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PROBING THE INTERACTIONS BETWEEN STERICALLY DEMANDING FERRIHEMES AND HISTIDINE ANALOGUES.  
I: 2-D NMR STUDIES OF NONPLANAR FERRIHEME / BIS(N-/2-METHYLIMIDAZOLE) COMPLEXES;  
II: SYNTHESIS OF A trans-DISUBSTITUTED TETRAARYLPORPHYRIN WITH A BULKY GROUP NEAR EACH POTENTIAL AXIAL LIGATION SITE

by
Hiroshi Ogura

A Dissertation Submitted to the Faculty of the  
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In the Graduate College  
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2000
As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Hiroshi Ogura entitled Probing the Interactions between Sterically Demanding Ferrihemes and Histidine Analogues. I: 2-D NMR Studies of Nonplanar Ferriheme / Bis(N-/2-Methylimidazole) Complexes; II: Synthesis of a trans-Disubstituted Tetraarylporphyrin with a Bulky Group near Each Potential Axial Ligand Site and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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ABSTRACT

In a hemoprotein, histidine and other aromatic amines that ligate to the heme may encounter steric interactions from the following: (1) the peptide chains that form the pocket; or (2) the porphyrin ring that may have been distorted by the protein matrix or a distal ligand. Models that mimic these steric interactions need to be synthesized and studied in detail. The results of two research projects — both dealing with porphyrins that interact sterically with the axially ligated aromatic amines — are presented in this dissertation.

In the first part, variable-temperature 2-D NMR studies of nonplanar ferriheme complexes are presented. Hemes are surprisingly flexible, and a significantly distorted conformation is accessible by the forces exerted by surrounding proteins and ligands. Iron(III) octaethyltetraphenylporphyrin ([Fe(III)OETPP]⁺) is a saddled macrocycle that could serve as a model for distorted hemes. Two low-spin [Fe(III)OETPP]⁺ complexes, one with two N-methylimidazole (N-Melm) molecules as the axial ligands and the other with two 2-methylimidazole (2-MeImH), have been made, and their NMR spectra recorded as a function of temperature. Spectral assignment for the bis(N-Melm) complex has been made through 2-D techniques. The NOESY spectra indicate that at about -40°C, the ring inversion halts while the axial ligand dissociation remains prevalent. The spectra of the bis(2-MeImH) complex at -85°C indicate that the dominant internal dynamics consists of ring inversion and a concerted rotation of the axial ligands.

In the second part, the synthesis of a porphyrin with two sterically large groups
near the potential sites of axial ligation is presented. In a bis(aromatic amine) / iron(III)
complex of the αβ-atropisomer of 5,15-di-p-tolyl-10,20-bis[2,3-[(((hydrotris(3,5-
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The product most likely consists entirely of the αβ-atropisomer because of the steric
constraint during the formation.
CHAPTER 1. INTRODUCTION: THE BIOLOGICAL IMPORTANCE OF IRON PORPHYRINS AND THE NEED FOR MODEL STUDIES

[The scientists] can develop the competence to convey the essence and importance of what we do, and the satisfaction we derive ... to those who ask the question, “So, what do you do for a living?” The challenge lies in creating ways to develop and reinforce these skills in scientists at large... Institutions could encourage graduate students ... to include an optional short chapter in their dissertations that summarizes their work for a nonspecialist reader, useful even for scientists outside the students’ specialities.


1.1 Porphyrians

Porphyrians are the ring-like molecules whose core consists of 20 carbon and 4 nitrogen atoms that are arranged as shown in Figure 1.1. They are found widely in virtually all living systems: they give rise to the red color of blood and tissue and the green color of plants, and the prevalence of these colors around us is an evidence of their ubiquity. They are versatile in the roles they play in the living systems.¹ Their functions range from the most general — carrying oxygen and harvesting the energy from sunlight — to the most specialized — carrying an agent for dilating blood vessels for an insect that feeds on blood.²

One type of porphyrin complex is particularly relevant to this dissertation: iron porphyrinate complex, also known as heme, the species that has an iron atom in the middle hole. Heme functions in one of the following capacities: (1) to bind a molecule so that it could be transported intact from one part of the body to another; (2) to bind a
molecule or an atom, and *modify* it chemically; or (3) to give or receive electrons as units of energy, in the way similar to a battery. Each of these three functions will be explained later in more detail, and a specific example will be given for each function (see Figure 1.2, adapted from References 3, 4, 5, and 6). In all cases, the iron atom serves as the binding site, while both the porphyrin ring and the iron atom serve together as the reservoir for the electrons.

1.2 Proteins that contain iron porphyrin

1.2.1 Oxygen Transporters: Hemoglobin and Myoglobin.

Hemoglobin and myoglobin are the most important of the hemoproteins that serve as transporters of molecules. Hemoglobin is a protein present in the red blood cells; it binds a molecule of oxygen gas so that it could be distributed throughout the body. Myoglobin is a protein present in all cells of the aerobic organisms; it stores an oxygen molecule that is to be used for cellular metabolism. They give the familiar red color to blood and tissue.

Hemoglobin and myoglobin are both made up of the units of folded protein whose structure is shown in Figure 1.2 (middle figure). Hemoglobin contains four of these units; myoglobin, only one (Figure 1.3). Iron porphyrin is present in the center of each unit in hemoglobin or myoglobin, serving as the binding site for oxygen. The structure around the binding site is shown in Figure 1.3. The oxygen gas molecule attaches onto the iron atom. The other side of the heme is occupied with a protein chain whose end is attached onto the iron: when the oxygen molecule is released, the chain pulls the iron
away from the center of the heme. The movement of the protein chain becomes transmitted throughout the unit, changing the latter's shape. In hemoglobin, the change in shape of one unit is transmitted to all the other units, resulting in a cooperative interaction in which all of the oxygen molecules are released at once. This massive release ensures that the oxygens are transported from hemoglobins to myoglobins instead of the other way around. The myoglobins store the oxygens until the tissue is ready to use them.

The detailed mechanism for the cooperative interaction within hemoglobin was proposed originally by Perutz. While the mechanism is fascinating, it is too complicated to be explained satisfactorily in this Chapter: the readers are advised to read the original paper or a beautifully illustrated monograph by Dickerson and Geis.

1.2.2 OXIDIZER PROTEINS: CYTOCHROME P450.

The proteins mentioned previously binds oxygen molecule and carries it intact until it is released. Another class of oxygen-binding hemoprotein exists; this one breaks down the oxygen molecule to create a reactive fragment that is used for a rapid oxidation. In particular, the protein called cytochrome P450, present in a large quantity in the liver, uses the reactive oxygen fragment to convert a molecule into a form more useful to the organism, or to destroy poisonous molecules and metabolic wastes. The protein has the structure as shown in Figure 1.2.

Cytochrome P450 works in a catalytic cycle, as long it does not destroy itself during the reaction (as it does when cyclopropylamines are involved). First, an oxygen
molecule adds onto the iron atom on the heme. Then, one of the two atoms in the molecule breaks off. The remaining atom, now a reactive fragment, is carried by the heme to a waste material: the reactive oxygen atom is transferred to the waste, making the latter more soluble to water for excretion. (Figure 1.4, adapted from Sono et al.9)

1.2.3 ELECTRON-TRANSFER PROTEINS: CYTOCHROMES B AND C.

In the hemoproteins known as cytochromes b and c (Figure 1.2) — which belong to a different class from cytochrome P450 in spite of the similarity in the name — the heme does not bind another molecule, but instead stores and releases electrons. These proteins act as mobile batteries inside living systems: instead of giving and receiving a current of electricity like man-made batteries do, they give and receive electricity one electron at a time. Cytochromes b and c form a part of the cellular respiration cycle, in which energy is produced by the oxidation of carbohydrates and fatty acids. The cytochromes shuttle electrons released by the oxidation to the ultimate acceptor, oxygen molecule.

Although hemoglobins are more visible to us than the cytochromes, the latter are more ubiquitous. Hemoglobins are present almost exclusively in vertebrates: invertebrates, plants, fungi, and bacteria mostly have oxygen carriers that are not heme-based. On the other hand, cytochromes are present in virtually all aerobic organisms,10 as well as in some anaerobic organisms.11 In the evolutionary process, the living systems have been capable of making a variety of oxygen carriers, but only one type of one-electron carriers for the respiratory process.
1.3 Model Studies

1.3.1 Defining Model Studies.

To study the porphyrins that are relevant to the living systems, one could study a hemoprotein; or one could study an isolated heme without surrounding protein. The latter — a study of the active site of a metalloprotein in isolated form — is called a model study. A model porphyrin does not necessarily have to be isolated from the hemoprotein: indeed, models studied usually are synthetic, designed so that they are easier to make or handle than the hemes from living systems.

1.3.2 Hemoproteins vs. Models — Advantages and Disadvantages.

The advantage of studying a model instead of a protein lies in the simplicity of execution and analysis. A study of an actual protein is tedious, time-consuming, and fraught with possibilities for errors. A sufficient quantity of the source for the desired protein must be collected: the raw material necessary to obtain a few hundred milligrams of the desired protein sometimes reaches a few tons that are harvested from millions of slaughtered animals. The sources must then be cut, cleaned, ground, and processed through many stages, in a preparation that often takes up to several continuous and consecutive days. The protein that is finally isolated is very fragile, and must be kept refrigerated; even then, its shelf life often is in the order of a year. Once an experiment begins, the protein matrix often interferes with the attempted study on the active site: the protein reacts with the chemical that was intended to react with the active site, or a measurement to see the change in the active site is overwhelmed by signals from the
On the other hand, model compounds are often inadequate in reproducing the physics and chemistry of metalloproteins; they may even have properties that had not been predicted. The protein provides a structure and an environment that enhances the reaction, or simply makes the reaction possible. One often encounters a difficulty in designing and making a model compound whose structure is sufficiently similar to the metalloprotein's active site. Some metalloproteins have a reaction center that is so difficult to model that the chemical and physical properties were elucidated almost completely using the protein, long before a suitable model became available. (The siroheme-based sulfite reductase is a notable example.\textsuperscript{13})

In the studies made for this dissertation, model compounds were used. No specific hemoprotein was targeted for our studies. Instead, we wished to understand the potential effects of some structural perturbations on porphyrins that occur in various hemoproteins. It would be easier to modify a porphyrin so that it would be perturbed in the way we wish, rather than to find a hemoprotein whose porphyrin ring shows exactly the type of perturbation that we wish to study.

1.3.3 PORPHYRINS USED MOST OFTEN IN MODEL STUDIES.

In model studies, the porphyrins most often used are the easily obtainable ones. One type of porphyrin used frequently is protoporphyrin IX (Figure 1.5). This is the porphyrin that is found most often in the living systems, and can be extracted easily from various organisms. This porphyrin however is not the most popular one used for model
studies. Its lack of symmetry makes the analysis of experimental data more complicated than necessary. Also, its close relation to the porphyrins in the living systems does not necessarily become an advantage. Although the living systems do use the shape of protoporphyrin IX to recognize and fit it in the cavity of the hemoproteins, the shape does not give rise to a particular chemical property of which an organism could take an advantage. Also, protoporphyrin IX is not as easy to modify as are the synthetic porphyrins that are described next.

The two types of synthetic porphyrins used often are octaethylporphyrin and tetraphenylporphyrin (Figure 1.5). Both of them are highly symmetrical, and the their spectral data are easy to interpret. Modified forms are easily prepared by varying the starting materials for the synthesis. These two porphyrins and their variants are the most widely studied in the model studies of hemes. In the studies for this dissertation, modified tetraphenylporphyrins were used.

1.4 Studies Performed for this Dissertation

We have already stated that studying the simplest of isolated hemes is not enough: at least some of the effect from the protein matrix must be replicated in the model. In a hemoprotein, the molecule that binds to the heme encounters various forces. (Figure 1.6) It may: (1) hydrogen-bond to a donor atom in the protein; or (2) sterically interact with the peptide chain that forms a cavity within the protein (the “pocket” effect). In addition, another interaction may be caused by a subtle effect: (3) the protein matrix may distort the heme, forming a pocket that interacts sterically.
In this dissertation, the effects (2) and (3) are addressed. The effect (1), although an important one, is outside of the scope here.

1.4.1 PORPHYRIN NONPLANARITY.

In almost all of the existing works on hemoproteins, the ring is assumed to be perfectly planar. The recent works on hemoproteins however suggest that in many proteins the hemes are not planar. In a study in which the crystal structures of cytochrome c were systematically checked, the observed structure showed a significant distortion of the heme. Also, it has been known that the heme in myoglobin and hemoglobin is distorted when the oxygen molecule is absent.7,8

One must find a suitable model in order to understand the effect of nonplanarity on the properties of porphyrins. Fortunately, there have already been successful syntheses of porphyrins whose rings are distorted out of plane.15,16,17,18 The crystal structures of many of these synthetic molecules have already been determined, and therefore the degree and type of distortion can be correlated with the spectroscopic data and chemical reactivity.

The porphyrin that was used for our study is octaethyltetraphenylporphyrin15 (Figure 1.7). The structural studies of this porphyrin shows that the macrocycle is distorted in “saddle” conformation. (In this porphyrin, the five-membered pyrrole rings constitute the apexes of the saddle. Another saddle-like distortion, in which the axis of distortion is rotated by 45°, i.e. the meso-carbons consitute the apexes, has a different name — “ruffle” conformation. For further discussion, see Scheidt and Lee23 and Jentzen
The study of this porphyrin with a coordinated iron atom have not been made extensively until recently. In this dissertation, we present the first detailed nuclear magnetic resonance (NMR) studies of iron(III) octaethyltetraphenylporphyrinate.

1.4.2 Relative Orientation of the Axial Ligands.

The molecule that is bound onto a heme perpendicularly to the plane of the ring is called the axial ligand. For example, the bound oxygen molecule in a hemoglobin is an axial ligand to the heme. An axial ligand is not limited to oxygen: a part of the protein chain is almost always attached to at least one side of the heme, as we have already seen with hemoglobin and myoglobin. The amino acid that is bound to the heme is usually histidine or the sulfur-containing cysteine or methionine (Figure 1.8). Cysteine- and methionine-bound hemes are widespread and very important to the living systems, cytochrome P450 (§1.2.2) and cytochrome c (§1.2.3) being the most prominent examples. Mimicking a sulfur-ligated heme, however, is difficult because of two reasons: (1) an iron(II)-sulfur bond is relatively weak; and (2) iron(III) oxidizes most organic sulfides into disulfides. On the other hand, histidine-bound hemes are easy to mimic because a iron-nitrogen bond is relatively robust, and also because the five-membered ring on histidine — called imidazole — is redox-stable.

In a biological iron(III) porphyrin complex bound to two histidines, the angle formed by the two imidazole ligands determine a significant portion of the environment around the metal. A calculation based on EPR and Mössbauer spectra and estimated values of iron(III) spin-orbit coupling have shown that the ability for a heme to carry
and release an electron may vary significantly as the imidazole-imidazole angle is changed. In simpler iron(III)-imidazole complexes, the axial ligands rotate freely around the iron-nitrogen bond. In order to prevent this free rotation, it is necessary to have a model in which the angle between the two axial ligands could be controlled.

Our target molecule, shown in Figure 1.9, is a modified tetraphenylporphyrin. Two of the four phenyls are modified to attach a sterically large group. The large groups protrude close to where the axial ligands bind, constraining their rotation. Since the two large groups are located across the porphyrin ring, the axial ligands are oriented in mutually parallel configuration. The synthesis of this molecule is described in this dissertation.
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Figure 1.2 Some hemoproteins. Top. Myoglobin. Middle. Cytochrome P450_{cam} (with a camphor molecule present in the reaction site). Bottom. Cytochrome b_{5}. 
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CHAPTER 2. NUCLEAR MAGNETIC RESONANCE
SPECTROSCOPY AS A TOOL FOR STUDYING NONPLANAR
FERRIHEMES

If you want to learn physics, don’t ever rely on professors or grad students. They’re useless! If you go up to them and ask questions, they’ll sneer at you and laugh at how stupid you are. You’ll learn physics only by reading, working out problems yourself, and cutting breezes with the other undergrads.

— A physics undergraduate at the University of Texas, ca. 1990.

2.1 Introduction

In this Chapter, the following are described: (1) octaethyltetraphenylporphyrinato-iron(III) ([Fe^III^OETPP]^+) (Figure 2.1) — its structure and relevance as a model for biological systems; and (2) the techniques in the nuclear magnetic resonance (NMR) that we used to study this iron porphyrin. This Chapter serves as an introduction to the next chapter — the account of the experimental work — and we attempt here to explain concisely and carefully the concepts central to the experiments. The synthetic aspect of [Fe^III^OETPP]^+ is de-emphasized: the focus will be on its structure, dynamics, and electronics. Likewise for NMR, the theoretical discussions will be kept as qualitative as possible, and only as a means to explain the specific techniques used in the experiments.

2.2 Octaethyltetraphenylporphyrin (H_2OETPP)

2.2.1 SYNTHESIS.

A related molecule, octamethyltetraphenylporphyrin, was first synthesized by Dolphin,\textsuperscript{21} by the reaction of 2,3-dimethylpyrrole and benzaldehyde in propionic acid at
room temperature, followed by the isolation and oxidation of the resulting porphyrinogen. Octaethyltetraphenylporphyrin (H$_2$OETPP) was first synthesized by Evans et al. In the improved procedure reported later by Barkigia et al., this porphyrin is synthesized from 2,3-diethylpyrrole and benzaldehyde by the procedure analogous to Dolphin's, but using CH$_2$Cl$_2$ / BF$_3$ as the solvent.

2.2.2 Static structure.

The crystal structure of H$_2$OETPP that was solved by Barkigia et al. shows a strong D$_{2d}$ distortion caused by the steric interaction between the ethyl groups in the β-positions and the phenyl groups in the meso-positions. This distortion, in which the pyrrole β-carbons are moved the farthest away from the porphine plane, is termed "saddle", and found also in the tetraarylporphyrins substituted at all the β-positions with halogens.

The metallocomplexes of OETPP preserve the saddle distortion. The grooves formed by the saddle distortion act as cavities for planar axial ligands. The crystal structures of [Cu$^{II}$(OETPP)(pyridine)]$^{1+}$pyridine and the molecular modeling and NMR studies of [Co$^{III}$(OETPP)L$_2$]$^{-}$ (L = pyridine, 1-methylimidazole) have indicated that the aromatic amines align themselves along the grooves formed by the saddle distortion; when two axial ligands are present, they align in mutually perpendicular orientation.

Axially bis-ligated aromatic amine complexes of the low-spin [Fe$^{III}$(OETPP)]$^{1+}$ have not been studied until now. Usually, the low-spin iron(III) porphyrinate complexes
ligate aromatic amines in mutually parallel orientation because of the Jahn-Teller effect.\textsuperscript{20}
(This electronic effect is discussed in detail in §2.2.4.) In [Fe\textsuperscript{III}(OETPP)(aromatic amine)\textsubscript{2}]\textsuperscript{+}, the steric and the electronic factors are expected to oppose each other in determining the orientation of the axial ligands. This interplay between the two opposing tendencies is investigated in the experimental works detailed in the next chapter.

2.2.3 INTERNAL DYNAMICS.

The distorted porphyrins are conformationally fluctional in their conformation, in a manner analogous to cyclohexanes. In a saddled porphyrin, one group of four $\beta$-carbons is located on one side of the porphyrin plane, and the other group is located on the other side. One group may move to the other side; but in order to do so, the other group must move simultaneously. This simultaneous movement of all the $\beta$-carbons results in another saddle conformation that appears to be rotated $90^\circ$ from the original conformation. (Figure 2.2) The saddle-saddle flip-flop has been studied by 1-D NMR spectroscopy in the free-base octaethyltetraphenylporphyrin\textsuperscript{15} and its zinc(II)\textsuperscript{15} and nickel(II)\textsuperscript{29} complexes. These studies have indeed indicated that the macrocycle fluctuates between two saddle conformations. The NMR spectra of [Co\textsuperscript{III}(OETPP)L\textsubscript{2}]\textsuperscript{+} (L = pyridine, N-methylimidazole)\textsuperscript{27,28} indicate that a similar fluctuation occurs in these 6-coordinate molecules. Theoretically, NMR spectroscopy may also be used for determining the axial ligands detaching from and reattaching to the metal (Figure 2.2): but none of the studies above have positively identified the ligand on-off dynamics.

All the studies reviewed above have been performed using 1-D NMR
spectroscopy. Studies of the dynamics can also be performed using 2-D NMR: this method may be used to obtain better information on the intermediates. All the dynamic studies in the next chapter were made using 2-D techniques.

2.2.4 ELECTRONIC STRUCTURE.

Theoretical studies\textsuperscript{25,30,31} indicate that in a porphyrin that is distorted out of plane, the energies of the molecular orbitals are different from the nondistorted counterparts: the HOMO and the second-highest occupied molecular orbital (SHOMO) are raised considerably, and the LUMO is raised to a smaller extent. The smaller size of the HOMO-LUMO and the SHOMO-LUMO gaps, predicted by these studies, has been confirmed through UV/visible spectroscopic studies.\textsuperscript{21,32,33,34,35} (Photoelectron spectroscopy has not yet been performed on nonplanar porphyrins, and therefore no experimental data exists on the \textit{absolute} orbital energies. An experiment is currently underway.) Red-shifting of the Soret and Q bands is expected to be found regardless of the choice of the coordinated metal or the axial ligands, and therefore UV/visible spectroscopic studies are not expected to reveal significantly new information on $[\text{Fe}^{III}(\text{OETPP})(\text{aromatic amine})]^{-}$.

In a low-spin iron(III) complex, another electronic gap exists, and the porphyrin distortion may affect its size. (Figure 2.3) In a planar ferriheme / bis(aromatic amine) complex where the ligands are oriented \textit{perpendicular} to each other, the overall symmetry is $D_{2d}$ if the ligands align on the porphyrin's $C_2$ axis, or $S_4$ otherwise. In either symmetry, a degenerate $E$ set exists for the metal-based orbital: $d_{xz}$ and $d_{yz}$. In the
corresponding bis(aromatic amine) complex of a ferriheme distorted in saddle or ruffle conformation, the lowest possible overall symmetry would be $S_4$: in this symmetry, the degeneracy of the E set is preserved. In a ferriheme complex with the $d_{xy}^2(d_{xz},d_{yz})^3$ configuration, the number of the electrons in each member of the E set is two and one: this unequal distribution of the electrons is an unstable configuration and is susceptible to a Jahn-Teller distortion.\(^\text{20}\)

In a planar ferriheme / bis(aromatic amine) complex in which the ligands are oriented parallel to each other, the overall symmetry would be $D_{2h}$ if the ligands are aligned to one of the porphyrin's $C_2$ axes, or $D_2$ otherwise. In either of the two symmetries, the $d_{xz}$ and $d_{yz}$ orbitals are not degenerate. Therefore, in a complex that has the $d_{xy}^2(d_{xz},d_{yz})^3$ configuration, a tendency exists for the axial ligands to become mutually parallel. The distortion of the porphyrin would not restore the degeneracy. In a complex containing a saddled or ruffled porphyrin, this driving force caused by the Jahn-Teller effect opposes the force caused by the steric interaction between the ligands and the porphyrin.

This gap is too small to be measured by UV/visible spectroscopy, but it can be measured by variable-temperature NMR spectroscopy under favorable conditions. In the next chapter, NMR spectroscopic studies are reported.

2.2.5 RELEVANCE TO BIOLOGICAL SYSTEMS.

The partially conjugated tetrapyrrolic systems such as vitamin $B_{12}$,\(^\text{37}\) chlorophyll,\(^\text{30}\) siroheme,\(^\text{13}\) and factor $F_{430}$\(^\text{38}\) are distorted because of the influence of the $sp^3$ carbons in
the ring. Vitamin B$_{12}$ contains an especially bulky axial ligand that distorts the ring even further.$^{37}$ Even some of the completely conjugated porphyrins are found distorted in the crystal structures of hemoproteins.$^{14}$ A systematic search$^{39}$ of the published crystal structures of hemoproteins has indicated that the tetrapyrrole rings in a significant quantity of the hemoproteins are distorted in the saddle conformation or the “ruffle” (a $D_{2d}$ distortion in which the meso-atoms are the farthest away from the tetrapyrrole plane) conformation, or a combination of the two.

Although nonplanar distortion in the tetrapyrrole macrocycle has been observed in many proteins, the role of the distortion has not yet become clear. The properties of distorted porphyrins, especially those containing low-spin iron(III) and an aromatic axial ligand, have not been studied thoroughly, and this is the motivation for the work in this dissertation.

2.3 NMR Concepts Relevant to this Investigation.

2.3.1 Paramagnetic Shift.$^{40}$

The observed chemical shift ($\delta_{\text{obs}}$) of a paramagnetic compound may be separated into two contributions.

$$\delta_{\text{obs}} = \delta_{\text{dia}} + \delta_{\text{para}}$$

The paramagnetic shift, $\delta_{\text{para}}$, may be divided into two contributions, contact ($\delta_{\text{con}}$) and the dipolar ($\delta_{\text{dip}}$).

$$\delta_{\text{para}} = \delta_{\text{con}} + \delta_{\text{dip}}$$
The origin of contact and dipolar shifts, and their temperature-dependence are discussed here.

2.3.1.1 Contact shift and Curie behavior.

Contact shift arises from the spin-spin interaction between the nucleus and unpaired electrons. An NMR transition in one nucleus is commonly represented as a two-level diagram, in which the change in the nuclear spin from $\alpha$ to $\beta$ is represented as a jump across the gap. A system that contains one nucleus with $I=1/2$ and one unpaired electron ($S=1/2$) is represented as a four-level diagram (Figure 2.4), in which each nuclear spin level is split by the electronic spin. The resulting two nuclear spin transitions (Transitions a and b in Figure 2.4) are not degenerate, because each level is perturbed by the nucleus-electron spin coupling $A$. If there were no other factors perturbing the system, the NMR spectrum would show two peaks that correspond to these two transitions.

$$h\nu_{a} = h\gamma_{N} H + \frac{1}{2} A$$
$$h\nu_{b} = h\gamma_{N} H - \frac{1}{2} A$$

($\gamma_{N}$: gyromagnetic ratio of the nucleus; $H$: strength of the external magnetic field; $A$: electron-nucleus spin coupling constant.)

Since electron spin has a finite relaxation time, Equation 3 is not valid. If the electron relaxation time is fast ($T_{1e} \leq 10^{-11}$ s), these two signals converge: this peak is observed at the average of the two, weighted by the Boltzmann distribution between the two electron spin states.
\[
\text{hν}_{\text{avg}} = \frac{1}{1 + \exp(-\langle g \rangle βH/kT)}(h\gamma_N H + \frac{1}{2}A) + \frac{1}{1 + \exp(\langle g \rangle βH/kT)}(h\gamma_N H - \frac{1}{2}A) \tag{4}
\]

\(\langle g \rangle\): average value of the anisotropic g-tensors; \(β\): Bohr magneton; \(k\): Boltzmann constant; \(T\): temperature. Note that the weight functions are normalized, i.e. \([1+\exp(-\langle g \rangle βH/kT)]^{-1} + [1+\exp(\langle g \rangle βH/kT)]^{-1} = 1.\)

Equation 4 may be simplified further. For small \(ν\), the following approximation may be made.

\[
e^ν = 1 + ν + \frac{ν^2}{2!} + \frac{ν^3}{3!} + \frac{ν^4}{4!} + \ldots
\]

\[≈ 1 + ν\] \tag{5}

Therefore, Equation 4 may be rewritten as follows.

\[
\text{hν}_{\text{avg}} = \frac{h\gamma_N H + \frac{1}{2}A}{2 - \langle g \rangle βH/kT} + \frac{h\gamma_N H - \frac{1}{2}A}{2 + \langle g \rangle βH/kT}
\]

\[= \frac{4h\gamma_N H + A\langle g \rangle βH/kT}{4 - (\langle g \rangle βH/kT)^2}\]

\[≡ \frac{4h\gamma_N H + A\langle g \rangle βH/kT}{4}\]

\[= h\gamma_N H + \frac{A\langle g \rangle βH}{4kT}\] \tag{6}

Thus, the contact shift in for a system with \(I=1/2, S=1/2\) may be expressed as:

\[
δ_{\text{con}} = \frac{\text{hν}_{\text{avg}} - \text{hν}_{\text{dia}}}{\text{hν}_{\text{dia}}} = \frac{A\langle g \rangle βH/4kT}{h\gamma_N H} = \frac{A\langle g \rangle β}{4h\gamma_N kT}\] \tag{7}

For cases in which \(S>1/2\), the expression for contact shift may be generalized as: \(^{41}\)
Spin coupling constant $A$, electron spin quantum number $S$, and π-electron spin density $\rho_c$ are related by the following equation.

$$A = \frac{Q\rho_c}{2S}$$  \hspace{1cm} (9)

(Q is commonly called McConnell Q factor, and is assumed to be constant for a given type of proton.) Equations 8 and 9 may be combined to give the following equation, which will be more useful when describing cases in which the spin density is temperature-dependent.

$$\delta_{\text{con}} = \frac{Q\rho_c (g)\beta (S + 1)}{6\gamma_N\hbar kT}$$  \hspace{1cm} (10)

All the terms in Equation 10 except $T$ are constant. Therefore, we may simplify the equation as follows.

$$\delta_{\text{con}} = K_{\text{con}} / T$$  \hspace{1cm} (11)

In other words, if the contact component of paramagnetic shift is isolated and plotted against $1/T$, the plot will be linear with the slope of $K_{\text{con}}$ and the y-intercept of 0 (Figure 2.6).

2.3.1.2 Dipolar shift and Curie behavior

The dipolar contribution $\delta_{\text{dip}}$ may be expressed as follows.
\[
\delta_{\text{dip}} = \frac{N_A}{12\pi} \left\{ \chi_{zz} - \frac{1}{2} (\chi_{xx} + \chi_{yy}) \frac{3\cos^2 \theta - 1}{r^3} + \frac{1}{2} (\chi_{xx} - \chi_{yy}) \frac{\sin^2 \theta \cos 2\Omega}{r^3} \right\} \tag{12}
\]

(\chi_{ii}: magnetic susceptibility anisotropy tensor; \(N_A\): Avogadro's number; \(\theta, \Omega, r\): position of the nucleus in polar coordinate (also called structure factor.))

