Electronic Supplementary Material (ESI) for ChemComm.

Supplementary information for

Drawing on biology to inspire molecular design: a redox-responsive MRI probe based on Gd(III)-nicotinamide

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Contents

Experimental methods

General remarks

All reagents were obtained from commercial sources and used without further purification. MilliQ water was used to prepare all aqueous solutions.

Electrochemical measurements were acquired using a PGSTAT12 AUTOLAB (Metrohm Autolab B.V., Netherlands) electrochemical analyser. A conventional three-electrode cell configuration was used consisting of a silver wire quasi reference electrode, a platinum gauze auxiliary electrode and a 3 mm diameter glassy carbon disc working electrode. The working electrode was polished with 0.3 μm and 0.05 μm alumina slurry on a BUEHLER Microcloth® and rinsed with Milli-Q water. Solutions were prepared at a concentration of 1 mM of GdNR1 in MilliQ water containing 0.1 M potassium chloride as a supporting electrolyte. All the potentials were referenced to the Ag^{+}/AgCl couple (1 mM) measured *in situ* as an internal standard. All the electrochemical experiments were performed under an argon atmosphere.

MR phantom images were acquired using a vertical 9.4 T Biospec MRI (Bruker, Ettlingen, Germany). Total time for imaging was 3 min 47 sec. The sample was imaged using a T1 weighted fast low angle shot (FLASH) imaging experiment and acquired using the following parameters: number of averages = 4; effective echo time = 4.1 ms; repetition time = 110.6 ms; field of view = 15.1×14.5 mm; matrix: 128×128; resolution: 118×113 μm with a slice thickness of 5 mm.

NMR spectra were obtained at 300 K on a Bruker Avance 300 or 400 spectrometer, equipped with a 5mm QNP probe with z-gradients or a 5 mm BBFO probe with z-gradients, respectively. All chemical shifts are reported relative to trace isotopic impurities as the internal reference. ^{1}H NMR data were collected at 300 or 400 MHz, and are reported as the downfield chemical shift () in parts per million (ppm), multiplicity, coupling constant (J) in Hz and relative integral. ^{13}C NMR data were collected at 75 or 100 MHz and are reported as the downfield chemical shift () in ppm. NMR data were analysed using TopSpin 3.5 software.

Low resolution mass spectrometry was performed using a Bruker amaZon SL mass spectrometer operating on Electrospray Ionisation (ESI) or a Thermo Finnigan LCQ Advantage mass spectrometer. High-resolution mass spectrometry was performed on a Bruker Apex-Ultra spectrometer operating on ESI using an Apollo II ESI/MALDI dual source. Accurate mass spectra were acquired on a quadrupole orthogonal acceleration time-of-flight mass spectrometer (Synapt G2 HDMS, Waters, Milford, MA). Samples were infused at 3 μL/min and spectra were obtained in positive ionization mode with a resolution of 15000 (FWHM) using leucine enkephalin as lock mass.

HPLC-ESI-MS Agilent 6110 quadrupole mass spectrometer: Grace prevail 3μ reverse phase C18 column, 150 mm length, 2.1 mm internal diameter. Isocratic mobile phase, water 10 mM ammonium acetate pH 7 95% : acetonitrile 5%. Run time 15 min.

TXRF measurements were performed on a Bruker S2 Picofox (Bruker, Berlin, Germany) with a molybdenum source. Lanthanide(III) solutions of approximately 250 ppm in milli-Q water were prepared and 50 µL of this solution was mixed with 50 µL of a 250 ppm Chem-Lab gallium standard solution (1000 µg/mL, 2-5% HNO3). 2 µL of this mixture with similar Ln(III)-Ga(III) concentrations was put on a Bruker AXS quartz glass sample plate for measurement.

Proton nuclear magnetic relaxation dispersion (NMRD) profiles were measured on a Stelar Spinmaster FFC, fast field cycling NMR relaxometer (Stelar, Mede (PV), Italy) over a magnetic field strength range extending from 0.24 mT to 0.7 T. Measurements were performed on 0.5 mL samples contained in 10 mm o.d. pyrex tubes. Additional relaxation rates at 20, 60 and 300 MHz were respectively obtained on a Minispec mq20, a Minispec mq60, and Bruker Avance 300 spectrometers (Bruker, Karlsruhe, Germany). The temperature was equal to 310K. Proton NMRD curves were fitted using data-processing software[1] including different theoretical models describing the nuclear relaxation phenomena (Minuit, CERN Library).[2]

The efficiency of an MRI contrast agent (r_1) results primarily from short-distance dipolar interactions between the Gd(III) centre and coordinated water molecules in exchange with bulk water known as the inner-sphere contribution. Paramagnetic relaxation theory takes into consideration the distance between the water protons and the paramagnetic centre (r), the residence time of coordinated water molecules (τ_M), the rotational correlation time (τ_R), the Gd(III) electronic relaxation time at zero field (τ_{S0}), and the correlation time that modulates the electronic relaxation (τ_V). Additionally, longer-distance dipolar interactions between the Gd(III) complex with water molecules diffusing in its close proximity are considered the outer-sphere contribution to the relaxivity. The outer-sphere effect shows little variation for all Gd(III) complexes and in the equations used to fit the NMRD data, is described by the distance of closest approach (*d*), the relative diffusion coefficient (*D*), as well as again, τ_{S0} and τ_V. In our fit, the parameters *d* and *r* are set to 0.36 and 0.31 nm, respectively (Solomon-Bloembergen-Morgan theory).[2] The water coordination number *q* is set to 2 or 0.3 for these fitting procedures as determined from the luminescence lifetime of the Eu(III) analogue and *D* is equal to 3.3×10^{-9} m^2 s^{-1} for Gd(III) complexes with molar masses that range from 500 to 1000.[3] For this small Gd^{3+} complex, the exchange between the coordinated water molecule and the bulk is expected to be fast so that τ_M was fixed to 100 ns during the fitting procedure. So, in our fitting, *q, d, D, r, and* τ_M are fixed and τ_R, τ_{S0}, and τ_V are variable.

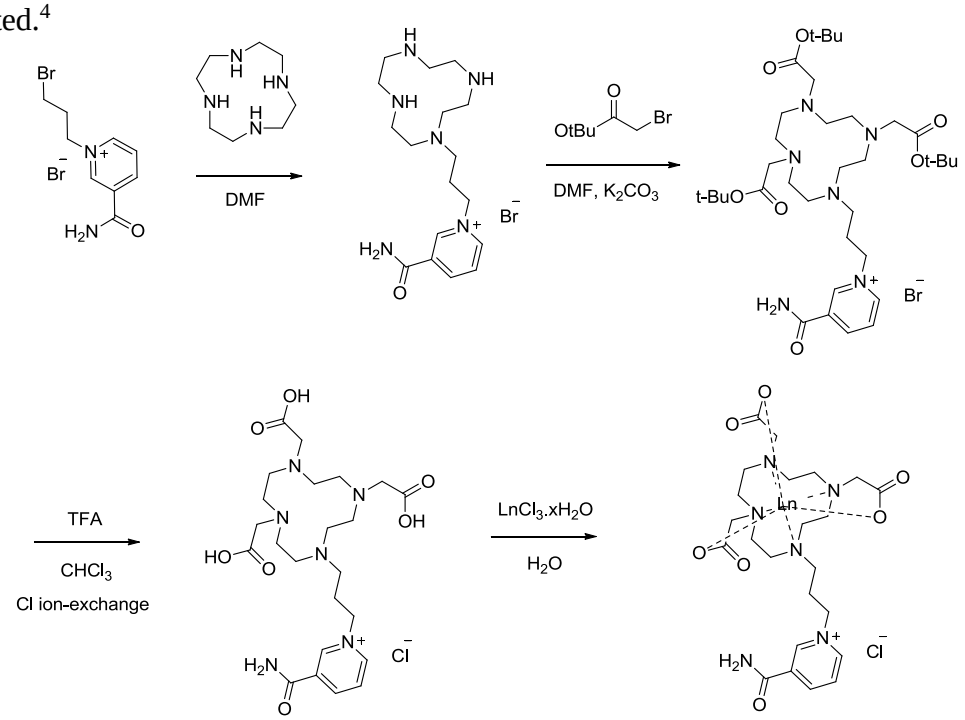
Emission spectra and luminescence decays of the Eu^{III} complex were recorded on an Edinburgh Instruments FS920 steady state spectrofluorimeter. This instrument is equipped with a 450W xenon arc lamp, a high energy microsecond flashlamp mF900H and an extended red-sensitive photomultiplier (185–1010 nm, Hamamatsu R 2658P). All spectra are corrected for the instrumental functions. Luminescence decays were determined under ligand excitation (400 nm) monitoring emission of the $^5D_0 \rightarrow {}^7F_2$ transition of the Eu^{III} complex. Luminescence decays were analyzed using Edinburgh software; lifetimes are averages of at least three measurements.

DLS measurements: Photon correlation spectroscopy was performed at 25 °C with a BIC multiangle laser light scattering system with a 90° scattering angle (Brookhaven Instruments Corporation, Holtsville, USA). The intensity weighted diameter was measured on 0.1 wt% diluted suspensions in Milli-Q water and calculated by a non-negatively constrained least-squares (multiple pass) routine.

