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Polymethylated DOTA Ligands. 1. Synthesis of Rigidified Ligands and Studies on the Effects of Alkyl Substitution on Acid–Base Properties and Conformational Mobility

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This work describes the synthesis and the conformational properties of new polymethylated macrocyclic ligands of potential interest for magnetic resonance imaging. M4cyclen, (2*S*,5*S*,8*S*,11*S*)-2,5,8,11-tetramethyl-1,4,7,10-tetraazacyclododecane, was obtained by cyclotetramerization of (2*S*)-1-benzyl-2-methylaziridine followed by catalytic hydrogenation. The ligands M4DOTA, [(2*S*,5*S*,8*S*,11*S*)-4,7,10-tris-carboxymethyl-2,5,8,11-tetramethyl- 1,4,7,10-tetraazacyclododecan-1-yl]acetic acid, and M4DOTMA, (*R*)-2-[(2*S*,5*S*,8*S*,11*S*)-4,7,10-tris-((*R*)-1-carboxyethyl)-2,5,8,11-tetramethyl-1,4,7,10-tetraazacyclododecan-1-yl]propionic acid, were prepared by carboxyalkylation of M4cyclen in the presence of Na_2CO_3. The triacetic ligand M4DO3A, [(2*S*,5*S*,8*S*,11*S*)-4,7-bis-carboxymethyl-2,5,8,11-tetramethyl-1,4,7,10-tetraazacyclododecan-1-yl]acetic acid, was obtained in good yields without traces of M4DOTA if $NaHCO_3$ was the acid scavenger when adding the carboxylic arms. In the same conditions, cyclen yielded M4DOTA in 82% yield. The difference between the reactivity of cyclen and M4cyclen is assigned to the high basicity of the substituted tetraamine as estimated by NMR titration. The one- and two-dimensional ^1H and ^{13}C NMR spectra of M4DOTA and M4DOTMA in the H_4L or H_6L^{2+} forms are interpreted as arising from a slow exchange between two elongated geometries in which the methyl substituents are in one of the two possible equatorial-like positions, either close to or away from the carboxylic arms. The axial-like positions are sterically too crowded and cannot be occupied by the methyl groups. An elongated conformation is also adopted by DOTMA, (*R*)-2-[4,7,10-tris-((*R*)-carboxyethyl)-1,4,7,10-tetraazacyclododecan-1-yl]propionic acid, in the H_6L^{2+} form. The rigidification of the polymethylated ligands allows a detailed NMR analysis that cannot be carried out on the parent unsubstituted ligand DOTA.

Introduction

The symmetric macrocyclic system DOTA, **1**, (4,7,10-tris-carboxymethyl-1,4,7,10-tetraaza-cyclododecan-1-yl)acetic acid (see Scheme 1), is now one of the most well-known and widely studied ligand systems in coordination chemistry.[1,2] This is mainly due to the ability of DOTA and its analogues to form stable chelates that are used extensively as safe and effective contrast agents for magnetic resonance imaging (MRI).[3,4] Notable examples of this application are the Gd^{3+} chelates of DOTA, **1** (Dotarem) and HPDO3A (Prohance).[5] DOTA-based chelates are also useful as in vivo NMR shift agents, radiopharmaceuticals, and luminescent probes.[3,6] DOTA is an attractive ligand for lanthanides because of its unusual steric requirements such as cavity size, ring conformation, and directionality of the donor atoms, which favor formation of thermodynamically stable and kinetically inert chelates.[7] These properties are required in applications meant for obtaining anatomical information using extracellular MRI agents, because any dissociation of the chelate and incor-

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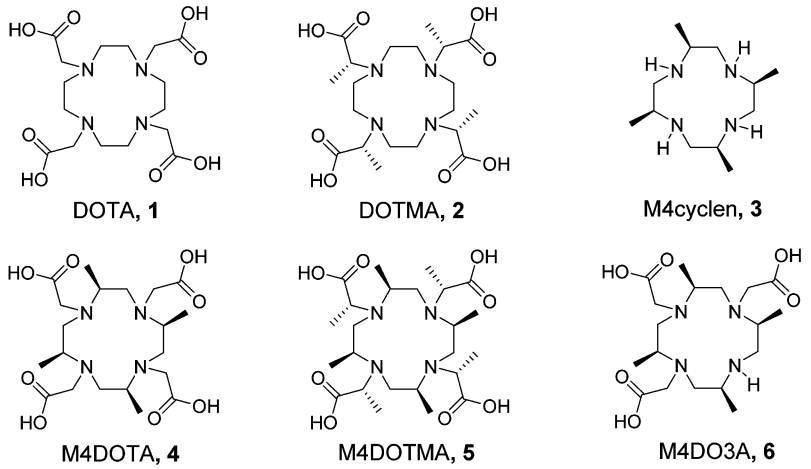
Scheme 1



poration of the freed Gd^{3+} ions into body tissues will cause unacceptable toxic effects. This requirement becomes more stringent when applying MRI to study physiological function or when using the chelates of metallic radionuclides. Conjugates of chelates with biological macromolecules that have affinity for specific tumors or tissues are employed for such studies. These conjugates are expected to reside longer in the body and thus have the potential to produce toxic effects, unless a higher degree of kinetic stability is assured. Furthermore, because the concentrations of receptors are typically in the low micromolar to nanomolar range, it would be important to achieve a several-fold increase in the relaxivity of the MRI chelates in order to achieve the required sensitivity.[8] To address these needs, we explored the hypothesis that rigidifying the macrocycle without altering its symmetry characteristics could create more effective and kinetically inert analogues of DOTA. An increase in the rigidity of the macrocycle would be expected if the ring carbon atoms and/or the donor-arm carbon atoms bear substituents. We already adopted[9] this approach in a study of the lanthanide chelates of the DOTMA ligand, **2**. The synthesis of the tetra-amine (2*S*,5*S*,8*S*,11*S*)-2,5,8,11-tetramethyl-1,4,7,10-tetraazacyclododecane, M4cyclen, **3**, and of the ligands M4DOTA, **4**, M4DOTMA, **5**, and M4DO3A, **6**, as reported herein allow us to extend our studies to a complete family of methylated DOTA derivatives. We describe here our successful synthesis of these macrocyclic compounds, and we report on the acid−base properties of **3** and on the conformational properties of the new ligands using NMR.

Experimental Section

Synthesis and Spectral Studies. General Information. Melting points are uncorrected. 1,4,7,10-Tetraazacyclododecane, cyclen, was provided by RCM, Bracco, Milan, Italy. (*R*)-2-[4,7,10-Tris-((*R*)-carboxyethyl)-1,4,7,10- tetraazacyclododecan-1-yl] propionic acid, DOTMA, **2**, was synthesized as reported previously.[9] Infrared spectra were obtained on KBr pellets. Flash column chromatography was carried out over silica gel. Unless otherwise specified, HPLC analyses were performed with a reverse phase C18-silica column (15 cm × 4.6 mm i.d.) using CH_3CN/H_2O at a flow rate of 0.5 mL/min, and the UV detector was set at 254 nm. The extent of hydration of the new compounds was determined by desorption or by Karl Fisher titration. All NMR experiments were performed on a Bruker Avance DRX-400 spectrometer equipped with a temperature control unit (±0.1 K) that was calibrated with ethylene glycol or on a Varian Unity Inova 500 spectrometer. The chemical shifts were referenced to the internal standard, sodium 3-(trimethylsilyl)-propionate in D_2O and TMS in organic solvents. An exponential apodization function with a line-broadening factor of 2 was applied to ^{13}C FIDs prior to their Fourier transformation. Two-dimensional COSY (basic or phase-sensitive), NOESY, and EXSY experiments were carried out using standard pulse programs. COSY spectra were obtained by Fourier transformation of two-dimensional 0° shifted sine bell apodized 1024 × 1024 time domain data points acquired with a typical COSY gradient pulse program (cosygp from the Bruker library). Nuclear Overhauser and exchange spectroscopy data (NOESY and EXSY) were recorded simultaneously by applying a phase-sensitive pulse sequence (TPPI phase encoding)[10] with a mixing time of 0.5 s. The data sets were apodized by using 90° shifted sine bell functions in both dimensions. Nuclear Overhauser and exchange cross-peaks could be distinguished in the Fourier transformed and phase corrected spectrum because of their phase that is, respectively, opposite or equal to that of the diagonal peaks. A $^1H-^{13}C$ heteronuclear correlation spectrum (1024 × 1024) was obtained with an inverse HMQC gradient pulse sequence (inv4gp from the Bruker library). A 180° shifted squared sine bell apodization function was applied in the t_2 dimension, while the t_1 data were multiplied by a 270° shifted sine bell prior to the 2D Fourier transform.

