

# Application of the Ugi four-component reaction to the synthesis of ditopic bifunctional chelating agents†

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The Ugi four-component reaction (Ugi 4CR) was exploited for the first time to obtain in a single synthetic step bifunctional ditopic chelators by using DOTA monoamide (DOTAMA) derivatives as amino and acid components. A number of ditopic systems in which the two DOTAMA units are connected by a central  $\alpha$ -acylaminoamide group were synthesized by reacting different aldehydes, isocyanides and two DOTAMA chelates containing amino and acid functionalities. Variation of the components allows the insertion of another functional group into the  $\alpha$ -acylaminoamide skeleton for further conjugation to biomolecules. The optimal reaction conditions were found by using methanol as solvent and ultrasound irradiation at a power of 60 W (20 kHz) for 3 h. The Gd(III) complexes of the dimeric ligands **L1** and **L2** (bearing a cyclohexyl ring and an octadecyl chain on the central  $\alpha$ -acylaminoamide moiety, respectively) were fully characterized in aqueous media by relaxometric techniques with varying temperature and magnetic field strength. The relaxivity of Gd<sub>2</sub>**L1** and Gd<sub>2</sub>**L2** (in the aggregated form), at 20 MHz and 310 K, are 5.6 and 20.0 mM<sup>-1</sup> s<sup>-1</sup>, respectively. The enhanced value found for Gd<sub>2</sub>**L2** indicates that this lipophilic complex forms micelles at concentrations <0.1 mM. Finally, the binding of Gd<sub>2</sub>**L2** to human serum albumin (HSA) was investigated by proton relaxometry, and the affinity constant of the complex and the relaxivity of the macromolecular adduct ( $r_{\text{ip}}^{\text{b}} = 38.1 \text{ mM}^{-1} \text{ s}^{-1}$ ; 20 MHz and 310 K) derived.

## Introduction

Multicomponent reactions (MCRs)<sup>1</sup> have experienced fast-growing interest in the last decade, mainly because they allow the assembly of complicated structures from simple building blocks in a single operation. One of the best-known MCRs is the Ugi four-component reaction (Ugi 4CR)<sup>2</sup> whose great potential in synthetic organic chemistry has been widely explored.<sup>3</sup> In fact, it is possible to synthesize a large number of different systems by the reaction of a number of carbonyl compounds, amines, isocyanides and carboxylic acids together to form  $\alpha$ -acylamino amides. These libraries have been investigated either with enzymes or living organisms to find new bioactive leads.<sup>4</sup> The exploitation of the Ugi 4CR provides the rapid generation of molecular complexity and diversity from simple and readily accessible starting materials. The one-pot preparation of variably substituted polyamides and/or of functionalized macrocyclic ligands allows one to avoid the time- and chemical-consuming steps needed to discriminate the chemically equivalent nitrogen atoms of di-, tri- or poly-amines.<sup>5</sup> Indeed, being capable of combining three or more reactants together in an ordered process, MCRs offer not

only great molecular complexity and diversity per step but also the possibility of introducing matched functionalities suitable for further transformations.<sup>3</sup> In fact, the combination of an MCR with an efficient post-transformation, for example the conjugation to macromolecules or biomolecules, could represent a powerful tool for the synthesis of molecular probes useful for biomedical applications.

In a parallel context, the synthesis of functionalized polyazacycloalkanes continues to see growing interest, since they represent a class of chelating agents able to form stable complexes with a large variety of metal ions, ranging from transition metals to lanthanides and other heavy metals. The uses of these chelates as contrast-enhancing agents (CAs) for magnetic resonance imaging (MRI)<sup>6</sup> and as radiopharmaceuticals for the diagnosis and therapy of tumours<sup>7</sup> represent the most relevant biomedical applications. An important challenge in the development of novel chelates is the improvement of their capability to target certain organs and tissues, in order to allow precise delivery to specific cellular targets.<sup>8</sup> The general strategy is the synthesis of a chelating unit linked to a suitable function through which it can be covalently bound to the specific biological carrier, leading to the so-called bifunctional chelating agents (BFCAs).<sup>9</sup> Macrocyclic BFCAs are often based on DOTA or DOTA monoamide (DOTA = 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid) derivatives bearing a large variety of functional groups available for conjugation, such as carboxyls, amines, aldehydes or ketones, isothiocyanates, maleimides, alkynes and vinylsulfone.<sup>10,11</sup>

In the search for the optimal Gd(III) chelate for MRI applications, one strategy is based on the increase of the number of Gd(III) units per CA through the formation of multimers, of

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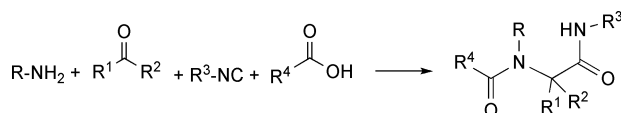
† Electronic supplementary information (ESI) available: <sup>1</sup>H relaxometric titration of Gd<sub>2</sub>**L2** with HSA (Fig. S1); VT <sup>1</sup>H NMR profile of Gd<sub>2</sub>**L2** conjugated to HSA (Fig. S2); 1/T<sub>1</sub> NMRD profiles of Gd<sub>2</sub>**L2** conjugated to HSA (Fig. S3). See DOI: 10.1039/b907932g

covalent or non-covalent conjugates between the paramagnetic chelate and slowly moving substrates, and the self-assembly of amphiphilic Gd(III) chelates to form micelles.<sup>12</sup>

In this context, the synthesis of multimeric BFCAs, which can combine the search for more efficient CA and the targeting of these CA to specific receptors, has never been pursued. Thus, although several ditopic cyclen-based ligands and their Ln(III) complexes have been reported in the literature,<sup>13</sup> none of them offers the possibility to anchor the dimeric system to vectors or biomolecules through orthogonally protected functional groups. We have therefore approached this problem through the use of the Ugi 4CR, which has permitted the synthesis of a series of dimeric ligands with a hydrophobic chain or different functional groups. The synthesis was achieved by the use of simple monomeric BFCAs containing a primary amino group in one case and a carboxylic acid in another and by changing the aldehyde and/or the isocyanide employed for the Ugi 4CR.

## Results and discussion

In the commonly accepted mechanism of the Ugi 4CR, the first step is the condensation of the primary amine (Scheme 1) with the carbonyl compound generating an intermediate imine,<sup>14</sup> protonated by the carboxylic acid moiety to give an iminium ion. The latter undergoes a nucleophilic attack by the isocyanide leading to the formation of a nitrilium ion intermediate, subsequently intercepted by the corresponding carboxylate anion. The resulting iminoanhydride typically proceeds through the irreversible transacylation known as a Mumm rearrangement<sup>15</sup> to give the final Ugi 4CR product.



