-cs124 ((Na$^+$)Tb^{3+}-DTPA-cs124). The complexation and purification

procedures followed the reported protocol with some modifications.[SR3-6] DTPA-cs124 chelator was prepared according to the reported procedure.[SR8] DTPA-cs124 chelator (100 mg, 0.182 mmol) was dissolved in 27 ml of distilled water. To this solution, 0.1 M aqueous NaOH was added until pH 7.0 was reached. An aqueous solution (3 ml) of $TbCl_3$-$6H_2O$ (68 mg, 0.182 mmol) was then added slowly, while a pH of 7 was maintained by addition of further aliquots of 0.1 M aqueous NaOH. The mixture was stirred for 1 h at room temperature and evaporated to dryness. The resulting residue was dissolved in distilled water and passed through an ion exchange column packed with Chelex 100 resin (BioRad) to remove any free metal. Eluent fractions containing the product were collected and further purified by reversed-phase chromatography (Chromatorex-ODS, Fuji Silysia Chemical Ltd.) (H_2O to 1 : 1 methanol / H_2O as eluent) to give $(Na^+)Tb^{3+}$-DTPA-cs124 (37 mg, 0.0509 mmol, 28% yield) as a colorless solid. IR (KBr): 3420 (broad), 2919, 1605, 1404, 1327, 1254, 1094, 995, 932, 868, 716 cm^{-1}. HPLC analysis: retention time, 8.7 min (purity, 97.1% integrated intensiry); Inertsil ODS-3 4.6 mm × 250 mm (Gl Sciences); eluent, a 20-min linear gradient, from 10% to 80% solvent B (solvent A, 0.1 M triethylammonium acetate (pH 6.5); solvent B, acetonitrile / H_2O = 4 : 1); flow rate, 1.0 ml/min; UV, 340 nm. HRMS (ESI^-): Calcd for $C_{24}H_{27}N_5O_{10}Tb^-$: 704.1011 ($M - Na^+$), found: 704.1007 ($M - Na^+$).

(References)

(SR1) Butera, J. A.; Spinelli, W.; Anantharaman, V.; Marcopulos, N.; Parsons, R. W.; Moubarak, I. F.; Cullinan, C.; Bagli, J. F. *J. Med. Chem.* **1991**, *34*, 3212-3228.

(SR2) Dadabhoy, A.; Faulkner, S.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 2* **2002**, 348-357.

(SR3) Hardcastle, K. I.; Botta, M.; Fasano, M.; Digilio, G. *Eur. J. Inorg. Chem.* **2000**, 971-977.

(SR4) Anelli, P. L.; Bertini, I.; Fragai, M.; Lattuada, L.; Luchinat, C.; Parigi, G. *Eur. J. Inorg. Chem.* **2000**, 625-630.

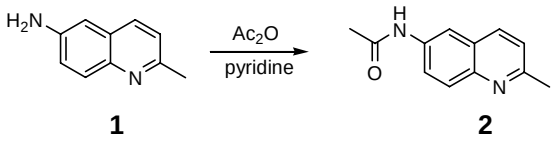
(SR5) Li, W.; Parigi, G.; Fragai, M.; Luchinat, C.; Meade, T. J. *Inorg. Chem.* **2002**, *41*, 4018-4024.

(SR6) Louie, A. Y.; Hüber, M. M.; Ahrens, E. T.; Rothbächer, U.; Moats, R.; Jacobs, R. E.; Fraser, S. E.; Meade, T. J. *Nat. Biotechnol.* **2000**, *18*, 321-325.

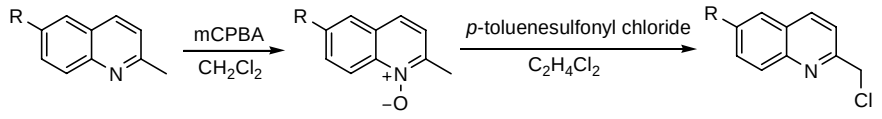
(SR7) Hanaoka, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 12470-12476.

(SR8) Li, M.; Selvin, P. R. *J. Am. Chem. Soc.* **1995**, *117*, 8132-8138.

Scheme S1. Synthetic scheme for luminescent Eu^{3+} complexes (R = H, F, CH_3, CH_3O, NHAc, NH_2 at the 6-position of the quinolyl sensitizing chromophore), and luminescent Tb^{3+} complex (Tb^{3+}-DTPA-cs124).



