Variation of water exchange dynamics with ligand structure and stereochemistry in lanthanide complexes based on 1,4-diazepine derivatives†

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Complexes of Gd, Eu and Yb(III) have been prepared with a series of heptadentate ligands related to the parent complex AAZTA, based on the 6-methyl-6-aminoperhydrodiazepine moiety. For (*RR*) and (*RS*)-diastereoisomers of a di-glutarate ligand, solution NMR studies revealed the presence of two major species that undergo water exchange rates at Gd differing by a factor of six. Comparison of solution hydration states for Eu(III) complexes reveals that each complex possesses two bound water molecules. The absence of a good correlation of ^{1}H NMR pseudo-contact shifts for Eu and Yb analogues is suggested to arise from a change in hydration state between Eu and Yb.

Coordination complexes of gadolinium(III) have been the subject of intense research activity over the past 25 years. Since 1988, they have been used as contrast agents for magnetic resonance imaging (MRI) and millions of scans are carried out at clinical centres each year following injection of a gadolinium complex to aid image contrast.[1,2] The factors that determine the efficacy of the contrast agent for this purpose are well understood.[3–6] Thus, the relaxivity (r_{1P}: units mM^{-1} s^{-1}) of a given complex is determined by the number of coordinated and second sphere water molecules,[3,4] the water exchange rate at the metal ion centre[4,5] and the rotational dynamics that define the extent of motional coupling between local water molecules and the tumbling motion of the overall complex or conjugate.[1,6,7]

The gadolinium(III) complexes are most often based on poly(aza-carboxylate) or poly(phenolic) ligands that exist in solution as nine or occasionally eight coordinate complexes. These complexes exist as coordination diastereoisomers, typically they are in slow exchange with respect to the water exchange rate. As each diastereoisomer usually possesses a different water exchange rate,[3,5] it is important to study this aspect in solution. Recently, there has been an upsurge of interest in di-aqua complexes of gadolinium, owing to the desire to maximise relaxivity.[8,9] Provided that displacement of the bound waters by endogenous anions[10] or protein[11] is suppressed and that sufficient kinetic stability with respect to premature loss of Gd(III) is retained, then di-aqua systems may offer distinct advantages. A recent example of such a system was introduced in 2004, with a heptadentate ligand based on the 6-alkyl-6-aminoperhydro-1,4-diazepine ring system, L^1. Thus, Gd(III) complexes of L^2 have been studied[4a] and $[Gd.L^1(H_2O)_2]$ or $[Gd\text{-}AAZTA]^{-1}$ exhibits a relaxivity of 7.1 mM^{-1} s^{-1} (298 K, 20 MHz), that does not vary significantly in the presence of added anions or protein.

With this background in mind, we set out to explore the behaviour of the lanthanide(III) complexes of the mono and di-glutarate analogues of H_4L^2 (AAZTA). Such systems offer the opportunity to allow conjugation of selected hydrophilic moieties, based on primary amines, leading to complexes of defined molecular volume. The extent to which the modulation of the ligand structure and stereochemistry affect exchange dynamics—and hence relaxivity—is analysed for complexes of L^2–L^5.

Ligand and complex synthesis and NMR characterisation

The synthesis of the 6-amino-1,4-perhydrodiazepine intermediate, L^1, and the ligand L^2 (or AAZTA), was carried out according to a literature method.[4a] Alkylation of L^1 occurs preferentially at the endocyclic ring nitrogen atoms, even with reactive electrophiles such as the α-bromo esters **1** and **2**, owing to the high steric demand at the 6-amino position. Reaction of L^1 with one equivalent of the orthogonally protected α-bromo ester, (*S*)-**1**, (K_2CO_3, MeCN), gave rise to the diastereoisomers, **3a** and **4a**, which were separated by fractional crystallisation from ethanol. The dialkylation product, **5a**, was also isolated from this procedure and could be obtained in a separate procedure in 70% yield by stoichiometric alkylation. Compounds **3a** and **4a** were distinguished by the ^{1}H NMR chemical shift non-equivalence of the benzylic CH_2 singlet ($\Delta\delta = 0.008$ ppm, $CDCl_3$, 295 K, 700 MHz), but their absolute configuration was not assigned. Subsequent reaction of **3a** or **5a** with *tert*-butylbromoacetate (K_2CO_3, Na_2SO_4, MeCN) afforded the esters **3b** and **5b**, which were purified by chromatography on neutral alumina. Stepwise reaction with trifluoroacetic acid and hydrogenolysis of the benzylic ester yielded the ligands H_6L^3 and H_5L^5. In a separate reaction pathway, Scheme 1, the two (*meso*)-diastereoisomers of H_6L^4 were obtained, possessing a pseudo-asymmetric centre at the quaternary C, involving a sequence of three alkylation reactions, using (*R*) and (*S*)-**1**.

For purposes of comparison, a statistical mixture of (*RR*)-L^3, (*SS*)-L^3 and (*meso*)-L^4 was also prepared. Reaction of L^1 with racemic α-bromodimethylglutarate afforded the ester **6a**

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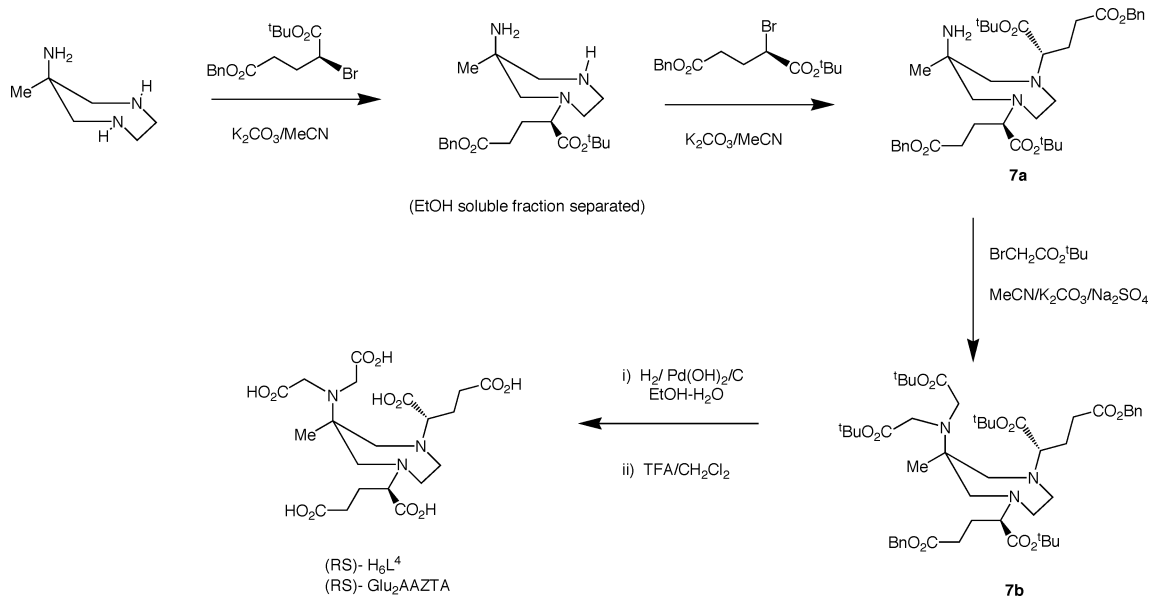
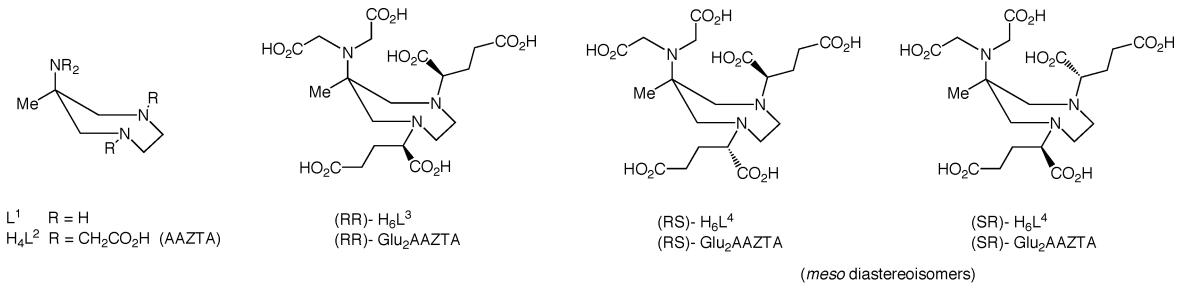
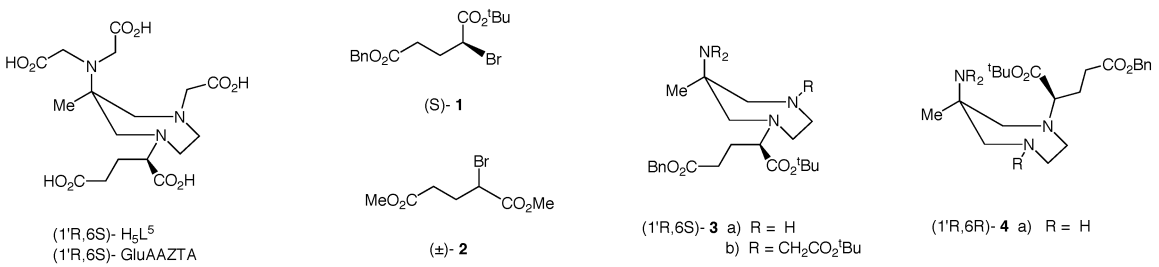
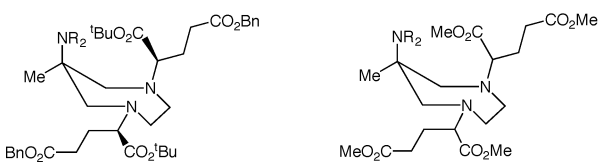
† Electronic supplementary information (ESI) available: ^{1}H NMR spectra of complexes, NMRD profiles of $[Gd.L^5]^{2-}$ and HPLC methods of analysis. See DOI: 10.1039/b818445c


K2CO3/MeCN
K2CO3/MeCN
(EtOH soluble fraction separated)
7a
BrCH2CO2tBu
MeCN/K2CO3/Na2SO4
i) H2/ Pd(OH)2/C
EtOH-H2O
ii) TFA/CH2Cl2
(RS)- H6L4
(RS)- Glu2AAZTA
7b

Scheme 1


L1 R = H
H4L2 R = CH2CO2H (AAZTA)
(RR)- H6L3
(RR)- Glu2AAZTA
(RS)- H6L4
(RS)- Glu2AAZTA
(SR)- H6L4
(SR)- Glu2AAZTA
(meso diastereoisomers)


(1'R,6S)- H5L5
(1'R,6S)- GluAAZTA
(S)- 1
(±)- 2
(1'R,6S)- 3 a) R = H
b) R = CH2CO2tBu
(1'R,6R)- 4 a) R = H


5 a) R = H
b) R = CH2CO2tBu
6 a) R = H
b) R = CH2CO2tBu

and subsequent treatment with *tert*-butylbromoacetate afforded the intermediate hexa-ester, **6b**. Stepwise ester hydrolysis (90% TFA–$CH_2CH_2CL_2$ then aq. KOH) afforded the mixture of (*RR*)–(*SS*)H_6L^3 and the two *meso* diastereoisomers of H_6L^4, following treatment with ion-exchange resin. Complexes of ligands L^2–L^5 with lanthanide(III) ions (Ln = Eu, Gd, Yb) were prepared at pH 5.5, using $LnCl_3 \cdot 6H_2O$ salts.

