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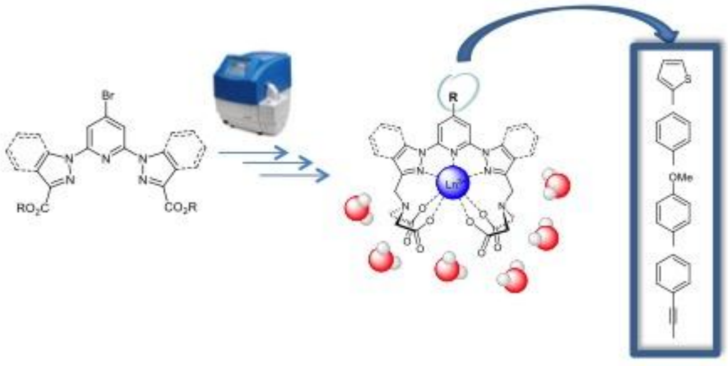
Graphical abstract



Highlights

- A versatile synthetic approach for best and most effective markers for bioassays is developed.
- This straightforward synthetic access to 4-substituted pyridines, allows easy optimization of the emission properties of lanthanides.
- The synthesis is based on microwave-assisted Pd cross-coupling reactions, to obtain photoactive push-pull donor-π-conjugated ligands.
- The ligands constructed on bispyrazolylpyridine and bisindazolylpyridine units formed stable complexes with Europium and Terbium in H_2O
- In an effort to shed light on the nature of the structure-property relationships, a study of the effect of different substituents on their photophysical behavior is presented.

Pd cross-coupling reactions in the access to bis-pyrazole and bis-indazole pyridine-based nona-coordinated ligands. Luminescence properties of their lanthanide complexes

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Abstract

A synthetic approach based on microwave-assisted Pd cross-coupling reactions is developed to obtain two series of push-pull donor-π-conjugated nonadentate coordinating ligands constructed on bispyrazolylpyridine and bisindazolylpyridine units. Both families of ligands formed stable complexes with Europium and Terbium in H_2O. To provide a basis to synthesize the best and most effective markers for bioassays, a study of the effect of different substituents on their photophysical behavior is presented in an effort to shed light on the nature of the structure-property relationships. Both families showed better emission properties for Tb(III) than for Eu(III). Bispyrazolylpyridine derivatives were revealed as better sensitizing ligands despite the promising higher absorptivity and red-shifted excitation wavelengths of their counterpart, bisindazolilpyridine. This straightforward synthetic access to 4-substituted pyridines allows easy optimization of the emission properties of lanthanides. In the case of 4-phenylethynyl substituted bispyrazolylpyridine, the emission properties of Eu(III) and Tb(III) complexes were similar, indicating that they can act as a promising long-lived luminescent dual probe in multiplex analysis.

Keywords

Energy transfer; Inner-sphere hydration number; Lanthanide(III) ions; Luminescence lifetime; Polyaminopolycarboxylate complexes

1. Introduction

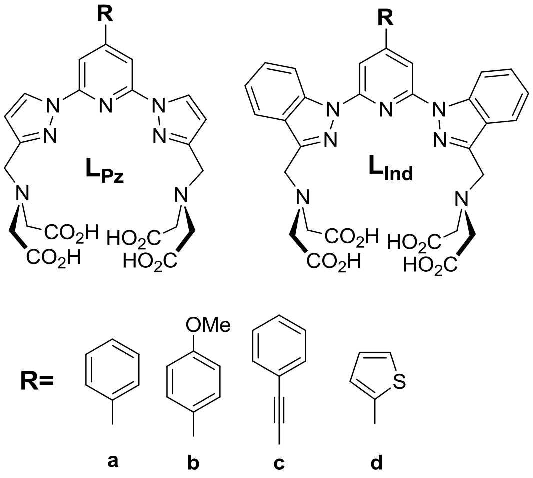
The luminescence emissions exhibited by lanthanide (III) complexes with organic chromophoric ligands have stimulated great interest in being indebted to the wide-ranging applicability of their narrow-band emissions as probes in a variety of biological applications [1], cell and tissue imaging [2] and Ln-based nanomaterials [3]. The attractive features of lanthanide ions when they are complexed with chromophoric organic ligands, are principally governed by their sensitivity and selectivity, attained by their line-like emission, large Stokes shift, high quantum yield, long luminescence lifetime and high photochemical stability [4, 5].

