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Terephthalamide-Containing Analogues of TREN-Me-3,2-HOPO

Kristy M. Clarke Jurchen and Kenneth N. Raymond*

Department of Chemistry, University of California at Berkeley, Berkeley, California 94720-1460

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A series of terephthalamide-containing analogues based on TREN-Me-3,2-HOPO have been prepared. These analogues contain one, two, or three bidentate 2,3-dihydroxyterephthalamide (TAM) units in place of the 3,2-hydroxypyridinone (HOPO) units on the parent hexadentate ligand. One representative ligand in the series, $TRENHOPOTAM_2$, and its gallium complex have been structurally characterized by X-ray diffraction. $TRENHOPOTAM_2$ crystallizes in the monoclinic space group $P2_1/c$ with cell parameters $a = 16.0340(17)$ Å, $b = 17.0609(18)$ Å, $c = 16.0695(17)$ Å, $\beta = 113.837(2)°$, and $Z = 4$. Ga[$TRENHOPOTAM_2$] also crystallizes in the monoclinic space group $P2_1/c$, with cell parameters $a = 16.3379(14)$ Å, $b = 15.2722(13)$ Å, $c = 19.4397(17)$ Å, $\beta = 91.656(2)°$, and $Z = 4$. The conformation of the $TRENHOPOTAM_2$ ligand structure suggests that the ligand is predisposed for metal ion binding. The aqueous protonation and ferric ion coordination chemistry of all ligands in the series were examined using potentiometric and spectrophotometric methods, giving log formation constants of 34.6(2) (β_{110}) and 38.8(2) (β_{111}) for the ferric $TRENHOPO_2TAM$ complexes, 41.0(3) (β_{110}) and 45.4(3) (β_{111}) for the ferric $TRENHOPOTAM_2$ complexes, and 45.2(2) (β_{110}) and 50.9(2) (β_{111}) for the ferric $TRENTAM_3$ complexes. These thermodynamic data confirm that adding terephthalamide units to a hydroxypyridinone-containing ligand tends to increase the stability of the resulting iron complex. The ferric $TRENTAM_3$ complex is one of the most stable iron complexes yet reported.

Introduction

The versatile ligand TREN-Me-3,2-HOPO (*N*,*N*′,*N*″-tris-[(3-hydroxy-1-methyl-2-oxo-1,2-didehydropyrid-4-yl)carboxamidoethyl]amine, Figure 1) has been investigated for use in several applications involving high oxidation MRI contrast enhancement,[2] and bacterial iron transport.[3] TREN-Me-3,2-HOPO has also shown potential as a therapeutic iron chelator. In a preliminary animal trial, TREN-Me-3,2-HOPO was effective at promoting iron excretion in rats when administered either by injection or by mouth.[4] TREN-Me-3,2-HOPO's therapeutic benefits arise from its low toxicity and its bioavailability.[1,4] This ligand is also able to rapidly remove iron from the iron transport protein transferrin, as demonstrated by an in vitro kinetic survey of TREN-Me-3,2-HOPO and other hydroxypyridinone ligands.[5] A recent study of TREN-Me-3,2-HOPO and several expanded scaffold analogues (Compounds **2**−**4**, Figure 1) found that ligands incorporating the TREN scaffold formed more stable iron complexes than ligands incorporating similar but larger scaffolds (Table 1).[6] An investigation of mixed hydroxypyridinone chelators containing terephthalamide or isophthalamide binding units in place of one of the hydroxypyridinone units (Compounds **5** and **6**, Figure 1) found that incorporating one isophthalamide group in place of hydroxypyridinone had little effect on the stability of the iron complex but that replacing a hydroxypyridinone unit with a terephthalamide unit resulted in a large increase in the iron complex formation constant (Table 1).[7]

In this program of research, we sought to probe this system further by investigating the effect on the iron complex stability of progressively replacing the hydroxypyridinone

* To whom correspondence should be addressed. E-mail: raymond@socrates.berkeley.edu.

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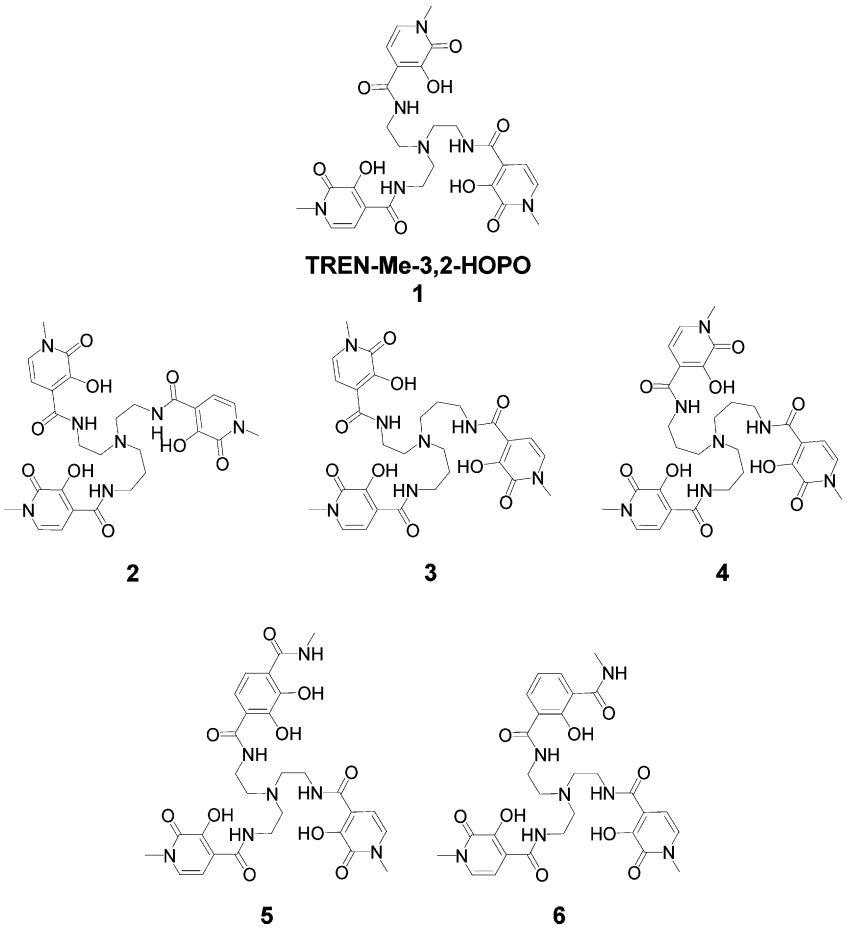

Figure 1. TREN-Me-3,2-HOPO and previously investigated analogues.

Table 1. Thermodynamic Ferric Complex Stability of TREN-Me-3,2-HOPO and Previously Investigated Analogs

param	TREN-Me-3,2-HOPO[6] (**1**)	**2**[6]	**3**[6]	**4**[6]	**5**[7]	**6**[7]
$\log \beta_{110}$	26.8(1)	26.2(2)	26.42(1)	24.48(1)	33.89(2)	26.89(3)
$\log \beta_{111}$	30.7(3)	30.8(1)	32.72(1)	32.2(6)	38.45(2)	31.16(6)
pM	26.8	26.6	25.8	23.8	30.2	26.7

(HOPO) units on TREN-Me-3,2-HOPO by terephthalamide (TAM) units. As a bidentate ligand, terephthalamide forms stronger iron complexes than bidentate hydroxypyridinone.[8] It was hypothesized that hexadentate ligands incorporating both hydroxypyridinone and terephththalamide units would combine the bioavailability of hydroxypyridinone with the greater iron affinity of terephthalamide, leading to ligands with overall increased chelation efficiency. For this purpose, a series of terephthalamide-containing analogues of TREN-Me-3,2-HOPO was designed (Figure 2) and evaluated for the thermodynamic stability of their iron complexes. A related series of ligands has been investigated previously for their Gd(III) complexing ability.[9] Future exploration will determine whether the terephthalamide-containing ligands are able to preserve the oral availability of the parent TREN-Me-3,2-HOPO ligand and whether the stronger terephthalamide iron-binding units contribute to a greater chelation efficiency. In this paper, we present the synthesis and solution thermodynamics of the three ligands in this series, as well as the structural characterization of a representative ligand, $TRENHOPOTAM_2$ (**8**), and its gallium complex by X-ray diffraction.

Results and Discussion

Synthesis. The synthesis of each member of this series of ligands followed one of two methods. The first step in Scheme 1 involves the selective preparation of the mono- or bis-substituted TREN amine intermediates **11** and **14**. In this reaction, a stoichiometric amount of HOPO thiaz (1 equiv in the case of **11**, 2 equiv in the case of **14**) is added slowly to a solution of the TREN amine. The thiazolide activated ester reacts selectively with primary amines.[10] The reaction proceeds slowly enough that the activated ester is distributed evenly throughout the rapidly stirred solution before the coupling reaction is complete, permitting preferential formation of the mono- or bis-HOPO TREN amine product, depending on the stoichiometry of HOPO thiaz used.

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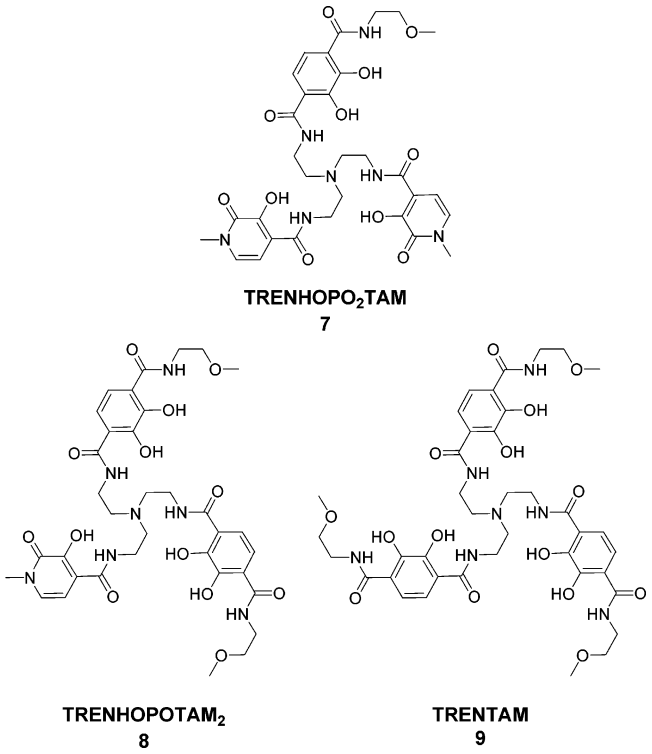


Figure 2. Terephthalamide-containing analogues of TREN-Me-3,2-HOPO.

In each case, a mixture of the singly, doubly, and triply coupled products is obtained, with the desired product comprising the largest fraction. The multiple products can be separated by column chromatography either at this point in the synthesis or after subsequent steps.

