Development of a Thorium Complex for Radiotherapeutic Applications

by

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A dissertation submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate Division of the University of California, Berkeley

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Abstract

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Chapter 1. A brief history of the use of ionizing radiation for cancer therapy is presented, as the motivation for modern clinical investigations on alpha therapy and the use and development of bifunctional chelators in targeted medicine. Recent studies on targeted therapy with alpha-emitters, such as $^{223}$Ra and $^{227}$Th, are described. The chemistry of Th(IV) is presented with a survey of important coordination compounds, with special emphasis on those developed by the Raymond group.

Chapter 2. The rational design of a ligand as a suitable bifunctional chelator for $^{227}$Th therapy is described in the context of ligands developed by the Raymond group for actinide sequestration. A novel $\Phi$ ligand topology incorporating macrocyclic and pendant terephthalamide binding groups aims to address the kinetic and thermodynamic requirements of targeted alpha therapy. The syntheses of the model ligands $\Phi(2,2)$moeTAM and $\Phi(3,3)$moeTAM are detailed, from which a derivative of $\Phi(2,2)$moeTAM is developed. Functionalization of a pendant terephthalamide of the model ligand gives the asymmetric bifunctional chelate $\Phi(2,2)$NBuTAM via the strategic use of protecting groups.

Chapter 3. The evaluation of the solution thermodynamic behavior of $\Phi(2,2)$moeTAM is extensively detailed. The protonation constants of the ligand and its affinity for Th(IV) is measured by spectrophotometric titration. The thorium complex was found to have a remarkably high thermodynamic stability, with a formation constant of $10^{54}$, surpassing the stabilities of previously measured complexes. Efforts to elucidate the effects of the $\Phi$ topology on the coordination of Th(IV) using thermodynamic studies of the linear analog 3,4,3-LiMeTAM as well as the shape analyses of the crystal structure of Th[$\Phi(2,2)$moeTAM] are described. The surprising geometry adopted by $\Phi(2,2)$moeTAM in the thorium complex relative to its structure as the free ligand is further investigated with DFT studies.

Chapter 4. While the measurement of the thermodynamic parameters of the thorium complex provides an assessment of its inertness, its formation kinetics are also of paramount importance due to the inherent time sensitivity of radiotherapeutic agents. Difficulties encountered in preliminary kinetic experiments with existing octadentate ligands and $\Phi(2,2)$moeTAM lead to the use of indirect kinetics. Dye-displacement kinetic studies with the $\Phi$ ligands, 3,4,3-LiMeTAM, and the aminocarboxylic acid ligands provide a useful qualitative comparison of the
rates of complexation of these ligands with Th(IV). Φ(2,2)moeTAM is observed to be a much more rapid chelator than the prevailing DTPA and DOTA, and its association (a second-order reaction with a rate constant of \( k_2 = 1.8(1) \times 10^4 \text{ M}^{-1}\text{s}^{-1} \)) can be directly measured using stopped-flow kinetics.

**Chapter 5.** The application of Φ(2,2)moeTAM toward the coordination of the lanthanide analogs cerium and praseodymium is presented. The crystal structure of the Ce(IV) complex with Φ(2,2)moeTAM is isomorphous to that of the Th(IV) complex, prompting solution thermodynamic and electrochemical studies to investigate the stability of the Ce(IV) complex. Spectrophotometric titration indicates an unprecedented formation constant on the order of \( 10^{61} \), and a Nernstian shift of the Ce(III)-(IV) couple of 2.0 V upon complexation is observed by cyclic voltammetry. The strong preference of Φ(2,2)moeTAM for the +4 oxidation state relative to the +3 is further highlighted by the solution thermodynamics of Pr(III).
ACKNOWLEDGEMENTS

As much as I have been looking forward to the end of this journey, it is with more sadness than joy that I reach it. I have thoroughly enjoyed my time at UC Berkeley and in the Bay Area. The Ph.D. program is the most difficult endeavor that I've undertaken, and I have hardly done it alone; there are many people who have helped me along the way and who have made it possible.

I have the deepest gratitude for Professor Ken Raymond, for letting me join his research group and work on this project. He has likened graduate school to a marathon, and the many obstacles and struggles that I've encountered have certainly made it feel as such. When it seemed like my progress had slowed to a glacial speed, Ken showed me encouragement, patience, and guidance. He never stopped pushing me as a scientist, giving me the opportunity to attend conferences and co-author a patent, for example. I've also appreciated the genuine care he has for the non-scientific development and well-being of his students; I hold very dear the times he and Barbara have taken me to the symphony and opened their house, cabin, and sailboat to me.

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I would not be who or where I am without the unfaltering strength and support of my mother. She trusted, loved, and supported me through my graduate career, and where she sacrificed, I had the opportunity to grow.
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CHAPTER 1

TOWARD TARGETED ALPHA-EMITTING THERAPEUTICS WITH BIFUNCTIONAL CHELATORS

Alpha Therapy

In 1934, Curie and Joliot accidentally discovered that the emission of positrons from boron persisted for months following its bombardment with helium nuclei. They proved the formation of a new isotope of nitrogen and effectively discovered artificial radioactivity, which spurred the production and identification of new radioisotopes by particle bombardment. Rutherford reported the existence of alpha and beta particles in 1898, but the application of these particles, which can destroy tissue, toward medical applications was made possible by the artificial production of their emitters. For example, $^{89}$Sr and $^{32}$P were reported in the early 1940s as effective agents for pain relief in leukemia patients with bone metastases. Most radioisotopes are produced in reactors or cyclotrons, and they have thus been central to the development of use of nuclear medicine.

A prominent aspect of nuclear medicine involves applying radioisotopes toward therapy, as they provide promising alternatives or additions to more widely used treatments. Prostate and breast cancer are the leading types of new cancer cases in American men and women, respectively. Over 50% of all patients with breast or prostate cancer eventually develop bone metastases, from which the complications—severe pain, spinal-cord compression, fractures—become primary concerns in managing the patient's quality of life. Among the various treatments for metastatic cancer, systemic administration of calcium-mimicking radioisotopes, such as $^{32}$P, $^{89}$Sr, $^{153}$Sm, $^{186}$Re, and $^{188}$Re, has been shown to be effective with milder side effects than with external beam radiation therapy. Overwhelmingly, beta-emitters have been used in systemic radionuclide therapy, but the therapeutic advantages of alpha particles over beta particles and X-rays have been investigated since the 1960s.

![Figure 1-1](image)

**Figure 1-1.** Comparison of relative biological effectiveness of alpha particles, beta particles, and X-rays, from studies by Barendsen et al. For a given dose of radiation, alpha particles effect the most cell damage.
These early, and subsequent studies, have shown that the shorter range and higher energy of alpha particles relative to beta particles results in greater cytotoxicity.\textsuperscript{9,10} The extent of cytotoxicity is related to the number of double-stranded DNA breaks caused by the radiation, which is in turn related to the linear energy transfer (LET) of the ionizing radiation. LET is defined as the energy deposited by the radiation per unit length (and is commonly in units of keV \(\mu\text{m}^{-1}\)):

\[ LET = \frac{d\varepsilon}{dl} \]

Alpha particles are more charged and 3-4 orders of magnitude more massive than beta particles, which results in the release of a large amount of energy over a short distance. They have a high LET of approximately 100 keV \(\mu\text{m}^{-1}\), which not only results in effective cell-killing but also in a more dose-dependent manner than beta particles.\textsuperscript{4,11}

<table>
<thead>
<tr>
<th>Particle</th>
<th>Energy (MeV)</th>
<th>Range ((\mu\text{m}))</th>
<th>LET (keV (\mu\text{m}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)</td>
<td>4 - 9</td>
<td>20 - 100</td>
<td>80 - 150</td>
</tr>
<tr>
<td>(\beta)</td>
<td>0.1 - 1</td>
<td>400 - 5000</td>
<td>0.1 - 3</td>
</tr>
</tbody>
</table>

However, clinical investigations of alpha therapeutics are fairly recent. To date, the only FDA-approved alpha-emitter is $^{223}\text{RaCl}_2$, a calcium mimic effective in the treatment of bone metastases.\textsuperscript{12} Coupling the short range of alpha particles with the precise targeting of a biomolecule, such as an antibody, could offer radiotherapeutics with both the advantages of versatility and specificity. Much of the current research in alpha therapy is directed toward using...
a variety of alpha emitters, e.g. $^{225}$Ac, $^{211}$At, $^{227}$Th, $^{213}$Bi, in conjunction with a ligand covalently attached to a biomolecule.

**Requirements for Bifunctional Chelators**

The concept of targeted therapy was introduced more than a century ago by Paul Ehrlich, who envisioned a selectivity made possible by the development of hybridoma technology for the preparation of monoclonal antibodies (mAb). Nine mAbs have been approved by the FDA for cancer therapy, and there are over 100 in clinical development. While monoclonal antibodies are the targeting moieties of choice for cell-surface receptors due to their high affinity and specificity, other potential targeting moieties are antibody fragments and peptides.

This approach has created a demand for suitable chelators, termed bifunctional because of their attachment to both the components responsible for the toxic ionization (the radioisotope) and targeting. Requirements for the ideal BFC include:

1. The formation of the radioisotope complex with high thermodynamic stability.
2. The formation of the radioisotope complex with high kinetic inertness under physiological conditions.
3. The rapid formation of the radioisotope complex, as radioactive drug is inherently time-sensitive.
4. The attachment of the BFC to the biomolecule without unwanted modifications.
5. The complex with the radioisotope should form under mild conditions, to avoid the denaturation of the mAb.
6. The BFC must be stable against radiolytic decomposition.

The rapid and irreversible formation of thermodynamically stable complexes with the radioisotope under physiological conditions is paramount in order to avoid the accumulation of the radioisotope in non-target organs. The first BFCs were derivatives of aminocarboxylic acids such as ethylene diamine tetraacetic acid (EDTA), diethylene triamine pentaacetic acid (DTPA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). The first FDA-approved radiolabeled BFC is a derivative of EDTA (tiuxetan) with the trade name Zevalin™, which is covalently linked to the anti-CD20 Ab ibritumomab. This BFC can be labeled with $^{111}$In for SPECT imaging, or with $^{90}$Y for treatment of non-Hodgkin’s lymphoma.
While these chelators have been available for several decades and still dominate current published studies, their effectiveness is in several respects quite limited, as they do not have both high thermodynamic stability and fast association. In general, macrocycles such as DOTA have both slow off and on rates, while acyclic ligands like DTPA display faster formation kinetics but form complexes of lower stability. For example, DOTA has a greater stability with lanthanides than DTPA by about two orders of magnitude, but the latter displays faster kinetics by four orders of magnitude.\textsuperscript{18}

Table 1-2. Selected FDA-approved target-specific radiopharmaceuticals using BFCs.

<table>
<thead>
<tr>
<th>Radiopharmaceutical</th>
<th>Trade name</th>
<th>Main uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{111}$In - Capromab penetide</td>
<td>ProtaScint®</td>
<td>Imaging of prostate cancer</td>
</tr>
<tr>
<td>$^{111}$In - pentetreotide</td>
<td>Octreoscan®</td>
<td>Imaging of neuroendocrine tumors</td>
</tr>
<tr>
<td>$^{111}$In - satumomab Pendetide</td>
<td>OncoScint®</td>
<td>Imaging of metastatic disease associated with colorectal and ovarian cancer</td>
</tr>
<tr>
<td>$^{99m}$Tc – Apcitide</td>
<td>AcuTect®</td>
<td>Synthetic peptide for imaging deep vein thrombosis</td>
</tr>
<tr>
<td>$^{99m}$Tc – Arcitumomab</td>
<td>CEA-Scan®</td>
<td>Monoclonal antibody for imaging colorectal cancer</td>
</tr>
<tr>
<td>$^{99m}$Tc – Depreotide</td>
<td>Neotect®</td>
<td>For imaging somatostatin receptor-positive tumors</td>
</tr>
<tr>
<td>$^{131}$I – Tositumomab</td>
<td>Bexxar®</td>
<td>For treatment of Non-Hodgkin's Lymphoma</td>
</tr>
</tbody>
</table>

Figure 1-4. Scheme of acyclic bifunctional chelator Zevalin\textsuperscript{TM}.
Thorium-227 Radiotherapeutic Agents

Algeta ASA, the producer of the commercialized $^{223}$RaCl$_2$, has pointed out the potential of $^{227}$Th, a beta decay product of $^{227}$Ac. This alpha-emitter decays to $^{223}$Ra with an 18.7 d half-life, a convenient time scale for manufacturing and shipping (Fig. 1-8). However, since Th(IV) lacks the inherent calcium-mimicking properties of Ra(II) and is insoluble as aqueous hydrolysis products, its chemistry requires its sequestration in a stable complex. If then attached to a targeting agent such as a monoclonal antibody, various $^{227}$Th therapeutic agents could be envisioned. The discovery and evaluation of appropriate BFCs are limiting and time-consuming steps in the development of radiotherapeutics, and the possibility of targeting different receptors.
by the mere choice of antibody is appealing. This ultimately provides more versatility to the $^{227}$Th radiotherapeutic, as one Th-chelate complex could be used to target different types of cancer.

The $^{227}$Th radiotherapeutic currently under investigation is the BFC consisting of a DOTA-derivative by Dahle et al. While in vivo trials have shown that the alpha-emitter is effective, the BFC is not optimal due to the slow association kinetics of DOTA evident with $^{64}$Cu$^{2+}$, $^{67/68}$Ga$^{3+}$, $^{44/47}$Sc$^{3+}$, $^{111}$In$^{3+}$, $^{177}$Lu$^{3+}$, $^{86/90}$Y$^{3+}$, $^{213}$Bi$^{3+}$, $^{212}$Pb$^{3+}$, $^{225}$Ac$^{3+}$ for other radiopharmaceuticals. Complexation of these metal ions require elevated temperatures (60-100 °C for up to 2 hrs), which is detrimental to the biomolecular targeting moiety. This problem limits the synthesis of the radiopharmaceutical to performing the bioconjugation after the complexation or using a vast excess of the DOTA derivative.
Thorium is the most naturally abundant radioactive element, but has not been studied as extensively as uranium and plutonium due to its more limited use as nuclear fuel and the lesser safety concern from its lower radioactivity. In nature it is found as the alpha-emitter $^{232}\text{Th}$ with a half-life of $1.4 \times 10^{10}$ years. $^{23}$ Its coordination chemistry has been investigated in the contexts of separation, such as the THOREX process involving phosphate complexes, $^{24}$ radioactive waste treatment, and decorporation. $^{25}$ It exhibits mainly the +4 oxidation state—exclusively so in water—and has thus been associated with hard cations with similar ionic radii (such as the +3 rare earths and the +4 transition metals) as well as with U(IV) and Pu(IV) as an actinide analog. $^{26}$

Since Th(IV) is the largest +4 cation, its coordination number is at least 6, $^{27}$ and has been observed to be as high as 15 with the small B-H donors of the $N,N$-dimethylaminodiboranate complex. $^{28}$ Thorium complexes have been reported with organic ligands with neutral oxygen donors, $^{29}$ neutral nitrogen donors, $^{30}$ and charged donors such as Schiff bases. $^{31}$ Thorium tetrakis(acetylacetonate) (Fig. 1-9a), an important thorium coordination compound and commonly used starting material, was first synthesized in 1898. $^{32}$ It can be sublimed, and subsequent structural characterization revealed an inner coordination environment of square

Figure 1-8. Decay scheme of $^{235}\text{U}$ family. $^{22}$

**Th(IV) Coordination Chemistry**

Thorium is the most naturally abundant radioactive element, but has not been studied as extensively as uranium and plutonium due to its more limited use as nuclear fuel and the lesser safety concern from its lower radioactivity. In nature it is found as the alpha-emitter $^{232}\text{Th}$ with a half-life of $1.4 \times 10^{10}$ years. $^{23}$ Its coordination chemistry has been investigated in the contexts of separation, such as the THOREX process involving phosphate complexes, $^{24}$ radioactive waste treatment, and decorporation. $^{25}$ It exhibits mainly the +4 oxidation state—exclusively so in water—and has thus been associated with hard cations with similar ionic radii (such as the +3 rare earths and the +4 transition metals) as well as with U(IV) and Pu(IV) as an actinide analog. $^{26}$

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antiprismatic geometry. Thorocene can be prepared by reaction of ThCl₄ or ThF₄ with K₂(COT)₂ or Mg(COT), and was found to be isomorphous to uranocene.³³ Photoelectron spectroscopy was used to probe the covalency of the bonding in thorocene, in which the interaction between the COT π and Th 6d orbitals was shown to be important.³⁴ Cyclopentadienyl complexes have also been reported with thorium, first in 1962 for Th(Cp)₄,³⁵ and subsequently as dihalides and permethylated Cp dihalides.³⁶ The first thorium phosphine complexes to be isolated were with bis(dimethylphosphino)ethane (dmpe): ThMe₄(dmpe)₂, from which Th(OPh)₄(dmpe)₂ and Th(CH₂Ph)₄(dmpe) (Fig. 1-9b) can be prepared, and Th(CH₂Ph)₄(dmpe) reacts with MeLi to give ThMe(CH₂Ph)₃(dmpe).³⁷

![Diagram of crystal structures of Th(acac)₄ and Th(CH₂Ph)₄(dmpe).](image)

**Figure 1-9.** Diagrams of crystal structures of (a) Th(acac)₄ and (b) Th(CH₂Ph)₄(dmpe).

The siderophore-inspired ligands developed by Raymond and coworkers have been used to complex actinides for applications in nuclear waste remediation and *in vivo* decorporation.²⁵ The Th(IV) complexes with catecholates,³⁸ hydroxypyridinonates,⁴⁰ alkyl hydroxamates,³⁹ terephthalates,⁴¹ and aminocarboxylates⁴² have been structurally characterized (Fig. 1-10). Complexes with catecholate-type ligands, where both donor oxygens on a bidentate moiety are negatively charged, are mainly eight-coordinate due to ligand-ligand repulsion. Ligands of lower charge, such as the hydroxypyridinonate, can form nine-coordinate complexes (Fig. 1-11).
Figure 1-10. ORTEP diagrams of eight-coordinate Th(IV) complexes and schemes of their protonated ligands. (a) [Th(catecholato)₄]⁴⁻, 38 (b) Th(1-hydroxy-2-pyridinonato)₄, 39 (c) Th(N-alkylalkanehydroxamato)₄, 40a (d) [Th(ethylterephthalato)₄]⁴⁻, 41 and (e) Th(edta). 42
Considerable effort has been dedicated to describing the solid-state coordination geometries of such structures in order understand their effects on the stabilities of the complexes and design more effective chelators. The positions of the donor oxygens around the thorium ion in tetrakis(catecholato)thorate form a geometry that is very close to the ideal trigonal-faced dodecahedron, while those in tetrakis(terephthalato)thorate lie in a geometry between bicapped trigonal prismatic and trigonal dodecahedral. Despite this distortion, the terephthalamide binding group has a very high binding affinity for Th(IV), and solution thermodynamic experiments provide a quantitative measure of complex stabilities. This latter approach forms the basis of the octadentate ligand development for the effective chelation of Th(IV) for the application of the $^{227}$Th complex as an alpha-emitting radiotherapeutic agent.

**Figure 1-11.** ORTEP diagrams of the nine-coordinate structure of Th(3-hydroxy-2-pyridinonato)$_4$·H$_2$O$^{40b}$ (a) View of one molecule. (b) Simplified structure of the linear coordination polymer formed in the solid state, with donor atoms overlaid onto idealized monocapped square antiprisms.
REFERENCES


CHAPTER 2
MACROCYCLIC LIGANDS DESIGNED FOR THE CHELATION OF Th(IV)

Ligand design
The siderophore-inspired ligands developed in the Raymond group have been used to complex actinides for applications in nuclear waste remediation and in vivo decorporation. While the design of a bifunctional chelator has different aims, the principles of metal-ligand stability and complexation kinetics are the same. Some of the binding groups used in these siderophore mimics are shown in Fig. 2-1; they are bidentate, with oxygen donors, and bind strongly to hard Lewis acids. These ligands are thus suitable for the coordination of $f$-block elements, which typically behave as hard Lewis acids.

The rational design of an ideal ligand for a radiotherapeutic thorium complex involved the consideration of the many requirements for an ideal bifunctional chelator (cf. Chap. 1). The terephthalamide (TAM) binding group was chosen for its high binding affinity for Th(IV), for the overall negative charge it imparts on the complex (neutral thorium complexes, such as those formed with HOPOs, have poor aqueous solubility), and because its two amide groups enable its incorporation in a novel ligand topology. The ligand $L^1$ (Fig. 2-2a) was intended to combine the favorable thermodynamics of macrocycles with the favorable kinetics of linear ligands. It features four binding units (at least eight atoms are required to fill the coordination sphere of Th(IV)): two TAMs in a macrocycle and two TAMs on pendant arms, connected by tris(2-aminoethyl)amine (tren) backbones. The multiple juxtapositional fixedness that is responsible for the formation of kinetically and thermodynamically inert complexes by macrocycles relative to their linear analogs is also responsible for their slow association kinetics. The aim was for the macrocyclic TAMs to confer thermodynamic stability
beyond that gained from the chelate effect, while the pendant TAMs would provide the flexibility and end groups necessary for fast chelation of the metal ion. Additionally, the pendant TAMs provide opportunities for further functionalization, e.g. for linking to a targeting moiety. In L¹ and L² they were functionalized with methoxyethyl groups to increase the water solubility of the untargeted ligand, while in L³, one of the pendant arms was instead functionalized with an amine, for covalent attachment to a targeting biomolecule. This chapter will detail the syntheses of Φ ligands (so named due to the resemblance of the overall shape to the Greek letter Φ) L¹ and its analogs L² and L³ (Fig. 2-2).

---

**Figure 2-2.** Chemical structures of Φ ligands synthesized. Octadentate, terephthalamide ligands with a topology incorporating a macrocycle with pendant binding units. a) Φ(2,2)moeTAM (L¹). b) Φ(3,3)moeTAM (L²). c) Φ(2,2)NBuTAM (L³).
Even though the synthesis of TAM from catechol is published, it is included here for completeness. It is worth noting that there are several methods to obtain the thiazolide 2-4. The first route followed, involving the conversion of the benzyl-protected terephthalic acid to the acid chloride prior to reaction with 2-mercaptotiazolide, was low-yielding, albeit only attempted once. The direct reaction of benzyl-protected terephthalic acid with 2-mercaptotiazolide in the presence of a coupling reagent is the preferred method, since it resulted in and increased yield and avoided the use of the water-sensitive and corrosive oxalyl chloride.

**Scheme 2-1. Synthesis of terephthalamide binding units.**

H.D. = high-dilution

**Synthesis of Φ(2,2)moeTAM (L)**

Even though the synthesis of TAM from catechol is published, it is included here for completeness. It is worth noting that there are several methods to obtain the thiazolide 2-4. The first route followed, involving the conversion of the benzyl-protected terephthalic acid to the acid chloride prior to reaction with 2-mercaptotiazolide, was low-yielding, albeit only attempted once. The direct reaction of benzyl-protected terephthalic acid with 2-mercaptotiazolide in the presence of a coupling reagent is the preferred method, since it resulted in and increased yield and avoided the use of the water-sensitive and corrosive oxalyl chloride.
Scheme 2-2. Synthesis of L¹.
The synthesis of $\mathbf{L}^1$ (Scheme 2-2) was adapted from known preparations of analogous ligands. For ease of purification the singly-protected tren $\mathbf{2-8}$ was accessed in three steps; the one-step synthesis$^5$ gave a mixture of singly- and doubly-protected amine that could not be separated. Two of the tren amines were protected with benzyloxycarbonyl (Cbz) groups (yielding $\mathbf{2-6}$), which were removed by hydrogenolysis following protection of the remaining amine with a $t$-butoxycarbonyl (Boc) group (forming $\mathbf{2-7}$). Another key intermediate was the macrocycle, which was formed with two high-dilution reactions to prevent undesired polymeric by-products.$^6$ Compound $\mathbf{2-9}$ was formed by the slow addition of mono-Boc tren ($\mathbf{2-8}$) into a large excess of the TAM derivative $\mathbf{2-4}$, while $\mathbf{2-10}$ was formed by the slow addition of the reactants, in equimolar amounts, into a large volume of solvent. Deprotection of the Boc groups in the presence of the benzyl protecting groups proved slightly problematic, since the benzyl groups were also susceptible to cleavage. Several small-scale ($\sim 0.03$ mmol) reactions were run in order to find the conditions that would yield the desired product (Table 2-1). Mass spectrometry of the mixture after reaction with 50% trifluoroacetic acid (TFA) in dichloromethane worked indicated the presence of product without Boc groups as well as without benzyl groups (with one to four benzyls missing). The selectivity of the reagent was most likely reduced by the rotary evaporation in a heated water bath after reaction completion. In order to facilitate purification by column chromatography, coupling with $\mathbf{2-5}$ was performed after deprotection in one pot. Given that TFA cleaves Boc groups before benzyl groups (as evidenced by the mass spectrum), quenching with triethylamine was performed following deprotection, before coupling. Reducing the concentration of TFA to 30%, drying in vacuo, and quenching with base did not solve the problem, however. Even after purification, NMR analysis of the products was difficult due to the large excess of triethylamine present (Trial 3). Attempts at isolating the free amine $\mathbf{2-11}$ were largely unsuccessful, since the product bound irreversibly to the alumina column (Trial 5). A small amount did elute, nonetheless, and the product was found to be a mixture of $\mathbf{2-11}$ ((+)-HR ESIMS calcd for C$_{56}$H$_{65}$O$_8$N$_8$ (M+H) = 977.4890; found m/z 977.4920) and mono-debenzylated $\mathbf{2-11}$. Although the amine could not be isolated, this indicated that the 30% TFA reaction condition was improving the selectivity. Deprotection at 0 °C was much too slow, as the formation of product was not observed even after five hours. Further trials using extraction of the reaction mixture (before column chromatography) with acidification of the aqueous layer to remove the excess triethylamine also yielded mixtures that contained the desired product but that could not be interpreted by NMR. Deprotection using trimethylsilyl chloride and phenol was attempted, but the phenol could not be extracted from the reaction mixture. Finally, successful deprotection conditions involved the dropwise addition of TFA at 0 °C, reaction at room temperature, drying in vacuo (to reduce the amount of base needed), and quenching with triethylamine. Subsequent coupling with $\mathbf{2}$, extraction, and purification yielded pure desired product $\mathbf{2-12}$. 
Table 2-1. Various Boc deprotection conditions for 2-10. Reaction progress was monitored by TLC (appearance of product near baseline).

