Fall 2008

Synthesis, characterization and inertness studies of gallium(III) and indium(III) complexes of dicarboxymethyl pendant-armed cross-bridged cyclam

Orjana Terova
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Synthesis, characterization and inertness studies of gallium(III) and indium(III) complexes of dicarboxymethyl pendant-armed cross-bridged cyclam

Abstract
Radiopharmaceuticals are drugs based on radioisotopes designed for diagnostic and therapeutic purposes. Radiometal complexes as radiopharmaceuticals for medical imaging have received increased attention in the past decades. Coordination chemistry plays an important role in the design of these target-specific radiopharmaceuticals. It is very important to correlate all aspects of a radiometal’s coordination chemistry with its in vivo behavior. Macrocyclic chelators are ligands that form stable metal complexes. In the 1990s Weisman, Wong et al. developed a new class of tetraazamacrocycles featuring ethylene cross-bridges linking nonadjacent nitrogens. These cross-bridged chelators form very inert complexes with a variety of metal cations. While most attention has been paid to their Cu(II) complexes, Ga(III) and In(III) are also potential radiometal candidates. In this work, previously inaccessible Ga(III) and In(III) complexes of a dicarboxylate cross-bridged cyclam ligand have been synthesized for the first time. Their kinetic inertness has been investigated by acid decomplexation studies. Finally, Cu(II) complexations have also been investigated incorporating microwave-assisted conditions.

Keywords
Chemistry, Inorganic

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SYNTHESIS, CHARACTERIZATION AND INERTNESS STUDIES OF
GALLIUM(III) AND INDIUM(III) COMPLEXES OF DICARBOXYMETHYL
PENDANT-ARMED CROSS-BRIDGED CYCLAM

BY

ORJANA TEROVA

B.S. Worcester State College, 2004

THESIS

Submitted to the University of New Hampshire
In Partial Fulfillment of
the Requirements of the Degree of

Master of Science

in

Chemistry

September, 2008
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Dr. Roy P. Planalp
Associate Professor of Chemistry

6/9/08
Date
DEDICATION

I dedicate this thesis to my husband Ilia for his continuous love and support. I would also like to dedicate this work to my amazing parents and brother for their inspiration, love and principles that shaped me into who I am today.
ACKNOWLEDGMENT

It will be very difficult to express in a paragraph the amount of gratitude that I feel for the people who helped me achieve this degree. Dr. Wong has had the most impact on me during my time here at UNH. I will forever have the deepest admiration and respect for him. He portrays the definition of a true mentor and I will always be grateful for his endless patience, guidance and genuineness. His passion for chemistry and innovative research has continuously inspired and motivated me. On a lighter note I will miss our conversations about sports, music, culture and life in general. Also, I have been fortunate to study under the guidance of Dr. Gary Weisman. I will always value his enthusiasm for teaching and research. I truly feel privileged to have studied under the guidance of these two great scientists.

I am thankful to Dr. Roy Planalp for his interest and his constructive suggestions about my research. I would like to thank all other UNH faculty who have played a very important role during my time here.

I can truly say that I have established a lifelong friendship with Antoinette Odendaal and Abraham Tucker. I want to thank them for their true values and character and for all the good times.

I would like to acknowledge all the graduate students in the department and the Wong-Weisman group members for their help and support.

I must recognize the UIC staff (Kathy Gallagher, Pat Wilkinson, and John Wilderman) and our chemistry librarian Bob Constantine for their help. Finally, I would like to give a special thanks to Cindi Rohwer and Peg Torch for all their help during my time at UNH.
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ABSTRACT

SYNTHESIS, CHARACTERIZATION AND INERTNESS STUDIES OF GALLIUM(III) AND INDIUM(III) COMPLEXES OF DICARBOXYMETHYL PENDANT-ARMED CROSS-BRIDGED CYCLAM

BY

ORJANA TEROVA

University of New Hampshire, September, 2008

Radiopharmaceuticals are drugs based on radioisotopes designed for diagnostic and therapeutic purposes. Radiometal complexes as radiopharmaceuticals for medical imaging have received increased attention in the past decades. Coordination chemistry plays an important role in the design of these target-specific radiopharmaceuticals. It is very important to correlate all aspects of a radiometal’s coordination chemistry with its \textit{in vivo} behavior. Macrocyclic chelators are ligands that form stable metal complexes. In the 1990s Weisman, Wong \textit{et al.} developed a new class of tetraazamacrocycles featuring ethylene cross-bridges linking nonadjacent nitrogens. These cross-bridged chelators form very inert complexes with a variety of metal cations. While most attention has been paid to their Cu(II) complexes, Ga(III) and In(III) are also potential radiometal candidates. In this work, previously inaccessible Ga(III) and In(III) complexes of a dicarboxylate cross-bridged cyclam ligand have been synthesized for the first time. Their kinetic inertness has been investigated by acid decomplexation studies. Finally, Cu(II) complexations have also been investigated incorporating microwave-assisted conditions.
CHAPTER I

INTRODUCTION

1. Coordination chemistry of tetraazamacrocycles

Polydentate macrocyclic ligands incorporate their donor atoms into the cyclic backbone. Macrocyclic ligands contain a minimum of nine atoms; at least three of which must be donors.¹ Macrocyclic host-guest complexation chemistry has been developed extensively. There has been intense investigation of this for a number of fundamental biological systems due to the enhanced kinetic and/or thermodynamic stabilities of the resulting metal complexes.¹⁻⁷ This has provided a great deal of impetus for the exploration of the metal-coordination chemistry of these systems.⁸,⁹

Tetraazamacrocycles are a class of macrocyclic ligands that contains nitrogen donor atoms (Figure 1.01).¹,² These ligands can form metal complexes with a variety of metal cations including alkali, alkaline earth, transition metals, and lanthanides.⁴,¹⁰ Metal complexes of macrocyclic tetraamines can potentially adopt several configurationally isomeric cis- or trans-coordination-modes.¹¹,¹² Increased thermodynamic stability and kinetic inertness are observed for the complexes of cyclic ligands versus the acyclic analogues.¹³ This increased stability is due to the macrocyclic effect as defined by Cabbiness and Margerum.¹³ As a result of their kinetic inertness, tetraazamacrocycle complexes have a wide variety of potential and realized applications in medical sciences.¹⁴,¹⁵
Figure 1.01: Common tetraazamacrocycles: Cyclen 1, Homocyclen 2, Cyclam 3

Cyclam metal complexes can adopt any of eight possible trans- or cis-folded configurations, depending on the arrangement at the nitrogen atoms (Figures 1.02, 1.04). The naming of the isomers is based on the nomenclature of Bosnich et al. The ligand can adopt any of these configurations about the metal depending on the size of the metal ion, because the selectivity of these ligands is mostly based on size match selectivity. It has been shown that the trans-III configuration is most frequently obtained when the macrocycle is coordinated to a metal ion that has the suitable size (i.e. Cu(II)). When the metal ion is too large, configurations such as trans-I (e.g. Hg(II), Cd(II)) or cis-V (e.g. Pb(II)) have been observed.

In general, cyclen metal complexes adopt the trans-I configuration (Figure 1.03) as supported by crystallographic data, as the trans-III configuration has a smaller cavity which is only suitable for very small metal cations like Be$^{2+}$. This configuration is higher in energy than the trans-I, which means that trans-III metal complexes of cyclen most likely will not be formed for normal d-cations.
Figure 1.02: Five possible trans-configurations for cyclam

Figure 1.03: Four possible configurations for cyclen

Figure 1.04: Cis coordination modes for cyclam
Some of the many realized and potential applications of cyclam complexes include catalysis, anti-HIV drugs as well as biomimetic chemistry.\textsuperscript{10,14,15,21,22} There has also been a great deal of research in investigating tetraazamacrocycles as potential bifunctional chelators in radiopharmaceutical-based applications due to the high stability of their complexes.\textsuperscript{21,22} A more detailed discussion of this will be given in Section 1.2.

1.1. Cross-bridged tetraamines and their coordination chemistry

In 1990 Weisman, Wong and co-workers were the first to report a class of novel cross-bridged tetraamine ligands, (Figure 1.05) which contain a bridging ethylene unit connecting two nonadjacent nitrogens.\textsuperscript{23,24} The ligand itself is quite flexible, but when it is coordinated with smaller metal cations, it adopts a \textit{cis-V} folded coordination geometry (Figure 1.06).\textsuperscript{25-30} The cross-bridged tetraamines have been designed to adopt low energy conformations in which all four nitrogen lone pairs are convergent upon a cleft.\textsuperscript{25-30} This is ideal for the complexation of smaller metal cations (e.g. Cu(II), Zn(II), Ga(III) with ionic radii 76 pm to 88 pm, data for coordination number of six).\textsuperscript{25-30} The cross-bridged ligands can further be functionalized with various pendant arms, which allows for fine tuning of metal coordination, completion of a coordination sphere, and neutralizing the positive charge of the metal cation with ionizable functionalities, all of which enhances the complex stability.
Among the transition metals used to complex several cross-bridged cyclam- and cyclen-based ligands that have been investigated by Niu are Cu(II), Zn(II), and Hg(II). Also Ga(III) metal complexes of ligands 5 and 6 and In(III) complexes of ligands 4 and 6 were synthesized by Niu. All of these display the favored cis-V folded coordination geometry.

Recently we have been interested in Cu(II), In(III), and Ga(III) complexes of these crossed-bridged ligands due to their potential as bifunctional chelators (BFCs) in radiometal-based pharmaceutical applications. It has been confirmed that crossed-bridged ligand 7, CB-TE2A, is a valuable bifunctional chelator (BFC) for $^{64}$Cu.
CB-TE2A has been bioconjugated to the somatostatin analogue Y3-TATE and superior behavior of its $^{64}$Cu-labeled bioconjugate has been observed as a result of its improved *in vivo* stability.\textsuperscript{32,33}

![Figure 1.07: Ligand 7-based bioconjugate](image)

### 1.2 Ga(III) and In(III) metal ions and their relevance in radiopharmaceutical applications

The use of radiometal complexes as radiopharmaceuticals for medical imaging is a relatively new area that is rapidly growing.\textsuperscript{21,22} Metallic radionuclides are of great interest because of their wide range of nuclear properties as well as their well established coordination chemistry. In 1959, Brookhaven National Laboratory developed the first $^{99}$Mo/$^{99m}$Tc generator, which marked a significant milestone for subsequent development of small molecule $^{99m}$Tc complex radiopharmaceuticals.\textsuperscript{36}

Coordination chemistry plays an important role in the design of target-specific radiopharmaceuticals. There are three general strategies in the radiopharmaceutical design: the integrated approach, the bifunctional approach (\textbf{Figure 1.08}), and the peptide-hybrid approach.\textsuperscript{35,40} In all three approaches inorganic chemistry is very significant, because it is the radiometal that distinguishes radiopharmaceuticals from
traditional therapeutic pharmaceuticals. When designing metal complex-based imaging agents it is very important to correlate aspects of coordination chemistry with subsequent in vivo behavior. Some of the factors that should be considered are the redox properties, stability, stereochemistry, charge and solubility of the metal complex.\textsuperscript{34,35}

There are three imaging modalities used in radiology: gamma scintigraphy, Positron Emission Tomography (PET), and Magnetic Resonance Imaging (MRI). Metal complexes are used in all three imaging modalities.\textsuperscript{34,35} For gamma scintigraphy and PET, radiopharmaceuticals labeled with metal radionuclides are injected into patients to diagnose medical problems such as cancer, infection, kidney and liver abnormalities, and cardiological and neurological disorders.\textsuperscript{34,37-40} There are several significant considerations when designing a radiopharmaceutical. These factors include the half-life of the radiometal, the mode of decay, and the cost and availability of the isotope.\textsuperscript{34,37-40} Radiometals for coordination complex-based radiopharmaceuticals in PET and gamma scintigraphy range in half-lives from about 10 min to several days.\textsuperscript{34,36} The desired half-life depends on the time required for the radiopharmaceutical to localize at the target tissue. Most of the radionuclides used in nuclear medicine for diagnostic imaging decay primarily by gamma emission.\textsuperscript{37-40} Radionuclides used in PET decay by positron emission.
Figure 1.08: A schematic representation of a radiopharmaceutical. CB-TE2A acts as a BFC where it complexes to a $^{64}$Cu radionuclide and also has a functional group to attach the chelated metal to a biomolecule.