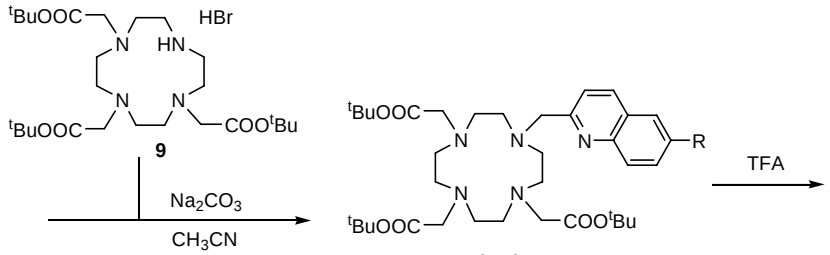
1 **2**



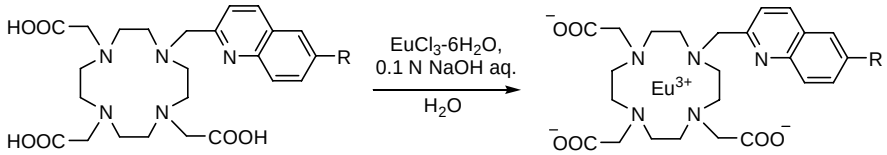
R: F (**3b**)
CH_3 (**3c**)
CH_3O (**3d**)
NHAc (**3e**)

F (**4b**)
CH_3 (**4c**)
CH_3O (**4d**)
NHAc (**4e**)

F (**5b**)
CH_3 (**5c**)
CH_3O (**5d**)
NHAc (**5e**)

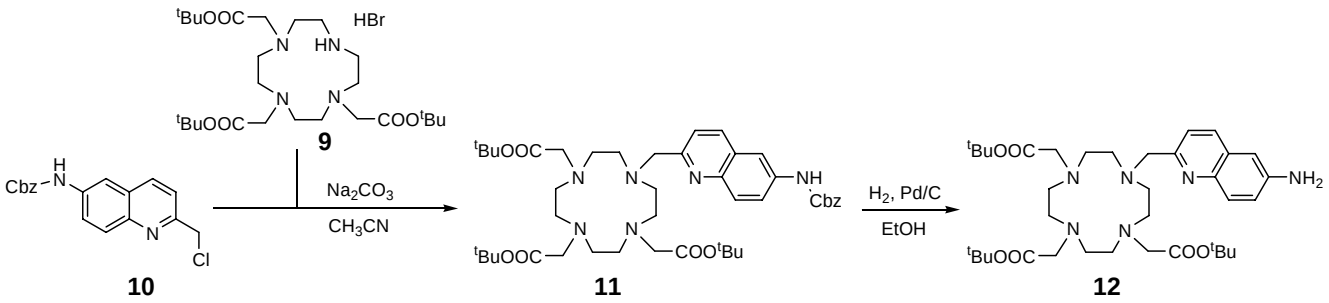


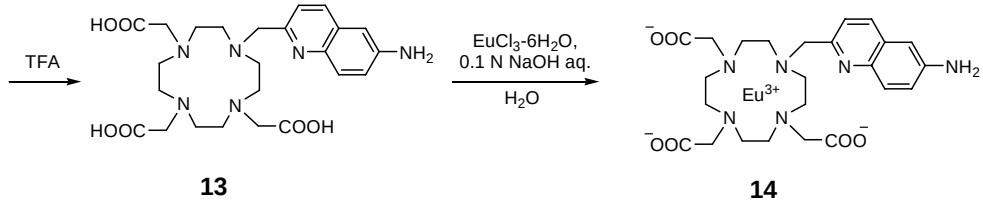
H (**6a**)
F (**6b**)
CH_3 (**6c**)
CH_3O (**6d**)
NHAc (**6e**)



H (**7a**)
F (**7b**)
CH_3 (**7c**)
CH_3O (**7d**)
NHAc (**7e**)

H (**8a**)
F (**8b**)
CH_3 (**8c**)
CH_3O (**8d**)
NHAc (**8e**)


tBuOOC
HBr
HN
N
COOtBu
9
Cbz
H
N
Cl
10
Na2CO3
CH3CN
NH
Cbz
11
H2, Pd/C
EtOH
NH2
12