NMR Titrations. Solutions (8 mM) of cyclen or M4cyclen were prepared in D_2O under a N_2 atmosphere at 22 °C. The required pD values were obtained by adjustment with either DCl or KOD. To keep the ionic strength constant for each sample, KCl was added to make the final concentration of KCl to be 80 mM. The final pH was determined with an Orion benchtop model SA 720 pH meter fitted with a microelectrode and corrected for the deuterium isotope effect using the relationship[11] pH = pD − 0.4. The HYPNMR program[12] was used to determine the pK_a values. The chemical shifts

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of the NMR peaks and the corresponding pH values were initially entered. The program converged after about 50 iterations provided reasonable initial estimates of the pK_a values were supplied to the program.

(2*S*)-2-(Benzylamino)propan-1-ol, 8. Method A: From *N*-Benzoyl-L-alanine, 7. To a solution of *N*-benzoyl-L-alanine, **7** (29.0 g, 150 mmol), in THF (200 mL) at 0 °C was added a solution of diborane (1 M solution, 800 mL) in THF, and the mixture was refluxed for 18 h. The excess of diborane was decomposed with methanol, and the solvents were removed under reduced pressure. The residue was dissolved in methanol (100 mL) and then treated with 6 N HCl (100 mL). The mixture was heated at 70 °C for 12 h, and the solvents were removed under reduced pressure. The residue was coevaporated with methanol (5 × 150 mL) and the product dissolved in water (50 mL), basified with 5 N NaOH to pH 12, and then extracted with ethyl acetate (3 × 200 mL). The combined organic layers were washed with saturated NaCl (150 mL), dried, and concentrated to a final volume of 100 mL. Hexane (300 mL) was added, and the solution cooled in the refrigerator overnight. The mixture was filtered, and the solid thus obtained was dried to obtain (2*S*)-2-(benzylamino)propan-1-ol, **8**, as white crystalline needles (21.6 g, 87%). Mp: 46−48 °C, (lit.:[13] mp 46−48 °C).

(2*S*)-2-(Benzylamino)propan-1-ol, 8. Method B: From (*S*)-2-Amino-1-propanol, 9. A solution of benzaldehyde (46.64 g, 0.44 mol) and (*S*)-2-amino-1-propanol, **9** (30.0 g, 0.4 mol), in dry toluene (500 mL) was heated under reflux under nitrogen until no more water separated in a Dean−Stark condenser (6 h). The solution was concentrated to a thick oil under vacuum, and the residue was dissolved in ethanol (500 mL) and cooled in an ice bath. Powdered sodium borohydride (38.8 g, 1 mol) was added in portions and the solution treated with 4 N HCl dropwise until the pH reached around 2−3 (1 h). The reaction mixture was stirred overnight (20 h) and then evaporated to dryness. The residue was dissolved in 1 N HCl (250 mL) and then extracted with dichloromethane (5 × 50 mL). The organic layer was discarded, and the aqueous layer was basified to pH 13.0. The basic solution was then extracted with dichloromethane (5 × 100 mL). The organic layers were combined and dried (Na_2SO_4). Evaporation of the solvent under reduced pressure yielded product **8** as a solid that was identical to the compound obtained by method A (56.0 g, yield: 85%).

(2*S*)-1-Benzyl-2-methylaziridine, 10b. To a stirred solution of (2*S*)-2-(benzylamino)propan-1-ol, **8** (33.0 g, 200 mmol), and triphenylphosphine (79.66 g, 300 mmol) in ether (500 mL), under nitrogen in an ice bath, was slowly added diethyl azodicarboxylate (DEAD, 95%, 50 mL, 30 mmol), and the solution was stirred at room temperature for 16 h. A crystalline precipitate (presumably a triphenylphosphine/diethyl hydrazine-1,2-carboxylate complex) was filtered off and washed with hexane/ether (1:1, 200 mL). The ether solution was extracted with 1 N HCl (2 × 100 mL) and the solution basified with 5 N NaOH, extracted with ether (3 × 150 mL), dried, and then concentrated to obtain the crude product as a yellow oily liquid. Further purification by distillation under reduced pressure afforded pure (2*S*)-1-benzyl-2-methylaziridine, **10b**, as a colorless liquid (24.3 g, 82.5%). Bp: 71−72 °C at 4 mm (lit.:[14] 58−60 °C at 2 mm).

(2*S*,5*S*,8*S*,11*S*)-1,4,7,10-Tetrabenzyl-2,5,8,11-tetramethyl-1,4,7,-10-tetraazacyclododecane, 11b. To a solution of (2*S*)-1-benzyl-2-methylaziridine, **10b** (19.1 g, 130 mmol), in ethanol (250 mL) was added *p*-toluenesulfonic acid (PTSA, 1.1 g, 6.5 mmol), and the mixture was stirred at room temperature for 64 h. At the end of this time, an additional 1.1 g (6.5 mmol) of PTSA was added, and the mixture was stirred for a further 48 h. Ethanol was removed in vacuo, and the product was purified by column chromatography over silica gel (400 g) using methanol as the eluent. Fractions containing the pure product were combined, and solvent removal afforded the PTSA salt of **11b** (5.8 g; yield 23%). This product was dissolved in methanol (100 mL) and basified with a concentrated ammonia solution. The precipitated solid was filtered, dried, and recrystallized from absolute ethanol to obtain compound **11b** as a colorless microcrystalline solid (2.8 g, yield 14%). Mp: 147−48 °C. IR(KBr, cm^{-1}): 2971, 1452, 1403, 731 cm^{-1}. ^1H NMR ($CDCl_3$): 0.88 (12 H, d, J = 6.6 Hz, CH_3), 1.98 (4 H, dd, J_{vic} = 5.2 Hz, J_{gem} = 13.2 Hz, ring protons), 3.11 (4H, dd, J_{vic} = 7.9 Hz, J_{gem} = 13.2 Hz, ring protons), 3.29−3.49 (12 H, m, NCH and benzylic CH_2); 7.10−7.60 (20 H, aromatic). ^{13}C NMR ($CDCl_3$): 12.37 (CH_3), 51.12 (NCH), 51.62, and 54.49 (NCH_2 and benzylic CH_2), 126.33, 127.83, 128.91 (ArCH), and 140.93 (ArC). MS: 589, $(M + H)^+$. Anal. Calcd for $C_{40}H_{52}N_4$: C, 81.59; H, 8.90; N, 9.51%. Found: C, 81.65; H, 8.97; N, 9.55%.

