

# Lanthanide complexes of new polyaminocarboxylates with the bis-pyrazolylpyrimidine chromophore

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**Abstract**—We describe the straightforward synthesis of a new polyacid ligand derived from the bis-pyrazolylpyrimidine motif that displayed excellent properties concerning its ability to sensitize the emission of lanthanides. Emission lifetimes were in the range of ns and quantum yields were 0.15 and 0.85 for Eu and Tb, respectively, among the largest measured by us in related compounds, thus making this ligand an excellent candidate for its application in multitagging time-resolved fluoroimmunoassays.

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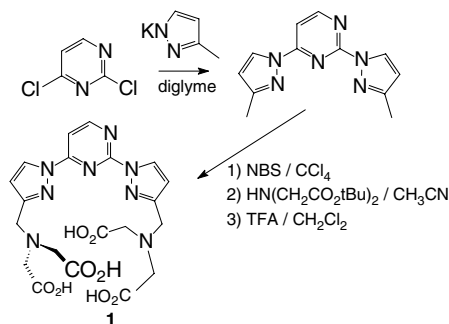
Potassium pyrazolate derivatives easily react with heteroaromatic halides to give N,C bonds by nucleophilic substitution. We have used this reaction in numerous occasions for the building of organic ligands adequate to achieve lanthanide sensitization.<sup>1</sup> The coordination chemistry of lanthanides has become increasingly significant in the last few years due to the wide variety of potential applications of their complexes in many important areas of chemistry,<sup>2</sup> biology,<sup>3</sup> medicine<sup>4</sup> and imaging.<sup>5</sup> Continuing our work in this field,<sup>6</sup> we describe in this paper the synthesis and the luminescence properties of the novel pyrimidine tetraacid **1** shown in Scheme 1, in the hope that it complies with the stringent elec-

tronic and structural properties required for the so-called efficient *antenna effect*, namely, good yields for intersystem crossing, reasonable matching between the chromophore first triplet state and the resonance level of the metal and, last but not least, a good isolation of the metal from water vibronic O–H deactivators.<sup>7</sup>

The reaction path (Scheme 1) is straightforward. Potassium 3-methylpyrazolate generated in situ was made to react with 2,4-dichloropyrimidine in diglyme at 100 °C for 15 h leading to 2,4-(bis-3-methylpyrazole)-pyrimidine in 53% yield. Bromination of both methyl groups by NBS using conventional methods led to a mixture of mono and polybrominated products from which the pursued 2,4-(bis-3-bromomethylpyrazole)-pyrimidine was isolated in 19% yield. The dibromide reacted with *tert*-butyl iminodiacetate in acetonitrile and sodium carbonate to render the tetraester in 75% yield which was finally cleaved to the tetraacid by the action of TFA in 80% yield.<sup>8</sup>

We tried to get good crystals of **1** but all attempts were unsuccessful in our hands. However, that was not the case for the precursor 2,4-(bis-3-methylpyrazolyl)-pyrimidine and its structure was thus resolved by X-ray diffraction (Fig. 1).<sup>9</sup>

While the molecule is practically planar as expected, the nitrogen atom N2 was found to be pointing to the opposite region where N3 and N6 were located. If the final tetraacid conserves this layout, the nitrogen atoms are not adequately arranged to co-operate for lanthanide



**Scheme 1.** Reactions leading to the preparation of 2,6-bis[*N*-3-(iminodiacetoxymethyl)pyrazolyl]pyrimidine studied in this work.

**Keywords:** Luminescence; Lanthanide chelates; Fluoroimmunoassay.

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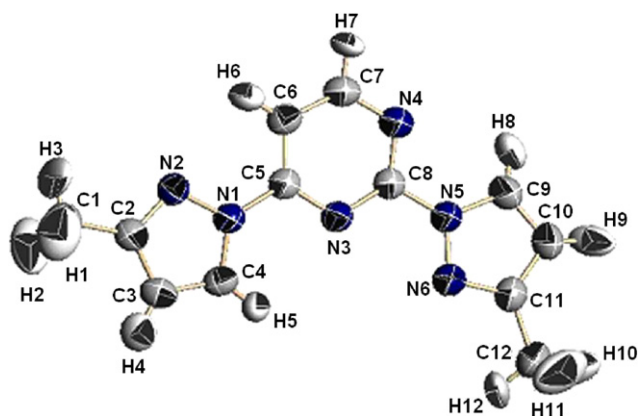


