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Synthesis of an hexadentate tricyclic tetraazadiacetic ligand as precursor for MRI contrast enhancement agents

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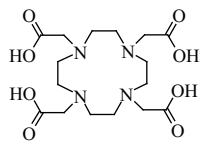
ABSTRACT

A tricyclic tetraazadiacetic compound, which is a rigidified derivative of **cyclo-PCTA12** ligand with an oxo-ethylene bridge replacing an ethylene one, was prepared. The synthetic route involved the macrocyclization between an activated amido-disulfonamide and the 2,6-bis(bromomethyl)pyridine. The acetate side chains were grafted on the macrocyclic backbone to lead to the highly rigid tricyclic ligand in 34% overall yield in four steps from the linear amido-disulfonamide precursor. The corresponding Gd(III) and Mn(II) complexes were then prepared in order to evaluate their potential as contrast agent for MRI.

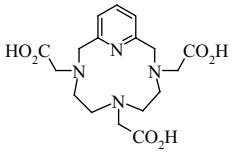
1. Introduction

Magnetic Resonance Imaging (MRI) is a non-invasive clinical modality widely used in the diagnosis of human diseases. Some MRI examinations require the use of contrast agents (CAs) to improve visualization of abnormal structures or lesions, and the most widespread pharmaceuticals routinely used for such purpose are gadolinium-based CAs.[1,2] They are complexes of Gd(III) with ligands designed to form kinetic and thermodynamic stable chelates thereby significantly reducing the toxicity of the free Gd(III) ion. It is now well-established that polyamino-polyacetate ligands can confer such a stability to the corresponding gadolinium complexes and that well-designed macrocyclic compounds, which are preorganized rigid rings of almost optimal size to cage the Gd(III) ion, generate Gd-complexes of higher stability.[3] In terms of performance, there is still a great effort to find new products endowed with higher relaxivity, so that the CA could be administered at a lower dose which is probably a key issue to move towards organ/tissue-specific agents and MRI molecular imaging.[4,5]

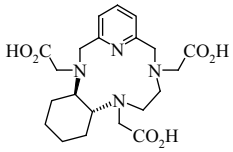
The relaxation theory of paramagnetic substances was discussed in details in previous reviews.[1,6,7] Relaxivity is a function of several parameters, the most important being the number of water molecules coordinated to the metal center (q, inner sphere term), the lifetime of the coordinated water molecule (τ_M), and the reorientational correlation time of the paramagnetic complex (τ_R).[1,6] Recently, it has been postulated that the rigidification of the chelate structure could be favourable to an higher longitudinal relaxivity.[8,9] The macrocyclic **PCTA12** ligand[10] is a rigidified analog of the well-known **DOTA** ligand (Fig. 1) and the Gd-complexes formed with **PCTA12** and with other pyridine-containing (PC-type) ligands are very stable and exhibit improved longitudinal relaxivity in comparison with that of the marketed gadolinium-based CAs.[1,11–13]



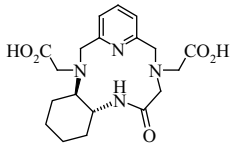
DOTA ligand



PCTA12 ligand



***rac*-cyclo-PCTA12** ligand



***rac*-1**

Figure 1. Some 12-membered polyamino-polyacetic macrocyclic ligands for the complexation of Gd^{3+} ion.

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With these structural aspects in mind, we have recently reported the synthesis of the even more rigid **cyclo-PCTA12** ligand (Fig. 1) and that of its Gd-complex.[14] We have shown that the rigidification of the scaffold did not alter the inertness of the complex toward transmetallation by Zn^{2+} ions and that the relaxivity was not quenched by endogeneous anions such as chloride, citrate, and phosphate.[15] Moreover, the constrained Gd-**cyclo-PCTA12** complex proved to have an higher longitudinal relaxivity than that of the parent Gd-**PCTA12** always above than those of the other gadolinium-based contrast agents used clinically at the present time. At last, this previous work showed that the rigidification induced the shortening of the residence lifetime of the inner-sphere water molecules (τ_M^{310}=34 ns versus 82 ns for Gd-**PCTA12**) thus approaching the optimal value required to attain high relaxivity once the chelate is immobilized by covalent or non-covalent binding to macromolecules.[7]

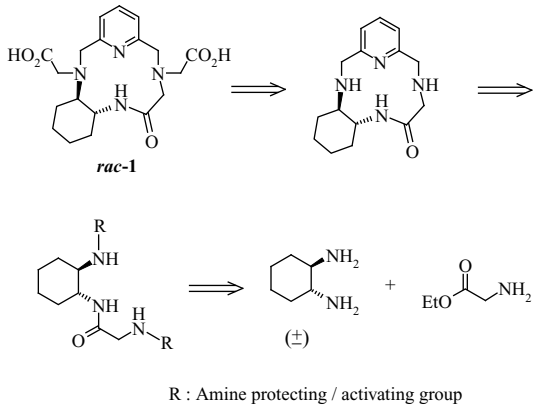
These results encouraged us to study the influence of structural modifications on **PCTA12** skeleton. We so decided to prepare the new **cyclo-PCTA12** derivative **1** in which the last ethylene bridge connecting two *N*-atoms is replaced by an oxo-ethylene bridge (Fig. 1). This macrocyclic ligand features four nitrogen atoms carrying two carboxymethyl side chains. This hexadentate ligand could give either a non-ionic complex with Mn^{2+} cation or a positively charged species upon complexation with Gd^{3+} cation. In the latter case, the positive charge may benefit to tissue/organ specificity for MRI purpose[16] and, as it was established for other hexadentate PC-type structures,[17] the lower denticity of the target ligand **1** may favour higher relaxivity by allowing three water molecules in the inner-sphere coordination.[1,16] This assumes that the *endo* amide subunit in ligand **1** is involved in the nine-coordinate ground state of gadolinium(III), probably by its carbonyl oxygen atom, together with the other three nitrogen atoms of the ring, and with the two carbonyl oxygen atoms of the carboxylate pendant arms. So, in ligand **1**, the *endo* amide subunit replaces and plays the coordinating role of one of the three amine subunits found in the parent ligands **PCTA12** and **cyclo-PCTA12** so that the presence of a carboxamide function should not be damageable in term of water exchange rate ($1/\tau_M$) as it was reported for ligands whose carboxylate pendant arms were replaced by carboxamide pendant arms.[31,32] The results presented here detail the synthetic route developed to prepare the tricyclic PC-type ligand **1**. Complexation with Gd(III) and Mn(II) is also reported in order to appreciate its potential activity as CA for MRI.