Information about the number of diastereoisomers present in solution was obtained by comparing ^{1}H NMR spectral data for the Eu(III) and Yb(III) complexes at 200, 500 and 700 MHz. First, the case of the Yb(III) complexes of (*RR*)-L^3, (*SS*)-L^3 (n.b. gives rise to identical NMR spectra for the enantiomeric series) and (*meso*)-L^4, present in the stereoisomeric mixture of ligands prepared from (±)-**2** was considered. Two diastereoisomeric complexes were observed in approximately a 9 : 4 ratio, (Fig. 1); each isomer was characterised by a separate set of dipolar shifts, exemplified by the resonance for the 6-methyl group at +10 and +14 ppm. The corresponding Eu(III) complex, derived from the same ligand mixture, gave a ^{1}H NMR spectrum of similar but not identical overall form, with two species in a ratio of ~2 : 1, with reduced dipolar shifts, in one of which the 6-Me group resonated at +6.6 ppm.

The assignment of these NMR spectra was aided by separate examination of ^{1}H NMR spectra from the enantiopure complexes, (*RR*)-[Yb.L^3]$^{3-}$ and (*RS*)-[Yb.L^4]$^{3-}$, (Fig. 2). In this case, the major species were observed in a ratio of 10 : 1, in which the 6-Me group resonated at +14 ppm. For (*RS*)-[Yb.L^4]$^{3-}$ the same sets of resonances were apparent, but in a ratio of 1 : 4, with the 6-Me group resonating at +10 ppm. Examination of the analogous Eu(III) complexes was also undertaken. In this case, the major isomer observed for the (*RR*)-[Eu.L^3]$^{3-}$ complex (in an 8 : 1 ratio) corresponded in chemical shift to the minor isomer for (*RS*)-[Eu.L^4]$^{3-}$ (ratio 1 : 3) and *vice versa*, (Fig. 3).

Comparison of the ^{1}H NMR spectra of Eu and Yb(III) complexes of a common ligand, *e.g.* (*RS*)-L^4, suggested that the two isomers observed had different solution coordination structures. The sense and sequence of NMR dipolar shifts differed in each case and most markedly for the complexes of (*RS*)-L^4. Previous work has established that the dipolar shifts of Eu/Yb(III) complexes of a common ligand, in which a constant coordination number and geometry are conserved, are strictly related.[5,12] In this case, given the rigidity of the ligand, the absence of a good correlation may be due to a change in complex hydration, reducing the coordination number from 9 (earlier Ln(III) ions) to 8 for the smaller ions, *e.g.* Yb(III). This may be manifested in a reduction in the number of coordinated waters from two (Eu) to one (Yb). Examples of this change in hydration state across the lanthanide(III) series have been frequently reported.[1,5,13–15] For example, in Ln(III) complexes based on DOTA (DOTA is 1,4,7,10-tetraazacyclododecane-tetracetate) and related phosphinate complexes, there is a reduction from mono-hydrated systems in the early and central Ln(III) ions, to species with no directly bound water molecules towards the end of the series.[14]

Complex hydration and structure

The solution hydration state of each europium complex was measured using a luminescence method[15] in which the radiative rate constant characterising depopulation of the Eu(III) excited was measured in water and D_2O. In each case (Table 1), data were consistent with the europium ion binding to two water molecules. Measurements of the paramagnetic relaxivity r_{1p} of the Gd(III) complexes at 298 K and 20 MHz gave values of about 8.0 mM^{-1} s^{-1} (Table 1), consistent with the expected value for a di-aqua complex of such a molecular volume.[2] It is evident that the 'mono-glutarate' complexes, (*R*)-[Eu.L^5]$^{2-}$ and (*R*)-[Gd.L^5]$^{2-}$, also form di-aqua

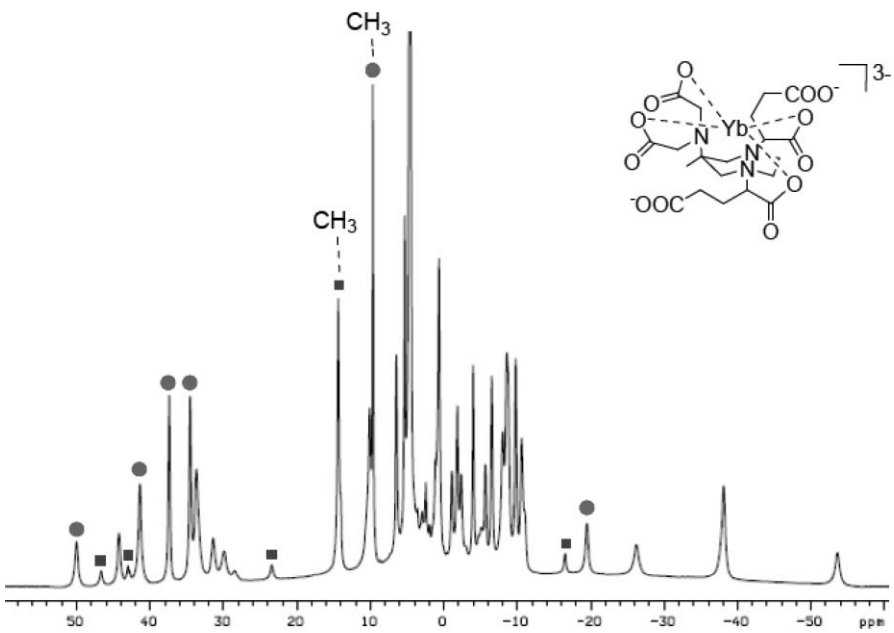


Fig. 1 ^{1}H NMR spectrum of the mixture of Yb(III) complexes derived from (*RR*)-L^3, (*SS*)-L^3 and (*meso*)-L^4, revealing a mixture of two major diastereoisomers in a ratio of 9 : 4 (295 K, pD 5.4, 200 MHz; major species—circles, minor species—squares).

Table 1 Radiative rate constants (ms^{-1}, ±10%) for europium(III) complexes in water and deuterium oxide (296 K, 1 mM complex, λ_{exc} 397 nm)[a]

Complex	$k^{Eu}_{H_2O}$ /ms^{-1}	$k^{Eu}_{D_2O}$ /ms^{-1}	q^b (±20%)
(*RR*/*SS*)-[EuL^3]$^{3-}$ + (*meso*)-[EuL^3]$^{3-}$ (statistical mixture)	3.20	1.20	2.1
(*RR*)-[Eu.L^3]$^{3-}$	3.40	1.15	2.4
(*RS*)-[Eu.L^4]$^{3-}$	2.94	1.27	1.7
(*R*)-[Eu.L^5]$^{2-}$	3.00	1.16	1.9
[Eu.L^2]$^-$	2.90	1.20	2.0

[a] [EuL^2]$^-$ was obtained from Bracco s.a. and was prepared as described in reference **4a**. [b] *q* represents the metal ion hydration number.[15]

complexes in aqueous solution. The ^{1}H NMR spectra of the Eu(III) and Yb(III) complexes (see ESI†) suggested the presence of one dominant solution species (8 : 1 for Yb; 6 : 1 for Eu) in each case. Again, the dipolar NMR shifts for the Eu/Yb(III) complexes of L^5 did not correspond (sequence/relative shift), consistent with a change in the local coordination number and ligand field, as you pass from Eu(III) to the smaller, more sterically demanding Yb(III) complex.

Analysis of water exchange rates at gadolinium

Previous studies have shown that in diastereoisomeric complexes of lanthanide(III) ions, the rate of water exchange may differ by up to two orders of magnitude.[3,5,15] Rates of water exchange at a gadolinium centre may be determined by analysing the temperature dependence of the transverse relaxation rate of the 17-O nucleus in water.[1,2,16,17] This data may then be used to enhance the fitting of NMRD profiles (r_{1p} as $f(B_o)$), in order to deduce appropriate relaxation terms. Details of such analyses have been extensively and thoroughly discussed.[1,2,5,15–17] Analysis of the data obtained, (Table 2), highlights the differing water exchange rates of the stereoisomeric Gd(III) complexes (Fig. 4), allowing the analysis of the variable temperature 1-H NMRD profiles (Fig. 5 and ESI†).

Plausible representations of the structures of the diastereoisomeric complexes of (*RR*)-[Ln.L^3]$^{3-}$ are given in Scheme 2; for (*RS*)-[Ln.L^4]$^{3-}$, analogous structures may be considered, differing only in configuration at one of the stereogenic centres in the glutarate arm. As was observed for the (*RRRR*), (*RRRS*), (*RSRS*) and (*RRSS*)-lanthanide(III) complexes of gDOTA,[3] each ligand stereoisomer gives rise to two common types of metal complex diastereoisomer, the relative proportion of which determines the overall water exchange dynamics. The ^{1}H NMR analysis

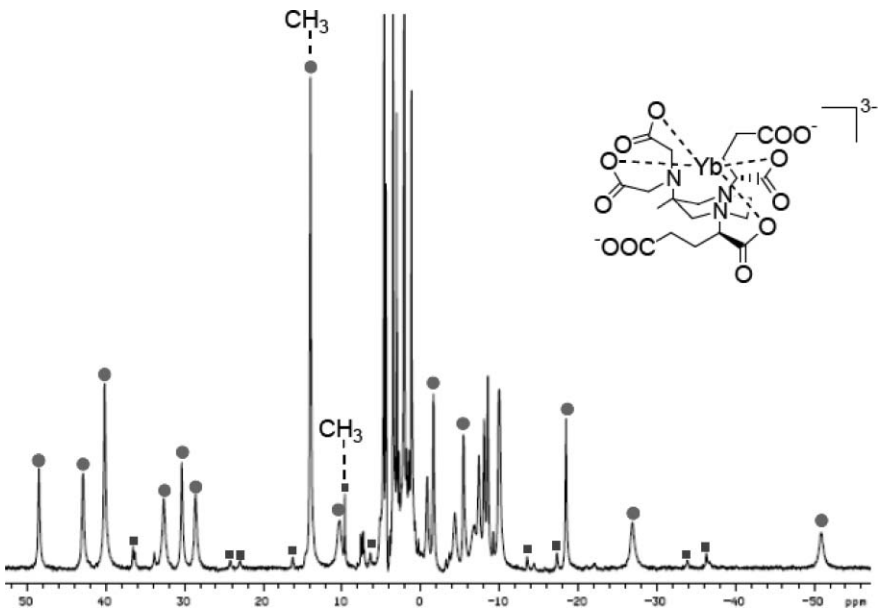


Fig. 2 ^{1}H NMR spectrum of (*RR*)-[Yb.L^3]$^{3-}$ (pD 5.4, 700 MHz, 295 K) showing selected assignments of the major (circles) and minor (squares) diastereoisomeric complexes in a ratio of ~10 : 1.

Table 2 Water exchange rates[a] (k^{298}_{ex}, s^{-1}), relaxivity values[b] and selected relaxation parameters obtained by analysis of variable temperature ^{17}O R_2 measurements[17] and by fitting of NMRD profiles[4a]

Complex	r_{1p}/mM^{-1} s^{-1}	k_{ex} (298 K) × 10^6 s^{-1}	Δ^2/s^{-2}	τ_v/ps
(*R*)-[Gd.L^5]$^{2-}$	7.3	8.70	3.4×10^{19}	21
(*RS*)-[Gd.L^4]$^{3-}$	7.5	4.12	1.6×10^{19}	28
(*RR*)/(*SS*) + (*meso*)-[Gd.L^3/L^4]$^{3-}$ (mix of stereoisomers)	8.0	2.42	2.7×10^{19}	28
(*RR*)-[Gd.L^3]$^{3-}$	8.6	1.39	2.2×10^{19}	63
[Gd.L^2]$^-$	7.1	11.1	2.1×10^{19}	31

[a] ^{17}O NMR data was measured at pH 7.4 at 14.1 T. [b] Relaxivity values are quoted at 298 K and 20 MHz. [c] τ_r values (298K) were estimated to be: [GdL^2]$^-$ 74 ps; [GdL^3]$^{3-}$ 111ps (83 ps at 310 K) and [GdL^5]$^{2-}$ 93 ps (75 ps at 310 K).