For a system for which the second-order Zeeman contribution to the magnetic susceptibility is negligible, the following equation is applicable. (This assumption holds for iron(III) tetraphenylporphyrinate / bis(imidazole), but not for cytochromes.)

\[
\chi_{ii} = \frac{\mu_0 \beta^2 S(S+1)}{3kT} g_{ii}^2 \tag{13}
\]

(\(\mu_0\): permittivity of free space; \(\beta\): Bohr magneton; \(g_{ii}\): g anisotropy tensor.)

Then Equation 12 may be rewritten in terms of g-tensors, which are numbers that may be obtained from an EPR spectrum of frozen solution.

\[
\delta_{\text{dip}} = \frac{\mu_0 \beta^2 S(S+1)}{72\pi kT} \left\{ 2g_{zz}^2 - \left( g_{xx}^2 + g_{yy}^2 \right) \frac{3\cos^2 \theta - 1}{r^3} + \frac{3}{2} \left( g_{xx}^2 - g_{yy}^2 \right) \frac{\sin^2 \theta \cos 2\Omega}{r^3} \right\} \tag{14}
\]

Again, all the terms except for \(T\) are constant, and therefore Equation 14 may be abbreviated as follows, to make the Curie relation more clear.

\[
\delta_{\text{dip}} = K_{\text{dip}} / T \tag{15}
\]

Equations 11 and 15 may be incorporated into Equation 2 to give the following.

\[
\delta_{\text{para}} = \left( K_{\text{con}} + K_{\text{dip}} \right) / T = K_{\text{para}} / T \tag{16}
\]
This equation indicates that a graph of paramagnetic shift vs. $1/T$ is linear if all the assumptions made previously do hold. It is possible to separate the contribution from the dipolar shift by either (1) calculating it from the g-tensors obtained from EPR data and the structural factors obtained from a crystal structure or a model; or (2) observing the protons whose paramagnetic shift consists almost exclusively of the dipolar contribution, e.g., protons on the meso-substituent for Fe(III) porphyrinate complexes with the $d_{xy}^2(d_{xz},d_{yz})^3$ configuration.42

2.3.1.3 Non-Curie behavior.

Systems exist in which the Curie plot has non-zero y-intercept, or is nonlinear. For these systems, an additional process must be considered, because the plot is actually a combination of two different plots.

Equation 16 is not valid if the paramagnetic shift does not obey the Curie law. The most common case is the presence of a low-lying excited state that is thermally accessible (i.e., $\Delta E$ is in the order of $kT$). This causes a deviation in both the contact and dipolar shifts, because the shifts are different between the excited and the ground states. For the contact shift, the deviation results from the difference in the spin density ($\rho_C$ in Equation 10) between the ground and excited states; for the dipolar shift, the deviation results from the difference in the $g$-tensors ($g_{ii}$ in Equation 14). In either case, the deviation is nonlinear with respect to $1/T$, because the population ratio of the two states vary according to $\exp(-C/T)$. If the paramagnetic shifts of proton $n$ in the pure ground and excited states are expressed as $\delta_{n1}$ and $\delta_{n2}$, then the observed paramagnetic shift $\delta_n$ at
a given temperature $T$ may be described as follows.

$$
\delta_n = \frac{\delta_{n1} W_1 + \delta_{n2} W_2 \exp(-\Delta E/kT)}{W_1 + W_2 \exp(-\Delta E/kT)}
$$

(17)

($W_1$, $W_2$: statistical weights of the ground and excited states (for a state with pure electron spin $S_i$, the condition $W_i=2S_i +1$ holds); $\Delta E$: energy difference between the ground and excited states.)

The paramagnetic shifts $\delta_{n1}$ and $\delta_{n2}$ at pure electronic states obey the Curie law. If the Curie constant for the pure ground and excited state are expressed as $K_{\text{para}, n1}$ and $K_{\text{para}, n2}$, Equation 17 may be rewritten as follows.

$$
\delta_n = \frac{\left(K_{\text{para}, n1}/T\right) W_1 + \left(K_{\text{para}, n2}/T\right) W_2 \exp(-\Delta E/kT)}{W_1 + W_2 \exp(-\Delta E/kT)}
$$

(18)

At the low temperature region where $kT \ll \Delta E$, the term $\exp(-\Delta E/kT)$ becomes approximately equal to 0, and therefore Equation 18 reduces to the following approximation.

$$
\delta_{n,\text{low}T} \approx \frac{1}{T} \frac{K_{\text{para}, n1} W_1}{W_1} = K_{\text{para}, n1}/T
$$

(19)

At the temperature region where $kT \gg \Delta E$, the term $\exp(-\Delta E/kT)$ becomes approximately equal to 1. If the temperature is at the intermediate region where the
condition $1/T>>0$ also applies, the following approximation can be made.

$$\delta_{n,\text{intermed}} \approx \frac{1}{T} \frac{K_{\text{para,n1}} W_1 + K_{\text{para,n2}} W_2}{W_1 + W_2} \quad (20)$$

Both Equations 19 and 20 are linear with respect to $1/T$, as long as the temperature range is small. However, their slopes are different. (Figure 2.6) Therefore, the line that connects these two graphs between the temperature regions cannot be linear: this is the origin of nonlinearity in the overall Curie plot. It should be noted that temperature at which NMR spectra are usually taken (220-370 K for nonbiological samples, 280-310 K for biological samples) usually lies exclusively in the nonlinear region: at very low temperature, the solvent freezes and therefore the resulting spectrum is anisotropic and difficult to interpret; and at very high temperature, either the solvent evaporates or the sample disintegrates.

Equation 18 is formulated for the overall paramagnetic shift, but analogous equations may be written separately for the contact and dipolar shift.

2.3.1.4 Nonlinearity in contact and dipolar shifts, and extracting $\Delta E$ from paramagnetic shift data.

The contact shift in both the purely ground and excited states can be written as follows, in the manner analogous to Equation 10.
\[ \delta_{n \text{con}} = \frac{Q\beta(g)(S+1)C_{n\text{i}}^2}{6\gamma_N\hbar kT} = \frac{F}{T}C_{n\text{i}}^2 \] (21)

\[ (F = Q\beta(g)(S+1)/6\gamma_N\hbar k) \]

(C\text{ni}: orbital coefficients in the ground (n1) and excited (n2) states; the other terms are defined after Equation 10. Notice that the $C_{n\text{i}}^2$ is equal to the $\pi$-spin density (written as $\rho_C$ in Equation 10) at the carbon adjacent to the hydrogen n.)

The overall contact shift $\delta_{n \text{con}}$ at the hydrogen $n$ at the temperature $T$ may be expressed as follows, in the manner analogous to Equation 18.

\[ \delta_{n \text{con}} = \frac{\delta_{n\text{i}1}W_1 + \delta_{n\text{i}2}W_2 \exp(-\Delta E/kT)}{W_1 + W_2 \exp(-\Delta E/kT)} = \frac{(F/T)C_{n\text{i}1}W_1 + (F/T)C_{n\text{i}2}W_2 \exp(-\Delta E/kT)}{W_1 + W_2 \exp(-\Delta E/kT)} \] (22)

Equation 22 indicates that the contact shift of a proton in the two-level system has non-Curie behavior. Moreover, the equation indicates that the energy difference $\Delta E$ may be determined by fitting variable-temperature NMR data. Before the curve-fitting is done, one must discuss the possible error in the calculation that results from the dipolar shift.

The dipolar shift $\delta_{n \text{dep}}$ at the temperature $T$ may be described by the following
equation, which is derived from Equations 14 and 18.

\[
\gamma^{\text{ dip}} = \frac{\mu_0 \beta^2 S(S+1)}{72\pi kT} \\
\cdot \left[ \left( \frac{3 \cos^2 \theta - 1}{r^3} \right) W_1 \left( 2g_{zz,1}^2 - \left( \frac{g_{xx,1}^2 + g_{yy,1}^2}{2} \right) \right) + W_2 \left( 2g_{zz,2}^2 - \left( \frac{g_{xx,2}^2 + g_{yy,2}^2}{2} \right) \right) \exp(-\Delta E/kT) \right] \\
+ \left[ \left( \frac{3 \sin^2 \theta \cos 2\Omega}{r^3} \right) W_1 \left( g_{xx,1}^2 - g_{yy,1}^2 \right) + W_2 \left( g_{xx,2}^2 - g_{yy,2}^2 \right) \exp(-\Delta E/kT) \right]
\]

(23)

The g-tensors or magnetic anisotropy tensors of the pure excited state cannot be determined independently by an experiment. However, Equation 23 may be simplified and the dipolar contribution estimated if we make assumptions that are specific to low-spin ferrihemes. In an iron(III) porphyrinate complex with the \(d^7(d^7,d_{yz})^3\) configuration, the ground and excited states differ because of the movement of one electron from to \(d_{yz}\). Since the movement affects the magnetic anisotropy along the x- and y- but not z-axis, \(\chi_{zz}\) (and therefore \(g_{zz}^2\)) is not expected to vary. Also, \(\chi_{xx} + \chi_{yy}\) (and therefore \(g_{xx}^2 + g_{yy}^2\)) is expected to remain constant. Therefore, the term that contains the factor \(3\cos^2 \theta - 1\) ("axial") is expected to be constant over temperature and not contribute to nonlinearity. The difference \(\chi_{xx} - \chi_{yy}\) (and therefore \(g_{xx}^2 - g_{yy}^2\)) is expected to vary and therefore the term that contains the factor \(\sin^2 \theta \cos 2\Omega\) ("rhombic") is expected to contribute to nonlinearity, but for iron(III)tetraarylporphyrinate / bis(imidazole) systems this term is relatively small — 1/5 to 1/10 of the contribution from contact shift.\(^7\)

Therefore, the axial and rhombic dipolar contributions may be calculated or measured and
subtracted from paramagnetic shift data, and the resulting "pure" contact shift data may be used for calculating $\Delta E$.

2.3.1.5 Axial ligand orientation, Jahn-Teller distortion, and the influence on contact shift.

In a ferriheme complex, two extreme cases exist for the mutual orientation of two aromatic amine axial ligands: perpendicular and parallel. For the reasons detailed in §2.2.4, the mutually perpendicular orientation causes the degeneracy of two $d$ orbitals, whereas this degeneracy is destroyed by the mutually parallel orientation. In most of these complexes, the electron configuration is $d_{xy}^2(d_{xz},d_{yz})^3$, and therefore the unpaired electron resides in one of the $d$-orbitals involved in this degeneracy. This electron causes the Jahn-Teller distortion that influences the mutual orientation of the axial ligands. In a complex with mutually nonperpendicular axial ligands, the removal of the degeneracy creates a gap between the $d_{xz}$ and $d_{yz}$ orbitals. This gap is in the order of $kT$, and therefore is not detectable using UV/visible spectroscopy. The gap however becomes apparent during variable-temperature studies of the NMR spectra: for the reasons detailed above, the plot of the contact shifts does not obey the Curie law. It is possible to determine the magnitude of the gap through such studies: Shokhirev has written a curve-fitting program for performing the necessary calculations.

2.3.1.6 Other factors that cause non-Curie behavior.

The calculation of the $d_{xz}$-$d_{yz}$ transition is possible only if the internal motion of the complex remains sufficiently constant over the temperature range so that the
McConnell Q values of the protons do not change.\textsuperscript{48} The temperature-variation of the Q values causes nonlinearity of the Curie plot that cannot be deconvoluted from that caused by the $d_{xz}$-$d_{yz}$ transition.

An assumption have been made that the electronic configuration does not change from $d_{xy}^2(d_{x^2-y^2})^3$. This assumption might not hold if the porphyrin ring is distorted. Wolowiec \textit{et al.}\textsuperscript{49} has synthesized an iron(III) imidazolate complex of a heavily saddled porphyrin; the NMR studies have indicated that as the temperature is decreased, the electron configuration changes from $d_{xy}^2(d_{x^2-y^2})^3$ to $d_{xz}^2d_{yz}^2d_{xy}$. Such transition would also cause a non-Curie behavior. A great caution must be observed during the interpretation of the Curie plots of nonplanar ferriheme complexes.

2.3.2 NOESY AND ROESY.

2.3.2.1 Overview.

Two 2-D NMR techniques are especially relevant to the studies of molecular fluctionality: NOESY (\textit{Nuclear Overhauser Effect Spectroscopy}) and ROESY (\textit{Rotational Overhauser Effect Spectroscopy}). In both techniques, the chemical exchange and the spatial proximity ("dipolar coupling") of the protons are detected.

In a NOESY spectrum taken above the point termed \textit{cross-over temperature} (which will be explained later), the cross-peaks caused by dipolar coupling have negative phase, while those caused by chemical exchange have positive phase. (Figure 2.7) The negative phase peaks may be used for structural studies, and the positive phase peaks for dynamic studies. In the spectrum taken below the transition temperature, all the cross-
peaks have positive phase: distinguishing the dipole-coupling peaks from the chemical-exchange peaks is difficult in this spectrum. In a ROESY spectrum, the phase relation of high-temperature NOESY spectra is maintained at all temperatures.

This is all the information necessary to understand the NOESY and ROESY studies described in Chapter 3. In this section, basic knowledge of the principles of the nuclear Overhauser effect is presented. Working knowledge of 2-D NMR is assumed for the readers.

2.3.2.2 Explanation of NOE through a four-level diagram.

A system that contains two protons (A and B) that are dipolar-coupled can be represented by a four-level diagram (Figure 2.8), in which the levels represent the following states: $\alpha_\alpha \alpha_B$, $\alpha_A \beta_B$, $\beta_A \alpha_B$, and $\beta_A \beta_B$ (henceforth abbreviated as $\alpha \alpha$, $\alpha \beta$, $\beta \alpha$, and $\beta \beta$, respectively). The population of the system at equilibrium obeys the Boltzmann law; but the population may be manipulated by selective irradiation. When the proton A is irradiated, transitions occur at $\alpha \alpha - \beta \alpha$ and $\alpha \beta - \beta \beta$, and the population becomes skewed accordingly. The tumbling of the system in liquid provides the energy for the usually forbidden transitions $\beta \beta - \alpha \alpha$ ("W_2", "double-quantum") and $\beta \alpha - \alpha \beta$ ("W_0", "zero-quantum") to occur. The $W_2$ transition causes the population difference at $\alpha \alpha - \alpha \beta$ and $\beta \alpha - \beta \beta$ to be greater than that at the equilibrium: when a $\pi/2$ pulse is given to the system and the signal is read and processed, the B signal in the resulting spectrum is found to be enhanced. Thus, $W_2$ transition results in a positive NOE. On the other hand, the $W_0$ transition enhances the population inversion at both $\alpha \alpha - \alpha \beta$ and $\beta \alpha - \beta \beta$. When a $\pi/2$ pulse
is given to the system and the signal is read and processed, the B signal in the resulting spectrum is found to be diminished, or even have negative phase in an extreme case. Thus, $W_0$ transition results in a negative NOE.

In a system in which the protons A and B exchange chemically, the labels interchange. Thus, the interchanges $\alpha_A\beta_B=\alpha_B\beta_A$ and $\beta_A\alpha_B=\beta_B\alpha_A$ occur. Both interchanges mimic the transition $\alpha\beta=\beta\alpha$, which is a $W_0$ transition. Thus, chemical exchange mimics a negative NOE.

The relative importance of $W_0$ and $W_2$ depends on the intensity of the molecular tumbling. If the intensity is small, $W_0$ dominates; and if the intensity is high, $W_2$ dominates. The population diagram shown in Figure 2.9 (adapted from Freeman\textsuperscript{50}) helps to illustrate the point.

The population density of the molecular tumbling changes with temperature. If the average angular frequency of tumbling is given as $\omega_0$, and correlation time $1/\omega_0$ is given as $\tau_c$, then the approximate expression is $\tau_c=4\pi\eta a^2/3kT$, where $\eta$ is the viscosity of the solvent, and $a$ the hydrodynamic radius of the molecule. (Within a small temperature interval, where viscosity $\eta$ may be regarded as constant. Qualitatively, lowering of the temperature results generally in higher viscosity and thus increases $\tau_c$ even more than where $\eta$ is constant.) At low temperature, the population is concentrated at low tumbling frequency. In the fast-tumbling region, the population is distributed throughout a wider range of frequency. For a molecule that tumbles with angular frequency above $\alpha\beta-\beta\alpha$ but below $\beta\beta-\alpha\alpha$, there is sufficient energy to cause the $W_0$ transition but not the $W_2$. 
Therefore, in the slow-tumbling region, $W_0$ is more important. For a molecule that tumbles with angular frequency above $\beta\beta-\alpha\alpha$, there is sufficient energy to cause both $W_0$ and $W_2$ transitions. If the tumbling is sufficiently faster than $\beta\beta-\alpha\alpha$, the magnitude of $W_2$ becomes greater than that of $W_0$.

The temperature where $W_2=W_0$ is called the *cross-over temperature*. At this temperature, no dipole-coupling cross-peak is observed in a NOESY spectrum. For the porphyrins reported in Chapter 3, the cross-over temperature is about -60°C.

### 2.3.2.3 NOESY, dipolar coupling, and chemical exchange.

The 1-D NOE experiments described above can be extended to two dimensions, using the pulse sequence $\pi/2-\tau_1-\pi/2-\tau_{mix}-\pi/2$-Acq. The resulting NOESY spectrum contains cross-peaks between the signals that are dipolar-coupled or chemically exchanging. A NOESY spectrum is usually phased so that the diagonal peaks are positive. In this spectrum, the phase is the opposite of the 1-D NOESY difference spectrum of the same molecule: above the cross-over temperature, the dipolar-coupling cross-peaks are negative, while the chemical-exchange cross-peaks are positive. At the temperatures below the cross-over, both dipolar-coupling and chemical-exchange peaks become positive.

Several explanations exist for the signs of the cross-peaks. According to Ernst *et al.*\textsuperscript{51}, the dipolar-coupling peaks are negative because the simultaneous change in two spins ($\beta\beta-\alpha\alpha$) increases the relaxation, whereas the chemical-exchange peaks are positive because energy is being exchanged between two states. On the other hand,
Neuhaus and Williamson explain the signs by making an analogy to the NOE difference spectroscopy. In the difference spectrum of a system in which the $W_2$ process dominates, the coupled peak is positive while the irradiated peak is negative. The signs may be reversed by phasing appropriately. The difference of the signs is reflected in the difference in the phases in the corresponding NOESY spectrum. A similar argument may be made for a system in which the $W_0$ process dominates.

2.3.2.4 **ROESY, and its advantages and disadvantages over NOESY.**

A small magnetization perpendicular to the main magnetic field $B_0$ can be created using the technique called "spin-locking." This small perpendicular field, often denoted $B_1$ and whose strength is usually 3-6 kHz (as opposed to that of $B_0$, which is usually 300-600 MHz), creates spin states analogous to those in the four-level diagrams shown for explaining NOE. (Figure 2.10) Because the field is small, the single-nucleus transition energies $v_A$ and $v_B$ and the energy required for the $W_2$ transition $(v_A+v_B)$ are likewise small. The transition temperature is so low that at practically the entire temperature range in which the solvent is liquid, the dipolar-coupling peaks are negative. ROESY (Rotational Overhauser Effect Spectroscopy) is the 2-D technique in which $B_1$ is applied to induce dipolar-coupling cross-peaks.

This technique offers an obvious advantage over NOESY. Since the dipolar-coupled and the chemical-exchange peaks have different signs at all temperatures, a ROESY spectrum is easier to interpret than the corresponding NOESY spectrum taken below the cross-over temperature. This advantage however is offset by several
disadvantages. The software and the electronics in the spectrometer must be capable of applying a spin-lock. Also, if B₁ is too strong, the spectrum becomes contaminated by J-coupling peaks (TOCSY). Therefore, the strength of B₁ must be controlled carefully.

2.4 Overview of the next Chapter

The complexes of [Fe¹¹⁺(OETPP)]⁺ with analogs of histidine were studied by NMR. The 2-D experiments were used to determine the structure and the dynamics of the molecules. The temperature-variation in the chemical shifts were used in an attempt to extract information about the electronic states; however, better information on the electronic states was obtained through EPR. The detailed account is provided in the next Chapter.
Figure 2.1  Top. Iron(III) octaethyltetraphenylporphyrin ([FeOETPP]⁺).  Bottom. The axial ligands studied in this dissertation.
Figure 2.2 Internal dynamics in axially ligated [Fe$^{III}$OETPP]$^+$. Left. Macrocycle flip-flop. Right. Ligand on-off.
Bonds defining the dihedral angle

Axial ligands perpendicular ($D_{2d}$)

Bonds defining the dihedral angle

Axial ligands parallel ($C_2$)

\[ \begin{align*}
\Delta & \quad \frac{\Delta}{d_{xz}} & \quad \frac{\Delta}{d_{yz}} \\
\Delta & \quad \frac{\Delta}{d_{xy}} & \quad \frac{\Delta}{d_{yz}} \quad -kT
\end{align*} \]

*Figure 2.3* Comparison of the d-orbital energy levels between the perpendicular and parallel ligand orientations. In the case in which the axial ligands are mutually parallel, the degeneracy between the $d_{xz}$ and $d_{yz}$ orbitals is lifted. The resulting energy gap is in the order of $kT$. 
Figure 2.4 Energy level diagram of a nucleus(I=1/2)-electron system.
Figure 2.5 Origin of contact shift. (A) Theoretical spectrum of the system in Figure 2.4, in which the electron spin is disregarded. (B) Theoretical spectrum of the same system, with the electron spin taken into account, but not the spin relaxation. (C) Theoretical spectrum, with the spin relaxation taken into account. As the temperature changes, the Boltzmann distribution changes and therefore the peak shifts.
Figure 2.6 Non-Curie behavior in ferriheme bis(aromatic amine) complexes.
Figure 2.7 Information that may be obtained from a NOESY experiment. *Top.* Through-space ("dipolar") coupling causes a cross-peak set whose phase is negative (shown here in red), if the experiment is done above the cross-over temperature. *Bottom.* Chemical exchange causes a set of positive cross-peaks (shown here in blue) at all temperatures.
Figure 2.8 Nuclear Overhauser effect (NOE) in which $W_2$ dominates (II), and the one in which $W_0$ dominates (III). The former results in an enhanced NOE difference signal of the nonirradiated peak (II-I), and the latter results in a diminished difference signal with concurrent diminishing of the irradiated signal (III-I).
Figure 2.9 Graph of spectral density from tumbling, adapted from Freeman. At the lower temperature, the tumbling provides only enough energy for the $W_0$ process. At the higher temperature, the tumbling becomes fast enough to provide energy for the $W_2$ process.
Figure 2.10 Comparison of the energy splitting caused by $B_0$, versus that caused by $B_1$ ("spin locking").
CHAPTER 3. VARIABLE-TEMPERATURE 2-D NMR SPECTRA OF HIGH- AND LOW-SPIN OCTAETHYLTETRAPHENYL-
PORPHORYRINATOIRON(III) COMPLEXES

...[T]his sensitivity encourages in these scientists what Kierkegaard has called the "paranoid leaps." Heightened sensitivity is accompanied in thinking by overalertness to relatively unimportant or tangential aspects of problems... It encourages highly individualized and autistic ways of thinking. Were this thinking not in the framework of scientific work, it would be considered paranoid.


3.1 Introduction

In this chapter, the NMR studies of the mono-chloride, bis(N-methylimidazole), and bis(2-methylimidazole) complexes of iron(III) octaethyltetraphenylporphyrin\(^{15}\) ([Fe(III)OETPP]) (Figure 3.1) are described. In this porphyrin, the ethyl groups are at the \(\beta\)-pyrrole positions, and the phenyl groups at the meso-positions. These substituent groups interact sterically, and cause the porphyrin to assume the saddle conformation. The same conformation is found in the tetraphenyl porphyrins with halogens at the \(\beta\)-pyrrole positions.\(^{31,54,55,56}\) These porphyrins have been studied more extensively than the \(\beta\)-alkylated counterparts, because their robustness against oxidation makes them more suitable as potential epoxidation catalysts.\(^{57}\) Nevertheless, a \(\beta\)-alkylated porphyrin was chosen for our studies because: (1) an alkyl group is closer in electronegativity than a halogen to the \(\beta\)-substituents of the physiological porphyrins; (2) an alkyl group contains protons that can be monitored by NMR spectroscopy, whereas halogens, with the
exception of fluorine, do not serve as convenient probes for NMR studies.

The axial ligands were chosen not only for their similarity to biological ligands but also for the ease of the preparation of the complexes. The chloride complex (FeOETPPCl) forms immediately upon the metallation of the porphyrin, and therefore is a convenient 5-coordinate high-spin species to study. A study of the one-dimensional variable-temperature NMR spectra of FeOETPPCl has already been published by Cheng et al., and some of our results are in agreement with theirs. The imidazoles serve as models for histidine, the most common axial ligand for hemes in biological systems. The N-methylated imidazole ligand mimics the N6 histidine ligation mode, while the 2-methylated imidazole mimics the N5 mode. Although the N6 ligation is sterically demanding, its possibility must be considered because it has been identified in a synthetic polypeptide-heme complex, and thus its existence in a biomolecule is probable although not yet confirmed. We present here the first NMR studies on [Fe(III)OETPP] complexes containing aromatic amines as the axial ligands.

3.2 Experimental

3.2.1 SAMPLE PREPARATION

Methylene chloride (Fisher Optima grade) was distilled over CaH2. All the other reagents — dimethylformamide (DMF) (Fisher), FeCl2·4H2O (Johnson-Matthey), NaCl (Johnson-Matthey), HCl (12 M, Fisher), N-MeIm (Aldrich), and 2-MeImH (Aldrich) — were used as purchased. Octaethyltetraphenylporphyrin (H2OETPP) was synthesized by Dr. Craig J. Medforth and Prof. Kevin M. Smith (Univ. of California – Davis) using the
previously reported method\textsuperscript{15} Iron was inserted into the porphyrin (17 mg, 0.020 mmol) by reacting it with 80 mg (0.40 mmol) of FeCl\textsubscript{2}-4H\textsubscript{2}O in 25 mL of refluxing DMF in the presence of air and absence of light. The reaction was monitored spectrophotometrically, and was complete within 30 min. The metallated porphyrin was then purified on a column of neutral alumina, using 10:1 CHCl\textsubscript{3}:CH\textsubscript{3}OH as the eluant. The solvent was removed from the chromatographed material using a rotary evaporator. The solid was then dissolved in 25 mL of CH\textsubscript{2}Cl\textsubscript{2} and treated with 100 mL of 1 M NaCl acidified with 1 mL of 12 M HCl, in order to cleave any μ-oxo dimer and to replace any axially ligated hydroxide with chloride.\textsuperscript{60} (The peaks at approximately 400 and 450 nm were unchanged after the treatment, suggesting that the μ-oxo dimer never did form.) The organic phase was washed with 3 × 25 mL of H\textsubscript{2}O. The aqueous phase was collected and washed with 3 × 10 mL of CH\textsubscript{2}Cl\textsubscript{2} to collect the suspended porphyrin solution. This organic layer was in turn washed with 3 × 10 mL of H\textsubscript{2}O. The organic phase was collected the solvent was removed using a rotary evaporator. The yield of FeOETPPCl was quantitative.

For the NMR measurements, FeOETPPCl (3 mg, 0.002 mmol) was dissolved in 0.5 mL of CD\textsubscript{2}Cl\textsubscript{2} (Cambridge Isotopes). For the preparation of the N-Melm complex, the ligand (3 equivalents relative to the porphyrin complex: 0.006 mmol, 0.5 mg) was introduced into the aforementioned sample with a Hamilton microsyringe. For the preparation of [FeOETPP(2-MeImH)\textsubscript{2}]\textsuperscript{+}, a batch of pre-weighed 2-MeImH (6 equivalents relative to the porphyrin complex: 0.012 mmol, 1.0 mg) was transferred quantitatively into an NMR sample tube, the solvent was removed, 3 mg (0.002 mmol) of FeOETPPCl
was introduced, and then the solids were dissolved in 0.5 mL of CD$_2$Cl$_2$. All NMR samples were treated with Ar prior to NMR measurement, in order to remove O$_2$.

3.2.2 Physical Measurement and Data Processing

The NMR spectra were recorded on a Varian Unity 300-MHz spectrometer at the University of Arizona, using an inverse probe. The length of the $\pi/2$ pulse was 8-9 $\mu$s. The temperature control hardware was calibrated using a methanol sample (Wilmad WGH-09). The VNMR v. 4.3 software (Varian) was used for the spectrometer control and the manipulation of the data for $T_1$ measurements. The $T_1$'s were measured by taking data using the inversion-recovery pulse sequence, and then fitting the data to a decreasing exponential. For magnitude-mode COSY-45 and phase-sensitive NOESY experiments, the standard pulse sequences available on the spectrometer were used.$^{61}$ For phase-sensitive ROESY, the pulse sequence used was $\text{Delay-}\pi/2-\tau-\pi/2-\text{Spinlock-}\pi/2-\text{Acq}$.

The Felix 95 software (Molecular Simulations) was used for the processing of the 2-D data. For the COSY data, a squared sine bell was applied before each of the two transformations. For the NOESY and ROESY data, Gaussian multiplication was applied before each transformation. Baseline correction was applied to each section in both dimensions, by determining the baseline points by FLATT$^{62}$ and then fitting these points to a 4$^{th}$-order polynomial. The macros used for processing these data are presented in Appendix A.

The EPR spectra were taken on frozen samples (4 K) in CD$_2$Cl$_2$ by Dr. Arnold M. Raitsimring (Univ. of Arizona) with a Bruker ESP-300E spectrometer equipped with an
3.3 Results and Discussion

3.3.1 Chloride Complex (FeOETPPCI)

3.3.1.1 1-D NMR Spectrum.

The spectrum, together with peak assignments, is shown in Figure 3.2: the peak positions and assignments are shown in Table 3.1. There are four methylene peaks, all of them shifted downfield from the expected diamagnetic positions. The number of the methylene peaks is consistent with the symmetry of the molecule (D2d). In one pair of pyrroles that are trans to each other, the β-ethyl groups point toward the same direction ("up") as the axial chloride ligand. In the other pair, the ethyl groups point away ("down") from the axial ligand. The buckling of the porphyrin would transpose the "inner-up" and the "outer-down" methylene protons, as it would the "outer-up" and the "inner-down" methylene protons. If the rate of the buckling were in the NMR fast-exchange regime, each of the two pairs would merge, yielding two methylene resonances. Nevertheless the observed spectrum shows no merging, and hence the data indicates that the rate of the rotation of the ethyl group — and thus the buckling of the macrocycle — remains within the slow-exchange region. The NOESY (EXSY) spectrum discussed below allows us to find the pairs of the signals from exchanging methylene protons.

