Inorganic Chemistry
Article

Inorg. Chem. **2009**, *48*, 10687–10696 **10687**
DOI: 10.1021/ic901424w

Luminescent Lanthanide Helicates Self-Assembled from Ditopic Ligands Bearing Phosphonic Acid or Phosphoester Units

Anne-Sophie Chauvin,*,† Steve Comby,† Matthias Baud,† Cyntia De Piano,† Céline Duhot,† and Jean-Claude G. Bünzli*,†,‡

†Laboratory of Lanthanide Supramolecular Chemistry, École Polytechnique Fédérale de Lausanne, BCH 1405, CH-1015 Lausanne, Switzerland, and ‡Department of Advanced Materials Chemistry, Korea University, 208 Seochong, Jochiwon, ChungNam 339-700, Republic of Korea

Received July 21, 2009

A series of hexadentate ditopic receptors incorporating benzimidazole moieties have been designed, which are fitted with phosphonic acid or phosphoethylester coordinating units. In addition, poly(oxyethylene) pendants have been introduced on the benzimidazole backbone of two ligands to increase water solubility. The ligands self-assemble with lanthanide ions under stoichiometric conditions, yielding triple-stranded homobimetallic helicates, as ascertained by mass spectrometry and UV−visible titrations. The helicates display large thermodynamic stability, for example, log $\beta_{23} \approx 21-24$ for all the Eu^{III} complexes. Photophysical measurements reveal sensitization of the metal-centered luminescence in the europium and terbium complexes, which is modulated by the nature of the ligand. Hydration numbers determined by the lifetime method are essentially zero. The $Eu(^5D_0)$ lifetimes are long and reach values up to 3.2 ms, while quantum yields as high as 25% are obtained in water at pH 7.4. Back transfer limits the sensitization efficiency for Tb^{III} luminescence, and both lifetimes and quantum yields are much smaller. The properties of the helicates are discussed with respect to those self-assembled from ligands bearing carboxylate coordinating units.

Introduction

Present interest in the design of lanthanide-containing luminescent probes[1−3] for analytical sensors,[4] medical analyses,[5−7] and live cell[8−12] or tissue[13,14] imaging makes complexes based on the dipicolinate framework quite attractive in view of their solubility in water,[15] the large quantum yield of the parent compounds,[16] and their proneness to multiphoton excitation.[17,18] Double lanthanide tags presenting definite advantages over mononuclear probes,[19] we have been developing bis(tridentate) ditopic receptors with a 2-picolinic acid core and a benzimidazole moiety grafted in the 6-position (Chart 1).[20] These ligands self-assemble with lanthanide ions to yield highly stable triple-stranded homo-[21] and/or hetero-[22] bimetallic helicates. More recently we have demonstrated that upon adequate derivatization, bimetallic helicates are viable alternatives to the

---

*To whom correspondence should be addressed. E-mail: anne-sophie.chauvin@epfl.ch (A.-S.C.), jean-claude.bunzli@epfl.ch (J.-C.B.).

(1) Moore, E. G.; Samuel, A. P. S.; Raymond, K. N. *Acc. Chem. Res.* **2009**, *42*, 542.

(2) Bünzli, J.-C. G. *Chem. Lett.* **2009**, *38*, 104.

(3) Eliseeva, S. V.; Bünzli, J.-C. G. *Chem. Soc. Rev.* **2010**, published on the web, September 11, 2009; DOI: 10.1039/b905604c.

(4) Dos Santos, C. M. G.; Harte, A. J.; Quinn, S. J.; Gunnlaugsson, T. *Coord. Chem. Rev.* **2008**, *252*, 2512.

(5) Laitala, V.; Ylikoski, A.; Raussi, H. M.; Ollikka, P.; Hemmilä, I. *Anal. Biochem.* **2007**, *361*, 126.

(6) Ghose, S.; Trinquet, E.; Laget, M.; Bazin, H.; Mathis, G. *J. Alloys Compd.* **2008**, *451*, 35.

(7) Nishioka, T., Fukui, K., Matsumoto, K. Lanthanide Chelates as Luminescent Labels in Biomedical Analyses. In *Handbook on the Physics and Chemistry of Rare Earths*; Gschneidner, K. A., Jr., Bünzli, J.-C. G., Pecharsky, V. K., Eds.; Elsevier Science B.V.: Amsterdam, 2007; Vol. *37*, pp 171−216.

(8) Faulkner, S.; Pope, S. J. A.; Burton-Pye, B. P. *Appl. Spectrosc. Rev.* **2005**, *40*, 1.

(9) Montgomery, C. P.; Murray, B. S.; New, E. J.; Pal, R.; Parker, D. *Acc. Chem. Res.* **2009**, *42*, 925.

(10) New, E. J.; Parker, D. *Org. Biomol. Chem.* **2009**, *7*, 851.

(11) Bünzli, J.-C. G.; Chauvin, A.-S.; Vandevyver, C. D. B.; Song, B.; Comby, S. *Ann. N.Y. Acad. Sci.* **2008**, *1130*, 97.

(12) Song, B.; Vandevyver, C. D. B.; Chauvin, A.-S.; Bünzli, J.-C. G. *Org. Biomol. Chem.* **2008**, *6*, 4125.

(13) Hess, B. A.; Kedziorski, A.; Smentek, L.; Bornhop, D. J. *J. Phys. Chem. A* **2008**, *112*, 2397.

(14) Song, B.; Sivagnanam, V.; Vandevyver, C. D. B.; Hemmilä, I. A.; Lehr, H.-A.; Gijs, M. A. M.; Bünzli, J.-C. G. *Analyst* **2009**, *134*, 1991.

(15) Gassner, A.-L.; Duhot, C.; Bünzli, J.-C. G.; Chauvin, A.-S. *Inorg. Chem.* **2008**, *47*, 7802.

(16) Aebischer, A.; Gumy, F.; Bünzli, J.-C. G. *Phys. Chem. Chem. Phys.* **2009**, *11*, 1346.

(17) Kadjane, P.; Starck, M.; Camerel, F.; Hill, D.; Hildebrandt, N.; Ziessel, R.; Charbonniere, L. J. *Inorg. Chem.* **2009**, *48*, 4601.

(18) Picot, A.; D'Aleo, A.; Baldeck, P. L.; Grichine, A.; Duperray, A.; Andraud, C.; Maury, O. *J. Am. Chem. Soc.* **2008**, *130*, 1532.

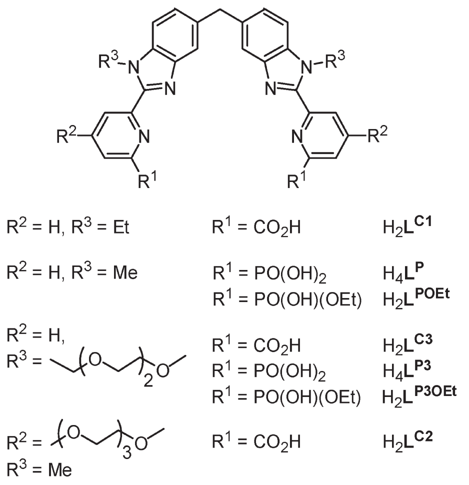
(19) Martin, L. J.; Hahnke, M. J.; Nitz, M.; Wohnert, J.; Silvaggi, N. R.; Allen, K. N.; Schwalbe, H.; Imperiali, B. *J. Am. Chem. Soc.* **2007**, *129*, 7106.

(20) Piguet, C. Bünzli, J.-C. G. Self-assembled Lanthanide Helicates: from Basic Thermodynamics to Applications. In *Handbook on the Physics and Chemistry of Rare Earths*; Gschneidner, K. A., Jr., Bünzli, J.-C. G., Pecharsky, V. K., Eds.; Elsevier Science, B.V.: Amsterdam, 2010; Vol. *40*, Chapter *247*, pp 351−553.

(21) Elhabiri, M.; Scopelliti, R.; Bünzli, J.-C. G.; Piguet, C. *J. Am. Chem. Soc.* **1999**, *121*, 10747.

(22) André, N.; Jensen, T. B.; Scopelliti, R.; Imbert, D.; Elhabiri, M.; Hopfgartner, G.; Piguet, C.; Bünzli, J.-C. G. *Inorg. Chem.* **2004**, *43*, 515.

Chart 1. Ligand Structures



existing lanthanide chelates for live cell imaging.[23–26] One of the key to their thermodynamic stability is the presence of the carboxylic acid group. However, other groups such as phosphonic acid are also strongly coordinating and their influence may be beneficial to the photophysical properties; this has for instance been demonstrated for complexes with cyclen derivatives for which the hydration is reduced with respect to DOTA complexes when carboxylate groups are replaced with phosphonate ones.[27,28] As we strive to the development of highly luminescent lanthanide bioprobes, we report herein the synthesis of a new series of four ligands derived from the $H_2\mathbf{L}^{\mathbf{Cx}}$ family in which the coordinating carboxylic acid moieties have been replaced by phosphonic acid or phosphonic acid ethyl ester (Chart 1). Poly(oxyethylene) arms have been grafted on two ligands to increase the solubility of the resulting helicates, as was demonstrated for $[Ln_2(L^{CX})_3]$ ($x = 2, 3$ versus $x = 1$).[23,24] The physicochemical and photophysical properties of the ligands and of their $[Ln_2(L^{Py})_3]^{i-}$ chelates ($y = 1, 3$ and $i = 1$; $y =$ OEt, 3OEt and $i = 0$) are presented along with a comparison with the corresponding helicates with H_2L^{CX} ligands.

Experimental Section

General Procedures. Solvents were purified by a non-hazardous procedure by passing them onto activated alumina columns (Innovative Technology Inc. system).[29] Chemicals were ordered from Fluka and Aldrich and used without further purification. The 2:3 complexes were prepared in situ by mixing 3 equiv of the desired ligand with 1 equiv of $Ln(ClO_4)_3 \cdot xH_2O$ (Ln = La, Lu, Gd, Tb, Eu, $x = 2.5-4.5$) in water or, when needed, in a Tris-HCl 0.1 M buffer solution (pH = 7.4). ^1H NMR spectra and ^{31}P NMR were performed on a Bruker Avance DRX 400 spectrometer and ^{13}C NMR spectra on a Bruker AV 600 MHz at 25 °C, using deuterated solvents as internal standards, and phosphoric acid in the case of phosphorus NMR. ESI-MS spectra were obtained on a Finningan SSQ 710C spectrometer using $10^{-5}-10^{-4}$ M solutions in acetonitrile/H_2O/acetic acid (50/50/1) or MeOH, a capillary temperature of 200 °C and an acceleration potential equal to 4.5 keV. The instrument was calibrated using horse myoglobin standard, and analyses were conducted in positive mode with a 4.6 keV ion spray voltage. Elemental analyses were performed by Dr. E. Solari at the École Polytechnique Fédérale de Lausanne and by Dr. Ilze Beetz, in Kronach (Germany). UV–visible spectra were measured in 0.2 cm quartz Suprasil cuvettes on a PerkinElmer Lambda 900 spectrometer.

