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A dual lanthanide probe suitable for optical (Tb^{3+} luminescence) and magnetic resonance imaging (Gd^{3+} relaxometry)

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Abstract—A new polyaminocarboxylate ligand derived from *N*,*C*-pyrazolylpyridine was synthesized. The luminescence and relaxometry properties of its Tb^{3+} and Gd^{3+} chelates were investigated in aqueous solutions. The Tb^{3+} chelate is strongly luminescent having remarkable lifetime and quantum yield ($\tau = 1.82$ ms and $\Phi = 0.42$). The $1/T_1$ proton relaxivity at 20 MHz and 25 °C ($5.3\ s^{-1}\ mM^{-1}$) of the Gd^{3+} chelate was found to be comparable to that of the clinically used Gd-DTPA.

The sustained research activity devoted to lanthanide complexes arises in part from their successful applications as diagnostic tools in biomedical analysis.[1] Gadolinium(III) complexes are the most popular magnetic resonance imaging (MRI) contrast agents and europium(III) or terbium(III) complexes are long-lived luminescent probes (ms range) which can efficiently exclude the fluorescence of biological materials by the use of time-resolved spectroscopy.[2,3] The search of bimodal systems that form both efficient luminescent Eu or Tb complexes and Gd relaxation agents shows great promise for a direct correlation of in vitro (time-resolved luminescence microscopy) and in vivo (MRI) imaging studies, because the structure of the two probes would be effectively identical.[4]

The design of lanthanide-containing bimodal agents is not an easy task because of the various and conflicting constraints associated to the chemical, relaxometric, and photophysical properties of the complexes. An efficient lanthanide complex should be obviously characterized by reasonable water solubility and high kinetic stability (with respect to dissociation) when biological applications are concerned. In order to obtain an efficient luminescent Eu or Tb label, the ligand needs to incorporate a chromophoric unit (antenna group), for absorbing light and transferring it to the metallic ion, thereby overcoming the intrinsic low absorption coefficients of the lanthanide ions.[5] Another important feature concerns the hydration state, that is, the number of directly coordinated water molecules to the Ln(III) ion. The proton relaxivity of gadolinium complexes is directly correlated to the number of metal-bound water molecules (inner-sphere contribution).[2c] In contrast, inner-sphere water molecules contribute to reduce emission lifetime and quantum yield for Tb^{3+} and especially Eu^{3+} luminescence.[5]

In this context, it appeared very attractive to synthesize the ligand **1** and to measure the luminescence and relaxivity properties of its Tb^{3+} and Gd^{3+} complexes, respectively. This ligand presents three main advantages: (i) its octadenticity allows the coordination of one water molecule in the inner-sphere of the metal (the preferred coordination number for Tb^{3+}, Gd^{3+} is 9); (ii) the bisheteroaryl group, *N*,*C*-pyrazolylpyridine may act as an efficient antenna in Tb^{3+} photosensitization;[6] (iii) the

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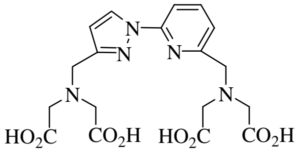
bis (iminodiacetate) unit is a very efficient chelating system for Ln^{3+} ions in aqueous solutions.[7]