**Scheme 1** Classic Ugi four-component reaction.

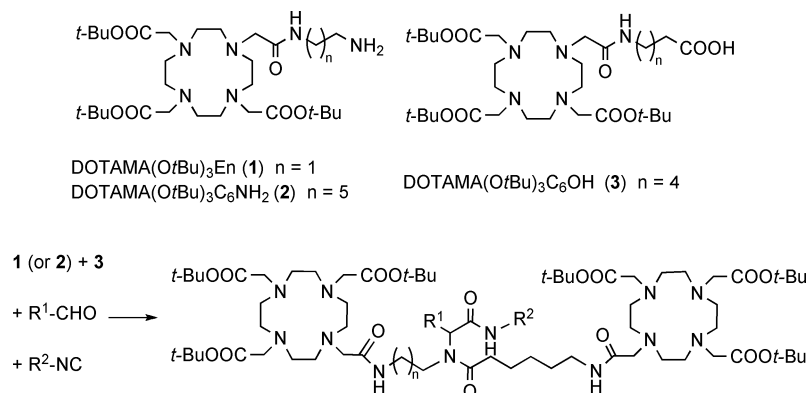
Although the Ugi 4CR has been one of the most used MCRs during the last decades, to the best of our knowledge this is the first report in which the Ugi 4CR is applied to poly(aminocarboxylate) ligands for the preparation of multimeric systems. Recently, S. Faulkner and coworkers reported the use of the Ugi reaction for preparing chromophore-appended DOTA-monoamide ligands.<sup>5</sup> We exploited the Ugi

4CR by using three bifunctional chelating agents<sup>11,16</sup> based on a DOTA-monoamide platform containing a primary amino group (DOTAMA(OtBu)<sub>3</sub>En (**1**) and DOTAMA(OtBu)<sub>3</sub>C<sub>6</sub>NH<sub>2</sub> (**2**)) or a carboxylic acid (DOTAMA(OtBu)<sub>3</sub>C<sub>6</sub>OH (**3**)) functional group, respectively (Scheme 2). The reacting functionality is separated from the DOTA monoamide moiety by a two-carbon spacer group in **1** and a six-carbon spacer in **2** and **3**. In detail, by using one of the BFCAs with the free NH<sub>2</sub> as the amino component and **3** as the acid component and varying the aldehyde and the isocyanide components, we were able to synthesize a series of ditopic systems in which the two chelating units are connected by a central  $\alpha$ -acylaminoamide group (Scheme 2).

Initially, we sought the best conditions to obtain the product with yields as high as possible. For this reason, we pursued the Ugi 4CR reaction either by using conventional heating or alternative activation tools, *i.e.*: microwaves (MW) and ultrasound. MW and ultrasound have in fact been shown to improve many organic reactions in terms of reaction times, product yields and purity substantially by reducing or even eliminating side reactions.


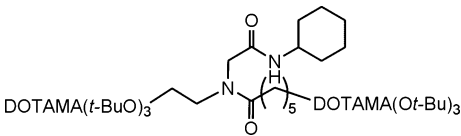

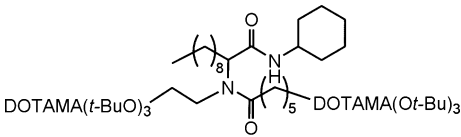

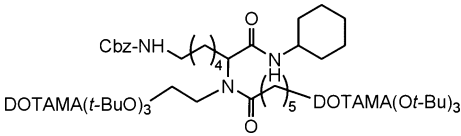
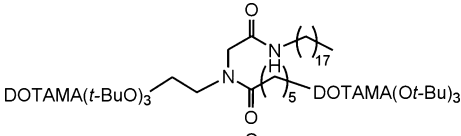
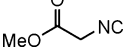
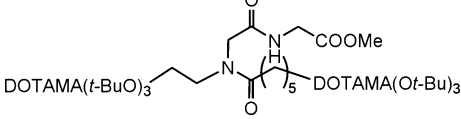
## Synthetic procedures

The first attempt was made by treating **1** with equimolar amounts of paraformaldehyde, **3**, and cyclohexylisocyanide in refluxing methanol. Indeed, we observed the formation of the Ugi 4CR product by ESI-MS, although only after 6 h with by-products and residual starting materials. The dimer **4** was isolated after column chromatography in quite low yield (18%). Either using other solvents (acetonitrile, THF) or increasing the reaction time did not improve significantly the yield. Switching the aldehyde component to decanal increased the yield slightly (28%), but this was not yet satisfactory. Replacing the amino component **1** with the ligand DOTAMA(OtBu)<sub>3</sub>C<sub>6</sub>NH<sub>2</sub> (**2**) gave the product in 20% yield. We surmised that the amino and acid functional groups on the DOTA monoamide derivatives **1** and **3** were not easily available for the multicomponent reaction. In fact, the NH<sub>2</sub> group can interact with carboxylate or amide oxygen atoms, and the carboxylic acid with nitrogen atoms of the macrocycle, thus wrapping the pendant arms and closing the molecule to the action of external reactants (molecular “pangolinization”). Therefore, we were prompted to explore alternative heating techniques such as MW and ultrasound in order to provide enough energy to dissociate these intramolecular H-bonds. Several examples of the



**Scheme 2** General synthesis of ditopic bifunctional chelating agents through Ugi 4CR (see Table 1 for different R<sup>1</sup> and R<sup>2</sup> groups employed).

**Table 1** Compounds obtained varying the aldehyde and/or isocyanide components

Amine	Acid	Aldehyde R <sup>1</sup> -CHO	Isocyanide R <sup>2</sup> -NC	Product	Yield (%)
1	3	(CH <sub>2</sub> O) <sub>n</sub>			48
1	3	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>8</sub> CHO			45
1	3	Cbz-NH(CH <sub>2</sub> ) <sub>5</sub> CHO			42
1	3	(CH <sub>2</sub> O) <sub>n</sub>	C <sub>18</sub> H <sub>37</sub> NC		52
1	3	(CH <sub>2</sub> O) <sub>n</sub>			38

use of MW in MCR have been reported to improve reaction rates and yields.<sup>17</sup> Due to the marked polarity of the functionalized polyaminocarboxylic substrates, we envisaged that the use of MW as source of power would be useful to the progress of the Ugi 4CR unfolding of the pendant arms. The reactions were carried out in a CEM Discover microwave oven using 10 mL Pyrex reaction vessels sealed with pressure caps. Thus, **1** was reacted with equimolar amounts of paraformaldehyde, **3**, and cyclohexylisocyanide for 1 h at 100 W and 2 bar in methanol. With conventional heating, the product was collected only in low yield (24%) after evaporation of the solvent and purification by column chromatography. No significant improvement in the yield was observed using decanal instead of paraformaldehyde. However, using MW, we observed the formation of a large amount of by-products, whereas the starting materials were still present in the reaction mixture when the reaction was carried out under conventional heating. This behavior may be ascribed to a stronger activation of reagents and intermediates by MW, leading to a reactivity enhancement and a resulting selectivity loss. Isocyanides and some of the reaction intermediates are indeed known to undergo side-reactions such as oligo- and polymerization.<sup>1</sup> Finally, we exploited ultrasound,<sup>18</sup> a tunable high-energy power source which might break up the intramolecular interactions and help the formation of the desired Ugi 4CR product. The reaction components (**1**, paraformaldehyde, **3**, and cyclohexylisocyanide) dissolved in methanol were therefore mixed in equimolar amounts in a cup horn and sonicated at a power of 60 W (20 kHz) for 3 h. Under these conditions, the product was obtained in moderately good yields (48%) after column chromatography and was characterized by ESI-MS, and <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy.

