6-Carboxamido-5,4-Hydroxypyrimidinones: A New Class of Heterocyclic Ligands and Their Evaluation as Gadolinium Chelating Agents

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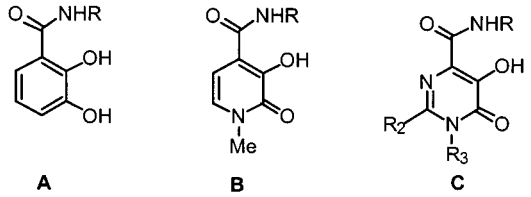
A previously unexplored class of heterocyclic bidentate chelating groups, 6-carboxamido-5,4-hydroxypyrimidinones (6-substituted-HOPYs), have been synthesized by two routes that provide a flexible entry into this ligand system. These are related to, but distinct from, the hydroxypyridonates and have been characterized in this study as a gadolinium chelating agent for magnetic resonance imaging (MRI) applications. The complex Gd[TrenHOPY] demonstrates high stability and high selectivity relative to other ions of biological interest, such as Zn(II) and Ca(II). These stability constants are comparable to those demonstrated by the previously studied 3,2-pyridinone analogues, however, the 5,4-pyrimidinones are at least an order of magnitude more soluble in water. The proton relaxation properties of Gd[TrenHOPY] in water were measured as a function of magnetic field, pH, and temperature. These results support the description of Gd[TrenHOPY] as a complex with two coordinated water molecules in fast exchange with bulk water. In addition, the influence of exogenous anions and blood serum proteins has been investigated. The favorable contrast agent properties emerging from these studies are discussed.

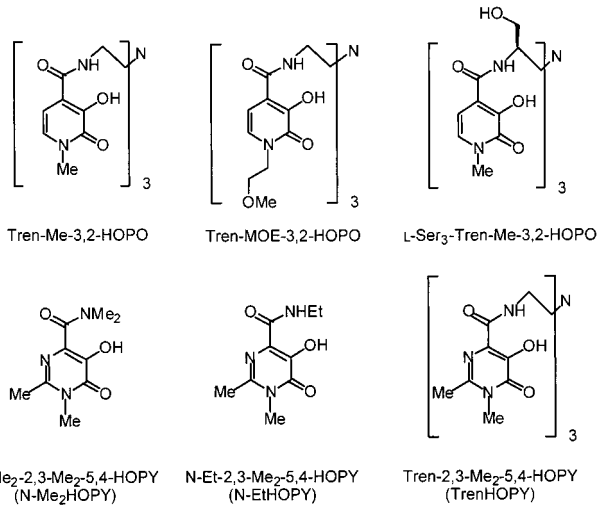
Introduction

The binding motif of the catecholamide chelate (CAM, Scheme 1, **A**), a bidentate catecholate stabilized by the H-bond of an ortho-amide, has been studied in great detail due to its importance as the chelating subunit of a number of siderophores.[1−3] The 4-carboxamido-3,2-hydroxypyridinone chelate (4-carboxamido-3,2-HOPO, Scheme 1, **B**) is a biomimetic analogue of CAM systems which incorporates the structural and H-bonding characteristics known to be important in the effectiveness of CAM siderophores. However, unlike the CAM chelating group, 3,2-HOPOs have a more acidic hydroxy group and are monoprotic. These characteristics make 3,2-HOPOs more versatile ligands than CAMs, and these properties have resulted in the investigation of multidentate 4-carboxamido-3,2-HOPO ligands as siderophore analogues,[4,5] actinide sequestering agents,[6−8] and lanthanide chelating agents.[9] The hexadentate HOPO system, 4-[tris(aminoethyl)amine]-Me-3,2-HOPO (Tren-Me-3,2-HOPO, Scheme 2) is particularly interesting for biological applications. Tren-Me-3,2-HOPO exhibits low acute toxicity, but high efficacy, in Am(III), Pu(IV), Np(V), and U(VI) removal

Scheme 1. The Structural Motif of Some Bidentate Chelating Groups Where an Ortho-Amide Provides Linkage to a Variety of Backbone Architectures



Scheme 2. The Chemical Structures of Ligands Discussed in This Paper



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in vivo.[6,7] Additionally, the very high affinity of Tren-Me-3,2-HOPO for gadolinium(III) compared to biologically available metal ions has prompted its investigation as a novel magnetic resonance imaging (MRI) contrast agent.[9] However, the limited solubility of uncharged metal complexes of Tren-Me-3,2-HOPO has circumscribed attempts fully to investigate the potential of such systems. While recently prepared derivatives of Tren-Me-3,2-HOPO have proved to increase the water solubility significantly (l-Ser_3-Tren-3,2-HOPO,[10] Tren-MOE-3,2-HOPO,[11] see Scheme 2), clinically useful solubilities are higher.

With the goal of expanding the pool of ligand subunits that display the H-bond stabilized, bidentate oxo-hydroxy donor system of **B**, a new heterocyclic ligand system has been developed. The pyrimidinone analogue of the HOPO class of ligands (**B**) are the 6-carboxamido-5,4-hydroxypyrimidinones (6-carboxamido-5,4-HOPY, Scheme 1, **C**). The HOPY system was expected to bring the structural and stability characteristics of the HOPOs to a more water soluble chelate that could be readily further functionalized at two ring positions, R_2 and R_3 (Scheme 1, **C**). *N*-Me_2-2,3-Me_2-5,4-HOPY (*N*-Me_2HOPY), *N*-Et-2,3-Me_2-5,4-EtHOPY (*N*-EtHOPY), and Tren-2,3-Me_2-5,4-HOPY (TrenHOPY) have been prepared in an initial study of this new heterocyclic system (Scheme 2).

Experimental Section

Solution Thermodynamics. General Methods. All solutions were prepared using distilled water that was further purified by passing through a Millipore Milli-Q cartridge system (resistivity = 18 MΩ cm) and then degassed by boiling for at least 30 min while bubbling with argon. Once prepared, solutions were protected from the ingress of oxygen and carbon dioxide by storing under a slight positive pressure of argon, which was purified by passing through an Ascarite II (A. H. Thomas) scrubber.

A solution of 0.100 M KCl was prepared from 99.99% KCl (Fisher Scientific) and was used to maintain constant ionic strength during all titrations. Carbonate-free 0.1 M KOH was prepared from Baker Dilut-It analytic concentrated KOH and standardized against potassium hydrogen phthalate to a phenolphthalein endpoint. Gadolinium(III) and zinc(II) solutions, each ~0.100 M in metal ion, dissolved in ~0.100 M HCl were prepared from anhydrous 99.99% chloride salts (Alpha). The metal ion content was checked by EDTA titration with Xylenol Orange as indicator using sodium acetate buffer. The proton concentration of the standard solutions was checked by titration of a known volume of metal ion solution and a slight excess of EDTA (~1.005 eq) to the equivalence point.[12] For all titrations, the observed pH was measured as $-\log[H^+]$. The glass electrode was calibrated in hydrogen ion concentration units by titrating 2.000 mL of standardized HCl diluted in 50.00 mL of 0.100 M KCl, with 4.200 mL of standardized KOH. The calibration titration data were analyzed by a nonlinear least-squares program.[13]

Potentiometric pH Titrations. As previously reported,[14] potentiometric titrations were performed using an automated apparatus consisting of a Accumet pH meter (models 925, 825MP or 15), a pH electrode (Orion Ross semi-micro combination, Cole Parmer semi-micro combination, or Corning high performance combination electrodes), an autoburet (Metrohm 665 Dosimat or 702 SM Titrino) fitted with a 5 mL piston exchange unit, and a jacketed Ar swept titration cell maintained at 25.0 °C by a Lauda K-2/R or Neslab RTE−111 constant temperature circulating bath. The electronic systems were integrated for automated collection with an IBM PC clone.

In this study, ligand and metal complex solutions were titrated from low to high pH and back again if possible. Titrations for the Zn/HOPY systems were not reversible, presumably due to the formation of mixed ML hydroxide complexes. In this case, titrations from low to high pH were carried out with differing point-by-point equilibration times (~45−120 s) to check for consistency in the determination. Formation constants calculated from the potentiometric titration data were determined with the aid of a FORTRAN nonlinear least-squares refinement program (BETA 90).[15,16] Due to low solubility of the neutral LH_3 species of Tren-Me_2-5,4-HOPY, titrations could not be carried out at ligand concentrations of >0.25 mM L^{-1}. Although this is a low concentration for potentiometric titrations, the buffer regions corresponding to the protonation steps are around neutral pH and, therefore, could still be determined.

Spectrophotometric pH Titrations. As previously reported,[17] spectrophotometric titrations were carried out in a custom-built automatic titration apparatus using an HP 8450A or HP 8452A spectrophotometer and the pH monitoring equipment mentioned above for potentiometric titrations. Solutions were titrated from low to high and high to low pH to ensure that equilibrium had been achieved. At least three data sets were collected, and the spectra (~50−100), pH values, and volumes were transferred to an IBM PC clone for analysis. Data from 230 to 400 nm were used in the refinement. Models used to fit the titration data and determine formation constants were refined using the factor analysis and least-squares refinement program REFSPEC.[14] The calculated spectral properties determined for Gd[TrenHOPY] (λ_{max} 251 nm (26000); 316 nm (24000)) and H_4[TrenHOPY] (λ_{max} LH_4: 306 nm (19000)) systems were independently confirmed from solutions of Gd[TrenHOPY] at pH 8 and H_4[TrenHOPY] at pH 2. The UV spectra of protonated GdL species were determined by calculation to be λ_{max} 325 nm (24000) for GdLH and λ_{max} 312 nm (23000) for $GdLH_2$.