Our interest in the coordination chemistry of gallium is in large part due to the potential application of $^{68}$Ga-labeled bioconjugates as PET imaging agents.\textsuperscript{34-40} We are also interested in exploring the coordination chemistry of indium due the wide use of $^{111}$In in gamma scintigraphy.\textsuperscript{37-40}

The most relevant oxidation state of gallium and indium for radiopharmaceutical chemistry is $^{+3}$, which is their most prevalent oxidation state in aqueous solution. In$^{3+}$ and Ga$^{3+}$ are hard acids and due to their high charge density they prefer chelators with hard donors such as amine-N, carboxylate-O, and phenolate-O. Depending on the ligand,
gallium can adopt coordination numbers of 3, 4, 5, and 6, but to maintain its *in vivo* stability $\text{Ga}^{3+}$ is often six-coordinated in a chelator. Indium(III) is a larger cation than $\text{Ga}^{3+}$ and can adopt up to 8-coordination geometries. Their aqueous chemistry is dominated by their ability to form strong complexes with $\text{OH}^-$ ion. Their hydrated $\text{Ga}^{3+}$ and $\text{In}^{3+}$ forms are only stable under acidic conditions. Once the pH is raised above 3, insoluble $\text{In(OH)}_3$ and $\text{Ga(OH)}_3$ will form. This is a great challenge that we face (particularly at pH>3) during radiolabeling in aqueous solution. Another important aspect that should be considered is that both $\text{Ga}^{3+}$ and $\text{In}^{3+}$ bind to transferrin protein (Tf) in the blood with high affinity, thus this protein has an important effect on the biodistribution of these radionuclides. Tf binding must be taken into account when designing new radiopharmaceuticals. Thus the majority of reported gallium and indium complexes employed as radiopharmaceuticals have very high thermodynamic stability or are kinetically inert to exchange with transferrin *in vivo*. Gallium and indium radioisotopes are summarized in Table 1.1.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>$T_{1/2}$ (hours)</th>
<th>Decay mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{65}\text{Ga}$</td>
<td>9.5</td>
<td>$\beta^+$ (56%) EC (44%)</td>
</tr>
<tr>
<td>$^{67}\text{Ga}$</td>
<td>78.26</td>
<td>EC (100%)</td>
</tr>
<tr>
<td>$^{68}\text{Ga}$</td>
<td>1.1</td>
<td>$\beta^+$ (90%) EC (10%)</td>
</tr>
<tr>
<td>$^{111}\text{In}$</td>
<td>67.9</td>
<td>EC (100%)</td>
</tr>
</tbody>
</table>

Table 1.1: Isotopes of gallium and indium.
Gallium has three radioisotopes with decay characteristic suitable for PET imaging or gamma scintigraphy (Table 1.1). $^{67}$Ga is more utilized due to its ability to identify both inflammations and soft tissue tumors. $^{68}$Ga is interesting because it is a positron emitter and thus used for PET imaging giving higher resolution and sensitivity. Also this radionuclide is generator produced with a very long-lived parent. $^{66}$Ga is also a positron emitter, but it has only a moderate positron efficiency.

A very important and the only clinically-used gallium radiopharmaceutical is $^{67}$Ga-labeled citrate/transferrin. This radiopharmaceutical exchanges in vivo with transferrin and is widely used for the clinical diagnosis of certain types of neoplasms, such as Hodgkin’s disease, lung cancer, melanoma, and leukemia.

The most widely-used radioisotope of indium is $^{111}$In (Table 1.1) which decays by electron capture with subsequent emission of gamma photons, suitable for gamma scintigraphy. $^{111}$In –DTPA-Octreotide (Octreoscan) was approved by the FDA in 1994 as a diagnostic imaging agent for neuroendocrine tumors.

1.2.1 Coordination chemistry of Ga(III) and In(III) of cyclam, cyclen and their derivatives

In section 1 the possible coordination geometries of cyclam and cyclen were described. There are actually few Ga(III) and In(III) complexes of cyclam, cyclen or their derivatives that are fully characterized in the literature. In 1999 Macke and coworkers reported the Ga(III) and In(III) complexes of a DOTA-functionalized somatostatin analogue, DOTATOC (Figure 1.09). The gallium complex adopts a pseudo-octahedral geometry with a cis-folded macrocyclic unit in a [2424]
conformation.\textsuperscript{41} The larger indium cation complexes in an eight-coordinated fashion in the solid state, but becomes seven-coordinate in solution.\textsuperscript{41}

Recently Liu and coworkers reported the crystal structures of In(III) and Ga(III) complexes of -DO3A-xy-TPP (Figure 1.10).\textsuperscript{42} In the indium complex the ligand acts as a heptadentate ligand and the coordination geometry is a monocapped octahedron. In the gallium complex the coordinated ligand is hexadentate due to its smaller size and the complex coordination geometry is described as distorted octahedral.\textsuperscript{42}

![Diagram of Ga(III) and In(III) complexes with DOTATOC](image)

**Figure 1.09: Ga(III) and In(III) complexes with DOTATOC**
1.2.2 Coordination chemistry of Ga(III) and In(III) with cross-bridged tetraamines

Weijun Niu was the first person to synthesize gallium and indium complexes of cross-bridged tetraamines. Specifically, Niu was able to prepare and fully characterize the Ga(III) complexes of ligands 5 and 6 and the In(III) complexes of ligands 4, and 6. The [GaCl$_2$-CB-Cyclam]Cl complex (Figure 1.11) revealed a *cis*-V coordination geometry where the conformation of the ligand is a [2323]/[2323] distorted diamond lattice. Gallium fits well into the ligand molecular cleft displaying a distorted octahedral coordination sphere. [InBr$_2$-CB-Cyclam]Br (Figure 1.12) also has a distorted octahedral geometry but in contrast to the [GaCl$_2$-CB-Cyclam]Cl complex, the larger In(III) does not fit as well in the ligand cleft. [InBr$_2$-CB-Cyclen]Br (Figure 1.13) has also been synthesized by Niu and it can be observed from the crystal structure that the geometry is pseudo-octahedral where In(III) has an even more distorted coordination
geometry than in [InBr$_2$-CB-Cyclam]Br.$^{31}$ This is a combination of both the large size of the In(III) cation along with the smaller ligand cavity in cyclen-based macrocycles.

Figure 1.11: X-ray structure of [GaCl$_2$-CB-Cyclam]$^+$

Figure 1.12: X-ray structure of [InBr$_2$-CB-Cyclam]$^+$
The structure of the \([\text{Ga-CB-DO2A}]\text{NO}_3\) complex (Figure 1.14) again reveals a distorted octahedral geometry around the Ga(III) coordination sphere. These results show that the smaller Ga(III) cation is a better fit for the cross-bridged chelators, whereas the larger In(III) yielded more distorted complexes. Wei-Chih Lee was able to synthesize \([\text{In-CB-DO2A}]\text{Cl}\) following the procedure developed by Niu for the \([\text{Ga-CB-DO2A}]\) complex. Direct attempts by Lee to synthesize complexes of In(III) and Ga(III) with 7 resulted only in protonated ligand. An indirect route was developed by Lee where \([\text{InBr}_2\text{-CB-Cyclam}]\text{Br}\) was alkylated with ethyl bromoacetate to synthesize \([\text{InBr}_2\text{-Et}_2\text{-CB-TE2A}]\text{Br}\) (Scheme 1.1). This was followed by hydrolysis of the two acetate arms \([\text{InBr}_2\text{-Et}_2\text{-TE2A}]\) to obtain \([\text{In-CB-TE2A}]^+\), which was not fully characterized. However Lee’s attempts to indirectly synthesize \([\text{Ga-CB-TE2A}]^+\) this way were not successful.
Scheme 1.1: Indirect route to synthesize [In-CB-TE2A]

Figure 1.14: X-ray structure of [Ga-CB-DO2A]+
1.3 Research Goals

The focus of this thesis is the synthesis and characterization of Ga(III) and In(III) complexes of dicarboxylate cross-bridged tetraamines. As mentioned above, Ga(III) and In(III) tend to form very stable complexes which makes them interesting for radiopharmaceutical-based applications. The *in vivo* stability of the complexes is a very important aspect; thus acid-decomplexation studies have been performed to gauge their kinetic inertness. The starting materials for preparing the desired complexes were synthesized according to previous procedures developed in the Weisman-Wong group (Scheme 1.2).\(^{25}\) We have expanded our efforts into exploring microwave-assisted coordination chemistry and several complexation reactions have been investigated using a microwave reactor.

![Scheme 1.2: General synthetic scheme to synthesize ligands 4, 5, 6, and 7](image-url)

Scheme 1.2: General synthetic scheme to synthesize ligands 4, 5, 6, and 7
CHAPTER II

GALLIUM(III) AND INDIUM(III) COMPLEXATIONS WITH A
DICARBOXYLATE-ARMED CROSS-BRIDGED CYCLAM LIGAND

2.1 Introduction

As discussed in Chapter I our interest in the complexation of Ga(III) and In(III) with ligand 7 (Figure 2.01) is due to the ability of this ligand to form kinetically inert complexes which can be used in radiopharmaceutical-based applications. Niu was the first to synthesize a Ga(III) complex with ligand 5. Lee successfully synthesized the In(III) complex with ligand 5, but no X-ray structural data were obtained. These were the only two Ga(III) and In(III) metal complexes with dicarboxylate-armed cross-bridged ligands when this work was undertaken. Acid decomplexation studies reveal remarkable kinetic inertness of these compounds.

![Figure 2.01: H2-CB-TE2A 7, a dicarboxylate cross-bridged cyclam ligand](image)

Figure 2.01: H2-CB-TE2A 7, a dicarboxylate cross-bridged cyclam ligand
There are many factors that will influence the ease of metal complexation with cross-bridged macrocycles. These include the nature of the solvent, steric and electrostatic effects as well as the ligand protonation state. One significant challenge to keep in mind is that in aqueous solution Ga$^{3+}$ and In$^{3+}$ tend to form the insoluble hydroxide species if the pH is higher than 3. This is a major limitation, which means that for effective complexation the reactions need to be performed at pH 3 or lower. This challenge now couples with another, because we must consider ligand protonation since under these conditions the ligand is at least twice inside-protonated. Metal complexation will be more challenging as a consequence of the additional electrostatic repulsion present. Solvent exchange can control formation kinetics which involves substitution of a ligand for a coordinated solvent molecule. Thus the water exchange rates for the hydrated Ga$^{3+}$ and In$^{3+}$ may be factors. The water exchange rates for these metal cations are relatively slow (rate constants are $10^7$ s$^{-1}$ for Ga$^{3+}$ and $10^6$ s$^{-1}$ for In$^{3+}$), which further slows down the metal complexation. It is imperative to keep in mind all of these factors and challenges when investigating Ga$^{3+}$ and In$^{3+}$ complexations with ligand 7.

Weijun Niu and Wei-Chih Lee attempted Ga(III) and In(III) complexations with ligand 7 under varying temperature, reaction times, aprotic solvents and strictly anhydrous conditions, but all their attempts resulted in only the protonated ligand.
2.2 Synthesis and characterization

2.2.1 Synthesis of the Ga(III) complex of ligand 7

Scheme 2.1: Ga(III) complexation with ligand 7

Ligand 7 (as an HCl salt from its diethyl ester) was dissolved in H$_2$O and to this solution an aqueous solution containing an equimolar amount of Ga(NO$_3$)$_3$ $\cdot$ H$_2$O was added. The pH of the resultant homogenous solution was adjusted to pH 3.6 with aqueous NaOH. After this the reaction was run in a closed (10 mL) microwave vessel for 4 hours at 180°C (Scheme 2.1). When the reaction was complete the supernatant was isolated from a small amount of precipitate via centrifugation. Evaporation of the supernatant to dryness yielded the complex as a yellow solid. The crude product was washed with methanol to afford the complex as a white precipitate. The resulting complex was then dissolved in a 80:20 methanol:water (v:v) solvent mixture and ether diffusion into this solution afforded colorless crystals of the complex (45%).

Many other approaches were tried including temperature and time variations, protic and aprotic solvents and a variety of bases. Most of these attempts were not successful. The conditions described above are optimized.
Previously various bases were used and the reactions were carried out in a strictly anhydrous environment in non-aqueous solvents, using a glove box with nitrogen atmosphere. Both thermal heating and microwave conditions were employed. However, all of these attempts were unsuccessful (Scheme 2.2)

Scheme 2.2: Previous attempts of Ga(III) complexation using anhydrous conditions and aprotic solvent

Other complexation conditions involved using buffered aqueous media. These reactions were run under thermal conventional as well as microwave-assisted conditions (Scheme 2.3). All the thermal reactions gave no product. Reactions utilizing a microwave reactor at pH=4 showed promising results from mass spectral data, but because of significant of the citrate buffer decomposition it was difficult to isolate or characterize the products.
Since buffer decomposition occurred, the reactions were subsequently run in water. Promising results were obtained at pH 3.5 at 150 °C for 30 minutes showing mixtures of the desired complex and starting material. This led to longer reaction times (30 hours) and higher temperatures (180 °C) (Scheme 2.4). It was noticed that at longer reaction times the pH became basic (9.2) when the reaction was complete. It is possible that because the starting material is a TFA salt, decomposition of TFA under such harsh reaction conditions was occurring, possibly releasing CF₃H (pKa~29) and OH⁻, which would account for the pH change. As a control, a TFA solution was placed in the microwave at 180 °C for 3 hours and the pH was indeed basic afterwards. HCl salts of the ligand were therefore used instead to avoid this problem. Unfortunately this problem still persisted, though reaction times were reduced to 4 hours.
2.2.2 Synthesis of In(III) complex with ligand 7

The same methodology developed for the Ga(III) complexation was used to prepare the In(III) complex of ligand 7 (Scheme 2.5). Ligand 7 prepared from HCl hydrolysis of its di-ethyl-ester was dissolved in 1 mL of water and added to an equimolar amount of In(NO$_3$)$_3$·5H$_2$O in 1mL of water. Aqueous NaOH was added to adjust the pH of the resulting clear solution to pH 3.9. The reaction was then run in a closed vessel for three hours in a microwave reactor at 180 °C. When the reaction was complete a very small amount of precipitate was removed by centrifugation. The supernatant was evaporated to dryness to yield a brown oily residue that solidified upon removal of residual solvent under reduced pressure.
Various attempts were tried to purify the complex but they were not successful. Along with the product, inorganic salts such as NaNO₃ and NaCl were found as byproducts. In order to simplify the reaction, InCl₃ was used as the metal salt. The reaction conditions were kept the same but the reaction time had to be increased to 7 hours (75% yield) (Scheme 2.6).

