Polymerizable Gd(III) building blocks for the synthesis of high relaxivity macromolecular MRI contrast agents†

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A new synthetic strategy for the preparation of macromolecular MRI contrast agents (CAs) is reported. Four gadolinium(III) complexes bearing either one or two polymerizable methacrylamide groups were synthesized, serving as monomers or crosslinkers for the preparation of water-soluble, polymeric CAs using Reversible Addition–Fragmentation Chain Transfer (RAFT) polymerization. Using this approach, macromolecular CAs were synthesized with different architectures, including linear, hyperbranched polymers and gels. The relaxivities of the polymeric CAs were determined by NMR relaxometry, revealing an up to 5-fold increase in relaxivity (60 MHz, 310 K) for the linear polymers compared with the clinically used CA, Gd-DOTA. Moreover, hyperbranched polymers obtained from Gd(III) crosslinkers, displayed even higher relaxivities up to 22.8 mM⁻¹ s⁻¹, approximately 8 times higher than that of Gd-DOTA (60 MHz, 310 K). A detailed NMRD study revealed that the enhanced relaxivities of the hyperbranched polymers were obtained by limiting the local motion of the crosslinked Gd(III) chelate. The versatility of RAFT polymerization of Gd(III) monomers and crosslinkers opens the doors to more advanced polymeric CAs capable of multimodal, bioresponsive or targeting properties.

Introduction

Magnetic Resonance Imaging (MRI) provides 2- and 3-dimensional anatomical information on tissues and organs in a non-invasive manner. MRI displays submillimeter spatial resolution, unlimited penetration depth, and excellent soft tissue contrast imaging, enhancing the diagnostic potential for neurological, cardiovascular and oncological imaging.1 However, MRI suffers from intrinsic low sensitivity, and image contrast can be enhanced by using contrast agents (CAs) to increase the relaxation rate of water protons. Most commercial CAs are based on discrete, low molecular weight gadolinium(III) complexes, such as Gd-DOTA and Gd-DTPA.2, 3 The ability of discrete Gd(III) complexes to enhance image contrast is measured by their relaxivity ($r_1$), which is determined by the number of water molecules coordinated to the metal (q), the water exchange lifetime ($\tau_M$) and the rotational correlation time ($\tau_R$). The majority of commercial CAs display relaxivities around 4–5 mM⁻¹ s⁻¹ (20 MHz, 298 K), far from the theoretical maximum value ($r_1 = 100$ mM⁻¹ s⁻¹) for complexes where q = 1).4

An exciting prospect in MRI CA design is the development of macromolecular systems that possess significantly higher relaxivities. Numerous strategies have been pursued,5–8 including the conjugation of one or several Gd(III) complexes to polymers,9–12 dendrimers,13–18 micelles19–23 or nanoparticles,24–31 or via the non-covalent association with biomolecules (e.g. serum albumin protein).32,33 or nano-assembled capsules.34,35 Some macromolecular systems have been shown to possess significantly higher relaxivities compared with commercial CAs (up to 40 mM⁻¹ s⁻¹), attributed predominantly to the slower tumbling of the macromolecules, and the incorporation of several Gd(III) complexes within a single system.4,6 Despite these advances, the challenge still remains to develop high molecular weight CAs wherein the global motion of the macromolecule is effectively coupled to the motion of the paramagnetic centre.34,36 One way to approach this challenge involves positioning a single Gd(III) chelate at the barycentre of the macromolecule (e.g. dendritic systems).17,37 The rotational correlation time of these high molecular weight Gd(III) chelates is thus defined by the motion of the macromolecule. Polymeric CAs have been developed which incorporate multiple Ln(III) chelates, linked via a single flexible arm to the polymeric scaffold. However, the use of flexible linkers permits local motion of
the paramagnetic centre, limiting the degree of coupling with the more slowly tumbling polymer.\textsuperscript{9-12,28}

A further challenge involves the development of new methods to control the macromolecular size, structure and shape, as this could lead to well-defined second spheres of hydration, whilst allowing fast-inner sphere water exchange. The majority of polymeric CAs are prepared by classical conjugation of appropriate ligands to a polymer bearing reactive pendant groups (e.g. maleimide group, ester activated monomers). Typically, a protected ligand is covalently attached to a synthesized polymer and the resulting conjugated ligand is deprotected, followed by complexation with Gd(III).\textsuperscript{9,10,39} In related work, Sherry and co-workers have presented a strategy for polymeric PARACEST agents in which non-metal containing complexes (\textit{cally stable, monomeric Gd(III) complexes within well-de

results of lanthanide(III) complexation. Furthermore, the removal of residual lanthanide(III) ions can be significantly improved by the addition of potassium iodide to the reaction mixture (K$_2$CO$_3$/acetonitrile), allowing iodide/bromide exchange.\textsuperscript{44} It is possible that partial racemisation of the alkylation agent 1 or racemisation during the alkylation reaction occurred,\textsuperscript{46} leading to the formation of the protected ligands as a mixture of stereoisomers. The methacrylamide arms were introduced by Cbz deprotection of the ornithine sidechains to give the bis-amine (e.g. macrocycle 4), followed by coupling with N-hydroxysuccinimide methacrylate ester 5. Next, the \textit{tert}-butyl esters were deprotected using trifluoroacetic acid, followed by the addition of GdCl$_3$ in water at pH 7, to afford the water soluble Gd(III) complexes Gd-L$_{1-3}$ after purification by preparative reverse-phase HPLC (Fig. S1–S3\textsuperscript{†}). The Gd(III) complexes of a given isomer of ligand L$_{1-3}$ will have further elements of chirality arising from the sign and torsion angles of the cyclen NCCN chelate rings, and the NCCO chelates defining the helicity of the pendant arms.\textsuperscript{39,48} As such, the Gd(III) complexes will exist as a mixture of stereoisomers in solution, which may interconvert by either cyclen ring inversion or arm rotation. The separation of stereoisomers was not attempted in this work.

The synthesis of Gd-L$_4$ involved initial bis-alkylation of \textit{cis}-DO$_3$A(O’Bu)$_2$ (8) with \textit{z}-bromoester 7, prepared from l-glutamic acid (Scheme S2\textsuperscript{†}) to give protected ligand 9. Again, it is possible that partial racemisation of \textit{z}-bromoester 7 occurred, or racemisation during the alkylation reaction, resulting in a mixture of stereoisomers of protected ligand 9. Subsequent deprotection

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Fig. 1 Structures of complexes Gd-L$_{1-4}$ developed in this study.
of the tert-butyl esters of 9 using trifluoroacetic acid, followed by
the addition of slight excess of GdCl₃ in water at pH 7, gave the
precursor Gd(III) complex 10. Finally, the methacrylamide
groups were introduced via coupling the terminal carboxylic
acids of 10 to N-(3-aminopropyl)-methacrylamide, using the
coupling reagent TNTU (2-(5-norborene-2,3-dicarboximido)-
1,1,3,3-tetramethyl-uronium tetrafluoroborate), to give the
water soluble complex Gd·L₄ after purification by reverse-phase
HPLC. Analysis of the purified complexes Gd·L₁₋₄ by analytical
reverse-phase HPLC revealed a single peak in each case, and
high-resolution mass spectral data confirmed formation of the
desired complexes (Fig. 2 and S1–S4†). A major signal corre-
sponding to the negatively charged molecular ion, [M]⁻, was
observed in each case, and the isotopic distribution was in
excellent agreement with the theoretical data.

Polymerization of monomeric and crosslinker complexes
Gd·L₁₋₄

RAFT polymerization was utilized to generate copolymers
incorporating the monomeric and crosslinker complexes
Gd·L₁₋₄. RAFT enables access to reproducible polymers with
low dispersity and control over the chain length, molecular
weight and polymer architecture.⁴⁶,⁴⁷ Additionally, RAFT can be
used in a wide range of conditions, including different
temperature, solvents, co-solvents, various additives, and with
different monomers (e.g. acrylates, methacrylates, acrylamides,
styrenes, vinyl esters and vinyl amides).⁴⁶–⁴⁹ Importantly, it has
been shown that RAFT is suitable for the polymerization of
charged monomers,⁵⁰ hence we postulated that direct poly-
merization of the Gd(III) complexes would be possible. Before
attempting polymerization with complexes Gd·L₁₋₄, we verified
that RAFT polymerization of N-acryloylmorpholine (NAM) was
possible in the presence of the negatively charged Gd(III)
complex, Gd-DOTA. NAM was chosen as the monomer because
P(NAM) displays several desirable properties for MRI applica-
tions, including good bio-compatibility, very low toxicity,
remarkable stealth properties, and prolonged blood residence
time.⁵¹,⁵² Pleasingly, RAFT polymerization of NAM in the pres-
ence of Gd-DOTA was well controlled, with the homopolymer
displaying low dispersity and close to target molecular weight
(Table 1, entry 1).

