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# New 2,6-Bis(5-phenyloxazolyl)pyridine Ligands for Luminescent LnIII Complexes †

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**Abstract:** Lanthanide (Ln(III)) luminescent complexes have been attracting a lot of interest for technological applications and molecular imaging. The luminescence of Ln(III)) ions is weak and depends on the use of light absorbing coordination ligands which sensitizes the lanthanide ion. A large variety of coordination ligands has been screened such as dipicolinates, oligopyridines, cyclen and crown ether derivatives, porphyrins, cryptands or calixarenes. In our research group we have developed an expeditious methodology to prepare bis(oxazolyl)pyridine ligands for LnIII from threonine and 2,6-pyridinedicarbonyl dichloride. In this work, two new pyridine-bis-oxazolyl ligands with an aromatic ring in position 5 of the oxazole ring were prepared from phenylserine and 2,6-pyridinedicarbonyl dichloride. The photophysical properties of compounds 1 and 2 were studied in acetonitrile and in Tris-HCl buffer (0.1 M, pH 7.1). These compounds were used for complexation with Eu(III) and/or Tb(III) ions, and the photophysical properties of the complexes studied. Luminescence titrations with anhydrous $EuCl_3$ and $TbCl_3$ allowed the determination of the stoichiometry of the complexes and of the stability constants.

**Keywords:** Pyridine-bis-(5-phenyloxazolyl) ligands; Lanthanides; Luminescence

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## 1. Introduction

Lanthanide chemistry is dominated by the oxidation state (III), Lanthanide(III) ions -$Ln^{3+}$, although oxidation states (II) and (IV) are also energetically accessible. The characteristic luminescence (fluorescence/phosphorescence) and magnetism (paramagnetism) properties of complexes of Lanthanide(III) ($Ln^{3+}$) make the complexes useful in diagnostic (Magnetic Resonance Imaging- MRI and optical imaging) and therapeutic applications. [1] Direct excitation of $Ln^{3+}$ ions is inefficient due to the forbidden nature of *f-f* electronic transitions (low molar absorptivity coefficients). However, $Ln^{3+}$ ions in complexes with organic ligands with high molar absorptivity can be excited indirectly—the antenna effect. The excitation energy of the ligand can be transferred to the $Ln^{3+}$ ions and result in emission centered on the $Ln^{3+}$ ions, producing larger Stokes shift, brighter visible emission, and longer lifetime. [2,3] Antenna ligands are usually planar conjugated aromatic organic molecules that efficiently absorb energy in the UV-Vis region of the electromagnetic spectrum. In the last decades, several derivatives of the pyridine moiety have been used as sensitizers for different metals of the LnIII group.[4–6] Throughout the reported pyridine derivatives, the remarkably high efficiency of energy transfer from the ligand to the metal is highlighted. In this work two new potential ligands for $Ln^{3+}$ ions were prepared and their complexes with Eu(III) and Tb (III) studied.

## 2. Materials and Methods

The $^1$H-NMR spectra were obtained using the Bruker Avance III 400 (400 MHz) equipment, using a solvent peak as an internal reference. The coupling constant (*J*) was obtained in Hertz and the chemical displacement (δ) in parts per million (ppm). The deuterated solvents used were dimethyl sulfoxide (DMSO-$d_6$) and chloroform ($CDCl_3$-$d_1$).

Thin layer chromatographies (TLC) were performed on Merck-Kieselgel 60 F254 plates and were developed in the ultraviolet (ν = 50 Hz) in a CN-6 ultraviolet light chamber. The petroleum ether used refers to the fraction with a boiling point of 40–60 °C. The organic phases were dried using anhydrous magnesium sulfate (Riedel) and anhydrous potassium carbonate (Merck).

The solutions were prepared using solvents with an HPLC grade. All measurements were performed at room temperature.

Absorbance measurements were performed on a Shimadzu UV-3101PC UV/ Vis/NIR spectrophotometer. The fluorescence measurements were performed on a Spex Fluorolog 2 spectrofluorometer equipped with double monochromators in excitation and emission. The fluorescence spectra were corrected for the instrumental response to the system.

