Moving the goal posts: enhancing the sensitivity of PARASHIFT proton magnetic resonance imaging and spectroscopy

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1. General Experimental and instrumentation

All solvents used were laboratory grade and anhydrous solvents, when required, were freshly distilled over the appropriate drying agent. Water was purified by the 'Purite$_{STILL}$plus' system, with conductivity of ≤ 0.04 μS cm^{-1}. All reagents used were purchased from commercial suppliers (Acros, Aldrich, Fluka, Fluorochem, Merck and Strem) and were used without further purification. Reactions requiring anhydrous conditions were carried out using Schlenk line techniques under argon.

^{1}H and ^{13}C NMR spectra were recorded in commercially available deuteriated solvents on a Varian Mercury-200 (^{1}H 199.975, ^{13}C 50.289), Varian Mercury-400 (^{1}H 399.960, ^{13}C 100.572), Bruker Avance-400 (^{1}H 400.052, ^{13}C 100.603), Varian Inova-500 (^{1}H 499.722, ^{13}C 125.671,), Appleby VNMRS-600 (^{1}H 599.832, ^{13}C 150.828), or Varian VNMRA-700 (^{1}H 699.731, ^{13}C 175.948 and ^{19}F 658.405) spectrometer. All chemical shifts are given in ppm and all coupling constants are reported in Hz. The

operating temperature of the spectrometers was measured with the aid of an internal calibration solution of ethylene glycol. The operating temperature of each spectrometer was measured before each set of measurements of relaxation data, using the calibration sample.

Longitudinal relaxation data were measured using the inversion-recovery technique. The recorded free induction decays were processed using backward linear prediction, optimal exponential weighting, zero-filling, Fourier transform, phasing and baseline correction (by polynomial fitting). The signals were integrated by Lorentzian line fitting. The proton relaxation rates of the series of complexes can be found in Table 1 (main text) and the fitting to obtain r and τ_r for the cited examples is given below.

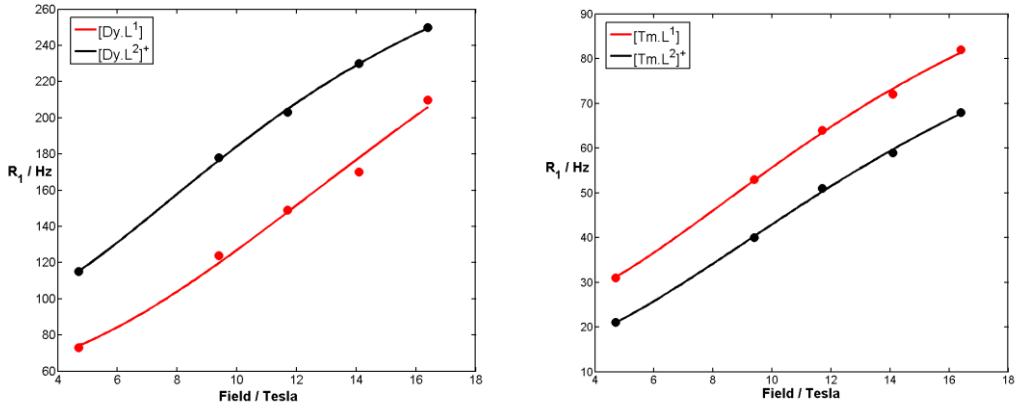


Figure S1: ^{1}H relaxation data (295 K, D_2O) and fitted curves for *left* [Dy.L^1], [Dy.L^2]$^+$ using a fixed μ_{eff} of 10.3 BM and *right* [Tm.L^1], [Tm.L^2]$^+$ using a fixed μ_{eff} of 7.6 BM. For [Dy.L^1]: fitted values minimised with: r = 6.38Å, τ_r = 190 ps, T_{1e} = 0.54 ps; for [Dy.L^2]$^+$: r = 6.58Å, τ_r = 280 ps, T_{1e} = 0.68 ps. For [Tm.L^1]: r = 6.35Å, τ_r = 252 ps, T_{1e} = 0.29 ps; for [Tm.L^2]$^+$: r = 6.50Å, τ_r = 234 ps, T_{1e} = 0.20 ps.

Table SI 1: Calculated values for r (Å), τ_r and T_{1e} (both ps) obtained through fitting of measured R_1 (Hz) at 5 different magnetic fields[a] to equation (4) for [Ln.L^1] and [Ln.L^2] using a fixed μ_{eff}[b] (BM) at 295 K (D_2O).

	Ln^{3+}	L^1			L^2		
	μ_{eff} / BM	r / Å	τ_r / ps	T_{1e} / ps	r / Å	τ_r / ps	T_{1e} / ps
Tb	9.8	**6.63**±0.01	**210**±5	**0.68**±0.04	**6.38**±0.02	**320**±4	**0.70**±0.03
Dy	10.3	**6.59**±0.03	**190**±4	**0.54**±0.03	**6.36**±0.01	**292**±3	**0.68**±0.02
Ho	10.4	**6.80**±0.02	**186**±2	**0.29**±0.03	**6.78**±0.02	**245**±1	**0.34**±0.03
Er	9.4	**6.68**±0.01	**149**±2	**0.22**±0.04	**n.d.**	**n.d.**	**n.d.**
Tm	7.6	**6.35**±0.02	**252**±2	**0.29**±0.03	**6.50**±0.02	**234**±2	**0.20**±0.05
Yb	4.3	**6.34**±0.10	**204**±2	**0.22**±0.05	**6.28**±0.03	**230**±3	**0.15**±0.03
Average		**6.65**±0.14	**197**±7		**6.57**±0.05	**247**±6	

[a] Fields used are 4.7, 9.4, 11.7, 14.1 16.5 Tesla [b] μ_{eff} was fixed to the values taken from literature.[1]

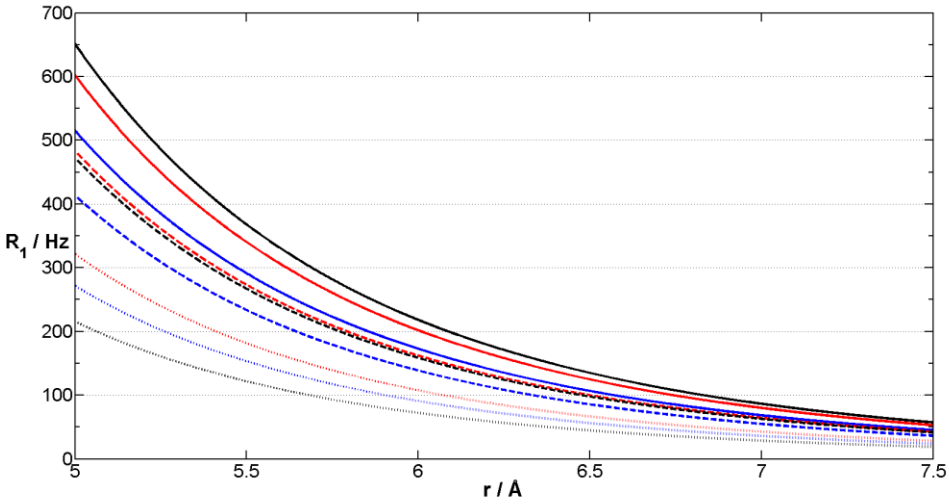


Figure S2: Calculated variation of the ^{1}H longitudinal relaxation rate R_1 with Ln-nuclear separation r; *black*: Ho complex, 295K, 9.4 T (solid), 7T (dash), 3T (dots), assuming μ_{eff} = 10.4 BM, T_{1e} = 0.25 ps, τ_r = 275 ps; *red*: analogous Tb complex; μ_{eff} = 9.8 BM, T_{1e} = 0.55 ps, τ_r = 275 ps; *blue*: analogous Er complex; μ_{eff} = 9.4 BM, T_{1e} = 0.50 ps, τ_r = 275 ps, 295K.

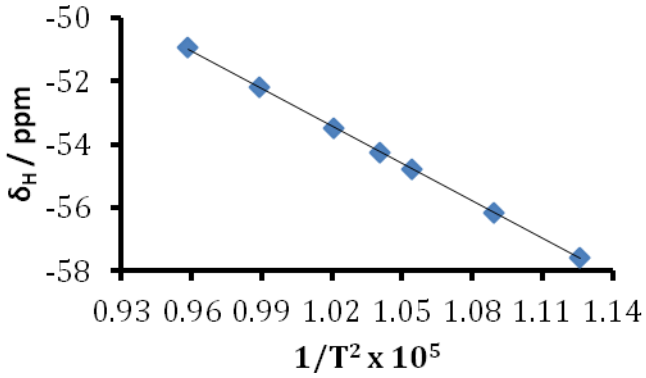


Figure S3: Variation in the tert-butyl resonance of **[Dy.L^3]** as a function of $1/T^2$ (D_2O, 14.1 T, 0.27 ppm K^{-1}).

The change in the linewidth (R_2) for $[Dy.L^2]^+$ observed in vivo (see main manuscript) in the muscle tissue) was examined in vitro, by assessing the variation with increasing serum albumin concentration, using albumin as a model protein. The changes observed (Fig. S4) were of a similar magnitude to those seen in vivo, suggesting that the origin of the greater R2 values may indeed be associated with reversible protein binding, e.g. to myosin in muscle tissue or to albumin in that region.

