This is to certify that the

dissertation entitled

SYNTHETIC PORPHYRINOIDS AS NATURAL
HEME MODELS AND PHOTODYNAMIC DYES

presented by

Craig M. Shiner

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Chemistry

Chi K. Chang
Major professor

Date May 6, 1999

# SYNTHETIC PORPHYRINOIDS AS NATURAL HEME MODELS AND PHOTODYNAMIC DYES

By

Craig M. Shiner

A DISSERTATION

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

1999

ABSTRACT

SYNTHETIC PORPHYRINOIDS AS NATURAL HEME MODELS AND PHOTODYNAMIC DYES

By

Craig M. Shiner

This dissertation describes the preparation of a series of novel porphyrinoids for use as natural heme models and photodynamic dyes. Chapters 2 and 3 describe our efforts into the synthesis of compounds designed to model the prosthetic groups of a number of bacterial hemoproteins. Chapter 2 presents synthetic studies of a chlorin diol compound used to investigate bacterial heme *d*. The required porphyrin intermediate was synthesized through the use of modified b-bilene chemistry. The further transformation of this porphyrin, including directed hydroxylation, resulted in the required chlorin model compound. Chapter 3 focuses on the preparation of synthetic models for the prosthetic group of P460. These compounds were prepared by the acid-catalyzed condensation of 1,19-unsubstituted biladienes and appropriately functionalized aldehydes. 2D NMR spectroscopy was then utilized in the comparison of the model compounds and naturally occurring P460. Chapter 4 describes the study of benzochlorin derivatives as potential photosensitizers. A series of hydrolysis experiments were initially carried out on a number of chlorin imminium salts, in order to investigate the relative stability of these compounds. The studies revealed that a benzochlorin imminium salt was by far the most

stable compound tested. Due to this unusual stability, investigations into the synthesis of similar benzochlorin derivatives were undertaken. The unreactive nature of the benzochlorin macrocycle towards direct functionalization, required the incorporation of more stepwise approaches in these syntheses. Octaethylporphyrin was derivatized with the appropriate substituents and then cyclized under acidic conditions to give a series of meso-substituted benzochlorins. Chapters 5 and 6 discuss the synthesis of interesting macrocycles that are potentially useful in the preparation of photodynamic sensitizers. Chapter 5 describes the directed synthesis of novel porphyrin isomers, through the use of "2 + 2" and "3 + 1" methodologies. The "2 + 2" technique involved the acid-catalyzed condensation of a 2,3'-dipyrrylmethane and a 2,2'-dipyrrylmethane dialdehyde to afford a "N-confused" porphyrin. The "3 + 1" synthesis involved the acid-catalyzed condensation a tripyrrane dicarboxylic acid and a diformyl pyrrole to produce a second "N-confused" porphyrin. Chapter 6 focuses on the synthesis of porphyrins possessing multiple β-unsubstituted positions. The acid-catalyzed condensation of a number of partially alkylated tetrapyrroles and several aldehydes, afforded a series of previously unreported tetra- and hexa-alkylated porphyrins.

To my family

# ACKNOWLEDGMENTS

First of all, I would like to thank Professor C.K. Chang for his encouragement and guidance throughout this work. I also particularly wish to thank Professor B. Borhan for serving as my second reader. Professors T. Pinnavaia and J. Allison are also acknowledged for serving as members of my guidance committee.

My appreciation goes out to Kermit and Long for their assistance in obtaining NMR spectra. I would also like to thank Rui for running all those mass spectrometry samples.

I would like to express my gratitude to the past and present members of Professor Chang's research group as well. I would especially like to thank Yongqi and Chen-Yu for their friendship.

I would also collectively like to thank all the other friends I have made throughout my stay at Michigan State. Mike and Tara however, deserve special mention and are particularly acknowledged for their friendship.

TABLE OF CONTENTS

LIST OF TABLES

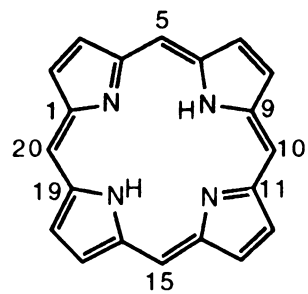
LIST OF FIGURES

LIST OF SCHEMES

# CHAPTER 1
# INTRODUCTION

## I. General

Porphyrins are an important class of aromatic macrocycles, which contain four pyrrole units connected together by methine bridges. The parent compound is known as porphin (1), and is numbered (IUPAC) as shown below. These structures also contain a conjugated pathway of 18 $\pi$ electrons and are generally planar. This conjugated double bond system results in a characteristic UV/visible absorption spectrum. In neutral organic solutions such as dichloromethane or chloroform, porphyrins display an intense absorption (Soret band) around 400 nm and four smaller absorption bands (Q bands) between 450 and 700 nm. The intensity of these smaller bands is dependent upon the nature of the substituents on the porphyrin ring.




1

The proton NMR spectra of these compounds provide evidence of a large aromatic ring current. The signals for the hydrogen atoms attached to the methine bridges (meso positions) are deshielded and appear at approximately

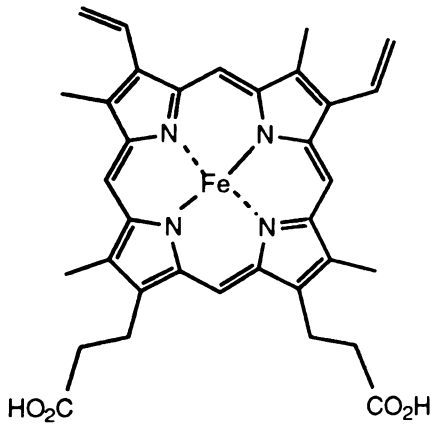
10 ppm, whereas the signals for the two internal pyrrolic hydrogens are strongly shielded and usually appear between -2 and -5 ppm.

The nitrogen atoms of a porphyrin are situated in such a manner as to allow complexation with a variety metals such as Mg(II), Fe(II), Cu(II), Co(II), Ni(II) and Zn(II). The porphyrins obtained from natural sources, are usually in the form of metal complexes. These complexes also display Soret bands in their UV/visible absorption spectra, but the Q bands are replaced by two absorptions known as the $\alpha$ and $\beta$ bands.

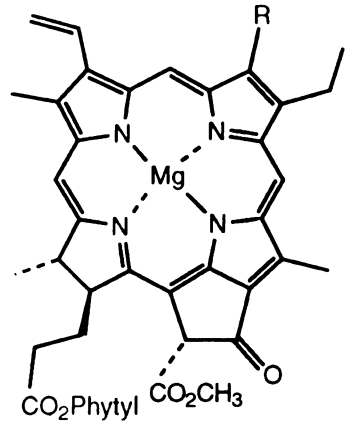
Porphyrins are involved in numerous biological processes. Heme (2) is a iron(II) porphyrin found in hemoglobin. Hemoglobin is a component of red blood cells and plays a vital role in transporting oxygen to bodily tissue. Heme and its closely related derivatives are also the prosthetic groups of many other hemoproteins. A few examples of other hemoproteins include myoglobin, cytochromes and catalases. Myoglobin is responsible for the storage of oxygen in muscle tissue, while cytochromes control electron transport in plants and animals. Catalases are a class of enzymes that catalyze the decomposition of hydrogen peroxide and in some cases the oxidation of organic compounds in plants, animals and aerobic bacteria. While heme is the most abundant prosthetic group found in nature, bacterial hemoproteins often contain interesting variations of the porphyrin core. The synthesis and model studies of a number of these bacteria-derived prosthetic groups will be described later in this report and include heme *d* (Chapter 2) and P460 (Chapter 3).

Chlorophylls are magnesium complexes of porphyrin derivatives known as chlorins, which have a saturated bond in one of the pyrrolic rings. There are several types of chlorophylls found in nature, including chlorophylls *a* (3a) and *b* (3b). Chlorophyll *a* is the most common naturally occurring chlorophyll and along with chlorophyll *b*, is found in higher plants and most green algae. These

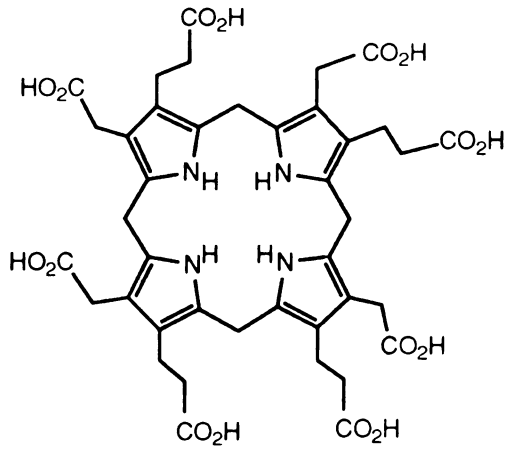
structures absorb light and provide the energy required for the oxidation and reduction processes associated with photosynthesis. Both heme and the chlorophylls have a common biochemical precursor, known as uroporphyrinogen III (4).




2



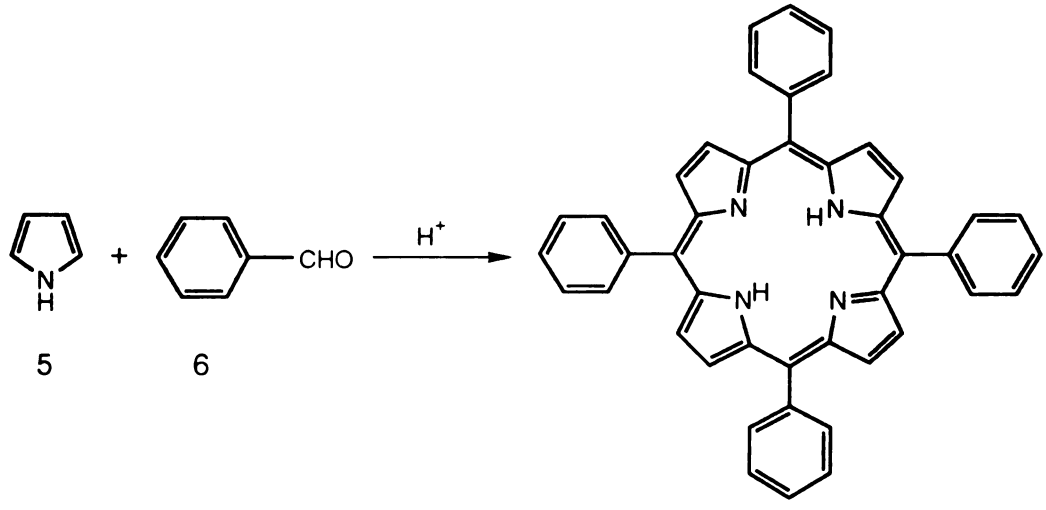

3a. R = $CH_3$ (chlorophyll a)
b. R = CHO (chlorophyll b)




4

## II. The Total Synthesis of Porphyrins and Porphyrinoids

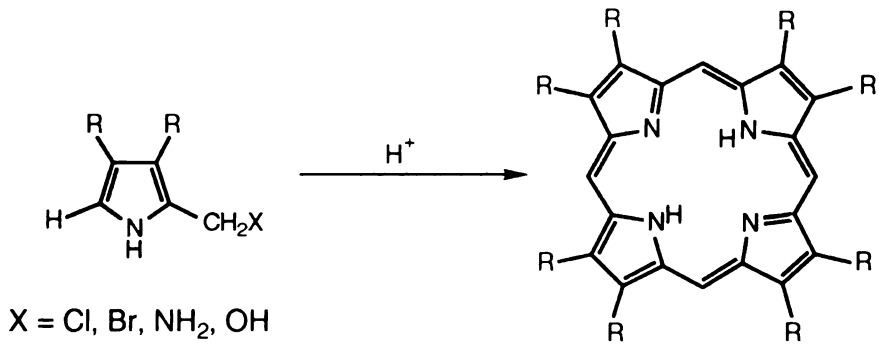
Many methods are available for the synthesis of porphyrins and the route selected is somewhat dependent on the substitution pattern desired. The preparation of symmetrical porphyrins is usually straightforward. In 1935, Rothemund described the synthesis of tetraphenylporphyrin (TPP) (7).[1] A mixture of pyrrole (5) and benzaldehyde (6) were heating in pyridine, to give low yields of (7) (Scheme 1). Since then, there have been various modifications of Rothemund's synthesis. Aldler reported that TPP was obtainable in 20-25% yield, when the reaction was carried out in refluxing propionic acid.[2] Lindsey and coworkers were able to achieve TPP yields of 30-40%, by utilizing boron trifluoride etherate.[3] Today, Lindsey's procedure is usually the preferred method of obtaining substituted tetraarylporphyrins. The slight modification of these reaction conditions have also resulted in the synthesis of a large number of ortho-substituted tetraarylporphyrins.[4]




5 6 7

Scheme 1. Synthesis of Tetraphenylporphyrin

Symmetrical porphyrins can also be prepared by the cyclotetramerization of pyrroles with active α-methylene substituents (8). This chemistry has been applied to the synthesis of a variety of porphyrins, including octaethylporphyrin (9) (R = $CH_2CH_3$) (Scheme 2).[5]

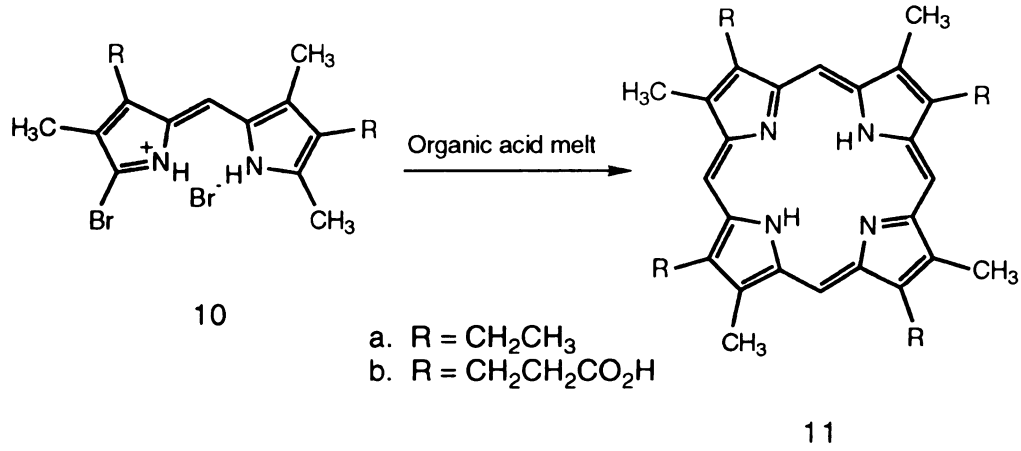



X = Cl, Br, $NH_2$, OH

8 9

Scheme 2. Synthesis of Octa-Alkyl Porphyrins

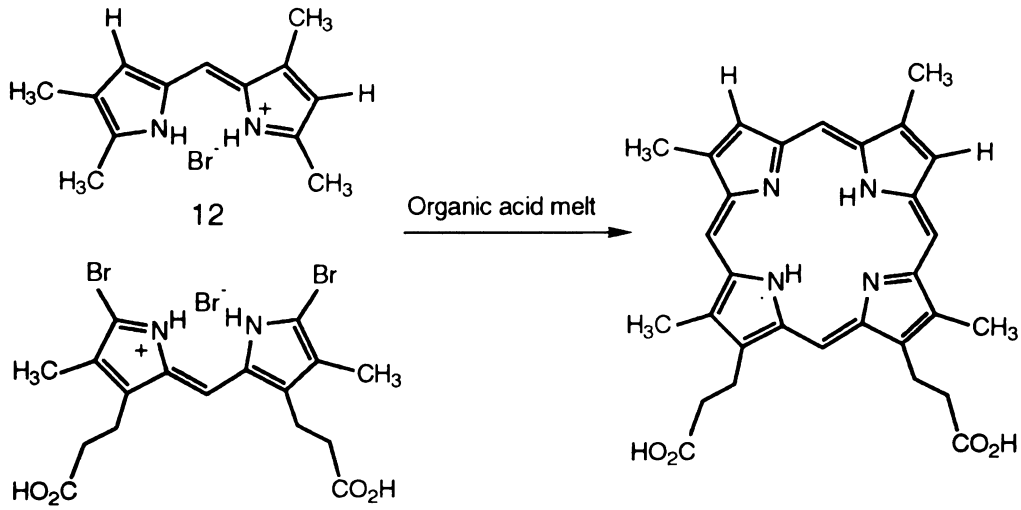
The synthesis of asymmetrically substituted porphyrins requires the use of more complex procedures. Most of these approaches employ a step-by-step assembly of the pyrrole subunits. The first procedure utilizing these techniques was developed by Fischer and coworkers.[6] The self-condensation of brominated dipyrromethenes (10a,b) in organic acid melts, afforded porphyrins (11a,b) in moderate yields (Scheme 3). Fischer expanded on this chemistry and discovered that dipyrromethene (12) and dibromodipyrromethene (13) would cyclize in organic acid melts, to give deuteroporphyrin IX (14) (Scheme 4).[7] Since then, a variety of compounds have been prepared by this method. Unfortunately, the strong reaction conditions can lead to low yields of porphyrin. One of the condensing dipyrromethenes must also be symmetrical, to ensure the formation of only one compound.




