Cyclen lanthanide-based micellar structures for application as luminescent [Eu(III)] and magnetic [Gd(III)] resonance imaging (MRI) contrast agents

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Contents:

1. General experimental procedures and characterisation

All solvents and chemicals were purchased from Sigma-Aldrich, Fluka, and Chematech, and unless otherwise stated, were used without further purification. Deuterated solvents used for NMR analysis were purchased from Apollo Scientific. Chromatographic columns were run on a Teledyne Isco Combiflash Rf200 Automatic machine using pre-packed silica columns. Melting points were obtained using an electrothermal 1A900 digital melting point apparatus. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer equipped with a universal ATR sampling accessory.

NMR spectra were recorded using either a Brüker DPX-400 Avance spectrometer, which operates at 400.13 MHz for ^{1}H NMR and 100.60 MHz for ^{13}C NMR, or a Brüker AV-600 spectrometer, operating at 600.10 MHz for ^{1}H NMR and 150.9 MHz for ^{13}C NMR. Tetramethylsilane (TMS) was used as an internal standard and chemical shifts were referenced relative to the internal solvent signals, with chemical shifts expressed in parts per million (ppm). Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), quintet (qu) and multiplet (m). All NMR spectra were carried out at 293 K, unless otherwise stated.

Electrospray mass spectra were acquired using a Bruker micrOTOF-Q III spectrometer interfaced to a Dionex UltiMate 3000 LC. A peak-matching method was used to determine accurate molecular weights using Agilent Technologies ESI-1 low concentration tuning mix as the internal lock mass. Maldi-Tof spectra were acquired using a Waters Maldi Q-Tof Premier, with the laser operating at 337 nm. High-resolution mass spectrometry was performed using [Glu] Fibrinopetide B as the internal reference (m/z = 1570.6774). All mass spectrometry was carried out using HPLC grade solvents and all accurate mass were reported within ±5 ppm of the expected mass.

Elemental analysis was performed on an Exter Analytical C3440 elemental analyser at the Microanalysis Laboratory, School of Chemistry and Chemical Biology, University College Dublin.

2. Photophysical measurements

Unless otherwise stated, all measurements were performed at 298 K in water, which was purified using a Millipore Milli-Q water purification system. UV-visible absorption spectra were measured in 3.0 cm quartz cuvettes (Hellma) on a Varian Cary 50 spectrophotometer with a wavelength range of 200-450 nm and a scan rate of 240 nm min^{-1}. Baseline correction measurements were applied to all spectra. Emission (fluorescence and phosphorescence) spectra and lifetime measurements were recorded on a Varian Cary Eclipse Fluorimeter in 3.0 cm quartz cells (Hellma), which are equipped with a 1.0 cm path length.

Luminescence Settings: Lanthanide Lifetime Studies

Luminescence lifetimes of the Eu($^{5}D_0$) excited state were measured in both H_2O and D_2O in time-resolved mode at 298 K. The average of three independent measurements was taken by monitoring the emission decay at 616 nm, which corresponds to the maxima of the Eu(III) $^{5}D_0 \rightarrow {}^{7}F_2$ transition,

justifying a 0.1 ms delay. All spectra were analyzed using OriginPro 8.1 and fitted with a mono-exponential fit. The number of water molecules bound directly to the metals inner sphere (q) for **1.Eu.Na** was calculated from Equation 1.1, where τ_{H_2O} and τ_{D_2O} are the lifetimes in H_2O and D_2O, respectively, with A = 1.2 and k_{corr} = 0.25 ms^{-1}.[1]

$$q^{Eu(III)} = A\left[\left(\frac{1}{\tau_{O\text{-}H}} - \frac{1}{\tau_{O\text{-}D}}\right) - k_{corr}\right] \qquad \textbf{Equation 1}$$

3. Relaxometric measurements

The frequency dependence of the ^1H relaxation for the aqueous suspensions was recorded over the frequency range of 0.01–40 MHz using a Spinmaster FFC-2000 Fast Field Cycling NMR Relaxometer (Stelar SRL, Mede, Italy). The system operated at a measurement frequency of 16.3 MHz for ^1H, at which frequency the 90° pulse was 7 μs. T_1 measurements were performed as a function of external field, B_0, with standard pulse sequences incorporating B_0 field excursions. The temperature of the samples was maintained at 25 ± 1 °C using a thermostatted airflow system. All of the ^1H magnetisation recovery curves were singly exponential within experimental error and the random errors in fitting T_1 were always less than 1%.

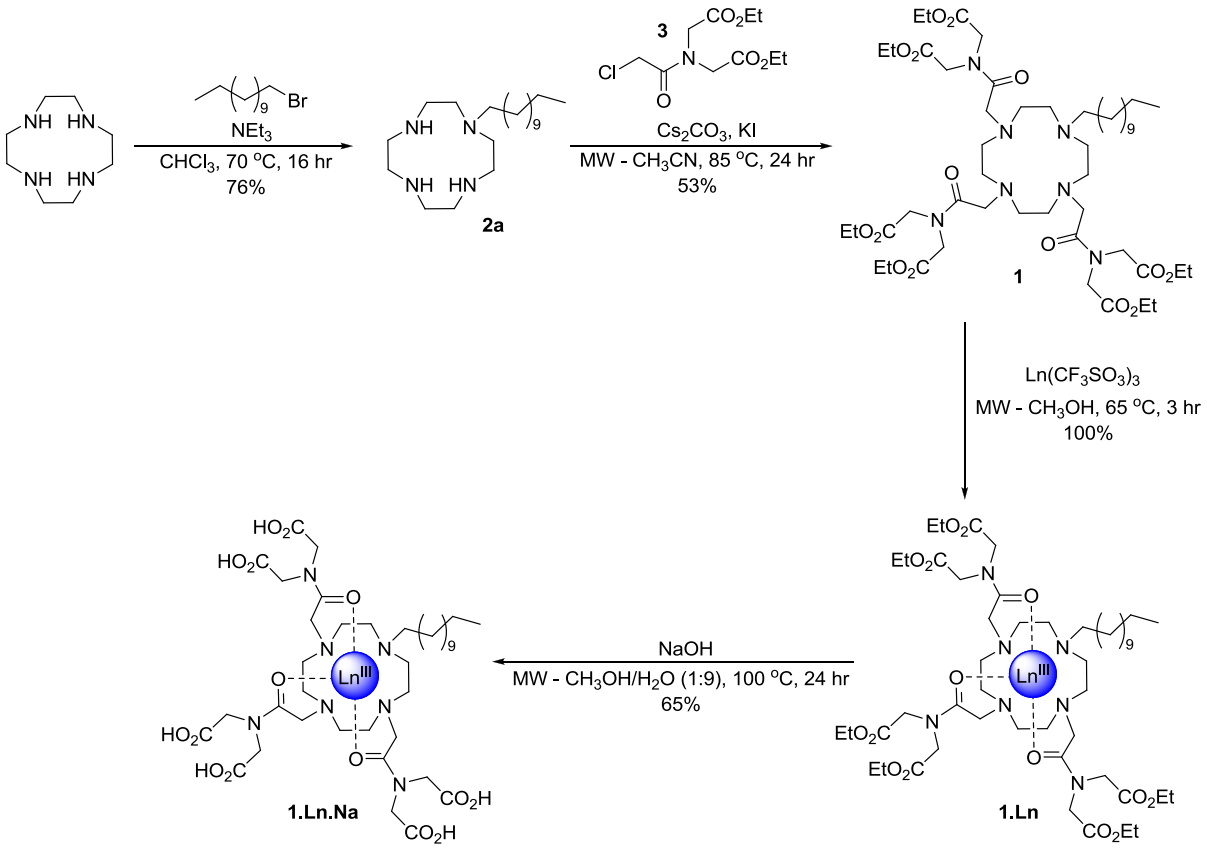
4. Protocol for preparing bovine tibiae specimens

Fresh bovine tibiae from animals slaughtered 2–3 days previously were supplied by a meat wholesaler. The soft tissue was fully removed and the bones then stored at −20 ºC until required. All bovine tibia specimens were prepared in accordance to previously reported protocols, in which several specimens were sectioned and polished using a diamond saw and P1200 grit sandpaper, respectively, in order to give mechanically smooth bone samples (15 mm × 5 mm × 1 mm) that mimicked the structure of bone *in vivo*.[2] A series of three scratches *ca.* 5 mm in length were made using a surgical scalpel. Each tibia specimen was then immersed in an aqueous solution of **1.Eu.Na** (4.5 mM or 10.0 mM) for initially 20 h after making the first scratch. Following their removal from solution, the second scratch was made, and the bone specimens were re-immersed for a further 4 h. The specimens were then removed a second time, and the last scratch was made. Any excess of **1.Eu.Na** was removed by rinsing each of the specimens with deionised water. The same specimens were finally immersed in an aqueous solution of the antenna **nta** (0.15 mM) for 30 s, and once again rinsed with deionised water. Two-photon excitation fluorescence microscopy was then utilised to view the microdamaged regions of the bone specimens. It should be noted that 0, 4 and 24 h refers to the period of time each microcrack was immersed in the micellar agent.

5. Two-Photon Excitation Fluorescence Microscopy

A Carl Zeiss 710 NLO microscope confocal microscope system coupled to a Coherent Chameleon Vision II Ti:Sapphire laser was used for two photon fluorescence microscopy (2PFM) imaging with a 10× water immersion objective (Carl zeiss W N-Achroplan 10x/0.3 W). The excitation wavelength of 750 nm was used and the two photon emission between 565-610 nm was collected with non-descanned detection. Carl Zeiss ZEN 2008 software was used for capturing images with Fiji and Volocity 3.7.0 (Improvision Ltd) was used to provide the processed images. Each image was obtained frame by frame with a dwell time of 1.15 µs and 17.000 µm interval in the *z*-axis with the axis of movement running perpendicular to the bone crack. Stack height varied as the depth of bone crack varied between samples, the top and bottom of each crack was assessed/defined visually.

6. General synthetic procedure



Scheme S1 Full synthetic schematic of **1 (**free ligand) and the corresponding complex **1.Ln**, followed by alkaline hydrolysis to give complex **1.Ln.Na.**

Procedure 1: Synthesis of Ln(III) complexes using $Ln(CF_3SO_3)_3$

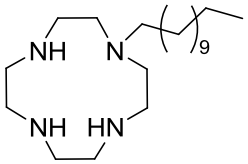
All Ln(III) complexes were prepared by refluxing, under microwave irradiation, the relevant ligand with 1.0 eq. of $Ln(CF_3SO_3)_3$ for 3 h in freshly distilled CH_3OH. The solvent was then reduced to *ca*. 1 mL and the relevant complexes were isolated by precipitation from swirling dry diethyl ether (200 mL). Following reaction completion the solvent was removed under reduced pressure to afford the

complexes. Owing to the paramagnetic nature of the Ln(III) ion, ^{1}H NMR spectra of the complexes consisted of broad resonances and, therefore, were not fully characterised in terms of integration. These paramagnetic properties also prevented ^{13}C NMR spectra from being recorded.