![Scheme 2.6: In(III) complexation with 7 using InCl₃](image)

Another approach that was taken to purify the [In-CBE2A]⁺NO₃⁻ complex was to use counterion exchange. Crude [InCBTE2A]⁺NO₃⁻ was stirred with a saturated aqueous solution of NH₄PF₆ to precipitate [In-CBTE2A]⁺PF₆⁻ as a white powder (67% yield) (Scheme 2.7). Slow evaporation from a 1:1 acetonitrile:methanol (v:v) solution of this product yielded colorless crystals of the complex.

![Scheme 2.7: Anion exchange using NH₄PF₆](image)
2.2.3 Spectral data

Solution $^1$H and $^{13}$C{$^1$H} NMR data

Figures 2.02 and 2.03 show the $^1$H and $^{13}$C{$^1$H} NMR spectra of the [Ga-CB-TE2A]$^+$ complex respectively. Both $^1$H and $^{13}$C{$^1$H} NMR spectra reveal that the complex is C$_2$-symmetrical. The proton spectrum reveals a doublet of pentets (δ 1.89) that is consistent with the equatorial protons on the β-ring carbons. The multiplet at δ 2.42-2.54 corresponds to the axial protons of the β-carbons. The carboxylate pendant arm methylene protons appear in an AX pattern with an additional long-range W-coupling. The $^{13}$C{$^1$H} NMR spectrum reveals eight carbon resonances suggesting that [Ga-CB-TE2A]$^+$ complex is C$_2$ symmetric on the NMR time scale. A 2D-HSQC (Figure 2.04) experiment was performed which showed that the downfield carbon resonance at 62.84 ppm correlates with the diastereotopic methylene protons on the pendant arms. Also the β methylene protons correlate with the β carbon resonance at 20.75 ppm.

![NMR Spectra](image)

Figure 2.02: $^1$H NMR (in D$_2$O using MeCN as reference at 2.06 ppm) of [Ga•7]$^+$NO$_3^-$
Figure 2.03: $^{13}$C{H} NMR (using MeCN as reference at 1.47 ppm) of [Ga-7]$^{+}$NO$_3^-$

Figure 2.04: HSQC NMR in D$_2$O (using MeCN as reference at 1.47 ppm) of [Ga-7]$^{+}$NO$_3^-$

The $^1$H NMR spectrum of [In-CB-TE2A]$^{+}$NO$_3^-$ (Figure 2.05) shows significant dynamic broadening at room temperature. Due to this significant broadening it is difficult to make assignments but the spectrum does show a doublet of multiplets at δ 1.85-1.95 and a broad quartet-like multiplet at 2.47-2.61 ppm that correspond to the equatorial and axial methylene protons on the β-carbon respectively.
Variable-temperature studies were performed by increasing the temperature from 25°C to 75°C (Figure 2.06). As 75°C was approached sharpening of the peaks occurred and the upfield doublet of multiplet became a doublet of pentets. Also the ~2.5 ppm multiplet gained better resolution. As the temperature was increased the distinct downfield AB corresponding to the diastereotopic methylene protons on the carboxylate arms can now be clearly discerned.
The ¹H and ¹³C{¹H} NMR spectra of [In-CB-TE2ACl] (Figures 2.07 and 2.08) show the same dynamic broadening observed for [In-CB-TE2A]⁺NO₃⁻. ¹H NMR does reveal the two diagnostic upfield multiplets; the doublet of multiplets and broad multiplet for the equatorial and axial β-methylene protons δ 1.75-1.87 and 2.43-2.57 ppm respectively. This points toward the dominance of a diamond-lattice structure. The ¹³C{¹H} NMR spectrum shows the β-carbon at δ 21.69 and the carbonyl carbon of the pendant arms at δ 175.93 as well as several broadened signals. High-temperature NMR experiments were performed over a range of temperatures (Figure 2.09). As the temperature was increased from 25 °C the peaks sharpened and once 80 °C was reached, the AX for the CH₂ protons on the pendant arms became resolved. The ¹³C{¹H} NMR spectrum at 80 °C (Figure 2.10) clearly revealed eight carbon resonances, which is consistent with a C₂-symmetric complex.

Figure 2.06: Variable-temperature ¹H NMR (in D₂O using MeCN as reference at 2.06 ppm) of [In-7]⁺NO₃⁻
One possible explanation for the dynamic broadening observed at room temperature is exchange with a species that has the counterion coordinating and filling the seventh coordination site. It is possible that nitrate or chloride can be coming on and off the indium coordination sphere at room temperature but at higher temperatures it may completely dissociate leaving a six-coordinate complex that is $C_2$ symmetric or the exchange might be fast on the NMR timescale. X-ray data have shown that the chloride is indeed coordinated in the solid state, confirming a seven-coordinate geometry. However this solid state data may not be relevant to actual solution speciation. Since addition of up to 50 equivalents of KCl did not alter the appearance of the room temperature spectrum, it is unlikely that counterion dissociation is responsible for the dynamic broadening. A fluxional seven-coordinate solution species may account for this temperature-dependent spectral behavior (Scheme 2.8).

![Figure 2.07: $^1$H NMR (in D$_2$O using MeCN as reference at 2.06 ppm) of [In-7]Cl at 25°C](image)

Scheme 2.8: Possible dynamic behavior of [In-7]Cl.
Figure 2.08: $^{13}$C{$^1$H} NMR (using MeCN as reference at 1.47 ppm) of [In-7Cl] at 25°C

Figure 2.09: Variable temperature {$^1$H} NMR (in D$_2$O using MeCN as reference at 2.06 ppm) of [In-7Cl]
Room temperature $^1$H and $^{13}$C$^1$H NMR spectra of [In-CB-TE2A]PF$_6$ are consistent with a C$_2$-symmetric complex in solution. The $^1$H NMR spectrum (Figure 2.11) reveals an upfield doublet of multiplets and a quartet-like multiplet for the equatorial and axial $\beta$-methylene protons at $\delta$ 1.91-1.99 and 2.49-2.62 ppm respectively. The AX of the pendant arm methylene protons appears at $\delta$ 3.62 and 3.89 ppm. The $^{13}$C$^1$H NMR spectrum (Figure 2.12) reveals seven instead of eight carbon resonances but two of the signals are likely isochronous making the intensity of the resonance at $\delta$ 58.92 ppm twice as large as the other resonances. The carbon resonances at $\delta$ 21.93 and $\delta$ 174.88 correspond to the $\beta$-carbon and the carbonyl carbon on the pendant arms respectively.

Interestingly there is no significant dynamic broadening observed in this case. This could be another indication that what was observed for the [In-CB-TE2A]NO$_3$ and [In-CB-TE2A]Cl complexes may indeed be due to anion coordination to give a seven-coordinate solution species. In [In-CB-TE2A]PF$_6$ where the counterion is non-coordinating, this dynamic broadening was therefore not observed.
Infrared and Mass Spectra

The IR (KBr) spectrum (Figure 2.13) of the [Ga-CB-TE2A]'NO$_3^-$ complex shows a band at 2970 cm$^{-1}$ which corresponds to the CH$_2$ stretch. There are also two sharp carboxylate bands at 1678 and 1650 cm$^{-1}$ from the coordinated carboxylate pendant arms. The FAB$^+$ mass spectrum (Figure 2.14) of [Ga-CB-TE2A]'NO$_3^-$ exhibits a major peak at approximately 409 which is consistent with the cation [Ga-CB-TE2A]$^+$ and composition of C$_{10}$H$_{20}$O$_4$N$_4$Ga. The most abundant peak at 100% intensity is followed by isotopologues at 30% intensity and then 70% intensity. These are confirmed by a spectral simulation (Figure 2.15).$^{60}$

The [In-CB-TE2A]$^+$Cl$^-$ IR spectrum (Figure 2.16) exhibits a carboxylate stretch at 1634 cm$^{-1}$ and CH$_2$ stretch at 2921 cm$^{-1}$. The FAB$^+$ Mass spectrum of
[In-CB-TE2A]^+Cl^- (Figure 2.17) exhibits a major peak around 455 which is consistent with the cation [In-CB-TE2A]^+ with the composition C_{16}H_{25}O_{4}N_{4}In. The peak at 455 is the major one at 100% intensity and the next peak is at 19.3% intensity, consistent with the predicted spectrum (Figure 2.18). Mass spectrometry has therefore been very useful in characterizing these Ga(III) and In(III) metal complexes due to the unique isotopic distribution of the respective metal cations.

![Figure 2.13: IR(KBr) spectrum of [Ga-7]^+NO_3^-](image)

![Figure 2.14: FAB^+ Mass spectrum of [Ga-7]^+NO_3^-](image)
Figure 2.15: Simulated spectrum of [Ga\textsuperscript{7}]NO\textsubscript{3}\textsuperscript{60}

Figure 2.16: IR(KBr) spectrum of [In\textsuperscript{7}]Cl
Figure 2.17: FAB$^+$ Mass spectrum of [In$^7$]Cl

Figure 2.18: Simulated spectrum of [In$^7$]Cl $^{60}$
The \([\text{In-CB-TE2A}]^+\text{PF}_6^-\) IR (KBr) spectrum (Figure 2.19) contains the carboxylate stretch at 1640 cm\(^{-1}\) and PF\(_6^-\) stretch at 835 cm\(^{-1}\). The FAB\(^+\) mass spectrum (Figure 2.20) of \([\text{In-CB-TE2A}]^+\text{PF}_6^-\) exhibits a major peak around 455 which is consistent with the cation \([\text{In-CB-TE2A}]^+\) and the composition of C\(_{16}\)H\(_{28}\)O\(_4\)N\(_4\)In. The isotopic distribution is the same as for \([\text{In-CB-TE2A}]^+\text{Cl}^-\). Table 2.1 summarizes all the IR data.

Figure 2.19: IR(KBr) spectrum of \([\text{In-7}]^+\text{PF}_6^-\)
Figure 2.20: FAB* Mass spectrum of [In-7]⁺PF₆⁻

<table>
<thead>
<tr>
<th>Complex</th>
<th>ν_CO₂ (cm⁻¹)</th>
<th>ν_CH₂ (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ga·7]⁺NO₃⁻</td>
<td>1678, 1650</td>
<td>2970</td>
</tr>
<tr>
<td>[In·7]⁺Cl⁻</td>
<td>1634</td>
<td>2921</td>
</tr>
<tr>
<td>[In·7]⁺PF₆⁻</td>
<td>1640</td>
<td>2923</td>
</tr>
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</table>

Table 2.1: IR data of the Ga(III) and In(III) complexes of 7
2.2.4 X-ray Structural Data

X-ray structural data for $[\text{Ga-7}]^+\text{NO}_3^-$, $[\text{In-7}]^+\text{Cl}^-$, and $[\text{In-7}]^+\text{PF}_6^-$ have been obtained. Important bond lengths and bond angles have been summarized in Tables 2.2, 2.3 and 2.4.

