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Part I. Syntheses, characterization, and evaluation of new poly-alcohol porphyrin compounds of manganese(III) as contrast enhancement media in magnetic resonance imaging. Part II. Model systems for the sulfite reductase active site: Toward a tetrasulfido-iron-cluster-porphyrin compound

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PART I: Syntheses, Characterization, and Evaluation of New Poly-Alcohol Porphyrin Compounds of Manganese(III) as Contrast Enhancement Media in Magnetic Resonance Imaging

PART II: Model Systems for the Sulfite Reductase Active Site: Toward a Tetralsulfido-Iron-Cluster-Porphyrin Compound

by

Joseph Earl Bradshaw

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE DOCTOR OF PHILOSOPHY

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May, 1993
ABSTRACT

PART I: Syntheses, Characterization, and Evaluation of New Poly-Alcohol Porphyrin Compounds of Manganese(III) as Contrast Enhancement Media in Magnetic Resonance Imaging

PART II: Model Systems for the Sulfite Reductase Active Site: Toward a Tetrasulfido-Iron-Cluster-Porphyrin Compound

by

Joseph Earl Bradshaw

PART I

A new series of water-soluble porphyrins and metalloporphyrins using poly-alcohol substituents to achieve water solubility have been synthesized and spectroscopically characterized. Two of these compounds, [Mn\textsuperscript{III}(TPPAS)Cl] and [Mn\textsuperscript{III}(TPPIS)Cl], are candidates for commercial MRI contrast enhancement agents. The proton magnetic relaxation rate (1 / T\textsubscript{1}) of [Mn\textsuperscript{III}(TPPAS)Cl] has been studied in aqueous solution as a function of the field strength over the range of Larmor frequencies from 0.01 to 30 MHz, and it shows a typical relaxation rate pattern similar to other manganese(III) porphyrin compounds over this frequency range. Conductivity and osmometry measurements for [Mn\textsuperscript{III}(TPPAS)Cl] in water demonstrate complete dissociation of chloride ion to yield a manganese(III) porphyrin species in solution of probable composition [Mn\textsuperscript{III}(TPPAS)(H\textsubscript{2}O)\textsubscript{2}]\textsuperscript{+}. This suggests that all other water-soluble manganese(III) porphyrin compounds studied to date as \textsuperscript{1}H NMR relaxation agents are also likely to be [Mn\textsuperscript{III}(porphyrin)(H\textsubscript{2}O)\textsubscript{X}]\textsuperscript{n} species. Additionally, variable-temperature NMR studies of the H\textsubscript{2}TPPAS and H\textsubscript{2}TPPIS free ligands have shown possible multiple forms of aggregation as indicated by two distinct but interrelated H\textsubscript{β} pyrrole proton signals. This multiple
aggregate/slow proton exchange process, occurring on the porphyrin periphery, provides insight as to the source of the higher than expected proton relaxation rate of manganese(III) porphyrin compounds, in general. With an acute toxicity of 0.1 mmole / kg, the [MnIII(TPPAS)Cl] compound is too toxic for clinical use. Actual magnetic resonance images of Sprague Dawley rats, to which [MnIII(TPPAS)Cl] had been administered, showed a significant increase in enhancement of contrast in the heart, liver, lungs, and gastrointestinal tract.

PART II

Three new unsymmetrical monothiophenyl-derivatized porphyrins have been synthesized and spectroscopically characterized. One of the compounds, H2TPP(Me)1, provides a precursor molecule for straight-forward removal of the methyl group to yield a desired monothiophenylporphyrin, H2TPP(SH)1. Based upon well-documented substitution reactions of the alkyl-thiolate arms in [Fe4S4(SR)4]2- clusters, the in situ reaction of H2TPP(SH)1 with [Fe4S4(SCH2CH3)4]2- has been monitored by 1H NMR spectroscopy. Substitution of the alkyl-thiolate arms (CH3CH2S-) of the parent cluster by the aryl-thiolate porphyrin (H2TPPS-) does indeed occur, and complete substitution of all four alkyl groups is accomplished by a porphyrin / cluster ratio of 5:1. This first successful covalent attachment of a porphyrin molecule to an [Fe4S4]2- cluster core to produce the [Fe4S4(H2TPPS)4]2- ion provides a promising method for the future development of model compounds for the sulfite reductase active site.
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Introduction

Clinical use of nuclear magnetic resonance (NMR) imaging has been practiced since 1980.\textsuperscript{1} While remarkable images have been obtained of the heart, brain, pelvis and other structures, investigators have suggested the use of contrast enhancement agents to further the diagnostic potential of the NMR technique.\textsuperscript{2,3} Unlike conventional radiography in which X-ray absorption provides contrast, magnetic resonance imaging (MRI) utilizes four parameters - proton density, $T_1$, $T_2$, and blood flow to obtain contrast intensity of the desired image. Despite this flexibility, certain organ systems and disease states cannot be imaged well. For example, the gastrointestinal tract cannot be identified consistently nor can tissue vascularity and function be observed directly. Additionally, MRI is sensitive to identification of abnormalities, but often not helpful in distinguishing among the possible causes of the abnormality.\textsuperscript{4} In these and other areas, the use of contrast enhancement agents may increase the diagnostic potential of MRI.

Contrast enhancement media for MRI differ from those iodinated agents used in radiography. Iodinated agents produce a contrast effect by the absorption of X-rays. NMR imaging agents enhance proton relaxation by altering the magnetic environment around them. This alteration is carried out by decreasing $T_1$ and $T_2$, the spin-lattice and spin-spin relaxation times, respectively. Thus, for MRI, contrast enhancement is produced by a compound's influence on water protons within a close proximity.

In the absence of an applied magnetic field, a water molecule's proton magnetic moments are randomly aligned. However, in an externally applied magnetic field these magnetic moments preferentially align with the magnetic field. Figure I-1 illustrates both the $T_1$, longitudinal, and $T_2$, transverse, decay processes. If one applies an RF pulse to the proton's net magnetization vector, this causes the proton's net magnetization to be left at an angle in the same direction as the applied RF field. The $T_2$ process describes the time
Figure I-1. The $T_1$, Longitudinal and $T_2$, Transverse Relaxation Processes.

(a) Depiction of the transverse relaxation process that shows how the $x$-$y$ component of magnetization decays to the $z$ axis with time constant $T_2$.

(b) Depiction of the longitudinal relaxation process that shows how the magnetization vector recovers to its equilibrium position parallel to the $z$ axis with time constant $T_1$. 
required for perturbed, in-phase spins to undergo dephasing with respect to one another. It is also called the transverse relaxation process since it describes the decay of the magnetization vector, \( M \), in the x-y plane which is by definition transverse or perpendicular to the z axis, the direction of the constant applied magnetic field, \( B_0 \) (Figure I-1(a)). \( T_1 \), on the other hand, is the time constant that describes the recovery of the perturbed magnetization vector \( M \) to its equilibrium value \( M_0 \) along the direction \( z \) of the applied magnetic field, \( B_0 \) (Figure I-1(b)). The local magnetic field produced by paramagnetic substances shortens both the spin-lattice and spin-spin relaxation times (\( T_1 \) and \( T_2 \)) of hydrogen nuclei in close proximity. This phenomenon is referred to as "proton relaxation enhancement".\(^5\)\(^6\)

Paramagnetic enhancement of nuclear relaxation was first described in 1946 by Bloch and co-workers\(^7\) when they demonstrated a convenient practice of shortening the time needed to observe water \(^1\)H \( T_1 \) by adding a paramagnetic solute, ferric nitrate. The strength of interaction between a paramagnetic center and the hydrogen nuclei led to a mathematic formulation described first by Solomon\(^8\) in 1955 and modified later by Bloembergen.\(^9\) Equations (1) and (2),

\[
\frac{1}{T_1} = \frac{2}{15} \left[ \frac{S(S+1) \gamma^2 I \mu_B^2}{r^6} \left( \frac{3\tau_e}{1 + \omega_1 \tau_e} + \frac{7\tau_e}{1 + \omega_2 \tau_e} \right) + \frac{2}{3} \frac{\mu_e^2}{\hbar} \left( \frac{\tau_e}{1 + \omega_2 \tau_e} \right) \right] \tag{1}
\]

and

\[
\frac{1}{T_2} = \frac{1}{15} \left[ \frac{S(S+1) \gamma^2 I \mu_B^2}{r^6} \left( 4\tau_e + \frac{3\tau_e}{1 + \omega_1 \tau_e} + \frac{13\tau_e}{1 + \omega_2 \tau_e} \right) + \frac{1}{3} \frac{\mu_e^2}{\hbar} \left( \frac{\tau_e}{1 + \omega_2 \tau_e} \right) \right] \tag{2}
\]

describe the relaxation effects pictured in Figure I-1, where \( S \) represents electron spin quantum number, \( g \) is the electronic \( g \) factor, \( \beta \) is the Bohr magneton, \( \omega_1 \) and \( \omega_2 \) are
Larmor frequencies for nuclear and electron spins, r is the ion-nucleus distance, A is the hyperfine coupling constant, and $\tau_c$ and $\tau_e$ are correlation times for dipolar and scalar interactions. It is apparent from equations 1 and 2 that a decrease in T$_1$ is dependent on the paramagnetic species' concentration and the square of its magnetic moment. Also of importance is the fact that the interaction of the paramagnetic center and the water proton is inversely related to the sixth power of the distance separating the two species.

Equation (3) represents the relationship between the correlation time for dipole-dipole interaction, $\tau_c$, and longitudinal electron-spin relaxation time, $\tau_{le}$, chemical exchange (water residence time), $\tau_m$, and rotational-tumbling time, $\tau_r$.

$$\frac{1}{\tau_c} = \frac{1}{\tau_{le}} + \frac{1}{\tau_m} + \frac{1}{\tau_r} \quad (3)$$

The shortest time of $\tau_{le}$, $\tau_m$, $\tau_r$ dominates the equation. If $\tau_c$ is too short, then interactions are too brief for effective relaxation enhancement. If $\tau_c$ is too long, then each interaction takes too long and is therefore inefficient.$^{10}$

A desirable contrast enhancement agent should 1) be paramagnetic with as much unpaired electron spin density as possible, 2) have sites for direct access to bulk water with "rapid" exchange of associated water and free water molecules, 3) be chemically versatile for structure modification, 4) be chemically stable in vivo and in vitro, 5) have low toxicity, and 6) be inexpensive.

In the selection of paramagnetic agents, one needs to consider the effective magnetic moment, $\mu$, since reduction of T$_1$ is directly proportional to $\mu^2$. The need for contrast enhancement agents to have facile exchange of water molecules, as well as, being chemically versatile has led to much investigation into the utilization of transition metal and lanthanide chelate complexes to accomplish MRI image enhancement. A list of
representative paramagnetic metal ions, the number of unpaired electrons, and their corresponding magnetic moments are shown in Table I-1.

One method for introducing the particular enhancement agent is orally. Several soluble metal complexes have been introduced into the body in this fashion. Young, et. al.\textsuperscript{11} used ferric chloride, FeCl$_3$·3 H$_2$O, to increase contrast of the stomach, however toxicity precludes the general application of this compound. Wesby, et. al.\textsuperscript{12} have utilized ferric ammonium citrate, a component of dietary iron supplements, with gastrointestinal side effects reduced. Insoluble colloidal suspensions of various transition metal and lanthanide compounds, such as gadolinium oxalate and iron sulfide have also been used to enhance MRI images of the alimentary tract.\textsuperscript{4}

A second method for introduction of imaging agents is that of inhalation. The agent generally used is that of molecular oxygen.\textsuperscript{14} Molecular oxygen contains two unpaired electrons of parallel spin and it is therefore paramagnetic with a triplet ground state. The utilization of molecular oxygen is limited, however, by O$_2$-hemoglobin saturation\textsuperscript{15}, and O$_2$ does not exhibit strong proton-relaxation in plasma or water.\textsuperscript{16}

The most common method for incorporation of MRI agents into the body is intravenously. One type of intravascular MRI enhancement agent is that of the stable nitrooxide free radicals investigated by Brasch and co-workers.\textsuperscript{17,18} The piperidylne and the pyrrolidine derivatives are two distinct classes of nitrooxide stable free radicals that have been used. The ring structures of both derivatives are shown in Figure I-2. These agents have paramagnetic properties because of the presence of an unpaired electron that is delocalized between the nitrogen and oxygen atoms. This delocalization and the steric hinderance provided by adjacent bulky organic groups stabilize the free radical \textit{in vitro}. Unfortunately, nitrooxide stable free radicals undergo degradation \textit{in vivo} by anti-oxidants.
Table 1-1. Typical Metal Ions Used as Contrast Enhancement Media.

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>Number of Unpaired electrons</th>
<th>Magnetic Moment (B.M.)</th>
<th>Electron Spin Quantum Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cr$^{3+}$</td>
<td>3</td>
<td>3.8</td>
<td>3/2</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>5</td>
<td>5.9</td>
<td>5/2</td>
</tr>
<tr>
<td>Fe$^{3+}$</td>
<td>5</td>
<td>5.9</td>
<td>5/2</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>1</td>
<td>1.7 - 2.2</td>
<td>1/2</td>
</tr>
<tr>
<td>Eu$^{3+}$</td>
<td>6</td>
<td>6.9</td>
<td>6/2</td>
</tr>
<tr>
<td>Gd$^{3+}$</td>
<td>7</td>
<td>7.9</td>
<td>7/2</td>
</tr>
<tr>
<td>Dy$^{3+}$</td>
<td>5</td>
<td>5.9</td>
<td>5/2</td>
</tr>
</tbody>
</table>
Figure I-2. The Piperidine-N-oxyl and Pyrrolidine-N-oxyl Nitroxide Stable Free Radicals (NSFR).
and enzyme systems.\textsuperscript{19} However, some success at a prolonged \textit{in vivo} half life has been achieved by Brasch et al.\textsuperscript{18}

Several transition metal and lanthanide chelate complexes have been investigated as possible MRI image enhancers and have been found to be stable \textit{in vivo}. One such complex is that of the Cr-EDTA anion, aquoethylenediaminetetraacetatochromium(III), shown in Figure I-3. Chromium-EDTA is an octahedral complex with one coordination site available for a water molecules. Experimentation as revealed that the \([\text{Cr}^{III}(\text{EDTA})(\text{H}_2\text{O})]^+\) anion decreases both \(T_1\) and \(T_2\) of solutions.\textsuperscript{20} Both of these processes are competitive and therefore both concentration and pulse sequence need to be optimized in the use of \([\text{Cr}^{III}(\text{EDTA})(\text{H}_2\text{O})]^+\). This particular paramagnetic agent has been used effectively to quantify renal function and enhance kidney contrast.\textsuperscript{21}

The contrast agent that has been investigated most widely has been that of aquodiethyltriaminepentaacetatogadolinium(III), \([\text{Gd}^{III}(\text{DTPA})(\text{H}_2\text{O})]^2-\), as shown in Figure I-4.\textsuperscript{23} Gadolinium(III) contains seven unpaired electrons and has a high effective magnetic moment (7.9 B.M.), making it, therefore, an ideal candidate for use as a nuclear magnetic resonance contrast enhancement agent. Gadolinium chelates can possibly assume coordination numbers from seven to nine. The \(\text{DTPA}\)\textsuperscript{5-} anion occupies up to eight of these possible sites, thereby allowing a maximum of one possible site for water exchange. An X-ray crystal structure and a solution determination of \(q\) (the number of exchangeable water molecules) has shown that for \([\text{Gd}^{III}(\text{DTPA})(\text{H}_2\text{O})]^2-\) \(q = 1\).

Clinical results of \([\text{Gd}^{III}(\text{DTPA})(\text{H}_2\text{O})]^2-\) have shown great promise, however the complex does not cross the blood brain barrier. Another drawback has been the consistent finding of a 15\% to 30\% increase in serum iron concentration up to 24 hours after initial injection. Increase in serum bilirubin has also been observed in a few cases.\textsuperscript{22} Most
Figure I-3. The Ethylenediaminetetraacetate anion and the Aquoethylenediaminetetraacetatochromium(III) Anion.

\[ \text{[EDTA]}^{4-} \quad \text{[Cr}^{III}\text{(EDTA)(H}_2\text{O)}]^{-} \]

(EDTA = ethylenediaminetetraacetic acid)
Figure I-4. The Diethylenetriaminepentaacetate anion and the Aquo(diethylenetriaminepentaacetato)gadolinium(III) Anion.

\[
\text{[DTPA]}^{5-}
\]

(DTPA = diethylenetriaminepentaacetic acid)

\[
\text{[Gd}^{III}\text{(DTPA)(H}_2\text{O)}_n\text{]}^{2-}
\]
recently, [GdⅢ(DTPA)(H₂O)]²⁻ has been associated with anaphylactic shock in some patients.²³

A new polyazamacrocyclic ligand system for gadolinium has recently been reported.²⁴ The polyazamacrocyclic ligand, 1,4,7,10-tetrazacyclododecane-1,4,7,10-tetraacetate anion, [DOTA]⁴⁻, and its related derivatives, as seen in Figure I-5, has an even higher association constant for gadolinium than the [DTPA]⁵⁻ anion (log Kₐ = 28 versus 22.5).²⁵ [GdⅢ(DOTA)(H₂O)]⁺ shows higher relaxivity than [GdⅢ(DTPA)(H₂O)]²⁻ by a factor of two over the range of magnetic fields studied.²⁴ The use of gadolinium(III) complexes, including [GdⅢ(DOTA)]⁺, may be somewhat limited due to toxicity.²⁶ [GdⅢ(HP-DO3A)(H₂O)₂]⁰ is a new neutrally-charged gadolinium(III) chelate that will soon be marketed by Bristol-Myers Squibb under the name ProHance®.

The final group of coordination compounds that have been considered is that of metalloporphyrins. The tendency of metalloporphyrins to become isolated in tumors,²⁷ combined with the ability of a paramagnetic metal center to increase the relaxation rates of water protons, makes certain metalloporphyrins prime candidates for tumor imaging.

Porphyrins are macrocyclic ring systems consisting of four pyrrole rings joined by four methine bridges. Such metallocotetrapyrrrole complexes are of interest because of the occurrence of the macrocyclic structure in biological systems such as the heme proteins, photosynthetic pigments, and vitamin B₁₂.²⁸ Most synthetic porphyrins are not water soluble and therefore not compatible with biological systems. However, porphyrins can be made water soluble by the incorporation of various substituents on the porphyrin core which are hydrophobic in nature, such as the N-methyl pyridyl and 4-sulfanatophenyl groups, as shown in Figure I-6.

Incorporation of various paramagnetic metal ions such as iron(III), manganese(III), and gadolinium(III) into these water soluble porphyrins has been accomplished and their
Figure 1-5. The Polyazamacrocyclic ligand, [DOTA]$^{4-}$, 1,4,7,10-tetrazacyclododecane-1,4,7,10-tetraacetate anion, and Related Derivatives.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Derivative</th>
<th>$R_1$</th>
<th>$R_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[DOTA]$^{4-}$</td>
<td>H</td>
<td>CH$_2$COO$^-$</td>
</tr>
<tr>
<td>[DO3A]$^{3-}$</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>[HP-DO3A]$^{3-}$</td>
<td>H</td>
<td>CH$_2$CHOHCH$_3$</td>
</tr>
<tr>
<td>[MCTA]$^{4-}$</td>
<td>CH$_3$</td>
<td>CH$_2$COO$^-$</td>
</tr>
</tbody>
</table>
Figure I-6. Water-Solubilizing Moieties Attached to a Metalloporphyrin Core.

\[ \text{R} = \begin{array}{c}
1) \quad \text{CO}_2^\text{H}^+ \\
2) \quad \text{SO}_3^\text{Na}^+ \\
3) \quad \text{N}^+\text{CH}_3\text{Cl}^- \\
4) \quad \text{N(CH}_3)_2\text{Cl}^- \\
\end{array} \]

TPPC, TPPS\textsubscript{4}, TMPyP, TAP
relaxation rates have been obtained. In the absence of solvent-solute interactions, relaxation rates are linearly dependent on the concentration of the paramagnetic species. The relaxivity is defined as the slope of this dependence.\textsuperscript{26} Reported relaxivities at pH 7.0 for [Mn\textsuperscript{III}(TPPS\textsubscript{4})\textsuperscript{3-}], the manganese(III) tetrakis(4-sulfophenyl)porphine ion, and 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrinatomanganese(III) chloride, [Mn\textsuperscript{III}(TPPC)Cl], are 10.3 and 15.9 (mM s\textsuperscript{-1}), respectively.\textsuperscript{29,30} The relaxivity for [Gd\textsuperscript{III}(TPPS\textsubscript{4})\textsuperscript{3-}] was measured at 22.8 (mM s\textsuperscript{-1}), but the gadolinium compound was found to dissociate metal rapidly in both plasma and water.\textsuperscript{29}

Although iron(III) compounds normally have five unpaired electrons, the relaxivity values found for all iron(III) porphyrins have been lower than the values for manganese(III) porphyrins which at most, possess four unpaired electrons.\textsuperscript{29} Additionally, the conclusion has been reached that iron(III) porphyrins will be unlikely candidates for MRI contrast agents due to the loss of paramagnetism at pH > 6, the physiological range of interest.\textsuperscript{30} This loss of paramagnetism arises from base-promoted formation of the \(\mu\)-oxo iron porphyrin dimer.

While Lyon, et al. concluded that neither gadolinium(III) nor iron(III) water-soluble metalloporphyrins are suitable as potential MRI contrast agents,\textsuperscript{29} manganese(III) water-soluble metalloporphyrins seemingly possess suitable stabilities and relaxivities. Thus, manganese derivatives of 4-sulfonatophenyl porphyrin, protoporphyrin IX, and mesoporphyrin have been thoroughly studied, with most attention being given to [Mn\textsuperscript{III}(TPPS\textsubscript{4})\textsuperscript{3-}].\textsuperscript{29-38}

Most recently, McMillan et al.\textsuperscript{39} have investigated manganese(III) uroporphyrin I as a potential contrast agent for MRI of tumors and have reported a "narrow window of efficacy" for tumor enhancement and suggests possible modification of chemical structure to overcome this barrier. These workers also supported further investigation into
manganese(III) porphyrin analogs as tumor enhancement agents. Lyon et al.\textsuperscript{29}, also proposed that it should be possible to find a manganese(III) water-soluble porphyrin which will be less toxic than those of [Mn\textsuperscript{III}(TPPS\textsubscript{4})]\textsuperscript{3-} and [Mn\textsuperscript{III}(TMPyP)\textsubscript{X}]. These researchers views have, thus, encouraged research into the design, synthesis and study of novel water-soluble manganese(III) porphyrins with large relaxivities and low toxicities.