10

11

Scheme 3. Fischer's Porphyrin Synthesis Utilizing Dipyrromethenes




12

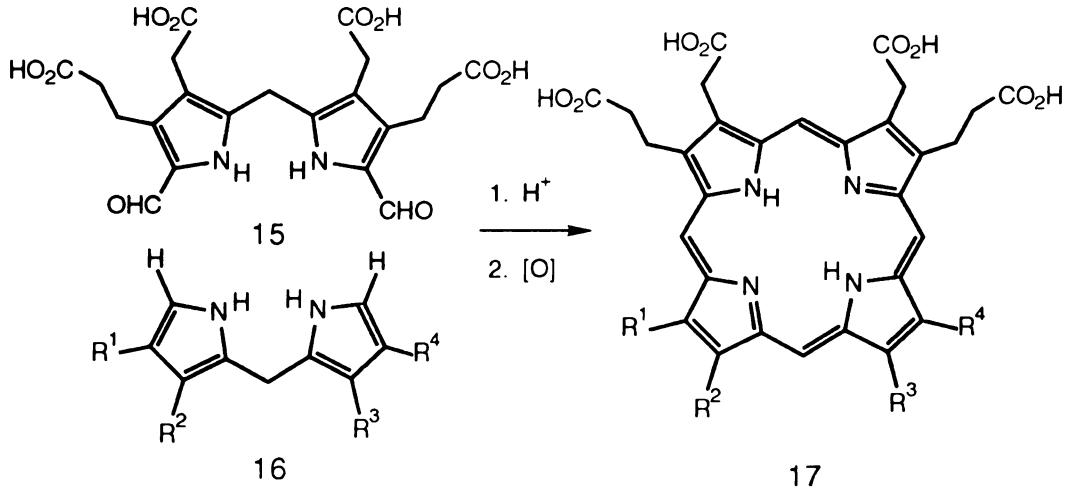
13

14

Scheme 4. Fischer's Synthesis of Deuteroporphyrin IX

In 1960, MacDonald and coworkers described a highly useful "2 + 2" cyclization technique. The acid-catalyzed condensation of 5,5'-diformyldipyrrylmethane (15) and 5,5'-diunsubstituted dipyrrylmethanes (16a-c), afforded uroporphyrin isomers (17a-c) in yields of 55-65% (Scheme 5).[8] Until this report, dipyrrylmethanes (16a-c) were thought to be too unstable for use in porphyrin syntheses. The main advantages of this "2 + 2"

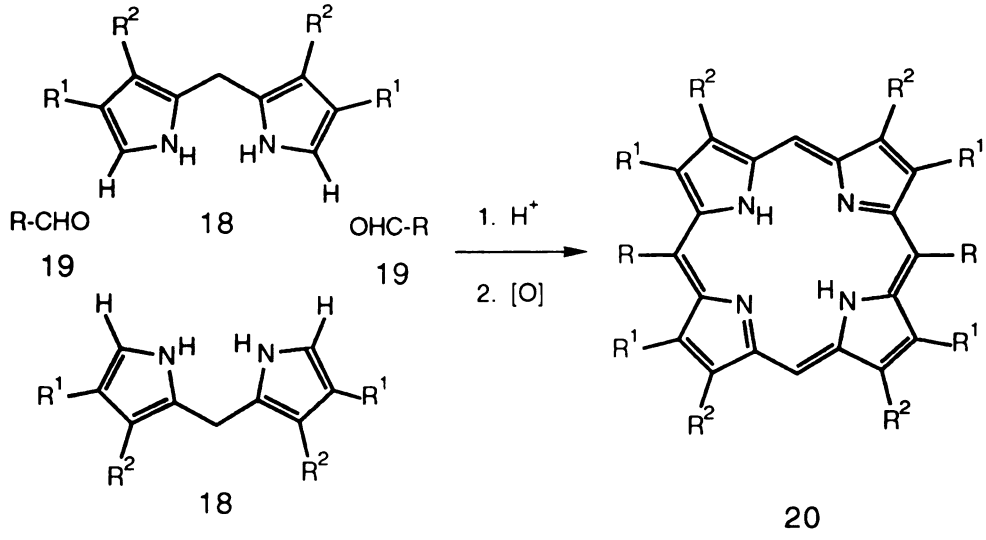
methodology over the use of dipyrromethenes include milder reaction conditions and higher yields. However, one of the dipyrrylmethanes must also be symmetrical in order to avoid a mixture of porphyrin isomers.




a. $R^1 = R^4 = CH_2CH_2CO_2H$, $R^2 = R^3 = CH_2CO_2H$
b. $R^1 = R^4 = CH_2CO_2H$, $R^2 = R^3 = CH_2CH_2CO_2H$
c. $R^1 = R^3 = CH_2CH_2CO_2H$, $R^2 = R^4 = CH_2CO_2H$

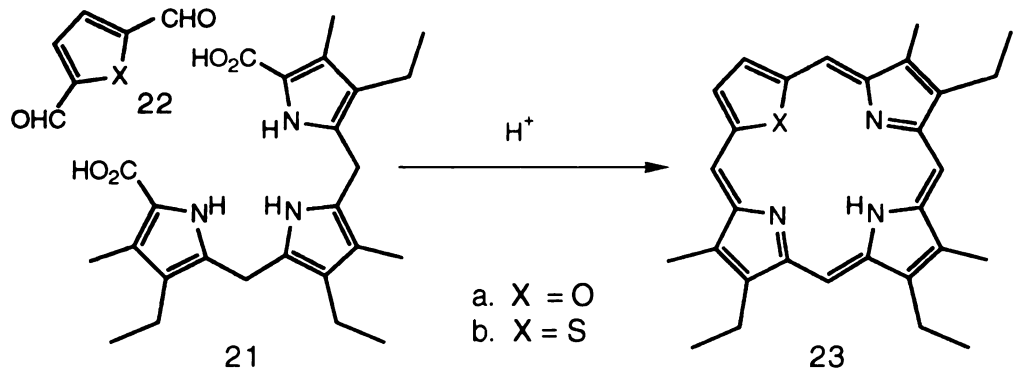
Scheme 5. MacDonald Synthesis of Uroporphyrin Isomers

A second type of condensation utilizing dipyrrylmethanes is shown in Scheme 6. The acid-catalyzed condensation of a 5,5'-diunsubstituted dipyrrylmethane (18) and aldehyde (19), results in the formation of a 5,15-disubstituted porphyrin (20). This procedure is particularly useful when the aldehyde is aromatic. Originally, propionic[9] and p-toluenesulfonic acid[10] were used in the condensations. Trifluoro[11] and trichloroacetic acid,[12] as well as boron trifluoride etherate,[13] have more recently been found to give high yields of 5,15-diaryl porphyrins.

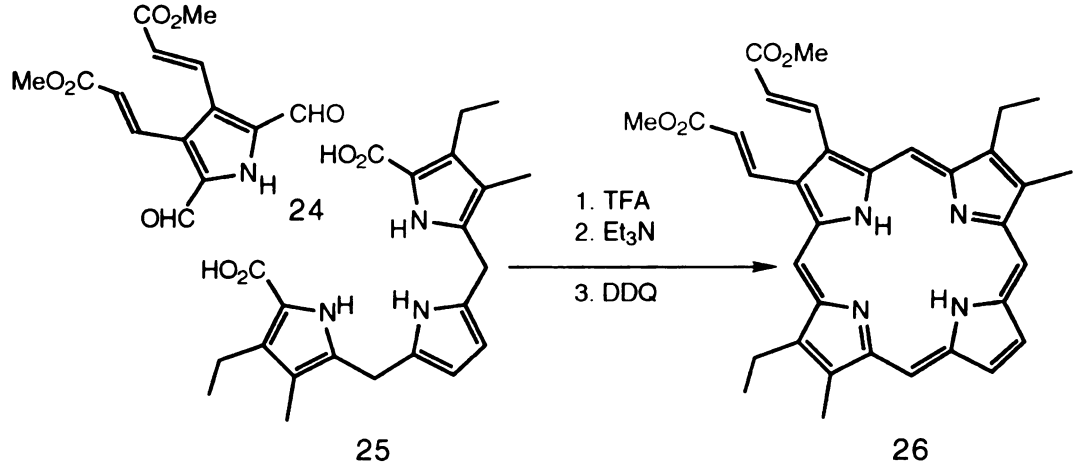



Scheme 6. Synthesis of 5,15-Disubstituted Porphyrins

Another synthetic method that has received a great deal of attention over the last several years is the so called "3 + 1" condensation. In 1971, Johnson and coworkers utilized this methodology to prepare porphyrin derivatives containing furan and thiophene subunits.[14] Tripyrrane (21) was condensed with dialdehydes (22a,b), to give porphyrin analogues (23a,b) in yields of 12-25% (Scheme 7). However, it was not until the 1990's that this methodology was applied to the synthesis of actual porphyrins. Boudif and Momenteau reported that the condensation of dialdehyde (24) and tripyrrane (25), in the presence of trifluoroacetic acid, gave porphyrin (26) in 33% yield (Scheme 8).[15] Since this report, a wide variety of porphyrins have been synthesized by this method. Several examples include porphyrins (27a-c)[16] and (28).[17] This "3 + 1" strategy has also been applied to the synthesis of benzo and pyrido porphyrin analogues. Berlin and Breitmaier[18] have synthesized porphyrin analogue (29), while Lash and coworkers[16,19] have prepared the related compounds (30a) and (30b).



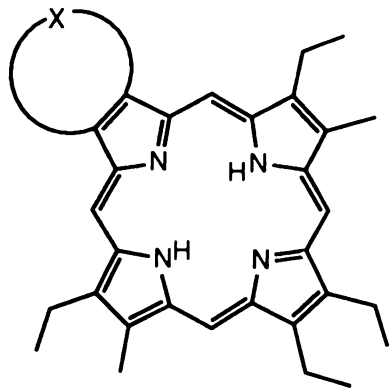

Scheme 7. Johnson's "3 + 1" Synthesis of Porphyrin Analogues

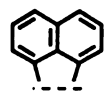
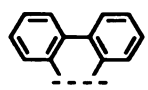
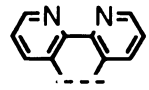



Scheme 8. Boudif and Momenteu's "3 + 1" Synthesis

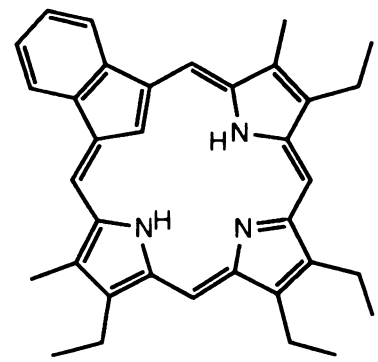
Typically, these condensation reactions are carried out in the presence of trifluoroacetic or hydrobromic acid. Recently however, Smith and coworkers described a variation of the "3 + 1" synthesis that utilized non-acidic conditions.[20] 2,5-Bis(dimethylaminomethyl)pyrrole (31) was condensed with tripyrranes (32a-c), in the presence of refluxing methanol and $K_3Fe(CN)_6$, to give porphyrins (33a-c) in yields of 17-31% (Scheme 9). Masaki and coworkers have recently synthesized imidazole analogues (34a,b), through a variation of Smith's procedure.[21] The "3 + 1" cyclization technique has also proven valuable in the preparation of porphyrins that are difficult to synthesize by other

methods. For example, the synthesis of porphyrin (27c) failed when attempted by a MacDonald "2 + 2" condensation. The utilization of "3 + 1" techniques however, afforded (27c) in yields of 72-83%.[16] As in the case of the "2 + 2" condensations, one of the components must be symmetrical in order to avoid a mixture of isomeric compounds.

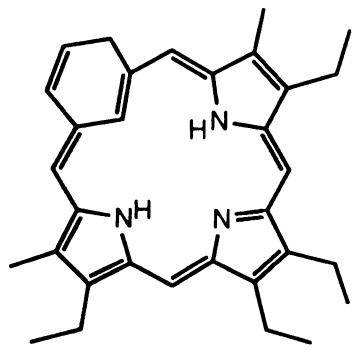



27 X =   

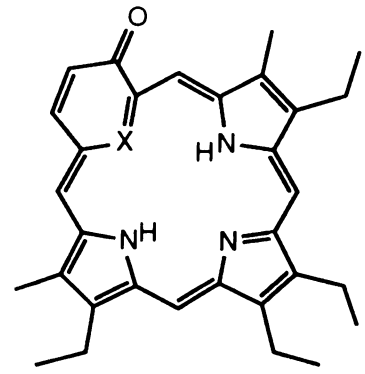

a. b. c.



