7. Lanthanide Complexes of Polyacid Ligands Derived from 2,6-Bis(pyrazol-1-yl)pyridine, Pyrazine, and 6,6′-Bis(pyrazol-1-yl)-2,2′-bipyridine: Synthesis and Luminescence Properties

by **Juan Carlos Rodríguez-Ubis***, **Rosa Sedano**, **Gemma Barroso**, **Olga Juanes**, and **Ernesto Brunet***

Departamento de Química Orgánica, Facultad de Ciencias C-I,
Universidad Autónoma de Madrid, E–28049 Madrid

(17.VIII.96)

The synthesis of three novel pyrazole-containing complexing acids, *N,N,N′,N′*-{2,6-bis[3-(aminomethyl)pyrazol-1-yl]-4-methoxypyridine}tetrakis(acetic acid) (**1**), *N,N,N′,N′*-{2,6-bis[3-(aminomethyl)pyrazol-1-yl]pyrazine}-tetrakis(acetic acid) (**2**), and *N,N,N′,N′*-{6,6′-bis[3-(aminomethyl)pyrazol-1-yl]-2,2′-bipyridine}tetrakis(acetic acid) (**3**) is described. Ligands **1**–**3** formed stable complexes with Eu^{III}, Tb^{III}, Sm^{III}, and Dy^{III} in H_2O whose relative luminescence yields, triplet-state energies, and emission decay lifetimes were measured. The number of H_2O molecules in the first coordination sphere of the lanthanide ion were also determined. Comparison of data from the Eu^{III} and Tb^{III} complexes of **1**–**3** and those of the parent trisheterocycle *N,N,N′,N′*-{2,6-bis[3-(aminomethyl)pyrazol-1-yl]pyridine}tetrakis(acetic acid) showed that the modification of the pyridine ring for pyrazine or 2,2′-bipyridine strongly modify the luminescence properties of the complexes. MeO Substitution at C(4) of **1** maintain the excellent properties described for the parent compound and give an additional functional group that will serve for attaching the label to biomolecules in bioaffinity applications.

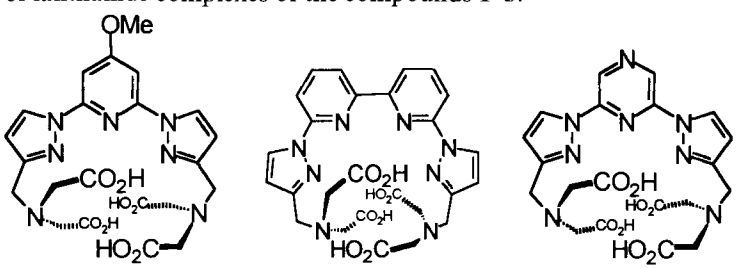
Introduction. – Immunological analyses based on luminescent labels and time-resolved fluorescence (TRFIA) are becoming highly competitive [1] compared to radioimmunoassays which present severe drawbacks mainly related to the radioactivity of the labels (expensive analysis, high risks for the analyzer, environmental problems, restrictive protocols for radioactive waste disposal, difficulties in obtaining legal handling licenses, *etc.*). In contrast, fluorimetric labeling with lanthanide complexes is comparatively inexpensive, not dangerous, and fluorescence can be quickly measured using automated and relatively simple equipment.

Present TRFIA labeling involves the attachment to the biomolecule of an organic moiety to which lanthanide ions are chelated but in a nonfluorescent form, followed by a development procedure in which the lanthanide ions are extracted into a so-called enhancer solution where the lanthanide forms a new chelate which is luminescent [2]. These manipulations limit sensitivity and avoid following the bioaffinity reaction *in situ*. For instance, this labeling technique cannot be utilized for the localized quantification of specific nucleic-acid sequences in individual cells by microscopy or flow cytometry, because the localization information is lost in the development step. The localized information is, thus, essential for the detection of, *e.g.*, small fractions of cancer or other rare cells in patient samples.

Therefore, TRFIA would highly increase its sensitivity and applicability if a second generation of lanthanide labels, luminescent on themselves, could be used, for which the aforementioned development step would be no longer necessary.

Proper design of a suitable organic ligand for such directly luminescent lanthanide complexes should make as effective as possible the energy conversion processes that take place in these systems [3]: *i*) intense absorption of UV/VIS light by the organic ligand, *ii*) efficient energy transfer from the excited ligand to the metal ion, and *iii*) intense emission of visible light by the metal ion. The organic ligand should hence form strong complexes with lanthanide metals (thermodynamic stability and kinetic inertness) and shield the metal from the environment, thus minimizing nonradiative deactivation processes of the metal excited states (H_2O is a particular problem, since it causes vibronic deactivation *via* the O–H oscillators [4] [5]). Finally, the organic ligand should also contain appropriate functionalization to be covalently attached to biomolecules in such a way that the binding must not alter its luminescent properties.

Although the number of new directly luminescent chelates described is increasing [6], very few applications of these systems have appeared, mainly based in crytates [7] and polyamine-poly(acetic acid) derivatives [8]. Within this aim, we are currently investigating the preparation of new lanthanide chelates. In a previous paper, we described the synthesis of Eu^{3+} and Tb^{3+} complexes of a polyacid derivative of 2,6-bis(*N*-pyrazolyl)pyridine [9] which displayed notable luminescent properties, namely long emission lifetimes and high quantum yields. In this paper, we report the synthesis and luminescence properties of lanthanide complexes of the compounds **1**–**3**.

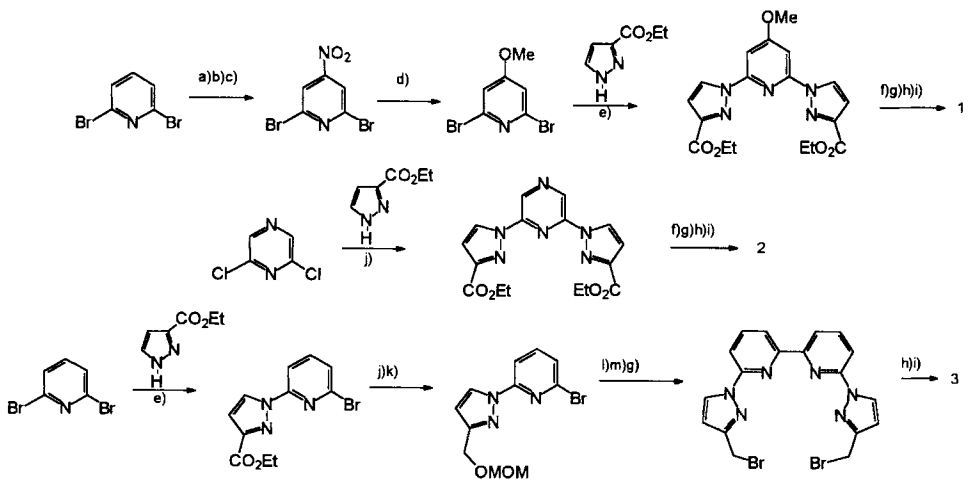


1 **2** **3**

Results and Discussion. – *Synthesis and Characterization of the Ligands. Scheme 1* depicts the reaction paths followed to obtain compounds **1**–**3**. In all cases the starting material is the corresponding 2,6-dihalo derivative. In the case of **1**, substitution of NO_2 by MeO deserves some comment. Although it is long known that NO_2 is a particularly good leaving group in aromatic nucleophilic substitution [10], we tried first to preserve it, because that could be an entry to a number of interesting derivatives with different substituents at C(4) of the pyridine ring. We, thus, reacted 2,6-dibromo-4-nitropyridine with 2 equiv. of potassium 3-(ethoxycarbonyl)pyrazolate (*Scheme 2*).

