NMR Studies of the Lanthanide(III) Complexes of 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrakis(methanephosphonic acid mono(2′,2′,2′-trifluoroethyl) ester)

Won D. Kim,[†] Garry E. Kiefer,[‡] Jurriaan Huskens,[†] and A. Dean Sherry*[,†,§]

Department of Chemistry, University of Texas at Dallas, P.O. Box 830688, Richardson, Texas 75083-0688, Designed Chemicals R & D, Dow Chemical Company, Freeport, Texas 77541, and The Rogers Magnetic Resonance Center, University of Texas Southwestern Medical Center, 5801 Forest Park Road, Dallas, Texas 75235-9085

Received February 12, 1997[⊗]

A new macrocyclic ligand 1,4,7,10,-tetraazacyclododecane-1,4,7,10-tetrakis(methanephosphonic acid mono(2′,2′,2′-trifluoroethyl) ester) (F-DOTPME) has been prepared and some of its metal binding properties examined. The ligand protonation constants (log $K_1 = 10.5$, log $K_2 = 6.7$) and its stability constant with Ca^{II} (log $K_{CaL} = 8.4$) were determined by pH potentiometry. The stability of the La^{III} complex could not be determined by potentiometry so it was evaluated in an EDTA competition experiment using ^{31}P NMR to monitor the reaction. The dissociation kinetics of La(F-DOTPME)$^-$ was slow, with a $k_{d,obs}$ of only 1.0×10^{-7} s^{-1}. A series of Ln(F-DOTPME)$^-$ complexes (Ln^{III} = La, Gd, Dy, Tm, and Yb) were examined by multinuclear NMR. The spectra show that these complexes exist in aqueous solution as a mixture of stereoisomers of nearly equal energy (energy differences of less than 1 kJ/mol). Several resolved ^{19}F resonances in the NMR spectra of the Dy(F-DOTPME)$^-$, Tm(F-DOTPME)$^-$, and Yb(F-DOTPME)$^-$ complexes have been assigned to specific diastereomers by comparing resonance integrals, assuming an interaction model between neighboring pendant arms, and the magnitude and direction of the hyperfine ^{19}F NMR shifts induced by the paramagnetic lanthanide cation. Cationic detergents added to two different Ln(F-DOTPME)$^-$ complexes altered the distribution of isomers in favor of the symmetrical Δ-*SSSS* isomer, while neutral polyethylene glycol affected the ^{19}F chemical shifts of some isomers without altering their populations. Gd(F-DOTPME)$^-$ displayed a water proton relaxivity (R_1) of 2.5 mM^{-1} s^{-1} at 25 °C and 40 MHz, typical of complexes lacking an inner-sphere-coordinated water molecule. ^{17}O NMR was used to confirm that Dy(F-DOTPME)$^-$ does not have an inner-sphere-coordinated water molecule. Addition of human serum albumin to aqueous solutions of Gd(F-DOTPME)$^-$ produced a 4-fold increase in water relaxivity, and an analysis of binding curves indicated the fluorinated complex binds to HSA with a binding constant of about 0.17 mM.

Introduction

Numerous derivatives of 1,4,7,10-tetraazacyclododecane (cyclen) have now been prepared because of widespread interest in the use of these ligands in biomedicine.[1,2] Two of the more widely studied derivatives include DOTA (acetate side arms) and DOTP (methanephosphonate side arms). The LnDOTA$^-$ complexes exist in solution as a mixture of at least two isomeric forms (the % population of each depends upon the size of the Ln^{3+} cation),[3−5] while the more highly charged LnDOTP^{5-} complexes appear to exist as a single species in solution regardless of cation size.[6] One further difference between these complexes is that most LnDOTA$^-$ complexes are nine-coordinate with one inner-sphere water molecule, while the LnDOTP^{5-} complexes appear to be eight-coordinate without an inner-sphere water molecule.[7] The highly charged surface offered by the four coordinated phosphonates of TmDOTP^{5-} makes this complex quite useful as a paramagnetic shift reagent (SR) for biological cations having NMR-active nuclei.[8−12]

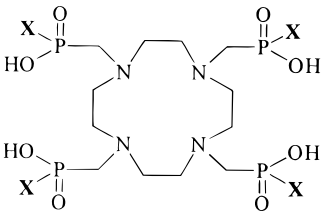
A few phosphinate and phosphonate ester derivatives of 1,4,7,10-tetraazacyclododecane have also been prepared and characterized.[13−16] Introduction of an alkyl or alkoxy functionality on the phosphorus of these systems introduces the additional possibility of four centers of chirality in their lanthanide complexes. Nevertheless, lanthanide complexes

[†] University of Texas at Dallas.
[‡] Dow Chemical Company.
[§] University of Texas Southwestern Medical Center.
[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1997.

(1) Desreux, J. F.; Barthelemy, P. P. *Nucl. Med. Biol*. **1988**, *15*, 9−15.
(2) Tweedle, M. F. Relaxation Agents in NMR Imaging. In *Lanthanide Probes in Life, Chemical and Earth Sciences*: *Theory and Practice*; Bünzli, J.-C. G., Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989; pp 127−79.
(3) Desreux, J. F. *Inorg. Chem*. **1980**, *19*, 1319−1324.
(4) Aime, S.; Botta, M.; Ermondi, G. *Inorg. Chem*. **1992**, *31*, 4291−4299.
(5) Marques, M. P. M.; Geraldes, C. F. G. C.; Sherry, A. D.; Merbach, A. E.; Powell, H.; Pubanz, D.; Aime, S.; Botta, M. *J. Alloys Compd*. **1995**, *225*, 303−307.
(6) Geraldes, C. F. G. C.; Sherry, A. D.; Kiefer, G. E. *J. Magn. Reson*. **1992**, *97*, 290−304.
(7) Sherry, A. D.; Ren, J.; Huskens, J.; Brucher, E.; Toth, E.; Geraldes, C. F. C. G.; Castro, M. M. C. A.; Cacheris, W. P. *Inorg. Chem*. **1996**, *35*, 4604−4612.
(8) Buster, D. C.; Castro, M. M. C. A.; Geraldes, C. F. G. C.; Malloy, C. R.; Sherry, A. D.; Siemers, T. C. *Magn. Reson. Med*. **1990**, *15*, 25−32.
(9) Bansal, N.; Germann, M. J.; Lazar, I.; Malloy, C. R.; Sherry, A. D. *Magn. Reson. Imaging* **1992**, *2*, 385−391.
(10) Bansal, N.; Germann, M. J.; Seshan, V.; Shires, G. T., III; Malloy, C. R.; Sherry, A. D. *Biochemistry* **1993**, *32*, 5638−5643.
(11) Xia, Z.-F.; Horton, J. W.; Zhao, P.-Y.; Bansal, N.; Babcock, E. E.; Sherry, A. D.; Malloy, C. R. *J. Appl. Physiol*. **1994**, *76*, 1507−1511.
(12) Seshan, V.; Germann, M. J.; Preisig, P.; Malloy, C. R.; Sherry, A. D.; Bansal, N. *Magn. Reson. Med*. **1995**, *34*, 25−31.
(13) Lazar, I.; Sherry, A. D.; Ramasamy, R.; Brucher, E.; Kiraly, R. *Inorg. Chem*. **1991**, *30*, 5016−5019.
(14) Aime, S.; Batsanov, A. S.; Botta, M.; Howard, J. A. K.; Parker, D.; Senanayake, K.; Williams, G. *Inorg. Chem*. **1994**, *33*, 4696−4706.
(15) Harrison, A.; Royle, L.; Walker, C.; Pereira, C.; Parker, D.; Pulukkody, K.; Norman, T. J. *Magn. Reson. Imaging* **1993**, *11*, 761.
(16) Pulukkody, K. P.; Norman, T. J.; Parker, D.; Royle, L.; Broan, C. J. *J. Chem. Soc., Perkin Trans*. **1993**, 605−620.