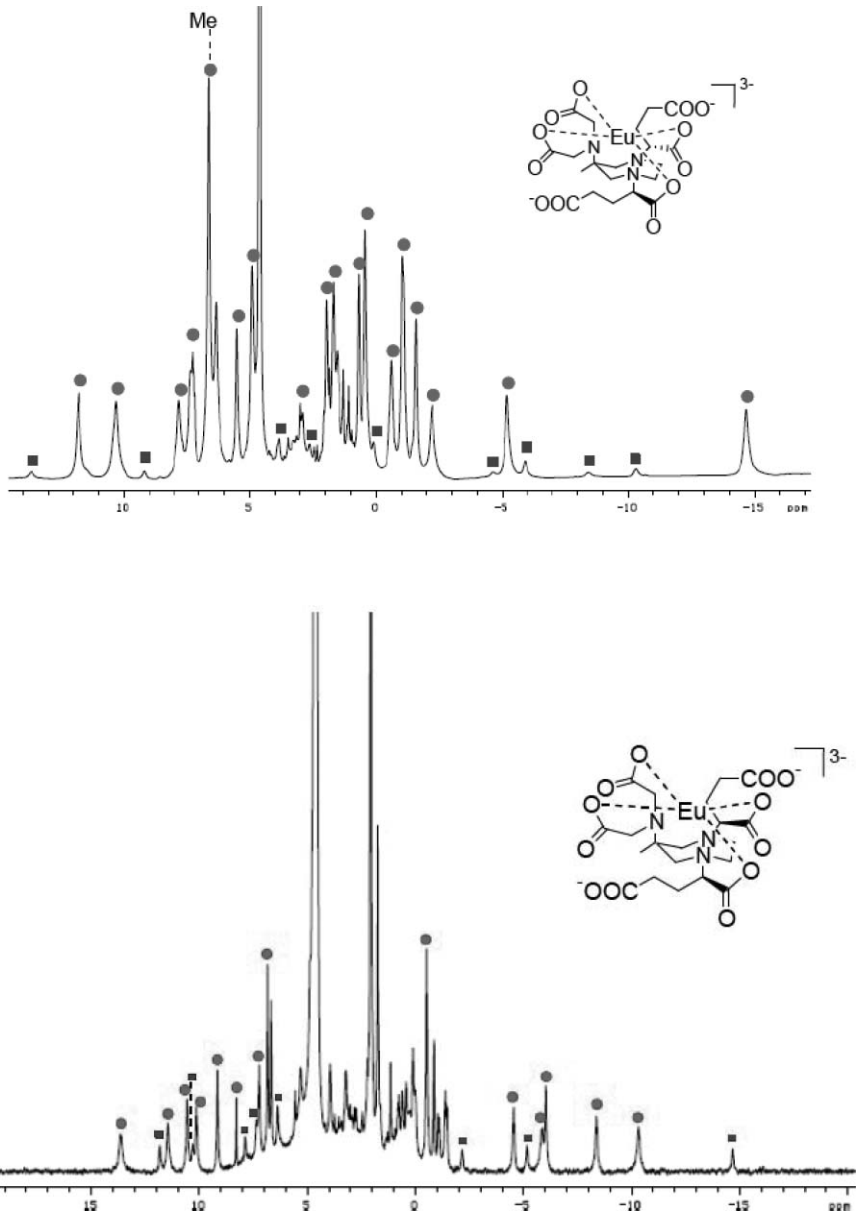
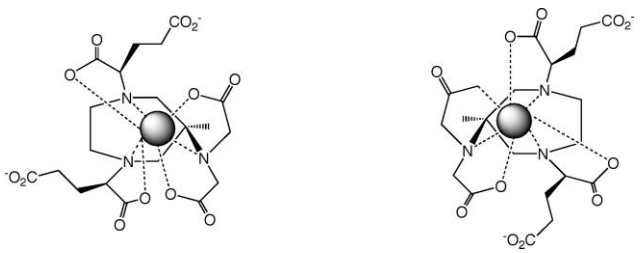


Fig. 3 ^{1}H NMR spectra for (*RR*)-[Eu.L^{3}]$^{3-}$ (upper) and (*RS*)-[Eu.L^{4}]$^{3-}$ (lower) revealing the 1 : 9 and 3 : 1 ratio of corresponding diastereoisomers (295 K, pD 5.4, 700 MHz; major species—circles, minor species—squares).



Scheme 2

of the corresponding Eu(III) complexes had suggested that for complexes of the (*RR*)-isomeric ligand, L^{3}, the ratio of solution diastereoisomers was 8 : 1; this ratio was 1 : 3 for the complexes of (*RS*)-L^{5}. It is an implicit assumption that this isomer ratio does not change significantly between europium and gadolinium; only small changes are expected based on earlier analyses of gDOTA complexes.[3]

Using these mole fractions to define the weighting of the contributions of each Gd(III) complex diastereoisomer to the observed rate, the rates of water exchange may be estimated by solving the simultaneous eqn (1) and (2), where x and y represent the unknowns:

$$k_{\mathrm{obs}}^{\mathrm{RR}}\ 1.39 \times 10^{6} = 0.88x + 0.11y \quad (1)$$

$$k_{\mathrm{obs}}^{\mathrm{RS}}\ 4.12 \times 10^{6} = 0.25x + 0.75y \quad (2)$$

hence: $x = 9.2 \times 10^{5}\ \mathrm{s}^{-1}$; $y = 5.4 \times 10^{6}\ \mathrm{s}^{-1}$.

The values obtained reveal a difference in water exchange rate of a factor of six and the data is consistent (±10%) with

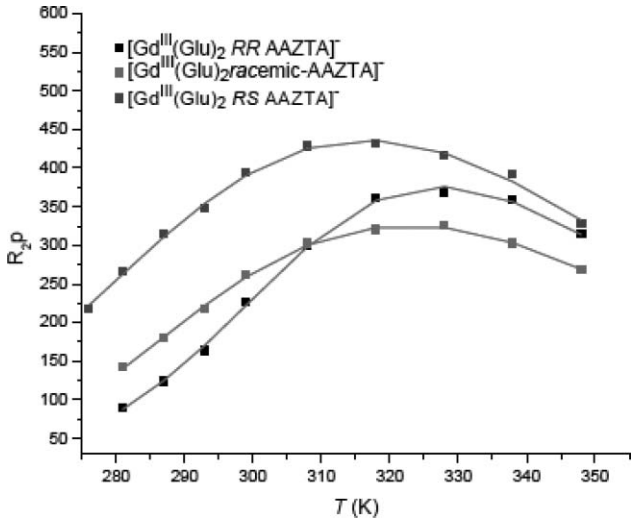


Fig. 4 Comparison of ^{17}O NMR R_{2p} *vs. T* profiles for the stated complexes (14.1 T, pH 7.4; see Table 2 for analysis), showing the fit (line) to the experimental data.

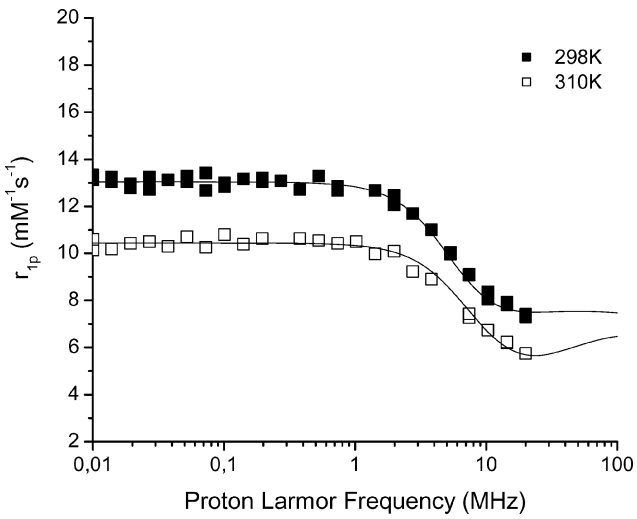


Fig. 5 Proton NMRD profile for (*RR*)-[GdL^3]$^{3-}$ at 298 K and 310 K (lower), showing the fit (line) to the experimental data; (profile for [GdL^5]$^{2-}$ is in the ESI†).

values measured for the stereoisomeric mixture of complexes (L^3–L^4). In (*RS*)-[Gd.L^4]$^{3-}$, the more abundant solution isomer possesses the faster water exchange rate and for (*RR*)-[Gd.L^3]$^{3-}$, the opposite is true. Examples of water exchange rates that differ for diastereoisomeric complexes of a given ligand have been defined previously.[2,3,5,15] In certain cases, these observations may reflect the differing activation energies to water exchange that arise from changes in local hydration.

The magnitude of these individual exchange rates is lower than required for most MRI applications. Faster rates—of the order of ten to one hundred times faster—are needed in derivatives of such systems, in order to avoid the 'quenching' of relaxivity gains in more slowly rotating conjugates, in the magnetic field range 0.5 to 3 T.

Conclusions

The measured water exchange rates for Gd(III) complexes of the mono and di-glutarate derivatives of AAZTA are less than 10^7 s^{-1} (298 K), and are lower than the value of 1.1×10^7 s^{-1} recorded for [Gd·AAZTA]$^-$ itself,[4a] and for related anionic di-aqua Gd(III) complexes of comparable molecular volume.[8,9] Faster exchange dynamics (×3) were observed for (*RS*)-[Gd.L^4]$^{3-}$ compared to (*RR*)-[Gd.L^3]$^{3-}$, reflecting the greater proportion of a diastereoisomer in solution that exchanges with a rate of 5.4×10^6 s^{-1}, compared to 9.2×10^5 s^{-1} for the other isomer. The introduction of substituents at each ring N increases the steric demand at the metal centre, and this increased steric crowding may be inhibiting the approach of water molecules, slowing the water interchange process.

Such behaviour suggests that MRI contrast agents, based on these glutarate derivatives, are unlikely to give rise to high relaxivity values in amide conjugates of greater molecular volume.

Experimental

All reagents were used as supplied by commercial sources unless otherwise stated. Solvents were dried over the appropriate drying agents when required. Water and H_2O refer to high purity water with conductivity $\leq$0.04 μScm^{-1}, obtained from the "Purite$_{STILL}$ Plus" purification system. Reactions requiring anhydrous conditions were carried out using Schlenk-line techniques under an atmosphere of dry argon. Anhydrous solvents when required were freshly distilled over the appropriate drying agent. Thin-layer chromatography was carried out on silica plates (Merck 5554) and visualised under UV light (254 nm) or by washing in a bath of ethanol–sulfuric acid 5% or permanganate or by staining with iodine. Preparative column chromatography was performed using neutral aluminium oxide (Merck Aluminium Oxide 90, activity II–III, 70–230 mesh) washed in ethyl acetate, or silica (Merck Silica Gel 60, 230–400 mesh). The HPLC analysis and separation were carried out on a Perkin Elmer system comprising a Perkin Elmer Series 200 Pump, Perkin Elmer Series 200 Autosampler and a Perkin Elmer Series 200 Fluorescence detector. A Gilson-FC203B fraction collector was used in separation procedures. The stationary phase was a Phenomenex Synergi 4 μ Fusion-RP 80, and the size of the column used was 150 × 4.6 mm (flow rate 1mL min^{-1}). Details of the HPLC methods used are given in the ESI.†

Electrospray mass spectra were recorded on a VG Platform II (Fisons Instruments), operating in positive or negative ion mode, with methanol as the carrier solvent. Accurate masses were recorded on a Thermo Finnigan LTQ instrument. Measurements of [Gd] using ICP-mass spectrometry were performed by Dr C Ottley (Durham University, Earth Sciences) following sample digestion in conc. HCl at 120 °C for 18 h.

