Cell Permeable Ln (III) Chelate Functionalized Quantum Dots as Multimodal Imaging Agents

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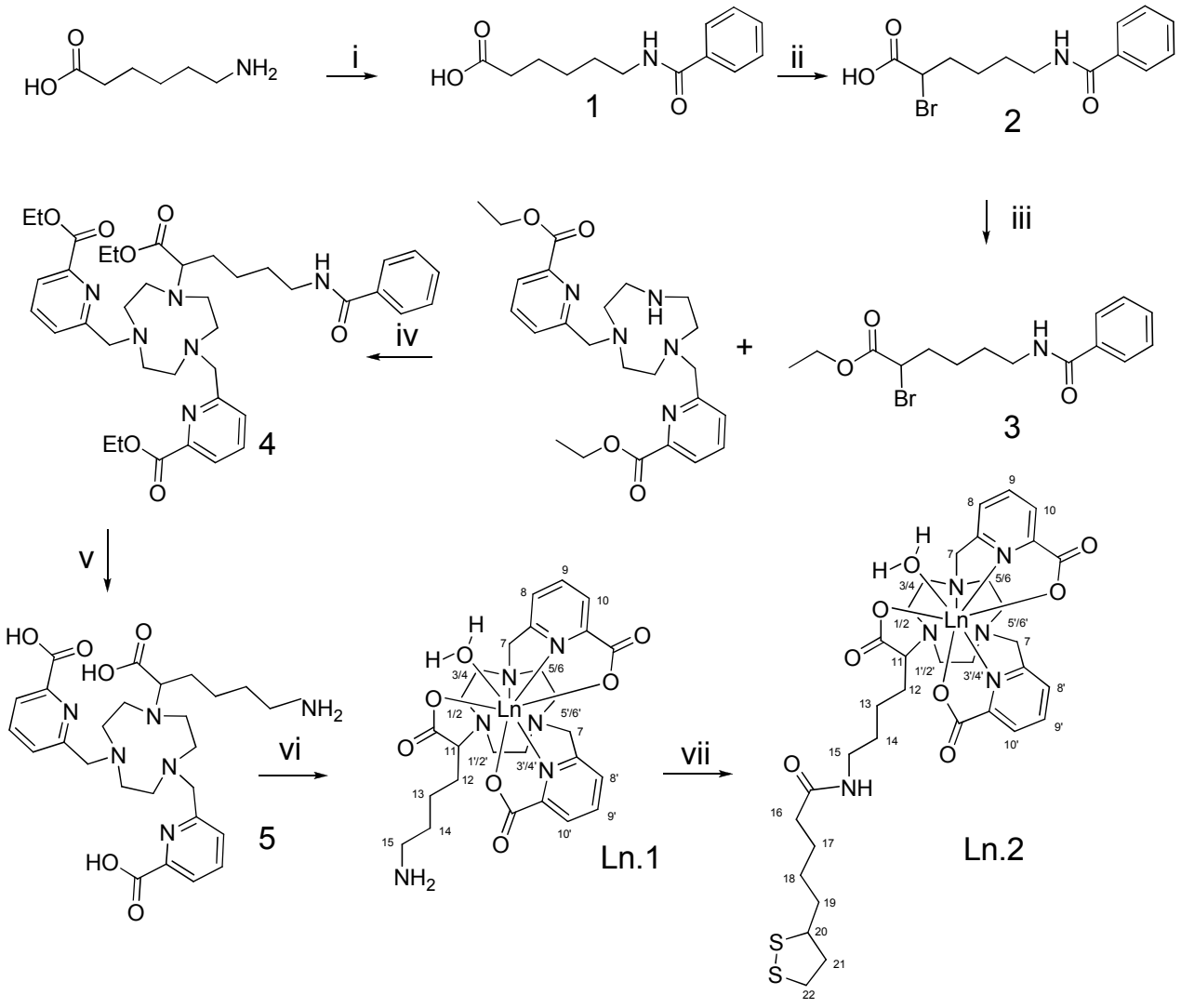
Ligand Synthesis



i) Benzoyl chloride, NaOH, H_2O. ii) Br_2, PBr_3, CCl_4. iii) EtOH, H_2SO_4. iv) K_2CO_3, KI, MeCN. v) HCl.
vi) $LnCl_3.6H_2O$, H_2O, pH 5.5. vii) DHLA, EDCI, DMAP, DMF/H_2O

