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Lanthanide DO3A-tropone complexes: efficient dual MR/NIR imaging probes in aqueous medium

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Abstract

In this work, we describe a novel DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) based ligand with a chromophoric tropone coordinating sidearm (**1**). Ln³⁺ complexes of **1** have one inner sphere water molecule. The r_1 relaxivity of Gd**1** is similar to that of the commercial Gd-based MRI agents. The neutral O-donor atom of the tropone moiety slows down the water exchange rate by a factor of 3 compared to GdDOTA. In addition, Nd**1** and Yb**1** complexes exhibit significant NIR emission in aqueous solutions indicating that the tropone unit is an efficient sensitizer for these Ln³⁺-ions. Therefore, this new ligand is a promising platform for the design of Ln³⁺ based dual MR/optical imaging probes.

COMMUNICATION

A DOTA derivative with a tropone coordinating sidearm forms monohydrated lanthanide complexes. The Gd^{3+} -chelate has the expected relaxivity while the Nd^{3+} and Yb^{3+} complexes exhibit bright NIR luminescence despite the quenching effect of the inner sphere water molecule.

Keywords

Lanthanide; MRI; Near-infrared; tropone; imaging

Lanthanide ions (Ln^{3+}) have been involved in a large number of applications as components of magnetic resonance and optical imaging agents as a result of their unique magnetic and optical properties. Gd^{3+} - and Eu^{3+} -complexes are well established as MR imaging contrast agents^[1] while several other Ln^{3+} have been explored for their luminescent properties in the visible or near-infrared (NIR) range.^[2] Ln^{3+} -based luminescent probes have several favorable properties for bio applications that include narrow emission bands, large difference between excitation and emission wavelengths and strong resistance towards photobleaching.^[2a-c] Current research is focusing on NIR probes because of the potential deep tissue penetration of NIR photons and low autofluorescence of biological tissues in this range.^[2c-f] Lanthanide ions share similar coordination chemistry, thus one can explore the entire lanthanide series with the same ligand. This versatility makes it possible to construct agents for combined or dual MR/optical imaging that contain Gd^{3+} for MRI and another lanthanide, such as Nd^{3+} or Yb^{3+} for NIR optical detection. The structural similarity of lanthanide complexes allows the co-localization of the MR and optical images due to similar biodistributions.^[3] Dual MR /optical imaging is advantageous because it combines the high spatial resolution of MRI at the anatomical level with the high sensitivity of optical detection at the cellular level.^[3,4] The ligand in such dual agents should fulfill several requirements: form thermodynamically stable and kinetically inert complexes with Ln^{3+} to prevent toxicity associated with free lanthanide ions, provide a mechanism for the efficient paramagnetic relaxation enhancement of the bulk water protons and sensitize characteristic emission of a luminescent lanthanide ion (antenna effect). This last point is particularly important since most of f-f- transitions of Ln^{3+} are Laporte-forbidden and exhibit very low molar absorption coefficients making their direct excitation difficult.^[2,3] However, this problem can be overcome if the organic ligand includes an appropriate chromophoric moiety that can absorb excitation energy and transfer it to the corresponding Ln^{3+} .^[2,3] Efficient relaxation of bulk water protons by Gd^{3+} requires at least one rapidly exchanging inner sphere water molecule,^[1] but this requirement appears to be incompatible with the design of optical agents, especially those emitting in the NIR range, because the overtones of O-H vibrations can quench the Ln^{3+} excited states through non-radiative deactivation.^[5] It has been shown, however, that in some Ln^{3+} -based complexes, the NIR emission can be efficiently sensitized despite the presence of several coordinated water molecules.^[6] Nevertheless, such examples remain very scarce.^[3,6] Here we report a new ligand (**1**, Figure 1), whose lanthanide complexes (Ln1 , $\text{Ln} = \text{Nd}, \text{Gd}, \text{Yb}$) have one inner-sphere water molecule and yet, Nd1 and Yb1 display bright luminescence in the NIR domain. The ligand contains a tropone (cyclohepta-2,4,6-trien-1-one) chromophore appended on a DO3A (1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) unit (Figure 1). Lanthanide chelates of DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) and related ligands have been

shown to have high *in vivo* stability and are frequently used for various biomedical applications.^[7] Tropolone (2-hydroxytropone) is a seven membered, non-benzenoid aromatic compound.^[8] Its pK_a is around 7 and the tropolonate anion can act as a bidentate ligand for alkaline earth, transition metal and lanthanide ions.^[8,9] The electronic structure of the coordinated tropolone moiety enables it to be an efficient sensitizer for several NIR-emitting Ln^{3+} ions in solution^[10] However, the thermodynamic stability and kinetic inertness of lanthanide tropolonato complexes is not sufficient for *in vivo* applications.^[9]