<table>
<thead>
<tr>
<th>Trial</th>
<th>Reagents</th>
<th>Temp.</th>
<th>Reaction time</th>
<th>Workup</th>
<th>Subsequent Reactions</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50% TFA, CH₂Cl₂</td>
<td>RT</td>
<td>55 min</td>
<td>rotary evap.</td>
<td>coupling, column</td>
<td>de-Bn, mixture</td>
</tr>
<tr>
<td>2</td>
<td>1M Me₃SiCl, 1M phenol, CH₂Cl₂</td>
<td>RT</td>
<td>45 min</td>
<td>H₂O, DIEA, extraction</td>
<td>phenol difficult to separate</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>30% TFA, CH₂Cl₂</td>
<td>RT</td>
<td>30 min</td>
<td>Et₃N</td>
<td>coupling, column</td>
<td>mixture</td>
</tr>
<tr>
<td>4</td>
<td>30% TFA, CH₂Cl₂</td>
<td>0 °C</td>
<td>&gt; 5 hrs</td>
<td>vacuum line</td>
<td>coupling, extraction, column</td>
<td>mixture</td>
</tr>
<tr>
<td>5</td>
<td>30% TFA, CH₂Cl₂</td>
<td>RT</td>
<td>4 hrs</td>
<td>vacuum line</td>
<td>amine not isolable</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>30% TFA, CH₂Cl₂</td>
<td>RT</td>
<td>2.5 hrs</td>
<td>Et₃N</td>
<td>coupling, extraction, column</td>
<td>mixture</td>
</tr>
<tr>
<td>7</td>
<td>30% TFA, CH₂Cl₂</td>
<td>0 °C addn, RT</td>
<td>30 min + 1 hr</td>
<td>vacuum line, Et₃N</td>
<td>coupling, extraction, column</td>
<td>desired product</td>
</tr>
</tbody>
</table>

MS and EA characterization of L¹ confirmed the identity of the ligand, but analysis of its NMR spectrum proved more difficult. While the ¹H NMR spectrum of the benzyl-protected ligand (2-12) can easily be assigned, such was not the case with the spectra of the deprotected in ligand. In DMSO-d₆ (Fig. 2-3), extensive hydrogen bonding breaks the symmetry and affords a complicated spectrum, as there are five O-H peaks, five N-H peaks, and four aromatic peaks, when there should be three of each. The alkyl peaks corresponding to the tren backbones are broad and overlapping. In D₂O with NaOD, the aromatic and alkyl peaks are well-resolved (Fig. 2-4), and can be assigned to a fully symmetric L¹. The presence of additional hydrogen-bonding in DMSO is further supported by the spectra of the ligand in D₂O with H₂O (Fig. 2-5). At high temperature, the aromatic peaks become more resolved and three amine proton peaks can be discerned. However, the alkyl region still is not as resolved as in the spectrum with D₂O/NaOD, indicating that there might still be some hydrogen bonding with the amines in the tren backbones. This behavior at elevated temperature is not observed in DMSO, as the O-H and N-H peaks significantly broaden and the aromatic peaks collapse into two multiplets, which cannot be assigned either. This indicates the presence of hydrogen bonding networks present in DMSO but not in D₂O, at 80 °C. These significant solvent effects observed with L¹ were not further investigated, but the ¹H spectrum in D₂O/NaOD was sufficient to characterize the ligand.
Figure 2-3. $^1$H NMR spectrum of $\textbf{L}^1$ (500 MHz) in DMSO-$d_6$. 
Figure 2-4. $^1$H NMR spectrum of L$^i$ (500 MHz) in D$_2$O (+ NaOD).
Figure 2-5. $^1$H NMR spectra of L1 (500 MHz) in D$_2$O (10% H$_2$O) at room temperature and at 80 °C. A gradient Watergate pulse sequence$^7$ was used to suppress the water signal.

1) 30% TFA, CH$_2$Cl$_2$
   $0^\circ$C, 40 min
   RT, 20 min
2) Et$_3$N
3) $\text{H}_2$ (1600 psi)
Pd/C
MeOH
RT, 27 h
Synthesis of Φ(3,3)moeTAM (L₂)

The tris(2-aminopropyl)amine (trpn) analog of L₁, L₂, was conceptualized following the somewhat surprising coordination mode of L₁ about Th(IV) in the crystal structure (cf. Figs. 3-14 and 3-15). Computational studies calculated a considerable energetic advantage for the pendant TAMs to coordinate the metal ion on the same side of the metal-macrocycle plane; this finding, in conjunction with the greater distortion of the macrocyclic TAMs from ideal dodecahedral coordination relative to the pendant TAMs suggest that the chelation of Th(IV) is constrained by the ring size (this is discussed in further detail in Chap. 3). The longer methylene linkers of L₂ was hypothesized to reduce the ring strain and enable the binding moieties to adopt a more ideally dodecahedral geometry about the metal ion. The synthesis of L₂ was directly analogous to that of L₁ and thus relatively straightforward.

Synthesis of Φ(2,2)NBuTAM (L₃)

In the first functionalization of L₁ into a BFC, the modification was performed at one of the pendant TAMs—rather than at the carbon backbone—to maintain the synthesis simple and most similar to that of L₁. A primary amine was chosen because it can be converted to the maleimide (by reaction with maleic anhydride) or the isothiocyanate (by reaction with thiophosgene, or from the decomposition of the thiocarbamate formed by treatment with carbon disulfide and triethylamine), which readily react with sulfuhydryl and amino groups on proteins. Of the many viable synthetic routes to L₂, one involves the formation of an asymmetric macrocycle using orthogonal amino protecting groups that can be cleaved separately for attachment of different pendant TAMs (Scheme 2-4a). A different route (Scheme 2-4b) avoids the use of protecting groups altogether by coupling the pendant TAMs to the tren backbone prior to cyclization. While the lack of protecting groups is an attractive feature, the former synthesis is more versatile due to the asymmetric macrocycle, the formation of which is the slowest step in the synthesis. The selection of suitable protecting groups and determination of repeatable formation and cleavage procedures would enable a variety of Φ ligands to be easily synthesized by the coupling of a variety of pendant binding units to this key intermediate.

However, the selection of a suitable amino protecting group was not trivial, given that it required orthogonality with the other protecting groups used in the synthesis of L₁: the Boc group for the amino group of tren, the benzyl group for the hydroxyl groups of TAM, the Cbz group used in the synthesis of the singly-protected tren. Based on reactivity charts, viable candidates (not requiring many synthetic steps prior to formation) are the formyl, acetamide, phthalimide, and 2,2,2-trichloroethoxycarbonyl groups. The protection of one amino group of tren with phthalic anhydride using a literature procedure for the amino protection of a nucleoside was not successful.
Scheme 2-4. Abbreviated synthetic routes to $L^3$, one with a) the formation of an asymmetrically protected macrocycle, and the other with b) no protecting groups.

Legend:

- $TAM$
- $thiaz$
- $PG$ = protecting group
The $^1$HNMR spectrum of the isolated product indicated the presence of some phthaloyl product, but with a significant amount of impurities and an alkyl region that could not be assigned, perhaps due to the presence of unreacted tren. Slow recrystallization from methanol-chloroform gave a more impure product. Since the phthaloyl group can be cleaved by ammonia, it was most likely removed by the excess of primary amines in the reaction. In an attempt to prevent the simultaneous presence of the phthaloyl and amino groups under heating, tren was doubly-protected with Cbz groups prior to protection with phthalic anhydride:

The mono-phthaloyl-bis-Cbz tren could be cleanly obtained in good yield (which was improved by the purification of phthalic anhydride by precipitation from CH$_2$Cl$_2$), but removal of the Cbz groups from this intermediate afforded a highly impure product. Elution through an ion-exchange column to remove carbonate resulted in the complete loss of the phthaloyl moiety. The unexpected facile cleavage of the phthalimido group under basic conditions was reported on by Astleford and Weigl, who offered a solution by reversibly opening the five-membered ring of the phthalimido with pyrrolidine, resulting in an $o$-pyrrolidinocarbonylbzamide (OPCB) that is stable under basic and nucleophilic conditions, as well as to hydrogenation. The OPCB could then be converted back to the phthalimido with mild acid (a slight excess of HCl or hydrofluoric-boric acid):

Small-scale reactions of the ring-opening with pyrrolidine proceeded in both CH$_2$Cl$_2$ and THF (albeit not to completion in 20 h, which meant that the product had to be separated from the starting material by silica column chromatography), but hydrogenation of the isolated OPCB gave an unidentifiable mixture, and endeavors to synthesize mono-phthaloyl-tren were abandoned.
From the harsher deprotection conditions required for the formyl group relative to the phthalimido group, the formyl group is seemingly more stable and was chosen next for the mono-protection of tren. Acetamido protection was not attempted because cleavage of this group could conceivably be detrimental to the amide groups in the macrocyclic intermediate. For ease of separation, the formyl group was introduced to the di-Cbz tren derivative rather than tren. Mono-formyl tren was successfully obtained by hydrogenolysis of the Cbz groups (Scheme 2-5).

The remaining steps in the synthesis of $L^2$ are detailed in Schemes 3 and 4. The formation of the key intermediate, the asymmetric macrocycle 8, was straightforward with the synthetic procedure established for the formation of the symmetrically Boc-protected macrocycle. 8 was then sequentially deprotected; the formyl group was cleaved and the exposed primary amine was coupled with a pendant TAM before cleavage of the Boc group and coupling to a different pendant TAM. The primary amine intermediates were not isolated.
Scheme 2-7. Synthesis of L³.
Cleavage of the formyl group was tested with hydrazine,\textsuperscript{12} methylamine (with the same procedure as for hydrazine; methylamine was chosen for ease of removal), NaOH,\textsuperscript{13} and UV irradiation.\textsuperscript{14} UV irradiation removed the benzyl instead of the formyl group, which was confirmed by MS. Monitoring of these reactions was difficult by TLC since the deformylated product had an R\textsubscript{f} close to that of the starting material; initial reactions with the bases were performed with too many equivalents of base and for too long. For the hydrazine and methylamine reactions, this resulted in decomposition of the starting material. However, with NaOH, the decomposition could be attributed to the cleavage of the Boc group (confirmed by $^1$HNMR and MS). While the formyl group had been completely cleaved, the presence of some product with a Boc group indicated that Boc cleavage occurred more slowly than formyl cleavage under these conditions. The deformylation by NaOH was then monitored at three different concentrations of NaOH by NMR to find conditions at which none of the Boc group would be cleaved. This was very straightforward, as the reaction could be clearly monitored by the disappearance of the formyl H and the persistence of the Boc methyl groups.

Another problematic step in the synthesis of L\textsuperscript{3} was the purification of 2-24 from the starting material 2-23. Without prior knowledge that the two compounds had the same R\textsubscript{f} by TLC, the Boc deprotection by TFA was quenched before it was complete and purification by silica column chromatography following pendant TAM coupling was difficult. This was elucidated with $^1$H and $^{13}$C NMR spectra of the column fractions. The $^1$H resonances of 9 and 10 all overlap, except for the Boc methyl peak in 2-23 and the macrocyclic TAM aromatic hydrogens, which are shifted upfield in 2-24 relative to 2-23 by 0.17 ppm. In the future, the Boc deprotection should be monitored by $^1$HNMR, as the absence of unreacted starting material would greatly simplify the purification of 2-24.

Following acid deprotection of the benzyl and Cbz groups, L\textsuperscript{3} was obtained in reasonably good yield and purity (94\%, as determined by HPLC). The next step lies in its bioconjugation to a targeting antibody and the evaluation of the \textit{in vivo} behavior of the antibody-Th(IV) complex conjugate.
EXPERIMENTAL DETAILS

Synthesis. All chemicals were used as supplied without further purification, unless otherwise noted. Characterization data were obtained at facilities at the University of California, Berkeley, except for the elemental analysis of L₁, which was also performed by Columbia Analytical Services (Tucson, AZ). NMR spectra were obtained at room temperature on Bruker AV-500 or AV-600 spectrometers at the College of Chemistry NMR Facility. Chemical shifts are reported in parts per million (ppm) relative to solvent residual signals. Mass spectra were obtained at the QB3/Chemistry Mass Spectrometry Facility, on a Finnigan LTQ FT high-resolution electrospray ionization (ESI) mass spectrometer. Yields indicate the amount of isolated material, and reactions were not optimized. Silica gel (230-400 mesh) was used for column chromatography purifications.

Synthesis of TAM binding unit
2,3-dihydroxyterephthalic acid (2-1). On a warm, sunny day (28 °C, 18% humidity), catechol (TCI, 120.033 g, 1.09 mol) and K₂CO₃ (316 g, 2.29 mol) were ground together, and the homogeneous mixture was poured into a 500-mL beaker, which was placed in a par-bomb. The bomb was purged three times with 600 psi CO₂ before being filled to 860 psi CO₂ and placed in an oil bath. The oil bath gradually increased in temperature to 225 °C in 3 h, with an accompanying increase in bomb pressure to 1170 psi. Within 1.25 h, the pressure decreased to 380 psi (250 °C), and the temperature was maintained at 230 °C for 15 hours. The bomb, at 160 psi, was let cool to RT (and placed in the freezer to facilitate opening) and opened. The reaction mixture, a hard gray rock with turquoise and white specks, was combined with 1.5 L water and 1 L conc. HCl. The mixture slowly bubbled and produced beige foam, and after 3 h, the solid chunks were broken apart with a stick and stirred with a magnetic stir bar for 2 h. The beige slurry was then filtered and left to dry on the house vacuum for 14 h. The solid light gray solid was washed with 4 L Millipore water, and left on the house vacuum for 9 days. The dried gray solid was ground to a powder and further dried on the vacuum line for 26 h (95%).

₁H NMR (400 MHz, MeOD, δ): 7.34 (s, Ar H, 2H).

₁³C NMR (101 MHz, MeOD, δ): 117.8, 120.1, 152.6, 173.2.


Dibenzyl 2,3-bis(benzyloxy)terephthalate (2-2). 2-1 (101.798 g, 0.514 mol), benzyl chloride (258 mL, 2.242 mol), KI (25.304 g, 0.140 mol), K₂CO₃ (664.270 g, 4.806 mol), and 1.3 L DMF were combined in a 5-L 3-neck round-bottom flask, yielding a mixture of dark brown solution and undissolved solid. The flask was fitted with a reflux condenser and mechanical stirrer, and placed in a heating mantle. The reaction mixture was heated at 67 °C for 3 d and filtered. The dark brown, clear filtrate was rotovapped to ~400 mL of opaque, brown, viscous oil, which was dissolved in ~600 mL DCM. The opaque, brown solution was filtered and extracted with water (5 x 500 mL). A small amount of brine was used to break up emulsions. The organic layer was dried with MgSO₄ and rotovapped to ~250 mL of dark brown oil. This oil was purified by silica column chromatography (0-5% MeOH in DCM) to yield a hardened brown oil (48%).

₁H NMR (500 MHz, CDCl₃, δ): 5.10 (s, Bn CH₂, 4H), 5.33 (s, COOBn H, 4H), 7.25-7.44 (m, Ph H, 20H), 7.58 (s, Ar H, 2H).

₁³C NMR (151 MHz, CDCl₃, δ): 67.8, 70.1, 76.9, 126.1, 128.6, 128.8, 128.9, 129.0, 129.1, 129.1, 131.0, 136.4, 137.5, 153.4, 165.6.
HRMS-ESI (m/z): [M + H]⁺ calcd for C₃₆H₃₁O₆, 559.2115; found, 559.2116.

2,3-bis(benzyloxy)terephthalic acid (2-3). 2-2 (56.970 g, 0.102 mol) was dissolved in 200 mL 1:1::EtOH:dioxane in a 1-L round-bottom flask, resulting in a dark orange, clear solution, to which a suspension of LiOH (7.315 g, 0.305 mol) in 75 mL water was added. The flask was fitted with a reflux condenser and magnetically stirred. The emulsion (containing undissolved LiOH) was heated to 60 °C for 12 h, and became a translucent, orange solution. The reaction mixture was filtered through a M glass frit, dissolved in 500 mL basic water, and washed with DCM (3 x 250 mL). The aqueous layer was acidified with ~80 mL conc. HCl, and the resulting white precipitate was filtered and washed with water. The solid was dried using house vacuum overnight, ground into a white powder, and dried on the vacuum line for 12 h (100%).

1H NMR (600 MHz, DMSO-d₆, δ): 5.03 (s, Bn CH₂, 4H), 7.33-7.41 (m, Ph H, 10H), 7.49 (s, Ar H, 2H), 13.31 (s, COOH, 2H).

13C NMR (151 MHz, DMSO-d₆, δ): 75.6, 124.9, 128.0, 128.2, 128.2, 131.0, 136.9, 151.4, 166.7.


(2,3-bis(benzyloxy)-1,4-phenylene)bis((2-thioxothiazolidin-3-yl)methanone) (2-4). An opaque, beige suspension of 2-3 (10.435 g, 27.58 mmol) in 80 mL dry toluene was stirred and degassed with N₂. Oxalyl chloride (8.00 mL, 94.5 mmol) was added via syringe, and the reaction mixture bubbled. The addition of 3 drops of DMF resulted in more vigorous bubbling. The next day, the reaction mixture was a still an opaque slurry, with no bubbling. 20 mL toluene was added to dilute the mixture, and as a few drops DMF did not result in bubbling, 3.0 mL (16 mmol) oxalyl chloride was added, and the reaction bubbled. The following day, 4.5 mL (24 mmol) oxalyl chloride was added to the reaction mixture, which became a translucent, yellow solution. The addition of another 2.0 mL (11 mmol) oxalyl chloride did not result in additional bubbling, and the reaction was let stir overnight. The following day, the reaction mixture had become a clear, orange solution containing a small amount of white solid settled at the bottom of the flask. The toluene and excess oxalyl chloride were removed by rotary evaporation, and the brown cake was dissolved in 50 mL DCM. This opaque, orange solution was dripped via teflon cannula into a stirring, 0 °C (ice-water bath) solution of 2-mercaptothiazoline (7.2682 g, 60.97 mmol) in 50 mL DCM and 14 ml triethylamine. Following the addition of the ice bath, the reaction was let stir at RT overnight. The dark orange, clear solution was filtered, washed with water (3 x 330 mL), and washed with aqueous 2M HCl (4 x 250 mL). The organic layer was dried with MgSO₄ and rotovapped to a brown oil, which was purified by silica column chromatography (DCM). The product, as a 20 mL yellow DCM solution, was precipitated from 1 L isopropanol at 4 °C. Filtration afforded a yellow solid (43%).

1H NMR (500 MHz, CDCl₃, δ): 3.04 (t, J = 5.8 Hz, NCH₂CH₂S, 4H), 4.35 (t, J = 6.0 Hz, NCH₂CH₂S, 4H), 5.10 (s, Bn CH₂, 4H), 7.18 (s, Ar H, 2H), 7.38 (m, Ph H, 10H).

13C NMR (151 MHz, CDCl₃, δ): 29.4, 56.2, 76.4, 124.8, 128.8, 128.9, 129.0, 133.8, 137.4, 149.5, 167.0, 202.0.

An alternative method performed is as follows. The benzyl-protected terephthalic acid (26.46 mmol) was combined with 2-mercaptothiazolide (58.10 mmol) and 60 mL CH₂Cl₂, forming a white and opaque slurry, which was placed on an ice-water bath for the addition of the diisopropylcarbodiimide (58.1 mmol) and dimethylaminopyridine (1.53 mmol). The resulting orange-brown reaction mixture was stirred at room temperature under nitrogen flow for 12 h. It was then filtered to remove white precipitate, and purified by silica column chromatography (2%
MeOH/CH$_2$Cl$_2$). Rotary evaporation of the pure fractions and precipitation of the impure fractions from isopropanol (the purification was not optimal) afforded the desired yellow solid (68%).

$BnTAM(thiaz)(OMeEtN)\ (2-5)$. A solution of 2-methoxy-ethanamine (Aldrich, 0.35 mL, 4.03 mmol) in 200 mL CHCl$_3$ (with four drops of triethylamine) was added dropwise to a stirring solution of $BnTAM(thiaz)_2\ (2-4)$ (30.010 g, 51.7 mmol), in 1000 mL CH$_2$Cl$_2$. The high-dilution slow addition was performed at room temperature over 16 hours. The reaction mixture was then directly applied onto a gradient silica column (0-10% methanol in dichloromethane), and the desired product was eluted with 8% methanol. Rotary evaporation gave a yellow, foamy solid (91%) that became an oil upon storage at 4°C.

$^{1}$H NMR (500 MHz, CD$_2$Cl$_2$): $\delta$ 3.03 (t, $J = 9.0$ Hz, 1H, methoxyethanamide CH$_2$), 3.24 (s, 3H, CH$_3$), 3.39-3.42 (m, 2H, thiaz CH$_2$), 3.47-3.51 (m, 2H, methoxyethanamide CH$_2$), 4.40 (t, $J = 9.3$ Hz, 2H, thiazolidine CH$_2$), 5.11 (s, 2H, Bn CH$_2$), 5.12 (s, 2H, Bn CH$_2$), 7.19 (d, $J = 10.5$ Hz, 1H, ArH), 7.35-7.43 (m, 10H, Ph), 7.84 (d, $J = 10.5$ Hz, 1H, ArH), 8.01 (br t, 1H, amide H).

$^{13}$C NMR (100 MHz, CD$_2$Cl$_2$): $\delta$ 28.8, 39.5, 55.7, 58.4, 70.8, 76.1, 76.7, 124.0, 126.4, 128.1, 128.4, 128.6, 128.6, 128.9, 130.3, 133.6, 135.9, 137.1, 149.6, 150.2, 163.9, 166.7, 201.7. HRMS-ESI ($m/z$): [M+H]$^+$ calcd for C$_{28}$H$_{29}$O$_5$N$_2$S$_2$, 537.1512; found, 537.1518.

Synthesis of $L1$

$Bis(Cbz)tren\ (2-6)$. Benzyl phenyl carbamate (Sigma-Aldrich, 20.5 mL, 103.8 mmol) was added dropwise to a stirring CH$_2$Cl$_2$ (50 mL, degassed) solution of tren (TCI, 7.0 mL, 46.8 mmol) in an ice bath. Following melting of the ice bath, the reaction was stirred at room temperature for 12 h. The clear, light yellow reaction mixture was reduced in vacuo to a yellow oil and purified by silica column chromatography (5-15% MeOH in DCM). The product fractions were rotary evaporated and further dried on the vacuum line for 8 h with light (50 °C) heating to a light yellow, translucent oil (72%).

$^{1}$H NMR (600 MHz, CDCl$_2$, $\delta$): 2.45-2.65 (m, -NC$_2$H$_2$NH$_2$ and -NC$_2$H$_2$CH$_2$NHBoc, 8H), 3.24 (s, -NC$_2$H$_2$CH$_2$NHBoc, 4H), 3.10 (br t, -NCH$_2$C$_6$H$_4$NHBoc, 2H), 3.19 (br t, -NCH$_2$C$_6$H$_4$NHCbz, 4H), 5.05 (s, Bn H, 4H), 5.10 (br s, RNHBCbz, 1H), 5.55 (br s, RNHBCbz, 2H), 7.28-7.32 (m, Ph H, 10H).

$^{13}$C NMR (151 MHz, CDCl$_2$, $\delta$): 39.7, 40.0, 49.5, 54.5, 57.3, 66.6, 116.2, 119.2, 128.2, 128.7, 128.8, 129.8, 137.7, 157.3, 158.4. HRMS-ESI ($m/z$): [M+H]$^+$ calcd for C$_{22}$H$_{31}$O$_4$N$_4$, 415.2340; found, 415.2333.

$Mono(Boc)-bis(Cbz)tren\ (2-7)$. A solution of NaCl (4.789 g, 81.95 mmol) and NaHCO$_3$ (2.997 g, 35.67 mmol) in 60 mL water was added to a solution of 2-6 (10.2754 g, 24.05 mmol) in 50 mL CH$_2$Cl$_2$. Di-tert-butyl dicarbonate (Sigma-Aldrich, 5.7373 g, 26.29 mmol) dissolved in 10 mL CH$_2$Cl$_2$ was added to the biphasic mixture, resulting in immediate bubbling. The reaction was let stir at room temperature overnight, filtered, and extracted with CH$_2$Cl$_2$ (3 x 40 mL). The combined organic extracts were reduced to a viscous, clear, light yellow oil that was purified by silica column chromatography (0-10% MeOH in CH$_2$Cl$_2$). The product fractions were rotary evaporated to a clear, yellow, viscous oil (84%).

$^{1}$H NMR (500 MHz, CDCl$_2$, $\delta$): 1.36 (t, $J = 9.0$ Hz, 9H), 2.51 (br t, -NCH$_2$CH$_2$NHBoc, 2H), 2.54 (br t, -NCH$_2$CH$_2$NHCBz, 4H), 3.10 (br t, -NCH$_2$CH$_2$NHBoc, 2H), 3.19 (br t, -NCH$_2$CH$_2$NHCBz, 4H), 5.05 (s, Bn H, 4H), 5.10 (br s, RNHBCbz, 1H), 5.55 (br s, RNHCBz, 2H), 7.28-7.32 (m, Ph H, 10H).
$^{13}$C NMR (151 MHz, CDCl$_3$, δ): 28.7, 39.2, 39.6, 54.6, 54.9, 66.9, 79.5, 128.4, 128.5, 128.9, 139.7, 157.2.

HRMS-ESI (m/z): [M+H]$^+$ calcd for C$_{37}$H$_{39}$O$_6$N$_4$$^+$, 515.2864; found, 515.2847.

**Mono(Boc)tren (2-8).** 2-7 (11.220 g, 20.20 mmol) was dissolved in 120 mL MeOH in a glass vessel containing a magnetic stir bar. A 5 mL MeOH suspension of Pd/C (Sigma-Aldrich, 10 wt%, type E101 NE/W; 1.1318 g) was added to the solution and the glass vessel was placed in a Parr bomb. The bomb was purged 3 times with 750 psi H$_2$ before being filled to 1250 psi H$_2$. The reaction was stirred at room temperature for 24 h with a sustained pressure of 1000 psi. The reaction mixture was then filtered through a fine glass frit and rotary evaporated to a light yellow, viscous oil. The oil was coevaporated with MeOH on the vacuum line and dried for 24 h. NMR showed the presence of carbonate adduct in the product, which was dissolved in water and purified by elution through an ion-exchange column (Dowex 21K XLT resin). Evaporation afforded a clear, yellow oil (90%), which was stored at 4°C as a CH$_2$Cl$_2$ solution containing 1 mol equivalent triethylamine.

$^{1}$H NMR (500 MHz, CDCl$_3$, δ): 1.38 (s, t-Bu, 9H), 2.44-2.49 (m, -NC$_2$H$_2$CH$_2$NH$_2$ and -NC$_2$H$_2$CH$_2$NHBoc, 6H), 2.66 (t, $J$ = 6.0 Hz, 4H), 3.10 (br q, -NCH$_2$C$_2$H$_2$NHBoc, 2H), 5.78 (br t, amine H, 1H).

$^{13}$C NMR (151 MHz, CDCl$_3$, δ): 28.7, 39.3, 40.0, 51.6, 57.8, 79.1, 156.8.

HRMS-ESI (m/z): [M+H]$^+$ calcd for C$_{11}$H$_{27}$O$_2$N$_4$$,^+$, 247.2129; found, 247.2127.

**Mono(Boc)-Bis(BnTAMthiaz)tren (2-9).** A solution of 2-8 (0.2792 g, 1.13 mmol) in 200 mL CHCl$_3$ (with three drops of triethylamine) was added dropwise to a stirring solution of 2-4 (27.7 g, 47.7 mmol) in 500 mL CH$_2$Cl$_2$ (with 5 drops triethylamine). The high-dilution slow addition was performed at room temperature over 18 hours. The reaction mixture was reduced in volume by rotary evaporation and separated by silica column chromatography (2-5 % MeOH in CH$_2$Cl$_2$). 5 was eluted with 4% methanol, and was isolated as a yellow foamy solid after removal of solvent (66%).

$^{1}$H NMR (500 MHz, CD$_2$Cl$_2$, δ): 1.36 (s, 9H, CH$_3$), 2.39 (t, $J$ = 6.5 Hz, 4H, tren CH$_2$), 2.46 (t, $J$ = 6.0 Hz, 2H, tren CH$_2$), 2.99 (d, $J$ = 6.0 Hz, 2H, tren CH$_2$), 3.03 (t, $J$ = 7.3, 4H, tren CH$_2$), 3.23 (q, $J$ = 6.2 Hz,4H, thiaz CH$_2$), 4.40 (t, $J$ = 7.5 Hz, 4H, thiaz CH$_2$), 5.031 (br t, 1H, amide H), 5.10 (s, 4H, Bn CH$_2$), 5.12 (s, 4H, Bn CH$_2$), 7.17 (d, $J$ = 8.0 Hz, 2H, ArH), 7.35-7.39 (m, 20H, Ph), 7.70 (d, $J$ = 8.0 Hz, 2H, ArH), 7.76 (br t, 2H, amide H).