^{1}H and ^{13}C NMR spectra were recorded on a Varian Unity-300 spectrometer (^{1}H at 299.908 MHz, ^{13}C at 75.412 MHz), Varian VXR 400 (^{1}H at 399.968 MHz, ^{13}C at 100.572 MHz), Bruker AMX 500 spectrometer or Varian Unity-700 spectrometer (^{1}H at 699.73 MHz). Spectra were referenced internally to the residual proton-solvent resonances and are reported in ppm relative to TMS, with coupling constants in Hz (typically ±0.4 Hz). Solution pD values stated are given as pH (meter reading) + 0.4.

Variable temperature ^{17}O NMR experiments were recorded at 14.1 T and 298 K and were performed and analysed as described in the recent literature; see ref. 4*a* and references therein. Measurements of 1-H NMRD profiles at 298 and 310 K in the range 0.001 to 20 MHz were made using a Stelar Spinmaster relaxometer. Profiles were fitted with standard iterative methods reported in ref. 4*a*, using the tau-m values derived from the 17-O analysis.

Luminescence spectra of the Eu^{III} complexes were recorded using a direct excitation of the Eu^{III} ion at 397 nm. Lifetime measurements of the Eu^{III} complex were recorded on a Perkin Elmer LS55 luminescence spectrometer using FL Winlab software. The Eu^{III} ion was excited directly at 397 nm, with an excitation slit width of 10 nm. Lifetime values were measured following excitation of the sample by a short pulse of light, monitoring the integrated intensity of light (613 nm for europium) emitted during a fixed gate time, t_g, a delay time, t_d, later. A gate time of 0.1 ms was used.

Ligand and complex synthesis

Racemic dimethyl-α-bromoglutarate was prepared as described in ref. 3; samples of L^2 and $[Eu.L^2(H_2O)_2]\,H_3O^+$ were obtained from Bracco s.a. and were prepared according to methods defined in ref. 4*a*.

1,4-Dibenzyl-6-methyl-6-nitroperhydro-1,4-diazepine. A suspension of *N*,*N*′-dibenzylethylenediamine (5.0 mL, 0.02 mol) and *para*-formaldehyde (1.91 g, 0.06 mmol) in EtOH (50 mL) was boiled under reflux for 4 h. Nitroethane (1.52 mL, 0.02 mol) was added dropwise, and the reaction mixture was boiled under reflux overnight, under an argon atmosphere. The progress of the reaction was monitored by TLC, and after 16 h, solvent was removed under reduced pressure and the residue partitioned between CH_2Cl_2 and saturated aqueous Na_2CO_3 solution. The organic extracts were washed with water, dried, filtered, evaporated and purified by column chromatography (SiO_2, CH_2Cl_2) to afford a light brown waxy solid (6.50 g, 96%). R_f (CH_2Cl_2, SiO_2) = 0.4 (UV). Mp 49.5–51 °C. δ_H ($CDCl_3$, 300 MHz): 1.34 (3H, s, $CH_3C_{(quat)}$), 2.59 (4H, m, $2\times CH_2N$), 2.95 (2H, d, 14.1), 3.60 (2H, d, $J = 14.1$), 3.65 (2H, d, $J = 13.2$), 3.78 (2H, d, $J = 13.2$), 7.26–7.33 (10H, m, $2\times$Ph-*H*). ES-MS: *m*/*z* 339.3 $[M]^+$, 340.3 $[M + H]^+$, 361.4 $[M + Na]^+$. Found: C, 70.6; H, 7.51; N, 12.2%. $C_{20}H_{25}N_3O_2$ requires: C, 70.8; H, 7.38; N, 12.4%.

6-Amino-6-methylperhydro-1,4-diazepine, L^1 [4a]. A suspension of the nitro compound (2.27 g, 6.69 mmol) in MeOH (10 mL) and 20% $Pd(OH)_2/C$ (1.88 g, 13.4 mmol) was hydrogenated overnight using a Parr hydrogenator (10 psi H_2). The reaction mixture was filtered over Celite, the solvent was evaporated under reduced pressure and a pale yellow oil (0.850 g, 98%) was obtained. R_f (CH_2Cl_2–MeOH 19%–conc. aq. NH_3 1%, SiO_2) = 0.10 (iodine). δ_H ($CDCl_3$, 400 MHz): 0.85 (3H, s, $CH_3C_{(quat)}$), 1.86 (br. s, 4H, exch. with D_2O), 2.50 (4H, m, $2\times CH_{2(ring)}$ H-5a, 5b and H-7a, 7b), 2.63–2.69 (2H, m, $CH_{2(ring)}$ H-2a, 3a), 2.74–2.80 (2H, m, $CH_{2(ring)}$ H-2b, 3b). δ_C ($CDCl_3$, 100.6 MHz): 26.81 ($CH_3C_{(quat)}$), 52.06 (*C-2* and *C-3*), 54.15 ($C_{(quat)}$), 62.42 (C-5 and C-7). ES-MS: *m*/*z* 130 $[M + H]^+$. Found: 130.1343; $C_6H_{16}N_3$ requires: 130.1344.

(Note: this compound reacts readily with carbon dioxide and should be stored as the hydrochloride salt and handled under dry argon or nitrogen.)

6-Amino-6-methylperhydro-1,4-bis(1′-methoxycarbonyl 3′methoxycarbonylpropyl)diazepine, 6a: (mixture of diastereoisomers). The racemic α-bromoglutarate ester, **2** (1 g, 4.18 mmol) dissolved in CH_3CN (10 mL) was added over a period of 10 min to a suspension of L^1 (0.257 g, 1.99 mmol) and K_2CO_3 (0.55 g, 3.98 mmol) in MeCN (10 mL). After stirring at 75 °C for 24 h, the solvent was removed under reduced pressure and the residue dissolved in EtOAc (15 mL), washed with water–brine (80 : 20 v/v, 3 × 15 mL) and dried over Na_2SO_4. Evaporation of the solvent to dryness gave a dark yellow oil (0.74 g, 1.66 mmol, 84%). R_f ($CHCl_3$–MeOH–conc. aq. NH_3 9 : 1 : 0.1, SiO_2) = 0.2 (UV, $KMnO_4$).

δ_H ($CDCl_3$, 399.96 MHz): 0.91 (3H, s, CH_3), 1.73–1.88 (2H, m, $2\times CH_aCH_2CO_2CH_3$), 1.89–2.04 (2H, m, $2\times CH_bCH_2CO_2CH_3$), 2.37–2.42 (4H, m, $2\times CH_2CO_2CH_3$), 2.42–2.80 (8H, br. m, $4\times CH_{2ring}$), 3.15–3.23 (1H, br. dd, CH_XN), 3.23–3.30 (1H, br. dd, CH_YN), 3.58–3.60 (12H, m, $4\times OCH_3$). δ_C ($CDCl_3$, 75 MHz): 24.42 (CH_3), 25.0–25.5 ($CH_2CH_2CO_2CH_3$), 30.8 ($CH_2CO_2CH_3$), 51.8 (OCH_3), 49.9–55.4 (CH_{2ring}), 67.41 (*C*HN), 173.0 (*C*=O), 173.6 (*C*=O). *m*/*z* (ES+): 446.3 $[M + H]^+$, 468.3 $[M + Na]^+$. (Found: $[M + H]^+$, 446.2493 $C_{20}H_{36}N_3O_8$ requires $[M + H]^+$, 446.2497).

6-Amino-bis(*tert*-butoxycarbonylmethyl)-6-methyl-1,4-bis(1′-methoxycarbonyl-3′methoxycarbonylpropyl)-diazepine, 6b. A suspension of **6a** (0.7 g, 1.57 mmol), *tert*-butylbromoacetate (0.66 g, 3.4 mmol) and K_2CO_3 (0.86 g, 6.20 mmol) in CH_3CN (15 mL) was cooled to 0 °C. The reaction mixture was allowed to warm to room temperature, Na_2SO_4 (0.20 g, 1.41 mmol) was added and the suspension boiled under reflux overnight. After cooling to room temperature, salts were filtered off and the mother liquor evaporated to give the crude product (0.85 g, 1.26 mmol). Purification by flash chromatography (SiO_2, 20% EtOAc in hexane → 50% EtOAc in hexane) gave a pale yellow oil (0.37 g, 0.55 mmol, 35%). R_f (hexane–EtOAc 7 : 3, SiO_2) = 0.2 (UV, iodine). δ_H ($CDCl_3$, 399.95 MHz): 0.95 (3H, s, CH_3), 1.34–1.35 (18H, s, $C(CH_3)_3$), 1.73–1.85 (2H, m, $2\times CH_{2a}CH_2CO_2CH_3$), 1.86–1.98 (2H, m, $2\times CH_{2b}CH_2CO_2CH_3$), 2.31–2.36 (4H, m, $2\times CH_2CO_2CH_3$), 2.36–3.05 (8H, br. m, $4\times CH_{2ring}$), 3.17–3.23 (1H, br. dd, CH_XN), 3.26–3.31 (1H, br. dd, CH_YN), 3.51 (2H, d, $J = 12.4$, $CH_2COOtBu$), 3.56 (2H, d, $J = 12.4$, $CH_2COOtBu$), 3.56–3.60 (12H, m, $4\times OCH_3$). δ_C ($CDCl_3$, 125.66 MHz): 23.9 (CH_3), 24.20 ($CH_2CH_2CO_2CH_3$), 28.3 ($CH_2CO_2CH_3$), 51.8 (OCH_3), 51.40–51.87 (CH_{2ring}), 54.03 ($C_{quat}CH_3$), 68.42 ($CH_2COOtBu$), 68.5 (*C*HN), 80.71 ($C_{quat}(CH_3)_3$), 172.6–172.9 (*C*=O), 173.19 (C=O), 173.75 (*C*=O). *m*/*z* (ES–): 674.2 $[M + H]^+$, 696.3 $[M + Na]^+$. (Found: $[M + H]^+$, 674.3858. $C_{32}H_{56}O_{12}N_3$ requires $[M + H]^+$, 674.3856; found: $[M + Na]^+$, 696.3678. $C_{32}H_{55}O_{12}N_3Na$ requires $[M + Na]^+$, 696.3676).

6-Amino-bis(carboxymethyl)-6-methyl-1,4-bis(1′-methoxycarbonyl-3′methoxycarbonylpropyl)-diazepine. A solution of the *tert*-butyl ester **6b** (0.2 g, 0.29 mmol) in TFA–CH_2Cl_2 (1 : 1, 3.0 mL) was stirred at room temperature for 24 h. The solvent was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 (3 mL) and the solution evaporated. This procedure

was repeated twice. The residue was washed twice with diethyl ether (2 × 2 mL) and the trifluoroacetate salt was obtained as a rather hygroscopic white precipitate (0.146 g, 0.26 mmol, 90%). δ_H ($CDCl_3$, 399.96 MHz): 1.2 (3H, br. s, CH_3), 1.96–2.11 (4H, m, 2 × CH_2CH$_2$CO$_2$CH$_3$), 2.38–2.55 (4H, m, 2 × CH_2CO$_2$CH$_3$), 2.80–3.35 (8H, br. m, 4 × CH_{2ring}), 3.46 (1H, br. dd, CH_XN), 3.48 (1H, br. dd, CH_YN), 3.6 (2H, br. d, 2 × CH_2COOH), 3.69–3.72 (12H, m, 4 × OCH_3). This was used directly in the next step (methyl ester hydrolysis, below) without further characterisation.

