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Abstract

The synthesis and characterization of two distinct sets of Manganese(II) based magnetic resonance imaging (MRI) contrast agents (CAs) are reported. The first set is comprised of a series of cyclen-based derivatives. Included in this series is a new, mixed pendant-arm macrocyclic ligand, 1,4,7,10-tetraazacyclododecane-1,7-bis(acetate)-4,10-bis(acetamide). $H_xDO2A^{2+}$, $H_xDO2AM^{X+}$, $H_xDO2A2AM^{(X-2)+}$ and their Mn(II) derivatives are characterized by solution state infrared spectroscopy. Full crystallographic details are reported for $[\text{Mn(DO2AM)(H}_2\text{O)}](\text{Cl}_{1.34}\text{Br}_{0.66})\cdot2\text{H}_2\text{O}$ (triclinic, $P\bar{1}; a=7.68140(10)$, $b=8.80980(10)$, $c=16.6027(2)$ Å; $\alpha=103.7880(10)^\circ$, $\beta=97.2190(10)^\circ$, $\gamma=100.9880(10)^\circ$; $Z=2$) and $[\text{Mn(DO2A2AM)}](\text{NH}_4\text{Cl})\cdot3.5\text{H}_2\text{O}$ (triclinic, $P\bar{1}; a=8.4976(5)$, $b=9.5396(2)$, $c=15.6492(8)$ Å; $\alpha=94.139(3)^\circ$, $\beta=103.306(5)^\circ$, $\gamma=90.393(3)^\circ$; $Z=2$), which are seven- and six-coordinate, respectively. Binding constants for five macrocyclic Mn(II) complexes are reported at 37°C, along with pH dependent $r_1$ relaxivity measurements. Finally, a temperature dependent study of the $^{17}$O transverse relaxivity of the three complexes is used to determine the number of bound waters ($q$) in each complex: $[\text{Mn(H}_x\text{DO2A)}]^{X+}$ ($q=0$), $[\text{Mn(DO2AM)}]^{2+}$ ($q=1$), and $[\text{Mn(H}_x\text{DO2A2AM)}]^{X+}$ ($q=0$).

The second set is comprised of two tris-pyridine amine carboxylic acid derivatives. The synthesis of the two ligands, TPAMA and TPADA, is reported. $pK_a$ values for the ligands and binding constants for their Mn(II) complexes are determined by potentiometry at 37°C. The pH dependent relaxivity profiles are reported and $r_1$ values at physiological pH are compared with other pyridine derived Mn(II) complexes.
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Chapter 1: Introduction

1.1 Magnetic Resonance Imaging Contrast Agents

Magnetic Resonance Imaging (MRI) is a non-invasive tool used for medicinal diagnostics. MRI is used for diagnosing tumors within the human body. MRI works based upon the same principles as nuclear magnetic resonance (NMR). MR imaging creates an image of a specific portion of the human body by manipulating the spins of protons in the bulk water of the body. In the absence of an external magnetic field the spins of the water protons are oriented in random directions. When an external field \( B_0 \) is applied, the spins will align in a direction parallel to the field or anti-parallel to the field and begin precessing at the Larmor frequency \( \nu_0 = -\left(\frac{\gamma}{2\pi}\right)B_0 \), which is dependent on the field strength of the magnet. Slightly more spins will align parallel to the applied magnetic field than anti-parallel because the parallel alignment is more energetically favorable.\(^1\) By applying radio frequency (RF) pulses, the spins can be knocked out of the alignment of the external magnetic field. The orientation of the spins is then probed at various times as they return to the equilibrium of the applied magnetic field. Because MRI uses a gradient field, protons in different parts of the body are easily distinguishable. MR images are generated by taking multiple scans throughout the area of the body that is being imaged. These scans are then transformed into the scan image via a Fourier Transform. Figure 1.1 shows an MR image of the brain.\(^2\)

MRI Contrast Agents (CAs) are aqueous paramagnetic compounds which enhance the MR image by either lightening or darkening the area which is to be studied depending on if they are \( T_1 \) or \( T_2 \) weighted, respectively. The contrast agent used in Figure 1.1 was a gadolinium(III) based \( T_1 \)-weighted contrast agent, thus the tumor that
was being imaged appears brighter than the original scan without a contrast agent. About 40% of all MRI scans use these CAs because they are largely beneficial as a diagnostic tool.³

![Figure 1.1. Two MR images of a human brain. The image on the left was taken without a contrast agent present. The image on the right was taken in the presence of a Gd(III) based contrast agent. The yellow arrow is pointing to the tumor that was the target of the MRI. Image was adapted from [Ref 2].](image)

**1.2 Gadolinium(III) vs. Manganese(II) CAs**

There are currently nine gadolinium(III) FDA approved contrast agents that are used clinically. Gd(III) is used in contrast agents due to its effective relaxometric properties. Gd(III) has a spin of 7/2 and a water exchange rate of $K_{\text{Gd(III)}} = 8.3 \times 10^8$ s⁻¹.⁴ These properties make Gd(III) complexes highly desirable as MRI contrast agents due to the high values of relaxivity that Gd(III) complexes possess (see below 1.3 and 1.4).

Free Gd(III) ions in the body are harmful. Gd(III) has been shown to be a calcium ion antagonist as it inhibits the activity of many Ca²⁺ based enzymes.⁵ Additionally, free Gd(III) ions in the body are the cause of a disease known as Nephrogenic Systemic Fibrosis (NSF). NSF causes a hardening of the skin and
connective tissues within the body, often leading to disability and death. Gadolinium(III) CAs are removed from the body via excretion through the kidneys. Though the FDA approved Gd(III) contrast agents are generally stable to demetallation these CAs cannot be used for patients with renal failure. About 14% of the US population currently experiences chronic kidney disease. Patients with this disease could greatly benefit from CAs, however Gd(III) based contrast agents cannot be used. Additionally, recent studies have shown that accumulation of gadolinium in the brain is harmful. These studies have shown that even in patients without renal failure gadolinium(III) can build up in the brain, causing complications.

Manganese(II) has been shown to be a less harmful metal and is biogenic. Recently, many Mn(II) complexes have been studied as potential alternatives to Gd(III) CAs. Manganese(II) has a relatively high spin (5/2) and fast water exchange rate (2.1x10^7 s⁻¹). While these values are not as high as those of Gd(III) complexes, it has been shown that Mn(II) complexes can have relaxivity values comparable to those of Gd(III) (see Table E, below). For these reasons and because Mn(II) is native to the body, its incorporation into CAs has been of interest for some time.

1.3 Relaxation Theory and $^1$H Longitudinal ($T_1$) Relaxivity

In the absence of an external magnetic field, the spins associated with the protons of a sample of bulk water occur in a random Boltzmann equilibrium. Upon application of an external magnetic field in the z-direction ($B_0$), the spins align to precess along the z-direction either parallel or anti-parallel to the field at the Larmor frequency ($\nu_0 = -{(\gamma/2\pi)}B_0$; where $\gamma$ is the gyromagnetic ratio associated with the system). In order for a $T_1$-weighted contrast agent to effectively enhance the efficacy
of an MRI, it must have a high $r_1$ relaxivity which is determined from the concentration of the sample and the $T_1$. This $T_1$ term, termed the longitudinal relaxation time, describes the rate constant associated with the return of the proton spins to their Boltzmann equilibrium after a 90° radio frequency (RF) pulse is applied to the system. Figure 1.2 shows a cartoon representation of this process.

![Figure 1.2](image.png)

**Figure 1.2.** Cartoon representation of the relaxation of proton spins to their Boltzmann equilibrium after a 90° radio frequency pulse. Adapted from [9].

Equation 1.1, below, details the factors that determine the $T_1$ of a complex according to Solomon-Bloembergen theory. This theory was originally used to describe the interaction of a nuclear and electronic spin—that of a $^1$H and a paramagnet.

$$\frac{1}{T_1} = \frac{1}{10} \left( \frac{\mu_0}{4\pi} \right) \frac{\hbar^2 \gamma_I^2 \gamma_S^2}{4\pi^2 r^6} \left( \frac{\tau_c}{1 + (\omega_I - \omega_S)^2 \tau_c^2} + \frac{3\tau_c}{1 + \omega_I^2 \tau_c^2} + \frac{6\tau_c}{1 + (\omega_I + \omega_S)^2 \tau_c^2} \right)$$ (1.1)

In this equation, $\mu_0$ is the permeability of free space in a vacuum; $\gamma_I$ and $\gamma_S$ are the nuclear and electronic gyromagnetic ratios; $r$ is the distance from the paramagnet to the exchangeable proton; $\omega_I$ and $\omega_S$ are nuclear and electronic Larmor frequencies (which are field dependent). And,

$$\frac{1}{\tau_c} = \frac{1}{\tau_m} + \frac{1}{\tau_R} + \frac{1}{\tau_{1e}} \quad \quad \frac{1}{\tau_{1e}} = \frac{1}{\tau_m} + \frac{1}{T_1}$$
where $\tau_c$ is the correlation time of the paramagnetic complex made up of three components: $\tau_m$ is the water exchange time of a water bound to the paramagnetic metal center; $\tau_R$ is the rotational correlation time associated with the molecular tumbling of the paramagnetic complex in solution; $T_{1e}$ is the longitudinal electronic relaxation time. When calculating $\tau_c$ the fastest of the $\tau_m$, $\tau_R$, or $T_{1e}$ terms will dominate. A pictorial representation of these different correlation times is given in Figure 1.3, below.²

![Figure 1.3](image)

**Figure 1.3.** Pictorial representation of three correlation times. $\tau_m$ is the water exchange time of a water bound to the paramagnetic metal center, $\tau_R$ is the rotational correlation time associated with the molecular tumbling of the paramagnetic complex in solution; $T_{1e}$ is the longitudinal electronic relaxation time. Adapted from [Ref 2]
In order for a contrast agent to have a high relaxivity (fast $T_1$), the paramagnetic complex must have bound and exchangeable inner sphere water molecules.\cite{11}

Equation 1.2, derived from SBM (Solomon-Bloembergen-Morgan) theory,\cite{10c,12} details the $T_1$ rate constant associated with only the inner sphere exchange mechanism:

$$\frac{1}{T_{1M}} = \frac{B}{r^6} \left( \frac{7\tau_{2c}}{1 + (\omega_S\tau_{2c})^2} + \frac{3\tau_{1c}}{1 + (\omega_I\tau_{1c})^2} \right) + \frac{2A^2S(S+1)}{3h^2} \left( \frac{\tau_{1e}}{1 + (\omega_S\tau_{2e})^2} \right)$$

(1.2)

where $B=2(\mu_0/(4\pi))^2\gamma_I^2\gamma_S^2h^2S(S+1)/15$; $\mu_0$, $\gamma_I$ and $\gamma_S$, $\tau_c$, $\omega_I$, and $\omega_S$ are detailed above; $A/h$ is the hyperfine coupling constant; $S$ is the electronic spin of the paramagnetic (5/2 for Mn$^{2+}$ and 7/2 for Gd$^{3+}$).

The efficacy of a $T_1$-weighted CA is determined by its $^1$H relaxivity, $r_1$. The $r_1$ value of a paramagnetic complex in solution is determined using Equation 1.3:\cite{8}

$$r_1 = \frac{1}{[M]} \left( \frac{1}{T_1} - \frac{1}{T_1^0} \right)$$

(1.3)

where $T_1$ is the relaxation time of the water in the presence of the paramagnetic complex, $T_1^0$ is the relaxation time of water, $[M]$ is the concentration of the sample, and $r_1$ is the calculated relaxivity. By subtracting the time constant associated with water ($T_1^0=3.5$ sec) from the $T_1$ of the water in the presence of a paramagnet and dividing by the concentration of the paramagnetic complex, the relaxivity value can be determined. The relaxivity of the complex at physiological pH (6.8-7.4) is especially important.
1.4 Temperature Dependent $^{17}$O Transverse ($T_2$) Relaxivity

The $r_1$ relaxivity of CAs is highly dependent upon the hydration state of the paramagnetic metal center (see above). Because of this, determining the number of waters that are bound to the metal center in the solution state is quite important. Previously, $^1$H $r_1$ relaxivity studies have been used in order to qualitatively determine the hydration state.$^{13}$ Recently, however, a more quantitative method for the determination of the hydration state of Mn(II) complexes has been proposed.$^{14}$ This method uses temperature dependent $^{17}$O NMR spectroscopy, measuring the $T_2$ value in increments of 5°C from 5°C to 95°C. Depending upon the hydration number of the Mn(II) complex, plotting these $r_2$ values against the reciprocal of the temperature gives a variety of different curves. Figure 1.3 depicts the temperature dependent curves for complexes with a number of bound waters ($q$) equal to 0, 1, 2, and 6. The number of bound waters can be determined based upon the $r_2^{\text{max}}$ of each curve, from Equation 1.4.$^{14}$

$$q = r_2^{\text{max}}[\text{H}_2\text{O}] \left( \frac{2}{S(S+1)A_0} \right) \cong \frac{r_2^{\text{max}}}{510} \quad (1.4)$$

where all of the terms have been defined previously. This equation is derived from Equations 1.5 and 1.6, below.$^1$ Specifically, the $T_{2p}$, and thus $r_2$, is dependent upon three separate correlation times: $\tau_m$ (defined above), $T_{2m}$ (the transverse relaxation rate of the bound water, defined below), and $T_{1e}$ (defined above). Equations 1.5 and 1.6 are defined below:

$^1$ For a more thorough derivation, see [Ref 15]
In Equation 1.6, $\tau_{sc}$ is termed the scalar relaxation correlation time.

\[
\frac{1}{T_{2_p}} = \frac{q[Mn]}{[H_2O]} \frac{1}{T_{2_m} + \tau_m} \quad (1.5)
\]

\[
\frac{1}{T_{2_m}} = \frac{S(S + 1)}{3} \left( \frac{A_o}{\hbar} \right)^2 \tau_{sc} \quad (1.6) \quad ; \quad \frac{1}{\tau_{sc}} = \frac{1}{T_{1e}} + \frac{1}{\tau_m}
\]

The peak in the curve $(r_2^{\text{max}})$ occurs due to a change in the dominating correlation time in the mechanism that dictates the transverse relaxivity value. A negative slope for the curve indicates that $T_{2_m} \gg \tau_m$. This is termed the fast exchange regime, wherein relaxivity is almost completely dependent upon the electronic

**Figure 1.4.** Curves of $r_2$ against $1000/T$ (K$^{-1}$) for $[\text{Mn}(H_2O)_6]^{2+}$ (circles), $[\text{Mn}(\text{PMPDA})(H_2O)_3]^{-}$ (diamonds), $[\text{Mn}(\text{CDTA})(H_2O)]^{2-}$ (triangles), and $[\text{Mn}(\text{DTPA})]^{3-}$ (squares) at two different fields, 9.4 T (closed symbols) and 11.7 T (open symbols). Adapted from [14]
relaxation as the bound water is exchanging faster than its protons can be relaxed. The peak occurs at the point at which $T_{2m} = \tau_m$. A positive slope indicates that the complex is in the slow exchange regime. The water exchange rate dominates the slow exchange regime. As temperature is increased, the inner sphere water can exchange with the bulk water at a faster rate, and so the $T_2$ increases.

1.5 Stability Constants and $\log K_{ML}$

In addition to relaxivity, the other important factor in determining the viability of a potential CA is the thermodynamic stability of the complex to demetallation. Though Mn(II) is biogenic and is cleared from the body relatively quickly, having free metal ions in the bloodstream is less than desirable. Long term exposure to large doses of free Mn(II) can lead to accumulation of the metal in the brain. If not treated, this can lead to Parkinson-like symptoms.\[^{16}\] While manganese poisoning can be treated with the injection of chelating agents,\[^{16b,c}\] prevention of an overabundance of Mn(II) in the bloodstream is still a major goal in the rational design of potential CAs. The measure of stability of the complex to demetallation is defined by the term $\log K_{ML}$. This term is generally defined by a binding equilibrium as follows,

$$\left[Mn(H_2O)_6]^{2+} + [L] \rightleftharpoons [Mn(L)]$$

and can be determined with Equation 1.8,

$$K_{MnL} = \frac{[MnL]}{[Mn][L]} \quad (1.8)$$
The current FDA approved Gd(III) CAs range in log$K_{\text{GdL}}$ from 17 to 25 (see Table 1.1, below). All of these CAs employ octadentate ligands. Such a binding motif leaves one open coordination site for water, as gadolinium(III) tends to have a coordination number of nine. Manganese(II) tends to have complexes with a coordination number of 6 or 7, however there are rare cases where it can bind octadentate to ligands. This unpredictability makes rationally designing Mn(II) complexes quite challenging. Additionally, Mn(II) complexes which incorporate a bound, inner-sphere water tend to be several orders of magnitude less stable than Mn(II) complexes without waters bound to their metal centers.

1.6 FDA Approved CAs

Nine of the FDA approved CAs are general use and consist of complexes with gadolinium(III) as the metal center. All of these Gd(III)-based CAs are shown in Scheme 1.1. The CAs fall into two categories: cyclen-based derivatives or DTPA-based derivatives. All of the Gd(III) metal centers in these complexes are octadentate.

<table>
<thead>
<tr>
<th>Complex</th>
<th>$r_1$ (mM$^{-1}$sec$^{-1}$)$^a$</th>
<th>log$K_{\text{GdL}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd-DOTA</td>
<td>4.2</td>
<td>25.3</td>
</tr>
<tr>
<td>Gd-HP-DO3A</td>
<td>4.4</td>
<td>23.8</td>
</tr>
<tr>
<td>Gd-BT-DO3A</td>
<td>5.3</td>
<td>20.8</td>
</tr>
<tr>
<td>Gd-DTPA</td>
<td>4.3</td>
<td>22.2</td>
</tr>
<tr>
<td>Gd-DTPA-BMA</td>
<td>4.6</td>
<td>16.8</td>
</tr>
<tr>
<td>Gd-GTPA-BMEA</td>
<td>5.2</td>
<td>16.8</td>
</tr>
<tr>
<td>Gd-BOPTA</td>
<td>6.7</td>
<td>18.4</td>
</tr>
<tr>
<td>Gd-EOB-DTPA</td>
<td>7.3</td>
<td>23.5</td>
</tr>
<tr>
<td>MS-325</td>
<td>19</td>
<td>23.2</td>
</tr>
</tbody>
</table>

$^a$In blood, 37°C, 1.5 T.
with respect to the ligand with one inner-sphere water molecule bound to the metal. This combination results in thermodynamically stable complexes with high relaxivities. Table 1.1 gives $\log K_{GdL}$ and relaxivity values for the Gd(III) contrast agents. The $\log K_{GdL}$ values for these complexes range from 16.8 to 25.3. For these complexes, the relaxivities range from 4.2 mM$^{-1}$sec$^{-1}$ to 19 mM$^{-1}$sec$^{-1}$ at 37°C and 1.5 T in human blood. The macrocyclic Gd(III) complexes tend to have higher stabilities, but lower relaxivities.

Scheme 1.1.

![Scheme 1.1](image-url)
The one Mn(II) based CA, [Mn(DPDP)]$^{3-}$ is solely targeted at the liver.\textsuperscript{19} Upon arriving at the liver, the complex breaks apart and the $T_1$ measurement is due only to [Mn(H$_2$O)$_6$]$^{2+}$(aq). This complex demonstrates both the potential high relaxivity of Mn(II) metal complexes and challenges of creating a stable complex that are inherent to working with manganese(II).

1.7 DOTA and DOTAM

Two tetra-pendant arm cyclen derivatives are shown in Scheme 1.2, below. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid (H$_4$DOTA) and its derivatives have been studied as potential contrast agents when bound to lanthanide metals due to their high relaxivity.\textsuperscript{20} [Gd(DOTA)(H$_2$O)]$^-$ is one of the FDA approved Gd(III) based CAs and two others are based on DOTA derivatives. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetamide (DOTAM) and its derivatives have been studied as potential MRI paraCEST (paramagnetic Chemical Exchange Saturation Transfer) and contrast agents when bound to various metals due to their high stability and inertness to demetallation.\textsuperscript{18, 21} [Ln(DOTAM)]$^{3+}$ complexes have been extensively studied for this reason.