The specific aim of this research was to synthesize and characterize a new family of water-soluble manganese(III) porphyrins with high relaxivities and low toxicities. These porphyrins and their metallated derivatives have been produced by incorporating new water-solubilizing poly-alcohol amine substituents on the periphery of the porphyrin core.\textsuperscript{40} These particular poly-alcohol substituents were suggested for use due to their successful (non-toxic) incorporation as water-solubilizing substituents for iodine X-ray contrast agents. The resulting molecules are neutrally charged, thereby decreasing ionic strength and vascular pain. In addition to analytical characterization, these new water-soluble manganese(III) porphyrin compounds have been clinically characterized as to their acute toxicity, and their NMR imaging potential has been evaluated in rats. The structures of the new manganese(III) porphyrin compounds of this study along with their abbreviations are shown directly below in Figure 1-7. Both [Mn\textsuperscript{III}(TPPIS)\textsubscript{Cl}] and [Mn\textsuperscript{III}(TPPAS)\textsubscript{Cl}] have had a patent application (U. S. Patent 733,568) completed for their possible use as new MRI contrast enhancement media.
Figure I-7. The New Water-Soluble Manganese(III) Porphyrin Compounds: Structures of 5,10,15,20-tetrakis[[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl]porphyrinatomanganese(III) Chloride, [Mn$^{III}$(TPPAS)Cl] and 5,10,15,20-tetrakis[[4-carboxylic acid-(2,3-dihydroxypropyl)amide]phenyl]porphyrinatomanganese(III) Chloride, [Mn$^{III}$(TPPIS)Cl].
Experimental

Materials and Methods

All solvents used were reagent grade or better. DMF (EM) was either spectral grade or freshly distilled under vacuum from Molecular Sieves after stirring for 1 hour. Methanol was distilled prior to use from Na metal under a N₂ blanket. H₂O was either deionized or triply distilled using a Millipore MILLI-Q system. 3-amino-1,2-propanediol was used as received from Bristol-Myers Squibb. 2-amino-1,3-propanediol was liberated from its oxalate salt which was supplied by Bristol-Myers Squibb. 4-carboxybenzaldehyde (Aldrich), diethyl ether (EM), MnCl₂·4 H₂O (Fisher) and ZnCl₂·4 H₂O (MCB) were used without further purification. Pyrrole (Aldrich) was distilled prior to use. SOCl₂ (Aldrich) was refluxed over flowers of sulphur and distilled under a blanket of N₂.

Column separations employed the use of several chromatographic columns (Ace Column #15) equipped with teflon coupling and a teflon Luer Drip adapter. Sephadex LH-20 and Sephadex G-50 were used as received. All reactions were carried out in Schlenk glassware, using N₂ or Ar to purge the reaction flask.

Spectroscopic, Analytical, and Magnetic Measurements

Solid state infrared spectra were recorded with either NaCl (range 4000-600 cm⁻¹), CsI (range 4000-200 cm⁻¹) plates or as KBr pellets (range 4000-600 cm⁻¹) using a Perkin-Elmer IR-1430 or Perkin-Elmer FTIR-1600 series spectrophotometer. Electronic spectra were recorded on a Cary-17 series spectrophotometer equipped with a Compaq personal computer and software for computerized data collection, using 1 cm pathlength matched quartz cells. The measurements were made using 10⁻⁴ and 10⁻⁵ M solutions scanning the range 900-250 nm. The ¹H and ¹³C NMR spectra were recorded on either an IBM/ Bruker
AF 250 or 300 MHz NMR spectrometer. Chloroform-d$_1$ (Cambridge Isotopes Laboratories) and D$_2$O (Cambridge Isotopes Laboratories) were 99.8%-D solvents used in the NMR studies. The molecular weights and fragmentation patterns of the material were confirmed by mass spectrometry using a Finnigan 9500 GC-MS instrument operating at 70 or 35 eV in the electron impact mode, or obtained commercially by the Analytical Chemistry Center at The University of Texas Medical School at Houston using fast-atom bombardment using a Kratos MS50TC mass spectrometer, calibrated with cesium iodide. The FAB mass spectra were obtained on compounds using a 3-nitrobenzyl alcohol (NBA) matrix. Solution conductivities were obtained using a Model 31 YSI conductivity bridge. Osmometry measurements were carried out at Bristol-Myers Squibb using a Wescor 5500 Vapor Pressure Osmometer.

Elemental analysis on key compounds were obtained from Galbraith Laboratories or in-house using a Carlo Erba Instruments NA 1500 series 2 analyzer with E.A.G.E.R. 100 software and card for an IBM personal computer. Metal analyses were obtained using a Perkin-Elmer Model 60 Atomic Absorption spectrophotometer.

Bulk samples sent to Bristol-Myers Squibb were further purified prior to toxicity and imaging experiments to remove trace manganese. This was accomplished either by use of a "Chelex" column (sodium form) using acetonitrile / H$_2$O as the eluent or by use of HPLC using a Rainin HPX Rapid HPLC interfaced with a Macintosh computer system using Dinamax software to run the HPLC. A Hamilton PRP-1 polymer based reverse phase column was used.

Magnetic susceptibilities of the solids at 293 K were obtained by the Faraday method, using a system described previously, except for having been modified with a Scientific Instruments Model 3800 temperature indicator/controller equipped with an LFE model 4427 voltmeter monitoring a calibrated Scientific Instruments Model Si-400 silicon
diode sensor. The diamagnetic corrections ($\chi_{dia}$ values given in cgs units x $10^6$) were those measured from the free porphyrin ligands: $[\text{Mn}^{III}(\text{TPPAS})\text{Cl} \cdot 2\text{H}_2\text{O}] = -464$;

$[\text{Mn}^{III}(\text{TPPIS})\text{Cl} \cdot 2\text{H}_2\text{O}] = -484$.

**Nuclear Magnetic Relaxation Rate Measurements**

The nuclear magnetic relaxation rates were measured over a range of magnetic field strengths corresponding to proton Larmor frequencies between 0.01 and 30 MHz using a field-cycling spectrometer described elsewhere.\textsuperscript{42,43} This instrument switches the current in a copper solenoidal magnet to vary the field according to a program that initially polarizes the spins at a high field, then switches to the magnetic field of interest (measured field) for a variable delay time, after which it switches to a resonance field corresponding to a proton Larmor frequency of 7.25 MHz where the remaining magnetization is measured by numerically integrating a spin echo or a free induction decay. Samples are contained in pyrex test tubes sealed with both a rubber stopper and a teflon screw cap. The sample chamber of the magnetic system is bathed in recirculating perchloroethylene that is thermostated using a Neslab RTE-8 temperature controller. Typically 32 points are taken on the magnetization decay curve and fitted to a single exponential using the software developed by Dr. Cathy C. Lester.\textsuperscript{44} The magnetic relaxation dispersion plots are generated by changing the measured field over the range of interest.

The $[\text{Mn}^{III}(\text{TPPAS})\text{Cl}]$ compound concentration was determined spectrophotometrically from the measured intensity of the 468 nm band. This concentration was then used in determining the relaxation rate as a function of field strength.

Relaxation rates at a constant field were measured using an IBM PC 20 MHz relaxometer. Acute toxicity, LD\textsubscript{50}, and actual animal magnetic resonance images were
obtained at Bristol-Myers Squibb using sprague dawley rats. Magnetic resonance images of the rats were obtained using a General Electric, 2 Tesla, 31 cm. bore magnetic resonance unit equipped with acquisition shielded gradients.

**Syntheses**

2-amino-1,3-propanediol or Serinol

Serinol oxalate 5.0 gm. (0.018 mole) was added over a period of 15 minutes in small portions with stirring at a temperature of 45 °C to a solution of 500 ml. isopropanol, 14.7 gm. sodium hydroxide (0.037 mole) and 30 ml. water. After addition was complete, the solution was maintained at 45 °C with stirring for 1.5 hours. During this time, the solution formed a suspension. This mixture was then cooled to 20 °C and the insoluble sodium oxalate was filtered off and subsequently washed 3 times (25 ml. each) with cool isopropanol. The filtrate and the washing were combined and the solvent removed under reduced pressure. The residue, a golden oil, was then dried under high vacuum at 55 °C for a period of 8 hours to obtain 2-amino-1,3-propanediol as a light yellow oil. Yield: 1.56 gm.; 95%.

$^1\text{H NMR (D}_2\text{O, 300 MHz) } \delta 2.73 (m, 1H), 3.37 (m, 4H)$. $^{13}\text{C DEPT NMR (D}_2\text{O, 300 MHz) } \delta 55.2, 65.2$ ppm.

3-amino-1,2-propanediol or Isoserinol

3-amino-1,2 propanediol was used as received from Bristol-Myers Squibb.

$^1\text{H NMR (D}_2\text{O, 300 MHz) } \delta 2.51 (m, 1H), 3.46 (m, 4H)$. $^{13}\text{C DEPT NMR (D}_2\text{O, 300 MHz) } \delta 45.2, 65.7, 75.1$ ppm.
5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin, H$_2$TPPC

The H$_2$TPPC acid derivative of H$_2$TPP was prepared according to the procedure of Longo, et al.\textsuperscript{45}. The synthetic scheme for the preparation of 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin, H$_2$TPPC, is outlined in Figure I-8. In a typical preparation, 1.39 ml. pyrrole (0.02 mole) was dissolved in 200 ml. propionic acid. To this solution, 3.00 gm. (0.02 mole) 4-carboxybenzaldehyde was added. The mixture was refluxed for 1 hour, cooled to room temperature, and placed in a freezer overnight. A brownish purple precipitate formed upon cooling. The resulting solid was washed 3 times (25 ml. each) with cool CH$_2$Cl$_2$. The solid was then air dried for a period of 1 hour. The solid was placed in a vial and dried under vacuum at 25° C over P$_2$O$_5$ for a period of 6 hours. Yield: 1.78 gm; 11%.

Anal. Caled. for C$_{48}$H$_{30}$N$_4$O$_8$·CH$_3$OH: C, 71.53; H, 4.16; N, 6.32. Found: C, 71.49; H 4.04; N, 6.32.

$^1$H NMR (CD$_3$OD, 300 MHz) $\delta$ 8.08 (m, 8H), 8.27 (m, 8H), 8.87 (br, 8H) (Figure A-3, Appendix A).

UV-vis in CH$_3$OH, nm ($\varepsilon$ in (M x 10$^3$ cm)$^{-1}$), 645 (6.73); 589 (8.59); 548 (13.8); 513 (26.1); 479 (7.79); 415 (307).

5,10,15,20-tetrakis(4-carboxyphenyl)porphyrinatomanganese(III) Chloride, [Mn$^{III}$(TPPC)Cl]

The metallation of the free porphyrin (Figure I-9) was accomplished via a modified procedure of Adler, et al.\textsuperscript{45} Originally, a 10:1 molar ratio of MnCl · 4 H$_2$O to H$_2$TPPC was employed as in Adler's work, but with consistently poor elemental analyses resulting from this protocol. Therefore, a 1:1 molar ratio of starting materials was used with success. Typically, 1.48 gm. H$_2$TPPC (1.87 x 10$^{-3}$ moles) and 0.38 gm. MnCl · 4 H$_2$O (1.91 x 10$^{-3}$ moles) were combined in 150 ml. DMF. The resulting mixture was refluxed
Figure I-8. The Preparation of 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin, $\text{H}_2\text{TPPC}$. 

\[
\begin{align*}
\text{H} & \quad + \quad \text{COOH} \\
\begin{array}{c}
\text{CHO} \\
\text{COOH}
\end{array} & \quad \uparrow \\
\text{Propionic Acid} \\
\text{Reflux, 2 hours} \\
\begin{array}{c}
\text{HOOC} \\
\text{COOH}
\end{array} & \quad \text{H}_2\text{TPPC}
\end{align*}
\]
Figure I-9. The Formation of 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrinato-
manganese(III) Chloride.

\[
\begin{align*}
\text{HOOC} & \quad \text{COOH} \\
\text{HOOC} & \quad \text{COOH}
\end{align*}
\]

\[
\begin{align*}
\text{MnCl}_2 \cdot 4\text{H}_2\text{O} & \quad \text{DMF} \\
\text{Reflux, 12 hours} & \quad \\
\text{HOOC} & \quad \text{COOH} \\
\text{HOOC} & \quad \text{COOH}
\end{align*}
\]

\[
[Mn^{III}(TPPC)Cl]
\]
overnight, the DMF then removed under reduced pressure, and the crude solid dried under vacuum. The solid was dissolved in methanol and filtered using a medium filter frit. The insoluble filtered material was discarded. The methanol filtrate was then evaporated under reduced pressure. The dark purple solid was collected and was dried over P₂O₅ at room temperature overnight on the vacuum line. Yield: 1.12 gm.; 68%.

**Anal. Calcd.** for C₄₈H₂₈N₄O₈MnCl·C₃H₇NO(DMF)·2 H₂O:  C, 61.98; H, 3.98; N, 7.09.  **Found:**  C, 62.09; H 3.97; N, 7.05.

UV- vis in CH₃OH, nm (ε in (M x 10⁻³ cm⁻¹)), 598 (1.85); 569 (2.05); 512 (1.65); 468 (23.3); 400 (13.2); 378 (13.3).

5,10,15,20-tetrakis[(4-carboxylic acid-(1,3-dihydroxyisopropyl)amide] phenyl]porphin, H₂TPPAS

The compound was prepared by reacting 0.26 gm. H₂TPPC (3.29 x 10⁻⁴ moles) with 0.25 ml. SOCl₂ (3.43 x 10⁻⁳ moles) in 250 ml. spectral grade DMF. This mixture was stirred at room temperature under a slow flow of N₂ for 3 hours to form the acid-chloride of the porphyrin. The DMF was then removed under reduced pressure. The remaining green solid (the acid-chloride porphyrin) was dried overnight under high vacuum at room temperature.

The acid-chloride porphyrin was then dissolved in 250 ml. methanol which had been freshly distilled from Na metal. Subsequently, 0.12 gm. of 2-amino-1,3-propanediol (serinol, 1.32 x 10⁻³ moles) was dissolved in freshly distilled methanol (ca. 10 ml.) and added to the acid chloride porphyrin solution. The reaction mixture was stirred at room temperature for 1.5 hours under a slow flow of N₂, after which the methanol was removed under reduced pressure. The resulting crude solid was dried overnight under high vacuum. The material was then purified by passing aliquots down a Sephadex LH-20 column using a 50:50 methanol:H₂O mixture as eluent. The pure, red band of H₂TPPAS followed a
blackish-green band. The reddish band was collected, reduced to dryness and recrystallized from methanol/diethyl ether to yield purple crystals. The purified material was dried under vacuum at 110 °C over P₂O₅ for a period of 24 hours. Yield: 0.09 gm.; 25%.

Anal. Calcd. for C₆₀H₅₈N₈O₁₂ · 3 CH₃OH · 5 H₂O:  C, 59.61; H, 6.35; N, 8.83.

Found:  C, 59.89; H, 5.47; N, 8.68.

¹H NMR (D₂O, 300 MHz) δ 3.24 (s, 4H), 3.55 (m, 16H), 7.28 (s, 8H), 8.06 (s, 8H), 8.55 (br, 8H) ppm. ¹³C DEPT NMR (D₂O, 300 MHz) δ 56.3, 60.9, 129.6, 137.5 ppm.

UV- vis in H₂O, nm (ε in (M x 10³ cm)⁻¹), 639 (5.18); 584 (6.61); 556 (8.60); 519 (10.8); 434 (42.6); 415 (351).

5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxypropyl)amide]phenyl)porphyrin, H₂TPPIS

The compound was prepared in generally the same manner as H₂TPPAS, but with slight modification. Typically, 0.15 gm. H₂TPPC (1.71 x 10⁻⁴ moles) was dissolved in 150 ml. spectral grade DMF under a slow flow of Ar. A reflux condenser was attached to the flask, 0.15 ml. SOCl₂ (3.79 x 10⁻³ moles) added to the solution, and the mixture was stirred with gentle heating (ca. 50°C) for 2 hours. The DMF was then removed under reduced pressure and the resulting green residue dried on the vacuum line.

The intermediate acid-chloride was then dissolved in 150 ml. methanol (freshly distilled from Na metal) under a slow flow of either Ar or N₂. Subsequently, 0.15 gm. of 3-amino-1,2-propanediol (isoserinol, 1.65 x 10⁻³ moles) was dissolved in a minimum amount of freshly distilled methanol and added to the reaction mixture. The mixture was stirred with gentle heating (ca. 50°C) for 2 hours, after which the methanol was removed under reduced pressure and the resulting dark purple solid dried on the vacuum line.

Purification of the porphyrin was accomplished utilizing a Sephadex LH-20 column with a
50:50 methanol:H₂O solvent system, followed by further purification by passing down a Sephadex G-50 column using H₂O as the eluent. The pure, reddish band of H₂TPPS followed a black band. The reddish band was collected, reduced to dryness and recrystallized from methanol/diethyl ether to yield purple crystals. The purified material was dried under vacuum at 110 °C over P₂O₅ for a period of 24 hours. Yield: 0.030 gm.; 32%.

Found: C, 62.07; H, 6.19; N, 9.63.

¹H NMR (D₂O, 300 MHz) δ 2.96 (m, 8H), 3.43 (s, 8H), 3.76 (s, 4H), 7.50 (s, 8H), 8.12 (s, 8H), 8.50 (br, 8H). ¹³C DEPT NMR (D₂O, 300 MHz) δ 44.3, 65.7, 70.4, 130.0, 137.8 ppm.

UV- vis in H₂O, nm (ε in (M x 10³ cm)⁻¹), 635 (4.51); 580 (5.94); 554 (7.00); 517 (12.3); 434 (64.5); 415 (337).

5,10,15,20-tetrakis([4-carboxylic acid-(1,3-dihydroxyisopropyl)amide] phenyl)porphyrinatomanganese(III) Chloride Dihydrate, [MnIII(TPPAS)Cl] · 2 H₂O

In a typical synthesis, 0.39 gm. [Mn(TPPC)Cl] (4.39 x 10⁻⁴ moles) was dissolved in 200 ml. spectral grade DMF. Thionyl chloride, 0.35 ml. (4.8 x 10⁻³ moles), was added slowly with stirring. The reaction mixture was then stirred for 3 hours under N₂ or Ar sparge. The DMF was removed under reduced pressure. The manganese acid-chloride porphyrin was then dried overnight under high vacuum.

This material was subsequently dissolved in 200 ml. of methanol which was freshly distilled from Na. A solution of 0.16 gm. of 2-amino-1,3-propanediol (1.8 x 10⁻³ moles) in freshly distilled methanol was added slowly with stirring. Again, the reaction flask was kept under a N₂ or Ar flow. The reaction was stopped and the methanol
removed by evaporation. The solid was then dried at room temperature over P₂O₅ overnight under high vacuum. This crude material was purified by passing aliquots down a Sephadex LH-20 column using a 50:50 methanol:H₂O mixture as eluent. Front and trailing fractions of the desired dark band were discarded. The desired manganese porphyrin was reduced to dryness and recrystallized from methanol/diethyl ether to yield dark crystals. The purified material was dried under vacuum at 110 °C over P₂O₅ for a period of 24 hours. Yield : 0.24 gm. ; 47%.

**Anal. Calcd.** for C₆₀H₅₆N₈O₁₂MnCl · 2 H₂O: C, 59.68; H, 5.01; N, 9.28; Mn, 4.55. **Found:** C 59.78; H, 4.91; N, 8.95; Mn, 4.71.

UV- vis in H₂O, nm (ε in (M x 10³ cm)⁻¹), 598 (8.89); 566 (11.1); 513 (7.57); 468 (84.2); 401 (53.2); 381 (53.0).

5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxypropyl)amide] phenyl)porphyrinatomanganese(III) Chloride Dihydrate, [Mn₃(PPPIS)Cl] · 2 H₂O

The compound was prepared in generally the same manner as [Mn₃(TPPAS)Cl], but with a few modifications. The acid-chloride intermediate was formed by the reaction of 0.25 gm. [Mn₃(TPPC)Cl] (2.84 x 10⁻⁴ moles) with 0.25 ml. SOCl₂ (3.41 x 10⁻³ moles) in 200 ml. spectral grade DMF. The reaction was carried out under a slow flow of Ar, with stirring and gentle heating (ca. 50°C), for 2 hours. The DMF was removed under reduced pressure, and the resulting solid was dried on the vacuum line.

The acid-chloride metalloporphyrin was then dissolved in 200 ml. methanol (freshly distilled from Na metal) and stirred under a slow flow of Ar. Subsequently, 0.15 gm. of 3-amino-1,2-propanediol (1.65 x 10⁻³ moles) was dissolved in a minimum amount of freshly distilled methanol and added to the reaction. The reaction was continued with gentle heating (ca. 40°C) for 2 hours, after which the methanol was removed under reduced
pressure and the resulting crude solid dried on the vacuum line. Purification was accomplished via a Sephadex LH-20 column employing a 50:50 methanol:H₂O solvent system. The metalloporphyrin eluted as a single dark band, from which the front and trailing fractions were discarded. The metalloporphyrin was further purified by passing aliquots down a Sephadex G-50 column using H₂O as the eluent. Again, front and trailing fractions were discarded. The desired manganese porphyrin was reduced to dryness and recrystallized from methanol/diethyl ether to yield dark crystals. The purified material was dried under vacuum at 110 °C over P₂O₅ for a period of 24 hours. Yield: 0.18 gm.; 54%.

**Anal. Calcd.** for C₆₀H₅₆N₄O₁₂MnCl·2 H₂O: C, 59.68; H, 5.01; N, 9.28; Mn, 4.55.
**Found:** C, 59.88; H, 5.22; N, 8.82; Mn, 4.69.

UV-vis in H₂O, nm (ε in (M x 10³ cm)⁻¹), 599 (6.06); 564 (7.44); 513 (7.32); 468 (31.4); 401 (26.4); 379 (27.2).

5,10,15,20-tetrakis(4-carboxyphenyl)porphyrinatozinc(II), [Zn(II)(TPPC)]

This material was synthesized analogously to 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrinatomanganese(III) chloride, but with the substitution of zinc chloride tetrahydrate for manganese chloride tetrahydrate. 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (H₂TPPC), 2.05 gm. (2.59 x 10⁻³ mole) and 0.70 gm. ZnCl₂·4 H₂O (5.14 x 10⁻³ mole) were combined in DMF and refluxed for 3 hours. DMF was then removed under reduced pressure with heating. The solid was dissolved in methanol and filtered. The filtrate evaporated to yield an oil, from which the desired [Zn(II)(TPPC)] was isolated by the addition of a large excess (ca. 500 ml.) of CH₂Cl₂ and subsequent filtration. The material was dried under vacuum at room temperature over P₂O₅ for a period of 24 hours. Yield: 1.10 gm.; 50%.
$^{1}$H NMR (CD$_3$OD, 300 MHz) δ 8.21 (m, 8H), 8.33 (m, 8H), 8.75 (s, 8H) (Figure A-4, Appendix A).

UV-vis in H$_2$O, nm ($\varepsilon$ in (M x 10$^3$·cm)$^{-1}$), 598 (2.60); 557 (5.74); 520 (1.32); 423 (161); 405 (14.0).

$5,10,15,20$-tetrakis[[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide] phenyl]porphyrinatozinc(II) Decahydrate, [Zn$^{II}$(TPPAS)]$\cdot$ 10 H$_2$O

The compound was prepared by dissolving 0.20 gm. [Zn$^{II}$(TPPC)] (2.34 x 10$^{-4}$ mole) in 150 ml. of spectral grade methanol. Thionyl chloride 0.20 ml. (2.74 x 10$^{-3}$ mole) was added under N$_2$ sparge. The solution immediately turned green. The solution was then stirred for 15 minutes, after which 2-amino-1,3-propanediol (serinol, neat) was added dropwise directly into the zinc-acid-chloride-porphyrin solution until the solution changed color to purple. The reaction was then stirred for one hour, after which the methanol was removed by evaporation under reduced pressure. The crude material was purified by chromatography using first a Sephadex LH-20 column with methanol:H$_2$O (50:50) as the eluent. The desired pink material trailed a dark colored band. The methanol/ water was removed, and the compound was run down a separate Sephadex G-50 column using only H$_2$O as the eluent. This desired purified material was collected after evaporation of H$_2$O and subsequent drying under vacuum at 110 °C over P$_2$O$_5$ overnight. Yield: 35 mg.; 4.3%.

Anal. Calcd. for C$_{60}$H$_{56}$N$_8$O$_{12}$Zn $\cdot$ 10 H$_2$O: C, 54.38; H, 5.78; N, 8.46; Zn, 4.82.

Found: C, 54.67; H, 5.32; N, 8.61; Zn, 4.73.

