Synthesis, Potentiometry, and NMR Studies of Two New 1,7-Disubstituted Tetraazacyclododecanes and Their Complexes Formed with Lanthanide, Alkaline Earth Metal, Mn^{2+}, and Zn^{2+} Ions[⊥]

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Two new 1,7-disubstituted-1,4,7,10-tetraazacyclododecane ligands, DO2P and DO2PME, and their complexes with Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}, Zn^{2+} and Ln^{3+} were prepared and characterized by pH potentiometry. The pH titration data showed that DO2P and DO2PME both form 1:1 M:L complexes with all divalent and trivalent metal ions. Protonated complexes did not appear to form with the bis(phosphonate ester) ligand, DO2PME, but were evident for all of the metal ion−DO2P complexes. The alkaline earth metal ion−DO2P complexes formed both ML and MHL complexes while the lanthanide ion (Ln^{3+}), Zn^{2+}, and Mn^{2+} complexes of DO2P formed ML, MHL, and MH_2L species. Zn^{2+} formed the most stable complex with both ligands. The stability (β_{101}) of the $LnDO2PME^{+}$ complexes increased by about 2 orders of magnitude along the lanthanide series (La^{3+} to Lu^{3+}) while the stability of the $LnDO2P^{-}$ complexes over this same series increased by over 3 orders of magnitude. The bis(phosphonate) ligand, DO2P, and some of its complexes formed with Ln^{3+} ions were further examined by NMR spectroscopy. ^{1}H and ^{31}P spectra of DO2P collected as a function of pH provided evidence that the first two protonations on the ligand take place largely at the tertiary nitrogens. The similarity of the ^{31}P chemical shifts of $EuDO2P^{-}$ and $EuDOTP^{5-}$ indicate that DO2P forms an "in-cage" complex with Eu^{3+} using all four macrocyclic ring nitrogens and the two phosphonate sidearms as ligands. ^{17}O NMR shifts of the water signal indicated that the $DyDO2P^{-}$ complex has two inner-sphere coordinated water molecules. In the presence of excess of DO2P, a 1:2 metal:ligand, $LnDO2P(HDO2P)^{4-}$, complex forms with the second ligand interacting only weakly with the coordination sites left vacant by the first DO2P. Both water proton relaxivity data for $GdDO2P^{-}$ and ^{31}P NMR spectra of $EuDO2P^{-}$ provide evidence for formation of an "out-of-cage" LnH_2DO2P^{+} complex at low pH values (<6.5) in which the two phosphonate groups of DO2P are only involved in bonding with the lanthanide cation.

Introduction

The successful application of several 1,4,7,10-tetraazacyclododecane (cyclen) macrocyclic lanthanide complexes as MRI contrast agents,[1,2] NMR shift reagents,[3,4] in vivo temperature reporters,[5−7] and RNA cleavage catalysts[8] has stimulated interest in new cyclen-based ligands with varying types and numbers of pendant arms in an attempt to find new ligands with differing chemical, biological, or catalytic properties. We have been exploring the effect of pendant-arm modification on the thermodynamic stability, dissociation kinetics, structural rigidity, metal selectivity, and water coordination behavior of related lanthanide complexes. As one of such effort, we recently reported the preparation and characterization of a number of 1,7-disubstituted cyclens,[9] including DO2P and DO2A. We anticipated that these potentially hexadentate ligands would form complexes with lanthanides having at least two more water molecules in the inner coordination sphere than in the corresponding octadentate tetrasubstituted analogues, DOTP and DOTA. Recently, the $LnDO2A^{+}$ complexes have been characterized by potentiometry, high-resolution NMR, and water proton relaxometry measurements.[10] It was found that, com-

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[⊥] Abbreviations used in the text: DO2P, 1,4,7,10-tetraazacyclododecane-1,7-bis(methanephosphonic acid); DO2PME, 1,4,7,10-tetraazacyclododecane-1,7-bis(methanephosphonic acid monoethyl ester); DO2A, (1,4,7,10-tetraazacyclododecane-1,7-bis(acetic acid); DOTP, (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methanephosphonic acid); DOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(acetic acid); DO3A, (1,4,7,10-tetraazacyclododecane-1,4,7-tris(acetic acid).

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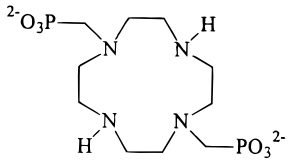
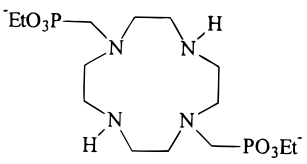
pared to that of DO3A and DOTA, the lower ligand denticity of DO2A resulted in $LnDO2A^+$ complexes of lower thermodynamic stability and structural rigidity, increased water proton relaxivity (the Gd^{3+} complex), and greater selectivity for the heavier lanthanide cations. The lower ligand denticity of DO2A also left open coordination sites for other molecules to bind, thereby allowing complexes with stoichiometry greater than 1:1 to also form.[10] In the present work, we report data for complexes formed by two other 1,7-disubstituted cyclen derivatives, the bis(phosphonate monoester) ligand, DO2PME, and the bis(phosphonate) ligand, DO2P, with lanthanide, alkaline earth metal, Mn^{2+}, and Zn^{2+} ions.



DO2P



DO2PME

Experimental Section

Chemical Reagents. 1,4,7,10-tetraazacyclododecane was purchased from Parish Chemical Co. (Orem, UT). All other reagents used were of analytical grade. Stock solutions of $LnCl_3$ were prepared from the lanthanide oxides (Ln_2O_3, 99.9%, Fluka). The concentrations of $MgCl_2$, $CaCl_2$, $SrCl_2$, $MnCl_2$, $ZnCl_2$, and $LnCl_3$ solutions were determined by complexometric titration using standardized Na_2H_2EDTA solution and Eriochrome black T ($CaCl_2$, $ZnCl_2$, $MnCl_2$), methylthymol blue ($SrCl_2$, $MgCl_2$), or xylenol orange ($LnCl_3$) as indicator. The triethyl phosphite was distilled prior to use. Elemental analyses were obtained from Galbraith Laboratories, Inc. (Knoxville, TN), or Oneida Research Services, Inc. (Whitesboro, NY).