Physicochemical Measurements. Protonation constants of the ligands were determined with the help of a J&M diode array spectrometer (Tidas series) connected to an external computer. All titrations were performed in a thermostatted (25.0 ± 0.1 °C) glass-jacketed vessel at $I = 0.1$ M (KCl). In a typical experiment 50 mL of a ligand solution ($[H_4L^P] = 1.34 \times 10^{-4}$ M, $[H_4L^{P3}] = 8.99 \times 10^{-6}$ M, $[H_2L^{POEt}] = 1.56 \times 10^{-5}$ M, $[H_2L^{P3OEt}] = 1.31 \times 10^{-5}$ M) were titrated by freshly prepared sodium hydroxide solutions at different concentrations (10, 4, 1, 0.1, and 0.01 M). After each addition, the pH of the solution was measured by a KCl-saturated electrode (Methrom No. 6.0224 100) and the UV–vis absorption spectrum was recorded using a 1 cm Hellma optrode immersed in the thermostatted titration vessel. Measurements were conducted in the pH range 1.1–12. Using the same equipment; conditional stability constants were determined by titration of the ligands 10^{-4} M by Ln^{III} 5×10^{-3} M (Ln = Eu, Tb) at fixed pH 7.4 (0.1 M Tris-HCl buffer). Factor analysis[30] and mathematical treatment of the spectrophotometric data were performed with the Specfit software.[31,32] Broad-band excited emission spectra were recorded on a Fluorolog FL-3–22 spectrometer from Horiba-Jobin-Yvon Ltd.; quartz cells with optical paths of 0.2 cm were used for room temperature (RT) spectra while 77 K measurements were carried out on samples put into quartz Suprasil capillaries. All spectra are corrected for the instrumental function. Absorbance of the samples and reference was measured three times and for three different absorbance values (≈0.1, ≈0.15, and ≈0.2). Quantum yields were determined in aerated water both by a comparative method with $[Eu_2(L^{C2})_3]$ (Q = 21%)[24] and $[Ln(dpa)_3]^{3-}$ (Q = 24%)[33] as standards and by an absolute method using a specially designed integration sphere.[16]

Synthesis of the Ligands. Synthesis of 4,4′-methanediyl-bis-(benzene-1,2-diamine) (**1a**) and 4,4′-methanediyl-bis(*N*-methyl-benzene-1,2-diamine) (**1b**) was performed according to a described procedure,[24,34] except for the purification of **1b**, which was finally dissolved in $CHCl_3$ and allowed to crystallize in the freezer (−18 °C). The crystals were kept in the mother liquid at low temperature; they were filtered and dried for 30 min under vacuum just before being used.

Synthesis of 1-Oxy-pyridine-2-carboxylic Acid Ethyl Ester. The procedure was adapted from ref 35. Ethylpicolinate (4 g, 26.6 mmol) was stirred for 12 h with *meta*-chloroperoxybenzoic acid (*m*-CPBA) (10 g, 58 mmol) in 200 mL of chloroform, then the solution was poured in 200 mL of half-saturated solution of K_2CO_3 and stirred for additional 10 min. The phases were separated, the organic layer was washed twice with 100 mL of

---

(23) Chauvin, A.-S.; Comby, S.; Song, B.; Vandevyver, C. D. B.; Bünzli, J.-C. G. *Chem.—Eur. J.* **2007**, *13*, 9515.

(24) Chauvin, A.-S.; Comby, S.; Song, B.; Vandevyver, C. D. B.; Bünzli, J.-C. G. *Chem.—Eur. J.* **2008**, *14*, 1726.

(25) Deiters, E.; Song, B.; Chauvin, A.-S.; Vandevyver, C. D. B.; Bünzli, J.-C. G. *New J. Chem.* **2008**, *32*, 1140.

(26) Deiters, E.; Song, B.; Chauvin, A.-S.; Vandevyver, C.; Bünzli, J.-C. G. *Chem.—Eur. J.* **2009**, *15*, 885.

(27) Rohovec, J.; Vojtisek, P.; Hermann, P.; Mosinger, J.; Zak, Z.; Lukes, I. *J. Chem. Soc., Dalton Trans.* **1999**, 3585.

(28) Kotkova, Z.; Pereira, G. A.; Djanashvili, K.; Kotek, J.; Rudovsky, J.; Hermann, P.; Elst, L. V.; Muller, R. N.; Geraldes, C. F. G. C.; Lukes, I.; Peters, J. A. *Eur. J. Inorg. Chem.* **2009**, 119.

(29) Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518.

(30) Malinowski,E. R.; Howery,D. G. *Factor Analysis in Chemistry*; John Wiley: New York, Chichester, Brisbane, Toronto, 1991.

(31) Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1986**, *33*, 943.

(32) Gampp, H.; Maeder, M.; Meyer, C. J.; Zuberbühler, A. D. *Talanta* **1985**, *32*, 257.

(33) Chauvin, A.-S.; Gumy, F.; Imbert, D.; Bünzli, J.-C. G. *Spectrosc. Lett.* **2004**, *37*, 517; erratum, 2006, 40, 193.

(34) Piguet, C.; Bernardinelli, G.; Bocquet, B.; Quattropani, A.; Williams, A. F. *J. Am. Chem. Soc.* **1992**, *114*, 7440.

(35) Agrawal, K. C.; Sartorelli, A. C. *J. Med. Chem.* **1978**, *21*, 218.

half-saturated K_2CO_3 solution, and then it was dried over Na_2SO_4. The product was purified by bulb-to-bulb distillation after removal of the solvent under reduced pressure (3.1 g, 75%). ESI-MS: m/z = 168.34 $[M+H]^+$ (Calcd 168.06), ^1H NMR (400 MHz, $CDCl_3$, ppm): δ =8.25 (dd, J = 6.6, 0.7 Hz, 1 H, H_{ar}), 7.58 (dd, J = 7.5, 2.0 Hz, 1 H, H_{ar}), 7.32 (m, 1 H, H_{ar}), 7.27 (m, 1 H, H_{ar}), 4.47 (d, J = 7.1 Hz, 2 H, $-OCH_2-$), 1.41 (t, J = 7.1 Hz, 3 H, $-CH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$, ppm): δ =161.84 (CO), 141.19 (C_{ar}), 127.70 (CH_{ar}), 126.98 (CH_{ar}), 125,20 (CH_{ar}), 62.95($-OCH_2-$), 14.42 (CH_3).

Synthesis of 6-(Diethoxy-phosphoryl)-pyridine-2-carboxylic Acid Ethyl Ester. The procedure was adapted from ref 36. 1-Oxy-pyridine-2-carboxylic acid ethyl ester (1.2 g, 7.17 mmol) was dissolved in 3 mL of toluene with a molecular sieve (4 Å), and 0.9 g of dimethylsulfate was added. The solution was stirred 30 min, and two layers appeared. Diethylphosphite (3.5 mL) was then added to homogenize the solution which was stirred for additional 10 min. Sodium (0.21 g) in 4 mL of diethylphosphite was poured under nitrogen atmosphere in a two-necked flask immerged in an ice bath and was then added dropwise under vigorous stirring to the solution of the N-oxo derivative. The reaction was exothermic, and a brown color appeared. The solution was stirred for 24 h under inert atmosphere and turned pale brown. Water was added (20 mL), and the organic phase separated. After washing of the aqueous phase twice with 50 mL of $CHCl_3$, the collected organic layers were dried over Na_2SO_4, the solvents were removed, and the excess of diethylphosphite was eliminated by distillation under reduced pressure. The product was purified by chromatography (Silicagel, pure ethyl acetate) to give 1.1 g of a yellow oil (64%). ESI-MS: m/z = 288.34 $[M+H]^+$ (Calcd 288.09), ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.19 (m, 1H, H_{ar}), 8.07 (m, 1H, H_{ar}), 8.93 (m, 1H, H_{ar}), 4.46 (q, 2H, J = 7.1 Hz, $-OCH_2-$), 4.30 (m, 4H, $-P(O)OCH_2-$), 1.41 (t, 3H, J = 7.1 Hz, $-CH_3$), 1.37 (t, 6H, $-CH_3$). ^{31}P NMR (162 MHz, $CDCl_3$, ppm): δ = 9.21.

Synthesis of 6-(Diethoxy-phosphoryl)-pyridine-2-carboxylic Acid (2a). 6-(Diethoxy-phosphoryl)-pyridine-2-carboxylic acid ethyl ester (1 g, 3.48 mmol) was dissolved in ethanol (5 mL), and sodium hydroxide 0.1 M (34.8 mL, 3.84 mmol, 1.1 equiv) was added dropwise for 30 min. The solution was stirred at RT for 1 h, and the disappearance of the starting product was followed by TLC (CH_2Cl_2/MeOH 98/2 v/v, R_f = 0.5 for starting material and 0 for the product). This basic aqueous phase was washed with CH_2Cl_2 (2 × 100 mL), the pH was adjusted to 2 with HCl 2M, and the product was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic phases were dried over Na_2SO_4, and the solvent was evaporated; **2a** was obtained as a pale yellow solid (815 mg, 90%). The same experiment could be conducted starting from the crude product 6-(diethoxy-phosphoryl)-pyridine-2-carboxylic acid ethyl ester before purification by chromatography, and provided (**2a**) in a 45% global yield with respect to the oxo-product. ESI-MS: m/z = 260.30 $[M+H]^+$ (Calcd 260.06). ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.34 (m, 1 H, H_{ar}), 8.17 (m, 1 H, H_{ar}), 8.06 (td, J = 7.7, 4.9 Hz, 1 H, H_{ar}), 4.27 (m, 4 H, $-OCH_2-$), 1.37 (t, J = 7.1 Hz, 6 H, $-CH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$, ppm): δ = 164.05 (CO), 150.60 (C$-$C), 147.75 (C$-$P), 139.07 (CH_{ar}), 131.79 (CH_{ar}), 126.54 (CH_{ar}), 63.92 ($-OCH_2-$), 16.78 (CH_3). ^{31}P NMR (162 MHz, $CDCl_3$, ppm): δ = 8.69.

Synthesis of $H_4L^P \cdot 2HCl$. Freshly prepared **1b** (260 mg, 0.1 mmol) was introduced with **2a** (585 mg, 0.23 mmol) and polyphosphoric acid (20 mL) in a two-neck 50 mL flask equipped with a thermometer and a distillation apparatus. The mixture was heated with a DrySyn apparatus at 180 °C, all the water removed by distillation, and then the temperature was increased to 210 °C. After stirring 6 h, the mixture was cooled to approximately 60 °C, and the viscous brown residue poured into 100 mL of water. A green solid formed, which was filtrated, and then dissolved in NaOH 5 M until pH 8. The brown solution was filtrated, and the pH was adjusted to 2 by addition of HCl 2.5 M; this resulted in the formation of a brown solid which was collected, rinsed with water, and dried under vacuum (2 mbar) for 12 h (350 mg, 53%). ^1H NMR (400 MHz, D_2O, ppm): δ = 7.80 (m, 1 H, H_{ar}), 7.71 (m, 1 H, H_{ar}), 7.58 (m, 1 H, H_{ar}), 7.52 (m, 1 H, H_{ar}), 7.35 (m, 1 H, H_{ar}), 7.21 (m, 1 H, H_{ar}), 4.07 (s, 1 H, $-CH_2-$), 3.92 (s, 3 H, $-CH_3$). ^{13}C NMR (150.9 MHz, D_2O) δ = 163.34 ($-C_{ar}$), 162.52 ($-C_{ar}$), 152.50 ($-C_{ar}$), 147.95 ($-C_{ar}$), 141.42 ($-C_{ar}$), 137.45 ($-C_{ar}$), 137.18 ($-C_{ar}$), 135.11($-C_{ar}$), 126.45 ($-CH_{ar}$), 126.44 ($-CH_{ar}$), 124.44 ($-CH_{ar}$), 124.48 ($-CH_{ar}$), 118.01 ($-CH_{ar}$), 110.76 ($-CH_{ar}$), 41.48 ($-CH_2-$), 32.11 ($-NCH_3-$).^{31}P NMR (162 MHz, D_2O, ppm): δ = 5.90. Anal. Calcd for L^P.2HCl ($C_{27}H_{26}Cl_2N_6O_6P_2$): C 48.88, H 3.95, Cl 10.69, N 12.67, O 14.47, P 9.34; found: C 48.91, H 3.98, N 12.69, Cl 10.76, O 14.43, P, 9.23%.