Two methyl peaks appear in the spectrum in Figure 3.2. Our observation of four methylene and two methyl peaks is consistent with that of Cheng et al. The two spectra differ in the positions of the methylene peaks. For example, in our spectrum taken at
10°C, the methylene peaks appear at 55, 41, 34, and 25 ppm; in contrast, in the spectrum of Cheng et al. taken at the same temperature, the methylene peaks appear at 51, 37, 32, and 20 ppm. The difference can be explained by the difference in the polarity and perhaps the concentration of residual chloride ion between the NMR solvents used: CD$_2$Cl$_2$ in our experiment, versus C$_2$D$_2$Cl$_4$ in that of Cheng et al.

3.3.1.2 2-D NMR Spectra.

Two pairs of methylene cross-peaks are found in the COSY spectrum (Figure 3.3). Each methylene proton in the complex is expected to be J-coupled only to its geminal counterpart and its adjacent methyl. The cross-peak pattern is consistent with the expected coupling between the methylenes. (The methyl protons relax at rates that are too different from the methylene protons to give rise to observable COSY cross-peaks.) In the NOESY spectrum (Figure 3.3), two pairs of methylene signals give rise to a different set of cross-peaks. These cross-peaks are positive, and therefore they do not arise from dipolar coupling between geminal protons, but instead from the chemical exchange of the methylene protons caused by the conformational fluctuation. In this case, the “inner-up” and the “outer-down” methylene protons interconvert, as do the “outer-up” and the “inner-down” methylene protons (Figure 3.4). Attempts were made to locate the through-space dipolar coupling peaks (NOE peaks) that are expected to have the same pattern as in the COSY spectrum: the number of scans was increased, and the temperature was lowered to decrease the ring flip flop. These attempts were not successful. The T$_1$’s of the methylene protons in this complex (~5 ms) were considerably
shorter than those in the low-spin counterparts (>20 ms) whose NOESY spectra are discussed in the later part of this Chapter. It is likely that the faster relaxation decreased the magnitude of the Overhauser effect build-up in this high-spin complex.

3.3.1.3 Curie Plot.

The Curie plot for the four methylene protons over the temperature range from -90°C to +20°C is shown in Figure 3.5. For two of the methylene signals, the plots are linear throughout the entire temperature range, and the y-intercepts are at the diamagnetic positions as expected. For the other two signals, the plots curve slightly away from each other at the low-temperature region. This deviation from linearity results from the restricted rotation of ethyl groups, which would cause an incomplete averaging of the McConnell Q’s of the individual methylene protons. This effect has been observed in iron porphyrin complexes whose rotation rate of the β-ethyl groups varies with temperature: the variation in the rotation rate changes the Q’s for the methylenes, and therefore the Q’s are no longer constant with respect to temperature. In these cases, the assumption for Equation 21 in Chapter 2 (p. 54) is not valid.

3.3.1.4 EPR Spectrum.

The X-Band EPR spectrum of FeOETPPCl taken at 4 K (Figure 3.6) shows three peaks at $g = 6.61, 5.33,$ and $2.01$. The signals at 6.6 and 2.0 are typical of typical of a high-spin iron(III) porphyrinate complex. The signal at 5.3 is similar to what Cheng et al. found in their study of FeOETPPCl; they attributed the signal to the $S=(3/2,5/2)$ admixed state, the position of the signal determined by the proportion into the mixing of
the states $S=3/2$ (which in pure state would give rise to a signal at $g=4$) and $S=5/2$ ($g=6$). If the two species with different spin states existed also in the NMR temperature range (-90 to +20°C), then the Curie plot from the variable temperature NMR experiments should show nonlinearity. The plot in Figure 3.5 shows two signals — those from methylenes $a$ and $d$ — behaving in nonlinear manner. This nonlinearity is most likely caused by the change in the rotation rate of the ethyl groups (§3.3.1.3). However, a transition between two (or more) spin states cannot be completely ruled out, even though the linear behavior of the other two methylene signals makes it unlikely that the spin densities at the β-carbons are changing with temperature.

3.3.2 BIS(N-MeIm) Complex ([FeOETPP(N-MeIm)]Cl).

3.3.2.1 1-D NMR Spectra.

The 1-D NMR spectra of [FeOETPP(N-MeIm)]Cl (Figure 3.7, taken at -30°C and -80°C) are more crowded than those of FeOETPPCl because of the additional peaks from the free and the ligated imidazoles. Consequently, the peaks could be assigned (Table 3.2) only after the 2-D spectra were examined. The logic behind the assignment will be presented in §3.3.2.2. The positions of the peaks is consistent with those of a low-spin Fe(III) complex in which the methylene peaks are found in the 6-12 ppm range instead of the much more downfield shift expected in a high-spin complex (25-50 ppm). The proton $T_1$’s were measured (Table 3.2), and can be categorized into two groups: short (about 50 ms) and long (about 300 ms). As will be shown later, the short $T_1$’s belong to the porphyrin methylene and the axial ligand protons, and the long $T_1$’s to the porphyrin
phenyl and methyl protons. The T1 data were used for optimizing the mixing times for
the NOESY experiments.

The peaks are designated from a to n according to their positions from down- to
upfield at -30°C. (The solvent signal at 5.32 ppm is omitted from the designation.)
Three peaks from the free N-Melm do not vary their chemical shift with temperature: 1-
Me (3.64 ppm, f), 2-H (7.39 ppm, d), and 4,5-H (6.92 ppm, e). All the other peaks in the
spectrum shift significantly as the temperature decreases. The peak k broadens
considerably as the temperature is decreased, until it becomes invisible by -60°C. All the
other peaks remain visible throughout the temperature range used here (-90—+20°C)
3.3.2.2 2-D NMR Spectra.

The NOESY spectrum taken at -30°C is shown in Figure 3.8. The presence of
negative phase cross-peaks in the spectrum indicates that the system is in the positive
NOE regime (§2.3.2.2, p. 58) at the average tumbling of the molecule in CD2Cl2 at this
temperature. The a-j, c-e, and d-k cross-peak sets have positive phase, and therefore these
are caused by chemical exchange. Because the peaks d, e, and j have already been
assigned to the protons of the free N-Melm, the peaks a, c, and k should be assigned to
the corresponding protons of the ligated N-methylimidazoles. Thus, the peak a arises
from the methyl protons, c from the 4 or 5-protons (most likely 5-H, since 4-H is
expected to be broadened considerably), and k from the 2-protons of the axially ligated
N-methylimidazoles. The axial ligand 2-H peak appears at about 2 ppm, less upfield
than the 2-H peaks of the iron(III) bis(N-MelIm) complexes of several β-octaalkylated
porphyrins (-2 to -5 ppm). The \( h-m \) cross-peak set has negative phase, indicating that the coupling arises from a through-space dipolar interaction. The peak \( m \) may be assigned to the porphyrin methyl protons because of its position at both -30°C and -80°C, and the relatively small change in the chemical shift with temperature. The \( h-m \) cross-peak set therefore may be assigned to the coupling between the methyls (\( m \)) and other protons that are nearby, namely the methylenes within the same ethyl group (\( h \)). The negative phase \( b-m \) cross-peak set indicates another methylene-methyl coupling, and therefore the existence of another set of methylene protons (\( b \)). The presence of the positive \( b-h \) set indicates an exchange between the two types of methylene protons. Through an analogy to FeOETPPCl, these two methylene sets are assigned to the "inner" and the "outer" protons. (In the NOESY spectrum taken at -40°C (Figure 3.9), the \( b-h \) set is negative. Its sign is different from the spectrum taken at -30°C because the lowering of the temperature has slowed down the exchange sufficiently so that only the NOE interaction has become observable.)

The spectrum also shows a distinctive cross-peak pattern between \( f, g, \) and \( i \). The pairs \( f-g \) and \( g-i \) each give rise to a set of dipolar cross-peaks while the pair \( f-i \) does not. This pattern is what is expected from phenyl protons, in which only the adjacent protons couple (i.e. ortho-meta and meta-para). Thus, the peak \( g \) is assigned to the phenyl meta protons; the peak \( f \), which is about half the size of the other two, to the para; and \( i \) to the ortho.
The dipolar $b$-$i$ cross-peak set results from an interaction between the methylene ($b$) and the phenyl ortho-protons ($i$, assigned in the next paragraph) and indicates that $b$ arises from the “outer” methylene protons, which are close to the meso-phenyl groups. Consequently, $h$, the other methylene signal, must arise from the “inner” protons.

The COSY spectrum, taken at -30°C (Figure 3.8), confirms the observations from the NOESY spectrum. Cross-peaks are observed between the pairs $b$-$m$ and $h$-$m$, the methylene-methyl pairs. The cross-peak set $b$-$h$ indicates that the methylene protons that give rise to these peaks are indeed geminal. The presence of the cross-peaks $f$-$g$ and $g$-$i$, together with the lack of interaction between $f$ and $i$, confirm that the signals arise from the phenyl protons.

The complete assignment of the peaks is summarized in Table 3.2. A notable feature in this spectrum is the presence of only two signals from the methylene protons and only one each from the phenyl ortho-, meta-, and para-protons. The number of peaks is consistent with either the axial ligands rotating freely around the metal-nitrogen bond — which is difficult to justify, in the light of the NMR and molecular dynamics studies on [Co$^{III}$OETPP(aromatic amine)$_2$]$^-$ by Medforth et al. — or the ligands being oriented in perpendicular configuration, positioned directly over either the nitrogen or the meso-carbon atoms of the porphyrin so that the environment around the porphyrin possesses $D_{2d}$ symmetry. In the latter configuration, all the “outer” methylene protons are equivalent, as are the “inner” methylene, the methyl, the ortho-, the meta-, and the para-protons. (It is assumed that the N-methyl group of each axial ligand is sufficiently distant
from the metal center so that it does not affect the symmetry of the porphyrin ring. Also, note that the distinction between “inner” and “outer” methylenes exist because the ethyl groups are not co-planar with the porphyrin ring, unlike in the case of [Fe
\text{III} \text{OEP(Im)}_2]^+.

A comparison between the NOESY data at -30°C and -40°C (Figure 3.9) shows that at the lower temperature, the axially bound and free imidazole resonances exchange while the porphyrin methylene protons do not. The dominant internal dynamics is illustrated in Figure 3.10. The contrast with the bis(2-MeImH) complex is discussed in §3.3.3.

Assuming that the ring inversion stops completely at about -35°C (238 K), the activation energy may be calculated to be about 1.98 kJ mol\(^{-1}\). Also, assuming that the inversion is a vibrational process — a valid assumption, in the light of the paper by Jentzen et al.\(^{39}\) — the IR/Raman absorption should be about 166 cm\(^{-1}\). This value is within the observable range of the modern IR and Raman instruments.

The NOESY spectrum taken at -80°C is shown in Figure 3.11. At this temperature, the nuclear Overhauser effect (NOE) is negative, and therefore the dipolar cross-peaks are positive, \(i.e.\) have the same sign as the diagonal and the chemical-exchange peaks.\(^{52}\) (The NOE crosses over from positive to negative in the vicinity of -60°C (see §2.3.2.2, p.58). The NOESY spectrum taken at this temperature contains no interpretable dipolar cross-peaks above the noise level, and is not shown herein.) A set of cross-peaks between \(b\) and \(h\) is apparent; however, it is impossible to learn from this spectrum alone if the set results from dipolar coupling or chemical exchange. From the
aforementioned observation that the \( b-h \) cross-peaks are negative at \(-40^\circ\text{C}\), an inference can be made that the \( b-h \) set observed at \(-80^\circ\text{C}\) results from dipolar coupling. A ROESY experiment would allow one to confirm the nature of the cross-peak, without resorting to a systematic variable-temperature study.

In ROESY, the field responsible for the magnetization transfer is not \( B_0 \), but the rotational-frame transverse field \( B_1 \). Since \( B_1 \) is several orders of magnitude smaller than \( B_0 \) (usually 3-5 kHz, compared to 200-700 MHz for \( B_0 \)), the Overhauser effect induced by \( B_1 \) has positive sign at all temperatures.\(^6\) For our studies, \( B_1 \) was set at about 10 kHz, a typical value for the spectral frequency range in our experiments performed on a 300-MHz spectrometer. Although the existing literature warns that TOCSY peaks could contaminate a ROESY spectrum if \( B_1 \) is set too high,\(^6\) we found no significant problem in our studies. The ROESY spectrum of the N-Melm complex, taken at \(-80^\circ\text{C}\) (Figure 3.11) indicates that the \( b-h \) set arises from the dipolar interaction. The spectrum shows no chemical exchange peaks, suggesting that the molecule is not fluctional on the NMR time scale at this temperature.

### 3.3.2.3 Curie Plot.

The Curie plot for \([\text{FeOETPP(N-MeIm)}_2]\text{Cl}\) is shown in Figure 3.12. The porphyrin methyl resonance shifts only slightly with temperature. The other peaks move significantly with temperature because the corresponding protons are closer to the delocalized unpaired electron on the porphyrin ring and thereby their paramagnetic shifts are large. Moreover, these plots show significant deviation from linearity. This
nonlinearity is not related to that of the ferriheme / bis(aromatic amine) complexes in which the thermally-induced electronic transition from $d_{xz}$ to $d_{yz}$ causes non-Curie behavior (§2.3.1.5, p. 56). It is tempting to interpret the curvature in the Curie plot of the N-MeIm complex as evidence that the ligands are not perpendicularly oriented and therefore are not perfectly aligned to the grooves of the saddled porphyrin. Nevertheless, other factors unrelated to the ligand orientation could cause similar deviation from linearity, and these factors are much more likely to be the true cause of the non-Curie behavior. For example, the temperature-dependent flexibility of the molecule would result in variation of the rotation rate of the methyl group, which in turn would make the McConnell Q factor into a temperature-dependent variable that cannot be factored out as in Equation 22, Chapter 2 (p. 54). The change of the ground state from $d_{xy}^2(d_{xz},d_{yz})^3$ to $d_{xz}^2d_{yz}^2d_{xy}$ with variation in temperature, postulated for some of the nonplanar iron(III) porphyrinate complexes, is ruled out by the EPR spectrum that is discussed in §3.3.2.4; however, this spectrum raises another possibility, in which two species with differing dihedral angle of the axial ligands are present and the equilibrium changes with temperature. These factors make the Curie plot unreliable as an indicator for the orientation of the ligands.

3.3.2.4 EPR Spectrum.

The X-band EPR spectrum of [FeOETPP(N-MeIm)$_2$]Cl in toluene at 4 K (Figure 3.13) shows a mixture of “large $g_{max}$” and rhombic signals. The rhombic signal appears at $g=2.72, 2.38, and 1.66, and is indicative of a $d_{xy}^2(d_{xz},d_{yz})^3$ heme center having axial ligand
planes in *nearly parallel* orientation. The “large \( g_{\text{max}} \)" signal appears at \( g=3.12 \), and is indicative of a \( d_{xy}^2(d_{xz},d_{yz})^3 \) heme center having axial ligands in *perpendicular* orientation. The spectrum indicates that there is a mixture of species with axial ligands in perpendicular and near-parallel orientations.\(^{20} \) It is reasonable to assume that there is a mixture of two species at the NMR temperatures also. Thus, the EPR spectrum has provided more clues than the NMR spectrum on the interplay between steric and electronic factors.

3.3.3 BIS(2-MeImH) COMPLEX ([FeOETPP(2-MeImH)\(_2\)]Cl).

3.3.3.1 1-D NMR Spectra.

A sample containing 1:6 FeOETPPCl / 2-MeImH was used for the NMR studies. (Figure 3.14) A resolved NMR spectrum could be seen only below \(-70^\circ\text{C}\), because of multiple chemical exchange processes resulting from the steric hindrance between the 2-methyl group and the porphyrin. The methylene peaks are located at 25 ppm or less, and therefore the spectrum is consistent with that of a low-spin species. The spectrum is also considerably more complicated than that of [FeOETPP(N-MeIm)\(_2\)]\(^+\) (Figure 3.7). The axial ligand 2-Me is close enough to the porphyrin ring to destroy the symmetry of the environment around the macrocycle, and hence makes the spectrum more complicated. The spectrum could be interpreted only after the 2-D spectra were obtained, just as in the case of [FeOETPP(N-MeIm)\(_2\)]\(^-\). (The assignments, some definite and some tentative, are shown in Table 3.3.) The \( T_1 \)'s became longer as the temperature was lowered, indicating that the relaxation rate was dominated by chemical exchange rather than the shortening of
the correlation time. However, the $T_1$'s were short even at -90°C, indicating that the exchange was significant even at the lowest temperature attainable with CD$_2$Cl$_2$ (mp -94°C) as the solvent.

3.3.3.2 2-D NMR Spectra.

The NOESY spectrum (the one taken at -85°C is shown in Figure 3.15) is crowded and difficult to interpret by itself. The problem is made even more complicated by the fact that the NOE is negative at the temperature range where this complex was studied. Since both the NOE and the chemical exchange peaks have the same sign, it is difficult to distinguish between them. We therefore resorted to the ROESY experiment, in order to circumvent this problem. Also, we halved the mixing time (from 20 to 10 ms), in order to observe only the most significant internal motion of the molecule, and also because the magnitude of the NOE cross-peaks in ROESY spectra increases twice as fast as that in the NOESY counterpart.

When we compare the NOESY and ROESY spectra taken at the same temperature (Figure 3.15), the advantage of the latter becomes clear. The ROESY spectrum is not only much more simplified, but also its chemical exchange peaks can be identified and traced so that the $8 \times 8$ matrix of crosspeaks seen in the NOESY spectrum can be shown to be caused by the chemical exchange between 8 different types of protons. The only group of 8 protons in the molecule that could exchange with one another is that of the methylene protons. (Figure 3.16) Not only can one assign these peaks, but one can also determine which protons are geminal pairs. For each methylene signal, there is a dipolar
cross peak to another methylene signal, most likely that of the geminal partner. We conclude that the geminal partners are \(a-f, b-e, c-h,\) and \(d-g.\) From the same ROESY spectrum, one can identify the axial ligands' methyl, N-, and 5-protons through the chemical exchange peaks with the free 2-MeImH.

Magnitude-mode COSY and phase-sensitive TOCSY spectra (Figure 3.17) were taken order to assign more peaks, but these proved to be ineffective. The COSY signals were too weak, and the TOCSY spectrum appeared to be contaminated with spin diffusion signals.

### 3.3.3.3 Curie Plot.

Because there was a considerable overlap in the 1-D spectra, the peaks used for the Curie plot had to be selected from the NOESY data taken at various temperatures between -73°C and -90°C. The plot for the methylene peaks (Figure 3.18) shows that a group of 4 peaks in the downfield region converges at higher temperature, as does another group of 4 peaks at the upfield region. The plot suggests that at a higher temperature, those 8 peaks would merge into 2, the same number of methylene peaks as in \([\text{FeOETPP(N-MeIm)}_2]^+\). From an analogy to the N-MeIm peaks, the downfield 4 peaks can be assigned to the "outer" methylene protons and the upfield 4 peaks to the "inner" methylene protons.

The additional knowledge about the methylene protons, combined with a re-inspection of the ROESY data in Figure 3.15, gives us insight into the mechanism of interconversion between these protons. The ROESY spectrum suggests that an "outer"
proton is converted predominantly to an "inner" proton; not only that, but also the conversion occurs exclusively with only two of the possible four "inner" protons. The analogous observation can be made for the conversion of the "inner" to the "outer" protons. The likeliest mechanism involves the inversion of the saddled macrocycle, accompanied by the concerted rotation of both axial ligands. (Figure 3.19) The spectrum indicates that the random dissociation / re-association of the axial ligands is a minor process: this is a surprising observation, considering that 2-MeImH has a sterically-hindering group that makes the ligand dissociation favorable thermodynamically.

Barkigia et al.⁶⁶ have shown through their studies of dodecacyclohexylporphyrin that as more stress is applied to a nonplanar porphyrin, the more flexible it becomes. The stress destabilizes the most stable conformation, but not the transition state between two conformations. This results in a decrease in the activation energy and thereby in a greater flexibility. We can explain the extensive ring flip-flop in the bis(2-MeImH) complex by the destabilization of the nonplanar conformation of the porphyrin, which is caused by the steric interaction with the 2-methyl groups. Although the free energy of formation is smaller in the bis(2-MeImH) complex than in the bis(N-MeIm), the formation constant of the former at -70°C and below is sufficiently large and hence the population of the non- or mono-ligated species is negligible. The only difference between the bis(2-MeImH) and the bis(N-MeIm) complexes at this temperature range is the steric stress exerted by the methyl groups in the former. This stress causes the greater flexibility of the bis(2-MeImH) complex. Since the inversion of the bis(2-MeImH) complex was observable
even at -90°C (183 K), the upper limit of the activation energy is set at 1.52 kJ mol⁻¹. One may compare this with the estimated activation energy of the bis(N-MelM) complex (p. 84).

3.3.3.4 EPR Spectrum.

The EPR spectrum of [Fe³⁺OETPP(2-MelM)₂]Cl at 4 K (Figure 3.20) is noisy, probably because of the rapid relaxation of the signal. The g-value at 3.09 is typical of a "large gₘₐₓ" spectrum, which is an evidence for the dₓ²(y²,dₓz,dᵧz)³ configuration in which the axial ligands are mutually perpendicular.⁶⁶ (Note that a "large gₘₐₓ" spectrum, in spite of its appearance, is not an axial spectrum. In the latter the gₓ and gᵧ peaks overlap to make the large feature. In contrast, in the former the large peak consists solely of the gₓ peak: the gᵧ peak is extremely broadened, and the gₓ peak is shifted to a very small g-value.)

This orientation of the axial ligands is expected from the bis(2-MelM) complex, in which the 2-methyl groups are expected to fit into the "grooves" formed by the saddle distortion. The crystal structure (unpublished) of [Fe³⁺OETPP(2-MelM)₂]⁺ by Barkigia et al.⁶⁸,⁶⁹ (Figure 3.21) do provide an evidence that the ligands are mutually perpendicular: however, the structure indicates that the ligands are not aligned perfectly with the grooves of the porphyrin, but instead are rotated by about 14°, assuming that the grooves are parallel to the trans-N-Fe-N axis. The reason for the relative rotation is unclear.

3.4 Conclusions

The two-dimensional and variable-temperature NMR data of the [Fe(III)OETPP]⁺
complexes give insights on the fluctional properties and the stereochemistry of these
complex ions. The 2-D spectra of the 5-coordinate FeOETPPCl show cross-peaks
consistent with the expected fluctional motion in solution. The 6-coordinate
[FeOETPP(N-MeIm)₂]⁻ shows a significantly more complicated peak pattern than
[FeOETPPCl] because of the extra signals from the free and the ligated imidazoles. A
complete peak assignment is however possible by a combination of NOESY and COSY.
The complex appears to be fluctional at -40°C or above, but its fluctionality becomes
negligible on the NMR time scale at -80°C. The 2-D data indicate that the axial ligand
orientation is perpendicular, and the Curie plot is affected by too many factors to help us
determine the orientation. The 6-coordinate [FeOETPP(2-MeImH)₂]⁻ shows even more
complicated peak pattern. A detailed assignment of the methylene peaks is possible by
the use of ROESY along with NOESY. The NMR spectra of the complex are resolved
only at low temperature, and they indicate that the complex is fluctional at all
temperatures in which NMR is useful.
Figure 3.1 Stereo diagrams of the [Fe(III)OETPP]⁺ complexes studied for this dissertation.
Figure 3.2 1-D $^1$H-NMR spectrum of Fe$^{III}$OETPPCl at room temperature. There are total of four CH$_2$ peaks: "inner-up", "inner-down", "outer-up", and "outer-down."
Figure 3.3 2-D NMR spectra of FeOETPPCl at 22°C. Left. NOESY. 256 x 80 complex points, 48 scans/increment, 10 ms mixing, 15 ms acquisition, 500 ms delay, 16.6 kHz spectral width. Right. Magnitude-mode COSY-45. 1024 x 128 real points, 400 scans/increment, 31 ms acquisition time, 250 ms delay, 16.5 kHz spectral width. Processed with squared sine bells, 10 ms (1st dimension), 4 ms (2nd dimension).
Figure 3.4 Interconversion of the methylene protons in FeOETPPCl. The "inner-up" and the "outer-down" protons (H_i and H_\text{IV}) interconvert, as do the "outer-up" and the "inner-down" protons (H_\text{II} and H_\text{III}).
Figure 3.5 Curie plot of the methylene proton peaks of FeOETPPCl.
Figure 3.6 EPR spectrum (X-band) of FeOETPPCl at 4 K.
Figure 3.7 1-D $^1$H-NMR spectra of FeOETPP(N-Melm)$_2$Cl in CD$_2$Cl$_2$ at -30°C and -80°C.

bis(N-Melm) complex
Figure 3.8 2-D spectra of FeOETPP(N-MeIm)$_2$Cl at -30°C. Left. NOESY. 512 $\times$ 64 complex points, 80 scans/increment, 50 ms mixing, 85 ms acquisition, 335 ms delay, 6.0 kHz spectral width. Right. Magnitude-mode COSY-45. 2048 $\times$ 100 real points, 80 scans/increment, 171 ms acquisition, 295 ms delay, 6.0 kHz spectral width. Processed with sine bell squared, 85 ms (1st dimension) and 8 ms (2nd dimension).
Figure 3.9  *Left.* NOESY spectrum of FeOETPP(N-Melm)$_2$Cl at -40°C. 512 × 64 complex points, 48 scans/incr, 60 ms mixing, 78 ms acquisition, 402 ms delay, 6.6 kHz sweep width. *Right.* Spectrum at -30°C, shown in Figure 3.8, reproduced here for comparison.
Figure 3.10 Dominant internal dynamics in [FeOETPP(N-Melm)₂]⁺ at -40°C, according to the NOESY data shown in Figure 3.9.
Figure 3.11 2-D spectra of FeOETPP(N-MeIm)₂Cl at -80°C. *Left.* NOESY. 512 × 64 complex points, 32 scans/incr, 36 ms mixing, 57 ms acquisition, 708 ms delay, 9.0 kHz spectral width. *Right.* ROESY. 512 × 64 complex points, 32 scans/incr, 18 ms mixing, 57 ms acquisition, 708 ms delay, 9.0 kHz spectral width, 9.0 kHz spin lock. This spectrum clearly shows that all the cross-peaks originate from dipolar coupling.
Figure 3.12 Curie plots from $^1$H-NMR spectra of [FeOETPP(N-Melm)$_2$]Cl. Left: paramagnetic region. Right: diamagnetic region.
Figure 3.13 EPR spectrum (X band) of $[\text{FeOETPP(Melm)}_2]\text{Cl}$ at 4 K.
Figure 3.14 NMR spectrum of [FeOETPP(2-MelmH)$_2$]Cl at $-85^\circ$C.
Figure 3.15 2D spectra of [FeOETPP(2-Mel)mH]Cl at -85°C. Left. NOESY. 512 x 160 complex points, 48 scans/increment, 20 ms mixing, 49 ms acquisition, 150 ms delay, 10.5 kHz spectral width. ROESY. 512 x 160 complex points, 96 scans/increment, 10 ms mixing, 49 ms acquisition, 160 ms delay, 10.5 kHz spectral width, 10.5 kHz spin lock.
Figure 3.16 Types of methylene protons in [FeOETPP(N-MelIm)_2]^+ (left) and [FeOETPP(2-MelImH)_2]^+ (right). The lower symmetry of the latter is manifested in the presence of 8 types of methylene protons, as opposed to only 2 types in the former.
Figure 3.17 Magnitude-mode COSY-45 (left) and phase-sensitive TOCSY (right) of [FeOETPP(2-MelH)2]Cl at -90°C.
Figure 3.18 Curie plot for the methylene proton NMR peaks of [FeOETPP(2-MeImH)₂]Cl from -73°C to -90°C, taken from NOESY data.
Figure 3.19 Intramolecular dynamics in [FeOETPP(2-MeImH)₂]^−, according to the ROESY data in Figure 3.15.
Figure 3.20 EPR spectrum (X band) of [FeOEtPP(2-MeImH)₂]Cl at 4 K.
Figure 3.21 Crystal structure of $\text{[Fe}^{III}\text{OETPP(2-MelmH)}_2]^{2/3 \text{Cl}^-\cdot 1/3 \text{SbF}_6^{-}}$ , courtesy of Dr. K. M. Barkigia and co-workers at the Brookhaven National Laboratory.58
Table 3.1 $^1$H-NMR peak positions and assignment for FeOETPPCl.

<table>
<thead>
<tr>
<th>Chemical Shift (ppm)</th>
<th>Assignment</th>
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<tbody>
<tr>
<td>45.69</td>
<td>Methylene $a$</td>
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<tr>
<td>38.85</td>
<td>Methylene $b$</td>
</tr>
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<td>35.85</td>
<td>Methylene $c$</td>
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</tr>
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<td>meta-Phenyl</td>
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<tr>
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<td>meta-Phenyl</td>
</tr>
<tr>
<td>10.77</td>
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<tr>
<td>8.21</td>
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<tr>
<td>7.06</td>
<td>para-Phenyl</td>
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<tr>
<td>3.75</td>
<td>Methyl</td>
</tr>
<tr>
<td>1.28</td>
<td>Methyl</td>
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</tbody>
</table>
Table 3.2 Chemical shifts and T<sub>1</sub>'s of <sup>1</sup>H-NMR spectra of FeOETPP(N-MeIm)<sub>2</sub>Cl at -30°C and -80°C, and the peak assignment.

| Peak | -30°C | | -80°C | | Assignment |
|------|-------|-------|-------|---------------|
|      | Shift (ppm) | T<sub>1</sub> (second) | Shift (ppm) | T<sub>1</sub> (second) | |
| a    | 17.18 | 0.178(8) | 22.53 | 0.0235(3) | Axial ligand N-Me |
| b    | 12.58 | 0.0619(4) | 14.15 | 0.0386(3) | Porphyrin methylene (outer) |
| c    | 12.38 | 0.099(8) | 14.15 | Hidden behind b | Axial ligand 5-H |
| d    | 7.39  | 0.045(2) | 7.39  | 0.436(1) | Free imidazole 2-H |
| e    | 6.92  | 0.055(2) | 6.92  | 0.686(3) | Free imidazole 4-/5-H |
| f    | 6.38  | 0.492(3) | 5.80  | 0.367(3) | Porphyrin para-phenyl |
| g    | 5.21  | 0.351(2) | 4.35  | 0.226(1) | Porphyrin meta-phenyl |
| h    | 4.39  | 0.0667(2) | 3.49  | 0.046(1) | Porphyrin methylene (inner) |
| i    | 3.83  | 0.0768(3) | 2.16  | 0.041(1) | Porphyrin ortho-phenyl |
| j    | 3.65  | 0.195(2) | 3.62  | 0.804(8) | Free imidazole N-Me |
| k    | 2.25  | 0.063(3) | Not observed | N/A | Axial ligand 2-H |
| l    | 1.21  | 0.057(2) | 1.15  | 0.0277(6) | Impurities |
| m    | 1.07  | 0.467(8) | 1.39  | 0.209(4) | Porphyrin methyl |
| n    | 0.84  | 0.65(4) | 0.80  | 0.273(4) | Impurities |
Table 3.3 $^1$H-NMR peak positions at -85°C and assignment for [FeOETPP(2-MeImH)$_2$Cl].