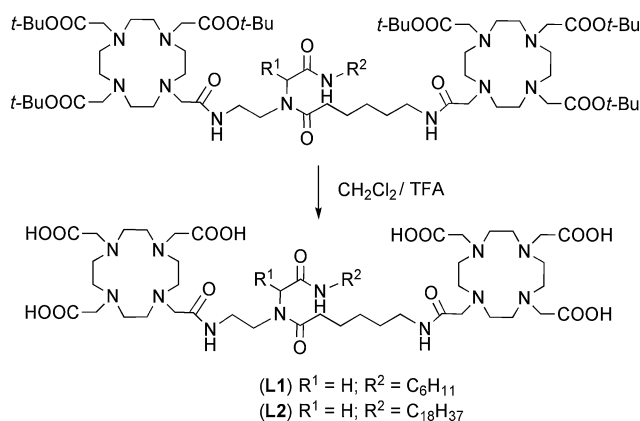
### Synthesis of ditopic BFCAs

Once we had found the best conditions for the Ugi 4CR, we checked the generality of this reaction for DOTA monoamide amino (**1**) and acid (**3**) derivatives, by varying the aldehyde and isocyanide components. Different components were studied in order to demonstrate the possibility of inserting a functional group into the α-acylaminoamide skeleton for further conjugation to biomolecules. In addition to paraformaldehyde (Table 1, line 1), alkanals (Table 1, line 2) and aminoaldehydes (Table 1, line 3) were successfully employed as the carbonyl compound.

Modification of the isocyanide was similarly performed by using octadecylisocyanide (Table 1, line 4) or methyl isocyanoacetate (Table 1, line 5) instead of cyclohexylisocyanide (Table 1, lines 1–3). The presence of protected amino (**6**) or carboxylate (**8**) groups in the dimeric system makes these compounds the first examples of dimeric BFCA. In fact, orthogonal removal of the Cbz protecting group from **6** by catalytic hydrogenation or alkaline hydrolysis of the methyl ester in **8** would yield products able to react respectively with carboxylic acid or amino functionalities of biomolecules. These systems, containing two chelates for the coordination of metal ions of medical interest and a suitable biological carrier, are of great interest for possible use in molecular imaging and therapy applications.

Moreover, with the idea to build a tetrameric system with four DOTA monoamide units in a one-pot reaction, we performed the Ugi 4CR with a diisocyanide and two molar equivalents of **1**, formaldehyde and **3**. Unfortunately, this attempt, which was performed using two different diisocyanides (1,4-diisocyanobenzene and 1,4-diisocyanohexane), did not succeed, since the reaction stopped at the dimeric systems, possibly due to steric hindrance of the DOTA units.

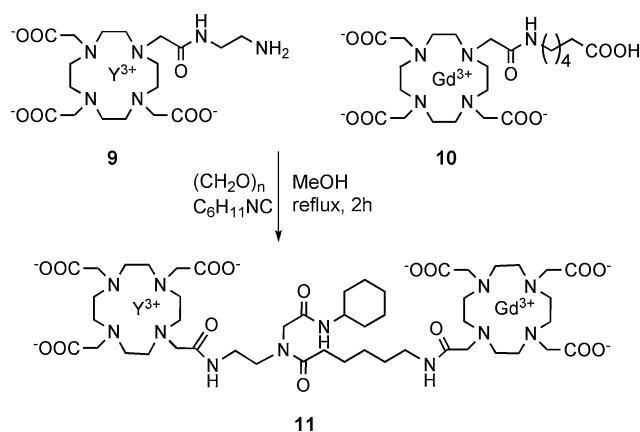
Finally, two of the synthesized compounds (**4** and **7**) were successfully deprotected by hydrolysis of the *t*-butyl esters with TFA, and the dimeric ligands **L1** and **L2** were obtained as analytically pure white solids by precipitation with diethyl ether (Scheme 3).



**Scheme 3** Synthesis of ditopic ligands **L1** and **L2**.

### Heteroditopic Y–Gd complex.

An additional advantage of this synthetic approach is the easy access to heteroditopic complexes. In fact, carrying out the Ugi 4CR directly on metal–DOTAMA complexes we were able to obtain heteroditopic complexes (Scheme 4). Thus, we synthesized the Y(III) complex of DOTAMAE<sub>n</sub> (**9**) and the Gd(III) complex of DOTAMAC<sub>6</sub>OH (**10**),<sup>16</sup> which were then reacted together with paraformaldehyde and cyclohexylisocyanide.



**Scheme 4** Synthesis of a heteroditopic Y–Gd complex by Ugi four-component reaction.

This reaction was performed both with ultrasound and with conventional heating but with different outcomes in respect of what was observed previously on the protected **1** and **3** derivatives. Thus, while using ultrasound, the Ugi 4CR product **11** was obtained only in low amounts due to the known problem of metal–ligand bond cleavage caused by ultrasound,<sup>18</sup> whereas by refluxing in methanol we observed the formation of **11** in good yields (47%). This is good evidence of our hypothesis of intramolecular interactions as the origin of the low yields in the conventional heating Ugi 4CR protocol using **1** and **3** derivatives. In fact,

intramolecular interactions cannot occur when all the donor atoms of the DOTA–monoamide cage are involved in coordination of the metal ion.

### Relaxometric characterization of the Gd(III) complexes

NMR relaxometry is a powerful tool for obtaining detailed structural and dynamic information on a paramagnetic system through the measurement and analysis of the nuclear magnetic longitudinal and transverse relaxation rates ( $R_1$  and  $R_2$ ) of the solvent nuclei as a function of the applied field strength, temperature and pH.<sup>19</sup> The water proton relaxation enhancement ability of a Gd(III) complex is expressed by the parameter relaxivity,  $r_{ip}$  ( $i = 1, 2$ ), which represents the increase of the water proton relaxation rates observed in a 1 mM solution of the paramagnetic system at a given temperature and magnetic field strength (typically 298/310 K and 20 MHz).

