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Polyazamacrocycles based on a tetraaminoacetate moiety and a (poly)pyridine intracyclic unit: direct synthesis and application to the photosensitization of Eu(III) and Tb(III) ions in aqueous solutions

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ABSTRACT

A series of five new 15-, 18- or 21-membered polyazamacrocycles ($\mathbf{L_1}$–$\mathbf{L_5}$) based on a pyridine, bipyridine or terpyridine unit and a triethylenetetraminetetraacetic acid (TTTA) skeleton is described. In ligands $\mathbf{L_4}$ and $\mathbf{L_5}$ the azaheterocycle contains an additional extracyclic functionality (ester group) suitable for covalently attachment to bioactive molecules. The synthetic procedure is based on the use of a linear tetra-*N*-alkylated tetramine synthon incorporating masked acetate arms and an efficient metal template ion effect, which controls the crucial macrocyclization step. In the case of $\mathbf{L_1}$–$\mathbf{L_3}$, the formation of lanthanide complexes with europium(III) and terbium(III) was investigated and the fluorescence characteristics of the complexes were established. In this series, the terbium(III) complex derived from the bipyridine ligand exhibits the highest lifetime and quantum yield values (τ=2.18 ms, Φ=26%).

1. Introduction

N-Functionalized polyazamacrocycles represent an important class of synthetic hosts, in particular when development of lanthanide complexes for biological and biomedical applications is considered. These 'predisposed ligands' feature an intermediate character between rigid receptors based on cryptands ('lock and key' principle) and flexible receptors based on podands ('induced fit' concept). They encapsulate Ln(III) ions in macrocyclic cavities, while additional donor groups on the flexible arms can provide further coordination to the metal. The large coordination number of Ln(III) ions in solution (typically 8–10) may be thus fulfilled, and as a consequence these Ln(III) complexes often exhibit high thermodynamic and kinetic stabilities that are essential for bioanalytical applications. In this respect, polyazamacrocycles with acetic acid side chains pendant from amino groups are excellent complexing agents for lanthanide ions. The aminoacetate group is an efficient complexing unit leading to five-membered chelate rings and formation of strong ionic bonds with the carboxylate unit. As a consequence lanthanide complexes derived from polyazamacrocycles fitted with acetic pendant groups have been fruitfully used for the design of contrast enhancing agents for magnetic resonance imaging (Gd^{3+}),[1] radiopharmaceuticals carrying radionuclides such as ^{86}Y, ^{90}Y, ^{153}Sm, ^{177}Lu for the diagnosis and therapy of tumours,[2] long-lived fluorescent probes (Eu^{3+}, Tb^{3+}) for bioanalyses.[3]

For the development of time-resolved fluorescent Ln(III) bioprobes, the ligand must enhance the fluorescence properties of the free metal ion by bearing a chromophoric unit, which balances the inherently weak metal centred absorption band thus yielding highly fluorescent Eu(III), Tb(III) species (antenna effect).[4] A survey of the literature highlights the use of macrocyclic fluorophores based on DOTA type derivatives (DOTA=1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate) where a chromophoric unit is substituted to one acetic side arm.[5] From our side, we focused our attention towards the design of polyazamacrocyclic ligands incorporating both an intracyclic chromophoric unit and exocyclic acetate groups. This choice is considered attractive for the following reasons: (i) the heterocyclic moiety is expected to increase the rigidity of the resulting complexes and thus their kinetic inertness in biological media, (ii) chromophoric-to-lanthanide photosensitization step occurs between partners in a rigid conformation that can improve the energy-transfer rates, (iii) functionalization of the heterocyclic unit for bioconjugation purposes is expected to have

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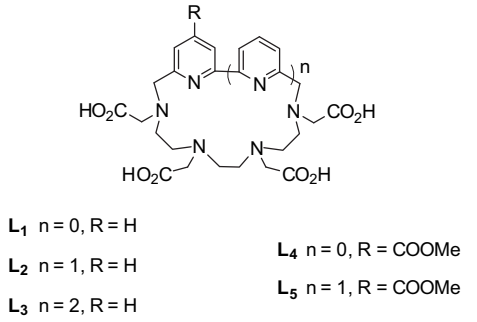
little effect on the chelation properties of the ligand. We have recently reported the synthesis and photophysical properties of europium and (or) terbium macrocyclic complexes derived from a diethylenetriaminetriacetic acid core (DTTA) and an intracyclic 2,2′-bipyridine, *N*,*C*-pyrazolylpyridine or 2,2′:6′,2″-terpyridine chromophore.[6] These complexes exhibited good thermodynamic stability (log K_{cond} ML>18 at pH 7.3) and kinetic inertness in serum media. They showed also promising properties as fluorescent probes or as dual lanthanide probes suitable for optical and magnetic resonance imaging. A pyridine analogue, the pyridine-containing 12-membered tetraazatriacetate ligand (H_3PCTA) has also been described by several groups and has some attractive features for use in biomedicine.[7] Stability constants of about 20.3 log *K* units were recently reported for the corresponding Ln(III) complexes.[7b] The presence of two water molecules in the inner coordination sphere of the metal in these complexes leads to a high water relaxivity for Gd-PCTA, but a weak fluorescence emission for Eu-,Tb-PCTA due to vibronic deactivation via the O–H oscillators of coordinated water molecules.

In the course of our research on the design of lanthanide-containing chelating systems with potential applications as fluorescent tags, we report here on the synthesis and characterization of five new macrocyclic polyamines comprising a triethylenetetraminetetraacetic acid (TTTA) core and an intracyclic chromophoric unit (pyridine, 2,2′-bipyridine, 2,2′:6′,2″-terpyridine). The structures of these 15-, 18- and 21-membered macrocyclic ligands are shown in Scheme 1.



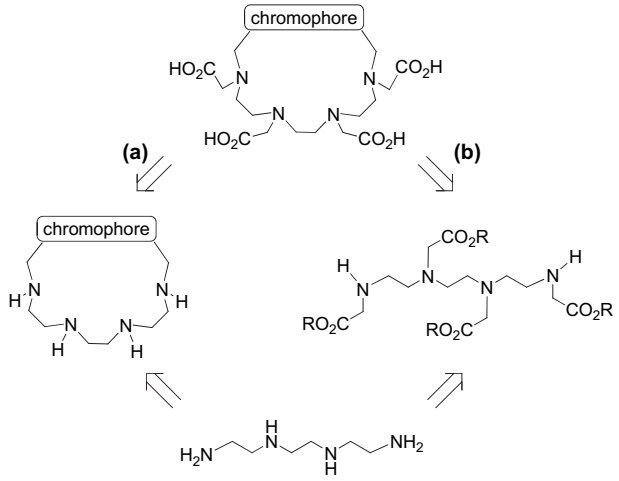
Scheme 1. Structure of the polyaminocarboxylate macrocyclic ligands.

To the best of our knowledge, the use of a TTTA core in the field of acyclic or macrocyclic chelating agents has not been reported until now. Only four patents claimed its potential use, but no data were reported.[8] On the other hand, these polypyridine chromophores are widely used as antenna groups for photosensitizing lanthanide ions, enabling us to compare the optical properties of Ln(III) complexes depending on the ligand structure. These ligands are potentially nona-, deca- or undecadentate: i.e., eight coordination sites from the TTTA host and 1–3 additional ones coming from heterocyclic nitrogen atoms. Assuming a coordination number of nine, commonly reported for Eu(III) and Tb(III) ions in aqueous solutions, these ligands may preclude the coordination of water molecules in the inner sphere of the metal and thereby avoiding a partial quenching of the metal fluorescence. In addition, two of them contain a pyridine ring functionalized with an ester function suitable for conjugation to biological materials. To have some insight into the fluorescence properties of the corresponding Ln(III) complexes, we have also investigated the formation of Eu(III) and Tb(III) complexes derived from the unfunctionalized ligands and established their photophysical properties in aqueous solutions. Preliminary results from this work have been published previously.[9]

2. Results and discussion

2.1. Synthetic strategies

As previously showed in the literature, two synthetic strategies (Scheme 2) may be envisaged for the preparation of polyazamacrocycles comprising an intracyclic heterocyclic unit and bearing appended acetic groups, depending on the stage at which the pendant groups are branched. In both strategies, efficient macrocyclization using two precursor molecules is a crucial step.



Scheme 2. General methodologies for the synthesis of polyazamacrocyclic ligands incorporating both an intracyclic chromophoric unit and exocyclic acetate groups.

The first and traditional route (Scheme 2, route a) begins with the formation of polyazamacrocyclic intermediates bearing secondary amine sites. In a second step, carboxyalkylation of these amine sites gives the desired ligand. In this strategy, representative examples of important cyclization procedures include reaction of polyamines and a dialdehyde fragment of the heterocycle,[10] a pernosylated amine with a dihydroxy derivative of the heterocycle[11] or a pertosylated (pernosylated) amine with a bifunctional heterocyclic electrophile (dibromide or ditosylate).[12] The last reaction, known as the Richman–Atkins cyclization[13] provides an efficient (cyclization yields up to 70% without resorting high dilution) and time tested means of preparing unsubstituted macrocyclic amines. On the other hand, per-N-carboxyalkylation of the unsubstituted nitrogens is not always straightforward, requiring optimization of reaction conditions or contributing to a drop in the overall yield of the target products.[11b,12d,e,14]

