AAZTA-based bifunctional chelating agents for the synthesis of multimeric/dendrimeric MRI contrast agents†

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Monomeric and dimeric bifunctional chelates based on the AAZTA (6-amino-6-methylperhydro-1,4-diazepinetetraacetic acid) platform bearing arylamino and isothiocyanate groups for conjugation to biomolecules were synthesised. Both systems were used for the preparation of high relaxivity dendrimeric (PAMAM G1) and multimeric octa-Gd^{III} complexes. These systems show enhanced relaxometric properties attributed to the presence of two coordinated, fast exchanging, water molecules ($q = 2$) on each metal ion and to a rather rigid and compact molecular structure.

Introduction

Complexes of transition and lanthanide(III) metal ions have found a broad range of applications in chemistry, biology and medicine such as diagnostic imaging,[1] molecular imaging,[2] tumour therapy[3] and luminescent materials.[4] Among all the chelating ligands reported so far, those based on a polyamino polycarboxylic structure are particularly useful and widely employed for their large versatility and strong coordination properties. Bifunctional chelating agents (BFCAs) are molecules containing two different moieties: a strong metal chelating unit and a suitable function able to link the probe to a given biomolecule.[5] The linkage must be stable under physiological conditions (*i.e.* hydrolytic, oxidizing, and reducing conditions in an aqueous solution near neutrality containing several anions, metal cations, small molecules, and enzymes) and possibly performed under mild conditions to avoid vector and/or probe degradation.[5] Moreover, the conjugation step should be quantitative, selective, fast and easy to perform. For example, BFCAs have been extensively used to label specific biological carriers or targeting vectors (*e.g.* peptides, proteins or antibody fragments) with a (radio)metal ion.[6,7]

Paramagnetic contrast agents (CAs) based on Gd^{III} chelates have proved essential in differentiating soft tissues in Magnetic Resonance Imaging (MRI) protocols.[1,8] However, as MRI is characterized by an intrinsically low sensitivity, the most innovative molecular or cellular imaging applications require highly efficient CAs that produce a sufficient relaxation effect by a limited mass of the agent with high local concentration. A possible strategy to obtain this effect is the delivery of a high number of imaging reporters at the site of interest by conjugating several Gd^{III} chelates to macromolecules or nanoparticles linked to a targeting vector.[2] Moreover, for high field MR scanners (1.5–4.7 T), multimeric Gd^{III} T_1 agents of medium molecular weight (~ 2–6 kDa) have been proposed as good candidates as they show excellent relaxation properties over a broad range of imaging field strengths.[9,10] Thus, the synthesis of dimeric BFCAs could be very useful both for increasing the payload of Gd^{III} chelates per unit of functional group on the biological or nano-sized carrier and for the straightforward preparation of multimeric systems for high field MRI or MR-molecular imaging applications.

Among the various polyamino polycarboxylic BFCAs employed for MRI studies, one of the most promising is represented by the heptadentate ligand AAZTA.[11] In fact, as $[GdAAZTA]^-$ shows excellent properties in terms of thermodynamic stability and relaxivity (*e.g.* two water molecules in the inner coordination sphere of Gd^{III} with a fast rate of exchange), a functionalised derivative of $[GdAAZTA]^-$ can be considered as a prototype for the development of new classes of high relaxivity multimeric agents.

We recently reported a general synthetic procedure towards a series of AAZTA bifunctional prochelators for coupling both to NH_2-containing biomolecules and to other reactive functional groups of relevance for biomedical applications.[12] Among these, the AAZTA-like chelator with a free hydroxymethyl group and the carboxylates protected as *t*-butyl esters (AAZTA-OH, Scheme 1) was used as starting material to react with succinic anhydride to form an ester or with thionyl chloride to form the alkyl chloride derivative. However, the versatility of these bifunctional chelators is limited by the formation of a cyclic 2-oxomorpholine derivative in both basic or acid conditions.

In order to prevent the intramolecular cyclization of AAZTA-OH and exploit this ligand as a basic unit for the synthesis of mono- and dimeric BFCAs, aromatic isocyanates were selected as they react with alcohols at neutral pH and room temperature forming carbamates. Using this procedure, mono- and dimeric AAZTA derivatives containing arylamino and arylisothiocyanate reacting groups were synthesised. The utility of these BFCAs was demonstrated by their attachment onto a polyamine or PAMAM G1 dendrimer to form octameric ligands. The synthesis of the octa-Gd^{III} complexes and their relaxivity is also reported.

Results and discussion

A detailed study on the thermodynamic and kinetic stabilities of AAZTA with several lanthanide ions was recently reported by Baranyai *et al.*[13] In the case of several GdDOTA and

