Coordination chemistry of amide-functionalised tetraazamacrocycles: structural, relaxometric and cytotoxicity studies

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Abstract

Three different tetraazamacrocyclic ligands containing four amide substituents that feature groups (namely allyl, styryl and propargyl groups) suitable for polymerisation have been synthesised. Gadolinium(III) complexes of these three ligands have been prepared as potential monomers for the synthesis of polymeric MRI contrast agents. To assess the potential of these monomers as MRI contrast agents, their relaxation enhancement properties and cytotoxicity have been determined. A europium(III) complex of one of these ligands (with propargyl substituents) is also presented together with its PARACEST properties. In addition, to gain further insight into the coordination chemistry of the tetra-propargyl substituted ligand, the corresponding zinc(II) and cadmium(II) complexes have been prepared. The X-ray crystal structures of the tetra-propargyl ligand and its corresponding gadolinium(III), zinc(II) and cadmium(II) complexes are also presented.

Introduction

Polyazamacrocycles substituted with coordinating pendant arms have shown to be excellent ligands for a wide range of metal ions.^{1,2} The resulting complexes are generally thermodynamically very stable, which has resulted in these systems being used in a range of different applications.³ For example, coordination of this type of ligand to metals such as ⁹⁰Y, ⁶⁸Ga and ¹¹¹In yields complexes that have been successfully used as radiopharmaceuticals for imaging and therapy.⁴⁻¹⁰ On the other hand, complexes of substituted polyazamacrocycles with lanthanides (e.g. Eu^{III}, Tb^{III} and Gd^{III}) have been shown to be excellent molecular probes for optical¹¹⁻¹⁵ and magnetic resonance imaging (MRI).¹⁶⁻¹⁹

There are currently five gadolinium(III) complexes approved by the FDA for use as MRI contrast agents in the US. From these, two (Dotarem® and Prohance®) are based on tetraazamacrocyclic derivatives (see Scheme 1). In spite of the great impact these complexes have had in the medical applications of MRI, their performance at clinically relevant concentrations is limited and well below what can be achieved theoretically.²⁰ Consequently, there has been interest in finding new types of complexes that optimise the various factors which affect the ability of gadolinium(III) to change the relaxation time of water molecules.²¹ Several approaches have been investigated such as increasing the number of water molecules coordinating to gadolinium (*q*); optimising the water exchange rate ($1/\tau_m$); increasing the rotational correlation time (τ_R) of the contrast agent. The latter is a promising approach and has been successfully employed by attaching gadolinium complexes to high molecular weight platforms such as cyclodextrins,^{22,23} dendrimers²⁴⁻²⁸ and less well defined polymers.^{21,29,30} Besides their potential beneficial effect in slowing tumbling, high molecular weight materials also have the advantage of increasing the load of gadolinium per molecule with the consequent increase in relaxivity.³¹ Another interesting approach that has been gaining terrain recently is the development of lanthanide-based MRI contrast agents that operate via the

chemical exchange saturation transfer (CEST) mechanism.³²⁻³⁴ These PARACEST agents are often based on Ln-DOTA-tetramide complexes (e.g. with Eu and Yb); this type of complex displays very slow water exchange kinetics which is ideal for the CEST phenomenon to take place. Also in this approach, there is interest in developing polymeric materials containing a large number of lanthanide centres to provide highly sensitive molecular probes.³⁵

For the reasons outlined above, we are interested in developing novel MRI contrast agents based on hyperbranched polymers. Therefore, we have synthesised a series of tetraazamacrocyclic ligands containing four amide substituents that feature functional groups suitable for free-radical, ADMET, cross-coupling and cycloaddition (e.g. 1,3-dipolar cycloadditions, “click”) polymerisation approaches (see Scheme 2).

Scheme 1 –Two examples, Dotarem® and Prohance®, of clinically approved MRI contrast agents that make use of the azamacrocyclic ligands DOTA and HPDO3A shown in this scheme.

Herein we present the synthesis and characterisation of three of such tetraazamacrocyclic ligands and their corresponding gadolinium(III) complexes (and in one case also a europium(III) complex). To assess their potential as MRI contrast agents (as part of a larger polymeric structure), their relaxation enhancement properties have been investigated as well

as their cytotoxicity at concentrations relevant for MRI. As per the europium(III) complex herein presented, its potential as PARACEST agent has been evaluated. In addition, to gain insight into the structural features of the ligands and complexes, X-ray crystallographic studies have been carried out of one of the ligands (the tetra-propargyl derivative) and its corresponding gadolinium(III), cadmium(II) and zinc(II) complexes.

Results and Discussion

Synthesis

The tetra-substituted tetraazamacrocyclic ligands **4-6** (see Scheme 2) were prepared by reacting cyclen with the corresponding chloro-acetamides (**1-3**). Compounds **4** and **6** have not been reported before while the synthesis of **5** (*via* a different route) was published by Antoni in the course of this investigation.³⁶ The three products were isolated as white or off-white solids in 58 to 74% yields and were fully characterised by spectroscopic and analytical techniques, and in one case (compound **5**) by X-ray crystallography (*vide infra*).

The three cyclen derivatives were then reacted in water with GdCl₃ to yield the new complexes **7**, **8** and **10** (see Scheme 2). In each case, after 4 hours, the corresponding reaction mixture was analysed for free Gd^{III} using the Methyl Thymol Blue (MTB) test. In all cases the test was negative indicating that all the gadolinium(III) had coordinated to the corresponding ligand. The solvent was then removed under reduced pressure and the solid residue washed thoroughly with CH₂Cl₂ or THF (to remove any unreacted ligand) yielding the three gadolinium(III) complexes as white solids. ESI(+) mass spectrometry of these complexes showed molecular peaks at 716, 708 and 964 a.m.u. for **7**, **8** and **10** respectively consistent with the formula [Gd+L-2H]⁺ (where L = **4**, **5** or **6**). The formulation of the complexes was confirmed by elemental analyses. In addition, one of the complexes (**8**) was

structurally characterised by single crystal X-ray crystallography (*vide infra*) confirming the octa-coordination of the gadolinium(III) centre by the tetra-substituted cyclen ligand.

Scheme 2 – Reaction scheme showing the syntheses of all ligands and metal complexes used in this study.

The europium complex **9** (see Scheme 2) was prepared using a similar synthetic methodology than that used for the gadolinium(III) complex **8** described above. Mass spectrometry, ^1H NMR spectroscopy and elemental analyses were all consistent with the formation of the correct complex.³⁷ Interestingly, in the region above 5 ppm (where the resonance for the axial ethylene unit appears) only one resonance at 24 ppm was observed. This resonance and the absence of any other between 5 and 12 ppm suggest that in solution complex **9** is present in a mono-capped square antiprismatic geometry and not as a mixture of isomers (the second being the mono-capped twisted square antiprismatic geometry) as is often the case with DOTA-tetramide lanthanide complexes.³⁷ In addition, the emission spectrum of this complex was recorded showing the expected transitions for cyclen-based europium(III) complexes³⁸ (see Figure 1).

Figure 1 – Emission spectrum of the Eu-tetraalkynyl complex **9** excited at 345 nm. The spectrum shows the characteristic $^5\text{D}_0 \rightarrow ^7\text{F}_J$ transitions ($\Delta J = 0, 1, 2, 3, 4$) for europium(III) complexes coordinated to cycle-based ligands.

