Optimizing the Relaxivity of Gd(III) Complexes Appended to InP/ZnS Quantum Dots by Linker Tuning.

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General procedures. NMR spectra were recorded on an Advance DMX 200 Brucker spectrometer. Chemical shifts are reported in ppm with solvent as internal reference. ESMS were recorded on a Thermo Scientific (LXQ) spectrometer. Electronic absorption spectra were recorded on a Varian CARY 50 probe UV/vis spectrometer.

Materials. Solvents and starting materials were obtained from Aldrich, Fluka, Acros, and Alfa. They were used without further purification unless otherwise stated. Methyl (S)-2-amino-4-(tert-butoxycarbonylamino)butanoate hydrochloride (b) was purchased from BACHEM (Weil am Rhein, Germany).Water and H_2O refer to high purity water with resistivity value of 18 MΩ·cm, obtained from the "Millipore/MilliQ" purification system. Lanthanide chloride salts were purchased from Aldrich. The precise metal ion content was determined by colorimetric titration in acetate buffer (pH = 4.5) using a standardized H_2Na_2edta solution (Merck) and Xylenol orange as indicator. 6-Chloromethylpyridine-2-carboxylic acid ethyl ester (a) was prepared according to literature procedure.[1]

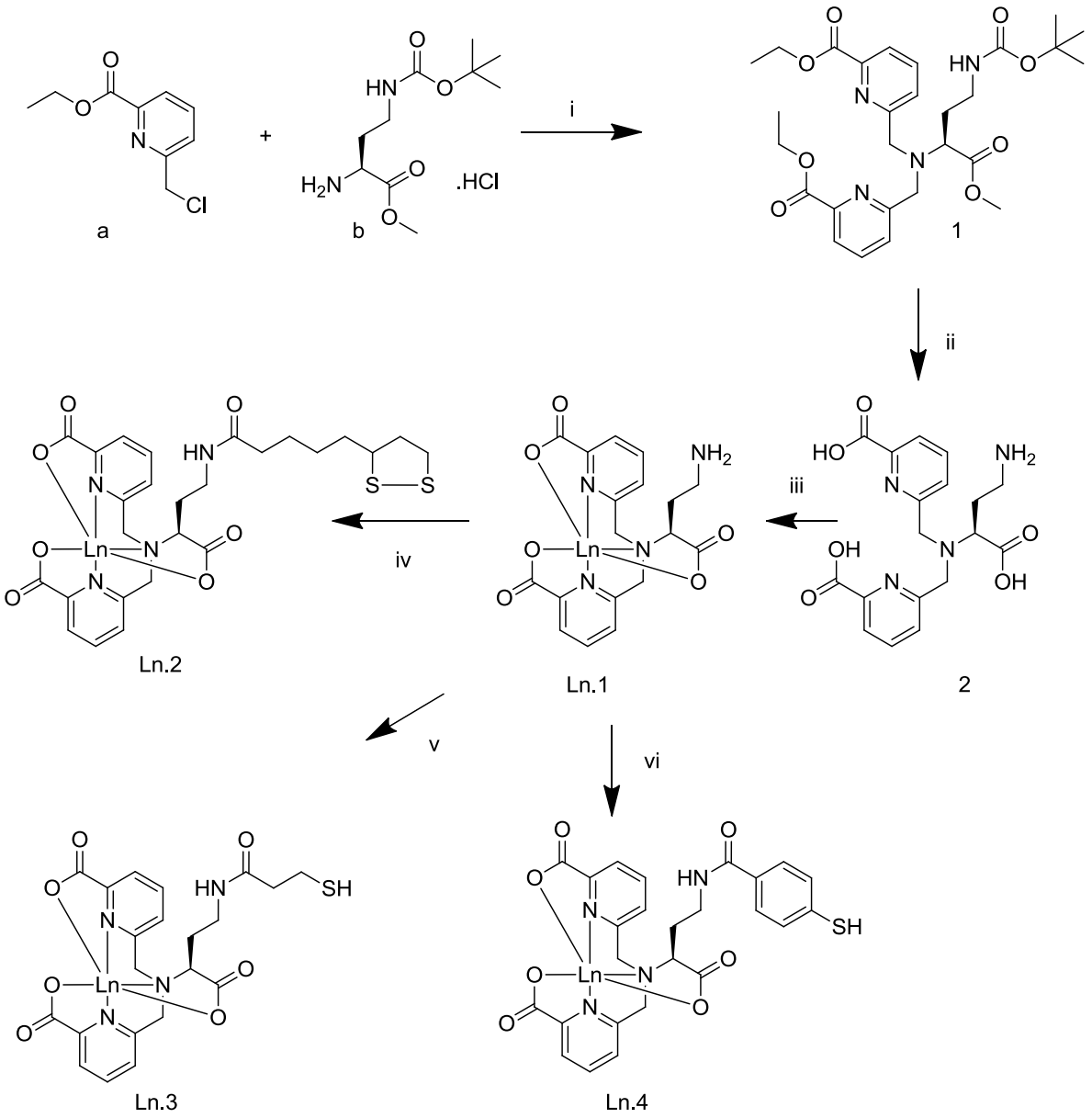
Relaxivity measurements. The Gd.1 was prepared *in situ* by mixing the appropriate amounts of ligand and $GdCl_3.6H_2O$ (99.99%; Aldrich) in H_2O followed by adjustment of the pH with NaOH aqueous solution (pH = 7.4). Gd.2 Gd.3 and Gd.4 where taken up in H_2O followed by adjustment of the pH with NaOH aqueous solution (pH = 7.4). The pH of the Gd2.QD, Gd.3.QD and Gd.4.QD suspension were prepared as above, for relaxivity measurements at 7.4. The resulting solutions/suspensions were placed in a 1.7 mm diameter capillary which was sealed. The absence of free gadolinium was checked in all samples by the xylenol orange