Next, the synthesis of linear P[NAM·r-Gd·L₁] copolymers was
investigated using different molar proportions of Gd·L₁,
ranging from 1 to nearly 17 mol%. The polymerizations were
conducted in a mixture of DMSO/water (80:20) at 80 °C
(Scheme 2), and the polymers were purified by dialysis through
a semi-permeable membrane against distilled water (15 MΩ cm⁻¹). Successful synthesis of the target linear P[NAM·r-
Gd·L₁] copolymers was confirmed by SEC analysis (Fig. 3 and
S7†): polymers with number average molecular weights (Mₙ)
between 7600 and 14 200 g mol⁻¹ were formed with low
discrepancy ($D$) values, ranging from 1.08 to 1.25 (Table 1, entries 2–8). Due to the charged nature of the Gd(III) complex and the difference between the PNAM and the standard used (PS), the polymers displayed lower molecular weights than the theoretical values. Standard $^{1}$H NMR analysis to confirm $M_n$ for the polymers was not possible due to severe line broadening imposed by the paramagnetic Gd(III) ion; however, polymerization of NAM in the presence of free Gd-DOTA resulted in polymeric MRI CAs using NAM and Gd-L1.4. For each sample and ICP-MS analysis of the copolymers a theoretical number of NAM and Gd-L1 units per chain depending on the initial polymerization reaction composition.

In the highest case, eight Gd-L1 units were incorporated per polymer chain (Tables 1, S2 and S3). As expected, the number of Gd-L1 units incorporated into the polymer increased with increasing molar ratio of Gd-L1/NAM monomers used for the polymerization. In the highest case, eight Gd-L1 units were incorporated per polymer chain (Table 1, entry 8).

Having demonstrated that linear copolymers can be prepared in a controlled manner using the monomers Gd-L1 and NAM, we turned our attention to the crosslinker complexes Gd-L2–4. In order to prevent gelation from occurring and based on some of our previous work and that of Flynn et al.,26,27 the crosslinked polymers were obtained by fixing the concentration ratio of the crosslinker [Gd-L2–4] relative to the chain transfer agent (CTA), such that [Gd-L2–4][CTA] = 0.9. Initially, a range of water soluble hyperbranched polymers were synthesized by RAFT polymerization of NAM and the crosslinker Gd-L1 at different initial concentrations (1–2 M) in DMSO, using poly(ethylene glycol) methyl ether 2-(dodecylthiocarbamo-thioylthio)-2-methyl-propionate as the CTA (Table 2, entries 1–4). For each reaction, SEC analysis of the molecular weight distribution indicated the formation of hyperbranched polymers, evident from the high molecular weight shoulder (Fig. 3, centre). The broad molecular weight distribution was reflected in the high dispersions, $D$, ranging between 4.2 and 19.9 (Table 2), consistent with crosslinking of the growing polymer chains during RAFT polymerization. Further evidence for crosslinking of Gd-L2 was given by analysis of the BMS shift and ICP-MS

### Table 1: Monomer conversions and SEC data of linear P(NAM-r-Gd-L1) copolymers

| Entry | $N_{0,NAM}^{NAM}$ | $N_{0,CTA}^{Gd}$ | NAM$^{b}$ conv.,% | Expected $M_n^{a}$, g mol$^{-1}$ | $M_n$ SEC, g mol$^{-1}$ | $D$ | $[Gd]^{F}$, μg L$^{-1}$ | $N_{Gd}^{b}$ |
|-------|-----------------|-----------------|-------------------|--------------------------|--------------------------|---|----------------|----------------|
| 1$^{a}$ | 100 | 0 + 4 Gd-DOTA | 97.6 | 14 181 | 13 900 | 1.08 | 0 | 0 |
| 2 | 99.1 | 0.9 | 98.2 | 15 632 | 11 800 | 1.18 | 7308 | 0.37 |
| 3 | 98.1 | 1.9 | 96.3 | 15 894 | 10 900 | 1.14 | 14 680 | 1.2 |
| 4 | 97.2 | 2.8 | 97.5 | 16 619 | 10 200 | 1.12 | 16 680 | 1.37 |
| 5 | 96.2 | 3.8 | 96.2 | 16 946 | 7 600 | 1.19 | 18 260 | 1.59 |
| 6 | 95.5 | 4.5 | 98.3 | 17 863 | 9 100 | 1.23 | 19 560 | 0.53 |
| 7 | 91.4 | 8.6 | 98.1 | 20 532 | 12.10 | 1.20 | 57 840 | 2.98 |
| 8 | 83.7 | 16.7 | N/D$^{c}$ | 25 335 | 14 200 | 1.25 | 62 680 | 8.02 |

$^{a}$ RAFT polymerization in the presence of Gd-DOTA. $^{b}$ Theoretical number of NAM and Gd-L1 units per chain depending on the initial polymerization reaction composition. $^{c}$ Conversion determined by $^{1}$H NMR spectroscopy. $^{d}$ Expected $M_n = [\text{NAM}]_0 \times M_{\text{NAM}} \times \left(\frac{\text{Gd-L1}}{\text{CTA}}\right) + M_{\text{CTA}}$, with $\text{Conv}_{\text{Gd-L1}} = 1$. $^{e}$ Obtained by SEC analysis (CH$_3$Cl/triethylamine 98:2 v/v, RID detectors). $^{f}$ Obtained by SEC analysis (H$_2$O/MeOH 80/20 v/v with 0.1 M NaNO3, RID detector). $^{g}$ Gd(III) concentration determined by ICP-MS based on mass spectral signal of 157Gd isotope. $^{h}$ N$_{Gd}^{chain}$ = number of Gd(III) ions per polymer chain, estimated from ICP-MS data (ESI, Section 2, Table S2). $^{i}$ Not determined due to the high content of Gd-L1.

### Scheme 2: Synthesis of linear (DP 100 and $m$ between 1 to 17) and crosslinked (DP 100 and $m = 0.9$) polymeric MRI CAs using NAM and Gd-L1–4.
Table 2. Conditions, monomer conversions and SEC data of hyperbranched copolymers P(NAM-\(r\)-Gd \(L_2\))\(_{2-4}\), prepared by RAFT polymerization of NAM and Gd \(L_2\)\(_{2-4}\)

| Entry | Complex | Gd \(L_2\) equiv. | \([\text{NAM}]_0\) \(\text{mol L}^{-1}\) | Expected \(M_n\) \(\text{g mol}^{-1}\) | \(\%\) NAM\(^{\text{b conv.}}\) | \(M_n\) SEC\(^{\text{c conv.}}\) \(\text{g mol}^{-1}\) | \(M_w\) SEC\(^{\text{c conv.}}\) \(\text{g mol}^{-1}\) | \(Đ\) | \([\text{Gd}]^{\text{d}}\) \(\mu g L^{-1}\) | \(M_{\text{Chain}}^{\text{d}}\) |
|-------|---------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----|-----------------|-----------------|
| 1     | Gd \(L_2\) | 0.9             | 1.00            | 15 817          | 99.1            | 33 200          | 138 600         | 4.2  | 7 128           | 7.0             |
| 2     | Gd \(L_2\) | 0.9             | 1.25            | 15 817          | 99.7            | 38 900          | 378 600         | 9.7  | 4 920           | 21.9            |
| 3     | Gd \(L_2\) | 0.9             | 1.50            | 15 817          | 98.8            | 30 800          | 592 700         | 19.2 | 6 276           | 30.5            |
| 4     | Gd \(L_2\) | 0.9             | 2.00            | 15 817          | 99.6            | 37 400          | 744 700         | 19.9 | 6 008           | 33.6            |
| 5     | Gd \(L_2\) | >1.0            | 2.00            | 15 884          | >95             | Gel             | Gel             | N/D  | N/D             | N/D             |
| 6     | Gd \(L_3\) | 0.9             | 2.00            | 15 817          | 100             | 26 600          | 302 800         | 11.4 | N/D             | N/D             |
| 7     | Gd \(L_4\) | 0.9             | 2.00            | 16 945          | 99.6            | 24 800          | 119 900         | 4.8  | N/D             | N/D             |

\(^{a}\) Expected \(M_n = (\text{DP} \times n \times M_{\text{NAM}}) + (\text{DP} \times m/100 \times M_{\text{Gd(L)}}) + M_{\text{CTA}}\), with Conv\(_{\text{Gd(L)}} = 1.\(^{b}\) Determined by \(^1\)H NMR spectroscopy. \(^{c}\) Determined by SEC (DMF with 5 mM NH\(_4\)BF\(_4\), RID/UV/LS detector). \(^{d}\) Estimated number of Gd(III) ions per polymer chain, determined by ICP-MS (ESI, Table S3).