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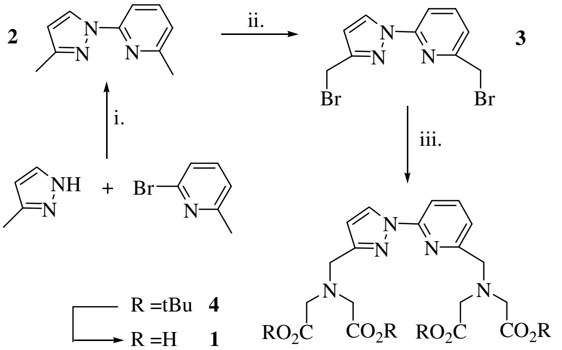
The synthesis of compound **1** is described in Scheme 1. The traditional method to synthesize pyridinylpyrazoles involves a direct reaction of potassium pyrazolate derivatives (using potassium metal as a base) with halogeno pyridines in diglyme at 130 °C.[8] In an effort to explore a more friendly alternative method for the preparation of the target compound, we were attracted by recent reports about catalysts derived from copper iodide and simple diamine which promote efficiently the N-arylation of nitrogen heterocycles.[9] The reaction between 2-bromo-6-methyl-pyridine and 3-methyl-pyrazole was carried out in the presence of 10 mol% CuI, 10 mol% of *trans*-1,2-cyclohexanediamine, and K_2CO_3 in refluxing dioxane and afforded the recently reported compound **2**[6] in a 39% isolated yield. Interestingly, besides the β-nitrogen substituted pyrazole **2**, a minor amount (2%) of the α-nitrogen substituted isomer was also isolated, indicating that, as expected, the coupling occurred preferentially at the less hindered nitrogen atom. The yield of this N-arylation of 3-methyl-pyrazole could presumably be improved through further optimization of the chelating amino ligand.[10] Activation of the methyl groups of **2** was achieved by a free radical procedure using NBS/AIBN under UV irradiation and gave the corresponding bis-bromomethyl derivative **3** with moderate yield. Finally the tetra-*tert*-butyl ester **4** was synthesized from **3** and di-*tert*-butyliminodiacetate, and hydrolyzed with CF_3COOH to give the target tetraacetic acid **1** in good yield.[11]



Scheme 1. Reagents and conditions: (i) 10 mol% CuI, 10 mol% *trans*-1,2-cyclohexanediamine, K_2CO_3, dioxane, reflux, 39%; (ii) NBS, AIBN, CCl_4, reflux, hυ, 20%; (iii) $NH(CH_2COOtBu)_2$, Na_2CO_3, CH_3CN, 72%; (iv) CF_3COOH, CH_2Cl_2, 96%.

Mononuclear complexes $[Tb.\mathbf{1}]^-$ and $[Gd.\mathbf{1}]^-$ were easily obtained by treating a solution of ligand **1** with a metal salt in aqueous solution.[12] The photophysical investigations of these complexes were carried out in HEPES buffer at pH 7.4 (Table 1). We note that the molar absorption coefficient at the maximum of the ligand-centered (LC) band ($\lambda = 290$ nm) is higher than $10^4\ M^{-1}\ cm^{-1}$, a favorable condition for an efficient antenna effect.

The $[Tb.\mathbf{1}]^-$ complex is very strongly luminescent in the green domain when excited into the lowest-energy LC absorption band, indicating that the energy is absorbed by the surrounding ligand and efficiently transferred to the chelated Tb^{3+} ion. Transitions were measured from the first excited level ($^5D_4$) to the first four components of the ground term (^7F_j, $j = 6$–3), with the transition $^5D_4 \rightarrow {}^7F_5$ (545 nm) as the most prominent band (Fig. 1). This complex exhibits excited-state lifetime (1.82 ms) that is among the longest reported for Tb(III) complexes in aqueous solution. The decay profile fits a single-exponential law, as expected for one discrete $[Tb.\mathbf{1}]^-$ species. From the difference in excited-state life-

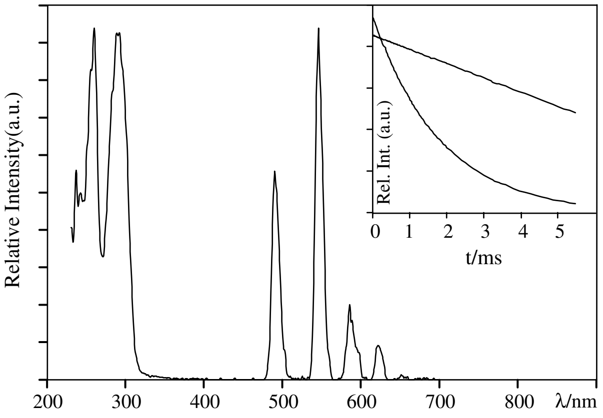


Figure 1. Corrected excitation (200–400 nm, $\lambda_{em} = 545$ nm) and emission (400–700 nm, $\lambda_{exc} = 290$ nm) of the $[Tb.\mathbf{1}]^-$ complex solution (2.5×10^{-6} M in HEPES buffer, pH 7.4). The insert shows the excited-state lifetime: luminescence decay curve (bottom plot) and ln(intensity) versus time (upper plot).