$^{13}$C NMR (100 Hz, CD$_2$Cl$_2$, δ): 28.2, 28.9, 37.6, 55.7, 76.2, 76.8, 124.1, 126.2, 128.1, 128.4, 128.6, 128.7, 128.8, 128.9, 130.7, 133.4, 136.0, 137.1, 149.5, 150.1, 164.2, 166.7, 201.7.

HRMS-ESI (m/z): [M+H]$^+$ calcd for C$_{61}$H$_{65}$O$_{10}$N$_6$S$_4$$,^+$, 1169.3640; found, 1169.3617.

**Mono(Boc)-Bis(BnTAM)2tren (2-10).** Two 450 mL CHCl$_3$ solutions, one of 2-9 (0.8503 g, 0.727 mmol) with 5 drops triethylamine and one of 2-8 (0.19981 g, 0.811 mmol) with 15 drops triethylamine, were combined by dropwise addition into a flask containing 1000 mL CH$_2$Cl$_2$. The high-dilution addition took place over 72 h at room temperature. The reaction mixture became progressively less yellow, as the starting material 2-9 is yellow, but thiazolidine and TAM are colorless in solution. Since the reaction was still yellow once the addition was complete, diethylenetriamine (1 mL, 9 mmol) was added to the reaction mixture to facilitate separation from remaining 5, as the of a primary amine renders its R$_f$ on silica much lower. After 1.5 hours, the reaction mixture had become colorless and was directly applied on a flash gradient silica column (0-3% MeOH in
CH$_2$Cl$_2$). The product eluted with 3% methanol and was dried *in vacuo* to afford a white solid (87%).

$^1$H NMR (500 MHz, CD$_2$Cl$_2$): $\delta$ 1.33 (s, 18H, CH$_3$), 2.45 (br t, 4H, tren CH$_2$), 2.54 (t, $J = 5.3$ Hz, 8H, tren CH$_2$), 2.99 (br q, 4H, tren CH$_2$), 3.38 (q, $J = 5.2$ Hz, 8H, tren CH$_2$), 4.92 (br s, 2H, amide H), 5.03 (s, 8H, Bn CH$_2$), 7.10 (s, 4H, ArH), 7.36 (m, 20H, Ph), 7.67 (br s, 4H, amide H).

$^{13}$C NMR (100 MHz, CD$_2$Cl$_2$): $\delta$ 28.1, 37.1, 37.7, 52.6, 76.7, 78.7, 125.1, 128.4, 128.5, 128.6, 131.8, 136.3, 150.4, 155.8, 165.7.

HRMS-ESI (m/z): [M+H]$^+$ calcd for C$_{66}$H$_{81}$O$_{12}$N$_{18}$+, 1177.5968; found, 1177.5962.

Tren$_2$(BnTAM)$_2$[BnTAM(OMeEtN)]$_2$ (2-12). A solution of 2-10 (0.26435 g, 0.225 mmol) in 7 mL CH$_2$Cl$_2$ was placed in an ice-water bath and stirred. 3 mL trifluoroacetic acid (TFA) was added dropwise over 5 min, and the reaction progress was monitored by TLC. The reaction mixture stirred at 0 °C for 10 min and at room temperature for 20 min. The reaction mixture was dried in *vacuo*, affording a pink-orange oil that was redissolved in 2 mL dichloromethane and subsequently dried in *vacuo* (this was performed twice). 1 mL triethylamine and 1 mL CH$_2$Cl$_2$ were added to the foamy oil at 0 °C, and the mixture was stirred at room temperature for 45 min. before the addition of a solution of 7 (0.56 g, 1.05 mmol) in 8 mL CH$_2$Cl$_2$. The reaction mixture was stirred at room temperature for 34 h. Separation of the product from triethylamine was achieved by extraction with 10 mL water. The aqueous layer was acidified to pH 8 (from pH 10) with HCl and extracted with CH$_2$Cl$_2$ (3 x 5 mL). The combined organic layers were rotary evaporated and applied onto a gradient silica column (1-5% MeOH in CH$_2$Cl$_2$). The product was eluted with 5% methanol and further purified on a silica chromatron plate. The by-products were eluted with 2.5% methanol in CH$_2$Cl$_2$, and a slow gradient to 6% was used to collect the desired product. Rotary evaporation resulted in a light yellow foamy solid (80%).

$^1$H NMR (500 MHz, CD$_2$Cl$_2$): $\delta$ 2.45 (t, $J = 8.0$ Hz, 4H, tren CH$_2$), 2.58 (t, $J = 6.0$ Hz, 8H, tren CH$_2$), 3.21 (s, 6H, CH$_3$), 3.23-3.28 (m, 4H, methoxyethanamide CH$_2$), 3.37-3.41 (m, 12H, methoxyethanamide CH$_2$, tren CH$_2$), 3.49 (q, $J = 6.7$ Hz, tren CH$_2$), 4.89 (s, 8H, Bn CH$_2$), 5.06 (s, 4H, Bn CH$_2$), 5.07 (s, 4H, ArH), 6.90 (s, 4H, ArH), 7.31-7.42 (m, 40H, Ph), 7.43 (d, $J = 10.5$ Hz, 2H, ArH), 7.49 (d, $J = 10.5$ Hz, 2H, ArH), 7.62 (t, $J = 6.5$ Hz, 2H, amide H), 7.69 (t, $J = 6.5$ Hz, 4H, ArH), 8.07 (t, $J = 5.5$ Hz, 2H, amide H).

$^{13}$C NMR (100 MHz, CD$_2$Cl$_2$): $\delta$ 36.7, 37.0, 39.6, 51.8, 52.4, 58.3, 70.6, 76.3, 76.8, 76.9, 124.7, 125.4, 125.8, 128.3, 128.5, 128.6, 128.6, 130.2, 132.0, 136.3, 136.7, 149.5, 150.5, 150.6, 150.9, 151.0, 164.3, 164.7, 164.7.

HRMS-ESI (m/z): [M+Na]$^+$ calcd for C$_{106}$H$_{110}$O$_{18}$N$_{10}$Na$^+$, 1833.7892; found, 1833.7866.

L$^1$. 5 mL 12.1 N HCl was added to a solution of 2-12 (0.32466 g, 0.179 mmol) dissolved in 5 mL glacial acetic acid. The solution was stirred at room temperature for 64 hours and dried in *vacuo*. The resulting light yellow cake was suspended in 10 mL MeOH and dried, 3 times, to give a light yellow solid. Further drying of this solid under vacuum overnight yielded a light gray solid (89%).

$^1$H NMR (500 MHz, D$_2$O + NaOD): $\delta$ 2.83 (t, $J = 7.3$ Hz, 4H, tren CH$_2$), 2.93 (t, $J = 6.0$ Hz, 8H, tren CH$_2$), 3.34 (s, 6H, CH$_3$), 3.41 (t, 6.3 Hz, 8H), 3.49-3.53 (m, 8H, tren CH$_2$, methoxyethanamide CH$_2$), 3.60 (t, $J = 5.5$ Hz, 4H, methoxyethanamide CH$_2$), 6.54 (s, 4H, ArH), 6.87 (s, 2H, ArH), 6.87 (s, 2H, ArH).

$^{13}$C NMR (125 Hz, DMSO-$d_6$): $\delta$ 34.3, 34.4, 39.5, 49.0, 50.4, 51.6, 51.9, 58.4, 70.5, 116.6, 116.7, 116.9, 117.0, 117.3, 118.4, 119.6, 149.5, 150.5, 150.6, 150.9, 169.0, 169.1, 170.8.
HRMS-ESI (m/z): [M+Na]$^+$ calcd for C$_{50}$H$_{62}$O$_{18}$N$_{10}$Na$,^+$ 1113.4136; found, 1113.4126; [M-H]$^-$ calcd for C$_{50}$H$_{61}$O$_{18}$N$_{10}$, 1089.4171; found, 1089.4170.

Anal. calcd % (found %) for C$_{50}$H$_{62}$O$_{18}$N$_{10}$·3HCl: C, 50.03 (50.38); H, 5.46 (5.58); N, 11.67 (11.51). Anal. calcd % (found %) for C$_{50}$H$_{62}$O$_{18}$N$_{10}$·3HCl (Columbia Analytical Services): C, 50.03 (50.01); Cl, 8.9 (7.0); H, 5.46 (5.64); N, 11.67 (11.30).

**Synthesis of L$_2$**

*DiCbz-trpn (2-13).* Benzyl phenyl carbonate (7.68 mL, 38.9 mmol; Aldrich) was added dropwise to a stirring and cold solution (on an ice-water bath) of trpn (3.50 mL, 17.7 mmol) in 20 mL degassed CH$_2$Cl$_2$. The ice-water bath was removed after 1.5 hr, and the capped solution was left to stir overnight. The resulting peach-colored solution was purified by column chromatography (5-15% MeOH in CH$_2$Cl$_2$ with 1% triethylamine). The fractions containing pure product were combined and evaporated on the vacuum line with light heating to obtain a clear, yellow oil (45%).

$^1$H NMR (500 MHz, CD$_2$Cl$_2$, $\delta$): 1.54 (quin, $J = 7.0$ Hz, -CH$_2$CH$_2$CH$_2$NH$_2$, 2H), 1.63 (quin, $J = 6.6$ Hz, -CH$_2$CH$_2$CH$_2$NH$_2$ and -CH$_2$CH$_2$NH$_2$Cbz, 6H), 2.37-2.41 (m, -C$_{14}$H$_{24}$CH$_2$CH$_2$NH$_2$, 6H), 2.67 (t, $J = 6.8$ Hz, -CH$_2$CH$_2$CH$_2$NH$_2$, 2H), 3.21 (q, $J = 6.2$ Hz, -CH$_2$CH$_2$NH$_2$Cbz, 4H), 5.07 (s, Bn CH$_2$, 4H), 6.05 (br s, amide NH), 7.29-7.35 (m, Ar H, 10H).

$^{13}$C NMR (151 MHz, CD$_2$Cl$_2$, $\delta$): 11.3, 27.0, 39.7, 40.3, 46.2, 66.1, 127.77, 127.8, 128.2, 128.4, 137.3, 156.5.

HRMS-ESI (m/z): [M + H]$^+$ calcd for C$_{25}$H$_{37}$O$_4$N$_4$, 457.2809; found, 457.2809.

*DiCbz-monoBoc-trpn (2-14).* A 50-mL round-bottom flask was charged with 2-13 (2.0544 g 2-13·0.20Et$_3$N·0.22MeOH, 4.23 mmol), 9 mL CH$_2$Cl$_2$ to dissolve the oil, an aqueous solution of NaHCO$_3$ (0.3843 g, 4.57 mmol), NaCl (0.980 g, 15.2 mmol), and a solution of di-tert-butyl carbonate (0.9859 g, 4.52 mmol) dissolved in 3 mL CH$_2$Cl$_2$. The reaction mixture bubbled for 20 min, and was left to stir overnight. It was then filtered, and the organic layer was separated from the filtrate. The aqueous layer was extracted with 10 and 5 mL CH$_2$Cl$_2$. The combined organic layers were purified by column chromatography (0-10% MeOH in CH$_2$Cl$_2$). The fractions containing pure product were rotary evaporated to yield a clear, colorless oil (92%).

$^1$H NMR (500 MHz, CD$_2$Cl$_2$, $\delta$): 1.41 (s, t-Bu CH$_3$, 9H), 1.55-1.59 (m, -CH$_2$CH$_2$CH$_2$NHBoc, 2H), 1.62 (quin, $J = 6.6$ Hz, -CH$_2$CH$_2$CH$_2$NHBoc, 4H), 2.37-2.41 (m, -CH$_2$CH$_2$CH$_2$NHBoc and -CH$_2$CH$_2$CH$_2$NH$_2$Cbz, 6H), 3.10 (br q, $J = 6.0$ Hz, -CH$_2$CH$_2$CH$_2$NH$_2$Cbz, 4H), 5.07 (s, Bn CH$_2$, 4H), 6.05 (br s, amide NH), 7.29-7.35 (m, Ar H, 10H).

$^{13}$C NMR (151 MHz, CD$_2$Cl$_2$, $\delta$): 27.0, 27.2, 28.1, 39.1, 39.6, 50.1, 51.7, 66.2, 78.6, 127.8, 128.3, 155.9, 156.4.

HRMS-ESI (m/z): [M + H]$^+$ calcd for C$_{30}$H$_{45}$O$_6$N$_4$, 557.3334; found, 557.3345.

*MonoBoc-trpn (2-15).* 2-14 (2.30 g of 2-14·0.89MeOH, 3.73 mmol) was dissolved in 30 mL MeOH in a glass vessel and mixed with a suspension of 0.400 g Pd/C (Pd 10% wt.) in 5 mL MeOH. A stir bar was added to the vessel, which was then placed in a pressure bomb. The bomb was pressurized with 1650 psi H$_2$ and placed on a stirring plate to stir the reaction for 27 h. The reaction mixture was filtered through a fine glass frit, and the filtrate was rotary evaporated and further dried on the vacuum line to afford a clear, colorless oil (100%).
1H NMR (500 MHz, CD2Cl2, δ): 1.41 (s, t-Bu CH3, 9H), 1.59 (quin, J = 6.8 Hz, –CH2CH2CH2NHBOc, 2H), 1.77 (br quin, J = 6.3 Hz, –CH2CH2CH2NH2, 4H), 2.42 (t, J = 6.8 Hz, –CH2CH2CH2NHBOc, 2H), 2.50 (t, J = 6.2 Hz, –CH2CH2CH2NH2, 4H), 2.93 (t, J = 6.8 Hz, –CH2CH2CH2NH2, 4H), 3.05 (br q, J = 5.5 Hz, –CH2CH2CH2NHBOc, 2H), 6.01 (br s, amide NH, 1H), 8.53 (s, NH2, 2H).

13C NMR (151 MHz, CD2Cl2, δ): 24.9, 26.7, 27.4, 38.1, 38.4, 48.4, 50.8, 50.9, 78.6, 157.2.

HRMS-ESI (m/z): [M + H]+ calcd for C14H33O2N4, 289.2598; found, 289.2596.

MonoBoc-diTAMBndithiaz-trpn (2-16). A solution of TAMBndithiaz (2-4) (50.603 g, 87.1 mmol) and 10 drops triethylamine in 1.2 L CH2Cl2 was degassed in a 2-L round-bottom flask. A degassed solution of 2-15 (0.9457 g 2-15•2.59MeOH, 2.34 mmol) and 7 drops triethylamine in 400 mL CH3Cl in a 500-mL round bottom flask was degassed and slowly dripped (through a capillary) into the stirring solution of TAMBndithiaz. The dripping (of about 2 drops/sec) was started by briefly flowing nitrogen into the 500-mL flask and took 38 h to complete. The reaction was left to stir for an additional 8 h, concentrated by rotary evaporation, and purified by column chromatography (0-10% MeOH in CH2Cl2). The fractions containing pure product were combined and rotary evaporated to yield a yellow foamy solid (74%).

1H NMR (600 MHz, CD2Cl2, δ): 1.39 (s, t-Bu CH3, 9H), 1.58-1.64 (m, –CH2C6H4CH2TAM and –CH2C6H4CH2NHBoc, 6H), 2.49 (t, J = 5.5 Hz, –CH2C6H4CH2TAM, 4H), 2.54 (t, J = 5.5 Hz, –CH2C6H4CH2NHBOc, 2H), 3.03 (t, J = 6.0 Hz, –CH2C6H4CH2TAM, 4H), 3.10 (br q, J = 4.5 Hz, –CH2C6H4CH2NHBOc, 2H), 3.24 (q, J = 5.5 Hz, –NCH2C6H4S–, 4H), 4.40 (t, J = 6.3 Hz, –NC6H4CH2S–, 4H), 5.10 (s, Bn CH2, 4H), 5.12 (s, Bn CH2, 4H), 7.17 (d, J = 6.5 Hz, TAM Ar H, 2H), 7.34-7.40 (m, Bn Ar H), 7.74 (d, J = 7.0, TAM Ar H, 2H), 7.84 (t, J = 4.8, TAM amide NH, 2H, 8.25 (s, NBoc amide NH, 1H).

13C NMR (151 MHz, CD2Cl2, δ): 28.1, 28.8, 37.67, 50.9, 55.6, 76.1, 76.8, 124.0, 126.1, 128.0, 128.3, 128.5, 128.7, 128.8, 128.9, 130.6, 133.5, 135.9, 137.0, 149.4, 150.1, 164.2, 166.6, 201.6.

HRMS-ESI (m/z): [M + H]+ calcd for C64H71O10N6S4, 1211.4109; found, 1211.4119.

Φ(3,3)-TAMBn-NBoc (2-17). 2-16 (1.6669 g, 1.38 mmol) was dissolved in 900 mL CH3Cl with 10 drops triethylamine in a 1-L round bottom flask, and 2-15 (0.5535 g 2-15•2.59MeOH, 1.37 mmol) was dissolved in 900 mL CH3Cl with 25 drops triethylamine in a separate 1-L round bottom flask. The contents of the two flasks were simultaneously dripped, through capillaries, into 2 L of stirring CH2Cl2 in a 3-necked round bottom flask. The dripping required approximately 6 d, after which the solution was left to stir for an additional day. 3 mL diisopropylethylamine was added to the reaction mixture to trap unreacted 2-15, and the reaction mixture was again left to stir for a day before concentration by rotary evaporation and purification by column chromatography (1-4% MeOH in CH2Cl2). Pure product (71%), as a yellow foamy solid, could only be obtained after elution from three silica columns due to the excess of triethylamine and diisopropylethylamine present in the reaction mixture.

1H NMR (500 MHz, CD2Cl2, δ): 1.38 (s, t-Bu CH3, 18H), 1.57 (t, J = 6.0 Hz, –CH2CH2CH2TAM and –CH2CH2CH2NHBOc, 12H), 2.28 (br s, –CH2CH2CH2TAM, 8H), 2.34 (br s, –CH2CH2CH2NHBOc, 4H), 3.08 (br q, J = 6.0 Hz, –CH2CH2CH2NHBOc, 4H), 3.32 (q, J = 6.2 Hz, –CH2CH2CH2TAM, 8H), 4.94 (s, Bn CH2, 8H), 7.29-7.36 (m, Bn Ar H and TAM Ar H, 24 H), 8.02 (t, J = 5.8 Hz, TAM amide NH, 4H).
13C NMR (151 MHz, CD2Cl2, δ): 27.2, 28.7, 38.1, 39.83, 42.2, 50.5, 51.2, 52.3, 54.4, 77.3, 79.0, 125.7, 129.0, 129.0, 129.2, 132.5, 137.0, 150.7, 156.4, 165.6.

HRMS-ESI (m/z): [M + H]⁺ calcd for C72H93O12N8, 1261.6907; found 1261.6908.

Φ(3,3)-TAMBn-moeTAMBn (2-18). A 50-mL round bottom flask was charged with 2-17 (0.5081 g, 0.403 mmol), 9 mL CH2Cl2, and a stir bar, and placed in an ice-water bath. Trifluoroacetic acid (4 mL, 52 mmol) was added dropwise to the stirring, cooled solution dropwise over 6 min. The reaction progress was monitored by TLC every 10-15 min. The water bath was removed 30 min following the addition, and the reaction mixture was left at room temperature for 25 min. It was then placed on the vacuum line to evaporate the solvent, side products, and excess acid (by coevaporation with CH2Cl2). The thick oil was placed on an ice-water bath, dissolved in approximately 8 mL CH2Cl2, and neutralized with 1.5 mL triethylamine. The reaction mixture was left to stir for 15 min, removed from the bath, and combined with a solution of TAMBnthiaz-moe (the synthesis of which was detailed in Progress Report #2) (0.880 g, 1.64 mmol) in 10 mL CH2Cl2. The solution was stirred for 18 h at room temperature, and transferred to a separatory funnel, to which 20 mL H2O was added. The aqueous layer was acidified with 1M HCl(aq) to pH 7, and extracted with 4 times with 5-10 mL CH2Cl2. The combined organic layers were combined, concentrated by rotary evaporation, and purified by column chromatography (1-10% MeOH in CH2Cl2). Purification with two columns and drying (by rotary evaporation and vacuum line) afforded an off-white solid (72%).

1H NMR (600 MHz, CD2Cl2, δ): 1.78 (br s, -CH2CH2CH2TAM, 8H), 2.21-2.42 (m, -CH2CH2C2H4TAM and -CH2CH2C2H4TAMmoe and -CH2CH2CH2TAMmoe, 14H), 3.22 (s, moe CH3, 6H), 3.23-3.28 (m, -CH2CH2C2H4TAMmoe and -C2H5CH2CH2TAM, 12H), 3.40 (t, J = 4.2 Hz, -CH2CH2OCH3, 6H), 3.50 (q, J = 4.5 Hz, -C2H5CH2OCH3, 4H), 4.93 (s, TAM Bn CH2, 8H), 5.12 (s, TAMmoe Bn CH2, 8H), 7.23 (br s, TAM Ar H, 4H), 7.28-7.44 (m, Bn Ar H), 7.77 (d, J = 6.5 Hz, TAMmoe Ar H, 2H), 7.85 (d, J = 7.0 Hz, TAMmoe Ar H, 2H), 7.94 (TAMmoe amide NH, 4H), 7.97 (t, J = 4.2 Hz, TAM amide NH, 4H).

13C NMR (151 MHz, CD2Cl2, δ): 27.1, 27.3, 38.1, 38.8, 40.2, 51.1, 51.9, 59.0, 71.4, 77.3, 77.6, 77.7, 125.8, 126.6, 126.8, 129.1, 129.1, 129.2, 129.2, 129.2, 129.2, 129.3, 129.3, 129.3, 129.4, 131.3, 136.6, 136.6, 137.0, 150.8, 151.1, 151.1, 164.5, 165.6.

HRMS-ESI (m/z): [M + 2H]²⁺ calcd for C112H124O18N10, 948.4542; found, 948.4546.

Φ(3,3)-TAM-moeTAM (L²). A 25-mL round-bottom flask (previously soaked in an EDTA bath) was charged with 2-18 (0.1981 g, 0.105 mmol), 5 mL glacial acetic acid, and a stir bar. After a few minutes and with stirring, the solid dissolved, and 5 mL concentrated HCl was added to the solution. The reaction mixture was left to stir at room temperature for 3 d, and put on the vacuum line with gentle heating, using a 50 °C water bath. The solution was coevaporated with several portions of H2O and MeOH, and left on the vacuum line at room temperature overnight. An off-white solid (85%) was obtained as the HCl salt of L².

1H NMR (600 MHz, DMSO-d6, δ): 1.91 (br m, -CH2CH2CH2TAM, 8H), 1.99 (br m, -CH2CH2CH2TAMmoe), 3.15 (br m, -CH2CH2CH2TAM and -CH2CH2CH2TAMmoe, 12H), 3.27 (s, moe CH3, 6H), 3.39-3.41 (m, -CH2CH2CH2TAM and -C2H5CH2OCH3, 10H), 3.46-3.50 (m, -CH2CH2OCH3 and -C2H5CH2OCH3 and -CH2CH2CH2TAMmoe, 10H), 7.24 (s, TAM Ar H, 4H), 7.36 (d, J = 7.5 Hz, TAMmoe Ar H, 2H), 7.40 (d, J = 7.5 Hz, TAMmoe Ar H, 2H), 8.97-8.99 (m, TAM amide NH and TAMmoe amide NH, 6H), 9.04 (t, J = 4.5 Hz, TAMmoe amide
NH, 2H), 12.54 (s, TAM OH, 2H), 12.55 (s, TAM OH, 2H), 12.58 (s, TAMmoe OH, 2H), 12.69 (s, TAMmoe OH, 2H).

$^{13}$C NMR (151 MHz, DMSO-$d_6$, δ): 22.8, 23.1, 36.0, 36.4, 49.1, 19.5, 57.9, 70.0, 115.8, 115.9, 116.0, 117.2, 117.3, 117.5, 149.9, 149.9, 150.0, 168.5, 168.8, 168.9.

HRMS-ESI (m/z): [M - 2H]^- calcd for C$_{56}$H$_{73}$O$_{18}$N$_{10}$, 1173.5110; found, 1173.5105.

Anal. calcd % (found %) for L$_2$-4HCl (C$_{56}$H$_{78}$Cl$_4$N$_{10}$O$_{18}$): C, 50.91(50.82); H, 5.95 (5.28), N, 10.60 (10.05).

**Synthesis of L$^3$**

**Mono-formyl-di-Cbz-tren (2-19).** 2-6 (3.45 mmol) was formylated with formic acid (6 mL, 159 mmol) in 3 mL acetic anhydride. Formic acid was added to a solution of the starting material 2-6 in acetic anhydride, and the reaction mixture was heated to 60 °C with a reflux condenser for 19 h (the reaction was verified by TLC at 9 h and incomplete). The reaction mixture was then rotary evaporated, redissolved in 10 mL water, and basified to pH 8-9 with 1 M NaOH, which resulted in immediate formation of a white precipitate. Recrystallization from ethanol-water was unsuccessful, yielding a small amount of oil. Instead, the product was extracted from water with 4 x 25 mL dichloromethane. Rotary evaporation of the organic layer afforded the product as a clear yellow oil (100%).

$^1$HNMR (600 MHz, CD$_2$Cl$_2$, δ): 2.44 (br t, tren CH$_2$, 2H), 2.50 (br t, tren CH$_2$, 4H), 3.14 (t, J = 5.1 Hz, tren CH$_2$, 2H), 3.18 (br tr, tren CH$_2$, 2H), 3.23 (br tr, tren CH$_2$, 2H), 5.01 (s, Bn CH$_2$, 4H), 5.81 (br s, NH, 2H), 6.96 (br s, NH, 1 H), 7.28-7.31 (m, Ph, 10H), 7.97 (s, -NC$_2$H$_2$O, 1H).

$^{13}$CNMR (125 MHz, CD$_2$Cl$_2$, δ): 35.8, 39.1, 54.3, 54.7, 66.4, 127.7, 127.8, 128.3, 137.0, 157.0, 161.4.

HRMS-ESI (m/z): [M + H]$^+$ calcd for C$_{21}$H$_{29}$O$_4$N$_7$+, 443.2276; found, 443.2281.

**Mono-formyl-tren (2-20).** 2-19 (3.96 mmol) was dissolved in methanol and combined with a suspension of Pd/C (0.4011 g, Sigma, 10% wt. on activated C) in methanol, for a total reaction volume of 30 mL. The reaction vessel was placed in a par-bomb, which was purged three times with at least 500 psi H$_2$ prior to filling to 1500 psi. The reaction was stirred at this H$_2$ pressure at room temperature for 15 h. Following pressure release, the reaction mixture was filtered through a fine glass frit and rotary evaporated to a light yellow oil. The oil was dissolved in water and eluted through an ion-exchange column (Dowex 21K XLT resin) to remove any carbonate. Approximately 800 mL of eluent was collected (at pH > 7) and rotary evaporated to afford a clear, light yellow oil (75%).

$^1$HNMR (600 MHz, CD$_2$Cl$_2$, δ): 2.47 (t, J = 5.7 Hz, -NC$_2$H$_2$CH$_2$NH$_2$, 4H), 2.53 (t, J = 6.0 Hz, -NC$_2$H$_2$CH$_2$NCHO, 2H), 2.68 (t, J = 6.0 Hz, -NC$_2$H$_2$CH$_2$NH$_2$, 4H), 3.25 (t, J = 5.7 Hz, -NC$_2$H$_2$CH$_2$NCHO, 2H), 8.08 (s, -NCHO, 1H).

$^{13}$CNMR (125 MHz, CD$_2$Cl$_2$, δ): 36.5, 39.7, 55.5, 57.1, 161.2.

HRMS-ESI (m/z): [M + H]$^+$ calcd for C$_7$H$_{19}$ON$_4$+, 175.1553; found, 175.1551.

