Regioselectively *N*-Functionalised 14-Membered Azapyridinomacrocycles Bearing Trialkanoic Acid Side Chains as Ligands for Lanthanide Ions

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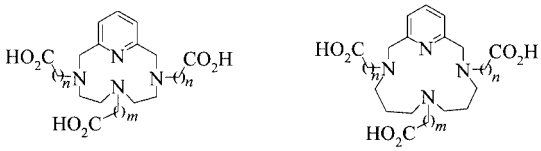
Four 14-membered azamacrocycles **2a–d**, each containing a pyridine moiety and three acetic and/or propionic acid pendent arms, were prepared from regioselectively functionalised polyamines. The stability constants of the lanthanide complexes (La, Pr, Nd, Eu, Gd, Tb, Er, Yb, Lu) formed with the triacetic acid compound **2a** were determined by spectroscopic methods (luminescence and/or UV/Vis spectroscopy). Other characteristics of the luminescent Eu^{III} and Tb^{III} complexes formed with the triacetic acid derivative **2a** were also evaluated.

Introduction

During the past decades, chelating agents based on azamacrocyclic backbones have proved to be extremely valuable for the generation of aqueous stable lanthanide complexes. In particular, aminocarboxylate derivatives have been shown to form highly stable lanthanide chelates,[1] and have consequently found valuable medical applications, either as diagnostic[2] or as therapeutic agents.[3] On the other hand, the separation of f-elements is of interest for the development of depollution processes, in particular for the backend of nuclear fuel cycle.[4] f-Element group separation (lanthanides versus actinides) and separations of individual members of the two groups are difficult, due to their very similar chemical properties in solution.[5] However, the radius size of the cation and/or its degree of hardness could constitute discriminating factors.

We have previously reported the synthesis of the 12-membered azapyridinomacrocycles **1a–d** (Figure 1) and a preliminary evaluation of their capability to chelate Eu^{3+} cation.[6] In the context of a program aimed at the synthesis of selective ionophores, we are interested in establishing the structural requirements that govern the formation of complexes with lanthanides in terms of kinetics and thermodynamics. In order to evaluate the influence of the macrocyclic size, together with the size of the chelation rings involving the cation, we report here the synthesis of the homologous 14-membered azapyridinomacrocycles **2a–d** (Figure 1). The complexing properties of compound **2a** towards various lanthanide cations were examined, together with further characteristics of the luminescent europium and terbium complexes.



1a $n = m = 1$
1b $n = m = 2$
1c $n = 1$ $m = 2$
1d $n = 2$ $m = 1$

2a $n = m = 1$
2b $n = m = 2$
2c $n = 1$ $m = 2$
2d $n = 2$ $m = 1$

Figure 1. Azapyridinomacrocycles as ligand for lanthanide cations

We identified macrocyclic pyridine derivatives **1** and **2** as valuable targets for the complexation of lanthanide cations for various reasons:

– the presence of seven potential O- or N-donor atoms would satisfy the electronic demand of lanthanide cations, known for having coordination numbers of 8 or 9;[7] moreover, the hard/hard interactions between the two components might ensure good stability of the corresponding complex,

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– the macrocyclic backbone, by diminishing the degree of free rotation, might preorganise the chelation sites, which could be valuable for rapid complexation kinetics,

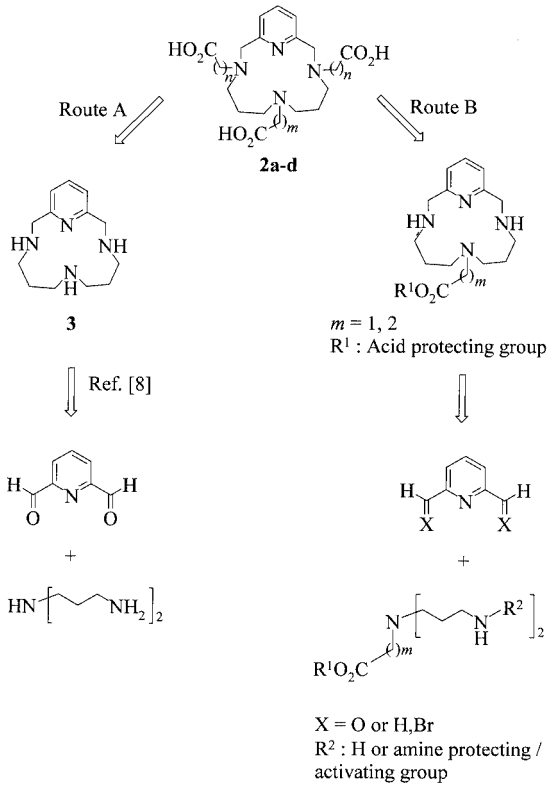
– this azapyridinomacrocyclic skeleton allows studies of the effect of small structural changes on the complexation properties; indeed, both the size of the macrocyclic cavity and the sizes of chelation cycles involving the cation, the nature of the side chains as ligating moiety and the nature of substitutions on the diaminoalkyl subunits could be studied, and

– the presence of a pyridine moiety (i) constitutes another element of rigidification, (ii) introduces a less hard nitrogen atom, which is a possible discriminating element for the selective f-element complexation, (iii) offers positions for the introduction of an anchor group, thus extending the application areas, and (iv) allows the evaluation of the complexing properties of the ligand by spectroscopic methods.

Results and Discussion

Synthesis of the 14-Membered Azapyridinomacrocyclic Ligands 2a–d

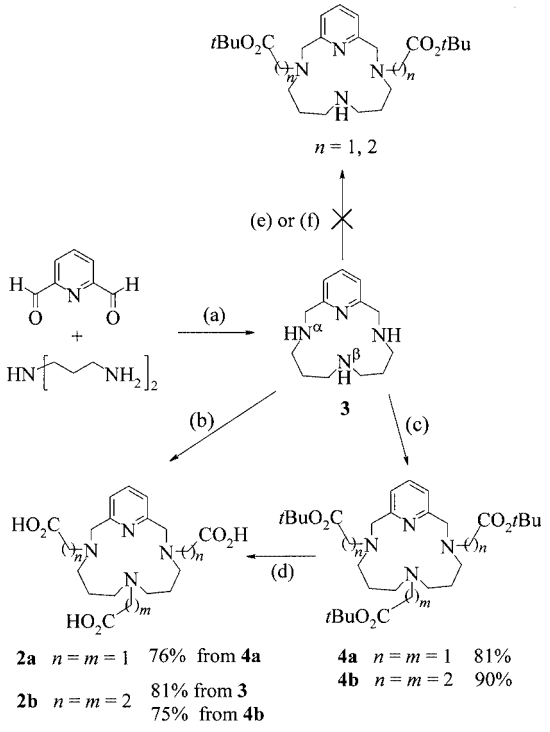
Two different routes for the synthesis of the targeted molecules **2a–d** can be envisaged, as shown in Scheme 1. Route **A** appears to be the more straightforward, as macrocycles **2a–d** would result from *N*-alkylation(s) of the known tetraazamacrocycle **3**.[8] This route is particularly well adapted to the synthesis of macrocycles **2a** and **2b**, with identical side chains ($n = m$), while for macrocycles **2c** and **2d**, with different side chains attached at nitrogen atoms, the synthetic challenge of Route **A** lies in the regioselective functionalisation of the two sets of secondary amines. Route **B** constitutes an alternative approach if parent macrocycle **3** cannot be alkylated or if the *N*-alkylation step is not regioselective, but this latter route lacks divergence as it requires the preliminary synthesis of different *N*-functionalised polyamine blocks.



Scheme 1

Our initial work followed the more obvious Route **A**. The parent macrocycle **3** was prepared as described previously[8] (Scheme 2). The synthesis of the azapyridinomacrocycle **2a**, bearing three acetic acid side chains, through acidic hydrolysis of the corresponding triethyl ester has been reported,[9a] but involved an *N*-alkylation step that occurred with 35% yield. The preparation of the corresponding tripotassium salt resulting from alkaline hydrolysis of the trimethyl ester has also been reported.[9b] We thus tried to obtain tris(carboxyalkyl)macrocycles **2a** and **2b** by direct alkylation with sodium chloroacetate or acrylic acid, respectively. On treatment of macrocycle **3** with a small excess of sodium chloroacetate (3.5 mol-equiv.) in a pH-controlled medium (pH ≥ 10) and at a temperature rising from 40 to 75 °C, the desired product **2a** was obtained together with a dialkylated one (**2a**/dialkylated product, 80:20). However, treatment of compound **3** with a large excess of acrylic acid in water at 70 °C gave the desired macrocycle **2b** in 81% yield.[10] In order to prepare compound **2a**, we have developed a new synthetic route involving the macrocyclic tri-*tert*-butyl ester **4a**. When the parent macrocycle **3** was treated with an excess of *tert*-butyl bromoacetate in the presence of K_2CO_3, the desired alkylated product could not be isolated. Replacement of the base by Ag_2CO_3 as halide scavenger, together with strict control of the reaction time (a prolonged time results in degradation products), provided the desired alkylated product **4a** in 81% yield. On the other hand, if a large excess of *tert*-butyl acrylate was heated at reflux in a concentrated methanolic solution of macrocycle **3**, the desired azapyridinomacrocycle **4b**, bearing three *tert*-butyl propanoate side chains, was isolated in 90% yield. Upon treatment with dry hydrogen chloride in diethyl ether, salt-free poly(aminocarboxylic acids) **2a** and **2b** were isolated in ca. 75% yields by simple filtration followed by an anion-exchange resin filtration step.

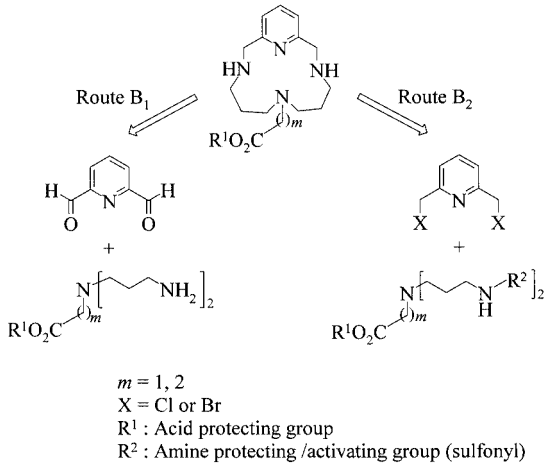
J. Costa and R. Delgado have determined the acidity constants of azamacrocycle **3**, and it appears that the secondary amines adjacent to the pyridine moiety NH_α are more basic than that opposed to the pyridine moiety NH_β (see Scheme 2).[11] As a consequence, we thought that a difference in nucleophilicity might be exploitable for the two sets of nitrogen atoms as well. The parent macrocycle **3** was thus treated with a twofold molar excess of *tert*-butyl bromoacetate in the presence of Ag_2CO_3. Monitoring of the reaction over 7 d revealed the coexistence of a large amount of starting material **3** and trialkylated compound **4a**, together with other product(s), probably partially alkylated. The NH_α regioselective alkylation of macrocycle **3**



Scheme 2. Synthesis of macrocycles **2a** and **2b** by Route **A**; reagents and conditions: (a) see ref.[8], (b) acrylic acid, H_2O, 70 °C, overnight; (c) for **4a**: *tert*-butyl bromoacetate (12 equiv.), Ag_2CO_3 (2.5 equiv.), THF, room temp., 1 h; for **4b**: *tert*-butyl acrylate (13 equiv.), MeOH, 70 °C, 40 h; (d) HCl, Et_2O; (e) *tert*-butyl bromoacetate (2 equiv.), Ag_2CO_3 (2 equiv.), THF, room temp., 7 d or *tert*-butyl bromoacetate (2.5 equiv.), NaOH (2.5 equiv.), CH_2Cl_2/H_2O, room temp., 16 h; (f) *tert*-butyl acrylate (2 equiv.), MeOH, 70 °C, 20h

with 2-(chloromethyl)pyridine[12] and benzyl chloride[13] in the presence of sodium hydroxide in a biphasic dichloromethane/water mixture had been reported to produce the targeted dialkylated derivatives in 75 and 55% yields, respectively. When we extended these conditions to the alkylation of compound **3** with a slight excess of *tert*-butyl bromoacetate the system proved to be more reactive than the previous one, completion of the reaction being reached after 16 h. Unfortunately, it lacked selectivity, as a mixture of at least three products, including the trialkylated **4a**, was recovered. An attempt to introduce two propanoic side chains selectively on the NH_α secondary amine by treatment with a twofold molar excess of *tert*-butyl acrylate also failed, as starting material **3** and trialkylated compound **4b** were recovered together with side products.