Stability constants and relaxivities of [Mn(H$_x$DOTA)]$^{X-2}$\textsuperscript{22} and [Mn(DOTAM)]$^{2+}$\textsuperscript{22c} have been previously reported. The log$K_{MnL}$ for [Mn(H$_x$DOTA)]$^{X-2}$ at 37°C is quite high at 20.42.\textsuperscript{22a} The complex, however, undergoes proton assisted decomplexation below the relatively high pH of 4.\textsuperscript{22c} [Mn(DOTAM)]$^{2+}$ has quite a low log$K_{MnL}$ value of 12.82, but does not undergo proton assisted decomplexation until the pH is lowered below 2. In addition, [Mn(DOTAM)]$^{2+}$ has a large kinetic stability.\textsuperscript{22c} A ligand that combines both of these features would be of interest as a potential contrast agent as
it would be stable to dissociation over a range of pH values and would not undergo decomplexation while within the patient.

1.8 DO2A, DO2AM, and DO2A2AM

DO2A and DO2AM, shown in Scheme 1.2, are the bis-pendant arm trans analogues for DOTA and DOTAM. There have been a number of studies of the characteristics for the manganese(II) complex of both cis\textsuperscript{,23a} and trans-DO2A\textsuperscript{13,23c,23d} (see chapter 8), as well as DO2A with other metals\textsuperscript{,23e,23f,23g} including their stability constants and relaxivity values. While DO2AM has been previously synthesized\textsuperscript{,24} its binding constants have not been determined, and no Mn(II) complex has been reported. The \textsuperscript{1}H and \textsuperscript{13}C NMR, melting point, IR, and ESI-MS data have been reported for the ligand\textsuperscript{,24b}.

The characterization of the Mn(II) complexes of these two ligands can give insights into the novel mixed pendant arm ligand, DO2A2AM. Composed of a cyclen ring with two acetate pendant arms and two amide pendant arms, the manganese(II) complex of this ligand has the potential for a high stability toward dissociation, combining the two traits from DOTA and DOTAM.

Scheme 1.2.

\[
\begin{align*}
\text{H}_2\text{DO2A}: R&=\text{OH} \\
\text{H}_2\text{DO2AM}: R&=\text{NH}_2 \\
\text{H}_2\text{DO2A2AM} \\
\text{H}_2\text{DOTA}: R&=\text{OH} \\
\text{DOTAM}: R&=\text{NH}_2
\end{align*}
\]
1.9 This Study

The goal of this study is to thoroughly characterize the novel ligand 1,4,7,10-tetraazacyclododecane-1,7-bis(acetate)-4,10-bis(acetamide) (H2DO2A2AM) and its manganese(II) derivative. Additionally, the bis-acetate cyclen derivative (1,7-DO2A) and the bis-amide cyclen derivative (1,7-DO2AM) and their manganese(II) complexes are used as analogues in order to more fully understand DO2A2AM. Understanding the solution state speciation and structure of potential contrast agents is paramount, as these CAs are designed for bodily injection. Because manganese(II) is paramagnetic, common NMR techniques cannot be used to probe solution state structure or dynamics. In addition, Mn(II) is colorless in solution and does not have any diagnostic transitions in the visible region. Instead, we used potentiometry in order to determine the pH dependent speciation of both the ligands and their complexes. Additionally, the binding constants for the Mn(II) complexes of the ligands were determined at 37°C and compared to previously reported binding constants for other Mn(II) pendant arm bearing cyclen derivatives. pH dependent solution state IR spectroscopy was used, as this technique is an effective means to further probe the solution structure of the compounds. Solution state IR can be very sensitive to protonation sites and can be used to determine the coordination environment of the Mn(II) metal center in the solution state. Crystal structures of [Mn(DO2A2AM)](NH4Cl)•3.5H2O and [Mn(DO2AM)(H2O)](Cl1.34)(Br0.66)•2H2O were used to determine the solid state structures of the two cyclen derivatives bearing amide pendant arms, and direct comparisons were made with previously reported crystal structures for the manganese(II) complexes of the tri-amide and tetra-amide cyclen derivatives.
Two different relaxometric techniques were employed. A pH dependent study of the $^1$H $r_1$ relaxivities of the Mn(II) macrocyclic complexes was used to determine both their efficacy as contrast agents at physiological pH and in order to qualitatively compare their susceptibility to proton assisted decomplexation at low pH values. Finally, a temperature dependent $^{17}$O transverse $T_2$ relaxation study was used to determine the number of inner-sphere water molecules that are bound to the Mn(II) metal center of each complex. The complete characterization of these compounds will lead to a greater understanding of the solution phase properties of the macrocyclic compounds, and others like them.
References:


Chapter 2: Materials and Methods

2.1 Chemicals

1,7-Bis(benzyloxy carbonyl)-1,4,7,10-tetraazacyclododecane (DO2A t-Bu ester) was either synthesized\(^1\) or purchased from Macrocyclics, Inc. 1,4,7,10-Tetraazacyclododecane (cyclen) and 1,4,7,10-tetraazacyclododecane-1,7-diacetic acid (DO2A) were purchased from Macrocyclics, Inc. D\(_2\)O (99.9% D) was purchased from Cambridge Isotopes Laboratories, Inc. Potassium nitrate was recrystallized from water prior to use in potentiometric titrations. All other chemicals were purchased from commercial sources and used without further purification.

2.2 Nuclear Magnetic Resonance

\(^1\)H and \(^{13}\)C NMR spectra were obtained on either a 300 MHz, 400 MHz, or 500 MHz Varian Spectrometer at 25°C.

2.3 Infrared Spectroscopy

Solid state IR spectra were collected in the form of KBr pellets. Solution IR spectra were collected using a cell with CaF\(_2\) windows with a path length of 0.1 mm. All solution IR spectra were taken in D\(_2\)O in order to avoid the overlapping bands of H\(_2\)O that are present in the carbonyl region of the spectra. Each pH value was taken, then the pD was determined by taking pD = pH(measured) + 0.44.\(^2\) The spectra were obtained using a Perkin-Elmer Spectrum BX FT-IR System.

2.4 Mass Spectrometry
ESI-MS data were obtained in H₂O:MeOH solutions using a Finnigan LCQ Advantage Max spectrometer.

2.5 Elemental Analysis

Elemental Analyses were performed by Robertson Microlit Laboratories, Ledgewood NJ.

2.6 X-Ray Crystallography

X-Ray crystallographic analyses were performed by Dr. Brandon Mercado of the Yale University X-Ray Crystallographic Facilities. Crystals of [Mn(DO2A2AM)](NH₄Cl)•3.5H₂O and [Mn(DO2AM)(H₂O)](Cl₁.₃₄)(Br₀.₆₆)•2H₂O were obtained by slow diffusion of isopropyl alcohol into a solution of the complex in water. First, each ligand was mixed with anhydrous MnCl₂ in H₂O in a slightly more than 1:1 ratio. Ammonium hydroxide was then added until a pH of 5.5 for MnDO2A2AM and 7.2 for MnDO2AM was achieved. Isopropyl alcohol vapor was diffused into this solution to produce single crystals suitable for X-ray analysis. Complete experimental details are given in Chapter 6.

2.7 Potentiometry

Potentiometric titrations were performed at 37°C under humidified N₂ in the presence of 0.1M KNO₃ in water (distilled from basic KMnO₄). All titrations were conducted at a 10 mmol concentration of both the ligand and the manganese(II) salt. The data were fit using Hyperquad08 or Hyperquad13 and speciation diagrams were generated using HySS. The pH measurements were taken using a Thermo Scientific
Orion Star A211 pH Meter. The standardization of all reagents used for potentiometry is covered in chapter 5.

2.8 $^1H$ $R_1$ Measurements

$^1H$ longitudinal proton relaxivity measurements were obtained on a Bruker Minispec mq20 spectrometer operating at 20 MHz and 37°C in deionized water distilled from basic KMnO$_4$. All proton relaxivity samples were allowed to equilibrate to 37°C for at least fifteen minutes prior to data collection. The pH value of each sample was determined just before the data were collected, and again just after to ensure stability. All $^1H$ samples were ~1mM in metal complex. $T_1$ data were obtained using a saturation recovery pulse sequence, $(90°-\tau-90°-AQ-D)_n$. All pH measurements were obtained using a Thermo Scientific Ag/AgCl semi-micro Orion pH probe attached to a Mettler Toledo MP225 pH meter.

Relaxivities were determined using equation 2.1$^4$

$$r_{1,2} = \frac{1}{[M]} \left( \frac{1}{T_{1,2}} - \frac{1}{T_{1,2}^0} \right) \quad (2.1)$$

where $T_{1,2}$ is the relaxation time of the water in the presence of the paramagnetic complex, $T_{1,2}^0$ is the relaxation time of water, [M] is the concentration of the paramagnetic, and $r_{1,2}$ is the calculated relaxivity.

2.9 $^{17}O$ Temperature Dependent $T_2$ Measurements
Temperature dependent $^{17}$O transverse $T_2$ measurements were obtained in natural abundance in $D_2O$ in intervals of $5^\circ C$ on a $400 \text{ MHz}$ Varian NMR spectrometer using a probe tuned to $54\text{MHz}$. The temperature was controlled using a Varian Mercury Model L600 Variable-Temperature Controller. A standard CPMG spin-echo pulse sequence was used for each measurement. A diagram of the pulse sequence is shown in Figure 2.1, below.$^5$

![Figure 2.1. CPMG pulse sequence used to obtain $^{17}$O $T_2$ data. Adapted from [Ref 5].](image)

Where $d_1$ (the relaxation delay) was set to $30 \text{ ms}$; $pw90^\circ$ (the $90 \text{ degree}$ pulse width) was set to $17\mu\text{s}$; variables within the brackets represent the arrays, set to $14-28$ arrays beginning at $0.01 \text{ ms}$ and ending at $28-54 \text{ ms}$; $n$ is the number of scans. The pH of each sample was adjusted using concentrated HCl in $H_2O$ for low pH values and $40\%$ KOH in $H_2O$ for the high pH values. The samples were allowed to equilibrate for at least ten minutes before the data were collected. All $^{17}$O samples were $\sim 0.06-0.08\text{mM}$.
References:


Chapter 3: Synthesis and Spectra

3.2 DO2A2AM

The synthesis of H$_2$DO2A2AM and its Mn(II) analogue was previously reported by Sarah Hensiek.$^1$ The reaction scheme for the H$_2$DO2A2AM ligand is given in Scheme 3.1. The synthesis was completed in high yield in two steps starting from the commercially available 1,7-DO2A-tBu-ester precursor. The first step was a nucleophilic addition of amide arms to 1,7-DO2A-tBu-ester. 2-Chloroacetamide has been substituted into the first step in place of 2-bromoacetamide, however, the substitution of this starting material decreases the yield and increases reaction time. Addition of the amide arms before the acetate arms was attempted, however, the addition of amide pendant arms to a bis-protected cyclen ring was quite slow and only low to moderate yields of the protected bis-amide cyclen were obtained.$^2$ After hydrolysis of 2, the product 3 was obtained in its fully protonated form. The neutral species was isolated by elution from a cation exchange column.

Scheme 3.1.

(i) 2 equiv. 2-bromoacetamide, 2 equiv. K$_2$CO$_3$, KI, 80˚C, 20 hrs.; (ii) TFA, CH$_2$Cl$_2$, rt, 5 hrs.

3.2a 1,4,7,10-Tetraazacyclododecane-1,7-bis(tert-butyl acetate)-4,10-bis(acetamide)

(2)
DO2A-tert-butyl ester (1) (0.4956 g; 1.24 mmol; 1 equiv.) was dissolved in 20 mL anhydrous acetonitrile (MeCN). To this solution was added K$_2$CO$_3$ (0.3935 g; mmol; 2.3 equiv.), 2-bromoacetamide (0.3926; 2.85 mmol; 2.3 equiv.), and a catalytic amount of KI (0.0843 g; 0.508 mmol). The reaction was stirred at 80°C for 20 hours under N$_2$. The solvent was removed by rotary evaporation. The solid residue was dissolved into 30 mL of chloroform and 30 mL of water. The organic layer was isolated, dried over Na$_2$SO$_4$, and the chloroform was removed by rotary evaporation yielding the desired product (2) an off-white solid powder (0.6184 g; 1.20 mmol). Yield 96.8%.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.74 (s, 2H, NH$_2$), 5.39 (s, 2H, NH$_2$), 3.14 (s, 4H, NC$_2$H$_5$), 3.07 (s, 4H, NC$_2$H$_5$), 2.61 (d, 16H, NC$_2$H$_5$), 1.46 (s, 18H, t-Bu).

3.2b 1,4,7,10-Tetraazacyclododecane-1,7-bis(acetate)-4,10-bis(acetamide) (H$_2$DO2A2AM) (3)

1,4,7,10-Tetraazacyclododecane-1,7-bis(tert-butylacetate)-4,10-bis(acetamide) (2) (0.5775 g; 1.12 mmol) was stirred with 3 mL trifluoroacetic acid and 3 mL dichloromethane (DCM) at room temperature for five hours. The solvent was removed by rotary evaporation to yield a viscous orange oil. This oil was dissolved in a minimum of deionized water. A column was prepared using Dowex 50X4-400 ion-exchange resin in 0.01 M HCl. The product was dissolved in water and loaded onto the column. The column was first eluted with 200 mL deionized water, until the pH of the eluate was approximately 5-6. The column was then eluted with 0.05 M NH$_3$ and fractions were collected. The fractions were collected as the pH of the eluate increased to 10. The solvent was removed by rotary evaporation to yield the product as a pale, yellow-gold solid (0.3657 g; 0.910 mmol). Yield: 81.3%. $^1$H NMR (400 MHz,
Figure 3.1. $^1$H NMR spectrum of DO2AM. The spectrum was taken in D$_2$O (4.8 ppm).

3.2c Mn(II)DO2A2AM

DO2A2AM (0.6591 g; 1.64 mmol) was mixed with anhydrous MnCl$_2$ (0.2100 g; 1.669 mmol; 1 equiv.) in a minimum of water and allowed to stir for two hours. This solution was added dropwise to stirring acetone to afford a white solid precipitate. The acetone was decanted off to give Mn(II)DO2A2AM (0.6922 g; 1.15 mmol). Yield: 70.4%
IR cm\(^{-1}\) (KBr pellet): 1676 \(\nu(\text{CONH}_2)\), 1618 \(\nu(\text{COO}^-)\). ESI-MS (50:50 MeOH:H\(_2\)O): 456 (MnHDO2A2AM\(^+\)).

3.3 DO2AM\(\cdot\)2HBr

The synthesis of DO2AM\(\cdot\)2HBr (8) has been previously reported,\(^3\) and all procedures were followed accordingly. Scheme 3.2 shows the structures throughout the reaction. The first step involved an addition to the cyclen (4) ring using a dehydration reaction whereupon the cyclen ring was protected with a glyoxal bridging group (5). Subsequent nucleophilic addition was used to attach two amide arms in the 1 and 7 positions, yielding 1,7-bis(acetamide) cyclen glyoxal (6). Deprotection of the

\[\text{Scheme 3.2.}\]

\[\text{i) 40\% Glyoxal in water, r.t., 4.5 hrs. (ii) 2 equiv. 2-bromoacetamide, r.t., 24 hrs. (iii) o-PDA, r.t., overnight, N}_2\]
cycloen ring occurred upon the addition of o-phenylenediamine, giving DO2AM•2HBr (7) as the pure product and a quinoxaline side product.

3.3a Cyclen Glyoxal (5)

Cyclen (4) (0.5089 g; 2.959 mmol) was dissolved into 50 mL MeOH. To this solution at 0 °C was added a 40% wt. solution of glyoxal in H2O (0.4293 g; 2.959 mmol; 1 equiv.). The reaction was then stirred at room temperature for 4.5 hours. The solvent was then removed by rotary evaporation to yield a white solid. This was dissolved into ether and filtered in order to remove residual polymers from the reaction. The filtrate was removed under vacuum to yield the product as a white solid (0.4385 g; 2.237 mmol). Yield: 75.6% 

13C NMR (400 MHz, CDCl3): δ 77.84, 51.10, 50.32

3.3b 1,7-Bis(acetamide) Cyclen Glyoxal Bis-hydrobromide (6)

Cyclen glyoxal (5) (0.4385 g; 2.237 mmol) was dissolved into 15-mL of dry MeCN. To this solution was added bromoacetamide (0.6507 g; 4.717 mmol; 2.1 equiv.) dissolved in 5-mL MeCN, and a catalytic amount of KI. The reaction was stirred for 24 hours at room temperature and an off-white solid precipitated out of the solution, marking the completion of the reaction. This solid was collected by filtration and washed with ether. The solid was then dissolved in H2O and centrifuged in order to separate any remaining organic starting materials. The solvent was removed under vacuum to yield the product as an off-white solid (0.6045 g; 1.938 mmol). Yield: 86.6%

13C NMR (400 MHz, D2O): δ 166.26, 165.72, 83.94, 71.70, 64.88, 64.50, 59.53, 57.78, 57.33, 51.94, 48.97, 48.67, 48.14, 47.83, 47.42, 44.10, 43.35

3.3c DO2AM•2HBr
1,7-Bis(acetamide) cyclen glyoxal di-hydrobromide (6) (1.6781 g; 3.555 mmol) was dissolved into 35 mL of methanol. To this solution was added, o-phenylenediamine (0.4382 g; 4.052 mmol; 1.1 equiv.). The reaction was stirred overnight under N₂ at room temperature, whereupon a precipitate was formed. The solvent was removed via roto evaporation, and 40 mL dry THF was added. The precipitate was collected by filtration, washed with 20 mL dry THF, and dried in vacuo to yield the solid as a white product (0.8689 g; 1.948 mmol). Yield: 54.8% ¹H NMR (500 MHz, D₂O): δ 3.26 (s, 4H, CH₂CONH₂), 2.80 (dt, 16H, NCH₂). Anal. Calc. for C₁₂N₆O₂H₂6Br₂: C, 32.16; H, 5.81; N, 18.76 Found C, 32.24; H, 6.35; N, 18.57.

Figure 3.2. ¹H NMR spectrum of DO2AM. The spectrum was taken in D₂O (4.8 ppm).
References:
Chapter 4: Potentiometry

Determining the speciation state of ligands at different pH values in solution is an important aspect of fully characterizing these compounds. Along with the qualitative speciation diagrams, quantitative pKₐ’s for the ligands can be generated. Additionally, potentiometry can determine the thermodynamic stability of metal-ligand complexes, which is important for determining the efficacy of a potential contrast agent. Specifically, determining if only one species of the complex is present at physiological pH (~ 6.8-7.4) is quite important.

4.1 Experimental

4.1a Standardization of KOH(aq) and HNO₃

Potassium hydroxide (40% w/v in water; 1.000 mL) was diluted to 100 mL with DI water which was distilled from basic KMnO₄ (distilled water). This solution was titrated against a solution of potassium hydrogen phthalate (KHP; 10 mL of 0.1051 M). A total of 8.6 mL of KOH was added and the concentration of KOH was determined to be 0.1222 M.

The previously standardized KOH solution was then used to standardize a HNO₃ solution (0.6 mL conc. HNO₃ diluted to 100 mL with distilled water). The HNO₃ solution (10 mL) was added to a cell jacket at 37°C under N₂ humified by bubbling through 0.1 M KNO₃. After the solution was allowed to reach thermal equilibrium, aliquots of KOH were added. A total of 7.76 mL of 0.1222 M KOH were added and the concentration of HNO₃ was determined to be 0.0948 M.

