Alkaline Earth Metal and Lanthanide(III) Complexes of Ligands Based upon 1,4,7,10-Tetraazacyclododecane-1,7-bis(acetic acid)

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The macrocyclic ligand DO2A (1,4,7,10-tetraazacyclododecane-1,7-bis(acetic acid)) was prepared and used as a building block for four new macrocyclic ligands having mixed side-chain chelating groups. These ligands and their complexes with Mg^{II}, Ca^{II}, and Ln^{III} were studied extensively by potentiometry, high-resolution NMR, and water proton relaxivity measurements. The protonation constants of all compounds compared well with those of other cyclen-based macrocyclic ligands. All Ca^{II} complexes were found to be more stable than the corresponding Mg^{II} complexes. Trends for the stabilities of the Ln^{III} complexes are discussed and compared with literature data, incorporating the effects of water coordination numbers, Ln^{III} contraction, and the nature of the side chains and the steric hindrance between them. ^1H NMR titrations of DO2A revealed that the first and second protonations take place preferentially at the secondary ring nitrogens, while the third and fourth involved protonation of the acetates. ^{17}O NMR shifts showed that the $DyDO2A^+$ complex had two inner-sphere water molecules. Water proton spin−lattice relaxation rates for the $GdDO2A^+$ complex were also consistent with water exchange between bulk water and two inner-sphere Gd^{III} coordination positions. Upon formation of the diamagnetic complexes of DO2A (Ca^{II}, Mg^{II}, La^{III}, and Lu^{III}), all of the macrocyclic ring protons became nonequivalent due to slow conformational rearrangements, while the signals for the acetate CH_2 protons remained a singlet.

Introduction

Recent interest in polyazamacrocyclic paramagnetic and radioactive metal ion chelates largely results from their biomedical applications such as (i) magnetic resonance imaging (MRI) contrast agents,[1,2] (ii) shift reagents for NMR-active cations,[3] and (iii) diagnostic and therapeutic radiopharmaceuticals.[4] As a result of intensive investigations of the chemical (thermodynamic, kinetic, structural, spectral, and electrochemical) and pharmacological properties of macrocyclic complexes, a number of paramagnetic Gd^{III} chelates are now used clinically as MRI contrast agents[5] and some $^{90}Y^{III}$ chelates are useful bioconjugates for monoclonal antibody radioactive labeling.[4,6−8]

The ligand DOTA (see Figure 1), derived from 1,4,7,10-tetraazacyclododecane (cyclen), forms one of the most thermodynamically stable and kinetically inert complexes with the trivalent lanthanide cations of any known chelate.[9−11] These properties make $GdDOTA^-$ one of the most effective and safest MRI contrast enhancement[5] agents available. $GdDOTA^-$, like all current commercially available MRI contrast agents, is a nonspecific extracellular agent, also known as "perfusion agent", which distributes throughout all extracellular space before being

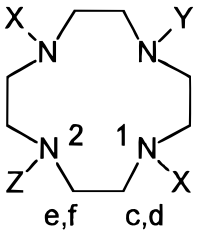

Figure 1. Macrocyclic ligands discussed in this study. Cyclen: X = Y = Z = H. DOTEP: X = Y = Z = $CH_2P(O)(Et)O^-$. X = CH_2COO^- for the following. DO2A: Y = Z = H. DO3A: Y = CH_2COO^-, Z = H. DOTA: Y = Z = CH_2COO^-. DO2A-2HE: Y = Z = $(CH_2)_2OH$. DO2A-2HP: Y = Z = $CH_2CH(CH_3)OH$. DO3A-HP: Y = CH_2COO^-, Z = $CH_2CH(CH_3)OH$. DO2A-2PME: Y = Z = $CH_2P(O)(OEt)O^-$. DO2A-2EP: Y = Z = $CH_2P(O)(Et)O^-$. Numbering is for DO2A (a,b: CH_2COO^- protons).

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excreted through the kidneys. This lack of tissue specificity means that a relatively high dosage of such perfusion agents is required for significant MRI contrast enhancement. The relatively high osmolality of $GdDOTA^-$ and other ionic contrast agents led to the development of neutral low-osmolality agents, which can be safely used at higher dose levels.[12,13] The nonionic Gd^{III} complex of DO3A-HP, a derivative of the heptadentate ligand DO3A, has favorable thermodynamic and kinetic properties[14,15] and is used clinically as an MRI agent.

A lower dose could be employed if the contrast agent were delivered to a specific organ, tissue, or cell type, resulting in a high localized concentration. The development of organ/tissue-specific radiopharmaceutical agents allows diagnostic evaluation of hepatobiliary and kidney function and brain, myocardial, and bone imaging,[16,17] mostly using ^{99}Tc-based complexes,[3,18−20] while radiotherapeutic agents involving $^{90}Y^{III}$-labeled monoclonal antibodies have also been developed.[4−8] Tissue-specific delivery of Gd^{III}-based MRI contrast agents could be designed through variation of the lipophilicity, molecular weight, and ionic charge of the chelate.[18−20]

Previous reports have described the synthesis, physicochemical characterization,[21] and biodistribution studies[22] of positively charged Gd^{III} chelates, which show promising bone-targeted specificity. One of them is the hexadentate DOTA analogue DO2A (see Figure 1). Preliminary reports have appeared describing the synthesis of DO2A[21] and some of the properties[22,23] of $GdDO2A^+$. The present work describes in detail the synthesis of the ligand DO2A, the characterization of its acid−base properties, and the thermodynamic stability constants and some structural features of its Mg^{II}, Ca^{II}, and Ln^{III} (La^{III}, Gd^{III}, Dy^{III}, Yb^{III}, and Lu^{III}) chelates in solution using potentiometry, multinuclear NMR spectroscopy, and water proton relaxometry. These properties are compared with those of the parent compound, cyclen, and of related tetraazamacrocyclic polycarboxylates, DO3A and DOTA. The effects of changing the number of macrocyclic pendant acetate arms on those chelate properties are also discussed. Furthermore, DO2A has been used as a building block for the synthesis of several new macrocyclic ligands with two acetate and two other side-chain chelating groups. These compounds are useful as potential ligands for MRI contrast agents, providing either positively charged Gd^{III} complexes using neutral side chains, such as hydroxyethyl (as in DO2A-2HE; see Figure 1) or hydroxypropyl (DO2A-2HP), or negatively charged complexes using negatively charged side chains, such as ethyl methylenephosphonate (DO2A-2PME) or methyleneethylphosphinate (DO2A-2EP). Their thermodynamic characteristics with Mg^{II}, Ca^{II}, and Gd^{III} were determined and compared with literature data on similar compounds.

Experimental Section

General Procedures and Reagents. 1,4,7,10-Tetraazacyclododecane (cyclen) tetrahydrochloride was purchased from Parish Chemical Co. Cation exchange resins, (*S*)-(−)-propylene oxide, phosphorous acid, paraformaldehyde, 37% w/w formaldehyde, diethyl phosphite, dichloroethylphosphine, gadolinium chloride hexahydrate, standardized disodium dihydrogen ethylenediaminetetraacetate (0.0499 M), xylenol orange, anhydrous solvents, D_2O, NaOD, and all other standard reagents were obtained from Aldrich Chemical Co. Volumetric standard solutions of NaOH (1.0 M), HCl (0.1 M), and KOH (0.1 M) were obtained from Ricca Chemical Co., Arlington, TX. Filtrations and extractions of air-sensitive or hygroscopic compounds were accomplished with a Schlenk-type apparatus under a nitrogen atmosphere. Infrared spectra were recorded on a Mattson 2025 FT-IR spectrometer. Elemental analyses were obtained from either Galbraith Laboratories, Inc., or Oneida Research Services, Inc.