$^{1}$H NMR (D$_2$O, 300 MHz) δ 3.40 (m, 4H), 3.75 (m, 16H), 8.20 (s, 8H), 8.25 (s, 8H), 8.88 (br, 8H). $^{13}$C DEPT NMR (D$_2$O, 300 MHz) δ 56.2, 61.0, 129.3, 134.1, 136.7 ppm.
UV-vis in H$_2$O, nm ($\varepsilon$ in (M x 10$^3$ cm)$^{-1}$), 598 (6.71); 557 (12.7); 519 (3.80); 422 (355); 405 (52.0).

5,10,15,20-tetrakis[[4-carboxylic acid-(2,3-dihydroxypropyl)amide] phenyl]porphyrinatozinc(II) Trihydrate, [Zn$^{II}$(TPPIS)] · 3 H$_2$O

The compound was synthesized and purified identically to that of 5,10,15,20-tetrakis[[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl]porphyrinatozinc(II), with the appropriate substitution of 3-amino-1,2-propanediol (isoserinol) for 2-amino-1,3-propanediol (serinol). The compound was dried under vacuum at 110 °C over P$_2$O$_5$ overnight. Yield: 45 mg.; 4.4%.

Anal. Calcd. for C$_{60}$H$_{56}$N$_8$O$_{12}$Zn · 3 H$_2$O: C, 60.10; H, 5.21; N, 9.34; Zn, 5.33.
Found: C, 59.85; H, 5.42; N, 9.03; Zn, 5.17.

$^1$H NMR (D$_2$O, 300 MHz) δ 2.98 (m, 4H), 3.16 (s, 4H), 3.57 (s, 8H), 3.94 (s, 4H), 7.42 (s, 8H), 7.60 (s, 8H), 8.16 (br, 8H). $^{13}$C DEPT NMR (D$_2$O, 300 MHz) δ 44.0, 65.4, 70.1, 129.7, 133.1, 137.7 ppm.

UV-vis in H$_2$O, nm ($\varepsilon$ in (M x 10$^3$ cm)$^{-1}$), 645 (2.95); 598 (8.15); 557 (14.1); 519 (6.91); 422 (354).
Results and Discussion

The synthesis of 5,10,15,20-tetrakis\{[4-carboxylic acid-(2,3-dihydroxyisopropyl)-amide]phenyl\}porphyrin, H$_2$TPPIS, and its structural isomer 5,10,15,20-tetrakis\{[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl\}porphyrin, H$_2$TPPAS, have been carried out to provide water-soluble porphyrins utilizing poly-alcohol amine functionalities rather than cationic or anionic groups to achieve water solubility. These two non-metallated porphyrins provide a means for the formation of their two new water-soluble manganese(III) complexes, which are possible novel magnetic resonance imaging contrast enhancement agents. In addition, the free ligand compounds provide a means to characterize their structures by $^1$H NMR spectroscopy, which is not possible with the manganese(III) derivatives.

5,10,15,20-tetrakis\{[4-carboxylic acid-(2,3-dihydroxyisopropyl)-amide]phenyl\}porphyrin, H$_2$TPPIS

The synthesis of 5,10,15,20-tetrakis\{[4-carboxylic acid-(2,3-dihydroxyisopropyl)-amide]phenyl\}porphyrin, H$_2$TPPIS, was carried out as illustrated in Figure I-10. The 5,10,15,20-tetrakis[4-carboxylphenyl]porphyrin, H$_2$TPPC, is reacted with thionyl chloride in dimethylformamide in order to form the acid-chloride porphyrin intermediate. This acid-chloride intermediate is subsequently reacted with 3-amino-1,2-propanediol, isoserinol, in freshly distilled methanol to yield the desired 5,10,15,20-tetrakis\{[4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl\}porphyrin, H$_2$TPPIS. Attempts to characterize H$_2$TPPIS by FAB mass spectroscopy did not reveal a parent ion.

H$_2$TPPIS was also characterized in the solid state by infrared spectroscopy. The infrared spectrum of neat 3-amino-1,2-propanediol using KBr plates is shown in Figure I-11 and that for 5,10,15,20-tetrakis[4-carboxylphenyl]porphyrin, H$_2$TPPC, as a KBr pellet is shown in Figure I-12; both are shown for comparison purposes. The infrared spectrum
Figure I-10. The Synthesis of 5,10,15,20-tetrakis[(4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl)porphyrin, H₂TPPIS.
Figure I-11. The FT-IR Spectrum of 3-amino-1,2-propanediol, Isosertanol, Neat Using KBr Plates.
Figure I-12. The FT-IR Spectrum of 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin, H_2TPPC, as a KBr Pellet.
of isoserinol reveals a broad O-H stretch between 3600-3000 cm\(^{-1}\). In addition, there is a C-O stretch at 1000 cm\(^{-1}\). The infrared spectrum of \(H_2TPPC\), shown in Figure I-12, contains the \(C_\beta-H\) pyrrole bending modes (1099 cm\(^{-1}\) and 796 cm\(^{-1}\)).\(^{46}\) As one would expect C=O stretching occurs at 1695 cm\(^{-1}\), and C-O stretching occurs at 1401 cm\(^{-1}\) for \(H_2TPPC\). The infrared spectrum of 5,10,15,20-tetrakis\{[4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl\}porphyrin, \(H_2TPPIS\), as a KBr pellet is shown in Figure I-13. The infrared spectrum of \(H_2TPPIS\) also contains a broad O-H stretch between 3600-3000 cm\(^{-1}\). The N-H stretching of porphyrins normally seen at \(ca.\) 3300 cm\(^{-1}\) is not observed due to this broad O-H stretch. However, a N-H bending mode is observed at 964 cm\(^{-1}\). All amides show a carbonyl absorption band known as the amide I band. Its position (1600 cm\(^{-1}\) - 1500 cm\(^{-1}\)) depends on the degree of hydrogen bonding and thus the physical state of the compound. Primary amides display a band or bands in the region 1650 - 1515 cm\(^{-1}\) primarily due to N-H bending; this is known as the amide II band.\(^{47}\) The infrared spectrum of \(H_2TPPIS\) contains both the amide I and amide II bands as fairly strong absorptions at 1585 cm\(^{-1}\) and 1538 cm\(^{-1}\), respectively. The \(C_\beta-H\) pyrrole bending modes (1077 cm\(^{-1}\) and 793 cm\(^{-1}\)) are also observed for \(H_2TPPIS\) in Figure I-13. The O-H in-plane bending vibration occurs in the general area of 1420-1330 cm\(^{-1}\).\(^{47}\) This shows up for \(H_2TPPIS\) as a strong absorption at 1385 cm\(^{-1}\), due in part to coupling with other components such as C-H wagging.\(^{47}\)

The \(^1H\) NMR and \(^{13}C\)-DEPT NMR spectra for 3-amino-1,2-propanediol in D\(_2\)O are shown in Figure I-14. There are two types of protons present for 3-amino-1,2-propanediol, those methylene protons attached to \(C_1\) and \(C_3\) which are centered at 3.46 ppm and also that methine proton attached to \(C_2\) centered at 2.51 ppm. As indicated above, the \(^{13}C\)-DEPT NMR of 3-amino-1,2-propanediol contains three distinct signals in the \(^{13}C\) NMR. \(^{13}C\)-DEPT NMR (Distortionless Enhancement by Polarization Transfer)\(^{48}\)
Figure I-13. The FT-IR Spectrum of 5,10,15,20-tetrakis[(4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl]porphyrin, H₂TPPIS, as a KBr Pellet.
Figure I-14. The $^1$H NMR Spectrum and $^{13}$C DEPT Spectrum of 3-amino-1,2-propanediol, Isoserinol, in $\text{D}_2\text{O}$. 

$^1$H NMR

$^{13}$C DEPT NMR
distinguishes between CH$_3$, CH$_2$ and CH protons via proton polarization. In this case, selective pulses and delays eliminate quaternary carbon atom signals. In addition to signal enhancement, this experiment can distinguish odd and even protonated carbons using variable delays which shift the phases of the observed signals by 180° [CH$_3$ (↑), CH(↑), and CH$_2$(↓)]. The carbon containing the methine proton, C$_2$ at 75.1 ppm, is distinguished in Figure I-14 by being 180° out of phase, from C$_1$ (65.7 ppm) and C$_3$ (45.2 ppm). C$_1$ and C$_3$ are assigned based on the attachment of C$_3$ to a nitrogen heteroatom.

The $^1$H NMR spectrum of H$_2$TPPIS in D$_2$O is shown in Figure I-15. The pyrrole H$_8$ protons are broadened virtually into the baseline. This broadening of the pyrrole protons will be discussed in detail later. Assignments of individual signals were based on other free porphyrin systems$^{49}$ and integration of the signal area. H$_2$ and H$_3$ signals are located at 8.12 ppm and 7.50 ppm. H$_6$ and H$_6'$ can be distinguished one from another though their assignments are random and their multiplets are centered at 2.92 ppm and 2.98 ppm, respectively, with an apparent coupling constant of 11 Hz. H$_7$ is a multiplet centered at 3.76 ppm. Protons H$_8$/H$_8'$, which are not distinguishable from each other, are centered at 3.43 ppm. Proton H$_7$ is coupled to H$_6'$ and H$_6$ with apparent coupling constants (J) of 2.3 Hz and 7.5 Hz.

The $^{13}$C-DEPT NMR spectrum for H$_2$TPPIS is shown in Figure I-16. Like that of 3-amino-1,2-propanediol, the $^{13}$C DEPT NMR spectrum of H$_2$TPPIS shows the carbon atom containing the methine proton, C$_7$ (70.4 ppm) being 180° out of phase with those carbon atoms containing methylene protons, C$_6$ (44.3 ppm) and C$_8$ (65.7 ppm). The $^{13}$C-DEPT NMR spectrum also shows carbon atoms associated with that of the phenyl ring of the porphyrin. C$_2$ and C$_3$ (Figure I-16) appear at 137.8 ppm and 130.0 ppm, respectively. There is no signal observed for C$_8$, the outer pyrrole carbon atoms. C$_m$, the meso carbon atoms (the carbon atom to which the phenyl group is attached) and C$_\alpha$, the carbon atoms
Figure I-15. The $^1$H NMR Spectrum of 5,10,15,20-tetrakis[(4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl]porphyrin, H$_2$TPPIS, in D$_2$O.
Figure I-16. The $^{13}$C-DEPT NMR Spectrum of 5,10,15,20-tetrakis{(4-carboxylic acid-(2,3-dihydroxyisopropyl)amide)phenyl}porphyrin, H$_2$TPPIS, in D$_2$O.
directly adjacent to \( C_m \) do not appear in the \(^{13}\text{C}-\text{DEPT} \) NMR due to their being quaternary carbon atoms. However, neither \( C_\beta \), \( C_m \), nor \( C_\alpha \) appear in a normal \(^{13}\text{C} \) NMR spectrum. These signals are normally broad in porphyrin systems and are occasionally unobserved.\(^{49}\)

The electronic absorption spectrum for \( \text{H}_2\text{TPPIS} \) in \( \text{H}_2\text{O} \) is shown in Figure I-17. Like other non-metallated porphyrins,\(^{50}\) there is the presence of an intense Soret band at 415 nm (\( \varepsilon = 337 \text{ mM}^{-1} \cdot \text{cm}^{-1} \)) due to a \( \pi \rightarrow \pi^* \) transition. Also present are the \( \alpha \) and \( \beta \) bands\(^{50}\) at 554 nm (\( \varepsilon = 7.0 \text{ mM}^{-1} \cdot \text{cm}^{-1} \)) and 517 nm (\( \varepsilon = 12.3 \text{ mM}^{-1} \cdot \text{cm}^{-1} \)). The electronic absorption spectrum for \( 5,10,15,20\)-tetrakis[4-carboxyphenyl]porphyrin, \( \text{H}_2\text{TPPC} \) in methanol is shown in Figure I-18, for comparison purposes. The Soret band is located again at 415 nm (\( \varepsilon = 307 \text{ mM}^{-1} \cdot \text{cm}^{-1} \)). The \( \alpha \) and \( \beta \) bands are located at 548 nm (\( \varepsilon = 13.9 \text{ mM}^{-1} \cdot \text{cm}^{-1} \)) and 513 nm (\( \varepsilon = 26.1 \text{ mM}^{-1} \cdot \text{cm}^{-1} \)), respectively.

\[ 5,10,15,20\text{-tetrakis}[[4\text{-carboxylic acid-(1,3-dihydroxyisopropyl)-amide}]\text{phenyl}]\text{porphyrin, H}_2\text{TPPAS} \]

The synthesis of \( \text{H}_2\text{TPPAS} \), \( 5,10,15,20\)-tetrakis[[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl]porphyrin was carried out analogously to that of \( \text{H}_2\text{TPPIS} \), with the substitution of serinol, 2-amino-1,3-propanediol, for isoserinol, 3-amino-1,2-propanediol. The synthesis of \( \text{H}_2\text{TPPAS} \), \( 5,10,15,20\)-tetrakis[[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl]porphyrin is outlined in Figure I-19.

Attempts at FAB mass spectroscopy of \( \text{H}_2\text{TPPAS} \) did not reveal a parent ion. The purified \( \text{H}_2\text{TPPAS} \) was characterized in the solid state by infrared spectroscopy. As with \( \text{H}_2\text{TPPIS} \), the infrared spectrum of \( 5,10,15,20\)-tetrakis[[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl]porphyrin, \( \text{H}_2\text{TPPAS} \), as a KBr pellet contains a very broad O-H stretch between 3600-3000 cm\(^{-1}\), as seen in Figure I-20. Again the N-H stretching characteristic of porphyrins at \( \sim \) 3300 cm\(^{-1}\) is not seen due to the broad O-H
Figure I-17. The Electronic Absorption Spectrum for 5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl)porphyrin, H$_2$TPPIS, in H$_2$O.
Figure I-18. The Electronic Absorption Spectrum for 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin, H$_2$TPPC, in CH$_3$OH.
Figure I-19. The Synthesis of 5,10,15,20-tetrakis([4-carboxylic acid-(1,3-dihydroxyisopropyl) amide]phenyl)porphyrin, $\text{H}_2\text{TPPAS}$. 

$\text{H}_2\text{TPPC}$ + DMF + $\text{SOCl}_2$ + Methanol → $\text{H}_2\text{TPPAS}$
Figure I-20. The FT-IR Spectrum of 5,10,15,20-tetrakis([4-carboxylic acid-(1,3-dihydroxypropyl)amide]phenyl)porphyrin, H$_2$TPPAS, as a KBr Pellet.
stretch. However, the N-H bending mode associated with porphyrins is seen at 964 cm\(^{-1}\). The C\(_\beta\)-H pyrrole bending modes (1056 cm\(^{-1}\) and 794 cm\(^{-1}\)) are also seen in Figure I-20. Infrared bands associated with the amide I and amide II vibrations are seen at 1584 cm\(^{-1}\) and 1540 cm\(^{-1}\), respectively. The large absorption at 1388 cm\(^{-1}\) is due to an in-plane O-H bending vibration, again most likely coupled to C-H wagging. The infrared spectrum for 2-amino-1,3-propanediol, serinol, neat on a KBr plate is shown for comparison in Figure I-21. As with isoserinol, infrared spectrum of 2-amino-1,3-propanediol contains the very large and broad O-H stretch ca. 3400 cm\(^{-1}\).

The \(^1\)H NMR and \(^13\)C-DEPT NMR spectra for 2-amino-1,3-propanediol in D\(_2\)O are shown in Figure I-22. There are only two different sets of protons present for 2-amino-1,3-propanediol, those methylene protons attached to C\(_1\) and C\(_3\) which are symmetry related and are represented by a complex multiplet centered at 3.37 ppm and the methine proton (multiplet) attached to C\(_2\) centered at 2.73 ppm. This represents a highly second order spin system, AA'BB'C system. The methylene and methine proton region of the \(^1\)H NMR spectra in D\(_2\)O for 2-amino-1,3-propanediol was analyzed, to a first approximation, by using the computer program PANIC\(^{51}\) calculations were performed on the ASPECT 3000 computer of the IBM/Bruker AF-300 spectrometer. Because a rigorous analysis of the spin system was beyond the scope of this study, the iterative feature of the program was not used. The chemical shift differences and coupling constants of these methylene and methine protons, initially obtained from comparison to AA'BB' system values\(^{52}\) and experimental data, were manually adjusted and ultimately gave excellent agreement with the observed spectra, thus permitting an approximation of coupling constants. Figure I-23 shows a comparison between the actual \(^1\)H NMR spectrum for 2-amino-1,3-propanediol in D\(_2\)O in this region and that calculated based on a AA'BB'C system.
Figure I-21. The FT-IR Spectrum of 2-amino-1,3-propanediol, Serinol, Neat Using KBr Plates.
Figure I-22. The $^1$H NMR Spectrum and $^{13}$C DEPT Spectrum of 2-amino-1,3-propanediol, Serinol, in D$_2$O.

$^1$H NMR

$^{13}$C DEPT NMR
Figure 1-23. A Comparison between the $^1$H NMR Spectrum of 2-amino-1,3-propanediol, Serinol, in D$_2$O and that of the PANIC Calculated Spectrum for an A'A'B'B'C System in the Methylene Region.
Unlike 3-amino-1,2-propanediol, 2-amino-1,3-propanediol (serinol) contains two symmetric carbon atom types and therefore only two distinct signals in the \( ^{13} \text{C} \) NMR or \( ^{13} \text{C}-\text{DEPT} \) NMR spectrum. First there is the carbon atom containing the methine proton, \( C_2 \) at 55.2 ppm, and secondly there is a signal due to the symmetry related \( C_1 \) and \( C_3 \) at 65.2 ppm.

The \( ^1 \text{H} \) NMR spectrum of 5,10,15,20-tetrakis\{[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl\}porphyrin, H\(_2\)TPPAS in D\(_2\)O is shown in Figure I-24. Assignments of individual signals were based on other free porphyrin systems\(^{49} \) and integration of the signal area. Two distinct \( \beta \) pyrrole protons, \( H_{\beta 1} \) and \( H_{\beta 2} \), are observed as broadened signals respectively, at 8.55 ppm and 6.80 ppm. This signal broadening will be discussed later. \( H_2 \) and \( H_3 \) signals are broad but located at 8.06 ppm and 7.28 ppm, respectively. The signal for \( H_6 \) is located at 3.24 ppm. Equivalent protons \( H_7 \) and \( H_7^* \) are represented by a complex multiplet centered at 3.55 ppm. Apparent J values for geminal coupling are \( \text{ca.} \) 11 Hz between \( H_7/H_7^* \) and 6 Hz for vicinal coupling to \( H_6 \).

The \( ^{13} \text{C} \)-DEPT NMR spectrum of 5,10,15,20-tetrakis\{[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl\}porphyrin, H\(_2\)TPPAS, in D\(_2\)O is shown in Figure I-25. Like the \( ^{13} \text{C} \)-DEPT NMR spectrum of 2-amino-1,3-propanediol, the \( ^{13} \text{C} \)-DEPT NMR spectrum of H\(_2\)TPPAS shows a carbon containing a methine proton \( C_6 \) (56.3 ppm) being 180° out of phase with \( C_7/C_7^* \) (60.9 ppm). Figure I-25 also shows carbon atoms associated with the phenyl ring of the porphyrin. \( C_2 \) and \( C_3 \) appear at 137.5 ppm and 129.6 ppm, respectively. Again as with H\(_2\)TPPIS, there are no signals observed for \( C_\beta \). However, \( C_m \) and \( C_\alpha \) in the \( ^{13} \text{C} \) NMR spectrum are observed at 119.9 and 142.6 ppm, respectively.

The electronic absorption spectrum for 5,10,15,20-tetrakis\{[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl\}porphyrin, H\(_2\)TPPAS, in H\(_2\)O as seen in Figure I-26 is
Figure I-24. The $^1$H NMR Spectrum of 5,10,15,20-tetrakis[[4-carboxylic acid-(1,3-dihydroxypropyl)amide]phenyl]porphyrin, $\text{H}_2\text{TPPAS}$, in D$_2$O.
Figure I-25. The $^{13}$C-DEPT NMR Spectrum of 5,10,15,20-tetrakis[(4-carboxylic-acid-(1,3-dihydroxypropyl)amide]phenyl)porphyrin, H$_2$TPPAS, in D$_2$O.
Figure I-26. The Electronic Absorption Spectrum for 5,10,15,20-tetrakis([4-carboxylic acid-(1,3-dihydroxypropyl)amidophenyl])porphyrin, H₂TPPAS, in H₂O.
very similar to that of H$_2$TPPIS. The Soret band for H$_2$TPPAS is located at 414 nm with $\epsilon = 350$ mM$^{-1}$ cm$^{-1}$. Table I-2 compares the electronic absorption spectra for these non-metallated porphyrins and their metallated (Zn and Mn) derivatives, and related compounds of interest. The metallated compounds are discussed in detail below.

5,10,15,20-tetrakis[{4-carboxylic acid-(2,3-dihydroxyisopropyl)amide}-phenyl]porphyrinatozinc(II) Trihydrate, [Zn$^{II}$(TPPIS)] $\cdot$ 3 H$_2$O

The synthesis of [Zn$^{II}$(TPPIS)], 5,10,15,20-tetrakis[{4-carboxylic acid-(2,3-dihydroxyisopropyl)amide}phenyl]porphyrinatozinc(II) Trihydrate, was carried out in order to have a metallated water-soluble porphyrin utilizing poly-alcohol amine functionalities which could still be characterized by NMR due to Zn$^{II}$ being diamagnetic. It was also believed that these water-soluble zinc(II) porphyrins would give insight for the determination of $\tau_T$, the rotation/tumbling constant for this system. The synthesis was carried out by first inserting zinc(II) into 5,10,15,20-tetrakis[4-carboxylphenyl]porphyrin, H$_2$TPPC, and subsequently reacting this metalloporphyrin to form first the acid chloride and reacting this intermediate immediately with 3-amino-1,2-propanediol to form the desired poly-alcohol amine substituted metalloporphyrin.

Again as with the non-metallated porphyrin, H$_2$TPPIS, [Zn$^{II}$(TPPIS)] contains a very broad signal at ca. 3400 due to O-H stretching, as seen in its infrared spectrum as a KBr pellet in Figure I-27. Both amide I and amide II bands are present at 1600 cm$^{-1}$ and 1533 cm$^{-1}$, respectively. The C$_{\beta}$-H pyrrole bending modes (1072 cm$^{-1}$ and 790 cm$^{-1}$) are also seen. An O-H in-plane bending mode is seen as a strong sharp signal at 1380 cm$^{-1}$ in Figure I-27.

The $^1$H NMR spectrum for 5,10,15,20-tetrakis[{4-carboxylic acid-(2,3-dihydroxyisopropyl)amide}phenyl]porphyrinatozinc(II) Trihydrate, [Zn$^{II}$(TPPIS)], in D$_2$O
Table I-2. Electronic Absorption Spectral Data for Compounds of Interest.