In order to gain more insight into the coordinating abilities of these ligands, one of them (ligand **5**) was also reacted with zinc(II) and cadmium(II) perchlorates. It has been previously shown that these metal ions (in particular cadmium(II)) often coordinate to cyclen derivatives in a similar fashion than gadolinium(III) does.³⁹ An advantage of using cadmium(II) and zinc(II) is that, having a closed shell (d^{10}), they are diamagnetic and therefore the resulting complexes can be studied by NMR spectroscopy. Thus, reaction of the tetra-propargyl ligand with $\text{Cd}(\text{ClO}_4)_2$ and $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ yielded complexes **11** and **12** respectively (see Scheme 2). Both complexes were obtained as white solids and characterised by spectroscopic, analytical and structural techniques. The ^1H NMR spectrum of cadmium complex **11** is generally broader as compared to that of the free ligand. The protons of cyclen are slightly more shielded and appear as a broad signal centred at 2.45 ppm (*cf.* a singlet at 2.62 ppm for the free ligand). The alkyne protons appear at 3.20 ppm as opposed to 3.05 ppm in the free ligand, while the amide protons appear downfield at 9.17 ppm (*cf.* 8.42 ppm in the free ligand). Upon coordination of zinc(II) to ligand **5**, the CH_2 protons of cyclen become unequivalent and two different signals (at 2.67 and 3.02, *c.f.* one signal at 2.61 for the free ligand) can be observed. The mass spectra of both these complexes showed peaks at 715 and 765 a.m.u. which correspond to $[\text{M}(\mathbf{5})\text{ClO}_4]^+$ ($\text{M} = \text{Cd}, \text{Zn}$).

X-ray crystal structures of compounds 5, 8, 11 and 12. The structures of the tetra-propargyl cyclen ligand (**5**), and its complexes with gadolinium (**8**), cadmium (**11**) and zinc (**12**) are shown in Figures 2 – 5; selected bond lengths and angles for the complexes are given in Tables 1, 2 and 3. It can readily be seen that whilst in the free ligand the four amide arms are spread out (Figure 2), in the presence of a metal ion coordinated to all four of the cyclen nitrogen centres, some or all of the arms wrap around and encapsulate the metal centre.

Figure 2 The structure of the C_7 -symmetric molecule **5**.

In the gadolinium and cadmium complexes **8** and **11**, the tetra-propargyl cyclen ligand adopts an octadentate coordination mode with the carbonyl oxygen atoms of all four amide arms binding to the metal (see Figure 3 and 4).

Fig. 3 (a) The molecular structure of the cation present in the crystals of **8**; (b) The coordination sphere around the gadolinium atom in the structure of **8** showing the mono-capped Archimedean anti-prismatic coordination geometry.

In the zinc complex **12** however, the ligand adopts a hexadentate coordination mode with only two of the amide arms (a pair opposite each other) linking to the metal (see Figures 5 and 6). The additional coordination of a water molecule [O(40)] in the gadolinium complex **8**

gives rise to a mono-capped Archimidean antiprismatic coordination geometry with O(40) in the capping position. The cadmium complex **11** has a coordination geometry intermediate between cubic and Archimidean antiprismatic, whilst the zinc complex **12** has an octahedral coordination geometry.

Fig. 4 (a) The molecular structure of the C_2 -symmetric cationic complex present in the crystals of **11**, viewed along the crystallographic C_2 axis direction. (b) The coordination sphere around the cadmium atom in the structure of **11** showing the “intermediate between cubic and Archimidean anti-prismatic” coordination geometry.

The change in conformation of the ligand arms between the free ligand and the three metal complexes is reflected in the X–N–C–C [where X is either the nitrogen lone pair (**5**)⁴⁰ or the coordinated metal atom (**8**, **11** and **12**)] and N–C–C–O torsion angles for each of the amide arms (Tables 4 and 5). For one of the nitrogen atoms of the macrocycle and its associated amide oxygen atom to bind to the same metal their X–N–C–C and N–C–C–O torsion angles must both be small so that the nitrogen lone pair or bonding pair and the oxygen atom point in approximately the same direction. The chelating arms in complexes **8**

and **11** all have X–N–C–C torsion angles with magnitudes in the range $35 - 43^\circ$, and N–C–C–O torsion angles with magnitudes in the range $23 - 36^\circ$ (the deviations from zero being due to the preferential coordination geometries of the particular metal atom). In the ligand **5**, the N(1)-based amide arm has X–N–C–C and N–C–C–O torsion angles of 25 and -167° respectively, showing that the O(14) oxygen atom is in the wrong position relative to N(1) for chelation. For the N(4)-based arm, by contrast, the X–N–C–C and N–C–C–O torsion angles of -45 and -5° respectively show that this unit is ideally arranged for chelation. However, significant rearrangement would still be required for both this unit and its centrosymmetrically related counterpart to chelate to the same metal as they are currently on opposites sides of the tetraaza macrocycle. The zinc complex **12** has two coordinated arms and two non-coordinated arms. For the coordinated arms the X–N–C–C and N–C–C–O torsion angles are 15 and -21° respectively, whilst for the non-coordinated arms they are *ca.* 168 and 43° respectively.

Fig. 5 The molecular structure of the C_2 -symmetric cationic complex present in the crystals of **12**, viewed along the crystallographic C_2 axis direction.

Fig. 6 The coordination sphere around the zinc atom in the structure of **12** showing the distorted octahedral coordination geometry.

Table 1. Selected bond lengths (Å) and angles (°) for **8**.

Gd–N(1)	2.652(4)	Gd–N(4)	2.664(4)
Gd–N(7)	2.677(4)	Gd–N(10)	2.623(4)
Gd–O(14)	2.387(3)	Gd–O(20)	2.393(3)
Gd–O(26)	2.363(3)	Gd–O(32)	2.424(3)
Gd–O(40)	2.437(3)		
N(1)–Gd–N(4)	68.12(12)	N(1)–Gd–N(7)	104.86(12)
N(1)–Gd–N(10)	68.85(12)	N(1)–Gd–O(14)	66.20(12)
N(1)–Gd–O(20)	71.99(12)	N(1)–Gd–O(26)	141.67(12)
N(1)–Gd–O(32)	132.78(12)	N(1)–Gd–O(40)	126.53(12)
N(4)–Gd–N(7)	67.65(12)	N(4)–Gd–N(10)	105.11(13)
N(4)–Gd–O(14)	130.77(12)	N(4)–Gd–O(20)	65.76(12)
N(4)–Gd–O(26)	74.35(12)	N(4)–Gd–O(32)	139.60(12)
N(4)–Gd–O(40)	126.76(12)	N(7)–Gd–N(10)	68.34(13)
N(7)–Gd–O(14)	142.18(12)	N(7)–Gd–O(20)	130.57(12)
N(7)–Gd–O(26)	66.26(12)	N(7)–Gd–O(32)	72.93(11)
N(7)–Gd–O(40)	128.59(12)	N(10)–Gd–O(14)	74.42(12)
N(10)–Gd–O(20)	140.14(12)	N(10)–Gd–O(26)	130.66(12)
N(10)–Gd–O(32)	66.82(11)	N(10)–Gd–O(40)	128.12(12)
O(14)–Gd–O(20)	83.43(11)	O(14)–Gd–O(26)	143.27(11)
O(14)–Gd–O(32)	86.80(11)	O(14)–Gd–O(40)	71.55(11)
O(20)–Gd–O(26)	86.00(11)	O(20)–Gd–O(32)	145.29(11)
O(20)–Gd–O(40)	72.04(11)	O(26)–Gd–O(32)	82.20(11)
O(26)–Gd–O(40)	71.73(11)	O(32)–Gd–O(40)	73.27(11)

Table 2. Selected bond lengths (Å) and angles (°) for **11**.

Cd–N(1)	2.4762(15)	Cd–N(4)	2.4380(15)
Cd–O(14)	2.3915(12)	Cd–O(20)	2.4024(12)
N(1)–Cd–N(4)	74.45(6)	N(1)–Cd–O(14)	68.26(5)
N(1)–Cd–O(20)	91.89(5)	N(1)–Cd–N(1A)	118.04(7)
N(1)–Cd–N(4A)	74.42(5)	N(1)–Cd–O(14A)	160.53(5)
N(1)–Cd–O(20A)	123.72(4)	N(4)–Cd–O(14)	124.44(4)
N(4)–Cd–O(20)	69.35(5)	N(4)–Cd–N(4A)	117.17(7)
N(4)–Cd–O(14A)	90.81(5)	N(4)–Cd–O(20A)	161.47(5)
O(14)–Cd–O(20)	72.26(4)	O(14)–Cd–O(14A)	112.44(6)
O(14)–Cd–O(20A)	70.70(4)	O(20)–Cd–O(20A)	110.33(6)

Table 3. Selected bond lengths (Å) and angles (°) for **12**.