(2*S*,5*S*,8*S*,11*S*)-2,5,8,11-Tetramethyl-1,4,7,10-tetraazacyclododecane, M4cyclen, 3. Method A: Catalytic Transfer Hydrogenolysis in Neutral Medium. To a solution of (2*S*,5*S*,8*S*,11*S*)-1,4,7,-10-tetrabenzyl-2,5,8,11-tetramethyl-1,4,7,10-tetraaza-cyclododecane, **11b**, (2.35 g, 4 mmol) in ethyl acetate (40 mL) and ethanol (400 mL) was added ammonium formate (2.52 g) and $Pd(OH)_2$ on carbon (20%, 2.35 g), and the mixture was stirred under reflux for 16 h. The solution was filtered to remove the catalyst, and the solvents were removed to obtain M4cyclen **3** as a light yellow solid (840 mg, yield 92%). Mp: 180−182 °C (decomp). IR (KBr, cm^{-1}): 3231, 2930, 1456, 1078 cm^{-1}. ^1H NMR (D_2O, ppm): 0.86 (d, 12H, CH_3); 2.24 (m, 4H, CH); 2.66−2.74 (m, 8H, CH_2). ^1H NMR ($CDCl_3$): 0.95 (12H, d, J = 5.94, CH_3), 2.3−2.8 (16H, m, NCH, NCH_2 and NH). ^{13}C NMR ($CDCl_3$): 18.25 (CH_3), 47.66 (NCH), 52.01 (NCH_2). MS: 229 $(M + H)^+$. HPLC: column PLRP-S; Cu method;[15] conditions 5% CH_3CN/50 mM $NH_4H_2PO_4$ (pH 4.5); UV at 290 nm; flow rate 0.5 mL/min.; t_R 7.7 min.

(2*S*,5*S*,8*S*,11*S*)-2,5,8,11-Tetramethyl-1,4,7,10-tetraazacyclododecane, M4cyclen, 3. Method B: Catalytic Hydrogenation in Acidic Medium. A solution of (2*S*,5*S*,8*S*,11*S*)-1,4,7,10-tetrabenzyl-2,5,8,11-tetramethyl-1,4,7,10-tetraaza-cyclododecane, **11b** (0.2 g, free base, 0.34 mmol), in methanol (20 mL) was treated with concentrated HCl (1 mL) and Pd/C (Pd content 10% by wt; 0.04 g). The solution was hydrogenated at 55 psi for 20 h. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure to a paste. The paste was crystallized from methanol to yield M4cyclen hydrochloride as a colorless solid. Yield: 0.094 g, mp > 200 °C (dec). To obtain the free amine, AG1-X2 resin (formate form, Biorad; 10 mL) was washed with H_2O (10×), 1 N NaOH (15×), and finally H_2O until the pH of the filtrate was 6. A sample of M4cyclen-HCl (50 mg) obtained as described was dissolved in H_2O (5 mL) and loaded onto the AG1-X2 column. The column was then eluted with H_2O. The fractions were checked by first adding 23 mM $CuCl_2$ solution to an aliquot and observing whether the blue colored Cu^{2+} complex was present. If this test was positive, the fraction was again analyzed by HPLC. The fractions containing the product were combined and lyophilized to obtain M4cyclen, **3**, as a white fluffy solid (30 mg; 42% overall yield). This compound was identical to the one obtained by method A.

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[(2*S*,5*S*,8*S*,11*S*)-4,7,10-Tris-carboxymethyl-2,5,8,11-tetramethyl-1,4,7,10-tetraazacyclododecan-1-yl]acetic Acid, M4DOTA, 4. To a solution of M4cyclen, **3** (570 mg, 2.5 mmol), in acetonitrile (200 mL) was added potassium carbonate (4.0 g) and *tert*-butyl bromoacetate (2.34 g, 12 mmol), and the mixture was stirred at room temperature for 18 h. The inorganic solid was filtered off, acetonitrile removed in vacuo, and the residue purified by column chromatography over silica gel (50 g), using chloroform/methanol mixtures, to obtain tetra-*tert*-butyl ester **13** (1.6 g). This intermediate was dissolved in TFA (150 mL), anisole (10 mL) was added, and the mixture was stirred at room temperature for 16 h. TFA was removed in vacuo, and anisole was removed by coevaporation with water (6 × 50 mL) to obtain the crude product. The residue was dissolved in water (100 mL) and purified by anion exchange column chromatography over AG1-X2 resin (150 mL). The column, after washing with water, was eluted with 1 M formic acid. The fractions were analyzed by HPLC,[15] and those containing the product were combined. Solvent removal followed by removal of traces of formic acid by coevaporation with water (5 × 50 mL) yielded M4DOTA (**4**) as a colorless glassy solid (550 mg, overall yield 48%). IR (KBr, cm^{-1}): 3500 (OH), 1680, 1632, 1379, 1252 cm^{-1}. ^1H NMR ($CDCl_3$): 0.95 and 1.25 (12 H, 2 broad doublets, CH_3); 2.80−3.75 (20 H, m, NCH and NCH_2). ^{13}C NMR ($CDCl_3$): 8.78 and 10.02 (CH_3); 48.35; 49.26; 49.36, 52.10, 53.79, and 56.93 (NCH_2, NCH_2-COOH and NCH); 170. 40 and 174.33 (COOH). MS: 461, (M + H); 499, $(M + K)^+$, 483 $(M + Na)^+$. Anal. Calcd for $C_{20}H_{36}N_4O_8 \cdot 2.3H_2O$ (water content determined by Karl Fisher titration): C, 47.78; H, 8.16; N, 11.14, O, 32.93%. Found: C, 47.51; H, 8.07; N, 10.70%.

(*R*)-2-[(2*S*,5*S*,8*S*,11*S*)-4,7,10-Tris-((*R*)-1-carboxyethyl)-2,5,8,-11-tetramethyl-1,4,7,10-tetraazacyclododecan-1-yl]propionic acid, M4DOTMA, 5. To a solution of M4cyclen, **3** (570 mg, 2.5 mmol), in acetonitrile (200 mL) was added potassium carbonate followed by (*S*)-2-trifluoromethanesulfonyloxypropionate benzyl ester[16] (3.9 g, 12.5 mmol), and the mixture was stirred at room temperature for 48 h. The inorganic salt was filtered off, acetonitrile was removed in vacuo, and the residue was purified by column chromatography over silica gel (100 g), using chloroform/methanol mixtures, to obtain the tetra-benzyl ester **14** (1.1 g). This material was dissolved in a mixture of ethanol (75 mL) and water (10 mL) and hydrogenated over 10% Pd/C (250 mg) for 18 h. The catalyst was filtered off and the solvent removed to obtain the crude product. This product was dissolved in water (100 mL) and purified by anion exchange column chromatography over AG1-X2 resin (150 mL). The column, after washing with water, was eluted with a gradient of 0−200 mM formic acid. The fractions were analyzed by HPLC, and those containing the product were combined. Solvent removal followed by removal of traces of formic acid by coevaporation with water (5 × 50 mL) furnished pure M4DOTMA, **5**, as a colorless glassy solid (340 mg, overall yield 26%). IR (KBr, cm^{-1}): 3500 (OH), 1680, 1632, 1379, 1252 cm^{-1}. ^1H NMR (D_2O, ppm): 0.79 (12H, d, J = 5.94, C*H*$_3$CHN); 1.18 (d, J = 6.60, 12H, C*H*$_3$-CHCOOH), 2.2−2.65 (m, 8H, NC*H*$_2$); 3.0−3.20 (4H, m, NC*H*); 3.3−3.5 (4H, q, J = 6.60, C*H*COOH). ^{13}C NMR (D_2O, ppm): 11.84 (CH_3), 16.86 (CH_3), 46.79 (NCH_2), 52.86 (NCH), 60.85 (CHCO), 183.13 (COOH). MS: 517, $(M + H)^+$; 539, $(M + Na)^+$. Anal. Calcd for $C_{24}H_{44}N_4O_8 \cdot 1.3H_2O$ (water content determined by Karl Fisher titration): C, 53.41; H, 8.70; N, 10.38. Found: C, 53.32; H, 9.13; N, 10.10%.

