Synthesis of Homochiral Tris(2-alkyl-2-aminoethyl)amine Derivatives from Chiral α-Amino Aldehydes and Their Application in the Synthesis of Water Soluble Chelators

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A novel synthesis of 3-fold symmetric, homochiral tris(2-alkyl-2-aminoethyl)amine (TREN) derivatives is presented. The synthesis is general in scope, starting from readily prepared chiral α-amino aldehydes. The optical purity of the *N*-BOC protected derivatives of tris(2-methyl-2-aminoethyl)amine and tris(2-hydroxymethyl-2-aminoethyl)-amine has been ascertained by polarimetry and chiral NMR chemical shift experiments. An X-ray diffraction study of the L-alanine derivative (tris(2-methyl-2-aminoethyl)amine•3 HCl, L-Ala_3-TREN) is presented: crystals grown from ether diffusion into methanol are cubic, space group $P2_13$ with unit cell dimensions $a = 11.4807(2)$ Å, $V = 1513.23(4)$ Å^3, and $Z = 4$. Attachment of the triserine derived backbone tris(2-hydroxymethyl-2-aminoethyl)amine (L-Ser_3-TREN) to three 3-hydroxy-1-methyl-2(1*H*)-pyridinonate (3,2-HOPO) moieties, followed by complexation with Gd(III) gives the complex Gd(L-Ser_3-TREN-Me-3,2-HOPO)$(H_2O)_2$, which is more water soluble than the parent Gd(TREN-Me-3,2-HOPO)$(H_2O)_2$ and a promising candidate for magnetic resonance imaging (MRI) applications. Crystals of the chiral ferric complex Fe(L-Ser_3-TREN-Me-3,2-HOPO) grown from ether/methanol are orthorhombic, space group $P2_12_12_1$, with unit cell dimensions $a = 13.6290(2)$ Å, $b = 18.6117(3)$ Å, $c = 30.6789(3)$ Å, $V = 7782.0(2)$ Å^3, and $Z = 8$. The solution conformation of the ferric complex has been investigated by circular dichroism spectroscopy. The coordination chemistry of this new ligand and its iron(III) and gadolinium(III) complexes has been studied by potentiometric and spectrophotometric methods. Compared to the protonation constants of previously studied polydentate 3,2-HOPO-4-carboxamide ligands, the sum of protonation constants (log β_{014}) of L-Ser_3-TREN-Me-3,2-HOPO (24.78) is more acidic by 1.13 log units than the parent TREN-Me-3,2-HOPO. The formation constants for the iron(III) and gadolinium(III) complexes have been evaluated by spectrophotometric pH titration to be (log *K*) 26.3(1) and 17.2(2), respectively.

Introduction

For a variety of reasons, the synthesis of chiral, C_3-symmetric ligands has recently been an area of active investigation.[1,2] Ligands exhibiting many of the common donor groups have been synthesized, including phosphorus donors,[3] tris(pyrazolyl)-hydro-borates,[4−6] alkoxides,[7,8] tri- and tetraamines[9,10] tri-amides,[11,12] and tripyridines.[13] Additionally, chiral tripodal ligands which do not contain 3-fold symmetry have been prepared, including tris(2-aminoethyl)amine (TREN) derivatives with a single chiral substituent,[14,15] or with three different tripodal "arms."[16]

TREN is among the most widely employed 3-fold symmetric ligands, and many derivatives have been made for use as metal-binding ligands for both transition metals[17] and main group elements.[18] TREN has also been used as a scaffold for the synthesis of many tripodal ligands, particularly those used as models of the siderophore enterobactin or for high stability metal sequestering agents.[19−21] Recently, we reported that TREN-Me-3,2-HOPO forms an extremely stable Gd(III) complex which shows promise as a new magnetic resonance imaging (MRI) contrast agent.[22] While investigating the synthesis of more water soluble derivatives of TREN-Me-3,2-HOPO we have developed

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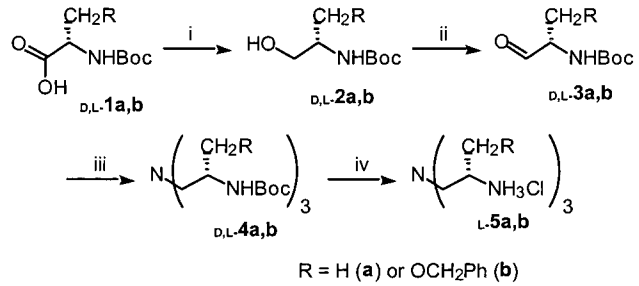
Scheme 1[a]



[a] Reagents and Conditions: (i) BH_3·THF, THF (50−80%); (ii) NaOCl, TEMPO (<1 mol %) (65−80%); (iii) NH_4OAc, $NaBH(OAc)_3$ (55−60%); (iv) HCl, Et_2O (ca. 100%).

a synthesis for the preparation of homochiral TREN tetraamines derived from optically pure amino acids. A preliminary account of this work as it relates to MRI contrast agent development has been communicated.[23]

While derivatives of TREN with chiral substituents at the nitrogen are not expected to exert good stereocontrol in catalytic reactions, since their conformation is flexible, TREN derivatives substituted at either methylene position should be more rigid and hence exert good control.[2] In light of this fact, it is surprising that very few methods have been reported for the synthesis of such derivatives, especially given the large number of derivatives substituted at the primary amine positions.[12,17,18] Two examples of such systems have been reported. The first involves the nucleophilic ring opening of amino acid derived aziridines,[10] and the second is a derivative of alanine which gives rise to a one arm chiral derivative.[14] Both of these syntheses are lengthy and low yielding, and neither can be used to produce *N*-unsubstituted tetraamines. Herein, we report a general route to the synthesis of mono- and tri- subsitituted derivatives that are functionalized at the β-carbon of TREN backbone. These compounds are synthesized from amino acids, thus facilitating access to a wide variety of stereospecifically substituted TREN derivatives. Such compounds are of added importance due to the burgeoning interest in the use of "chiral pool" agents for the synthesis of optically pure materials,[24] rather than extensive enantioselective synthesis. The optical purity of the subsitituted TREN compounds has been confirmed by chiral shift NMR experiments, polarimetry, and X-ray crystallography. We expect that this methodology will find wide use in the preparation of new ligands for chiral metal complexation.

Results and Discussion

Synthesis. The general procedure for the synthesis of β-carbon substituted TREN compounds is outlined in Scheme 1. The homochiral tris(2-methyl)-TREN is prepared from alanine and their derivatives (Scheme 1, R = H). Reaction of commercially available L-alininal (*N*-BOC-alininal) (**L-3a**) with ammonium acetate under reductive amination conditions affords the protected tris(methyl)TREN compound (**L-4a**) in 97% yield. The opposite enantiomer is prepared starting with D-*N*-BOC-alanine (**D-1a**), which is reduced with borane in THF to afford the amino alcohol (**D-2a**) in 58% yield following standard procedures.[25,26] Transformation of the alcohol to the aldehyde (**D-3a**) is carried out by TEMPO catalyzed bleach oxidation[27,28] (rather than the previously reported pyridinium dichromate oxidation procedure)[25] giving an isolated yield of 65%. The biphasic, free radical-catalyzed oxidation works quite well as long as the buffered bleach solution is freshly prepared for each reaction. Vigorous stirring is critical for the highest possible yield. Due to concern over the optical stability of **D-3a**, the product is used immediately in the subsequent reductive amination reaction to afford the desired trimethyl-substituted TREN derivative (**D-4a**) in about 50% yield. Both of the BOC-protected amine derivatives (**D-**, **L-4a**) are isolated as viscous pale oils. Deprotection of the L-enantiomer is achieved using standard acidic treatment,[29] giving the tetraamine (**L-5a**) as the white, powdery trihydrochloride salt in essentially quantitative yield.

Similarly, reduction of D- or L-*N*-BOC-*O*-benzyl-serine (**D-** or **L-1b**, R = OCH_2Ph) to the alcohol (**D-** or **L-2b**) proceeds in 75−80% yield.[25,26] The ^1H and ^{13}C NMR spectra of the product alcohol are consistent with those previously reported.[30,31] Oxidation to the aldehyde (**D-** or **L-3b**) by standard procedures[28] proceeds in about 80% yield for both enantiomers; the observed NMR spectra again are in agreement with previously reported data.[32] Reductive coupling to ammonium acetate occurs in 55−60% yield to give the *N*-BOC protected tris(benzyloxy)TREN derivatives (**D-4b** and **L-4b**). Acidic BOC deprotection[29] of the L-enantiomer proceeds in essentially quantitative yield to afford the tris(benzyloxy)TREN derivative (**L-5b**). Interestingly, the benzyl deprotected derivative of **L-5b** has been previously reported,[33] but its synthesis has not been described. The isopropyl substituted derivatives of compounds **2**, **3**, **4** and **5** (starting with protected L-valine) have been similarly prepared, demonstrating the generality of the procedure [data not shown].