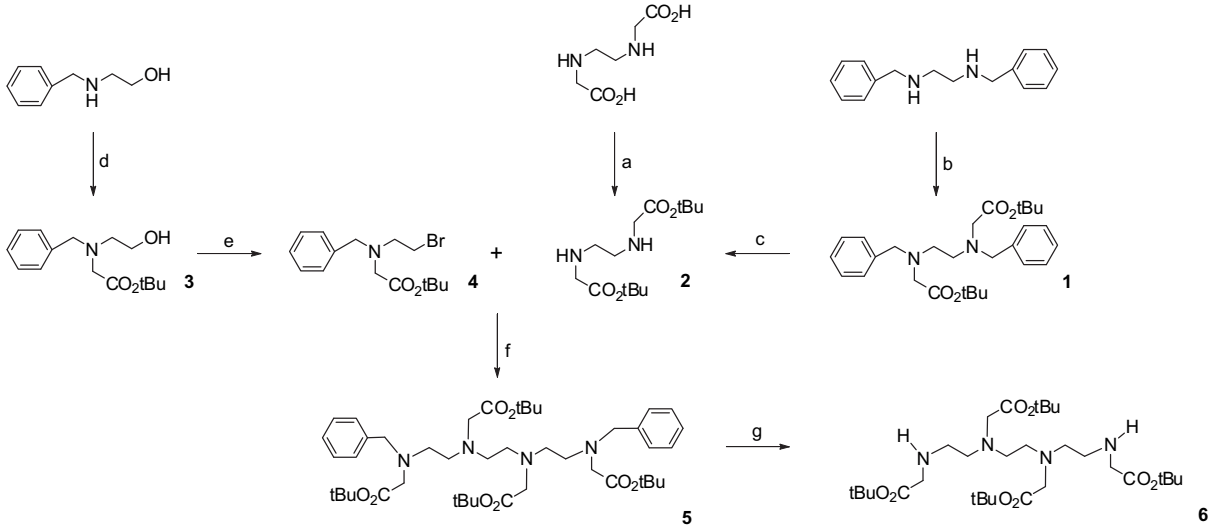
The second strategy (Scheme 2, route b) involves the use of linear polyamines having pendant alkyl acetate groups as precursor molecules for the macrocyclization process. In previous reports, this methodology exploits a metal template effect on the macrocyclization process with dibromo derivatives of heterocycles, affording macrocyclic rings in 35–65% yield.[6,15] An important advantage of this approach is that the acetate arms are incorporated prior to cyclization, eliminating the need for a protection/deprotection sequence and subsequent N-carboxyalkylation reaction on polyamine macrocycles. We decided to develop the second strategy, which allows variations on the nature of chromophoric antenna without the reengineering of the whole macrocyclic molecule.

2.2. Starting material: tetramine bearing four acetate pendant arms

In this work, the key building block for the synthesis of all compounds shown in Scheme 1 is the tetramine **6** bearing four *tert*-

butyl acetate side chains and two terminal secondary amine groups available for subsequent ring closure reactions with a dihalide (Scheme 3). For the preparation of **6**, prealkylated precursor molecules, *N*,*N*′-dialkylated ethylenediamine derivative **2** and *N*,*N*-dialkylated bromide **4** were used. In this approach, *tert*-butyl ester and benzyl groups were selected as protection for the acetic acid chains and amine functions, respectively. The use of bulky *tert*-butyl ester prevents intramolecular cyclization yielding six-membered lactams during the course of alkylation reactions. As a matter of fact, it is well established that such side reaction occurs in the case of polyamines bearing secondary amine sites and more reactive esters, such as methyl, ethyl or benzyl esters.[16] On the other hand, benzyl protective groups of amine functions can be efficiently removed in neutral conditions by hydrogenolysis, avoiding purification of charged species.

The secondary amine groups of **2** were alkylated with 2 equiv of bromide **4** to give tetraester **5** in high yield (92%) after purification by column chromatography. Debenzylation of **5** was readily achieved in a quantitative yield by catalytic hydrogenation at rt under hydrogen pressure, in methanol, using Pd/C (10%) as catalyst. The total yield of this reaction sequence (Scheme 3) was 75% in compound **6**, starting from commercial materials. Preparation of compound **6** following a more direct route involving a tetra alkylation of *N*,*N*‴-dibenzyltriethylenetetramine was less successful. In this case, formation of polyalkylated by-products, which are difficult to separate from the desired product by chromatographic purification limited the yield (9% overall yield in **6** from commercial triethylenetetramine).[9]



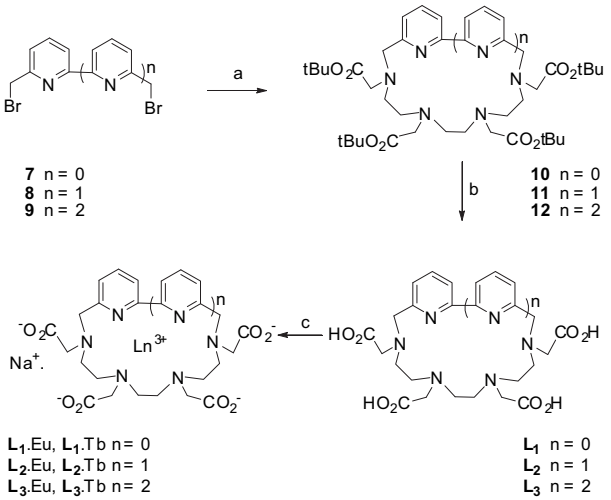
Scheme 3. Synthesis of tetramine **6**. Reagents and conditions: (a) CH_3COOt-Bu, $HClO_4$, rt, 12 d, 43%; (b) $BrCH_2CO_2t$-Bu (2 equiv), K_2CO_3, CH_3CN, reflux, 24 h, 100%; (c) Pd/C (10%), H_2 (5 bar), MeOH, rt, 12 h, 100%; (d) $BrCH_2COOt$-Bu, *i*-Pr_2NEt, DMF, rt, 24 h, 100%; (e) NBS/PPh_3, CH_2Cl_2, rt, 4 h, 82%; (f) **4** (2 equiv), K_2CO_3, CH_3CN, reflux, 24 h, 92%; (g) Pd/C (10%), H_2 (5 bar), MeOH, rt, 12 h, 100%.

Although C.G. Pitt described a three-step method for the preparation of di-*tert*-butyl ester **2**, the procedure is cumbersome (use of isobutene) and gives a poor yield (15%) of the final product, starting from commercial ethylenediamine-*N*,*N*′-diacetic acid.[17] By using the same starting material, we have prepared **2** in a one-step procedure by utilizing a transesterification process commonly used in peptide chemistry.[18] Preparation of **2** was realized by reacting ethylenediamine-*N*,*N*′-diacetic acid in *tert*-butyl acetate as solvent and in the presence of perchloric acid. The reaction occurred at rt and the desired diester **2** was isolated by simple extraction procedure in 43% yield with satisfying purity allowing to use the crude product. We have also employed an alternative method, starting from commercial *N*,*N*′-dibenzylethylenediamine. A combination of alkylation with *tert*-butyl bromoacetate followed by a deprotection step of benzyl protecting group by catalytic hydrogenation produced the target compound **2** in 100% yield for the two steps. A report that appeared after the completion of the work described here outlines that this procedure can be adapted to a large-scale preparation of **2** (multigram scale).[19]

The second precursor molecule **4** was prepared in two steps from commercially available *N*-benzyl ethanol amine using modified published procedure.[20] Briefly, alkylation of the amine group of *N*-benzyl ethanolamine was performed with *tert*-butyl bromoacetate in the presence of *N*,*N*-diisopropylethyl amine (DIPEA) as a base. The use of this hindered organic base prevents totally a lactonization side reaction forming 4-benzyl-morpholin-2-one.[9,21] The hydroxyl group of the resulting compound **3** was then transformed into the bromo derivative **4** by treatment with NBS/PPh_3 at rt. In this way, compound **4** can be prepared in 82% overall yield.

2.3. Synthesis of ligands L_1–L_5

With the tetramine precursor **6** in hand, the macrocyclization step for getting the 15-, 18- and 21-membered macrocycles $\mathbf{L_1}$, $\mathbf{L_2}$ and $\mathbf{L_3}$, respectively, was then envisaged (Scheme 4). Because ease



Scheme 4. Synthesis of ligands $\mathbf{L_1}$–$\mathbf{L_3}$ and their Eu(III) and Tb(III) complexes. Reagents and conditions: (a) **6** (1 equiv), Na_2CO_3, CH_3CN, reflux, 24 h, [reactants]=2.7×10^{-3} M, 54% (**10**), 58% (**11**) and 39% (**12**); (b) HCOOH, 60 °C, 24 h, 100% ($\mathbf{L_1}$) and 98% ($\mathbf{L_2}$) or CF_3COOH, CH_2Cl_2, rt, 24 h, 92% ($\mathbf{L_3}$); (c) $EuCl_3\cdot6H_2O$ or $TbCl_3\cdot6H_2O$, H_2O, rt, 24 h.

of ring closure may be dependent on the size of macrocycle, we started the optimization of the macrocyclization process with the 18-membered macrocycle derived from 2,2′-bipyridine.

Treatment of the tetramine **6** with 6,6′-bis(bromomethyl)-2,2′-bipyridine **8**[22] was carried out in refluxing acetonitrile and in the presence of an excess of sodium carbonate and without using high-dilution techniques (reactant concentration: 2.7×10^{-3} M). ^{1}H, ^{13}C NMR and MS analyses of the crude reaction product evidenced the presence of a major species, characterized as the sodium monomeric complex **11**·Na. Attempted purification by column chromatography led to the isolation of a mixture of **11**·Na and free ligand **11** (45:55 ratio), as a result of alumina-mediated dissociation. Treatment of this mixture of **11**·Na and **11** with an excess of NaCl in CH_3CN or with a saturated EDTA aqueous solution furnished cleanly as single species **11**·Na or **11**, respectively. It can be noticed that these two species can be readily distinguished by their ^{1}H NMR spectra (Fig. 1). Especially, these spectra exhibited significant differences for the heteroaromatic and *tert*-butyl hydrogens. The heterocyclic moiety related resonances cover a chemical shift range 1.5 fold larger in **11**·Na than in **11** and the signals of the *tert*-butyl protons are shifted upfield by $\Delta\delta>0.12$ ppm upon complexation by Na^{+} ion. It is interesting to note that the positions of H-3,3′ heteroatomic hydrogens (7.86 and 7.94 ppm) indicate that the orientation of the bipyridinyl moiety is approaching a *syn* conformation in these two species, and thus support the monomeric cyclic structure.[12b] On the other hand, complexation by Na^{+} was accompanied by a reduction in the frequency of the carbonyl infrared band (5 cm^{-1}) and a bathochromic shift of the maximum in the bipyridine ultraviolet spectrum (6 nm), suggesting that these two groups are involved in complexation. Finally, when the mixture of **11** and **11**·Na species was treated with saturated EDTA solution, the free macrocycle **11** was readily obtained in 58% yield.