Since the first tetracarboxylic podands were published by our research group in 1993 [6], there have been many efforts to synthesize new nona-coordinated ligands due to the good luminescence properties shown by their complexes with lanthanide ions [7-9], making them excellent tools in homogeneous bioassays [10,11].

These ligands are constituted by a chromophore frame based on bispyrazolylpyridine system, and two iminodiacetic acid units, which act as complexing agents. The cavity created is able to host effectively the lanthanide ion isolating its coordination sphere from the surrounding interactions. As a result of the effectiveness in coordination these ligands exhibit good quantum yields for their complexes with Eu (III) and especially for those of Tb (III). Other approaches to this type of structure have been synthesized by our research group where different modifications of the chromophore or complexing units were introduced to study their influence [12,13].

However, the synthetic approach to obtaining systematic variation on the pyridine ring on the polyheterocyclic chromophore presented some limitations; pyrazoles were introduced by nucleophilic substitution of 2,6-dibromopyridines in harsh conditions and these bromines limit the number of possible functions that can be introduced in the pyridine ring.

Recently the easy substitution of fluorine atoms developed by Slosscher [14,15] has been employed by other groups to widen the synthesis for these type of compounds by means of 4-bromo-2,6-difluoropyridine [9,16]. Taking advantage of the favorable reaction conditions offered by this trihalogenated molecule for the orthogonal access to trisubstituted pyridines, herein we report the synthesis of two families of tris-heterocyclic ligands based on 2,6-bis(pyrazol-1-yl)pyridine (*1-bpp*) and 2,6-bis(indazol-1-yl)pyridine (*1-bip*) with different substitution at the C4 position of the pyridine ring obtained by means of Pd cross-coupling reactions (Scheme 1). The possibility of introducing almost unlimited aromatic substituents on the 4 position of the pyridine by means of a universal reaction is an interesting synthetic challenge that could help to tune the sensitization properties of the chromophoric unit, enhancing the luminescent emission of lanthanide complexes with these ligands.



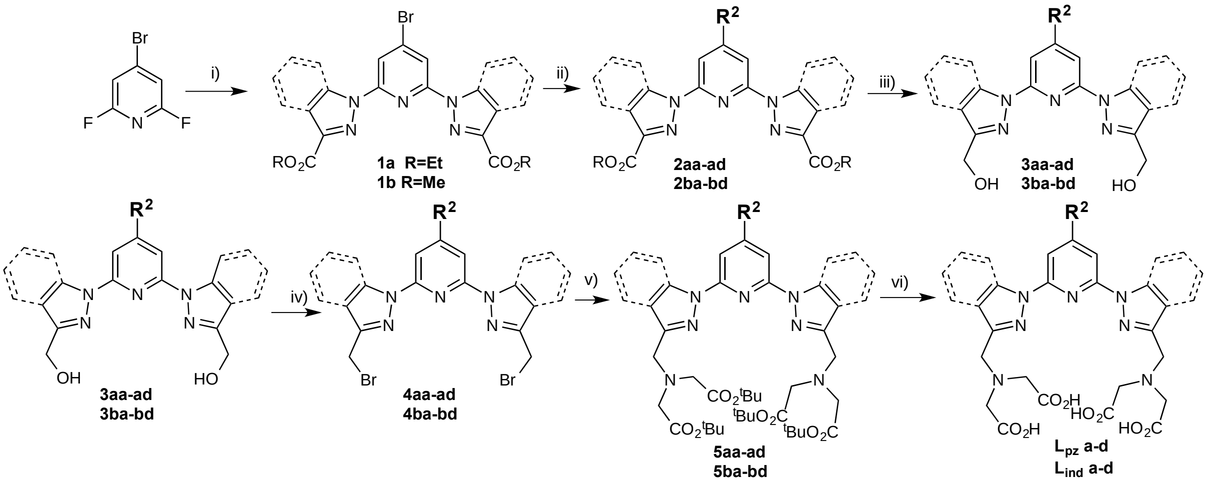
Scheme 1: Ligands described in this work

Furthermore, to extend these ideas, indazolyl groups are newcomers to these families of antenna ligands and we consider it interesting to compare both heterocycles, pyrazole and indazole as sensitizers and chelating points of lanthanide ions in a series of compounds described here, in which we can compare the effect of these rings with same aromatic substituents at C-4 introduced by Suzuki-Miyaura and Sonogashira reactions. These substituents are an example of the variety of push-pull donor-π-conjugated aromatic groups that could be introduced to these systems. They could be extended in the future to a wide variety of systems for tuning the sensitization capabilities toward other lanthanides in an effort to obtain new chelates for the simultaneous detection of multiple analytes; therefore, enhancing the efficacy of bioassays by conducting just a single multiplexed bioanalysis.