Treatment of **L-4b** with trifluoroacetic acid for several hours removes the BOC protecting groups (Scheme 2). Subsequent neutralization with base affords the neutral tetraamine (**L-5c**) in good yield. Addition of benzyl protected HOPO-thiaz (3-(benzyloxy)-1-methyl-4-[(2-thioxothiazolidin-1-yl)carbonyl]-2(1*H*)-pyridinone) to the tetraamine, which is not isolated, results in slow conversion to the benzyl protected (L-BnSer)$_3$-TREN-Me-3,2-HOPO ligand (**L-6**) in moderate yield. Some epimerization of one of the chiral centers is observed (ca. 10%), but the RRS derivative can be separated by chromatography. All six benzyl groups can be removed from the protected ligand by aqueous acidic treatment over the period of several days, resulting in the free L-Ser$_3$-TREN-Me-3,2-HOPO ligand (**L-7**) in essentially quantitative yield. Metalation of the ligand was carried out by treatment with ferric acetylacetonate in methanol. The iron complex (**L-8**) precipitates as a red-black solid that can be purified by chromatography and is isolated in 90% yield.

Methodology for the synthesis of monosubstituted TREN-Me-3,2-HOPO ligands has also been developed, as outlined in Scheme 3. Treatment of the benzyl protected serinal **L-3b** with 1,7-bis(*tert*-butoxycarbonyl)diethylenetriamine[34] under reductive amination conditions similar to those used in the preparation

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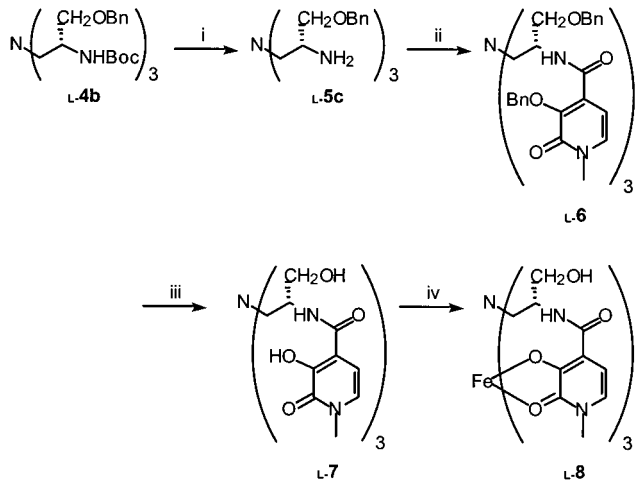
Table 1. Crystal Data and Structure Refinement

	L-5a	L-8
formula	$C_9H_{27}N_4Cl_3$	$FeC_{30}H_{36}N_7O_{12}\cdot 0.5\ C_4H_{10}O\cdot 0.25CH_3OH\cdot 2.75H_2O$
formula weight	297.70	837.13
crystal system	cubic	orthorhombic
space group (#)	$P2_13$ (no. 198)	$P2_12_12_1$ (no. 19)
T (°C)	−111	−135
λ,(Å)	0.71072	0.71072
a (Å)	11.4807(2)	13.6290 (2)
b (Å)		18.6117 (3)
c (Å)		30.6789 (3)
V (A^3)	1513.23(4)	7782.0 (2)
Z	4	8
D_{calc}, g cm^{-1}	1.307	1.429
μ_{calc} (cm^{-1})	5.9	16.64
transmission coeff	0.99−00.89	0.879−0.744
R_1[a]	0.016	0.0470
R_{w2}[b]		0.0509
$wR2$[c]	0.020	

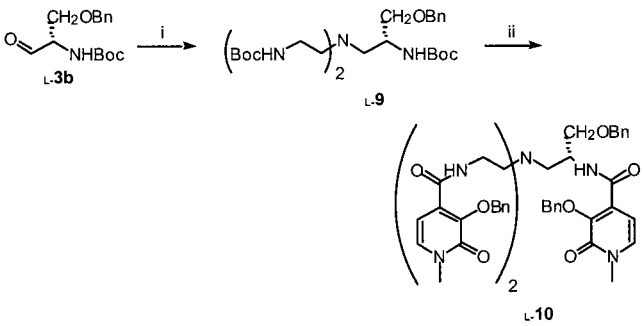
[a] $R_1 = \sum||F_o| - |F_c||/\sum|F_o|$. [b] $R_w = \{\sum[w(|F_o| - |F_c|)^2]/\sum(wF_o^2)\}^{1/2}$. [c] $wR2 = \{\sum[w(F_o^2 - F_c^2)^2]/\sum[w(F_o^2)^2]\}^{1/2}$.

Scheme 2[a]



[a] Reagents and Conditions: (i) (1) TFA, (2) KOH, H_2O (94%); (ii) thiaz-HOPO, CH_2Cl_2 (74%); (iii) HBr/HOAc (99%); (iv) $Fe(acac)_3$, CH_2Cl_2 (89%).

Scheme 3[a]



[a] Reagents and Conditions: (i) $(BOCNHCH_2CH_2)_2NH$, $NaBH(OAc)_3$, THF (ca. 100%); (ii) 1) TFA, CH_2Cl_2; 2) K_2CO_3, Me-3,2-HOPO thiazolide, CH_2Cl_2, (36%).

of the trisubstituted derivatives results in essentially quantitative formation of the monosubstituted product **L-9**. Deprotection of the primary amines with trifluoroacetic acid proceeds to the ammonium salt, which is not isolated but instead treated with 3 equiv of benzyl protected HOPO-thiaz and potassium carbonate. After several days, the benzyl protected ligand **L-10** can be isolated in low yield. Further chemistry of the monosubstituted derivatives, including optimization of the procedures and substitution of the unique alcohol, is currently being explored; although such chemistry has not yet been explored, the synthetic methodology here should also be applicable to the synthesis of bis-substituted TREN ligands, beginning with a mono-*N*-protected ethylenediamine.

X-ray Crystal Structures.[35] To prove the conservation of chirality in the synthesis, an X-ray crystal diffraction study was carried out for the highly crystalline L-alanine tri-hydrochloride (**L-5a**). The salt crystallizes in the cubic space group $P2_13$ with $Z = 4$ (Table 1). The absolute configuration was established by the method of Flack.[36] A structural diagram (ORTEP) of this compound, clearly demonstrating retention of configuration for all three methyl groups, is shown in Figure 1. The homochiral substitution imposes two notable features to the tetraamine structure. First, the three crystallographically identical ethylamine arms are poised in a parallel fashion, creating a predisposed binding pocket for metal binding. The second important feature is that the methyl substituents give the arms of the tetraamine a counterclockwise twist (when viewed down the "binding pocket" toward the apical nitrogen). These features suggest that this ligand will generate a similarly chiral environment around a bound metal ion.

The iron complex of L-Ser_3-TREN-Me-3,2-HOPO (L-**8**) was also examined by single-crystal X-ray diffraction. The complex crystallizes in the orthorhombic space group $P2_12_12_1$ with $Z = 8$ (Table 1). The absolute configuration was established by the method of Flack.[36] A structural diagram (ORTEP) of this compound is shown in Figure 2. The structure clearly shows a Δ coordination environment about the iron atom, with retention of configuration at the three β-carbons of the TREN backbone. The hydrogen bonding present between the amide hydrogen (on N3, N5, and N7) and the hydroxyl oxygens (O2, O6, and O10) is typical of 4-carboxamide-3,2-HOPO ligands,[37] suggesting that the new trihydroxy ligand will retain the high stability seen for TREN-Me-3,2-HOPO with iron and gadolinium. The average

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(35) Crystallographic data (excluding structure factors) for **L-5a** and **L-8** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 121766 and CCDC 121437. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax, +44 1223 336033; e-mail, deposit@ccdc.cam.ac.uk), 1999.