Syntheses. The derivatives 1,7-bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane and 1,7-bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane-4,10-bis(acetic acid *tert*-butyl ester) were prepared as described previously.[21]

1,4,7,10-Tetraazacyclododecane-1,7-bis(acetic acid) (DO2A). 1,7-Bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane-4,10-bis(acetic acid *tert*-butyl ester) (4.21 g, 6.30 mmol) was dissolved in 100 mL of 20% hydrochloric acid and refluxed for 1 day. The hydrochloric acid was removed by rotary evaporation to give a white solid. Absolute ethanol (50 mL) and then ether (10 mL) were added, and the white precipitate was filtered off onto a Schlenk filter, washed with an ethanol−ether (1:1) mixture (20 mL) and with ether (3 × 20 mL), and then dried in a stream of nitrogen to give 2.30 g (90%) of a white solid. Anal. Calcd (found) for $C_{12}H_{24}N_4O_4 \cdot 2.78HCl \cdot 1.11H_2O$ (fw = 409.47): C, 35.20 (35.12); H, 7.13 (7.12); N, 13.68 (13.72); Cl, 24.03 (24.03). ^1H NMR (D_2O), δ (ppm): 3.57 (s, 4H, CH_2COOH), 3.22, 3.10, 2.95 (br, 16H, NCH_2CH_2N). ^{13}C NMR (D_2O), δ (ppm): 176.45 (CH_2COOH), 55.31 (CH_2COOH), 50.78 (CH_2NCH_2COOH), 44.32 ($HNCH_2$).

4,10-Bis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane-1,7-bis(acetic acid) (DO2A-2HE). DO2A (729 mg, 1.88 mmol) was dissolved in 5 mL of water, with stirring, and the pH was adjusted to between 10.5 and 11.0 by addition of NaOH (5.4 mL of 1.483 M, 8.0 mmol). The reaction mixture was cooled to 2−5 °C, and 0.215 mL (4.3 mmol, 14% excess) of ethylene oxide was added. After slow warming to room temperature, the reaction course was followed by ^1H NMR. After 4 h, no DO2A remained. The mixture was acidified with hydrochloric acid to pH 1.0 and evaporated to near dryness *in vacuo* to remove the excess ethylene oxide. The product was dissolved in 50 mL of distilled water, and the solution was loaded onto a 30 mL 50X8-200 cation exchange column (2.8 cm column diameter). The column was eluted consecutively with 100 mL of distilled water, 100 mL of 0.5 M HCl, 100 mL of 1.0 M HCl, 100 mL of 1.5 M HCl, and 1000 mL of 2.0 M HCl. The product came off in the 2.0 M HCl fractions, while the earlier fractions contained inorganic salts (NaCl) and impurities. All fractions containing product were evaporated to dryness *in vacuo* and coevaporated three times with 250 mL of distilled water to remove the excess HCl. The product was lyophilized to yield 750 mg (1.44 mmol, 76.3%) of pure DO2A-2HE as a powder. Anal. Calcd (found) for $C_{16}H_{32}N_4O_6 \cdot 4HCl$ (fw = 522.31): C, 36.79 (36.74); H, 6.95 (6.94); N, 10.72 (10.23). ^1H NMR (D_2O/TSP), δ (ppm): 3.98 (t, 4H, NCH_2CH_2OH, $^3J_{HH}$ = 7 Hz), 3.61−3.44 (m, 20H, ring CH_2 and NCH_2COOH), 3.18 (t, 4H, NCH_2CH_2OH). ^{13}C NMR (D_2O/TSP), δ (ppm): 173.03 (COOH), 54.00, 53.79 (NCH_2COOH, NCH_2CH_2OH), 52.04 (NCH_2CH_2OH), 49.53, 46.93 (ring CH_2).

4,10-Bis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,7-bis(acetic acid) (DO2A-2HP). A 1.084 g (2.803 mmol) sample of DO2A was dissolved in a 1.483 M NaOH solution (8.0 mL, 11.86 mmol), yielding a pH of 11.0. The reaction mixture was cooled to 5 °C, and 0.450 mL (373 mg, 6.42 mmol, 15% excess) of (*S*)-(−)-propylene oxide was added. The reaction mixture was stirred for 6 h at room temperature. A ^1H NMR spectrum of the reaction mixture

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was recorded every hour until the DO2A ring proton resonance at 2.61 ppm had disappeared. The crude product was purified on a 50X8-200 cation exchange column (36 mL of resin, 2.8 cm column diameter). The column was eluted consecutively with 120 mL of water, 120 mL of 0.5 M HCl, 120 mL of 1.0 M HCl, 120 mL of 1.5 M HCl, 500 mL of 2.0 M HCl, and 250 mL of 2.5 M HCl. The product came off in the 2.0 and 2.5 M HCl fractions. All fractions containing the product were evaporated to dryness *in vacuo* and coevaporated three times with 250 mL of water to remove the excess HCl. Lyophilization gave 1.234 g (2.383 mmol, 85.0%) of pure DO2A-2HP as a powder. Anal. Calcd (found) for $C_{18}H_{36}N_4O_6 \cdot 3.11HCl$ (fw = 517.74): C, 41.77 (41.73); H, 7.62 (7.61); N, 10.83 (10.37). ^{1}H NMR (D_2O/TSP), δ (ppm): 4.27 (m, 2H, $NCH_2CH(CH_3)OH$), 3.61 (d, 4H, $NCH_2CH(CH_3)OH$, $^3J_{HH}$ = 10 Hz), 3.54 (s, 4H, NCH_2COOH), 3.34−3.19 (br m, 16H, ring CH_2), 1.21 (d, 6H, $NCH_2CH(CH_3)OH$). ^{13}C NMR (D_2O/TSP, pD = 11), δ (ppm): 181.00 (COOH), 66.74, 65.92 ($NCH_2CH(CH_3)OH$), 61.42 (NCH_2COOH), 52.43−51.40 (br, ring CH_2), 22.86 ($NCH_2CH(CH_3)$-OH).

1,4,7,10-Tetraazacyclododecane-1,7-bis(acetic acid)-4,10-bis(ethyl methylenephosphonate) (DO2A-2PME). A 926 mg (2.39 mmol) sample of DO2A was dissolved in 10 mL of water and the pH adjusted to 10.6 by addition of 1 M NaOH. Water was removed *in vacuo* and the product dried under high vacuum for 3 h. The resulting product was dissolved in 2.512 mL (33.52 mmol, 600% excess) of 37% formaldehyde, and the solution was stirred for 30 min at room temperature. A 1.260 mL (9.585 mmol, 100% excess) portion of diethyl phosphite was then added to the reaction mixture and stirred for 16 h at room temperature. The product was evaporated to dryness and coevaporated with water to remove the excess formaldehyde. The mixture, dissolved in 20 mL of water, was washed three times with 50 mL of methylene chloride and five times with 25 mL of diethyl ether to remove the excess diethyl phosphite. The aqueous layer contained the diethyl ester product, which was hydrolyzed to the monoethyl ester with 25 mL of 1 M NaOH (522% excess) under reflux until judged complete by NMR (4 h). The product was loaded onto a 50X4-200 cation exchange column (100 mL) and eluted with 500 mL of water followed by 250 mL of 0.5 M HCl. The fractions containing the product were combined, and the solvent was removed *in vacuo*. After coevaporation with three 250 mL portions of water to remove excess HCl, the product was redissolved in water and precipitated by addition of ethanol. After filtration and washing with diethyl ether, 624 mg (1.17 mmol, 48.9%) of pure DO2A-2PME was obtained as a white, hygroscopic powder. Anal. Calcd (found) for $C_{18}H_{38}N_4O_{10}P_2$ (fw = 532.46): C, 40.60 (40.51); H, 7.19 (7.18); N, 10.52 (10.21). ^{1}H NMR (D_2O), δ (ppm): 3.98 (qn, 4H, $P(O)OCH_2CH_3$, $^3J_{HH} = {}^3J_{PH} = 6.5$ Hz), 3.65−3.08 (br m, 24H, ring CH_2, NCH_2COOH, and NCH_2P), 1.31 (t, 6H, $P(O)OCH_2CH_3$). ^{13}C NMR (D_2O/TSP), δ (ppm): 175.45 (COOH), 65.09 ($P(O)OCH_2CH_3$, $^2J_{PC}$ = 6 Hz), 54.72 (NCH_2COOH), 51.38, 43.79 (ring CH_2), 48.64 (NCH_2P, $^1J_{CP}$ = 148 Hz), 16.36 ($P(O)OCH_2CH_3$).