2. Synthesis of Ligands

Starting from the 4-Bromo-2,6-difluoropyridine, disubstituted pyridines **1a-b** were obtained by nucleophilic substitution of the fluorine atoms with the sodium salts of 3-ethoxycarbonylpyrazole or 3-methoxycarbonylindazole obtained by the treatment of the corresponding esters with sodium hydride (Scheme 2). The reaction requires mild conditions for pyrazole substitution where the reaction completes in 8h at room temperature in THF, whereas for indazole substitution, the reaction requires 50ºC for 4h in DMF as a solvent in a similar manner, as described in [16]. The introduction of the first indazole ring deactivates the second fluorine atom substitution, requiring more severe conditions. In our hands we obtained the monosubstituted 2-(3-methoxycarbonylindazol-1-yl)-4-bromo-6-fluoropyridine as the only product for the reaction between indazole and pyridine in THF at room temperature; this monosubstituted product has been employed for access to new families of unsymmetrically substituted pyridines used as antenna ligands in the bioanalysis of DNA [17].

The brominated position was, at this stage, employed for the introduction of different aromatic groups by Pd coupling reactions. Two kinds of reactions were performed, as described in the Scheme 2; Suzuki-Miyaura coupling with phenyl, thienyl and 4-methoxyphenyl boronic acids and Sonogashira coupling with ethynylbenzene. Both reactions were optimized using microwave-assisted conditions, which drastically reduce the reaction times without affecting the reaction yields compared to conventional methods; this methodology has been extended to other ethynyl derivatives and boronic acids by our group [17].



Scheme 2: i) 2 equiv ethoxycarbonylpyrazole, NaH (2.2 equiv), THF, r.t., 4h for $\mathbf{1_a}$, or 2 equiv methoxycarbonylindazole, NaH (2.2 equiv), DMF, 40ºC, 5h for $\mathbf{1_b}$ ii) R^2-$B(OH)_2$, THF, KOH, $Pd(PPh_3)_4$, 130ºC MW or ethynylbenzene, THF, Et_3N, $PdCl_2(PPh_3)_2$, CuI, MW, 125ºC. iii) $LiAlH_4$, THF. iv) PBr_3, CH_3CN; v) $NH(CH_2CO_2{}^tBu)_2$, CH_3CN; vi) TFA/ CH_2Cl_2.

Transformation of esters **2aa-d** and **2ba-d** to the tetracarboxyloic ligands (**L$_{pz\text{-}ind}$a-d**) was accomplished as depicted in Scheme 2, following the procedures previously reported by us [6,12,18].

3. Results and Discussion

Lanthanide complexes of the tetraacids **L$_{pz}$a-d** and **L$_{ind}$a-d** were prepared by mixing equimolecular amounts of ligand and lanthanide salts ($LnCl_3 \cdot 6H_2O$) in borate buffer (pH = 8.1). Tables 1-3 summarize the main photophysical properties of the complexes obtained.

3.1 UV-Vis absorption spectra

The UV-Vis absorption spectra of ligands show a similar appearance with same substituent at position C-4 of the pyridine, in both families of compounds, *1-bpp* and *1-bip*. Absorption maxima are red shifted by about 10-30 nm for *1-bip* related to *1-bpp* as a consequence of the more conjugated indazole ring and planar disposition of the bis-indazolylpyridine heterocyclic system. This value is especially interesting as less energetic excitation maxima could drive to a better match of the excited states of ligand and resonance energy level of lanthanide, mainly in Eu (III). In addition, lower excitation energy is also interesting as it reduces the cost of quartz materials associated with UV range measurements.

The absorption maxima for the complexes in 1-bpp derivatives **L$_{pz}$a-d** show a bathochromic shift of the absorption maxima related to the free ligands, whereas this bathochromic effect is less pronounced for the ligands 1-bip **L$_{ind}$a-d**. Assuming that the relative disposition of the three heterocyclic rings around the lanthanide ion must be very similar in the complexes of both families, the different bathochromic shift found probably has its origin in the more coplanar original disposition of the bis-indazole ligands compared with their bis-pyrazole equivalents.