Cellular studies for viability used the DLD-1 cells. Cells were maintained in exponential growth as monolayers at 37 ºC in 5% CO_2 humidified Dulbecco's modified eagle medium (DMEM) was used supplemented with 2.5 mM glutamine, 10% fetal calf serum and antibiotics as per supplier's instructions.

Synthetic procedures

The starting material, 1-(3-bromopropyl)-3-carbamoylpyridin-1-ium bromide, was synthesized as reported.[4]



Scheme S1. Synthesis of ligand and complex.

1-(3-(1,4,7,10-Tetraazacyclododecan-1-yl)propyl)-3-carbamoylpyridin-1-ium bromide

Step 1 was both isolated for characterisation and carried over directly into step 2 on different occasions.

Cyclen (2 eq., 1.063 g, 6.17 mmol) was dissolved in dry DMF (100 mL) and 1-(3-bromopropyl)-3-carbamoylpyridin-1-ium bromide (1 eq., 1 g, 3.09 mmol) was added in one portion and the reaction placed under nitrogen atmosphere. To assist with dissolution, the reaction was placed in an ultrasonic bath at room temperature for 30 min followed by stirring for 3 d, during which time a dark yellow/brown precipitate formed. The DMF was removed by

rotary evaporation and the remaining solid was washed with chloroform and diethyl ether and dried. Yield: 1.28 g, quantitative. ^{1}H NMR (300 MHz, CD_3OD), δ = 9.61 (1H, s, Ar), 9.30 (1H, d, Ar), 9.02 (1H, d, Ar), 8.28 (1H, t, Ar), 4.79 (under solvent, 2H, t, CH_2 propyl), 2.88-2.74 (16H,br, CH_2 ring), 2.35 ppm (2H, m, propyl CH_2). ESI-MS, positive mode, found *m/z*, 355.22 $[M]^+$; calculated for $[C_{17}H_{31}N_6O]^+$ *m/z*, 355.26.

When carried over directly to step 2, the solid was not isolated and following reactants were added directly to the reaction mixture.

3-Carbamoyl-1-(3-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)propyl)pyridin-1-ium bromide

Dry DMF (100 mL) was added to 1-(3-(1,4,7,10-tetraazacyclododecan-1-yl)propyl)-3-carbamoylpyridin-1-ium bromide (1 eq., 1.28 g, 3.09 mmol), and potassium carbonate (8 eq., 3.41 g, 24.69 mmol) and the mixture was placed in an ultrasonic bath at room temp under nitrogen atmosphere. To this mixture, tert-butyl 2-bromoacetate (8 eq., 4.82 g, 24.69 mmol) was added and the reaction was stirred for 3 days. Chloroform (200 mL) was added and the reaction was filtered and the solvents were removed by rotary evaporation. The remaining solid material was dissolved in a minimum amount of methanol and added to water in a separating funnel. The aqueous layer was washed with diethyl ether and ethyl acetate until the organic extractions were clear and no trace of mass peak 651 was visible by ESI-MS. The water was collected and evaporated yielding a red/brown solid. Yield: 30%, 0.63 g. ^{1}H NMR (300 MHz, CD_3OD, 300 K), δ = 9.62 (1H, s, Ar), 9.35 (1H, d, Ar), 9.02 (1H, d, Ar), 8.29 (1H, t, Ar), 4.76 (under solvent, 2H, t, CH_2 propyl), 3.88-2.74 (24H,br, CH_2 ring), 2.38 (2H, br, propyl CH_2), 1.55, 1.53 ppm (18H, s, t-Bu). ^{13}C NMR (75 MHz, CD_3Cl, 300 K), δ = 172.3 (2xC=O), 171.79 (C=O), 162.55 (NH_2C=O), 147.33 (Ar), 145.02 (Ar), 144.50 (Ar), 133.59 (Ar), 128.10 (Ar), 82.02 (t-Bu <u>C</u>O), 60.21 (propyl CH2), 57.49 (br, N<u>C</u>H_2CO), 50.70 (br, ring CH_2N and propyl CH_2), 28.06 (t-Bu <u>C</u>H_3), 25.69 (propyl-CH_2) ppm. ESI-MS, positive mode, found *m/z*, 677.45 $[M]^+$, 339.21 $[M+H]^{2+}$; calculated for $[C_{17}H_{31}N_6O]^+$ *m/z*, 677.46, $[C_{35}H_{62}N_6O_7]^{2+}$ *m/z*, 339.23.

3-Carbamoyl-1-(3-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)propyl)pyridin-1-ium bromide

3-Carbamoyl-1-(3-(4,7,10-tris(2-(tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)propyl)pyridin-1-ium chloride (0.5 g) was dissolved in chloroform (5 mL) under nitrogen atmosphere, TFA (5 mL) was added and stirred overnight at room temp. After which the solvents were evaporated and the solid resuspended and evaporated a few times in dichloromethane before final drying. The resulting ligand was dissolved in water and passed through a chloride ion exchange column.

Yield: Quantitative. ^{1}H NMR (400 MHz, D_2O, 300 K), δ = 9.310 (1H, d, Ar), 9.00 (1H, d, Ar), 8.85 (1H, d, Ar), 8.16 (1H, t, Ar), 4.57 (2H, br, CH_2 propyl), 3.8-2.90 (24H,br, CH_2 ring), 2.35 ppm (2H, br, propyl CH_2). ^{13}C NMR (75 MHz, CD_3OD, 300 K), δ = 165.39 (2xC=O), 162.85 (C=O), 162.38 (NH_2C=O), 148.07 (Ar), 146.38 (Ar), 145.74 (Ar), 136.06 (Ar), 129.78 (Ar), 60.58 (propyl CH_2), 55.62 (br, N<u>C</u>H_2CO), 54.67 (propyl CH_2), 50.86 (br, ring CH_2N), 26.8

(propyl CH_2) ppm. ESI-MS, positive mode, found *m/z*, 509.23 $[M]^+$, 274.07 $[M+K]^{2+}$; calculated for $[C_{23}H_{37}N_6O_7]^+$ *m/z*, 509.27, $[C_{23}H_{37}KN_6O_7]^{2+}$ *m/z*, 274.12.

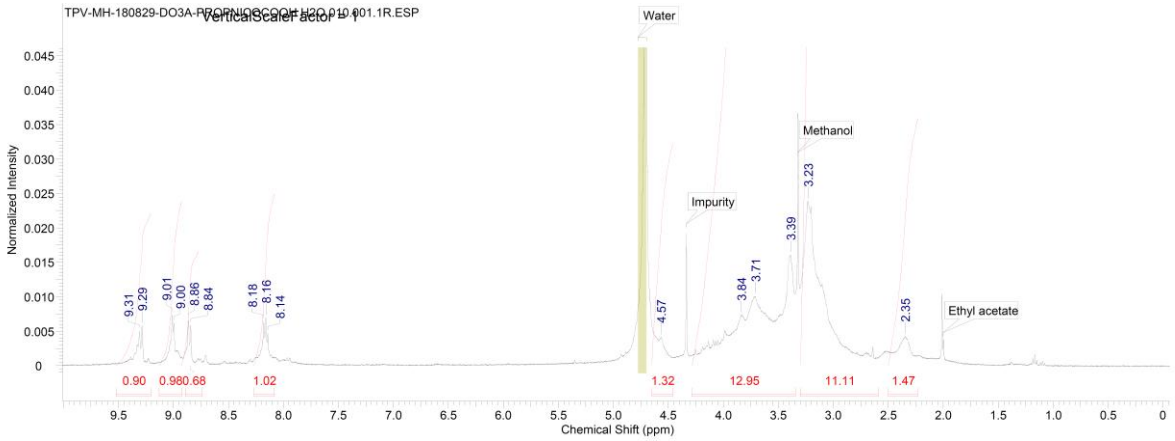


Figure S1. 400 MHz 1H spectrum of 3-carbamoyl-1-(3-(4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododecan-1-yl)propyl)pyridin-1-ium bromide.

Lanthanide(III) complexes

The free ligand (1 eq., 100 mg, 0.183 mmol) was dissolved in deionized water (5 mL) and the pH was adjusted to 6.5 with a few drops of 1 mM sodium hydroxide solution. An aqueous solution of the relevant hydrated lanthanide(III) chloride salt (1.1 eq., ~0.05 g) was added while monitoring the pH and adjusting if needed. After stirring for 3 h, the water was removed my rotary evaporation. The final solid material was washed liberally with methanol and dried.

^{157}Gd(III)-complex. Yield: 0.1 g, 78% . ESI-MS, positive mode, found *m/z*, 664.11 $[M]^+$; calculated for $[C_{23}H_{34}GdN_6O_7]^+$ *m/z*, 664.17. MADLI-TOF, found *m/z*, 664.129 $[M]^+$; calculated for $[C_{23}H_{34}GdN_6O_7]^+$ *m/z*, 664.172. HRMS, found 664.1724 $[M]^+$; calculated for $[C_{23}H_{34}GdN_6O_7]^+$ *m/z*, 664.1724.

^{152}Eu(III)-complex. Yield: 0.11 g, 86%. ESI-MS, positive mode, found *m/z*, 659.13 $[M]^+$; calculated for $[C_{23}H_{34}EuN_6O_7]^+$ *m/z*, 659.17. MADLI-TOF, found *m/z*, 659.190 $[M]^+$; calculated for $[C_{23}H_{34}EuN_6O_7]^+$ *m/z*, 659.170.