Scheme S1. Synthesis of amino appended bptacn ligand

6-benzamidohexanoic acid(1)

A solution of NaOH (16.8 g, 0.419 moles) in water (17 mL) was added to a solution of 6-aminohexanoic acid (25.0 g, 0.191 moles) in of water (500 mL). The solution temperature was lowered to 10^{o}C and benzoyl chloride (29.5 g, 24.30 mL, 0.201 moles), was added dropwise. The desired product precipitated out of the solution as a white powder and was collected and washed 3 times with water

followed by 3 times with petroleum ether and then dried under vacuum overnight. Yield = 30.45 g, 68 %. δ_H (MeOD; 200 MHz, 298 K) 7.78 (m, 2H, H_{ar}), 7.45 (m, 3H, H_{ar}), 3.42 (t, $^3J_{HH}$ = 6.9 Hz, 2H, NHCH_2), 2.24 (t, $^3J_{HH}$ = 7.6 Hz,2H, CH_2CO_2H), 1.66 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$), 1.46 (m, 2H, $CH_2CH_2CH_2CH_2$); *m*/*z* (ESMS) $[M+H]^+$ 236.5

6-benzamido-2-bromohexanoic acid (2)

6-benzamidohexanoic acid (2.4 g, 0.010 moles) and phosphorous tribromide (3.03 g, 1.06 mL, 0.012 moles) were dissolved under argon in CCl_4 (20 mL), in a 250 mL three neck round bottom flask, equipped with a reflux condenser bearing a calcium chloride drying tube. Bromine (3.56 g, 1.15 mL, 0.022 moles), was added slowly in three portions to the reaction mixture over 30 minutes at 0°C. After this period the reaction was heated to reflux (85°C – 90°C) for 18 hours. After cooling, the reaction mixture was added to 10 mL of chloroform and then was poured carefully into approx 50 ml of methanol and then water. The solvents were evaporated to dryness and the crude mixture was dissolved again in 100 mL water. The solution was cooled to 0°C to afford a white precipitate which was collected and recrystallized from 20 mL of 5% water:ethanol solution at -85°C to yield the desired monohydrated carboxylic acid (2.15 g, yield 67%). δ_H (MeOD; 200 MHz, 298 K) 7.82 (m, 2H, H_{ar}), 7.52 (m, 3H, H_{ar}), 4.34 (t, $^3J_{HH}$ = 7.1 Hz, 1H, CHBrCO$_2$H), 3.43 (t, $^3J_{HH}$ = 6.0 Hz, 2H, NHCH_2), 2.07 (m, 2H, $CH_2CH_2CH_2CH_2$), 1.67 (m, 4H, $CH_2CH_2CH_2CH_2CH_2$); *m*/*z* (ESMS) $[M+H]^+$ = 314.5.

Ethyl 6-benzamido-2-bromohexanoate (3)

6-benzamido-2-bromohexanoic acid (1.35 g, 4.29 mmoles), was dissolved in ethanol (100 mL). H_2SO_4 (15 mL, 97%) was then added and the solution was heated to reflux for one hour. The solution was evaporated to dryness and the residue was extracted with 3 x 50 mL dichloromethane as a brown oil (.0.78 g, yield 77 %). δ_H ($CDCl_3$; 200 MHz, 298 K) 7.81 (m, 2H, H_{ar}), 7.51 (m, 3H, H_{ar}), 6.23 (broad s, 1H, NH) 4.25 (m, 3H, BrCH and CH_3CH_2O), 3.53 (q, J = 6.7 Hz, 2H, NHCH_2), 2.12 (bm, 2H, $CH_2CH_2CH_2CH_2$), 1.68 (bm, 4H, $CH_2CH_2CH_2CH_2CH_2$), 1.33 (t, J = 7.0 Hz 3H, CH_2CH_3); *m*/*z* (ESMS) $[M+H]^+$ 344.1.