test.[5] The NMRD profiles were measured at 298K in the range 0.1 and 35Mz, by using a Spinmaster FFC (fast field cycling) NMR relaxometer (Stelar, Italy). The $1/T_1$ measurements were performed on a Bruker Avance 200 spectrometer (200MHz). The $1/T1$ measurements at 45 MHz (1.06 T) and 298K were performed on a BrukerMinispec "mqvar" ND2318. At higher magnetic fields relaxation measurements were performed on conventional high resolution NMR spectrometers.

Luminescence Spectroscopy. Luminescence measurements (spectra and lifetimes) were recorded using a Perkin Elmer LS50B luminescence spectrometer (using FLWINLAB for Windows v2.2) and using a modular Fluorolog FL 3- 22 spectrometer from Horiba-Jobin Yvon-Spex. It is equipped with a double grating excitation monochromator and a iHR320 imaging spectrometer coupled to a R928P Hamamatsu photomultiplier for visible measurement. For measurements in the NIR spectral range, the spectrometer was fitted with a second photomultiplier Hamamatsu R5509. The excitation source was a 450W Xe arc lamp and all spectra were corrected for detection and optical spectral response (instrumental functions) of the spectrofluorimeter. Phosphorescence lifetimes were measured in time-resolved mode. They are averages of three independent measurements that were taken by monitoring the decay at the maxima of the emission spectra. The monoexponential decays were analyzed by using the package Origin® 7.5 and a home program on Labview® 7. The quantum yields were determined at room temperature through an absolute method using a home-modified integrating sphere coupled to the modular Fluorolog FL 3-22 spectrofluorimeter. The values reported are the average of three independent determinations for each sample. The absolute quantum yield was calculated using the following expression:

$$\Phi = \frac{E_c}{L_a - L_c} = \frac{E_c}{L_a \cdot \alpha} \quad \text{and} \quad \alpha = \frac{L_a - L_c}{L_a}$$

Where *Ec* is the emission spectra in the emission range of the sample; *Lc* is the emission spectra of the excitation wavelength of the sample; *La* is the emission spectra of the excitation wavelength of the reference. Quartz capillaries tube 4 mm in diameter filled with the solvent and the complex solutions are used.



Scheme S1: Synthesis of **Ln.1, Ln.2, Ln.3 and Ln.4** : i) K_2CO_3, KI, CH_3CN, ii) HCl (6M) iii) $LnCl_3$, H_2O, pH 5.5 (iv) thioctic acid, EDC, DMAP, DMF/H_2O, (v) 3-mercaptopropionic acid, EDC, DMAP, DMF/H_2O, (vi) 4-mercaptobenzoic acid, EDC, DMAP, DMF/H_2O.