Procedure 2: Base hydrolysis of the diethyliminodiacetate functional groups

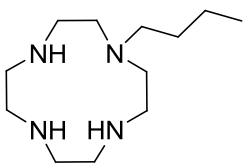
Alkaline hydrolysis was carried out by refluxing, under microwave irradiation, the relevant Ln(III) complex with 6.0 eq. of NaOH for 24 h in a mixed CH_3OH/H_2O (1:9 v/v) solution. After reaction completion, the solvent was reduced to *ca*. 5 mL and acidified to pH 3 using 2M HCl. The supernatant was then decanted and the protonated product was dissolved in either CH_3CN or IPA. The precipitated salts were subsequently removed by centrifugation and the hydrolysed Ln(III) complex, in its acid form, was afforded upon removal of the solvent by reduced pressure. Lastly, the corresponding sodium carboxylate form of the complex was then given by adding 6.0 eq of aqueous NaOH.

1-Dodecyl-1,4,7,10-tetraazacyclododecane (2a)[3]



Cyclen (2.00 g, 11.61 mmol) was dissolved along with NEt_3 (0.35 g, 3.48 mmol) in freshly distilled $CHCl_3$ (50 mL). After stirring the solution for 5 min, 1-bromododecane (0.72 g, 2.90 mmol) was added and the resulting solution was refluxed for 16 hr under an inert atmosphere. After cooling to room temperature, the organic solution was washed with 1 M NaOH (3 x 20 mL) to remove the excess cyclen and with water (3 x 10 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure to yield the product **2a** (0.91 g, 2.67 mmol, 92 % yield) as a colourless oil. HRMS (*m/z,* ESI^+): Calculated for $C_{20}H_{45}N_4$ *m/z* = 341.3644 $[M+H]^+$. Found *m/z* = 341.3657; ^{1}H NMR δ_H (400 MHz, $CDCl_3$) 2.80-2.51 (16H, m, cyclen $\underline{CH_2}$), 2.41 (2H, t, *J* = 7.20, $N\underline{CH_2}$), 1.45 (2H, m, $NCH_2\underline{CH_2}$), 1.25 (18H, m, 9 x $\underline{CH_2}$), 0.87 (3H, t, *J* = 6.97 $(CH_2)_9\underline{CH_3}$); ^{13}C NMR δ_C (400 MHz, $CDCl_3$): 54.64 (CH_2), 51.64 (CH_2), 47.16 (CH_2), 46.20 (CH_2), 45.30 (CH_2), 31.98 (CH_2), 29.75 (CH_2), 29.72 (CH_2), 29.71 (CH_2), 29.61 (CH_2), 29.41 (CH_2), 27.56 (CH_2), 27.39 (CH_2), 22.74 (CH_2), 14.18 (CH_3); IR ν_{max} (cm^{-1}): 2921, 2852, 1458, 1350, 1271, 1115, 1049, 933, 747.

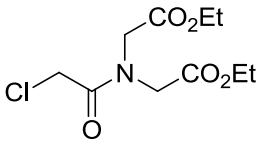
1-Butyl-1,4,7,10-tetraazacyclododecane (2b)[3]



Cyclen (2.52 g, 14.63 mmol) was dissolved along with NEt_3 (0.44 g, 4.38 mmol) in freshly distilled $CHCl_3$ (50 mL). After stirring the solution for 5 min, 1-bromobutane (0.50 g, 3.65 mmol) was added and the resulting solution was refluxed for 16 hr under an inert atmosphere. After cooling to room temperature, the organic solution was washed with 1 M NaOH (3 x 20 mL) to remove the excess cyclen and with water (3 x 10 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent

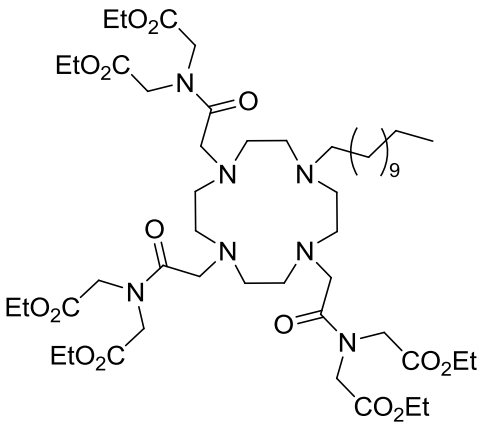
removed under reduced pressure to yield the product **2b** (0.79 g, 3.46 mmol, 95 % yield) as a colourless oil. HRMS (*m/z,* ESI^+): Calculated for $C_{12}H_{29}N_4$ *m/z* = 229.2392 $[M+H]^+$. Found *m/z* = 229.2396; ^1H NMR δ_H (400 MHz, $CDCl_3$) 2.76-2.50 (16H, m, cyclen $\underline{CH_2}$), 2.39 (2H, t, *J* = 7.20, $N\underline{CH_2}$), 1.44 (2H, m, $NCH_2\underline{CH_2}$), 1.29 (2H, m, $\underline{CH_2}CH_3$), 0.87 (3H, t, *J* = 7.30, $(CH_2)_3\underline{CH_3}$); ^{13}C NMR δ_C (400 MHz, $CDCl_3$): 54.30 (CH_2), 51.64 (CH_2), 47.11 (CH_2), 46.17 (CH_2), 45.24 (CH_2), 29.66 (CH_2), 20.75 (CH_2), 14.18 (CH_3); IR ν_{max} (cm^{-1}): 2929, 2872, 2803, 1460, 1377, 1352, 1305, 1274, 1201, 1116, 1084, 1038, 1018, 934, 916, 799, 748, 712, 658.

Diethyl 2,2-(2-chloroacetylamino)diacetate (3)[2a]



Diethyl iminodiacetate (3.00 g, 15.86 mmol) and NEt_3 (2.55 g, 25.20 mmol) were dissolved in CH_2Cl_2 (50 mL). The clear solution was cooled to 0 °C and chloroacetyl chloride (3.54 g, 31.34 mmol) was added slowly along with CH_2Cl_2 (30 mL) using a pressure equalised dropping funnel. The resulting solution was then stirred at room temperature for 24 hr. Following reaction completion, the pale yellow solution was washed with water (2 x 30 mL), 0.1 M HCl (2 x 30 mL), and brine (2 × 30 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure to afford **3** as an orange oil (3.87 g, 14.57 mmol, 92% yield). HRMS (*m/z,* ESI^+): Calculated for $C_{10}H_{16}NO_5NaCl$ *m/z* = 288.0615 $[M+Na]^+$. Found *m/z* = 288.0611; ^1H NMR δ_H (400 MHz, $CDCl_3$) 4.22 (8H, m, $CO_2\underline{CH_2}CH_3$), 4.12 (2H, s, $CO\underline{CH_2}Cl$), 1.30 (3H, t, *J* = 7.20, $CH_2\underline{CH_3}$), 1.27 (3H, t, *J* = 7.20, $CH_2\underline{CH_3}$); ^{13}C NMR δ_C (400 MHz, $CDCl_3$): 168.64 (qt), 168.38 (qt), 167.87 (qt), 62.14 (CH_2), 61.68 (CH_2), 50.48 (CH_2), 48.71 (CH_2), 40.60 (CH_2), 14.10 (CH_3), 14.08 (CH_3); IR ν_{max} (cm^{-1}): 2986, 1738, 1661, 1548, 1463, 1407, 1375, 1299, 1260, 1188, 1063, 1021, 966, 931, 866, 796, 736.

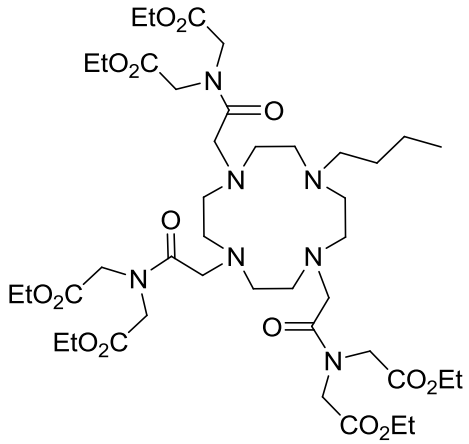
1,4,7-Tris-(N,N-bis(ethoxycarbonylmethyl)-10-[dodec-1-yl]-1,4,7,10 tetraazacyclododecane (1)



2a (0.10 g, 0.29 mmol) was dissolved in freshly distilled CH_3CN (20 mL) in the presence of Cs_2CO_3 (0.33 g, 1.01 mmol) and KI (0.17 g, 1.02 mmol). **3** (0.25 g, 0.94 mmol) was then added and the resulting solution was exposed to microwave irradiation for 24 hr at 85 °C with 30 s pre-stirring. After cooling, removal of the inorganic salts was achieved by centrifugation and the solvent was then removed by reduced pressure. The resulting brown oil was purified by flash silica column chromatography using a gradient elution of 100 to 80:20 CH_2Cl_2:CH_3OH. The desired product was obtained as a brown viscous oil (0.63 g, 0.61 mmol, 53% yield). HRMS (*m/z,* MALDI): Calculated for $C_{50}H_{89}N_7O_{15}Na$ *m/z* = 1050.6314 $[M + Na]^+$. Found *m/z* = 1050.6316; ^1H NMR δ_H (600 MHz, $CDCl_3$): 4.31-3.99 (24H,

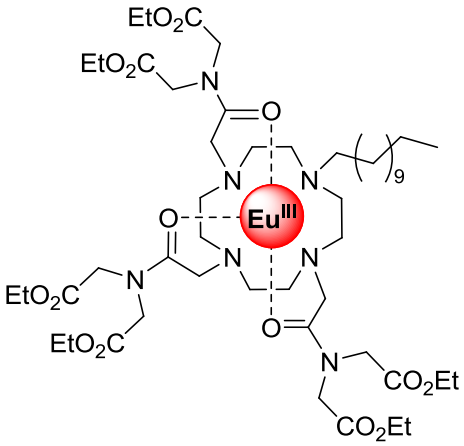
m, ($\underline{CH_2}CO_2\underline{CH_2}CH_3)_2$), 3.78-3.40 (24H, m, cyclen-$\underline{CH_2}$, $N\underline{CH_2}CO$, $N\underline{CH_2}(CH_2)_{10}$), 1.65 (2H, broad s, $NCH_2\underline{CH_2}(CH_2)_9$), 1.28 (36H, m, $(\underline{CH_2})_9CH_3$), $(CO_2CH_2\underline{CH_3})_2$), 0.87 (3H, t, J = 7.01 $(CH_2)_9\underline{CH_3}$)); ^{13}C NMR δ_C (600 MHz, $CDCl_3$): 67.77 (CH_2), 62.43 (CH_2), 62.38 (CH_2), 61.92 (CH_2), 61.19 (CH_2), 60.13 (CH_2), 55.04 (CH_2), 53.60 (CH_2), 53.10 (CH_2), 52.72 (CH_2), 50.55 (CH_2), 49.64 (CH_2), 49.16 (CH_2), 48.57 (CH_2), 48.42 (CH_2), 48.35 (CH_2), 47.39 (CH_2), 47.25 (CH_2), 32.24 (CH_2), 29.97 (CH_2), 29.67 (CH_2), 23.01 (CH_2), 14.55 (CH_3), 14.46 (CH_3); Calculated for $C_{50}H_{89}N_7O_{15}.1.5CHCl_3.1.5CH_2Cl_2$: C, 47.69; H, 7.06; N, 7.35. Found C, 47.54; H, 7.06; N, 7.31; IR ν_{max} (cm^{-1}): 2926, 2858, 1740, 1660, 1524, 1462, 1425, 1379, 1298, 1189, 1098, 1022, 854, 725, 662.