^{139}La(III)-complex. Yield: 0.08 g, 57%. ^{1}H NMR (300 MHz, D_2O), δ = 9.34 (1H, s, Ar), 9.03 (1H, d, Ar), 8.88 (1H, d, Ar), 8.18 (1H, t, Ar),), 4.63 (under water suppression, 2H, t, CH_2 propyl), 3.8-2.71 (24H,br, CH_2 ring), 2.29 ppm (2H, br, propyl CH_2). ^{13}C NMR (75 MHz, D_2O, 300 K), δ = 180.05 (C=O), 165.69 (NH_2C=O), 146.55 (Ar), 144.46 (Ar), 144.33 (Ar), 134.14 (Ar), 128.68 (Ar), 60.41 (Br), 53,66 (Br), 48.97 (Br), 47.88 (Br), 22.78 (propyl CH_2) ppm. ESI-MS, positive mode, found *m/z*, 645.10 $[M]^+$; calculated for $[C_{23}H_{34}LaN_6O_7]^+$ *m/z*, 645.15. MADLI-TOF, found *m/z*, 645.114 $[M]^+$; calculated for $[C_{23}H_{34}LaN_6O_7]^+$ *m/z*, 645.155.

Supplementary data

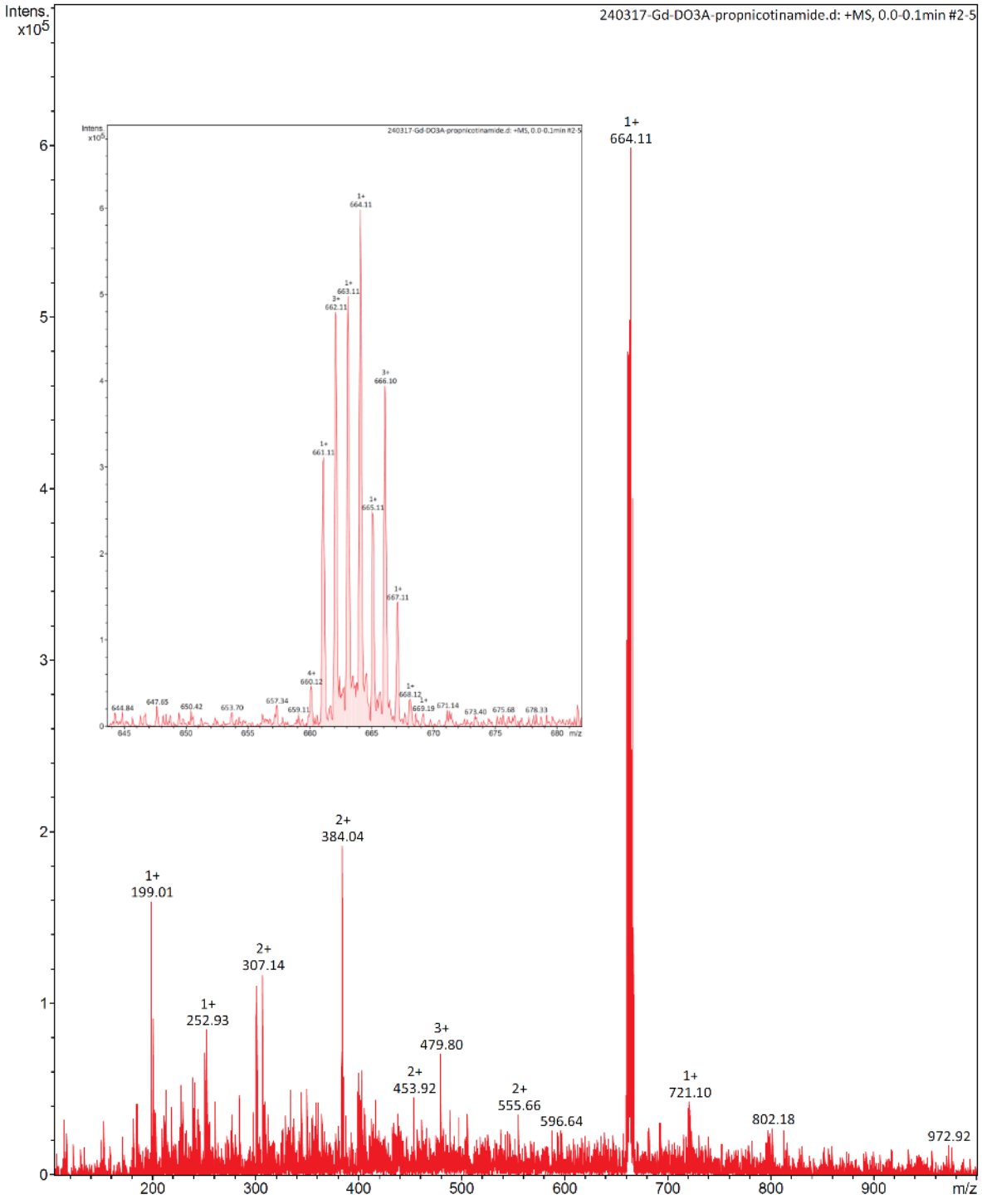


Figure S2. ESI-MS GdNR1. Inset: expansion of isotopic pattern.

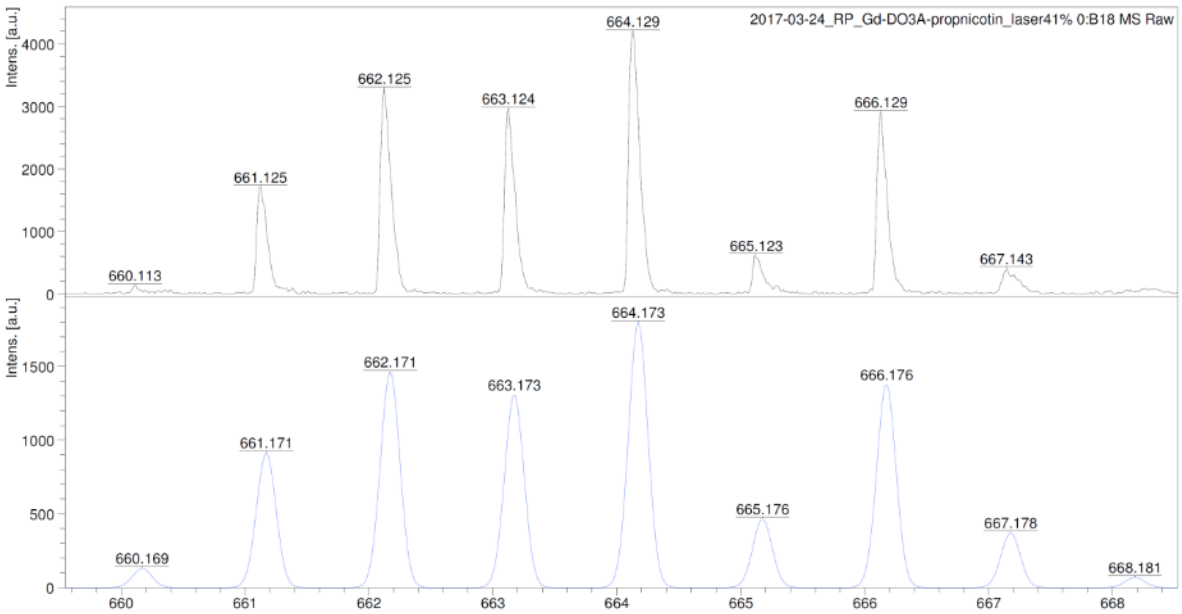


Figure S3. MALDI-TOF spectrum GdNR1. Upper: measured spectrum. Lower: predicted spectrum.