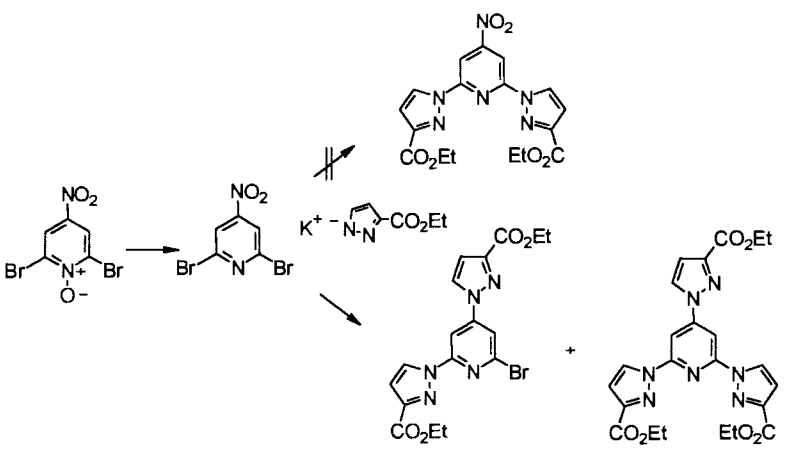
The outcome of this reaction, a mixture of bis- and tris(pyrazol-1-yl)pyridine, clearly indicated that the NO_2 group was faster replaced than at least the second bromide. On the other hand, reaction of 2,6-dibromo-4-nitropyridine with NaOMe gave exclusively 2,6-dibromo-4-methoxypyridine in 95% yield. This is an explicit evidence that the C-atom *ipso* to NO_2 is more reactive than those to the Br-atom in 2,6-dibromo-4-nitropyridine. Besides, this fact made the synthesis of 2,6-dibromo-4-methoxypyridine quite straightforward compared to the method previously described by *Neumann* and *Vögtle* [11] (*Scheme 3*), where one more step and separation of 4- and 6-methoxy derivatives were necessary. We are currently investigating the substitution of NO_2 with other nucleophiles.

Scheme 1

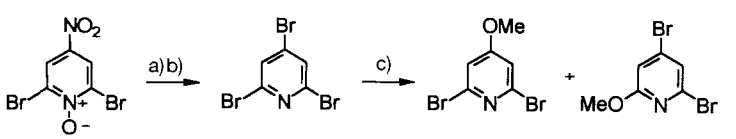


a) H_2O_2/TFA. b) $HNO_3/H_2SO_4 \cdot SO_3$. c) $PBr_3/CHCl_3$. d) NaMeO/THF. e) K/Diglyme (reflux, 96 h). f) $LiAlH_4$. g) PBr_3. h) Di(*tert*-butyl) iminodiacetate/K_2CO_3. i) CF_3COOH. j) K/THF (60°, 48 h). k) $MeOCH_2O$ (excess). l) $NiCl_2 \cdot 6\,H_2O/PPh_3$/Zn/DMF. m) HCl/MeOH.

Scheme 2



Scheme 3



a) AcOH/AcBr. b) $PBr_3/CHCl_3$. c) NaOMe.

The reaction between 2,6-dibromo-4-methoxypyridine and potassium 3-(ethoxycarbonyl)pyrazolate (step *e* leading to **1**; *Scheme 1*) was carried out under vigorous conditions similar to those used for the non-methoxylated product [9], observing also comparable results. Unfortunately, the yield was even lower (10%) probably due to the deactivating effect of the MeO group towards nucleophilic substitution. In turn, 2,6-dibromopyrazine reacted with potassium 3-(ethoxycarbonyl)pyrazolate under milder conditions (step *j* leading to **2**; *Scheme 1*) and with satisfactory yield (55%). Reduction of carboxylates (step *f* leading to **2**; *Scheme 1*) had to be carefully performed, because either an excess of reducing agent (> 2 mol) or an increase of temperature (> 0°) led to the partial reduction of pyrazine ring.

Reaction of 2,6-dibromopyridine with 1 equiv. of potassium 3-(ethoxycarbonyl)-pyrazolate gave the monosubstituted product in good yield (step *e* leading to **3**; *Scheme 1*). The self-coupling of the pyridine ring has been attempted at different stages of the reaction following the method described in [12]. It was successful with the 2-bromo-6-[3-(ethoxycarbonyl)pyrazolyl]pyridine and the corresponding 2-bromo-6-[3-(hydroxymethyl)pyrazolyl]pyridine, but subsequent reduction of the ester led to cleavage of the Py–Py bond, and the dimerized alcohol was impossible to separate in our hands from its starting material. We then decided to reduce first the ester group and protect the resulting alcohol (steps *f* and *j* leading to **3**; *Scheme 1*). The Py–Py coupling was thus effected in reasonable yield, and the usual transformation of the alcohol into the iminodiacetic-acid group finally led to **3**.

Lanthanide Complexes. The complexes of Eu^{3+}, Tb^{3+}, Dy^{3+}, and Sm^{3+} with **1** and **2** were prepared in H_2O solution but, in the case of **3**, due to its low solubility, they had to be prepared from a solution of the tetraacid in a buffer. In all cases, spectrometric titration of ligands with increasing amounts of $EuCl_3$ or $TbCl_3$ evidenced that complexes with 1:1 stoichiometry were formed. Measurements were performed in a borate buffer (pH 8.5). To evaluate the stability constants of the complexes, we carried out potentiometric titrations. The results showed that stability constants were too high ($\log \beta > 20$) to be measured by this method. On the other hand, competitive measurements with ethylenediaminetetraacetic acid (EDTA) confirmed these high values which were corroborated by the emission properties of the complexes that remained constant with ligands **1** and **2** over a wide pH range (4–10). With the less soluble ligand **3**, the pH range was relatively more limited (5.5–10). All the complexes resulted photostable upon long-term storage.

The UV spectra of all studied complexes were very similar to those of their parent ligands **1**–**3**, showing in all cases a small bathochromic shift of *ca.* 10 nm (*Table 1*). This suggests that, while the pyrazoles are in the free ligand presumably out of plane containing the neighboring pyridine or pyrazine ring, the almost planar meridional coordination of the metal to the N-atoms, expected for the complexes, should force the pyrazoles to coplanarity, therefore, increasing conjugation.

In case of the 4-MeO-substituted ligand **1**, hypso- and hypochromic shifts of the absorption maxima are observed (*Table 1*) compared to the unsubstituted parent ligand.