Zn–N(1)	2.231(2)	Zn–N(4)	2.182(2)
Zn–O(14)	2.0920(15)		
N(1)–Zn–N(4)	82.08(9)	N(1)–Zn–O(14)	78.10(7)
N(1)–Zn–N(1A)	126.08(12)	N(1)–Zn–N(4A)	79.75(7)
N(1)–Zn–O(14A)	152.27(8)	N(4)–Zn–O(14)	92.09(7)
N(4)–Zn–N(4A)	139.23(13)	N(4)–Zn–O(14A)	119.02(8)
O(14)–Zn–O(14A)	83.03(8)		

Table 4. Comparative X–N–C–C torsion angles (°) for each amide arm of the tetra-propargyl cyclen ligand in the structures of **5**, **8**, **11** and **12**.^[a]

	5	8	11	12
X–N(1)–C–C	25	–35	–42	15
X–N(4)–C–C	–45	–40	–43	168
X–N(7)–C–C	n/a	–37	n/a	n/a
X–N(10)–C–C	n/a	–38	n/a	n/a

^[a] X is either the nitrogen lone pair or the coordinated metal atom.⁴⁰

Table 5. Comparative N–C–C–O torsion angles (°) for each amide arm of the tetra-propargyl cyclen ligand in the structures of **5**, **8**, **11** and **12**.

	5	8	11	12
N(1)–C–C–O(14)	–167	23	30	–21
N(4)–C–C–O(20)	–5	36	30	43
N(7)–C–C–O(26)	n/a	30	n/a	n/a
N(10)–C–C–O(32)	n/a	31	n/a	n/a

Determination of relaxivity of gadolinium complexes 7 and 8. Once the gadolinium complexes **7**, **8** and **10** were prepared and fully characterised, it was of interest to determine their potential as MRI contrast agents. Therefore relaxivity determinations were carried out using two of the complexes (**7** and **8**). Complex **10** showed to have limited solubility in water and therefore was excluded of any further relaxivity and cell viability (see next section) studies. Relaxation rates were measured at 25°C using 2.35 T (100 MHz) and 9.4 T (400 MHz) systems. Solutions of the corresponding complexes at 0.1–2.5 mM concentration range in double distilled water were prepared and the relaxivities determined (see Table 6).

Table 6 Longitudinal (r_1) and transversal (r_2) relaxivities calculated for complexes **7** and **8** at different magnetic field strengths (B).

<i>Compound</i>	<i>B = 2.35 T</i>		<i>B = 9.4 T</i>	
	r_1 ($\text{mM}^{-1} \text{s}^{-1}$)	r_2 ($\text{mM}^{-1} \text{s}^{-1}$)	r_1 ($\text{mM}^{-1} \text{s}^{-1}$)	r_2 ($\text{mM}^{-1} \text{s}^{-1}$)
7	2.84	3.74	2.25	3.41
8	2.86	2.47	2.83	4.79

The r_1 values obtained for these compounds are comparable to other gadolinium(III) complexes with amide-functionalised DOTA ligands. For example, the relaxivities for Gd-DOTAM,⁴¹ Gd-DOTTA⁴¹ and Gd-DOTA-benzyl-amide⁴² (measured at 0.47 T, corresponding to a proton Larmor frequency of 20 MHz) have been previously reported to be 2.5, 3.0 and 1.88 $\text{mM}^{-1} \text{s}^{-1}$ respectively. The transversal relaxivities (r_2) of complexes **7** and **8** were also determined by using a spin-echo technique. This parameter is rarely reported for

Gd-based contrast agents and indeed no transversal relaxivity values were found in the literature for any Gd-DOTA-tetraamide complexes making any comparison difficult. As a reference, the previously reported r_2 value (at $B = 2.35$ T) for Gd-DOTA is $4.9 \text{ mM}^{-1} \text{ s}^{-1}$.⁴³

CEST spectrum of PARACEST agent 9. The PARACEST spectrum of the europium complex **9** was recorded (see Figure 7) by applying selective saturation between +100 and –100 ppm. The CEST profile of this complex is consistent with analogous DOTA-tetraamide europium(III) complexes showing intermediate to slow water exchange between the Eu-bound water species (*ca.* $\delta = 50$ ppm) and that of bulk water (*ca.* $\delta = 0$ ppm).^{37,44}

Figure 7. CEST spectrum of a 20 mM solution of europium(III) complex **9** at neutral pH ($B_0 = 9.4$ T, 294 ± 1 K, irradiation time 2s, $B_1 = 22.1 \mu\text{T}$).

Cell viability studies of gadolinium complexes. The toxicity of complexes **7** and **8** was investigated by incubating ST14A stem cells⁴⁵ (an immortalized neural stem cell line derived from embryonic striatum at developmental stage E14) in solutions containing different concentrations of the corresponding complex, at 33°C and 5% CO₂ atmosphere for 24 hours. Solutions of **7** and **8** ranging from 0.01 to 10 mM were prepared in DMEM. Incubation was then carried out with these solutions, after which the cells were washed with EBSS and tested for viability. Cells were first checked under an inverted microscope to assess morphological changes as possible occurrence of cell death. Figure 8 shows examples of the morphology of cells after being exposed to different concentrations of the complex (in this case complex **7**). Whereas 1 mM concentration of compound **7** does not overtly affect cell morphology as compared to controls (Figure 8A-B), most cells seem to be dying by 24 hours in the presence of a 10 mM concentration of compound **7** (Figure 8C).

Figure 8. Optical images of cells as seen under an inverted microscope after exposure to different concentrations of gadolinium(III) complex **7**. A) Untreated control cells. B) Cells incubated with a 1 mM concentration of compound **7** displayed a similar morphology to the healthy untreated control cells (A). C) Cells treated with a 10 mM concentration of compound **7**. Note the round morphology typical of dead or dying cells.

For a quantitative analysis of the effect of compounds **7** and **8** on cell survival, change in cell metabolism was assessed using the Alamar Blue (Figure 9 and Table 7)⁴⁶ and Methylene Blue⁴⁷ assays (Figure 10 and Table 7). These methods utilize dyes that detect either changes in the redox state of the culture medium resulting from cell growth (Alamar Blue assay), or that bind to living cell membranes (Methylene Blue assay). Across the range of concentrations, both complexes showed similar metabolic activity and cytotoxicity as the control cells which were not exposed to gadolinium complexes. In the Alamar Blue assay, a small, though statistically different, reduction in cell number appeared to be induced by treatment with compound **8** at 0.1 mM and 0.01 mM (Figure 7; $p=0.015$). In the Methylene Blue assay, a concentration-dependent effect on cell viability was observed, and cell number at 3 mM using compound **8** was statistically lower than in control cells (Figure 8; $p=0.001$). Microscopy indicated that cells at the range of concentrations tested in the viability assays (0.01-3 mM) were not visibly undergoing cell death, suggesting that the results of the Alamar Blue and Methylene Blue assays may reflect an effect of the compounds on cell metabolism and proliferation, respectively, rather than in cell survival. Whereas the Methylene Blue assay provides a measure of the dye bound to living cell membranes, and its binding is an indicator of the number of cells in culture,⁴⁷ the Alamar Blue assay detects dye colour changes due to oxidation–reduction activity, providing a measure of cell metabolic activity. Altogether our results suggest that compound **7** is non-toxic at and below 3 mM, while at 3 mM cell numbers decrease for compound **8**.

