Synthesis of a Ligand Based upon a New Entry into the 3-Hydroxy-*N*-alkyl-2(1*H*)-pyridinone Ring System and Thermodynamic Evaluation of Its Gadolinium Complex

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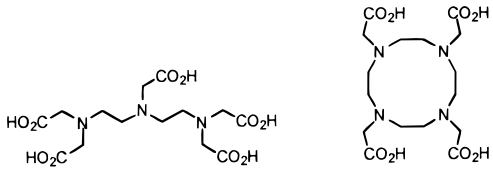
The synthesis of a new, more water soluble derivative of TREN-Me-3,2-HOPO {tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]amine} is presented. The synthesis starts with the condensation reaction of (*N*-methoxyethylamino)acetonitrile hydrochloride and oxalyl chloride to give 3,5-dichloro-*N*-(methoxyethyl)-2(1*H*)-pyrazinone. The 3-position is readily substituted with a benzyloxy group, and the pyrazinone is converted to ethyl 3-(benzyloxy)-*N*-(methoxyethyl)-2(1*H*)-pyridinone-4-carboxylate by a Diels−Alder cycloaddition with ethyl propiolate. Basic deprotection of the ester followed by activation, coupling to tren, and acidic deprotection of the benzyl groups gives the ligand TREN-MOE-3,2-HOPO {tris[(3-hydroxy-1-(methoxyethyl)-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]amine}. The gadolinium complex of TREN-MOE-3,2-HOPO was prepared by metathesis, starting from gadolinium chloride. The solubility of the new metal complex is significantly enhanced. The four protonation constants (determined by potentiometry) for TREN-MOE-3,2-HOPO ($\log K_{a1} = 8.08$, $\log K_{a2} = 6.85$, $\log K_{a3} = 5.81$, $\log K_{a4} = 4.98$) are virtually identical to those reported for the parent ligand. The stability constants for the gadolinium complex of TREN-MOE-3,2-HOPO determined by potentiometry ($\log \beta_{110} = 19.69(2)$, $\log \beta_{111} = 22.80(2)$) and by spectrophotometry ($\log \beta_{110} = 19.80(1)$, $\log \beta_{111} = 22.88(1)$, $\log \beta_{112} = 25.88(1)$) differ slightly from those for the parent ligand; this follows from a change in the complexation model in which a new diprotonated species, $[Gd(TREN\text{-}MOE\text{-}3,2\text{-}HOPO)(H)_2]^{2+}$, was included. The presence of this extra species was demonstrated by factor analysis, comparison of spectral data, and nonlinear least-squares refinement. Significant formation of this species is observed between pH 3 and pH 1.5.

Introduction

Magnetic resonance imaging (MRI) is a noninvasive procedure which does not use ionizing radiation yet permits high-quality images of internal organs and tissues to be obtained.[1,2] Signals can be enhanced through the use of paramagnetic contrast agents which speed the longitudinal (R_1) and transverse (R_2) relaxation rates (and thereby the signal) of tissue-derived water protons. The increase in signal augments the high-resolution images obtained by traditional MRI methods.[3] Two examples of ligands (DTPA and DOTA) whose Gd(III) complexes are currently used as commercial contrast agents are shown in Chart 1. Gadolinium(III) is used because it has the highest isotropic magnetic moment ($S = {}^7/_2$) of any element. Typically octadentate poly(amino carboxylate) ligands are used, thereby providing a high thermodynamic stability with Gd(III) while permitting an open coordination site to be retained for water. The thermodynamic stability of Gd(III) with these ligands, as measured by their pGd value ($pGd = -\log [Gd]_{free}$ at pH 7.4, $[Gd]_{total} = 1 \times 10^{-6}$ M, and $[ligand]_{total} = 1 \times 10^{-5}$ M), is quite high at ∼19 for both ligands.[4−11]

Chart 1[a]



[a] The Gd(III) complexes of these ligands are used commercially as contrast agents for MRI. The thermodynamic stability of these complexes, as measured by their pGd values, are 19.1 (DTPA)[4,11] and 18.9 (DOTA).[5−10]

The complex Gd(TREN-Me-3,2-HOPO) {TREN-Me-3,2-HOPO = tris[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyridine-4-carboxamido)ethyl]amine}was earlier reported as a new type of MRI contrast agent.[12] The ligand is unique in that it only possesses six coordinating atoms with two coordination

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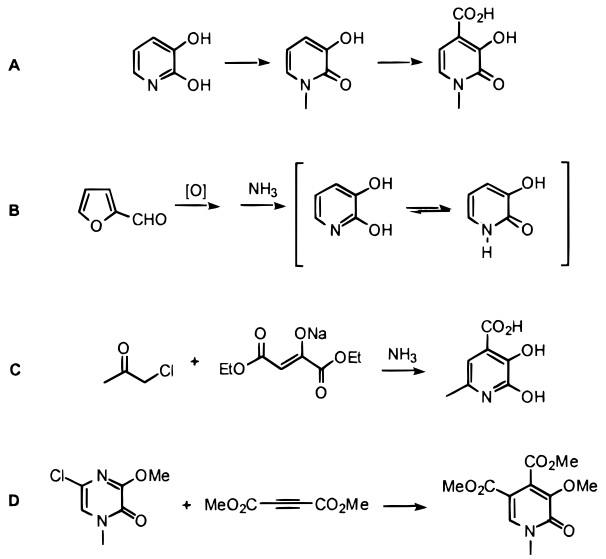
Scheme 1. Four Plausible Routes into the 3-Hydroxy-1-methyl-2(1*H*)-pyridinone-4-carboxylate Ring System[a]



[a] Method A was used in the original synthesis of TREN-Me-3,2-HOPO. Methods B and C have not yet been applied successfully. A modification of method D was used to prepare TREN-MOE-3,2-HOPO.

sites occupied by water. This enhances the relaxivity (a measure of signal intensity) 2.5-fold over other contrast agents, since water exchange can occur via an associative mechanism that is fast due to the relatively low energy difference between 8- and 9-coordinate gadolinium.[13] The increase in relaxivity due to the lower coordination number of the ligand does not come at the cost of thermodynamic stability. In fact, Gd(TREN-Me-3,2-HOPO) has a pGd value of 20.3, higher by approximately 1 log unit than that observed for either DTPA or DOTA.

A series of derivatives of TREN-Me-3,2-HOPO have been prepared to address the relatively low solubility of the ligand and its complexes in water. The first efforts were toward functionalization of the TREN cap and resulted in the discovery of new synthetic methodology for homochiral trisubstituted TREN derivatives.[14,15] The gadolinium complex of the best ligand has a solubility of approximately 5−10 mM, making it between 1 and 2 orders of magnitude more soluble in water than the parent TREN-Me-3,2-HOPO complex. This is not quite sufficient for diagnostic applications, which led to the search for even more water-soluble 3,2-HOPO derivatives. The synthesis of new 3,2-HOPO derivatives must not involve a strong change in the electronic structure of the ligand subunit, which would dramatically affect the stability of the resulting complex. Therefore, the preparation of *N*-alkyl-substituted 3,2-HOPO derivatives was undertaken in the current work.