As representative examples, we have considered the Gd(III) complexes of two ditopic chelators, **L1** and **L2**, differing in the chemical nature and size of the central moiety. Whereas for the ligand **L1** the central pendant moiety is a cyclohexyl group, for **L2** it is a long  $\text{C}_{18}$  alkyl chain that provides the corresponding complex with the possibility to form aggregates (micelles) and to interact with the hydrophobic pockets of HSA.<sup>12</sup> All the ligand subunits of these systems are octadentate, and thus the corresponding Gd(III) complexes are expected to be coordinated by a single water molecule ( $q = 1$ ). The longitudinal relaxivity values (20 MHz, 310 K and pH = 7.2; per Gd) of the two ditopic complexes are 5.6 ( $\text{Gd}_2\text{L1}$ ) and  $20.0 \text{ mM}^{-1} \text{ s}^{-1}$  ( $\text{Gd}_2\text{L2}$ ).

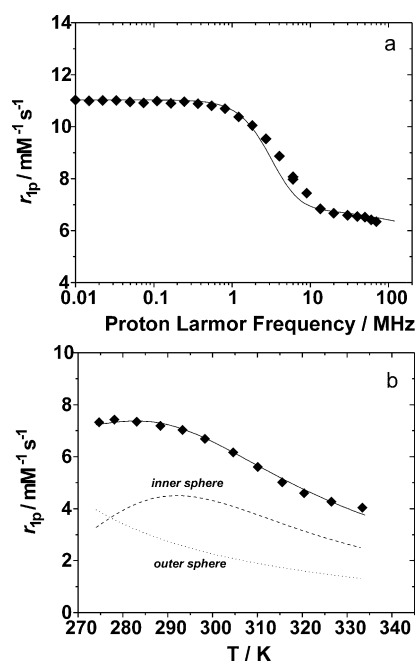
The NMRD (Nuclear Magnetic Relaxation Dispersion) profile of  $\text{Gd}_2\text{L1}$ , measured at 298 K and neutral pH and reported in Fig. 1a, is characterized by the typical shape and amplitude of mono-aqua, low molecular weight complexes, featuring a single dispersion around 4–6 MHz. The relaxivity of  $\text{Gd}_2\text{L1}$  (per Gd) is higher than that of a typical monomeric  $\text{GdDOTAMA}$  complex (*i.e.*  $3.9 \text{ mM}^{-1} \text{ s}^{-1}$  at 310 K)<sup>20</sup> to indicate that the difference is due to the different rotational dynamics of the two complexes. The best fit parameters, listed in Table 2, have been obtained using the standard equations for the inner sphere (IS) and outer sphere (OS) relaxation contributions and by fixing the values of the following parameters: the hydration number ( $q = 1$ ), the Gd– $\text{H}_w$  distance ( $r = 3.0 \text{ \AA}$ ), the distance of closest approach of the bulk water

**Table 2** Selected relaxation parameters for  $\text{Gd}_2\text{L1}$  and  $\text{Gd}_2\text{L2}$  derived from the analysis of  $^1\text{H}$  relaxivity as a function of temperature (20 MHz) and magnetic field strength (NMRD)<sup>a</sup>

Parameter	$\text{Gd}_2\text{L1}$	$\text{Gd}_2\text{L2}$
$^{310}r_{ip} (\text{mM}^{-1} \text{ s}^{-1})$	5.6	20.0
$^{298}k_{ex} (10^6 \text{ s}^{-1})$	$1.1 \pm 0.2$	$1.4 \pm 0.2$
$\Delta H_M (\text{kJ mol}^{-1})$	$39.3 \pm 1.8$	$38.2 \pm 2.1$
$\Delta^2 (10^{19} \text{ s}^{-2})$	$2.2 \pm 0.1$	$0.60 \pm 0.03$
$^{298}\tau_V (\text{ps})$	$22 \pm 1$	$19 \pm 2$
$^{298}\tau_{Rg} (\text{ns})$	$156 \pm 4$	$3710 \pm 203$
$\Delta H_{Rg} (\text{kJ mol}^{-1})$	$19 \pm 1$	$21.2 \pm 1.8$
$^{298}\tau_{Ri} (\text{ns})$	—	$400 \pm 28$
$\Delta H_{Ri} (\text{kJ mol}^{-1})$	—	$17 \pm 1$
$S^2$	—	$0.36 \pm 0.02$

<sup>a</sup> For the parameters  $q$ ,  $r$ ,  $a$ ,  $\Delta H_V$  and  $^{298}D$ , values of 1,  $3.0 \text{ \AA}$ ,  $4.0 \text{ \AA}$ ,  $2.0 \text{ kJ mol}^{-1}$  and  $2.24 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$  were used, respectively.



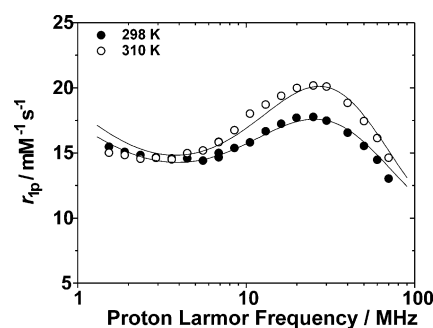


**Fig. 1** a)  $1/T_1$   $^1\text{H}$  NMRD profile for  $\text{Gd}_2\text{L1}$  at 298 K and pH = 7.2. The solid line represents the best-fit to the experimental points (see Table 2). b) Proton relaxivity as a function of temperature for  $\text{Gd}_2\text{L1}$  at 20 MHz. The solid line represents the best-fit to the experimental data (Table 2), whereas the dotted and dashed lines represent the calculated outer- and inner-sphere contributions to the relaxivity.

molecules ( $a = 4.0 \text{ \AA}$ ) to the metal ion, and the relative diffusion coefficient ( $D = 2.4 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ ).<sup>6</sup> For  $\text{Gd}_2\text{L1}$ , due to its rather low solubility, the information on the water exchange rate ( $k_{\text{ex}} = 1/\tau_{\text{M}}$ ) was extracted from the analysis of the temperature dependence of the  $^1\text{H}$  relaxivity at 20 MHz (as detailed in ref. 20). As shown in Fig. 1b, the  $r_{1p}$  increases by lowering the temperature from 335 to ca. 290 K, then it flattens and begins to decrease below ~280 K. This behavior is consistent with an intermediate rate of exchange of the bound water molecule. The overall behavior is the results of the different temperature-dependence of the IS and OS contributions to  $r_{1p}$ : the OS relaxivity decreases with increasing temperature, whereas the behavior of the IS term depends on the relative values of the longitudinal relaxation time of the bound water proton,  $T_{1\text{M}}$ , and its mean residence lifetime,  $\tau_{\text{M}}$ .<sup>21</sup> When  $\tau_{\text{M}} < T_{1\text{M}}$ ,  $r_{1p}$  increases with decreasing temperature ( $>300 \text{ K}$  in Fig. 3), whereas when  $\tau_{\text{M}} > T_{1\text{M}}$  ( $<285 \text{ K}$  in Fig. 3) the relaxivity has the opposite behavior, and the contribution of the IS term diminishes with decreasing temperature.