6-Amino-bis(carboxymethyl)-6-methyl-1,4-bis(1′-carboxy-3′-carboxypropyl)-diazepine: L^3–L^4 as a statistical mixture of *RR*/*SS* and the *RS* and *SR* isomers. KOD (1 M solution in D_2O, 1 mL) was added to the methyl ester (0.15 g, 0.27 mmol) and the solution stirred at 40 °C. The reaction progress was checked by ^{1}H NMR. After 7 days, the measured pH was adjusted to 7 and solvent removed under reduced pressure. The residue was treated with freshly activated Dowex H^+ resin, and the acid eluted following addition of 20% acetic acid solution. The white glassy solid that was obtained was used directly for complexation.

δ_H (D_2O, 399.96 MHz): 1.1 (3H, s, CH_3), 1.6–1.88 (4H, m, 2 × CH_2CH$_2$CO$_2$H), 2.03–2.15 (4H, m, 2 × CH$_2$CH_2CO$_2$H), 2.45–3.32 (8H, br. m, 4 × CH_{2ring}), 3.55 (1H, br. dd, CH_XN), 3.67 (1H, br. dd, CH_YN), 3.7 (4H, br. d, 2 × CH_2COOH). *m*/*z* (ES): 504.2 [M − H]$^-$. Found: [M − H]$^-$, 504.1834. $C_{20}H_{30}N_3O_{12}$ requires [M − H]$^-$, 504.1836.

[Ln^{III}(Glu)$_2$*Racemic*-AAZTA]$^{3-}$ (*i.e.* a mixture of (*RR*/*SS*)-L^3 and (*RS*/*SR*)-L^4). An aqueous solution of $LnCl_3 \cdot 6H_2O$ (0.1 mmol, 0.95 eq.) was added dropwise to a solution of H_6L^3–L^4 (0.1 mmol, 1 eq,) dissolved in the minimum volume of H_2O. The pH was adjusted to ~5.5 with aqueous KOH solution (1 M) and the mixture was left to stir at 50 °C. After 48 h, the reaction mixture was cooled to room temperature and the pH of the solution raised to ~10 (using aqueous KOH, 1 M). The white powder that precipitated was isolated by centrifugation and the pH of the supernatant readjusted to ~5.5. Freeze drying of the liquid gave the complex as a colourless crystalline solid, together with residual salts. The properties of the complex were examined in the presence of the salts.

[Yb^{III}L$^{3/4}$]$^{3-}$. *m*/*z* (TOF MS ES–): 672.5 [M]$^-$. (Found: [M]$^-$, 668.4998. $C_{20}H_{27}N_3O_{12}{}^{170}Yb_1$ requires [M]$^-$, 668.4998.

[Gd^{III}L$^{3/4}$]$^{3-}$. *m*/*z* (TOF MS ES–): 659.0 [M]$^-$. (Found: [M]$^-$, 659.0304 $C_{20}H_{27}N_3O_{12}{}^{158}Gd_1$ requires [M − H]$^-$, 659.0309; [M]$^-$, 655.0465 $C_{20}H_{27}N_3O_{12}{}^{154}Gd_1$ requires [M − H]$^-$, 655.0470; [M]$^-$, 656.0506 $C_{20}H_{27}N_3O_{12}{}^{155}Gd_1$ requires [M − H]$^-$, 656.0510). r_{1p} = 8.02 $mM^{-1}s^{-1}$ (20 MHz, 298 K). The [Gd^{3+}] was determined following mineralization with 37% HCl at 120 °C overnight and then ICP-MS analysis was used to determine [Gd].

[Eu^{III}L$^{3/4}$]$^{3-}$. *m*/*z* (TOF MS ES–): 652.2 [M]$^-$. (Found: [M]$^-$, 652.0795 $C_{20}H_{27}O_{12}N_3{}^{151}Eu$ requires [M]$^-$, 652.0798; [M]$^-$, 654.0808 $C_{20}H_{27}O_{12}N_3{}^{153}Eu$ requires [M]$^-$, 654.0813).

(1′*R*,6*S*)-(1′-*tert*-Butoxycarbonyl-3′-benzyloxycarbonylpropyl)-6-amino-6-methylperhydro-1,4-diazepine, 3a; (1′*R*,6*R*)-1′-*tert*-butoxycarbonyl-3′-benzyloxycarbonylpropyl)-6-amino-6-methylperhydro-1,4-diazepine, 4a; (*R*,*R*)-1,4-bis(1′-*tert*-butoxycarbonyl-3′-benzyloxycarbonylpropyl)-6-amino-6-methylperhydro-1,4-diazepine, 5a: (arbitrary assignment of configuration for 3a). A suspension of (*S*)-**1** (2.25 g, 6.32 mmol), L^1 (0.54 g, 4.2 mmol) and K_2CO_3 (0.87 g, 6.3 mmol) in MeCN (80 mL) was stirred at room temperature for 18 h. The solvent was removed under reduced pressure and the residue dissolved in EtOAc (80 mL), washed with brine (20%, 2 × 70 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in EtOAc (50 mL), washed with hydrobromic acid solution (1 M) (3 × 40 mL), dried over Na_2SO_4 and evaporated to dryness, to give **5a**, a pale brown oil (1.19 g, 1.76 mmol, 28%).

Concentrated aqueous ammonia (5.2 mL) was added dropwise to the aqueous phase (until pH ~ 9), which was then extracted with EtOAc (4 × 40 mL). The organic phase was washed with H_2O–brine (4 : 1 v/v, 3 × 50 mL), dried over Na_2SO_4 and evaporated to dryness to give an oil (1.5 g, 3.7 mmol). Crystallization from EtOH and washing of the precipitated solid with CH_3CN gave **3a** as a yellow oil (1.02 g, 2.52 mmol, 40%) and **4a** as a semi-crystalline white solid (0.25 g, 0.61 mmol, 10%).

(*R*,*R*)-1,4-Bis(1′-*tert*-butoxycarbonyl-3′-benzyloxycarbonylpropyl)-6-amino-6-methylperhydro-1,4-diazepine, 5a. This compound could be obtained directly in 70% yield using 2.2 equivalents of (*S*)-**1** using the reaction conditions and work-up described above, where it was isolated as a pale yellow oil from the ethyl acetate extraction of the residue.

R_f ($CHCl_3$–EtOH–NH_3 95 : 5 : 0.1, SiO_2) = 0.2 (UV, $KMnO_4$). HPLC (Chromatographic method A2): t_r: 14 min. δ_H ($CDCl_3$, 399.9 MHz): 0.91 (3H, s, CH_3), 1.40 (9H, s, C(CH_3)$_3$), 1.42 (9H, s, C(CH_3)$_3$), 1.82–2.19 (4H, br. m, 2 × CH_2CHN), 2.35–2.72 (8H, m, CH_{2ring}), 2.88–3.0 (4H, m, 2 × CH_2COOBn), 3.44–3.5 (2H, m, 2 × CHN), 5.10 (4H, d, J = 7.2, 2 × CH_2Ph), 7.29–7.35 (10 H, m, 2 × Ph–*H*). δ_C ($CDCl_3$, 75 MHz): 25.10 (*C*H$_3$), 26.08 (*C*H$_2$CHN), 28.56 (C(*C*H$_3$)$_3$), 32.11 (*C*H$_2$COOBn), 49.10 (*C*H$_{2(ring)}$N), 51.75 (*C*H$_{2(ring)}$C$_{quat}$), 66.54 (*C*H$_2$Ph), 68.33 (*C*HN), 128.5–129.08 (*C*$_{arom}$), 136.33 (*C*$_{quat\ arom}$), 171.98 (*C*$_{quat}$NH$_2$), 173.21 (*C*=O$_{Bn}$), 174.00 (*C*=O$_{tBu}$). *m*/*z* (ES+): 682.4 [M + H]$^+$, 704.3 [M + Na]$^+$, 626.33 [M − tBu]$^+$. (Found: C, 64.3; H, 8.15; N, 5.90%; $C_{38}H_{55}N_3O_8 \cdot 3/2\ H_2O$ requires: C, 64.4; H, 8.19; N, 5.93%).

(1′*R*,6*S*)-(1′-*tert*-Butoxycarbonyl-3′-benzyloxycarbonylpropyl)-6-amino-6-methylperhydro-1,4-diazepine, 3a diastereoisomer soluble in EtOH (arbitrary assignment of configuration). R_f ($CHCl_3$–MeOH–NH_3 86 : 12 : 1, SiO_2) = 0.35 (UV, $KMnO_4$). HPLC (Chromatographic method A2): t_r: 5 min. δ_H ($CDCl_3$, 699.73 MHz): 0.94 (3H, s, CH_3), 1.42 (9H, s, C(CH_3)$_3$), 1.81–1.91 (1H, m, CH_{2a}CHN), 1.96–2.03 (1H, m, CH_{2b}CHN), 2.45–2.54 (2H, m, CH_2COOBn), 2.61–2.91 (8H, m, H$_{ring}$), 3.14–3.19 (1H, m, CHN), 5.09 (2H, s, CH_2Ph), 7.31–7.33 (5H, m, Ph–*H*). δ_C ($CDCl_3$, 125.67 MHz): 25.42 (*C*H$_2$CHN), 25.7 (*C*H$_3$), 28.44 (C(*C*H$_3$)$_3$), 31.26 (*C*H$_2$COOBn), 49.42 (*C*H$_2$N), 54.3 (*C*H$_2$N), 56.8 (C$_{quat}$), 56.9 (*C*H$_2$C$_{quat}$), 60.0 (*C*H$_2$C$_{quat}$), 66.73 (*C*H$_2$Ph), 68.7 (*C*HN), 82.6 (*C*(CH$_3$)$_3$), 128.4–128.84 (*C*$_{arom}$), 136.0 (*C*$_{quat\ arom}$), 171.01 (*C*=O$_{Bn}$), 173.0 (*C*=O$_{tBu}$). *m*/*z* (ES+): 406.2 [M + H]$^+$, 428.3 [M + Na]$^+$.

(1′*R*,6*R*)-(1′-*tert*-Butoxycarbonyl-3′-benzyloxycarbonylpropyl)-6-amino-6-methylperhydro-1,4-diazepine, 4a crystalline solid insoluble in EtOH (arbitrary assignment of configuration at the quaternary centre). R_f ($CHCl_3$–MeOH–NH_3 86 : 12 : 1, SiO_2) = 0.35 (UV, $KMnO_4$). HPLC (Chromatographic method A2): t_r: 5 min. Mp 119–121 °C. δ_H ($CDCl_3$, 699.73 MHz): 0.98 (3H, s, CH_3), 1.44 (9H, s, C(CH_3)$_3$), 1.82–1.89 (1H, m, CH_{2a}CHN),

2.0–2.07 (1H, m, CH_{2b}CHN), 2.49–2.52 (2H, td, ^3J = 2.8, CH_2COOBn), 2.62–2.68 (m, 5H, H_{ring}), 2.71–2.75 (m, 1H, H_{ring}), 2.79–2.83 (m, 1H, H_{ring}), 2.91–2.95 (m, 1H, H_{ring}), 3.17 (1H, dd, ^3J = 6.3, ^3J = 9.1, CHN), 5.11 (2H, s, CH_2Ph), 7.33–7.35 (5H, m, Ph–H). δ_C ($CDCl_3$, 175.95 MHz): 25.73 (CH_2CHN), 26.41 (CH_3), 28.51 (C(CH_3)$_3$), 31.19 (CH_2COOBn), 51.84 (CH_2N), 53.62 (CH_2N), 55.5 (C_{quat}), 62.46 (CH_2C_{quat}), 66.54 (CH_2C_{quat}), 66.87 (CH_2Ph), 68.91 (CHN), 81.46 (C(CH_3)$_3$), 128.48–128.8 (C_{arom}), 136.13 ($C_{quat\ arom}$), 171.95 (C=O_{Bn}), 173.32 (C=O_{tBu}). m/z (ES+): 406.2 [M + H]$^+$, 428.3 [M + Na]$^+$.