Synthesis:

(S)-Methyl-2-[bis((6-(ethoxycarbonyl)pyridin-2-yl)methyl)amino]-4-(tert-butoxycarbonylamino)butanoate (1)

Methyl (S)-2-amino-4-(*tert*-butoxycarbonylamino)butanoate hydrochloride (0.499 g, 1.86 mmol) was dissolved in anhydrous acetonitrile (30 ml) under argon atmosphere. Ethyl 6-(chloromethyl)pyridine-2-carboxylate (0.817 g, 4.092 mmol) and K_2CO_3 (0.821 g, 5.952 mmol) were successively added. After stirring for 2h at room temperature, KI (0.680g, 4.092 mmol) was added. The reaction mixture was stirred at reflux for 18h. After filtration and evaporation of the reaction mixture, the resulting brown oil (2.7 g) was chromatographed over alumina activity III (150 g; CH_2Cl_2/EtOH gradient from 100/0 to 95/5). The desired compound 1 was obtained as an orange oil (0.712 g, 69%).

^{1}H NMR (CD_3CN, 200 MHz): 1.28 (s, 9H, $(CH_3)_3COC(O)NH$), 1.37 (t, J=7.1 Hz, 6H, $pyCOOCH_2CH_3$), 1.8-2.0 (m, 2H, $NHCH_2CH_2CHN$), 3.07-3.22 (m, 2H, $NHCH_2CH_2CHN$), 3.44 (dd, J=6.2, 9.1 Hz, 1H, $NHCH_2CH_2CHN$), 3.72 (s, 3H, $CHCOOCH_3$), 3.97-4.06 (AB system, J=15.2 Hz, 4H, $N(CH_2py)_2$), 4.38 (q, J=7.1 Hz, 4H, $pyCOOCH_2CH_3$), 5.92 (s broad, , 1H, $(CH_3)_3COC(O)NH$), 7.72-7.80 (m, 2H, Hpy), 7.81 (t, J=6.9 Hz, 2H, Hpy), 7.91 (dd, J= 2.1, 6.9 Hz, 1H, Hpy).

ES-MS (m/z): 559.3 $[M+H]^+$.

(S)-2-[bis((6-(carboxy)pyridin-2-yl)methyl)amino]-4-aminobutyric acid (2)

Methyl (S)-2-[bis((6-(ethoxycarbonyl)pyridin-2-yl)methyl)amino]-4-(*tert*-butoxycarbonylamino)butanoate 1 (0.151 g, 0.268 mmol) was dissolved in 30 ml of 6M HCl and the solution was heated at reflux for 24 hours. The solvent was removed and the residue was dissolved in 20 ml water. The pH was raised to 4.5 and the aqueous solution was washed 3 times with 20 ml diethyl ether. The resulting brown oil (quantitative yield, 0.104 g) was used without further purification in the next step.

^{1}H NMR (D_2O, 200 MHz): 2.40-2.51 (m, 2H, $NH_2CH_2CH_2CHN$), 3.28-3.36 (m, 2H, $NH_2CH_2CH_2CHN$), 4.11 (t, J=7.0 Hz, 1H, $NH_2CH_2CH_2CHN$), 4.72 (, 4H, $N(CH_2py)_2$), 7.72 (dd, J= 1.2, 7.8 Hz, 2H, Hpy), 7.98 (dd, J= 1.2, 7.8 Hz, 2H, Hpy), 8.12 (t, J= 7.8 Hz, 2H, Hpy).

ES-MS (m/z): 389.6 $[M+H]^+$.

Ln (S)-2-[bis((6-(carboxylato)pyridin-2-yl)methyl)amino]-4-aminobutanoate (Ln.1)

(S)-2-[bis((6-(carboxy)pyridin-2-yl)methyl)amino]-4-aminobutyric acid (0.104 g, 0.268 mmoles) was dissolved in water (2 mL) and the pH was adjusted to 5.5 with small aliquots of 1.0 M NaOH. $LnCl_3.6H_2O$ (0.384 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 eluting 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. Gd = 0.143 g, 69%, *m*/*z* (ESMS) $[M+H]^+$ 544.3, Tb = 0.173 g, 83%, *m*/*z* (ESMS) $[M+H]^+$ 545.4.