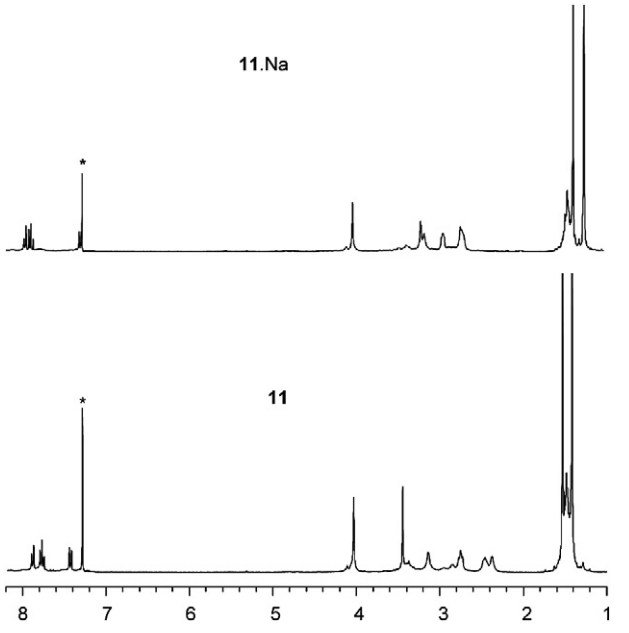
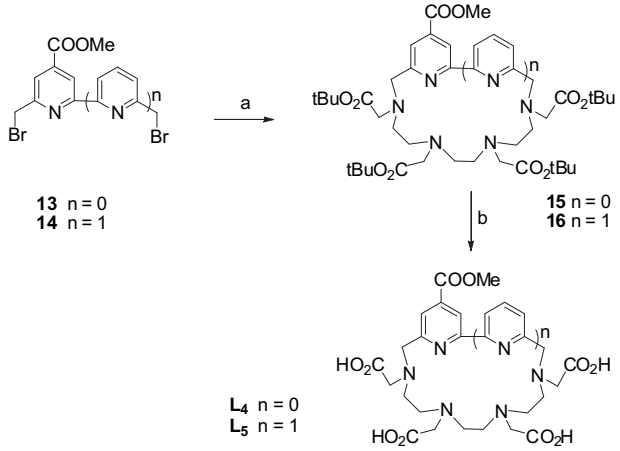


Fig. 1. ^{1}H NMR spectra of compounds **11** and **11**·Na in $CDCl_3$ at 300 MHz (*=solvent peak).

The 15- and 18-membered macrocycles **10** and **12** were obtained by treating **6** with 2,6-bis(bromomethyl)pyridine **7**[23] or 6,6″-bis (bromomethyl)-2,2′:6′,2″-terpyridine **9**.[6c] This macrocyclization was carried out under batch-wise procedure and in a heterogeneous reaction in refluxing CH_3CN with Na_2CO_3, as described for **11**. As in the case of **11**, the analyses of the crude reaction mixtures highlighted the formation of a single species, **10**·Na, while a more complex mixture was observed for **12**. This suggests that the sodium template effect is less efficient for **12**, probably due to the size of the macrocycle to be formed (21-membered cycle). K_2CO_3 was also tested in the preparation of **12**, but the use of this alkaline carbonate did not improve the selectivity of the macrocyclization reaction towards the monomeric structure. Purification by column chromatography on alumina associated with EDTA treatment afforded macrocycles **10** and **12** in 54 and 39% isolated yields, respectively.

Fifteen- and eighteen-membered macrocycles **15** and **16** (Scheme 5) tethered to a methyl ester group were also prepared by using this synthetic approach. This aromatic ester group was selected because its subsequent transformation to various functionalities allows further grafting of biological material by classical methods or click chemistry.[24] In the cyclization step we used the dibromide compounds **13** and **14**. The preparation of **13** was carried out following a standard synthetic scheme starting from commercial 2,6-dimethylpyridine-*N*-oxide. This was achieved in five steps according to literature procedures.[25] As previously described,[24] an efficient access to **14** required the use of a palladium-catalyzed cross coupling using a modified Negishi procedure and a Boeckelheide rearrangement, starting from 2-chloro-6-methyl-isonicotinic acid. Condensation of tetramine **6** with dibromide **13** or **14** was carried out in CH_3CN and by using Na_2CO_3 as previously described. The free ligand **15** was isolated in 56% yield and the sodium complex **16**·Na in 40% yield. For the later, repeated treatments with EDTA did not allow to get the free ligand **16** with a suitable purity degree.



Scheme 5. Synthesis of ligands $\mathbf{L_4}$ and $\mathbf{L_5}$. Reagents and conditions: (a) **6** (1 equiv), Na_2CO_3, CH_3CN, reflux, 24 h, [reactants]=2.7×10^{-3} M, 56% (**15**) and 40% (**16**·Na); (b) CF_3COOH, CH_2Cl_2, rt, 24 h, 100% ($\mathbf{L_4}$) or HCOOH, 60 °C, 24 h, 91% ($\mathbf{L_5}$).

Hydrolysis of *tert*-butyl ester groups of **10**–**12** under acidic conditions with trifluoroacetic acid (at rt) or with formic acid (at 60 °C) gave cleanly tetraacids $\mathbf{L_1}$–$\mathbf{L_3}$ in high yields. Selective deprotection of *tert*-butyl ester groups of **15** and **16** (vs methyl ester group) was also carried in the same experimental conditions.

It is important to note that tetraacid macrocycle $\mathbf{L_1}$ was previously prepared by using the Richman–Atkins methodology for the macrocyclization step and was isolated in 17% overall yield starting from tetratosylated triethylenetetramine.[12a] Following our direct method, $\mathbf{L_1}$ can be prepared in 54% overall yield, starting from tetramine **6**. This result highlighted the relevance of using route b (Scheme 2) for the synthesis of such macrocycles.

2.4. Photophysical properties of $\mathbf{L_{1-3}}$·Eu and $\mathbf{L_{1-3}}$·Tb complexes

Next, we assessed the photophysical properties of complexes of the ligands $\mathbf{L_1}$–$\mathbf{L_3}$ with Eu(III) and Tb(III), lanthanide ions of choice

in the design of luminescent probes. The complexes were prepared by the addition of a stoichiometric amount of the lanthanide salt ($LnCl_3 \cdot 6H_2O$) to an aqueous solution of the corresponding ligand (Scheme 4). These solutions were adjusted in Tris buffer (50 mM, pH 7.4) at a final concentration of 1×10^{-4} M for absorption and 1×10^{-6} M for emission spectroscopies, respectively. These complexes were characterized by UV, MS, HPLC and ligand-sensitized Ln(III) fluorescence techniques. ESI mass spectrometry analyses allowed a 1:1 ligand/metal stoichiometry to be established in aqueous solutions.

Both Ln(III) complexes gave at rt classical europium- or terbium-centred luminescence spectra, with the strongest transition at 620 nm ($^5D_0 \rightarrow {}^7F_2$ transition) or 545 nm ($^5D_4 \rightarrow {}^7F_5$ transition), respectively, when photoexcited in the lowest energy absorption of the heterocyclic chromophore. Representative emission spectra are shown in Fig. 2 for $\mathbf{L_2}\cdot$Eu and $\mathbf{L_2}\cdot$Tb complexes.

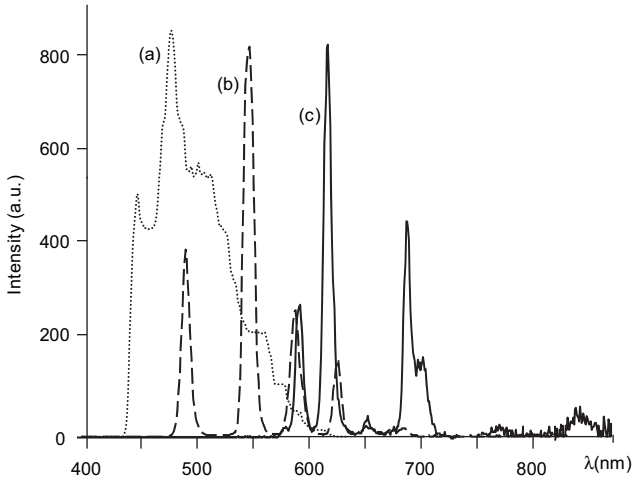


Fig. 2. Normalized (a) phosphorescence (…) spectrum of $\mathbf{L_2}\cdot$Gd and (b), (c) fluorescence spectra of $\mathbf{L_2}\cdot$Tb (- - -) and $\mathbf{L_2}\cdot$Eu (—) complexes. The phosphorescence spectrum was measured at 77 K in MeOH/EtOH (4:1 v/v) glassy matrix and the fluorescence spectra at 298 K in Tris buffer (50 mM, pH 7.4).

The similarity between the absorption and excitation spectra proves an energy transfer from the excited states of these ligands to the Ln(III) emission states. The temporal decay of the emission of both complexes was rigorously monoexponential, suggesting the presence of one discrete $\mathbf{L}\cdot$Ln species. The lifetimes data are reported in Table 1, along with quantum yields, calculated hydration numbers (q) and triplet-state energies.

An inspection of these luminescence data reveals several points of significance:

Table 1
Absorption and excitation maxima (λ_{max} and λ_{exc} in nm), luminescence lifetimes (τ in ms), quantum yields (Φ, %), hydration states (q) and triplet-state energies (E_T in cm^{-1}) for $\mathbf{L_{1-3}}\cdot$Eu and $\mathbf{L_{1-3}}\cdot$Tb complexes.[a]

	λ_{max}	λ_{exc}	$\tau_H^{298\ K}$	$\tau_D^{298\ K}$	$\tau_H^{77\ K}$	$\tau_D^{77\ K}$	$\Phi_H^{298\ K}$	$\Phi_D^{298\ K}$	q[b]	E_T[c]
$\mathbf{L_1}\cdot$Eu	266	268	0.67	1.80	0.92	1.97	0.6	1.6	0.8	27,550
$\mathbf{L_1}\cdot$Tb	266	268	1.54	3.00	1.94	3.00	8.5	13	1.3	
$\mathbf{L_2}\cdot$Eu	305	310	1.13	1.81	1.21	1.93	5	6	0.1	22,550
$\mathbf{L_2}\cdot$Tb	305	310	2.18	2.35	2.34	2.87	26	27	−0.1	
$\mathbf{L_3}\cdot$Eu	320	326	0.76	1.73	0.87	1.86	4.5	10	0.6	22,200
$\mathbf{L_3}\cdot$Tb	320	326	0.89	1.25	1.85	2.78	9.2	14	1.3	

[a] Data obtained in aerated Tris buffer (50 mM, pH 7.4), H_2O (H) or D_2O (D) solutions.
[b] Number of coordinated H_2O molecules calculated using the equation $q=1.2((1/\tau_H)-(1/\tau_D)-0.25)$ for $\mathbf{L_{1-3}}\cdot$Eu and $q=5((1/\tau_H)-(1/\tau_D)-0.06)$ for $\mathbf{L_{1-3}}\cdot$Tb.[27]
[c] From the structured phosphorescence profiles of $\mathbf{L_{1-3}}\cdot$Gd complexes recorded at 77 K in a MeOH/EtOH (4:1 v/v) mixture. The energy level E_T was derived in correspondence to the highest energy band maximum.