There are only four convenient routes into the 3-hydroxy-1-methyl-2(1*H*)-pyridinone-4-carboxylate ring system, Scheme 1. Method A, used to prepare TREN-Me-3,2-HOPO, starts by *N*-alkylating 2,3-dihydroxypyridine with methyl iodide, followed by carbonylation under high pressure.[12] Modification of this route to prepare other *N*-alkyl derivatives has not been straightforward; the carbonylation reaction cannot be carried out successfully with *N*-substitution other than methyl or ethyl. Method B involves ring expansion of furfural by oxidative cleavage, followed by ring closure with an amine.[16] However, this route suffers from the same carbonylation problems as the first when higher amines are used. Method C is the condensation reaction of chloroacetone, sodium diethyloxalacetate, and ammonia.[17] It may be possible to use this route to prepare other derivatives of 3,2-HOPO; investigations of this pathway are currently underway. Finally, there is the Diels−Alder addition followed by retro Diels−Alder elimination of substituted 5-chloro-3-alkoxy-*N*-alkyl-2(1*H*)-pyrazinones developed by Hoornaert and co-workers, method D.[18] There is a high degree of flexibility in the synthesis of the pyrazinone precursor, allowing for substitution of the *N*-alkyl group. Following the work of collaborating researchers,[19] we sought to explore this chemistry further to develop a synthesis of more water-soluble *N*-alkyl-3,2-HOPO ligands. This paper describes the results of our initial exploitation of this synthetic method.[20]

Results and Discussion

Synthesis. The general procedure for the synthesis of *N*-alkyl-3,2-HOPO derivatives is outlined in Scheme 2. Condensation of glycolonitrile[21] with *N*-methoxyethylamine gives (*N*-methoxyethylamino)acetonitrile,[22] which is converted to its hydrochloride salt with anhydrous hydrogen chloride. The hydrochloride condenses with excess oxalyl chloride in chlorobenzene at room temperature over several days to give 3,5-dichloro-*N*-(methoxyethyl)-2(1*H*)-pyrazinone (**1**) in 40−50% yields as a white to pale yellow solid.[23,24] Substitution of the chlorine in the 3-position is carried out with sodium benzyloxide (generated in situ from benzyl alcohol and sodium hydride) in THF; compound **2** is isolated in 70% yield as a beige solid.

The Diels−Alder reaction of complexes related to **2** with symmetrical acetylenes has been reported.[18] However, to prepare the desired ligand, unsymmetrical acetylenes had to be used, resulting in the formation of geometric isomers. Treating **2** with an excess of neat ethyl propiolate at 140 °C resulted in clean conversion to a 3.5:1 ratio of **3a** and **3b**, the 4- and 5-substituted pyridinones, respectively. This ratio is approximately the same as was originally observed in the reaction of ethyl propiolate with *N*-methyl-5-chloro-3-methoxy-2(1*H*)-pyrazinone,[25] suggesting that the methoxyethyl substituent has a limited electronic effect on the pyrazinone ring system in **2**. The ^{1}H NMR spectrum of the mixture shows that the major isomer exhibits a coupling between the two aromatic protons of 7 Hz, suggesting

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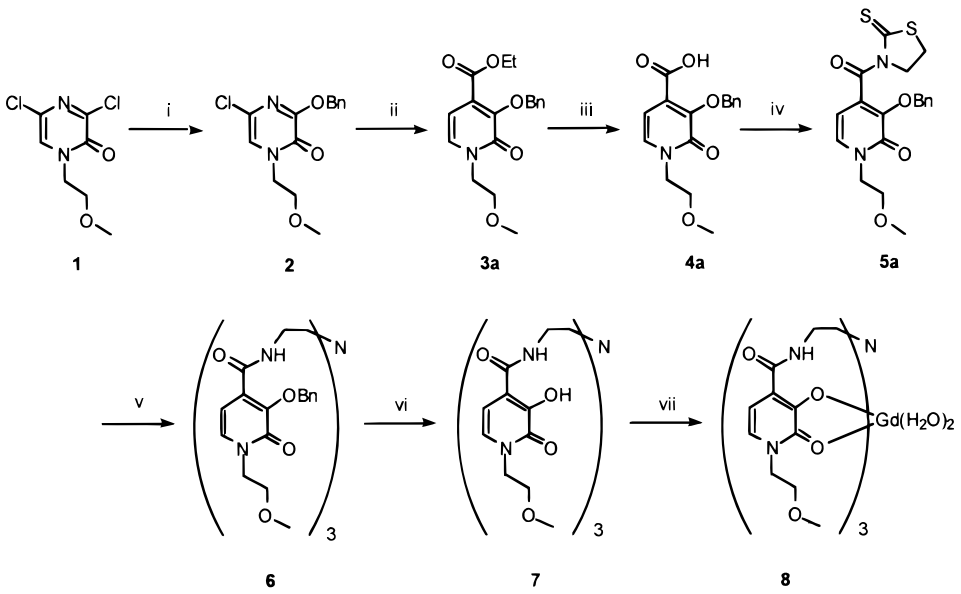
Scheme 2[a]



[a] Reagents and conditions: (i) NaOBn, THF, 70%; (ii) ethyl propiolate, 140 °C, 3.5:1 **3a**:**3b**; (iii) KOH/H_2O/THF, 7.5:1 **4a**:**4b**; (iv) 2-mercaptothiazoline, DCC, DMAP, CH_2Cl_2, 81% **5a**; 4% **5b**; (v) tren, CH_2Cl_2, 93%; (vi) HOAc, concentrated HCl, 87%; (vii) $GdCl_3$, 15% MeOH/THF, 93%.

an ortho relationship. The two aromatic protons in the minor isomer do not show a resolved coupling constant. It has not been possible to separate **3a** and **3b** by chromatography, but saponification of the mixture with dilute aqueous base in THF gives the free acid as a mixture of 4- and 5-isomers (**4a** and **4b**, respectively) in a 7.5:1 ratio. An increase in the isomer ratio to favor the desired compound suggests that the saponification reaction does not proceed at the same rate for both **3a** and **3b** and that it may be possible to chemically separate unreacted **3b** from the product **4a** by using a deficiency of base. Recent results support this hypothesis in a related system.[26] Compound **4a** also exhibits a proton NMR coupling of 7 Hz between the two aromatic protons, indicating an ortho relationship.

The mixture of acids **4a** and **4b** is activated by standard procedures to form the thiaza compound.[12] It is possible to separate the 5-isomer by selective crystallization, leaving the desired activated acid **5a** in 81% yield as a thick yellow oil. The proton NMR spectrum of **5a** shows a coupling of 7 Hz between the two aromatic hydrogens at 7.17 and 7.04 ppm. The 5-isomer (**5b**) is contaminated with the urea byproduct, DCU, but recrystallization from ethyl acetate or methylene chloride/ether gives the compound in pure form as a yellow powder (4%). The proton NMR spectrum of **5b** shows a coupling of 2.5 Hz between the two aromatic hydrogens at 7.78 and 7.00 ppm. This small coupling constant is consistent with a meta relationship between the two protons.

Coupling of thiaza-activated **5a** with tris(2-aminoethyl)amine (tren) is straightforward if a deficiency of tren (0.25 equiv) is used. The benzyl-protected compound **6** is isolated in 93% yield as a pale yellow oil after chromatography. Incomplete conversion to **6** is observed when a stoichiometric amount of tren (0.33 equiv) is used. Compound **6** has not been isolated in analytically pure form, but NMR spectroscopy and high-resolution mass spectral data support the proposed formulation. Benzyl deprotection is carried out by dissolving **6** in a 1:1 mixture of acetic acid and concentrated hydrochloric acid and stirring for 3 days. The free ligand **7** (TREN-MOE-3,2-HOPO) is isolated as a white solid in 87% yield. Metalation of **7** with gadolinium chloride provides the Gd(III) complex **8**. The improved solubility of this metal complex is exhibited during its synthesis; the complex does not precipitate from a methanol solution if that is used for its preparation, as was the case for Gd(TREN-Me-3,2-HOPO). Instead, the mixed-solvent system of 15% methanol/THF is used to prepare the complex. Complex **8** precipitates from this solvent system and can be isolated by centrifugation in about 95% yield. The solubility of the gadolinium complex in water is on the order of 1 mM (at pH 7, in 0.1 M KCl, and at 25 °C), an increase over the parent TREN-Me-3,2-HOPO system of approximately 10-fold.

Gd^{3+} Coordination Chemistry. Protonation and Gd(III) formation constants for the ligand TREN-MOE-3,2-HOPO were determined. To completely remove the metal from the ligand, it was necessary to titrate the system down to a pH of approximately 1.5. Thus, when necessary, the glass-bulb pH electrode was calibrated using a method which incorporates a correction for variation in the junction potential at the liquid−liquid interface between the test solution and the electrode reference cell compartment.