Ln (S)-2-[bis((6-(carboxylato)pyridin-2-yl)methyl)amino]-4-(5-(1,2-dithiolan-3-yl)pentanamido)butanoate (Ln.2)

Ln (S)-2-[bis((6-(carboxylato)pyridin-2-yl)methyl)amino]-4-aminobutanoate (0.039 g, 0.073mmoles), thioctic acid (0.043 g, 0.222 mmoles), EDC (0.042 g, 0.222 mmoles) and DMAP (0.027 g, 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 and the desired complexes were obtained as a hygroscopic solid. Gd = 0.031 g, 60%, *m*/*z* (ESMS) $[M+H]^+$ 732.3. Tb = 0.042 g, 81%, *m*/*z* (ESMS) $[M+H]^+$ 733.5.

Ln (S)-2-[bis((6-(carboxylato)pyridin-2-yl)methyl)amino]-4-(3-mercaptopropanamido)butanoate (Ln.3)

Ln (S)-2-[bis((6-(carboxylato)pyridin-2-yl)methyl)amino]-4-aminobutanoate (0.039 g, 0.073mmoles), 3-Mercaptopropionic acid (0.032 g, 0.222 mmoles), EDC (0.042 g,0.222 mmoles) and DMAP (0.027 g, 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 and the desired Ln.3 complexes were obtained as hygroscopic solids. Gd = 0.034 g, 75%, *m*/*z* (ESMS) $[M+H]^+$ 632.1, Tb = 0.038 g, 83%, *m*/*z* (ESMS) $[M+H]^+$ 633.4.

Ln (S)-2-[bis((6-(carboxylato)pyridin-2-yl)methyl)amino]-4-(4-mercaptobenzamido)butanoate(Ln.4)

Ln (S)-2-[bis((6-(carboxylato)pyridin-2-yl)methyl)amino]-4-aminobutanoate (0.039 g, 0.073mmoles), 4-Mercaptobenzoic acid (0.034 g, 0.222 mmoles), EDC (0.042 g, 0.222 mmoles) and DMAP (0.027 g, 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 and the desired Ln.4 complexes were obtained as hygroscopic solids. Gd = 0.032 g, 65%, *m*/*z* (ESMS) $[M+H]^+$ 680.5, Tb = 0.040 g, 81%, *m*/*z* (ESMS) $[M+H]^+$ 681.2.

Quantum Dot Synthesis

The synthesis follows the protocol given in Ref. 2. Briefly, the indium precursor (1 eq. of indium myristate in 1-octadecene) was reacted under Ar atmosphere with *in situ* generated PH_3 gas at 250°C for 60 min to form the core InP QDs. For growth of the ZnS shell, 10 eq. of zinc stearate are added into the dispersion of the InP NCs and heated for 4 hrs to 280°C to form a Zn^{2+}-rich surface before injecting slowly 2.5 eq. of zinc ethylxanthate at 210°C during 30 min.

Synthesis of penicillamine–capped QDs (QD-pen)

Thorough purification of the initial QDs assuring the removal of excess hydrophobic ligands is crucial for the successful phase transfer. 5 mL of the QDs in organic solvent are mixed with anhydrous ethanol (1:3) and centrifuged at 10 000 rpm (rotations per minute) for 6 minutes. The clear solution of supernatant is discarded and the precipitate is re-dispersed in 15 mL of a 1:3 chloroform:ethanol mixture and centrifuged again. The precipitate is dispersed in a minimum amount of chloroform. The concentration is estimated from the first excitonic peak in the UV-Vis spectrum.[3] A 0.2 M solution of penicillamine in degassed MilliQ water (1mL) with pH adjusted at 9 with 0.5 M TMAH, is mixed with a ~5 μM dispersion of the QDs in chloroform (1.5 mL). The resulting biphasic mixture is stirred vigorously at 1400 rpm for 2 hrs to afford a fine aqueous suspension of QD-pen which is separated from the organic layer (yield: 60%).