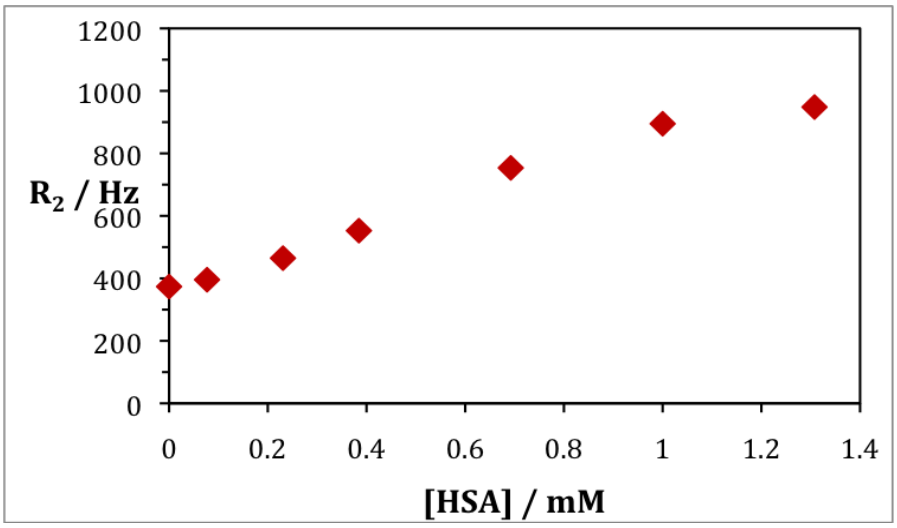


Figure S4 Variation in R_2 (s^{-1}), as a function of added human serum albumin (295K, 9.4T) for **[Dy.L^2]Cl.**

Electrospray mass spectra were recorded on a Fisons VG Platform II, Waters Micromass LCT or Thermo-Finnigan LTQ FT instrument operating in positive or negative ion mode as stated, with MeOH as the carrier solvent. Accurate mass spectra were recorded using the Thermo-Finnigan LTQ FT mass spectrometer. LCMS analyses were performed on a Waters system comprising a 3100 Mass Detector and a 2998 Photodiode array detector. Melting points were recorded using a Gallenkamp (Sanyo) apparatus and are uncorrected.

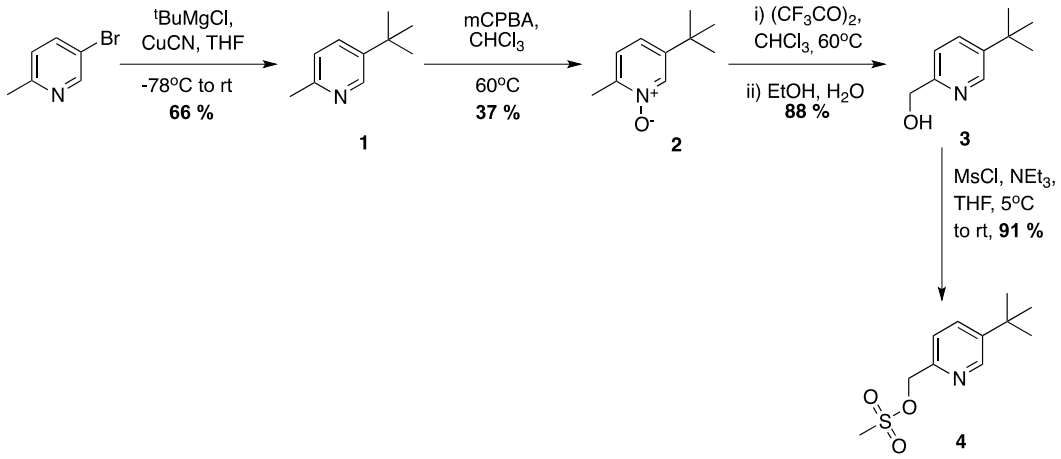
Thin layer chromatography was performed on neutral aluminium sheet silica gel plates (Merck Art 5554) and visualised under UV irradiation (254 nm), or using Dragendorff reagent staining. Preparative column chromatography was performed using silica gel (Merck Silica Gel 60, 230-400 mesh).

Reverse phase analytical HPLC traces were recorded at 298 K using Waters Mass Directed Auto Preparation (MDAP) system. XBridge C18 4.6 x 100 mm, i.d. 5 μm analytical column and XBridge C18 OBD 19 x 100 mm, i.d. 5 μm semi-preparative columns were used to analyse and purify the complexes. A gradient elution with a solvent system composed of H_2O + 0.1% HCOOH/MeOH + 0.1% HCOOH was performed for a total run time of 20 min, flow rate= 1mL/ min. The separation details are tabulated below.

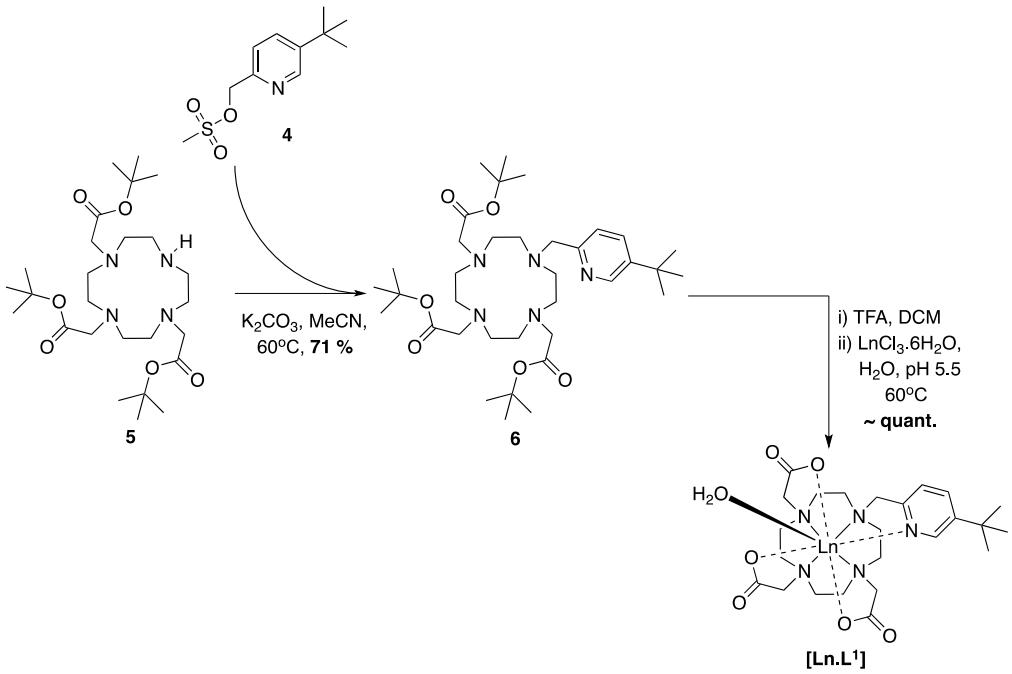
Time	**% Water (+ 0.1 % Formic acid)**	**% MeOH (+ 0.1 % Formic acid)**	**Curve**
0	90	10	0
10	5	95	6
13	5	95	6
13.5	90	10	6
16.5	90	10	1

Reverse phase HPLC conditions for the analysis of complexes.

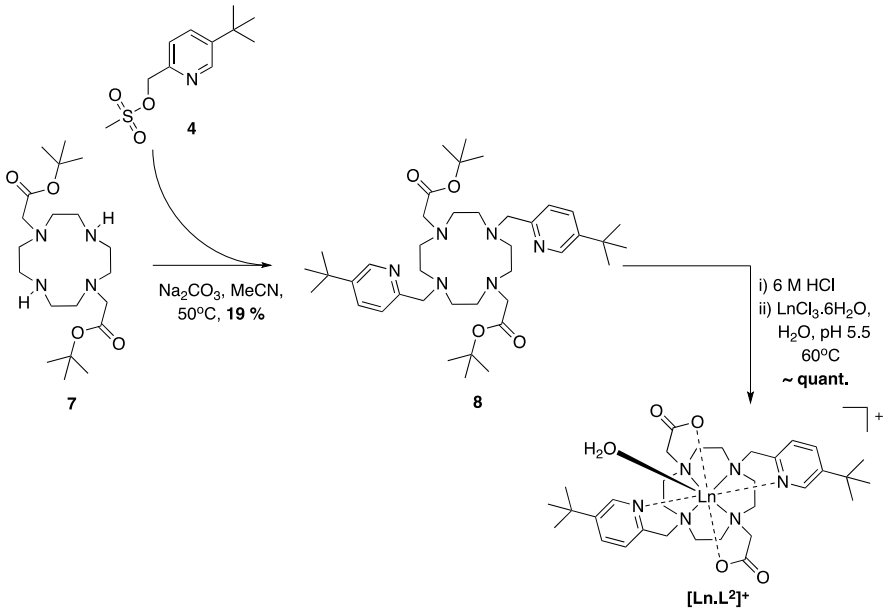
2. Reaction schemes



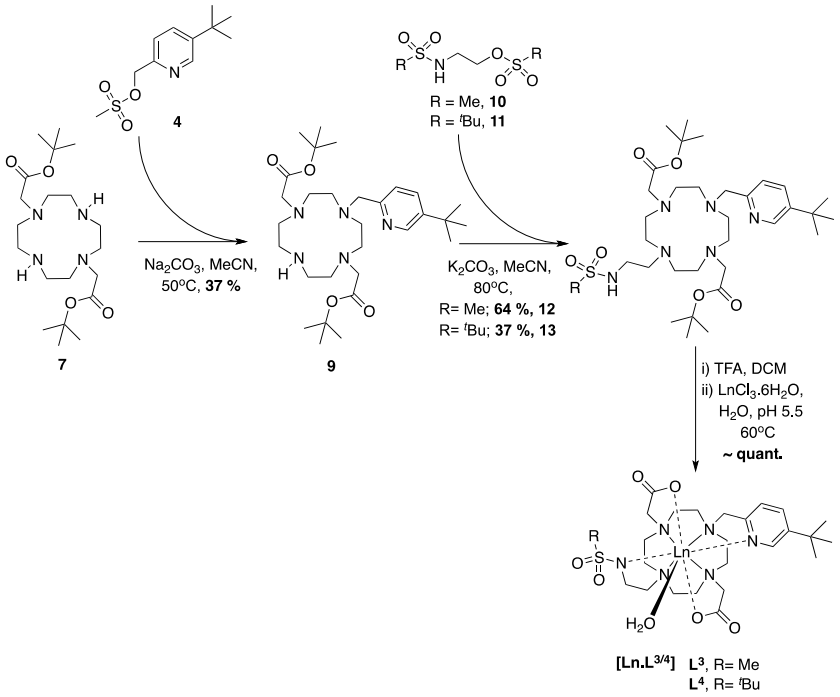
Scheme S1. Synthesis of mesylate precursor.