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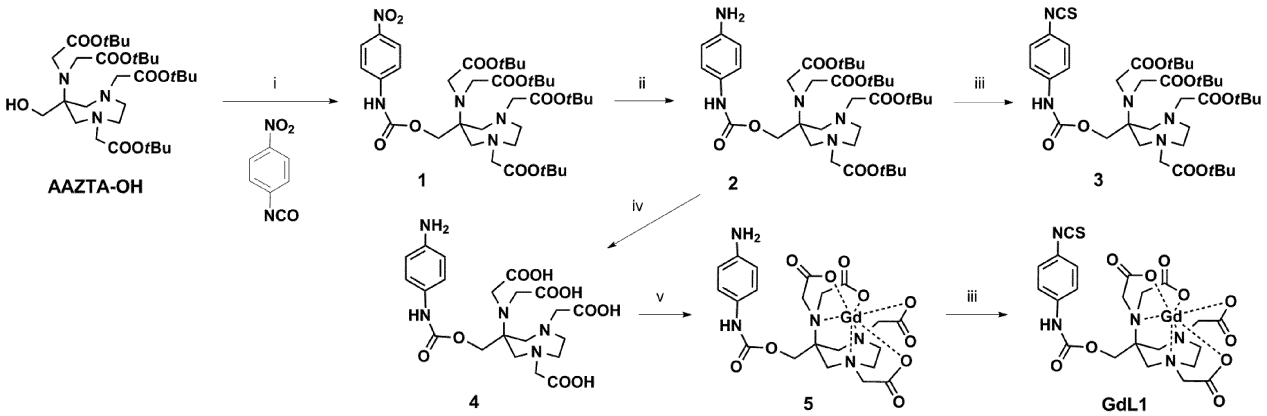
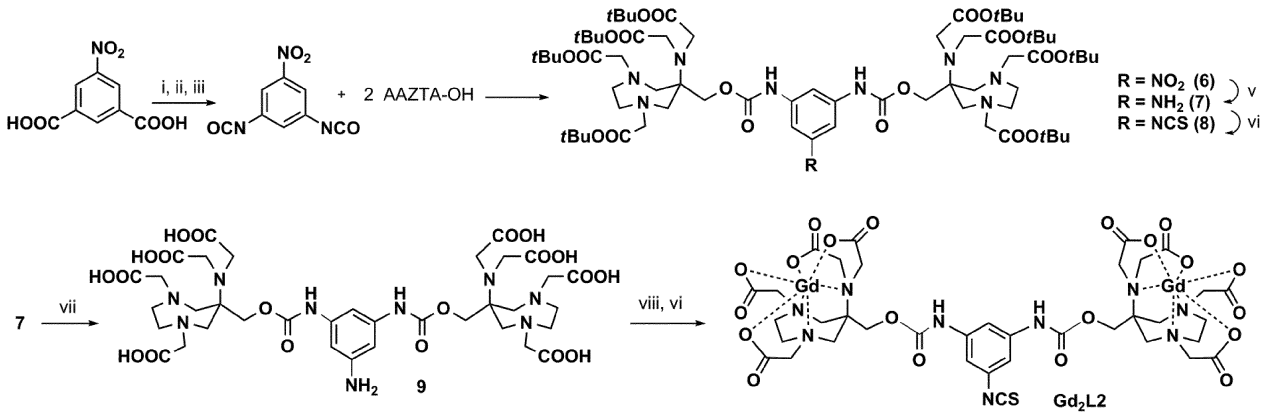


Scheme 1 Synthesis of monomeric AAZTA and GdAAZTA BFCAs. *Reagents and conditions*: i: CH_2Cl_2, rt, 2 h; ii: H_2, Pd/C 10%, MeOH, rt, 18 h; iii: $CSCl_2$, CH_2Cl_2/$KHCO_3$ sat. (1 : 1 v/v), rt, 2 h; iv: TFA/CH_2Cl_2 (1 : 1 v/v), rt, 18 h; v: $GdCl_3$, H_2O, pH = 7.

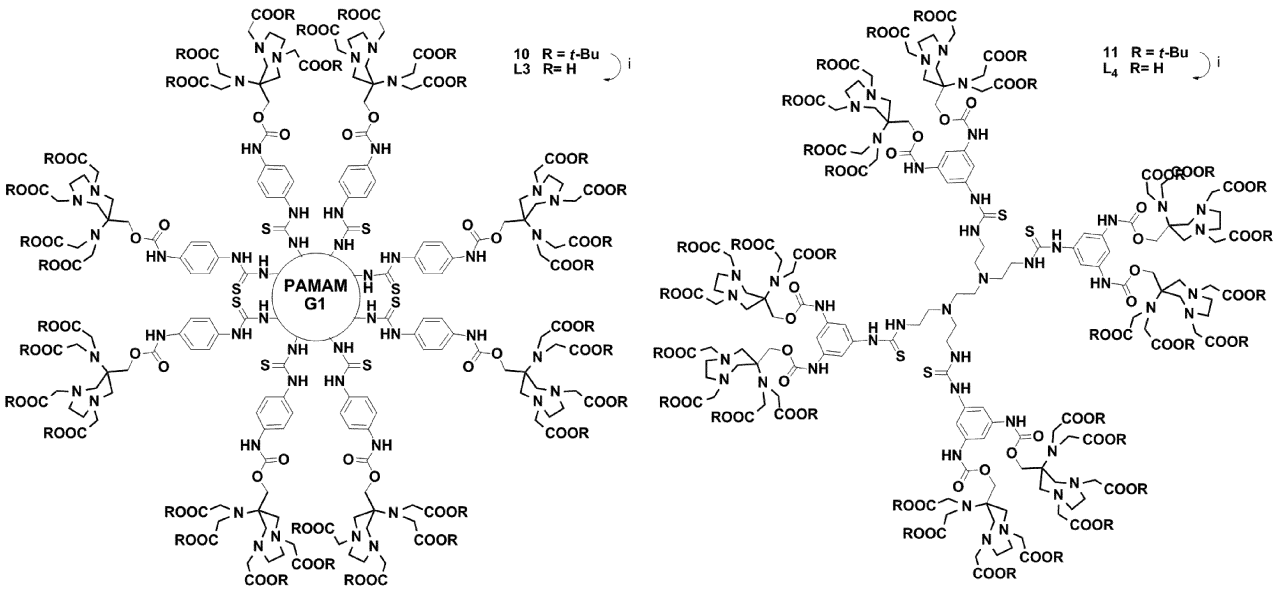


Scheme 2 Synthesis of dimeric AAZTA-based BFCAs. *Reagents and conditions*: i: $SOCl_2$, reflux, 18 h; ii: NaN_3, NaOH 1 M/CH_2Cl_2, rt, 1 h; iii: Toluene, reflux, 3 h; iv: CH_2Cl_2, rt, 2 h; v: H_2, Pd/C 10%, MeOH, rt, 18 h; vi: $CSCl_2$, CH_2Cl_2/$KHCO_3$ sat. (1 : 1 v/v), rt, 2 h; vii: TFA/CH_2Cl_2 (1 : 1 v/v), rt, 18 h; viii: $GdCl_3$, H_2O, pH = 7, to obtain Gd_2(**10**).