3.2 Lanthanide Emission Spectra

In all complexes, excitation into the longest absorption maxima wavelength results in the observation of the distinctive emission patterns of the Eu(III) or Tb(III) ions (Figure 1). The excitation spectra were in perfect agreement with the absorption observed.

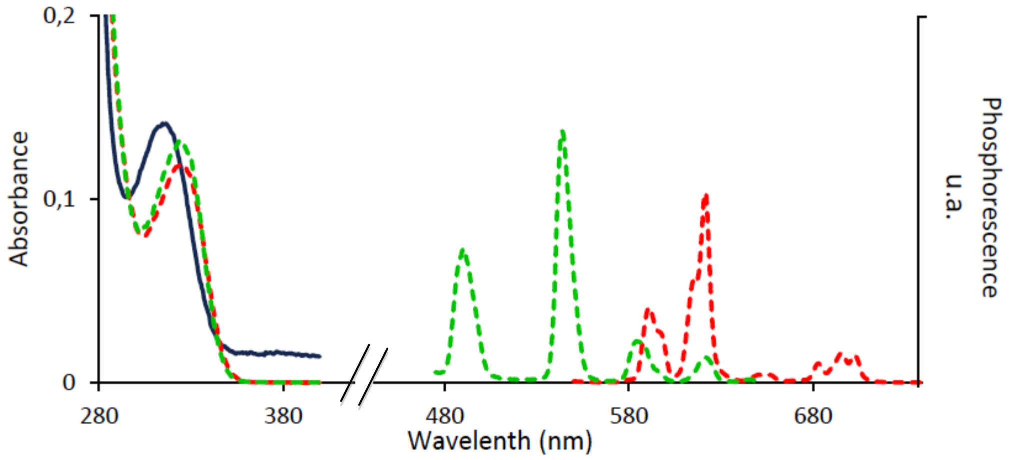


Figure 1: UV/Vis absorption spectrum of free ligand $\mathbf{L_{pz}a}$ (solid black line), excitation/emission (λ_{em}=616 nm, λ_{exc}=320 nm) spectra of Eu-$\mathbf{L_{pz}a}$ (red line) and excitation/emission (λ_{em}=545 nm, λ_{exc}=320 nm) spectra of Tb-$\mathbf{L_{pz}a}$ (green line) in borate buffer.

Observed emission intensities are generally stronger for the Tb(III) complexes compared with their Eu(III) counterpart. The quantum yield described by Yuan's group for Tb-$\mathbf{L_{pz}a}$ (ϕ =1) [7] is confirmed in our hands. The excellent availability of this compound by the synthetic pathway herein described gives a tremendous opportunity for the application of this ligand as a luminescent probe.

Another ligand whose synthesis has also been described by Yuan's group was thienyl derivative **L_{pz}d** which presents with Eu(III) a quantum yield in our hands, of only 0.02 slightly lower than previously described [19], and the only ligand where the Eu(III) emission is higher than for Tb(III). Despite the good sensitization properties of this thienyl aromatic heterocycle described for lanthanide complexes of thenoyltrifluoroacetone, our thienyl ligands **L_{pz}d** and **L_{ind}d** present low emission intensities with the bisindazole compound being the lowest in the series.

The lower emission of the **L_{ind}a-d** complexes is a constant in all of the products described here, which shows that despite their promising lower-energy excitation band, these ligands are not as good sensitizers as the corresponding bispyrazolyl **L_{pz}a-d**. A similar behavior was observed by Charbonniere et al. for the only example of these type of 1-bisindazole pyridine described until now [16].

Analyzing the measured lifetimes for these complexes, presented in Table 1, we can obtain some explanations about the emissions observed. The lifetimes measured at room temperature show the normal behavior observed for Eu(III) and Tb(III) complexes where longer values correspond to the Tb(III) complexes. The values increase when the lifetimes were measured in D_2O at room temperature and at 77K. The lifetime dependence on temperature evinced the deactivation by thermal coupling, both radiant and non-radiant, which is non-negligible.

Table 1: Absorptivity (ε) and Lifetime data in H_2O and D_2O at rt and 77 K (ms).