1, 4, 7, 10-Tetraazacyclododecane-1, 7-bis(acetic acid)-4, 10-bis-(methyleneethylphosphinate) (DO2A-2EP). With vigorous stirring, 2.1 mL (20.2 mmol) of dichloroethylphosphine was added dropwise to 4.2 mL of cold (5 °C) water. The reaction mixture was allowed to warm to room temperature. The presence of ethylphosphinic acid was confirmed by ^{1}H NMR. In a 50 mL three-neck round-bottom flask fitted with a reflux condenser, a stirring bar, an inlet and an outlet for nitrogen, and addition tubing, DO2A (915 mg, 2.37 mmol) was dissolved in the freshly prepared ethylphosphinic acid solution. The reaction mixture was refluxed gently, and 6.6 mL (34.5 mmol) of a paraformaldehyde solution (157 mg/mL, 6.0 M HCl) was added at a rate of 0.55 mL/h over 12 h, after which the reaction mixture was refluxed for an additional 6 h. The reaction mixture was cooled to room temperature, evaporated to dryness, and coevaporated three times with 100 mL of water to remove the excess HCl. The crude product was purified by passing it over a column of 150 mL 50X4-200 cation exchange resin (2.8 cm column diameter), eluted with 250 mL of distilled water followed by 250 mL of 0.66 M HCl. The fractions containing the product (by ^{1}H NMR) were evaporated to dryness. After uptake in water, the product was precipitated by addition of ethanol. The product was collected by filtration under nitrogen. After being washed once with 10 mL of ethanol and three times with 15 mL of diethyl ether, the product was dried *in vacuo* to yield 486 mg (0.905 mmol, 38.2%) of pure DO2A-2EP as a white powder. Anal. Calcd (found) for $C_{18}H_{38}N_4O_8P_2 \cdot HCl$ (fw = 536.92): C, 40.26 (40.21); H, 7.32 (7.31); N, 10.44 (10.19). ^{1}H NMR (D_2O/TSP), δ (ppm): 3.83 (s, 4H, NCH_2COOH), 3.27 (d, 4H, $NCH_2P(Et)(O)OH$, $^2J_{PH}$ = 10 Hz), 3.18−2.95 (br m, 16H, ring CH_2), 1.53 (dq, 4H, $NCH_2P(O)(OH)CH_2CH_3$), 0.75 (dt, 6H, $NCH_2P(O)(OH)CH_2CH_3$, $^3J_{PH}$ = 14 Hz, $^3J_{HH}$ = 8 Hz).

Potentiometry. All potentiometric titrations were performed in a jacketed vessel at 25.0 ± 0.1 °C under a N_2 atmosphere. The ionic strength of all samples was adjusted to 0.1 M prior to titration using Me_4NCl or KCl. Hydrogen ion concentrations were calculated from the measured pH values using a pK_w of 13.81 and a H^+ activity coefficient of 0.82, determined in separate titrations containing known amounts of acid or base. Unless stated otherwise, the titrations were evaluated using a spreadsheet program described previously.[24] All titrations were performed at least twice.

The protonation constants of DO2A were determined by potentiometric titration of 5 mM DO2A (I = 0.1 M, Me_4NCl or KCl) with either 0.1 M Me_4NOH or 0.1 M KOH. Since all ligands in this study form complexes with Ca^{II} and Mg^{II} relatively quickly, the stability constants of these systems could be determined by direct potentiometric titration of 1:1 metal:ligand solutions. Such titrations were carried out on 5−10 mL solutions containing 1.5−2.5 mM ligand and metal ion (three separate titrations for each metal ion). The stability constant of CaDO2A was also determined by titrating a solution containing 5 mM $(Me_4N)_2$DO2A and 5.5 mM $CaCl_2$ with 0.1 M HCl. In this case, sample equilibration required about 2−5 min after each addition of acid.

Gd^{III}, however, forms complexes with these ligands too slowly for direct potentiometric titration. Therefore, these stability constants were evaluated in two different ways. First, an "out-of-cell" potentiometric titration was performed by preparing 15 samples of Gd^{III} and ligand and adding different amounts of 0.1 M KOH to each sample. The pH was measured in each sample daily until no further changes were detected, indicating that the sample had reached equilibrium (7−10 days). The volume of KOH added to each sample and the final pH readings were used to calculate the stability constants with a Simplex/Marquardt algorithm program.[25] The stability constants of $LnDO2A^+$ (Ln = La^{III}, Gd^{III}, Yb^{III}) were also determined in potentiometric competition experiments in which 2.0 mM $LnCl_3$ + 2.0 mM $(Me_4N)_2$-DO2A + 2.0 mM $(Me_4N)_4$EDTA was titrated with 0.1 M HCl. The protonation constants of $EDTA^{4-}$ as well as the stability constants of $LnEDTA^-$ in 0.1 M Me_4NCl were obtained from the literature.[26] These titrations were performed with allowance of up to 15 min equilibration after each addition of titrant, but equilibration was generally reached within 5 min.

NMR Experiments. NMR spectra were obtained on either a Varian Unity 500, a Bruker (GE) GN-500, or a JEOL FX-200 spectrometer. Probe temperatures in all instruments were accurate to ±1 °C. ^{1}H NMR spectra were recorded in $CDCl_3$ (*versus* TMS) or D_2O (*versus* sodium 3-(trimethylsilyl)propanesulfonate (TSP) or HDO (4.80 ppm)). ^{13}C-{^{1}H} NMR spectra were recorded in $CDCl_3$ (*versus* $CDCl_3$ (77 ppm)) or D_2O (*versus* TSP (0 ppm) or CH_3 of *tert*-butyl alcohol (31.2 ppm)).

Solutions of DO2A (0.02 M) for NMR pH titrations were prepared in D_2O, and the pD was adjusted with DCl, NaOD, or a 1.106 M standardized KOD solution. The final pH was determined with a Crison 2002 micro-pH meter fitted with a combined Ingold 405 M3 microelectrode and calibrated at 21 ± 0.5 °C with two standard buffers at pH 4.000 and 7.020 and corrected for the deuterium isotope effect using pH = pD − 0.4.[27,28] The ^{1}H NMR spectra were recorded as a function of added base using a single solution below pH 12. Above this pH, individual solutions were prepared from a DO2A stock solution at pH 12 by adding known amounts of NaOD or KOD solution. The p[H] was calculated for each solution by assuming that the ligand is

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Table 1. Protonation Constants of DO2A and Its Derivatives Compared with Those of Other Macrocyclic Ligands at $I = 0.1$ M and 25 °C

ligand	electrolyte	protonation constant[a] log K_1	log K_2	log K_3	log K_4
cyclen[b]		10.6	9.6	1.5	0.7
DO2A[c]	Me_4NCl	11.38(2)	9.62(3)	3.95(3)	2.62(4)
	KCl	10.91(14)	9.45(13)	4.09(8)	3.18(16)
DO3A[d]	Me_4NCl	11.59(3)	9.24(3)	4.43(2)	3.48(3)
	KCl	11.55(8)	9.15(9)	4.48(2)	
	NaCl	10.51(1)	9.08(4)	4.36(13)	
DOTA	Me_4NCl[d]	11.73(3)	9.40(2)	4.50(4)	4.19(6)
	Me_4NNO_3[e]	12.09(4)	9.68(1)	4.55(1)	4.13(1)
	Me_4NCl[f]	11.22(1)	9.64(2)	4.86(2)	3.68(1)
	KCl[d]	11.14(7)	9.50(1)	4.61(9)	4.30(9)
	KNO_3[e]	11.22(3)	9.75(1)	4.37(1)	4.36(1)
	KCl[f]	11.14(1)	9.69(2)	4.85(2)	3.95(1)
	NaCl[d]	9.37(3)	9.14(8)	4.63(12)	3.91(7)
DO2A-2HE[c]	KCl	10.71(5)	8.98(13)	4.06(4)	2.73(4)
DO2A-2HP[c]	KCl	12.23(16)	8.92(9)	4.04(3)	3.00(5)
DO3A-HP[d]	Me_4NCl	11.96	9.43	4.30	3.26
DO2A-2PME[c]	KCl	10.60(9)	9.25(5)	4.41(4)	3.56(3)
DO2A-2EP[c]	KCl	10.84(1)	8.61(23)	3.96(13)	2.89(7)
DOTEP[g]	KNO_3	10.94(3)	8.24(3)	3.71(3)	

[a] $K_i = [H_iL]/[H][H_{i-1}L]$; errors represent standard deviations for two or three separate titrations. [b] Reference 29. [c] Present work. [d] Reference 15. [e] Reference 30. [f] Reference 31. [g] Reference 33.

completely deprotonated at these high pH values. ^{1}H NMR spectra for the pH titrations were recorded on the Varian Unity 500 spectrometer at probe temperatures of 25 and 75 °C.

