



## A bifunctional chelator featuring the DOTAM-Gly-L-Phe-OH structural subunit: *en route* toward homo- and heterobimetallic lanthanide(III) complexes as PARACEST MRI contrast agents

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### ABSTRACT

A new synthesis of a bifunctional chelator possessing DOTAM-Gly-L-Phe-OH and DO3A chelating cages interconnected by an oligoamide chain has been achieved via HBTU-mediated coupling from easily accessible building blocks. Both homo- and heterobimetallic lanthanide(III) complexes derived from this bifunctional chelator have been prepared in moderate yields. The CEST spectrum acquired for homobimetallic Eu<sup>3+</sup> complex showed this molecule to be a promising PARACEST MRI contrast agent whereas the Eu<sup>3+</sup>/Tm<sup>3+</sup> heterobimetallic complex lacked a useful CEST signal.

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Since its first synthesis in 1961,<sup>1</sup> the chemistry of cyclen,<sup>2</sup> its derivatives, and their complexes with transition metals, rare earth elements, actinides, and lanthanides has experienced vigorous development. These complexes are suitable for a variety of applications in modern chemistry, biochemistry, and medicine. As such, cyclen-based complexes are nowadays being studied and used as antimicrobial agents, synthetic nucleases, metal sensors, fluorescent, and luminescent probes as well as contrast agents (CAs) in positron emission tomography (PET) or magnetic resonance (MR) imaging.

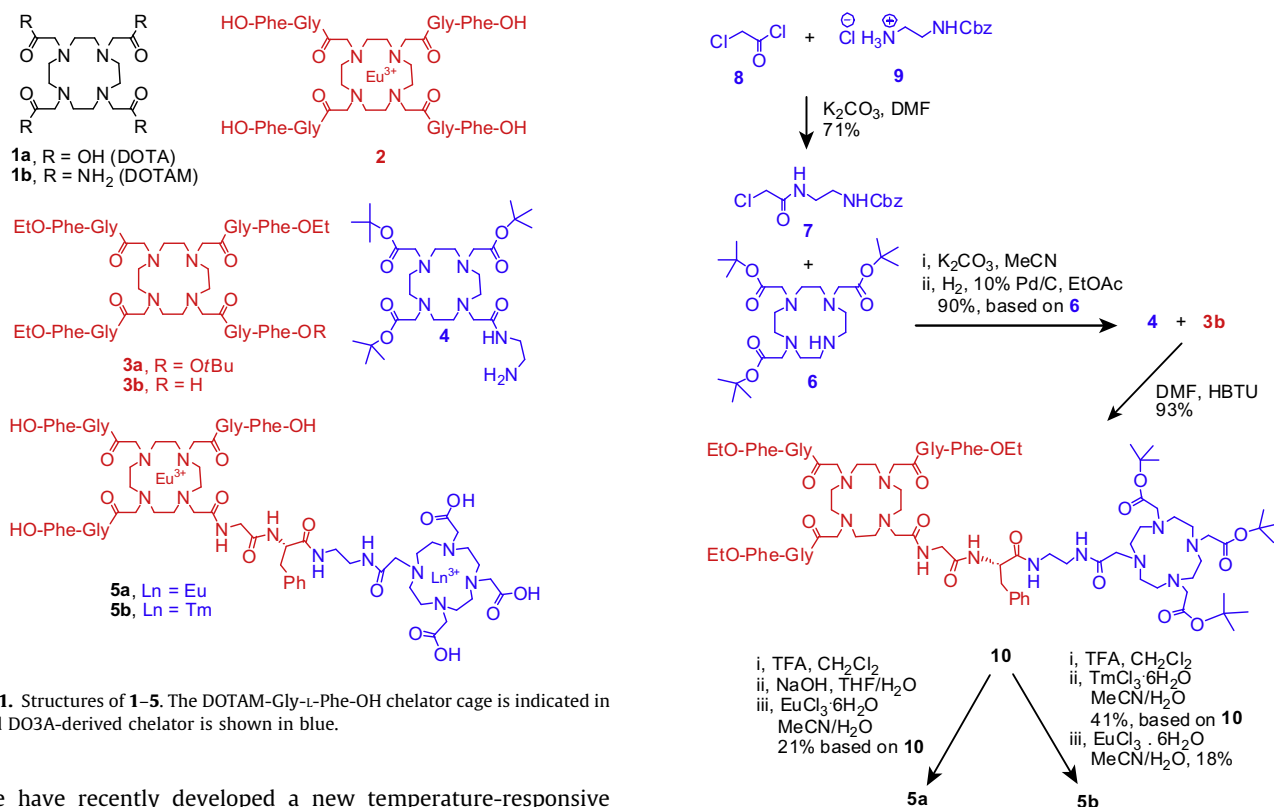
A major category of MRI contrast agents are paramagnetic complexes of Gd<sup>3+</sup> which are capable of altering the T<sub>1</sub> of water protons, resulting in increased brightness in T<sub>1</sub>-weighted images.<sup>3</sup> More recently, a new group of MRI CAs was introduced that modulate signal by chemical exchange saturation transfer (CEST).<sup>4</sup> Subsequently a large number of paramagnetic ion-containing lanthanide(III) complexes have been prepared which were capable of inducing large hyperfine shifts of coordinated water protons as well as other exchangeable protons present in proximity to the lanthanide(III) center.<sup>5</sup> The term PARACEST<sup>6</sup> MRI CAs has been coined to describe these molecules.