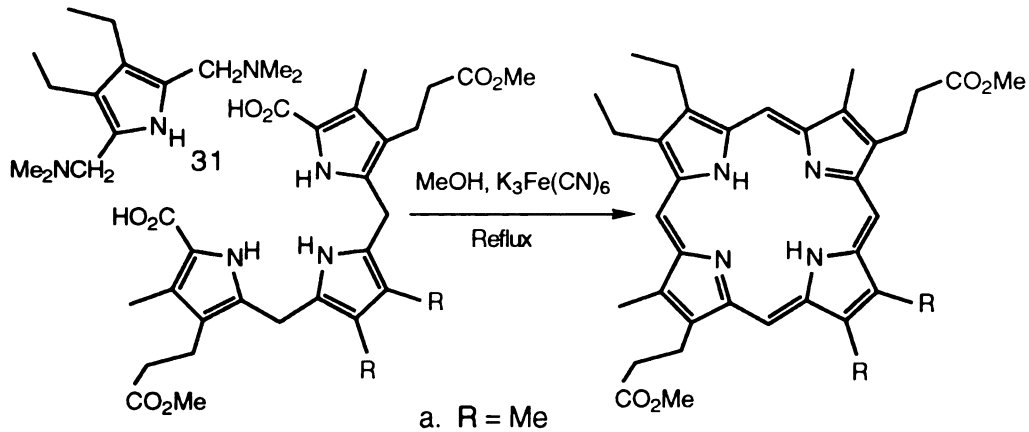

28




29

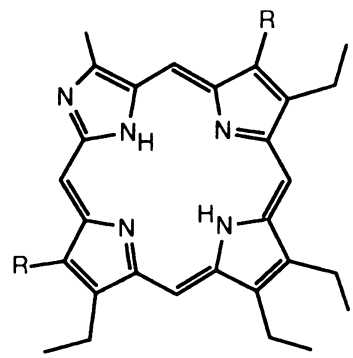



30a. X = CH
b. X = N



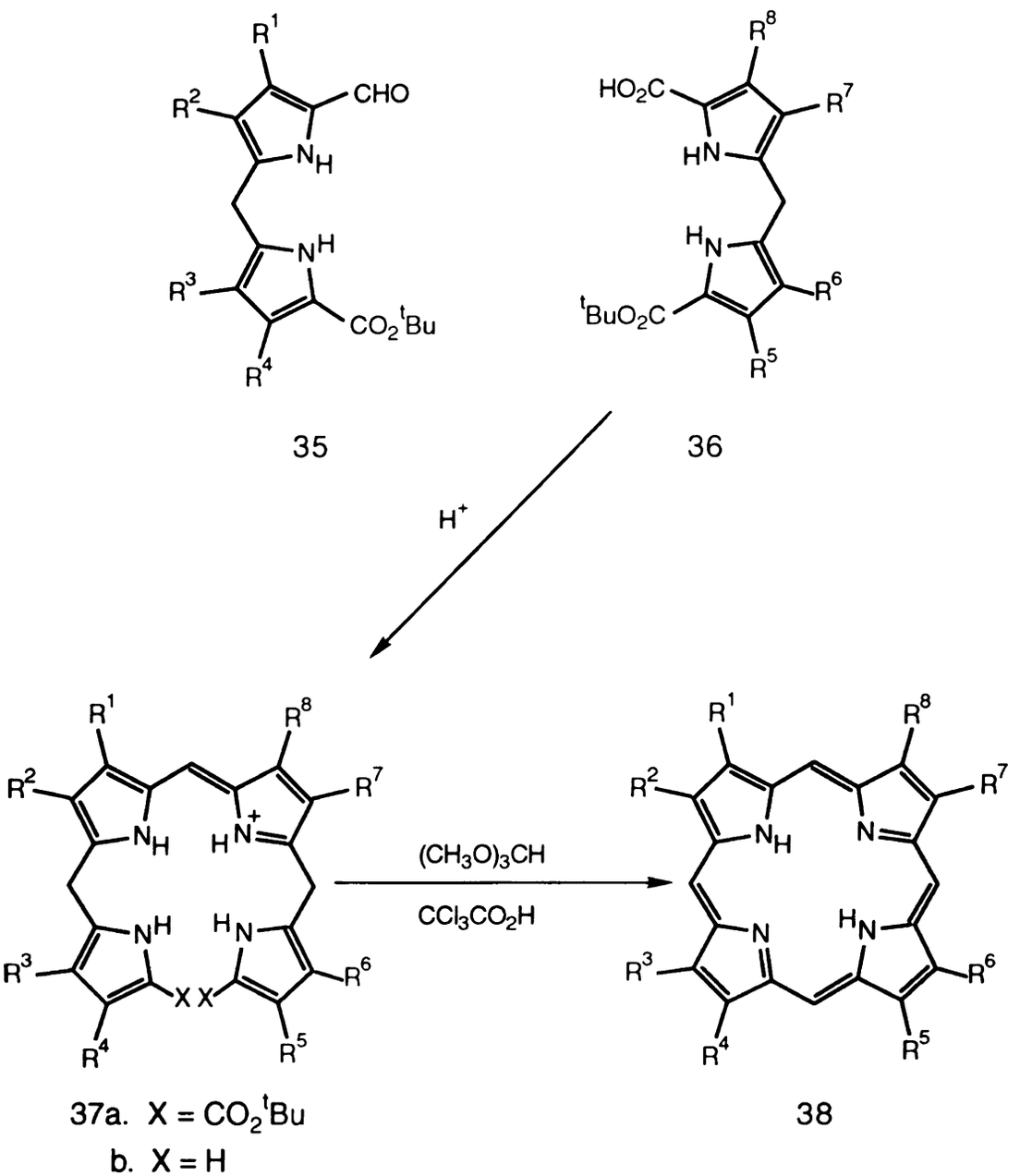

32 a. R = Me b. R = Ph c. R = -$(CH_2)_4$- 33

Scheme 9. Smith's "3 + 1" Synthesis




34a. R = $CH_2CH_3$
b. R = $CH_3$

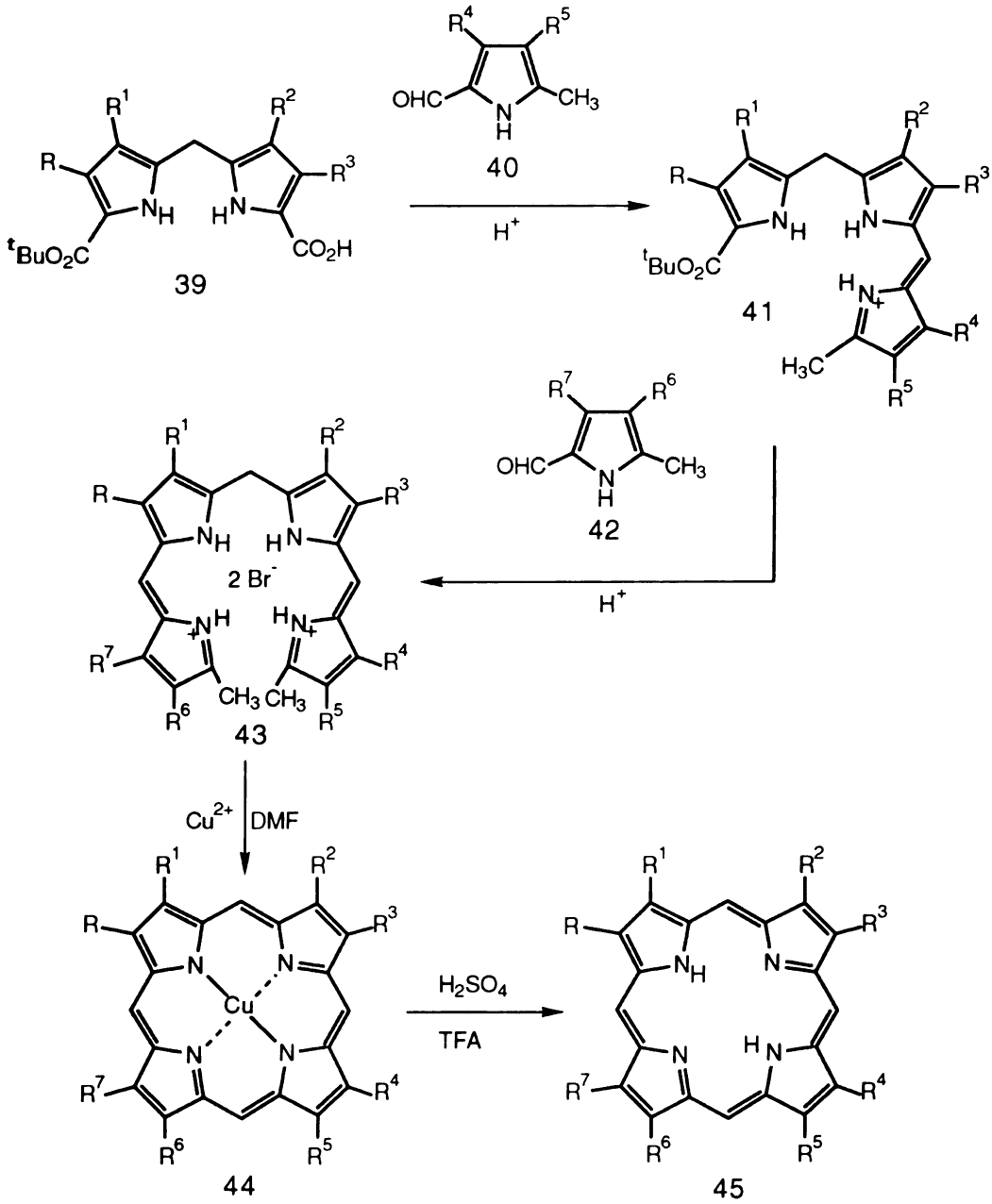
The synthesis of totally asymmetrical porphyrins requires the use of different methodologies. One well known procedure involves the synthesis and cyclizations of b-bilenes (Scheme 10).[22] The acid-catalyzed condensation of a 5-formyldipyrrylmethane (35) and a dipyrrylmethane-5-carboxylic acid (36), results in b-bilene (37a). The treatment of (37a) with trifluoroacetic acid, affords compound (37b). The subsequent cyclization of (37b), in the presence of trimethyl orthoformate and trichloroacetic acid, results in porphyrin (38).



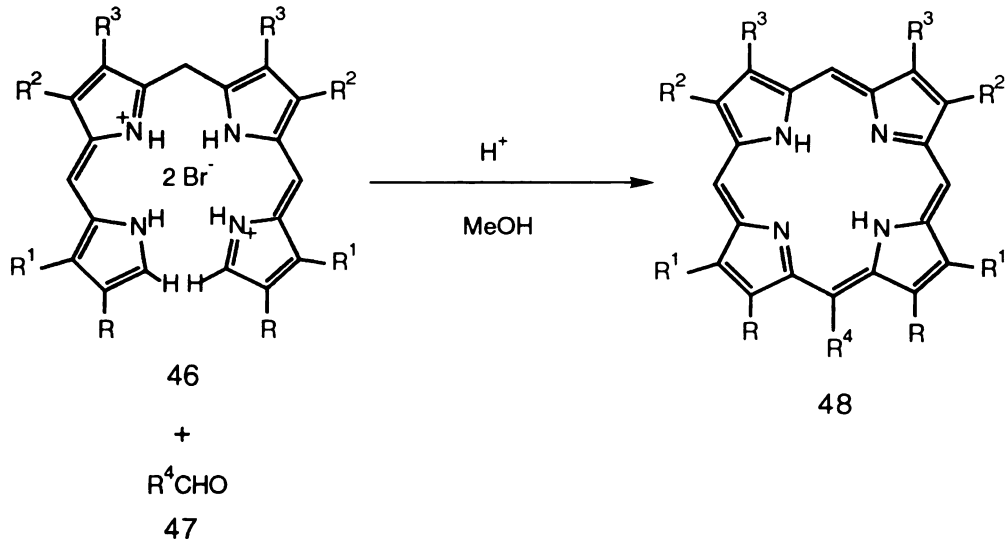

Scheme 10. Porphyrin Syntheses Utilizing the b-Bilene Approach

In the mid 1970's, Kenner and coworkers devised the tripyrrene-a,c-biladiene approach to porphyrin synthesis (Scheme 11).[23] The the acid-catalyzed condensation of dipyrrylmethane carboxylic acid (39) and pyrrole aldehyde (40), affords tripyrrene salt (41). The treatment of (41) with trifluoroacetic acid (TFA) and subsequent condensation with a different pyrrole aldehyde (42), leads to unsymmetrical a,c-biladiene (43). The a,c-biladiene is cyclized, in the presence of copper(II) salts, to give porphyrin (44). Compound (44) can then be treated with a mixture of sulfuric/trifluoroacetic acid, to afford porphyrin (45).

Another synthetic method that utilizes biladienes is shown in Scheme 12. The condensation of 1,19-diunsubstituted biladiene (46) and an aldehyde (47), in the presence of hydrobromic acid or hydrogen bromide, affords the corresponding meso-substituted porphyrin (48). The tetrapyrrolic salts used in these condensations were initially prepared as intermediates in the synthesis of corroles, but it was discovered that cyclization with formaldehyde also afforded porphyrin.[24] Johnson and coworkers expanded this chemistry to include the condensation of the biladienes with various para-substituted benzaldehydes.[25] Porphyrins containing meso-alkyl and meso-ester substituents, have also been prepared in a similar fashion. The major advantage of this procedure is that the condensations usually lead directly to the desired porphyrin. This is of value when transformations of the meso substituent are difficult after incorporation into the porphyrin. One potential disadvantage of this technique is possible steric interactions between the tetrapyrrole and aldehyde, which could inhibit cyclization. In such cases, it may be beneficial to prepare the desired porphyrin through a MacDonald "2 + 2" condensation.




Scheme 11. Porphyrin Syntheses Utilizing the Tripyrene-a,c-Biladiene Approach




Scheme 12. Synthesis of Mono, Meso-Substituted Porphyrins

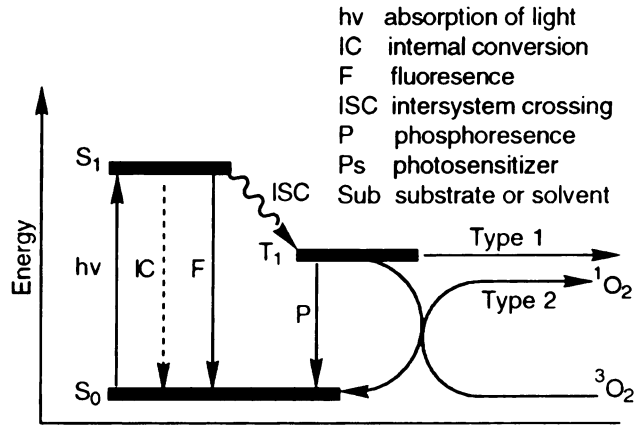
As it can been seen, there are a wide variety of synthetic techniques available for the preparation of porphyrins. Unfortunately, many target molecules often require the use of specialized reaction conditions. Subsequently, there are usually numerous variations and modifications for each general type of porphyrin synthesis. The MacDonald condensation was one of the major developments that allowed for the practical synthesis of large numbers of porphyrins. Two of the most versatile condensation techniques involve the use of b-bilenes and tripyrrene-a,c-biladienes. However, along with this versatility comes the added expense of preparing greater numbers of pyrrolic intermediates. The recent emergence of the "3 + 1" synthesis, has proven valuable in the preparation of unusual porphyrinoid structures.

## II. Photodynamic Therapy

Porphyrins have been utilized in various areas of research for many years. These compounds are often designed to study important biological functions such as oxygen binding,[26] electron transfer processes,[27] hydrogen bonding[28] and molecular recognition.[29] Supramolecular chemistry is another current area of interest.[30] Synthetic porphyrins are also valuable in the analysis of naturally occurring compounds.[31]

Porphyrins and porphyrin derivatives have also been extensively investigated in the treatment of cancer by a process known as photodynamic therapy (PDT). Photodynamic therapy involves the use of photosensitizing compounds that are introduced into the body and absorbed by malignant cells. These compounds are then photo-excited with light, which leads to the destruction of the diseased cells. The photophysical processes involved in this type of therapy are shown in Figure 1. The photosensitizer can initially absorb a photon to produce a short-lived excited singlet state. The excited photosensitizer can then return to the ground state by loss of energy through fluorescence, or it can be converted to an excited triplet state through intersystem crossing. The photosensitizer can undergo two types of processes in the triplet state, which lead to cellular damage. One process involves the direct reaction of the triplet state with the substrate or solvent to form radicals or radical ions. These radical species can then react with oxygen to give oxygenated products such as hydroxy radicals or superoxide anion radicals, which destroy the cells (Type 1 reaction).[32,33] The other process involves a direct energy transfer from the triplet state of the photosensitizer to oxygen, which produces singlet oxygen. The singlet oxygen can then react with important biomolecules such as cholesterol, tryptophan and guanine, which

leads to death of the cells (Type 2 reaction).[32,33] It is generally believed that most porphyrin sensitizers proceed through a Type 2 reaction mechanism. Whichever mechanism a sensitizer goes through, it is apparent that majority of the PDT processes are oxygen dependent.[34,35]




Type 1 $^3Ps + Sub \longrightarrow Ps^{+\cdot} + Sub^{-\cdot}$ (electron transfer)

$Sub^{-\cdot} + {}^3O_2 \longrightarrow Sub + O_2^{-\cdot} \longrightarrow HO^- + HO^\cdot$

Type 2 $^3Ps + {}^3O_2 \longrightarrow Ps + {}^1O_2$ (energy transfer)

$^1O_2$ + biomolecules $\longrightarrow$ cell death

Figure 1. Modified Jablonski Diagram for a Typical Photosensitizer

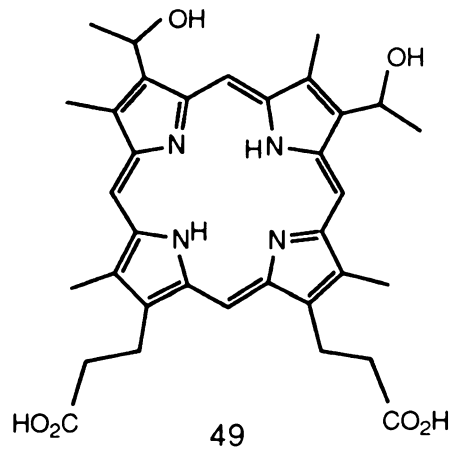
The mechanisms responsible for photosensitizer accumulation in diseased tissue are not readily understood. However, various hypotheses have been proposed.[36] One widely held theory suggests that the photosensitizer may be delivered to the cell through low density lipoproteins.[37] The actual method of tumor destruction has also been a topic of much discussion and is often dependent upon the type of photosensitizer utilized. There appears to be three different mechanisms by which tumor destruction can occur. These include direct damage to the tumor cells, damage to the vascular system of the

tumor, and macrophage mediated infiltration of the tumor.[36] The destruction pathway may also be dependent of how the photosensitizer is administered, as well as on the type of diseased tissue being treated.

The most widely studied synthetic photosensitizer is hematoporphyrin derivative (HpD).[38] This compound has been under extensive investigation since the early 1970's,[39] and can be readily prepared from a hematoporphyrin (49) in two steps. HpD is a complex mixture of porphyrins and higher molecular weight material, consisting of dimeric[40] and oligomeric[41] porphyrins. These dimers and oligomers contain ether,[42] ester,[43] and carbon-carbon[44] interporphyrin linkages. Also, the dimeric and oligomeric compounds are believed to be the most PDT effective components of HpD. HpD is enriched in these fractions and marketed under the name Photofrin.[45] The advantages of Photofrin include easy preparation and good water solubility. Unfortunately, it is also difficult to characterize all the components of this complex mixture. Another disadvantage is that Photofrin's longest wavelength absorption (630 nm) is weak, which hampers efficient utilization of the light during treatment. Skin phototoxicity may also last up to six weeks after injection.

There are several characteristics that are desirable in photodynamic compounds. These include long wavelength absorbances (>600 nm), low toxicity, high yielding singlet oxygen production, wide range effectiveness against various cancers, and selective tumor accumulation. A great deal of research has focused on the synthesis of compounds with these properties. Strong absorbances above 600 nm are desired, because the penetration of light through tissue increases with increasing wavelength. The interfering absorbances of biological compounds such as hemoglobin, are also minimal above 600 nm.[46] Therefore, compounds that absorb in the near infrared should ideally be more effective than Photofrin.[47] Consequently, new diode lasers are

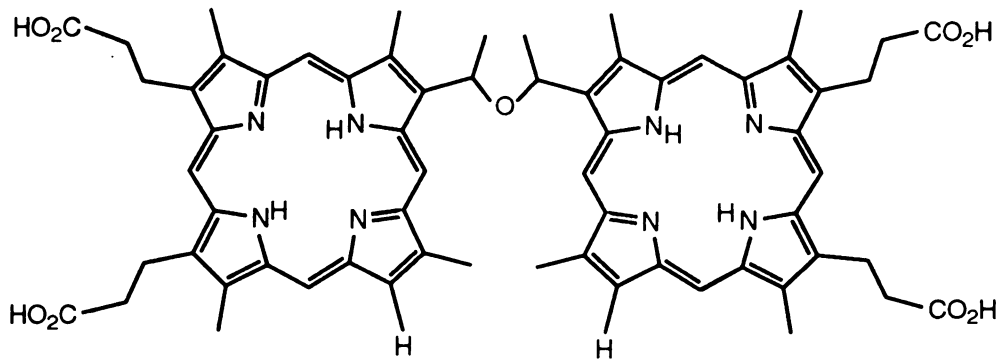
being designed to operate from >600 nm to 800 nm. These diode lasers also offer safety, cost, and ease of operation advantages over the argon-dye lasers previously used in photodynamic therapy.




49

As mentioned above, it is desirable to develop photosensitizers that have a high affinity for a wide variety of cancers and little uptake or retention in healthy cells. Unfortunately, these are probably the least understood and most difficult properties to manipulate in the development of PDT compounds. The majority of sensitizers studied to date are retained to some extent in normal tissue, which presents the potential for healthy cell damage.

Since dimeric compounds are believed to be one of the most effective constituents of Photofrin, many types of porphyrin dimers have been synthesized and tested in photodynamic therapy. In 1988, Pandey and Dougherty described the synthesis and photosensitizing activity of diporphyrin ether (50).[48] It was discovered that this compound possessed effective *in vivo* PDT activity, against SMT-F murine tumors. In general, dimers containing ether linkages have been found to be more active than ester-linked porphyrins of similar construction.