2. Results and discussion

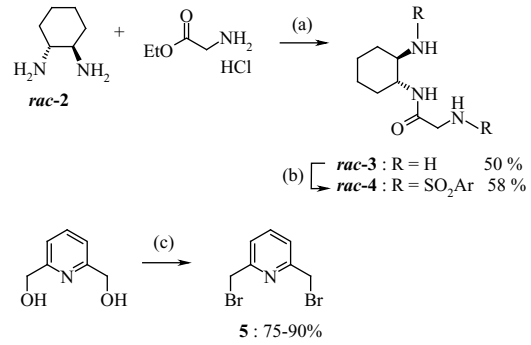
2.1. Preparation of the ligand

It has been previously shown in the literature that two synthetic routes could be envisaged for the preparation of such **PCTA** derivatives depending on the stage at which the arms are grafted: either on a pyridine-containing macrocyclic intermediate bearing functionalizable secondary amine sites, or on a linear triamine precursor before the macrocyclization process.[14,16,18–21] By comparison with other **PCTA12** derivatives, the target macrocycle **1** bears only two acetate arms attached on amino sites a priori no so crowded so that we decided to develop the first strategy which allows variations on the nature of ligating chains (Scheme 1). The construction of the linear amido-diamine precursor **3** could result from the monoamidification of *trans*-1,2-diamino-cyclohexane with a glycine derivative (Scheme 2). Moreover, we planned to apply the Fukuyama's 2-nitro-phenylsulfonamide strategy (R=(2-NO_2)C_6H_4-SO_2) to control and activate the macrocyclization process.[22]

The mono-*N*-functionalizations of *trans*-1,2-diamino-cyclohexane **2** are usually controlled by reacting a three fold excess of the unexpensive diamine **2** relative to the derivatizing agent (Boc_2O, $ArSO_2Cl$)[23,24] without any acid scavenger. In such conditions, there is no, or a very few amount of, competitive di-*N*,*N*′-functionalized compound formed and the mono-*N*-functionalized compound is always isolated with high yields superior to 70%. In our case, by allowing to react an equimolecular mixture of hydrochloric salt of ethyl glycinate and *trans*-1,2-diamino-cyclohexane **2** for three days in refluxing ethanol, diamine **2** was selectively transformed in the desired amido-diamine **3**. Moreover, amido-diamine **3** crystallizes during the process and was isolated by simple filtration in 50% yield with a satisfying purity allowing to use the crude product. It should be noted that this process has been scaled up to a large scale (14 g), once limited at the laboratory scale by the prolonged reflux together with the dilution which proved to be an important parameter. A 1.0 M concentration for both reagents led to an important precipitation that prevented an effective stirring. A dilution to 0.25 M induced a decreased yield of the expected monocondensed product **3** (38%). The best results were obtained with a 0.5 M concentration for both reagents. In that case, a GC–MS analysis of the residual alcoholic surnageant revealed the presence of the desired amido-diamine **3** together with the unreacted starting diamino compound **2**. As the glycinate reagent was totally consumed, we added an excess of glycinate reagent in order to improve the yield. Unfortunately, when a three fold excess of glycinate derivative was added, either in one portion or in three successive portions, as usually practised for monofunctionalization,[23,24] the expected amido-diamine **3** did not precipitate from the reactional medium. The compound **3** was recovered in solution together with unreacted starting diamine **2** and polyamido compounds. Furthermore, when the ethyl glycinate derivative was replaced by the more reactive methyl derivative, the reaction occurred in refluxing



Scheme 1. Synthetic pathway envisaged for the preparation of the target pyridine-containing macrocycle **1**.



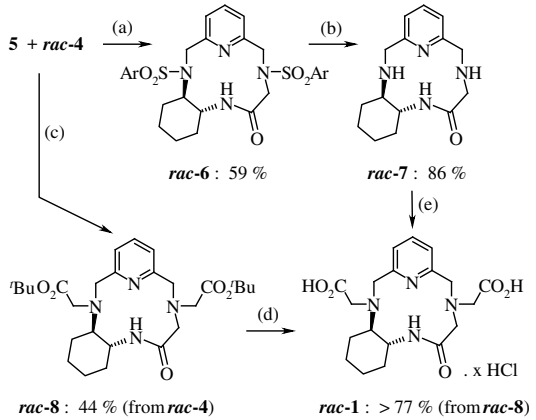
Scheme 2. Preparations of the precursors for macrocyclization *rac*-4 and **5**; reagents and conditions: Ar=(2-NO_2)C_6H_4; (a) EtOH, reflux, 3 days; (b) Ar-SO_2-Cl (2.7 equiv), Na_2CO_3 (3 equiv), Et_2O/THF/H_2O (63/20/17), rt, 1.5 day; (c) (i) HBr (aq, 48%) (16 equiv), H_2SO_4 (aq, 96%) (3.6 equiv), reflux, 31 h; (ii) HBr (aq, 48%) (16 equiv), H_2SO_4 (aq, 96%) (3.6 equiv), reflux, 63 h; (iii) Na_2CO_3.

methanol and, after 3 days, no conversion of diamino reagent **2** was observed while the glycinate reagent was totally converted to piperazine-2,5-dione. Even if all attempts to improve the moderate yield failed, this protocol presents several advantages as it involves commercially available and inexpensive reagents, a benign solvent allowing the reaction to be conducted at high concentation, and it affords a pure crude product easily isolated by filtration.

Subsequent activation/protection was realized by reacting the previous triamino compound **3** with a small excess of 2-nitrophenylsulfonyl chloride in the presence of Na_2CO_3 as a non nucleophile acid scavenger. The reaction occurred at room temperature and, once again, the crude product isolated by filtration in 58% yield was pure enough to avoid any purification step. It should be noted that the analysis of the residual filtrate revealed the presence of the desired product **4** so that the yield could be increased by treatment of this filtrate.

The quite expensive 2,6-bis(bromomethyl)pyridine **5** reagent was prepared from the 2,6-bis(hydroxymethyl)pyridine whose bromination has been reported with hydrobromic acid in water under reflux with[26] or without[25] an additional portion of concentrated sulfuric acid (Scheme 2). In both cases, the presence of mono-brominated side-product had required an effective purification step by column chromatography[25] or by recristallization.[26] After optimization, two successive additions of both concentrated hydrobromic and sulfuric acids, and a prolonged reflux in water (at least 4 days), led to the desired product **5** which crystallized in the crude mixture once cooled to room temperature. In these conditions, the proportion of mono-brominated compound recovered fell down to 1.5% (molar proportion), and yields up to 90% of the expected di-brominated compound **5** were obtained on a large scale (>20 g).

With the two precursors **4** and **5** in hand, the macrocyclization step was then envisaged (Scheme 3). As previously observed,[14,20,21] optimization of the conditions was necessary to minimize the amounts of competitive adducts such as cyclic [2+2] adducts, uncyclized [2+1] adducts (two amido-disulfonamides **4** condensed on the bis-bromo compound **5**), and other oligomers. By slow addition of a slight excess of 2,6-bis(bromomethyl)pyridine **5** to a warmed DMF solution of amido-disulfonamide **4** in the presence of an excess of K_2CO_3, an immediate condensation occurred with the formation of the desired macrocycle **6** isolated in 59% yield after chromatography. We studied the influence of both the nature of the carbonate and that of the polar solvent used. Indeed, when K_2CO_3 was replaced by Na_2CO_3, the crude mixture was much more



Scheme 3. Route to the target macrocycle **1**; reagents and conditions: Ar=(2-NO_2)C_6H_4; (a) K_2CO_3 (4 equiv), DMF, 100 °C, 75 min; (b) PhSH (2.8 equiv), K_2CO_3 (3.2 equiv), acetonitrile, 70 °C, 4 h; (c) (i) K_2CO_3 (4 equiv), DMF, 100 °C, 2 h; (ii) PhSH (2.8 equiv), K_2CO_3 (+2 equiv), rt, overnight; (iii) *tert*-butyl bromoacetate (2.2 equiv), TEA (2.4 equiv), THF, reflux, 7 h; (d) (i) HCl_g (ca. 80 equiv), Et_2O, rt, 5 days; (ii) HCl_g (ca. 80 equiv), Et_2O, reflux, overnight; (e) Ref. 25 (i) sodium bromoacetate (6.6 equiv), NaOH (pH 10), MeOH/H_2O (1/6), 80 °C, 3 days; (ii) HCl_{aq}.

complex with a larger proportion of uncyclized [2+1] adducts, while replacing DMF by CH_3CN had also a dramatic effect for both carbonates with higher proportions of competitive [2+2] or [2+1] adducts.[27]

The ^1H and ^{13}C NMR spectra of the macrocyclic disulfonamide **6** revealed the presence of two forms in 80/20 or 67/33 ratio in $CDCl_3$ or DMSO-d_6 solution respectively. By increasing the temperature, the ^1H NMR spectrum of a DMSO-d_6 solution showed coalescence for all signals of both isomers between 393 and 413 K. It is interesting to notice that we previously observed such a splitting for the less rigid **cyclo-PCTA12** analog,[14] attributed to the presence, at room temperature, of a mixture of rotamers probably due to prevented free rotations generated by the bulky 2-nitro-phenylsulfonyl and/or cyclohexyl subunits.