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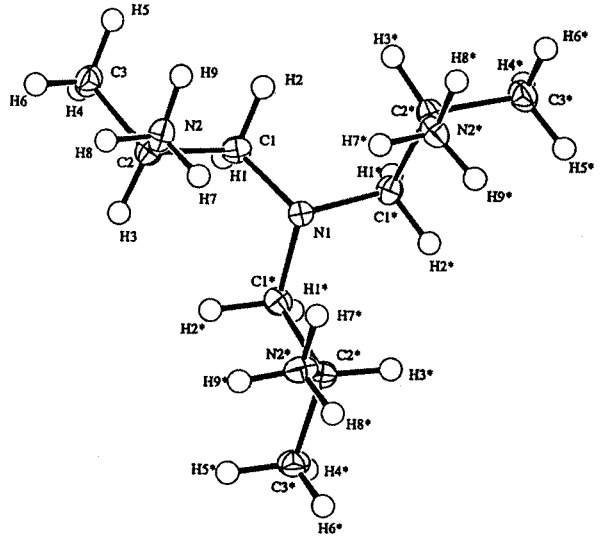

Figure 1. The structure of the chiral TREN derivative L-Ala_3 TREN, **L-5a** (ORTEP, 50% probability ellipsoids). Selected bond lengths (Å): N1−C1, 1.479(2); C1−C2, 1.523(2); C2−C3, 1.525(2); C2−N2, 1.500-(2). Selected bond angles (deg): N1−C1−C2, 114.6(1); C1−C2−C3, 111.2(1); C1−C2−N2, 109.5(1); N2−C2−C3, 107.7(1); C1−N1−C1*, 110.02(9).

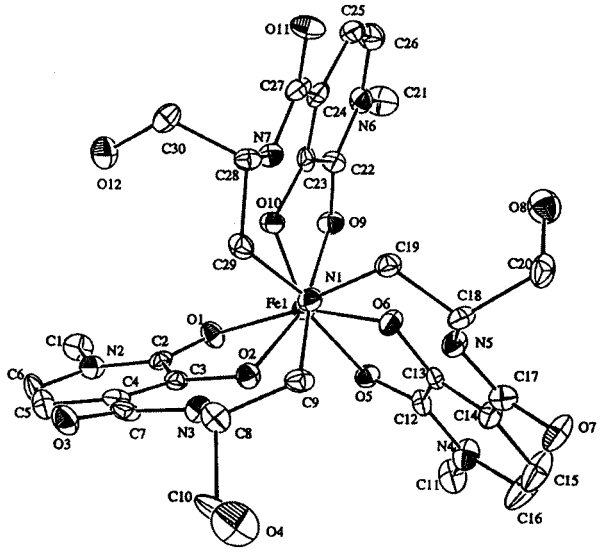

Figure 2. The structure of the ferric complex Fe(L-Ser-TREN-Me-3,2-HOPO), **L-8** (ORTEP, 50% probability ellipsoids). Selected bond lengths (Å): Fe1−O1, 2.022(4); Fe1−O2, 2.041(4); Fe1−O5, 2.034-(4); Fe1−O6, 1.991(4); Fe1−O9, 2.043(4); Fe1−O10, 1.988(4). Selected bond angles (deg): O1−Fe1−O9, 94.0(2); O1−Fe1−O5, 92.2-(2); O5−Fe1−O9, 90.5(1); O2−Fe1−O10, 86.3(1); O2−Fe1−O6, 83.5(1); O6−Fe1−O9, 106.4(2); O1−Fe1−O2, 79.0(1); O5−Fe1−O6, 79.9(2); O9−Fe1−O10, 79.9(1).

Fe−O bond length for the 3-hydroxy oxygen is shorter by 0.03 Å than for the 2-carbonyl oxygen, typical of an iron hydroxypyridinonate complex.[37]

It is interesting to compare the structure of **L-8** with that previously reported for Fe(TREN-Me-3,2-HOPO).[38] That molecule has an essentially identical geometry, except that it exists as a mixture of Δ and Λ isomers in the solid state. The amide hydrogen bonds serve to enhance the rigidity of the backbone in both structures. The pendant hydroxymethyl groups in **L-8** do not significantly alter either the coordination geometry, bond lengths, or complexation behavior (vide infra). We conclude from this similarity that TREN-Me-3,2-HOPO should also serve as a good model for the coordination behavior of **L-7** with gadolinium. Gd(TREN-Me-3,2-HOPO) is eight-coordinate, with two coordinated water molecules, in a distorted bicapped trigonal prismatic geometry.[22] It is anticipated that Gd(**L-7**) has a similar structure in the solid state. Attempts to prepare X-ray quality crystals of this complex have not been successful to date.

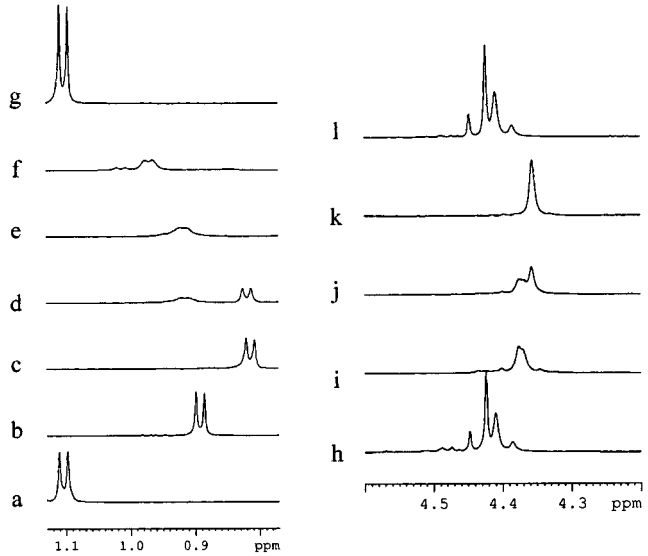

Figure 3. Effect of chiral shift reagents on trialanine derivative (**D-** and **L-4a**) and triserine derivative (**D-** and **L-4b**). See text for details.

Solution Stereochemistry. Chiral shift NMR experiments were performed to demonstrate that the bulk material had the same optical purity as the isolated crystalline material.[39−41] Figures 3a−c show the effect of (*R*)-(−)-2,2,2-Trifluoro-1-(9-anthryl)ethanol on the chemical shift of the methyl group in NMR spectra of **D-4a**. The spectra show that addition of the shift reagent results in a change in the position of the resonance, but no diastereomeric splitting is observed. Addition of 0.10 mmol (3.6 equiv) of shift reagent resulted in a 0.21 ppm upfield shift of the methyl resonance (Figure 3b). Increasing the concentration of the chiral reagent (0.29 mmol, 6.6 equiv) only slightly increases the upfield movment of this resonance a further 0.09 ppm (Figure 3c). The L-isomer displays similar but not identical shifts of the methyl resonance upon addition of the chiral reagent, Figures 3e−g. The shift occurs with a concomitant broadening of the resonance, but again no diastereomeric splitting is observed. Figure 3d shows a spectrum taken after the samples corresponding to Figure 3c and e were mixed.

A similar experiment was carried out with both **D-4b** and **L-4b**, further confirming the retention of chirality in the synthesis of these substituted TREN ligands. Figure 3 shows that addition of 4.3 equiv of *S*-(+)-2,2,2-Trifluoro-1-(9-anthryl)ethanol to either **D-4b** (Figure 3h−i) or **L-4b** (Figure 3k−l) causes an upfield shift in the benzyl methylene resonance. The complex splitting also collapses to a broad singlet. Figure 3j shows a spectrum taken after the samples corresponding to Figure 3i and k were mixed. Both the D- and L-isomers of **4b** were also examined by polarimetery. The D- isomer exhibits a specific optical rotation ($[\alpha]^{20}_{589}$) of +0.111°, while the L- isomer exhibits an optical rotation of −0.117°.

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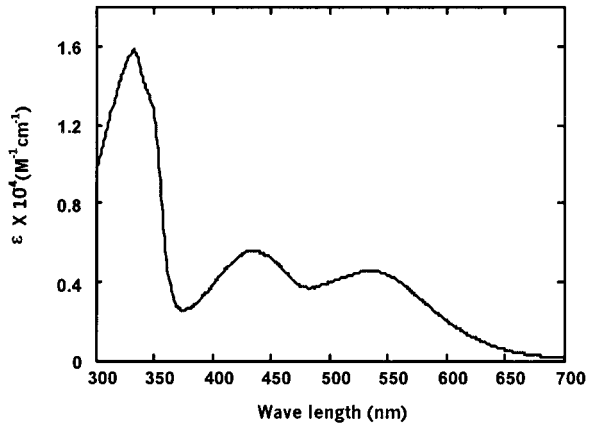

Figure 4. The UV−vis absorption spectrum of Fe(L-Ser$_3$-TREN-Me-3,2-HOPO) in pure methanol.