The $[\text{Ga-7}]^+\text{NO}_3^-$ complex (Figure 2.21) has a slightly distorted octahedral geometry around gallium. This cation is encapsulated within the ligand molecular cleft. The conformation of the ligand is a distorted diamond lattice [2323]/[2323] with the familiar cis-folded coordination geometry. The axial and equatorial angles N-Ga-N are 179.10 (12)$^\circ$ and 85.39 (11)$^\circ$ respectively. The axial Ga-N bond distance is 2.065 (3) Å and equatorial Ga-N bond distance is 2.086 (3) Å and the average Ga-O bond distance is 1.950 (2) Å. Niu was able to prepare the Ga(III) complex of ligand 5, CB-DO2A, and he was able to obtain X-ray structural data for it. When comparing these two complexes it can be noted that in the Ga-7 complex gallium fits much better in the cyclam-based ligand molecular cleft than in that of Ga-5, which is cyclen-based. For Ga-CB-DO2A the N-Ga-N axial and equatorial bond angles are 164.56 (9)$^\circ$ and 86.24 (9)$^\circ$ respectively. The axial and equatorial Ga-N bond lengths are almost the same as for the Ga-7 complex, 2.078 (15) Å and 2.046 (15) Å respectively and the Ga-O bond length is of 1.942 (13) Å (Table 2.2). These results show that Ga(III) is a much better fit in ligand 7.
Figure 2.21: X-ray structure of [Ga\textsuperscript{7}]\textsuperscript{+}

<table>
<thead>
<tr>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
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<td>O(1)-Ga(1)-N(4)</td>
<td>91.98(10)</td>
</tr>
</tbody>
</table>

Table 2.2: Selected bond angles (deg) and bond lengths (Å) of [Ga\textsuperscript{7}]\textsuperscript{+}NO\textsubscript{3}
The \([In\cdot7]^+Cl^-\) complex (Figure 2.22) has a capped octahedral coordination geometry around the indium. Ligand 7 acts as a hexadentate donor with a N₄O₂ donor set. The chloride is also coordinated giving indium a seven-coordinate geometry. Indium is also larger than gallium thus the fit is poorer than in the Ga-7 case. The axial N(2)-In-Cl(1) and the equatorial N(1)-In-N(4) angles are 160.21 (6) ° and 81.46 (11) ° respectively. The average In-N bond length is 2.360 (2) Å and the average In-O bond length is 2.258 (17) Å. Table 2.3 has a summary of the important bond lengths and bonds angles of this complex. The coordination of the chloride counterion is not surprising. It would be interesting to look at the coordination chemistry with a non-coordinating anion. Thus as mentioned above the \([In\cdot7]^+PF_6^-\) was also prepared and its structural data (Figure 2.23) confirm a distorted octahedral complex. The crystal structure actually shows a dimer where two complexes are bridged through two sodium cations via both carbonyl oxygen atoms of the pendant arms. Ligand 7 binds to indium in a hexadentate fashion with a N₄O₂ donor set. The cation is encapsulated within the ligand molecular cleft. The conformation of the ligand is again a distorted diamond lattice [2323]/[2323] with a cis-folded coordination geometry. The axial and equatorial bond angles of N-In-N are 174.3 (3)° and 80.3 (3)° respectively (Table 2.4). The average In-O bond length is 2.136 (6) Å and the average In-N bond length is 2.231 (8) Å. Comparing this structure with that of \([In\cdot7]^+Cl^-\), the cation fit is better in this case because of the lower coordination number. It is important to note that the \([In\cdot7]^+PF_6^-\) complex shows the best fit so far for In(III) compared to octahedral complexes of In-CB-cyclam (164.2° (12) axial angle and 78.8° (9) equatorial angle) and In-CB-cyclen (143.9° (12) axial angle and 76.7° (12) equatorial angle. Also when comparing this six-coordinate \([In\cdot7]^+PF_6^-\) crystal structure with the
analogous [Ga·7]⁺NO₃⁻ structure, Ga(III) still fits better into the ligand molecular cleft due to its superior size match. The larger six-coordinate In(III) ionic radius of 0.94 Å compared with Ga(III)’s 0.76 Å accounts reasonably well for the longer observed In-O and In-N bond distances.

Figure 2.22: X-ray structure of [In·7Cl]

Figure 2.23: X-ray structure of [In·7]⁺
<table>
<thead>
<tr>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
<th>Bond</th>
<th>Length (Å)</th>
</tr>
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<tbody>
<tr>
<td>In(1)-N(1)</td>
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<td>In(1)-N(2)</td>
<td>2.333(2)</td>
<td></td>
<td></td>
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<tr>
<td>In(1)-N(3)</td>
<td>2.335(2)</td>
<td>In(1)-N(4)</td>
<td>2.388(2)</td>
<td></td>
<td></td>
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<tr>
<td>In(1)-O(1)</td>
<td>2.1964(17)</td>
<td>In(1)-O(3)</td>
<td>2.3196(19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In(1)-Cl(1)</td>
<td>2.4766(7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N(1)-In(1)-N(2)</td>
<td>78.05(8)</td>
<td>N(4)-In(1)-N(3)</td>
<td>76.73(8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O(1)-In(1)-O(3)</td>
<td>69.93(7)</td>
<td>O(1)-In(1)-N(2)</td>
<td>83.90(7)</td>
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<td></td>
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<tr>
<td>O(3)-In(1)-N(2)</td>
<td>120.13(8)</td>
<td>O(1)-In(1)-N(3)</td>
<td>122.89(8)</td>
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<tr>
<td>O(3)-In(1)-N(3)</td>
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<td>73.00(8)</td>
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<td></td>
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<tr>
<td>O(3)-In(1)-N(1)</td>
<td>135.79(7)</td>
<td>N(3)-In(1)-N(1)</td>
<td>154.34(8)</td>
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<tr>
<td>N(1)-In(1)-N(4)</td>
<td>81.46(11)</td>
<td>O(1)-In(1)-Cl(1)</td>
<td>105.94(5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O(3)-In(1)-Cl(1)</td>
<td>79.62(5)</td>
<td>N(2)-In(1)-Cl(1)</td>
<td>160.21(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N(3)-In(1)-Cl(1)</td>
<td>104.18(6)</td>
<td>N(1)-In(1)-Cl(1)</td>
<td>88.32(6)</td>
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<td></td>
</tr>
</tbody>
</table>

Table 2.3: Selected bond angles (deg) and bond lengths (Å) of [In·7]⁺Cl⁻
### Table 2.4: Selected bond angles (deg) and bond lengths (Å) of [In·7]+PF₆⁻

<table>
<thead>
<tr>
<th>Bond</th>
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<th>Length (Å)</th>
</tr>
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<tr>
<td>In(1)-N(1)</td>
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<td>2.216(8)</td>
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</tr>
<tr>
<td>In(1)-O(1)</td>
<td>2.147(6)</td>
<td>In(1)-O(3)</td>
</tr>
<tr>
<td>N(1)-In(1)-N(2)</td>
<td>84.0(3)</td>
<td>N(4)-In(1)-N(3)</td>
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<tr>
<td>O(1)-In(1)-O(3)</td>
<td>94.0(3)</td>
<td>O(1)-In(1)-N(1)</td>
</tr>
<tr>
<td>O(3)-In(1)-N(1)</td>
<td>102.9(3)</td>
<td>O(1)-In(1)-N(2)</td>
</tr>
<tr>
<td>O(3)-In(1)-N(2)</td>
<td>171.6(3)</td>
<td>O(3)-In(1)-N(4)</td>
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<tr>
<td>O(1)-In(1)-N(3)</td>
<td>105.8(2)</td>
<td>O(3)-In(1)-N(3)</td>
</tr>
<tr>
<td>N(1)-In(1)-N(3)</td>
<td>174.3(3)</td>
<td>N(2)-In(1)-N(3)</td>
</tr>
<tr>
<td>N(4)-Ga(1)-N(3)</td>
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<td>O(1)-In(1)-N(4)</td>
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<tr>
<td>N(2)-In(1)-N(4)</td>
<td>80.3(3)</td>
<td>N(3)-In(1)-N(1)</td>
</tr>
</tbody>
</table>

#### 2.3 Conclusion

Three Ga(III) and In(III) complexes with ligand 7 have been prepared. Microwave-assisted conditions have been shown to be successful for the synthesis of [Ga·7]+NO₃⁻, [In·7]+Cl⁻ and [In·7]+PF₆⁻. All these complexes have been fully characterized and X-ray structural data have been obtained. In solution both the [Ga·7]+NO₃⁻ and [In·7]+PF₆⁻ complexes have C₂-time averaged symmetry on the NMR time scale, while [In·7]+Cl⁻ shows dynamic broadening at room temperature. This is most likely due to the higher coordination number in [In·7Cl] as a result of chloride...
coordination as confirmed in its solid-state structure. X-ray structural data further show that the smaller Ga(III) is a better fit than In(III) for ligand 7.
CHAPTER III

KINETIC INERTNESS STUDIES OF Ga-CB-TE2A AND In-CB-TE2A

3.1 Introduction

Coordination chemistry plays an important role in the design of target-specific radiopharmaceuticals.\(^{35,36,38,61-63}\) When designing metal complex-based imaging agents it is very important to correlate aspects of their coordination chemistry with \textit{in vivo} behavior.\(^{35,36,38,61-63}\) The stability of these complexes in vivo is crucial because if transchelation occurs the released radiometal can bind to serum proteins such as transferrin (Ga, In) or may build up in radiation-sensitive organs such as the bone/bone marrow.\(^{35,36,38,61-63}\) The build up of significant amounts of the radiometal in these organs can have harmful consequences.\(^{61-64}\)

Acid-dissociation studies have been used to investigate the kinetic inertness of selected Cu(II) and Zn(II) metal complexes of macro-cyclic amines.\(^{31,44,65,67}\) Acid-decomplexation studies of various Cu(II), Zn(II), Ga(III) and In(III)metal complexes with cross-bridged tetraamines have been investigated.\(^{31,43,44,46,65,67}\) Kinetic studies have shown that metal complexes of the cross-bridged ligands can demonstrate significant kinetic inertness as compared to the non-bridged complexes.\(^{44,46,65,66}\) However, kinetic inertness data for Ga(III) and In(III) complexes with carboxymethyl pendant-armed derivatives of tetraazamacrocycles are rare. Niu and Lee were able to obtain data for several Cu(II), Zn(II), Ga(III) and In(III) complexes of cross-bridged tetraamines (Table
3.1) as a useful indicator of viability of these complexes *in vivo*. Further studies are described in this chapter.

<table>
<thead>
<tr>
<th>Complex</th>
<th>1N DCI/D$_2$O, 90°C</th>
<th>5N DCI/D$_2$O, RT</th>
<th>5N DCI/D$_2$O, 90°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-CB-Cyclen</td>
<td>59(6) minutes</td>
<td>43 hours</td>
<td>&lt; 5 minutes</td>
</tr>
<tr>
<td>In-CB-Cyclam</td>
<td>&gt; 3 weeks</td>
<td>&lt; 20 hours</td>
<td></td>
</tr>
<tr>
<td>In-CB-DO2A</td>
<td>&gt; 2 months</td>
<td>poor solubility</td>
<td>poor solubility</td>
</tr>
<tr>
<td>Ga-CB-DO2A</td>
<td>&gt; 3 months</td>
<td>poor solubility</td>
<td>poor solubility</td>
</tr>
<tr>
<td>Zn-CB-TE2A</td>
<td>46(6) minutes</td>
<td>104 hours</td>
<td>&lt; 5 minutes</td>
</tr>
<tr>
<td>Cd-CB-TE2A</td>
<td>-</td>
<td>&lt; 5 minutes</td>
<td>-</td>
</tr>
<tr>
<td>Hg-CB-TE2A</td>
<td>-</td>
<td>&lt; 5 minutes</td>
<td>-</td>
</tr>
<tr>
<td>Cu-CB-TE2A</td>
<td>&gt; 2.5 x 10$^4$ hrs</td>
<td>154(6) hours</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.1: Half-lives of selected metal complexes with cross-bridged tetraamines in acidic media.**

3.2 Results and Discussion

3.2.1 Kinetic inertness studies of Ga(III) and In(III) complexes with ligand 7

The synthesis and characterization of [Ga·7]NO$_3$ was discussed in Chapter II.

Acid-decomplexation $^1$H NMR studies were performed in 1.02 N and 5.04 N DCI/D$_2$O at 25 °C and also in 1.02 N DCI/D$_2$O at 90 °C with substrate concentrations of 21.4 mM.

All these results showed no decomplexation for a period of at least 11 months.
(Figure 3.01). Note that in 1.02 N DCl/D₂O at 90 °C, the AX that corresponds to the diastereotopic protons on the carboxylate arms has disappeared. This is due to H/D exchange whereas at 25 °C no exchange was observed.

**Figure 3.01:** ¹H NMR spectra in 1.02 N DCl/D₂O: a) Ligand 7 in 1.02 N DCl/D₂O at 90°C, b) Complex Ga·7 in 1.02 N DCl/D₂O at 90°C, c) Ligand 7 in 1.02 N DCl/D₂O at 25°C, d) Complex Ga·7 in 1.04 N DCl/D₂O at 25°C after 11 months.