Solutions of the diamagnetic metal ion (Mg^{II}, Ca^{II}, La^{III}, and Lu^{III}) complexes of DO2A for NMR measurements contained 10 mM DO2A and 1 equiv of the Mg^{II}, Ca^{II}, or lanthanide(III) chloride in D_2O adjusted to pH 8−9. All pH adjustments for these samples were made with DCl or NaOD. Proton 1D and 2D COSY spectra of the complexes were obtained at various temperatures on the Varian Unity 500 spectrometer, using decoupler presaturation to suppress the residual solvent signal.

^{17}O NMR spectra were recorded on the GE 500 spectrometer at 60 °C. To a solution of 30 mM DO2A (pH 10) in 10% D_2O in H_2O was added $DyCl_3$ in 12 equal aliquots up to 50 mM. The pH was measured after each addition and adjusted to above 9 if necessary. Then, EDTA was added in three aliquots, up to 50 mM, and 1.5 mL of the sample was diluted by the addition of 1.5 mL of 10% D_2O in H_2O. This procedure was repeated four times.

Water Proton Relaxation Rates. Water proton ($1/T_1$) relaxation rates were measured as a function of [GdL] (0.5−8 mM) on 100 mL samples of complex at pH = 6.8 (PIPES buffer), at 25 °C using an inversion recovery pulse sequence on a spin-lock pulsed NMR instrument (Model CPS-2) operating at 40 MHz. Since equilibrium was not reached immediately upon mixing Gd^{III} with ligand, T_{null} was measured each day until changes could no longer be detected. After equilibrium had been established, the paramagnetic contributions to the water relaxation rates ($1/T_{1P}$) were evaluated for each complex by subtracting the diamagnetic water relaxation rate from each observed paramagnetic relaxation rate. The slope of a plot of ($1/T_{1P}$) *vs* [GdL] (obtained by linear regression) provided a measure of the relaxivity (R_1) of each complex.

Results and Discussion

Ligand Protonation Studies. (a) Potentiometric Titrations. The protonation constants of DO2A (log $K_i = 11.38$, 9.62, 3.95, and 2.62) were determined by titration of the ligand with Me_4NOH (see Table 1). The protonation constants of DO2A in 0.1 M KCl were quite similar, and protonation of DO2A followed the same trend, two protonations above neutral pH and the remaining below, as for cyclen,[29] DO3A,[15] and DOTA.[15,30,31] It can be concluded that, although derivatization of the cyclen ring nitrogens with pendant acetate functionalities increases the log K_1 value from 10.6 for cyclen to 11.4−11.7 for the derivatives, a change of the number of bound pendant acetates from 2 to 4 has virtually no effect on the second protonation constant of the ligand. The third and fourth protonation constants of DO2A are similar to the analogous values for DO3A and DOTA. An interesting question now arises as to where the first two protonations occur in DO2A, at the secondary or tertiary nitrogens or some combination thereof. Secondary nitrogens typically have better electron-donating capabilities, but protonation at a tertiary nitrogen in systems such as these is often assisted by hydrogen bonding with the directly bonded acetate side chain.[32] Although potentiometry cannot provide these microscopic details, ^{1}H NMR spectra of such ligands collected as a function of pH are usually quite informative (see below).

The protonation constants measured for DO2A-2HE and DO2A-2HP follow trends similar to those seen for DO3A-HP[15] and the acetate derivatives described above. The protonation constants of DO2A-2PME and DO2A-2EP were comparable to those reported earlier for DOTEP.[33] In each case, both the first and second protonation constants tend to be lower for the methylenephosphonate ester and phosphinate derivatives compared to the analogous acetate derivatives. It is remarkable that the third protonation constant of DO2A-2EP is only little larger than the corresponding value for DOTEP, even though this step likely involves protonation of an acetate group in the former and protonation of an ethylphosphinate side chain in the latter compound.

(b) NMR Titrations. To study the microscopic protonation sequence of the ligands, we performed a ^{1}H NMR pH titration of DO2A at 75 °C using K^+ as the counterion and compared this (see Supporting Information) with analogous data for DOTA[34] and other tetraazamacrocyclic amino polycarboxylates.[35] At this temperature, the proton NMR spectrum of DO2A consisted of three sharp resonances at all pH values: the acetate methylene protons (a,b) appeared as a singlet, and the macrocyclic methylenic protons (c,d and e,f) appeared as two complex multiplets. NMR pH titrations of macrocyclic ligands are usually performed[32,34,35] in the presence of K^+, as this cation does not bind significantly to macrocyclic ligands of this type at high pH. Chemical shift *versus* pH curves collected in the presence of Na^+ instead of K^+ were shifted somewhat at high pH values, due to the formation of the $NaDO2A^-$ complex at high pH, analogous to the binding of Na^+ to other tetraazacyclododecane derivatives.[32,36]

The NMR titration curve of DO2A shows the effect of successive protonations of the various basic sites of the molecule. The first two inflections at high pH (11.0−13.0 and 9.0−11.0) were observed only for the ring protons e,f and, to a smaller extent, c,d. These correspond to the first and second protonations, as obtained by potentiometry (Table 1). Another inflection was observed at lower pH values (pH 2.0−4.0) which had a greater effect upon the acetate protons (a,b). Qualitatively, these shifts parallel the third and fourth protonations as

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Table 2. Thermodynamic Metal−Ligand Stability Constants (K_{ML}) of Ln^{III}, Mg^{II}, and Ca^{II} with DO2A and Its Derivatives Compared with Those for Other Macrocyclic Ligands at $I = 0.1$ M and 25 °C and Conditional Stability Constants, K_c, at pH 7.4 and Relaxivities, R_1 (mM^{-1} s^{-1}), of the Gd^{III} Complexes at pH 7, 40 MHz, and 25 °C

	log K_{ML}[a]						
ligand	La	Gd	Yb	Mg	Ca	log K_c[b]	R_1
cyclen					3.1[c]		
DO2A	16.6(2)[d]	19.4(1), 19.1(3)[d]	20.6(3)[d]	5.40(5)	7.8(1)	13.2	6.49
DO3A	19.7(4) (Ce)[e]	21.0[e]	23.0(2) (Lu)[e]		11.74(2)[f]	15.0	4.85[g]
DOTA	22.9[h]	24.6,[h] 25.3(6)[e,i]	24.9[h]	11.92[j]	17.23[j]	19.0	3.60,[g] 4.60[k]
DO2A-2HE		21.1(1)		7.0(3)	10.1(3)	16.2	4.38
DO2A-2HP		22.5(1)		8.0(1)	11.0(4)	16.1	4.16
DO3A-HP		23.8[e]			14.83(9)[f]	17.2	3.65[g]
DO2A-2PME		16.8(1)		7.5(1)	9.4(1)	11.7	5.75
DO2A-2EP		15.8(1)		7.63(5)	9.7(1)	11.1	5.87
DOTEP		16.50(5)		4.41(5)	9.39(5)	12.1	5.10[k]