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<sup>a</sup> in CH₃OH
<sup>b</sup> in H₂O
Figure I-27. The FT-IR Spectrum of 5,10,15,20-tetrakis[[4-carboxylic acid-(2,3-dihydroxypropyl)amide]phenyl]porphyrinatozinc(II), [Zn\textsuperscript{II}(TPPIS)], as a KBr Pellet.
Figure I-28. The $^1$H NMR Spectrum of 5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxypropyl)amide]phenyl)porphyrinatozinc(II), [Zn$^{II}$(TPPIS)] in D$_2$O.
is seen in Figure I-28. Assignments of individual signals were based on other zinc(II) porphyrin systems$^{50}$ and integration of the signal area. The H$_{8}$ pyrrole protons are seen as a broad signal centered at 8.16 ppm. PRESAT, a solvent supression routine was used to diminish the signal due to H$_{2}$O at ca. 4.6 ppm. The H$_{2}$ and H$_{3}$ proton signals are also shifted from their non-metallated positions of 8.12 ppm and 7.50 ppm to 7.60 ppm and 7.42 ppm, respectively. This is not unexpected as these signals shift in other zinc(II) porphyrin systems.$^{50}$ There are multiplets centered at 3.94 ppm, 3.57 ppm 3.16 ppm, and 2.98 ppm corresponding to H$_{7}$, H$_{8}$/H$_{9}$, H$_{6}'$ and H$_{6}$, respectively. Again as in the non-metallated porphyrin, H$_{2}$TPPIS, H$_{6}$ and H$_{6}'$ are assigned randomly, however, the assignment for [Zn$^{II}$(TPPIS)] remains consistent with that of H$_{2}$TPPIS. The apparent coupling constants between H$_{6}$ and H$_{6}'$ is ca. 10 Hz and both protons are coupled to H$_{7}$ with an apparent value of ca. 4 Hz. One also notes that the H$_{8}$ pyrrole protons are again somewhat broadened.

The $^{13}$C-DEPT NMR spectrum for 5,10,15,20-tetrakis{[4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl}porphyrinatozinc(II), [Zn$^{II}$(TPPIS)], in D$_{2}$O (Figure I-29) reveals that the C$_{8}$ carbon atom is seen at 133.1 ppm. As with the shift of H$_{8}$ and H$_{2}$ and H$_{3}$ protons in $^{1}$H NMR of zinc(II) porphyrins, sharpening of the C$_{8}$ signal in $^{13}$C NMR is seen in other metalloporphyrin systems as well.$^{53}$ C$_{2}$ and C$_{3}$ are observed at 135.5 ppm and 127.5 ppm, respectively. The $^{13}$C NMR spectrum of 5,10,15,20-tetrakis{[4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl}porphyrinatozinc(II), [Zn$^{II}$(TPPIS)], in D$_{2}$O seen in Figure I-30 shows C$_{m}$ is located at 119.2 ppm and C$_{o}$ also become a sharp signal at 148.2 ppm. The $^{13}$C-DEPT NMR spectrum of [Zn$^{II}$(TPPIS)] (Figure I-29) shows the carbon atom containing the methine proton C$_{7}$ at 70.1 ppm which is 180° out of phase with those carbon atoms containing methylene protons C$_{6}$ (44.0 ppm) and C$_{8}$ (65.4 ppm).
Figure I-29. The $^{13}$C-DEPT NMR Spectrum of 5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl)porphyrinatozinc(II), [Zn$^{II}$(TPPS)], in D$_2$O.
Figure I-30. The $^{13}$C NMR Spectrum of 5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxypropyl)amide]phenyl)porphyrinatozinc(II), [Zn$^{II}$(TPPIS)], in D$_2$O.
The electronic absorption spectrum for 5,10,15,20-tetrakis{[4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl}porphyrinatozinc(II) Trihydrate, [Zn^{II}(TPPIS)], in H_{2}O (Figure I-31) contains the porphyrin Soret band at 422 nm (ε = 353 mM^{-1}·cm^{-1}), representing a slight red shift relative to H_{2}TPPIS. There is also the α band at 596 nm (ε = 8.2 mM^{-1}·cm^{-1}) and the β band at 557 nm (ε = 14.1 mM^{-1}·cm^{-1}). The α / β band ratio is one method of determining whether a zinc(II) porphyrin is four or five coordinate. The fifth coordination site arises from the possible axial coordination of a neutral or anionic species. However, for [Zn^{II}(TPPIS)] the α / β ratio is 0.58 and the Soret band is not significantly red shifted indicating that the complex is indeed only four coordinate in D_{2}O.

5,10,15,20-tetrakis{[4-carboxylic acid-(1,3-dihydroxypropyl)amide]phenyl}porphyrinatozinc(II) Decahydrate, [Zn^{II}(TPPAS)] · 10 H_{2}O

The synthesis of [Zn^{II}(TPPAS)] was carried out analogously to that of [Zn^{II}(TPPIS)], with the appropriate substitution of 2-amino-1,3-propanediol (serinol), for 3-amino-1,2-propanediol (isoserinol). The low yields of both [Zn^{II}(TPPAS)] and [Zn^{II}(TPPIS)] are attributable to the fact that both were carried out entirely in methanol. The use of a single solvent (methanol) throughout the reaction process for the formation of the zinc(II) porphyrins was carried out in order to see if the number of steps in a scale-up of production of metallated product could be accomplished. It was clear that the reaction of non-metallated porphyrin with thionyl chloride to form the acid chloride porphyrin was hindered due to the large amount of water-insoluble material recovered after completion of the reaction supposedly [Zn^{II}(TPPC)]. This obviously indicates that the first reaction to form the acid-chloride intermediates should be carried out in a non-reacting solvent such as dimethylformamide (DMF), and thus a two solvent system using both DMF and methanol should be used in any scale-up of production of metallated material.
Figure I-31. The Electronic Absorption Spectrum for 5,10,15,20-tetrakis[4-carboxylic acid-(2,3-dihydroxypropyl)amide]phenyl]porphyrinato-zinc(II), [ZnII(TPPIS)], in H2O.
Like infrared spectrum of [Zn\textsuperscript{II}(TPPIS)], the infrared spectrum of [Zn\textsuperscript{II}(TPPAS)] contains the broad O-H stretching band at ca. 3400 cm\textsuperscript{-1}, Figure I-32. Both amide I and amide II bands are present at 1605 cm\textsuperscript{-1} and 1533 cm\textsuperscript{-1}, respectively. The C\textbeta-H pyrrole bending modes (1051 cm\textsuperscript{-1} and 795 cm\textsuperscript{-1}) are also seen. An O-H in-plane bending mode is seen at as a strong sharp signal at 1379 cm\textsuperscript{-1}. Table I-3 summarizes the infrared spectral results for the zinc(II) porphyrins, [Zn\textsuperscript{II}(TPPIS)] and [Zn\textsuperscript{II}(TPPAS)], their unmetallated precursors and also their inanganese(III) complexes.

The \textsuperscript{1}H NMR spectrum for 5,10,15,20-tetrakis([4-carboxylic acid-(1,3-dihydroxyisopropyl) amide]phenyl)porphyrinatozinc(II), [Zn\textsuperscript{II}(TPPAS)], in D\textsubscript{2}O is seen in Figure I-33. The H\textbeta pyrrole protons are seen as a broad signal centered at 8.55 ppm. Again, PRESAT, a solvent supression routine was used to diminish the signal due to H\textsubscript{2}O ca. 4.6 ppm. As is the case with [Zn\textsuperscript{II}(TPPIS)], the H\textsubscript{2} and H\textsubscript{3} protons for [Zn\textsuperscript{II}(TPPAS)] are also shifted from their non-metallated positions of 8.06 ppm and 7.28 ppm to 8.25 ppm and 8.20 ppm, respectively. The chemical shift for H\textsubscript{6} is a multiplet centered at 3.40 ppm compared to a value of 3.24 ppm for the free base porphyrin. The protons labeled H\textgamma/H\textgamma are again represented by the complex multiplet centered at 3.75 ppm (3.55 in the non-metallated precursor). Again, both of these zinc(II) porphyrins are complex spin systems represented by AA'BB'C. Apparent J values for geminal coupling of the H\textgamma/H\textgamma spin system is ca. 12 Hz and for vicinal coupling to H\textsubscript{6} ca. 5 Hz. Table I-4 summarizes the \textsuperscript{1}H NMR spectra for H\textsubscript{2}TPPIS, H\textsubscript{2}TPPAS, and their corresponding zinc derivatives and other relative compounds.

The \textsuperscript{13}C-DEPT NMR spectrum for [Zn\textsuperscript{II}(TPPAS)] in D\textsubscript{2}O is shown in Figure I-34. Like the \textsuperscript{13}C-DEPT spectra of 2-amino-1,3-propanediol and H\textsubscript{2}TPPAS, the \textsuperscript{13}C-DEPT NMR spectrum for [Zn\textsuperscript{II}(TPPAS)] contains a signal at 56.2 ppm, which is 180° out of phase with the signal at 61.0 ppm, which corresponds to the symmetrically equivalent
Figure I-32. The FT-IR Spectrum of 5,10,15,20-tetrakis([4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl)porphyrinatozinc(II), [Zn^{II}(TPPAS)], as a KBr Pellet.
Table I-3. Summary of Infrared Spectral Results (in cm$^{-1}$) as KBr Pellets for Compounds of Interest.

<table>
<thead>
<tr>
<th>Infrared Band</th>
<th>$H_2$TPPIS</th>
<th>[Zn$^{II}$TPPIS]</th>
<th>[Mn$^{III}$TPPIS]$Cl$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu$ O-H</td>
<td>3600-3300</td>
<td>3600-3300</td>
<td>3600-3300</td>
</tr>
<tr>
<td>Amide I</td>
<td>1585</td>
<td>1600</td>
<td>1605</td>
</tr>
<tr>
<td>Amide II</td>
<td>1538</td>
<td>1533</td>
<td>1539</td>
</tr>
<tr>
<td>$\delta$ C$\beta$-H</td>
<td>1077</td>
<td>1072</td>
<td>1077</td>
</tr>
<tr>
<td>$\delta$ C$\beta$-H</td>
<td>793</td>
<td>790</td>
<td>805</td>
</tr>
<tr>
<td>$\delta$ N-H</td>
<td>964</td>
<td>964</td>
<td>--</td>
</tr>
<tr>
<td>$\delta$ O-H</td>
<td>1385</td>
<td>1380</td>
<td>1384</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Infrared Band</th>
<th>$H_2$TPPAS</th>
<th>[Zn$^{II}$TPPAS]</th>
<th>[Mn$^{III}$TPPAS]$Cl$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu$ O-H</td>
<td>3600-3300</td>
<td>3600-3300</td>
<td>3600-3300</td>
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<tr>
<td>Amide I</td>
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<td>1605</td>
<td>1605</td>
</tr>
<tr>
<td>Amide II</td>
<td>1540</td>
<td>1533</td>
<td>1539</td>
</tr>
<tr>
<td>$\delta$ C$\beta$-H</td>
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<td>1051</td>
<td>1056</td>
</tr>
<tr>
<td>$\delta$ C$\beta$-H</td>
<td>794</td>
<td>795</td>
<td>805</td>
</tr>
<tr>
<td>$\delta$ N-H</td>
<td>964</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>$\delta$ O-H</td>
<td>1388</td>
<td>1379</td>
<td>1384</td>
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Figure I-33. The $^1$H NMR Spectrum of 5,10,15,20-tetrakis[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl]porphyrinatozinc(II), [Zn$^{II}$(TPPAS)], in D$_2$O.
Table I-4. $^1$H NMR Spectral Chemical Shifts (in ppm) Relative to D$_2$O for the Compounds of Interest.

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<td>Methine H</td>
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<tr>
<td>3.37</td>
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2-amino-1,3-propanediol

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<th>Assignment</th>
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<tbody>
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<td>3.46</td>
<td>Methylene H's</td>
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3-amino-1,2-propanediol

H$_2$TPPAS

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<td>3.55</td>
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<td>6.80(br)</td>
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<tr>
<td>7.28</td>
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</tr>
<tr>
<td>8.06</td>
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<tr>
<td>8.55 (br)</td>
<td>H$_{61}$</td>
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H$_2$TPPIS

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<td>H$<em>{62}$/H$</em>{62}'$</td>
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<td>3.76</td>
<td>H$_7$</td>
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<td>H$_2$</td>
</tr>
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<td>8.50(br)</td>
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</table>
Table I-4 (Continued).  

$^{1}H$ NMR Spectral Chemical Shifts (in ppm) Relative to $D_2O$ for the Compounds of Interest.

![Chemical Structures]

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<th>$\delta$</th>
<th>Assignment</th>
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<td>3.75</td>
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<td>H$_6'$</td>
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<td>8.20</td>
<td>H$_3$</td>
<td>3.57</td>
<td>H$_g$/H$_g'$</td>
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<tr>
<td>8.25</td>
<td>H$_2$</td>
<td>3.94</td>
<td>H$_7$</td>
</tr>
<tr>
<td>8.88 (br)</td>
<td>H$_{8}$</td>
<td>7.42</td>
<td>H$_3$</td>
</tr>
<tr>
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<td>7.60</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>8.16 (br)</td>
<td>H$_{8}$</td>
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Figure I-34. The $^{13}$C-DEPT NMR Spectrum of 5,10,15,20-tetrakis[[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl]porphyrinatozinc(II), [Zn$^{II}$(TPPAS)], in D$_2$O.
carbon atoms C7/C7. The 13C NMR spectrum for [ZnII(TPPAS)] in D2O is shown in Figure I-35. The Cα again appears as a sharp signal at 149.5 ppm. C2, Cβ and C3 are seen at 135.2 ppm, 131.9 ppm, and 127.0 ppm, these also correspond to those signals at 136.7 ppm, 134.1 ppm, and 129.3 ppm in the 13C-DEPT NMR (Figure I-34). Chemical shifts for C1, C4 and Cm appear at 145.4 ppm, 134.4 ppm, and 120.2 ppm, respectively, in the 13C NMR for [ZnII(TPPAS)] in Figure I-35. Table I-5 and Table I-6 summarizes the 13C-DEPT NMR and 13C NMR, respectively, for H2TPPIS, H2TPPAS, and their corresponding zinc derivatives and other related compounds.

The electronic absorption spectrum for [ZnII(TPPAS)] in H2O shown in Figure I-36 is very similar to that of [ZnII(TPPIS)], however, there are some differences. First there is no absorption band at 645 nm for [ZnII(TPPAS)], whereas [ZnII(TPPIS)] contains a band at this position with (ε = 2.95 mM⁻¹ cm⁻¹). Secondly, the ε value for the peak at 519 for [ZnII(TPPAS)] is approximately one half of the ε value for [ZnII(TPPIS)] (3.8 mM⁻¹ cm⁻¹ versus 6.8 mM⁻¹ cm⁻¹). These subtle differences in spectra are again most likely due to the structural differences of the differing functionalities on the periphery of the porphyrin, since there are no other differences in the two compounds. The Soret bands for both [ZnII(TPPAS)] and [ZnII(TPPIS)] in H2O are nearly identical in both position and ε value (Table I-2). The α band is located at 597 nm (ε = 6.7 mM⁻¹ cm⁻¹) and the β band is located at 557 (ε = 12.7 mM⁻¹ cm⁻¹). The α / β ratio is 0.53. This value taken with the fact that the electronic spectrum of [ZnII(TPPAS)] is not red shifted indicates that [ZnII(TPPAS)] is four coordinate in H2O.54

5,10,15,20-tetakis{[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl}porphyrin atomanganese(III) Chloride Dihydrate, [MnIII(TPPAS)Cl] · 2 H2O

The synthesis of 5,10,15,20-tetakis{[4-carboxylic acid-(1,3-dihydroxyisopropyl)-amide]phenyl}porphyrin atomanganese(III) Chloride Dihydrate, [MnIII(TPPAS)Cl] · 2 H2O
Figure I-35. The $^{13}$C NMR Spectrum of 5,10,15,20-tetrakis[[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl]porphyrinatozinc(II), [Zn$^{II}$TPPAS], in D$_2$O.
Table I-5. $^{13}$C-DEPT NMR Spectral Chemical Shifts (in ppm) for the Compounds of Interest in D$_2$O.

### 2-amino-1,3-propanediol

<table>
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<tr>
<th>δ</th>
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<tbody>
<tr>
<td>55.2</td>
<td>C$_2$</td>
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<tr>
<td>65.2</td>
<td>C$_1$ / C$_3$</td>
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### 3-amino-1,2-propanediol

<table>
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<td>75.1</td>
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### H$_2$TPPAS

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<td>60.9</td>
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### H$_2$TPPIS

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Table I-5 (Continued).  

$^{13}$C-DEPT NMR Spectral Chemical Shifts (in ppm) for the Compounds of Interest in D$_2$O.

![Chemical Structures](image)

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<tbody>
<tr>
<td>56.2</td>
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<tr>
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<td>C$_3$</td>
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Table I-6. $^{13}$C NMR Spectral Chemical Shifts (in ppm) for the Compounds of Interest in D$_2$O.

2-amino-1,3-propanediol

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<tbody>
<tr>
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<td>65.2</td>
<td>C$_1$ / C$_3$</td>
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3-amino-1,2-propanediol

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![H$_2$TPPAS](image1)

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<td>C$_m$</td>
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![H$_2$TPPIS](image2)

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Table I-6 (Continued). $^{13}$C NMR Spectral Chemical Shifts (in ppm) for the Compounds of Interest in D$_2$O.

![Chemical Structures]

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</table>
Figure I-36. The Electronic Absorption Spectrum for 5,10,15,20-tetrakis[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl]porphyrinato-zinc(II), [Zn^{II}(TPPAS)], in D_{2}O.
was carried out as shown in Figure I-37. Two different approaches were attempted for the synthesis of [Mn$^{III}$(TPPAS)Cl]. The initial approach taken was that in which 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrinatomanagenese(III) Chloride, [Mn$^{III}$(TPPC)Cl], was used as the starting material and formation of the final product carried out as outlined in Figure I-37. Typical yields after purification using Sephadex LH-20 chromatography were ca. 50% utilizing this method. The second approach taken was that of the insertion of manganese(III) into the unmetallated water-soluble H$_2$TPPAS by refluxing H$_2$TPPAS and MnCl$_2$·4H$_2$O in dimethylformamide. After purification by Sephadex LH-20 chromatography, the yield of [Mn$^{III}$(TPPAS)Cl] was ca. 25%.

[Mn$^{III}$(TPPAS)Cl], 5,10,15,20-tetrakis{[4-carboxylic acid-(1,3-dihydroxyisopropyl)amide]phenyl}porphyrinatomanagenese(III) Chloride Dihydrate, was characterized in the solid as a KBr pellet by infrared spectroscopy (Figure I-38). The infrared spectrum of 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrinatomanagenese(III) Chloride, [Mn$^{III}$(TPPC)Cl], as a KBr pellet is shown in Figure I-39 for comparison. As with the infrared spectra of both the water-soluble unmetallated and zinc porphyrins, [Mn$^{III}$(TPPAS)Cl], shows a broad signal at ca. 3400 cm$^{-1}$ due to O-H stretching. The amide I and amide II bands are present at 1605 cm$^{-1}$ and 1540 cm$^{-1}$, respectively. The C$_{\beta}$-H pyrrole bending modes are located at 1056 cm$^{-1}$ and 805 cm$^{-1}$. Again an O-H in-plane bending mode is seen at 1384 cm$^{-1}$. For [Mn$^{III}$(TPPC)Cl], the C$_{\beta}$-H pyrrole bending modes are located at 1080 cm$^{-1}$ and 801 cm$^{-1}$. In addition, an attempt was made to characterize [Mn$^{III}$(TPPAS)Cl] in the solid-state by FAB mass spectrometry. Several attempts were made using various sample matrices with no mass greater than ca. 900 m/e being observed. However, while no parent ion (M$^+$ = 1172.2 m/e) is seen, a fragment corresponding to [M$^+$-2H-Cl] (M = 1133.2 m/e) was observed using 1% trifluoroacetic acid in a 3-nitrobenzylalcohol matrix (Figure
Figure I-37. The Synthesis of 5,10,15,20-tetrakis[(4-carboxylic acid-(1,3-dihydroxyisopropyl)amide)phenyl]porphyrinatomanganese(III) Chloride, [Mn$^{III}$(TPPAS)Cl].
Figure I-38. The FT-IR Spectrum of 5,10,15,20-tetrakis([4-carboxylic acid-(1,3-dihydroxypropyl)amide]phenyl)porphyrinomanganese(III) Chloride, [Mn$^{III}$(TPPAS)Cl], as a KBr Pellet.
Figure I-39. The FT-IR Spectrum of 5,10,15,20-tetrakis(4-carboxyphenyl) porphyrinatomanganese(III) Chloride, [Mn^{III}(TPPC)Cl], as a KBr Pellet.
A measurement of the ionic strength of a $5 \times 10^{-2}$ M solution of [Mn$^{III}$(TPPAS)Cl] resulted in a value of 120 mOsmol/kg-H$_2$O. The expected value was 60 mOsmol/kg-H$_2$O for a non-dissociated axial chloride anion. This value implies that the porphyrin is a 1:1 electrolyte in aqueous solution$^{55}$ and thus the [Mn$^{III}$(TPPAS)]$^+$ cation exists in H$_2$O and not [Mn$^{III}$(TPPAS)Cl]. The osmolality of blood is ca. 300 mOsmol/kg-H$_2$O. The osmolality of the "pain threshold" for vascular pain is generally considered to be ca. 600 - 700 mOsmol/kg-H$_2$O. Clinical doses of 0.5 M NMG$_2$[Gd$^{III}$(DTPA)] have an osmolality significantly above this ca. 2.0 x $10^3$ mOsmol/kg-H$_2$O. Thus, [Mn$^{III}$(TPPAS)Cl] should not cause vascular pain when injected.

This manganese(III) porphyrin thus allows for a six-coordinate manganese(III) center in H$_2$O providing two trans sites for H$_2$O molecule coordination and exchange, i.e. [Mn$^{III}$(TPPAS)(H$_2$O)$_2$]$^+$ in this case. This situation becomes possible if the chloride ion is dissociated in aqueous solvents. There are several examples of six-coordinate manganese(III) porphyrins whose crystal structures are known including a bis-methanol complex where the methanol groups are bound to the manganese(III) center trans to one another.$^{65}$ Electrical conductivity measurements at 25$^\circ$ C for a 1 x $10^{-3}$ M solution gave a molar conductivity of 143 mho $\cdot$ M$^{-1}$. Again, this indicates that [Mn$^{III}$(TPPAS)Cl] is a univalent electrolyte in aqueous solution, suggesting [Mn$^{III}$(TPPAS)(H$_2$O)$_2$]$^+$. A corresponding molar conductance of a $10^{-3}$ M KCl solution yielded a result of 157 mho $\cdot$ M$^{-1}$. A saturated solution of [Mn$^{III}$(TPPAS)Cl] was also prepared in order to determine an appropriate maximum solubility. The measured absorption of the 599 nm band [Mn$^{III}$(TPPAS)Cl] yielded a solubility of 63 mmol / L.

The oxidation state [Mn$^{III}$(TPPAS)Cl] has been confirmed by a magnetic susceptibility measurement. The magnetic moments of manganese(III) porphyrins are
generally in the 4.8 B.M. - 5.0 B.M. range, a value consistent with a high-spin d^4 (S=2) metal center.\textsuperscript{56} The measured room temperature (298 K) magnetic moment of a solid sample of [Mn\textsuperscript{III}(TPPAS)Cl] was 4.85 B.M. Again, this value is entirely consistent with high-spin d^4 manganese(III).