Calibration of the Glass-Bulb Electrode. Two distinct techniques were used. The "standard technique" was used for measurements over the pH range 2.5−11.5, and a Nernstian relationship, eq 1, was assumed for the electrode response. This

Nernstian response

$$E = E^\circ + s \log [H^+] \quad (1)$$

Nernstian response plus junction potential

$$E = E^\circ + s \log [H^+] + j_p[H^+] \quad (2)$$

gave the parameters E°, the cell constant, and s, the electrode slope. To collect measurements in strongly acidic media (below pH 2.5) the j_p coefficient was also determined as in eq 2. In both cases, the electrode response was calibrated in terms of hydrogen ion *concentration*, $[H^+]$, as opposed to activity, $\{H^+\}$. The two techniques produced values for E° and s that were in agreement within experimental error.

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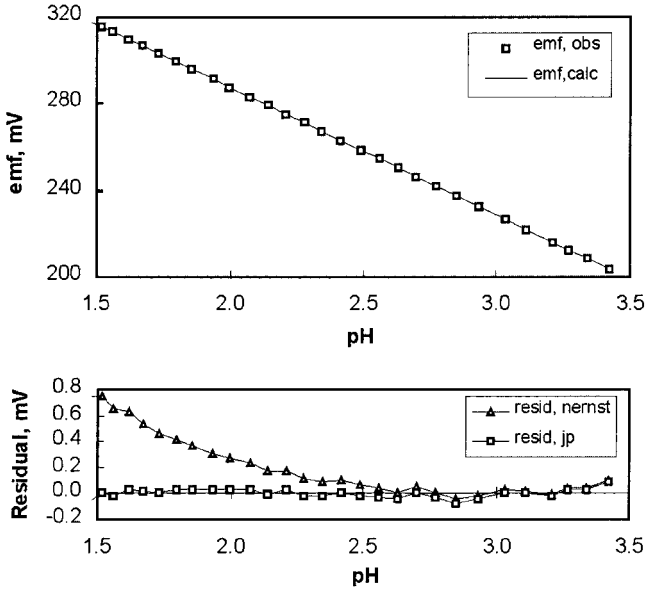

Figure 1. Typical calibration of the glass-bulb electrode to low pH showing the benefit of applying a "junction potential" correction (square symbols). Triangular symbols indicate the residuals when only a Nernstian electrode response is assumed.

The generation of an electrochemical potential at the junction between solutions of differing compositions is a well-known phenomenon.[27,28] Indeed, these potentials are universally present in all potentiometric experiments which rely on an ionic bridge between solutions of differing electrolytic compositions. Normally, the junction potential remains constant, but a correction must be applied for its variation when there is a significant variation in the composition of the test solution's electrolyte. In the present case, there is substantial replacement of potassium ions, K^+, by hydronium ions, H^+. The effect of such changes has been the subject of a relatively recent theoretical treatment.[29] This review also gives a compilation of reported values for the correction term (j_p). Typical values reported when H^+ is substituted for any of the common alkali metal cations (Li^+, Na^+, or K^+) vary between 20 and 60 mV L mol^{-1}. In the present work, values of the j_p coefficient were obtained between 20 and 30 mV L mol^{-1} which are consistent with the literature values.

A typical calibration to low pH is illustrated in Figure 1. Note that when the correction for the junction potential is included, the residuals fall to $\pm$0.1 mV. When allowance is not made for variation in the junction potential (the residuals obtained using only a Nernstian model (eq 1)), the error becomes significant below pH 2.5. The effect of the variation in junction potential is relatively minor; even at the most extreme point in the experiment, pH 1.5, the junction potential correction is less than 1 mV, which corresponds to a correction of 0.017 pH unit.

Spectrophotometry. The low-pH calibration method is crucial to the successful characterization of the coordination chemistry since it allows the smooth transition of data collection into the region where junction potentials become significant. At such low pH, the concentration of free protons far exceeds the concentrations of both the metal and the ligand; hence, the protons liberated by the coordination reaction make an insignificant contribution to the total acid concentration. For this reason, it is necessary to combine the potentiometric measurements with spectrophotometric data since the complexation reaction cannot be accurately measured by potentiometry alone.

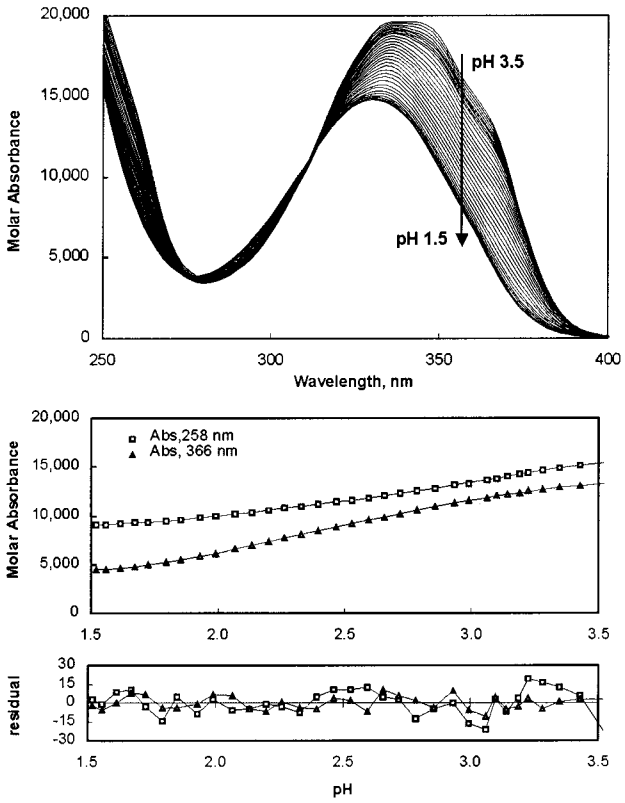

Figure 2. Spectral titration data set for the system Gd^{3+}/TREN-MOE-3,2-HOPO, collected over the pH range 3.5−1.5. In all plots, the raw spectral data have been corrected for dilution by conversion to molar absorbance (units M^{-1} cm^{-1}). The two lower diagrams illustrate the nonlinear least-squares fits to the data at two wavelengths. Data at all 70 wavelengths between 250 and 390 nm were included in the refinements. The symbols in the bottom residual bar correspond to those in the middle plot.

A spectral data set from one of the titrations is shown in Figure 2. The scans have been corrected for dilution effects by conversion to molar absorbance (M^{-1} cm^{-1}). Also illustrated in this figure are the results of least-squares fitting of the data. The random trend and low absolute values in the residual bar indicate the high quality of fit. This figure illustrates the results at just two wavelengths (258 and 366 nm); similarly good results were obtained at all 70 wavelengths (250−390 nm at 2 nm intervals) which were incorporated into the refinements. The resulting formation constants determined for this system are summarized in Table 1 along with a comparison with those published for the Gd^{3+}/TREN-Me-3,2-HOPO system.[12]

Potentiometry. In addition to the low-pH spectrophotometric experiments, a set of conventional potentiometric experiments were performed. The latter experiments were limited to pH values greater than or equal to 2.5 for the reasons elaborated above; i.e., the relatively low metal and ligand concentrations compared to the high concentration of free protons below pH 2.5 precludes the study of complexation by potentiometric measurements alone, even when a correction for variation in junction potentials is used. The initial pH of 2.5 means that the β_{112} formation constant could not be determined in these experiments since the corresponding species is fully formed at this point. This formation constant was held as a fixed parameter in the refinements using the value obtained in the spectrophotometric experiments. Inclusion of this species was necessary

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Table 1. Determined Formation Constants and Calculated pM Values for the Gd/TREN-MOE-3,2-HOPO System[a]

		log β_{MLH} (std dev)		
		TREN-MOE-3,2-HOPO		TREN-Me-3,2-HOPO[b]
species	MLH	spectrophotometric	potentiometric	
$[LH]^{2-}$	011		8.08 (1)	8.20 (1)
$[LH_2]^{-}$	012		14.93 (1)	15.15 (3)
$[LH_3]$	013		20.74 (1)	20.95 (3)
$[LH_4]^{+}$	014		25.69 (2)	25.91 (5)
[GdL]	110	19.84 (1)	19.69 (2)	20.3 (2)
$[GdLH]^{+}$	111	22.93 (1)	22.80 (2)	23.8 (1)
$[GdLH_2]^{2+}$	112	25.88 (1)	25.88 (fixed)	
	pM	19.8		20.3

[a] Figures in parentheses give the standard deviations in the least significant figures as determined by the least-squares refinements. pM values give the free metal concentration calculated using the formation constants in the table and the conditions of pH 7.4, [M] = 1×10^{-6} M, and [L] = 1×10^{-5} M. [b] From reference 12.