[a] $K_{ML} = [ML]/[M][L]$; errors represent standard deviations for two or three separate titrations. [b] $K_{c,GdL} = [GdL]/[Gd][L]_f$, $[L]_f = \sum[H_nL]$. [c] Reference 26. [d] By competition with EDTA; see text. [e] Reference 15. [f] Reference 42. [g] Reference 57; pH 7, 20 MHz, 40 °C. [h] Reference 9. [i] Reference 14. [j] Reference 30. [k] Reference 33; pH 7, 40 MHz, 25 °C.

determined by potentiometry (Table 1). It is quite clear that the first two protonations occur almost exclusively at the secondary ring nitrogens despite the fact that the acetate groups are capable of assisting protonation at the tertiary nitrogens by hydrogen bonding.[32] The next two protonations occur almost exclusively at the carboxylate groups. A more elaborate and quantitative description using the empirical procedure of Sudmeier and Reilley[37] is given in the Supporting Information. Except for the asymmetry of the protonation of the ring nitrogens in DO2A, its protonation scheme is quite similar to that previously described for the symmetric ligand DOTA.[34,35]

At temperatures below 75 °C, the macrocyclic ring methylenic ^1H resonances of DO2A are quite broad below pH 9. Between pH 9 and 4, with DO2A in its diprotonated form, H_2L, the ring ^1H resonances appear as two broad signals of relative intensity 3:1 at 25 °C (see Supporting Information). This indicates that the H_2L species is locked into a rigid conformation, probably assisted by internal hydrogen bonds within the macrocyclic ring. The acetate ^1H resonance remains a sharp singlet, however, indicating that these groups remain conformationally mobile. At lower pH values, the larger of the two broad resonances splits into three broad signals, indicating that protonation of the acetate groups to produce the H_3L and H_4L species results in an even more rigid, asymmetric macrocyclic ring conformation. Such rigid conformations have previously been detected for protonated forms of asymmetric macrocyclic ligands[32] but not for the symmetric ligand DOTA.[34]

Complexation Studies. (a) Potentiometric Titrations. The stabilities of MgL and CaL (see Table 2) for all ligands studied here could be determined from potentiometric titration data for the ligand in the presence of 1 equiv of $MgCl_2$ or $CaCl_2$. For the Ln^{III} complexes, this procedure was impractical due to extremely slow equilibration at low pH values. Therefore, about 15 samples were prepared separately per ligand at different pH values and these were allowed to equilibrate over several days. After the pH stabilized, final readings were taken and used to evaluate the stability constants. The stability constant obtained for $GdDO2A^+$ using this "out-of-cell" potentiometric method was confirmed by competition experiments performed using EDTA as a second ligand. This allowed direct potentiometric titrations to be performed at high pH (9−12), where equilibration is much faster than at low pH. A similar procedure was successfully applied previously for the determination of the stability of $LaDOTP^{5-}$.[38] This method, in which a 1:1:1 Ln^{III}:DO2A:EDTA mixture was titrated with HCl from pH 12 to 9, relies on the differences between the protonation constants of the two ligands, on the fact that equilibration is generally faster at higher pH, and on the requirement that no mixed-ligand complexes or complexes with stoichiometry M_mL_n ($m, n > 1$) were formed. The last requirement might be more problematical than in the competition between DOTP and DTPA because DO2A and EDTA are only hexadentate and, therefore, do not saturate the first coordination sphere of the Ln^{III} ion. Similar to that in the experiments with $LaDOTP^{5-}$,[38] equilibration was reasonably fast (within 5 min) at pH >9. Below pH 9, equilibration appeared to be slower. Assuming that $LnDO2A^+$ and $LnEDTA^-$ are the only complexes formed, log K_{ML} values for the DO2A complexes of La^{III}, Gd^{III}, and Yb^{III} obtained in this way were 16.6 ± 0.2, 19.1 ± 0.3, and 20.6 ± 0.3, respectively. The standard deviations in the stability constants determined by this method are fairly large only because the differences between the highest protonation constants (K_1 values) of DO2A and EDTA are small while the difference between the second protonation constants (K_2 values) cannot be optimally exploited because the pH has to be > 9 for kinetic reasons. For EDTA, polynuclear[39] and hydroxy[40] complexes are known, but these barely form at the concentrations (2 mM) used in these experiments. The value for $GdDO2A^+$ by competition compared favorably with that determined by the "out-of-cell" potentiometric method in the absence of EDTA, supporting the assumption that mixed Ln−EDTA−DO2A complexes were not formed under the conditions exploited here. Further evidence in support of this assumption was provided by ^{17}O NMR measurements (see below).

Table 2 summarizes the stability constants determined in this work for a variety of metal ions with DO2A and several DO2A derivatives. Also shown are data for the analogous DO3A and DOTA complexes reported elsewhere.[9,14,15,30,41,42] As expected, the K_{MgL} values are lower than the corresponding K_{CaL} values for all ligands reported in Table 2. This reflects the effect of macrocyclic ring size where all 1,4,7,10-tetraazacyclododecane derivatives show a preference for the larger Ca^{II} ion while 1,4,7-triazacyclononane compounds generally bind more strongly to Mg^{II}.[43,44] The log K_{ML} values for the Ca^{II} complexes with DO2A, DO2A-2HE, and DO2A-2HP were consistently 11−11.5 log K units lower than those of the corresponding Gd^{III}

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complexes. Kumar et al.[42] recently reported a linear relationship between the stability constants of GdL and the corresponding CaL systems, for a large number of five- and six-membered chelates. This relationship, $\log K_{GdL} = (1.4 \pm 0.1) \log K_{CaL} + (2.5 \pm 1.2)$, predicted that $\log K_{CaL}$ should be 12, 13.3, and 14.3 for DO2A, DO2A-2HE, and DO2A-2HP, respectively, compared to measured values of 7.8, 10.1, and 11.0. Thus, the differences between calculated and observed stability constants for Ca^{II} complexes with these three ligands were 4.2, 3.2, and 3.3 log *K* units. Since the $\log K_{ML}$ value for $GdDO2A^+$ agrees well with linear correlations between $\log K_{GdL}$ and $\sum \log K_i$ values (see below), we assume that these differences reflect a problem with Ca^{II} binding in these systems. Similar descrepancies[43] were reported previously for the ligands DTPA, EGTA, and TTHA and ascribed to the occupation of less coordination sites by these ligands for binding to Ca^{II} compared to Ln^{III}. If this is correct, our results suggest that DO2A, DO2A-2HE, and DO2A-2HP, unlike DOTA, do not use all possible ligand donor groups in forming complexes with Ca^{II} in aqueous solution.

A comparison of the stability constants of the Gd^{III} complexes of the three tetraazamacrocyclic amino carboxylates illustrates clearly the order DOTA > DO3A > DO2A, approximately paralleling the differences in the number of donor atoms and five-membered chelate rings formed in these complexes. The Ln^{III} chelates of DO2A show a marked increase in stability (4 log *K* units) from the beginning of the series (La^{III}) to the end (Yb^{III}), somewhat larger than that observed previously for the DO3A complexes (3.3 log *K* units)[14] and a factor of 2 larger than that observed previously for the DOTA complexes (2 log *K* units).[9] Thus it appears that the decrease in ionic radius of the Ln^{III} ions across the lanthanide series has a more pronounced effect on chelate stability for the $LnDO2A^+$ and LnDO3A complexes than for the $LnDOTA^-$ complexes. This is in contrast to the generally observed phenomenon that the increase in LnL stability constants across the Ln^{III} series is larger for ligands with a higher coordination number due to the larger formation entropies for the heavier Ln^{III} ions.[45]

For comparison of the Gd^{III}-binding strengths, the conditional binding constant K_c at physiological pH (7.4) is commonly regarded as a better measure than K_{GdL}. Listed in Table 2 are $\log K_c$ values at pH 7.4, as calculated from the protonation constants (in Me_4NCl where available) given in Table 1 and the K_{GdL} values from Table 2. Interestingly, the loss of one acetate group from DOTA to DO3A has a much larger effect on the conditional stability constant ($\Delta(\log K_c) = 4.0$) than the loss of a second group from DO3A to DO2A ($\Delta(\log K_c) = 1.8$). Substitution of an acetate group on DOTA by a hydroxypropyl group to give DO3A-HP results in only a small decrease in complex stability ($\Delta(\log K_c)$ again is 1.8), despite the lower overall charge. Substitution of a second acetate group by a second hydroxypropyl side chain to give DO2A-2HP again has a smaller effect ($\Delta(\log K_c) = 1.1$) when compared with Gd(DO3A-HP)). The $Gd(DO2A\text{-}2HE)^+$ and $Gd(DO2A\text{-}2HP)^+$ complexes are about 1 order of magnitude more stable than GdDO3A at pH 7.4, indicating that the additional chelate bonds in the eight-coordinate DO2A-2X derivatives contribute more to the stability of the complexes than the additional negative charge on DO3A.