1,4,7-Tris-(N,N-bis(ethoxycarbonylmethyl)-10-[butyl]-1,4,7,10 tetraazacyclododecane (4)



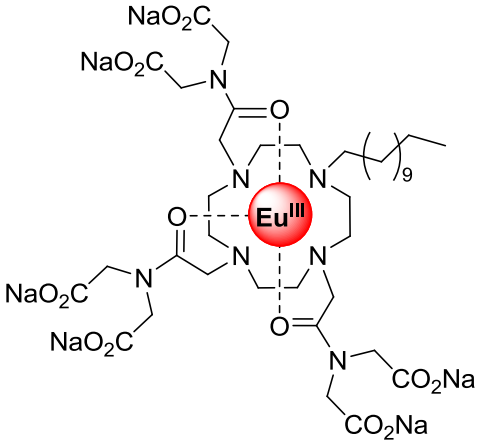
2b (0.25 g, 1.09 mmol) was dissolved in freshly distilled CH_3CN (20 mL) in the presence of Cs_2CO_3 (1.16 g, 3.56 mmol) and KI (0.59 g, 3.56 mmol). **3** (0.92 g, 3.46 mmol) was then added and the resulting solution was exposed to microwave irradiation for 24 hr at 85 ^oC with 30 s pre-stirring. After cooling, removal of the inorganic salts was achieved by centrifugation and the solvent was then removed by reduced pressure. The resulting orange oil was purified by flash silica column chromatography using a gradient elution of 100 to 80:20 CH_2Cl_2:CH_3OH. The desired product was obtained as a viscous orange oil (0.42 g, 0.46 mmol, 42% yield). HRMS (*m/z,* ESI^+): Calculated for $C_{42}H_{74}N_7O_{15}$ *m/z* = 916.5234 $[M + H]^+$. Found *m/z* = 916.5245; ^{1}H NMR δ_H (600 MHz, $CDCl_3$): 4.23-3.98 (24H, m, ($\underline{CH_2}CO_2\underline{CH_2}CH_3)_2$), 3.77-2.57 (24H, bm, cyclen-$\underline{CH_2}$, $N\underline{CH_2}CO$, $N\underline{CH_2}(CH_2)_3$), 1.27 (22H, m, $(\underline{CH_2})_2CH_3$), $(CO_2CH_2\underline{CH_3})_2$), 0.87 (3H, m, $(CH_2)_3\underline{CH_3}$)); ^{13}C NMR δ_C (600 MHz, $CDCl_3$): 172.63 (qt), 172.16 (qt), 169.12 (qt), 169.03 (qt), 168.91 (qt), 168.74 (qt), 62.11 (CH_2), 61.38 (CH_2), 61.28 (CH_2), 55.12 (CH_2), 54.85 (CH_2), 54.12 (CH_2), 53.62 (CH_2), 50.28 (CH_2), 49.93 (CH_2), 49.65 (CH_2), 48.61 (CH_2), 48.27 (CH_2), 28.07 (CH_2), 20.84 (CH_2), 14.31 (CH_3), 14.26 (CH_3), 14.05 (CH_3; IR ν_{max} (cm^{-1}): 2962, 2824, 1737, 1656, 1590, 1468, 1408, 1373, 1300, 1260, 1187, 1113, 1100, 1023, 971, 906, 870, 837, 806, 781, 732, 700, 661.

Complex 1.Eu



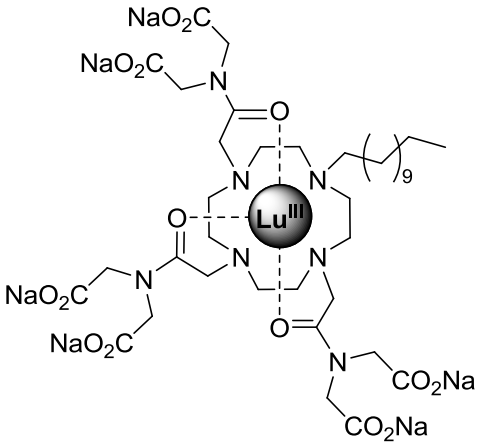
Complex **1.Eu** was synthesised according to **Procedure 1** using ligand **3** (0.20 g, 0.19 mmol) and $Eu(CF_3SO_3)_3$ (0.13 g, 0.21 mmol). A fine orange solid was obtained (0.30 g, 0.18 mmol, 96% yield). M.P: decomposed above 270 °C; HRMS (*m/z,* MALDI): Calculated for $C_{51}H_{89}N_7O_{18}F_3SEu$ *m/z* = 1329.5149 $[M + CF_3SO_3]^+$. Found *m/z* = 1329.5194; ^1H NMR δ_H (600 MHz, CD_3OD): 33.99, 29.15, 25.94, 24.97, 24.04, 22.18, 21.20, 19.62, 19.02, 17.81, 17.20, 16.37, 14.51, 10.97, 4.93, 4.28, 3.75, 3.37, 3.34, 1.84, 1.32, 0.92, -6.60, -8.04, -9.67, -10.97, -11.76, -12.37, -13.11, -14.37, -15.44, -16.97, -18.18, -19.39, -20.04, -21.67, -23.81, -35.89; IR ν_{max} (cm^{-1}): 2932, 2863, 1736, 1633, 1563, 1511, 1446, 1383, 1221, 1172, 1025, 842, 722, 628.

Complex 1.Eu.Na



Complex **1.Eu.Na** was synthesised according to **Procedure 2** using complex **1.Eu** (0.092 g, 0.057 mmol) and NaOH (0.014 g, 0.35 mmol). The desired product was obtained as a yellow solid (0.056 g, 0.038 mmol, 68% yield). M.P: decomposed above 280 °C; HRMS (*m/z,* MALDI): Calculated for $C_{38}H_{61}N_7O_{15}Eu$ *m/z* = 1008.3438 $[M - 6Na+2H]^-$. Found *m/z* = 1008.3467; ^1H NMR δ_H (600 MHz, D_2O):8.32, 7.89, 7.23, 4.71, 4.14, 4.05, 3.81, 3.72, 3.49, 3.35, 3.22, 3.17, 3.13, 3.08, 2.69, 2.24, 2.11, 1.79, 1.16, 0.03, -0.06, -2.72; IR ν_{max} (cm^{-1}): 3411, 2927, 2856, 1591, 1541, 1506, 1401, 1258, 1170, 1102, 1036, 917, 839, 718, 627.

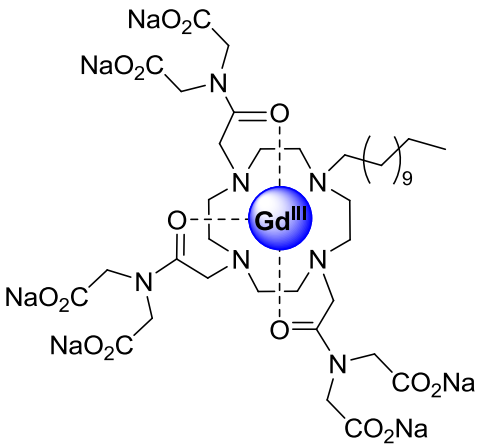
Complex 1.Lu.Na



Complex **1.Lu.Na** was synthesised according to **Procedure 2** using complex **1.Lu** (0.045 g, 0.027 mmol) and NaOH (0.0067 g, 0.17 mmol). The desired product was obtained as a yellow solid (0.024g, 0.016 mmol, 60% yield). M.P: decomposed above 280 °C; HRMS (*m/z,* MALDI): Calculated for $C_{38}H_{61}N_7O_{15}Lu$ *m/z* = 1030.3634 $[M - 6Na+2H]^-$. Found *m/z* = 1030.3610; ^1H NMR δ_H (600 MHz, D_2O): 4.27-2.77 (36H, broad m, cyclen-$\underline{CH_2}$,

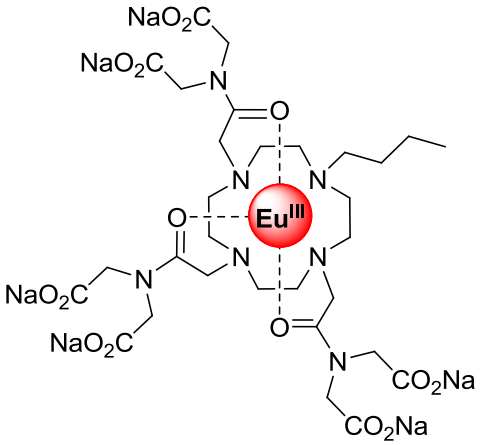
($\underline{CH_2}CO_2\underline{Na})_2$, $N\underline{CH_2}CO$, $N\underline{CH_2}(CH_2)_{10}$, 1.28 (20H, m, $NCH_2(\underline{CH_2})_{10}$), 0.88 (3H, m, $(CH_2)_{10}\underline{CH_3}$); ^{13}C NMR δ_C (600 MHz, D_2O): 176.41 (qt), 175.73 (qt), 173.49 (qt), 173.01 (qt), 59.44 (CH_2), 53.79 (CH_2), 51.95 (CH_2), 51.04 (CH_2), 50.86 (CH_2), 49.98 (CH_2), 47.80 (CH_2), 32.07 (CH_2), 31.25 (CH_2), 29.89 (CH_2), 29.48 (CH_2), 28.60 (CH_2), 25.94 (CH_2), 22.74 (CH_2), 22.13 (CH_2), 13.89 (CH_3), 13.55 (CH_3); IR ν_{max} (cm^{-1}): 3448, 2929, 2857, 1602, 1545, 1507, 1404, 1252, 1170, 1102, 1036, 920, 848, 716, 631.

Complex 1.Gd.Na



Complex **1.Gd.Na** was synthesised according to **Procedure 2** using complex **1.Gd** (0.056 g, 0.034 mmol) and NaOH (0.0084 g, 0.21 mmol). The desired product was obtained as a yellow solid (0.036g, 0.025 mmol, 71% yield). M.P: decomposed above 280 °C; HRMS (*m/z,* MALDI): Calculated for $C_{38}H_{61}N_7O_{15}Gd$ *m/z* = 1013.3467 $[M - 6Na+2H]^-$. Found *m/z* = 1013.3434; IR υ_{max} (cm^{-1}): 3411, 2927, 2856, 1591, 1541, 1506, 1401, 1258, 1170, 1102, 1036, 917, 839, 718, 627.

Complex 4.Eu.Na



Complex **4.Eu.Na** was synthesised according to **Procedure 2** using complex **4.Eu** (0.24 g, 0.16 mmol) and NaOH (0.045 g, 1.13 mmol). The desired product was obtained as an orange solid (0.14g, 0.10 mmol, 66% yield). M.P: decomposed above 260 °C; HRMS (*m/z,* MALDI): Calculated for $C_{30}H_{47}N_7O_{15}Eu$ *m/z* = 898.2346 $[M - 6Na+4H]^+$. Found *m/z* = 898.2313; ^1H NMR δ_H (600 MHz, D_2O): 11.82, 7.03, 6.50, 5.69, 5.52, 3.79, 2.87, 2.66, 2.40, 2.17, 1.92, 1.75, 1.30, 0.94, 0.33, 0.24, −0.09, −0.26, −0.51, −0.62, −1.46, −1.82, −2.55, −4.34, −5.51; IR υ_{max} (cm^{-1}): 3478, 2933, 2872, 1598, 1395, 1257, 1170, 1081, 1036, 977, 907, 855, 716, 672.