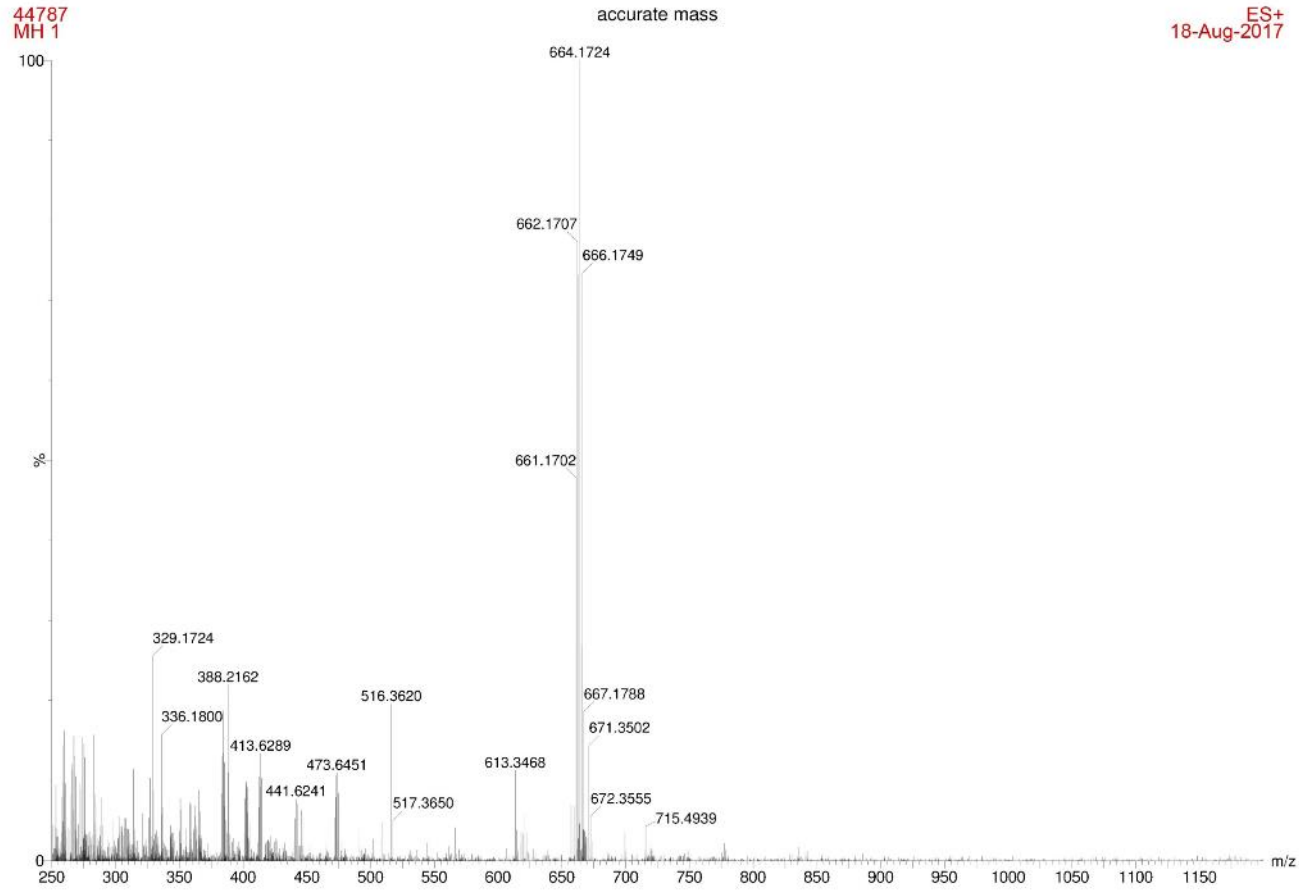


Figure S4. HRMS of GdNR1.

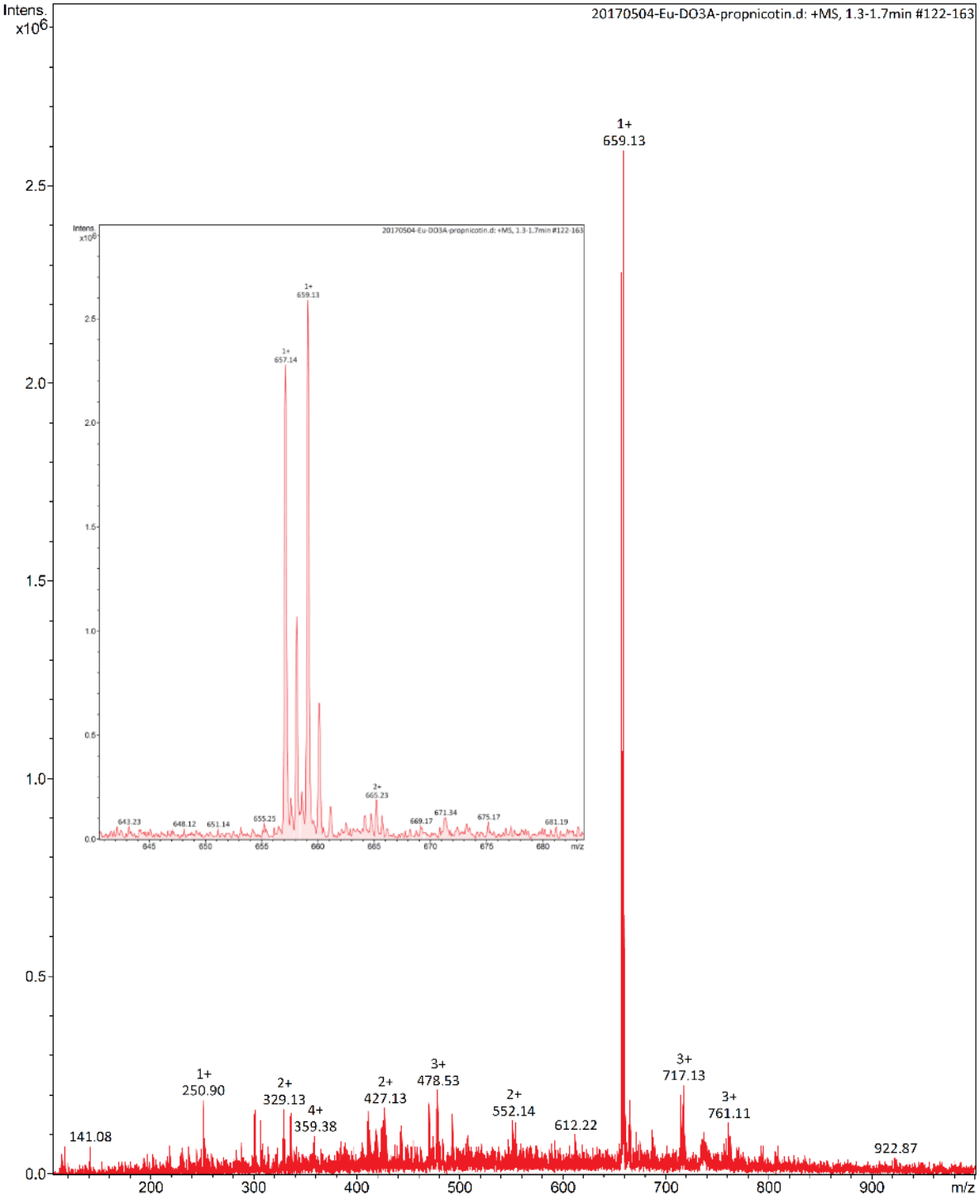


Figure S5. ESI-MS Eu-complex. Inset: expansion of isotopic pattern.

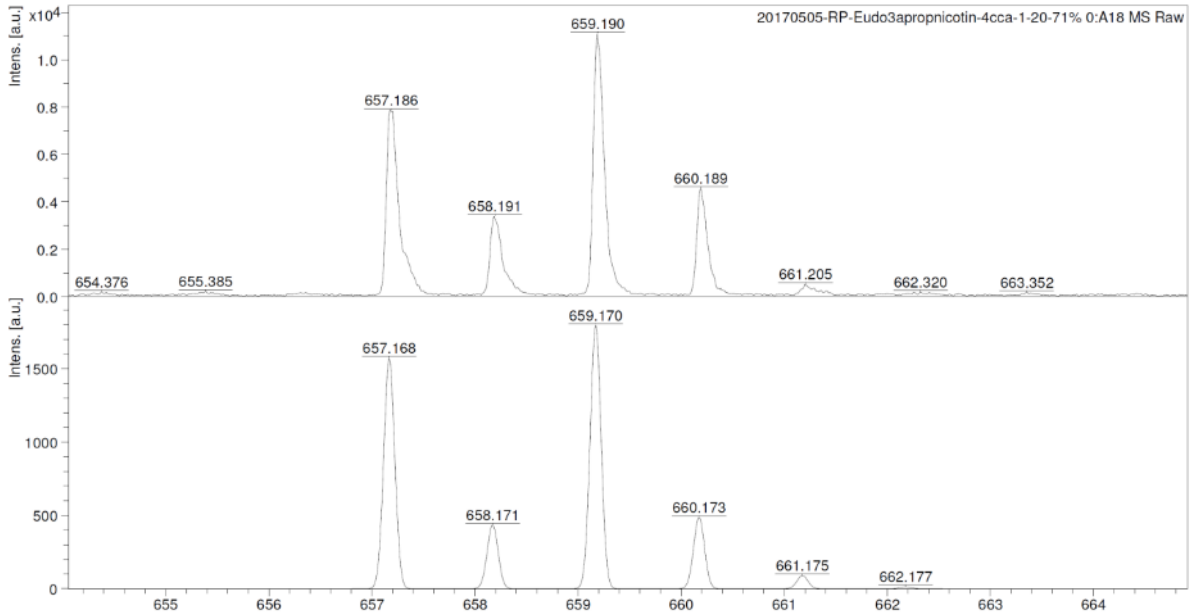


Figure S6. MALDI-TOF spectrum of Eu-complex. Upper: measured spectrum. Lower: predicted spectrum.

