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Direct Access to Terpyridine-Containing Polyazamacrocycles as Photosensitizing Ligands for Eu(III) Luminescence in Aqueous Media

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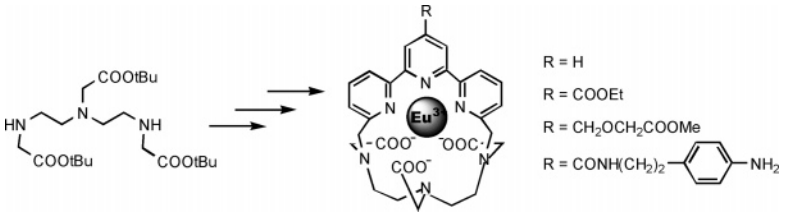

The synthesis of new 18-membered hexaazamacrocycles containing a functionalized 2,2′:6′,2″-terpyridine moiety as part of the cyclic backbone and three acetate pendant arms is described. The reported synthetic procedure is based on the use of an efficient metal template ion effect which controls the macrocyclization step. This procedure is compatible with some functional groups present in the macrocyclic structure. The photophysical properties of the Eu(III) complexes derived from these ligands were examined in aqueous solutions. Their luminescence lifetimes ($\tau \approx 1$ ms) and quantum yields ($13\% < \Phi < 18\%$) on one hand, their high kinetic inertness on the other hand, and the presence of additional functionality allowing their covalent conjugation to biomolecules seriously nominate these complexes as very promising candidates for luminescent labeling of biological materials.

Introduction

Fluorometry is a very powerful technology and one of the fastest growing and most widespread analytical tools in biosciences. Consequently, the design of fluorophores which are suitable for direct determination of analytes in complex biological matrixes is a topical subject and an area of active investigation. In this respect, Ln(III) organocomplexes (Ln = Eu and Tb especially) offer a number of advantageous features as fluorescent labels over conventional organic dyes, including (i) long emission lifetimes (millisecond range) under ambient conditions, the signal can thus be easily distinguished from light scattering and short-lived (nanosecond range) fluorescence background, (ii) no self-quenching, allowing enhancement of the detection sensitivity by multilabeling of biomolecules, and (iii) excellent solubility in aqueous solvents.[1,2] Several europium-complex-based detection systems for time-resolved fluoroimmunoassays are available for routine biomedical diagnoses.[3] The use of these luminescent labels in hybridization, enzyme or receptor assays, cell sorting, bioactive ion detection, the luminescence resonance energy transfer (LRET) technique, and optical microscopy has also been reported.[4]

To design an efficient luminescent Ln(III) edifice, it is well established that the ligand should possess a strong binding domain which ensures a high kinetic stability with respect to metal ion dissociation and a chromophoric domain which collects the excitation light and channels it to the lanthanide ion.[4a] Moreover, it must have a high denticity to avert direct coordination of water molecules, which leads to radiationless energy transfer to the aqueous solvent shell. To avoid ligand exchange processes and generate high kinetic inertness of Ln(III) complexes in aqueous solutions and biological media, the same basic ligand systems which have been developed for the elaboration of Gd(III) contrast agents for magnetic resonance imaging[5] were exploited for use in luminescent Ln(III) bioprobe research. A survey of the literature

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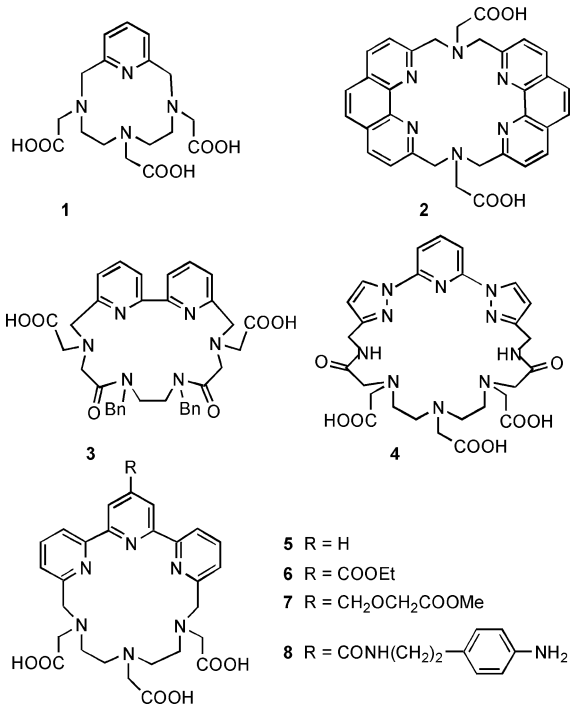
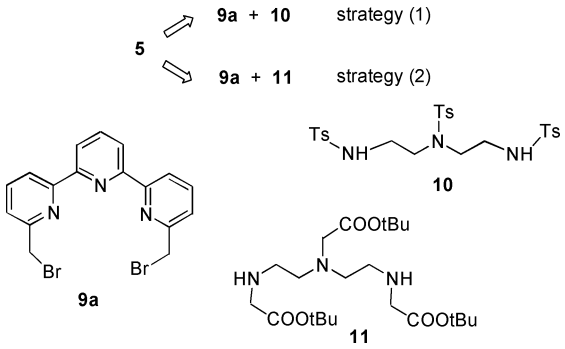
SCHEME 1



highlights the use of aminopolycarboxylic acyclic chain ligands[6] and DOTA-type macrocyclic derivatives where a chromophoric unit is substituted on one acetic side arm (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate).[7] We have noticed only a few reports about lanthanide binding by macrocyclic compounds incorporating both an intracyclic chromophoric unit and pendant carboxylate groups. In this family of ligands, compounds **1**−**4** (Scheme 1) incorporating a pyridine,[8] phenanthroline,[9] bipyridine,[10] or bis(pyrazol-1-yl)pyridine[11] unit as the heterocyclic moiety into 12−24-membered macrocyclic structures are the main representative examples.

We report here our results concerning the synthesis and the main photophysical properties of a new series of Eu(III) macrocyclic receptors containing a terpyridine moiety as part of a polyamine ring with N-pendant acetate groups (compounds **5**−**8**, Scheme 1). Acting as a photosensitizer antenna to enhance the luminescence of Eu(III) ion in biological media, the terpyridine group is a very suitable energy-absorbing and energy-transferring moiety. It can be excited efficiently ($\epsilon \approx 10000\ M^{-1}\ cm^{-1}$) at a wavelength of ca. 330 nm, which is a convenient excitation wavelength for instrumental reasons related to UV transmission of glass slides, for the use of nitrogen laser excitation, and for reducing the amount of fluorescence caused by the surrounding tissue. Its lowest excited state ($E(^3\pi\pi^*) = 23000\ cm^{-1}$) is sufficiently high in energy to be able to transfer excitation to the luminescence levels of Eu(III) ion (^5D_j, $j = 0-2$).[12] In addition, the nine coordination sites reported for Eu(III) ions in aqueous solutions may be entirely occupied by the nonadentate structure of these ligands, preventing coordination of other ligands (solvent or anion). Another key point to be put forward is that the terpyridine group may be easily functionalized at the 4-position of the central ring. As a matter of fact, for luminescent complexes of practical use the ligand must contain appropriate functionalization to be covalently attached to bioactive molecules without altering its binding properties.[13] For this purpose, compounds **6**−**8** contain an ester or amino extracyclic moiety and are potential precursors of amine-, acid-, or thiol-reactive complexes. Preliminary results concerning compound **5** have been published previously.[14]

SCHEME 2



Results and Discussion

Synthesis. As evidenced in numerous reports on the synthesis of polyazamacrocyclic ligands, the crucial step in the preparation of the target ligands is the macrocyclization reaction.[15] In this study, we investigated two strategies for the preparation of the 18-membered macrocyclic ligand **5** (Scheme 2). Our first strategy was based on the "Richman−Atkins" method, which is a time-tested and probably the most widely used process for macroring closure. In this procedure, the macrocyclization reaction was conducted by reacting disodium salts of tosylated polyamines with dihalogeno fragments in dimethylformamide at elevated temperature and did not require

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(7) For examples see: (a) Quici, S.; Marzanni, G.; Cavazzini, M.; Anelli, P. L.; Botta, M.; Gianolio, E.; Accorsi, G.; Armaroli, N.; Barigelletti, F. *Inorg. Chem.* **2002**, *41*, 2777−2784. (b) Beeby, A.; Bushby, L. M.; Maffeo, D.; Williams, J. A. G. *J. Chem. Soc., Dalton Trans.* **2002**, 48−54. (c) Dadabhoy, A.; Faulkner, S.; Sammes, P. G. *J. Chem. Soc., Perkin Trans. 2* **2000**, 2359−2360. (d) Lowe, M. P.; Parker, D. *Chem. Commun.* **2000**, 707−708.

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(13) For a recent example, see: (a) Manning, H. C.; Goebel, T.; Marx, J. N.; Bornhop, D. J. *Org. Lett.* **2002**, *4*, 1075−1078. (b) Manning, H. C.; Goebel, T.; Thompson, R. C.; Price, R. R.; Lee, H.; Bornhop, D. J. *Bioconjugate Chem.* **2004**, *15*, 1488−1495.