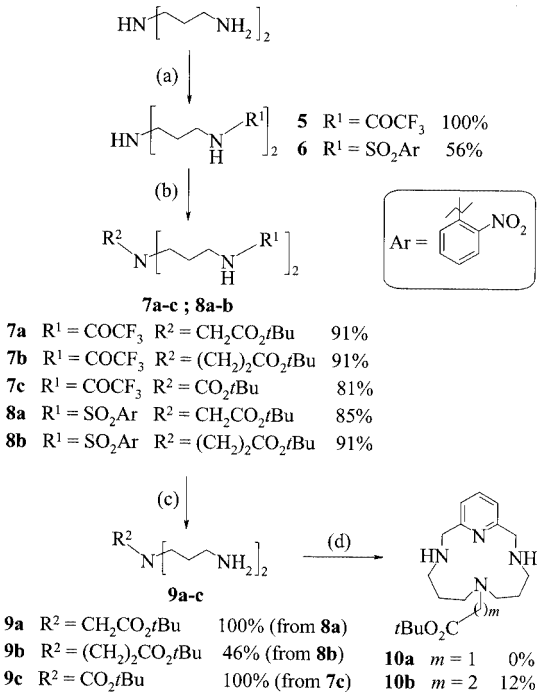
Given that Route **A** appeared unsuitable for the preparation of macrocycles **2c** and **2d** bearing different side chains attached to nitrogen atoms, we turned our attention to Route **B**, involving a prior chemoselective *N*-functionalisation of the polyamine skeleton. Two macrocylisation processes, equivalent in terms of number of steps, can be envisaged as shown in Scheme 3: a reductive templated amination involving copper(II) ion, which takes place in aqueous medium[8,11,14] (Route **B1**), or a nucleophilic substitution requiring a polar solvent and relatively high dilution conditions[6a,9b] (Route **B2**). We first considered the more convenient route **B1** to obtain macrocycles selectively *N*-alkylated on the nitrogen atom opposite to the pyridine moiety.



Scheme 3. Macrocyclisation processes envisaged for the preparation of regioselectively *N*-functionalised azapyridinomacrocycles **2c** and **2d**

The trifluoroacetyl group has been widely used as amine protecting group, thanks to its ease of removal under mild conditions and its selective introduction (primary versus secondary amines) through the use of ethyl trifluoroacetate as a mild transferring agent.[15] For our purpose, it appeared to be a protecting group of choice, as it can be selectively cleaved in the presence of the *tert*-butyl ester protection we planned to use on the polyamino acid skeleton. The diacetamide **5** was thus obtained in a quantitative yield by treatment of 1,7-diamino-4-azaheptane with 2 mol-equiv. of ethyl trifluoroacetate (Scheme 4). The alkylation of the resulting secondary amine was then achieved with *tert*-butyl bromoacetate or *tert*-butyl acrylate, to afford the corresponding aminoalkanoates **7a** and **7b** in high yields (91%).

Unfortunately, under standard conditions ($NaBH_4$/EtOH or NH_3/MeOH/H_2O or K_2CO_3/MeOH/H_2O), all attempts to cleave the acetamide groups of compounds **7a** and **7b** gave mixtures of two or three polar products inseparable by normal-phase chromatography. It should be noted that the complexity of the mixture was a function of reaction temperature and time. An analogous trifluoroacetamide bearing an *N*-*tert*-butoxycarbonyl group had been reported to be quantitatively cleaved with a methanolic aqueous ammonia solution.[15b] We thus prepared compound **7c** by treatment of trifluoroacetamide **5** with a stoichiometric amount of 2-(*tert*-butoxycarbonyloximino)-2-phenylacetonitrile (BOC-ON) in the presence of triethylamine. Under the previously reported conditions,[15b] the trifluoroacetamide cleavage occurred to provide the diamine **9c** in a quantitative yield. This was evidence that the alkanoic side chains of compounds **7a** and **7b** were involved in the formation of side products during the acetamide cleavage step. In order to identify the natures of these side products, we decided to treat the crude mixtures arising from **7a** with an



Scheme 4. Synthesis by route **B1**; reagents and conditions: (a) for **5**: CF_3CO_2Et (2 equiv.), CH_3CN, room temp., 15 h; for **6**: see ref.[16], (b) for **7a**: *tert*-butyl bromoacetate (1.3 equiv.), DIPEA (1.3 equiv.), CH_3CN, reflux, 5 h; for **7b**: *tert*-butyl acrylate (3.5 equiv.), MeOH, room temp., 1 d; for **7c**: BOC−ON (1.2 equiv.), TEA (1.5 equiv.), THF, room temp., 1 d; for **8a**: *tert*-butyl bromoacetate (3 equiv.), TEA (6 equiv.), THF, reflux, 1 d; for **8b**: *tert*-butyl acrylate (large excess), CH_2Cl_2/MeOH (3:1), 70 °C, 1 d; (c) from **7a**−**c**: aq. NH_3, MeOH or from **7a** and **7b**: $NaBH_4$, EtOH or K_2CO_3, MeOH/H_2O; from **8a** and **8b**: PhSH (3 equiv.), Na_2CO_3 (8 equiv.), DMF, room temp., overnight; (d) see ref.[8]

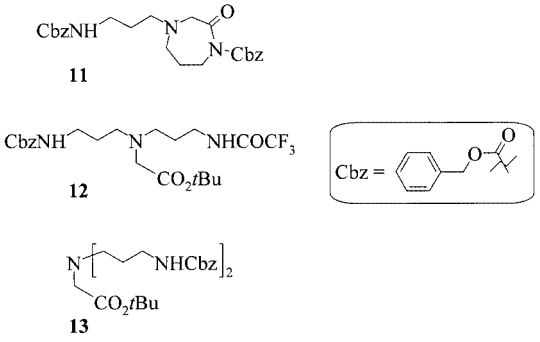


Figure 2. Derivatised side products recovered from the trifluoroacetamides **7a** and **7b** cleavage step

excess of benzyloxycarbonyl chloride in the presence of triethylamine. The resulting mixtures were composed of products **11**−**13**, separable by normal-phase chromatography (Figure 2). For a prolonged reaction time in the acetamide cleavage step (1 d at reflux), the two major products isolated were identified as compounds **11** and **13**, while a shorter reaction time (3 h at reflux were necessary for the starting material to be consumed) resulted in the isolation of compounds **11**, **12** and **13**. This latter experiment, showing the coexistence of side product **11** and partially deprotected product **12** just after the total consumption of starting material, convinced us to abandon the trifluoroacetyl protecting group in favour of the 2-nitrobenzenesulfonyl one. Indeed, we had previously reported a procedure for the selective transformation of a primary amine into the corresponding sulfonamide with a secondary amine remaining unchanged.[16] Moreover, the cleavage conditions for such sulfonamides proved to be compatible with *tert*-butyl alkanoate subunits, as they enabled us to prepare regioselectively functionalised azapyridinomacrocycles **1c** and **1d**.[6]

Alkylation of disulfonamide **6** was achieved either with *tert*-butyl bromoacetate or with *tert*-butyl acrylate and yielded the corresponding aminoalkanoates **8a** and **8b** in 85 and 91% yields, respectively (see Scheme 4). Subsequent treatment with thiophenol in the presence of sodium carbonate gave the functionalised primary diamines **9a** and **9b** in medium (not optimised) to quantitative yields. The reductive copper(II)-templated amination attempts with 2,6-pyridinedicarboxaldehyde were unsuccessful in producing the mono-*N*-alkylated macrocycles **10a** and **10b** efficiently. The lack of reactivity of primary diamines **9a** and **9b** could be attributable to their coordinating alkanoate side chains, which may prevent the suitable coordination of dialdehyde and α,ω-diamine around the templating metal ion that would favour the cyclisation.

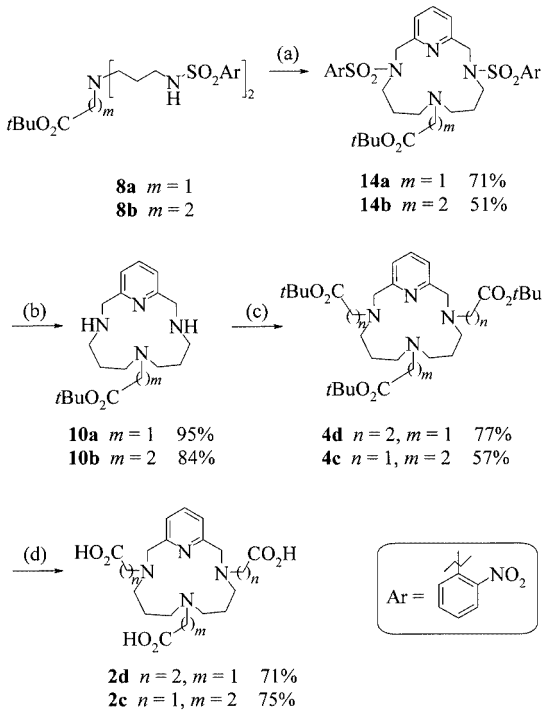
The failure of route **B1** (see Scheme 3) to produce the macrocyclic intermediates for the preparation of the regioselectively *N*-functionalised azapyridinomacrocycles **2c** and **2d** prompted us to envisage route **B2**, involving the previously prepared disulfonamides **8a** and **8b** (Scheme 5). When these disulfonamides were treated with 2,6-bis-(bromomethyl)pyridine according to the previously reported macrocyclisation procedure,[6] the corresponding expected macrocycles **14a** and **14b** were isolated in 71 and 51% yields, respectively. Cleavage of the sulfonamide subunits with thiophenol in the presence of sodium carbonate gave the monofunctionalised compounds **10a** and **10b**, bearing transannular secondary amine functions, in high yields.

The versatile compounds **10a** and **10b** were further alkylated with *tert*-butyl acrylate and *tert*-butyl bromoacetate, respectively, to give the corresponding macrocyclic tris(*tert*-butyl carboxylates) **4d** and **4c** in 57 and 77% yields. Final cleavage of the *tert*-butyl carboxylate functions with dry hydrogen chloride afforded the desired regioselectively functionalised azapyridinomacrocycles **2c** and **2d**.

Complexing Properties of the Azamacrocycle 2a

A. Preliminary Assays

In buffered aqueous medium (pH = 8.0), the chromophoric ligand **2a** is characterised in the UV region by an absorption band due to π-π* transitions, with $\lambda_{max.}$ = 261.5 nm and $\varepsilon_{max.}$ = 3918 $dm^3 \cdot mol^{-1} \cdot cm^{-1}$ (Figure 3, dotted line). In the presence of $EuCl_3$, a red shift of the π-π* transitions was observed, and is indicative of perturbation produced by interaction between the coordinating metal ion and ligand **2a** (Figure 3, solid line).