4.1b Titration Procedure
DO2AM·2HBr (0.0460 g; 0.1027 mmol) was dissolved into 10 mL of distilled DI water. This solution was added to a cell jacket kept at a constant 37°C under humidified N₂. To this solution was added 12.500 mL of distilled water and 2.500 mL of 1.00 M KNO₃, which was recrystallized from distilled water prior to use. The solution was allowed to reach thermal equilibrium and the pH was determined to be 6.59. Addition of 4.000 mL of previously standardized HNO₃ (0.0948 M) decreased the pH to 2.01. Previously standardized KOH(aq) (0.1222 M) was added in increments ranging from 0.020 mL to 0.200 mL. The system was allowed to reach equilibrium for ~30 seconds before the pH was taken after each addition of base. 4.900 mL of KOH was added and the final pH was 10.68.

This procedure was followed for all potentiometric titrations with slight modifications for titrations containing Mn(II) complexes. The titration for [Mn(HₓDO2A2AM)]ₓ⁺ was performed with preformed complex, while those for [Mn(HₓDO2A)]ₓ⁺ and [Mn(DO2AM)]²⁺ were performed with complex that was generated in situ. For Mn(II) complexes which were not isolated, a 1:1 metal:ligand ratio was made in situ within the cell jacket. For such titrations, the amount of time between the addition of base and measurement of pH was increased to one minute to allow the system to reach equilibrium.

4.1c Titration Data and Fitting for (HₓDO2AM)ₓ⁺

The programs Hyperquad09 and Hyperquad13 were used to abstract pKₐ values and binding constants from the potentiometric data acquired from the titrations.¹ Hyperquad uses a non-linear, least-squares fit to model titration data. These models are based upon the titration data and all of the associated titration conditions, including
temperature, concentration of reagents, and error in the volume and pH readings. Figure 4.1 shows the fitting program for the potentiometric titration of (H$_2$DO2AM)$_x^x$.

The open blue diamonds are data that are included within the model, while the open red diamonds are data that are not included in the fit. These data are not included because they are within a region at which there is only one species. Because the speciation is not changing within such a pH range, the data represent the titration of a strong acid (HNO$_3$) with a strong base (KOH) and do not contribute to the model. The red dotted line represents the fit generated via Hyperquad. Each of the solid colored lines represent the concentration of specific species as a function of progression of the titration.

![Figure 4.1](image.png)

**Figure 4.1.** The model fit for (H$_2$DO2AM)$_x^x$ generated by Hyperquad. Data were obtained at 37°C and [L]=0.1 mM. Blue diamonds indicate data used in the fit, while red indicate data that were omitted from the fit (see text). The red dotted line indicates the fit generated by Hyperquad.

From these models logβ (pK$_a$) values are be abstracted, and overall binding constants can be determined. These values can be used to generate speciation diagrams using the HySS program. Fits for all potentiometric titrations are given in Appendix A.
4.2 Speciation diagram for \([1,7-H_xDO2A]^{X-2+}\)

The speciation diagram,\(^2\) or predominance diagram, for \([1,7-H_xDO2A]^{X-2+}\) is given in Figure 4.2, below. The x-axis represents pH and the y-axis is % species based upon the fit from the potentiometry titration. At values of over 50%, the line indicates the dominant species in solution at that specific pH.

At high pH values the ligand is completely deprotonated and has a (2-) charge. As the pH is lowered two of the cyclen ring nitrogen amines become protonated. In the region from pH 4 to pH 9 the neutral species dominates. This is a recurring theme for tetraamine macrocyclic ligands that contain carboxylic acid pendant arms, such as \(H_4\)DOTA\(^3\) and \(H_2\)DO2A2AM (see below). As the pH is further lowered, the carboxylate arms become protonated and the compound becomes positively charged. The specific sites of protonation for the ligands and their Mn(II) complexes are explored in

![Speciation diagram for \((H_xDO2AM)^{(X-2)}\) at 37°C, [L]=0.1 mM, 0.1 M KNO₃. Adapted from [Ref 2].](image)

**Figure 4.2.** Speciation diagram for \((H_xDO2AM)^{(X-2)}\) at 37°C, [L]=0.1 mM, 0.1 M KNO₃. Adapted from [Ref 2].
Chapter 5. The pK\textsubscript{a} values, with experimental error, for five macrocyclic compounds are given in Table 4.1.

4.3 Speciation diagram for [Mn(1,7-H\textsubscript{x}DO2A)]\textsuperscript{X+}

The speciation diagram for the Mn(II) complex of DO2A is given below in Figure 4.3. Above a pH of six, the complex is fully formed. At this pH in the solution state, Mn(DO2A) is hexadentate with the Mn(II) metal center binding to the four ring amines and the two carboxylate arms, see below. As the pH is decreased below this, one of the carboxylate pendant arms becomes protonated. The Mn(II) metal center falls out of the ring as the pH is further decreased until the complex is fully dissociated at around a pH of 2.5.

The pK\textsubscript{a} values of [Mn(H\textsubscript{x}DO2A)]\textsuperscript{X+} have previously been reported.\textsuperscript{6} These values were abstracted from titrations performed at 25°C, whereas the titrations

![Figure 4.3. Speciation diagram for [Mn(H\textsubscript{x}DO2A)]\textsuperscript{X+} at 37°C, [L]=[M]=0.1 mM, 0.1 M KNO\textsubscript{3}.](image-url)
Where the pKₙ values, the logKₘₙₗ value, and the pKₘₙₗ value are defined in Equations 4.1, 4.2, and 4.3, respectively.

$$K_{LH_{n}} = \frac{[H_nL]}{[H][H_{n-1}L]} \quad (4.1)$$

$$K_{MnL} = \frac{[MnL]}{[Mn][L]} \quad (4.2)$$

$$K_{MnH_{n}L} = \frac{[MnH_{n}L]}{[H][MnH_{n-1}L]} \quad (4.3)$$
reported herein (Table 4.1) were performed at 37°C. The log $K_{\text{MnL}}$ for the complex is 14.93±0.08, close to the values from previous titrations ($pK_a=14.54$ at 25°C). The log $K_{\text{MnL}}$ is a measure of the thermodynamic stability of a complex and is one of the most important factors in determining the efficacy of a potential contrast agent as it describes the strength of the ligand:metal bonding. The stability constant for $[\text{Mn(H}_x\text{DO2A)}]^{X+}$ is relatively low for six-coordinate macrocyclic Mn(II) complexes.\(^6\) Indeed, $[\text{Mn(DOTA)}]$, the manganese(II) complex of the tetra-acetate cyclen derivative, has a log $K_{\text{MnL}}=20.42$.\(^3\) Though the two additional acetate arms present in ligand do not bind to the metal center, as $[\text{Mn(DOTA)}]^{(X-2)+}$ is six-coordinate in solution (see chapter 5), they increase the stability of the complex by more than five orders of magnitude. The complex has one additional $pK_a$, log $K_{\text{MnHL}}=4.53±0.14$. This protonation occurs at one of the carboxylic pendant arms. This value is higher than the $pK_a$ values for the acetate pendant arms in $[\text{Mn(H}_x\text{DOTA)}]^{2-X+}$ (see Table 4.1). This is likely due to the lower overall stability of the DO2A complex. Indeed, only one of these pendant arms can become protonated before the metal center decomplexes from the ligand.

### 4.4 Speciation diagram for $[1,7-H_x\text{DO2AM}]^{X+}$

Figure 4.4 shows the speciation diagram for $[1,7-H_x\text{DO2AM}]^{X+}$. At high pH values DO2AM has the same speciation profile as other tetraamide macrocycles. The two protonation sites as the pH is lowered, with $pK_a$ values of 10.15 and 8.54,
represent the protonation of two cyclen ring amines, see chapter 6 for protonation sites. Below a pH of 7 there are no sites on the molecule that can be protonated as there are no acetate arms. Each of these pK\textsubscript{a} values is about one order of magnitude lower than those of \((H\textsubscript{x}DO2A)^{(X-2)+}\).

4.5 Speciation diagram for \([Mn(DO2AM)(H\textsubscript{2}O)]^{2+}\)

Figure 4.5 shows the speciation diagram for \([Mn(DO2AM)(H\textsubscript{2}O)]^{2+}\). As the pH is decreased, the complex begins to undergo proton assisted decomplexation starting around a pH of six. As the pH is lowered below 3.5, the metal has completely decomplexed from the ligand and the only species present are free Mn(II) and \((H\textsubscript{2}DO2AM)^{2+}\). Unlike \([Mn(H\textsubscript{x}DO2A)]^{X+}\), the DO2AM ligand has no acetate arms to be protonated. Due to this, there are no protonation sites and, thus, no pK\textsubscript{a} values.
Because of this, only the binding constant could be abstracted from the potentiometric titration. Previously, the $pK_a$ values of bound waters have been reported, however, all of these deprotonations occur at pH$>$10. When the pH of a solution containing $[\text{Mn(DO2AM)(H}_2\text{O)}]^2^+ \text{ is raised above } 9 \text{ the manganese(II) falls out the ring and precipitates as the insoluble Mn(OH)}_2^\text{, so none of the } [\text{Mn(DO2AM)(OH)}]^+ \text{ species should be present in solution. The } \log K_{\text{MnL}} \text{ for } [\text{Mn(DO2AM)(H}_2\text{O)}]^2^+ \text{ is } 10.92 \pm 0.02, \text{ which is almost four magnitudes lower than that of } [\text{Mn(H}_x\text{DO2A)}]^X$. This difference is due to two factors. Firstly, the acetate arms of $[\text{Mn(H}_x\text{DO2A)}]^X$ bind more strongly to the metal center than do the amide arms of DO2AM due to their increased electrostatic interactions with the metal center. In addition, MnDO2AM has a bound water in the solution state (see chapter 7), which destabilizes the complex, as the bulk water can access the Mn(II) metal center more easily. This ease of access for the water decreases thermodynamic stability. $[\text{Mn(DO2AM)(H}_2\text{O)}]^2^+ \text{ has the lowest}

\textbf{Figure 4.5.} Speciation diagram for $[\text{Mn(DO2AM)}]^2^+$ at 37°C, $[\text{L}]=[\text{M}]=0.1 \text{ mM, 0.1 M KNO}_3$. 

Because of this, only the binding constant could be abstracted from the potentiometric titration. Previously, the $pK_a$ values of bound waters have been reported, however, all of these deprotonations occur at pH$>$10. When the pH of a solution containing $[\text{Mn(DO2AM)(H}_2\text{O)}]^2^+ \text{ is raised above } 9 \text{ the manganese(II) falls out the ring and precipitates as the insoluble Mn(OH)}_2^\text{, so none of the } [\text{Mn(DO2AM)(OH)}]^+ \text{ species should be present in solution. The } \log K_{\text{MnL}} \text{ for } [\text{Mn(DO2AM)(H}_2\text{O)}]^2^+ \text{ is } 10.92 \pm 0.02, \text{ which is almost four magnitudes lower than that of } [\text{Mn(H}_x\text{DO2A)}]^X$. This difference is due to two factors. Firstly, the acetate arms of $[\text{Mn(H}_x\text{DO2A)}]^X$ bind more strongly to the metal center than do the amide arms of DO2AM due to their increased electrostatic interactions with the metal center. In addition, MnDO2AM has a bound water in the solution state (see chapter 7), which destabilizes the complex, as the bulk water can access the Mn(II) metal center more easily. This ease of access for the water decreases thermodynamic stability. $[\text{Mn(DO2AM)(H}_2\text{O)}]^2^+ \text{ has the lowest}
binding constant of all of the cyclen derived Mn(II) complexes detailed herein. The binding constant is almost two orders of magnitude lower than that of \([\text{Mn(DOTAM)}]^{2+}\), the tetra-amide cyclen derivative, though \([\text{Mn(DOTAM)}]^{2+}\) is eight-coordinate in the solution state.

4.6 Speciation diagram for \([\text{H}_x\text{DO2A2AM}]^{(X-2)+}\)

The speciation diagram for \([\text{H}_x\text{DO2A2AM}]^{(X-2)+}\) is shown below, in Figure 4.6. The protonation scheme is quite similar to that of \((1,7-\text{H}_x\text{DO2A})^{X-2+}\). At high pH values, the ligand is completely deprotonated and holds a \((2-)\) charge. As the pH is decreased, the tertiary ring amines become protonated. These protonations account for the two highest \(pK_a\) values (11.87±0.05 and 8.87±0.06, respectively). The neutral form of the ligand dominates in the pH range from 3-8.5. Then, as the pH is lowered past 3, the carboxylic acid groups become protonated, accounting for the two lower \(pK_a\) values of 2.37±0.06 and 1.91±0.06, respectively. These values are considerably lower than

![Speciation diagram for \((\text{H}_x\text{DO2A2AM})^{(X-2)+}\) at 37°C, \([\text{L}]=0.1 \text{mM}, 0.1 \text{M KNO}_3\).](image-url)
those of both \((H_xDO2A)^{(X-2)+}\) and \((H_xDOTA)^{(X-2)+}\). This decrease in \(pK_a\) is likely due to a proton shuttling mechanism that takes place as the pH is lowered below 3.5 (see Section 5.4). In the pH range from 3.5 to 8, the protons reside on the acetate appended tertiary amine sites in the cyclen ring. Because the carboxylate pendant arms are deprotonated at this pH, they are able to form intramolecular hydrogen bonds with the protonated amine sites. As the pH is decreased below 3 the carboxylic acid pendant arms become protonated and the protons migrate to the amide appended tertiary amine sites. Such a transfer process is not seen in \((H_xDO2A)^{(X-2)+}\) or \((H_xDOTA)^{(X-2)+}\). In \((H_xDO2A)^{(X-2)+}\) the protonation sites on the ring occur almost exclusively at the secondary amine sites (see Section 5.2). In \((H_xDOTA)^{(X-2)+}\) the first two protonations (at \(pK_1=12.04\pm0.14\) and \(pK_2=10.54\pm0.15\)) occur at ring amine sites that are trans to each other. The deprotonated carboxylic acid pendant arms that are attached to these amine sites form intermolecular hydrogen bonds to the protonated amine sites. The next two protonation sites occur at the two carboxylate pendant arms that are not attached to the previously protonated amine sites. Because these pendant arms are attached to neutral amines, their \(pK_a\) values (\(pK_3=4.36\pm0.12\) and \(pK_4=3.49\pm0.17\)) are quite close to that of acetic acid (\(pK_1=4.8\)), and markedly higher than those for DO2A2AM.

Ethylenediamine tetraacetic acid (EDTA) is an open chain chelating agent that similarly has two tertiary amine sites that can be protonated. These amine sites each have two appended acetate pendant arms which can be protonated. The \(pK_a\) values (\(pK_3=2.60\) and \(pK_4=2.00\)) for these pendant arms are much lower than those of DOTA due to their proximity to the positively charged tertiary amine sites. Thus, because the two carboxylate pendant arms of DO2A2AM are attached to positively charged amine
ring sites, their pK\textsubscript{a} values (pK\textsubscript{3}=2.37±0.06 and pK\textsubscript{4}=1.91±0.06) are much closer to those of EDTA and much higher than those of DOTA.

4.7 Speciation Diagram for [Mn(H\textsubscript{x}DO2A2AM)]\textsuperscript{X+}

The speciation diagram for [Mn(H\textsubscript{x}DO2A2AM)]\textsuperscript{X+} is shown in Figure 4.7. This speciation diagram looks quite similar to that of [Mn(1,7-H\textsubscript{x}DO2A)]\textsuperscript{X+} but shifted toward lower pH values. The complex has a logK\textsubscript{MnL}=17.07±0.03. This is six magnitudes higher than that for [Mn(DO2AM)]\textsuperscript{2+}, though both complexes bind to the Mn(II) metal center through their amide pendant arms. This is almost the same difference between binding constants as that seen for [Mn(DO2A)] and [Mn(DOTA)]\textsuperscript{2-}. The addition of acetate arms to the cyclen ring considerably raises the overall stability of the complex, even when these arms do not bind (see Chapter 5). Similarly to [Mn(H\textsubscript{x}DO2A)]\textsuperscript{X+}, only one of the acetate arms becomes protonated as the pH is lowered. The

![Figure 4.7. Speciation diagram for [Mn(H\textsubscript{x}DO2A2AM)]\textsuperscript{X+} at 37°C, [L]=[M]=0.1 mM, 0.1 M KNO\textsubscript{3}.]
pK_{\text{MnHL}} = 3.13 \pm 0.03$ (see equation 4.3), which is lower than the equivalent pK_a values in [Mn(H_xDO2A)]^{X+} and [Mn(DOTA)]^{(2-X)+}. This lower value is likely due to the lower pK_a values of the acetate pendant arms in the (H_xDO2A2AM)^{(X-2)+} ligand compared with those of the (H_xDO2A)^{(X-2)+} or (H_xDOTA)^{(X-2)+} (see Table 4.1). While the deprotonated acetate pendant arms do not bind to the metal center, their presence in the complex noticeably increases the stability constant by some interaction, possibly electrostatic. Likely, when the unbound acetate arm becomes protonated, this interaction decreases significantly. At pH values at which both acetate arms are protonated, Mn(DO2A2AM)^{2+} then closely resembles Mn(DO2AM)^{2+}, and the metal decomplexes from the ring.
References:
Chapter 5: Solution State IR Spectroscopy

5.1 Introduction

While potentiometry can give insight into the total proton content for a compound at different pH values in solution, it is of interest to determine the specific sites of protonation at these pH values. Additionally, pH dependent solution state IR spectra of macrocyclics can be sensitive to the sites of protonation. This technique is especially effective when used in concert with acid/base NMR titrations, such as described previously for DO2A.¹

Determining the solution state structure, especially with respect to the coordination environment of the metal, of potential CAs is a challenge. Due to their paramagnetic nature traditional NMR techniques offer no insight. In addition, Mn(II) complexes have no diagnostic visible absorption transitions. Indeed, ligand field spectra are generally not particularly useful for probing structure in the solution state. By looking at the carbonyl region of the infrared spectra of these macrocyclic complexes the binding structure can be elucidated. When one of the pendant arms binds to the metal center, the $C=O$ stretching frequency is red-shifted. If the stretching frequencies of the unbound arms are known, this shift indicates the binding of the pendant arm. It has been successfully shown that the coordination environment of the metal in $[M(DOTAM)]^{2+}$ (where $M=$Cu, Ni, Zn, Co, Mn) in both the solid and solution state can be determined. Additionally, the bonding environment from the solid state to the solution state does not vary much.²

5.2 Solution State IR of $[D_xDO2A]^{x-2+}$
Solution state IR data for the carbonyl region of $[\text{D}_x\text{DO2A}]^{x-2+}$ are given in Table 5.1. The table details the pD at which each spectrum was taken, the dominant species at that pD value, and the frequency of the peaks that are present in the spectrum with their assignment. The dominant species was determined based upon the speciation diagram for the respective ligand, see Chapter 4.

