Self-assembly of a helical zinc-europium complex: speciation in aqueous solution and luminescence

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INTRODUCTION

Helical structures have attracted chemists’ attention when Linus Pauling published a seminal series of papers at the beginning of the 1950’s dealing with the secondary structures of proteins (Pauling et al., 1951). The demonstration that helical structures can also be engineered at the molecular level by taking advantage of stereochemical properties of metal ions had to wait until 1987 when Jean-Marie Lehn isolated 3:2 CuI:L double-stranded helical complexes that he named helicates, where L is an oligo-bipyridine ligand (Lehn et al., 1987). A few years later, Claude Piguet applied the same concept to trivalent lanthanide ions and successfully self-assembled the first LnIII dinuclear triple-stranded helicate, [Eu2(L3)3]6+ (*Scheme 1*), the crystal structure of which evidences a stabilization of the molecular architecture by π–π stacking interactions between the ligand strands (Bernardinelli et al., 1992). The two 9-coordinate metal ions lie on a pseudo-C3 axis of symmetry (**Figure 1**); in solution the average symmetry of the edifice is D3h on NMR time scale (Piguet et al., 1993). This initial work paved the way for the development of several series of lanthanide polynuclear and polymetallic complexes including heterobimetallic nd-4f (Piguet et al., 1995a) and 4f-4f* (André et al., 2004) chelates, as well as tri- and tetranuclear homometalic and heterometallic entities (Piguet et al., 2000; Piguet and Bünzli, 2010). Interestingly, the helicates are quite stable in solution despite large Coulomb repulsion between two neighboring cations which lie about 9 Å apart; careful thermodynamic considerations for 3d-4f and 4f-4f-4f helicates indeed show that the cation-cation repulsive energy (≈700 kJ·mol\(^{-1}\)) is largely compensated by favorable solvation energy (Canard and Piguet, 2007). Furthermore, soluble helicates [Lu2(L2)3] can be assembled in water and are highly stable, with logβ3 on the order of 26–30 (Elhabiri et al., 1998). Crystal structures revealed triple-stranded helicates with 9-coordinate metal ions (Ln = Eu, Tb) well-imbedded into the edifice and displaying interesting photophysical properties (Elhabiri et al., 1998; Gonçalves e Silva et al., 2002). Subsequent molecular engineering led to the series of the more water-soluble [Lu2(L2)3] helicates and their bioconjugates which proved to be adequate luminescent bioprobes for live cell staining (Song et al., 2008; Chauvin et al., 2013) and for specific detection of biomarkers expressed by cancerous cells (Fernandez-Moreira et al., 2010).

One fascinating aspect of the polymetallic helical molecular edifices is the possibility of controlling the optical and/or magnetic properties of one ion by the other, through communication along the pseudo C3 axis. Examples are the tuning of the spin-crossover temperature in [FeLn(L1)3]3+ (Piguet et al., 1995b; Edder et al., 2000, 2001), (*Scheme 1*) or the lengthening of the excited state lifetimes of NdIII and YbIII in [CrLn(L1)3]6+ (Torelli et al., 2005). Such tunability would be of great help in the design of specific biosensors and stains, especially that [EuZn(L2)3]3+ proved to be quite luminescent in water with a quantum yield of 15% (Edder et al., 1997; Piguet and Bünzli, 2010). Bioprobes need to be water soluble and amenable to bioconjugation; unfortunately, helicates with the carboxylic acid derivatives HL3 and HL4 do not show enough water solubility for this purpose. In this paper, we apply to HL3 and HL4 the successful strategy used in going from H2LC1 to H2LC2 in the hope of gaining access to luminescent and soluble 3d-4f helicates with ligands HL3 and HL4. More specifically, and as a first step toward engineering bioprobes based...
strategy based on a modified Phillips reaction for the formation of the benzimidazole rings. However, the diethylamino groups are replaced by 2-[2-(2-methoxyethoxy)ethoxy]-N-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]-ethanamine groups. Following our previous work (Deiters et al., 2009), the latter have been grafted on the key intermediate (6), the synthesis of which is depicted on Scheme 2 while the routes for accessing ligands HL 5 and HL 6 are summarized on Scheme 3. Regarding sulfonation, the absence of directional electronic effects favoring electrophilic substitution in the ditopic ligands and the large number of carbon atoms amenable to sulfonation implies that the corresponding group has to be inserted in one of the starting building blocks, namely (3).

Intermediate (6) was prepared in 7 steps and 23% yield from commercially available 2-picoline, benzylamine and triethylene glycol monomethyl ether (TEGOMe). The first two steps involve the synthesis of 2-[2-(2-methoxyethoxy)ethoxy]-N-[2-[2-(2-methoxyethoxy)ethoxy]ethyl]-ethanamine (2): two TEGOMe arms are grafted on the primary benzylamine by reaction with BrTEGOMe in presence of a weak base (reaction i, Scheme 2) followed by selective and quantitative cleavage of the benzylamino group by continuous flow hydrogenation (reaction ii). Two other steps are necessary for preparing (3) from 2-picoline by treatment with oleum and subsequent oxidation with permanganate, according to a previously described procedure (Delarge, 1965). The sulfonate function of (3) is then activated by chlorination (reaction iii), in presence of a PCl 5/POCl 3 mixture to give the corresponding sulfonyl chloride (4). Coupling between (2) and (4) is subsequently achieved under standard conditions (reaction iv) to yield sulfonamide (5). Finally, oxidation of the methyl group of (5) into a carboxy group conducted in presence of an excess of SeO 2 in refluxing pyridine affords synthon (6) in almost quantitative yield (reaction v).

Preparation of the segmental ligands HL 5,6 is divided in fourth steps. Firstly, sequential Phillips coupling reactions (reactions vi/vii, Scheme 3) with successive introduction of different pyridine arms, (6), (7), and (9), are performed on the central dinitro, bis-(N-methylamino)diphenylmethane synthon (8). Secondly, the resulting bis-amides (11) and (14) are reduced (reactions vii, ix) in presence of a large excess of iron to form the bis(benzimidazole) intermediates (12) and (15). Finally, basic hydrolysis of both ethylenes functions (reactions x) leads to the targeted segmental ligands with overall yields (steps iii-x in Schemes 2, 3) of 18.8 and 18.4% for HL 5 and HL 6, respectively.