Figure 1. X-ray structure of 2,4-(bis-3-methylpyrazole)pyrimidine.

chelation. In fact, AM1 calculations (Hyperchem) predicted that the conformer resulting from the X-ray analysis is ca. 4 kcal/mol more stable than that where N2 and N3 would be *syn*. The higher energy of the latter conformer should be due to the strong steric demand between H5 and H6 which would be directed towards each other. Therefore, the formation of the most stable lanthanide complex comprising the interaction between the metal and the maximum possible number of heteroatoms will require a 180° turn around the N1–C5 bond and the corresponding energy compensation. Yet, this should not be difficult since the formation of stable lanthanide complexes was clearly evidenced in the pyridine analogue, where N4 of Figure 1 has to be replaced by a C–H group which would be in a high steric demand with H8 (cf. Fig. 2).

The strong bathochromic shift displayed by the pyridine derivative upon complexation (Fig. 3) was taken as solid evidence of the proposed conformational change.

Figure 4 shows that the changes in the absorption maxima of the pyrimidine derivative were in turn much more moderate, in agreement with the suggestion that the free

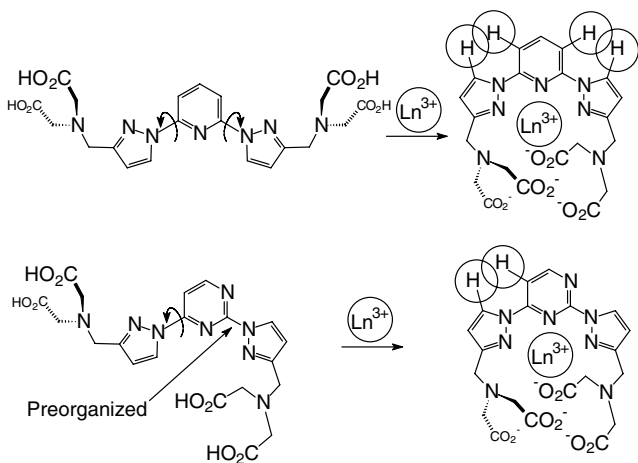


Figure 2. Conformational changes presumably suffered by the bis-pyrazolyl-pyridine (top) and pyrimidine (bottom) tetraacids upon complexation with lanthanides.

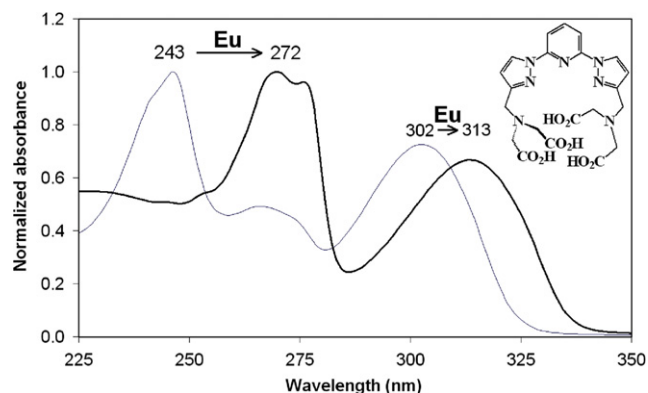


Figure 3. UV-vis spectra of the indicated compound (pyridine derivative) free (narrow line) and complexed with  $\text{Eu}^{3+}$ .

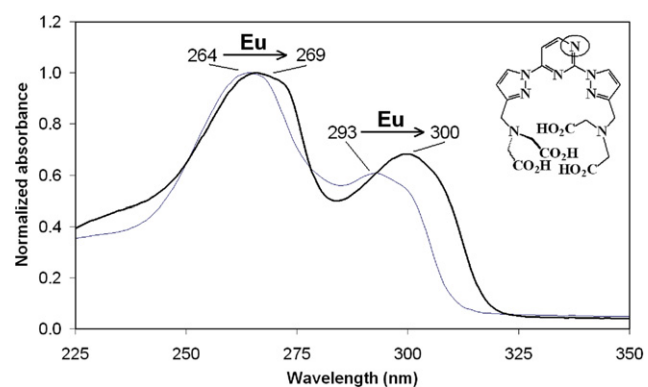


Figure 4. UV-vis spectra of the indicated compound (pyrimidine derivative) free (narrow line) and complexed with  $\text{Eu}^{3+}$ .

compound was already in a conformation closer to that appropriate for complexation than its pyridine counterpart (Fig. 2).