The cleavage of the 2-nitro-phenylsulfonamides was realized following a conventional method,[28] by treatment with thiophenate in slightly warmed CH_3CN. In such conditions, the desulfonylated macrocycle **7** was isolated in 86% yield as hydrochloride. It should be interesting to note that this step can also be envisaged in DMF, from the crude DMF solution of previous macrocycle **6**. In that case, the reaction medium of previous step was twice concentrated and the reaction occurred overnight at room temperature with similar yield.

The subsequent di-*N*-alkylation step was then performed in conditions previously described for the parent **cyclo-PCTA12** series,[14] with a stoechiometric amount of *tert*-butyl bromoacetate in the presence of a slight excess of triethylamine in refluxing THF. The desired product **8** was isolated after purification by column chromatography on silica gel in 44% overall yield (3 steps from the linear disulfonalmide **4**). At last, when treated with anhydrous hydrogen chloride, the diester **8** was converted to the target macrocyclic diacetic acid **1** as the HCl salt with high yield (77 to 90% with $1<x<3$). We verified that the desired macrocycle **1** could also result from direct di-*N*-alkylation of macrocyclic diamine **7** with an excess of sodium bromoacetate in alcaline aqueous medium (pH 10). In such conditions, the conversion was complete after a prolonged warming (3 days) and no trace of per-alkylated products was detected.

2.2. Preparation of the gadolinium and manganese complexes

With the hexadentate ligand **1** in hand, we decided to prepare the corresponding gadolinium (III) and manganese (II) complexes in order to evaluate them as potential contrast agents for MRI. Several examples of gadolinium complexes formed with PC-type ligands exhibited a 1/1 stoichiometry.[11,12,13,16,29,30] We postulated a similar stoichiometry for both target complexes and, by mixing equimolar amounts of ligand **1** with gadolinium or manganese chloride, in water, at pH 5.7–5.8, the expected Gd–**1** and Mn–**1** complexes were formed. Both complexes were isolated from alcaline water solutions (KOH), then characterized in the solid state (IR) and in solution (mass spectrometry). In both cases, the complexation induced a shift of the CO stretching vibration bands from 1740 and 1687 cm^{-1} to lower wave numbers (1660–1540 cm^{-1}). Moreover, by mass spectrometry, only species of 1/1 stoichiometry were detected together with mono-hydrated form(s) in the case of the gadolinium complex.

3. Conclusion

In our course to increase the relaxivity of Gd(III) complexes used as CAs for MRI, we have synthesized a rigidified derivative of the previously prepared **cyclo-PCTA12** ligand, bearing an amide function in the skeleton. The synthetic route envisaged provided the desired target ligand in 34% overall yield in four steps from the linear amino precursor **4**. The corresponding gadolinium and

manganese complexes of 1/1 stoichiometry were then prepared in order to evaluate their potentiality as CA for MRI.

4. Experimental section

4.1. General information

4.1.1. Chemicals

All reactions were monitored for completion by thin layer chromatography (TLC) analyses performed on aluminium sheets precoated with silica gel Si60 (F_{254}, Merck, Ref 1.05554.0001) or with reverse phase silica gel RP18 (F_{254s}, Merck, Ref 1.05559.0001). Visualization was accomplished by irradiation with UV light at 254 nm and/or developed in an iodine chamber or by spraying with ninhydrin or with Dragendorf reagent (bismuth subnitrate—potassium iodate). When warming is necessary, the temperature mentioned is that of the oil bath. Preparative chromatography was performed by elution from columns of silica gel 60 (particle size 0.063–0.200 mm, Merck, Ref 1.07734.2500). The solution of anhydrous HCl in Et_2O 2.0 M was purchased from Sigma-Aldrich Co (Ref 45,518–0). Desionized water was produced on exchange resin with Millipore system (Simplicity®). Unless stated otherwise, all reagents were purchased from commercial sources and used without additional purification.

4.1.2. Physical measurements

^1H and ^{13}C NMR spectra were acquired on a Bruker AM400 spectrometer (400 MHz), at room temperature, in $CDCl_3$ ($CHCl_3$ at δ=7.26 ppm and $CDCl_3$ at δ=77.1 ppm (central shift) as internal standards for ^1H and ^{13}C NMR spectra respectively), in D_2O (HOD at δ=4.80 ppm as internal standard for ^1H NMR spectra and 3-trimethylsilyl-1-propanesulfonic acid sodium salt as external reference for ^{13}C NMR spectra), or in DMSO-d_6 (DMSO-d_6 at δ=2.50 ppm (central shift) and at δ=39.5 ppm (central shift) as internal standards for ^1H and ^{13}C NMR spectra respectively). ^{13}C spectra reported are proton decoupled. For the assignments of the NMR signals, we chose to use the convention presented in Figure 2. Chemical shifts δ are given in ppm. Coupling constants J are measured in Hz. For a given ^1H NMR spectrum, $J_{H,H}(1)$, $J_{H,H}(2)$, … can be used for easier association of protons coupled each together. Splitting patterns are designed as follows: s, singlet; d, doublet; t, triplet; ddd, doublet of a doublet of doublet; m, multiplet. Concerning the two diastereotopic protons of an AB system: (i) when $\delta_A \approx \delta_B$, the group of four neighbouring signals with respective chemical shifts δ_1, δ_2, δ_3 and δ_4 integrates for 2 protons; in this case, the AB system is described by giving the two extreme chemical shifts δ_1 and δ_4 together with the corresponding $^2J_{H,H}$ calculated between δ_1 and δ_2 (or δ_3 and δ_4); (ii) when $\delta_A << \delta_B$, each proton A and B of the AB system is described by a 'doublet' (d of AB); in this case, the 'doublets' are described by giving δ_1 and δ_2 for the first one, and δ_3 and δ_4 for the second one.

Infrared spectra were measured as KBr discs with a Nicolet FTIR Avatar 320 spectrometer.

Melting points (Mp) were obtained using a Büchi Tottoli apparatus.