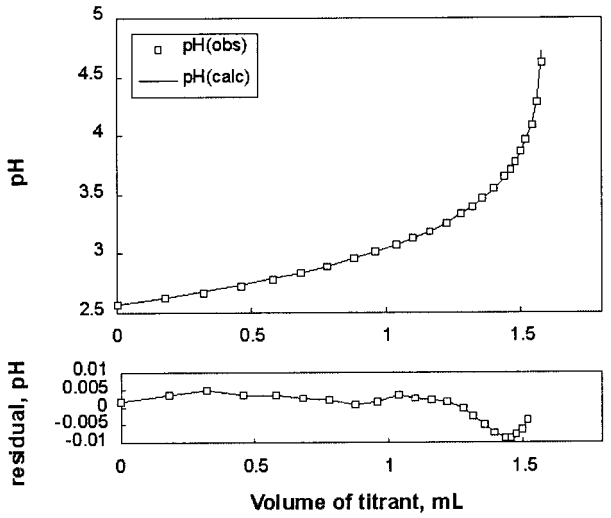

Figure 3. Potentiometric titration of the Gd^{3+}/TREN-MOE-3,2-HOPO system. The lower bar indicates the residuals in pH.

to obtain a good fit to the data. An illustration of one of these experiments is given in Figure 3. The refined values for the constants β_{110} and β_{111} (Table 1) show good agreement with the values determined by spectrophotometry.

The benefit of performing this additional set of experiments is that the total data set is extended to cover a wider range of experimental conditions, including higher concentrations of metal and ligand, as well as a slightly higher pH range (2.5−4). The combined data set of spectrophotometric and potentiometric results covers ligand concentrations over the range 3.1 $\times 10^{-5}$ to 5.3 $\times 10^{-4}$ M and ligand-to-metal ratios ranging from 1.1:1 to 5.3:1. The finding of equal values for the formation constants over this range of concentrations increases confidence in the model and reduces the possibility that species of different stoichiometries (e.g., M_2L_2 type complexes) have been overlooked.

A species distribution diagram is provided for the Gd^{3+}/TREN-MOE-3,2-HOPO system in Figure 4.

Choice of Complexation Model. The Gd^{3+}/TREN-MOE-3,2-HOPO data were fit with a complexation model slightly different from that used in the previously published study of Gd^{3+}/TREN-Me-3,2-HOPO coordination. In particular, a new species was added; the doubly protonated complex $[GdLH_2]^{2+}$ (the ligand is represented as "L"). The presence of this extra species was established by factor analysis of the spectral data. Also, the calculated spectra for each of the complexes were not

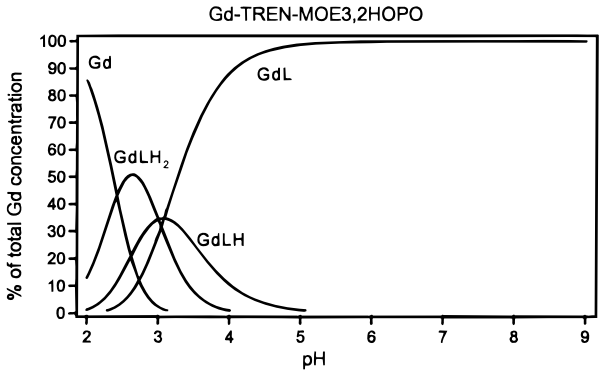

Figure 4. Species distribution diagram for the Gd^{3+}/TREN-MOE-3,2-HOPO system, calculated for 1×10^{-6} M metal and 1×10^{-5} M ligand. The ligand is represented as "L" in the diagram.

correct and the quality of fit in both the spectrophotometric and potentiometric data sets was poor unless this species was included in the model.

Factor analysis of the spectral data was achieved using the program HYPERQUAD. The numerical basis of this analysis is explained in the documentation/help files of this program and has been reviewed in the literature.[30,31] Briefly, it is important to note that this analysis treats the data with a singular-value decomposition, allowing the number of colored species present during the titration to be established. For the Gd^{3+}/TREN-MOE-3,2-HOPO system, four different species were found to contribute to the observed spectral changes between pH 3.5 and 1.5. In the course of the nonlinear least-squares refinement, it was established that these four colored components were the species $[LH_4]^{+}$ (β_{014}), $[GdL]^{0}$ (β_{110}), $[GdLH]^{+}$ (β_{111}), and $[GdLH_2]^{2+}$ (β_{112}). Inclusion of these four species in the complexation model gave the lowest global σ values and the lowest standard deviations in the formation constants (note that the factor analysis was performed on titrations in which the triprotonated ligand species, $[LH_3]^{0}$, never formed more than 0.4% of the total distribution of ligand species throughout the experiments).

The speciation in the Gd^{3+}/TREN-MOE-3,2-HOPO system is completely static over the pH range 5.5−10 (see Figure 4). This is confirmed by the observation that there is no detectable change in the absorbance of the complex over this range. Thus the molar absorbance of the [GdL] species (corresponding to β_{110}) may be measured with confidence. The calculated spectrum for this species, resulting from the nonlinear least-squares refinements, did not match the measured spectrum unless the $[GdLH_2]^{2+}$ species (corresponding to β_{112}) was included in the complexation model.

A duplicate set of experiments were performed with the TREN-Me-3,2-HOPO ligand.[32] These experiments showed spectral changes and factor analysis results virtually identical to those observed with the ligand TREN-MOE-3,2-HOPO. A full nonlinear least-squares analysis of this second data set indicated that, in fact, an equivalent $[GdLH_2]^{2+}$ species is also formed.

Physical Interpretation. The formation constants β_{110}, β_{111}, and β_{112} define two protonation steps for the Gd(III)−ligand chelate. The differences between the log values of these constants give log K_a values for these steps as 3.09 (GdL^{0} + H^{+} ↔ $GdLH^{+}$) and 2.95 ($GdLH^{+}$ + H^{+} ↔ $GdLH_2^{2+}$)

(30) Tauler, R.; Smilde, A.; Kowalski, B. *J. Chemom.* **1995**, *9*, 31−58.
(31) Geladi, P.; Smilde, A. *J. Chemom.* **1995**, *9*, 1−2.
(32) O'Sullivan, B.; Raymond, K. N. Unpublished results, 1999.

(calculated using the spectrophotometrically determined constants). These two values are almost coincident and suggest that the protonation steps are not independent and occur in a concerted manner.

The crystallographic structure for the parent complex, Gd(III)−TREN-Me-3,2-HOPO, has been reported[12] and shows that in the neutral complex, $[GdL]^0$, the capping nitrogen of the ligand scaffold points into the central cavity of the chelate, its lone pair associated with the three amide protons by hydrogen bonding. To accept a proton from the solvent medium, this nitrogen must switch to an "out" conformation, a transition which is apparently prevented by the rigidity of the Gd(III) chelate. Hence there is the concerted 2-fold process with concurrent protonation of the capping amine and one of the chelating arms of the ligand.

Conclusions

The discovery that Gd(TREN-Me-3,2-HOPO) has excellent water relaxation properties has prompted a synthetic effort toward functionalization of the parent complex to improve its aqueous solubility and to understand its properties. A new procedure, incorporating a Diels−Alder cyclization reaction, has been used to prepare functionalized derivatives of the 3,2-HOPO ring system. The synthetic procedure is relatively short and high yielding and allows for substitution of the *N*-methyl group by a short-chain alkyl group.

The formation of geometric isomers during the reaction is not problematic, as the desired isomer forms in a greater than 3:1 ratio. Separation of the two compounds can be effected by selective crystallization of the minor isomer. Coupling of the *N*-(methoxyethyl)-3,2-HOPO subunit to tren yields the new ligand TREN-MOE-3,2-HOPO, whose gadolinium complex is about 10 times more water soluble than the parent *N*-methyl compound, with approximately millimolar solubility at pH 7.4. Clinical applications typically require 0.5 M solubility or greater.