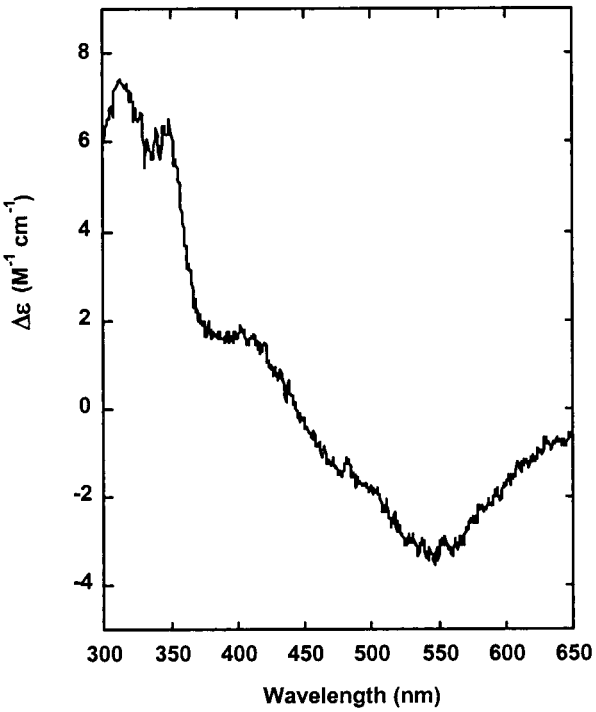

Figure 5. Circular dichroism spectrum of Fe(L-Ser$_3$-TREN-Me-3,2-HOPO) in pure methanol.

The visible absorption spectrum of **L-8** (Figure 4) shows two ligand-to-metal charge transfer (LMCT) transitions centered at 434 nm (ϵ = 5590 M^{-1} cm^{-1}) and at 536 nm (ϵ = 4580 M^{-1} cm^{-1}). The band at 340 nm is presumably due to the chiral TREN scaffold. The energy and intensity of the bands are typical for pseudooctahedral ferric trishydroxypyridinonate complexes. The chirality of the ferric complex was probed by circular dichroism spectroscopy. Figure 5 shows the CD spectrum recorded in pure methanol. Two transitions are observed in the visible region at 434 and 536 nm. These bands arise from LMCT transitions and are therefore sensitive to the chirality at the metal center. The size and magnitude of the LMCT transitions match those reported previously for a Δ coordination geometry of 3,2-HOPO ligands around iron,[37] indicating that a single enantiomer is present in solution.

Protonation Constants. The protonation constants for L-Ser$_3$-TREN-Me-3,2-HOPO were determined by potentiometric titration; the speciation plot is shown in Figure 6, and a comparison to the parent TREN-Me-3,2-HOPO protonation constants is found in Table 2. The average protonation constant,

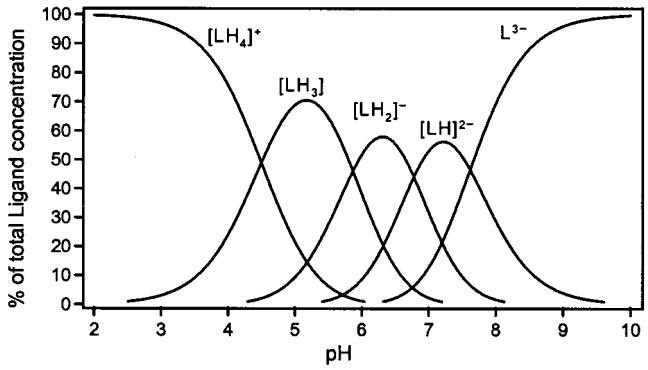

Figure 6. Species distribution for the protonation of L-Ser$_3$TREN-HOPO, [L] = 1 mM, μ = 0.1 M (KCl), T = 25 °C.

Table 2. Formation Constants for L-Ser$_3$-TREN-Me-3,2-HOPO (**L-7**)[a] and TREN-Me-3,2-HOPO[b] with H^+, Gd^{3+}, and Fe^{3+}

ligand	L-7	TREN-Me-3,2-HOPO
log K_1	7.62(3)	8.20(1)
log K_2	6.78(3)	6.95(3)
log K_3	5.87(4)	5.80(3)
log K_4	4.50(10)	4.96(5)
log β_{014}	24.78	25.91
log β_{110}(Gd)	17.2(2)	20.3(2)
log β_{111}	20.7(2)	23.8(1)
log β_{112}	24.5(2)	
pGd	17.7	20.3
log β_{110}(Fe)	26.3(1)	26.8(1)[c]
log β_{111}		30.7(3)[c]
pFe	26.8	26.8[c]

[a] This work. [b] Values from ref 22. [c] Xu, J.; reference 47.

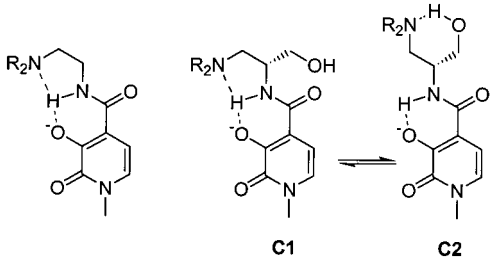

Figure 7. Possible hydrogen-bonding schemes for TREN-Me-3,2-HOPO and L-Ser$_3$-TREN-Me-3,2-HOPO. The R substituents are homostructural to the TREN arm depicted in full in each case. The two conformations for L-Ser$_3$-TREN-Me-3,2-HOPO (C1, C2) are discussed in the text.

is notably more acidic by (0.28 log units) in L-Ser$_3$-TREN-Me-3,2-HOPO than in TREN-Me-3,2-HOPO. The Me-3,2-HOPO moieties in these two hexadentate ligands should have similar acidities; therefore the increased acidity of L-Ser$_3$-TREN-Me-3,2-HOPO may originate from stronger intramolecular hydrogen-bonding afforded by the substituted TREN cap. Conformational restrictions imposed upon the TREN-cap by the hydroxymethyl substituent of L-Ser$_3$-TREN-Me-3,2-HOPO may stabilize hydrogen bonding structures such as shown in Figure 7 with the resulting increase in acidity.

Formation Constants. The formation constant β_{110} of Fe^{3+} or Gd^{3+} with L (L = L-Ser$_3$-TREN-Me-3,2-HOPO) is defined by eq 1.

$$mM^{3+} + lL^{n-} + hH^{+} \leftrightarrow M_mL_lH_h^{3m-nl+h}$$

$$\beta_{mlh} = \frac{[M_mL_lH_h]^{3m-nl+h}}{[M^{3+}]^m[L^{n-}]^l[H^+]^h} \quad (1)$$

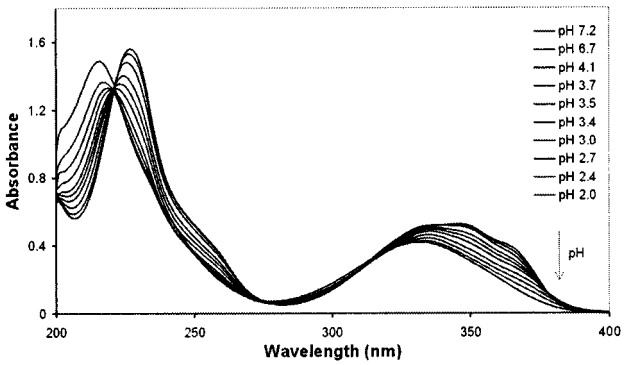

Figure 8. Spectrophotometric titration of Gd^{3+} ($[Gd^{3+}]_T = 0.026$ mM) and [L-Ser_3-TREN-Me-3,2-HOPO] ($[L]_T = 0.026$ mM) as a function of pH, $\mu = 0.1$ M (KCl), T = 25 °C.

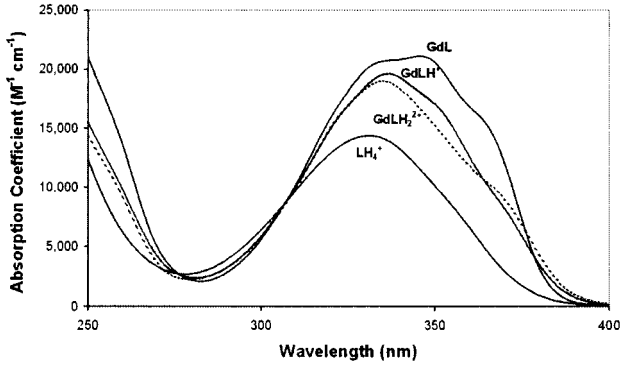

Figure 9. Calculated spectra for the species present in the pH range of 2−7 for Gd[L-Ser_3-TREN-Me-3,2-HOPO].