The electronic absorption spectrum of [Mn\textsuperscript{III}(TPPAS)Cl] in H\textsubscript{2}O shown in Figure I-40 displays split Soret bands typical of manganese(III) porphyrins at ca. 400 nm and 460 nm. Actual ε values for [Mn\textsuperscript{III}(TPPAS)Cl] Soret bands are 84.1 mM\textsuperscript{-1} cm\textsuperscript{-1} (λ = 468 nm) and 39.4 mM\textsuperscript{-1} cm\textsuperscript{-1} (λ = 419 nm) (See Table I-2). The electronic absorption spectrum for [Mn\textsuperscript{III}(TPPCl)] in CH\textsubscript{3}OH is shown in Figure I-41 for comparison. The Soret band of [Mn\textsuperscript{III}(TPPS\textsubscript{4})]\textsuperscript{3-}, the manganese(III)tetrakis(4-sulfophenyl)porphine ion, in H\textsubscript{2}O is located at 466 nm.\textsuperscript{30}

The absorption spectrum of manganese(III) porphyrins can be attributed to a strong metal-porphyrin π interaction.\textsuperscript{56} For metal ions with effective D\textsubscript{4h} symmetry, the e\textsubscript{g} (d\textsubscript{xy},d\textsubscript{yz}) orbital is of the proper symmetry to interact with the e\textsuperscript{*}g(π) orbital of the porphyrin. There is strong sigma interaction between the metal and the four pyrrole nitrogen donors. However, π back-bonding from the metal to the porphyrin does occur. Theoretical calculations predict that manganese d orbitals and porphyrin π energy levels match in energy. Therefore, manganese(III) porphyrins can show strong metal e\textsubscript{g}(π) porphyrin e\textsuperscript{*}g(π) mixing. A qualitative molecular orbital energy level diagram taken from Boucher\textsuperscript{56} is shown in Figure I-42. The visible absorption band assignments for [Mn\textsuperscript{III}(TPPAS)Cl] for the Soret band (Band V) at 468 nm corresponds to an a\textsubscript{2u},b\textsubscript{2u} → e\textsubscript{g} charge transfer. The assignments of the bands at 419 nm and 401 nm, respectively, are based on a\textsubscript{1u},a\textsubscript{2u} → e\textsuperscript{*}g and a\textsuperscript{′}2u, b\textsubscript{2u} → e\textsubscript{g} transitions.\textsuperscript{56}
Figure I-40. The Electronic Absorption Spectrum for 5,10,15,20-tetrakis[[4-carboxylic acid-(1,3-dihydroxypropyl)amide] phenyl]porphyrinato-manganese(III) Chloride, [Mn^{III}(TPPAS)Cl], in D_2O.
Figure I-41. The Electronic Absorption Spectrum for 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrinatomanganese(III) Chloride, [Mn$^{II}$(TPPCl)], in CH$_3$OH.
Figure I-42. Qualitative Molecular Orbital Level Diagram and Visible Absorption Assignments for Manganese(III) Porphyrins.\textsuperscript{56}

\begin{align*}
\text{Band I} & \quad a_{2u}, a_{1u} \rightarrow e_g \\
\text{Band II} & \quad a_{2u} \rightarrow a_{1g} \\
\text{Band III} & \quad a_{2u}, a_{1u} \rightarrow e_g \\
\text{Band IV} & \quad a_{2u}, a_{1u} \rightarrow e^* g \\
\text{Band V} & \quad b_{2u}, a'_{2u} \rightarrow e_g \\
\text{Band V a} & \quad a'_{2u} \rightarrow a_{1g} \\
\text{Band VI} & \quad a_{2u}, a_{1u} \rightarrow e^* g \quad b_{2u}, a'_{2u} \rightarrow e_g
\end{align*}
5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]-phenyl)porphyrinatomanganese(III) Chloride Dihydrate, [Mn^{III}(TPPIS)Cl] \cdot 2 \text{H}_2\text{O} \\

The synthesis of 5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]-phenyl)porphyrinatomanganese(III) Chloride Dihydrate, [Mn^{III}(TPPIS)Cl] \cdot 2 \text{H}_2\text{O}, was carried out analogously to that of [Mn^{III}(TPPAS)Cl] with the substitution of 3-amino-1,2-propanediol (isoserinol) for that of 2-amino-1,3-propanediol (serinol). The synthetic scheme for the formation of [Mn^{III}(TPPIS)Cl] is shown in Figure I-43. Based on the information obtained regarding product yield of [Mn^{III}(TPPAS)Cl] for the two different synthetic approaches, the synthesis of [Mn^{III}(TPPIS)Cl] originated from [Mn^{III}(TPPC)Cl] rather than the non-metallated precursor, \text{H}_2\text{TPPIS}. No attempt was made to insert manganese(III) into the non-metallated water-soluble porphyrin, \text{H}_2\text{TPPIS}. Typical yields of [Mn^{III}(TPPIS)Cl] using the outlined protocol after purification by Sephadex LH-20 / G-50 chromatography is ca. 50%.

[Mn^{III}(TPPIS)Cl], 5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]-phenyl)porphyrinatomanganese(III) Chloride Dihydrate, was characterized in the solid state as a KBr pellet by infrared spectroscopy (Figure I-44). The infrared spectrum of [Mn^{III}(TPPIS)Cl] contains the broad O-H stretch at ca. 3400 cm\(^{-1}\). The spectrum also contains both the amide I and amide II bands at 1605 cm\(^{-1}\) and 1539 cm\(^{-1}\), respectively. The \text{C} = \text{H} pyrrole bending modes are seen at 1056 cm\(^{-1}\) and 805 cm\(^{-1}\).

[Mn^{III}(TPPIS)Cl] was also characterized in the solid state by FAB mass spectrometry. Like [Mn^{III}(TPPAS)Cl], no parent ion (M\(^{+}\) = 1172.2 m/e) was seen for [Mn^{III}(TPPIS)Cl], however, a fragment corresponding to [M\(^{+}\) - 2\text{H}-\text{Cl}] = 1133.2 m/e was finally observed using a 1% trifluoroacetic acid / NBA matrix (Figure A-2, Appendix A).

As was the case with [Mn^{III}(TPPAS)Cl], an electrical conductivity measurement at 25 \(^\circ\)C for a 1 \times 10^{-3} \text{M} solution of [Mn^{III}(TPPIS)Cl] yielded a result of 139 mho \cdot \text{M}^{-1}. This value indicates that [Mn^{III}(TPPIS)Cl] is a univalent electrolyte in aqueous solution.
Figure I-43. The Synthesis of 5,10,15,20-tetrakis[[4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl]porphyrinatomanganese(III) Chloride, [Mn\textsuperscript{III}(TPPIS)Cl].

\[
\begin{align*}
\text{[Mn(TPPC)Cl]} + \\
\text{DMF} + \text{SOCl}_2 \\
\text{50\degree C} + \text{Ar} \\
\rightarrow \\
\text{Methanol} + \\
\text{40\degree C} + \text{Ar} \\
\rightarrow \\
\text{[Mn\textsuperscript{III}(TPPIS)Cl]}
\end{align*}
\]
Figure 1-44. The FT-IR Spectrum of 5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl)porphyrinomanganese(III) Chloride, [Mn^{III}(TPPIS)Cl], as a KBr Pellet.
(10^{-3} \text{ M KCl} = 157 \text{ mho} \cdot \text{M}^{-1}). \text{ No measurement of the osmolality of } [\text{Mn}^{III}(\text{TPPIS})\text{Cl}] \text{ was carried out, however it is pointed out that the osmolality should not be dramatically different from } [\text{Mn}^{III}(\text{TPPAS})\text{Cl}] \text{ in light of the conductivity results for } [\text{Mn}^{III}(\text{TPPIS})\text{Cl}]. \text{ A tentative maximum solubility measurement was determined to be } 65 \text{ mmol/L in a manner analogous to that for } [\text{Mn}^{III}(\text{TPPAS})\text{Cl}].

The magnetic susceptibility for a solid sample of [Mn^{III}(TPPIS)Cl] measured at room temperature (298 K) was 4.89 B.M. This value is again entirely consistent with high-spin d^4 manganese(III).

The electronic absorption spectrum of [Mn^{III}(TPPIS)Cl] in H_2O shown in Figure 1-45 displays the typical manganese(III) porphyrin split Soret bands at ca. 400 nm and 460 nm. Actual \( \varepsilon \) values for [Mn^{III}(TPPIS)Cl] Soret bands are 31.4 mM \cdot cm^{-1} (\( \lambda = 468 \text{ nm} \)) and 23.4 mM \cdot cm^{-1} (\( \lambda = 418 \text{ nm} \)), other \( \lambda_{max} \) values are seen in Table I-2. As with [Mn^{III}(TPPAS)Cl], the assignment of the Soret band for [Mn^{III}(TPPIS)Cl] at 468 nm. corresponds to an \( a'_{2u},b_{2u} \rightarrow e_g \) charge transfer.

**Variable-Temperature ^1H NMR Study**

The variable-temperature ^1H NMR spectrum of the H_8-pyrrole and phenyl region for H_2TPPIS in D_2O is shown in Figure I-46. Note that as temperature was increased the shoulder (H_81) on the side of the signal at ca. 8.0 ppm (H_2) became more distinct in the temperature range of 280 - 295 K. This shoulder appears to be most prominent at 290 K. With increasing temperature, it was observed that this shoulder collapsed into the larger signal (H_2). This larger signal's base broadened (H_2) while the overall appearance sharpens as the temperature is increased. Also, with increasing temperature the chemical shift assigned to H_82 at ca. 6.0 ppm sharpened to 295 K and then as the temperature was
Figure I-45. The Electronic Absorption Spectrum for 5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxyisopropyl)amide]phenyl)porphyrinato-manganese(III) Chloride, [Mn^{III}(TPPIS)Cl], in H_{2}O.
Figure I-46. 300-MHz Variable-Temperature $^1$H NMR Spectra of the Phenyl Region for 5,10,15,20-tetrakis([4-carboxylic acid-(2,3-dihydroxypropyl)amide]-phenyl)porphyrin, H$_2$TPPIS, in D$_2$O.
further increased this signal broadened into the baseline. Reappearance of the H_p2 signal was observed as the sample was cooled back to 295 K.

The chemical shift signal associated with H_3 was broadened relative to porphyrin phenyl proton signals in other solvents, but this signal does not become increasingly broad nor sharpened over the temperature range studied. Therefore, the slight broadness of this signal is most probably due to solvent interaction.

The extreme broadening and shifting of the H_8 protons cannot be due to nuclear quadrupolar relaxation because of both the distance of the H_8 protons from a possible 14N quadrupolar nucleus and the fact that an opposite temperature effect is usually observed for nuclear quadrupolar relaxation processes (the spectrum should sharpen as temperature increases.)\textsuperscript{57} Another possibility for this broadening includes some slow exchange process, since the only observable effect in the NMR spectrum of slow exchanging systems is the line broadening of the resonances involved in the exchange. The effect of an "intermediate" exchange rate is first to broaden the individual lines and then to draw them closer to eventually coalesce into a single peak. Finally this single signal sharpens as the exchange rate increases.\textsuperscript{58} This "intermediate" exchange rate cannot be the case for H_2TPPIS or H_2TPPAS as the peak at ca. 8.0 ppm did not sharpen as the temperature was increased (increasing the exchange rate). A final possibility, which seems the most likely, is that there exists multiple forms of aggregation among the porphyrin molecules. Upon initial observation of the variable temperature NMR spectra, Figures I-46 and I-47, it was noted that the H_{P1} and H_{P2} signals were diminished in intensity at the same rate. It was first believed that H_{P2} was perhaps the amide (N-H) proton. However, upon further investigation, a single frequency saturation NMR experiment at 295 K revealed that irradiation of the signal H_{P1} led to a diminishment of intensity of H_{P2}, and vice versa for both H_2TPPIS or H_2TPPAS. Also, the irradiation of the H_8 protons did not result in any
other changes in the $^1$H NMR spectrum. This leads to the conclusion that the $H_{B1}$ and $H_{B2}$ signals are somehow interrelated. Kellar et. al.\textsuperscript{59}, have recently investigated the effect of Mn(III) porphyrin aggregates on NMRD relaxation rates where aggregation becomes possible at higher concentrations. Analogously, aggregation could be occurring at the concentrations of these variable temperature $^1$H NMR experiments on these free porphyrins\textsuperscript{60}, thus providing at least two aggregate types and two distinct $\beta$ pyrrole proton types, $H_{B1}$ and $H_{B2}$ (Figure I-46 and Figure I-47). This reasoning would explain the two separate signals disappearing and reappearing in concert with one another with changing temperature. In addition, two different types of aggregation would explain the results of the single frequency saturation transfer experiments. Finally, the concept of multiple forms of aggregation would explain the broadness of signals which are observed for $H_{B1}$ and $H_{B2}$ and not the other signals present. A second rational for the broadness of the $H_{B1}$ and $H_{B2}$ signals could be due to a slow exchange rate between aggregate type $H_{B1}$ and $H_{B2}$ or possible intermolecular hydrogen bonding of these two aggregate forms.

Similar results are more dramatically seen in the variable temperature $^1$H NMR of $H_2$TPPAS in D$_2$O (Figure I-47). The $H_{B1}$ signal (8.3 ppm), while broad, was the sharpest at 290 K broadening both as temperature was increased and decreased. As with $H_2$TPPIS, the $H_{B2}$ signal at ca. 6.3 ppm for $H_2$TPPAS disappeared as temperature was increased, and was shown by a single frequency saturation NMR experiment to be related to $H_{B1}$. The explanation utilizing multiple forms of aggregation involving the $\beta$ pyrrole protons does seem to hold for both $H_2$TPPIS and $H_2$TPPAS in D$_2$O.

**Proton Relaxation Study**

The data for water proton relaxation rate versus $^1$H Larmor magnetic field strength over the frequency range 0.01 MHz to 30 MHz for [Mn$^{III}$TPPASCl] are presented in
Figure I-47. 300-MHz Variable-Temperature $^1$H NMR Spectra of the Phenyl Region for 5,10,15,20-tetraakis[(4-carboxylic acid-(1,3-dihydroxypropyl)amide]-phenyl]porphyrin, H$_2$TPPAS, in D$_2$O.
Figure I-48. The water proton relaxation rate for [Mn\textsuperscript{III}(TPPAS)Cl] increases with increasing magnetic field strength over the range investigated. It should be remembered that the species for which the relaxivity is being measured is most likely the [Mn\textsuperscript{III}(TPPAS)(H\textsubscript{2}O)\textsubscript{2}]\textsuperscript{+} cation.

The anomalously high relaxivity of manganese(III) porphyrins has been suggested to arise from three effects: (1) a favorable anisotropy of the electronic wavefunction associated with manganese(III)-proton interactions, (2) a relatively long value of $\tau_{e}$ that causes $\tau_{e}$ to increase significantly above 2 MHz., and (3) a longer than expected value of $\tau_{\text{iso}}$ for a non-S-state configuration.\textsuperscript{38}

This increase of relaxation rate over increasing field strength is a clear indication that the electron relaxation rate makes a dominate contribution to the effective correlation time for the electron-nuclear coupling. Of those factors affecting Equation 3, only the electron-spin relaxation time, $\tau_{e}$, is a function of the magnetic field strength. The electron relaxation rate decreases with increasing magnetic strength. The consequences of this rate decrease is that when the electron relaxation dominates the low magnetic field relaxation rate, the observed water proton relaxation rate increases with increasing field strength.\textsuperscript{38,61}

The relaxation rate for [Mn\textsuperscript{III}(TPPS\textsubscript{4})\textsuperscript{3-}] is also presented in Figure I-48 for comparison. The relaxation rate for [Mn\textsuperscript{III}(TPPAS)Cl] is clearly less than that of [Mn\textsuperscript{III}(TPPS\textsubscript{4})\textsuperscript{3-}] but [Mn\textsuperscript{III}(TPPAS)Cl] exhibits the same relaxation rate pattern over the given frequency range. Ligands such as [EDTA]\textsuperscript{4-}, [DTPA]\textsuperscript{5-} can induce rapid exchange of coordinated water molecules when compared to their parent ions.\textsuperscript{62} The possibility exists for these manganese(III) porphyrin systems to undergo this rapid exchange of coordinated water molecules. The inner-sphere contribution, but not the outer-sphere one will decrease if water exchange becomes rate limiting. Tweedle, et. al.\textsuperscript{62} has concluded that the total outer-sphere contribution to the relaxation rate can be approximately as large as
Figure I-48. The Water Proton Nuclear Spin-Lattice Relaxation Rate for 5,10,15,20-tetrakis([4-carboxylic acid-(1,3-dihydroxyisopropyl)-amido]phenyl)porphyrinatomanganese(III) Chloride, \([\text{Mn}^{III}(\text{TPPAS})\text{Cl}]\), and the Manganese(III) Tetrakis(4-sulfophenyl)porphine Ion, \([\text{Mn}^{III}(\text{TPPS}_4)]^{3-}\), at pH 7 Plotted Against Magnetic Field Strength Expressed as the Proton Larmor Frequency.
the inner sphere contribution per coordinated water molecule, but, on a per water basis, the outer-sphere relaxivity is less that the inner-sphere relaxivity by a factor of approximately five. Outer-sphere relaxation rates may be limited by the lifetime of the water molecule in the secondary coordination sphere. The possibility exists for the outer-sphere contribution for relaxation to be quite high for these manganese(III) porphyrins due to the high number of hydroxyl groups on the periphery of the metalloporphyrin core. In addition, the possible slow exchange of the H_β protons for the free ligand in D_2O provides evidence that there may be some type of influence from the metal center through the π-network of the metalloporphyrin core to water molecules in the outer-coordination sphere. Also, the fact that the axial chloride anion is dissociated in water may allow for inner / outer-sphere exchange to occur at two *trans* positions thus increasing the relaxation rate. Therefore, all water-soluble manganese(III) porphyrins are likely to be [Mn^{III}(porphyrin)(H_2O)_κ]^n species in H_2O. Thus, different measured relaxation rates could arise due to different second-sphere contributions from varying substituents on the porphyrin core.

**Toxicity Study**

Determining LD_{50} values for new substances always requires that the injected samples be pure. When the substance is a metal complex, the problem of sample impurity is magnified due to the possibility that starting materials used in the synthesis can be a source of toxicity.\(^{62}\) The LD_{50} values for [Mn^{III}(TPPAS)Cl] is ca. 0.10 mmole/kg. A laboratory rat injected with this dose died immediately, while one injected with 0.05 mmole/kg survived. LD_{50} values for various compounds of interest are shown in Table 1-7 for comparison. [Mn^{III}(TPPAS)Cl] is ca. 100 times as toxic as those gadolinium(III) complexes currently in use as clinical MRI contrast agents. Comparing [Mn^{III}(TPPAS)Cl] to other manganese(III) porphyrins shows it to be somewhat more toxic than
Table I-7. LD<sub>50</sub> Values as a Measure of Intravenous Acute Toxicity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; (mmol / kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMG&lt;sub&gt;2&lt;/sub&gt;[Gd&lt;sup&gt;II&lt;/sup&gt;(DTPA)]</td>
<td>8.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na&lt;sub&gt;2&lt;/sub&gt;[Gd&lt;sup&gt;III&lt;/sup&gt;(DTPA)]</td>
<td>&gt;10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NMG[Gd&lt;sup&gt;III&lt;/sup&gt;(DOTA)]</td>
<td>&gt;10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na[Gd&lt;sup&gt;III&lt;/sup&gt;(DOTA)]</td>
<td>&gt;10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gd&lt;sup&gt;III&lt;/sup&gt;Cl&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.38&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gd&lt;sup&gt;III&lt;/sup&gt;(OH)&lt;sub&gt;3&lt;/sub&gt;</td>
<td>0.10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>NMG&lt;sub&gt;2&lt;/sub&gt;H&lt;sub&gt;2&lt;/sub&gt;DOTA</td>
<td>0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>VΜG&lt;sub&gt;3&lt;/sub&gt;H&lt;sub&gt;2&lt;/sub&gt;DTPA</td>
<td>0.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>[Gd&lt;sup&gt;III&lt;/sup&gt;(DO3A)]</td>
<td>10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na[Gd&lt;sup&gt;III&lt;/sup&gt;(MCTA)]</td>
<td>15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>[Mn&lt;sup&gt;III&lt;/sup&gt;(TPPS&lt;sub&gt;4&lt;/sub&gt;)Cl]</td>
<td>0.30&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>[Mn&lt;sup&gt;III&lt;/sup&gt;(uroporphyrin I)]</td>
<td>0.18&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>[Mn&lt;sup&gt;III&lt;/sup&gt;(TPPAS)Cl]</td>
<td>0.10&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>From Reference 62.
<sup>b</sup>From Reference 63.
<sup>c</sup>From Reference 32.
<sup>d</sup>From Reference 39.
<sup>e</sup>This Work.
[Mn^{III}(TPPS_4)]^{3-} which has a reported LD_{50} value of 0.30 mmole/kg and slightly more toxic than [Mn^{III}(uroporphyrin I)] with a LD_{50} value of 0.18 mmole/kg. The serinol substituent itself has been shown to be non-toxic by its incorporation into iodinated X-ray contrast agents to achieve water solubility. Thus, the porphyrin core itself must be responsible for the observed toxicity. However, significant contrast effects in other manganese(III) porphyrin systems are seen down to a dose of 0.05 μmole/kg. Additionally, small structural changes such as those associated with changing from [Mn^{III}(TPPAS)Cl] to [Mn^{III}(TPPIS)Cl] may have a dramatic effect on toxicity. The toxicity of [Mn^{III}(TPPIS)Cl] has not yet been determined.

**Animal Imaging Study**

[Mn^{III}(TPPAS)Cl] in solution has been evaluated as a magnetic resonance imaging contrast enhancement media in rat heart, liver and gastrointestinal tract Figures 1-49, I-50 and I-51, respectively. Images of heart and liver were obtained by injecting a 0.05 mmole/kg dose into two sprague dawley rats with ECG and respiratory gating used respectively.

Figure I-49 shows the transverse cross-section of the rats body with the dorsal surface downward. Heart beat rates are ca. 140 beats per minute using ECG gating, except Figure I-49(6) which is at ca. 200 beats per minute. The image was obtained using $\tau_K = 420$ ms and employing a spin-echo technique with $\tau_E = 21$ ms.

The small round organ in the mid-upper-left corner which was enhanced in Figure I-49(3) is the myocardium of the heart. Also, seen enhanced to some extent was that of the right ventricle seen to the right of the myocardium. The left ventricle (the smaller inner-circle within the myocardium) was also seen as being somewhat enhanced in Figure I-49(3) five minutes after injection. The muscle tissue along the spine was also seen to be
Figure I-49. MR-Images of a Transverse Section of a Sprague Dawley Rat with the Dorsal Surface Downward Showing the Heart Using [Mn^{III}(TPPAS)Cl] as the Contrast Enhancement Agent.

(1) Control, Prior to injection. (2) Post-injection, 0 minutes. (3) Post-injection, 5 minutes. (4) Post-injection, 10 minutes. (5) Post-injection, 15 minutes. (6) Post-injection, 20 minutes.
Figure I-50. MR-Images of a Sagittal Section of a Sprague Dawley Rat with the Dorsal Surface Downward Showing the Lungs, Liver and Kidneys Using $\text{Mn}^{III}(\text{TPPAS})\text{Cl}$ as the Contrast Enhancement Agent.

Figure I-51. MR-Images of a Sagittal Section of a Sprague Dawley Rat with the Dorsal Surface Downward Showing the Gastrointestinal Tract Using [Mn$^{III}$](TPPAS)Cl as the Contrast Enhancement Agent.

Rat gastrointestinal tract images were obtained before and after ingestion of 10 ml. of 1 mM [Mn$^{III}$](TPPAS)Cl solution.

1A, 1B, 1C are slice offset = 0; 2A, 2B, 2C are slice offset = -3; 3A, 3B, 3C are slice offset = -6; 4A, 4B, 4C are slice offset = -9. 1A, 2A, 3A, 4A are all controls for the various slice offsets. Time after ingestion for other images is as follows: 4B, 0 minutes. 3B, 4 minutes. 2B, 10 minutes. 1B, 15 minutes. 1C, 20 minutes. 2C, 25 minutes. 3C, 30 minutes. 4C, 35 minutes.
enhanced near the spinal cord (Figure I-49(4)) ten minutes after injection.

Figure I-50 shows the sagittal section of the rat's body with the dorsal surface downward. Respiration was gated and the image obtained again using a spin-echo technique with $\tau_r = ca. 500$ ms and $\tau_e = 21$ ms. The results indicate that there exists fairly good signal enhancement in the rat's liver at the given dose. Muscle and kidneys (bean-shaped objects in Figure I-50) were also enhanced indicating that $[\text{Mn}^{III}(\text{TPPAS})\text{Cl}]$ was being taken out of the blood circulation and eliminated through the kidneys.