High-resolution mass spectra (HRMS) analyses have been done on samples whose purity was checked by HPLC and/or ^1H NMR (≥ 98%); chemical ionizations (CI-HRMS) using CH_4 as the ionizing gas were performed with a Jeol MS700 mass spectrometer; ionizations by electrospray (ESI-HRMS) were acquired on a Thermo-Fisher Scientific hybrid linear ion trap LTQ-Orbitrap mass spectrometer. Low-resolution mass spectra (LRMS) resulting from ionization by electronic impact (EI-LRMS) were acquired on an Agilent 5973 Network MSD mass spectrometer; ionizations by electrospray (ESI-LRMS) were performed on a Waters Micromass ZQ2000 mass spectrometer. The mass analyses of both complexes were made by direct introduction carried out by infusion over 2 min of an aqueous solution of the sample (1 mg/mL) within a flow rate of 30 μL/min. The abundance indicated for each mass number (m/z values) is given in percentage relative to the strongest peak of 100% abundance (base peak). The isotopic distributions due to the presence of Br, Cl, or Gd elements were described by giving the more abondant m/z value of the distribution; the abundance given corresponds to that major peak and is relative to the base peak.

The HPLC analyses used a Waters 996 Photodiode Array Detector (210–400 nm); all the detections occurred at 260 nm; moreover, an isocratic system of elution was always used. The analyses were realized either with normal phase columns: A (Lichrosorb Si60, length: 25 cm, diameter: 4.6 mm, stationary phase: 5 μm); B (Hypersil Si60, length: 15 cm, diameter: 4.6 mm, stationary phase: 5 μm) or with reverse phase columns: C (Kromasil C8, length: 15 cm, diameter: 4.6 mm, stationary phase: 5 μm); D (Kromasil 100-5C18 Akzo-Nobel, length: 25 cm, diameter: 4.6 mm, stationary phase: 5 μm). The retention times t_R are expressed in minutes in the decimal system.

The GC analyses were performed on an Agilent 6890 N gas chromatograph using an apolar column SGE-BP1 (length: 15 m, diameter: 0.22 mm, film thickness: 0.25 μm). The injector temperature was fixed at 280 °C; the He flow was constant and fixed to 1 mL/min; the column oven temperature programming ramps were 50 °C (1 min) then 50 °C to 300 °C (20 °C/min) then 300 °C (1 min). The retention times t_R are expressed in minutes in the decimal system.

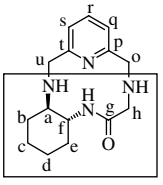


Figure 2. Convention adopted to assign signals of ^1H and ^{13}C NMR spectra.

4.2. (±) *trans* 2-Amino-*N*-(2-amino-cyclohexyl)-acetamide 3

The hydrochloride ethyl glycinate (22.85 g, 164 mmol, 1 equiv) was added in one portion to a solution of (±) *trans*-1,2-diaminocyclohexane **2** (19 mL, 158 mmol) in absolute ethanol (300 mL). The resulting suspension was refluxed for 3 days during which the insoluble matter increased. After cooling to room temperature, the white precipitate was filtered on büchner, washed with EtOH (3×20 mL), then dried to give the title compound **3** as a white solid (13.63 g, 79.6 mmol, Yield: 50%) whose purity was checked by ^1H RMN (≥98%). GC: t_R=7.8 min. TLC: Si60, MeOH/NH_4OH, 9:1 v/v, R_f=0.45–0.55, ninhydrin. Mp >260 °C. ^1H NMR (D_2O): δ=1.27–1.45 (m, 3H, C*H*H_c, C*H*H_d, and $H_{e,ax}$,), 1.45–1.56 (m, 1H, $H_{b,ax}$), 1.75–1.85 (m, 2H, C*H*H_c and C*H*H_d), 1.90–2.00 (m, 1H, $H_{e,eq}$), 2.07–2.15 (m, 1H, $H_{b,eq}$), 3.11 (ddd, $^3J_{H,H}(1)=^3J_{H,H}(2)$=11.3 Hz, $^3J_{H,H}(3)$=4.2 Hz, 1H, H_a), 3.74 (s, 2H, H_h), 3.80–3.95 (m, 1H, H_f) ppm. ^{13}C NMR (D_2O): δ=25.7 (C_c or C_d), 26.2 (C_c or C_d), 31.9 (C_b), 33.3 (C_e), 43.3 (C_h), 53.6 (C_f), 57.1 (C_a), 170.2 (*C*O) ppm. IR (KBr): ν=3202, 3018 (br), 2935 (br), 2865, 1682, 1563, 1501 cm^{-1}. EI-LRMS: m/z 171 $[M]^+$ (1%), 141 $[M-CH_2NH_2]^+$ (10%), 97 $[M-H_2NCH_2CONH_2]^+$ (100%), 82 $[C_6H_{10}]^+$ (12%), 81 (14%), 69 (22%), 56 $[C_4H_8]^+$ (40%). CI-HRMS: m/z calcd for $C_8H_{18}N_3O$, 172.1450; found, 172.1454 $[M+H]^+$.

4.3. (±) *trans* 2-(2-Nitro-phenylsulfonylamino)-*N*-[2-(2-nitro-phenylsulfonylamino) cyclohexyl]-acetamide 4

A solution of 2-nitro-phenylsulfonyl chloride (11.36 g; 49.72 mmol, 1.1 equiv/NH_2) in a mixture of THF (125 mL) and diethyl ether (400 mL) was added dropwise (over ca. 30 min) to a solution of diamino-acetamide **3** (4.00 g; 23.36 mmol) and Na_2CO_3 (7.42 g, 70.01 mmol, 1.5 equiv/NH_2) in water (110 mL). The

mixture was stirred at room temperature. After one day, a cristallization had occurred and the monitoring of the surnageant phase (TLC), showed a partial conversion of the starting diamino compound **3**. A second portion of 2-nitro-phenylsulfonyl chloride (2.59 g; 11.34 mmol, 0.25 equiv/NH_2) was added in one solid portion and the reaction was stirred at room temperature overnight. The white solid formed was filtered on büchner, washed with THF (2×10 mL), then dried to give the title compound **4** as a white solid (7.36 g, 13.59 mmol, Yield: 58%) whose purity was checked by RMN (≥ 98%). HPLC: Column A, EtOAc, 1.0 mL/min, t_R=5.3 to 5.4 min, λ_{max} <255 nm. TLC: Si60, EtOAc/cyclohexane, 6:4 v/v, R_f=0.2–0.25, irradiation with UV light. Mp 204 °C. ^{1}H NMR (DMSO-d_6): δ=1.00–1.35 (m, 4H, $H_{b,ax}$, $H_{e,ax}$, and 2H[$]), 1.45–1.70 (m, 4H, $H_{b,eq}$, $H_{e,eq}$, and 2H[$]), 2.95–3.10 (m, 1H, H_a), 3.27 and 3.31 (d of AB, $^2J_{H,H}$=16.6 Hz, 1H, C*H*H$_h$), 3.49 and 3.53 (d of AB, $^2J_{H,H}$=16.6 Hz, 1H, C*H*H$_h$), 3.43–3.55 (m, 1H, H_f), 7.68 (d, $^3J_{H,H}$=9.0 Hz, 1H, N*H*CO), 7.75–7.91 (m, 6H, 5C*H*$_{ar}$, and N*H*SO$_2$), 7.94–8.01 (m, 2H, C*H*$_{ar}$), 8.01–8.06 (m, 1H, C*H*$_{ar}$), 8.10 (br s,[£] 1H, N*H*CH$_2$) ppm. [$]Uncertain assignment: 2H=1H of H_c+1H of H_d, or 2H of H_c, or 2H of H_d. [£]This signal can have a broad triplet shape with $^3J_{H,H}$ ≈ 5.5 Hz; in this case, another coupling pattern appears for each 'd of AB' relative to H_h. ^{13}C NMR (DMSO-d_6): δ=23.8 (C_c or C_d), 23.9 (C_c or C_d), 31.4 (C_e), 32.1 (C_b), 45.0 (C_h), 51.2 (C_f), 56.6 (C_a), 124.0, and 124.4 (*C*H$_{ar}$CNO$_2$), 129.6, and 129.7 (*C*H$_{ar}$CSO$_2$), 132.4, 132.6, 133.7, and 133.9 (*C*H$_{ar}$), 132.9, and 134.2 (C_{quat}SO$_2$), 147.2, and 147.5 (C_{quat}NO$_2$), 166.7 (*C*O) ppm. IR (KBr): ν=3357 (br), 3098, 2935, 2860,1672, 1541, 1443, 1420, 1362, 1167 cm^{-1}. CI-HRMS: *m*/*z* calcd for $C_{20}H_{24}N_5O_9S_2$, 542.1015; found, 542.1014 $[M+H]^+$.