GdDTPA derivatives and of their mono- and bisamide analogs the stability constants of the complexes vary only marginally with the modification of the basic ligand structure. This is because the coordination cage of the paramagnetic metal ion remains unaffected. In accordance with these observations, the modifications of the ligand AAZTA reported herein occur on the exocyclic methyl group and therefore are not expected to influence the coordinating ability towards lanthanide(III) ions as also demonstrated by the relaxometric data reported for the AAZTA-OH precursor.[12]

The synthesis of the monomeric BFCAs **2**, **3** and Gd**L1** started from the reaction of AAZTA-OH with 4-nitrophenyl isocyanate as shown in Scheme 1 to obtain the 4-nitrophenyl carbamate **1** in high yield. Interestingly, carbamates are known to be stable both at the acidic pH values necessary for *tert*-butyl ester deprotection and under physiological conditions. The nitro group was then reduced to amine (**2**) by using hydrogen at ambient pressure and 10 wt% Pd/C catalyst, and finally the amine was reacted with thiophosgene to form almost quantitatively the isothiocyanate derivative (**3**). This latter can readily react with a variety of amino-containing biomolecules. It is worth noting that derivative **2** is also a BFCA that can be coupled to carboxylic acids by forming stable amide bonds. Interestingly, deprotection of the *t*-butyl esters of **2** yielded the ligand **4** which was complexed with $GdCl_3$ and subsequently reacted with thiophosgene to give Gd**L1**, a BFCA that can react with NH_2 groups also in aqueous media (Scheme 1)

Then, a functionalised AAZTA dimer was designed as a multimeric BFCA for use in the construction of higher oligomers. The synthesis was carried out employing a nitro-isophthalic acyl azide transformed into the isocyanate through Curtius rearrangement by a modification of the reported procedures.[14] In particular, nitro-isophthalic acid was converted into acyl chloride with thionyl chloride and then into acyl azide with sodium azide in a mixture of NaOH (1.0 mol L^{-1}) and CH_2Cl_2 (Scheme 2). Heating to reflux (3 h) in toluene quantitatively yielded the nitro isophthaloyl diisocyanate that was then reacted with two equivalents of AAZTA-OH to obtain the dimeric ligand (**6**) after column chromatographic purification. The nitro group was converted into amine and then to isocyanate following the analogous procedure reported to obtain **3** from **1**. As for the monomeric system, deprotection of the *t*-butyl esters of **7** yielded the ligand **9** which was complexed with $GdCl_3$ and subsequently reacted with thiophosgene to give Gd_2**L2**, a dimeric Gd-based bifunctional chelate that can react with amines also in aqueous media. It might be worth outlining that derivatives **7**, **8**, Gd_2(**10**) and Gd_2**L2** (Scheme 2) are among the first examples of dimeric bifunctional chelating agents.[15]



Scheme 3 Synthesis of octameric ligands **L3** and **L4**. *Reagents and conditions*: i: TFA/CH_2Cl_2 (1 : 1 v/v), rt, 18 h.

As an exemplification of the use of **3** and **8**, the reaction with aliphatic primary polyamines was carried out in CH_2Cl_2 in order to obtain two octameric ligands (Scheme 3). While **3** was reacted with octaamino PAMAM G1 dendrimer, for the dimeric ligand **8** the reaction was carried out with *N*,*N*′-bis-(2-aminoethyl)-ethylenediamine, prepared as described in the literature.[16] Octameric protected ligands were purified by crystallization from diethyl ether/petroleum ether. Then, the *t*-butyl esters were deprotected in a 1 : 1 mixture of CH_2Cl_2 and trifluoroacetic acid to obtain the octameric ligands **L3** and **L4**. Both ligands were also purified and desalted by passing through a sephadex G25 gel filtration column.

The Gd^{III} complexes of these octameric ligands were prepared by adding small volumes of a stock solution of $GdCl_3$ to a solution of the ligand and maintaining the pH at 6.5 with diluted NaOH. The complexation process was monitored by measuring the change in the longitudinal water proton relaxation rate (R_1) at 20 MHz as a function of the concentration of gadolinium(III).

The relaxivity, r_1, per Gd of Gd_8**L3** and Gd_8**L4** is 21.9 and 24.4 mM^{-1} s^{-1} (20 MHz, 298 K), respectively. To the best of our knowledge, Gd_8**L3** is the first example of a dendrimer conjugated to Gd-complexes possessing two water molecules (q = 2) in the first coordination sphere.[17] The higher relaxivity of Gd_8**L4**, composed of four dimeric AAZTA units, can be attributed to its more compact and rigid structure that affects the effective reorientational correlation time, τ_R. To account for this difference, mass relaxivities or densities of relaxivity have been defined as the enhancement of the relaxation rate by a unit mass (g L^{-1}) of CA.[10] High density of relaxivity is required for applications such as cell imaging in which a sufficient relaxation effect has to be produced by a limited mass of the agent. The values obtained for Gd_8**L2** and Gd_8**L3** are 24.7 and 36.5 $(g\ L^{-1})^{-1}\ s^{-1}$, respectively (20 MHz, 298 K). The highest density of relaxivity so far reported is that of "metallostar" a supramolecular system based on the self assembly of a dimeric Gd-DTTA-bipyridine complex into a Fe(III) complex (DTTA = diethylenetriaminotetraacetic acid).[10] The resulting hexameric Gd-complex has a density of relaxivity at 20 MHz and 298 K of 43.3 $(g\ L^{-1})^{-1}\ s^{-1}$. This value is more than double that of Gadomer17, an intravascular dendrimeric MRI contrast agent under development (Bayer Schering Pharma AG) for MR angiography, containing 24 Gd-DOTA-monoamide units (22.7 $(g\ L^{-1})^{-1}\ s^{-1}$).[18] Thus, our octameric Gd-complexes Gd_8**L3** and Gd_8**L4** show densities of relaxivity intermediate between these multimeric/dendrimeric systems. In addition, these systems show enhanced relaxometric properties when compared to systems of similar size/molecular weight because of the concomitant occurrence of fast rate of water exchange and high hydration number (q = 2).

Conclusions

In conclusion, the synthesis of monomeric and dimeric BFCAs offers the possibility to design and construct a series of CAs containing a large payload of highly efficient Gd^{III} chelates by a modular synthetic approach. These modules have been proved to be useful for the synthesis of multimeric/dendrimeric systems endowed with high relaxivity both per Gd and per molecule. They can also be very versatile since by varying linkers or scaffold it is possible to design multimeric systems suitable for different requirements. At the same time, these oligomeric BFCAs may be of interest for increasing the number of Gd^{III} units attached to various types of particles and thus for obtaining high relaxivity nano-sized systems. Also direct conjugation to targeting vectors would permit the synthesis of multimeric imaging probes in few steps to allow the delivery of highly efficient CAs at the target site.