TFA
HOOC
COOH
NH2
13
EuCl3-6H2O,
0.1 N NaOH aq.
H2O
−OOC
COO−
Eu3+
14

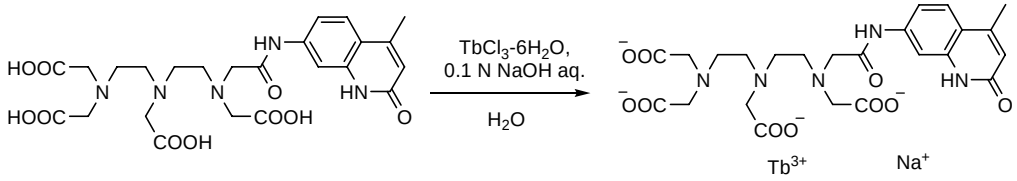

HOOC
COOH
HN
O
DTPA-cs124
TbCl3-6H2O,
0.1 N NaOH aq.
H2O
−OOC
COO−
Tb3+
Na+
Tb3+-DTPA-cs124

Figure S1. Photograph of 5 μM solutions of luminescent Eu^{3+} complexes (R = H, F, CH_3, CH_3O, NHAc, NH_2). Aqueous solutions except R = NH_2 are brightly luminescent upon excitation with a TLC plate reader lamp (312 nm). The pink luminescence seen in the photograph is typical of Eu^{3+}.

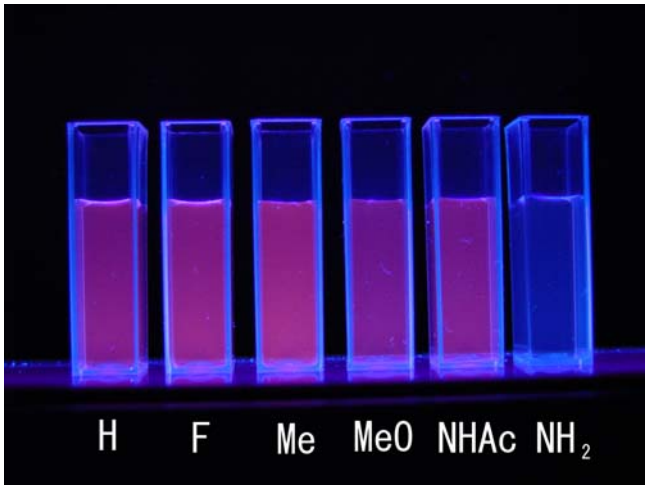


Figure S2. Photobleaching profiles of solutions of Eu^{3+} complex (R = NHAc or CH_3O) or fluorescein in 100 mM HEPES buffer (pH 7.4) during continuous illumination with a xenon lamp excitation source (325 nm (1.57 mW) for Eu^{3+} complexes or 492 nm (2.07 mW) for fluorescein): Eu^{3+} complex (R = NHAc) (■), Eu^{3+} complex (R = CH_3O) (●), fluorescein (▲). The wavelength of the irradiation light can be easily adjusted with this irradiation system.