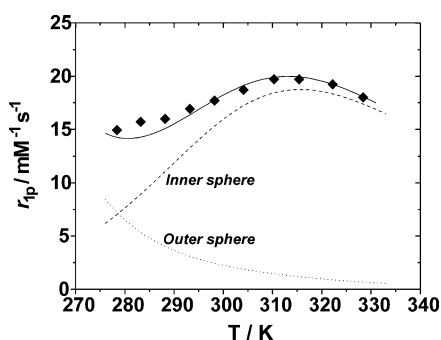
The relaxivity of  $\text{Gd}_2\text{L2}$  is significantly higher than that of  $\text{Gd}_2\text{L1}$ . This is the result of the presence of an octadecyl aliphatic chain that has the tendency to aggregate and form micellar systems of large dimension and thus slow tumbling rate (large  $\tau_{\text{R}}$  value). The observed proton relaxation rate of  $\text{Gd}_2\text{L2}$  (20 MHz; 298 K) shows a linear dependence from the concentration in the range 0.10–1.0 mM, and so an accurate determination of the critical micellar concentration (cmc) was not possible. However, cmc values lower than 0.10 mM are in agreement with typical values of previously investigated systems conjugated to long lipophilic chains.<sup>12,22</sup> Then, all the experimental data relative to  $\text{Gd}_2\text{L2}$  refer

to its aggregated form. The NMRD profiles (298 and 310 K) were recorded in the frequency range 1–70 MHz and are shown in Fig. 2. The profiles are characterized by different amplitudes but present a common general shape with a broad hump centered at ~25–30 MHz, typical of slowly tumbling systems. The analysis of the NMRD profile takes into account the relatively fast local rotation of the complex about the main axis of the aliphatic chain superimposed on the global motion of the micellar aggregate (Lipari–Szabo approach).<sup>23</sup> This model allows one to separate the contribution of the overall global rotation of the paramagnetic micelle ( $\tau_{\text{RG}} = 3710 \text{ ps}$ ) from the contribution of a faster local motion ( $\tau_{\text{RL}} = 400 \text{ ps}$ ) due to the rotation of the coordination polyhedron about the pendant arm. The large difference between the two correlation times indicates that the system is highly flexible and its relaxivity strongly limited by the relatively short value of  $\tau_{\text{RL}}$ . The correlation of the two types of motions is described by the parameter  $S^2$ , whose value is between zero (completely independent motions) and one (totally correlated motions). Data relative to micellar assemblies formed by ditopic complexes are reported here for the first time and can only be compared to those for corresponding aggregated monomeric complexes.<sup>22</sup> Larger values for the parameters  $\tau_{\text{RG}}$  and  $\tau_{\text{RL}}$  are calculated for  $\text{Gd}_2\text{L2}$  to indicate both the presence of larger aggregates and a more restricted local rotational motion. This is supported by the larger value of  $S^2$  and can be interpreted as the consequence of larger steric interactions involving the bulkier dimeric units. It is worth noting that the relaxivity values at 310 K are higher than those at 298 K over the entire range of frequencies. This indicates some limiting effects arising from the non-optimal value of the rate of water exchange.



**Fig. 2**  $1/T_1$   $^1\text{H}$  NMRD profiles of  $\text{Gd}_2\text{L2}$  at 298 (●) and 310 K (○). The solid lines are calculated with the parameters of Table 2.

The temperature dependence of the proton relaxivity was also measured in order to obtain an independent evaluation of the residence lifetime of the coordinated water molecule, as the value of this parameter is generally not determined with high accuracy from the NMRD profiles. The profile is characterized by a broad peak centered at about 310–315 K (Fig. 3): this implies that around physiological temperatures the relaxivity reaches the maximum value and it is not limited by the relatively slow exchange of the bound water molecule. A good fit of the data was obtained with a  $^{298} \tau_{\text{M}}$  value of 700 ns (Table 2), to confirm that this parameter is not significantly affected by the aggregation process. Finally, it is worth noting that the satisfactory fit of the calculated curve to the



**Fig. 3** Variable-temperature  $^1\text{H}$  NMR relaxation data for  $\text{Gd}_2\text{L2}$  at 20 MHz normalized to 1 mM concentration of gadolinium. The solid line represents the best results of the fitting to the experimental data (see Table 2), whereas the dotted and dashed lines represent the calculated outer- and inner-sphere contributions to the overall relaxivity.

experimental data provides further support to the results of the NMRD study.

**Binding to HSA.** The presence of a long-chain alkyl group offers to  $\text{Gd}_2\text{L2}$  the possibility to form supramolecular adducts with HSA characterized by enhanced relaxivity.<sup>12</sup> The non-covalent hydrophobic interaction was investigated by titrating a dilute solution of the complex (0.07 mM) with the protein up to a 1.1 mM concentration, and by following the resulting increase of the observed longitudinal proton relaxation rate,  $R_1$ , at 20 MHz and 298 K (Fig. S1†). A pronounced enhancement of  $R_1$  is observed at low concentration of the protein, indicating a rather strong affinity of the complex for the hydrophobic sites of HSA. The binding isotherm was analyzed in terms of the PRE equations to yield a value of  $2.6 \times 10^5 \text{ M}^{-1}$  for the association constant,  $K_A$ , and a value of  $34.1 \text{ mM}^{-1} \text{ s}^{-1}$  for the relaxivity of the protein-conjugate,  $r_{1p}^b$ .<sup>12</sup> The  $K_A$  value provides just a simple indication of the binding strength since different binding sites of different affinity are likely to be present on the protein.<sup>24</sup> However, the value is quite similar to that found for the interaction with high-affinity fatty acid sites on HSA of  $\text{GdDOTA-C}_{11}$ <sup>24</sup> and  $\text{GdAAZTA-C}_{17}$  derivatives.<sup>12</sup>

The value of  $r_{1p}^b$  is in the upper range of values of the HSA-conjugates of complexes with  $q = 1$ , where the relaxivity is limited by both the presence of a fast internal motion and a relatively long residence lifetime of the bound water molecule. In agreement with the results obtained on the micellar system, the  $r_{1p}^b$  of the HSA-conjugated complex appears to be limited mostly by the high flexibility of the alkyl chain. In fact, the  $r_{1p}^b$  increases with temperature up to ca. 310 K and then it decreases (Fig. S2†) again. The maximum of relaxivity is then at physiological temperatures, where it has a value of  $38.1 \text{ mM}^{-1} \text{ s}^{-1}$ . The NMRD profiles do not add further information. The relaxivity presents a broad peak centered around 25 MHz, typical of this type of slowly tumbling macromolecular systems, whose value is higher than those found for several other  $\text{Gd(III)}$  chelates conjugated to HSA, suggesting a more restricted internal rotation of the dimeric unit about the alkyl chain.