Relaxivity Measurements. Water proton relaxation measurements were carried out at 20 MHz with a Stelar Spinmaster spectrometer (Mede, Pv, Italy) on 0.5−2 mM L^{-1} solutions of the Gd(III) complex. Spin−lattice relaxation times T_1 were measured by the standard inversion recovery method with typical 90° pulse width of 3.5 ms, 16 experiments of 4 scans each. The reproducibility of the data is ±1%. The temperature was controlled by a Stelar VTC-91 air-flow heater equipped with a copper−constantan thermocouple (uncertainty ±0.1 °C). The $1/T_1$ nuclear magnetic relaxation dispersion (NMRD) profiles of water protons were measured from 0.00024 to 1.2 T (corresponding to the range 0.01−50 MHz of proton Larmor frequencies) at 15, 25, and 39 °C using 1.5 mM L^{-1} solutions of the complex on the field-cycling Koenig−Brown relaxometer of the University of Torino (Italy). The temperature was controlled by circulating freon from an external bath and measured by a thermometer inserted into the freon close to the sample. The reproducibilities of the measured T_1 values were estimated to be ±2%. Technical details of the instrument and data acquisition procedure are given elsewhere.[18] The sample for the NMRD profile in blood serum was prepared by dissolving a 1 mM L^{-1} solution of the Gd(III) complex in a lyophilized serum of human origin (Seronorm™, Nycomed) from controlled voluntary blood donors of Scandinavian blood banks.

Variable-temperature ^{17}O NMR measurements were recorded on a JEOL EX-400 (9.4 T) spectrometer, equipped with a 5 mm probe, using D_2O for external lock of the magnetic field. Experimental settings were: spectral width 10000 Hz, pulse width 7 μs, acquisition time 10 ms, 1000 scans, and no sample spinning. The solution used contained ^{17}O enriched water (2.6%, Yeda, Israel). The observed transverse relaxation rates (R^{O}_{2obs}) were calculated from the line width of the resonance at half height.

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Single-Crystal X-ray Diffraction. Diffraction quality crystals of *N,N*-dimethyl-6-carboxamido-2,3-dimethyl-5,4-hydroxypyrimidinone were grown by diffusion of ether into an ethanol solution at room temperature. Collection details and structural parameters in CIF format can be found in the Supporting Information.

Physical Measurements. The NMR spectra were recorded on Bruker AMX 300, AMX 400, or DRX 500 spectrometers. Chemical shifts (δ) are reported in ppm referenced to residual protio-solvent resonances. Melting points were obtained on a Buchi apparatus and are uncorrected. Electronic absorption spectra were recorded on an HP 8450A or HP 8452A UV−vis diode array spectrophotometer with 1 cm quartz cells. Elemental analyses were performed by the analytical services laboratory, College of Chemistry, University of California, Berkeley, CA. Mass spectra (FAB^+ and EI) were obtained by the mass spectrometry laboratory at the College of Chemistry, University of California, Berkeley, CA.

Preparation of Compounds. Unless otherwise noted, all solvents and starting materials were obtained commercially and used without further purification. Water was doubly distilled and further purified by a Millipore cartridge system (resistivity 18 MΩ cm). Syntheses were typically performed under an N_2 atmosphere, although none of the compounds showed particular air or moisture sensitivity during routine manipulations. Benzyl benzyloxyglycolate was prepared as previously reported,[19] and THP-protected ethyl glycolate was prepared by a modified literature procedure[20] as outlined below.

Preparation 1. Tetrahydropyran-2-yloxy-acetic Acid Ethyl Ester. To a stirred solution of ethyl glycolate (35.3 g, 0.339 mol) containing a few crystals of *p*-toluenesulfonic acid, 3,4-dihydropyran (30.0 g, 0.357 mol) was added dropwise (15 g over 1 h followed by 15 g over 30 min). After stirring overnight at room temperature, the mixture was diluted with diethyl ether (80 mL) and washed with a $NaHCO_3$ solution (from 30 mL saturated $NaHCO_3$ and 10 mL water). The organic layer was separated and dried (Na_2SO_4); this was followed by evaporation of the ether. The residue was distilled under high vacuum to give 58.4 g (91.5%) of **1** as a clear liquid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.29 (t, 3H, J = 7.1 Hz, CH_3), 1.53−1.95 (m, 6H, 3,4,5-THP−CH_2's), 3.50−3.55 (m, 1H, 6-THP−CH_2), 3.83−3.89 (m, 1H, 6-THP−CH_2), 4.19 (s, 2H, OCH_2CO_2R), 4.20 (t, 2H, J = 7.1 Hz, CH_2Me), 4.75 (m, 1H, THP−CH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 18.7, 25.2, 30.0, 60.7, 61.9, 63.8, 170.4.

2-Methyl-3*H*-5-tetrahydropyran-2-yloxy-6-tetrahydropyran-2-yloximethyl-4-pyrimidinone (2). THPO−ethyl glycolate (34.0 g, 0.195 mol) in ether (180 mL) was stirred with Na shot (2.24 g, 0.0974 mol) for 20 h under an N_2 atmosphere, resulting in a yellow solution. NaH (0.54 equiv) can be used in place of Na, but usually requires the addition of 2−5% m/m EtOH to promote the reaction. The ether was removed and the residue was covered with absolute ethanol (20 mL). An acetamidine solution was prepared from acetamidine·HCl (9.32 g, 0.0989 mol), which was stirred for 2 h in sodium ethoxide in ethanol (130 mL, by addition of 2.36 g of Na). This suspension was filtered onto the ethanol-covered residue from above, and the filter cake was washed with ethanol (5 mL). The reaction mixture was then stirred and heated at reflux 3.5 h, cooled to room temperature, and the solvent evaporated. The residue was dissolved in CH_2Cl_2 (80 mL), and HOAc was added (to pH 6, wet pH paper). After washing with water (2 × 80 mL), the organic layer was dried (Na_2SO_4), and most of the solvent was removed. To the viscous CH_2Cl_2 solution, hexanes (100 mL) were added, producing a white precipitate which was filtered and washed with hexanes to afford **2**, 20.6 g (65.2%). Mp: 113−114 °C. (+)-FABMS: m/z 325 $[MH]^+$. ^{1}H NMR ($CDCl_3$, 300MHz): δ 1.4−2.0 (m, 12H, 3,4,5-THP−CH_2), 2.42 (s, 3H, 2- CH_3), 3.50−3.56 (m, 2H, 6-THP−CH_2), 3.86−3.96 (m, 2H, 6-THP−CH_2), 4.44−4.81 (4 d's, 2H, CH_2−OTHP), 4.79 (br m, 1H, THP−CH), 5.83 (br m, 1H, THP−CH), 11.44 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 10.5, 18.5, 18.5, 19.1, 19.3, 21.1, 25.0, 25.38, 25.40, 29.9, 30.3, 30.4, 61.9, 62.2, 62.70, 62.74, 63.6, 63.8, 82.8, 98.4, 98.6, 98.63, 98.7, 138.58, 138.63, 152.4, 152.9, 161.6. Anal. Calcd (found) for $C_{15}H_{24}N_2O_5$: C, 59.24 (59.10); H, 7.46 (7.51); N, 8.64 (8.94).

2,3-Dimethyl-5-tetrahydropyran-2-yloxy-6-tetrahydropyran-2-yloximethyl-4-pyrimidinone (3). The THP-protected pyrimidine **2** (17.9 g, 54.9 mmol) dissolved in DMF (70 mL) was dripped into a slurry of NaH (2.30 g, 60% in oil, 57.5 mmol) in DMF (100 mL) maintaining a gentle effervescence. The reaction was stirred a for another 10min then MeI (3.45 mL, 55.4 mmol) was added. After stirring 18 h a few drops of methanol were added, followed after a few minutes by evaporation of the solvent under reduced pressure. The residue was dissolved in CH_2Cl_2 (150 mL) and washed with water (3 × 150 mL). The solvent was removed and the residue dissolved in acetonitrile, then washed with hexanes (50 mL). After removal of the solvent this afforded 16.2−18.6 g (87−100%) of the crude product (>90% pure by ^{1}H NMR). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.52−1.89 (m, 12H, 3,4,5-THP−CH_2), 2.49 (s, 3H, 2-CH_3), 3.49 (s, 3H, N-Me), 3.49−3.55 (m, 2H, 6-THP−CH_2), 3.92−3.96 (m, 2H, 6-THP−CH_2), 4.39−4.82 (m, 3H, CH_2−OTHP + THP−CH), 5.77 (br s, 1H, THP−CH).

2,3-Dimethyl-5-hydroxy-6-(hydroxymethyl)-4-pyrimidinone·HCl (4·HCl). Method A. The protected pyrimidine **3** (3.38 g, 10 mmol) was dissolved in ^{i}PrOH (20 mL), diethyl ether (20 mL), and concentrated HCl (1 mL). After standing at room temperature for 4 h, the crystallizing solution was refrigerated (0 °C) overnight. After filtration and washing with 1:1 ^{i}PrOH/Et_2O (3 × 5 mL) then Et_2O (10 mL), the white solid was dried under high vacuum at room temperature to give 1.83 g (88.8%) of **4·HCl**.