Tb	ε [$10^3 \cdot M^{-1} \cdot cm^{-1}$] ($\lambda_{max}$ [nm])^a	τ_{H2O} [ms]	τ_{D2O}^{b} [ms]	$\tau_{D2O\ (77k)}$^{b} [ms]
L$_{pz}$a	11.5 (320)	2.68	2.92	3.68
L$_{pz}$b	24.5 (306)	1.66	2.29	3.22
L$_{pz}$c	19.7 (312)	2.43	2.59	2.88
L$_{pz}$d	32.9 (317)	1.93	2.3	2.31
L$_{ind}$a	9.4 (323)	1.01	1.48	2.82
L$_{ind}$b	26.1 (329)	1.45	1.86	1.87
L$_{ind}$c	17.9 (338)	1.02	1.47	2.74
L$_{ind}$d	26.7 (321)	1.52	1.87	2.75

Eu	**I$_{ems}$*ε** (λ_{max} [nm])^a	τ_{H2O} [ms]	τ_{D2O}^{b} [ms]	$\tau_{D2O\ (77k)}$^{b} [ms]
L$_{Pz}$a	11.3 (320)	1.35	2.63	3.77
L$_{pz}$b	25.1 (306)	1.38	2.26	2.52
L$_{pz}$c	19.1 (312)	1.35	2.19	2.29
L$_{Pz}$d	34.5 (317)	1.32	2.31	2.66
L$_{ind}$a	9.2 (323)	1.41	2.03	2.36
L$_{ind}$b	25.1 (329)	1.38	1.81	1.95
L$_{ind}$c	20.4(338)	1.16	1.93	2.21
L$_{ind}$d	28.1 (321)	0.8	1.64	1.99

[a] Excitation λ_{max}, [b] Measured in 0.05M $NaHCO_3$-D_2O buffer

From these values it is possible to also extract the number of coordinated water molecules around the first coordination sphere of the Ln(III) ion. These values have been calculated according to the three main methods described in literature, those of Horrocks [20,21], Parker [22] and Kimura [23], and are presented in Table 2. These methods take into account different deactivation parameters, but in most cases all show similar values. In our case Horrocks' and Parker's values show very similar records, with less than one coordinated water molecule, except for **L$_{ind}$a** and **L$_{ind}$c** which show two anomalously higher values with more than one water molecule. The similar coordinating geometry around the lanthanide for all of the ligands described suggests that these values are artifacts with a distinct origin than simple deactivation by O-H thermal coupling. Kimura's equation takes into account only lifetimes in water at r.t.; these values are of special interest when comparing ligands with very similar geometries, as is our case, as deactivation by temperature or vibronic coupling with O-H oscillators must be very similar in all cases. The values obtained from Kimura's equations show large discrepancies with the other two methods, mainly for the Tb(III) complexes, where in most cases, values much higher than for one water molecule were found. Only **L$_{pz}$a** and **L$_{pz}$c** show lower values more coherent with the other methods.

Table 2: Number of water molecules calculated by indicated method

Tb	Horrocks[a]	Parker[b]	Kimura[c]
L$_{pz}$a	0.1	0.1	0.6
L$_{pz}$b	0.7	0.8	1.6
L$_{pz}$c	0.1	0.1	0.8
L$_{pz}$d	0.4	0.4	1.2
L$_{ind}$a	1.3	1.5	3.1
L$_{ind}$b	0.6	0.7	1.9
L$_{ind}$c	1.3	1.4	3.1
L$_{ind}$d	0.5	0.6	1.8

Eu	Horrocks[a]	Parker[b]	Kimura[c]
L$_{pz}$a	0.4	0.2	0.3
L$_{pz}$b	0.3	0.1	0.3
L$_{pz}$c	0.3	0.1	0.3
L$_{pz}$d	0.3	0.1	0.4
L$_{ind}$a	0.2	0.0	0.3
L$_{ind}$b	0.2	0.0	0.3
L$_{ind}$c	0.4	0.2	0.5
L$_{ind}$c	0.7	0.5	0.9

[a] According to Ref. [20]; [b] According to Ref. [22]; [c] According to Ref. [23]

These results provide evidence that deactivation could also have its origin in other processes. Among them and mainly for the Tb(III) complexes a back-transfer process is a common cause of deactivation. This is produced when the ligand excited state is next in energy with the $^5D_4$ resonance emissive level of Tb. In order to study this back-transfer process, the energy measurement of the triplet excited state was determined from the phosphorescence spectra of the Gd(III) complexes at 77K, whose values are presented in Table 3.