A new model for the thermodynamic stability constants of the Gd/TREN-Me-3,2-HOPO system is presented. This model requires the presence of a diprotonated species, written as $[GdLH_2]^{2+}$. With this modification, the affinity of the TREN-MOE-3,2-HOPO ligand for Gd(III) at physiological pH is calculated to be slightly lower than that previously reported for the TREN-Me-3,2-HOPO ligand (i.e., pGd = 19.8 compared with 20.3 for the former ligand). Nonetheless, it is clear that tripodal, 3,2-HOPO-based ligands have a high affinity for Gd(III), which equals or betters that of current commercially available contrast agents. The protonation constants of TREN-MOE-3,2-HOPO are essentially unchanged from those of the parent system. Accurate examination of the solution chemistry of this system required the acquisition of pH measurements below pH 2.5. For these measurements, a standard glass-bulb electrode was used with a calibration method which incorporated a correction for variation in the liquid−liquid junction potential.

Experimental Section

General Considerations. All chemicals were obtained from commercial suppliers (Aldrich or Fisher) and were used as received. Reactions were carried out under an atmosphere of nitrogen. Glycolonitrile was prepared by a modification of the literature method.[21] (*N*-Methoxyethylamino)acetonitrile was prepared by standard methods.[22] The 3,5-dichloropyrazinones were prepared by a substantial modification of the literature procedure.[23,24] Diels−Alder cyclizations were carried out using the procedures developed previously.[18,25] Flash silica gel chromatography was performed using Merck 40−70 mesh silica gel. Microanalyses were performed by the Microanalytical Services Laboratory, College of Chemistry, University of California, Berkeley, CA. Mass spectra were recorded at the Mass Spectrometry Laboratory, College of Chemistry, University of California, Berkeley, CA. Unless otherwise specified, all NMR spectra were recorded at ambient temperature on Bruker DRX 500, AMX 400, and AMX 300 spectrometers. Melting points were taken on a Buchi melting point apparatus and are uncorrected.

Glycolonitrile. Glycolonitrile was prepared by a modification of the literature preparation.[21] **Safety note:** Both sodium and hydrogen cyanide are potent and fast-acting toxins. Gaseous HCN may be liberated from acidic media. All reactions should be performed in a well-ventilated hood and adequate personal safety equipment should be worn. Sodium cyanide (4.99 g, 102 mmol) was dissolved in water (20 mL), and the solution was cooled to 0 °C. A solution of formaldehyde (37% in water, 8.17 g, 101 mmol) was added dropwise, and the reaction mixture was stirred for 30 min. The pH of the cold stirred solution was adjusted to 2 by addition of sufuric acid (7.5 N, ca. 15 mL). Then, sodium carbonate was added to bring the pH to 5. Copious amounts of a white precipitate (presumably Na_2SO_4) formed, and additional water was added to dissolve it. The aqueous fraction was washed with ether (4 × 70 mL), and the organic fraction was evaporated to dryness, yielding a thin colorless oil (4.40 g, 77.1 mmol, 77%). The oil was used immediately without further purification. Standing at room temperature for 24 h resulted in extensive decomposition. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.30 (d, 2H), 2.8 (br t, 1H).

3,5-Dichloro-1-(methoxyethyl)-2(1*H*)-pyrazinone (1). (*N*-Methoxyethylamino)acetonitrile (7.397 g, 64.80 mmol) was dissolved in ether (100 mL), and the solution was cooled to 0 °C. Hydrogen chloride was bubbled through the solution for 10 min, causing a white precipitate to form. The solvent was removed in vacuo, and the resulting solid was redissolved in chlorobenzene (65 mL) under an atmosphere of nitrogen. A solution of oxalyl chloride (24 mL, 280 mmol, 4.3 equiv) in chlorobenzene (80 mL) was added dropwise over 25 min, whereupon the solution became golden yellow. The reaction mixture was stirred for 44 h, at which point the solution was dark brown, and the reaction was then carefully quenched by dropwise addition of water. The organic layer was separated from the mixture, and the aqueous layer was washed with methylene chloride (2 × 100 mL). The combined organic phases were evaporated to dryness, leaving a dark brown-red oil, which was subjected to column chromatography on silica (90 mL, eluted with 1.5 L of methylene chloride). The product was obtained as a pale yellow oil which crystallized on standing (6.754 g, 30.28 mmol, 47%). Analytically pure material could be obtained by running a second column to give a colorless crystalline solid. Mp: 61−62 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.30 (s, 1H), 4.03 (dd, J = 4.8, 4.8 Hz, 2H), 3.55 (dd, J = 5.0, 4.8 Hz, 2H), 3.20 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.70, 146.31, 128.59, 123.11, 68.84, 58.93, 50.77. FAB-MS(+), m/z (%): 223.1 (100) [MH^+]. Anal. Calcd (found) for $C_7H_8N_2ClO_2$: C, 37.69 (37.50); H, 3.62 (3.75); N, 12.56 (12.26).

3-Benzyl-5-chloro-1-(methoxyethyl)-2(1*H*)-pyrazinone (2). Sodium hydride (0.150 g, 6.25 mmol) was dissolved in THF (15 mL) under an atmosphere of nitrogen. Benzyl alcohol (480 μL, 4.64 mmol) was added, gas evolution was observed, and the resulting solution was stirred for 30 min. 3,5-Dichloro-1-(methoxyethyl)-2(1*H*)-pyrazinone (**1**; 1.004 g, 4.50 mmol) dissolved in THF (20 mL) was added dropwise to the stirring solution over about 30 min. The reaction mixture was stirred in the dark for 18 h, during which the solution became red-purple. Hydrogen chloride (1 M in ether, 10 mL) was added, and the solvent was removed in vacuo. Methylene chloride (100 mL) and water (100 mL) were added, and the layers were separated. The organic layer was washed with brine (100 mL), dried (Na_2SO_4), and evaporated to dryness. The resulting solid was slurried in methylene chloride/hexane (5:95), and a beige solid was collected by filtration. Additional material was obtained by column chromatography on silica (40 mL) by eluting with a methanol gradient in methylene chloride (0.932 g, 3.16 mmol, 70%). Mp: 120−121 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46−7.50 (m, 2H), 7.10−7.38 (m, 3H), 6.94 (s, 1H, Ar H), 5.37 (s, 2H, CH_2Ph), 4.02 (dd, J = 5.0, 5.0 Hz, 2H), 3.62 (dd, J = 5.0, 5.0 Hz, 2H), 3.31 (s, 3H, OCH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.28, 150.16, 135.27, 128.80, 128.46, 128.37, 122.05, 120.98, 69.61, 69.59,

58.99, 49.42. FAB-MS(+), *m*/*z* (%): 295.1 (100) [MH^+]. Anal. Calcd (found) for $C_{14}H_{15}N_2ClO_3$: C, 57.05 (56.70); H, 5.13 (5.25); N, 9.50 (9.51).

Ethyl 3-(Benzyloxy)-1-(methoxyethyl)-2(1*H*)-pyridinone-4-carboxylate (3a). 3-Benzyl-5-chloro-1-(methoxyethyl)-2(1*H*)-pyrazinone (**2**; 2.63 g, 8.92 mmol) was mixed with ethyl propiolate (5.2 mL, 51 mmol, 5.8 equiv) under an atmosphere of nitrogen, and the mixture was heated at 140 °C for 5 h. The resulting mixture was subjected to column chromatography on silica (160 mL) with a methanol/methylene chloride gradient as the eluent. It was not possible to separate the 4-carboxylate (**3a)** and 5-carboxylate (**3b**) isomers. Proton NMR spectroscopy suggested that the 4-substituted isomer was present in a 3.5:1 excess. The crude material, which was isolated as a yellow oil (2.94 g, 8.87 mmol), was used without further purification. ^{1}H NMR for **3a** (400 MHz, $CDCl_3$): δ = 7.49 (m, 2H), 7.2−7.3 (m, 3H), 7.14 (d, J = 7.2 Hz, 1H), 6.29 (d, J = 7.1 Hz, 1H), 5.28 (s, 2H), 4.27 (overlapped q, 2H), 4.1 (overlapped t, 2H), 3.7 (overlapped t, 2H), 3.3 (overlapped s, 3H), 1.27 (t, J = 7.14 Hz, 3H). ^{1}H NMR for **3b** (400 MHz, $CDCl_3$): δ = 7.87 (m, 1H), 7.45 (m, 2H), 7.2−7.3 (m, 3H), 7.20 (m, 1H), 5.10 (s, 2H), 4.27 (overlapped q, 2H), 4.1 (overlapped t, 2H), 3.7 (overlapped t, 2H), 3.3 (overlapped s, 3H), 1.333 (t, J = 7.12 Hz, 3H). ^{13}C NMR for **3a** (100 MHz, $CDCl_3$): δ = 165.11, 159.55, 147.55, 137.11, 133.13, 130.72, 128.61, 128.30, 128.05, 103.58, 73.99, 70.11, 61.69, 59.04, 50.01, 14.12.