(1′*R*,6*S*)-(1′-*tert*-Butoxycarbonyl-3′-benzyloxycarbonylpropyl)-4–6-tris(*tert*-butoxycarbonylmethyl)-6-amino-6-methylperhydro-1,4-diazepine, 3b. *tert*-Butyl bromoacetate (0.26 mL, 1.75 mmol) was added dropwise to a stirred solution of **3a** (0.20 g, 0.5 mmol), K_2CO_3 (0.27 g, 1.95 mmol) and Na_2SO_4 (0.064 g, 0.45 mmol) in CH_3CN (10 mL) cooled at 0 °C. The reaction mixture was allowed to warm to room temperature, boiled under reflux for 6 h and then left stirring overnight at room temperature. The mixture was evaporated under reduced pressure and the residue treated with petroleum ether–EtOAc 8 : 2 (20 mL). Salts were filtered off and the mother liquor evaporated to give the crude product (0.6 g). Purification by flash chromatography (SiO_2, 10% EtOAc in petroleum ether to 20% EtOAc in petroleum ether) gave the product as a yellow oil (0.22 g, 0.3 mmol, 60%). R_f (petroleum ether–EtOAc 8 : 2, SiO_2) = 0.3 (UV, $KMnO_4$). HPLC (Chromatographic method A2): t_r: 19 min. δ_H ($CDCl_3$, 699.73 MHz): 0.99 (3H, s, CH_3), 1.42–1.47 (36H, m, C(CH_3)$_3$), 1.81–1.91 (1H, m, CH_{2a}CHN), 1.94–2.04 (1H, m, CH_{2b}CHN), 2.45–2.76 (8H, m, 4 × CH_{2ring}), 3.0–3.07 (2H, m, CH_2COOBn), 3.13 (1H, dd, J = 5.6, J = 10.5, CHN), 3.26 (1H, d, J = 14, C$H_{2a}N_{ring}$), 3.33 (1H, d, J = 14, C$H_{2b}N_{ring}$), 3.67 (2H, s, CH_2COOtBu), 3.68 (2H, s, CH_2COOtBu), 5.11 (2H, s, CH_2Ph), 7.31–7.35 (5H, m, Ph–H). δ_C ($CDCl_3$, 125.67 MHz): 24.38 (CH_3), 25.11 (CH_2CHN), 28.4 (C(CH_3)$_3$), 31.15 (CH_2COOBn), 51.84 ($C_{ring}H_2$N), 52.56 ($C_{ring}H_2$N), 60.1 (CH_2C_{quat}), 61.24 (C_{quat}), 62.07 (NCH_2COOtBu), 66.52 (CH_2Ph), 67.33 (CH_2COOtBu), 69.33 (CHN), 80.46 (C(CH_3)$_3$), 80.94 (C(CH_3)$_3$), 81.21 (C(CH_3)$_3$), 128.44–128.77 (C_{arom}), 136.18 ($C_{quat\ Ph}$), 171.24 (C=O_{Bn}), 172.07 (C=O_{Bn}), 172.88 (C=O_{tBu}), 173.47 (C=O_{tBu}). m/z (ES+): 748.4 [M + H]$^+$, 770.4 [M + Na]$^+$.

(*R*,*R*)-1,4-Bis(1′-*tert*-butoxycarbonyl-3′-benzyloxycarbonylpropyl)-6-bis(*tert*-butoxycarbonlymethyl)-amino-6-methylperhydro-1,4-diazepine, 5b. *tert*-Butyl bromoacetate (0.13 mL, 0.88 mmol) was added to a stirred solution of **5a** (0.24 g, 0.35 mmol) and K_2CO_3 (0.1 g, 0.7 mmol) in CH_3CN (5 mL) cooled to 0 °C. The reaction mixture was allowed to warm to room temperature and Na_2SO_4 (0.025 g, 0.17 mmol) was added. The suspension was boiled under reflux overnight and stirred at 60 °C for a further 8 h. After addition of more *tert*-butyl bromoacetate (0.13 mL, 0.88 mmol), the reaction mixture was boiled under reflux again overnight, then cooled to room temperature and left to stir for 5 h. The suspension was filtered, the solvent removed under reduced pressure and the residue was treated with petroleum ether–EtOAc 8 : 2 (10 mL). Salts were filtered off and the mother liquor evaporated to give a crude product (0.37 g) that was purified by flash chromatography (SiO_2, 5% EtOAc in petroleum ether → 15% EtOAc in petroleum ether) to give **5b** as a yellow oil (0.2 g, 0.23 mmol, 65%). R_f (petroleum ether–EtOAc 8 : 2, SiO_2) = 0.5 (UV, $KMnO_4$). HPLC (Chromatographic method A2): t_r: 21 min. δ_H ($CDCl_3$, 699.74 MHz): 0.99 (3H, s, CH_3), 1.40–1.43 (36H, m, 4 × C(CH_3)$_3$), 1.82–1.87 (1H, m, C$H_{2a}$$CH_X$N), 1.88–1.94 (1H, m, C$H_{2b}$$CH_X$N), 1.98–2.05 (2H, m, C$H_{2a,b}$$CH_Y$N), 2.35–2.59 (8H, m, CH_{2ring}), 2.71 (1H, d, J = 14.7, CH_{2a}COOBn), 2.74 (1H, d, J = 12.6, CH_{2b}COOBn), 2.90 (1H, d, J = 12.6, CH_{2b}COOBn), 3.00 (1H, d, J = 14.7, CH_{2a}COOBn), 3.14 (1H, dd, J = 5.6, J = 10, CH_XN), 3.25 (1H, dd, J = 5.6, J = 10, CH_YN), 3.57 (2H, d, J = 17.5, CH_{2b}COOtBu), 3.67 (2H, d, J = 17.5, CH_{2a}COOtBu), 5.10 (4H, m, $J$$H_aH_b$ = 15, C$H_{2(a,b)}$Ph + C$H_{2(a',b')}$Ph), 7.31–7.35 (10H, m, 2 × Ph–H). δ_C ($CDCl_3$, 125.67 MHz): 25.23 (CH_3), 26.14 (CH_2CHN), 28.32–28.53 (C(CH_3)$_3$), 31.13 (CH_2COOBn), 51.7–54.2 (CH_{2ring}), 61.74 ($C_{quat}$$CH_3$), 66.6 (C$H_2$Ph), 68.13 (C$H_2COOt$Bu), 69.23 (C$H$N), 80.58 (C(C$H_3$)$_3$), 81.26 (C(C$H_3$)$_3$), 81.33 (C(C$H_3$)$_3$), 128.44–128.79 ($C_{arom}$), 136.15 ($C_{quat\ Ph}$), 172.0 (C=$O_{Bn}$), 172.21 (C=$O_{Bn}$), 172.64 (C=$O_{tBu}$), 173.38 (C=$O_{tBu}$). m/z (ES+): 910.5 [M + H]$^+$, 932.5 [M + Na]$^+$. (Found: C, 62.2; H, 8.10; N, 4.11%; $C_{50}H_{75}N_3O_{12}$·3H_2O requires: C, 62.3; H, 8.41; N, 4.36%).

(*R*,*S*)-1,4-Bis(1′-*tert*-butoxycarbonyl-3′-benzyloxycarbonylpropyl)-6-amino-6-methylperhydro-1,4-diazepine, 7a. (*R*)-**1** (0.73 g, 2.05 mmol) dissolved in CH_3CN (10mL) was added dropwise to a stirred solution of **3a** (0.83 g, 2.05 mmol), K_2CO_3 (0.28 g, 2.05 mmol) and Na_2SO_4 (0.064 g, 0.45 mmol) in CH_3CN (10 mL) cooled at 0 °C. The reaction mixture was allowed to warm to room temperature and left stirring for 18 h. The solvent was removed under reduced pressure and the residue dissolved in EtOAc (30 mL), washed with water–brine (80 : 20, v/v, 2 × 30 mL), dried over Na_2SO_4 and evaporated to dryness. The residue was dissolved in EtOAc (20 mL), washed with H_2O–HBr (1 M) (10 : 1 v/v, 3 × 20 mL), dried over Na_2SO_4 and evaporated to dryness, to give **7a** as a yellow oil (0.97 g, 1.42 mmol, 70%). R_f ($CHCl_3$–EtOH–NH_3 95 : 5 : 0.1, SiO_2) = 0.2 (UV, $KMnO_4$). δ_H ($CDCl_3$, 400.13 MHz): 1.40–1.46 (21H, br. s, 2 × C(CH_3)$_3$ + CH_3), 1.83–2.03 (2H, m, CH_{2a}CHN), 2.08–2.17 (2H, m, CH_{2b}CHN), 2.33–3.10 (12H, m, 2 × CH_2COOBn + H_{ring}), 3.46 (2H, dd, J = 6, J = 8.8, 2 × CHN), 5.10–5.12 (2H, s + s, 2 × CH_2Ph), 7.28–7.34 (10 H, m, 2 × Ph–H). δ_C ($CDCl_3$, 100 MHz): 24.72 (CH_3), 27.72 (C(CH_3)$_3$), 29.71 (CH_2CHN), 31.63 (CH_2COOBn), 48.8 (C$H_{2(ring)}$N), 52.61 (C$H_{2(ring)}C_{quat}$), 66.54 (CH_2Ph), 68.21 (CHN), 127.0–128.75 (C_{arom}), 136.0 ($C_{quat\ arom}$), 168.29 (C=O_{Bn}), 173.01 (C=O_{tBu}), 173.13 ($C_{quat}NH_2$). m/z (ES+): 682.4 [M + H]$^+$, 704.3 [M + Na]$^+$, 626.33 [M − tBu]$^+$. Found: 682.4069; $C_{38}H_{56}N_3O_8$ requires: 682.4067.