Gd(L-Ser_3-TREN-Me-3,2-HOPO) was found to dissociate at low pH, allowing for direct evaluation of the Gd(**L-7**) formation and protonation constants. Spectra collected over the pH range of 2−7 (Figure 8) were refined using REFSPEC,[42] a nonlinear least-squares spectrophotometric titration analysis program to provide β_{mlh} values. The calculated spectra for the species present in the titration are shown in Figure 9.

Since Fe(L-Ser_3-TREN-Me-3,2-HOPO) does not dissociate sufficiently at low pH for this equilibrium to be evaluated directly, spectrophotometric competition titrations were employed. The competition equilibrium constant (eq 2) can be used to determine the value of β_{110} using known values for the protonation constants for L-Ser_3-TREN-Me-3,2-HOPO (defining α^{L}) and the protonation constants and Fe^{3+} formation constants for EDTA (defining α^{EDTA} and β', respectively).[43]

$$\mathrm{FeL} + \mathrm{EDTA} \leftrightarrow \mathrm{FeEDTA} + \mathrm{L}$$

$$K_{comp} = \frac{[\mathrm{FeEDTA}]_{tot}[\mathrm{L}]_{tot}}{[\mathrm{FeL}][\mathrm{EDTA}]_{tot}} = \frac{\beta'_{110}\alpha^{\mathrm{FeEDTA}}\alpha^{\mathrm{L}}}{\beta_{110}\alpha^{\mathrm{EDTA}}} \qquad (2)$$

The competition titration was performed over the approximate pH range of 2−7. The exchange rates between Fe^{3+} with L-Ser_3-TREN-Me-3,2-HOPO and EDTA were sufficiently fast that equilibrium of the ferric species was reached without the need for batch titration techniques. A representative titration is shown in Figure 10. β_{110} was refined using REFSPEC.[42]

The Gd(L-Ser_3-TREN-Me-3,2-HOPO) pH dependent UV−vis data were successfully refined with a model containing four

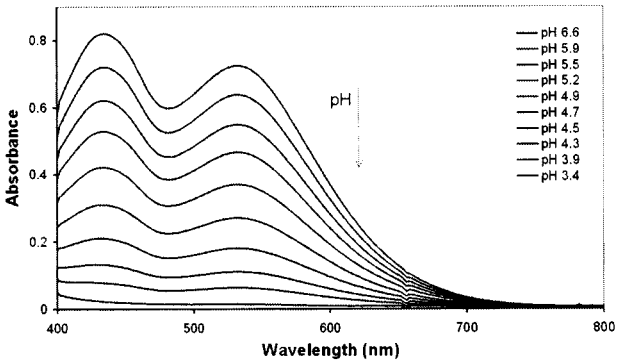

Figure 10. Spectrophotometric competition titration of Fe^{3+} ($[Fe^{3+}]_T = 0.15$mM) and [L-Ser_3-TREN-Me-3,2-HOPO] ($[L]_T = 0.15$ mM) in the presence of a competing ligand, EDTA ($[EDTA]_T = 0.5$ mM) as a function of pH, $\mu = 0.1$ M (KCl), T = 25 °C. Spectrum 1 corresponds to Fe[L-Ser_3-TREN-Me-3,2-HOPO], $\lambda_{max} = 434$ nm ($\epsilon = 5300$ M^{-1} cm^{-1}), $\lambda_{max} = 532$ nm ($\epsilon = 4600$ M^{-1} cm^{-1}).

components: LH_4^+, GdL, $GdLH^+$ and $GdLH_2^{2+}$. This is similar to the solution behavior of other TREN-3,2-HOPO derivatives although the parent complex, Gd(TREN-Me-3,2-HOPO), was not initially reported as possessing a diprotonated species.[22] In fact this species is also formed in the parent system, as has been revealed by recent investigations. These latter studies used a combination of spectral factor analysis,[45,46] comparison of calculated and observed spectral band-shapes, and superior potentiometric calibration procedures[44] to more accurately examine the solution speciation. A close derivative of the parent compound with improved water-solubility has been prepared and thermodynamic analysis of its Gd^{3+} coordination chemistry has established the presence of an equivalent $GdLH_2$ species.[44] The cumulative formation constants (eq 1) for the gadolinium complexes of L-Ser_3-TREN-Me-3,2-HOPO present over the pH range studied are described by β_{110}, β_{111}, and β_{112} (Table 2). The overall formation constant of $\log\beta_{110} = 17.2$ is approximately 3 log units lower than that of the Gd(TREN-Me-3,2-HOPO). This lower stability may be partially a consequence of the increased acidity of the ligand. Other factors including enthalpic and entropic contributions from solvation changes and conformational constraints arising from the substitution on the TREN-cap may contribute to the lower stability of Gd(**L-7**). The Gd(L-Ser_3-TREN-Me-3,2-HOPO) complex was found to protonate twice before dissociating into free Gd(III) and ligand at low pH. The equilibrium constants for the protonation of Gd(**L-7**) can be determined from the differences between log β_{111} and log β_{110} (for the first protonation constant log K_{111}) and between log β_{112} and log β_{111} (for logK_{112}). These values ($\log K_{111} = 3.5$, $\log K_{112} = 3.8$) are essentially the same within the experimental uncertainty, which suggests a cooperative process in which the first protonation step leads to a structural change in the metal−ligand system that leads to immediate subsequent reaction to the diprotonated species. The capping amine is known from crystal structures to be in the "in" conformation for Gd(TREN-Me-3,2-HOPO), which presumably must be in the "out" conformation when protonated. Protonation of the capping amine may lead to disruption of the TREN-cap structure such that a Gd−HOPO ring interaction is destabilized, facilitating a coincident protonation and dissociation behavior.

The formation constant of Fe(L-Ser_3-TREN-Me-3,2-HOPO) ($\log \beta_{110}(Fe) = 25.3$) is similar to that of the parent Fe[TREN-

(42) Turowski, P. N.; Rodgers, S. J.; Scarrow, R. C.; Raymond, K. N. *Inorg. Chem.* **1988**, *27*, 474.
(43) Martell, A. E.; Smith, R. M. *Critical Stability Constants*; Plenum Press: New York, 1974−1981; Vol. 1−5.
(44) Johnson, A. R.; O'Sullivan, B.; Raymond, K. N. *Inorg. Chem.* **2000**, *39*, 2652.
(45) Tauler, R.; Smilde, A.; Kowalski, B. *J. Chemometrics* **1995**, *9*, 31.
(46) Geladi, P.; Smilde, A. *J. Chemometrics* **1995**, *9*, 1.

Me-3,2-HOPO] complex (log β_{110}(Fe) = 26.7).[47] As mentioned above, the crystal structures of Fe(L-Ser_3-TREN-Me-3,2-HOPO) and Fe(TREN-Me-3,2-HOPO) are very similar, and the solution formation constants for these two complexes support the idea that substitution of the TREN backbone has little affect on the nature of iron(III) coordination.

For use in therapeutic procedures, the stability and charge of metal complexes at neutral pH is important. The effectiveness of these ligands as metal chelators at a biologically relevant pH can be evaluated by calculation of the pM values (pM = −log [M] at pH 7.4 where [M] = 1 μM and [L] = 10 μM). For both L-Ser_3-TREN-Me-3,2-HOPO and TREN-Me-3,2-HOPO the pFe values are 26.7, while the pGd values are 18.2 and 20.3, respectively. Thus, at biologically relevant pH, Fe(III) and Gd(III) form remarkably stable neutral complexes with L-Ser_3-TREN-Me-3,2-HOPO.

The gadolinium complex of ligand **L-7** (as prepared in the titration apparatus) is approximately an order of magnitude more soluble than the parent TREN-Me-3,2-HOPO complex, with a solubility on the order of 5−10 mM.[23] While not soluble enough for diagnostic applications, the increase in solubility has enabled more accurate determination of parameters related to MRI efficacy.[46]

Conclusions

Our requirement for highly functionalized tris-(2-aminoethyl)-amine (TREN) derivatives has led to the development of a new synthetic methodology. The key step involves reductive amination of aldehydes, which are prepared in accordance with previously reported literature methods. The procedures we describe are a convenient method to prepare homochiral TREN derivatives with a variety of amino acid derived alkyl substituents. They are general in scope and are unprecedented in that they allow for the preparation of methylene-substituted, *N*-unsubstituted tetraamines. Elaboration of the TREN scaffold by coupling activated carboxylic acid derivatives is straightforward. We have found that the tris-hydroxymethyl substituted L-Ser_3-TREN-Me-3,2-HOPO ligand increases the solubility of the resulting Gd(III) complex and causes some changes to the protonation and formation constants although the overall stability remains within a therapeutically useful range. The improved solubility of the complex brings it much closer to the range required for medical applicability.