### *2.1. Synthesis of Compounds*

#### 2.1.1. Compound 1

Dipicolinic acid (0.514 g, 3.08 mmol) and the phenyl serine methyl ester (2.2 eq., 1.57 g) were added to acetonitrile (50 mL). The flask was placed in an ice bath and $Et_3N$ (1.5 eq., 1.4 mL), HOBt and DCC (2.2 eq., 1.4 g) were added. The reaction was left stirring at room temperature for 18 h. The reaction mixture was filtered and solvent removed under reduced pressure. The residue was recovered in acetone (50 mL) and the solution was left in the freezer for 18 h. The precipitated urea was filtered off and the solvent was removed. The residue was recovered in ethyl acetate (150 mL) and washed with sodium bicarbonate (3x50 mL) and with saturated sodium chloride solution (2 × 50 mL). The organic phase was dried with anhydrous magnesium sulfate. After removal of the solvent, compound 1 (1.88 g, 2.88 mmol) was obtained in 93% yield.

$^1$H-NMR (400 MHz, $CDCl_3$ δ): (mixture of diastereomers) 3.71 and 3.72 (2s, 6H, $OCH_3$); 5.00–5.09 (m, 4H, αH + OH); 5.45 (broad s, 2H, βH); 7.19–7.45 (m, 10H, ArH); 7.70–8.10 (m, 3H, ArH); 8.87–8.94 (m, 2H, NH) ppm.

#### 2.1.2. Compound 2

Compound 1 (2.88 mmol, 1.50 g) was dissolved in dry acetonitrile (25 mL) and $Boc_2O$ (2.2 eq. 1.38 g) and DMAP (0.1 eq., 71 mg) were added and left stirring. The reaction was followed by $^1$H NMR. After checking the disappearance of compound 1, TMG (4% V/V) was added. The solvent was removed, and the residue was recovered in ethyl acetate (50 mL). Washed with 1M $NaHCO_3$ (3 × 15 mL) and saturated sodium chloride (3 × 15 mL). The organic phase was dried with anhydrous magnesium sulfate and the solvent removed under reduced pressure. The compound 2 was purified by “dry-flash” using ethyl acetate / petroleum ether 40–60 as eluent. Compound 2 was obtained in 33% yield (0.95 mmol, 0.46 g)

$^1$H NMR (400 MHz, $CDCl_3$ δ): 3.87 (s, 6H, $OCH_3$); 7.28–7.37 (m, 6H, ArH); 7.49 (s, 2H, βCH); 7.50–7.52 (m, 4H, ArH); 8.09 (t,J = 8.0 Hz, 1H, ArH); 8.40 (d, J = 8.0 Hz, 2H, ArH); 9.40 (s, 2H, NH) ppm.

#### 2.1.3. Compound 3

1,2-dichloroethane was treated with 100 mL of 10% $H_2SO_4$. To 5 mL of the 1,2-dichloroethane solution, compound 2 (142 mg, 0.292mmol) was added with $BF_3 \cdot O\ (CH_2CH_3)_2$ (4 equivalents, 1.17 mmol, 150μL). The mixture was refluxed with heating and then PIDA (244 mg, 0.760 mmol, 2.6 equivalents) was added, dissolved in 2 mL of $CH_2Cl_2$. A yield of approximately 26% (0.076 mmol, 37 mg) was obtained.

$^1$H NMR (400 MHz, $CDCl_3$ δ): 4.01 (s, 6H, 2 $OCH_3$); 7.53-7.57 (m, 6H, ArH); 8.04 (t, J=7.6 Hz, 1H, ArH); 8.24-8.26 (m, 4H, ArH); 8.41 (d, J=7.6 Hz, 2H, ArH) ppm.

2.1.4. Compound 4

All compound 3 (0.076 mmol, 37 mg) obtained previously was dissolved in dioxane (7ml) and NaOH 1 (M) was added. The reaction was followed by TLC. Then the solvent was evaporated. Water was added, acidified to pH 2-3 and the aqueous phase was extracted. The organic phase was washed with $H_2O$ (3 × 15 mL) and saturated NaCl solution (3 × 15 mL). The organic phase was dried with anhydrous MgSO4 and the solvent was removed. The reaction yield was 80% (0.061 mmol, 28 mg).