Characterisation:

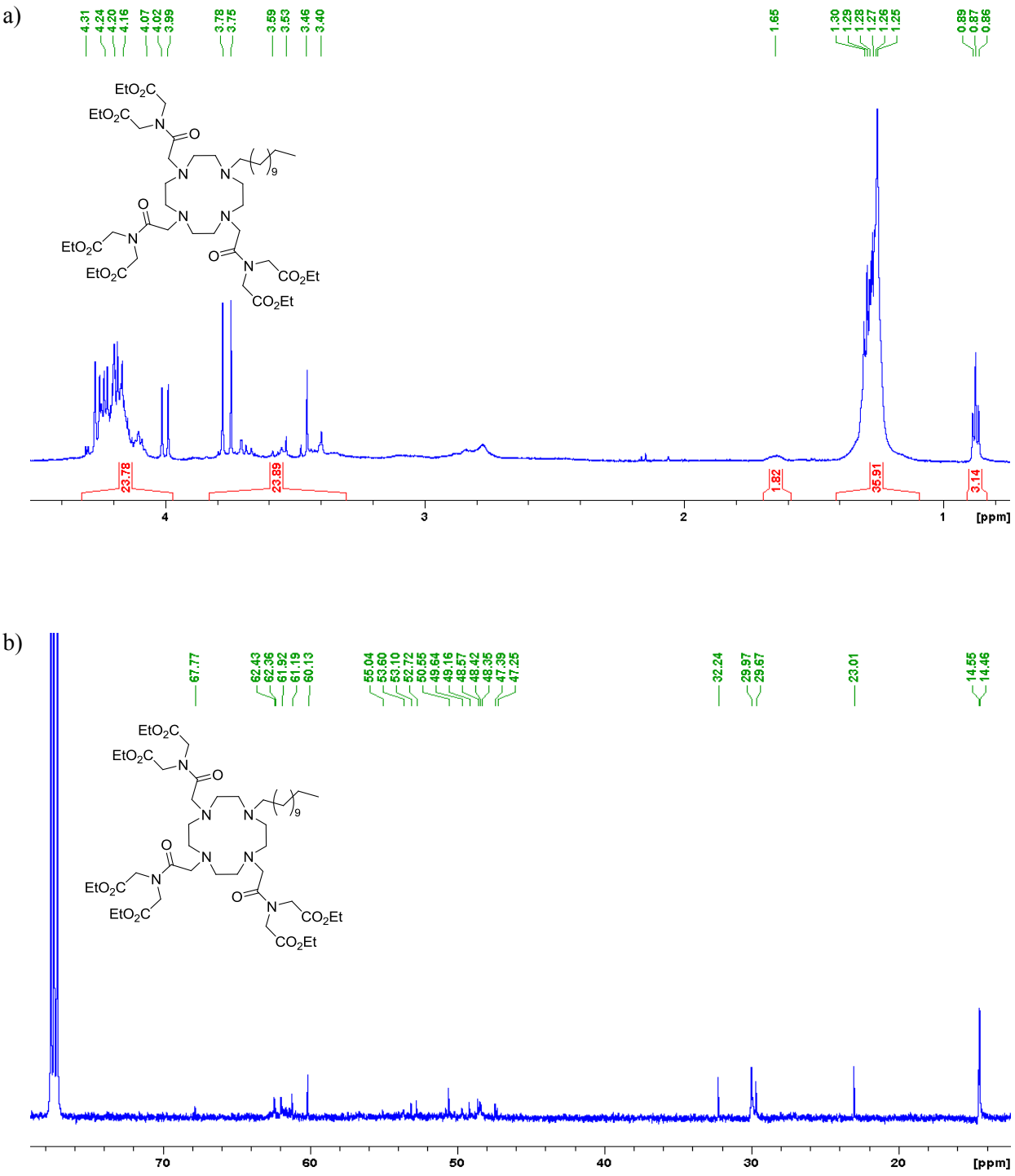


Figure S1. The a) ^{1}H NMR (600 MHz) and b) ^{13}C NMR (600 MHz) spectrum of ligand **1** in $CDCl_3$.

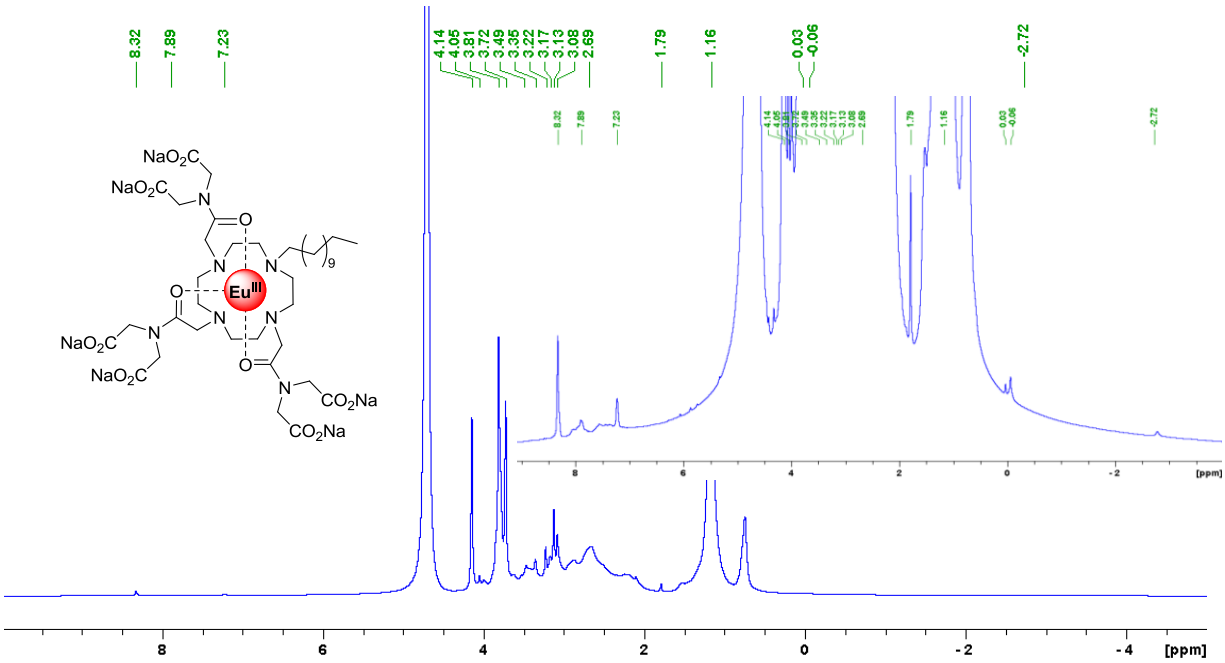


Figure S2. The ^{1}H NMR (600 MHz) spectrum of complex **1.Eu.Na** in D_2O.

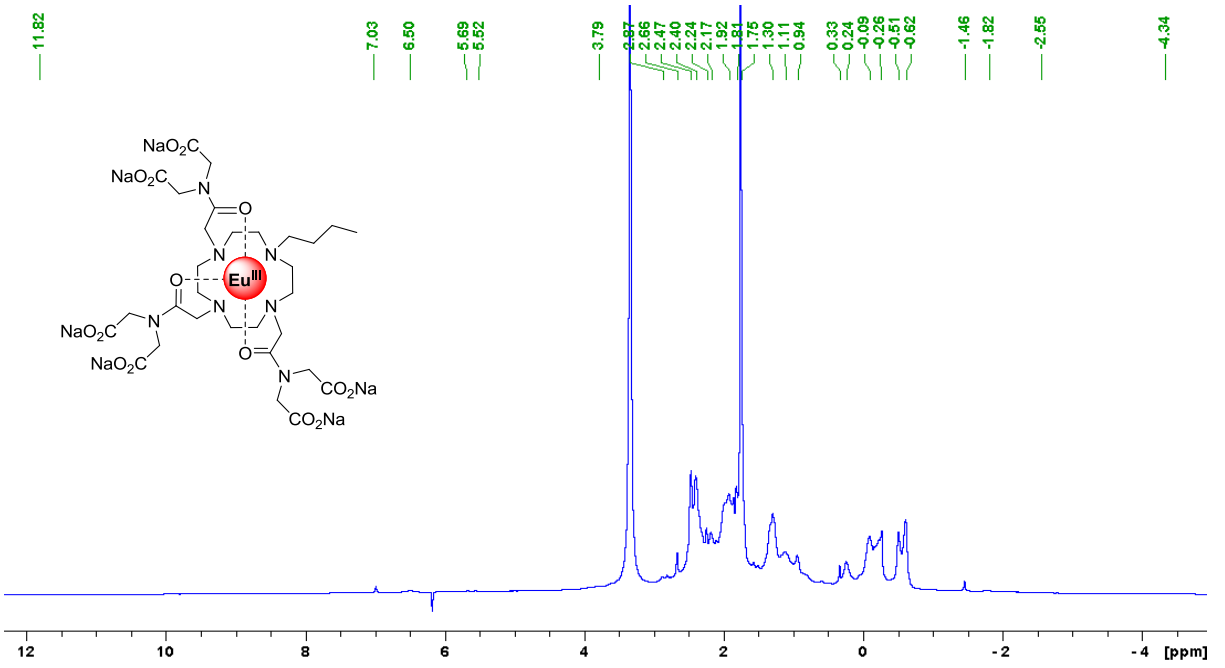


Figure S3. The ^{1}H NMR (600 MHz) spectrum of complex **4.Eu.Na** in D_2O.

