Supplementary Material (ESI) for Chemical Communications

Supporting Information for

A New Quinoline Sensitizer-Centered Lanthanide Chelate and Its Use for Protein Labling on Ni-NTA Beads for TR LRET Assays

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Materials and Methods

$POCl_3$, Cs124, 10% Pd/C, diBoc anhydride, pTsCl, NaN_3, DTPA dianhydride, trifluoroacetic acid, terbium(III) chloride hexahydrate, europium(III) chloride hexahydrate, and other solvent were purchased from Aldrich Inc, St. Louis. Maleimidopropionic acid NHS ester was purchased from PIERCE Inc. Melting points were determined with a Thomas melting point apparatus without correction. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Inova-500 spectrometer at 500 and 126 MHz with deuterated solvents. All NMR spectra used tetramethyl silane as a internal standard. MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time Of Flight) mass analysis (2,5-dihydroxybenzoic acid, DHB, as a matrix) and electrospray ionization mass spectra were obtained using Voyager-DE™ STR and a Q-TOF Ultima API (Waters Co. Ltd), respectively. Fluorescence experiments were performed using black 96-well microplates (Molecular Devices) on a Wallac Victor^2 V plate-reading fluorometer (Perkin Elmer). A 50-μs counting delay was used with a 1000-μs window and a 1500-μs counting cycle. All data were analyzed with Origin 7.5 (OriginLab Co., Massachusetts, USA).

Protein expression, purification, and labeling of ERα-417 with 9-Tb^{3+} or 9-Eu^{3+}, *and SRC3 with fluorescein*

N-terminally His-tagged constructs in pET15b plasmids for ERα-417 and SRC3 were prepared as done previously.[1] The ligand-binding domain of ERα-417 (amino acids 304-554), mutated to retain only a single reactive cysteine at C417, or the nuclear receptor domain of SRC3 encompassing three nuclear receptor (NR) boxes (amino acids 627-829) were transformed into *E. coli* BL21(DE3)pLysS, grown at 37 °C to OD_{600} ~0.5., induced with 1 mM IPTG, and grown for 4 hours at 28 °C according to previously reported protocols. [2]

To isolate and purify the proteins, a cell pellet was suspended in 5 mL of buffer (50 mM Tris buffer, pH 7.5, 10% glycerol, 0.1 mM TCEP) per gram of cell

paste. The resuspended pellet was sonicated (Vibra cell sonicator with a micro probe; Sonic Materials, Inc., Danbury, CT) for 10 s at 60% power to shear the DNA. The cell debris and pellet fraction was separated from the supernatant by centrifugation for 30 min at 30000*g*. This supernatant was purified to near-homogeneity by batchwise adsorption onto a nickel-charged nitrilotriacetic acid-agarose resin (Ni-NTA-agarose; Qiagen Inc, Santa Clarita, CA), following standard protocols. [3]

Site-specific labeling of the estrogen receptor was accomplished using 30:1 equivalents of a **9-Tb^{3+}** or **9-Eu^{3+}** while the purified His$_6$-tagged ERα-417 LBD was immobilized on the Ni-NTA resin. The SRC3-NRD was labeled in the same manner using the recommended equivalents of a thiol-reactive fluorescein derivative (5-iodoacetamidofluorescein, Invitrogen). These labeling reactions were incubated overnight at 4 °C in Tris-glycerol buffer (50 mM Tris pH 7.0, 10% glycerol, 0.1 mM TCEP). Excess fluorophore was removed by washing the protein-bound resin complex with wash buffer (50 mM Tris buffer, pH 7.5, 10% glycerol, and 10 mM mercaptoethanol) before eluting the labeled receptor.

1. Tamrazi, A.; Carlson, K. E.; Rodriguez, A. L.; Katzenellenbogen, J. A. *Mol Endocrinol* **2005,** 19, (6), 1516-28.
2. Carlson, K. E.; Choi, I.; Gee, A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *Biochemistry* **1997,** 36, (48), 14897-905.
3. Tamrazi, A.; Katzenellenbogen, J. A. *Methods Enzymol*. **2003**, *364*, 37.

Fluorescence Resonance Energy Transfer (FRET) Measurements

The buffer used throughout the assays (TR-FRET buffer) contained 50 mM Tris, pH 7.5, and 10% glycerol. Measurements were performed using black 96-well microplates (Molecular Devices) on a Wallac Victor^2 V plate-reading fluorometer (Perkin Elmer). A 50-μs counting delay was used with a 1000-μs window and a 1500-μs counting cycle. All FRET measurements were expressed as a ratio of the acceptor/donor and normalized. The resulting dose-reponse curves were analyzed and graphed using GraphPad 4.0 software.