<table>
<thead>
<tr>
<th>Shift (ppm)</th>
<th>Assignment</th>
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<tr>
<td>25.49</td>
<td>Axial ligand NH</td>
</tr>
<tr>
<td>19.21</td>
<td>Axial ligand 5-H</td>
</tr>
<tr>
<td>19.10</td>
<td>Porphyrin methylene $a$</td>
</tr>
<tr>
<td>16.67</td>
<td>Porphyrin phenyl?</td>
</tr>
<tr>
<td>16.11</td>
<td>Porphyrin methylene $b$</td>
</tr>
<tr>
<td>13.34</td>
<td>Free imidazole NH</td>
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<tr>
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<tr>
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<tr>
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</tr>
<tr>
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<td>Porphyrin methylene $g$</td>
</tr>
<tr>
<td>0.42</td>
<td>Porphyrin methylene $h$</td>
</tr>
</tbody>
</table>
CHAPTER 4. *trans*-BIS-MOLYBDATED TETRAARYLPORPHYRIN:

WHY AND HOW

You don’t owe me nothin’ and as far as I know,
Lord, I don’t owe nothin’ to You
But I ain’t askin’ for a miracle, Lord
Just a little bit of luck would do

— Steve Earle, “Tom Ames’ Prayer”

4.1 Introduction

4.1.1 TARGET MOLECULE

In Chapters 5 and 6, the synthesis of 5,15-bis[2,3-[((hydrotris(3,5-dimethyl-
pyrazolyl)borato)oxomolybdenio)dioxy]phenyl-10,20-di-p-tolylporphyrin (Figure 4.1) is
detailed. We wished to obtain a heme whose axial ligands are rotationally fixed in either
mutually perpendicular or parallel configuration, in order to see how the dihedral angle of
axial ligands relate to EPR spectrum and redox potential. The hint for this molecule was
obtained from 5-[2,3-[((hydrotris(3,5-dimethylpyrazolyl)borato)oxomolybdenio)dioxy]-
phenyl-10,15,20-tri-p-tolylporphyrin (Figure 4.1), which was reported by Basu et al.60,70
Iron(III) was inserted into this porphyrin, aromatic amines were axially ligated onto the
iron, and the resulting complex was observed by 'H-NMR: the resulting spectrum
indicated that at least one of the two axial ligands had stopped rotating. Since one bulky
MoO(Me₂Pyz)₃BH group was capable of hindering the rotation of one axial ligand, it was
apparent that two sterically large groups would hinder both axial ligands. (As shown in
p. 123, it is possible to design two types of such complex: one with the axial ligands
mutually parallel, and the other with the ligands mutually perpendicular. In fact, it is desirable to synthesize both of these. The degree of the difficulty of synthesis however puts a constraint on the type of complex to be synthesized, as it will be shown in Chapters 5 and 6.)

In this Chapter, the works of Basu, Enemark, and co-workers are reviewed in order to provide a background to our work. Also, the techniques for synthesis of tetraarylporphyrins are reviewed, since a successful preparation of the porphyrin with the desired stereochemistry is the most crucial part of our synthesis.

4.1.2 Nomenclature.

For most part, the IUPAC guidelines for organic and inorganic compounds, the International Union of Biochemistry’s (IUB) guideline for biochemical compounds, and the ACS guideline for inorganic compounds, are followed for the nomenclature used in Chapters 4, 5, and 6. Exceptions were made, however, when the nomenclature used in the literature relevant to our studies was judged to be superior to that recommended by the guidelines. The generic names “arenecarbaldehyde” and “arenecarboxylic halide” conform to the IUPAC organic guideline. The name “dipyrrromethane” conforms to the IUB guideline. The name “tripyrrane” has been used in the literature for the synthetic methods most relevant to our studies. The numbering systems used for the substitutions in porphyrins and tripyrranes conform to the IUB guideline. The “α/β” designation for atropisomers was first used by Collman and co-workers in their landmark papers on the “picket-fence” porphyrins. The substituent name “catecholyl”
was derived from the trivial name “catechol” by applying the following rule in the IUPAC inorganic guideline: “Radicals or groups derived formally by the removal of one hydrogen atom from any position of a molecular hydride are named by adding the suffix -yl to the name, with the elision of any final ‘e’. ” In this dissertation, “catecholyl” is used when the phenyl carbon-substituent bond is emphasized (e.g. “catecholyl-porphyrin”); on the other hand, “catecholato” is used when the oxygen-substituent bond is emphasized (e.g. “catecholatomolybdenum”). The name “5,15-bis[2,3-(((hydrotris-(3,5-dimethylpyrazolyl)borato)oxomolybdenio)dioxy]phenyl-10,20-di-p-tolylporphyrin” was adapted from the name used by Basu et al. for the compounds closely related to ours, albeit with one modification that is explained in the next paragraph.

Only one of our conventions deviates from both the official guidelines and the relevant literature. In naming our porphyrins, the oxygen-bearing aryl groups have taken precedence over the methyl-bearing ones. Thus, we have “dimethoxyphenyltolyl-porphyrin” instead of “tolyldimethoxyphenylporphyrin”. Although this does not conform to the IUPAC organic guideline nor the convention used by Basu et al., it helps to emphasize the groups that are functionalized in our work.

4.1.3 Abbreviations.

TLC: thin-layer chromatography. DDQ: 2,3-dichloro-5,6-dicyanoquinone. TCQ: 2,3,5,6-tetrachloroquinone (p-chloranil). TPB: tris(pyrazolyl)borate. HB(Me₂Pyz)₃: hydrotris(3,5-dimethylpyrazol-1-yl)borate. TPP: meso-tetraphenylporphyrinate. TMP: meso-tetramesitylporphyrinate. N-Melm: N-methylimidazole. 2-MelmH: 2-methyl-
imidazole.

4.2 Mono-Molybdated Porphyrin — Previous Studies

The mono-molybdated iron porphyrin was first synthesized by LaBarre as a spectroscopic model for sulfite oxidase. The plan was to measure the magnetic dipole interaction between the unpaired electrons in Mo and Fe, relate the magnitude of the interaction with the Mo-Fe distance, and use the resulting equation to determine the distance between the two metal centers in sulfite oxidase. (The plan was carried out successfully, but this is out of the scope for this dissertation.) Dihydroxybenzaldehyde (2,3- or 3,4-), p-tolualdehyde, and pyrrole were reacted in refluxing propionic acid to form a mixture of porphyrins, from which dihydroxyphenyltritolylporphyrin was isolated by column chromatography. This porphyrin was subsequently reacted with HB(Me₂Pyz)₃MoVo(OCH₂CH₂O) to form the mono-molybdated porphyrin. Later, Basu substituted dihydroxybenzaldehyde with the corresponding dimethoxybenzaldehyde in order to prevent any loss by unnecessary oxidation, and added a demethylation step before the molybdation.

Basu et al. found evidence that when two aromatic amines are ligated axially to the iron, one of them is hindered from rotating. When N-methylimidazole was added to a solution of [5-[2,3-][(hydrotris(3,5-dimethylpyrazolyl)borato)oxomolybdenio)dioxyphenyl-10,15,20-tri-p-tolylporphyrinatoiron(III)]⁺ (“[2,3-Mo-Fe₃Por]⁺”), and the resulting complex was studied by ¹H-NMR at room temperature, seven of the eight β-pyrrole signals were found resolved and spread throughout a range 13 ppm. On the other
hand, when the N-methylimidazole complex of $[5\text{-}[3,4\text{-}[[((\text{hydrotris}(3,5\text{-}\text{dimethylpyrazolyl})\text{borato})\text{oxomolybdenio})\text{dioxy}]\text{phenyl-10,15,20-tri-}p\text{-}\text{tolyporphyrinato-iron(III)}]\text{]}^-$ ("$[3,4\text{-}\text{Mo-Fe}^\text{III}\text{Por}]^-$") was prepared and its NMR spectrum was taken at room temperature, the $\beta\text{-pyrrole}$ signals were found to have collapsed into three resolved peaks which were clustered in a range of 3 ppm. The results were interpreted as follows. In $[2,3\text{-}\text{Mo-Fe}^\text{III}\text{Por(N-MeIm)}_2]^-$, the MoO(Me$_2$Pyz)$_3$BH fragment is in contact with one of the axial ligands, hindering the rotation of the latter. Meanwhile, in $[3,4\text{-}\text{Mo-Fe}^\text{III}\text{Por(N-MeIm)}_2]^-$, the MoO(Me$_2$Pyz)$_3$BH fragment is located too far away to interact sterically with the closest axial ligand, and therefore the ligand rotates freely.

The EPR spectra of the molybdated porphyrins showed a superposition of molybdenum and iron signals, in which the latter was consistent with the $(d_{xy})^2(d_{xz},d_{yz})^3$ configuration. The Fe$^\text{III}$ signal of $[2,3\text{-}\text{Mo-Fe}^\text{III}\text{Por(N-MeIm)}_2]^-$ was rhombic: the peaks from $g_x$, $g_y$, $g_z$ were discernible. The Fe$^\text{III}$ signal of $[2,3\text{-}\text{Mo-Fe}^\text{III}\text{Por(2-MeImH)}_2]^-$ however was of the "large $g_{\text{max}}$" type, in which only one strong signal is seen at $g=3$.

We had conjectured that the rotation of both axial ligands could be stopped by placing another MoO(Me$_2$Pyz)$_3$BH fragment at the other side of the plane in the porphyrin. The resulting orientation of the axial ligands can be determined by analyzing the EPR spectrum. For a ferriheme complex in which the electron configuration is $d_{xy}^2(d_{xz},d_{yz})^3$, if the relative orientation is parallel, the pattern would be rhombic. If the relative orientation is perpendicular, the pattern would be "large $g_{\text{max}}$". (See p. 91 for the difference between a large $g_{\text{max}}$ and an axial spectrum.) If an aromatic amine that
normally induces a large $g_{\text{max}}$ EPR spectrum is placed on a heme that forces the mutually parallel conformation, the spectrum of the resulting complex would be rhombic.

Conversely, with an amine that normally induces a rhombic spectrum is placed on a heme that forces the mutually perpendicular conformation, the resulting spectrum would be of the large $g_{\text{max}}$ type.

4.3 Mutual Orientation of the Axial Ligands — The Relevance to Cytochrome $b$

One of the hemoproteins that contain two ligated histidines is cytochrome $b$ (Figure 1.2, Page 33). It is postulated that in this protein, the mutual orientation of the axial ligands may play a role in determining the redox potential: a calculation based on EPR and Mössbauer spectra and an estimated value of iron(III) spin–orbit coupling has indicated that two ferrihemes, one with the axial ligands mutually perpendicular and the other parallel, would differ in the oxidation potentials by as much as 50 mV. In order to test this hypothesis, two very similar ferriheme complexes with different dihedral angles of the axial ligands must be made.

In a low-spin ferriheme / bis(aromatic amine) complex whose lowest d-orbital is $d_{xy}$, the singly-occupied molecular orbital (SOMO) changes in energy as the dihedral angle of the axial ligands is varied. (§2.2.4, p. 44) When the ligands are mutually perpendicular, the $d_{xz}$ and $d_{yz}$ orbitals are degenerate. This degeneracy is removed when the dihedral angle deviates from 90°. As the angle becomes closer to 0°, $d_{xz}$ becomes more stabilized: at the same time, $d_{yz}$ — which is the SOMO — becomes destabilized. The destabilization of the $d_{yz}$ orbital in the ferriheme results in an increase in the
reduction potential and a decrease in the oxidation potential, assuming that the energy of
the reduced species do not depend as much on the dihedral angle.

If one is to test this out, one bis-Mo-porphyrin is not enough: two such
porphyrins, one being trans-disubstituted (i.e. 5,15-disubstituted) and the other cis- (i.e.
5,10-disubstituted), must be synthesized so that the comparison of the redox potential
becomes meaningful. Making both of them, however, is a non-trivial task. The cis-
isomer is less symmetric than the trans-, and therefore more difficult to synthesize rationally. Setting the first goal on the synthesis of the trans-isomer is more prudent than attempting to synthesize both of them rationally at the same time. Once this goal is reached, one may apply the knowledge gained to make an attempt at the cis-isomer.

4.4 Synthetic Approaches — Especially Porphyrins

In order to make a porphyrin with two MoO(Me₂Pyz)₃BH groups, an
appropriately substituted porphyrin containing two dimethoxyphenyl groups must be
synthesized. Two approaches can be made: (1) synthesize the desired disubstituted
porphyrin in a rational manner; or (2) synthesize a mixture of porphyrins using a simple
method, then isolate the desired porphyrin by column chromatography. The feasibility
of the two approaches is discussed after the methods for porphyrin synthesis are
summarized.

4.4.1 Synthetic Methods for Symmetric Tetraarylporphyrins.

Tetraarylporphyrins are always synthesized from the corresponding arene-
carbaldehyde and pyrrole. (Figure 4.2) The reactants are polymerized by dehydration in
an acidic medium, the oligomer is cyclized to form a partially saturated tetramer called *porphyrinogen*, and then the porphyrinogen is then oxidized to form the desired porphyrin. Because an equilibrium exists between porphyrinogen and the other oligomers, the porphyrin yield is always low: about 50% for tetraphenylporphyrin, and 5-25% for other tetraarylporphyrins.

Of the following methods, the Adler and Lindsey syntheses are the most commonly known. The other methods are relatively new and therefore obscure to the community of porphyrin researchers. One of them (the Bonar-Law synthesis) however has turned out to be crucial to our work, affording us success in the synthetic step where the Adler and the Lindsey methods had failed.

4.4.1.1 Adler synthesis.

The method used by Rothemund and co-workers\(^{88}\) for the first synthesis of tetraphenylporphyrin was inefficient and impractical, and no further discussion of it will be made here. The first practical method for the synthesis of tetraarylporphyrins was reported by Adler *et al.*\(^{89}\) in 1967. In this method, an arenecarbaldehyde and pyrrole (both \(10^{-1}\) M) are reacted in refluxing alkanecarboxylic acid; the resulting porphyrinogen is oxidized by atmospheric oxygen. When propionic acid is used, the product usually precipitates from the reaction mixture and thus makes the recovery easy. No such precipitation occurs when acetic acid is used.\(^{89}\)

Until the development of the Lindsey synthesis, the Adler synthesis was the only practical preparative method for tetraarylporphyrins. The development of the method
enabled the synthesis of various models for heme-containing biological systems. As the method became more popular, various modifications have been developed.

Influenced by the report by Ball et al. on the Rothemund synthesis, many workers began to add transition metal cations to the reaction mixture to increase the yield, even though Adler et al. had found no significant increase in the yield when they applied the cation addition to their synthesis in refluxing acetic acid. Rocha Gonsalves et al. substituted nitrobenzene in the place of propionic acid with good results. Dolphin, by performing the reaction at room temperature, formed and isolated porphyrinogens with sp3 meso-carbons: this study, along with that of Smith et al., are so far the only ones in which a meso-tetraarylporphyrinogen has been isolated.

The advantage of the Adler synthesis is its simplicity. The solvent, usually propionic acid, does not need to be purified after being purchased commercially. (This presents a strong contrast to the Lindsey synthesis, as we will see.) The reaction need not to be performed in an inert atmosphere (and should not be!) If the porphyrin is symmetric, it usually precipitates out of the reaction mixture and therefore can be collected easily by filtration. The disadvantage is the necessity of refluxing the solvent in order to complete the reaction in reasonable time. The high temperature destroys temperature-sensitive substituents on the aryl groups: this limits the types of arene-carbaldehydes that can be used. The high temperature also causes the formation of viscous tar, which complicates the recovery of the product if the latter remains dissolved in the reaction mixture.
4.4.1.2 Lindsey synthesis.

In Adler synthesis, the porphyrinogen formation and the oxidation are performed in one step. Dolphin was the first one to use two separate steps for the porphyrinogen formation and the oxidation. Lindsey et al. later made more systematic study of such partition, and in the process discovered (and reported in 1986) a new synthetic method of tetraarylporphyrin. The best porphyrin yield was obtained when the concentration of each reactants were small ($10^{-2}$ M), and the following reagents were used: CH$_2$Cl$_2$ or CHCl$_3$ as the solvent, BF$_3$OEt$_2$ or trifluoroacetic acid as the catalyst, and TCQ or DDQ as the oxidant.

Some modifications have been made on the Lindsey synthesis. In the synthesis of tetramesitylporphyrin by Lindsey and Wagner, ethanol was added to the reaction mixture in order to counter the excessive affinity of BF$_3$ toward the carbonyl oxygen of the aldehyde. Lindsey et al. increased the concentration of the reactants ($10^{-1}$ M) without decreasing the yield, by simultaneously increasing the concentration of the acid. Shinoda et al. used solid porous aluminosilicates in the place of BF$_3$ or trifluoroacetic acid: this material can be separated by filtration and regenerated by calcination. Addition of some inorganic salts into the reaction mixture was found to increase the yield, although transition metal ions had no effect.

The Lindsey synthesis has found wide application. Since the reaction is performed at room temperature, arenecarbaldehydes that contain temperature-sensitive substituents are not destroyed. Unlike in the Adler synthesis, no viscous tar is produced
during the reaction, and therefore the reaction mixture can easily be put through a rotary evaporator, and the resulting solid processed by chromatography without plugging the column. The disadvantage lies in the necessity of low reactant concentration: even a reaction scaled up to 1000-mL can produce only about 100 mg of a porphyrin; in contrast, with the Adler synthesis, a gram of the product can be produced in a 1000-mL scale reaction. Another disadvantage lies in the necessity of rigorous control of the reaction. The solvent must be dried, since the reaction is sensitive to even a trace of water. Also, the reaction must be performed under inert gas to exclude the detrimental effect of the atmospheric moisture and dioxygen.

4.4.1.3 Bonar-Law synthesis.

This method was reported by Bonar-Law⁹⁸ in 1996. It resembles the Lindsey synthesis, except for the use of water as a component of the solvent. Aqueous sodium dodecyl sulfate (0.5 M) at room temperature is used as the solvent, aqueous HCl is used as the catalyst, and TCQ or DDQ as the oxidant. After the oxidation, the porphyrin is recovered by precipitating the surfactant and then extracting the product from the precipitate.

Aside from the advantages from the low temperature used in the reaction, the Bonar-Law synthesis has an additional advantage in the ease of purification of the reagents. Because the reaction is performed in an aqueous solution, the drying of the solvent is unnecessary (obviously!). Also, moisture-free atmosphere is not needed during the reaction. The method, however, suffers from difficulty in scaling up the reaction,
since the concentration of the reactants is low. Also, the extraction of the product from the precipitated surfactant can become difficult because of the paste-like property of the precipitate. The data that Bonar-Law has presented indicate that ortho-substituted aryls cannot be easily incorporated into porphyrins by this method.

The method is relatively new, and it has not yet found a widespread use. Perhaps the greatest hurdle to popularity of this method is the use of water in the method: the sensitivity of the Lindsey synthesis to water has been ingrained in the mind of many researchers, and the skepticism against any use of water in porphyrin synthesis remains very strong. The utility of this method will be demonstrated in Chapter 6.

4.4.1.4 Other methods (not used for the work in this dissertation).

The formation of porphyrinogen from aldehyde and pyrrole always involves a simultaneous formation of water. LeChatelier's principle indicates that removing this water would drive the reaction forward. Adler and co-workers, in their initial exploration of porphyrin synthesis, successfully applied the equilibrium-shifting by azeotropic removal. They however did not pursue this method further after they discovered the propionic acid method. DiMagno et al. and then Crossley et al. rediscovered and reported the synthetic method of tetraalkyl- and tetraarylporphyrins in which azeotropic removal of water is applied. Refluxing benzene or toluene is used as the solvent, methyl-sulfonic acid is used as the catalyst, and either the atmospheric oxygen, DDQ, or TCQ is used as the oxidant. The azeotrope is removed by a Dean-Stark trap. The major advantage of this method is its efficiency in preparing tetraalkylporphyrins, which are
difficult to prepare using the Adler or the Lindsey synthesis.

Solvent is not an absolute requirement in the synthesis of tetraarylporphyrins. Drain and Gong\textsuperscript{101} has prepared tetraarylporphyrins by reacting arenecarbaldehyde and pyrrole in the gas phase in a hot quartz tube in the presence of oxygen. The recovery of the product is simplified by the lack of a solvent. The reaction condition is obviously too harsh for labile aldehydes. If, however, the reactants do withstand the condition, the ease of product recovery makes this method attractive.

4.4.2 Strategies for preparing disubstituted tetraarylporphyrins.

The porphyrin that we wish to make is \textit{trans}-disubstituted at the \textit{meso}-positions (Figure 4.1): the methods described above must be modified in order to make such porphyrin. Two general modifications exist: (1) one-pot reaction between pyrrole and a mixture of aldehydes, followed by the chromatographic separation of the statistical mixture (Figure 4.3); (2) synthesis of pyrrole-arenecarbaldehyde oligomers, followed by the formation of the desired porphyrin by fusing the oligomers together (Figure 4.4).

4.4.2.1 Mixed-aldehyde porphyrin synthesis / chromatographic separation.

This method is suited for making mono-substituted tetraarylporphyrins, especially when the polarity of the two arenecarbaldehydes are dissimilar. Basu \textit{et al.}\textsuperscript{60} prepared their mono-molybdated complex by using the mixed-aldehyde Adler synthesis. This method however is more difficult to apply to the preparation of a disubstituted species, because of the difficulty in separating the \textit{trans}- and \textit{cis}-isomers.\textsuperscript{102} Either of the isomers would be useful for making a complex in which the rotation of the axial ligands is
hindered, but the separation of these two is required for a meaningful and interpretable study.

The two arenecarbaldehydes required for the synthesis of our complex — 2,3-dimethoxybenzaldehyde and p-tolualdehyde — are both capable of withstanding the boiling temperature of propionic acid, and therefore the Adler synthesis is the method of choice. If the separation of the trans- and cis-isomers is successful, it is possible to prepare two types of porphyrin complex: one in which the axial ligands are oriented in mutually parallel manner, and the other in which the ligands are oriented in mutually perpendicular manner.

4.4.2.2 Rational synthesis.

Theoretically, a trans-disubstituted tetraarylporphyrin can be prepared by first forming a dipyrrolic species that bears arene A, and then reacting it with the aldehyde that bears arene B. Similarly, a cis-disubstituted tetraarylporphyrin can be prepared by forming a tripyrrolic species that bears two arenes A and a monopyrrolic species that bears two arenes B, and then combining the two together. The preparation of the trans-isomer is much easier than the cis- because of the former’s higher symmetry: if the synthesis of the trans-isomer is unsuccessful, the synthesis of the cis- would likely be unsuccessful also. Therefore, it is more prudent to attempt the synthesis of the trans-isomer first.

The use of a dipyrrolic species for porphyrin synthesis is commonly termed the “MacDonald ‘2+2’ synthesis” after the worker who pioneered such synthesis for meso-
unsubstituted, \( \beta \)-alkylated porphyrins. The MacDonald synthesis of disubstituted tetraarylporphyrins was attempted by Wallace et al. using the reaction condition of the Adler synthesis. Their success, however, was only partial because of an extensive rearrangement found in the product. The first successful MacDonald synthesis of tetraarylporphyrins was reported by Lee and Lindsey in 1994. Aryldipyrromethanes were synthesized by reacting an excess of pyrrole with the desired arenecarbaldehyde; then, each dipyrromethane was reacted with an arenecarbaldehyde under the mild condition of the Lindsey synthesis. Although Lee and Lindsey reported that they did not observe any scrambling in their synthesis, Lindsey later reported in 1999, together with his co-workers, that they had observed a significant amount of scrambling. Setsune et al. reported success in the synthesis of a disubstituted tetraarylporphyrin using a modified Adler condition. In their study, the reaction mixture was kept at room temperature for a day; then it was refluxed only during the last two hours for oxidizing the porphyrinogen. No scrambling was observed in their synthesis.

The methods that could conceivably be used for the MacDonald synthesis without decomposing the dipyrromethane or the porphyrinogen are the Lindsey, the Bonar-Law, and the (low-temperature) Adler syntheses. The other synthetic methods involve high temperature that could cause decomposition of the dipyrromethane and rearrangement of the porphyrinogen. Because the Lindsey “2+2” synthesis is susceptible to rearrangement, an alternative condition for affording a clean reaction must be found.
4.4.3 MOLYBDATION.

After the disubstituted porphyrin is successfully prepared, it can be molybdated using the method of Basu et al.\textsuperscript{50} (Figure 4.5) The bis(dimethoxyphenyl)ditolylporphyrin is demethylated using BBr\textsubscript{3}; then, the resulting bis(catecholyl)ditolylporphyrin is reacted with HB(Me\textsubscript{2}Pyz)\textsubscript{3}Mo\textsuperscript{V}O(OCH\textsubscript{2}CH\textsubscript{2}O). Iron(III) can be inserted into the bis-molybdated porphyrin using FeCl\textsubscript{2} in CH\textsubscript{2}Cl\textsubscript{2} under the oxygenated atmosphere (no μ-oxo dimer is expected to form because of the sterics of the tris(pyrazolyl)borate group), then the desired aromatic amine can be added immediately before the spectroscopic study.

4.5 Scope of the Next Two Chapters

We had already determined that the synthesis of the disubstituted porphyrin-oxo-molybdenum tris(pyrazolyl)borate framework would take so much effort that the subsequent studies of the iron(III)-coordinated species would be out of scope of this dissertation. In the next two Chapters, the synthesis of bis(dimethoxyphenyl)ditolylporphyrin is described. In Chapter 5, the mixed-aldehyde Adler synthesis and the subsequent attempt at chromatographic separation are recounted. In Chapter 6, the MacDonald “2+2” synthesis of the trans-isomer is detailed: this approach was found to be the successful one. Also in that Chapter, the molybdation of the trans-isomer and the characterization of the product are described.
Figure 4.1  Top left. The target complex ("trans-bis-Mo-Porph") to be synthesized for this dissertation.  Top right. Stereo diagram of trans-bis-Mo-Porph with centrally coordinated Fe^{III} atom and two pyridines as axial ligands.  Bottom left. The complex ("Mo-Porph") synthesized by Basu et al.  Bottom right. Stereo diagram of Mo-Porph with centrally coordinated Fe^{III} atom and two N-methylimidazoles as axial ligands.
Figure 4.2 Synthetic methods for tetraarylporphyrins.
Figure 4.3 Scheme for obtaining a trans-disubstituted tetraarylporphyrin from mixed-aldehyde synthesis. A success in the chromatographic separation step is crucial.
Figure 4.4 Scheme for the rational synthesis of (top) trans- and (bottom) cis-disubstituted tetraarylporphyrins from appropriately substituted precursors.
Figure 4.5 Scheme for molybdatation of a catechol.
CHAPTER 5. MIXED-ALDEHYDE ADLER SYNTHESIS AND
CHROMATOGRAPHIC SEPARATION OF TOLYLDIMETHOXY-
PHENYLPORPHYRINS

Try again. Fail again. Fail better.

— Samuel Beckett, *Worstward Ho*

5.1 Introduction

In the last Chapter, two general approaches to the synthesis of disubstituted porphyrins were discussed. The more straightforward of these two would seem to be the mixed-aldehyde synthesis / chromatographic separation. This method, the most widely used one in the synthesis of asymmetric tetraarylporphyrins, was indeed used in one of our attempts at the synthesis of the disubstituted porphyrin. This attempt, which had limited success, is described in this Chapter and summarized in Figure 5.1.

The works described in this Chapter was performed concurrently with those in the next Chapter. More specifically, it was performed between the unsuccessful Lindsey and the moderately successful low-temperature Adler “2+2” syntheses. Although the goal of making a disubstituted porphyrin consisting of only one regioisomer was not met, the works made during the attempt helped in establishing the protocols for chromatography and demethylation that were used later in the successful syntheses of the desired trans-disubstituted tetraarylporphyrins.

_Nomenclature and abbreviations._ Please refer to §4.1.2 (p. 118) and §4.1.3
(p.119) for the nomenclature and abbreviations used in this Chapter.

5.2 Experimental

5.2.1 REAGENTS AND PHYSICAL MEASUREMENTS.

Propionic acid (Aldrich) and ethyl acetate (Fisher) were used without further purification. Methylene chloride (Fisher, Optima grade) was dried over CaH$_2$ and distilled under N$_2$. Toluene (Fisher) was dried over Na and distilled under N$_2$. Pyrrole (Aldrich) was dried over CaH$_2$ and fractionally distilled under N$_2$ or Ar. para-Tolualdehyde (4-methylbenzaldehyde, Aldrich) was fractionally distilled under vacuum. Triethylamine (Lancaster) was passed through a silica gel column before use, to remove impurities (A. Pacheco and A. M. Shachter, personal communication). Zinc acetate dihydrate (Mallinkrodt), and 2,3-dimethoxybenzaldehyde (Aldrich) were used as purchased. The silica gel used in the preparation was J. T. Baker 60-200 mesh unless noted otherwise. The TLC plates used for the analyses were J. T. Baker Silica Gel IB2. The $^1$H-NMR spectra were taken and processed using a Bruker AM 250-MHz instrument.