SPECIATION IN SOLUTION
Conditional stability constants of both homometallic (M = Zn II, La III, Eu III, Lu III) and heterometallic (M 1 = Zn II, M 2 = La III, Eu III, Lu III) complexes have been determined in Tris-HCl 0.1 M (pH 7.4) at 295 K by spectrophotometric titrations of the ligands (1.43 × 10 -5 M for HL 5 and 1.62 × 10 -5 M for HL 6, corresponding to an absorbance of about 0.5) with concentrated solutions of the metal perchlorates: 2.5–5.0 × 10 -3 M for homometallic titrations and 2 × 10 -4 M for each cation in the case of heterometallic titrations, in view of the poorer solubility of the hetero species. Titrations were performed batch wise for 20–25 [M i ]/[HL i ], (i = 5.6) ratios ranging between 0 and 2 on such compounds, complex formation in the Eu III-Zn II-HL 6 system and associated luminescent properties are investigated. Spectroscopically silent Zn II has been chosen because it allows studying the coordination environment of the Eu III ion without interference from the M II ion.

RESULTS AND DISCUSSION
LIGAND SYNTHESIS
The underlying principle of the synthesis of HL 5 and HL 6 is the same as the one adopted for preparing ligands L 1,2 (Edder et al., 2000), and HL 3 (Edder et al., 1997), namely a multistep
SCHEME 2 | Synthesis of the new precursor (6): (i) \( \text{CH}_3(OCH_2CH_2)_3\text{Br} \) (2.2 eqs), \( \text{Na}_2\text{CO}_3 \) (2.2 eqs), \( \text{CH}_3\text{CN} \) (62°C, 76 h); (ii) \( \text{Pd/C}, \text{H}_2 \) (70 bar, 45°C); (iii) \( \text{PCl}_5 \) (1.5 eqs), \( \text{POCl}_3 \) (reflux, 3 h); (iv) \( \text{[CH}_3(OCH_2CH_2)_3\text{]}_2\text{NH} \) (1.2 eqs), \( \text{NEt}_3 \) (5.0 eqs), \( \text{CH}_2(OCH_2CH_2)_3\text{Br} \) (2.2 eqs), \( \text{Na}_2\text{CO}_3 \) (2.2 eqs), \( \text{CH}_3\text{CN} \) (62°C, 76 h); (v) \( \text{SeO}_2 \) (4.5 eqs); pyridine (reflux, 23 h).

SCHEME 3 | Synthesis of HL\(^5\) and HL\(^6\): (vii) (6), (7), or (9) (1.0 eq.), \( \text{SOCl}_2 \) (10 eqs), \( \text{DMF} \) (0.5 eq.), \( \text{CH}_2\text{Cl}_2 \) (reflux, 2 h); (viii) (8) (0.9 eq.), (10) (0.6 eq.) or (13) (0.4 eq.), \( \text{NEt}_3 \), \( \text{CH}_2\text{Cl}_2 \) (reflux, 16 h); (ix) \( \text{EtOH/H}_2\text{SO}_4 \) (reflux, 4 h); (x) (12) or (15) (1.0 eq.), \( \text{Fe} \) (30 eqs), \( \text{EtOH/H}_2\text{O/HCl} \) (reflux, 16 h); (xi) \( \text{EtOH/H}_2\text{SO}_4 \) (reflux, 4 h); (xii) (12) or (15) (1.0 eq.), \( \text{NaOH} \) (1.2 eqs), \( \text{EtOH/H}_2\text{O} \) (60°C, 16 h).
Recalculated spectra are heavily correlated (Figures S1, S2, Supplementary Material), which explains the difficulties in the fitting procedure. The corresponding conditional stability constants are listed in Table 1. In the case of ZnII, the main species at 2:3 Zn:L stoichiometric ratio is the dinuclear complex (Figure 2, top) while the 1:3 complex remains a minor species (maximum speciation: 21% at $R = 0.20$); when $R$ further increases, the 2:3 complex transforms into a 2:2 species. This behavior is in line with our previous results (Piguet and Bünzli, 2010). The tridentate-bidentate compartmental ligand HL6 is not well-suited for building triple-stranded helicates with LnIII ions (two tridentate coordination units would be required) and this is seen in the corresponding speciation diagram: the dominant species is a 1:3 complex, with a 75% speciation for $R = 0.33$ (Figure 2, middle). For this ratio, only a small quantity of the 2:3 species is present (8%). Absorbance values extracted at different wavelengths for the titrations with ZnII and EuIII (Figures S1, S2, Supplementary Material) are compatible with the initial formation of 1:3 species.

The titration with a mixture of metal ions has been conducted in a slightly different way, due to reduced solubility of the formed products, the concentration of each metal ion has been set to $2.00 \times 10^{-4}$ M only. Again fit of the data was difficult in view of the correlated spectra (Figure S3, Supplementary Material), so that the extracted data and corresponding discussion have to be taken with care. Indeed, log$\beta_{23}$ for $\left[\text{Zn}_{2}(L_{6})_{2}\right]^{2+}$ extracted from this titration amounts to 18.7(3) whereas a value of 21.7(4) was found from the homometallic titration. We think, however, that the salient features are correct: contrary to what was expected, and found for other ZnII-LnIII helicates in acetonitrile, for instance with $L^2$ (Edder et al., 2000), the 1:1:3 species is not the dominant one, accounting for only 38% of the speciation at the 1:1:3 stoichiometric ratio. Another species is present in sizeable quantity (18%), namely the ZnII 2:2 complex which is less stable than the 1:1:3 species by less than two orders of magnitude. So it seems there is competition between ZnII and EuIII for the tridentate coordination unit of $(L_{6})^{-}$. This competition is further demonstrated by an experiment in which ZnII was added to a 1:3 Eu:(L6)$^{-}$ stoichiometric solution 5.4 $\mu$M in EuIII up to an Eu:Zn ratio equal to 1. The EuIII luminescence intensity clearly decreases while ligand fluorescence centered at 450 nm increases (Figures S4, S5, Supplementary Material). Moreover, the ES-MS spectra discussed below point to other species being present in solution and the low solubility exhibited by this mixed system could well reflect the formation of polymeric (hydroxide?) species as well.

In order to substantiate the speciation determined by UV-visible titrations and, also, to determine if lighter and heavier lanthanides would lead to the same species in solution, ES-MS
high-resolution scan of which matches well the calculated isotopic distribution (Figure 3). As for neodymium, an ytterbium 1:3 species is present, as well as the 1:2 zinc complex. In both cases, no 2:2 zinc complex was identified though, contrary to UV-visible titration data; we note, however that the solvent is different and that the conditions in the spectrometer may lead to dissociation of this species.