$\Phi$(2,2)-TAMOBn-Boc-Formyl (2-22). A CHCl$_3$ solution of 2-9 (0.937 mmol) with eight drops triethylamine and a CHCl$_3$ solution of 2-8 (1.039 mmol) with 20 drops triethylamine, each of 750 mL, were slowly added dropwise (by capillary action) into a 5-L round-bottom flask containing 1.5 L CH$_2$Cl$_2$. This high-dilution reaction was stirred at room temperature and required five days to deplete the CHCl$_3$ solutions. Since the reaction mixture was still yellow, indicating the presence of unreacted 2-9, 0.103 mmol of the amine, 2-8, in 80 mL CHCl$_3$, was added dropwise
over 11 h (at which point the reaction was still yellow) and let stir for an additional 10 h (previously, a sacrificial amine, such as diisopropylethylamine, was added to the reaction mixture instead). The reaction had become colorless, and was purified as is (without removal of solvent) by silica column chromatography. The desired product eluted with 4% MeOH/CH₂Cl₂, which was rotary evaporated to yield a clear, faintly yellow oil (60%).

\[ ^1 \text{H} \text{NMR (600 MHz, CD}_2\text{Cl}_2, \delta): 1.34 \text{ (s, t-Bu CH}_3, 9\text{H}), 2.43 \text{ (t, } J = 6.3 \text{ Hz, R}_2\text{NCH}_2\text{CH}_2\text{NH}, 4\text{H}), 2.49 \text{ (t, } J = 6.3 \text{ Hz, R'RNC}_2\text{H}_2\text{NHTAM, 4H}), 2.54 \text{ (t, } J = 6.3 \text{ Hz, R'RNC}_2\text{H}_2\text{NHTAM, 4H}), 3.04 \text{ (br q, } J = 6.6 \text{ Hz, R}_2\text{NCH}_2\text{CH}_2\text{NH}, 2\text{H}), 3.15 \text{ (q, } J = 6.2 \text{ Hz, R}_2\text{NCH}_2\text{CH}_2\text{NH}, 2\text{H}), 3.32 \text{ (q, } J = 6.4 \text{ Hz, R'RNC}_2\text{H}_2\text{NHTAM, 4H}), 3.37 \text{ (q, } J = 6.2 \text{ Hz, R'RNC}_2\text{H}_2\text{NHTAM, 4H}), 5.01 \text{ (s, Bn CH}_2, 4\text{H}), 5.02 \text{ (s, Bn CH}_2, 4\text{H}), 5.14 \text{ (br s, amide NH, 1H), 6.84 (br s, amide NH, 1H), 7.13 (s, Ar H, 4H), 7.35-7.37 (m, Ph, 20H), 7.47 (br s, amide NH, 1H), 7.56 (br s, amide NH, 1H), 7.90 (s, -NC CH O, 1H).} \]

\[ ^{13} \text{C} \text{NMR (125 MHz, CD}_2\text{Cl}_2, \delta): 28.1, 35.3, 37.1, 37.1, 52.3, 52.8, 76.5, 76.7, 78.6, 124.7, 124.8, 128.3, 128.3, 128.4, 128.4, 128.5, 128.6, 128.6, 131.3, 132.2, 136.5, 136.6, 150.4, 150.5, 155.9, 161.0, 165.5, 165.6. \]

HRMS-ESI (m/z): [M + H]⁺ calcd for C₆₂H₇₃O₁₁N₈⁺, 1105.5393; found, 1105.5390.

\[ \Phi (2,2)\text{-TAMOBn-Boc-TAMmoe (2-23). Starting material 2-22 (0.401 mmol) and 9.6 mL 5M NaOH (48 mmol) were dissolved in 100 mL ethanol and 8.4 mL water. The reaction mixture was lightly refluxed at 85 °C for 75 min.} \]

\[ ^1 \text{H} \text{NMR of a 2 mL aliquot of the reaction mixture indicated 97% deformylation and an intact Boc group. The reaction mixture was further refluxed for 10 min and rotary evaporated to a tan foam, which was redissolved in 50 mL water and 40 mL brine and extracted with 4 x 50 mL CH₂Cl₂. The organic fractions were dried over MgSO₄ and reduced to 80 mL by rotary evaporation. To this solution was added 0.463 mmol TAMOBN-thiaz (2-5) and 2 drops of triethylamine. The reaction mixture was stirred at room temperature for 25 h, rotary evaporated, and purified by silica column chromatography (1-5% MeOH/CH₂Cl₂). The product eluted with 5% MeOH and was a clear, light yellow oil (71%) upon removal of the solvent.} \]

\[ ^1 \text{H} \text{NMR (600 MHz, CD}_2\text{Cl}_2, \delta): 1.35 \text{ (s, t-Bu CH}_3, 9\text{H}), 2.39 \text{ (t, } J = 6.3 \text{ Hz, R}_2\text{NCH}_2\text{CH}_2\text{NH}, 2\text{H}), 2.49-2.53 \text{ (m, R'RNC}_2\text{H}_2\text{NHTAM + R}_2\text{NCH}_2\text{CH}_2\text{NHTAMmoe, 6H}), 2.59 \text{ (br s, R'RNC}_2\text{H}_2\text{NHTAM, 4H}), 3.02 \text{ (br q, } J = 5.4 \text{ Hz, TAMC}_2\text{H}_2\text{OMe, 2H}), 3.20 \text{ (m, R}_2\text{NCH}_2\text{CH}_2\text{NHBoc, 2H}), 3.21 \text{ (s, moe CH}_3, 3\text{H}), 3.35-3.42 \text{ (m, R'RNC}_2\text{H}_2\text{NHTAM + R}_2\text{NCH}_2\text{CH}_2\text{NHTAMmoe, 10H}), 3.48 \text{ (q, } J = 4.8 \text{ Hz, TAMCH}_2\text{CH}_2\text{OMe, 2H}), 4.96 \text{ (s, Bn CH}_2, 4\text{H}), 4.96 \text{ (s, Bn CH}_2, 4\text{H}), 5.04 \text{ (s, Bn CH}_2, 4\text{H}), 5.05 \text{ (s, Bn CH}_2, 4\text{H}), 7.00 \text{ (s, Ar H, 4H), 7.30-7.39 (m, Ph, 30H), 7.44 (d, } J = 7.8 \text{ Hz, Ar H, 2H), 7.57 (br s, amide H, 1H), 7.67 (d, } J = 6.0 \text{ Hz, amide H, 4H), 8.08 (t, } J = 5.1 \text{ Hz, amide H, 1H).} \]

\[ ^{13} \text{C} \text{NMR (125 MHz, CD}_2\text{Cl}_2, \delta): 28.1, 36.7, 37.1, 37.7, 39.6, 52.1, 52.2, 52.4, 52.6, 53.8, 58.4, 70.7, 76.4, 76.5, 76.8, 76.9, 78.6, 124.7, 125.0, 125.4, 125.8, 128.3, 128.4, 128.5, 128.6, 128.6, 130.3, 131.6, 131.8, 132.3, 136.1, 136.3, 136.7, 150.3, 150.4, 150.5, 150.5, 155.8, 164.3, 164.7, 165.5, 165.8. \]

HRMS-ESI (m/z): [M + H]⁺ calcd for C₄₈H₄₆O₁₃N₉⁺, 1494.7020; found, 1494.6994.

\[ \Phi (2,2)\text{-TAMOBn-TAMBuN-TAMmoe (2-24). 2-23 (0.285 mmol) was dissolved in 7 mL CH₂Cl₂ and cooled in an ice-water bath. 2 mL trifluoroacetic acid (TFA) was added dropwise to the starting material solution with stirring over 10 min. The reaction progress was monitored by TLC every 10-15 min; after 70 min, the solvent was removed on the vacuum line. The resulting light} \]
orange oil was redissolved in 5 mL CH$_2$Cl$_2$ and dried again, a procedure that was repeated twice more. The oil was then dissolved in 5 mL CH$_2$Cl$_2$ and neutralized with 2 mL triethylamine, which was added dropwise to the solution on an ice bath until no more vapor was evolved. A CH$_2$Cl$_2$ solution of 2-21 (0.285 mmol, a gift from Dr. Sylvie Pailloux) was added to the amine solution (for a total reaction volume of 25 mL). The reaction mixture was let stir at room temperature for 18 h, and transferred to a separatory funnel with 15 mL water. The aqueous layer was acidified to pH 7-8 and extracted with 3 x 25 mL CH$_2$Cl$_2$. The organic fractions were rotary evaporated and purified by silica column chromatography (1-4%MeOH/CH$_2$Cl$_2$). The desired product eluted with 4% MeOH/CH$_2$Cl$_2$, part of which was impure (¹HNMR and MS indicated the presence of starting material 2-23). The impure fraction was further purified using the chromatron (silica plate, 2-8% MeOH/CH$_2$Cl$_2$). The content of the fractions were determined by ¹HNMR, since the product, 2-24, and starting material, 2-23, have the same TLC $R_f$. Rotary evaporation of the pure product fractions afforded a clear, yellow oil (61%).

¹HNMR (500 MHz, CD$_2$Cl$_2$, $\delta$): 1.34-1.37 (m, TAMCH$_2$CH$_2$CH$_2$NH)$_2$Cbz, 4H), 2.40-2.46 (m, R'RNCH$_2$CH$_2$NH-, 4H), 2.55 (s, R'RNC$_2$H$_2$CH$_2$NHTAM, 8H), 3.06 (q, $J$ = 6.5 Hz, TAMC$_2$H$_2$CH$_2$OMe, 2H), 3.21-3.26 (m, TAMC$_2$H$_2$CH$_2$CH$_2$C$_6$H$_5$NHCbz + R'RNCH$_2$C$_6$H$_5$NH-, 6H), 3.37 (d, $J$ = 5.0 Hz, R'RNC$_2$H$_2$CH$_2$NHTAM + R'RNCH$_2$C$_6$H$_5$NH-, 10H), 3.47 (q, $J$ = 5.3 Hz, TAMC$_2$H$_2$CH$_2$OMe, 2H), 4.88 (s, Bn CH$_2$, 4H), 4.88 (s, Bn CH$_2$, 4H), 5.05 (s, Bn CH$_2$, 4H), 5.05 (s, Bn CH$_2$, 4H), 5.08 (s, Cbz CH$_2$, 2H), 6.83 (d, $J$ = 1.5 Hz, Ar H, 4H), 6.85-7.37 (m, Ph, 45H), 7.41 (d, $J$ = 8.5 Hz, Ar H 2H), 7.48 (t, $J$ = 8.0 Hz, Ar H, 2H), 7.60-7.66 (m, amide H, 7H), 7.80 (br t, amide H, 1H).

¹³CNMR (125 MHz, CD$_2$Cl$_2$, $\delta$): 26.5, 27.3, 36.7, 36.8, 37.1, 39.3, 39.6, 51.8, 52.4, 52.5, 58.3, 70.6, 76.4, 76.8, 76.9, 76.9, 124.6, 125.4, 125.8, 125.8, 127.8, 127.9, 128.3, 128.3, 128.3, 128.4, 128.5, 128.5, 128.5, 128.6, 128.6, 128.6, 128.7, 128.7, 128.7, 128.7, 130.0, 130.2, 132.0, 132.1, 136.1, 136.1, 136.3, 136.3, 136.7, 136.7, 150.3, 150.3, 150.3, 150.3, 150.4, 164.2, 164.3, 164.7, 164.8, 165.7.

HRMS-ESI ($m/z$): [M + H]$^+$ calcd for C$_{115}$H$_{120}$O$_{19}$N$_{11}$+, 1958.8756; found, 1958.8761.

Φ(2,2)-TAMBuN-TAMmoe (L$^3$). 2-24 (0.0811 mmol) was dissolved in 7 mL glacial acetic acid in a round-bottom flask that had been soaked in an EDTA bath overnight. 7 mL 12.1 N HCl was added to the starting material solution, and the reaction mixture was stirred at room temperature for 3 days. The acid was removed on the vacuum line, and the resulting off-white solid was resuspended in methanol three times for coevaporation. The solid was dissolved in methanol and precipitated with the addition of ether. This off-white product was filtered and dried on the vacuum line (93%).

¹HNMR (600 MHz, D$_2$O/NaOD, $\delta$): 1.48-1.53 (m, TAMCH$_2$CH$_2$CH$_2$CH$_2$NH)$_2$Cbz, 2H), 1.58-1.63 (m, TAMCH$_2$CH$_2$CH$_2$CH$_2$NH)$_2$Cbz, 2H), 2.93 (t, $J$ = 7.2 Hz, R$_2$NCH$_2$CH$_2$NH-, 4H), 2.96 (t, $J$ = 6.0 Hz, R'RNC$_2$H$_2$CH$_2$NHTAM, 8H), 3.35 (t, $J$ = 7.2 Hz, TAMC$_2$H$_2$CH$_2$OMe, 2H), 3.39 (s, moe CH$_3$, 3H), 3.45 (t, $J$ = 5.7 Hz, TAMC$_2$H$_2$CH$_2$CH$_2$NH)$_2$Cbz + R$_2$NCH$_2$CH$_2$NH-, 8H), 3.54-3.61 (m, R'RNCH$_2$C$_6$H$_5$NH, 8H), 3.64 (t, $J$ = 5.4 Hz, TAMC$_2$H$_2$OMe, 2H), 6.59 (s, Ar H, 4H), 6.92 (d, $J$ = 3.6 Hz, Ar H, 4H).

¹³CNMR (125 MHz, D$_2$O/NaOD, $\delta$): 26.4, 29.5, 29.6, 36.4, 36.5, 38.4, 38.8, 39.5, 40.4, 52.3, 58.1, 71.0, 110.9, 111.4, 115.7, 116.2, 116.3, 116.4, 116.5, 116.7, 165.1, 165.5, 165.7, 165.8, 172.0, 172.1.

HRMS-ESI ($m/z$): [M - H]$^-$ calcd for C$_{51}$H$_{64}$O$_{17}$N$_{11}$, 1102.4487; found, 1102.4468.
Anal. calcd % (found %) for C$_{51}$H$_{70}$N$_{11}$ O$_{17}$·5HCl: C, 47.62 (49.98); H, 5.48 (5.50); N, 11.98 (11.62).
Analytical HPLC (CH$_3$CN/0.1%TFA): rt 13.555 min, 94.4%.

[ThL$_3$]$_4$PyH • L$_3$ (0.0102 g, 0.0077 mmol) was dissolved in 4 mL MeOH in a 10-mL round-bottom flask (previously soaked in an EDTA bath) containing a stir bar. Th(NO$_3$)$_4$•4H$_2$O (0.0042, 0.0076 mmol) dissolved in 1 mL MeOH was added dropwise to the ligand solution, resulting in the immediate color change of the reaction mixture from colorless to yellow as well as the formation of fine, white precipitate. 2 drops of pyridine (~0.01 mL, 0.1 mmol) were added to the reaction mixture, producing more precipitation. The flask was attached to a condenser and the reaction mixture was refluxed, while stirring, in an oil bath for 4.5 h. It was then cooled to room temperature and filtered to isolate a light brown solid, which was dried in vacuo overnight (76%).

HRMS-ESI (m/z): [M + 3H]$^-$ calcd for C$_{56}$H$_{69}$O$_{18}$N$_{10}$Th$_1$, 1401.5177; found, 1401.5191.
REFERENCES


CHAPTER 3

SOLUTION THERMODYNAMIC BEHAVIOR OF $\Phi(2,2)$MOETAM AND ITS TH(IV) COMPLEX

Introduction

Ultimately, since any alpha therapeutic thorium complex will be intravenously administered, it is imperative that it remain complexed in vivo. Released Th(IV) would cause radiation toxicity, since it forms hydroxides and colloids that can react with many proteins, amino acids, and other biological molecules to form stable complexes that aggregate primarily in the liver, bone, spleen, and kidneys.\(^1\) Even if the picomolar concentrations used in alpha therapy were to render this risk negligible, the low aqueous solubility of these thorium hydroxides and colloids would cause precipitation of $^{227}$Th and prevent its specific targeting. In vivo stability is not merely a thermodynamic characteristic—experimental conditions can be vastly simplified relative to those in the blood, and an understanding of the pharmacokinetics requires extensive studies in in vivo models. Nonetheless, the metal-ligand binding constant is an important physical property, and one that is lacking for many Th(IV) complexes. For instance, the stability of the widely used macrocycle DOTA has not been measured with Th(IV), despite the extensive research already performed on the evaluation of a Th-DOTA conjugate for radiotherapeutic applications.\(^2\) The measurement of the protonation constants of $L$\(^1\) and its solution thermodynamic stability with Th(IV) will be detailed in this chapter.

Solution Thermodynamics and Determination of Stability Constants

The binding affinity of the ligands was determined by solution thermodynamic studies, where the cumulative formation constants $\beta_{mlh}$ for the equation below were determined.

\[
mM^{a+} + mL^{b-} + hH^+ \rightleftharpoons M_nL_h^{(ma-lb+h)+}
\]

The cumulative formation constants for ligand-proton equilibria are simply the products of the protonation constants, or $pK_a$'s, as detailed in the equations below (the equations in blue are highlighted to emphasize key substitutions). Formation constants for metal-ligand-proton equilibria follow directly analogous equations. In this example, the determination of the binding constant $\beta_{110}$ requires prior knowledge of the protonation constants $\beta_{011}$ to $\beta_{01h}$. Formation constants are experimentally determined by titration of the system with solutions of base and acid, where the data collected are pH and added volume (for the number of protons removed or added, respectively) or pH and absorbance, when the changes observed are too slight. For a given set of protonation and formation constants—the model—an absorbance can be calculated at every pH from the total concentrations of metal and ligand. Non-linear least-squares refinement, via a computerized algorithm, calculates the best model to fit the data.
Chapter 3

Protonation Constants

Due to the high number of $pK_a$'s and moderate water solubility, UV-Vis spectrophotometric titrations were performed to determine the protonation and constants of $L_1$. A large spectral change (as the UV absorption shifts from 340 to 380 nm and increases in intensity) arising from the $\pi \rightarrow \pi^*$ transition of the TAMs upon deprotonation allows the $pK_a$'s to be determined spectrophotometrically. Although the $pK_a$'s of the hydroxyl groups of a bidentate TAM unit are 6.1 and 11.0,$^3$ those in $L_1$ range from 3.95 to 12.79 (Table 3-1). Statistical factors alone would result in $pK_a$ differences of log 2 in $L_1$ relative to those of a bidentate TAM. For example, if the structural symmetry of the two TAMs in the macrocycle were maintained in the

\[
L + H \rightleftharpoons HL \quad K_1 = [LH] / [H][L]
\]

\[
[\text{LH}] = K_1 [H][L] = \beta_{011} [L][H]
\]

\[
HL + H \rightleftharpoons H_2L \quad K_2 = [LH_2] / [L][H]
\]

\[
[\text{LH}_2] = K_2 [L][H] = K_1 K_2 [L][H]^2 = \beta_{012} [L][H]^2
\]

\[
M + L \rightleftharpoons ML \quad K_1 = [ML] / [M][L]
\]

\[
[\text{ML}] = K_1 [M][L] = \beta_{110} [M][L]
\]

\[
ML + H \rightleftharpoons MLH \quad K_2 = [MLH] / [ML][H]
\]

\[
[\text{MLH}] = K_2 [ML][H] = K_1 K_2 [M][L][H] = \beta_{111} [M][L][H]
\]

\[
\beta_{111} = K_1 K_2 
\] \[
\log \beta_{111} = \log K_1 + \log K_2
\]

\[
c_L = [\text{L}] + [\text{LH}] + [\text{LH}_2] + [\text{LH}_3] + \ldots 
= [\text{L}] + \beta_{011} [\text{L}][H] + \beta_{012} [L][H]^2 + \beta_{013} [M][L][H]^3 
= [\text{L}] (1 + \beta_{011} [H] + \beta_{012} [H]^2 + \beta_{113} [H]^3) 
= [\text{L}] \left( 1 + \sum_{i = 1 \to 3} \beta_{0i} [H]^i \right)
\]

\[
\frac{[\text{L}]}{c_L} = \frac{1}{1 + \sum_{i = 1 \to 3} \beta_{0i} [H]^i}
\]

\[
[\text{LH}] = \beta_{011} [H][L] / c_L = \beta_{011} [H][L] / c_L
\]

\[
[\text{LH}] / c_L = \beta_{011} [H][L] / c_L
\]

\[
\beta_{111} = K_1 K_2 
\] \[
\log \beta_{111} = \log K_1 + \log K_2
\]

\[
c_M = [\text{M}] + [\text{ML}] + [\text{MLH}] + [\text{MLH}_2] 
= [\text{M}] + \beta_{110} [M][L] + \beta_{111} [M][L][H] + \beta_{112} [M][L][H]^2 
= [\text{M}] \left( 1 + \beta_{110} [L] + \beta_{111} [L][H] + \beta_{112} [L][H]^2 \right)
\]

\[
\frac{[\text{M}]}{c_M} = \frac{1}{1 + \sum_{i = 1 \to 3} \beta_{0i} [H]^i}
\]

\[
[\text{M}] / c_M = \frac{1}{1 + \beta_{110} [L] + \beta_{111} [L][H] + \beta_{112} [L][H]^2}
\]

Protonation Constants of $\Phi(2,2)$mocTAM ($L_1$)
deprotonation equilibria, $pK_a$'s of 5.8 and 6.4 would be observed for the sites where they would be 6.1 in an isolated TAM unit. The considerably larger range exhibited by the $pK_a$'s indicate further interactions between the various deprotonated states. As is apparent in the representative titration shown in Figure 3-1, a large pH range was required to attain all the protonation states of $L$, and the UV-Vis spectra of the individual species display significant overlap.

Table 3-1. Log $pK_a$ Values for $L$.

<table>
<thead>
<tr>
<th>$pK_a$</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$pK_{a1}$</td>
<td>12.79(2)</td>
</tr>
<tr>
<td>$pK_{a2}$</td>
<td>11.9(4)</td>
</tr>
<tr>
<td>$pK_{a3}$</td>
<td>11.1(2)</td>
</tr>
<tr>
<td>$pK_{a4}$</td>
<td>9.3(4)</td>
</tr>
<tr>
<td>$pK_{a5}$</td>
<td>7.57(9)</td>
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<tr>
<td>$pK_{a6}$</td>
<td>6.1(6)</td>
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<td>$pK_{a7}$</td>
<td>5.73(6)</td>
</tr>
<tr>
<td>$pK_{a8}$</td>
<td>3.95(4)</td>
</tr>
<tr>
<td>$pK_{a9}$</td>
<td>3.30(7)</td>
</tr>
<tr>
<td>$pK_{a10}$</td>
<td>1.9(2)</td>
</tr>
</tbody>
</table>
The determination of the ligand $pK_a$'s was the most challenging aspect of the solution thermodynamics for this system, as the high number of parameters and incremental spectral changes rendered the refinement rather difficult. This is apparent from the rather large correlation coefficients between the refined protonation constants (Table 3-2). While such a model is not optimal, it was unavoidable given that the ten different protonation states of $L^1$ have UV spectra very similar to one another, with only slight shifts in $\lambda_{\text{max}}$ values (Fig. 3-5). Simplifying the model with the assumption that the symmetry in the ligand reduces the number of distinct $pK_a$'s by half was not a viable option. In early titrations, the pH range attained was not sufficiently wide, and a seemingly acceptable fit was reached with a model with five $pK_a$'s. Increasing the range, however, made it possible to refine all ten constants, which are not five pairs of identical.

Figure 3-1. Spectrophotometric titration of $L^1$. Starting conditions: 70 $\mu$M $L^1$, 0.1 M KCl, and 1 mM each of MES, HEPES, and CHES (25°C). a) $A$ vs. wavelength plot at varying pH (data abridged for clarity; spectra normalized for dilution). b) $A$ vs. pH at 380 nm (experimental data points are blue circles, red crosses are calculated absorbances) overlaid onto speciation.
constants. Evidently the symmetry is not preserved in the partially deprotonated $L^1$, and the deprotonations from chemically equivalent sites are not equivalent.

Table 3-2. Correlation Coefficients for $L^1$ Protonation Constants.

<table>
<thead>
<tr>
<th></th>
<th>$L^1H_2$</th>
<th>$L^1H_3$</th>
<th>$L^1H_4$</th>
<th>$L^1H_5$</th>
<th>$L^1H_6$</th>
<th>$L^1H_7$</th>
<th>$L^1H_8$</th>
<th>$L^1H_9$</th>
<th>$L^1H_{10}$</th>
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<tbody>
<tr>
<td>$L^1H_1$</td>
<td>0.92</td>
<td>0.977</td>
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<td></td>
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<tr>
<td>$L^1H_2$</td>
<td></td>
<td></td>
<td>0.843</td>
<td>0.876</td>
<td>0.917</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>$L^1H_3$</td>
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<td></td>
<td>0.624</td>
<td>0.633</td>
<td>0.667</td>
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<td>$L^1H_4$</td>
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<td>0.834</td>
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<td></td>
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<td>0.384</td>
<td>0.383</td>
<td>0.402</td>
<td>0.536</td>
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<tr>
<td>$L^1H_6$</td>
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<td></td>
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<td>$L^1H_7$</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>$L^1H_9$</td>
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<td></td>
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<tr>
<td>$L^1H_{10}$</td>
<td>0.335</td>
<td>0.345</td>
<td>0.36</td>
<td>0.428</td>
<td>0.378</td>
<td>0.285</td>
<td>0.725</td>
<td>0.929</td>
<td>0.855</td>
</tr>
</tbody>
</table>

The lowest $pK_a$'s correspond to the deprotonations of the tertiary amines. While these are many order of magnitude lower than the $pK_a$ of triethylamine, large shifts were also observed in analogous ligands with tren backbones. These are not associated with large spectral changes like with the TAM hydroxyl deprotonations, as can be seen from the isolated spectra of $L^1H_{10}$, $L^1H_9$, and $L^1H_8$ (Fig. 3-5). The $pK_a$'s for $L^1H_{10}$ and $L^1H_9$ species were then verified by potentiometric titration (Fig. 3-2), with reasonable agreement. The difficulty in refining for all of the $pK_a$'s of $L^1$ using this method is obvious in the smoothness of the potentiometric titration curve.
Measurement of the Th(IV) stability constant required the use of a competing ligand in excess, otherwise ThL\(^4\) would already be formed at the start of the titration at pH 2. In the presence of DTPA, the Th-DTPA complex instead forms at this low pH, and at approximately pH 4, the [ThL\(^4\)H\(^3\)]\(^3\)- complex begins to form, which can be monitored spectrophotometrically. The largest change in absorbance occurs at pH 5-6, when the [ThL\(^4\)H\(^3\)]\(^3\)- becomes deprotonated to form [ThL\(^4\)]\(^4\)- (Fig. 3-3).

**Figure 3-2.** Potentiometric titration of L\(^1\). (a) Starting conditions: 0.5 mM L\(^1\), 0.1 M KCl, 5% DMSO (25°C). The ligand solution was acidified with HCl to pH 1.8 prior to titration with 0.09981 M KOH. (b) Protonation constants for pK\(_{a9}\) and pK\(_{a10}\) refined from potentiometric titration.

