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Modulating water-exchange rates of lanthanide(III)-containing polyaminopolycarboxylate-type complexes using polyethylene glycol

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Abstract

We have synthesized a series of Ln^{III}-containing polyethylene glycol conjugates and studied the structural and electronic properties of these complexes. These studies demonstrate that polyethylene glycol can be used to fine-tune water-exchange rates of Ln^{III}-containing polyaminopolycarboxylate-type complexes; this control is desirable in developing Ln^{III}-containing contrast agents for magnetic resonance imaging.

The efficiency of Ln^{III}-containing complexes as conventional (T_1 -reducing) and paramagnetic chemical exchange saturation transfer (PARACEST) contrast agents for magnetic resonance imaging (MRI) is governed by the interactions among the structural and electronic properties of these complexes and by the interactions of these complexes with the environment.^{1,2} The optimal value for a specific parameter is a moving target that varies as a function of magnetic field strength and the other parameters.^{1–4} Of these parameters, the exchange rate between coordinated and bulk water, k_{ex} , plays a critical role in establishing contrast-enhancing efficiency.^{1,2} Hence, the ability to precisely control the water-exchange rates of Ln^{III}-containing complexes is imperative to achieving optimal efficiencies of contrast agents for both conventional and PARACEST imaging.

For example, conventional, Gd^{III}-containing, small molecular-weight agents require a fast water-exchange rate ($\sim 10^8 \text{ s}^{-1}$) to achieve optimal efficiency at the clinically relevant field strength of 1.5 T,^{3,5} and macromolecular Gd^{III}-based agents require a faster water-exchange rate ($\sim 10^{10} \text{ s}^{-1}$).⁴ However, these target values change with changes in field strength. Different from conventional agents, Ln^{III}-based PARACEST agents require slow-to-intermediate water-exchange rates on the NMR time scale, and the optimal value for the water-exchange rate depends on the frequency of the pulse used to presaturate the exchangeable protons.⁶ The estimated optimal water-exchange rate for PARACEST agents is between 0.3×10^3 and $1 \times 10^3 \text{ s}^{-1}$ at clinically relevant pulse frequencies (50–100 Hz).⁶

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[†]Electronic Supplementary Information (ESI) available: general experimental and synthetic procedures; water-proton relaxation rate, ¹⁷O NMR, and luminescence-decay data; ¹H and ¹³C NMR and electron paramagnetic resonance spectra; estimations of rotational correlation time and expected relaxivity; and high performance liquid chromatography chromatograms. See DOI: 10.1039/b000000x/

The water-exchange rates of clinically approved Gd^{III}-containing small molecular-weight contrast agents are slower (1×10^6 to 4×10^6 s⁻¹) than the optimal value,⁷ and the water-exchange rates of most Ln^{III}-based complexes developed as potential PARACEST agents are faster (0.3×10^4 to 1×10^4 s⁻¹) than the optimal value.⁶ Consequently, there is a need to tune the water-exchange rates of Ln^{III}-containing complexes to achieve optimum efficiencies for conventional and PARACEST agents. This need has been the focus of a great deal of research. Tuning water-exchange rates of Ln^{III}-containing complexes has been achieved by modifying (1) the charge of the Ln^{III}-based complex;⁸ (2) the accessibility of the metal center to bulk water;^{7,9-11} (3) the mechanism of water exchange;¹² and (4) the ratio between twisted square anti-prism and square anti-prism isomers for Ln^{III}-based complexes with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-type ligands.¹³ These studies demonstrate that small modifications in ligand structure can have large impacts on the water-exchange rates of Ln^{III}-containing complexes, but most of these studies are specific to the system being studied.

We envisioned that a modular and tuneable system of modifications could be incorporated into Ln^{III}-based DOTA-type systems to tune water-exchange rates. We hypothesized that hydrophilic oligomers of polyethylene glycol (PEG) could be used to modulate water-exchange rates by altering the accessibility of bulk water to the Ln^{III}-center through both steric interactions and hydrogen bonding in a systematic fashion based on oligomer length. Here, we report the influence of PEG length on the molecular parameters, including water-exchange rate, of Ln^{III}-containing DOTA-type complexes.