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[(2*S*,5*S*,8*S*,11*S*)-4,7-Bis-carboxymethyl-2,5,8,11-tetramethyl-1,4,7,10-tetraazacyclododecan-1-yl]acetic Acid, M4DO3A, 6. To a solution of M4cyclen, **3** (456 mg, 2 mmol), in acetonitrile (150 mL) was added sodium bicarbonate (4.0 g) and *tert*-butyl bromoacetate (2.34 g, 12 mmol), and the mixture was stirred at room temperature for 48 h. The inorganic salts were filtered off, and acetonitrile was removed in vacuo to obtain the tris-*tert*-butyl ester **15**. This intermediate was dissolved in TFA (25 mL), anisole (1 mL) was added, and the mixture was stirred at room temperature for 12 h. TFA was removed in vacuo, and anisole was eliminated by coevaporation with water (5 × 50 mL) to obtain a gummy residue. This crude product was dissolved in water (60 mL) and purified by anion exchange column chromatography over AG1-X2 resin (100 mL). The column, after washing with water, was eluted with a formic acid gradient (0 to 50 mM formic acid). The fractions were analyzed by HPLC, and those containing the product were combined. Solvent removal followed by elimination of traces of formic acid by coevaporation with water (5 × 50 mL) afforded M4-DO3A, **6**, as a colorless glassy solid (320 mg, overall yield 40%). IR (KBr, cm^{-1}): 3500 (OH), 1680, 1632, 1379, 1252 cm^{-1}. ^1H NMR ($CDCl_3$): 0.95−1.25 (12 H, m, CH_3); 2.20−3.85 (18H, m, NCH and NCH_2). MS: 403 (M + H); 425 $(M + Na)^+$. Anal. Calcd for $C_{20}H_{36}N_4O_8 \cdot 1.3H_2O$ (water content determined by Karl Fisher titration): C, 50.70; H, 8.66; N, 13.14. Found: C, 51.15; H, 8.43; N, 12.69; H_2O, 5.61%.

Results and Discussion

Synthesis of M4cyclen, 3. A direct and simple route to access the parent ring M4cyclen, **3**, was needed in order to achieve the synthesis of ligands **4**−**6**. On the basis of a literature survey, we decided that cyclo-tetramerization of suitably substituted aziridines offered the most facile entry into the tetra-methyl-substituted cyclen system in which the substituents are symmetrically located. Cyclo-tetramerization of *N*-benzyl-aziridine, **10a**, catalyzed by *p*-toluenesulfonic acid (PTSA), has been reported[17] to give 1,4,7,10-tetrabenzyl-1,4,7,10-tetraazacyclodecane, **11a**, in high yield. Ham et al.[18] also described the PTSA-catalyzed oligomerization of N-substituted aziridines to obtain the corresponding cyclic tetramers, along with lower and higher cyclic oligomers. Using $BF_3 \cdot Et_2O$ as the catalyst, Tsuboyama et al.[19] cyclo-tetramerized (*R*)-1-benzyl-2-ethylaziridine, **10c**, in 20−30% yields, depending on the solvent used. The oligomerization of *N*-benzyl-aziridine, **10a**, has also been reported by other authors,[20,21] and a mechanism has been proposed very recently for this reaction.[22] In light of these reports, we prepared[23] M4cyclen, **3**, as shown in Scheme 2. (*S*)-*N*-Benzoylalanine, **7**, was reduced with diborane in THF to obtain (2*S*)-2-(benzylamino)-propan-1-ol, **8**, in 90% yield. To avoid the use of diborane while working with large

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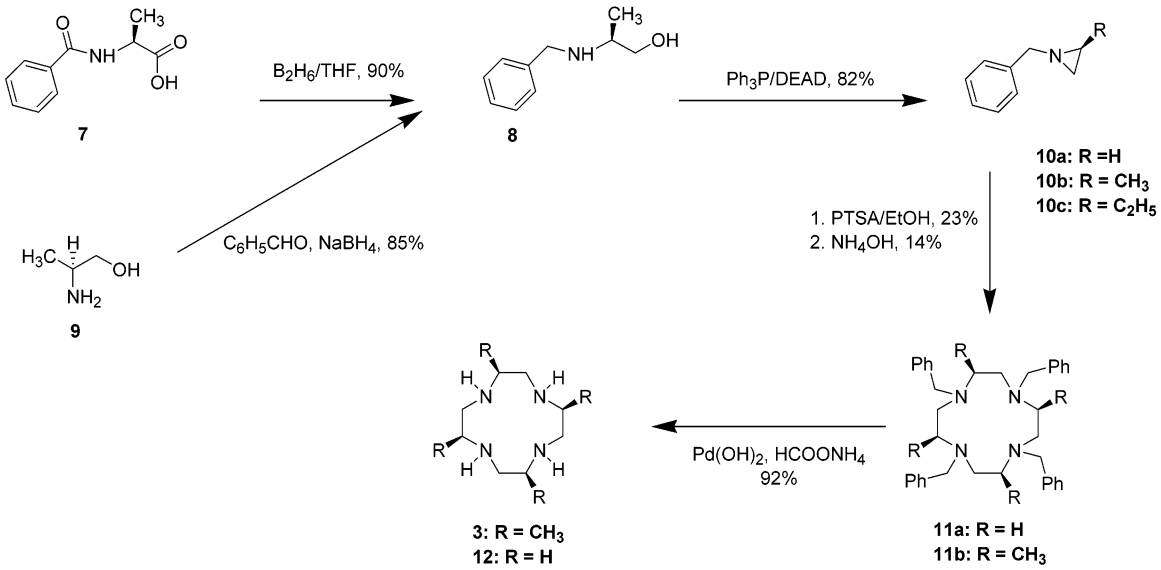
Scheme 2



quantities, we also prepared compound **8** by the reductive alkylation of (2*S*)-2-aminopropan-1-ol, **9**, with benzaldehyde in the presence of sodium borohydride in 87% yield. Intramolecular dehydration of **8** under Mitsunobu conditions using triphenylphosphine and diethyl azodicarboxylate provided (2*S*)-1-benzyl-2-methylaziridine, **10b**,[14] in 75% yield. PTSA-catalyzed cyclo-tetramerization of **10b** furnished a large number of oligomeric products, as per TLC, one of which had an R_f value similar to that of 1,4,7,10-tetrabenzyl-1,4,7,10-tetraazacyclododecane, **11a**. Isolation of this fraction by silica gel column chromatography furnished a product, presumed to be the mono-PTSA salt of (2*S*,5*S*,8*S*,11*S*)-1,4,7,-10-tetrabenzyl-2,5,8,11-tetramethyl-1,4,7,10-tetraazacyclododecane, **11b** (yield 23%). Neutralization of this salt by treatment with an excess of ammonia provided free base **11b** as a crystalline solid in 14% yield from **10b** after complete purification. While this work was in progress,[23] it was reported[22] that the cyclization of benzyl aziridine substituted by a hydroxymethyl group is improved by the addition of a photosensitizer but the reaction yields remain low whatever the procedure used. Catalytic hydrogenation of **11b** over Pd/C in methanol, in the presence of HCl, provided the tetrahydrochloride salt of M4cyclen, **3**, in 42% yield. Ion exchange over AG1-X2 resin (OH^- form) provided free base **3** in quantitative yield. This debenzylation reaction could also be accomplished by catalytic transfer hydrogenolysis of **11b** using $Pd(OH)_2$ on carbon and ammonium formate to obtain **3** as the free base in 92% yield.

