Synthesis and NMR Studies of New DOTP-like Lanthanide(III) Complexes Containing a Hydrophobic Substituent on One Phosphonate Side Arm

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Three derivatives of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonic acid) (DOTP) containing a hydrophobic substituent on one side chain were prepared and their lanthanide complexes examined by NMR. The new ligands include 1-(1-octyl-methyl-phosphonic acid)-4,7,10-tris(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane (C_8-DOTP), 1-(1-undecyl-methyl-phosphonic acid)-4,7,10-tris(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane (C_{11}-DOTP), and 1-(1-4-nitro-phenyl-methyl-phosphonic acid)-4,7,10-tris(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane (NO_2-Ph-DOTP). ^{1}H NMR spectra of the ytterbium(III) complexes were assigned by using a combination of COSY spectroscopy and a fitting procedure that matches experimental NMR hyperfine shifts with those estimated from a MMX-derived structure. The analysis showed that a single isomer is present in solution and that the bulky hydrophobic substituent occupies the less sterically demanding H_6 equatorial position in the YbL^{5-} complexes. Although the YbL^{5-} complexes have lower symmetry due to the added substituent, the average ^{1}H hyperfine shifts are 5−10% larger in these complexes compared to $YbDOTP^{5-}$. This was magnified further in the hyperfine ^{23}Na NMR shifts of ion-paired sodium ions where the extracellular Na^+ signal in perfused rat hearts displayed a 28% larger hyperfine shift in the presence of $Tm(C_{11}\text{-DOTP})^{5-}$ than with an equivalent amount of $TmDOTP^{5-}$.

Introduction

The thulium complex of DOTP (Chart 1), $TmDOTP^{5-}$, has been widely applied as a hyperfine frequency shift reagent (SR) in resolving the NMR resonances of Na^+ in extracellular and intracellular compartments.[1,2] The intrinsic negative charge on the complex ($HTmDOTP^{4-}$ is the predominant anionic species at pH 7.4[3]) prevents it from crossing cell membranes, and thus the complex forms ion-pair complexes with Na^+ in all extracellular space. Since it was first introduced in the mid-1980s, $TmDOTP^{5-}$ has been widely used to separate the intra- and extracellular ^{23}Na resonances from isolated cells,[4−6] perfused organs,[7−14] and intact animals[15−19] by ^{23}Na NMR. This SR is currently considered the best available for in vivo use even though some of its chemical properties could be improved upon. Notably, $TmDOTP^{5-}$ forms strong ion pairs with Ca^{2+}, Mg^{2+}, and other metal cations,[20] and this binding competition not only reduces the efficiency of the SR but also tends to reduce mean arterial blood pressure (∼20%) when infused into laboratory rats.[16] $TmDOTP^{5-}$ quickly distributes throughout all extracellular space and is filtered by the kidneys with a time constant of about 12 min.[18] This relatively short renal clearance time requires that the SR is infused continually throughout a ^{23}Na NMR study to maintain an adequate shift separation between the intra- and extracellular Na^+ resonances.

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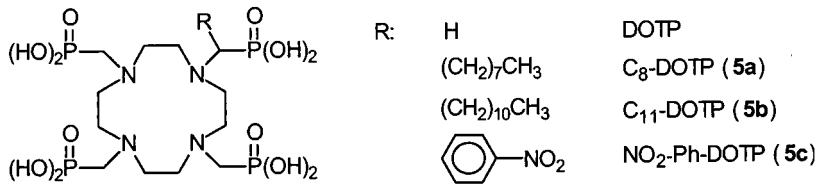
Chart 1



There are three steps one might take to improve the properties of $TmDOTP^{5-}$: (1) reduce its affinity for Ca^{2+} and Mg^{2+}; (2) increase the magnitude of the ^{23}Na hyperfine shift so that less SR is required; and (3) reduce its in vivo clearance rate. The first is difficult to solve because any SR with a reduced affinity for divalent ions would likely also have reduced affinity for sodium.[21] In this work, we have attempted to address the second and third approaches using a single ligand modification. It has been reported that the hyperfine shifts in DOTA-like complexes increase with increasing ligand rigidity.[22,23] This suggested that introduction of a substituent somewhere in the ligand backbone might make the complex more rigid and hence magnify the hyperfine shifts. Second, a hydrophobic substituent could help to prolong the in vivo lifetime of a SR through noncovalent or covalent interaction with plasma proteins.

In this work, we report the synthesis and characterization of new DOTP-like ligands having an extended functional group on a methylenic carbon of a phosphonate pendant arm. The new ligands (Chart 1) included 1-(1-octyl-methyl-phosphonic acid)-4,7,10-tris(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane (C_8-DOTP), 1-(1-undecyl-methyl-phosphonic acid)-4,7,10-tris(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane (C_{11}-DOTP), and 1-(1-4-nitro-phenyl-methyl-phosphonic acid)-4,7,10-tris(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane (NO_2-Ph-DOTP). The latter derivative is particularly interesting since the NO_2 substituent on the phenyl group can be reduced to NH_2 and this provides a site for further modification of the ligand. Paramagnetic lanthanide complexes of these ligands were prepared and investigated by NMR spectroscopy, and some preliminary ^{23}Na NMR shifts were measured in a perfused heart preparation.

Experimental Section

General. 1,4,7,10-Tetraazacyclododecane (cyclen) and DOTP were obtained from Macrocyclics (Dallas, TX). BSA (fatty acid free and regular) was purchased from Sigma and was used without further purification. Lanthanide chloride stock solutions (~0.2 M) were standardized with EDTA (0.005 M) using xylenol orange as the endpoint indicator in NaAC−HOAC buffer (pH = 5.2). All other chemicals and solvents were purchased from commercial sources and were used without further purification. Silica gel (200−400 mesh, 60 Å) for column chromatography was purchased from Aldrich. TLC was conducted on Whatman precoated silica gel on polyester plates. All hydrogenation reactions were carried out in a Parr hydrogenation apparatus. pH measurements were made at room temperature with a Fisher Scientific Accumet 925 pH/ion meter and a calibrated Orion 8103 Ross combination pH electrode. Molecular models and molecular mechanics (MM+) calculations were conducted using HyperChem 5.0 (Hypercube Inc., Gainesville, FL). Elemental analysis was performed by Galbraith Laboratories (Knoxville, TN).

Preparation of Lanthanide Complexes. Lanthanide complexes used in relaxometric and Na^+ shift experiments were prepared by mixing stoichiometic amounts of lanthanide chloride stock solutions with 5−10% excess ligand. Complexes prepared for high-resolution NMR studies (^1H, ^{13}C, ^{31}P, $^1H-^1H$ COSY) were prepared by reacting a ligand with 10−15% excess lanthanide chloride. The ligands were suspended in water and were solubilized by adding either tetramethylammonium hydroxide (for the Tm^{3+} complex) or sodium hydroxide (for the other lanthanide complexes) to pH ~ 8−9. Lanthanide chloride solutions were added in one portion, and the mixture was heated to ~65 °C. The pH of the mixture was maintained at ~8−9 during complexation. After the reaction was completed, as judged by stabilization of the solution pH, the pH of the complex solution was adjusted to the desired value. When excess lanthanide chloride was used, the mixture was centrifuged to remove unreacted $Ln(OH)_3$ precipitate before adjusting the pH. The complexes were then lyophilized to a powdery solid and redissolved into water or buffers as necessary.

NMR Experiments. NMR spectra of all synthetic intermediates, the final products, and the lanthanide complexes were recorded on a Varian INOVA 500 spectrometer. ^1H, $^{13}C\{^1H\}$, ^{23}Na, and ^{31}P spectra were obtained using a 5 mm tunable broad-band probe at 499.8, 125.7, 132.2, and 202.3 MHz, respectively, at 25 °C. $^1H-^1H$ COSY spectra were acquired using an ID/PFG probe. ^1H and ^{13}C NMR chemical shifts were referenced to either TMS or solvent resonances (organic solvents) and to *tert*-butyl alcohol (^1H at 1.24 ppm, ^{13}C at 30.29 ppm) in aqueous solution. ^{31}P NMR chemical shifts were referenced to external 85% aqueous H_3PO_4 solution ($\delta = 0$ ppm) while ^{23}Na NMR chemical shifts were referenced to external 140 mM NaCl solution in D_2O ($\delta = 0$ ppm).