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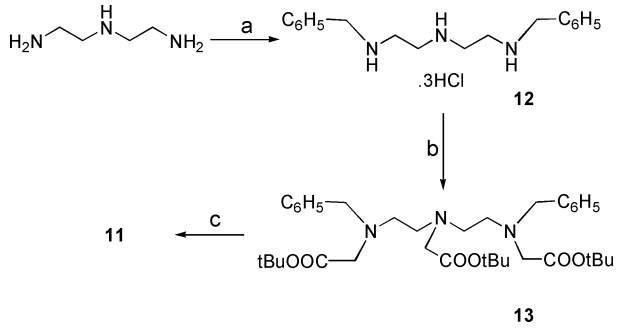
SCHEME 3[a]

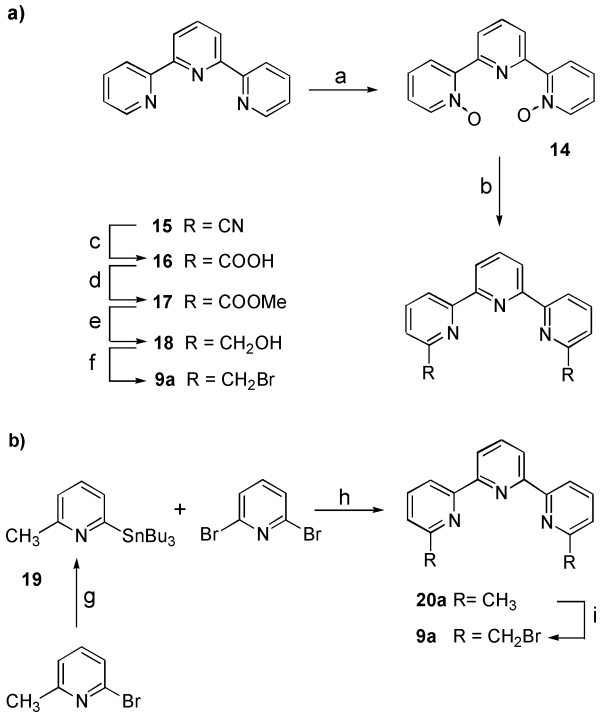


[a] Reagents and conditions: (a) (i) C_6H_5CHO, EtOH, 50 °C, (ii) $NaBH_4$, EtOH, 50 °C, (iii) HCl(aq) (37%), CH_2Cl_2; (b) $BrCH_2COO$-tBu, K_2CO_3, CH_3CN, reflux; (c) Pd/C (10%), H_2 (3 bar), MeOH, rt.

a template ion or the use of high-dilution techniques.[16] Recent studies on the sulfonamide ring-closure process have shown that the reaction can be more conveniently carried out by using tosylated polyamines, potassium carbonate as a base, and anhydrous acetonitrile as a solvent under heterogeneous conditions.[17] However, in this strategy, it is still necessary to carry out further alkylations on the deprotected macrocycle to obtain the desired three-armed systems. The second strategy is an alternative procedure and involves the triamine **11**, which presents coordinating function groups that may interact with a metal ion. Thus, it may be possible to control the formation of the macroring by a metal "template" ion effect. An important advantage of this method is that the acetate arms are incorporated prior to cyclization, eliminating the need for a protection/deprotection sequence.

Starting Materials. The tosylated triamine **10** was obtained according to the literature,[18] and the synthetic procedure of the versatile building block **11** is reported in Scheme 3. In this route, the protection of primary amine functions of diethylenetriamine was performed by a one-pot reductive amination sequence.[19] By using a simplified procedure, the HCl salt of the resulting polyamine **12** was isolated in a 94% yield. The three secondary amine groups of **12** were alkylated with an excess of *tert*-butylbromoacetate, in refluxing acetonitrile and in the presence of K_2CO_3 as base to give **13** in 79% yield. A complete trialkylation of **12** was also obtained by using an organic base ($^iPr_2NEt$) in DMF at room temperature (63% yield). Debenzylation of **13** was readily achieved in a quantitative yield by catalytic hydrogenation at room temperature under hydrogen pressure, in methanol, using Pd/C (10%) as catalyst. Removal of the *N*-benzyl groups was also effective by using catalytic transfer hydrogenation with ammonium formate as the hydrogen source, but this method suffered from time-consuming purification steps related to the formation of side products.

SCHEME 4[a]



[a] Reagents and conditions: (a) 3-$ClC_6H_4CO_3H$, CH_2Cl_2, rt, 12 h; (b) Me_3SiCN, PhCOCl, CH_2Cl_2, rt, 12 h; (c) (i) KOH, EtOH–H_2O, reflux,12 h, (ii) H_2SO_4–CH_3COOH, reflux, 5 h; (d) $SOCl_2$, MeOH, reflux, 5 h; (e) $NaBH_4$, EtOH, reflux, 12 h; (f) PBr_3, DMF, rt, 12 h; (g) (i) nBuLi, THF, −78 °C, (ii) Bu_3SnCl, −78 °C, 4 h; (h) 2 equiv of **19**, $Pd(PPh_3)_4$, toluene, reflux, 4 days; (i) NBS, AIBN, benzene, *hν*, reflux, 9 h.

The preparation of 6,6″-bis(bromomethyl)-2,2′:6′,2″-terpyridine (**9a**) was carried out following the classical procedure depicted in the Scheme 4a, starting from commercial 2,2′:6′,2″-terpyridine. The introduction of cyano groups on the terminal pyridine moieties was performed through the *N*,*N*″-dioxide derivative and by using the modified Reissert–Henze reaction.[20] These first two steps were carried out according to the literature[21] with improved yields. Successive treatments of dicarbonitrile **15** with KOH in EtOH/H_2O and then with a mixture of sulfuric acid and acetic acid (1:1) gave the dicarboxylic derivative **16** in 92% yield. This latter compound was converted to its methyl ester, and the corresponding diol **18** was finally brominated with phosphorus tribromide, using standard methodology. **9a** was thus obtained with an overall yield of 37%.

Compound **9a** was also prepared by another synthetic route, using a Stille-type coupling procedure (Scheme 4b). In this route, 2,6-dibromopyridine was treated with 2 equiv of 2-methyl-6-(tributylstannyl)pyridine (**19**)[22] in the

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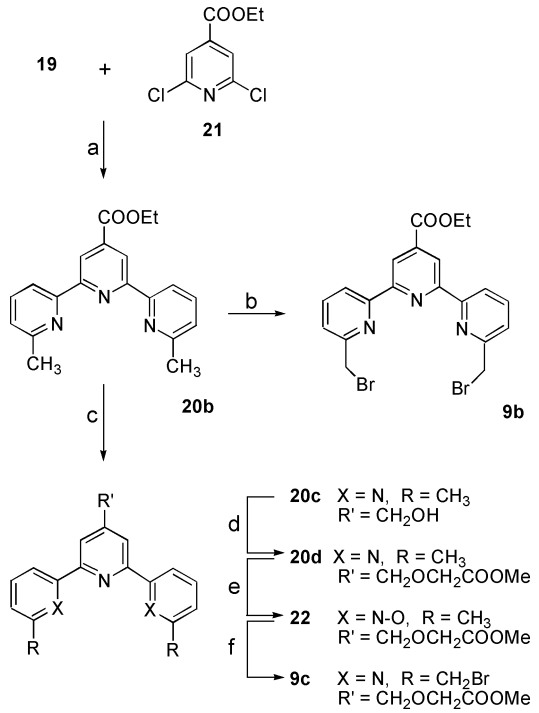
SCHEME 5[a]



[a] Reagents and conditions: (a) 2 equiv of **19**, $Pd(PPh_3)_4$, toluene, reflux, 4 days; (b) NBS, AIBN, benzene, *hν*, reflux, 3 h; (c) $NaBH_4$, EtOH, reflux, 24 h; (d) NaH, $BrCH_2COOMe$, THF, reflux, 24 h; (e) 3-$ClC_6H_4CO_3H$, CH_2Cl_2, rt, 12 h; (f) (i) $(CF_3CO)_2O$, $CHCl_3$, 40 °C, 5 h, (ii) LiBr, THF−DMF, rt, 40 h.

presence of $(PPh_3)_4Pd$ for 4 days in toluene. Very recently, Schubert reported that this coupling reaction afforded mainly 6-bromo-6′-methyl-2,2′-bipyridine, resulting from a single coupling step.[23] It was suggested that the activity of the bromo function was reduced in this intermediate due to the steric and electronic influence of the pyridine ring at the 6-position. In our hands, this reaction was clean and the desired 6,6″-dimethyl-2,2′:6′,2″-terpyridine (**20a**) was isolated by liquid chromatography on alumina in high yield (75%). The methyl groups of **20a** were monobrominated via classical radical conditions (NBS, AIBN, and under irradiation). In this reaction, the use of benzene as solvent[24] limits multiple bromination, especially the formation of an unsymmetrical (dibromomethyl)methyl derivative, making the purification process easier. In this route and starting from commercially available 2-bromo-6-methylpyridine, compound **9a** was obtained with an overall yield of 41%.

The Stille-type cross-coupling procedure was also used for the introduction of an ester group into the central 4′-position of 6,6″-dimethyl-2,2′:6′,2″-terpyridine (Scheme 5). The use of 2,6-dichloroisonicotinic ethyl ester (**21**) as the central building block instead of the corresponding dibromo derivative[25] led to the 6,6″-dimethyl-2,2′:6′,2″-terpyridine 4′-ethyl ester (**20b**) in similar yield (63% and 55%,[25] respectively). This is worth noting since the dichloro derivative is more easily available than its dibromo counterpart.[26,27] The methyl groups of **20b** were then brominated via NBS and AIBN in a benzene/water mixture as previously described.[25]

In contrast, the attempts to synthesize compound **20d** by coupling 2 equiv of organotin compound **19** with 2,6-dichloropyridine functionalized in the 4-position with a CH_2OCH_2COOMe chain using the standard Pd(0) catalytic conditions failed. The target terpyridine **20d** was obtained in two steps, by reduction of the 4′-ethyl ester group of **20b** with an excess of $NaBH_4$ in ethanol and subsequent alkylation of the hydroxy function by methyl bromoacetate (72% and 58% yield, respectively). Although several methods were utilized, it was not possible to achieve the functionalization of the methyl groups of **20d** by a free radical bromination. This failure was apparently due to competitive cleavage of the side chain. For example, when **20d** was reacted with Br_2 in a biphasic medium (C_6H_6/H_2O), 6,6″-dimethyl-2,2′:6′,2″-terpyridine-4′-carbaldehyde was the only reaction product isolated after column chromatography (45% yield). Thus, dimethyl functionalization of **20d** was achieved by employing an N,N″-oxidation process.[28] The selective oxidation of **20d** by 4 equiv of *m*-CPBA in dichloromethane gave the corresponding 1,1″-dioxide derivative **22** (Scheme 5) in 81% yield. Treatment of this compound with trifluoroacetic anhydride and an excess of anhydrous LiBr in THF afforded the bis(bromomethylated) compound **9c** in 13% yield. The conditions for both reaction and purification steps could be optimized and the yield improved.