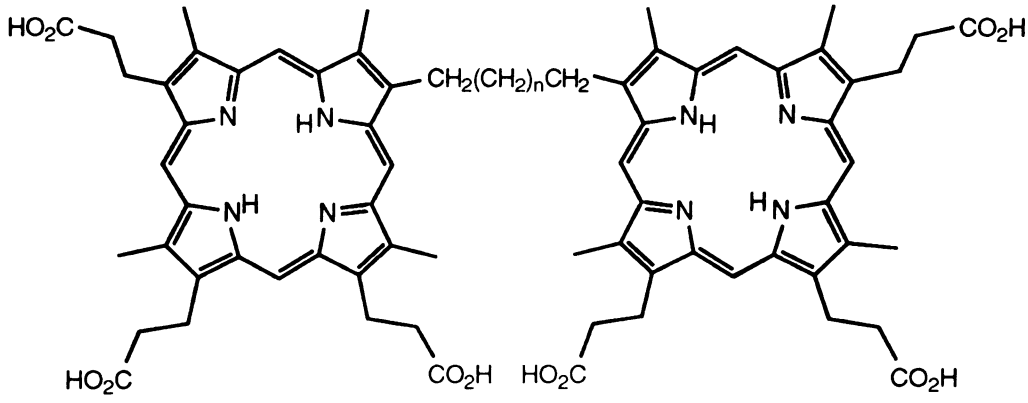


50

Porphyrin dimers (51a-c), have been tested against SMT-F murine neoplasms.[49] The experiments revealed that dimer (51b) was the most effective sensitizer. Compound (51b) was also determined to be shorter-lived than Photofrin. Dimer (51a) showed little or no sensitizing ability. A more recent report utilizing the radiation induced fibrosarcoma (RIF) tumor system however, revealed dimer (51c) as the most efficient photosensitizer of the three.[50] It was suggested that different mechanistic pathways were involved in the destruction of each tumor, which could explain the conflicting results.

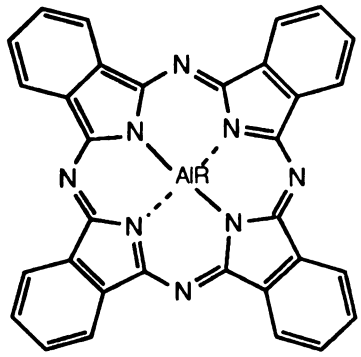
There have been many attempts to produce compounds that absorb light above 630 nm. Several examples include phthalocyanines, naphthalocyanines, chlorins and expanded porphyrin systems. Oleinick and coworkers have extensively studied phthalocyanines as photosensitizers in photodynamic therapy. Recently, several aluminum and silicon phthalocyanines were prepared and their phototoxicity toward hamster lung fibroblast cells were studied.[51] It was determined that compound (52a) and the methylated derivative (52b) were as effective as (52c) in destroying the cells, while compound (53) was more effective. Currently these researchers are continuing to develop other amine containing phthalocyanines, including cationic species. Phthalocyanine (52d) was recently found to be effective in the

killing of acute myeloid leukemia cells, *in vitro*.[52] The authors noted that there was preferential and significant destruction of the leukemia cells, as compared to the normal cells. It was suggested that compound (52d) may also have potential as a bone marrow purging agent.

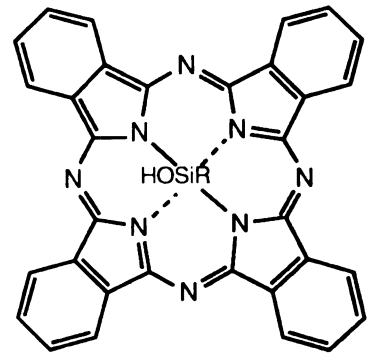



51a. n = 1 ($C_3$)
b. n = 4 ($C_6$)
c. n = 9 ($C_{13}$)

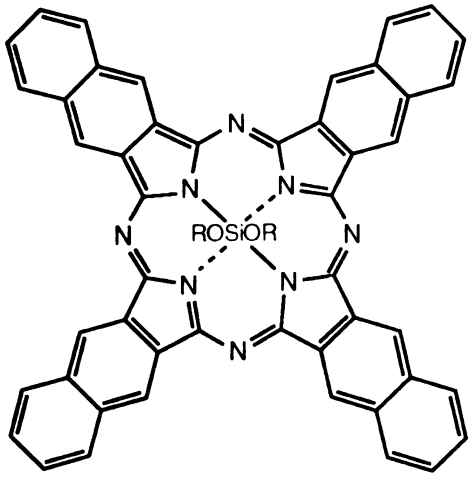
In 1987, Rogers and Kenney introduced naphthalocyanine (54a) as a possible PDT compound.[53] The photodynamic properties of the related naphthalocyanine (54b) have also been investigated.[54] It was discovered that this compound produced results comparable Photofrin, against murine foot tumors, at only 6% of the concentration (0.3 mg/kg vs 5 mg/kg). However, compound (54b) also possessed several of the negative traits associated with Photofrin. Zinc naphthocyanines (55a-d), have also been investigated.[55] The *in vivo* PDT efficacy of these compounds, against lung cancer cells, was found to follow the order (55a), (55b)>>(55c)>>(55d). It was also noted however, that the hydrophobic nature of these compounds required their incorporation into liposomes before injection.



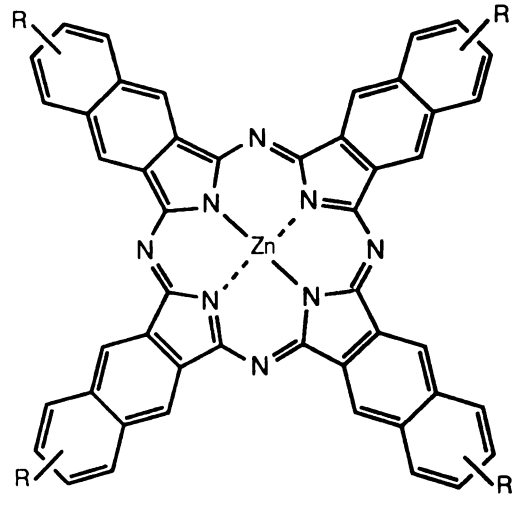

52a. R = $OSi(CH_3)_2(CH_2)_3N(CH_3)_2$
b. R = $OSi(CH_3)_2(CH_2)_3N^+(CH_3)_3I^-$
c. R = OH
d. R = Cl




53 R = $OSi(CH_3)_2(CH_2)_3N(CH_3)_2$

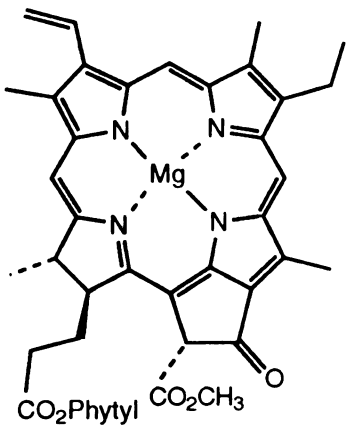



54a. R = $Si(n\text{-}C_6H_{13})_3$
b. R = $Si(CH_3)_2(CH_2)_3CO_2Me$

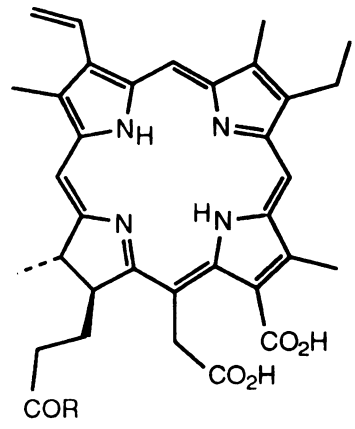



55a. R = $OCH_3$
b. R = $NHCOCH_3$
c. R = H
d. R = $NH_2$

Various chlorin structures have been examined as possible PDT agents, and many different synthetic approaches have been utilized in their preparation. Also, these compounds usually have strong absorbances between 650 and 700 nm. Naturally occurring chlorophyll *a* (3a) is not an ideal PDT photosensitizer, due to its limited stability. However, several derivatives of chlorophyll *a* have been prepared and studied for potential PDT activity. Chlorin $e_6$ (56a), which is produced by the treatment of chlorophyll *a* with base, has been found to have moderate *in vivo* activity against a number of transplantable rat tumors.[56] The highest photosensitizer concentration in these tumors was found to occur 12 to 18 hours after injection. Various derivatives of chlorin $e_6$ have also been investigated. Monoaspartyl chlorin $e_6$ (MACE) (56b) has been shown to be an effective PDT agent in animal studies.[57] Recent *in vivo* studies revealed that MACE was more effective than HpD in the treatment of human cholangiocarcinoma cells, at all concentrations studied (0.2-10 mg/Kg).[58] The duration of skin phototoxicity was also found to be shorter than HpD. Clinical testing of MACE is currently under investigation.

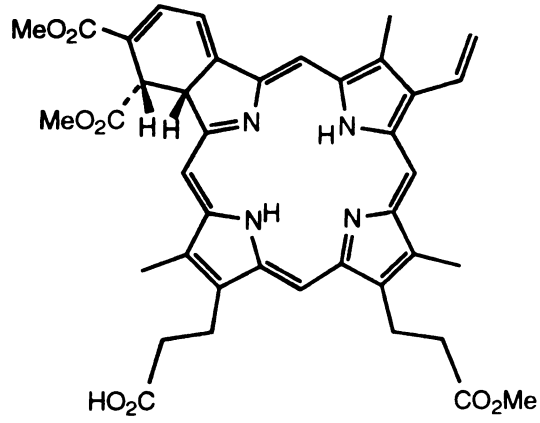



3a.

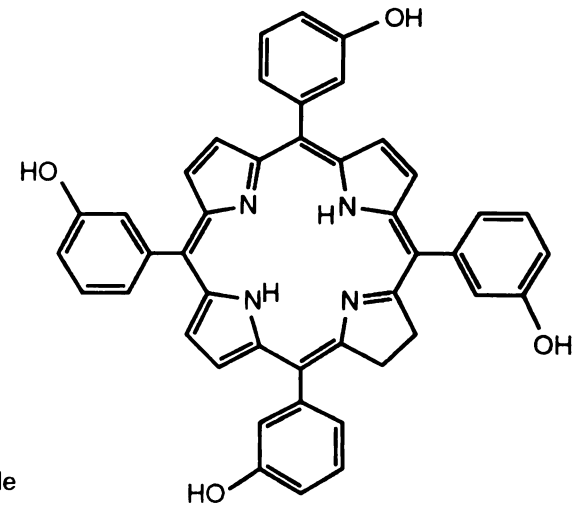



56a. R = OH
b. R = (L)-NHCH($CO_2H$)$CH_2CO_2H$

Amphiphilic chlorin sensitizers have also received a great deal of attention, due to the fact that this class of compounds has given rise to some of the most potent PDT compounds. Two major examples are benzoporphyrin derivative monoacid ring A (BPD-MA) (57) and meso-tetrakis(m-hydroxyphenyl) chlorin (m-THPC) (58). The *in vitro*[59] and *in vivo*[60] studies that have been carried out on BPD-MA, have shown it to be a promising photosensitizer. Currently, BPD-MA is undergoing phase II clinical trials for the treatment of skin lesions. It has also recently been shown that BPD-MA may be useful in the treatment of rheumatoid arthritis.[61]




57




58

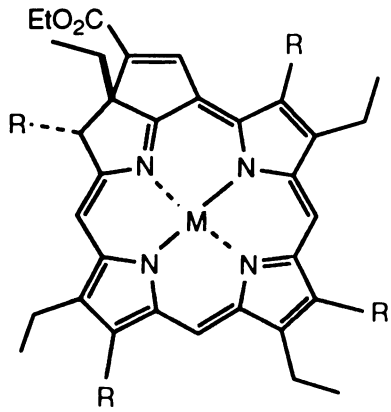
In the 1980's, Bonnet and coworkers began to investigate meso-tetraaryl porphyrins as possible PDT agents. The limited success obtained with these porphyrins prompted investigations into meso-tetraaryl chlorins and isobacteriochlorins.[62] The comparison of over 100 photosensitizers, based upon tumor selectivity and PDT efficacy, revealed m-THPC (58) as the most promising compound.[63] Advantages of m-THPC include a strong absorption at 652 nm, low dark phototoxicity, and non mutagenic properties. It can also be

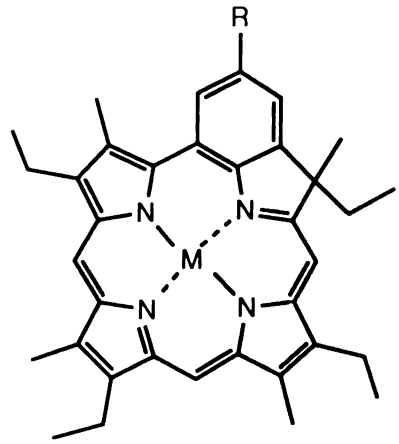
readily prepared from pyrrole and 3-methoxybenzaldehyde in three steps. The formulation of m-THPC for delivery is also simple and consists of dissolving the chlorin in a mixture of ethanol, polyethylene glycol and water. The skin phototoxicity associated with m-THPC is also less than Photofrin. The results from experimental and clinical trials are encouraging and have shown m-THPC to be an effective photosensitizer.[64] Phase II clinical trials are presently in progress for the treatment of patients afflicted with chest malignancies[65] and cancers of the head and neck.[66] Recently, m-THPC has also been shown to be effective in the treatment of patients with oral cancers.[67]

Morgan and coworkers have examined purpurins (59a-d) for PDT activity.[68] Animal studies revealed that the efficacy of these compounds followed the order (59a)>(59b)>(59c)>(59d). Also, the maximum therapeutic effect of (59a) was found to be 24 hours after injection. Skin phototoxicity was also less than with HpD. Currently, compound (59a) is undergoing clinical testing for the treatment of various skin cancers[69] and AIDS-associated skin lesions.[70]

Kessel has examined the photobiological properties of etiobenzochlorins (60a-c) in cell cultures.[71] It was found that chlorin-sulfonate (60a) was the most damaging to the cells (murine leukemia), while tin chlorin-sulfonate (60b) was much less active. The parent chlorin (60c), was found to have almost no activity.

Expanded porphyrins are also being investigated in photodynamic therapy. Compounds in this category include sapphyrins and heterosapphyrins (61a-c).[72] These compounds usually have absorbances above 700 nm, which is ideally suited for PDT. However, the results of biological testing are currently limited. This is due in part to the fact that it has only been in the last several years that these compounds have been produced in efficient manners.



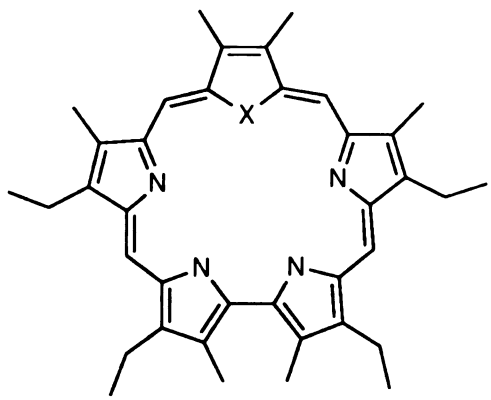

59a. R = $CH_3$, M = Sn•2Cl
b. R = $CH_2CH_3$, M = Sn•2Cl
c. R = $CH_3$, M = Zn
d. R = $CH_2CH_3$, M = Zn

60a. R = $SO_3H$, M = 2H
b. R = $SO_3H$, M = Sn•2Cl
c. R = H, M = 2H

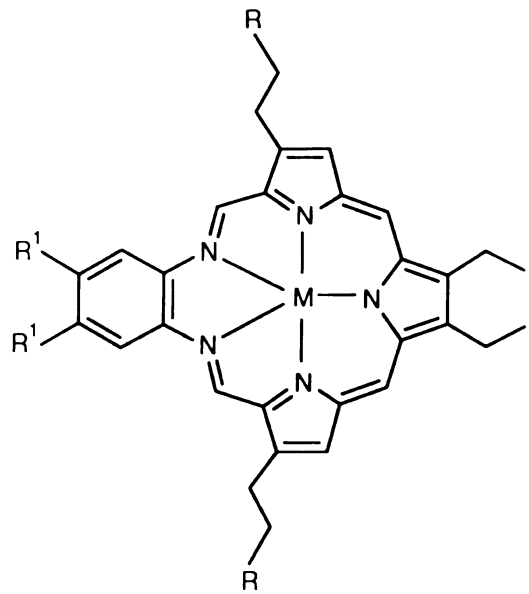
The most widely studied expanded porphyrins, are pentaaza texaphyrins such as (62a,b).[73] These compounds possess long wavelength absorbances above 700 nm and contain internal cores that are approximately 20% larger than porphyrin. This larger internal cavity allows for the complexation of metals such as Lu(III) and Gd(III), that are too big to be inserted into porphyrin. Other metals that can also bind with texaphyrin include Cd(II), Zn(II), Hg(II), La(III), Ce(III), and Eu(III). The favorable properties associated with texaphyrins, have driven investigations into their use in such areas as magnetic resonance imaging (MRI) and photodynamic therapy. For example, gadolinium texaphyrin (62a) has been shown to be an effective MRI contrasting agent in a number of animal studies.[74] The studies also revealed that the doses required for efficient tumor and target organ enhancement, did not result in any serious toxicity.

Lutetium texaphyrins have shown potential in photodynamic therapy. Texaphyrin (62b) is a water soluble photosensitizer that has a strong absorption at 732 nm. This compound has been tested in laboratory mice, against STM-F

tumors of varying size.[75] The PDT efficacy was found to be greatest when photoirradiation was administered three to five hours after injection. In addition, no skin phototoxicity was observed in any of the animals. Texaphyrin (62b) has also shown effective *in vivo* destruction of transplantable colon cancer tumors,[76] and is currently undergoing clinical trials in patients with metastatic cancers.[77] Several advantages that expanded macrocycles have over porphyrins include longer wavelength absorbances, and the ability to complex to a wider variety of metals. However, these compounds are usually harder to synthesize than their porphyrin counterparts.