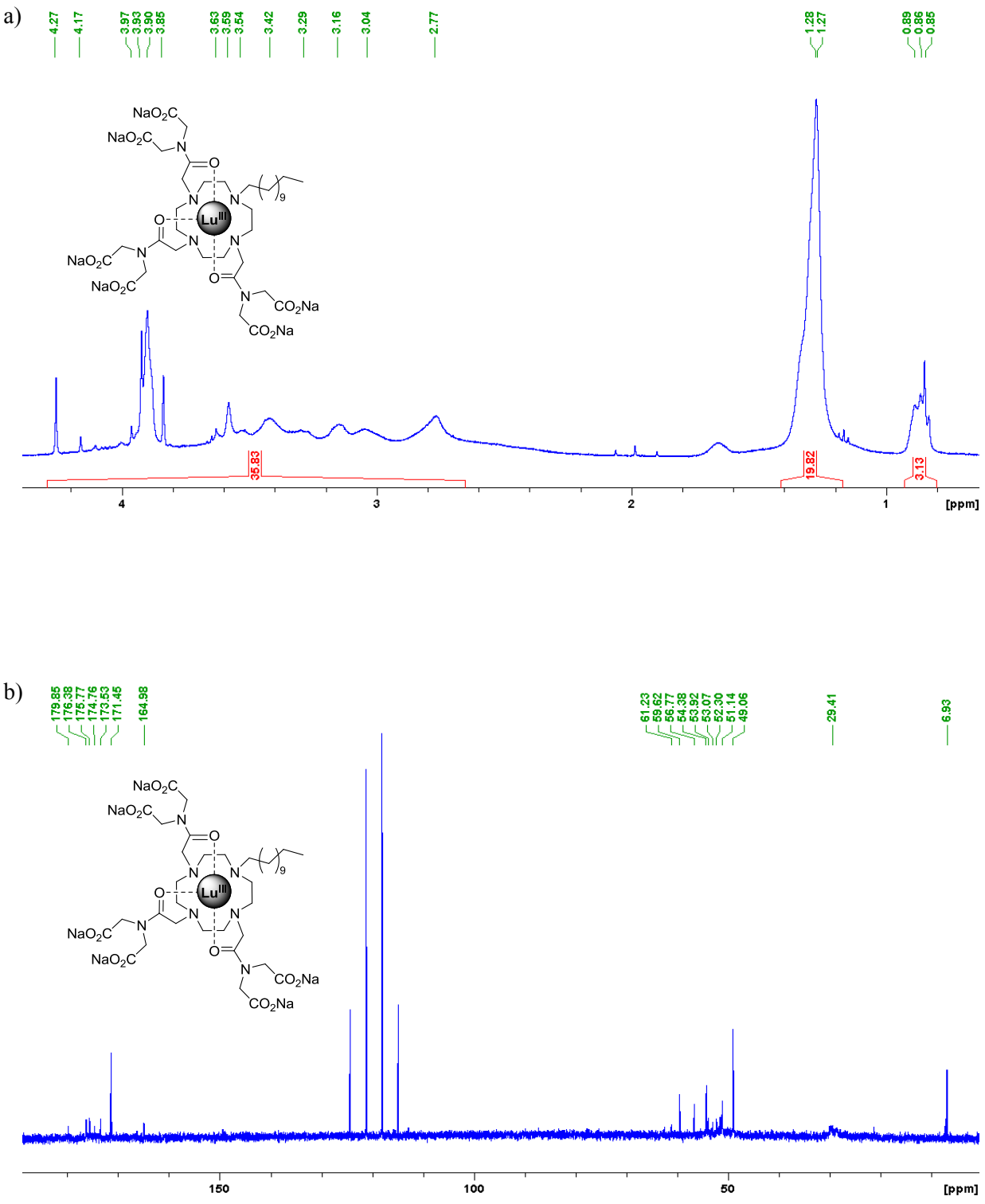


Figure S4. The a) ^{1}H NMR (600 MHz) and b) ^{13}C NMR (600 MHz) spectrum of complex **1.Lu.Na** in D_2O.

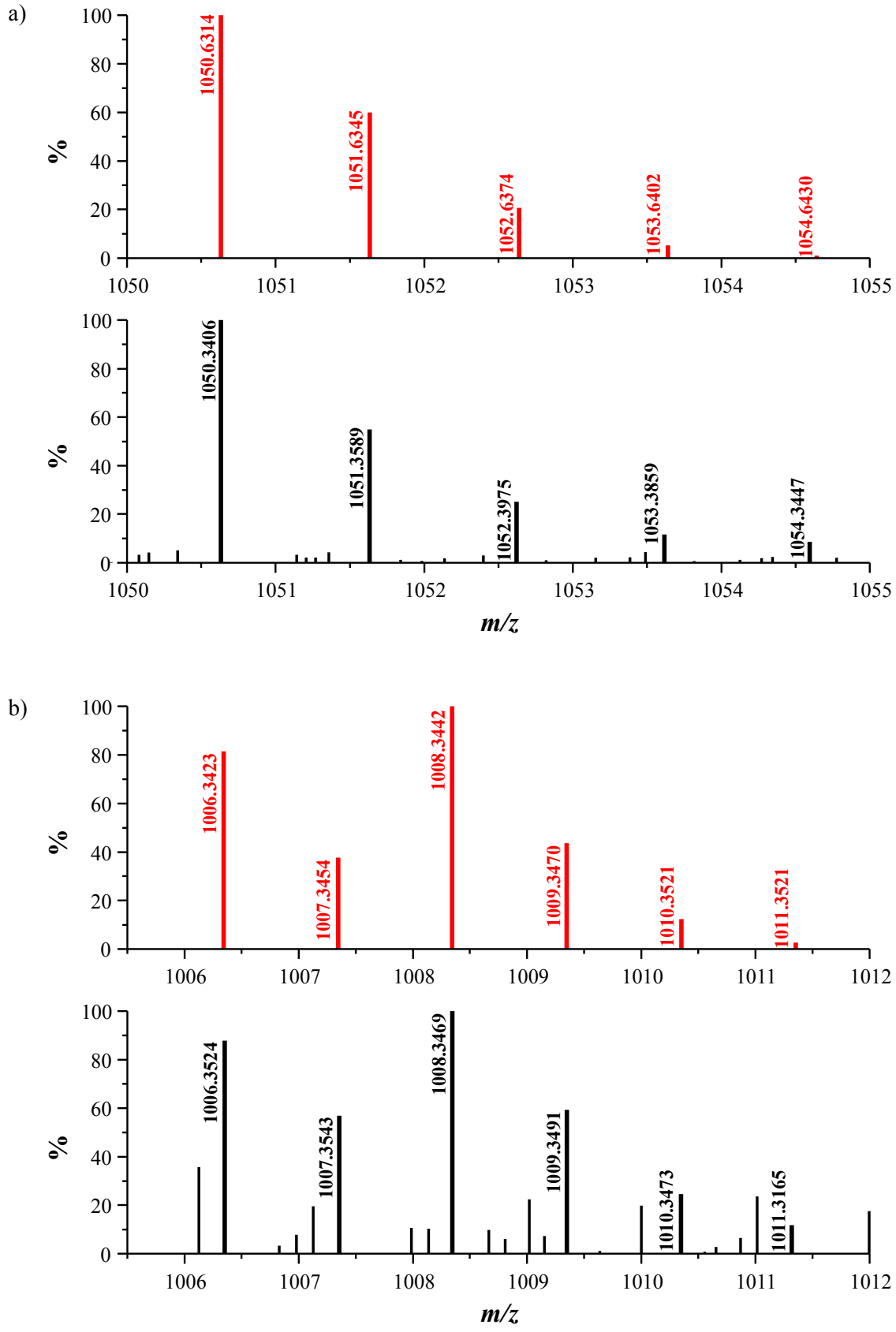


Figure S5. The calculated (red) and the observed (black) HRMS isotopic distribution pattern for a) ligand **1** and b) complex **1.Eu.Na**.

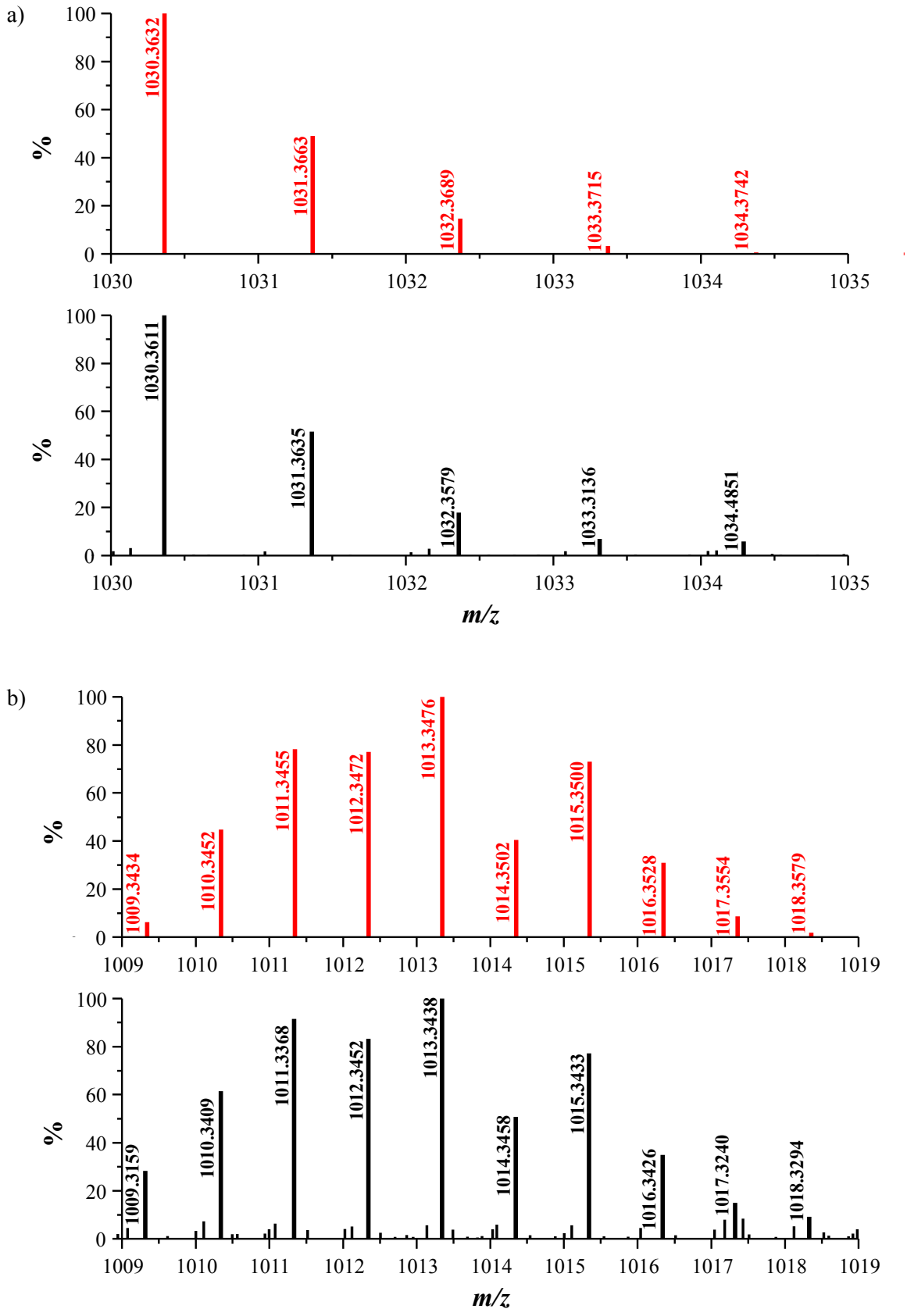


Figure S6. The calculated (red) and the observed (black) HRMS isotopic distribution pattern for a) complex **1.Lu.Na** and b) complex **1.Gd.Na.**