Figure 5.1 shows the solution IR spectra for $[\text{D}_x\text{DO2A}]^{x-2+}$ at three different pD values, along with the dominant protonation microstate corresponding to each spectrum. The IR spectrum for the fully protonated form of the ligand, $[\text{D}_4\text{DO2A}]^{2+}$, consists of a single peak at 1710 cm$^{-1}$ (Figure 5.1a). This peak is assigned to the stretching frequency from the protonated acetate pendant-arms ($\text{COOH}$). As the pD is increased, the acetate arms become deprotonated and the peak at 1716 cm$^{-1}$ decreases in intensity while a peak at 1584 cm$^{-1}$, characteristic of the deprotonated acetate side-arm stretching frequency ($\text{COO}^-$), becomes evident in the spectrum (see Figure 5.1b). This peak grows in intensity until both pendant arms are deprotonated, and the intensity stays constant as the pD is raised above 5. For $\text{D}_2\text{DO2A}$ and $[\text{DDO2A}]^+$ the protonation sites are at the secondary amine nitrogen atoms in the

<table>
<thead>
<tr>
<th>pD$^a$</th>
<th>Dominant Species$^b$</th>
<th>Frequencies (cm$^{-1}$) and Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.69</td>
<td>($\text{D}_4\text{L}$)$^{2+}$</td>
<td>1710 $\nu(\text{COOH})$</td>
</tr>
<tr>
<td>3.54</td>
<td>($\text{D}_3\text{L}$)$^+$</td>
<td>1716 $\nu(\text{COOH})$; 1584 $\nu(\text{COO}^-)$</td>
</tr>
<tr>
<td>4.51</td>
<td>($\text{D}_3\text{L}$)$^+$; ($\text{D}_2\text{L}$)</td>
<td>1716 $\nu(\text{COOH})$; 1584 $\nu(\text{COO}^-)$</td>
</tr>
<tr>
<td>5.36</td>
<td>($\text{D}_2\text{L}$)</td>
<td>1584 $\nu(\text{COO}^-)$</td>
</tr>
<tr>
<td>10.54</td>
<td>($\text{DL}$)$^-$</td>
<td>1583 $\nu(\text{COO}^-)$</td>
</tr>
</tbody>
</table>

$^a$Taken to be pD = pH (measured) + 0.44 [Ref 3]. $^b$According to speciation diagrams for the given ligand, see above.
The protonation/deprotonation of these sites has no significant effect on the stretching frequency of the pendant arms and thus the tertiary ring nitrogen atoms are not protonated in the pD range from 2 to 11. Additionally, the stretching frequency of a deprotonated carboxylic acid adjacent to a protonated secondary amine (i.e. $^+\text{NHR}_2\text{CH}_2\text{COO}^-$) has previously been reported at 1632 cm$^{-1}$. This frequency is not

Figure 5.1. Carbonyl region of the solution state IR spectra for $(\text{D}_x\text{DO2A})^{(X^-)^{2+}}$ at a variety of pD values, top. Representative structures are given for each pD value, bottom.
present in any spectra of DO2A across the varying pD ranges. Thus, it is not the tertiary cyclen amines which become protonated.

These results are in good agreement with those obtained from an NMR titration that was previously reported. The NMR spectrum of DO2A has three peaks. By monitoring these peaks as the pH is varied, the specific sites of protonation can be determined. As the pH is lowered from a very high pH value (pH= ~ 13), there is a change only in the methylene ring protons. This change represents the protonation of the secondary ring nitrogens. As the pH is further lowered past the final two protonations the acetate pendant arm methylene peak shifts, indicating the protonation of the carboxylic acids.

5.3 Solution State IR of \([\text{D}_{\text{X}}\text{DO2AM}]^{\text{X}+}\)

Table 5.2 gives the peak frequencies in the carbonyl region in the solution state IR for \([\text{D}_{\text{X}}\text{DO2AM}]^{\text{X}+}\) at a variety of pD values. The dominant species at each pD value were determined based upon the speciation diagram for \([\text{D}_{\text{X}}\text{DO2AM}]^{\text{X}+}\), see above.

<table>
<thead>
<tr>
<th>pD</th>
<th>Dominant Species</th>
<th>Frequencies (cm(^{-1})) and Assignment</th>
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</thead>
<tbody>
<tr>
<td>6.93</td>
<td>(D(_2)L)(^{2+})</td>
<td>1644 (\nu(\text{CONH}_2))</td>
</tr>
<tr>
<td>13.44</td>
<td>(L)</td>
<td>1632 (\nu(\text{CONH}_2))</td>
</tr>
</tbody>
</table>

\(a^{\text{Taken to be pD = pH (measured) + 0.44 [Ref 3]. \quad b^{\text{According to speciation diagrams for the given ligand, see above.}}}}\)

The assignment of the protonation sites of DO2AM by solution state IR is made simpler by the lack of protonation sites on the pendant arms. As the pD is raised deprotonation of the ligand occurs at the secondary cyclen ring amine sites, the sole carbonyl peak
in the spectrum gradually shifts from 1644 cm$^{-1}$ to 1632 cm$^{-1}$ (see Figure 5.2). It has been previously observed that the carbonyl stretching frequency for an amide bound to a positively charged ring amine (i.e. $^{+}$NHR$_2$CH$_2$CONH$_2$) is ~ 1680 cm$^{-1}$, while that of an amide bound to a neutral ring amine (i.e. NR$_2$CH$_2$CONH$_2$) is ~ 1645-1630 cm$^{-1}$. The observed shift in frequency for DO2AM is thus assigned to protonation of the secondary amine.

**Figure 5.2.** Carbonyl region of the solution state IR spectra for (D$_x$DO2AM)$^{x+}$ at a variety of pD values, top. Representative structures are given for each pD value, bottom.
5.4 Solution State IR of (DₓDO2A2AM)(ₓ-2)⁺

The protonation sequence of the DO2A2AM ligand is more complex than that of either DO2A or DO2AM since the only ring sites that can be protonated are the tertiary nitrogen amines. The protonation of an amine site in the ring has a large effect on the stretching frequency of the carbonyl group to which it is attached, whether it is an amide or carboxylate. The carbonyl portion of the spectrum for the fully protonated species, (D₄DO2A2AM)²⁺ is shown in Figure 5.3a, along with the assigned protonation scheme. There are two peaks in the carbonyl region, at 1723 cm⁻¹ and 1680 cm⁻¹. The peak at 1723 cm⁻¹ is assigned to the protonated carboxylic acid pendant arm stretch, ν(COOH) and the peak at 1680 cm⁻¹ corresponds to the C=O stretch of an amide adjacent to a protonated ring nitrogen (⁺NHR₂CH₂CONH₂).⁴ Thus, the solution IR is consistent with protonation at the amide substituted ring nitrogen, not the carboxylate substituted. Figure 5.3b shows the carbonyl region of the IR spectrum at a pD value of 3.37. According to the speciation diagram (see above), there are two species present in roughly equal concentrations at this pD, (D₃DO2A2AM)⁺ and (D₂DO2A2AM). The (D₃L)⁺ species exhibits two peaks that are present in the fully protonated species, indicating both a fully protonated carboxylic acid and an amide adjacent to a protonated ring nitrogen. In addition to these peaks, there is a new frequency at 1635 cm⁻¹, which is representative of a deprotonated carboxylic acid adjacent to a protonated nitrogen in the cyclen ring.⁴ The (D₃L)⁺ species also gives rise to the new peak at 1650 cm⁻¹, which is assigned to the C=O stretch of the amide ν(CONH₂) with an unprotonated adjacent nitrogen.⁴ In the pD range from ~ 3-8, where the (D₂L) species is dominant, the carbonyl region of the IR spectrum (Figure 5.3c) contains peaks assigned to an amide adjacent to an unprotonated amine (1650 cm⁻¹)
and to a carboxylate adjacent to a protonated amine (1635 cm$^{-1}$). As the pD is raised above a value of 8 (Figure 5.3d), a peak at 1580 cm$^{-1}$ grows in intensity while the peak at 1635 cm$^{-1}$ decreases. This peak at 1580 cm$^{-1}$ corresponds to the stretching frequency of a deprotonated carboxylic acid with a deprotonated ring nitrogen, similar to that observed at high pD values for (D$_x$DO2A)$^{(X-2)+}$.

At intermediate pH values (4-8), two of the ring amine sites are protonated while the carboxylate groups are deprotonated. The frequency of the peak for the carboxylate pendant arms (1628 cm$^{-1}$) indicates that the ring nitrogen to which they are adjacent is protonated (i.e. $^+$NHR$_2$CH$_2$COO$^-$). It has been previously shown that such macrocyclic systems containing carboxylate pendant arms can display intramolecular hydrogen bonding.$^5$ As the pH value is lowered, the carboxylate arms become deprotonated and can no longer form hydrogen bonds with their adjacent protonated ring amines. This results in proton shuttling in which the protons move

### Table 5.3. Solution State IR data in the carbonyl region for (D$_x$DO2A2AM)$^{(X-2)+}$ at varying pD values

<table>
<thead>
<tr>
<th>pD$^a$</th>
<th>Dominant Species$^b$</th>
<th>Frequencies (cm$^{-1}$) and Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.28</td>
<td>(D$_4$L)$^{2+}$</td>
<td>1723 $\nu$(COOH); 1680 $\nu$(NHR$_2$CH$_2$CONH$_2$)$^c$</td>
</tr>
<tr>
<td>2.27</td>
<td>(D$_4$L)$^{2+}$</td>
<td>1724 $\nu$(COOH); 1679 $\nu$(NHR$_2$CH$_2$CONH$_2$)$^c$</td>
</tr>
<tr>
<td>3.41</td>
<td>(D$_3$L)$^+$</td>
<td>1723 $\nu$(COOH); 1680 $\nu$(NHR$_2$CH$_2$CONH$_2$)$^c$; 1650 $\nu$(CONH$_2$); 1635 $\nu$(NHR$_2$CH$_2$COO)$^c$</td>
</tr>
<tr>
<td>4.88</td>
<td>(D$_2$L)</td>
<td>1645 $\nu$(CONH$_2$); 1628 $\nu$(NHR$_2$CH$_2$COO)$^c$</td>
</tr>
<tr>
<td>6.83</td>
<td>(D$_2$L)</td>
<td>1645 $\nu$(CONH$_2$); 1628 $\nu$(NHR$_2$CH$_2$COO)$^c$</td>
</tr>
<tr>
<td>8.30</td>
<td>(D$_2$L); (DL)$^-$</td>
<td>1645 $\nu$(CONH$_2$); 1628 $\nu$(NHR$_2$CH$_2$COO)$^c$; 1578 $\nu$(COO)$^c$</td>
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<td>9.38</td>
<td>(D$_2$L); (DL)$^-$</td>
<td>1645 $\nu$(CONH$_2$); 1629 $\nu$(NHR$_2$CH$_2$COO)$^c$; 1584 $\nu$(COO)$^c$</td>
</tr>
<tr>
<td>10.15</td>
<td>(DL)$^-$</td>
<td>1638 $\nu$(CONH$_2$); 1582 $\nu$(COO)$^c$</td>
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</table>

$^a$Taken to be pD = pH (measured) + 0.44 [Ref 3]. $^b$According to speciation diagrams for the given ligand, see above. $^c$For cation analysis, see text.
from the ring amines bearing the carboxylate pendant arms to those bearing the amide pendant arms.

![Image](image.png)

**Figure 5.3.** Carbonyl region of solution IR spectra of [DxDO2A2AM]^{(X-2)+} as a function of pD and absorbance (arbitrary units), above, with corresponding protonation states of the ligand, below.

### 5.5 Solution State IR of Macroyclic Mn(II) Complexes

The coordination environment of the metal center of complexes in solution is particularly difficult to determine for paramagnetic metal ions. While X-ray crystallography gives detailed information for solid-state complexes, the coordination environment can change substantially when the complex becomes solvated. Solution IR is an effective diagnostic tool since the technique is not affected by the
paramagnetic nature of the Mn(II) metal center. Table 5.4, below, gives the frequencies corresponding to the ligand carbonyl stretches in their manganese complexes and their corresponding assignments.

The carbonyl regions of the IR spectra for [Mn(DO2A)] and [Mn(DO2AM)]\(^{2+}\), at pD values of 6.05 and 5.05 respectively, are shown in Figure 5.4 and display peaks at 1603 cm\(^{-1}\) and 1635 cm\(^{-1}\), respectively. These peaks are assigned to the pendant-arm carbonyl stretches when bound to the Mn(II) metal center. These peaks are at similar frequencies as those for the tetra-substituted analogues of the manganese complexes, [Mn(D\(\times\)DOTA)]\(^{(X-2)+}\) \(^6\) and [Mn(DOTAM)]\(^{2+}\) \(^2\) which have been reported previously. On the basis of the observed solution IR, both [Mn(DO2A)] and [Mn(DO2AM)]\(^{2+}\) are hexadentate to the ligand in the solution state.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Frequencies (cm(^{-1})) and Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Mn(DO2A)]</td>
<td>1603 (\nu(\text{COO}))(^a)</td>
</tr>
<tr>
<td>[Mn(DO2AM)](^{2+})</td>
<td>1635 (\nu(\text{CONH}_2))(^a)</td>
</tr>
<tr>
<td>[Mn(DO2A2AM)]</td>
<td>1645 (\nu(\text{CONH}_2))(^a); 1586 (\nu(\text{COO}))(^b)</td>
</tr>
<tr>
<td>[Mn(DOTA)](^{2-}) (^c)</td>
<td>1616 (\nu(\text{COO}))(^a); 1584 (\nu(\text{COO}))(^b)</td>
</tr>
<tr>
<td>[Mn(DOTAM)](^{2+}) (^d)</td>
<td>1642 (\nu(\text{CONH}_2))(^a)</td>
</tr>
</tbody>
</table>

\(^a\)Bound pendant arm. \(^b\)Unbound pendant arm. \(^c\)[Ref 6] \(^d\)[Ref 2]

Two peaks are present in the IR spectrum for [Mn(DO2A2AM)], shown in Figure 5.4b, at a pD value of 4.23, where the fully-complexed species dominates (see Figure 4.7, above). The peak at 1645 cm\(^{-1}\) corresponds to the amide pendant arms bound to the metal center, while the peak at 1586 cm\(^{-1}\) corresponds to the unbound, deprotonated carboxylic acid groups. The observation of only these two frequencies indicates that DO2A2AM\(^{2-}\) binds to Mn(II) as a hexadentate ligand. The structure of
the mixed ligand manganese center is especially interesting when compared to that of 
\([\text{Mn}(\text{DOTA})]^2\) and [\(\text{Mn(DOTAM)}\)]^2+. [\(\text{Mn}(\text{DOTA})\)]^2 has a hexadentate metal center in

\[ \text{[Mn(DOTA)]}^2 \]

\[ \text{[Mn(DOTAM)]}^{2+} \]

\[ \text{[Mn(DO2A2AM)]} \]

\[ \text{[Mn(DO2AM)]}^{2+} \]

Figure 5.4. Solution state IR spectra of the Mn(II) complexes with corresponding structures. The y-axis is absorption (arbitrary units).

This is evident based upon the two peaks present within the spectrum of the complex at pD=4.23 at frequencies of 1645 cm\(^{-1}\) and 1586 cm\(^{-1}\). At this pH, only the neutral [Mn(DO2A2AM)] species should be present, indicating that the two acetate arms are deprotonated. The
peak with the frequency of 1645 cm\(^{-1}\) corresponds to the carboxylate stretch of an amide arm bound to the Mn(II) metal center. This value is in agreement with the frequencies seen for [Mn(DO2AM)]\(^{2+}\) (1635 cm\(^{-1}\)) and [Mn(DOTAM)]\(^{2+}\) (1642 cm\(^{-1}\)),\(^2\) which correspond to the bound amide pendant arms. The peak at a frequency of 1584 cm\(^{-1}\) corresponds to the carboxylate stretch for unbound, deprotonated acetate pendant arms. This same frequency is seen in the spectrum for [Mn(DOTA)] at similar pD values. It is likely that electrostatic repulsions between the unbound acetate pendant arms and the bound pendant arms (either acetate as with MnDOTA or amide as with MnDO2A2AM) prevent more than two pendant arms binding to the manganese center. Thus, the bonding environment in the solid-state structure determined via X-ray crystallography is maintained in aqueous solution (see Chapter 6).
References:
Chapter 6: X-Ray Crystallography

In addition to characterizing complexes in the solution state, determining the solid state coordination environment of the metal center can give invaluable insights into hydration number and coordination number. X-Ray crystallography can be used to exactly determine the characteristics of these complexes.

6.1 Experimental

X-Ray crystallographic data were collected and analyzed by Dr. Brandon Mercado of the Yale University X-Ray Crystallographic Facilities.

\[ \text{[Mn(DO2A2AM)](NH}_4\text{Cl)}\cdot3.5\text{H}_2\text{O} \]

Low-temperature diffraction data (ω-scans) were collected on a Rigaku MicroMax-007HF diffractometer coupled to a Dectris Pilatus3R detector with Mo Kα (λ = 0.71073 Å). The diffraction images were processed and scaled using Rigaku Oxford Diffraction software.\(^1\) The structure was solved with SHELXT and was refined against \( F^2 \) on all data by full-matrix least squares with SHELXL.\(^2\) All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the U value of the atoms to which they are linked (1.5 times for methyl groups). The difference map contains evidence of a chloride on a crystallographic special position at 0.5 occupancy. There are two water molecules with oxygen atoms O7 and O8. There is also a solvent void which contains an additional 49 e/Å\(^3\). This residual electron density is disordered, but one large difference map peak (9.5 e as found by refinement with SHELXL) was modeled as a disordered 0.5 occupancy chloride. The remaining peaks were modeled as disordered water positions (O9A at 0.59(3) occupancy; O9B at 0.41(3); O10A at
0.3; O10B at 0.2). The small amount of electron density associated with site O10 required that the thermal parameters of those atoms be constrained as identical to O8. One low angle reflection was improperly recorded and omitted from the least square refinement.

\[[\text{Mn(DO2AM)}(\text{H}_2\text{O})](\text{Cl}_{1.34})(\text{Br}_{0.66})\cdot 2\text{H}_2\text{O}\]

Low-temperature diffraction data (ω-scans) were collected on a Rigaku MicroMax-007HF diffractometer coupled to a Dectris Pilatus3R detector with Mo Kα (λ = 0.71073 Å). The diffraction images were processed and scaled using Rigaku Oxford Diffraction software.¹ The structure was solved with SHELXT and was refined against \(F^2\) on all data by full-matrix least squares with SHELXL.² All non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included in the model at geometrically calculated positions and refined using a riding model. The isotropic displacement parameters of all hydrogen atoms were fixed to 1.2 times the U value of the atoms to which they are linked (1.5 times for methyl groups). The hydrogen atoms associated with heteroatoms in the Mn complex were found in the difference map and freely refined. The hydrogen atoms associated with water were geometrically generated and refined as riding atoms. The counter ion sites have mixed occupancies of Cl/Br. The site with atoms Cl1 and Br1 have split occupancies of 0.659(1) and 0.341(1), respectively. The atoms sites with Cl2 and Br2 have split occupancies of 0.681(1) and 0.319(1), respectively. The thermal parameters and positional parameters were constrained to be identical between the Cl1/Br1 and Cl2/Br2.
6.2 Parameter Table

<table>
<thead>
<tr>
<th>Chemical Formula</th>
<th><a href="NH%E2%82%84Cl">Mn(DO2A2AM)</a>•3.5H₂O</th>
<th><a href="Cl%E2%82%81.%E2%82%83%E2%82%84(Br%E2%82%80.%E2%82%86%E2%82%86)%E2%80%A22H%E2%82%82O">Mn(DO2AM)(H₂O)</a></th>
</tr>
</thead>
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<tr>
<td>Empirical formula</td>
<td>C₁₆H₃₈ClMnN₇O₉.₅₀</td>
<td>C₁₂H₃₂Br₀.₆₆Cl₁.₃₄MnN₆O₅</td>
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<td>9₃(2) K</td>
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<td>P₁</td>
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<td>a = 7.₆₈₁₄₀(10) Å</td>
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<td>b = 8.₈₈₀₉₈₀(10) Å</td>
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<td>c = 1₆.₆₀₂₇(₂) Å</td>
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<td>1₀₅₃.₇₆(₂) Å³</td>
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<td>1.₅₆₂ Mg/m³</td>
</tr>
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<td>2.₀₈₁ mm⁻¹</td>
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<td>5₁₄</td>
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<td>0.₂₀₀ x 0.₁₀₀ x 0.₀₅₀ mm³</td>
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<td>Dectris Pilatus 3R</td>
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<td>3.₀₃₂ to 3₀.₅₀₅°</td>
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<td>-₁₀₅sh₁₀, -₁₁₁s₁₂, -₂₃₁s₂₁</td>
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<td>2₅₈₀₇</td>
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<td>6₃₆₀ [R(int) = 0.₀₂₂₆]</td>
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<td>₅₆₈₀</td>
</tr>
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<td></td>
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<td>₉₉.₉ % (2₅.₂₄₂°)</td>
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<td>Semi-empirical from equivalents</td>
<td>Semi-empirical from equivalents</td>
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<td>1.₀₀₀₀₀₀ and ₀.₈₁₁₅₁₈</td>
</tr>
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<td>₆₃₆₀ / ₁₇ / ₂₇₅</td>
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<tr>
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<td>₁.₀₃₀</td>
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<td>Final R indices</td>
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<td>R₁ = ₀.₀₂₃₀, wR₂ = ₀.₀₄₇₉</td>
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<td></td>
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<td>R indices (all data)</td>
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<td>R₁ = ₀.₀₂₈₆, wR₂ = ₀.₀₄₉₃</td>
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<tr>
<td>Largest diff. peak and</td>
<td>₁.₅₁₆ and -₁.₀₀₄ e.Å⁻³</td>
<td>₀.₄₅₁ and -₀.₃₂₇ e.Å⁻³</td>
</tr>
</tbody>
</table>

The full crystallographic parameters are given in Table 6.1.