Syntheses. 1,7-Bis(benzyloxycarbonyl)- and 1,7-bis(ethoxycarbonyl)-1,4,7,10-tetraazacyclododecane were prepared as described previously.[9]

1,7-Bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane-4,10-bis(methanephosphonic acid diethyl ester). Paraformaldehyde (0.355 g, 11.83 mmol; 6.6% excess) was added to a mixture of 2.44 g (5.55 mmol) of 1,7-bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane and 2.21 g (19 mmol; 20% excess) of triethyl phosphite. The mixture was stirred at room temperature for 2 days and then at 40 °C for 1 day. The resulting clear oil was placed under high vacuum at 80 °C for 8 h to remove volatile impurities. The residue was dissolved in ether (50 mL), and the ethereal solution was extracted with water (2 × 20 mL), 2% NaOH solution (2 × 20 mL), and water (2 × 20 mL). The ether layer was dried over Na_2SO_4, and the solvent was removed under vacuum. The residue was loaded on a silica gel column (300 g), and the product was eluted with methanol. The fractions containing the product were combined, and the methanol was removed in vacuum to give 3.10 g (75%) of colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$/TMS), δ (ppm): 7.33 (br, 10H, aromatic protons), 5.13 (s, 4H, benzyl protons), 4.06 (m, 8H, $POCH_2CH_3$), 3.48 (br, 8H, $CONCH_2CH_2NCH_2P$), 2.91 (br, 12H, overlapping resonances of $CONCH_2CH_2NCH_2P$ and NCH_2P), 1.28 (t, 12H, $POCH_2CH_3$). ^{13}C NMR (50.10 MHz, $CDCl_3$/TMS), δ (ppm): 156.39 (*C*OO), 136.78, 128.42, 127.90 (aromatic carbons), 66.98 $CH_2C_6H_5$, 61.47 (d, J_{PC} = 7.33 Hz, $POCH_2CH_3$), 55.35 (br, $CONCH_2CH_2NCH_2P$), 49.86 (d, J_{PC} = 149.42 Hz, NCH_2P), 46.23 (br, $CONCH_2CH_2NCH_2P$), 16.42 (d, J_{PC} = 4.39 Hz, $POCH_2CH_3$). ^{31}P NMR (202.40 MHz, $CDCl_3/H_3PO_4$), δ (ppm): 26.22. Anal. Found (calcd) for $C_{34}H_{54}N_4O_{10}P_2$: C, 54.39 (55.11); H, 7.50 (7.35); N, 7.53 (7.57).[11]

1,4,7,10-Tetraazacyclododecane-1,7-bis(methanephosphonic acid) (DO2P). 1,7-bis(benzyloxycarbonyl)-1,4,7,10-tetraazacyclododecane-1,7-bis(methanephosphonic acid diethyl ester) (2.84 g, 3.83 mmol) was dissolved in 20% hydrochloride acid (60 mL) and the solution was refluxed for 24 h. After cooling to room temperature, the mixture was extracted with ether (4 × 50 mL). The aqueous layer was removed, and the solution was evaporated to dryness in vacuum. The solid residue was taken up in absolute ethanol (25 mL), and ether (25 mL) was added. The white solid was filtered off and dried in a stream of nitrogen to give 1.67 g of product. The crude product was dissolved in water (5 mL), and ethanol (25 mL) and later ether (25 mL) were added. The crystalline product was filtered off, washed with ethanol−ether (1:1) and ether (three times), and dried in a stream of nitrogen to give 1.48 g of product (90%). ^{1}H NMR (200 MHz, D_2O/DSS, pD ~2), δ (ppm): 3.21, 3.14, 2.90 (br, $NCH_2CH_2NCH_2P$), 2.90 (d, J_{PH} = 10.99 Hz, NCH_2P). ^{13}C NMR (50.10 MHz, H_2O-D_2O, pH ~2), δ (ppm): 52.47 (d, J_{PC} = 161.14 Hz, PCH_2N), 52.34 (d, J_{PC} = 7.32 Hz, $PCH_2NCH_2CH_2NH$), 44.35 ($PCH_2NCH_2CH_2NH$). ^{31}P NMR (202.40 MHz, D_2O-H_2O/H_3PO_4, pH ~2), δ (ppm): 22.27. Anal. Found (calcd) for $C_{10}H_{26}N_4O_6P_2 \cdot 1.65H_2O \cdot 1.0HCl$: C, 28.32 (28.17); H, 7.11 (7.16); N, 13.07 (13.14); Cl, 8.35 (8.31).[11]

1,7-Bis(ethoxycarbonyl)-1,4,7,10-tetraazacyclododecane-4,10-bis(methanephosphonic acid diethyl ester). Paraformaldehyde (0.195 g, 6.49 mmol, 3% excess) was added to a mixture of 1,7-bis(ethoxycarbonyl)-1,4,7,10-tetraazacyclododecane (1.00 g, 3.16 mmol) and triethyl phosphite (1.16 g, 6.95 mmol; 10% excess). The mixture was stirred at room temperature for 4 days. The resulting clear oil was placed under high vacuum at room temperature for 8 h and then at 50 °C for 8 h to remove any volatile impurities. The residue is a pale yellow oil; 1.88 g, 96%. ^{1}H NMR (200 MHz, $CDCl_3$/TMS), δ (ppm): 4.12 (m, 12H, CO_2CH_2 and $POCH_2$), 3.47 (br, 8H, $CONCH_2CH_2N$), 3.02 (d, J_{PH} = 9.77 Hz, 4H, NCH_2P), 2.91 (br, 8H, $CONCH_2CH_2NCH_2P$), 1.33 (t, 12H, $POCH_2CH_3$), 1.25 (t, 6H, $CO_2CH_2CH_3$). ^{13}C NMR (50.1 MHz, $CDCl_3$/TMS), δ (ppm): 156.36 (CO_2), 61.27 (d, J_{PC} = 7.32 Hz, $POCH_2CH_3$), 60.84, ($CO_2CH_2CH_3$), 55.29 (br, $CONCH_2CH_2NCH_2P$), 49.65 (d, J_{PC} = 152.35 Hz, NCH_2P), 45.88 (br, $CONCH_2CH_2NCH_2P$), 16.14 (d, J_{PC} = 5.86 Hz, $POCH_2CH_3$), 14.33 ($CO_2CH_2CH_3$). ^{31}P NMR (202.4 MHz, $CDCl_3/H_3PO_4$), δ (ppm): 25.44. Anal. Found (calcd) for $C_{24}H_{50}N_4O_{10}P_2$: C, 45.94 (46.75); H, 8.20 (8.17); N, 9.14 (9.17).[11]