5.2.2 MIXED-ALDEHYDE ADLER SYNTHESIS AND THE SEPARATION OF THE PORPHYRIN MIXTURE.

A 1000-mL three-neck flask was equipped with a reflux column and a 250-mL pressure-equilibrating addition funnel. Propionic acid (500 mL) was placed in the flask together with a stir bar, and was heated to boiling. A mixture of pyrrole (13.4 g, 0.200 mol), $p$-tolualdehyde (11.7 g, 0.097 mol), 2,3-dimethoxybenzaldehyde (16.7 g, 0.100 mol), and propionic acid (100 mL) was placed in the dropping funnel and was
added to the boiling acid. The resulting black mixture was heated for 1 h, then cooled and left overnight. The amorphous purple precipitate was recovered by filtration. This precipitate was recrystallized in CHCl₃/EtOAc. The recrystallization product was chromatographed in a silica gel column: it was found to consist largely of tetratolylporphyrin and the monosubstituted porphyrin (5-(2,3-dimethoxyphenyl)-10,15,20-tritolylporphyrin). The mother liquor, which still contained an appreciable amount of porphyrins, was dried. Two portions, about 5 mg each, was taken from this solid, and each was chromatographed in a column: one using coarse-mesh (60-200) silica gel; and the other using fine-mesh (200-400). The two chromatographic separations gave essentially the same result. The solid from the dried mother liquor contained larger quantity of the monosubstituted porphyrin, but only little of the porphyrins with higher substitution. The NMR spectra of the chromatographically separated products are shown in Appendix B.

From the black, tarry reaction mixture that had been filtered, the solvent was removed by drying; then the resulting black solid (~10 g) was put through a silica gel column (7 × 35 cm) using CH₂Cl₂/EtOAc (9:1) as the eluant. The band containing porphyrins was identified by fluorescence from long-wavelength UV and collected in a round-bottom flask. The collected layer, which still contained a significant amount of impurities, was dried with a rotary evaporator; and the resulting solid was chromatographed using the identical condition as in the preceding process. The solvent was removed from the layer containing porphyrins.
Tetratolylporphyrin was chromatographically separated from the resulting purple solid using a silica gel column (7 × 35 cm) and hexanes / CH₂Cl₂ (1:3). (The resulting solid was named "Fraction 1".) The further attempt to separate more polar porphyrins using solvent ramping resulted in a severely cracked column. The porphyrins remaining in the column was eluted together using CH₂Cl₂ / EtOAc (9:1); the solvent was removed, and the resulting solid ("Fraction 2") was separated further using a smaller silica gel column (4 × 20 cm). The monosubstituted porphyrin (5-(2,3-dimethoxyphenyl)-10,15,20-tritolylporphyrin) ("Fraction 2-1") was separated using hexane / CH₂Cl₂ (1:3); two fractions ("Fractions 2-2 and 2-3"), eluted with hexanes / CH₂Cl₂ (1:100) and CH₂Cl₂ / EtOAc (100:2) respectively, consisted mostly of the disubstituted porphyrins (bis(2,3-dimethoxyphenyl)ditolylporphyrin, trans- and cis-) and the trisubstituted porphyrin (5,10,15-tris(2,3-dimethoxyphenyl)-20-tolylporphyrin). Fraction 2-2 was put through a column (4 × 15 cm) of 200-400 mesh silica gel, using CH₂Cl₂ / EtOAc (100:0.2). (This fraction was chosen over 2-3 because it contained a larger proportion of the disubstituted porphyrins.) The first fraction ("2-2-1") consisted of the monosubstituted porphyrin. The second fraction ("2-2-2", CH₂Cl₂) consisted of the disubstituted porphyrins (trans- and cis-): since this band eluted before the other band of disubstituted porphyrins, this band very likely consisted of the αβ-atropisomer . The third fraction ("2-2-3", CH₂Cl₂ / EtOAc 100:3) consisted of the αα-atropisomer of the disubstituted porphyrins, and one of the atropisomers of the trisubstituted porphyrin. The fourth fraction ("2-2-4", CH₂Cl₂ / EtOAc 9:1) consisted of the trisubstituted porphyrin. Fraction 2-2-3 was put through a
4 x 15 cm column of 200-400 mesh silica gel. The first fraction ("2-2-3-1", CH₂Cl₂) consisted of the αβ-disubstituted porphyrins. The second fraction ("2-2-3-2", CH₂Cl₂ / EtOAc 100:1) consisted of the αα-disubstituted porphyrins with no trisubstituted species. The NMR spectra of all the separated products are shown in Appendix B.

All of the separated products were placed in vials. They are currently stored in Old Chemistry 248.

5.2.3 Demethylation of the mixture of trans- and cis-disubstituted porphyrins.

In a nitrogen-filled 100-mL Schlenk flask equipped with a stirring magnet, 5 mL of freshly distilled CH₂Cl₂ and then 2 mL of BBr₃ were introduced using cannulae. The flask was then immersed in a dry ice / isopropanol bath. In a 50-mL Schlenk flask filled with N₂, a mixture of the trans- and cis-disubstituted porphyrins synthesized from the previous procedure (125.6 mg, 0.1646 mmol, mixture of Fractions 2-2-3-1 and 2-2-3-2) was placed, and then 5 mL of freshly distilled CH₂Cl₂ was introduced using a cannula. The porphyrin / CH₂Cl₂ mixture was introduced into the BBr₃ mixture. The resulting green reaction mixture was stirred in the bath for 2 h, with constant replenishing of dry ice; the coolant was then left to sublime, the flask was removed from the bath, and the mixture was stirred overnight. After the reaction was finished, the flask was immersed in an ice water bath. Degassed H₂O (30 mL) was slowly added using a syringe. Degassed Et₃N (30 mL) was then added to neutralize the acid. The resulting precipitate was then collected by and washed with water. The mother liquor, which had an intense red color, was treated with 100 mL of heptane to produce a large quantity of precipitate. The red-
brown, amorphous solids were collected and weighed 85.3 mg. The NMR spectrum of the product indicated the presence of impurities by signals at δ0-1 and δ7-8. The porphyrin was chromatographed through a short (4 × 2 cm) column of silica gel using 1:1 CH₂Cl₂ / MeOH. After removing the solvent, red-brown, amorphous solid was collected. The final yield was not determined, since this procedure was performed as a test before the demethylation of a sample of pure trans-disubstituted porphyrin (see the next Chapter).

5.2.4 COLUMN CHROMATOGRAPHY OF THE DEMETHYLATED PORPHYRIN.

Di-p-tolyl-bis(2,3-dihydroxymethylphenyl)porphyrin (5 mg, 0.007 mmol) was dissolved in CH₂Cl₂ and placed over a 4 × 20 cm column of silica gel. The eluant was varied slowly from CH₂Cl₂ to EtOAc and then to MeOH. The porphyrin remained at the top of the column, streaking only slightly.

5.3 Results

5.3.1 MIXED-ALDEHYDE ADLER SYNTHESIS.

The literature method for the Adler synthesis was used in the reaction of 2,3-dimethoxybenzaldehyde and p-tolualdehyde with pyrrole. Purple, microcrystalline precipitate was found in the mixture after the reaction; but when the solid was chromatographed, it was found to consist mostly of tetratolylporphyrin and the monosubstituted species. The solvent was removed from the tar (in a process complicated by the viscosity of the tar and the tendency for it to form a film on the surface), and the remaining solid was chromatographed and the resulting bands analyzed
by NMR; the analysis indicated that most of the desired disubstituted porphyrin was located in the tar.

5.3.2 Column Chromatography of the Mixed Porphyrins.

A total of six chromatography columns were necessary to separate the porphyrin mixture into the non-, mono-, di-, and trisubstituted components. The large number of columns was necessitated by the following: (1) the large quantity of impurities in the dried tar; (2) the cracking of the column caused by a drastic change in eluant; and (3) the difficulty in separating one atropisomer of the disubstituted porphyrin with one of the trisubstituted. (The tetrasubstituted porphyrin was not found in the mixture.)

Two columns were necessary to remove the black impurities in the dried tar. In the first column, the matrix blackened so intensely that the bands could not be identified under the standard room lighting: the porphyrin bands had to be identified by the fluorescence from long-wavelength UV light. In the columns that were used subsequently, the nonsubstituted porphyrin (tetratolylporphyrin) was separated using 1:1 toluene / CH$_2$Cl$_2$; the monosubstituted, using 1:3 toluene / CH$_2$Cl$_2$; and the αβ atropisomer of the disubstituted, using CH$_2$Cl$_2$. The extent of the substitution was determined by the ratio of the integration of the tolyl methyl (δ2.7) with that of either the ortho- (δ4.1) or the meta- (δ3.8) methoxy protons (see the figures in Appendix B (p. 240ff)).

A problem arose during the separation of the αα atropisomer of the disubstituted porphyrin. The mixture of 100:1 CH$_2$Cl$_2$ / EtOAc, suitable for the elution of the desired
species, was found also to elute one atropisomer of the trisubstituted porphyrin at the same time. Several unsuccessful attempts were made to separate these two species using 60-200 mesh silica gel. The two species were separated successfully only after the matrix was changed to finer silica gel (200-400 mesh).

Two isomers of the disubstituted porphyrins were separated during the chromatographic process; they interconverted between chromatographic runs. The interconversion indicated that these isomers were not trans- and cis-, but αα and αβ atropisomers. The efforts to separate the trans-isomer from the cis- was unsuccessful.

5.3.3 Demethylation of the disubstituted porphyrins.

To demethylate the methoxy groups, the porphyrin was treated with BBr3. Basu and co-workers have noted that adding Et3N to the reaction mixture causes the product to precipitate. We have observed that part of the product does indeed precipitate, but a significant quantity of the product remains in the liquid phase. Addition of heptane was necessary to make sure that all of the product precipitated. The precipitated product was found by NMR to be impure, and had to be chromatographed using silica gel to produce a reasonably pure product. The chromatography was performed on a short column (4 × 2 cm) to prevent losses. After the chromatography, the column was found to be strongly tinted, indicating that a significant quantity of the porphyrin had adhered to the silica gel. The tendency of the bis(dihydroxyphenyl)ditolylporphyrin to adhere to silica had been noted earlier, when it was found that the Schlenk flask used for the demethylation became highly colored.
5.3.4 CHROMATOGRAPHY.

We had hoped that the demethylation of the methoxy groups and the subsequent increase in polarity would aid the separation of isomers by silica gel chromatography. Unfortunately, when column chromatography was attempted on the demethylated porphyrin using a 4 × 20 cm column and solvent ramping, the porphyrin adhered to the matrix and could not be removed even with MeOH.

5.4 Discussion

5.4.1 MIXED-ALDEHYDE ADLER SYNTHESIS — ISOLATING THE PRODUCT FROM THE REACTION MIXTURE.

In the Adler synthesis involving only one type of arenecarbaldehyde, the product porphyrin usually precipitates out of the propionic acid mixture. In this case, the product can be isolated by filtration, which is an easy and fast method. In our mixed-aldehyde Adler synthesis however, we found that most of our desired product — bis-(dihydroxyphenyl)ditolylporphyrin ("disubstituted") — remained in the propionic acid mixture without precipitating. Precipitate did form after the reaction, but this precipitate consisted mostly of tetratolylporphyrin ("unsubstituted") and dihydroxyphenyltritolylporphyrin ("monosubstituted"). The more symmetrical porphyrins — tetratolyl and monosubstituted — crystallized easily. The disubstituted porphyrin however is more difficult to pack into a crystal lattice because of its asymmetry, especially the αα atropisomer. In an earlier study, Setsune et al. had to extract their product from the propionic acid reaction mixture in their low-temperature Adler synthesis of a trans-
disubstituted porphyrin that is recovered as a mixture of four atropisomers.

The removal of the tar from the mixture was accomplished by using a large (7 × 30 cm) silica gel column. In retrospect, such a large column was not necessary: a much shorter column of about 3 cm in length could have been used, and the number of the chromatographic runs increased.

5.4.2 Separation of the Porphyrins Synthesized by the Mixed-Aldehyde Adler Synthesis.

By using an extensive solvent-ramping on a silica-gel column, the product of the mixed-aldehyde synthesis can be separated by the number of the dimethoxyphenyl substituents on the meso-positions. The 1H-NMR spectra of all the separated components are shown in Appendix B. For the di- and trisubstituted porphyrins, the atropisomers can also be separated. Of the disubstituted species, the trans- and the cis-isomers cannot be separated using this method: because of this difficulty, the mixed-aldehyde method cannot be used for the multi-step synthesis of the desired sterically demanding porphyrins described in §4.1.1 (p. 117).

The difficulty of separation of the trans- and cis-isomers of a meso-disubstituted tetraarylporphyrin has been noted earlier. Of the few known examples of successful separations, all of them involve either nitrophenyl or pyridyl groups on the meso-positions. The difficulty lies in the mechanism of molecular recognition by silica gel. This matrix is extremely polar, and therefore it interacts very strongly with a polar group such as dimethoxyphenyl; it however does not interact with a nonpolar group such as the
porphine core. Therefore, its binding ability is sensitive to the number and the relative orientation of the dimethoxyphenyl groups, but insensitive to the change of the induced polarity in the porphine core.

Perhaps, in order to separate the trans-isomer from the cis-, a nonpolar matrix is required. Reverse-phase silica gel, tailored for either preparative column chromatography or HPLC, is available commercially. Polystyrene gel matrix, which has been used in separation of C_{60} from C_{70},\textsuperscript{111} is another alternative. One should attempt HPLC first, in order to see if the trans-cis separation is indeed possible. Once the possibility is verified, one may proceed to column chromatography to check the feasibility of a 100 mg-scale separation.

5.4.3 Demethylation of the Dimethoxyphenyl Groups and the Subsequent Column Chromatography.

The demethylation of the dimethoxyphenyl groups was performed under inert atmosphere in order to minimize the oxidation of the pendant catecholato group. The product was precipitated; after that, it was handled under the regular atmosphere. Uyeda and Therien,\textsuperscript{112} upon testing the procedure of Basu et al.\textsuperscript{108} for Inorganic Syntheses, proposed and carried out an alternative method for the extraction of the demethylated product: extraction of the reaction mixture with CH_2Cl_2 under the regular atmosphere. This method exposes the catecholyporphyrin to oxygen while it is in solution state and therefore makes it more susceptible to loss by oxidation. Nevertheless, the method is the preferable one when the quantity of product is so small that loss by adsorption onto the
filter paper becomes significant.

Basu and co-workers, after they demethylated the monosubstituted porphyrin, crystallized the product to purify it. The disubstituted porphyrin is less suited for crystallization because it exists as two atropisomers. Therefore, it had to be purified through column chromatography. The column chromatography on silica gel is an undesirable method, not only because the product is exposed to oxygen, but also the dihydroxyphenyl groups have a strong affinity to the silica gel. The four hydroxy groups on the product coordinate to SiO₂, causing the product to adhere onto the matrix. In order to avoid the loss by adhesion as much as possible, a short column (2 cm in height) was used and the chromatography was performed rapidly.

We had hoped that the high polarity of the dihydroxyphenyl groups would allow the separation of the trans- and the cis-isomers. The attempt at the separation however was thwarted by the affinity of the group to silica. If such separation is attempted in future, a non-acidic medium such as reverse-phase silica gel or Sephadex should be used. If one insists on using silica gel as the matrix, a treatment of the matrix with a small quantity of acid is recommended: the acidity might keep the oxygen moieties protonated, and thus prevent them from binding to the matrix.

5.5 Future Directions

If the mixed-aldehyde Adler method is to be used for the preparation of the rotation-hindering porphyrin described earlier in this dissertation, a method for the trans-cis separation must be developed. One should use a reverse-phase matrix for an attempt
at the separation of either the methylated or the demethylated species. A use of HPLC would be desirable over a simple column chromatography. The samples from our attempt are stored in Old Chemistry 248, and these may be used for trying out these methods.

5.6 Conclusions

A mixed-aldehyde Adler synthesis was performed using pyrrole, $p$-tolualdehyde, and 2,3-dimethoxybenzaldehyde. The substituted porphyrins mostly remained in the reaction mixture without precipitating, and had to be isolated by chromatographing the dried tar. The porphyrin mixture could be separated according to the number of substitution and the atropisomerism, but not to the trans-cis isomerism. The attempt at separating the trans- and the cis-isomers of bis(dihydroxyphenyl)ditolylporphyrin was unsuccessful because of the affinity of this dihydroxyphenyl group to silica gel.
Figure 5.1 Summary of the reactions described in Chapter 5.
CHAPTER 6. MACDONALD “2+2” SYNTHESIS OF trans-BIS(2,3-DIMETHOXYPHENYL)DI-p-TOLYLPOURPHIRIN IN AQUEOUS ANIONIC SURFACTANT, AND THE SUBSEQUENT PERIPHERAL LIGATION TO TWO Mo^O(Me2Pyz)3BH GROUPS

Let be be the finale of seem.

— Wallace Stevens, “The Emperor of Ice Cream”

6.1 Introduction

As we indicated in the last Chapter, the mixed-aldehyde synthesis / chromatographic separation did not provide the desired trans-disubstituted tetraarylporphyrin. Therefore, in order to make the target molecule described in §4.1.1 (p. 117), we must perform a rational synthesis of the tetraarylporphyrin. The method for the MacDonald “2+2” synthesis of trans-disubstituted tetraarylporphyrins was first described by Lee and Lindsey. This method, however, is susceptible to scrambling of the substituents: this was later documented by Lindsey himself, together with his co-workers. It was necessary for us to develop a new method for the “2+2” synthesis.

In this Chapter, the application of Bonar-Law’s method of synthesis to the “2+2” synthesis is described. Bonar-Law first described the synthesis of tetraarylporphyrins in aqueous anionic surfactant in 1996. Our work is the first one in which his method has been applied to the “2+2” synthesis. The subsequent molybdation of the disubstituted porphyrin is also described. The reactions are summarized in Figure 6.1.
Nomenclature and abbreviations. Please refer to §4.1.2 (p. 118) and §4.1.3 (p.119) for the nomenclature and abbreviations used in this Chapter.

6.2 Experimental

6.2.1 REAGENTS.

Propionic acid (Aldrich) and ethyl acetate (Fisher) were used without further purification. Methylene chloride (Fisher, Optima grade) was dried over CaH₂ and distilled under N₂. Toluene (Fisher) was dried over Na and distilled under N₂. Tetrahydrofuran (THF, Mallinkrodt) was dried using potassium / benzophenone and distilled under N₂. Pyrrole (Aldrich) was dried over CaH₂ and fractionally distilled under N₂. \textit{p}ara\text{-Tolualdehyde} (4-methylbenzaldehyde, Aldrich) was fractionally distilled under vacuum. Triethylamine (Lancaster) was passed through a silica gel column before use, to remove the impurities. Trifluoroacetic acid (EM Science), DDQ (Fluka), TCQ (Aldrich), zinc acetate dihydrate (Mallinkrodt), 2,3-dimethoxybenzaldehyde (Aldrich), sodium dodecyl sulfate (sodium lauryl sulfate, Sigma, 95% assay), and concentrated (12 M) HCl (Fisher) were used as received. The silica gel used in the preparations was J. T. Baker 60-200 mesh unless noted otherwise. The TLC plates used for the analyses were J. T. Baker Silica Gel IB2.

6.2.2 PHYSICAL MEASUREMENTS

The one-dimensional NMR spectra were taken and processed with a Bruker AM spectrometer operating at 250 MHz. The two-dimensional NMR spectra were taken with a Varian Unity 300-MHz spectrometer, and processed with Varian VNMR v. 4.3
software. The EPR spectra was taken by Dr. Arnold M. Raitsimring (Univ. of Arizona) with a Bruker ESP-300E spectrometer equipped with an Oxford CF935 cryostat. The fast-atom bombardment mass spectra (FAB-MS) was taken by the Mass Spectrometer Facility (Dept. of Chemistry, Univ. of Arizona) with a JEOL HX-110A spectrometer; for the matrix, a mixture of glycerol and m-nitrobenzyl alcohol was used.

6.2.3 2,3-DIMETHOXYPHENYLDIPYRRMETHANE AND 5,10-BIS(2,3-DIMETHOXYPHENYL)-TRIPYRRANE (LARGE-SCALE SYNTHESIS).

In a 200-mL Schlenk flask equipped with a stirring magnet, 50 mL (48 g, 0.72 mol) of distilled pyrrole was placed. The solvent was degassed three times by the freeze-pump-thaw method, then the flask was filled with N₂. 2,3-Dimethoxybenzaldehyde (1.7061 g, 0.01027 mol) was added to the solvent while stirring. After the mixture became homogeneous, 100 μL of trifluoroacetic acid was added. The light yellow reaction mixture was stirred for 8 h; then the reaction was quenched by diluting the mixture with 20 mL of CH₂Cl₂ and then washing it with 25 mL of 0.1 N NaOH. The organic layer was washed with 3 × 25 mL of water, dried over 5 g of Na₂SO₄, and decanted. Methylene chloride was removed by using a rotary evaporator, and then the excess pyrrole was removed by subjecting the mixture to the vacuum from a Schlenk line. (N. B. : The excess pyrrole can be collected and recycled for subsequent use.) The remaining brown oil was chromatographed using a 4 × 40 cm column of silica gel. Fractions of 125 mL were collected and were tested for the desired products by TLC. Cyclohexane / EtOAc / Et₃N (80:20:1) was used to elute the desired dipyrromethane, and
cyclohexane / EtOAc / Et₃N (50:50:1) was used to elute the desired tripyrrane. From each fraction, the solvent was removed by using a rotary evaporator to obtain lumps of white crystals contaminated by brown or red oxidation products. This product was recrystallized from MeOH / H₂O and dried, after which 1.5548 g (54% yield) of the dipyrromethane was obtained as white-yellow microcrystals. The yield for the tripyrrane was not measured. In a different preparation, a smaller quantity of the aldehyde was used (0.8386 mg, 0.005047 mol), while those of the other reagents were kept the same. The yield of the dipyrromethane was smaller (0.8976 g), but the percentage yield was greater (63%).

2,3-Dimethoxyphenyldipyrromethane. ¹H-NMR (in CDCl₃): δ8.33 (s, 2H, pyrrole NH); δ6.98 (t, J = 8 Hz, 1H, phenyl 5-H); δ6.83 (d, J = 8 Hz, 1H, phenyl 4-H); δ6.78 (d, J = 8 Hz, 1H, phenyl 6-H); δ6.65 (m, 2H, pyrrole(1,9)-H); δ6.13 (m, 2H, pyrrole(2,8)-H); δ5.92 (m, 2H, pyrrole(3,7)-H); δ5.69 (s, 1H, meso-H); δ3.86 (s, 3H, 3-OCH₃); δ3.49 (s, 3H, 2-OCH₃). ¹³C-NMR (in CDCl₃): δ152.9 (phenyl 2-C); δ146.1 (phenyl 3-C); δ136.6 (phenyl 1-C); δ132.5 (pyrrole(4,6)-C); δ124.2 (phenyl 5-C); δ121.5 (phenyl 6-C); δ116.7 (pyrrole(1,9)-C); δ111.1 (phenyl 4-C); δ108.1 (pyrrole(2,8)-C); δ106.7 (pyrrole(3,7)-C); δ60.3 (3-OCH₃); δ55.6 (2-OCH₃); δ39.3 (meso-C).

5,10-bis(2,3-dimethoxyphenyl)tripyrrane. ¹H NMR (in CDCl₃): δ8.34 (s, 2H, pyrrole(α,γ) NH); δ8.21 (m, 1H, pyrrole(β) NH); δ6.97 (m, 2H, phenyl 5-H); δ6.81 (d, 2H, phenyl 4-H); δ6.72 (d, 2H, phenyl 6-H); δ6.62 (s, 2H, pyrrole α(1,14)-H); δ6.09 (s, 2H, pyrrole β(2,13)-H); δ5.87 (s, 2H, pyrrole β(3,12)-H); δ5.75 (m, 2H, pyrrole β(7,8)-H)
δ5.67 (s, 2H, meso-H); δ3.83 (s, 6H, 3-OCH₃); δ3.48 (s, 6H, 2-OCH₃). ¹³C NMR: δ152.7 (phenyl 2-C); δ146.1 (phenyl 3-C); δ136.6 (phenyl 1-C); δ132.5 (pyrrole α(4,11)-C); δ131.7 (pyrrole α(6,9)-C); δ124.0 (phenyl 5-C); δ121.3 (phenyl 4-C); δ116.6 (pyrrole α(1,14)-C); δ111.0 (phenyl 5-C); δ107.9 (pyrrole β(2,13)-C); δ106.7 (pyrrole β(7,8)-C); δ106.5 (pyrrole β(3,12)-C); δ60.3 (2-OH₂C); δ55.6 (3-OH₂C); δ38.5 (meso-C).

6.2.4 ATTEMPTS AT THE LINDSEY “2+2” SYNTHESIS OF 5,15-BIS(2,3-DIMETHOXYPHENYL)-10,20-DI-p-TOLYLPORPHYRIN.

In a 100-mL Schlenk flask equipped with a stirring magnet, freshly distilled CH₂Cl₂ (50 mL) was introduced using a cannula; then p-tolualdehyde (21 µL, 21 mg, 0.18 mmol) was added using a micropipette. 2,3-Dimethoxyphenyl dipyrromethane (51.3 mg, 0.182 mmol) was placed in the flask. The mixture was degassed by the freeze-pump-thaw method. Trifluoroacetic acid (15 µl) was added to start the reaction. The reaction mixture immediately turned light brown. The reaction was monitored through spectrophotometry by the following procedure. The reaction was run for about 60 minutes, and then DDQ (89 mg, 0.40 mmol) was added to quench the reaction.

The solvent was removed from the black mixture using a rotary evaporator. The solid mixture was chromatographed on a slurry-packed silica gel column (4 × 20 cm) several times. First, the solid was chromatographed using 10:1 CHCl₃ / EtOAc to separate the porphyrins from all the other products: the porphyrins separated first (identified by the orange fluorescence from long-wavelength UV light). Second, the porphyrins were chromatographed using CH₂Cl₂: two fast moving fractions and one slow-
moving fractions were separated. One of the two first fractions gave an NMR spectrum that was consistent with $A_2B_2$ porphyrins. Third, the slow-moving fraction was chromatographed using 100:1 CH$_2$Cl$_2$ / MeOH. Three fractions separated. NMR spectra indicated that these products consisted mostly of the tri-substituted tris(dimethoxyphenyl)tolylporphyrin. The yields were not determined.

Later, another reaction was run using the same reagents and reaction conditions, but cutting the reaction time to 20 minutes. In this reaction mixture, no monosubstituted porphyrin was found; however, a considerable amount of the trisubstituted porphyrin was found along with the disubstituted porphyrins. The disubstituted porphyrins were separated, and the yield was determined to be 8.1%.

6.2.5 Low-temperature Adler synthesis of (5,15-bis(2,3-dimethoxyphenyl)-10,20-di-$p$-tolylporphyrinato)Zinc(II).

In a 50-mL round-bottom flask equipped with a stirring magnet, 5 mL of propionic acid and 0.1 g (0.5 mmol) of Zn(OAc)$_2$·2H$_2$O were placed. The flask was then placed in an ice bath. 2,3-Dimethoxyphenyldipyrromethane (99.5 mg, 0.352 mmol) and a mixture of $p$-tolualdehyde (60.9 mg, 0.507 mmol) and 2 mL of CH$_2$Cl$_2$ were added, and then a drying tube equipped with CaSO$_4$ was placed on top of the flask. The mixture was stirred in the ice bath for 2.5 h, and then at room temperature overnight. Dichlorodicyanoquinone (223 mg, 0.982 mmol) in 5 mL of CH$_2$Cl$_2$ and 5 mL of propionic acid was then added dropwise to the resulting inhomogeneous dark brown mixture. To make sure that the metallation went to completion, 0.9 g (4 mmol) of Zn(OAc)$_2$·2H$_2$O was
added. The solvent was then removed with a rotary evaporator. The resulting solid was dissolved in CH₂Cl₂ and chromatographed on a 4 × 20 cm column of silica gel. Two bands were identified. The first band (purple) was recovered from the column with CH₂Cl₂; the NMR spectrum of the dried solid identified it as the αβ atropisomer. The second band (purple) was recovered with CH₂Cl₂ / EtOAc (100:3). The NMR spectrum identified it as the αα atropisomer. No products of scrambling were detected. The combined yield of the two atropisomers was 12.3 mg (8.4%). ¹H-NMR (αα in CDCl₃): δ8.92 (d, J = 4 Hz, 4H, pyrrole β-H); δ8.90 (d, J = 4 Hz, 4H, pyrrole β-H); δ8.14 (d, J = 8 Hz, 2H, MePh o-H); δ8.02 (d, J = 8 Hz, 2H, MePh o-H); δ7.68 (d, 2H, (MeO)₂Ph o-H); δ7.52 (t, 4H, (MeO)₂Ph p- and m-H); δ7.36 (m, 4H, MePh m-H); δ4.11 (s, 6H, m-OCH₃); δ3.13 (s, 6H, o-OCH₃); δ2.69 (s, 6H, p-CH₃).

6.2.6 **Bonar-Law Synthesis of 5,15-bis(2,3-dimethoxyphenyl)-10,20-di-p-tolyl-porphyrin.**

In a 200-mL Schlenk flask, a stirring magnet and 18 g of sodium dodecyl sulfate were placed. The flask was evacuated and refilled with argon three times. Using a cannula, 100 mL of degassed, distilled H₂O was introduced. The mixture was stirred until it became homogeneous. Distilled p-tolualdehyde (47 µL, 0.399 mmol) and then uncocrystallized 2,3-dimethoxyphenyldipyrromethane (100 mg, 0.354 mmol) in lumps were added to the mixture while stirring. After an hour, while some of the dipyrromethane remained undissolved and the mixture had turned light brown, 50 µL of 12 M HCl was added to initiate the formation of porphyrinogen. (The concentration of HCl in the
reaction mixture was 6 mM.)