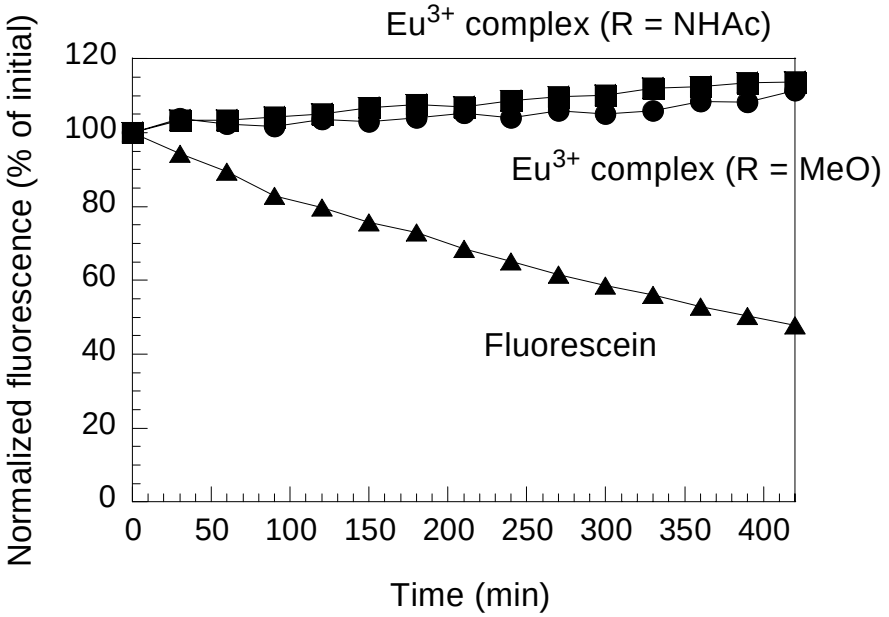
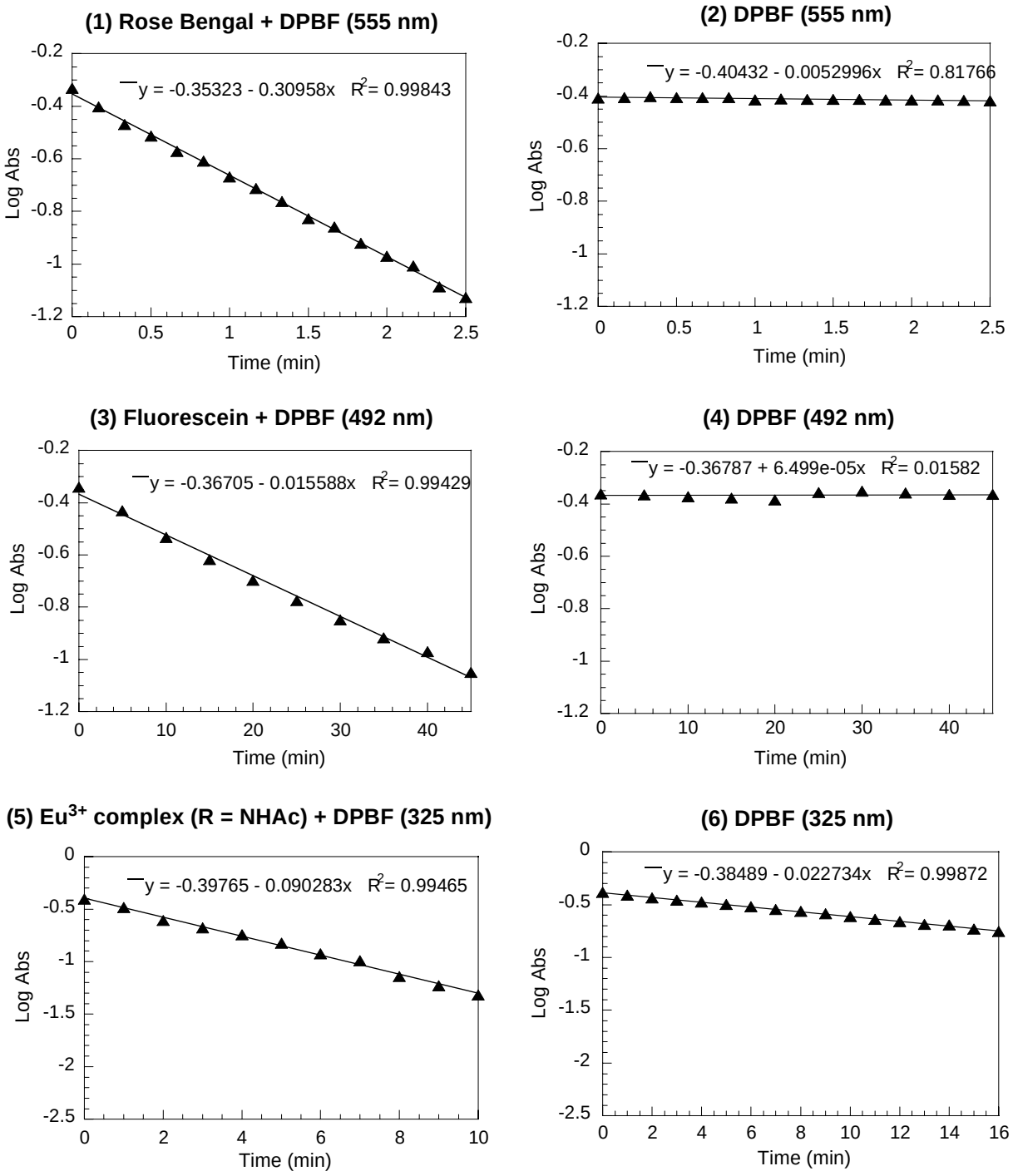
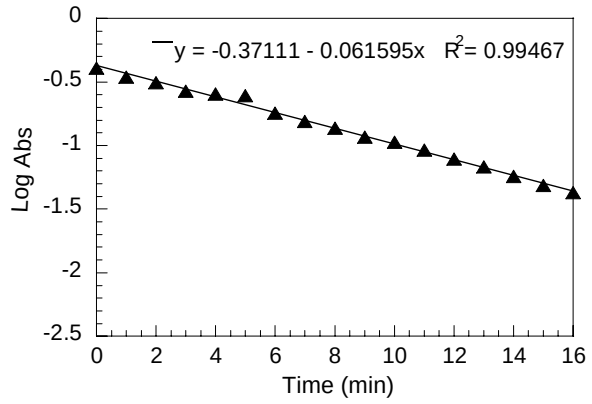