## Conclusions

The Ugi four-component reaction has been exploited to obtain, in a single pot, bifunctional ditopic chelators by using DOTA monoamide derivatives as amino and acid components. This synthetic approach offers several advantages: i) quick reaction times with reasonable yields; ii) the possibility of modulating the chemical properties of the chelator (ligating groups, charge, denticity) and the pendant central functional moiety; and iii) the improved possibility of rapidly generating a large number of derivatives to meet different chemical needs. In addition, this synthetic approach may involve the use of  $\text{Ln(III)}$  complexes as reactants and this allows the easy preparation of a variety of heterodimetallic derivatives. The wide variety of magnetic, optical and nuclear properties of the lanthanide cations could then be exploited to prepare homo- and heterodimetallic systems for both diagnostic and therapeutic applications. Two representative  $\text{Gd(III)}$  complexes,  $\text{Gd}_2\text{L1}$  and  $\text{Gd}_2\text{L2}$ , were investigated in detail by relaxometric techniques in aqueous solution.  $\text{Gd}_2\text{L1}$  shows the typical properties of ditopic complexes: a higher relaxivity (per Gd) with regard to the monomer as a consequence of the slower rotation.  $\text{Gd}_2\text{L2}$  forms micelles at concentrations  $< 0.1 \text{ mM}$ , whose relaxivity is ca. 25% higher (per Gd) than that of corresponding monomeric complexes in the aggregated form.<sup>12,21</sup> This enhanced relaxivity appears to be the consequence of the combined effect of i) a larger molecular dimensions of the micelles and ii) more hindered local rotation of the dimer about the alkyl chain. Then, micellar aggregates formed by multimeric complexes have the favorable property of being characterized by a larger number of paramagnetic units (higher molecular relaxivity) and by a lower anisotropic rotational dynamics (higher relaxivity per Gd). Finally, it is worth noting that this approach has general validity, and it could be easily applied to the preparation of a large variety of bifunctional ditopic metal chelates characterized by different size, charge, coordination polyhedra and hydration number.

## Experimental

### Materials and instrumentation

All chemicals were purchased from Sigma-Aldrich Co. and were used without purification unless otherwise stated. NMR spectra were recorded on a JEOL Eclipse Plus 400 (operating at 9.4 Tesla). ESI mass spectra were recorded on a Waters Micromass ZQ. Infrared (IR) spectra were recorded in the range  $4000\text{--}400 \text{ cm}^{-1}$  at  $4 \text{ cm}^{-1}$  resolution using a Bruker Equinox 55 spectrometer. MW-promoted reactions were carried out in a CEM Discover oven. Sonochemical apparatus used in the present work was developed by Prof. G. Cravotto's group (Department of Drug Science and Technology, University of Torino) in collaboration with Danacamerini s.a.s. (Torino).<sup>25</sup>  $\text{DOTAMA}(t\text{-BuO})_3\text{C}_6\text{OH}$ ,  $\text{DOTAMA}(t\text{-BuO})_3\text{En}$  and  $\text{DOTAMA}(\text{OtBu})_3\text{C}_6\text{NH}_2$  were prepared following a reported procedure.<sup>11</sup>

### General Ugi 4CR – Conventional heating protocol

A solution of  $\text{DOTAMA}(\text{OtBu})_3\text{En}$  (61.4 mg; 0.1 mmol), aldehyde (0.1 mmol),  $\text{DOTAMA}(\text{OtBu})_3\text{C}_6\text{OH}$  (68.6 mg; 0.1 mmol) and isocyanide (0.1 mmol) in MeOH (3 mL) was refluxed for 6 h. Then, the solvent was evaporated *in vacuo* and the residue was purified

by column chromatography (silica gel, eluent: CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 95:5, v/v) to give a light yellow oil.

#### General Ugi 4CR – Microwave protocol

A solution of DOTAMA(OtBu)<sub>3</sub>En (61.4 mg; 0.1 mmol), aldehyde (0.1 mmol), DOTAMA(OtBu)<sub>3</sub>C<sub>6</sub>OH (68.6 mg; 0.1 mmol) and isocyanide (0.1 mmol) in MeOH (4 mL) was irradiated with microwaves at 100 W ( $P_{\max}$  = 2 bar) for 1 h. The solvent was then evaporated *in vacuo* and the residue was purified by column chromatography (silica gel, eluent: CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 95:5, v/v) to give a light yellow oil.

#### General Ugi 4CR – Power ultrasound protocol

A solution of DOTAMA(OtBu)<sub>3</sub>En (61.4 mg; 0.1 mmol), aldehyde (0.1 mmol), DOTAMA(OtBu)<sub>3</sub>C<sub>6</sub>OH (68.6 mg; 0.1 mmol) and isocyanide (0.1 mmol) in MeOH (4 mL) was sonicated in an ultrasound reactor at 60 W (20.3 kHz) for 3 h. Then, the solvent was evaporated *in vacuo* and the residue was purified by column chromatography (silica gel, eluent: CH<sub>2</sub>Cl<sub>2</sub>–MeOH, 95:5, v/v) to give a light yellow oil.

#### 1,15-Bis[4,7,10-tris(*t*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododec-1-yl]-6-[(*N*-cyclohexyl)carboxamidomethyl]-2,7,14-trioxo-3,6,13-triazapentadecane (4)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 8.58 (d, 1H,  $J$  = 8.0 Hz), 8.09 (t, 2H,  $J$  = 5.5 Hz), 3.76–1.91 (m, 57H), 1.86 (m, 2H), 1.72 (m, 2H), 1.63–1.08 (m, 14H), 1.42 (s, 36H), 1.41 (s, 18H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 175.1, 172.6, 172.5, 171.4, 169.5, 82.0, 81.9, 56.8, 56.3, 55.8, 55.7, 53.5–48.0 (bs), 38.7, 37.6, 33.3, 32.9, 32.6, 29.7, 28.5, 28.1, 28.0, 26.3, 25.5, 25.4, 25.2, 24.8, 24.5, 22.7, 14.2. ESI-MS ( $m/z$ ): found 1422.56 (M + H<sup>+</sup>, 10%), (calc. 1422.00), found 1444.53 (M + Na<sup>+</sup>, 5%), (calc. 1443.98), found 711.85 [(M + 2H<sup>+</sup>)/2, 100%], (calc. 711.50). IR spectrum (KBr disk): 3245, 2978, 2930, 2880, 1748, 1668, 1367, 1231, 1165, 1108 cm<sup>−1</sup>. Elemental analysis calcd (%) for C<sub>72</sub>H<sub>132</sub>N<sub>12</sub>O<sub>16</sub>: C, 60.82; H, 9.36; N, 11.82; found: C, 60.76; H, 9.21; N, 11.65.