The reaction was monitored using two methods: UV/visible spectroscopy for monitoring the yield of the porphyrin, and TLC for monitoring the possible rearrangement products. In a 5-mL volumetric flask, 200 μL of the reaction mixture was mixed with 40 μL of 2.5% w/v DDQ solution in THF and then with 40 μL of pyridine. The solution was made up to volume with THF. In a 10-mm quartz cuvette, 200 μL of the porphyrin-THF solution was mixed with 3 mL of CH₂Cl₂, and the optical spectrum of the mixture was measured. From the remaining porphyrin-THF solution, the solvent was removed; and the resulting solid was suspended in toluene. The suspension was applied to a silica gel TLC plate, which in turn was developed using Et₂O. The disubstituted porphyrin appears at Rₖ=0.6; the mono- and the trisubstituted porphyrins appear close to the disubstituted, but they do separate clearly (ΔRₖ=0.03).

The reaction was complete in two days. To the reaction mixture, 220 mg (0.895 mmol) of TCQ was added. The mixture turned cherry red, then brown in a gradual manner. The oxidation was monitored using UV/visible spectroscopy, using the procedure above for sample preparation but omitting the DDQ solution. The oxidation was complete in two days. In a 500-mL beaker, 0.5 g of KOH, 1 g of pH 7 phosphate buffer powder (Na₂HPO₄ + KH₂PO₄), and 1 g of KCl was mixed with 20 mL of H₂O and 50 mL of toluene. The reaction mixture was then placed in the beaker to precipitate the detergent. The inhomogeneous dark-brown mixture was filtered using a 500-mL separatory funnel whose narrow end was plugged with glass fiber; pressure was applied
to the mixture in order to increase the rate of filtration. The precipitate was washed with toluene until it became light gray. The organic layer was washed with $3 \times 25$ mL of H$_2$O, then the solvent was removed.

The resulting brown solid was dissolved in CH$_2$Cl$_2$ and loaded onto a silica gel column (4 x 20 cm, slurry-packed with CH$_2$Cl$_2$). Two bands were identified: the $\alpha\beta$ atropisomer, removed with CH$_2$Cl$_2$; and the $\alpha\alpha$ atropisomer, removed with CH$_2$Cl$_2$/EtOAc (100:1). The total yield of the trans-disubstituted porphyrins was 34 mg (25%). (If the solid is dissolved in toluene before loading, three bands are identified. The first band consists of the $\alpha\beta$ atropisomer. The second and the third bands both consist of the $\alpha\alpha$ atropisomer: the component of one of them is suspected to be a porphyrin-toluene $\pi$-complex.) $^1$H-NMR ($\alpha\alpha$ in CDCl$_3$): 88.81 (s, 8H, $\beta$-pyrrole H); 88.12 (d, J = 7 Hz, 2H, MePh o-H); 88.01 (d, J = 7 Hz, 2H, MePh o-H); 87.65 (d, 2H, (MeO)$_2$Ph o-H); 87.51 (m, 4H, (MeO)$_2$Ph m- and p-H); 87.34 (d, 4H, MePh m-H); 84.09 (s, 6H, m-OMe); 83.13 (s, 6H, o-OMe); 82.67 (s, 6H, p-Me); -2.76 (s, 2H, pyrrole NH). (In the spectrum of the $\alpha\beta$ atropisomer, the signals from MePh o-H collapses at 88.07. Also, the signal from o-OMe shifts to 83.16.)

6.2.7 LARGE-SCALE BONAR-LAW SYNTHESIS.

In a 2000-mL three-necked round bottom flask, 1000 mL of distilled H$_2$O was degassed. A stirring magnet and 150 g of sodium dodecyl sulfate were placed in the flask and the mixture was stirred until it was homogeneous. Recrystallized powdery 2,3-dimethylphenylidipyrromethane (1002 mg, 3.55 mmol) was placed in the flask and the
mixture was stirred until homogeneous. \( p \)-Tolualdehyde (420 \( \mu \)L, 428 mg, 3.56 mmol) was transferred into the flask. Concentrated HCl (500 \( \mu \)L) was added. The mixture was monitored for 12 h, but no reaction was observed. Another 150 g of sodium dodecyl sulfate was added and the reaction was monitored for another 12 h, but no reaction was apparent. A test with pH paper indicated that the mixture was neutral (pH = 7). Concentrated HCl (2500 \( \mu \)L) was added in batches of 500 and 2000 \( \mu \)L, until the mixture had turned brown and the reaction was apparent by its UV spectrum. The reaction was complete in 6 h after the last addition of concentrated HCl. An analysis by TLC showed no evidence of the mono- or the tri-substituted porphyrin. The reaction was quenched with 2200 mg (9.0 mmol) of TCQ.

In a 2000-mL beaker, 200 mL of \( \text{H}_2\text{O} \), 1 g of \( \text{NaH}_{2}\text{PO}_4 \cdot \text{H}_2\text{O} \), 1 g of \( \text{Na}_2\text{HPO}_4 \), 2 g of \( \text{NaOH} \), 5 g of \( \text{NaCl} \), and 200 mL of toluene were placed. The reaction mixture was poured into the beaker and the mixture was stirred. The resulting precipitate was so fine that it went through a base-resistant filter paper. More toluene, \( \text{H}_2\text{O} \), and \( \text{NaOH} \) were added randomly, in an attempt to form precipitate amenable to filtration. Finally, KCl was found to cause the formation of such precipitate: by then, the volume of the mixture had increased to about 6000 mL. The mixture was filtered through a Hirsch funnel with packed glass fiber in place of a filter paper. The filtration was made difficult by the large quantity of precipitate blocking the filter. The precipitate was washed with toluene; the solvent was separated from the precipitate by filtration. The organic layer was separated, and the solvent was removed. The brown residue was dissolved in toluene and was
chromatographed on a 7 × 35 cm column of silica gel, varying the eluant from toluene to CH₂Cl₂ to ethyl acetate. No scrambling products were found. The solvent was removed from the fractions containing the porphyrin. Total yield: 230 mg (16%).

6.2.8 (5,15-Bis(2,3-dimethoxyphenyl)-10,20-di-p-tolyIporphyrinato)Zn(II) FROM THE CORRESPONDING FREE-BASE PORPHYRIN.

In a 500-mL round-bottomed flask, 100 mL of DMF and a stirring magnet were placed. While stirring, 30 mg (0.039 mmol) of 5,15-bis(2,3-dimethoxyphenyl)-10,20-di-p-tolyIporphyrin (both atropisomers) and 1000 mg (5 mmol) of Zn(OAc)₂·2H₂O were added. The mixture was stirred at room temperature. The reaction was monitored by UV/visible spectroscopy. After 24 h, 100 mL of H₂O was added dropwise to precipitate the porphyrin. The solid was collected by filtration and was chromatographed using a 4 × 20 cm silica gel column. The αβ atropisomer was collected using CH₂Cl₂; the αα, using CH₂Cl₂ / EtOAc 100:1 mixture. Yield: 18 mg (57%).

6.2.9 5,15-Bis(2,3-dihydroxyphenyl)-10,20-di-p-tolyIporphyrin.

In a dinitrogen-filled 200-mL Schlenk flask, 2 mL of BBr₃ (5 g, 20 mmol) and 5 mL of freshly distilled CH₂Cl₂ were placed, and the entire flask was placed in a dry ice bath. In a dinitrogen-filled 100-mL Schlenk flask, 5,15-bis(2,3-dimethoxyphenyl)-10,20-di-p-tolyIporphyrin (230 mg, 0.278 mmol) and 3 mL of freshly distilled CH₂Cl₂ were placed. The porphyrin / CH₂Cl₂ mixture was transferred to the BBr₃ / CH₂Cl₂ mixture using a cannula. The resulting green solution was stirred in the dry ice bath for 2 h. The dry ice was left to sublime while the flask was still in the bath; the flask was then
removed from the bath and the stirring was continued overnight. The flask was then placed in an ice bath, and 30 mL of degassed H₂O was *slowly* added using a syringe. Degassed 0.1-N NaOH (10 mL) was added to ensure that the boron-oxygen bonds were cleaved completely. Degassed Et₃N (40 mL) was added to neutralize the acid. Degassed heptane (100 mL) was added then to precipitate the product. The precipitate plugged the sintered-glass funnel. The mixture was placed in a 500 mL separatory funnel and was extracted with 3 × 50 mL of CH₂Cl₂. The organic layer was washed with 3 × 10 mL of pH 7 buffer (Na₂HPO₄ / KH₂PO₃), then with 3 × 10 mL of H₂O. The solvent was removed, and the remaining purple-brown solid was chromatographed through a very short (4 × 2 cm) column of silica gel using 1:1 CH₂Cl₂ / MeOH. The solvent was removed, and the purple amorphous solid was recovered. Yield: 173 mg (81%). The product is moderately soluble in CH₂Cl₂, and very soluble in DMF. ¹H-NMR (αα-αβ mixture in CD₂Cl₂): δ 8.87 (m, 8H, pyrrole β-H); δ 8.09 (m, 4H, MePh o-H); δ 7.58 (m, 8H, MePh m-H and o,m-H); δ 7.21 (m, 6H, (HO)$_2$Ph o,m,p-H); δ 2.71 (s, 6H, m-CH₃), δ -2.96 (s (very broad), 2H, pyrrole NH).

6.2.10 5,15-Bis[2,3-][(HYDROTRIS(3,5-DIMETHYPYRAZOLYL)BORATO)OXOMOLYBDENIO]DIOXY]PHENYL-10,20-DI-p-TOLYLPOPHRIN.

In a dinitrogen-filled 250-mL Schlenk flask, 173 mg (0.245 mmol) of bis(2,3-dihydroxyphenyl)di-p-tolylporphyrin and 494 mg (1.053 mmol) of hydrotris(3,5-dimethylpyrazol-1-yl)borato-1,2-dioxyethyloxomolybdenum(V) were placed together with a stirring magnet. Freshly distilled and degassed toluene (100 mL) was introduced
into the flask with a cannula. The flask was placed in an oil bath at room temperature, the magnetic stirrer was turned on, and the bath was slowly warmed to 63°C. The reaction was monitored by TLC on silica-gel plates, using 10:1 CH$_2$Cl$_2$ / EtOAc as the eluant. The reactant appeared at $R_f$=0.0-0.3, and was strongly fluorescent; whereas the product appeared at $R_f$=0.7, and showed no fluorescence. The stirring was continued for 3 days, after which no more change was apparent on the TLC plates. The flask was removed from the oil bath and cooled to room temperature, and then placed on a rotary evaporator to remove the solvent. The brown solid was dissolved in 20 mL of toluene, and was chromatographed on a short column (4 × 2 cm) of silica gel, changing the eluant from toluene to CH$_2$Cl$_2$ and then to 10:1 CH$_2$Cl$_2$ / EtOAc. The solvent was removed from the fraction containing the products. The resulting purple-brown solid was dissolved in toluene, and was chromatographed on a 4 × 20 cm column of silica gel, changing the eluant from toluene to CH$_2$Cl$_2$ and then to 10:1 CH$_2$Cl$_2$ / EtOAc. The first band was recovered with toluene. The second band streaked considerably: the initial part of the band was recovered with 100:3 toluene / CH$_2$Cl$_2$, and the final part was recovered with CH$_2$Cl$_2$. The third band was recovered with 10:1 CH$_2$Cl$_2$ / EtOAc. The solids from the first and the third band were too small in quantity for identification.

The solvent was removed from the second band. The purple solid was dissolved in minimal quantity of CH$_2$Cl$_2$ in a test tube, and then MeOH was added to promote precipitation. The test tube was centrifuged and the purple mother liquor was decanted. The powdery tan-purple solid was washed twice with MeOH. The solvent was removed
from the mother liquor, and the solid was recrystallized twice with CH$_2$Cl$_2$ / MeOH. The solids were combined in a vial. Yield: 45 mg (12%). Mass spectrum (FAB, positive ion detection): Calculated (M+1, weighted average): 1522.01. Found (weighted average): 1522.03.

6.3 Results

6.3.1 Dipyrromethane and Tripyrrane.

The procedure of Lee and Lindsey$^{102}$ was applied to the synthesis of 2,3-dimethoxyphenyldipyrromethane: 2,3-dimethoxybenzaldehyde was dissolved in pyrrole and reacted in the presence of trifluoroacetic acid. (Figure 6.2) The synthesis proceeded cleanly: when the aldehyde / pyrrole mole ratio of 1:70 was used, the dipyrromethane was obtained in 54% yield. The $^1$H-NMR spectrum of the product shows the peak pattern and the integration values consistent with the expected product (Figure 6.3) The peaks can be assigned by the integration values and the J-coupling patterns. The $^{13}$C-NMR spectrum shows the number of peaks consistent with the types of carbons that are present in the molecule. (The total of 13 types — 6 phenyl, 4 pyrrole, 1 meso, and 2 methoxy.) The peaks in the carbon spectrum can be assigned by using the HETCOR experiment (Figure 6.3). The 2-D spectrum unambiguously identifies the two different types of aromatic carbons — phenyl and pyrrole. A significant side product is 5,10-bis(2,3-dimethoxyphenyl)tripyrane, which is isolated by chromatography. The identity of this product was confirmed by $^1$H and $^{13}$C 1-D NMR, DQF-COSY, NOESY, and HETCOR (Figures 6.4 and 6.5) The yield percentage of dipyrromethane is increased with the proportion of
pyrrole to aldehyde that is used in the reaction. When the aldehyde / pyrrole ratio was decreased to 1:140, the yield percentage increased to 63%. (The pyrrole used in the reaction can be recycled by washing with water and distilling fractionally.)

The dipyrromethane, when recovered by drying the fraction from the chromatography column, is slightly brown, because it undergoes oxidation easily in solution. The oxidation product can be removed by crystallization. The dipyrromethane is dissolved in MeOH, and then a large amount of water (greater than 10 times the volume of MeOH) is added. Crystallization is complete after half a day. One must wait for the oil to crystallize completely before attempting to filter, or else the remaining oil will block the filter paper. The dipyrromethane oxidizes easily at room temperature, but is indefinitely stable at -5°C.

6.3.2 Lindsey “2+2” Synthesis.

In the Lindsey “2+2” synthesis, the condensation of the dipyrromethane with p-tolualdehyde is performed in CH₂Cl₂ or CHCl₃, with either BF₃ or trifluoroacetic acid as the catalyst. In our attempt at the Lindsey synthesis, the use of trifluoroacetic acid instead of BF₃ was crucial. In an earlier attempt at the porphyrin synthesis, BF₃·OEt₂ was used. No porphyrin was formed, and the NMR spectrum showed that an intractable mixture of side products was present. Since BF₃ binds strongly to oxygen and becomes unavailable for catalysis, it does not serve as a good catalyst in the presence of an alkoxy group.¹¹³

Several chromatographic steps were needed to separate the products. The
porphyrins first had to be separated from the other products (mostly dipyrrromethenes). First, some $AB_3$ ("monosubstituted") porphyrin was recovered. Then a small amount of $A_2B_2$ ("disubstituted") porphyrins was separated. The spectrum shows two methoxy peaks ($\delta 4.1$ and $\delta 3.1$) and one methyl peak ($\delta 2.7$), whose integration ratio is 1:1:1, which is consistent with the desired disubstituted porphyrin. The remaining porphyrins were separated with another chromatography run. The NMR spectrum of these porphyrins had much larger integration value for the methoxy peaks: the sample contained a large quantity of $A_3B$ ("trisubstituted") porphyrin. (Figure 6.6)

In the later attempt, in which the reaction time was cut from 60 to 20 minutes, no monosubstituted porphyrin was observable. However, a considerable quantity of the trisubstituted porphyrin was present, along with the desired disubstituted porphyrins. Although the disubstituted species was recovered in a tolerable yield, its regioisomeric purity is highly questionable.

6.3.3 Low-temperature Adler and Bonar-Law syntheses.

In two of the synthetic procedures carried out here — the modified Adler\textsuperscript{107} and the Bonar-Law\textsuperscript{98} syntheses (Figure 6.7) — the acidity was kept low in order to minimize the rearrangement of the desired porphyrinogen. In this respect, both procedures have been successful: no mono- or trisubstituted porphyrin was observed in the product. The yield was acceptable for the low-temperature Adler synthesis (8.8%), and it is possible that the reaction could be optimized further: in our attempt at this synthetic procedure, the progress was not monitored, and it is possible that the reaction did not go to completion.
In the Bonar-Law synthesis, the yield was excellent (25%), and no further optimization is necessary. In this study, the "2+2" synthesis of porphyrins using an aqueous anionic detergent has been shown to be not only feasible, but also effective.

The porphyrins synthesized using the low-temperature Adler method are metallated with zinc, because the procedure calls for the addition of zinc acetate in the reaction mixture. In contrast, the porphyrins synthesized using the Bonar-Law method are not metallated. The free-base porphyrins can be metallated with zinc easily by mixing it with zinc acetate in DMF at room temperature for 24 hours. The product is recovered by adding water slowly into the mixture and then isolating the resulting solid by filtration.

6.3.4 ATROPISOMERISM.

The two atropisomers of the free-base porphyrins (Figure 6.8) are easily separable using a silica gel column. Also, the solubilities of the atropisomers are significantly different from each other. The αβ isomer is very sparingly soluble in CH₂Cl₂ and CHCl₃, and moderately soluble in toluene and DMF; the αα isomer is very soluble in all four solvents. When recovered from a column and dried, the αβ isomer forms much more compact solid than the αα, giving an appearance that the former is formed in lower yield than the latter. When the isomers are weighed however, it is found out that the yield is the same for both. The atropisomers of the Zn-complex are also easily separable. Their solvation properties parallel those of the free-base porphyrins. Both atropisomers of the Zn-complex are soluble in THF.
The NMR spectra (Figure 6.9) of the free-base porphyrins indicate that the products are indeed disubstituted species: the expected 1:1:1 ratio of the $m$-methoxy / $o$-methoxy / $p$-methyl signals is exactly what is found. The tolyl $o$-H signals are different in the spectra of the two atropisomers. In the spectrum of the $\alpha\beta$ isomer, the $o$-H signal is a single peak at $\delta 8.07$; this is a reflection of the magnetic equivalence of the $o$-protons. In the spectrum of the $\alpha\alpha$ isomer however, the $o$-phenyl signal is split into two peaks at $\delta 8.12$ and $\delta 8.01$. This isomer has two types of $o$-protons: the ones on the same side as the methoxy groups, and the ones on the opposite side. Also, the position of the $o$-methoxy signal is slightly different in the spectra of the two isomers ($\delta 3.16$ in $\alpha\beta$, $\delta 3.13$ in $\alpha\alpha$). In the other respects, the spectra of the two atropisomers are identical.

6.3.5 **Solvent-dependent shift.**

The spectra of the trans-disubstituted porphyrin taken in toluene-$d_8$ (Figures 6.10 and 6.11) are considerably different from those taken in CDCl$_3$, CD$_2$Cl$_2$, or DMF-$d_6$. The positions of the ortho- and meta-methoxy and para-methyl signals are shifted: the $m$-OMe signal shifts from $\delta 4.09$ in CDCl$_3$ to $\delta 3.57$ in toluene-$d_8$; $o$-OMe, from $\delta 3.13$ to $\delta 3.26$; and $p$-Me, from $\delta 2.67$ to $\delta 2.40$. (These numbers are for the $\alpha\alpha$ atropisomer, but those for the $\alpha\beta$ isomer are almost equal.) The peak from pyrrole NH is shifted even further, from $\delta 2.76$ to $\delta 2.14$. Also, the signals from the $\beta$-protons are different: whereas the signal was a singlet ($\delta 8.81$) in the spectrum taken in CDCl$_3$, it was two doublets ($\delta 8.98$ and $\delta 8.91$, $J = 4$ Hz for both signals) in the spectrum taken in toluene-$d_8$.

Formation of a porphyrin-toluene $\pi$-complex is suspected. Solvent-dependent shift is
also observed in the spectra of the Zn(II)-metallated species (Figure 6.12), taken in DMF-
$d$- and CDCl$_3$. Here, coordination of the oxygen atom in DMF to zinc is suspected.

The solvent-dependent shift was useful in establishing even more firmly the
identity of the product. While the pyrrole $\beta$-signals were unresolved in the spectrum
taken in CDCl$_3$, they were cleanly resolved in the one taken in toluene-$d_6$. The two
doublets, whose coupling constants are equal, confirmed that the product was $trans$ and
not $cis$.

6.3.6 Demethylation.

The demethylation of the disubstituted porphyrin (both atropisomers) with BBr$_3$
(Figure 6.1) was fraught with difficulties. A precipitate formed after the reaction plugged
the filter paper, and therefore the solid had to be recovered by extraction. A significant
quantity of brown solid that was insoluble in either water or CH$_2$Cl$_2$ made the extraction
difficult. (Later, after the demethylated porphyrin had been used for the next synthetic
step, this solid was tested for solubility: it was found to be soluble in acetone.) The
product recovered after the extraction was found by NMR to be impure, and therefore
purification by chromatography was necessary. After the chromatography, the silica gel
column had become intensely colored, indicating that a significant quantity of the desired
product was lost. (The demethylated product was found to have a strong affinity to SiO$_2$:
all the glassware that was used for handling the demethylated porphyrin became heavily
colored, and the color persisted even after washing.) The spectrum of the product after
the chromatography indicates that the purification was successful (Figure 6.13). The
yield was calculated to be about 80%, but this number is suspect because of the impurities that could still have been present.

6.3.7 Molybdation.

The procedure of Basu et al.\textsuperscript{60,108} was used for the reaction of the bis(catecholy)porphyrin with HB(Me\textsubscript{2}Pyz\textsubscript{3}OMo\textsuperscript{V}(OCH\textsubscript{2}CH\textsubscript{2}O) (Figure 6.1). The reaction temperature (63°C) was set slightly lower than that of Basu et al. (>70°C) in order to minimize possible side reactions. The reaction took 3 days to reach completion, considerably longer than the typical time used by Basu et al. (6 hours). The TLC analysis showed that the reaction was not clean: besides the spots for the reactant and the product, the ones for side products were observed at R\textsubscript{T}=0.4-0.6. (These spots are not a result of the acidolysis of the product on silica gel. After the product was purified by column chromatography, it was analyzed by TLC: the plate did not show any extraneous spot.) The yield of the product was 12%: the low yield could have been the result of the aforementioned side reaction, or the low purity of the bis(catecholy)porphyrin. Only one major band was identified during the column chromatography of the product, indicating that only one of the possible two atropisomers had formed. The major product is very likely the αβ atropisomer (see §6.4.8). Fast-atom bombardment mass spectroscopy (FAB-MS) confirmed the identity of the product. (Figure 6.14) The EPR spectrum (Figure 6.15) was found to be very similar to that of HB(Me\textsubscript{2}Pyz\textsubscript{3}Mo\textsuperscript{V}(o-cathecolate).\textsuperscript{114}
6.4 Discussion

6.4.1 Synthesis of 5,10-bis(2,3-dimethoxyphenyl)dipyromethane and the simultaneous formation of tripyrrane.

The synthetic procedure for dipyrrromethane by Lee and Lindsey\textsuperscript{102} can be used with various substituted arenecarbaldehydes. Our gram-scale synthesis proceeded with an acceptable yield and an ease of separating the main and the side products. Dolphin \textit{et al.}\textsuperscript{79} and Lindsey \textit{et al.}\textsuperscript{80} have reported that the chromatographic step can be replaced by sublimation or distillation: we did not try this procedure, but it is a promising one to attempt in future.

Most of the side product consisted of the corresponding tripyrrane.\textsuperscript{79,80} If one wishes to minimize the side product, the reaction condition can be easily manipulated: in the condensation of an arenecarbaldehyde with pyrrole, the percent yield becomes greater as the proportion of the latter is increased. In our future studies however, the optimization of the yield is not necessary, since the side product is potentially useful: a 5,10-diaryltripyrane is a potential starting material for a \textit{cis}-disubstitued tetraarylporphyrin (see Figure 4.4, p. 136). The reactant for providing the complementary fragment, a 2,5-bis(arylhydroxymethyl)pyrrole, can be synthesized either by (1) reaction of pyrrole Grignard with the arenecarbonyl halide, followed by reduction with NaBH\textsubscript{4},\textsuperscript{115} or (2) reaction of pyrrole with the aryloxathioliun tetrafluoroborate, followed by cleavage with HgO and reduction with NaBH\textsubscript{4}.\textsuperscript{115,116} The synthesis of \textit{cis}-bis(dimethoxyphenyl)ditolylporphyrin must be attempted in the near future.
6.4.2 Fragmentation in the Lindsey condition and the necessity of low acidity.

In our attempt at the “2+2” condensation of dipyrromethane under the Lindsey condition, the stereochemistry of the product could not be controlled because of a considerable rearrangement of the porphyrinogen.

The side products consisted of the trisubstituted porphyrin, often at the exclusion of the monosubstituted species. This selectivity in rearrangement offers a clue to the mechanism. (Figure 6.16) The \textit{trans}-bis(dimethoxyphenyl)ditolylporphyrinogen disintegrates to either of the following fragment pairs: (1) tolylpyrrilium cation, and a tripyrrrole fragment bearing two dimethoxyphenyls and one tolyl; or (2) dimethoxyphenylpyrrilium cation, and a tripyrrrole fragment bearing one dimethoxyphenyl and two tolyls. In either case, the tripyrrrole fragment combines with dimethoxyphenyl dipyrromethane, then eliminates a pyrrrole to cyclize into a porphyrinogen. If the porphyrinogen fragmentation occurs as in (2), the resulting porphyrinogen is \textit{trans}-bis(dimethoxyphenyl)ditolylporphyrinogen — the starting disubstituted species. In (1) however, the resulting porphyrinogen is tris(dimethoxyphenyl)tolylporphyrinogen — the trisubstituted species that gives rise to the trisubstituted porphyrin.

In the efforts by other workers\textsuperscript{105,107} in which condensation of a dipyrromethane containing \textit{ortho-} or \textit{para-}methoxy group was attempted, rearrangement was also observed. The methoxy group is an electron-donating group, and it is expected to stabilize a positive charge in the benzyl position. The stabilization of the pyrrilium cation
(either the mono- or tripyrrolic species) facilitates the fragmentation of porphyrinogens. Lee and Lindsey\textsuperscript{102} have avoided the problem by protecting the oxido groups not with methyls, but pentafluorobenzyls, whose electron-withdrawing capability counteract the electron-donating capability of the oxy lone pairs.

The fragmentation can be minimized by slowing down the reaction. Inherently, the formation of a porphyrinogen is faster than the fragmentation — if not, then the porphyrinogen would have never been observed in the first place. When the formation is slowed down, the fragmentation is slowed down even more. The effectiveness of this strategy was demonstrated by Setsune \textit{et al.}\textsuperscript{107} who performed the “2+2” condensation of (2-methoxynaphth-1-yl)dipyrromethane and 2-nitrobenzaldehyde in propionic acid at room temperature over 15 hr. Also, Littler \textit{et al.}\textsuperscript{80} found that slowing down the condensation of \textit{p}-tolylidipyrromethane and benzaldehyde minimizes scrambling, albeit with decrease in the percent yield.

When we attempted the condensation of 2,3-dimethoxyphenyldipyrromethane and \textit{p}-tolualdehyde in propionic acid at room temperature, no mono- or trisubstituted porphyrins were found. The yield was 8%, an acceptable but unsatisfactory value. We had much better success at the attempt using the Bonar-Law conditions\textsuperscript{105} under low acidity (6 mM); this will be discussed in detail below.

6.4.3 \textsc{Porphyrin synthesis in aqueous anionic surfactant (Bonar-Law synthesis)}.

When Lindsey and co-workers first outlined the synthetic method that currently
bears his name,\textsuperscript{93} they observed that the yield decreased considerably if the solvent was not dried carefully: even an increase in H$_2$O concentration by no more than 13 mM caused a decrease in yield by one-third. The observation was reasonable, considering that the formation of porphyrinogen necessitates a simultaneous formation of water. Therefore, when Bonar-Law\textsuperscript{98} reported the synthetic method for tetraarylporphyrins in an aqueous medium, it was largely ignored. (As of 14 July 1999, the Science Citation Index\textsuperscript{117} indicates that Bonar-Law’s article has been cited only 4 times.)

In Bonar-Law synthesis, pyrrole (10 mM) and arenecarbaldehyde (10 mM) are mixed in aqueous sodium dodecyl sulfate (0.5 M), the mixture is acidified with HCl (concentration in the mixture: 120 mM) to start the reaction, and the resulting porphyrinogen is oxidized with TCQ or DDQ. The surfactant is then precipitated and the porphyrin extracted from the precipitate. Bonar-Law speculated that the micelles acted as templates for the formation of porphyrinogen.

We speculated that this method would be mild enough to be applied to the “2+2” synthesis involving dipyrromethane, and flexible enough to be adapted for a dilute-acid condition. Also, we speculated that the purported template effect would keep the porphyrinogen from fragmenting. We reacted 2,3-dimethoxyphenyldipyrromethane and $p$-tolualdehyde in aqueous sodium dodecyl sulfate, using the acid concentration (6 mM) that was 1/20 of the one used typically in Bonar-Law’s studies. The yield was 25%, and no scrambling was observed.
6.4.4 Micelles as the partitioning device between reactants and intermediates.

How much of the success of this method is due to the low acidity and how much is due to the micelles? Some insights were gained in the large-scale (200 mg final product) reaction run. In this run, the acid concentration was increased to 40 mM, and yet no scrambling was observed. This indicates that the micelles do play a role in preventing the fragmentation of the porphyrinogen. The template effect, however, is not necessary to explain the anti-fragmentation activity. Instead, the activity can be explained by the partition of the acid, the reactants, the intermediates, and the product within a micelle.

Experimental evidences in literature for such partitioning by micelles is abundant. Fendler and Fendler,\textsuperscript{118} in their monograph on micellar catalysis, have compiled the cases in which the materials dissolved in micelles are distributed in the manner consistent with their polarity.\textsuperscript{119} Also, Evans and Bolton\textsuperscript{120,121} have performed a series of experiments in which organic radicals of varying polarities were dissolved in aqueous sodium dodecyl sulfate, and the NMR spectra of the solutions were compared for the broadening of the surfactant signals by the radicals. The high-polarity radicals broadened most prominently the signals from the protons closest to the head group; the medium-polarity radicals, the signals from the protons second closest; and the low-polarity radicals, the signals from the rest of the hydrocarbon chain. These experiments indicate the existence of a gradient in polarity inside a micelle.