**PHOTOPHYSICAL PROPERTIES OF THE SOLUTIONS**

Absorption spectra of ligand (L₆)⁻ and various solutions containing LnIII ions (Ln = Eu, Gd) or an equimolar EuIII/ZnII mixture are reported on Figure 4. The ligand absorption band at 319 nm can be assigned to a π → π⁺ transition involving intramolecular electron transfer from the benzimidazole units to the pyridine and carboxylic groups. This band is red-shifted to 326.5–327 nm in the solutions containing LnIII ions only while the presence of ZnII results in a slightly larger shift, to 329.5 nm. The molar absorption coefficients of the 1:3 solutions are, within experimental errors, equal to three times the molar absorption coefficient of the free ligand, while they are marginally smaller (−3.5%) for the 2:3 and 1:1:3 solutions (Table 3).

Upon excitation into the 319-nm absorption band, ligand fluorescence emission is seen as a broad feature with maximum at 466 nm (Figure 5) and the corresponding excitation spectrum matches the absorption spectrum. At 77 K and upon enforcing a 50-μs delay time, weak phosphorescence is detected with a maximum at 506 nm. For the 1:3 EuIII solution, fluorescence of the ligand is still seen, representing 37% of the total emission of the sample; this is consistent with the fact that the solution contains about 17% of free ligand (Figure 2, middle). In addition characteristic f-f emission from the Eu(5D₀) level is detected. A striking feature is that this spectrum is quite typical of a species with pseudo D₃ symmetry and is quasi identical to the one recorded for the [Eu₂(L⁵C₂₃)₃] helicate (Chauvin et al., 2008). In particular, the branching ratios expressed with respect to the intensity of the magnetic dipole transition, I(⁵D₀ → ⁷F₅)/I(⁵D₀ → ⁷F₁) and I(⁵D₀ → ⁷F₃) are very similar for the two samples (data for [Eu₂(L⁵C₂₃)₃] are between parentheses): 0.02 (0.01), 1.00 (1.00), 0.87 (0.95), 0.16 (0.13), 1.62 (1.72), and 0.05 (n.a.). The splitting of the ⁵D₀ → ⁷F₁ transition is also very similar, 170 vs. 161 cm⁻¹. These data point to luminescence arising from a coordination environment made up of 3 NNO moieties and very similar to the sites in [Eu₂(L⁵C₂₃)₃]; if some coordination were to occur through the bidentate site, then the coordination sphere would be completed by water molecules, leading to a poorly luminescent species. The solution also contains 8% of the 2:3 species, featuring two different metal ion sites (NNO)₃ and (NN)₃; the first one will give a spectrum identical to the one of the 1:3 complex, while the second will be poorly luminescent and therefore its contribution to the emission spectrum can be neglected. As an additional proof, the decay curve of the Eu(⁵D₀) emission is perfectly monoexponential, with a lifetime of 2.7 ms (2.4 ms for [Eu₂(L⁵C₂₃)₃]), confirming that emission essentially originates from very similar coordination environments. Emission spectra of solutions with stoichiometric ratios Eu:(L₆)⁻:Zn = 2:3 and Zn:Eu:(L₆)⁻ = 1:1:3 display spectra identical to the one of the 1:3 solution (Figure S6, Supplementary Material), consistent with the speciation reported in Figure 2; in particular,
the emission intensity of the heterometallic solution is weak, due to the low concentration in 1:1:3 species (38%, which translates in 19% with respect to the europium site).

Low-temperature emission spectra are presented on Figure 6 for the ligand, a GdII-containing solution and the Eu:Zn 1:1 sample. Upon enforcing a 50-μs delay time, fluorescence of the ligand almost disappears to the benefit of a phosphorescence band centered at 509 nm. In the Gd:(L6)1:1:3 sample, this band is red shifted at 525 nm and presents a vibrational structure (463, 525, 559 nm) with μ sample. Upon enforcing a 50-

| Table 2 | Main peaks observed in the ESI-MS spectra of heterometallic solutions containing ZnII, NdIII or YbIII, and (L6) in stoichiometric ratio 1:1:3 in acetonitrile/H2O/formic acid 49.5/49.5/1. |
|----------|----------|----------|----------|----------|
| Solution | Species   | m/z (exp.) | Int.     | Assignment |
| Zn:Nd:(L6)−1:3 | [NdZn(L6)3] | 808.53 | 20 | [M + 2H]2+/3 | 808.53 |
| Zn:Yb:(L6)−1:3 | [YbZn(L6)3] | 1087.73 | 25 | [M + H]2+/3 | 1087.38 |
| Zn:Eu:(L6)−1:3 | [Zn(L6)3] | 1062.38 | 35 | [M + 2Na]2+/3 | 1062.38 |

| Table 3 | Photophysical data of the free and coordinated (L6)− ligand at 298 K in Tris-HCl (pH 7.4). Energies are reported in cm−1. |
|----------|----------|----------|----------|----------|
| (L6)−     | Eu:(L6)− | Gd:(L6)− | Eu:(L6)− | Zn:Eu:(L6)− |
| 1:3       | 1:3      | 2:3      | 1:1:3    | 1:1:3    |
| E1/2π(π−π) | 31,450   | 30,630   | 30,580   | 30,630   | 30,350   |
| Logε      | 4.50      | 4.97     | 4.98     | 4.96     | 4.96     |
| E1/2(π−π)  | 21,450   | 22,350   | 22,350   | 22,450   | 21,950   |

a From absorption spectra, maximum of band envelope.
b From fluorescence spectra, maximum of band envelope.
with the largest concentration of the 2:3 (or 1:1:3) species, in 2.46–2.69 ms, the shortest figure corresponding to the solution.}
Table 4 | Observed and radiative lifetimes (τ), intrinsic and absolute quantum yields (Q) of the Eu(6D0) level, as well as apparent ligand sensitization (ηsens) for various samples in Tris-HCl 0.1 M (pH 7.4) with [([L8]−)1 = 16.2 μM, as determined at 298 K under ligand excitation (326–329 nm).

| Solution | Speciation a | τobs/μs | τrad/μs b | QEu%/ | QEu%/ | ηsens%/ |
|----------|--------------|--------|-----------|--------|--------|---------|
| Eu:[L5]−:1:3 | 1:3 75%, 2:3 8% | 2.69 ± 0.02 | 7.5 ± 0.7 | 36 ± 4 | 8 ± 1 | 22 ± 4 |
| Eu:[L5]−:2:3 | 1:3 53%, 2:3 39% | 2.46 ± 0.06 | 7.7 ± 0.8 | 32 ± 4 | 8 ± 1 | 25 ± 5 |
| Zn:Eu:[L5]−:1:1:3 | 1:1:3 38% | 2.54 ± 0.15 | 7.5 ± 0.7 | 34 ± 4 | 5 ± 1 | 15 ± 3 |

a From Figure 2
b Calculated with eq. (6).
c In frozen solution at 77 K.

complexes in which the lanthanide ion is coordinated to the tridentate chelating unit of ligand HL6 a, a judged by the crystal field splitting and other photophysical parameters which reflect the peculiar signature of the Eu(NNO)3 environment. In particular, the radiative lifetimes and intrinsic quantum yields match those of the previously reported helicates [Eu2(LC1)3] and [Eu2(LC2)3] with bis(tridentate) ligands.