Although several examples of multimeric PARACEST MRI CAs appeared recently,<sup>7</sup> the vast majority of these molecules contain

only one type of chelator unit capable of binding one lanthanide(III) cation. Almost all of these complexes are derivatives of DOTA (**1a**, Fig. 1) or DOTAM (**1b**, Fig. 1). Curiously, to the best of our knowledge no heterometallic MRI CAs containing two or more different lanthanide(III) cations have been studied so far.

Based on the limited number of literature reports, the design and synthesis of cyclen-derived lanthanide(III)-based heterometallic complexes appears to be a challenging task. The first example was described in 2003 by Faulkner and Pope.<sup>8</sup> The Tb<sup>3+</sup> complex of DO3A was first formed and two equivalents of this complex was treated with DTPA-like chelator, followed by the introduction of Yb<sup>3+</sup>. In the more recent work that appeared in 2006, Tremblay and Sames employed selective sequential metalation based on the differences in kinetic stability of DOTA and DTPA-derived complexes.<sup>9</sup> Faulkner's group has also accomplished the synthesis of heterotetrametallic complex containing two different lanthanide(III) cations. For this work, two homobimetallic complexes served as precursors, which were joined by an azo (–N=N–) linkage.<sup>10</sup> The same group of authors also investigated the possibilities of the orthogonal protection/deprotection strategy applied to the bifunctional DOTA-derived ligand; followed by a sequential metalation using two different lanthanide(III) salts.<sup>11</sup> Although not strictly lanthanide(III) containing, it is worth mentioning that very recently the Ugi multicomponent reaction has been used to prepare a heterobimetallic complex (DO3A-based) containing Gd<sup>3+</sup> and Y<sup>3+</sup> cations.<sup>12</sup>