Macrocyclization Reaction. The initial route to obtain the 18-membered macrocyclic ligand **5** involved the treatment of the tosylated triamine **10** with dibromide **9a** in the presence of K_2CO_3 and MeCN (Scheme 6). This reaction did not give the 1:1 cyclization product, but it mainly afforded the dimeric 36-membered macrocycle **23** in 20% yield. Furthermore, no evidence for the monomeric 18-membered macrocycle was observed by modifying the nature of the base metal ion (Na_2CO_3 instead of K_2CO_3); only higher amounts of acyclic products were obtained. This orientation of the sulfonamide cyclization reaction is in marked contrast with the predominant 1:1 macrocycle formation observed by Aime[17a] or us[29] for analogous macrocycles where pyridine or 2,2′-bipyridine moieties are substituted for the terpyridine moiety. Following the same procedure, the corresponding 12- or 15-membered macrocycles were isolated in 90% and 77% yield, respectively. These results support the earlier observations of Iwata and Kuzuhara[30] indicating that the chain length of the two starting reagents is one of the major factors playing a role in this macrocyclization process. These authors pointed out that the best results were obtained by coupling the electrophile containing the shortest chain length with the nucleophile containing the longest chain.

In the second strategy (Scheme 6), a marked ion effect was found facilitating the macrocyclization process in

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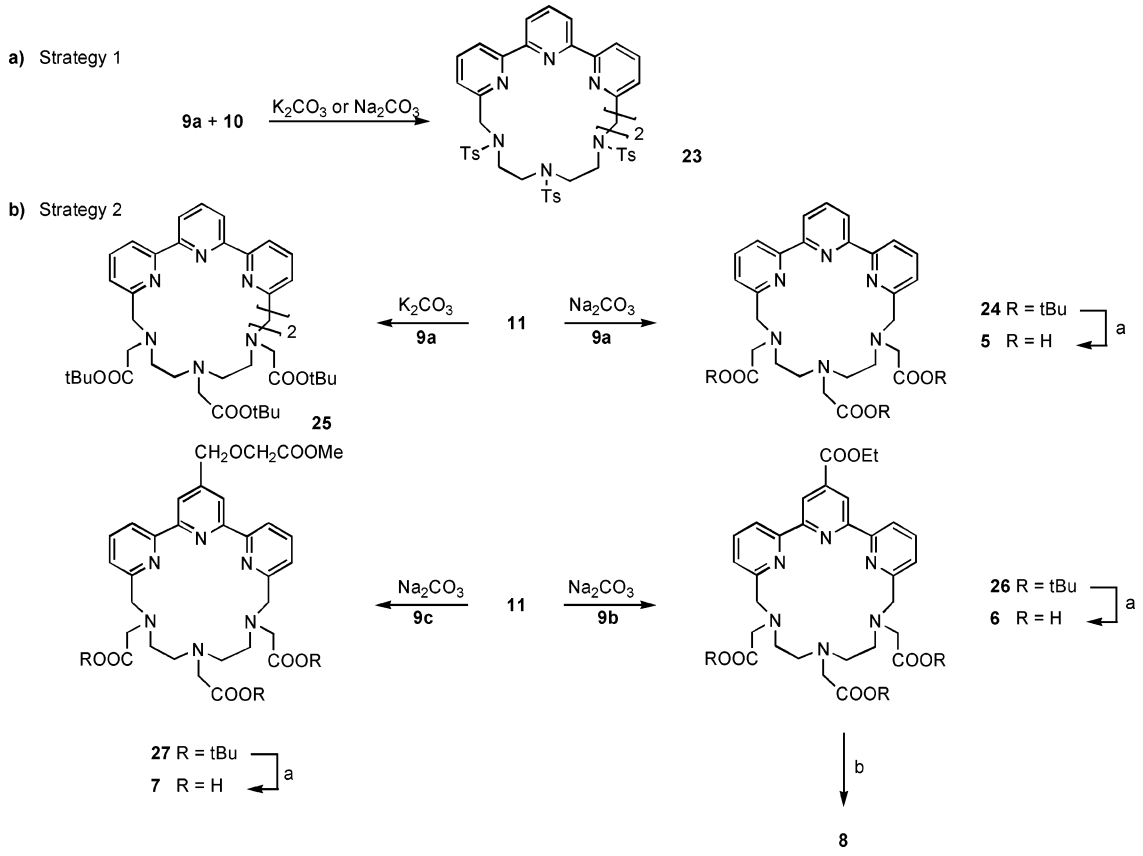
SCHEME 6[a]



[a] Reagents and conditions: macrocyclization reactions, [reactants] = 2.2×10^{-3} M, M_2CO_3, CH_3CN, reflux, 12 h; (a) CF_3COOH, CH_2Cl_2, rt, 12−24 h; (b) (i) 2-(4-aminophenyl)ethylamine, NaCN (catalytic), MeOH, 100 °C, 4 days, (ii) NaOH, EtOH−H_2O, rt, 24 h.

favor of the targeted macrocycle. As a matter of fact, condensation of the building block **11** with dibromide **9a** was tested with various metal carbonates (M_2CO_3, M = Li, Na, or K) in refluxing CH_3CN and without the use of high-dilution techniques ([reactants] = 2.3×10^{-3} M). The procedure using Na_2CO_3 as a base generated mainly the desired 18-membered macrocycle **24**, which was isolated, after column chromatography, as its NaBr complex in 56% yield. When a lithium ion was used, the 1:1 macrocyclization process was also favored, but this reaction was accompanied by polymerization to a higher extent. Under similar reaction conditions and by using K_2CO_3, formation of the 18-membered monomer structure was not observed, but the 36-membered dimer structure free of potassium ion **25** was isolated in 37% yield. These experimental results suggest a metal template ion effect as a dominant factor in this 1:1 cyclization reaction, as recently reported[31] in a macrobicyclization procedure leading to polyamine cryptands incorporating bipyridine units. The best result obtained with Na_2CO_3 can be probably explained by an optimum fit between Na^+ and the 18-membered cavity. This is supported by the ability of this molecular cavity to accommodate Eu^{3+} (vide infra), an ion of size similar to that of Na^+.

The proposed monomer (M) and dimer (2M) structures for **24** and **25**, respectively, were supported by a mass spectrometry study. The ESI^+ mass spectrum of **24** was dominated by peaks at *m/z* 703.5 and 725.5 (base peak) corresponding to the species $[M + H]^+$ and $[M + Na]^+$, respectively. The fragmentations of these two ions are governed by the usual rupture of the *tert*-butyl ester bond, which leads to the observation of one, two, and three successive losses of 56 Da (C_4H_8 fragment). In the ESI^+ MS spectrum of **25**, recorded in the same conditions as that of **24**, dimeric monocharged ions $[2M + H]^+$ and $[2M + Na]^+$ and dimeric doubly charged ion $[2M + 2H]^{2+}$ (base peak) were successfully detected at *m/z* 1406, 1428, and 703.5, respectively. The dimeric structure of **25** was confirmed by the observation in the same spectrum of a series of six peaks corresponding to the loss of one to six 28 Da units from the doubly charged ion at *m/z* 703.5, accordingly with the presence of six *tert*-butyl ester functions in this compound. These 1:1 and 2:2 macrocycles may also be easily distinguished by their ^1H NMR and UV spectra. Their ^1H NMR spectra exhibited significant differences for the heteroatomic hydrogens. Especially, the protons at the 3,3″-positions of the terpyridine unit are shifted to higher field in **24** as compared to the corresponding dimer **25** (7.99 vs 8.32 ppm). This suggests that the orientation of the terpyridinyl moiety in the smaller macrocycle **24** is approaching a cis−cis conformation.[22] The observation of two distant UV absorption bands in MeOH for **24** (λ = 281 and 307 nm) and only one for **25** (λ = 286 nm) is also consistent with a preferential cis−cis conformation of the terpyridine unit in **24**. This was suggested by the previous study of UV

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spectra of 2,2′:6′,2″-terpyridine and bisannealed derivatives.[32] These results are consistent with the participation of the three heterocyclic nitrogen atoms of **24** in sodium binding. On the other hand, complexation by Na^+ was accompanied by a reduction in the carbonyl stretching frequency of only 5 cm^{-1}, indicating very weak or no ligation of the ester functions of this ligand.

Finally, acidic treatment of compound **24** gave the triacid **5** in 77% isolated yield.

Functionalized Macrocycles. Eighteen-membered macrocycles **6** and **7** tethered to an ester group were also prepared by using this new synthetic approach. Condensation of the triamine **11** with dibromide **9b** or **9c** by using Na_2CO_3 gave the NaBr complexes of the corresponding macrocycle **26** or **27**. Na−**26** and Na−**27** were isolated in 34% and 43% yield, respectively, after purification by column chromatography. Mild hydrolysis of the *tert*-butyl esters with trifluoroacetic acid at room temperature gave the triacid derivatives **6** and **7** in 87% and 65% yield, respectively. The terpyridine macrocycle tethered to an aromatic amino group, compound **8**, was synthesized starting with tetraester macrocycle **26** (Scheme 6). For the aminolysis of the ethyl ester group of **26**, we took advantage of the method reported by Hoegberg et al.[33] In this method, the cyanide anion was used as a mild nucleophilic catalyst in the aminolysis of aromatic esters with primary aliphatic amines. Reaction of **26** with 2-(4-aminophenyl)ethylamine was carried out in methanol and in the presence of a catalytic amount of NaCN. Subsequent saponification of the remaining three *tert*-butyl ester functions was conducted on the crude condensation product and afforded the targeted macrocycle **8** after column chromatography (29% yield, two steps).

