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# The Population of SAP and TSAP Isomers in Cyclen-Based Lanthanide(III) Chelates Is Substantially Affected by Solvent

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## Abstract

The square antiprism/twisted square antiprism ratio in LnDOTA-tetraamide chelates is a critical parameter in governing water-exchange kinetics and ultimately the utility of a chelate as a PARACEST MRI contrast agent. In LnDOTA-tetraamide chelates with tertiary amides, this ratio and the rate of interconversion between these two structural isomers are found to be dramatically dependent upon the solvent and possibly other local environmental factors.

The realization that $Ln^{3+}$ chelates with slowly exchanging inner-sphere water molecules could induce magnetic resonance imaging (MRI) contrast through a paramagnetic chemical exchange saturation transfer (PARACEST) mechanism has given rise to a recent upsurge in interest in LnDOTA-tetraamide chelates.[1,2] The large paramagnetic hyperfine chemical shifts induced by anisotropic 4f electrons effectively shift the resonance of the inner-sphere water far from that of solvent water. However, because unchelated $Ln^{3+}$ ions are toxic, suitable chelating ligands must be employed that both eliminate the toxic effects[3] of these ions and appropriately slow water exchange.[1,2,4,5] Ligands derived from cyclen have been widely accepted in the development of MRI contrast agents,[3] and the related DOTA-tetraamide ligands (Chart 1) are now widely studied as PARACEST agents. Although DOTA-tetraamide chelates are kinetically inert, retaining the $Ln^{3+}$ ion throughout the in vivo residence of the chelate,[6,7] the use of DOTA-tetraamide ligands in vivo can be problematic. It has been shown that only when the cationic nature of these chelates is offset by anionic substituents, such as carboxylates, can the severe toxic effects intrinsic to cationic chelates be avoided at the relatively high dosing levels required for MRI.[6] A chelate incorporating four glycinateamide substituents has been shown to be safe for in vivo use.[6,7] The unique water-exchange characteristics of these chelates make them attractive as MRI sensors of various biological species such as glucose, $Zn^{2+}$, or protons (pH),[8–11] but this normally requires the addition of side-chain substituents that act as binding sites for such

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Supporting Information Available: Experimental details and additional NMR and luminescence data. This material is available free of charge via the Internet at http://pubs.acs.org.

species. In such situations, it may be desirable to incorporate the offsetting negative charges as tertiary amide substituents, leaving the sensing secondary amide substituents in place.

However, DOTA-tetraamides with tertiary amide pendant arms have not been widely studied as PARACEST agents. Of particular relevance is the coordination chemistry of these chelates. LnDOTA-tetraamide chelates can adopt both a monocapped square antiprismatic (SAP) and a monocapped twisted square antiprismatic (TSAP) coordination geometry,[4,5] and it has been shown that water exchange in complexes that form a TSAP isomer is 1–2 orders of magnitude faster than that in complexes that form a SAP isomer.[4,5,12] Given the requirement for slow exchange kinetics in putative PARACEST agents, it is clearly preferable for the SAP coordination geometry to predominate. Indeed, to our knowledge, the rate of exchange observed in the TSAP isomers of these complexes is so fast that CEST arising from this species has not been observed.

The only substantive literature contributions concerning tertiary LnDOTA-tetraamides are reports about the simple dimethyl ligand, DOTTA (Chart 1).[4,5] This ligand is notable in that, unlike other simple tetraamides such as DOTAM and DTMA, it was reported to exhibit a strong (2:1) preference for the TSAP coordination geometry in $D_2O$. However, in secondary amide systems, larger substituents have generally been found to have an overwhelming preference for the SAP isomer.[13,14] This raised the question of what happens in tertiary DOTA-tetraamide systems in which larger substituents are employed. In response, the structural isomerism of some symmetrically substituted tertiary DOTA-tetraamide chelates are examined herein.