(i) For both series of complexes, the excitation wavelength (Table 1) is in agreement with the spectral signature of the pyridine, *cis* 2,2′-bipyridine and *cis-cis* 2,2′:6′,2″-terpyridine moieties. As expected, these excitation wavelengths increase with an increasing number of pyridine rings and are similar to those reported for Ln(III) complexes derived from (poly)pyridine antennae and bis(iminodiacetate) or DTTA chelating moieties.[6b,c,26–28] On the other hand, no transition corresponding to the own Eu(III) and Tb(III) absorptions levels, especially the $^7F_0 \rightarrow {}^5L_6$ (393 nm) and $^7F_6 \rightarrow {}^5L_{10}$ (369 nm) transitions are observable in the excitation spectra, demonstrating efficiency of energy transfer (antenna effect) in these complexes.

(ii) Lifetimes are in the millisecond range, in particular, the metal luminescence lifetime of $\mathbf{L_2}\cdot$Tb at rt is about 2.2 ms, a remarkably high value in comparison with those found in aqueous solutions for Tb(III) complexes of ligands containing bipyridine-type chromophore.[4,6b,22,29] On the other hand, upon solvent deuteration, the lifetimes of some complexes are increased by a factor two, indicative of some coupling between the metal ion and O–H oscillators of the solvent, which favour radiationless deactivation of the metal excited state. Using this well-established isotope effect and the empirical relations of Parker allowed an estimation of the apparent hydration state q of the complexes.[30] These analyses indicate that there is about one-metal bound water molecule in $\mathbf{L_1}\cdot$Ln and $\mathbf{L_3}\cdot$Ln and no bound water molecule in $\mathbf{L_2}\cdot$Ln. The non-integer values observed can be arise from the uncertainty of these empirical formulae or from the presence of two species with different degrees of solvation in exchange faster than the Ln(III) lifetime. Thus the introduction of a TTTA core (vs DDTA) in the macrocyclic systems derived from pyridine and bipyridine chromophores displaces one water molecule from the first coordination sphere of the lanthanide ion. In contrast, when the TTTA core is substituted for the DTTA core in macrocyclic terpyridine system, a water molecule is allowed to bind the metal ion. In this case, the expansion of the cavity size (21-membered vs 18-membered) is an unfavourable factor for the shielding of Ln(III) ions, despite the presence of two additional coordination sites.

(iii) The sensitization pathway, which seems to be general in Eu(III) and Tb(III) complexes, proceeds through an intramolecular energy transfer from the triplet state of the antenna chromophore to the closest emitting level of the metal. Except for $\mathbf{L_3}\cdot$Tb, the Eu ($^5D_0$) and Tb ($^5D_4$) lifetimes remain approximately constant between 298 K and 77 K, indicating that thermally activated processes are negligible for the investigated complexes. For $\mathbf{L_3}\cdot$Tb, the temperature dependence of the Tb ($^5D_4$) lifetime is larger ($k_{nr}(T)=400\ s^{-1}$),[31] which suggests a back-transfer process taking place between the ligand triplet-state and the $^5D_4$ level, a deactivation pathway, which is commonly observed for photosensitized terbium complexes.[4,26] This was supported by the triplet-state energies, measured as usual from the ligand phosphorescence spectra of the corresponding Gd(III) complexes (Fig. 2, Table 1). For $\mathbf{L_3}\cdot$Tb, the energy gap between the triplet state of the ligand (22,200 cm^{-1}) and the resonance level of Tb(III) (20,500 cm^{-1}) is 1700 cm^{-1}, which is slightly below the minimum value of 1850 cm^{-1} proposed by Latva et al.[26] to prevent such a metal–ligand reversible process.

(iv) The emission quantum yields are higher for the Tb(III) complexes than for the Eu(III) complexes. The higher susceptibility of Eu(III) (vs Tb(III)) luminescence towards quenching by the hydroxyl groups of the solvent cannot account for these reduced emission quantum yields. As a matter of fact, a similar trend was observed in D_2O. These results can be explained either by a less efficient ligand-to-metal energy transfer or by the presence of ligand-to-metal charge transfer (LMCT) excited

states, the latter being in agreement with the fact that Eu(III) can be easily reduced. The best ligand for sensitizing the Tb(III) luminescence is **L2** with an overall quantum yield of 26%. In moving to **L1** and **L3**, the quantum yield decreases to 8.5 and 9.2%, respectively. In these macrocyclic TTTA-based ligands, the (poly)pyridine chromophore sensitizes the Eu(III) ion less efficiently than in open-chain (bis iminodiacetic core) or macrocyclic (DTTA core) analogues.[6b,c,26,32,33] Particularly, although the long lifetime observed, **L2**·Eu displays a relatively weak emission (Φ=5%) despite the fact that in this complex, coordination around the metal ion is saturated by the ligand. For example, the emission quantum yield found for the monoaquo Eu(III) complex of bpyCTA[15], a macrocyclic ligand based on 2,2′-bipyridine and DTPA moieties, is two times higher.[6b] On the other hand, the luminescence quantum yield of **L2**·Tb is among the largest values reported in aqueous solution for Tb(III) complexes containing one or more 2,2′-bipyridine chromophore.[6b,22,26,29,34] Moreover, it is interesting to note that this quantum yield is close to the one reported for terbium-based commercial luminescent probe, DTPA-Cs124 (Φ=32%).[35]

As far as the stability of these complexes are concerned, no change in their luminescence data (emission intensity, lifetime) in aqueous solutions (Tris buffer, 50 mM, pH 7.4) was observed after several days at rt, indicating that the complexes are resistant to dissociation in this medium. We have also studied the resistance of the Eu(III) complexes at pH 7.4 (Tris buffer) in the presence of chelating agents such EDTA (log K_{cond} EDTA·Eu=14.4 at pH 7.4). The dissociation of the complexes was determined by luminescent experiments, that is, by monitoring the disappearance of the $^5D_0 \rightarrow {}^7F_2$ peak at 620 nm as a function of time (Fig. 3). **L3**·Eu was nearly completely dissociated after two days in the presence of a 10-fold excess of EDTA, suggesting that the EDTA·Eu complex has been formed preferentially. **L1**·Eu and **L2**·Eu complexes were 28 and 22% dissociated, respectively, after two days under the same conditions. These behaviours indicate that these complexes based on a TTTA macrocyclic system have a weaker kinetic stability in comparison to their DTTA macrocyclic analogues.[6b,c,7b] From these experiments and by using the Verhoeven analysis,[36] log K_{cond} (pH 7.4) was measured to be 16.6 for the formation of **L2**·Eu complex in water.[37] This indicates a reasonable physiological stability, compared to the lowest log K_{cond} value (14.9 at pH 7.4) found in commercially used MRI agents.[38] Similar K_{cond} was found for **L2**·Tb and additional experiments showed that this complex does not dissociate in human serum and is also an efficient emitter in 50 mM HEPES (pH 7.3) and phosphate (pH 8) buffers.

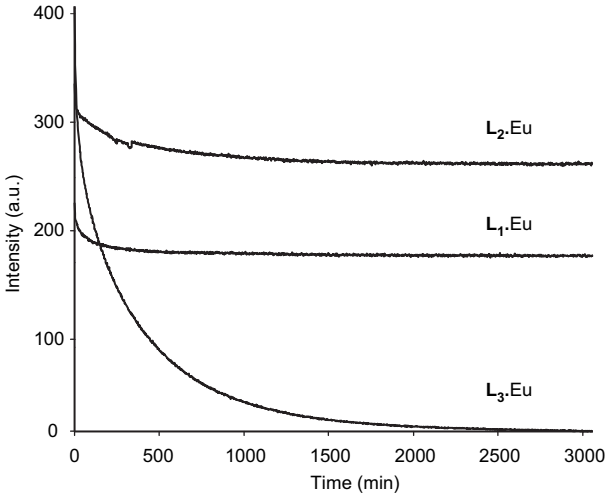


Fig. 3. Plot of emission intensity of the $^5D_0 \rightarrow {}^7F_2$ transition (620 nm) of the complexes **L1**·Eu, **L2**·Eu and **L3**·Eu in the presence of 10 mol equiv of EDTA added in Tris buffer (50 mM, pH 7.4).

Among this series of Ln(III) complexes, **L2**·Tb displays high luminescence efficiency, long luminescence lifetime and reasonable kinetic stability in aqueous and biological media. The properties of this complex are compatible with the stringent requirements of a lanthanide luminescent bioprobe.

3. Conclusion

In conclusion, we present here a direct and efficient approach for the synthesis of a new class of polyazamacrocycles containing four acetate pendant arms and an intracyclic heterocyclic unit. The synthetic route involved the macrocyclization between a dibromo fragment of the heterocycle and a tetramine incorporating four masked acetate arms and two secondary amine groups. The last compound, readily available, promises to be useful synthetic tool not only for polyazamacrocycles with other heterocyclic units, but also for a wider range of acyclic compounds, which may possess interesting properties for chelating lanthanide and related ions. On the other hand, the photophysical properties of Eu(III) and Tb(III) complexes reported here will help in rationalizing the ligand design of luminescent probes for bioanalytical applications.