The reactants and the products in our porphyrinogen synthesis have different
polarities. Bonar-Law\textsuperscript{98} demonstrated by capillary micelle chromatography that the polarity of the reactants (the aldehyde and the pyrrole) was significantly higher than that of the product (the porphyrinogen). Based on this evidence, and on the previous experiments on micelles, the following speculation can be made. (Figure 6.17) Protons, the most polar of the species involved in the reaction, remain at the surface. The aldehydes and the dipyrrromethanes reside at the level below the surface. As the reaction proceeds, the intermediates become less polar and move toward the core. The porphyrinogens, the least polar species, settle close to the core. The protons and the porphyrinogens become separated thus within a micelle, and the acidolysis is prevented.

6.4.5 Micelles as a Stabilizer of Reactive Intermediates — Phase Transfer Catalysis?

Charged micelles are known to stabilize species of opposite charge. Experiments on various pH indicators in aqueous surfactants have indicated that the equilibrium shifts toward the negatively charged species in the cationic micelles and the positively charged species in the anionic micelles.\textsuperscript{122} Therefore, it is safe to assume that anionic micelles can activate the aldehyde by aiding the addition of proton to the carbonyl oxygen, making the carbonyl carbon more susceptible to the nucleophilic attack by the dipyrrromethane.

During the small-scale synthesis, the order of the addition into the surfactant solution was: the aldehyde, the dipyrrromethane, and then the acid. The dipyrrromethane was in the form of large lumps that were difficult to dissolve: a significant quantity of the dipyrrromethane had remained undissolved at the time when HCl was added. At that
moment, the solute in the micelles presumably consisted mostly of the aldehyde. Immediately before the acid was added, it was noted that the mixture had turned brown, indicating that the reaction had started without HCl. The micelles can apparently activate the aldehyde significantly even in the absence of a strong acid.

During the large-scale synthesis, the order of addition of the reactants was: the dipyrrromethane, the aldehyde, and then concentrated HCl. The dipyrrromethane used in this reaction had been previously recrystallized, and it was in form of microcrystals that dissolved rapidly in the surfactant solution. The pH of the mixture was measured shortly after the addition of first two batches of HCl into the surfactant-reactant mixture: the pH was found to be about 7, while the nominal HCl concentration was calculated to be about 10 mM. At that point, reaction had not started: it did not start until the nominal HCl concentration was increased to 40 mM. The unexpectedly large pH and the lack of reactivity can be explained by the protonation of both reactants. The aldehyde is not the only species that can become protonated: the dipyrrromethane can also become protonated, and it does so at the expense of the activated aldehyde when it is present in the micelles before the other reactant. The resulting dipyrrromethane-proton σ-complex is deactivated because the added positive charge renders it less nucleophilic. In the presence of an arencarbaldehyde and a dipyrrromethane, protons cause the formation of both activated aldehyde and deactivated dipyrrromethane. A large quantity of acid becomes necessary to counter the deactivation.

That the reaction begins more easily when the aldehyde is added first is
reminiscent of phase transfer catalysis. In phase transfer catalysis, an anionic salt of one of the reactants is mixed with water and a salt with lipophilic cation such as tetraalkylammonium halide. The anionic nucleophile / lipophilic cation / water mixture is then placed into an organic solvent containing the other reactant. The lipophilic cation enters into the organic phase, taking the anionic nucleophile with it to maintain the overall charge neutrality. The activated nucleophile reacts rapidly with the other reactant. Our procedure parallels that of phase transfer catalysis, except for the substitution of the cationic surfactant for an anionic counterpart, and the absence of an organic solvent. In our procedure, the cationic electrophile / lipophilic anion / water mixture is used; and during the reaction, the electrophile and the anion are transported toward the dipyrrromethane.

In general, phase transfer catalysis is considered to be different from micellar catalysis. Whereas the reaction occurs inside the micelles in the latter, the reaction occurs in the organic phase in the former. No formation of micelles is necessary in phase transfer catalysis, and therefore poor surfactants often turn out to be very good phase transfer catalysts (e.g. tetraphenylammonium salts). When a good surfactant is used as a phase transfer catalyst however, the distinction between these two mechanisms becomes blurred.

6.4.6 Precipitation of the Surfactant from the Reaction Mixture.

In Bonar-Law’s procedure, the surfactant is precipitated from the reaction mixture, and then the product is extracted from the precipitate. The procedure calls for
the following reagents as the precipitants: (1) strong base (KOH); (2) organic solvent (ethyl acetate, methylene chloride, or toluene); and (3) potassium salt (KCl). *None of these three must be omitted.* When the base or the organic solvent is omitted, no precipitate is formed. When a sodium salt is substituted for the potassium salt, the resulting precipitate is so fine that it goes through a base-resistant filter paper.

Precipitation of dodecyl sulfate by potassium cation has been used in the recovery of nucleic acids \(^{125}\) and proteins \(^{126,127}\) from aqueous solutions. At room temperature, the solubility of potassium dodecyl sulfate is lower than its critical micelle concentration (defined as the minimum surfactant concentration at which the formation of micelles is possible). \(^{128}\) Therefore, the monomers of potassium dodecyl sulfate precipitates out of the solution without forming micelles. The role of the buffer, the strong base, and the organic solvent in the precipitation of dodecyl sulfate is not clear.

The extraction was performed by passing an organic solvent through the paste-like precipitate. Increasing the quantity of the precipitate slows down the process. One could conceivably bypass this bottleneck by performing the extraction by mixing the precipitate and the solvent in a large centrifuge tube, and then separating the precipitate by using a large centrifuge. If such a device is unavailable, scaling up the Bonar-Law synthesis to gram-scale would be nearly impossible.

### 6.4.7 Demethylation.

Two methods exist for demethylation of alkyl phenyl ether: treatment with BBr\(_3\) at room temperature, \(^{129}\) and treatment with pyridyl chloride at the melt temperature
The former method was used because the latter one was deemed too harsh in the presence of catecholyl groups. Boron tribromide is known to demethylate only one of the two methoxy groups in a meta- or para-dimethoxyaryl, however, its demethylating activity is complete when the two methoxy groups are ortho. The BBr₃ method has been used successfully by Basu et al. in their demethylation of dimethoxyphenyltritolylporphyrin.

After the demethylation, the porphyrin can be separated from the reaction mixture by either precipitation or extraction. The precipitation can be performed easily under inert atmosphere; however, a significant amount of the product could become trapped in the filter paper and thus become lost. The extraction usually is performed in air and therefore is not a good procedure for an air-sensitive compound, but virtually all of the product can be recovered. (Extraction was attempted several times on the Schlenk line: it was found very difficult to remove a layer completely using a cannula, and also the cannula clogged up very easily. No attempt was made to perform an extraction in a dry box, since the mixture to be extracted contained CH₂Cl₂, a substance that poisons the deoxygenating catalyst irreversibly.) No matter how the product is recovered from the reaction mixture, it usually must purified by chromatography in air: this offsets the advantage of precipitation. We opted for extraction: this is the method that Uyeda and Therien recommended upon checking the procedure of Basu et al. for Inorganic Synthesis.

The hydrolyzed reaction mixture was made basic with triethylamine before the
recovery of the product by extraction. In retrospect, the treatment with base could have been delayed until a later part in the process, in order to minimize the oxidation of the catecholyl group that is accelerated by the deprotonation of the hydroxyls. The product could have been extracted while still fully protonated, and then treated with base under inert atmosphere.

The product recovered after the extraction was shown to be impure by NMR spectroscopy. Judging from overlapping broad peaks at δ8.5-9.0, most of the impurities were porphyrins that were not the desired product. There are several possibilities for the identity of these impurities: salts of the catecholate anion, porphyrins with benzoquinone groups produced by oxidation, N-alkylated species produced by the reaction with CH₃Br, or species with boron still attached to the catecholate oxygen. The product was chromatographed using a short column of silica gel. The NMR spectrum of this purified product show much simpler pattern at δ8.5-9.0. The integration values at δ7.0-8.0 correspond to four extra protons besides the expected phenyl protons: this is consistent with all of the four deprotected oxido groups being protonated. The spectrum also shows a very broad peak at δ-2.8, whose integration value corresponds very roughly to two protons.

Purifying the product with silica gel chromatography is an inefficient process because of the strong affinity of the ortho-dihydroxyl groups to the matrix. In the future, a matrix that is not Lewis-acidic must be considered for column chromatography. A size-exclusion matrix that can withstand organic solvents, such as lipophilic Sephadex, is a
possibility. If one insists on using silica gel as the matrix, then it is recommended that
the column be acidified in order to protonate the oxygen moieties on the porphyrin
completely and thus minimize the adsorption. Recrystallization is not expected to give a
good result because of the presence of two atropisomers: one of the isomers could
crystallize preferentially, leaving the other isomer in the mother liquor and thereby
caus[ing loss of the product.

The yield of the demethylation was calculated to be 80%. This number, however,
is suspect: a significant quantity of impurities could have been present because of the
necessity of a short column for purification. The presence of impurities could account for
the relatively low yield in the next step.

6.4.8 Molybdation of bis(catecholyl)porphyrin.

Our procedure for molybdation was adapted from that of Basu et al.\textsuperscript{108}, which in
turn was adapted from the procedure of Cleland et al.\textsuperscript{114} for the reaction of HB(Me\textsubscript{2}Pyz)\textsubscript{3}O
OMo\textsuperscript{V}(OCH\textsubscript{2}CH\textsubscript{2}O) with catechol. The plates from the TLC analyses performed on the
reaction mixture showed extra spots besides that for the reactants and the product. This
indicates that side reactions occurred during the synthesis, most likely fragmentation of
the tris(pyrazolyl)borate species. The yield could probably be improved from 12% if a
method is found to suppress the side reaction, and perhaps also if a better method for
purifying the bis(catecholyl)porphyrin can be found. A high yield is essential if an
extensive study of the centrally coordinated Fe-species is to be performed.

Only one main product was observed: this is almost certainly the $\alpha\beta$ atropisomer.
The formation of the αα atropisomer is hindered because of the steric factor encountered by two tris(pyrazolyl)borate groups at close distance. Another type of stereoisomerism is possible with the bis-molybdated porphyrin: the presence of two (TPB)OMo groups gives rise to two diastereomers: one with both (TPB)OMo groups having the same chirality (Λ-Λ or Δ-Δ) and the other with the two groups having different chirality (meso). Because the chiral centers are surrounded by very bulky groups, the crystal packing of the two diastereomers are expected to be virtually the same, and therefore the major product is expected to contain both diastereomers in equal proportion.

The main product tailed considerably during the column chromatography with toluene / CH₂Cl₂ as the eluant: the band was very diffuse until the proportion of CH₂Cl₂ was increased to more than half of the eluant. This behavior during the elution is consistent with the low solubility of the complex in toluene and the high solubility in CH₂Cl₂. The tail end of the band overlapped with another band containing a blue impurity: this impurity was easily removed with recrystallization from CH₂Cl₂ / MeOH. The recrystallized product was powdery and compact, but it did have a regular structure when seen through the test tube during the purification. A study is needed to see if a crystal suitable for a structural analysis could be grown.

Fast-atom bombardment (FAB) mass spectrometry (Figure 6.14) has confirmed that the main product was the desired bis-molybdated porphyrin. The spectrum however has also indicated the presence of a bis-molybdated species whose molecular weight is higher by either 15 or 16 than the desired product. Assuming that this side product
contained one extra methyl (m=15), then one possibility exists for the identity of this heavier side product: an incompletely demethylated porphyrin which is monomethylated at one of the phenyl oxygens. This possibility however is not supported by the NMR spectrum in Figure 6.13. Assuming that the side product contained one oxygen (m=16), one could point at two possible sites for the oxygenation: (1) a Mo-catecholate O bond, into which an oxygen atom could insert and form a peroxide; (2) one of the borons, which can release the hydride and coordinate a hydroxide (in the manner analogous to BH₄⁻ converting into B(OH)₃ in the presence of water). Neither would have been detected by NMR of the bis(catecholyl)porphyrin, since the oxygenation would have occurred during or after the molybdation. (Another possible site for oxygenation exists. One of the molybdenums can increase its oxidation state to +6, and coordinate one extra oxo group.¹³⁶ For this to happen however, one of the catecholate oxygen must detach to make space for the new oxo group. The resulting oxido group is basic enough to add a proton. The net increase in mass therefore is 16+1=17, greater than what our data would allow.)

An extraneous "M+15" or "M+16" peak (the exact increase in mass is difficult to determine, because of the presence of many peaks from the isotopes of molybdenum) in mass spectra of molybdenum compounds are prevalent, especially in the bis-molybdated species.¹³⁷ In the studies of these species, the most common of the possible contaminants is not methyl but oxygen. We conclude that the extraneous peak in the mass spectrum of our bis-molybdated porphyrin was caused by oxygenation, not methylation.

The EPR spectrum of the complex (Figure 6.15) shows a nearly axial pattern, with
the peaks corresponding to \( g_1 \) and \( g_2 \) nearly overlapping and the one corresponding to \( g_3 \) (\( g_3 \) appearing at higher frequency than the other two. This spectrum is typical of a 6-coordinate \( \text{Mo}^{\text{V}}\text{O}(\text{TPB}) \) complex whose remaining two ligands are coordinated by their oxygens.\(^{114,134,135}\) An ELDOR (Electron-electron Double Resonance) experiment\(^{138}\) was attempted to detect the dipole-dipole interaction between the two unpaired electrons on the molybdenums. (The Mo-Mo distance in the \( \alpha\beta \) atropisomer is about 15.0 Å; in the \( \alpha\alpha \), about 12.7 Å) No signal was found. The lack of an ELDOR signal cannot be attributed to aggregation of the complex, because no spin diffusion was detected. Instead, the experiment indicates that the electron-electron dipolar interaction in a bis-molybdenum complex is very strong, even when the Mo-Mo distance is as great as 15 Å.

6.5 Future Studies

The success in the synthesis of the \textit{trans}-bis-molybdated porphyrin has opened up many paths.

6.5.1 IMMEDIATE FUTURE.

6.5.1.1 Optimization of the yield of the \textit{bis}-molybdated porphyrin.

One should purify the \textit{bis}(catecholyl)porphyrin more rigorously. If the yield does not increase, one should increase the temperature to make sure that the reaction goes to completion. One should also see if the product is decomposing due to the acidity of the chromatographic matrix.

6.5.1.2 Determination of crystal structures.

Crystal structures must be determined for both the molybdated and the non-
molybdated bis(catecholyl)porphyrin. The non-molybdated porphyrin should be centrally metallated with zinc: this stiffens the ring and slows down the rotation of the catechol moieties. In the preliminary studies, it was found that toluene / cyclohexane system produces crystals that appear to be the most suitable. As for the molybdated species, a crystallographic study is more crucial. Although the mass spectrum has confirmed the composition of the product, it offers no clue about the stereochemistry. Although the circumstantial evidences indicate that the product is the αβ atropisomer of the mixed diastereomers, definite proof must be provided. Unlike for the non-molybdated species, NMR spectroscopy does not provide the answer because of severe broadening of the peaks. A crystallographic study is the only physical method that would establish the stereochemistry unequivocally (but see the following section).

6.5.1.3 Study of the centrally Fe-coordinated species.

An iron can be inserted into the bis-molybdated porphyrins using the method of Basu et al. The resulting ferriheme should be put through a column to see if a μ-oxo dimer forms: while the αα-atropisomer is expected to form such dimer, the αβ-atropisomer is not, because of the steric interaction between two hemes. The presence of a μ-oxo dimer can be monitored by EPR. (Figure 6.18) The heme should then be reacted with an aromatic amine that is known to coordinate axially to unhindered ferrihemes in mutually perpendicular manner (see the next paragraph for some examples); then, the resulting 6-coordinate complex should be studied by EPR, to see if the resulting spectrum is rhombic instead of the “large $g_{\text{max}}$” type.
The aromatic amines that coordinate in mutually *perpendicular* conformation usually (1) contains a large substituent next to the coordinating nitrogen (*e.g.* 2-methylimidazole), or (2) do so only with a tetraarylporphyrin that has large substituents at the *o*-phenyl positions (*e.g.* tetramesitylporphyrin).\(^\text{86,87}\) Exceptions do exist however. 3,4-Dimethylpyridine\(^\text{86}\) and unsubstituted pyridine,\(^\text{86,139}\) whose substituents at the positions next to the coordinating nitrogen are too small to interact with the heme, do form a 6-coordinate complex with [Fe\(^{III}\)TPP]\(^+\) whose EPR spectrum show large \(g_1\) (~3.4).

A "large \(g_{\text{max}}\)" spectrum is a characteristic of a \((d_{xy})^2(d_{xz},d_{yz})^1\) whose axial ligands are mutually *perpendicular*. In the presence of the two bulky MoO(Me\(_2\)Pyz)\(_2\)BH groups nearby the axial coordination sites, the two aromatic amines would be forced to take a mutually parallel conformation. The resulting spectrum EPR spectrum should be rhombic \((g_1=2.7-2.9, g_2=2.2-2.3, g_3=1.5-1.7)\) even for the complexes of the aforementioned pyridines.

Also, a bis(substituted imidazole) complex should be made and its redox potentials obtained, so that the data could be compared in future with that of the *cis*-bismolybdated porphyrin (§6.5.2.2).

6.5.2 LONG-TERM STUDIES.

6.5.2.1 Further exploration of Bonar-Law's method applied to "2+2" synthesis.

The MacDonald "2+2" synthesis of porphyrin in aqueous surfactant is still novel and its applicability involving various substituted aryl groups is unexplored. One should attempt to make various *trans*-\(A_2B_2\) porphyrins using Bonar-Law's method, using aryl
groups with varied substituents (halogens, -NO₂, esters, ethers, amides) and substitution patterns. One should especially look for potential rearrangement, which can be monitored by mass spectrometry. One could also vary the acid catalyst. For example, scandium triflate, which has been applied successfully in organic syntheses in aqueous micelles, could be used. Also, a high-acidity buffer (pH = 2) such as potassium citrate-hydrochloric acid could be used to counter the increase in pH with the reaction time (see §6.2.7).

6.5.2.2 Synthesis of cis-bis(catecholyl)porphyrin.

The cis-bis-molybdated porphyrin is required for the study of a heme that contains axial ligands oriented perpendicularly to each other. A cis-bis(catecholyl)porphyrin is more difficult to make than the corresponding trans-substituted one, because of the longer synthetic steps required by the lower symmetry. A “3+1” approach is necessary, and this requires a tripyrrolic and a monopyrrolic species, each bearing two phenyl groups. Such tripyrrolic species can be made in the synthetic procedure designed for aryldipyrromethane (§6.2.3), and the synthetic procedure for a monopyrrolic species has been well established. If the “3+1” procedure is not susceptible to the fragmentation / rearrangement that has plagued the Lindsey “2+2” synthesis, then the synthesis of a cis-bis(catecholyl)porphyrin is viable and imperative.

6.5.2.3 Diamagnetic bulky group.

Oxomolybdenum(V) is not only paramagnetic but also has slow electron relaxation time. Consequently, the NMR signals the porphyrin protons close to the
bulky group are severely broadened. In the studies of the mono-molybdated heme, the porphyrin protons distant from the molybdenum center were detectable by NMR and therefore available for detailed studies. In a bis-molybdated heme however, most of the heme protons are expected to be broadened too much for a practical NMR studies. The development of a diamagnetic bulky group is necessary.

One of the possibilities is to replace molybdenum(V) with niobium(V), a $d^0$ species. Oxoniobium(V) is known to form a stable complex with both catecholates and tris(3,5-dimethylpyrazolyl)borate. The synthetic procedure of an oxoniobium(V) complex that contains both of these ligands is not known yet: Dhawan has reported that refluxing $[\text{HB(Me}_2\text{Py)}_3\text{ONb}^V]\text{Cl}_2$ and catechol in THF resulted in an intractable material. One could however attempt a similar reaction, using the ethylene-glycolate or dimethoxide complex instead of the dichloride, and performing the reaction in a nonbasic solvent such as toluene at a temperature well below the boiling point. If the synthesis of $[\text{HB(Me}_2\text{Py)}_3\text{ONb}^V](\text{o-catecholate})$ is successful, one must attempt the synthesis of the mono- and bis-niobiated porphyrins.

6.6 Conclusions

5,15-Bis(2,3-dimethylphenyl)-10,20-di-$p$-tolylporphyrin has been synthesized by reacting 2,3-dimethylphenylidipyrromethane and $p$-tolualdehyde in aqueous sodium dodecyl sulfate acidified with HCl, and then oxidizing the resulting porphyrinogen with DDQ. The yield (25%) was excellent for a porphyrin synthesis, and no rearrangement was observed. The synthesis was scaled up to 200-mg, although at that scale the
extraction of the product from the surfactant proved difficult.

5,15-Bis[2,3-((((hydrotris(3,5-dimethylpyrazolyl)borato)oxomolybdenio)dioxy]-phenyl-10,20-di-p-tolylporphyrin was synthesized from the aforementioned tetrapyrrrole. This porphyrin, when centrally metallated with Fe, is expected to coordinate any pair of axial ligands in mutually perpendicular orientation.
Figure 6.1 Summary of the reactions described in Chapter 6.
Figure 6.2 Scheme for the synthesis of 2,3-dimethoxyphenyldipyromethane. 5,10-(2,3-dimethoxyphenyl)tripyrane is produced as a minor but significant byproduct.
Figure 6.3 $^1$H (left) and $^{13}$C (top) NMR and HETCOR spectrum of 2,3-dimethoxy-phenyldipyrrromethane.
Figure 6.4 Conventional (top) and long-range (bottom) HETCOR of 5,10-bis(2,3-dimethoxyphenyl)tripyrane, together with $^1$H (left) and $^{13}$C (top) NMR spectra.
Figure 6.5 NOESY (left) and DQF-COSY (right) of 5,10-bis(2,3-dimethoxyphenyl)tripyrane.
Figure 6.6 Lindsey "2+2" reaction resulted in a significant amount of the trisubstituted porphyrin.
Figure 6.7 "2+2" synthesis using the low-temperature Adler condition (top) and the Bonar-Law condition (bottom). The amount of the dipyrromethane used was 100 mg for both cases.
Figure 6.8 Atropisomerism in bis(2,3-dimethoxyphenyl)di-p-tolylporphyrin.
Figure 6.9 NMR of trans-bis(2,3-dimethoxyphenyl)di-p-tolylporphyrin (both atropisomers). The spectrum of αβ is noisier because of its poor solubility.
Figure 6.10 Solvent-dependent shifts. Top. NMR spectrum of *trans*-bis(2,3-dimethoxy-methyl)di-*p*-toly1porphyrin (aa atropisomer) in toluene. Bottom. Spectrum in CDCl$_3$. 
Figure 6.11 NMR spectra of the αβ atropisomer. Top. Toluene-\(d_7\). Bottom. CDCl\(_3\).
Figure 6.12 NMR spectra of zinc-metallated trans-disubstituted porphyrin. Top. αα in DMF-d$_7$. Bottom. αβ in CDCl$_3$. Insets. Signals of the pyrrole protons.
Figure 6.13 NMR spectrum of *trans*-bis(2,3-dihydroxymethyl)di-*p*-tolylporphyrin (mixture of both atropisomers) in CD$_2$Cl$_2$. 
Figure 6.14 FAB(+) - MS of the molybdation product. Top. m/z 0-2000. Bottom. Enlarged region. Inset. Theoretical isotopic distribution.
Figure 6.15 EPR spectrum (X band) of the bis-molybdated porphyrin at 77 K.
Figure 6.16 A possible mechanism for the observed scrambling.
Figure 6.17 A proposed scheme for the protection of the product from a further degradation by acid.
Figure 6.18  Schematic plan for the insertion of iron(III) and the subsequent determination of the atropisomerism.
APPENDIX A.

CODES AND TIPS FOR PROCESSING 2-D NMR DATA

As soon as we started programming, we found to our surprise that it wasn't as easy to get programs right as we had thought. Debugging had to be discovered. I can remember the exact instant when I realized that a large part of my life from then on was going to be spent in finding mistakes in my own programs.

— Maurice Wilkes discovers debugging, Cambridge University, 1949

A.1 Introduction

A.1.1 Importance of Computer Programming in NMR

The modern NMR spectroscopic analysis relies heavily on Fourier transforms and digital filtering, which can be accomplished efficiently only by the use of computers. Those who use softwares specializing in NMR analysis often need to write software-specific programs ("macros") in order to manipulate various parameters and make sure that the right calculations are performed in right order. Also, since the raw and processed data are stored as computer files, manipulating the data often requires programming.

The programs used for processing the raw data into the spectra found in Chapter 3 are collected here. It is hoped that the readers find them useful as starting points for their own programs.

A.1.2 Felix 95 — Macros

Felix 95 was used for processing all the data in Chapter 3. This software was chosen over VNMR Version 4.3, which was packaged with the spectrometer, because of the former's flexibility which will be discussed later. Unfortunately, Felix is also much
more user-hostile. Not only that, but also the accompanying manual is diminished in its utility by its haphazardly planned index, which lists nothing but the names of the macros.

In order to take advantage of Felix's flexibility, the users must be capable of writing macros. Because the manual is only marginally useful, the potential macro programers must learn the language by examining sample macros that others have written and (successfully) run. The macros included here is meant to protect the users of Felix from potential psychosis. Many of the macros here are based on those written by Neil E. Jacobsen, the current director of the NMR facility in this Department. Copies of his own macros may be obtained directly from him.

All the Felix macros have the suffix .mac. To run a macro from inside Felix 95, click on Users on the menu bar to pull down a list of items, then click on Run user macro. A dialogue box will open. Type in the file name of the macro and hit return.

The calculations and data manipulations associated with the commands in the Felix macro language, such as linear prediction and baseline correction, are explained thoroughly in an excellent book by Hoch and Stern.145

A.1.3 UNIX OPERATING SYSTEM — SHELL SCRIPTS

Felix 95 is run on the Unix operating system, a command-oriented system that is superficially similar to MS-DOS. The Unix commands themselves can be used as a programming language: a series of commands is put together in a file, and then the name of this file is invoked to run the commands. Such series of commands is called a shell script. For file manipulation, a shell script is usually more convenient than a Felix
macro.

The shell scripts presented in this Appendix do not have a suffix. In order to make a script file functional, an "executable" attribute has to be given to it. For a detailed description, please see the specification for the command *chmod* in the Unix manual.

A.1.4 Compiled commands — C codes

A large shell script executes slowly, because a great amount of time is spent by the computer on reading the commands line-by-line and interpreting them. The same task can be accomplished much faster by using a code written in machine language. Although it is difficult to program directly in machine language, it is easy to write a code written in a readable language and then convert it into an equivalent machine-language code. The program that does such conversion is called a *compiler*.

A compiler for Unix shell scripts do not exist; however, many good compilers are available for popular programming languages such as FORTRAN and Pascal. For this work, the C programming language was used to write the codes to be compiled. This language is used for writing the entire Unix operating system, and is superior to either FORTRAN or Pascal for detailed manipulation of the contents of a file.

The C codes in this Appendix have the suffix .c, and have been compiled by the command *cc* in the Unix system. Please see the manual for this command for the specific usage.
A.2 Converting from VNMR or Aspect to Felix: Unix Shell Script

A.2.1 SIMPLE WAY — VNMRFELIX, AMFELIX

Before a data from a Varian Unity or a Bruker AM spectrometer can be processed by Felix, it must be converted to the corresponding file format. Programs do exist for making such conversions — vnmr2felix and am2felix. In a Unix machine, the usage for these programs are:

vnmr2felix -if: inputfilename -of: outputfilename

and

am2felix -if: inputfilename -of: outputfilename

The conversion strips the data of valuable information such as spectral width, spectrometer frequency, peak reference, phase angles, and title text. These have to be typed in manually at the later stages of the data processing.

A.2.2 CUSTOMIZED WAY

The commands vnmr2felix and am2felix suffers from a shortcoming: because the flags -if: and -of: precedes the file names without a space, the Unix feature for file name completion that is activated by the tab or esc key cannot be used. Also, the name for the output file is never optional, even when it has the same root as that for the input file (e.g. sample.fid for the input file, sample.dat for the output file). These result in more keys typed than necessary, and thus more chances for error.

The following Unix shell scripts — var2felix and bru2felix — were written to overcome these problems. The usages are described within the scripts. Because no flag precedes either the input or the output file names, the file completion feature may be
used. If no name for the output file is specified, the scripts will automatically create one by deleting the suffix from the input file name and then appending .dat.

A.2.2.1 Varian VNMR files — var2felix

```bash
#!/bin/sh
if [ $# = 0 ]
then
    echo Usage: var2felix input [output]
    exit 1
fi
if [ $# = 1 ]
then
    echo $1
    tempoutput="echo $1 |
                awk ' $1 !~ /\....$/ { printf "%s\1\n\1$1 ~ /\....$/ { gsub(/\....$/", ".dat", $1) print $1
                }
        input="-if:$1"
        output="-of:$tempoutput"
    fi
if [ $# = 2 ]
then
    input="-if:$1"
    output="-of:$2"
fi
vn2felix $input $output
input=
output=
tempoutput=
exit 0
```

A.2.2.2 Bruker Aspect files — bru2felix

(This script will also create a file that contains the following data: spectrometer frequency, spectral width, and frequency offset. These data are useful when inputting the parameters for the Felix macros used in the further processing.)

```bash
#!/bin/sh
if [ $# = 0 ]
```
then
    echo Usage: am2felix input [output [parameter]]
    exit 1
fi
if [ $# = 1 ]
then
    input="-if:$1"
    output="-of:$1.dat"
    parameter="$1.prm"
fi
if [ $# = 2 ]
then
    input="-if:$1"
    output="-of:$2"
    parameter="$2.prm"
fi
if [ $# = 3 ]
then
    input="-if:$1"
    output="-of:$2"
    parameter="$3"
fi
am2felix $input $output 2> $parameter
cat $parameter
input=
output=
parameter=
exit 0

A.3 Data Processing

When processing a 2-D NMR data, it is necessary to do the Fourier transform in both the directly-detected and the indirectly-detected dimensions. In addition, it is often necessary to correct the baseline, again in both dimensions. These four tasks may be performed by using only one macro if so desired; however, if these tasks are divided among several macros, experimenting with the parameters becomes much easier. For example, if one finds the second Fourier transform unsatisfactory, the backup data file can be recalled and another second transform with different parameters can be performed on it.

Four Felix macros are presented here, one for each of the tasks mentioned in the
beginning of the last paragraph. These macros may be re-used for different data by
modifying the parameters. It is however recommended that a separate set of macros be
kept for each data, since they would also serve to store experimental parameters such as
spectral width and spectrometer frequency. (Remember that the format conversion strips
these parameters from the data.)