$^1$H NMR (400 MHz, D2O δ): 7.42–7.48 (m, 6H, 4H ArH); 7.94–7.96 (m, 4H, ArH); 8.05-8.12 (m, 3H, ArH) ppm.

## 3. Results and Discussion

### *3.1. Synthesis*

Two oxazolylpyridine derivatives were prepared from the methyl ester of β-hydroxyphenylalanine and dipicolinic acid (Figure 1). The synthetic strategy to obtain compound **3** involved a coupling reaction, followed by a dehydration and a cyclization. The cyclization of compound **2** was carried out by treatment with a hypervalent iodine reagent [(diacetoxyiodo)benzene, PIDA]. The basic hydrolysis of compound **3** afforded compound **4**.

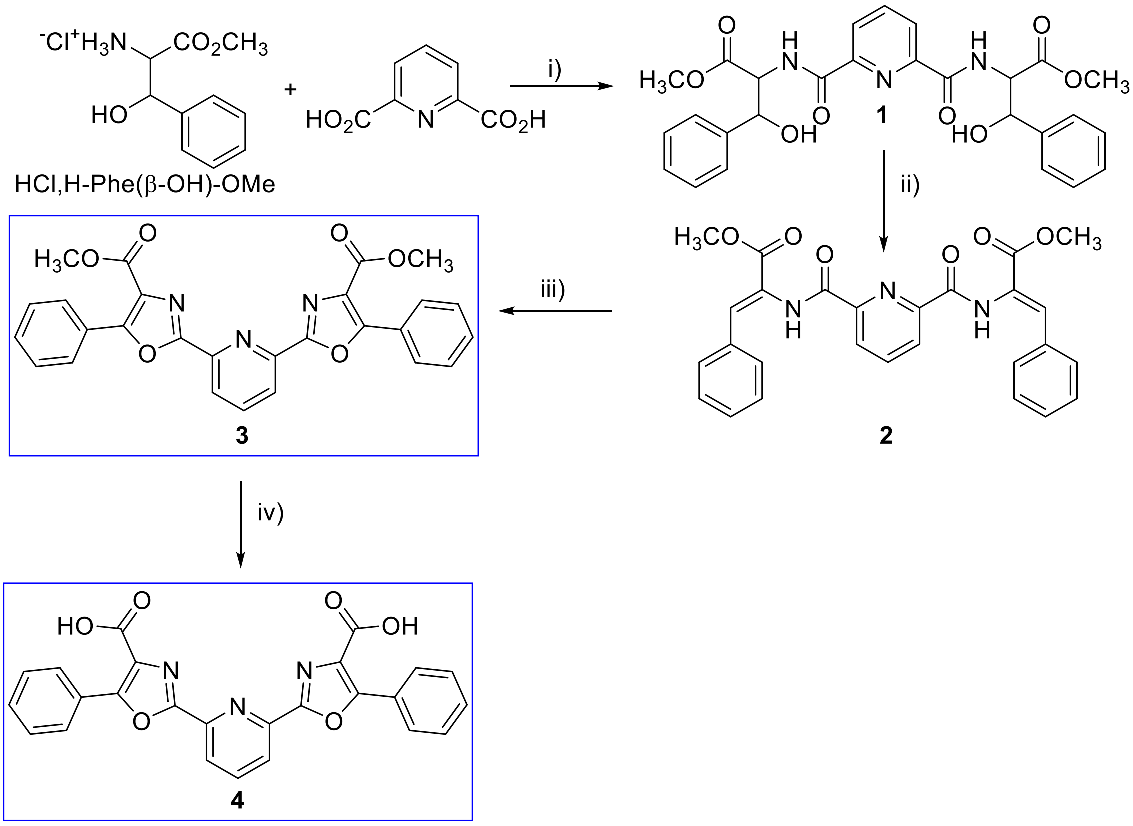


**Figure 1.** Synthesis of bis-(phenyloxazolyl)piridine derivatives **3** and **4**. i) DCC/HOBT; ii) 1. $Boc_2O$/DMAP, 2. TMG (2%); iii) PIDA, $BF_3$; iv) NaOH 1M.