Finally, it is worth noting that, as AAZTA is a highly efficient ligand for the coordination of lanthanide or other trivalent metal ions, such systems could also be useful for the complexation of other diagnostic (^{111}In, $^{67/68}Ga$) or therapeutic (^{90}Y, ^{177}Lu) radiometals for nuclear medicine applications.

Experimental

General experimental conditions

All reactants were used as supplied from commercial sources unless stated otherwise. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL ECP 400 spectrometer. Mass spectra with electrospray ionization (ES) were recorded on a SQD 3100 Mass Detector (Waters). The HPLC-MS analysis was carried out on a Waters system equipped with Waters 1525 binary HPLC Pump and Waters 2489 UV/vis and Waters SQD 3100 detectors. The water proton longitudinal relaxation rates of aqueous solutions of Gd_8**L3** and Gd_8**L4** were measured by using a Stelar Spinmaster spectrometer (Mede, Italy) operating at 0.5 T and 298 K. Infrared (IR) spectra were recorded in the range 4000–400 cm^{-1} using a Bruker Equinox 55 spectrometer. Elemental analysis were performed on a EuroVector EA 3000 instrument. AAZTA-OH [1,4-bis(*t*-butoxycarbonylmethyl)-6-[bis(*t*-butoxycarbonylmethyl)]amino-6-hydroxymethylperhydro-1,4-diazepine] was synthesised as described previously.[12]

1. A solution of AAZTA-OH (600 mg; 1.00 mmol) in dry CH_2Cl_2 (5 mL) was added under N_2 to a solution of *p*-nitrophenylisocyanate (165 mg; 1.00 mmol) in dry CH_2Cl_2 (5 mL). The reaction mixture was stirred for 2 h at rt and then evaporated *in vacuo*. The crude product was purified on silica gel chromatography column (petroleum ether/ethyl acetate 8/2) yielding **1** as pale yellow solid (734 mg; 96%). TLC (silica gel 60 F_{254}, petroleum ether/EtOAc 8 : 2, detection: UV 254 nm): R_f 0.45. ^{1}H-NMR ($CDCl_3$) 400 MHz δ = 8.53 (bs, 1H, N*H*), 8.12 (d, 2H, J = 9.2 Hz, C*H*), 7.57 (m, 2H, C*H*), 4.21 (s, 2H, CH_2OCO), 3.65 (s, 4H, CH_2CO), 3.22 (s, 4H, CH_2CO), 3.05 (d, 2H, J = 14.3 Hz, CH_2C), 2.76–2.65 (m, 6H, CH_{2cyclo}), 1.40 (s, 18H, CH_3), 1.39 (s, 18H, CH_3).^{13}C-NMR ($CDCl_3$) 100 MHz δ = 172.8, 170.7 (*C*O), 153.1 (NH*C*OO), 144.7, 142.7 (*C*), 125.2, 117.7 (*C*H), 81.0, 80.9 (CCH_3), 68.0 (CH_2OCO), 63.3 (*C*), 62.0, 60.4 (CH_2CO), 59.1, 51.8 (CH_{2cyclo}), 28.2, 28.1 (CH_3). ESI-MS (*m*/*z*): 766.55 (M + H^+); calc for $C_{37}H_{60}N_5O_{12}$: 766.42. IR spectrum (KBr disk): 3323, 2979, 2932, 1745, 1513, 1383, 1223, 1167 cm^{-1}.

2. A suspension of 10% Pd/C (70 mg) in water (1 mL) was added to a solution of **1** (730 mg; 0.95 mmol) in MeOH (10 mL). The mixture, under H_2 atmosphere, was stirred overnight and then filtered over celite. The filtrate was evaporated *in vacuo* affording pure **2** (683 mg; 93%). TLC (silica gel 60 F_{254}, petroleum ether/EtOAc 8 : 2, detection: UV 254 nm): R_f 0.13. ^{1}H-NMR ($CDCl_3$) 400 MHz δ = 7.22 (s, 2H, J = 5.9 Hz, C*H*), 6.64 (d, 2H, J = 5.9 Hz, C*H*), 4.16 (s, 2H, CH_2OCO), 3.71 (s, 4H, CH_2CO), 3.40 (bs), 2.88 (bs) (12H, CH_2CO + CH_{2cyclo}), 1.43 (s, 36H, CH_3).^{13}C-NMR ($CDCl_3$) 100 MHz δ = 172.7, 170.6 (bs) (*C*O), 153.6 (NH*C*OO), 140.0, 130.1 (*C*), 120.5, 115.7 (*C*H), 81.2 (bs) (CCH_3), 67.4 (bs) (CH_2OCO), 61.7 (bs), 58.8 (bs), 51.8 (bs) (*C*, CH_2CO, CH_{2cyclo}), 28.2 (CH_3). ESI-MS (*m*/*z*): 736.25 (M + H^+); calc for $C_{37}H_{62}N_5O_{10}$: 736.45. IR spectrum (KBr disk): 3364, 2971, 1729, 1527, 1218, 1144 cm^{-1}.

3. A solution of thiosphogene (83 μL; 1.09 mmol) in dry CH_2Cl_2 (2 mL) was added drop wise in 2 min to a ice-bath cooled mixture of **2** (670 mg; 0.91 mmol) in saturated potassium bicarbonate (5 mL) and CH_2Cl_2 (5 mL), under vigorous magnetic stirring. The ice-bath was removed after 5 min and the suspension was stirred for 2 h at rt. The organic phase was separated and extracted with water (3 × 10 mL); then the organic phase was dried over $NaSO_4$, filtered and evaporated to yield a yellow solid. TLC (silica gel 60 F_{254}, petroleum ether/EtOAc 8 : 2, detection: UV 254 nm): R_f 0.41. ^{1}H-NMR ($CDCl_3$) 400 MHz δ = 7.41 (bs, 2H, C*H*), 7.20 (bs, 2H, C*H*), 7.15 (bs, 1H, N*H*), 4.20 (s, 2H, CH_2OCO), 3.72 (s, 4H, CH_2CO), 3.21 (s, 4H, CH_2CO), 3.12 (d, 2H, J = 14.3 Hz, CH_2C), 2.87 (d, 2H, J = 14.3 Hz, CH_2C), 2.70–2.64 (m, 4H, CH_2CH_2), 1.44 (s, 18H, CH_3), 1.43 (s, 18H, CH_3).^{13}C-NMR ($CDCl_3$) 100 MHz δ = 172.9, 170.4 (*C*O), 153.3 (NH*C*OO), 140.0 (*C*), 128.0 (*C*), 110.3 (*C*H), 106.7 (*C*H), 81.4, 81.2 (CCH_3), 67.6 (CH_2OCO), 63.4 (*C*), 62.2, 62.3 (CH_2CO), 59.0, 51.6 (CH_{2cyclo}), 28.2, 28.0 (CH_3). ESI-MS (*m*/*z*): 778.47 (M + H^+); calc for $C_{38}H_{60}N_5O_{10}S$: 778.41. IR spectrum (KBr disk): 3322, 2971, 2111, 1739, 1548, 1218, 1155 cm^{-1}.