To investigate the influence of PEG oligomers on the water-exchange rates of Ln^{III}-containing complexes, we designed and synthesized a PEG-containing model system (**1a-d** and **2a-d** in Fig. 1) to modulate the water-exchange rates of Ln^{III}-DOTA-based complexes. We expected that PEG would hydrogen bond to water through the large number of oxygen-based hydrogen-bond acceptors, thereby changing the extent of the hydrogen-bonding network as PEG length is varied. Our hypothesis is consistent with reports of changes in water-exchange rates being observed as the length of PEG was varied with PEG-conjugates of Gd^{III}-containing hydroxypyridonate (HOPO) complexes.¹¹ The HOPO-based systems have a water-coordination number of two and display fast water-exchange rates ($\sim 10^8$ s⁻¹). The PEG moiety in those systems led to slower water-exchange rates and a decrease in water-coordination number from two to one. We expected that PEG could be used to fine-tune the water-exchange rates of other systems including Ln^{III}-containing DOTA-type complexes with water-coordination numbers of one and relatively slow water-exchange rates ($\sim 10^6$ s⁻¹).

Our model system includes Ln^{III}-containing complexes without PEG (**1a** and **2a**) as well as three complexes with different length oligomers of PEG (**1b-d** and **2b-d**). The synthetic route to complexes **1a-d** and **2a-d** is shown in Scheme 1. Briefly, the α -chloroamide derivative of *tert*-butoxycarbonyl-protected ethylenediamine, **4**, was reacted with tri-*tert*-butyl-1,4,7,10-tetraazacyclododecane-1,4,7-triacetate, **5**, to obtain protected ligand **6** that was treated with HCl to yield deprotected ligand **7**. Metallation was carried out using metal chlorides to obtain Gd^{III}- and Eu^{III}-containing complexes **8** and **9**, respectively. Complexes **8** and **9** were reacted with succinimidyl ester derivatives of PEG or acetate (**a**, **b**, **c**, or **d**) to

yield **1a–d** or **2a–d**. The yttrium analogues, **3a–d**, were synthesized as diamagnetic controls for the variable-temperature ^{17}O NMR experiments following the same procedures.

The water-coordination number, q , was obtained for Eu^{III} -containing complexes **2a–d** using luminescence-decay measurements (Table 1).¹⁴ Values of q were close to 1, and these values agree with what is expected for Ln^{III} -complexes coordinated to octadentate ligands. Water-coordination number data for **2a–d** suggest that steric blocking has little if any influence on the properties of the complexes.

To investigate the influence of PEG on tuning water-exchange rates, we performed variable-temperature ^{17}O NMR experiments for Gd^{III} -containing conjugates **1a–d**, and the fitted molecular parameters are summarized in Table 1: the residence lifetime of coordinated water, τ_m^{298} (water-exchange rate, k_{ex}^{298} , is $1/\tau_m^{298}$; the longitudinal electronic relaxation time, T_{1e}^{298} ; and the enthalpy change for the water-exchange process, H . Based on the results of the ^{17}O NMR experiments, a gradual decrease in water-exchange rates was observed with increasing length of PEG from **1a** (control without PEG) to **1d** (long PEG). Moreover, 1.8-, 3.3-, and 4.0-fold decreases in water-exchange rates were observed from **1a** to **1b**, **1c**, and **1d**, respectively. The observed decrease in water-exchange rates with increasing length of PEG was supported by an increase in H from **1a** to **1d**. This increase in enthalpy suggests that the exchange between coordinated and bulk water molecules becomes difficult as PEG length is increased, possibly due to a larger hydrogen-bond network. The increase in H and decrease in water-exchange rates from **1a** to **1d** support our hypothesis that PEG is able to modify the water-exchange properties of Gd^{III} -containing DOTA-type complexes.

To investigate the influence of PEG on the efficiency of complexes **1a–d** as conventional contrast agents, we carried out relaxivity, r_1 , measurements of **1a–d** at 37 °C and 1.4 T in phosphate-buffered saline (pH = 7.4). The r_1 values of **1a–d** were obtained from the slopes of the plots of $1/T_1$ versus Gd concentration (Table 1). A 2.5-fold increase in relaxivity was observed as the length of PEG was increased from **1a** to **1d**. This increase in relaxivity corresponds to the increase in molecular weight (MW, Table 1) from **1a** to **1d**. As the molecular weight increases, complexes tend to tumble more slowly in solution leading to higher relaxivity values.¹⁵