### *3.2. Fluorescence Properties of Ligands*

The photophysical properties of compounds **3** and **4** were studied in acetonitrile and Tris-HCl buffer (0.1 M, pH 7.1). Figure 1 shows the normalized absorption and emission spectra of these compounds. The maximum absorption ($\lambda_{abs}$) and emission wavelengths ($\lambda_{em}$), molar absorption coefficients (ε) and fluorescence quantum yields ($\Phi_F$) are presented in Table 1. Both compounds **3** and **4** absorb strongly in the UV, with maxima at $\lambda$ = 280 nm.These bis-oxazoles are also fluorescent, with emission quantum yields around 50% for compound **3** and 30% for compound **4**. The fluorescence quantum yields of ligands 3 and 4 are significantly lower than fluorescence quantum yields of analogous ligands with a methyl group at the 5-position of the oxazole ring.[7] A red-shift in emission is observed for compound **4** relative to compound **3** (Table I) which could be attributed, not only to solvent effects, but also to the additional intramolecular charge transfer (ICT) character of the excited state of bis-oxazole **4**. This was also observed for other 5-substituted bis-oxazolylpyridine derivatives.[7]

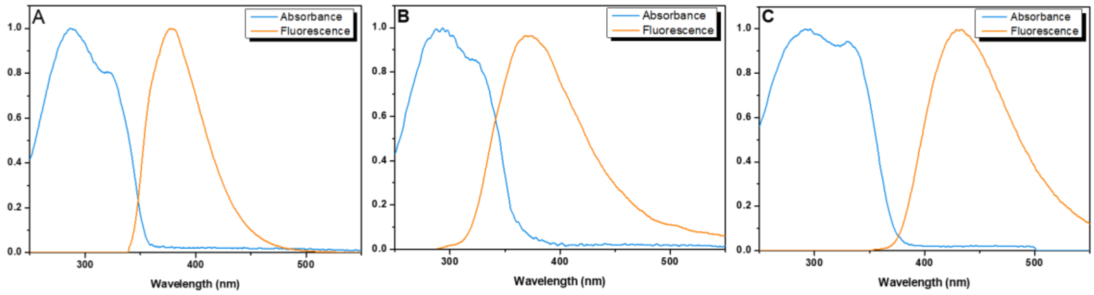


**Figure 2.** Normalized absorption and fluorescence spectra ($\lambda_{exc}$ = 280 nm) of compounds **3** and **4**. A) Ligand **3** in MeCN; B) Ligand **4** in MeCN; C) Ligand **4** in Tris-HCl Buffer (0.1M; pH = 7.1).

**Table 1.** Maximum absorption ($\lambda_{abs}$) and emission wavelengths ($\lambda_{em}$), molar absorption coefficients ($\varepsilon$) and fluorescence quantum yields($\Phi_F$) for compounds **3** and **4**.

| Ligand | Solvent | $\lambda_{abs}$ (nm) ($\varepsilon$ $M^{-1}cm^{-1}$) | $\lambda_{em}$ (nm)* (Stokes shift, nm) | $\phi_F$** |
|---|---|---|---|---|
| **3** | Acetonitrile | 280 ($3.47 \times 10^4$) | 376 (96) | 0.49 |
| **4** | Acetonitrile | 280 ( 488.7) | 364 (84) | 0.32 |
| **4** | Tris-HCl buffer 0.1M, pH= 7.1 | 280 ($7.28 \times 10^3$) | 425 (145) | 0.35 |

* $\lambda_{exc}$ = 280 nm; ** using tryptophan as standard, $\phi_F$= 0.13 [8].

Fluorescence titration of the ligand **4** in acetonitrile with a solution of Eu(III) trifluoromethanesulfonate ($Eu(Tf)_3$) in acetonitrile (Figure 3A) was carried out to determine the stoichiometry of the complex.