In the next step of synthetic method 1, the TAM moiety is coupled onto the free pendant amine (or amines) of the TREN backbone. To avoid reaction of both thiazolide groups of the terephthalamide unit, the reaction is performed in the presence of a large excess of TAM thiaz (**17**), and the TREN−HOPO amine solution is added slowly to the reaction mixture. This gives complete coupling of the remaining TREN arm(s) while preserving the second TAM thiazolide group for later reactions. When the crude product from step 1 is used in this reaction without further purification, the side products resulting from coupling of the HOPO moiety to one, two, or three of the TREN amine arms are all present in this TAM coupling reaction, and the desired intermediate (**12** or **15**) must be isolated by column chromatography.

A slight excess of 2-methoxyethylamine is used to treat the purified product. The amine couples with the remaining thiazolide group on the TAM unit(s), affording the desired benzyl-protected ligand **13** or **16**, again easily purified by column chromatography. Other substituents may be introduced by coupling different amines to the thiazolide intermediate, and determining the effects on biological activity and chelation strength of modifying the ligand system by varying the substituents attached to the TAM subunits will be the subject of future work.

Treating the protected ligand with a 1:1 mixture of glacial acetic acid and a concentrated mineral acid removes the benzyl protecting groups. Because these ligands are destined for use in vivo, it is necessary to choose carefully the mineral acid to be used in the deprotection reaction, as the capping TREN nitrogen is protonated during the course of the reaction and certain halide salts are toxic. Thus, concentrated hydrochloric acid was used as the mineral acid, and the final ligands were isolated as the innocuous hydrochloride salts.

Method 1 was used to prepare the HOPO-containing ligands TRENHOPO$_2$TAM (**7**) and TRENHOPOTAM$_2$ (**8**). The overall yield for TRENHOPO$_2$TAM (**7**) is good: 52.3% from the TREN amine. Unfortunately, the yield for TRENHOPOTAM$_2$ (**8**) was poor by comparison, 14.1% from TREN. If large amounts of these ligands are to be prepared for in vitro testing, greater yields will be required. Although this strategy provides increased flexibility in the final ligand by permitting the coupling of different amines onto the pendant thiazolides midway through the synthesis, the greater number of side products from the first coupling step with TREN makes selective isolation of the TRENtris(TAM thiaz) intermediate difficult, making this synthetic strategy impractical for the preparation of large quantities of TRENTAM (**9**).

For the synthesis of TRENTAM (**9**), and an improved synthesis of TRENHOPOTAM$_2$ (**8**), an alternative strategy was developed (Scheme 2). In this case, BnTAM thiaz (**17**) is used as the initial starting material. In the first step, a large excess of **17** is treated with 2-methoxyethylamine to selectively produce the monosubstituted TAM thiaz product **18**, easily separable by chromatography from the excess starting material **17** as well as from the small amount of disubstituted product afforded by the reaction.

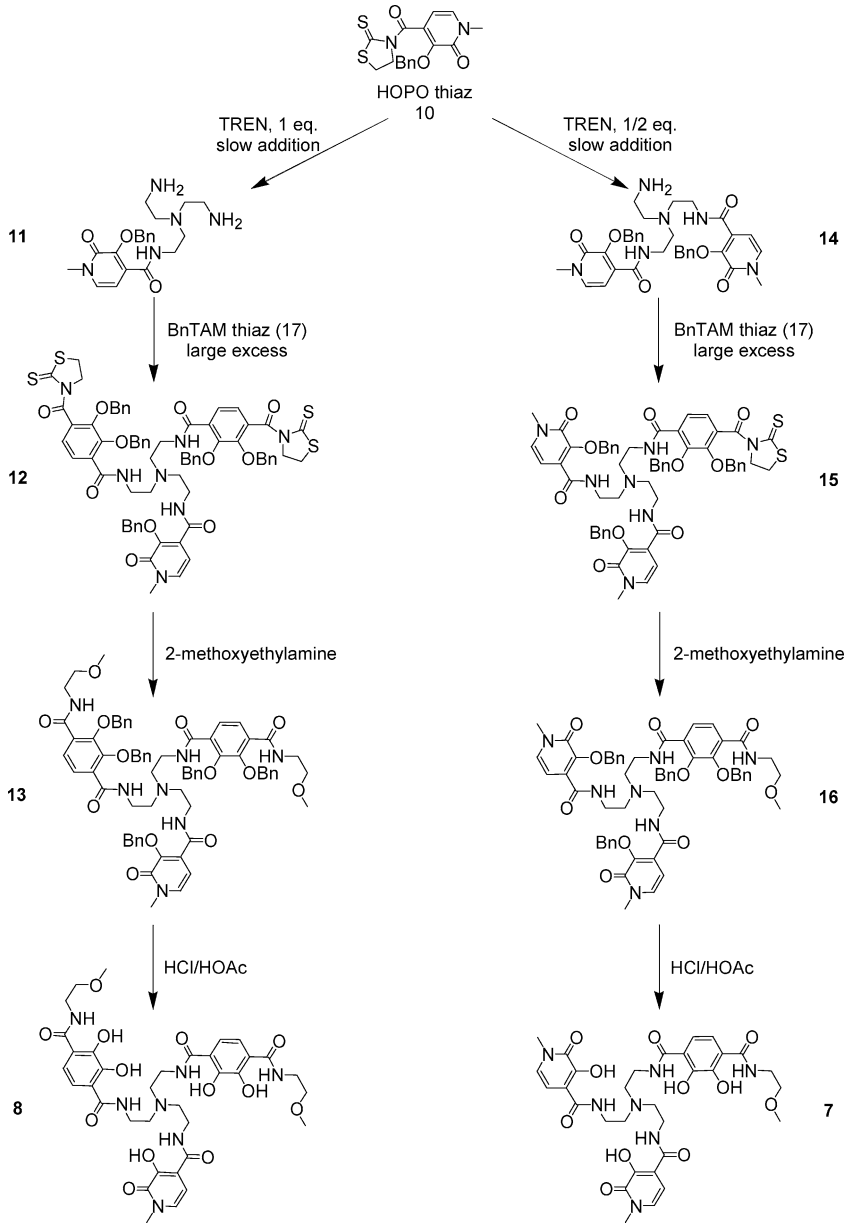
Subsequently, a stoichiometric amount of the mono-thiaz mono-meg terephthalamide **18** is added dropwise to a rapidly stirred solution of the TREN backbone. When 3 equiv of **18** is used, this second step yields the protected ligand **21**. With the addition of 2 equiv of **18**, the bis-terephthalamide TREN amine **20** is formed preferentially, along with a small amount of the mono- and tris-substituted side products **19** and **21**. Attempts to separate the multiple products at this point in the synthesis were not successful. They must be separated after the next step in the synthesis.

The amine intermediate **20** is treated with a slight excess of HOPO thiaz (**10**). The HOPO couples to the remaining, unreacted primary amines, giving a mixture of protected TRENHOPO$_2$TAM (**16**), TRENHOPOTAM$_2$ (**13**), and TRENTAM (**21**) ligands, with **13** comprising the largest fraction of the product mixture. At this point, the multiple products can be separated cleanly by column chromatography, eluting with acetone. With this technique, the three protected ligands **13**, **16**, and **21** have been isolated from the same product mixture.

As described above, treatment of the protected ligand (**13**, **16**, or **21**) with glacial acetic acid and concentrated HCl affords the hydrochloride salt of the final ligand (**7**, **8**, or **9**). The overall yield of TRENHOPOTAM$_2$ (**8**) from TREN was greatly improved using this synthetic strategy, nearly double at 27% (24.3% from **17**). The yield of TRENTAM was also very good, at 70% from TREN (56% from **17**).

Metal complexes (iron and gallium) of the three ligands in this series were prepared. The complexation reaction is

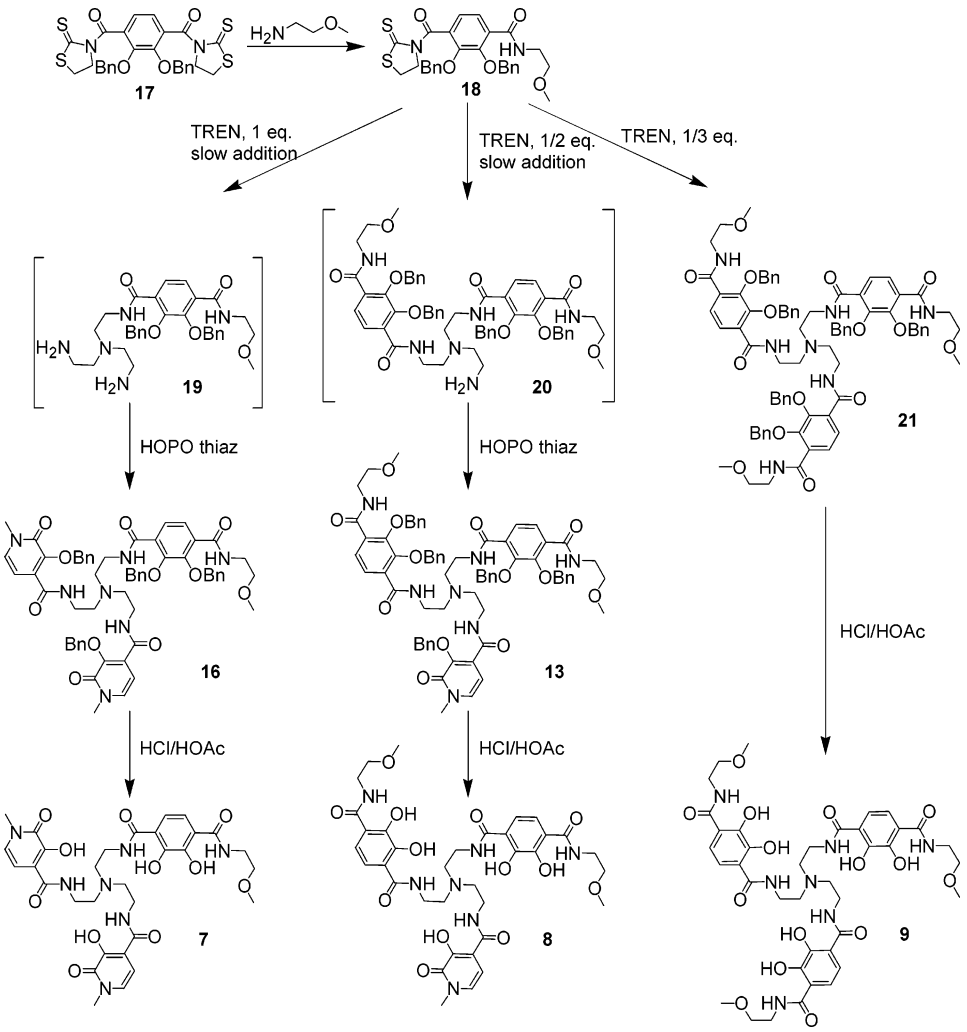
Scheme 1. TREN Ligand Synthesis Method 1



carried out in much the same way for each individual synthesis. The ligand, H_nL, is suspended in methanol and treated with $n - 3$ equiv of KOH. A 1 equiv amount of metal acetylacetonate ($Fe(acac)_3$ or $Ga(acac)_3$) is then dissolved in a small amount of methanol and added to the ligand solution. After the mixture was stirred for at least 1 h to ensure complete reaction, the concentration of the complex solution and precipitation from ether or acetone affords the analytically pure metal complex.