The calculated structure of complex  $1\cdot\text{Eu}^{3+}$  by AM1/MM+ methods (Hyperchem) is shown in Figure 5. It

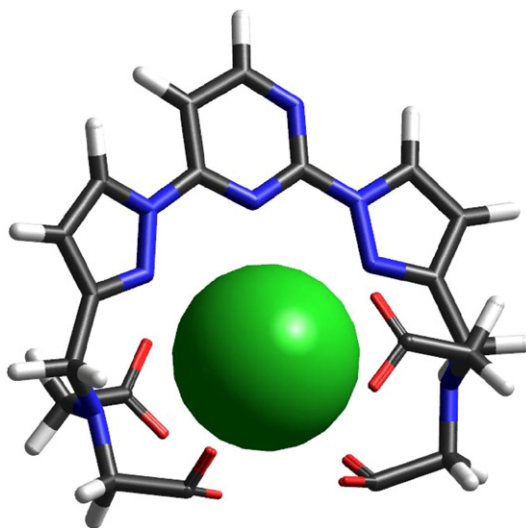
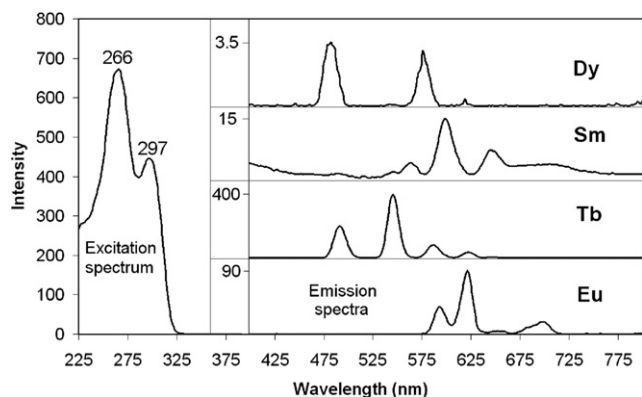


Figure 5. Calculated structure by AM1/MM+ methods of complex  $1\cdot\text{Eu}^{3+}$ .

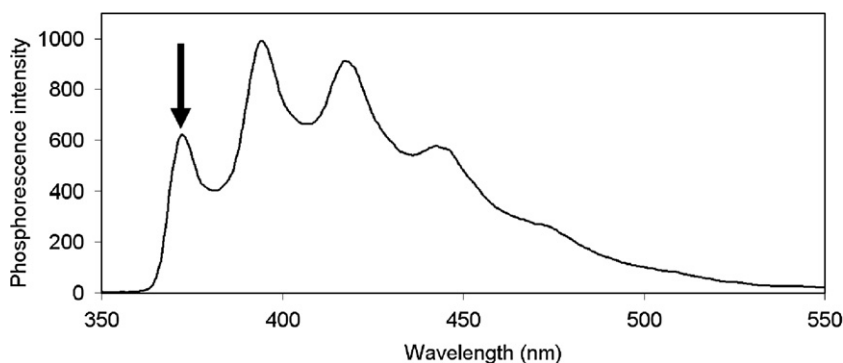


**Figure 6.** Excitation and emission spectra of **1** complexed with the indicated lanthanides(III).

can be seen that the pyrazole ring in the left hand side of the model is predicted to be slightly out of co-planarity with respect to the pyridine ring in order to avoid the aforementioned interaction between the hydrogen atoms equivalent to those labelled as H5 and H6 in the X-ray structure of **Figure 1**.