Synthesis of Ln.2.QD

Solutions of Ln.2 (0.25 mL, $2.1 \cdot 10^{-3}$ M) and TCEP (0.046 mL, 0.5 M) in degassed water were added to the suspension of QD-pen (0.5 mL, $5.2 \cdot 10^{-6}$ M) in degassed water and the pH of the resulting suspension was adjusted to 9. The mixture was shaken at 800 rpm overnight at 20°C. The resulting fine suspension was purified portion-wise (200 μL) using PES 30K centrifuge filters at 1400 rpm for 60 seconds. The obtained nanoparticles were washed with 3 x 150 μl (degassed H_2O/PBS) to remove any un-grafted complex. The nanoparticle portions were combined and concentrated to 100 μl, giving a fine orange suspension.This procedure was repeated several times. A gadolinium content in the range 100-110 complexes per QD was determined for independent syntheses by combined magnetic susceptibility measurements and UV-Vis spectroscopy.

Synthesis of Ln.3.QD

Solutions of Ln.3 (0.25 mL, $2.1 \cdot 10^{-3}$ M) and TCEP (0.046 mL, 0.5 M) in degassed water were added to the suspension of QD-pen (0.5 mL, $5.2 \cdot 10^{-6}$ M) in degassed water and the pH of the resulting suspension was adjusted to 9. The mixture was shaken at 800 rpm overnight at 20°C. The resulting fine suspension was purified portionwise (200 μL) using PES 30K centrifuge filters at 1400 rpm for 60 seconds. The obtained nanoparticles were washed with 3 x 150 μl (degassed H_2O/PBS) to remove

any un-grafted complex. The nanoparticle portions were combined and concentrated to 100 µl, giving a fine orange suspension. This procedure was repeated several times. A gadolinium content in the range 50-60 complexes per QD was determined for independent syntheses by combined magnetic susceptibility measurements and UV-Vis spectroscopy.

Synthesis of Ln.4.QD

Solutions of Ln.4 (0.25 mL, $2.1 \cdot 10^{-3}$ M) and TCEP (0.046 mL, 0.5 M) in degassed water were added to the suspension of QD-pen (0.5 mL, $5.2 \cdot 10^{-6}$ M) in degassed water and the pH of the resulting suspension was adjusted to 9. The mixture was shaken at 800 rpm overnight at 20°C. The resulting fine suspension was purified portion-wise (200 µL) using PES 30K centrifuge filters at 1400 rpm for 60 seconds. The obtained nanoparticles were washed with 3 x 150 µl (degassed H_2O/PBS) to remove any un-grafted complex. The nanoparticle portions were combined and concentrated to 100 µl, giving a fine orange suspension. This procedure was repeated several times. A gadolinium content in the range 70-80 complexes per QD was determined for independent syntheses by combined magnetic susceptibility measurements and UV-Vis spectroscopy.