3-(Benzyloxy)-1-(methoxyethyl)-2(1*H*)-pyridinone-4-carboxylic Acid (4a). Crude Ethyl 3-(benzyloxy)-1-(methoxyethyl)-2(1*H*)-pyridinone-4-carboxylate (**3a**; 2.94 g, 8.87 mmol) was dissolved in THF (60 mL), and the solution was cooled to 0 °C. An aqueous solution of KOH (14.3 mL, 0.932 M, 13.3 mmol, 1.5 equiv) was added, and the reaction mixture was stirred for 18 h. An ethereal solution of HCl (20 mL, 1 M) was then added, the reaction mixture was stirred for 1 h, and the solvent was removed in vacuo, leaving a beige solid. Methylene chloride (100 mL) and water (100 mL) were added, causing a color change of the organic phase to a dark reddish brown. The organic layer was separated from the mixture, washed with brine (100 mL), dried ($MgSO_4$), and filtered. The filtrate was evaporated to dryness, yielding a red-brown oily solid (2.484 g, 8.19 mmol, 92%). Proton NMR spectroscopy suggested a 4-isomer (**4a**) to 5-isomer (**4b**) ratio of 7.5:1. ^{1}H NMR fir **4a** (500 MHz, $CDCl_3$): δ = 7.43−7.45 (m, 2H), 7.30−7.35 (m, 3H), 7.19 (d, J = 7.0 Hz, 1H), 6.59 (d, J = 7.0 Hz, 1H), 5.50 (s, 2H, CH_2Ph), 4.14 (t, J = 5.0 Hz, 2H), 3.67 (t, J = 5.0 Hz, 2H), 3.29 (s, 3H, OMe). ^{13}C NMR for **4a** (125 MHz, $CDCl_3$): δ = 164.37, 158.64, 147.43, 134.98, 133.64, 129.26, 129.11, 128.75, 104.07, 75.30, 69.79, 58.96, 50.13.

4-((2-Thioxothiazolidin-1-yl)carbonyl)-3-(benzyloxy)-1-(methoxyethyl)-2(1*H*)-pyridinone (5a). Crude 3-(Benzyloxy)-1-(methoxyethyl)-2(1*H*)-pyridinone-4-carboxylic acid (**4a**; 2.803 g, 9.24 mmol) was dissolved in methylene chloride (60 mL) under an atmosphere of nitrogen, and 2-mercaptothiazoline (1.274 g, 10.69 mmol, 1.16 equiv), 4-(dimethylamino)pyridine (ca. 20 mg), and dicyclohexylcarbodiimide (2.190 g, 10.61 mmol, 1.15 equiv) were added. The reaction mixture was stirred for 24 h, at which point a white solid was removed by filtration. The organic phases were diluted with methylene chloride (50 mL), washed with aqueous KOH (4%, 50 mL), and passed through a column of silica (60 mL) with 1% methanol/methylene chloride to give a thick yellow oil. The 5-isomer (**5b**) and additional DCU were separated by precipitation from hot ethyl acetate, leaving a yellow oil (3.03 g, 7.49 mmol, 81%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.35−7.4 (m, 2H), 7.2−7.35 (m, 3H), 7.17 (d, J = 7.0 Hz, 1H), 7.04 (d, J = 6.5 Hz, 1H), 5.29 (s, 2H, CH_2Ph), 4.28 (t, J = 7.3 Hz, 2H), 4.10 (t, J = 5.0 Hz, 2H), 3.65 (t, J = 4.5 Hz, 2H), 3.29 (s, 3H, OMe), 2.85 (t, J = 7.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 200.91. 165.89, 158.55, 143.69, 137.55, 133.66, 132.93, 128.35, 128.27, 128.08, 103.31, 73.93, 70.49, 59.05, 55.19, 49.74, 29.01. FAB-MS(+), *m*/*z* (%): 405.1 (40) [MH^+]. Anal. Calcd (found) for $C_{19}H_{20}N_2O_4S_2$: C, 56.42 (56.28); H, 4.98 (4.95); N, 6.93 (6.73).

5-((2-Thioxothiazolidin-1-yl)carbonyl)-3(benzyloxy)-1-(methoxyethyl)-2(1*H*)-pyridinone (5b). A reaction identical to that for the preparation of **5a** was used. With crude 3-(benzyloxy)-1-(methoxyethyl)-2(1*H*)-pyridinone-5-carboxylic acid (**4b**; 2.452 g, 8.084 mmol) used as the starting material, a yellow powder was obtained from methylene chloride/ether (2:50) at −40 °C after the column treatment (0.140 g, 0.324 mmol, 4%). Mp: 153−160 °C dec. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.78 (d, J = 2.5 Hz, 1H), 7.2−7.5 (m, 5H, Bn), 7.00 (d, J = 2.5 Hz, 1H), 5.09 (s, 2H, CH_2Ph), 4.41 (t, J = 7.0 Hz, 2H), 4.16 (m, 2H), 3.65 (m, 2H), 3.41 (t, J = 7.5 Hz, 2H), 3.30 (s, 3H, OMe). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 201.88, 168.05, 157.97, 147.28, 138.04, 135.75, 128.61, 128.20, 127.57, 113.98, 110.85, 71.15, 69.74, 58.98, 56.67, 50.30, 29.70. FAB-MS(+), *m*/*z* (%): 405.1 (65) [MH^+]. Anal. Calcd (found) for $C_{19}H_{20}N_2O_4S_2 \cdot 1.25\ H_2O$: C, 53.44 (53.05); H, 5.05 (5.05); N, 6.43 (6.43).

Tribenzyl-TREN-MOE-3,2-HOPO (6). 4-((2-Thioxothiazolidin-1-yl)carbonyl)-3-(benzyloxy)-1-(methoxyethyl)-2(1*H*)-pyridinone (**5a**; 1.289 g, 3.187 mmol, 4.1 equiv) was dissolved in methylene chloride (40 mL), and a solution of tren (115 μL, 0.769 mmol, 1.0 equiv) in methylene chloride (10 mL) was added dropwise. The reaction mixture was stirred for several days until TLC showed no further reaction. Additional methylene chloride (50 mL) was added, and the organic phases were washed with aqueous KOH (4%, 50 mL) and brine (50 mL). The solvent was removed, leaving a yellow oil, which was subjected to column chromatography on silica (40 mL) with methanol/methylene chloride as the eluent. The desired product was obtained as a pale yellow oil (0.718 g, 0.716 mmol, 93%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.78 (m, 3H, NH), 7.32−7.35 (m, 6H), 7.20−7.28 (m, 9H), 7.13 (d, J = 7.3 Hz, 3H), 6.65 (d, J = 7.3 Hz, 3H), 5.27 (s, 6H, CH_2Ph), 4.11 (t, J = 5.0 Hz, 6H), 3.65 (t, J = 5.0 Hz, 6H), 3.27 (s, 9H, OMe), 3.06 (m, 6H), 2.24 (t, J = 6.9 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.25, 159.08, 146.18, 136.27, 132.79, 130.78, 128.88, 128.70, 128.61, 104.13, 74.64, 70.00, 58.93, 52.24, 49.80, 37.26. FAB-MS(+), *m*/*z* (%): 1002.5 (40) [MH^+]. HRMS (FAB), *m*/*z*: calcd, 1002.4613; found, 1002.4599.