S0020-1669(97)00169-9 CCC: $14.00

formed with the tetrabenzyl phosphinate ligand, DOTBP, exist in solution as one predominant isomer[14] with no inner-sphere water molecule. High-resolution NMR examination of some



X = OH	**DOTP**
OCH_2CH_3	**DOTPME**
OCH_2CF_3	**F-DOTPME**
CH_2CH_3	**DOTEP**
CH_3	**DOTMP**
CH_2Bz	**DOTBP**

paramagnetic lanthanide complexes formed with the DOTP monoesters indicate that multiple stereoisomers are present in solution (Ball, L. M.S. Thesis, UT-Dallas, 1993). Water relaxivity measurements on some of the Gd^{III} complexes indicate multiple species may be present with differing water coordination numbers, depending upon the length of the ester functionality.[17] Animal biodistribution results also show that these complexes distribute differently in tissues, once again depending upon the length of the ester side chains.[17] Lanthanide complexes of the monoethyl ester (DOTPME) clear from animals via renal filtration, while complexes of the monobutyl ester (DOTPMB) tend to clear via the hepatobiliary system.

We now wish to report data for a fluorinated ethyl ester analog, F-DOTPME, which offers considerable new insight into the coordination behavior of these interesting ligand systems. The CF_3 groups of this ligand proved to be excellent ^{19}F NMR probes of the solution structures of the $Ln(F\text{-}DOTPME)^-$ complexes. The fluorine atoms in these complexes are situated far enough away from the paramagnetic centers so that line broadening effects were minimal, yet close enough so that lanthanide induced shifts (LIS) were sufficiently large to resolve individual resonances. We report here pK_a values for F-DOTPME, thermodynamics and kinetics of complex formation for $Ca(F\text{-}DOTPME)^{2-}$ and $La(F\text{-}DOTPME)^-$, an analysis of the ^{19}F NMR spectra of three paramagnetic $Ln(F\text{-}DOTPME)^-$ complexes, showing that all six possible coordination stereoisomers are nearly equally populated, and the changes in structure that occur upon addition of cationic detergents and neutral polyethylene glycol to these complexes.

Experimental Section

Materials and Methods. All reagents and the lanthanide trichloride salts were obtained from commercial suppliers and used without further purification. Lanthanide chloride stock solutions (0.1 M in deionized water) were standardized by titration with EDTA (0.0499 M) using xylenol orange as an endpoint indicator. Cyclen was prepared as described in a recent patent (Athey, P. S.; Kiefer, G. E. U.S. Patent 5,587,451, issued 12/24/96). Paraformaldehyde and tris(2,2,2-trifluoroethyl) phosphite were obtained from Aldrich Chemical Co. (Milwaukee, WI). Elemental analyses were performed by Galbraith Laboratories, Inc., (Knoxville, TN) and FAB mass spectroscopy was done at Dow Analytical (Plaqueman, LA). A stock solution (0.1 M) of the ligand F-DOTPME was prepared in deionized water and standardized by potentiometric titration with standard KOH (0.1 M) solution.

Synthesis of 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetrakis-(methanephosphonic acid bis(2′,2′,2′-trifluoroethyl) ester) (F-DOTPME). To a THF suspension (70 mL) of 1,4,7,10-tetraazacyclododecane (10 g, 58 mmol) and paraformaldehyde (7.4 g, 246 mmol) was added tris(2,2,2-trifluoroethyl) phosphite (81 g, 246 mmol) in one portion. The solution was stirred for 24 h under a N_2 atmosphere, then concentrated *in vacuo* to give a viscous orange oil. The resulting oil was dissolved in acetone (50 ml) and passed through an alumina column (basic form) by eluting with acetone. Upon evaporation of the eluent fractions containing product, the perester was obtained as a viscous, moisture-sensitive oil (60 g, 86%). This product was immediately hydrolyzed without further characterization. To an aqueous dioxane solution (100 mL H_2O/70 mL dioxane) containing potassium hydroxide (25 g, 0.45 mol) was added the perester (60 g, 50 mmol). This solution was stirred at reflux for 1 h, then cooled to room temperature. Upon concentration of the aqueous reaction mixture, the desired tetrapotassium salt product crystallized as an off-white solid and was filtered (15 g, 15 mmol, 30%). ^{13}C NMR (D_2O): δ (ppm) 52.95 (d, CH_2, $^1J_{PC}$ = 119.5 Hz), 53.88 (s, CH_2), 63.60 (qd, CH_2, $^2J_{FC}$ = 36.4 Hz, $^2J_{PC}$ = 5.6 Hz), 126.49 (qd, CF_3, $^1J_{FC}$ = 277.3 Hz, $^3J_{PC}$ = 8.0 Hz). ^{31}P NMR (D_2O): δ (ppm) 22.69. ^{19}F NMR (D_2O): δ (ppm) 3.51. The potassium salt form of the product was passed through a cation-exchange colum (Dowex-50 X4-400) which had been conditioned with 1 N HCl. Freeze drying of the aqueous eluant yielded the free acid as a floculant, white powder. Anal. Calcd for $C_{20}H_{36}F_{12}N_4O_{12}P_4 \cdot H_2O$ (fw = 894.42): C, 26.86; H, 4.28; N, 6.26. Found: C, 26.83; H, 4.37; N, 6.42. MS, *m/e* (FAB): 877.3 ($M^+ + 1$).