Figure 9. Effect of compounds **7** and **8** on cell viability assessed by Alamar Blue assay.

Figure 10. Effect of compounds **7** and **8** on cell viability assessed by Methylene Blue assay.

Table 7 Summary of the effect (+ = positive; – = negative) on cell viability for Gd-complexes **7** and **8**.

Compound	Concentration (mM)						
	0.01	0.1	0.5	1.0	2.0	3.0	10.0
7	+	+	+	+	+	+	-
8	+	+	+	+	+	-	-

Conclusions

Three tetra-amide substituted ligands and the corresponding gadolinium(III) complexes have been prepared. In one case (with ligand **5**) the corresponding europium(III) complex has also been synthesised. These ligands were designed to feature readily polymerisable groups (i.e. terminal alkenes or alkynes) for their potential use as monomers in the synthesis of cyclen-containing hyperbranched polymers. Two of the new gadolinium(III) complexes, were further studied to determine their relaxivities (r_1 and r_2) and cytotoxicity. Both these complexes show longitudinal relaxivities comparable to the values previously reported for other Gd^{III} complexes with tetraamide-DOTA ligands. It should be noted that Gd-DOTA-tetramide complexes generally have very slow water exchange kinetics in comparison to Gd-DOTA (i.e. the tetraacid complex). This is not ideal for their application as MRI contrast agents. However, as indicated above, our aim is to polymerise these monomeric units to generate materials that would provide a higher payload of gadolinium per molecule increasing the relaxivity this way. This polymeric materials could also see a further enhancement of the relaxivity due to the slower tumbling of the resulting molecules, although this effect is minimised in systems where the water exchange is slow as is likely to be the case here (in spite of this, second coordination sphere interactions can overcome this problem – see for example the recent publication by Sherry⁴⁸). The cytotoxicity studies indicate that one of the compounds under study (**7**) is non-toxic at and below 3 mM, while a slight cytotoxicity was observed for compound **8** at 3 mM (but not at lower concentrations). We also studied the PARACEST properties of europium(III) complex **9** which showed to have an intermediate to slow water exchange between the Eu-bound water species and bulk water, as expected. In addition to these studies – directed towards the development of novel MRI contrast agents – we also carried out a detailed structural study of one of the ligands (with propargyl substituents) and the corresponding Gd^{III}, Cd^{II} and Zn^{II} complexes. The single crystal X-ray

crystallographic studies showed the ligand to be octadentate for the gadolinium and cadmium complexes and hexadentate in the case of zinc. This is consistent with the coordination chemistry expected in each case.

We are currently investigating the formation of hyperbranched polymers from these monomers; the resulting species will in turn be loaded with gadolinium(III) to yield new MRI contrast agents. These results will be reported in due course.

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Experimental

Materials and methods. All reactions requiring anhydrous conditions or involving moisture sensitive reactants were performed under an atmosphere of dry nitrogen using oven dried (80°C) and/or flame dried glassware. Reactants, reagents and special solvents were obtained from commercial suppliers and were used without further purification. All common solvents were of Analar[®] grade from BDH and were dried in accordance with established protocols.

All reaction temperatures reported indicate the temperature of the bath in contact with the reaction vessel. The presence of free (i.e. unreacted) gadolinium(III) ions during complexation reactions was checked by adding a small volume (approximately 0.2 ml) of reaction mixture to a sample tube containing 0.2 ml of a 0.2% aqueous solution of methylthymol blue and 2 ml of a pH 6 urotropine buffer (prepared from a 1 M solution of urotropine and by dropwise addition of 0.1 M HCl until a pH value of 6 was reached). Flash column chromatography was performed using Merck-Kieselgel BDH F₂₅₄ (230-400 mesh). Elemental analyses were performed by the Elemental Analysis Service at London Metropolitan University. All ¹H NMR spectra were recorded as solutions in specified deuterated solvents on a Bruker 2 channel DRX-400 spectrometer using the residual protic solvent signal as internal reference. ¹³C NMR spectra were recorded on a Bruker 2 channel DRX-400 spectrometer as solutions in specified deuterated solvents. FT-IR spectra were recorded on a Perkin-Elmer Spectrum RX FT-IR spectrometer with built-in internal calibration. The compound to analyse was either pressed together with fine anhydrous potassium bromide to obtain a disk, or added as a thin layer between KBr pellets. Mass spectra were recorded under CI⁺ or FAB⁺ conditions on a Micromass AutoSpec Premier instrument, or ESI⁺ conditions using a Micromass LCT Premier instrument. Fluorescence emission spectra were obtained using a Horiba Jobin Yvon, Fluorolog-3 spectrofluorimeter, using 10 x 10 mm quartz cuvettes.

***N*-allyl-2-chloroacetamide, 1.** Chloroacetyl chloride (0.80 ml, 10 mmol) in dry THF (2 ml) was cooled down to -10 °C on an ice bath. A solution of dry allylamine (0.75 ml, 10 mmol) and dry Et₃N (1.39 ml, 10.0 mmol) in dry THF (2 ml) was added dropwise over 1 hour at -10 °C. The mixture was left stirring for 1 h. After removal of the solvent under

reduced pressure, 0.1 M HCl (15 ml) was added, and the product was extracted with CHCl₃. The organic phase was dried over anhydrous K₂CO₃. After evaporation of the solvent under reduced pressure, the resulting brown oil was distilled under reduced pressure at 100°C, and the pure product was collected as a colourless oil in 67% yield (0.90 g, 6.7 mmol). Elemental analysis: calculated C 44.96%, H 6.04%, N 10.49%; found C 45.01%, H 6.11%, N 10.51%. CI⁺ MS, m/z: 151 ([M+NH₄]⁺), 134 ([M+H]⁺); HSMS: calculated MW 134.0373 a.m.u., found 134.0375 a.m.u.). IR (film on KBr pellets), cm⁻¹: 3296, 3083, 1664, 1545, 1419, 1324, 1261, 1240, 1154, 990, 923, 788, 696, 651, 565. ¹H NMR (400 MHz, 25°C, CDCl₃), δ (ppm): 6.71 (br s, 1H, NH), 5.82 (m, 1H, NHCH₂CH), 5.18 (m, 2H, NHCH₂CHCH₂), 4.05 (s, 2H, ClCH₂CO), 3.92 (m, 2H, NHCH₂). ¹³C NMR (100 MHz, 25°C, CDCl₃), δ (ppm): 165.7 (CO), 133.2 (NHCH₂CH), 116.9 (NHCH₂CHCH₂), 42.5 (ClCH₂), 42.0 (NHCH₂).

***N*-(prop-2-ynyl)-2-chloroacetamide, 2.** Chloroacetyl chloride (4.03 ml, 50.6 mmol) in dry THF (30 ml) was cooled down to -5 °C on an ice bath. A solution of dry propargylamine (3.47 ml, 50.6 mmol) and dry Et₃N (7.05 ml, 50.6 mmol) in dry THF (30 ml) was added dropwise over 1 hour at -5 °C. The mixture was left stirring for 1 h. After removal of the solvent under reduced pressure, 0.1 M HCl (40 ml) was added, and the product was extracted with CHCl₃ (3x20 ml). The organic phase was dried over anhydrous K₂CO₃. After evaporation of the solvent under reduced pressure, the resulting beige solid was purified by flash chromatography (SiO₂, EtOAc/*c*-Hex 50:50 v/v, R_f 0.40), leading to the desired product as a white solid in 49% yield (3.27 g, 24.8 mmol). Mp: 68 °C. Elemental analysis: calculated C 45.65%, H 4.60%, N 10.65%; found C 45.76%, H 4.68%, N 10.73%. CI⁺ MS, m/z: 149 ([M+NH₄]⁺), 132 ([M+H]⁺; calculated 132.0216, found 132.0219), 280 ([2M+NH₄]⁺), 263 ([2M+H]⁺). IR (KBr disk), cm⁻¹: 3417, 3277, 3057, 2997, 2953, 2122, 1677, 1639, 1618, 1544, 1447, 1414, 1384, 1318, 1236, 1077, 1004, 931, 783, 696, 659. ¹H NMR (400 MHz, 25

°C, CDCl₃), δ (ppm): 6.77 (br s, 1H, NH), 4.11 (m, 2H, NHCH₂), 4.07 (s, 2H, ClCH₂CO), 2.28 (t, ³J_{HH} = 2.6 Hz, 1H, C≡CH). ¹³C NMR (100 MHz, 25 °C, CDCl₃), δ (ppm): 165.6 (CO), 78.5 (C≡CH), 72.2 (C≡CH), 42.3 (ClCH₂), 29.6 (NHCH₂).