X-ray Crystallography. The free ligand TRENHOPOTAM$_2$ (**8**) crystallizes in space group $P2_1/c$ as the hydrochloride monohydrate, with four ligands/unit cell. All three pendant arms fall to one side of the protonated capping amine (Figure 3), with the HOPO ring and one of the terephthalamide rings oriented parallel to each other in a manner suitable for pi stacking (centroid−centroid distance = 3.756 Å; nearest atom distance = 3.363 Å). The second terephthalamide ring lies at an angle of ∼70° to the other two rings. This (presumed) preferred geometry is the result of numerous hydrogen-bonding interactions. The TAM phenol protons hydrogen bond with the adjacent amide carbonyl groups (average O−O distance = 2.51 Å), holding the extended terephthalamide units in a planar geometry. The HOPO ring is coplanar with its amide group due to hydrogen bonding of the amide proton to the phenolate oxygen (N−O distance = 2.664 Å). The phenol proton H-bonds in turn with the adjacent HOPO oxo group (O−O distance 2.702 Å). Finally, the entire HOPO unit is held in place by a hydrogen bond between the capping amine proton and the amide carbonyl oxygen (N−O distance = 2.691 Å). In the extended structure, the ligands are also held in their orientation with respect to each other by hydrogen bonds. Two chloride counterions are bound cooperatively by the eight TAM amide protons on two adjacent ligands (Figure 4, average H−Cl bond = 2.50 Å). In addition to H-bonding

Scheme 2. TREN Ligand Synthesis Method 2



with the adjacent oxo group, the HOPO phenol proton hydrogen-bonds with a TAM phenol oxygen on a neighboring ligand.

There has been some discussion in the literature about predisposed versus preorganized ligands.[11−15] In general, a ligand is considered to be preorganized if the atoms in the molecule stay in virtually the same place upon metal coordination. A good example of this phenomenon is found in the tetradentate hydroxamate siderophore alcaligin and its ferric complex.[13] The structures of the ligand alone and of the ligand within the metal complex are virtually superimposable, indicating that the bonding oxygen atoms in the ligand are already in the optimum position for binding iron. A predisposed ligand has its binding groups in the correct region of space for metal binding; however, unlike a preorganized ligand, it must undergo some conformational change to bring the binding atoms to the correct position to coordinate a metal ion. This is the situation found in metal complexes of analogues of the siderophore enterobactin[12] and is proposed for the siderophore itself on the basis of structural studies of the vanadium complex.[16] In the most stable conformation of enterobactin, the three catecholamide groups lie on one side of the trilactone backbone (Figure 5). Due to hydrogen bonding between the phenol hydrogen and carbonyl oxygen on each catecholamide subunit, the binding atoms in the fully protonated ligand point away from the central ligand cavity, and the ligand must undergo a conformational change, twisting the binding groups by 180°, for iron binding to occur.

The question of whether the TREN-based ligands are predisposed or preorganized for iron binding can be answered by comparing the TRENHOPOTAM$_2$ ligand crystal structure to the structure of Ga−TRENHOPOTAM$_2$, which also crystallizes in the monoclinic space group $P2_1/c$ with $Z = 4$. The complex displays the expected tripodal geometry, with the capping amine in the "in" conformation (Figure 6). The −2 overall charge of the complex is offset by two potassium counterions: K1 is bound by the phenol protons opposite

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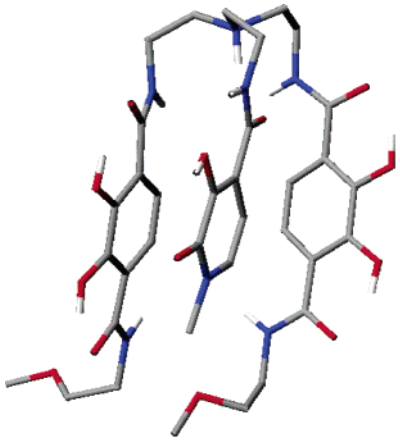

Figure 3. TRENHOPOTAM$_2$ (**8**) crystal structure (cation only).

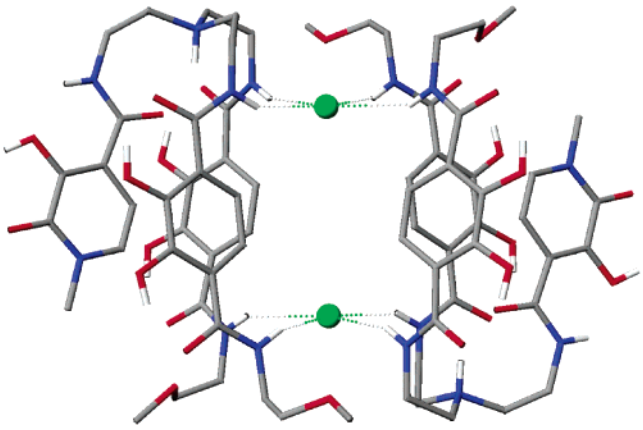

Figure 4. Cooperative chloride binding by TRENHOPOTAM$_2$ ligand cations.

the capping amine, by the two ether side chain oxygens, and by two carbonyl oxygens on neighboring complexes. K2 is bound by three carbonyl oxygens on neighboring complexes and by disordered solvent and is bridged to another K2 atom by two of the carbonyls and one DMF oxygen. The gallium ion coordination is best described as pseudooctahedral; the average twist angle of the complex (defined as the angle between the two Ga−O vectors in a binding unit projected onto a plane perpendicular to the pseudo-3-fold axis of the complex) is 45.24°, smaller than the expected 60° octahedral twist angle but consistent with the coordination geometry seen in the metal complexes of other TREN-based ligands.[6,7] The average Ga−O bond is 1.97 Å, with a range of 1.92 Å for the Ga−O_{TAM} bonds nearest the capping amine, to 2.057 for the bond between Ga and the HOPO ketone. As is common in terephthalamide and HOPO amide coordination complexes,[7] the amide protons H-bond with the nearest phenolate oxygen atoms (average N−O distance = 2.69 Å). The three amide protons nearest the amine cap also engage in weak H-bonding with the capping amine with an average N_{amide}−N_{cap} distance of 3.06 Å.

Upon examination of the superimposed structures of the ligand and of the metal complex (Figure 7), it becomes apparent that the appropriate description for TRENHOPOTAM$_2$ is predisposed. In the ligand, all three binding units have the same general orientation on one side of the amine as they do in the complex, but to actually bind a metal ion the binding groups need to twist up to a full 180° to bring the binding oxygens into the correct position.

Solution Thermodynamics. Ligand Protonation Constants. The protonation constants of the phenolic oxygens and capping TREN amines for each ligand in this series were determined by potentiometric titration. Each titration (forward and back) was repeated three times, with the final equilibrium constants determined by simultaneous refinement of all of the accumulated data using the program Hyperquad.[17] For each ligand, a constant pH buffering region was observed throughout the pH range 5.0−9.0 used in the titrations. Analysis of the titration curves indicated four sequential protonation equilibria corresponding to $-\log K_a$ values of approximately 5, 6, 7, and 8 (exact values given in Table 2). Speciation diagrams for these ligands in their various protonation states are shown in Figure 8. On the basis of previous work,[6] the lowest proton dissociation constant at ∼5 is assigned to protonation/deprotonation of the capping TREN nitrogen atom, with the higher three dissociation constants corresponding to sequential removal of one proton from each of the three binding groups. The $-\log K_a$ values determined for this series of ligands correlate well with $-\log K_a$ values determined for a similar series of mixed HOPO/TAM ligands.[9] The equilibrium constants for removal of a second proton from each terephthalamide unit were not refined. The dissociation constant for each of these protons was assigned a value of 11.1 for refinement purposes, on the basis of previous work.[7]

Metal Complexation Equilibria. Determination of the iron complex formation constant for any ligand requires the establishment of equilibrium between the metal complex and a species of known thermodynamic stability. Because the iron complexes of the multidentate ligands in this series do not dissociate according to the proton-dependent equilibrium $Fe^{3+} + H_nL \rightleftharpoons FeL^{(3-n)+} + nH^+$ except at negative pH values, the iron complex formation constants were determined via competition titration with a ligand of known iron affinity.[18] EDTA is the ligand of choice for these competition experiments, as its protonation and iron complexation equilibria are well-characterized and both the ligand and its iron complex are transparent above 420 nm.[19] Thus, when the equilibrium $FeEDTA^- + H_nL \rightleftharpoons FeL^{(3-n)+} + H_2EDTA^{2-} + (n-2)H^+$ is monitored at several different values of $[H^+]$ (i.e., pH), the formation of the HOPO/TAM ligand−iron complex can be followed by monitoring the charge-transfer bands between 420 and 650 nm that give the complexes their reddish or purplish color. The above equilibrium is accessible in the general pH range 2.5−6.0 for HOPO/TAM ligands. EDTA competition titrations are commonly performed in two general ways, either incrementally or in batch titrations.[7] In incremental titrations, a single bulk solution is used, and its pH is incrementally perturbed by the addition of standardized

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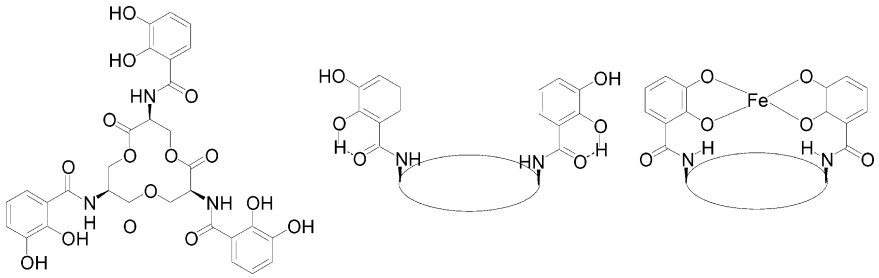

Figure 5. Enterobactin and schematic models of the proposed predisposed ligand and iron complex. One of the catecholamide arms has been omitted from the schematic for clarity.

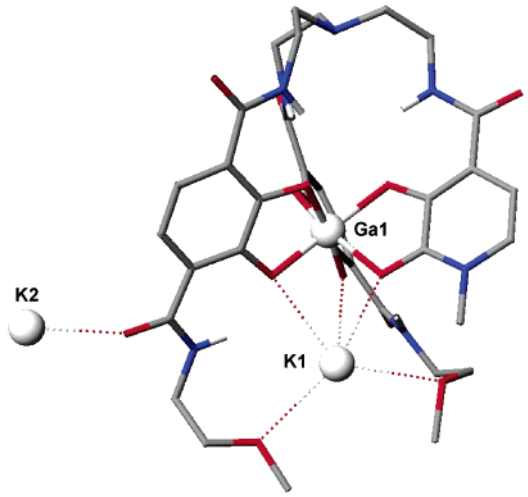

Figure 6. Gallium(III) TRENHOPOTAM$_2$, showing potassium counterions.

acid or base solution. The solution is allowed to equilibrate for 2 h after the pH is adjusted, at which time the pH is redetermined and a UV−visible spectrum is taken. A batch titration technique can also be used to confirm the accuracy of formation constants found using the incremental technique. In this case, aliquots are removed from the bulk solution, and the pH of each aliquot is adjusted individually. The aliquots are then allowed to reach equilibrium over a period of 48 h or more, at which time the pH of each aliquot is redetermined and a UV−visible spectrum is taken.