SRC3-FI was titrated in a 1:10 fashion into Tris buffer and then diluted again 1:10 into TR-FRET buffer. 10 µL of each titration point were placed in 96-well black microplate. 10 µL of a pre-made mixture were added to each well to make a final assay volume of 20 µL with final concentrations as follows: ERα-417 (5 nM), and ligand (1 µM). The mixture was allowed to incubate at room termperature for 20 minutes before reading. The terbium chelate was excited using a 340/20 nm filter and the emissions of the terbium donor and the fluorescein acceptor were measured at 488/20 and 525/20 nm, respectively.

Life time measurements

Time-gated luminescence measurements were performed with laboratory-built spectrometer described previously, adopting a 5 ns excitation pulse at 337 nm followed by time-gated detection of lanthanide emission.[1]

1. Xao, M. and Selvin, P. R. *Rev. Sci. Instrum.* **1999**, *70*, 3877.

MALDI-TOF spectrum of Tb^{3+} or Eu^{3+} chelate labeled ERα

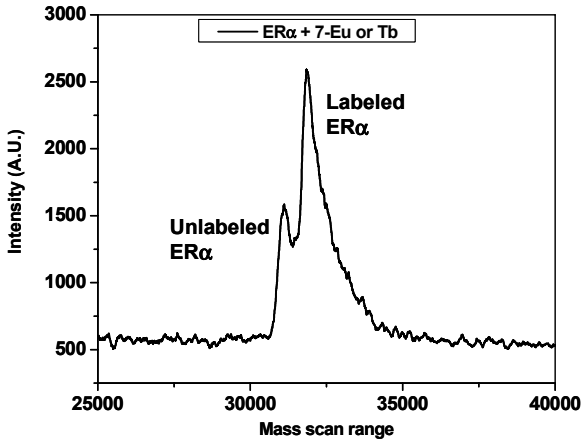
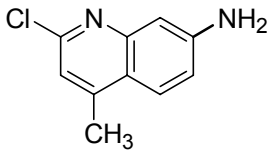


Figure 1. MALDI-TOF spectra of **9-Tb^{3+} or 9-Eu^{3+}** labeled ERα**.** (Sample for MALDI-TOF were taken directly from the NTA-bead at the end of labeling process.)

Chemical Synthesis

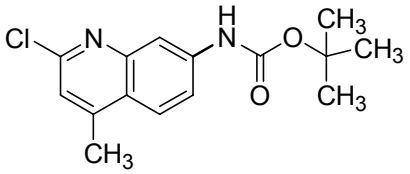
2-Chloro-4-methyl-7-aminoqunoline



A mixture of Cs124 (1.74 g, 10 mmol) and $POCl_3$ (10 ml) was refluxed until Cs124 was dissolved into $POCl_3$. The excess $POCl_3$ was removed under reduced pressure using a rotary evaporator. The pH of a residue was carefully adjusted to pH 7 with conc. NH_4OH solution. At pH 7, a pale yellowish solid precipitated; it was collected by filtration and dried to give 1.66 g (86%) of the desired product as a yellowish powder.

Mp 65-66 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.56 (s, 3H), 4.11 (s, 2H, NH_2), 6.93-6.97 (m, 2H, C3 and C6), 7.09 (d, J = 2.4 Hz, 1H, C8), 7.71 (d, J = 8.8 Hz, 1H, C5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.68, 109.48, 118.28, 119.29, 120.71, 125.28, 147.56, 148.61, 149.82, 151.07; LRMS (ESI) m/z 193.1 (M^++1, 100%) 195.1 (30%); HRMS (ESI) Calcd. for $C_{10}H_{10}N_2Cl$ (M^++1) 193.0533, found 193.0528.

*[2-(2-chloro-4-methyl-quinoline-7-yl)]-carbamic acid t-butyl ester (**2**)*

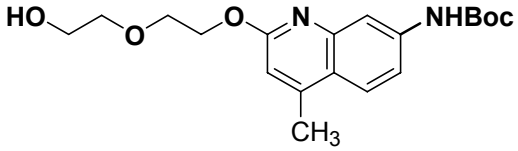


To the solution of amino quinoline compound (1.00 g, 5.21 mmol) in pyridine (10 ml) was added diBoc anhydride (1.20 g, 5.50 mmol). After stirring for 1 hr at rt, solvent was evaporated under reduced pressure to produce the desired product as yellowish solid (1.50 g, 100%).