***N*-(4-vinylphenyl)-2-chloroacetamide (3).** Chloroacetyl chloride (2.00 ml, 25.1 mmol) in dry THF (15 ml) was cooled down to -5 °C on an ice bath. A solution of 4-aminostyrene (2.94 ml, 25.1 mmol) and dry Et₃N (3.50 ml, 25.1 mmol) in dry THF (15 ml) was added dropwise over 1 hour at -5 °C. The mixture was left stirring for 1 h. After removal of the solvent under reduced pressure, 0.1 M HCl (30 ml) was added, and the product was extracted with chloroform (3x15 ml). The organic phase was dried over anhydrous K₂CO₃. After evaporation of the solvent under reduced pressure, the resulting beige solid was purified by flash chromatography (SiO₂, EtOAc/*c*-Hex 40:60 v/v, R_f 0.49), leading to the desired product as a white solid in 55% yield (2.70 g, 13.8 mmol). Mp: 138 °C. Elemental analysis: calculated C 61.39%, H 5.15%, N 7.16%; found C 61.38%, H 5.07%, N 7.14%. FAB⁺ MS, *m/z*: 196 ([M+H]⁺). IR (KBr disk), cm⁻¹: 3474, 3123, 1670, 1615, 1546, 1511, 1403, 1343, 1297, 1255, 1195, 986, 964, 898, 865, 838, 792, 737, 691. ¹H NMR (400 MHz, 25 °C, CDCl₃), δ (ppm): 8.24 (br s, 1H, NH), 7.52 (d, ³J_{HH} = 8.6 Hz, 2H, H^o), 7.40 (d, ³J_{HH} = 8.6 Hz, 2H, H^m), 6.68 (dd, ³J_{HH} = 17.6, ³J_{HH} = 10.9 Hz, 1H, CH=CH₂), 5.71 (d, ³J_{HH} = 17.6 Hz, 1H, CH=CHH^{*cis*}), 5.23 (d, ³J_{HH} = 10.9 Hz, 1H, CH=CHH^{*trans*}), 4.19 (s, 2H, ClCH₂). ¹³C NMR (100 MHz, 25 °C, CDCl₃), δ (ppm): 163.6 (CO), 136.1 (CONHC^{*ipso*}), 135.9 (CH=CH₂), 134.6 (C^{*p*}), 126.9 (C^{*m*}), 120.0 (C^{*o*}), 113.6 (CH=CH₂), 42.8 (ClCH₂).

1,4,7,10-tetrakis(allylcarbamoylmethyl)-1,4,7,10-tetraazacyclo-dodecane (4).

Cyclen (0.100 g, 0.581 mmol), K₂CO₃ (0.401 g, 2.90 mmol) and **1** (0.388 g, 2.90 mmol) were suspended in dry MeCN (20 ml) and heated at reflux (85 °C) for 18 h. After removal of the

solvent under reduced pressure, the residue was suspended in distilled water (20 ml) and the product extracted with CHCl_3 (5x10 ml). The organic phase was dried over anhydrous K_2CO_3 and the solvent was evaporated under reduced pressure. The beige solid residue was purified by double precipitation from DCM with cyclohexane, yielding the desired product in 58% yield (0.189 g, 0.337 mmol). M.p.: 167 °C. Elemental analysis: calculated C 59.98%, H 8.63%, N 19.98%; found C 60.02%, H 8.57%, N 19.99%. FAB⁺ MS, m/z: 561 ([M+H]⁺; calculated 561.3877, found 561.3871), 583 ([M+Na]⁺). IR (KBr disk), cm⁻¹: 3242, 2954, 2822, 1666, 1541, 1452, 1427, 1368, 1308, 1263, 1227, 1109, 993, 972, 926, 807, 780, 722, 647, 584, 557. ¹H NMR (400 MHz, 25 °C, CDCl_3), δ (ppm): 7.06 (br s, 4H, NH), 5.82 (m, 4H, NHCH_2CH), 5.15 (m, 8H, $\text{NHCH}_2\text{CHCH}_2$), 3.88 (m, 8H, NHCH_2), 3.08 (s, 8H, NCH_2CO), 2.70 (s, 16H, $\text{NCH}_2\text{CH}_2\text{N}$). ¹³C NMR (100 MHz, 25 °C, CDCl_3), δ (ppm): 41.6 (NHCH_2), 53.6 ($\text{NCH}_2\text{CH}_2\text{N}$), 59.2 (NCH_2CO), 116.4 ($\text{NHCH}_2\text{CHCH}_2$), 134.3 (NHCH_2CH), 170.4 (CO).

1,4,7,10-tetrakis((prop-2-ynyl)carbamoylmethyl)-1,4,7,10-tetraazacyclododecane

(5). In the course of this investigation, the synthesis of this compound was reported. Our synthetic methodology differs slightly from the reported and is therefore reported herein. Cyclen (1.66 g, 9.66 mmol), Cs_2CO_3 (13.85 g, 42.51 mmol) and KI (7.06 g, 42.5 mmol), all anhydrous, were suspended in dry DMF (100 ml). Compound **2** (5.59 g, 42.51 mmol) was added, and the suspension was stirred at 50 °C for 2 days. After removing the solvent under reduced pressure, the brown coloured solid was suspended in H_2O (100 ml) and filtered under vacuum. The solid residue was washed with H_2O until the filtrate was colourless (80 ml). The white solid was purified by recrystallisation from a 95:5 v/v mixture of DMF/ H_2O , yielding 3.95 g (7.15 mmol, 74%) of the desired product. Mp (°C): 204. Elemental analysis: calculated C 60.85%, H 7.30%, N 20.27%; found C 60.98%, H 7.21%, N 20.19%. ESI MS, m/z: 553

($[M+H]^+$; calculated 553.3251, found 553.3257), 575 ($[M+Na]^+$), 277 ($[M+2H]^{2+}$). IR (KBr disk), cm^{-1} : 3283, 3069, 2948, 2825, 2122, 1657, 1532, 1450, 1424, 1360, 1326, 1300, 1259, 1224, 1143, 1105, 1081, 1044, 1021, 996, 978, 917, 890, 803, 759, 685, 595, 575, 536, 312. ^1H NMR (400 MHz, 25 °C DMSO- d_6), δ (ppm): 8.41 (t, $^3J_{\text{HH}} = 5.5$ Hz, 4H, NH), 3.87 (m, 8H, NHCH_2), 3.05 (t, $^3J_{\text{HH}} = 2.4$ Hz, 4H, $\text{C}\equiv\text{CH}$), 3.04 (s, 8H, NCH_2CO), 2.61 (s, 16H, $\text{NCH}_2\text{CH}_2\text{N}$). ^{13}C NMR (100 MHz, 25 °C, DMSO- d_6), δ (ppm): 170.4 (CO), 81.2 ($\text{C}\equiv\text{CH}$), 72.8 ($\text{C}\equiv\text{CH}$), 57.4 (NCH_2CO), 53.2 ($\text{NCH}_2\text{CH}_2\text{N}$), 27.8 (NHCH_2).