Luminescence Studies. Ln^{III} Chelates give narrow-band emission of the metal ion under UV/VIS excitation. In the so-called *antenna effect* [13], the light energy absorbed by the organic chromophore is firstly transferred to a triplet state of the ligand by intersystem crossing and then intramolecularly passed to the Ln^{III} ion. Excitation of the solutions of our complexes into the lowest-energy ligand-centered (LC) absorption band gave rise

Table 1. *Absorption Data for Ligands* **1**–**3** *and Their* Eu^{3+}, Tb^{3+}, Sm^{3+}, *and* Dy^{3+} *Complexes* ($c = 10^{-5}$–10^{-6})

Compound	λ_{max} [nm][b])	ε [$dm^3 \cdot mol^{-1} \cdot cm^{-1}$][c])
parent ligand[a])	245, *300*	12200
parent-Eu^{3+}	270, *313*	8100
parent-Tb^{3+}	270, *314*	7850
parent-Sm^{3+}	270, *313*	8400
parent-Dy^{3+}	269, *313*	9050
1	249, *287*	8750
1-Eu^{3+}	*296*	6250
1-Tb^{3+}	267, *296*	5930
1-Sm^{3+}	267, *297*	6460
1-Dy^{3+}	267, *296*	6810
2	245, 323	17500
2-Eu^{3+}	275, *336*	8400
2-Tb^{3+}	274, *336*	11100
2-Sm^{3+}	275, *336*	8700
2-Dy^{3+}	275, *336*	9500
3	265, 312	13250
3-Eu^{3+}	262, *329*	7100
3-Tb^{3+}	263, *325*	7620
3-Sm^{3+}	262, *329*	9050
3-Dy^{3+}	262, *323*	8500

[a]) *N,N,N',N'*-[2,6-Bis(3-aminomethylpyrazolyl)pyridine]tetrakis(acetic acid); see [9]. [b]) Values in italics correspond to the excitation maxima, employed in luminescence studies. [c]) Value corresponding to the maxima in italics.

to the well-structured emission of the lanthanide cations. This suggests that energy transfer did take place from ligand-centered (LC) to metal-centered (MC) levels. The excitation spectra were in perfect agreement with the absorption spectra.

Ligands **2** and **3** exhibited fluorescence, showing a radiative pathway that, in their respective complexes, could compete with the energy transfer to the lanthanide ions. However, the Ln^{III} complexes prepared with these ligands showed a drastic reduction of that fluorescence (*ca.* 30 times), and the residual signal did not interfere with the metal luminescence, because the much longer lifetimes of the latter for Eu^{3+} and Tb^{3+} (*vide infra*) allowed to resolve the short-lived fluorescence emission.

Excitation maxima (λ_{exc}), luminescence lifetimes (τ), quantum yields (ϕ), and the measured energy of the triplet-state levels (E) of the studied Ln^{3+} complexes are collected in *Table 2*.

It can be seen that Ln^{III} complexes exhibited two excitation maxima. Although not always the $n \rightarrow \pi^*$ transition had higher ε than the $\pi \rightarrow \pi^*$, we found more convenient, due to instrumental reasons (UV transmission of lenses, filters, cuvettes, and glass slides) to excite the complexes at the wavelength of the former transition ($n \rightarrow \pi^*$). On the other hand, we observed that excitation maxima were the same for all complexes of a given ligand, indicating that the coordination geometry of the organic moiety should be very similar regardless of the metal considered.

Considering quantum yields (*Table 2*), the complexes displayed very different values depending on lanthanide ion and, most important, on ligand type. Eu^{3+} and Tb^{3+} chelates

Table 2. *The Triplet-State Energy Level (E), Excitation Maxima (λ_{exc}), Luminescence Decay Times (τ), Luminescence Yields (ϕ), and Number of Coordinated Molecules (q) of Lanthanide(III) Chelates of Unsubstituted Pyridine Ligand*[a]) *and Ligands* **1**–**3** *Prepared in This Work*[b])

Compound[c])	E [cm^{-1}][d])	λ_{exc} [nm][e])	Emission [nm][f])	τ [ms][g])	ϕ (10^4)	q[h])
parent[a])-Eu^{3+}	23250	320	622	1.30	1300	0.4
parent-Tb^{3+}		320	545	2.75	6000	
parent-Sm^{3+}		313	601	0.03	6	
parent-Dy^{3+}		313	578	0.04	49	
1-Eu^{3+}	25750	300	622	1.38	230	0.4
1-Tb^{3+}		300	545	2.88	5100	
1-Sm^{3+}		297	601	0.03	8	
1-Dy^{3+}		[i])	[i])	[i])	[i])	
2-Eu^{3+}	25150	336	621	1.37	300	0.5
2-Tb^{3+}		336	546	2.10	5800	
2-Sm^{3+}		336	600	0.02	20	
2-Dy^{3+}		336	578	0.03	120	
3-Eu^{3+}	21600	329	613	1.19	1200	0.3
3-Tb^{3+}		329	546	0.13	290	
3-Sm^{3+}		329	601	0.03	17	
3-Dy^{3+}		321	576	0.01	1	

[a]) *N,N,N′,N′*-[2,6-Bis(3-aminomethylpyrazolyl)pyridine]tetrakis(acetic acid); see [9]. [b]) The values described have been determined in borate buffer solution (pH 8.5). [c]) Concentrations; $8.6 \cdot 10^{-7}$ for **1** and **2**, and $1.7 \cdot 10^{-7}$ for **3**. [d]) [15]. [e]) Wavelengths correspond to the λ_{exc} used in the emission study. [f]) Assigned to the most intense emission band ($^5D_0$–$^7F_2$, $^5D_4$–$^7F_5$5, $^4G_{5/2}$–$^6H_{7/2}$, and $^4F_{9/2}$–$^6H_{15/2}$ transitions for Eu^{3+}, Tb^{3+}, Sm^{3+}, and Dy^{3+} complexes, respectively). [g]) Experimental uncertainties: lifetimes, < 10%; luminescence yields, < 15%. [h]) Number of coordinate H_2O molecules around the Eu^{3+} complexes, calculated from decay emission lifetimes measured in D_2O (see text). [i]) Very low signal.

behaved, as expected, as the most efficient emitters, but, compared to the parent unsubstituted compound [9], the MeO substitution at C(4) of pyridine **1** reduced the excellent quantum yield described for the Tb^{3+} complex of parent unsubstituted compound; the Eu^{3+} complex shows also a lower value. The values for Eu^{3+} and Tb^{3+} of pyrazine derivative **2** are only slightly higher than those of **1** (0.03 *vs.* 0.02 for Eu^{3+}, and 0.58 *vs.* 0.51 for Tb^{3+}). In the case of 2,2′-bipyridine derivative **3**, the value for Eu^{3+} and Tb^{3+} complexes were higher and lower, respectively, than those of **1** and **2**. The chelate **3**-Eu^{3+} bore an excellent quantum yield (0.12), comparable to that of the parent pyridine complex and other reported good Eu labels [14].