1,4,7,10-Tetraazacyclododecane-1,7-bis(methanephosphonic acid monoethyl ester sodium salt) (DO2PME). 1,7-Bis(ethoxycarbonyl)-1,4,7,10-tetraazacyclododecane-4,10-bis(methanephosphonic acid diethyl ester) (1.82 g, 2.95 mmol) was dissolved in ethanol (10 mL) in a Teflon flask. Sodium hydroxide (2.5 g) dissolved in water (10 mL) was added, and the ethanol was boiled off with periodic addition of water. The solution was then refluxed for 5 days. Removal of the water by rotary evaporation gave a solid residue containing the product and excess sodium hydroxide. The product was extracted into CH_2Cl_2 (4 × 20 mL). The solution was dried over Na_2SO_4 and filtered. Removal of the solvent by rotary evaporation gave a solid residue, which was taken up with ether (50 mL); the solid was filtered off on a Schlenk filter and washed with ether (3 × 50 mL). It was dried in a stream of nitrogen to give 1.05 g (72%) of a white, very hygroscopic solid. ^{1}H NMR (200 MHz, D_2O/DSS), pD ~11), δ (ppm): 3.91 (m, 4H, OCH_2), 2.83 (d, J_{PH} = 8.55 Hz, 4H, NCH_2P), 2.79 (br, 8H, PCH2NCH_2CH_2NH), 2.63 (br, 8H, $PCH_2NCH_2CH_2NH$), 1.26 (t, 6H, OCH_2CH_3). ^{13}C NMR (50.1 MHz, D_2O/DSS), δ (ppm): 61.97 (d, J_{PC} = 5.86, $POCH_2$), 52.66 (d, J_{PC} = 4.40 Hz, $PCH_2NCH_2CH_2NH$), 52.01 (d, J_{PC} = 143.56 Hz, PCH_2N), 45.37 ($PCH_2NCH_2CH_2NH$), 17.76 (d, J_{PC} = 5.85 Hz, $POCH_2CH_3$). ^{31}P NMR (D_2O/H_3PO_4), δ (ppm): 22.19. Anal. Found (calcd) for $C_{14}H_{32}N_4O_6P_2Na_2 \cdot 2H_2O$: C, 33.52 (33.88); H, 7.36 (7.31); N, 11.12 (11.29).[11]

pH Potentiometry and UV Spectrophotometry. The protonation constants of H_4DO2P and $H_2DO2PME$ ligands were determined by pH potentiometric titration of 0.005 M ligand solutions. The first protonation constant of DO2P was confirmed by UV spectrophotometry. The absorbance values of a 3×10^{-4} M ligand solution were determined

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(11) The carbon analyses were consistently lower than theory for the phosphonate diester ligands (for reasons yet unexplained, even though they were clean by TLC and NMR), yet the carbon analyses for the hydrolyzed products, $H_4DO2P \cdot HCl$ and $Na_2DOTPME$, agreed well with theory.

at eight wavelengths between 240 and 260 nm while the OH^- ion concentration was varied between 0.03 and 0.1 M (the sum of the KOH and KCl concentrations was kept constant at 0.1 M). All spectrophotometric measurements were carried out using a Varian DMS 100 spectrophotometer.

The stability constants of DO2PME and DO2P complexes formed with Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}, and Zn^{2+} were determined by direct pH potentiometry. The titrations were carried out using 15 mL sample solutions in a thermostated (25 °C) glass-jacketed vessel in an atmosphere of N_2. The metal ion concentrations were typically 1 mM whereas the ligand concentrations were varied. The stability constants of the $LnDO2P^-$ complexes were determined using the out-of-cell technique because complex formation was slow. For each Ln^{3+}, 18−20 separate 1.0 mL samples were prepared and equilibrated at room temperature for 4−5 days. The $LnCl_3$ concentration was 0.002 M whereas ligand concentration was either 0.002 or 0.004 M.

pH Potentiometric titrations were carried out with a Radiometer ABU-80 autoburet and a Radiometer PHM-85 pH-meter equipped with G202B glass and K401 calomel electrodes. For measuring the pH in the separate samples, a GK2421C combined electrode was used; 0.05 M KH−phthalate (pH = 4.008) and 0.01−0.01 M Na_2HPO_4−KH_2PO_4 (pH 6.865) buffers were used to calibrate the electrodes. The electrode system was calibrated by titrating a HCl solution of known concentration with KOH. The difference between each measured and calculated pH value (over the pH range 2−3) was used to calculate the H^+ ion concentration from the pH values obtained in the titration experiments.[12] The ion product of water was determined from the same HCl−KOH titration over the pH range 11−12 (pK_w = 13.91). All equilibrium measurements were carried out at 25 °C at constant ionic strength (0.1 M KCl).

The stepwise protonation constants of the ligands are defined as

$$K_i = [H_iL]/[H_{i-1}L][H^+]$$

$$K^{H}_{MH_{i-1}L} = [MH_iL]/[MH_{i-1}L][H^+]$$

while the overall equilibrium constants describing all metal−ligand species, $M_pH_qL_r$, are defined as

$$\beta_{pqr} = [M_pH_qL_r]/[M]^p[H^+]^q[L]^r$$

Thus, $\beta_{101} = K_{ML}$. The program PSEQUAD[13] was used to calculate all protonation and metal ion−ligand stability constants.