4.4. 2,6-Bis(bromomethyl)pyridine 5[25,26]

CAUTION: Lachrymator compound! A concentrated (96%) aqueous solution of sulphuric acid (20 mL, 0.36 mol, 3.6 equiv) was added to a solution of 2,6-bis(hydroxymethyl)pyridine (15.054 g, 0.11 mol) in a concentrated (48%) aqueous solution of hydrobromic acid (200 mL, 1.76 mol, 16 equiv). The mixture was refluxed for 31 h. Second portions of both concentrated aqueous solution of hydrobromic acid (200 mL, 1.76 mol, 16 equiv) and concentrated aqueous solution of sulphuric acid were then added (20 mL, 0.36 mol, 3.6 equiv) and the reflux was maintained for 63 h.

A cristallization occurred when the mixture cooled to room temperature. The orange acidic surnageant was collected. The white solid remaining was solubilized in water (900 mL) and the solution was basified (pH ≥10) by portionwise addition of solid Na_2CO_3. At basic pH, a cristallization occurred. The whitish solid formed was filtered on büchner, washed with water (2×30 mL), then taken up with diethyl ether (700 mL) and water (200 mL). The ethereal layer was dried, then concentrated to obtain a first crop of the title compound **5** (16.308 g; 0.062 mol, Yield (1st crop): 56%) as a white solid whose purity was checked by ^{1}H NMR (≥98%). The acidic surnageant was similarly treated. The combined ethereal layers were dried, then concentrated to obtain a grey solid (5.280 g) that revealed to be a mixture of the desired title compound **5** and of the intermediate 2-bromomethyl-6-hydroxymethyl-pyridine in 94:6 molar proportions respectively (^{1}H NMR estimation). This second part was purified by filtration on silica gel (diethyl ether/petroleum ether 40–60 °C, 1:9 v/v) to give a second crop of the title compound **5** as a white solid (5.087 g, 0.0192 mol) whose purity was checked by ^{1}H NMR (≥98%). Yield (1st+2nd crops): 74%. GC: t_R=6.8 min. TLC: Si60, CH_2Cl_2, R_f=0.6, irradiation with UV light. Mp 86 °C. ^{1}H NMR ($CDCl_3$): conform to reported data.[25,26] EI-LRMS: *m*/*z* 265 $[M]^+$ (21%), 184, 186 $[M-Br]^+$ (100%), 157, 159 $[M-2\ CH_2Br+HBr]^+=[C_5H_4BrN]^+$ (5%), 144, 146 $[C_5H_5Br]^+$ (2%), 105 $[M-Br_2]^+$ (29%), 104 (25%), 78 $[M-2\ CH_2Br+H]^+=[C_5H_4N]^+$, (16%), 77 $[M-2\ CH_2Br]^+=[C_5H_3N]^+$ (15%), 63 $[C_5H_3]^+$ (7%), 51 $[C_4H_3]^+$ (6%).

4.5. (±) *trans* 3,13-Bis(2-nitro-phenylsulfonyl)-3,10,13,19-tetraaza-tricyclo[13.3.1.0^{4,9}]nonadeca-1(19),15,17-trien-11-one 6

A solution of 2,6-bis(bromomethyl)pyridine **5** (0.323 g, 1.221 mmol, 1.2 equiv) in DMF (18 mL) was added dropwise (over ca. 65 min) to a suspension of disulfonamido-acetamide **4** (0.546 g, 1.009 mmol) and anhydrous K_2CO_3 (0.559 g, 4.049 mmol, 4 equiv) in DMF (18 mL) previously warmed at 100 °C. At the end of the addition, heating and stirring were maintained for an additional 10 min. The crude mixture was allowed to cool to room temperature; the insoluble matter was filtered off and washed with CH_2Cl_2. The filtrate was concentrated and the residue obtained was purified by chromatography on silica gel (EtOAc/cyclohexane, 7:3 to 10:0 v/v) to give the title compound **6** (0.383 g, 0.594 mmol, Yield: 59%) as a light yellow solid whose purity was checked by HPLC (95%). HPLC: Column B, EtOAc/MeOH 98:2 v/v, 1.0 mL/min, t_R=5.4 min, λ_{max} <255 nm. TLC: Si60, EtOAc, R_f=0.2–0.4, irradiation with UV light, spot became yellow on standing at daylight. Mp 145–148 °C. ^{1}H NMR ($CDCl_3$)[£,$]: δ=1.04–1.22 (m, 3H, C*H*H$_c$, C*H*H$_d$, and $H_{e,ax}$), 1.48–1.72 (m, 4H, $H_{b,ax}$, $H_{b,eq}$, C*H*H$_c$, and C*H*H$_d$), 2.10–2.22 (m, 1H, $H_{e,eq}$), 2.94–3.03 (m, 1H, H_f), 3.71 (ddd, $^3J_{H,H}(1)=^3J_{H,H}(2)$=11.4 Hz, $^3J_{H,H}(3)$=3.7 Hz, 1H, H_a), 3.87 and 3.96 (AB, $^2J_{H,H}(1)$=16.1 Hz, 2H, H_β), 4.28 and 4.32 (d of AB, $^2J_{H,H}(2)$=14.3 Hz, 1H, C*H*H$_\gamma$), 4.29 and 4.33 (d of AB, $^2J_{H,H}(3)$=17.3 Hz, 1H, C*H*H$_\alpha$), 4.46 and 4.50 (d of AB, $^2J_{H,H}(2)$=14.3 Hz, 1H, C*H*H$_\gamma$), 5.02 and 5.06 (d of AB, $^2J_{H,H}(3)$=17.3 Hz, 1H, C*H*H$_\alpha$), 7.11 (d, $^3J_{H,H}(4)$=7.7 Hz, 1H, H_q or H_s), 7.14 (d, $^3J_{H,H}(5)$=7.7 Hz, 1H, H_q or H_s), 7.31 (d, $^3J_{H,H}(6)$=4.7 Hz, 1H, N*H*), 7.55 (dd, $^3J_{H,H}(4)=^3J_{H,H}(5)$=7.7 Hz, 1H, H_r), 7.58–7.77 (m, 6H, C*H*$_{ar}$), 7.96–8.02 (m, 1H, C*H*$_{ar}$), 8.14–8.20 (m, 1H, C*H*$_{ar}$) ppm. ^{13}C NMR ($CDCl_3$)[£,$]: δ=23.9 (C_c or C_d), 25.4 (C_c or C_d), 29.9 (C_b), 33.4 (C_e), 47.8 (C_α), 52.0 (C_f), 53.0 (C_β), 55.5 (C_γ), 60.0 (C_a), 120.8 (C_q or C_s), 122.9 (C_q or C_s), 124.1, and 124.8 (*C*H$_{ar}$CNO$_2$), 131.3, 131.5, 131.7, 131.9, 133.79, and 133.83 (*C*H$_{ar}$), 132.7, and 133.9 (C_{quat}SO$_2$), 137.6 (C_r), 147.7, and 148.3 (C_{quat}NO$_2$), 154.0 (C_p or C_t), 154.7 (C_p or C_t) 168.3 (C_g) ppm. [£]In $CDCl_3$, there is two distinct forms in 80/20 molar proportions; the following description refers to the major form. [$]Signals relative to the three populations of NC*H*$_2$ nuclei could not be certainly assigned so that α, β, γ were used to design these positions. IR (KBr): ν=3378 (br), 2930, 2857, 1670, 1543, 1439, 1373, 1163 cm^{-1}. CI-HRMS: *m*/*z* calcd for $C_{27}H_{29}N_6O_9S_2$, 645.1437; found, 645.1448 $[M+H]^+$.