NMR Spectra and pK_a of cyclen, 12, and M4cyclen, 3. In addition to the NH or the water peak, the room temperature ^{1}H NMR spectrum of cyclen, **12**, in DMF or D_2O exhibits a single peak at 2.6 ppm. This peak is due to the methylene groups that are all equivalent. There is thus a rapid equilibration among the various conformers of cyclen, and only an averaged spectrum is observable. The highly symmetrical nature of compound **3** is evidenced by its ^{13}C NMR spectrum in which the 12 sp^3 carbon atoms appear as three well resolved peaks at 18.25, 47.66, and 52.01 ppm, standing respectively for methyl, methylene, and methine carbon atoms. The room temperature ^{1}H NMR spectrum of M4cyclen, **3**, in D_2O, whether as a free base (Figure 1) or in the protonated form, shows a total of four resonances in addition to the HDO peak at 4.75 ppm. On the basis of the peak integration and the COSY spectrum (Figure S1 in the Supporting Information), the doublet at 0.9 ppm stands for the methyl protons, and the resonance at 2.8 ppm is clearly due to the CH proton (H_a in Scheme 3, top). The doublets of doublets at 2.3 and 2.7 ppm with *J*-couplings of 13.5 and 10.9 Hz and 13.5 and 2.4 Hz, respectively, are assigned to the methylene protons that are diastereotopic. The Karplus relationship[24] indicates that orienting two vicinal protons at a dihedral angle of 180° leads to one large $^3J_{ax-ax}$ and one small $^3J_{ax-eq}$ in addition to one large ^2J. A coupling pattern with one large ^2J and two small ^3J would be obtained for a gauche arrangement of the vicinal protons. The most stable conformations of **3** must thus essentially feature *trans* arrangements of the vicinal protons as in Scheme 3, top.

Approximate pK_a values of M4cyclen (**3**) were determined using ^{1}H NMR spectrometry and the computer program HYPNMR.[12] The ^{1}H NMR spectra for the two polyamines **3** and **12** were run at pH values in the range 7.5−11.7 in D_2O under N_2 at 22 °C adjusting pD using DCl or KOD and keeping the ionic strength at 80 mM using KCl. pD values were finally expressed as pH values using the relationship[11] pH = pD − 0.4. The data obtained are given in Figure S2 in the Supporting Information. Using the pK_a values reported[25] for cyclen, **12**, as starting values, the HYPNMR program could not generate any result in the case of M4cyclen, **3**. This program was then modified to set the pK_{a1} value as a constant in the range 12−13. With this modification, the program was able to generate a pK_{a2} value. Irrespective of the estimated pK_{a1} value entered, the pK_{a2} value always converged around 10.35−10.40. The best value

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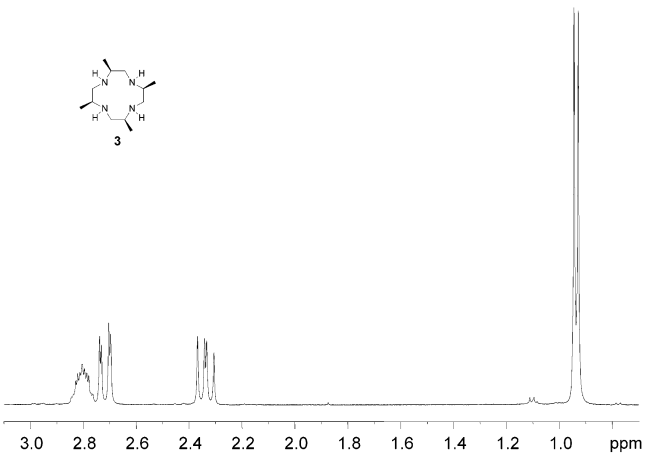
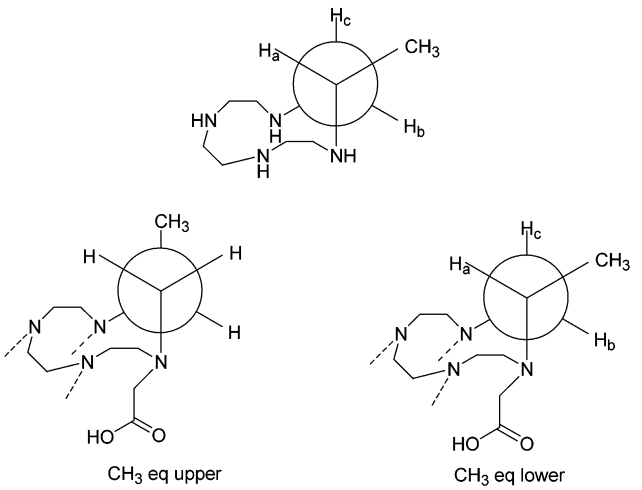


Figure 1. Proton NMR spectrum of unprotonated M4cyclen, **3**, in D_2O at 298 K.

Scheme 3



for the pK_{a2} value for M4cyclen, **3**, is 10.4 ± 0.2, which makes it a stronger base than cyclen, **12**. As mentioned later, this higher basicity has to be taken into account prior to the selection of an acid scavenger for the carboxyethylation of **3**.

Synthesis of Ligands M4DOTA, 4, M4DOTMA, 5, and M4DO3A, 6. Conversion of **3** into ligands **4**−**6** was achieved as depicted in Scheme 4. Tetra-carboxymethylation of **3** by treatment with excess *tert*-butyl bromoacetate in the presence of Na_2CO_3 in acetonitrile led to tetra-*tert*-butyl ester **13**, which upon deprotection with TFA, followed by ion-exchange purification, provided M4DOTA, **4**, in 48% overall yield. Tetra-1-carboxyethylation of **3** by treatment with (L)-benzyl 2-trifluoromethanesulfonyloxy-propionate[16] afforded tetra-benzyl ester **14** which was subjected to catalytic hydrogenolytic deprotection and ion-exchange purification to obtain M4DOTMA, **5**, in 26% overall yield. In attempting to prepare M4DO3A, **6**, we observed an interesting difference between cyclen, **12**, and M4cyclen, **3**, in their ability to undergo selective alkylation as a function of the base used. When using Na_2CO_3 as an acid scavenger in CH_3CN and a 6-fold excess of *tert*-butyl bromoacetate as the alkylating agent, both **3** and **12** behaved similarly giving rise to tetra-alkylated products in comparable yields. However, when $NaHCO_3$ was used as the acid scavenger, keeping the rest of the parameters unchanged, **12** gave 82% of the corresponding tetra-alkylated product, while **3** gave only a mixture of the di-alkylated (31%) and the tri-alkylated (**15**, 40%) products. No tetra-alkylated product could be detected. As discussed later, an explanation for these findings could rest in the difference in the basicity between **12** and **3**.