Heart Perfusions. Male Sprague−Dawley rats weighing 350−400 g were anesthetized in an air/diethyl ether chamber. Hearts were rapidly excised and placed in ice-cold phosphate-free Krebs−Henseleit (KH) perfusate. After cannulation of the aorta, perfusion was started at a constant pressure of 100 cm of water. Hearts were initially perfused with KH buffer containing 147 mM Na^+, 124.6 mM Cl^-, 29 mM HCO_3^-, 5 mM K^+, 1.5 mM Ca^{2+}, 1.2 mM Mg^{2+}, 1.2 mM SO_4^{2-}, and 10 mM glucose. For Ca^{2+}-free perfusions an identical buffer without Ca^{2+} was used. The perfusion apparatus had two buffer chambers to allow easy switching from one perfusate to another without interruption of flow. Buffer was recirculated, bubbled continuously with 95% O_2−5% CO_2 and maintained at 37 °C. The perfusate was filtered on each pass through a 45 μm filter. After an initial stabilization period with KH buffer (15−20 min), the perfusate was switched to Ca^{2+}-free KH buffer containing 1.8 mM $TmDOTP^{5-}$ and a ^{23}Na spectrum was recorded. The perfusate was then switched to a second Ca^{2+}-free KH buffer containing 1.8 mM $Tm(C_{11}\text{-DOTP})^{5-}$, and a second spectrum was recorded. Triple quantum filtered (TQF) ^{23}Na NMR spectra were acquired using the parameters described previously by Seshan.[19]

Ligand Synthesis. Diethyl (±)-(1-Hydroxynonyl)-phosphonate (1a). Diethyl phosphite (15.338 g, 11.11 mmol) was added to nonyl aldehyde (15.046 g, 105.78 mmol), followed by the addition of triethylamine (1.07 g, 10.6 mmol). The mixture was heated at 65 °C for 26 h. The crude product was subjected to high vacuum at 65 °C overnight to afford product **1a** as a pale yellow oil, which solidified into an off-white waxlike solid (27.2 g, 91.7%). The product was used in the next step without further purification. ^1H ($CDCl_3$): δ 4.16 (m, 4H, OCH_2), 3.85 (m, 1H, HOCHP(O)), 1.34 (tt, 6H, OCH_2CH_3), 1.72 (m), 1.62 (b), 1.30 (b), 0.88 (t) (17H, $(CH_2)_7CH_3$). ^{13}C ($CDCl_3$): δ 67.62 (d, 1C, HOCHP(O)), 62.43 (dd, 2C, OCH_2), 16.38 (dd, 2C, OCH_2CH_3), 31.75, 31.25, 29.35, 29.22, 29.15, 25.64, 22.54, 13.97 (8C, $(CH_2)_7CH_3$).

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Diethyl (±)-(1-Hydroxydodecyl)-phosphonate (1b). The title compound was prepared following the method described above by mixing dodecyl aldehyde (12.71 g, 68.96 mmol), diethyl phosphite (10.0 g, 72.41 mmol), and triethylamine (0.733 g, 7.24 mmol). The product was obtained as an off-white solid (17.3 g, 84.6%) and was used in the next step without further purification. ^{1}H ($CDCl_3$): δ 4.15 (m, 4H, OCH_2), 3.84 (m, 1H, HOCHP(O)), 1.35 (t, 6H, OCH_2CH_3), 1.71 (m), 1.62 (b), 1.28 (b), 0.88 (t) (23H, $(CH_2)_{10}CH_3$). ^{13}C ($CDCl_3$): δ 67.72 (d, 1C, HOCHP(O)), 62.47 (dd, 2C, OCH_2), 16.43 (dd, 2C, OCH_2CH_3), 31.85, 31.29, 29.58, 29.56, 29.54, 29.44, 29.30, 29.12, 25.68 (d), 22.62, 14.04 (11C, $(CH_2)_{10}CH_3$).

Diethyl (±)-(1-Hydroxy-1-(4-nitro-phenyl)-methyl)-phosphonate (1c). The title compound was prepared following a method similar to that described above by mixing 4-nitrobenzyl aldehyde (20.017 g, 132.458 mmol), diethyl phosphite (19.207 g, 139.08 mmol), and triethylamine (1.407 g, 13.9 mmol) at 70 °C for 38 h. The crude product was dissolved in CH_2Cl_2 (250 mL). Diethyl ether (200 mL) was added, and the mixture was filtered to remove unreacted aldehyde. The removal of the solvent gave the compound **1c** as a brown solid (35.42 g, 92.50%). The product was used in the next step without further purification. ^{1}H ($CDCl_3$): δ 8.21, 8.18, 7.69 (d), 7.67 (d) (4H, Ar), 5.19 (d, HOCHP(O)), 4.58 (b, OH), 4.12 (m, 4H, OCH_2), 1.29 (t, 6H, CH_3). ^{13}C ($CDCl_3$): δ 147.33 (d), 144.65 (d), 127.65 (d), 123.14 (6C, Ar), 69.86 (d, 1C, (HO)CHP(O)), 63.47 (dd, 2C, OCH_2), 16.27 (t, 2C, CH_3).

Triflate of Diethyl (±)-(1-Hydroxynonyl)-phosphonate (2a). Compound **1a** (27.2 g, 99.56 mmol) was dissolved in CH_2Cl_2 (160 mL). Pyridine (10.9 g, 139.4 mmol) was added, and the mixture was cooled in liquid nitrogen to near freezing. Triflic anhydride (32.3 g, 114.5 mmol) was added dropwise with stirring. The mixture was warmed to 0 °C in an ice bath for 2 h. The solvent was removed, and the residue was extracted with diethyl ether (4 × 50 mL). The ether extracts were filtered, washed successively with cold H_2O (2 × 30 mL) and 0.5 N HCl (2 × 30 mL), dried (Na_2SO_4), and filtered. The solvent was removed under reduced pressure to afford a red-brown oil (34.5 g, 84.1%). The product was used in the next step without further purification. ^{1}H ($CDCl_3$): δ 5.0 (m, 1H, CHOTf), 4.23 (m, 4H, OCH_2), 1.38 (t, 6H, OCH_2CH_3), 2.02 (b, m), 1.28 (b), 0.88 (t) (17H, $(CH_2)_7CH_3$). ^{13}C ($CDCl_3$): δ 118.25 (q, 1C, CF_3), 82.02 (d, 1C, CHOTf), 63.54 (dd, 2C, OCH_2), 16.16 (dd, 2C, OCH_2CH_3), 31.62, 30.15, 28.97, 28.94, 28.82, 24.90 (d), 24.47, 13.88 (8C, $(CH_2)_7CH_3$).

Triflate of Diethyl (±)-(1-Hydroxydodecyl)-phosphonate (2b). Compound **1b** (10.0 g, 31.02 mol) was dissolved in CH_2Cl_2 (100 mL). Pyridine (3.9 g, 50.0 mmol) was added, and the mixture was cooled in liquid nitrogen to near freezing. Triflic anhydride (10.06 g, 35.67 mmol) was added dropwise with stirring. The reaction was warmed to 0 °C in an ice bath for 2.5 h. The solvent was removed under reduced pressure, and the residue was extracted with pentane (4 × 50 mL). The pentane extracts were filtered, washed successively with cold H_2O (2 × 20 mL) and 0.5 N HCl (1 × 20 mL), dried (Na_2SO_4), and filtered. Pentane was removed under reduced pressure to yield a red-orange oil (12.56 g, 89.1%). The product was used in the next step without further purification. ^{1}H ($CDCl_3$): δ 4.99 (m, 1H, CHOTf), 4.23 (m, 4H, OCH_2), 1.38 (t, 6H, OCH_2CH_3), 2.04 (m), 1.56 (b), 1.46 (b), 1.29 (b), 1.26 (b), 0.88 (t) (23H, $(CH_2)_{10}CH_3$). ^{13}C ($CDCl_3$): δ 118.36 (q, 1C, CF_3), 82.11 (d, 1C, CHOTf), 63.70 (dd, 2C, OCH_2), 16.26 (dd, 2C, OCH_2CH_3), 31.84, 30.22, 29.50, 29.48, 29.35, 29.25, 29.09, 28.90, 24.98 (d), 22.61, 14.02 (11C, $(CH_2)_{10}CH_3$).