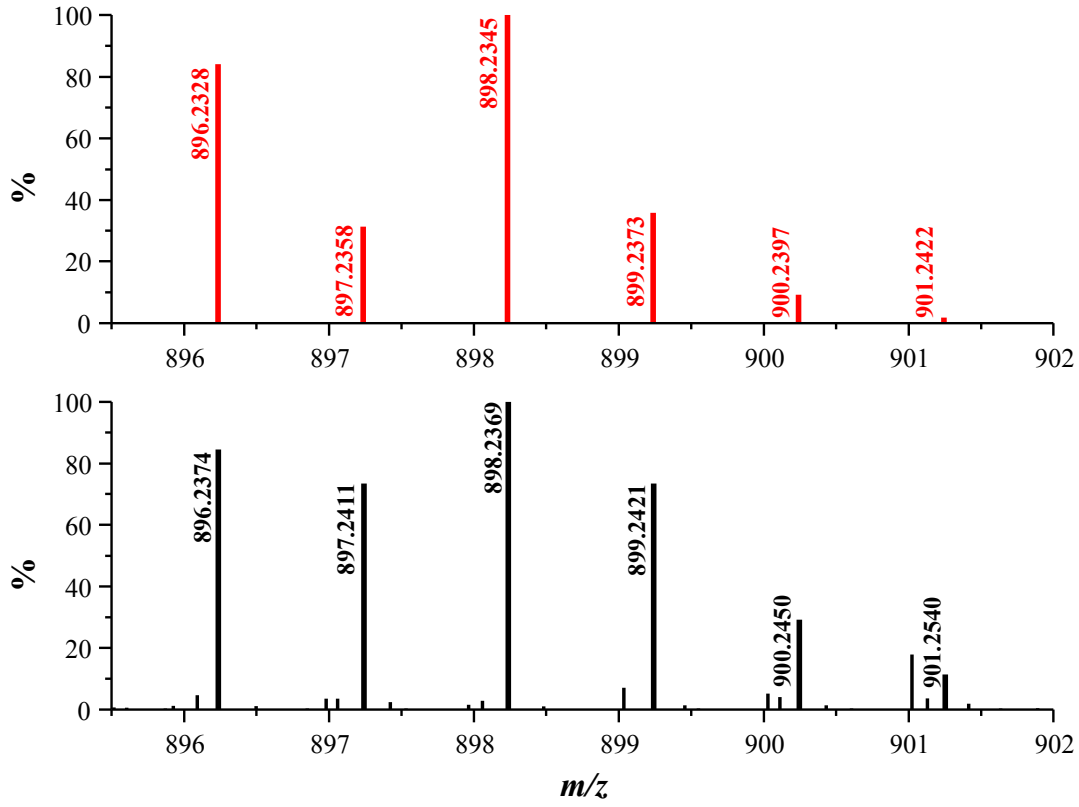


Figure S7. The calculated (red) and the observed (black) HRMS isotopic distribution pattern for complex **4.Eu.Na**.

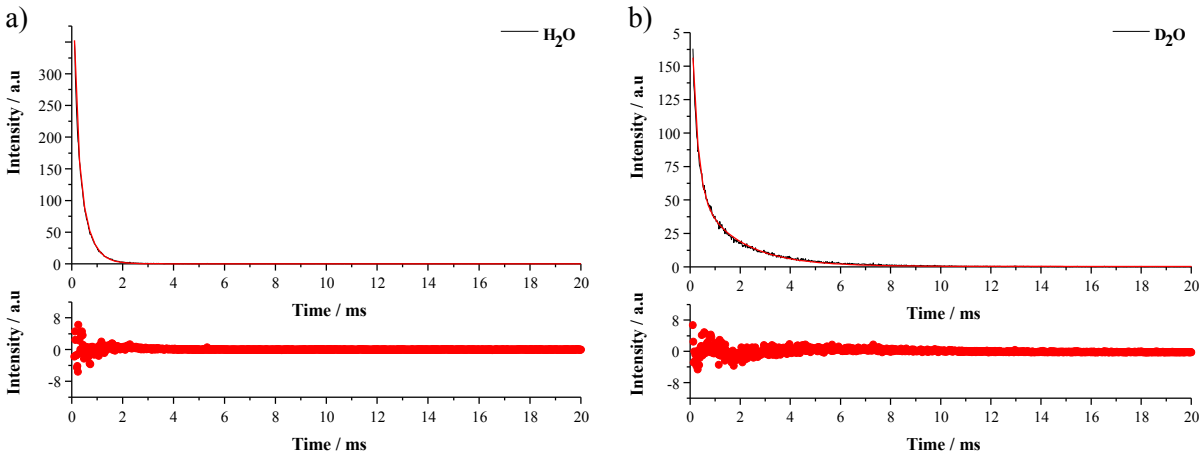


Figure S8. Luminescence lifetime decay of **1.Eu.Na** fitted with a mono-exponential function in a) H_2O and b) D_2O.

Calculation of critical micelle concentration (CMC) from UV/vis, Fluorescence, Resonance Rayleigh Scattering and Relaxometric Titrations

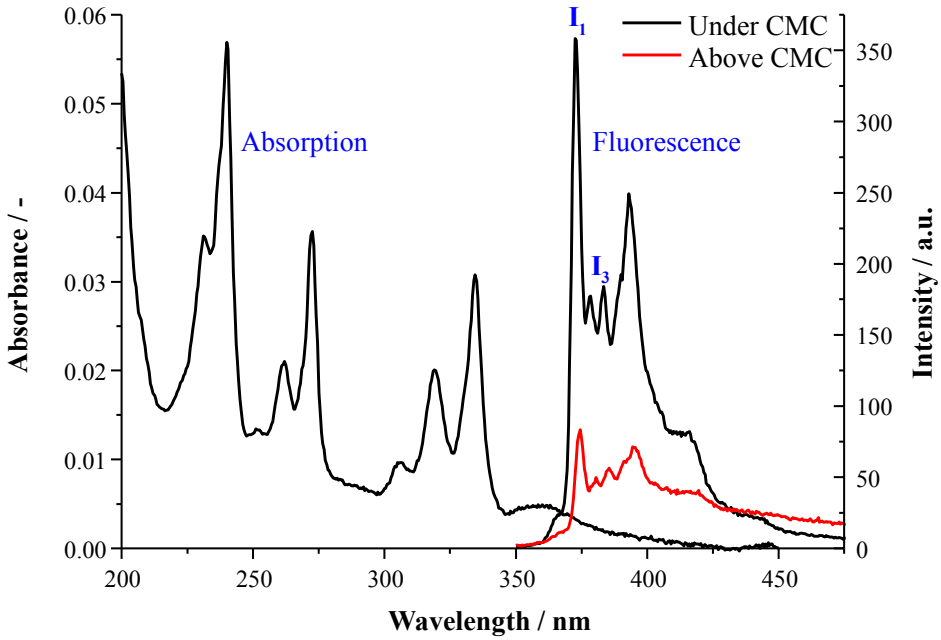


Figure S9. Absorption spectra fluorescence spectra of pyrene (2 μM) in its monomeric form, measured in an aqueous solution of **1.Lu.Na** above and below the cmc at 298 K (λ_{exc} = 334 nm).

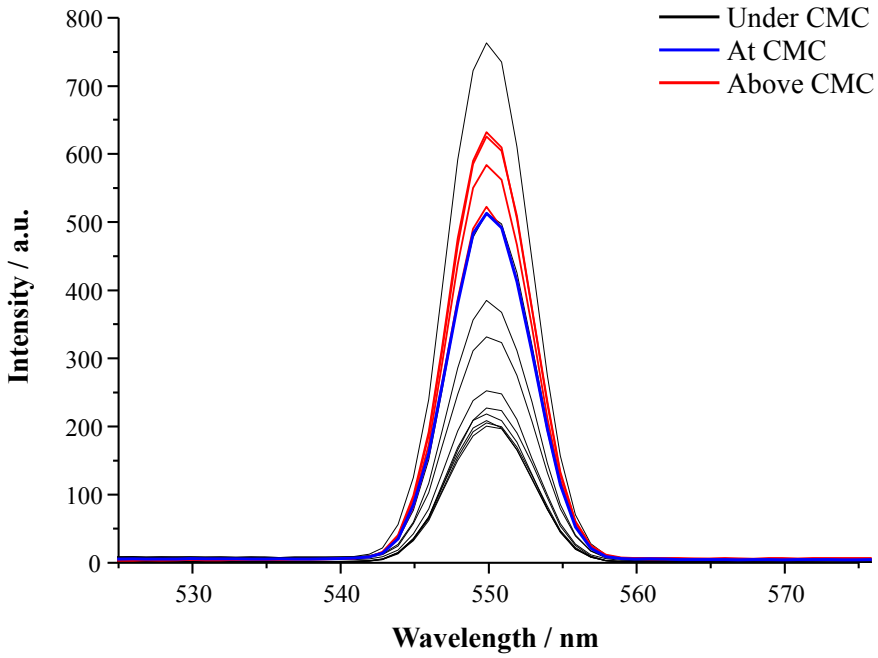


Figure S10. The RRS spectra of different concentrations of **1.Lu.Na** (0.0 → 10.0mM) in H_2O at 298 K (λ_{exc} = 550 nm).

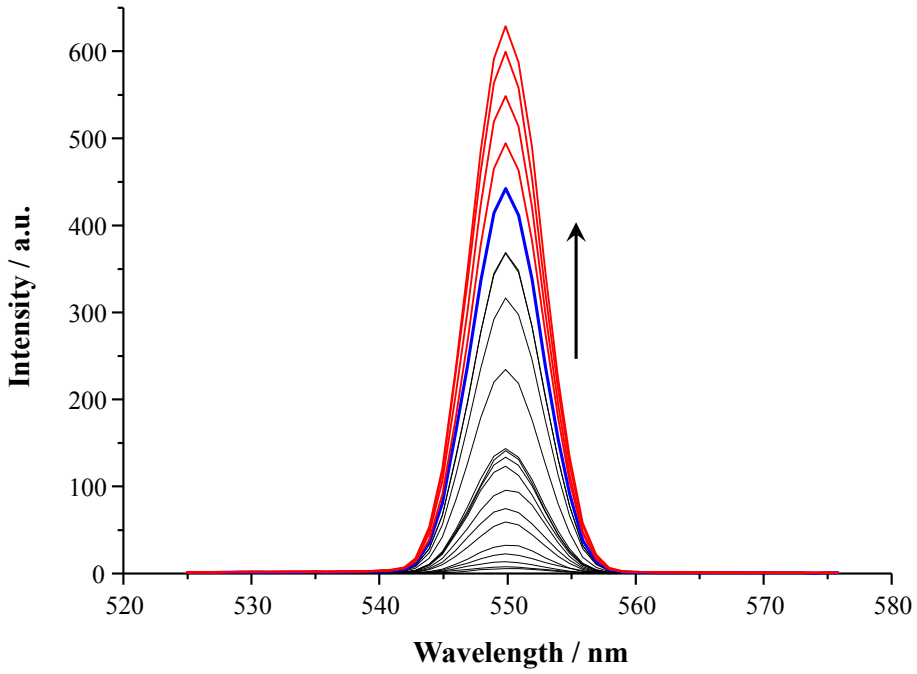


Figure S11. The RRS spectra of different concentrations of **4.Eu.Na** (0.0 → 10.0mM) in H_2O at 298 K (λ_{exc} = 550 nm).