Method B. A hydrogen chloride/dioxane solution (50 mL, 4M) was dripped into a solution of the protected pyrimidine **3** (30.1 g, 89 mmol) in EtOH (20 mL). A white solid rapidly precipitated and, after standing for a few hours at room temperature, the solid was filtered and washed with dioxane (3 × 10 mL) and diethyl ether (2 × 10 mL). Drying in vacuo overnight at 40 °C gave 16.08 g (88.8%) of **4·HCl**. This absorbs 1equiv of H_2O upon standing exposed in air. Mp: >200 °C (dec). (+)-FABMS: m/z 171 $[MH]^+$. ^{1}H NMR (DMSO-d_6, 300 MHz): δ 2.68 (s, 3H, 2-Me), 3.49 (s, 3H, N-Me), 4.47 (s, 2H, CH_2O). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 18.7, 31.8, 54.1, 132.9, 138.0, 155.2, 157.2. Anal. Calcd (found) for $C_7H_{13}ClN_2O_4$: C, 37.43 (37.65); H, 5.83 (5.91); N, 12.47 (12.46).

2,3-Dimethyl-5-benzyloxy-6-(hydroxymethyl)-4-pyrimidinone (5). In a flask protected from direct light, DMF (300 mL), the above pyrimidine, **4·HCl** (14.14 g, 68.43 mmol), and K_2CO_3 (20.0 g, 145 mmol) were mechanically stirred for 1 h at 65 °C; this was followed by addition of BnCl (8.30 mL, 72.1 mmol) in one portion. Additional BnCl was added as the reaction progressed if TLC indicated it to be necessary. After stirring for 8 h at 65 °C, the reaction mixture was cooled to room temperature, filtered (washing the cake with 2 × 20 mL DMF), and the solvent removed. The residue, in CH_2Cl_2 (100 mL), was filtered again and concentrated to ∼20 mL, at which point the cooled solution began to deposit a white crystalline mass. Dilution with diethyl ether (∼100 mL) afforded slightly off-white crystals (10.1 g, 56.7%) of **5**. Mp: 93−95 °C. (+)FABMS: m/z 261 $[MH]^+$. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.49 (s, 3H, 2-Me), 3.19 (br s, 1H, OH), 3.53 (s, 3H, N-Me), 4.37 (s, 2H, CH_2O), 5.17 (s, 2H, CH_2Ph), 7.28−7.40 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.9, 31.3, 58.8, 73.2, 128.3, 128.4, 128.6, 136.7, 137.5, 150.6, 153.9, 158.9. Anal. Calcd (found) for $C_{14}H_{16}N_2O_3$: C, 64.60 (64.62); H, 6.20 (6.04); N, 10.76 (10.82).

2,3-Dimethyl-5-benzyloxy-6-carboxy-4-pyrimidinone (6). The above pyrimidine, **5** (9.00 g, 34.6 mmol), TEMPO catalyst (55 mg), Adogen-464 phase transfer catalyst (690 mg), and NaBr (360 mg, 3.50 mmol) were combined in CH_2Cl_2 (400 mL) and water (10 mL). After cooling to 0 °C, the reaction was stirred at 1500 rpm while adding a cooled (10 °C), buffered bleach solution (125 mL commercial bleach, 125 mL water, and 12.5 g $NaHCO_3$) keeping the reaction <4 °C (takes approximately 15 min). After a further 5 min, 2 M NaOH solution was added until a solution of pH 10 was obtained. The CH_2Cl_2 layer was separated and extracted with basic water (pH 10, 100 mL). The combined aqueous solutions were washed with CH_2Cl_2 (50 mL). The aqueous phase was concentrated to ∼200−250 mL and carefully acidified (concentrated HCl) to pH 2, concomitant with precipitation. After standing at 5 °C overnight, the white solid was filtered, washed with water, and dried under high vacuum to give 5.20 g (54.9%) of **6**. Mp: 180−181 °C. (+)FABMS: m/z 275 $[MH]^+$. ^{1}H NMR ($CDCl_3$,

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300 MHz): δ 2.53 (s, 3H, 2-Me), 3.55 (s, 3H, N-Me), 5.46 (s, 2H, $BnCH_2$), 7.30−7.38 (m, 3H, Ph), 7.49−7.52 (m, 2H, Ph).^{13}C NMR (DMSO-d_6, 100 MHz): δ 22.6, 31.2, 73.3, 128.0, 128.2, 128.3, 136.9, 139.1, 143.0, 155.7, 159.2, 165.7. Anal. Calcd (found) for $C_{14}H_{14}N_2O_4$: C, 61.31 (61.36); H, 5.14 (5.06); N, 10.21 (10.30).

2,3-Dimethyl-5-benzyloxy-6-carboxy-4-pyrimidinone Ethyl Ester (10). To a stirred suspension of **6** (0.380 g, 1.39 mmol) in CH_2Cl_2 (20 mL) was added carbonyl diimidazole (0.230 g, 1.42 mmol). The suspension rapidly dissolved, and after ∼3 min the solution was diluted with ethanol (20 mL) and stirred overnight. After chromatography (SiO_2, 2%MeOH/CH_2Cl_2) and recrystallization from ether/hexanes, **14** (0.19 g, 45%) was afforded as a white powder. Mp: 110−111.5 °C. (+)-FABMS: *m*/*z* 303 ([MH]$^+$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.29 (t, 3H, J = 7.1 Hz, ethyl-Me), 2.52 (s, 3H, 2-Me), 3.55 (s, 3H, NCH_3), 4.32 (q, 2H, J = 7.1 Hz, OCH_2), 5.23 (s, 2, $BnCH_2$), 7.30−7.36, 7.45−7.47 (m, 3+2H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 13.9, 23.0, 31.5, 61.8, 74.0, 128.1, 128.2, 128.4, 136.5, 141.2, 141.6, 154.1, 159.8, 164.2. Anal. Calcd (found) for $C_{16}H_{18}N_2O_4$: C, 63.56 (63.78); H, 6.00 (6.09); N, 9.27 (9.24).

Preparation 2. 2-Methyl-3*H*-5-benzyloxy-6-carboxy-4-pyrimidinone Ethyl Ester (9). Benzyl benzyloxyacetate (14.13 g, 55.13 mmol), diethyloxalate (8.060 g, 55.13 mmol), and ethanol (0.2 mL) were stirred in dry THF (100 mL) with NaH (2.34 g, 60% in oil, 58.5 mmol) at room temperature for 24 h. The THF was removed on a rotary evaporator, and the residue was dissolved in ethanol (100 mL) followed by addition of sodium ethoxide (3.75 g, 55.1 mmol) and acetamidine hydrochloride (5.21 g, 5.11 mmol). After stirring at 60 °C for 1.5 h, the resulting suspension was cooled to room temperature, and the solvent was removed. The resulting oil was partitioned between CH_2Cl_2 (80 mL) and water (50 mL), and the pH was adjusted to ∼6. Filtration was performed if necessary. The CH_2Cl_2 was separated and combined with a CH_2Cl_2 (30 mL) wash of the aqueous phase. The CH_2Cl_2 solution was washed with water, separated, dried (Na_2SO_4), and the volume reduced until a thick oil was obtained. This was immediately shaken with diethyl ether (30 mL), and a white solid precipitated. After dilution with hexanes (∼10 vol %), the solution was filtered and the cake washed with cold ether (3 × 10 mL). After drying, 7.2 g (45%) of **9** was obtained as a pale powder. Mp: 125−126 °C. (+)FABMS: *m*/*z* 289 ([MH]$^+$). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.31 (t, 3H, J = 7.1 Hz, ethyl-Me), 2.50 (s, 3H, 2-Me), 4.35 (q, 2H, J = 7.1 Hz, OCH_2), 5.25 (s, 2, NCH_3), 7.32−7.38, 7.44−7.46 (m, 3 + 2H, Ph), 13.14 (br t, 1H, amide NH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.1, 21.3, 62.2, 74.5, 128.4, 128.5, 136.4, 141.9, 144.9, 153.6, 162.2, 164.2. Anal. Calcd (found) for $C_{15}H_{16}N_2O_4$: C, 62.49 (62.51); H, 5.59 (5.62); N, 9.72 (9.71).

2,3-Dimethyl-5-benzyloxy-6-carboxy-4-pyrimidinone (6) via 2,3-Dimethyl-5-benzyloxy-6-carboxy-4-pyrimidinone Ethyl Ester (10). To a stirred suspension of NaH (0.745 g, 60% in oil, 18.6 mmol) in dry DMF (30 mL) was added dropwise a solution of **9** (4.88 g, 16.9 mmol) in DMF (20 mL) over ∼20 min (maintaining gentle effervescence). After H_2 evolution had ceased, methyl iodide (1.27 mL, 20.4 mmol) was added in one portion, and the reaction was stirred at ambient temperature. Fine crystals deposited, and after ∼2 h the reaction was complete (by TLC, silica gel, 4%MeOH/CH_2Cl_2). The excess hydride was quenched with ethanol (2 mL), and the DMF was removed by rotary evaporation. Addition of water produced an oily solid which became an off-white crystalline mass upon further shaking. This solid was separated by filtration, dried briefly, and then washed with hexanes (3 × 20 mL) to afford 3.0 g (∼10 mmol) of crude **10** (>95% pure by NMR). The spectroscopic properties of this material corresponded to those of **10** synthesized in Preparation 1. This crude product was dissolved in methanol (50 mL) and KOH (0.660 g, 11.8 mmol) and stirred at room temperature. After 6 h the hydrolysis was complete, and a fine white precipitate had formed. After evaporation of the solvent under reduced pressure, the residue was dissolved in water (20 mL), filtered, and slowly acidified with concentrated HCl. A small amount of yellowish, sticky solid initially precipitated and the solution was decanted from this. Acidification continued (to pH 2) and a white solid was isolated by filtration; an aqueous wash followed (2 × 10 mL) to give, after drying in vacuo, 2.63 g of **6** (57% from **9**). This material had identical spectroscopic properties to **6** synthesized in Preparation 1. Mp. 179−180 °C. Anal. Calcd (found) for $C_{14}H_{14}N_2O_4$: C, 61.31 (61.50); H, 5.14 (5.13); N, 10.21 (10.30).