A linear relationship between ligand basicity and complex stability has been reported for numerous polyaza polycarboxylate ligands of this type. A plot of $\log K_{GdL}$ *versus* $\sum \log K_i$ values for a limited number of ligands is shown in Figure 2. Only the *n* most basic sites were included in the $\sum \log K_i$ values for each ligand in this plot, where n = the number of protonations required to yield a neutral ligand. The data for DOTA, DO3A, DO2A, and two other macrocyclic diacetate ligands, PC2A and BP2A,[22] exhibit a very good linear relationship, with a slope identical to that reported earlier for a much larger number of linear polyaza polycarboxylate ligands.[14] The data for DO2A-2HE, DO2A-2HP, and DO3A-HP all appear to fall above this linear relationship, suggesting that these complexes are more stable than expected on the basis of ligand basicity alone. This agrees with the previously observed phenomenon for polycarboxylate compounds containing neutral-oxygen donor sites, which also form more stable Ln^{III} complexes when compared to polycarboxylate ligands lacking these neutral-oxygen donor sites.[45]

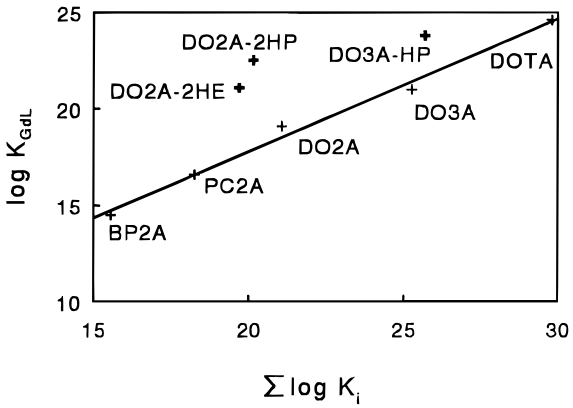

Figure 2. $\log K_{GdL}$ *versus* $\sum \log K_i$ for various macrocyclic ligands.

Substitution of two acetate groups of DOTA by ethyl phosphonate or ethylphosphinate side-chain functionalities actually decreases the stability of the complexes. In this case, not only are the Gd^{III} complexes of DO2A-2PME and DO2A-2EP less stable than DO2A, but the latter is even less stable than the fully substituted $GdDOTEP^-$. This suggests that steric hindrance in complexes with these mixed side-chain functionalities is greater than in complexes with only one type of coordinating side chain. In fact, the plane of coordinating oxygens has a different twist angle relative to the plane of ring nitrogens in $LnDOTA^-$ (40°) compared to the Y^{III} complex of the tetrasubstituted benzylphosphinate derivative (29°),[46] which probably has a structure similar to that of $LnDOTEP^-$. This indicates that, in ligands with two acetates and two phosphinate side chains, these side chains will have conflicting geometric needs in order to accommodate the Ln^{III} ion, resulting in lower stabilities or even partial coordination as suggested by the results presented here.

(b) Water Coordination Numbers for $LnDO2A^+$. The number of water molecules in the first coordination sphere of $DyDO2A^+$ was determined by Dy^{III}-induced ^{17}O shift measurements of the bulk water resonance, as described previously.[47] This method relies on the fact that the water molecules in the first coordination sphere of Dy^{III} generally exchange rapidly with the bulk water, so that one averaged ^{17}O signal is obtained using only the natural ^{17}O abundance. As shown before,[47] the bound shift of a coordinated water molecule is approximately 2000 ppm, arising mainly from a contact interaction between the ^{17}O water nucleus and the unpaired electrons of the Dy^{III} ion.

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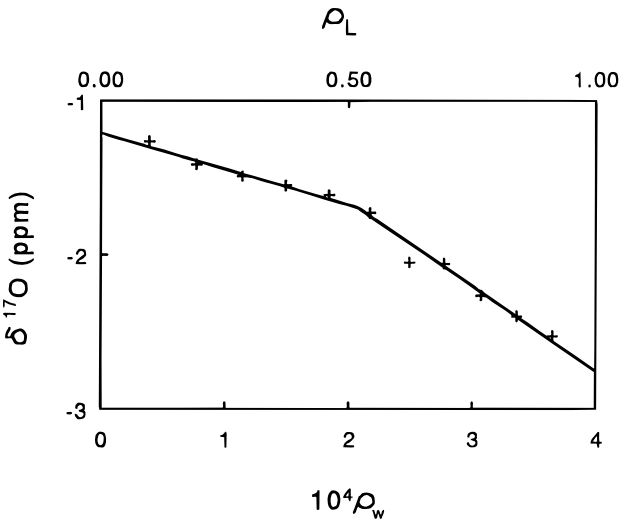

Figure 3. Dy^{III}-induced ^{17}O NMR shifts for a sample containing 30 mM DO2A and 0−30 mM $DyCl_3$ *versus* $\rho_w = [Dy]/[H_2O]$ (lower *x* axis) and $\rho_L = [Dy]/[DO2A]$ (upper *x* axis), as measured at 67.8 MHz, 60 °C, and pH >9.

The water coordination number of $DyDO2A^+$ was determined by measuring the ^{17}O NMR shifts as a function of the amount of added $DyCl_3$ for a sample containing 30 mM DO2A and 10% D_2O for locking purposes. A temperature of 60 °C was maintained to provide a sharper ^{17}O resonance and to ensure rapid exchange of the water molecules between bound and bulk water on the NMR time scale. The pH was checked and kept above pH 9, since the potentiometric titrations had shown that the exchange between bound and free DO2A became slow at pH <9 (see above). Figure 3 shows the Dy^{III}-induced ^{17}O water shift as a function of $\rho_w = [Dy]/[H_2O]$ (lower *x* axis) and $\rho_L = [Dy]/[L]$ (L = DO2A; upper *x* axis). At $\rho_L = 1$, a total bound shift of 4000 ppm was calculated, leading to a water coordination number of 2. This agrees with the observation that the Dy^{III} aquo ion has eight water molecules in the first coordination sphere,[48,49] so complexation by the hexadentate ligand, DO2A, results in removal of six water molecules from the first coordination sphere. For $\rho_L = 0-0.5$, the bound shift was only 2000 ppm, corresponding to a single bound water molecule. This is consistent with formation of a ML_2 complex which may consist of a nine-coordinate Dy^{III} ion complexed by a six-coordinate DO2A ligand, a two-coordinate DO2A ligand (using either two acetates or one acetate and one ring nitrogen), and one water molecule. An expansion of the Dy^{III} coordination sphere from 8 to 9 is known to occur when two or more (negatively charged) ligands are coordinated.[40] For $\rho_L = 0.5-1$, the addition of Dy^{III} leads to redistribution of DO2A from ML_2 to ML.