Ligands **1**, **2**, and DOTTA were prepared by condensation of the appropriate secondary amine with a haloacetyl halide ($CH_2Cl_2/K_2CO_3$); the resulting haloacetamides were then used to alkylate cyclen under standard conditions.[5,13] After purification by either column chromatography or reversed-phase high-performance liquid chromatography (RP-HPLC), as appropriate, $Ln^{3+}$ complexes of each ligand were prepared from the corresponding $Ln(OTf)_3$ salt. Because the nature of the counterion in this type of complex is known to affect the structure and water-exchange properties of the complex,[15] triflate counterions were used throughout this work to eliminate changes in behavior arising from differences in the counterion. Our initial hypothesis was that the SAP/TSAP ratio across the Ln series could be examined by $^1H$ NMR by taking advantage of the known difference in the chemical shifts of the axial ring protons ($ax^S$) to both identify and quantify each coordination isomer.[16,17] However, the SAP and TSAP isomers are in dynamic equilibrium, interconverting through either pendant arm rotation or flipping of the macrocyclic ring conformation.[18,19] If this interconversion is fast on the NMR time scale, then identifying and quantifying the two isomeric forms of a chelate can only be achieved by lowering the temperature, something that may necessitate changing solvents. To resolve two isomers of EuDOTTA in $CD_3CN$, the temperature was lowered to 233K. Once resolved (Figure 1), it is clear that the distribution of SAP and TSAP isomers in $CD_3CN$ (mole fraction of the SAP isomer, $x^{SAP}$=0.67) is dramatically different from that published previously ($D_2O$, 273 K), $^5x^{SAP}$=0.33. It has been reported that a change in the solvent from $D_2O$ to $CD_3CN$ can increase $x^{SAP}$ of EuDOTAM from 0.80 to 0.97.[5] However, such a change is relatively modest in comparison to that observed here for EuDOTTA in which the dominant isomer is opposite in the two solvents. In addition to a change in the the isomeric distribution, a change in the solvent also substanitally affects the rate of isomer interconversion and hence the low-temperature requirement to resolve the two isomeric forms.

The rate of isomeric interconversion was also found to be accelerated for Yb**1** upon a change of the solvent from$D_2O$ to $CD_3CN$. However, this change in the solvent had an effect upon the mole fraction $x^{SAP}$ opposite to that observed with EuDOTTA, dropping from 0.82 in

$D_2O$ to 0.72 in $CD_3CN$ (Figure 1). This suggests that the nature of the trend with a change in the solvent may be $Ln^{3+}$ ion specific, given the strong relationship between the SAP/TSAP ratio and the $Ln^{3+}$ ionic radius. Nonetheless, the strong solvent dependency was found to hold for all $Ln^{3+}$ chelates tested with this ligand.

Eu**2** is not readily soluble in water, but spectra could be obtained in $CD_3OD$ and $CD_3CN$. In this tetraamide system, the TSAP isomer predominated in $CD_3CN$ ($x^{SAP}$ = 0.05), while the SAP isomer predominated ($x^{SAP}$=0.70) in the protic solvent, $CD_3OD$(Figure 2). The dependence of the isomeric distribution upon the nature of the solvent is also not confined to LnDOTA-tetraamide chelates. For example, in the cyclen tetrapyridyl complexes, Ln**3**, the crystal and solution state structures of which have been described elsewhere,[20,21] was also found to be highly affected by the nature of the solvent. $x^{SAP}$ for Eu**3** was found to drop from 0.85 to 0.50 upon a change in the solvent from$D_2O$to$CD_3CN$(Figure 3).

The origins of these profound solvent-dependent changes in the isomeric distribution are unclear. Because the counterion was triflate in all cases, the nature of the counterion can at least be ruled out. However, a change in the solvent may alter the interaction between a cationic chelate and its anions, which may translate into changes in isomerism. However, the fact that a change from a protic to an aprotic solvent does not result in the same consistent trend in isomerism tends to suggest that the underlying cause is more complex than the simple disruption of ionic interaction.

The luminescent properties of $Eu^{3+}$ provide a valuable means of probing its coordination environment and, in particular, the nature of the capping axial ligand. Axial ligands of differing polarizability can substantially alter Bleaney's $B_0^2$ ligand-field parameter,[22] giving rise to changes in the $^1H$ NMR hyperfine shifts and the splitting of the $^5D_0 \rightarrow {}^7F_1$ emission band.[23] SAP/TSAP isomerism has a similar effect on both of these parameters;[24,25] however, incorrectly assigning either isomeric form as a result of such changes can be discounted because both isomeric forms can be identified in each case. Nonetheless, the commonality of this effect frustrated our attempts to probe the emission spectra of these $Eu^{3+}$ chelates for clues as to the origin of the changes in isomerism with a change in the solvent. The emission spectra of these chelates are provided in the Supporting Information for further examination (Figures S1 and S2). This leaves only the hydration state of the $Eu^{3+}$ ion available for exploration. $H_2O$ and $D_2O$ were titrated into separate samples of Eu**2** and Eu**3** in dry $CH_3CN$, and the hydration state of the $Eu^{3+}$ ion was determined by a modified Horrocks' method.[26] The plot of $q$ versus [$H_2O$] (Figure S3 in the Supporting Information) obtained for Eu**2** resembles a classical binding curve but curiously plateaus with a $q$ value of ~0.4. The curve obtained for Eu**3** is more complex still (Figure S4 in the Supporting Information), possibly because the counterion may become involved in the direct binding to the $Eu^{3+}$ center, a phenomenon observed in some crystal structures of this chelate.[20] Nonetheless, each chelate demonstrates an increase in the hydration state as water is added to the $CH_3CN$ solution of the chelate, strongly indicative of substitution of the axial ligand as the solvent is changed.[27]