The hexadentate TREN-based ligands are capable of saturating iron's coordination sphere, and complexes of stoichiometries other than 1:1 are not expected to appear in solution. For each ligand, the iron complex formation constant was determined by means of three incremental EDTA competition titrations and confirmed by a single batch competition titration. In each competition experiment, the ferric EDTA complex predominated at low pH, while at higher pH the concentration of the HOPO/TAM iron complex increased, changing the color of the solution from colorless to red or purple. In the collected spectra, the charge-transfer bands for the complexes can be seen growing between 400 and 600 nm (Figures 9−11). Factor analysis of the spectra collected over the course of the competition experiment for each ligand indicated the presence of two absorbing species in the solution. These two species were modeled as the FeL and FeLH complexes. Refinement of these values also provided the protonation constant for the iron complex, as the difference between the formation constants of the two

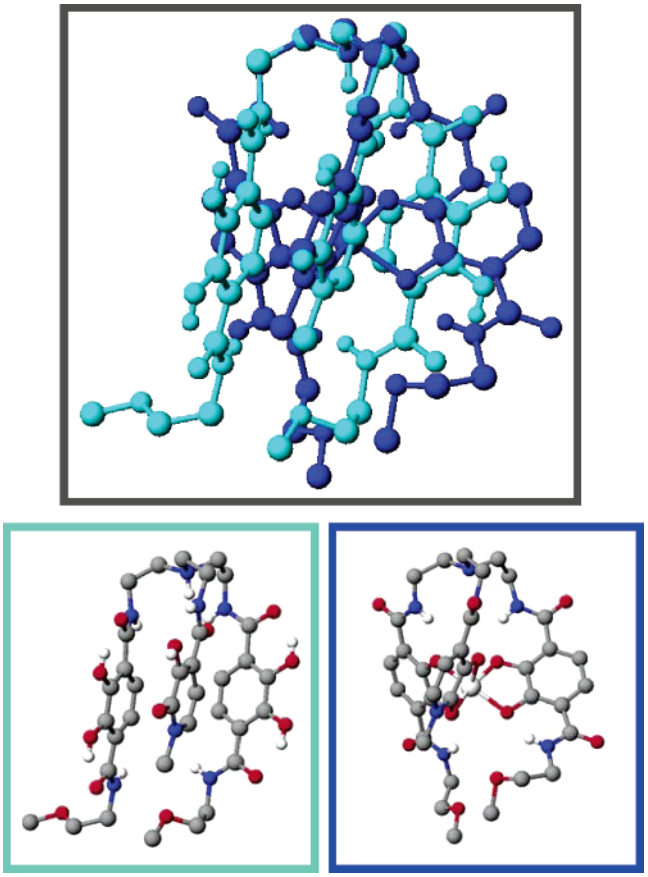

Figure 7. Top: Superimposed TRENHOPOTAM$_2$ and Ga[TRENHOPOTAM$_2$] crystal structures. The ligand is shown in cyan, and the complex in blue. Bottom: TRENHOPOTAM$_2$ and Ga[TRENHOPOTAM$_2$] crystal structures in the same orientation as in the superimposed picture.

Table 2. Summary of Solution Thermodynamics of TREN-Based Ligands

param	TRENHOPO[6]	TRENHOPO$_2$TAM	TRENHOPOTAM$_2$	TRENTAM
$-\log K_{a_1}$	4.97(1)	5.06(1)	5.11(1)	5.17(1)
$-\log K_{a_2}$	5.83(1)	5.79(1)	5.86(1)	6.00(1)
$-\log K_{a_3}$	6.97(1)	6.97(1)	6.99(1)	6.98(1)
$-\log K_{a_4}$	8.19(2)	8.07(1)	8.10(1)	8.03(1)
$\log \beta_{110}$	26.8(1)	34.6(2)	41.0(5)	45.2(2)
$\log \beta_{111}$	30.7(3)	38.8(2)	45.4(6)	50.9(2)
pM(Fe^{III})	26.8	30.9	33.6	34.2

species. For TRENHOPOTAM$_2$ (**8**) and TRENHOPO$_2$TAM (**7**), the protonated iron complexes were not soluble enough to permit independent confirmation of the iron complex protonation constant. In contrast, the protonated TRENTAM (**9**) iron complex was soluble enough to permit a potentiometric titration of the complex to confirm the refined

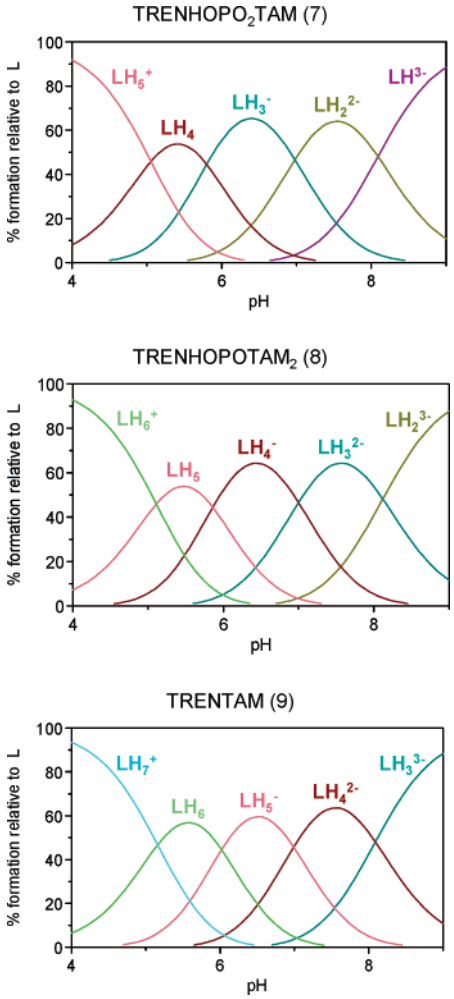

Figure 8. Speciation diagrams for the TREN ligand series.

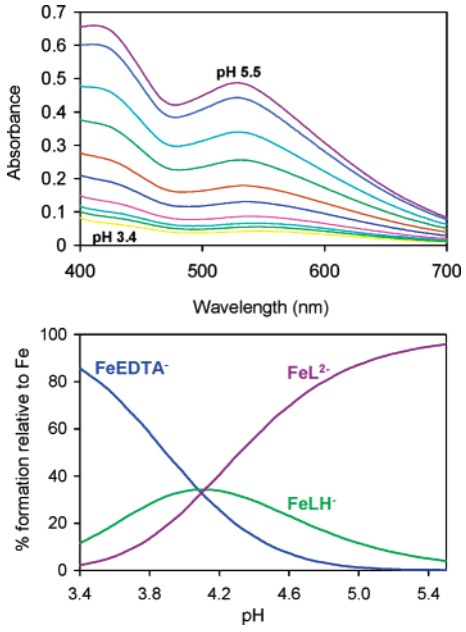

Figure 9. Sample spectra and speciation diagram for TRENHOPO$_2$TAM (**7**)− EDTA competition.

protonation constant for Fe[TRENTAM]. The value obtained by potentiometric titration was nearly identical with the value

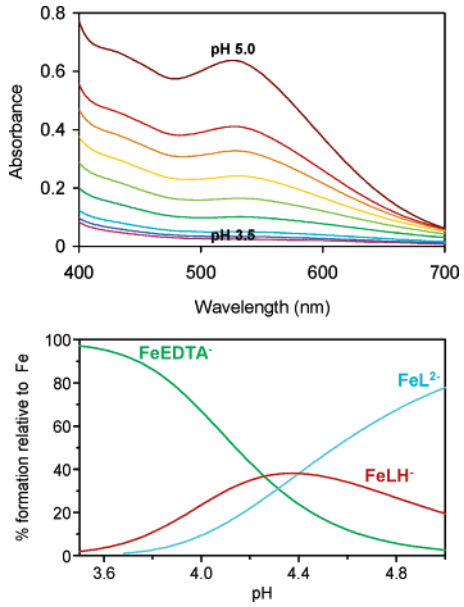

Figure 10. Sample spectra and speciation diagram for TRENHOPOTAM$_2$ (**8**)−EDTA competition.

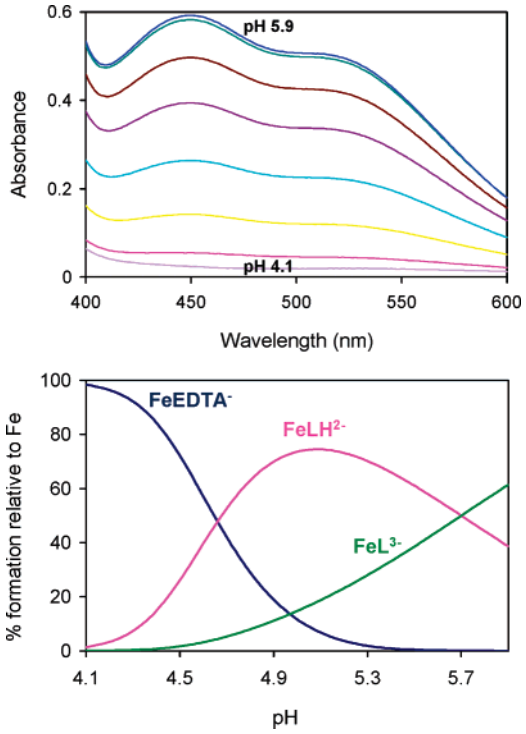

Figure 11. Sample spectra and speciation diagram for TRENTAM (**9**)− EDTA competition.

obtained by refinement of the two iron complex formation constants using data from the EDTA titrations.

On the basis of the iron complex formation constants and ligand protonation constants, a pM value was calculated for each ligand. The pM is defined as the negative log of the free iron concentration at physiological pH with 1 μM total iron concentration and 10 μM total ligand concentration. A higher pM indicates a stronger iron complex. The results of the solution thermodynamics studies of the TREN-based ligand series, as well as the analogous results of previous

studies of the parent ligand TREN-Me-3,2-HOPO (**1**), are summarized in Table 2.

Examination of the results in Table 2 reveals that the original hypothesis was correct; specifically, the incorporation of additional terephthalamide units into the TREN ligand design leads to ligands with increased affinity for iron. In fact, TRENTAM, with a K_f of $10^{45.2}$ and a pM of 34.2, possesses one of the highest iron affinities reported for any chelator. Only enterobactin ($K_f = 10^{49}$, pM = 35.5)[20,21] and a macrobicyclic mesityl-capped tris(catecholate) ligand[22,23] have been reported to have higher iron complex formation constants.