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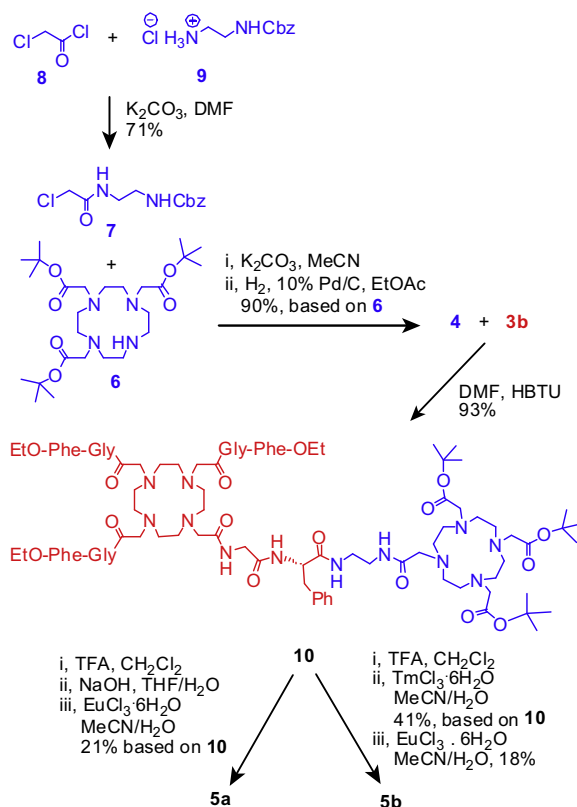


**Figure 1.** Structures of **1**–**5**. The DOTAM-Gly-L-Phe-OH chelator cage is indicated in red and DO3A-derived chelator is shown in blue.

We have recently developed a new temperature-responsive PARACEST MRI CA,  $\text{Eu}^{3+}$  DOTAM-Gly-L-Phe-OH<sup>13</sup> (**2**, Fig. 1) exhibiting a unique sensitivity in the physiological temperature range (36–40 °C). As a part of our broad research program devoted to the development of new PARACEST MRI CAs, we have also prepared several asymmetrically substituted cyclen derivatives based on the parent structure of **2**.<sup>14</sup> Among them, the carboxylic acid derivative **3b**, obtained after orthogonal deprotection of an ester **3a** (Fig. 1), is well suited for amide bond formation via coupling with primary amines. The accessibility of an amine counterpart **4** (Fig. 1), prepared by modifying a recently described synthetic protocol,<sup>15</sup> prompted us to investigate the synthesis of bifunctional chelator and its homo- (**5a**, Fig. 1) and heterobimetallic (**5b**, Fig. 1) complexes. The results of these synthetic studies along with the magnetic (PARACEST) properties of both complexes (Fig. 1) are described herein.

The synthesis of the building block **3b** has been accomplished by a synthetic methodology developed in our laboratory.<sup>14</sup> A modified synthesis of amine **4** was developed as indicated in Scheme 1 and described in Supplementary data. Acylation of mono-*N*-Cbz ethylenediamine hydrochloride (**9**) with chloroacetyl chloride (**8**) resulted in the formation of electrophile **7** in 71% yield. Alkylation of commercially available DO3A *tert*-butyl ester (**6**) with electrophile **7**, followed by a reductive removal of the Cbz group proceeded with the formation of amine **4** in high yield (94%, based on **6**, Scheme 1) and purity sufficient for the subsequent reaction. With building blocks **3b** and **4** in hand, we investigated the possibilities of the amide bond formation. HBTU in warm (60 °C) DMF in the presence of a base (DIPEA) afforded superior results, and orthogonally protected bifunctional ligand **10** was prepared in 93% yield as indicated in Scheme 1 and described in Supplementary data.

An oligoamide linker joining the two chelating cages present in **10** was found to be sensitive toward both acid and base-catalyzed hydrolysis. The unwanted cleavage occurred on the amidic bond connecting the phenylalanine unit with ethylene diamine side chain. A similar behavior was previously observed with cystamine-modified DOTAM-Gly-L-Phe-OH. In fact, significant portions



of chelator **10** have been cleaved after subjecting the ligand **10** to TFA-mediated removal of *tert*-butyl ester functionalities. Despite this unexpected problem, we were able to prepare homo- and heterobimetallic complexes **5** in moderate yield.