To more rigorously unite our ^{17}O NMR and relaxivity data, we performed electron paramagnetic resonance spectroscopy measurements to obtain the electronic Landé g factor, g_L , and the transverse electronic relaxation rate, $1/T_{2e}$, of **1a–d**. These parameters together with r_1 , k_{ex} , T_{1e} , and q were used with the Solomon–Bloembergen–Morgan equations, which describe the factors affecting the efficiency of contrast agents for MRI,³ to obtain estimated rotational correlation times, τ_R , for **1a–d** (Table 1 and calculations in the ESI). The values of τ_R seem lower than expected likely due to the internal motion of the PEG moieties as suggested for the HOPO-based system,¹¹ due to the ineffective coupling between the motion of Gd-water vector and rotational motion of the entire molecule,¹⁶ or a combination of the two. An increase in the τ_R values was observed from **1a** to **1d**, which is consistent with the increase in molecular weight as the length of PEG is increased. This increase in τ_R is likely the cause of the observed increase in relaxivity from **1a** to **1d**. However, the increase in

relaxivity observed from **1a** to **1d** is lower-than-expected based solely on the variation in τ_R from **1a** to **1d** (see ESI for calculations), and this observation is a likely result of the decrease in water-exchange rates as PEG length increases from **1a** to **1d**.

We have studied the influence of PEG oligomer length on the water-exchange rates and other properties that contribute to the efficiency of Ln^{III}-containing DOTA-type contrast agents. Based on our findings, PEG can be used to fine-tune, toward slower rates, the water-exchange rates of Ln^{III}-containing DOTA-type complexes. Our results demonstrate a similar magnitude of slowing of water-exchange rates as was reported with HOPO-based systems,¹¹ but without the associated change in water coordination number. Consequently, PEG is able to slow the water-exchange rates of Ln^{III}-containing complexes regardless of water-coordination number, demonstrating that conjugation of PEG represents a modular and tuneable strategy for slowing water-exchange rates that is general for Ln^{III}-containing complexes. We expect that these findings will be useful in the design of Ln^{III}-based contrast agents that require slow water-exchange rates on the NMR time scale.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

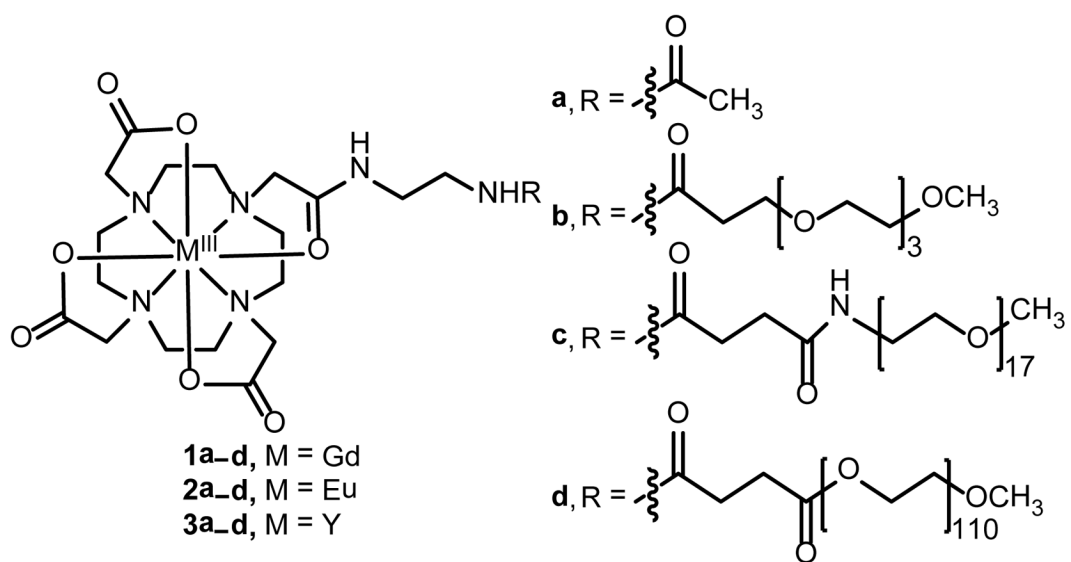
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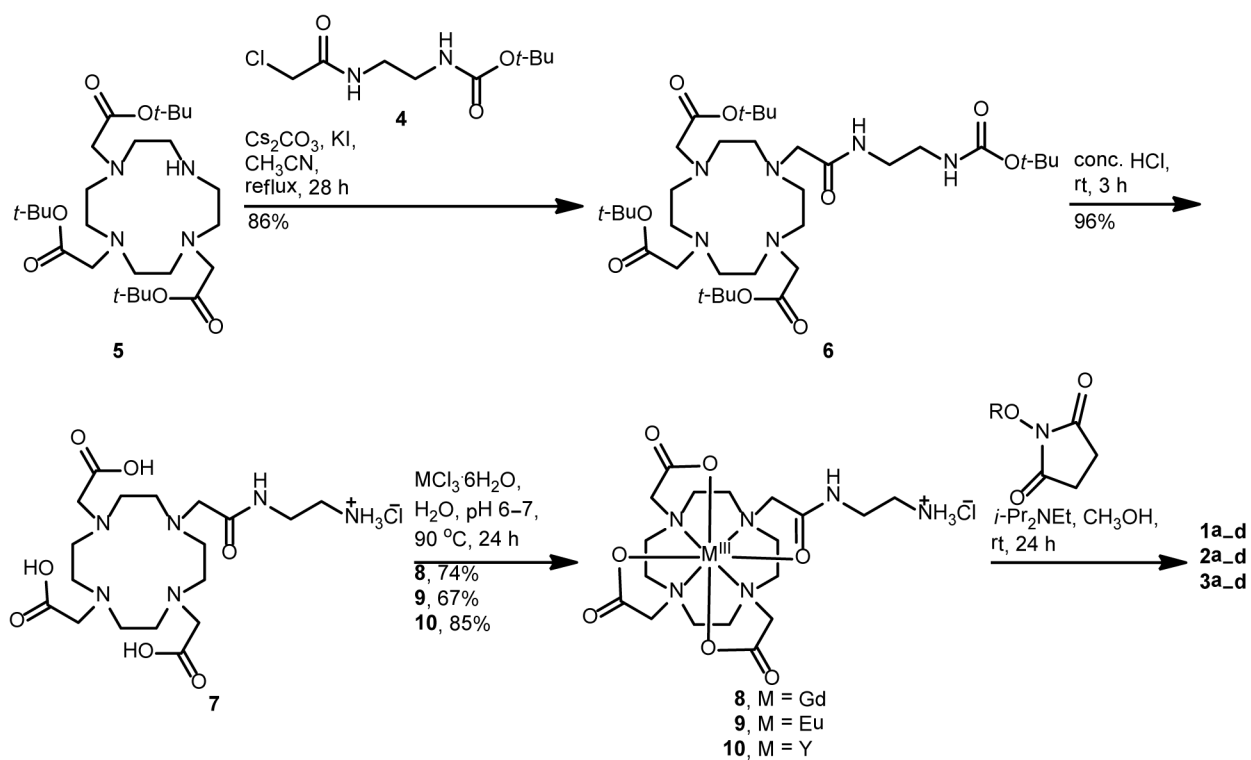
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**Fig. 1.**