8a $m = 1$
8b $m = 2$

14a $m = 1$ 71%
14b $m = 2$ 51%

10a $m = 1$ 95%
10b $m = 2$ 84%

4d $n = 2, m = 1$ 77%
4c $n = 1, m = 2$ 57%

2d $n = 2, m = 1$ 71%
2c $n = 1, m = 2$ 75%

Scheme 5. Synthesis of azapyridinomacrocycles **2c** and **2d** by route **B2**; reagents and conditions: (a) 2,6-bis(bromomethyl)pyridine (1.2 equiv.), Na_2CO_3 (5 equiv.), DMF, 100 °C, 1 d; (b) PhSH (3.3 equiv.), Na_2CO_3 (10 equiv.), DMF, room temp., 5−15 h; (c) for **4d**: *tert*-butyl acrylate/MeOH (6:1), 70 °C, overnight; for **4c**: (i) *tert*-butyl bromoacetate (5.5 equiv.), Ag_2CO_3 (0.3 equiv.), THF, room temp., 1 h; (ii) HCl; (iii) Na_2S; (d) HCl, Et_2O, room temp., overnight

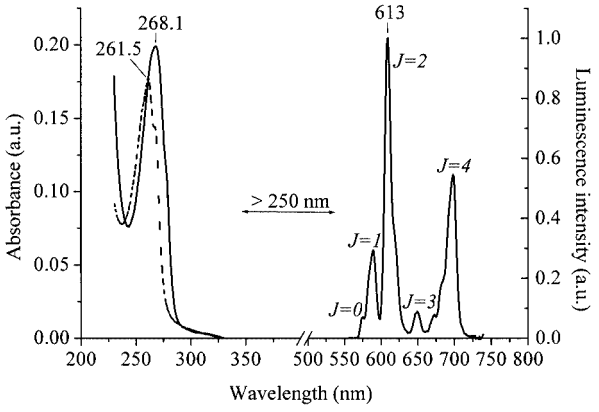


Figure 3. Absorption spectra of free ligand **2a** (dotted line) and of ligand **2a** in the presence of $EuCl_3$ (solid line, 200−320 nm) in HEPES-buffered aqueous medium (pH = 8.0); sensitised emission spectrum (solid line, 550−750 nm) obtained by irradiation at 262 nm of the previous buffered aqueous solution of ligand **2a** and $EuCl_3$

In the absence of the antenna ligand **2a**, irradiation at 262 nm (ligand-centred transition) of a buffered aqueous Eu^{III}-containing solution did not induce Eu^{III} luminescence. However, the introduction of the chromophoric ligand **2a** gave rise to the well-known structured Eu^{III} emission spectrum (see Figure 3, solid line), thus providing evidence of in situ formation of an europium chelate that gives rise to a through-space energy transfer from the excited chromophore to the adjacent complexed lanthanide (antenna effect[17]). The use of pyridine as luminophore is responsible for a large Stokes shift (> 250 nm), so that there is no overlap of the metal-centred emission bands with the ligand-centred absorption band (Figure 3). As would be expected, all emissions arise from the $^5D_0$ state, and the most intense bands corresponding to the $^5D_0 \rightarrow {}^7F_j$ ($\Delta J = 0, 1, 2, 3, 4$) transitions are observed. The spectrum is dominated by the $^5D_0 \rightarrow {}^7F_2$ transition centred on the 613 nm peak and integrating to 46% of total emission.

A similar study was carried out with the propionate derivative **2b**, which shows an absorption band in the UV region with $\lambda_{max.} = 262.5$ nm and $\varepsilon_{max.} = 3294$ $dm^3 \cdot mol^{-1} \cdot cm^{-1}$. Unfortunately, it proved to be ineffective for complexation of Eu^{III} ion, as reflected in an absence of UV-spectrum deformation together with the absence of luminescence emission on excitation at $\lambda_{max.}$. This dramatic loss of complexing ability with enlargement of the *N*-appended arms from acetate to propionate encouraged us to characterise only the all-acetate derivative **2a**.

B. Stoichiometry of the Eu^{III} Complex Formed with Triacetate Ligand 2a

Since the validity of the stability constant evaluation depended both on the correctness of the assumptions made concerning the species in solution and on the attainment of equilibrium at the time of the measurement, the stoichiometry of the previously examined europium chelate was first determined and all solutions were subsequently allowed to equilibrate until no more spectroscopic variation was detectable.

The stoichiometry of the Eu^{III} complex formed with ligand **2a** was determined by a mol ratio method.[18] In order to furnish a constant amount of excitation energy to the system, a set of HEPES-buffered aqueous samples containing ligand **2a** with increasing amounts of $EuCl_3$ was irradiated at the isosbestic point (262 nm). Through monitoring of the luminescence intensity (615 nm) as a function of the amount of $EuCl_3$, a binding curve was obtained (Figure 4). Since the ligand concentration is well below the stability constants known for such complexes,[9b,19] the titration curve should exhibit an abrupt change of slope corresponding to the stoichiometric ratio. As expected, the curve exhibited a break when an equimolar amount of $EuCl_3$ (relative to initial ligand concentration) was added, thus being consistent with a 1:1 stoichiometry for the Eu^{III} chelate formed with ligand **2a**.[20]

C. Determination of the Stability Constants of Ln^{III} Complexes Formed with Ligand 2a

In a general case, the complexation of a lanthanide cation Ln^{3+} with a trianionic ligand L^{3-} is described by Equation (1).

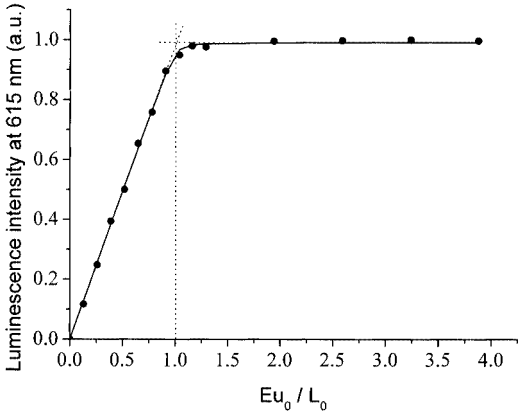


Figure 4. Binding curve resulting from monitoring of the luminescence intensity (615 nm) of samples containing 37.4 μM of ligand **2a** (L_0) and varying amounts of $EuCl_3$ (Eu_0: 0−150 μM) in 0.085 M of KCl and 0.015 M HEPES-buffered medium (pH = 8.0); the luminescence results from a UV light excitation at 262 nm; the full circles represent actual data points and the solid line represents the theoretical fit of data with log K^{Eu} = 9.54 and $I_{max.}$ = 0.997

$$Ln^{3+} + L^{3-} \overset{K^{Ln}}{\rightleftharpoons} LnL \qquad (1)$$

with $K^{Ln} = [LnL] / [Ln^{3+}].[L^{3-}]$

In the case of acid compounds such as ligand **2a**, however, the complexation of the lanthanide cation Ln^{3+} is the result of an ion-exchange process with the protons of the ligand as described by Equation (2), with $0 \leq i \leq 4$ as only four of the seven basic centres of ligand **2a** could be protonated in such a medium.[9a]

$$Ln^{3+} + LH_{4-i}^{(1-i)+} \rightleftharpoons LnL + (4-i)\,H^+ \qquad (2)$$

Hence, depending on its intrinsic basicity, each ligand has a different response to the proton competition, which limits the metal ion binding providing the Ln^{III} chelate, represented as LnL.

1. Eu^{III} Complex: Stability Constants Determined by Sensitised Eu^{III} Luminescence

In the case of europium (Ln = Eu) and under the condition that a constant amount of energy is furnished to the studied system, the luminescence intensity I is proportional to the concentration of the Eu^{III} chelate EuL.[21] Furthermore, if it assumed that there is no hydrolysis of the (aquo)-metal ion, no participation of the buffer in the cation complexation, and a unique Eu^{III} chelate, the intensity can be expressed as a function of the pH and K^{Eu} by Equation (3), with $\alpha_H^{-1} = 1 + K_{a1}\,10^{-pH} + K_{a1}\,K_{a2}\,10^{-2pH} + K_{a1}\,K_{a2}\,K_{a3}\,10^{-3pH} + K_{a1}\,K_{a2}\,K_{a3}\,K_{a4}\,10^{-4pH}$, where K_{ai} represents the stepwise protonation constant of the fully deprotonated ligand **2a**,[9a] Eu_0 and L_0 stand for the initial concentrations of $EuCl_3$ and ligand, respectively, and $I_{max.}$ is the luminescence intensity for total complexation of the ligand.

$$I = \frac{I_{max}}{2.L_0}\left(Eu_0 + L_0 + \frac{1}{\alpha_H.K^{Eu}} - \sqrt{\left(Eu_0 + L_0 + \frac{1}{\alpha_H.K^{Eu}}\right)^2 - 4.Eu_0.L_0}\right) \qquad (3)$$

Equation (3) shows that, for a given complex and for fixed initial concentrations of lanthanide salt and ligand, the luminescence intensity I is a function only of the pH. In order to determine the stability constant of the complex studied (K^{Eu}), we thus prepared a set of samples at different pH values. Then, in order to furnish a constant amount of excitation energy to the system, we determined the isosbestic point from the absorption spectra of this set of samples (Figure 5). The presence of such a point was consistent with the assumption of a two-state chromophoric system: the free ligand and a unique mononuclear Eu^{III} chelate as previously examined. The set of samples was thus irradiated at the isosbestic point (262 nm) and gave a binding curve (Figure 6). The fitting of the theoretical curve obtained from Equation (3) to the experimental data provided a log K^{Eu} value of 9.54.

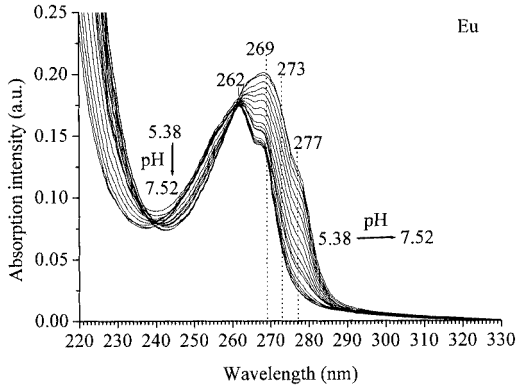


Figure 5. UV absorbance spectra of ligand **2a** (50.00 μM) in the presence of $EuCl_3$ (56.25 μM) in MES-buffered aqueous medium (0.015 M) and KCl (0.085 M); the pH varies from 5.38 to 7.52

2. Ln^{III} Complexes (Ln: La, Pr, Nd, Eu, Gd, Tb, Er, Yb, Lu): Stability Constants Determined by UV-Light Absorption Spectroscopy

Given that luminescence properties characterised only a few lanthanide cations, we were interested in developing an alternative method of evaluation of stability constants.

The deformation of the absorption spectrum of ligand **2a** induced by the addition of a lanthanide salt (see Figure 3) is consistent with the implication of the chromophoric group in the complexation process. As a consequence, UV/Vis absorption spectroscopy appeared to be a method of choice to determine stability constant of complexes, as it requires less unconventional laboratory equipment.