As mentioned above, the emission of the lanthanide ion is induced by intramolecular energy transfer from the triplet-state level of the ligand to the resonance level of the metal ion. It, thus, seems reasonable to assume that, among other factors, the energy matching of both energy levels would be paramount in attaining a good quantum yield. Therefore, it is of primary importance in the development of a good luminescent label of this kind for bioaffinity assays that the energy of the ligand triplet state could be measured [15]. Chelated Eu^{3+} and Tb^{3+} are able to accept energy to all of their ^5D_j levels, depending on the donor's triplet state energy, but the quantum yield should be highest, when the energy is transferred from the triplet-state level of the ligand directly to the lowest excited state of Ln^{III}. Following this reasoning, a clear correlation could be obtained between the ob-

served quantum yields of the chelates and the measured energy values for the triplet states of the ligands. For Eu^{3+} (see *Table 2*), those chelates with parent unsubstituted pyridine and **3**, whose triplet levels are below 24000 cm^{-1}, gave acceptable quantum yields, whereas the efficiencies of those complexes of ligands **1** and **2** (triplet levels above 24000 cm^{-1}) were lower than 10%. In the latter two cases, the unmatching between appropriate levels probably favors alternate mechanisms for energy transfer, namely ligand-to-metal-charge transfer processes (LMCT) very favorably for Eu^{3+} complexes due to the relatively ease of reduction Eu^{3+}/Eu^{2+}, which drastically reduces quantum yields of metal emission [16].

For Tb^{3+} chelates, unlike Eu^{3+}, should the triplet state of the ligand be slightly above the metal emitting level (20500 cm^{-1}), the latter easily returns the energy back to the ligand [16], and the emission efficiency and its lifetime are severely diminished. This is the case of ligand **3** ($\phi = 0.03$, $\tau = 0.13$ ms), whose triplet level (21600 cm^{-1}) results too close to the resonance level of the metal (20500 cm^{-1}). The other ligands bore similar, higher triplet levels (23250–25750 cm^{-1}), therefore, displayed alike, good quantum yields ($\phi = 0.51$–0.69) and longer lifetimes ($\tau = 1.3$–1.38 for Eu^{3+} and 2.10–2.88 for Tb^{3+}).

Decay times (τ) deserve some comment. The large lifetimes observed for Eu^{3+} and Tb^{3+} chelates should be attributed to the excellent isolation of the metal from the surrounding H_2O which causes vibronic deactivation *via* the O–H oscillators. This exceptional isolation should be provided by the iminodiacetic subunits which presumably play two different roles: as coordinating groups, leaving less room for the coordination of H_2O molecules to the metal, and improving complex stability. The average number of H_2O molecules in the first coordination sphere of the metal can be calculated following the method described by *Hooroks* and *Sudnick* [17], in which the lifetimes in D_2O should also be measured.

We carried out this study on complexes of Eu^{3+} and Tb^{3+}, and it resulted (see *Table 2*) that only 0.3–0.5 H_2O molecules surround the metal. This finding is specially interesting in the case of ligand **3**, for which the distance between pyrazole rings should be higher than in the other cases because of the extra length of the bipyridine moiety, and, so, the change for H_2O molecules to coordinate to the metal should in principle be higher. A tight, helical arrangement of the heterocyclic rings around the metal and the disposition of the iminodiacetic subunits in opposite faces in a somewhat perpendicular plane to that containing the bipyridine bond can preclude H_2O molecules to coordinate to the metal.

Finally, we investigated complexation with Sm^{3+} and Dy^{3+}, and ligands **1**–**3** also formed strong chelates with these metals. Although their ϕ and τ values (*Table 2*) were much lower than those of Eu^{3+} and Tb^{3+}, their different emission wavelengths may still make them useful as labels in dual labeling systems [18].

In conclusion, the described complexes showed remarkable luminescence properties (decay times, quantum yields, excitation wavelengths), and high stability in aqueous solutions. Replacement of the pyridine ring by other heterocyclic systems or its functionalization with appropriate groups do affect the luminescence properties of the corresponding chelates, but can be suitably governed, providing a way to get them attached to biomolecules. These systems may thus be used as lanthanide markers in bioaffinity assays.

We would like to thank *H. Takalo* and *V.-M. Mukkala* for a generous loan of the ligand *N,N,N′,N′*-[6,6″-(aminomethyl)-4′-phenyl-2,2′:6′,2″-terpyridine]tetrakis(acetic acid) used for quantum-yield determination. Financial support from *Wallac Oy* and *Comisión Interministerial de Ciencia y Tecnología* (*CICYT*), grants No. PB90-0176, PB93-0264, PB95-0172, is gratefully acknowledged.

Experimental Part

General. ^{1}H- and ^{13}C-NMR spectra: on *Bruker* instruments *AC-200* (Organic Chemistry Deptartment, OCD) and *AMX-300* (Servicio Interdepartamental de Investigación, SIdI). δ in ppm relative to TMS as internal reference, and J in Hz; abbreviations for assignments: Py: pyridine, Pz: pyrazole, Pzn: pyrazine. MS: *VG Autospec* spectrometer (SIdI) in electronic impact mode unless otherwise indicated. Absorption spectra: *Lambda 6 Perkin-Elmer* spectrophotometer (OCD). Excitation and emission spectra: *LS-50 Perkin-Elmer* spectrofluorimeter (OCD). The excitation spectra were automatically corrected, and the emission spectra were corrected according to the instrument guidebook. Elemental analyses were measured on *Perkin-Elmer* automatic analyzers (SIdI). All solvents were purified prior to their use following standard methods [19]. Lanthanide chlorides were purchased from *Aldrich* and used as received.

General Procedures. Reduction of Diesters with $LiAlH_4$. $LiAlH_4$ (3 equiv.) was added in small portions to a soln. of diester (1 equiv.) in 40 ml of THF at 0°. 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 with enough purity (NMR) to be used in the next step without further manipulation.

Br Substitution in Diols. PBr_3 (*ca.* 5 equiv.) was added to a soln. of diol (1 equiv.) in 60 ml of MeCN at 40°. 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 × 50 ml). Usual workup of the extracts yieded the expected dibromide.

*Reaction of Dibromides with Di(*tert*-butyl) Iminodiacetate.* A mixture of Na_2CO_3 (*ca.* 9 equiv.) and the dibromo 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 24 h at r.t. and filtered. The filtrate was evaporated to dryness and redissolved 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).

*Hydrolysis of Tetra(*tert*-butyl) Tetraesters.* 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 solid residue was thoroughly washed once with CH_2Cl_2 and several times with hot MeOH.

2,6-Dibromo-4-methoxypyridine. MeONa (473 mg, 8.76 mmol) was added in small portions to a soln. of 2,6-dibromo-4-nitropyridine (824 mg, 2.92 mmol) in 20 ml of anh. THF. The mixture was stirred at 40° for 44 h and the solvent was removed. The solid residue was suspended and stirred in CH_2Cl_2 and the precipitate was filtered off: yield: 92%. White crystals. M.p. 135–136° ([11]: 136–137°).