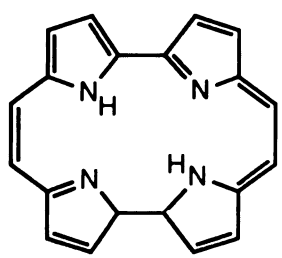

61a. R = $CH_2CH_3$, X = NH
b. R = H, X = O
c. R = H, X = S



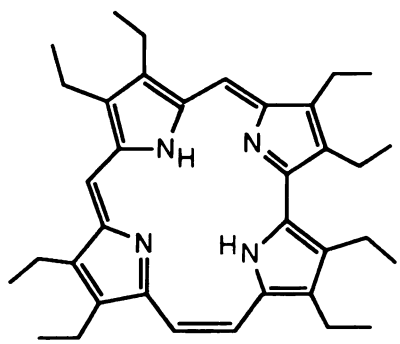

62a. R = $CH_2OH$, $R^1$ = $O(CH_2)_3OH$
M = Gd•$(OAc)_2$
b. R = $CH_2OH$, $R^1$ = $(OCH_2CH_2)_3OCH_3$
M = Lu•$(OAc)_2$

Isomeric porphyrin structures are also currently receiving considerable attention as possible photosensitizers. Of the seven possible porphyrin isomers that contain an $N_4$ coordination site, three have been successfully synthesized.

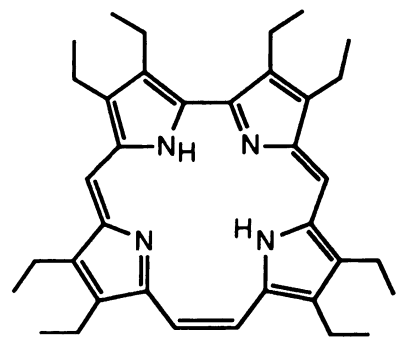
These include porphycene (63),[78] hemiporphycene (64)[79] and corrphycene (65).[80] Porphycene is the most widely studied isomer to date, and over the last several years a wealth of information has been gathered on these compounds.[81] This includes a recent report on the photophysical properties of close to forty derivatized porphycenes.[82] Porphycenes generally possess the characteristics common to efficient PDT sensitizers, which include strong absorbances above 600 nm and high yields of singlet oxygen. Several porphycenes have also shown efficient tumor localization properties.[83]



63



64

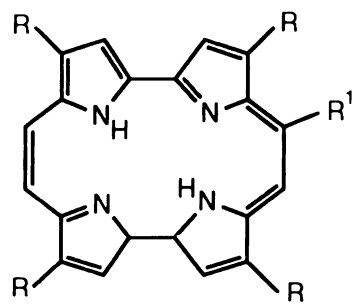


65

One porphycene showing promise as a photosensitizer is 9-acetoxy-2,7,12,17-tetrakis(β-methoxyethyl)-porphycene (ATMPn) (66a). ATMPn has exhibited fast intracellular uptake and high photodynamic efficacy against human cancer cells, *in vitro*.[84] The incorporation of β-methoxyethyl substituents, had previously been shown to enhance the photodynamic activity of a number compounds.[85] Recent *in vivo* studies using amelantonic melanomas, revealed ATMPn to be superior to Photofrin under the same dosage concentrations (2.8 μmole/Kg).[86] Maximum tumor destruction occured when irradiation was carried out one minute after injection. Also, the majority of the photosensitizer was found to be localized in the tumor microcirculation. The fast pharmacokinetics of ATMPn should make it possible to conduct a full PDT

treatment cycle within one session. ATMPn may also have potential as a topical photosensitizer.

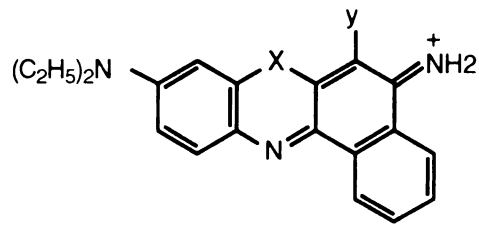
A second example is tetraphenylporphycene (TPPo) (66b). This compound has an absorption maxima at 659 nm (log $\varepsilon$ = 4.70) and photoproduces singlet oxygen with a quantum yield of approximately 0.25.[87] TPPo has also been found to be destructive to cancer cells, while possessing low dark phototoxicity. Further research into similar porphycenes will likely involve the optimization of the photophysical properties, through the derivatization of the phenyl substituents.




66a. R = $CH_2CH_2OCH_3$, $R^1$ = $OCOCH_3$
b. R = Ph, $R^1$ = H

As mentioned above, it is advantageous to design photosensitizing compounds that selectively accumulate in diseased tissue. Cationic dyes are one class of sensitizers that are showing potential in this area. Non-porphyrin compounds such as Nile Blue A (NBA)(67a) and derivatives (67b,c), have been shown to be retained preferentially in the mitochondria of cancer cells.[88] The selective accumulation is believed to arise from the attraction of these positive species, to the negatively charged cell and mitochondrial membranes of the diseased tissue. These compounds also absorb between 620 and 660 nm, which is the most common region utilized in PDT therapy. NBA itself is not an

effective photosensitizer, due to its low ability to produce singlet oxygen. NBA derivatives such as (67b,c), possess improved singlet oxygen yields and increased *in vitro* PDT activity. A number of these compounds have also shown relatively selective subcutaneous tumor destruction in rodents, as compared to overlying skin.[89] However, it was also noted that tumor destruction was often incomplete.

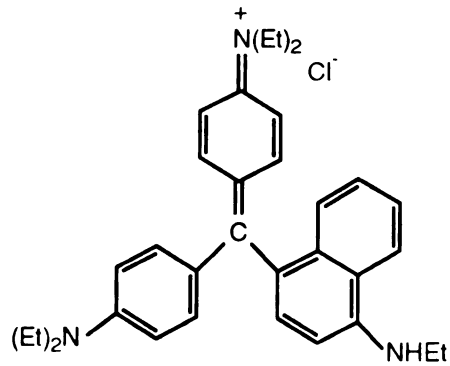



67a. X = O, Y = H
b. X = O, Y = Br
c. X = S, Y = I

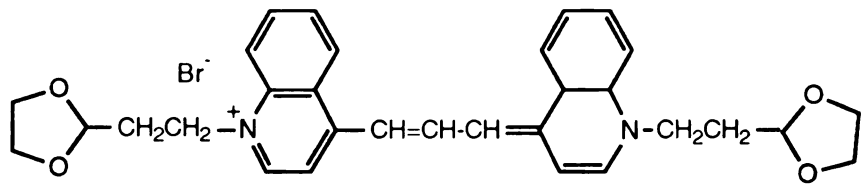
Other non-porphyrin cationic dyes that may be useful in photodynamic therapy include triaryl methanes such as Victoria Blue BO (68), and cyanines such as N,N'-bis(2-ethyl-1,3-dioxalene)kryptocyanine (EDKC) (69). Dye (68) has shown 99% effectiveness against two human leukemia cell lines.[90] Compound (69) appears to have oxygen independent phototoxicity, but also is relatively unstable in solution.[91]

The PDT properties of several cationic porphyrins have also been investigated. Porphyrin (70) was found to have moderate *in vivo* activity against murine fibrosarcoma tumors.[92] The mode of tumor destruction was attributed to damage of the vascular support. Meso-tetra(4N-methylpyridyl)porphine ($T_4$MPyP) (71) was found to cause substantial tumor necrosis in mice transplanted with fibrosarcoma tissue.[93] The destruction facilitated by $T_4$MPyP

was characterized by a slow development of damage to the malignant cells and vascular system.

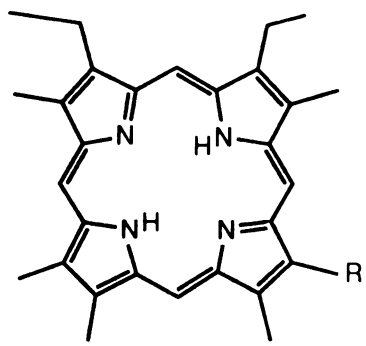


68

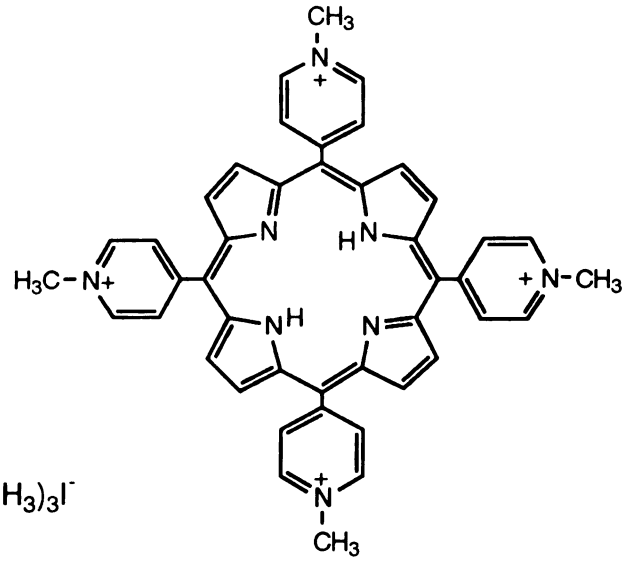


69

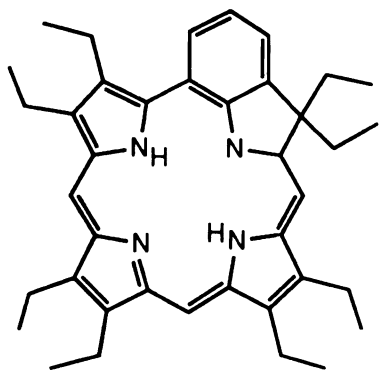
Morgan and coworkers have described a series of interesting cationic photosensitizers derived from benzochlorin (72a).[94] For example, copper imminium salt (73c) contains a strong absorption at 755 nm and is unexpectedly stable towards hydrolysis. Chlorin (73c) has also been shown to be an effective photosensitizer against subcutaneous tumors in rats.[94] In these studies, the mode of destruction was attributed to a rapid decrease in tumor blood flow. This compound was also found to be only a minimal photosensitizer of healthy tissue. The results for chlorin (73c) are promising, since it appears to be one of the first selective *in vivo* cationic photosensitizers. It is also interesting to note that compound (73) requires molecular oxygen to facilitate cellular death, even though the triplet lifetime is too short to produce singlet oxygen.[95]



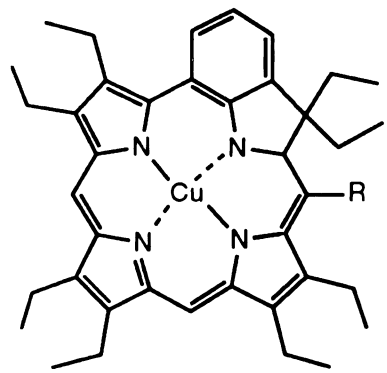
70 R = $(CH_2)_2CONH(CH_2)_2N^+(CH_3)_3I^-$



71



72a.



73c. R = $CH{=}N^+(CH_3)_2Cl^-$

Photodynamic therapy is a ever expanding area of chemistry and biology. A continuing goal in photodynamic therapy, is the development of selectively accumulating compounds that absorb well into the red region of the visible spectrum. Cationic structures may prove valuable in the eventual fulfillment of this goal. New cationic structures are also expected to be more water soluble than previous PDT drugs, which should allow for greater efficiency in the delivery of the photosensitizer.

## IV. Objectives of the present work

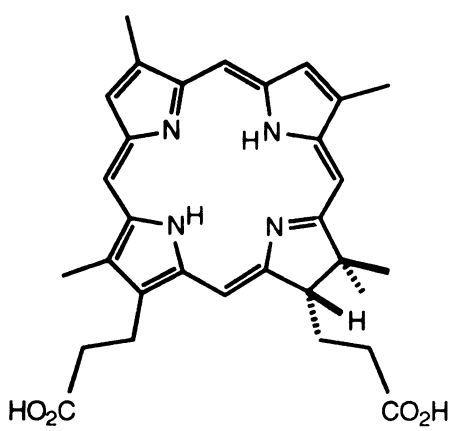
The principal objectives of this work were to design a variety of porphyrinoid structures, for utilization as heme model compounds and potential PDT photosensitizers. Through the use of the various synthetic methods described above, the preparation of a number of chlorins (Chapters 2 and 4), meso-substituted porphyrins (Chapters 3 and 6) and porphyrin analogues (Chapter 5) are described in this study. As a secondary goal, it was envisioned that these investigations would lead to new and improved methodologies for the synthesis of porphyrins and porphyrin-type compounds. The results reported in this study should give further insights into a number of natural heme compounds, and interesting porphyrinoid structures for use in photodynamic therapy.

# CHAPTER 2

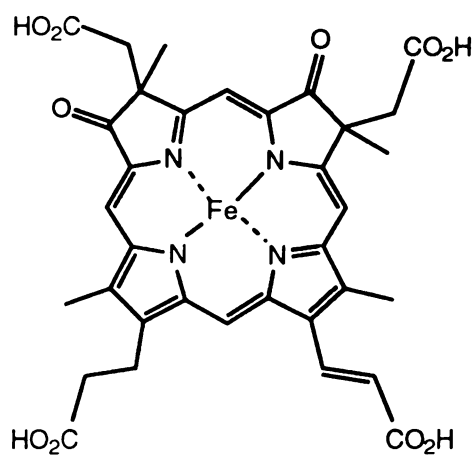
# SYNTHETIC MODELS OF HEME D

## I. Introduction

As stated in Chapter 1, the majority of prosthetic groups found in naturally occurring hemoproteins are iron porphyrins closely related to protoheme IX. However, an increasing number of animals and aerobic bacteria have been found to contain tetrapyrrolic macrocycles based upon C-substituted chlorins and isobacteriochlorins. Several examples include bonellin (74) isolated from the worm *Bonellia viridis*,[96] heme $d_1$ (75) from *Pseudemonas aeruginesa*,[97] Faktor I (76) from $B_{12}$ producing *Clostridium tetanomorphum* [98] and heme *d* (77?) from *Escherichia coli* and various other bacteria.[99]



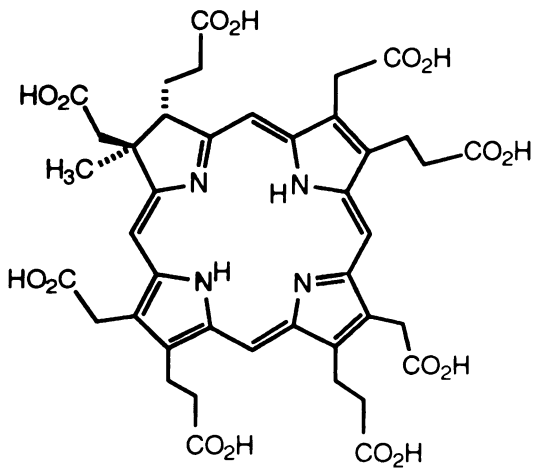

74



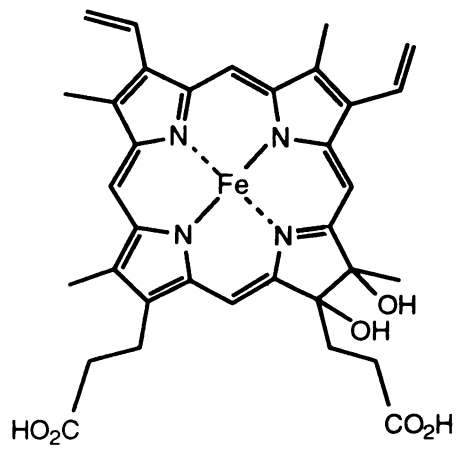

75

Heme $d_1$ and heme *d* have been extensively studied. Heme $d_1$ is involved in the reduction of nitrite to nitric oxide, while heme *d* is known to catalyze the reduction of oxygen to water. However, the exact structure of heme

*d* has still remained uncertain seventy years after its first observation.[100] In the 1950s, Barrett obtained heme *d* from *Aerobacter aerogenes* and proposed compound (78) as the structure.[101] For nearly thirty years, chlorin (78) was generally accepted as the correct structure and served as a comparison model for other green bacterial hemes.