NMR Measurements. ^1H, ^{13}C, ^{31}P, and ^{17}O NMR spectra were recorded using a 5 mm broad-band tunable probe on either a Bruker GN500 or a JEOL FX-200 NMR spectrometer. ^1H NMR spectra were recorded in $CDCl_3$ or D_2O using TMS or DSS, respectively, as an internal reference. ^{13}C NMR spectra were recorded in $CDCl_3$ or in D_2O using the methyl carbon of *tert*-butyl alcohol as the internal reference at 31.2 ppm. ^{31}P shifts were referenced to external 85% H_3PO_4 while ^{17}O shifts were referenced to water. Longitudinal water proton relaxation times (T_1) were measured using an MRS-4 NMR spectrometer (Jozef Stefan Institute, Ljubljana, Slovenia) operating at 9 MHz. The sample holder was kept at 25 °C by an air stream. T_1 values were measured using the inversion recovery method.

Samples of DO2P (10 mM) for NMR pD titrations were prepared in D_2O (99.9%, Aldrich) containing 0.1 M NaCl to maintain constant ionic strength and used for NMR pD titrations. For ^{31}P NMR complexation titrations, solutions of 40 mM DO2P were prepared in D_2O containing varying amounts of $EuCl_3$ (99.9% from Sigma). The pD (=pH + 0.40) was adjusted with DCl or NaOD and measured using an Accumet 925 pH meter. An Orion 8103 Ross combination electrode used in the NMR pH titrations allowed a reliable and accurate determination of pH over an extended range. A sample of $DyDO2P^-$ was used for determination of the number of inner-sphere water molecules using the method of Alpoim et al.[14] In that experiment, the chemical shift of the ^{17}O NMR signal of bulk water was monitored while [$DyDO2P^-$] was varied from 0−20 mM. The observed shifts were corrected for bulk susceptibility effects using the ^1H signal of internal *tert*-butyl alcohol.

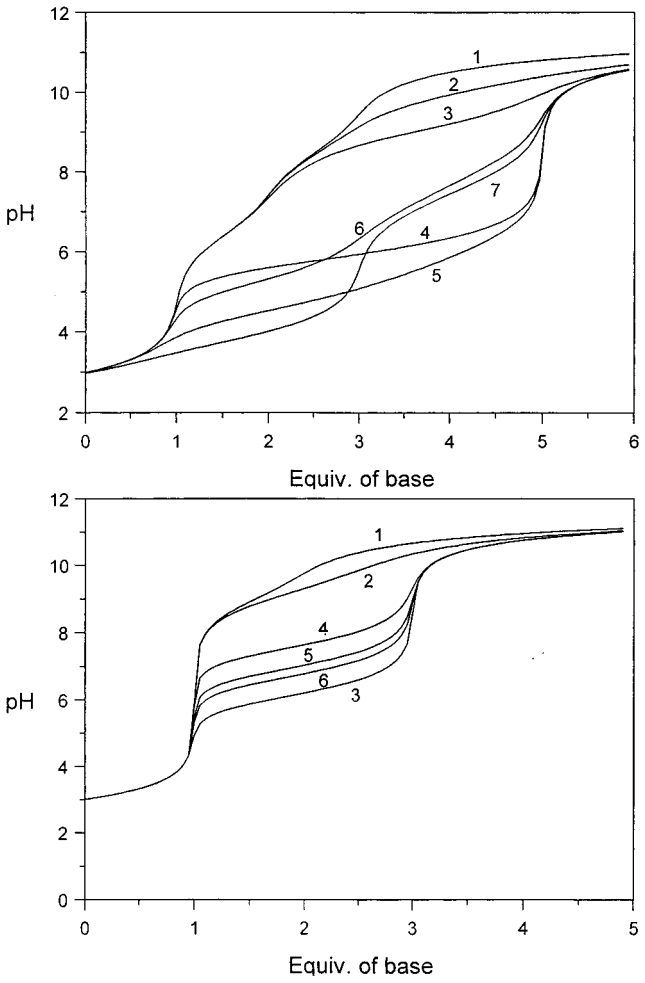

Figure 1. Top panel: Titration curves of DO2P in the absence (1) and presence of Sr^{2+} (2), Ca^{2+} (3), Mn^{2+} (4), Zn^{2+} (5), La^{3+} (6), and Er^{3+} (7). Bottom panel: Titration curves of DO2PME in the absence (1) and the presence of Ca^{2+} (2), Mn^{2+} (3), La^{3+} (4), Nd^{3+} (5), and Lu^{3+} (6). In all cases, the concentrations of metal ion and ligand were 0.001 M. (One equivalent of acid was added to the samples before titration.)

Results and Discussion

Protonation constants. Given the known protonation constants of other tetraazacyclododecane ligands,[15−17] one would anticipate that DO2P would have two high protonation constants corresponding to two macrocyclic ring nitrogens and two protonation constants near neutrality corresponding to the two phosphonate groups. A pH potentiometric titration of DO2P over the pH range 2−12 gave accurate values for the second (10.92), third (8.47), and fourth (6.39) protonation constants (see top panel of Figure 1). However, the first protonation constant was too high to be accurately determined by potenti-

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Table 1. Protonation Constants of Ligands (25 °C, 0.1 M KCl)

	DO2P	DO2PME	EDBP[a]	DO2A[b]
log K_1	12.8 (0.08)[c]	11.14 (0.015)	10.60	10.91(14)
log K_2	10.92 (0.02)	8.94 (0.02)	7.72	9.45(13)
log K_3	8.47 (0.02)		5.74	4.09(8)
log K_4	6.39 (0.02)		4.58	3.18(16)

[a] Reference 17. [b] Reference 10. [c] Determined by UV spectroscopy (see text).