Photophysical Properties of Europium Complexes. To investigate the potentiality of these ligands to photosensitize the europium ion and to determine the extent of its protection against water interactions, equivalent amounts of europium (in the form of $EuCl_3{\cdot}6H_2O$) were added to 1×10^{-5} M solutions of **5**−**8** in aqueous solution (borate buffer, pH 8.6). Both Eu−(**5**−**8**) complexes gave classical europium-centered luminescence spectra, with the strongest transition at 620 nm ($^5D_0$-to-$^7F_2$ transition) when photoexcited in the lowest energy absorption of the heterocyclic chromophore. The similarity between the absorption and excitation spectra proves energy transfer from the excited states of the ligand to the Eu(III) emission states. Representative excitation and emission spectra are shown in Figure 1 for Eu−**6**. The intensity ratios of the $^5D_0 \rightarrow {}^7F_2$ transition and the $^5D_0 \rightarrow {}^7F_1$ transition, which are a good measure of the nature and symmetry of the first coordination sphere,[1] are identical in the four investigated complexes, suggesting that the binding sites are the same in all cases. All the emission lifetimes of the Eu ($^5D_0$) excited level measured for these complexes are monoexponential, as expected for one discrete Eu−(**5**−**8**) solution species and are in the millisecond range (Table 1).

On the other hand, the great sensitivity of Eu^{3+} luminescence toward quenching by O−H oscillators of the solvent provides an experimental tool to determine the degree to which these new ligands are capable of shielding the ion from the environment. By comparison of the luminescence lifetimes in protonated and deuterated water and by using the conventional "Horrocks equation",[34] the hydration number (i.e., the number of coordinated water molecules) was determined to be in the range 0.37−0.42. These noninteger q values represent the quenching contribution of unbound but closely diffusing water molecules.[35] Quite recently, a refined equation taking into account the effect of second-sphere water molecules has been proposed for Eu(III) complexes, with an estimated uncertainty on q of ±0.1.[36] The values of q using this analysis are reduced to 0.1. These results clearly indicate that water is expelled from the inner coordination sphere of the europium ion in these complexes and suggests that the nine binding sites provided by the ligands **5**−**8**, which wrap about the metal ion uniformly, coordinate the metal ion. The overall quantum yields of the metal-centered luminescence, Φ, upon excitation of the terpyridine group are given in Table 1. The Φ value found for the unsubstituted terpyridinyl chelate **5** is relatively high and quite competitive with those recently reported in the literature for Eu(III)

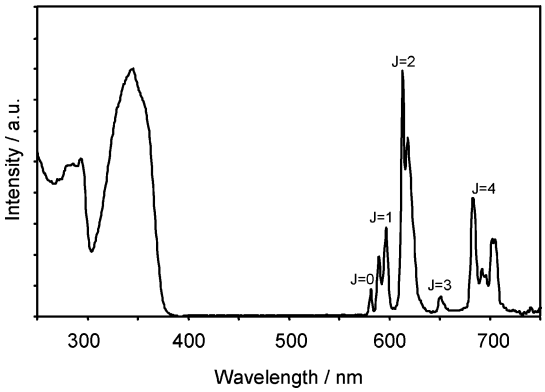

FIGURE 1. Corrected excitation (250−450 nm, $\lambda_{em} = 618$ nm) and emission (550−750 nm, $\lambda_{ex} = 349$ nm) spectra of the Eu−**6** complex solution (1×10^{-6} M in borate buffer, pH 8.6). Excitation and emission band-passes 2.5 nm, delay time 0.1 ms, gate time 0.4 ms. The emission bands arise from the $^5D_0 \rightarrow {}^7F_J$ transitions; the J values are shown on the spectrum.

TABLE 1. Luminescence Data[a] for Complexes Eu−(5−8) and Hydration Numbers *q*

compd	λ_{exc} (nm)	τ_{H_2O} (ms)	τ_{D_2O} (ms)	Φ_{H_2O} (%)	I_{F_2}/I_{F_1}[b]	q_{H_2O}[c]	q_{H_2O}[d]
Eu−**5**	334	1.06	1.70	17.5	3.1	0.37	0.05
Eu−**6**	349	1.07	1.77	13.4	3.4	0.39	0.07
Eu−**7**	329	0.92	1.47	15.0	3.4	0.43	0.11
Eu−**8**	344	0.45	0.55	4.0	3.3	0.42	0.10

[a] In aerated borate buffer solution (0.05 M, pH 8.6) at 295 K. [b] I_{F_2}/I_{F_1} is the ratio of the integrated emissions from the $^5D_0 \rightarrow {}^7F_2$ and $^5D_0 \rightarrow {}^7F_1$ transitions. [c] Calculated by using the following equation: $q = 1.05(1/\tau_{H_2O} - 1/\tau_{D_2O})$.[34] [d] Calculated by using the following equation: $q = 1.11(1/\tau_{H_2O} - 1/\tau_{D_2O} - 0.31)$.[36]

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complexes derived from podands,[37] macrocycles,[38] cryptands,[39] or polyaminocarboxylic ligands[40] and sensitized by various chromophores. More significantly, the functionalization of the terpyridine group in ligands **6** and **7** does not change the capacity of the antenna to sensitize efficiently the luminescence of the europium ion in aqueous solution. By comparison, at pH 8.6, a europium complex derived from the amino-substituted ligand **8** exhibited a weaker luminescence. This is expected, since simple amino groups are known to quench the luminescence of Eu(III) complexes by a photoinduced electron transfer (PET) process. This PET activity is efficient even if the amino groups are not directly attached to excited aromatic moieties.[41] It is also well established that further modification of the NH_2 group into an activated group (isocyanato derivative) or protonation of the amino substituent suppresses this quenching.[42] Effectively, when the amino group of **8** is protonated (pH 2.0), the luminescent properties of Eu−**8** are restored, with an emission lifetime of 0.85 ms and a proton-induced luminescence enhancement factor of 3.6.

Because of our interest in synthesizing lanthanide complexes that are kinetically robust to metal ion release under physiological conditions, we have studied, by luminescence spectroscopy, the resistance of the Eu−**5** complex to dissociation at various pHs and in the presence of competing cations or ligands. No change in its luminescence properties was observed after several days at room temperature in aerated water (examined pH range 6−9), in the presence of an excess of abundant human serum cations (especially Ca^{2+} and Zn^{2+}), and in the presence of proteins (BSA).[43] On the other hand, when Eu−**5** was challenged in the presence of a 5-fold excess of DOTA[44] at pH 7.4, 35% of the complex was dissociated after 20 days. By using Verhoeven's method,[45] a conditional stability constant for Eu−**5** was estimated to be 10^{18}. These preliminary data demonstrate that we have in hand complexes which are thermodynamically and kinetically stable species.

In conclusion, we present here a direct approach for the synthesis of a new class of hexaazamacrocycles containing an intracyclic terpyridine moiety and acetate pendant arms. The reported methodology constitutes an alternative to the original or related Richman−Atkins procedures,[17,46] and, in principle, permits substantial variations of the pendant groups, but also the azaheterocycle subcyclic unit.[47] These ligands form stable lanthanide complexes in aqueous solutions and sensitize efficiently the luminescence of the Eu(III) ion in a pertinent excitation wavelength region (around 337 nm). Moreover, these complexes feature amino or ester groups that can be linked to biomolecules by classical procedures. We can also notice that the net charge of these complexes is neutral, an important factor to take into account, since positively or negatively charged complexes may lead to nonspecific binding in biological media.

Experimental Section

General experimental data are given in the Supporting Information.

1,2-Ethanediamine, *N*-(Phenylmethyl)-*N′*-[2-[(phenylmethyl)amino]ethyl]-, Trihydrochloride (12). This compound was prepared according to a previously published procedure[19] with the following modifications: Benzaldehyde (4 mL, 39.4 mmol) and diethylenetriamine (1.93 mL, 17.9 mmol) were dissolved in 140 mL of absolute ethanol, and the mixture was heated at 50 °C for 2 h. After the mixture was cooled to ambient temperature, $NaBH_4$ (3.36 g, 88.8 mmol) was added, and the resulting mixture was stirred at 50 °C for 2 h and then at room temperature for 15 h. The solvent was removed by rotary evaporation, and the residue was diluted with aqueous NaOH (1 M, 100 mL) and extracted with dichloromethane (3 × 100 mL). The combined organic phases were washed with aqueous NaOH (1 M, 100 mL), concentrated to $^1/_2$, and acidified with aqueous HCl (concentrated, 5 mL). The resulting precipitate was collected by vacuum filtration and washed with dichloromethane and diethyl ether to give after drying in vacuo at 50 °C a white powder (6.60 g, 94%): mp > 250 °C (lit.[19] mp 280 °C dec); ^{1}H NMR (250 MHz, D_2O) δ 3.38 (s, 8H), 4.26 (s, 4H), 7.44 (s, 10H); ^{13}C NMR (62.5 MHz, D_2O) δ 45.7, 46.3, 54.2, 132.0, 132.5, 132.6, 132.7.