Structures of Ln^{III} -containing PEG conjugates **1a-d** and **2a-d** and Y^{III} -containing conjugates **3a-d**.



Scheme 1.

Synthetic route to Ln^{III}-containing PEG conjugates **1a-d** and **2a-d** and Y^{III}-containing conjugates **3a-d**.

Table 1Molecular parameters of complexes **1a–d**.

Parameter	1a	1b	1c	1d
$q^{a,b,c}$	0.90 ± 0.01	0.89 ± 0.01	0.96 ± 0.02	0.76 ± 0.02
$\tau_m^{298} \times 10^{-6}$ (s) ^a	0.37 ± 0.01	0.66 ± 0.01	1.2 ± 0.1	1.5 ± 0.07
$k_{ex}^{298} \times 10^6$ (s ⁻¹) ^d	2.7 ± 0.03	1.5 ± 0.02	0.83 ± 0.08	0.67 ± 0.05
$T_{1e}^{298} \times 10^{-9}$ (s) ^a	440 ± 30	61 ± 0.7	12 ± 0.3	4.0 ± 0.04
H (kJ mol ⁻¹) ^a	36 ± 0.8	50. ± 0.6	71 ± 4	90. ± 1.5
r_1 (mM ⁻¹ s ⁻¹) ^a	2.5 ± 0.1	4.0 ± 0.1	5.3 ± 0.1	6.3 ± 0.1
MW (Da)	641	817	1463	5222
g_L	1.98	1.98	1.99	1.98
$1/T_{2e} \times 10^8$ (s ⁻¹)	17.8	8.11	6.51	5.03
$\tau_R \times 10^{-12}$ (s)	46	79	110	220

^a reported as mean ± standard error,^b from complexes **2a–d**,^c the error associated with q determination is ±0.1 water molecules,¹⁴^d error represents relative uncertainty