General procedure for *t*-butyl ester deprotection

To a solution of polyaminocarboxylate-*t*-butyl esters in CH_2Cl_2 (*ca.* 0.4 N) was gradually added trifluoroacetic acid (equal volume) and the mixture was stirred at rt overnight. The solution was then evaporated *in vacuo* and the product was recovered with excess diethyl ether, isolated by centrifugation, washed thoroughly with diethyl ether and dried *in vacuo* obtaining pure desired product as the trifluoroacetate salt. Final products were purified from salts and low molecular weight impurities by gel filtration on a Sephadex G25 column (30 cm × 1.6 cm) (Pharmacia Biotech, Uppsala, Sweden) using H_2O as eluent. Analytical HPLC-MS runs on final ligands (10 μL of a 2 mg mL^{-1} solution in H_2O) were carried out using Method 1 or 2 (see Electronic Supplementary Information†) and H_2O–TFA 0.1% (A) and CH_3CN–TFA 0.1% (or CH_3OH–TFA 0.1%) (B) as eluents.

4. HPLC-MS, method 1, retention time 4.80 min, purity 95%. ^{1}H-NMR (D_2O) 400 MHz δ = 7.43 (d, 2H, J = 8.4 Hz, C*H*), 7.31 (d, 2H, J = 8.4 Hz, C*H*), 4.11 (s, 2H, CH_2OCO), 3.81 (s, 4H, CH_2CO), 3.77 (s, 4H, CH_2CO), 3.49–3.43 (m, 8H, CH_{2cyclo}). ^{13}C-NMR ($CDCl_3$) 100 MHz δ = 176.9, 171.4 (*C*O), 154.5 (NH*C*OO), 138.2, 125.5 (*C*), 122.9, 121.0 (*C*H), 66.1 (CH_2OCO), 61.7 (*C*), 59.0, 53.3 (CH_2CO), 58.1, 51.7 (CH_{2cyclo}). ESI-MS (*m*/*z*): 512.20 (M + H^+); calc for $C_{21}H_{30}N_5O_{10}$: 512.20. IR spectrum (KBr disk): 3407, 2919, 2610, 1729, 1659, 1198, 1123 cm^{-1}.

General procedure for gadolinium complex preparation

The Gd^{III} complexes were prepared by mixing stoichiometric amounts of the ligands and $GdCl_3$ solution (final concentration *ca.* 1 mM). Unchelated Gd^{3+} ions were eliminated by precipitation of the hydroxide at basic pH by adding aliquots of a concentrated NaOH solution.

[GdL1]. A solution of thiosphogene (54 μL; 0.70 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise in 2 min to a solution of **4** (100 mg; *ca.* 0.14 mmol) in H_2O (5 mL). The mixture was vigorously stirred for 2 h at rt. Then, after adjustment of the pH to 5.5, the water solution was washed 3 times with CH_2Cl_2 (5 mL) and then evaporated *in vacuo*. ESI-MS (*m*/*z*): 708.06 (M + H^+); calc for $C_{22}H_{24}N_5O_{10}SGd$: 708.05 (100,0%), (isotopic distribution consistent with Gd complex).

1-Nitro-3,5-benzenedicarbonyldiazide. A solution of 1-nitroisophthaloyl dichloride (1.168 g; 4.73 mmol) in dry CH_2Cl_2

(4 mL) was added drop wise in 2 min to an ice-bath cooled mixture of NaN_3 (3.075 g; 47.3 mmol) in 1.0 mol L^{-1} NaOH solution (10 mL) and CH_2Cl_2 (8 mL), under vigorous magnetic stirring. The ice-bath was removed after 5 min and the mixture was stirred for 45 min at rt. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL); then, all organic phases were collected, dried over $NaSO_4$, filtered and evaporated to yield a white solid (1.045 g, 83%). ^{1}H-NMR ($CDCl_3$) 400 MHz δ = 9.0 (d, 2H, J = 1.8 Hz), 8.9 (t, 1H, J = 1.8 Hz). ^{13}C-NMR ($CDCl_3$) 100 MHz δ = 169.7 (*C*O), 148.8 (*C*), 135.1 (*C*H), 133.3 (2*C*), 128.8 (2*C*H).