Ligand Syntheses from HOPY Acid (6). Tris[(2,3-Dimethyl-5-benzyloxy-6-carboxamido-4-pyrimidinone) ethyl]amine (7a). To a slurry of HOPY acid **6** (1.99 g, 7.26 mmol) in CH_2Cl_2 (20 mL), carbonyl diimidazole (1.25 g, 7.71 mmol) was added in ∼0.3 g portions over 10 min. After a further 5 min, tris(2-aminoethyl)amine (0.350 g, 2.39 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 5 min, and the reaction was stirred overnight. The CH_2Cl_2 was removed and the resultant oil shaken into ethanol (20 mL). Upon standing, a white crystalline mass separated, which was filtered and washed with ethanol (5 × 5 mL). After drying under high vacuum at 40 °C, 1.53 g (69%) of **(7a)** was isolated. Mp: 110−113 °C. (+)FABMS: *m*/*z* 915 [MH]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.28 (s, 3H, 2-Me), 2.64 (t, 2H, J = 5.8 Hz, cap NCH_2), 3.34−3.37 (m, 3 + 2H, NCH_3 + CH_2NHCOR), 5.10 (s, 2H, $BnCH_2$), 7.27−7.35, (m, 3H, Ph), 7.50−7.52 (m, 2H, Ph), 7.79 (br t, 1H, amide NH).^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.8, 31.9, 37.8, 53.3, 74.6, 128.3, 128.4, 129.0, 136.7, 141.1, 141.4, 153.4, 160.1, 162.9. Anal. Calcd (found) for $C_{48}H_{54}N_{10}O_9\cdot H_2O$: C, 61.79 (61.48); H, 6.05 (5.98); N, 15.01 (14.98).

***N*-Ethyl-2,3-dimethyl-5-benzyloxy-6-carboxamido-4-pyrimidinone (7b):** synthesized by the method given above for **7a** and isolated as a white solid in 59% yield after chromatography (SiO_2, 2%MeOH/CH_2Cl_2). (+)FABMS: *m*/*z* 302 [MH]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.08 (t, 3H, J = 7.3 Hz, ethyl-Me), 2.49 (s, 3H, 2-Me), 3.33 (m, 2H, CH_2NHCOR), 3.50 (s, 3H, NMe), 5.22 (s, 2H, $BnCH_2$), 7.27−7.35, 7.38−7.40 (m, 3+2H, Ph), 7.41 (br s, 1H, amide NH).^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.4, 23.1, 31.6, 34.3, 74.7, 128.4, 128.9, 136.3, 140.6, 141.9, 153.1, 160.3, 162.4. Anal. Calcd (found) for $C_{16}H_{19}N_3O_3$: C, 63.77 (63.84); H, 6.36 (6.27); N, 13.94 (14.00).

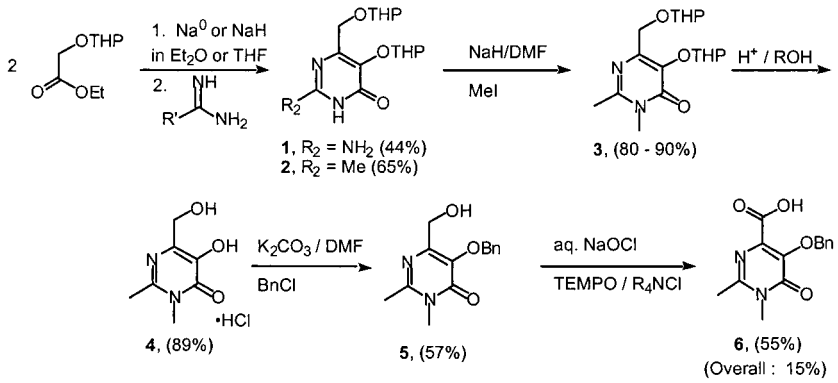
***N*,*N*-Dimethyl-2,3-dimethyl-5-benzyloxy-6-carboxamido-4-pyrimidinone (7c):** synthesized by the method given above for **7a** and isolated as a white solid in 68% yield after chromatography (SiO_2, 2%MeOH/CH_2Cl_2). (+)FABMS: *m*/*z* 302 [MH]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.48 (s, 3H, 2-Me), 2.74 (s, 3H, amide N-Me), 2.99 (s, 3H, amide N-Me), 3.52 (s, 3H, NMe), 5.16 (s, 2H, $BnCH_2$), 7.26−7.33, 7.38−7.40 (m, 3+2H, Ph).^{13}C NMR ($CDCl_3$, 100 MHz): δ 23.04, 31.43, 34.30, 37.50, 74.02, 128.11, 128.27, 128.43, 136.83, 138.43, 145.98, 154.95, 159.50, 165.56. Anal. Calcd (found) for $C_{16}H_{19}N_3O_3$: C, 63.77 (64.08); H, 6.36 (6.35); N, 13.94 (13.90).

Tris[(2,3-dimethyl-5-hydroxy-6-carboxamido-4-pyrimidinone)-ethyl]amine (8a). The benzyloxypyrimidinone **(7a)** (1.12 g, 1.22 mmol) was dissolved in acetic acid (10 mL) and 5%Pd/C (60 mg) was added. The reaction mixture was stirred under an atmosphere of H_2 for 2 h at room temperature. The Pd/C was removed by filtration, and the solution was concentrated to a thick oil. Dilution with MeOH (5 mL) then water (25 mL) led to precipitation of the product as a white powder, which was dried in vacuo to afford 0.70 g of **8a** (86%). Mp: 217−219 °C (melts and resolidifes), 242−244 °C (dec). (+)FABMS: *m*/*z* 665 [MH]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.63 (br s, 2H, water), 2.36 (br s, 3H, 2-Me), 2.81 (br t, 2H, J = 6 Hz, cap NCH_2), 3.48 (br t + s, 2 + 3H, CH_2NHCOR + NCH_3), 7.83 (br t, 1H, amide NH), 11.91 (s, 1H, OH).^{13}C NMR ($CDCl_3$, 100 MHz): δ 22.2, 31.3, 37.1, 52.6, 125.1, 145.4, 149.0, 157.5, 168.1. Anal. Calcd (found) for $C_{27}H_{36}N_{10}O_9\cdot H_2O$: C, 48.94 (48.69); H, 5.78 (5.89); N, 21.14 (20.78)

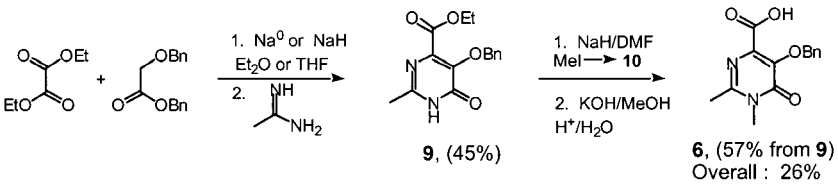
***N*-Ethyl-2,3-dimethyl-5-hydroxy-6-carboxamido-4-pyrimidinone (8b):** synthesized by the method given above for **8a** as a white solid in 95% yield after crystallization of the crude filtrate from 2-propanol/ether. Mp: 138−139 °C. (+)FABMS: *m*/*z* 212 [MH]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.24 (t, 3H, J = 7.3 Hz, ethyl-Me), 2.44 (s, 3H, 2-CH_3), 3.43 (m, 2H, CH_2NCOR), 3.52 (s, 3H, NCH_3), 7.61 (br s, 1H, amide NH), 12.06 (s, 1H, OH).^{13}C NMR ($CDCl_3$, 100 MHz): δ 14.5, 22.5, 31.6, 33.9, 125.2, 146.4, 147.9, 158.6, 168.0. Anal. Calcd (found) for $C_9H_{13}N_3O_3$: C, 51.18 (51.40); H, 6.20 (6.10); N, 19.89 (19.79).

***N*,*N*-Dimethyl-2,3-dimethyl-5-hydroxy-6-carboxamido-4-pyrimidinone (8c):** synthesized by the method given above for **8a** as a white solid in 92% yield after crystallization of the crude filtrate from ethanol. Mp: 203−204 °C. (+)FABMS: *m*/*z* 212 [MH]$^+$. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.42 (s, 3H, 2-Me), 3.04 (br s, 3H, amide NCH_3), 3.22 (br s, 3H, amide NCH_3), 3.50 (s, 3H, NCH_3), 9.98 (s, 1H, OH).^{13}C

Scheme 3. Synthetic Outline of Preparation 1 for the Synthesis of a HOPY System



Scheme 4. Preparation of HOPY Ligands from Precursor **6**



NMR ($CDCl_3$, 100 MHz): δ 22.5, 31.6, 36.2, 38.7, 130.4, 143.2, 147.5, 159.2, 167.2. Anal. Calcd (found) for $C_9H_{13}N_3O_3$: C, 51.18 (51.10); H, 6.20 (6.08); N, 19.89 (19.88).

Results and Discussion

Synthesis. Syntheses of compounds with the HOPY motif, **C**, have been previously reported,[21−23] albeit as unexpected products in the study of other heterocyclic systems. Difficulties with reproducibility,[23] preparative scale-up,[22] or substrate specificity (which precluded the development of a general synthesis)[21,22] dissuaded us from the use of these synthetic routes. Synthesis of the desired class of functionalized HOPYs was achieved by modifying and extending the 5,4-hydroxypyrimidinone synthesis of Davoll and Laney,[20] where **1** was reported (Scheme 3).