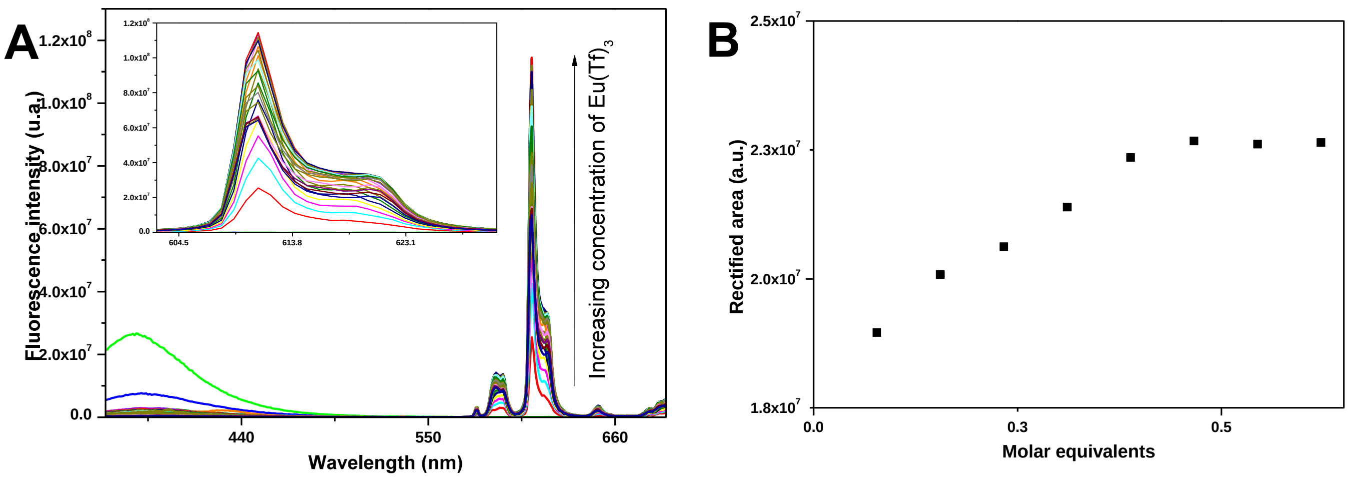


**Figure 3. A**) Fluorescence titration ($\lambda_{exc}$= 280 nm) of a solution of ligand **4** (1.24x$10^{-5}$ M in acetonitrile, 2 mL) with a solution of $Eu(Tf)_3$ (9.68x$10^{-4}$ M, in acetonitrile) **B)** Representation of the rectified area of the emission band of the Eu(**4**)$_x$ complex at 614 nm ($\lambda_{exc}$ = 280 nm) as a function of the number of molar equivalents of $Eu(Tf)_3$ added.

As can be seen in Figure 3A, the fluorescence emission band of the ligand **4** at 364 nm (green curve) undergoes a progressive decrease of intensity with the addition of $Eu(Tf)_3$. Simultaneously, new bands appear at 578, 592, 614, and 652 nm, which undergo progressive intensification with the addition of $Eu(Tf)_3$. This phenomenon indicates the formation of a Eu complex with ligand **4** and the sensitization of the $Eu^{3+}$ ion fluorescence by ligand **4**. The bands at 578, 592, 614, and 652 nm have a characteristic shape, dependent on the symmetry of the complex and can be attributed to transitions $^5D_0$-$^7F_J$ (J = 0, 1, 2, 3, 4. The progressive intensification of the more intense band at 614 nm (hypersensitive transition) with the addition of $Eu(Tf)_3$ allows estimation of the stoichiometry of the complex.

The stabilization of the curve after the addition of approximately 0.5 molar equivalents of $Eu(Tf)_3$ suggests the formation of a 2:1 complex. The formation of a complex is also likely to cause significant changes in the absorption spectrum of the ligand in the UV-Vis region of the electromagnetic spectrum.