#### 1,15-Bis[4,7,10-tris(*t*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododec-1-yl]-6-[(*N*-cyclohexyl)(1-nonyl)carboxamidomethyl]-2,7,14-trioxo-3,6,13-triazapentadecane (5)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 8.65 (d, 1H,  $J$  = 5.5 Hz), 8.16 (t, 1H,  $J$  = 5.5 Hz), 8.06 (t, 1H,  $J$  = 5.1 Hz), 3.86–1.89 (bs, 56H), 1.83 (m, 2H), 1.74–1.34 (m, 20H), 1.44 (s, 36H), 1.43 (s, 18H), 1.32–1.08 (m, 12H), 0.85 (t, 3H,  $J$  = 7.0 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 172.9, 172.7, 172.5, 172.2, 171.6, 81.9, 81.8, 56.4, 55.9, 55.8, 55.7, 53.5, 58.9–47.5 (bs), 34.0, 32.9, 32.6, 31.9, 29.8, 29.6, 29.4, 28.2, 28.1, 28.0, 26.6, 26.5, 26.3, 26.2, 25.6, 25.4, 25.3, 25.2, 25.1, 24.9, 24.7, 24.6, 22.7, 14.2. ESI-MS ( $m/z$ ): found 1548.46 (M + H<sup>+</sup>, 6%), (calc. 1548.14), found 1570.49 (M + Na<sup>+</sup>, 3%), (calc. 1570.12), found 775.27 [(M + 2H<sup>+</sup>)/2, 100%], (calc. 774.57). IR spectrum (KBr disk) 3439, 3258, 2974, 2925, 2852, 1723, 1665, 1367, 1230, 1165, 1109 cm<sup>−1</sup>. Elemental analysis calcd (%) for C<sub>81</sub>H<sub>150</sub>N<sub>12</sub>O<sub>16</sub>: C, 62.84; H, 9.77; N, 10.86; found: C, 62.91; H, 9.88; N, 10.94.

#### 1,15-Bis[4,7,10-tris(*t*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododec-1-yl]-6-[(*N*-cyclohexyl-1-(*N*-Cbz-5-aminopentyl)carboxamidomethyl)-2,7,14-trioxo-3,6,13-triazapentadecane (6)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 8.56 (d, 1H,  $J$  = 7.3 Hz), 8.05 (t, 2H,  $J$  = 5.8 Hz), 7.30 (m, 5H), 5.03 (s, 2H), 3.67–1.72 (m, 58H), 1.68 (m, 2H), 1.66–0.74 (m, 22H), 1.41 (s, 36H), 1.40 (s, 18H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 175.1, 172.6, 172.5, 172.1, 171.5, 156.6, 156.4, 128.6, 128.5, 128.1, 81.9, 81.8, 66.5, 66.4, 56.3, 55.8, 55.8, 53.5, 52.7–48.3 (bs), 40.9, 38.8, 38.6, 38.4, 37.6, 32.8, 32.6, 32.5, 29.7, 29.6, 29.4, 28.1, 28.0, 26.4, 26.0, 25.4, 25.2, 24.7, 22.7, 14.2. ESI-MS ( $m/z$ ): found 1642.45 (M + H<sup>+</sup>, 8%), (calc. 1641.19), found 1663.37 (M + Na<sup>+</sup>, 3%), (calc. 1663.10), found 821.79 [(M + 2H<sup>+</sup>)/2, 100%], (calc. 821.06). IR spectrum (KBr disk) 3240, 3062, 2973, 2925, 2861, 1731, 1674, 1544, 1455, 1367, 1230, 1165, 1108 cm<sup>−1</sup>. Elemental analysis calcd (%) for C<sub>85</sub>H<sub>149</sub>N<sub>12</sub>O<sub>16</sub>: C, 62.21; H, 9.15; N, 11.09; found: C, 62.16; H, 9.21; N, 10.97.

#### 1,15-Bis[4,7,10-tris(*t*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododec-1-yl]-6-[(*N*-octadecyl)carboxamidomethyl]-2,7,14-trioxo-3,6,13-triazapentadecane (7)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 8.68 (t, 1H,  $J$  = 5.5 Hz), 8.06 (t, 1H,  $J$  = 5.8 Hz), 7.99 (t, 1H,  $J$  = 5.5 Hz), 3.67–2.00 (m, 60H), 1.80–1.45 (m, 8H), 1.41 (s, 36H), 1.39 (s, 18H), 1.19 (m, 30H), 0.82 (t, 3H,  $J$  = 7.0 Hz). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 175.1, 172.6, 172.5, 171.4, 171.1, 170.4, 169.3, 82.0, 81.9, 81.8, 64.3, 56.3, 55.8, 55.7, 53.1–47.6 (bs), 39.8, 39.4, 38.7, 33.1, 33.0, 31.9, 29.8, 29.4, 28.7, 28.2, 28.1, 28.0, 27.2, 26.4, 25.4, 24.6, 22.7, 14.2. ESI-MS ( $m/z$ ): found 1592.50 (M + H<sup>+</sup>, 5%), (calc. 1592.20), found 1614.52 (M + Na<sup>+</sup>, 2%), (calc. 1614.18), found 797.30 [(M + 2H<sup>+</sup>)/2, 25%], (calc. 796.60), found 531.66 [(M + 3H<sup>+</sup>)/3, 100%], (calc. 531.40). IR spectrum (KBr disk) 3444, 3271, 2970, 2931, 2848, 1725, 1660, 1367, 1231, 1163, 1110 cm<sup>−1</sup>. Elemental analysis calcd (%) for C<sub>84</sub>H<sub>158</sub>N<sub>12</sub>O<sub>17</sub>: C, 63.36; H, 10.00; N, 10.56; found: C, 63.56; H, 10.11; N, 10.64.

#### 1,15-Bis[4,7,10-tris(*t*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododec-1-yl]-6-[(*N*-methoxycarbonylmethyl)carboxamidomethyl]-2,7,14-trioxo-3,6,13-triazapentadecane (8)

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 8.75 (d, 1H,  $J$  = 7.4 Hz), 8.06 (t, 2H,  $J$  = 5.5 Hz), 3.72 (s, 3H), 3.68–2.10 (m, 60H), 1.95–1.55 (m, 6H), 1.42 (s, 36H), 1.41 (s, 18H). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 175.1, 172.6, 172.5, 171.4, 170.2, 169.5, 81.9, 81.9, 56.8, 56.3, 55.8, 55.7, 53.5–48.0 (bs), 41.8, 38.7, 37.6, 33.3, 32.9, 29.7, 28.5, 28.1, 28.0, 26.3, 25.5, 25.4, 25.2, 24.5, 14.2. ESI-MS ( $m/z$ ): found 1412.55 (M + H<sup>+</sup>, 8%), (calc. 1411.94), found 1434.52 (M + Na<sup>+</sup>, 10%), (calc. 1433.92), found 706.81 [(M + 2H<sup>+</sup>)/2, 100%], (calc. 706.47). IR spectrum (KBr disk) 3252, 2975, 2935, 2862, 1728, 1666, 1365, 1229, 1164, 1107 cm<sup>−1</sup>. Elemental analysis calcd (%) for C<sub>69</sub>H<sub>126</sub>N<sub>12</sub>O<sub>18</sub>: C, 58.70; H, 9.00; N, 11.91; found: C, 58.86; H, 9.07; N, 12.04.