Figure S3. Comparative singlet oxygen generation plots of Rose Bengal (1 μM), fluorescein (1 μM) and Eu^{3+} complexes (R = NHAc and CH_3O) (100 μM). Rates of oxygenation of 1,3-diphenylisobenzofuran (DPBF) were estimated by comparison of the rates of consumption of DPBF at the initial stage of each experiment. Reaction of DPBF with singlet oxygen was monitored in terms of the reduction in intensity of the absorbance at 410 nm.



(7) Eu^{3+} complex (R = CH_3O) + DPBF (325 nm)



Relative Rates of Singlet Oxygen Generation = the rates of consumption of DPBF at the initial stage (min^{-1}) / the absorbance of the solution at the irradiation wavelength / the energy of the irradiation light (mW)

From **(1)** and **(2)**,

Relative rate (Rose Bengal) = (0.3096 – 0.0053) min^{-1} / 0.077 / 3.0 mW = 1.32 ($min^{-1} \cdot mW^{-1}$)

From **(3)** and **(4)**,

Relative rate (Fluorescein) = (0.0156 – 0.0000) min^{-1} / 0.068 / 3.09 mW = 0.0742 ($min^{-1} \cdot mW^{-1}$)

From **(5)** and **(6)**,

Relative rate (Eu^{3+} complex (R = NHAc)) = (0.0903 – 0.0227) min^{-1} / 0.402 / 2.55 mW = 0.0659 ($min^{-1} \cdot mW^{-1}$)