Synthesis of 6-Chloropicolinic Acid (2b). The procedure developed by Novartis was adapted:[37] 6-hydroxy picolinic acid (4 g, 28.8 mmol) was suspended in a mixture of $POCl_3$ (1.7 g, 66.2 mmol) and PCl_5 (20 g, 96 mmol) and refluxed for 12 h at 100 °C. Formic acid (1.4 mL) was carefully added at RT (exothermic reaction), and the solvents were removed under reduced pressure. The crude product was suspended in water (25 mL), and K_2CO_3 (5.6 g 40 mmol) in 25 mL of water was carefully added to the previous solution. After 10 min, the solution was washed with CH_2Cl_2 (3 × 50 mL), and HCl 0.5 M was added until precipitation of the desired product occurred (pH $\approx$ 2). The precipitate was filtrated, rinsed, and dried under vacuum to provide **2b** as a yellow solid (3.6 g, 80%). ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.16 (dd, J = 7.5, 0.7 Hz, 1 H, H_{ar}), 7.93 (t, J = 7.9, Hz, 1 H, H_{ar}), 7.61 (dd, J = 7.9, 0.7 Hz, 1 H, H_{ar}). Anal. Calcd for $C_6H_4ClNO_2$: C 45.74, H 2.56, N 8.89; found: C 46.04, H 2.65, N, 9.16%.

Synthesis of 5,5′-Methanediylbis[2-(6-chloropyridin-2-yl)-1*H*-benzimidazole] (3a). The same procedure as for H_4L^P was used, starting from **1a** (540 mg, 2.2 mmol), **2b** (700 mg, 4.5 mmol), and polyphosphoric acid (20 mL). After 6 h, the crude product was poured in water, filtered and triturated in NaOH 5 M, filtered, and dried. The solid (560 mg, 54%) consisted in a mixture of chloro- and monohydroxy-substituted products which could not be separated and were used without further purification. ESI-MS: m/z = 471.38 $[M+H]^+$ (calcd 471.09); 453.36 $[M+H]^+$ (calcd 453.13, OH substituted product); ^1H NMR (400 MHz, DMSO-d6, ppm) δ = 8.25 (dd, J = 0.9 Hz, 0.5 Hz, 1 H, H_{ar}), 8.00 (m, 1 H. H_{ar}), 7.49 (m, 3 H, H_{ar}), 7.13 (m, 1 H, H_{ar}), 4.10 (s, 1 H, $-CH_2-$), 3.59 (s, 4 H, NH_2); additional peaks corresponding to the OH substituted product (25%): 6.92 (d, 1 H, H_{ar}), 6.26 (d, 1 H, H_{ar}), 4.16 (s, 1 H, $-CH_2-$).

Synthesis of 5,5′-Methanediylbis[2-(6-chloropyridin-2-yl)-1-methyl-1*H*-benzimidazole] (3b). The same procedure as for H_4L^P was used, starting from **1b** (570 g, 2.2 mmol), **2b** (700 mg, 4.5 mmol), and polyphosphoric acid (20 mL), except that the crude product was poured in ethanol instead of water, filtered and triturated in NaOH 5 M, then filtered and dried. (**3b**) was obtained has a brown solid (690 mg, 62%). ESI-MS: m/z = 499.31 $[M+H]^+$ (calcd 499.12); 250.35 $[M+2H]^+/2$ (calcd 250.06); ^1H NMR (400 MHz, $CDCl_3$, ppm) δ = 8.34 (d, J = 7.68 Hz, 1 H, H_{ar}), 7.78 (t, J = 7.87 Hz, 1 H. H_{ar}), 7.68 (s, 1 H, H_{ar}), 7.34 (t, J = 7.87 Hz, 2 H, H_{ar}), 7.25 (dd, J = 8.42 and 1.46 Hz, 1 H, H_{ar}), 4.28 (s, 1 H, $-CH_2-$), 4.26 (s, 3 H, $-CH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$, ppm) δ = 150.84 ($-C_{ar}$), 150.11 ($-C_{ar}$), 148.57 ($-C_{ar}$), 142.67 ($-C_{ar}$), 139.37 ($-C_{ar}$), 136.63 ($-C_{ar}$), 135.93 ($-C_{ar}$), 125.25 ($-CH_{ar}$), 124.12 ($-CH_{ar}$), 122.86 ($-CH_{ar}$), 119.98 ($-CH_{ar}$), 109.96 ($-CH_{ar}$), 42.20 ($-NCH_3$), 32.88 ($-CH_3$).

---

(36) Boduszek, B. *J. Prakt. Chem. Chem. Z.* **1992**, *334*, 444.

(37) Altmann, E., Beerli, R., Gerspracher, M., Renaud, J., Weiler, S. Patent WO 2004/056365 A2, 2004.

5,5′-Methanediylbis[2-(6-chloropyridin-2-yl)-1-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]-1*H*-benzimidazole] (4). Diamine **3a** (500 g, 3.5 mmol) was dissolved in THF (150 mL) in presence of triethyleneglycol monomethylether (1.1 mL, 6.4 mmol), and triphenylphosphine (1.7 g, 6.4 mmol). Azodicarboxylic acid diisopropyl ester (1.3 g, 6.4 mmol) was added dropwise at RT. The orange solution was refluxed for 12 h, the solvent was removed under reduced pressure, and the residue was redissolved in CH_2Cl_2 (100 mL), washed with aqueous HCl 0.1 M (100 mL) and with water (2 × 100 mL). The organic phase was dried over Na_2SO_4, evaporated, and the resulting crude solid was purified by column chromatography (silica gel; CH_2Cl_2/MeOH, 100→98.2 v/v) to give the disubstituted product **4** as an orange oil (400 mg, 50%). ESI-MS: *m*/*z* = 763.29 $[M+H]^+$ (calcd 763.28.); 382.36 $[M+2H]^+/2$ (calcd 382.64); ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.34 (d, *J* = 7.5 Hz, 1 H, H_{ar}), 7.79 (t, *J* = 7.9 Hz, 1 H, H_{ar}), 7.66 (s, 1 H, H_{ar}), 7.46 (d, *J* = 8.4 Hz, 1 H, H_{ar}), 7.35 (d, *J* = 7.5 Hz, 1 H, H_{ar}), 7.22 (d, 1 H, H_{ar}), 4.90 (q, 2 H, *J* = 5.9 Hz, $-OCH_2-$), 4.26 (d, *J* = 5.1 Hz, 1 H, $-CH_2-$), 3.98 (t, *J* = 5.9 Hz, 2 H, $-CH_2-$), 3.61 (d, *J* = 5.1 Hz, 2 H, $-CH_2-$), 3.51 (m, 4 H, $-CH_2-$), 3.44 (m, 2 H), 3.32 (s, 3 H, $-CH_3$).

Tetraethyl[methanediylbis[(1-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]-1*H*-benzimidazole-5,2-diyl)pyridine-6,2-diyl]]bis(phosphonate) (5a). Under anhydrous atmosphere, compound **4** (306 mg, 0.4 mmol), triphenyl phosphine (200 mg, 0.76 mmol), ethyldiisopropyl-amine (0.6 mL, 3.5 mmol) were dissolved in dry toluene (3 mL). A solution of diethylphosphite (0.13 mL, 10.16 mmol) and palladium(0) tetrakis(triphenylphosphine) (9.2 mg, 0.2 equiv) in toluene (2 mL) was added dropwise, and the solution was heated at 80 °C for 12 h. The solvents were removed under vacuum, the crude product was redissolved in dichloromethane, washed twice with water (10 mL), dried over Na_2SO_4, evaporated, and the resulting crude solid was purified by column chromatography (silica gel; CH_2Cl_2/MeOH, 100→98.2 v/v) to give **5a** as a brown solid (135 mg, 40% yield). ESI-MS: *m*/*z* = 967.40 $[M+H]^+$ (calcd 967.42) and 484.40 $[M+2H]^{2+}/2$ (calcd 484.21). ^1H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.50 (d, *J* = 7.7 Hz, 1 H, H_{ar}), 7.92 (m, 2 H, H_{ar}), 7.68 (dd, *J* = 8.4, 4.6 Hz, 1 H, H_{ar}), 7.46 (m, 1 H, H_{ar}), 7.18 (m, 1 H, H_{ar}), 4.92 (m, 2 H, $-OCH_2-$), 4.24 (s, 1 H, $-CH_2-$), 4.16 (m, 4H, $POCH_2-$), 3.94 (m, 2 H, $-CH_2-$), 3.47 (m, 4 H, $-CH_2-$), 3.39 (m, 4 H, $-CH_2-$), 3.25 (s, 3 H, $-OCH_3$),1.31 (t, *J* = 7.0 Hz, 6 H, $-CH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$, ppm) 151,46 (C_{ar}), 151,27 (C_{ar}), 151,04 (C_{ar}), 149,18 (C_{ar}), 148,62 (C_{ar}), 142,54 (C_{ar}), 140,91 (C_{ar}), 137,57 (C_{ar}), 136,95 (C_{ar}), 136,33 (C_{ar}), 135,91 (C_{ar}), 127,83 (CH_{ar}), 127,58 (CH_{ar}), 126,55 (CH_{ar}), 125,03 (CH_{ar}), 124,42 (CH_{ar}), 119,74 (CH_{ar}), 119,59 (CH_{ar}), 111,04 (CH_{ar}), 110,96 (CH_{ar}), 71,67 ($-CH_2-$), 70,63 ($-CH_2-$), 70,59 ($-CH_2-$), 70,34 ($-CH_2-$), 70,17 ($-CH_2-$), 62,67 ($-POCH_2-$), 62,47 ($-POCH_2-$), 58,77 ($-OCH_3$), 45,66 ($-NCH_2-$), 42,40 ($-CH_2-$), 16,32 ($-CH_3$). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 10.13. Anal. Calcd for $(\mathbf{5a})\cdot 0.75H_2O$ ($C_{47}H_{64}N_6O_{12}P_2\cdot 0.75H_2O$): C 56.73, H 6.63, N 8.45; found: C 56.69, H 6.65, N, 8.08%.