#### Synthesis of ligands L1 and L2

The compound obtained by the Ugi four-component reaction was dissolved in a mixture of CH<sub>2</sub>Cl<sub>2</sub> and trifluoroacetic acid (1:1 v/v,



2 mL) and stirred at room temperature overnight. The solution was then evaporated *in vacuo* and the product was precipitated with excess diethyl ether, isolated by centrifugation, washed thoroughly with diethyl ether and dried *in vacuo*, to give the pure desired product as amorphous white solid.

**1,15-Bis[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododec-1-yl]-6-[(N-cyclohexyl) carboxamidomethyl]-2,7,14-trioxo-3,6,13-triazapentadecane (L1)**

$^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 400 MHz):  $\delta$  = 4.11–3.20 (m, 54H), 2.48 (m, 1H), 2.28 (m, 2H), 1.84 (m, 2H), 1.75 (m, 2H), 1.68–1.17 (m, 14H).  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 100 MHz):  $\delta$  = 175.7, 175.2, 169.9, 169.0, 65.6, 54.8–52.6 (bs), 51.1–49.0 (bs), 48.3, 48.1, 39.0, 37.5, 37.1, 32.6, 33.3, 28.7, 27.2, 26.6, 26.1, 25.2, 24.8, 24.6, 24.4, 14.1. ESI-MS ( $m/z$ ): found 1086.01 ( $\text{M} + \text{H}^+$ , 8%), (calc. 1085.62), found 1108.05 ( $\text{M} + \text{Na}^+$ , 10%), (calc. 1107.60), found 543.49 [ $(\text{M} + 2\text{H}^+)/2$ , 100%], (calc. 543.31). IR spectrum (KBr disk) 3361, 3199, 2917, 2853, 1649, 1463, 1383, 1092  $\text{cm}^{-1}$ . Elemental analysis calcd (%) for  $\text{C}_{48}\text{H}_{84}\text{N}_{12}\text{O}_{16}\cdot 4\text{CF}_3\text{COOH}$ : C, 43.64; H, 5.75; N, 10.90; found: C, 43.35; H, 5.51; N, 10.75.

**1,15-Bis[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraazacyclododec-1-yl]-6-[(N-octadecyl) carboxamidomethyl]-2,7,14-trioxo-3,6,13-triazapentadecane (L2)**

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 400 MHz):  $\delta$  = 4.16–3.04 (m, 58H), 2.49 (t, 1H,  $J$  = 7.0 Hz), 2.30 (m, 1H,  $J$  = 7.0 Hz), 1.62–1.39 (m, 8H), 1.28 (s, 30H), 0.89 (t, 3H,  $J$  = 7.0 Hz).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 100 MHz):  $\delta$  = 175.1, 175.5, 170.2, 169.9, 54.9–53.1 (bs), 51.1–49.0 (bs), 50.0, 49.5, 39.4, 39.3, 39.0, 32.5, 32.2, 31.8, 29.5, 29.2, 28.6, 26.8, 26.2, 26.1, 24.5, 24.3, 22.4, 13.2. ESI-MS ( $m/z$ ): found 1256.25 ( $\text{M} + \text{H}^+$ , 10%), (calc. 1255.81), found 628.63 [ $(\text{M} + 2\text{H}^+)/2$ , 70%], (calc. 628.41), found 419.57 [ $(\text{M} + 3\text{H}^+)/3$ , 100%], (calc. 419.28). IR spectrum (KBr disk) 3375, 3187, 2925, 2862, 1655, 1471, 1375, 1096  $\text{cm}^{-1}$ . Elemental analysis calcd (%) for  $\text{C}_{60}\text{H}_{110}\text{N}_{12}\text{O}_{16}\cdot 4\text{CF}_3\text{COOH}$ : C, 47.72; H, 6.71; N, 9.82; found: C, 47.44; H, 6.59; N, 9.65.

### Synthesis of the heteroditopic Y–Gd complex

A solution of Y–DOTAMAE<sub>n</sub> (10 mg; 0.017 mmol), paraformaldehyde (0.5 mg, 0.017 mmol), Gd–DOTAMAC<sub>6</sub>OH (11.5 mg; 0.017 mmol) and cyclohexylisocyanide (2.1 mg, 0.017 mmol) in MeOH (2 mL) was refluxed for 4 h. Then, the solvent was evaporated *in vacuo* and the residue was purified by LC-MS (Waters FractionLynx autopurification system equipped with Waters 2996 diode array; Waters Atlantis RPC18 19/100 column, final purity >90%). A white powder was obtained (11.3 mg, 47% yield); ESI-MS  $m/z$ , main signals: found 1325.04, 1326.02, 1327.06 ( $\text{M} + \text{H}^+$ ), (calc. for  $\text{C}_{48}\text{H}_{79}\text{GdN}_{12}\text{O}_{16}\text{Y}$ : 1326.37), 1347.00, 1348.05, 1349.08 ( $\text{M} + \text{Na}^+$ ), (calc. 1348.36), isotopic distribution consistent with Y–Gd complex.

### $^1\text{H}$ relaxation measurements

The water proton longitudinal relaxation rates as a function of the magnetic field strength were measured with a Stelar Spinmaster Spectrometer FFC-2000 (Mede, Pv, Italy) on about 0.5–2.5 mM gadolinium solutions in non-deuterated water. The exact

concentrations of gadolinium were determined by measurement of bulk magnetic susceptibility shifts of a *t*BuOH signal and/or ICP-MS. The  $^1\text{H}$   $T_1$  relaxation times were acquired by the standard inversion recovery method with typical 90° pulse width of 3.5 ms, 16 experiments of 4 scans. The reproducibility of the  $T_1$  data was  $\pm 5\%$ . The temperature was controlled with a Stelar VTC-91 airflow heater equipped with a calibrated copper–constantan thermocouple (uncertainty of  $\pm 0.1^\circ\text{C}$ ). The data were measured in the range of magnetic fields from 0.00024 to 1.6 T (corresponding to 0.01–70 MHz proton Larmor frequencies).

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