On the other hand, the synthetic strategy applied for the preparation of tridentate-bidentate compartmental ligands aimed at assembling 3d-4f binuclear complexes proved to be valuable in that the ligands are obtained in reasonable yields given the number of steps needed. Moreover, the strategy can be adapted to graft other substituents on the sulfonate groups through modification of the key building block 6 (Scheme 2), so that this class of ligands represent a valuable addition to the chemistry of 3d-4f complexes.

MATERIALS AND METHODS
SYNTHESIS OF THE LIGANDS
Sulfonation of 2-picoline (Delarge, 1965) and bromination of TEGOMe (Deiters et al., 2009) have been previously reported so that these steps are not described here, except for NMR and ESI-MS characterization of (3). Substituted pyridines (7) and (9) (Li et al., 2008; Deiters et al., 2009) and bis-(N-methylamino)diphenylmethane (8) (Piguet et al., 1992) were prepared according to literature procedures.

Starting materials and general procedures
Chemicals and solvents were purchased from Fluka A.G or Aldrich. Solvents were purified by passing them through activated alumina columns from Innovative Technology Inc. (Pangborn et al., 1996). Complexes were studied in solution only. Stock solutions of lanthanides were prepared just before use in freshly boiled, doubly distilled water from the corresponding Ln(ClO4)3·xH2O salts (Ln = La, Eu, Gd, Lu; x = 2.5–4.5). These salts were prepared from their oxides (Rhône-Poulenc, 99.99% and Catalysis or Research Chemicals, Phoenix, AZ) in the usual way (Bünzli and Mabillard, 1986). The concentrations of the solutions were determined by complexometric titrations using a standardized Na2H2EDTA in urotropine buffered medium and with xylenet orange as indicator (Schwarzenbach, 1957).

N-benzyl-2-[2-(2-methoxyethoxy)ethoxy]-N-[2-(2-methoxyethoxy)ethyl]ethanamine (1)
Benzylamine (2.5 g, 23.3 mmol), 1-bromo-2-[2-(2-methoxyethoxy)ethoxy]ethane (11.59 g, 51.3 mmol) and Na2CO3 (5.44 g, 51.3 mmol) were heated and stirred in anhydrous CH3CN (30 mL) under inert atmosphere at 62°C for 76 h. After cooling, the reaction mixture was filtered and the white precipitate of Na2CO3 was washed with Et2O (about 50 mL). The resultant solution was evaporated under reduced pressure. The residue so obtained was re-dissolved in 1 hydrochloric acid solution (50 mL, 2 M) and extracted with Et2O (250 mL). The pH of the aqueous phase was then increased by addition of NaHCO3 up to saturation and the resulting solution was extracted with Et2O (3 × 250 mL). The three organic phases were combined, dried over Na2SO4, filtered, and concentrated under reduced pressure. The crude material was purified by column chromatography (silica gel, CH2Cl2/MeOH 100:0 → 97:3) to give pale yellow oil (6.32 g, 68% yield).1H NMR (400 MHz, 298 K, CD3CN) δ (ppm): 2.66 (t, J = 6.2 Hz, 4H, NCH2CH2), 3.28 (s, 6H, OCH3), 3.44 (m, 4H, 4H, CH2, NCH2CH2), 3.48–3.54 (m, 16H, OCH2), 3.67 (s, 2H, CH2), 7.24 (m, 1H, HPh1), 7.30 (m, 2H, HPh2), 7.33 (m, 3H, 2H, HPh3).13C NMR (800 MHz, 298 K, CDCl3) δ (ppm): 35.86 (NCN), 59.20 (OCH3), 59.86 (CH2), 74.24 (m, 1H, HPh1), 7.30 (m, 2H, HPh2), 7.33 (m, 3H, 2H, HPh3). ESI-MS m/z calcd for [M + H+] (found): 400.27 (400.04).

2-[2-(2-methoxyethoxy)ethoxy]-N-[2-(2-methoxyethoxy)ethoxy]ethyl]ethanamine (2)
Continuous flow hydrogenation of an ethanolic solution (0.05 M, 250 mL) of (1) (5.00 g, 12.5 mmol) was conducted with a safety H-Cube device from Thales Nanotechnology equipped with a HPLC pump under the following conditions: flow rate: 1 mL/min, catalyst cartridge: Pt/C; H2 pressure: 70 bar; temperature: 45°C. Ethanol and generated toluene were removed under reduced pressure and (2) obtained as a yellow oil was subsequently dried under vacuum (3.87 g, 100% yield). Note: one run was enough to fully convert (1) into (2) under the conditions mentioned above.1H NMR (400 MHz, 298 K, CD3CN) δ (ppm): 2.73 (t, J = 6.2 Hz, 4H, NCH2CH2), 3.30 (s, 6H, OCH3), 3.46–3.57 (m, 20H, OCH2).13C NMR (800 MHz, 298 K, CDCl3) δ (ppm): 49.30 (NCH2CH2), 70.68 (OCH2), 70.78 (OCH2), 72.06 (OCH2), 126.96 (CHPh3), 128.27 (CHPh1), 128.97 (CHPh2), 139.85 (CHPh3,quat.). ESI-MS m/z calcd for [M + H+](found): 205.27 (204.04).
59.21 (OCH 3), 70.47 (OCH 3), 70.63 (OCH 2), 70.67 (OCH 2), 72.05 (OCH 2). ESI-MS m/z calcd for [M + H + ] + (found): 310.23 (309.94).