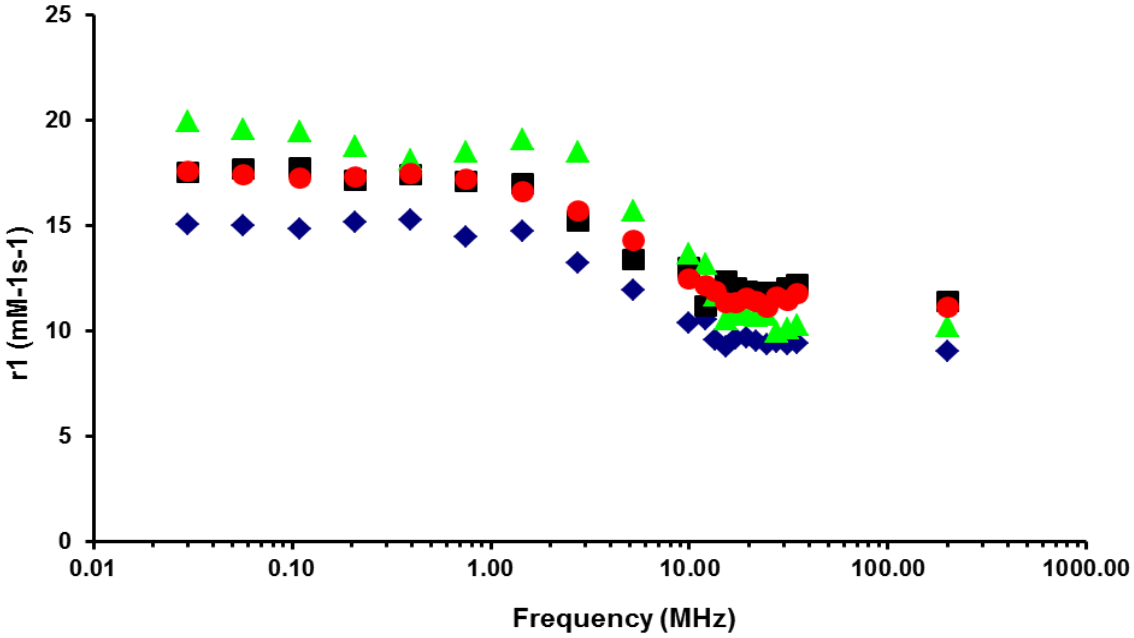


Figure S1: NMRD profile for Gd.1 (green triangles), Gd.2 (red circles), Gd.3 (blue diamonds) and Gd.4 (Black Squares), relaxivity per chelate in H_2O at 298 K

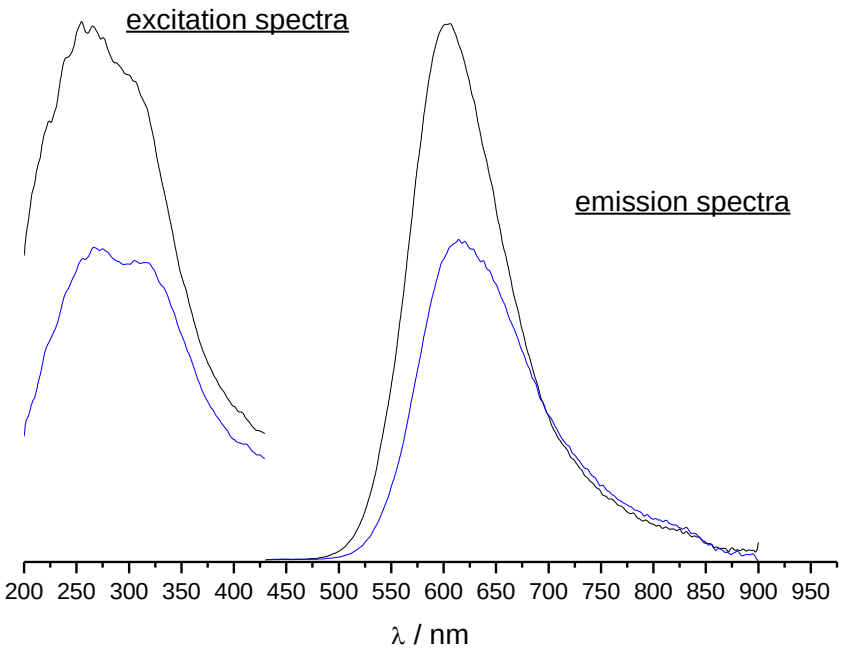


Figure S2: Excitation (λ_{em} = 620 nm) and emission (λ_{ex} = 320 nm spectra of QD-penicilamine (black) and QD.Gd (blue).