LnDOTA-tetraamide chelates that possess tertiary amides demonstrate a greater predisposition for adopting a TSAP coordination geometry than their secondary amide counterparts, even with very large amide substituents. Yet, the monobenzylic analogue of Eu**2**, which is observed to predominate as the SAP isomer in $CD_3OD$ ($x^{SAP}$>0.99; Figure S5 in the Supporting Information), also exhibits a change in the isomer ratio upon a change in the solvent to $CD_3CN$ ($x^{SAP}$ = 0.77), a change that is coupled with an acceleration in the rate of isomer interconversion. Although this change is not as a dramatic as that observed for Eu**2**, it does raise questions about the assumptions that are made regarding isomeric ratios when such systems are to be applied in vivo. In particular, if tertiary amide systems come to

be viewed as a solution to offsetting the cationic nature of DOTA-tetraamide chelates, then the question of how local environmental factors affect structural isomerism in these chelates will become critical. Although the solvent for all in vivo applications is aqueous, it is not homogeneous throughout extracellular space. Compartmentalization of water within tissue leads to differences in the constituents of the solvent throughout tissue. Some components, such as phosphate, phospholipids, and phosphorylated metabolites, may be capable of substituting the axial ligand of tertiary LnDOTA-tetraamide chelates, which it seems plays some role in influencing the isomer distribution. They may also affect the very nature of the water itself, perturbing its inter- and intramolecular interactions and ultimately its interactions with these chelates. If either of these alter the proportion of SAP isomer present or the hydration state of the chelate, it would concomitantly affect the CEST contrast observed in an imaging experiment. This may be an Achilles heel or an advantage that can be exploited to gain additional information, but in either case, it is necessary to understand these effects thoroughly before these systems can be applied in vivo.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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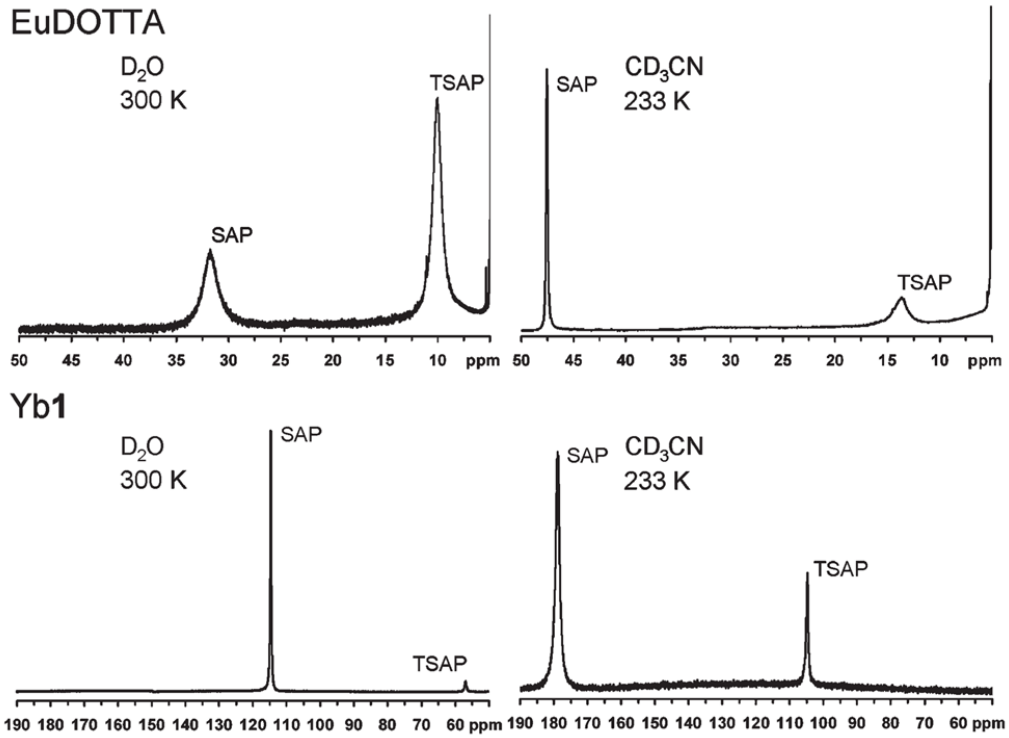