Fig. 3 SEC molecular weight (MW) distribution of selected linear and hyperbranched copolymers demonstrating: (left) low \(D\) of linear copolymers and increasing MW as the ratio of Gd \(L_1\) : NAM increases; (centre) high MW shoulder and broad distribution of MW when a crosslinker is introduced; (right) increase in high MW content as initial concentration of monomer is increased.

Analysis (Tables 2 and S3\(^{\dagger}\)), which confirmed an increasing number of crosslinked polymer chains at higher initial monomer concentration. For example, when \([\text{NAM}]_0\) was 1.00 M, the average number of crosslinker Gd \(L_1\) units was estimated to be 7.0 per hyperbranched polymer chain, whereas this increased to approximately 34 units per chain when \([\text{NAM}]_0\) was increased to 2.00 M. A higher initial monomer concentration led to higher molecular weight and dispersity, with values up to \(M_n = 744 000 \text{ g mol}^{-1}\) and \(Đ = 19.9\) when \([\text{NAM}]_0 = 2.00 \text{ M}\). However, polymerization conducted at higher initial monomer concentration, such that \([\text{Gd(L)}]/[\text{CTA}] > 1\), consistently led to the formation of gels (Table 2, entry 5). This is in accordance with the work of Perrier and co-workers, who showed that EGDMA/CTA ratios of over 1 led to gelation in a RAFT system.\(^{54}\)

The optimal conditions found for the synthesis of the hyperbranched P(NAM-\(r\)-Gd \(L_2\)) polymers were \([\text{NAM}]_0 = 2.00 \text{ M}, [\text{Gd(L)}]/[\text{CTA}] = 0.9\) (entry 4). These parameters were applied to the synthesis of hyperbranched polymers using crosslinkers Gd \(L_1\) and Gd \(L_4\), bearing cis-related polymerizable arms (entries 6 and 7). Hyperbranched polymers P(NAM-\(r\)-Gd \(L_1\)) and P(NAM-\(r\)-Gd \(L_4\)) were successfully formed. Notably, they displayed lower molecular weights and dispersities compared with those obtained using Gd \(L_2\) under similar conditions ([NAM])\(_0 = 2.00 \text{ M}, [\text{Gd}] / [\text{CTA}] = 0.9\), suggesting that these polymerizations could be achieved at higher initial monomer concentration (>2.00 M).

The average hydrodynamic diameter \(D_h\) and dispersity of representative examples of linear and hyperbranched polymeric CAs were estimated by diffraction light scattering (DLS)
measurements. From the number weighted particle size distribution, the linear polymer P[NAM-2%-Gd-L1] has a $D_h$ of 4.3 ± 0.9 nm. Crosslinked systems with Gd-L$_3$ or Gd-L$_4$ display $D_h$ of 13.2 ± 4.6 nm and 15.1 ± 4.3 nm, respectively (Fig. 4). This indicates that the polymers exist primarily as unimers in solution. The sizes of the hyperbranched polymers are comparable to previously reported hydrophilic and charged hyperbranched polymers ($D_h$ between 10 to 20 nm for MW between 100 to 500 kDa). The Gd(III) monomers are charged and therefore hydrophilic, and as expected, do not direct the assembly of these polymers into higher order structures.

$^1$H and $^{17}$O NMR relaxometric studies

The millimolar water proton longitudinal relaxation rates ($r_1 = (R_{1,obs} - R_{1,dia})/\{GD(III)-chelate\}$) of a Gd(III)-chelate, both in monomeric or polymeric forms, depends on the magnetic field strength, temperature and on several important structural and dynamic molecular parameters that describe the magnetic coupling between the water protons and the paramagnetic ion. As shown in Table 3, the $r_1$ values for the discrete complexes Gd-L$_{1-4}$ were found to be in the range 4.5–6.6 mM$^{-1}$s$^{-1}$ at 60 MHz (310 K, pH 7.4), each higher than that measured for Gd-DOTA under the same experimental conditions (2.9 mM$^{-1}$s$^{-1}$). This increase in relaxivity is consistent with the slightly higher molecular weights of Gd-L$_{1-4}$ relative to Gd-DOTA, and hence the increase in the rotational correlation time, $\tau_R$.

Upon copolymerization of Gd-L$_4$ with NAM by RAFT, the resulting linear polymers P[NAM-r-Gd-L1] possessed significantly higher relaxivities (Table 4) in the range 12.6–13.5 mM$^{-1}$s$^{-1}$ at 60 MHz, and 14.3–15.4 mM$^{-1}$s$^{-1}$ at 20 MHz (310 K, pH 7.4), up to ca. 5 times higher than Gd-DOTA. These relaxivity
values are similar to those obtained previously by Davis, Boyer and coworkers, who for both discrete core crosslinked star polymers and hyperbranched polymers, each containing Gd(III) chelates attached via a single pendant arm.

The hyperbranched polymers P(NAM-r-Gd-L2-4) obtained using crosslinkers Gd-L2-4, possessed even higher relaxivities in the range 18.6-22.8 mM⁻¹ s⁻¹ at 60 MHz, and 23.0-33.5 mM⁻¹ s⁻¹ at 20 MHz (310 K, pH 7.4) (Table 4). The enhancements in relaxivity of the polymeric CAs relative to the reference agent Gd-DOTA are shown in Fig. 6. Notably, we observe a substantial 9 to 10-fold increase in relaxivity for the crosslinked polymers based on Gd-L2 and Gd-L3, relative to Gd-DOTA at 20 MHz, and a 2-fold increase relative to the linear polymers. Such high gains in relaxivity can be ascribed to the role of Gd-L3 crosslinkers, which reduce the rate of tumbling of the Gd(III) chelate in the resulting hyperbranched polymers. This limited rotational flexibility leads to much higher relaxivity. It is also clear from Table 3 that the gains in relaxivity for the linear polymers are essentially the same at 20 and 60 MHz, whereas for the crosslinked polymers based on Gd-L2 and Gd-L4, the relaxivity gains are greater when measured at 20 MHz. In contrast, for the crosslinked polymer based on Gd-L4 the relaxivity enhancement at 20 MHz is less substantial. This can be explained by the faster local tumbling motion of the paramagnetic centre of Gd-L4, due to the longer and more flexible crosslinking arms.