Potentiometry. All potentiometric titrations were performed in a jacketed vessel at 25.0 ± 0.1 °C under a N_2 atmosphere. pH measurements were performed using an Accumet 925 pH meter (Fisher) and an Orion 8103 Ross combination electrode, after calibration with Ricca high-precision buffers at pH 4 and 10. The ionic strength of all samples was adjusted to 0.1 M prior to titration using KCl. Hydrogen ion concentrations were calculated from the measured pH values using a pK_w of 13.79 and a H^+ activity coefficient of 0.82, determined in separate titrations containing known amounts of acid or base. The titrations were evaluated using a spreadsheet program described previously.[18] All titrations were performed at least twice.

The protonation constants of F-DOTPME were determined by potentiometric titration of 2 mM ligand (pH 12) with 0.1 M HCl. Since the ligand formed complexes with Ca^{II} relatively quickly, the stability constant of this complex could be determined by direct potentiometric titration of a solution containing 1:1 metal:ligand (2 mM, three separate titrations). Ln^{III} ions, however, form complexes with this ligand too slowly for direct potentiometric titration. Therefore, the stability constant for $La(F\text{-}DOTPME)^-$ was evaluated by a competition experiment with EDTA, monitored by ^{31}P NMR (see below).

NMR Experiments. All NMR spectra of the complexes (^{17}O, ^{19}F, ^{31}P) were obtained on a Bruker GN-500 spectrometer at 298 K, using a tunable broad-band probe. NMR spectra of all intermediates in the synthesis of the ligand and the final product were recorded on a Bruker AC-250 spectrometer equipped with a multinuclear quad probe (^1H, ^{13}C, ^{31}P, and ^{19}F) at 297 K unless otherwise indicated. ^1H NMR spectra were obtained in D_2O utilizing homonuclear solvent suppression to eliminate residual water. ^1H NMR spectra were referenced to either residual chloroform (in $CDCl_3$) at 7.26 ppm or externally referenced to dioxane (in D_2O) at 3.55 ppm. $^{13}C\{^1H\}$ NMR spectra were referenced to external dioxane at 66.7 ppm. ^{31}P NMR chemical shifts were referenced to external phosphoric acid and ^{19}F NMR chemical shifts to 2,2,2-trifluoroethanol. ^{13}C, ^{31}P, and ^{19}F spectra were broad-band proton decoupled unless otherwise specified. The lanthanide(III) complexes were prepared by mixing the ligand and lanthanide(III) stock solutions and slowly adjusting the pH to 7.4, first with concentrated KOH and then final adjustment with dilute KOH. This procedure resulted in a stock solution of about 40 mM. For NMR measurements, a small amount of D_2O was added to provide field lock (to compensate bulk magnetic susceptibility effects) and to provide the necessary dilutions. An off-line NMR data processing program Nuts (Acorn NMR, Fremont, CA) was used to measure the integrals of overlapping ^{19}F resonances using the line-fitting routine supplied with that package.

The chemical shift of the bulk water ^{17}O NMR resonance (67.8 MHz, 298 K) was measured as a function of the concentration of $Dy(F\text{-}DOTPME)^-$ to evaluate the number of water molecules in the first

(17) Geraldes, C. F. G. C.; Sherry, A. D.; Lazar, I.; Miseta, A.; Bogner, P.; Berenyi, E.; Sumegi, B.; Kiefer, G. E.; McMillan, K.; Maton, F.; Muller, R. N. *Magn. Reson. Med.* **1993**, *30*, 696−703.

(18) Huskens, J.; van Bekkum, H.; Peters, J. A. *Comput. Chem.* **1995**, *19*, 409−416.

coordination sphere as described previously.[19] These experiments were performed using 0−40 mM complex. The ^{17}O resonance of a water sample (20% D_2O, for locking purposes) without the complex was used as an external standard.

A competition experiment between F-DOTPME and EDTA for complexation with La^{III} was monitored by ^{31}P NMR at 298 K. Two samples were prepared that contained F-DOTPME (5 mM), EDTA, and La^{III} in a 1:1:1 or 1:2:1 ratio. The ionic strength was adjusted to 0.1 M KCl. In the first sample F-DOTPME was added to the already present $LaEDTA^-$ complex (final pH 9), while in the second sample EDTA was added to the already present $La(F\text{-}DOTPME)^-$ complex (final pH 7.5). ^{31}P NMR spectra were recorded from time-to-time during a period of 5 months. The protonation constants of EDTA as well as the stability constant of $LaEDTA^-$ in 0.1 M KCl were obtained from the literature.[20]

Water Proton Relaxation Rates. Water proton ($1/T_1$) relaxation rates were measured as a function of $Gd(F\text{-}DOTPME)^-$ concentration (0.5−8 mM) on 100 μL samples of complex at pH = 6.8 (PIPES buffer), 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 contribution 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 [$Gd(F\text{-}DOTPME)^-$] (obtained by linear regression) provided a measure of the water proton relaxivity (R_1) of the complex. Water proton relaxation rates were also determined for samples containing human serum albumin (HSA), at 25 °C 40 MHz and on samples containing 0.2 mM $Gd(F\text{-}DOTPME)^-$ as the concentration of HSA (M_w ~60,000) was increased from 0 to 2 mM.

Results and Discussion

Thermodynamics and La^{III} Complex Dissociation Kinetics. The protonation constants of F-DOTPME were obtained by potentiometric titration of the free ligand (potassium salt) with 0.1 M HCl at 298 K and an ionic strength of 0.1 M (KCl). Only two protonation constants (log $K_1 = 10.48 \pm 0.09$, log $K_2 = 6.68 \pm 0.04$; $K_i = [H_iL]/[H_{i-1}L][H]$) were evident from the data collected between pH 2−12. These two protonations were tentatively assigned to two macrocyclic ring nitrogens, based upon the numerous other cyclen derivatives similar to this that have been examined.[7,13,21−23] Assuming this assignment is correct, we can conclude that protonation of the $P-O^-$ groups of this fluorinated ligand must occur well below pH 2. The low values of the first two protonation constants of the ligand (compared to other systems of this type[7,13,21−23]) must be attributed to the strong electron-withdrawing nature of the CF_3 groups.

