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# Study on luminescent ternary EuEDTA complexes with a set of substituted 4-phenylethynyl and 4-aryl pyridine-2,6-dicarboxylic acids

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## ABSTRACT

A set of pyridine-2,6-dicarboxylic acids (dipicolinic acids, DPAD, Lx) were prepared to study the photo-physical properties of their ternary complexes with Eu(III) and ethylenediaminetetraacetic acid (EDTA or Y). The stability constant of the formation of EuY-L1 (L1 = dipicolinic acid, measured  $\log K_{\text{ter}} = 5.38$ ) was determined and found to be comparable to the formation constant of  $\text{Eu(L1)}_3$  ( $\log K_3 = 5.51$ ). All the studied ternary complexes exhibit two predominant luminescence decay times. The origin of the shorter lifetime was shown to be due to the dissociation of carboxylate group(s) and their replacement by water molecules. The presence of the short-lived component decreases the quantum yields compared those reported for complexes of  $\text{Eu(Lx)}_3$ . However, the use of ternary complexes of  $\text{EuY-Lx}$  offers a simple comparison method between different dipicolinic acids (Lx). Eight of the studied ligands Lx form brighter ternary complexes than the currently used ligand in the duplex PCR assay, with quantum yields over 20%, and the most luminescent one has three times higher signal level compared to the currently used ligand.

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## 1. Introduction

Lanthanide chelates, especially the chelates of europium and terbium, are widely used as labels in various bioanalytical assays due to the long decay times of their luminescence. The unique properties of lanthanide ions place special requirements on the chelating compounds. The low absorption cross-section or absorptivity of lanthanide ions means that the ligand should have both a high absorptivity and efficient energy transfer from the excited state of the “antenna” ligand to the proper energy level of the lanthanide ion. The lanthanide coordination occurs predominantly via electrostatic interactions, meaning that the coordination has little or no directionality and the steric constraints have a strong influence on the stability of the complexes. Most trivalent lanthanide ions form octa- or nonacoordination complexes and at least six electronegative donors in the chelating ligand are needed to guarantee the stability of the complex in aqueous solutions. The remaining free coordination sites of the lanthanide cation are filled with water molecules unless molecules of higher affinity for lanthanide ions are present. In this case a

ternary complex or mixed-ligand complex is formed.

Tendency of lanthanide cations to form ternary complexes has been utilized in various analytical techniques. For instance, a suitable complexing agent may be added just for displacing water from the inner coordination sphere of the lanthanide cation. Water is very effective in quenching luminescence by multiphonon deactivation and hence considerable enhancement of intensity is obtained. Other applications, for example, are molecular recognition and chirality sensing using luminescence and NMR techniques [1]. It is obvious that there are two essentially different ways to apply the ternary complexation. Either the lanthanide cation forms a relatively stable complex, (a) with an antenna-type ligand and the second molecule binds to the remaining free coordination sites, or (b) with a non-absorbing ligand and an antenna-type ligand binds to the remaining coordination sites. In the first case (a) the chelate is already luminescent without the addition of the second ligand. The second ligand may change the luminescent properties and becomes characterized or recognized on this basis. In the case (b) the chelate becomes luminescent only after coordination of the second ligand. It is this case which is of our interest in this work.

A ternary complex of terbium cation has been applied already about 25 years ago in a DNA-hybridization assay [2]. In this assay a specific nucleic acid sequence is identified by using two oligonucleotide sequences, one labelled by a Tb(III) complex of

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diethylenetriamine pentaacetate and the other by aminosalicylate. The oligo sequences have been chosen to be complementary to the DNA strand in such a way that, when hybridized, the labels are in direct proximity to form a ternary complex where the aminosalicylate moiety functions as an antenna for Tb(III). The assay is then accomplished by measuring the intensity of the long-lived Tb(III) emission. The method has been later improved by replacing Tb(III) by Eu(III) and aminosalicylate by an aromatic  $\beta$ -diketone [3]. Even higher sensitivity was obtained by Karhunen et al. by using a dipicolinic acid derivative as the light-harvesting antenna [4]. The method, called “switchable lanthanide probe technique”, was further successfully extended into homogeneous PCR assays [5a,5b].

The purpose of the carrier ligand is to keep the lanthanide ion strongly complexed even in very dilute solutions. Hence the stability constant should be high or at least the dissociation kinetics should be slow. Various aminopolycarboxylic acids have been used, both acyclic and macrocyclic compounds. A common feature is that the compounds do not absorb in the near UV region and consequently, the chelates are practically nonfluorescent. On the other hand, the carrier ligand should not be more than heptadentate because at least two coordination sites should be left for the antenna ligand. The light-harvesting antenna ligand should have a high absorptivity at a suitable wavelength and it should transfer energy effectively to the lanthanide ion. The formation constant of the ternary complex should not be too high because otherwise the background signal would be observable even without the DNA hybridization.