Figure I-51 shows the sagittal section of the rodent's gastrointestinal tract at four different slice offsets. Rat gastrointestinal tract images were obtained before and after ingestion of 10 ml of 1 mM $[\text{Mn}^{III}(\text{TPPAS})\text{Cl}]$ solution. $[\text{Mn}^{III}(\text{TPPAS})\text{Cl}]$ has a dramatic effect on enhancement of contrast in the intestinal tract. The dark region in the middle of the intestinal tract may be caused by a high $T_2$ of $[\text{Mn}^{III}(\text{TPPAS})\text{Cl}]$. No significant absorption of $[\text{Mn}^{III}(\text{TPPAS})\text{Cl}]$ into the kidney was observed during 30 minutes and no further study was carried out.

The most interesting findings of these animal toxicity and imaging studies were first the "long" residence time, signal enhancement seems to remain for a longer period of time than does ProHance® $[\text{Gd}^{III}(\text{HP-DO3A})]$, and secondly the higher than expected toxicity. These two factors may be interrelated and the contrast enhancement media $[\text{Mn}^{III}(\text{TPPAS})\text{Cl}]$ may indeed be attacking certain cells immediately, such as those of the respiratory tract (Figure I-50), perhaps causing death at these low doses.
Conclusions

1. A new series of porphyrins and metallocorphyrins utilizing two different poly-alcohol substituents to achieve water-solubility have been synthesized and spectroscopically characterized. These compounds have been the subject of an application for patent (U. S. Patent 733,568) due to two of the compounds, [MnIII(TPPAS)Cl] and [MnIII(TPPIS)Cl], being candidates for commercial MRI contrast enhancement agents.

2. Conductivity and osmometry measurements for [MnIII(TPPAS)Cl] in water indicate complete dissociation of chloride ion to yield a manganese(III) porphyrin species of composition [MnIII(TPPAS)(H2O)x]+ with x = 1 or 2. By analogy, all other water-soluble manganese(III) porphyrin compounds in the literature that have been studied as 1H NMR relaxation agents are also likely to be [MnIII(porphyrin)(H2O)x]+ species.

3. The proton magnetic relaxation rate (1 / T1) for [MnIII(TPPAS)Cl] has been studied as a function of the field strength over the range of Larmor frequencies from 0.01 to 30 MHz in aqueous solution. The compound shows a typical relaxation rate pattern with similar rates as for other manganese(III) porphyrins over this frequency range.

4. Variable-temperature 1H NMR studies of the free porphyrin ligands, H2TPPAS and H2TPPIS, have shown possible multiple forms of aggregation as indicated by two distinct but interrelated Hβ signals. This provides some evidence for the "anomalously high" relaxation rates of manganese(III) porphyrins arising from the unusually large outer-sphere contributions at the porphyrin periphery.
5. The acute toxicity study of [Mn\textsuperscript{III}(TPPAS)Cl] has shown that [Mn\textsuperscript{III}(TPPAS)Cl] is much more toxic than the gadolinium(III) compounds that are currently under clinical development for MRI contrast use and somewhat more toxic than other manganese(III) porphyrins which have been investigated. Clearly [Mn\textsuperscript{III}(TPPAS)Cl] is too toxic for clinical use; the toxicity of [Mn\textsuperscript{III}(TPP1S)Cl] has yet to be determined.

6. Actual magnetic resonance images of sprague dawley rats with [Mn\textsuperscript{III}(TPPAS)Cl] as a contrast enhancement medium showed an increase of enhancement in the heart, liver, lungs and gastrointestinal tract with prolonged residence times.
References


51. PANIC: Parameter Adjustment in NMR by Iteration Calculation. PANIC is a minicomputer version of the Laocoon-type programs used in large computer systems and is available in the ASPECT 3000 Computer Program Library.


55. Tweedle, M. F. Personal Communication.


60. This is not the case, however, for [MnIII(TPPAS)Cl] and [MnIII(TPPIS)Cl] at low concentration (1 x 10^{-2} mM) as a Beer's law plot has indicated no aggregation is present.


PART II: Model Systems for the Sulfite Reductase Active Site: Toward a Tetrasulfido-Iron-Cluster-Porphyrin Compound
Introduction

Porphyrin-based model compounds which aim to mimic the structure and function of metalloprotein active sites have been the subject of intense investigation over the past twenty-five years.\(^1\) The guiding principle in these studies has been to correlate the structural, magnetic, and spectroscopic properties of smaller synthetic model compounds to those of the more complex metal-containing active sites to further elucidate chemical or structural information about the sites that may not be clearly understood in the absence of such model compounds.

One such metalloprotein system for which model compounds seem especially suitable is that of sulfite reductase which was first isolated from a variety of plant sources over two decades ago.\(^2\) Sulfite reductase catalyzes the six-electron reduction of SO\(_3^{2-}\) to S\(^2-\) and of NO\(_2^-\) to NH\(_3\). Nitrite reductase also catalyzes these same reactions. One can propose the following one- and two-electron reduction steps for the two substrates, although none of the proposed intermediates are detected during turnover of the enzymes:

\[
\begin{align*}
\text{SO}_3^{2-} & \rightarrow \text{SO}_2^- \rightarrow S = O \rightarrow S^0 \rightarrow S^2^- \\
\text{NO}_2^- & \rightarrow NO \rightarrow HNO \rightarrow NH_2OH \rightarrow NH_3
\end{align*}
\]

The enzyme's physiological role has been shown, by genetic studies, to be the provision of reduced sulfur for biosynthesis.\(^3\) In this capacity, sulfite reductase (SiR) is termed an "assimilatory" sulfite reductase, and this type of SiR exists, for example, in \textit{E. coli}. A second class, the "dissimilatory" sulfite reductases, is involved in the use of SO\(_3^{2-}\) as a terminal electron acceptor in certain chemolithotrophs.
In *E. coli*, the enzyme is a complex hemoflavinprotein (685 kDa) of subunit composition αβ4. The α subunit (59 kDa) has the NADPH binding site and is associated with two prosthetic groups, FAD and FMN, with a stoichiometry of one each per two subunits. The β subunit (55 KDa) is a hemoprotein having an isobacteriochlorin-type heme called "siroheme" and an [Fe₄S₄]²⁻ cluster. Siroheme has two adjacent pyrrole rings that are reductively methylated, allowing for easier out-of-plane bending than is the case for porphyrin. In addition, siroheme has eight carboxylic acid substituents which makes it highly hydrophilic. Unlike other biological heme groups, siroheme is not derived from protoporphyrin IX but rather from an intermediate in corrin (vitamin B₁₂) biosynthesis. Siroheme can be extruded from the protein as an intact prosthetic group using HCl/acetone but without the [Fe₄S₄]²⁻ cluster. The structure of the siroheme is shown in Figure II-1.

The fully-functioning enzyme contains 4 FMD, 4 FAD, 4 sirohemes, and 4 [Fe₄S₄]²⁻ clusters between which electrons flow in minimum sequence: NADPH → FAD → FMN → siroheme → SO₃²⁻. The [Fe₄S₄]²⁻ cluster is involved in the reductive mechanism, but its position in the pathway is presently unclear. Sulfite and assimilatory nitrite reductases from organisms other than *E. coli* also possess siroheme and [Fe₄S₄]²⁻ clusters, but not necessarily the α subunits. The β subunits alone can catalyze the reduction of sulfite to sulfide if a suitable electron donor (e.g. methyl viologen) is available, although it cannot use NADPH without the α subunit.

The siroheme is the site of ligand, and presumably, substrate binding since typical ligands such as CN⁻ or CO bind in a mutually exclusive manner with SO₃²⁻ and also perturb the siroheme electronic spectrum. Epr, optical, ENDOR and Mössbauer spectroscopy all indicate that the siroheme and cluster unit are "exchange-coupled", but the magnitude and mechanism of this magnetic exchange-coupling is uncertain, although it is
Figure II-1. The Structure of Siroheme, an Isobacteriochlorin.
thought to exist in all oxidation states exhibited by the active site (SiR$^0$, SiR$^1$- and SiR$^2$-).\textsuperscript{12-17} Furthermore, epr and $^{57}$Fe Mössbauer spectroscopy and magnetic susceptibility results for the site continue to exhibit inconsistencies.\textsuperscript{18}

Contrary to many other enzyme systems where little is known about the active site, there is little guess work associated with the active site structure of SiR, since a 3 Å resolution x-ray structure of \textit{E. coli} sulfite reductase in its fully-oxidized state has been reported.\textsuperscript{19} From the x-ray structure, the prosthetic groups of \textit{E. coli} SiR have been shown to be a siroheme and an [Fe$_4$S$_4$]$^{2-}$ cluster together, in close proximity, to form a polymetallic active site as shown in Figure II-2. Both the cluster and the siroheme are found near the surface of the protein, with the two groups packed next to one another, apparently with a common ligand consistent with a cysteine sulfur atom shared by the siroheme Fe center and one of the cluster apical Fe atoms. The distance from the siroheme Fe and the center of the cluster is reported to be 5.5 Å, with the distance from the siroheme Fe to the closest cluster Fe being 4.4 Å. Furthermore, the edge of the siroheme macrocycle is described as being in Van der Waals contact with a cubane sulfur atom of the cluster on the proximal side of the siroheme. The distal side (the side opposite the cluster) of the siroheme is unoccupied and open to solvent molecules or ligands, including SO$_3^{2-}$. All eight carboxylic acid residues of the siroheme are disposed toward the distal side of the molecule, forming a strongly hydrophilic pocket for potential ligands. However, the authors confess\textsuperscript{19} that, "the data (ENDOR and x-ray taken together) are quite consistent with a cysteine-S$_\gamma$ as the bridging ligand, but do not rule out ligands of similar size such as a serine oxygen." However, this structure confirms that the siroheme iron and the iron-sulfur cluster have physical contact that allows for exchange coupling.

Not only may there be a common bridge between the cluster and the siroheme, but the two groups are in close contact. This contact may allow for important electronic
Figure II-2. The Structure for the Active Site of Sulfite Reductase Showing a Proposed Bridging Cysteine Sulfur Atom between the Siroheme and the \([Fe_4S_4]^{2-}\) Cluster.
interactions between the cluster and the $\pi$-orbitals of the siroheme. The ligand binding site is likely the siroheme iron. The openness of the distal sixth position makes it the most appealing site, but other possibilities exist for the catalytically poised enzyme with its reduced cluster and reduced Fe(II) siroheme. Young, et al.\textsuperscript{20} further suggest that the lack of information regarding the exact confirmation of the active site has led to the difficulty in discernment of the electron-transfer mechanism.

Herskovitz, et al.\textsuperscript{21} first reported the synthesis of the iron-sulfur cubane cluster, $[\text{Fe}_4\text{S}_4(\text{SR})_4]^2-$ in 1972. The $[\text{Fe}_4\text{S}_4]^2-$ cluster has a slight distortion from being exactly cubic. Angles between S-Fe-S* range in synthetic $[\text{Fe}_4\text{S}_4]^2-$ clusters from 100.2° to 135.7° depending on the -SR functionality bonded to the apical Fe atom. This distortion from 90° is due to steric considerations and packing forces. Mean Fe-S, Fe-S* distances are on the order of 2.25 Å, and 2.23 Å, respectively. Mean Fe-Fe distances are 2.73 Å.\textsuperscript{22} This information together with comparative spectroscopic evidence demonstrated that the synthetic $[\text{Fe}_4\text{S}_4(\text{SR})_4]^2-$ complexes are analogs of the active sites of some iron-sulfur proteins.

In order to form reasonable synthetic model compounds for other iron-sulfur proteins, such as sulfite reductase in which an $[\text{Fe}_4\text{S}_4]^2-$ core is in close proximity of siroheme, modification of the $[\text{Fe}_4\text{S}_4(\text{SR})_4]^2-$ surrounding ligand environment must be accomplished. While ligand substitution reactions\textsuperscript{22} and use of various cluster-capping ligands\textsuperscript{23-27} have demonstrated the ability of the -SR functionalities to undergo exchange, any previous attempt to combine the iron-sulfur cluster with Fe(III) porphyrins has resulted in failure. The end result of these attempts is apparently the decomposition of the iron-sulfur cluster.\textsuperscript{28} Along similar lines, Liu, et al.\textsuperscript{29} have shown that the $[\text{Fe}_4\text{S}_4]^2-$ cluster can be coupled to another Fe(II) center using thiolpyridines as bridging ligands; while no physical evidence has been provided, the authors claim to have demonstrated the binding of
[Fe^{II}(OEP)], octaethylporphyriniron(II), to the [Fe_4S_4]^{2-} cluster. Therefore to date no physical evidence has been provided for the attachment of a porphyrin molecule to an [Fe_4S_4]^{2-} cluster core.

The specific goal of this research has been to synthesize and characterize a new unsymmetrical monothiolphenylporphyrin, H_2TPP(SH)_1, and investigate its reaction of this porphyrin with an [Fe_4S_4(SR)_4]^{2-} cluster to yield such products as those shown in Figure II-3 and Figure II-4. The present investigation has demonstrated, for the first time, the feasibility of covalently attaching [Fe_4S_4]^{2-} clusters to porphyrin molecules via a porphyrin aryl-thio atom. As a consequence, this approach seemingly holds great promise for the future development of well-defined sulfite reductase model compounds.
Figure II-3. A Possible Reaction Product between Equal Molar Concentrations of the New Mono-Substituted Thioporphyrin, 5-(4-thiophenyl)-10,15,20-triphenyl porphyrin and [Fe₄S₄(SCH₂CH₃)₄]²⁻.
Figure II-4. A Possible Reaction Product between an Excess Concentration of the New Mono-Substituted Thiolporphyrin, 5-(4-thiophenyl)-10,15,20-triphenyl porphyrin and [Fe₄S₄(SCH₂CH₃)₄]²⁻.
Experimental

Materials and Methods

All solvents used were reagent grade or better. Pyrrole (Aldrich), benzaldehyde (Mallinckrodt), and 4-methylthiobenzaldehyde (Aldrich) were distilled prior to use. Propionic acid (Aldrich, Mallinckrodt), ferric chloride (Aldrich), sodium methoxide (Aldrich), ethanethiol (Aldrich), tetraethyl ammonium chloride (Aldrich), triethylamine (Mallinckrodt), 3-chloroperoxybenzoic acid [MCPBA] (Aldrich), and trifluoroacetic anhydride [TFAA] (Aldrich) were used as received. Toluene (EM), heptane (EM), methanol (EM), dichloromethane (EM) were normally used without drying. Chloroform (EM) was distilled from CaH2 prior to use. NaSH was synthesized as described by Eibeck and stored under vacuum.

Column separations employed standard glass chromatography columns using teflon stopcocks. Silica gel (mesh 60-200) was dried for a minimum of six hours and cooled to room temperature under vacuum in a desiccator prior to use. All manipulations involving air-sensitive or hygroscopic compounds were performed in an inert atmosphere (O2 < 5 ppm) either within a dry box (a Vacuum Atmosphere Drlab with a HE-35 Dri-Train) or on a Schlenk line [Argon passed through CaSO4 desiccant and through a R3-11 catalyst (Chemical Dynamic Co.) heated to 70 °C] using standard techniques. All solvents used in air-sensitive reactions were either degassed with argon or purged using the freeze-thaw-degas method.

Spectroscopic Measurements

Solid state infrared spectra were recorded with either NaCl (range 4000-600 cm⁻¹), CsI (range 4000-200 cm⁻¹) plates or as KBr pellets (range 4000-600 cm⁻¹) using a Perkin-Elmer IR-1430 or Perkin-Elmer FTIR-1600 series spectrophotometer. Routine electronic
spectra were recorded on a Cary-17 series spectrophotometer equipped with a Compaq personal computer and software for computerized data collection, using 1 cm pathlength matched quartz cells. The spectral measurements were made using $10^{-4}$ and $10^{-7}$ M solutions scanning the range 700 nm. - 300 nm. Electronic absorption spectra of the \textit{in situ} \( \text{H}_2\text{TPP(SH)}_1 / [\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_3)_4]^{2-} \) reactions were run scanning the range 700 nm. - 285 nm. using 1 mm. quartz cells on a Hewlett Packard 8452A Diode Array Spectrophotometer with a Hewlett Packard 9000 Series 300 computer system equipped with a Hewlett Packard 9153 disk drive utilizing UV/vis Chem Station software. Spectra of the reactions were plotted using a Hewlett Packard ColorPro plotter. The \( ^1\text{H} \) and \( ^{13}\text{C} \) NMR spectra were recorded on either an IBM/ Bruker AF 250 or 300 MHz NMR spectrometer. Chloroform-\( d_1 \) (Cambridge Isotopes Laboratories) and \( d_6\)-DMSO (Cambridge Isotopes Laboratories) were 99.8\%-D solvents used in the NMR studies. The molecular weights and fragmentation patterns of the material were confirmed by mass spectra using a Finnigan 9500 GC-MS instrument operating at 70 or 35 eV in the electron impact mode, or obtained commercially by the Analytical Chemistry Center at The University of Texas Medical School at Houston using a Kratos MS50TC fast-atom bombardment mass spectrometer, calibrated with cesium iodide. The FAB mass spectra were obtained on compounds using a 3-nitrobenzyl alcohol (NBA) matrix unless otherwise indicated. The solution molecular weight determinations were carried out commercially by Galbraith Laboratories.

X-band Electron Paramagnetic Resonance (EPR) spectra were recorded at 77 K on a Varian E-Line EPR Spectrometer using DMSO as the solvent glass. DPPH, 2,2-diphenyl-1-picrylhydrazyl hydrate, was used both as the field calibrant and as the free radical standard.
Elemental analysis on key compounds were obtained from Galbraith Laboratories or in-house using a Carlo Erba Instruments NA 1500 series 2 analyzer using E.A.G.E.R. 100 software and card for a IBM personal computer. Metal analyses were obtained using a Perkin-Elmer Model 60 Atomic Absorption spectrophotometer.

**Syntheses**

**5,10,15,20-tetraphenylporphyrin**

$\text{H}_2\text{TPP}$: 5,10,15,20-tetraphenylporphyrin ($\text{H}_2\text{TPP}$) was prepared according to literature methods.$^{32}$

**5-(4-methylthiophenyl)-10,15,20-triphenylporphyrin**

$\text{H}_2\text{TPP(SMe)}_1$: This porphyrin was prepared after the synthetic scheme of Adler, et al.$^{32}$ Typically, 2.66 ml. 4-methylthiobenzaldehyde ($2.0 \times 10^{-2}$ mole) and 6.11 ml. benzaldehyde ($6.0 \times 10^{-2}$ mole) (a 3:1 molar mixture) were stirred together to ensure thorough mixing; then, ca. 200 ml. of propionic acid was added in a 500 ml. round-bottom flask. To this mixture, 5.54 ml. pyrrole ($8.0 \times 10^{-2}$ mole) was added with stirring. This solution was then refluxed for a period of three hours and allowed to cool to room temperature. The propionic acid was then removed under reduced pressure with heating. The black-purple residue was dissolved in a minimum amount of $\text{CH}_2\text{Cl}_2$ (ca. 75 ml.) and a large excess of methanol (ca. 300 ml.) is added. The solution was then filtered using a course fritted filter. The purple solid was washed three times with 100 ml. methanol or until the filtrate became light brown in color. The porphyrin was then air dried overnight. Typically a yield of 5.50 gm. (10.4 %) of the crude product was obtained at this point. The desired monothiomethyl porphyrin isomer was then isolated chromatographically using
silica gel slurried in a 50:50 toluene/heptane. The mixture of isomers (1.0 gm.) was dissolved in a minimum of CH$_2$Cl$_2$ and placed on a chromatography column (2.5 cm. x 30 cm.) using the 50:50 toluene/heptane solution as the eluent. The first 150 ml. of colored eluent was discarded. The desired mono-methylthiophenyl porphyrin isomer was then isolated in the subsequent 500 ml. of eluent. The eluent color was substantially decreased in intensity in this fraction. The solvent was then removed under reduced pressure with heating. Separation of the desired isomer was confirmed by taking the NMR spectrum of the eluent. The isolated solid was then passed down a fresh column using then same conditions, with the exception that only the first 50 ml. was discarded. The eluent was again evaporated under reduced pressure and the sample of H$_2$TPP(SMe)$_1$ collected and dried under vacuum at 110 °C using P$_2$O$_5$. Yield: 320 mg., 5.8% yield. Mass Spectrum: Mp$^+$ 661. Electronic spectral data: See Table II-1. $^1$H and $^{13}$C data: See Tables II-2, and II-3.

**Anal. Calcd.** for C$_{45}$H$_{32}$N$_4$S$\cdot$2 H$_2$O: C, 77.56; H, 5.21; N, 8.04; S, 4.60. **Found:** C, 77.74; H 5.21; N, 7.71; S, 4.47.

**5-(4-thiolphenyl)-10,15,20-triphenylporphyrin**

H$_2$TPP(SH)$_1$: Typically, 0.0512 gm. H$_2$TPP(SMe)$_1$ (7.7 x $10^{-5}$ mole) was dissolved in 50 ml. of freshly distilled CHCl$_3$. 3-chloroperoxybenzoic acid, [MCPBA], 0.1707 gm. (9.8 x $10^{-4}$ mole), was dissolved in 100 ml. of CHCl$_3$. This MCPBA solution was then added dropwise over 1.5 hours to the porphyrin solution at -58 °C (xylenes/dry ice bath). The reaction was monitored by thin-layer chromatography. After 30 minutes, the reaction color changed from purple to a dark brown. At this point, however, thin-layer chromatography revealed no change. After addition of MCPBA was completed and, after an additional 30 minutes of reaction time, 0.20 ml. of triethylamine (TEA) was added to
reduce any excess oxidant. The solution was warmed up to room temperature, during which time the color changed back to purple. The solvent was then evaporated to one half its original volume (ca. 100 ml.) under reduced pressure, and the solution was filtered with a medium filter frit. Trifluoroacetic anhydride (TFAA), 10.0 ml. was then added with stirring. The reaction at this point turned green. This reaction was stirred at room temperature for 30 minutes. The volatile components were then removed by evaporation. A 50:50 triethylamine/ methanol solution was then added and stirred for 15 minutes and subsequently evaporated to dryness. The residue was dissolved in CH₂Cl₂ and extracted with an NH₄Cl-saturated H₂O solution. Purification of the desired porphyrin was accomplished via chromatography by removing any unreacted starting material and any degradation products using a silica gel column with CH₂Cl₂ as the eluent. The overoxidized sulfone-porphyrin (see below) was removed by shifting the eluent to 5% diethyl ether and 95% CH₂Cl₂. The desired monothiophenyl porphyrin was isolated by using acetone as the eluent. The material was further purified on a second column by repeating the same procedure. Yield: 28.8 mg., 22%

Mass Spectrum: Mp⁺ 646. Electronic spectral data: See Table II-1. ¹H and ¹³C data: See Tables II-2, and II-3.