2,6-Bis[3-(ethoxycarbonyl)pyrazol-1-yl]-4-methoxypyridine. K (965 mg, 24.7 mmol) was added in small portions at 70° to a soln. of 3-(ethoxycarbonyl)pyrazol (3.46 g, 24.7 mmol) in 15 of diglyme. Once the metal was dissolved, 2,6-dibromo-4-methoxypyridine (2 g, 7.49 mmol) was added, and the mixture was refluxed for 4 d. The solvent was removed, and the residue was treated with H_2O and extracted with CH_2Cl_2 (3 × 25 ml). Usual workup of the extracts afforded a mixture of the expected diester and the monoester 2-bromo-6-[3-(ethoxycarbonyl)pyrazol-1-yl]4-methoxypyridine which were separated by FC (AcOEt/hexane 1:3). Yield of the diester 10%. White crystals. M.p. 183–185°. ^{1}H-NMR ($CDCl_3$): 8.61 (*d*, J = 2,6, 2 H, H–C(5) of Pz); 7.61 (*s*, 2 H, Py); 6.98 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.52 (*q*, J = 7.2, 2 MeCH_2); 1.45 (*t*, J = 7.1, 2 *Me*CH_2). ^{13}C-NMR ($CDCl_3$): 170.3 (*C*=O); 150.8 (C(3) of Pz; C(2), C(6) of Py); 141.7 (C(4) of Py); 128.6 (C(5) of Pz); 110.3 (C(4) of Pz); 97.7 (C(3), C(5) of Py); 61.4 (CH_2); 56.6 (MeO); 14.4 (Me). MS: 386 (100, M^+), 340 (48), 312 (4). Anal. calc. for $C_{18}H_{19}N_5O_5$: C 56.10, H 4.97, N 18.17; found: C 55.78, H 4.92, N 17.84.

Yield of monoester: 10%. White crystals. M.p. 162°. ^{1}H-NMR ($CDCl_3$): 8.60 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 7.63 (*d*, J = 1.5, H–C(5) of Py); 7.00 (*d*, J = 1.5, H–C(3) of Py); 6.98 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.50 (*q*, J = 7.2, MeCH_2); 1.49 (*t*, J = 7.1, *Me*CH_2). MS: 327 (88), 325 (89, M^+), 282 (94), 280 (100), 255 (42), 253 (43).

2,6-Bis[3-(hydroxymethyl)pyrazol-1-yl]-4-methoxypyridine. It was prepared from 2,6-bis[3-(ethoxycarbonyl)pyrazol-1-yl]-4-methoxypyridine following the *General Procedure* (*vide supra*). Yield: 92%. White crystals. M.p. 178–180°. ^{1}H-NMR ($CDCl_3$): 8.60 (*d*, 2 H, J = 2.6, H–C(5) of Pz); 7.29 (*s*, 2 H of Py); 6.52 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.72 (*s*, 2 CH_2); 3.99 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 169.8 (C(3) of Pz); 155.3 (C(2), C(6) of Py); 151.0 (C(4) of Py); 128.3 (C(5) of Pz); 106.4 (C(3), C(5) of Py); 95.1 (C(4) of Pz); 55.9 (MeO); 50.0 (CH_2). MS: 301 (100, M^+), 282 (32), 254 (21).

2,6-Bis[3-(bromomethyl)pyrazol-1-yl]-4-methoxypyridine. It was prepared from 2,6-bis[3-(hydroxymethyl)-pyrazol-1-yl]-4-methoxypyridine following the *General Procedure* (*vide supra*). Yield: 52%. White crystals. M.p. 194–196°. ^{1}H-NMR ($CDCl_3$): 8.48 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 7.26 (*s*, 2 H of Py); 6.51 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.56 (*s*, 2 CH_2); 4.00 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 150.8 (C(3) of Pz); C(2), C(6) of Py); 141.7 (C(4) of Py); 128.6 (C(5) of Pz); 108.0 (C(4) of Pz); 96.0 (C(3), C(5) of Py); 56.2 (MeO); 24.8 (CH_2). MS: 429 (13), 427 (26), 425 (13, M^+), 346 (100), 267 (51).

Tetra(tert-*butyl)* N,N,N′,N′-{*2,6-Bis[3-(aminomethyl)pyrazol-1-yl]-4-methoxypyridine*}*tetrakis(acetate)*. It was prepared from 2,6-bis[3-(bromomethyl)pyrazol-1-yl]-4-methoxypyridine in 78% yield following the *General Procedure* (*vide supra*). ^{1}H-NMR ($CDCl_3$): 8.49 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 7.35 (*s*, 2 H of Py); 6.58 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.05 (*s*, 2 CH_2N); 3.99 (*s*, MeO); 3.51 (*s*, 4 NCH_2CO); 1.50 (*s*, 4 *t*-Bu). ^{13}C-NMR ($CDCl_3$): 170.5 (C=O); 169.8 (C(3) of Pz); 153.1 (C(4) of Py); 151.4 (C(2), C(6) of Py); 128.0 (C(5) of Pz); 112.9 (C(3), C(5) of Py); 108.2 (C(4) of Pz); 81.0 (Me_3C); 56.9 (NCH_2); 55.3 (MeO); 51.1 (CH_2CO); 28.2 (Me_3C). MS (FAB+): 756 (MH^+).

N,N,N′,N′-{*2,6-Bis[3-(aminomethyl)pyrazol-1-yl]-4-methoxypyridine*}*tetrakis(acetic Acid)* (**1**). It was prepared from the corresponding tetra(*tert*-butyl) tetraester following the *General Procedure* (*vide supra*). Yield: 52%. White crystals. M.p. 207–209°. ^{1}H-NMR ($CDCl_3$): 8.82 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 7.21 (*s*, 2 H of Py); 6.53 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.00 (*s*, CH_2N); 3.91 (*s*, MeO); 3.48 (*s*, 4 NCH_2CO). ^{13}C-NMR ((D_6)DMSO): 172.3 (C=O); 169.6 (C(3) of Pz); 153.2 (C(4) of Py); 150.9 (C(2), C(6) of Py); 129.0 (C(5) of Pz); 108.3 (C(3), C(5) of Py); 95.5 (C(4) of Pz); 56.3 (NCH_2); 53.8 (MeO); 50.7 (CH_2CO). MS (FAB+): 532 (MH^+). Anal. calc. for $C_{22}H_{25}N_7O_9$: C 48.89, H 4.85; found: C 48.82, H 4.83.

2,6-Bis[3-(ethoxycarbonyl)pyrazol-1-yl]pyrazine. K (524 mg, 13.4 mmol) was added in small portions at 60° to a soln. of 3-(ethoxycarbonyl)pyrazol (1.97 g, 14.1 mmol) in 15 ml of anh. THF. Once the metal was dissolved, a soln. of 2,6-dibromopyrazine (1 g, 6.7 mmol) in 10 ml of anh. THF was added, and the mixture was refluxed for 2 d. The solvent was removed, and the residue was treated with H_2O and extracted with CH_2Cl_2 (3 × 25 ml). Usual workup of the extracts afforded a mixture of the expected diester and the monoester 2-bromo-6-[3-(ethoxycarbonyl)pyrazol-1-yl]pyrazine which were separated by FC (AcOEt/hexane 1:3). Yield of diester: 60%. White crystals. M.p. 191–193°. ^{1}H-NMR ($CDCl_3$): 9.42 (*s*, H–C(3), H–C(5) of Pzn); 8.55 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 7.07 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.47 (*q*, J = 7.2, 2 MeCH_2); 1.45 (*t*, J = 7.1, 2 *Me*CH_2). ^{13}C-NMR ($CDCl_3$): 161.5 (C=O); 147.5 (C(3) of Pz); 144.2 (C(2), C(6) of Pzn); 133.3 (C(3), C(5) of Pzn); 128.7 (C(5) of Pz); 111.0 (C(4) of Pz); 61.5 (CH_2); 14.2 (Me). MS: 356 (100, M^+), 311 (48), 283 (4). Anal. calc. for $C_{16}H_{16}N_6O_4$: C 53.93, H 4.53, N 23.58; found: C 53.64, H 4.42, N 23.25.