1,4,7,10-tetrakis((4-vinylphenyl)carbamoylmethyl)-1,4,7,10-

tetraazacyclododecane (6). Compound **3** (0.931 g, 4.76 mmol) and dry Et_3N (0.66 ml, 4.8 mmol) were dissolved in dry DMF (2 ml). A solution of cyclen (0.200 g, 1.16 mmol) in distilled water (4 ml) was added dropwise in 1 hour at 50 °C. After 2 hours, the white solid precipitate was filtered and washed with distilled water. After drying under reduced pressure and in a freeze dryer, the desired product was obtained in 63% yield (0.60 g, 0.74 mmol). Mp: decomposes. Elemental analysis: calculated C 71.26%, H 6.98%, N 13.85%; found C 71.27%, H 7.07%, N 13.76%. FAB^+ MS, m/z : 809 ($[M+H]^+$), 831 ($[M+Na]^+$), 847 ($[M+K]^+$). IR (KBr disk), cm^{-1} : 3239, 3085, 3039, 2976, 2811, 1901, 1674, 1627, 1605, 1514, 1421, 1403, 1317, 1250, 1180, 1103, 1068, 991, 955, 902, 839, 717, 495. ^1H NMR (400 MHz, 25 °C, DMSO- d_6), δ (ppm): 9.97 (s, 4H, NH), 7.55 (d, $^3J_{\text{HH}} = 8.6$ Hz, 4H, H^o), 7.33 (d, $^3J_{\text{HH}} = 8.6$ Hz, 4H, H^m), 6.63 (dd, $^3J_{\text{HH}} = 17.6$ Hz, $^3J_{\text{HH}} = 11.0$ Hz, 4H, $\text{CH}=\text{CH}_2$), 5.67 (d, $^3J_{\text{HH}} = 17.6$ Hz, 4H, $\text{CH}=\text{CHH}^{\text{cis}}$), 5.14 (d, $^3J_{\text{HH}} = 11.0$ Hz, 4H, $\text{CH}=\text{CHH}^{\text{trans}}$), 3.27 (s, 8H, NCH_2CO), 2.87 (s, 16H, $\text{NCH}_2\text{CH}_2\text{N}$). ^{13}C NMR (100 MHz, 25 °C, DMSO- d_6), δ (ppm): 169.5 (CO), 138.4 ($\text{CONHC}^{\text{ipso}}$), 136.2 ($\text{CH}=\text{CH}_2$), 132.1 (C^p), 126.5 (C^m), 119.2 (C^o), 112.7 ($\text{CH}=\text{CH}_2$), 58.0 (NCH_2CO), 52.7 ($\text{NCH}_2\text{CH}_2\text{N}$).

Gd-1,4,7,10-tetrakis(allylcarbamoylmethyl)-1,4,7,10-tetraazacyclododecane (7).

Compound **4** (0.408 g, 0.727 mmol) was dissolved in 30 ml of distilled water. $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ (0.180 g, 0.485 mmol) was added, and the mixture was heated at 50 °C. After 3 hours the test with MTB for the presence of free Gd^{III} ions was negative. The solvent was removed under reduced pressure, and the residue was suspended in dry DCM, refluxed for 2 h and filtered, yielding 0.327 g of white solid (0.388 mmol, 80% respect to the $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$). Mp: >270 °C (decomposition). Elemental analysis: calculated ($\text{C}_{28}\text{H}_{48}\text{N}_8\text{O}_4 \cdot \text{GdCl}_3 \cdot \text{H}_2\text{O}$) C 39.92%, H 5.98%, N 13.30%; found C 39.79%, H 6.01%, N 13.36%. FAB⁺ MS, m/z: 716 ($[\text{Gd}(\mathbf{3}-2\text{H})]^+$). IR (KBr disk), cm^{-1} : 1627, 1485, 1462, 1433, 1412, 1388, 1363, 1324, 1297, 1268, 1243, 1156, 1083, 996, 972, 952, 918, 882, 831, 796, 721, 666, 566, 486.

Gd-1,4,7,10-tetrakis((prop-2-ynyl)carbamoylmethyl)-1,4,7,10-

tetraazacyclododecane, (8). Compound **5** (0.200 g, 0.362 mmol) was suspended in distilled water (30 ml). $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ (0.090 g, 0.241 mmol) was added, and the mixture was heated at 50 °C. After 4 hours the test with MTB for the presence of free Gd^{III} ions was negative. The solvent was removed under reduced pressure, and the residue was washed in a Soxhlet with dry DCM for 2 days, resulting in 0.172 g (0.207 mmol) of a white solid (yield: 86% respect to the $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$). Mp: decomposition. Elemental analysis: calculated ($\text{C}_{28}\text{H}_{40}\text{N}_8\text{O}_4 \cdot \text{GdCl}_3 \cdot \text{H}_2\text{O}$) C 40.31%, H 5.07%, N 13.43%; found C 40.41%, H 5.15%, N 13.34%. ESI(+) MS, m/z: 708.2290 ($[\text{Gd}(\mathbf{5}-2\text{H})]^+$; calculated 708.2257). IR (KBr disk), cm^{-1} : 3450, 3227, 3085, 2951, 2865, 2109, 1621, 1567, 1462, 1426, 1358, 1324, 1292, 1276, 1085, 1029, 976, 931, 832, 723, 689. Crystals of complex **8** suitable for X-ray crystallographic analysis were obtained by slow diffusion of acetone into an aqueous solution of the gadolinium complex.

Eu-1,4,7,10-tetrakis((prop-2-ynyl)carbamoylmethyl)-1,4,7,10-

tetraazacyclododecane, (9). 1,4,7,10-tetrakis(2-propynylcarbamoylmethyl)-1,4,7,10-tetraazacyclododecane (0.200 g, 0.36 mmol) was suspended in 30 ml of distilled water. $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ (0.073 g, 0.24 mmol) was added, and the mixture was heated at 50°C for 20 hours. The free ligand was filtered and washed with 20 ml of distilled water. The filtrate was freeze-dried and re-crystallized from ethanol affording 0.157 g (0.207 mmol) of white solid (yield: 80% in respect to the $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$). Elemental analysis: calculated ($\text{C}_{28}\text{H}_{40}\text{N}_8\text{O}_4 \cdot \text{EuCl}_3 \cdot 2\text{H}_2\text{O}$): %C 39.88, %H 5.51, %N 13.06; found: %C 39.70, %H 5.24, %N 13.23. ESI(+) MS, m/z: 703 $[\text{M}(\mathbf{5}-2\text{H})]^+$. ^1H NMR (500 MHz, 10% D_2O in H_2O , 25°C): δ 24.0 (s, 4H, cyclen ring CH_2 axial), -2.9 (s, 4H, ring CH_2 equatorial), -4.4 (s, 4H, ring CH_2 equatorial), -8.7 (s, 4H, ring CH_2 axial), -9.1 (s, 4H, CH_2CON), -12.4 (s, 4H, CH_2CON). The protons of alkyne and NHCH_2 are expected at ~ 2-3 ppm. These are masked by water peak.

Gd-1,4,7,10-tetrakis((4-vinylphenyl)carbamoylmethyl)-1,4,7,10-

tetraazacyclododecane (10). Compound **6** (0.300 g, 0.371 mmol) was suspended in a 1:1 v/v mixture of distilled water and DMF (60 ml). $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ (0.092 g, 0.25 mmol) was added, and the mixture was heated at 50 °C. After 3 hours the test with MTB (see General) for the presence of free Gd^{III} ions was negative. The solvent was removed under reduced pressure, and the residue was washed in a Soxhlet with dry THF for 2 days, resulting in 0.231 g (0.211 mmol) of white solid (yield: 86% respect to the $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$). Mp: decomposition. Elemental analysis: calculated ($\text{C}_{48}\text{H}_{56}\text{N}_8\text{O}_4 \cdot \text{GdCl}_3 \cdot \text{H}_2\text{O}$) C 52.86%, H 5.36%, N 10.27%; found C 52.95%, H 5.48%, N 10.23%. ESI(+) MS, m/z: 1000 $[\text{Gd}(\mathbf{6}-\text{H})\text{Cl}]^+$. IR (KBr disk), cm^{-1} : 3416, 2976, 1631, 1553, 1511, 1458, 1426, 1407, 1384, 1364, 1323, 1247, 1081, 991, 963, 908, 844, 714.