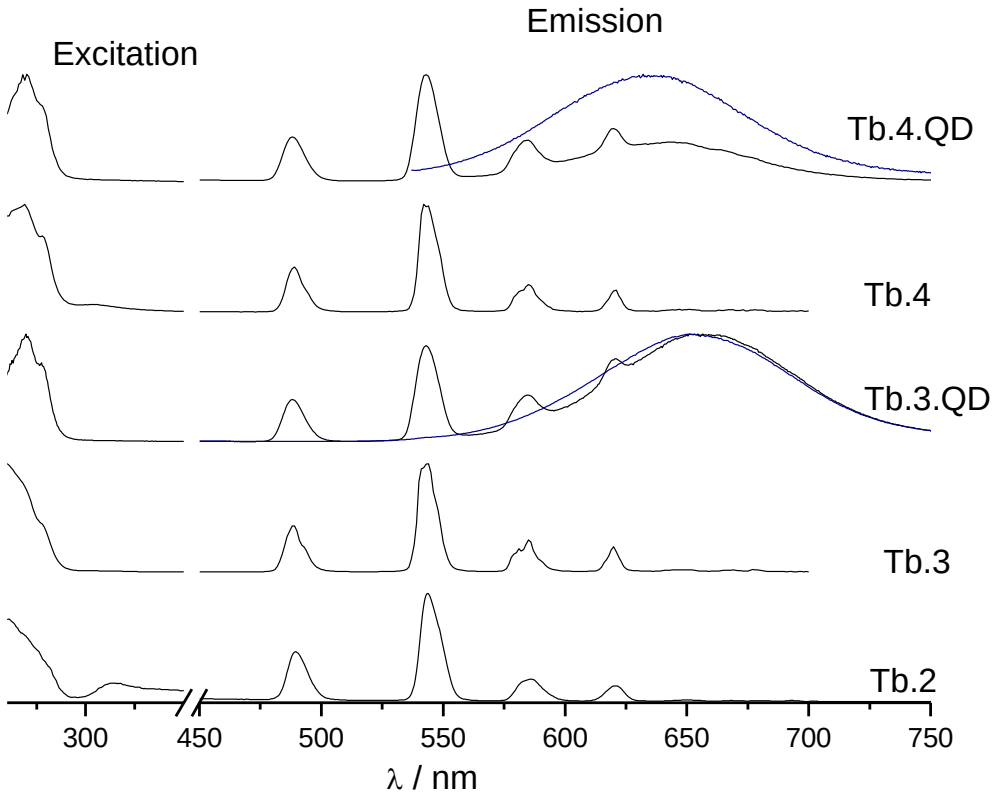


Figure S3: Luminescence of Tb.2, Tb.3, Tb.4; Tb.3.QD and Tb.4.QD. (Left) Excitation spectra with λ_{em} = 543-546 nm (Right) Emission spectra with λ_{ex} = 275 (black) and 340 nm (Blue).

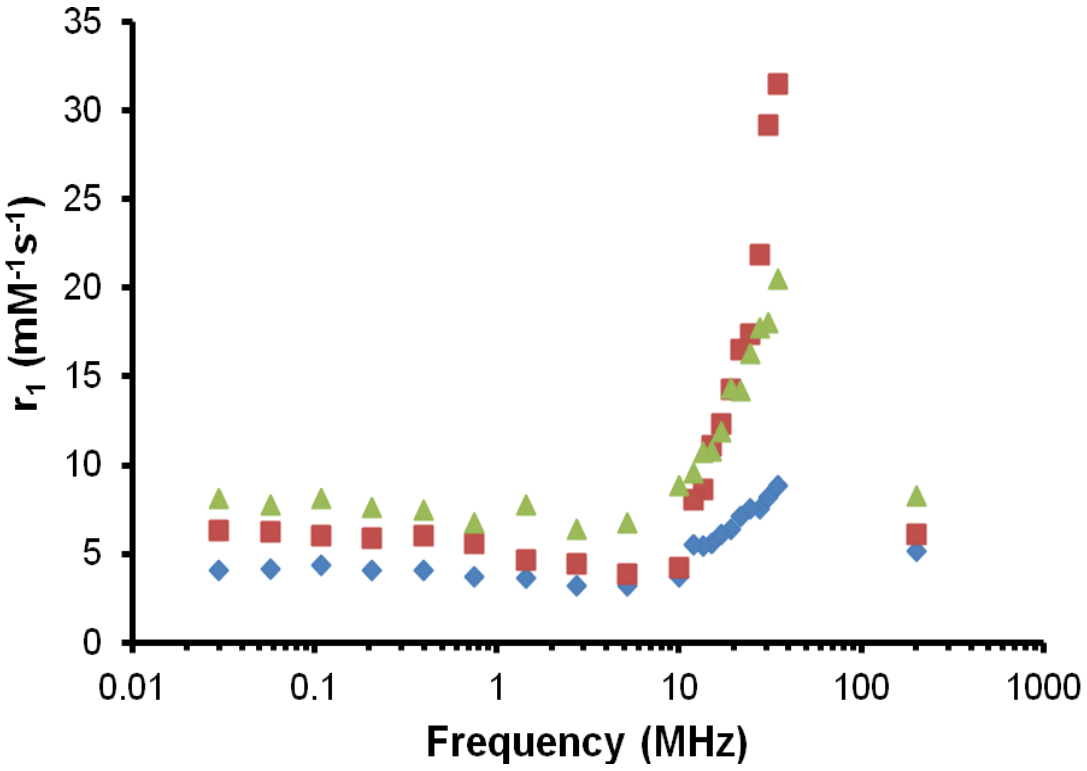


Figure S4: NMRD profile for Gd.2.QD (Green triangles), Gd.3.QD (Blue diamonds), and Gd.4.QD (red squares), relaxivity per-Gd in H_2O at 298 K