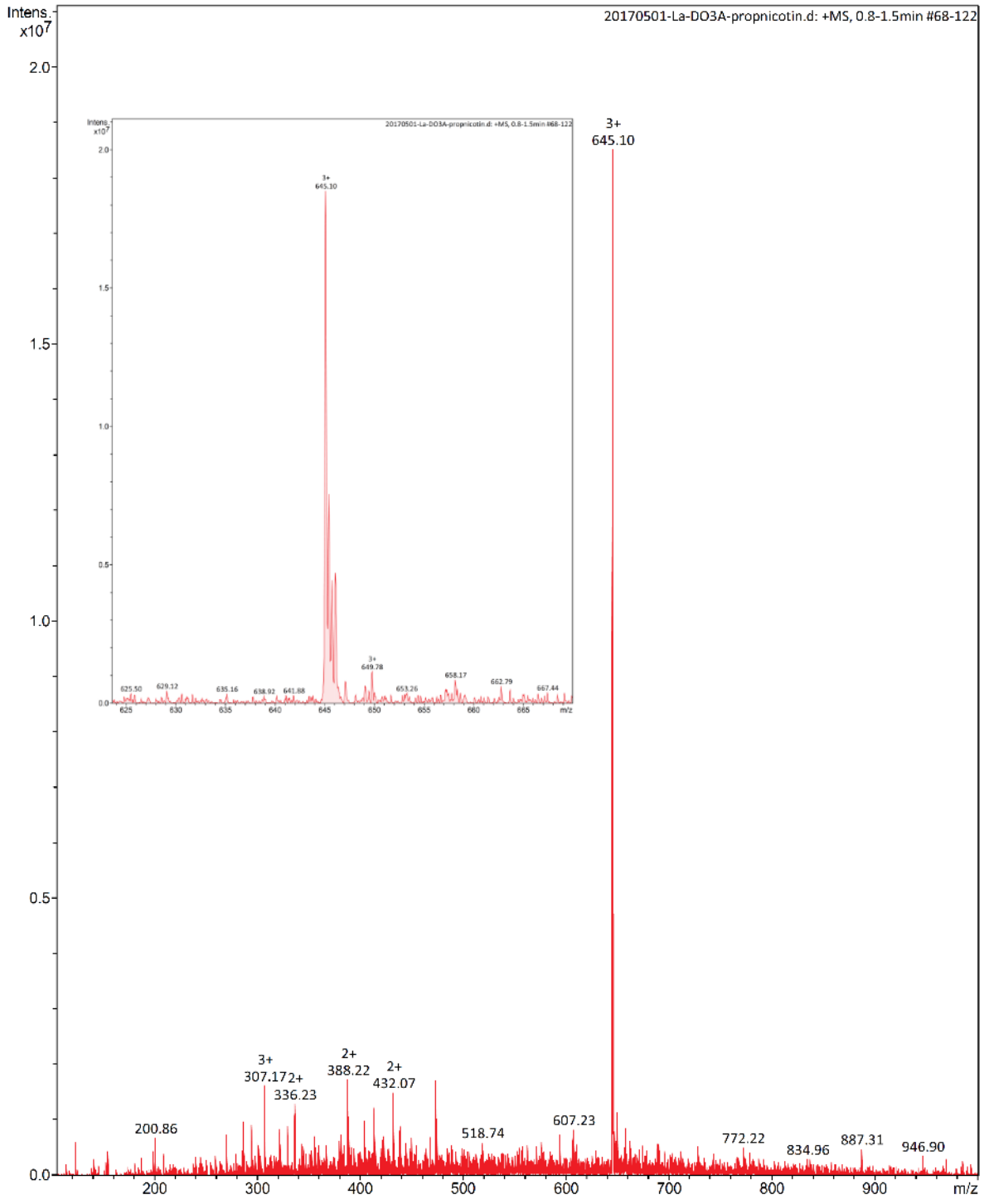


Figure S7. ESI-MS of La-complex. Inset: expansion of isotopic pattern.

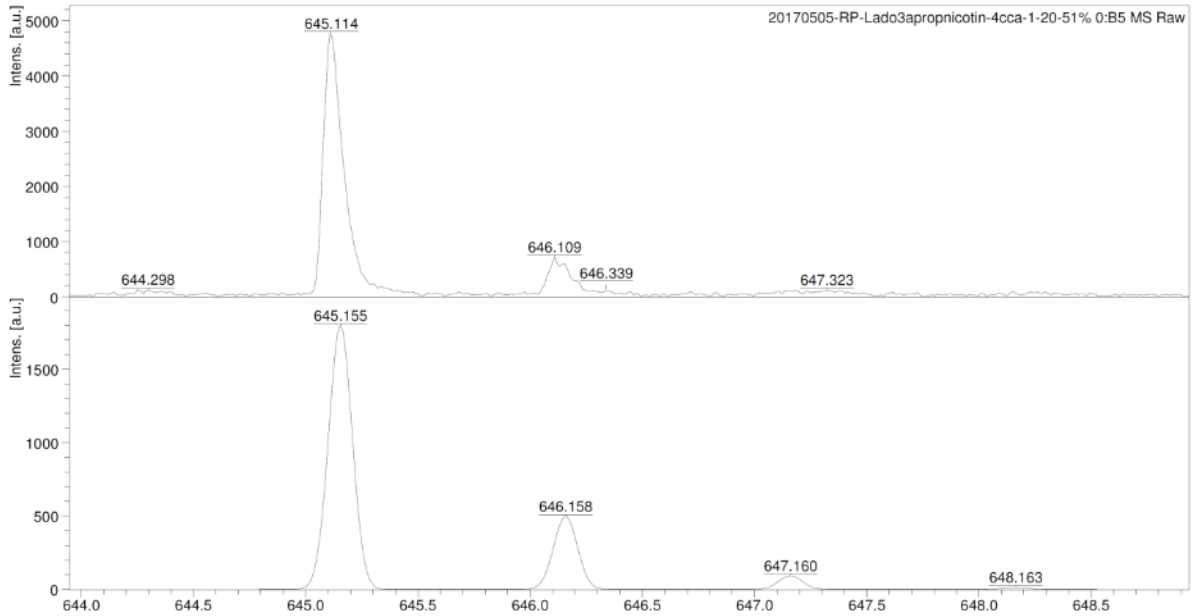


Figure S8. MALDI-TOF La-complex. Upper: measured spectrum. Lower: predicted spectrum.

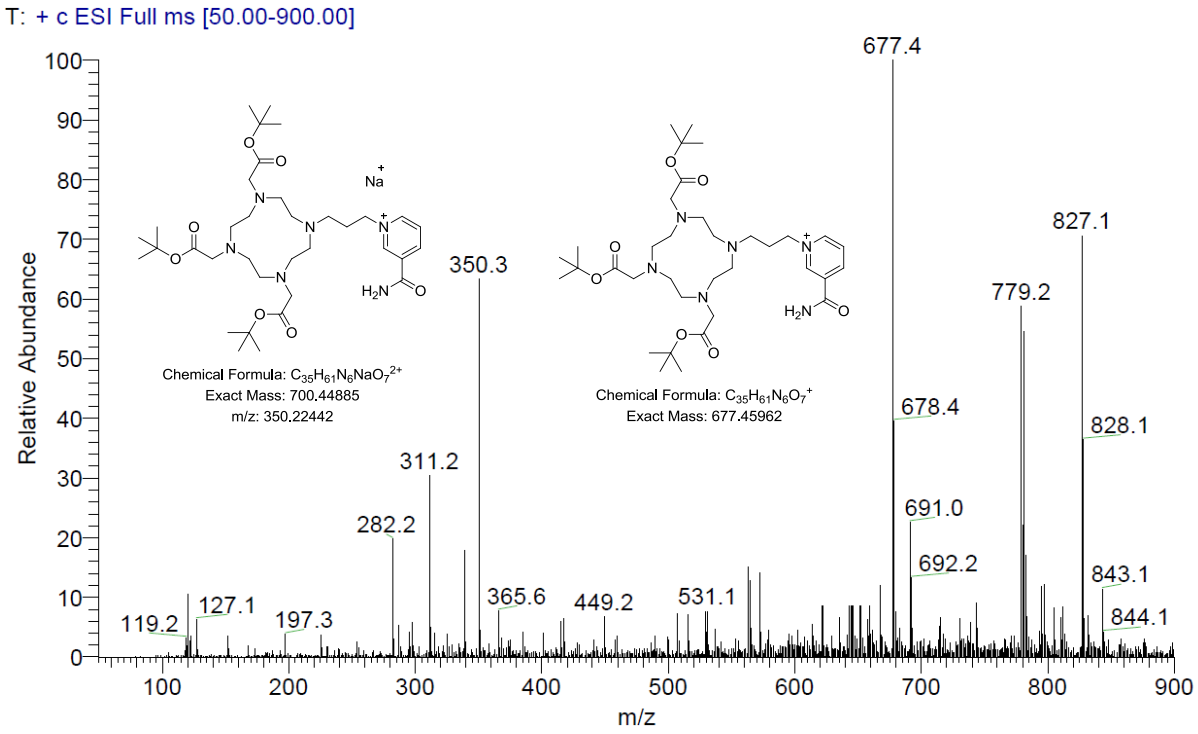


Figure S9. ESI-MS of DO3A-propnicotinCOO^{t}Bu before $Na_2S_2O_4$ addition.