6.3 [Mn(DO2A2AM)](NH₄Cl)•3.5H₂O

MnDO2A2AM has a crystal structure in which the manganese binds in a hexadentate manner to the ligand through the amide arms. The unit cell of the crystal contains two complex molecules along with seven water molecules which co-
crystallized. An NH₄Cl formula unit also co-crystallized with each of the complexes. The NH₄⁺ ion was present in the solution due to the use of NH₄OH(aq) to increase the pH to a value at which the complex was fully formed. The Cl⁻ was present from the manganese source, MnCl₂, and occupies a crystallographically special position on the a axis of the unit cell at (0.5, 1.0, 1.0). The NH₄⁺ ion forms extensive hydrogen bonds with the amide arms of both complexes in the unit cell. The Cl⁻ ion forms a 179.88° angle with the Mn(II) and the NH₄⁺ (Cl⁻-Mn²⁺-NH₄⁺). Though the space group of the crystal is P1 (a centrosymmetric space group) there is a pseudo-C2 axis running through these three ions. The viewpoint of Figure 6.1b is down this pseudo-C2 axis.

Figure 6.1. Two views of the crystal structure for [Mn(DO2A2AM)]. Figure 6.1a, left, shows the metal-ligand complex with hydrogen atoms. The coordination environment for the metal center is shown in figure 6.2b, right, along with the labeling scheme. Only amide protons are shown in Figure 6.2b for clarity. Black spheres represent protons; Grey ellipsoids represent carbon; Purple represent nitrogen; Red represent oxygen; Blue represent manganese. Ellipsoids are shown at 90% probability.
Figure 6.1 gives two views of the structure of the [Mn(DO2A2AM)] complex. The crystal structure, with the disordered solvent molecules and NH\textsubscript{4}\textsuperscript{+} and Cl\textsuperscript{−} omitted, is shown in Figure 6.1a, while the bonding environment of the metal center, with labeling scheme included, is shown in Figure 6.1b. The metal center binds to the ligand through the four cyclen ring nitrogen atoms and the two amide oxygen groups, which are trans to each other across the cyclen ring. The manganese can be best described as having a distorted octahedral geometry, similar to the metal center of Mn(H\textsubscript{2}DOTA).\textsuperscript{3} Also similarly to the crystal structure of Mn(H\textsubscript{2}DOTA), Mn(DO2A2AM) adopts a syn configuration in which all of the pendant arms are on one side of the macrocyclic plane.

Figure 6.2. The two sets of five-membered rings which determine the helicity of Mn(DO2A2AM). The bonds shown in blue correspond to the five-membered rings formed with the amide pendant arms while bonds shown in red correspond to those formed with the cyclen ring. The purple bonds are used in both sets of five-membered rings.
This orientation of the Mn(II) within the complex leads to 6 five-membered rings. The amide pendant arms binding to the manganese account for 2 of these rings, while the other 4 arise from the Mn(II) binding to the cyclen ring. Each of these two sets of five-membered rings can give rise to different helicities. The five-membered rings are shown in Figure 6.2. The bonds shown in blue correspond to the five-membered rings formed with the amide pendant arms while bonds shown in red correspond to those formed with the cyclen ring. The purple bonds are used in both sets of five-membered rings.

Within the crystal structure shown, the 4 five-membered rings formed by the binding of the Mn(II) with the nitrogen atoms in the cyclen ring all adopt the same conformation, leading to the absolute conformations (δδδδ). These absolute conformations are defined by the orientation of the carbon-carbon bond of one of the five-membered ethylene diamine rings to the plane of the Mn(II) and the nitrogen atoms in the cyclen ring. Figure 6.3 shows the δ and λ conformations of one of these five-membered rings. These rings can also invert when the complex is in solution going from the δ conformation to the λ conformation. Full inversion of all 4 rings gives the enantiomer in the absolute configuration (λλλλ). The 2 five-membered rings formed by the manganese and the pendant arms can adopt the absolute configuration Λ or Δ, which denote two different optical enantiomers. The Mn(DO2A2AM) molecule shown in Figures 6.1 and 6.2 adopts the absolute configuration of Λ(δδδδ). The other Mn(DO2A2AM) molecule in the unit cell has the opposite absolute configuration of Δ(λλλλ) as the two molecules are related by inversion.

In solution these five-membered rings are able to invert, however, at room temperature this inversion most likely occurs faster than the NMR timescale. A
previous study\textsuperscript{5} probed the kinetics and thermodynamics of such a process in a series of [M(DOTAM)]\textsuperscript{2+} complexes (where M\textsuperscript{2+}=Zn, Hg, Cd, Ca, and Pb) by obtaining the \textsuperscript{13}C NMR spectrum of the [M(DOTAM)]\textsuperscript{2+} complex at a range of temperatures from 218 K to 295 K. At lower temperatures the carbon peaks assigned to the cyclen ring decoalesce into two separate peaks, each indicating a different absolute configuration. The rate constant and free energy of activation for the change in helicity were determined from this data. These rates of change are based upon the size of the metal in each complex. Complexes with larger metals undergo inversion of helicity more readily than those with smaller metals.\textsuperscript{5} Unfortunately, due to the paramagnetic nature of Mn(II), such studies cannot be undertaken with the complexes present herein, however, based upon the size dependence trend, the inversion of helicity for these Mn(II) complexes should occur at temperatures lower than 0\textdegree C.

\textbf{Figure 6.3.} The $\delta$ (left) and $\lambda$ (right) configurations for one of the five-membered ethylene diamine rings formed by the Mn(II) metal center and the cyclen ring in Mn(DO2A2AM).

Bond distances and bond angles for the Mn(II) center are given in Table 6.2. The average Mn-O bond length (2.25 \AA) is similar to that of [MnDO2AM(H\textsubscript{2}O)]\textsuperscript{2+} (2.22 \AA) (see below) and shorter than that of MnDOTAM (2.31 \AA).\textsuperscript{3} These are noticeably larger than the Mn-O bond length in Mn(H\textsubscript{2}DOTA) (2.16 \AA).\textsuperscript{3} This difference is likely
due to the electrostatic interaction between the carboxylate pendant arm and the Mn(II) ion. The acetate arms in Mn(H₂DOTA) that bind to the metal center are deprotonated, and thus are negatively charged. The interaction between the negatively charged, acetate pendant arms and the Mn(II) metal center creates a shorter bond than the interaction between neutrally charged, amide pendant arms and the metal center. The average Mn-N bond length is 2.37 Å, which is only slightly higher than those of Mn(H₂DOTA), at 2.36 Å. Indeed, the average N-Mn-N bond angle for MnDO2A2AM, at 75.88°, and Mn(H₂DOTA), at 75.86°, are also quite close in value.³ Though the metal center in Mn(DO2A2AM) binds to the ligand through the amide pendant-arms.

<table>
<thead>
<tr>
<th>Table 6.2. Selected bond lengths and bond angles for [Mn(DO2AM)(H₂O)]<a href="Br%E2%82%80.66">Cl₁.34</a>·2H₂O and <a href="NH%E2%82%84Cl">Mn(DO2A2AM)</a>·3.5H₂O.</th>
<th></th>
<th></th>
</tr>
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<td></td>
</tr>
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<td>Bond Lengths (Å)</td>
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<td></td>
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<td>Mn—N</td>
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<td>2.378(2)</td>
</tr>
<tr>
<td>2.2964(9)</td>
<td>2.401(2)</td>
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</tr>
<tr>
<td>2.4470(10)</td>
<td>2.341(2)</td>
<td></td>
</tr>
<tr>
<td>2.2844(10)</td>
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<td>2.2220(19)</td>
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<td>2.2225(8)</td>
<td>2.286(2)</td>
<td></td>
</tr>
<tr>
<td>Mn—OH₂</td>
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<td>Bond Angles (°)</td>
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<td>75.57(3)</td>
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<td>75.29(3)</td>
<td>75.87(8)</td>
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<tr>
<td>O—Mn—N</td>
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<td>70.94(7)</td>
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<td>72.43(3)</td>
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<tr>
<td>O—Mn—O</td>
<td>85.79(3)</td>
<td>106.38(7)</td>
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</tbody>
</table>

³Numbers in parentheses indicate values of uncertainty.
while that of Mn(H₂DOTA) binds through acetate pendant arms, the configurations of the metal centers are quite similar.

6.4 [Mn(DO2AM)(H₂O)](Cl₁.₃₄)(Br₀.₆₆)·₂H₂O

The Mn(II) complex of DO2AM has the crystallographic formula of [Mn(DO2AM)(H₂O)](Cl₁.₃₄)(Br₀.₆₆)·₂H₂O. The metal center binds hexadentate to the ligand, and has a bound inner-sphere water molecule. There are two halide counter ions with mixed occupancy per MnDO2AM unit in order to balance out the (2+) charge. The source of manganese was MnCl₂, while the ligand was the hydrobromide salt, leading to both halides crystallizing with the complex. There has previously been reported a mixed halide crystal structure of [Co(DOTAM)]²⁺, with mixed occupancy Cl⁻ and Br⁻ counter ions.⁶

There is considerably less hydrogen bonding present within the MnDO2AM crystal lattice compared to the MnDO2A2AM lattice. This can be rationalized due to the lack of unbound pendant arms in MnDO2AM. Additionally, unlike MnDO2A2AM,

**Figure 6.4.** Two views of the crystal structure for [Mn(DO2AM)]. Figure 6.4a, left, shows the metal-ligand complex with hydrogen atoms. The coordination environment for the metal center is shown in figure 6.4b, right, along with the labeling scheme. Only amide protons are shown in figure 6.4b for clarity. Black spheres represent protons; Grey ellipsoids represent carbon; Purple represent nitrogen; Red represent oxygen; Blue represent manganese. Ellipsoids are shown at 90% probability.
the MnDO2AM crystal does not contain an NH$_4^+$ ion. Much of the hydrogen bonding that occurs within the MnDO2A2AM lattice is due to the acetate pendant arms and the NH$_4^+$. There is inter-complex hydrogen bonding between amide arms on different complexes.

Two views of the structure including the labeling scheme for [Mn(DO2AM)(H$_2$O)]$^{2+}$ are shown in Figure 6.4. The Mn(II) metal center bonds to the DO2AM ligand at six sites. There is also an inner-sphere water bound to the manganese(II), thus, the metal center is heptadentate. Due to this bound water, Mn(DO2AM) has the oxygen atoms of the amide pendant arms cis to the N$_1$—Mn—N$_3$ plane. Both coordinating amide pendant arms are on the same side of the macrocyclic ring unit, adopting a syn conformation. All of the reported crystal structures of manganese(II) complexes with pendant arm cyclen derivatives adopt the syn configuration.$^{3,7}$

The crystal structure for MnDO2AM$^{2+}$ is quite similar to that of the MnDO3AM crystal, replacing the bound water, in MnDO2AM$^{2+}$, with a third amide pendant arm, in MnDO3AM$^{2+}$. While Mn(II) metal center in MnDO3AM is a distorted capped trigonal prism,$^3$ the MnDO2AM$^{2+}$ complex can best be described as a distorted pentagonal bipyramid, with O$_3$ and N$_4$ as the axial ligands. Other pentagonal bipyramidal Mn(II) complexes have previously been reported.$^8$ The majority of these other pentagonal bipyramidal complexes generally consist of the Mn(II) metal center binding to a ligand in an equatorial pentadentate manner with two ligands (water or Cl$^-$) occupying the axial positions.

Because the two amides of MnDO2AM$^{2+}$ are in different positions only the five-membered rings created by the Mn(II) metal center binding to the cyclen ring have
absolute configurations. The absolute configuration for the [Mn(DO2AM)]^{2+} shown is \((\lambda\lambda\lambda\lambda\lambda)\). The other \([(\text{Mn(DO2AM)})^{2+}\) molecule present within the unit cell has the absolute configuration of \((\delta\delta\delta\delta\delta)\) due to the \(\text{P}\bar{1}\) space group of the crystal. Like Mn(DO2A2AM), in the solution state \([(\text{MnDO2AM})^{2+}\) has the potential to invert its configuration, however due to the paramagnetic nature of the complex probing this inversion cannot be done with simple NMR techniques.

The distances between the metal center and the ligand are given in Table 6.2, along with selected bond angles. The conformation of the amide arms pulls the Mn(II) away from the nitrogen atoms adjacent to the amide pendant arms. Thus, there are sets of distinct Mn-N bond lengths, with an average of 2.42 Å and 2.28 Å. Despite the difference in Mn-N bond length, the angle formed between the metal center and any two adjacent nitrogen atoms is relatively constant, at around 75°. The three Mn-O bonds are similar in length (~2.22 Å) and are shorter than the average bond length for MnDOTAM (~2.31 Å). This is most likely because MnDOTAM is eight-coordinate. The bonds are necessarily longer in order to reduce the steric crowding around the metal center. There are three distinct Mn-O bond lengths of 2.162(4), 2.238(3), and 2.297(4) for \([\text{Mn(DO3AM)}]^{2+}\). The three Mn-O bond lengths for MnDO2AM are much more uniform with the bond between Mn(II) and the two amide pendant arms at 2.2107(8) and 2.2225(8), and the Mn(II)-water bond length at 2.2347(9).
References:

Chapter 7: $^1$H Longitudinal $r_1$ and $^{17}$O Transverse $r_2$ Measurements

7.1 $^1$H Relaxivity of Mn(II) complexes of cyclen derivatives

Due to their previous use as potential contrast agents in binding lanthanide metals, the relaxivities of a variety of Mn(II) complexes of pendant arm cyclen derivatives have been previously reported. Many of these complexes exhibit relatively low $r_1$ values due to their lack of an inner sphere water molecule binding to the metal center of the complex, see Table 7.1. Indeed, only one such cyclen derived Mn(II) complex, [Mn(DO1A)]$^+$, contains a water that is consistently bound to the metal center in the solution state. It has been previously reported that [Mn(1,4-DO2A)] has 0<$q$<1 while in the solution state. Such an intermediate q value indicates an equilibrium between $q$=0 and $q$=1 at a pH value at which the complex should be fully formed. This analysis was solely based upon the trend of $^1$H relaxivity values for such complexes. Since the publication of that study an efficient method for the determination of hydration state has been proposed (see Sections 1.4 and 7.6).

<table>
<thead>
<tr>
<th>Complex</th>
<th>$r_1$ (mM$^{-1}$sec$^{-1}$)</th>
<th>q (bound waters)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Mn(1,7-DO2A)]</td>
<td>1.3 (37°C)</td>
<td>0</td>
<td>This work</td>
</tr>
<tr>
<td>[Mn(1,7-DO2AM)]$^{2+}$</td>
<td>1.7 (37°C)</td>
<td>0&lt;$q$&lt;1</td>
<td>This work</td>
</tr>
<tr>
<td>[(MnDO2A2AM)]$^+$</td>
<td>1.5 (37°C)</td>
<td>0</td>
<td>This work</td>
</tr>
<tr>
<td>[Mn(DOTA)]$^{2+}$</td>
<td>1.1 (37°C)</td>
<td>0</td>
<td>[2]</td>
</tr>
<tr>
<td>[Mn(DOTAM)]$^{2+}$</td>
<td>0.9 (37°C)</td>
<td>0</td>
<td>[2]</td>
</tr>
<tr>
<td>[Mn(1,4-DO2A)]</td>
<td>2.1 (25°C)</td>
<td>0&lt;$q$&lt;1</td>
<td>[3]</td>
</tr>
<tr>
<td>[Mn(1,7-DO2A)]</td>
<td>1.5 (25°C)</td>
<td>0</td>
<td>[3]</td>
</tr>
<tr>
<td>[Mn(DO3A)]$^+$</td>
<td>1.3 (25°C)</td>
<td>0</td>
<td>[3]</td>
</tr>
<tr>
<td>[Mn(DO1A)]$^+$</td>
<td>2.4 (25°C)</td>
<td>1</td>
<td>[3]</td>
</tr>
<tr>
<td>[Mn(EDTA)]$^{2-}$</td>
<td>3.3 (25°C)</td>
<td>1</td>
<td>[3]</td>
</tr>
<tr>
<td>[Mn(12-pyN₄A)]$^+$</td>
<td>2.4 (25°C)</td>
<td>1</td>
<td>[6]</td>
</tr>
<tr>
<td>[Mn(PyC3A)]$^+$</td>
<td>2.1 (37°C)</td>
<td>1</td>
<td>[7]</td>
</tr>
</tbody>
</table>
The pH dependent relaxivity profiles for \([\text{Mn(H}_x\text{DOTA)}]^{(X-2)+}\) and \([\text{Mn(DOTAM)}]^{2+}\) at 37°C are given in Figures 7.1 and 7.2, respectively. For \([\text{Mn(H}_x\text{DOTA)}]^{(X-2)+}\) at low pH values (<2) the only manganese(II) containing species is \([\text{Mn(H}_2\text{O)}_6]^{2+}\), which has an \(r_1\) value of ~7.1. As the pH is increased, the Mn(II) binds to the DOTA ligand and the relaxivity drops until all of the manganese(II) is fully complexed to the ligand. \([\text{Mn(DOTA)}]^{2+}\), the only species present at physiological pH, has an \(r_1\) value of 1.1 mM\(^{-1}\)sec\(^{-1}\). This is a relatively low relaxivity value compared to the majority of other pendant arm cyclen derivatives, see Table 7.1 above. \([\text{Mn(H}_x\text{DOTA)}]^{(X-2)+}\) has no bound waters (q=0) and so the only mechanism that the complex has for relaxation is the outer sphere mechanism which is not as effective at relaxing proton spins as the inner sphere water exchange mechanism.

![Figure 7.1. pH dependent relaxivity profile for \([\text{Mn(H}_x\text{DOTA)}]^{(X-2)+}\) at 20 MHz, 37°C. The \(r_1\) value at physiological pH is 1.1 mM\(^{-1}\)sec\(^{-1}\). Error Bars are shown in black. Adapted from [Ref 2].](image)
The pH dependent relaxivity profile for [Mn(DOTAM)]^{2+} is quite similar to that of [Mn(H_2DOTA)]^{X-2+}, however, that of [Mn(DOTAM)]^{2+} is shifted toward a lower pH by a considerable margin. The Mn(II) is completely decomplexed from the ligand below a pH of 1, and [Mn(H_2O)_6]^{2+} is the only manganese(II) containing species, with a relaxivity of 7.1 mM^{-1}\text{sec}^{-1}. As the pH is raised the complex begins to form and above a pH of 3.5, all of the Mn(II) has bound to the DOTAM ligand. [Mn(DOTAM)]^{2+} has a relaxivity value of 0.9 mM^{-1}\text{sec}^{-1} at physiological pH, which is the lowest of any of the Mn(II) cyclen derivatives reported herein. Neither the [Mn(DOTA)]^{2-} nor [Mn(DOTAM)]^{2+} species contain a bound inner sphere water. The difference in relaxivity values for these two species, therefore, are completely due to outer sphere relaxation mechanisms. This difference in relaxivity is due to the switch between acetate pendant arms and amide pendant arms from the DOTA ligand to the DOTAM ligand.