Tropones with a leaving group (-Cl, -Br or -OTs) at position 2 easily undergo aromatic nucleophilic substitution.^[8] We have chosen DO3A-tris(tert-butyl ester) as starting material, which reacted cleanly with the commercially available tropolone-*p*-toluenesulfonate to give the expected *tert*-butyl protected product (Scheme S1, Supporting Information). Acidic hydrolysis of the *tert*-butyl groups gave the desired ligand as free acid in good yield. Ligand **1** is expected to form neutral chelates with trivalent metal ions. The lanthanide complexes **Ln1** were prepared by adding LnCl_3 ($\text{Ln} = \text{Gd}, \text{Eu}, \text{Nd}$ and Yb) to the ligand at pH 6 in the presence of OH-form anion exchange resin as a base. ^1H NMR spectra (Figure S1) indicated that the structure of the Nd^{3+} complex adopts a twisted square antiprism (TSAP) geometry. **Eu1** is present in solution predominantly as the square antiprism (SAP) isomer with approximately 20 % of the TSAP form while and Yb^{3+} chelate exclusively formed the SAP isomer. This behavior is analogous to that of the corresponding DOTA complexes and can be explained to the lanthanide contraction.^[11]

The relaxivity (Figure S2) of the **Gd1** complex was found to be $3.46 \text{ mM}^{-1}\text{s}^{-1}$ at 1.4 T and 37 °C in water. This value falls in the range that is expected for a small molecular weight complex with one water molecule bound to the Gd^{3+} .^[1a] The number of inner-sphere water molecules in **Gd1** was measured by ^{17}O NMR bulk magnetic susceptibility water shift measurements ($q = 1$) (Supporting Information). Since the tropone sidearm has a neutral oxygen donor atom, we anticipated that this would significantly decrease the exchange rate compared to a negatively charged carboxylate coordinating group.^[1b] Variable temperature ^{17}O NMR measurements revealed that the exchange rate of the inner sphere water molecule in **Gd1** was indeed about 3 times slower than that in the parent complex **Gd-DOTA** (Figures 2, S3 and Table S1).

While this value is not optimal, it did not affect the r_1 significantly because it is still in the range where the relaxivity is limited by the short rotational correlation time of the complex^[1a]. However, slowing down the tumbling rate should result in an increase of relaxivity.^[1a] We anticipated that the complex might bind to albumin (HSA) because of the presence of the lipophilic tropone moiety. Therefore, the binding of **Gd1** to albumin was studied by adding increasing amounts of albumin to the complex and measuring the relaxation rate of the solution. The r_1 did not improve dramatically in the presence of albumin. Fitting of the data revealed a weak binding to HSA with an affinity constant of $K_A = 0.6 \text{ mM}^{-1}$, which does not significantly increase the r_1 value of the complex in the concentration range relevant for MR imaging (Figure S4 and Table S2).

In vivo MR imaging experiments with Gd**1** revealed that as expected, the agent was excreted by the kidneys and the liver. The presence of the intact complex in urine was confirmed by MS. Gd**1** did not show any toxicity at the injected doses (Figure S5).