3-Oxa-6,9,12-triazatetradecan-14-oic Acid, 9-[2-(1,1-Dimethylethoxy)-2-oxoethyl]-2,2-dimethyl-4-oxo-6,12-bis-(phenylmethyl)-, 1,1-Dimethylethyl Ester (13). To a mixture of **12** (3 g, 7.6 mmol) and K_2CO_3 (31.8 g, 230 mmol) in anhydrous acetonitrile (80 mL) was added dropwise a solution of *tert*-butylbromoacetate (6.72 mL, 45.5 mmol) in anhydrous acetonitrile (50 mL). The mixture was heated at reflux for 2 h and stirred at room temperature overnight. Subsequently, the reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was purified by chromatography on silica gel, eluting first with dichloromethane and then with a gradient of dichloromethane−acetonitrile (v/v 80:20), to afford 3.76 g (79%) of the title compound as a yellow oil: R_f = 0.22 (silica, CH_2Cl_2/CH_3CN, 80:20); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.45 (s, 18H), 2.76 (s, 8H), 3.23 (s, 4H), 3.31 (s, 2H), 3.77 (s, 4H), 7.31 (m, 10H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 28.2, 52.0, 52.6, 55.2, 56.0, 58.4, 80.6, 80.7, 127.0, 128.2, 129.0, 139.2, 170.9, 171.0; IR (neat, cm^{-1}) 1733; MS (ESI$^+$) *m/z* (rel intens) 648.7 ([M + Na]$^+$, 19), 626.7 ([M + H]$^+$, 100). Anal. Calcd for $C_{36}H_{55}N_3O_6$: C, 69.09; H, 8.86; N, 6.71. Found: C, 68.78; H, 9.18; N, 6.71.

3-Oxa-6,9,12-triazatetradecan-14-oic Acid, 9-[2-(1,1-Dimethylethoxy)-2-oxoethyl]-2,2-dimethyl-4-oxo-, 1,1-Dimethylethyl Ester (11). A mixture of **13** (1.5 g, 2.4 mmol) and 10% Pd/C (300 mg) in methanol (40 mL) was stirred overnight under H_2 (3 bar). The reaction mixture was filtered over Celite, and the filtrate was concentrated in vacuo.

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(43) In these experiments, the Eu−**5** complex (0.01 mM) was incubated for 10 days at 37 °C and physiological pH (HEPES buffer, pH 7.4) in the presence of cations [Ca^{2+} (126 mM), Mg^{2+} (0.8 mM), Zn^{2+} (0.1 mM), Na^+ (140 mM), or K^+ (5 mM)] or in the presence of proteins (BSA, 5 mg/mL).
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Compound **11** (1.07 g) was obtained as a pale yellow oil in quantitative yield: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.44 (s, 18H), 2.67 (t, J = 4.0 Hz, 4H), 2.81 (t, J = 4.0 Hz, 4H), 2.91 (s, 2H), 3.32 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.1, 28.2, 47.2, 51.2, 53.6, 55.7, 80.9, 81.2, 171.0, 171.2; IR (neat, cm^{-1}) 3328, 1734; MS (ESI$^+$) *m/z* (rel intens) 468.3 ([M + Na]$^+$, 5), 446.3 ([M + H]$^+$, 100). Anal. Calcd for $C_{22}H_{43}N_3O_6$, 0.5H_2O: C, 58.13; H, 9.76; N, 9.24. Found: C, 58.15; H, 9.89; N, 9.08.

2,2′:6′,2″-Terpyridine-6,6″-dicarbonitrile (15). 15 was synthesized from **14**[21a] in 92% yield according to the published procedure:[21b] ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.75 (dd, J = 1.0 Hz, 8.0 Hz, 2H), 8.01 (t, J = 7.9 Hz, 2H), 8.05 (t, J = 7.9 Hz, 1H), 8.57 (d, J = 7.8 Hz, 2H), 8.82 (dd, J = 1.0 Hz, 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 117.5, 122.8, 124.4, 128.6, 133.6, 138.1, 138.8, 153.7, 157.6; IR (KBr, cm^{-1}) 2238, 1577, 1562.

2,2′:6′,2″-Terpyridine-6,6″-dicarboxylic Acid (16). To a solution of **15** (300 mg, 1.06 mmol) in ethanol (19 mL) and water (4 mL) was added KOH (594 mg, 10.6 mmol) as a solid. The reaction mixture was refluxed for overnight, and then the solvent was evapored under vacuum. The residue was dissolved in water (5 mL), and the pH was adjusted to 4 with aqueous HCl (1 M). The precipitate was removed by filtration and washed with cold water and acetonitrile. Subsequently, the solid material was heated to reflux in a mixture of concd H_2SO_4/concd CH_3COOH (9 mL, 1:1) for 5 h, and the reaction mixture was then poured onto ice (45 mL). The product was filtered and washed with cold water and acetonitrile. Diacid **16**, which was not further purified, was obtained in 312 mg yield (92%) as a white solid: mp > 250 °C; ^{1}H NMR (250 MHz, DMSO-d_6) δ 8.15 (dd, J = 1.5 Hz, 7.6 Hz, 2H), 8.21 (t, J = 7.6 Hz, 3H), 8.65 (d, J = 7.6 Hz, 2H), 8.87 (dd, J = 1.5 Hz, 7.6 Hz, 2H), 13.26 (s, 2H); IR (KBr, cm^{-1}) 3451, 1709, 1580, 1566; MS (FAB$^+$) *m/z* (rel intens) 344.2 ([M + Na]$^+$, 100).

2,2′:6′,2″-Terpyridine-6,6″-dicarboxylic Acid Dimethyl Ester (17). $SOCl_2$ (1 mL, 13.7 mmol) was dropped slowly into cooled methanol (50 mL). After the mixture was stirred for 10 min at room temperature, **16** (1.21 g, 3.8 mmol) was added and the mixture refluxed for 5 h. After evaporation, the residue was dissolved in $CHCl_3$ (100 mL), methanol (10 mL), and aqueous $NaHCO_3$ (saturated, 100 mL). The organic layer was separated, and the residual aqueous layer was extracted with $CHCl_3$ (3 × 30 mL). The combined organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo to give white crystals (1.25 g, 95% yield) of compound **17**: mp 254−255 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.06 (s, 6H), 8.01 (t, J = 7.7 Hz, 3H), 8.18 (d, J = 7.7 Hz, 2H), 8.62 (d, J = 7.7 Hz, 2H), 8.80 (d, J = 7.9 Hz, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 53.1, 122.3, 124.5, 125.3, 138.1, 138.5, 147.8, 154.7, 156.5, 166.1; IR (KBr, cm^{-1}) 1723, 1579, 1567. Anal. Calcd for $C_{19}H_{15}N_3O_4$, 2H_2O: C, 59.22; H, 4.97; N, 10.90. Found: C, 59.55; H, 5.36; N, 10.52.

2,2′:6′,2″-Terpyridine-6,6″-dimethanol (18). $NaBH_4$ (610 mg, 16.1 mmol) was added to a suspension of **17** (1.25 g, 3.6 mmol) in absolute ethanol (50 mL), and the mixture was refluxed for overnight. After removal of the solvent, aqueous $NaHCO_3$ (saturated, 30 mL) was added, and the mixture was brought to boiling. A 50 mL portion of water was added, and then the cold mixture was neutralized with aqueous HCl (1 M). The precipitate was filtered off and washed with H_2O and CH_3CN, providing 780 mg (74%) of the title compound as a white solid: mp 140−142 °C (lit.[48] mp 142−143 °C); R_f = 0.2 (silica, CH_2Cl_2/MeOH, 95:5); ^{1}H NMR (250 MHz, DMSO-d_6) δ 4.69 (d, J = 5.8 Hz, 4H), 5.53 (t, J = 5.9 Hz, 2H), 7.57 (d, J = 7.7 Hz, 2H), 8.01 (t, J = 7.8 Hz, 2H), 8.08 (t, J = 7.6 Hz, 1H), 8.43 (d, J = 7.8 Hz, 2H), 8.49 (d, J = 7.8 Hz, 2H); IR (KBr, cm^{-1}) 3415, 1572.

2,2′:6′,2″-Terpyridine, 6,6″-Dimethyl- (20a). A mixture of 2,6-dibromopyridine (3.1 g, 13 mmol), 2-methyl-6-(tributylstannyl)pyridine[22] (11.1 g, 29 mmol), and $Pd(PPh_3)_4$ (0.76 g, 0.66 mmol) in degassed toluene (90 mL) was refluxed under argon for 4 days. The resulting solution was filtered over Celite and evaporated under reduced pressure. The brown residue was treated with aqueous HCl (15 mL, 6 M), and the aqueous suspension was extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were washed with aqueous HCl (6 M), and the combined aqueous layers were brought to pH 9 with aqueous NH_4OH (concentrated). The precipitate was filtered off and dissolved in CH_2Cl_2, and the solution was dried over Na_2SO_4. After evaporation of the solvent, the solid residue was purified by recrystallization from cyclohexane to afford the title compound (2.55 g, 75%) as a white solid: mp 170−171 °C (lit.[23] mp 171 °C); R_f = 0.30 (alumina, toluene); ^{1}H NMR (200 MHz, $CDCl_3$) δ 2.65 (s, 6H), 7.18 (d, J = 7.6 Hz, 2H), 7.73 (t, J = 7.7 Hz, 2H), 7.92 (t, J = 7.8 Hz, 1H), 8.40 (d, J = 7.8 Hz, 2H), 8.45 (d, J = 7.8 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.2, 121.8, 124.3, 127.3, 140.0, 144.0, 148.9, 149.6, 156.1.

2,2′:6′,2″-Terpyridine-4′-carboxylic Acid, 6,6″-Dimethyl-, Ethyl Ester (20b). A mixture of 2,6-dichloroisonicotinic acid ethyl ester[26,27] (3.2 g, 14.5 mmol), 2-methyl-6-(tributylstannyl)pyridine[22] (11.1 g, 29 mmol), and $Pd(PPh_3)_4$ (1.67 g, 1.45 mmol) in degassed toluene (100 mL) was refluxed under argon for 3 days. The resulting solution was filtered over Celite and evaporated under reduced pressure. HCl (40 mL, 6M) was added, and the solution was washed with CH_2Cl_2 (3 × 50 mL). The aqueous phase was neutralized with solid $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were dried ($MgSO_4$) and evaporated. The residue was chromatographed on alumina, eluting with CH_2Cl_2, to afford 3.05 g (63%) of the title compound as a white solid: mp 134−135 °C (lit.[25] mp 134−135 °C); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.47 (t, J = 7.1 Hz, 3H), 2.67 (s, 6H), 4.50 (q, J = 7.1 Hz, 2H), 7.21 (d, J = 7.6 Hz, 2H), 7.75 (t, J = 7.7 Hz, 2H), 8.40 (d, J = 7.8 Hz, 2H), 8.97 (s, 2H).