4. Experimental

4.1. General methods

2,6-Bis(bromomethyl)pyridine **7**,[23] 6,6′-bis(bromomethyl)-2,2′-bipyridine **8**,[22] 6,6″-bis(bromomethyl)-2,2′:6′,2″-terpyridine **9**,[6c] 4-carbomethoxy-2,6-bis(bromomethyl)pyridine **13**[25] and 4-carbomethoxy-6,6′-bis(bromomethyl)-2,2′-bipyridine **14**[24] were prepared according to literature procedures. Reactions requiring an inert atmosphere were run under Argon. Acetonitrile was freshly distilled from P_2O_5, and CH_2Cl_2 was freshly distilled from CaH_2. Diisopropylamine was dried and distilled over KOH. Thin-layer chromatography was performed on Merck silica or alumina plates with a fluorescence indicator. Column chromatography was carried out on silica gel (Merck, 60–200 μm, porosity 60 Å) and on alumina (Macherey-Nagel, activity IV, 50–200 μm).

Melting points were taken with a Büchi mel-temp apparatus. Infrared spectra were recorded on a Perkin–Elmer FTIR 1725x spectrophotometer. Samples were prepared as KBr pellets (solid sample) or applied to NaCl plates (liquid sample). Selected characteristic absorption frequencies are reported in cm^{-1}. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer; chemical shifts are given in parts per million according to the solvent peak. Electrospray (ES) mass spectra were obtained on a Q TRAP Applied Biosystems Spectrometer and high-resolution mass spectra (HRMS) on an LCT Premier Waters spectrometer. DCI, NH_3 or CH_4, mass spectra were obtained on a DSQ II Thermo Fisher or a GCT Premier Waters spectrometer, respectively. Elemental analyses were carried out by the 'Service d'Analyse', Laboratoire de Chimie de Coordination (Toulouse). Absorption measurements were done with a Hewlett Packard 8453 temperature-controlled spectrophotometer.

The ligands (**L1**–**L5**) and lanthanide complexes (**L1–3**·Eu and **L1–3**·Tb) were analyzed by RP-HPLC using a Waters Alliance 2695 system with a PDA 2996 detector and using a reversed-phase (RP) C_8 column (Phenomenex Luna C8(2), 5 μm 100 Å, 150×4.6 mm). The flow rate was 1 mL/min with UV monitoring at 260 nm for ligands and complexes derived from a pyridine unit or 315 nm for ligands and complexes constructed on the basis of a bipyridine or a terpyridine unit. Two analytical procedures were developed as

following. System A: solvents were 10 mM pH 4 ammonium formate buffer (solvent A) and acetonitrile (solvent B); the compounds were analyzed using the HPLC gradient system beginning with a solvent composition of 100% A and following a linear gradient up to 80% A:20% B from 0 to 18 min. System B: solvents were H_2O containing 0.1% TFA (solvent A) and acetonitrile (solvent B); analyses were performed using the HPLC gradient system beginning with a solvent composition of 95% A:5% B and following a linear gradient up to 80% A: 20% B from 0 to 35 min.

4.1.1. Luminescence measurements. Fluorescence and phosphorescence spectra were obtained with a LS-50B Perkin–Elmer and a Cary Eclipse spectrofluorimeters equipped with a Xenon flash lamp source and a Hamamatsu R928 photomultiplier tube. The measurements were carried out at pH 7.4 in Tris buffer (50 mM) and all samples were prepared with an absorbance between 0.01 and 0.05 at the excitation wavelength in order to prevent the inner-filter effect. Phosphorescence spectra at 77 K of gadolinium complexes were carried out in a MeOH/EtOH (4:1 v/v) mixture and recorded with the LS-50B Perkin–Elmer spectrofluorimeter equipped with the low-temperature accessory No. L2250136. Spectra were corrected for both the excitation light source variation and the emission spectral response. Lifetimes τ (uncertainty $\leq$5%) are the average values from at least five separate measurements covering two or more lifetimes made by monitoring the decay at a wavelength corresponding to the maximum intensity of the emission spectrum, following pulsed excitation. The luminescence decay curves were fitted by an equation of the form $I(t)=I(0)\exp(-t/\tau)$ by using a curve-fitting program. High correlation coefficients were observed in each cases (higher than 0.999). The luminescence quantum yields (uncertainty±10%) were determined by the method described by Haas and Stein,[39] using as standards $[Ru(bpy)_3]^{2+}$ in aerated water (Φ=0.028)[40] for the Eu(III) complexes or quinine sulfate in 1 N sulfuric acid (Φ=0.546)[41] for the Tb(III) complexes.

4.2. Di-*tert*-butyl ethylenediamine-*N,N′*-diacetate (2)

4.2.1. Route A. To a stirred solution of ethylenediamine-*N,N′*-diacetic acid (3 g, 17 mmol) in *tert*-butyl acetate (135 mL), at 0 °C, was added dropwise $HClO_4$ (70%, 4.5 mL). The mixture was then allowed to warm up to rt, and stirring was continued for 12 days. The resulting milky solution was cooled to 0 °C and washed with HCl (0.5 N, aqueous) (5×60 mL). The combined aqueous phases were neutralized with solid $NaHCO_3$, extracted with ether (6×100 mL). The combined organic phases were dried (Na_2SO_4), and the solvent was removed under reduced pressure. The resulting yellow oil was dissolved in ether (100 mL), washed with water (3×15 mL) and dried. Evaporation of the solvent afforded the title compound **2** (2.1 g, 7.28 mmol, yield 43%) as a yellow oil. IR ν_{max}: 1733 (C=O ester). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.48 (s, 18H), 2.71 (s, 4H), 3.30 (s, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.1 (CH_3), 48.9 (CH_2), 51.6 (CH_2), 81.1 (Cq), 171.8 (Cq). MS (DCI/NH_3): *m*/*z* (%) 289.5 (100) $[M+H]^+$.

4.2.2. Route B.

*4.2.2.1. Di-tert-butyl N,N′-dibenzylethylenediamine-N,N′-diacetate (**1**).* To a solution of *N,N′*-dibenzylethylenediamine (3 g, 12.48 mmol) in anhydrous acetonitrile (300 mL) was added solid K_2CO_3 (17 g, 123 mmol). The suspension was refluxed for 1 h, then *tert*-butyl bromoacetate (4.87 g, 25 mmol) was added dropwise, and the mixture was stirred at reflux for 24 h before filtration. The solvent was removed under reduced pressure, then the solid residue was treated with CH_2Cl_2 and the insoluble fraction was eliminated by filtration. The filtrate was evaporated to dryness to give **1** (5.85 g, 12.48 mmol, yield 100%) as a white solid. Mp: 70–71 °C. R_f (silica gel, CH_2Cl_2/MeOH 99:01): 0.24. IR ν_{max}: 1718 (C=O ester). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.45 (s, 18H), 2.80 (s, 4H), 3.25 (s, 4H), 3.78 (s, 4H), 7.22–7.30 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.2 (CH_3), 51.7 (CH_2), 55.2 (CH_2), 58.4 (CH_2), 80.7 (Cq), 127.0 (CH), 128.2 (CH), 129.0 (CH), 139.3 (Cq), 171.0 (Cq). MS (ESI$^+$): *m*/*z* (%) 491.4 (7) $[M+Na]^+$, 469.3 (100) $[M+H]^+$, 413.4 (16) $[(M-C_4H_8)+H]^+$, 357.3 (14) $[(M-2\times C_4H_8)+H]^+$. Anal. Calcd for $C_{28}H_{40}N_2O_4$: C 71.76, H 8.60, N 5.98. Found: C 71.50, H 8.30, N 5.89.

*4.2.2.2. Compound (**2**).* A mixture of **1** (540 mg, 1.15 mmol) and 10% Pd/C (180 mg) in methanol (20 mL) was stirred overnight at rt under H_2 (5 bar). The reaction mixture was filtered over Celite and the Celite pad was washed with methanol. The filtrate was concentrated in vacuo to give pure **2** (330 mg, 1.15 mmol, yield 100%) as a yellow oil, which was identical with the product obtained by route A.

4.3. *tert*-Butyl 2-(benzyl(2-hydroxyethyl)amino)acetate (3)

To a stirred solution of *N*-benzyl ethanolamine (500 mg, 3.3 mmol) and *N,N*-diisopropylethylamine (426 mg, 3.3 mmol) in anhydrous DMF (4 mL), at 0 °C, was added dropwise *tert*-butyl bromoacetate (644 mg, 3.3 mmol). The reaction mixture was allowed to warm up to rt and stirring was continued for 24 h. The solvent was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 (50 mL) and washed with water (4×20 mL). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to provide **3** (875 mg, 3.3 mmol, yield 100%) as a yellow oil. R_f (silica gel, CH_2Cl_2/petroleum ether 50:50): 0.08. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.45 (s, 9H), 2.92 (t, 2H, *J*=6), 3.27 (s, 2H), 3.63 (t, 2H, *J*=6), 3.88 (s, 2H), 7.29–7.35 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.1 (CH_3), 55.4 (CH_2), 56.6 (CH_2), 58.6 (CH_2), 59.0 (CH_2), 81.4 (Cq), 127.4 (CH), 128.5 (CH), 129.0 (CH), 138.4 (Cq), 171.1 (Cq). MS (DCI/CH_4): *m*/*z* (%) 266.2 (23) $[M+H]^+$, 210.1 (100) $[(M-C_4H_8)+H]^+$.

4.4. *tert*-Butyl 2-(benzyl(2-bromoethyl)amino)acetate (4)

To a stirred solution of **3** (3.2 g, 12 mmol) and *N*-bromosuccinimide (2.5 g, 14.0 mmol) in CH_2Cl_2 (50 mL), at 0 °C, was added portionwise triphenylphosphine (3.7 g, 14.1 mmol) over 1 h. The resulting mixture was stirred at 0 °C for 30 min, then it was allowed to warm up to rt, and stirring was continued for 4 h. The solvent was removed under reduced pressure and the crude product was purified by chromatography over silica gel (petroleum ether/diethyl ether 95:5) to give bromide **4** (3.23 g, 9.84 mmol, yield 82%) as a yellow oil. R_f (silica gel, CH_2Cl_2/petroleum ether 50:50): 0.61. IR ν_{max}: 1733 (C=O ester). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.47 (s, 9H), 3.16 (t, 2H, *J*=6), 3.30 (s, 2H), 3.38 (t, 2H, *J*=6), 3.88 (s, 2H), 7.29–7.34 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.2 (CH_3), 30.5 (CH_2), 55.2 (CH_2), 55.9 (CH_2), 58.1 (CH_2), 81.2 (Cq), 127.3 (CH), 128.4 (CH), 128.8 (CH), 138.7 (Cq), 170.6 (Cq). MS (DCI/CH_4): *m*/*z* (%) 330.1 (20)/328.1 (25) $[M+H]^+$, 274.0 (80)/272.0 (85) $[(M-C_4H_8)+H]^+$.