We have previously shown that a two-state chromophoric system occurs; as a consequence, for a fixed wavelength λ, the total absorbance of the solution A^{λ} is the sum of the absorbance of the uncomplexed chromophoric ligand $LH_{(4-i)}^{(1-i)+}$ ($0 \leq i \leq 4$) and that of the lanthanide chelate

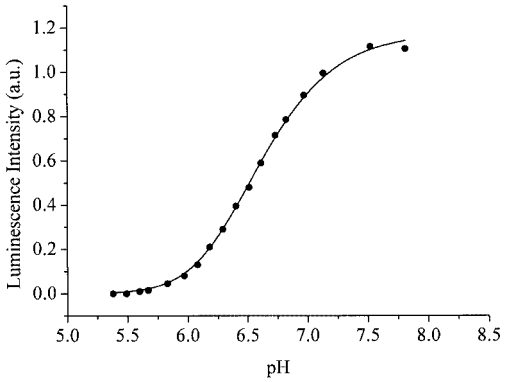


Figure 6. Binding curve resulting from monitoring of the luminescence intensity (615 nm) of samples containing 50.00 μM of ligand **2a** and $EuCl_3$ (56.25 μM) as a function of the pH (0.015 M MES-buffered solutions with 0.085 M of KCl); the luminescence results from UV light excitation at 262 nm; the full circles represent actual data points and the solid line represents the theoretical fit of data with log K^{Eu} = 9.54

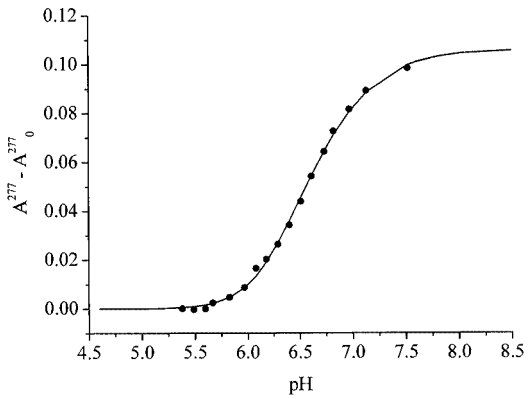


Figure 7. Binding curve resulting from monitoring of the relative absorbance at 277 nm ($A^{277} - A_0^{277}$) of samples containing 50.00 μM of ligand **2a** and $EuCl_3$ (56.25 μM) as a function of the pH (0.015 M MES-buffered solutions with 0.085 M of KCl); the full circles represent actual data points and the solid line represents the theoretical fit of data with log K^{Eu} = 9.59

LnL (see Equation (2)]. With the same assumptions as made before, and in addition that all species obey Beer's law and that all the uncomplexed forms of ligand $LH_{(4-i)}^{(1-i)+}$ have the same extinction coefficient, the absorbance A^{λ} can be expressed as a function of pH and K^{Ln} as given in Equation (4), where α_H^{-1} is the parameter defined in Equation (3), A_0^{λ} is the absorption of free ligand, A_{max}^{λ} is the maximum absorption corresponding to the total complexation of the ligand, and Ln_0 and L_0 are the initial concentrations of lanthanide salt $LnCl_3$ and ligand, respectively.

$$A^{\lambda} - A_0^{\lambda} = \frac{A_{max}^{\lambda} - A_0^{\lambda}}{2.L_0}\left(Ln_0 + L_0 + \frac{1}{\alpha_H.K^{Ln}} - \sqrt{\left(Ln_0 + L_0 + \frac{1}{\alpha_H.K^{Ln}}\right)^2 - 4.Ln_0.L_0}\right) \quad (4)$$

For a fixed wavelength λ, by plotting the variation of the absorption ($A^{\lambda} - A_0^{\lambda}$) as the function of the pH, a binding curve should be obtained and, by fitting of the theoretical curve obtained from Equation (4) to the experimental data, the sought-after log K^{Ln} value should be determined. To validate this approach, we first carried out the experiment with the Eu^{3+} cation (Figure 7). The agreement of the log K^{Eu} value found by the emission spectroscopic method and by the new technique encouraged us to determine the stability constants of other lanthanide complexes formed with ligand **2a**.

Analogous experiments were thus carried out with eight other lanthanide salts $LnCl_3$ (Ln: La, Pr, Nd, Gd, Tb, Er, Yb, Lu), and the results are listed in Table 1. On the whole, the affinity of Ln^{3+} cations for ligand **2a** rises slowly along the lanthanide series (Figure 8). The greatest difference occurs between the two extreme elements lanthanum and ytterbium, with a difference of about 1.5 in log K^{Ln} values. This result is consistent with those already reported, as the difference in log K^{Ln} obtained with macrocyclic polyaminoacetates never exceed 3.5.[1b,1c,22]

Table 1. Stability constants of the complexes formed with ligand **2a** and some lanthanide cations, determined at ambient temperature

Ln	pH range	Number of samples	Isosbestic point [nm]	log K^{Ln} [a]
La	7.57−5.64	13	261.0	8.7(2)
Pr	5.66−7.35	15	261.5	9.50(8)
Nd	5.53−7.53	15	262.0	9.50(2)
Eu	5.38−7.52	17	262.0	9.59(4)[b]
Gd	5.29−7.61	14	262.0	9.34(6)
Tb	7.54−5.66	12	261.0	9.68(6)
Er	7.56−5.58	14	262.0	10.00(6)
Yb	7.75−5.06	15	261.5	10.24(8)
Lu	7.58−5.52	12	261.5	10.07(7)

[a] Unless mentioned, the number in parentheses represents the error in the last digit based on nine experiments. [b] Error based on three experiments.

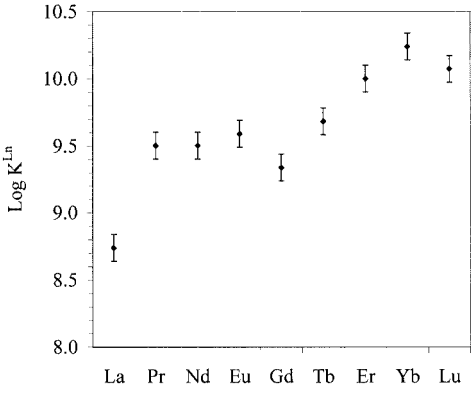


Figure 8. Stability constants of lanthanide complexes formed with ligand **2a**

Further Characterisation of the Luminescent Eu^{III} and Tb^{III} Complexes Formed with Ligand 2a

The rate constants for depopulation of the excited state and the overall quantum yields for Eu^{III} and Tb^{III} species were measured in H_2O and D_2O and are given in Table 2.

Table 2. Other properties of the complexes formed with ligand **2a** and the luminescent Eu^{3+} or Tb^{3+} cations

Complex	Rate constants [ms^{-1}]				Hydration state		Luminescence quantum yield (exp. error 10%)	
	k_{H2O} [a]	k_{D2O} [a]	Δk[b]	Δk_{corr} [c]	q[d]	q'_{corr} [e]	Φ_{H2O}	Φ_{D2O}
[Eu ⊂ **2a**]	2.56	0.64	1.92	1.67	2.0	2.00	0.015	0.064
[Tb ⊂ **2a**]	0.66	0.31	0.35	0.29	1.5	1.44	0.215	0.391

[a] Experimental error 5%. [b] $\Delta k = k_{H2O} - k_{D2O}$. [c] See ref.[23b]: corrections of -0.25 ms^{-1} and -0.06 ms^{-1} have been applied for europium and terbium species, respectively, to allow for the quenching effect of closely diffusing OH oscillators (outer-sphere water molecules). [d] See ref.[23a]: $q = A_{Ln} \cdot \Delta k$ with $A_{Eu} = 1.05$ ms and $A_{Tb} = 4.2$ ms; the uncertainty in q is approximately ± 0.5 water molecules. [e] See ref[23b]: $q'_{corr} = A_{Ln'} \cdot \Delta k_{corr}$ with $A_{Eu'} = 1.2$ ms and $A_{Tb'} = 5$ ms.

The measurement of the rates of depopulation allows the estimation of the hydration state of the lanthanide complexes in solution.[23] For the europium species, the values obtained with empirical equations established by Horrocks or Parker are consistent with the presence of two coordinated water molecules. The nonintegral values found for the terbium species suggest that other considerations should be taken into account in order to establish the correction factor. However, the presence of at least one water molecule in the first coordination sphere is consistent with the higher value of the luminescence quantum yield measured in D_2O than in H_2O, as it reflects the deactivating effect of vibrational quenching by OH oscillators coordinated to the metal ion. Finally, both of these results are in agreement with the expected hepta-coordinating nature of ligand **2a**, since lanthanide cations prefer coordination numbers of 8 or 9.[7] Moreover, these complexes appears to be potential candidates for labelling purposes, as the luminescence quantum yields are consistent with those reported for related macrocyclic triacetic ligand species.[24,25]

Conclusion

In order to evaluate the influence of the size of the chelation rings on the stability of complexes formed between lanthanide cations and azapyridinomacrocycles bearing three alkanoic acid side chains, four 14-membered macrocycles **2a**−**d** were synthesised. Compounds **2a** and **2b**, with identical side chains, were straightforwardly obtained by alkylation of the parent macrocycle **3**, while compounds **2c** and **2d**, with mixed pendent arms, required the preparation of appropriate triamine blocks.

The characterisation of these ligands in terms of complexing ability in aqueous medium revealed the dramatic effect of the enlargement of the pendent arms. Indeed, the acetate derivative **2a** was able to form stable complexes with lanthanide cations ($8.7 \leq \log K^{Ln} \leq 10.2$), while no complexation occurred with the homologous propionate **2b**. Moreover, ligand **2a** exhibited a slight selectivity of complexation along the lanthanide series ($\Delta \log K^{Ln} \approx 1.5$). This result encouraged us to pursue this project; the study of the influence of the nature of the ligating side chains is now in progress.

Experimental Section

General Remarks

Syntheses of the Ligands: All reactions were monitored for completion by thin layer chromatography (TLC) performed on precoated silica gel plates (Merck 60 F_{254}); TLC plates were viewed under UV and in an iodine chamber. Preparative chromatography was performed by elution from columns of Silica Gel 60 (particle size 0.040−0.063 mm). ^1H and ^{13}C NMR spectra were recorded with a Bruker AM 400 spectrometer; external references: for solutions in $CDCl_3$ and CD_3OD: TMS (δ =0.00 ppm for both ^1H and ^{13}C); for solutions in D_2O, dioxane (δ = 67.4 ppm for ^{13}C) and 3-trimethylsilyl-1-propanesulfonic acid sodium salt (δ =0.00 ppm for ^1H). Proton signal assignments were made by first-order analysis of the spectra and analysis of 2D ^1H-^1H correlation maps (COSY). The ^{13}C NMR assignments were supported by 2D ^{13}C-^1H correlation maps (HETCOR). In the assignments of these NMR signals. "Nos" is used as the abbreviation for 2-nitrobenzenesulfonyl. Coupling constants J are measured in Hz. Coupling patterns are described by abbreviations: s (singlet), d (doublet), t (triplet), dd (doublet of a doublet), m (multiplet), bs (broad singlet). High-resolution mass spectra were taken in the positive-ion mode either by chemical ionisation (CI-HRMS) with CH_4 as the ionising gas or by fast atom bombardment (FAB-HRMS) with "Magic Bullet" as the matrix. Unless stated otherwise, all reagents were purchased from commercial sources and were used without additional purification.

Characterisation of Ligand 2a: The UV/Vis spectra were recorded with a Cary-100 Scan spectrophotometer operating with Cary-WinUV software. The UV-light-induced Eu^{III} luminescence measurements were recorded with an SLM Aminco 8100 spectrofluorimeter; corrected spectra were obtained with account being taken of the wavelength-dependent response of the instrument. The data analyses were performed by use of an iterative least-squares fitting procedure; this was done with the Solver function of Microsoft Excel® which applies a nonlinear regression method. The protonation constants of ligand **2a** used in these calculations were 10.27, 7.90, 5.18 and 2.4.[9a] The fluorescence quantum yields were measured at 293 K and determined by comparison with that of quinine sulfate in aqueous sulfuric acid solution (1 N), for which a reference yield of 0.55 was taken.[26] The luminescence lifetimes measurements were carried out at 293 K and were determined at 600 nm by excitation with a B.M. Industries frequency-quadrupled Nd-YAG laser (266 nm, pulse duration 8 ns FWHM) with use of a Tektronix 9310 oscilloscope to monitor the output signal of the photomultiplier. The water was distilled and then deionised with a Millipore system, and this was used throughout. All glassware was thoroughly rinsed with such a treated water. All $LnCl_3$ salts were

purchased from Aldrich Chemical Co as their purest hexa-or heptahydrated form (≥ 99.9%) and were used as received. [4-(2-*H*ydroxy*e*thyl)-1-*p*iperazine*e*thane*s*ulfonic acid (HEPES) and 2-(*N*-*m*orpholino)*e*thane*s*ulfonic acid (MES) were purchased as sodium salts from Aldrich Chemical and Sigma Co, respectively. KCl was purchased from Aldrich Chemical Co in its purest form. The pH values of the solutions were measured with a pHM 220 Radiometer Copenhagen pH-meter. Stock solutions (ca. 10 mM) of $LnCl_3$ (Ln = La, Pr, Nd, Eu, Gd, Tb, Er, Yb, Lu) were titrated by ICP−AES at the CNRS Service Central d'Analyse (Département d'Analyse Elémentaire, 69390 Vernaison, France). The concentration of the ligand stock solution (ca. 0.1 M) was determined by titration with standardised Eu^{III} solution at pH = 8 on equilibrated samples through sensitised Eu^{III} luminescence.