6-methylpyridinium-3-sulfonate (3)

This compound was synthesized according to a procedure described in the literature (Delarge, 1965); NMR and ESI-MS data are however reported here for the first time. 1H NMR (400 MHz, 298 K, D2O) δ (ppm): 2.81 (s, 3H, CH3), 7.97 (d, J = 8.5 Hz, 1H, Hpy), 8.69 (d, J = 8.5 Hz, 1H, Hpy), 8.97 (s, 1H, Hpy) 13C NMR (800 MHz, 298 K, D2O) δ (ppm): 19.15 (CH3), 128.55 (CHpy), 138.35 (CHpy), 140.16 (Cpy, quat.), 142.95 (CHpy), 156.86 (Cpy, quat.). ESI-MS m/z calcd for [M+H + ] + (found): 174.02 (173.91); calcd [M + CH3CN + H + ] + (found): 215.05 (214.87).

6-methylpyridinium-3-sulfonyl chloride (4)

Synth (3) (1.00 g, 5.78 mmol) and PCl5 (1.91 g, 9.25 mmol) were suspended in POCl3 (5 mL) and stirred at room temperature for 48 h. Then, POC13 was rotor-evaporated and the residue was dissolved in CH2Cl2 (25 mL). Crushed ice (about 5 g) was slowly added. After separation, the aqueous phase was extracted with CH2Cl2 (3×). The combined organic phases were washed with dilute NaHCO3 solution, dried over Na2SO4, filtered and concentrated under reduced pressure. The crude material was charged and eluted on a chromatography column (silica gel, EltO/ petroleum ether 50:50 → 90:10) to give a white solid (0.766 g, 70% yield). 1H NMR (400 MHz, 298 K, CDCl3) δ (ppm): 2.73 (s, 3H, CH3), 7.42 (d, J = 8.5 Hz, 1H, Hpy), 8.18 (dd, J = 8.5 Hz, J' = 2.3 Hz, 1H, Hpy), 9.12 (d, J = 2.3 Hz, 1H, Hpy) 13C NMR (800 MHz, 298 K, DMSO-d6) δ (ppm): 19.36 (CH3), 127.68 (CHpy), 138.23 (CHpy), 142.49 (CHpy), 144.30 (Cpy, quat.), 154.30 (Cpy, quat.). ESI-MS m/z calcd for [M+H + ] + (found): 191.99 (191.81).

N,N-bis[2-(2-methoxyethoxy)ethoxy]ethyl-6-methylpyridine-3-sulfonamide (5)

Compound (4) (0.900 g, 4.71 mmol) was dissolved in dry CH2Cl2 (about 20 mL) under N2 stream. The heterogeneous mixture was refluxed for 24 h and filtered through Celite® after cooling. Celite® was further washed with Et2O (about 100 mL) and the solvents were removed under reduced pressure. The residue was dissolved in distilled H2O (about 20 mL) and the pH was increased to 10 by addition of aqueous NaOH (5%). The aqueous phase was then extracted with CH2Cl2 (3× 100 mL). The aqueous phase was acidified to pH 3 by adding aqueous hydrochloric acid (25%) and the resulting solution was extracted again with CH2Cl2 (3× 100 mL). The organic phases were combined, reduced to a volume of about 1 mL, dried over Na2SO4, filtered, and rotor-evaporated under reduced pressure. After drying, (6) was obtained as an amber oil (1.83 g, 96% yield). 1H NMR (400 MHz, 298 K, acetone-d6) δ (ppm): 3.29 (s, 6H, OCH3), 3.45–3.49 (m, 12H, OCH2), 3.54 (m, 4H, OCH2), 3.58 (m, 4H, OCH2), 3.64 (m, 4H, OCH2), 8.32 (d, J = 8.1 Hz, 1H, Hpy), 8.50 (dd, J = 8.1 Hz, J' = 2.2 Hz, 1H, Hpy), 9.09 (d, J' = 2.2 Hz, 1H, Hpy) 13C NMR (800 MHz, 298 K, CDCl3) δ (ppm): 47.71 (NCCH2), 59.16 (OCH2), 67.21 (OCH2), 69.37 (OCH2), 70.46 (OCH2), 70.57 (OCH2), 72.06 (OCH2), 123.97 (CHpy), 137.71 (CHpy), 141.33 (CHpy), 147.19 (CHpy), 148.35 (Cpy, quat.), 163.18 (COOH). ESI-MS m/z calcd for [M+H + ] + (found): 495.20 (495.00).

Ethyl 6-(methyl-[4-[4-(methylamino)-3-nitrobenzyl]-2-nitrophenyl]carbamoyl)pyridine-2-carboxylate (10)

A mixture of 2,6-pyridinecarboxylic acid monoethyl ester (7) (1.00 g, 5.13 mmol), freshly distilled SOCl2 (6.10 g, 51.3 mmol), and dry DMF (200 μL, 2.56 mmol) were refluxed in dry CH2Cl2 (50 mL) under an inert atmosphere for 2 h. After evaporation and pumping for 2 h, the pale yellow solid formed was re-dissolved in dry CH2Cl2 (50 mL) and a saturated aqueous solution of NH4Cl (100 mL) was added. After separation, the aqueous phase was extracted with CH2Cl2 (3× 50 mL). The organic phases were combined, reduced to a volume of about 10 mL, dissolved in dry CH2Cl2 (50 mL) under an inert atmosphere for 2 h. The resulting solution was extracted again with CH2Cl2 (3× 100 mL). The organic phases were combined, reduced to a volume of about 10 mL, dried over Na2SO4, filtered, and rotor-evaporated under reduced pressure. After drying, (6) was obtained as an amber oil (1.83 g, 96% yield). 1H NMR (400 MHz, 298 K, acetone-d6) δ (ppm): 3.29 (s, 6H, OCH3), 3.45–3.49 (m, 12H, OCH2), 3.54 (m, 4H, OCH2), 3.58 (m, 4H, OCH2), 3.64 (m, 4H, OCH2), 8.32 (d, J = 8.1 Hz, 1H, Hpy), 8.50 (dd, J = 8.1 Hz, J' = 2.2 Hz, 1H, Hpy), 9.09 (d, J' = 2.2 Hz, 1H, Hpy) 13C NMR (800 MHz, 298 K, CDCl3) δ (ppm): 47.71 (NCCH2), 59.16 (OCH2), 67.21 (OCH2), 69.37 (OCH2), 70.46 (OCH2), 70.57 (OCH2), 72.06 (OCH2), 123.97 (CHpy), 137.71 (CHpy), 141.33 (CHpy), 147.19 (CHpy), 148.35 (Cpy, quat.), 163.18 (COOH). ESI-MS m/z calcd for [M+H + ] + (found): 495.20 (495.00).
H\textsubscript{Benz},) 7.84 (d, J = 8.8 Hz, 1H, H\textsubscript{Py}), 7.89 (d, J = 9.2 Hz, 1H, H\textsubscript{Py}), 7.91 (s, 1H, H\textsubscript{Benz}), 7.98 (dd, J = 9.2 Hz, J = 8.8 Hz, 1H, H\textsubscript{Py}). \textsuperscript{13}C NMR (800 MHz, 327 K, DMSO-d\textsubscript{6}) δ (ppm): 13.66 (OCH\textsubscript{2}CH\textsubscript{3}), 29.43 (NHC\textsubscript{3}), 37.47 (NCH\textsubscript{3}), 37.92 (CH\textsubscript{2}), 60.73 (OCH\textsubscript{2}CH\textsubscript{3}), 114.44 (CH\textsubscript{Benz}), 124.85 (CH\textsubscript{Benz}), 125.16 (CH\textsubscript{Benz}), 125.21 (CH\textsubscript{Py}), 126.34 (CH\textsubscript{Py}), 126.75 (CH\textsubscript{Py}), 130.59 (C(Benz, quat.), 131.10 (C(Benz, quat.), 134.45 (CH\textsubscript{Benz}), 135.84 (C(Benz, quat.), 137.02 (CH\textsubscript{Benz}), 138.32 (CH\textsubscript{Benz}), 141.95 (C(Benz, quat.), 144.60 (C(Benz, quat.), 145.03 (C(Benz, quat.), 145.62 (C(Py, quat.), 151.96 (C(Py, quat.), 163.57 (CONMe), 165.49 (COOEt). ESI-MS m/z calc for [M + H\textsuperscript{+}] (found): 970.36 (970.20); calc for [M + Na\textsuperscript{+}] (found): 992.34 (992.17).