(*RS*)-1,4-Bis(1′-*tert*-butoxycarbonyl-3′-benzyloxycarbonylpropyl)-6-bis(*tert*-butoxycarbonylmethyl)-amino-6-methylperhydro-1,4-diazepine, (*RS*)-7b. *tert*-Butyl bromoacetate (0.53 mL, 3.56 mmol) was added to a stirred solution of **7a** (0.97 g, 1.42 mmol) and K_2CO_3 (0.57 g, 4.12 mmol) in CH_3CN (10 mL) cooled to 0 °C. The reaction mixture was allowed to warm to room temperature, Na_2SO_4 (0.025 g, 0.17 mmol) was added and then boiled under reflux overnight and stirred for a further 6 h at 60 °C. The solvent was removed under reduced pressure and the residue treated with petroleum ether–EtOAc 8 : 2 (15 mL). Salts were filtered off and the mother liquor evaporated to give the crude product (1.05 g). Purification by flash chromatography (SiO_2, 5% EtOAc in petroleum ether to 20% EtOAc in petroleum ether) gave **7b** as a yellow oil (0.52 g, 0.57 mmol, 40%). R_f

(petroleum ether–EtOAc 8 : 2, SiO_2) = 0.4 (UV, $KMnO_4$). δ_H ($CDCl_3$, 400.13 MHz): 1.01 (3H, s, CH_3), 1.43–1.46 (36H, s + s, 4 × C(CH_3)$_3$), 1.89–1.95 (2H, m, CH_2CHN), 1.98–2.10 (2H, m, CH_2CHN), 2.36–2.57 (8H, m, CH_{2ring}), 2.62–2.87 (1H, m, 2 × CH_2COOBn), 3.01–3.12 (2H, m, 2 × CHN), 3.63 (2H, d, J = 17.6, CH_2COO*t*Bu), 3.69 (2H, d, J = 17.6, CH_2COO*t*Bu), 5.13 (4H, d, J = 2.4, C$H_{2(a,b)}$Ph + C$H_{2(a',b')}$Ph), 7.30–7.37 (10H, m, 2 × Ph–H). δ_C ($CDCl_3$, 125.67 MHz): 25.22 (*C*H$_3$), 25.8 (*C*H$_2$CHN), 28.31–28.52 (C(*C*H$_3$)$_3$), 31.40 (*C*H$_2$COOBn), 31.52 (*C*H$_2$COOBn), 51.1–54.1 (*C*H$_{2ring}$), 61.74 (*C*$_{quat}$CH$_3$), 66.51 (*C*H$_2$Ph), 66.73 (*C*H$_2$Ph), 68.13 (*C*H$_2$COO*t*Bu), 68.38 (*C*H$_2$COO*t*Bu), 69.23 (*C*HN), 69.3 (*C*HN), 80.44 (*C*(CH$_3$)$_3$), 80.57 (*C*(CH$_3$)$_3$), 81.26 (*C*(CH$_3$)$_3$), 81.33 (*C*(CH$_3$)$_3$), 128.43–128.78 (*C*$_{arom}$), 136.15 (*C*$_{quatPh}$), 136.20 (*C*$_{quatPh}$), 171.73 (*C*=O$_{Bn}$), 172.0 (*C*=O$_{Bn}$), 172.64 (*C*=O$_{tBu}$), 173.38 (*C*=O$_{tBu}$). m/z (ES+): 910.3 [M + H]$^+$, 932.4 [M + Na]$^+$. (Found: MH$^+$, 910.5427. $C_{50}H_{76}N_3O_{12}$ requires MH$^+$, 910.5423; Found: [M + Na]$^+$, 932.5250. $C_{50}H_{75}N_3O_{12}Na$ requires [M + Na]$^+$, 932.5243).

(1′*R*,6*S*)-6-[Bis(carboxymethyl)]-4-(carboxymethyl)-1-(1′-carboxy-3′-carboxypropyl)-tetrahydro-6-amino-6-methyl-1*H*-1,4-diazepine, Glu-AAZTA, L^5. 10% Pd/C (0.005 g) was added to a solution of compound **3b** (0.05 g, 0.067 mmol) in EtOH (4 mL) and H_2O (0.5 mL). The reaction mixture was stirred under a hydrogen atmosphere for 48 h (30 psi H_2). The catalyst was filtered over Celite and the solvent evaporated under reduced pressure, to afford a white glassy solid (0.047 g, 0.07 mmol, 96%). δ_H (D_2O, 199.99 MHz): 1.05 (3H, br. s, CH_3), 1.45 (9H, s C(CH$_3$)$_3$), 1.92–2.01 (2H, m, CH_2CHN), 2.42–2.49 (2H, br. m, CH_2COOH), 3.06–3.57 (9 H, br. m, CHN + 4 × CH$_{2ring}$), 3.67 (4H, br. s, 2 × CH_2COOH), 3.7 (2H, br. s, CH_2COOH). m/z (ES-MS): 703 [M + Na]$^-$.

The acid prepared above (0.047 g, 0.07 mmol) was treated with TFA–CH_2Cl_2 (1 : 1, 2.5 mL) and stirred at room temperature for 24 h. The solvent was removed under reduced pressure and the residue taken up with CH_2Cl_2 (3 × 4 mL) and the solution evaporated under reduced pressure each time. The residue was washed twice with diethyl ether (2 × 2 mL) and a colourless precipitate was obtained of the bis-trifluoroacetate salt of H_5L^5 (0.03 g, 0.06 mmol). δ_H (D_2O, 399.96 MHz): 1.04 (3H, br. s, CH_3), 1.91–1.93 (2H, m, CH_2CHN), 2.41 (2H, br. m, CH_2COOH), 3.0–3.49 (9 H, br. m, CHN + 4 × CH$_{2ring}$), 3.62 (4H, br. s, 2 × CH_2COOH), 3.82 (2H, br. s, CH_2COOH). m/z (ES+): 456.4 [M + Na]$^+$. Found: 456.1596; $C_{17}H_{27}O_{10}N_3Na$ requires: 456.1594.

(*RR*)-6-[Bis(carboxymethyl)]-1,4-bis(1′-carboxy-3′-carboxypropyl)-tetrahydro-6-amino-6-methyl-1*H*-1,4-diazepine, L^3. This compound could be obtained by stepwise de-benzylation followed by TFA removal of the *tert*-butyl groups or *vice versa*. Only the first step is shown for the first of these routes.

Pearlman's catalyst (0.008 g) was added to a solution of compound **5b** (0.08 g, 0.12 mmol) in EtOH (10 mL) and H_2O (1 mL). The reaction mixture was stirred under a hydrogen atmosphere for 48 h (30 psi H_2). The catalyst was filtered over Celite and the solvent evaporated under reduced pressure, to afford the de-benzylated product as a white glassy solid (0.08 g, 0.11 mmol, 92%). δ_H (CD_3OD, 699.73 MHz): 1.18 (3H, br. s, CH_3), 1.48 (18H, s, C(CH_3)$_3$), 1.56 (18H, m, C(CH_3)$_3$), 1.96–1.99 (2H, m, CH_2CHN), 2.07–2.1 (2H, m, CH_2CHN), 2.1–2.18 (4H, m, 2 × CH_{2ring}), 2.47–2.58 (4H, m, 2 × CH_{2ring}), 2.65 (2H, dd, J = 14, CH_2COOH), 2.96 (1H, d, J = 14, CH_{2a}COOH), 3.10 (1H, d, J = 14, CH_{2b}COOH), 3.25–3.28 (2H, m, 2 × CHN), 3.50 (2H, d, J = 17.5, CH_2COO*t*Bu), 3.61 (2H, d, J = 17.5, CH_2COO*t*Bu). δ_C (CD_3OD, 125.67 MHz): 25.8 (*C*H$_3$), 26.9 (*C*H$_2$CHN), 27.04–27.31 (C(*C*H$_3$)$_3$), 29.92 (*C*H$_2$COOH), 30.17 (*C*H$_2$COOH), 50.68 (*C*H$_{2ring}$), 65.40 (*C*$_{quat}$CH$_3$), 67.41–67.61 (*C*H$_2$COO*t*Bu), 82.23 (*C*(CH$_3$)$_3$), 82.54 (*C*(CH$_3$)$_3$), 167.45 (*C*=O$_{tBu}$), 170.8 (*C*=O$_{tBu}$), 174.7 (*C*=OOH), 174.9 (*C*=OOH). m/z (ES+): 730.3 [M + H]$^+$. m/z (ES–): 728.5 [M – H]$^-$. (Found: MH$^+$, 730.4493. $C_{36}H_{64}N_3O_{12}$ requires MH$^+$, 730.4485).

Alternate route. A solution of compound **5b** (0.07 g, 0.077 mmol) in TFA and CH_2Cl_2 (1 : 1, 2 mL) was stirred at room temperature for 24 h. The mixture was then evaporated, the residue taken up with CH_2Cl_2 (2 mL) and the solution evaporated under reduced pressure. This operation was repeated three times, then the residue was washed twice with diethyl ether (2 × 2 mL) to yield a white solid (0.06 g, 0.087 mmol). δ_H (D_2O, 399.96 MHz): 1.05 (3H, s, CH_3), 1.98–2.06 (4H, m, 2 × CH_2CHN), 2.50–2.80 (4H, m, 2 × CH_2COOBn), 3.0–3.75 (10H, br. m, 4 × CH_{2ring} + 2 × CHN), 3.80–3.90 (4H, m, NCH_2COOH), 5.14 (4H, d, J = 4, 2 × CH_2Ph), 7.33–7.36 (10H, m, 2 × Ph–H). This trifluoroacetate salt was used directly without further characterisation.

Pearlman's catalyst (0.006 g) was added to a solution of the acid obtained above (0.06 g, 0.087 mmol) in EtOH (3 mL) and H_2O (0.4 mL). The reaction mixture was stirred under a hydrogen atmosphere for 24 h (30 psi H_2). The catalyst was filtered over Celite and the solvent evaporated under reduced pressure, to afford the bis-trifluoroacetate salt of H_6L^3 as a white glassy solid (0.044 g, 0.08 mmol). δ_H (D_2O, 399.96 MHz): 1.06 (3H, s, CH_3), 1.8–2.05 (4H, m, 2 × CH_2CHN), 2.40–2.55 (4H, m, 2 × CH_2COOH), 2.95–3.40 (10H, br. m, 4 × CH_{2ring} + 2 × CHN), 3.62–3.75 (4H, m, 2 × NCH_2COOH). m/z (ES): 504.2 [M – H]$^-$. Found: [M – H]$^-$, 504.1838. $C_{20}H_{30}N_3O_{12}$ requires [M – H]$^-$, 504.1836.

(*RS*)-6-[Bis(*tert*-butoxycarbonylmethyl)]-1,4-bis(1′-*tert*-butoxycarbonyl-3′-carboxypropyl)-tetrahydro-6-amino-6-methyl-1*H*-1,4-diazepine. 10% Pd/C (0.03 g) was added to a solution of compound **7b** (0.30 g, 0.33 mmol) in EtOH (8 mL) and H_2O (1 mL). The reaction mixture was stirred under a hydrogen atmosphere for 48 h using a hydrogenator (30 psi H_2). The catalyst was filtered over Celite and the solvent evaporated under reduced pressure, to afford the tetra-*tert*-butyl ester as a colourless glassy solid (0.48 g, 0.35 mmol, 94%). δ_H (CD_3OD, 499.77 MHz): 1.09 (3H, s, CH_3), 1.48–1.50 (36H, m, 4 × C(CH_3)$_3$), 1.85–1.89 (2H, m, CH_2CHN), 1.95–2.0 (2H, m, CH_2CHN), 2.36–2.48 (4H, m, 2 × CH_{2ring}), 2.66–2.77 (4H, m, CH_{2ring}), 3.0 (2H, d, J = 14, CH_2COOH), 3.16 (2H, d, J = 14, CH_2COOH), 3.26–3.36 (2H, m, 2 × CHN), 3.6 (2H, d, J = 17.5, CH_2COO*t*Bu), 3.75 (2H, d, J = 17.5, CH_2COO*t*Bu). δ_C (CD_3OD, 125.67 MHz): 25.7 (*C*H$_3$), 26.04 (*C*H$_2$CHN), 27.26–27.43 (C(*C*H$_3$)$_3$), 31.0 (*C*H$_2$COOH), 51.5–53.68 (*C*H$_{2ring}$), 64.55 (*C*$_{quat}$CH$_3$), 68.8–69.3 (*C*H$_2$COO*t*Bu), 67.9 (*C*HN), 80.73 (*C*(CH$_3$)$_3$), 80.9 (*C*(CH$_3$)$_3$), 81.13 (*C*(CH$_3$)$_3$), 81.37 (*C*(CH$_3$)$_3$), 171.73 (*C*=O$_{tBu}$), 171.95 (*C*=O$_{tBu}$), 173.10 (*C*=OOH). m/z (ES+): 730.3 [M + H]$^+$, 752.4 [M + Na]$^+$. (Found: MH$^+$, 730.4475. $C_{36}H_{64}N_3O_{12}$ requires MH$^+$, 730.4484; Found: MNa$^+$, 752.4303. $C_{36}H_{63}N_3O_{12}Na$ requires MNa$^+$, 752.4304).