Conclusion

Several terephthalamide-containing analogues of TREN-Me-3,2-HOPO have been prepared. Solution thermodynamic evaluation of the ligands has confirmed the hypothesis that adding terephthalamide units to a HOPO-containing ligand will give a ligand that forms a stronger iron complex. The all-terephthalamide analogue TRENTAM possesses one of the highest iron affinities reported for any ligand. Structural studies of TRENHOPOTAM$_2$ and gallium TRENHOPOTAM$_2$ have indicated that the TREN ligands are predisposed rather than preorganized for metal complexation.

Experimental Section

Ligand and Metal Complex Synthesis. General Methods. Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Dropwise additions were performed under N_2 atmosphere using a pressure-equalizing addition funnel. Organic solutions were dried over anhydrous magnesium sulfate. ^{1}H NMR and proton-decoupled ^{13}C NMR spectra were obtained in $CDCl_3$ solutions using a Bruker FT-NMR spectrometer operating at either 400 or 500 MHz (500 MHz unless noted otherwise), referenced to residual solvent protons. Elemental analyses and mass spectrometry were performed in the Analytical Facility in the College of Chemistry. The starting materials 3-benzyloxy-1-methyl-4-(2-thioxothiazolidin-1-yl)carbonyl-2(1*H*)-pyridinone (HOPO thiaz, **10**)[24,25] and 2,3-bis(benzyloxy)-1,4-bis-(2-thioxothiazolidin-1-yl)terephthalamide (BnTAM thiaz, **17**)[9] were prepared according to procedures described in the literature.

Preparation of TRENTAM (9). TAMmeg thiaz (18). BnTAM thiaz (20 g, 35 mmol) was placed in a 250-mL round-bottom flask and suspended in CH_2Cl_2 (30 mL). 2-Methoxyethylamine (1.2 mL, 15 mmol) was dissolved in CH_2Cl_2 (125 mL), and the solution was added dropwise to the suspension of **17**. The product mixture was poured into 2-propanol (1 L) to precipitate the majority of the excess **17**. The mother liquor was condensed and applied to a silica column to separate the starting material and multiple products. CH_2Cl_2 was used to elute **17** and the free 2-mercaptothiazoline. Ethyl acetate was then used to separate and elute the mono- and bis(methoxyethylamide) (meg) products. **18** was isolated as a thick yellow oil (6.3 g, 80%): ^{1}H NMR δ 2.94 (t, $J = 7.3$, 2H, CH_2), 3.25 (s, 3H, CH_3), 3.42 (t, $J = 5.3$, 2H, CH_2), 3.52 (q, br, 2H, CH_2), 4.38 (t, $J = 7.3$, 2H, CH_2), 5.10 (s, 4H, $ArCH_2$), 7.22 (d, $J = 8.2$, 1H, ArH), 7.37 (m, 10H ArH), 7.92 (d, $J = 8.2$, 1H ArH), 8.11 (t, br, 1H NH); ^{13}C NMR δ 28.60, 39.57, 55.48, 58.55, 70.74, 76.06, 124.32, 126.81, 127.92, 128.33, 128.55, 128.61, 128.67, 128.77, 130.04, 133.45, 135.71, 136.98, 149.34, 150.01, 164.17, 166.81. Anal. Calcd (found) for $C_{28}H_{28}N_2O_5S_2$: C, 62.67 (62.63); H, 5.26 (5.30); N, 5.22 (5.04).

TRENTAM, Protected (21). Tris(2-aminoethyl)amine (TREN, 0.344 g, 2.35 mmol) was dissolved in CH_2Cl_2 (20 mL). **18** (3.81 g, 7.10 mmol) was dissolved in CH_2Cl_2 (100 mL), and the solution was added to the TREN solution. The product mixture was washed with 1 M KOH in brine (40 mL), dried and condensed. The residue was applied to a silica column (2 × 20 cm) and eluted with ethyl acetate to remove the starting material. The product was then eluted with acetone. The product fractions were evaporated to dryness to give a yellowish foam (2.97 g, 90.3%): ^{1}H NMR δ 2.37 (t, $J = 6.8$, 6H, CH_2), 3.22 (q, br, 6H, CH_2), 3.24 (s, 9H, CH_3), 3.43 (t, br, 6H, CH_2), 3.55 (q, br, 6H, CH_2), 5.08 (s, 6H, $ArCH_2$), 5.09 (s, 6H $ArCH_2$), 7.27−7.41 (m, 30H, ArH), 7.68 (d, $J = 8.3$, 3H, ArH), 7.73 (t, br, 3H, NH), 7.79 (d, $J = 8.3$, 3H, ArH), 8.08 (t, br, 3H, NH); ^{13}C NMR δ 30.88, 37.58, 39.60, 52.88, 58.55, 70.71, 76.77, 126.04, 126.33, 128.47, 128.58, 128.65, 128.68, 128.76, 128.83, 130.64, 130.85, 135.76, 135.82, 150.34, 150.40, 164.31, 164.48. Anal. Calcd (found) for $C_{81}H_{87}N_7O_{15}$: C, 69.56 (69.23); H, 6.27 (6.39); N, 7.01 (6.87).

TRENTAM, HCl Salt (9). 21 (2.968 g, 2.122 mmol) was dissolved in glacial acetic acid (10 mL) and concentrated HCl (10 mL) and stirred under N_2 for 2 days. The solution was evaporated to dryness and coevaporated with MeOH (2 × 15 mL). The residue was redissolved in MeOH (10 mL) and precipitated from rapidly stirring acetone (100 mL). The ligand was isolated by filtration as a light tan powder (1.463 g, 77.1%): ^{1}H NMR δ 3.26 (s, 9H, CH_3), 3.39 (m, 6H, CH_2), 3.47 (m, 6H, CH_2), 3.52 (m, 6H, CH_2), 3.76 (m, 6H, CH_2), 7.37 (s, 6H, ArH), 8.97 (t, br, 3H, NH), 9.19 (t, br, 3H, NH); ^{13}C NMR δ 34.67, 38.91, 54.26, 57.54, 70.35, 116.29, 116.95, 117.37, 118.00, 139.37, 148.26, 148.71, 169.99. Anal. Calcd (found) for $C_{39}H_{51}N_7O_{15}\cdot$HCl: C, 52.38 (52.07); H, 5.86 (5.99); N, 10.96 (10.66).

Preparation of TRENHOPOTAM$_2$ (8). Method 1. TRENHOPO Diamine (11). Tris(2-aminoethyl)amine (TREN, 0.521 g, 3.56 mmol) was dissolved in CH_2Cl_2 (25 mL) in a round-bottom flask. HOPO thiaz (**10**) (1.28 g, 3.56 mmol) was dissolved in CH_2Cl_2 (75 mL), and the solution was added by slow dropwise addition to the TREN solution. TLC of the product solution (15% MeOH in CH_2Cl_2 on silica pacified with 10% triethylamine in CH_2Cl_2) shows 2-mercaptothiazoline ($R_f = 0.8$), TRENHOPO$_2$ monoamine ($R_f = 0.3$), and TRENHOPO diamine (baseline). The solution was condensed and used immediately in the next reaction without separating the multiple products.

TRENHOPO(TAMthiaz)$_2$ (12). 11 was dissolved in CH_2Cl_2 (125 mL) and added dropwise to a solution of BnTAM thiaz (**17**) (12 g, 21 mmol) in CH_2Cl_2 (25 mL). The solution was filtered, washed with brine (40 mL) and 1 M KOH in brine (2 × 40 mL), dried, and condensed. The residue was applied to a silica column (2 × 25 cm) and eluted with CH_2Cl_2 to remove the excess TAM thiaz. The multiple products were eluted with 5% MeOH in CH_2Cl_2, and the impure fractions were separated on a second column. The product fractions were combined and condensed to yield a dark yellow foam (1.68 g, 35.8% from TREN): ^{1}H NMR δ 2.33 (m, 6H, CH_2), 2.93 (t, $J = 7.3$, 4H, CH_2), 3.16 (m, 6H, CH_2), 3.54 (s, 3H, CH_3), 4.37 (t, $J = 7.3$, 4H, CH_2), 5.06 (s, 4H, CH_2), 5.08 (s,

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4H, CH_2), 5.33 (s, 2H, CH_2), 6.57 (d, J = 7.2, 1H, ArH), 7.05 (d, J = 7.2, 1H, ArH), 7.18 (d, J = 8.2, 2H, ArH), 7.26−7.40 (m, 25H, ArH), 7.70 (t, br, 2H, NH), 7.73 (d, J = 8.2, 2H, ArH), 7.88 (t, br, 1H, NH); ^{13}C NMR δ 28.64, 37.43, 37.62, 52.62, 53.41, 55.53, 74.67, 76.87, 104.58, 124.32, 126.39, 127.92, 128.33, 128.56, 128.69, 128.73, 128.76, 128.80, 128.89, 130.53, 130.58, 132.20, 133.15, 135.84, 136.31, 136.99, 146.25, 149.26, 149.94, 159.42, 163.26, 164.33, 166.82, 201.25. Anal. Calcd (found) for $C_{70}H_{67}N_7O_{11}S_4 \cdot 0.5\ H_2O$: C, 63.71 (64.00); H, 5.19 (5.12); N, 7.43 (7.04).

TRENHOPOTAM$_2$, Protected (13). 12 (1.68 g, 1.28 mmol) was dissolved in CH_2Cl_2 (50 mL), and 2-methoxyethylamine (1.0 g, 13 mmol) was added under N_2. After TLC indicated complete reaction, the product solution was washed with 1 M KOH in brine (2 × 40 mL), dried, and condensed. The residue was applied to a silica column (2 × 25 cm) and eluted with 5% MeOH in CH_2Cl_2. The product fractions were combined and evaporated to dryness to yield a light-colored foam (0.8537 g, 19.6% from TREN): ^{1}H NMR δ 2.32 (m, 6H, CH_2), 3.15 (m, 6H, CH_2), 3.23 (s, 6H, OCH_3), 3.42 (m, 4H, CH_2), 3.52 (s, 3H, NCH_3), 3.55 (m, 4H, CH_2), 5.06 (s, 4H, CH_2), 5.11 (s, 4H, CH_2), 5.27 (s, 2H, CH_2), 6.47 (d, J = 7.2, 1H, ArH), 6.99 (d, J = 7.2, 1H, ArH), 7.24−7.42 (m, 25H, ArH), 7.64 (t, br, 2H, NH), 7.68 (d, J = 8.4, 2H, ArH), 7.80 (d, J = 8.4, 2H, ArH), 7.86 (t, br, 1H, NH), 8.07 (t, br, 2H, NH); ^{13}C NMR δ 37.24, 37.45, 37.55, 39.59, 52.45, 52.68, 58.54, 70.71, 74.62, 77.11, 104.49, 125.89, 126.29, 128.45, 128.54, 128.63, 128.71, 128.75, 128.85, 130.45, 131.23, 132.23, 135.86, 135.92, 136.29, 146.15, 150.33, 150.36, 159.32, 163.29, 164.36, 164.58. Anal. Calcd (found) for $C_{70}H_{75}N_7O_{13}$: C, 68.78 (68.77); H, 6.18 (6.20); N, 8.02 (8.03).