**Ethyl 6-[[4-[(5-[[bis-[2-[2-methoxyethoxy]ethoxy]ethoxy]ethyl]sulfamoyl]pyridin-2-yl]-1-methyl-1H-benimidazol-5-yl](methyl)-2-nitrophenyl](methyl)carbamoyl]pyridine-2-carboxylate (11)**

A mixture of (6) (700 mg, 1.42 mmol), freshly distilled SOCl\textsubscript{2} (3.37 g, 28.3 mmol), and dry DMF (55 L, 0.708 mmol) were refluxed in dry CH\textsubscript{2}Cl\textsubscript{2} (50 mL) under an inert atmosphere for 2 h. After evaporation and pumping for 2 h, the brown oil formed was re-dissolved in dry CH\textsubscript{2}Cl\textsubscript{2} (25 mL) and NEt\textsubscript{3} (2.00 mL) was added. This mixture was refluxed and a solution of dry CH\textsubscript{2}Cl\textsubscript{2} (5 x 100 mL). The organic phases were combined, dried over Na\textsubscript{2}SO\textsubscript{4}, filtered, and evaporated to dryness, resulting in a brown crude solid which was purified by column chromatography (silica gel; CH\textsubscript{2}Cl\textsubscript{2} → CH\textsubscript{2}Cl\textsubscript{2}/MeOH 96:4) to give a pale yellow solid (532 mg, 79% yield). \textsuperscript{1}H NMR (400 MHz, 298 K, acetone-d\textsubscript{6}) δ (ppm): 1.43 (t, J = 6.8 Hz, 3H, OCH\textsubscript{2}CH\textsubscript{3}), 3.25 (s, 6H, OCH\textsubscript{3}), 3.44 (s, 4H, NCH\textsubscript{2}), 3.44 (s, 3H, OCH\textsubscript{2}CH\textsubscript{3}), 3.50–3.54 (m, 12H, OCH\textsubscript{2}), 3.59 (m, 4H, OCH\textsubscript{2}), 3.66 (m, 4H, OCH\textsubscript{2}), 4.29 (s broad, 2H, CH\textsubscript{2}), 4.35 (s, 3H, NCH\textsubscript{3}), 4.43 (s, 3H, NCH\textsubscript{3}), 4.44 (q, J = 6.8 Hz, 2H, OCH\textsubscript{2}CH\textsubscript{3}), 5.97 (s, 1H, 1H, H\textsubscript{Benz}), 5.97 (s, 1H, 1H, H\textsubscript{Benz}), 6.66 (d, J = 1.3 Hz, 1H, H\textsubscript{Benz}), 6.68 (d, J = 1.3 Hz, 1H, H\textsubscript{Benz}), 8.15 (d, J = 4.4 Hz, 2H, H\textsubscript{Py}), 8.42 (dd, J = 8.5 Hz, J = 1.3 Hz, 1H, H\textsubscript{Benz}), 7.55 (d, J = 8.5 Hz, 1H, H\textsubscript{Benz}), 7.55 (d, J = 8.5 Hz, 1H, H\textsubscript{Benz}), 7.66 (d, J = 1.3 Hz, 1H, H\textsubscript{Benz}), 7.68 (d, J = 1.3 Hz, 1H, H\textsubscript{Benz}), 8.15 (d, J = 4.4 Hz, 2H, H\textsubscript{Py}), 8.42 (dd, J = 8.5 Hz, J = 2.4 Hz, 1H, H\textsubscript{Py}), 8.59 (d, J = 8.5 Hz, 1H, H\textsubscript{Py}), 8.62 (t, J = 4.4 Hz, 1H, H\textsubscript{Py}), 9.12 (d, J = 2.4 Hz, 1H, H\textsubscript{Py}).