Yield of monoester: 7%. White crystals. M.p. 94–95°. ^{1}H-NMR ($CDCl_3$): 9.15 (*s*, H–C(3) of Pzn); 8.40 (*s*, H–C(5) of Pzn); 8.34 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 6.84 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.30 (*q*, J = 7.2, MeCH_2); 1.29 (*t*, J = 7.1, *Me*CH_2). ^{13}C-NMR ($CDCl_3$): 160.9 (C=O); 147.1 (C(3) of Pz); 146.2 (C(2) of Pzn); 145.3 (C(6) of Pzn); 141.8 (C(3) of Pzn); 132.8 (C(5) of Pz); 128.7 (C(5) of Pzn); 110.6 (C(4) of Pz); 61.1 (CH_2); 13.9 (Me).

2,6-Bis[3-(hydroxymethyl)pyrazol-1-yl]pyrazine. It was prepared from 2,6-bis[3-(ethoxycarbonyl)pyrazol-1-yl]pyrazine following the *General Procedure* (*vide supra*). Yield: 85%. White crystals. M.p. 218°. ^{1}H-NMR ($CDCl_3$/CD_3OD): 8.98 (*s*, H–C(3), H–C(5) of Pzn); 8.44 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 6.53 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.65 (*s*, CH_2). MS: 272 (100, M^+), 253 (25), 225 (28).

2,6-Bis[3-(bromomethyl)pyrazol-1-yl]pyrazine. It was prepared from 2,6-bis[3-(hydroxymethyl)pyrazol-1-yl]-pyrazine following the *General Procedure* (*vide supra*). Yield: 65%. White crystals. M.p. 186°. ^{1}H-NMR ($CDCl_3$): 9.17 (*s*, H–C(3), H–C(5) of Pzn); 8.46 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 6.61 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.59 (*s*, CH_2). ^{13}C-NMR ($CDCl_3$): 131.7 (C(3), C(5) of Pzn); 128.7 (C(5) of Pz); 109.0 (C(4) of Pz); 24.1 (CH_2). MS: 398 (M^+). Anal. calc. for $C_{12}H_{10}N_6Br_2$: C 36.31, H 2.53, N 21.21; found: C 36.38, H 2.50, N 20.47.

Tetra(tert-*butyl)* N,N,N′,N′-{*2,6-Bis[3-(aminomethyl)pyrazol-1-yl]pyrazine*}*tetrakis(acetate)*. It was prepared from 2,6-bis[3-(bromomethyl)pyrazol-1-yl]pyrazine following the *General Procedure* (*vide supra*). Yield: 88%. White crystals. ^{1}H-NMR ($CDCl_3$): 9.11 (*s*, H–C(3), H–C(5) of Pzn); 8.47 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 6.62 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.05 (*s*, 2 CH_2N); 3.50 (*s*, 4 NCH_2CO); 1.49 (*s*, 4 *t*-Bu). ^{13}C-NMR: 170.1 (C=O); 154.4 (C(3) of Pz); 144.7 (C(2), C(6) of Pzn); 130.9 (C(3), C(5) of Pzn); 128.0 (C(5) of Pz); 109.1 (C(4) of Pz); 80.8 (Me_3C); 55.2 (NCH_2); 50.7 (CH_2CO); 28.0 (Me_3C). MS: 726 (M^+).

N,N,N′,N′-{*2,6-Bis[3-(aminomethyl)pyrazol-1-yl]pyrazine*}*tetrakis(acetic Acid)* (**2**). It was obtained from the corresponding tetraester following the *General Procedure* (*vide supra*). Yield: 52%. White crystals. M.p. 195°. ^{1}H-NMR (D_2O): 8.85 (*s*, H–C(3), H–C(5) of Pzn); 8.50 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 6.53 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.45 (*s*, 2 CH_2N); 3.82 (*s*, 4 NCH_2CO). ^{13}C-NMR (D_2O): 164.7 (C=O); 148.2 (C(3), C(5) of Pzn); 132.9 (C(5) of Pz); 113.5 (C(4) of Pz); 59.8 (NCH_2Pz); 54.0 (CH_2CO). MS (FAB+): 503 (MH^+). Anal. calc. for $C_{20}H_{22}N_8O_8$ requires: C 47.81, H 4.41, N 22.30; found: C 48.12, H 4.25, N 22.80.

2-Bromo-6-[3-(ethoxycarbonyl)pyrazol-1-yl]pyridine. We followed a modification of our previously described method [9]: K (860 mg, 22 mmol) was added in small portions to a soln. of 3(5)-(ethoxycarbonyl)pyrazol (3 g, 21 mmol) in diglyme (20 ml) at 70°. When the metal was dissolved, freshly recrystallized 2,6-dibromopyridine (5.07 g, 21 mmol) was added in one portion, and the mixture was then stirred at 130° for 4 d. The solvent was removed *in vacuo*, and H_2O was added to the residue. Extraction with CH_2Cl_2 (3 × 25 ml) and workup of the

extracts yielded the expected product together with a small amount (6%) of 2,6-bis[3-(ethoxycarbonyl)pyrazol-1-yl]pyridine, which were separated by FC on silica gel (AcOEt/hexane 1:4). Yield: 28%. Anal. data: *cf.* [9].

2-Bromo-6-[3-(hydroxymethyl)pyrazol-1-yl]pyridine. $LiAlH_4$ (250 mg, 6.7 mmol) was added in small portions to a soln. of 2-bromo-6-[3-(ethoxycarbonyl)pyrazol-1-yl]pyridine (1 g, 3.4 mmol) in 80 ml of THF at 0°. The mixture was stirred at r.t. for 90 min and treated with 0.18 ml of H_2O, 0.18 ml of 15% aq. NaOH, and 0.55 ml of H_2O. The soln. was filtered and the solvent evaporated. The white solid residue was the pure alcohol (quant. yield) which was used in the following steps without further purification. M.p. 108°. ^{1}H-NMR ($CDCl_3$): 8.50 (*d*, J = 2.6, H–C(5) of Pz); 7.90 (*d*, J = 8, H–C(5) of Py); 7.65 (*t*, J = 8, H–C(4) of Py); 7.35 (*d*, J = 8, H–C(3) of Py); 6.50 (*d*, J = 2.6, H–C(4) of Pz); 4.80 (*d*, J = 5.4, CH_2); 2.10 (*d*, J = 5.4, OH). ^{13}C-NMR ($CDCl_3$): 155.4 (C(3) of Pz); 139.9 (C(2) of Py); 128.7 (C(5) of Pz); 125.0 (C(3), C(5) of Py); 110.6 (C(4) of Py); 106.6 (C(4) of Pz); 59.2 (CH_2). MS: 255 (95), 253 (100, M^+).