Scheme S2. Synthesis of complexes **[Ln.L^1]**.



Scheme S3. Synthesis of complexes **[Ln.L^2]$^+$**.



Scheme S4. Synthesis of complexes **[Ln.L$^{3/4}$]**, where R= Me for **L^3** and R= ^{t}Bu for **L^4.**

3. Variation of terbium emission intensity with pH for [Tb.L^3]

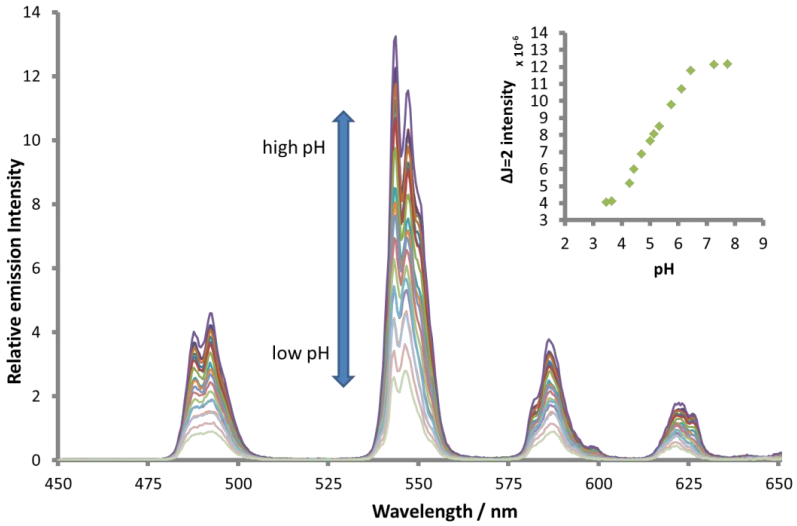


Figure S5 Tb(III) emission spectra at varying pH and change in the ΔJ = 1 band intensity as a function of pH for **[Tb.L^3]**, with a calculated pK_a of 5.09 ±0.07 (H_2O, 0.1 M NaCl, 298 K, λ_{exc} = 270 nm).

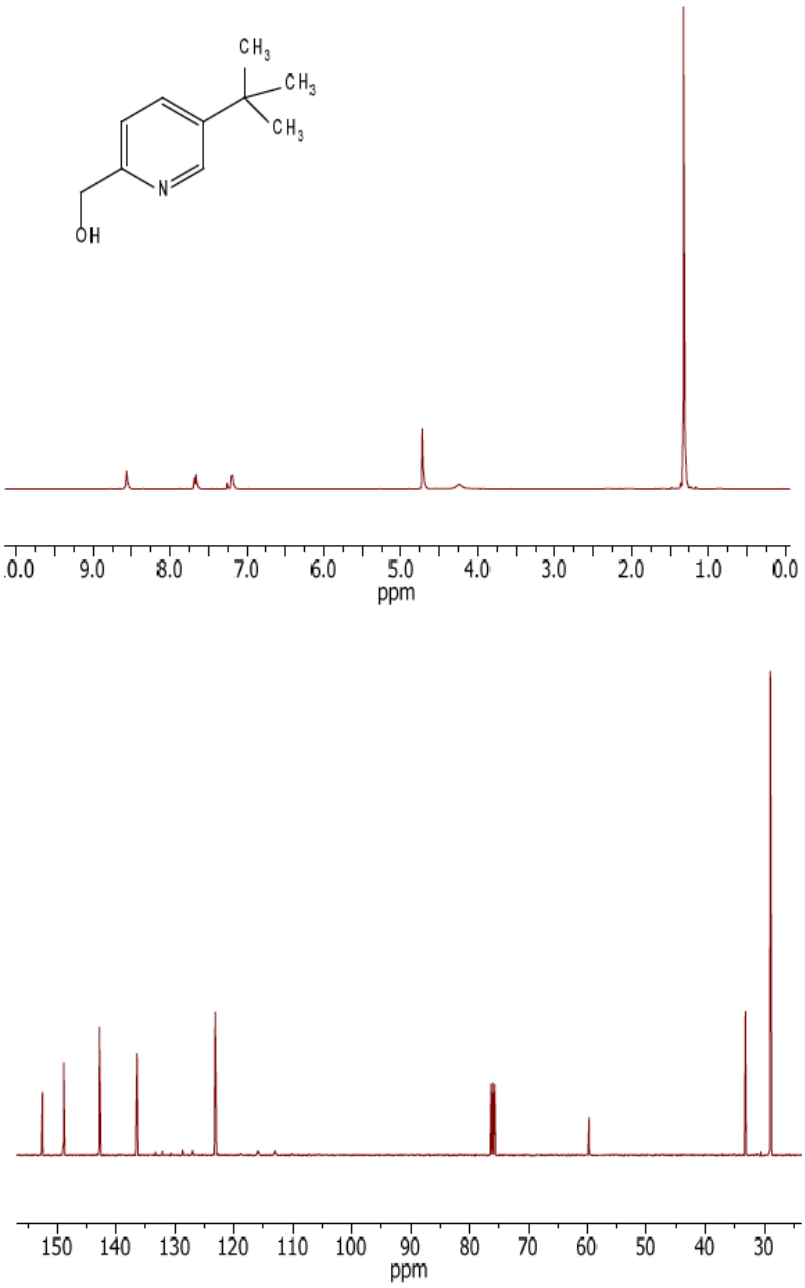
4. Ligand and Complex Synthesis

5-*tert*-Butyl-2-methylpyridine (1). Copper(I) cyanide (10 mg) was added to anhydrous diethyl ether/THF (40+10 mL) and the resulting suspension cooled to -78°C. To this was added *tert*-butylmagnesium chloride (14.5 mL, 2.0 M solution in diethyl ether, 29.1 mmol) and the mixture was maintained at -78°C, stirred for 20 min under argon and 5-bromo-2-methylpyridine (2.5 g, 14.5 mmol) added. The reaction was stirred for 3 h at -78°C before being allowed to warm to rt and stirred for a further 18 h. Upon completion of the reaction, sat. $NH_4OH_{(aq)}$ was added dropwise to quench any excess Grignard reagent remaining in solution. This mixture was extracted with diethyl ether (3 x 50 mL), the organic layers combined, dried over $MgSO_4$ and solvent removed under reduced pressure. The resulting yellow liquid was purified by silica gel column chromatography, eluting with 50 % ethyl acetate/hexane, to yield a volatile pale yellow liquid, (1.42 g, 66 %). R_f (50 % ethyl acetate/hexane) = 0.35; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (d, J = 2 Hz, 1H, H^6), 7.63 (dd, J = 8, 2 Hz, 1H, H^4), 7.10 (d, J = 8 Hz, 1H, H^3), 2.39 (s, 3H, Me), 1.24 (s, 9H, ^{t}Bu); ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.6 (C^2), 148.0 (C^6), 146.5 (C^5), 137.4 (C^4), 126.1 (C^3), 36.2

($\underline{C}(CH_3)_3$), 33.2 ($C(\underline{C}H_3)$), 23.6 ($\underline{C}H_3$); ESI-LRMS (+) *m/z* 150.2 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{10}H_{16}N$ 150.1283, found 150.1279.

5-*tert*-Butyl-2-methylpyridine 1-oxide (2). *m*CPBA (2.17 g, 12.6 mmol) was added to a solution of 5-*tert*-butyl-2-methylpyridine (1.25 g, 8.39 mmol) in chloroform (30 mL). The resulting solution was stirred at rt for 18 h under argon, before being quenched with sat. $Na_2SO_{4(aq)}$ (10 mL) and stirred for 10 min. The organic layer was extracted and washed with sat. $NaHCO_{3(aq)}$ (30 mL). The aqueous layer was extracted with ethyl acetate (3 x 25 mL); the organic layers were combined, dried over Na_2SO_4, and the solvent removed under reduced pressure. The resulting yellow liquid was purified by silica gel column chromatography, eluting with a gradient starting from 100 % DCM to 5 % MeOH/DCM to yield a pale yellow liquid (0.51 g, 37 %). R_f (10 % MeOH/DCM) = 0.39; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.32 (d, *J* = 2 Hz, 1H, H^6), 7.21 (dd, *J* = 8, 2 Hz, 1H, H^4), 7.18 (d, *J* = 8 Hz, 1H, H^3), 2.50 (s, 3H, Me), 1.31 (s, 9H, ^{t}Bu); ^{13}C NMR (101 MHz, $CDCl_3$): δ 147.4 (C^2), 145.7 (C^6), 137.4 (C^5), 125.7 (C^3), 123.7 (C^4), 33.6 ($\underline{C}(CH)_3$), 30.6 ($C(\underline{C}H)_3$), 17.3 ($\underline{C}H_3$); ESI-LRMS (+) *m/z* 166.2 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{10}H_{16}NO$ 166.1232, found 166.1268.