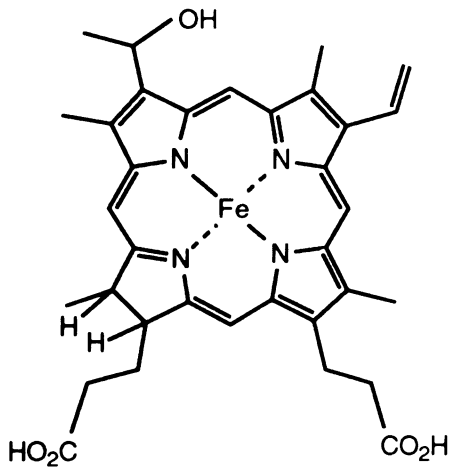



76

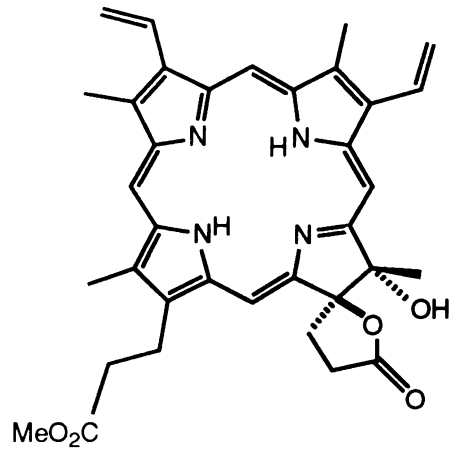



77

In 1985 however, Timkovitch and coworkers isolated the terminal oxidase protein from *Eschesichia coli* and tentatively assigned compound (79) as the structure of the heme *d* prosthetic group.[99] This structural elucidation was based upon $^1H$ NMR, IR, UV/visible and mass spectral analysis of the esterified and demetallated heme. Evidence for the unique spiro-γ lactone was based largely on an intense IR absorption at 1782 $cm^{-1}$. However, the authors also pointed out that there was uncertainty on whether or not the lactone was actually a result of isolation techniques. Further studies revealed that this intense IR absorption was absent, when the natural compound was not subjected to esterification or demetallation.[102] This lended support to compound (77) as the actual structure of heme *d*.

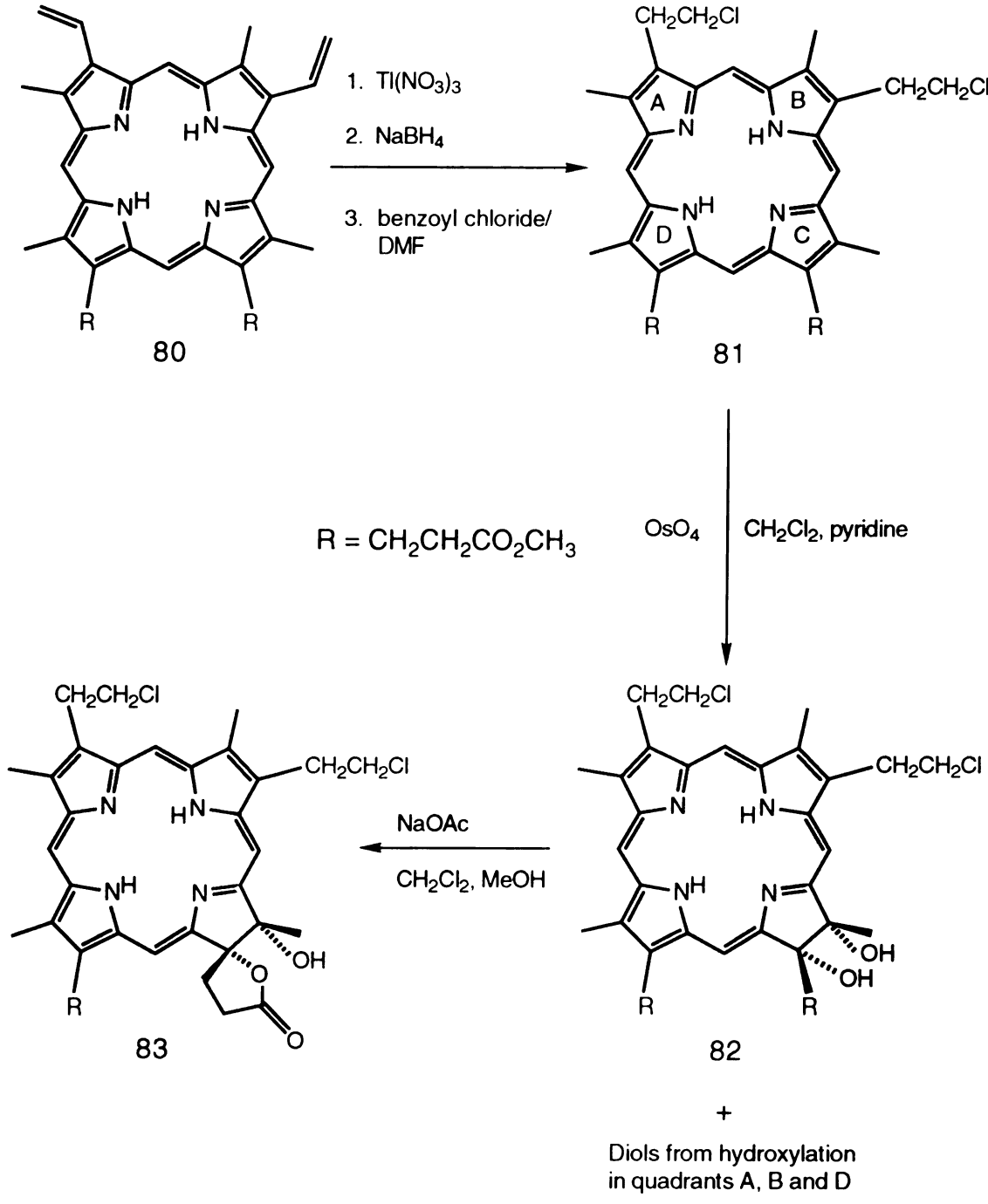



78

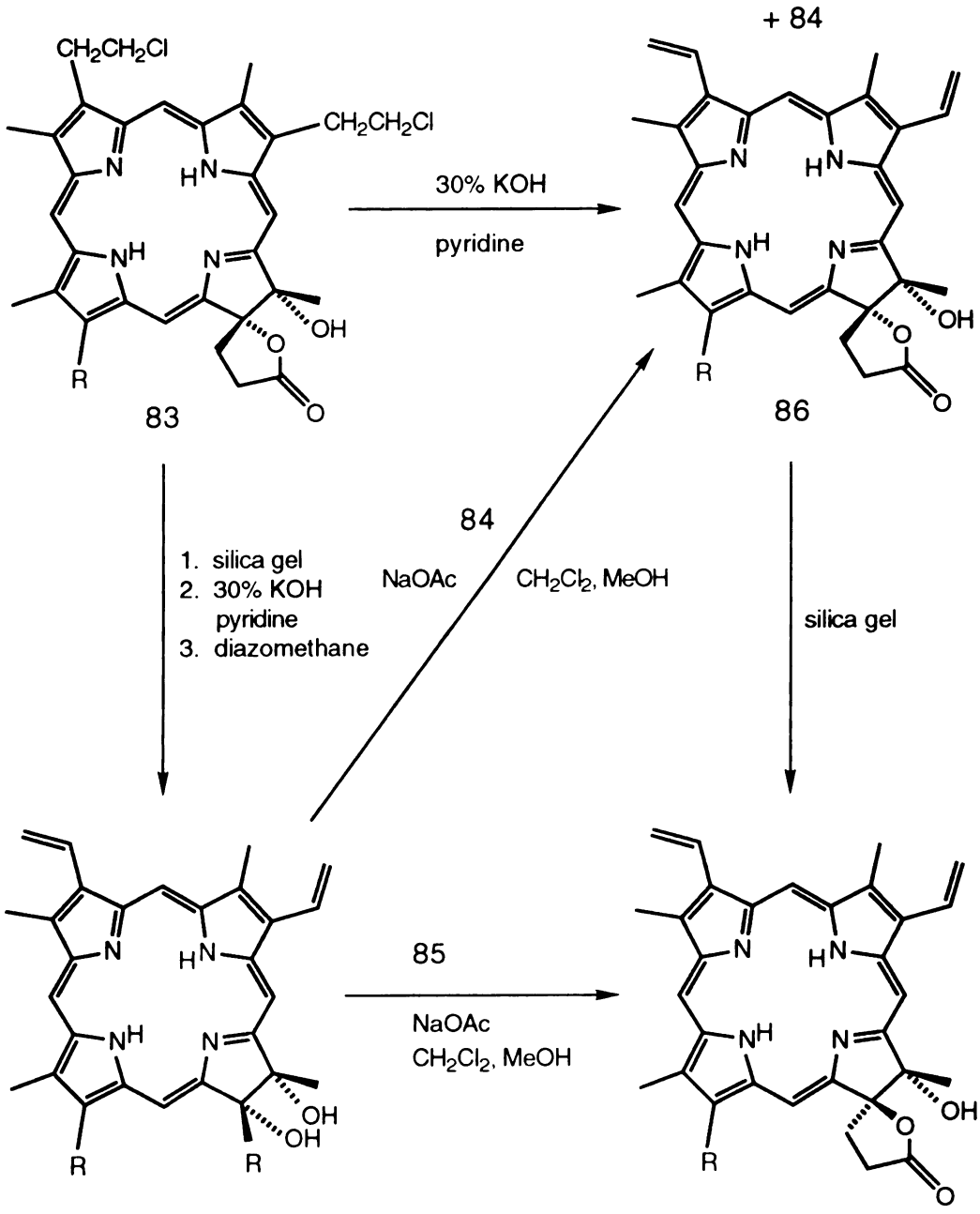



79

Lactochlorins (79) and (86), as well as the nonlactonized forms (84) and (85), were previously prepared by the syntheses outlined in Schemes 13 and 14.[103] Protoporphyrin IX methyl ester (80), was initially transformed into compound (81). Porphyrin (81) was then treated with osmium tetroxide to give a mixture of four hydroxylated products, including chlorin (82). The separation of these compounds by chromatography and subsequent lactonization of compound (82) with sodium acetate, afforded chlorin (83) (Scheme 13). Compound (83) was treated with pyridine/KOH to hydrolyze the lactone and then with diazomethane to give (84). The trans-diastereomer (85) was produced by the epimerization of compound (83) with silica gel, followed by basic hydrolysis of the lactone and subsequent esterification with diazomethane. Compounds (84) and (85) were then lactonized with sodium acetate to give chlorins (86) and (79), respectively. Alternatively, the treatment of compound (83) with pyridine/KOH led to a mixture of compounds (84) and (86). The subsequent separation of these chlorins and epimerization of (86) with silica gel, also afforded (79) (Scheme 14).




Scheme 13. Synthesis of Chlorin (83)



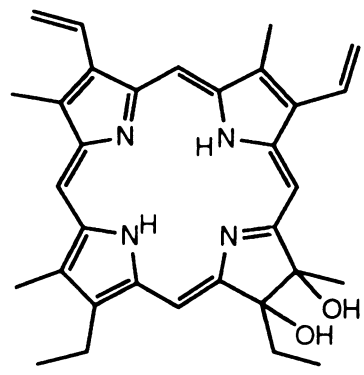

**84** (shown; cis) steps 2 and 3
**85** (not shown; trans) steps 1-3

$R = CH_2CH_2CO_2CH_3$

Scheme 14. Synthesis of Chlorins (79) and (84-86)

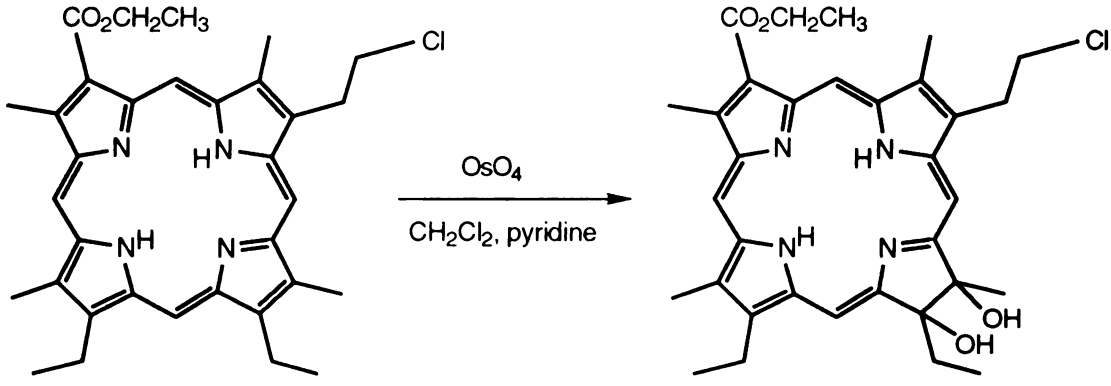
The NMR and HPLC data of compound (79) was basically indistinguishable from Timkovich's lactochlorin, which confirmed the proposed structure. Unfortunately, the limited quantities of chlorins (79) and (84-86) prevented detailed investigations into these compounds and their metal complexes. Also, standard metallation techniques would have most likely induced lactonization in chlorins (84) and (85). If sufficient amounts of material were available, it may have been possible to metallate the spirolactones and then proceed with the lactone hydrolysis. However, purification and/or esterification of the resulting complexes would have probably been difficult without reforming the spirolactones. As mentioned above, the IR data of the unesterified heme *d* seemed to suggest that the spirolactone was a result of isolation techniques.

The continued uncertainty in the actual structure of heme *d*, made it necessary to proceed with investigations into the synthesis of possible model compounds.[104] To alleviate the problem of lactone formation, it was determined that a model compound without the propionic acid side chains should be prepared. Chlorin (99) was chosen as a suitable candidate due to its structural similarity to compound (77). It was expected that studies of this compound would give further insights into the actual structure of heme *d*.




99

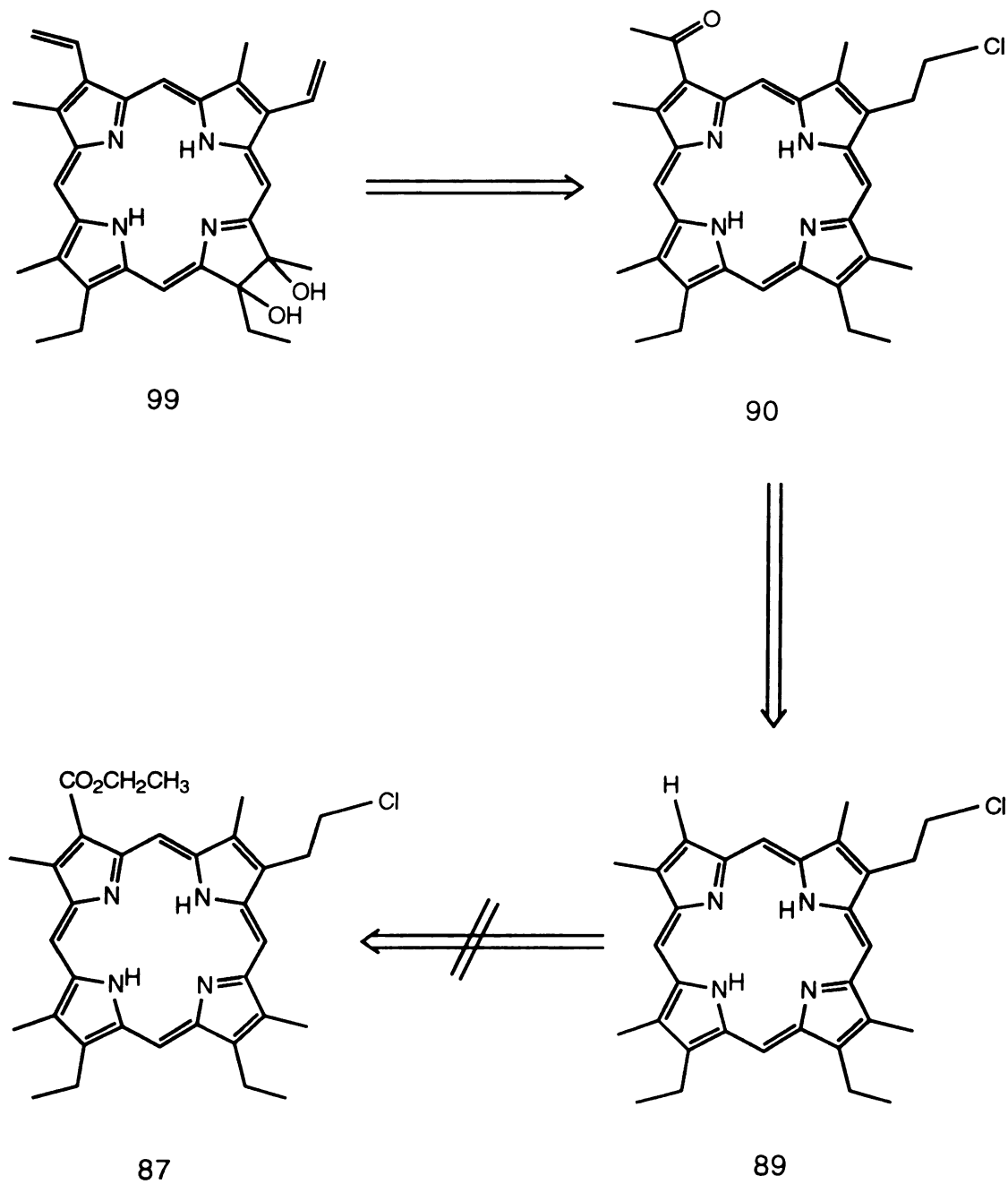
As stated above, the hydroxylation of (81) resulted in a mixture of four products. In order to avoid a similar situation during the preparation of the (99), directed hydroxylation techniques were initially investigated for use in the synthesis. Smith has reported that an electron withdrawing group, will direct osmium tetroxide hydroxylation to the quadrant opposite the one containing that group.[105] This phenomena was tested using compound (87) (Scheme 15).[104] It was determined that the electron withdrawing nature of the ester group did lead to regiospecific hydroxylation and exclusively gave compound (88), as shown by NOE experiments.