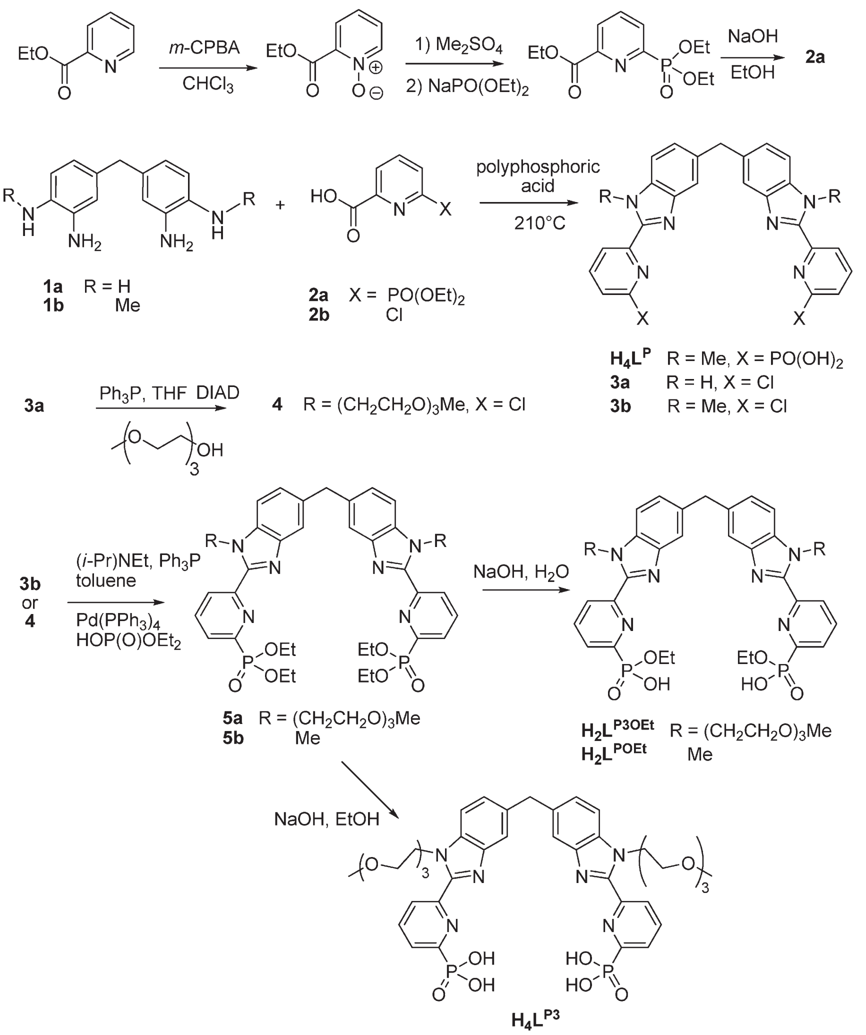
Diethyl[methanediylbis[(1-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]-1*H*-benzimidazole-5,2-diyl)pyridine-6,2-diyl]]bis[hydrogen (phosphonate)], H_2L^{P3OEt}. Compound **5a** (130 mg, 0.13 mmol) was dissolved in water (50 mL) in presence of NaOH 0.1 M (2.6 mL, 0.26 mmol) and the solution was stirred at RT for 30 min. The aqueous phase was washed with CH_2Cl_2 (2 × 50 mL, this organic phase contained starting material **5a** which could be recovered and reused). The pH was then lowered with HCl 1 M until formation of a pale yellow solid which was collected, rinsed with cold water and dried under vacuum for 48 h (70 mg, 57%). ESI-MS: *m*/*z* = 911.31 $[M+H]^+$ (calcd 911.36) and 456.30 $[M+2H]^{2+}/2$ (calcd 456.18). ^1H NMR (400 MHz, D_2O, ppm): δ = 7.78 (m, 1 H, H_{ar}), 7.69 (d, *J* = 5.7 Hz, 1 H, H_{ar}), 7.39 (m, 1 H, H_{ar}), 7.12 (d, *J* = 5.7 Hz, 1 H, H_{ar}), 7.04 (d, *J* = 5.7 Hz, 1 H, H_{ar}), 6.93 (m, 1 H, H_{ar}), 4.55 (s, 1 H, $-CH_2-$), 3.76 (m, 4 H, $-CH_2-$ and $POCH_2-$), 3.65 (m, 4 H, $-CH_2-$), 3.07 (d, *J* = 2.4 Hz, 2 H,$-CH_2-$), 2.92 (s, 3 H, OCH_3), 2.80 (m, 4H, $-CH_2-$), 1.06 (q, 3 H, $-CH_3$). ^{13}C NMR (150.9 MHz, D_2O, ppm) δ = 156.72 ($-C_{ar}$), 154.62 ($-C_{ar}$), 149.63 ($-C_{ar}$), 149.30 ($-C_{ar}$), 148.92 ($-C_{ar}$), 148.70 ($-C_{ar}$), 141.08 ($-C_{ar}$), 139.25 ($-C_{ar}$), 137.48 ($-C_{ar}$), 137.38 ($-C_{ar}$), 136.71 ($-C_{ar}$), 134.85 ($-C_{ar}$), 127.16 ($-CH_{ar}$), 126.94 ($-CH_{ar}$), 124.94 ($-CH_{ar}$), 124.42 ($-CH_{ar}$), 118.41 ($-CH_{ar}$), 117.83 ($-CH_{ar}$), 111.46 ($-CH_{ar}$), 110.91 ($-CH_{ar}$), 70.58 ($-CH_2-$), 70.33 ($-CH_2-$), 70.00 ($-CH_2-$), 69.83 ($-CH_2-$), 69.30 ($-CH_2-$), 69.23 ($-CH_2-$), 69.03 ($-CH_2-$), 61.47 ($-OCH_2-$), 57.68 ($-OCH_2-$), 45.06 ($-CH_2-$), 42.03 ($-CH_2-$), 41.86 ($-CH_2-$), 15.98 ($-CH_3$). ^{31}P NMR (162 MHz, D_2O, ppm): δ = 8.23. Anal. Calcd for $C_{43}H_{56}N_6O_{12}P_2\cdot 4.2HCl$: C, 48.54; H, 5.70; N, 7.90. Found: C, 48.55; H, 5.50; N, 8.10%.

{Methanediylbis[(1-[2-[2-(2-methoxyethoxy)ethoxy]ethyl-1*H*-benzimidazole-5,2-diyl)pyridine-6,2-diyl]]bis(phosphonic acid), H_4L^{P3}. Compound **5a** (130 mg, 0.13 mmol) was dissolved in ethanol (50 mL) in presence of NaOH 0.1 M (7 mL, 0.7 mmol), and the solution was stirred overnight at RT. After completion of the reaction, ethanol was evaporated, and the crude product was purified by chromatography (silica gel, MeCN, NH_4OAc, 100→ 80/20 v/v) to give the tri sodium salt of H_4L^{P3} as a pale yellow solid (80 mg, 70%). ESI-MS: *m*/*z* = 855.36 $[M+H]^+$ (calcd 855.29) and 427.27 $[M+2H]^{2+}/2$ (calcd 428.15). ^1H NMR (600 MHz, MeOD) δ = 6.53 (m, 1 H, H_{ar}), 6.30 (m, 2 H, H_{ar}), 6.00 (m, 1 H, H_{ar}), 5.90 (m, 1 H, H_{ar}), 5.62 (d, *J* = 4.6 Hz, 1 H, H_{ar}), 3.33 (m, 2 H, $-OCH_2-$), 2.59 (m, 1 H, $-CH_2-$), 2.29 (m, 2 H, $-CH_2-$), 1.77 (m, 2 H, $-CH_2-$), 1.68 (m, 2 H, $-CH_2-$), 1.63 (m, 4 H, $-CH_2-$), 1.57 (s, 3 H, $-CH_3$). ^{13}C NMR (150.9 MHz, MeOD) δ = 161.72 ($-C_{ar}$), 160.33 ($-C_{ar}$), 151.26 ($-C_{ar}$), 150.19 ($-C_{ar}$), 142.44 ($-C_{ar}$), 140.79 ($-C_{ar}$), 140.68 ($-C_{ar}$), 139.25 ($-C_{ar}$), 138.39 ($-C_{ar}$), 137.90 ($-C_{ar}$), 136.54 ($-C_{ar}$), 127.35 ($-CH_{ar}$), 126.35 ($-CH_{ar}$), 125.94 ($-CH_{ar}$), 125.62 ($-CH_{ar}$), 125.54 ($-CH_{ar}$), 119.57 ($-CH_{ar}$), 119.36 ($-CH_{ar}$), 112.89 ($-CH_{ar}$), 112.78 ($-CH_{ar}$), 72.72 ($-CH_2-$), 71.59 ($-CH_2-$), 71.26 ($-CH_2-$), 71.12 ($-CH_2-$), 58.97 ($-OCH_3$), 46.84 ($-CH_2$), 46.72 ($-CH_2$), 43.55 ($-CH_2-$), 43.30 ($-CH_3$).^{31}P NMR (162 MHz, MeOD): δ = 6.50. Anal. Calcd for $C_{39}H_{46}Na_2N_6O_{12}P_2\cdot 2.9NH_4OH\cdot 0.5\,H_2O$: C, 46.16; H, 5.86; N, 12.28. Found: C, 46.18; H, 5.96; N, 12.51%.

Tetraethyl {Methanediylbis[(1-methyl-1*H*-benzimidazole-5,2-diyl)pyridine-6,2-diyl]}-bis(phosphonate) (5b). The synthesis of **5b** was performed as for **5a**, starting from **3b** (250 mg, 0.5 mmol) and gave **5b** as a brown solid (145 mg, 42%). ESI-MS: *m*/*z* = 703.36 $[M+H]^+$ (calcd 703.26) and 352.32 $[M+2H]^{2+}/2$ (calcd 352.13). ^1H NMR (600 MHz, $CDCl_3$) δ = 8.51 (d, *J* = 7.5 Hz, 1 H, H_{ar}), 7.93 (m, 2 H, H_{ar}), 7.62 (m, 1 H, H_{ar}), 7.38 (m, 1 H, H_{ar}), 7.22 (m, 1 H, H_{ar}), 4.28 (s, 3 H, $-NCH_3$), 4.22 (m, 5 H, $-CH_2-$ and $-OCH_2-$), 1.35 (t, *J* = 7.0 Hz, 6 H, $-CH_3$). ^{13}C NMR (150.9 MHz, $CDCl_3$) δ = 151.70 ($-C_{ar}$), 151.35 ($-C_{ar}$), 151.11 ($-C_{ar}$), 149.13 ($-C_{ar}$), 148.99 ($-C_{ar}$), 142.63 ($-C_{ar}$), 137.35 ($-C_{ar}$), 136.99 ($-C_{ar}$), 136.88 ($-C_{ar}$), 136.53 ($-C_{ar}$), 135.87 ($-C_{ar}$), 127.73 ($-CH_{ar}$), 127.49 ($-CH_{ar}$), 126.57 ($-CH_{ar}$), 125.17 ($-CH_{ar}$), 109.89 ($-CH_{ar}$), 109.88 ($-CH_{ar}$), 62.91 ($-OCH_2-$), 42.09 ($-CH_2-$), 32.83 ($-NCH_3-$), 16.37 ($-CH_3$). ^{31}P NMR (162 MHz, $CDCl_3$): δ = 10.29. Anal. Calcd for $C_{35}H_{40}N_6O_6P_2$ 3.2 MeOH: C, 56.98; H, 6.61; N, 10.44. Found: C, 56.95; H, 6.26; N, 10.46%.