1. Preparation of the Functionalised Triamines 8a and 8b

***N*-{3-[3-(2-Nitrobenzenesulfonylamino)propylamino]propyl}-2-nitrobenzenesulfonamide (6):** A solution of 2-nitrobenzenesulfonyl chloride (21.28 g, 96.0 mmol, 2 equiv.) in THF (350 mL) was added dropwise at 0 °C under argon (over 5 h) to a suspension of 1,7-diamino-4-azaheptane (7 mL, 49.1 mmol) and anhydrous $NaHCO_3$ (16.3 g, 194 mmol) in anhydrous THF (350 mL). The resulting mixture was warmed to room temperature and stirred overnight. The crude mixture was concentrated under reduced pressure and then taken up in water (300 mL) and CH_2Cl_2 (800 mL). The aqueous layer was further extracted with CH_2Cl_2 (2 × 200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was chromatographed on silica gel (CH_2Cl_2/MeOH, 92:8 to 90:10) and gave the title compound **6** as a white solid (13.9 g, 27.65 mmol, 56%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.72 [m, 4 H, NH($CH_2CH_2CH_2NHNos)_2$], 2.71 [t, $^3J_{H,H}$ = 6.1 Hz, 4 H, NH($CH_2CH_2CH_2NHNos)_2$], 3.20 [t, $^3J_{H,H}$ = 6.1 Hz, 4 H, NH($CH_2CH_2CH_2NHNos)_2$], 7.69−7.76 (m, 4 H, CH_{Ar}), 7.82−7.84 (m, 2 H, CH_{Ar}), 8.12−8.14 (m, 2 H, CH_{Ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 28.6 [NH($CH_2CH_2CH_2NHNos)_2$], 43.2 [NH($CH_2CH_2CH_2NHNos)_2$], 48.1 [NH($CH_2CH_2CH_2NHNos)_2$], 125.2, 131.2, 132.7, 133.4 (CH_{Ar}), 133.5, 148.0 ($C_{quat,Ar}$) ppm. CI-HRMS: *m*/*z* calcd. for $C_{18}H_{24}N_5O_8S_2$ 502.1066, found 502.1064 $[M + H]^+$.

***tert*-Butyl {Bis[3-(2-nitrobenzenesulfonylamino)propyl]amino}acetate (8a):** *tert*-Butyl bromoacetate (7.5 mL, 46.14 mmol, 3 equiv.) was added to a solution of triamine **6** (7.83 g, 15.61 mmol) and triethylamine (13 mL, 101.5 mmol) in THF (120 mL). The reaction mixture was heated at reflux for 24 h and was then concentrated under reduced pressure. The crude oil obtained was purified by chromatography on silica gel (*n*-heptane/EtOAc, 5:5 to 2:8); the title compound **8a** was obtained as a yellow oil (8.17 g, 13.27 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.40 [s, 9 H, C($CH_3)_3$], 1.71 [m, 4 H, N($CH_2CH_2CH_2NHNos)_2$], 2.59 [t, $^3J_{H,H}$ = 6.3 Hz, 4 H, N($CH_2CH_2CH_2NHNos)_2$], 3.15 (s, 2 H, NCH_2CO_2tBu), 3.19 [broad m, 4 H, N($CH_2CH_2CH_2NHNos)_2$], 6.45 (broad m, 2 H, N*H*), 7.67−7.74 (m, 4 H, CH_{Ar}), 7.79−7.81 (m, 2 H, CH_{Ar}), 8.10−8.13 (m, 2 H, CH_{Ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 26.7 [N($CH_2CH_2CH_2NHNos)_2$], 28.1 [C($CH_3)_3$], 42.7 [N($CH_2CH_2CH_2NHNos)_2$], 52.2 [N($CH_2CH_2CH_2NHNos)_2$], 55.5 (NCH_2CO_2tBu), 81.5 [$C(CH_3)_3$], 125.1, 131.0, 132.7, 133.3 (CH_{Ar}), 133.7, 148.0 ($C_{quat,Ar}$), 170.4 (CO_2tBu) ppm. CI-HRMS: *m*/*z* calcd. for $C_{24}H_{34}N_5O_{10}S_2$ 616.1747, found 616.1735 $[M + H]^+$.

***tert*-Butyl 3-{Bis[3-(2-nitrobenzenesulfonylamino)propyl]amino}-propionate (8b):** A solution of *tert*-butyl acrylate (50 mL, 0.34 mol, 30 equiv.) in a 2:1 mixture of CH_2Cl_2/MeOH (30 mL) was added to a solution of triamine **6** (5.68 g, 11.32 mmol) in a 1:1 mixture of CH_2Cl_2/MeOH (15 mL). After 24 h of stirring at 70 °C, the crude mixture was concentrated under reduced pressure. The orange oil obtained was purified by chromatography on silica gel (EtOAc/*n*-heptane, 5:5 to 10:0) and yielded the title compound **8b** as a yellow oil (6.49 g, 10.31 mmol, 91%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.41 [s, 9 H, C($CH_3)_3$], 1.70 [m, 4 H, N(CH_2CH_2-$CH_2NHNos)_2$], 2.30 (t, $^3J_{H,H}$ = 7.3 Hz, 2 H, $NCH_2CH_2CO_2tBu$), 2.46 [t, $^3J_{H,H}$ = 6.3 Hz, 4 H, N($CH_2CH_2CH_2NHNos)_2$], 2.71 (t, $^3J_{H,H}$ = 7.3 Hz, 2 H, $NCH_2CH_2CO_2tBu$), 3.14 [t, $^3J_{H,H}$ = 6.3 Hz, 4 H, N($CH_2CH_2CH_2NHNos)_2$], 6.24 (broad m, 2 H, N*H*), 7.70−7.75 (m, 4 H, CH_{Ar}), 7.82−7.84 (m, 2 H, CH_{Ar}), 8.10−8.12 (m, 2 H, CH_{Ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 26.3 [N($CH_2CH_2CH_2NHNos)_2$], 28.1 [C($CH_3)_3$], 32.1 ($NCH_2CH_2CO_2tBu$), 42.8 [N($CH_2CH_2CH_2NHNos)_2$], 48.8 ($NCH_2CH_2CO_2tBu$), 51.6 [N($CH_2CH_2CH_2NHNos)_2$], 80.7 [$C(CH_3)_3$], 125.2, 131.0, 132.7, 133.5 (CH_{Ar}), 133.6, 148.0 ($C_{quat,Ar}$), 171.9 (CO_2tBu) ppm. CI-HRMS: *m*/*z* calcd. for $C_{25}H_{36}N_5O_{10}S_2$ 630.1904, found 630.1891 $[M + H]^+$.

2. Preparation of the Triamines 9a and 9b. General Procedure for the Cleavage of Sulfonamide Subunits from Functionalised Triamines 8a and 8b: In a typical procedure, a solution of triamine **8a** or **8b** (3.06 mmol) in anhydrous DMF (50 mL) was cannulated into a three-necked flask containing anhydrous Na_2CO_3 (2.57 g, 24.25 mmol). Thiophenol (1.0 mL, 9.74 mmol) was then added and the suspension was stirred at room temperature under argon overnight. The reaction mixture was concentrated under reduced pressure, and the obtained crude orange oil was pre-purified by filtration on a silica gel pad [CH_2Cl_2/MeOH, 10:0 to 0:10 then MeOH/aq. NH_3 (30%), 7:3]. The resulting oil was further purified by chromatography on silica gel [CH_2Cl_2/MeOH, 5:5 to 0:10 then MeOH/aq. NH_3 (30%), 7:3].

***tert*-Butyl [Bis(3-aminopropyl)amino]acetate (9a):** The title compound was obtained from amine **8a** as an amorphous, white solid (0.751 g, 3.06 mmol, quantitative yield). ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 1.47 [s, 9 H, C($CH_3)_3$], 1.66 [m, 4 H, N($CH_2CH_2CH_2NH_2)_2$], 2.58 [t, $^3J_{H,H}$ = 6.9 Hz, 4 H, N($CH_2CH_2CH_2NH_2)_2$], 2.76 [t, $^3J_{H,H}$ = 6.7 Hz, 4 H, N($CH_2CH_2CH_2NH_2)_2$], 3.22 (s, 2 H, NCH_2CO_2tBu) ppm. ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 28.4 [C($CH_3)_3$], 29.7 [N($CH_2CH_2CH_2NH_2)_2$], 40.9 [N($CH_2CH_2CH_2NH_2)_2$], 53.5 [N($CH_2CH_2CH_2NH_2)_2$], 57.1 (NCH_2CO_2tBu), 82.5 [$C(CH_3)_3$], 173.0 (CO_2tBu) ppm. CI-HRMS: *m*/*z* calcd. for $C_{12}H_{28}N_3O_2$ 246.2182, found 246.2187 $[M + H]^+$.

***tert*-Butyl 3-[Bis(3-aminopropyl)amino]propionate (9b):** The title compound was obtained from amine **8b** as an amorphous, white solid (0.365 g, 1.41 mmol, 46%). ^{1}H NMR (400 MHz, CD_3OD, 25 °C): δ = 1.45 [s, 9 H, C($CH_3)_3$], 1.62 [m, 4 H, N($CH_2CH_2CH_2NH_2)_2$], 2.38 (t, $^3J_{H,H}$ = 7.1 Hz, 2 H, $NCH_2CH_2CO_2tBu$), 2.47 [t, $^3J_{H,H}$ = 7.2 Hz, 4 H, N($CH_2CH_2CH_2NH_2)_2$], 2.65 [t, $^3J_{H,H}$ = 7.0 Hz, 4 H, N($CH_2CH_2CH_2NH_2)_2$], 2.73 (t, $^3J_{H,H}$ = 7.1 Hz, 2 H, $NCH_2CH_2CO_2tBu$) ppm. ^{13}C NMR (100 MHz, CD_3OD, 25 °C): δ = 28.4 [C($CH_3)_3$], 31.0 [N($CH_2CH_2CH_2NH_2)_2$], 34.3 ($NCH_2CH_2CO_2tBu$), 41.0 [N($CH_2CH_2CH_2NH_2)_2$], 50.6 ($NCH_2CH_2CO_2tBu$), 52.6 [N($CH_2CH_2CH_2NH_2)_2$], 81.6 [$C(CH_3)_3$], 173.8 (CO_2tBu) ppm. CI-HRMS: *m*/*z* calcd. for $C_{13}H_{30}N_3O_2$ 260.2338, found 260.2337 $[M + H]^+$.