Table 1. Photophysical properties of **1**, $[Tb.\mathbf{1}]^-$, and $[Gd.\mathbf{1}]^-$ in aerated HEPES buffer (pH 7.4) solutions at 25 °C

	1	$[Tb.\mathbf{1}]^-$	$[Gd.\mathbf{1}]^-$
λ_{abs} (nm)	254, 289	256, 261, 292	255, 261, 293
ε ($10^3\ M^{-1}\ cm^{-1}$)	12.4, 12.1	11.5, 11.6, 11.7	11.7, 11.8, 12.0
τ_H (τ_D)[a] (ms)	—	1.82 (3.85)	—
Φ_H (Φ_D)[b] (10^2)	—	42 (76)	—

[a] H and D refer to H_2O and D_2O solutions, respectively. Luminescence decays were investigated by direct excitation of the ligand and by recording the intensity of the emitted light of the $^5D_4 \rightarrow {}^7F_5$ transition.

[b] Measured by excitation from the lowest-energy ligand-centered absorption band.

times obtained in H_2O and D_2O solutions, and use of Parker's equation,[13] only one metal-bound water molecule was detected ($qH_2O = 1.15$). This suggests that all eight donor groups of **1** are coordinated to the lanthanide ion, in agreement with the experimentally determined coordination number of 9 of aqueous Tb^{3+} ion ($Tb(H_2O)_9$ species).[14] Ligand **1** is an excellent sensitizer of the terbium luminescence. The value of the overall quantum yield upon direct excitation of the ligand (42%) is among the highest values found up to now in water for a mono–aquo terbium complex.[15]

These photophysical results (τ, Φ) are consistent with a compound in which the triplet energy level (26,400 cm^{-1}) matches the $^5D_4$ energy level of Tb^{3+} (20,500 cm^{-1}) properly and precludes a back-energy transfer between the metal $^5D_4$ and ligand $^3\pi\pi^*$ excited states, a deactivation pathway which is commonly observed for photosensitized terbium complexes.[7,16] [Tb.**1**]$^-$ displays an appreciable luminescence intensity ($\varepsilon\Phi$) that makes it very competitive as luminescent probe in relation to previously reported lanthanide bimodal agents.[4]

At 20 MHz (a still clinically relevant field strength) the proton relaxivity was measured to be 5.3 s^{-1} mM^{-1} at 25 °C (4.0 s^{-1} mM^{-1} at 37 °C) for [Gd.**1**]$^-$, a value which is strongly indicative of the presence of one coordinated water molecule in the inner coordination sphere of Gd^{3+}.[17] This value is larger than those found in mono–aquo contrast agents [Gd-(DTPA)]$^{2-}$ (4.3 s^{-1} mM^{-1} at 25 °C) and [Gd-(DOTA)]$^-$ (4.2 s^{-1} mM^{-1}) which are currently used in clinical practice.[2c] A preliminary study of the temperature dependence on the relaxivity indicates that the water-residence lifetime (τ_M), that is, the residence time describing the exchange between coordinated and bulk water, is not a limiting factor for the relaxivity of [Gd.**1**]$^-$ at temperatures $\geqslant$25 °C. As a matter of fact, [Gd.**1**]$^-$ exhibits a continuous increase in r_1 when the temperature is lowered (Fig. 2) like [Gd-(DTPA)]$^{2-}$ or [S-Gd-(C_4BzDTPA)]$^{2-}$ for which τ_M values determined by oxygen-17 relaxometry are equal to 143 ± 25 ns and 86 ± 8 ns at 37 °C, respectively, and to 331 ± 60 ns and 195 ± 18 ns at 25 °C, respectively.[18] The experimental proton NMRD profiles were analyzed using the classical innersphere[19,20] and outersphere[21] contributions to the paramagnetic relaxation rate. Some parameters were fixed during the fitting procedure: q, the number of coordinated water molecules ($q = 1$), d, the distance of closest approach ($d = 0.36$ nm), D, the relative diffusion constant ($D = 3.3\ 10^{-9}\ m^2/s$)[22], and r, the distance between the Gd(III) ion and the proton nuclei of water ($r = 0.31$ nm). By comparison with the τ_M of Gd-DTPA and S-Gd-(C_4BzDTPA), the water-residence time at 37 °C was set to 100 ns, a value which has no influence on the relaxivity, and to 200 or 300 ns at 25 °C. The results of these fittings are shown in Table 2 and Figure 3. Both fittings performed at 25 °C are very similar and agree with a τ_R value close to 105 ps.