**Figure 6** contains the excitation and emission spectra of the complexes of **1** with  $\text{Eu}^{3+}$ ,  $\text{Tb}^{3+}$ ,  $\text{Sm}^{3+}$  and  $\text{Dy}^{3+}$ . The profiles of the excitation spectra were coincident, within experimental error, with the absorption spectrum of the complex (**Fig. 4**). This finding evidenced that the energy emitted by the metal was solely that collected by the organic moiety (antenna effect). The overall energy-transfer process occurred in an efficient manner because the complex displayed high absorptivity coefficients  $\epsilon$  (an average of  $20.5 \pm 0.5 \times 10^3$  and  $13.7 \pm 0.5 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$  for the absorptions at 269 and 300  $\text{cm}^{-1}$ , respectively, for all the studied metals) and excellent quantum yields of emission (vide infra).

The energy of the triplet state measured in the phosphorescence spectrum of complex **1**· $\text{Gd}^{3+}$  (**Fig. 7**) was  $26,666 \text{ cm}^{-1}$ , which gives  $\Delta E$  values of 9416 and  $6166 \text{ cm}^{-1}$  with the emissive metal levels  $^5\text{D}_0$  for  $\text{Eu}^{3+}$  ( $17,250 \text{ cm}^{-1}$ ) and  $^5\text{D}_4$  for  $\text{Tb}^{3+}$  ( $20,500 \text{ cm}^{-1}$ ), respectively. Emission lifetimes in water were (ms) 1.35 (**1**· $\text{Eu}^{3+}$ ), 2.74 (**1**· $\text{Tb}^{3+}$ ) and 0.02 (**1**· $\text{Sm}^{3+}$  and **1**· $\text{Dy}^{3+}$ ). These rather long values suggested an excellent isolation



**Figure 7.** Phosphorescence spectrum of the complex **1**· $\text{Gd}^{3+}$  for the measurement of the triplet state assigned to the indicated band.

from the deactivating solvent. In fact, the number of water molecules in the first coordination sphere, as calculated by the Horrocks, Parker and Kimura methods,<sup>10</sup> did not exceed 0.5 for all the studied metals. Each and every one of these parameters should be responsible for the exceptionally high quantum yields measured (0.15 and 0.85 for  $\text{Eu}^{3+}$  and  $\text{Tb}^{3+}$ , respectively) which were among the largest measured by us in similar compounds.

In conclusion we have described the straightforward synthesis of a new polyacid ligand derived from the bis-pyrazolylpyrimidine motif that displayed excellent properties concerning its ability to sensitize the emission of lanthanides. The long emission lifetimes in the ms range and the high quantum yields displayed by the lanthanide complexes of this ligand make it an excellent candidate for bioconjugation and thus its application in multitagging time-resolved fluoroimmunoassays.

### Acknowledgements

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  - $^1\text{H}$  NMR (300 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 8.85–8.82 [3H, m, Pym(H6), 2-Pz(H5), 4-Pz(H5)]; 7.71 [1H, d,  $J = 5.1$  Hz, Pym(H5)]; 6.65 [1H, d,  $J = 2.7$  Hz, 2-Pz(H4)]; 6.59 [1H, d,  $J = 2.7$  Hz, 4-Pz(H4)]; 4.02 [2H, s, 2-PzCH<sub>2</sub>N]; 3.99 [2H, s, 4-PzCH<sub>2</sub>N]; 3.55 [4H, s, 2  $\times$  NCH<sub>2</sub>CO<sub>2</sub>H]; 3.52 [4H, s, 2  $\times$  NCH<sub>2</sub>CO<sub>2</sub>H].  $^{13}\text{C}$  NMR (50 MHz, DMSO- $d_6$ )  $\delta$  (ppm): 171.8 [CO<sub>2</sub>H]; 161.5 [PymC6]; 157.5 [PymC2]; 154.8 [PymC4]; 153.3 [Pz(C3)]; 131.4 [4-Pz(C5)]; 129.7 [2-Pz(C5)]; 110.3 [2-Pz(C4)]; 109.4 [4-Pz(C4)]; 105.8 [PymC5]; 54.0 [NCH<sub>2</sub>CO<sub>2</sub>H]; 51.1 [2-PzCH<sub>2</sub>N]; 50.9 [4-PzCH<sub>2</sub>N]. MS (L–SIMS+): 525 (M+Na<sup>+</sup>, 6), 503 (M+H<sup>+</sup>, 25). Exact mass for C<sub>20</sub>H<sub>22</sub>N<sub>8</sub>O<sub>8</sub>: 503.1638. Found: 503.1642.
  - CCDC 628205 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif).
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