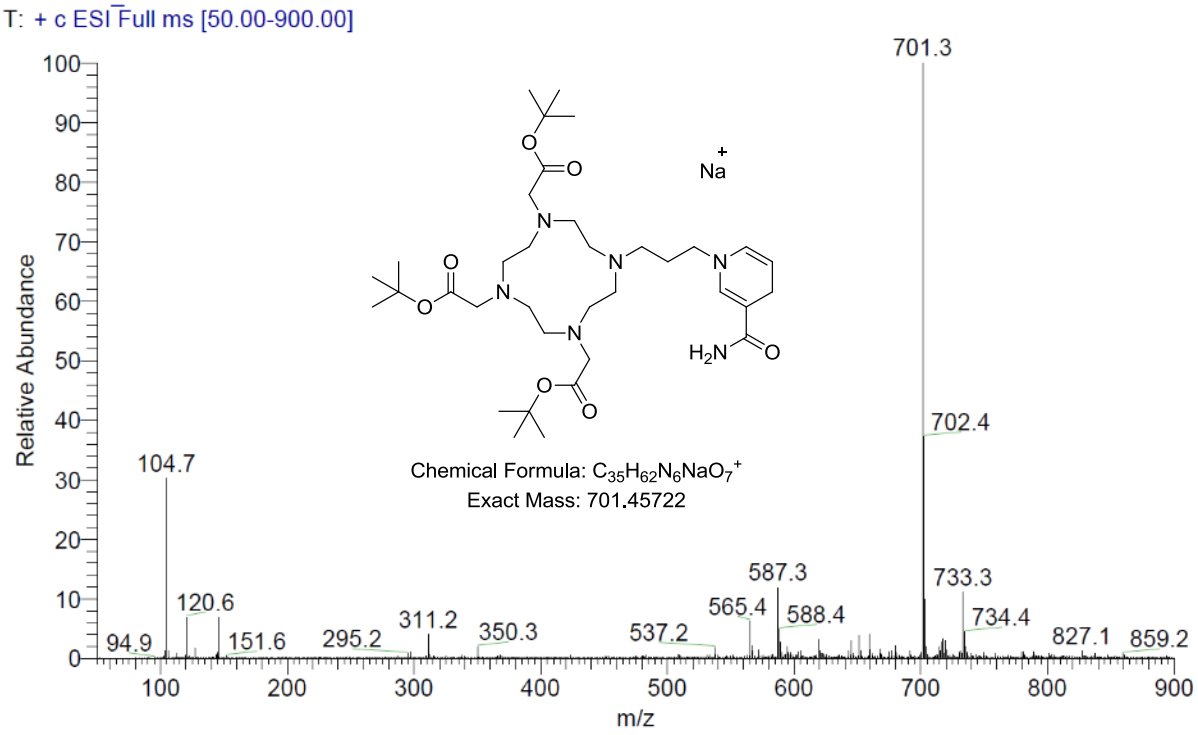


Figure S10. ESI-MS of DO3A-propnicotinCOOtBu after $Na_2S_2O_4$ addition.

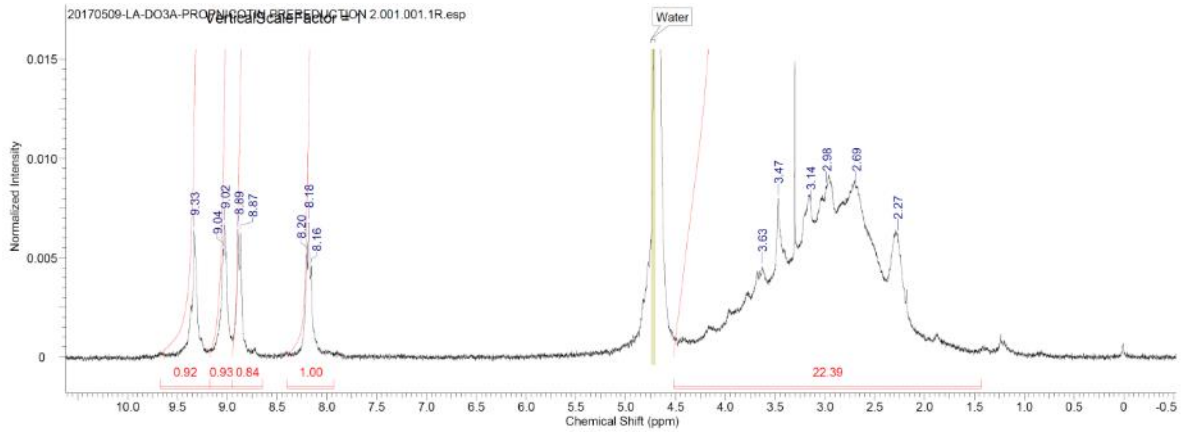


Figure S11. ^{1}H NMR spectrum of La-complex.

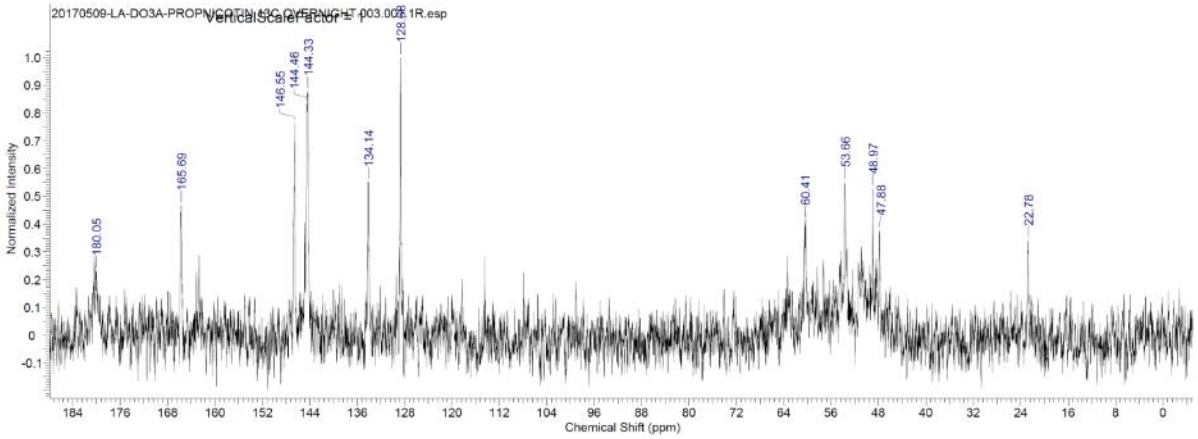


Figure S12. ^{13}C NMR spectrum of La-complex.

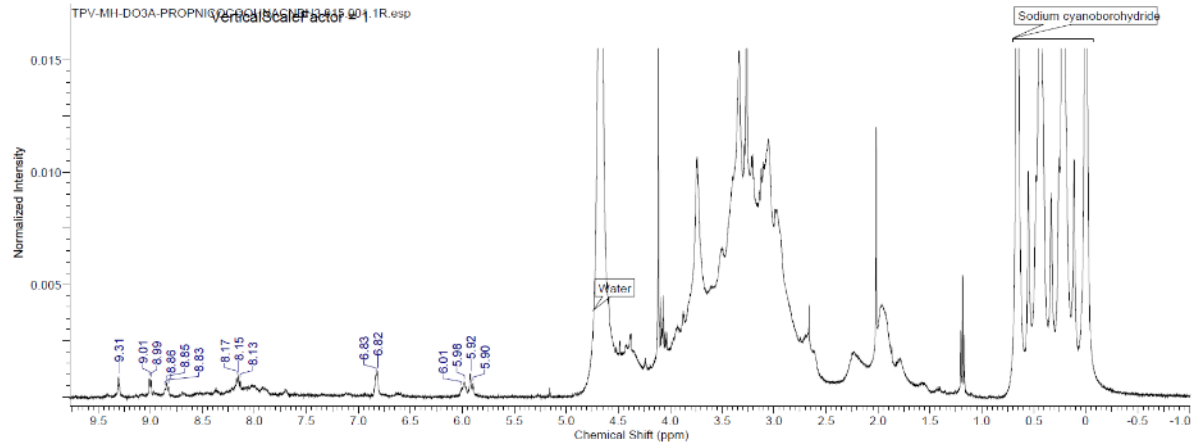


Figure S13. ^{1}H NMR spectrum of the free ligand DO3A-propnicotin COOH after sodium cyanoborohydride reduction.

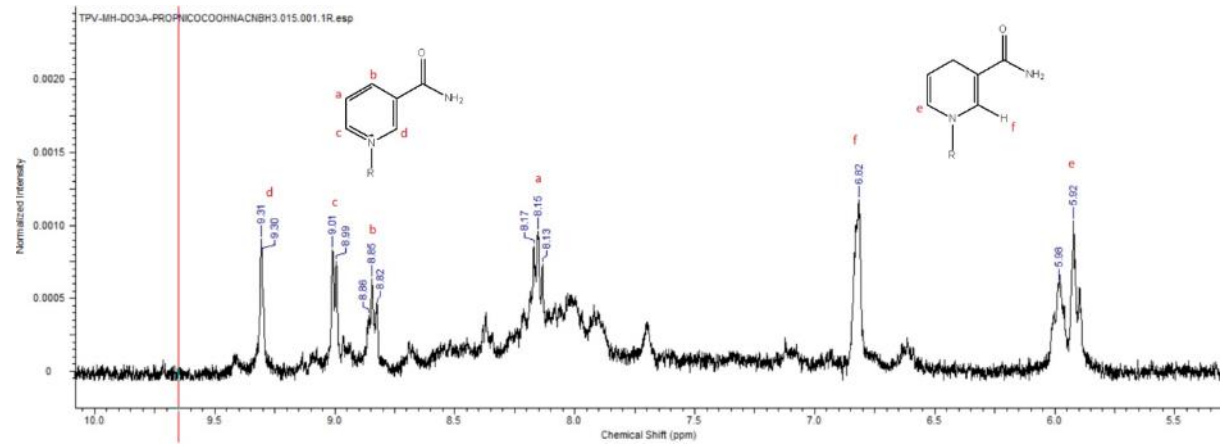


Figure S14. Zoom of ^{1}H NMR spectrum of the free ligand DO3A-propnicotin COOH after sodium cyanoborohydride reduction showing peak assignment.