(*RS*)-6-[Bis(carboxymethyl)]-1,4-(bis-1′-carboxy-3′-carboxypropyl)-tetrahydro-6-amino-6-methyl-1*H*-1,4-diazepine: (*RS*)-Glu$_2$AAZTA, L^4. A solution of the *tert*-butyl ester (0.24 g, 0.33 mmol) in TFA–CH_2Cl_2 (1 : 1, 4.0 mL) was stirred at

room temperature for 24 h. The solvent was removed under reduced pressure, the residue taken up with CH_2Cl_2 (4 mL) and the solution evaporated under reduced pressure. This procedure was repeated three times, then the residue was washed twice with diethyl ether (2 × 2 mL) and a colourless solid was obtained of the bis-trifluoroacetate salt (0.18 g, 0.34 mmol). δ_H (D_2O, 499.77 MHz): 1.05 (3H, s, CH_3), 1.92–2.06 (4H, m, 2 × CH_2CHN), 2.36–2.50 (4H, m, 2 × CH_2COOH), 3.02–3.32 (8H, m, CH_{2ring}), 3.54–3.58 (2H, m, 2 × CHN), 3.59 (2H, d, J = 11.5, NCH_2COOH), 3.65 (2H, d, J = 11.5, NCH_2COOH). δ_C (D_2O, 125.67 MHz): 22.01 (CH_3), 24.37 (CH_2CHN), 30.65 (CH_2COOH), 50.65–51.61 (CH_{2ring}), 61.61 ($C_{quat}CH_3$), 66.17 (NCH_2COOH), 67.81 (CHN), 173.08 (CHC=OOH), 176.1 (NCH$_2C$=OOH), 177.0 ((CH$_2$)$_2C$=OOH). m/z (ES–): 504.3 [M – H]$^-$. (Found: [M – H]$^-$, 504.1835. $C_{20}H_{30}N_3O_{12}$ requires [M – H]$^-$, 504.1836).

[Ln^{III}L^5]$^{2-}$. An aqueous solution of $LnCl_3{\cdot}6H_2O$ (0.1 mmol, 0.95 eq.) was added dropwise to a solution of $H_5L^5.(CF_3CO_2)_2$ in H_2O–MeOH 5% solution (0.1 mmol), dissolved in the minimum volume of solvent. The pH was adjusted to ~5.5 with aqueous KOH solution (1 M) and the mixture was left to stir at 50 °C, maintaining the pH at about 5.5 by additional additions of base. After 48 h, the reaction mixture was cooled to room temperature and the pH of the solution raised to ~10 (using aqueous KOH solution, 1 M). Any white solid that precipitated was isolated by centrifugation and the pH of the supernatant readjusted to ~5.0. Freeze drying of the liquid gave a white crystalline solid, together with potassium salts. The properties of the complex were examined in the presence of the salts.

[Yb.GluAAZTA]$^{2-}$ or [Yb.L^5]$^{2-}$. m/z (ES–): 603.07 [M – H]$^-$ (Found: [M – H]$^-$, 599.0737. $C_{17}H_{23}N_3O_{10}{}^{170}$Yb requires [M – H]$^-$, 599.0737 and [M – H]$^-$, 603.0777. $C_{17}H_{23}N_3O_{10}{}^{174}$Yb requires [M – H]$^-$, 603.0777). $\delta_{H\,(Major\ isomer)}$ (D_2O, 499.79 MHz): –36.05, –28.62, –8.72, –5.69, –5.47, –3.84, –3.38, –2.21, –1.07, –1.17, 5.65, 6.01, 8.88, 10.66, 27.26, 29.54, 30.92, 32.51, 36.09, 42.33. $\delta_{H\,(Minor\ isomer)}$ (D_2O, 499.79 MHz): –41.85, –25.32, –8.14, –6.31, –3.82, –3.81, –3.62, –2.50, –1.11, –0.89, 0.70, 7.62, 9.1, 12.03, 28.43, 33.85, 34.25, 36.23.

[Gd.GluAAZTA]$^{2-}$ or [GdL^5]$^{2-}$. m/z (ES–): 587.1 [M – H]$^-$ (Found: [M – H]$^-$, 587.0618. $C_{17}H_{23}N_3O_{10}{}^{158}$Gd requires [M – H]$^-$, 587.0630; [M – H]$^-$, 583.0590. $C_{17}H_{23}N_3O_{10}{}^{154}$Gd requires [M – H]$^-$, 583.0597); [M – H]$^-$, 584.0607. $C_{17}H_{23}N_3O_{10}{}^{155}$Gd requires [M – H]$^-$, 584.0615).

Relaxivity value found by NMRD analysis: r_{1p} = 7.3 mM^{-1}s^{-1} (20 MHz, 298 K). The [Gd^{3+}] have been determined by mineralization with 37% HCl at 120 °C overnight, followed by ICP-MS analysis of [Gd]. Fitting of the ^{17}O NMR R_{2p} *vs.* T (K) profile gave τ_M = 115 ns.

[Eu.GluAAZTA]$^{2-}$ or [Eu.L^5]$^{2-}$. $\tau_{(H_2O)}$ = 0.33, $\tau_{(D_2O)}$ = 0.86; $\delta_{H\,(Major\ isomer)}$ (D_2O, 499.79 MHz): –14.03, –12.96, –12.34, –5.95, –5.14, –4.96, –3.33, –2.55, –2.34, –1.20, –0.97, –0.86, –0.23, 0.28, 0.67, 1.24, 5.40, 5.45, 6.34, 6.84, 7.92, 8.88, 10.51, 11.72, 14.31. $\delta_{H\,(Minor\ isomer)}$ (D_2O, 499.79 MHz): –10.71, –7.72, –7.23, –3.09, –1.98, 6.74, 7.08, 7.21, 9.43, 9.66, 15.01.

[Eu.Glu$_2$AAZTA]$^{3-}$ or [Eu.L^3]$^{3-}$. m/z (ES+): 652.2 [M]$^-$. (Found: [M]$^-$, 652.0794. $C_{20}H_{27}O_{12}N_3{}^{151}$Eu requires [M]$^-$, 652.0798; [M]$^-$, 654.0806 $C_{17}H_{27}O_{10}N_3{}^{153}$Eu requires [M – H]$^-$, 654.0813). $\tau_{(H_2O)}$ = 0.28, $\tau_{(D_2O)}$ = 0.87. $\delta_{H\,(Major\ isomer)}$ (D_2O, 699.73 MHz): –14.65, –5.19, –2.21, –1.59, –1.03, –1.59, –1.03, –0.62, 0.09, 0.43, 0.69, 1.86, 1.96, 4.90, 6.63, 7.29, 7.79, 10.29, 11.77. $\delta_{H\,(Minor\ isomer)}$ (D_2O, 499.79 MHz): –10.27, –8.39, –5.89, –4.64, 0.09, 0.89, 2.32, 2.46, 3.84, 5.52, 9.23, 13.76.

[Gd.L^3]$^{3-}$. The Gd complex was prepared in an analogous manner; m/z (ES+): 658.9 [M – H]$^-$ (Found: [M – H]$^-$, 659.0831. $C_{20}H_{27}N_3O_{12}{}^{158}Gd_1$ requires [M – H]$^-$, 659.0841; [M – H]$^-$, 655.0801. $C_{17}H_{27}N_3O_{10}{}^{154}$Gd requires [M – H]$^-$, 655.0809; [M – H]$^-$, 656.0817. $C_{17}H_{27}N_3O_{12}{}^{155}$Gd requires [M – H]$^-$, 584.0826). r_{1p} = 8.65 mM^{-1} s^{-1} (20 MHz, 298 K). The [Gd^{3+}] was determined by mineralization with 37% HCl at 120 °C overnight. Fitting of the ^{17}O NMR R_{2p} *vs.* T (K) profile gave τ_M = 720 ns.

[Yb^{III}L^3]$^{3-}$. $\delta_{H\,(Major\ isomer)}$ (D_2O, 699.73 MHz): –50.83, –26.87, –18.45, –17.30, –9.92, –8.53, –8.08, –7.41, –5.43, –4.29, –1.62, –0.79, 10.41, 14.03, 28.65, 30.38, 32.69, 40.18, 42.95, 48.55. $\delta_{H\,(Minor\ isomer)}$ (D_2O, 499.79 MHz): –36.32, –33.80, –22.23, –14.76, –13.55, –9.22, 9.64, 16.31, 22.93, 24.27, 33.87, 36.58.

[Gd.L^4]$^{3-}$. Crude yield: 50%. m/z (ES–): 659.2 [M – H]$^-$. (Found: [M – H]$^-$, 659.0845. $C_{20}H_{27}N_3O_{12}{}^{158}$Gd requires [M – H]$^-$, 659.0841). r_{1p} = 7.5 mM^{-1} s^{-1} (20 MHz, 298 K).

Fitting of the ^{17}O NMR R_{2p} *vs.* T (K) profile gave τ_M = 246 ns.

[Yb^{III}L^4]$^{3-}$. Crude yield: 60%. m/z (ES–): 675.3 [M]$^-$. (Found: [M]$^-$, 675.0996. $C_{20}H_{27}N_3O_{12}{}^{174}$Yb requires [M – H]$^-$, 675.0989). $\delta_{H\,(Major\ isomer)}$ (D_2O, 699.73 MHz): –51.48, –37.01, –35.99, –26.08, –19.10, –16.28, –11.24, –9.67, –9.27, –7.18, –4.77, –2.69, –1.48, 0.23, 0.64, 0.82, 1.88, 3.56, 7.25, 10.10, 10.98, 14.85, 24.39, 28.16, 33.13, 35.07, 37.44, 43.18, 47.42, 50.24. $\delta_{H\,(Minor\ isomer)}$ (D_2O, 699.73 MHz): –55.84, –49.92, –43.70, –38.62, –34.93, –32.15, –21.73, –19.96, –17.60, –12.45, –10.03, –7.99, –5.87, –4.4, –0.53, 6.14, 9.00, 10.32, 14.56, 17.59, 25.82, 29.22, 31.49, 38.50, 41.43, 44.32, 52.14.

[Eu^{III}L^4]$^{3-}$. Crude yield: 45%. $\tau_{(H_2O)}$ = 0.38, $\tau_{(D_2O)}$ = 1.27. $\delta_{H\,(Major\ isomer)}$ (D_2O, 699.73 MHz): –10.32, –8.37, –6.03, –5.86, –4.54, –1.46, –1.38, –0.85, –0.51, 0.11, 1.75, 2.07, 3.21, 3.95, 5.31, 6.38, 6.84, 7.23, 8.29, 9.17, 10.14, 10.57, 11.45, 13.64. $\delta_{H\,(Minor\ isomer)}$ (D_2O, 699.73 MHz): –14.66, –5.16, –2.16, –1.09, –1.04, 5.56, 8.29, 11.83.

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