TRENHOPOTAM$_2$, HCl Salt (8). 13 (0.854 g, 0.699 mmol) was dissolved in glacial acetic acid (5 mL) and concentrated HCl (5 mL), and the solution was stirred overnight under N_2. The solution was evaporated to dryness and coevaporated with MeOH (4 × 4 mL). The residue was redissolved in MeOH (2 mL) and precipitated from rapidly stirring ether (50 mL). The product was collected by filtration as a light beige powder (0.415 g, 14.1% from TREN): ^{1}H NMR (DMSO-d_6) δ 3.26 (s, 6H, OCH_3), 3.37 (m, 6H, CH_2), 3.44 (s, 3H, NCH_3), 3.48 (m, 12H, CH_2), 3.74 (m, 6H, CH_2), 6.43 (d, J = 8.3, 1H, ArH), 7.10 (d, J = 8.3, 1H, ArH), 7.37 (s, 4H, ArH), 8.71 (t, br, 1H, NH), 8.98 (t, br, 2H, NH), 9.19 (t, br, 2H, NH), 10.26 (s, br, 1H, OH), 12.23 (s, br, 2H, OH), 12.72 (s, br, 2H, OH); ^{13}C NMR: (CD_3OD) δ 14.06, 34.39, 35.21, 37.08, 38.91, 53.92, 53.96, 57.84, 65.68, 70.33, 116.65, 117.03, 117.06, 118.19, 148.03, 148.46, 164.13, 167.89, 168.61, 170.25. Anal. Calcd (found) for $C_{35}H_{45}N_7O_{13} \cdot HCl \cdot H_2O$: C, 50.88 (51.00); H, 5.86 (5.88); N, 11.87 (11.82).

Method 2. TRENHOPOTAM$_2$, Protected (13). Tris(2-aminoethyl)amine (TREN, 0.366 g, 2.50 mmol) was dissolved in CH_2Cl_2 (40 mL) in a round-bottom flask. **18** (2.8 g, 5.2 mmol) was dissolved in CH_2Cl_2 (80 mL), and the solution was added dropwise to the TREN solution. The bis-TAM amine intermediate was not isolated. HOPO thiaz (**10**) (0.90 g, 2.5 mmol) was added to the reaction mixture. The product solution was washed with 2 M KOH in brine (2 × 40 mL), dried, and condensed. The resulting mixture of **13**, **16**, and **21** was separated on a silica column (2.5 × 30 cm) eluted with acetone (R_f values: **21**, 0.7; **13**, streak 0.1−0.63; **16**, 0.06). The product **13** was used immediately in the next reaction: ^{1}H NMR δ 2.32 (m, 6H, CH_2), 3.15 (m, 6H, CH_2), 3.23 (s, 6H, OCH_3), 3.42 (m, 4H, CH_2), 3.52 (s, 3H, NCH_3), 3.55 (m, 4H, CH_2), 5.06 (s, 4H, CH_2), 5.11 (s, 4H, CH_2), 5.27 (s, 2H, CH_2), 6.47 (d, J = 7.2, 1H, ArH), 6.99 (d, J = 7.2, 1H, ArH), 7.24−7.42 (m, 25H, ArH), 7.64 (t, br, 2H, NH), 7.68 (d, J = 8.4, 2H, ArH), 7.80 (d, J = 8.4, 2H, ArH), 7.86 (t, br, 1H, NH), 8.07 (t, br, 2H, NH).

TRENHOPOTAM$_2$, HCl Salt (8). 13 was dissolved in a 1:1 mixture of concentrated HCl and glacial acetic acid (20 mL), and the solution was stirred overnight. The product solution was evaporated to dryness and coevaporated with MeOH (4 × 20 mL). The residue was redissolved in methanol (3 mL) and precipitated from rapidly stirring acetone. The product was isolated by filtration as an off-white powder (0.546 g, 27.0% from TREN): ^{1}H NMR (DMSO-d_6) δ 3.26 (s, 6H, OCH_3), 3.37 (m, 6H, CH_2), 3.44 (s, 3H, NCH_3), 3.48 (m, 12H, CH_2), 3.74 (m, 6H, CH_2), 6.43 (d, J = 8.3, 1H, ArH), 7.10 (d, J = 8.3, 1H, ArH), 7.37 (s, 4H, ArH), 8.71 (t, br, 1H, NH), 8.98 (t, br, 2H, NH), 9.19 (t, br, 2H, NH), 10.26 (s, br, 1H, OH), 12.23 (s, br, 2H, OH), 12.72 (s, br, 2H, OH). Anal. Calcd (found) for $C_{35}H_{45}N_7O_{13} \cdot HCl$: C, 52.01 (51.80); H, 5.74 (5.84); N, 12.13 (11.94).

Preparation of TRENHOPO$_2$TAM (7). Method 1. TRENHOPO$_2$ Monoamine (14). Tris(2-aminoethyl)amine (TREN, 0.44 g, 3.01 mmol) was dissolved in dry CH_2Cl_2 (100 mL). HOPO thiaz (**10**) (2.16 g, 5.99 mmol) was dissolved in dry CH_2Cl_2 (200 mL), and the solution was added to the TREN solution dropwise over a period of 15 h. The product mixture was condensed and used immediately in the next reaction.

TRENHOPO$_2$TAM Thiaz, Benzyl Protected (15). 14 was prepared as described above, and the crude product was dissolved in CH_2Cl_2 (80 mL). BnTAM thiaz (**17**) (20 g, 34 mmol) was partially dissolved in CH_2Cl_2 (40 mL). The amine solution was added dropwise to the solution of **17**. When addition was complete, the product solution was washed with 2 M KOH in brine (2 × 40 mL) and dried. 2-Propanol (100 mL) was added to precipitate the excess **17**. The solution was filtered and condensed. The residue was applied to a silica column (2 × 30 cm) and eluted with 3% MeOH/CH_2Cl_2. The product fractions were combined and evaporated to dryness to afford a yellow solid (2.15 g, 65.5% from TREN): ^{1}H NMR (400 MHz) δ 2.25 (m, 6H, CH_2), 2.90 (t, J = 7.3, 2H, CH_2), 3.09 (m, 6H, CH_2), 3.53 (s, 6H, NCH_3), 4.34 (t, J = 7.3, 2H, CH_2), 5.03 (s, 2H, $ArCH_2$), 5.07 (s, 2H, $ArCH_2$), 5.26 (s, 4H, $ArCH_2$), 6.61 (d, J = 7.2, 2H, ArH), 7.05 (d, J = 7.2, 2H, ArH), 7.15 (d, J = 8.2, 1H, ArH), 7.23−7.35 (m, 20H, ArH), 7.64 (t, br, 1H, NH), 7.74 (d, J = 8.2, 1H, ArH), 7.81 (t, br, 2H, NH); ^{13}C NMR (400 MHz) δ 28.69, 37.34, 37.68, 52.10, 52.40, 53.51, 55.57, 74.71, 76.12, 104.65, 124.30, 126.41, 127.97, 128.39, 128.61, 128.71, 127.78, 128.95, 130.64, 132.23, 133.20, 135.88, 136.36, 137.05, 146.34, 149.31, 149.96, 159.51, 163.32, 164.37, 166.85. Anal. Calcd (found) for $C_{59}H_{59}N_7O_{10}S_2$: C, 65.00 (64.97); H, 5.45 (5.56); N, 8.99 (8.79).

TRENHOPO$_2$TAM, Benzyl Protected (16). 15 was dissolved in CH_2Cl_2 (50 mL) in a 100-mL flask. 2-Methoxyethylamine (1.0 g, 13 mmol) was added, and the solution was stirred until the yellow color faded. The product solution was washed with 2 M KOH in brine (2 × 40 mL), dried, and condensed. The residue was applied to a silica column (2 × 20 cm) and eluted with 5% MeOH/CH_2Cl_2. The product fractions were combined and evaporated to dryness to yield a pale yellow foam (2.0 g, 63% from TREN): ^{1}H NMR δ 2.25 (m, 4H, CH_2), 2.31 (m, 2H, CH_2), 3.08 (m, 4H, CH_2), 3.14 (m, 2H, CH_2), 3.23 (s, 3H, OCH_3), 3.42 (t, br, 2H, CH_2), 3.52 (m, 2H, CH_2), 3.55 (s, 6H, NCH_3), 5.06 (s, 2H, $ArCH_2$), 5.13 (s, 2H, $ArCH_2$), 5.28 (s, 4H, $ArCH_2$), 6.59 (d, J = 7.1, 2H, ArH), 7.05 (d, J = 7.1, 2H, ArH), 7.26−7.39 (m, 20H, ArH), 7.57 (t, br, 1H, NH), 7.65 (d, J = 8.3, 1H, ArH), 7.78 (d, J = 8.3, 1H, ArH), 7.80 (t, br, 2H, NH), 8.09 (t, br, 1H, NH); ^{13}C NMR δ 37.16, 37.20, 37.23, 37.29, 37.32, 37.66, 39.64, 52.01, 52.33, 53.46, 58.60, 70.77, 74.75, 77.27, 100.18, 104.65, 125.80, 126.27, 128.51, 128.56, 128.67, 128.71, 128.78, 128.96, 130.31, 130.60, 132.22, 136.04, 136.36, 146.33, 150.32, 150.44, 159.48, 163.33, 164.46, 164.73.

Anal. Calcd (found) for $C_{59}H_{63}N_7O_{11}\cdot 2H_2O$: C, 65.48 (65.55); H, 6.24 (6.24); N, 9.06 (9.26).

TRENHOPO$_2$TAM (7). 16 (2.00 g, 1.91 mmol) was dissolved in concentrated HCl (5 mL) and glacial acetic acid (5 mL) and stirred under N_2 for 2 days. The solution was evaporated to dryness and coevaporated with methanol (3 × 5 mL). The residue was dissolved in methanol (4 mL) and added dropwise to rapidly stirring acetone (150 mL). A fluffy, off-white precipitate (the HCl salt of the product) formed and was collected by filtration and dried under vacuum (1.208 g, 52.3% from TREN): ^{1}H NMR (DMSO-d_6) δ 3.26 (s, 3H, CH_3), 3.44 (s, 6H, NCH_3), 3.47 (m, 8H, CH_2), 3.73 (m, 8H, $NHCH_2$), 6.46 (d, J = 7.3, 2H, ArH), 7.11 (d, J = 7.3, 2H ArH), 7.37 (d. J = 8.7, 2H, ArH), 7.40 (d, J = 8.7, 1H, ArH), 8.76 (t, br, 2H, NH), 8.99 (t, br, 1H NH), 9.25 (t, br, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 34.44, 39.24, 51.94, 58.35, 70.45, 98.46, 103.14, 116.43, 116.66, 117.20, 117.58, 117.76, 128.02, 147.99, 150.33, 158.41, 166.55, 169.02, 169.58. Anal. Calcd (found) for $C_{31}H_{39}N_7O_{11}\cdot HCl\cdot 2.5H_2O$: C, 48.53 (48.56); H, 5.91 (6.18); N, 12.78 (12.77).