2-Bromo-6-[3-(methoxymethoxy)pyrazol-1-yl]pyridine. Dimethoxymethane (71.2 mg, 0.82 mmol), LiBr (71.2 mg, 0.82 mmol), and TsOH (77.9 mg, 0.41 mmol) were added to a soln. of 2-bromo-6-[3-(hydroxymethyl)pyrazol-1-yl]pyridine (1.04 mg, 4.1 mmol) in 5 ml of $CHCl_3$ at r.t. with vigorous stirring. Another portion of LiBr was added after 12 h, and the reaction was followed by TLC (hexane/Et_2O 1:6). After 24 h, the solvent was removed, the residue was treated with 5 ml of brine, and the mixture was extracted with $CHCl_3$ (3 × 15 ml). Workup of the extracts afforded a white solid which was purified by precipitation in hexane. Yield: 28%. ^{1}H-NMR ($CDCl_3$): 8.50 (*d*, J = 2.6, H–C(5) of Pz); 7.90 (*d*, J = 8, H–C(5) of Py); 7.65 (*t*, J = 8, H–C(4) of Py); 7.35 (*d*, J = 8, H–C(3) of Py); 6.50 (*d*, J = 2.6, H–C(4) of Pz); 4.80 (*s*, CH_2); 4.70 (*s*, OCH_2O); 3.45 (*s*, MeO). ^{13}C-NMR ($CDCl_3$): 140.6 (C(2) of Pz); 128.5 (C(5) of Pz); 125.1 (C(3), C(5) of Py); 110.9 (C(4) of Py); 107.8 (C(4) of Pz); 95.9 (OCH_2O); 62.9 (CH_2); 55.5 (Me).

6,6′-Bis[3-(methoxymethoxy)pyrazol-1-yl]-2,2′-bipyridine. Zn (74 mg, 1.14 mmol) was added to a soln. of $NiCl_2 \cdot 6\,H_2O$ (271 mg, 1.14 mmol) and Ph_3P (1.19 g, 4.56 mmol) in 5 ml of DMF at 50°. The mixture was stirred for 1 h, and a soln. of 2-bromo-6-[3-(methoxymethoxy)pyrazol-1-yl]pyridine (339 mg, 1.14 mmol) in 10 ml of DMF was then added. The mixture was treated with 20 ml of 10% NH_4OH and 25 ml of CH_2Cl_2. The two layers were separated, and the aq. layer was extracted again with CH_2Cl_2 (3 × 25 ml). Workup of the org. extracts afforded a residue from which the product was separated in 40% yield by FC (3% MeOH in CH_2Cl_2). ^{1}H-NMR ($CDCl_3$): 8.70 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 8.40 (*d*, J = 8, 2 H, H–C(5) of Py); 8.00 (*m*, 4 H, H–C(3), H–C(4) of Py); 6.50 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.80 (*s*, CH_2); 4.70 (*s*, 2 OCH_2O); 3.45 (*s*, 2 MeO).

6,6′-Bis[3-(hydroxymethyl)pyrazol-1-yl]-2,2′-bipyridine. 6,6′-Bis[3-(methoxymethoxy)pyrazol-1-yl)-2,2′-bipyridine (197 mg, 0.45 mmol) was dissolved in 25 ml of MeOH containing traces of HCl, and the soln. was refluxed for 6 h. The mixture was neutralized with sat. $NaHCO_3$, and the resulting precipitate was filtered. Yield: 90%. ^{1}H-NMR ($CDCl_3$/CD_3OD 9:1): 8.70 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 8.40 (*d*, J = 8, 2 H, H–C(5) of Py); 7.80 (*m*, 4 H, H–C(3), H–C(4) of Py); 6.59 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.70 (*s*, 2 CH_2).

6,6′-Bis[3-(bromomethyl)pyrazol-1-yl]-2,2′-bipyridine. It was obtained from 6,6′-bis[3-(hydroxymethyl)pyrazol-1-yl]-2,2′-bipyridine following the *General Procedure* (*vide supra*). Yield: 57%. White solid. ^{1}H-NMR ($CDCl_3$/CD_3OD 9.1): 8.70 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 8.40 (*d*, J = 8, 2 H, H–C(5) of Py); 7.80 (*m*, 4 H, H–C(3), H–C(4) of Py); 6.59 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.60 (*s*, 2 CH_2).

*Tetra(*tert*-butyl) N,N,N′,N′-{6,6′-Bis[3-(aminomethyl)pyrazol-1-yl]-2,2′-bipyridine}tetrakis(acetate).* It was obtained from 6,6′-bis[3-(bromomethyl)pyrazol-1-yl]-2,2′-bipyridine following the *General Procedure* (*vide supra*). Yield: 42%. ^{1}H-NMR ($CDCl_3$): 8.70 (*d*, J = 2.6, 2 H, H–C(5) of Pz); 8.35 (*d*, J = 8, 2 H, H–C(5) of Py); 8.00 (*m*, 4 H, H–C(3), H–C(4) of Py); 6.60 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.05 (*s*, 2 CH_2N); 3.5 (*s*, 4 NCH_2CO); 1.5 (*s*, 4 *t*-Bu). ^{13}C-NMR ($CDCl_3$): 1.70 (C=O); 153 (C(2) of Py); 152 (C(6) of Py); 150 (C(3) of Pz); 139 (C(4) of Py); 127 (C(5) of Pz); 117 (C(5) of Py); 112 (C(3) of Py); 108 (C(4) of Pz); 80 (Me_3C); 55 (CH_2); 51 (NCH_2CO); 28 (Me_3C). MS (FAB+): 803.6 (MH^+). Anal. calc. for $C_{42}H_{60}N_8O_9$: C 61.45, H 7.35, N 13.65; found: C 61.27, H 7.10, N 13.31.

N,N,N′,N′-{*6,6′-Bis[3-(aminomethyl)pyrazol-1-yl]-2,2′-bipyridine*}*tetrakis(acetic Acid)* (**3**). It was obtained from the corresponding tetraester following the *General Procedure* (*vide supra*). Yield: 69%. White solid. ^{1}H-NMR (D_2O: NaOD 9:1) 9.00 (*d*, J = 2.6, 2 H, H–C(5), of Pz); 8.60 (*d*, J = 8, 2 H, H–C(5) of Py); 8.15 (*m*, 4 H, H–C(3), H–C(4) of Py); 6.80 (*d*, J = 2.6, 2 H, H–C(4) of Pz); 4.70 (*s*, 2 CH_2N); 4.2 (*s*, 4 NCH_2CO). ^{13}C-NMR (D_2O: NaOD 9:1): 180 (C=O); 154 (C(2) of Py); 153 (C(6) of Py); 151 (C(3) of Pz); 141 (C(4) of Py); 120 (C(3), C(5) of Py); 108 (C(4) of Pz); 80 (Me_3C); 55 (CH_2); 51 (NCH_2CO); 28 (Me_3C). MS (FAB+): 803.6 (MH^+).