Triflate of Diethyl (±)-(1-Hydroxy-1-(4-nitro-phenyl)methyl)-phosphonate (2c). Compound **1c** (10.0 g, 34.6 mmol) was dissolved in CH_2Cl_2 (100 mL). Pyridine (3.78 g, 48.4 mmol) was added, and the mixture was cooled in liquid nitrogen to near freezing. Triflic anhydride (11.2 g, 39.8 mmol) was added dropwise with stirring. The mixture was warmed to 0 °C in an ice bath for 80 min. The solvent was removed under reduced pressure, and the residue was extracted with diethyl ether (3 × 50 mL). The ether extracts were filtered, washed with cold H_2O (2 × 20 mL) and cold 0.5 N HCl (2 × 20 mL), dried (Na_2SO_4), and filtered. The solvent was removed, and the crude product was purified by silica gel flash column chromatography (250 g) eluting successively with CH_2Cl_2 and $CH_2Cl_2/(C_2H_5)_2O$ (4:1) to afford a light yellow solid (7.07 g, 48.52%). ^{1}H ($CDCl_3$): δ 8.34, 8.29, 7.75 (d), 7.71 (d) (4H, Ar), 5.99 (d, 1H, TfOCHP(O)), 4.33−4.02 (m, 4H, OCH_2), 1.31 (q, 6H, CH_3). ^{13}C ($CDCl_3$): δ 148.86, 137.7, 128.66, 124.10 (6C, Ar), 118.26 (q, 1C, CF_3), 80.76 (d, 1C, TfOCHP(O)), 64.80 (dd, 2C, OCH_2), 16.36 (dd, 2C, CH_3).

1-(1-Octyl-methyl-phosphonate)-1,4,7,10-tetraazacyclododecane (3a). Cyclen (23.545 g, 136.65 mmol) was dissolved in $CHCl_3$ (70 mL). The solution was cooled in an ice bath, and compound **2a** (11.271 g, 27.33 mmol) in $CHCl_3$ (50 mL) was added dropwise. After the addition was completed, the mixture was stirred at room temperature for 2 days. The solvent was removed under reduced pressure, and the residue was extracted with diethyl ether (4 × 100 mL). The ether extracts were combined and washed with H_2O (3 × 10 mL). Ether was separated, dried (Na_2SO_4), and filtered, and the removal of the solvent yielded a red-brown oil. The crude product was purified by silica gel flash column chromatography (80 g, the oil was first soaked onto 10 g of silica gel) eluting successively with $(C_2H_5)_2O/CH_3OH$ (60:40) and $(C_2H_5)_2O/CH_3OH/NH_4OH$ (42:50:8) to afford a yellow oil (5.496 g, 48.3%). The product was dissolved in 2 N NaOH (30 mL) and extracted with $CHCl_3$ (3 × 50 mL). The $CHCl_3$ extracts were combined, dried (Na_2SO_4), filtered, and concentrated to give a yellow oil (4.817 g, 40.55%). ^{1}H ($CDCl_3$): δ 4.11 (m, 4H, OCH_2), 2.97−2.49 (m, b, 16H, NCH_2CH_2N), 1.32 (tt, 6H, OCH_2CH_3), 1.67 (m, b), 1.29 (b), 0.87 (t) (17H, $(CH_2)_7CH_3$). ^{13}C ($CDCl_3$): δ 60.99 (dd, 2C, OCH_2), 58.33 (d, 1C, CHP(O)), 49.30 (d), 47.25 (b), 45.84 (b), 45.53 (8C, NCH_2CH_2N), 16.43 (dd, 2C, OCH_2CH_3), 31.71, 29.34, 29.19, 29.08, 27.92 (d), 27.39 (d), 22.52, 13.93 (8C, $(CH_2)_7CH_3$).

1-(1-Undecyl-methyl-phosphonate)-1,4,7,10-tetraazacyclododecane (3b). Cyclen (9.5 g, 55.0 mmol) was dissolved in $CHCl_3$ (75 mL), and the solution was cooled in an ice bath. Compound **2b** (12.5 g, 27.5 mmol) in $CHCl_3$ (75 mL) was added dropwise. After the addition, the mixture was stirred at room temperature for 3 days. The solvent was removed under reduced pressure, and the residue was extracted with diethyl ether (4 × 100 mL). The ether extracts were combined and washed with H_2O (5 × 4 mL). Ether was separated, and the aqueous extracts were combined and extracted with diethyl ether (5 × 100 mL). The ether extracts from the above two processes were combined, dried (Na_2SO_4), and filtered, and the removal of ether gave a red-brown oil (11.964 g, 91%). Purification by silica gel flash column chromatography (120 g, the crude product was first soaked onto 20 g of silica gel) eluting successively with $(C_2H_5)_2O/CH_3OH$ (70:30), $(C_2H_5)_2O/CH_3OH/NH_4OH$ (55:40:5) yielded a yellow oil. The product was dissolved in 3 N NaOH (20 mL) and extracted with $CHCl_3$ (3 × 50 mL). The $CHCl_3$ was separated, combined, dried (Na_2SO_4), filtered, and concentrated to afford a yellow oil (8.01 g, 60.93%). ^{1}H ($CDCl_3$): δ 4.11 (m, 4H, OCH2), 2.97−2.50 (m, b, 16H, NCH_2CH_2N), 1.32 (t, 6H, OCH_2CH_3), 1.68 (m), 1.26 (b), 0.88 (t) (23H, $(CH_2)_{10}CH_3$). ^{13}C ($CDCl_3$): δ 61.0 (dd, 2C, OCH_2), 58.29 (d, 1C, CHP(O)), 16.45 (dd, 2C, OCH_2CH_3), 49.34, 47.31 (b), 45.88, 45.60 (8C, NCH_2CH_2N), 31.74, 29.52, 29.46, 29.42, 29.19, 27.98 (d), 27.40 (d), 22.51, 13.95 (11C, $(CH_2)_{10}CH_3$).

1-(1-4-Nitro-phenyl-methyl phosphonate)-1,4,7,10-tetraazacyclododecane (3c). Cyclen (2.16 g, 12.5 mmol) was dissolved in $CHCl_3$ (15 mL), to which compound **2c** (4.8 g, 11.4 mmol) in $CHCl_3$ (25 mL) was added dropwise over 4 h. After 5 days the solvent was removed, acetone (150 mL) was added, and the unreacted cyclen was filtered off. While the solvent was being removed, the product started to precipitate. The crude product was dissolved in H_2O/acetone and soaked onto ~10 g of silica gel, and the solvent was removed by rotary evaporation. The silica gel was loaded on top of another 65 g of silica gel. The column was eluted successively with $(C_2H_5)_2O/CH_3OH$ (60:40) and $(C_2H_5)_2O/CH_3OH/NH_4OH$ (55:40:5). Fractions containing the product were combined, and the removal of the solvent afforded product **3c** as a light yellow solid (4.6 g, 91%). ^{1}H ($CDCl_3$): δ 8.23, 8.22, 7.76, 7.74 (4H, Ar), 4.30 (d, 1H, CHP(O)), 4.25 (m), 4.0 (m), 3.87 (m) (4H, OCH_2), 3.25 (b), 2.80 (b), 2.66 (b), 2.52 (b, m) (16H, NCH_2-CH_2N), 1.38 (t, 3H, CH_3), 1.10 (t, 3H, CH_3). ^{13}C ($CDCl_3$): δ 147.45, 140.89 (d), 131.24 (d), 123.26 (6C, Ar), 62.34 (dd, 2C, OCH_2), 60.79 (d, 1C, CHP(O)), 49.06 (b), 47.20, 45.98, 45.06 (8C, CH_2CH_2N), 16.3 (dd, 2C, CH_3).