Experimental Section

General Considerations. The reagents BH_3·THF (1 M in THF), TEMPO, (*R*)-(−)-2,2,2-Trifluoro-1-(9-anthryl)ethanol, (*S*)-(+)-2,2,2-Trifluoro-1-(9-anthryl)ethanol, and $NaHB(OAc)_3$ were obtained from Aldrich Chemical Co. *N*-BOC-*O*-benzyl-L-serine (**L-1b**), *N*- BOC-*O*-benzyl-D-serine (**D-1b**), and *N*-BOC-L-alininal (**L-3a**) were purchased from Sigma Chemical Co. 3-(Benzyloxy)-1-methyl-4-[(2-thioxothiazolidin-1-yl)carbonyl]2(1*H*)-pyridinone (Me-3,2-HOPO thiazolide) was prepared by previously described methods.[22,48] *N*-BOC-*O*-benzyl-serinol (**D-** or **L-1b**) was synthesized according to the procedure of Kanellis and co-workers[25,26] with minor modification. *N*- BOC-*O*-benzyl-serinal (**L-3b**)[27,28] and 1,7-bis(*tert*-butoxycarbonyl)diethylenetriamine[34] were prepared by standard procedures. THF was freshly distilled from sodium benzophenone-ketyl prior to its use. All air and/or moisture sensitive compounds were manipulated under an atmosphere of either nitrogen or argon using standard high vacuum line, Schlenk, or cannula techniques. 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 Brüker DRX 500, AMX 400, or AMX 300 spectrometers. Melting points were taken on a Büchi melting apparatus and are uncorrected. Polarimetry was carried out using a Perkin-Elmer 210 instrument with a sodium lamp (589 nm). Circular dichroism spectra were recorded using a quartz cell of 1 cm optical path length (Hellma, Suprasil) on a Jasco J500C spectrometer which was equipped with an IF-500 II A/D converter and controlled by a microcomputer.

L-Ala_3-BOC_3-TREN (L-4a). *N*-BOC-L-Alanal (**L-3a**, 1.00 g, 5.77 mmol, 3 equiv), ammonium acetate (0.164 g, 1.92 mmol, 1 equiv), and $NaHB(OAc)_3$ (1.83 g, 8.66 mmol, 4.5 equiv) were placed in a flask under an atmosphere of argon, and THF (20 mL) was added via cannula. The reaction was allowed to stir overnight, at which point it was quenched with 10% HOAc/MeOH. The solvent was removed in vacuo, and the residue was dissolved in methylene chloride (40 mL) and washed with KOH (4%, 2 × 40 mL). The organics were washed with brine (40 mL), dried (Na_2SO_4), and evaporated to dryness leaving a foamy solid (0.915 g, 1.87 mmol, 97%). The material was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.82 (br s, 3H), 3.561 (br s, 3H), 2.38 (m, 3H), 2.15 (m, 3H), 1.33 (s, 27H), 0.99 (d, J = 6.3 Hz, 9H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 156.50, 78.84, 60.48, 44.19, 28.39, 19.29.

L-Ala_3-TREN-trihydrochloride (L-5a). Protected amine **L-4a** (0.915 g, 1.85 mmol) was dissolved in ethyl acetate (40 mL) and sparged with argon. Anhydrous HCl gas was bubbled through the solution for a few minutes until a vigorous gas evolution was observed, and then a white precipitate formed. The reaction was stirred under argon for 45 min, at which point the solid was collected by filtration, washed with ether (3 × 50 mL), and dried under high vacuum, yielding a white powder (0.536 g, 1.81 mmol, 98%). ^{1}H NMR (500 MHz, D_2O): δ = 4.829 (s, 6H), 3.54 (m, 3H), 2.78 (m, 3H), 2.68 (m, 3H), 1.32 (d, J = 6.5 Hz, 9H). Anal. Calcd (Found) for $C_9H_{28}N_4Cl_4$: C, 32.35 (32.87); H, 8.45 (8.95); N, 16.77 (16.97).

***N*-BOC-*O*-benzyl-L-serinal (3b).** Both enantiomers of the aldehyde were synthesized according to the published procedures.[25−28] In each case the syntheses begin with reduction of *N*-BOC-*O*-benzyl-serine (**D-** or **L-1b**) to the corresponding amino-alcohol (**D-** or **L-2b**), which is then selectively oxidized to **D-** or **L-3b**. A pale yellow oil was obtained as product, the observed NMR spectra matched those reported previously.[25,27] The aldehyde (**L-3b**) is used immediately for next step reaction in order to minimize racemization.

L-$BnSer_3$-BOC_3-TREN (L-4b). *N*-BOC-*O*-benzyl-L-serinal (**L-3b**, 3.13 g, 11.2 mmol. 4 equiv), ammonium acetate (0.216 g, 2.80 mmol, 1 equiv), and $NaHB(OAc)_3$ (3.56 g, 16.8 mmol, 6 equiv) were placed in a flask under argon, and THF (100 mL) was added via cannula. After 24 h of stirring, the solvent was removed in vacuo and the resulting oil was dissolved in methylene chloride (50 mL) and KOH (4%, 50 mL). The layers were separated, and the aqueous phase was washed with additional CH_2Cl_2 (50 mL). The combined organic phases were washed with brine (50 mL), dried ($MgSO_4$), and evaporated to give 2.80 g of crude product as a mixture of the desired amine, **L-4b**, and the alcohol, **L-2b**, as a side product arising from direct aldehyde reduction. The mixture was separated by flash chromatography on silica gel ($MeOH/CH_2Cl_2$) yielding a pale oil (1.22 g, 1.51 mmol, 54%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.30 (m, 15H), 5.00 (br s, 3H), 4.43 (m, 6H), 3.69 (m, 3H), 3.49 (m, 3H), 3.31 (m, 3H), 2.70 (m, 3H), 2.49 (m, 3H), 1.43 (s, 18H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 155.2, 137.7, 128.2, 127.5, 127.4, 78.87, 73.08, 69.39, 55.76, 48.45, 28.30. ESI-MS (+) m/z(%): 807(80) $[MH]^+$. Anal. Calcd (Found) for $C_{45}H_{66}N_4O_9$: C, 66.97 (66.66); H, 8.24 (8.39); N, 6.94 (7.09).

D-$BnSer_3$-BOC_3-TREN (D-4b). *N*-BOC-*O*-Bn-D-serinal (**D-3b**, 3.06 g, 10.9 mmol, 3 equiv), ammonium acetate (0.282 g, 3.65 mmol, 1 equiv), and $NaHB(OAc)_3$ (3.10 g, 14.6 mmol, 4 equiv) were dissolved in THF under an atmosphere of argon and stirred for 18 h. The workup and column were analogous to those for **L-4b**, yielding a pale oil (1.78 g, 2.21 mmol, 61%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.3−7.5 (m,

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(48) Xu, J.; Kullgren, B.; Durbin, P. W.; Raymond, K. N. *J. Med. Chem.* **1995**, *38*, 2606.

15H), 5.00 (br s, 3H), 4.4−4.5 (m, 6H), 3.702 (br s, 3H), 3.49 (m, 3H), 3.31 (br s, 3H), 2.71 (m, 3H), 2.50 (br s, 3H), 1.44 (s, 27H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 155.38, 137.97, 128.33, 127.74, 127.61, 79.06, 73.26, 69.60, 55.90, 48.62, 28.40.

L-BnSer$_3$-TREN·3HCl·H$_2$O (L-5b). L-BnSer$_3$−BOC$_3$-TREN (**L-4b**, 1.22 g, 1.51 mmol) was dissolved in trifluoroacetic acid (50 mL) and stirred for 1 h. The acid was removed in vacuo and the resulting oil was partitioned in methylene chloride (50 mL) and water (50 mL). While stirring, the biphasic mixture was adjusted to pH 10 by slow addition of NaOH (20%, 7 mL). The organic layer was separated, and the aqueous layer was washed with additional methylene chloride (2 × 50 mL). The combined organic phases were dried (Na_2SO_4) and filtered to obtain a pale yellow solution. To this solution, anhydrous HCl was bubbled through for about 7 min, at which point a white precipitate formed. The solid was collected by filtration, yielding a tan solid (0.787 g, 1.24 mmol, 82%). The material was recrystalized by ether diffusion into methanol to give a white solid. ^{1}H NMR (500 MHz, D_2O/CD_3OD): δ = 7.3−7.5 (m, 15H), 4.42 (dd, J = 77, 15 Hz, 6H), 3.1−3.3 (m, 9H), 2.77 (m, 3H), 2.55 (m, 3H). ^{13}C NMR (125 MHz, D_2O/CD_3OD): δ = 137.77, 129.82, 129.62, 129.58, 74.28, 66.71, 53,-35, 49.57. Anal. Calcd (Found) for $C_{30}H_{46}N_4O_3Cl_4\cdot H_2O$: C, 56.83 (56.73); H, 7.47 (7.39); N, 8.84 (8.84).