ometry (the titration data indicated a log K_1 value of ~12.7). Because the UV absorption band of the DO2P ligand showed a red shift between pH 12 and 13, the value of log K_1 was refined by spectrophotometry. Thirteen samples containing constant DO2P (3×10^{-4} M) and varying amounts of KOH (0.03−0.1 M) were prepared, and the UV absorptions were determined at eight wavelengths between 240 and 263 nm. The analytical OH^- concentration was used in calculating the value of log K_1 from the UV spectrophotometric data, since $[OH^-] \sim [DO2P_t]$. Although the concentration of the diprotonated ligand, H_2L^{2-}, is very low even at the lowest OH^- concentration used (0.03 M), the measured absorbance values were taken as the sum of the absorbances of H_2L^{2-}, HL^{3-}, and L^{4-}. In calculating the molar abosorptivities (ϵ) of all three ligand species, we included the protonation constant, log K_2 (as obtained by pH potentiometry), in the fitting procedure. The program PSEQUAD[13] was used to minimize the difference between measured and calculated absorbances at all eight wavelengths (this difference was never more than 0.004). The calculated molar absorptivities of H_2L^{2-}, HL^{3-}, and L^{4-} were 106.6, 746.7, and 1811 M^{-1} cm^{-1} (at 243 nm), respectively, and 10.9, 310.3, and 1125 M^{-1} cm^{-1} (at 252 nm), respectively, while the log K_1 value was 12.8 ± 0.08 (the error represents the average standard deviation given by the program).

The protonation constants of DO2P are summarized in Table 1 and compared with literature values for the acyclic ligand EDBP[18] (ethylenediamine-*N*,*N*′-bis(methanephosphonate) and the analogous diacetate ligand DO2A.[10] While EDBP has the same type of donor atoms as DO2P, the protonation constants of the open-chain ligand are lower since the nitrogen donor atoms are closer to each other than they are in DO2P. Substitution of methanephosphonate groups (DO2P) for acetate groups (DO2A) increases the overall negative charge on the ligand and hence increases all protonation constants, analogous to the differences seen between DOTA and DOTP.[15,16] The first protonation constant of DO2P was relatively high compared to that seen for DOTA (log K_1 = 12.09[16]) but lower than that reported for the more highly charged ligand DOTP (log K_1 = 13.7[15]).

The protonation constants of DO2PME were also determined by pH potentiometry (see bottom panel of Figure 1). The fully deprotonated DO2PME has only two negative charges, and as a result, its first two protonation constants are significantly lower than those of DO2P. The oxygen atom of the $P(O)(OEt)O^-$ group is very weakly basic, and consequently its protonation occurs only at low pH values (<2).

^1H and ^{31}P NMR spectra of 10 mM DO2P were recorded as a function of pD in D_2O. The titration curves of Figure 2 can be divided into three regions: a high-pH region (pH 10−13) corresponding to two protonations, a middle-pH region (pH 6−10) corresponding to two protonations, and a low-pH region (below pH 2). The ^1H NMR spectrum of DO2P at pD 13 shows two well-resolved triplets for the ring ethylene protons and a

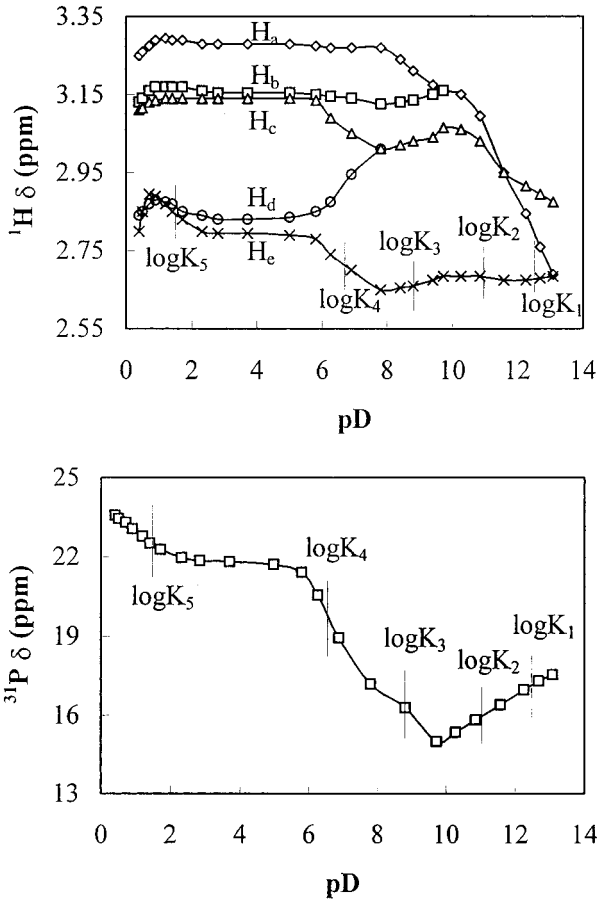

Figure 2. ^1H and ^{31}P NMR chemical shifts of DO2P as a function of pD. The pK_a values determined by potentiometry are shown on the plots for comparison purposes.

doublet for the methanephosphonate protons, consistent with rapid inversion of the macrocyclic ring nitrogens. Addition of 2 equiv of DCl resulted in deshielding of the ring protons, similar to that observed for the macrocyclic ligands NOTP (1,4,7-triazacyclononane-1,4,7-tris(methanephosphonate)), DOTRP (1,5,9-triazacyclododecane-1,5,9-tris(methanephosphonate)), and DOTP (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methanephosphonic acid).[17] The proton pairs nearest the tertiary amine groups (H_a and H_b) were deshielded more than the proton pairs closer to the secondary amine groups (H_c and H_d) over this pH range, indicating that the first two protonations take place at the tertiary nitrogens. Although tertiary nitrogens are known to be more basic than the secondary nitrogens,[17−20] the opposite was found for the ligand DO2A.[10] The ^{31}P resonances shifted upfield upon protonation of the nearby tertiary amine groups, possibly reflecting dissociation of Na^+ from the phosphonate groups.[17] Addition of an additional 2 equiv of DCl resulted in a lifting of the magnetic degeneracy of the two ethylene proton pairs and a deshielding of the ^{31}P nuclei. This is consistent with the third and fourth protonation steps occurring at the two phosphonate groups, accompanied by proton transfer from the tertiary to the secondary nitrogens and formation of hydrogen bonds between the phosphonate groups and the tertiary nitrogens. As a result of increased ligand rigidity brought about by the nitrogen−phosphonate hydrogen bonds, nitrogen inversion is restricted and each of the two geminal proton pairs becomes magnetically nonequivalent ($\Delta\delta_{ab}$ = 0.3 ppm and $\Delta\delta_{cd}$