As far as the stability of [Ln.**1**]$^-$ is concerned, no change in luminescence data of [Tb.**1**]$^-$ (emission intensity, lifetime) in water (examined pH range 6.6–8.6) was observed after several days at room temperature, indicating that this complex is highly resistant to dissociation in this medium. In the presence of a 5-fold excess of EDTA at pH 7.4 (HEPES buffer), 40% of the [Tb.**1**]$^-$ complex was dissociated after 2 days (Fig. 4). From this experiment and by using the Verhoeven analysis,[23] $\log K_{\text{cond}}$ (pH 7.4) was measured to be 16.3 for the formation of the terbium complex with **1** in water. This indicates a reasonable physiological stability, compared to the lowest $\log K_{\text{cond}}$ Gd value (14.9 at pH 7.4) found in commercially used MRI agents.[2c] The kinetic inertness of this complex in the presence of competing metal ions which may be involved in a cation-promoted dissociation pathway is under investigation.

Table 2. Fitting parameters obtained from analysis of NMRD profile for [Gd.**1**]$^-$

Parameters	25 °C	25 °C	37 °C
τ_R (ps)	104 ± 2	106 ± 2	61 ± 2.4
τ_M (ns)	200[a]	300[a]	100[a]
τ_{SO} (ps)	73 ± 1	74 ± 10	80 ± 3
τ_V (ps)	21 ± 1.4	21 ± 1.4	21 ± 2.7

[a] Fixed value.

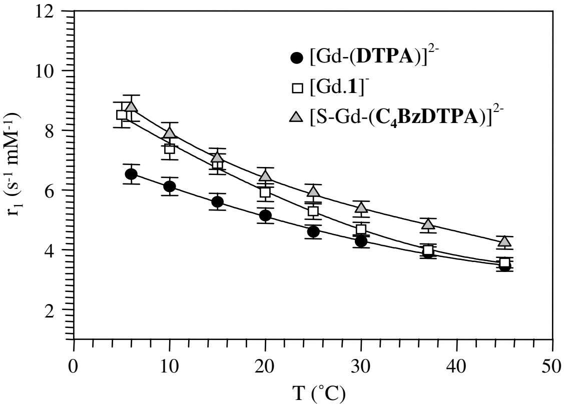


Figure 2. Temperature dependence of the proton longitudinal relaxivity of [Gd.**1**]$^-$ complex (at 20 MHz). Data reported for Gd-DTPA and *S*-Gd-C_4BzDTPA are shown for comparison.[18]

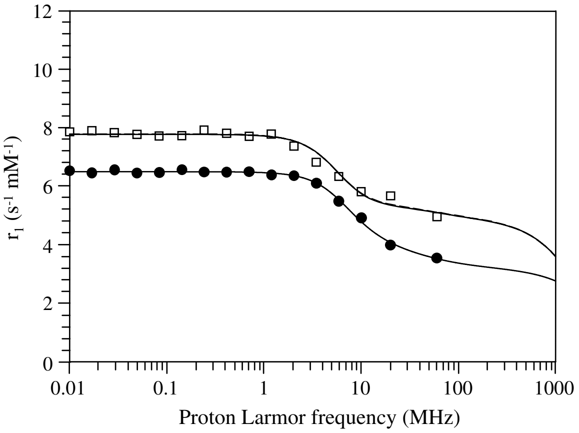


Figure 3. ^{1}H NMRD relaxivity profile of [Gd.**1**]$^-$ complex in water at 37 °C (closed circles) and 25 °C (open squares).