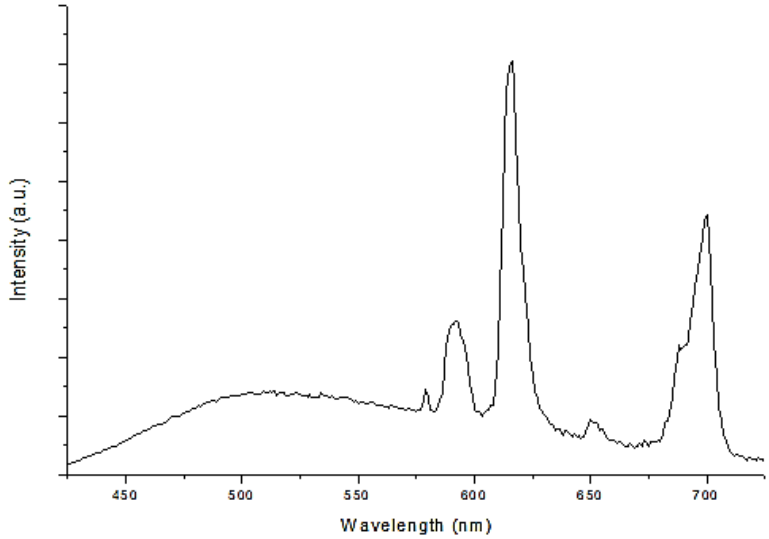


Figure S15. Eu-complex luminescence in water (400 nm excitation, MES 20 mM, pH 7).

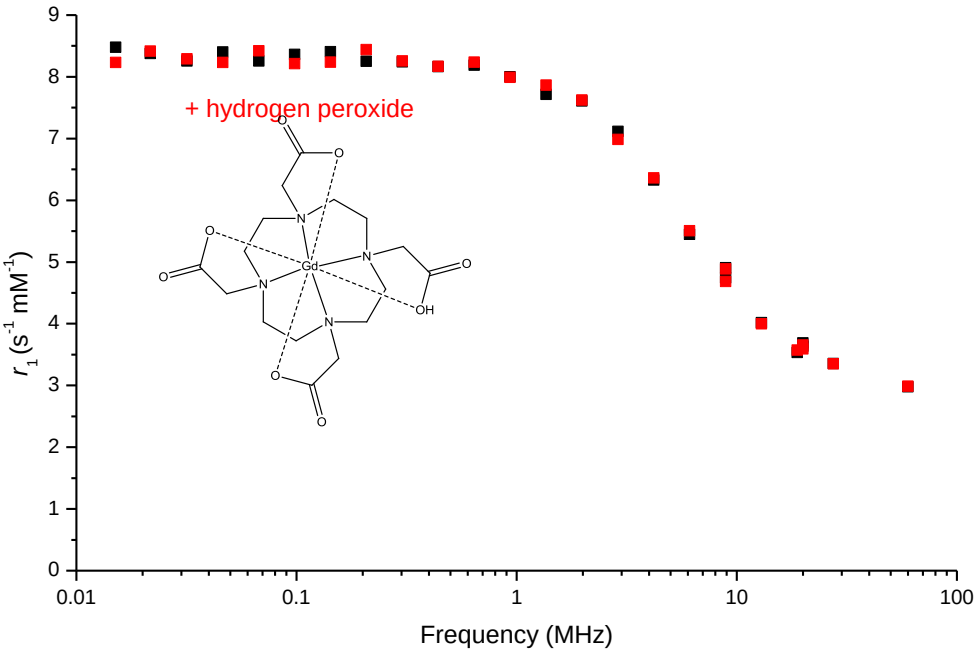


Figure S16. DOTAREM control with H_2O_2 (red) and without (black).

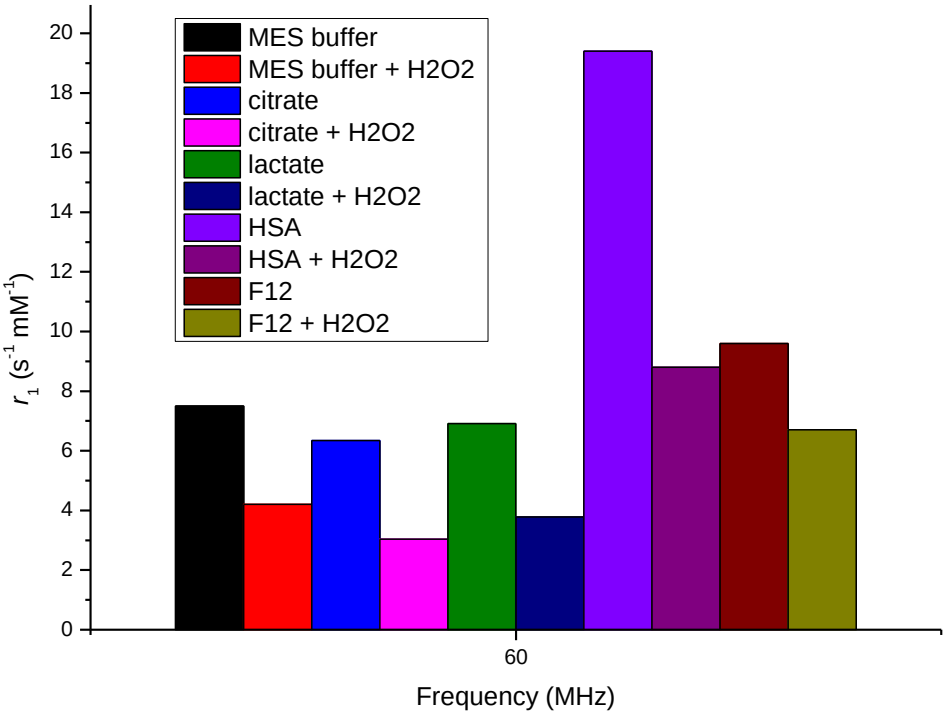


Figure S17. Relaxivity of GdNR1 (1 mM) in different conditions. Samples in non-degassed water (MES buffer 50 mM) at 37 °C with added citrate (0.13 mM), lactate (2.3 mM) or HSA (4 wt%), F12 cell-medium with 10% FBS, with and without addition of 10 eq. hydrogen peroxide, (100 eq. hydrogen peroxide for F12).

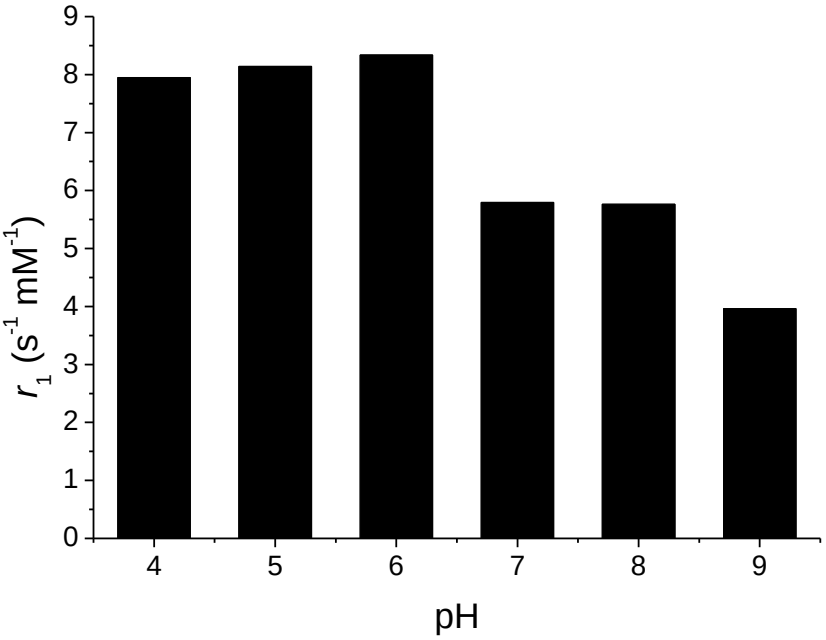


Figure S18. Relaxivity of oxidized GdNR1 in non-degassed water at different pH values measured at 60 MHz and 37 °C. The pH was adjusted using either aqueous HCl or NaOH.