6. 1-Nitro-3,5-benzenedicarbonyldiazide (100 mg; 0.38 mmol) was heated to reflux for 3 h in dry toluene, under N_2. After cooling to rt, a solution of AAZTA-OH (460 mg; 0.76 mmol) in dry CH_2Cl_2 (3 mL) was added under N_2 atmosphere. The reaction mixture was stirred overnight at rt, and then evaporated *in vacuo*. The crude product was purified on a silica gel chromatography column (petroleum ether/ethyl acetate 75/25) yielding pure **6** as a pale yellow solid (472 mg, 88%). TLC (silica gel 60 F_{254}, petroleum ether/EtOAc 8 : 2, detection: UV 254 nm): R_f 0.35. ^{1}H-NMR ($CDCl_3$) 400 MHz δ = 8.03 (s, 2H, C*H*), 7.93 (bs, 2H, N*H*), 7.79 (s, 1H, C*H*), 4.23 (s, 4H, CH_2OCO), 3.68 (s, 8H, CH_2CO), 3.25 (s, 4H, CH_2CO), 3.24 (s, 4H, CH_2CO), 3.08 (d, 4H, J = 14.3 Hz, CH_2C), 2.78 (d, 4H, J = 14.3 Hz, CH_2C), 2.80–2.63 (m, 8H, CH_2CH_2), 1.42 (s, 36H, CH_3), 1.41 (s, 36H, CH_3).^{13}C-NMR ($CDCl_3$) 100 MHz δ = 172.8, 170.7 (*C*O), 153.2 (NH*C*OO), 149.3 (*C*), 140.0 (2*C*), 113.0 (*C*H), 107.6 (2*C*H), 81.0, 80.8 (*C*CH$_3$), 68.1 (*C*H$_2$OCO), 63.3 (*C*), 62.3, 62.1 (*C*H$_2$CO), 59.0, 51.7 (*C*H$_{2cyclo}$), 28.3, 28.2 (*C*H$_3$). ESI-MS (m/z): 1408.78 (M + H$^+$); calc for $C_{68}H_{114}N_9O_{22}$: 1408.81. IR spectrum (KBr disk): 3331, 2973, 2942, 1729, 1549, 1370, 1212, 1158 cm^{-1}.

7. 10% Pd/C (50 mg) suspended in water (1 mL) was added to a solution of **6** (472 mg; 0.33 mmol) in MeOH (10 mL). The mixture was stirred overnight under H_2 atmosphere and then filtered. The filtrate was evaporated *in vacuo* affording pure **7** (430 mg; 93%). TLC (silica gel 60 F_{254}, petroleum ether/EtOAc 8 : 2, detection: UV 254 nm): R_f 0.05. ^{1}H-NMR ($CDCl_3$) 400 MHz δ = 7.12 (bs, 2H, N*H*), 6.79 (bs, 1H, C*H*), 6.61 (bs, 2H, C*H*), 4.14 (s, 4H, CH_2OCO), 3.66 (s, 8H, CH_2CO), 3.22 (s, 8H, CH_2CO), 3.04 (d, 4H, J = 14.3 Hz, CH_2C), 2.74 (d, 4H, J = 14.3 Hz, CH_2C), 2.87–2.61 (m, 8H, CH_2CH_2), 1.37 (s, 36H, CH_3), 1.36 (s, 36H, CH_3).^{13}C-NMR ($CDCl_3$) 100 MHz δ = 172.8, 170.6 (*C*O), 153.2 (NH*C*OO), 148.4 (*C*), 139.5 (2*C*), 100.1 (2*C*H), 98.7 (*C*H), 81.0, 80.8 (*C*CH$_3$), 67.5 (*C*H$_2$OCO), 63.1 (*C*), 62.1–62.0 (*C*H$_2$CO), 58.7, 51.4 (*C*H$_{2cyclo}$), 28.2, 28.1 (*C*H$_3$). ESI-MS (m/z): 1379.43 (M + H$^+$); calc for $C_{68}H_{116}N_9O_{20}$: 1378.83. IR spectrum (KBr disk): 3360, 2965, 1745, 1513, 1208, 1124 cm^{-1}.

8. A solution of thiosphogene (31 µL; 0.4 mmol) in dry CH_2Cl_2 (2 mL) was added drop wise in 2 min to a ice-bath cooled mixture of **7** (430 mg; 0.31 mmol) in saturated $KHCO_3$ (5 mL) and CH_2Cl_2 (5 mL), under vigorous magnetic stirring. The ice-bath was removed after 5 min and the suspension was stirred for 2 h at rt. The organic phase was separated and extracted with water (3 × 10 mL); then the organic phase was dried over $NaSO_4$, filtered and evaporated to yield a yellow solid. TLC (silica gel 60 F_{254}, petroleum ether/EtOAc 8 : 2, detection: UV 254 nm): R_f 0.29. ^{1}H-NMR ($CDCl_3$) 400 MHz δ = 7.41 (bs, 2H, C*H*), 7.20 (bs, 1H, C*H*), 7.15 (bs, 2H, N*H*), 4.21 (s, 4H, CH_2OCO), 3.69 (s, 8H, CH_2CO), 3.25 (s, 8H, CH_2CO), 3.09 (d, 4H, J = 14.3 Hz, CH_2C), 2.87 (d, 4H, J = 14.3 Hz, CH_2C), 2.70–2.64 (m, 8H, CH_2CH_2), 1.43 (s, 36H, CH_3), 1.42 (s, 36H, CH_3).^{13}C-NMR ($CDCl_3$) 100 MHz δ = 172.8, 170.4 (*C*O), 153.1 (NH*C*OO), 140.0 (*C*), 128.0 (*C*), 110.3 (*C*H), 106.7 (*C*H), 81.3, 81.0 (*C*CH$_3$), 67.5 (*C*H$_2$OCO), 63.2 (*C*), 62.3, 62.1 (*C*H$_2$CO), 59.0, 51.6 (*C*H$_{2cyclo}$), 28.2, 28.0 (*C*H$_3$). ESI-MS (m/z): 1421.38 (M + H$^+$); calc for $C_{69}H_{114}N_9O_{20}S$: 1420.79. IR spectrum (KBr disk): 3320, 2968, 2109, 1739, 1548, 1208, 1155 cm^{-1}.

9. HPLC-MS, method 1, retention time 6.51 min, purity 92%. ^{1}H-NMR (D_2O) 400 MHz δ = 7.10 (bs, 3H, C*H*), 4.06 (s, 4H, CH_2OCO), 3.80 (s, 8H, CH_2CO), 3.67 (s, 8H, CH_2CO), 3.49–3.40 (m, 16H, CH_{2cyclo}).^{13}C-NMR ($CDCl_3$) 100 MHz δ = 177.1, 171.2 (*C*O), 154.5 (NH*C*OO), 137.1, 125.0 (*C*), 122.5, 121.0 (*C*H), 66.2 (*C*H$_2$OCO), 61.5 (*C*), 59.4, 53.2 (*C*H$_2$CO), 58.4, 51.7 (*C*H$_{2cyclo}$). ESI-MS (m/z): 930.45 (M + H$^+$); calc for $C_{36}H_{52}N_9O_{20}$: 930.33. IR spectrum (KBr disk): 3329, 2957, 2586, 1720, 1410, 1187, 1137 cm^{-1}.