TREN-MOE-3,2-HOPO (7). Tribenzyl-TREN-MOE-3,2-HOPO (**6**; 0.662 g, 0.661 mmol) was dissolved in a mixture of acetic acid (9 mL) and hydrochloric acid (concentrated, 9 mL) in the dark under nitrogen. The reaction mixture was stirred for 3 days, at which point the solvent was removed in vacuo. Methanol (5 × 25 mL) was added to the residue and removed by evaporation until an oily pale yellow solid formed. This solid was taken up in a minimum quantity of methanol (ca. 5 mL), and this solution was added to a stirring solution of ether (100 mL), causing the precipitation of a white solid, which was collected by filtration (0.440 g, 0.573 mmol, 87%). Mp: 120−125 °C dec. ^{1}H NMR (500 MHz, $(CD_3)_2SO$): δ = 8.66 (br m, 3H, NH), 7.10 (d, J = 7 Hz, 3H), 6.46 (d, J = 7 Hz, 3H), 4.07 (t, J = 5 Hz, 6H), 3.69 (br s, 6H), 3.58 (t, J = 5 Hz, 6H), 3.44 (br s, 6H), 3.21 (s, 9H, OCH_3). ^{13}C NMR (100 MHz, CD_3OD): δ = 168.94, 159.76, 150.00, 129.04, 117.68, 104.06, 70.94, 59.15, 53.68, 50.72, 35.57. FAB-MS(+), *m*/*z* (%): 732.4 (16) [MH^+]. Anal. Calcd (found) for $C_{33}H_{45}N_7O_{12} \cdot HCl \cdot 2.5H_2O$: C, 48.73 (48.88); H, 6.20 (6.22); N, 12.06 (12.03).

Gd(TREN-MOE-3,2-HOPO)(H_2O)$_n$ (8). TREN-MOE-3,2-HOPO (**7**; 0.212 g, 0.267 mmol) was dissolved fully with some heating in 15% methanol/THF (20 mL). $GdCl_3 \cdot 6H_2O$ (0.090 g, 0.242 mmol) dissolved in 15% methanol/THF (3 mL) was added dropwise, whereupon a fine white precipitate formed. Pyridine (0.5 mL) was added, and the reaction mixture was refluxed for 18 h. The precipitate was collected by centrifugation, washed with additional 15% methanol/THF (20 mL), and centrifuged to yield a white powder (0.257 g, 0.250 mmol, 93%). Mp: >200 °C dec. FAB-MS(+), *m*/*z* (%): 887.2 (30) [MH^+]; the calculated and observed molecular ion envelopes are in agreement. HRMS (FAB), *m*/*z*: calcd for $C_{33}H_{42}N_7O_{12}{}^{160}Gd$, 887.221 08; found, 887.221 84. Anal. Calcd (found) for $C_{33}H_{42}N_7O_{12}Gd \cdot 8.2H_2O$: C, 38.32 (38.33); H, 5.70 (5.10); N, 9.40 (9.08).

Solutions for Thermodynamic Studies. All solutions were prepared with "CO_2-free" water. For this, MilliQ water (resistivity >18 MΩ cm^{-1}) was boiled and cooled under a stream of nitrogen. The electrolyte solution (0.1 M KCl) was prepared by dissolution of ACS grade KCl (Fisher). Standard acid (HCl) and base (KOH) solutions (approximately 0.1 M) were prepared by dilution of Baker Dilut-it ampules. All solutions were allowed to stand in their storage vessels (glass) for 24 h before use. The base and electrolyte solutions were permanently stored under a slight positive pressure of ascarite-scrubbed argon gas to reduce the ingression of CO_2 from the atmosphere.

The acid solution was standardized against a weighed amount of Borax ($Na_2B_4O_7 \cdot 10H_2O$, SigmaUltra grade, Sigma) using methyl red (sodium salt, ACS reagent grade, Sigma) as a visual end-point indicator. The base solution was standardized in a similar manner against potassium hydrogen phthalate using phenolphthalein (1 drop of a 1% solution in ethanol) as a visual indicator. In both cases, a sufficient amount of the primary standard (dissolved in 25 mL of CO_2-free electrolyte) was used to give an end point of approximately 4 mL, far exceeding the precision of the autoburet. Each solution was restandardized until its concentration was known to within 0.2% (typically requiring six replications). The standardizations were conducted manually using the same apparatus as used for the potentiometric titrations. In effect, this approach calibrated the volumetric scale of the autoburets.

A stock solution of the metal was prepared by dissolution of $GdCl_3$ (99.99% REO, Ultra-dry anhydrous, Alfa Aesar) in the standard (~0.1 M) HCl solution prepared as above. This solution was standardized by titration against a standard solution of EDTA (Na_2 EDTA, Biochemika grade, Fluka) using an adapted method.[33] For this, an aliquot of the Gd^{3+} stock was taken with a calibrated glass-bulb pipet and diluted into 25 mL of 0.05 M ammonium acetate (ACS grade, Sigma) to give a pH of ~2.5. This solution was titrated with the standard EDTA solution. Three drops of an Arsenazo III (sodium salt, hydrate, 98%, Aldrich) solution was used as a visual end-point indicator.

The EDTA solution itself was standardized by potentiometric titration from pH 5 to 9 and back to pH 5 using the standard acid/base solutions described above.

Titration Equipment. The titration cell was built "in-house" and is composed of glass with a Teflon lid. The lid has openings for insertion of the electrode, buret tip, and gas intake/outlet. The cell was held under a positive pressure of argon to eliminate the ingression of CO_2. A water jacket around the cell provided temperature control (25 ± 0.1 °C) by means of circulating water from a thermostated water bath (Neslab). For the collection of spectral data, an opening was cut into the side of the cell. A quartz curvette was cut open and attached to the opening in the titration cell using silicone rubber glue (General Electric RTV 102). This entire apparatus, including a water-powered magnetic stirrer, was small enough to be housed within the bay of the spectrophotometer with the quartz curvette intercepting the light path.

Fully automated instrumentation was used for titrant delivery and the collection of potentiometric and/or spectrophotometric data. Metrohm autoburets (Titrino 702 SM and Dosimat 665) were used for titrant delivery. Either an Accumet pH-meter (model AR15 or 15) or the Metrohm Titrino was used for electrode potential measurements. A high-performance glass-bulb electrode (Corning combination reference, catalog no. 476146) was used. Both the autoburets and pH meters were controlled via a serial (RS-232C) interface. A Hewlett-Packard 8452a spectrophotometer (diode array), controlled via a GPIB interface, was used for the collection of absorbance data. This instrument was always powered-on for at least 1 h before use to allow the lamp to come to operating temperature. The electrolyte solution was used for the blank-reference scan. Remote control of these instruments from a personal computer was achieved using modules of the LABVIEW programming environment.[34] These modules were either written "in-house" or obtained from National Instruments (drivers for the Dosimat 665 autoburet[35] and the HP 8452a spectrophotometer[36] are available for download).

Standard Electrode Calibration. In this method, 2.000 mL of standardized HCl (ca. 0.1 M) was combined with 50.0 mL of KCl solution (0.1 M), and the solution was titrated with standardized KOH (approximately 0.1 M) to pH 11.6 (final titer 4.4 mL). Incremental additions were selected to give an approximately linear variation in pH throughout the course of the calibration, with approximately 30 points being collected over the pH ranges 2.4−4 and 10.8−11.4. The data were analyzed using the program GLEE,[37,38] allowing refinement of the parameters $E°$, s, and γ. A fixed value of 13.78 was used for pK_w.[39,40] Typically, the residuals between observed and calculated electrode potentials fell within a tolerance of 0.1 mV with rms values of ~0.05 mV.

Low-pH Calibration with Junction Potential Correction. The strategy for this procedure was to dilute up to 25 mL of HCl (~0.1 M) in 50 mL of KCl (0.1 M), giving a final pH of ~1.5. Incremental additions of HCl were selected so as to give an approximately linear variation in pH throughout the course of the calibration, with approximately 30 points being collected by the end. This simple procedure has the advantages that it requires only one standardized solution, the HCl, and the ionic strength remains constant (0.1 M) although there is substantial substitution of K^+ for protons, H^+, hence the variation in junction potentials.