4.6. (±) *trans* 3,10,13,19-Tetraaza-tricyclo[13.3.1.0^{4,9}]nonadeca-1(19),15,17-trien-11-one 7

Thiophenol (0.850 mL, 8.278 mmol, 1.4 equiv/NSO_2Ar) was added in one portion to a suspension of macrocyclic disulfonamide **6** (1.909 g, 2.961 mmol) and anhydrous K_2CO_3 (1.308 g, 9.466 mmol, 1.6 equiv/NSO_2Ar) in acetonitrile (30 mL). The resulting mixture was warmed up to 70 °C for 4 h. The crude mixture was then allowed to cool to room temperature then it was diluted with CH_2Cl_2 (30 mL) and H_2O (27 mL). The heterogeneous mixture was vigorously stirred then acidified by addition of an aqueous solution of HCl 6 M (3 mL, 18 mmol). The aqueous layer was repeatedly washed with CH_2Cl_2 (9×30 mL). The washed aqueous layer was then basified by addition of an aqueous solution of NaOH 6 M (5 mL, 30 mmol) then extracted with CH_2Cl_2 (3×100 mL). The combined organic layers were dried and concentrated to give the title compound **7** (0.699 g, 2.547 mmol, Yield: 86%) as a sticky light yellow solid whose purity was checked by ^{1}H NMR and HPLC (≥98%). HPLC: Column C, MeOH/H_2O 95:5 v/v+HCO_2H 0.1%, 0.8 mL/min, t_R=1.4 to 1.5 min, λ_{max}=261.6 nm. TLC: RP18, MeOH, R_f=0–0.2 (trail), irradiation with UV light, I_2, ninhydrin. ^{1}H NMR ($CDCl_3$)[$]: δ=0.93–1.10 (m, 2H, $H_{c,ax}$ or $H_{d,ax}$, and $H_{e,ax}$), 1.17–1.32 (m, 2H, $H_{b,ax}$, and $H_{c,ax}$ or $H_{d,ax}$), 1.54–1.68 (m, 2H, $H_{c,eq}$ and $H_{d,eq}$), 1.87 (ddd, $^3J_{H,H}(1)=^3J_{H,H}(2)$=10.7 Hz, $^3J_{H,H}(3)$=4.2 Hz, 1H, H_a),

1.94–2.09 (m, 2H, $H_{b,eq}$, and $H_{e,eq}$), 2.70 (br s, 2H, N*H*), 3.34 and 3.39 (d of AB, $^2J_{H,H}(1)$=17.3 Hz, 1H, C*H*H$_\alpha$), 3.59–3.70 (m, 1H, H_f), 3.69 and 3.73 (d of AB, $^2J_{H,H}(2)$=16.8 Hz, 1H, C*H*H$_h$), 3.71 and 3.75 (d of AB, $^2J_{H,H}(1)$=17.3 Hz, 1H, C*H*H$_\alpha$), 3.89 and 3.93 (d of AB, $^2J_{H,H}(3)$=15.7 Hz, 1H, C*H*H$_\beta$), 3.94 and 3.98 (d of AB, $^2J_{H,H}(2)$=16.8 Hz, 1H, C*H*H$_h$), 4.08 and 4.12 (d of AB, $^2J_{H,H}(3)$=15.7 Hz, 1H, C*H*H$_\beta$), 6.90 (d, $^3J_{H,H}(4)$=7.7 Hz, 1H, H_q or H_s), 6.94 (d, $^3J_{H,H}(5)$=7.7 Hz, 1H, H_q or H_s), 7.49 (dd, $^3J_{H,H}(4)$=$^3J_{H,H}(5)$=7.7 Hz, 1H, H_r), 8.05 (br s, 1H, N*H*) ppm. [$]In the following description, signals relative to nuclei designed by o and u letters (Fig. 2) could not be certainly assigned so that α, β were used to design these positions. ^{13}C NMR ($CDCl_3$): δ=24.6 (C_c or C_d), 24.9 (C_c or C_d), 32.6 (C_e), 33.5 (C_b), 51.3 (C_h), 52.2 (C_f), 56.2 (C_o or C_u), 56.3 (C_o or C_u), 62.4 (C_a), 119.3 (C_q or C_s), 120.8 (C_q or C_s), 136.6 (C_r), 159.9 (C_p and C_t),[£] 173.7 (C_g) ppm. [£]This unique signal accounts for the two populations of $C_{quat,ar}$. IR (KBr): ν=3327 (br), 2925, 2852, 1658, 1650, 1575, 1526, 1448, 1431 cm^{-1}. CI-HRMS: *m*/*z* calcd for $C_{15}H_{23}N_4O$, 275.1872; found, 275.1872 $[M+H]^+$.

4.7. (±) *trans tert*-Butyl (13-*tert*-butoxycarbonylmethyl-11-oxo-3,10,13,19-tetraaza-tricyclo[13.3.1.0^{4,9}]nonadeca-1(19),15,17-trien-3-yl)acetate 8