1-(1-Octyl-methyl-phosphonate)-4,7,10-tris(methylene phosphonate)-1,4,7,10-tetraazacyclododecane (4a). To compound **3a** (4.817 g, 11.083 mmol) were added paraformaldehyde (1.166 g, 36.573 mmol)

and triethyl phosphite (6.353 g, 38.263 mmol). The mixture was stirred at room temperature for 4 days and then subjected to high vacuum first at room temperature and again at 65 °C overnight to remove excess reactants and volatile impurities. The resultant brown oil was purified by silica gel flash column chromatography (200 g, the crude product was first soaked onto 15 g of silica gel) eluting successively with $(C_2H_5)_2O/CH_3OH$ (70:30) and $(C_2H_5)_2O/CH_3OH/NH_4OH$ (65:30:5) to give a yellow oil (4.673 g, 47.65%). ^1H ($CDCl_3$): δ 4.18 (m, 4H, OCH_2), 4.11 (m, 12H, OCH_2), 3.91 (d, 1H, CHP(O)), 2.97 (d, 2H, $CH_2P(O)$), 2.94 (d, 4H, $CH_2P(O)$), 3.01−2.83 (m, b, 16H, NCH_2CH_2N), 1.33(t, 24H, OCH_2CH_3), 1.61 (m, b), 1.45 (b), 1.35 (t), 1.27 (b), 0.88 (t) (17H, $(CH_2)_7CH_3$). ^{13}C ($CDCl_3$): δ 61,45 (m, 6C, OCH_2), 60.87 (d, 2C, OCH_2), 60.19 (d, 1C, CHP(O)), 50.17 (d, 1C, $CH_2P(O)$), 50.06 (d, 2C, $CH_2P(O)$), 54.55 (d), 53.16 (d), 52.73(b), 50.25(b) (8C, NCH_2CH_2N), 16.43 (m, 8C, OCH_2CH_3), 31.78, 29.51, 29.44 (d), 29.26, 27.27 (d), 22.57, 14.0 (8C, $(CH_2)_7CH_3$).

1-(1-Undecyl-methyl-phosphonate)-4,7,10-tris(methylene phosphonate)-1,4,7,10-tetraazacyclododecane (4b). The title compound was prepared following the method described above by mixing compound **3b** (8.0 g, 16.75 mmol), paraformaldehyde (1.682 g, 55.53 mmol), and triethyl phosphite (9.184 g, 55.27 mmol). Purification with silica gel flash column chromatography (150 g) eluting with $(C_2H_5)_2O/CH_3OH$ (60:40) afforded compound **4b** as a brown oil (11.795 g, 75.7%). ^1H ($CDCl_3$): δ 4.21−4.07 (m, 16H, OCH_2), 3.91 (d, 1H, CHP(O)), 2.97 (d, 2H, $CH_2P(O)$), 2.94 (d, 4H, $CH_2P(O)$), 3.02−2.82 (m, 16H, NCH_2CH_2N), 1.33 (t, 24H, OCH_2CH_3), 1.61 (m, b), 1.45 (b), 1.35 (t), 1.26 (b), 0.88 (t) (23H, $(CH_2)_{10}CH_3$). ^{13}C ($CDCl_3$): δ 61.39 (m, 6C, OCH_2), 60.80 (d, 2C, OCH_2), 60.16 (d, 1C, CHP(O)), 50.16 (d, 1C, $CH_2P(O)$), 50.04 (d, 2C, $CH_2P(O)$), 54.53 (d), 53.15 (d), 52.72 (d), 50.22 (b) (8C, NCH_2CH_2N), 31.79, 29.61, 29.56, 29.53, 29.47, 29.23, 27.91 (d), 27.26 (d), 25.55, 13.99 (11C, $(CH_2)_{11}CH_3$), 16.46 (m, 8C, OCH_2CH_3).

1-(1-4-Nitro-phenylmethyl phosphonate)-4,7,10-tris(methylene phosphonate)-1,4,7,10-tetraazacyclododecane (4c). The title compound was prepared following the method described above by mixing compound **3c** (3.0 g, 6.76 mmol), paraformaldehyde (0.679 g, 7.102 mmol), and triethyl phosphite (3.709 g, 7.441 mmol). Purification with a silica gel flash column (70 g, $(C_2H_5)_2O/CH_3OH$ (4:1)) afforded the product **4c** as a brown oil (3.504 g, 58.0%). ^1H ($CDCl_3$): δ 8.21, 8.19, 7.86, 7.84 (4H, Ar), 4.36 (d, CHP(O)), 4.27−4.17 (m, 6H, OCH_2), 4.15−4.05 (m, 18H, OCH_2), 3.23 (b, m), 2.99−2.76 (m), 2.65 (m, b) (16H, NCH_2CH_2N), 1.37−1.28 (m, 24H, CH_3). ^{13}C ($CDCl_3$): δ 147.23, 142.30 (d), 131.31 (d), 122.97 (6C, Ar), 61.16 (d, 1C, CHP(O)), 62.26 (d, 2C, OCH_2), 61.45 (m, 6C, OCH_2), 50.24 (d, 2C, $CH_2P(O)$), 50.2 (d, 1C, $CH_2P(O)$), 53.72 (d), 53.54 (d), 53.39 (d), 49.64 (8C, NCH_2CH_2N), 16.82 (d, 8C, CH_3).

1-(1-Octyl-methyl-phosphonic acid)-4,7,10-tris(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane (5a, C_8-DOTP). A portion of compound **4a** (4.027 g, 4.551 mmol) was dissolved in a 30 wt % hydrogen bromide solution in acetic acid (40 mL). The solution was stirred at room temperature for 40 h. The solvent and excess hydrogen bromide were removed under reduced pressure. The crude product was purified by recrystallization in ethanol (75 mL) and H_2O (15 mL) mixture. The precipitate was filtered, washed with diethyl ether, and dried under vacuum to give the product **5a** as an off-white solid (2.71 g, 67.3%). A sample for elemental analysis was prepared by reprecipitating the product from base by addition of acid. ^1H NMR (D_2O, pH = 7.4) δ 3.47−2.79 (b, m, 16H, NCH_2CH_2N), 1.63 (b), 1.43 (b), 1.28 (b), 1.16 (b), 0.74 (b) (17H, $(CH_2)_7CH_3$). ^{13}C (D_2O, pH = 7.4) δ 58.37 (d, b, 1C, CHP(O)), 52.42 (d, 1C, $CH_2P(O)$), 52.05 (d, b, 2C, $CH_2P(O)$), 52.9 (b), 51.8 (b), 49.65 (b), 47.58 (b) (8C, NCH_2CH_2N), 31.83, 29.93, 29.28, 29.14, 28.58, 25.47, 22.68, 14.11 (8C, $(CH_2)_7CH_3$). Anal. Calcd for $C_{20}H_{48}N_4O_{12}P_4 \cdot 2.84H_2O$: C, 33.72; H, 7.54; N, 7.87. Found: C, 34.0; H, 7.62; N, 7.84.