Nuclear Magnetic Resonance Dispersion (NMRD) profiles, i.e. the variation of relaxivity (r1) as a function of the applied magnetic field strength, were measured at 298 and 310 K and at pH 7.4 in the proton Larmor frequency range 0.01-70 MHz (0.000234-1.64 T). Representative examples of the NMRD profiles of the monomeric complexes, and of the linear and crosslinked polymers are presented in Fig. 5. The monomeric complexes Gd-L1-4 displayed profiles typical for fast tumbling, low molecular weight complexes, each characterized by a steady decrease in their relaxivity at low magnetic field (<1 MHz), a drop in relaxivity between 1 MHz to 10 MHz, followed by a second plateau in the high magnetic field region (>10 MHz), governed by the rotational correlation time, τR. In the high field region, the relaxivity is similar for the four monomers Gd-L1-4 as expected, since their similar molecular weight, size and charge results in similar rotational dynamics. Raw data and

Table 3  Relaxivity and best fitting parameters obtained for the fitting of discrete complexes NMRD profiles at 298 K and ¹⁷O NMR data (1.75 T)  

| Parameters | Gd-L1 | Gd-L2 | Gd-L3 | Gd-L4 |
|------------|-------|-------|-------|-------|
| 298 K, 20 MHz/mM⁻¹ s⁻¹ | 6.5 ± 0.1 | 8.4 ± 0.1 | 7.6 ± 0.1 | 9.1 ± 0.1 |
| 310 K, 20 MHz/mM⁻¹ s⁻¹ | 5.1 ± 0.1 | 6.6 ± 0.1 | 5.9 ± 0.1 | 7.1 ± 0.1 |
| 298 K, 60 MHz/mM⁻¹ s⁻¹ | 5.6 ± 0.1 | 7.6 ± 0.1 | 7.1 ± 0.1 | 8.6 ± 0.1 |
| 310 K, 60 MHz/mM⁻¹ s⁻¹ | 4.5 ± 0.1 | 6.1 ± 0.1 | 5.5 ± 0.1 | 6.6 ± 0.1 |
| D²/10¹² cm² s⁻² | 9.4 ± 0.1 | 8.1 ± 0.1 | 8.2 ± 0.1 | 7.0 ± 0.1 |
| 298 K/s/ps | 32.5 ± 0.3 | 48.8 ± 0.4 | 29.6 ± 0.4 | 35.1 ± 0.4 |
| 298 K/s/pS | 106 ± 1 | 161 ± 1 | 142 ± 1 | 187 ± 1 |
| 310 K/s/ns | 154 ± 2 | 166 ± 2 | 125 ± 1 | 119 ± 3 |
| ΔH²/0/kJ mol⁻¹ | 43.8 ± 0.4 | 43.1 ± 0.4 | 43.5 ± 0.3 | 39 ± 1 |

* To fit the ¹H NMRD data at 298 K, the following parameters were fixed: γ = 1, γGd-H = 3.0 Å, αGd-H = 4 Å, γGd-H = 2.25 × 10⁻³ cm² s⁻¹. From the fitting of the ¹⁷O NMR data, with the fixed value EV = 1 kJ mol⁻¹ and ER = 20 kJ mol⁻¹.

Table 4  Relaxivity and best fitting parameters obtained for linear and hyperbranched polymer NMRD profiles at 298 K*  

| Parameters | Linear polymers P(NAM-r-X% Gd-L2) | Hyperbranched polymers P(NAM-r-0.9% Gd-L2) |
|------------|------------------------------------|------------------------------------------|
| 298 K, 20 MHz/mM⁻¹ s⁻¹ | 18.6 ± 0.2 | 17.0 ± 0.2 | 17.1 ± 0.2 | 17.6 ± 0.2 |
| 310 K, 20 MHz/mM⁻¹ s⁻¹ | 15.4 ± 0.2 | 14.3 ± 0.1 | 14.4 ± 0.2 | 15.0 ± 0.1 |
| 298 K, 60 MHz/mM⁻¹ s⁻¹ | 15.8 ± 0.2 | 14.8 ± 0.2 | 14.8 ± 0.2 | 15.2 ± 0.2 |
| 310 K, 60 MHz/mM⁻¹ s⁻¹ | 13.5 ± 0.2 | 12.6 ± 0.1 | 12.9 ± 0.1 | 13.2 ± 0.1 |
| D²/10¹² cm² s⁻² | 4.37 ± 0.06 | 5.94 ± 0.07 | 5.61 ± 0.04 | 5.31 ± 0.05 |
| 298 K/s/ps | 42.1 ± 0.6 | 38.1 ± 0.5 | 38.2 ± 0.3 | 41.8 ± 0.4 |
| 298 K/s/pS | 342 ± 6 | 369 ± 5 | 313 ± 3 | 346 ± 4 |
| 310 K/s/ns | 2680 ± 90 | 2870 ± 110 | 2420 ± 50 | 2500 ± 60 |
| S² | 0.075 | 0.118 | 0.163 | 0.159 |
| 298 K/ns | 330 ± 5 | 330 ± 5 | 330 ± 5 | 330 ± 4 |
| 304 ± 0.4 | 32.7 ± 0.5 | 32.0 ± 0.4 | 32.7 ± 0.5 | 32.0 ± 0.4 |
| 307 ± 0.4 | 33.5 ± 0.5 | 32.0 ± 0.4 | 33.5 ± 0.5 | 32.0 ± 0.4 |
| 320 ± 0.3 | 32.1 ± 0.2 | 30.9 ± 0.2 | 32.1 ± 0.2 | 30.9 ± 0.2 |
| 320 ± 0.3 | 22.8 ± 0.2 | 18.6 ± 0.2 | 22.8 ± 0.2 | 18.6 ± 0.2 |
| 320 ± 0.3 | 6.2 ± 0.2 | 6.3 ± 0.4 | 5.7 ± 0.3 |
| 320 ± 0.3 | 16.1 ± 1 | 10.4 ± 0.5 | 12.6 ± 0.3 | 16.1 ± 1 | 10.4 ± 0.5 | 12.6 ± 0.3 |
| 320 ± 0.3 | 501 ± 45 | 418 ± 99 | 447 ± 34 |
| 320 ± 0.3 | 6366 ± 486 | 6668 ± 812 | 3810 ± 256 |
| 320 ± 0.3 | 0.500 | 0.601 | 0.403 |
| 320 ± 0.3 | 326 ± 8 | 308 ± 12 | 315 ± 9 |

* To fit the ¹H NMRD data at 298 K, the following parameters were fixed: γ = 1, γGd-H = 3.0 Å, αGd-H = 4 Å, γGd-H = 2.25 × 10⁻³ cm² s⁻¹. Estimated from VT NMR relaxivity profiles (Fig. 5) at fixed magnetic field (20 MHz).
fitted NMRD profiles are reported in the ESI (Tables S5–S8 and Fig. S9†).

The NMRD profiles of Gd·L1–4 were fitted according to the established theory of paramagnetic relaxation expressed by the Solomon–Bloembergen–Morgan (SBM) and Freed’s equations for the inner- (IS) and outer-sphere (OS) proton relaxation mechanisms, respectively (see ESI, eqn (S1)–(S11)†). Certain parameters were fixed to reasonable values according to previously reported examples.55,65,66 A hydration number of q = 1 was assumed, and the distance between the Gd(III) ion and a bound water molecule proton, rGdH, was set to 3.0 Å, based on crystallographic data for Gd-DOTA. The closest approach of the bulk water molecules, rwater, was set to 4.0 Å, and the diffusion coefficient of a water proton away from the Gd(III) centre was assumed equal to Dwater = 2.25 × 10−5 cm2 s−1 at 298 K, or 3.1 × 10−5 cm2 s−1 at 310 K.

To provide better estimates of the rotational correlation times of Gd·L1–4, by fitting of the NMRD profiles, the water residence lifetimes (τm = 1/kex) were determined by 17O NMR relaxometry. Thus, the temperature dependence of the transverse relaxation rate (R2) and chemical shifts (ΔDQ) were determined at 11.75 T at neutral pH using relatively concentrated solutions of Gd·L1–4 (Fig. 5 and S10, Table S9–S12†) and the profiles were fitted according to the Swift–Connick theory for 17O relaxation.65,66 For complexes Gd·L1–4 the τm values were similar (τm = 119–166 ns) and in line with the values determined for similar Gd-DOTAGA (DOTAGA = 2-(4,7,10-tris-carboxymethyl-1,4,7,10-tetraazacyclododecan-1-yl)pentanoic acid) derivatives under the same conditions.65 However, it appears that the complexes Gd·L3 and Gd·L4, bearing cis-methacrylamide arms, exhibit slightly faster water exchange.

The NMRD profiles obtained for the linear and crosslinked polymers revealed a significant increase in relaxivity over the entire proton Larmor frequency range (Fig. 5), compared with monomeric complexes Gd·L1–4 (raw data and fitted NMRD profiles are reported in Tables S13–S19 and Fig. S11 and S12†). The profile shapes were also distinctly different, indicating successful incorporation of the monomeric complexes into higher molecular weight macromolecules. The profiles of the linear copolymers P[NAM-r-Gd·L1] displayed a broad relaxivity peak in the Larmor frequency range of 10–60 MHz, whereas in the case of the hyperbranched (crosslinked) polymers, the relaxivity peak was sharper in the 20–50 MHz region, indicating slower tumbling of the crosslinked polymers compared with the linear polymers.