Luminescence Measurements. The luminescence parameters were analyzed in borate buffer solns., pH 8.5, prepared by dissolving the ligand in 0.05M borate buffer soln. ($c = 8.6 \cdot 10^{-7}$ for **1** and **2**, and $1.7 \cdot 10^{-7}$ for **3**). The emission quantum yields were measured by a relative method using the Eu^{3+} and Tb^{3+} complexes of *N,N,N′,N′*-[6,6″-bis(aminomethyl)-4′-phenyl-2,2′:6′,2″-terpyridine]tetrakis(acetic acid) as a standard. The total luminescence intensities of chelates were determined by integrating the emissions of each lanthanide chelate as described in [15].

REFERENCES

[1] W. DeW. Horrocks, Jr., D. R. Sudnick, *Acc. Chem. Res.* **1981,** *14,* 384; S. E. Burroughs, G. Eisenman, W. DeW. Horrocks, Jr., *Biophys. Chem.* **1992,** *42,* 249; A. Chartier, C. G. Fox, J. Georges, *Analusis* **1992,** *20,* 269; E. Soini, T. Lovgren, *CRC Crit. Rev. Anal. Chem.* **1987,** *18,* 105; E. Soini, I. Hemmila, P. Dahlen, *Ann. Biol. Clin.* **1990,** *48,* 567; T. Lovgren, I. Hemmila, K. Peterson, P. Halonen, in 'Alternative Immunoassays', Ed. W. P. Collins, Wiley, London, 1985, Chapt. 12; O. Prat, E. Lopez, G. Mathis, *Anal. Biochem.* **1991,** *195,* 283; E. P. Diamandis, T. K. Christopoulos, *Anal. Chem.* **1990,** *62,* 1149A.

[2] I. Hemmilä, S. Dakubu, V.-M. Mukkala, H. Siitari, T. Lövgren, *Anal. Biochem.* **1984,** *137,* 335.

[3] F. S. Richardson, *Chem. Rev.* **1982,** *82,* 541; G. Blasse, *Photochem. Photobiol.* **1990,** *52,* 417; W. DeW. Horrocks, Jr., M. Albin, *Prog. Inorg. Chem.* **1984,** *31,* 1.

[4] A. P. B. Sinha, *Spectrosc. Inorg. Chem.* **1971,** *2,* 255; W. T. Carnall, in 'Handbook on the Physics and Chemistry of Rare Earths', Eds. K. Gschneidner And L. Eyring, North-Holland, Amsterdam, 1979, Vol. 3, p. 171.

[5] F. K. Freed, *Topics Curr. Chem.* **1976,** *15,* 23; G. Stein, E. Wurzberg, *J. Chem. Phys.* **1975,** *62,* 208; Y. Haas, G. Stein, *ibid.* **1971,** *75,* 3677.

[6] U. Kallistratos, H. Münder, *Chimika Chronika, New Series* **1982,** *11,* 249; N. Sabbatini, M. Guardigli, A. Mecati, V. Balzani, R. Ungaro, E. Ghidini, A. Casnati, A. Pochini, *J. Chem. Soc., Chem. Commun.* **1990,** 878; G. Marriot, R. M. Clegg, D. J. Arndt-Jovin, T. M. Jovin, *Biophys. J.* **1991,** *60,* 1374; N. Sato, M. Gato, S. Matsumoto, S. Shinkai, *Tetrahedron Lett.* **1993,** *34,* 4847; G. Marriot, M. Heidecker, E. P. Diamandis, Y. Yan-Marriot, *Biophys. J.* **1994,** *67,* 957; P. R. Selvin, T. M. Rana, J. E. Hearst, *J. Am. Chem. Soc.* **1994,** *116,* 6029; V.-M. Mukkala, P. Liitti, I. Hemmilä, H. Takalo, C. Matachescu, J. Kankare, *Helv. Chim. Acta* **1996,** *79,* 295.

[7] E. Lopez, C. Chypre, B. Alpha, G. Mathis, *Clin. Chem.* **1993,** *39,* 196; G. Mathis, *ibid.* **1993,** *39,* 1953.

[8] M. P. Bailey, B. F. Rocks, C. Riley, *Analyst* **1984,** *109,* 1449; S. Dakubu, R. Hale, A. Lu, J. Quick, D. Solas, J. Weinberg, *Clin. Chem.* **1988,** *34,* 2337.

[9] M. Remuiñán, H. Román, M. T. Alonso, J. C. Rodríguez-Ubis, *J. Chem. Soc., Perkin Trans. 2* **1993,** 1099.

[10] J. D. Luodon, N. Shulman, *J. Chem. Soc.* **1941,** 722; Suhr, *Chem. Ber.* **1964,** *97,* 3268; J. R. Beck, *Tetrahedron* **1978,** *34,* 2057.

[11] U. Neumann, F. Vögtle, *Chem. Ber.* **1989,** *122,* 589.

[12] M. Tiecco, L. Testaferri, M. Tingoli, D. Chianelli, M. Montanucci, *Synthesis* **1984,** 736.

[13] N. Sabbatini, S. Perathoner, V. Balzani, B. Alpha, J.-M. Lehn, in 'Supramolecular Photochemistry', Ed. V. Balzani, Reidel, Dordrecht, 1987, p. 187; B. Alpha, R. Ballardini, V. Balzani, J.-M. Lehn, N. Sabbatini, *Photochem. Photobiol.* **1990,** *52,* 299.

[14] V.-M. Mukkala, P. Liitti, I. Hemmilä, H. Takalo, C. Matachescu, J. Kankare, *Helv. Chim. Acta* **1992,** *75,* 1621; V.-M. Mukkala, M. Helenius, I. Hemmilä, J. Kankare, H. Takalo, *ibid.* **1993,** *76,* 1361; H. Takalo, E. Hänninen, J. Kankare, *ibid.* **1993,** *76,* 877; M. Pietraszkiewicz, K. Kairpuk, A. K. Rout, *Pure Appl. Chem.* **1993,** *65,* 566.

[15] M. Latva, H. Takalo, V.-M. Mukkala, C. Matachescu, J. C. Rodríguez-Ubis, J. Kankare, unpublished results.

[16] P. G. Sammes, G. Yahioglu, *Nat. Prod. Reports* **1996,** 1; W. T. Carnall, in 'Handbook on the Physics and Chemistry of Rare Earths, Eds. K. Gschneidner and L. Eyring, North-Holland, Amsterdam, 1979, Vol. 3, p. 171.

[17] W. DeW. Horrocks, Jr., D. R. Sudnick, *J. Am. Chem. Soc.* **1979,** *101,* 334.

[18] Y.-Y. Xu, I. Hemmilä, V.-M. Mukkala, S. Holttinen, T. Lövgren, *Analyst* **1991,** *116,* 1155.

[19] D. D. Perrin, W. L. F. Armarego, D. R. Perrin, 'Purification of Laboratory Chemicals', 2nd edn., Pergamon Press, Oxford, 1980.