The formation and dissociation of the $Ca(F\text{-}DOTPME)^{2-}$ complex appeared to be relatively fast, so its stability constant (log $K_{CaL} = 8.4 \pm 0.2$) could be obtained by direct potentiometric titration of a 1:1 mixture of F-DOTPME:Ca^{II} from pH 12 to 2 by the addition of HCl. The formation of $Ln(F\text{-}DOTPME)^-$ complexes by addition of a Ln^{III} salt to a solution of F-DOTPME at high pH was reasonably fast. At low pH or in the presence of an additional Ln^{III} chelating ligand, the kinetics of $Ln(F\text{-}DOTPME)^-$ formation was much slower. Potentiometric competition titrations at high pH (where equilibration between competing ligands has been shown to be rapid) have been used recently with other macrocyclic ligand systems ($LaDO2A^+$ and $LaDOTP^{5-}$),[7,24] but this also proved to be too slow to evaluate the stabilities of the $Ln(F\text{-}DOTPME)^-$ complexes. These phenomena prohibited the use of direct potentiometric titration techniques for the evaluation of K_{LnL}. Thus, K_{LaL} was assessed by competition experiments between F-DOTPME and EDTA for complexation with La^{III}, while monitoring free and bound F-DOTPME by ^{31}P NMR. One sample was prepared by adding La^{III} to a solution of F-DOTPME at pH 10 to form the 1:1 complex, followed by the addition of 2 equiv of EDTA and adjustment of the pH to 7.6. The ^{31}P NMR spectrum of this sample initially showed one large resonance representing $La(F\text{-}DOTPME)^-$ (19.5 ppm) and a very small resonance representing the free ligand (15.1 ppm). The intensity of the free ligand peak increased with time and reached a value of about 70% of the total peak area after 5 months at room temperature (although the sample was still not at equilibrium). During this period, the pH increased slowly to about 8.0. In a second sample, La^{III} and EDTA (1:1) were mixed initially at pH 11 and after the addition of 1 equiv of F-DOTPME the pH was adjusted to 10.0. The ^{31}P NMR spectrum of this sample initially showed a large resonance for uncomplexed F-DOTPME and only a very small one for the La^{III} complex. The intensity of the complex resonance gradually increased with time until it reached about 22% of the total ^{31}P peak area after about 1 month, after which it did not change substantially. The pH of this sample decreased to about 8.8. The ratio between bound and free F-DOTPME was used to calculate "apparent" (nonequilibrium) log K_{LaL} values as a function of time for both samples (Figure 1). A fit of these curves to monoexponential decay functions gave log K_{LaL} = 15.1 and 14.7 as the final equilibrium values for sample 1 and 2, respectively. Therefore, it is concluded that log $K_{LaL} = 14.9 \pm 0.2$.

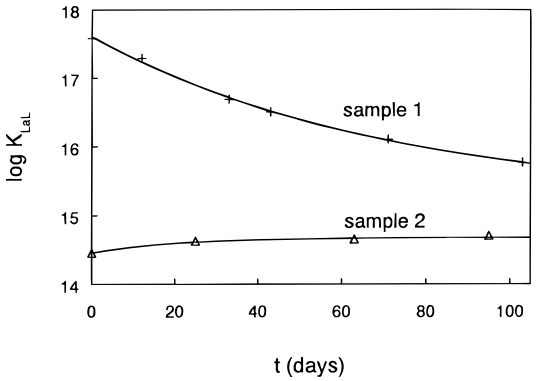


Figure 1. The apparent log K_{LaL} of $La(F\text{-}DOTPME)^-$ as a function of time as determined by ^{31}P NMR competition experiments with EDTA, either starting from the $La(F\text{-}DOTPME)^-$ complex (sample 1) or the $La(EDTA)^-$ complex (sample 2).

The K_{ML} values found here for $Ca(F\text{-}DOTPME)^{2-}$ and $La(F\text{-}DOTPME)^-$ are quite similar to the stability constants reported previously for these same metal ions (except Gd^{III} was reported instead of La^{III}) with the macrocyclic phosphinate ligand DOTEP.[13] Nevertheless, the lower protonation constants for F-DOTPME compared to DOTEP (10.94, 8.24, 3.71) lead to a remarkable thermodynamic stability of $Ln(F\text{-}DOTPME)^-$ at low pH. The presence of only two protonation constants

(19) Alpoim, M. C.; Urbano, A. M.; Geraldes, C. F. G. C.; Peters, J. *J. Chem. Soc., Dalton Trans.* **1992**, 463−467.
(20) Martell, A. E.; Smith, R. M.; Motekaitis, R. J. *NIST Critical Stability Constants of Metal Complexes*; NIST Standard Reference Data: Gaithersburg, MD, 1993.
(21) Kumar, K.; Chang, C. A.; Francesconi, L. C.; Dischino, D. D.; Malley, M. F.; Gougoutas, J. Z.; Tweedle, M. F. *Inorg. Chem.* **1994**, *33*, 3567−3575.
(22) Delgado, R.; Siegfried, L. C.; Kaden, T. A. *Helv. Chim. Acta* **1990**, *73*, 140−148.
(23) Cole, E.; Parker, D.; Ferguson, G.; Gallagher, J. F.; Kaitner, B. *J. Chem. Soc., Chem. Commun.* **1991**, 1473−1475.
(24) Huskens, J.; Torres, D. A.; Kovacs, Z.; Andre, J. P.; Geraldes, C. F. G. C.; Sherry, A. D. *Inorg. Chem.* **1997**, *36*, 1495−1503.

above pH 2 results in a conditional stability constant, K_{cond} ($= [ML]/[M][L]_f$, where $[L]_f = \sum[H_nL]$), that decreases only gradually with increasing acidity. This is in contrast to DOTA where the additional third and fourth ligand protonations (log $K_3 = 4.50$ and log $K_4 = 4.19$)[21] destabilize the Ln^{III} complexes below pH 2.5. Similarly, the tetraphosphonate ligand DOTP has six protonation constants[7] above pH 5, and consequently the $LnDOTP^{5-}$ complexes begin to dissociate, albeit slowly, below pH 6.