(5-*tert*-Butylpyridin-2-yl)methanol (3). Trifluoroacetic anhydride (10 mL) was added to a solution of 5-*tert*-butyl-2-methylpyridine 1-oxide (450 mg, 2.73 mmol) in $CHCl_3$ (15 mL). The resulting mixture was heated at 60°C for 36 h under an inert atmosphere. After this time, the solvent was removed under reduced pressure and reaction completion to the trifluoroacetate intermediate was confirmed by ^{1}H NMR analysis. The resulting bright yellow oil was stirred in a mixture of EtOH (5 mL) and H_2O (5mL) for 1 h. The solution was concentrated (ca. 2 mL) and extracted with ethyl acetate (3 x 10 mL). The organic layers were combined, washed dilute aqueous sodium hydroxide solution (pH 9)dried over $MgSO_4$, and the solvent removed under reduced pressure to yield a yellow oil (396 mg, 88 %). R_f (10 % MeOH/DCM) = 0.42; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.57 (s, 1H, H^6), 7.71 (d, *J* = 8 Hz, 1H, H^4), 7.18 (d, *J* = 8 Hz, 1H, H^3), 5.74 (br s, 1H, OH),4.77 (s, 2H, $C\underline{H}_2OH$), 1.34 (s, 9H, ^{t}Bu); ^{13}C NMR (101 MHz, $CDCl_3$): δ 153.4 (C^2), 148.5 (C^6), 141.6 (C^5), 137.8 (C^3), 123.7 (C^4), 59.5 ($\underline{C}H_2OH$), 33.4 ($\underline{C}(CH)_3$), 29.5 ($C(\underline{C}H)_3$); ESI-LRMS (+) *m/z* 166.2 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{10}H_{16}NO$ 166.1232, found 166.1248.



(5-*tert*-Butylpyridin-2-yl)methyl methanesulfonate (4). (5-*tert*-Butylpyridin-2-yl)methanol (396 mg, 2.40 mmol) was dissolved in THF (10 mL) and cooled to 5°C. Triethylamine (0.67 mL, 4.79 mmol) and mesyl chloride (0.28 mL, 3.60 mmol) were added dropwise to this solution. Once addition was complete, the reaction mixture was allowed to warm to rt and stirred for 2 h, before the solvent was removed under reduced pressure. The residue was treated with brine (10 mL) and extracted with

DCM (2 x 10 mL). The organic layers were combined, dried over $MgSO_4$, and the solvent removed under reduced pressure to yield a pale orange oil, which was used immediately (530 mg, 91 %). R_f (10 % MeOH/DCM) = 0.76; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.68 (d, J = 2 Hz, 1H, H^6), 7.89 (dd, J = 8, 2 Hz, 1H, H^4), 7.52 (d, J = 8 Hz, 1H, H^3), 5.39 (s, 2H, $C\underline{H}_2OMs$), 3.12 (s, 3H, $SO_2C\underline{H}_3$), 1.37 (s, 9H, ^{t}Bu); ESI-LRMS (+) *m/z* 244.2 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{11}H_{17}NO_3S$ 244.1007, found 244.1020.

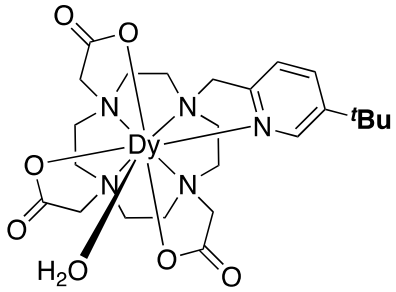
1,4,7-Tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (5). *Tert*-butyl bromoacetate (11.5 mL, 78.3 mmol) was added to a stirred solution of 1,4,7,10-tetraazacyclododecane (5.00 g, 29.0 mmol) and $NaHCO_3$ (6.50 g, 78.3 mmol) in anhydrous CH_3CN (190 mL) and the resulting mixture stirred for 18 h under argon. The reaction mixture was filtered and concentrated *in vacuo*. The resulting white residue was dissolved in a minimal amount of toluene, with heating, and upon cooling a white solid precipitated out of solution (6.70 g, 45 %). R_f (10 % CH_3OH/CH_2Cl_2) = 0.29; m.p. 184-185 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.03 (br s, 1H, NH), 3.37 (s, 4H, CH_2), 3.29 (s, 2H, CH_2), 3.04-3.15 (br m, 4H, cyclen CH_2), 2.78-2.98 (br m, 12H, cyclen CH_2), 1.46 (s, 18H, $C(CH_3)_3$), 1.45 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.5, 168.7, 80.9, 80.7, 57.2, 50.3, 48.2, 46.6, 27.2, 27.1; ESI-LRMS (+) *m/z* 515.7 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{26}H_{51}N_4O_6$ 515.3809, found 515.3804.

***tert*-Butyl-2,2',2''-(10-((5-*tert*-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (6).** A stirred mixture of *tert*-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (DO3A) (19 mg, 0.038 mmol), (5-*tert*-butylpyridin-2-yl)methyl methanesulfonate (11 mg, 0.045 mmol), and K_2CO_3 (9 mg, 0.065 mmol) in anhydrous MeCN (5 mL) was heated at 60°C for 18 h under argon. After this time, the reaction mixture was cooled and filtered before the solvent was removed under reduced pressure. The resulting yellow oil was purified by silica gel column chromatography, eluting with a gradient starting from 100 % DCM to 3 % MeOH/DCM to yield a pale yellow oil (18 mg, 71 %). R_f (10 % MeOH/DCM) = 0.40; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28 (d, J = 3 Hz, 1H, H^6), 7.65 (dd, J = 8, 3 Hz, 1H, H^4), 7.09 (d, J = 8 Hz, 1H, H^3), 3.07 (br m, 12H, $NC\underline{H}_2$-COO^tBu/cyclen-$C\underline{H}_2$/$NC\underline{H}_2$py), 2.31 (br m, 12H, $NC\underline{H}_2COO^tBu$/cyclen-$C\underline{H}_2$/$NC\underline{H}_2$py), 1.49 (s, 9H, py-$C(C\underline{H}_3)_3$), 1.39 (br s, 18H, $COOC(C\underline{H}_3)_3$), 1.29 (s, 9H, $COOC(C\underline{H}_3)_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 172.7, 155.8, 145.8, 144.2, 133.7,

122.0, 81.8, 58.3, 55.4, 33.3, 31.0, 27.9; ESI-LRMS (+) *m/z* 662.6 $[M+H]^+$, 684.6 $[M+Na]^+$; ESI-HRMS (+) calcd for $C_{36}H_{64}N_5O_6$ 662.4857, found 662.4852.

2,2',2''-(10-((5-*tert*-Butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (L^1). *tert*-Butyl-2,2',2''-(10-((5-*tert*-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (15 mg, 0.02 mmol) was dissolved in DCM (1 mL) with stirring. To this was added TFA (1 mL) and the mixture stirred at rt for 18 h. After this time, the solvent was removed under reduced pressure to give a yellow oil. This yellow oil was repeatedly washed with DCM to yield the TFA salt of $\mathbf{L^1}$ as a pale yellow oil (11 mg, 99 %). ^{1}H NMR (400 MHz, D_2O): δ 8.57 (d, *J* = 2 Hz, 1H, H^6), 8.40 (dd, *J* = 8, 2 Hz, 1H, H^4), 7.85 (d, *J* = 8 Hz, 1H, H^3), 3.97 (br s, 2H, NC$\underline{H}_2$COO^{t}Bu/cyclen-C$\underline{H}_2$/NC$\underline{H}_2$py), 3.72 (br m, 2H, NC$\underline{H}_2$-COO^{t}Bu/cyclen-C$\underline{H}_2$/NC$\underline{H}_2$py), 3.50 (br m, 4H, NC$\underline{H}_2$COO^{t}Bu/cyclen-C$\underline{H}_2$/NC$\underline{H}_2$py), 3.32 (br m, 6H, NC$\underline{H}_2$COO^{t}Bu/cyclen-C$\underline{H}_2$/NC$\underline{H}_2$py), 3.21 (br m, 2H, NC$\underline{H}_2$-COO^{t}Bu/cyclen-C$\underline{H}_2$/NC$\underline{H}_2$py), 2.94 (br m, 4H, NC$\underline{H}_2$COO^{t}Bu/cyclen-C$\underline{H}_2$/NC$\underline{H}_2$py), 2.78 (br m, 4H, NC$\underline{H}_2$COO^{t}Bu/cyclen-C$\underline{H}_2$/NC$\underline{H}_2$py), 1.22 (s, 9H, C(C$\underline{H}_3$)$_3$); ESI-LRMS (+) *m/z* 494.4 $[M+H]^+$, 517.4 $[M+Na]^+$; ESI-HRMS (+) calcd for $C_{24}H_{40}N_5O_6$ 494.2979, found 494.2970.