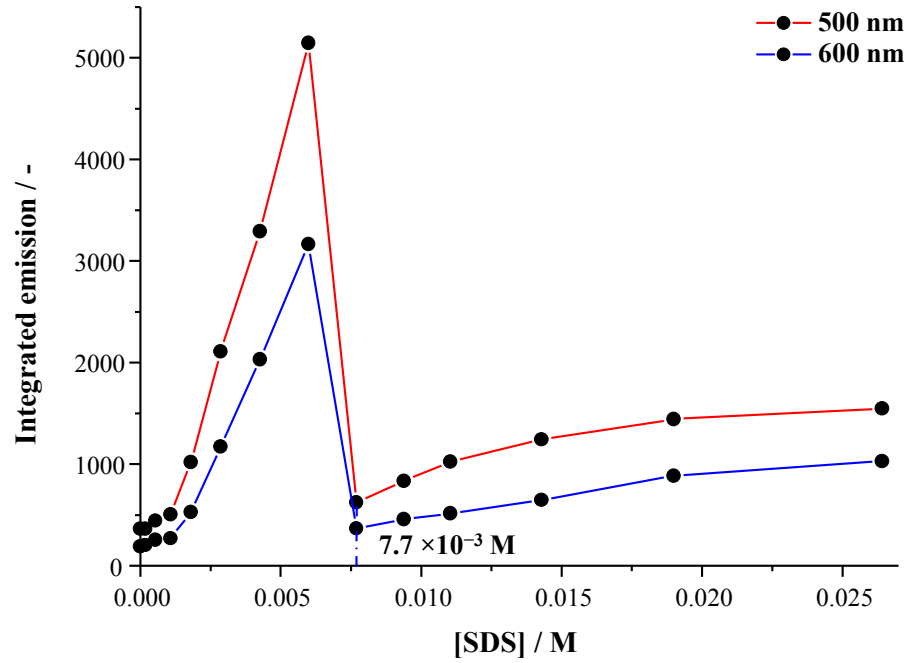


Figure S12. Changes in the RRS intensity ($\lambda_{exc} = \lambda_{em}$) upon excitation at 500 and 600 nm as a function of SDS concentration. Blue dashed line indicates the CMC value of SDS.

Table S1. Tau values calculated from the luminescence lifetime decay and the corresponding *q* value.

τ_{H2O}	τ_{D2O}	*q value*
0.39	**1.33**	**1.89 ± 0.5**

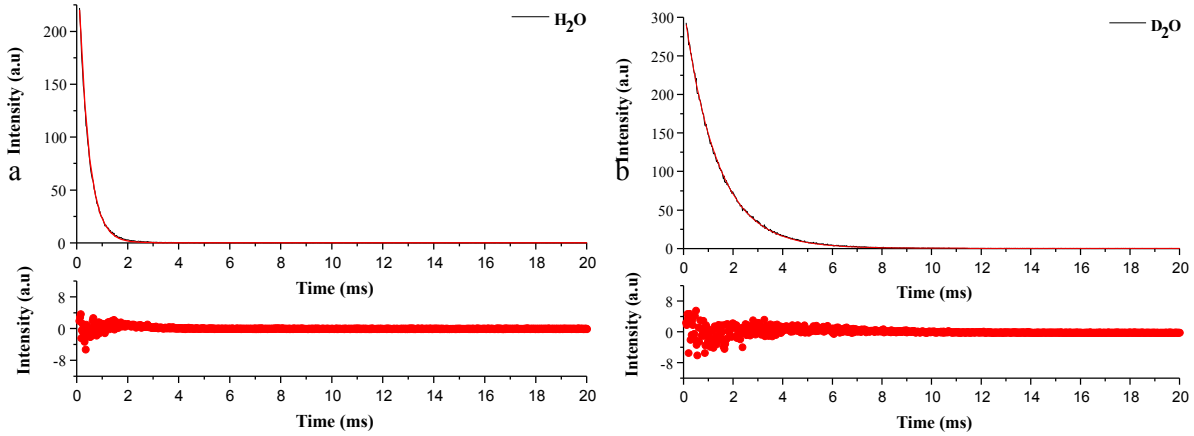


Figure S13. Luminescence lifetime decay of **4.Eu.Na** fitted with a mono-exponential function in a) H_2O and b) D_2O.

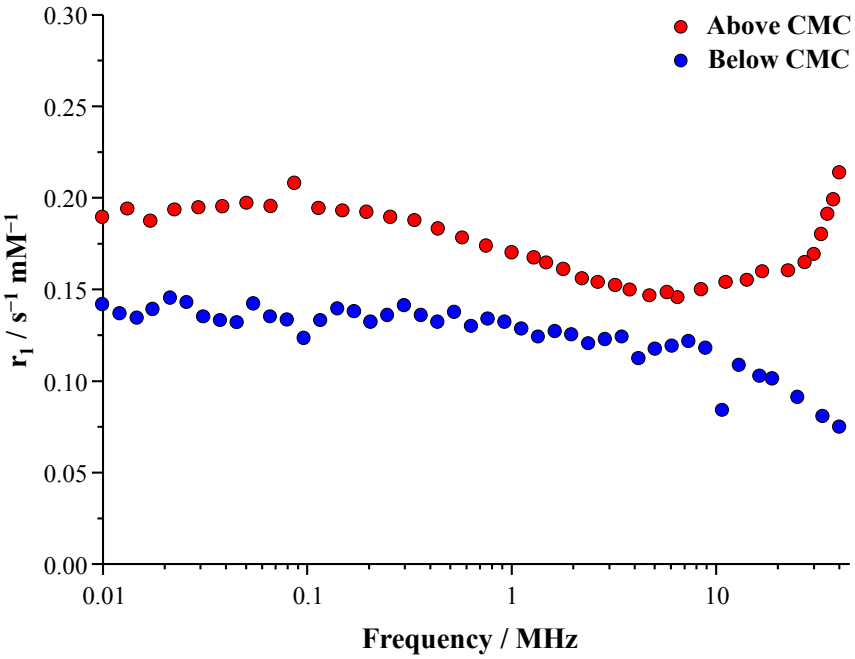


Figure S14. ^{1}H NMRD profiles of **1.Gd.Na** suspensions in the monomeric (blue circles, 3 mM) and micellar (red circles, 27 mM) state in H_2O at 298 K, measured between 0.01–40.00 MHz.

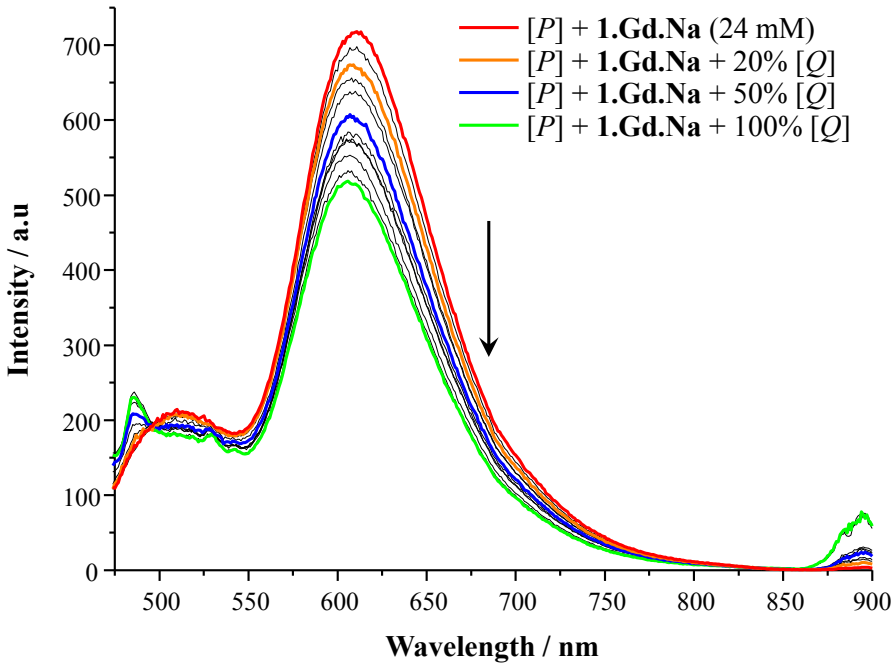


Figure S15. The luminescent response of $[Ru(bpy)_3]^{2+}$ ([*D*] = 0.00693 mM) upon titrating with 9-methylanthracene (0.0 → 0.6 mM, 0 → 100% [*Q*]) in H_2O at 298 K (λ_{exc} = 450 nm), in the presence of **1.Gd.Na** (24 mM).

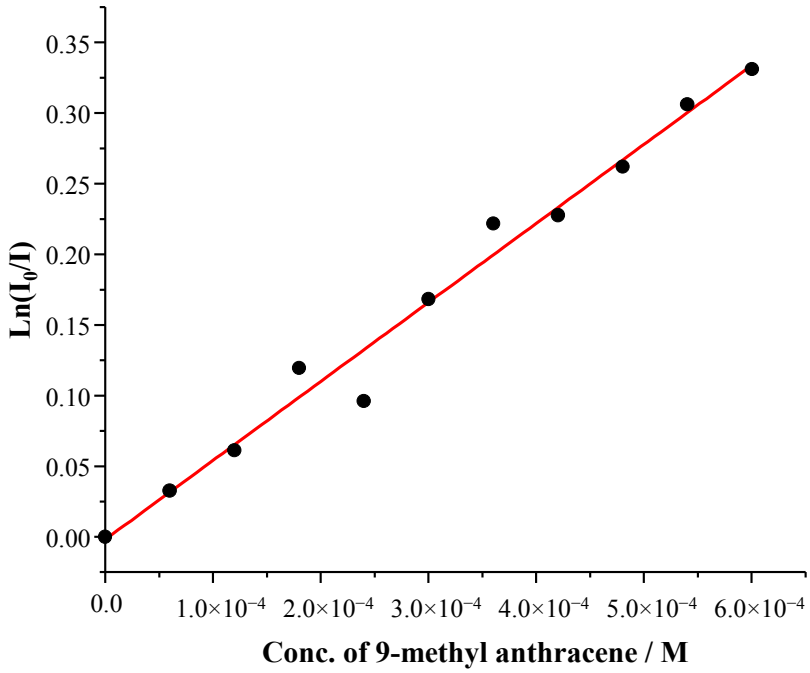


Figure S16: Changes in the Ru(II) luminescence when in the presence of **1.Gd.Na**, as a function of quencher (9-methylanthracene) concentration, measured at 625 nm. Red line indicates the linear fit.

$$(I/I_0) = e^{(-[Q]/[M])} \qquad \text{Equation 2}$$

$$[M] = ([S] - CMC) / \tilde{n} \qquad \text{Equation 3}$$

Where:

[Q] = Quencher concentration (9-methyl anthracene)

[M] = Micellar concentration

[S] = Bulk concentration of either **1.Gd.Na** or **SDS**

CMC = Critical micellar concentration

$\tilde{n}$ = Mean aggregation number

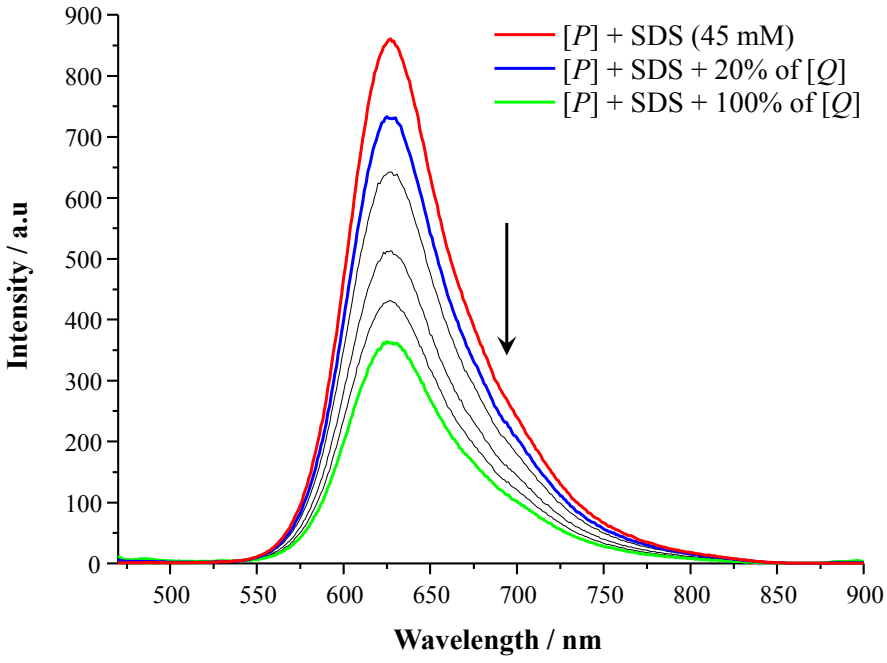


Figure S17: The luminescent response of $[Ru(bpy)_3]^{2+}$ ([*D*] = 0.00693 mM) upon titrating with 9-methylanthracene (0.0 → 0.6 mM, 0 → 100% [*Q*]) in H_2O at 298 K (λ_{exc} = 450 nm), in the presence of **SDS** (45 mM).