L-BnSer$_3$-TREN (L-5c). The BOC-amine (**L-4b**, 1.43 g, 1.87 mmol) was taken up in a minimum amount of trifluoroacetic acid and the solution was allowed to stir for 1.5 h. After evaporation, the residue was taken up in CH_2Cl_2 and evaporated several times in order to remove excess acid. The salt was redissolved in methylene chloride (100 mL), and water (100 mL) was added. While stirring, the biphasic solution was adjusted to pH 10 by slow addition of 20% KOH (ca. 4 mL). The layers were separated and the aqueous fraction washed with additional methylene chloride (50 mL). The combined organic phases were dried ($MgSO_4$) and evaporated to give a pale yellow oil which was used immediately in the next procedure (847 mg, 1.82 mmol, 97%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.2−7.35 (m, 5H, Ar); 4.45−4.50 (m, 2H, CH_2Ph); 3−3.5 (m, 3H); 2.3−2.4 (m, 2H); 2.9 (br s, 1H, NH).

L-Bn$_3$-BnSer$_3$-TREN-Me-3,2-HOPO (L-6). To a stirring solution of **L-5c** (847 mg, 1.82 mmol) in CH_2Cl_2 (100 mL) was added thiazoide activated 4-carboxy-1-methyl-3-benzyloxy-2(1*H*) pyridinone and DMAP (ca. 10 mg). The mixture was stirred for 10 days, after which all the HOPO starting material had been consumed and TLC was consistent with one major product. The product was isolated by flash chromatography on silica gel (MeOH/CH_2Cl_2) to give **L-6** as a substantially pure product containing ca. 10% impurity (presumably the epimer (***RRS*)-6**). The mixture could be cleanly separated by chromatography on silica gel (1.61 g, 1.36 mmol, 74%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.30 (d, J = 10 Hz, 3H), 7.17−7.41 (m, 30H), 7.05 (d, J = 9 Hz, 3H), 6.74 (d, J = 9 Hz, 3H), 5.36 (d, J = 14 Hz, 3H), 5.28 (d, J = 14 Hz, 3H), 4.20 (m, 6H), 4.00 (m, 3H), 3.55 (s, 9H), 3.29 (d, J = 9 Hz, 3H), 3.12 (d, J = 9 Hz, 3H), 2.75 (m, 3H), 2.22 (3H, d, J = 13 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.4, 159.5, 146.1, 137.7, 135.9, 131.8, 130.8, 129.3, 128.6, 128.2, 127.8, 127.5, 104.8, 74.1, 73.1, 68.2, 54.6, 48.1, 37.6. ESI-MS (+) *m*/*z*(%): 1230(60) [MH^+].

L-Ser$_3$-TREN-Me-3,2-HOPO·HBr (L-7). Benzyl deprotection was carried out by allowing **L-6** (350 mg, 0.295 mmol) to stir for 2 days in a mixture of 48% HBr (10 mL) and glacial acetic acid (10 mL). The volatiles were removed in vacuo and the residue was coevaporated with methanol (3 × 5 mL). The product was isolated by precipitation from methanol with ether as a free flowing light yellow powder (203 mg, 0.279 mmol, 94%). ^{1}H NMR (500 MHz, D_2O): δ = 6.82 (d, J = 9.2 Hz, 3H), 6.13 (d, J = 9.2 Hz, 3H), 4.62 (m, 3H), 3.96 (m, 3H), 3.77 (m, 6H), 3.45 (2, 9H), 3.42 (m, 3H). FAB-MS (+) *m*/z(%): 698(4) [MHD_9+]. Anal. Calcd (Found) for $C_{30}H_{39}N_7O_{12}\cdot$HBr·2 H_2O: C, 44.67 (44.25); H, 5.50 (5.52); N, 12.16 (11.66).

Fe(L-Ser$_3$-TREN-Me-3,2-HOPO) (L-8). To a solution of L-Ser$_3$-TREN-Me-3,2-HOPO (**L-7**, 94 mg, 0.11 mmol) in methanol (20 mL) was added a solution of iron acetylacetonate (35 mg, 0.1 mmol) in methanol (50 mL) while stirring. The mixture was refluxed overnight under nitrogen, during which time the complex deposited as a deep red precipitate. This was collected, dissolved in minimum amount of methylene chloride, loaded on a flash silica column, and eluted with 5% methanol in methylene chloride. The main red fraction, which shows only one spot on TLC plate, was collected and evaporated to dryness, yielding the desired complex as a red-black solid (63 mg, 0.081 mmol, 82%). Anal. Calcd. (Found) for $FeC_{30}H_{36}N_7O_{12}\cdot 2H_2O$: C, 47.38 (47.31); H, 5.30 (5.26); N, 12.89 (12.48).

L-BnSer-BOC$_3$-TREN (L-9). Sodium tris-acetoxyborohydride (8.41 g, 39.68 mmol) was slurried in THF (50 mL) under nitrogen in a Schlenk flask, and aldehyde **L-3b** (6.69 g, 23.9 mmol) in THF (200 mL) was added via cannula. 1,7-bis(*tert*-butoxycarbonyl)diethylenetriamine (6.09 g, 20.1 mmol) in THF (150 mL) was added dropwise over about 2 h and the reaction mixture was stirred overnight. The reaction was quenched with 10% acetic acid in methanol (50 mL) added over a 15 min period, and the solvent was then removed in vacuo. The resulting solid was dissolved in ethyl acetate (250 mL), and the organic phases were washed with 4% KOH (2 × 125 mL) and brine (125 mL) and dried (Na_2SO_4) and evaporated in vacuo to yield a thick oil (11.5 g). The oil was subjected to column chromatography on silica (7 × 12 cm) and eluted with 5−10% methanol in methylene chloride, resulting in a pale yellow oil (9.60 g, 16.9 mmol, 84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.24−7.34 (m, 5H, Ar); 5.38 (br s, 2H, NH); 4.99 (br s, 1H, NH); 4.45 (s, 2H, CH_2Ph); 3.71 (br s, 1H); 3.57 (d, 4H, J = 8.6 Hz, CH_2OBn); 3.45 (dd, 2H, J = 8.9, 3.5 Hz, CH_2OBn); 3.17 (br s, 2H); 3.07 (br s, 2H); 2.51 (br s, 6H, CH_2); 1.428 (s, 9H, BOC); 1.415 (s, 18H, BOC). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.35, 155.92, 137.83, 128.45, 127.89, 127.84, 79.55, 78.95, 73.41, 70.15, 56.66, 54.61, 49.02, 38.59, 28.43, 28.37. FAB-MS (+) *m*/*z* (%): 567.4(100)[MH^+]. Anal. Calcd (Found) for $C_{29}H_{50}N_4O_7$: C, 61.46 (61.06); H, 8.89 (9.04); N, 9.89 (9.63).

L-BnSer-BOC$_3$-TREN-Me-3,2-HOPO (L-10). BOC$_3$BnSerTREN (**L-9**, 0.677 g, 1.19 mmol) was dissolved in methylene chloride (100 mL) and cooled to 0 °C. Trifluoroacetic acid (10 mL, 110 equiv) was added, and the reaction was stirred for 17 h. The solvent was removed in vacuo to give a pale yellow-brown oil, which was dissolved in THF (10 mL). Potassium carbonate (3.02 g, 21.8 mmol, 18 equiv) and Me-3,2-HOPO thiazolide (1.28 g, 3.56 mmol, 3.0 equiv) were added, and the reaction mixture was stirred for 3 days. The solvent was removed in vacuo, the resulting solids were dissolved in water (50 mL) and methylene chloride (50 mL), and the layers were separated. The aqueous layer was washed with methylene chloride (50 mL), and the combined organic phases were dried ($MgSO_4$) and evaporated to dryness. The resulting yellow oil was subjected to column chromatography on silica (50 mL) and eluted with 0−4% methanol/methylene chloride. Fractions were combined resulting in a colorless oil (0.420 g, 0.424 mmol, 36%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.205 (d, J = 7.95, 1H, NH); 7.809 (t, J = 5.43, 2H, NH); 7.07−7.37 (m, 20H, Ph); 7.087 (d, J = 5.5 Hz, 2H); 7.073 (d, J = 5.5 Hz, 1H); 6.717 (d, J = 7.0 Hz, 1H); 6.703 (d, J = 7.0 Hz, 2H); 5.26−5.33 (m, 8H, CH_2Ph); 3.92 (m, 1H); 3.576 (s, 3H, NMe); 3.573 (s, 6H, NMe); 3.37 (m, 1H); 3.21 (m, 1H); 3.09−3.19 (m, 4H); 2.14−2.45 (m, 6H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 163.23, 162.71, 159.54, 159.31, 146.36, 146.23, 137.82, 136.26, 136.07, 132.03, 131.82, 130.94, 130.31, 129.09, 128.91, 128.70, 128.64, 128.55, 128.49, 128.30, 127.81, 127.64, 104.80, 104.73, 74.61, 74.21, 73.14, 68.71, 53.97, 52.83, 48.25, 37.64, 37.61, 37.23. FAB-MS (+) *m*/*z* (%): 990.6(58) [MH^+]. HRMS: calcd, 990.4401, found, 990.4397.