Method 2. TRENHOPO$_2$TAM, Protected (16). Tris(2-aminoethyl)amine (TREN, 0.146 g, 1.00 mmol) was dissolved in CH_2Cl_2 (40 mL) in a round-bottom flask. **18** (0.54 g, 1.0 mmol) was dissolved in CH_2Cl_2 (80 mL), and the solution was added dropwise to the TREN solution. HOPO thiaz (**10**) (0.90 g, 2.5 mmol) was then added to the reaction mixture. The product solution was washed with 2 M KOH in brine (2 × 40 mL), dried, and condensed. The crude product was purified on a silica column (2.5 × 30 cm) eluted with acetone. The product fractions were combined and evaporated to yield a white foam (0.255 g, 23.6%): ^{1}H NMR δ 2.25 (m, 4H, CH_2), 2.31 (m, 2H, CH_2), 3.08 (m, 4H, CH_2), 3.14 (m, 2H, CH_2), 3.23 (s, 3H, OCH_3), 3.42 (t, br, 2H, CH_2), 3.52 (m, 2H, CH_2), 3.55 (s, 6H, NCH_3), 5.06 (s, 2H, $ArCH_2$), 5.13 (s, 2H, $ArCH_2$), 5.28 (s, 4H, $ArCH_2$), 6.59 (d, J = 7.1, 2H, ArH), 7.05 (d, J = 7.1, 2H, ArH), 7.26−7.39 (m, 20H, ArH), 7.57 (t, br, 1H, NH), 7.65 (d, J = 8.3, 1H, ArH), 7.78 (d, J = 8.3, 1H, ArH), 7.80 (t, br, 2H, NH), 8.09 (t, br, 1H, NH).

TRENHOPO$_2$TAM, HCl Salt (7). 16 was dissolved in a 1:1 mixture of concentrated HCl and glacial acetic acid (20 mL), and the solution was stirred overnight. The product solution was evaporated to dryness and coevaporated with MeOH (4 × 20 mL). The residue was redissolved in methanol (3 mL) and precipitated from rapidly stirring acetone. The product was isolated by filtration as an off-white powder (0.156 g, 20.3% from TREN): ^{1}H NMR (CD_3OD) δ 3.26 (s, 6H, OCH_3), 3.37 (m, 6H, CH_2), 3.44 (s, 3H, NCH_3), 3.48 (m, 12H, CH_2), 3.74 (m, 6H, CH_2), 6.43 (d, J = 8.3, 1H, ArH), 7.10 (d, J = 8.3, 1H, ArH), 7.37 (s, 4H, ArH).

Metal Complexes, General Procedure. A weighed portion of the ligand was dissolved in MeOH (10 mL) and degassed by applying a slight vacuum. KOH (0.5 M solution in MeOH) was added to the ligand solution with a glass syringe, and the solution was degassed again. Fe(acac)$_3$ or Ga(acac)$_3$ was dissolved in MeOH (4 mL) and added to the ligand solution. The solution was degassed again and stirred for at least 1 h. The product solution was condensed to 2−4 mL and added dropwise to 20−50 mL of ether. The precipitated metal complex was isolated by centrifugation. After the supernatant was decanted, the solid was resuspended in 5 mL of ether and collected by filtration.

Fe−TRENHOPO$_2$TAM. The complex was prepared as described above using **7** (0.0391 g, 0.05 mmol), KOH (0.10 mL of a 0.5 M solution in MeOH, 0.05 mmol), and Fe(acac)$_3$ (0.018 g, 0.05 mmol). Yield: 0.0341 g (92.4%). Anal. Calcd (found) for $KC_{31}H_{35}N_7O_{11}Fe\cdot 4H_2O$: C, 43.87 (43.42); H, 5.11 (4.94); N, 11.55 (11.37). ESI-MS: m/z 737.2 (M^-).

Fe−TRENHOPOTAM$_2$. The complex was prepared as described above using **8** (82.6 mg, 0.100 mmol), KOH (0.6 mL of a 0.5 M solution in MeOH, 0.3 mmol), and Fe(acac)$_3$ (35.3 mg, 0.100 mmol). Yield: 65 mg, 66.8%. Anal. Calcd (found) for $K_2C_{35}H_{40}N_7O_{13}Fe\cdot 4H_2O$: C, 43.21 (43.42); H, 4.97 (4.90); N, 10.08 (10.18). ESI-MS: m/z 862.2 ($M^{2-}\cdot K^+$), 845.2 ($M^{2-}\cdot Na^+$), 823.2 ($M^{2-}\cdot H^+$), 411.1 (M^{2-}).

Fe−TRENTAM. The complex was prepared as described above using **9** (0.150 g, 0.168 mmol), KOH (1.0 mL of a 0.5 M solution in MeOH, 0.5 mmol), and Fe(acac)$_3$ (0.059 g, 0.17 mmol). Yield: 0.174 g, 91.7%. Anal. Calcd (found) for $K_3C_{39}H_{45}N_7O_{15}Fe\cdot 6H_2O$: C, 41.34 (41.14); H, 5.07 (4.81); N, 8.65 (8.51). ESI-MS: m/z 947.3 ($M^{3-}\cdot K^+\cdot H^+$), 473.1 ($M^{3-}\cdot K^+$), 454.2 ($M^{3-}\cdot H^+$).

Ga−TRENHOPO$_2$TAM. The complex was prepared as described above using **7** (99.7 mg, 0.130 mmol), KOH (0.52 mL of a 0.5 M solution in MeOH, 0.26 mmol), and Ga(acac)$_3$ (47.7 mg, 0.130 mmol). Yield: 81.7 mg, 70%. ^{1}H NMR (CD_3OD): δ 2.47 (m, 4H, CH_2), 3.30 (m, 4H, CH_2), 3.39 (s, 3H, CH_3), 3.52 (m, 4H, CH_2), 3.60 (m, 4H, CH_2), 3.77 (s, 3H, CH_3), 6.93 (d, J = 7, 2H, ArH), 7.00 (d, J = 7, 2H, ArH), 7.08 (d, J = 8.5, 1H, ArH), 7.12 (d, J = 8.5, 1H, ArH), 10.35 (t, br, 2H, NH), 10.45 (t, br, 1H, NH), 10.82 (t, br, 1H, NH). ^{13}C NMR (CD_3OD): δ 37.15, 54.13, 57.66, 70.98, 110.07, 113.90, 113.97, 116.10, 116.37, 116.42, 116.61, 121.29, 153.08, 153.09, 155.76, 155.81, 162.78, 162.79, 165.53, 165.63, 169.02.

Ga−TRENHOPOTAM$_2$. The complex was prepared as described above using **8** (85.7 mg, 0.104 mmol), KOH (0.6 mL of a 0.5 M solution in MeOH, 0.3 mmol), and Ga(acac)$_3$ (38.5 mg, 0.105 mmol). Yield: 96 mg, 95%. ^{1}H NMR (CD_3OD + D_2O): δ 2.45 (s, br, 6H, CH_2), 3.31 (m, 4H, CH_2), 3.36 (s, 6H, CH_3), 3.50 (m, 6H, CH_2), 3.63 (m, 4H, CH_2), 3.75 (s, 3H, CH_3), 6.87 (d, J = 7.5, 1H, ArH), 6.95 (d, J = 7.5, 1H, ArH), 7.06 (d, J = 8.5, 2H, ArH), 7.08 (d, J = 8.5, 2H, ArH), 11.07 (t, br, 2H, NH). ^{13}C NMR (CD_3OD + D_2O): δ 38.60, 48.49, 57.75, 70.93, 109.62, 113.59, 115.67, 116.08, 156.55, 160.00, 169.51, 169.79. Anal. Calcd (found) for $K_2C_{35}H_{40}N_7O_{13}Ga\cdot 3H_2O$: C, 43.40 (43.34); H, 4.79 (4.98); N, 10.12 (10.42).

Ga−TRENTAM. The complex was prepared as described above using 9 (91.9 mg, 0.103 mmol), KOH (0.8 mL of a 0.5 M solution in MeOH, 0.4 mmol), and Ga(acac)$_3$ (38.0 mg, 0.104 mmol). Yield: 106 mg, 92.2%. ^{1}H NMR (CD_3OD): δ 2.35 (s, br, 6H, CH_2), 3.19 (s, 9H, CH_3), 3.20 (m, 6H, CH_2), 3.49 (m, 12H, CH_2), 6.89 (d, J = 9, 3H, ArH), 6.92 (d, J = 9, 3H, ArH), 11.27 (t, br, 3H, NH). ^{13}C NMR (CD_3OD): δ 13.99, 38.28, 48.39, 57.43, 65.46, 70.84, 115.50, 115.65, 157.29, 158.25, 169.77, 170.16. Anal. Calcd (found) for $K_3C_{39}H_{45}N_7O_{15}Ga\cdot 4H_2O$: C, 42.17 (42.31); H, 4.81 (4.48); N, 8.83 (8.65).

X-ray Crystallography. TRENHOPOTAM$_2$·HCl crystallizes in the monoclinic space group $P2_1/c$, grown as colorless prisms from methanol diffused with diethyl ether. Potassium Ga−TRENHOPOTAM$_2$ also crystallizes in $P2_1/c$, grown as pale yellow plates from a DMF solution diffused with diethyl ether.

Selected crystals of each compound were mounted in Paratone N oil on quartz capillaries and frozen in place under a cold N_2 stream (110−150 K, maintained throughout data collection). The crystallographic data sets were collected on a Siemens SMART X-ray diffractometer equipped with a CCD area detector using Mo Kα radiation (λ = 0.710 72 Å, graphite monochromator). An arbitrary hemisphere of data was collected for each crystal using ω scans of 0.3°/CCD area detector frame and a total measuring time of 25 s for Ga−TRENHOPOTAM$_2$ and 35 s for TRENHOPOTAM$_2$. The intensity data to a maximum 2θ range of ∼50° (specific 2θ different for each crystal) were integrated using SAINT

Table 3. $TRENHOPOTAM_2$ and $Ga[TRENHOPOTAM_2]$ Crystal Data

param	$TRENHOPOTAM_2$·HCl	$Ga[TRENHOPOTAM_2]$
fw	826.26	1059.73
temp, K	124	147
max 2θ, deg	49.44	50
cryst system	monoclinic	monoclinic
space group	$P2_1/c$	$P2_1/c$
unit cell dimens		
a, Å	16.0340(17)	16.3379(14)
α, deg	90	90
b, Å	17.0609(18)	15.2722(13)
β, deg	113.837(2)	91.656(2)
c, Å	16.0695(17)	19.4397(17)
γ, deg	90	90
V, Å^3	4020.91(84)	4848.48(94)
Z	4	4
calcd density, g cm^{-3}	1.36	1.45
cryst size, mm	0.45 × 0.20 × 0.15	0.40 × 0.30 × 0.15
abs coeff, μ, mm^{-1}	0.166	0.81
reflcns collcd	17 635	21 206
indep reflcns	6622	8148
data-to-param ratio	12.6	12.9
goodness-of-fit on F^2	1.167	1.067
final R indices ($I > 2\sigma(I)$)		
R1	0.0760	0.0700
wR2	0.1959	0.1913
R indices (all data)		
R1	0.1490	0.1184
wR2	0.2212	0.2245
largest diff peak and hole, e$^-$/Å^3	0.82 and −0.31	1.3 and −1.04

with box size parameters of 1.6 × 1.6 × 0.6.[26] The data were corrected for Lorentz and polarization effects. An empirical absorption correction for each crystal was based on the measurement of redundant and equivalent reflections using an ellipsoid model for the absorption surface and was applied using SADABS.[27] Equivalent reflections were merged, and the space groups were determined using XPREP[28] on the basis of lattice symmetry and systematic absences.