Cd-1,4,7,10-tetrakis((prop-2-ynyl)carbamoylmethyl)-1,4,7,10-

tetraazacyclododecane (11). Compound **5** (200 mg, 0.361 mmol) was suspended in 80% methanol and refluxed for 10 minutes. After this time $\text{Cd}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (152 mg, 0.361 mmol) in 6 ml of methanol was added drop-wise to the reaction mixture. After an additional 2h of reflux, the solution was cooled to room temperature and ethanol (30 ml) was added. The solution was stirred at RT overnight. The volume was reduced to half under reduced pressure and the white powder which precipitated was separated by filtration and then recrystallized from water and dried under vacuum. (Yield: 165 mg, 53%), Anal. Calc. for $[\text{C}_{28}\text{H}_{40}\text{Cl}_2\text{CdN}_8\text{O}_{12}]$: % C, 38.9; % H, 4.67; %N, 12.97 found %C, 39.01; %H, 4.62; %N, 13.04. ESI(+) MS, m/z: 765, $[\text{Cd}(\mathbf{5})\text{ClO}_4]^+$; ^1H -NMR (400 MHz, DMSO-d_6 , 25 °C), δ (ppm): 2.45 (br, 16H, $\text{NCH}_2\text{CH}_2\text{N}$), 3.09 (br, 8H, NCH_2CO), 3.11 (m, 4H, $\text{C}\equiv\text{CH}$), 4.02 (m, 8H, NHCH_2), 9.17 (t, $J_{\text{HH}}=5.38$ Hz, 4H, NH). ^{13}C -NMR (400 MHz, DMSO-d_6 , 25 °C), δ (ppm): 29.1 (NHCH_2), 55.3 ($\text{NCH}_2\text{CH}_2\text{N}$), 74.4 ($\text{C}\equiv\text{CH}$), 80.2 ($\text{C}\equiv\text{CH}$), 172.1 (CO). Crystals of complex **11** suitable for X-ray crystallographic analysis were obtained from re-crystallization as stated above.

Zn-1,4,7,10-tetrakis((prop-2-ynyl)carbamoylmethyl)-1,4,7,10-

tetraazacyclododecane (12). Compound **5** (0.500 g, 0.905 mmol) was suspended in 80% MeOH (50 ml), and the suspension was heated to reflux (80 °C). A solution of $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (0.337 g, 0.905 mmol) in MeOH (10 ml) was added dropwise, and the mixture was refluxed for further 2 hours. After removal of the solvents under reduced pressure, a white solid was obtained, that was suspended in distilled H_2O and heated to 50 °C for 1 hour. The suspension was cooled down to room temperature and filtered. The filtrate was dried and purified by precipitation from MeOH with Et_2O , leading to a white solid in 87% yield (0.643 g, 0.787 mmol). Elemental analysis: calculated C 41.17%, H 4.94%, N

13.72%; found C 41.28%, H 4.91%, N 13.62%. ESI(+) MS, m/z : 308 ($[\text{Zn}(\mathbf{4})]^{2+}$), 615 ($[\text{Zn}(\mathbf{4-H})]^+$; calculated 615.2386, found 615.2397), 715 ($[\text{Zn}(\mathbf{4})\text{ClO}_4]^+$). IR (KBr disk), cm^{-1} : 3411, 3270, 3079, 2930, 2123, 2025, 1631, 1560, 1463, 1431, 1384, 1365, 1311, 1247, 1145, 1117, 1086, 998, 973, 926. ^1H NMR (400 MHz, 25 °C, D_2O), δ (ppm): 4.00 (d, $^3J_{\text{HH}} = 2.4$ Hz, 8H, NHCH_2), 3.43 (br s, 4H, NCH_2CO), 2.95 (br m, 8H, $\text{NCH}_2\text{CH}_2\text{N}$), 2.70 (br m, 8H, $\text{NCH}_2\text{CH}_2\text{N}$), 2.56 (t, $^3J_{\text{HH}} = 2.4$ Hz, 4H, $\text{C}\equiv\text{CH}$). ^{13}C NMR (100 MHz, 25 °C, D_2O), δ (ppm): 171.7 ($\text{C}=\text{O}$), 79.2 ($\text{C}\equiv\text{CH}$), 72.2 ($\text{C}\equiv\text{CH}$), 55.6 (NCH_2CO), 50.3 ($\text{NCH}_2\text{CH}_2\text{N}$), 29.0 (NHCH_2). Crystals of complex **12** suitable for X-ray crystallography were obtained by slow diffusion of acetone into an aqueous solution of the compound.

X-ray crystallography. *Crystal data for 5:* $\text{C}_{28}\text{H}_{40}\text{N}_8\text{O}_4$, $M = 552.68$, monoclinic, $P2_1/c$ (no. 14), $a = 9.3718(2)$, $b = 8.1181(2)$, $c = 19.6489(6)$ Å, $\beta = 101.157(3)^\circ$, $V = 1466.66(7)$ Å³, $Z = 2$ [C_i symmetry], $D_c = 1.251$ g cm⁻³, $\mu(\text{Mo-K}\alpha) = 0.086$ mm⁻¹, $T = 173$ K, colourless blocks, Oxford Diffraction Xcalibur 3 diffractometer; 4946 independent measured reflections ($R_{\text{int}} = 0.0479$), F^2 refinement, $R_1(\text{obs}) = 0.0400$, $wR_2(\text{all}) = 0.0927$, 2906 independent observed absorption-corrected reflections [$|F_o| > 4\sigma(|F_o|)$, $2\theta_{\text{max}} = 65^\circ$], 189 parameters. CCDC 756369.

Crystal data for 8: $[\text{C}_{28}\text{H}_{42}\text{GdN}_8\text{O}_5](\text{Cl})_3 \cdot 4\text{H}_2\text{O} \cdot \text{C}_3\text{H}_6\text{O}$, $M = 964.44$, triclinic, $P\bar{1}$ (no. 2), $a = 11.1042(3)$, $b = 13.8935(4)$, $c = 15.2301(4)$ Å, $\alpha = 111.233(2)$, $\beta = 102.814(2)$, $\gamma = 100.096(2)^\circ$, $V = 2049.39(11)$ Å³, $Z = 2$, $D_c = 1.563$ g cm⁻³, $\mu(\text{Cu-K}\alpha) = 12.781$ mm⁻¹, $T = 173$ K, colourless plates, Oxford Diffraction Xcalibur PX Ultra diffractometer; 7848 independent measured reflections ($R_{\text{int}} = 0.0476$), F^2 refinement, $R_1(\text{obs}) = 0.0473$, $wR_2(\text{all}) = 0.1341$, 6887 independent observed absorption-corrected reflections [$|F_o| > 4\sigma(|F_o|)$, $2\theta_{\text{max}} = 143^\circ$], 480 parameters. CCDC 756370.

Crystal data for 10: $[\text{C}_{28}\text{H}_{40}\text{CdN}_8\text{O}_4](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$, $M = 882.00$, orthorhombic, $Pbcn$ (no. 60), $a = 20.47188(8)$, $b = 8.61724(3)$, $c = 20.27469(8)$ Å, $V = 3576.68(2)$ Å³, $Z = 4$ [C_2 symmetry], $D_c = 1.638$ g cm⁻³, $\mu(\text{Cu-K}\alpha) = 6.918$ mm⁻¹, $T = 173$ K, colourless blocks, Oxford Diffraction Xcalibur PX Ultra diffractometer; 3476 independent measured reflections ($R_{\text{int}} = 0.0283$), F^2 refinement, $R_1(\text{obs}) = 0.0251$, $wR_2(\text{all}) = 0.0745$, 3263 independent observed absorption-corrected reflections [$|F_o| > 4\sigma(|F_o|)$, $2\theta_{\text{max}} = 143^\circ$], 245 parameters. CCDC 756371.