Mp 73-74 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H), 2.61 (s, 3H), 6.90-7.05 (m, 1H), 7.08 (s, 1H), 7.73 (s, 1H), 7.80-7.90 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.73, 28.52, 81.39, 115.40, 119.54, 121.23, 123.28, 124.91, 140.71, 147.64, 148.62, 151.45, 152.69; MS (ESI) m/z 137.2 (M^++1) (100); LRMS (ESI)

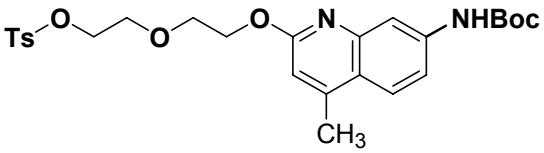
m/z 293.1 (M^++1, 100%), 295.1 (30%); HRMS (ESI) Calcd. for $C_{15}H_{18}N_2O_2Cl$ (M^++1) 293.1057, found 293.1064.

*{2-[2-(Hydroxy-ethoxy)-ethoxy]-4-methyl-quinoline-7-yl}-carbamic acid t-butyl ester (**3**)*



To the solution of **2** (1.00 g, 3.41 mmol) in diethylene glycol (20 ml) was added 240-mg lump of sodium. This solution was stirred for overnight at 80 °C and then diluted with 100 ml DI water. After collection of the precipitate by filtration and drying, a colorless solid was obtained. This mixture was dissolved in methanol (50 ml). To this solution was added diBoc anhydride (650 mg, 3.0 mmol). After stirring for one hour, solvent was removed under reduced pressure, and the residue was loaded on a silica gel column. The excess diBoc anhydride was removed by elution with n-hexane-ethyl acetate (1:1, v/v). Elution with ethyl acetate afforded the desired product as a sticky liquid (1.02 g, 83 %).
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.54 (s, 9H), 2.54 (d, 3H, J = 1.0 Hz), 3.67 (t, 2H, J = 5.0 Hz), 3.75 (t, 2H, J = 5.0 Hz), 3.89 (t, 2H, J = 5.) Hz), 4.61 (t, 2H, J = 5.0 Hz), 6.65 (d, 1H, J = 1.0 Hz), 6.78 (s, 1H), 7.43 (td, 1H, J = 9.0, 2.0 Hz), 7.74 (d, 1H, J = 9.0 Hz), 7.80 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 18.73, 28.54, 52.85, 64.78, 69.97, 70.18, 80.91, 111.79, 115.00, 116.35, 121.72, 124.57, 139.62, 146.71, 147.55, 152.91, 162.30; MS (ESI) 363.2 (M^++1); HRMS (ESI) Calcd. for $C_{19}H_{27}N_2O_5$ (M^++1) 363.1920, found 363.1916.

*{2-[2-(p-Toluenesulfonyl-ethoxy)-ethoxy]-4-methyl-quinoline-7-yl}-carbamic acid t-butyl ester (**4**)*

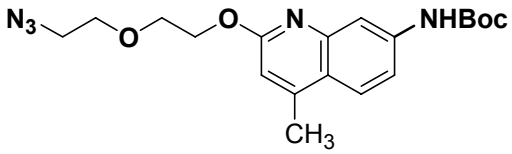


To alcohol **3** (150.0 mg, 0.41 mmol) in pyridine (5 ml) was added p-toluenesulfonyl chloride (94.5 mg, 0.50 mmol) at -20 °C. After stirring for 30 min, the reaction mixture warmed to room temperature and stirred for and additional 2 hr. Solvent was concentrated under vacuum and poured into 0.5 N HCl water. The suspended solid was extracted with ethyl acetate (20 ml x 3) and washed 0.5 N HCl (10 ml x 4). The extract was dried over $MgSO_4$ and evaporation gave the desired product as a colorless solid (203 mg, 96%).