The photophysical properties of Gd**1**, Nd**1** and Yb**1** were studied by recording the absorption, excitation and emission spectra (Figure 3). The absorption spectra of aqueous solutions of Ln**1** chelates were found to be fairly similar to each other and display broad bands in the range 250–400 nm due to ligand-centered $\pi^* \leftarrow \pi$ electronic transitions with the lower energy absorption maxima located around 330 nm. Upon excitation of the ligand-centered bands at 340 nm, the Yb³⁺ and Nd³⁺ complexes exhibited characteristic emission in the NIR arising from the $^2F_{5/2} \rightarrow ^2F_{7/2}$ and $^4F_{3/2} \rightarrow ^4I_J$ ($J = 9/2, 11/2, 13/2$) transitions, respectively. The excitation spectra of Yb**1** and Nd**1** recorded upon monitoring the main transitions at 980 and 1064 nm, respectively, displayed broad bands in the range 250–400 nm. This confirms that the sensitization of these ions occurs through the ligand-centered levels (antenna effect).^[12] The energy position of the ligand triplet state was determined from the phosphorescence spectrum of Gd**1** as a 0-0 transition at 17,460 cm⁻¹ (572.5 nm) (see discussion and Figure S5, Supporting Information). This value is slightly higher but still comparable to the reported value for tropolone, 16,800 cm⁻¹.^[12] Thus, the energy of the ligand triplet state is higher than the corresponding emitting levels of Nd³⁺ and Yb³⁺ ($E^{\text{Nd}}(^4F_{3/2}) = 11,460 \text{ cm}^{-1}$, and $E^{\text{Yb}}(^2F_{5/2}) = 10,300 \text{ cm}^{-1}$).^[13] facilitating their population through ligand-to-metal energy transfer. The experimental luminescence decays (Figure S6) were best fitted by monoexponential functions reflecting the presence of only one Ln³⁺-containing emitting species in solutions. Comparison of the luminescence lifetimes in H₂O and D₂O buffered solutions and the use of phenomenological equations^[5, 14] allowed the estimation of the number of inner sphere water molecules (Figure S7). The value of $q = 0.8 \pm 0.2$ was found for both Yb**1** and Nd**1**, which is in a good agreement with the one obtained for Gd**1** from susceptibility measurements. The absolute quantum yields measured in HEPES at pH 7.4 and D₂O under ligand excitation at 340 nm are summarized in Table 1. While these quantum yields are several orders of magnitude lower in comparison to those reported for organic fluorophores, they are fairly high for hydrated lanthanide chelates.^[15] The higher quantum yield values and longer luminescence lifetimes in D₂O indicate the higher probability of non-radiative deactivation of Ln³⁺ ions through overtones of O-H (3,600 cm⁻¹) rather than O-D (2,200 cm⁻¹) vibrations.^[16]

In conclusion, we have synthesized a novel DOTA-based ligand with a tropone sidearm. The Ln³⁺ complexes have one inner sphere water molecule. The Gd-complex has nearly the same r_1 relaxivity as GdDOTA, which is generally considered as the “gold standard” for MRI contrast agents. The neutral O-donor atom of the tropone moiety slows down the water exchange rate by a factor of 3 relative to GdDOTA.

Experimental Section

Experimental Details are given in the Supporting Information

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Structure of DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid), tropolone and DO3A-tropolone (**1**) discussed in this work.

Figure 2. Temperature dependence of the reduced ^{17}O NMR transverse relaxation rates of Gd1 (●) and GdDOTA (■) as reference.

Figure 3.

UV-vis absorption (left), excitation (middle) and) NIR emission (right) spectra of Nd1 and Yb1 in HEPES buffer (200 μ M, pH 7.4, 25 $^{\circ}$ C).

Table 1

Absolute quantum yields and observed luminescence lifetimes (200 μM in HEPES buffer at pH7.4, or D_2O , 25 $^\circ\text{C}$).

Ln1	Solvent	Quantum yield [%]	Lifetime [μs]
Nd1	HEPES	$5.5(2) \cdot 10^{-3}$	0.084(2)
Nd1	D_2O	$2.68(1) \cdot 10^{-2}$	0.342(2)
Yb1	HEPES	$6.7(2) \cdot 10^{-3}$	0.864(2)
Yb1	D_2O	$8.29(2) \cdot 10^{-2}$	7.75

It is worth noting that the tropone coordinating group could therefore be used to fine tune the water exchange rate of a lanthanide complex for applications where slow water exchange is preferred.^[1b] Photophysical measurements revealed that the tropone unit of the ligand **1** is an efficient sensitizer for Nd^{3+} and Yb^{3+} allowing the detection of characteristic bright NIR emission in aqueous solutions. The favourable photophysical properties of the tropone chromophore combined with the DOTA framework offers a novel platform for the design of lanthanide-based dual MR/optical imaging agents for *in vivo* applications.