Again we can observe a dependence of the lifetime values for the Tb(III) complexes on the triplet state energy levels; once again the best emissive complexes with **L$_{pz}$a** and **L$_{pz}$c** showing the higher values of $^3E_{00}$ (23365 and 23474cm^{-1}) and τH_2O (2.68 and 2.43ms). These energy levels are situated well above the $^5D_4$ emissive levels of Tb and an efficient energy transfer takes place as revealed by their quantum yields. Other interesting examples are those of **L$_{pz}$b** and **L$_{ind}$c** whose $^3E_{00}$ level although lower (22075 and 22124cm^{-1}) also show high quantum yields (62 and 32%); their low lifetimes at room temperature in water is recovered in D_2O and also at 77K showing a more sensitive behavior towards OH coupling vibrations and temperature.

The remaining $^3E_{00}$ levels are well below 22000cm^{-1}; these energy values are recognized as the limits when the energy of the lowest triplet state of the ligand is at the borderline for competitive back-transfer energy process [24]. Four ligands, **L_{pz}d**, **L_{ind}a**, **L_{ind}b** and **L_{ind}d** present values under this limits and their quantum yields suffer from this competitive process.

Table 3: Photophysical data of LnL in buffer borate

	$^3E_{00}$	ϕ TbL[%]	ϕ EuL [%][a]
L_{pz}a	23365	100	10
L_{pz}b	22075	62	9
L_{pz}c	23474	19	17
L_{pz}d	20619	0.8	2
L_{ind}a	21413	24	1
L_{ind}b	21322	3	1
L_{ind}c	22124	32	4
L_{ind}d	21231	0.6	0.1

[a] Quantum yields were determined using $Cs_3[Eu(dpa)_3]$ (($\Phi = 0.24$) as a standard [25].

For the Eu(III) complexes the number of coordinated water molecules is always less than one and is very similar independent of the equation employed in the calculation, which corroborated the good isolation of the lanthanide ion and supports the deactivation process of their equivalent Tb(III) complexes as an explanation for the anomalous q values observed for this ion.

The $^3E_{00}$ energy range contains the europium emissive levels ^5D_i and the Eu(III) luminescence deactivation is mainly governed by non-radiative transitions. The strong isotopic variation of lifetimes shows the coupling with O–H vibronic oscillators as the main deactivation route with temperature being less important. From the luminescence lifetimes at rt and 77K and in H_2O and D_2O by means of the equations stated by Sabattini et al [26], the values of radiative (k_r) and non-radiative (k_{nr}) rate constants, as well as ligand–metal energy transfer efficiency (η) and lanthanide quantum yield (ϕM) were estimated (the most relevant ones are collected in the supporting information file).

The variation of the $^3E_{00}$ energy values, attending to the nature of substituents **a-d** in both series $\mathbf{L_{pz}}$ and $\mathbf{L_{ind}}$ show more variation in the case of 1-bpp than for the 1-bip coordinating units. In the case of the 1-bpp unit, phenyl (**a**) and phenylethynyl (**c**) substituents, 23365 and 23474cm^{-1} respectively, show higher similar energies whereas electro-donating substituents (**b**) and (**d**) decrease the energy of the triplet state; a small reduction for p-methoxyphenyl (**b**) (22075cm^{-1}) and a very low value of 20619cm^{-1} for thienyl substituent (**d**) is observed. However, values with small variation <900 cm^{-1} (22124-21231cm^{-1}) are observed in $\mathbf{L_{ind}}$ series. This small range for the 1-bip triplet levels suggests that they are centered on the 1-bip frame with low influence on the electron demanding nature of the substituent at position 4 of the pyridine ring.

4. Conclusion

In summary the synthesis proposed opens the way to access an enormous variation of trisubstituted pyridine ligands by microwave assisted cross-coupling reactions in an easy and straightforward way.

From both families of compounds reported, the luminescent properties of the ligands based on the 2,6-bispyrazolyl-1-pyridine pattern are quite sensitive to pyridine substitution, whereas its counterpart 2,6-bisindazolyl-1-pyridine ligands show more uniform triplet state levels, with these levels being centered in the tris-heterocyclic motif. Despite the expectations generated by the introduction of units, which offer greater conjugation to the structure, such as indazole rings, these did not improve their luminescent properties as expected.

All data suggest that Tb(III) ion is better sensitized by both families of ligands than Eu(III) although ligand **L$_{pz}$c** shows similar quantum yield for both ions and might act as a promising long-lived luminescent dual probe in multiplexing analysis.