In the presence of $NaHCO_3$, it is likely that the stronger base, **3**, might exist predominantly in the diprotonated form, which presumably undergoes selective alkylation to the di- and tri-alkylated stages only. The weaker base, **12**, might exist predominantly in the monoprotonated form that is likely to undergo less selective alkylation to the tetra-alkylated stage. Such pH controlled selective reactions are not without precedence in the literature.[26] The end result of this finding was that we were able to uncover a direct route to prepare M4DO3A, **6**. TFA deprotection of tris-*tert*-butyl ester **15**, followed by ion-exchange purification, afforded compound **6** in 40% overall yield from **3**. In contradistinction, to prepare DO3A efficiently from **12**, a multistep protection-deprotection strategy had to be used.[27]

Conformational Studies on Ligands M4DOTA (4) and M4DOTMA (5) by NMR. The ^{1}H NMR spectrum of DOTA, **1**, in its tetra-protonated form is not very informative because it features only one acetate and one ethylenic peak at room temperature.[29] This ligand undergoes fast exchange processes that are somewhat slowed at 278 K and in strongly acidic media as shown in Figure S3. In these conditions, the pH is lower than 1, and DOTA is thus entirely in the H_6L^{2+} form where L designates the fully deprotonated ligand.[25,29] The ^{1}H spectrum then displays two broad acetate peaks that are partially overlapping with four large ethylenic resonances, all with the same relative area. Some degree of rigidification is thus brought about by the hexa-protonation of DOTA and by lowering the temperature, but the spectral resolution is still too poor to allow one to unravel the dynamic properties of the ligand. The ^{1}H spectrum of M4DOTA, **4**, in the H_4L form is better resolved and much more complex at room temperature (Figure S4) and is most amenable to analysis if DCl is added to reach pH < 1 as all resonances are well separated (see Figure 2). It should be emphasized here that the lowering of the pH does not seem to modify the number and global shapes of the resonances even if some overlappings are taking place in the H_4L form and not in the H_6L form. The COSY, HMQC, and NOESY spectra are reproduced in Figures S6−S9, and positive and negative peaks in the NOESY experiment are separated in Figures S8 and S9 to show separately exchange and nOe cross-peaks.[30] The methyl protons appear as two well-resolved doublets. Moreover, 10 acetate and ethylenic multiplets, all with the same relative area, are found between 2.5 and 4.3 ppm. The poorly

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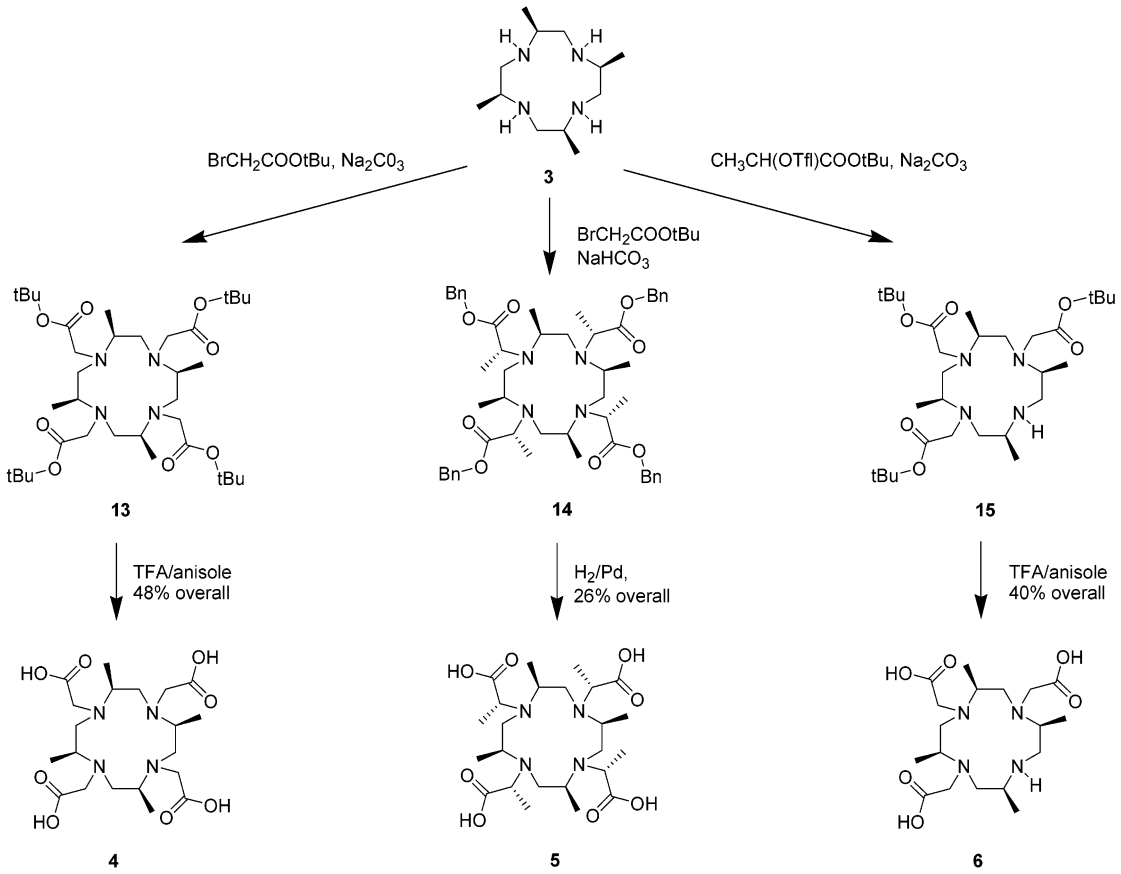
Scheme 4



resolved peaks at 3.78 and 3.46 ppm are coupled to the methyl peaks in the COSY spectrum and are thus assigned to methine protons. The COSY and EXSY spectra also clearly show the connections inside two different $CH-CH_2$ groups as shown in Figure 2. It thus appears that M4DOTA, **4**, adopts a geometry of 2-fold symmetry and not the 4-fold symmetry that is found for all lanthanide DOTA chelates[28] and in the solid state structure of DOTA[31] in the $H_5L^+ \cdot H_3O^+$ form. The 2-fold symmetry of hexa-protonated M4DOTA is also deduced from its ^{13}C spectrum as it features 10 intense resonances (Figure S5). Indeed, the methyl groups are displayed as two ^{13}C peaks at 11.6 and 12.8 ppm rather than as a single peak as anticipated for a fully symmetric configuration. In addition, four CH_2 and two CH peaks are found between 49 and 61 ppm (assignment based on the DEPT spectrum), and two C═O resonances are observed at 171.0 and 177.1 ppm. The HMQC spectrum (Figure S7) is in complete accord with the ^1H COSY and EXSY connections shown in Figure 2. As already noted for M4cyclen, the coupling pattern in the tetraaza ring depends on the orientation of the methyl group that can be either in a pseudoaxial or a pseudoequatorial position. The spectrum in Figure 2 obviously corresponds to the "CH_3 pseudoequatorial" solution, and the peak assignments are indicated accordingly in the caption of this figure. Indeed, two doublets of doublets with large coupling constants appear at 2.69 and 3.67 ppm (J = 12 and 16 Hz), and two other doublets of doublets with one large and one small J constant (J = 12 or 16 Hz and <3 Hz) are found at 2.9 and 3.3 ppm as should be expected for two different $-(CH_3)CH-CH_2-$ units with the methyl groups in the equatorial-like position.

Several weak resonances are also noted in the ^1H and ^{13}C spectra, and two rigid species thus seem to coexist in solution. The exact number of ^{13}C and ^1H resonances due to the minor isomer cannot be determined accurately because of peak overlappings, but one can safely assume that the numbers of intense and weak peaks are identical. Indeed, the minor species displays five small ^{13}C peaks between 49 and 61 ppm, and a sixth one is probably hidden under one of the major peaks. Moreover, a small ^{13}C methyl peak is found at 12.2 ppm, and a second one is probably also hidden under one of the two more intense methyl peaks. Finally, a small shoulder on the 177.1 ppm peak is due to one of the C═O groups of the minor species. Resonances originating from this species are also noted in the ^1H spectrum. For instance, each of the intense methyl resonances is accompanied at room temperature by a smaller partially overlapping broad peak at a somewhat lower field (see Figure 2). The low-field methyl doublet of the minor isomer is well resolved at 291 K, and the doublet of doublets due to one of its pseudoequatorial protons is clearly visible at 2.5 ppm (Figure S10).