**Stability Constants of \(\Phi(2,2)\text{moeTAM} \ (L^1)\) with Th(IV)**

Stability constants of the ligand L\(^1\) with Th(IV) are given in Table 3-1.
The log $\beta_{110}$ was found to be 53.7(5), 24 orders of magnitude greater than that of DTPA (28.78, Table 3-3). Its greater log $\beta_{110}$ value relative to the log $\beta_{140} = 45.54$ for ethyl-TAM suggests that the higher denticity and/or macrocyclic topology confers substantial thermodynamic stability to the ThL$_1$ complex. A measure of the complex stability is also provided by the pTh value, or the -log of the free thorium concentration at a specific pH and $[\text{Th}]_{\text{tot}} = 1 \mu\text{M}$ and $[\text{L}]_{\text{tot}} = 10 \mu\text{M}$. This value demonstrates the effect of protonation on metal-
ligand equilibria, providing a direct comparison of the complexing abilities of various ligands, which have different protonation constants, at physiologically relevant conditions. While DTPA has a higher pTh at low pH, due to its greater acidity, L\(^1\) has a remarkably higher pTh at neutral and higher pH, signifying negligible Th dissociation from L\(^1\) in vivo.

Table 3-3. Log \(\beta_{mlh}\) and pTh Values.

<table>
<thead>
<tr>
<th>Log value(^a)</th>
<th>L</th>
<th>Ligand</th>
<th>ETAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta_{1ll})</td>
<td>53.7(5)</td>
<td>28.78</td>
<td>45.54</td>
</tr>
<tr>
<td>(\beta_{1ll}; \ pK_a)</td>
<td>58.9(6); 5.2(2)</td>
<td>30.94; 2.16</td>
<td>—</td>
</tr>
<tr>
<td>pTh(^b)</td>
<td>pH 3.0</td>
<td>12.0</td>
<td>15.4</td>
</tr>
<tr>
<td>pH 7.4</td>
<td>39.1</td>
<td>25.6</td>
<td>19.4</td>
</tr>
<tr>
<td>pH 9.0</td>
<td>45.4</td>
<td>28.2</td>
<td>24.7</td>
</tr>
</tbody>
</table>

\(^a\)Refined from DTPA competition titrations

\(^b\)pTh = -log([Th\(^{4+}\)]\(_{tot}\) - [Th\(^{4+}\)]\(_{tot}\) = 10\(^{-6}\) M, [L]\(_{tot}\) = 10\(^{-5}\) M
The thorium stability constant measured with the DTPA competition titrations was verified with analogous experiments using EDTA as the competing ligand (Fig. 3-6). The results from the EDTA titrations are in very good agreement with those using DTPA, but the definitive value for the formation constant was refined solely from the DTPA titrations since EDTA does not have enough donor atoms to fill the coordination sphere of the Th(IV) ion. It is then possible for ternary complexes of Th-EDTA to form, which would be difficult to accurately detect by UV-Vis. A specie of Th-EDTA-L may be responsible for the deviation from the model at pH 4.5 (Fig. 3-6b), where the absorbance is larger than expected.

Figure 3-5. Molar absorbances of species of L and ThL.
Figure 3-6. Spectrophotometric competition titration of $\text{ThL}^4$ with EDTA. Starting conditions: 50 $\mu$/M $\text{L}^4$, 50 $\mu$/M Th, 500 $\mu$/M EDTA (Z), and 0.1 M KCl (25°C). a) $A$ vs. wavelength plot at varying pH (data abridged for clarity). b) $A$ vs. pH at 378 nm (experimental data points are blue circles, red crosses are calculated absorbances) overlaid onto speciation. c) Formation constants refined from EDTA titrations.

<table>
<thead>
<tr>
<th>log $\beta_{110}$</th>
<th>54(1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log $\beta_{111}$</td>
<td>59.9(8)</td>
</tr>
<tr>
<td>pM</td>
<td>42</td>
</tr>
</tbody>
</table>
Solution Thermodynamics with the Linear Analog 3,4,3-LiMeTAM (L⁴)

More extensive solution thermodynamic studies have been performed with the linear analog (Fig. 3-7) of Φ(2,2)-moeTAM (L¹), in an effort to understand the role of the topological features of the macrocycle in the high stability of the Th complex.

![Chemical structures of 3,4,3-LiMeTAM (L⁴).](image)

**Figure 3-7.** Chemical structures of 3,4,3-LiMeTAM (L⁴).

The protonation constants of L⁴ (Table 3-4) were determined solely by UV-Vis titration (Fig. 3-8a); the lack of tertiary amines precluded the need for potentiometric titration. The lowest pKₐ (3.9) is the same as the lowest hydroxyl pKₐ of L¹ (whose most acidic protons are those on the tertiary amines). The most basic constant of L⁴ is higher than that of L¹, and since it was too high to access, it was set to a constant value of 13.0 when refining for the remaining seven values. This fully deprotonated ligand was thus not observed in the titrations, which accounts for its noisy refined spectrum (Fig. 3-8b).

![UV-Vis titration of L⁴.](image)

**Figure 3-8.** UV-Vis titration of L⁴. Starting conditions: 50 μM L⁴, 1 mM each of MES, HEPES, CHES, 0.1 M KCl, 5% DMSO (25°C). pH 2.0-12.9. A vs. pH plot of forward titration at 376 nm, overlaid on speciation diagram. Blue diamonds are experimental data, red x's are calculated points.
Table 3-4. Log pK\textsubscript{a} Values for L\textsuperscript{4}.

<table>
<thead>
<tr>
<th>pK\textsubscript{a1}</th>
<th>(13.0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pK\textsubscript{a2}</td>
<td>12.1</td>
</tr>
<tr>
<td>pK\textsubscript{a3}</td>
<td>12.0</td>
</tr>
<tr>
<td>pK\textsubscript{a4}</td>
<td>9.9</td>
</tr>
<tr>
<td>pK\textsubscript{a5}</td>
<td>8.3</td>
</tr>
<tr>
<td>pK\textsubscript{a6}</td>
<td>7.6</td>
</tr>
<tr>
<td>pK\textsubscript{a7}</td>
<td>5.9</td>
</tr>
<tr>
<td>pK\textsubscript{a8}</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Value in parentheses was set constant in the refinement.
Figure 3-9. UV-Vis titration of ThL with EDTA. Starting conditions: 50 μM L, 50 μM Th, 500 μM EDTA, 0.1 M KCl, 5% DMSO (25°C). pH 3.2-11.6. a) Absorption spectra at different pH values. Indicated values are those at which the spectra stop changing. b) Absorbance vs. pH plot of backward titration at 382 nm, overlaid on speciation diagram. Blue diamonds are experimental data, red x's are calculated points.

The protonation constants were used to determine the stability constants of L with Th(IV). EDTA competition titrations (Fig. 3-9) could only be fit to a more complex model than with L (Table 3-9), suggesting the presence of a mixed ligand specie at pH 4-7. This is suggestive of the less effective encapsulation of Th(IV) by the linear ligand compared to the macrocycle. These titrations refined formation constant values log β and log β higher than those for the macrocycle, but the pM value is the same, as the higher complex formation constants are offset by its higher protonation constants. DTPA competition titrations confirmed these β's (Fig. 3-10, Table 3-5), with a model that does not include the presence of a mixed-ligand specie. The model fit is far from perfect, however, and these competition titrations are still a work in progress. The poor solubility of L at low pH (despite the use of 5% DMSO) made it difficult to observe the formation of the ThLH complex, which exhibits an already small spectroscopic change relative to the protonated ligand, as observed in the L titrations.
Figure 3-10. UV-Vis titration of ThL₄ with DTPA. Starting conditions: 50 μM L⁴, 50 μM Th, 500 μM DTPA (Y), 0.1 M KCl, 5% DMSO (25°C), pH 4.3-11.5. A vs. pH plot at of backward titration at 386 nm, overlaid onto speciation diagram. Blue diamonds are experimental data, red x's are calculated points.

Figure 3-11. Molar absorbances of species of L⁴ and ThL₄.
Table 3-5. Formation constants of ThL⁴ with and pTh values, as determined by spectrophotometric competition titration with either EDTA or DTPA.

<table>
<thead>
<tr>
<th>Species</th>
<th>EDTA Titration</th>
<th>DTPA Titration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>log β</td>
<td>pTh</td>
</tr>
<tr>
<td>[ThL⁴]⁺</td>
<td>56.0</td>
<td>38.9</td>
</tr>
<tr>
<td>[ThL⁴H]⁻</td>
<td>62.0</td>
<td>46.5</td>
</tr>
<tr>
<td>[ThL⁴H₂]²⁻</td>
<td>68.0</td>
<td>58.3</td>
</tr>
<tr>
<td>[ThL⁴H₃]⁻</td>
<td>75.0</td>
<td>65.8</td>
</tr>
<tr>
<td>[ThL⁴ZH₄]⁺</td>
<td>90.0</td>
<td>78.0</td>
</tr>
</tbody>
</table>

*pTh = -log[Th⁴⁺]free; [Th⁴⁺]₂ = 10⁻⁶ M, [L]₅ = 10⁻⁵ M
Z = EDTA⁴⁻

Figure 3-12. Speciation diagram for ThL⁴: 10 μM L⁴ and 1 μM Th(IV).

EDTA and DTPA competition titrations suggest that the pTh value with L¹ is the same as that with L⁴. While this is not surprising since both ligands have the same number of identical binding moieties, it is somewhat disappointing that the macrocycle does not confer a stability advantage over the linear analog. Perhaps the pendant arms of the macrocycle—which give it the same number of end groups as the linear ligand—reduce its thermodynamic stability relative to ligand of purely macrocyclic topology. However, these studies have demonstrated that L¹, encapsulate the Th(IV) ion more thoroughly than L⁴. In titrations with EDTA as the competing ligand, a Th-L-EDTA ternary complex was observed with L⁴ but not with L¹ (and it was not observed in titrations with DTPA with either ligand). This is potentially a structural advantage in vivo, where a Th(IV) complex with L¹ is much less likely to bind biomolecules other than the target receptor, which could affect the binding, transport, and excretion of the complex. Even though the speciation diagrams of L¹ (Fig. 3-4) and L⁴ (Fig. 3-12) indicate that the formation of
the protonated complex occurs at the same pH with both ligands, this is only one aspect of the solution-state behavior of this system.

Structural Characterization of $L^1$ and Th$L^1$

Single crystals suitable for XRD were grown by the vapor diffusion of acetonitrile into an aqueous solution of the free ligand. It crystallized as the hydrochloride tetrahydrate in $P2_1/n$ space group, with an inversion center inside the molecule (the asymmetric unit is half of the ligand). Without any metal ion to complex, $L^1$ adopts a structure that maximizes the $\pi$-stacking (3.309 Å) between the aromatic TAMs as well as the hydrogen bonding between the phenol protons and the adjacent carbonyl oxygens, as observed in crystal structures of previous TAM ligands (Fig. 3-13, Table 3-6). The XRD data was of sufficiently high quality to locate the hydrogens; the hydrogen bond distances between the phenol hydrogens and the adjacent carbonyls range from 1.596 to 1.882 Å. However, the phenol groups closest to the tren backbone on the pendant TAMs do not adopt this geometry, with their protons pointing to the adjacent phenol instead. The planarity of the TAM is maintained, but the nitrogen of the amide rather than the oxygen is oriented toward the hydroxyl group. This is due to the hydrogen bonding between the amide oxygen and the protonated tertiary amine (1.675 Å). Each chloride counterion is loosely bound by interactions with two amide protons (2.399 Å from the pendant TAM and 2.491 Å from the ring TAM of the same ligand molecule) and one water molecule (2.256 Å).

The thorium complex (Fig. 3-14, Table 3-6) was in turn crystallized from a methanol and dimethylformamide solution, into which diethyl ether and tetrahydrofuran were diffused. In principle, the macrocyclic TAMs of the ligand could chelate a metal ion in two possible configurations, either equatorially, with the pendant units on opposite sides of the metal-macrocycle plane (pseudo-$C_{2h}$ symmetry), or in a configuration of lower symmetry (pseudo-$C_2$ symmetry), where the pendant arms are adjacent to one another (Fig. 3-15). As the protonated ligand, $L^1$ crystallizes with inversion symmetry (as in the pseudo-$C_{2h}$), but was observed to adopt the $C_2$-symmetric conformation around the metal center when bound to Th(IV). When deprotonated and coordinated to the metal ion, the main hydrogen-bonding interaction in $L^1$ observed is between the amide proton and the bound phenol oxygen, which is observed in all catecholamide metal complexes.

DFT-level calculations (B3LYP/6-31G, SDD(f))$^{10}$ of the complex in these two possible conformations suggest that the observed mode is the more thermodynamically stable one. The calculated $C_2$-symmetric structure with adjacent pendant units is 22 kcal/mol lower in energy than the corresponding $C_{2h}$-symmetric structure, a stabilization that can be attributed to the macrocyclic strain induced by the large diameter of Th(IV). Increasing the ring size with tris(2-aminopropyl)amine (trpn) instead of tren in the ligand backbones (as in $L^2$) reduces this calculated energy difference to 13 kcal/mol. The inner coordination environment of the crystallized complex was analyzed with a quantitative shape measure (SM),$^{11}$ which compares the dihedral angles of adjacent faces in the crystal structure's coordination polyhedron to those of idealized polyhedra most common in eight-coordinate complexes: the bicapped trigonal prism ($C_{2v}$), trigonal dodecahedron ($D_{2d}$), and square antiprism ($D_{4d}$). The coordination of Th$L^1$ about
the metal center is intermediate between the ideal $C_{2v}$ and $D_{2d}$ geometries [SM = 5.3 ($C_{2v}$), 7.7 ($D_{2d}$), 11.1 ($D_{4d}$)].

Figure 3-13. ORTEP diagrams of the protonated ligand L$^1$H$_{10}$·2HCl. (a) Entire molecule, generated by an inversion operation of the asymmetric unit. (b) Showing hydrogen bond distances on one pendant TAM and one macrocyclic TAM. Ellipsoids are shown at 50% probability (gray C, red O, blue N, green Cl). Water molecules are omitted for clarity.
Figure 3-14. ORTEP diagram of the [ThL\textsuperscript{1}]\textsuperscript{4+} complex. Potassium ions, solvent molecules, and hydrogens in are omitted for clarity. Ellipsoids are shown at 50% probability (gray C, red O, blue N, lime-green Th).

Figure 3-15. Diagrams illustrating possible chelation modes of Th(IV) by L\textsuperscript{1}. a) Pseudo-$C_{2h}$ symmetric structure, where the pendant TAMs lie on opposite sides of the equatorially-bound macrocyclic TAMs. b) Pseudo-$C_{2}$ symmetric structure as observed in the crystal structure, where the pendant TAMs are on the same side of the macrocycle. (Images rendered using POV-Ray)
Chapter 3

ThL\textsuperscript{1} Crystal Structure: Analysis of the Coordination Polyhedron

The shape analysis previously mentioned does not provide a very descriptive measure of the coordination polyhedron about the Th(IV) center. Despite considerable distortion, the TAM binding units can be viewed as spanning the edges of a trigonal dodecahedron. Further analysis was carried out to determine whether the TAMs span the $g$ or the $m$ edges of the dodecahedron (Fig. 3-16a) described by Hoard and Silverton.\textsuperscript{12} While spanning of the $m$ edges in dodecahedral eight-coordinate complexes is much more common, a Ce(BrMaltol)$_4$ crystal structure that has been reported features the unusual spanning of the $g$ edges.\textsuperscript{13}

Table 3-6. Crystallographic data and structure refinement for L\textsuperscript{1}H\textsubscript{10}2HCl\textsubscript{4}H\textsubscript{2}O and ThL\textsuperscript{1}K\textsubscript{4}(DMF)\textsubscript{2}(MeOH)\textsubscript{2}·THF.

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<tr>
<th>Empirical formula</th>
<th>C\textsubscript{50}H\textsubscript{72}Cl\textsubscript{2}N\textsubscript{10}O\textsubscript{22}</th>
<th>C\textsubscript{62}H\textsubscript{84}K\textsubscript{4}N\textsubscript{12}O\textsubscript{23}Th</th>
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<tbody>
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<td>1753.85 g/mol</td>
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</tr>
<tr>
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<td>$P\bar{T}$</td>
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<td>$a = 11.347(2)$ Å $\alpha = 79.129(2)^\circ$</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>$c = 29.9278(15)$ Å $\gamma = 90^\circ$</td>
<td>$c = 20.044(3)$ Å $\gamma = 79.929(2)^\circ$</td>
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<td>3638.8(8) Å$^3$</td>
</tr>
<tr>
<td>$Z$</td>
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<td>2</td>
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<tr>
<td>$\rho_{calcd}$</td>
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<td>1.551 g/cm$^3$</td>
</tr>
<tr>
<td>$\mu_{calcd}$</td>
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<td>2.357 mm$^{-1}$</td>
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<tr>
<td>$F(000)$</td>
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<td>1719</td>
</tr>
<tr>
<td>Crystal size</td>
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<td>0.21 x 0.09 x 0.06 mm$^3$</td>
</tr>
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<td>1.03 to 50.74$^\circ$</td>
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<td>45683</td>
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<td>Independent reflections</td>
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<td>13322 [R(int) = 0.0928]</td>
</tr>
<tr>
<td>Completeness to $\theta = 25.00^\circ$</td>
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<td>99.9 % Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>0.8367 and 0.2593</td>
<td>0.8715 and 0.6374</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.386 and 0.203</td>
<td>Full-matrix least-squares on $F^2$</td>
</tr>
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<td>Refinement method</td>
<td>Full-matrix least-squares on $F^2$</td>
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<td>Data / restraints / parameters</td>
<td>5013 / 0 / 432</td>
<td>13322 / 102 / 954</td>
</tr>
<tr>
<td>Goodness-of-fit on $F^2$</td>
<td>1.037</td>
<td>1.038</td>
</tr>
<tr>
<td>Final $R$ indices [$I &gt; 2\sigma(I)$]</td>
<td>$R_1 = 0.0355$, $wR_2 = 0.0919$</td>
<td>$R_1 = 0.0709$, $wR_2 = 0.1711$</td>
</tr>
<tr>
<td>$R$ indices (all data)</td>
<td>$R_1 = 0.0432$, $wR_2 = 0.0965$</td>
<td>$R_1 = 0.1147$, $wR_2 = 0.1973$</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>0.673 and -0.268 e·Å$^{-3}$</td>
<td>1.568 and -1.564 e·Å$^{-3}$</td>
</tr>
</tbody>
</table>
In order to determine whether the binding moieties are closer to the \( m \) edges or the \( g \) edges, an idealized \( m \)-edge-spanned dodecahedron was generated and the angles between the actual TAMs and the ideal ones was calculated. The coordinates of the oxygen atoms (translated to place Th at the origin) were first converted into a spherical coordinate system (the numbering scheme used is shown in the diagram in Fig. 3-16). In order to align the \( z \)-axis with the \( S_4 \) axis of the molecule, a fictional atom O9 was placed at the midpoint between O4 and O5. Since O9 was already quite close to the \( x \)-axis, the entire system was rotated such that O9 would coincide with it.

<table>
<thead>
<tr>
<th>Atom</th>
<th>Coordinates translated to place Th at (0,0,0)</th>
<th>Spherical coordinates</th>
<th>Rotated to align O9 with x-axis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( x )</td>
<td>( y )</td>
<td>( z )</td>
</tr>
<tr>
<td>O1</td>
<td>-2.03145</td>
<td>-0.52067</td>
<td>-1.30124</td>
</tr>
<tr>
<td>O2</td>
<td>-0.34182</td>
<td>-2.30284</td>
<td>-0.48447</td>
</tr>
<tr>
<td>O3</td>
<td>0.897894</td>
<td>-0.28006</td>
<td>-2.18886</td>
</tr>
<tr>
<td>O4</td>
<td>0.24499</td>
<td>2.044808</td>
<td>-1.26188</td>
</tr>
<tr>
<td>O5</td>
<td>-1.67966</td>
<td>1.408628</td>
<td>0.968</td>
</tr>
<tr>
<td>O6</td>
<td>-0.97625</td>
<td>-0.8428</td>
<td>2.05637</td>
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<tr>
<td>O7</td>
<td>2.26218</td>
<td>-0.6384</td>
<td>0.544854</td>
</tr>
<tr>
<td>O8</td>
<td>1.103703</td>
<td>1.422192</td>
<td>1.584021</td>
</tr>
<tr>
<td>O9</td>
<td>-0.71734</td>
<td>1.726718</td>
<td>-0.14694</td>
</tr>
</tbody>
</table>

The spherical coordinates were converted back to Cartesian coordinates in order to exchange the \( x \)- and \( z \)-axes, and then back to spherical coordinates to calculate the angle of the binding moieties from the vertical.

<table>
<thead>
<tr>
<th>Atom</th>
<th>Cartesian coordinates</th>
<th>Cartesian coordinates with axis change</th>
<th>Spherical coordinates</th>
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</thead>
<tbody>
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<td>( z )</td>
</tr>
<tr>
<td>O1</td>
<td>0.312118</td>
<td>2.170289</td>
<td>-1.13294</td>
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</table>

\[Figure 3-16.\] Ideal trigonal dodecahedra. (a) \( m \) edges are highlighted in red, while 4 of the 8 \( g \) edges are highlighted in blue. (b) Diagram depicting numbering scheme used in analysis.
For a given edge, e.g. the one defined by O1 and O2, its angle off the vertical was calculated as $d\phi$, the difference between the $\phi$ of O1 (or O2) and that of the average $\phi$ of O1 and O2 (Fig. 3-17). This difference was then converted into degrees, and the required sweep angle for the edge to be vertical is twice this value.

<table>
<thead>
<tr>
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<th>Spherical coordinates</th>
<th>Angles off the vertical</th>
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</thead>
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<td>r</td>
<td>$\theta$</td>
</tr>
<tr>
<td>O1</td>
<td>2.468022</td>
<td>1.443992</td>
</tr>
<tr>
<td>O2</td>
<td>2.377949</td>
<td>2.537303</td>
</tr>
<tr>
<td>O3</td>
<td>2.382388</td>
<td>1.778504</td>
</tr>
<tr>
<td>O4</td>
<td>2.415285</td>
<td>0.68203</td>
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<tr>
<td>O5</td>
<td>2.396357</td>
<td>0.67426</td>
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<tr>
<td>O6</td>
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<td>O8</td>
<td>2.397898</td>
<td>1.219189</td>
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</table>

Figure 3-17. Diagram showing the "straightening" of the edges of the dodecahedron.

Two of the binding moieties (O1-O2 and O5-O6) are only slightly slanted from the vertical, whereas the remaining two are more greatly slanted, with angles of 14.7° and 19.5°. However, the binding moieties are still closest to the $m$ edge, as angles of approximately 60° (moving from one side of a trigonal face to the other) would be required for them to be on the $g$ edge. In order to visualize the discrepancies quantified by these angles, an idealized dodecahedron was generated by applying the $S_4$ rotation matrix onto a the "straightened" O1-O2 edge (from the Cartesian coordinates of the spherical coordinates in the previous table) and repeatedly onto subsequently generated edges. "Straightened" spherical coordinates for O1 and O2 were generated using average r and $\phi$ values, and the $\theta$ value was calculated as the sum of the original
\( \theta \) and \( d\phi \) to account for the shortening of the edge resulting from averaging the \( \varphi \) values of \( O1 \) and \( O2 \) (Fig. 3-17).

<table>
<thead>
<tr>
<th></th>
<th>Spherical coordinates</th>
<th>&quot;Straightened&quot; coordinates</th>
<th>Cartesian coordinates, ( S_4 )-generated dodecahedron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r ) ( \theta ) ( \varphi )</td>
<td>( r ) (avg) ( \theta ) (( \theta + d\varphi )) ( \varphi ) (avg)</td>
<td>( x ) ( y ) ( z )</td>
</tr>
<tr>
<td>( O1 )</td>
<td>2.468022 1.443992 -0.48111</td>
<td>2.422986 1.427085 -0.49802</td>
<td>2.115826 -1.15044 0.265745</td>
</tr>
<tr>
<td>( O2 )</td>
<td>2.377949 2.537303 -0.51493</td>
<td>2.422986 2.55421 -0.49802</td>
<td>1.238903 -0.67363 -1.97033</td>
</tr>
<tr>
<td>( O6 )</td>
<td></td>
<td></td>
<td>1.150444 2.115826 -0.26574</td>
</tr>
<tr>
<td>( O5 )</td>
<td></td>
<td></td>
<td>0.673632 1.238903 1.97033</td>
</tr>
<tr>
<td>( O8 )</td>
<td></td>
<td></td>
<td>-2.11583 1.150444 0.265745</td>
</tr>
<tr>
<td>( O7 )</td>
<td></td>
<td></td>
<td>-1.2389 0.673632 -1.97033</td>
</tr>
<tr>
<td>( O3 )</td>
<td></td>
<td></td>
<td>-1.15044 -2.11583 -0.26574</td>
</tr>
<tr>
<td>( O4 )</td>
<td></td>
<td></td>
<td>-0.67363 -1.2389 1.97033</td>
</tr>
</tbody>
</table>

\( S_4 \) rotation matrix =

\[
\begin{pmatrix}
0 & 1 & 0 \\
-1 & 0 & 0 \\
0 & 0 & -1
\end{pmatrix}
\]

Figure 3-18. Experimental edges of \( \text{ThL}^1 \) dodecahedron (black, with red oxygen atoms) overlaid on idealized, \( S_4 \)-generated dodecahedron \( m \) edges(gray). The angles in the front are between (a) \( O1 \) and \( O2 \), (b) \( O3 \) and \( O4 \), (c) \( O5 \) and \( O6 \), (d) \( O7 \) and \( O8 \). (Images rendered using POV-Ray)
The TAMs that are closest to the \( m \) edge are the pendant ones; it is likely that they can achieve this more favorable coordination geometry because they are less physically constrained than the macrocyclic TAMs. The macrocyclic binding units have the largest deviation angles, providing further evidence for the overall chelation mode of the ligand being attributable to ring strain.
EXPERIMENTAL DETAILS

Solution Thermodynamics. All spectrophotometric titrations were carried out with constant stirring and a blanket of Ar flow in a jacketed cell connected to a recirculating water bath to maintain the temperature at 25°C. The ionic strength of all solutions was maintained at 0.1 M with 0.1 M KCl in titrand solutions and 0.1 M acid and base titrants. A standardized stock solution of 0.0010(1) M Th(IV) was prepared by dissolving Th(NO₃)₄·4H₂O (Alfa Products) in Millipore water with concentrated HNO₃ (79 mM, pH 1.4). This solution was complexometrically titrated with Na₂H₂EDTA (volumetric standard, Sigma Aldrich) to the yellow endpoint, using pyrocatechol violet as the indicator. The ligand was added as a 50 mM DMSO solution, prepared by dissolution of the solid ligand, weighed on an analytical balance accurate to 0.01 mg. The HCl and KOH solutions were prepared by dilution of Dilut-It (J.T. Baker, ampoules) concentrated solutions with degassed Millipore water. The 0.1 M HCl solution was standardized by the potentiometric or colorimetric (using bromocresol green as indicator) titration of tris(hydroxymethyl)aminomethane, and the 0.1 M KOH solution was standardized by potentiometric titration of potassium hydrogen phthalate or the standardized HCl solution. The KOH solution was stored under a blanket of Ar flow and standardized before every titration. The glass electrode (Metrohm Microtrode) used for the pH measurements was calibrated by the titration of 1.000 mL standardized 0.1 M HCl in 25.0 mL 0.1 M KCl with standardized 0.1 M KOH to pH 11.6. The titration was analyzed using the program GLEE to refine for E° and the slope. This calibration was also performed prior to each titration. The automated titration system was controlled by a Metrohm Titrando 907 and the program Tiamo® light. 2 mL Dosino 800 burets dosed the titrant into the titration vessel (5-90 mL). UV-Vis spectra were acquired with an Ocean Optics USB4000-UV-Vis spectrometer equipped with a dip probe (set to a 10 mm path length) and a DH-2000 light source (deuterium and tungsten lamps), using the program Spectra Suite. For titrations with L⁴, spectra were acquired with a diode-array HP8452 spectrometer, and 5% DMSO was used in the electrolyte and titrant solutions. Titrations were performed at least in triplicate.