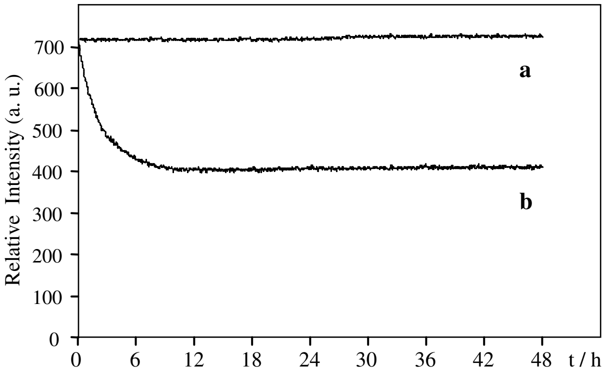


Figure 4. Luminescence (excited at 290 nm, monitored at 545 nm) of a solution of [Tb.1]$^-$ in HEPES buffer (2.5×10^{-6} M, pH 7.4): (a) no added EDTA and (b) five molar equivalents of EDTA added.

In summary, we have described a new octadentate ligand, based on a *N*,*C*-pyrazolylpyridine moiety and iminodiacetate units, which leads to lanthanide complexes with high thermodynamic stability in aqueous solutions. The properties of its Tb^{3+} and Gd^{3+} chelates make this system a very good candidate for the development of efficient bimodal agents (luminophore and paramagnetic contrastophore) for biological applications. The introduction of additional functionality in the pyridine ring, in order to generate targeted molecular imaging agent, is in progress.

Acknowledgments

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11. Selected data of ligand **1**: oil. ^{1}H NMR (D_2O)300 MHz: δ (ppm) 4.00 (s, 4H), 4.02 (s, 4H), 4.61 (s, 2H), 4.64 (s, 2H), 6.72 (d, $J = 2.7$ Hz, 1 H, pz), 7.40 (d, $J = 6.9$ Hz, 1H, py), 7.92 (d, $J = 8.1$ Hz, 1H, py), 8.03 (t, $J = 7.8$ Hz, 1H, py), 8.67 (d, $J = 2.7$ Hz, 1H, pz). ^{13}C NMR (D_2O) 75.5 MHz: δ (ppm) 51.7 (CH_2), 55.4 (CH_2), 56.4 (CH_2), 57,9 (CH_2), 110.6 (CH), 113.2 (CH), 122.5 (CH), 130.3 (CH), 141.5 (CH), 144.2 (Cq), 148.2 (Cq), 150.1 (Cq), 169.2 (Cq), 169.7 (Cq). MS (ES^-): *m*/*z* 472.4 $[M{+}K{-}2H]^-$, 434.4 $[M{-}H]^-$. Analytical HPLC (column CC125-3MN C18 HD 5 μm, solvent: 0.1% TFA in H_2O/MeOH 95/5, flow rate 0.6 mL/mn): $t_R = 7.8$ min.
12. The Ln chelates were prepared by stirring ligand **1** with $LnCl_3.6H_2O$ (1.1 equiv) at pH 6 and precipitating the excess of Ln by adjusting the pH to 8 using NaOH solution. The solvent was removed, the resulting solid was dissolved in a minimum of MeOH, and Et_2O was added to precipitate the compound. The absence of free Ln ions was verified using the Arsenazo test. [Tb.**1**] Na: yield 88%. MS (ESI^-): *m*/*z* 590.3 $[M{-}Na]^-$. Analytical HPLC (column Atlantis dC18 3 μm, solvent: ammonium acetate 25 mM, pH 6.8, flow rate 0.6 mL/mn): $t_R = 3.2$ min. [Gd.**1**]Na: yield 85%. MS (ESI^-, H_2O): *m*/*z* 589.3 $[M{-}Na]^-$.
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