**Intermediate (12)** (525 mg, 0.602 mmol) was dissolved in an absolute EtOH/H\textsubscript{2}O mixture (20:20 mL) containing NaOH (28.9 mg, 0.721 mmol). The mixture was stirred at 60°C for 16 h.
After completion of the reaction, the solvents were evaporated. The residue was dissolved in distilled water (50 mL) and the resulting aqueous solution was acidified to pH = 2 by addition of 0.02 M hydrochloric acid. The acidic solution was then extracted with CH₂Cl₂ (5 × 100 mL), dried over Na₂SO₄ and evaporated. The crude product was triturated with hexane (100 mL), filtered, and dried under vacuum to give a pale yellow solid (498 mg, 98% yield). ¹H NMR (400 MHz, 298 K, acetone-d₆) δ (ppm): 3.25 (s, 6H, OCH₃), 3.43 (m, 4H, NCH₂CH₂), 3.50–3.54 (m, 12H, OCH₂CH₂), 3.59 (m, 4H, OCH₂), 3.66 (m, 4H, OCH₂), 4.30 (s (broad), 2H, CH₂), 4.35 (s, 3H, NCH₃), 4.38 (s, 3H, NCH₃), 7.32 (d, J = 8.1 Hz, 1H, H₇Benz), 7.34 (d, J = 8.5 Hz, 1H, H₈Benz), 7.55 (d, J = 8.5 Hz, 1H, H₉Benz), 7.68 (s, 1H, H₆Benz), 7.68 (s, 1H, H₉Benz), 8.20 (d, J = 6.4 Hz, 1H, H₈Py), 8.21 (d, J = 4.7 Hz, 1H, H₉Py), 8.41 (dd, J = 8.5 Hz, J = 0.6 Hz, 1H, H₇Py), 8.60 (d, J = 8.5 Hz, 1H, H₉Py), 8.60 (dd, J = 6.4 Hz, J = 4.7 Hz, 1H, H₈Py), 9.12 (d, J = 0.6 Hz, 1H, H₉Py). ¹³C NMR (800 MHz, 298 K, CDCl₃) δ (ppm): 13.33 (OCH₂C₂H₅), 38.63 (CH₂), 71.79 (OCH₂), 112.53 (CHPy), 113.40 (CH₂, H₇Benz), 113.73 (CHBenz), 114.30 (CH₂, H₉Benz), 124.17 (CHBenz), 124.42 (CHBenz), 125.82 (CH₂Py), 125.86 (CH₂Py), 126.64 (CH₂Py), 135.51 (C₈Benz, quat), 135.69 (CH₂Py), 136.21 (C₉Benz, quat), 136.53 (C₉Benz, quat), 145.03 (C₈Benz, quat), 145.57 (C₉Benz, quat), 146.34 (C₉Benz, quat), 147.20 (CH₂Py), 148.66 (CH₂Py), 149.11 (C₈Benz, quat), 153.47 (C₉Benz, quat). ESI-MS m/z calc for [M + H⁺] (found): 423.68 (423.75). Anal. Calcd for C₄₂H₅₁N₇O₁₀S: ΔH = −7.82 kJ mol⁻¹, ΔS = 47.46 J K⁻¹ mol⁻¹. ESI-MS m/z calc for [M + H⁺] (found): 656.26 (656.06).

Ethyl 6-[[5-(bis-[2-(2-methoxyethoxy)ethoxy]ethyl) sulfamoyl]-[pyridin-2-yl]-carbonyl[methyl]amino]-3-nitrobenzyl]-2-nitrophenyl[methyl]carbamoyl]-4-[2-(2-methoxyethoxy)ethoxy]ethoxy]-pyridine-2-carboxylate (14)

A mixture of (6) (1.12 g, 2.26 mmol), freshly distilled SOCl₂ (5.38 g, 45.2 mmol), and dry DMF (87 μL, 1.13 mmol) were refluxed in dry CH₂Cl₂ (30 mL) under an inert atmosphere for 2 h. After evaporation and pumping for 2 h, the brown oil formed was re-dissolved in dry CH₂Cl₂ (25 mL) and NEt₃ (2.00 mL) was added. This mixture was refluxed and a solution of dry CH₂Cl₂ (25 mL) containing (13) (336 mg, 8.17 mmol) was added dropwise over a period of 30 min. The resulting solution was kept under reflux for 16 h. After evaporation. The crude residue was re-dissolved in CH₂Cl₂ (100 mL) and washed with half-saturated NH₄Cl solution (100 mL). After filtration, the aqueous phase was extracted with CH₂Cl₂ (2 × 100 mL). The combined organic phases were dried over Na₂SO₄ and evaporated. The crude material was purified by column chromatography (silica gel, CH₂Cl₂/MeOH 99:1 → CH₂Cl₂/MeOH 95:5) to afford brown powder (725 mg, 79% yield). ¹H NMR (400 MHz, 843 K, DMSO-d₆) δ (ppm): 1.28 (t, J = 6.8 Hz, 3H, OCH₂CH₂), 3.27 (s, 3H, OCH₃), 3.28 (s, 6H, OCH₃), 3.39 (s, 3H, NCH₃), 3.40 (s, 3H, NCH₃), 3.40 (m, 4H, NCH₂CH₃), 3.45–3.50 (m, 14H, OCH₂), 3.51–3.58 (m, 12H, OCH₂), 3.61 (m, 2H, OCH₂), 3.80 (m, 2H, OCH₂), 4.17 (s (broad), 2H, CH₂), 4.23 (q, J = 6.8 Hz, 2H, OCH₂CH₃), 4.33 (m, 2H, OCH₂), 7.38 (d, J = 2.4 Hz, 1H, H₉Py), 7.43 (d, J = 2.4 Hz, 2H, H₈Py), 7.49–7.52 (d, J = 8.2 Hz, 3H, H₉Benz), 7.80 (d, J = 8.2 Hz, 1H, H₈Benz), 7.86 (s, 1H, H₇Benz), 7.91 (s, 1H, H₉Benz), 8.23 (dd, J = 8.2 Hz, J = 1.7 Hz, 1H, H₇Py), 8.64 (s, 1H, H₈Py). ¹³C NMR (800 MHz, 327 K, DMSO-d₆) δ (ppm): 13.66 (OCH₂CH₂), 37.45 (NCH₃), 38.27 (NCH₂CH₂), 47.23 (NCH₂CH₂), 47.39 (CH₂), 57.81 (OCH₂), 60.79 (OCH₂CH₂), 68.03 (OCH₂), 68.24 (OCH₂), 68.33 (OCH₂), 68.47 (OCH₂), 69.39 (OCH₂), 69.42 (OCH₂), 69.57 (OCH₂), 69.81 (OCH₂), 71.09 (OCH₂), 111.83 (CH₂Py), 112.76 (CH₇Py), 123.88 (CHBenz), 124.82 (CHBenz), 125.18 (CH₈Py), 131.12 (CHBenz), 131.19 (CHBenz), 134.38 (CHBenz), 134.50 (CHBenz), 135.76 (CHBenz), 136.21 (C₉Benz, quat), 136.68 (CH₂Py), 140.50 (C₈Benz, quat), 141.39 (C₇Benz, quat), 145.03 (C₈Benz, quat), 145.77 (C₇Benz, quat), 145.77 (C₇Benz, quat), 145.77 (C₇Benz, quat), 153.67 (C₇Benz, quat).
Ethyl 6-[5-[[2-[[2-(2-methoxyethoxy)ethoxy]ethoxy]ethyl]sulfamoyl]pyridin-2-yl]-1-methyl-1H-benzimidazol-5-yl]methyl]-1-methyl-1H-benzimidazol-2-yl]-4-[2-[2-(2-methoxyethoxy)ethoxy]ethoxy]ethoxy]pyridine-2-carboxylic acid (HL$_3^\circ$)