[Dy.L^1]. Dy(III)Cl_3.6H_2O (4.6 mg, 0.012 mmol) was added to a stirred solution of 2,2',2''-(10-((5-*tert*-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (5.0 mg, 0.010 mmol) in water (2 mL) and the pH adjusted to 5.5 using $NaOH_{(aq)}$. The reaction was left stirring at 50°C overnight. After cooling to rt, the pH was increased to 10 and precipitated metal hydroxide removed by centrifugation. The pH was lowered to 5.5, before solvent was removed under reduced pressure and the residue taken up into ethanol. Any metal hydroxide precipitate and other insoluble material was again removed by centrifugation. The solvent was removed under reduced pressure to yield a white solid (6.3 mg, 97 %). ^{1}H NMR (376 MHz, D_2O, pD 6.5): δ -20.5 (^{t}Bu); ESI-LRMS (+) *m/z* 654.3 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{24}H_{36}{}^{160}DyN_5NaO_6$ 673.1815, found 673.1824.



[Dy.L^1]

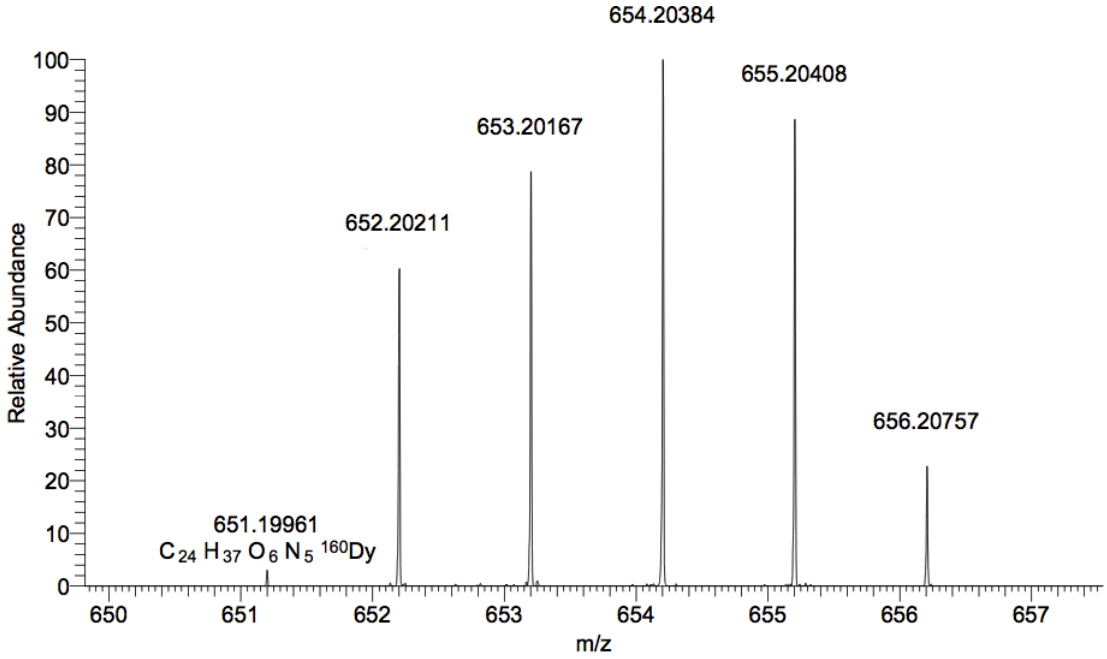


Figure S6 Observed ESI-HRMS (+) spectrum of **[Dy.L^1]**; $[C_{24}H_{37}O_6N_5{}^{160}Dy]^+$ requires 651.1990, found 651.1996, error 0.9 ppm.

[Tm.L^1]. An analogous procedure to that described for the synthesis of **[Dy.L^1]** was followed using 2,2',2''-(10-((5-*tert*-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (4.0 mg, 0.0081 mmol) and Tm(III)Cl_3.6H_2O (3.7 mg, 0.0097 mmol) to give a white solid (5.2 mg, 98 %). ^{1}H NMR (376 MHz, D_2O, pD 6.5): δ +10.8 (^{t}Bu); ESI-LRMS (+) *m/z* 682.3 $[M+Na]^+$; ESI-HRMS (+) calcd for $C_{24}H_{36}{}^{169}TmN_5NaO_6$ 682.1906, found 682.1880.

1,4,7,10-Tetraazacyclododecane-1,7-dicarboxylic acid dibenzyl ester. 1,4,7,10-Tetraazacyclododecane (3.5 g, 20.3 mmol) was dissolved in a solution of distilled H_2O:dioxane (50:20 mL), followed by addition of Na_2HPO_4 (9.8 g, 69.0 mmol). The pH of the solution was adjusted to 2.5 by careful addition of conc. HCl (aq). Benzyl chloroformate (7.3 mL, 50.8 mmol) in dioxane (10 mL) was added dropwise over a period of 30 min and the solution left to stir for a further 18 h at room temperature,

yielding a colourless solution with a white precipitate. The solvent was evaporated under reduced pressure and the residue dissolved in H_2O (100 mL), followed by adjustment of the pH to 7 by addition of conc. KOH (aq). The aqueous phase was extracted with Et_2O (2 x 100 mL), followed by CH_2Cl_2 (2 x 100 mL). The organic phases were combined, dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure to give a clear viscous oil. The oil was dissolved in CH_2Cl_2 (10 mL) and to it cold Et_2O (100 mL) was added, causing a white solid to precipitate (5.80 g, 65 %). m.p. 115-117 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29–7.42 (m, 10H, Ar), 5.17 (s, 4H, Cbz CH_2), 3.27–4.13 (br m, 8H, cyclen CH_2), 2.76–3.22 (br m, 8H, cyclen CH_2), 1.70 (br s, 2H, NH); ^{13}C NMR (176 MHz, $CDCl_3$): δ 156.2, 128.0, 128.4, 128.5, 128.7, 135.8, 67.8, 67.9, 49.0, 50.5; ESI-LRMS (+) *m/z* 441.6 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{24}H_{33}O_4N_4$ 441.2502, found 441.2514.

4,10-Bis-*tert*-butoxycarbonylmethyl-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylic acid dibenzyl ester. 1,4,7,10-Tetraazacyclododecane-1,7-dicarboxylic acid dibenzyl ester (5.8 g, 13.2 mmol) and *tert*-butyl bromoacetate (4.5 mL, 30.4 mmol) were dissolved in anhydrous CH_3CN (70 mL), followed by the addition of Cs_2CO_3 (12.9 g, 39.6 mmol). The mixture was heated at 80^{o}C for 18 h. The caesium salts were filtered off, and the solvent removed under reduced pressure. The resulting dark yellow oil was purified by silica gel column chromatography, eluting with a gradient starting from 100% CH_2Cl_2 to 2% CH_3OH/CH_2Cl_2 to yield a dark yellow oil (8.0 g, 91 %). R_f (10 % CH_3OH/CH_2Cl_2) = 0.53; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28-7.38 (10H, m, Ar), 5.12 (4H, s, Cbz CH_2), 3.14-3.51 (12H, cyclen CH_2/CH_2CO), 2.74-2.96 (8H, br s, CH_2 ring), 1.42 (18H, s, $C(C\underline{H}_3)_3$); ^{13}C NMR ($CDCl_3$, 101 MHz): δ 169.6, 155.5, 135.9, 127.5, 127.0, 126.9, 80.0, 66.0, 55.0, 52.9-53.7, 45.7-46.1, 27.2; ESI-LRMS (+) *m/z* 669.3 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{36}H_{53}O_8N_4$ 669.3863, found 669.3888.

(7-*tert*-Butoxycarbonylmethyl-1,4,7,10-tetraazacyclododec-1-yl)-acetic acid *tert*-butyl ester (7). 4,10-Bis-*tert*-butoxycarbonylmethyl-1,4,7,10-tetraazacyclododecane-1,7-dicarboxylic acid dibenzyl ester (2.0 g, 2.99 mmol) was dissolved in CH_3OH (10 mL) to which $Pd(OH)_2/C$ (Pd content 10 %, 50 mg) was added. The vessel was then loaded onto a Parr hydrogenator (pressure 40 bar H_2) and the reaction mixture agitated for 3 days. After this time, the catalyst was filtered off and the solvent removed under reduced pressure to yield a glassy yellow oil (1.06 g, 89 %). ^{1}H NMR

(400 MHz, $CDCl_3$): δ 3.28 (4H, s, CH_2CO), 2.81 (8H, br m, cyclen CH_2), 2.65 (8H, br m, cyclen CH_2), 1.36 (18H, s, $C(C\underline{H}_3)_3$); ^{13}C NMR (101MHz, $CDCl_3$): δ 170.7, 80.7, 60.1, 45.8, 49.1, 51.1, 56.3, 27.6; ESI-LRMS (+) *m/z* 401.3 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{20}H_{41}O_4N_4$ 401.3128, found 401.3109.