[GdL2]. A solution of thiosphogene (31 µL; 0.40 mmol) in dry CH_2Cl_2 (5 mL) was added dropwise in 2 min to a solution of **9** (100 mg; *ca.* 0.08 mmol) in H_2O (5 mL). The mixture was then stirred for 2 h at rt. After adjustment of the pH to 5.5, the water solution was washed 3 times with CH_2Cl_2 (5 mL) and then evaporated *in vacuo*. ESI-MS (m/z): 1279.09 (M + H$^+$); calc for $C_{38}H_{46}N_9O_{19}SGd_2$: 1279.11 (100,0%), (isotopic distribution consistent with bis-Gd complex).

General procedure for octameric ligands

To a solution of the appropriate poly primary amine and triethylamine (2 eq for each amino group) in dry CH_2Cl_2 (10 mN), under N_2 atmosphere, cooled at $T \leq 10$ °C with an ice-bath, a solution of **3** or **8** (1.2 eq for each amino group) in dry CH_2Cl_2 (2 mM) was added dropwise. The reaction mixture was stirred overnight at rt and then washed with water (3 × 5 mL), HCl 0.1 mol L^{-1} (2 × 5 mL) and saturated $NaHCO_3$ (2 × 5 mL). The organic phase was dried over $NaSO_4$, filtered and evaporated to yield the crude product, that was purified through crystallizations from diethyl ether with petroleum ether.

10. (yield 81%, calculated on polyamine). TLC (silica gel 60 F_{254}, petroleum ether/EtOAc 1 : 1, detection: UV 254 nm): R_f 0.22. ^{1}H-NMR ($CDCl_3$) 400 MHz δ = 7.36 (bs, 32H, C*H*), 4.20 (s, 16H, CH_2OCO), 3.84 (bs, 8H, CH_2) 3.71 (s, 32H, CH_2CO), 3.40 (bs, 16H, CH_2), 3.26 (s, 32H, CH_2CO), 3.09 (d, 16H, J = 13.9 Hz, CH_2C), 2.92 (bs, 8H, CH_2), 2.83–2.63 (m, 32H, CH_{2cyclo}), 2.64 (bs, 20H, CH$_2$), 2.54 (bs, 24H, CH_2), 2.38 (bs, 24H, CH_2), 1.42 (s, 288H, CH_3).^{13}C-NMR ($CDCl_3$) 100 MHz δ = 173.6, 172.8, 170.9 (*C*O), 153.5 (NH*C*OO), 136.2, 133.0 (bs, *C*), 125.7, 119.1 (bs, *C*H), 81.0–80.8 (*C*CH$_3$), 67.7 (*C*H$_2$OCO), 63.3 (*C*), 62.3 (*C*H$_2$CO), 59.0–51.6 (*C*H$_{2cyclo}$), 55.2, 51.7, 50.4, 44.3, 39.1, 37.6, 34.0 (*C*H$_2$), 28.3–28.2 (*C*H$_3$). IR spectrum (KBr disk) 3391, 3241, 3002, 1746, 1641, 1522, 1356, 1221, 1148, 1073 cm^{-1}. Elemental analysis calcd (%) for $C_{366}H_{600}N_{66}O_{92}S_8$: C, 57.44; H, 7.90; N, 12.08; S, 3.35. found: C, 57.10; H, 7.75; N, 11.89; S, 3.21.

L3. HPLC, method 2, retention time 13.90 min, purity 85%. ^{1}H-NMR (D_2O) 400 MHz δ = 7.36 (bs, 16H, C*H*), 7.17 (bs, 16H, C*H*), 4.10 (s, 16H, CH_2OCO), 3.68 (bs, 16H, CH_2) 3.51 (s, 64H,

CH_2CO), 3.46 (bs, 16H, CH_2), 3.27 (bs, 64H, CH_{2cyclo}), 3.05 (bs, 32H, CH_2), 2.27 (bs, 12H, CH_2), 2.54 (bs, 24H, CH_2). ^{13}C-NMR (D_2O) 100 MHz δ = 180.0, 175.2, 173.9 (*C*O), 154.8 (NH*C*OO), 136.7, 132.4 (bs, *C*), 126.8, 120.7 (bs, *C*H), 67.2 (CH_2OCO), 63.2 (*C*), 60.8 (CH_2CO), 58.1, 53.4 (CH_{2cyclo}), 52.7, 49.8, 44.0, 39.1, 36.1, 31.4 (CH_2). IR spectrum (KBr disk) 3259, 2897, 1739, 1633, 1527, 1389, 1293, 1129, 1048 cm^{-1}. Elemental analysis calcd (%) for $C_{238}H_{325}Cl_5N_{66}Na_{24}O_{92}S_8$: C, 43.52; H, 4.99; N, 14.07; S, 3.91. found: C, 43.35; H, 4.85; N, 13.97; S, 3.82.

11. (yield 24%, calculated on polyamine). TLC (silica gel 60 F_{254}, petroleum ether/EtOAc 5 : 5, detection: UV 254 nm): R_f 0.20. ^{1}H-NMR ($CDCl_3$) 400 MHz δ = 7.78 (bs, 12H, C*H*), 4.19 (s, 16H, CH_2OCO), 3.98 (bs, 2H, CH_2NHCS), 3.71 (s, 32H, CH_2CO), 3.26 (s, 32H, CH_2CO), 3.09 (d, 16H, J = 13.9 Hz, CH_2C), 2.77–2.63 (m, 48H, CH_{2cyclo}), 2.16 (bs, 20H, CH_2N), 1.42 (s, 288H, CH_3).^{13}C-NMR ($CDCl_3$) 100 MHz δ = 172.8, 170.9 (*C*O), 153.3 (NH*C*OO), 140.1 (bs, *C*), 120.1 (bs, *C*H), 80.9, 80.7 (CCH_3), 67.9 (CH_2OCO), 63.3 (*C*), 62.3 (CH_2CO), 59.0–51.6 (CH_{2cyclo}), 51.8 (CH_2NHCS), 62.3, 53.5, 41.8 (CH_2), 28.3, 28.2 (CH_3). IR spectrum (KBr disk): 3357, 2984, 1710, 1627, 1422, 1220, 1073 cm^{-1}. Elemental analysis calcd (%) for $C_{286}H_{480}N_{42}O_{80}S_4$: C, 58.07; H, 8.18; N, 9.94; S, 2.17. found: C, 57.90; H, 8.03; N, 9.87; S, 2.09.