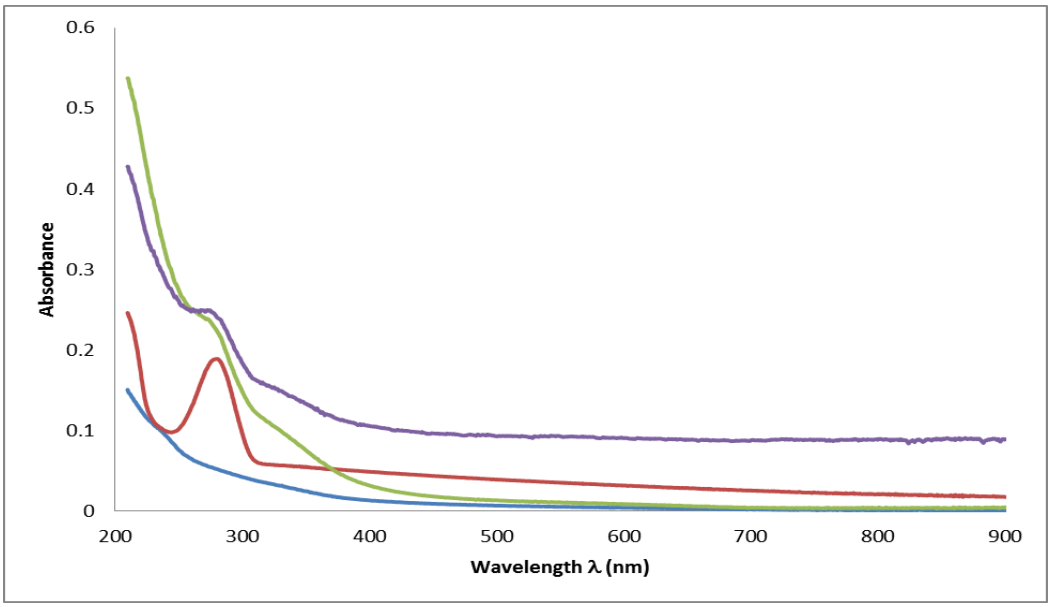


Figure S5. Uv-Vis spectra for QD.penicilamine (blue),Gd.2.QD (red), Gd.3.QD (Green) and Gd.4.QD (purple)

Table S1. Lifetimes measured by direct excitation (275 nm), *q*-values calculated and quantum yields

sample	τH_2O	τD_2O	q	Φ H_2O (%)
Tb.2.QD	1.02(5)	2.72(3)	2.8	
Tb.3.QD	1.04(3)	2.65(2)	2.6	
Tb.4.QD	0.93(4)	2.42(13)	3.0	
Pen.QD sample 1				5.9*
Pen.QD sample 2				6.5
Pen.QD sample 3				6.3
Gd.2.QD				4.4*
Gd.3.QD				2.5
Gd.4.QD				3.9

* The quantum yields vary depending on the synthesis process, the quality of the quantum dots and the phase transfer via surface ligand exchange from chloroform to water.

References:

1) A. Nonat, C. Gateau, P. H. Fries and M. Mazzanti, *Chem. Eur. J.*, **2006**, 12, 7133-7150.
2) L. Li, M. Protière, P. Reiss, *Chem. Mater.* **2008**, *20*, 2621-2623.
3) Reiss P.; Protière M.; Li L. *Small*, **2009**, *5*, 154–168.
4) D. M. Corsi, C. Platas-Iglesias,H. van Bekkum and J. A. Peters, *Magn Reson. Chem.*, **2001**, **3**9, 723–726
5) G. Brunisholz and M. Randin, *Helv. Chim. Acta*, **1959**, 42, 1927–1938