The extremely slow kinetic behavior of La(F-DOTPME)$^-$ is also quite unique (the curve for sample 1 of Figure 1 represents dissociation of La(F-DOTPME)$^-$). One can assume that the formation rate was negligible in the beginning of this experiment since [La] was very low due to immediate uptake by EDTA. A plot of these same data as $\ln([LaL]_0/[LaL])$ *versus* time was linear up to 110 days (data not shown), the slope of which gave a dissociation rate constant, $k_{d,obs}$, of $(1.04 \pm 0.02) \times 10^{-7}$ s^{-1}. Since the observed pH changed from 7.6 to 8.0 during this experiment, the excellent linearity of this plot indicated that the dissociation rate was independent of pH, at least over this narrow pH range. Therefore, dissociation of the complex appears not be be catalyzed by either H^+ or OH^- under these conditions, so $k_{d,obs}$ may be approximated as $k_{d,0}$. This value can be compared directly to $k_{d,0}$ values obtained for other macrocyclic complexes. This comparison shows that the dissociation rate observed here for La(F-DOTPME)$^-$ is several orders of magnitude slower than for dissociation of LnDO3A, Ln(HP-DO3A), LnNOTA, and LnDETA and is similar to the estimated upper limit of 5×10^{-8} s^{-1} for $GdDOTA^-$.[25] In contrast to other dissociative kinetic studies, the La(F-DOTPME)$^-$ dissociation rate was measured near physiological pH, thereby providing direct evidence that this compound might be safe for *in vivo* applications. Although more studies are needed at lower pH values to investigate possible H^+-assisted dissociation pathways, it can be argued that the kinetic stability at a low pH is likely to be stronger for Ln(F-DOTPME) than for Ln-(DOTPME). This is rationalized by the lower protonation constants for the fluorinated ligand so that protonation of the complex, which is the first step in a H^+-assisted dissociation pathway, is also more difficult.

NMR Studies. The ^{19}F NMR spectrum of F-DOTPME consisted of a single triplet ($^3J_{FH} = 8.5$ Hz) due to spin coupling with the neighboring CH_2 groups. The measured T_1 and T_2 values for the free ligand were 966 and 312 ms, respectively, and the line width of each component of the triplet was about 1.5 Hz. The ^{19}F NMR resonance of La(F-DOTPME)$^-$ appeared as a broad singlet with a non-Lorentzian line width of 136 Hz at 25 °C, while the corresponding spectrum of Gd(F-DOTPME)$^-$ appeared as a single broad peak ($\nu_{1/2} = 1300$ Hz, $T_2^* = 0.2$ ms) with a T_1 of 0.4 ms (T_2 was too short to be measured using spin-echo techniques). The ^{19}F NMR spectra of three other paramagnetic complexes Yb(F-DOTPME)$^-$, Tm(F-DOTPME)$^-$, and Dy(F-DOTPME)$^-$ were much more informative (in this case, only singlets were observed due to paramagnetic broadening, see Figure 2). Each of these complexes showed multiple ^{19}F resonances, indicating that the near-chemical-shift equivalence of the CF_3 groups in these complexes had been lifted by the paramagnetic centers. High-resolution ^{31}P NMR spectra of these same complexes also had multiple resonances (data not shown), but the smaller chemical shift dispersion did not allow a more detailed analysis.

The first feature common to the ^{19}F NMR spectra shown in Figure 2 were the two groups of resonances, one group of eight

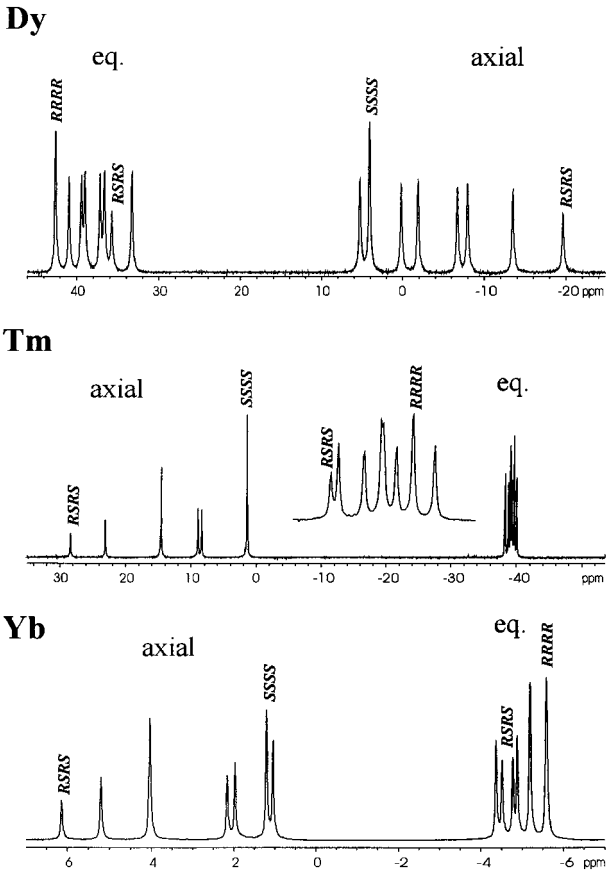


Figure 2. ^{19}F NMR spectra of 40 mM Yb(F-DOTPME)$^-$, Tm(F-DOTPME)$^-$, and Dy(F-DOTPME)$^-$ at 25 °C. The inset in the middle spectrum is an expansion of the upfield (low-frequency) group of resonances. The labeled resonances were assigned to the indicated isomer as described in the text.

resonances shifted mostly downfield and another group of eight resonances shifted mostly upfield relative to their diamagnetic position (note, the full complement of eight resonances in each group were evident only in the spectrum of Dy(F-DOTPME)$^-$ in Figure 2). The direction of the lanthanide-induced paramagnetic shifts for each complex provided information about the orientation of the CF_3 groups relative to the metal center and its principle magnetic axis. The group of high-frequency resonances in the spectrum of Dy(F-DOTPME)$^-$ represented ^{19}F nuclei positioned near the equatorial region of the complex perpendicular to the principal magnetic axis of the dysprosium ion.[26] These paramagnetic shifts are in the same direction as those observed previously for the bridging $N-CH_2-P$ protons and the ^{31}P resonance of $DyDOTP^{5-}$.[6] Conversely, the group of eight resonances shifted to low frequency in the spectrum of Dy(F-DOTPME)$^-$ reflect CF_3 groups positioned axially. These are in the same direction as the shifts observed for Na^+ ion-paired with the highly charged phosphonate groups of $DyDOTP^{5-}$.[6] Since the direction of a pseudocontact shift induced by Tm^{III} or Yb^{III} is expected to be opposite that induced by Dy^{III},[26] the group of high-frequency ^{19}F resonances in the spectra of Tm(F-DOTPME)$^-$ and Yb(F-DOTPME)$^-$ corresponded to axially-positioned CF_3 groups while the group of lower frequency resonances corresponded to equatorially-positioned CF_3 groups.