2,2′:6′,2″-Terpyridine-4′-methanol, 6,6″-Dimethyl- (20c). Sodium borohydride (3.29 g, 87 mmol) was added to **20b** (4.82 g, 14.5 mmol) in ethanol (150 mL) cooled at 0 °C. The mixture was then refluxed for 24 h. After evaporation under reduced pressure, 80 mL of water was poured onto the crude material, followed by an acidification using HCl (1 M), and then neutralized with solid $NaHCO_3$. The organic layer was extracted with CH_2Cl_2, dried over $MgSO_4$, and evaporated. A chromatography column (alumina, elution CH_2Cl_2/petroleum ether/AcOEt, 1:1:0 → 9:0:1) gave 3.05 g (72%) of a white solid: mp 125−126 °C; R_f = 0.15 (alumina, CH_2Cl_2/AcOEt, 9:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.64 (s, 6H), 4.80 (s, 2H), 7.17 (d, J = 7.6 Hz, 2H), 7.71 (t, J = 7.6 Hz, 2H), 8.34 (d, J = 7.7 Hz, 2H), 8.36 (s, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 24.3, 63.0, 118.3, 118.5, 123.2, 136.9, 152.5, 155.4, 155.7, 157.7; IR (KBr, cm^{-1}) 3390, 1582; MS (ESI$^+$) *m/z* (rel intens) 314 ([M + Na$^+$], 95), 292 ([M + H$^+$], 100). Anal. Calcd for $C_{18}H_{17}N_3O$: C, 74.20; H, 5.88; N, 14.42. Found: C, 74.46; H, 6.03; N, 14.39.

2,2′:6′,2″-Terpyridine, 6,6″-Dimethyl-4′-[[(methoxycarbonyl)methoxy]methyl]- (20d). A mixture of **20c** (700 mg, 2.4 mmol) and NaH (95%, 182 mg, 7.2 mmol) in THF (50 mL) was refluxed. After 30 min, methyl bromoacetate (228 μL, 2.4 mmol) was added and the reflux continued for 24 h. After dilution of the crude solution in ethyl acetate (50 mL), a saturated solution of NH_4Cl (10 mL) and water (20 mL) were used to neutralize the media. The organic layer was extracted with AcOEt, dried, and evaporated under reduced pressure. A chromatography column (alumina, elution CH_2Cl_2/petroleum ether, 1:1 → 1:0) led to 500 mg (58%) of a white solid: mp 98−99 °C; R_f = 0.30 (alumina, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.64 (s, 6H), 3.78 (s, 3H), 4.22 (s, 2H), 4.83 (s, 2H), 7.18 (d, J = 7.6 Hz, 2H), 7.72 (t, J = 7.6 Hz, 2H), 8.39 (d, J = 7.6 Hz, 2H), 8.46 (s, 2H); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 24.6, 52.0, 67.8, 72.3, 118.4, 119.3, 123.4, 137.1, 148.1, 155.5, 156.0, 157.9, 177.0; IR (KBr, cm^{-1}) 1751, 1581; MS (ESI$^+$) *m/z* (rel intens) 386 ([M + Na$^+$], 79), 364 ([M + H$^+$], 100). Anal. Calcd for $C_{21}H_{21}N_3O_3$: C, 69.41; H, 5.82; N, 11.56. Found: C, 69.05; H, 5.91; N, 11.73.

(48) El-ghayoury, A.; Ziessel, R. *J. Org. Chem.* **2000**, *65*, 7757−7763.

2,2′:6′,2″-Terpyridine, 6,6″-Dimethyl-4′-[[(methoxycarbonyl)methoxy]methyl]-, 1,1″-Dioxide (22). Compound **20d** (363 mg, 1 mmol) was added to a solution of 3-chloroperbenzoic acid (673 mg, 3.9 mmol) in CH_2Cl_2 (7 mL). The mixture was protected from light and stirred at room temperature for 12 h. The resulting solution was neutralized with an aqueous saturated solution of $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were washed with water, dried over $MgSO_4$, and evaporated under reduced pressure. Silica chromatography (CH_2Cl_2/MeOH, 1:0 → 9:1) yielded 320 mg (81%) of a yellow oil: $R_f = 0.40$ (silica, CH_2Cl_2/MeOH, 9:1); ^{1}H NMR (250 MHz, $CDCl_3$) δ 2.60 (s, 6H), 3.76 (s, 3H), 4.21 (s, 2H), 4.81 (s, 2H), 7.23−7.33 (m, 4H), 7.98 (dd, $J = 7.3$, 2.1 Hz, 2H), 8.84 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.6, 52.1, 67.9, 72.1, 124.4, 125.3, 126.0, 126.2, 147.1, 147.4, 150.1, 150.5, 170.7; IR (KBr, cm^{-1}) 1753, 1564, 1265, 850; MS (ESI$^+$) *m/z* (rel intens) 434 ([M + K$^+$], 26), 418 ([M + Na$^+$], 100), 396 ([M + H$^+$], 28).

2,2′:6′,2″-Terpyridine, 6,6″-Bis(bromomethyl)- (9a). Route A. A mixture of dry DMF (8 mL) and PBr_3 (150 μL, 1.58 mmol) was stirred for 15 min at room temperature. The diol **18** (132 mg, 0.45 mmol) was added, and the mixture was heated at 60 °C for 1 h and then stirred at room temperature overnight. After neutralization with aqueous $NaHCO_3$ (saturated), the precipitate was filtered and washed with cold water and acetonitrile. The solid was purified by recrystallization from CCl_4 to afford the title compound (141 mg, 75%) as white crystals: mp 203−205 °C dec; $R_f = 0.4$ (alumina, toluene); ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.65 (s, 4H), 7.49 (d, $J = 7.7$ Hz, 2H), 7.85 (t, $J = 7.8$ Hz, 2H), 7.95 (t, $J = 7.8$ Hz, 1H), 8.51 (d, $J = 7.8$ Hz, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 34.1, 120.3, 121.5, 123.4, 137.8, 137.9, 154.9, 156.0, 156.3. Anal. Calcd for $C_{17}H_{13}N_3Br_2 \cdot 0.5H_2O$: C, 47.69; H, 3.30; N, 9.81. Found: C, 48.05; H, 2.85; N, 9.99.

Route B. A mixture of **20a** (500 mg, 1.9 mmol), NBS (850 mg, 4.78 mmol), and AIBN (20 mg, 0.12 mmol) in benzene (50 mL) was refluxed and lightened using a halogen lamp (150 W). The mixture was allowed to stir for 6 h, when TLC indicated incomplete bromination of the starting material. Therefore, more NBS (350 mg, 2 mmol) and AIBN (10 mg, 0.06 mmol) were added at this time, and the reaction mixture was allowed to stir at reflux for another 3 h. The solvent was evaporated 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, and the residue was purified by recrystallization from $CHCl_3$ to afford **9a** (460 mg, 57%) as white crystals, which was identical with the product obtained by route A.

2,2′:6′,2″-Terpyridine,4′-[[(Methoxycarbonyl)methoxy]methyl]-6,6″-bis(bromomethyl)- (9c). To a cooled (0 °C) solution of **22** (263 mg, 0.66 mmol) in $CHCl_3$ (5.5 mL) was added dropwise trifluoroacetic anhydride (4.5 mL, 31.9 mmol). The mixture was warmed at 40 °C for 5 h and evaporated. A solution of LiBr (577 mg, 6.64 mmol) in THF/DMF (5.5 mL/50 μL) was then added. The resulting mixture was stirred at room temperature for 40 h and evaporated. After addition of CH_2Cl_2 (15 mL) and water (6 mL), the extracted organic layer was washed with H_2O (3 × 6 mL), dried over $MgSO_4$, and evaporated under reduced pressure. The crude material was chromatographed over silica (CH_2Cl_2 → CH_2Cl_2/AcOEt, 9:1) to give 46 mg (13%) of a yellow oil: $R_f = 0.40$ (alumina, CH_2Cl_2); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.80 (s, 3H), 4.26 (s, 2H), 4.65 (s, 4H), 4.84 (s, 2H), 7.49 (d, $J = 7.6$ Hz, 2H), 7.84 (t, $J = 7.9$ Hz, 2H), 8.49−8.51 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.4, 52.3, 68.1, 72.4, 120.0, 120.7, 123.8, 138.1, 148.6, 155.5, 155.9, 156.5, 170.8; IR (KBr, cm^{-1}) 1748, 1586; MS (ESI$^+$) *m/z* (rel intens) 542/544/546 ([M + Na$^+$], 50/100/50), 520/522/524 ([M + H$^+$], 8/17/8). Anal. Calcd for $C_{21}H_{19}N_3O_3Br_2$: C, 48.39; H, 3.67; N, 8.06. Found: C, 48.25; H, 3.95; N, 8.10.