¹H NMR spectra of Ligand 7 and Ga·7 complex were also investigated in 5.04 N DCl/D₂O at 25°C with substrate concentrations of 21.4 mM and again it was noticed that no decomplexation occurred under those conditions (Figure 3.02) either.
Since these conditions were not harsh enough to obtain decomplexation data, NMR investigations in 4.97 N DCl/D₂O at 90°C with substrate concentrations of 21.4 mM were performed. It was observed that within 24 hours there was a 13.2% change but then over a period of 5 months there was only a further 3% decrease of starting material. These ¹H NMR spectra were obtained over appropriate periods of time. The furthest upfield doublet of multiplets that corresponds to the β-CH₂-eq protons was used for integrations to calculate the amount of the original and the amount of “decomplexed” material.
Figure 3.03: $^1$H NMR spectra of Ga-7 complex in 4.97 N DCl/D$_2$O at 90°C, time (hours).

$^1$H NMR spectra (Figure 3.03) revealed that the doublet of multiplets corresponding to the starting material is at ~1.87 ppm. Two new additional doublets of multiplets appeared upfield at ~1.81 and ~1.73 ppm respectively after 24 hours. Figure 3.04 (a) and 3.04 (b) shows the kinetic plot of ln(N/No) vs time where N is the concentration at time (t) and No is the initial concentration. The data reveals an initial drop after 24 hours and then equilibrium is reached because no significant change was noticed (Table 3.2 a, b). This suggests that we might be dealing with a first-order reaction approaching equilibrium. By analyzing the data during the initial hours of the experiment we can
obtain half-lives. The half-life for Ga-7 in 4.97N DCI at 90°C is 161 (± 3) hours (Figure 3.04 a, b).

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>% Complex Left</th>
<th>Ln (N/N₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.00</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>86.80</td>
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</tr>
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<td>66.08</td>
<td>86.71</td>
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<td>141.83</td>
<td>86.36</td>
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</tr>
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<td>545.49</td>
<td>85.86</td>
<td>-0.152</td>
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<td>881.49</td>
<td>87.77</td>
<td>-0.160</td>
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<tr>
<td>2105.49</td>
<td>83.27</td>
<td>-0.188</td>
</tr>
<tr>
<td>3607.49</td>
<td>83.27</td>
<td>-0.188</td>
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</tbody>
</table>

Table 3.2 a: Data for figure 3.04 a

<table>
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<tr>
<th>Time (hours)</th>
<th>% Complex Left</th>
<th>Ln (N/N₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
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<td>2.27</td>
<td>96.86</td>
<td>-0.0319</td>
</tr>
<tr>
<td>4.27</td>
<td>95.83</td>
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<tr>
<td>8.27</td>
<td>94.92</td>
<td>-0.0522</td>
</tr>
<tr>
<td>16.27</td>
<td>92.34</td>
<td>-0.0796</td>
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</table>

Table 3.2 b: Data for figure 3.04 b

Figure 3.04: Plot of \( \ln(N/No) \) vs time(hours) of complex Ga-7 "decomplexation" data in 4.97 N DCI/D₂O at 90°C: (a) approaching equilibrium; b) measuring half-life

There are several questions that arose from these results. Why did the decomplexation stop after an initial period? Are there two or three species observed? If this was a first-order decomplexation we should observe the complexed species as well as the protonated free ligand. This would lead to only one new doublet of multiplets. The
appearance of two new doublets of multiplets implied that apart from the starting complex, free ligand and some other species are present. Alternatively, it could also mean that there is only a single new species that has a lower symmetry than C2 which would account for the two new doublets of multiplets.

An experiment that was performed to try and identify the new species was by spiking the sample with free ligand. $^1$H NMR spectra (Figure 3.05) were obtained and it was noticed that the doublet of multiplets of the protonated ligand actually grew in the middle of the new two sets of multiplets. This suggested that free ligand may not be present in the sample. $^{13}$C NMR data (Figure 3.06) of the spiked sample were also obtained and it was noticed that multiple species are present along with the free ligand. The upfield carbon resonance at 19.88 corresponds to the β-carbon of the free ligand and the one at 20.90 ppm corresponds to the complex but there are other carbon resonances noticed between these peaks with much lower intensity. Also multiple carbonyl resonances are noticed which suggests that there are multiple species present.

MALDI-TOF mass spectra were also obtained. Prior to the spiking, the sample mass spectrum showed only the complexed material with a major peak at 412.7 amu (Figure 3.07) and no free ligand peak. FAB$^+$ mass spectrum was also obtained for this sample which revealed a peak with exact mass of 413 amu, consistent with the simulation (Figure 3.09). After spiking the sample the mass spectrum showed the complex peak at 412.7 amu as well as the free ligand peak at 343 amu (Figure 3.08). It should be noted that complex [Ga-7]$^+$ should have a major peak at 409 amu, but H/D exchange at the pendant arm methylenes has occurred under the reaction conditions. At time zero the AX of the methylene protons of the carboxylate arms were present, but after heating the
sample at 90°C, within 24 hours it was noticed that this AX had disappeared confirming H/D exchange. Simulation of the deuterated complex spectrum has been carried out (Figure 3.09). Also a mass spectrum of the complex in 5M HCl indeed yielded a major peak at 409 amu (Figure 3.10) as expected.

![Figure 3.05: Top spectrum: $^1$H NMR of sample spiked with free ligand 7.](image)

Figure 3.05: Top spectrum: $^1$H NMR of sample spiked with free ligand 7.

![Figure 3.06: $^{13}$C NMR of sample spiked with free ligand 7 (arrows are pointing to the free ligand peaks).](image)

Figure 3.06: $^{13}$C NMR of sample spiked with free ligand 7 (arrows are pointing to the free ligand peaks).
Figure 3.07: MALDI mass spectrum of complex Ga-7 in 4.97 N DCl/D₂O prior to spiking with free ligand 7.

Figure 3.08: MALDI mass spectrum of complex Ga-7 in 4.97 N DCl/D₂O after spiking the sample with free ligand 7.
Figure 3.09: Simulated mass spectrum of complex Ga-7 after H/D exchange.

Figure 3.10: MALDI mass spectrum of complex Ga-7 in 5.03 M HCl.

Similar results were obtained when the experiments were performed in 9.01 N DCl/D₂O at 90 °C. Ga-CB-TE2A was dissolved in a 9.01 N DCl/D₂O solution with an
initial concentration of 23.4 mM. $^1$H NMR spectra (Figure 3.11) were taken after specific time intervals and the doublet of multiplets at 1.87 ppm integrated to calculate the amount of original vs new species. The results showed that after 24 hours there are again three sets of doublet of multiplets: one corresponds to the starting complex and two new sets belonging to an unsymmetrical species that is still complexed to gallium. This unsymmetrical species must be a gallium complex because mass spectral results (Figure 3.12) again did not reveal any free ligand peaks.

![Figure 3.11: $^1$H NMR spectra of complex Ga\(\cdot\)7 in 9.01 N DCI/D\(_2\)O at 90\(^\circ\)C, time (hours).](image)

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Figure 3.12: MALDI mass spectrum of Ga·7 complex in 9.01 N DCI/D2O.

Figure 3.13 shows the kinetic plot of ln(N/No) vs time where N is the concentration at time (t) and No is the initial concentration. The data reveals an initial drop of 19.7% change over a period of 48 hours, but afterwards little further change occurred. This suggests again that we might be dealing with a first-order reaction approaching equilibrium.

Figure 3.13: Plot of ln(N/No) vs time(hours) of complex Ga·7 “decomplexation” data in 9.01 N DCI/D2O at 90°C.
Apart from obtaining kinetic inertness data in acidic conditions, our interest was expanded into studying the kinetic inertness in basic conditions as well. It is very important to perform studies under basic conditions to observe if Ga(OH)$_3$ precipitation or Ga(OH)$_4^-$ formation would occur under highly basic conditions. NMR investigations of Ga-CB-TE2A complex in 1.31 N NaOD at an initial concentration of 22.3 mM at 25 °C were performed. After a period of one month no decomposition of the complex occurred (Figure 3.14)

![NMR spectra](image)

Figure 3.14: Kinetic inertness studies of Ga·7 in 1.3 N NaOD at 25°C: a) Ligand 7, b) Ga·7 complex.

As mentioned in Chapter II, two In(III) complexes with ligand 7 were also synthesized, [In·7Cl] and [In·7]PF$_6$. X-ray structural data revealed that [In·7Cl] is a seven-coordinate complex while [In·7]PF$_6$ is a six-coordinate complex. It would be very interesting to investigate the acid-promoted behavior of both of these complexes in order to correlate their structure with their kinetic inertness. Which complex will be more kinetically inert, the seven- or the six-coordinated one?
The acid-decomplexation studies of the [In-7Cl] complex were performed in 1.03 N DC1/D2O solution at 25°C with an initial concentration of 40.7 mM. 1H NMR spectroscopy was used to monitor these experiments over appropriate periods of time. Again the upfield doublet of multiplet assigned to the β-CH2-eq protons was used for integrations to calculate the amount of original and the amount of new species. 

1H NMR spectra (Figure 3.15) revealed that the doublet of multiplets that corresponded to starting material is at ~1.83 ppm. An additional doublet of multiplets appeared upfield at ~1.78 ppm even at time zero. This interpretation may be oversimplistic because all peaks were broadened and there may well be other species buried under them. Figure 3.16 is a kinetic plot of ln (N/No) vs time where N is the concentration at time (t) and No is the initial concentration. The data showed that at time zero (i.e. sample preparation time) there was already a 22.5 % change (Table 3.3). Within 45 minutes there was a 40.2 % change and within two days the loss of starting material was 54.7%. Yet again from two days out to 50 days there was little further change occurring, with only another 1.5% loss. This suggests that we might be dealing with a first-order reaction approaching equilibrium. By analyzing the data during the initial hours of the experiment we can obtain half-lives. The half-life for In-7Cl in 1.03 N at 25°C is 165 (± 28) minutes (Figure 3.16).

Mass spectrometry was used to identify the new species. In this case a major peak at 455 (m/z) which corresponded to the starting complex as well as one at 343 corresponding to the free ligand were observed (Figure 3.17).
Figure 3.15: $^1$H NMR spectra of [ln-7Cl] in 1.03 N DCl/D$_2$O at 25°C, time (minutes).

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>% Complex</th>
<th>Left</th>
<th>ln (N/No)</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>5</td>
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</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>20</td>
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<td>45</td>
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<tr>
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Table 3.3: Data for figure 3.16
Figure 3.16: Plot of ln(N/No) vs time(minutes) of [ln-7Cl] decomplexation data in 1.03 N DCI/D₂O at 25°C.

Figure 3.17: MALDI mass spectrum of complex [ln-7Cl] in 1.03 N DCI/D₂O after 3 months.
To gauge the importance of chloride on acid-decomplexation, NMR studies of the [In·7]PF₆ complex were performed in 1.02 N DCIO₄/D₂O solution at 25 °C with an initial concentration of 41.6 mM. ^1H NMR spectra were obtained over appropriate periods. Again the upfield doublet of multiplets corresponding to the methylene β-CH₂-eq protons was integrated to calculate the amount of starting and new species. ^1H NMR spectra (Figure 3.18) revealed that the doublet of multiplets corresponding to [In·7]PF₆ material was at ~1.87 ppm. An additional set of doublet of multiplets appeared upfield at ~1.81 ppm after 24 hours, though there was a shoulder next to the major doublet of multiplets which may indicate other species buried under these resonances. Figure 3.19 is a kinetic plot of ln(N/No) where N is the concentration at time (t) and No is the initial concentration. The data showed that after 24 hours there was a 13.1 % loss of starting material (Table 3.4). No further significant change occurred from day one to day three, though after six days there is an additional 5.2 % loss. It was observed that after six days out to 2 months, there was no major change occurring, with only 2.3 % further loss of the original material. This suggests that we might be dealing with a first-order reaction approaching equilibrium. By analyzing the data during the initial hours of the experiment we can obtain half-lives. The half-life for In·7Cl in 1.03 N at 25°C is 630 (±71) hours (Figure 3.19).

Mass spectrometry was again used to identify the new species. A major peak at 455 (m/z) which corresponds to the complex was observed, but no free ligand peak at 343 amu was found (Figure 3.20).
Figure 3.18: $^1$H NMR spectra of $[\text{In-7}]PF_6$ in 1.02 N $\text{DClO}_4/\text{D}_2\text{O}$ at 25°C, time (hours).

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>% Complex Left</th>
<th>ln (N/No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100.0</td>
<td>0.000</td>
</tr>
<tr>
<td>86</td>
<td>86.89</td>
<td>-0.145</td>
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</tr>
<tr>
<td>374</td>
<td>78.91</td>
<td>-0.237</td>
</tr>
<tr>
<td>446</td>
<td>78.91</td>
<td>-0.237</td>
</tr>
<tr>
<td>2160</td>
<td>78.89</td>
<td>-0.237</td>
</tr>
</tbody>
</table>

Table 3.4: Data for figure 3.19
Figure 3.19: Plot of ln(N/No) vs time(hours) of [In·7]PF$_6^-$ “decomplexation” data in 1.02 N DCIO$_4$/D$_2$O at 25°C.