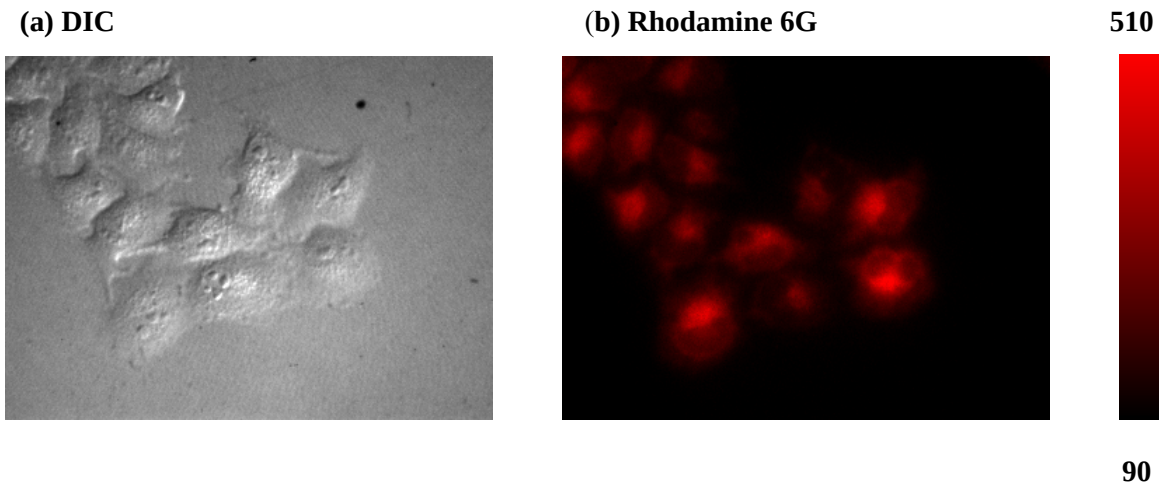
From **(7)** and **(6)**,

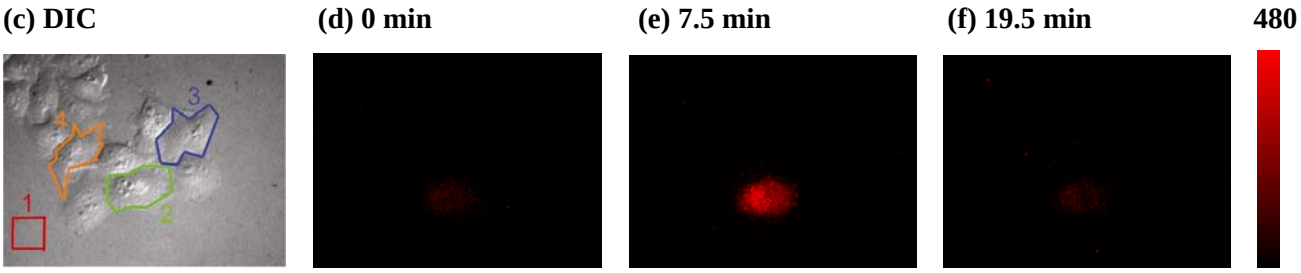
Relative rate (Eu^{3+} complex (R = CH_3O)) = (0.0616 – 0.0227) min^{-1} / 0.405 / 2.55 mW = 0.0377 ($min^{-1} \cdot mW^{-1}$)

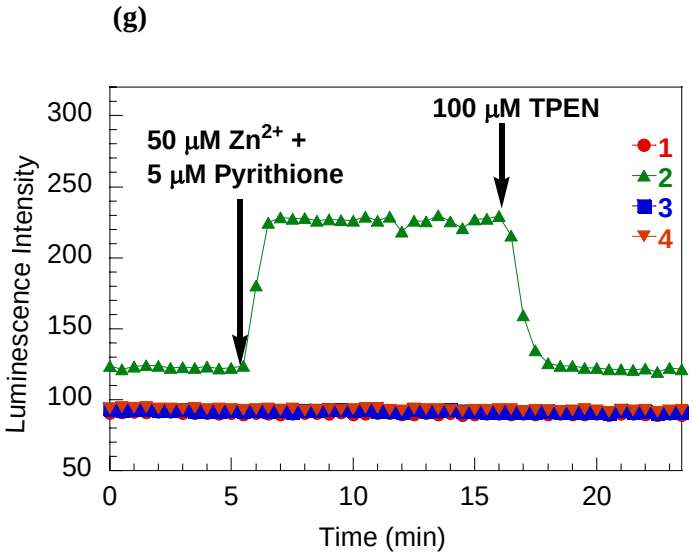
Relative Rates of Singlet Oxygen Generation:

Rose Bengal : Fluorescein : Eu^{3+} complex (R = NHAc) : Eu^{3+} complex (R = CH_3O) = **18** : **1** : **0.89** : **0.51**

Figure S4. Bright-field transmission images, prompt fluorescence images and time-resolved long-lived luminescence images of intracellular Zn^{2+} in HeLa cells which were stained with rhodamine 6G. The fluorescence at 617 ± 37 nm was measured with excitation at 360 ± 40 nm for both prompt fluorescence imaging and time-resolved long-lived luminescence imaging. The cells were cultured in DMEM supplemented with 10% fetal bovine serum, 1% penicillin and 1% streptomycin at 37 ˚C in a 5% CO_2/95% air incubator. The cells were washed with HBSS buffer twice and stained with rhodamine 6G for 30 min at room temperature. Then, they were washed with HBSS buffer twice and injected with **[Eu-7]** solution. (a) Bright-field transmission image (0 min). (b) Prompt fluorescence image of (a) without a time resolution process (0 min). This fluorescence is mainly derived from rhodamine 6G. (d) Time-resolved long-lived luminescence image of (a) (0 min). (e) Time-resolved long-lived luminescence image (7.5 min) following addition of 5 μM pyrithione (zinc ionophore) and 50 μM $ZnSO_4$ to the medium at 5.5 min. (f) Time-resolved long-lived luminescence image (19.5 min) following addition of 100 μM TPEN to the medium at 16 min. Time-resolved long-lived luminecence images (d-f) correspond to the luminescence intensity data in (g), which shows the average intensity of the corresponding area or cell area in (c) (1, extracellular region; 2, intracellular region of **[Eu-7]**-injected cell; 3, 4, intracellular regions of **[Eu-7]**-noninjected cells.).




(c) DIC
(d) 0 min
(e) 7.5 min
(f) 19.5 min
480
100
1
2
3


(g)
100 μM TPEN
50 μM Zn2+ +
5 μM Pyrithione
1
2
3
4
Luminescence Intensity
300
250
200
150
100
50
0
5
10
15
20
Time (min)