Diethyl 6,6'-((7-(6-benzamido-1-ethoxy-1-oxohexan-2-yl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))dipicolinate (4)

Diethyl 6,6'-((1,4,7-triazacyclononane-1,4-diyl)bis(methylene))dipicolinate (310 mg, 0.687 mmoles), was dissolved in dry acetonitrile (20 mL) in the presence of dry K_2CO_3 (107 mg, 0.776 mmoles) under argon. A solution of ethyl 6-benzamido-2-bromohexanoate (266 mg, 0.776 mmoles) in dry acetonitrile (10 mL) was then added to the solution under argon and the reaction mixture was heated to reflux for 48 hours. Upon cooling, the mixture was filtered over celite to remove K_2CO_3, the solvent was evaporated and the residue was purified *via* silica column chromatography using graduated dichloromethane:methanol as eluent (graduated from 0% to 10 % ethanol). Brown oil = 251 mg, yield 51%. δ_H (CDCN; 200 MHz, 298 K) 7.89 (br m, 8H), 7.43 (br m, 3H), 4.35 (q, $^3J_{HH}$ = 7.1 Hz, 4H,CH_3CH_2O), 4.06 (q, $^3J_{HH}$ = 7.2 Hz, 2H, CH_3CH_2O), 3.79 (s, 4H, NCH_2C), 3.36 (q, $^3J_{HH}$ = 6.1 Hz, 2H, CH_2CH_2NH), 3.24 (t, $^3J_{HH}$ = 6.4 Hz, 1H, $NCHCH_2$), 2.82 (bm, ring 12H), 1.61 (bm, 6H $CH_2CH_2CH_2$), 1.35 (t, 3JHH 7.1 Hz, 6H, CH_2CH_3), 1.16 (t, $^3J_{HH}$ = 7.1 Hz, 3H CH_2CH_3); *m*/*z* (ESMS) $[M+H]^+$ 717.6.

6,6'-((7-(5-amino-1-carboxypentyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))dipicolinic acid (5)

Diethyl6,6'-((7-(6-benzamido-1-ethoxy-1-oxohexan-2-yl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))dipicolinate (200 mg, 0.279 mmoles) were dissolved in 6M HCl (30 mL) and the solution was heated at reflux for 24 hours. The solvents were removed and the residue was dissolved in water (20 ml). The pH was raised to 4.5 and the resulting aqueous solution was washed 3 times with 20 mL diethyl ether. δ_H (D_2O; 200 MHz, 298 K) 8.01 (t, $^3J_{HH}$ = 7.8 Hz 2H), 7.91 (d, $^3J_{HH}$ = 7.8, 2H), 7.62 (d, $^3J_{HH}$ = 7.9, 2H), 4.79 (s, 4H, NCH_2C), 3.73 (m, 13H, $NCHCH_2$ and *ring*), 2.84 (t, $^3J_{HH}$ = 7.1,Hz 2H, CH_2NH_2), 1.52 - 1.49 (broad m, 6H, $CH_2CH_2CH_2$); *m*/*z* (ESMS) $[M+H]^+$ 529.4, elemental analysis (%) calcd. for $C_{26}H_{41}Cl_5N_6O_6 \cdot 3H_2O$: C 40.82, H 6.19, N 10.99; found C 41.09, H 6.27, N 11.17.