**Figure 7.2.** pH dependent relaxivity profile for [Mn(DOTAM)]^{2+} at 20 MHz, 37°C. The $r_1$ value at physiological pH is 0.9 mM^{-1}\text{sec}^{-1}. Error Bars are shown in black. Adapted from [Ref 2].
7.2 $^1$H Relaxivity measurements and profile for $[\text{Mn}(1,7-\text{H}_x\text{DO2A})]^{\text{X}^+}$

A 3mM stock solution of $[\text{Mn(DO2A)}]$ was obtained by dissolving DO2A•4HCl (0.0326 g; 0.075 mmol) and MnCl$_2$ (0.0094 g; 0.075 mmol) into 25 mL of dd DI water. Each sample used 1 mL of the stock solution and was ~3 mL in total, making each sample 1 mM in concentration. The pH of each sample was adjusted using either NH$_4$OH (to increase the pH) or trifluoro acetic acid (TFA) (to decrease the pH). Each sample was allowed to reach thermal equilibrium in a water bath set to 37°C for at least fifteen minutes prior to data collection. The pH of each sample was determined immediately preceding and immediately following the data collection in order to ensure that the sample had reached equilibrium.

![Figure 7.3. pH dependent relaxivity profile for $[\text{Mn}(1,7-\text{H}_x\text{DO2A})]^{\text{X}^+}$ at 37°C. The $r_1$ value at physiological pH is 1.3 mM$^{-1}$sec$^{-1}$. Error Bars are shown in black.](image)

The pH dependent relaxivity profile for $[\text{Mn}(1,7-\text{H}_x\text{DO2A})]^{\text{X}^+}$ at 37°C is given in Figure 7.3, above. At low pH values (1-4), the Mn$^{2+}$ is completely decomplexed from the ligand. The contribution to relaxivity is only from $[\text{Mn(H}_2\text{O)}_6]^{2+}$, which has an $r_1$ of...
7.1±0.1 mM⁻¹sec⁻¹. As the pH is increased above a value of 4, the MnDO2A complex begins to form and the relaxivity drops. Above a pH of 6 the complex is fully formed and no more free [Mn(H₂O)₆]²⁺ is in solution (see Chapter 4). At a pH of 7 (physiological pH) the relaxivity value is 1.34 mM⁻¹sec⁻¹. The relaxivity does not change as the pH is further increased, however, at pH values above 9 the Mn(II) falls out of the ring and precipitates from solution as Mn(OH)₂. This low of an r₁ value is indicative of a complex containing no bound waters in the solution state.³ This is consistent with data from the temperature dependent ¹⁷O T₂ study (see below). Because there are no inner-sphere waters bound to the metal center the relaxivity of the complex is dominated by outer-sphere and prototropic exchange of the acetate pendant arm protons (see above).

A pH dependent study of the ¹H relaxivity of [Mn(1,7-H₂DO2A)]³⁺ has previously been reported,³ however, that study was conducted at 25°C. At lower temperatures ¹H relaxivity values are higher. Indeed, at 25°C for Mn(DO2A) r₁=1.5 mM⁻¹sec⁻¹.³ The r₁ value for [Mn(DO3A)]⁻ was also reported in the same study.³ This value of 1.3 mM⁻¹sec⁻¹ is even lower than that of Mn(DO2A). Additionally, the relaxivity for [Mn(DOTA)]²⁻ has previously been reported (see above).² At 1.1 mM⁻¹sec⁻¹ the relaxivity is the lowest out of all the acetate pendant arm cyclen derivatives, however, the data were obtained at 37°C, so at 25°C it could be more comparable to that of MnDO3A. As the q value is constant across this series of acetate pendant arm derivatives the differences in the relaxivity for their Mn(II) complexes are accorded to outer sphere relaxation mechanisms. Two Mn(II) complexes of acetate containing cyclen derivatives do contain inner sphere water molecules bound to the Mn(II) metal center. [Mn(DO1A)(H₂O)]⁺ and [Mn(1,4-DO2A)(H₂O)] have markedly higher relaxivity
values, 2.4 mM$^{-1}$sec$^{-1}$ and 2.1 mM$^{-1}$sec$^{-1}$ respectively.$^3$ There are likely small changes in the relaxivities of these complexes as compared to those already discussed which are due to changes in the outer sphere contribution to relaxivity and small differences in the water exchange rate. The largest change, however, is due to the additional mechanism of relaxation that the bound water molecule offers to the Mn(II) complex. Because Mn(II) has a relatively fast water exchange rate ($k_{ex} = 2.1 \times 10^7$ sec$^{-1}$),$^8$ this is an efficient mechanism for relaxation.

7.3 Relaxivity profile for [Mn(DO2AM)(H$_2$O)]$^{2+}$

The pH dependent relaxivity profile for [Mn(DO2AM)(H$_2$O)]$^{2+}$ at 37°C is given below, in Figure 7.4. At low pH values the $r_i$ is consistently ~ 7.2 mM$^{-1}$sec$^{-1}$. According to the speciation diagram (see chapter 4) the only manganese(II) containing species is [Mn(H$_2$O)$_6$]$^{2+}$. The manganese(II) does not become complexed to the DO2AM ligand until the pH is raised above five. At a pH of 6.5 the Mn(II) is fully complexed and [Mn(DO2AM)(H$_2$O)]$^{2+}$ is the only species. The relaxivity for [Mn(DO2AM)(H$_2$O)]$^{2+}$ at physiological pH (7.22) is 1.7 mM$^{-1}$sec$^{-1}$. In the solid state, Mn(DO2AM)$^{2+}$ has an inner sphere water bound to the metal center. There is also evidence from an $^{17}$O temperature dependent study (see below) that in the solution state the water remains bound to the metal center. This relaxivity value is rather low for complexes with $q=1$, see Table 7.1 above.

[Mn(1,4-DO2A)(H$_2$O)] is the other di-substituted cyclen derivative with a bound inner sphere water molecule, with an $r_i=1.7$ mM$^{-1}$sec$^{-1}$ at 37°C based upon the NMRD (Nuclear Magnetic Resonance Dispersion) curve.$^3$ The structure of [Mn(DO2AM)(H$_2$O)]$^{2+}$ is similar to that of [Mn(1,4-DO2A)(H$_2$O)] (see chapter 5), with
the only difference being the pendant arm substituents. In this case, there is no
difference between the relaxivity values for two complexes with different pendant
arms. Most likely this is due to the additional relaxation mechanism that these two
complexes can go through—inner sphere water exchange—which tends to dominate.

![Image]

**Figure 7.4.** pH dependent relaxivity profile for [Mn(1,7-DO2AM)]²⁺ at 37°C. The $r_1$ value
at physiological pH is $1.7 \text{mM}^{-1}\text{sec}^{-1}$. Error Bars are shown in black.

7.4 Relaxivity profile for [Mn(HₓDO2A2AM)]³⁺

Figure 7.5, below, gives the pH dependent $^1$H relaxivity profile for
[Mn(HₓDO2A2AM)]³⁺ at 37°C. The relaxivity profile for MnDO2A2AM looks quite
similar to that of MnDOTA. Below a pH of 2, [Mn(H₂O)₆]²⁺ is the only species that
contains Mn(II). The relaxivity at this pH is $7.1 \text{mM}^{-1}\text{sec}^{-1}$, which is consistent with data
from the other macrocyclic complexes at low pH values. Similarly to [Mn(HₓDOTA)]¹⁻⁻³⁺,²⁺ as the pH is raised the Mn(II) begins to bind to the ligand, until at above a pH of 4
the Mn(II) is fully bound to the ligand and the [Mn(DO2A2AM)] complex is formed.
These data are consistent with the speciation diagram for \([\text{Mn}(\text{H}_x\text{DO2A2AM})]^x^+\), see above. At physiological pH the \(r_1 = 1.5 \text{ mM}^{-1}\text{sec}^{-1}\), which is significantly higher than that of Mn(DOTA) at a similar pH value. In fact, Mn(DO2A2AM) has the highest \(^1\text{H}\) relaxivity value of all the cyclen derivatives that do not have inner sphere water molecules.

7.5 Temperature Dependent \(^{17}\text{O} T_2\) Determination of Hydration State

Figure 7.6 gives the temperature dependent \(^{17}\text{O} r_2\) curves for \([\text{Mn}(1,7-\text{H}_x\text{DO2A})]^x^+\) at two different pD values, 6.72 and 2.87, as well as the curve\(^8\) for \([\text{Mn}(\text{H}_2\text{O})_6]^{2+}_{(aq)}\). The pD values were chosen such that \([\text{Mn}(1,7-\text{H}_x\text{DO2A})]^x^+\) would either be fully formed, at high pD, or fully decomplexed, at low pD, according to its
speciation diagram (see Chapter 4). At low pD, the only Mn(II) containing species is 
\[ \text{Mn}(\text{H}_2\text{O})_6^{2+}(\text{aq}) \]. This reflected quite well by the $^{17}\text{O}$ curve, which matches that of the 
sample containing only Mn$^{2+}$. The $r_2^{\text{max}}$ for this curve is $3043\pm225 \text{ mM}^{-1}\text{sec}^{-1}$, which

corresponds to $q=5.97\pm0.44$. This is exactly the expected hydration number for free
Mn$^{2+}$ ($q=6$) within experimental error. At high pD, the $r_2$ curve over the temperature
range does not resemble any of those depicted in Figure 1.4. It most resembles the
line that occurs for $q=0$ complexes. Indeed, the average $r_2$ value is $178 \text{ mM}^{-1}\text{sec}^{-1}$,
which corresponds to $q=0$. A Mn(DO2A) complex with no bound waters agrees with
previous results, and with the low $r_1$ value that it exhibits (see above).

Figure 7.6. Temperature Dependent $^{17}\text{O}$ Study for $[\text{Mn}(\text{H}_2\text{O})_{\text{X}}\text{DO2A})]^{X+}$.
The temperature dependent $^{17}\text{O}$ relaxivity curve for $[\text{Mn}(\text{H}_2\text{O})_6]^{2+}(\text{aq})$ (red circles) is included for reference.
The blue squares represent data acquired at a lower pH where the Mn(II) has completely
decomplexed from the ligand (pD=2.87. The green triangles represent data acquired at a
pD of 6.72.
Figure 7.7 gives the temperature dependent $^{17}$O $r_2$ curves for [Mn(1,7-HDO2AM)]$^{2+}$ at two different pH values, 7.10 and 3.23, as well as the curve for [Mn(H$_2$O)$_6$]$^{2+}$(aq). The curve for the complex at low pH is, again, similar to that of the free Mn$^{2+}$ species. The $r_2^{\text{max}}=2887\pm193$ mM$^{-1}$sec$^{-1}$, which corresponds to $q=5.66\pm0.38$. This value is lower than the expected hydration state, however, it still falls within error of the value for free Mn$^{2+}$. At a pH at which the complex is fully formed (7.10), the

![Temperature Dependent $^{17}$O Study for [Mn(DO2AM)]$^{2+}$](image)

Figure 7.7. Temperature Dependent $^{17}$O Study for [Mn(DO2AM)]$^{2+}$. The temperature dependent $^{17}$O relaxivity curve for [Mn(H$_2$O)$_6$]$^{2+}$(aq) (red circles) is included for reference. The blue squares represent data acquired at a lower pH where the Mn(II) has completely decomplexed from the ligand (pH=3.23. The green triangles represent data acquired at a pH of 7.10.

curve resembles that of species with $q=1$ quite well, with an $r_2^{\text{max}}=290\pm21$ mM$^{-1}$sec$^{-1}$ (see Figure 1.4). While this only corresponds to $q=0.57\pm0.04$, this study did not go as
low in temperature as that of the curve depicted in Figure 1.4. These data indicate that Mn(DO2AM)$^{2+}$ does, in fact, have a water bound to the metal center, however, they are inconclusive as to whether 0<q<1. Based upon the $r_1$, it is likely that there is an equilibrium between q=0 and q=1, similar to that of [Mn(1,4-DO2A)]. This in good agreement with both the H$^+$ relaxivity data, and the crystal structure (see above).

![Temperature Dependent $^{17}$O Study for [Mn(H$_x$DO2A2AM)]$^{X^+}$](image)

**Figure 7.8.** Temperature Dependent $^{17}$O Study for [Mn(H$_x$DO2A2AM)]$^{X^+}$. The temperature dependent $^{17}$O relaxivity curve for [Mn(H$_2$O)$_6$]$^{2+}$ (red circles) is included for reference. The blue squares represent data acquired at a lower pH where the Mn(II) has completely decomplexed from the ligand (pD=2.42). The green triangles represent data acquired at a pH of 8.05.

The temperature dependent $^{17}$O $r_2$ curves for [Mn(H$_x$DO2A2AM)]$^{X^+}$ are given in Figure 7.8, above. The pD values were chosen such that the complex would either
be fully formed at high pD (8.05), or fully decomplexed at low pD (2.42). The low pD curve matches that of \([\text{Mn(H}_2\text{O)}_6]^{2+}\)\text{(aq)}, though the \(r^2_{\text{max}}\) value is higher (3223±322 mM\(^{-1}\text{sec}^{-1}\)). This value corresponds to \(q=6.32±0.63\), which is noticeably higher than that of free Mn(II), however, this q value still falls within the range of experimental error for free manganese. At high pD (8.05), the Mn(II) has fully complexed to the ligand and no free metal is present in solution (see Section 4.7). The temperature dependent curve at this pD closely resembles that of a q=0 complex. While the \(r^2_{\text{max}}\) is not equal to zero \((r^2_{\text{max}}=136±10\ \text{mM}^{-1}\text{sec}^{-1})\), this value is negligible and there is little change in the overall \(r_2\) as the temperature is varied. These results are in agreement with the crystal structure, as in the solid state there are no inner sphere water molecules bound to the manganese(II) metal center.
References:
Chapter 8: Pyridine Derived Contrast Agents

8.1 Pyridine Derived CAs

Recently, there have been a number of studies\textsuperscript{1,2,3,4} concerning potential CAs containing pyridine derivatives. The majority of these ligands contain picolinate groups and all of them are derived from amine backbones (see Scheme 8.1).

![Scheme 8.1](image-url)
While these pyridine derived CAs tend to have relatively high relaxivities, mostly due to the number of inner sphere water molecules bound to the metal center, their stabilities are lower than desired, often falling between \(\log K_{\text{MnL}}=8\text{-}10\) (see Table 8.1). Two of these complexes stand out, \([\text{Mn(PyC3A)(H}_2\text{O)}]^{\text{2-}}\) and \([\text{Mn(DPAAA)(H}_2\text{O)}]^{\text{2-}}\). The two complexes have \(\log K_{\text{MnL}}\) values of 14.14 and 13.19, respectively. In addition, due to the bound inner sphere water present, they have relatively high relaxivity values, 2.1 mM\(^{-1}\)sec\(^{-1}\) and 2.7 mM\(^{-1}\)sec\(^{-1}\), respectively. Both of these complexes contain Mn(II) metal centers that coordinate to the corresponding ligand in a hexa-coordinate manner. Additionally, they both contain one inner sphere water molecule bound to the metal center. Because of this bound water, these pyridine containing complexes have a higher potential as contrast agents than the cyclen derivatives previously discussed.

### Table 8.1. Relaxivities and Binding Constants for a Series of Manganese(II) Complexes of Pyridine Derived ligands

<table>
<thead>
<tr>
<th>Mn(^{2+}) Complex of:</th>
<th>(n) (mM(^{-1}) sec(^{-1}))</th>
<th>(\log K_{\text{MnL}}) (25°C)</th>
<th>q</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-PyN(_3)O(_2)</td>
<td>3.6 (25°C)</td>
<td>7.18</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>15-PyN(_5)</td>
<td>3.1 (25°C)</td>
<td>10.89</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>DPAAA(^3-)</td>
<td>2.7 (37°C)</td>
<td>13.19</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>DPAPhA(^2-)</td>
<td>5.1 (37°C)</td>
<td>9.55</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>DPAMeA(^2-)</td>
<td>4.2 (37°C)</td>
<td>10.13</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>BCPE(^2-)</td>
<td>1.2 (37°C)</td>
<td>10.63</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>PyC3A</td>
<td>2.1 (37°C)</td>
<td>14.14\pm0.01</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

respectively. In addition, due to the bound inner sphere water present, they have relatively high relaxivity values, 2.1 mM\(^{-1}\)sec\(^{-1}\) and 2.7 mM\(^{-1}\)sec\(^{-1}\), respectively. Both of these complexes contain Mn(II) metal centers that coordinate to the corresponding ligand in a hexa-coordinate manner. Additionally, they both contain one inner sphere water molecule bound to the metal center. Because of this bound water, these pyridine containing complexes have a higher potential as contrast agents than the cyclen derivatives previously discussed.

### 8.1a TPADA and TPAMA

Two ligands—6,6’-(pyridin-2-ylmethylazanediyl)bis(methylene)dipicolinic acid (TPADA)\(^5\) and 6-(dipyridin-2-ylmethylazanediyl)-(methylene)picolinic acid (TPAMA)\(^6\)—that are of interest for their potential as contrast agents when bound to Mn(II) are shown below in Scheme 8.2. A crystal structure, shown in Figure 8.1 below,
for the manganese(II) TPADA complex has previously been published. The ligand binds to the Mn(II) hexadentate, and the two deprotonated carboxylic acid substituents act as bridging ligands between the manganese and a hydrated calcium ion. Due to their role as bridging ligands, the negative charges are shared between the manganese and the calcium. The resulting positive charge on the MnTPADA complex is balanced by a chloride counter-ion which binds directly to the Mn(II) metal center, leaving the whole calcium/manganese complex with an overall positive charge. If the MnTPADA complex was to be crystallized without the hydrated calcium ion, the chloride counter-ion would no longer be necessary and there would be the potential for an inner-sphere water bound to the manganese metal center. Such a complex would be quite similar to the two promising Mn(II) based CAs discussed above.

Scheme 8.2

The TPAMA ligand has the potential to bind to a Mn(II) metal center in a pentadentate manner. Though it is quite difficult to predict the hydration number of a Mn(II) complex based solely upon the ligand configuration, TPAMA has the potential for two open coordination sites in the manganese that could be filled with inner-sphere
water molecules. Other penta-dentate pyridine complexes previously reported contain two inner sphere water molecules (see Table 8.1). These complexes exhibit very large relaxivity values ($4.2 \text{ mM}^{-1} \text{ sec}^{-1}$ and $5.1 \text{ mM}^{-1} \text{ sec}^{-1}$), which is quite desirable but they have low thermodynamic stabilities ($\log K_{MnL} \approx 10$) causing them to be discarded as potential CAs.