At $\rho_L = 1$, EDTA was added to the sample to investigate the possibility of formation of mixed-ligand complexes (the pH was constant at 10.4). Indeed, addition of 1 equiv of EDTA led to an almost complete reversal of the Dy^{III}-induced ^{17}O shift, suggesting formation of a 1:1:1 complex with no bound water molecules. This complex may consist of a hexadentate DO2A and a tridentate EDTA, the latter probably via an IDA unit (*i.e.*, one nitrogen and its attached acetates), coordinated to a nine-coordinate Dy^{III} ion. Dilution of this sample by a factor 16 (in increments of factors of 2) led to an initial increase in ^{17}O shift followed by a decrease due to the dilution. Assuming that $DyDO2A^+$, $DyEDTA^-$ (both with two coordinated water molecules), and $Dy(DO2A)(EDTA)^{3-}$ (without any water molecules) were the only complexes present, about 50% of the Dy^{III} appeared to be in the mixed-complex form at $M_{tot} = 2$ mM (60 °C, pH 10.4). The stability of this complex is probably overestimated with about 0.6 log *K* unit, since a decrease of the protonation constants ($\Delta H_{HL} = -35$ kJ mol^{-1} for DOTA and −33 kJ mol^{-1} for cyclen)[26] and a concomitant increase of the apparent complex stability are usually the main effect of an increase in temperature.[43] Therefore, under the conditions exploited in the potentiometric competition experiments (25 °C, $M_{tot} = 2$ mM, Gd^{III}:DO2A:EDTA = 1:1:1), less than 10% contribution of this mixed complex was estimated at pH 9−10. The presence of Ln(DO2A)(EDTA) in the potentiometric competition titrations would cause an overestimation of the $LnDO2A^+$ stability constants, and a variation of K_{LnL} over the pH range exploited should have been observed. Since this was not the case and the calculated K_{GdL} is even lower than the value determined by the regular potentiometric method without EDTA, we conclude that the influence of the mixed-ligand complex on the potentiometric competition experiment was negligible.

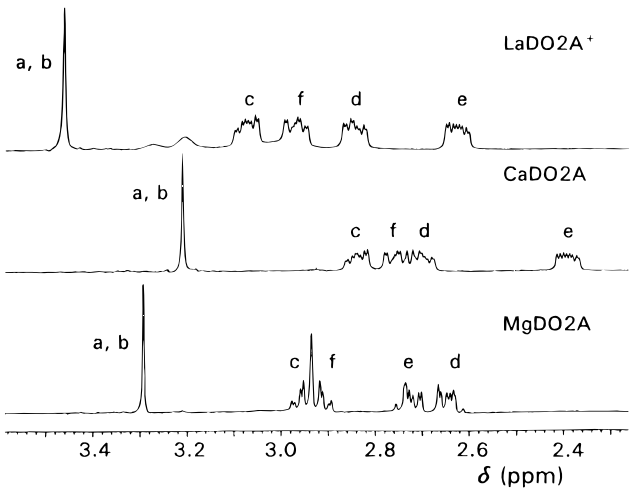

Figure 4. ^1H NMR spectra of 20 mM DO2A with 1 equiv of Mg^{II} (pH 9.5), Ca^{II} (pH 9.5), or La^{III} (pH 7.0), as measured at 500 MHz and 75 °C.

(c) NMR Studies of DO2A Complexes with Diamagnetic Ions. The conformations of the 1:1 complexes formed in aqueous solution between the macrocyclic chelate DO2A and the diamagnetic cations Mg^{II}, Ca^{II}, La^{III}, and Lu^{III} were studied by one- and two-dimensional ^1H NMR techniques. When less than a stoichiometric amount of cation was added to a DO2A solution or, in the cases of the cations Mg^{II} and Ca^{II}, when stoichiometric solutions were used at some pH values, resonances for the free and bound ligands were detected in the ^1H NMR spectra. Figure 4 shows typical spectra for MgDO2A, CaDO2A, and $LaDO2A^+$ obtained at 348 K. The acetate protons (H_a, H_b) appear as a singlet in these complexes. This contrasts with the $MDOTA^-$ complexes (M = Y^{III}, La^{III}, Lu^{III}) which show AB type patterns for the acetate protons.[50,51] In the latter complexes, this reflects the maintenance of the screw conformation of the plane of coordinating carboxylate oxygens compared to the plane of the ring nitrogens, due to the steric demands of the four acetates. A twist of these planes is slow on the NMR time scale in the $MDOTA^-$ complexes. In the MDO2A complexes, however, the presence of the acetate singlet

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Table 3. ^{1}H NMR Chemical Shifts of Aqueous Diamagnetic Complexes of DO2A and DOTA

		proton chemical shifts, δ (ppm)[a]					
ligand	cation	H_a	H_b	H_c	H_d	H_e	H_f
DO2A[b]	*c*	3.20		2.78		2.63	
	Mg^{II}	3.29		2.95	2.64	2.72	2.91
	Ca^{II}	3.20		2.84	2.70	2.39	2.75
	La^{III}	3.46		3.07	2.84	2.62	2.96
	Lu^{III}	3.54		3.11	3.07	2.84	2.81
DOTA	*c*	3.15		2.55			
	Mg^{II} [d]	3.12		2.84	2.48	2.84	2.48
	Ca^{II} [d]	3.42	2.68	3.06	2.24	2.08	2.68
	La^{III} [e]	3.75	3.05	3.42	2.48	2.36	2.98
	Lu^{III} [e]	3.55	3.24	3.40	2.68	2.44	2.76
	Y^{III} [f]	3.57	3.19	3.38	2.73	2.40	2.73

[a] Proton chemical shifts, taken as the central value of the observed multiplets, relative to TSP-d_4. [b] This work; at 348 K and at pH 7.0 for the Mg^{II} and Ca^{II} complexes and pH 9.4 for the La^{III} and Lu^{III} complexes. [c] Shifts for the free ligand, pH >12. [d] Reference 52; 293 K. [e] Reference 51; 273 K (the shifts for $LaDOTA^-$ are of the minor isomer, and those for $LuDOTA^-$ are of the major isomer). [f] Reference 50; 273 K.

suggests that the two acetates can rearrange quickly and independently from each other. The four protons of each ethylene ring moiety (H_c, H_d, H_e, H_f) of the DO2A complexes appear as four octet resonances, corresponding to a first-order ADMX spectrum. This was most easily seen in the spectrum of $LaDO2A^+$. The assignment of these proton signals was based upon standard 2D homonuclear correlated (COSY) spectra of the complexes (see Supporting Information). These spectra showed strong cross-peaks for the geminal-coupled protons (c,d and e,f) and the single vicinal coupling between protons located close to a trans conformation (e.g. c,f), whereas the vicinal couplings close to a gauche conformation gave very weak or no cross-peaks.

Table 3 summarizes the ^{1}H chemical shifts for the DO2A complexes and compares them with similar data for the DOTA complexes[50,51] of La^{III}, Lu^{III}, and Y^{III} and with our own results[52] for the Mg^{II} and Ca^{II} complexes. The 12-membered macrocyclic ring of DO2A, like that of DOTA,[53] likely has an enantiomeric square {3333} conformation in aqueous solution, similar to that found for cyclododecane.[54,55] Thus, in the corresponding metal complexes, there are two enantiomeric conformations of the macrocycle in solution, Δ and Λ, each one corresponding to a distinct combination of the δ and λ configurations of the four rings formed by the ethylenediamine bridges bound to the metal ion within the chelate (see structures **I** and **II** of Figure 5). The $\Delta \leftrightarrow \Lambda$ conformational interconversion process of the macrocycle is caused by interconversions of conformations **I** and **II** of each ethylenediamine bridge. In the case of the DOTA complexes, as the four nitrogens are chemically equivalent, that fast interconversion process causes an exchange between the H_c and H_e protons and between the H_d and H_f protons, making them magnetically equivalent. This was the case found experimentally for the $MgDOTA^{2-}$ complex at 298 K, which gives an AA′XX′ type spectrum (see Table 3).[52] However, the Ca^{II}, La^{III}, Lu^{III}, and Y^{III} complexes of DOTA give ADMX type ^{1}H spectra for the ring moiety, indicating that this interconversion process is slow on the NMR time scale between 298 and 373 K.[50−53] This increased rigidity, with a rather high energy barrier of the interconversion process between the two conformations of the macrocycle, is responsible for the nonequivalence of the corresponding protons detected in their NMR spectra (see Table 3). The observed broadening of those proton resonances at higher temperatures reflects a faster $\Delta \leftrightarrow \Lambda$ interconversion.[50−53] However, in the case of the DO2A complexes, the chemical nonequivalence of the two types of macrocyclic ring nitrogens, N1 and N2, leads to a rather different situation. Even if the $\Delta \leftrightarrow \Lambda$ interconversion is fast on the NMR time scale, the proton pairs H_c, H_e and H_d, H_f do not become magnetically equivalent. This fast interconversion process was found experimentally at 348 K for all the DO2A complexes, as the observed broadening of their ring resonances as the temperature is lowered to 323 and 298 K reflects the slowing down of that dynamic process.