Self-condensation of tetrahydropyran protected ethyl glycolate[20] provided the corresponding β-ketoester, which was combined in situ with acetamidine in ethanol to afford **2**. The ^{1}H and ^{13}C NMR spectra of **2** indicate that it is an equal mixture of two diastereomers, resulting from chirality at the two THP acetal carbons. Methylation of **2** with NaH/MeI/DMF[24] gave **3** as a thick oil of >90% purity. Deprotection of **3** (4M HCl·dioxane/2-propanol) provides pure **4·HCl**, alleviating the necessity to further purify **3**, the oily precursor. Reprotection of the 5-hydroxy group ($BnCl/K_2CO_3$/DMF) gave **5**. Compound **5** should be a useful intermediate in the preparation of multidentate HOPY ligands where the electronic and structural influences of an amide group are not desired. Oxidation of **5** using bleach with TEMPO catalyst under phase transfer conditions[25] provided analytically pure **6** after careful acidification of the crude reaction extracts. The overall yield of the synthetic sequence described is ~15% for multigram syntheses. The reactions were optimized to be run on this scale without the need for chromatography in preparing the key ligand precursor, HOPY carboxylic acid, **6**.

A second, more direct preparation of **6** was also developed, as outlined in Scheme 4. The Claisen condensation product of ethyl oxalate and 2-benzyloxy benzyl acetate was reacted with acetamidine in ethanol to provide **9**. Chromatography provides analytically pure material, although crystallization provides material of sufficient purity for subsequent use. *N*-Methylation by the previously described method (MeI/NaH/DMF) provides the N3 alkylated isomer (**10**), which, after hydrolysis gives the HOPY acid **6**, which is identical in spectroscopic properties to **6** prepared from Preparation 1 (Scheme 3).

Further synthesis of several common products using materials from Preparations 1 and 2 confirms that the same heterocyclic system is formed in both cases. Thus, **10** (an intermediate in the synthesis of **6** in Preparation 2) was also made from HOPY acid, **6** (from Preparation 1), by ethanolysis of the CDI activated ester of **6**. Also **7a** (and then **8a**) was prepared from **6** (from Preparation 2) and found to be identical to **7a** and **8a** previously made from Preparation 1. Synthesis of **6** via Preparation 2 does not give access to precursors such as **5**, but gives access to HOPY systems (such as **9**) that need not be alkylated at the N3 position, thus providing access to a further series of HOPY ligands.

Conversion of **6** into an activated ester with CDI and in situ reaction with the appropriate amines gave **7a**−**c** in 70−90% yield. Deprotection of the benzyl protecting group and recrystallization afforded the free ligands **8**, which were obtained as analytically pure white solids. TrenHOPY, **8a**, *N*-EtHOPY, **8b**, and *N*-Me_2HOPY, **8c**, were prepared in this manner. Anhydrous **8a** absorbs 1 equiv of H_2O after a few hours of exposure to air and is then stable in this hydration state. *N*-EtHOPY and *N*-Me_2-HOPY are air stable as anhydrous materials.

The spectroscopic properties of products **2**−**8** are consistent with the structures as formulated. However, for further confirmation of structure, single crystals of *N*-Me_2HOPY suitable for analysis by X-ray diffraction were obtained. *N*-Me_2HOPY crystallized in $P\bar{1}$ with the asymmetric unit consisting of a hydrogen bonded dimer. No solvents of crystallization were found in the lattice. All hydrogen atoms were found and their

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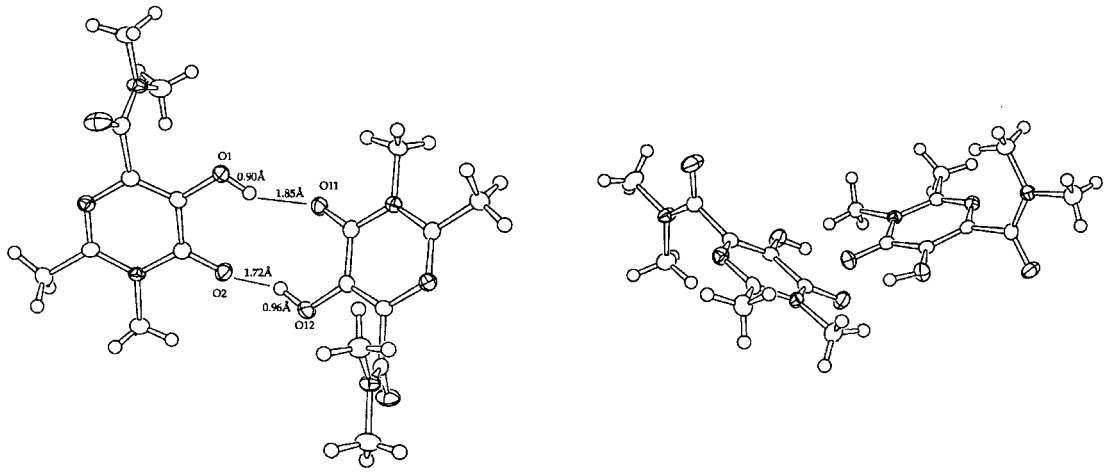

Figure 1. The H-bonded dimer of *N*-Me_2HOPY in the solid-state view from two perspectives. Molecule 1 is on the left, molecule 2 on the right for each view of the dimer.

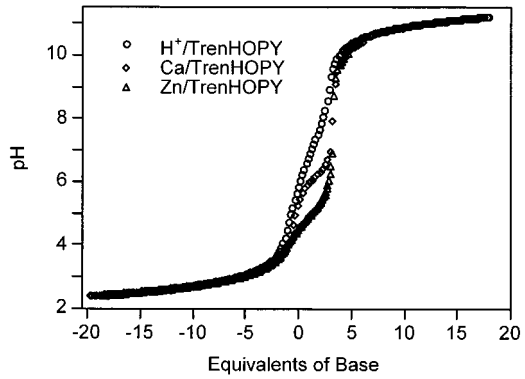

Figure 2. Potentiometric titration curve for H^+, Ca^{2+}, and Zn^{2+} with TrenHOPY, for M^{2+}, 1:1 M:L, $[M^{2+}] = 0.25$ mM L^{-1}, for H^+, $[L] = 0.20$ mM L^{-1}, $\mu = 0.1$ M (KCl), T = 25 °C.

coordinates refined. Each *N*-Me_2HOPY is structurally similar and consists of the planar N_2C_4 HOPY core (0.01 Å rms deviation from a least squares plane in both molecules), with methyl groups at the 2,3 positions as expected (Figure 1). The dimethyl amide function is rotated out of the heterocyclic plane by 67 and 78° for molecules 1 and 2, respectively. The dimethylamide carbonyl oxygen is not within H-bonding distance of any H-bond donors and clearly does not form a six-membered ring intermolecular hydrogen bond to the 5-hydroxy position. The hydrogen bonding between the two independent units has no unusual metric parameters, consisting of a 10 membered ring with intermolecular OH····O distances of 1.72 and 1.85 Å and an identical OHO angle of 157°.

Ligand Protonation Constants. The protonation constants for **8a**, **8b**, and **8c** were determined by potentiometric titrations (Figure 2). These constants are defined by eq 1.

$$H_{n-1}L + H^+ \leftrightarrow H_nL \quad K_n = \frac{[H_nL]}{[H_{n-1}L][H^+]} \qquad (1)$$

The HOPY amide derivatives, *N*-EtHOPY (**8b**) and *N*-Me_2-HOPY (**8c**), provide simple systems for which comparisons can be made to 6-carboxamido-3,2-HOPO ligands. For a wide variety of analogous pyridine and pyrimidine structures, the pyrimidines, by virtue of substituting a carbon atom for a nitrogen in the heterocyclic ring, possess the more acidic protonation constant.[26] However, for *N*-EtHOPY, the protonation constant (log $K_H = 6.48$) is more basic than those for similar HOPO compounds (e.g., *N*-PrHOPO, log$K_H = 6.12$). When comparing the protonated forms of these HOPOs and HOPYs, a significant difference is apparent. *N*-EtHOPY has a conformer in which a hydrogen bond can be formed between the amide-NH and the N_1 pyrimidine nitrogen, stabilizing the protonated form with bicyclic H-bonded chelates

This assertion, that the internal H-bond of *N*-EtHOPY is responsible for the otherwise surprising relative protonation constants of *N*-EtHOPY and *N*-PrHOPO, can be further tested by consideration of the tertiary amide derivatives (Scheme 5). The protonation constants of the dimethylamide derivatives of these heterocycles, *N*-Me_2HOPY and *N*-Me_2HOPO, display the expected trends in ligand basicities; the log protonation constants are 6.68 and 7.69, respectively. This comparison demonstrates that the HOPY system is indeed not more electron rich than the HOPO system, but that the pyrimidinone N_1−HN hydrogen bond lends stability to the protonated HOPY form. Additionally, as can be see by comparing *N*-Me_2HOPO and *N*-PrHOPO, the hydrogen bond of the ortho amide contributes over 1.5 log units to acidifying the OH group of the HOPO core. This is in accord with the general trend observed in HOPO and CAM units in which the NH amide group contributes to significant acidification of the heterocyclic OH group. By contrast, *N*-EtHOPY is found to be only 0.2 log units more acidic than *N*-Me_2HOPY. Hence, the N_1 to amide NH hydrogen bond clearly competes against the normal (acidifying) action of the amide NH on the 5-hydroxy substituent. The N_1−HN hydrogen bond probably does not contribute significantly to the solution structure of the deprotonated form of *N*-EtHOPY, since the heterocyclic alkoxide group participating in a six-membered H-bond to the amide NH affords the stronger electrostatic interaction. From this consideration, the deprotonated HOPY systems are expected to be structurally akin to the deprotonated HOPO systems. Therefore, the deprotonated ligands are a lesser consideration in explaining the differences in protonation constants between the HOPY and HOPO systems.