The solution behavior of M4DOTA, **4**, is obviously not as simple as that of DOTA, **1**, as two topomers of protonated M4DOTA are clearly present in solution. No J couplings,

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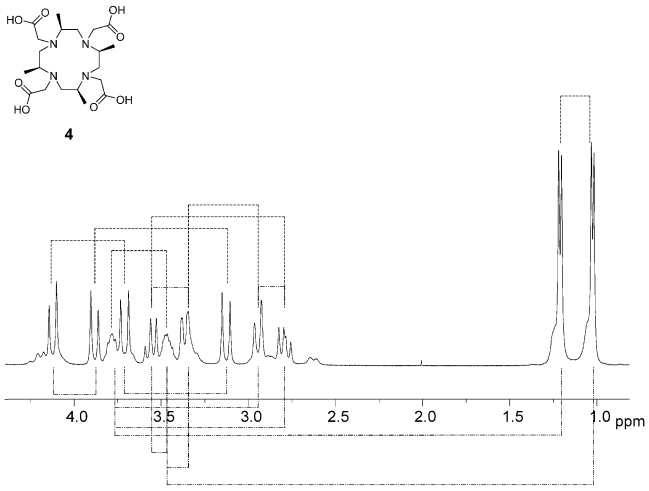

Figure 2. Proton NMR spectrum of M4DOTA, **4**, in acidic medium at 298 K. The COSY and EXSY connections are indicated by -••- and - - - - lines, respectively. The peak assignments from 4.1 to 2.6 ppm are ac, ac, C*H*-CH_3, ac, H_{ax}, CH, H_{eq}, ac, H_{eq}, H_{ax} (ac = acetate, eq = pseudoequatorial, ax = pseudoaxial).

exchange patterns, or nOe effects were found between the most intense and the weakest resonances. On the basis of the numbers of ^{1}H and ^{13}C resonances that are most probably the same for the minor and the major species, it thus appears that M4DOTA in the H_4L or H_6L^{2+} forms is present in aqueous solutions as two topomers differing by the orientation of the methyl groups that are either "eq upper" or "eq lower" relative to the acetate groups as shown in Scheme 3. There cannot be a distinction between these two positions in the case of M4cyclen, **3**, because of rapid inversion of the nitrogen atoms. However, the difference between the "eq upper" and "eq lower" positions of the methyl groups is clearly apparent in the NMR spectra of M4DOTA, **4**, that is more rigid presumably because of the protonation of two amine functions[29,31] and of hydrogen bonds between the carboxylic and amino groups.

One of the equatorial-like conformations of M4DOTA must be sterically more crowded than the other one and hence less abundant. At 313 K, the spectrum of the major topomer is still partially resolved while the peaks assigned to the minor isomer are extremely broad (Figure S11). At 363 K, rapid conformational exchanges start to take place in each isomer which then exhibits a well-resolved methyl doublet (Figure S12). An exchange is thus taking place at high temperature inside each topomer but not between them. In keeping with these measurements, EXSY peaks are observed between the two intense methyl peaks and between the two weak ones but not between the two types (Figure S8). Finally, it is noteworthy that the two major H_{ax} peaks are exchanging between themselves, as are the two major H_{eq} resonances. By contrast, an exchange is taking place between axial and equatorial protons in the axially symmetric DOTA metal chelates.[28] The exchange patterns are thus totally different in the metal DOTA complexes and in the protonated M4DOTA ligand.

In the solid state, diprotonated DOTA adopts a square [3.3.3.3] conformation[28] of 4-fold symmetry with all the carboxylic arms located on the same side of the tetra-aza ring.[31] Such a highly symmetrical geometrical arrangement could not lead to the NMR spectra recorded for M4DOTA as the ^{1}H and ^{13}C NMR spectra of each isomer would feature twice fewer resonances. Moreover $H_{ax}-H_{eq}$ exchanges would be observed rather than the $H_{ax}-H_{ax}$ and $H_{eq}-H_{eq}$ exchanges noted in the COSY spectrum of M4DOTA. An elongated conformation of this ligand in acidic media accounts for the spectroscopic observations. As shown in Figure 3, an elongation of the tetraazacyclododecane ring leads to a C_2 symmetry arrangement that features two different types of pseudoaxial, pseudoequatorial, and methyl protons and thus the number of ^{1}H and ^{13}C resonances observed in the NMR spectra. Moreover, a breathing movement, that is, an exchange between two identical forms elongated in perpendicular directions, brings about an exchange between protons of the same type (ax−ax and eq−eq) as indeed found in the EXSY spectra. It is noteworthy that an elongated conformation of the 12-membered cycles is not unknown: molecular modeling analyses indicate that the lowest energy conformers of the diprotonated form of cyclen[32] and even of unprotonated cyclen[33] are not exact S_4 geometries but rather C_2 arrangements elongated along a line joining two CH_2 groups as in Figure 3. Furthermore, uncomplexed 1,4,7,10-tetraoxacyclododecane in the solid state does not adopt the expected [3.3.3.3] conformation but rather an elongated [6.6] conformation.[34−35] Molecular mechanics calculations in the gas phase at the MM2 level indicate that the strain energy of the elongated form shown in Figure 3 follows the following order: methyl groups in the position "eq upper" ≈ "eq lower" ≪ "ax lower" ≪ "ax upper". The axial-like positions are sterically so crowded that no elongated topomer with methyl groups in these positions could exist. Exchanges between the methyl groups in the "eq upper" and "eq lower" positions are totally prevented at room temperature because this would imply a nitrogen inversion that is thwarted by hydrogen bonds in the ligands and by the rigidification effect of the methyl groups. Two topomers with pseudoequatorial methyl groups either "upper" or "lower" thus coexist in

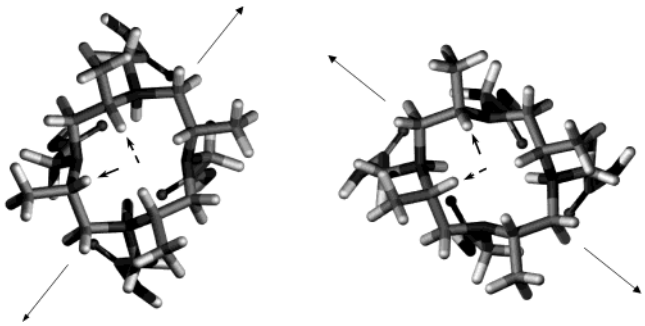

Figure 3. View of the geometries proposed for the rapidly exchanging elongated forms of M4DOTA, **4**, (*SSSS* isomer with the methyl groups in the "eq lower" position). The solid and dashed arrows show the two types of "ax upper" protons that are exchanging.

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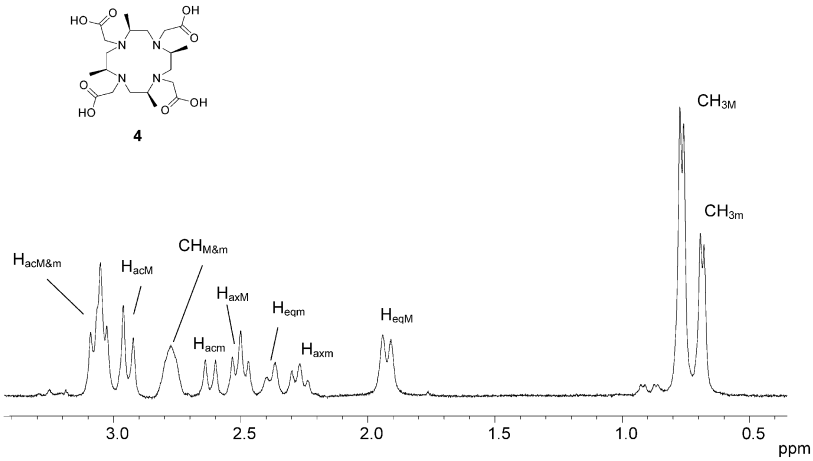

Figure 4. Proton NMR spectrum of M4DOTA, **4**, in basic (LiOD) medium at 298 K. M = major isomer; m = minor isomer (the small peaks at ≈ 0.9 ppm and at low fields are due to an impurity).