Mp 55-56 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.54 (s, 9H), 2.37 (s, 3H), 2.54 (d, 3H, J = 1.0 Hz), 3.73 (t, 2H, J = 5.0 Hz), 3.78 (t, 2H, J = 5.0 Hz), 4.19 (t, 2H, J = 5.) Hz), 4.50 (t, 2H, J = 5.0 Hz), 6.63 (d, 1H, J = 1.0 Hz), 6.77 (s, 1H), 7.26 (d, 2H, J = 8.5 Hz), 7.41 (td, 1H, J = 9.0, 2.0 Hz), 7.73 (d, 1H, J = 9.0 Hz), 7.78 (d, 2H, J = 8.5 Hz), 7.81 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 18.75, 21.78, 28.54, 64.76, 68.82, 69.48, 70.10, 80.99, 111.76, 114.95, 116.34, 121.74, 124.62, 128.21, 130.02, 133.14, 139.60, 145.04, 146.75, 147.56, 152.83, 162.26; MS (ESI) 517.2 (M^++1); HRMS (ESI) Calcd. for $C_{26}H_{33}N_2O_7S$ (M^++1) 517.2008, found 517.2018.

*{2-[2-(Amino-ethoxy)-ethoxy]-4-methyl-quinoline-7-yl}-carbamic acid t-butyl ester (**5**)*

1. {2-[2-(Azido-ethoxy)-ethoxy]-4-methyl-quinoline-7-yl}-carbamic acid t-butyl ester

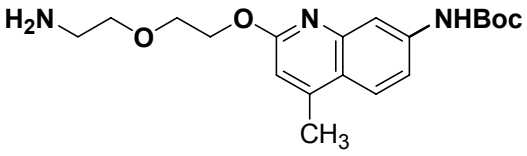


The mixture of tosylate **4** (120 mg, 0.23 mmol) and sodium azide (200 mg, 3.1 mmol) in DMF (1 ml) was stirred at 60 ^{o}C for 4 hr. When tosylate was no longer detected on TLC, water (10 ml) was added to the reaction mixture. Extraction with ethyl acetate (5 ml x 3), drying over $MgSO_4$, and evaporation afforded the desired product as a sticky liquid (85 mg, 96%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.52 (s, 9H), 2.50 (s, 3H), 3.38 (t, 2H, J = 5.0 Hz), 3.71 (t, 2H, J = 5.0 Hz), 3.87 (t, 2H, J = 5.) Hz), 4.59 (t, 2H, J = 5.0 Hz), 6.63 (s, 1H), 6.93 (s, 1H), 7.38 (td, 1H, J = 9.0, 2.0 Hz), 7.69 (d, 1H, J = 9.0 Hz), 7.83 (s,

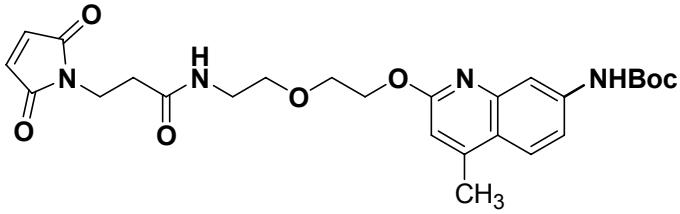
1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 18.71, 28.52, 50.86, 64.79, 69.99, 70.15, 80.90, 111.79, 115.00, 116.35, 121.72, 124.57, 139.62, 146.71, 147.55, 152.91, 162.30. MS (ESI) 388.2 (M^++1); HRMS (ESI) Calcd. for $C_{19}H_{26}N_5O_4$ (M^++1) 388.1985, found 388.1996.

*2. {2-[2-(Amino-ethoxy)-ethoxy]-4-methyl-quinoline-7-yl}-carbamic acid t-butyl ester (**5**)*



To the azide compound prepared above (80 mg, 0.21 mmol) in methanol (10 ml) was added 5 mg 10% Pd on carbon. The mixture was agitated under 30 psi hydrogen pressure using Parr shaker at rt for 4 hr. The reaction mixture was passed through Celite to remove Pd on carbon. Evaporation of solvent afforded the desired product as an off-white solid powder (74 mg, 100 %).
Mp 64-65 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.50 (s, 9H), 2.45 (s, 3H), 3.06 (t, 2H, J = 5.0 Hz), 3.68 (t, 2H, J = 5.0 Hz), 3.79 (t, 2H, J = 5.) Hz), 4.50 (t, 2H, J = 5.0 Hz), 5.56 (s, 3H), 6.56 (d, 1H, J = 1.0 Hz), 7.46 (td, 1H, J = 9.0, 1.5 Hz), 7.63 (d, 1H, J = 9.0 Hz), 7.72 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 18.67, 28.52, 40.64, 53.67, 64.82, 69.76, 80.80, 111.55, 114.84, 116.45, 121.62, 124.51, 139.87, 146.91, 147.30, 153.05, 162.20. MS (ESI) 362.2 (M^++1); HRMS (ESI) Calcd. for $C_{19}H_{28}N_3O_4$ (M^++1) 362.2080, found 362.2086.