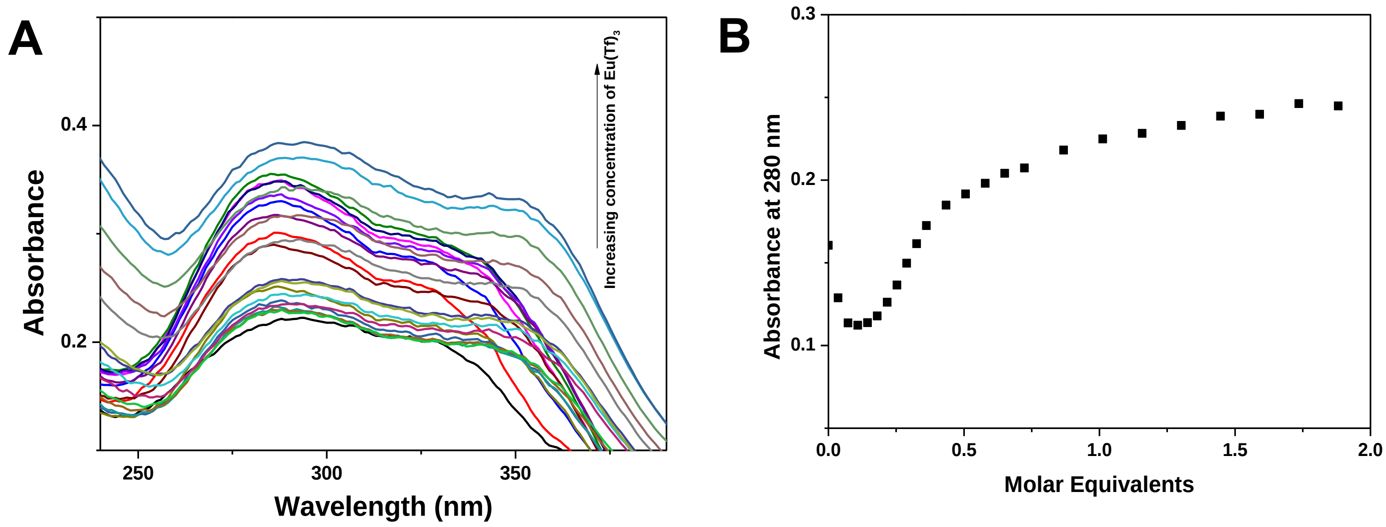


***Figure 4.*** **A)** Spectrophotometric titration of a solution of ligand **4** ($1.34 \times 10^{-4}$ M in acetonitrile, 1 mL) with Eu $(Tf)_3$ ($9.68 \times 10^{-4}$ M, in acetonitrile) **B)** Dependence of the intensity of the absorption band at 280 nm of a solution of ligand **4** ($1.34 \times 10^{-4}$ M, in acetonitrile) with the number of molar equivalents of $Eu(Tf)_3$ added.

Addition of $Eu(Tf)_3$ causes significant changes in the absorption spectrum of ligand **4** indicating the formation of a complex.

There is initially a decrease in the band intensity at 280 nm, followed by an increase in the intensity with tendency to stabilize after addition of approximately one molar equivalent of $Eu^{3+}$ ions. The decrease in the slope of the curve after the addition of approximately 0.5 molar equivalents of $Eu(Tf)_3$ also suggests the formation of a complex with stoichiometry of 1:2 Eu(**4**)$_2$ which may continue to react more $Eu(Tf)_3$ to give another complex, possibly with M:L (1:1) Eu(**4**) stoichiometry.

Ligand **4** is not very soluble in acetonitrile but soluble in aqueous medium. The formation of a water-soluble Eu(**4**)$_x$ luminescent complex would be of great interest for biological studies as a cell marker, sensor or molecular imaging.

Fluorescence titrations ($\lambda_{exc}$ = 280 nm) of aqueous solutions (0.1M Tris-HCl buffer, pH=7.1) of ligand **4** with $EuCl_3.6H_2O$ revealed no change in the emission spectrum of the ligand nor the appearance of bands attributed to electronic transitions of $Eu^{3+}$ ion. However, the spectrophotometric titration reveals that there is interaction between the ligand **4** and the $Eu^{3+}$ ion in aqueous solution (Figure 5A).