Figure 3.20: MALDI mass spectrum of complex [In·7]PF$_6^-$ in 1.02 N DCIO$_4$/D$_2$O.
3.3 Summary and Discussion

The acid-decomplexation studies of [Ga•7]NO₃, [In•7Cl] and [In•7]PF₆ were investigated. For [Ga•7]NO₃ experiments were performed in 1.02 N DCI/D₂O at 25 °C and 90 °C as well as 5.04 N DCI/D₂O at 25 °C. All these results revealed that no decomplexation had occurred after a period of 11 months. In order to obtain useful half-life data for this complex, experiments in 4.97 DCI/D₂O at 90 °C were performed. These suggested that the reaction might be a first-order approach to an equilibrium. The half-life for this reaction is 161 (± 3) hours. After plotting the data it was observed that there is a 13.2 % “decomplexation” after a period of 24 hours, an equilibrium being established between the starting material and a new unsymmetrical species. There was no further change over a period of 5 months. The equilibrium is apparently established between the starting complex which is C₂ symmetric (gives rise to only one upfield doublet of multiplets) and another complex with lower symmetry (which would account for two upfield sets of doublet of multiplets). One possibility is that one of the arms may be uncoordinated with chloride filling the empty coordination site. The sample was spiked with free ligand and ¹H NMR results suggested that the new species observed was not free ligand. Further mass spectral data also confirmed that no free ligand was present.

Acid-decomplexation studies of [Ga•7]NO₃ were also performed in 9.01 N DCI/D₂O at 90 °C. The data suggested that this again be a first-order reaction approaching equilibrium. There was a 19.7 % change over 48 hours, but afterwards there was no further change, suggesting that an equilibrium has been established. Again the equilibrium is established between the original complex and an unsymmetrical C₁ complex.
The acid-decomplexation studies of the [In\textbullet 7Cl] complex were performed in 1.03 N DCl/D$_2$O solution at 25°C. The data suggested that this again may be a first-order reaction approaching equilibrium. At time zero there was already a 22.5 % change, and within two days the decrease of the starting complex reached 54.7 %. After two days out to 50 days, there was very little further change occurring. The half-life for this reaction is 165 (± 28) minutes. Mass spectrometry revealed a major starting complex peak at 455 (m/z) and also one at 343 corresponding to the free ligand. This confirms partial acid decomplexation only of the starting complex.

The acid-decomplexation studies of the [In\textbullet 7]PF$_6$ complex were performed in absence of chloride in 1.02 N DCIO$_4$/D$_2$O solution at 25°C. The data for this complex again suggested a first-order reaction approaching equilibrium with the half-life of this reaction being 630 (± 71) hours. After 24 hours there was 13.1 % loss of starting material but from then till 2 months later less than 8% further loss occurred. These studies were performed in the absence of chloride while no free ligand is observed. Mass spectra also revealed a major starting material peak at 455 (m/z) but no free ligand. It is possible that one arm has dissociated from the indium coordination sphere and a water molecule is filling the sixth coordination site or simply a lowering from C$_2$ to C$_1$ symmetry of the complex without pendant arm dissociation had occurred.
3.4 Conclusion

The data for the [Ga-7]NO₃ complex suggested that this complex is remarkably stable under very acidic conditions even at high temperature. The data for [In-7Cl] and [In-7]PF₆ showed that in absence of chloride [In-7]⁺ is more kinetically inert than [In-7Cl]. For [In-7Cl] in DCI, actual decomplexation to free ligand was confirmed by mass spectral data. These results can be correlated to the solid state structures of the two In(III) complexes. Comparing the six-coordinate [In-7]PF₆ complex geometry to the seven-coordinate [In-7Cl] geometry, the cation fit is significantly better in the former because of its lower coordination number. Finally, both of these In(III) complexes are less kinetically inert than [Ga-7]NO₃ complex since Ga(III) is a clearly better fit for ligand 7 than In(III).

3.4 Experimental section for kinetic studies

Complexes used for acid decomplexation studies were: [In-CBTE2ACl], [In-CBTE2A]PF₆, and [Ga-CBTE2A]NO₃. These complexes were prepared according to the procedures described in Chapter V. Proton NMR spectra of these complexes recorded at specific time intervals were used to monitor their decomplexation in acidic media (1.02 N, 4.97 N, 9.01N DCI/D₂O and 1.03 N DCIO₄) at room temperature and 90°C. All sample solutions of the [Ga-CBTE2A]NO₃ complex were securely-sealed using Wilmad screw-cap NMR tubes. For acid-promoted studies of the [Ga-CBTE2A]NO₃ complex in 1.02 N, 4.97 N, at 25°C and 90°C, substrate concentrations were 21.4 mM. For acid promoted studies of the [Ga-CBTE2A]NO₃ complex in 9.01N DCI/D₂O at 90°C
substrate concentrations were 23.4 mM. Initial concentration for [In-CB-TE2ACl] acid
decomplexation in 1.05M DCl at 25°C was 40.7mM. Initial concentration for [In-CB-
TE2A]PF₆ acid decomplexation in 1.05M DCIO₄ at 25°C was 41.6mM. Regular capped
NMR tubes were used for this experiment sealed with parafilm and teflon tape.
[In-CBTE2ACl] acid-promoted studies at 25°C were directly acquired on the 500 MHz
NMR spectrometer (Varian Inova-500). Other high-temperature data were collected on
batch samples placed in a constant-temperature water-bath. The integration of the
decreasing signals for the complexed material versus the increasing signals for the new
species were used to monitor the decomplexation.
CHAPTER IV

OPTIMIZING MICROWAVE-ASSISTED COPPER(II), GALLIUM(III) AND INDIUM(III) COMPLEXATIONS WITH CROSS-BRIDGED TETRAAMINE LIGANDS 5-7

4.1 Introduction

The application of microwaves to synthetic chemistry is a research area that has attracted a large amount of attention in the past fifteen years. In 1986 Gedye, Giguere and Majetich were the first to report the use of microwave in accelerating organic reactions. After these reports in the late 1980s and early 1990s there was still a lack of interest. This can be attributed to the lack of control and reproducibility and also with the lack of understanding of the basics of microwave dielectric heating. There were also many concerns regarding flammability of organic solvents in a microwave field and the lack of available systems to control pressure and temperature. There has been an increase of publications since 1995 on microwave-assisted organic synthesis (MAOS) due to the development of application-optimized microwave instruments designed with adequate temperature and pressure control compared to the modified domestic microwave ovens used in the 1980s. Many research facilities and academic research groups began using MAOS as an advanced technology for efficient synthesis resulting in shorter reaction times and higher yields.
Microwave radiation is electromagnetic radiation in the frequency range of 0.3 to 300 GHz. The mechanism by which matter absorbs microwave energy is called dielectric heating, thus the mobility of the dipoles and the ability to orient them according to the direction of the electric field is very important. Molecules that have permanent dipole moments are able to align themselves through rotation completely or at least partly with the direction of the field. Phase shifts and dielectric losses are the results. Field energy is transferred to the medium and is converted to kinetic or thermal energy. Molecular friction is often cited as a model for this behavior. For polar substances dielectric losses are observed in the microwave range. For example, the water molecule will rotate due to the fast-changing electric field of the microwave radiation. Due to this process, “internal friction” takes place in a polar medium which leads to a direct and almost even heating of the reaction mixture. Because the change in polarity of the electric field is faster than the rotation rate of the water molecules around its dipole centre, a phase shift occurs and energy is absorbed from the electric field.

Synthetic organic chemistry reactions are typically carried out by conductive heating with an external heating source (i.e. oil baths). This is a slow method to transfer energy into the system, since it depends on the thermal conductivity of the various materials that have to be penetrated, and results in the temperature of the reaction vessel being higher than that of the reaction mixture. Microwave irradiation produces efficient internal heating (in-core volumetric heating) by direct coupling of microwave energy with the molecules that are present in the reaction mixture. The observed rate accelerations when using microwaves compared to oil bath experiments have led to
the development of terminology such as “specific” or “non-thermal” microwave effects.\textsuperscript{74,84,85} Most people in the scientific community now agree that in the majority of cases the reason for the observed rate enhancements is a purely thermal/kinetic effect; that is, a consequence of the high reaction temperatures that can rapidly be attained when irradiating polar materials in a microwave field.\textsuperscript{73,74,84,85} For example, a reaction mixture using water or methanol, which are high microwave absorbing solvents, can be rapidly superheated at temperatures higher than 100 °C above their boiling points when irradiated under microwave conditions in a sealed vessel.\textsuperscript{73,74,84,85} These temperature profiles are difficult to be achieved and reproduced by standard thermal heating. Dramatic rate enhancements have been observed for reactions performed in high temperature microwave-heated processes versus the standard thermal heating processes. Baghurst and Mingos have used the Arrhenius equation \((k=A\exp(-E_a/RT))\) to rationalize these enhancements.\textsuperscript{73} Based on this equation they observed that a transformation that requires 68 days to reach 90% conversion at 27 °C, will show the same degree of conversion within 1.61 seconds when performed at 227 °C.\textsuperscript{73} The rapid heating and extreme temperatures observed in microwave chemistry means that many of the reported rate enhancements can be rationalized by simple thermal/kinetic effects.\textsuperscript{73,74,84,85}

As described in Chapter II our interest in microwave chemistry developed when we were faced with the excruciatingly slow kinetics of Ga(III) and In(III) complexations with ligand 7. Indeed our results show that microwave-assisted conditions have enhanced the reaction rates and ultimately gave successful complexations versus the unsuccessful thermal reactions. These slow kinetics were also noticed with Cu(II) complexations of our cross-bridged ligands. As mentioned in Chapter I, one major goal
of this research is to use the ligands as bifunctional chelators for diagnostic PET imaging. Rapid complexation is a concern when radiolabeling is performed. $^{64}$Cu has a desirable half-life for our purposes but faster radiolabeling would be a significant advantage. After the 1986 reports about using microwaves in organic reactions, the first successful microwave-accelerated radiopharmaceutical syntheses in solution were reported by Hwang et al. The importance of generating an intense microwave field in samples led to the first use of a single-mode cavity to perform these transformations in solution. Monomodal devices designed for the space limitations of radiolabeling environments were also constructed, some of which have subsequently become available. There have been a few reports on using microwaves for radiolabeling and there are significant advantages related with this such as shorter reaction times and higher radiochemical purity. Furthermore some reactions are only achievable using microwave heating. Also, these microwave-assisted conditions have shown that the amount of starting material required driving the pseudo-first order labeling reaction could sometimes be reduced, which can save precious precursors and simplify clean-up procedures and decrease competing reactions at other reactive sites in the starting material.
4.2 Synthesis of Cu(II) complexes with ligands 5-7

![Chemical structures of ligands 5, 6, and 7]

**Figure 4.01: Cross-bridged ligands 5-7**

The Cu(II) complexations with ligands 5 and 6 were previously performed by Niu and the Cu(II) complex with ligand 7 was prepared by Wong. These reactions were run in organic solvents and for ligands 5 and 7 (Figure 4.01) a base is needed to assist deprotonation of the ligand for effective complexation. Our goal is to ultimately be able to run these reactions in aqueous buffered media and realize shorter reaction times especially considering possible biological applications. Thus our interest expanded to investigating these complexations using microwave-assisted conditions. The first Cu(II) complexation that was investigated was with ligand 7.

![Scheme 4.1: Cu(II) complexation with ligand 7 using microwave-assisted conditions]

Scheme 4.1: Cu(II) complexation with ligand 7 using microwave-assisted conditions
The reactions were run in aqueous buffered media using 0.2 M aqueous citrate buffer at pH 6.31. Ligand 7 was dissolved in this citrate buffer solution. To this solution another citrate buffered aqueous solution containing an equimolar amount of CuCl₂ · 2H₂O was added. The pH of the resultant blue homogenous solution (19mM) remained at 6.31. The reaction was run in the microwave closed-vessel with simultaneous cooling for 10 minutes at 80 °C (Scheme 4.1). When the reaction was complete the supernatant was analyzed using UV-Vis spectroscopy (Figure 4.02). These spectra showed a λₘₐₓ at 642 nm, which is consistent with the authentic complex. These results have been the most successful ones. Other trials were attempted to see if the reaction can proceed at lower temperatures. Table 4.1 is a summary of these attempts. Unfortunately, performing the complexation at temperatures lower than 85 °C showed incomplete results. The published conditions involved 95% EtOH as a solvent and also required a base like NaOH for deprotonation of the ligand to facilitate the complexation. The reaction also had to be refluxed for 2 hours. The microwave conditions are not only faster, the yields are also higher (85% for the microwave reaction and 61% for the conventional methods). The published radiolabeling was performed at 75 °C for 4 hours. Though this temperature is slightly lower than the microwave conditions the reaction time is considerably longer. This could potentially be an improved way to perform the radiolabeling.
Table 4.1: Unsuccessful attempts for Cu'7 complexations at lower temperatures using microwave assistance

<table>
<thead>
<tr>
<th>Temp(°C)</th>
<th>Rxn time</th>
<th>$\lambda_{\text{max}}$(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>55</td>
<td>10min</td>
<td>732.1</td>
</tr>
<tr>
<td>65</td>
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<tr>
<td>70</td>
<td>20min</td>
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<tr>
<td>70</td>
<td>30min</td>
<td>broad trace ~ 686</td>
</tr>
<tr>
<td>85</td>
<td>5min</td>
<td>broad trace ~ 673</td>
</tr>
</tbody>
</table>

Figure 4.02: Visible electronic absorption spectrum of Cu'7 in aqueous citrate buffer.