**Figure 1.**

$^{1}$H NMR spectra of EuDOTTA (top) and Yb**1** (bottom) focusing on the highly shifted *ax*$^{S}$ resonance. Spectra were acquired at 400MHz in$D_2O$ at 300K(left) and in $CD_3CN$ at 233K (right). Note that the hyperfine shifts of these resonances cannot be compared from one solvent to another because of temperature differences.

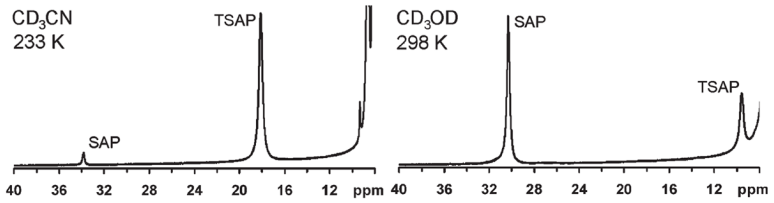


**Figure 2.**

$^{1}$H NMR spectra of Eu**2** focusing on the highly shifted *ax*$^{S}$ resonance. Spectra were acquired at 400 MHz in $CD_3CN$ at 233 K (left) and in $CD_3OD$ at 298 K (right).

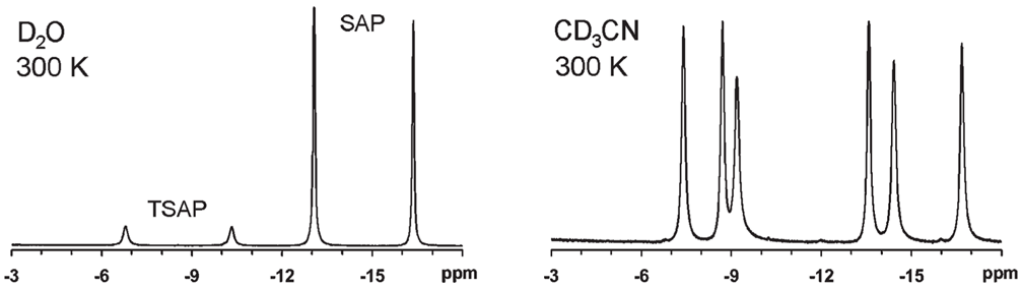


**Figure 3.**

$^{1}$H NMR spectra of Eu**3** focusing on the highly shifted *ax*$^{S}$ resonance. Spectra were acquired at 400MHz and 300K in $D_2O$ (left) and $CD_3CN$ (right).

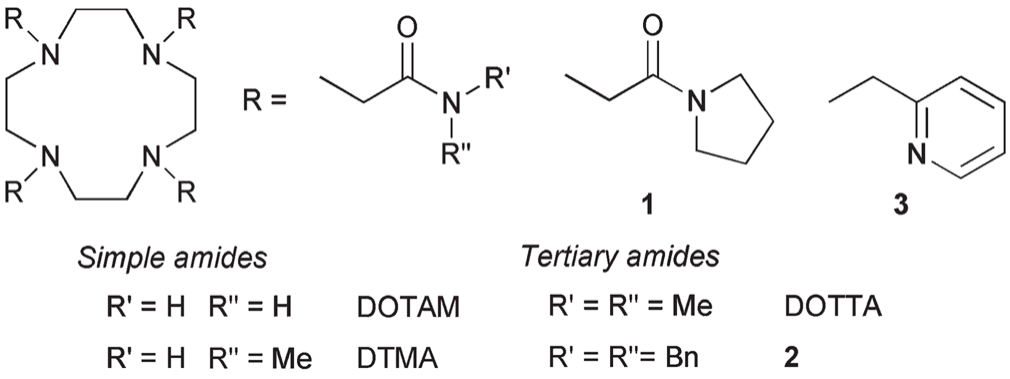


**Chart 1.**
Structures of Macrocyclic Ligands Discussed in This Work