3. General Procedure for Macrocyclisations. Treatment of 2,6-Bis-(bromomethyl)pyridine with Disulfonamides 8a and 8b: In a typical procedure, a suspension of the disulfonamide **8a** or **8b** (3.89 mmol) and anhydrous Na_2CO_3 (2.15 g, 20.3 mmol) was stirred in anhy-

drous DMF (100 mL) at 100 °C under argon for 2 h. A solution of 2,6-bis(bromomethyl)pyridine (1.31 g, 4.86 mmol) in anhydrous DMF (100 mL) was then added dropwise (during 5 h) and the mixture was stirred for an additional 1 h. The crude mixture was concentrated and the residue was taken up in CH_2Cl_2 (250 mL). The organic phase was washed with an aqueous solution of NaOH (0.1 M, 2 × 70 mL) and was then dried with sodium sulfate and concentrated.

***tert*-Butyl {3,11-Bis(2-nitrobenzenesulfonyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-7-yl}acetate (14a):** The title compound was obtained from the sulfonamide **8a** as a light brown, amorphous solid (1.99 g, 2.77 mmol, 71%) after chromatography on silica gel (*n*-heptane/EtOAc, 4:6 to 0:10). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.37−1.45 [m, 13 H, C(CH_3)$_3$ and N(CH$_2$CH_2CH$_2$NNos)$_2$], 2.39 [t, $^3J_{H,H}$ = 6.7 Hz, 4 H, N(CH_2CH$_2$CH$_2$NNos)$_2$], 3.00 (s, 2 H, NCH_2CO$_2$*t*Bu), 3.30 [t, $^3J_{H,H}$ = 7.6 Hz, 4 H, N(CH$_2$CH$_2$CH_2NNos)$_2$], 4.56 (s, 4 H, C$_{Pyr}$CH_2NNos), 7.45 [d, $^3J_{H,H}$ = 7.7 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 7.62−7.73 [m, 7 H, CH_{Ar} (Nos) and *p*-CH_{Ar} (Pyr)], 8.00−8.03 [m, 2 H, CH_{Ar} (Nos)] ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 26.7 [N(CH$_2$*C*H$_2$CH$_2$NNos)$_2$], 28.2 [C(*C*H$_3$)$_3$], 47.0 [N(CH$_2$CH$_2$*C*H$_2$NNos)$_2$], 51.2 [N(*C*H$_2$CH$_2$CH$_2$NNos)$_2$], 54.2 (C$_{Pyr}$*C*H$_2$NNos), 57.8 (N*C*H$_2$CO$_2$*t*Bu), 80.9 [*C*(CH$_3$)$_3$], 123.2 [*m*-*C*H$_{Ar}$ (Pyr)], 124.2, 130.6, 131.8 [*C*H$_{Ar}$ (Nos)], 133.1 [C$_{quat,Ar}$ (Nos)], 133.6 [*C*H$_{Ar}$ (Nos)], 138.3 [*p*-*C*H$_{Ar}$ (Pyr)], 148.1 [C$_{quat,Ar}$ (Nos)], 155.9 [C$_{quat,Ar}$ (Pyr)], 170.6 (*C*O$_2$*t*Bu) ppm. CI-HRMS: *m*/*z* calcd. for $C_{31}H_{39}N_6O_{10}S_2$ 719.2169, found 719.2171 [M + H]$^+$.

***tert*-Butyl 3-{3,11-Bis(2-nitrobenzenesulfonyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-7-yl}propionate (14b):** The title compound was obtained from sulfonamide **8b** as a light orange oil (1.46 g, 1.99 mmol, 51%) after chromatography on silica gel (*n*-heptane/EtOAc, 4:6 to 0:10). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.31 [s, 9 H, C(CH_3)$_3$], 1.39 [m, 4 H, N(CH$_2$CH_2CH$_2$NNos)$_2$], 2.13−2.17 [m, 6 H, N(CH_2CH$_2$CH$_2$NNos)$_2$(CH$_2$CH_2CO$_2$*t*Bu) or N(CH$_2$CH$_2$CH_2NNos)$_2$(CH$_2$CH_2CO$_2$*t*Bu)], 2.48 (t, $^3J_{H,H}$ = 6.8 Hz, 2 H, NCH_2CH$_2$CO$_2$*t*Bu), 3.26 [t, $^3J_{H,H}$ = 7.4 Hz, 4 H, N(CH_2CH$_2$CH$_2$NNos)$_2$ or N(CH$_2$CH$_2$CH_2NNos)$_2$], 4.54 (s, 4 H, C$_{Pyr}$CH_2NNos), 7.43 [d, $^3J_{H,H}$ = 7.7 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 7.62−7.73 [m, 7 H, CH_{Ar} (Nos) and *p*-CH_{Ar} (Pyr)], 8.00−8.02 [m, 2 H, CH_{Ar} (Nos)] ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 26.2 [N(CH$_2$*C*H$_2$CH$_2$NNos)$_2$], 28.1 [C(*C*H$_3$)$_3$], 33.8 (NCH$_2$*C*H$_2$CO$_2$*t*Bu), 47.3, 50.7 [N(*C*H$_2$CH$_2$*C*H$_2$NNos)$_2$], 51.1 (N*C*H$_2$CH$_2$CO$_2$*t*Bu), 54.4 (C$_{Pyr}$*C*H$_2$NNos), 80.2 [*C*(CH$_3$)$_3$], 123.1 [*m*-*C*H$_{Ar}$ (Pyr)], 124.2, 130.6, 131.8 [*C*H$_{Ar}$ (Nos)], 133.0 [C$_{quat,Ar}$ (Nos)], 133.7 [*C*H$_{Ar}$ (Nos)], 138.2 [*p*-*C*H$_{Ar}$ (Pyr)], 148.1 [C$_{quat,Ar}$ (Nos)], 156.0 [C$_{quat,Ar}$ (Pyr)], 171.8 (*C*O$_2$*t*Bu) ppm. CI-HRMS: *m*/*z* calcd. for $C_{32}H_{41}N_6O_{10}S_2$ 733.2326, found 733.2326 [M + H]$^+$.

4. General Procedure for the Cleavage of Sulfonamide Subunits from Functionalised Macrocycles 14a and 14b: In a typical procedure, a solution of macrocycle **14a** or **14b** (2.96 mmol) in anhydrous DMF (200 mL) was cannulated into a three-necked flask containing anhydrous Na_2CO_3 (3.20 g, 30.22 mmol). Thiophenol (1 mL, 9.74 mmol) was then added.

***tert*-Butyl {3,7,11,17-Tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-7-yl}acetate (10a):** After stirring overnight at room temperature, the reaction mixture was concentrated and then taken up in CH_2Cl_2 (200 mL). The organic layer was washed with water (100 mL). The aqueous layer was further extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried (Na_2SO_4) and then concentrated under reduced pressure. The crude product was purified by chromatography on silica gel [CH_2Cl_2/MeOH, 10:0 to 0:10, then MeOH/aq. NH_3 (32%), 7:3]. The chromatographed product was dissolved in CH_2Cl_2 (100 mL) and the cloudy solution was filtered. The filtrate was concentrated, yielding the title compound **10a** as a light brown oil (0.990 g, 2.81 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.42 [s, 9 H, C(CH_3)$_3$], 1.74 [m, 4 H, N(CH$_2$CH_2CH$_2$NH)$_2$], 2.50 [t, $^3J_{H,H}$ = 5.8 Hz, 4 H, N(CH$_2$CH$_2$CH_2NH)$_2$], 2.55 [t, $^3J_{H,H}$ = 5.8 Hz, 4 H, N(CH_2CH$_2$CH$_2$NH)$_2$], 3.14 (s, 2 H, NCH_2CO$_2$*t*Bu), 3.88 (s, 4 H, C$_{Pyr}$CH_2NH), 6.98 (d, $^3J_{H,H}$ = 7.6 Hz, 2 H, *m*-CH_{Ar}), 7.52 (dd, $^3J_{H,H}$ = $^3J_{H,H'}$ = 7.6 Hz, 1 H, *p*-CH_{Ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 27.0 [N(CH$_2$*C*H$_2$CH$_2$NH)$_2$], 28.2 [C(*C*H$_3$)$_3$], 46.3 [N(CH$_2$CH$_2$*C*H$_2$NH)$_2$], 52.0 [N(*C*H$_2$CH$_2$CH$_2$NH)$_2$], 54.3 (C$_{Pyr}$*C*H$_2$NH), 55.3 (N*C*H$_2$CO$_2$*t*Bu), 80.9 [*C*(CH$_3$)$_3$], 120.4 (*m*-*C*H$_{Ar}$), 136.4 (*p*-*C*H$_{Ar}$), 159.1 (C$_{quat,Ar}$), 170.7 (*C*O$_2$*t*Bu) ppm. CI-HRMS: *m*/*z* calcd. for $C_{19}H_{33}N_4O_2$ 349.2604, found 349.2599 [M + H]$^+$.

***tert*-Butyl 3-{3,7,11,17-Tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-7-yl}propionate (10b):** After 5 h of stirring at room temperature, the Na_2CO_3 was filtered off through a Celite® pad and washed with CH_2Cl_2. The filtrate was concentrated and the crude product was purified by chromatography on neutral activated aluminium oxide (CH_2Cl_2/MeOH, 10:0 to 8:2) to yield the title compound **10b** as an orange oil (0.901 g, 2.48 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.32 [s, 9 H, C(CH_3)$_3$], 1.73 [m, 4 H, N(CH$_2$CH_2CH$_2$NH)$_2$], 2.24 (t, $^3J_{H,H}$ = 7.2 Hz, 2 H, NCH$_2$CH_2CO$_2$*t*Bu), 2.41 [t, $^3J_{H,H}$ = 5.7 Hz, 4 H, N(CH_2CH$_2$CH$_2$NH)$_2$], 2.53 [t, $^3J_{H,H}$ = 6.2 Hz, 4 H, N(CH$_2$CH$_2$CH_2NH)$_2$], 2.65 (t, $^3J_{H,H}$ = 7.2 Hz, 2 H, NCH_2CH$_2$CO$_2$*t*Bu), 3.90 (s, 4 H, C$_{Pyr}$CH_2NH), 6.99 (d, $^3J_{H,H}$ = 7.6 Hz, 2 H, *m*-CH_{Ar}), 7.53 (dd, $^3J_{H,H}$ = $^3J_{H,H'}$ = 7.6 Hz, 1 H, *p*-CH_{Ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 27.2 [N(CH$_2$*C*H$_2$CH$_2$NH)$_2$], 28.0 [C(*C*H$_3$)$_3$], 31.5 (NCH$_2$*C*H$_2$CO$_2$*t*Bu), 46.1 [N(CH$_2$CH$_2$*C*H$_2$NH)$_2$], 47.7 (N*C*H$_2$CH$_2$CO$_2$*t*Bu), 51.4 [N(*C*H$_2$CH$_2$CH$_2$NH)$_2$], 53.9 (C$_{Pyr}$*C*H$_2$NH), 80.2 [*C*(CH$_3$)$_3$], 120.7 (*m*-*C*H$_{Ar}$), 136.7 (*p*-*C*H$_{Ar}$), 158.6 (C$_{quat,Ar}$), 172.1 (*C*O$_2$*t*Bu) ppm. CI-HRMS: *m*/*z* calcd. for $C_{20}H_{35}N_4O_2$ 363.2760, found 363.2757 [M + H]$^+$.