Complex Synthesis

Ln 6,6'-((7-(5-amino-1-carboxypentyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))dipicolinic acid (Ln.1)

6,6'-((7-(5-amino-1-carboxypentyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))dipicolinic acid (0.076 mmoles) was dissolved in water (2mL) and the pH was adjusted to 5.5 with small aliquots of 1.0 M NaOH. $LnCl_3.6H_2O$ (0.076 mmoles) was dissolved in 1 ml of water at pH 5.5. The two solutions were combined and the pH re-adjusted to 5.5. The solution was then stirred for 30 minutes. The solvent was removed to give a hygroscopic white powder, this was purified on a Sephadex G25 resin (equilibrated with water) to remove inorganic salts. The resulting aliquots containing the complex were combined and the solvent removed, yielding the desired Ln.1 complexes as white hygroscopic solids in yield for Ln=Gd; 69% for Ln=Eu 79% and for Ln = Tb 75%.

Gd: *m*/*z* (ESMS) $[M+H]^+$ 684.3

Eu: *m*/*z* (ESMS) $[M+H]^+$ 679.31 H NMR (200 MHz, D2O, 278 K,): 25.7 (s, 1H; H1'/H2'), 14.89 (s, 1H; H7'), 9.15(s,1H; H8),8.89(s, 1H; H8'), 8.17 (s,1H; H9), 7.97 (s, 1H; H9'), 7.65 (s, 2H; H10/H10'), 5.44 (s, 1H; H7) , 3.72 (s, 1H; H11), 3.19 (s, 2H; H5'/H6'), 2.81 (s, 2H; H12), 2.41 (s, 2H; H7), 1.69 (s, 2H; H15), 1.36 (s, 2H; H13), 1.03 (s,1H; H7), 0.32 (s, 2H; H14), -0.86 (s, 1H; H3'/H4'), -1.16 (s, 1H; H5/H6), -2.35 (s, 1H; H3'/H4'), -4.18 (s, 1H; H7), -4.7 (s, 1H; H1/H2), -8.02 (s, 1H; H1'/H2'), -10.19 ppm (s, 1H; H5/H6), -13.48, (s, 1H; H3/H4) -15.05(s, 1H; H1/H2)

Tb:*m*/*z* (ESMS) $[M+H]^+$ 685.3

Ln 6,6'-((7-(13-12-dithiolanyl-5-pentaamido-1-carboxypentyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))dipicolinic acid (Ln.2)

Ln. 6,6'-((7-(5-amino-1-carboxypentyl)-1,4,7-triazacyclononane-1,4-diyl)bis(methylene))dipicolinic acid (0.073mmoles), thoctic acid (0.043 0.222 mmoles), EDCI (0.222 mmoles) and DMAP (0.222

mmoles) were dissolved in water:DMF (5 mL, 1:1). A precipitate formed, corresponding to the DMAP chloride salt. The solution was stirred for 18 hours and filtered. The volume was then concentrated to exactly 1 mL, the complex purified on a Sephadex G-25 size exclusion column, eluting with water. The aliquots containing the complex were combined as the desired Ln.2 complexes as a hygroscopic solids, in yield for Ln=Gd; 60% for Ln=Eu 59% and for Ln = Tb 61%. Lanthanide complexes are used without further purification;

Gd.2. *m*/*z* (ESMS) $[M+H]^+$ 874.3.

Eu.2: *m*/*z* (ESMS) $[M+H]^+$ 868.3. H NMR (200 MHz, D2O, 278 K,): 25.7 (s, 1H; H1'/H2'), 14.89 (s, 1H; H7'), 9.15(s,1H; H8),8.89(s, 1H; H8'), 8.17 (s,1H; H9), 7.97 (s, 1H; H9'), 7.65 (s, 2H; H10/H10'), 5.44 (s, 1H; H7) , 3.72 (s, 1H; H11), 3.19 (s, 2H; H5'/H6'), 2.94 (bm, 7H; H12, H17, H19, H20), 2.72 (bm, H16, H18), 2.41 (s, 2H; H7), 1.69 (s, 2H; H15), 1.36 (s, 2H; H13), 1.03 (s,1H; H7), 0.92 (bm, 4H, H21, H22), 0.32 (s, 2H; H14), -0.86 (s, 1H; H3'/H4'), -1.16 (s, 1H; H5/H6), -2.35 (s, 1H; H3'/H4'), -4.18 (s, 1H; H7), -4.7 (s, 1H; H1/H2), -8.02 (s, 1H; H1'/H2'), -10.19 ppm (s, 1H; H5/H6), -13.48, (s, 1H; H3/H4) -15.05(s, 1H; H1/H2)

Tb.2 *m*/*z* (ESMS) $[M+H]^+$ 875.3.