L4. HPLC, method 2, retention time 14.77 min, purity 81%. ^{1}H-NMR (D_2O) 400 MHz δ = 7.63–7.42 (bs, 12H, C*H*), 4.37 (s, 16H, CH_2OCO), 3.60 (s, 32H, CH_2CO), 3.43 (s, 32H, CH_2CO), 3.09–2.84 (m, 64H, CH_{2cyclo}), 2,18, 1.95 (bs, 20H, CH_2N). ^{13}C-NMR (D_2O) 100 MHz δ = 180.6, 179.4 (*C*O), 155.1 (NH*C*OO), 139.7 (bs, *C*), 103.5 (bs, *C*H), 66.2 (CH_2OCO), 64.3 (*C*), 63.0 (CH_2CO), 60.6, 56.6 (CH_{2cyclo}), 51.8 (CH_2NHCS), 64.3, 57.3, 36.6 (CH_2). IR spectrum (KBr disk) 3418, 1618, 1410, 1218, 1070 cm^{-1}. Elemental analysis calcd (%) for $C_{158}H_{201}ClN_{42}Na_{24}O_{80}S_4$: C, 40.51; H, 4.33; N, 12.56; S, 2.74. found: C, 40.39; H, 4.25; N, 12.44; S, 2.63.

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References

1 V. Kubicek and E. Toth, *Adv. Inorg. Chem.*, 2009, **61**, 63; C. F. G. C. Geraldes and S. Laurent, *Contrast Media Mol. Imaging*, 2009, **4**, 1.
2 S. Aime, D. Delli Castelli, S. Geninatti Crich, E. Gianolio and E. Terreno, *Acc. Chem. Res.*, 2009, **42**, 822; S. Aime, S. Geninatti Crich, E. Gianolio, G. B. Giovenzana, L. Tei and E. Terreno, *Coord. Chem. Rev.*, 2006, **250**, 1562.
3 D. Chen, V. Milacic, M. Frezza and Q. P. Dou, *Curr. Pharm. Des.*, 2009, **15**, 777; M. J. Heeg and S. S. Jurisson, *Acc. Chem. Res.*, 1999, **32**, 1053.
4 S. V. Eliseevaa and J.-C. G. Bünzli, *Chem. Soc. Rev.*, 2010, **39**, 189; L. Armelao, S. Quici, F. Barigelletti, G. Accorsi, G. Bottaro, M. Cavazzini and E. Tondello, *Coord. Chem. Rev.*, 2010, **254**, 487.
5 G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, 1996.
6 M. Woods and A. D. Sherry, *Chemistry Today*, 2005, **23**, 31; M. L. De León Rodriguez and Z. Kovacs, *Bioconjugate Chem.*, 2008, **19**, 391; A. Barge, L. Tei, D. Upadhyaya, F. Fedeli, L. Beltrami, R. Stefania, S. Aime and G. Cravotto, *Org. Biomol. Chem.*, 2008, **6**, 1176.
7 S. Liu, *Adv. Drug Delivery Rev.*, 2008, **60**, 1347; J. Fichna and A. Janecka, *Bioconjugate Chem.*, 2003, **14**, 3.
8 P. Caravan, J. J. Ellison, T. J. McMurry and R. B. Lauffer, *Chem. Rev.*, 1999, **99**, 2293; S. Aime, M. Botta and E. Terreno, *Adv. Inorg. Chem.*, 2005, **57**, 173.
9 P. Caravan, C. T. Farrara, L. Frullano and U. Ritika, *Contrast Media Mol. Imaging*, 2009, **4**, 89; Y. Song, E. K. Kohlmeir and T. J. Meade, *J. Am. Chem. Soc.*, 2008, **130**, 6662.
10 J. B. Livramento, E. Toth, A. Sour, A. Borel, A. E. Merbach and R. Ruloff, *Angew. Chem., Int. Ed.*, 2005, **44**, 1480; J. B. Livramento, A. Sour, A. Borel, A. E. Merbach and E. Toth, *Chem.–Eur. J.*, 2006, **12**, 989.
11 S. Aime, L. Calabi, C. Cavallotti, E. Gianolio, G. B. Giovenzana, P. Losi, A. Maiocchi, G. Palmisano and M. Sisti, *Inorg. Chem.*, 2004, **43**, 7588; G. B. Giovenzana, G. Palmisano, M. Sisti, C. Cavallotti, S. Aime and L. Calabi, *PCT Int. Appl.*, 2003, WO0308390.
12 G. Gugliotta, M. Botta, G. B. Giovenzana and L. Tei, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 3442.
13 Z. Baranyai, F. Uggeri, G. B. Giovenzana, A. Benyei, E. Brucher and S. Aime, *Chem.–Eur. J.*, 2009, **15**, 1696.
14 M. Yoneno, S. Abe and K. Namba, *Kogyo Kagaku Zasshi*, 1967, **70**, 1442; V. D. Jadhav and F. P. Schmidtchen, *J. Org. Chem.*, 2008, **73**, 1077.
15 L. Tei, G. Gugliotta, S. Avedano, G. B. Giovenzana and M. Botta, *Org. Biomol. Chem.*, 2009, **7**, 4406; Z. Zhang, J. C. Amedio, P. D. Caravan, S. Dumas, A. Kolodziej and T. J. McMurry, US Patent 2003/0180222.
16 K. N. Raymond, T. M. Corneillie, J. Xu, US Patent 2008/0213917.
17 S. Langereis, A. Dirksen, T. M. Hackeng, M. H. P. van Genderen and E. W. Meijer, *New J. Chem*, 2007, **31**, 1152; V. J. Venditto, A. I. S. Regino and M. V. Brechbiel, *Mol. Pharmaceutics*, 2005, **2**, 302.
18 G. M. Nicolle, E. Toth, H. Schmitt-Willich, B. Radüchel and A. E. Merbach, *Chem. Eur. J.*, 2002, **8**, 1040.