1-(1-Undecyl-methyl-phosphonic acid)-4,7,10-tris(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane (5b, C_{11}-DOTP). The title compound was prepared following a method similar to that described above by dissolving compound **4b** (11.795 g, 12.67 mmol) in a 30 wt % hydrogen bromide solution in acetic acid (80 mL). The crude product was dissolved in ethyl alcohol (150 mL). H_2O (20 mL) was added dropwise to the above solution to induce precipitation while stirring. After the addition, diethyl ether (100 mL) was added. The product was filtered, washed with diethyl ether (4 × 20 mL), and dried under vacuum to give compound **5b** as a pale yellow solid (7.6 g, 84.8%). A sample for elemental analysis was prepared by reprecipitating the product from base by addition of acid. ^1H NMR (D_2O, pH = 7.4): δ 3.45−2.78 (b, m, 16H, NCH_2CH_2N), 1.63 (b), 1.44 (b), 1.30 (b), 1.21 (b), 1.17 (b), 0.75 (t) (23H, $(CH_2)_{10}CH_3$). ^{13}C (D_2O, pH = 7.4): δ 58.35 (d, b, 1C, CHP(O)), 52.41 (d, 1C, $CH_2P(O)$), 52.03 (d, b, 2C, $CH_2P(O)$), 53.2 (b), 51.4 (b), 49.62 (b), 47.48 (b), 45.52 (b) (8C, NCH_2CH_2N), 31.85, 29.97, 29.48, 29.44, 29.34, 29.18, 28.64 (d), 25.47, 22.70, 14.1 (11C, $(CH_2)_{10}CH_3$). Anal. Calcd for $C_{23}H_{54}N_4O_{12}P_4 \cdot 2.56H_2O$: C, 36.87; H, 7.90; N, 7.48. Found: C, 36.97; H, 8.14; N, 7.49.

1-(1-4-Nitro-phenyl-methyl-phosphonic acid)-4,7,10-tris(methylene phosphonic acid)-1,4,7,10-tetraazacyclododecane (5c, NO_2-Ph-DOTP). The title compound was prepared following a method similar to that described above by dissolving compound **4c** (3.504 g, 3.92 mmol) in 30 wt % hydrogen bromide in acetic acid (35 mL). The crude product was recrystallized in a mixture of H_2O (6 mL), ethyl alcohol (45 mL), and diethyl ether (30 mL). The precipitate was filtered, washed with diethyl ether, and dried under vacuum to give **5c** as a pale yellow powder (2.30 g, 87.7%). The product was further purified by acidifying the basic solution of the ligand. ^1H (D_2O, pH = 7.4): δ 8.17, 8.15, 7.67, 7.66 (4H, Ar), 4.23−2.51 (b, m, 16H, NCH_2CH_2N), 4.08 (d, 1H, CHP(O)). ^{13}C (D_2O, pH = 7.4): δ 149.42, 145.28, 133.98 (d), 125.82 (6C, Ar), 64.07 (d, 1C, CHP(O)), 54.29 (d, 2C, $CH_2P(O)$), 53.31 (d, 1C, $CH_2P(O)$), 54.60, 52.75, 52.11, 51.0, 50.94, 48.51, 47.28 (8C, NCH_2CH_2N). Anal. Calcd for $C_{18}H_{35}N_5O_{14}P_4 \cdot 6.2H_2O$: C, 27.65; H, 6.07; N, 8.96. Found: C, 27.64; H, 5.80; N, 9.01.

Results and Discussion

Ligand Synthesis. The synthetic route for preparation of the ligands is shown in Scheme 1. The method of Kluge[24] was used to prepare the alcohol phosphonates, **1a**−**1c**. The reaction proceeded by a Pudovik mechanism in which triethylamine catalyzed the reaction by deprotonating diethyl phosphite.[25] All attempts to purify the product by the standard distillation techniques were accompanied by extensive decomposition. No attempt was made to separate the enantiomers. Conversion of **1a**−**1c** into the corresponding triflates **2a**−**2c** was accomplished using a literature method.[26]

Reactions of **2a**, **2b** with cyclen to make the monoalkylated cyclens **3a**, **3b** were complicated by competitive elimination of the triflates to form diethyl alkenephosphonates. The elimination reaction could be suppressed by using excess cyclen but could not be totally eliminated even with a 500% excess of cyclen. The monoalkylated products were purified from cyclen on a silica column.

3a−**3c** were converted to **4a**−**4c** using paraformaldehyde and triethyl phosphite in a Mannich-type reaction. Conversion of phosphonates **4a**−**4c** into phosphonic acids **5a**−**5c** was achieved in 30 wt % HBr/glacial acetic acid (hydrolysis in 6 N hydrochloric acid cleaved the pendant arm to give DO3P). The ligands were purified to >95% by the recrystallization of the hydrolysis products in a mixture of ethanol and water. ^{31}P NMR spectra of recrystallized **5a**−**5c** showed that a small amount of DO3P (<5%) remained. These products could be further purified by dissolving in aqueous base followed by reacidification, but this resulted in substantially reduced yields (~50%) for **5a**, **5b**. The yield was higher for ligand **5c** (66.5%).

NO_2-Ph-DOTP (**5c**) was reduced to NH_2-Ph-DOTP in a Parr hydrogenation apparatus using palladium/activated carbon as catalyst in H_2O. A ^1H NMR spectrum of the product indicated

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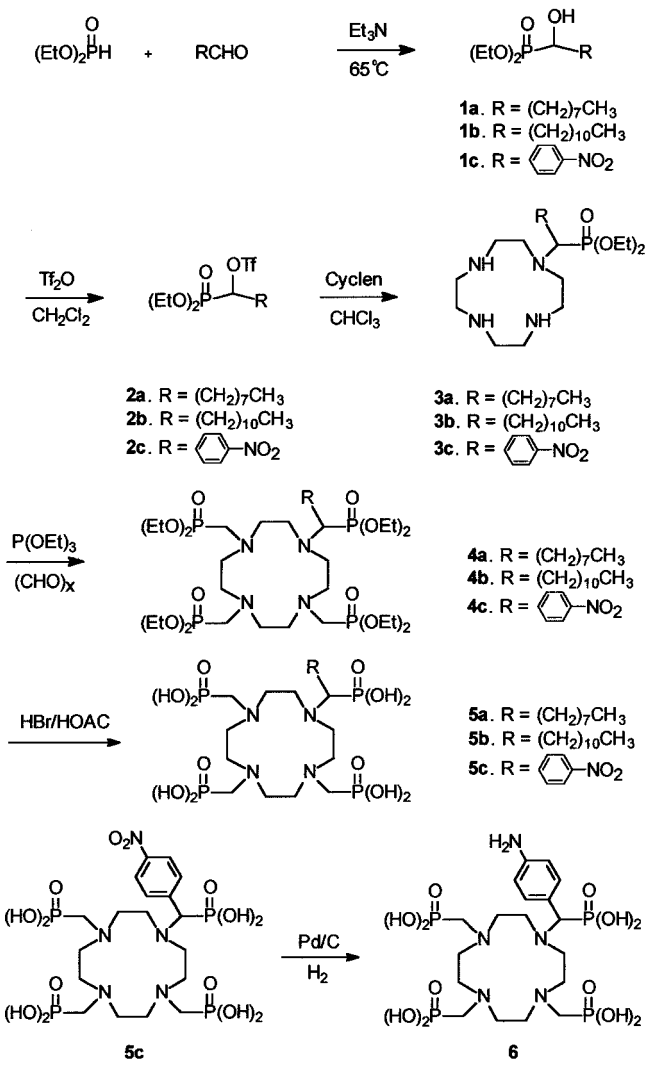
Scheme 1



that the nitro group was completely reduced but that a second product was also present. ^{31}P NMR spectra collected as a function of time showed that NH_2-Ph-DOTP gradually decomposed to DO3P in aqueous solution. A subsequent experiment revealed that Ln^{3+} complexes of **5c** could be reduced to the corresponding $Ln(NH_2\text{-Ph-DOTP})^{5-}$ complex without further degradation.