The protonation constant behavior described for the simple HOPY and HOPO systems also extends to a comparison of the hexadentate systems TrenHOPY and TrenHOPO. Table 1 summarizes protonation constant data for TrenHOPY; the values

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Scheme 5. *N*-Me_2HOPO, *N*-PrHOPO, *N*-Me_2HOPY, and *N*-EtHOPY, Demonstrating the H-Bonding Scheme of the Protonated Ligands

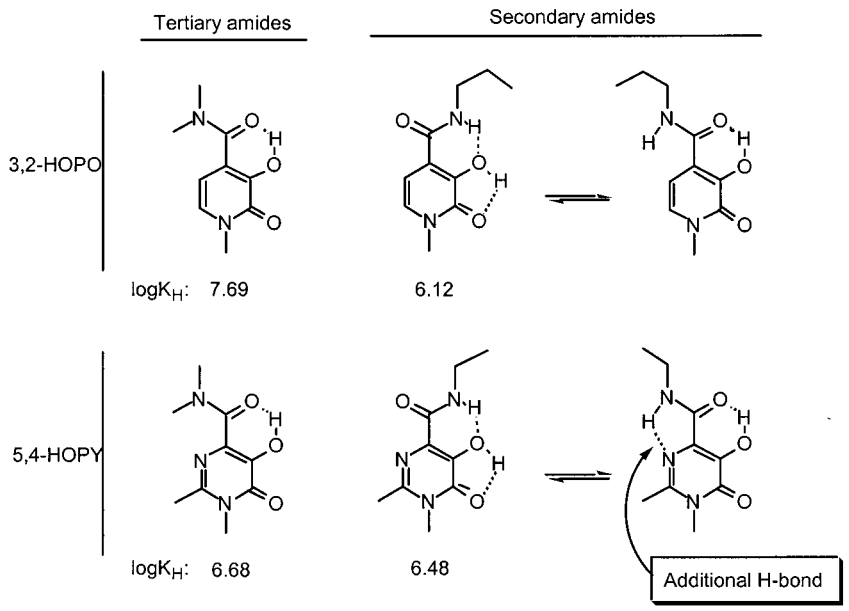

Table 1. A Comparison of the Protonation Constants Determined for TrenHOPY and Tren-MOE-3,2-HOPO

	TrenHOPY	Tren-MOE−3,2-HOPO[a]
logK_1	8.27(1)	8.08
logK_2	7.23(2)	6.85
logK_3	6.46(2)	5.81
logK_4	5.61(1)	4.95
logβ_{014}	27.38	25.69
logβ_{014} − logK$_1$	19.11	17.61
Mean[b]	6.37	5.87

[a] Values from reference 11. [b] The average of the heterocyclic protonation constants: (logβ_{014} − logK_1)/3.

determined for Tren-MOE-3,2-HOPO[11] are included for the purposes of comparison.

The protonation constant of the TrenHOPY amine cap (log K_H 8.27) is more basic than the amine cap of Tren-MOE-3,2-HOPO (log K_H 8.08). The average log protonation constant of the TrenHOPY chelate units is 6.37, which is more acidic than the simple bidentate 5,4-hydroxypyrimidinone, *N*-EtHOPY, **8b**, (logK_H 6.48). This is consistent with trends observed in the HOPO systems, where the average protonation constant for the hexadentate Tren-MOE-3,2-HOPO chelating groups is 5.87; this is also more acidic than those of simple *N*-alkyl 3,2-HOPO ligands, which typically have a logK_H of ∼6.1.[8] As expected from the protonation constants of *N*-EtHOPO and *N*-PrHOPO, TrenHOPY is more basic than the TrenHOPO systems.

Gd(III), Zn(II), and Ca(II) Formation Constants for Tren-Me_2-5,4-HOPY. For the spectrophotometric determination of formation constants for Tren-Me_2-5,4-HOPY with gadolinium, spectra were collected over a pH range of 2−8 (Figure 3). The formation constants are defined by eq 2.

$$mM + lL + hH \leftrightarrow M_mL_lH_h \quad \beta_{mlh} = \frac{[M_mL_lH_h]}{[M]^m[L]^l[H]^h} \quad (2)$$

The Gd[TrenHOPY] data were successfully refined with a model containing four components: LH_4^+, GdL, $GdLH^+$, and $GdLH_2^{2+}$. This speciation is similar to the solution behavior of Tren-MOE-3,2-HOPO, although the parent complex, Gd(Tren-Me-3,2-HOPO), was not formerly reported as possessing a

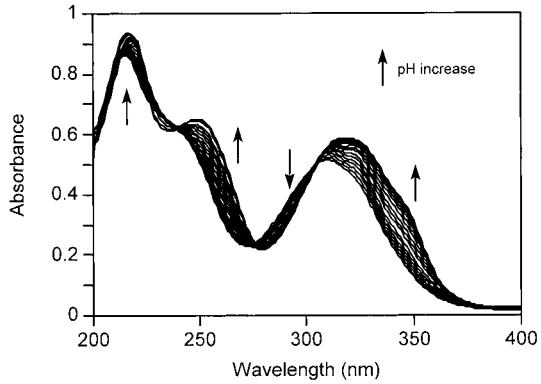

Figure 3. UV spectra of a 1:1 solution of Gd^{3+} and TrenHOPY as a function of pH (2.0−8.0) from a spectrophotometric titration, [TrenHOPY] = 0.025 mM L^{-1}, μ = 0.1 M (KCl), T = 25 °C.

Table 2. Formation Constants[a] for TrenHOPY and Tren-MOE-3,2-HOPO with Gd^{3+}

species	MLH	TrenHOPY	Tren-MOE−3,2-HOPO[b]
[GdL]	110	18.2(2)	19.8
$[GdLH]^+$	111	22.4(1)	22.9
$[GdLH_2]^{2+}$	112	26.6(1)	25.9
	pGd	18.0	19.8

[a] Determined by spectrophotometric titration. [b] From ref 11.

diprotonated species.[11] The cumulative formation constants (eq 2) for the gadolinium complexes of TrenHOPY present over the pH range studied are described by β_{110}, β_{111}, and β_{112} (Table 2). The overall formation constant of logβ_{110} = 18.2 is approximately 2 log units lower than that of Gd(Tren-MOE-3,2-HOPO). This lower stability is to be expected; although the protonation constants are higher for the secondary amide HOPY systems, HOPOs are the more electron rich systems. The Gd-[TrenHOPY] complex was found to protonate twice before dissociating into free Gd(III) and protonated ligand at low pH. The equilibrium constants for the protonation of Gd[Tren-Me_2-5,4-HOPY] can be determined from the differences between logβ_{111} and logβ_{110} (for the first protonation constant logK_{111}) and between logβ_{112} and logβ_{111} (for logK_{112}). These values (logK_{111} = logK_{112} = 4.2) occur at the same pH. This

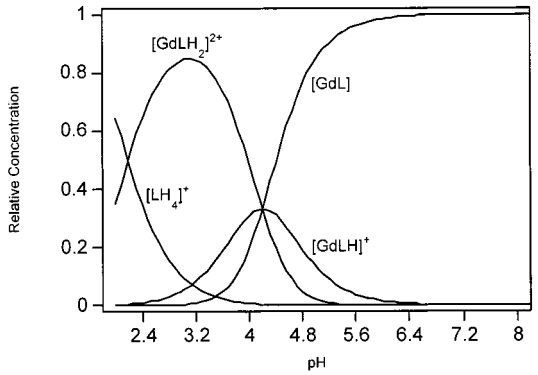

Figure 4. Species distribution of the Gd−TrenHOPY system, 1:1 Gd^{3+}: TrenHOPY, $[Gd^{3+}] = 1$ mM L^{-1}, $\mu = 0.1$ M (KCl), T = 25 °C.

protonation behavior is essentially identical to that found for the TrenHOPO systems where $\log K_{111} = \log K_{112}$. The proximity of the two protonation steps in TrenHOPO systems was attributed to a process in which the first protonation step leads to a structural change in the metal−ligand system that leads to immediate subsequent reaction to the diprotonated species.[11] The capping amine is known from crystal structures to be in the "in" conformation for metal complexes of TrenHOPO ligands, including Gd(Tren-Me-3,2-HOPO),[9] and in the "out" conformation when protonated. Protonation, therefore, leads to disruption of the Tren-cap structure in both the HOPO and HOPY chelate systems such that the Gd(III) donor atom chelate interaction is destabilized, facilitating a coincident protonation and dissociation behavior for an arm of the trigonal cap.

$\text{Log}K_{\text{ML}}$ values reflect the metal−ligand affinity for deprotonated ligand in the reaction M + L ⇌ ML. The pM value is one way to make an allowance for the competition for the ligand by protons in real solutions, and thus, a more complete picture of the effectiveness of the ligand in chelating the metal is given. The pGd values in Table 2 are defined by eq 3

$$\text{pGd} = -\log[\text{Gd}]_{\text{free}} \quad \text{at pH 7.4 for } [\text{Gd}] = 1\mu\text{M}, [\text{L}] = 10\mu\text{M} \quad (3)$$

A pM value can be calculated for many conditions, but in this case the conditions are chosen to help deliberate biological considerations. As expected from the near neutral protonation constants of the heterocyclic groups, the pGd values (Table 2) are comparable to the formation constants. However, for the same reason that the protonation constants of TrenHOPY are more basic than expected (see above), the ML complex is more basic, and the Gd[TrenHOPY] complex is less stable in acidic solution than the gadolinium TrenHOPO systems. The pH dependent speciation behavior for TrenHOPY with gadolinium is shown in Figure 4.