Diethyl [Methanediylbis[(1-methyl-1*H*-benzimidazole-5,2-diyl)pyridine-6,2-diyl]]bis[hydrogen (phosphonate)], H_2L^{POEt}. The synthesis of H_2L^{POEt} was performed as for H_2L^{P3OEt}, starting from **5b** (130 mg, 0.2 mmol). The crude product was purified by chromatography (silica gel, MeCN, NH_4OAc, 100→ 80/20 v/v) and gave the desired product as a brown solid (75 mg, 62%). ESI-MS: *m*/*z* = 647.30 $[M+H]^+$ (calcd 647.19) and 324.29 $[M+2H]^{2+}/2$ (calcd 324.10). ^1H NMR (600 MHz, MeOD) δ = 8.19 (d, *J* = 7.5 Hz,

Scheme 1. Synthesis of the Ligands



1 H, H$_{ar}$), 7.99 (m, 2 H, H$_{ar}$), 7.57 (s, 1 H, H$_{ar}$), 7.51 (d, J = 7.5 Hz, 1 H, H$_{ar}$), 7.29 (d, J = 7.5 Hz, 1 H, H$_{ar}$), 4.29 (s, 3H, NCH$_3$), 4.04 (m, 3 H, $-$CH$_2-$ and $-$OCH$_2-$), 1.24 (t, J = 7.0 Hz, 3 H, $-$CH$_3$). NMR (150.9 MHz, CDCl$_3$) δ = 160.87 ($-$C$_{ar}$), 159.49 ($-$C$_{ar}$), 150.76 ($-$C$_{ar}$), 139.63 ($-$C$_{ar}$), 138.26 ($-$C$_{ar}$), 136.13 ($-$C$_{ar}$), 130.87 ($-$C$_{ar}$), 128.63 ($-$CH$_{ar}$), 127.32 ($-$CH$_{ar}$), 126.05 ($-$CH$_{ar}$), 118.39 ($-$CH$_{ar}$), 112.41 ($-$CH$_{ar}$), 62.27 ($-$OCH$_2-$), 42.81 ($-$CH$_2-$), 33.78 ($-$NCH$_3-$), 17.13 ($-$CH$_3$). ^{31}P NMR (162 MHz, CDCl$_3$): δ = 6.39. Anal. Calcd for $C_{31}H_{30}N_6O_6P_2Na_2$ 2.3NH$_4$OH: C, 48.28; H, 5.42; N, 15.08. Found: C, 48.18; H, 5.69; N, 15.20%.

Results and Discussion

Synthesis of the Ligands. The synthesis of the H$_i$**L**$^{\mathbf{Py}}$ ligands essentially follows the same route as for H$_2$**L**$^{\mathbf{Cx}}$ (Scheme 1). The diethoxy-phosphoryl substituent was grafted in the 6-position of the pyridine-2-carboxylic acid ethyl ester after activation into its *N*-oxo form, followed by addition of sodium diethylphosphonate.[38] Then, 6-(diethoxy-phosphoryl)-pyridine-2-carboxylic acid ethyl ester could be selectively hydrolyzed into its carboxylic form **2a**, under basic conditions. In fact, ethylpicolinate is not hydrolyzed under mild experimental conditions so that only **2a** was isolated after acido-basic treatment.

For the formation of the benzimidazole rings, we initially followed the strategy previously applied in our laboratory, which consists in a modified double Philips coupling between 4,4′-methanediylbis(*N*-methyl-2-nitroaniline) and a picolinic acid derivative, followed by cyclization under reductive conditions. However, the coupling reaction failed with **2a** so that we turned to another synthetic pathway: 4,4′-methanediyl-bis(*N*-methylbenzene-1,2-diamine) was first reduced to *N*-methyl-1,2-phenylenediamine (**1b**) and this product was reacted with **2a** in polyphosphoric acid at 210 °C for 5 h, to directly provide the expected ligand H$_4$**L**$^{\mathbf{P}}$ with reasonable yield. The synthesis of the mono phosphoester-containing ligands was somewhat more complicated since the phosphoester functions had to be introduced after the condensation in presence of polyphosphoric acid to avoid hydrolysis. In a first attempt, we applied the same synthetic pathway than the one described for H$_4$**L**$^{\mathbf{P}}$: the aromatic cycle was obtain in one step, by condensation of **1a** with picolinic acid, then the nitrogens of the pyridine ring were converted to the *N*-oxo form, but the reaction with sodium diethylphosphonate failed. The successful alternative strategy implied introducing a chloro substituent in the ortho position of the pyridine ring of **2b**. After formation of the bis(benzimidazole) ring (**3a**, during which part of the

---

(38) Redmore, D. *J. Org. Chem.* **1970**, *35*, 4114.

Table 1. Acidity Constants of the Ligands in KCl 0.1 M, at 298 K

	H_2L^{POEt}	H_2L^{P3OEt}	H_4L^{P3}	H_4L^{P}	H_2L^{C3a}
pK_{a1}	3.3 (2)	2.6 (6)	}4.6 (2)	n.a.	}3.5 (1)[b]
pK_{a2}	4.1 (2)	3.9 (6)		n.a.	
pK_{a3}	7.1 (2)	6.1 (2)	4.2 (1)	n.a.	3.2 (1)
pK_{a4}	}17.5 (1)[c]	8.2 (3)	4.6 (1)	n.a.	4.3 (1)
pK_{a5}		n.a	}14.3 (1)[d]	n.a.	7.6 (1)
pK_{a6}	10.9 (1)	n.a		n.a.	10.3 (1)
pK_{a7}			9.0 (1)	10.1 (1)	
pK_{a8}			12.5 (1)	12.0 (1)	

[a] From ref 23. [b] pK_{a1} + pK_{a2}. [c] pK_{a4} + pK_{a5}. [d] pK_{a5} + pK_{a6}.

chlorine converted to alcohol, thus reducing the yields), the chlorines were converted to phosphodiester groups thanks to a palladium coupling reaction. Two intermediates (**5a** and **5b**) have been obtained thanks to this strategy, one of them bearing the poly(oxyethylene) arm grafted on the benzimidazole ring by a Mitsunobu reaction. The phosphodiester functions were finally hydrolyzed into monophosphoester groups ($H_2\mathbf{L}^{\mathbf{POEt}}$ and $H_2\mathbf{L}^{\mathbf{P3OEt}}$) or completely hydrolyzed to provide the diphosphonic acid $H_4\mathbf{L}^{\mathbf{P3}}$ ligand.

Acido-Basic Properties of the Ligands. Acidity constants of the new ligands were determined by UV−visible spectrophotometric titrations in the spectral range 200−500 nm and pH range 1.2 to 13, except for ligand $H_4\mathbf{L}^{\mathbf{P}}$ which was insufficiently soluble in acidic medium so that only the two most basic acidity constants could be determined. The evolution of the experimental spectra of $H_4\mathbf{L}^{\mathbf{P3}}$ and $H_2\mathbf{L}^{\mathbf{P3OEt}}$, recalculated spectra, and distribution diagrams are given in Figures S1 to S7 (Supporting Information). Experimental data were fitted with Specfit to the following set of equations with $n = 8$ for of $H_4\mathbf{L}^{\mathbf{P3}}$ and $n = 6$ for $H_2\mathbf{L}^{\mathbf{POEt}}$ and $H_2\mathbf{L}^{\mathbf{P3OEt}}$ (Table 1).

$$(H_n\mathbf{L}^{PX})^{4+} \leftrightharpoons (H_{n-1}\mathbf{L}^{PX})^{3+} + H^+ \qquad pK_{a1} \qquad (1)$$

$$(H_{n-1}\mathbf{L}^{PX})^{3+} \leftrightharpoons (H_{n-2}\mathbf{L}^{PX})^{2+} + H^+ \qquad pK_{a2} \qquad (2)$$

$$(H_{n-2}\mathbf{L}^{PX})^{2+} \leftrightharpoons (H_{n-3}\mathbf{L}^{PX})^{+} + H^+ \qquad pK_{a3} \qquad (3)$$

$$(H_{n-3}\mathbf{L}^{PX})^{+} \leftrightharpoons (H_{n-4}\mathbf{L}^{PX}) + H^+ \qquad pK_{a4} \qquad (4)$$

$$(H_{n-4}\mathbf{L}^{PX}) \leftrightharpoons (H_{n-5}\mathbf{L}^{PX})^{-} + H^+ \qquad pK_{a5} \qquad (5)$$

$$(H_{n-5}\mathbf{L}^{PX})^{-} \leftrightharpoons (H_{n-6}\mathbf{L}^{PX})^{2-} + H^+ \qquad pK_{a6} \qquad (6)$$

$$(H_{n-6}\mathbf{L}^{PX})^{2-} \leftrightharpoons (H_{n-7}\mathbf{L}^{PX})^{3-} + H^+ \qquad pK_{a7} \qquad (7)$$

$$(H_{n-7}\mathbf{L}^{PX})^{3-} \leftrightharpoons (\mathbf{L}^{PX})^{4-} + H^+ \qquad pK_{a8} \qquad (8)$$

The $H_4\mathbf{L}^{\mathbf{P3}}$ ligand possesses 8 protonation sites: four phosphonic acid protons (pK_{a1}−pK_{a2} and pK_{a5}−pK_{a6}), two pyridinium (pK_{a3}−pK_{a4}) and two imidazolium moieties (pK_{a7}−pK_{a8}), whereas ligands $H_2\mathbf{L}^{\mathbf{POEt}}$ and $H_2\mathbf{L}^{\mathbf{P3OEt}}$ have only two phosphonic acid protons so that pK_{a5} and pK_{a6} are then referring to the two imidazolium moieties. The spectra of $H_2\mathbf{L}^{\mathbf{P3OEt}}$ are heavily correlated so that only four pK_a values could be extracted. The acidity constants of $H_4\mathbf{L}^{\mathbf{P3}}$ were assigned by comparison with $H_2\mathbf{L}^{\mathbf{C3}}$,[23] which has a similar structure but for the carboxylic acid functions; its pK_a values are somewhat higher compared to the carboxylic acid ligand, in particular with respect to the dissociation of the benzimidazolium (ΔpK_a = 1.4 and 2.2). In addition, the first pK_a values, corresponding to the sum of the first two carboxylic acid or phosphonic acid dissociation constants, also differ by 1 order of magnitude. On the other hand, these values are smaller compared to the ones for ligands bearing phosphonic acid monoester moieties (pK_{a1} + pK_{a2} ≈ 6.5−7.4), in line with the larger electron-withdrawing character of the phosphonic ester. The same observation is made for the values corresponding to pyridinium acidity constants, with an increased basicity related to the electrostatic stabilization due to the presence of phosphonic ester compared to phosphonic or carboxylic acid. Finally, acidity constants of the $H_2\mathbf{L}^{\mathbf{POEt}}$ ligand are more basic than the ones of $H_2\mathbf{L}^{\mathbf{P3OEt}}$, pointing to the influence of the poly(oxyethylene) arms. The distribution diagram for $H_4\mathbf{L}^{\mathbf{P3}}$, Supporting Information, shows that the major species at physiological pH are $(H_2\mathbf{L}^{\mathbf{P3}})^-$ + $(H_3\mathbf{L}^{\mathbf{P3}})^{2-}$ (since the pK value found corresponds to pK_{a5} + pK_{a6}) accounting for 73%; there is also a non negligible amount of $H_4\mathbf{L}^{\mathbf{P3}}$ (26%), and traces of $(H\mathbf{L}^{\mathbf{P3}})^{3-}$ (1%). For $H_2\mathbf{L}^{\mathbf{POEt}}$, two main species are present, $(H_3\mathbf{L}^{\mathbf{POEt}})^+$ (34%) bearing one phosphonate moiety and $(H_3\mathbf{L}^{\mathbf{POEt}})^{2+}$ (66%, see Figure S8, Supporting Information).