***tert*-Butyl 2,2'-(4,10-bis((5-*tert*-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (8).** (5-*tert*-Butylpyridin-2-yl)methyl methanesulfonate (530 mg, 2.18 mmol) and (7-*tert*-butoxycarbonylmethyl-1,4,7,10-tetraazacyclododec-1-yl)-acetic acid *tert*-butyl ester (DO2A) (874 mg, 2.18 mmol) were dissolved in anhydrous MeCN (15 mL) under an atmosphere of argon. To this was added Na_2CO_3 (231 mg, 2.18 mmol) and the reaction mixture stirred at 50°C for 48 h. After this, the reaction mixture was filtered before the solvent was removed under reduced pressure. The resulting orange oil was purified by silica gel column chromatography, eluting with a gradient starting from 100 % DCM to 10 % MeOH/DCM to yield a yellow-orange sticky solid (284 mg, 19 %). R_f (15 % MeOH/DCM) = 0.68; ^1H NMR (600 MHz, $CDCl_3$): δ 8.36 (d, *J* = 2 Hz, 2H, H^6), 7.63 (dd, *J* = 8, 2 Hz, 2H, H^4), 7.07 (d, *J* = 8 Hz, 2H, H^3), 3.68 (br s, 4H, $NC\underline{H}_2py$), 2.76 (br m, 10H, $NC\underline{H}_2CO_2{}^tBu$/cyclen-$C\underline{H}_2$), 2.37 (br m, 8H, cyclen-$C\underline{H}_2$), 2.14 (br s, 2H, cyclen-$C\underline{H}_2$), 1.27 (s, 18H, ^tBu), 1.23 (s, 18H, ^tBu); ^{13}C NMR (151 MHz, $CDCl_3$): δ 172.0, 155.1, 146.0, 144.5, 133.7, 122.1, 81.7, 58.1, 57.2, 53.4, 50.3, 33.3, 31.0, 27.9; ESI-LRMS (+) *m/z* 696.1 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{40}H_{67}N_6O_4$ 695.5224, found 695.5197.

2,2'-(4,10-Bis((5-*tert*-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (L^2). *tert*-Butyl 2,2'-(4,10-bis((5-*tert*-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (18.3 mg, 0.026 mmol) was dissolved in HCl (6 M, 3 mL) and stirred for 18 h at 90°C. The solvent was removed under reduced pressure and the residue washed repeatedly with DCM (3 x 5 mL), before being dissolved in EtOH. The resulting white precipitate was removed by filtration and the solvent removed under reduced pressure to yield the hydrochloride salt of $\mathbf{L^2}$ as a pale yellow gum (12.7 mg, 84 %). ^1H NMR (400 MHz, D_2O): δ 8.73 (d, *J* = 2 Hz, 2H, H^6), 8.51 (dd, *J* = 8, 2 Hz, 2H, H^4), 7.89 (d, *J* = 8 Hz, 2H, H^3), 3.81 (br s, 4H, $NC\underline{H}_2py$), 3.46 (br s, 4H, $NC\underline{H}_2CO_2{}^tBu$), 3.35 (br s, 4H, cyclen-$C\underline{H}_2$), 3.17 (br s, 4H, cyclen-$C\underline{H}_2$), 2.99 (br s, 4H, cyclen-$C\underline{H}_2$), 2.74 (br m, 4H, cyclen-$C\underline{H}_2$), 1.27 (s, 18H, ^tBu); ESI-LRMS (+) *m/z* 583.9 $[M+H]^+$.

[Dy.L^2]$^+$. Dy(III)Cl_3.6H_2O (19 mg, 0.052 mmol) was added to a stirred solution of 2,2'-(4,10-bis((5-*tert*-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (25 mg, 0.043 mmol) in water (2 mL) and the pH adjusted to 5.5 using $NaOH_{(aq)}$. The reaction was left stirring at 50°C overnight. After cooling to rt, the pH was increased to 10 and precipitated metal hydroxide removed by centrifugation. The pH was lowered back to 5.5, before the solvent was removed under reduced pressure and the residue taken up into ethanol. Any remaining metal hydroxide precipitate and other insoluble material was again removed by centrifugation. The solvent was removed under reduced pressure to yield a white solid (30.5 mg, 96 %). ^{1}H NMR (376 MHz, D_2O, pD 6.5): δ -17.8 (^{t}Bu); ESI-LRMS (+) *m/z* 744.9 [M]$^+$; ESI-HRMS (+) calcd for $C_{32}H_{48}{}^{160}DyN_6O_4$ 740.2989, found 740.2997.

[Tm.L^2]$^+$. An analogous procedure to that described for the synthesis of **[Dy.L^2]$^+$** was followed using 2,2'-(4,10-bis((5-*tert*-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (10.5 mg, 0.019 mmol) and Tm(III)Cl_3.6H_2O (8.7 mg, 0.023 mmol) to give a white solid (13.5 mg, 95 %). ^{1}H NMR (376 MHz, D_2O, pD 6.5): δ +6.2 (^{t}Bu); ESI-LRMS (+) *m/z* 750.0 [M]$^+$; ESI-HRMS (+) calcd for $C_{32}H_{48}{}^{169}TmN_6O_4$ 749.3079, found 749.3090.

tert-Butyl 2,2'-(4-((5-tert-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (9). (5-*tert*-Butylpyridin-2-yl)methyl methanesulfonate (530 mg, 2.18 mmol) and (7-*tert*-butoxycarbonylmethyl-1,4,7,10-tetraazacyclododec-1-yl)-acetic acid *tert*-butyl ester (DO2A) (874 mg, 2.18 mmol) were dissolved in anhydrous MeCN (15 mL) under an atmosphere of argon. To this was added Na_2CO_3 (231 mg, 2.18 mmol) and the reaction mixture stirred at 50°C for 48 h. After this, the reaction mixture was filtered before the solvent was removed under reduced pressure. The resulting orange oil was purified by silica gel column chromatography, eluting with a gradient starting from 100 % DCM to 10 % MeOH/DCM to yield a yellow sticky solid (470 mg, 37 %). R_f (15 % MeOH/DCM) = 0.56; ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.82 (d, *J* = 2 Hz, 1H, H^6), 7.60 (dd, *J* = 8, 2 Hz, 1H, H^4), 7.14 (d, *J* = 8 Hz, 1H, H^3), 3.65 (s, 2H, NC$\underline{H}_2$py), 3.07 (s, 12H, NC$\underline{H}_2$COO/cyclen-C$\underline{H}_2$), 2.78 (br m, 4H, cyclen-C$\underline{H}_2$), 2.57 (br s, 4H, cyclen-C$\underline{H}_2$), 1.38 (s, 18H, COOC(C$\underline{H}_3$)$_3$), 1.32 (s, 9H, pyC(C$\underline{H}_3$)$_3$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 170.5, 154.2, 147.8, 145.0, 133.3, 123.2, 81.3, 56.6, 54.0, 53.4, 50.6, 50.3, 46.6,

33.5, 31.0, 28.1; ESI-LRMS (+) *m/z* 549.0 $[M+H]^+$, 576.9 $[M+Na]^+$; ESI-HRMS (+) calcd for $C_{30}H_{54}N_5O_4$ 548.4176, found 548.4176.

2-Methanesulphonate-*N*-methanesulphonylethylamine (10). Methane sulfonyl chloride (5.1 mL, 65.4 mmol) was added carefully to a stirred solution of ethanolamine (2 mL, 32.7 mmol), pyridine (5.8 mL, 72.0 mmol) and anhydrous THF (15 mL), at 5 °C. When addition was complete the solution was allowed to warm to rt and was subsequently stirred for 1 h. After this time, the reaction mixture was poured onto ice and extracted with CH_2Cl_2 (4 x 30 mL). The organic layers were combined and washed with 10 % $CuSO_4$ solution (50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure to yield a yellow solid (1.23 g, 17 %). m.p. 45-47 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.85 (br s, 1H, NH), 4.36 (t, ^3J = 5, 2H, OCH_2), 3.51 (q, ^3J = 5, 2H, NCH_2), 3.08 (s, 3H, SCH_3), 3.02 (s, 3H, SCH_3); ^{13}C NMR (101 MHz, $CDCl_3$): δ 68.7 (OC), 42.4 (NC), 41.0 (SC), 37.6 (SC); ESI-LRMS (+) *m/z* 218.0 $[M+H]^+$; ESI-HRMS (+) calcd for $C_4H_{12}NO_5S_2$ 218.0157, found 218.0149.

***tert*-Butyl 2,2'-(4-((5-*tert*-butylpyridin-2-yl)methyl)-10-(2-(methylsulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (12).** A solution of tert-butyl 2,2'-(4-((5-tert-butylpyridin-2-yl)methyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (110 mg, 0.201 mmol), 2-methanesulphonate-*N*-methanesulphonylethylamine (44 mg, 0.201 mmol), and K_2CO_3 (56 mg, 0.402 mmol) in anhydrous MeCN (10 mL) were heated at 80°C for 18 h, before the solution was cooled to rt and filtered. The solvent was removed under reduced pressure to produce a yellow-brown oil that was purified by silica gel column chromatography, eluting with a gradient starting from 100 % DCM to 7 % MeOH/DCM to yield a yellow sticky solid (86 mg, 64 %). R_f (10 % MeOH/DCM) = 0.18; ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.66 (d, J = 2 Hz, 1H, H^6), 7.60 (dd, J = 8, 2 Hz, 1H, H^4), 7.02 (d, J = 8 Hz, 1H, H^3), 3.62 (s, 2H, $NC\underline{H}_2$py), 3.32 (m, 2H, $CH_2C\underline{H}_2NHSO_2$), 3.15 (br m, 4H, $NC\underline{H}_2COO$), 2.99 (s, 3H, $SO_2C\underline{H}_3$), 2.78 (br m, 6H, $NC\underline{H}_2CH_2NHSO_2$/cyclen-$C\underline{H}_2$), 2.50 (br m, 10H, cyclen-$C\underline{H}_2$), 1.35 (s, 18H, $COOC(C\underline{H}_3)_3$), 1.30 (s, 9H, $pyC(C\underline{H}_3)_3$); ^{13}C NMR (151 MHz, $CDCl_3$): δ 172.1, 154.3, 147.7, 144.9, 133.6, 122.0, 82.2, 58.6, 56.7, 53.6, 53.4, 50.3, 40.3, 38.7, 33.5, 30.9, 28.1; ESI-LRMS (+) *m/z* 670.0 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{33}H_{61}N_6O_6S$ 669.4373, found 669.4362.