5. *N*-Alkylations of Macrocyclic Intermediates

***tert*-Butyl {3,11-Bis(*tert*-butoxycarbonylmethyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-7-yl}acetate (4a):** *tert*-Butyl bromoacetate (2.3 mL, 15.58 mmol, 12 equiv.) was added to a suspension of compound **3** (0.301 g, 1.28 mmol) and Ag_2CO_3 (0.899 g, 3.26 mmol, 2.5 equiv.) in anhydrous THF (10 mL). After 1 h of stirring at room temperature, the reaction mixture was cooled and acidified to pH = 1 with an aqueous solution of HCl (ca. 6 M, 2.5 mL, ca. 15 mmol). The mixture was stirred at 0 °C for an additional 0.5 h, and $Na_2S·9H_2O$ (10.6 g, 44.1 mmol) was then added. After dilution with H_2O (5 mL), the basic suspension was stirred for 3 h and was then warmed to room temperature. The black solid was filtered through a Celite® pad and was then washed with CH_2Cl_2. The filtrate was concentrated and the resulting aqueous phase was extracted with CH_2Cl_2 (5 × 40 mL). The organic phase was dried (Na_2SO_4) and concentrated to give a crude orange oil that was purified by chromatography on neutral activated aluminium oxide (*n*-heptane/EtOAc, 7:3 to 5:5) to give the title compound **4a** as a yellow oil (0.603 g, 1.04 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.34 [s, 9 H, N(CH$_2$CH$_2$CH$_2$-NCH$_2$CO$_2$*t*Bu)$_2${CH$_2$CO$_2$C(CH_3)$_3$}], 1.39−1.44 [m, 22 H, N{CH$_2$CH_2CH$_2$NCH$_2$CO$_2$C(CH_3)$_3$}$_2$], 2.45 [broad t, 4 H, N(CH_2CH$_2$CH$_2$NCH$_2$CO$_2$*t*Bu)$_2$ or N(CH$_2$CH$_2$CH_2NCH$_2$CO$_2$*t*-Bu)$_2$], 2.55 [t, $^3J_{H,H}$ = 7.3 Hz, 4 H, N(CH_2CH$_2$CH$_2$NCH$_2$CO$_2$*t*Bu)$_2$ or N(CH$_2$CH$_2$CH_2NCH$_2$CO$_2$*t*Bu)$_2$], 2.92 [s, 2 H, N(CH$_2$-

$CH_2CH_2NCH_2CO_2tBu)_2(CH_2CO_2tBu)$], 3.33 [s, 4 H, $N(CH_2CH_2CH_2NCH_2CO_2tBu)_2$], 3.86 (s, 4 H, $C_{Pyr}CH_2N$), 7.19 [d, $^3J_{H,H}$ = 7.6 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 7.54 [dd, $^3J_{H,H}$ = $^3J_{H,H'}$ = 7.6 Hz, 1 H, *p*-CH_{Ar} (Pyr)] ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 25.4 [$N(CH_2CH_2CH_2NCH_2CO_2tBu)_2$], 28.1, 28.2 [$N\{CH_2CH_2CH_2NCH_2CO_2C(CH_3)_3\}_2\{CH_2CO_2C(CH_3)_3\}$], 50.6, 51.5 [$N(CH_2CH_2CH_2NCH_2CO_2tBu)_2$], 56.6 [$N(CH_2CH_2CH_2NCH_2CO_2tBu)_2(CH_2CO_2tBu)$], 57.3 [$N(CH_2CH_2CH_2NCH_2CO_2tBu)_2$], 60.6 ($C_{Pyr}CH_2N$), 80.4, 80.8 [$\{N(CH_2CH_2CH_2NCH_2CO_2C(CH_3)_3\}_2\{CH_2CO_2C(CH_3)_3\}$], 122.8 (*m*-$CH_{Ar}$), 136.6 (*p*-$CH_{Ar}$), 158.1 ($C_{quat,Ar}$), 170.7, 170.9 [$N(CH_2CH_2CH_2NCH_2CO_2tBu)_2(CH_2CO_2tBu)$] ppm. FAB-HRMS: *m*/*z* calcd. for $C_{31}H_{53}N_4O_6$ 577.3965, found 577.3959 $[M + H]^+$.

***tert*-Butyl 3-{3,11-Bis[2-(*tert*-butoxycarbonyl)ethyl]-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-7-yl}propionate (4b):** *tert*-Butyl acrylate (50 mL, 341 mmol) was added to a solution of compound **3** (6.01 g, 25.65 mmol, 13 equiv.) in MeOH (7 mL). After 40 h of stirring at 70 °C with protection from light, the crude mixture was concentrated under reduced pressure. The obtained crude dark brown oil was purified by chromatography on silica gel (CH_2Cl_2/EtOAc, 10:0 to 0:10), and the title compound **4b** was isolated as an orange oil (14.37 g, 23.22 mmol, 90%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.29−1.34 {m, 13 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2[CH_2CH_2CO_2C(CH_3)_3]\}$, 1.42 {s, 18 H, $N[CH_2CH_2CH_2NCH_2CH_2CO_2C(CH_3)_3]_2\}$, 2.07 (t, $^3J_{H,H}$ = 7.3 Hz, 2 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2(CH_2CH_2CO_2tBu)$, 2.16 [t, $^3J_{H,H}$ = 6.2 Hz, 4 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 2.39−2.46 [m, 10 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2(CH_2CH_2CO_2tBu)$], 2.89 [t, $^3J_{H,H}$ = 7.2 Hz, 4 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 3.66 (s, 4 H, $C_{Pyr}CH_2N$), 7.15 [d, $^3J_{H,H}$ = 7.6 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 7.51 [dd, $^3J_{H,H}$ = $^3J_{H,H'}$ = 7.6 Hz, 1 H, *p*-CH_{Ar} (Pyr)] ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 24.7 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 28.1, 28.2 $\{N[CH_2CH_2CH_2NCH_2CH_2CO_2C(CH_3)_3]_2[CH_2CH_2CO_2C(CH_3)_3]\}$, 33.6 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2(CH_2CH_2CO_2tBu)$], 33.9 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 50.6 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 51.0 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2(CH_2CH_2CO_2tBu)$], 51.5 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 51.8 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 60.5 ($C_{Pyr}CH_2N$), 79.8, 80.2 $\{N[CH_2CH_2CH_2NCH_2CH_2CO_2C(CH_3)_3]_2[CH_2CH_2CO_2C(CH_3)_3]\}$, 122.7 (*m*-$CH_{Ar}$), 136.4 (*p*-$CH_{Ar}$), 158.4 ($C_{quat,Ar}$), 172.1 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2(CH_2CH_2CO_2tBu)$] ppm. FAB-HRMS: *m*/*z* calcd. for $C_{34}H_{59}N_4O_6$ 619.4435, found 619.4432 $[M + H]^+$.

***tert*-Butyl 3-{3,11-Bis(*tert*-butoxycarbonylmethyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-7-yl}propionate (4c):** *tert*-Butyl bromoacetate (0.6 mL, 4.06 mmol) was added to a suspension of compound **10b** (0.266 g, 0.734 mmol) and Ag_2CO_3 (0.347 g, 1.258 mmol) in THF (20 mL). After 1 h of stirring at room temperature, the crude mixture was cooled and acidified with an aqueous solution of HCl (3 M, 1 mL). $Na_2S{\cdot}9H_2O$ (0.6 g, 2.50 mmol) was then added, the mixture was stirred for 3 h, and the black solid was filtered through a Celite® pad and then washed with CH_2Cl_2. The filtrate was concentrated and the crude product was purified by chromatography on neutral activated aluminium oxide (gradient: CH_2Cl_2 then *n*-heptane/EtOAc, 10:0 to 7:3) to give the title compound **4c** as a yellow oil (0.247 g, 0.418 mmol, 57%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.37 [s, 9 H, $NCH_2CH_2CO_2C(CH_3)_3$], 1.39−1.53 {m, 22 H, $N[CH_2CH_2CH_2NCH_2CO_2C(CH_3)_3]_2\}$; 2.12 (t, $^3J_{H,H}$ = 7.3 Hz, 2 H, $NCH_2CH_2CO_2tBu$), 2.25 [t, $^3J_{H,H}$ = 6.0 Hz, 4 H, $N(CH_2CH_2CH_2NCH_2CO_2tBu)_2$], 2.50 (t, $^3J_{H,H}$ = 7.3 Hz, 2 H, $NCH_2CH_2CO_2tBu$), 2.56 [t, $^3J_{H,H}$ = 7.4 Hz, 4 H, $N(CH_2CH_2CH_2NCH_2CO_2tBu)_2$], 3.38 (s, 4 H, NCH_2CO_2tBu), 3.92 (s, 4 H, $C_{Pyr}CH_2N$), 7.24 [d, $^3J_{H,H}$ = 7.6 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 7.58 [dd, $^3J_{H,H}$ = $^3J_{H,H}$ = 7.6 Hz, 1 H, *p*-CH_{Ar} (Pyr)] ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 25.2 [$N(CH_2CH_2CH_2NCH_2CO_2tBu)_2$], 28.1, 28.3 [$NCH_2CO_2C(CH_3)_3$ and $NCH_2CH_2CO_2C(CH_3)_3$], 33.8 ($NCH_2CH_2CO_2tBu$), 50.7 ($NCH_2CH_2CO_2tBu$), 51.0 [$N(CH_2CH_2CH_2NCH_2CO_2tBu)_2$], 51.6 [$N(CH_2CH_2CH_2NCH_2CO_2tBu)_2$], 57.5 ($NCH_2CO_2tBu$), 60.6 ($C_{Pyr}CH_2N$), 80.9 [$NCH_2CO_2C(CH_3)_3$ and $NCH_2CH_2CO_2C(CH_3)_3$], 122.8 (*m*-CH_{Ar}), 136.6 (*p*-CH_{Ar}), 158.2 ($C_{quat,Ar}$), 170.8 (NCH_2CO_2tBu and $NCH_2CH_2CO_2tBu$) ppm. CI-HRMS: *m*/*z* calcd. for $C_{32}H_{55}N_4O_6$ 591.4122, found 591.4124 $[M + H]^+$.

***tert*-Butyl 3-{11-[2-*tert*-Butoxycarbonyl)ethyl]-7-(*tert*-butoxycarbonylmethyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-3-yl}propionate (4d):** *tert*-Butyl acrylate (12 mL, 81.9 mmol) was added to a solution of compound **10a** (2.086 g, 5.986 mmol) in MeOH (2 mL). The solution was stirred at 70 °C overnight and was then concentrated under reduced pressure. The obtained crude orange oil was purified by chromatography on silica gel (EtOAc) to provide the title compound **4d** as a yellow oil (2.783 g, 4.601 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.33−1.40 {m, 13 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2[CH_2CO_2C(CH_3)_3]\}$; 1.45 [s, 18 H, $NCH_2CH_2CO_2C(CH_3)_3$], 2.41−2.49 [m, 12 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 2.90−2.93 [m, 6 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2(CH_2CO_2tBu)$], 3.68 (s, 4 H, $C_{Pyr}CH_2N$), 7.19 [d, $^3J_{H,H}$ = 7.6 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 7.56 [dd, $^3J_{H,H}$ = $^3J_{H,H'}$ = 7.6 Hz, 1 H, *p*-CH_{Ar} (Pyr)] ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 25.1 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 28.1, 28.2 [$NCH_2CO_2C(CH_3)_3$ and $NCH_2CH_2CO_2C(CH_3)_3$], 33.8 ($NCH_2CH_2CO_2tBu$), 50.7 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$ or $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 51.4 ($NCH_2CH_2CO_2tBu$), 51.6 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$ or $N(CH_2CH_2CH_2NCH_2CH_2CO_2tBu)_2$], 56.2 ($NCH_2CO_2tBu$), 60.4 ($C_{Pyr}CH_2N$), 80.3 [$NCH_2CO_2C(CH_3)_3$ and $NCH_2CH_2CO_2C(CH_3)_3$], 122.8 (*m*-CH_{Ar}), 136.5 (*p*-CH_{Ar}), 158.4 ($C_{quat,Ar}$), 171.0, 172.2 (NCH_2CO_2tBu and $NCH_2CH_2CO_2tBu$) ppm. CI-HRMS: *m*/*z* calcd. for $C_{33}H_{57}N_4O_6$ 605.4278, found 605.4272 $[M + H]^+$.