^{31}P NMR of the Ligands. Figure 1 shows temperature-variable ^{31}P NMR spectra of ligands **5b**, **5c** in D_2O at pH = 7.4 without proton decoupling (spectra of **5a** were nearly identical to those of **5b**). For **5a** and **5b** three phosphorus peaks were observed with an area integration ratio of 1:1:2. The broad peaks of ligand **5b** indicate that the molecular tumbling rate is slowed by the long aliphatic chain substituent. The peaks sharpened at higher temperatures but remained too broad to reveal H−P couplings. In comparison, the ^{31}P NMR resonances of ligand **5c** were much sharper, likely due to faster rotational averaging. Four phosphorus peaks were observed for **5c** at 25 °C with a 1:1:1:1 area integration ratio. At higher temperature, two of the four ^{31}P peaks coalesced to give three phosphorus peaks with a 1:1:2 area ratio. The spectrum at 80 °C allows the observation of the full H−P coupling pattern. The doublet at 14.4 ppm ($^2J_{HP}$ = 25.3 Hz) could be directly assigned to the pendant arm with the nitrophenyl substituent. The chiral center at this pendant arm caused the nonequivalency of the two methylene protons at the other three pendant arms as reflected by the different H−P coupling constants. The phosphonate pendant arm trans to the chiral center gave rise to a quartet (a doublet of doublets) at 17.4 ppm ($^2J_{H(1)P}$ = 14.6 Hz, $^2J_{H(2)P}$ = 7.3 Hz). The other two phosphonate pendant arms trans to each other gave rise to the pseudotriplet at 7.2 ppm ($^2J_{HP(1)} \approx {}^2J_{HP(2)}$ = 9.8 Hz) at 80 °C.

NMR Spectra. ^{1}H NMR spectra of several ytterbium complexes are compared in Figure 2. The spectra of $Yb(C_8\text{-DOTP})^{5-}$ and $Yb(C_{11}\text{-DOTP})^{5-}$ had nearly identical chemical shifts although the resonances of the later complex were somewhat broader apparently due to slower tumbling of the larger chelate. The spectra of $Yb(NO_2\text{-Ph-DOTP})^{5-}$ and $Yb(NH_2\text{-Ph-DOTP})^{5-}$ were similar as well.

The ^{1}H NMR spectrum of $YbDOTP^{5-}$ shows one set of H_1−H_6 proton resonances due to C_4 symmetry. The assignments were reported previously.[27] The single substitution on one pendant arm of the DOTP-like ligands reported here lowers symmetry of the complex, resulting in 4 sets of H_1−H_5 resonances (this is most evident for the most highly shifted H_4 resonances) but only 3 H_6 resonances. This indicates that the single R substituent in these complexes occupies a normal H_6 equatorial position. The H_1, H_4, H_5, and H_6 protons were easily identified by comparison of their chemical shifts with the reference compound $YbDOTP^{5-}$. The H_2 and H_3 protons are less highly shifted and cannot be identified by simple inspection. Like the $LnDOTP^{5-}$ and $LnDOTBP^{-}$ complexes,[27,28] the DOTP-like complexes shown here exist as a single coordination isomer, likely in a twisted square antiprismatic geometry.[3]

Further assignments of the proton resonances came from COSY spectra (the spectrum of $Yb(C_8\text{-DOTP})^{5-}$ is illustrated in Figure 3). The geminal (H_3−H_4 and H_1−H_2) and vicinal (H_1−H_4, H_2−H_3) cross peaks established the correlations necessary to assign each of the individual ethylene fragments within the macrocyclic backbone. Only three H_5−H_6 correlation peaks were observed, further confirming the absence of the fourth H_6 due to the substitution by the hydrophobic side group. From the cross peaks in the COSY spectrum, the connectivity information between individual H_1−H_4 groups and between individual H_5−H_6 groups was determined. Nonetheless, the H_1−H_4 and H_5−H_6 groupings cannot be provided by COSY due to the lack of the *J* couplings between the protons in these two groups. A complete assignment of all protons was accomplished by the observed hyperfine shifts with those calculated from a MMX-derived model of these complexes.

^{1}H Hyperfine Shift Analysis. The observed chemical shift (Δ_{obs}) of a ligand nucleus in a paramagnetic lanthanide complex can be expressed by

$$\Delta_{obs} = \Delta_p + \Delta_{dia} \quad (1)$$

where Δ_p is the paramagnetic shift contribution and Δ_{dia} the diamagnetic shift contribution. The Δ_{dia} term represents the inductive and conformational effects that occur upon complexation; this term is normally evaluated from the chemical shift difference between free ligand and that of diamagnetic lanthanum or lutetium complexes. In this study we did not attempt to assign all ^{1}H chemical shifts in spectra of the La^{3+} or Lu^{3+} complexes due to the complexity of those spectra, so a Δ_{dia} value of 3 ppm, as was reported previously,[29] was used to

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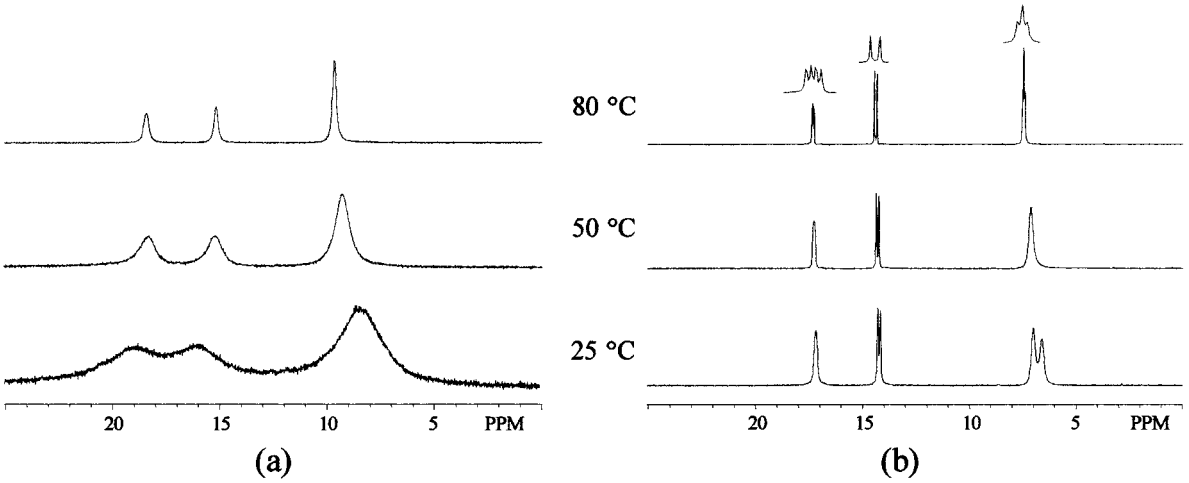

Figure 1. Temperature-variable ^{31}P NMR spectra of (a) C_{11}-DOTP and (b) NO_2-Ph-DOTP in D_2O at pH 7.4. The spectra were obtained without P{H} decoupling. C_{11}-DOTP was obtained by acid purification.