Table 2. Mass Spectrometric Data for H_2L^{POEt} and Stoichiometric Ln:H_2L^{POEt} 2:3 Solutions in Water/Acetonitrile 90/10 (Ln = La, Eu, Gd, Tb)[a]

	m/z (obs)	*m/z* (calcd)	species
L^{POEt}	324.10	324.10	$[L^{POEt} + 2H]^{2+}$
	647.17	647.19	$[L^{POEt} + H]^{+}$
	669.20	669.18	$[L^{POEt} + Na]^{+}$
La	1106.69	1106.67	$[La_2(L^{POEt})_3 + 2H]^{2+}$
	1117.70	1117.66	$[La_2(L^{POEt})_3 + H + Na]^{2+}$
	1128.72	1128.65	$[La_2(L^{POEt})_3 + 2\,Na]^{2+}$
Eu	1119.76	1119.18	$[Eu_2(L^{POEt})_3 + 2H]^{2+}$
	1130.26	1130.17	$[Eu_2(L^{POEt})_3 + H + Na]^{2+}$
	1141.27	1141.16	$[Eu_2(L^{POEt})_3 + 2\,Na]^{2+}$
Gd	1125.49	1125.18	$[Gd_2(L^{POEt})_3 + 2H]^{2+}$
	1136.48	1136.17	$[Gd_2(L^{POEt})_3 + H + Na]^{2+}$
Tb	1126.98	1126.68	$[Tb_2(L^{POEt})_3 + 2H]^{2+}$
	1137.98	1137.68	$[Tb_2(L^{POEt})_3 + H + Na]^{2+}$
	1149.00	1148.67	$[Tb_2(L^{POEt})_3 + 2\,Na]^{2+}$

[a] Observed and calculated values refer to the most abundant peak.

Formation of the Binuclear Helicates. Formation of the helicates in water was detected by electrospray mass spectrometry in the case of $H_2\mathbf{L}^{\mathbf{POEt}}$ only. No signal was observed for $H_4\mathbf{L}^{\mathbf{P3}}$ and $H_2\mathbf{L}^{\mathbf{P3OEt}}$ while $H_4\mathbf{L}^{\mathbf{P}}$ was not soluble enough to carry out the experiment. Stoichiometric 2:3 Ln:L solutions were prepared, starting from the perchlorate salt of lanthanide (Ln = La, Eu, Gd, Tb) and the free $H_2\mathbf{L}^{\mathbf{POEt}}$ ligand 7×10^{-4} M in water containing 10% of acetonitrile to induce ionization. Several peaks were observed, corresponding to +2 charged species with hydrogen or sodium adducts (see Table 2). The shapes of these peaks as well as the isotopic distributions were found to be in agreement with theoretical predictions (Figure 1). No other species than the 2:3 chelate and

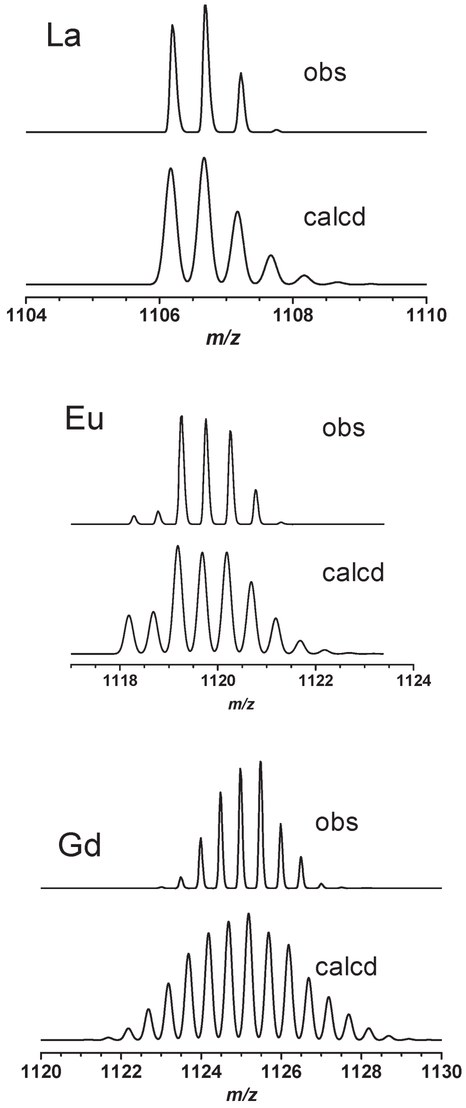


Figure 1. Parts of the observed electrospray mass spectra of solutions containing 2:3 stoichiometric amounts of Ln^{III} and H_2L^{POEt}, $[H_2L^{POEt}] = 7 \times 10^{-4}$ M in water/acetonitrile 90/10; calculated spectra are given for comparison.

its adducts were evidenced, pointing to the helicate being the major species present in solution. Therefore, substitution of a carboxylic acid in $H_2\mathbf{L}^{\mathbf{Cx}}$ by an phosphonic ester moiety in $H_2\mathbf{L}^{\mathbf{POEt}}$ does not affect the self-assembly of binuclear helicates in water. In addition, the spectra did not change at all after one week, indicating that the mono phosphoester ligand is not hydrolyzed into phosphonate in these experimental conditions.

We then turned to spectrophotometric titration of the ligands (9×10^{-6}–10^{-5} M) with lanthanide perchlorate solutions (5×10^{-3} M) up to a total concentration ratio $R = [Ln^{III}]_t/[L]_t = 4$ at pH 7.4 to quantify the interaction. Because of precipitation problems, the titration of H_4L^P could not be performed. The same problem occurred, but to a lesser extent, with H_4L^{P3} so that the spectra could be fitted only up to $R = 2$. In the case of H_2L^{P3OEt} the spectra were heavily correlated, and the results should be considered with care. On the other hand, the UV−visible spectra of H_2L^{POEt} in presence of europium perchlorate display well-defined isosbestic points at 321 and 244 nm (see Figure 2 and Figures S9 to S11 in the Supporting Information) with evolving factors analysis pointing to the presence of four absorbing species. The best model for fitting the data turned out to be the one taking 1:3, 2:3, and 2:1 species into account. Relevant stability constants are listed in Table 3.

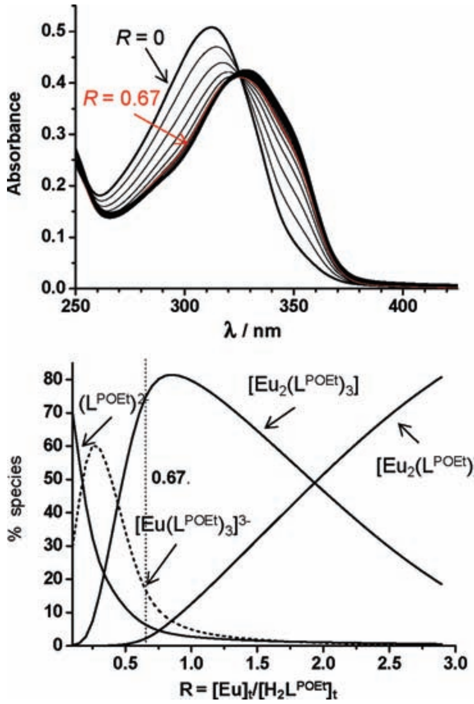


Figure 2. Top: UV−vis absorption spectra of H_2L^{POEt} 10^{-5} M in Tris-HCl 0.1 M (pH = 7.4) upon addition of increasing amounts of $Eu(ClO_4)_3$; bottom: distribution diagram drawn with the conditional stability constants reported in Table 3.

Table 3. Conditional Stability Constants for the Helicates at pH 7.4 (Tris-HCl 0.1 M) and at 298 K; Standard Deviation (2σ) are Given Within Parentheses

	$\log \beta_{13}$	$\log \beta_{23}$	$\log \beta_{21}$
$[Eu_2(\mathbf{L}^{\mathbf{C3}})_3]^a$	16.8(1)	23.4(1)	11.4(1)
$[Eu_2(\mathbf{L}^{\mathbf{P3}})_3]$	18.3^b	24.3(1)	11.2(1)
$[Eu_2(\mathbf{L}^{\mathbf{P3OEt}})_3]$	n.d.	21.4(4)	n.d.
$[Eu_2(\mathbf{L}^{\mathbf{POEt}})_3]$	17.9^b	24.6(1)	11.6(2)
$[Tb_2(\mathbf{L}^{\mathbf{POEt}})_3]$	18.3(2)	24.4(2)	11.6(1)

[a] From ref 23. [b] Value fixed, otherwise convergence was not reached.

Except for $[Eu_2(\mathbf{L}^{\mathbf{P3OEt}})_3]$ for which $\log \beta_{23}$ is smaller by two units compared to that of the other helicates, but for which spectra are heavily correlated, all the extracted stability constants are comparable within the series, indicating that replacement of the diphosphonate coordinating unit by an ethyl ester phosphonate has apparently no effect on the interaction with lanthanide ions. On the other hand, β_{23} values are 1 order of magnitude larger compared to $H_2\mathbf{L}^{\mathbf{C3}}$, indicating that introduction of phosphonate groups induces a slightly enhanced stabilization of the 2:3 complexes. Such an effect has been previously observed when comparing bipyridine bearing $PO(OH)_2$ or CO_2H substituents.[39] In addition, replacing europium by terbium has little effect on the stability constants of the complexes with $H_2\mathbf{L}^{\mathbf{POEt}}$. Finally, it seems that

---

[39] Comby, S.; Imbert, D.; Chauvin, A.-S.; Bünzli, J.-C. G.; Charbonnière, L. J.; Ziessel, R. F. *Inorg. Chem.* **2004**, *43*, 7369.