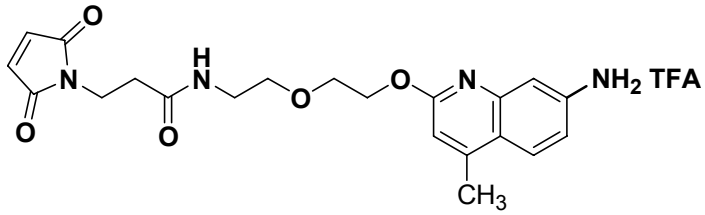
{2-[2-(3-Maleimidopropionyl-ethoxy)-ethoxy]-4-methyl-quinoline-7-yl}-carbamic acid t-butyl ester



The mixture of amine compound **5** (20 mg, 0.055 mmol), maleimidopropionic acid N-hydroxysuccinicimide ester (18 mg, 0.066 mmol), and diisopropyl ethyl amine (20 µl) in DMF (1 ml) at rt was stirred and monitored by TLC until the

amine spot disappeared. The reaction mixture was concentrated under reduced pressure and poured into water (5 ml). After extraction with ethyl acetate (5 ml x 3), the extract was washed with sat. aqueous $NaHCO_3$ solution, dried over $MgSO_4$, and evaporated to afford 24 mg of the desired product as an off-white solid that was sufficiently pure to use directly in the next reaction.
Mp 69-70 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.54 (s, 9H), 2.43 (t, 2H, J = 9.0 Hz), 2.55 (s, 3H), 3.43 (q, 2H, J = 7.0 Hz), 3.59 (t, 2H, J = 7.0 Hz), 3.79 (t, 2H, J = 9.0) Hz), 3.83 (t, 2H, J = 5.0 Hz), 4.59 (t, 2H, J = 5.0 Hz), 6.06 (s, 1H, CONH), 6.65 (s, 1H), 6.66 (s, 2H), 6.82 (s, 1H), 7.41 (td, 1H, J = 9.0, 1.5 Hz), 7.73 (d, 1H, J = 9.0 Hz), 7.82 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 18.82, 28.57, 34.44, 34.81, 39.45, 64.68, 69.75, 69.79, 81.05, 111.68, 114.88, 116.38, 121.74, 124.66, 134.39, 139.67, 146.95, 147.51, 152.82, 162.30, 169.86, 170.70. MS (ESI) 513.2 (M^++1); HRMS (ESI) Calcd. for $C_{26}H_{33}N_4O_7$ (M^++1) 513.2349, found 513.2343.

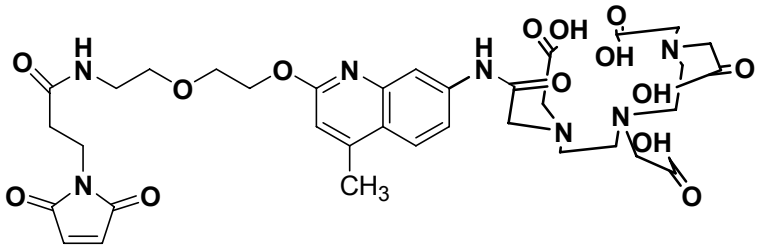
*2-[2-(3-Maleimidopropionyl-ethoxy)-ethoxy]-4-methyl-7-amino-quinoline TFA salt (**6**)*



The Boc-protected compound prepared above (15 mg, 0.03 mmol) was treated with 5 ml 50% TFA-CH_2Cl_2 for 1 hr at rt. Solvent was evaporated under reduced pressure, and diethyl ether was added to form precipitate. The collected solid was dissolved in dichloromethane, and solvent was evaporated again to produce the desired product as an off-white solid, which was dried under vacuum for 10 hr.
Mp 94-95 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.54 (t, 2H, J = 7.0 Hz), 2.66 (s, 3H), 3.45 (q, 2H, J = 5.0 Hz), 3.64 (t, 2H, J = 5.0 Hz), 3.74 (t, 2H, J = 7.0) Hz), 3.89 (t, 2H, J = 5.0 Hz), 4.60 (t, 2H, J = 5.0 Hz), 6.62 and 6.63 (s, s, 1H, 1H), 6.92 (d, 1H, J = 9.0 Hz), 7.10 (s, 1H), 7.52 (s, 1H), 7.67 (dd, 1H, J = 9.0, 1.5 Hz) 8.00-8.60 (s, 3H, NH3); ^{13}C NMR (126 MHz, $CDCl_3$) δ 20.03, 34.47, 34.55, 39.45,

68.45, 69.84, 70.79, 98.95, 103.33, 114.69, 116.62, 118.16, 126.64, 134.35, 139.53, 153.39, 158.34, 159.81, 160.88, 170.75, 172.29. MS (ESI) 413.2 (M^++1); HRMS (ESI) Calcd. for $C_{21}H_{25}N_4O_5$ (M^++1) 413.1825, found 413.1837.