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Table 2. Equilibrium Constants of the Complexes Formed with DO2P and DO2PME (25 °C, 0.1 M KCl) and with EDTP (25 °C, 0.1 M KNO_3)

	DO2P			DO2PME	EDTP[a]
	log K_{ML}	log K^H_{ML}	log K^H_{MHL}	log K_{ML}	log K^H_{ML}
Mg^{2+}	7.9 (0.02)	9.5 (0.03)		<3	10.07
Ca^{2+}	9.0 (0.02)	8.5 (0.06)		4.55 (0.03)	9.45
Sr^{2+}	7.1 (0.02)	9.6 (0.04)		3.38 (0.03)	10.08
Mn^{2+}	18.1 (0.02)	6.4 (0.03)	5.7 (0.05)	11.03 (0.05)	
Zn^{2+}	21.2 (0.03)	6.3 (0.03)	5.3 (0.03)	14.98 (0.02)	

	DO2P				DO2PME	EDTP[b]	
	log K_{ML}	log K^H_{ML}	log K^H_{MHL}	log β_{112}	log K_{ML}	log K^H_{ML}	log K^H_{MHL}
La^{3+}	16.0 (0.08)	8.2 (0.19)	7.1 (0.11)	34.4 (0.3)	8.16 (0.04)	7.1	7.6
Nd^{3+}	17.2 (0.1)	8.0 (0.16)	7.2 (0.08)	36.5 (0.2)	9.37 (0.06)	7.19	6.68
Eu^{3+}	18.3 (0.08)	7.9 (0.10)	7.1 (0.06)	38.1 (0.2)	9.75 (0.08)	7.31	6.25
Gd^{3+}	18.2 (0.09)	8.1 (0.1)	7.0 (0.06)	37.9 (0.2)	9.81 (0.07)	7.35	6.19
Er^{3+}	19.2 (0.1)	7.9 (0.14)	7.0 (0.07)	39.2 (0.4)	9.68 (0.06)	7.36	6.09
Lu^{3+}	19.3 (0.2)	8.2 (0.2)	7.2 (0.1)	38.7 (0.5)	9.88 (0.08)	7.42	5.9

[a] Reference 21. [b] Reference 23.

= 0.15 ppm). The chemical shifts observed in the low-pD range are consistent with the fifth and perhaps sixth protonations occurring at the phosphonate groups.[17]

Stability Constants. Complex formation equilibria of DO2P and DO2PME were studied with Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+}, and Zn^{2+} and with some lanthanide(III) ions. The complexes of DO2PME were formed sufficiently fast and the direct pH potentiometric titration could be performed (Figure 1) (0.5−1 min was required to reach equilibrium at the lower pH values for the $LnDO2PME^+$ complexes). Three parallel titrations were carried out using 1:1, 1:3, and 1:5 metal:ligand ratios. Complex equilibria could be described by assuming formation of only ML complexes. The stability constants are summarized in Table 2.

Complexation equilibria in the Mg^{2+}−, Ca^{2+}−, Sr^{2+}−, Mn^{2+}−, and Zn^{2+}−DO2P systems were also studied by direct titration, while formation of $LnDO2P^-$ complexes was studied by using the out-of-cell technique, both at 1:1 and 1:2 metal:ligand ratios. The Mg^{2+}−, Ca^{2+}−, and Sr^{2+}−DO2P equilibria were best described by the formation of MHL and ML complexes, while the Mn^{2+}, Zn^{2+}, and Ln^{3+} potentiometric data were best fit by assuming the formation of MH_2L, MHL, and ML complexes. Diprotonated complexes are particularly stable in the case of lanthanides, where the LnH_2L complexes predominate in the pH range 3−6 (when 1−3 equiv of KOH was added; see Figure 1). In the presence of excess ligand, Ln^{3+} ions also form LnL(HL) complexes at pH values higher than about 8, likely a species $Ln(DO2P)(HDO2P)^{4-}$ (see ^{31}P NMR results below). When the amount of KOH added to the samples was increased, a small pH increase could be observed at around the seventh equivalent of base, indicating coordination of the species $HDO2P^{3-}$ to LnL. The second ligand is probably weakly bound, since the Ln^{3+} ion has only two (or three) free coordination sites in the $LnDO2P^-$ complex. This weak interaction does not promote dissociation of the proton from the ligand, $HDO2P^{3-}$, which possesses a very high protonation constant. In the calculation of stability constants, the best fit was obtained when species LnH_2L, LnHL, LnL, and LnL(HL) were assumed to be present. The stability constants of all complexes as determined by this model are summarized in Table 2 (the standard deviations for the LnH_xL constants are higher than the others, typical of titrations using "out-of-cell" techniques). These constants indicate that DO2P forms significantly more stable complexes than the monophosphonate ester ligand, DO2PME, consistent with the weakly basic $P(O)(OEt)O^-$ groups. Zn^{2+}, a cation that forms strong bonds with nitrogen donors, formed the most stable complex with both ligands. The stability constants of the DO2P complexes formed with La^{3+} and Gd^{3+} are about 1 log K unit less than those of corresponding DO2A complexes.[10]

The protonation constants of the alkaline earth metal−DO2P complexes, K^H_{ML} (representing the extent of the reaction, ML + H^+), are all quite high. The values of K^H_{ML} determined here are slightly lower than the protonation constants of the corresponding alkaline earth metal complexes with EDTP (ethylenediaminetetrakis(methanephosphonic acid)),[21] a ligand with higher charge and a higher first protonation constant, log K_1, than those of DO2P. Sawada et al.[23] have reported that the first protonation of the alkaline earth metal−EDTP complexes occurs at a nitrogen atom of the ligand and not at a coordinated phosphonate oxygen. The protonation constants found here for $SrDO2P^{2-} \sim MgDO2P^{2-} > CaDO2P^{2-}$ fall in the order of the complex stabilities, consistent with protonation at a nitrogen atom as well.