Table 4. Ligand-Centered Photophysical Data for the Ligands and for 2:3 Ln:L Solutions (Ln = Gd or Lu) in Tris-HCl 0.1 M (pH 7.4)

species	$E(^1\pi\pi^*)$/cm^{-1a}	$E(^3\pi\pi^*)$/cm^{-1b}	$\Delta E(^5D_0)^c$	$\Delta E(^5D_4)^c$
$H_2\mathbf{L}^{\mathbf{C3}d}$	26600	20900, *22100*		
$[Gd_2(\mathbf{L}^{\mathbf{C3}})_3]^d$	26450	19850, *22100*	4850	1650
$H_4\mathbf{L}^{\mathbf{P3}}$	26630	20865, *22000*		
$[Gd_2(\mathbf{L}^{\mathbf{P3}})_3]$	26040	19745, *21400*	4150	950
$H_2\mathbf{L}^{\mathbf{P3OEt}}$	29500	20000, *21000*		
$[Gd_2(\mathbf{L}^{\mathbf{P3OEt}})_3]$	28500, *28750*	20000, *21250*	4000	800
$H_4\mathbf{L}^{\mathbf{P}}$	25450	n.a.		
$[Lu_2(\mathbf{L}^{\mathbf{P}})_3]$	25000	21190, *22570*	5320	2120
$H_2\mathbf{L}^{\mathbf{POEt}}$	25500	19250		
$[Gd_2(\mathbf{L}^{\mathbf{POEt}})_3]$	24250, *26300*	19950, *21400*	4150	950

[a] Maximum of the fluorescence band at 295 K and 0-phonon component in italics, λ_{ex} = 388 nm. [b] Maximum of the phosphorescence band at 77 K (time-delay 0.05 ms, λ_{ex} = 388 nm) and 0-phonon transition (italicized). [c] Energy difference between $E_{T(0-0)}$ and the emissive levels of Eu^{III} ($^5D_0$, 17250 cm^{-1}) and Tb^{III} ($^5D_4$, 20450 cm^{-1}). [d] Taken from ref 23.

the grafting of poly(oxyethylene) arms on the benzimidazole rings has a detrimental effect on the stability, log β_{23} = 21.4 versus 24.6 for $[Eu_2(\mathbf{L}^{\mathbf{P3OEt}})_3]$ versus $[Ln_2(\mathbf{L}^{\mathbf{P3}})_3]$, much as has been previously observed between $[Eu_2(\mathbf{L}^{\mathbf{C3}})_3]$ and $[Eu_2(\mathbf{L}^{\mathbf{C1}})_3]$.[23] In conclusion, the stability of Eu^{III} helicates with the $H_4\mathbf{L}^{\mathbf{P3}}$ and $H_2\mathbf{L}^{\mathbf{POEt}}$ ligands, log $\beta_{23} \approx 24.5$, is intermediate between the stability of $[Eu_2(\mathbf{L}^{\mathbf{C3}})_3]$ (log β_{23} = 23.4) and $[Eu_2(\mathbf{L}^{\mathbf{C2}})_3]$ (log β_{23} = 25.5).[24]

Ligand-Centered Photophysical Properties. The absorption spectra of the deprotonated ligands feature two main bands located around 310 (32260 cm^{-1}), 217 nm (46080 cm^{-1}), and a shoulder at 240 nm (41670 cm^{-1}) mainly involving orbitals located on the aromatic rings and phosphoryl groups. At 295 K, UV excitation in the $\pi \rightarrow \pi^*$ absorption bands of the ligands results in a broad emission band with a maximum between 25500 and 29500 cm^{-1} (see Table 4 and Supporting Information, Table S1). The singlet state energy is the same for $H_2\mathbf{L}^{\mathbf{C3}}$ and $H_2\mathbf{L}^{\mathbf{P3}}$ but replacing the phosphonic acid moiety to yield $H_2\mathbf{L}^{\mathbf{P3OEt}}$ results in a blue shift of about 2900 cm^{-1}; on the other hand, a red shift of about 1000 cm^{-1} is observed for $H_2\mathbf{L}^{\mathbf{P}}$ and $H_2\mathbf{L}^{\mathbf{POEt}}$. The ligand-centered luminescence of the 2:3 Ln:L solutions (Ln = La, Gd, Lu) essentially displays the same features as the free ligands (Figure 3, Table 4; Supporting Information, Table S1, Figures S12 to S14), but for a bathochromic shift of about 20 nm for $[Ln_2(\mathbf{L}^{\mathbf{P3}})_3]$ and $[Ln_2(\mathbf{L}^{\mathbf{POEt}})_3]$. Upon lowering the temperature to 77 K and enforcement of a time delay the fluorescence band vanishes confirming its origin from a ligand $^1\pi\pi^*$ state. Under time-resolved conditions, only the long-lived phosphorescence is observed as large and intense bands extending from 25000 cm^{-1} to 14000 cm^{-1}. The maxima of these bands are comparable with the one observed with $H_2\mathbf{L}^{\mathbf{C3}}$ ligand, except for $H_2\mathbf{L}^{\mathbf{POEt}}$ (blue shift of about 1000 cm^{-1}). The vibrational progressions due to a ring-breathing mode are well resolved in the Gd^{III} complexes and amount to 1340 ± 50 cm^{-1} for $[Gd_2(\mathbf{L}^{\mathbf{POEt}})_3]$ and 1410 ± 50 cm^{-1} for $[Gd_2(\mathbf{L}^{\mathbf{P3}})_3]$. The presence of an ester moiety has little influence on the energy of the triplet levels so that using phosphonic acid or ester phosphonic acid coordinating moieties does not affect the photophysical properties of the ligand. The triplet state decays at 77 K are biexponential in water (see Supporting Information, Table S2), in line with the presence of two aromatic systems on the ligand, as previously observed for the helicates with the $H_2\mathbf{L}^{\mathbf{Cx}}$ ligands.[24]

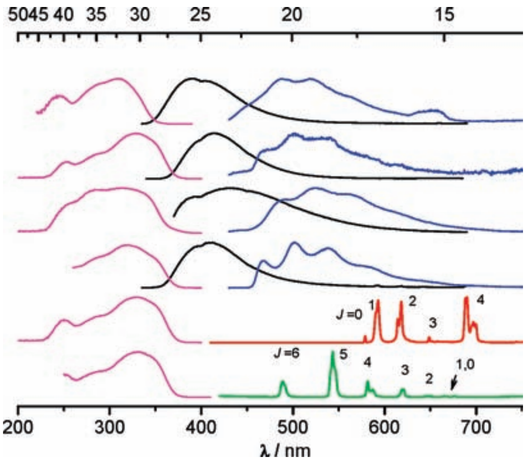


Figure 3. Absorption spectra (violet lines) of $(L^{POEt})^{2-}$ and its 2:3 complexes as well as the luminescence spectra at 295 K (black, red, green lines, $[(L^{POEt})^{2-}] = 10^{-4}$ M, Tris-HCl 0.1 M, pH = 7.4, no delay) and 77 K (blue lines, 0.05 ms delay, Tris-HCl 0.1 M containing 10% glycerol); λ_{ex} = 330 nm.

The residual $^3\pi\pi^*$ emission of the ligand is extremely faint for the complexes of Eu^{III} and Tb^{III} with ligands $H_2\mathbf{L}^{\mathbf{POEt}}$ and $H_4\mathbf{L}^{\mathbf{P}}$, pointing to substantial ligand-to-metal energy transfer. On the contrary, the triplet state emission is still dominating in the helicates with $H_2\mathbf{L}^{\mathbf{P3OEt}}$ and $H_4\mathbf{L}^{\mathbf{P3}}$. One of the main energy transfer paths usually goes through the ligand triplet state, and some empirical rules have been proposed linking the overall quantum yield to the energy gap between the 0-phonon component of the triplet state, $E_{T(0-0)}$, and the emissive level: 2500 cm^{-1} < ΔE ($^3\pi\pi^*-^5D_J$) < 3500−4000 cm^{-1} for sizable ligand-sensitized luminescence.[3] Taking the energy of the triplet state in the Gd^{III} (or Lu^{III}) complex at 77 K for $E_{T(0-0)}$, one finds ΔE between 4000 and 5300 cm^{-1} for Eu^{III} and between 800 and 2100 cm^{-1} for Tb^{III}. From these arguments, one would predict the new ligands being adequate, although not ideal, for the sensitization of Eu^{III}, but not for that of Tb^{III}.

Metal-Centered Photophysical Properties. Metal-centered luminescence has been quantified by determining the lifetimes of the emissive levels, as well as the quantum yields on 2:3 Ln:L stoichiometric solutions with $[L]_t = 10^{-4}$ M (Table 5 and Table 6). The luminescence decays for the Eu^{III} and Tb^{III} helicates are monoexponential, except with $H_2\mathbf{L}^{\mathbf{P3OEt}}$ for which a second, shorter lifetime was detected. The Eu($^5D_0$) lifetimes are long, between 1.9 and 3.2 ms, clearly pointing to the absence of coordinated water in the inner coordination sphere. Using the phenomenological equations proposed by Supkowsky and Horrocks[40] on one hand and by Beeby et al.[41] on the other hand, hydration numbers of 0.1 and −0.2 are indeed found, respectively. We note that longer lifetimes are obtained when the ligands are devoid of the poly(oxyethylene) pendants, the effect being substantial with increases of 0.6 and 1.2 ms in going from $[Eu_2(\mathbf{L}^{\mathbf{P3}})_3]$ to $[Eu_2(\mathbf{L}^{\mathbf{P}})_3]$ and from $[Eu_2(\mathbf{L}^{\mathbf{P3OEt}})_3]$ (longer lifetime) to $[Eu_2(\mathbf{L}^{\mathbf{POEt}})_3]$, respectively. This effect may be traced

---

(40) Supkowski, R. M.; Horrocks, W. d., Jr. *Inorg. Chim. Acta* **2002**, *340*, 44.

(41) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 493.

Table 5. Photophysical Data for the Eu^{III}−Centered Luminescence in Stoichiometric 2:3 (Eu:L) Solutions Tris-HCl 0.1M; $[L]_t = 10^{-4}$ M[a]

cmpnd	τ_{obs} (H_2O)/ms	τ_{obs} (H_2O)/ms (77 K)	τ_{obs} (D_2O) /ms	$Q^{Eu}{}_L$/ %	τ_{rad} /ms [b]	$Q^{Eu}{}_{Eu}$/ % [c]	η_{sens}/% [d]
$[Eu_2(\mathbf{L}^{C3})_3]$	2.2 (1), 90% 0.54 (5), 10%	2.6 (1)	4.0 (1)	11 ± 2	6.2	36	30
$[Eu_2(\mathbf{L}^{P3})_3]$	1.9 (2)	1.9(2)	2.45 (1)	6 ± 2	4.2	45	13
$[Eu_2(\mathbf{L}^{P3OEt})_3]$	2.1 (2), 75% 0.17(2), 25%	n.d.	2.8 (2)	2.5 ± 0.3	n.d.	n.d.	n.d.
$[Eu_2(\mathbf{L}^{P})_3]$	2.54 (2)	n.d.	n.d.	4.6 ± 0.7	n.d.	n.d.	n.d.
$[Eu_2(\mathbf{L}^{POEt})_3]$	3.23 (1)	3.61(2)	4.47 (1)	25 ± 2	4.8	67	37

[a] All data at 295 K unless otherwise stated; $\lambda_{exc} = 330$ nm. [b] Estimated error ± 5%. [c] Estimated error ± 10%. [d] Estimated error ± 15%.