The structures were solved by direct methods (SHELXS-86).[29] After most of the atoms had been located, the data set was refined using the SHELXTL-97 software package.[28] All nondisordered nonhydrogen atoms were refined anisotropically. Unless otherwise noted, hydrogen atoms were assigned to idealized positions. In the structure of $TRENHOPOTAM_2$ HCl, the phenol, amide, and capping amine protons were found in a Fourier difference map before being included in idealized positions. Additional experimental details for the two structures are summarized in Table 3.

Solution Thermodynamics. General Methods. The titration apparatus has been described in detail by O'Sulllivan et al.[6,7,30] Corning high-performance combination glass electrodes, whose response to [H^+] was calibrated before each titration,[31] were used in concert with either an Accumet pH meter or a Metrohm Titrino to measure the pH of the experimental solutions. Metrohm autoburets (Dosimat or Titrino) were used for incremental addition of acid or base standard solutions to the titration cell. UV−visible spectra for incremental titrations were recorded on a Hewlett-Packard 8452a spectrophotometer (diode array). UV−visible spectra for batch titrations were recorded on a Varian Cary 300 Scan UV−visible spectrophotometer. The titration instruments were fully automated and controlled using LabView software.[32] Titrations were performed in 0.1 M KCl supporting electrolyte under positive Ar gas pressure. The temperature of the experimental solution was maintained at 25 °C by an external circulating water bath. Solid reagents were weighed on a Metrohm analytical balance accurate to 0.05 mg. Milli-Q purified water was used to prepare all solutions and was degassed prior to use by simultaneously boiling and purging with Ar gas. Standard solutions of 0.1 M HCl and 0.1 M KOH were prepared from JT Baker DILUT-IT ampules using freshly degassed Milli-Q purified water. Precise acid concentration was determined by titration of a sodium tetraborate solution to the Methyl Red end point. Precise base concentration was determined by titration of a potassium hydrogen phthalate solution to the phenolphthalein endpoint. Stock solutions of EDTA were obtained by dissolving disodium EDTA (Fisher) in freshly degassed Milli-Q water. Precise ligand concentration was determined by performing potentiometric titrations of the ligand solution (pH 3.5 to 8.0 and back to 3.5) in triplicate and solving for total moles of ligand using the program Hyperquad,[17] by calculating the buffering capacity of the solution based on published protonation constants.[19] Stock solutions of ferric ion were obtained by dissolving solid $FeCl_3$·$6H_2O$ in standardized 0.1 M HCl. The actual ferric ion concentration was determined by titration with a standardized EDTA solution to the Variamine Blue end point.[33]

Incremental Titrations. Initial titration solutions were assembled from the constituent reagents in ratios determined previously by modeling using estimated formation constants and the modeling program Hyss.[34,35] In the case of the EDTA competition titrations, a trial titration solution was assembled and systematically varied in pH to confirm the appropriate conditions. The solutions were incrementally perturbed by the addition of either acid (HCl) or base (KOH) titrant, followed by a time delay for equilibration (90 s for protonation studies; 2 h for EDTA competition titrations). All titrations were conducted in pairs: first a forward titration from low to high pH (or vice versa) and then a reverse titration back to the starting pH. The data for the two titrations comprising each experiment were pooled for calculation of formation constants. All absorbance measurements used for calculation of formation constants were less than 1.05 absorbance units.

Batch Titrations. Bulk titration solutions were assembled from the constituent reagents as described above. Aliquots of the bulk solution were removed with a volumetric pipet and stored in plastic centrifuge tubes. The pH of each aliquot was adjusted individually using acid (HCl) or base (KOH) titrant. The aliquots were allowed to equilibrate at 25 °C for at least 48 h, at which time the final pH was determined, and a UV−visible spectrum was taken of each aliquot. All absorbance measurements used for calculation of formation constants were less than 1.05 absorbance units.

Protonation Constants. The protonation constants of $TRENHOPO_2TAM$, $TRENHOPOTAM_2$, and TRENTAM were determined by potentiometric titration (no spectral data collected). Solutions were assembled from a weighed portion of ligand and the supporting electrolyte solution, with resulting ligand concentrations between 0.2 and 0.5 mM. The solutions were titrated from pH 4.0 to 9.5 and back to 4.0. An average of 60−90 data points

(26) *SAINT, SAX Area-Detector Integration Program*, 4.024 ed.; Siemens Industrial Automation, Inc.: Madison, WI, 1994.
(27) *SMART Area-Detector Software Package*; Siemens Industrial Automation, Inc.: Madison, WI, 1994.
(28) *SHELXTL, Crystal Structure Analysis Determination Package*, 5.10 ed.; Siemens Industrial Automation, Inc.: Madison, WI, 1997.
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Table 4. Protonation Constants of TREN-Based Ligands

ligand	TRENHOPO$_2$TAM	TRENHOPOTAM$_2$	TRENTAM
$-\log K_{a_1}$	5.06(1)	5.11(1)	5.17(1)
$-\log K_{a_2}$	5.79(1)	5.86(1)	6.00(1)
$-\log K_{a_3}$	6.97(1)	6.99(1)	6.98(1)
$-\log K_{a_4}$	8.07(1)	8.10(1)	8.03(1)
replicates	3	4	3
global σ	0.044	0.060	0.061
largest corr	0.64	0.66	0.65

were collected in each pair of titrations (forward and back), each data point consisting of a volume increment and a pH reading, and the titrations were repeated at least three times to provide at least 180 data points for final refinement of the ligand protonation constants. Refinement of the protonation constants was accomplished using the program Hyperquad,[17] which allows simultaneous refinement of the data from multiple titration curves. Determination of the first protonation constant for the bidentate terephthalamide units ($\log \beta_{011} \approx 11.1$) was not attempted. For the purpose of refining the other protonation constants, the value of $\log \beta_{011}$ was held constant at 11.1. For TRENHOPOTAM$_2$, containing two terephthalamide units, $\log \beta_{012}$ was also held constant at 22.2, and for TRENTAM (3 terephthalamides), $\log \beta_{013}$ was also held constant at 33.3. Global σ values between 0.028 and 0.075 were obtained for the refinement of the ligand protonation constants, and the largest correlation between two protonation constants for any ligand was 0.8 (most were 0.6 or less). The protonation constants were refined in two ways: as association constants (corresponding to stepwise association of protons with a fully deprotonated ligand) and as dissociation constants (corresponding to stepwise dissociation of protons from a fully protonated ligand). Virtually identical $-\log K_a$ values were obtained using the two methods, and the refined values are summarized in Table 4. In the course of the refinement, the molar amount of ligand used in each titration was refined. In every case, the average molecular weight calculated from these molar amounts corresponded closely with that determined by elemental analysis.

Iron Complexation Constants Determined by EDTA Competition Titrations. Each EDTA competition experiment consisted of a pair of titrations: forward vs KOH and reverse vs HCl. At the lower pH end of the titration, the colorless iron−EDTA complex dominated, but as the pH was raised, the solution turned pink to red, corresponding to the gradual formation of the iron−ligand complex. The following concentration ranges were used for iron, ligand, and EDTA in the competition titrations: Fe, 0.05−0.15 mM; ligand, 0.06−0.16 mM; EDTA, 0.6−1.0 mM. In every case, the EDTA and ligand were present in at least 5% excess over the iron to prevent formation of insoluble iron species. In each experiment, an average of 24 points were collected over a period of 48 h (~2 h equilibration time/point). Each data point consisted of a pH measurement and an absorbance spectrum over at least 80 different wavelengths between 400 and 650 nm. The data were imported into the refinement program pHab[36] and analyzed by nonlinear least-squares refinement. The previously determined ligand protonation constants were included as constants, as were the literature values for the iron complex formation and protonation constants of EDTA.[19]

(36) Gans, P.; Sabatini, A.; Vacca, A. *Ann. Chim. (Rome)* **1999**, *89*, 45−49.

TRENTAM EDTA Competitions. The data from three incremental EDTA competition titrations and one batch titration over the pH range 4.1−5.9 were used to determine the Fe[TRENTAM] complex formation constants. As the pH was raised, the charge-transfer bands for an Fe(TAM)$_3$ complex could be seen growing in at 450 and 506 nm (broad shoulder). During refinement of the data from these experiments, factor analysis of the collected spectra indicated the presence of two absorbing species over the pH range of the experiment. These absorbing species were modeled as the FeL^{3-} and $FeLH^{2-}$ species. The refined formation constants for the incremental and batch titrations were very similar and were combined to give final values of 45.2(2) for $\log \beta_{110}$ and 50.9(2) for $\log \beta_{111}$.

TRENHOPOTAM$_2$ EDTA Competitions. Three forward-and-back incremental EDTA competition titrations and a single batch titration were performed over the pH range 3.5−5.2. As the pH was raised, the charge-transfer bands for the ligand-iron complex could be seen growing in at 420 nm (shoulder of a band below 400 nm) and at 526 nm. During refinement, factor analysis of the collected spectra indicated the presence of two absorbing species in the wavelength range under investigation. The absorbing species were modeled as FeL^{2-} and $FeLH^{-}$ species. Refinement of the data with this model gave values of $\log \beta_{110} = 41.0(5)$ and $\log \beta_{111} = 45.4(6)$.

TRENHOPO$_2$TAM EDTA Competitions. Three forward-and-back incremental EDTA competition titrations and a single batch titration were performed over the pH range 2.9−5.5. As the pH was raised, the charge-transfer bands for the ligand−iron complex could be seen growing in at 412 nm and at 528 nm. During refinement, factor analysis of the collected spectra indicated the presence of two absorbing species in the wavelength range under investigation. The absorbing species were modeled as the FeL^{-} and FeLH species. Refinement of the data with this model gave values of $\log \beta_{110} = 34.53(20)$ and $\log \beta_{111} = 38.65(25)$.

TRENTAM FeL ⇌ FeLH Potentiometric Titration. The UV−visible spectra of Fe−TRENTAM and monoprotonated Fe−TRENTAM are very similar. To confirm the value of the ferric complex protonation constant obtained from the EDTA competition data, a separate potentiometric titration of the iron complex was performed at an iron complex concentration of 0.25 mM. The solution was titrated forward-and-back over the pH range 5.5−7.5. The collected data were refined in Hyperquad with the total FeL concentration held constant. A value for the complex protonation constant of 5.75(20) was obtained. This is within error of the value of 5.6(3) obtained by refinement of the EDTA competition titration data.

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