In order to be able to mimic more closely radiolabeling conditions, increasing ratios of ligand to metal were explored. These reactions led to decreasing complex
concentrations, which made the results hard to monitor using UV-Vis spectroscopy. HPLC was thus employed, because it offers better sensitivity compared to UV-VIS. The HPLC conditions and the detection wavelength were the same for all the reaction mixtures analyzed. The absorbance window was changed from sample to sample in order to see clearly whether or not the complex formed. The following conditions were developed by Xiaoxuan Shen in the Tomellini group.

**Mobile Phase:** 23/77% (v/v) Methanol/30 mM Citric acid at pH 2.36

**Flow rate:** 0.4 mL/min (Pressure 99-102 bar)

**Column:** Hypercarb (100 × 3mm; 5μ)

**Loop size:** 10 μL

**UV-VIS Detection λ:** 280 nm.

**Temperature:** 22°C

**Retention time for Cu-CB-TE2A:** 3.9 minutes

All reactions were run closed-vessel at 85 °C for 10 minutes. The first reaction had a concentration of 1:10 Cu(II)/Ligand ratio (0.68mM /6.8mM) with an absorbance window set at 0.5 (a.u.f.s.). Also, reactions with a concentration of 1:100 Cu(II)/Ligand (0.068mM /6.8mM) with an absorbance window set at 0.025 (a.u.f.s.) and with concentration of 1:1000 Cu(II)/Ligand (0.0068mM /6.8mM) with an absorbance window set at 0.005 (a.u.f.s.) were performed. The reaction mixtures were injected prior to the reaction and no major peaks were seen. After the reaction was complete the mixture was injected and a peak corresponding to the product Cu-CB-TE2A appeared. A peak with retention time of 3.8 minutes was present in all of the above reactions, which is consistent with the authentic material.
Figure 4.03: Chromatograms of Cu·7 with: (left) 1:10 Cu(II)/Ligand ratio, (middle) 1:100 Cu(II)/Ligand ratio, (right) 1:1000 Cu(II)/Ligand ratio with retention time of Cu·7 is 3.9 minutes, the first peak corresponds to the citrate (arrows are pointing at the product peak).

Also reactions at 75 °C for 10 minutes were tried at lower concentration. The reaction mixture was injected prior to the reaction and no major peaks were seen. After the reaction was complete the mixture was injected and a peak corresponding to the Cu-CB-TE2A appeared. The peak height was lower than expected leading to the conclusion that the reaction was not complete. The reaction was run for an additional 20 minutes. The peak height corresponding to the Cu-CB-TE2A nearly doubled proving the hypothesis that the reaction was not complete. Even at a total time of 30 minutes the reaction was not complete.

The same investigation was performed for Cu(II) complexation with ligand 6.
Scheme 4.2: Cu(II) complexation with ligand 6 using microwave-assisted conditions

The reactions were run in aqueous citrate buffer at pH 6.71 at a concentration of 0.2 M. Ligand 6 was dissolved in this solution and a solution containing an equimolar amount of CuCl₂ · 2H₂O in the same buffer solution was added. The pH of the resultant blue homogenous solution (20 mM) remained at 6.71. The reaction was run in the microwave in closed-vessel mode for 10 minutes at 80 °C (Scheme 4.2). When the reaction was complete the supernatant was analyzed with UV-Vis spectroscopy (Figure 4.04). These results showed a peak at λₘₐₓ 604 nm, which is consistent with the authentic material.

These results have been the most successful ones. Other attempts were made at milder temperatures. Table 4.2 is a summary of these less successful complexations. The published conventional conditions were performed in refluxing MeOH for 2 hours. The microwave-assisted complexations were run in aqueous buffered media and the reaction times were much shorter. The yields were also higher (87% yield for the microwave-assisted complexations vs. the 63% yield of the conventional method). The previous radiolabeling conditions for this complexation were in EtOH and NaOH was used as base at 75°C for 1 hour. Again although the microwave-assisted conditions were at a higher temperature (85°C), the reaction time was shorter.
Table 4.2: Unsuccessful attempts for Cu*6 complexations at lower temperatures using microwave assistance

<table>
<thead>
<tr>
<th>Temp(°C)</th>
<th>Rxn time</th>
<th>λmax(nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>10min</td>
<td>broad trace at 614.0</td>
</tr>
<tr>
<td>75</td>
<td>25min</td>
<td>broad trace at 607.0</td>
</tr>
</tbody>
</table>

Different metal/ligand ratios were explored on this reaction as well. The HPLC conditions followed were developed by Ilia Terova in the Tomellini group.\textsuperscript{94}
The conditions are as follows:

**Mobile Phase:** 30/70 (v/v) Methanol/0.8M potassium acetate at pH 5.3

**Flow rate:** 1.0 mL/min

**Column:** Strong cation exchange column (250 × 4.6mm; 10μ)

**Loop size:** 10 μL

**Temperature:** 45°C

**UV-VIS Detection λ:** 280 nm.

**Retention time for Cu-CB-Cyclam:** 9.5 minutes

This retention time is consistent with authentic material. All the reactions were done at 85°C for 10 minutes. The reaction Cu(II)/Ligand ratio is (1.0mM /10.0mM) with an absorbance window was set at 0.2 (a.u.f.s.). Also reactions with a 1:100 Cu(II)/Ligand ratio (0.1mM /10.0mM) and absorbance window set at 0.02 (a.u.f.s.), and reactions at concentration 1:1000 Cu(II)/Ligand (0.001mM /10.0mM) ratio with an absorbance window set at 0.002 (a.u.f.s.) were performed. A peak with retention time of 9.5 minutes was present in all of the above reactions which is consistent with formation of authentic material (Figure 4.05).
Figure 4.05: Chromatograms of CuCl$_2$·6 with: (left) 1:10 Cu(II)/Ligand ratio, (middle) 1:100 Cu(II)/Ligand ratio, (right) 1:1000 Cu(II)/Ligand ratio with retention time of Cu·7 is 9.5 minutes, the first peak corresponds to the citrate (arrows are pointing on the complex peak)

Another Cu(II) complexation investigated was with ligand 5. Usually cyclen-based complexations are faster than the cyclam-based ones. Our interest in this complexation was to confirm that this reaction would be faster and in milder conditions than the one with ligand 7.
Scheme 4.3: Cu(II) complexation with ligand 5 using microwave-assisted conditions

The reactions were run in aqueous citrate buffer at pH 6.61 with a concentration of 0.2 M. Ligand 6 was dissolved in this citrate buffer aqueous solution, to this solution an equimolar amount of CuCl₂ · 2H₂O in the same buffer was added. The pH of the resultant blue homogenous solution (18 mM) remained at 6.61. The reaction was run in the microwave closed-vessel mode for 10 minutes at 70 °C (Scheme 4.3). When the reaction was complete the supernatant was analyzed using UV-Vis spectroscopy (Figure 4.06). These spectra showed a λ<sub>max</sub> at 637 nm, which is consistent with the authentic material. These results were the most successful. Other attempts were made at milder temperatures. Table 4.3 is a summary of these less successful complexations.

<table>
<thead>
<tr>
<th>Temp(°C)</th>
<th>Rxn time</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt;(nm)</th>
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</thead>
<tbody>
<tr>
<td>65</td>
<td>10min</td>
<td>Broad trace ~ 666</td>
</tr>
<tr>
<td>55</td>
<td>10min</td>
<td>Broad trace ~ 677</td>
</tr>
</tbody>
</table>

Table 4.3: Unsuccessful attempts for Cu·5 complexations at lower temperatures using microwave assistance
These results show that the Cu(II) complexation with ligand 5 can indeed be performed under slightly milder conditions than the complexation with ligands 6 and 7. The published conditions were performed at refluxing MeOH for 8 hours using NaOH as the base for the deprotonation of the ligand. The microwave-assisted complexations were run in aqueous buffered media and the reaction times are much shorter.
4.3 Synthesis of Ga(III) and In(III) complexes with ligand 5

The In(III) and Ga(III) complexations with ligand 5 were also investigated using microwave-assisted conditions.

Ligand 5 was dissolved in H₂O and to this solution an aqueous solution containing equimolar amounts of Ga(NO₃)₃ • H₂O was added. The pH of the resultant homogenous solution was adjusted to 3.71 with aqueous NaOH. The reaction was then run in the microwave closed-vessel mode for 30 minutes at 85 °C using instantaneous cooling (Scheme 4.4). When the reaction was complete the supernatant was isolated from a small amount of precipitate via centrifugation. Evaporating the supernatant to dryness yielded the complex as a yellow solid. ¹H NMR spectroscopy was used to analyze the product and the results confirmed formation of authentic material. The previous method was performed in MeOH with refluxing for 2 days. The microwave-assisted conditions showed completion of the complexation in 30 minutes at 85 °C, though the yields were not significantly different (83-87 % yield, which are comparable to the conventional reactions).

![Scheme 4.4: Ga(III) complexation with ligand 5 using microwave-assisted conditions](image)

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The same methodology was applied to the complexation of In(III) with ligand 5, which was previously reported by Lee using conventional heating. These conditions involved the use of NaOAc as base to assist deprotonation of the ligand with refluxing in MeOH for 4 days. In this work, the complexation was investigated under microwave-assisted conditions where the reaction was run for 30 minutes at 150 °C. Ligand 5 was dissolved in water and to this solution an aqueous solution containing an equimolar amount of InCl₃ was added. The pH of the resultant homogenous solution was adjusted to pH 3.76 with aqueous NaOH. After this the reaction was run in the microwave closed-vessel mode for 30 minutes at 150 °C (Scheme 4.5).

Scheme 4.5: In(III) complexation with ligand 5 using microwave-assisted conditions

Lee's attempts to obtain X-ray quality crystals of this complex were not successful. Many other attempts using various recrystallization methods failed. I was able to purify the [In-CB-D02A]⁺Cl⁻ complex using counterion exchange. Crude [InCB-D02A]⁺Cl⁻ was stirred with a saturated solution of NH₄PF₆ to precipitate "[In-CB-D02A]⁺PF₆⁻" as a white powder (Scheme 4.6, Figure 4.07). Slow evaporation of this from a 1:1 acetonitrile:methanol (v:v) solution yielded colorless crystals of the complex.
Scheme 4.6: Counter ion-exchange using NH$_4$PF$_6$

Figure 4.07: $^1$H NMR spectrum (in D$_2$O using MeCN as reference at 2.06 ppm) of [In·5Cl]

Figure 4.08: X-ray structure of [In·5Cl]·2NaPF$_6$
X-ray structural data indicate a seven-coordinate [In-5Cl] complex (Figure 4.08) with a capped octahedral coordination geometry around the indium coordination sphere. The ligand is hexadentate with a N₄O₂ donor set. The chloride is also coordinated giving indium a seven-coordination geometry. Two equivalents of NaPF₆ are present with one sodium coordinated to the two carboxylate oxygens on the pendant arms and also bonded to the other sodium are two PF₆⁻'s. Since In(III) is larger than Ga(III) the ligand cleft fit is slightly poorer than in the Ga-CB-DO2A case. Further, in this case the In(III) coordination number is 7 instead of 6. The axial N(1)-In-Cl(1) and the equatorial N(1)-In-N(2) angles are 171.61(15)°C and 77.71(19)°C respectively. The average In-N bond length is 2.32 Å and the average In-O bond length is 2.34 Å. Table 2.3 shows a summary of the important bond lengths and bond angles of this complex.