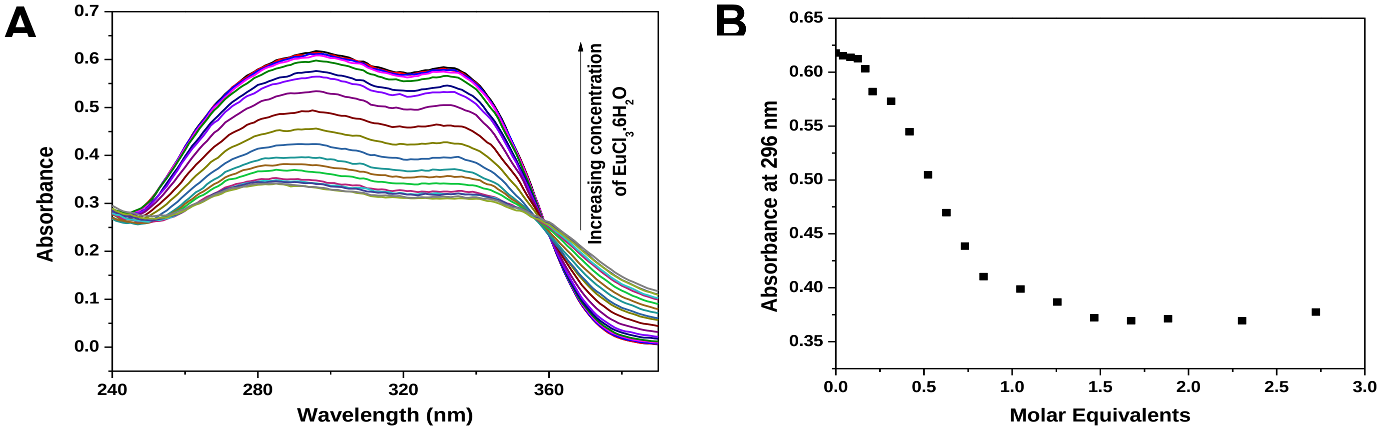


**Figure 5. A)** Spectrophotometric titration of a solution of ligand **4** ($8.29 \times 10^{-5}$ M, in 0.1 M Tris-HCl buffer, pH = 7.1) with $EuCl_3.6H_2O$ ($1.74 \times 10^{-3}$ M, in 0.1M Tris-HCl buffer), pH = 7.1) **B)** Dependence of the intensity of the absorption band at 296 nm on the number of $Eu^{3+}$ molar equivalents in the titration of an aqueous solution (0.1 M Tris-HCl buffer, pH=7.1) of ligand **4** ($8.29 \times 10^{-5}$ M) with an aqueous solution of $EuCl_3.6H_2O$ ($1.74 \times 10^{-3}$M).

The dependence of the absorption band intensity at 296 nm on the number of $Eu^{3+}$ molar equivalents clearly indicates the formation in aqueous solution of an Eu(**4**) complex with M:L (1:1) stoichiometry.

While ligand **4** forms a fluorescent complex with $Eu^{3+}$ in acetonitrile, the respective complex with $Tb^{3+}$ ions is non-fluorescent in acetonitrile. To investigate the complexation of ligand **4** with $Tb^{3+}$ ions in aqueous medium a spectrophotometric titration was performed (Figure 6A).