Table 6. Photophysical Data for the Tb^{III}−Centered Luminescence in Stoichiometric 2:3 (Tb:L) Solutions Tris-HCl 0.1 M; $[L]_t = 10^{-4}$ M[a]

cmpnd	τ_{obs} (H_2O) /ms	obs (H_2O) / ms (77 K)	τ_{obs} (D_2O) /ms	$Q^{Ln}{}_L$/ %
$[Tb_2(\mathbf{L}^{P3})_3]$	n.d.	1.4(2)	n.d.	0.2 ± 0.07
$[Tb_2(\mathbf{L}^{P3OEt})_3]$	0.46 (1), 85% 0.03(2), 15%	n.d.	1.36 (1)	1.6 ± 0.6
$[Tb_2(\mathbf{L}^{P})_3]$	0.22 (5)	n.d.	n.d.	2.7 ± 0.4
$[Tb_2(\mathbf{L}^{POEt})_3]$	0.13 (1)	3.29(1)	0.18 (1)	2.5 ± 0.4

[a] All data at 295 K unless otherwise stated; $\lambda_{exc} = 330$ nm.

back to the conformational lability of the pendants which contribute to non-radiative deactivation. A similar effect, although far less pronounced, has been observed when going from $[Eu_2(\mathbf{L}^{C1})_3]$ (2.43 ms) to $[Eu_2(\mathbf{L}^{C3})_3]$ (2.20 ms).[23] Comparing the nature of the coordinating tridentate unit, data reported in Table 5 point to a favorable effect when the phosphonate unit is replaced with a phosphoester one, in particular in absence of poly(oxyethylene) pendant; we tentatively trace this back to the larger hydrophilicity of the phosphonate unit which could generate more H-bonding with proximate water molecules. Finally, the $Eu(^5D_0)$ lifetimes appear to have only a slight temperature dependence, excluding substantial vibronic-assisted contributions to non-radiative deactivation.

The case of the Tb^{III} helicates is quite different, with very short lifetimes, in the range 0.1 to 0.5 ms at room temperature, which increase substantially at 77 K, up to 3.3 ms for $[Tb_2(\mathbf{L}^{POEt})_3]$. This is typical of vibronically assisted back transfer processes taking place in these helicates in view of the small energy gap (Table 4); in this respect the new Tb^{III} helicate have a behavior which is quite similar to the ones with the $H_2\mathbf{L}^{Cx}$ ligands. As a consequence, the phenomenological equations for calculating the hydration number cannot be applied since deactivation by O−H vibrators is not the main actor of non-radiative transitions.

The biexponential decays observed for $[Ln_2(\mathbf{L}^{P3OEt})_3]$ (Ln = Eu, Tb) are consistent with β_{23} for this species being 2 orders of magnitude smaller compared to the other helicates (Table 3). At the concentrations used for the photophysical measurements, the proportion of helicate in solution calculated from the reported value of β_{23} is 85%, which is perfectly in line with the observation of a short lifetime corresponding to populations of 15−25%. Given the experimental uncertainties on both β_{23} because of correlated spectra and on the mathematical decomposition of the luminescence decay, the agreement between luminescence and spectrophotometric data is good, as was previously documented in the case of $[Eu_2(\mathbf{L}^{C3})_3]$.[23] The minor species obviously correspond to a heavily hydrated species with q estimated to be around 6; therefore it could correspond to a tricoordinate Eu^{III} ion, either by one ligand strand or by several partly coordinated ones.

As expected from the above discussion, quantum yields for Eu^{III} helicates are substantially larger than those of the Tb^{III} helicates. Compared to $H_2\mathbf{L}^{C3}$ ($Q^{Eu}{}_L = 11 \pm 1\%$ and $Q^{Tb}{}_L = 0.34 \pm 0.04\%$), replacement of the carboxylate groups by phosphonate ones results in a decrease in quantum yield by a factor 2, approximately. The situation for the ligands devoid of poly(oxyethylene) pendants, which have to be compared to $H_2\mathbf{L}^{C1}$ ($Q^{Eu}{}_L = 24 \pm 2\%$ and $Q^{Tb}{}_L = 1.2 \pm 0.2\%$),[11] is different in that $Q^{Eu}{}_L$ decreases by a factor ≈5 while $Q^{Tb}{}_L$ increases by a factor ≈2. Among the helicates containing phosphoester groups, $[Eu_2(\mathbf{L}^{POEt})_3]$ has by far the largest quantum yield, 25%, that is as large as that of $[Eu_2(\mathbf{L}^{C1})_3]$. This ligand also sensitizes the luminescence of Tb^{III}, but to an extent which remains modest and which is comparable to the effect generated by the other ligands, barring $H_4\mathbf{L}^{P}$.

The overall quantum yield depends on two factors, the efficacy of the energy transfer from the ligand to the metal ion (η_{sens}) and the intrinsic quantum yield (eq 9), $Q^{Ln}{}_{Ln}$, which reflects the ratio of radiative to non-radiative deactivation processes going on in the coordination sphere. In view of the faint absorbances of the f-f transition, $Q^{Ln}{}_{Ln}$ is difficult to measure experimentally. However it can be estimated from the observed and radiative lifetimes. In turn, for Eu^{III}, the radiative lifetime can be simply estimated from the emission spectrum (eq 10):[3,42]

$$Q^{Eu}_{L} = \eta_{sens} \times Q^{Eu}_{Eu} = \eta_{sens} \times (\tau_{obs}/\tau_{rad}) \qquad (9)$$

$$1/\tau_{rad} = A_{MD,0} \times n^3 \times (I_{tot}/I_{MD}) \qquad (10)$$

Relevant data for the two most luminescent Eu^{III} chelates are listed in Table 5. The radiative lifetimes for $[Eu_2(\mathbf{L}^{P3})_3]$ and $[Eu_2(\mathbf{L}^{POEt})_3]$ are about 1.5-fold shorter than for the carboxylates $[Eu_2(\mathbf{L}^{Cx})_3]$ (x = 1−3: 6.2−6.9 ms) resulting in $Q^{Eu}{}_{Eu}$ values which are substantially larger, particularly for $[Eu_2(\mathbf{L}^{POEt})_3]$ (67% compared to an average of 36% for the dicarboxylates). On the other hand, the sensitization efficiencies for $[Eu_2(\mathbf{L}^{POEt})_3]$ and $[Eu_2(\mathbf{L}^{C3})_3]$ (30%) are comparable, so that the improved quantum yield of the former (25% versus 11%) essentially arises from the larger value of $Q^{Eu}{}_{Eu}$, which could reflect the larger hydrophobicity of the

---

(42) Bünzli, J.-C. G.; Eliseeva, S. V. In Basics of Lanthanide Photophysics. In *Springer Series on Fluorescence, Vol. 7, Lanthanide Spectroscopy, Materials, and Bio-applications*; Hänninen,P., Härmä,H., Eds.; Springer Verlag: Berlin, 2010; 2, in press.

phosphonate groups compared to carboxylates.[43] Conversely, the poorer emissive properties of [$Eu_2(\mathbf{L}^{\mathbf{P3}})_3$] stem essentially from a much smaller sensitization efficiency, 13%, compared to [$Eu_2(\mathbf{L}^{\mathbf{C3}})_3$], while the 4-fold decrease in $Q^{Eu}{}_{L}$ compared to [$Eu_2(\mathbf{L}^{\mathbf{POEt}})_3$] is due to both a decrease in energy transfer efficacy (2.8-fold) and a decrease in $Q^{Eu}{}_{Eu}$ (1.9-fold).

Conclusion

In this study, we have investigated the influence of substituting a carboxylate moiety with either a phosphonate or a phosphoester group ($H_4\mathbf{L}^{\mathbf{P}}$ and $H_2\mathbf{L}^{\mathbf{POEt}}$ vs $H_2\mathbf{L}^{\mathbf{Cx}}$ ligands), as well as grafting a poly(oxyethylene) arm on the benzimidazole ring ($H_4\mathbf{L}^{\mathbf{P3}}$ and $H_2\mathbf{L}^{\mathbf{P3OEt}}$ vs $H_4\mathbf{L}^{\mathbf{P}}$ and $H_2\mathbf{L}^{\mathbf{POEt}}$ ligands). Whatever the tridentate coordinating unit is, the self-assembly process of three ligand strands with two lanthanide ions leads to the formation of stable triple-stranded binuclear helicates. Regarding the solubility of the latter, we have previously shown that the grafting of poly(oxyethylene) arms enhances the solubility of the chelates with $H_2\mathbf{L}^{\mathbf{C2}}$ and $H_2\mathbf{L}^{\mathbf{C3}}$ compared to those with $H_2\mathbf{L}^{\mathbf{C1}}$. In the present study, chelates with $H_2\mathbf{L}^{\mathbf{P3}}$ revealed to be more soluble than with $H_4\mathbf{L}^{\mathbf{P}}$, but precipitation problems occurred in both cases. On the other hand, providing phosphonate moieties was detrimental to the solubility, while the presence of phosphoester functions induced large solubility, independently of the presence of poly(oxyethylene) pendants. As a consequence, introducing the latter is not necessary for solubility reasons if phosphoester coordinating groups are used, which is a welcome simplification in the synthetic procedure. Moreover, the presence of the pendants is somewhat detrimental to the stability of the final helicates.

With respect to photophysical properties, only one ligand proved to yield adequate sensitization efficiency of the europium luminescence. Looking at Table 4, one realizes that the simple phenomenological rule based on the energy gap between the triplet state and the Eu^{III} emissive level does not operate with the present series of helicates in that for instance both [$Eu_2(\mathbf{L}^{\mathbf{P3}})_3$] and [$Eu_2(\mathbf{L}^{\mathbf{POEt}})_3$] have the same triplet state energy but overall quantum yields differing by a factor 4. In fact a more detailed analysis, as presented above, has to be conducted to decipher the contribution of both the energy transfer efficacy and the intrinsic quantum yield which reflects the proportion of non-radiative deactivation occurring within the chelate. In turn, $Q^{Ln}{}_{Ln}$ depends on the radiative lifetime which has been found to be significantly shorter for the phosphonate helicates compared to the carboxylate ones. A shorter radiative lifetime means that emission of light can better compete with non-radiative deactivation. It also indicates that the f-f transitions are “less forbidden”, that is, that the 4f orbitals are less pure owing to more mixing with ligand orbitals, a phenomenon made easier if the symmetry of the coordination sphere is lower. This is the case for the phosphonato helicates compared to the dicarboxylato chelates, as ascertained by the overall shape of the emission spectra.

In conclusion, the $H_i\mathbf{L}^{\mathbf{Py}}$ series of ligands acts similarly to $H_2\mathbf{L}^{\mathbf{CX}}$ regarding the coordination of the lanthanide ion, and substitution of the carboxylates by a phosphoester substituent on the pyridine ring is a valuable alternative to the grafting of a poly(oxyethylene) arm with respect to solubility. Moreover, the photophysical properties of [$Eu_2(\mathbf{L}^{\mathbf{POEt}})_3$] are compatible with the stringent requirements of a lanthanide luminescent bioprobe, a fact that we are now testing for live cell imaging. Preliminary results indicate that [$Eu_2(\mathbf{L}^{\mathbf{POEt}})_3$] indeed penetrates into live HeLa cells.

Acknowledgment. This work is supported through a grant from the Swiss National Science Foundation (200020-119866). We thank Dr. Song Bo, Frédéric Gumy, and Frédéric Thomas for their help in cell imaging experiments, luminescence measurements, and ligand synthesis, respectively. J.-C.B. thanks the World Class University (WCU) program from the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (Grant R31-10035).

Supporting Information Available: Figures S1−S11 show the UV−visible titration of the ligands and corresponding spectra and distribution diagrams, Figure S12−S14 displays luminescence spectra of the different ligands and their complexes (10 pages). Table S1 reports the ligand-centered photophysical data of these complexes and Table S2 the lifetimes of the triplet stated of the ligands and their complexes with Lu, La or Gd. This material is available free of charge via the Internet at http://pubs.acs.org.

---

(43) Rohovec, J.; Lukes, I.; Hermann, P. *New J. Chem.* **1999**, *23*, 1129.