To obtain more accurate fitting of the NMRD profiles of the polymeric systems, τm values were estimated by fitting of the variable temperature 1H NMR profiles at 20 MHz for the linear polymers containing between 1–17 mol% Gd·L1 theoretically, and for all hyperbranched polymers (Table S22–S24†). In fact, the relatively low concentration of Gd(III) in the polymeric systems prevented the acquisition of 17O NMR data, which typically require Gd(III) concentrations in excess of 5 mM. The water exchange rate of the coordinated water molecule of the polymerized Gd(III) complexes were determined to be two times slower than the corresponding monomeric complexes (τm values between 300–330 ns). Slower water exchange kinetics has been observed previously for other macromolecular Gd(III) systems,25,27 and in the current study this may be tentatively ascribed to weak non-covalent interactions between the Gd(III) chelate and polymer backbone. Alternatively, a reduced rate of water diffusion through the polymer could also contribute to the slower water exchange rate.16,64 For linear polymers with 5 and 9 mol% of Gd·L1, the water residence lifetime was assumed to be 330 ns, since these polymers have similar molecular weights, dimensions and relaxivities to those containing 1 and 17 mol% Gd·L1.

The NMRD profiles for the linear and crosslinked polymers were fitted based on SBM theory and modified with the Lipari–Szabo approach for the description of the rotational dynamics of Gd(III) chelates covalently linked to macromolecules (see equations in Section 5).64,65 In particular, the contributions of the fast local tumbling motion of the Gd-chelate (τRL) were separated from the slower global tumbling of the macromolecule (τRG). τRL and τRG are associated with the order parameter, S2, which describes the level of interconnectivity between the local and the global motions (i.e., if S2 = 0 the motions are independent, if S2 = 1 the motions are fully linked).

The NMRD profiles of the linear polymers were fitted over the entire range of magnetic fields investigated (0.01 to 70 MHz, Fig. 5-B2 and S11†), whereas for the hyperbranched systems, the profiles were better fitted using only the high field data, i.e. above 1 MHz (Fig. 5-B3 and S12†), as commonly performed for large macromolecular MRI CAs.

Compared with the discrete complexes Gd·L1–4, the linear polymers displayed slower local (τRL greater than 2-fold longer) and slow global (τRG greater than 20-fold longer) reorientation correlation times (Table 4). This can be ascribed to the incorporation of Gd·L1 into polymer chains via the pendant arm of the macroyclic ligand. The order parameter, S2, obtained for the linear polymers ranged between 0.12 and 0.18, which is reasonable for macromolecules containing Gd-chelates conjugated via a single flexible linker, which allows relatively fast local tumbling. The hyperbranched polymers containing Gd·L2–4 showed a large increase in relaxivity (18.6–22.8 mM−1 s−1 at 60 MHz), primarily attributed to the slower tumbling of the crosslinked Gd(III) complexes. In fact, both τRL and τRG increased relative to the linear polymers (Table 4), and the order parameter S2, was also much higher than for the linear polymers (S2 = 0.60 for P[NAM] containing 0.9% Gd·L3), consistent with a more restricted motion of the Gd(III) chelate within the crosslinked systems.

Further inspection of the relaxivity data revealed that for the crosslinked polymers prepared from Gd·L2 and Gd·L3, there is very little difference in relaxivity at 298 K and 310 K (Fig. 5-A3). This cannot be explained by differences in water exchange rate, since this parameter is very similar for the different polymers synthesised (Table 4). Rather, this can be attributed to more effective coupling of the local and global tumbling motion (S2 up to 0.60) in the crosslinked systems, hence we do not lose any relaxivity gains at higher temperature. In the case of the crosslinked polymer synthesised from Gd·L4, the local and global motion is less effectively coupled (S2 = 0.40), and consequently the relaxivity at 310 K is approximately 11% lower than at 298 K.
Comparison with previous macromolecular CAs

A comparison of the relaxivities and NMRD parameters obtained in this work with previously reported linear and crosslinked systems (acquired at the same magnetic field, temperature and pH) is given in Table 5. Our linear polymers display similar or slightly higher relaxivities (12.6–13.5 mM⁻¹ s⁻¹ at 60 MHz) and similar NMRD parameters to comparable macromolecular or nanoscale systems (11–13 mM⁻¹ s⁻¹), involving Gd(III) complexes attached via single flexible linkers.²⁵,²⁷

The hyperbranched polymers containing Gd-L₂₋₄ showed a large increase in relaxivity (18.6–22.8 mM⁻¹ s⁻¹ at 60 MHz). Interestingly, the values of the rotational correlation times and of S² are comparable to those reported for micellar aggregates obtained by self-assembly of a Gd-DOTAGA₃ complex bearing two C₁₂ aliphatic chains in cis-position,²⁵ as seen in Table 5. Also in that example, the restricted local motion of the Gd(III) complex was responsible for a strong relaxivity enhancement with respect to analogous micelles embedding a Gd(III) complex bearing only one aliphatic chain. Our hyperbranched polymers also displayed similar relaxivities and NMRD parameters to those obtained for hyperbranched dendrimers, conjugated with Gd-DOTAPBN via a single arm (τ₁ ≈ 25 mM⁻¹ s⁻¹, 298 K).⁶⁷

Comparing our hyperbranched polymers at 60 MHz and 310 K (Table 4), the system prepared from crosslinker Gd-L₁, bearing the shortest pendant arms in a cis orientation, displayed a higher relaxivity (22.8 mM⁻¹ s⁻¹), than systems prepared from the trans-oriented crosslinker Gd-L₂ (20.7 mM⁻¹ s⁻¹) or the cis-oriented crosslinker Gd-L₄ with longer arms (18.6 mM⁻¹ s⁻¹). This indicates that the combination of shorter crosslinker arms in a cis-geometry is most ideal for limiting the local motion of the Gd(III) complex within the hyperbranched macromolecules.

Of the very few reported macromolecular CAs wherein the Gd(III) complex behaves as a crosslinker,⁶⁸–⁷⁰ our system is the only example which takes advantages of the crosslinking to efficiently reduce the local rotational tumbling. This leads to higher relaxivity than those previously reported (e.g. crosslinked acrylamide nanogels⁷¹ bearing Gd-DOTA or DTPA like ligands show r₁ ≈ 9.7–17.6 mM⁻¹ s⁻¹, 60 MHz, 310 K). To the best of our knowledge, only one other crosslinked system displays a slightly higher relaxivity (24.1 mM⁻¹ s⁻¹ at 60 MHz, 310 K)⁶⁹ which we propose is due to the slower global tumbling of the nanoparticles (average diameter of 65 nm), and faster water exchange (since all the Gd(III) complexes are on the outside of the nanoparticle), despite exhibiting faster local tumbling.

Conclusions

We have developed a strategy for the efficient synthesis of high molecular weight macromolecular contrast agents, via RAFT polymerization of kinetically stable Gd(III) monomers and crosslinkers Gd-L₁₋₄, each based on a DOTA-like core bearing one or two pendant methacrylamide arms.