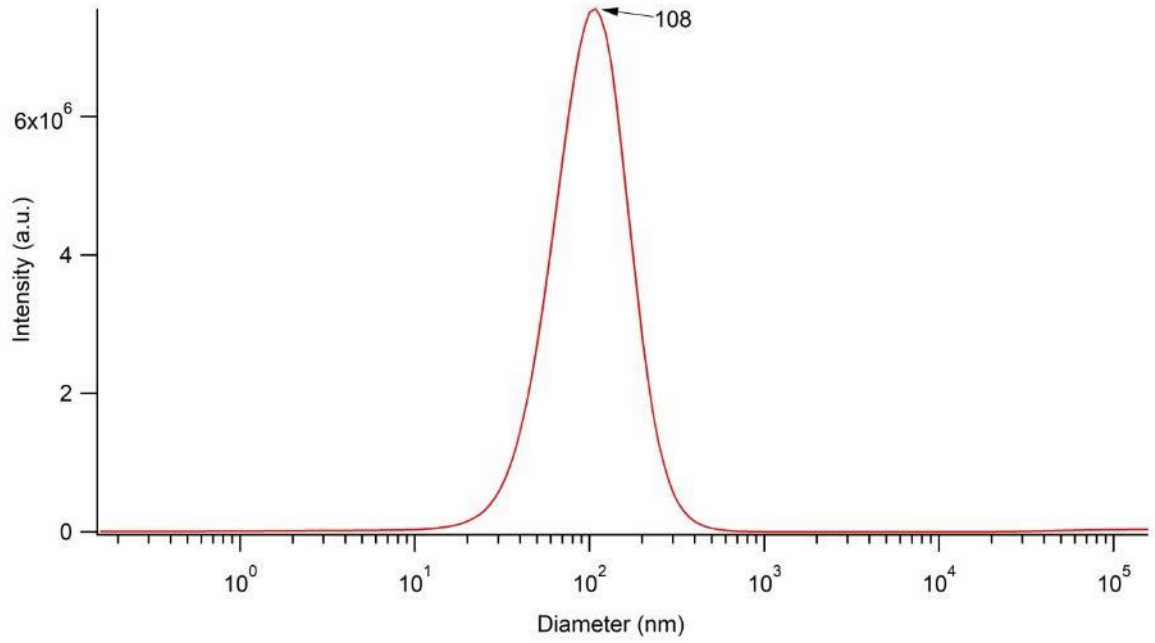


Figure S19. DLS measurement of GdNR1 in MES buffer (20 mM) and hydrogen peroxide addition (10 eq.)

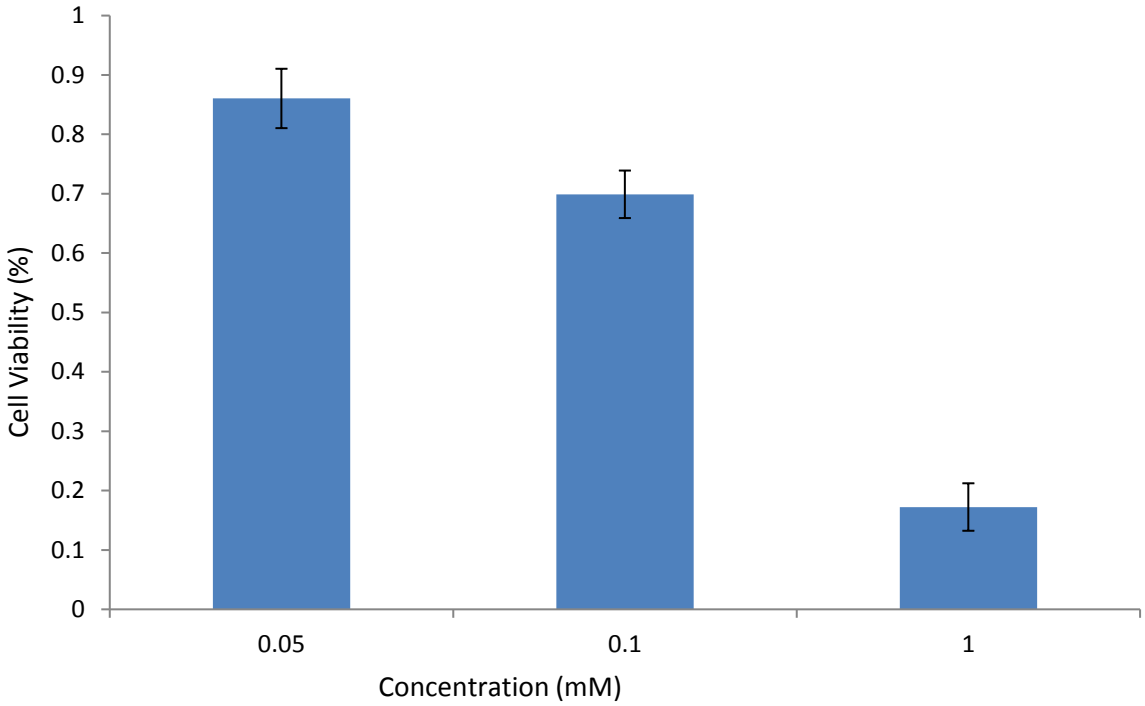


Figure S20. DLD-1 cell viability by alamar blue assay in the presence of GdNR1. Incubation time was 72 hours (n = 3).

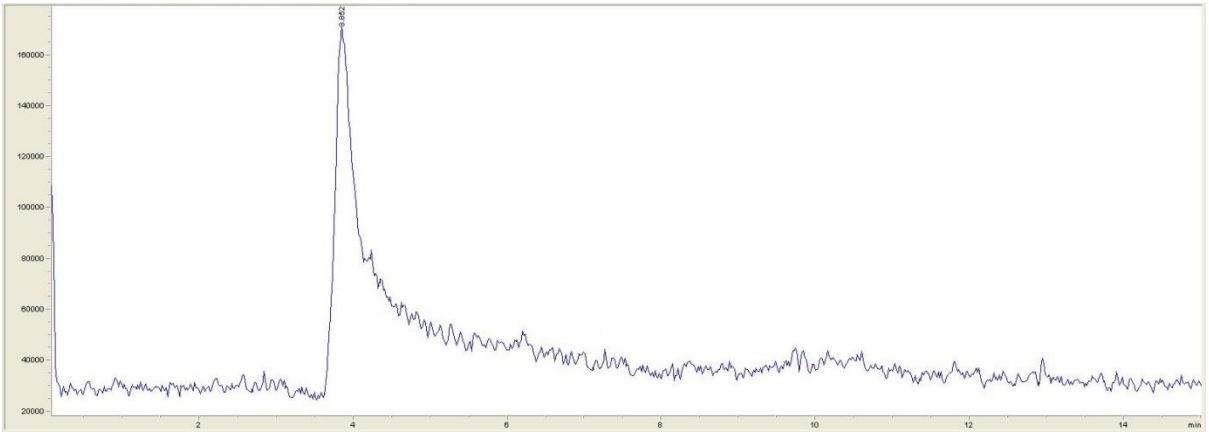


Figure S21. GdNR1 HPLC total ion chromatogram.

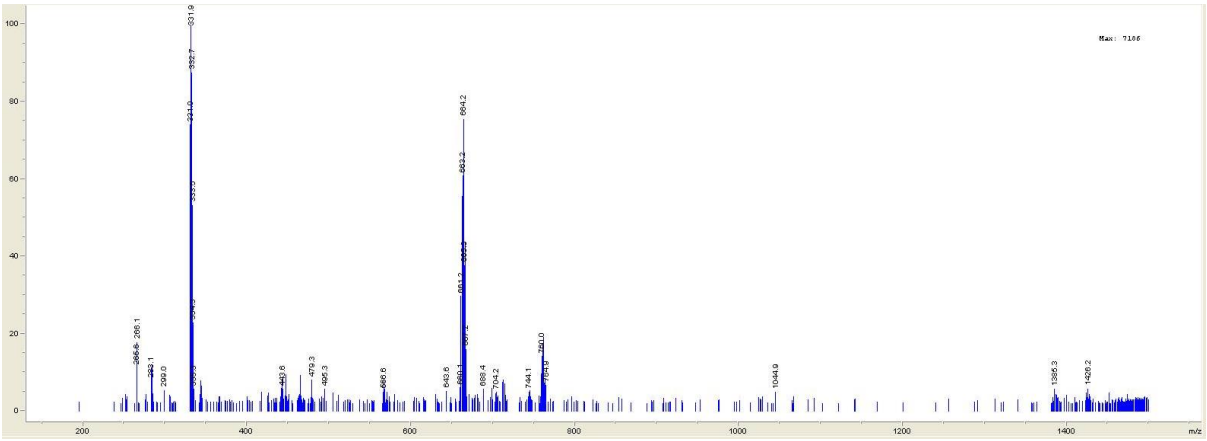


Figure S22. GdNR1 HPLC-MS; mass spectrum at retention time 3.9 min.

Table S1. Relaxivity data of GdNR1, r_1 (s^{-1} mM^{-1}), at 60 MHz (37 °C) in water (degassed), HCO_3^- (10 mM), non-degassed buffered water (20 mM) and addition of 10 eq. oxidant or reductant.

	r_1 (s^{-1} mM^{-1} @ 60 MHz)
water	7.6
water + HCO_3^-	2.8
HEPES	6.9
MES	7.5
HEPES + H_2O_2	4.6
MES + H_2O_2	4.6
HEPES + $Na_2S_2O_4$	8.8
MES + $Na_2S_2O_4$	6.8

Table S2. Parameters obtained by the theoretical fitting of the proton NMRD data in degassed water and non-degassed MES buffer (20 mM, pH 7) at 37 °C.

	GdNR1	GdNR1 (MES 20 mM)	GdNR1 (MES 20 mM + H_2O_2)
d (nm)	0.36	0.36	0.36
r (nm)	0.31	0.31	0.31

τ_R (ps)	92.4	83	3550
q	2	2	0.3
τ_M (ns)	100	100	100
τ_{S0} (ps)	171	62	38
τ_V (ps)	66	20	17

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