CAUTION: the purified isolated product **8** must be stored at −18 °C. A solution of 2,6-bis(bromomethyl)pyridine **5** (1.778 g, 6.711 mmol, 1.2 equiv) in DMF (50 mL) was added dropwise (over 70 min) to a suspension of disulfonamido-acetamide **4** (3.004 g, 5.547 mmol) and anhydrous K_2CO_3 (3.080 g, 22.283 mmol, 4 equiv) in DMF (50 mL) previously warmed at 100 °C. At the end of the addition, heating and stirring were maintained for an additional 50 min. The crude mixture was concentrated to reduce the volume twofold. A second portion of anhydrous K_2CO_3 (1.506 g, 10.898 mmol, 1 equiv/NSO_2Ar) was then added followed by the addition of thiophenol (1.6 mL, 15.582 mmol, 1.4 equiv/NSO_2Ar). The resulting suspension was stirred at room temperature overnight. The crude mixture was then diluted with CH_2Cl_2 (50 mL) and H_2O (40 mL). The heterogeneous mixture was vigorously stirred then acidified by addition of an aqueous solution of HCl 6 M (10 mL). The upper aqueous layer was repeatedly washed with CH_2Cl_2 (12×50 mL). The washed aqueous layer was then basified by addition of solid NaOH (3 g) then extracted with CH_2Cl_2 (3×50 mL). The combined organic layers were dried and concentrated to give macrocyclic diamine **7** as a light yellow solid (1.190 g, ≤4.337 mmol, Yield (crude, 2 steps) ≤78%). *tert*-Butyl bromoacetate (1.4 mL, 9.603 mmol, 1.1 equiv/NH) was added to a solution of the crude macrocyclic diamine **7** (1.190 g, 4.337 mmol) and triethylamine (1.4 mL, 10.044 mmol, 1.2 equiv/NH) in tetrahydrofuran (45 mL). The mixture was refluxed for 7 h (after 10–15 min, a white precipitate was formed and remained until the end of the reaction). The crude mixture was concentrated, then taken up in CH_2Cl_2 (100 mL) and water (30 mL). The organic layer was further washed with water (2×30 mL), dried and concentrated. The residue obtained was purified by chromatography on silica gel (EtOAc/cyclohexane, 5:5 v/v) to give the title compound **8** as a white solid (1.215 g, 2.417 mmol, 44% overall yield from **4** (3 steps)) whose purity was checked by HPLC (≥94%). HPLC: Column D, MeOH/H_2O 75:25 v/v+HCO_2H 0.1%, 0.7 mL/min, t_R=4.2 min (br), λ_{max}=262.7, and 263.9 nm. TLC: Si60, EtOAc, R_f=0.1–0.6 (trail), irradiation with UV light, I_2, Dragendorf. Mp 126–128 °C. ^{1}H NMR ($CDCl_3$)[$]: δ=0.98–1.15 (m, 1H, $H_{e,ax}$), 1.18–1.32 (m, 3H, $H_{b,ax}$, $H_{c,ax}$, and $H_{d,ax}$), 1.40 (s, 9H, C*H*$_3$), 1.47 (s, 9H, C*H*$_3$), 1.57–1.66 (m, 1H, $H_{c,eq}$ or $H_{d,eq}$), 1.72–1.80 (m, 1H, $H_{c,eq}$ or $H_{d,eq}$), 2.03–2.10 (m, 1H, $H_{b,eq}$), 2.40–2.55 (m, 1H, $H_{e,eq}$), 2.72–2.85 (m, 1H, H_a), 2.91 and 2.95 (d of AB, $^2J_{H,H}(1)$=18.1 Hz, 1H, C*H*H$_\alpha$), 3.13 and 3.17 (d of AB, $^2J_{H,H}(2)$=17.6 Hz, 1H, C*H*H$_\gamma$), 3.23 and 3.28 (d of AB, $^2J_{H,H}(1)$=18.1 Hz, 1H, C*H*H$_\alpha$), 3.39 (s large, 2H, H_β), 3.46–3.59 (m, 2H, H_f and C*H*H$_\gamma$), 4.00–4.15 (m, 2H, C*H*H$_\delta$, and C*H*H$_\varepsilon$), 4.25 and 4.29 (d of AB, $^2J_{H,H}(3)$=16.6 Hz, 1H, C*H*H$_\delta$), 4.34 and 4.38 (d of AB, $^2J_{H,H}(4)$=16.1 Hz, 1H, C*H*H$_\varepsilon$), 6.93 (d, $^3J_{H,H}(1)$=7.5 Hz, 1H, H_q or H_s), 6.97 (d, $^3J_{H,H}(2)$=7.5 Hz, 1H, H_q or H_s), 7.52 (dd, $^3J_{H,H}(1)$=$^3J_{H,H}(2)$=7.5 Hz, 1H, H_r), 10.75 (br s, 1H, N*H*) ppm. ^{13}C NMR ($CDCl_3$): δ=24.4 (C_c or C_d), 26.0 (C_c or C_d), 28.0 (*C*H$_3$), 28.2 (*C*H$_3$), 31.6 (C_b),[£] 33.2 (C_e), 47.6 (C_α), 51.6 (C_f), 56.3 (C_β), 58.7 (C_γ and C_δ),[#] 60.3 (C_ε), 68.2 (C_a), 80.3 (*C*(CH$_3$)$_3$), 81.3 (*C*(CH$_3$)$_3$), 120.3 (C_q or C_s), 120.6 (C_q or C_s), 136.9 (C_r), 158.8 (C_p or C_t), 160.0 (C_p or C_t), 171.2 (*C*O), 172.6 (*C*O), 172.8 (*C*O) ppm. [$]In this description, signals relative to the five populations of NC*H*$_2$ nuclei could not be certainly assigned so that α, β, γ, δ, and ε were used to design these positions. [£]This signal has an uncommon shape: very small and broad. [#]This unique signal accounts for the two populations of *C*H$_2$. IR (KBr): ν=3329, 3210, 3065, 2972, 2931, 2851, 1736, 1675, 1577, 1556, 1451, 1365, 1215, 1147 cm^{-1}. ESI-HRMS: *m*/*z* calcd for $C_{27}H_{43}N_4O_5$, 503.32280; found, 503.32255 $[M+H]^+$.

4.8. (±) *trans* 11-Oxo-3,10,13,19-tetraaza-tricyclo[13.3.1.0^{4,9}]-nonadeca-1(19),15,17-triene-3,13-diacetic acid hydrochloride 1

*4.8.1. From macrocyclic diester **8***

A solution of anhydrous HCl in Et_2O (2.0 M, 15 mL, 30 mmol, 77 equiv) was poured over the macrocyclic di-*tert*-butyl ester **8** (0.195 g, 0.387 mmol). A white precipitate appeared after several minutes. The mixture was vigorously stirred at room temperature for 5 days, then a second portion of anhydrous HCl in Et_2O (2.0 M, 15 mL, 30 mmol, 77 equiv) was added and the suspension was refluxed overnight. The precipitate was then filtered and washed with Et_2O to give the title compound **1** as a white powder (0.149 g, 0.299 mmol for *x*=3 or 0.350 mmol for *x*=1, 77%<Rdt<90%). Its purity was checked by HPLC (97%). HPLC: Column D, MeOH/H_2O 75:25 v/v+HCO_2H 0.1%, 0.7 mL/min, t_R=3.2–3.6 min (br), λ_{max}=262.7 nm. TLC: RP18, MeOH, R_f=0.1–0.5 (trail), irradiation with UV light, I_2, Dragendorf. Mp 214 °C (decomposition). ^{1}H NMR (D_2O, pD≈1): δ=1.07–1.41, 1.41–1.75, and 1.75–2.00 (3 m, 7H, C*H*H$_b$, H_c, H_d, and H_e), 2.10–2.35 (m, 1H, C*H*H$_b$), 3.05, and 3.21–3.34 (br s and m, 0.63H and 0.37H, H_a)[£], 3.46–3.60, and 3.73 (m and br s, 0.37H and 0.63H, H_f)[£], 3.85–4.10 (m, 2H), 4.12–4.35 (m, 4H), 4.36–4.50 (m, 1H), 4.56–4.80 (m, 3H)[$], 7.50–7.70 (m, 2H, H_q, and H_s), 8.02–8.15 (m, 1H, H_r) ppm. [£]Presence of two forms in aqueous solution; 63/37 molar proportions. [$]A part of this signal is under the HOD one (seen by HOD irradiation experiment). ^{13}C NMR (D_2O, pD≈1)[*]: δ=23.39[*], 23.40[*], 23.45, 23.53[*], 23.64[*] (C_{β}, C_{ε}, C_d, C_{α} and C_{ε}), 24.1 (C_b), 29.4 (C_c), 31.6 (C_e), 48.9*, and 49.6 (C_f), 50.1, 51.7, 56.1, 56.6, 57.7, 58.1, 58.9, 59.5, 61.5 (C_h, C_o, C_u, and *C*H$_2$CO$_2$H), 67.5, and 68.3[*] (C_a), 123.5, 124.6, 125.5, 126.1 (C_q, and C_s), 141.2, 142.0 (C_r), 148.8, 149.7, 150.2, 150.6 (C_p, and C_t), 167.8, 168.9, 169.5, 169.8, 170.5, 171.1 (*C*OO, and *C*ONH). [*]Nearly all the ^{13}C signals are doubled due to the presence of two forms in aqueous solution; signals marked referred to the minor form. IR (KBr): ν=3361 (br), 3216 (br), 3000, 2944, 2865, 1740, 1687, 1595, 1576, 1564, 1453, 1408 (br) cm^{-1}. ESI-HRMS: *m*/*z* calcd for $C_{19}H_{25}N_4O_5$ 389.18304, found 389.18207 $[M-H]^-$; *m*/*z* calcd for $C_{19}H_{26}ClN_4O_5$ 425.15972, found 425.15874 $[M+Cl]^-$.