Footnote: The X-ray study revealed a stoichiometry of “[In-5Cl]Na₂(PF₆)₃·6H₂O”. For charge balance an additional cation, most likely a proton must also be present. This may be on one carboxylate arm or a water of hydration. An examination of the C-O bond lengths did not reveal any evidence for protonation.
Table 4.4: Selected bond angles (deg) and bond lengths (Å) of [In-5Cl]  

<table>
<thead>
<tr>
<th>Bond</th>
<th>Angles (deg)</th>
<th>Bond</th>
<th>Angles (deg)</th>
</tr>
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<td>2.277(5)</td>
<td>In(1)-N(2)</td>
<td>2.338(6)</td>
</tr>
<tr>
<td>In(1)-N(3)</td>
<td>2.348(4)</td>
<td>In(1)-N(3)#1</td>
<td>2.348(4)</td>
</tr>
<tr>
<td>In(1)-O(2)#1</td>
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<td>In(1)-O(2)</td>
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4.4 Conclusion  

In this research the use of microwave-assistance for several Cu(II), Ga(III) and In(III) complexations with ligands 5-7 was studied. In all cases the results showed faster reaction times and comparable yields using the microwave reactor. Cu(II) complexations with ligands 5-7 achieved faster reaction times, under mild conditions that should be transferable to radiolabeling. Also different stoichiometries of excess ligand to Cu(II)
were explored without affecting the process. The Ga(III) and In(III) complexations with ligand 5 were also performed using microwave-assisted conditions. The reactions times for these complexations were also shorter than the conventional conditions giving comparable yields. Finally, X-ray-quality crystals of the previously synthesized In-CB-DO2A complex were obtained and a seven-coordinate structure found.
CHAPTER V

EXPERIMENTAL SECTION

General Methods and Materials

$^1$H NMR and $^{13}$C{$^1$H} NMR spectra were obtained from Varian Inova-400 and Varian Inova-500 spectrometers. Acetonitrile was used as internal reference for samples run in D$_2$O. Mass spectral data were collected using a Shimadzu Axima-CFR MALDI-TOF and a ThermoFinnigan LCQ-ESI (using positive mode) mass spectrometer at the University of New Hampshire. Also samples for high resolution mass spectrometry were sent to the University of Notre Dame where FAB$^+$ mass spectrometry was performed. All reactions at reflux were run under a nitrogen atmosphere with magnetic stirring. Microwave reactions were performed in a CEM Discover microwave reactor in a closed pressurized vessel mode with power setting at 300W. Solvent removal was by rotary evaporation under reduced pressure and trace solvent removal from solids was by vacuum pump. All solvents were reagent grade and were dried as needed. Cross-bridged ligands were prepared according to literature procedure (Scheme 1.2). Metal salts were obtained commercially and used without further purification. X-ray crystal structures were solved by Prof. Arnold L. Rheingold and his group at UC San Diego.

$[\text{Ga•7]}\text{NO}_3$. Ligand 7 (53.2 mg, 0.108 mmol) prepared from its diethyl-ester and followed by HCl hydrolysis was dissolved in 1mL of water and added to a solution of
Ga(NO$_3$)$_3$·H$_2$O (37.7 mg, 0.147 mmol) in 1 mL of the same solvent. Aqueous NaOH was added to adjust the pH of the clear solution to pH 3.6. The reaction was run in the closed-vessel (10 mL vessel) mode for 4 h in the microwave reactor at 180 °C.

When the reaction was complete the supernatant was isolated from a small amount of precipitate by centrifugation. Evaporating the supernatant to dryness yielded the complex as a yellow solid (70.5 mg, contaminated with NaCl and NaNO$_3$). This crude product was washed with methanol to afford the desired complex as a white solid (23.3 mg, 45%). X-ray crystals were obtained by dissolving a sample in a 80% methanol: 20% water solvent mixture followed by ether diffusion into this solution to yield colorless crystals.

Elemental analysis: Found: C, 40.20; H, 5.94; N, 14.83. Calc for C$_{16}$H$_{28}$N$_2$O$_4$Ga: C, 40.70; H, 5.98; N, 14.83. IR: $\nu_{\max}$ (solid, KBr)/cm$^{-1}$ 2970, 1678, 1650, 1384. $^1$H NMR (400 MHz, D$_2$O): $\delta_H$ 1.89 (2H, dp, $J$ 17.7 Hz, 2.9 Hz, $\beta$-CH$_{eq}$H), 2.42-2.54 (2H, qm, $J$ 12.9 Hz, $\beta$-CH$_{ax}$H), 2.98 (2H, dd, $J$ 14.9 Hz, 4.4 Hz), 3.09-3.25 (8H, m), 3.35-3.55 (6H, m), 3.62 and 3.92 (AB of apparent $ABX$, CH$_2$COO, $J_{AB}$ 17.5 Hz, $J_{AX}$ 1.5 Hz, 4H), 3.72-3.82 (4H, m). $^{13}$C ($^1$H) NMR (500 MHz, D$_2$O) $\delta_C$ 20.75, 49.82, 51.58, 57.71, 57.99, 59.65, 62.84, 174.61.

[In$^7$]Cl. Ligand 7 (81.3 mg, 0.166 mmol) prepared from its diethyl-ester and followed by HCl hydrolysis was dissolved in 1mL of water and added to a solution of InCl$_3$ (40.1 mg, 0.181 mmol) in 1 mL of the same solvent. Aqueous NaOH was added to adjust the pH of the clear solution to pH 3.8. The reaction was run in the closed-vessel mode for 7 h in the microwave reactor at 180°C. When the reaction was complete, the supernatant was isolated from a small amount of precipitate by centrifugation.
Evaporating the supernatant to dryness yielded the crude complex as a white solid (93.9 mg, contaminated with NaCl). Purification was effected by (3x3mL) hexafluoroisopropanol washings to yield 61.2 mg (75%) of the product as white solid after evaporating the supernatant to dryness. A sample of this was dissolved in hexafluoroisopropanol followed by ether diffusion to give colorless crystals of X-ray-quality crystals. Elemental analysis: Found: C, 29.96; H, 4.27; N, 7.56; Cl, 11.01; F, 13.51. Calc for C_{19}H_{30}Cl_3 F_5InN_4Na_2O_5 C, 30.16; H, 4.00; N, 7.40; Cl, 14.06; F, 12.55. IR: \nu_{\text{max}}(\text{solid, KBr})/\text{cm}^{-1} 2921, 1634. ^1H NMR(500 MHz, D_2O): \delta_H 1.75-1.87 (2H, dm, J 17.5 Hz, \beta-\text{CH}_3\text{H}), 2.43-2.57 (2H, qm, J 13.6 Hz, \beta-\text{CH}_2\text{H}), 2.71-2.79 (4H, m), 2.86-3.04 (6H, m), 3.23-3.28 (2H, m), 3.40-3.57 (8H, m), 3.70-3.77 (2H, m). ^13C\{^1H\} NMR (500 MHz, D_2O) \delta_C 21.69, 48.85, 49.48, 57.34, 57.56, 62.42, 175.93.

[In•7]PF_6. Ligand 7 (78.8 mg, 0.161 mmol) prepared from its diethyl-ester followed by HCl hydrolysis was dissolved in 1mL of water and added to a solution of In(NO_3)_3•5H_2O (66.4 mg, 0.169 mmol) in 1 mL of the same solvent. Aqueous NaOH was added to adjust the pH of this clear solution to pH 3.9. The reaction was run using the closed-vessel mode for 3 h in the microwave reactor at 180°C. The resulting reaction mixture supernatant was isolated from a small amount of precipitate by centrifugation. Evaporating the supernatant to dryness yielded the crude complex as a yellow residue. This was dissolved in 1mL H_2O and stirred with a 1mL saturated solution of NH_4PF_6 in the same solvent to precipitate the desired product as a white powder (65.2 mg, 67% yield). Slow evaporation of a sample from a 1:1 acetonitrile:methanol (v:v) solution yielded colorless crystals suitable for X-ray diffraction studies. Elemental analysis:
Found: C, 23.03; H, 4.00; N, 9.08; F, 34.09. Calc for C_{16}H_{32.5}F_{15}InN_{5.5}O_{4}P_{2.5} C, 22.79; H, 3.89; N, 9.14; F, 33.80. IR: \nu_{\text{max}}(\text{solid, KBr})/\text{cm}^{-1} 1640, 835. \text{ }^1\text{H NMR}(500 \text{ MHz, D}_2\text{O}): \delta_H 1.91-1.99 (2H, dm, J 18.0 Hz, \beta-CH_{eq}H), 2.49-2.62 (2H, qm, J 12.9 Hz, \beta-CH_{ax}H), 2.97-3.23 (10H, m), 3.34-3.49 (6H, m), 3.62 (2H, X of AX, CH_2COO, J_{ax} 17.3 Hz), 3.69-3.79 (4H, m), 3.89 (2H, A of AX, CH_2COO, J_{ax} 17.3 Hz, 2H). \text{ }^{13}\text{C}({}^1\text{H}) \text{ NMR} (100.5 \text{ MHz, D}_2\text{O}) \delta_C 21.93, 49.06, 51.63, 57.52, 58.92 (overlapping signals), 62.65, 174.88.

[Ga•5]NO_3. Ligand 5 (41.3 mg, 0.131 mmol) prepared from its tert-butyl-ester and followed by TFA deprotection was dissolved in 1mL of water and added to a solution of Ga(NO_3)_3\cdot\text{H}_2\text{O} (47.2mg, 0.184 mmol) in 1 mL of the same solvent. Aqueous NaOH was added to adjust the pH of the clear resulting solution to pH 3.7. The reaction was run in the closed vessel mode for 30 min in the microwave reactor at 85°C. When the reaction was complete the supernatant was isolated from a small amount of precipitate by centrifugation. Evaporating the supernatant to dryness yielded the complex as a white-yellowish solid (52.4 mg, 87.1%). The NMR spectra of this product are consistent with those of the authentic material first prepared by Niu.

[In•5]Cl. Ligand 5 (50.3 mg, 0.159 mmol) prepared from its diethyl-ester and followed by HCl hydrolysis was dissolved in 1mL of water and added to a solution of InCl_3 (40.1 mg, 0.181 mmol) in 1 mL of the same solvent. Aqueous NaOH was added to adjust the pH of the clear solution to pH 3.8. The reaction was run in the closed-vessel mode for 25 min in the microwave reactor at 150°C. When the reaction was complete,
supernatant was isolated from a small amount of precipitate by centrifugation. Evaporating the supernatant to dryness yielded the crude complex as a yellow oil (64.3 mg, 84.7 %). Product spectra were consistent with those of an authentic material first prepared by Lee.44

\[ \text{[In}\text{•5Cl}]\text{-2NaPF}_6 \]. Complex \([\text{In}\text{•5}]\text{Cl}\) (31.2 mg, 0.065 mmol) was dissolved in 1mL of water and stirred with a 1mL aqueous saturated solution of \(\text{NH}_4\text{PF}_6\) to precipitate the desired product as a white powder (21.9 mg, 57.3 %). Slow evaporation of the sample from a 1:1 acetonitrile methanol solution yielded colorless crystals suitable for X-ray diffraction studies.

\[ \text{[Cu}\text{•7}]\]. Ligand 7 (14.0 mg, 0.0286 mmol) prepared from its diethyl-ester and followed by HCl hydrolysis was dissolved in 0.8 mL of 0.2 M citrate buffer (pH 6.3) solution and added to another 0.2 M citrate buffer solution of CuCl\(_2\) (5.6 mg, 0.0328 mmol) in 0.7 mL with pH of the resulting homogeneous reaction mixture remaining at 6.3. The reaction was run in the closed vessel mode for 10 min in the microwave reactor at 85°C. When the reaction was complete, the supernatant was analyzed by UV-Vis giving a \(\lambda_{max}\) at 642 nm, (9.8 mg, 85 % when removing the solvent) which is consisted with authentic material.

\[ \text{[Cu}\text{•6}]\]. Ligand 6 (9.5 mg, 0.0419 mmol) was dissolved in 1mL of 0.2 M citrate buffer (pH 6.7) solution and added to another 0.2 M citrate buffer solution of CuCl\(_2\) (7.0 mg, 0.0410 mmol) in 1mL with pH of the resulting homogeneous reaction mixture
remaining at 6.7. The reaction was run in the closed-vessel mode for 10 min in the microwave reactor at 85°C. When the reaction was complete the supernatant was analyzed by UV-Vis with a $\lambda_{\text{max}}$ appearing at 604 nm, (14.4 mg, 87 % when removing the solvent) which is consistent with that of authentic material.

[Cu•5]. Ligand 5 (10.5 mg, 0.0271 mmol) prepared from its diethyl-ester and followed by HCl hydrolysis was dissolved in 0.8 mL of 0.2 M citrate buffer (pH 6.6) solution and added to another 0.2 M citrate buffer solution of CuCl$_2$ (5.5 mg, 0.0322 mmol) in 0.8 mL with pH of the resulting homogeneous reaction mixture remaining at 6.6. The reaction was run in the closed-vessel mode for 10 min in the microwave reactor at 70°C. When the reaction was complete the supernatant was analyzed by UV-Vis revealing a $\lambda_{\text{max}}$ at 637 nm, (8.7 mg, 85 % when removing the solvent) which is consistent with that of an authentic sample.
APPENDIX
Solvent: D$_2$O
(MeCN set at 2.06)
Solvent: D$_2$O

MeCN set at 1.47
Solvent: D$_2$O

(MeCN set at 2.06)
Solvent: D$_2$O

(MeCN set at 1.47)
Solvent: D$_2$O

(MeCN set at 2.06)
Solvent: D$_2$O

(MeCN set at 1.47)
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