87 88

Scheme 15. Synthesis of Diol (88)

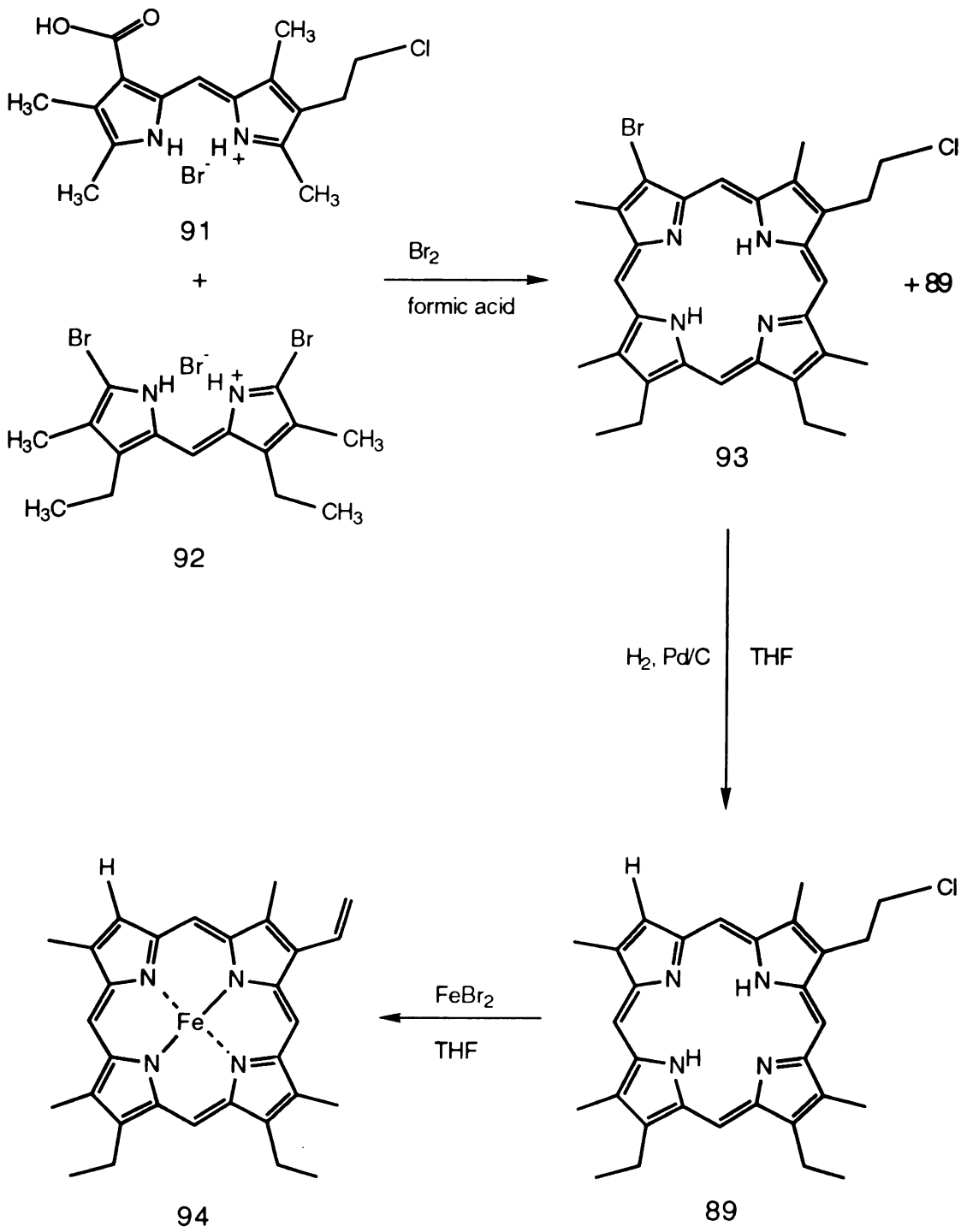
It was envisioned that chlorin (99) could be prepared from the compounds shown in Scheme 16. Porphyrin (90) possessed all the features required for the synthesis of (99). The main purpose of acetyl group was to direct hydroxylation. Also, the acetyl and chloroethyl groups were suitable precursors to the vinyl substituents. As shown in the reaction scheme, porphyrin (89) was required for the synthesis of (90). Unfortunately, compound (89) could not be prepared through the hydrolysis of (87). In order to synthesize chlorin (99) by this method, an alternate route to compound (89) was required.



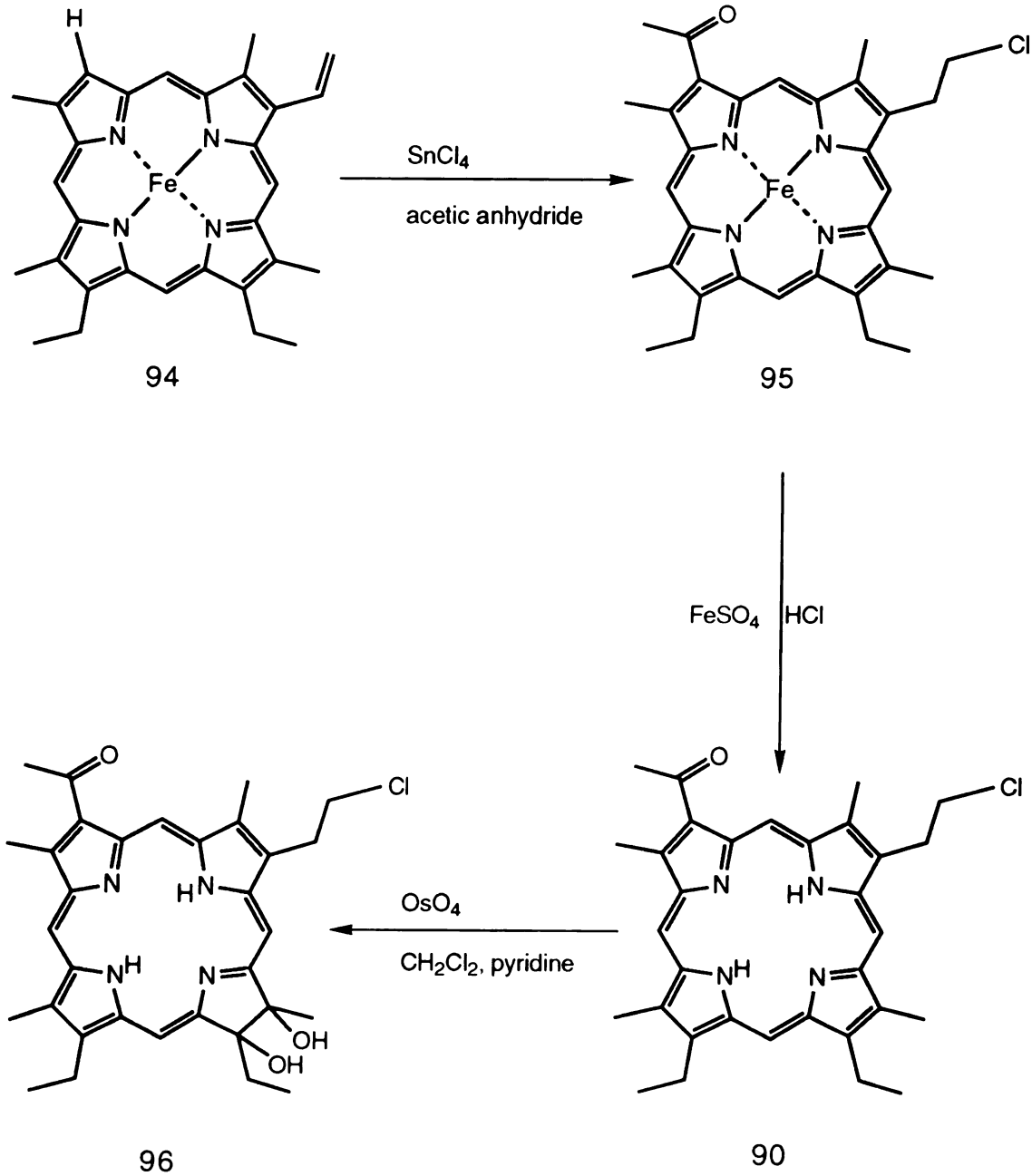

Scheme 16. Retrosynthetic Analysis of Chlorin (99)

The original synthetic routes used to prepare porphyrin (89) and divinyl chlorin (99), are outlined in Schemes 17-19.[104] Dipyrrylmethenes (91) and (92) were refluxed in a mixture of formic acid and bromine, to give porphyrin (93) and a small amount of porphyrin (89). The bromine substituent of porphyrin (93) was then deliberately cleaved, in the presence of hydrogen and palladium/charcoal, to give (89). The subsequent treatment of (89) with ferric bromide, afforded iron porphyrin (94) (Scheme 17). The iron complex was acetylated with stannic chloride and acetic anhydride to give (95). The iron was then removed with ferric sulfate to afford compound (90). Porphyrin (90) was treated with osmium tetroxide to give chlorin (96) (Scheme 18). Compound (96) was then heated in a mixture of DBU and DMF to eliminate the chloroethyl group and afford chlorin (97). The acetyl group of (97) was reduced with sodium borohydride to give chlorin (98). Compound (98) was then heated in DMF, which tentatively afforded the desired chlorin (99) (Scheme 19).

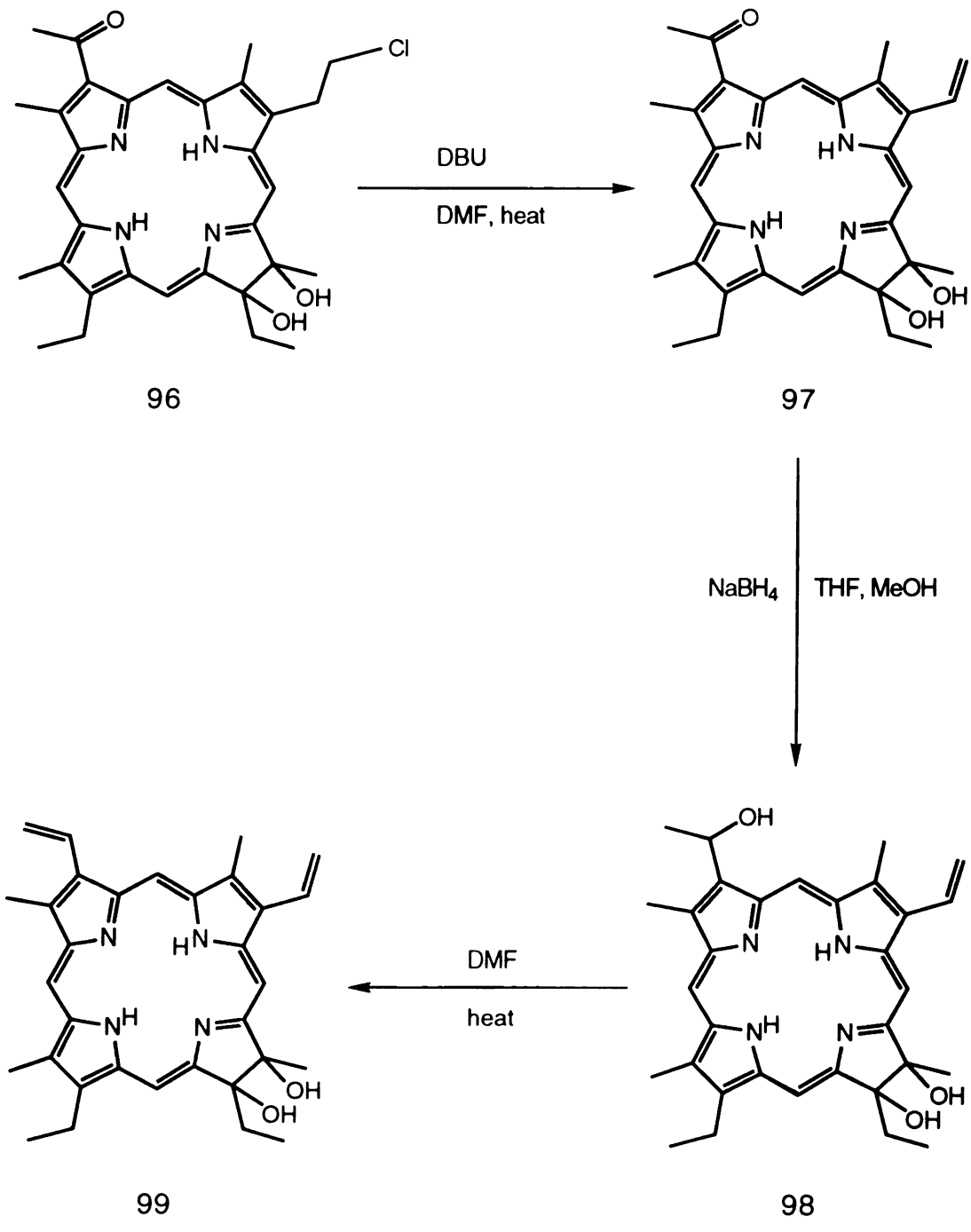
As it turns out, there were several problems associated with this reaction sequence. The condensation of compounds (91) and (92), afforded (93) in only 6% yield. The electron withdrawing nature of the carboxylic acid group contained in (91), most likely hampered the reaction and resulted in the low yield.[106] The hydrogenolysis of (93) to remove the bromine, also proved troublesome and rarely proceeded to completion. Porphyrinogen was produced during the reaction, which had to be oxidized back to porphyrin. This led to only a 49% yield of compound (89) after chromatography. As a result, only a small amount of porphyrin was available for the successive transformations of the reaction sequence. This precluded the optimization of the reaction conditions, and the full characterization of divinyl compound (99).




Scheme 17. Synthesis of Porphyrin (94)

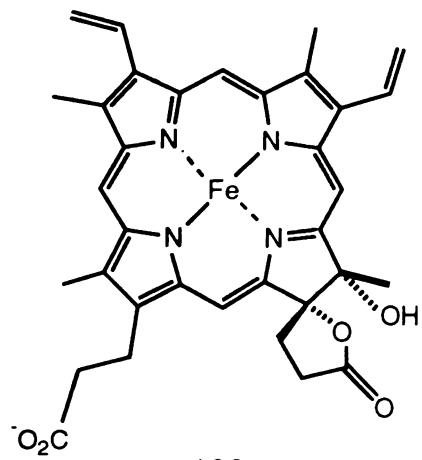



Scheme 18. Synthesis of Chlorin (96)

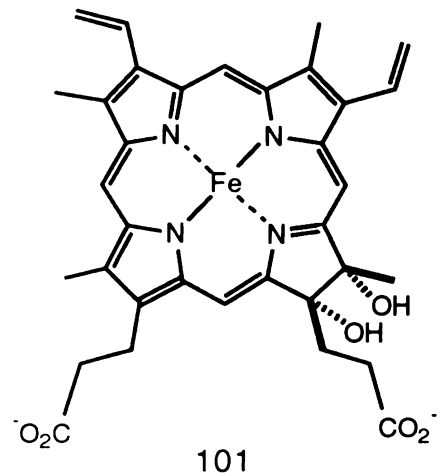



Scheme 19. Initial Synthesis of Chlorin (99)

Since the original studies of compound (99) were carried out, information has been presented that suggests heme *d* does in fact contain a spiro-γ lactone substituent. In 1996, Murshudov and coworkers[107] were able to obtain X-ray crystal structures of the enzymes *Penicillium vitale catalase* (PVC) (native enzyme and 3-amino-1,2,4-triazole complex) and *Eschericha coli* catalase hydroperoxidase II (HP II) (azide complex). The data revealed that both enzymes contained heme *d* prosthetic groups consistent with structure (100). Until this report, it was assumed that the heme *d* species of HP II was *cis*-diol (101).[108] Even though the crystal structures show the presence of spiro-lactones, it is possible that the hemes were altered during the preparation of the enzymes for X-ray analysis. Therefore, diols such as (99) are still useful as heme *d* model compounds. We then decided to investigate alternate routes to chlorin (99).



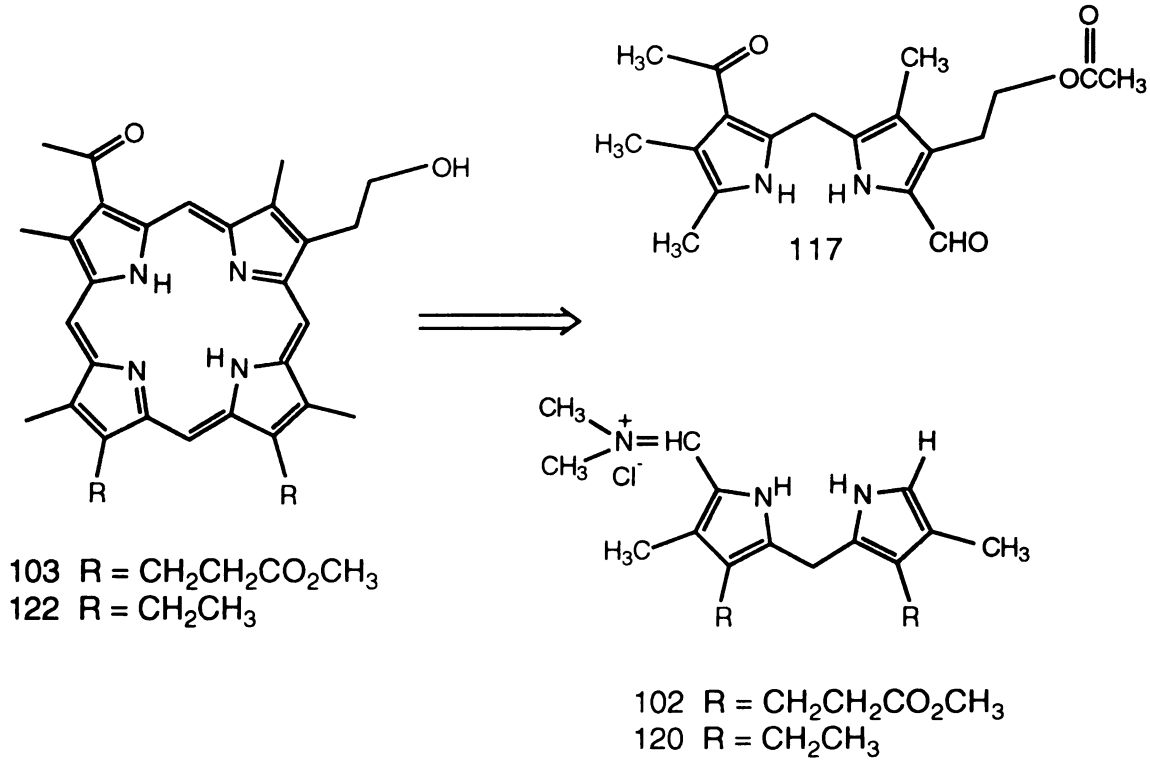

100




101

## II. Results and Discussion

One of the initial goals in our investigations was to synthesize compound (90), through more efficient methods than those originally developed in the study of chlorin (99). Fortunately, we discovered that porphyrin (103) contained a framework similar to that of compound (90).[109] It was envisioned that the replacement of the southern half of porphyrin (103) with dipyrrole (120), would lead to porphyrin (122) (Scheme 20). We thought that this compound could then be converted to porphyrin (90) and further transformed into chlorin (99), through the use of the techniques described above.