TEM image of Gd.2.QD

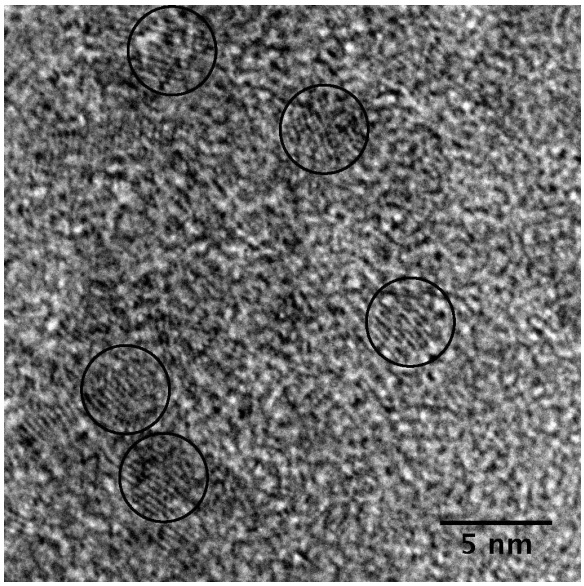


Figure S1: TEM images of Gd.2.QD. The circles drawn in the image show individual InP/ZnS Gd.2.QDs

Relaxivity measurements

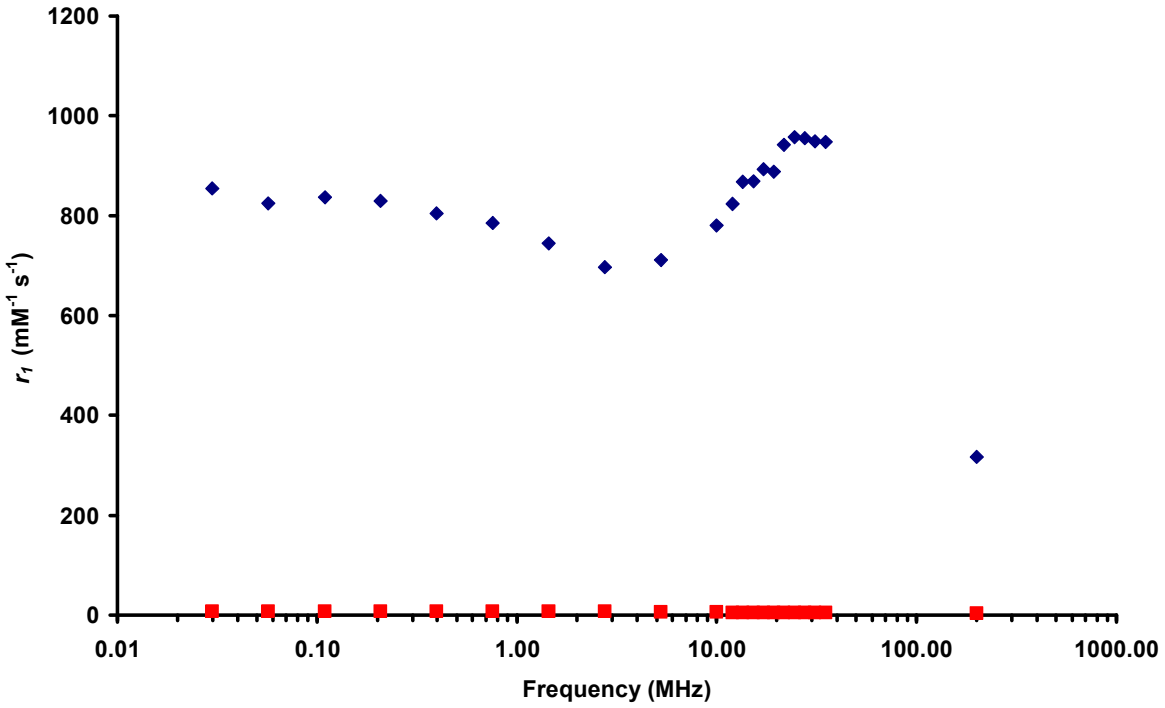


Figure S2: NMRD profile for Gd.2 (red squares) and Gd.2.QD (80 chelates) (Blue diamonds), relaxivity per object in H_2O at 298 K