Copolymerization of Gd-L₁, bearing a single methacrylamide arm, with NAM led to the formation of linear polymers with higher relaxivities (12.6–13.5 mM⁻¹ s⁻¹ at 60 MHz) and slower tumbling compared with the discrete monomeric complexes

Table 5 Comparison of NMRD parameters obtained in this work with previously reported linear and crosslinked systems

| Name       | This work | Ref. 25 | Ref. 27 | This work | Ref. 25 | Ref. 67 |
|------------|-----------|---------|---------|-----------|---------|---------|
| P(NAM-Gd-L₁) | 15.8     | 13–14.5 |       | 22.1       | 27–29      | 25     |
| Gd-DOTAGA₁₂ | 13.5     | 11–11.5 | 13     | 22.8       | 23–25      |       |
| Gd-DOTAGA₁₂ | 43.7 ± 0.06 | 4.9 | 7     | 6.3 ± 0.4  | 5.2       |       |
| Gd-DOTAGA₁₂ | 342 ± 6  | 210    | 150    | 10.4 ± 0.5 | 13       |       |
| Gd-DOTAGA₁₂ | 2680 ± 90 | 2900  | 2800   | 418 ± 99   | 820      | 530    |
| Gd-DOTAGA₁₂ | 0.175    | 0.14   | 0.25   | 6668 ± 812 | 4700     | 4000   |
| Gd-DOTAGA₁₂ | 330 ± 5 | 220   | 350    | 0.601      | 0.7      | 0.36   |
| Gd-DOTAGA₁₂ | 308 ± 12 | 297   | 152    |           |          |       |

a r₁ values are given at 60 MHz. Relaxivities of literature examples were estimated from NMRD profiles. For ref. 25, a range of relaxivities is given, accounting for differences observed depending on the type of assembly formed (e.g. micelles or liposomes). b Water residence times (τₛ) were estimated from VT NMR relaxivity profiles at fixed magnetic field (20 or 40 MHz).
Gd-L₄. Moreover, hyperbranched polymers prepared via the incorporation of crosslinked Gd(III) chelates Gd-L₄ displayed significantly higher relaxivities and slower tumbling compared with the linear polymers. Analysis of the NMRD profiles revealed that the higher relaxivities of the hyperbranched polymers is due to the restricted motion of the crosslinked Gd(III) chelates, which approximately doubles the local and global reorientation correlation times relative to the linear polymers containing Gd-L₄. Crucially, the global motion of the hyperbranched polymers was more effectively coupled to the motion of the paramagnetic centre. This is apparent from an increase in the order parameter, S², relative to the linear polymers, thus showing that the rotational flexibility was significantly reduced for polymers containing crosslinkers Gd-L₂₋₄.

Hyperbranched polymers prepared from Gd-L₄ displayed the highest relaxivity (22.8 mM⁻¹ s⁻¹ at 60 MHz), suggesting that the combination of shorter polymerizable arms in a cis-orientation is optimal for limiting the local motion of the Gd(III) complex within a hyperbranched polymer. In comparison, hyperbranched polymers prepared from Gd-L₂, bearing more flexible arms in a cis-geometry, or from Gd-L₁ bearing two trans-related polymerizable arms, showed lower relaxivities (18.6–20.7 mM⁻¹ s⁻¹ at 60 MHz). The results obtained herein will guide the design of second generation Gd(III) monomers possessing three or four polymerizable arms, in order to access macromolecules with even higher relaxivities.

Our synthetic approach to macromolecular CAs combines the simplicity of a single polymerization step (with no post-polymerization modification) and scalability. The monomeric complexes Gd-L₄ serve as building blocks for the construction of more complex polymeric MRI CAs possessing responsive or theragnostic properties. Further, polymeric CAs capable of in vivo targeting may be addressed through the copolymerization of Gd-L₄ with monomers containing water-soluble sugar moieties or small peptide sequences, which mediate the second sphere of hydration. Work in this regard is ongoing in our laboratories.

**Experimental**

**Synthesis of Gd-L₂ and Gd-L₄**

**tert-Butyl**

(S)-5-(((benzlyoxy)carbonyl)amino)-2-bromopentanoate (1). A solution of sodium nitrite (2.59 g, 70.0 mmol, 1.00 equiv.) in water (5 mL) was added dropwise over two hours to a cold (−10 °C) stirred solution of (S)-2-amino-5-(((benzlyoxy)carbonyl)amino)pentanoic acid (5.00 g, 18.8 mmol, 1.00 equiv.), potassium bromide (8.27 g, 69.6 mmol, 3.70 equiv.) bromohydric acid (9.40 mL, 47.0 mmol, 2.50 equiv., 48% w/w in water) in water (100 mL). The resulting yellow solution was stirred at room temperature overnight. The reaction mixture was then extracted with diethyl ether (3 × 100 mL) and the combined organic phases were washed with brine (100 mL), dried over MgSO₄, filtered and concentrated under reduced pressure to give a viscous yellow liquid. The crude product was dissolved in tert-butyl acetate (60.0 mL) and an aqueous solution of HClO₄ (70% w/w in H₂O, 70 μL, 0.81 mmol, 0.05 equiv.) was added and the reaction mixture was stirred at room temperature for 18 hours. Diethyl ether (100 mL) and water (50 mL) were added to the reaction mixture. The organic phase was isolated and washed with a saturated aq. solution of Na₂CO₃ until neutral pH is obtained. The organic layer was then dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, Pet. ether/EtOAc, 2 : 1 v/v) to give compound 1 as a colourless oil (3.32 g, 8.60 mmol, 46% over 2 steps). IR (νmax/cm⁻¹, neat): 3341, 2940, 1705, 1528, 1250, 1141, 714, 694. Rf (Pet. ether/EtOAc v/v 70 : 30): 0.42. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.49–7.28 (m, 5H, CH aromatic), 5.08 (s, 2H, CH₃Ph), 4.12 (t, 2J = 7.2 Hz, 1H, CH), 3.22 (app. q, 2J = 6.7 Hz, 2H, NHCH₂), 2.15–1.80 (m, 2H, CH₂CH₂Br), 1.75–1.50 (m, 2H, CH₂CH₂CH₂), 1.46 (s, 9H, CH₃). N–H signals are not observed. ¹³C NMR (101 MHz, CDCl₃) δ (ppm): 168.8 (CO₂Bu), 156.5 (NHCO₂Bu), 136.6 (C¹V aromatic), 128.7, 128.3 (CH aromatic), 82.7 (C(CH₃)₃), 66.9 (CH₂Ph), 47.2 (CH), 40.3 (NHCH₂), 32.1 (CH₂CH₂Br), 27.9 (CH₃ and CH₂CH₂CH₂). HRMS (ESI⁺, m/z) calculated for M = C₁₇H₂₄O₂⁺Na⁺: 308.0790, found 308.0780.