6. General Procedure for the Cleavage of *tert*-Butyl Groups from Macrocyclic Tri-*tert*-butyl Esters 4a−d: In a typical procedure, a solution of HCl in Et_2O (2.5 M, 250 mL) was added to a solution of macrocyclic tri-*tert*-butyl ester **4a**, **4b**, **4c** or **4d** (4.60 mmol) in Et_2O (25 mL) and the reaction mixture was stirred overnight. The white solid formed was filtered and washed with Et_2O.

3,7,11,17-Tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-triene-3,7,11-triacetic Acid (2a): The crude solid was purified by chromatography on an ion-exchange resin (Dowex® 1X8-50, formate form, elution with 0.02 M formic acid) to provide the title compound as a white solid that became deliquescent on standing under ambient conditions (1.43 g, 3.50 mmol, 76%). ^{1}H NMR (400 MHz, D_2O, pD ≈ 3−4, 25 °C): δ = 2.50 [m, 4 H, $N(CH_2CH_2CH_2NCH_2CO_2H)_2$], 3.44 [t, $^3J_{H,H}$ = 6.6 Hz, 4 H, $N(CH_2CH_2CH_2NCH_2CO_2H)_2$ or $N(CH_2CH_2CH_2NCH_2CO_2H)_2$], 3.58 [t, $^3J_{H,H}$ = 7.7 Hz, 4 H, $N(CH_2CH_2CH_2NCH_2CO_2H)_2$ or $N(CH_2CH_2CH_2NCH_2CO_2H)_2$], 3.99 [s, 6 H, $N(CH_2CH_2CH_2NCH_2CO_2H)_2(CH_2CO_2H)$], 4.77 (s, 4 H, $C_{Pyr}CH_2N$), 7.72 [d, $^3J_{H,H}$ = 7.8 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 8.12 [dd, $^3J_{H,H}$ = $^3J_{H,H'}$ = 7.8 Hz, 1 H, *p*-CH_{Ar} (Pyr)] ppm. ^{13}C NMR (100 MHz, D_2O, pD ≈ 3−4, 25 °C): δ = 20.6 [$N(CH_2CH_2CH_2NCH_2CO_2H)_2$], 51.5, 54.0 [$N(CH_2CH_2CH_2NCH_2CO_2H)_2$], 58.9, 59.0 [$C_{Pyr}CH_2N$ and

$N(CH_2CH_2CH_2NCH_2CO_2H)_2(CH_2CO_2H)$ or $N(CH_2CH_2CH_2NCH_2CO_2H)_2(CH_2CO_2H)$], 59.9 [$N(CH_2CH_2CH_2NCH_2CO_2H)_2(CH_2CO_2H)$ or $N(CH_2CH_2CH_2NCH_2CO_2H)_2(CH_2CO_2H)$], 129.1 (*m*-$CH_{Ar}$), 143.2 (*p*-$CH_{Ar}$), 152.1 ($C_{quat,Ar}$), 171.7, 172.0 [$N(CH_2CH_2CH_2NCH_2CO_2H)_2(CH_2CO_2H)$] ppm. FAB-HRMS: *m*/*z* calcd. for $C_{19}H_{29}N_4O_6$ 409.2087, found 409.2117 $[M + H]^+$.

3-{3,11-Bis(2-carboxyethyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-7-yl}propionic Acid (2b): The crude solid was purified by chromatography on an ion-exchange resin (Dowex® 1X8-50, formate form, elution with 0.02 M formic acid) to provide the title compound as a white solid that became deliquescent on standing under ambient conditions (1.55 g, 3.45 mmol, 75%). ^1H NMR (400 MHz, D_2O, pD ≈ 3−4, 25 °C): δ = 2.49 [m, 4 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2$], 2.73−2.81 [m, 6 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2(CH_2CH_2CO_2H)$], 3.44−3.59 [m, 14 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2(CH_2CH_2CO_2H)$], 4.71 (s, 4 H, $C_{Pyr}CH_2N$), 7.69 [d, $^3J_{H,H}$ = 7.8 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 8.08 [dd, $^3J_{H,H} = {}^3J_{H,H'}$ = 7.8 Hz, 1 H, *p*-CH_{Ar} (Pyr)] ppm. ^{13}C NMR (100 MHz, D_2O, pD ≈ 3−4, 25 °C): δ = 20.4 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2$], 32.4, 32.5 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2(CH_2CH_2CO_2H)$], 50.4, 52.6, 54.0, 55.4 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2(CH_2CH_2CO_2H)$], 59.3 ($C_{Pyr}CH_2N$), 128.7 (*m*-$CH_{Ar}$), 143.1 (*p*-$CH_{Ar}$), 152.5 ($C_{quat,Ar}$), 178.6, 179.3 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2(CH_2CH_2CO_2H)$] ppm. FAB-HRMS: *m*/*z* calcd. for $C_{22}H_{35}N_4O_6$ 451.2557, found 451.2538 $[M + H]^+$.

3-{3,11-Bis(carboxymethyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-7-yl}propionic Acid (2c): The crude solid was purified by gel filtration (Sephadex® G-10 eluting with water) to provide the title compound as a white solid that became deliquescent on standing under ambient conditions (1.47 g, 3.47 mmol, 75%). ^1H NMR (400 MHz, D_2O, pD ≈ 1, 25 °C): δ = 2.49 [m, 4 H, $N(CH_2CH_2CH_2NCH_2CO_2H)_2$], 2.97 (t, $^3J_{H,H}$ = 6.7 Hz, 2 H, $NCH_2CH_2CO_2H$), 3.47−3.60 [m, 10 H, $N(CH_2CH_2CH_2NCH_2CO_2H)_2(CH_2CH_2CO_2H)$], 4.16 (s, 4 H, NCH_2CO_2H), 4.78 (s, 4 H, $C_{Pyr}CH_2N$), 7.71 [d, $^3J_{H,H}$ = 7.8 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 8.10 [dd, $^3J_{H,H} = {}^3J_{H,H'}$ = 7.8 Hz, 1 H, *p*-CH_{Ar} (Pyr)] ppm.^{13}C NMR (100 MHz, D_2O, pD ≈ 1, 25 °C): δ = 19.7 [$N(CH_2CH_2CH_2NCH_2CO_2H)_2$], 31.4 ($NCH_2CH_2CO_2H$), 50.1, 53.2, 53.5 [$N(CH_2CH_2CH_2NCH_2CO_2H)_2(CH_2CH_2CO_2H)$], 58.5, 58.6 ($NCH_2CO_2H$ and $C_{Pyr}CH_2N$), 129.3 (*m*-CH_{Ar}), 143.0 (*p*-CH_{Ar}), 151.8 ($C_{quat,Ar}$), 170.9, 176.0 [$N(CH_2CH_2CH_2NCH_2CO_2H)_2(CH_2CH_2CO_2H)$] ppm. FAB-HRMS: *m*/*z* calcd. for $C_{20}H_{31}N_4O_6$ 423.2244, found 423.2243 $[M + H]^+$.

3-{11-(2-Carboxyethyl)-7-(carboxymethyl)-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),13,15-trien-3-yl}propionic Acid (2d): The crude solid was purified by chromatography on an ion-exchange resin (Dowex® 1X8-50, formate form, elution with 0.02 M formic acid) to provide the title compound as a white solid that became deliquescent on standing under ambient conditions (1.43 g, 3.27 mmol, 71%). ^1H NMR (400 MHz, D_2O, pD ≈ 3−4, 25 °C): δ = 2.50 [m, 4 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2$], 2.68 (t, $^3J_{H,H}$ = 6.2 Hz, 4 H, $NCH_2CH_2CO_2H$), 3.44 [t, $^3J_{H,H}$ = 6.3 Hz, 4 H, $N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2$ or $N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2$], 3.49−3.56 [m, 8 H, $NCH_2CH_2CO_2H$ and $N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2$ or $N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2$], 3.85 (s, 2 H, NCH_2CO_2H), 4.70 (s, 4 H, $C_{Pyr}CH_2N$), 7.68 [d, $^3J_{H,H}$ = 7.8 Hz, 2 H, *m*-CH_{Ar} (Pyr)], 8.06 [dd, $^3J_{H,H} = {}^3J_{H,H'}$ = 7.8 Hz, 1 H, *p*-CH_{Ar} (Pyr)] ppm. ^{13}C NMR (100 MHz, D_2O, pD ≈ 3−4, 25 °C): δ = 20.4 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2$], 32.3 ($NCH_2CH_2CO_2H$), 51.1, 52.2, 55.5 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2$], 59.2, 59.3 ($NCH_2CO_2H$ and $C_{Pyr}CH_2N$), 128.4 (*m*-CH_{Ar}), 142.8 (*p*-CH_{Ar}), 152.3 ($C_{quat,Ar}$), 172.1, 179.5 [$N(CH_2CH_2CH_2NCH_2CH_2CO_2H)_2(CH_2CO_2H)$] ppm. FAB-HRMS: *m*/*z* calcd. for $C_{21}H_{33}N_4O_6$ 437.2400, found 437.2397 $[M + H]^+$.

7. Preparation of the Samples for Spectroscopic Studies on Ligand 2a

a. Stoichiometry of the Eu^{III} Complex: The pH of an aqueous stock solution containing HEPES (sodium salt, 0.015 M), KCl (0.085 M) and ligand **2a** ($3.74\ 10^{-5}$ M) was adjusted to pH = 8.1 with an aqueous solution of HCl. A series of 15 samples was prepared in separate sealed containers with various concentration of $EuCl_3$ ranging from 0 to $1.5\ 10^{-4}$ M. After incubation at 60 °C overnight, the samples were allowed to cool to room temperature and were monitored by absorption spectroscopy (λ = 269 nm) until no further spectral change was detected (typically 24 h). In order to furnish a constant amount of excitation energy to the system, the set of samples was irradiated at their isosbestic point (262 nm) and the luminescence intensity of each sample was measured at 615 nm (dominant emission peak corresponding to the $^5D_0 \rightarrow {}^7F_2$ transition) at ambient temperature.

b. Determination of the Stability Constants of the Ln^{III} Complexes: An aqueous stock solution containing MES (sodium salt, 0.015 M), KCl (0.085 M) and ligand **2a** (5.00×10^{-5} M) was prepared. For each lanthanide, a slight excess of $LnCl_3$ salt was added (5.5×10^{-5} to 6.3×10^{-5} M) and a series of samples (12 to 17) was prepared in separate sealed containers at various pH values by addition of an aqueous solution of HCl. After incubation at 60 °C for 24 h, the samples were cooled to room temperature. **Eu^{III}-Sensitised Luminescence:** The samples were monitored by Eu^{III} luminescence emission spectroscopy measured at 615 nm ($^5D_0 \rightarrow {}^7F_2$ transition) until no further spectral change was detected (typically 7 d). When the equilibrium was reached, the samples were irradiated at their isosbestic point (262 nm) and the luminescence intensity of each sample was measured at 615 nm at ambient temperature. The series was analysed twice. **Absorption Spectroscopy:** The samples were monitored by absorption spectroscopy (269 nm) until no further spectral change was detected (typically 7 d). When the equilibrium was reached, the absorption spectra were recorded at ambient temperature in the 200−320 nm range. Each series of samples was analysed three times, and the determination of the stability constant was carried out each time at three wavelengths chosen in the spectral region exhibiting the highest absorption intensity amplitude (269, 273 and 277 nm).

C. Lifetime and Quantum Yield Measurements of the Luminescent Eu^{III} and Tb^{III} Complexes: Samples containing HEPES (sodium salt, 0.015 M), KCl (0.085 M), ligand **2a** ($5.23\ 10^{-5}$ M) and $LnCl_3$ (Ln = Eu, Tb; $7.90\ 10^{-5}$ M) were prepared in H_2O (D_2O). The pH (pD) was adjusted with an aqueous solution of HCl (DCl) to 8.0 (8.4).

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