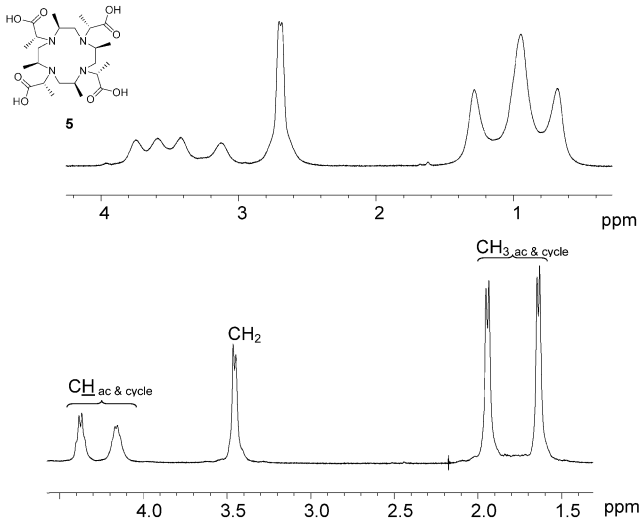

Figure 5. Proton NMR spectrum of tetraprotonated M4DOTMA, **5**, at 273 K (top) and 333 K (bottom).

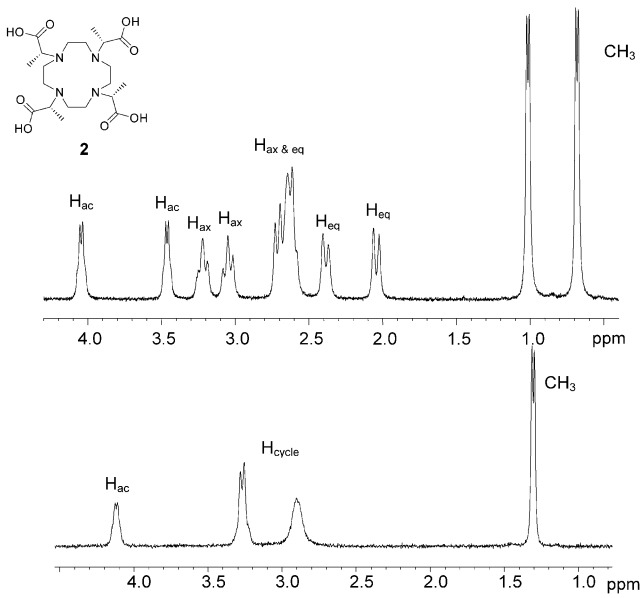

Figure 6. Proton NMR spectrum of DOTMA, **2**, at low acidity and at 278 K (top) or 368 K (bottom).

solution and are not involved in intermolecular exchange processes. One cannot reliably assign one of these conformations to one of the topomers on the basis of the simple molecular modeling calculations and the NMR data presently available. The rigidity of these topomers probably arises from hydrogen bonds as confirmed by the drastic changes brought about by the addition of LiOD to a solution of protonated M4DOTA (Figure 4). A major and a minor species are still present, but the number of their ^{1}H and ^{13}C resonances (Figure S13) is halved as either a rapid breathing movement leads to an averaged spectrum for each isomer or axially symmetric conformations are adopted. In both cases, an exchange between the methyl groups in "eq upper" and "eq lower" positions remains hindered.

The proton spectra of M4DOTMA (**5**) in the tetraprotonated form at 273 K and at 333 K are presented in Figure 5. At 273 K, there are four partially overlapping broad resonances at high fields that are due to the methyl groups and four broad peaks at low fields that are due to the methine protons. Finally, the peak at 2.70 ppm is assigned to the ring methylene protons that are all resonating at about the same frequency. This spectrum greatly simplifies when the temperature is increased, and at 333 K, the spectrum features two methyl doublets, two enlarged methine quartets, and one broad methylene resonance. The peaks are broad at all temperatures below 350 K, and it is thus difficult to ascertain whether both the "eq upper" and "eq lower" isomers are present in solution. However, one of the two isomers is clearly the major species in solution and undergoes a slow exchange at room temperature between two elongated conformations as in the case of the M4DOTA ligand.

The question then arises as to whether methyl substituents on the tetraaza ring of DOTA are required to obtain rigidified elongated conformations in solution. An analysis of the NMR spectra of the DOTMA ligand, **2**, clearly indicates that an elongated arrangement is also adopted if methyl substituents are located on the acetate arms. The ^{1}H spectrum of DOTMA, **2**, in the H_4L form features single broad methine and methyl resonances and three featureless methylene peaks (Figure S14). The spectral resolution is considerably improved if the pH is lowered below 1 as shown in Figure 6, and the assignment of the resonances is easily made by taking

into account the splitting patterns and the COSY cross-peaks. The spectrum at 278 K displays two methyl peaks, two methine peaks, and eight methylene peaks, four of which are overlapping. The ^{13}C spectrum shows two methyl and two C═O resonances, and DOTMA thus adopts a conformation of 2-fold symmetry. In addition, exchange cross-peaks between the two methyl peaks and between the two methine peaks are noted in the EXSY spectrum as must be expected for an elongated structure of the type illustrated in Figure 3 undergoing a breathing movement. However, the exchange peaks between the methylene protons are very weak and difficult to interpret because of spectral overlappings. Raising the temperature to 368 K accelerates the breathing movement, and the spectrum simplifies as already observed for the other methylated DOTA ligands. For instance, a single methyl doublet occurs at 1.3 ppm as shown in Figure 6.

Conclusion

The ligand DOTA is probably one of the most thoroughly studied chelating agents.[3] Its tetraaza ring has been substituted in a number of ways, for instance, by replacing the acetate groups by amide or phosphonate moieties or by adding anchor groups for tethering to macromolecules. However, the synthesis of DOTA derivatives featuring four or eight stereogenic centers on the macrocyclic ring required the development of new synthetic methods.[22,23] The synthesis of polymethylated DOTA ligands is described here, and the conformational properties of these ligands are investigated by NMR. The rigidification brought about by substitution on the macrocyclic ring and/or on the acetate arms allows one to obtain conformational information that could not be deduced from a study of the parent unsubstituted DOTA ligand. The preferred geometry in solution is an elongated conformation of 2-fold symmetry that undergoes a slow breathing movement. Although a detailed analysis could not be carried out in the case of DOTA, it is very likely that this ligand also adopts an elongated geometry in solution even if the solid state structure is axially symmetric.[31]

Acknowledgment. We gratefully acknowledge the assistance from Dr. Alberto Vacca for the HYPNMR program. J.F.D. and V.J. gratefully acknowledge the Fonds National de la Recherche Scientifique and the Institut Interunivesitaire des Sciences Nucléaires of Belgium for their financial support. V. J. is Chercheur Qualifié at the FNRS. J.F.D. and V. J. also thank COST D18 for support.

Supporting Information Available: ^{1}H and ^{13}C spectra of **2**−**5** in different conditions of acidity and temperature. COSY, HMQC, EXSY, and NOESY spectra of **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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