The equatorial resonances, in general, displayed a smaller range of chemical shifts, indicating that these CF_3 groups

(25) Wang, X.; Jin, T.; Comblin, V.; Lopez-Mut, A.; Merciny, E.; Desreux, J. F. *Inorg. Chem.* **1992**, *31*, 1095−1099.

(26) Sherry, A. D.; Geraldes, C. F. G. C. Shift Reagents in NMR Spectroscopy. In *Lanthanide Probes in Life, Chemical and Earth Sciences: Theory and Practice*; Bünzli, J.-C. G., Choppin, G. R., Eds.; Elsevier: Amsterdam, 1989; pp 93−126.

Table 1. Diastereomeric Complexes and Their Contributions to ^{19}F NMR Spectra and the Interactions between the Pendant Arms

	statistical abundance	no. of nonequivalent ^{19}F nuclei	no. of equatorial resonances	no. of axial resonances	relative intensities	interaction energy[a]
Δ-*RRRR*	1	1	1		4	$4E_{RR}$
Δ-*RRRS*	4	4	3	1	1:1:1:1	$2E_{RR} + 2E_{RS}$
Δ-*RRSS*	4	4	2	2	1:1:1:1	$2E_{RR} + 2E_{RS}$
Δ-*RSRS*	2	2	1	1	2:2	$4E_{RS}$
Δ-*RSSS*	4	4	1	3	1:1:1:1	$2E_{RR} + 2E_{RS}$
Δ-*SSSS*	1	1		1	4	$4E_{SS}$
total	16	16	8	8		

[a] According to neighboring pendant arm interactions only, see text.

experienced similar magnetic environments both in terms of distance and orientation from the metal center. The group of axial resonances, on the other hand, displayed a wider range of chemical shifts, and the absolute magnitude of the hyperfine shifts of the axial resonances tended to be smaller (this was most obvious in the spectra of Dy(F-DOTPME)$^-$ and Tm(F-DOTPME)$^-$ in Figure 2). This indicated that these CF_3 groups were on average situated relatively close to the dipolar shift cone (~54° from the presumed 4-fold magnetic axis of symmetry) resulting in small absolute shifts but large variations in magnitude, as expected for a collection of CF_3 groups near the magic angle.

Since coordination of each $-CH_2-PO_2^--O-CH_2-CF_3$ group of the ligand to Ln^{III} produces an asymmetric center at each phosphorus, the 16 ^{19}F resonances observed in the spectra of the paramagnetic complexes reflect the large number of stereoisomers that are possible in these complexes.[27] If the *R*- or *S*-orientation of each group is independent of the conformation of the other coordinated phosphonates, it is easily seen that six diastereomers are possible, *RRRR*, *RRRS*, *RRSS*, *RSRS*, *RSSS*, and *SSSS*. Of course, both clockwise (Δ) and counterclockwise (Λ) rotation of the cyclododecane {3333} ring conformation is also possible in these complexes so that the Δ-*RRRR* and Λ-*SSSS* stereoisomers represent a pair of enantiomers not distinguishable by NMR spectroscopy, although they are distinguishable from the enantiomeric pair Δ-*SSSS*/Λ-*RRRR*. Thus, for the remainder of this paper we will consider only the clockwise (Δ) isomers and *RRRR* will refer to the Δ-*RRRR*/Λ-*SSSS* enantiomeric pair, *RRRS* represents the Δ-*RRRS*/Λ-*SSSR* pair, etc. An inspection of the molecular models of the six possible stereoisomeric complexes reveals that eight CF_3 groups would be positioned axially with respect to the 4-fold principal magnetic axis of symmetry of the LnN_4O_4 coordination sphere while eight would be positioned equatorially.

Statistically, one would expect the diastereomeric complexes would not be equally abundant since, e.g., *RRRR* occurs only once among the 16 possibilities while *RRRS* occurs four times. The number of peaks and the relative intensities that a complex exhibits in the ^{19}F NMR spectrum depends upon its structure; *RRRR* is expected to appear as a single resonance in the equatorial region, while *RRRS* would have four resonances in a 1:1:1:1 ratio, three in the equatorial region and one in the axial region. Obviously, the single *RRRR* resonance would be four times as intense as the individual *RRRS* resonances if the two isomers were present in equimolar amounts. Table 1 summarizes all possible diastereomers, their relative abundances, the expected number of resonances, their distribution over the two regions, and their relative intensities. The data in Table 1 shows that 16 resonances are expected, and if one multiplies the expected abundance of each diastereomer by the peak intensities, it also shows that all 16 resonances would display the same intensity if the stereochemistry at each coordinating phosphonate is statistically determined rather than determined by the stereochemistry of the neighboring phosphonates.

Figure 2 shows that the 16 resonances were not equally populated, but each grouping of 8 resonances had 6 resonances of approximately equal intensity joined by one more intense and one less intense resonance. From Table 1, it is clear that the two most intense and the two least intense resonances could only arise from the *RRRR*, *SSSS*, and *RSRS* stereoisomers while the remaining 12 resonances of intermediate intensity must be assigned to the *RRRS*, *RRSS*, and *RSSS* isomers. The small differences in intensities between the latter 12 resonances must reflect deviations from the statistical result due to small differences in interaction energies between the pendant arms. Since either the two most intense or the two least intense resonances must be assigned to *RSRS*, the remaining pair must be assigned to *RRRR* and *SSSS*. This means that the *RRRR* and *SSSS* isomers must be nearly equally populated and that the stereochemistry of the pendant arms is not influenced by the configuration of the macrocyclic ring carbons.