**Di-tert-butyl-2,2’-(4,10-bis((tert-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)bis(5-((benzlyoxy)carbonyl)amino)pentanoate** (3). trans-DI2OAc(OC₂Bu) (ref. 43) (200 mg, 2.00 mmol, 1.00 equiv.), K₂CO₃ (1.66 g, 12.0 mmol, 5.00 equiv.), 5-(((benzyloxy)carbonyl)-amino)pentanoic acid (5.00 g, 20.7 mmol, 1.00 equiv.), and potassium iodide (663 mg, 4.00 mmol, 2.00 equiv.) were added to a mixed solvent (diethyl ether/tert-butyl acetate (60.0 mL) and an aqueous solution of HClO₄ (70% w/w in H₂O, 70 μL, 0.81 mmol, 0.05 equiv.) was added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was then extracted with diethyl ether (3 × 100 mL) and the combined organic phases were washed with a saturated aq. solution of Na₂CO₃ until neutral pH is obtained. The organic layer was then dried over MgSO₄, filtered and concentrated under reduced pressure to give a yellow oil. The crude material was then purified by column chromatography (silica gel, pure CH₂Cl₂ to CH₂Cl₂/CH₃OH, 92 : 8 v/v, with an increment of 2%) to yield compound 3 (1.76 mg, 1.74 mmol, 87%) as a yellow solid. IR (νmax/cm⁻¹, neat): 3254, 2975, 2933, 2837, 1711, 1517, 1227, 1154, 1113. Rf (CH₂Cl₂/CH₃OH, v/v 90 : 10): 0.32. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 7.40–7.26 (m, 10H, CH aromatic), 5.06 (s, 4H, CH₂Ph), 3.42 (d, 2J = 17.1 Hz, 2H, CO₂CH₂CH₃), 3.32 (m, 2H, CH₃), 3.19 (m, 4H, NHCH₂CH₂), 3.07 (app. t, Japp = 13.3 Hz, 2H, CH₂ cyclen), 2.94 (app. t, Japp = 13.3 Hz, 2H, CH₂ cyclen), 2.76 (d, 2J = 17.1 Hz, 2H, CO₂CH₂CH₃), 2.65–2.25 (m, 8H, 4 × CH₂ cyclen), 2.20 (app. d, Japp = 13.3 Hz, 2H, CH₂ cyclen), 2.08 (app. d, Japp = 13.3 Hz, 2H, CH₂ cyclen), 1.86 (m, 2H, CH₂CH₂CH₂), 1.69 (m, 2H, CH₂CH₂Br), 1.53–1.65 (m, 4H, overlap CH₂CH₂Br + CH₂CH₂CH₂), 1.53–1.30 (m, 36H, CH₃). N–H signals are not observed. ¹³C NMR (101 MHz, CDCl₃) δ (ppm): 174.9, 172.9 (CO₂Bu), 156.6 (PhCO₂CH₂), 136.8 (C¹V aromatic), 128.5, 128.0 (CH aromatic), 82.1, 81.9 (C(CH₃)₃), 66.4 (CH₂Ph), 61.0 (CH), 56.0 (NCH₂CH₂), 52.7, 48.8, 47.3, 44.6 (CH₂ cyclen), 40.8, 40.7 (NHCH₂CH₂), 29.8 (NHCH₂CH₂), 28.3, 28.2, 27.9 (CH₃), 22.0 (CH₂CH₂). HRMS (ESI⁺, m/z) calculated for M = C₁₇H₂₄O₂⁺Na⁺: 308.0780, found 308.0780.
reaction mixture was stirred overnight at room temperature and the catalyst was removed by filtration through a pad of Celite® and rinsed with CH₂OH. The resulting solution was filtered through a syringe filter (220 nm cut-off) and the filtrate was concentrated under reduced pressure to give compound 4 (35.0 mg, 0.47 mmol, 95%) as a yellow oil. IR (ν(max)/cm⁻¹, neat): 3368, 2977, 2932, 2844, 1718, 1577, 1455, 1367, 1227, 1155. ¹H NMR (400 MHz, CDCl₃) δ (ppm): 3.41 (d, J = 17.3 Hz, 2H, CO₂CH₂N), 3.37–3.33 (m, 2H, CH₂), 3.22 (app. t, J app = 13.4 Hz, 2H, CH₂ cyclen), 2.92 (app. t, J app = 13.0 Hz, 2H, CH₂ cyclen), 2.75 (d, J = 17.3 Hz, 2H, CO₂CH₂N), 2.67 (m, 4H, CH₂–NH₂), 2.57–2.31 (m, 8H, 4 × CH₂ cyclen), 2.26 (app. d, J app = 13.6 Hz, 2H, CH₂ cyclen), 2.09 (app. d, J app = 14.0 Hz, 2H, CH₂ cyclen), 1.90–1.53 (m, 8H, CH₂CH₂CH₂), 1.50–1.35 (m, 36H, CH₃). N–H signals are not observed. ¹³C NMR (101 MHz, CDCl₃) δ (ppm): 174.8, 174.0, 173.2, 172.7 (CO₂C(CH₃)₃), 83.0, 82.3, 81.8, 81.7 (C(CH₃)₃), 61.0 (CH), 56.3, 55.9, 55.8 (NCH₂CO₂), 52.6, 48.7, 47.1, 46.9, 44.5 (CH₂ cyclen), 41.7 (NH₂ CH₂), 32.9 (CH₂CH₂CH₂), 28.1, 27.7, 27.6 (CH₃), 22.1 (CH₂CH₂). HRMS (ESI⁺, m/z) calculated for M = C₉H₁₁O₈N₆ [M + H⁺]: 743.5641, found 743.5648.

Gadolinium(III)(2,2'-tetra-azacyclododecane-1,4-diyl)bis(5-methacrylamidopentanoate) (Gd L₁)

Part A – synthesis of di-tert-butyl-2,2’-[(4,10-bis(carboxymethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)bis(5-methacrylamidopentanoate)] (Gd L₁). Aqueous HClO₄ (70% w/w, 125 µL, 1.42 mmol, 0.07 equiv.) was added to a solution of NaNO₂ (10.8 g, 157 mmol, 2.50 equiv.) in water (150 mL). The reaction mixture was stirred at room temperature over-night. A solution of NaNO₂ (10.8 g, 157 mmol, 2.50 equiv.) at room temperature. The reaction mixture was stirred at room temperature overnight. H₂SO₄ (4.00 mL) was added slowly and the resulting aqueous solution was extracted with Et₂O (3 × 25 mL). The combined organic layers were washed with brine (2 × 75 mL), dried over MgSO₄, filtered, and the supernatant was centrifuged twice, and the supernatant was filtered through a syringe filter (220 nm cut-off). The crude product was lyophilized and the resulting solid was redissolved in deionized water, centrifuged twice, and the supernatant was filtered through a syringe filter (220 nm cut-off). The crude product was purified by preparative HPLC (gradient 0–100% acetonitrile in 25 mM NH₄HCO₃ over 10 minutes) to give Gd L₁ (103 mg, 128 µmol, 24% over 3 steps) as a white powder. IR (ν(max)/cm⁻¹, neat): 3286, 2980, 2866, 1592, 1389. HRMS (ESI⁺, m/z) calculated for M = C₉H₁₇C₂O₇N₆O₁₀, [M⁺]: 808.2511, found 808.2543. HPLC (gradient 0–100% acetonitrile in 25 mM NH₄HCO₃ over 10 minutes): tR = 5.591 minutes.

Di-tert-butyl(5)-2-bromopentanedioate (7)

Part B. 5-2-Bromopentanedioic acid. 5-2-Bromopentanedioic acid was synthesized according to literature. ¹H NMR (ν(max)/cm⁻¹, neat): 3286, 2980, 2866, 1592, 1389. HRMS (ESI⁺, m/z) calculated for M = C₈H₁₄O₄Br₂N₆, [M⁺]: 559.0671, found 559.0677. The crude product was used directly in the next step without further purification.

Part B. Aqueous HClO₄ (70% w/w, 125 µL, 1.42 mmol, 0.07 equiv.) was added to a solution of (S)-2-bromopentanedioic acid (4.00 g, 19.0 mmol, 1.00 equiv.) in tert-butyl acetate (130 mL). The reaction mixture was stirred at room temperature overnight. Diethyl ether (100 mL) and water (50 mL) were added to the reaction mixture, the layers were separated, and the organic phase was washed with a saturated aqueous solution of Na₂CO₃ solution to reach pH 7. The organic layer was then dried over MgSO₄, filtered and concentrated under reduced pressure. The crude (S)-2-bromopentanedioic acid (4.00 g) was used directly in the next step without further purification.

Part C. Deprotected ligand 6b (354 mg) as a white solid, which was used in the next step with no further purification. HRMS (ESI⁺, m/z) calculated for M = C₉H₁₅NO₂N₆O₁₂, [M + Na⁺]: 655.3631, found 655.3680.

Part C. Deprotected ligand 6b (354 mg, 0.54 mmol, 1.00 equiv.) and gadolinium(III)chloride hexahydrate (221 mg, 0.60 mmol, 1.10 equiv.) were stirred in deionized water (10.0 mL) for 24 hours at room temperature. Over the course of the reaction, the pH was adjusted to 7 by addition of aq. HCl or aq. NaOH. The reaction mixture was lyophilized and the resulting solid was redissolved in deionized water, centrifuged twice, and the supernatant was filtered through a syringe filter (220 nm cut-off). The crude product was purified by preparative HPLC (gradient 100% acetonitrile in 25 mM NH₄HCO₃ over 10 minutes) to give (S)-DO₂-A(OBu₂)₈ (8) (500 mg, 1.25 mmol, 1.00 equiv.) was synthesized from a modified literature procedure,34 and was added to K₂CO₃ (1.04 g, 7.49 mmol, 6.00 equiv.), arm 7 (1.13 g, 3.50 mmol, 2.80 equiv.) and potassium iodide (414 mg, 2.50 mmol, 2.00 equiv.)
in anhydrous CH₂CN (10 mL) and the reaction mixture was stirred at 80 °C overnight. Potassium salts were removed by centrifugation, and the supernatant was concentrated under reduced pressure to obtain an oil. The crude residue was purified by column chromatography (silica gel, neat CH₂Cl₂ to CH₂Cl₂/CH₃OH 90 : 10 v/v, with an increment of 2%) to give compound 7 (823 mg, 0.93 mmol, 75%) as a yellow amorphous solid. IR (νmax/cm⁻¹, neat): 3232, 2974, 2923, 2832, 1718, 1366, 1205, 1128. HRMS (ESI⁺) calculated for M⁰ [M⁺] = 549.2402, found 549.2402.