2,2'-(4-((5-tert-butylpyridin-2-yl)methyl)-10-(2-(methylsulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (L^3). *tert*-Butyl 2,2'-(4-((5-*tert*-butylpyridin-2-yl)methyl)-10-(2-(methylsulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (45 mg, 0.67 mmol) was dissolved in DCM (3 mL) with stirring. To this was added TFA (1 mL) and the mixture stirred at rt for 18 h. After this time, the solvent was removed under reduced pressure to give a yellow oil, which was repeatedly washed with DCM to yield the TFA salt of $\mathbf{L^3}$ as a pale yellow oil (37 mg, 98 %). ^{1}H NMR (600 MHz, D_2O): δ 8.63 (d, *J* = 2 Hz, 1H, H^6), 8.43 (dd, *J* = 8, 2 Hz, 1H, H^4), 7.87 (d, *J* = 8 Hz, 1H, H^3), 3.94 (s, 2H, NC$\underline{H}_2$py), 3.70 (br m, 2H, NC$\underline{H}_2$COO), 3.50 (br s, 2H, NC$\underline{H}_2$COO), 3.43 (br m, 4H, cyclen-C$\underline{H}_2$), 3.30 (br m, 6H, cyclen-C$\underline{H}_2$), 3.15 (m, 2H, $CH_2C\underline{H}_2NHSO_2$), 3.03 (br m, 2H, cyclen-C$\underline{H}_2$), 2.96 (m, 2H, NC$\underline{H}_2CH_2NHSO_2$), 2.94 (s, 3H, $SO_2C\underline{H}_3$), 2.79 (br m, 2H, cyclen-C$\underline{H}_2$), 2.72 (br m, 2H, cyclen-C$\underline{H}_2$), 1.22 (s, 9H, pyC(C$\underline{H}_3$)$_3$); ESI-LRMS (+) *m/z* 557.8 $[M+H]^+$.

[Dy.L^3]. Dy(III)$Cl_3.6H_2O$ (8.5 mg, 0.0226 mmol) was added to a stirred solution of 2,2'-(4-((5-*tert*-butylpyridin-2-yl)methyl)-10-(2-(methylsulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (10.5 mg, 0.0189 mmol) in water (2 mL) and the pH adjusted to 5.5 using $NaOH_{(aq)}$. The reaction was left stirring at 50°C overnight. After cooling to rt, the pH was increased to 10 and precipitated metal hydroxide removed by centrifugation. The pH was lowered back to 5.5, before the solvent was removed under reduced pressure and the residue taken up into ethanol. Any remaining metal hydroxide precipitate and other insoluble material was again removed by centrifugation. The solvent was removed under reduced pressure to yield a white solid (13 mg, 97 %). ^{1}H NMR (400 MHz, D_2O, pD 6.5): δ -58.9 (^{t}Bu); ESI-LRMS (+) *m/z* 740.8 $[M-H+Na]^+$; ESI-HRMS (+) calcd for $C_{25}H_{41}{}^{160}DyN_6O_6SNa$ 736.1958, found 736.1945.

[Tm.L^3]. An analogous procedure to that for the synthesis of **[Dy.L^3]** was followed using 2,2'-(4-((5-*tert*-butylpyridin-2-yl)methyl)-10-(2-(methylsulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (8.3 mg, 0.015 mmol) and Tm(III)$Cl_3.6H_2O$ (6.9 mg, 0.018 mmol) to give a white solid (10.5 mg, 97 %). ^{1}H NMR (400 MHz, D_2O, pD 6.5): δ +44.3 (^{t}Bu); ESI-LRMS (+) *m/z* 745.8 $[M-H+Na]^+$; ESI-HRMS (+) calcd for $C_{25}H_{41}{}^{169}TmN_6O_6SNa$ 745.2048, found 745.2066.

2-(2,2-Dimethylpropylsulfonamido)ethyl 2,2-dimethylpropane-1-sulfonate (11). 2,2-Dimethylpropane-1-sulfonyl chloride (2.0 g, 11.7 mmol) in pyridine (10ml) was added dropwise to a solution of ethanolamine (0.36 g, 5.86 mmol) in pyridine (30ml) cooled to -40°C. The reaction mixture was maintained at -10°C and stored in the fridge overnight. The reaction mixture was then poured onto crushed ice. The resulting slurry was extracted using DCM (10 mL), washed with water (3 x 10 mL) and dried using $MgSO_4$ before the solvent was removed under reduced pressure. The resulting oily residue was purified by silica gel column chromatography, eluting with a gradient starting from 100% DCM to 5 % MeOH/DCM to yield a pale yellow solid (0.74 g, 54 %). R_f = 0.42 (5 % MeOH/DCM); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.03 (1H, s, NH), 4.28 (2H, t, ^1J = 12, OCH_2), 3.41 (2H, dd, ^1J = 12, ^2J = 4, NCH_2), 3.09 (2H, s, SCH_3), 2.98 (2H, s, SCH_3), 1.16 (9H, s, $C(CH_3)_3$), 1.15 (9H, s, $C(CH_3)_3$; ^{13}C NMR (101 MHz, $CDCl_3$): δ 68.63 (2C, SC, SC), 64.78 (NC), 62.08 (OC), 31.69 ($\underline{C}(CH_3)_3$), 31.42 ($\underline{C}(CH_3)_3$), 29.73 ($C(\underline{C}H_3)_3$), 29.53 ($C(\underline{C}H_3)_3$); ESI-LRMS (+) *m/z* 330.1 $[M+H]^+$, 352.2 $[M+Na]^+$; ESI-HRMS (+) calcd for $C_{12}H_{28}NO_5S_2$ 330.1409, found 330.1401.

Di-*tert*-Butyl 2,2'-(4-((5-*tert*-butylpyridin-2-yl)methyl)-10-(2- (2,2 dimethylpropyl sulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (13). 2-(2,2-dimethylpropylsulfonamido)ethyl 2,2-dimethylpropane-1-sulfonate (0.09 g, 0.27 mmol) was added to a solution of tert-butyl 2,2'-(4-((5-tert-butylpyridin-2-yl)methyl)-1,4,7,10- tetraazacyclododecane-1,7-diyl) diacetate (0.15 g, 0.27 mmol) and K_2CO_3 (0.08 g, 0.55 mmol) in dry MeCN (25 mL). The reaction mixture was heated at 80°C under argon for 18 h, before being filtered and concentrated *in vacuo*. The resulting oil was purified by silica gel column chromatography, eluting with a gradient starting from 100 % DCM to 15 % MeOH/DCM, 2 % NH_4OH to yield a colourless oil (0.08 g, 37 %). R_f = 0.42 (15% MeOH/DCM, 2 % NH_4OH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.59 (1H, s, H^6), 7.63 (1H, d, ^{2}J = 12, H^4), 7.34 (1H, d, ^{2}J =12, H^3), 3.37 (4H, br s, NCH_2CO), 3.09-3.22(14H, br, NCH_2, cyclen CH_2), 3.00 (2H, s, $C\underline{H}_2C$), 2.83 (8H, br s, cyclen $C\underline{H}_2$), 1.39 (18H, s, ^{t}Bu $C\underline{H}_3$), 1.30 (s, 9H, $pyC(C\underline{H}_3)_3$), 1.15 (9H, s, $CH_2C(C\underline{H}_3)_3$); ^{13}C NMR (101 MHz, $CDCl_3$): δ 170.62, 154.3, 147.7, 144.9, 133.6, 122.0, 82.9, 82.6, 68.7, 63.7, 63.4, 58.5, 55.9, 55.6, 55.37, 53.48, 53.18, 49.7, 33.5, 30.8, 29.8, 29.4; ESI-LRMS (+) *m/z* 725.5 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{37}H_{69}N_6O_6S$ 725.4989, found 725.4985.

2,2'-(4-((5-*tert*-Butylpyridin-2-yl)methyl)-10-(2- (2,2 dimethylpropyl sulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (L^4). Di-*tert*-Butyl 2,2'-(4-((5-*tert*-butylpyridin-2-yl)methyl)-10-(2- (2,2 dimethylpropyl sulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (0.08 g, 0.11 mmol) was dissolved in DCM (5 mL) and to this was added TFA (2 mL). The resulting mixture was stirred at rt for 18 h. The solvent was removed under reduced pressure and any residual TFA was removed by adding small portions (3 x 1 mL) of DCM which was removed each time under vacuum. The resulting residue was dissolved in DCM (10 mL) and was stirred for 10 min, before the solvent was decanted and dried under vacuum to yield a glassy solid (0.06 g, 89%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.66 (1H, dd, ^2J =4, H^6), 7.95 (1H, dd, ^2J = 8 , ^3J = 4, H^4), 7.42 (1H, d, ^2J = 8, H^3), 3.56 (4H, br s, NCH_2CO), 3.41-3.69 (14H, br m, NCH_2, cyclen CH_2), 3.09 (2H, s, CH_2CO), 2.98-3.4 (8H, br s, cyclen CH_2), 1.37 (s, 9H, pyC($C\underline{H}_3)_3$), 1.17 (9H, s, $CH_2C(C\underline{H}_3)_3$); ESI-LRMS (+) *m/z* 613.37 $[M+H]^+$; ESI-HRMS (+) calcd for $C_{29}H_{53}N_6O_6S$ 613.3747, found 613.3761.