Ligand Titrations. 10 mL solutions of 50 μM ligand and 20 equivalents (1 mM) each of MES, HEPES, and CHES, were titrated forward and backward between pH 1.8 and 12.7 with standardized 0.1 M KOH and 0.1 M HCl. Data points (pH readings and UV-Vis spectra) were collected following 0.040-1.000 mL titrant additions, with an equilibration time of 180 sec. The 1 mL titrant additions were performed above pH 11 due to the buffering capacity of water at high pH, and to minimize the exposure of the electrode to basic solution. 0.1 M HCl was added to the solution prior to the forward titration to start at a pH below 2. All absorbance measurements used in the refinement were no more than 1.0 absorbance units. Spectra at 250-450 nm (at intervals of approximately 0.1 nm) were analyzed (simultaneously) in the program Hypspec. (Hypspec, from the Hyperquad suite of programs by Peter Gans, is an updated version of pHAb, previously used in the group.) For potentiometric titrations, 10 mL solutions of 0.5 mM L¹ were titrated forwards and backwards between pH 1.8 and 12.5. The data were analyzed using the Hyperquad 2008 program.

Th(IV) Competition Titrations. 10 mL solutions of 50 μM ligand, 50 μM Th(IV), 500 μM DTPA (Pharm-Eco) or EDTA (volumetric standard, Sigma) were titrated forward and backward with between pH 1.8 and 10.0 with 0.1 M KOH and 0.1 M HCl. The Th(IV) was added to a solution of DTPA at pH 7.4, and this solution was allowed to equilibrate for at least 24 h prior to the addition of ligand. The data points (pH readings and UV-Vis spectra) were collected.
following 0.040-0.100 mL titrant, with an equilibration time of 10 min following KOH additions (forward titrations) or 20 min following HCl titrations (backward titrations). 0.1 M HCl was added to the solution prior to the forward titration to start at a pH below 2. All absorbance measurements used in the refinement were no more than 1.1 absorbance units. Spectra of 250-450 nm (at intervals of approximately 0.1 nm) were analyzed (simultaneously) in the program Hypspec. The following values for the log $\beta$ for the formation of Th hydroxides were included in the refinement: [ThOH]$^{3+}$, -2.5; [Th(OH)$_2$]$^{2+}$, -6.2; Th(OH)$_6$, -17.4; [Th$_2$(OH)$_4$]$^{6+}$, -5.9; [Th$_2$(OH)$_5$]$^{5+}$, -6.8; [Th$_4$(OH)$_8$]$^{8+}$, -20.4; [Th$_4$(OH)$_{12}$]$^{4+}$, -26.6; [Th$_6$(OH)$_{14}$]$^{10+}$, -36.8; [Th$_6$(OH)$_{15}$]$^{9+}$, -36.8. The following protonation and stability constants for DTPA and the Th-DTPA complex were also included: log $\beta_{011}$, 10.4; log $\beta_{012}$, 18.95; log $\beta_{013}$, 23.23; log $\beta_{014}$, 25.93; log $\beta_{015}$, 27.93; log $\beta_{016}$, 29.53; log $\beta_{017}$, 30.23; log $\beta_{110}$, 28.78; log $\beta_{111}$, 30.94; log $\beta_{1111}$, -8.88. Spectra and protonation constants of the free ligand, refined from the ligand titrations, were set constant in the refinement.

[Th$L^1$]4K. $L^1$ (13.65 mg, 0.0114 mmol) was suspended in 7 mL methanol at 45 °C. A solution of Th(NO$_3$)$_4$·4H$_2$O (Alfa, 6.12 mg, 0.0111 mmol) in 1 mL MeOH was added dropwise to the ligand solution while stirring, causing an immediate color change to yellow. Naturally-occurring 232Th, which has a half-life of 1.4 x 10$^{10}$ years, was used for ease of handling. A stoichiometric amount of 1M KOH in methanol (0.09 mL, 0.0910 mmol) was added to the ligand suspension dropwise, to a pH of 8, solubilizing the reaction mixture. (The thorium solution can alternatively be added after the base.) The reaction mixture was refluxed under nitrogen flow for 3 h. Once cooled to room temperature, the product was precipitated by addition of diethyl ether and concentration of the reaction mixture. The tan precipitate was filtered and dried overnight under vacuum, resulting in a light brown solid (15.11 mg, 93%). 1H NMR (500 MHz, D$_2$O + NaOD + DMSO-$d_6$): 2.264 (br t, 2H, tren CH$_2$), 2.365 (br t, 4H, tren CH$_2$), 2.647 (br t, 4H, tren CH$_2$), 3.069 (s, 6H, CH$_3$), 3.133-3.259 (m, 14H, tren CH$_2$, methoxyethanamide CH$_2$), 3.510 (d, $J$ = 10 Hz, 4H, methoxyethanamide CH$_2$), 3.743 (d, $J$ = 11.5 Hz, tren CH$_2$), 6.662-6.681 (d, 2H, ArH; s, 4H, ArH), 6.785 (d, $J$ = 8.5 Hz, ArH). 13C NMR (125 Hz, DMSO-$d_6$): δ 36.2, 38.2, 38.8, 40.3, 56.1, 58.3, 58.4, 58.5, 70.8, 72.2, 109.6, 109.8, 114.5, 114.6, 114.7, 168.3, 168.5, 168.9, 169.0, 171.0, 171.2, 171.4. HRMS-ESI ($m/z$): [M+H]$^+$ calcd for C$_{50}$H$_{54}$O$_{18}$N$_{10}$Th$_3^-$, 438.1338; found 438.1355.

X-Ray Crystallography. Single crystals of $L^1$H$_8$ suitable for XRD were grown by the vapor diffusion of acetonitrile into an aqueous solution of the protonated ligand. Single crystals of Th$L^1$K$_4$ were grown by the vapor diffusion of 1:1::diethyl ether::tetrahydrofuran into a solution of the complex in 1:20::dimethylformamide::methanol. Selected crystals were mounted in Paratone N oil at the end of a capstan loop and frozen in place under a low-temperature nitrogen stream. The data were collected on Bruker MicroSTAR-H X8 APEX-II CCD with Cu Ka radiation ($L^1$H$_8$) and SMART APEX-I CCD with Mo Ka radiation (Th $L^1$K$_4$) X-ray diffractometers. Intensity data were extracted from the frames with the program APEX2. The data were corrected for Lorentz and polarization effects, and an empirical absorption correction was applied using the SADABS program. The structures were solved by direct methods and refined using full-matrix least squares refinements based on $F^2$. Crystallographic analyses were performed using the WinGX system of programs. All non-hydrogen atoms were refined anisotropically, while hydrogen atoms were assigned to idealized positions (with the exception of the hydrogens in $L^1$H$_8$ bound to heteroatoms and aromatic rings). The free ligand crystallized with two chlorine atoms (balancing the charge on the
protonated tertiary amines) and four water molecules per ligand molecule. The thorium complex crystallized with four potassium atoms, as well as two dimethylformamide, one tetrahydrofuran, and two methanol molecules per asymmetric unit. Disordered solvent molecules in this crystal structure (all but one methanol) were modeled with occupancies of less than one. One of the methoxyethyl substituents (atoms C37, O18, C38 and the attached hydrogens) on the pendant terephthalamide units showed some disorder, which could be satisfactorily modeled over two positions. The structure diagrams in Fig. 3-13 and 3-14 were created using ORTEP-3.22

**Computational Studies.** DFT calculations were performed at the UC Berkeley Molecular Graphics and Computation Facility with Gaussian 09 software and the GaussView graphical user interface.23 The geometries and energies of the complexes were optimized at the B3LYP level with the 6-31G basis set for all atoms except the thorium atoms, for which the Stuttgart/Dresden ECP60MWB_SEG basis set was used to model a 60-electron small core pseudopotential, incorporating quasi-relativistic effects.10
APPENDIX

\(^1\)H NMR titrations of L\(^1\)

\(^1\)H NMR titrations were performed (Fig. A3-1) in an attempt to measure the last protonation constants (which affect all of the others, since formation constants are cumulative protonation constants) of L\(^1\). The assignment of the peaks is unambiguous at high pH (i.e. greater than pH 12), but it becomes much more difficult at lower pH, as the hydrogen bonding network between hydroxyl, amide, and amine groups break the symmetry exhibited by the fully deprotonated ligand. At pH 3.2, where the majority of the ligand is LH\(_9\) and LH\(_{10}\), the tertiary amines are partially protonated, and the methylene protons adjacent to them appear at \(~3.73\) ppm. At pH 7.5, they are highly shifted upfield, to \(~2.74\) ppm. The methylene protons are thus significantly shifted by hydrogen bonding of nearby amines, but they appear as overlapping, broad peaks that are difficult to deconvolute and assign. The aromatic protons, which would be affected by the hydroxyl groups, indicate that the pendant arms are the last to be deprotonated (most basic), as the most downfield aromatic resonance (corresponding to the pendant arms because it is a doublet) shows changes at pH < 12.3, in both shift and multiplicity. This is expected, since the presence of a hydrogen-bonded proton would change both the electron shielding and the symmetry of the nearby atoms.

Figure A3-1. \(^1\)H NMR titration of L\(^1\) (3.000 mM L in H\(_2\)O, pH adjusted with KOH; acetonitrile internal standard). The water signal was suppressed in the spectra shown.
Atomic Coordinates for Crystal and Calculated (DFT) Structures

Table A3-1. Atomic coordinates \( \times 10^4 \) and equivalent isotropic displacement parameters \( \text{Å}^2 \times 10^3 \) for \( \text{L}^1\text{H}_{10} \cdot 2\text{HCl} \cdot 4\text{H}_2\text{O} \). \( U_{eq} \) is defined as one third of the trace of the orthogonalized \( U_{ij} \) tensor.

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<th>z</th>
<th>U(eq)</th>
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Chapter 3

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Figure A3-2. UV-Vis spectrum of ThL (geometry optimization and frequency calculation from crystal structure coordinates) calculated from TD-DFT. $\lambda_{\text{max}} = 391.7$ nm (12000 M$^{-1}$cm$^{-1}$), in good agreement with the experimental value of 377 nm (17039 M$^{-1}$cm$^{-1}$). The geometry and energy of the complex were optimized at the B3LYP level with the 6-31G basis set for all atoms except the thorium atoms, for which the Stuttgart/Dresden ECP78MWB_AVDZ basis set was used to model a 78-electron small core pseudopotential, incorporating quasi-relativistic effects.\textsuperscript{10a-g,23,24}
REFERENCES


11. An algorithm for calculating the dihedral angles in a coordination polyhedron (only taking into account the metal and donor atoms) and minimizing their variance from those of idealized polyhedra was written some years ago (Xu, J.; Radkov, E.; Ziegler, M.; Raymond, K.N. Inorg. Chem. 2000, 39, 4156-4164) and recently updated (Tatum, D.; Raymond, K.N. "Automated δ dihedral angle shape analysis for coordination numbers four through nine." Manuscript in preparation.). The values stated here were calculated using the updated algorithm.


CHAPTER 4

SOLUTION KINETIC BEHAVIOR OF THE TH(IV) WITH Φ(2,2)MOETAM AND RELATED LIGANDS

Introduction

Radiotherapeutics are inherently time-sensitive, as the radioisotope must be fully sequestered by the chelate as fast as possible to minimize decay prior to administration to the patient. In addition to entropic effects, the high thermodynamic stability of macrocycles with metal ions is attributed to the preorganization of the donor atoms and multiple juxtapositional fixedness, which describes the greater difficulty with which the donor atoms dissociate due to the lack of end groups. However, the rigidity of the ligand causing this stability can also hinder its kinetics of complexation. For example, the polyaminocarboxylic acid macrocycles DOTA and HEHA have slow formation rates with Ln(III) ions. Despite this shortcoming, DOTA is among the most commonly studied macrocycles in radioactive medical applications, but an ideal bifunctional chelator would form the final complex quickly under mild conditions, to avoid damaging the targeting biomolecule.

Preliminary Studies and Challenges

UV-Vis spectroscopy was used to investigate the kinetics of complex formation between Th(IV) and various octadentate tetracatecholate ligands in aqueous solution at physiological pH (a generous gift from Dr. Jide Xu, Fig. 4-1). The formation rate of metal complexation by a ligand can be studied by following the changes in absorbance relative to the spectrum of the free ligand. However, this proved rather challenging due to the subtle differences in UV spectrum of the complex relative to that of the ligand. For example, in the case of the complexation of iron with 3,4,3-LI-CAM(S), the spectrum of the complex shows an absorbance peak in the visible region, where the ligand has zero absorbance (Fig. 4-2). This feature enables iron complexation to be conveniently characterized by pseudo-first order kinetics studies, where the ligand is in excess, since the increase in absorbance of the ligand does not interfere with the monitored wavelengths. This is not the case with thorium complexes, as the lack of a ligand-to-metal charge transfer band makes them colorless, and any UV changes upon complexation are ligand-based: a slight red-shift and increase in UV absorbance are observed for all ligands, as with 3,4,3-LI-CAM(S). These changes become even more subtle in the presence of excess ligand, conditions necessary for pseudo-first order kinetics. Upon combination of the ligand and Th, a rate constant \( k \) can be calculated from absorbance vs. time data using the following equation:

\[
\ln \frac{A_\infty - A}{A_\infty - A_0} = -kt
\]

where \( A_\infty \) is the absorbance at the final time point, \( A_0 \) is the absorbance at the first time point, and \( A \) is the absorbance at any given time \( t \). Wavelengths at which there was the greatest difference in absorbance between the free ligand and the complex were those observed in the kinetics.
experiments. However, in the presence of excess ligand, thorium complexation did not display first-order kinetics, but rather suggested the occurrence of complicated equilibria. With some ligands, an increase in absorbance was followed by a decrease, while others showed a slow gradual increase (Fig. 4-3).
When the ligand and metal were mixed in a 1:1 ratio, the absorbance curves were entirely different (Fig. 4-4). For all the ligands studied, a fast reaction occurred within the first 30 minutes, followed by a much less drastic change in absorbance than with the 10:1::L:Th conditions. The curves could not be fit with first- or second-order rate laws, suggesting the presence of multiple steps and the buildup of intermediates. The large discrepancy in kinetics between the 10:1 and 1:1 ligand to metal ratios could be caused by the slow formation of polynuclear products in the presence of excess ligand following the formation of an intermediate (perhaps an ML complex). This is not unlikely, as the nine-coordinate Th(PR-1,2-HOPO)₄·0.5H₂O complex was crystallized as a linear coordination polymer,⁴ thorium is known to form polymeric structures with halides and hydroxides,⁵ and a ternary complex was observed in titrations with 3,4,3-LI-MeTAM (cf. Chap. 3).

**Figure 4-2.** UV-Vis spectra of 3,4,3-LI-CAM(S) complexed with Fe(III) and Th(IV). ([L] = 50 μM, [Fe] = 50 μM, [Th] = 50 μM, pH 7.4)

When the ligand and metal were mixed in a 1:1 ratio, the absorbance curves were entirely different (Fig. 4-4). For all the ligands studied, a fast reaction occurred within the first 30 minutes, followed by a much less drastic change in absorbance than with the 10:1::L:Th conditions. The curves could not be fit with first- or second-order rate laws, suggesting the presence of multiple steps and the buildup of intermediates. The large discrepancy in kinetics between the 10:1 and 1:1 ligand to metal ratios could be caused by the slow formation of polynuclear products in the presence of excess ligand following the formation of an intermediate (perhaps an ML complex). This is not unlikely, as the nine-coordinate Th(PR-1,2-HOPO)₄·0.5H₂O complex was crystallized as a linear coordination polymer,⁴ thorium is known to form polymeric structures with halides and hydroxides,⁵ and a ternary complex was observed in titrations with 3,4,3-LI-MeTAM (cf. Chap. 3).

**Figure 4-3.** Absorbance vs. time data for 3,4,3-LI-CAM(C) and 3,4,3-LI-CAM(S) mixed with Th(IV) in a 10:1 ratio. ([L] = 50 μM, [Th] = 5 μM, pH 7.4)
Chapter 5

The kinetics of complexation of 3,4,3-LI-CAM(S) were also studied at higher pH (pH = 9, 10, and 11) to determine whether the protonation state of the ligand upon mixing with thorium affects the kinetics of formation. Increasing the buffer pH did not change the kinetics relative to those at pH 7.4, as the absorbance curves very similar to those at pH 7.4. This implies that at this pH, the complete deprotonation of the ligand before complexation with Th is not necessary, which is conceivable given that the metal binding shifts the pKa of the binding unit.

The preliminary studies highlighted the challenges faced with measuring the rates of Th(IV) complexation:
1) Since the spectroscopic changes upon complex formation are mainly due to ligand deprotonation, they are small and difficult to precisely monitor.
2) Even though Th⁴⁺ is the largest tetravalent cation and the most resistant to hydrolysis, hydroxides of Th are very likely to form in aqueous solution at physiological pH. Above pH 3.1, more than 50% of the Th in solution is hydrolyzed ([ThOH]⁻, [Th(OH)₂]²⁻, [Th₄(OH)₁₂]⁴⁺, etc.), and increasingly more so at higher pH values (Fig. 4-5). The thorium species coordinated by the ligand at pH 7.4 is most certainly a hydroxide, but it is unclear whether the fact that it is not a naked Th⁴⁺ ion affects the formation of the desired product or how the dissociation of the hydroxide ion(s) plays into the mechanism.

**Figure 4-4.** Absorbance vs. time data for 3,4,3-LI-CAM(C), H(2,2)-CAM(C), and 3,4,3-LI-CAM(S) mixed with Th(IV) in a 1:1 ratio. ([L] = 50 μM, [Th] = 50 μM, pH 7.4)
Dye-Displacement Studies: Indirect Kinetics

In order to observe the complexation of Th(IV) while addressing the problems mentioned above, an indirect method using azo dyes was used to quantify the relative rates of complexation with different ligands. Two azo dyes (Fig. 4-6) were used because of their selectivity for Th (among other elements such as U, Zr, Hf, Ph, Sc, Pa, Np, Pu, Am, C, rare earth elements) and because of their large color change upon binding of thorium.7 Arsenazo III (Az) changes from pink to green in the presence of thorium, while chlorophosphonazo III (Cz) changes from blue to green. The addition of ligand displaces the dye, and the formation of a thorium-ligand complex can be monitored by disappearance of the thorium-dye complex (or the appearance of the free dye). The reaction is effectively the ligand exchange described by the following general equation:

\[ \text{ThD} + \text{L} \rightarrow \text{ThL} + \text{D} \]

This was performed by complexing thorium with the dye under acidic conditions (since the dye would not have to compete with hydroxide ion, as would be the case at high pH where Th(IV) hydrolysis is significant), and observing the disappearance of the complex upon addition of a ligand. To ensure that all of the thorium is bound by the dye, the appropriate stoichiometry between the dye and metal must be determined. Job plots were performed to find the stoichiometry with the highest absorbance of the complex, but this proved more difficult than expected.

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Figure 4-5. Speciation diagram of Th(IV) hydrolysis.6b
Initially, Job plots indicated a thorium-Az complex stoichiometry of 1:2, but commercially available Az is known to contain significant impurities, and purification of the dye by reverse-phase HPLC (detailed in the Experimental Details and further in the Appendix) gave different results. Previous work supports the existence of both the 1:1 and 1:2 Th:Az complexes, depending on the pH and ratio of reagents, but these studies were not performed at pH 7.4. A Job plot with twice-purified dye (Fig. 4-7) indicates the predominance of a 1:1 complex at this pH.
pH, but since the exact stoichiometry of the complex was not crucial for this indirect kinetic study, an excess of dye was used to ensure that all of the thorium was complexed. The Th-dye complex was formed at acidic pH to avoid the presence of thorium hydroxide species, and buffered to pH 7.4 prior to mixing with the ligand. Based on the pKₐ's of Az (which has eight dissociable protons)¹⁰ and the finding that only one of each of the phenols and arsonic acids of an Arsenazo III molecule bind the metal,⁷ᵇ the thorium-dye complex is more precisely [ThAzH₂]²⁻ and the free dye [AzH₃]⁵⁻ at the buffered pH. The aim was to extrapolate a second-order rate constant by performing this displacement reaction under pseudo-first-order conditions at different concentrations of excess ligand. The analogous Job plot was constructed with purified Chlorophosphonazo III (Fig. 4-8, supporting the predominance of the 1:2 complex),⁷ᶜ but the kinetic experiments were performed most thoroughly with Arsenazo III, since the spectral differences were greater than with Chlorophosphonazo III.

**Figure 4-8.** Job plot of Th(IV) with Chlorophosphonazo III. (a) UV-Vis spectra of solutions of purified Chlorophosphonazo III and Th(IV) at different mole fractions of Chlorophosphonazo III, indicated in the legend ([Cz]ₜₒₜ + [Th]ₜₒₜ = 50 μM, pH 7.4, 100 mM HEPES, 0.1 M KCl). (b) Job plots (absorbance vs. mole fraction of Chlorophosphonazo III) at wavelengths characteristic of the Th-dye complex.
Figure 4-9 shows examples of the curves obtained from pseudo-first order kinetic experiments where an excess of ligand causes the dissociation of the Th-Az complex. While the first-order fits are good, the calculated second-order rate constants decrease with increasing concentrations of \( L^1 \) (Table 4-1). Even though using freshly purified Az improved the reproducibility of the kinetic experiments (and produced a Th-Az Job plot with a well-defined isosbestic point), it is clear that the mechanism of ligand association is not as simple as expected.

Table 4-1. First- and second-order rate constants for the displacement of Arsenazo III (Az) by \( L^1 \), calculated from monitoring at 669 nm (pH 7.4, 100 mM HEPES, 0.1 M KCl, 25 °C).

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<th>Equiv. ( L^1 )</th>
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The first-order rate constant does not show the same decrease, but rather, a lack of correlation to the ligand concentration, as the slope (Fig. 4-10) of regression line of the $k_{\text{obs}}$ vs. $[L^1]$ plot is close to 0 and the $R^2$ value is low.

![Graph of first-order rate constant for absorbance decay at 669 nm upon addition of L^1 to Th-Az complex (using freshly purified Az) vs. total concentration of L^1. (with pH 7.4, 0.1 M KCl, 25 °C).](image)

Figure 4-10. Graph of first-order rate constant for absorbance decay at 669 nm upon addition of L^1 to Th-Az complex (using freshly purified Az) vs. total concentration of L^1. (with pH 7.4, 0.1 M KCl, 25 °C).

Using the same method to investigate the behavior of L^2 under pseudo-first order kinetics, the same puzzling trend is observed. Both the first- and second-order rate constants decrease with increasing L^2 equivalents (Table 4-2, Fig. 4-11).

![Table 4-2. First- and second-order rate constants for the displacement of Arsenazo III (Az) by L^2, calculated from monitoring at 669 nm (using freshly purified Az). (pH 7.4, 0.1 M KCl, 25 °C) ](image)

Table 4-2. First- and second-order rate constants for the displacement of Arsenazo III (Az) by L^2, calculated from monitoring at 669 nm (using freshly purified Az). (pH 7.4, 0.1 M KCl, 25 °C)

<table>
<thead>
<tr>
<th>[Th-Az] (μM)</th>
<th>Equiv. L^2</th>
<th>$k_{\text{obs}}, 669$ nm (s$^{-1}$)</th>
<th>$k_{2,\text{obs}}, 669$ nm (s$^{-1}$ M$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>10</td>
<td>0.00366</td>
<td>73.2</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.00408</td>
<td>81.7</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.00412</td>
<td>41.2</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>0.00340</td>
<td>22.7</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>0.00374</td>
<td>18.7</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>0.00345</td>
<td>13.8</td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>0.00318</td>
<td>10.6</td>
</tr>
</tbody>
</table>
With both Φ ligands, the kinetic data obtained were quite inconsistent. For example, repeating the kinetic experiment with 5 μM Th-Az and 10 equivalents of L1 gives values of $k_{2,\text{obs}}$ that range from 130 - 168 s$^{-1}$M$^{-1}$ (where the standard deviation is 13% of the average). Rates obtained from different wavelengths were even more inconsistent. Data refinement in the regression modeling program SpecFit, which analyzes the kinetics by factor analysis, was unsuccessful due to the lack of a good model. The displacement of an Az from ThAz by a Φ ligand $L$ can be described by various mechanisms. For an associative mechanism, the ligand binds ThAz, and subsequent dissociation of the dye leads to the Th$L$ product. The spectral change observed would not provide any information about the formation of Th$L$ if the dye dissociation is rate-limiting. The dye is still bound to Th(IV) upon initial binding of the ligand, which complicates the kinetics because it is unclear whether the ternary Th-$L$-Az complex has a spectrum distinct from ThAz. In a dissociative mechanism, the ligand could be reacting with a Th(IV) partially bound to Az, a complex from which the complete dissociation of the dye might not be immediate or indicative of the association of the ligand.

A reasonable hypothesis to rationalize this behavior is that system saturation is reached above 10 equivalents of ligand, and the ligand is inhibiting itself, causing a slower reaction at higher concentrations. Ligand self-inhibition could be the result of dimerization or self-catalyzed decomposition, possibilities that seem unlikely. It was ensured that the other components in the reaction were not significantly skewing the results with several control experiments:
1) The ligands alone in buffer (pH 7.4) were monitored by UV-Vis over 12 hours (with a spectrum taken every 4 minutes) with no spectral change. The ligands are thus stable in the aqueous conditions of the kinetic experiments over time and to light.
2) The same control experiment with Th-Az alone in buffer also shows no spectral change.
3) The concentration of DMSO in solution varies slightly, from 0.5 to 3% (v/v), in runs with different concentrations of ligand (since the ligand is stored as a DMSO solution). Two experiments with the same concentration of Th-Az and ligand (5 μM and 10 equivalents, 

![Figure 4-11. Graph of first-order rate constant for absorbance decay at 669 nm upon addition of $L^2$ to Th-Az complex (using freshly purified Az) vs. total concentration of $L^2$. (with pH 7.4, 0.1 M KCl, 25 °C) y = -2.710x + 0.004 R² = 0.566](image)
respectively) but with 0.5 or 3% DMSO gave nearly identical first-order rates at 669 nm (0.00408 s\(^{-1}\) for 0.5% and 0.00407 s\(^{-1}\)).

4) The same experiments were carried without any ligand (5 \(\mu\)M Th-Az, and 0.5 or 3% DMSO), and no spectral change was observed. Therefore, the DMSO is not interfering with any of the components in solution, and its different concentrations within the range used in the kinetics experiments is not affecting the observed rate.

Given the lack of published data on the kinetics of association in water of Th(IV) and a ligand, the dye displacement kinetics method was applied to the complexation of the Th(IV) by the commercially available polyaminocarboxylic acid ligands DOTA and DTPA (Fig. 4-12), as well as with 3,4,3-LiMeTAM (\(L^4\)). This provided context in which to evaluate the kinetic data obtained for the \(\Phi\) ligands.

Using the pseudo-first order kinetic conditions of 10 equivalents of ligand, both DTPA and DOTA were significantly slower—by two orders of magnitude—than either of the \(\Phi\) ligands at displacing Az from its Th complex (Table 4-3). The half-life of the reaction with the \(\Phi\) ligands is on the order of a few minutes (1.6 min. for \(L^1\), 2.9 min. for \(L^2\)), but with DTPA, it is 6.33 hours. DOTA was even slower, as no reaction was observed at room temperature for over a month. Increasing the pH to 9 (using CHES instead of HEPES as buffer) did not produce any spectral change after 3 days. Instead, heating the reaction to 100°C (reflux at pH 7.4, and on a 3-mL scale instead of the 100 \(\mu\)L needed for a cuvette) allowed the DOTA to react with the dye complex within a practical time. Despite this energetic advantage, the half-life of this reaction was relatively high: 4.11 hours.