5. Experimental

5.1. General

^{1}H-NMR, ^{13}C-NMR: Bruker AV-300 spectrometer (Departamento de Química Orgánica, QO). MS: VG Autospec in EI FAB and FAB-HRMS modes (L-SIMS+), ABSciex QSTAR (ESI+, HRMS), Bruker ULTRAFLEX III (MALDI-TOF/TOF) (HRMS) spectrometers (Servicio Interdepartamental de Investigación, SIdI). Elemental analyses : Perkin-Elmer CHN 2400 automatic analyzers (SIdI). All reagents used were of reagent grade, the solvents were purified prior to their use. Lanthanide chlorides were purchased from Aldrich and used as received. Absorption spectra: JASCO V-660 spectrophotometer (QO). Excitation and emission spectra: Varian Cary Eclypse (SIdI), Jasco FP-8600 (QO), L50 Perkin-Elmer spectrofluorimeters (QO). The excitation spectra were automatically corrected and the emission spectra were corrected according to the instruments' guidebooks. The emission quantum yields were measured by a relative method using $Cs_3[Eu(dpa)_3]$ ($\Phi = 0.24$) as a standard according to literature [25].

5.2. General Synthetic Procedures.

Microwave assisted Suzuki-Miyaura cross coupling reaction with boronic acids.

A Microwave vial under argon containing **1a** or **1b** (0.5mmol), the corresponding boronic acid (0.51mmol) and $Pd(PPh_3)_4$ (0.02mmol) dissolved in anhydrous THF (3.5 mL) was stirred at room temperature for 10 min, then a solution of 0.5mL KOH (1M) was added to the mixture. The mixture was heated at 130°C for 11 min. in a Microwave oven (Biotage Initiator 2.5). The solvent was removed under vacuum to yield a black residue which was dissolved in 20 mL of CH_2Cl_2 and washed with 10 mL of water. The aqueous phase was extracted with CH_2Cl_2 (2x20 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, filtered and concentrated to dryness. Flash chromatography in CH_2Cl_2:hexane 1:1 yield the corresponding **2aa-c** or **2ba-c** compounds**.**

Microwave assisted Sonogashira cross coupling reaction with Phenylacetylene.

In a Microwave vial under argon containing **1a** or **1b** (0.46mmol), $PdCl_2(PPh_3)_2$ (0.061 eq.) and CuI (0.061 eq.) dissolved in a mixture of anhydrous THF (2.5mL) and freshly distilled NEt_3 (2.2mL), was stirred for 5 minutes. Then phenylacetylene (1.2 eq.) was added and the mixture was heated at 120°C for 13 min. in a Microwave oven (Biotage Initiator 2.5). The solvent was removed under reduced pressure to yield a dark residue which was dissolved in 20 mL of CH_2Cl_2 and washed with 15 mL of water. The aqueous phase was extracted with CH_2Cl_2 (2x20 mL). The combined organic phases were washed with brine (20 mL), dried over Na_2SO_4, vacuum filtered and concentrated to dryness. Flash column chromatography, CH_2Cl_2:hexane 9:1, yield the corresponding **2ac** or **2bc** compounds.

Diesters Reduction. In a similar manner as described in [6, 12, 18], $LiAlH_4$, (3 equiv.) was added in small portions to a soln. of diester (**1** equiv.) in 40 ml of THF at 0ºC**.**

The mixture was stirred at r.t. for 90 min and treated with 0.18 ml of H_2O, 0.18 ml of 15 % NaOH, and 0.55 ml of H_2O. The mixture was filtered and the filtrate concentrated yielding the diol.

Bromination of bishydroxymethyl derivatives. In a similar manner as described in [6, 12, 18], PBr_3, (ca. 5 equiv.) was added to a soln. of diol (1 equiv.) in 60 ml of MeCN at 40ºC. The mixture was refluxed for 90 min and the solvent removed. The residue was treated with 10 ml of a sat. soln. of Na_2CO_3 and extracted with CH_2Cl_2 (2 x 50 ml). The organic phase was washed with water (3 x 50 ml) and dried over Na_2SO_4 and concentrated to dryness to yield the expected bisbromomethyl derivatives with enough purity (NMR) to be used in the next step without further manipulation.

Reaction of Bisbromomethyl compounds with Di(tert-butyl)Iminodiacetate. In a similar manner as described in [6, 12, 18], a mixture of Na_2CO_3, (ca. 9 equiv.) and the bisbromomethyl derivative (1 equiv.) in 40 ml of dry MeCN was added to a soln. of di(tert-butyl)iminodiacetate (2 equiv.) in 12 ml of dry MeCN. The mixture was stirred for 24h at r.t. and filtered. The filtrate was evaporated to dryness and dissolved in 25 ml of CH_2Cl_2. The org. soln. was washed with H_2O and worked up as usual. The resulting oily residue was purified by flash chromatography (AcOEt/hexane 1:3).