[Dy.L^4]. 2,2'-(4-((5-*tert*-Butylpyridin-2-yl)methyl)-10-(2- (2,2 dimethylpropyl sulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (0.04 g, 0.07 mmol) and Dy(III)$Cl_3.6H_2O$ (0.035 g, 0.07 mmol) were dissolved in distilled water (10 mL) and the pH adjusted to 5.5 using $NaOH_{(aq)}$. The reaction mixture was heated at 90°C for 18 h. After this, the pH was raised to 10 using $NaOH_{(aq)}$, which caused the precipitation of a white solid. This solid was removed by centrifugation, the solvent isolated, the pH adjusted to 7 and the solvent removed by lyophilisation. The resulting white solid was extracted using 50 % MeOH/DCM and the solvent was removed under vacuum to yield a white solid (0.04 g, 80 %). ^{1}H NMR (400 MHz, D_2O, pD 10.0): δ -15.1 (^{t}Bu), -54.2 (py^{t}Bu); ESI-LRMS (+) *m/z* 774.28 $[M^+]$; ESI-HRMS (+) calcd for $C_{29}H_{50}O_6N_6S^{160}Dy$ 770.2762, found 770.2762; HPLC t_R = 9.9 min, *m/z* 774.28 $[M]^+$.

[Tm.L^4]. An analogous procedure to that described for the synthesis of **[Dy.L^4]** was followed using 2,2'-(4-((5-*tert*-Butylpyridin-2-yl)methyl)-10-(2- (2,2 dimethylpropyl sulfonamido)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (0.05, 0.08 mmol) and Tm(III)$Cl_3.6H_2O$ (0.035 g, 0.08 mmol) to give a white solid (0.05 g, 79 %). ^{1}H NMR (400 MHz, D_2O, pD 10.0): δ 22.5, 23.9 (^{t}Bu), 37.4 (py^{t}Bu); ESI-LRMS

(+) *m/z* 779.28 [M^+]; HRMS Calcd for $C_{29}H_{50}O_6N_6S^{169}Tm$ 779.2854, found 779.2874; HPLC t_R = 9.5 min, *m/z* 779.28 $[M]^+$.

5. Imaging experiments: phantom studies and in vivo experiments

Imaging studies were carried out by Prof. Andrew Blamire and Dr. Ian Wilson at Newcastle Magnetic Resonance Centre, using a 7 T Varian Unity Inova microimaging/Pre-clinical system equipped with broadband capability and actively shielded gradients. For the ^{1}H MRI studies, *in vitro* experiments were performed with a 30 mm i.d. birdcage volume coil (Rapid Biomedical) and *in vivo* experiments were performed with a 39 mm i.d. birdcage volume coil. All coils were used for both signal excitation and reception.

For phantom studies, dilute aqueous samples (2-5 mM) were placed in Eppendorf tubes or cut down (2-3 mm) NMR tubes and positioned on the axis of the coil. For the *in vivo* studies, nude mice bearing a HT29 colorectal tumour xenograft were anaesthetised with oxygen/1-2 % isofluorane. Complexes were administered intravenously *via* the tail vein as an 0.3mL saline solution, with doses typically of 0.03 mmol/kg.

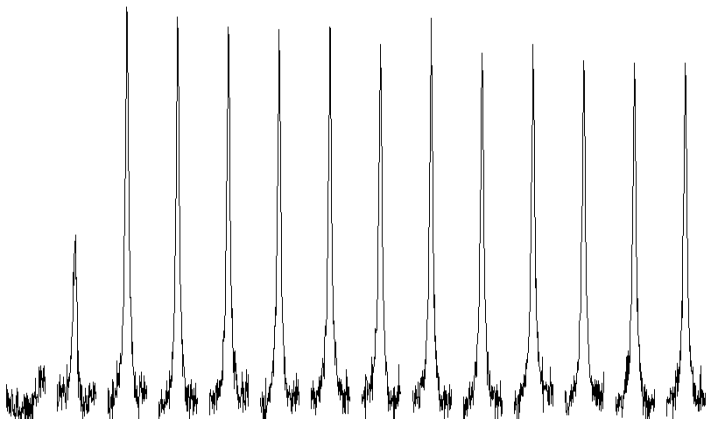


Figure S7 Time series of the observed t-Bu signal following administration of [**Dy.L^2**]+ in a mouse (0.12 mmol/kg) bearing an HT-29 tumour, taken at zero, 10, 60 and thence every 21s following injection.

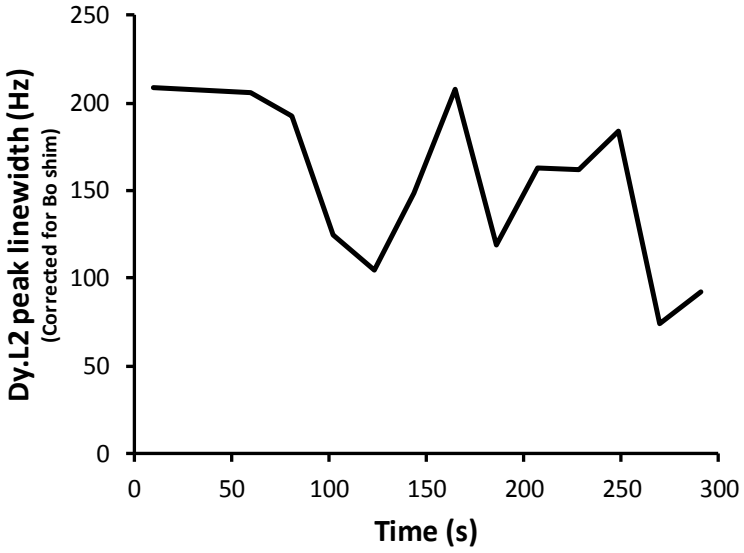


Figure S8 Variation of the linewidth of the observed t-butyl signal following injection of [**Dy.L^2**]$^+$, showing a gradual diminution over the 5 minute period.

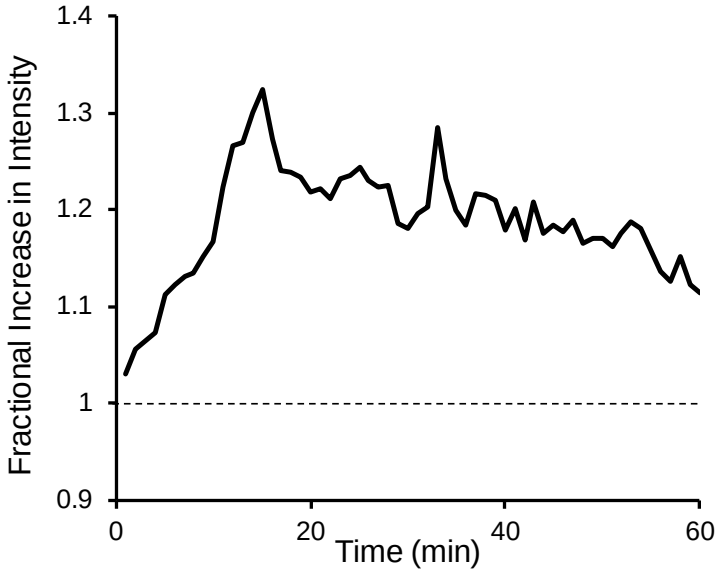


Figure S9 Relative T_1-weighted ^{1}H MRI water signal intensity as a function of time, within HT29 tumours of SCID male mice (mean of 3) following intravenous administration of **[Gd.L^2]$^+$**(0.03 mM/kg)

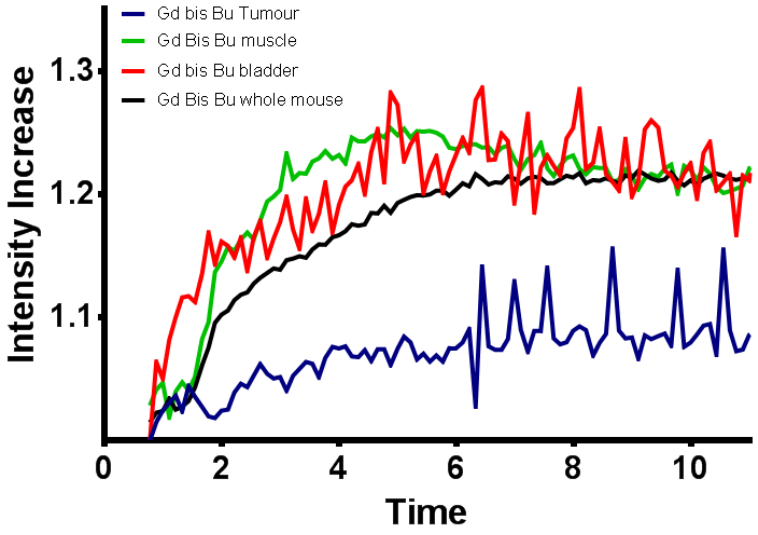


Figure S10 Signal intensity changes in a contrast enhanced MRI experiment observing the water proton signal following injection of 0.09mmol/kg of $[Gd.L^2(H_2O)]^+$.

[1] (a) Bertini, I.; Luchinat, C.; Parigi, G. *Solution NMR of Paramagnetic Molecules*; Elsevier: Amsterdam, 2001. (b) Gignoux, D.; Schmitt, D.; Zeguine, M. *J. Magn. Magn. Mater*. **1987**, 66, 373. (c) Chevalier, B.; Tence, S.; Andre, G.; Matier, S. F.; Gueden, E. *J. Phys. Conf. Ser*. **2010**, 032012.