**Anal. Calcd.** for C₄₄H₃₀N₄S: C, 81.77; H, 4.67; N, 8.66; S, 4.96. **Found:** C, 81.40; H, 4.71; N, 7.83; S, 5.06.

**5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin**

H₂TPP(SO₂Me)₁: The H₂TPP(SMe)₁ compound was treated similarly to that for the formation of the 5-(4-thiophenyl)-10,15,20-triphenylporphyrin, H₂TPP(SH)₁. H₂TPP(SMe)₁, 0.0508 gm. (7.7 x 10⁻⁵ mole) was dissolved in 50 ml. of freshly distilled CHCl₃. A large excess of 3-chloroperoxybenzoic acid, [MCPBA], 0.50 gm. (2.9 x 10⁻³
mole), was dissolved in 100 ml. of CHCl₃. This MCPBA solution was then added to the porphyrin solution at 25 °C. After addition of MCPBA the volatile components were then removed by evaporation. A 100 ml. solution of methanol / triethylamine (50:50) was then added and then solution returns to a deep purple color. The residue was dissolved in CH₂Cl₂ and extracted with an NH₄Cl saturated-H₂O solution. Purification of the desired porphyrin was accomplished via chromatography by removing any unreacted starting H₂TPP(SMe)₁ and degradation products using a silica gel column with CH₂Cl₂ as the eluent. The desired monosulfonephenyl porphyrin was removed by shifting the eluent to 5% diethyl ether and 95% CH₂Cl₂. The material was again purified on a second column employing the same conditions. Recrystallization of the desired material was carried out using acetone and methanol. Yield: 47.8 mg., 90%

Mass Spectrum: Mpf 693. Electronic spectral data: See Table II-1. ¹H and ¹³C data: See Tables II-2, and II-3.

**Anal. Caled.** for C₄₅H₃₂N₄S₂O₂·1 CH₃COCH₃: C, 76.78; H, 5.10; N, 7.46; S, 4.27.
**Found:** C, 76.69; H, 4.99; N, 7.76; S, 4.24.

**Tetra[ethylmercapto-μ₃-sulfoido-iron] cluster**

\[((C₂H₅)₄N)₂[Fe₄S₄(SC₂H₅)₄]\]: This iron-sulfur cluster was synthesized as according to Averill, et. al.³³, but with a few exceptions. All reactions were carried out utilizing the Schlenk-line with Ar as earlier described. Solvents were freshly distilled from the appropriate drying agents prior to use. Sodium methoxide 4.86 gm. (9.0 x 10⁻² mole) was dissolved in 200 ml. of methanol. To this stirred solution, freshly distilled and freeze-thaw-degassed ethanethiol 6.5 ml. (9.0 x 10⁻² mole) was added. Anhydrous ferric chloride 4.87 gm. (3.0 x 10⁻² mole) was dissolved in 100 ml. methanol and filtered. The ferric chloride and the sodium methoxide/ethanethiol solutions were combined with stirring. A solution of 1.68 gm. sodium hydrosulfide (3.0 x 10⁻² mole) and 1.62 gm. sodium
methoxide (3.0 x 10^{-2} mole) in 100 ml. was then immediately added. This mixture was stirred for 18 hours and the precipitate was allowed to settle for a period of one hour to facilitate filtration. A solution of 4.15 gm. tetraethylammonium chloride (2.5 x 10^{-2} mole) in 100 ml. of methanol was then added to the filtrate. Methanol was evaporated under reduced pressure to a volume of ca. 100 ml. Approximately, 500 ml. of diethyl ether was then layered on top of the methanol solution and the mixture was allowed to set for 12 hours. NaCl was removed by filtration, leaving a dark brown-black oil. The volume of this oil was reduced to a minimum and again, 500 ml. of diethyl ether is layered on top. The mixture was allowed to set overnight prior to filtering. Filtration yielded a dark black crystalline solid. Yield: 1.05 gm., 41%

^{1}H data: See Table II-2. Electronic Spectral data: See Results and Discussion Section.

**Anal. Calcd.** for Fe_{4}S_{8}C_{24}H_{60}N_{2}: C, 33.65; H, 7.06; N, 3.27; S, 29.94; Fe, 26.08.

**Found:** C, 32.12; H, 5.71; N, 3.63; S, 32.66; Fe, 25.76.

**Observation of the In Situ Reaction between H_{2}TPP(SH)_{1} and [(C_{2}H_{5})_{4}N]_{2}[Fe_{4}S_{4}(SC_{2}H_{5})_{4}] to Produce [Fe_{4}S_{4}(H_{2}TPPS)_{4}]^{2-}**

Stock solutions (2 ml.) of both H_{2}TPP(SH)_{1} and [(C_{2}H_{5})_{4}N]_{2}[Fe_{4}S_{4}(SC_{2}H_{5})_{4}] were made up at 8.2 x 10^{-3} M in d_{6}-DMSO. Reactions of monothiophenylporphyrin and [Fe_{4}S_{4}]^{2-} cluster were carried out by mixing various ratios, ranging from 0.5:1 to 5:1 of porphyrin:cluster, in different NMR tubes. The total volume used in the NMR tubes varied, but all reaction volumes were between 0.40 ml. and 0.60 ml. A series of blank [Fe_{4}S_{4}]^{2-} cluster dilutions from 0.40 ml. to 0.80 ml. was carried out to verify that the ^{1}H NMR spectra did not change due to dilution alone. ^{1}H NMR spectra (300 MHz) of the monothiophenylporphyrin / [Fe_{4}S_{4}]^{2-} cluster reactions were taken within 30 minutes of mixing.
Results and Discussion

5-(4-methylthiophenyl)-10,15,20-triphenylporphyrin, $\text{H}_2\text{TPP}(\text{SMe})_1$

The unsymmetrical mono-CH$_3$S-substituted 5-(4-methylthiophenyl)-10,15,20-triphenylporphyrin, $\text{H}_2\text{TPP}(\text{SMe})_1$, has been synthesized and purified; its structure is shown in Figure II-5. The $\text{H}_2\text{TPP}(\text{SMe})_1$ ligand has been characterized by $^1\text{H}$ NMR, $^{13}\text{C}$ NMR, electronic and infrared spectroscopies as well as FAB (Fast Atom Bombardment) mass spectrometry and elemental analysis.

The desired mono-thioanisole isomer was synthesized using a modification of the Adler-Longo procedure.$^{32}$ In this reaction a 1:3 molar ratio of 4-methylthiobenzaldehyde/benzaldehyde was used. This modified synthesis is shown in Figure II-5. This figure displays the desired mono-thioanisole isomer, although five different isomers (no, mono-, di-, tri-, tetra-substituted) are present in the crude reaction mixture. This crude material was purified using silica gel column chromatography with toluene/hexanes (50:50) as the eluent to obtain the desired mono-substituted isomer.

$\text{H}_2\text{TPP}(\text{SMe})_1$ was characterized in the solid state by FAB mass spectrometry $M^+ = 661$ m/e (Figure B-1, Appendix B) and infrared spectroscopy. The infrared spectrum was interpreted by comparison with the unsubstituted 5,10,15,20-tetraphenylporphyrin, $\text{H}_2\text{TPP}$, and appropriate literature descriptions of porphyrin infrared absorption frequencies.$^{34}$ The distinctive absorptions of $\text{H}_2\text{TPP}$ are the N-H stretching (3303 cm$^{-1}$) and bending modes (964 cm$^{-1}$ and 692 cm$^{-1}$) and the pyrrole C$_8$-H bending modes (1071 cm$^{-1}$ and 800 cm$^{-1}$) as seen in Figure II-6. The infrared spectrum of $\text{H}_2\text{TPP}(\text{SMe})_1$ can be seen in Figure II-7. It too has the characteristic porphyrin modes: N-H stretching (3313 cm$^{-1}$) and bending (964 cm$^{-1}$ and 697 cm$^{-1}$) and the pyrrole C$_8$-H bending modes (1071 cm$^{-1}$).
Figure II-5. The Synthetic Scheme for 5-(4-methylthiophenyl)-10,15,20-triphenyl porphyrin, $\text{H}_2\text{TPP(SMe)}_1$. 

\[ \begin{align*} 
4 \text{N} &+ 3 \text{H-C-} \text{C-} \text{C} \text{H} \text{C} \text{H} \text{SCH}_3 \\
\text{C}_2\text{H}_5\text{COOH} &\text{ at } 90 \text{ min.} \\
\text{H}_2\text{TPP(SMe)}_1 
\end{align*} \]
Figure II-6. The FT-IR Spectrum of 5,10,15,20-tetraphenylporphyrin, H₂TPP, as a KBr Pellet.
Figure II-7. The FT-IR Spectrum of 5-(4-methylthiophenyl)-
10,15,20-triphenylporphyrin, H$_2$TPP(SMe)$_1$, as a KBr Pellet.
cm\(^{-1}\) and 795 cm\(^{-1}\)). In addition, a \(\delta_s\) (symmetrical bending) CH\(_3\) mode occurs at 1344 cm\(^{-1}\) with a \(\delta_{as}\) (asymmetrical bending) CH\(_3\) mode at 1436 cm\(^{-1}\).

\(\text{H}_2\text{TPP(SMe)}_1\) was further characterized by \(^1\text{H}\) NMR spectroscopy. Again, the \(^1\text{H}\) NMR spectrum of \(\text{H}_2\text{TPP}\) is shown in Figure II-8 for comparison. Assignments of the \(^1\text{H}\) NMR spectrum were made based on comparison with \(\text{H}_2\text{TPP}\) and appropriate literature descriptions of substituted porphyrin \(^1\text{H}\) NMR chemical shifts.\(^{35}\) The most notable differences in the \(^1\text{H}\) NMR spectrum of \(\text{H}_2\text{TPP(SMe)}_1\) (Figure II-9) was first the appearance of the methyl signal at 2.74 ppm and secondly the splitting of the protons in the phenyl region of the spectrum. This splitting arises due to the now unsymmetrical nature of the porphyrin. The spectrum integrated for the correct ratio (8:3) of H\(_8\) to CH\(_3\) protons. As indicated in Figure II-9, it was the case that \(\text{H}_2\text{O}\) shows a broad resonance at 1.5 ppm (X) and there was a small non-integral amount of toluene (X') from the chromatography. An expanded plot of the \(^1\text{H}\) NMR of \(\text{H}_2\text{TPP(SMe)}_1\) of the region from 10 ppm to 7 ppm also revealed that the H\(_8\) protons were split into a multiplet with a coupling constant \((J)\) of 6.0 Hz. Protons labeled H\(_2\) and H\(_3\) on the substituted phenyl ring have a coupling constant of 8.2 Hz.

The \(^{13}\text{C}\) NMR spectrum of \(\text{H}_2\text{TPP(SMe)}_1\) is quite complex as shown in Figure II-10. This spectrum was interpreted based on information obtained from the \(^{13}\text{C}\)-DEPT NMR and \(^{13}\text{C}\)-QUATD NMR spectra for \(\text{H}_2\text{TPP(SMe)}_1\) seen in Figures II-11 and II-12, respectively. \(^{13}\text{C}\)-DEPT NMR (Distortionless Enhancement by Polarization Transfer)\(^{36}\) spectroscopy distinguishes between CH\(_3\), CH\(_2\) and CH protons via proton polarization. In this case, selective pulses and delays eliminate quaternary carbon atom signals. In addition to signal enhancement, this experiment can distinguish odd and even protonated carbons using variable delays which shift the phases of the observed signals by 180° [CH\(_3\) (↑),}
Figure II-8. The $^1$H NMR Spectrum of 5,10,15,20-tetraphenylporphyrin, H$_2$TPP, in CDCl$_3$. 
Figure II-9. The $^1$H NMR Spectrum of 5-(4-methylthiophenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SMe)$_1$, in CDCl$_3$. 
Figure II-10. The $^{13}$C NMR Spectrum of 5-(4-methylthiophenyl)-10,15,20-triphenylporphyrin, $\text{H}_2\text{TPP(SMe)}_1$, in CDCl$_3$. 
Figure II-11. The $^{13}$C-DEPT NMR Spectrum of 5-(4-methylthiophenyl)-
10,15,20-triphenylporphyrin, H$_2$TPP(SMe)$_1$, in CDCl$_3$. 
CH(↑), and CH₂(↓). ¹³C-QUATD NMR spectroscopy on the other hand distinguishes those carbon atoms which are quaternary (no H's) from those which contain protons.

Since H₂TPP(SMe)₁ has no CH₂ (methylene) groups the ¹³C-DEPT NMR and ¹³C-QUATD NMR spectra taken together are equivalent to the original ¹³C NMR spectrum. The ¹³C NMR spectrum of H₂TPP(SMe)₁ reveals the CH₃ signal is at 15.6 ppm. This position is common for methyl signals attached to heteroatoms.³⁷ The C₈ signal is actually two separate broad signals C₈ at 131 ppm and C₈' at 130.4 ppm. Structurally in H₂TPP(SMe)₁ there are also two distinguishable meso carbon types. The meso carbon positions are those carbon atoms on the porphyrin core at which the phenyl groups are attached. Cₘ' and Cₘ are seen in Figure II-12 at 120.1 ppm and 119.5 ppm, respectively. While ¹³C spectra are not integratable, one is able to distinguish that from the ¹³C-QUATD NMR spectrum for H₂TPP(SMe)₁ that C₁ and C₁' and Cₘ and Cₘ' are in an approximate 1:3 ratio (Figure II-12). The CH₃S-substituted phenyl-ring carbons are also distinguishable from the non-substituted phenyl carbons in the ¹³C-DEPT NMR spectra (Figure II-11). Again, C₂ and C₃ are in an approximate 1:3 ratio to those of C₂' and C₃', respectively. Table II-1 summarizes the ¹³C NMR chemical shifts for 5-(4-methyl-thiophenyl)-10,15,20-triphenylporphyrin, H₂TPP(SMe)₁.

The electronic absorption spectrum for H₂TPP(SMe)₁ in CHCl₃ is shown in Figure II-13. The electronic absorption spectrum for H₂TPP in CHCl₃ is presented in Figure II-14 for comparison with H₂TPP(SMe)₁ and the other free porphyrin ligands which follow. H₂TPP has the characteristic porphyrin Soret band located at 418 nm (ε = 577 mM⁻¹·cm⁻¹). Smith³⁸ reports λₓₓₓ of 419 nm (ε = 430 mM⁻¹·cm⁻¹) for the Soret band of H₂TPP in benzene. H₂TPP(SMe)₁ like H₂TPP has the Soret band at 420 nm (ε = 345 mM⁻¹·cm⁻¹). In general, all of the extinction coefficients for H₂TPP(SMe)₁ are decreased relative to H₂TPP, accounting for the decrease in intensity of color in solution of
Figure II-12. The $^{13}$C-QUATD NMR Spectrum of 5-(4-methylthiophenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SMe)$_1$, in CDCl$_3$. 
Table II-1. Summary of $^{13}$C NMR Chemical Shifts for H$_2$TPP(SMe)$_1$ in CDCl$_3$.

![Chemical Structure](image)

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Figure II-13. The Electronic Absorption Spectrum for 5-(4-methylthiophenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SMe)$_1$, in CHCl$_3$. 
Figure II-14. The Electronic Absorption Spectrum for 5,10,15,20-tetraphenylporphyrin, H$_2$TPP, in CHCl$_3$. 
H$_2$TPP(SMe)$_1$ compared to H$_2$TPP. The positions of the absorption maxima in the electronic spectrum of are not dramatically shifted, as expected, since neither conjugated functionality groups have been added to the porphyrin core nor to the phenyl substituents.$^{37,39}$

5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SO$_2$Me)$_1$

The synthesis of 5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SO$_2$Me)$_1$, was produced as an over-oxidized product in the Pummerer rearrangement of H$_2$TPP(SMe)$_1$ (see below). Figure II-15 shows the reaction scheme for the formation of H$_2$TPP(SO$_2$Me)$_1$. The purified H$_2$TPP(SO$_2$Me)$_1$ was characterized also in the solid state by mass spectroscopy and infrared spectroscopy. Conventional ionization mass spectrometry showed the parent ion $M^+ = 693$ m/e in the solid state (Figure B-2, Appendix B).

The infrared spectrum of H$_2$TPP(SO$_2$Me)$_1$ as a KBr pellet is shown in Figure II-16. Infrared spectra of sulfones show strong absorption bands between 1350-1300 cm$^{-1}$ and between 1160-1120 cm$^{-1}$. These bands are due to $\nu_{\text{as}}$ and $\nu_{\text{s}}$ SO$_2$ stretching modes, respectively.$^{37}$ These bands are present in the infrared spectrum of H$_2$TPP(SMe)$_1$ as a $\nu_{\text{as}}$ SO$_2$ stretching mode at 1308 cm$^{-1}$ and a $\nu_{\text{s}}$ SO$_2$ stretching mode at 1149 cm$^{-1}$, and the high frequency band is split, as is often the case for the solid state.$^{37}$ The infrared spectrum H$_2$TPP(SO$_2$Me)$_1$ also displays a $\delta_{\text{s}}$ CH$_3$ stretching mode at 1354 cm$^{-1}$ and a $\delta_{\text{as}}$ CH$_3$ stretching mode at 1441 cm$^{-1}$. The other distinctive porphyrin infrared bands are also seen as $\nu_{\text{N-H}}$ (3318 cm$^{-1}$), $\delta_{\text{N-H}}$ (946 cm$^{-1}$ and 703 cm$^{-1}$) and the pyrrole C$_\beta$-H bending modes (1067 cm$^{-1}$ and 800 cm$^{-1}$) as seen in Figure II-16.

H$_2$TPP(SO$_2$Me)$_1$ was further characterized by $^1$H NMR spectroscopy (Figure II-17). $^1$H NMR assignments were made by analogy to those assignments for the $^1$H NMR
Figure II-15. The Synthetic Scheme for the Formation of 5-\((4'\text{-methylsulfonephenyl})\)-10,15,20-triphenyl porphyrin, H$_2$TPP(SO$_2$Me)$_1$. 

1) Excess MCPBA
2) CH$_3$OH/TEA
Figure II-16. The FT-IR Spectrum of 5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H₂TPP(SO₂Me)₁, as a KBr Pellet.
Figure II-17. The $^1$H NMR Spectrum of 5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SO$_2$Me)$_1$, in CDCl$_3$. 
spectrum of H$_2$TPP(SMe)$_1$. The CH$_3$ signal is located at 3.38 ppm (a downfield shift of 0.64 ppm from that of H$_2$TPP(SMe)$_1$ as was expected due to deshielding of the sulfone oxygen atoms). Again, there is splitting of the protons of the unsymmetrical phenyl substituent. H$_2$ is located further downfield than H$_3$, 8.45 ppm and 8.35 ppm, respectively, with a J value of 8.3 Hz. Also, the H$_6$ protons are split into three distinct types having a ratio of 2:4:2 with an apparent coupling constant of 4.8 Hz. One can then assume the four protons which are distinct from the two sets of two are those protons on the opposite side of the porphyrin core than the 4-methylsulfonephenyl functionality. Those H$_6$ protons which are directly next to the meso carbon atom containing the substituted phenyl ring (C$_m$) are those which give rise to the doublet further upfield at 8.74 ppm. Therefore, the H$_6$ protons which are further away from the 4-methylsulfone functionality lie downfield at 8.90 ppm.

As was the case with H$_2$TPP(SMe)$_1$, the $^{13}$C NMR spectrum of H$_2$TPP(SO$_2$Me)$_1$ was complex as is shown in Figure II-18. Again the $^{13}$C NMR spectrum was interpretable based on $^{13}$C-DEPT NMR and $^{13}$C-QUATD NMR spectra. As was the case with the $^1$H NMR, the CH$_3$ signal was shifted downfield in both the $^{13}$C NMR and the $^{13}$C-DEPT NMR spectra (44.7 ppm). This shift of the CH$_3$ signal (29.1 ppm relative to H$_2$TPP(SMe)$_1$) is due to the presence of the sulfone functionality. Also of note is that the C$_\alpha$ signal at 148 ppm is sharp whereas in H$_2$TPP(SMe)$_1$ it is very broad. There also is a broad signal at ca. 150 ppm for H$_2$TPP(SO$_2$Me)$_1$. This may be an indication that one is now able to distinguish the C$_\alpha$ atoms close to the 4-methylsulfone functionality from those C$_\alpha$ atoms near the unsubstituted phenyl rings. As was the case with H$_2$TPP(SO$_2$Me)$_1$, the $^{13}$C-DEPT NMR spectrum for H$_2$TPP(SO$_2$Me)$_1$ (Figure II-19) shows both sets of phenyl carbon atoms; C$_2$, C$_2'$ and C$_3$, C$_3'$; which are in an approximate 1:3 ratio. From the $^{13}$C-QUATD of H$_2$TPP(SO$_2$Me)$_1$, Figure II-20, it was also observed that C$_4$ is shifted ca. 18
Figure II-18. The $^{13}$C NMR Spectrum of 5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SO$_2$Me)$_1$, in CDCl$_3$. 
Figure II-19. The $^{13}$C-DEPT NMR Spectrum of 5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SO$_2$Me)$_1$, in CDCl$_3$. 
Figure II-20. The $^{13}$C-QUATD NMR Spectrum of 5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SO$_2$Me)$_1$, in CDCl$_3$. 
ppm from 138 ppm in H₂TPP(SMe)₁ to 120 ppm in H₂TPP(SO₂Me)₁, again due to the electronic shielding effects of the sulfone functionality. Table II-2 summarizes the ¹³C NMR chemical shifts for 5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H₂TPP(SO₂Me)₁.

The electronic absorption spectrum for H₂TPP(SO₂Me)₁ in CHCl₃ is shown in Figure II-21. The absorption maxima and positions differ slightly from H₂TPP(SMe)₁, however, the band positions and intensities are nearly identical to that of H₂TPP. Table II-3 summarizes the electronic spectral results for these and other compounds of interest.

5-(4-thiophenyl)-10,15,20-triphenylporphyrin, H₂TPP(SH)₁

Several methods were attempted to form a mono-4-thiophenylporphyrin from various starting materials. First, it has been documented for various organic compounds that nucleophilic attack of NaSCH₃ on methylthioether substituents followed by treatment with acid yields the desired thiol.⁴⁰ Another attempt to remove the methyl protecting group from the -SCH₃ substituted porphyrin ring was carried out using Na metal in liquid ammonia.⁴¹ Both of these methods, while reporting high yields of desired thiol for organic compounds, resulted in failure for H₂TPP(SMe)₁.

Third, an attempt was carried out to synthesize a desired thiol derivative by starting with the 5,10,15,20-tetra(4-bromophenyl) porphyrin and reacting with H₂S. Replacement of bromine is a standard direct method of synthesis for some thiols.⁴² While the product would be the tetrathiophenylporphyrin, the results could be extended to the mono-substituted 5-(4-bromophenyl)-10,15,20-triphenylporphyrin. While a mass spectrum of the tetrathiophenylporphyrin reaction product indicated some success, the product was virtually insoluble in most solvents and this protocol was abandoned.
Table II-2. Summary of $^{13}$C NMR Chemical Shifts for H$_2$TPP(SO$_2$Me)$_1$ in CDCl$_3$.

![Chemical Structure](image)

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Figure II-21. The Electronic Absorption Spectrum for 5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SO$_2$Me)$_1$, in CHCl$_3$. 

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</table>
The fourth method attempted for the conversion of thioanisoles (C₆H₅SCH₃) to the corresponding aryl thiol (C₆H₅SH) takes advantage of the ability of aryl sulfoxides to undergo a facile Pummerer rearrangement to provide hemithioacetal acetates which are hydrolyzed to provide the desired aryl thiol. Young, et. al.⁴³ reported nearly quantitative conversion to the thiol for most of the organic thioanisole compounds attempted. Initial attempts to extend this procedure to the porphyrin system, H₂TPP(SMe)₁, resulted in complete conversion of the 4-thioanisole group to the 4-methylsulfone functionality as discussed earlier. However, subtle modifications of the procedure by Young, et al.⁴³ did finally result in a successful synthesis of the desired 5-(4-thiophenyl)-10,15,20-triphenylporphyrin, H₂TPP(SH)₁.