**Table 4-3.** Second-order rate constants for the displacement of Arsenazo III (Az) by various ligands, calculated from monitoring at 669 nm. The conditions were as follows: 5 \(\mu\)M Th-Az, 10 equivalents ligand, pH 7.4, 0.1 M KCl, and 25 °C or *100°C.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>(k_{2,obs}, 669) (\text{nm (s}^{-1}\text{M}^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\Phi(2,2)-\text{moeTAM}, L^1)</td>
<td>147</td>
</tr>
<tr>
<td>(\Phi(3,3)-\text{moeTAM}, L^2)</td>
<td>79</td>
</tr>
<tr>
<td>3,4,3-LiMeTAM, (L^4)</td>
<td>72</td>
</tr>
<tr>
<td>DTPA</td>
<td>0.61</td>
</tr>
<tr>
<td>DOTA*</td>
<td>0.93</td>
</tr>
</tbody>
</table>
The high kinetic inertness of DOTA has been explained by the existence of a stable, diprotonated intermediate\textsuperscript{11} with Ln(III) ions, where the transverse tertiary nitrogens are protonated (Fig. 4-13). The macrocyclic topology of the ligand hinders the complete complexation, since inversions of the nitrogens would be required for their lone pairs to coordinate to the Th ion. The physical constraints imposed by the ring connecting the coordinating atoms means that the reorganization requires a greater amount of energy relative to that of the complexation by a linear ligand.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{figure.png}
\caption{Scheme of hypothesized structure of ThDOTAH\textsubscript{2}\textsuperscript{2+} complex.}
\end{figure}

Despite the uncertain mechanism in these dye displacement experiments— the rate-limiting step is most likely dye dissociation rather than ligand association in the presence of a fast ligand—\textit{L}\textsuperscript{1} and \textit{L}\textsuperscript{2} are clearly much faster thorium chelators than the macrocyclic DOTA and the linear DTPA. These faster kinetics can be attributed to the greater affinity of TAM relative to the O and N donors in DTPA and DOTA, but likely also to the partially macrocyclic \(\Phi\) topology. Much like a linear ligand, the pendant arms afford greater flexibility that enable faster association to the metal ion. Interestingly, \textit{L}\textsuperscript{1} demonstrates faster indirect association kinetics than both the larger \textit{L}\textsuperscript{2} and the linear \textit{L}\textsuperscript{4}. While the differences in the rates of the \(\Phi\) ligands and 3,4,3-LiMeTAM are not nearly as large as those of the aminocarboxylic ligands from the others, a conservative conclusion is that they are approximately the same. Neither the linear or larger \(\Phi\) topologies of \textit{L}\textsuperscript{4} and \textit{L}\textsuperscript{2} (whose pendant arms would be closer to a linear ligand relative to \textit{L}\textsuperscript{1}) respectively, provide them with a kinetic advantage over \textit{L}\textsuperscript{1}. Perhaps this is because the (2,2) pendant arms of \textit{L}\textsuperscript{1} are sufficient for the initial association, with a longer arm being no faster, but the (2,2) ring provides a preorganization that is more advantageous for fast encapsulation of the Th(IV) ion. It has been shown that the \(\Phi\) (3,3) topology of \textit{L}\textsuperscript{2} makes it less thermodynamically stable with thorium (\textit{cf.} computational studies in Chap. 3), but this detriment does not result in faster complex formation kinetics.
Th-L\textsuperscript{1} Association Studies: Direct Kinetics

As mentioned previously, the direct study of the kinetics of association is challenging due to the spectroscopic silence of Th(IV), as well as its propensity to hydrolyze. Despite the small spectral differences between the L\textsuperscript{1} and the ThL\textsuperscript{1} complex (Fig. 4-14a), the complexation could be accurately monitored with a stopped-flow apparatus. In order to simplify the mechanism of complexation by L\textsuperscript{1}, the metal was maintained as mainly free Th(IV) in acidic solution (pH 1.4, in 79 mM HNO\textsubscript{3}) until mixing with L\textsuperscript{1}, which was buffered such that mixing with the Th(IV) solution resulted in a reaction mixture at pH 7.4. The reaction studied was then:

\[
\text{Th}^{4+} + \text{LH}_{n}^{8+n} \rightarrow [\text{ThL}]^{4+} + n\text{H}^{+}
\]

At this pH, most of the ligand is present as LH\textsubscript{4}\textsuperscript{4-} and LH\textsubscript{5}\textsuperscript{3-} (the species present in greater than 1% are LH\textsubscript{4}\textsuperscript{4-}, LH\textsubscript{5}\textsuperscript{3-}, and LH\textsubscript{6}\textsuperscript{2-}, at 39.1, 57.8, and 2.6%, respectively). The stopped-flow apparatus allowed UV spectra to be measured immediately following the fast mixing of the metal and ligand solutions. With various excess ligand concentrations, the complexation was performed under pseudo-first order kinetic conditions, from which a second-order rate constant could be calculated. The observed first-order rate constants (determined with Specfit to fit the changes in absorbance (Table 4-4) at a range of wavelengths) were linearly dependent on the ligand concentration, suggesting a second-order reaction with a rate constant of \(k_2 = 1.8(1) \times 10^4\) M\textsuperscript{-1}s\textsuperscript{-1} (the slope of the linear regression in Fig. 4-15). With increasing concentrations of ligand, the UV absorbance increased, and the spectral region with sufficient changes became narrower as the magnitude of the spectral changes (and thus the signal-to-noise ratio) decreased. This problem was partially addressed by increasing the intensity of light into the sample, but the error at 30 equivalents of ligand was still too large. The data from the kinetic runs at this concentration of ligand were omitted.

The half-life of the complexation of 1 \(\mu\)M Th and 1 \(\mu\)M L\textsuperscript{1} is then 57 s, a more than adequately short time period for radiotherapeutic applications of L\textsuperscript{1}. The direct comparison of the \(k_2\) value with those of other ligands is not possible given the lack of published work on the rate of complexation of Th(IV) by relevant ligands. However, it is evident that the complexing ability of L\textsuperscript{1} far exceeds that of the widely used DOTA in association rate and temperature requirement. In published studies on \(^{227}\text{Th-p}-\text{benzyl-DOTA-rituximab, the complex is synthesized by mixing Th(IV) at a concentration range of 2.8 - 0.04 \text{ uM with an } 1.7 \times 10^3 - 3.9 \times 10^5\) excess of DOTA derivative at 55-60 °C for 40 min.\textsuperscript{13} Not only does L\textsuperscript{1} have adequate complexation kinetics for protocols currently in use, the milder conditions under which it achieves this would not damage the targeting biomolecule (an antibody in this case), providing the possibility of performing the bioconjugation before the radiolabeling.
Table 4-4. First-order rate constants obtained with stopped-flow kinetics experiments with ThL$_1$. The $k_{obs}$ obtained from experiments with 30 equivalents of L$_1$ was omitted in the calculation of the $k_2$.

<table>
<thead>
<tr>
<th>[L$_1$] (μM)</th>
<th>Equiv. L$_1$ rel. to Th(IV)</th>
<th>Average $k_{obs}$ (s$^{-1}$)</th>
<th>Spectral region analyzed (nm)</th>
<th>$k_2$ (M$^{-1}$s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>5</td>
<td>0.15(6)</td>
<td>305-454</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>10</td>
<td>0.7(1)</td>
<td>305-454</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>15</td>
<td>1.1(2)</td>
<td>305-454</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>1.6(4)</td>
<td>350-454</td>
<td>1.8(1) x 10$^4$</td>
</tr>
<tr>
<td>125</td>
<td>25</td>
<td>2.6(5)</td>
<td>350-454</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>30</td>
<td>12(5)</td>
<td>370-405</td>
<td></td>
</tr>
</tbody>
</table>

Figure 4-15. Observed first-order rate constants (error bars are standard deviations) calculated at varying concentrations of L$_1$ relative to thorium. The second-order rate constant was extrapolated from the slope of the linear regression ($R^2 = 0.9718$, intercept = 0).
EXPERIMENTAL DETAILS

Preliminary Studies
Thorium complexes were formed with a variety of ligands (previously synthesized by Dr. Jide Xu) in situ and characterized by mass spectrometry:

\[ \text{[Th(3,4,3-LI-CAMS)]}8\text{K}. \] (-)-HR ESIMS calcd for C\(_{32}\)H\(_{34}\)O\(_{24}\)N\(_4\)S\(_4\)Th [M+5H]\(^+\): 322.5212; found m/z 322.5209.

\[ \text{[Th(3,4,3-LI-CAMC)]}8\text{K}. \] (-)-HR ESIMS calcd for C\(_{42}\)H\(_{35}\)O\(_{20}\)N\(_4\)Th [M+5H]\(^+\): 382.4081; found m/z 382.4080.

\[ \text{[Th(H2,2-CAMC)]}4\text{K}. \] (-)-HR ESIMS calcd for C\(_{42}\)H\(_{37}\)O\(_{20}\)N\(_6\)Th [M+5H]\(^+\): 392.4153; found m/z 392.4151.

\[ \text{[Th(3,4,3-LI-1,2HOPO)]}. \] (+)-HR ESIMS calcd for C\(_{34}\)H\(_{34}\)O\(_{12}\)N\(_8\)NaTh [M+Na]\(^+\): 1001.2569; found m/z 1001.2562.

\[ \text{[Th(DFO-1,2HOPO)]}. \] (+)-HR ESIMS calcd for C\(_{31}\)H\(_{47}\)O\(_{11}\)N\(_7\)KTh [M+K]\(^+\): 964.3346; found m/z 964.3327.

Indirect Kinetics
Dye purification. Arsenazo III (Sigma-Aldrich) and Chlorophosphonazo III (Dojindo Laboratories) were twice purified by HPLC prior to use. 100 mg of each dye was dissolved in Millipore water, filtered through a 0.22 μM nylon syringe filter, and separated on a preparatory Varian Dynamax 250 x 41.1 mm C\(_{18}\) column. A solvent gradient of 0-18% acetonitrile/H\(_2\)O (0.1 % TFA) was used to collect three fractions with intense UV absorbances. The fraction with the most intense absorbance (rt 15-16 min) was collected as the main product, without the front or back tailing compound. This fraction was rotary evaporated, and the dark purple-green solid was resuspended in methanol before drying under vacuum overnight.

HRMS-ESI (m/z): [M-2H]\(^2-\) calcd for C\(_{22}\)H\(_{16}\)As\(_2\)O\(_{14}\)N\(_4\)S\(_2\)\(^2-\) (Arsenazo III), 386.9274; found 386.9279. Anal. calcd % (found %) for C\(_{22}\)H\(_{18}\)As\(_2\)O\(_{14}\)N\(_4\)S\(_2\)·2H\(_2\)O: C, 32.53 (32.34); H, 2.73 (2.73); N, 6.90 (6.71).

HRMS-ESI (m/z): [M-2H]\(^2-\) calcd for C\(_{22}\)H\(_{14}\)Cl\(_2\)O\(_{14}\)N\(_4\)S\(_2\)\(^2-\) (Chlorophosphonazo III), 376.9406; found 376.9411.

Th-dye displacement kinetics. UV-Vis spectra were acquired on a Hewlett-Packard 8453 diode array spectrometer at 25 °C. The temperature was controlled by a recirculating water bath connected to the jacketed cell holder. Samples were measured in small volume (50 μL) quartz cuvettes with 1 cm path lengths. Solutions were buffered with 100 mM HEPES at pH 7.4 (at 25 °C, with 0.1 M KCl). Complexation of the thorium and dye was performed by combining aqueous stock solutions of standardized Th(IV) in nitric acid (pH 1.4, 1 mM, diluted from a 10 mM solution, the standardization is described in the experimental details of Chap. 3) and an excess of purified dye (1 mM). The solution was let sit on the bench at room temperature for 30 min, diluted in buffer, and let sit at room temperature for an hour. The absorbance was monitored starting from the addition of ligand, as a solution of DMSO ligand stock solution (5 mM) and buffer (equilibrated for 15 min. beforehand). DOTA was purchased from Sigma-Aldrich and DTPA from Eco-Pharma. The \(A\ vs. t\) plots were fit with a first-order decay equation in Origin® 6.1 at wavelengths corresponding to the appearance of free dye (550, 560 nm) and the disappearance of thorium-dye complex (614, 669, 700 nm).
Stopped-flow Kinetics
The stopped-flow kinetic experiments were performed using a stopped-flow apparatus equipped with an OLiS rapid-scanning monochromator 1000 and a 75 W Xe lamp. Upon electronic activation, the apparatus (powered by Ar flow) mixes 100 μL of each solution in the two syringes into a mixing chamber. This mixture is injected into a separate chamber, where the flow is stopped and UV spectra are taken. One syringe contained 10 μM Th⁴⁺ in 77.9 mM HNO₃ (pH 1.4), while the other held a solution of 50-300 μM ligand, in 200 mM HEPES (pH 8.3) and 10% DMSO. The 1:1 mixture of these solutions was 5 μM Th, 25-150 μM ligand, 100 mM HEPES (pH 7.4). UV spectra in the 305-454 nm range were collected for 1.5-10 s, depending on the concentrations of the starting materials, at rates of 31-1000 scans/s. The data were analyzed using the program SpecFit to simultaneously use the absorbance at all of the wavelengths to obtain a second-order rate constant.
APPENDIX

Preliminary Studies
In order to shed some light onto the poorly understood kinetics, the characterization of the complexes formed in solution was attempted. Job plots were constructed to determine the stoichiometry of the thorium complexes. The Job plot for 3,4,3-LI-CAM(C) (Fig. A4-1) suggests that this ligand forms a Th$_3$L$_2$ complex, the formation of which is most likely multi-step. This would be consistent with the hypothesis that the final products of the complexations in solution are polynuclear. The Job plot for the other ligands studied showed ill-defined isosbestic points and greater overlap between the complex and free ligand spectra.

Figure A4-1. Job plot for Th(IV) with 3,4,3-LI-CAMC. a) UV spectra of solutions of 3,4,3-LI-CAMC and Th(IV), with varying mole fractions of ligand indicated in the legend ([L]$_{tot}$ + [Th]$_{tot}$ = 0.1 mM, pH 7.4, 100 mM HEPES, 0.1 M KCl). b) Job plot from data in (a), taken at a wavelength where the complex absorbs more predominantly.
Dye-Displacement Studies: Indirect Kinetics

The azo dyes were purified by prep-scale HPLC using a C_{18} column. The peak with rt 13.014 min was isolated as the pure Arsenazo III (Fig. A4-2a) and that with rt 14.046 min was isolated as pure Chlorophosphonazo III (Fig. A4-2b) on a super-preparatory HPLC. Job plots of Th with the azo dyes were generated with unpurified and purified dyes. The $\lambda_{\text{max}}$ of Arsenazo III shifted by 10 nm relative to that of the unpurified dye, and the isosbestic point in the Job plot actually became less clearly defined. Kinetics with this dye still could not be explained, as an inverse trend was also observed (first-order rate constants of 7.80E-3, 7.73E-3, 6.63E-3, 6.61E-3, 5.99E-3 s^{-1} for 10, 20, 30, 40, and 50 equivalents of ligand for 5 μM thorium-dye complex. at 669 nm). In order to ensure that the system was adequately buffered, since the ligand was introduced at the hydrochloride salt, the kinetic data were collected at buffer concentrations of 10 mM and 100 mM HEPES (both with 100 mM KCl), with negligible differences. Re-examination of the purity of the dye revealed that it had been compromised. Within 21 days, HPLC indicated the presence of a new impurity (Fig. A4-3a) at rt 1.078 min. The low retention time of this compound is puzzling because the expected adulterant in arsenazo III is arsenazo I, which would not have a drastically different retention time. A new batch of arsenazo III was thus purified by HPLC, twice, in order to yield the dye with the HPLC trace in Fig. A4-3b, with a purity of 98.8% (from the area of the main peak with rt 13.215 min). The HPLC trace of this dye must be collected periodically for signs of decomposition.
Figure A4-2. Analytical HPLC traces of unpurified azo dyes (a) Arsenazo III and (b) Chlorophosphonazo III. (Gradient elution of 2-18% MeCN/H₂O in 20 min followed by 18-100% in 5 min, with 0.1% TFA).
Figure A4-3. Analytical HPLC traces of purified Arsenazo III, (a) 21 days following purification, and (c) twice purified. (Gradient elution of 2-18% MeCN/H₂O in 20 min, with 0.1% TFA)
REFERENCES


CHAPTER 5
COMPLEXATION OF OTHER METAL IONS WITH Φ(2,2)moeTAM

Introduction

Although lanthanides and actinides can exhibit significant differences in chemical behavior, most notably due to the greater covalency of the actinides, the use of lanthanides as actinide analogs has enabled the advancement of 5f-element chemistry and continues to be useful.¹ The remarkable stability of Φ(2,2)moeTAM (L¹) with Th(IV) has prompted its application toward the stabilization of high oxidation states in the other actinide ions, particularly those that are typically unstable under aqueous conditions, such as Am(IV). The easily accessible +4 oxidation state of cerium makes it an unusual lanthanide, and it was used as a surrogate for the Am(III)-(IV) system with L¹. The aqueous reduction potential of Am(IV)/Am(III) is estimated to be 2.62 V,² which means that Am(IV) oxidizes water or most other ligands. However, a remarkable stability of Am(IV) with L¹ relative to Am(III) would shift this redox potential, according to the Nernst equation:

\[ E_{n/n-1}^\circ = E_{n/n} + \left( \frac{RT}{F} \right) (\ln K_{M^{n-1}L} - \ln K_{M^nL}) \]

Such potential shifts have been extensively observed with the Ce(IV)-Ce(III) couple,³ and can be envisioned with lanthanide ions with larger potentials as well (Table 5-1). However, considering the potential range accessible with a typical electrochemical experimental setup, the readily measurable +4/+3 redox couples are those of Ce, Am, and Bk.
Central to this study is the measurement of the affinity difference of $L_1$ for the +4 and +3 oxidation states of the metal ion, a measurable selectivity that has applications in metal ion sequestration for separation chemistry as well as medicine. In addition to electrochemical studies, solution thermodynamic studies will be detailed with the lanthanide analogs Ce(IV) and Pr(III).

**CeL$_1$ Crystal Structure**

The Ce(IV) complex was successfully synthesized with the complexation of either Ce(IV) (from a solution of the sulfate salt) or Ce(III) (as the chloride solution) followed by air oxidation. The dark purple complex shows an intense and broad absorption at $\lambda_{\text{max}} = 522$ nm, which is the LMCT band (Fig. 5-1). Single crystals suitable for X-ray diffraction were grown by the vapor diffusion of diisopropyl ether into a 1:4::DMF:MeOH solution of the isolated potassium salt of the Ce(IV) complex (Fig. 5-2, Table 5-2). As with the Th(IV) complex, various other countercations were used for the crystallization (alkylammonium salts) but only the potassium salt crystallized adequately. The presence of the ordered and coordinated potassium ions suggests that they have a role in stabilizing the complex in a more rigid conformation. This is especially important given that the ligand is so large and flexible--one of the methoxyethyl substituents on the pendant arms exhibits some disorder, which was modeled over two positions (this was also seen in the Th structure). Solvent molecules were not ordered, and were accounted for with SQUEEZE to achieve a stable refinement. Other than with the shorter Ce-O bond lengths (Ce(IV) has an ionic radius of 97 pm, whereas Th(IV) is 105 pm), the crystal structure of the Ce complex is very similar to that of the Th complex in space group, unit cell, and inner coordination environment (Table 5-3).

<table>
<thead>
<tr>
<th>Ln or An</th>
<th>$E_{4/3}^0$ (V)</th>
<th>$E_{ML}(V)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ce$^5$</td>
<td>1.37</td>
<td>-0.53</td>
</tr>
<tr>
<td>Pr</td>
<td>2.96</td>
<td>1.06</td>
</tr>
<tr>
<td>Nd</td>
<td>4.76</td>
<td>2.86</td>
</tr>
<tr>
<td>Tb</td>
<td>2.86</td>
<td>0.96</td>
</tr>
<tr>
<td>Dy</td>
<td>4.96</td>
<td>3.06</td>
</tr>
<tr>
<td>U</td>
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<td>-2.77</td>
</tr>
<tr>
<td>Np</td>
<td>-0.09</td>
<td>-1.99</td>
</tr>
<tr>
<td>Pu</td>
<td>0.73</td>
<td>-1.17</td>
</tr>
<tr>
<td>Am$^2$</td>
<td>2.62</td>
<td>0.72</td>
</tr>
<tr>
<td>Cm</td>
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<td>0.96</td>
</tr>
<tr>
<td>Bk</td>
<td>1.4</td>
<td>-0.50</td>
</tr>
</tbody>
</table>

*a*Potentials are vs. SCE, and from Ref. 4, unless noted otherwise.

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**Table 5-1.** Standard reduction potentials for various lanthanides and actinides, with estimated Nernstian shift of 1.9 V upon complexation with $L_1$.
Figure 5-1. UV-Vis spectrum of \([\text{CeL}^+]\) in aqueous solution: 100 mM HEPES (pH 7.4), 100 mM KCl.

Table 5-2. Crystallographic data and structure refinement for \(\text{CeL}^+\text{K}_4\).

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical formula</td>
<td>(\text{C}<em>{50}\text{H}</em>{54}\text{CeK}<em>4\text{N}</em>{10}\text{O}_{18})</td>
</tr>
<tr>
<td>(M_r)</td>
<td>1379.05 g/mol</td>
</tr>
<tr>
<td>Temperature</td>
<td>100(2) K</td>
</tr>
<tr>
<td>Wavelength</td>
<td>0.71073 Å</td>
</tr>
<tr>
<td>Crystal system</td>
<td>Triclinic</td>
</tr>
<tr>
<td>Space group</td>
<td>(\text{P}\bar{1})</td>
</tr>
<tr>
<td>Unit cell dimensions</td>
<td>(a = 11.3510(7)) Å, (a = 79.136(2)°)</td>
</tr>
<tr>
<td></td>
<td>(b = 16.4110(12)) Å, (\beta = 86.923(2)°)</td>
</tr>
<tr>
<td></td>
<td>(c = 20.1170(14)) Å, (\gamma = 80.58(2)°)</td>
</tr>
<tr>
<td>(V)</td>
<td>3632.1(4) Å³</td>
</tr>
<tr>
<td>(Z)</td>
<td>2</td>
</tr>
<tr>
<td>(\rho_{\text{calc}})</td>
<td>1.261 mg/m³</td>
</tr>
<tr>
<td>(\mu_{\text{calc}})</td>
<td>0.919 mm⁻¹</td>
</tr>
<tr>
<td>(F(000))</td>
<td>1403</td>
</tr>
<tr>
<td>Crystal size</td>
<td>1.00 x 0.03 x 0.01 mm</td>
</tr>
<tr>
<td>2(\theta) range for data collection</td>
<td>1.49 to 25.52°</td>
</tr>
<tr>
<td>Index ranges</td>
<td>-13&lt;=h&lt;=13, -19&lt;=k&lt;=19, -23&lt;=l&lt;=24</td>
</tr>
<tr>
<td>Reflections collected</td>
<td>25169</td>
</tr>
<tr>
<td>Independent reflections</td>
<td>12747 ([R(\text{int}) = 0.0316])</td>
</tr>
<tr>
<td>Completeness to (\theta = 25.00°)</td>
<td>95.3 %</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Semi-empirical from equivalents</td>
</tr>
<tr>
<td>Max. and min. transmission</td>
<td>0.9909 and 0.4601</td>
</tr>
<tr>
<td>Refinement method</td>
<td>Full-matrix least-squares on (F^2)</td>
</tr>
<tr>
<td>Data / restraints / parameters</td>
<td>12747 / 29 / 760</td>
</tr>
<tr>
<td>Goodness-of-fit on (F^2)</td>
<td>1.134</td>
</tr>
<tr>
<td>Final (R) indices [(I &gt; 2\sigma(I))]</td>
<td>(R1 = 0.0815, wR2 = 0.2353)</td>
</tr>
<tr>
<td>(R) indices (all data)</td>
<td>(R1 = 0.1036, wR2 = 0.2614)</td>
</tr>
<tr>
<td>Largest diff. peak and hole</td>
<td>5.405 and -0.872 e.Å⁻³</td>
</tr>
</tbody>
</table>
Chapter 5

The angles of the TAM binding units about the Ce(IV) coordination polyhedron were measured using the same analysis applied to the Th(IV) crystal structure (Chap. 3). With both

<table>
<thead>
<tr>
<th>Crystal system</th>
<th>[ThL₁]K₄</th>
<th>[CeL₁]K₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Space group</td>
<td>PT</td>
<td>PT</td>
</tr>
<tr>
<td>a = 11.3472(15) Å</td>
<td>a = 11.3510(7) Å</td>
<td></td>
</tr>
<tr>
<td>b = 16.549(2) Å</td>
<td>b = 16.4110(12) Å</td>
<td></td>
</tr>
<tr>
<td>c = 20.044(3) Å</td>
<td>c = 20.1170(14) Å</td>
<td></td>
</tr>
<tr>
<td>α = 79.129(2)°</td>
<td>α = 79.136(2)°</td>
<td></td>
</tr>
<tr>
<td>β = 87.126(2)°</td>
<td>β = 86.923(2)°</td>
<td></td>
</tr>
<tr>
<td>γ = 79.929(2)°</td>
<td>γ = 80.854(2)°</td>
<td></td>
</tr>
<tr>
<td>V = 3638.8(8) Å³</td>
<td>V = 3632.1(4) Å³</td>
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</tr>
</tbody>
</table>

*Final R indices [I>2σ(I)]*

<table>
<thead>
<tr>
<th>R1 = 0.0709, wR2 = 0.1711</th>
<th>R1 = 0.0815, wR2 = 0.2353</th>
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<tr>
<td>2.468(6)</td>
<td>2.416(7)</td>
</tr>
<tr>
<td>2.378(7)</td>
<td>2.382(6)</td>
</tr>
<tr>
<td>2.396(6)</td>
<td>2.399(6)</td>
</tr>
<tr>
<td>2.427(7)</td>
<td>2.412(7)</td>
</tr>
<tr>
<td>2.400(5)</td>
<td>2.344(5)</td>
</tr>
<tr>
<td>2.312(5)</td>
<td>2.325(5)</td>
</tr>
<tr>
<td>2.315(4)</td>
<td>2.363(5)</td>
</tr>
<tr>
<td>2.368(5)</td>
<td>2.343(5)</td>
</tr>
</tbody>
</table>

The angles of the TAM binding units about the Ce(IV) coordination polyhedron were measured using the same analysis applied to the Th(IV) crystal structure (Chap. 3). With both
Thorium and cerium complexes, the binding units of $L^1$ coordinate the metal center in a geometry that is distorted from the ideal trigonal dodecahedron, but they still span the $m$ edges. The smaller radius of Ce(IV) relative to Th(IV) does not seem to alleviate any speculated ring strain in the ligand, since the deviation angles in the two complexes are very close (Table 5-4). This suggests that the asymmetric binding mode of the ligand and the distortion of the binding groups are not energetically disfavored.

Table 5-4. Calculated vectors between experimental TAM O-O vectors and edges of ideal trigonal dodecahedron.