13,16,19,37,40,43,49,50,51,52,53,54-Dodecaazaheptacyclo-[43.3.1.12.6.17.11.121.25.126.30.131.35]tetrapentaconta-1(49),2,4,6(54),7,9,11(53),21,23,25(52),26,28,30(51),31,33,-35(50),45,47-octadecaene, 13,16,19,37,40,43-Hexakis[(4-methylphenyl)sulfonyl]- (23). To a solution of 1,4,7-tris(*p*-tolylsulfonyl)-1,4,7-triazaheptane (**10**)[18] (61 mg, 0.11 mmol) in anhydrous acetonitrile (50 mL) was added K_2CO_3 (115 mg, 0.83 mmol). The suspension was refluxed for 1 h and 30 min, then **9a** (45 mg, 0.11 mmol) was added in one portion, and the mixture was stirred at reflux overnight before filtration. The solvent was removed by rotary evaporation, and the residue was purified by chromatography on silica gel, eluting first with dichloromethane and then with dichloromethane−methanol (v/v 99:1), to afford 18 mg (20%) of the 2 + 2 macrocycle **23** as a white solid: mp 179−180 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.82 (s, 6H), 2.27 (s, 12H), 2.98−3.04 (m, 8H), 3.37−3.43 (m, 8H), 4.57 (s, 8H), 6.67 (d, $J = 8.1$ Hz, 4H), 7.02 (d, $J = 8.2$ Hz, 4H), 7.18 (d, $J = 8.2$ Hz, 8H), 7.42 (d, $J = 7.2$ Hz, 4H), 7.62 (t, $J = 8.2$ Hz, 2H), 7.69 (d, $J = 8.2$ Hz, 8H), 7.82 (t, $J = 7.75$ Hz, 4H), 8.14 (d, $J = 7.8$ Hz, 4H), 8.52 (d, $J = 7.4$ Hz, 4H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 21.2, 21.5, 48.3, 48.7, 54.6, 119.9, 121.2, 123.6, 126.7, 127.3, 129.5, 129.8, 134.6, 136.1, 137.7, 137.8, 143.3, 143.4, 154.7, 155.6, 155.7, 155.8; IR (KBr, cm^{-1}) 1598, 1569, 1343, 1161; MS (FAB$^+$) *m/z* (rel intens) 1684 ([M + K]$^+$, 13), 1668 ([M + Na]$^+$, 100), 1646 ([M + H]$^+$, 42). Anal. Calcd for $C_{84}H_{84}N_{12}O_{12}S_6 \cdot 2CH_3OH$: C, 60.40; H, 5.42; N, 9.83; S, 11.25. Found: C, 60.44; H, 4.89; N, 9.45; S, 11.22.

13,16,19,25,26,27-Hexaazatetracyclo[19.3.1.12.6.17.11]-heptacosa-1(25),2,4,6(27),7,9,11(26),21,23-nonaene-13,16,-19-triacetic Acid Tris(1,1-dimethylethyl) Ester (24). To a solution of **11** (298 mg, 0.67 mmol) in anhydrous acetonitrile (300 mL) was added Na_2CO_3 (707 mg, 6.7 mmol). The suspension was refluxed for 1 h, **9a** (280 mg, 0.67 mmol) was added in one portion, and the mixture was stirred at reflux overnight before filtration. The solvent was removed by rotary evaporation, and the residue was purified by chromatography on alumina, eluting first with chloroform and then with chloroform−methanol (v/v 98:2 → 95:5), to afford 302 mg (56%) of the 1 + 1 macrocycle **24** as its NaBr complex: colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.09 (s, 9H), 1.22 (s, 18H), 2.77 (m, 4H), 2.90 (m, 4H), 3.17 (s br, 6H), 4.08 (s, 4H), 7.35 (d, $J = 7.1$ Hz, 2H), 7.92 (t, $J = 7.6$ Hz, 2H), 7.99 (d, $J = 7.2$ Hz, 2H), 8.09 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.9, 28.1, 53.1, 53.9, 56.7, 58.1, 82.0, 82.2, 120.6, 121.8, 124.0, 138.5, 139.1, 155.3, 155.5, 158.3, 171.6, 172.4; IR (neat, cm^{-1}) 1728, 1570; MS (ESI$^+$) *m/z* (rel intens) 725.5 ([M + Na]$^+$, 100), 703.5 ([M + H]$^+$, 50), 669.4 ([(M − C_4H_8) + Na]$^+$, 25), 647.4 ([(M − C_4H_8) + H]$^+$, 14), 613.2 ([(M − 2C_4H_8) + Na]$^+$, 10), 591.2 ([(M − 2C_4H_8) + H]$^+$, 7), 557.2 ([(M − 3C_4H_8) + Na]$^+$, 7), 535.2 ([(M − 3C_4H_8) + H]$^+$, 4). Anal. Calcd for $C_{39}H_{54}N_6O_6NaBr$: C, 58.13; H, 6.75; N, 10.43. Found: C, 57.93; H, 6.77; N, 10.21.

13,16,19,37,40,43,49,50,51,52,53,54-Dodecaazaheptacyclo-[43.3.1.12.6.17.11.121.25.126.30.131.35]tetrapentaconta-1(49),2,4,6(54),7,9,11(53),21,23,25(52),26,28,30(51),31,33,-35(50),45,47-octadecaene-13,16,19,37,40,43-hexaacetic Acid Hexakis(1,1-dimethylethyl) Ester (25). To a solution of **11** (155 mg, 0.35 mmol) in anhydrous acetonitrile (150 mL) was added K_2CO_3 (480 mg, 3.5 mmol). The suspension was refluxed for 1 h, then **9a** (146 mg, 0.35 mmol) was added in one portion, and the mixture was stirred at reflux overnight before filtration. The solvent was removed by rotary evaporation, and the residue was purified by chromatography on silica gel, eluting first with dichloromethane and then with dichloromethane−methanol (v/v 98:2 → 95:5), to afford 91 mg (37%) of the 2 + 2 macrocycle **25** as a pale yellow oil: ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.42 (s, 36H), 1.48 (s, 18H), 3.25 (m, 4H), 3.52−3.63 (m, 24H), 3.87 (s, 4H), 4.48 (s, 4H), 7.32 (d, $J = 7.6$ Hz, 4H), 7.66 (t, $J = 7.6$, 2H), 7.76 (t, $J = 7.3$ Hz, 4H), 8.18 (d, $J = 7.6$ Hz, 4H), 8.32 (d, $J = 7.6$ Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.2, 28.4, 52.3, 52.5, 56.4, 60.4, 81.0, 81.5, 119.3, 119.5, 120.8, 121.2, 123.0, 123.3, 137.2, 137.5, 155.2, 155.3, 155.6, 158.8, 170.9, 171.0; IR (neat, cm^{-1}) 1730, 1570; MS (ESI$^+$) *m/z* (rel intens) 1427.7 ([M + Na]$^+$, 7), 1405.8 ([M + H]$^+$, 62), 703.6 ([M + 2H]$^{2+}$, 100), 675.5 ([(M − C_4H_8) + 2H]$^{2+}$, 53), 647.5 ([(M −2C_4H_8) + 2H]$^{2+}$, 50), 619.5 ([(M − 3C_4H_8) + 2H]$^{2+}$, 48), 591.3

([(M − $4C_4H_8$) + 2H]$^{2+}$, 41), 563.3 ([(M − $5C_4H_8$) + 2H]$^{2+}$, 31), 535.3 ([(M − $6C_4H_8$) + 2H]$^{2+}$, 25). Anal. Calcd for $C_{78}H_{108}N_{12}O_{12}$: C, 66.64; H, 7.74; N, 11.96. Found: C, 66.32; H, 8.03; N, 11.77.

13,16,19,25,26,27-Hexaazatetracyclo[19.3.1.12.6.17.11]-heptacosa-1(25),2,4,6(27),7,9,11(26),21,23-nonaene-13,16,-19-triacetic Acid, 4-(Ethoxycarbonyl)-, Tris(1,1-dimethylethyl) Ester (26). To a suspension of **11** (173 mg, 0.39 mmol) and Na_2CO_3 (410 mg, 3.9 mmol) in anhydrous acetonitrile (170 mL) was added dropwise a solution of **9b**[25] (190 mg, 0.39 mmol) in anhydrous acetonitrile (10 mL), and then the mixture was stirred at reflux for 48 h. After filtration, the solvent was removed by rotary evaporation, and the residue was chromatographed on alumina, eluting with chloroform−methanol (v/v 99:1 → 90:1). The combined fractions were evaporated and triturated with petroleum ether to afford a residue that was purified by recrystallization from toluene, yielding 116 mg of the 1 + 1 macrocycle **26** as its NaBr complex (34% yield): white powder; mp 185−187 °C; R_f = 0.23 (alumina, $CHCl_3$/MeOH, 95:5); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.02 (s, 9H), 1.16 (s, 18H), 1.45 (t, J = 7.1 Hz, 3H), 2.73 (m, 4H), 2.87 (m, 4H), 3.12 (s, 6H), 4.05 (s, 4H), 4.49 (q, J = 7.1 Hz, 2H), 7.38 (d, J = 7.0 Hz, 2H), 7.92 (t, J = 7.4 Hz, 2H), 7.99 (d, J = 7.4 Hz, 2H), 8.49 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.4, 27.7, 27.9, 52.9, 53.8, 56.5, 57.8, 62.6, 81.8, 82.0, 120.5, 123.7, 124.4, 138.5, 140.6, 154.2, 156.5, 158.4, 164.6, 171.4, 172.5; IR (KBr, cm^{-1}) 1724, 1577; MS (ESI$^+$) *m/z* (rel intens) 797.5 ([M + Na]$^+$, 100), 775.5 ([M + H]$^+$, 64), 741.5 ([(M − C_4H_8) + Na]$^+$, 15), 719.5 ([(M − C_4H_8) + H]$^+$, 9), 685.4 ([(M − $2C_4H_8$) + Na]$^+$, 6), 663.4 ([(M − $2C_4H_8$) + H]$^+$, 4), 629.4 ([(M − $3C_4H_8$) + Na]$^+$, 3), 607.3 ([(M − $3C_4H_8$) + H]$^+$, 3). Anal. Calcd for $C_{42}H_{58}N_6O_8NaBr$: C, 57.47; H, 6.66; N, 9.57. Found: C, 57.73; H, 6.67; N, 9.73.