Potentiometric titrations of TrenHOPY with zinc and calcium ions were performed to provide a comparison to the high selectivity for Gd(III) afforded by the TrenHOPO system.[9] Solutions of a 1:1 ratio of metal ion to ligand were titrated over a pH range of 2.4−11. The titration curves are given in Figure 2, and the speciation diagrams for the calcium and zinc systems are presented in Figures 5 and 6. For both the Ca^{2+} and Zn^{2+} systems, low concentrations (∼0.25 mM L^{-1}) were required to avoid precipitation in the pH region of 5.5−6.5. The formation constants for calcium and zinc with TrenHOPY, along with those for Tren-Me-3,2-HOPO,[9] are summarized in Table 3. The formation constants with calcium are essentially the same for the two ligands. The formation constant for zinc with TrenHOPY is 1.7 log units less than that found for zinc with

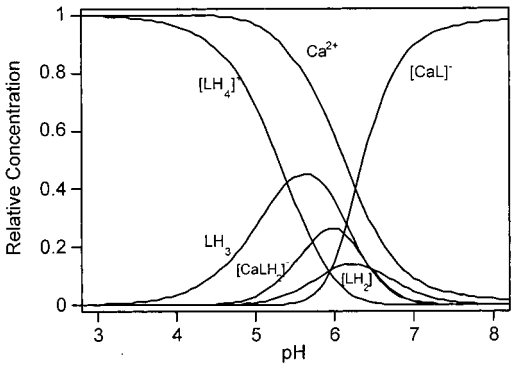

Figure 5. Species distribution of the Ca−TrenHOPY system, 1:1 Ca^{2+}: TrenHOPY, $[Ca^{2+}] = 0.25$ mM L^{-1}, $\mu = 0.1$ M (KCl), T = 25 °C.

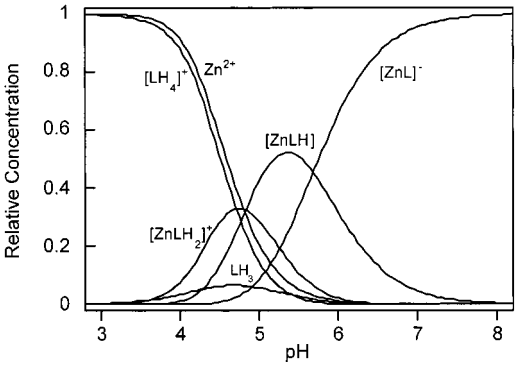

Figure 6. Species distribution of the Zn−TrenHOPY system, 1:1 Zn^{2+}: TrenHOPY, $[Zn^{2+}] = 0.25$ mM L^{-1}, $\mu = 0.1$ M (KCl), T = 25 °C.

Table 3. Formation Constants for Calcium and Zinc with TrenHOPY and Tren-Me-3,2-HOPO

species	MLH	TrenHOPY	Tren-Me-3,2-HOPO[a]
$[CaL]^-$	110	7.51(8)	7.6
[CaLH]	111	−	13.7
$[CaLH_2]^+$	112	19.67(7)	18.9
$[ZnL]^-$	110	11.41(4)	13.1
[ZnLH]	111	17.05(5)	18.08
$[ZnLH_2]^+$	112	21.85(1)	22.55

[a] Reference 9.

TrenHOPO. This indicates an improvement in selectivity for Gd(III) with the HOPY system compared to the HOPO system.

Preliminary examination indicates that the solubility of Gd-[TrenHOPY], the gadolinium complex of **8a**, is greater than 100 mM L^{-1}, which is a significant improvement in solubility compared to the TrenHOPO complexes (<10 mM L^{-1}solubility). The greater solubility of trivalent metal complexes and a ligand synthesis amenable to the preparation a variety of substituted HOPYs are key advantages of the HOPY systems compared to their HOPO analogues.

Relaxometric Properties. Water Proton Relaxation. The efficiency of a paramagnetic complex as a possible MRI contrast agent depends on its ability to catalyze the nuclear magnetic relaxation rate of solvent protons.[27−29] This property, which is measured in terms of relaxivity, r_{1p}, is defined as the enhancement of the water proton longitudinal relaxation rate induced by a 1 mM L^{-1} solution of the paramagnetic compound at a

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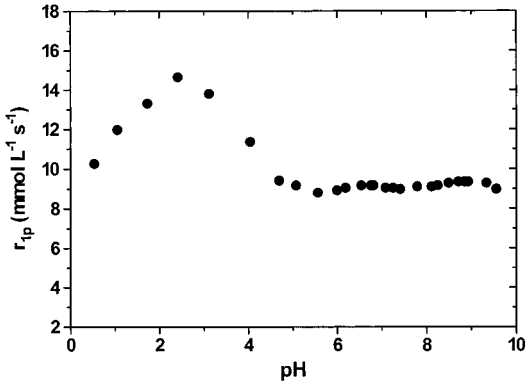

Figure 7. Plot of ^{1}H relaxivity (mM L^{-1} s^{-1}) at 20 MHz and 25 °C versus pH for Gd[TrenHOPY].

given temperature and magnetic field strength.[30] A complete discussion of the theory (with all the equations) can be found in several recent publications.[30−32]

Enhanced relaxivity can, in principle, be attained by slowing down the rotational mobility of the complex through covalent or noncovalent binding to macromolecular substrates.[30,33,34] This approach, of particular importance for the current research toward tissue targeted MRI agents, has so far been only partially successful because of the limiting factor of the slow water exchange rate (τ_M ca. 0.1−1 μs at 298 K) of the monoaquo, ennacoordinated Gd(III) complexes.[35,36] In such systems the coordinated water molecule exchanges with the bulk through a dissociative mechanism endowed with a large activation energy. A preliminary step toward optimized relaxivity is then the synthesis of Gd(III) complexes with low τ_M, whose optimal value can be set to about 30 ns.[9,30] The Gd(III) complexes of HOPO systems are very promising candidates since they possess high thermodynamic stability and high relaxivity due to the presence of two coordinated water molecules characterized by a fast rate of exchange.[9,31] The Gd[TrenHOPY] complex should maintain these favorable properties in addition to providing a higher solubility, an important requisite for modern applications of MRI techniques. The relaxivity of the complex measured at 20 MHz, 25 °C, and pH = 7.2 is 9.0 mM L^{-1} s^{-1}, a value about two times higher than that of the currently used contrast agents based on polyaminocarboxylate systems and very close to that of the Gd[Tren-Me-3,2-HOPO] system, which is of similar molecular weight. This increase in relaxivity is likely associated with the presence of two inner sphere water molecules and a higher molecular weight, which implies a longer value of the reorientational correlation time, τ_R. The relaxivity is constant in the pH range 6−10, increases below pH 5, reaches a maximum of 14.6 mM L^{-1} s^{-1} at pH 2.5, and then decreases at lower pH (Figure 7). By comparing the plot of r_{1p} versus pH with the thermodynamic speciation diagram in Figure 4, we may note a very close correlation. Above pH 5−6, only the unprotonated neutral species GdL exists in solution, which shows a pH independent behavior. Between pH 5 and 3, two concomitant protonation steps occur that probably involve the partial disruption of the chelate structure and the formation of an additional site for water coordination. Thus, the relaxivity peak near pH 3 results from the presence in solution of the species $[GdLH_2]^{2+}$ (ca. 85%) with $q = 3$ and a small amount of the free aquo ion (r_{1p} = 12.98 mM L^{-1} s^{-1}).

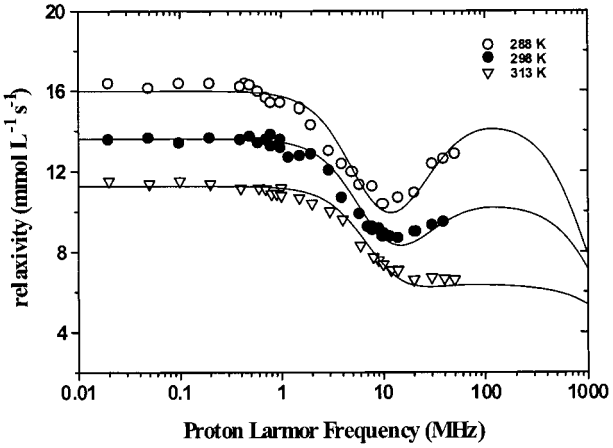

Figure 8. Nuclear magnetic relaxation dispersion (NMRD) profiles for Gd[TrenHOPY] at 15 (triangles), 25 (filled circles), and 39 °C (squares), at pH = 7.2.

The proton relaxivity has been also measured between 273 and 333 K in order to obtain a first qualitative evaluation of the water exchange rate. The relaxivity increases exponentially with decreasing temperature, unlike the case of the enneacoordinated polyaminocarboxylate Gd(III) complexes. This is a result of the lengthening of τ_R with decreasing temperature, which induces an increase of T_{1M}^H, the longitudinal relaxation time of the bound water protons, and thus of r_{1p}. This result also implies that, over the entire range investigated, Gd[TrenHOPY] is in the fast exchange regime ($\tau_M \ll T_{1M}^H$) and that the water exchange lifetime is significantly lower than 100 ns (at 298 K).