### 8.1b Hammett Parameters

A useful tool for probing electronic effects within aromatic rings is the Hammett Parameter. By placing a substituent meta or para to the nitrogen of a pyridine ring, the electronic effects of the pyridine ring can be changed. A recent study\(^8\) synthesized a variety of meta and para substituted pyridinamine derivatives for incorporation into potential positron emission tomography (PET) Cu\(^{2+}\) complexes. By varying the substituent in the para position of the pyridine ring, the $pK_a$ of the pyridine ring was modified. The Cu(II) complexes containing para-substituted $\alpha$-methoxy pyridine rings were found to be the most thermodynamically stable when compared to other para-substituted complexes.
The Hammett Parameter is designated by the variable $\sigma$. The sign and magnitude of the Hammett parameter are based on Reaction 8.1 (the deprotonation of benzoic acid):$^9$

**Reaction 8.1.**

\[
\begin{align*}
\text{Benzoi acid} + \text{H}_2\text{O} & \rightleftharpoons K_X \text{Benzoate ion} + \text{H}_3\text{O}^+ \\
\end{align*}
\]

The $\sigma$ value is determined according to Equation 8.1, below.

\[
\log \left( \frac{K_X}{K_H} \right) = \sigma_X \quad 8.1
\]

<table>
<thead>
<tr>
<th>Substituent</th>
<th>Hammett $\sigma$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>-H</td>
<td>0</td>
</tr>
<tr>
<td>-NO$_2$</td>
<td>0.778</td>
</tr>
<tr>
<td>-Cl</td>
<td>0.227</td>
</tr>
<tr>
<td>-OMe</td>
<td>-0.268</td>
</tr>
<tr>
<td>-NH$_2$</td>
<td>-0.66</td>
</tr>
</tbody>
</table>

Table 8.2 gives some of the Hammett parameters for a para-substituted aromatic ring. Electron withdrawing groups (EWGs) have a positive $\sigma$ value, while electron donating groups (EDGs) have a negative $\sigma$ value. Higher $\sigma$ values correspond to lower $pK_a$ values (i.e. the pyridine ring becomes more acidic), while lower $\sigma$ values correspond to the opposite. Adding a para substituent on one of the
pyridine rings will have an effect on both the stability, by modifying the pKₐ of the pyridine ring, and the relaxivity, by potentially modifying the water exchange rate, of a ligand-Mn(II) complex. By studying the effects of changing these aromatic substituents, both the stability and the relaxivity of Mn(TPADA) and Mn(TPAMA) can be optimized in order to synthesize a better potential CA.

8.2 Synthesis

TPADA and TPAMA

The syntheses of TPADA⁵ and TPAMA⁶ have been previously reported. Some of the steps in the syntheses were modified in order to increase yield. The first step in the synthesis used thionyl chloride to create an in situ pyridine diacyl chloride, which quickly reacted to form dimethyl pyridine dicarboxylate (9).¹¹ The second step reduced one of the carboxylate groups to a secondary alcohol with the addition of the weak reducing agent sodium borohydrdride to yield (10).¹² The secondary alcohol was then converted to a bromide upon treatment with phosphorous tribromide to yield (11).¹² MeTPADA (14) and MeTPAMA (15) were synthesized⁵ using a nucleophilic substitution by adding the brominated methyl picolinate building block (11) to either 2-picolylamine (12) or di-(2-picolyl)amine (13), which were purchased from commercial sources. The methyl esters, (14) and (15), were hydrolyzed¹³ by heating the respective compound to a reflux in 6M HCl(aq) to yield the corresponding carboxylic acid derivative, TPADA (16) and TPAMA (17).

Dimethyl-2,6-pyridine-dicarboxylate (9)
2,6-Pyridine dicarboxylic acid (8) (3.1657 g; 18.9 mmol) was dissolved into 80 mL of MeOH. At 0°C, SOCl₂ (25 mL; 0.345 mol; 18.25 equiv.) was added dropwise. Upon complete addition, the solution was heated to a reflux at 80°C for six hours. After cooling to room temperature, the solvent was removed in vacuo. The resulting solid was quenched with 40mL of a saturated NaHCO₃. The aqueous layer was extracted with EtOAc (5x40 mL). The organic layers were combined and dried over Na₂SO₄. The solvent was removed to yield the product as an off-white powder (3.6042 g; 18.5 mmol). Yield: 97.9% 

\[
\text{1H NMR (300 MHz, CDCl₃): } \delta 8.32 \text{ (d, 2H, } H_{\text{pyr}}), 8.03 \text{ (t, 1H, } H_{\text{pyr}}), 4.04 \text{ (s, 6H, COOCH₃)}
\]

Scheme 8.3

Methyl 6-(hydroxymethyl)picolinate (10)

Dimethyl-2,6-pyridine-dicarboxylate (9) (3.6919 g; 18.9 mmol) was dissolved into 50 mL MeOH. At 0°C, NaBH₄ (0.9510 g; 25.1 mmol; 1.3 equiv.) was added portion
wise to the stirring mixture. The reaction was stirred at ambient temperature for five hours before being quenched with 40 mL of a saturated NH₄Cl solution. The aqueous layer was extracted with CHCl₃ (3x40mL). The crude product was purified via silica gel column chromatography (1:1 EtOAc:hexanes, then 2:1 EtOAc:Hexanes). The fractions containing the product were combined and the solvent was removed in vacuo to yield the pure product as a white powder (2.3876 g; 15.1 mmol). Yield: 80.0% ¹H NMR (400 MHz, CDCl₃): δ 8.02 (d, 1H, Hₚ), 7.84 (t, 1H, Hₚ), 7.53 (d, 1H, Hₚ), 4.86 (s, 2H, CH₂OH), 3.98 (s, 3H, COOC₃H₇)

*Methyl 6-(bromomethyl)picolinate (11)*

Methyl 6-(hydroxymethyl)picolinate (10) (1.3836 g; 8.7 mmol) was dissolved into 35 mL DCM. At 0°C, PBr₃ (1.081 mL; 3.082 g; 11.4 mmol; 1.3 equiv) dissolved in DCM (3 mL) was added dropwise. The reaction mixture was stirred at ambient temperature for five hours whereupon 40 mL of a saturated K₂CO₃ solution was added to quench the reaction. Chloroform (100 mL) was added and the organic layer was separated, dried over Na₂SO₄, and removed under vacuum to yield the pure product as an off-white solid (1.5785 g; 7.2 mmol). Yield: 81.9% ¹H NMR (400 MHz, CDCl₃): δ 8.06 (d, 1H, Hₚ), 7.86 (t, 1H, Hₚ), 7.68 (dd, 1H, Hₚ), 4.64 (s, 2H, CH₂Br), 4.01 (s, 3H, COOC₃H₇)

*Dimethyl 6,6'-(pyridine-2-ylmethylazanediyl)bis-(methylene)dipicolinate [MeTPADA] (14)*

Methyl 6-(bromomethyl)picolinate (11) (1.5335 g; 6.97 mmol) and K₂CO₃ (1.0013 g; 7.26 mmol) were dissolved into 30 mL dry acetonitrile. To this solution
was added 2-picolyamine (0.2886 mL; 0.3027 g; 2.80 mmol) dissolved into 3 mL MeCN. The reaction was heated to 70°C for 18 hours. After cooling to ambient temperature, the mixture was filtered over Celite to remove excess salt. The solvent was removed and the resulting orange oil was dissolved into 20 mL DCM. The organic phase was washed with water (40 mL) and brine (2x40 mL) and dried over Na₂SO₄. The solvent was removed via roto-vap to yield the product as a dark red oil (0.8208 g; 2.02 mmol). Yield: 72.1% ¹H NMR (300 MHz, CDCl₃): δ 8.48 (dt, 1H, Hpyr), 7.94 (dd, 2H, Hpyr), 7.82 (dd, 2H, Hpyr), 7.77 (t, 2H, Hpyr), 7.60 (td, 1H, Hpyr), 7.49 (d, 1H, Hpyr), 7.10 (m, 1H, Hpyr), 3.97 (s, 4H, CH₂), 3.93 (s, 6H, COOCH₃), 3.84 (s, 2H, CH₂)

Scheme 8.4

Scheme 8.4. (i) 2 equiv. K₂CO₃, MeCN, 70°C, 18 hrs.

6,6’-(Pyridin-2-ylmethylazanediy)bis(methyl-ene)dipicolinc acid (TPADA) (15)

MeTPADA (0.8208 g; 2.02 mmol) was dissolved into 25 mL 6M HCl. This solution was heated to a reflux for 12 hours. After being cooled to ambient temperature, the solvent was removed under reduced pressure. The resulting oil was dissolved in MeOH (2x5 mL), which was then removed via roto-vap to yield the product as a grey solid (1.0206 g; 1.84 mmol). Yield: 91.2%. Elemental analysis: C₂₀N₄O₄H₁₈•3HCl•H₂O. Found: C:48.08%. H:4.71%. 10.73%. Calc: C:47.70%.
H: 4.57%. N: 11.10% ¹H NMR (400 MHz, D₂O): δ 8.70 (d, 1H, Hpyr), 8.32 (t, 1H, Hpyr), 8.02 (t, 2H, Hpyr), 7.91 (t, 2H, Hpyr), 7.82 (d, 1H, Hpyr), 7.77 (t, 1H, Hpyr), 7.63 (d, 2H, Hpyr), 4.42 (s, 2H, CH₂), 4.25 (s, 4H, CH₂) EMI-MS (50:50 MeOH:H₂O, m/z +): 379.93 (H₃TPADA⁺), 401.07 (NaH₂TPADA⁺)

**Figure 8.2.** ¹H NMR spectrum of TPADA. The spectrum was taken in D₂O (4.8 ppm).

*Methyl 6-(Dipyridin-2-ylmethylazanediyl)-(methylene)picolinate (MeTPAMA) (16)*

Methyl 6-(Bromomethyl)picolinate (0.5757 g; 2.1 mmol) and K₂CO₃ (0.2183 g; 2.1 mmol) were dissolved into 30 mL dry acetonitrile. To this solution was added di-(2-picolyl)amine (0.378 mL; 0.4179 g; 2.1 mmol) dissolved into 3 mL MeCN. The reaction was heated to 70°C for 18 hours. After cooling to ambient temperature, the mixture was filtered over Celite to remove excess salt. The solvent was removed and the resulting yellow oil was dissolved into 20 mL DCM. The organic phase was washed...
with water (40 mL) and brine (2x40 mL) and dried over Na$_2$SO$_4$. The solvent was removed via roto-vap to yield the product as a brown oil (0.5625 g; 1.62 mmol). Yield: 77.1% ¹H NMR (300 MHz, CDCl$_3$): δ 8.33 (d, 2H, $H^{\text{pyr}}$), 7.79 (d, 1H, $H^{\text{pyr}}$), 7.71 (d, 1H, $H^{\text{pyr}}$), 7.63 (t, 1H, $H^{\text{pyr}}$), 7.47 (td, 2H, $H^{\text{pyr}}$), 7.38 (d, 2H, $H^{\text{pyr}}$), 6.95 (t, 2H, $H^{\text{pyr}}$), 3.83 (s, 2H, $CH_2$), 3.78 (s, 3H, COOC$_{\text{H}_3}$), 3.71 (s, 4H, $CH_2$)

6-(Dipyridin-2-ylmethylanediyl)-(methylene)picolinic acid (TPAMA) (17)

MeTPAMA (0.6620 g; 1.9 mmol) was dissolved into 40 mL 6M HCl. This solution was heated at reflux for 12 hours. After being cooled to ambient temperature, the solvent was removed under reduced pressure to yield the product as an orange (0.8995g; 1.74 mmol). Yield: 91.6% Elemental analysis: C$_{19}$N$_4$O$_4$H$_{18}$•4HCl•H$_2$O. Found: C:43.75%. H:5.32%. N:10.55%. Calc: C:44.16%. H:5.04%. N: 10.85% ¹H NMR (300 MHz, D$_2$O): δ 8.51 (d, 2H, $H^{\text{pyr}}$), 8.26 (td, 2H, $H^{\text{pyr}}$), 7.89 (m, 2H, $H^{\text{pyr}}$), 7.80 (d, 2H, $H^{\text{pyr}}$), 3.83 (s, 2H, $CH_2$), 3.78 (s, 3H, COOC$_{\text{H}_3}$), 3.71 (s, 4H, $CH_2$)

**Figure 8.3.** ¹H NMR spectrum of TPAMA. The spectrum was taken in D$_2$O (4.8 ppm).
$H^N$, 7.70 (t, 2H, $H^N$), 7.57 (dd, 2H, $H^N$), 4.27 (s, 4H, $CH_2$), 4.05 (s, 2H, $CH_2$) ESI-MS (50:50 MeOH:H$_2$O, m/z +): 335.67 (H$_2$TPAMA$^+$)

p-Chloro-TPADA

The synthesis of the pyridine building blocks has been previously reported,$^{8a}$ however, the TPADA derivative has not been previously synthesized. First, 2-picolinic acid (16) was chlorinated in the para position and in the same step an acyl chloride was appended onto the methyl group in the ortho position. This was then converted into the methyl ester by the \textit{in situ} addition of methanol. Addition of sodium borohydride reduced the methyl ester to a methyl hydroxy group. The methyl hydroxy was then converted into a primary amine via one-pot Mitsunobu and Staudinger reactions. First, the hydroxy group was converted into an azide in a Mitsunobu reaction using diphenylphosphoryl azide (DPPA) in the presence of triphenylphosphine (TPP) and diisopropyl azodicarboxylate (DIAD). This \textit{in situ} azide reacted with TPP and water to form the amine. Two equivalents of methyl 6-(bromomethyl)picolinate (11) were added to this product, 4-chloropicolylamine (19), in nucleophilic addition reaction to product p-chloro MeTPADA (), according to Scheme 8.5. The methyl ester substituents were hydrolyzed in 6 M HCl to produce the final product, p-chloro TPADA (23).

\textit{Methyl 4-chloropicolinate (19)}

SOCl$_2$ (25 mL) was heated to 55°C under N$_2$. To this solution was added 1 mL DMF and the mixture was stirred under N$_2$. After 10 minutes, 2-picolinic acid (18) (8.1280 g; 66 mmol) was added and the temperature was raised to 70°C. The reaction was stirred at 70°C for 2 days. After cooling to ambient temperature, toluene (50 mL)
was added, and the mixture was concentrated \textit{in vacuo}. The solid was dissolved into toluene (100 mL) and cooled to 0°C whereupon methanol (5 mL) was added dropwise. An orange precipitate formed, which was collected via filtration. This solid was dissolved into 100 mL chloroform and quenched with portion-wise addition of NaHCO$_3$ (3x100 mL). The organic layer was isolated, dried over Na$_2$SO$_4$, and removed under reduced pressure to yield the product as a brown oil (5.0786 g; 29.6 mmol). Yield 44.9%. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 8.69 (ds, 1H, $H_{\text{pyr}}$), 8.13 (t, 1H, $H_{\text{pyr}}$), 7.49 (m, 1H, $H_{\text{pyr}}$), 4.00 (s, 3H, COOCH$_3$)

\begin{equation}
\text{Scheme 8.5}
\end{equation}

(i) SOCl$_2$, DMF, N$_2$, 70°C, 2 days. (ii) MeOH, r.t. (iii) NaBH$_4$, CaCl$_2$, MeOH/THF, 0°C, 2 hrs. (iv) PPh$_3$, DIAD, Ph$_2$PON$_3$, THF, 0°C-r.t., overnight.

(4-Chloropyridin-2-yl)methanol (20)

Methyl-4-chloropicolinate (19) (5.0786 g; 29.6 mmol) was dissolved into a mixture of 30 mL MeOH and 20 mL dry THF. To this solution was added finely ground CaCl$_2$ (13.9818 g; 0.126 mol). At 0°C, NaBH$_4$ (2.2238 g; 58.8 mmol) was added
portion-wise. The reaction mixture was stirred at 0°C for 1.5 hours, whereupon 50 mL distilled water was added to quench the reaction. This suspension was stirred for another 2 hours at ambient temperature and afterward extracted with EtOAc (3x50 mL). The combined organic layers were dried over Na₂SO₄, and the solvent was removed under reduced pressure to yield the product as a yellow solid (2.6718 g; 18.6 mmol). Yield: 62.8% ¹H NMR (500 MHz, CDCl₃): δ 8.19 (d, 1H, Hpyr), 7.32, (s, 1H, Hpyr), 7.02 (t, 1H, Hpyr), 4.61 (s, 2H, CH₂OH)

4-Chloropicolylamine (21)

(4-Chloropyridin-2-yl)methanol (20) (0.5902 g; 4.1 mmol) was dissolved into 20 mL dry THF. To this solution at 0°C under N₂ was added triphenyl phosphine (3.2670 g; ), DIAD (1.215 mL; 1.247 g; 6.17 mmol), and diphenylphosphorylazide (1.064 mL; 1.358 g; 4.936 mmol). The reaction was stirred at 0°C for 15 hours. Then, the reaction was heated for one hour at 55°C, whereupon 5 mL of distilled water was added and the reaction was stirred at 55°C for another hour. The solvent was removed and the residue was taken up into 20 mL DCM and 20 mL water. The pH of the water was lowered to 3 with conc. H₂SO₄ and the aqueous layer was isolated and washed with 20 mL DCM. The pH of the aqueous layer was raised to 12 with 40% KOH(aq) and the aqueous layer was extracted with DCM (3x30 mL). The organic layer was dried over Na₂SO₄, and concentrated to afford the product as a yellow oil (0.2218 g; 1.6 mmol). Yield: 37.8% ¹H NMR (300 MHz, CDCl₃): δ 8.20 (d, 1H, Hpyr), 7.11 (d, 1H, Hpyr), 6.94 (dd, 1H, Hpyr), 3.73 (s, 2H, CH₂), 1.97 (s, 2H, NH₂)

Dimethyl 6,6'-(4-chloro-pyridin-2-ylmethylanediyl)bis-(methylene)dipicolinate
(p-chloro-MeTPADA) (22)

Methyl 6-(bromomethyl)picolinate (11) (0.6970 g; 3.17 mmol) and K₂CO₃ (0.4263 g; 3.08 mmol) were dissolved into 30 mL dry acetonitrile. To this solution was added 4-chloropicolylamine (21) (0.2218 g; 1.56 mmol) dissolved into 3 mL MeCN. The reaction was heated to 70°C for 18 hours. After cooling to ambient temperature, the mixture was filtered over Celite to remove excess salt. The solvent was removed and the resulting yellow oil was dissolved into 20 mL DCM. The organic phase was washed with water (40 mL) and brine (2x40 mL) and dried over Na₂SO₄. The solvent was removed via roto-vap to yield the product as a dark red oil (0.5396 g; 1.22 mmol).

Yield: 78.2 % ¹H NMR (300 MHz, CDCl₃): δ 8.38 (d, 1H, Hpyr), 7.97 (q, 2H, Hpyr), 7.79 (d, 2H, Hpyr), 7.78 (s, 2H, Hpyr), 7.60 (d, 1H, Hpyr), 7.12 (dd, 1H, Hpyr), 3.98 (s, 4H, CH₂), 3.96 (s, 6H, COOC₃H₇), 3.84 (s, 2H, CH₂)

6,6’-(4-Chloro-pyridin-2-ylmethylazanediyl)bis-(methylenedipicolinic acid

(p-chloro-TPADA) (23)

p-Chloro MeTPADA (0.5396 g; 1.22 mmol) was dissolved into 25 mL 6M HCl. This solution was heated to a reflux for 12 hours. After being cooled to ambient temperature, the solvent was removed under reduced pressure. The resulting oil was dissolved in MeOH (2x5 mL), which was then removed via roto-vap to yield the product as a red solid (0.5513g; 1.0 mmol). Yield: 82.0% Elemental analysis: C₂₀N₄O₄Cl₁₇•3HCl•H₂O. Found: C:44.39%. H:4.33%. 10.10%. Calc: C:44.69%. H:4.10%. N: 10.43% ESI-MS (50:50 MeOH:H₂O, m/z +): 414.13 (p⁻³⁵Cl H₃TPADA), 416.13 (p⁻³⁷Cl H₃TPADA), 436.27 (p⁻³⁵Cl NaH₂TPADA), 438.33 (p⁻³⁷Cl NaH₂TPADA).
8.3 Speciation of TPADA, TPAMA, and Their Manganese(II) Derivatives

The speciation diagram for the \((H_XTPADA)^{(X-2)^+}\) ligand is given in Figure 8.5. There are six potential protonation sites on the ligand. The amine in the ligand backbone, all of the pyridine nitrogen sites, and the two carboxylic acid substituents can become protonated. Such a protonation scheme is different than that of the macrocyclic ligands discussed above. The cyclen ring is locked into a specific configuration in which the nitrogens cannot move away from each other. Only two protons can be placed within this ring at pH values above zero (see chapter 4). The
pyridine rings within the TPAA series are able to freely move away from each other, allowing all of the nitrogen sites to be protonated. The $pK_a$ values for the ligands are given in Table 8.3. The highest $pK_a$ (at 10.7) is assigned to the tertiary amine in the ligand backbone. This value is similar to those seen for the cyclen ring amine sites in the macrocyclic ligands discussed above ($pK_a\sim8.5-12$). The next $pK_a$ is almost four orders of magnitude lower than the first, at 7.0. This $pK_a$ is assigned to the nitrogen site on the pyridine ring without the carboxylate derivatives. The next two $pK_a$’s are assigned to the nitrogen sites on the two pyridine rings with the carboxylic acid substituents. The final two $pK_a$ values correspond to the protonation of the carboxylic

![Figure 8.5](image_url)  

**Figure 8.5.** The speciation diagram for $(H_xTPADA)_n^{(X-2)+}$ at 37°C. $[L]=.099$ mM, in 0.1 M KNO$_3$. 

...
acid groups on the pyridine rings. These pK\textsubscript{a}'s (3.2 and 2.7 respectively) fall within the range seen within the carboxylic acid groups in the cyclen derivative series (1.7-4.3, see Chapter 4). Though the Hyperquad fit for the titration for (H\textsubscript{x}TPADA)\textsuperscript{(X-2)+} qualitatively matches the data well, the errors associated with the pK\textsubscript{a} values abstracted from this fit are an order of magnitude larger than those from any other fit performed herein.