13,16,19,25,26,27-Hexaazatetracyclo[19.3.1.12.6.17.11]-heptacosa-1(25),2,4,6(27),7,9,11(26),21,23-nonaene-13,16,-19-triacetic Acid, 4-[[(Methoxycarbonyl)methoxy]methyl]-, Tris(1,1-dimethylethyl) Ester (27). A mixture of **11** (34 mg, 7.7 × 10^{-5} mol) and Na_2CO_3 (82 mg, 77 × 10^{-5} mol) in CH_3CN (30 mL) was refluxed for 30 min, and then **9c** (40 mg, 7.7 × 10^{-5} mol) in acetonitrile (5 mL) was added. The reflux was continued for 48 h, and then the solution was evaporated to dryness. Purification over a reversed-phase column (Hyperprep, 0.1% TFA in H_2O/CH_3CN, gradient from 30:70 to 10:90, flow rate 15 mL/min) afforded 27 mg (43%) of the desired product as its NaBr complex: colorless oil; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.07 (s, 9H), 1.20 (s, 18H), 2.60−3.00 (m, 8H), 3.12 (s, 6H), 3.53 (s, 3H), 4.03 (s, 4H), 4.32 (s, 2H), 4.88 (s, 2H), 7.29 (d, J = 7.6 Hz, 2H), 7.86 (t, J = 7.6 Hz, 2H), 7.98 (d, J = 7.6 Hz, 2H), 8.07 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.7, 28.1, 51.9, 52.9, 53.7, 56.5, 57.9, 68.1, 71.7, 81.8, 82.0, 119.7, 120.4, 123.7, 138.2, 149.9, 155.0, 155.6, 158.0, 171.3, 172.2; MS (ESI$^+$) *m/z* (rel intens) 827.6 ([M + Na$^+$], 100), 805.5 ([M + H$^+$], 78); HRMS-FAB$^+$ [M + Na$^+$] *m/z* calcd for $C_{43}H_{60}N_6O_9Na$ 827.43195, found 827.43238. Anal. Calcd for $C_{43}H_{60}N_6O_9NaBr$: C, 56.89; H, 6.66; N, 9.26. Found: C, 56.55; H, 6.45; N, 9.57.

13,16,19,25,26,27-Hexaazatetracyclo[19.3.1.12.6.17.11]-heptacosa-1(25),2,4,6(27),7,9,11(26),21,23-nonaene-13,16,-19-triacetic Acid (5). Compound **24** (85 mg, 0.1 mmol) was dissolved in a mixture of CH_2Cl_2/CF_3COOH (10 mL, 1:1). The solution was stirred for 15 h at room temperature. After evaporation of the solvent and the excess of acid, the residue was dissolved in methanol (5 mL), and diethyl ether was added, resulting in the formation of a precipitate, which was isolated after centrifugation: white solid; yield 41 mg (77%); analytical HPLC (column CC70-3MN C18 HD, solvent 0.1% HCOOH in H_2O/CH_3OH, 95:5, flow rate 0.6 mL/min) t_R = 1.42 min (98% of total chromatogram integration); ^{1}H NMR (200 MHz, D_2O) δ 3.0−4.0 (m, 14H), 4.85 (s, 4H), 7.7 (m, 2H), 8.27−8.40 (m, 4H), 8.59 (m, 3H); ^{13}C NMR (100 MHz, D_2O) δ 51.5, 53.2, 55.0, 55.8, 58.3, 124.2, 125.4, 127.9, 141.5, 149.0, 151.4, 171.0, 177.8; IR (KBr, cm^{-1}) 3427, 1680; MS (ESI$^+$) *m/z* (rel intens) 573.0 ([M + K]$^+$, 11), 557.1 ([M + Na]$^+$, 65), 535.1 ([M + H]$^+$, 100); HRMS-FAB$^+$ [M + H]$^+$ *m/z* calcd for $C_{27}H_{31}N_6O_6$ 535.23051, found 535.23189.

13,16,19,25,26,27-Hexaazatetracyclo[19.3.1.12.6.17.11]-heptacosa-1(25),2,4,6(27),7,9,11(26),21,23-nonaene-13,16,-19-triacetic Acid, 4-(Ethoxycarbonyl)- (6). Compound **26** (35 mg, 0.04 mmol) was dissolved in a mixture of CH_2Cl_2/CF_3COOH (5 mL, 1:1). The solution was stirred for 24 h at room temperature. After evaporation of the solvent and the excess of acid, the residue was purified by chromatography on reversed-phase silica (0.1% TFA in H_2O/CH_3OH, 80:20) to afford 21 mg (87%) of **6** as a colorless oil: ^{1}H NMR (250 MHz, D_2O) δ 1.40 (t, J = 7.1 Hz, 3H), 3.44 (m, 4H), 3.56 (m, 4H), 3.61 (s, 4H); 3.72 (s, 2H), 4.43 (s, 4H), 4.50 (q, J = 7.3 Hz, 2H), 8.01 (d, J = 6.8 Hz, 2H), 8.64 (m, 4H), 8.92 (s, 2H); ^{13}C NMR (50 MHz, D_2O) δ 15.8, 53.0, 54.6, 56.3, 57.0, 58.2, 66.4, 127.5, 127.7, 131.1, 145.7, 149.2, 150.9, 152.1, 154.8, 167.0, 172.4, 176.0; MS (FAB$^+$) *m/z* (rel intens) 629 ([M + Na]$^+$, 100), 607 ([M + H]$^+$, 21); HRMS-FAB$^+$ [M + Na]$^+$ *m/z* calcd for $C_{30}H_{34}N_6O_8Na$ 629.23358, found 629.23341.

13,16,19,25,26,27-Hexaazatetracyclo[19.3.1.12.6.17.11]-heptacosa-1(25),2,4,6(27),7,9,11(26),21,23-nonaene-13,16,-19-triacetic Acid, 4-[[(Methoxycarbonyl)methoxy]methyl]- (7). Compound **27** (28 mg, 3.1 × 10^{-5} mol) was solubilized in CH_2Cl_2/TFA (1:1, 14 mL) and the resulting solution stirred at room temperature overnight. After evaporation, the crude material was dissolved in a minimal amount of methanol and poured onto THF. The precipitate was centrifuged and washed with THF before being evaporated to dryness, yielding 13 mg (65%) of a brown oil: analytical HPLC (column CC125-3 MN C18 HD 5 μm, solvent 0.1% TFA in H_2O/MeOH, 85:15, flow rate 0.6 mL/mn): t_R = 5.6 min (95% of total chromatogram integration); ^{1}H (250 MHz, D_2O) δ 3.38 (m, 4H), 3.64 (m, 6H), 3.80 (s, 4H), 3.88 (s, 4H), 4.47 (s, 3H), 4.76 (s, 2H), 5.09 (s, 2H), 7.89 (d, J = 7.9 Hz, 2H), 8.37 (t, J = 7.9 Hz, 2H), 8.51 (d, J = 7.9 Hz, 2H), 8.66 (s, 2H); MS (FAB$^+$) *m/z* (rel intens) 675 ([M + K$^+$], 78), 661 ([M − CH_2 + K$^+$], 100); HRMS-FAB$^+$ [M + K]$^+$ *m/z* calcd for $C_{31}H_{36}N_6O_9K$ 675.21809, found 675.21856.

13,16,19,25,26,27-Hexaazatetracyclo[19.3.1.12.6.17.11]-heptacosa-1(25),2,4,6(27),7,9,11(26),21,23-nonaene-13,16,-19-triacetic Acid, 4-[[*N*-[2-(4-Aminophenyl)ethyl]amino]-carbonyl]- (8). A mixture of compound **26** (150 mg, 0.17 mmol) with NaCN (catalytic) and 2-(4-aminophenyl)ethylamine (125 μL, 0.95 mmol) in methanol (2 mL) was heated at 100 °C for 100 h in a sealed tube. After evaporation to dryness, the residue was chromatographed on silica gel, eluting with chloroform−methanol (v/v 80:20). The combined fractions were evaporated and triturated with petroleum ether to afford 56 mg of a white solid: R_f = 0.22 (silica, $CHCl_3$/MeOH, 90:10); MS (ESI$^+$) *m/z* (rel intens) 887.5 ([M + Na]$^+$, 100), 865.6 ([M + H]$^+$, 44). A solution of this crude product in a mixture of H_2O/MeOH (10 mL, 5:5) with NaOH (0.2 g) was stirred at room temperature for 24 h. After evaporation to dryness, the residue was purified by chromatography on reversed-phase silica (0.1% TFA in H_2O/CH_3OH, 80:20 to 50:50) to afford 35 mg (29% over two steps) of **8** as a colorless oil: ^{1}H NMR (250 MHz, D_2O) δ 3.01 (t, J = 6.2 Hz, 2H), 3.53−3.73 (m, 12H), 3.76 (m, 4H), 4.52 (s, 4H), 7.30 (d, J = 8.2 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.96 (d, J = 6.1 Hz, 2H), 8.53 (m, 6H); ^{13}C NMR (62.5 MHz, D_2O) δ 36.7, 43.9, 53.6, 54.7, 56.5, 57.6, 58.7, 125.7, 125.9, 127.5, 130.8, 133.2, 143.0, 148.0, 149.3, 152.0, 153.2, 154.5, 168.8, 173.3, 175.8; MS (ESI$^+$) *m/z* (rel intens) 735 ([M + K]$^+$, 60); HRMS-FAB [M + Na]$^+$ *m/z* calcd for $C_{36}H_{40}N_8O_7Na$ 719.29177, found 719.29092.

Procedure for the Preparation of the Europium Complexes: The Eu(III) neutral complexes of macrocycles **5**−**8** were prepared by the addition of the $EuCl_3$·$6H_2O$ salt (1 equiv) to the aqueous solution of the ligand. This solution was then adjusted at a final concentration of 1 × 10^{-6} M in borate buffer (0.05 M, pH 8.6).

Supporting Information Available: General experimental data, MS and ^{1}H NMR spectra for compounds **24** and **25**, and ^{1}H NMR spectra for compounds **12**, **16**, **18**, **20a**, **20b**, **22**, **6**, and **8** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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