The hydrogen ion concentration at each point was determined by back-calculation from the dilution of the HCl standard. This allowed calculated values of the electrode potential, E_{calc}, to be found for each point of the calibration using eq 2 and estimated values for the electrode parameters $E°$, s, and j_p. These estimates were refined by nonlinear least-squares calculations to minimize the sum of squared residuals between the calculated potentials and the observed electrode readings, E_{obs}. For this task, the SOLVER.XLA add-in for Microsoft Excel was used. Once the refined values of the electrode parameters had been obtained, they were used to convert the electrode readings recorded in the course of a metal/ligand titration into pH values. For this second task, the "Goal seek" function of Microsoft Excel was used. An exemplary spreadsheet is available for download.[41]

Data Treatment. All equilibrium constants were determined by refinement with the program HYPERQUAD[42,43] and are defined as cumulative formation constants, β_{pqr}, according to eq 3; note that the ligand is designated as "L". The ligand protonation constants, K_{an}, may

$$pGd^{3+} + qL^{3-} + rH^+ \rightleftharpoons Gd_pL_qH_r^{(3p-3q+r)+}$$

$$\beta_{pqr} = \frac{[Gd_pL_qH_r]}{[Gd]^p[L]^q[H]^r} \quad (3)$$

$$K_{an} = \frac{[LH_n]}{[H][LH_{n-1}]} = \frac{\beta_{01n}}{\beta_{01(n-1)}} \quad (4)$$

be derived from these cumulative constants according to eq 4; note that this definition describes proton *association* constants. For titrations involving metal−ligand coordination, the constant for the metal−ion hydrolysis species $GdOH^{2+}$ was included as a fixed parameter in the refinements ($\log \beta_{(10-1)} = -8.37$).[29]

The HYPERQUAD program allows treatment of potentiometric and/or spectrophotometric data and the simultaneous treatment of multiple titration curves. An important element in the treatment of data acquired from different measurement techniques (i.e., absorbance and potentiometric measurements) is the consideration of experimental uncertainties, since these affect the relative weighting of each observation in the refinements. For potentiometric observations, the error terms were given as 0.002 mL and 0.002 pH unit and are based on manufacturer specifications for the pH-meters and autoburets. For absorbance measurements, the error terms were determined using repeat spectral

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Table 2. Summary of Solution Equilibrium Studies

conditions	25 °C, 0.1 M KCl
equipment, techniques	glass-bulb potentiometry calibrated against $[H^+]$ UV spectrophotometry data analysis by nonlinear least-squares refinement (HYPERQUAD)
number of titrations	protonation studies: 4 titrations, ~280 points spectrophotometric: 5 titrations, ~150 points, 70 wavelengths at each point potentiometric: 6 titrations, ~150 points
concentration ranges	protonation studies: 5×10^{-4} to 7.5×10^{-4} M ligand: 3.1×10^{-5} to 5.3×10^{-4} M metal: 1.6×10^{-5} to 4.4×10^{-5} M ligand to metal ratios: 1.1:1 to 5.3:1 (ligand present in excess)
global values	protonation studies: 1.1−2.7 spectrophotometry: 0.9−1.7 potentiometry: 1.4−1.7

scans of a holmium oxide glass filter as directed by the program HYPERQUAD.[42,43]

Titrations. A summary of the solution thermodynamic investigations is provided in Table 2. For the ligand-only titrations, an equilibration time of 1 min was used. This was extended to 3 min in the presence of Gd. At the completion of the appropriate equilibration time, potentiometric measurements were recorded at 3 s intervals; the mean value was recorded once 10 measurements had been obtained in which the standard deviation in measured electrode potential was less than 0.05 mV. If this criterion was not reached within 5 min, then "drift" was declared to be present and the point was omitted from the titration data set. Absorbance measurements were collected using a 10 s integration period, and spectra were recorded before and after collection of potentiometric data, the whole cycle being repeated until the absorbance measurements agreed to within a tolerance of 0.001 absorbance unit. All absorbance measurements were less than or equal to 1.1 absorbance units. A typical titration data set contained 30 points with a potentiometric measurement of pH and an absorbance spectrum at each point. A selection of 70 wavelengths (250−390 nm at 2 nm intervals) was taken from the spectral data and incorporated into the refinements, giving an absorbance matrix containing ~2100 data points for each titration.

Protonation Studies. The protonation constants of TREN-MOE-3,2-HOPO were determined in two potentiometric experiments. The standard electrode calibration technique was used. For each experiment, a known volume of electrolyte (0.1 M KCl, taken by an A-grade glass-bulb pipet) was combined with a weighed portion of the ligand (giving a concentration of either 0.5 or 0.75 mM). The resulting solutions were titrated twice, first against 0.1 M KOH from pH 3 to 10.2 and then in reverse against 0.1 M HCl back down to pH 3. This gave four titrations with an average of 70 points being collected from each for a total of 280 measurements. The results from each pair of titrations were combined for nonlinear least-squares refinement with the program HYPERQUAD,[42,43] giving the values and standard deviations for the protonation constants as listed in Table 1. Both the proton and ligand concentrations were admitted as a refineable parameters, giving refined concentrations that were within 3% of the expected values. The global σ values were 1.1 and 2.7.

Gd Complexation (Spectrophotometric). Gd complexation was examined in five titrations, each of which covered the pH range 3.5−1.5. The electrode calibration technique, which incorporates a correction for variation in junction potentials, was used. Spectral data covered the wavelength range 250−390 nm. Over the five titrations, the ligand concentration varied from 3.1×10^{-5} to 6.6×10^{-5} M and the metal concentration was varied over the range $(1.6-4.8) \times 10^{-5}$ M, giving ligand-to-metal ratios varying from 1.1:1 to 5.3:1. Each titration was performed by dissolving a weighed amount of the ligand in 50.0 mL of 0.1 M KCl, adding an aliquot of the metal stock solution, and titrating with up to 25 mL of standardized HCl (~0.1 M). This large addition of titrant was required to take the titrations to the very low pH value of 1.5. By the end of the titration, a volume of titrant equal to half the initial volume of titrand had been used; thus it was not practical to conduct these titrations in the reverse direction; i.e., the Gd−ligand solutions were only titrated with acid and not with base. Approximately 30 points were recorded in each titration.

The data from each titration were imported into the program HYPERQUAD[42,43] and treated by nonlinear least-squares refinement. Observations recorded at all 70 wavelengths (250−390 nm at 2 nm intervals) were incorporated into the refinements. The species which were defined to be "colored" were the Gd−ligand complexes and the fully protonated form of the ligand, $[LH_4]^+$. The triprotonated form of the ligand, $[LH_3]^0$, was defined as not colored in titrations where it formed less than 0.5% of the total distribution of ligand species at all points. This species was significantly formed in titrations with a higher ligand-to-metal ratio (up to 5% formation in the titration with a 5.3:1 ratio), and in these cases, it was defined as colored using a fixed spectrum taken from a separate, ligand-only, spectrophotometric titration. A diagonal weighting scheme was selected, and the proton was the only species whose concentration was refined. The resulting formation constants are given in Table 1. The global σ values for refinement of the five titrations varied between 0.9 and 1.7.

Gd Complexation (Potentiometric). The standard electrode calibration technique was used. Three experiments were performed. In each case, a metal/ligand solution was titrated first with base and then in the reverse direction with acid, giving six titrations in all. The metal concentration was varied over the range $(0.9-4.4) \times 10^{-4}$ M and the ligand concentrations were in the range $(1-5.3) \times 10^{-4}$ M, with ligand-to-metal ratios from 1.1:1 to 1.2:1. An average of 25 points were collected from each titration, giving a total of 150 measurements over the six titrations. The results from each pair of titrations were combined for nonlinear least-squares refinement with the program HYPERQUAD,[42,43] giving the values and standard deviations for the formation constants β_{110} and β_{111} as listed in Table 1. The β_{112} formation constant was incorporated as a fixed parameter using the value determined in the spectrophotometric experiments. The proton was the only species whose concentration was admitted as a refineable parameter; other concentrations were fixed at values calculated from the dilution of the standardized Gd stock solutioin and from the weight of ligand taken. The global σ values were in the range 1.4−1.7.

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