The protonation constants, K^H_{ML} and K^H_{MHL}, of the $LnDO2P^-$ complexes are also relatively high (Table 2), around 8 and 7, respectively, for all Ln^{3+} complexes examined. These values are nearly identical to those found for the $LnDOTP^{5-}$ complexes[22] but are about 1 order of magnitude higher than the protonation constants of the $LnEDTP^{5-}$ complexes[23] (summarized in Table 2). We have shown by ^{31}P NMR that the $LnDOTP^{5-}$ complexes[22] undergo four protonations at phosphonate oxygens without disruption of a Ln−N bond. Similarly, Sawada et al.[21] have suggested that the first protonation of the $LnEDTP^{5-}$ complexes occurs at a coordinated phosphonate oxygen of the ligand (unlike the case of alkaline earth metal−EDTP complexes), while the second protonation occurs at a ligand nitrogen. This conclusion was based upon the insensitivity of log K^H_{ML} to the identity of the Ln^{3+} cation and decreasing log K^H_{MHL} values with decreasing Ln^{3+} ionic radius (see Table 2). Thus, the lack of sensitivity we observe in the protonation constants of the $LnDO2P^-$ complexes with changing Ln^{3+} ionic radius suggests that both the first and second protonations occur at phosphonate oxygens and not at coordinated nitrogens.

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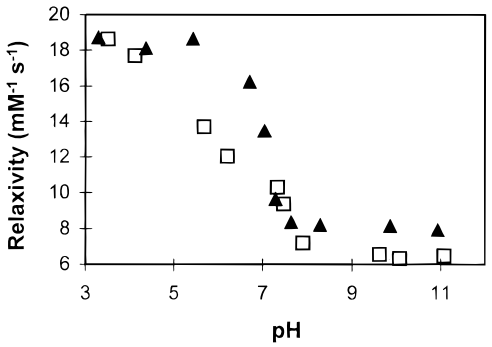

Figure 3. Water proton relaxivity values (9 MHz, 25 °C) of 1 mM GdDO2P^{-} (▲) as a function of solution pH. The (□) data are for a sample of 1 mM GdDO2P^{-} containing excess DO2P (1 mM).

Water Coordination: ^{17}O NMR Shifts and Water Proton Relaxation Rate Measurements. The water proton relaxation rates of solutions containing 1.0 mM Gd^{3+} and 1.0 mM or 2.0 mM DO2P are shown as a function of pH in Figure 3. There was an abrupt decrease in relaxivity above pH ~5.5 for solutions containing the 1:1 complex. Above pH 8, the measured relaxivity was constant and equal to 8.6 mM^{-1} s^{-1} (25 °C, 9 MHz), somewhat higher than those observed for GdDOTA^{-} (7.2 mM^{-1} s^{-1}, 23 °C, 10 MHz)[24] and GdDTPA^{2-} (5.6 mM^{-1} s^{-1}, 23 °C, 10 MHz)[24] with $q = 1$ and similar to that observed for GdNOTA (7.5 mM^{-1} s^{-1}, 25 °C, 9 MHz) with $q = 3$.[25] The water coordination number of the of DyDO2P^{-} at high pH was evaluated by ^{17}O NMR.[14] The chemical shift of the water ^{17}O NMR signal was linearly proportional to [DyDO2P^{-}] at pH 10, with a slope of -4000 M^{-1} ppm ($r^2 = 0.979$), corresponding to $q = 2$ for this complex.[14] If one assumes GdDO2P^{-} has the same structure as DyDO2P^{-}, then its water relaxivity appears to be enhanced somewhat over that expected for a system with two inner-sphere water molecules, perhaps due to a greater outer-sphere contribution from water molecules hydrogen-bonding to the coordinated phosphonate groups of GdDO2P^{-} as reported for GdDOTP^{5-}.[26]

The species distribution curves show that, between pH 5 and 6, the GdH_2DO2P^{+} species predominates, while at pH 8.5 both GdHDO2P (50%) and GdDO2P^{-} (50%) are present. These results and the high relaxivity values below pH ~5.5 suggest that the Gd^{3+} ion is in an "out-of-cage" position in the GdH_2-DO2P^{+} complex, perhaps with the Gd^{3+} ion only coordinated to the two phosphonate groups. As the GdHDO2P species is formed above pH ~6, the relaxivity decreases significantly, reflecting movement of the Gd^{3+} ion to an "in-cage" position. This is consistent with the idea that the proton in GdHDO2P is coordinated to a phosphonate oxygen. The abrupt decrease in relaxivity values between pH 6 and 8 indicates that the relaxivities of the complexes GdHDO2P and GdDO2P^{-} do not differ significantly. In the presence of excess ligand, formation of the binary ligand species, GdDO2P(HDO2P)$^{4-}$, begins at lower pH values, as evidenced by a nearly linear decrease in R_1 values from pH 3 to pH 7.4. At pH >9, GdDO2P(HDO2P)$^{4-}$ predominates and the relaxivity value is smaller by about 2 mM^{-1} s^{-1} compared with the value observed for the 1:1 sample. The relaxivity of GdDO2P(HDO2P)$^{4-}$ is similar to that of GdDOTP^{5-} ($R = 6.6$ mM^{-1} s^{-1}),[25,27] a system which has no inner-sphere coordinated H_2O's.[22,26]

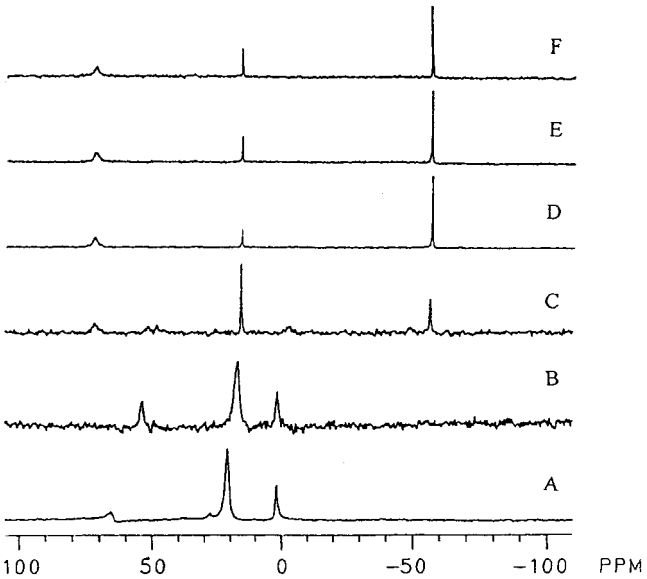

Figure 4. ^{31}P NMR spectra of 40 mM DO2P solutions containing 20 mM Eu^{3+} at pD values of 5.0 (A), 6.5 (B), 9.3 (C), 9.8 (D), 10.1 (E), and 10.5 (F), respectively.