NMRD Profiles. The water proton relaxivities as a function of the magnetic field strength and at 278, 298, and 312 K are shown in Figure 8. The experimental data were fitted to the equations of both inner and outer sphere relaxivities. These two contributions are determined by several dynamic and structural parameters (Δ^2, τ_V, τ_M, τ_R, q, r, a, and D) and a (chemically) reasonable choice needs to be made for some of them. The parameters a and D were fixed to typical values for small Gd(III) chelates, and $q = 2$ was assumed. There is a significant covariance between a and τ_R, but it must be noted that τ_R changes only ±4% by varying a of ±0.2 Å. The profiles do not show any dependence on the water exchange lifetime τ_M (whose value cannot be assessed and was fixed to 10 ns). The shape of the profiles shows a dispersion between 3 and 6 MHz and a hump between 100 and 200 MHz, indicative of a relatively long τ_R and a fast electronic relaxation, as compared to the profiles of commercial polyaminocarboxylate Gd(III) complexes. The best fit parameters are reported in Table 4 and confirm the above qualitative analysis. The reorientational correlation time is about 50% higher than that for the DOTA and DTPA Gd(III) complexes,[34] whereas the mean square of the zero field splitting energy (Δ^2) value of Gd[Tren-Me$_2$-5,4-HOPY] (1.1×10^{20} s^{-2}) is much higher than for $[Gd(DOTA)]^-$ (1.6×10^{19} s^{-2}) and slightly higher than for linear complexes, indicating a significantly less symmetric instantaneous symmetry

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Table 4. Best Fitting Parameters Determined by Analysis[a] of NMRD Profiles for Gd[Tren-Me$_2$-5,4-Hopy]$^-$

parameter	15 °C	25 °C	39 °C
Δ^2 ($s^{-2} \times 10^{20}$)	1.0 ± 0.4	1.1 ± 0.2	1.1 ± 0.2
τ_V (ps)	13 ± 2	12 ± 1	11 ± 1
τ_R (ps)	166 ± 4	114 ± 2	68 ± 2
D ($cm^2\,s^{-1} \times 10^{-5}$)[b]	1.65	2.24	3.15

[a] In each case, $q = 2$, $r = 3.0$ Å, and a, the distance of the closest approach of diffusing water, was set at 3.8 Å. [b] Values fixed in the best fitting procedure.

of the complex.[34] As a consequence, in the high field region the overall correlation time τ_C has a contribution from the electronic relaxation time which increases with increasing magnetic field strength and originates the broad relaxivity peak in the profiles.[29]

The favorable relaxation properties of Gd[Tren-Me$_2$-5,4-HOPY] in aqueous solution might not be observed in a physiological medium due to the possible displacement of one or both of the coordinated water molecules by endogenous anions, such as carbonate, lactate, malonate, citrate, etc. This phenomenon has been observed in the case of Gd(III) complexes with $q = 2$, such as GdDO3A[37,38] and DOTA triamide derivatives.[39] Recently, the displacement of inner sphere water molecules by coordinating groups on the surface of human serum albumin has been reported for two Gd(III) complexes with DO3A derivatives[40] To examine this possibility, we titrated a 1 mM L^{-1} solution of Gd[Tren-Me$_2$-5,4-HOPY] with acetate, lactate, and malonate, at 20 MHz, 298 K and pH = 7.2. We did not observe any change in the solvent relaxation rate, even in the presence of a 100-fold excess of the anions, to indicate the absence of binding interactions. Tight coordination of the ligand donor groups probably results in a high steric constraint at the water binding sites which hinders accessibility by anions. This result is of particular importance because it suggests that the complex may maintain its high relaxivity in vivo. Further proof of the absence of ternary complex formation with anionic substrates was gained by recording the NMRD profile in blood serum (Figure 9). The relaxivity, at 298 K, is significantly higher (ca. 35% at 20 MHz) than in pure water over the entire magnetic field range. The different microviscosity of the two media (which affects the value of τ_R) cannot cause the difference since much lower effects are found for both $[Gd(DTPA)]^{2-}$ and $[Gd(DOTA)]^-$. Rather, this suggests a certain degree of interaction with the plasma proteins. Indeed, a titration of the complex (0.1 mM L^{-1}, 20 MHz, 298 K) with human serum albumin at pH = 6.8 indicates the presence of a weak interaction (K_A ca. 100 M^{-1}) that accounts for the observed relaxivity enhancement.

Variable Temperature ^{17}O NMR. The NMRD profiles have been found to be in the fast exchange regime, a condition that precludes the assessment of the water exchange rate from the analysis of the magnetic field dependence of the proton relaxivities. The value of τ_M, a crucial parameter for the evaluation of the efficency of a contrast agent, can be independently obtained by a variable temperature, proton decoupled ^{17}O NMR measurement of the water nuclear transverse relaxation rate (R_2) using a well-established procedure (Figure 10)[32,36] The R_2 values are dominated by the scalar relaxation mechanism, which depends on k_{ex} and its temperature dependence (ΔH_M), the electronic relaxation rate and its temperature dependence (Δ^2, τ_V, ΔH_V), and the hyperfine coupling constant A/h. In the analysis, we used a standard value of -3.8×10^6 rad s^{-1} for the hyperfine coupling constant and the values obtained by the analysis of the NMRD profiles for electron relaxation (Δ^2 and τ_V). From fitting the data to the Swift−Connick equations, we obtained for the parameters the values reported in Table 5. The high value of k_{ex} calculated ($4.9 \times 10^8 \pm 1$ s^{-1} at 298 K) indicates a very small free energy difference between the 8- (ground) and 9-coordinated states and confirms the presence of a high steric constraint at the water binding sites, as suggested by the absence of ternary complexes with small anionic substrates. Analogous steric effects on the water exchange rate were observed in the case of the 9-coordinated monoaquo complexes $[Gd(EGTA)]^-$.[41]

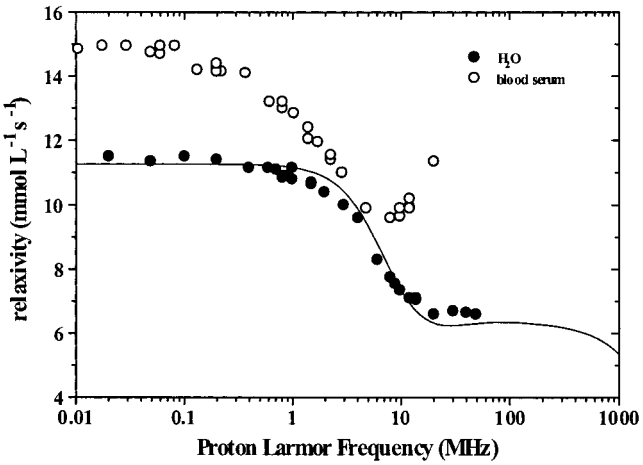

Figure 9. Nuclear magnetic relaxation dispersion (NMRD) profiles for Gd[TrenHOPY] in water (open circles) and in blood serum (filled circles) at 39 °C and pH = 7.2.

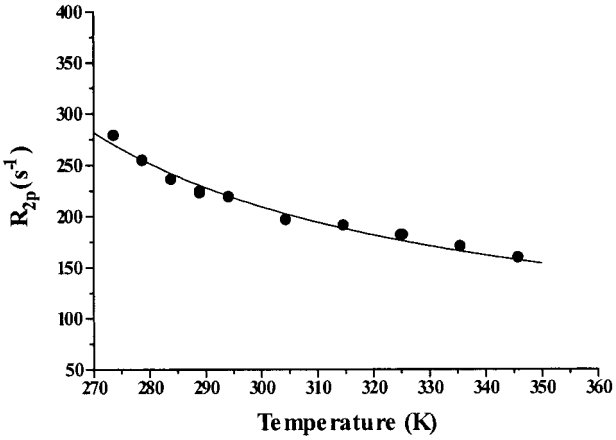

Figure 10. Temperature dependence of the paramagnetic contribution (R_{2p}) to the transverse ^{17}O water relaxation rate of a 38 mM L^{-1} solution of Gd[TrenHOPY] (9.4 T, pH = 7.2).

Table 5. ^{17}O NMR Best Fitting Parameters for Gd[Tren-Me$_2$-5,4-Hopy]$^-$

k_{ex}^{298} ($\times 10^8$ s^{-1})	ΔH_M (kJ/mol)	τ_v^{298} (ps)	ΔH_V (kJ/mol)	Δ^2 ($s^{-2} \times 10^{20}$)[a]
4.9 ± 0.2	1.0 ± 0.1	12 ± 1	1.8 ± 0.1	1.1

[a] Fixed in the fitting (from NMRD analysis, Table 4).

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The high thermodynamic stability, the excellent water solubility, the presence of two inner sphere water molecules which are not displaced by coordinating anions, and the rapid water exchange rate are important characteristics that make Gd[Tren-Me_2-5,4-HOPY] a promising candidate for the preparation of macromolecular MRI contrast agents with enhanced relaxivity.

Conclusions

The 6-substituted-5,4-hydroxypyrimidinones are a new class of heterocyclic bidentate ligands that show promise as lanthanide chelating agents. The gadolinium(III) complexes offer greater water solubility than the corresponding HOPO analogues, although form slightly less stable complexes with more acidic, harder metal ions. The relaxivity of Gd[TrenHOPY] is significantly higher than that of the commercially available MRI contrast agents; this is consequence of the presence of two water molecules in the inner coordination sphere of the metal ion and a slower tumbling rate of the complex in solution. Unlike the commercial contrast agents, the coordinated water molecules are in fast exchange with the bulk ($k_{ex} = 4.9 \times 10^8 \pm 1\ s^{-1}$ at 298 K), an essential feature for attaining high relaxivity through a linkage to macromolecular substrates. Significantly, the relaxivity in blood serum is higher than in pure water, which indicates: i) a certain degree of binding to plasma proteins and ii) the absence of displacement of the bound water molecules by endogenous coordinating anions. All these favorable relaxation properties and the high water solubility make the neutral Gd[TrenHOPY] complex a promising candidate for the development of more effective contrast agents for the new and emerging applications of MRI.[42−44]

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Supporting Information Available: Crystallographic details of the structural determination of NMe_2-2,3-Me_2-HOPY in CIF format are included. This material is available free of charge via the Internet at http://pubs.acs.org.

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