*7-{[(7,7,4,1-tetraacetic acid)-1,4,7-triaza-heptanyl]-1-methylcarbonyl}amino-4-methyl-2-[(3-Maleimidopropionyl-ethoxy)-ethoxy]-quinoline (**7**)*



Amine-TFA salt **6** (2 mg, 3.8 μmol) was dissolved in anhydrous DMSO (0.5 ml). To this was added DTPA di-anhydride (4.1 mg, 11.4 μmol) and heated up to 60 ^oC. After stirring overnight, the reaction mixture was added dropwise to ethyl acetate (30 ml). The precipitate that formed was collected by filtration and then was suspended in water to remove the excess, unreacted DTPA that remained. After the water layer was decanted, the residue was dissolved into pH 7.0 $NaHCO_3$ water and loaded on a Sep-Pak C-18 Vac 6cc Cartridges (packed with 1 g C-18 silica gel in 6 cc syringe, Waters Co.) that had been preactivated with acetonitrile and washed again with water. The first elution with water gave additional DTPA. The second elution with 10% MeOH-water afforded, after evaporation of eluant, 2.1 mg hygroscopic powder. This reaction was also scaled up to 500 mg of **6** and 1.0 g of DTPA anhydride. Product (211 mg) was collected using C-18 reversed phase column chromatography (1.5 x 10 cm) with the 10% MeOH-water eluant.

Mp 111-112.5 °C ; ^1H NMR (500 MHz, D_2O) δ 2.12 (t, 2H, J = 8.0 Hz), 2.44 (s, 3H), 3.14 (t, 2H, J = 6.5 Hz), 3.15 (t, 2H, J = 5.0 Hz), 3.19, 3.27, and 3.32 (broad t, 6H), 3.38 (t, 2H, J = 6.5 Hz), 3.48 and 3.54 (broad t, 4H), 3.65 (s, 4H), 3.70-3.82 (m + s, 6H), 3.92 (s, 2H), 4.44 (s, 2H), 6.26 (s, 2H), 6.89 (s, 1H), 7.32 (d, 1H, J = 11.0), 7.65 (d, 1H, J = 11.0), 7.88 (s, 1H); ^{13}C NMR (126 MHz, D_2O) δ 16.87, 19.10, 34.49, 34.75, 39.13, 50.63, 51.87, 52.14, 54.68, 56.30, 57.44, 58.39, 58.90, 68.15, 69.00, 69.93, 108.47, 108.86, 119.40, 120.56, 126.31,

134.34, 138.23, 141.23, 157.92, 160.63, 168.39, 170.47, 172.32, 172.49, 173.05, 173.49. MS (ESI) 788.2 (M^++1); HRMS (ESI) cald. for $C_{35}H_{46}N_7O_{14}$ (M^++1) 788.3103, found 788.3098.

Formation of 7-{[(7,7,4,1-tetraacetic acid)-1,4,7-triaza-heptanyl]-1-methylcarbonyl}amino-4-methyl-2-[(3-Maleimidopropionyl-ethoxy)-ethoxy]-quinoline Terbium(III) or Europium(III) complex

To the DTPA derivatized quinoline **7** (1.5 mg, 1.9 μmol) in pH 7.0 0.5 M ammonium acetate buffer (0.3 ml) was added 2 ml (either 1 mM terbium(III) chloride hexahydrate or 1 mM europium(III) chloride hexahydrate). The reaction mixture was stirred for 4 hr at rt. The methanol was evaporated by rotary evaporatoration. Lyophilization of the residue afforded off-white powders that were used without further purification. Free DTPA was not detected by the mass spectrometric determination using ESI.
MS (ESI) Tb^{3+}-complex m/z 943.2 (M^++1, 100%); Eu^{3+}-complex m/z 935.2 (M^++1, 91%) and 937.2 (M^++3, 100%).