*4.8.2. From macrocyclic diamine **7***

An aqueous solution of NaOH (3 M, 1.9 mL, 5.7 mmol, 5.7 equiv) was added in one portion to a solution of macrocyclic diamine **7** (0.276 g, 1.00 mmol) in MeOH/H_2O 1/1 v/v (4 mL). The resulting basic solution (pH ≈ 10) was warmed at 80 °C and a freshly prepared solution of sodium bromoacetate (bromoacetic acid (0.309 g, 2.22 mmol, 2.2 equiv) and NaOH (0.127 g, 3.17 mmol, 3.1 equiv)) in water (2 mL) was added in one portion. After 22 h, the monitoring of the reaction (TLC on RP18) revealed an uncomplete conversion of compound **7**. Three portions of sodium bromoacetate were successively added (4.3 equiv in all, after 22 h, 29 h, and 2.5 days of warming). The crude mixture was then allowed to cool to room temperature, washed with CH_2Cl_2 (3×10 mL), then acidified (pH≈1) by addition of a molar aqueous solution of HCl in the

presence of CH_2Cl_2 (10 mL). The acidic aqueous layer was repeatedly washed with CH_2Cl_2 (3×10 mL). The washed aqueous layer was concentrated to give a deliquescent light-brown solid (1.724 g) whose HPLC analysis showed a chromatogram identical to that previously obtained from the deprotection of *tert*-butyl diester **8**. The excess of crude matter may result from the excesses of both NaOH introduced to insure alkaline medium, and bromoacetic acid, and from a partial elimination of water.

4.9. Gadolinium complexation

The initial pH 1.0 of a solution of macrocyclic ligand **1** (0.132 g, 0.265 mmol (if x=3) to 0.310 mmol (if x=1)) in deionized H_2O (5 mL) was adjusted at pH 5.7 with an aqueous solution of KOH 0.5 M. Gadolinium(III) chloride hexahydrate (0.115 g, 0.310 mmol) was added in one portion which induced a decrease of pH down to pH 4.2 value so that pH was readjusted up to 5.7 (KOH 0.5 M). The resulting mixture (final volume 10 mL) was warmed at 80 °C for 24 h. The pH was then raised to 8.7 value (KOH 0.5 M). A white precipitate appeared after a prolonged stirring at room temperature, and persisted by decreasing the pH down to 8.2 value. The precipitate was filtered off on a 0.2 μm PES filter (Waters, WAT200539). The clear yellow aqueous filtrate was concentrated (2.5 mL) and CH_3CN (15 mL) was then added. A solid sticked to the reaction flask was formed. The filtrate was removed and a second portion of CH_3CN (15 mL) was poured. After vigorous stirring, the powder formed was filtered on an hardened ashless filter paper (Whatman, 542) to give the gadolinium complex as a whitish solid (0.134 g). IR (Solid): ν=3385 (br), 2934, 2860, 1581, 1545, 1397, 1212, 1154 cm^{-1}. ESI-LRMS: m/z:[$] 371.1 $[M-CH_2CO_2H+H+K]^+$ (100%), 546.1 $[C_{19}H_{24}{}^{158}GdN_4O_5]^+$=$[M-2H+{}^{158}Gd]^+$, 564.1 $[M-2H+{}^{158}Gd+H_2O]^+$, 584.1 $[M-3H+{}^{158}Gd+K]^+$, 602.1 $[M-3H+{}^{158}Gd+K+H_2O]^+$, 620.1 $[M-3H+{}^{158}Gd+K+2H_2O]^+$ and/or $[M-2H+{}^{158}Gd+KCl]^+$, 658.0 $[M-3H+{}^{158}Gd+K+KCl]^+$, 696.0 $[M-2H+{}^{158}Gd+2KCl]^+$, 734.0 $[M-3H+{}^{158}Gd+K+2KCl]^+$, 770.0 $[M-2H+{}^{158}Gd+3KCl]^+$, 807.9 $[M-3H+{}^{158}Gd+K+3KCl]^+$, 881.9 $[M-3H+{}^{158}Gd+K+4KCl]^+$. [$]The relative abundances of the other mass numbers were not mentioned as variable from one analysis to another.

4.10. Manganese complexation

The initial pH 1.0 of a solution of macrocyclic ligand **1** (0.052 g, 0.104 mmol (if x=3) to 0.122 mmol (if x=1)) in deionized H_2O (2 mL) was adjusted at pH 5.7 with an aqueous solution of KOH 0.5 M. Manganese(II) chloride tetrahydrate (0.024 g, 0.124 mmol) was added in one portion which induced a decrease of pH down to 3.5 value so that pH was readjusted to 5.8 (KOH 0.5 M). The resulting mixture (final volume 6 mL) was stirred at room temperature for 24 h. The pH was then raised to 8.5 (KOH 0.5 M) and the fine brown precipitate formed was filtered off on a 0.2 μm PES filter (Waters, WAT200539). The grey coloured filtrate became purple coloured by standing at room temperature. The clear grey-purple aqueous filtrate was concentrated (5 mL), and CH_3CN (25 mL) was then added. The solid obtained after slow evaporation to dryness was taken up in CH_3CN (20 mL) and the resulting powder was filtered on an hardened ashless filter paper (Whatman, 542) to give the manganese complex as a whitish fine powder. IR (Solid): ν=3420 (br), 3227, 2934, 2860, 1660, 1590, 1390, 1206, 1151 cm^{-1}. ESI-LRMS: m/z: 333.2 $[M-CH_2CO_2H+2H]^+$ (7–10%), 371.1 $[M-CH_2CO_2H+H+K]^+$ (4–10%), 482.0 $[C_{19}H_{24}N_4O_5MnK]^+$=$[M-2H+Mn+K]^+$ (100%), 520.0 $[M-3H+Mn+2K]^+$ (15%), 556.0 $[M-2H+Mn+K+KCl]^+$ (10%), 629.9 $[M-2H+Mn+K+2KCl]^+$ (25–30%), 925.4 $[2M-4H+2Mn+K]^+$ (8%), 999.4 $[2M-4H+2Mn+K+KCl]^+$ (10%).

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