Luminescence Spectroscopy

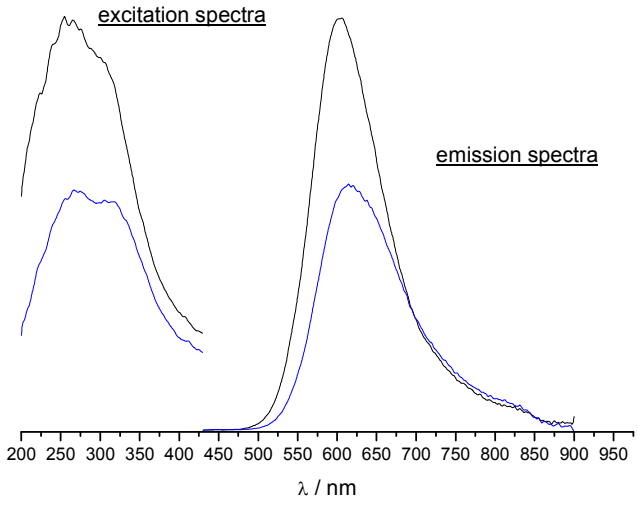


Figure S3: Excitation (λ_{em} = 620 nm) and emission (λ_{ex} = 320 nm spectra of QD-penicilamine (black) and QD.Gd (blue).

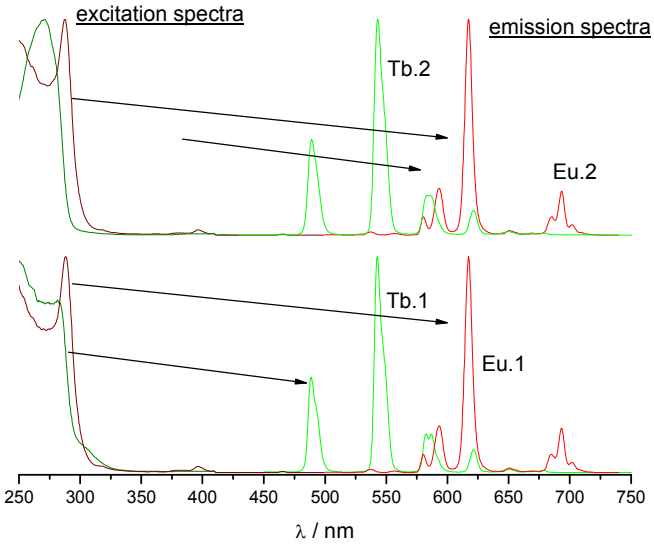


Figure S4: Excitation (λ_{em} = 546 or 612 nm) and emission (λ_{ex} = 275 nm spectra Eu.2, Tb.2 (top) Eu.1, Tb.1 (bottom)