X-ray Crystal Structure of L-Ala$_3$-TREN-trihydrochloride (L-5a).[35] Crystals of the hydrochloride salt were obtained from a methanol solution by vapor diffusion of ether. A crystal of approximate dimensions 0.20 mm × 0.17 mm × 0.08 mm was mounted on a quartz fiber using Paratone N hydrocarbon oil. All measurements were made on a Siemens SMART[49] diffractometer equipped with a CCD area detector with graphite-monochromated Mo Kα radiation. The data were collected at −111 °C using the ω scan technique with a total frame collection time of 20 s. Data were integrated by the program SAINT,[50] and data analysis was performed using the program XPREP.[51] An empirical absorption correction based on comparison of redundant and

(49) *SMART Area Detector Software Package*; Siemens Industrial Automation, Inc.: Madison, WI, 1995.
(50) *SAINT SAX Area Detector Integration Program 4.024*; Siemens Industrial Automation, Inc.: Madison, WI, 1995.
(51) *XPREP SHELXTL, Crystal Structure Determination Package*; Siemens Industrial Automation, Inc.: Madison, WI, 1995.

equivalent reflections was applied using SADABS[52] (ellipsoidal model, T_{max}=0.99, T_{min}=0.89).

The structure was solved by direct methods (SIR 92)[53] using the program teXsan.[54] The absolute configuration was established based on a comparison of F_o and F_c for 953 reflections of which 793 had Friedel mates. After all the atoms were located, the data set was refined using the SHELXTL software package.[51] The structure was refined on F in the cubic space group $P2_13$ (#198) using full-matrix least squares. All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined isotropically. The final cycle of refinement converged to $R_1 = 0.016$ and $wR_2 = 0.020$ for 85 paramaters and 882 reflections.

X-ray structure of Fe(L-Ser$_3$-TREN-Me-3,2-HOPO) (L-8).[35] Dark red plates of **L-9** were obtained by slow diffusion of ether into a wet methanol solution over a period of three months. The initially formed microneedle red complex underwent a slow phase transfer process, finally becoming red-black plate crystals. A crystal of approximate dimensions 0.40 mm × 0.17 mm × 0.12 mm was mounted on a quartz fiber in a droplet of Paratone N hydrocarbon oil. All measurements were made a Siemens SMART[49] diffractometer equipped with a CCD area detector with graphite-monochromated Mo Kα radiation. The data were collected at −135 °C using the ω scan technique with a total frame collection time of 30 s. Data analysis was performed using Siemens XPREP program.[51] No decay correction was applied.

The structure was initially solved using SIR92[53] using the program teXsan.[54] The absolute configuration was established by the comparison of F_o and F_c as mentioned above.[36] The structure was refined on F in the orthorhombic space group $P2_12_12_117$ using full-matrix least squares. All non-hydrogen atoms in the molecule were refined anisotropically. Hydrogen atoms were calculated in fixed positions. The final cycle of refinement converged to $R_1 = 0.0470$ and $R_w = 0.0509$ for 1048 parameters and 9446 reflections.

NMR Chiral Shift Experiments. Ala$_3$-BOC$_3$-TREN: The compound of interest (D- or **L-4a**, 14 mg, 0.029 mmol) was dissolved in $CDCl_3$ and examined by ^{1}H NMR spectroscopy. (*R*)-(−)-2,2,2-Trifluoro-1-(9-anthryl)ethanol (30 mg, 0.10 mmol, 3.6 equiv) was added to the NMR tube, and the solution was reexamined. Additional shift reagent (25 mg, 0.086 mmol, 6.4 equiv total) was added, and the solution was reexamined. The two solutions were then mixed together, and a final spectrum was obtained. **Ser$_3$-BOC$_3$-TREN:** The compound of interest (**D-** or **l-4b**, 10 mg, 0.012 mmol) was dissolved in $CDCl_3$ and examined by ^{1}H NMR spectroscopy. (*S*)-(+)-2,2,2-Trifluoro-1-(9-anthryl)ethanol (15 mg, 0.052 mmol, 4.3 equiv) was added to the NMR tube, and the solution was reexamined. The two solutions were then mixed together, and a final spectrum was obtained.

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

A solution of 0.100 M KCl was prepared from 99.99% KCl (Fisher Scientific) and was used to maintain constant ionic strength during all titrations. Carbonate-free 0.1 M KOH was prepared from Baker Dilut-It analytic concentrated KOH and was standardized against potassium hydrogen phthalate to a phenolphthalein endpoint. Ferric solutions (∼0.100 M in ∼0.1000 M HCl) were prepared from ferric chloride hexahydrate and standardized by EDTA titration with variamine blue B as indicator. Gadolinium(III) solutions (∼0.100 M in ∼0.1000 M HCl) were prepared from gadolinium chloride and standardized by EDTA titration with Xylenol Orange as indicator in sodium acetate buffer. For all titrations, the observed pH was measured as $-\log [H^+]$. The glass electrode was calibrated in hydrogen ion concentration units by titrating 2.000 mL of standardized HCl diluted in 50.0 mL of 0.100 M KCl, with 4.200 mL of standardized KOH. The calibration titration data were analyzed by a nonlinear least-squares program.[55]

Potentiometric pH Titrations. As previously reported,[42] potentiometric titrations were performed using an automated apparatus consisting of a Accumet pH meter (models 925, 825MP or 15), a pH electrode (Orion Ross semi-micro combination, Cole Parmer semi-micro combination or Corning high performance combination electrodes), an autoburet (Metrohm 665 Dosimat or 702 SM Titrino) fitted with a 5 mL piston exchange unit, and a jacketed Ar swept titration cell maintained at 25.0 °C by a Lauda K-2/R or Neslab RTE−111 constant temperature circulating bath. The electronic systems were integrated for automated collection with a IBM PC clone.

Solutions of L-Ser$_3$-TREN-Me-3,2-HOPO (∼1 mM) in 50.00 mL KCl (0.100 M) were titrated from low pH (∼2.4) to high pH (∼11.2), or in the opposite pH direction in four independent determinations. Proton association constants were determined with the aid of a FORTRAN nonlinear least-squares refinement program (BETA 90).[56,57]

Spectrophotometric pH Titrations. As previously reported,[58] spectrophotometric titrations were carried out in a custom-built automatic titration apparatus using a HP 8450A or HP 8452A spectrophotometer using a 1.0 dm UV−vis cuvette and the pH monitoring equipment mentioned above for potentiometric titrations. The formation constants of ferric L-Ser$_3$-TREN-Me-3,2-HOPO were determined by competition with EDTA. Solutions of ferric ion (∼0.15 mM), ligand (∼0.15 mM), and EDTA (∼0.5 mM) were titrated from low to high and high to low pH to ensure equilibrium had been achieved. The formation constants for Gd[L-Ser$_3$-TREN-Me-3,2-HOPO] were determined by direct pH titration using equimolar solutions of Gadolinium and ligand (∼0.03 mM). The spectra (∼45−60), pH values (range, 2.0−7.0), and corresponding volumes were transferred to an IBM PC clone for analysis. Three data sets were used to determine the final average. The model used to fit the titration data and determine the formation constant was refined using the factor analysis and least-squares refinement program REFSPEC.[42]

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Supporting Information Available: Tables of atomic coordinates and equivalent isotropic displacement parameters, bond lengths and angles, anisotropic displacement parameters, hydrogen coordinates, and isotropic displacement parameters for **L-5a** and **L-8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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