If one assumes that neighboring interactions dominate these small energy differences, four interaction energies are possible; E_{RR}, E_{RS}, E_{SR}, and E_{SS} represent interaction energies between neighboring *RR*-, *RS*-, *SR*-, and *SS*-orientations, respectively. Since *RRRR* and *SSSS* were equally populated, we assume that $E_{RR} \approx E_{SS}$ and, consequently, $E_{RS} \approx E_{SR}$. It is also reasonable to assume that $E_{RR} < E_{RS}$ (the presence of two groups with the same orientation is favored) since neighboring *RS*-orientations should produce more steric hindrance between the ester groups and electronic repulsions between the partially charged oxygens. The total interaction energies for this model are summarized in the last column of Table 1. This model suggests that the two most intense resonances reflect the *RRRR* and *SSSS* isomers, while the two least intense resonances reflect the *RSRS* isomer. It is now also obvious that equal probabilities are expected for the *RRRS*, *RRSS*, and *RSSS* isomers, giving rise to 12 resonances of nearly equal intensity.

A line-shape analysis of the ^{19}F NMR spectrum of Dy(F-DOTPME)$^-$ indicated that the peak areas of the 12 resonances of intermediate intensity were indeed very close to each other ($\sigma = 7\%$). If the average area of one such resonance is set to 1, the average area of the two most intense resonances was 1.53 while the two less intense resonances had areas of about 0.72. The difference in interaction energies between the *RRRR*/*SSSS*, *RRRS*/*RRSS*/*RSSS*, and *RSRS* groups of isomers according to the neighboring interaction model described above is $\Delta E = 2E_{RS} - 2E_{RR}$. This equal energy difference agrees reasonably well with the relative peak areas, a factor of about 1.5 between the first two groups *versus* a factor of 1.4 between the latter two groups. The Boltzmann distribution at 298 K led to a value for ΔE of 0.94 ± 0.18 kJ/mol or to an interaction difference of $E_{RS} - E_{RR} = {}^1/_2\Delta E = 0.47 \pm 0.09$ kJ/mol. There appeared to be no trend in the area differences for the 12 equal intermediate

(27) Aime, S.; Botta, M.; Parker, D.; Williams, J. A. G. *J. Chem. Soc., Dalton Trans.* **1995**, 2259−2266.

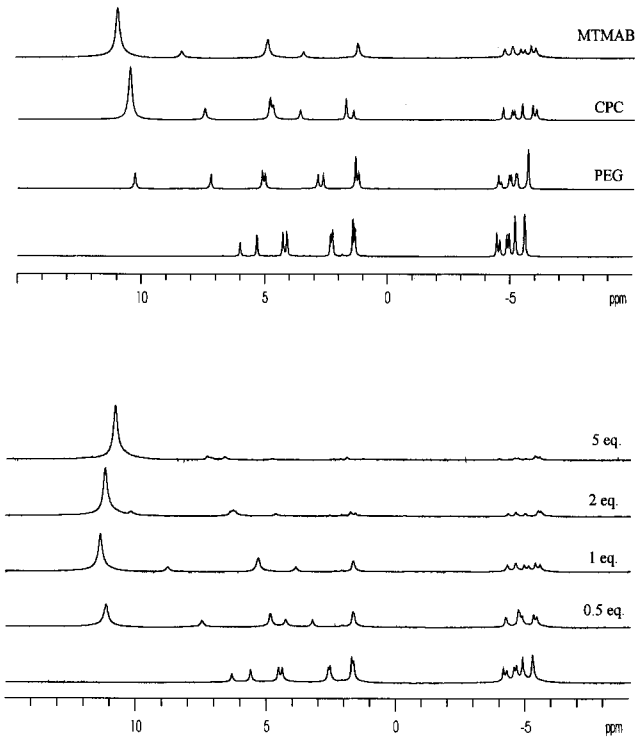


Figure 3. ^{19}F NMR spectra of 40 mM Yb(F-DOTPME)$^-$ (bottom) before and after incremental additions of myristyltrimethylammonium bromide (MTMAB) and (top) after addition of 1 molar equiv of either polyethylene glycol (PEG), cetylpyridinium chloride (CPC), or MTMAB.

resonances, so further assignment of the resonances of Dy(F-DOTPME)$^-$ (Figure 3) was not attempted.

Not all 16 resonances were resolved in the ^{19}F NMR spectra of Tm(F-DOTPME)$^-$ and Yb(F-DOTPME)$^-$ at room temperature. These spectra did show six to seven resolved resonances in both the low-field (the axial group) and high-field regions (the equatorial group). Cooling these samples to near 10 °C did result in complete resolution of all eight resonances in each group, similar to that seen in the spectrum of Dy(F-DOTPME)$^-$ at room temperature. The relative intensities of the resonances did not change significantly upon cooling from 25 to 10 °C, so the populations of the stereoisomers also did not change significantly over this temperature range. The resonance assignments for the Tm(F-DOTPME)$^-$ and Yb(F-DOTPME)$^-$ complexes shown in Figure 2 were made using the same arguments as outlined above for Dy(F-DOTPME)$^-$.

Addition of Detergents or PEG to Ln(F-DOTPME)$^-$ Complexes. Figure 3 shows the effect of adding 1 equiv of cationic surfactant MTMAB (myristyltrimethylammonium bromide, $CH_3(CH_2)_{13}N^+(CH_3)_3$ Br^-), CPC (cetylpyridinium chloride, $(C_5H_5N)^+(CH_2)_{15}CH_3Cl^-$), or neutral PEG (polyethylene glycol, $H(OCH_2CH_2)_nOH$, $n \approx 200$) on the ^{19}F NMR spectrum of Yb(F-DOTPME)$^-$. The two cationic detergents clearly alter both the chemical shift and the distribution of stereoisomers. The neutral polymer, PEG, had a similar influence on the ^{19}F chemical shifts of some of the isomers (particularly the CF_3 groups positioned axially) but had little effect on the apparent distribution of those isomers. After the addition of about 5 equiv of MTMAB (see lower panel of Figure 3), only a single stereoisomer of Yb(F-DOTPME)$^-$ remained with a chemical shift of about 11 ppm. This single ^{19}F resonance must be assigned to the *SSSS* isomer since this is the only isomer that would yield a single resonance (all four CF_3 groups are magnetically equivalent) *and* have the CF_3 groups positioned