**Synthesis of linear and hyperbranched polymeric CAs**

Representative example of the synthesis of linear P(NAM-r-Gd-L₁) with a DP = 100 and molar ratio NAM : Gd : L₁ : CTA equal to 95 : 5 : 1. 4-Acryloylmorpholine (67.1 mg, 0.60 µmol, 0.55 mmol, 95.0 equiv.), Gd-1 (17.1 mg, 25.0 µmol, 5 equiv.), tetracyclen, 33.7 (CO₂H), 28.3, 28.2, 28.1, 27.9 (3), 27.8 (6) (CH₃). HRMS (ESI⁺, m/z) calculated for M = C₆H₄N₂O₁₂, [M + H⁺]: 885.6159, found 885.6158.

**Gadolinium(m)[2,2′-((7,10-bis(carboxylatometilyl)-1,4,7,10-tetraazacyclododecane-1,4-diyl)bis(5-(3-methacrylamidopropylamino)-5-oxopentanoate)] (10)**

Part A – synthesis of 2,2′-(7,10-bis(carboxylatometilyl)-1,4,7,10-tetraazacyclododecane-1,4-diyl)-diglutaric acid (9). Ligand 8 (750 mg, 0.85 mmol, 1.00 equiv.) was stirred in TFA (10.0 mL) and H₂O (1.00 mL) at room temperature for 3 days until complete destruction (LC-MS analysis). The mixture was concentrated under reduced pressure and residual TFA was removed by successive co-evaporation with CH₂Cl₂. The resulting residue was dissolved in deionised water, centrifugated and filtered through a syringe filter (cut-off 220 nm). The filtrate was lyophilised to afford a white solid. The crude product 9 was used directly in the next step without further purification. IR (νmax/cm⁻¹, neat): 3084, 2933, 2556, 1945, 1708, 1662, 1390, 1177, 1130. HRMS (ESI⁺, m/z): calculated for M = C₂₂₃H₃₆N₄O₁₂, [M + H⁺]: 549.2402, found 549.2402.

Part B. Deprotected ligand 9 (400 mg, 0.73 mmol, 1.00 equiv.) and gadolinium(m)chloride hexahydrate (454 mg, 0.88 mmol, 1.20 equiv.) were stirred in water (10.0 mL) at room temperature for 24 hours. Over the course of the reaction, the pH was adjusted to 7 by addition ofaq. HCl oraq. NaOH. The reaction mixture was lyophilised and the resulting solid was dissolved in deionised water, centrifuged twice and filtered through a syringe filter (cut-off 220 nm cut-off). The supernatant was then lyophilised to afford the Gd complex 10 as a white amorphous solid. The crude product was directly engaged in the next reaction step without further purification. IR (νmax/cm⁻¹, neat): 3400, 2980, 2838, 1684, 1600, 1205, 1128. HRMS (ESI⁺, m/z): calculated for M = C₂₂₃H₃₆Gd₆N₁₂O₁₂, [M + H⁺]: 702.1258, found 702.1216.

**Gadolinium(m) [2,2′-((7,10-bis(carboxylatometilyl)-1,4,7,10-tetraazacyclododecane-1,4-diyl)bis(5-(3-methacrylamidopropylamino)-5-oxopentanoate))] (Gd-L₁), Gd(m) complex 10** (200 mg, 0.29 mmol, 1.00 equiv.) and 2-(5-norbornene-2,3-dicarboxyimido)-1,1,3,3-tetramethylurea tetrafluoroborate or TUNT (219 mg, 0.60 mmol, 2.10 equiv.) were dissolved in DMF (5.00 mL) at room temperature. The solution was stirred for 30 minutes at 40 °C and DIPEA (199 µL, 1.14 mmol, 4.00 equiv.) was added, followed by N-(3-aminopropyl)methacrylamide hydrochloride (107 mg, 0.60 mmol, 2.10 equiv.). The reaction mixture was stirred for 1 hour at 40 °C and then at room temperature overnight. The desired Gd(m) complex was precipitated in cold diethyl ether and collected by filtration. The solid was washed with cold Et₂O and dried under vacuum. The crude residue was dissolved in water and purified by reverse phase preparative HPLC (gradient 0–100% acetonitrile in 25 mM NH₄HCO₃ over 10 minutes) to afford complex Gd-L₂ (68.2 mg, 71.8 µmol, 25% over 3 steps) as a white amorphous solid. IR (νmax/cm⁻¹, neat): 3280, 3076, 2928, 2870, 1596, 1538, 1436, 1381, 1085. HRMS (ESI⁺, m/z): calculated for M = C₅₉H₆₅Gd₆N₁₂O₁₂, [M⁺]: 950.3259, found 950.3257. Analytical HPLC (gradient 0–100% acetonitrile in 25 mM NH₄HCO₃ over 10 minutes): t_R = 5.533 minutes.
Dynamic light scattering (DLS)

DLS measurements were performed with a Malvern Zetasizer Nano ZS using Zetasizer software (version 7.12). The Zetasizer system uses a Diode-pumped solid-state laser operating at a wavelength of 473 nm and an avalanche photodiode (APD) detector. The scattered light was detected at an angle of 175°. The temperature was stabilized to ±0.1 °C of the set temperature (25 °C). All aqueous polymer solutions were filtered prior to measurement, using a nylon syringe filter with 220 nm cut-off.

Relaxometry measurements

NMRD. The observed water protons longitudinal relaxation rate constant ($R_1^\text{obs}$) values were measured as a function of the magnetic field strength in non-deuterated aqueous solutions on a Fast Field-Cycling Stelar SmarTracer relaxometer over a continuum of magnetic field strengths from 0.00024 to 0.25 T (corresponding to 0.01–10 MHz proton Larmor frequencies) at 25 and 37 °C by using the standard inversion recovery pulse sequence with 4 scans for each acquired data point. The relaxometer operates under computer control with an absolute uncertainty in $1/T_1$ of ±1%. To complete the data set, 6 ESI data† points were obtained by measurements at higher magnetic fields (precisely 20, 30, 40, 50, 60 and 70 MHz) on a Stelar relaxometer with a Spinmaster console connected to a Bruker WP-80 magnet (80 MHz/2 T) adapted to variable-field measurements. The temperature was set and controlled with a Stelar VTC-91 airflow heater and measured by a substitution technique using a copper-constantan thermocouple (error ± 0.1 °C). The exact concentration of Gd(III) was determined by measurement of bulk magnetic susceptibility shifts of a BuOH signal,73 or by inductively coupled plasma mass spectrometry. The variable temperature $^1$H NMR profiles were obtained by measuring the relaxation rate at different temperature from 5 to 75 °C (12 to 16 acquisition points) at a fixed magnetic field intensity (20 MHz or 30 MHz) using an inversion recovery method with a 90° pulse.

$^{17}$O NMR measurements. Variable-temperature $^{17}$O NMR measurements were recorded on a 500 MHz Bruker Avance III spectrometer (11.75 T) equipped with a 5 mm probe and standard temperature was regulated by air or nitrogen flow controlled by a Bruker BVT 3200 control unit. The samples were analyzed at 278 K and from 280 to 350 K with a 5 K increment (16 measurements). Concentrated aqueous solutions of complexes (10–20 mM) at physiological pH (7.4) and containing 2.0% of the $^{17}$O isotope (Cambridge Isotope) were used. The observed transverse relaxation rates ($1/T_2$) were measured from the peak width at half-height. The fitting parameters were $\Delta^2$, $\gamma_r$, the $T_M$ value at 298 K, its enthalpy of activation $\Delta H_M$, and the scalar Gd-$^{17}$O coupling constant $A/h$.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by Loughborough University PhD Studentship (TRB) and a Doctoral College Travel Fund (TRB). The authors would like to thank Prof. David Parker and Dr Kanthi Senanayake (Durham University) for access to and support with the Bruker minispec spectrometer. We also thank Dr Elena Perin (Università del Piemonte Orientale) for conducting ICP measurements, and Matthieu Miclotte and Prof. Rachel O’Reilly (University of Birmingham) for support with SEC analysis. Thanks to Jordan Roe, Dr Colum Breen and Caty Marsden for help with conducting DLS measurements.

Notes and references

† NMRD profiles for the hyperbranched systems were also fitted over the entire Larmor frequency range (0.01–100 MHz) and are provided in the ESI (Fig. S13 and Table S21). The resulting NMRD parameters are very similar to those obtained from the high field fitting, presented in Fig. 5.

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