4.5. Tetra-*tert*-butyl *N,N‴*-dibenzyltriethylenetetramine *N,N′,N″,N‴*-tetraacetate (5)

To a stirred solution of diamine **2** (680 mg, 2.36 mmol) in anhydrous acetonitrile (85 mL) was added K_2CO_3 (3.3 g, 23.9 mmol). The suspension was refluxed for 1 h, then bromide **4** (1.55 g, 4.72 mmol) was added in one portion and the mixture was stirred at reflux for 24 h. After cooling to rt, the reaction mixture was filtered, and the filtrate was concentrated in vacuo. The resulting oily residue was purified by chromatography on silica gel (CH_2Cl_2/

MeOH 100:0 then 98:02) to give tetramine **5** (1.7 g, 2.17 mmol, yield 92%) as a yellow oil. R_f (silica gel, CH_2Cl_2/MeOH 98:02): 0.34, R_f (alumina, CH_2Cl_2): 0.66. IR ν_{max}: 1733 (C=O ester). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.43 (s, 18H), 1.45 (s, 18H), 2.68 (m, 4H), 2.75 (m, 8H), 3.23 (s, 4H), 3.29 (s, 4H), 3.78 (s, 4H), 7.28–7.31 (m, 10H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.2 (CH_3), 52.1 (CH_2), 52.6 (CH_2), 55.2 (CH_2), 56.1 (CH_2), 58.4 (CH_2), 80.6 (Cq), 80.7 (Cq), 126.9 (CH), 128.3 (CH), 128.9 (CH), 139.2 (Cq), 170.9 (Cq), 171.0 (Cq). Anal. Calcd for $C_{44}H_{70}N_4O_8$: C 67.49, H 9.01, N 7.15. Found: C 67.10, H 9.15, N 7.10. MS (DCI/NH_3): m/z (%) 783.6 (100) $[M+H]^+$.

4.6. Tetra-*tert*-butyl *N*,*N*‴-triethylenetetramine *N*,*N*′,*N*″,*N*‴-tetraacetate (6)

A mixture of **5** (313 mg, 0.40 mmol) and 10% Pd/C (106 mg) in methanol (14 mL) was stirred overnight at rt under H_2 (5 bar). The reaction mixture was filtered over Celite and the Celite pad was washed with methanol. The filtrate was concentrated in vacuo to give pure **6** (241 mg, 0.4 mmol, yield 100%) as a yellow oil. R_f (alumina, CH_2Cl_2/MeOH 98:02): 0.4. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.44 (s, 18H), 1.46 (s, 18H), 2.81 (m, 4H), 3.01 (m, 8H), 3.40 (s, 4H), 3.59 (s, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 28.2 (CH_3), 28.3 (CH_3), 45.5 (CH_2), 49.0 (CH_2), 50.2 (CH_2), 51.3 (CH_2), 55.3 (CH_2), 81.0 (Cq), 82.5 (Cq), 168.5 (Cq), 171.25 (Cq). MS (ESI$^+$): m/z (%) 603.5 (100) $[M+H]^+$.

4.7. General synthetic procedure for the macrocyclization reaction

To a stirred solution of tetramine **6** in anhydrous acetonitrile (2.7×10^{-3} M) was added solid Na_2CO_3 (10 equiv). The suspension was refluxed for 1 h under Argon, then dibromide derivative of the heterocycle (1 equiv) was added in one portion and the mixture was stirred at reflux for 24 h. The mixture was then cooled to rt, filtered and concentrated in vacuo. The residue was treated with CH_2Cl_2, filtered again and the solvent was removed under reduced pressure to give a residue that was further purified as indicated below for individual compounds.

4.8. 3,6,9,12,18-Pentaazabicyclo[12.3.1]octadeca-1(18),14,16-triene-3,6,9,12-tetraacetic acid, 3,6,9,12-tetrakis(1,1-dimethylethyl) ester (10) and its sodium complex 10·Na

*4.8.1. Compound **10**.* The reaction was carried out using tetramine **6** (150 mg, 0.25 mmol) and 2,6-bis(bromomethyl)pyridine **7** (66 mg, 0.25 mmol). The residue was purified by chromatography on alumina ($CH_2Cl_2\rightarrow CH_2Cl_2$/MeOH, 50:50) to give 100 mg of a mixture of sodium complex **10**·Na and free ligand **10** (25:75 ratio). This mixture was dissolved in $CHCl_3$ (50 mL) and sequentially washed with saturated Na_2EDTA aqueous solution (2×50 mL) and water (2×50 mL). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give **10** (95 mg, 0.135 mmol, yield 54%) as a yellow oil. R_f (alumina, CH_2Cl_2/MeOH 99:01): 0.4. IR ν_{max}: 1733 (C=O ester). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.41 (s, 18H), 1.47 (s, 18H), 2.47 (s, 4H), 2.50–2.80 (m, 8H), 3.15 (s, 4H), 3.38 (s, 4H), 3.87 (s, 4H), 7.33 (d, 2H, J=7.7), 7.60 (t, 1H, J=7.7). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.1 (CH_3), 28.2 (CH_3), 50.8 (CH_2), 51.8 (CH_2), 51.9 (CH_2), 57.5 (CH_2), 57.7 (CH_2), 60.4 (CH_2), 80.7 (Cq), 80.9 (Cq), 123.0 (CH), 136.6 (CH), 158.4 (Cq), 170.7 (Cq), 170.8 (Cq). MS (ESI$^+$): m/z (%) 728.8 (28) $[M+Na]^+$, 260.5 (100) $[(M-4\times C_4H_8)+H+K]^{2+}$.

*4.8.2. Compound **10**·Na.* To a stirred solution of free ligand **10** in acetonitrile was added solid NaCl (5 equiv). The suspension was refluxed for 1 h, then cooled to rt, filtered and the filtrate was concentrated in vacuo. The residue was treated with $CHCl_3$, filtered again and the solvent was removed under reduced pressure to give quantitatively the sodium complex **10**·Na as a pale yellow solid. Mp >250 °C. IR ν_{max}: 1728 (C=O ester). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.38 (s, 18H), 1.41 (s, 18H), 2.66 (m, 12H), 3.09 (s, 4H), 3.28 (s, 4H), 3.81 (m, 4H), 7.15 (d, 2H, J=7.7), 7.65 (t, 1H, J=7.7). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.9 (CH_3), 28.1 (CH_3), 53.5 (CH_2), 53.6 (CH_2), 54.1 (CH_2), 54.4 (CH_2), 58.4 (CH_2), 60.4 (CH_2), 81.8 (Cq), 81.9 (Cq), 122.2 (CH), 138.1 (CH), 157.7 (Cq), 171.3 (Cq), 171.6 (Cq). MS (ESI$^+$): m/z (%) 728.8 (100) $[M+Na]^+$.

4.9. 8,11,14,17,23,24-Hexaazatricyclo[17.3.1.12,6]tetracosa-1(23),2,4,6(24),19,21-hexaene-8,11,14,17-tetraacetic acid, 8,11,14,17-tetrakis(1,1-dimethylethyl) ester (11) and its sodium complex 11·Na

*4.9.1. Compound **11**.* The reaction was carried out using tetramine **6** (150 mg, 0.25 mmol) and 6,6′-bis(bromomethyl)-2,2′-bipyridine **8** (85 mg, 0.25 mmol). The residue was purified by chromatography on alumina ($CH_2Cl_2\rightarrow CH_2Cl_2$/MeOH, 50:50) to give 113 mg of a mixture of sodium complex **11**·Na and free ligand **11** (45:55 ratio). This mixture was dissolved in $CHCl_3$ (50 mL) and sequentially washed with saturated Na_2EDTA aqueous solution (2×50 mL) and water (2×50 mL). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure to give **11** (114 mg, 0.145 mmol, yield 58%) as a yellow oil. R_f (alumina, CH_2Cl_2/MeOH 98:02): 0.3. IR ν_{max}: 1733 (C=O ester). UV/vis (CH_3CN): λ_{max} (ε, L mol^{-1} cm^{-1})= 237 (13,200), 289 (14,900) nm. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.38 (s, 18H), 1.49 (s, 18H), 2.34–2.43 (m, 4H), 2.70–2.74 (m, 8H), 3.00–3.30 (m, 4H), 3.42 (s, 4H), 4.00 (s, 4H), 7.40 (d, 2H, J=7.5), 7.75 (t, 2H, J=7.6), 7.86 (d, 2H, J=7.5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.1 (CH_3), 28.2 (CH_3), 51.4 (CH_2), 52.5 (CH_2), 52.7 (CH_2), 56.2 (CH_2), 58.1 (CH_2), 60.5 (CH_2), 80.6 (Cq), 81.0 (Cq), 120.7 (CH), 124.0 (CH), 137.0 (CH), 156.6 (Cq), 158.9 (Cq), 170.8 (Cq). MS (ESI$^+$): m/z (%) 805.6 (67) $[M+Na]^+$, 783.7 (19) $[M+H]^+$, 299.4 (100) $[(M-4\times C_4H_8)+H+K]^{2+}$.

*4.9.2. Compound **11**·Na.* To a stirred solution of free ligand **11** in acetonitrile was added solid NaCl (5 equiv). The suspension was refluxed for 1 h, then cooled to rt, filtered, and the filtrate was concentrated in vacuo. The residue was treated with $CHCl_3$, filtered again and the solvent was removed under reduced pressure to give quantitatively the sodium complex **11**·Na as a pale yellow solid. Mp >250 °C. R_f (alumina, CH_2Cl_2/MeOH 98:02): 0.20. IR ν_{max}: 1728 (C=O ester). UV/vis (CH_3CN): λ_{max} (ε, L mol^{-1} cm^{-1})=242 (7800), 295 (9100) nm. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.24 (s, 18H), 1.37 (s, 18H), 2.60–2.80 (m, 8H), 2.83–2.96 (m, 4H), 3.15–3.19 (m, 8H), 4.01 (s, 4H), 7.28 (d, 2H, J=7.5), 7.87 (t, 2H, J=7.5), 7.94 (d, 2H, J=7.5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.0 (CH_3), 28.2 (CH_3), 52.0 (CH_2), 52.6 (CH_2), 56.0 (CH_2), 56.7 (CH_2), 81.8 (Cq), 82.2 (Cq), 120.3 (CH), 123.7 (CH), 138.5 (CH), 154.7 (Cq), 157.8 (Cq), 170.8 (Cq), 171.4 (Cq). MS (ESI$^+$): m/z (%) 805.7 (100) $[M+Na]^+$, 783.8 (17) $[M+H]^+$, 411.6 (92) $[(M+H)+K]^{2+}$, 383.6 (20) $[(M-C_4H_8)+H+K]^{2+}$, 355.5 (25) $[(M-2\times C_4H_8)+H+K]^{2+}$, 327.4 (21) $[(M-3\times C_4H_8)+H+K]^{2+}$, 299.5 (54) $[(M-4\times C_4H_8)+H+K]^{2+}$.