To prepare a homobimetallic complex **5a**, we first deprotected the *tert*-butyl ester functionalities and then subjected the partially deprotected molecule to saponification conditions in order to remove ethyl ester protecting groups. The mixture of the desired deprotected ligand along with the products of the hydrolysis on the oligoamide chain has been subjected to exhaustive metalation with  $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ . The homobimetallic complex **5a** was purified by HPLC and was obtained in 21% yield, based on the ligand **10** (Scheme 1). The absence of free  $\text{Eu}^{3+}$  in the product was confirmed by Xylenol Orange test.<sup>16</sup> Complex **5a** was characterized by HR-ESI-MS as described in Supplementary data.

To overcome the problem associated with the oligoamide linker hydrolysis, we decided to retain the ethyl ester protecting groups while trying to prepare heterobimetallic complex **5b**. Thus, *tert*-butyl ester-deprotected ligand was subjected to metalation with one equivalent of  $\text{TmCl}_3 \cdot 6\text{H}_2\text{O}$  (Scheme 1). This reaction was carried out at low temperature (0 °C) and monometallic complex was obtained in 41% yield after HPLC purification. Notably, no trace of the bimetallic complex was found (UPLC-ESI-HRMS). Treatment of the monometallic complex with 2.5 equiv of  $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ , followed by HPLC purification afforded heterobimetallic complex **5b** in 18% yield; overall yield based on the ligand **10** was 7% (Scheme 1). The product was characterized by HR-ESI-MS and it did not contain any free lanthanide(III) cations as indicated by Xylenol Orange test.<sup>16</sup>

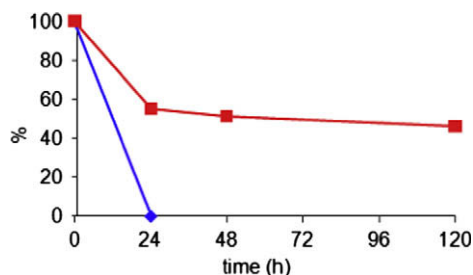
To confirm that the sequential metalation took place as indicated above, that is that DO3A unit was metalated first, we initially turned to <sup>1</sup>H NMR spectrometry. As depicted in Supplementary data, the spectra of both molecules, the unmetalated ligand and the corresponding monometalated  $\text{Tm}^{3+}$  complex, exhibit

extensive signal broadening and are therefore uninformative. In fact, in both spectra only three groups of broad unresolved signals at ca. 1.00–1.50 ppm, 2.50–4.50 ppm, and 7.00–8.50 ppm can be observed, precluding any structural assignment. It is also important to point out that none of the characteristic resonances<sup>17</sup> due to the presence of paramagnetic  $Tm^{3+}$  were observed.

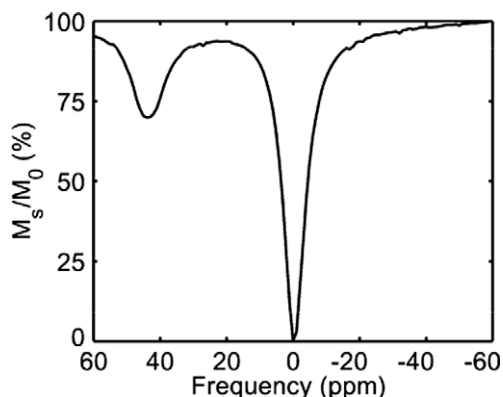
The problem was solved by performing a time course study (Fig. 2). DOTA (**1**) as a model compound for the DO3A chelator along with ligand **3b** was treated with one equivalent of  $TmCl_3 \cdot 6H_2O$  at 0 °C in aq MeCN. Samples were withdrawn after 24, 48, 72, and 120 h and were analyzed by HR-ESI-MS. It was found that the metalation of DOTA (**1**) was completed after 24 h, whereas significant portions (46%) of ligand **3b** remained unmetalated even after 120 h (Fig. 2). Based on the results of this kinetic study it may be reasonably assumed that the first site of the metalation in ligand **10** is the DO3A cage. This observation is consistent with our experience with the metalation of a disulfide dimer derived from DOTAM-Gly-L-Phe-OH was found to be quite sluggish.<sup>7f</sup>