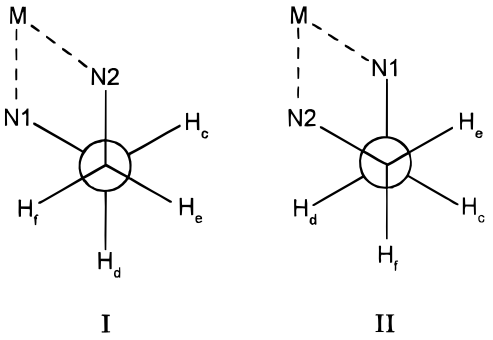

Figure 5. Newman projection along the C−C bond of the two staggered conformations of the ethylenediamine moieties of metal-bound DO2A and DOTA.

The proton coupling constants observed for the DO2A complexes at 348 K are also indicative of a fast conformational interconversion between the **I** and **II** forms shown in Figure 5. The values of those couplings are quite similar for all the DO2A complexes studied, so we report here only those found for $LaDO2A^+$. Two geminal couplings of −13.5 and −13.9 Hz and four vicinal couplings of 7.8, 7.6, 3.2, and 3.0 Hz were observed, consistent with gauche−gauche or trans−gauche averaging according to Figure 5. No large trans coupling, characteristic of a rigid conformation such as between the axial protons H_c and H_f of form **I** in Figure 5, was seen for the DO2A complexes, as opposed to a trans coupling of 14.2 Hz, reported for $LaDOTA^{2-}$, characteristic of a rigid macrocyclic ring.[51]

Comparison of the proton chemical shifts of the DO2A complexes with those of the deprotonated ligand (Table 3) shows that the acetate protons H_a and H_b and the axial ring protons H_c and H_f are consistently deshielded upon complexation, while the equatorial protons have complexation shifts of variable sign. In the case of the DOTA complexes, only the protons H_a, H_c, and H_f have positive complexation shifts. These complexation shifts must be dominated by electric field effects from the negatively charged oxygens of the ligand carboxylates as well as the metal ion polarization of the C−H bonds.[56]

(d) Water Proton Relaxivities. Table 2 shows the relaxivity values of the Gd^{III} complexes of DO2A and the four derivatives of DO2A containing mixed side-chain ligating arms, all measured at pH 6.8, 25 °C, and 40 MHz. These values are compared with literature values[33,57] for $GdDOTA^-$, $GdDOTP^{5-}$, $GdDOTEP^-$, GdDO3A, $GdDTPA^{2-}$, and Gd(DO3A-HP). A linear relationship between the relaxivity (R_1, 20 MHz, 40 °C) and water coordination number (q) has been reported for a series

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of linear[58] and macrocyclic[57] amino polycarboxylate complexes of Gd^{III}. The R_1 value reported in Table 2 for $GdDO2A^+$ can be adjusted downward to correct for the lower temperature and higher frequency at which this measurement was made by comparison to the two values for $GdDOTA^-$ reported in Table 2 (one at 20 MHz, 40 °C, and another at 40 MHz, 25 °C). If one assumes that the frequency dependence of R_1 for small complexes such as these is quite small between 20 and 40 MHz, then the primary effect would be due to temperature. The R_1 value of $GdDO2A^+$ corrected to 40 °C would be about 4.77, corresponding to approximately two inner-sphere water molecules. This value is quite similar to the R_1 value reported previously for GdDO3A.[57] The smaller R_1 values for GdDO2A-2HE$^-$ and GdDO2A-2HP$^-$ were consistent with a change in q from 2 to 1. However, the observation that both GdDO2A-2PME$^-$ and GdDO2A-2EP$^-$ have higher relaxivities than either $GdDOTA^-$ or $GdDOTEP^-$ suggests that the mixed side-chain ligands form complexes with Gd^{III} with fewer than eight coordination sites, consistent with the low stability constants of these complexes. A more thorough analysis of these data must await measurement of the frequency dependence of R_1 for these complexes.

Conclusions

The hexadentate macrocylic ligand, DO2A, forms kinetically inert and thermodynamically stable complexes with the trivalent lanthanide cations. The thermodynamic stability of $GdDO2A^+$ was found to be about 2.5−3 orders of magnitude lower than that of GdDO3A and about 5 orders of magnitude lower than that of $GdDOTA^-$. The Gd^{III} stability constants for this series of tetraazacyclododecanepolycarboxylate ligands correlated nicely with $\sum \log K_i$. The Gd^{III} complexes with the DO2A-2X ligands containing two acetates and two 2-hydroxyethyl or 2-hydroxypropyl groups were more stable than similar macrocyclic ligands with only acetate side chains, as predicted by the relationship between $\log K_{ML}$ and $\sum \log K_i$ (Figure 2). This property was also the key to the success of HP-DO3A as an MRI contrast agent; not only is Gd(HP-DO3A) uncharged so that higher doses can be administered but its thermodynamic stability is also barely compromised compared to that of $GdDOTA^-$.

Interestingly, the $LnDO2A^+$ complexes showed a much greater increase in stability with decreasing metal ionic radius along the Ln^{III} series than found previously for the LnDO3A and $LnDOTA^-$ complexes. Although this observation was based solely on data from three Ln^{III} cations (La^{III}, Gd^{III}, and Yb^{III}), this trend probably reflects more pronounced steric hindrance or electrostatic repulsion between the acetate side chains with decreasing cationic radius for DOTA and DO3A than for DO2A, thereby offsetting the expected increase in stability due to an increase in charge density across the Ln^{III} series. This is also reflected in the water coordination numbers of these complexes, with $GdDOTA^-$ having one inner-sphere water (total CN of 9)[57] and GdDO3A having two inner-sphere waters (total CN of 9),[57] while $GdDO2A^+$ also appears to have two inner-sphere waters with a total coordination number of 8. This shows that the less crowded DO2A ligand can more easily encapsulate a Ln^{III} cation, remove more waters of hydration, and respond more readily to changes in cation size along the series. The equivalence of the acetate CH_2 protons in $LaDO2A^+$ shows that steric hindrance between the two acetate groups is small and that their decoordination and recoordination occur independently of each other. This is in contrast to the behavior of the $LnDOTA^-$ complexes, where the nonequivalence of the CH_2 protons is attributed to the steric demands of the four acetates, resulting in the presence of a square of coordinating oxygens that is twisted with respect to the square of coordinating ring nitrogens and in the formation of two distinctly different isomers.[59]

The Gd^{III} complexes of the ligands with two acetates and two ethyl phosphonate or ethylphosphinate groups were less stable than both parent complexes $GdDOTA^-$ and $GdDOTEP^-$. This may be attributed to greater steric hindrance between the side chains of the mixed side-chain ligands than for the ligands with four equivalent groups. This effect may be so severe that coordination of these mixed side-chain ligands may not be complete, as suggested by the high relaxivities and lower thermodynamic stabilities of their Gd^{III} complexes. The structure of these complexes and their dynamic behavior might be very interesting and deserves further investigation, since Ln^{III} complexes with uncoordinated side chains and higher water coordination numbers could coordinate with other metal ions and thereby have interesting, new relaxation properties that may be useful in some MRI applications.

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Supporting Information Available: Text, a table, and a figure describing the analysis of the ^1H NMR shift *versus* pH titration data for DO2A and figures showing the ^1H NMR spectra of DO2A at pD 2.5 and 5.3 (25 °C) and the COSY spectrum of $LaDO2A^+$ (6 pages). Ordering information is given on any current masthead page.

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