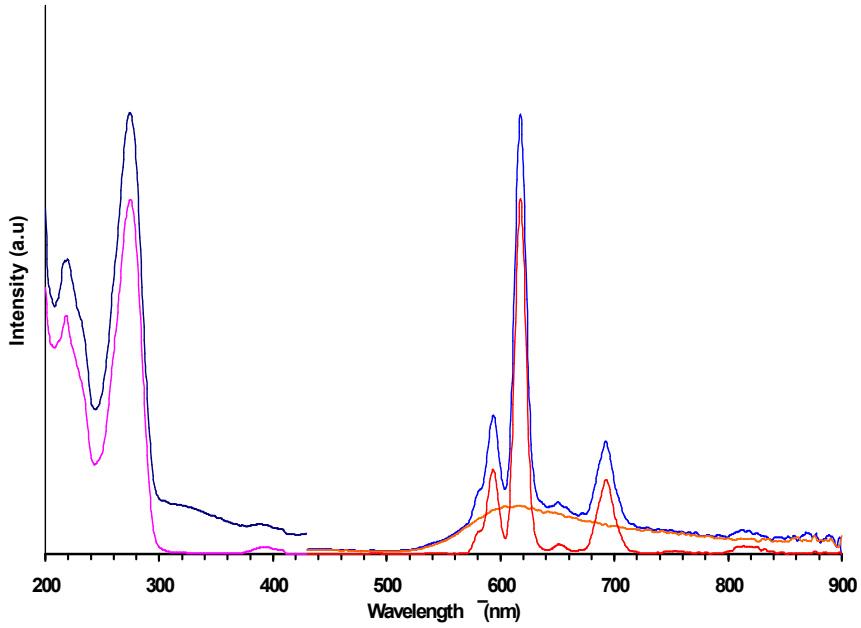


Figure S5. Luminescence spectra for Eu.2.QD, (Left) excitation spectra with λ_{em} = 617 nm (dark blue : 0 ms and pink : 0.05 ms delay, respectively); (Right) : emission spectra with λ_{ex} = 275 nm (blue : 0 ms delay and red 0.05 ms delay, respectively) and λ_{ex} = 320 nm (orange 0 ms delay)

UV-vis measurements

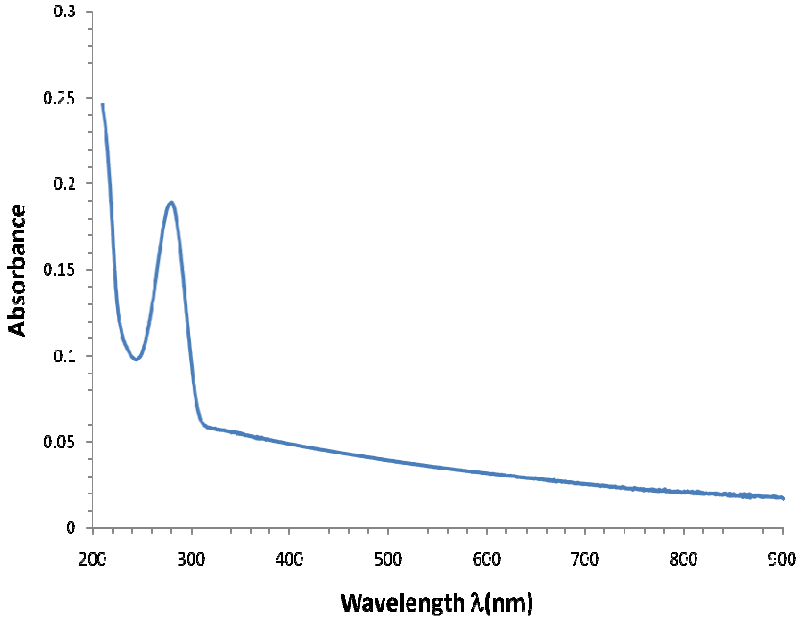


Figure S6. Uv-Vis spectra for Eu.2.QD.

Lifetime Measurements

Lifetimes measured by direct excitation (275 nm) and *q*-values calculated

Complex	***t*(H_2O)**	***t*(D_2O)**	***q***
Eu .1	1.5	0.52	1.2
Eu .2	1.51	0.53	1.2
Tb.1	1.34	1.95	1.1
Tb.2	1.35	2.26	1.2

DLS measurements

Table S1.

Quantum dot	**Size (nm)**
Quantum dot Penicilamine	6.9
Eu Grafted Quantum dot	9.2
Gd Grafted Quantum dot	8.6