The speciation diagram for the Mn(II) complex of TPADA is given in Figure 8.6. The binding constant for [Mn(H\textsubscript{x}TPADA)]\textsuperscript{X+} is quite high, log\textsubscript{K\textsubscript{MnL}}=19.8. This is by far the highest binding constant for a manganese(II) species containing a bound inner sphere water (see Table 7.1 and Table 8.1). Indeed, this value is about five magnitudes higher than any other such species, with [Mn(PyC3A)(H\textsubscript{2}O)]\textsuperscript{1+} (14.14)\textsuperscript{4} and Mn(CDTA) (14.32)\textsuperscript{14} being the closest. The magnitude of this binding constant is especially large when this complex is compared to other pyridyl amine derived ligands. DPAAA, which replaces the unsubstituted pyridine ring bound to the tertiary amine with a carboxylic acid arm, has log\textsubscript{K\textsubscript{MnL}}=13.2.\textsuperscript{2} The binding constant for MnTPADA is on the same order as that of Mn(DOTA), which itself is quite high for a potential CA.

The complex is relatively stable to proton assisted decomplexation at low pH values too. The complex has a pK\textsubscript{MnHL}=3.19±0.04. This protonation most likely occurs at one of the carboxylic acid arms, and has a similar pK\textsubscript{a} to the protonation of one of the carboxylic acid pendant arms within the Mn(II) complex of DO2A2AM. Additionally, though there are two carboxylate groups which can be protonated, the is no second pK\textsubscript{a} for MnTPADA, again similarly to MnDO2A2AM. Likely, the protonation of this second carboxylate group destabilizes the complex to such an extent that the metal goes through proton assisted decomplexation. However, the manganese(II) does not
begin to decomplex until the pH is lowered below 3, which is lower than any of the Mn(II) macrocyclic complexes except [Mn(DOTAM)]^{2+}. Such a thermodynamic stability coupled with its relatively high relaxivity ($r_1=2.3 \text{ mM}^{-1}\text{sec}^{-1}$, see below) makes this complex an ideal candidate for an MRI contrast agent. Additionally, only one species is present in solution at physiological pH values (6.8-7.4), which is desirable when designing potential CAs as multiple species can be in equilibrium with each other.

**Figure 8.6.** The speciation diagram for [Mn(H$_x$TPADA)]$^{x+}$ at 37ºC. [L]=[$\text{Mn}^{2+}$]=.099 mM, in 0.1 M KNO$_3$. 

\text{Figure 8.6}. The speciation diagram for [Mn(H$_x$TPADA)]$^{x+}$ at 37ºC. [L]=[$\text{Mn}^{2+}$]=.099 mM, in 0.1 M KNO$_3$. 

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The speciation diagram for the mono-carboxylic acid derivative, \((\text{H}_x\text{TPAMA})^{(x-1)+}\), is shown in Figure 8.7, below. The TPAMA ligand has five potential protonation sites, specifically the tertiary amine in the ligand backbone, the three nitrogens in the pyridine rings, and the carboxylic acid. The \(\text{p}K_a\) values for \([\text{H}_x\text{TPAMA}]^{(x-1)+}\) are given in Table 8.3. Three of these protonation sites are unique, while the two unsubstituted pyridine rings are equivalent. The highest \(\text{p}K_a\) (13.80±0.06) is assigned to the protonation of the tertiary amine. This \(\text{p}K_a\) is relatively similar to those previously seen within cyclen derivatives (see above). The next protonation occurs at \(\text{p}K_a=6.24\), corresponding to one of the pyridine rings. This protonation site is most likely the substituted pyridine ring, as the other two pyridine rings should have roughly the same protonation constants as they have the same chemical environments. The lowest \(\text{p}K_a\) is assigned to the carboxylic acid, based upon previous titrations of picolinate species.\(^2\)

\[\text{Figure 8.7. Speciation diagram for } (\text{H}_x\text{TPAMA})^{(x-1)+} \text{ at } 37^\circ\text{C}, [L]=0.1 \text{ mM, 0.1 M KNO}_3.\]
The speciation diagram for $[\text{Mn}(H_x \text{TPAMA})]^{(X+1)+}$ is shown in Figure 8.8, below. This speciation diagram looks quite similar to the speciation diagram of $[\text{Mn}(H_x \text{TPADA})]^X$. At high pH values (5-8), the deprotonated species, $[\text{Mn}(\text{TPAMA})]^+$, dominates. The binding constant for this species is quite high ($\log K_{\text{MnL}} = 16.55 \pm 0.06$), though it is still smaller than that of the di-carboxylic analogue ($\log K_{\text{MnL}} = 19.71 \pm 0.04$) as would be expected. Indeed, this is exactly the same increase in stability seen between the Mn(II) complexes of DPAmE-A$^2-$ ($\log K_{\text{MnL}} = 9.55$) and DPAAA$^3-$ ($\log K_{\text{MnL}} = 13.19$) where the only change in the addition of an extra coordinating carboxylic acid (see Scheme 8.1). This binding constant of $16.55 \pm 0.06$ is six magnitudes higher than any other Mn(II) bis-hydrated pyridine derived complex previously reported (see Table 8.1). The carboxylic acid arm of $[\text{Mn}(H_x \text{TPAMA})]^{(X+1)+}$ has $pK_{\text{MnHL}} = 3.13 \pm 0.06$, quite similar to that of the di-carboxylic acid derivative, $pK_{\text{MnHL}} = 3.19 \pm 0.04$.

**Figure 8.8.** Speciation diagram for $[\text{Mn}(\text{HTPAMA})]^{(1+X)+}$ at $37^\circ\text{C}$, $[L]=0.1 \text{ mM}$, 0.1 M KNO$_3$. 

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For a more detailed representation of the data in Table 8.1, please refer to the original text or the full dataset.
The pH dependent relaxivity curve for $[\text{Mn}(H\_X\text{TPADA})]^{X+}$ is given below in Figure 8.9. Below a pH of 3, the complex has begun to dissociate and there is free Mn$^{2+}$ present in the solution, which accounts for the rise in the relaxivity value. At pH values from 3 to 8.2, the complex is fully formed and the relaxivity in this pH range is 2.3 mM$^{-1}$sec$^{-1}$ at 37°C. This value is similar to the relaxivity values of other mono-hydrated pyridine derived complexes (see Table 8.1), and higher than any of the cyclen based Mn(II) complexes discussed earlier. The relaxivity value for $[\text{Mn}(H\_X\text{TPADA})]^{X+}$ is lower than many of the other mono-hydrated pyridine complexes. This is most likely due to the geometries of the complexes in solution, which can affect the water exchange rate, as less steric hinderance around the metal center can grant better access for the exchanging water molecules. The Mn(II) metal centers of two five-coordinate, mono-hydrated pyridine derived Mn(II) complexes, $[\text{Mn}(15-\text{PyN}_3\text{O}_2)(\text{H}_2\text{O})\text{Cl}]$ and $[\text{Mn}(15-\text{PyN}_3)(\text{H}_2\text{O})\text{Cl}]$, adopt a bi-pyramidal geometry binding

### Table 8.3. Stability constants for TPADA, TPAMA, and their Mn(II) Complexes determined by potentiometric titration. All titrations were performed at 37°C, $[\text{L}]=0.1$ mmol for ligands, $[\text{L}]=[\text{M}]=0.1$ mmol for complexes, 0.1 M KNO$_3$.

<table>
<thead>
<tr>
<th>$\text{[H}_X\text{TPADA}^{(X-2)+}$</th>
<th>$\text{[H}_X\text{TPAMA}^{(X-1)+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$pK_1$ [HL]$^a$</td>
<td>10.71±0.40</td>
</tr>
<tr>
<td>$pK_2$ [H$_2$L]$^a$</td>
<td>7.00±0.39</td>
</tr>
<tr>
<td>$pK_3$ [H$_3$L]</td>
<td>4.49±0.33</td>
</tr>
<tr>
<td>$pK_4$ [H$_4$L]</td>
<td>3.57±0.34</td>
</tr>
<tr>
<td>$pK_5$ [H$_5$L]</td>
<td>3.20±0.42</td>
</tr>
<tr>
<td>$pK_6$ [H$_6$L]</td>
<td>2.67±0.24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\text{[Mn(H}_X\text{TPADA})]^{X+}$</th>
<th>$\text{[Mn(H}_X\text{TPAMA})]^{(X+1)+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\log K_{\text{MnL}}^b$</td>
<td>19.71±0.04</td>
</tr>
<tr>
<td>$pK_2$ [MnHL]$^c$</td>
<td>3.19±0.04</td>
</tr>
</tbody>
</table>

$^a$Determined from Equation 4.1  $^b$Determined from Equation 4.2  $^c$Determined from Equation 4.3

### 8.4 $^1$H Relaxivities of $[\text{Mn}(H\_X\text{TPADA})]^{X+}$ and $[\text{Mn}(H\_X\text{TPAMA})]^{(X+1)+}$

The pH dependent relaxivity curve for $[\text{Mn}(H\_X\text{TPADA})]^{X+}$ is given below in Figure 8.9. Below a pH of 3, the complex has begun to dissociate and there is free Mn$^{2+}$ present in the solution, which accounts for the rise in the relaxivity value. At pH values from 3 to 8.2, the complex is fully formed and the relaxivity in this pH range is 2.3 mM$^{-1}$sec$^{-1}$ at 37°C. This value is similar to the relaxivity values of other mono-hydrated pyridine derived complexes (see Table 8.1), and higher than any of the cyclen based Mn(II) complexes discussed earlier. The relaxivity value for $[\text{Mn}(H\_X\text{TPADA})]^{X+}$ is lower than many of the other mono-hydrated pyridine complexes. This is most likely due to the geometries of the complexes in solution, which can affect the water exchange rate, as less steric hinderance around the metal center can grant better access for the exchanging water molecules. The Mn(II) metal centers of two five-coordinate, mono-hydrated pyridine derived Mn(II) complexes, $[\text{Mn}(15-\text{PyN}_3\text{O}_2)(\text{H}_2\text{O})\text{Cl}]$ and $[\text{Mn}(15-\text{PyN}_3)(\text{H}_2\text{O})\text{Cl}]$, adopt a bi-pyramidal geometry binding
**Figure 8.9.** pH dependent relaxivity profile for Mn(TPADA) at 20 MHz, 37°C. At a physiological pH value, 6.99, the $r_1 = 2.3 \text{ mM}^{-1} \text{ sec}^{-1}$.

**Figure 8.10.** pH dependent relaxivity profile for Mn(TPAMA) at 20 MHz, 37°C. At a physiological pH value, 7.01, the $r_1 = 4.4 \text{ mM}^{-1} \text{ sec}^{-1}$.
equatorially to the ligand in a penta-dentate manner.\textsuperscript{1} The two axial positions are capped with a water molecule and a chloride counter-ion. Such a geometry offers little steric hinderance to bulk water molecules attempting to access the metal center. As such, the complexes have significantly higher relaxivity values ($r_1=3.6$ mM$^{-1}$ sec$^{-1}$ and $3.1$ mM$^{-1}$ sec$^{-1}$, respectively). These complexes, however, have quite low binding constants ($\log K_{\text{MnL}}=7.18$ and 10.89, respectively), partly due to the ease with which water can access the metal center.

Figure 8.10, above, gives the pH dependent $r_1$ curve for $[\text{Mn(H}_x\text{TPAMA})]^{(1+x)^+}$. This complex begins to dissociate at a slightly higher pH than $[\text{Mn(H}_x\text{TPADA})]^{x^+}$ (see above), but at a lower pH than the majority of the macrocyclic Mn(II) compounds discussed herein. When fully formed, this complex has an $r_1=4.4$ mM$^{-1}$ sec$^{-1}$ at 37ºC. This is quite a large $r_1$ value for a Mn(II) metal complex and is indicative of a complex with two inner-sphere water molecules bound to the Mn(II) metal center. Only one previously reported\textsuperscript{2} Mn(II) pyridine derived complex has a higher value ($r_1=5.1$ mM$^{-1}$ sec$^{-1}$, see Table 8.1), however, this complex has a very low binding constant ($\log K_{\text{MnL}}=9.55$), which is seven orders of magnitude weaker than that of $[\text{Mn(TPAMA})]^+$. 
References:
**General Conclusions**

While the efficacy of a potential Magnetic Resonance Imaging (MRI) contrast agent (CA) is generally only dependent upon the thermodynamic stability of the complex and the magnitude of its relaxivity, it is not enough to simply synthesize, test, and catalogue these complexes. Understanding the solution state structures, and the reason for these structures, is indispensable when attempting to rationally design new, more efficient potential CAs.

The solution state structures of three macrocyclic cyclen-based manganese(II) complexes—\([\text{Mn}(H_x\text{DO2A})]^X^+\), \([\text{Mn}(\text{DO2AM})]^{2+}\), and \([\text{Mn}(H_x\text{DO2A2AM})]^X^+\)—have been extensively studied. Potentiometry was used in order to determine the binding constants of these three complexes, \(\log K_a = 14.93 \pm 0.14\), \(10.94 \pm 0.02\), and \(17.07 \pm 0.01\) for the complexes, respectively. Furthermore, the coordination environments of the manganese(II) metal centers were probed using solution state IR spectroscopy, and were determined to be 6 for all of the complexes. The relaxometric properties of the three complexes were explored using a pH dependent \(^1\text{H}\) relaxivity study. The three complexes have \(r_1\) values that are relatively low, \([\text{Mn}(\text{DO2A})]: 1.3 \text{ mM}^{-1}\text{sec}^{-1}\), \([\text{Mn}(\text{DO2AM})]^{2+}: 1.7 \text{ mM}^{-1}\text{sec}^{-1}\), and \([\text{Mn}(\text{DO2A2AM})]: 1.5 \text{ mM}^{-1}\text{sec}^{-1}\). Finally, the number of inner-sphere waters bound to the metal center of each complex using temperature dependent \(^{17}\text{O}\) NMR spectroscopy was determined to be \(q=0\) for \([\text{Mn}(\text{DO2A})]\), \(q=1\) for \([\text{Mn}(\text{DO2AM})(\text{H}_2\text{O})]^{2+}\), and \(q=0\) for \([\text{Mn}(\text{DO2A2AM})]\). Ultimately, these three complexes are not good candidates as potential contrast agents. \([\text{Mn}(H_x\text{DO2A})]^X^+\) has a moderate thermodynamic stability, but quite a low relaxivity. \([\text{Mn}(\text{DO2AM})(\text{H}_2\text{O})]^{2+}\) has a bound water which lends the complex a moderate relaxivity, however, its binding constant is very low for a macrocyclic complex.
[Mn(DO2A2AM)]^{2+} has a relatively high binding constant, however, because there are no bound waters, the relaxivity is not high enough for the complex to function as a contrast agent.

The two pyridine complexes discussed herein, [Mn(H_xTPADA)]^{X+} and [Mn(H_xTPAMA)]^{(1+X)+}, are ideal candidates for use as MRI contrast agents. [Mn(H_xTPADA)]^{X+} has a high thermodynamic stability (logK_{MnL}=19.71±0.04) and a relatively high relaxivity ($r_1=2.3$ mM$^{-1}$sec$^{-1}$) at 37°C. [Mn(H_xTPAMA)]^{(1+X)+} is, expectedly, less stable (logK_{MnL}=16.55±0.06), however, the relaxivity is almost twice that of its di-carboxylic acid derivative ($r_1=4.4$ mM$^{-1}$sec$^{-1}$) at 37°C. The Mn(II) complex of TPADA is four orders of magnitude more stable than any other mono-hydrated potential contrast agent, while still retaining a relaxivity that makes it desirable as such. The Mn(II) complex of TPAMA is even more striking. It has by far the highest stability of any previously reported bis-hydrated CA. Additionally, its relaxivity is of a comparable magnitude to some FDA approved Gd(III)-based contrast agents that are currently in use.

**Further Directions**

It has previously been shown that pyridine rings can easily be substituted in order to study the strength of the pyridine bonding with copper(II). The two pyridine derived ligands reported herein, TPADA and TPAMA, make compelling targets for such an approach to be applied to manganese(II) as they already possess desirable characteristics as potential contrast agents. Incorporation of para substituted pyridine rings into TPADA and TPAMA is a relatively straight-forward process, requiring only the modification of the pyridine precursors. In this study, the synthesis of a para
A substituted chloro-TPADA derivative was carried out. In addition, syntheses aimed at incorporating other substituents (i.e. OMe, NO₂, and N(CH₃)₂) in the para position of the pyridine rings.

After synthesizing these derivatives, binding constants will be used in order to compare the stabilities of their manganese(II) derivatives, and probe the influence of the substituent groups. Relaxivity profiles for the manganese(II) derivatives will be used to determine. Additionally, quantum mechanical calculations will be carried out in order to study the character of the bonding of the substituted pyridine with the metal center.
Appendix A: Hyperquad Fits

Figure A1. Hyperquad fit for the potentiometric titration of \([\text{Mn}(\text{H}_x\text{DO2A})]^X_+\) at 37°C, \([L]=[M]=0.1\) mM.

Figure A2. Hyperquad fit for the potentiometric titration of \([\text{Mn}(\text{DO2AM})]^2_+\) at 37°C, \([L]=[M]=0.1\) mM.
Figure A3. Hyperquad fit for the potentiometric titration of \((H_xDO2A2AM)^{(X-2)+}\) at 37°C, [L]=[M]=0.1 mM.

Figure A4. Hyperquad fit for the potentiometric titration of \([Mn(H_xDO2A2AM)]^{X+}\) at 37°C, [L]=[M]=0.1 mM.
Figure A5. Hyperquad fit for the potentiometric titration of (H$_x$TPADA)$^{(X-2)+}$ at $37^\circ$C, [L]=[M]=0.1 mM.

Figure A6. Hyperquad fit for the potentiometric titration of [Mn(H$_x$TPADA)]$^{X+}$ at $37^\circ$C, [L]=[M]=0.1 mM.
Figure A7. Hyperquad fit for the potentiometric titration of (H\textsubscript{x}TPAMA\textsuperscript{(X-1)+} at 37°C, [L]=[M]=0.1 mM.

Figure A8. Hyperquad fit for the potentiometric titration of [Mn(H\textsubscript{x}TPAMA)\textsuperscript{(X+1)+} at 37°C, [L]=[M]=0.1 mM.