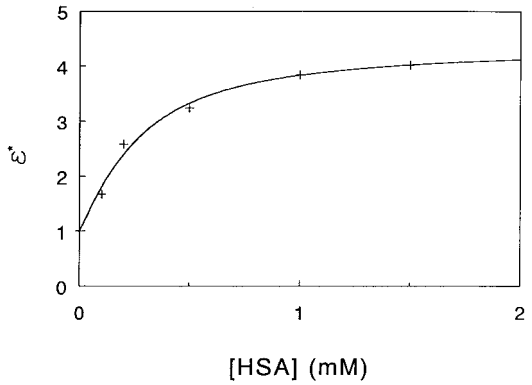


Figure 4. The relaxivity enhancement, $\epsilon^* = (T_{1P}^{-1})_{HSA}/(T_{1P}^{-1})_0$, as a function of the concentration of HSA, at 298 K and 40 MHz. The solid line represents the best fit (see text).

axially. The same experiment performed on Eu(F-DOTPME)$^-$ yielded similar results (data not shown).

Water Relaxation and Coordination Number. The ^{17}O NMR shift of the bulk water resonance was measured while adding Dy(F-DOTPME)$^-$ (0−40 mM) to 20% D_2O in water. No change in the ^{17}O chemical shift of the water resonance was observed, indicating that water did not have access to the first coordination sphere of the Dy^{III} ion.[19] Gd(F-DOTPME)$^-$ had a water proton T_1 relaxivity of 2.5 mM^{-1} s^{-1} (40 MHz), and this is also consistent with complexes having no inner-sphere water.[28] Thus, the absence of an inner-sphere water coordination site appears to be a general characteristic of lanthanide complexes of tetraazacyclododecane derivatives with either methanephosphinate, methanephosphonate, or phosphonate ester pendant functionalities (DOTBP and DOTMP;[14] DOTEP, DOTPME, and DOTPMB;[17] DOTP[7]).

The addition of human serum albumin (HSA) had a pronounced effect on the water relaxivity of the Gd(F-DOTPME)$^-$ complex, similar to that observed with Gd(DOTBP)$^-$.[14] The relaxivity of Gd(F-DOTPME)$^-$ increased as [HSA] was varied from 0 - 2 mM (Figure 4). Making the assumption that HSA has only a single (strong) binding site for Gd(F-DOTPME)$^-$, these data were fit to yield an apparent dissociation constant (K_d) of 0.17 mM and a water relaxivity enhancement of 4.41. Addition of HSA to a solution of Yb(F-DOTPME)$^-$ induced considerable line broadening in the ^{19}F NMR spectrum of the complex, but no change in the ratios of the stereoisomers was evident.

Conclusions

We have examined the ^{19}F NMR spectra of a series of Ln(F-DOTPME)$^-$ complexes (where Ln = La, Gd, Eu, Dy, Tm, and Yb) and found that these complexes exist in aqueous solution as a mixture of diastereomers. This was apparent even in the ^{19}F NMR spectrum of diamagnetic La(F-DOTPME)$^-$ near room temperature. This single resonance was broader than expected for a diamagnetic complex of this molecular weight, apparently reflecting the near chemical shift equivalency of several stereoisomers. Evidence for mixtures of stereoisomers was much more dramatic in the ^{19}F NMR spectra of the paramagnetic Ln(F-DOTPME)$^-$ complexes. We were able to assign several of the shifted ^{19}F resonances in the spectra of Dy(F-DOTPME)$^-$, Tm(F-DOTPME)$^-$, and Yb(F-DOTPME)$^-$ based upon hyperfine shifts of two groups of resonances (axially-*versus* equatorially-positioned CF_3 groups) and by comparison of the integrals of those resonances with a simple neighboring

(28) Zhang, X.; Chang, C. A.; Brittain, H. G.; Garrison, J. M.; Telser, J.; Tweedle, M. F. *Inorg. Chem.* **1992**, *31*, 5597−5600.

group interaction model. The analysis showed that the coordination stereochemistry of each phosphonate ester groups was determined largely by the stereochemistry at each neighboring coordination site (cis groups) and not by interactions between the phosphonate ester functions and the macrocyclic ring carbons.

PEG altered the chemical shifts of CF_3 groups positioned axially in Yb(F-DOTPME)$^-$ more than the CF_3 groups positioned equatorially but did not appear to substantially alter the populations of the various Yb(F-DOTPME)$^-$ stereoisomers. This is consistent with some of the known effects of PEG on solutes dissolved in water. Polyethylene glycol has been widely used to fractionally precipitate water-soluble proteins by altering the solvation properties of water,[29] thereby inducing self-association and aggregation.[30] Our observation that the axial CF_3 groups in these complexes displayed a wider range of ^{19}F chemical shifts prior to addition of PEG suggested that these CF_3 groups experienced a wider range of solvent exposure than the CF_3 groups equatorially coordinated. Thus, the change in water activity associated with the addition of PEG changed the chemical environment of those CF_3 groups more exposed to water than those more in contact with other organic portions of the molecule. Ionic detergents, on the other hand, dramatically altered both the populations of the various stereoisomers and their ^{19}F NMR chemical shifts. This indicates that the distribution of stereomers in these complexes is quite dynamic and can be altered by other solutes. As the ^{19}F NMR spectra of Figure 3 were collected at detergent concentrations well above the critical micelle concentrations of both MTMAB and CPC,[31] we conclude that micelles specifically stabilize the *SSSS* isomer of Yb(F-DOTPME)$^-$, likely by aligning the four $-CH_2CF_3$ groups of each complex close to one another into the hydrophobic portions of the micelle while maintaining the negatively charged chelate near the cationic head groups.

Acknowledgment. This work was supported in part from grants from the Robert A. Welch Foundation (Grant No. AT-584), the NIH NCRR Biomedical Research Technology Program (Grant No. P41-RR02584), and Dow Chemical Co. We thank Don Smithhisler for performing of some preliminary potentiometric titrations.

IC970169M

(29) Ingham, K. C. *Arch. Biochem. Biophys.* **1977**, *184*, 59−68.
(30) Reinhart, G. D.; Hartleip, S. B. *Arch. Biochem. Biophys.* **1987**, *258*, 65−76.
(31) Fendler, J. H.; Fendler, E. J. *Catalysis in micellar and macromolecular systems*, 1st ed.; Academic Press: New York, 1975; pp 26−29.