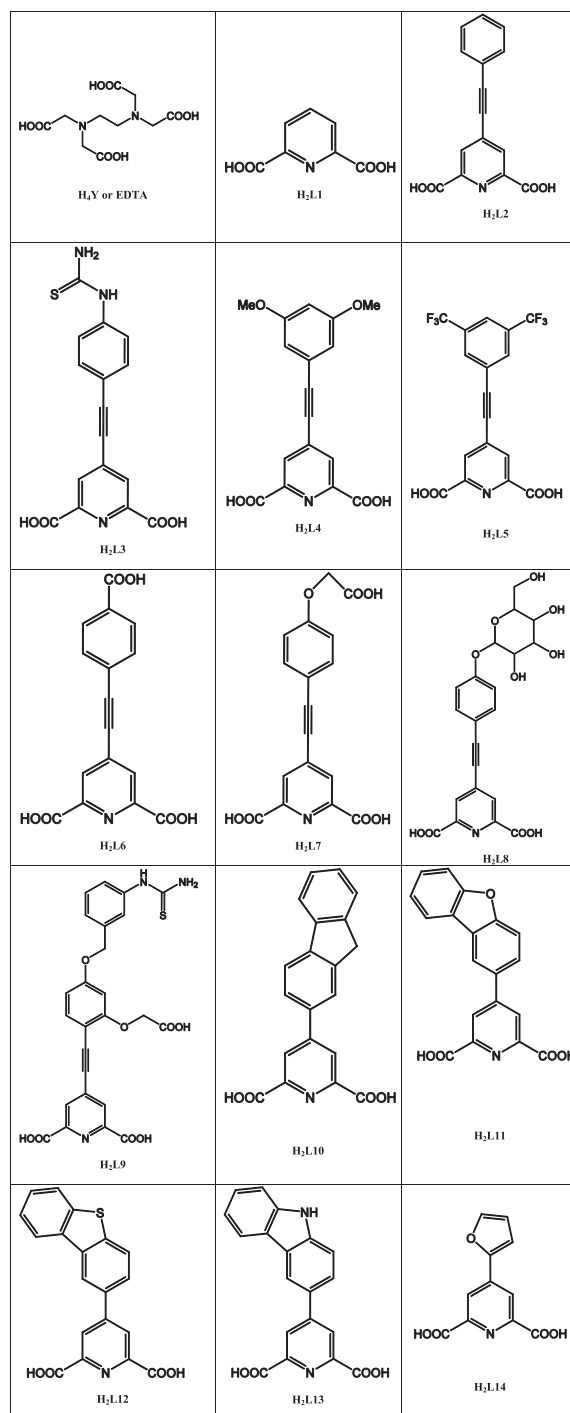
Derivatives of dipicolinic acid (DPAD) are tridentate and, together with a hexadentate carrier ligand, such as ethylenediaminetetraacetic acid (EDTA or Y in this work), interesting model systems for ternary complexes and their applications are formed. The aim of the present work is to study the ternary complexes of Eu(III) with a number of dipicolinic acid derivatives as antenna ligands and ethylenediaminetetraacetic acid as a carrier ligand. The choice of the DPADs was based on our previous experience with 4-phenylethynylpyridine-2,6-dicarboxylic acids as antenna ligands [6a-b]. In addition to eight phenylethynyl compounds, five aryl or heteroaryl substituted DPAs were included.

## 2. Experimental

### 2.1. Materials

Reverse osmosis water was distilled twice in quartz vessels before use. TRIS (tris(hydroxy-methyl)aminomethane) (p.a.), sodium acetate, EDTA (Titriplex III) (p.a.) and Xylenol orange were purchased from Merck. DPA ( $\geq 99.5\%$ ),  $\text{Eu}_2\text{O}_3$  (99.95%) and  $\text{Gd}_2\text{O}_3$  ( $\geq 99.99\%$ ) were from Aldrich. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) ( $> 99\%$ ) was from Biochemical. All the chemicals used in the synthesis were from Aldrich or Acros. The starting materials 4-[(4-isothiocyanatophenyl)ethynyl]-pyridine-2,6-dicarboxylic acid [4], 4-iodobenzene-1,3-diol [7], ethyl 4-ethynylbenzoate [8], diethyl 4-bromopyridine-2,6-dicarboxylate [9], 4-ethynylphenoxy 2,3,4,6-tetra-*O*-acetyl- $\alpha$ -galactopyranoside [10], ethyl (4-ethynylphenoxy)acetate [11], 3-bromodibenzofuran [12] and 3-bromodibenzothiophene [13] as well as the ligand L2 [9] were prepared according to the literature. Scheme 1 shows the abbreviations and structures of the present ligands. The general synthetic pathways used for the ligands L4-L14 are shown in Schemes 2 and 3. The more detailed synthetic procedures and the results of the spectroscopic product characterization are presented in the Supporting information.

The 100  $\mu\text{M}$  EuY stock solution in TRIS buffer was made by mixing one-to-one molar ratio the 1.00 mM EDTA solution and the 0.848 mM Eu solution made by dissolving europium oxide in dilute perchloric acid. EuY-Lx sample solutions were prepared using



**Scheme 1.** The abbreviations and structures of the ligands used.

the stock solutions of the ligands Lx and EuY in either TRIS or  $\text{NH}_3$  buffers ( $C_{\text{TRIS}/\text{NH}_3} = 50 \text{ mM}$ ,  $\text{pH} = 7.75$ ; L3 and L9 in  $\text{NH}_3$  buffer). The concentration of the Eu(III) solution was determined by titrating it with EDTA using xylenol orange as the indicator [14].

### 2.2. Methods

The excitation and emission spectra and quantum yields were determined on a Varian Cary Eclipse fluorescence spectrometer. The luminescence lifetimes of the europium complexes were determined by using the frequency-domain method with a UV-LED (UVTOP285 or UVTOP335, Roithner Lasertechnik GmbH, Vienna) for excitation. Data treatment was done as described previously [15,16]. The

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