A.3.1 FOURIER TRANSFORM IN THE DIRECT DIMENSION — F2.*MAC

(In the Unix operating system, asterisk (*) is used as the wildcard character. In
this Appendix, asterisk is used to denote that one may replace it with the name of the data
file.)

c1 ;reset any open serial file
cmx ;close any open matrix files
ty Building Matrix ;type message to the text window
bl d ho970322d 2 1024 1024 0 y
 ;build 2D matrix of 1024x1024 reals, overwrite
mat ho970322d write ;open matrix with write permission
rmx 1 299.957 10498.7 3 663.9839 5.32 D1
 ;reference the first dimension
rmx -1 sfreq swidth axtype refpt refsh dummy
 ;set everything
def phase0 81.9 ;define 0th order phase correction
def phase1 -40.8 ;define 1st order phase correction
;def swidth 104 98.7 ;define sweep width
;def sfreq 299.957 ;define spectrometer frequency
def pennum 1 ;white pen
def cycle 1 ;use only one pen
for row 1 320 ;loops over matrix rows 1 to 192
esc out ;assign value of 1 to out if 'esc' button hit
if &out ne 0 finish ;if value of out is not 0, branch to 'finish'
re ho970322d ;read next FID from serial file
bc 0.5 ;baseline correction
zf 1024 ;zero-fill to 1024 pts
lpf 512 20 20 2 ;linear predict first few data points
;lpl 512 16 16 513 1024 1
 ;linear predict to twice the data size
gmh 60 ;gaussian multiplication in Hz
ft ;Fourier transform
ph ;apply phase correction to
;pol 3 ;polynomial baseline correction
red ;discard the imaginary part
sto 0 &row
dr
A.3.2 FOURIER TRANSFORM IN THE INDIRECT DIMENSION — *$F1*. MAC

```
cl ; reset any open serial file to the start
cmx ; close any open matrix files
mat ho970322d write ; open matrix with write permission
rmx 2 299.957 10498.7 3 663.9839 5.32 D2
; reference the second dimension
rmx -2 sfreq swidth axtype refpt refsh dummy
    ; set everything
def phase0 0 ; define 0th order phase
def phasel 0 ; define 1st order phase
for col 1 1024 ; loop over matrix rows 1 to 1024
    esc out ; assign value of 1 to out if 'esc' button hit
    if &out ne 0 finish ; if value of out is not 0, branch to 'finish'
    loa &col 0 ; load next column from matrix
    def datsiz 320 ; truncate everything that is not data
    ; dr
    bc 0.5
    def datatype 1 ; data type is complex
    zf 1024 ; zero-fill
    lpf 160 16 16 1 ; linear predict first few data points
    ; ipl 160 20 20 161 640 1 ; linear predict to twice the data size
    ; zf 1024
    ; dr
    ; def swidth 10498.7 ; define sweep width
    ; def sfreq 299.957 ; define spectrometer frequency
    ; em -5 ; line-narrow 5 Hz
    gmh 120 ; gaussian multiplication in Hz
    ; dr
    ft ; Fourier transform
    ph ; phase correction
    ; pol 3 ; baseline correction
    ; csp ; cubic spline baseline correction
    red
    rev ; reverse
    ; mul -1 ; invert the spectrum
    sto &col 0
    ty col = &col $
    dr
next
```

```
ty F1 transform finished
def pennum 1 ; white pen
def cycle 2 ; two colors in the plot
def posneg 1 ; enable negative contour
finish:
```
A.3.3 Baseline correction in the direct dimension

Baseline curvature is found often in a spectrum that contains one or two particularly strong signals, usually that of the protonated solvent. To correct this distortion, a theoretical baseline is constructed by fitting a curve to the regions of the spectrum which does not contain a peak, and then the curve is subtracted from the spectrum. In our studies, the most severe baseline curvatures are found along the diamagnetic (0-10 ppm) region of our NOESY and ROESY spectra. (Figure A.1). When we started our studies, we corrected the baseline by constructing the theoretical baseline from the no-peak regions in the 1-D spectrum, and then subtracting this from each of the components across the indirect dimension. This method turned out to be deficient: unsightly artefacts were often found close to the diamagnetic region. (Figure A.1)

In order to correct the deficiency, a theoretical baseline had to be constructed separately for each segment. For the segments that contained only sparse signals in the diamagnetic region, the theoretical baseline should be significantly different from the components that contained dense signals in the diamagnetic region. In order to keep the processing time acceptably short, the detection for the baseline points had to be made automatically: this was beyond the capability of Varian’s VNMR software. Felix 95, for all of its shortcomings, is sophisticated enough for this task, and this is the greatest reason for the choice of this software in our study. The spectrum corrected by this method (Figure A.1) is superior to that corrected by the conventional method.
We used the procedure FLATT,\textsuperscript{62} incorporated into Felix 95, to detect the baseline points, and then fitted a polynomial to the 4\textsuperscript{th} order to construct the theoretical baseline. (FLATT can also be used to construct the theoretical baseline — it uses a sum of sine and cosine functions instead of a polynomial\textsuperscript{62} — but Felix does not allow the users to control these functions. One should \textit{never} use cubic spline, which is the default feature in VNMR, and is also available in Felix. This function corrects the baseline too aggressively, resulting in an unnatural-looking spectrum) The macros below displays the baseline-corrected 1-D slices, along with the baseline points. The parameters were determined by experimentation, and it is expected that the users of this code do the same for their own data.

\textsect{Direct dimension} — bcf2.*.mac

```
c; reset any open serial file
cl

c; close any open matrix files
cmx

ty Correcting baseline F2

c; type message to the text window
mat ho970322d write

for row 1 1024

esc out

if &out ne 0 finish

loa 0 &row

chi 10

abp 10 &chi 30 2

for morepts 0 10

bas add (&first+&morepts)

bas add (&last-&morepts)

next

pol 4

; polynomial baseline correct

; abl 5 50

; automatic convolution baseline flattening

sto 0 &row

dr

bas show

ty row = &row chi = &chi $

next

ty F2 baseline correction finished

finish:

ex return

end
```
A.3.3.2 Indirect dimension — bcfl.*.mac

cl ; reset any open serial file
cmx ; close any open matrix files
ty Correcting baseline F1
; type message to the text window
mat ho970322d write ; open matrix with write permission
for col 1 1024 ; loops over matrix columns
  esc out ; assign value of 1 to out if 'esc' button is hit
  if &out ne 0 finish ; if value of out is not 0, branch to 'finish'
  loa &col 0 ; load next column from matrix
  chi 10 ; calculate minimum chi square
  abp 10 &chi 4 0 2 ; select baseline point using FLATT
  for morepts 0 10 ; the first and the last points are baselines
    bas add (&first+&morepts)
  next
  pol 4 ; polynomial baseline correct
  ; abl 5 50 ; automatic convolution baseline flattening
  sto &col 0
  dr
  bas show
  ty col = &col chi = &chi $
next
ty F1 baseline correction finished
finish:
ex return
end

A.4 Formatting spectra for printing

After a 2-D spectrum is processed, it has to be formatted into a form suitable for printing or a further manipulation through a graphics software. All of our printable files were in the postscript format, which was compatible with the CorelDraw v. 7 graphics software.

For this dissertation, the phase-sensitive 2-D spectra were printed in color. For our paper however, they were printed in black-and-white: the procedure turned out to be much trickier than that for color. The macros used for both procedures are presented below.
A.4.1 COLOR

A.4.1.1 Set color — bluered.mac

The color spectra in this dissertation were printed so that the positive peaks appear blue and the negative peaks red. To set the pen color, the following macro is run.

; For contour plots
; Blue for positive, red for negative
pen 8 0 0 255 256 ; blue
pen 9 255 0 0 256 ; red
ex &return
end

From Felix 95, the pulldown menu is activated so that the frame was in 2D Mode. Then, the menu is activated in the following order: Display → Plot Parameters → Data Attribute. This produces the window “Data Attribute”. The variable “Negative Levels” is set to “On”, “Color Number” to 8, and “Color Cycle” to 2. The “Set” button is then activated. The menu is then activated in the order Display → Plot Type → Contour Plot to display the plot. If the contour threshold and the level multiplier needs to be changed, one may go back to the “Data Attribute” window to change them.

A.4.1.2 Create a postscript file

The pulldown menu is activated in the order File → Hardcopy to display a window. The variables are then set as follows: “Plot Device” to “Postscript”, “X Origin” to 1.5, “Y Origin” to 2, “X Size” to 5, “Y Size” to 7, “Orientation” to “Portrait”, “Font Style” to “Internal”, and “Color Style” to “RGB”. The name for the output file is set to whatever that the user desires. The “Print” button is then activated to create a postscript file.

If desired, a 1-D spectrum could be displayed above the 2-D spectrum. To create
an appropriate file, the x-axis and the box around the plot must be turned off by activating the pulldown menu in the order Display → Plot Parameters → General Appearance and then adjusting the appropriate variable in the window. The size and the positioning may be set by opening the window for the plot parameters (see above) and setting the following variables: “Plot Device” to “Postscript”, “X Origin” to 1.5, “Y Origin” to 7.25, “X Size” to 5, “Y Size” to 3, and “Orientation” to “Portrait”. The resulting postscript file and that of the 2-D spectrum are combined using the program combine.c, which is described in detail in §A.4.2.4.

A.4.2 Black-and-white

A.4.2.1 Problems particular to black-and-white

Because the *Journal of the American Chemical Society* charges $300 for each illustration in color,¹⁴⁹ it is advisable to make plots in black-and-white for publication. Creating a black-and-white plot of a phase-sensitive 2-D spectrum turns out to be more difficult than a corresponding plot in color. In order to distinguish between the positive and the negative peaks, these two must be drawn in different formats: for example, the positive peaks in contour, and the negative peaks in intensity. Unfortunately, Felix 95 does not provide a method to make such a plot in one step. Therefore, this plot must be made in a multiple step as follows: (1) create a contour plot for the positive peaks; (2) create an intensity plot for the negative peaks; (3) combine these plots, together with an appropriate 1-D spectrum. The macros and programs used for accomplishing these three steps are presented here.
A.4.2.2 Creating a graph with positive peaks — pos.mac

Creating a contour plot of the positive peaks is relatively straightforward. First, the following macro, named pos.mac, is run.

def posneg 0 ;positive levels only
def nlevel 2 ;2 levels
def clmode 1 ;geometric spacing
def conmod 8 ;large contour spacing modifier
def pennum 1 ;white pen
def cycle 1 ;only one color
cp ;contour plot
ex return
end

After this, one may modify the contour threshold and the level multiplier from the "Data Attributes" window (see p. 221). The postscript plot is then created by the method shown in §A.4.1.2, except that "Color Style" is changed to "Black-and-White". The resulting plot is shown in Figure A.2.

A.4.2.3 Creating a graph with negative peaks — invert.mac, neg.mac, and invert.c

To create a plot with the negative peaks, the entire matrix file is inverted — that is, the positive peaks are turned negative; and the negative, positive. When the following macro is being used, it prompts the user for the name of the matrix.

invert.mac:

cl ;reset any open serial file to the start
cmx ;close any open matrix files
get 'Filename without .mat: ' name
mat &name write ;open matrix with write permission
for col 1 &dsiz ;loop over matrix rows 1 to 1024
  esc out ;assign value of 1 to out if 'esc' button hit
  if &out ne 0 finish ;if value of out is not 0, branch to 'finish'
  loa &col 0 ;load next column from matrix
  mul -1 ;change the sign of the spectrum
  sto &col 0
  ty col = &col $
dr
next
ty Sign inversion finished
After the inversion is finished, grayscale levels for the intensity plot is set up by
the following macro.

\textit{neg.mac:}

\begin{verbatim}
; ; gray colorramp - 16 colors
; pen 133 0 0 0 256 ;black
pen 134 15 15 15 256
pen 135 31 31 31 256
pen 136 63 63 63 256
pen 137 79 79 79 256
pen 138 95 95 95 256
pen 139 111 111 111 256
pen 140 127 127 127 256
pen 141 143 143 143 256
pen 142 159 159 159 256
pen 143 175 175 175 256
pen 144 191 191 191 256
pen 145 207 207 207 256
pen 146 223 223 223 256
pen 147 239 239 239 256
pen 148 255 255 255 256 ;white

def posneg 0 ;positive levels only
def nlevel 16 ;16 levels
def clmode 1 ;geometric spacing
def conmod 1.2 ;small contour spacing modifier
def pennum 133 ;start gray ramp
def cycle 16 ;use all colors
ip ;intensity plot
ex return
end
\end{verbatim}

Again, the contour threshold and spacing are modified as necessary. When the
plot is redrawn and plotted using the dimensions specified in §A.4.1.2, the resulting plot
has peaks that are doughnut-like: the fringe is dark, while the middle is light (Figure A.3).

This is the exact opposite of what is intended. Rewriting \textit{neg.mac} so that the grayscale
levels are inverted results in the same plot. This is one of many features in Felix 95 that
put its users in the anthropomorphic mode and make them believe in the willful
malevolence of the software itself. This particular feature however may be countered by a program that reverses the effect. The following C program, `invert.c`, reverses the grayscale intensity.

`invert.c`:

```c
/*
In a postscript file, converts "setgray" from x to 1-x.
*/
#include <string.h>
#include <stdio.h>
#include <stdlib.h>

main()
{
    double grayness; /* intensity of grayscale */
    char line[201]; /* line to be examined in the postscript file */
    FILE *oldfile; /* postscript file to be read */
    char oldfile_name[25]; /* name of "oldfile" */
    FILE *newfile; /* postscript file to be written */
    char newfile_name[25]; /* name of "newfile" */

    /* Get the names of the input & output files */
    printf("Enter name of input file: ");
    scanf("%24s", oldfile_name);
    printf("Enter name of output file: ");
    scanf("%24s", newfile_name);

    /* Check if the input file can be read, the output file can be written */
    if ( ( oldfile = fopen(oldfile_name, "r") ) == NULL ) {
        printf("Can't open %s for reading.
", oldfile_name);
        return EXIT_FAILURE;
    } else if ( ( newfile = fopen(newfile_name, "w") ) == NULL ) {
        printf("Can't open %s for writing.
", newfile_name);
        fclose(oldfile);
        return EXIT_FAILURE;
    }

    /* If the file permissions OK, then go! */
    else {
        while ( fgets(line, 200, oldfile) != NULL ) {
            if ( strstr(line, "setgray") != NULL ) {
                grayness = atof(line); /* converts string into number */
                grayness = 1.0 - grayness; /* inverts setgray */
            }
        }
    }

    return EXIT_SUCCESS;
}
```

The resulting plot is shown in the right side of Figure A.3. The peaks now look as intended. The axes are inverted from black to white, making them invisible: this is not a problem, since they are restored when this plot is combined with that of the positive peaks.

A.4.2.4 Combining the positive and the negative graphs, and incorporating the appropriate 1-D spectrum — combine.c

Now that we have the plots for the positive and the negative peaks, along with that for the 1-D spectrum, we are ready to put them together. The following C program, combine.c, prompts for the names of the three plots, and creates the combined plot. The resulting plot is shown in the right side of Figure A.2. (This program may also be used for making the combined plot of a 1-D and a color 2-D spectra. When prompted for the name of the negative plot, hit space and return. Likewise, the program may also be used when a corresponding 1-D spectrum is unavailable.)

/*
Create a postscript file for a 2-D phase-sensitive spectrum
*/

#include <string.h>
#include <stdio.h>
#include <stdlib.h>

main() {

  fprintf(newfile, "%.6f setgray\n", grayness); /* prints the new line */
  } else {
    fputs(line, newfile); /* copies the line to the new file */
  }
}
fclose(oldfile);
fclose(newfile);
return EXIT_SUCCESS;
}
```c
int counter=0; /* generic counter */
char line[201]; /* line read from a postscript file */
FILE *posfile; /* ps file containing positive peaks */
char posfile_name[25]; /* name for "posfile" */
FILE *negfile; /* ps file containing negative peaks */
char negfile_name[25]; /* name for "negfile" */
FILE *onedimfile; /* ps file containing ld spectrum */
char onedimfile_name[25]; /* name for "onedimfile" */
FILE *newfile; /* ps file to be created */
char newfile_name[25]; /* name for "newfile" */

/* Get the names of the input & output files */
printf("Enter name of file with positive peaks (Default: pos.ps)\n");
counter = 0;
do {
    posfile_name[counter] = getchar();
} while (posfile_name[counter++] != '\n');
posfile_name[counter-1] = NULL;
if (posfile_name[0] == NULL)
    strcpy(posfile_name, "pos.ps");

printf("Enter name of file with negative peaks\n");
printf("(Default: neg.ps. If there is no file, hit spacebar then enter)\n");
counter = 0;
do {
    negfile_name[counter] = getchar();
} while (negfile_name[counter++] != '\n');
negfile_name[counter-1] = NULL;
if (negfile_name[0] == NULL)
    strcpy(negfile_name, "neg.ps");

printf("Enter name of file with 1-D spectrum\n");
printf("(Default: Id.ps. If there is no file, hit spacebar then enter)\n");
counter = 0;
do {
    onedimfile_name[counter] = getchar();
} while (onedimfile_name[counter++] != '\n');
onedimfile_name[counter-1] = NULL;
if (onedimfile_name[0] == NULL)
    strcpy(onedimfile_name, "Id.ps");

printf("Enter name of file to be created (Default: combined.ps)\n");
counter = 0;
do {
    newfile_name[counter] = getchar();
} while (newfile_name[counter++] != '\n');
ewfile_name[counter-1] = NULL;
if (newfile_name[0] == NULL)
    strcpy(newfile_name, "combined.ps");
```
/* Check if the input files can be read and the output files can be written */

if ( ( posfile = fopen(posfile_name, "r") ) == NULL ) {
    printf("Can't open %s for reading.\n", posfile_name);
    return EXIT_FAILURE;
}

if ( negfile_name[0] != ' ' ) {
    if ( ( negfile = fopen(negfile_name, "r") ) == NULL ) {
        printf("Can't open %s for reading.\n", negfile_name);
        exit(EXIT_FAILURE);
    }
}

if ( onedimfile_name[0] != ' ' ) {
    if ( ( onedimfile = fopen(onedimfile_name, "r") ) == NULL ) {
        printf("Can't open %s for reading.\n", onedimfile_name);
        exit(EXIT_FAILURE);
    }
}

if ( ( newfile = fopen(newfile_name, "w") ) == NULL ) {
    printf("Can't open %s for writing.\n", newfile_name);
    exit(EXIT_FAILURE);
}

/* If the file permissions are OK, then proceed */

/* Copy the header of posfile to newfile */

while ( strstr(fgets(line, 200, posfile), "%%EndPageSetup") == NULL )
    if ( feof(posfile) ) {
        printf("Header incomplete: %s possibly invalid.\n", posfile_name);
        exit(EXIT_FAILURE);
    } else {
        fputs(line, newfile);
    }

fputs(line, newfile);

/* Set the line width of newfile */

fprintf(newfile, " 2 setlinewidth\n");

/* Copy the main part of onedimfile to newfile, if the former exists */

if ( onedimfile_name[0] != ' ' ) {
    while ( strstr(fgets(line, 200, onedimfile), "%%EndPageSetup") == NULL ) {
        if ( feof(onedimfile) ) {
            /* Copy the main part of onedimfile to newfile, if the former exists */
        }
    }
}
printf("Main part not found: %s possibly invalid.\n", onedimfile_name);
exit(EXIT_FAILURE);
}
}
while ( strstr(fgets(line, 200, onedimfile), "showpage") == NULL ) {
    if ( feof(onedimfile) ) {
        printf("Main part incomplete: %s possibly invalid.\n", onedimfile_name);
        exit(EXIT_FAILURE);
    }
    else if ( !strstr(line, "setlinewidth") ) {
        fputs(line, newfile);
    }
}

/* Copy the main part of negfile to newfile, if the former exists */
if ( negfile_name[0] != ' ' ) {
    while ( strstr(fgets(line, 200, negfile), "%%EndPageSetup") == NULL ) {
        if ( feof(negfile) ) {
            printf("Main part not found: %s possibly invalid.\n", negfile_name);
            exit(EXIT_FAILURE);
        }
        while ( strstr(fgets(line, 200, negfile), "showpage") == NULL ) {
            if ( feof(negfile) ) {
                printf("Main part incomplete: %s possibly invalid.\n", negfile_name);
                exit(EXIT_FAILURE);
            }
            else if ( !strstr(line, "setlinewidth") ) {
                fputs(line, newfile);
            }
        }
    }
}

/* Copy the rest of posfile to newfile */
while ( fgets(line, 200, posfile) != NULL ) {
    if ( !strstr(line, "setlinewidth") ) {
        fputs(line, newfile);
    }
}

/* Close all files, exit */
exit(EXIT_SUCCESS);
A.5 Miscellaneous subjects

A.5.1 AXIS ON 1-D PLOT — HOW TO FOOL FELIX

Felix 95 allows better control of 2-D plotting than that of 1-D. When the program is in the 2-D mode, the methods for directly controlling the tickmarks and the fonts are available. These methods however become unavailable when the program is put into the 1-D mode: this is yet another manifestation of the perverse design of Felix 95. When a 1-D spectrum is plotted without any control of the axis format, the tickmarks are placed in awkward positions, and the axis do not cover the entire spectral width (Figure A.4).

The axis on a 1-D plot can be controlled by “fooling” Felix into “thinking” that it is working on a 2-D data. First, put the program into the 2-D mode and create a 2-D matrix file. Give this file a generic name: this will be the dummy file. Activate the “Tickmarks” window and set the tickmarks (major and minor) and the font size. Then, put the program into the 1-D mode and open the desired 1-D data file. When this data is Fourier-transformed and the spectrum plotted, the resulting plot will have not only the marks and the font that one desires, but also the axis will cover the entire spectral width (Figure A.4).

A.5.2 WINDOW FUNCTION AND DISAPPEARING COSY CROSS-PEAKS

COSY data are processed using a window function that is enhancing at the beginning and decaying at the end: such function is chosen because it matches the envelope of an FID from COSY. Unfortunately, this type of function could make some cross-peaks disappear if chosen carelessly. Take, for an example, a signal has a short T₂.
Let us say that the window function is chosen so that it matches the other signals which have long $T_2$’s. In this case, the function enhances the desired signal to the point where its post-decay noise is also enhanced. The resulting frequency-domain spectrum will have no visible peak from the desired signal, since it is buried in the noise.

In Figure A.5, two spectra are shown: they were processed from same FID data — that of FeOETPPCl at room temperature — but using sine-squared bells of different size. The methylene-methylene cross-peaks are severely affected by the choice of the window function, since the methylene signals have very short $T_2$’s (~5 ms) compared to the other signals. The spectrum processed with a sine-squared bell covering 8 ms shows visible methylene-methylene cross-peaks. However, that processed with a sine-squared bell covering 24 ms shows no methylene-methylene cross-peaks. Such obliteration of the cross-peaks of rapidly-decaying signals is very easy to make, since a window function is usually chosen by considering the entire FID, thus emphasizing the slowly-decaying signals at the expense of the others.

A.5.3 To linear-predict, or not to linear-predict: a question of “disappearing” cross-peaks

Linear prediction is a method for reconstructing a truncated or an imperfect FID. It is useful in correcting the first few data points, which usually are inaccurate because of the limit of the response time of the spectrometer electronics. All of the spectra presented in Chapter 3 had the first two points of the directly-detected dimension corrected by linear prediction.
The method has found a great interest among those who do an extensive amount of 2-D work. Because of the constraints on experiment time, 2-D data are usually very heavily truncated, especially on the indirectly-detected dimension. Apodization for removing the truncation artifact results in linewidth much larger than the limit from the decay time. Through linear prediction, it is possible to extrapolate the missing tails of both the FIDs and the interferograms. However, as we shall see, relying heavily on LP extrapolation may bring out some problems that had been masked by the apodization.

In Figure A.6, two spectra are shown. Both were processed from the ROESY data of \([\text{FeOETPP(2-MeImH)\text{Cl}}] \) at -85°C. The spectrum at left is the one seen in Chapter 3: no LP extrapolation was performed, and Gaussians of 60 Hz in the first dimension and 120 Hz in the second dimension were applied before the respective transforms. For the spectrum at right, both the FIDs and the interferograms were extrapolated by linear prediction to twice the original size, and then Gaussians of 15 Hz (first dimension) and 30 Hz (second dimension) were applied. In this spectrum, the diamagnetic region is much more clearly resolved. Some of the cross-peaks however appear to have diminished in intensity, particularly the one indicated by the arrows. The slices through this peak indicates that the intensity does not vary. The noise level however is much greater in the LP-enhanced spectrum. In other words, the cross-peaks appear to have diminished because the noise have become almost as big as the signal, and therefore the contour level cannot be lowered to where the signal becomes visible in the 2-D spectrum. This problem is not seen in the spectrum at left, because the apodization
also filters out a considerable amount of noise.

If small cross-peaks were to be visible in a LP-extrapolated 2-D spectrum, the number of transients must be sufficiently high. A lesson here is that extrapolation by linear prediction is not a substitute for reduction of signal-to-noise ratio.
Figure A.1 Left. Spectrum whose baseline was corrected using VNMR. The baseline was defined by the points not used for integration in the 1-D spectrum above. Note the presence of trenches in the diamagnetic region, along the direct dimension.

Right. The same spectrum, the baseline corrected using Felix 95. For each slice in both dimensions, the baseline was automatically detected and the baseline corrected accordingly. Note the lack of trenches.
Figure A.2  Left. Contour plot of the positive peaks. Right. Plot depicting both the positive and the negative peaks, and the corresponding 1-D spectrum.
Figure A.3  Left. Intensity plot of the negative peaks, as produced by Felix. The intensity grayscale is the opposite of what was intended.  Right. The same plot, after being processed using \textit{invert.c}. The grayscale is as intended. The axes are now invisible; but when combined with the plot with the positive peaks, the latter provides the axes.
Figure A.4  Top. 1-D spectrum generated with Felix 95, with the axis and tickmarks automatically formatted by the program. The axis does not extend fully, and the tickmarks are placed in awkward positions. Bottom. Same spectrum, with the axis and tickmarks formatted by manipulating the parameters in the 2-D mode. The appearance is much better.
Figure A.5 COSY spectrum of FeOETPPCI at 22°C, processed using sine squared bells (SSQ) of different size on the directly-detected dimension. *Left.* SSQ: 8 ms. The cross peak on the slice (*arrow*) is visible. *Right.* SSQ: 24 ms. The cross peak (*arrow*) has disappeared.
Figure A.6 Comparison in extrapolation by linear prediction. *Left.* Unextrapolated spectrum, apodized by Gaussians of 60 Hz (1st dimension), 120 Hz (2nd dimension). *Right.* Extrapolated spectrum, apodized by Gaussians of 15 Hz and 30 Hz. The diamagnetic region is resolved more clearly, but the cross-peak indicated by the arrow has disappeared. The slices (top) indicate that the intensity of the cross-peak has not diminished, but the overall noise level has increased.
APPENDIX B. \textit{\textsuperscript{1}H-NMR SPECTRA OF THE PRODUCTS FROM THE MIXED-ALDEHYDE ADLER SYNTHESIS IN CHAPTER 5}

No me pregunte más. ¿Su clasificación? En la tarjeta dice amor, felicidad, lo que sea. No importa. Nunca fue viable. Un feto en su frasco de alcohol. Es decir, un poema del libro del que usted hará el elogio.

— Rosario Castellanos, \textit{Entrevista de prensa}.

B.1 Introduction

The spectra of the products from the mixed-aldehyde Adler synthesis described in Chapter 5 are collected here. The samples have been placed in labeled glass vials, and are currently stored in Old Chemistry 248. To locate them, please consult the members of the laboratory.

The synthetic and chromatographic procedure and the convention for naming the samples are described in §5.2.2 (p. 139). The spectra were taken on Bruker AM 250-MHz spectrometer and was processed on the software on the machine. The hard copies were scanned in as a bitmap file, which in turn was incorporated into this document. Although it is possible to produce more aesthetically pleasing figures by converting the data into the Felix 95 format and then processing them on the software, it was chosen not to do so: the file conversion strips the title text that contains the valuable information about each sample.
B.2 Spectra

Figure B.1 Precipitated product, recrystallized, Fraction 1.

Figure B.2 Precipitated product, recrystallized, Fraction 2.
Figure B.3 Fraction 3, precipitated product, recrystallized.

Figure B.4 Fraction 4, precipitated product, recrystallized.
Figure B.5 Precipitated product, recrystallized, Fraction 5.

Figure B.6 Mother liquor from recrystallization, coarse-mesh column, Fraction 1.
Figure B.7 Mother liquor from precipitation, coarse-mesh column, Fraction 2.

Figure B.8 Mother liquor from precipitation, coarse-mesh column, Fraction 3.
Figure B.9 Mother liquor from recrystallization, coarse mesh column, Fraction 4.

Figure B.10 Mother liquor from recrystallization, coarse mesh column, Fraction 5.
Figure B.11 Mother liquor from recrystallization, fine mesh column, Fraction 1.

Figure B.12 Mother liquor from recrystallization, fine mesh column, Fraction 2.
Figure B.13 Mother liquor from recrystallization, fine mesh column, Fraction 3.

Figure B.14 Mother liquor from recrystallization, fine mesh column, Fraction 4.
Figure B.15 Mother liquor from recrystallization, fine mesh column, Fraction 5.

Figure B.16 Extract from tar, Fraction 1.
Figure B.17 Extract from tar, Fraction 2.

Figure B.18 Extract from tar, Fraction 2-1.
Figure B.19 Extract from tar, Fraction 2-2.

Figure B.20 Extract from tar, Fraction 2-3.
Figure B.21 Extract from tar, Fraction 2-4.

Figure B.22 Extract from tar, Fraction 2-2-1.
Figure B.23 Extract from tar, Fraction 2-2-2.

Figure B.24 Extract from tar, Fraction 2-2-3.
Figure B.25 Extract from tar, Fraction 2-2-4.

Figure B.26 Extract from tar, Fraction 2-2-3-1.
Figure B.27 Extract from tar, Fraction 2-2-3-2.

Figure B.28 Extract from tar, Fraction 2-2-3-3.
Figure B.29 Extract from tar, Fraction 2-2-3-4.
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