Crystal data for 11: $[\text{C}_{28}\text{H}_{40}\text{N}_8\text{O}_4\text{Zn}](\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$, $M = 834.97$, orthorhombic, $Pbcn$ (no. 60), $a = 21.0162(3)$, $b = 9.01749(12)$, $c = 18.5277(2)$ Å, $V = 3511.25(8)$ Å³, $Z = 4$ [C_2 symmetry], $D_c = 1.579$ g cm⁻³, $\mu(\text{Mo-K}\alpha) = 0.928$ mm⁻¹, $T = 173$ K, colourless blocks, Oxford Diffraction Xcalibur 3 diffractometer; 5990 independent measured reflections ($R_{\text{int}} = 0.0472$), F^2 refinement, $R_1(\text{obs}) = 0.0466$, $wR_2(\text{all}) = 0.1313$, 3840 independent observed absorption-corrected reflections [$|F_o| > 4\sigma(|F_o|)$, $2\theta_{\text{max}} = 65^\circ$], 317 parameters. CCDC 756372.

Magnetic resonance studies. Relaxation rate measurements were carried out using a 2.35 T horizontal magnet with a 120 mm bore (Oxford Instruments Eynsham, UK), interfaced to a Surrey Medical Imaging Systems (SMIS, UK) console. The samples were also scanned at a higher magnetic field using a 9.4 T horizontal 20 cm bore Varian VNMRS system. Reference samples for MRI scanning were made from commercially available DOTAREM[®] (Gadoterate meglumine) injection by Guerbet. At 2.35T, T_1 was measured at 25 °C using a spin-echo pulse sequence with an echo-time TE of 28 ms and an array of repetition-times TR of 100, 110, 130, 150, 180, 200, 250, 300, 400, 500, 600, 700, 800, 1000, 1200, 1500, 1800 and 2500 ms, FOV 100x40 mm, slice thickness 2 mm, resolution 128x64 voxels, 1 averages

(1 scan/voxel). 300 μ l solutions of each contrast agent in a 0.1-2.5 mM concentration range in double distilled water were prepared and scanned. T_2 was measured at 21 °C using a saturation recovery sequence with a fixed TR of 1000 ms and an array of TE of 28, 30, 40, 50, 60, 80 and 100 ms, FOV 30x30 mm, slice thickness 2 mm, resolution 128x64 voxels, 1 averages (1 scan/voxel). Data Analysis was performed using a Matlab programme (J. Wells, ICH, UCL) to calculate T_1 and T_2 values by selecting a ROI on the MR image and analysing the mean pixel intensity values within that ROI at different TR s and fixed TE (for T_1) and at different TE s and fixed TR (for T_2).

At 9.4T, T_1 was calculated using an inversion recovery sequence with the following parameters: $TE = 30$ ms, $TR = 17500$ ms, IR array = 15; 30; 60; 100; 150; 200; 300; 450; 600; 900; 1500; 3000; 6000; 17000 ms, FOV 100x40 mm, slice thickness 2 mm, resolution 64x64 voxels, 1 averages (1 scan/voxel). Acquisition time = 4 h 20 min. T_2 weighted images were acquired using a spin-echo sequence with fixed $TR = 2000$ ms, TE array = 20; 30; 40; 50; 60; 90; 120; 180; 250 ms, FOV 100x40 mm, slice thickness 2 mm, resolution 64x64 voxels, 1 averages (1 scan/voxel). Acquisition time = 20 min. Data Analysis was performed on ImageJ by processing the T_1 and T_2 maps obtained by the 9.4 T software. T_1 and T_2 values were calculated from the intensity of the pixels in the ROIs we selected on the T_1 and T_2 maps.

CEST NMR experiment. CEST measurements were carried out by following a previously reported procedure.⁴⁹ The sample for CEST study was prepared by dissolving the appropriate amount of complex **9** in H₂O:D₂O 9:1 to yield a 20 mM solution. NMR spectra were recorded on a Bruker Avance NMR spectrometer equipped with a narrow-bore 9.4 T superconducting magnet. 1D ¹H NMR measurements were carried out without water suppression. Complex data points (128 k) were acquired with a dwell time of 12.4 μ s. Prior to Fourier transform, the data were apodized with an exponential filter (line broadening = 5 Hz).

All spectra were calibrated relative to the H₂O frequency. CEST spectra were recorded using standard continuous-wave irradiation (2 s pulse duration; 22.1 μ T pulse amplitude). This was done to selectively presaturate the exchangeable-proton resonances. 160 individual 1D ¹H NMR spectra were acquired at different values of the pre-saturation offset frequency (500 Hz intervals from 40 kHz to -40 kHz). The data were stored in a single 2D NMR data set and reconstructed as CEST spectrum by integrating the water signal of each individual spectrum in the 2D data set and plotting it as a function of the pre-saturation offset frequency.

Incubation for cell viability tests. ST14A cells were cultured on adherent flasks (TPP) in DMEM (Dulbecco's modified Eagle medium, Invitrogen) with 10% foetal calf serum (Gibco) and 1% Penicillin-Streptomycin. Cells were incubated at 33°C with 5% CO₂ and 100% humidity. Cells were passaged every 48 hours, using 1X Trypsin-EDTA to detach cells from adherent flasks, collected and centrifuged in 10ml culture medium, replated 1:3 in fresh culture medium, and returned to the incubator. The ST14A cell line was established by immortalisation of foetal striatal cells from developmental stage E14 through retroviral transduction of the SV40 Large T Antigen oncogene.⁴⁵ The cells proliferate at 33°C, and proliferation is inactivated at 39°C. Cells were incubated in solutions containing different concentrations of the corresponding complex (0-10mM) for 24 hours, then contrast agents were removed and cells washed in EBSS prior to cell viability testing.

Alamar Blue assay.⁴⁶ Alamar Blue is a non-toxic oxidation-reduction (redox) indicator dye for the quantification cell metabolism and growth. Reduction of NADH occurs during cell metabolism and growth, and the Alamar Blue dye changes colour in a reduced

environment. After washing with EBSS (Earl's balanced salt solution, Invitrogen), cells were seeded into the wells of a 96-well plate (100 µl/well) with culture medium containing 10% w/v Alamar Blue, 10% w/v FCS and 1% penicillin-streptomycin in DMEM. Cells were plated at a density of 10^4 cells per well, and 8 wells per treatment group. Incubation was carried out at standard conditions of 5% CO₂ in air, 33°C and 100% humidity. As negative control, wells with Alamar Blue solution were also prepared with no cells and with cells previously incubated without a contrast agent. After 24 hours, 3 absorbance values for each dish were measured at 570 nm with an ELISA plate reader (Revelation).

Methylene Blue assay.⁴⁷ Cells were washed with EBSS and fixed onto a 96-well plate. 100 µl of 1% w/v Methylene Blue in 0.01 M borate buffer (pH 8.4) was added to each well. After 30 minutes the plates were washed with borate buffer and dried at room temperature. The dye was solubilised adding 100 µl of 0.1 M HCl to each well, and the absorbance at 650 nm was measured for each well with an ELISA plate reader.

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Text and Figure for Table of Contents

Three gadolinium(III) complexes with tetraazamacrocyclic ligands containing four amide substituents that feature groups suitable for polymerisation have been synthesised. The relaxation enhancement properties and cytotoxicity of two of these complexes have been determined. The X-ray crystal structures of one of the ligands and its corresponding gadolinium(III), zinc(II) and cadmium(II) complexes have been determined (the one with gadolinium(III) is pictured below).