<table>
<thead>
<tr>
<th>TAM oxygen atoms</th>
<th>Angle from ideal (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Th$L^1$</td>
</tr>
<tr>
<td>O1-O2</td>
<td>1.94</td>
</tr>
<tr>
<td>O3-O4</td>
<td>29.31</td>
</tr>
<tr>
<td>O5-O6</td>
<td>5.37</td>
</tr>
<tr>
<td>O7-O8</td>
<td>38.97</td>
</tr>
</tbody>
</table>

Solution Thermodynamics with Ce(IV)

The charge-transfer band of Ce$L^1$ in the visible region makes the measurement of its stability constant by spectrophotometric titration especially practicable (Fig. 5-3). The high stability constant predicted necessitated the use of a competing ligand; nitrilotriacetic acid (NTA) was used because of the stable and colorless complex it forms with Ce(IV), with a log $\beta^{120} = 38.6(8)$, which is greater than the log $\beta^{110}$ with DTPA. In addition to the shift of the ligand deprotonation to a lower pH (Fig. 5-4b), the formation of the complex can be monitored by the appearance of this band centered at 522 nm (Fig. 5-4a). As with the Th(IV) titrations, the main equivalence point observed corresponds to the deprotonation of the MLH$^3$ to the ML$^4$ species, the refined spectrum (Fig. 5-5) of which correlates well with that of the synthesized complex (Fig. 5-1). Refinement of the NTA competition titrations gave a log $\beta_{110} = 61(2)$ and a pH$\alpha = 5.5(7)$ (Table 5-5). The rather large standard deviations of these values are partly due to the errors in the NTA stability constant and $L^1$ protonation constants; moreover, the measurement of such a high affinity is inherently uncertain.
A high stability constant of $\log \beta_{110} = 41.5(2)$ was measured for the Ce(IV) complex with the hydroxypyridinone ligand 3,4,3-LI-1,2-HOPO, but the stability of CeL is 20 orders of magnitude greater. This thermodynamic stability surpasses that of the ThL complex by over 7 orders of magnitude, which is surprising, but perhaps attributable to the greater charge-to-radius ratio of Ce(IV) relative to Th(IV). The thermodynamic stability of CeL is among the highest measured, and indicates promise for the use of L in chelating other high-valent metal ions for medical applications, such as Zr(IV) for PET. The remarkably low amount of metal release by the ligand—given the pM value of 46—is especially appealing in its applications as a bifunctional chelator.

Figure 5-3. UV-Vis spectra of spectrophotometric competition titration of CeL with NTA. Starting conditions: 50 μM Ce(IV), 50 μM L, 1 mM NTA, 1mM MES, 1mM HEPES, and 0.1 M KCl (25°C). A vs. wavelength plot at varying pH values.
Figure 5-4. Plots of $A$ vs. pH of spectrophotometric competition titration of CeL$^1$ with NTA. Starting conditions: 50 $\mu$M Ce(IV), 50 $\mu$M L$^1$, 1 mM NTA (nta), 1mM MES, 1mM HEPES, and 0.1 M KCl (25°C). $A$ vs. pH at (a) 522 nm, the $\lambda_{max}$ of the LMCT band, and (b) 370 nm, the $\lambda_{max}$ of the ligand $\pi \rightarrow \pi^*$ transition, (experimental data points are blue circles, red crosses are calculated absorbances) overlaid onto speciation.
Figure 5-5. Molar absorbances of species of CeL⁺ (spectrum of the fully deprotonated L⁺ included for comparison).

Figure 5-6. Speciation diagram at [L⁺] = 10 μM and [Ce] = 1 μM.
Solution Thermodynamics with Pr(III)

The thermodynamic stability of TAM ligands for Ln(III) ions has mostly been measured for six-coordinate gadolinium complexes for applications in MRI contrast agents. A close analog is the hexadentate ligand TREN-TAM₃, which has a log $\beta_{110} > 17$ with Gd(III). Even though $L^1$ has the same binding units, it is difficult to predict its stability with a +3 lanthanide ion given the difference in coordination number and the entirely different topology of the ligands.

The direct verification of a Ce(IV)-Ce(III) redox potential shift due to complexation by the independent measurement of the Ce(III) stability constant was not possible due the overwhelming preference of +4 oxidation state of $L^1$. Even with the exclusion of oxygen in the titration experimental setup, titrations with Ce(III) resulted in its oxidation to Ce(IV) upon complexation. Based on ionic radii comparisons, Pr(III) is a closer analog to Ce(III) than Gd(III); spectrophotometric titrations with Pr(III) and $L^1$ were then performed (Fig. 5-7). The shift in the ligand deprotonation to lower pH values, indicative of complexation, was accompanied by two main equivalence points. This was refined to the formation of two dominant species in solution, the mono- and di-protonated praseodymium complexes with log $\beta_{110} = 32.02(2)$ and $pK_{a1} = 8.1(2)$ and $pK_{a1} = 7.0(2)$ (with the spectra shown in Fig. 5-8). The MLH₂ species is observed with Pr(III) but not with Ce(IV) and Th(IV), most likely due to the higher charge of the complex with Pr.

The stability of the Pr$L^1$ complex provides a quantitative assessment of the selectivity of $L^1$ for +4 over +3 metal ions. The binding affinity of $L^1$ for Pr(III) is considerably greater than that of DTPA ($\log \beta_{110} = 21.10$) but its $pM$ value is about the same (Table 5-5, 18.0 for $L^1$ and 17.6 for DTPA). Treating the early lanthanides and actinides as analogs, it can be concluded that the 29-order magnitude difference in binding constants between Pr(III) and Ce(IV) signifies that $L^1$ exhibits a strong preference for Ln(IV) and An(IV) ions, and is not an exceptional chelator for the sequestration of Ln(III) and An(III) ions.
Figure 5-7. Spectrophotometric titration of PrL$^1$. Starting conditions: 50 μM Pr(III), 50 μM L$^1$, 1 mM MES, 1mM HEPES, 1 mM CHES and 0.1 M KCl (25°C). a) $A$ vs. wavelength plot at varying pH (data abridged for clarity; spectra normalized for dilution). b) $A$ vs. pH at 390 nm (experimental data points are blue circles, red crosses are calculated absorbances) overlaid onto speciation.
Figure 5-8. Molar absorbances of species of PrL, (spectrum of the fully deprotonated L included for comparison).

Figure 5-9. Speciation diagram at [L] = 10 μM and [Pr] = 1 μM.
Chapter 5

Table 5-5. Stability Constants and pM Values for Complexes with L₁ and DTPA.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Metal ion</th>
<th>Log β₁₁₀, log Kₛ</th>
<th>pM</th>
<th>Ionic Radius (pm),⁶ CN = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>φ(2,2)moeTAM</td>
<td>Th(IV)</td>
<td>53.7(5), 5.2(2)</td>
<td>39.1</td>
<td>105</td>
</tr>
<tr>
<td>(L₁)</td>
<td>Ce(IV)</td>
<td>61(2), 5.5(7)</td>
<td>46</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Pr(III)</td>
<td>32.02(2), 8.1(2), 7.0(2)</td>
<td>18.0</td>
<td>113</td>
</tr>
<tr>
<td>DTPA⁸,¹¹</td>
<td>Th(IV)</td>
<td>28.78, 2.16</td>
<td>26.8</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>Ce(IV)</td>
<td>34.04</td>
<td>30.59</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Pr(III)</td>
<td>21.10, 2.38</td>
<td>17.6</td>
<td>113</td>
</tr>
</tbody>
</table>

Electrochemistry of Complexed Ce(IV)-Ce(III)

The electrochemical studies of the +3/+4 redox couple of the CeL₁ was undertaken with optimism, given the literature precedent for the successful measurement of the redox activity of the Ce(IV) complex with a variety of ligands, including catechols.³,¹²

However, no reversible redox couple for the Ce center could be observed using cyclic voltammetry (CV) under a variety of conditions (water, acetonitrile; NBu₄PF₆, KNO₃, KCl electrolytes; 1:1, 1:2, 1:3 Ce:L concentrations; pH 5-11) with the glassy carbon working electrode. Whether the complex was measured by the dissolution of isolated complex or by the in situ formation, only a nonreversible reduction was observed around -0.6 V (Fig. 5-10 and 5-11). The presence of the Ce(III) complex was ensured by maintaining the pH of the solution above 9 (based on the speciation of the Pr(III) complex, Fig. 5-9), either by basifying with KOH or buffering with phosphate or triethylamine.

Figure 5-10. Cyclic voltammogram of an aqueous solution of 0.5 mM Ce(IV), 0.5 mM L₁, in 0.1 M KCl, pH 10.5 at a scan rate of 50 mV/s with the glassy carbon working electrode.
Control experiments with Fe(II)-(III) redox couples as well as the Ce(IV)-Ce(III) couple with DTPA were performed to verify that problem was not with the experimental setup. Since DTPA has been repeatedly shown to be a vastly different ligand than $L^1$, more similar ligands (Fig. 5-12) were used to probe the effect of the Φ backbone of $L^1$ on the cerium electrochemistry.

The tetradentate ligand 5LiO-iPrTAM has terephthalamide binding moieties as in $L^1$, and the observation of a reversible redox couple at -224 mV (vs. NHE) was promising (Fig. 5-13). Increasing the similarity to $L^1$ with the octadentate H(2,2)-MeTAM Ce complex was studied. This last complex behaved very similarly to the Ce-$L^1$ system, as no reversible Ce redox couple could be observed with the same conditions previously applied. Studies on Ce systems with rare earth/alkali metal/BINOL frameworks have demonstrated the importance that ligand reorganization can have in the reversibility of the Ce redox couple.13 This phenomenon could be
responsible for the lack of reversibility in the electrochemical behavior of Ce(IV) when it is complexed by the octadentate $L^1$ and H(2,2)-MeTAM but not the tetradentate analog 5LiO-iPrTAM.

Oxidation following the reduction of the Ce$L^1$ complex was successfully observed with a hanging drop mercury electrode (HDME). Cyclic voltammograms demonstrating nearly reversible behavior at scan rates of 250-2000 mV/s were obtained for the system (as an aqueous solution of the isolated complex) in 1M KCl (Fig. 5-14) and 1M KNO$_3$ (Fig. 5-16). The current intensity at cathodic peaks is linearly proportional to the square root of the scan rate, suggesting a diffusion-limited process. Yet, upon examination of the cyclic voltammograms (Table 5-5), it is apparent that the redox behavior is not fully reversible for several reasons:

1) The voltage separation between the current peaks is greater than 59 mV in KCl and smaller in KNO$_3$. Moreover, voltage separations were closer to 59 mV at higher scan rates, suggesting the presence of a different slower process observable at lower scan rates. Still, the average voltage separations are sufficiently close to the ideal value to estimate that the metal center is undergoing the one-electron process of the +4/+3 redox couple.

2) The positions of both the cathodic and anodic peak voltages change with increasing scan rate. With increasing scan rates, the cathodic and anodic peaks shift to more reductive and oxidative potentials, respectively. This effect has been attributed to the slow equilibrium at the electrode surface relative to the higher scan rates.

3) The ratio of the peak currents is greater than one, with the cathodic current intensity being consistently greater than the anodic current. This is possibly the result of the lower stability of the reduced cerium complex, and thus its greater susceptibility to side reactions. At 100 mV/s, the oxidation is not even observed.

Figure 5-13. Cyclic voltammograms of an aqueous solution of 1 mM Ce, 6 mM 5LiO-iPrTAM in 1M KNO$_3$, pH 10, glassy carbon working electrode.
Despite these imperfections, the reversibility of the system using the HDME working electrode was satisfactory in order to calculate the redox shift of the induced by the complexation of Ce(IV) by $L_1$. Subtracting the average of the peak separations from the appropriate standard cerium potentials gave Nernstian shifts of 1.92 V (KCl) and 2.06 V (KNO₃). It is unclear why different values were obtained as a result of the change in electrolyte.

Figure 5-14. CV of CeL₁ in 1 M KCl and the HDME working electrode. a) Cyclic voltammograms of 1 mM CeL₁K₄ at scan rates of 100-2000 mV/s, initial scan direction: cathodic. Background taken at 100 mV/s. (b) Linear dependence of the current intensity at cathodic peak currents vs. the square root of the scan rate.
The calculated Nernstian shifts can be converted to a difference in log $\beta$ for the Ce(III) and Ce(IV) complexes of 32.4-34.9. This range is not in agreement with the difference in log $\beta$ values of Pr(III) and Ce(IV) measured by titration ($\log \beta_{CeL} - \log \beta_{PrL} = 29.0$). The slight discrepancy in the measured stabilities of the two methods demands further investigation, and perhaps the direct measurement of the binding affinity of $L^1$ for Ce(III).

Figure 5-15. CV of CeL$^1$ in 1 M KNO$_3$ and the HDME working electrode. a) Cyclic voltammograms of 1 mM CeL$^1$K$_4$ at scan rates of 100-2000 mV/s, initial scan direction: cathodic. Background taken at 100 mV/s. (b) Linear dependence of the current intensity at cathodic peak currents vs. the square root of the scan rate.
Table 5-5. CV data for the CeL¹ complex in 1 M KCl or 1M KNO₃ and the HDME working electrode. Values are averages from scan rates of 250-2000 mV/s.

<table>
<thead>
<tr>
<th>Average values</th>
<th>Electrolyte</th>
<th>1 M KCl</th>
<th>1 M KNO₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>E&lt;sub&gt;1/2&lt;/sub&gt; (V)</td>
<td>-0.651(9) vs. Ag/AgCl</td>
<td>-0.648(4) vs. Ag/AgCl</td>
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</tr>
<tr>
<td></td>
<td>-0.696 vs. SCE</td>
<td>-0.693 vs. SCE</td>
<td></td>
</tr>
<tr>
<td>ΔE (V)</td>
<td>0.0683</td>
<td>0.0548</td>
<td></td>
</tr>
<tr>
<td>i&lt;sub&gt;p&lt;/sub&gt;²/i&lt;sub&gt;p&lt;/sub&gt;¹</td>
<td>-2.8</td>
<td>-2.4</td>
<td></td>
</tr>
<tr>
<td>Shift from E&lt;sup&gt;o&lt;/sup&gt; (V)</td>
<td>1.92</td>
<td>2.06</td>
<td></td>
</tr>
</tbody>
</table>
EXPERIMENTAL DETAILS

Lanthanide Complexes

[CeL\textsuperscript{1/4}K\textsubscript{4}. L\textsubscript{1}H\textsubscript{8}-3HCl (29.18 mg, 0.0197 mmol) was suspended in 4 mL methanol in a 10-mL round-bottom flask (that had been soaked in an EDTA bath overnight). A solution of CeCl\textsubscript{3}-7H\textsubscript{2}O (7.26 mg, 0.0195 mmol) in 1 mL methanol was added dropwise to the ligand solution while stirring, resulting in a yellow suspension. A stoichiometric amount of 0.2 M KOH in water (0.985 mL, 0.197 mmol) was added to the ligand suspension dropwise, to a pH of 8, and the reaction mixture immediately became dark purple, indicating the oxidation of Ce(III) to Ce(IV). The reaction mixture was refluxed under nitrogen flow for 3 h, and once cooled to room temperature, was dropped into 30 mL diethyl ether, producing a fine precipitate. The dark purple precipitate was filtered and dried overnight under vacuum (35.90 mg, 94%).

\textsuperscript{1}H NMR (500 MHz, MeOD-\textsubscript{d4}): 2.310 (br d, 2H, tren CH\textsubscript{2}), 2.618 (d, \textit{J} = 12.5 Hz, 6H, tren CH\textsubscript{2}), 2.820 (br q, \textit{J} = 12.5 Hz, 6H, tren CH\textsubscript{2}), 2.905-2.932 (m, 4H, tren CH\textsubscript{2}), 3.175-3.483 (m, 16H, tren CH\textsubscript{2}, CH\textsubscript{3}, methoxyethanamide CH\textsubscript{2}), 3.681 (br t, 2H, tren CH\textsubscript{2}), 3.983 (d, \textit{J} = 14.5 Hz, 2H, tren CH\textsubscript{2}), 6.845 (d, \textit{J} = 9.0 Hz, 3H, ArH), 6.885 (d, \textit{J} = 8.5 Hz, 3H, ArH), 7.079 (d, \textit{J} = 8.5 Hz, 2H, ArH).

(-)-HR ESIMS calcd for C\textsubscript{50}H\textsubscript{55}O\textsubscript{18}N\textsubscript{10}Ce (CeL\textsubscript{1}H)\textsuperscript{3-}: 407.7589; found \textit{m/z} 407.7582.

Anal. calcd for C\textsubscript{50}H\textsubscript{54}O\textsubscript{18}N\textsubscript{10}Ce·5KCl·12H\textsubscript{2}O: C, 30.51; H, 3.99; N, 7.12. Found: C, 30.76; H, 3.98; N, 7.00.

A selected crystal of CeL\textsubscript{1}K\textsubscript{4} was mounted in Paratone N oil at the end of a captan loop and frozen in place under a low-temperature nitrogen stream. The data were collected on a Bruker APEX-II CCD X-ray diffractometer with Mo K\textsubscript{α} radiation in the X-Ray Facility in the University of California, Berkeley College of Chemistry. Intensity data with a maximum 2θ range of 51.04° were extracted from the frames with the program APEX2. The data were corrected for Lorentz and polarization effects, and an empirical absorption correction was applied using the SADABS program. The structure was solved by direct methods and refined using full-matrix least squares refinements based on \textit{F}2 in SHELXL-97. Crystallographic analyses were performed using the WinGX system of programs. All non-hydrogen atoms were refined anisotropically, while hydrogen atoms were assigned to idealized positions. Disordered solvent molecules were treated with the SQUEEZE procedure included in PLATON. A void of 922 Å\textsuperscript{3} containing 287 electrons was found.

[LnL\textsuperscript{1}/5K. L\textsubscript{1}H\textsubscript{8}-3HCl (20.84 mg, 0.01736 mmol) was suspended in 4 mL methanol in a 10-mL round-bottom flask (that had been soaked in an EDTA bath overnight). A solution of Ln salt (PrCl\textsubscript{3}-6H\textsubscript{2}O, Ventron Alfa Products, 6.07 mg, 0.0172 mmol; TbCl\textsubscript{3}-6H\textsubscript{2}O, Strem, 5.19 mg, 0.0139 mmol; or NdCl\textsubscript{3}, ROC/RIC, 3.09 mg, 0.0123 mmol) in 1 mL methanol was added dropwise to the ligand solution while stirring, resulting in a yellow suspension. A stoichiometric amount of 0.4896 M KOH in methanol (11 equiv.) was added to the ligand suspension dropwise, to a pH of 8, and the reaction mixture gradually became clear and yellow-green (Pr) or yellow (Tb, Nd). The reaction mixture was refluxed under nitrogen flow for 5 h, a time during which it became a dark red (Pr), orange-red (Tb), or orange (Nd). Upon cooling to room temperature and precipitation with the addition of 2-5 mL diethyl ether, a green (Pr), brown (Tb), or gray (Nd) solid was filtered with a glass frit. The solid was dried for 6h under vacuum (19.0 mg PrL\textsubscript{1}5K, 77%; 20.7 mg TbL\textsubscript{1}5K, 97%; 18.8 mg NdL\textsubscript{1}5K, 100%).
Chapter 5

(-)-HR ESIMS calcd for C₅₀H₅₈O₁₈N₁₀Pr (PrL₁H₄⁻)⁻: 1227.3063; found m/z 1227.3008.
(-)-HR ESIMS calcd for C₅₀H₅₇O₁₈N₁₀Tb (TbL₁H₃)²⁻: 622.1553; found m/z 622.1559.
(-)-HR ESIMS calcd for C₅₀H₅₇O₁₈N₁₀Nd (NdL₁H₃)²⁻: 613.6464; found m/z 613.6465.

The presence of the Fe(III) complex was detected in the mass spectra of these compounds, and was most likely responsible for the dark color changes observed during the synthesis.

**Solution Thermodynamics.** All spectrophotometric titrations were carried out with constant stirring and a blanket of Ar flow in a jacketed cell connected to a recirculating water bath to maintain the temperature at 25°C. The ionic strength of all solutions was maintained at 0.1 M with 0.1 M KCl in titrand solutions and 0.1 M acid and base titrants. A solution of 0.25 N Ce(IV) sulfate was used as purchased. A stock solution of Pr(III) was prepared by the dissolution of PrCl₃·6H₂O (Ventron Alfa Products) in water. The ligand was added as a 50 mM DMSO solution, prepared by dissolution of the solid ligand, weighed on an analytical balance accurate to 0.01 mg. The HCl and KOH solutions were prepared by dilution of Dilut-It (J.T. Baker, ampoules) concentrated solutions with degassed Millipore water. The 0.1 M HCl solution was standardized by the potentiometric titration of tris(hydroxymethyl)aminomethane, and the 0.1 M KOH solution was standardized by potentiometric titration of potassium hydrogen phthalate or the standardized HCl solution. The KOH solution was stored under a blanket of Ar flow and standardized before every titration. The glass electrode (Metrohm Microtode or Orion Pinnacle) used for the pH measurements was calibrated by the titration of 1.000 mL standardized 0.1 M HCl in 25.0 mL 0.1 M KCl with standardized 0.1 M KOH to pH 11.6. The titration was analyzed using the program GLEE¹⁵ to refine for E° and the slope. This calibration was also performed prior to each titration. The automated titration system was controlled by a Metrohm Titrando 907 and the program Tiamo® light. 2 mL Dosino 800 burets dosed the titrant into the titration vessel (5-90 mL). UV-Vis spectra were acquired with an Ocean Optics USB4000-UV-Vis spectrometer equipped with a dip probe (set to a 10 mm path length) and a DH-2000 light source (deuterium and tungsten lamps), using the program Spectra Suite. Alternatively, Metrohm Brinkmann 665 Dosimat instruments were the automated burettes used to dispense the titrant, the pH meter was a Fisher Accumet AR 15, and the UV-Vis spectrometer a Hewlett-Packard 8452A. The computer program Labview was the interface from which all these components were orchestrated to run the titrations. Titrations were performed at least in triplicate.

**Ce(IV) L¹ Competition Titration.** 20 mL solutions of 50 μM L¹, 50 μM Ce(IV), 1 mM NTA (Eastman Organic Chemicals) were titrated forward and backward with between pH 2.9 and 10.0 with 0.1 M KOH and 0.1 M HCl. 1 mM MES and 1 mM HEPES were used to buffer the system, which was maintained at an ionic strength of 0.1 M with KCl. The Ce(IV) was added to a solution of NTA and buffer, and this clear, light yellow solution was allowed to equilibrate for at least 24 h prior to the addition of ligand and used as the blank spectrum. The data points (pH readings and UV-Vis spectra) were collected at approximately 0.4 pH increments, with an equilibration time of 10 min following KOH additions (forward titrations) or 20 min following HCl titrations (backward titrations). 0.1 M HCl was added to the solution prior to the forward titration to reach the starting pH. All absorbance measurements used in the refinement were no more than 1.1 absorbance units. Spectra of 280-800 nm were analyzed (simultaneously) in the program Hypspec. The following values for the log β for the formation of cerium hydroxides were included in the refinement: [CeOH]³⁺, -0.56; [Ce₂(OH)₃]⁵⁺, -0.44; [Ce₂(OH)₄]⁴⁺, -0.62; [Ce₆(OH)₁₂]²⁺.¹¹ The following protonation and stability constants for NTA and the Ce(NTA)² complex were also included: log β₀₁₁, 9.84; log β₀₁₂, 12.36; log β₀₁₃, 14.17; log β₀₁₄, 15.17; log
Spectra and protonation constants of the free ligand, refined from the ligand titrations, were set constant in the refinement.

\[ \beta_{120}, 38.6 \] Spectra and protonation constants of the free ligand, refined from the ligand titrations, were set constant in the refinement.

Pr(III)\(L^1\) Titration. 20 mL solutions of 50 \(\mu\)M \(L^1\) and 50 \(\mu\)M Pr(III) were titrated forward and backward with between pH 2.6 and 11.0 with 0.1 M KOH and 0.1 M HCl. 1 mM each of MES, HEPES, and CHES were used to buffer the system, which was maintained at an ionic strength of 0.1 M with KCl. The data points (pH readings and UV-Vis spectra) were collected at approximately 0.4 pH increments, with an equilibration times of 10 min. 0.1 M HCl was added to the solution prior to the forward titration to reach the starting pH. All absorbance measurements used in the refinement were no more than 1.1 absorbance units. Spectra of 250-450 nm were analyzed (simultaneously) in the program Hypspec. The following value for the log \(\beta\) for the formation of pradegydmyium hydroxide was included in the refinement: \([\text{PrOH}]^{2+}\). 7.1 Spectra and protonation constants of the free ligand, refined from the ligand titrations, were set constant in the refinement.

Electrochemistry
Cyclic voltammograms using a glassy carbon electrode were obtained using a BAS100A potentiostat. The other electrodes were silver/silver chloride (reference) and platinum wire (auxiliary). In order to ensure that this setup functioned properly, a 2 mM K₃Fe(CN)₆ aqueous solution in 1 M KNO₃ was measured, and a reversible redox potential was observed at 245 mV (vs. Ag/AgCl) at scan rates of 20-200 mV/s. The glassy carbon working electrode was polished before every scan, and a resistance correction was applied to the cyclic voltammograms using the resistance measurement of the electrolyte solution. Solutions were purged with nitrogen flow prior to each measurement, stirred between scans, and kept under a blanket of nitrogen during measurements.

Cyclic and square wave voltammograms using the hanging drop mercury electrode were obtained with the help of Dr. Michael Nippe in the Long Group, with an SP-200 potentiostat. Electrochemical grade mercury was used as purchased, and nine drops were grown at the capillary for each measurement. The other electrodes were silver/silver chloride (reference) and platinum wire (auxiliary). A resistance correction was applied to the cyclic voltammograms using the resistance measurement of the electrolyte solution. Solutions were purged with argon flow prior to each scan and kept under a blanket of argon during scans.
**APPENDIX**

**Lanthanide Complexes with \( \mathbf{L}^1 \)**

In addition to cerium and praseodymium, neodymium and terbium are also lanthanides with metastable +4 oxidation states.\(^{1d}\) \( \text{Tb(IV)} \) and \( \text{Pr(IV)} \) in water have even been observed experimentally as the carbonate complexes,\(^{15}\) but reproducing these results with \( \mathbf{L}^1 \) has not been successful. With carbonate, \( \text{Tb(IV)} \) and \( \text{Pr(IV)} \) have absorption peaks centered at 380 and 300 nm, respectively, and similar transitions with the \( \mathbf{L}^1 \) system would be obscured by the ligand absorption. The absorption spectra of the synthesized \( \text{Pr}, \text{Nd}, \) and \( \text{Tb} \) complexes (Fig. A5-1) do not show any LMCT bands. Furthermore, the cyclic voltammograms of the \( \text{Pr} \) and \( \text{Tb} \) complexes only show waves characteristic of the ligand.

![UV-Vis spectra of Ln complexes with \( \mathbf{L}^1 \). Isolated solid complexes were dissolved in 0.1 M HEPES, 0.1 M KCl, at pH 7.2.](image)

**Figure A5-1.** UV-Vis spectra of Ln complexes with \( \mathbf{L}^1 \). Isolated solid complexes were dissolved in 0.1 M HEPES, 0.1 M KCl, at pH 7.2.

**Ce\( \mathbf{L}^1 \) Electrochemistry**

Upon basification of the aqueous solution of Ce\( \mathbf{L}^1 \) (whose pH is approximately neutral following the dissolution of the solid complex), the cathodic and anodic peaks were observed to completely disappear (Fig. A5-2), while a reduction of high current appeared below -0.7 V. This peculiar behavior was reproducible and should be further investigated.

The reversible Ce(IV)-Ce(III) couple was also observed by square wave voltammetry (Fig. A5-3). The higher sensitivity of this technique gave good resolution of the reduction and oxidation of the system, but these voltammograms were merely preliminary, and further experiments are required for a more thorough analysis.
Figure A5-2. CV of CeL1 in 1 M KNO3 and the HDME working electrode. Cyclic voltammograms of 1 mM CeL1K4 at a scan rates of 500 mV/s, before (blue) and after (orange) adjusting the pH of the solution with KOH. Initial scan direction: cathodic.

Figure A5-3. Square wave voltammogram of CeL1 in 1 M NaClO4 and the HDME working electrode. For scans shown, the pulse height was 50 mV and the step height was -10 mV, with the indicated pulse widths.
REFERENCES