Cleavage of Tetra(tert-butyl)esters. In a similar manner as described in [6, 12, 18], CF_3COOH (ca. 9 equiv.) was added to a soln. of tetraester (1 equiv.) in CH_2Cl_2. The mixture was stirred for 4 h at r.t. and the solvent removed. The resulting oil was triturated several times in Et_2O and washed once with hot MeOH, leading to a white precipitate.

4-Bromo-2,6-bis-(3-diethoxycarbonyl-1-pyrazolyl)pyridine, 1a

In a round bottom Schlenk flask under argon 2,6-difluoro-4-bromopyridyne (1 g, 5.15mmol) and 3-ethoxycarbonyl pyrazole (1.44 g, 10.3mmol) were dissolved in 30 mL of dry THF. The solution was cooled to 0ºC and NaH (60% in oil, 452 mg, 11.3mmol) was added. The reaction mixture was left at rt. for 4 h, after which 20 mL of CH_2Cl_2 was added. The organic phase was washed with water (20 mL) and the aqueous phase was extracted with CH_2Cl_2 (3x10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated to dryness to afford a solid that was purified by flash chromatography (CH_2Cl_2/hexane 1:1) to a white solid 2g, 89%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 8.55 (2H, d, *J*= 2.6 Hz), 8.28 (2H, s), 7.01 (2H, d, *J*= 2.6 Hz), 4.46 (4H, q, *J*= 7.1 Hz), 1.44 (6H, t, *J*= 7.2 Hz).ppm; ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 161.7; 149.7; 146.9; 137.4; 128.6; 114.6; 110.7; 61.5; 14.3 ppm. Anal. Calc. for $C_{17}H_{16}BrN_5O_4$: C 47.02, H 3.71, N 16.13, found: C 46.82, H 3.57, N 15.92; MS (EI+): m/z= 433.0 ($[M+H]^+$, 100%), 435.0 ($[M+H]^+$+2, 97%)

4-Bromo-2,6-bis-(3-methoxycarbonyl-1-indazolyl)pyridine, 1b

Analogous as described in [16], in a round bottom Schlenk flask under argon 2,6-difluoro-4-bromopyridyne (680mg, 3.5mmol) and 3-methoxycarbonyl indazol (1.46 g, 8.3mmol) were dissolved in 30mL of freshly distilled DMF. The solution was cooled to 0ºC and NaH (60% in oil, 364mg, 9.1mmol) was added. The reaction mixture was left at rt then heated at 40ºC for 5 h, after which a white precipitate formed. The solid was isolated by centrifugation and addition of water to the liquid phase gave a second precipitate, isolated by centrifugation. The combined solids were washed with of warm hexane (40mL) and water (40mL), 2.19g, 81%. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 8.57 (2H, d, *J*= 8.6 Hz), 8.31 (2H, d, *J*=7.8 Hz), 8.24 (2H, s), 7.51 (2H, t, *J*=8.6 Hz), 7.42 (2H, t, *J*=7.8 Hz), 4.08 (3H, s) ppm.; ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: 162.1, 151.9, 140.0, 139.2, 136.5, 128.8, 124.8, 125.1, 122.5, 115.8, 114.7, 52.5 ppm. FAB-HRMS m/z found: 506.0468, calculated for $C_{23}H_{17}BrN_5O_4$: 506.0464.

5.3 Luminescence Measurements

The luminescence properties of Eu(III), Tb(III) and Gd(III) chelates were measured using equimolecular mixtures of the ligands **L_{pz}a-d** and **L_{ind}a-d**, and the corresponding $LnCl_3$ in borate buffer (pH 8.1). The concentrations used were between 10^{-5} and 10^{-6} mol dm^3. In some cases a 2% attenuator in the emission path was necessary in order to avoid signal saturation, and to keep concentration of sample and standards in the same range. The energy measurement of the triplet excited state was determined from the phosphorescence spectra of the Gd(III) complexes at 77K.

6. Supporting Information

Full synthetic procedures and characterization details for the final products and their intermediates. Tables of photophysical data of Eu and Tb complexes of Ligands **L_{pz}a-d** and **L_{ind}a-d**.

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