4.10. 13,16,19,22,28,29,30-Heptaazatetracyclo[22.3.1.12,6.17,11]triacosa-1(28),2,4,6(29),7,9,11(30),24,26-nonaene-13,16,19,22-tetraacetic acid, 13,16,19,22-tetrakis(1,1-dimethylethyl) ester (12)

The reaction was carried out using tetramine **6** (199 mg, 0.33 mmol) and 6,6″-bis(bromomethyl)-2,2′:6′,2″-terpyridine **9** (138 mg, 0.33 mmol). The residue was dissolved in CH_2Cl_2 (100 mL) and sequentially washed with saturated Na_2EDTA aqueous solution (3×100 mL) and water (1×100 mL). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by chromatography on alumina ($CH_2Cl_2\rightarrow CH_2Cl_2$/MeOH, 50:50) to give **12** (110 mg, 0.128 mmol, yield 39%) as a yellow oil. R_f (alumina, CH_2Cl_2/MeOH 95:05): 0.3. IR ν_{max}: 1733 (C=O ester). ^{1}H

NMR (300 MHz, $CDCl_3$) δ: 1.36 (s, 9H), 1.48 (s, 27H), 2.47 (br s, 4H), 2.67–2.76 (m, 4H), 2.80–2.85 (m, 4H), 3.10 (s, 2H), 3.47 (br s, 6H), 4.09 (br s, 4H), 7.48–7.54 (m, 2H), 7.77–7.90 (m, 5H), 8.50 (br s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.0 (CH_3), 28.2 (CH_3), 51.6 (CH_2), 52.3 (CH_2), 52.7 (CH_2), 55.9 (CH_2), 56.9 (CH_2), 59.7 (CH_2), 80.5 (Cq), 80.9 (Cq), 120.8 (CH), 121.7 (CH), 123.1 (CH), 137.1 (CH), 137.4 (CH), 156.3 (Cq), 157.2 (Cq), 159.8 (Cq), 170.9 (Cq). MS (ESI$^+$): *m*/*z* (%) 898.4 (20) $[M+K]^+$, 882.5 (100) $[M+Na]^+$, 860.5 (80) $[M+H]^+$. HRMS (ESI$^+$) calcd for $C_{47}H_{69}N_7O_8+H^+$=860.5286, found 860.5261 (100%).

4.11. 3,6,9,12,18-Pentaazabicyclo[12.3.1]octadeca-1(18),14,16-triene-3,6,9,12-tetraacetic acid,16-(methoxycarbonyl)-, 3,6,9,12-tetrakis(1,1-dimethylethyl) ester (15)

The reaction was carried out using tetramine **6** (150 mg, 0.25 mmol) and 4-carbomethoxy-2,6-bis(bromomethyl)pyridine **13** (80 mg, 0.25 mmol). The residue was dissolved in CH_2Cl_2 (100 mL) and sequentially washed with saturated Na_2EDTA aqueous solution (3×100 mL) and water (1×100 mL). The organic layer was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by chromatography on alumina ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH, 50:50) to give **15** (107 mg, 0.140 mmol, yield 56%) as a yellow oil. R_f (alumina, CH_2Cl_2): 0.2. IR ν_{max}: 1733 (C=O ester). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.43 (s, 18H), 1.50 (s, 18H), 2.50–2.90 (m, 12H), 3.20–3.34 (m, 4H), 3.40 (s, 4H), 3.94 (s, 3H), 3.99 (s, 4H), 7.86 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 28.2 (CH_3), 50.9 (CH_2), 51.9 (CH_2), 52.0 (CH_2), 52.5 (CH_2), 56.1 (CH_2), 57.4 (CH_2), 57.5 (CH_2), 60.1 (CH_2), 80.9 (Cq), 81.1 (Cq), 122.1 (CH), 138.2 (Cq), 159.9 (Cq), 165.9 (Cq), 170.6 (Cq). MS (ESI$^+$): *m*/*z* (%) 786.8 (43) $[M+Na]^+$, 764.9 (100) $[M+H]^+$.

4.12. 8,11,14,17,23,24-Hexaazatricyclo[17.3.1.12,6]tetracosa-1(23),2,4,6(24),19,21-hexaene-8,11,14,17-tetraacetic acid, 4-(methoxycarbonyl)-, 8,11,14,17-tetrakis(1,1-dimethylethyl) ester, sodium complex (16·Na)

The reaction was carried out using tetramine **6** (150 mg, 0.25 mmol) and 4-carbomethoxy-6,6′-bis(bromomethyl)-2,2′-bipyridine **14** (100 mg, 0.25 mmol). The residue was purified by chromatography on alumina ($CH_2Cl_2 \rightarrow CH_2Cl_2$/MeOH, 50:50) to give 100 mg of a mixture of sodium complex **16**·Na and free ligand **16** (70/30). This mixture was dissolved in CH_3CN (9 mL), solid NaCl (73 mg, 1.25 mmol) was added and the suspension was refluxed for 1 h. The suspension was then cooled to rt, filtered, and the filtrate was concentrated in vacuo. The residue was treated with $CHCl_3$, filtered again and the solvent was removed under reduced pressure to give **16**·Na (97 mg, 0.1 mmol, yield 40%) as a pale yellow solid. Mp >250 °C. R_f (alumina, CH_2Cl_2/MeOH 98:02): 0.25. IR ν_{max}: 1729 (C=O ester). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 1.22 (s, 9H), 1.23 (s, 9H), 1.36 (s, 9H), 1.37 (s, 9H), 2.60–2.80 (m, 8H), 2.90–3.10 (m, 4H), 3.10–3.30 (m, 8H), 4.01 (s, 3H), 4.04 (s, 2H), 4.12 (s, 2H), 7.35 (d, 1H, *J*=7.8), 7.80 (s, 1H), 7.92 (t, 1H, *J*=7.8), 7.96 (d, 1H, *J*=7.5), 8.41 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 27.90 (CH_3), 27.95 (CH_3), 52.1 (CH_2), 52.9 (CH_3), 55.9 (CH_2), 56.0 (CH_2), 56.9 (CH_2), 81.8 (Cq), 81.9 (Cq), 82.3 (Cq), 119.4 (CH), 120.4 (CH), 122.7 (CH), 124.3 (CH), 138.6 (CH), 139.7 (Cq), 153.9 (Cq), 156.0 (Cq), 158.2 (Cq), 159.4 (Cq), 165.1 (Cq), 170.4 (Cq), 170.7 (Cq), 171.8 (Cq). MS (ESI$^+$): *m*/*z* (%) 863.5 (100) $[M+Na]^+$, 841.6 (7) $[M+H]^+$, 328.1 (65) $[(M-4\times C_4H_8)+H+K]^{2+}$.

4.13. General synthetic procedure for hydrolysis of *tert*-butyl ester functions

4.13.1. Procedure A. The protected ligand precursor was added to formic acid 99% (concentration 25×10^{-3} M) and the mixture was stirred at 60 °C for 24 h. The solution was concentrated in vacuo and the residue was re-dissolved three times in MeOH, then in water and then rotary evaporated. The residue was then dissolved in the minimum volume of MeOH and Et_2O was added dropwise, resulting in the formation of a precipitate, which was isolated after centrifugation and dried under vacuum.

4.13.2. Procedure B. The protected ligand precursor was added to a 1:1 mixture of trifluoroacetic acid/CH_2Cl_2 (concentration 25×10^{-3} M) and the mixture was stirred at rt for 24 h. The mixture was then treated as described in procedure A.

4.14. 3,6,9,12,18-Pentaazabicyclo[12.3.1]octadeca-1(18),14,16-triene-3,6,9,12-tetraacetic acid (L_1)

The reaction was carried out applying the procedure A on **10** (35 mg, 0.05 mmol). L_1 was isolated as a pale yellow powder (24 mg, 0.05 mmol, yield 100%). Mp >250 °C. HPLC (System A): t_R=6.83 min. UV/vis (Tris buffer, 50 mM, pH 7.4): λ_{max} (ε, L mol^{-1} cm^{-1})=264 (4300) nm. ^{1}H NMR (300 MHz, D_2O) δ: 3.42 (m, 4H), 3.55–3.66 (m, 8H), 3.71 (s, 4H), 3.87 (s, 4H), 4.67 (s, 4H), 7.59 (d, 2H, *J*=7.8), 8.05 (t, 1H, *J*=7.8). ^{13}C NMR (75 MHz, D_2O) δ: 51.0 (CH_2), 51.6 (CH_2), 53.0 (CH_2), 54.8 (CH_2), 56.7 (CH_2), 58.1 (CH_2), 124.7 (CH), 140.6 (CH), 150.4 (Cq), 170.3 (Cq), 172.3 (Cq). MS (ESI$^+$): *m*/*z* (%) 558.3 (100) $[M-H+2K]^+$, 542.3 (59) $[M-H+Na+K]^+$. HRMS (ESI$^+$) calcd for $C_{21}H_{31}N_5O_8+H^+$=482.2251, found 482.2244 (100%).