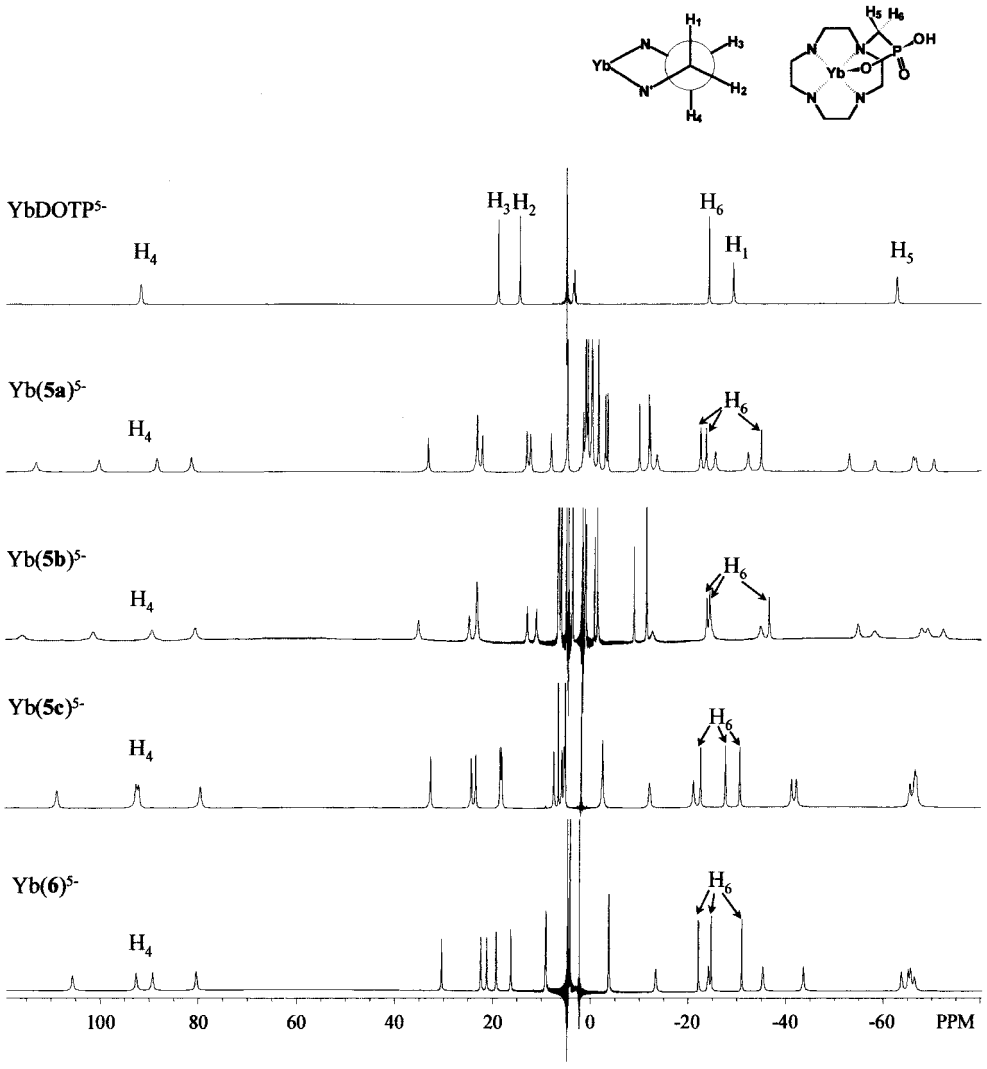

Figure 2. High-resolution ^{1}H NMR spectra of $YbDOTP^{5-}$ (pH = 9.0) and the YbL^{5-} complexes at pH = 10.0 and 25 °C.

represent the average of ^{1}H shifts in the diamagnetic complexes. The paramagnetic shift contribution (Δ_p) can be further divided into contact (Δ_{p-c}) and pseudocontact components (Δ_{p-pc}) depending on the nature of the electron−nucleus interactions between the lanthanide ion and the observed nucleus. It is generally accepted that the paramagnetic shifts observed in the ^{1}H spectrum of an ytterbium complex are largely pseudocontact in origin and can thereby be correlated to complex structure by[30]

$$\Delta_{p-pc} = D_1[(3\cos^2\theta - 1)/r^3] + D_2[(\sin^2\theta\cos^2\varphi)/r^3] \quad (2)$$

where D_1 and D_2 are proportional to the magnetic susceptibility differences, $\chi_{zz} - {}^1/_3(\chi_{xx} + \chi_{yy} + \chi_{zz})$, and $\chi_{xx} - \chi_{yy}$, respectively.

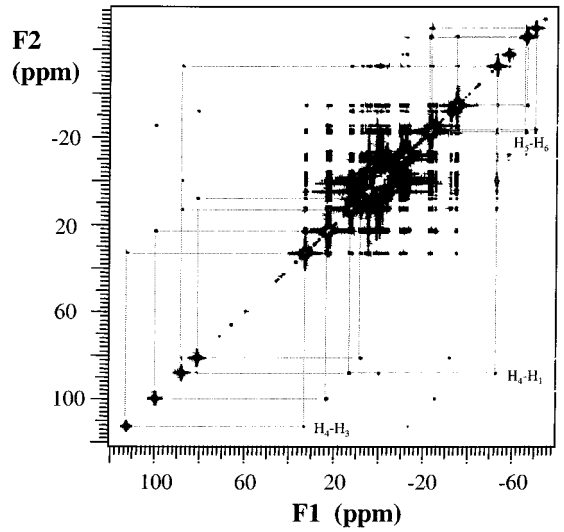

Figure 3. $^1H-^1H$ COSY spectrum of Yb(C_8-DOTP)$^{5-}$ at 0.223 M in D_2O at pH = 10.0 and 25 °C. For clarity, only one H_1-H_4 connectivity was drawn.

θ, φ, and r are the polar coordinates of the ligand nucleus with Ln(III) at the origin and with the principal magnetic axis of the system as the z axis.

An analysis of ^1H hyperfine shifts was carried out using a method described by Forsberg.[31] First, a molecular model of the chelate with Yb^{3+} bound to four macrocyclic nitrogens and four phosphonic oxygens was built using HyperChem 5.0. It was assumed that Yb^{3+} does not have an inner-sphere water molecule as reported for the LnDOTP^{5-} complexes.[3] The structure of the molecule was then optimized by molecular mechanics (MMX), holding the $Yb^{3+}-N$ and $Yb^{3+}-O$ bond distances fixed during energy minimization while allowing all the remaining atomic lengths and bond angles of the molecule to vary. The $Yb^{3+}-N$ (2.63 Å) and $Yb^{3+}-O$ (2.26 Å) distances were fixed to those reported in the crystal structure of TmDOTP^{5-}.[32]

Table 1. Summary of Axial (D_1) and Nonaxial (D_2) Susceptibility Terms Obtained by Analysis of Proton Hyperfine NMR Shifts of the Yb^{3+} Complexes

Yb^{3+} complex	D_1	D_2
DOTP	2931 ± 54	7.2 ± 54
C_8-DOTP	3101 ± 101	460 ± 104
C_{11}-DOTP	3187 ± 135	403 ± 139
NO_2-Ph-DOTP	3021 ± 108	141 ± 112

Figure 4 illustrates the MMX-minimized structure of Yb(C_8-DOTP)$^{5-}$. The H_1-H_6 protons were divided into four subgroups as indicated by superscripts. The COSY spectrum reports which H_1-H_4 protons are in the same subgroup, but does not identify the subgroup number. Furthermore, the H_5-H_6 can be identified on the basis of the magnitude of their Δ_{obs} values (in comparison to YbDOTP^{5-}) but cannot be correlated to a particular H_1-H_4 subgroup (with the exception of H_5^1, which can be assigned from the COSY spectrum). Therefore, the coordinates of all protons and ytterbium (from MMX) and the $\Delta_{p,exp}$ (=Δ_{obs} − 3 ppm) values were compared using Forsberg's method, allowing reassignment of all H_1-H_6 subgroups (while satisfying all the proton connectivities established by the COSY spectrum) until a best agreement between $\Delta_{p,exp}$ and $\Delta_{p,cal}$ for all protons was found. The agreement between calculated and observed hyperfine shifts was good for all three complexes (R^2 < 1%, see Supporting Information). The numerical values of D_1 and D_2 found by this analysis are summarized in Table 1. The nonaxial magnetic susceptibility terms, D_2, were larger for the asymmetric YbL^{5-} complexes, as expected, while the symmetric D_1 terms for the new complexes were slightly higher (3−9%) than the value found for YbDOTP^{5-}. Interestingly, the D_1 values