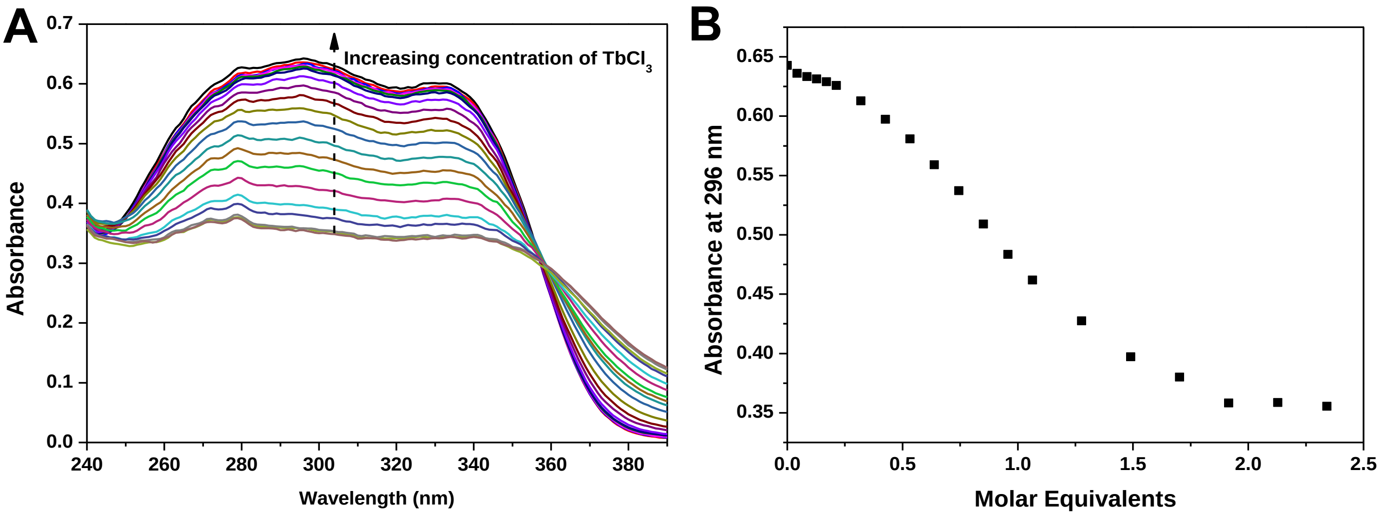


**Figure 6. A)** Spectrophotometric titration of a solution of ligand **4** ($8.29 \times 10^{-5}$ M, in 0.1 M Tris-HCl buffer, pH = 7.1) with $TbCl_3.6H_2O$ ($1.54 \times 10^{-3}$ M, in 0.1 M Tris-HCl buffer, pH = 7.1) **B)** Dependence of the intensity of the absorption band at 296 nm on the number of $Tb^{3+}$ molar equivalents in the titration of an aqueous solution (0.1 M Tris-HCl buffer, pH = 7.1) of ligand **4** ($8.29 \times 10^{-5}$ M) with an aqueous solution of $TbCl_3.6H_2O$ ($1.54 \times 10^{-3}$).

The dependence of the absorption band intensity at 296 nm on the number of molar equivalents of $Tb^{3+}$ also suggests the formation in aqueous solution of a Tb(4) complex with M:L stoichiometry (1:1) (Figure 6B).

## 4. Conclusion

In this work, two new ligands of the 2-6-bis(oxazolyl)-pyridine type were synthesized and characterized. The photophysical properties of these ligands were studied in acetonitrile and in aqueous medium (Tris-HCl buffer). The complexation of $Ln^{3+}$ ions by ligands **3** and **4** was studied in acetonitrile and in aqueous medium through fluorescence titrations and spectrophotometric titrations (UV-Vis). Ligand **4** forms a weakly fluorescent Eu(**4**)$_2$ complex in acetonitrile with M:L stoichiometryof 1:2. In aqueous medium, ligand **4** forms a non-fluorescent complex with M:L stoichiometry of 1: 1. These results suggest that the ligand structure, due to geometric or stereorestrictions, prevents the formation of a complex with M:L stoichiometry of 1:3. It also appears that in the M:L complexes of 1:2 and 1:1 coordination occurs through the $N_3$ tridentate motif of the pyridine-bis-oxazole nucleus leaving free positions in the metal coordination sphere, which may be occupied by molecules of solvent, with fluorescence quenching. The phenyl group at position 5 of the oxazole ring appears to have a negative effect on the quantum fluorescence yield, the coordinating capacity of the ligands for $Ln^{3+}$ ions and the efficiency of the $Ln^{3+}$ ion fluorescence sensitization process.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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