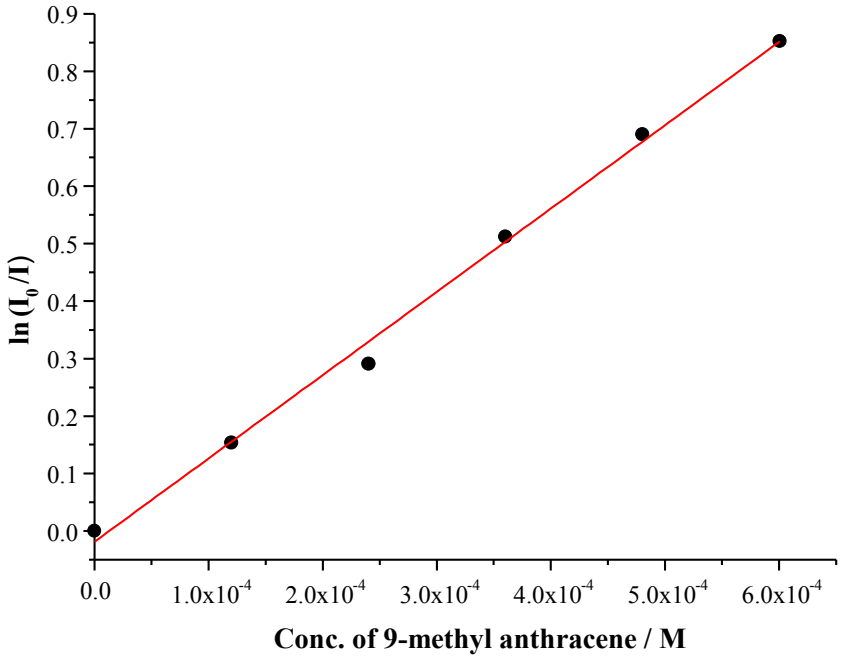


Figure S18: Changes in the Ru(II) luminescence when in the presence of **1.Gd.Na**, as a function of quencher (9-methylanthracene) concentration, measured at 625 nm. Red line indicates the linear fit.

Table S2: Summary of the calculated relaxivity values per micelle, based on each micelle being composed of 11 monomeric **1.Gd.Na** chelates, measured at 25 ˚C in H_2O.

Frequency / MHz	r_1 / $s^{-1}mM^{-1}$	r_1 per micelle / $s^{-1}mM^{-1}$
0.01	0.19	2.08
0.1	0.19	2.13
1.0	0.17	1.87
10	0.15	1.65
20	0.16	1.76
30	0.17	1.86
40	0.21	2.35

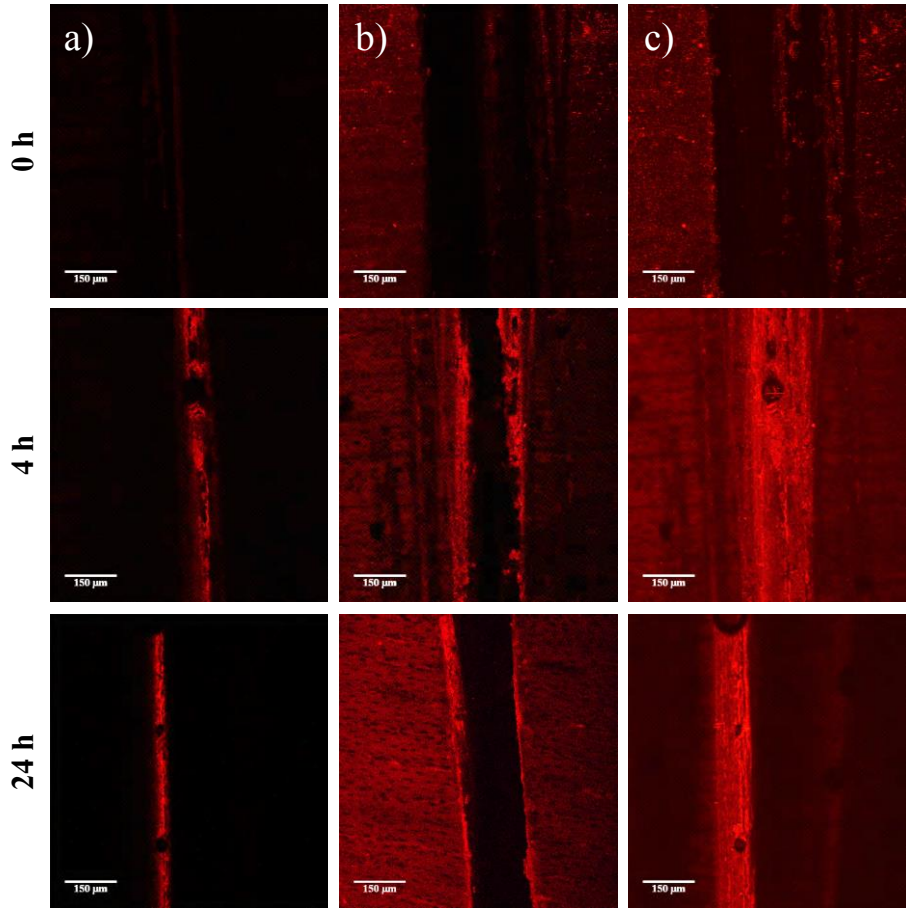


Figure S19: TPE images of scratched bovine bone specimens after being immersed for 4 and 24 hr in an aqueous solution of **1.Eu.Na** (4.5 mM), followed by immersion in an aqueous solution of **nta** (0.15 mM) for 30 s. The presented images show the a) bottom, b) top, and c) whole projection of the microcrack. Acquisition: Ti-Sapphire laser (λ_{exc} = 750 nm); all scale bars = 150 µm.

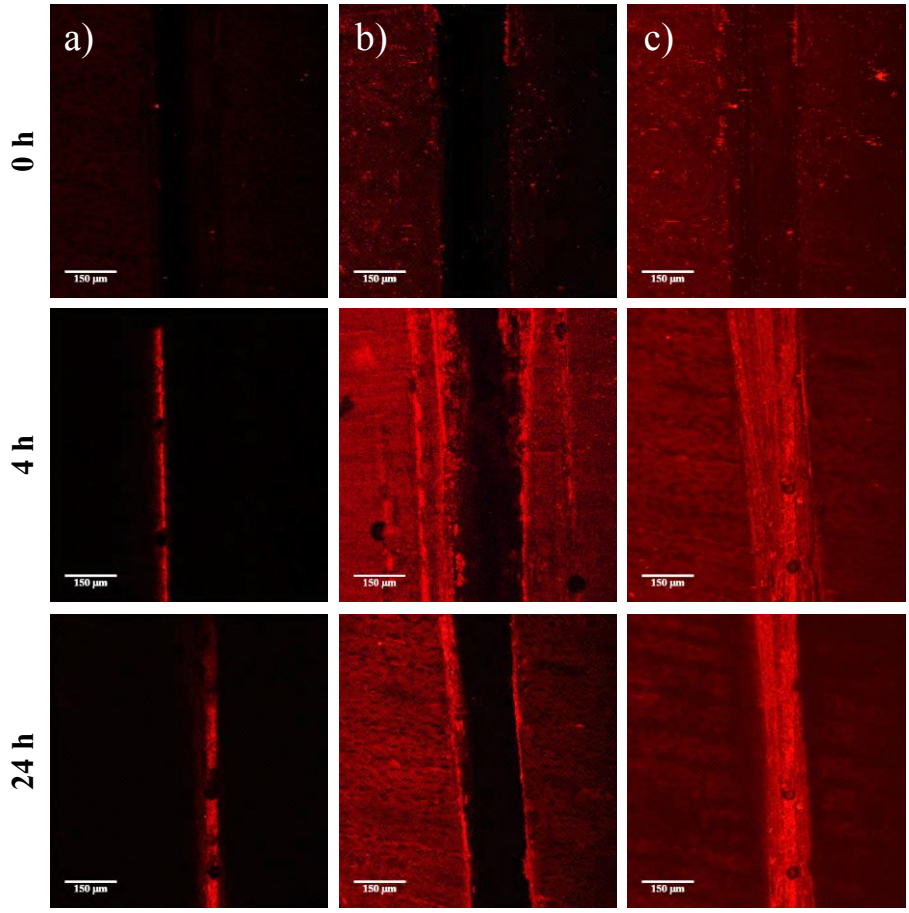


Figure S20: TPE images of scratched bovine bone specimens after being immersed for 4 and 24 hr in an aqueous solution of **1.Eu.Na** (10 mM), followed by immersion in an aqueous solution of **nta** (0.15 mM) for 30 s. The presented images show the a) bottom, b) top, and c) whole projection of the microcrack. Acquisition: Ti-Sapphire laser (λ_{exc} = 750 nm); all scale bars = 150 µm.

References

1 (a) A. M. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. Gareth Williams, M. Woods, *J. Chem. Soc., Perkin Trans. 2*, 1999, 493; (b) W. D. Horrocks, D. R. Sudnick, *J. Am. Chem. Soc.*, 1979, **101**, 334; (c) W. D. Horrocks, D. R. Sudnick, *Acc. Chem. Res.*, 1981.

2 (a) B. McMahon, P. Mauer, C. P. McCoy, T. C. Lee, T. Gunnlaugsson, *J. Am. Chem. Soc.*, 2009, **131**, 17452; (b) B. McMahon, P. Mauer, C. P. McCoy, T. C. Lee, *Aust. J. Chem.,* 2011, **64**, 600; (c) R. Parkesh, S. Mohsin, T. C. Lee, T. Gunnlaugsson, *Chem. Mater.*, 2007, **19**, 1656; (d) F. J. O'Brien, D. Taylor, T. C. Lee, *J. Orthop. Res.*, 2005, **23**, 475.

3 (a) J. Massue, S. E. Plush, C. S. Bonnet, D. A. Moore, T. Gunnlaugsson, *Tetrahedron Lett.,* 2007, **48**, 8052; (b) J. Massue, S.J. Quinn, T. Gunnlaugsson, *J. Am. Chem. Soc.,* 2008, **130**, 6900.