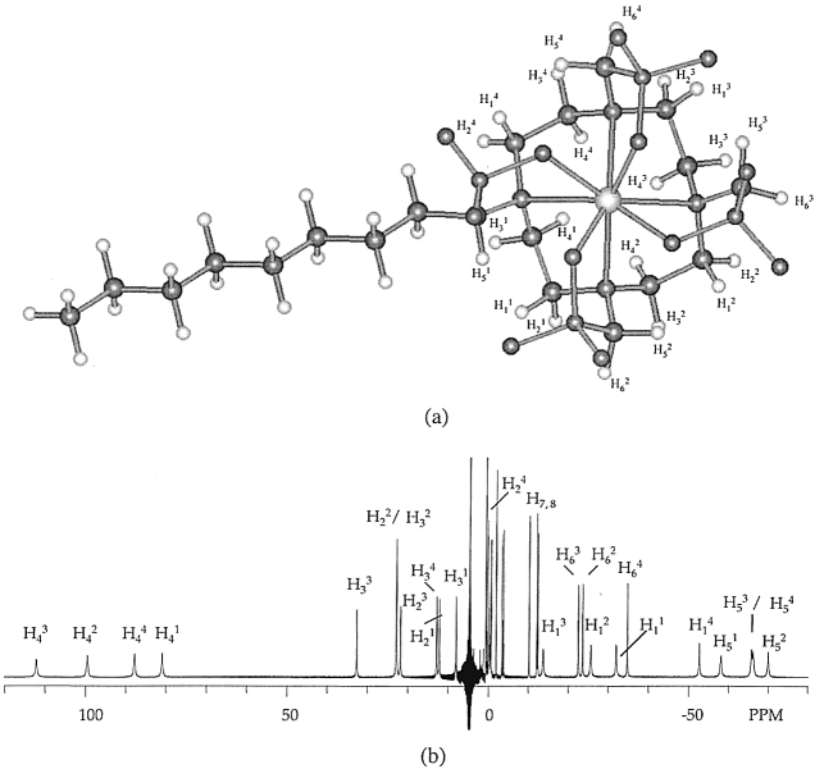

Figure 4. (a) MMX-minimized molecular model of Yb(C_8-DOTP)$^{5-}$. H_1-H_6 protons were divided into four subgroups, and the superscript denotes the subgroup number. (b) Tentative assignment of the protons of the Yb(C_8-DOTP)$^{5-}$ spectrum, a result of the combination of 2D COSY spectrum and the proton shift analysis.

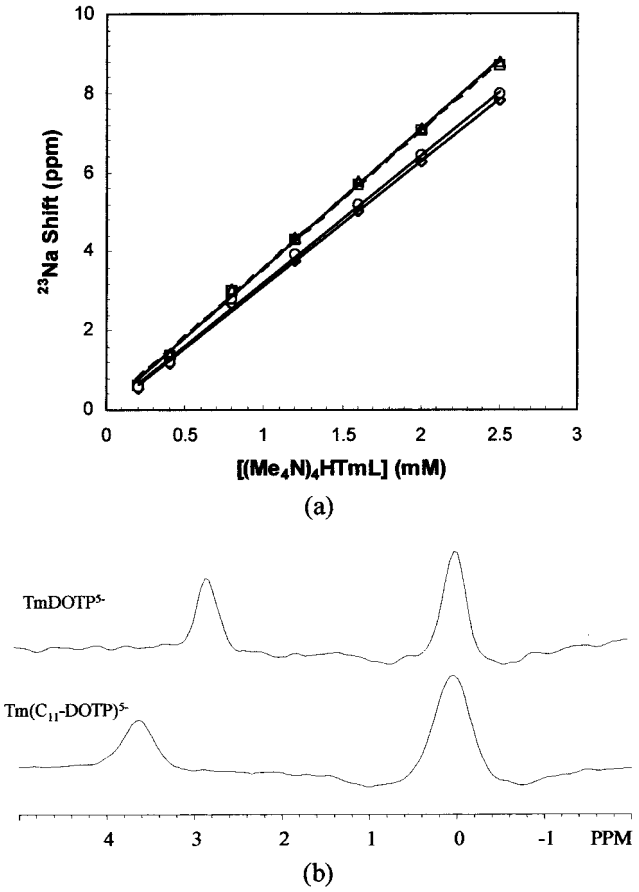

Figure 5. (a) ^{23}Na shift as a function of $(Me_4N)_4HTmL$ in aqueous buffer: (□) $(Me_4N)_4HTm(C_8\text{-DOTP})$, (△) $(Me_4N)_4HTm(C_{11}\text{-DOTP})$, (○) $(Me_4N)_4HTm(NO_2\text{-Ph-DOTP})$; (◇) $(Me_4N)_4HTmDOTP$. The lines correspond to the linear regression of the experimental data. Buffer: 118 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 10 mM glucose, pH = 9.0. (b) Triple quantum filtered (TQF) ^{23}Na spectra collected from an in vitro rat heart perfusion experiment first with $TmDOTP^{5-}$ and then with $Tm(C_{11}\text{-DOTP})^{5-}$ as the shift reagent.

correlated well with the hyperfine shifts (average of four values) of the H_4 protons of these complexes, suggesting that the nonaxial D_2 term contributes little to the observed hyperfine ^{1}H shifts.

^{23}Na NMR. As the hyperfine shifts of the H_4 protons were consistently larger in the new YbL^{5-} complexes compared to $YbDOTP^{5-}$ (the average downfield increases were 2.9, 5.2, and 0.1 ppm for $Yb(C_8\text{-DOTP})^{5-}$, $Yb(C_{11}\text{-DOTP})^{5-}$, and $Yb(NO_2\text{-Ph-DOTP})^{5-}$, respectively), we expected that the hyperfine shifts for ion-paired Na^+ ions might also be larger because both nuclei are positioned near the highest-fold symmetry axis of the complex (the H_4 protons and the presumptive Na^+ binding sites are located within the same dipolar shift cone but ~180° apart[20]). To test this, sodium hyperfine shifts were measured as a function of TmL^{5-} concentration in aqueous buffer and compared to those measured for an equivalent amount of $TmDOTP^{5-}$ (Figure 5). All ^{23}Na hyperfine shifts increased linearly with SR concentration, with the slopes falling in the order $Tm(C_{11}\text{-DOTP})^{5-}$ (3.6) > $Tm(C_8\text{-DOTP})^{5-}$ (3.5) > $Tm(NO_2\text{-Ph-DOTP})^{5-}$ (3.2) > $TmDOTP^{5-}$ (3.1). This demonstrates that the Na^+ shifting ability correlates with the magnitude of the hyperfine shifts observed for the H_4 protons and implies that the number of Na^+ binding sites and binding constants is similar in the new TmL^{5-} complexes as compared to $TmDOTP^{5-}$. Since Ca^{2+} reduces the efficiency of $TmDOTP^{5-}$ as a SR by direct competition with Na^+ for sites on the SR,[20] we also examined the effects of adding Ca^{2+} to the new SRs. Nearly identical decreases in ^{23}Na shift were observed when each of the new SRs and $TmDOTP^{5-}$ were titrated with Ca^{2+} (data not shown). This suggests that, like Na^+, Ca^{2+} has a similar binding affinity for the new TmDOTP-like systems. $Tm(C_8\text{-DOTP})^{5-}$ and $Tm(C_{11}\text{-DOTP})^{5-}$ also precipitated from solution upon addition of Ca^{2+} at a much lower concentration than required for precipitation of $TmDOTP^{5-}$, consistent with a lower water solubility for the $Ca:TmL^{5-}$ ternary complexes.

Triple quantum filtered (TQF) ^{23}Na spectra from a single rat heart perfused with two different SRs are also shown in Figure 5. ^{23}Na NMR signals were collected first in the presence of $TmDOTP^{5-}$ and then, after a brief washout period, collected again in the presence of $Tm(C_{11}DOTP)^{5-}$. Interestingly, the larger ^{23}Na hyperfine shift provided by $Tm(C_{11}\text{-DOTP})^{5-}$ in aqueous solution appears to be magnified in the tissue experiment (compare 3.6 versus 3.1, a 16% difference in H_2O with 3.6 versus 2.8, a 29% difference in the tissue preparation). The origin of these differences is unknown at present but could reflect an accumulation of the hydrophobic reagent in hydrophobic tissue regions thereby providing a higher "effective" concentration of this SR in the extracellular space. Similar results were obtained for hearts perfused first with $TmDOTP^{5-}$ followed by $Tm(C_8\text{-DOTP})^{5-}$ (data not shown). Although the origins of the enhanced hyperfine shifts in perfused tissue versus aqueous solution are not known and will require further experimentation, the nearly 30% increase in Na^+ hyperfine shift observed for these systems should prove advantageous in future in vivo ^{23}Na NMR experiments.

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Supporting Information Available: Tables S1 and S2 summarizing observed and calculated ^{1}H hyperfine shifts for Yb^{3+} complexes of DOTP, C_8-DOTP, C_{11}-DOTP, and NO_2-Ph-DOTP. This material is available free of charge via the Internet at http://pubs.acs.org.

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