4.15. 8,11,14,17,23,24-Hexaazatricyclo[17.3.1.12,6]tetracosa-1(23),2,4,6(24),19,21-hexaene-8,11,14,17-tetraacetic acid (L_2)

The reaction was carried out applying the procedure A on **11** (40 mg, 0.05 mmol). L_2 was isolated as a pale yellow powder (27.5 mg, 0.049 mmol, yield 98%). Mp >250 °C. HPLC (System A): t_R=6.03 min. UV/vis (Tris buffer, 50 mM, pH 7.4): λ_{max} (ε, L mol^{-1} cm^{-1})=240 (7820), 304 (8950), 315sh (3340) nm. ^{1}H NMR (300 MHz, D_2O) δ: 3.20–3.40 (m, 8H), 3.49–3.60 (m, 4H), 3.63 (s, 4H), 3.67 (s, 4H), 4.63 (s, 4H), 7.88 (d, 2H, *J*=7.4), 7.94 (t, 2H, *J*=7.4), 8.49 (d, 2H, *J*=7.8). ^{13}C NMR (75 MHz, D_2O) δ: 51.0 (CH_2), 51.2 (CH_2), 51.9 (CH_2), 54.5 (CH_2), 55.6 (CH_2), 57.1 (CH_2), 123.7 (CH), 128.0 (CH), 147.9 (Cq), 151.4 (Cq), 165.7 (Cq), 172.6 (Cq). MS (ESI$^+$): *m*/*z* (%) 619.3 (61) $[M-H+Na+K]^+$, 597.5 (100) $[M+K]^+$. HRMS (ESI$^+$) calcd for $C_{26}H_{34}N_6O_8+K^+$=597.2075, found 597.2070 (50%).

4.16. 13,16,19,22,28,29,30-Heptaazatetracyclo[22.3.1.12,6.17,11]triacosa-1(28),2,4,6(29),7,9,11(30),24,26-nonaene-13,16,19,22-tetraacetic acid (L_3)

The reaction was carried out applying the procedure B on **12** (65 mg, 0.075 mmol). L_3 was isolated as a pale yellow powder (44 mg, 0.069 mmol, yield 92%). Mp >250 °C. HPLC (System A): t_R=11.68 min. UV/vis (Tris buffer, 50 mM, pH 7.4): λ_{max} (ε, L mol^{-1} cm^{-1})=230 (14,900), 287 (10,700), 302 (9850) nm. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.60–3.30 (m, 12H), 3.30–3.70 (m, 8H), 3.90–4.20 (m, 4H), 7.53 (m, 2H), 7.80–8.26 (m, 5H), 8.45 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 50.2 (CH_2), 50.5 (CH_2), 51.5 (CH_2), 55.0 (CH_2), 55.7 (CH_2), 59.2 (CH_2), 122.3 (CH), 123.5 (CH), 124.5 (CH), 138.7 (CH), 139.1 (CH), 156.5 (Cq), 157.3 (Cq), 158.7 (Cq), 170.8 (Cq), 172.6 (Cq). MS (ESI$^+$): *m*/*z* (%) 696.4 (73) $[M-H+Na+K]^+$, 674.5 (100) $[M+K]^+$, 658.5 (36) $[M+Na]^+$. HRMS (ESI$^+$) calcd for $C_{31}H_{37}N_7O_8+H^+$=636.2782, found 636.2754 (75%).

4.17. 8,11,14,17,23,24-Hexaazatricyclo[17.3.1.12,6]tetracosa-1(23),2,4,6(24),19,21-hexaene-8,11,14,17-tetraacetic acid, 4-(methoxycarbonyl) (L_4)

The reaction was carried out applying the procedure B on **15** (50 mg, 0.065 mmol). L_4 was isolated as a pale yellow powder

(35 mg, 0.065 mmol, yield 100%). Mp >250 °C. HPLC (System A): t_R=6.68 min. ^{1}H NMR (300 MHz, D_2O) δ: 3.34 (s, 4H), 3.49 (m, 4H), 3.60 (m, 4H), 3.70 (s, 4H), 3.90 (s, 4H), 3.92 (s, 3H), 4.62 (s, 4H), 7.95 (s, 2H). ^{13}C NMR (75 MHz, D_2O) δ: 50.7 (CH_2), 51.6 (CH_2), 52.5 (CH_2), 53.5 (CH_3), 53.7 (CH_2), 55.4 (CH_2), 58.0 (CH_2), 123.7 (CH), 140.6 (Cq), 152.4 (Cq), 166.0 (Cq), 169.8 (Cq), 171.5 (Cq). HRMS (ESI$^+$) calcd for $C_{23}H_{33}N_5O_{10}$+H^+=540.2306, found 540.2304 (100%).

4.18. 8,11,14,17,23,24-Hexaazatricyclo[17.3.1.12,6]tetracosa-1(23),2,4,6(24),19,21-hexaene-8,11,14,17-tetraacetic acid, 4-(methoxycarbonyl) (L_5)

The reaction was carried out applying the procedure A on **16**·Na (33 mg, 0.035 mmol). $\mathbf{L_2}$ was isolated as a pale yellow powder (19.5 mg, 0.032 mmol, yield 91%). Mp >250 °C. HPLC (System B): t_R=9.54 min. UV/vis (Tris buffer, 50 mM, pH 7.4): λ_{max} (ε, L mol^{-1} cm^{-1})=243 (9500), 315 (8050) nm. ^{1}H NMR (300 MHz, D_2O) δ: 3.00–3.24 (m, 4H), 3.26–3.43 (m, 4H), 3.44–3.58 (m, 4H), 3.63 (s, 2H), 3.70 (s, 2H), 3.75–3.93 (m, 4H), 4.04 (s, 3H), 4.52 (s, 2H), 4.87 (s, 2H), 7.90 (d, 1H, J=5.1), 8.20 (br s, 1H), 8.51 (br s, 2H), 8.77 (br s, 1H). ^{13}C NMR (75 MHz, D_2O) δ: 50.2 (CH_2), 50.9 (CH_2), 52.0 (CH_2), 52.5 (CH_2), 53.6 (CH_3), 53.8 (CH_2), 54.6 (CH_2), 55.1 (CH_2), 55.5 (CH_2), 55.9 (CH_2), 57.1 (CH_2), 58.2 (CH_2), 63.6 (CH_2), 122.5 (CH), 124.0 (CH), 125.9 (CH), 127.3 (CH), 129.7 (CH), 141.2 (Cq), 152.2 (Cq), 152.3 (Cq), 162.9 (Cq), 163.1 (Cq), 165.7 (Cq), 170.7 (Cq), 174.2 (Cq). MS (ESI$^+$): m/z (%) 639.3 (80) [M+Na]$^+$, 617.3 (100) [M+H]$^+$. HRMS (ESI$^+$) calcd for $C_{28}H_{36}N_6O_{10}$+Na^+=639.2391, found 639.2374 (100%).

4.19. In situ preparation of the lanthanide complexes

To a solution of ligand ($\mathbf{L_1}$–$\mathbf{L_3}$) in water (2×10^{-3} M) was added an equimolar amount of $LnCl_3\cdot6H_2O$ in water (2×10^{-3} M), controlling the pH at 6.5 by simultaneous addition of diluted aqueous NaOH solution. The reaction mixture was then allowed to stir for 24 h at rt and was then adjusted with Tris buffer (50 mM, pH 7.4) at a final concentration of 1×10^{-4} M for absorption and 1×10^{-6} M for emission spectroscopies.

4.19.1. Complex $\mathbf{L_1}$·Eu. MS (ESI$^-$): m/z (%) 630.1 (100) [($\mathbf{L_1}$–3H) Eu–H]$^-$. HPLC (System A): t_R=8.77 min. UV/vis (Tris buffer): λ_{max} (ε, L mol^{-1} cm^{-1})=266 (4900) nm. Luminescence (Tris buffer, λ_{exc}=268 nm): λ_{em} (relative intensity, corrected spectrum), 580 (4), 593 (34), 615 (100), 652 (7), 694 (85) nm.

4.19.2. Complex $\mathbf{L_1}$·Tb. MS (ESI$^-$): m/z (%) 636.1 (100) [($\mathbf{L_1}$–3H) Tb–H]$^-$. HPLC (System A): t_R=8.73 min. UV/vis (Tris buffer): λ_{max} (ε, L mol^{-1} cm^{-1})=266 (4900) nm. Luminescence (Tris buffer, λ_{exc}=268 nm): λ_{em} (relative intensity, corrected spectrum), 488 (41), 544 (100), 584 (32), 620 (26) nm.

4.19.3. Complex $\mathbf{L_2}$·Eu. MS (ESI$^-$): m/z (%) 707.1 (100) [($\mathbf{L_2}$–3H) Eu–H]$^-$. HPLC (System A): t_R=7.05 min. UV/vis (Tris buffer): λ_{max} (ε, L mol^{-1} cm^{-1})=243 (7820), 305 (8950), 318sh (3450) nm. Luminescence (Tris buffer, λ_{exc}=310 nm): λ_{em} (relative intensity, corrected spectrum), 580 (2), 593 (31), 616 (100), 652 (5), 694 (72) nm.

4.19.4. Complex $\mathbf{L_2}$·Tb. MS (ESI$^-$): m/z (%) 713.1 (100) [($\mathbf{L_2}$–3H) Tb–H]$^-$. HPLC (System A): t_R=7.17 min. UV/vis (Tris buffer): λ_{max} (ε, L mol^{-1} cm^{-1})=243 (6100), 305 (7820), 318sh (4465) nm. Luminescence (Tris buffer, λ_{exc}=310 nm): λ_{em} (relative intensity, corrected spectrum), 490 (40), 544 (100), 585 (33), 621 (24) nm.

4.19.5. Complex $\mathbf{L_3}$·Eu. MS (ESI$^-$): m/z (%) 784.3 (100) [($\mathbf{L_3}$–3H) Eu–H]$^-$. HPLC (System A): t_R=8.52 min. UV/vis (Tris buffer): λ_{max} (ε, L mol^{-1} cm^{-1})=284 (9200), 292 (9500), 320 (7900) nm. Luminescence (Tris buffer, λ_{exc}=326 nm): λ_{em} (relative intensity, corrected spectrum), 580 (2), 593 (31), 616 (100), 652 (5), 694 (76) nm.

4.19.6. Complex $\mathbf{L_3}$·Tb. MS (ESI$^-$): m/z (%) 790.3 (100) [($\mathbf{L_3}$–3H) Tb–H]$^-$. HPLC (System A): t_R=8.38 min. UV/vis (Tris buffer): λ_{max} (ε, L mol^{-1} cm^{-1})=284 (8000), 292 (8000), 320 (6800) nm. Luminescence (Tris buffer, λ_{exc}=326 nm): λ_{em} (relative intensity, corrected spectrum), 488 (40), 544 (100), 585 (32), 621 (17) nm.

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