Complexes Formed between Eu^{3+} and DO2P As Detected by ^{31}P NMR. Below pD 4, exchange between free unbound DO2P and Eu^{3+}-bound DO2P is relatively fast, as evidenced by a single ^{31}P resonance that shifted with increasing [Eu^{3+}]. Plots of the Eu^{3+}-induced ^{31}P shifts as a function of $[Eu^{3+}]_T/[DO2P]_T$ ratio at pD 1.0 and 2.8 (data not shown) were fit to a 1:1 binding model ($Eu^{3+} + H_4DO2P \rightleftharpoons EuH_4DO2P^{3+}$) to give a limiting shift of -55 ppm and equilibrium constants of 50 M^{-1} (pD 1.0) and 81 M^{-1} (pD 2.8). The magnitude of these equilibrium constants suggests that Eu^{3+} binds only to the phosphonate groups of the ligand below pD ~3.

Figure 4 shows ^{31}P NMR spectra of 40 mM DO2P solutions containing 20 mM Eu^{3+} at several pD values. Three major resonances were observed under these experimental conditions. The peak at about 20 ppm showed the same pD dependence as free, uncomplexed DO2P (Figure 2). The small resonance near 4 ppm in the pD 5.0 and 6.5 spectra may be assigned to a species in which DO2P is coordinated to Eu^{3+} through the two phosphonate groups, forming an "out-of-cage" species. Such a structure was also observed recently for a EuDOTP^{5-} reaction intermediate under similar conditions (J. Ren, unpublished data). A previous lanthanide-induced-shift study of the LnDOTP^{5-} complexes showed that Eu^{3+} induces a hyperfine shift to high frequency in the ^{31}P resonance when the Eu^{3+} is coordinated to the four ring nitrogens and the four pendant methanephosphonate groups of DOTP.[22] The ^{31}P resonance of the "in-cage" EuDOTP^{5-} complex shifted upfield with increasing pH, from 56.8 ppm at pH 5.5 to 48.8 ppm at pH 10, largely reflecting an increase in the magnitude of the pseudocontact shift.[22] This allows us to tentatively assign the resonance at 65 ppm in the spectrum of EuH_2DO2P^{+} recorded at pD 5 to a species in which Eu^{3+} ion is coordinated to all four ring nitrogens and the two pendant methanephosphonate groups. On the basis of the relaxivity data and ^{17}O NMR results reported above, this "in-cage" species is expected to have two (or perhaps three, since Eu^{3+} is a larger cation) water molecules occupying its coordina-

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tion sphere. As with $EuDOTP^{5-}$, the ^{31}P resonance of the "in-cage" EuH_2DO2P^+ complex shifted to 56 ppm at pD 6.5, and finally disappeared at pD 9.3. At pD 9.3, a new peak emerged at 75 ppm, indicating that a major structural change occurred in solution. Given the ^{31}P chemical shift of this new species, it most likely reflects an "in-cage" species with the phosphonate groups of the second DO2P ligand occupying the coordination sites normally occupied by water molecules at lower pH. If the second ligand binds Eu^{3+} along the 2-fold symmetry axis of the complex base formed by the first ligand and the metal ion, the ligand at the "capping" position would display a high-field Eu-induced shift, likely dominated by a pseudocontact contribution ($3\cos^2\theta - 1 > 0$). A sharp ^{31}P resonance was observed at -55 ppm with an integral equal to that of the high-frequency 75 ppm peak, thus supporting the assignment of a $EuDO2P(HDO2P)^{4-}$ species with one ligand encapsulating the ion and the second capping the ion. Slight precipitation of europium hydroxide and appearance of free DO2P were noted in solutions above pD 10. The formation of a $EuDO2P(HDO2P)^{4-}$ species is consistent with the potentiometric data and the decrease in water relaxivity observed for $GdDO2P(HDO2P)^{4-}$ over the pH range 3−7.

In summary, the structures of complexes formed with the fully deprotonated ligands $DO2P^{4-}$ and $DO2PME^{2-}$ appear to be similar to those of $LnDOTA^-$ and $LnDOTP^{5-}$, with the metal ion positioned in a coordination cage formed by the four ring nitrogens and the two phosphonate oxygens.[28] Two coordination sites are presumably occupied by H_2O molecules (at least for the heavier Ln^{3+} complexes). The first two protonation constants of the $LnDO2P^-$ complexes did not vary with the ionic radius of the Ln^{3+} cation, suggesting that both protonations occur at coordinated phosphonate oxygens, similar to that seen for the $LnDOTP^{5-}$ complexes.[22] However, water relaxivity data for $GdDO2P^-$ and ^{31}P NMR data for $EuDOTP^-$ both suggest that the first protonation occurs at a phosphonate oxygen while maintaining the Ln^{3+} ion at an "in-cage" position in LnHDO2P, while the Ln^{3+} cation is likely bound to only two phosphonate groups (an "out-of-cage" position) in diprotonated species, LnH_2DO2P^+. This suggests that the second protonation step may indeed take place at a coordinated phosphonate and this "in-cage" LnH_2DO2P^+ species then rearranges, perhaps slowly, to the more stable "out-of-cage" LnH_2DO2P^+ species. This differs from the situation seen in the $H_2LnDOTP^{3-}$ complexes[22] because the coordination cage of LnH_2DO2P^+ is not as protected from solvent, so hydration of the Ln^{3+} cation may become the driving force in movement of the Ln^{3+} cation from an "in-cage" to "out-of-cage" position.

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