The infrared spectrum of 5-(4-thiophenyl)-10,15,20-triphenylporphyrin (Figure II-22) revealed typical porphyrin N-H stretching (3323 cm⁻¹) and bending (964 cm⁻¹ and 701 cm⁻¹) modes and a C₈ bending mode at 800 cm⁻¹. However, the infrared spectrum of H₂TPP(SH)₁ did not reveal a S-H stretching mode (ca. 2550 cm⁻¹) nor did it show a weak S-S stretch (600 cm⁻¹ - 400 cm⁻¹) upon close inspection of these spectral regions. Both S-H and S-S stretching bands are weak in general.³⁷ A broad band distinctly different from both H₂TPP(SMe)₁ and H₂TPP(SO₂Me)₁ was, however, located at ca. 1100 cm⁻¹.

5-(4-thiophenyl)-10,15,20-triphenylporphyrin, H₂TPP(SH)₁, has been characterized by FAB mass spectrometry, [M⁺-H] = 646 m/e (Figure B-3, Appendix B). No mass corresponding to disulfide (1292 m/e) was observed, suggesting either that the compound was indeed the desired mono-thiophenyl porphyrin or perhaps that cleavage of the disulfide porphyrin occurs readily under the conditions used to obtain the mass spectrum. A molecular weight determination in acetone using vapor phase osmometry resulted in a determined molecular weight value of 642 g/mole. Together the solution
Figure II-22. The FT-IR Spectrum of 5-(4-thiophenyl)-10,15,20-triphenylporphyrin, $\text{H}_2\text{TPP(SH)}_1$, as a KBr Pellet.
molecular weight and the FAB mass spectrometry data demonstrated that the porphyrin was indeed 5-(4-thiophenyl)-10,15,20-triphenylporphyrin rather than a porphyrin disulfide.

\( \text{H}_2\text{TPP(SH)}_1 \) was also characterized in CHCl\(_3\) by electronic spectroscopy (Figure II-23). The \( \varepsilon \) values at all \( \lambda_{\text{max}} \) positions were approximately one-half of the \( \varepsilon \) values for \( \text{H}_2\text{TPP(SMe)}_1 \). The Soret band at 420 nm has a value of 174 \text{mM}^{-1}\cdot\text{cm}^{-1} \) for \( \text{H}_2\text{TPP(SH)}_1 \) versus 345 \text{mM}^{-1}\cdot\text{cm}^{-1} \) for \( \text{H}_2\text{TPP(SMe)}_1 \). This difference of electronic spectra could be seen physically as a difference in color between the two species (brownish-purple for \( \text{H}_2\text{TPP(SH)}_1 \) and red-purple for \( \text{H}_2\text{TPP(SMe)}_1 \)). The lower \( \varepsilon \) values (Table II-3) for both \( \text{H}_2\text{TPP(SMe)}_1 \) and \( \text{H}_2\text{TPP(SH)}_1 \) relative to \( \text{H}_2\text{TPP} \) must be attributed to the substituted functionality on the single phenyl ring as there are no other structural differences, nor is there conjugation involvement to affect the electronic spectra.\(^{37}\) These lower \( \varepsilon \) values are seen in other porphyrin systems.\(^{39}\)

The \(^1\text{H}\) NMR spectrum for \( \text{H}_2\text{TPP(SH)}_1 \) (Figure II-24) was complicated by the presence of a small amount of a free-radical contaminant which seemed to change slightly in amount from reaction to reaction (See Figure B-5 and Figure B-6, Appendix B). However, it is pointed out that the -CH\(_3\) signal (from \( \text{H}_2\text{TPP(SMe)}_1 \)) was greatly diminished relative to the pyrrole H\(_8\) and phenyl (H\(_2\) and H\(_3\)) signals for \( \text{H}_2\text{TPP(SH)}_1 \) in all reactions. H\(_2\) and H\(_3\) (Figure II-24) are those phenyl proton signals on the phenyl rings that are not substituted with -SH. The H\(_8\), H\(_2\) and H\(_3\) resonances are located at 8.85 ppm, 8.22 ppm, and 7.77 ppm, respectively. The inner-pyrrole protons (N-H) are located at -2.80 ppm. The small signals on either side of H\(_2\) (Figure II-24) could possibly be H\(_2\) and H\(_3\) of the -SH substituted phenyl ring. H\(_8\) does also contain a small shoulder which could be those H\(_8\) protons on the side of the porphyrin core where the -SH functionality is located. However, due to the broadness of the signals no assignments can be definitive. The absence of the -CH\(_3\) signal indicated that cleavage of the methyl group was indeed
Figure II-23. The Electronic Absorption Spectrum for 5(4-thiophenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SH)$_1$, in CHCl$_3$. 
carried out. The large signal (X') in Figure II-24 at 1.25 ppm has not been assigned. The signal represented by (X) is due to H$_2$O and that of (X'') is due to stopcock grease. Addition of trifluoroacetic acid to the sample did not change the $^1$H NMR spectrum, thus a disulfide structure is unlikely. Interference of $^1$H NMR by radical contaminants was also seen with the $^1$H NMR spectrum of oxophlorins.$^{44}$ This free-radical contaminant of oxophlorins was attributed to tautomerism between two species, one of which is a stable radical. This type of free radical interference in the NMR spectrum has been seen for another thiolporphyrin system$^{44}$, and it is apparently observed for H$_2$TPP(SH)$_1$ as well. A quantitative EPR integration experiment revealed that the sample of H$_2$TPP(SH)$_1$ in a frozen DMSO glass at 77 K contained less than 5% of an epr-detectable paramagnetic species with $g = 2$. While small, the amount of radical impurity would be expected to broaden the $^1$H NMR spectrum in a manner as is experimentally observed.

Due to the small amount of H$_2$TPP(SH)$_1$ available and the presence of a small amount of paramagnetic impurity $^{13}$C NMR was not attempted.

**Tetra[ethylmercapto-µ$_3$-sulfido-iron] cluster; [(C$_2$H$_5$)$_4$N]$_2$[Fe$_4$S$_4$(SC$_2$H$_5$)$_4$]**

The synthesis of [N(CH$_2$CH$_3$)$_4$]$_2$[Fe$_4$S$_4$(SCH$_2$CH$_3$)$_4$] was carried out like that of Averill, et al.$^{33}$, with substitution of the tetraethylammonium cation for the tetraphenylarsonium cation. It was assumed that the phenyl proton signals of the tetraphenylarsonium cation would mask those proton signals of the phenyl rings of the porphyrin when the [Fe$_4$S$_4$(SCH$_2$CH$_3$)$_4$]$^{2-}$ cluster would be reacted with H$_2$TPP(SH)$_1$, and thus, substitution of the [N(CH$_2$CH$_3$)$_4$]$^+$ counter-ion for [Fe$_4$S$_4$(SCH$_2$CH$_3$)$_4$]$^{2-}$ was thought desirable.

The [Fe$_4$S$_4$(SCH$_2$CH$_3$)$_4$]$^{2-}$ cluster was characterized by FAB mass spectrometry. FAB mass spectrometry revealed a large base peak at 818 m/e. The expected mass of the
Figure II-24. The $^1$H NMR Spectrum of 5-(4-thiolphenyl)-10,15,20-triphenylporphyrin), H$_2$TPP(SH)$_1$, in CDCl$_3$. 
compound is 856 m/e. The base peak seen is probably due to the compound reacting with the solvent matrix and subsequent ion phase dissociation within the instrument. The expected computer-generated isotope pattern for the cluster and counter ion is seen in Figure B-4 (Appendix B). The same isotope pattern is also observed in the FAB mass spectrum base peak at 818 m/e, indicating that this rearranged fragment contains a nearly identical set of isotopes as for \([N(CH_2CH_3)_4]_2[Fe_4S_4(SCH_2CH_3)_4]\).

The \([N(CH_2CH_3)_4]_2[Fe_4S_4(SCH_2CH_3)_4]\) cluster was also characterized by \(^1H\) NMR spectroscopy in \(d_6\)-DMSO as seen Figure II-25. The chemical shift of the \(\alpha\)-CH\(_2\) groups (those attached to the \([Fe_4S_4]^{2-}\) center) is a broad signal located at 12.5 ppm. This was in agreement with a literature value of 12.5 ppm for \([(C_6H_5)_2As]_2[Fe_4S_4(SCH_2CH_3)_4]\).\(^45\) The \(\alpha\)-CH\(_3\) chemical shift is a broadened signal located at 2.20 ppm. The \(\alpha\)-CH\(_2\) and \(\alpha\)-CH\(_3\) signals are broadened due to the slight paramagnetism of these \([Fe_4S_4]^{2-}\) clusters (0.23-1.04 B.M. at -103 °C to 23 °C) due to a low-lying excited state.\(^45\) The methylene and methyl signals associated with the cation are located at 3.32 ppm and 3.09 ppm, respectively.

The electronic absorption spectrum of \([N(CH_2CH_3)_4]_2[Fe_4S_4(SCH_2CH_3)_4]\) was measured in DMSO as shown in Figure II-26. The \([Fe_4S_4(SCH_2CH_3)_4]^{2-}\) cluster has two principle absorption features with one located at 420 nm (\(\varepsilon = 3.18 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}\)) and the second located at 298 nm (\(\varepsilon = 1.47 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}\)). There is also a broad shoulder located at \(ca.\) 340 nm (\(ca. \varepsilon = 9 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}\)). The electronic spectrum of the \([Fe_4S_4(SCH_2CH_3)_4]^{2-}\) cluster with tetra-n-propylamine as the counter ion in DMF is similar with \(\varepsilon\) values of 1.72 \(\times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}\) and 2.33 \(\times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}\) for the 420 nm and 298 nm bands respectively.\(^46\) No additional characterization of \([N(CH_2CH_3)_4]_2[Fe_4S_4(SCH_2CH_3)_4]\) was carried out.
Figure II-25. The $^1$H NMR Spectrum of the Tetra[ethylmercapto-13-sulfido-iron] cluster, [(C$_2$H$_5$)$_4$N)$_2$[Fe$_4$S$_4$(SC$_2$H$_5$)$_4$], in d$_6$-DMSO.
Figure II-26. The Electronic Absorption Spectrum for the Tetra[ethylmercapto-μ3-sulfido-iron] cluster, [(C$_2$H$_5$)$_4$N)$_2$[Fe$_4$S$_4$(SC$_2$H$_5$)$_4$], in DMSO.
The In Situ Reaction between H2TPP(SH)1 and [(C2H5)4N]2[Fe4S4(SC2H5)4] to Produce [Fe4S4(H2TPPS)4]2-

The [Fe4S4(SR)4]2- cluster with R = CH2CH3 was reacted with varying ratios of 5-(4-thiophenyl)-10,15,20-triphenylporphyrin, H2TPP(SH)1. These reactions were performed based upon well-documented ligand substitution reactions of other aryl thiols replacing coordinated alkyl thiolates in [Fe4S4(SR)4]2- clusters.22,46 The reactions were monitored by 1H NMR spectroscopy in d6-DMSO and by electronic spectroscopy. 1H NMR spectral changes due to the addition of varying ratios of H2TPP(SH)1 to [Fe4S4(SR)4]2- cluster are shown in Figure II-27 and again in expanded form in Figure II-28. The signal due to DMSO is labeled with (X) in Figure II-27. The methylene and methyl group resonances of the tetraethylammonium cation have been assigned and labeled β-CH2 and β-CH3 for distinction from those due to the [Fe4S4(SCH2CH3)4]2- cluster (α-CH2 and α-CH3). An increase in the H2TPP(SH)1: [Fe4S4(SR)4]2- cluster ratio first resulted in the appearance of a new signal at ca. 13.2 ppm, presumably due to the substitution of increasing amounts of H2TPP(SH)1 into the cluster with liberation of increasing amounts of ethanethiol. This substitution of would produce a loss of symmetry in the cluster and therefore the possible appearance of additional α-CH2 resonances. As the concentration of H2TPP(SH)1 was further increased, the appearance of porphyrin Hβ-pyrrole signals (8.83 ppm), the phenyl H2 and H3 signals (8.21 ppm and 7.83 ppm, respectively, and a signal due to the inner-pyrrole protons (N-H at -2.80 ppm) occurred concatenate with the appearance of the new signal at 13.2 ppm and with the signal at 12.5 ppm remaining suggesting that the [Fe4S4(SR)4]2- cluster was still intact (Figure II-27). The phenyl protons (H2 and H3) on the ring directly attached through the sulfur atom to the [Fe4S4]2- cluster were expected to have different chemical shifts and to be broadened (relative to those phenyl rings of the porphyrin which are not directly attached to the
Figure II-27. The 300 MHz $^1$H NMR Spectrum for the Substitution Reaction between [(C$_2$H$_5$)$_4$N]$_2$[Fe$_4$S$_4$(SC$_2$H$_5$)$_4$] and H$_2$TPP(SH)$_1$ in d$_6$-DMSO at 25 °C.

Numerical values at the left are the mole ratios of added H$_2$TPP(SH)$_1$ to original [Fe$_4$S$_4$(SCH$_2$CH$_3$)]$^{2-}$ cluster.
Figure II-27. The 300 MHz $^1$H NMR Spectrum for the Substitution Reaction between (Continued) [(C$_2$H$_5$)$_4$N]$_2$[Fe$_4$S$_4$(SC$_2$H$_5$)$_4$] and H$_2$TPP(SH)$_1$ in d$_6$-DMSO at 25 °C.

Numerical values at the left are the mole ratios of added H$_2$TPP(SH)$_1$ to original [Fe$_4$S$_4$(SCH$_2$CH$_3$)]$^{2-}$ cluster.
Figure II-27. The 300 MHz $^1$H NMR Spectrum for the Substitution Reaction between (Continued) $[(\text{C}_2\text{H}_5)_4\text{N}]_2[\text{Fe}_4\text{S}_4(\text{SC}_2\text{H}_3)_4]$ and $\text{H}_2\text{TPP(SH)}_1$ in d$_6$-DMSO at 25 °C.

Numerical values at the left are the mole ratios of added $\text{H}_2\text{TPP(SH)}_1$ to original $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_3)]^{2-}$ cluster.
Figure II-28. The 300 MHz $^1$H NMR Expanded Spectrum for the Substitution Reaction between [(C$_2$H$_5$)$_4$N]$_2$[Fe$_4$S$_4$(SC$_2$H$_5$)$_4$] and H$_2$TPP(SH)$_1$ in d$_6$-DMSO at 25 °C.

Numerical values at the left are the mole ratios of added H$_2$TPP(SH)$_1$ to original [Fe$_4$S$_4$(SCH$_2$CH$_3$)]$^{2-}$ cluster.
Figure II-28. The 300 MHz $^1$H NMR Expanded Spectrum for the Substitution Reaction (Continued) between $[(\text{C}_2\text{H}_5)_4\text{N}]_2[\text{Fe}_4\text{S}_4(\text{SC}_2\text{H}_5)_4]$ and $\text{H}_2\text{TPP(SH)}_1$ in d$_6$-DMSO at 25 °C.

Numerical values at the left are the mole ratios of added $\text{H}_2\text{TPP(SH)}_1$ to original $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_3)]^{2-}$ cluster.
Figure II-28. The 300 MHz $^1$H NMR Expanded Spectrum for the Substitution Reaction (Continued) between $[(C_2H_5)_4N]_2[Fe_4S_4(SC_2H_5)_4]$ and $H_2TPP(SH)_1$ in $d_6$-DMSO at 25 °C.

Numerical values at the left are the mole ratios of added $H_2TPP(SH)_1$ to original $[Fe_4S_4(SCH_2CH_3)]^{2-}$ cluster.
cluster, H₂' and H₃') due to transfer of unpaired spin density of the paramagnetic [Fe₄S₄]²⁻ core into the π-network of the unique phenyl ring. Que et al.²² have reported that the ortho protons on a p-dimethylaminobenzezenethiol substituted [Fe₄S₄]²⁻ cluster gave a very broad signal located at 5.69 ppm. The meta phenyl protons for the p-dimethylaminobenzezenethiol system were broad but yielded a more intense signal observed at 7.54 ppm. For the present [Fe₄S₄(SR)₄]²⁻ cluster substitution reactions the ortho protons of the phenyl rings attached via the sulfur atom to the [Fe₄S₄]²⁻ cluster (H₂) were not directly evident. However, the upfield broadness of the porphyrin H₃ signal at 7.83 ppm indicated the presence of the meta proton signal of a phenyl ring attached to the [Fe₄S₄]²⁻ core at ca. 7.5 ppm (Figure II-28). This result is in close agreement with that of the p-dimethylaminobenzezenethiol substitution reaction reported by Que et al.²² As the H₂TPP(SH)₁ : [Fe₄S₄(SR)₄]²⁻ cluster ratio was increased to 4:1, the signals at 13.2 ppm and 12.5 ppm diminished significantly, and with excess H₂TPP(SH)₁ (5:1 ratio) no evidence of either of the 13.2 ppm or 12.5 ppm signals was observed, indicating complete substitution of porphyrin anion (H₂TPPS⁻) for thiolate (CH₃CH₂S⁻). Accompanying ¹H NMR spectral changes (Figure II-27) involved the disappearance of the α-CH₃ signal (located at 2.20 ppm for the non-substituted [Fe₄S₄(SR)₄]²⁻ cluster) with increasing concentrations of H₂TPP(SH)₁. Concatenate with the appearance of porphyrin H₆, H₂', and H₃' signals, two signals also appear at ca. 1.0 ppm and ca. 1.2 ppm, respectively, presumably arising from free ethanethiol in solution. These two signals eventually broadened and merged with excess H₂TPP(SH)₁ concentration.

As a final characterization of this [Fe₄S₄(SCH₂CH₃)₄]²⁻ cluster substitution reaction, solution electronic spectra were determined in the 700 nm - 285 nm region in DMSO. The change of the electronic absorption spectra for the H₂TPP(SH)₁ : [Fe₄S₄(SCH₂CH₃)₄]²⁻ reactions is shown for the 500 nm - 280 nm region of the spectrum
in Figure II-29. The position of the Soret band, as well as other bands associated with 
H₂TPP(SH)₁ did not noticeably change in position with addition of [Fe₄S₄(SCH₂CH₃)₄]²⁻ 
cluster. This lack of change in the position of the Soret band was not surprising since the 
[Fe₄S₄(SCH₂CH₃)₄]²⁻ cluster contains only two relatively weak absorption bands, the first 
of which occurs at 420 nm (the same location as the porphyrin Soret band) and the second 
at 298 nm. Also, as was observed with the three different thio-containing porphyrins 
synthesized in this work, a change of the functional group on the phenyl ring does not 
significantly effect the positions of the porphyrin absorption bands. This relative 
insensitivity of position of spectral absorption bands (compared to H₂TPP) is observed for 
other substituted-tetraphenylporphyrins. 39 Thus, while electronic electronic spectroscopy 
cannot be used constructively to monitor the *in situ* reaction of H₂TPP(SH)₁ with the 
[Fe₄S₄(SCH₂CH₃)₄]²⁻ cluster, the above ¹H NMR spectral data offers conclusive proof of 
a reaction to produce the desired [Fe₄S₄(H₂TPPS)₄]²⁻ cluster as shown in Figure II-4. As 
such, [Fe₄S₄(H₂TPPS)₄]²⁻ represents the first successful covalent attachment of a 
porphyrin moiety to the [Fe₄S₄]²⁻ cluster core. It is anticipated that the present study will 
soon lead to the isolation and characterization of a compound containing the 
[Fe₄S₄(H₂TPPS)₄]²⁻ ion as a first generation model compound for the sulfite reductase 
active site. Even in the absence of this connection with the sulfite reductase site, isolation 
of such porphyrin / cluster species promises a wealth of exciting new inorganic chemistry.
Figure II-29. The Electronic Absorption Spectrum of the Soret Region for the Substitution Reaction between [{(C₂H₅)₄N}₂Fe₄S₄(SC₂H₅)₄] and H₂TPP(SH)₁ in DMSO at 25 °C.

Numerical values at the left are the mole ratios of added H₂TPP(SH)₁ to original [Fe₄S₄(SCH₂CH₃)]²⁻ cluster.
Conclusions

1. Three new monothiophenylporphyrins, each containing three unsubstituted phenyl rings and one substituted phenyl ring (in the 4 position) with -SCH₃, -SO₂CH₃ and -SH, respectively, have been synthesized and spectroscopically characterized here for the first time.

2. The synthesis and characterization of $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_3)_4]^2^-$ with the $[\text{N}(\text{CH}_2\text{CH}_3)_4]^+$ counter-ion has been carried out for the first time.

3. The production of H₂TPP(SH)₁ provides a feasible way for covalent attachment of $[\text{Fe}_4\text{S}_4]^2^-$ clusters to porphyrins via an aryl thiolate atom. As demonstrated by an *in situ* $^1$H NMR study of the reaction between H₂TPP(SH)₁ and $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_3)_4]^2^-$; substitution of H₂TPPS⁻ for the alkyl thiolate arms (CH₃CH₂S⁻) in the cluster readily proceeds, and complete substitution of all four thiolate groups occurs with a H₂TPP(SH)₁ : $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_3)_4]^2^-$ ratio of 5:1. This approach has yielded the first successful covalent attachment of a porphyrin moiety to an $[\text{Fe}_4\text{S}_4]^2^-$ cluster core and thus, a promising route toward model compounds for the sulfite reductase active site.
References


18. For example, $\chi_M$ vs $T^{-1}$ (2-200 K) data is Curie in nature for SiR$^0$ ($S = 5/2$ siroheme and $S = 0$ cluster). There is no evidence in the magnetic data for an $S = 1$ excited state for the cluster, although Mössbauer spectroscopy shows evidence for "spin-coupling" between $S = 5/2$ siroheme and $S = 1$ cluster. In addition, early spectroscopic data suggested the possibility of $S = 1$ siroheme in SiR$^-$, although recent full-temperature magnetochemical measurements clearly demonstrate an $S = 2$ center. Magnetic data information from E. P. Day, private communication.


Appendix A

Supplemental Figures for Part I
Figure A.1. The FAB Mass Spectrum of 5,10,15,20-tetrakis[(4-carboxylic acid-(1,3-dihydroxypropyl)amide]phenyl]porphyrinatomanganese(III) Chloride, [Mn(TPPAS)Cl].
Figure A-2. The FAB Mass Spectrum of 5,10,15,20-tetrakis{[4-carboxylic acid-(2,3-dihydroxypropyl)amide]phenyl}porphyrinatomanganese(III) Chloride, [Mn(TPPIS)Cl].
Figure A-3. The $^1$H NMR Spectrum of 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin, H$_2$TPPC, in CD$_3$OD.
Figure A-4. The $^1$H NMR Spectrum of 5,10,15,20-tetrakis(4-carboxy-phenyl)porphyrinatozinc(II), [Zn$^{II}$(TPPC)], in CD$_3$OD.
Appendix B

Supplemental Figures for Part II
Figure B-1. The FAB Mass Spectrum and Ionization Mass Spectrum of 5-(4-methylthiophenyl)-10,15,20-triphenylporphyrin, $H_2$TPP(SMe)$_1$. 
Figure B-2. The Ionization Mass Spectrum of 5-(4-methylsulfonephenyl)-10,15,20-triphenylporphyrin, H$_2$TPP(SO$_2$Me)$_1$. 
Figure B-3. The FAB Mass Spectrum and Ionization Mass Spectrum of 5-(4-thiolphenyl)-10,15,20-triphenylporphyrin), H₂TPP(SH)₁.
Figure B-4. The FAB Mass Spectrum of the Tetra[ethylmercapto-μ3-sulfido-iron] cluster, [(C₂H₅)₄N]₂[Fe₄S₄(SC₂H₅)₄], in d₆-DMSO and Its Calculated Isotopic Distribution Pattern.
Figure B-5. The $^1$H NMR Spectrum of 5-(4-thiophenyl)-10,15,20-triphenylporphyrin), H$_2$TPP(SH)$_1$, in CDCl$_3$. 
Figure B-6. The $^1$H NMR Spectrum of 5-(4-thiophenyl)-10,15,20-triphenylporphyrin), H$_2$TPP(SH)$_1$, in CDCl$_3$. 