After we obtained the desired bimetallic complexes **5**, we evaluated the PARACEST effect. A CEST spectrum of the homobimetallic complex **5a** was acquired following a previously established experimental protocol.<sup>13b</sup> The CEST effect (ca. 30%) due to the bound water<sup>18</sup> was observed at ca. 45 ppm (Fig. 3), which is consistent with the data for the parent CA **2**.<sup>13b</sup> It is important to point out that the presence of the second  $Eu^{3+}$  slightly decreased the overall CEST effect associated with **5a**. Since  $Eu^{3+}$ -DOTA does not have a detectable off-resonance CEST effect,<sup>19</sup> it was therefore expected that **5a** would not have a greater CEST effect than **2**.

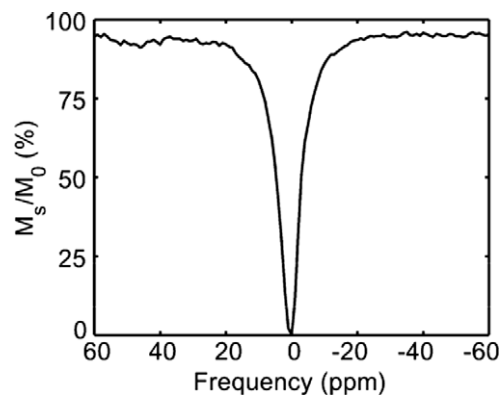
The CEST spectrum of the heterobimetallic complex **5b** shows a marked reduction in the signal due to bound water at the  $Eu^{3+}$  center (ca. 45 ppm), Figure 4. It is evident that the chelated  $Tm^{3+}$  has a large effect on the signal generated by water coordinated at the



**Figure 2.** Amount of unmetalated material present (%) in the reaction mixture after metalation of DOTA (**1**, blue curve,  $\blacklozenge$ ) and ligand **3b** (red curve,  $\blacksquare$ ) with  $TmCl_3 \cdot 6H_2O$ .



**Figure 3.** CEST spectrum of the homobimetallic complex **5a** (10 mM in water, 37 °C, saturation power  $B_1$  14  $\mu T$ , presaturation time TS 10 s).



**Figure 4.** CEST spectrum of heterobimetallic complex **5b** (20 mM, water, rt, saturation power  $B_1$  14  $\mu T$ , presaturation time TS 10 s).

$Eu^{3+}$  center. This may occur by acceleration of the rate of relaxation of water at the  $Eu^{3+}$  center by the proximal  $Tm^{3+}$ , but determination of the mechanism for the observed effect will require further study. This result highlights that the choice of lanthanides and their chelating ligand in the heterobimetallic complexes can profoundly affect the resulting CEST properties.

In summary we have developed a concise synthetic strategy toward a bifunctional chelator featuring DOTAM-Gly-L-Phe-OH subunit. We were able to prepare homo- and heterobimetallic lanthanide(III) complexes derived from this chelator in moderate chemical yield.

One of the drawbacks of our current design is the unwanted hydrolysis of the oligoamide chain connecting the two chelating units. Other possibilities (e.g.,  $Cu^{+}$ -catalyzed Huisgen 'click' dipolar cycloaddition<sup>20</sup>) are being explored in order to interconnect the two chelating subunits with a structural element that is not prone to facile hydrolysis.

The PARACEST spectrum of the  $Eu^{3+}$ -containing homobimetallic complex **5a** was determined. The results were consistent with those for the parent CA **2**, although the signal due to bound water was slightly diminished. In contrast, the signal due to bound water at the  $Eu^{3+}$  center was obliterated by the presence of a proximal coordinated  $Tm^{3+}$ . The synthesis of other heterobimetallic complexes and evaluation of their PARACEST properties are currently in progress and will be reported in due course.

#### Acknowledgment

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#### Supplementary data

Supplementary data (full experimental details and spectroscopic characterization of new compounds **5a**, **5b**, **7**, and **10**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.086.

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