Intermediate (16) (575 mg, 5.56 × 10$^{-1}$ mmol) was dissolved in an absolute EtOH/H$_2$O solution (20/5 mL) containing NaOH (26.7 mg, 0.667 mmol). This mixture was stirred at 60°C for 16 h. After completion of the reaction, the solvents were evaporated. The residue was dissolved in distilled water (50 mL) and the resulting aqueous solution was acidified to pH = 2 by addition of 0.02 M hydrochloric acid. The acidic solution was then extracted with CH$_2$Cl$_2$ (5 × 100 mL), dried over Na$_2$SO$_4$, and evaporated. The crude product was triturated with hexane (100 mL), filtered and dried under vacuum to give a pale yellow solid (560 mg, 100% yield).

1H NMR (400 MHz, 298 K, acetone-$_d_6$) δ (ppm): 1.42 (t, 3J = 7.2 Hz, 3H, OCH$_2$CH$_2$), 3.25 (s, 3H, NCH$_3$), 3.26 (s, 6H, OCH$_3$), 3.34 (m, 4H, NCH$_2$), 3.44 (m, 2H, OCH$_2$), 3.50–3.55 (m, 2H, OCH$_2$), 3.93 (m, 2H, OCH$_2$), 4.29 (s (broad), 2H, CH$_2$), 4.97 (s (broad), 1H, H benzene), 7.31 (dd, 3J = 8.5 Hz, 4J = 1.4 Hz, 1H, H benzene), 7.33 (dd, 3J = 8.5 Hz, 4J = 1.4 Hz, 1H, H benzene), 7.35 (dd, 3J = 8.5 Hz, 4J = 1.4 Hz, 1H, H benzene), 7.48 (dd, 3J = 1.4 Hz, 1H, H benzene), 7.60 (dd, 3J = 1.4 Hz, 1H, H benzene), 7.63 (dd, 3J = 1.4 Hz, 1H, H benzene), 7.67 (dd, 3J = 1.4 Hz, 1H, H benzene).

Analytical and Spectroscopic Measurements

Elemental analyses were performed by Dr. E. Solari, Elementary Analysis Laboratory of the Institute of Chemical Sciences and Engineering, EPFL. NMR spectra were measured on Bruker Avance DRX 400 (1H, 400 MHz), AV 600 (13C, 150.864 MHz), and AV 800 (13C, 201.54 MHz) spectrometers. Spectra of organic compounds were recorded in CDCl$_3$ (99.8%), CD$_3$CN (99.8%), acetone-$d_6$ (99.5%), DMSO-$d_6$ (99.8%), and D$_2$O (99.9%), all from Aldrich Chemicals. Deuterated solvents were taken as internal standards; chemical shift values are given in ppm with respect to TMS and J values are reported in Hz. The ESI-MS spectra of the organic compounds were obtained on a Finnigan TSQ 7100 spectrometer using 10$^{-3}$–10$^{-4}$ M solutions in acetonitrile/H$_2$O/formic acid (49.5/49.5/1) or MeOH.
the capillary temperature was set to 180°C and the ion spray voltage to 3.5 kV. The instrument was calibrated using horse myoglobin and the analyses were conducted in positive mode. Phosphoric acid was used for mass calibration in the range 500–2000 m/z. Data were acquired and processed with Masslynx version 4.0. Electro spray conditions were as follows: capillary voltage, 3 kV; source temperature, 80°C; cone voltage, 35 V; source block temperature, 150°C. The ESI nebulization and drying gas was nitrogen. The sample was introduced through a syringe pump operating at 20 μL·min⁻¹. Simulation of spectra was achieved with Molecular Weight Calculator 6.42®. UV/Vis absorption spectra were measured in 1.0 cm quartz Suprasil® cells on a Perkin-Elmer Lambda 900 spectrometer. Stability constants were determined by spectrophotometric titration of (L₄)⁻ by Eu(III) or Eu(III)/Zn(II) (1:1) in Tris-HCl 0.1 M (pH 7.4) under N₂ atmosphere. All titrations were performed batch wise in thermostated (25.0 ± 0.1°C) 1-cm quartz cuvettes. Factor analysis (Malinowski and Howery, 1991) and mathematical treatment of the spectrophotometric data were performed with the Specfit® software (Gampp et al., 1986). Luminescence spectra and lifetimes were collected either on a Horiba-Jobin Yvon FL 3-22 fluorometer or (Gampp et al., 1986). Luminescence spectra and lifetimes were measured by an absolute method using a specially designed integration sphere (Aebischer et al., 2009).

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/Inorganic_Chemistry/10.3389/fchem.2013.00015/abstract

Figure S1 | (Top) Re-calculated spectra from the titration of HL⁶ with zinc perchlorate at 295 K and pH 7.4. (Bottom) Absorbance values extracted at different wavelengths during the titration compared with theoretical prediction from the stability extracted from the fit procedure (Table 1).

Figure S2 | (Top) Re-calculated spectra from the titration of HL⁶ with europium perchlorate at 295 K and pH 7.4. (Bottom) Absorbance values extracted at different wavelengths during the titration compared with theoretical prediction from the stability extracted from the fit procedure (Table 1).

Figure S3 | (Top) Re-calculated spectra from the titration of HL⁶ with zinc and europium perchlorate (1:1) at 295 K and pH 7.4. (Bottom) Absorbance values extracted at different wavelengths during the titration compared with theoretical prediction from the stability extracted from the fit procedure (Table 1).

Figure S4 | Effect on luminescence spectra of the addition of Zn(II) to a stoichiometric 1:3 Eu(III)·(L⁶)⁻ solution 16.4 μM in ligand, pH 7.4.

Figure S5 | Stoichiometric solution 1:3 Eu(III)·(L⁶)⁻ (left) and 1:1:3 Eu(III)/Zn(II)·(L⁶)⁻ under irradiation at 366 nm; total ligand concentration: 16.4 μM in ligand, pH 7.4.

Figure S6 | Emission spectra of solutions in Tris-HCl 0.1 M (pH 7.4) with different stoichiometries; λₐₑₓ = 3.7 nm; [(L⁶)³⁻] = 16.2 μM; the star denotes an artifact (2nd order Rayleigh band from excitation beam).

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