103 R = $CH_2CH_2CO_2CH_3$
122 R = $CH_2CH_3$

102 R = $CH_2CH_2CO_2CH_3$
120 R = $CH_2CH_3$

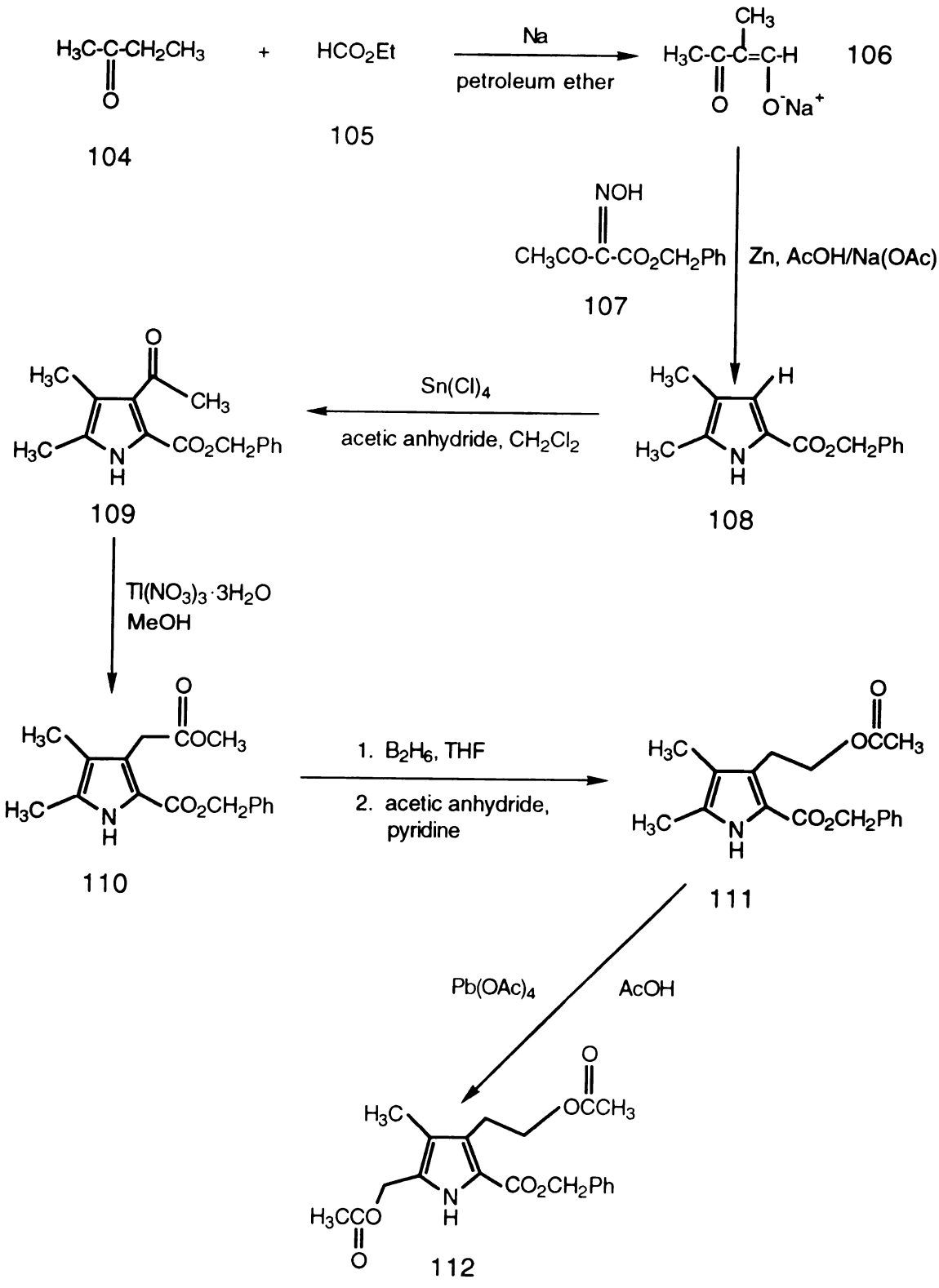
Scheme 20. Retrosynthetic Analysis of Porphyrins (103) and (122)

Dipyrrylmethanes (117) and (120) were the major components of porphyrin (122). The synthesis of dipyrrole (117) was initiated by the formation of acetoxy pyrrole (112) (Scheme 21). Methyl ethyl ketone (104) was reacted with sodium and ethyl formate (105) to give salt (106). Compounds (106) and (107) were then condensed, in the presence of zinc and buffered acetic acid, to give pyrrole (108) in 21.6% yield. The acetylation of (108) with tin(IV) chloride and acetic anhydride, afforded pyrrole (109) in 94.6% yield. Pyrrole (109) was then treated with thallium(III) nitrate, which gave compound (110) in 85.3% yield. The diborane reduction of (110) and subsequent acetylation of the resulting hydroxyethyl pyrrole with acetic anhydride in pyridine, afforded (111) in an overall yield of 85.6%. Compound (111) was then reacted with lead tetraacetate to give the corresponding acetoxy derivative (112) in 87.1% yield.

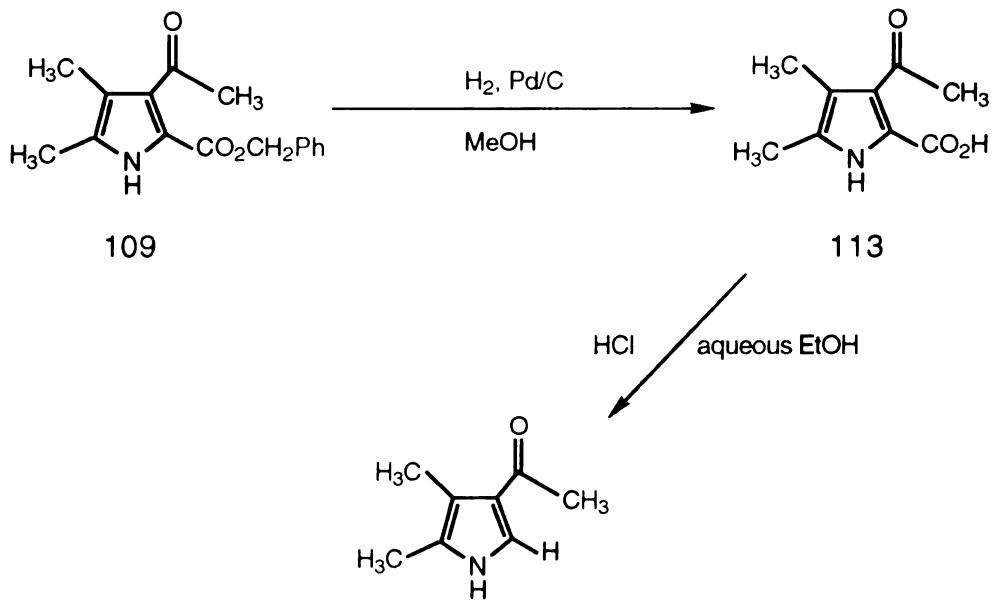
Pyrrole (114) was also required in the synthesis of (117). The hydrogenolysis of (109), in the presence of palladium/charcoal, gave carboxylic acid (113). The treatment of (113) with hydrochloric acid and aqueous ethanol, afforded the required $\alpha$-free pyrrole (114) in an overall yield of 51.0% (Scheme 22).

The condensation of acetoxy compound (112) and $\alpha$-free pyrrole (114) in refluxing pyridine, gave dipyrrole (115) in 69.5% yield. The hydrogenolysis of (115), in the presence of palladium/charcoal, furnished carboxylic acid (116). Compound (116) was then treated with trifluoroacetic acid and triethyl orthoformate to afford dipyrrole aldehyde (117) in a two-step yield of 83.4% (Scheme 23).

The synthesis of compound (120) proved to be straightforward. Dipyrrole (118) was decarboxlayted with sodium hydroxide and ethylene glycol to give (119). This compound was treated with benzoyl chloride and DMF to afford monoimine salt (120), in an overall yield of 54.0% (Scheme 24).




Scheme 21. Synthesis of Acetoxy Pyrrole (112)

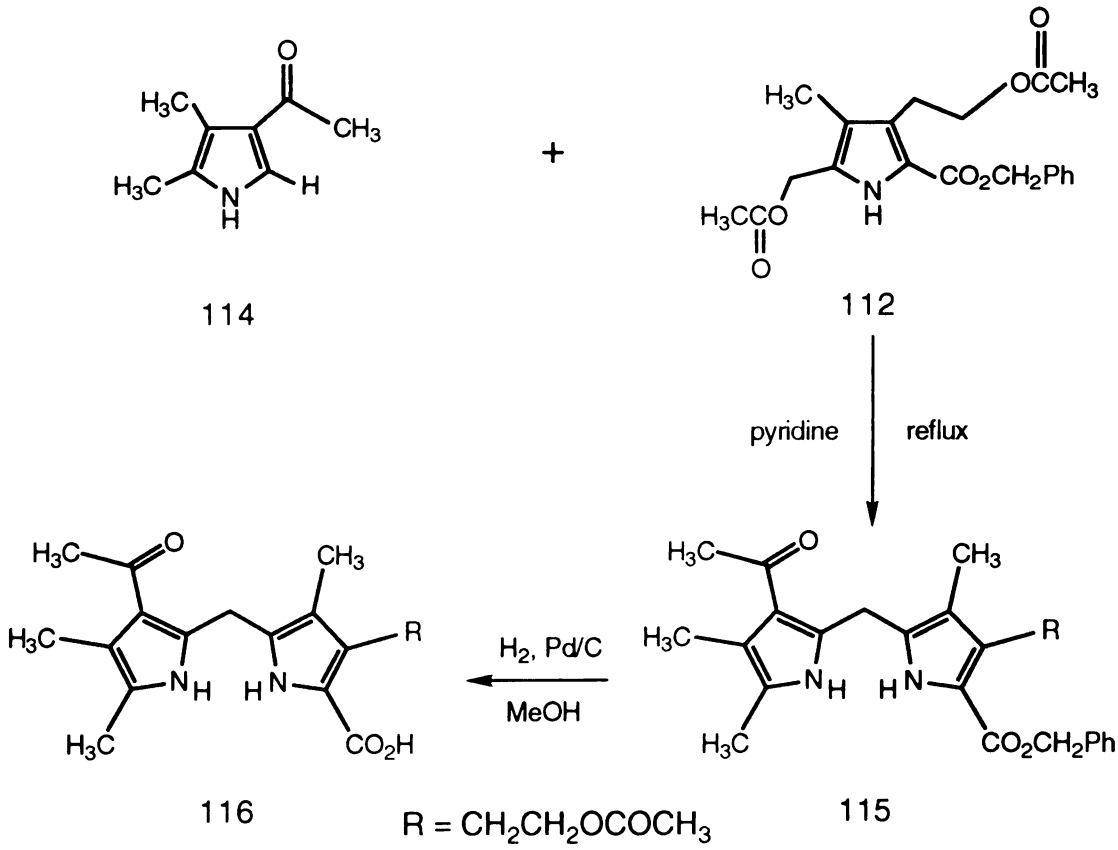



109 113 114

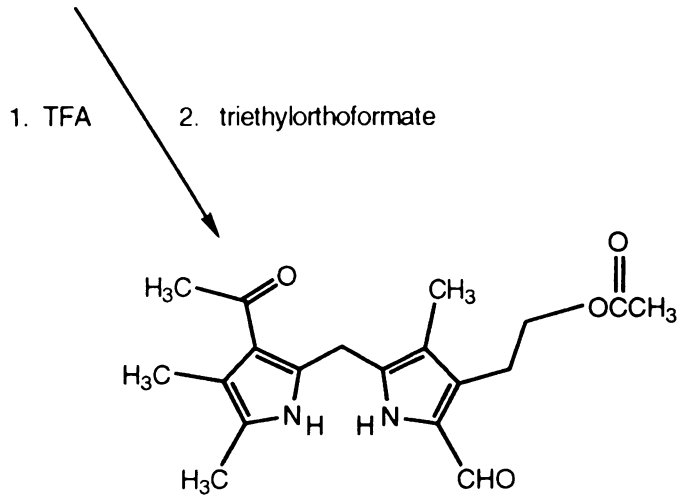
Scheme 22. Synthesis of Pyrrole (114)

With the required dipyrrolic pieces in hand, it was then possible to synthesize porphyrin (90) (Scheme 25). The acid-catalyzed condensation of formyl dipyrrylmethane (117) and imine (120) produced an intermediate b-bilene salt, which was cyclized *in situ* with copper(II) acetate to give porphyrin (121). Compound (121) was subsequently treated with sulfuric acid to afford porphyrin (122) in an overall yield of 32.1%. The treatment of porphyrin (122) with benzoyl chloride and DMF,[110] gave compound (90) in 82.3% yield (Scheme 25).

After porphyrin (90) was obtained, it was then possible to attempt the synthesis of model compound (99). The treatment of porphyrin (90) with one and a half equivalents of osmium tetroxide, gave chlorin (96) in 85.2% yield. The acetyl group of chlorin (96) was then reduced with sodium borohydride to give compound (123). The subsequent treatment of compound (123) with DBU, afforded the monovinyl compound (98).

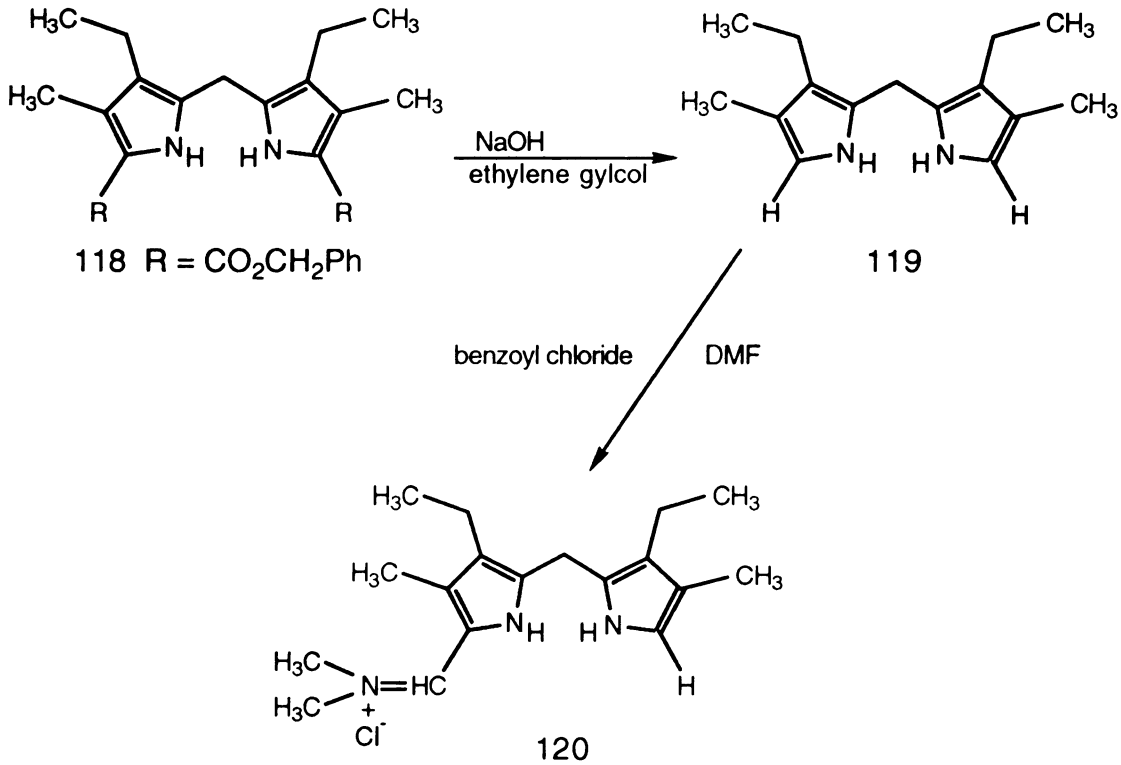



114 112 116 115




117

Scheme 23. Synthesis of Dipyrrole (117)




118 R = $CO_2CH_2Ph$

119

120

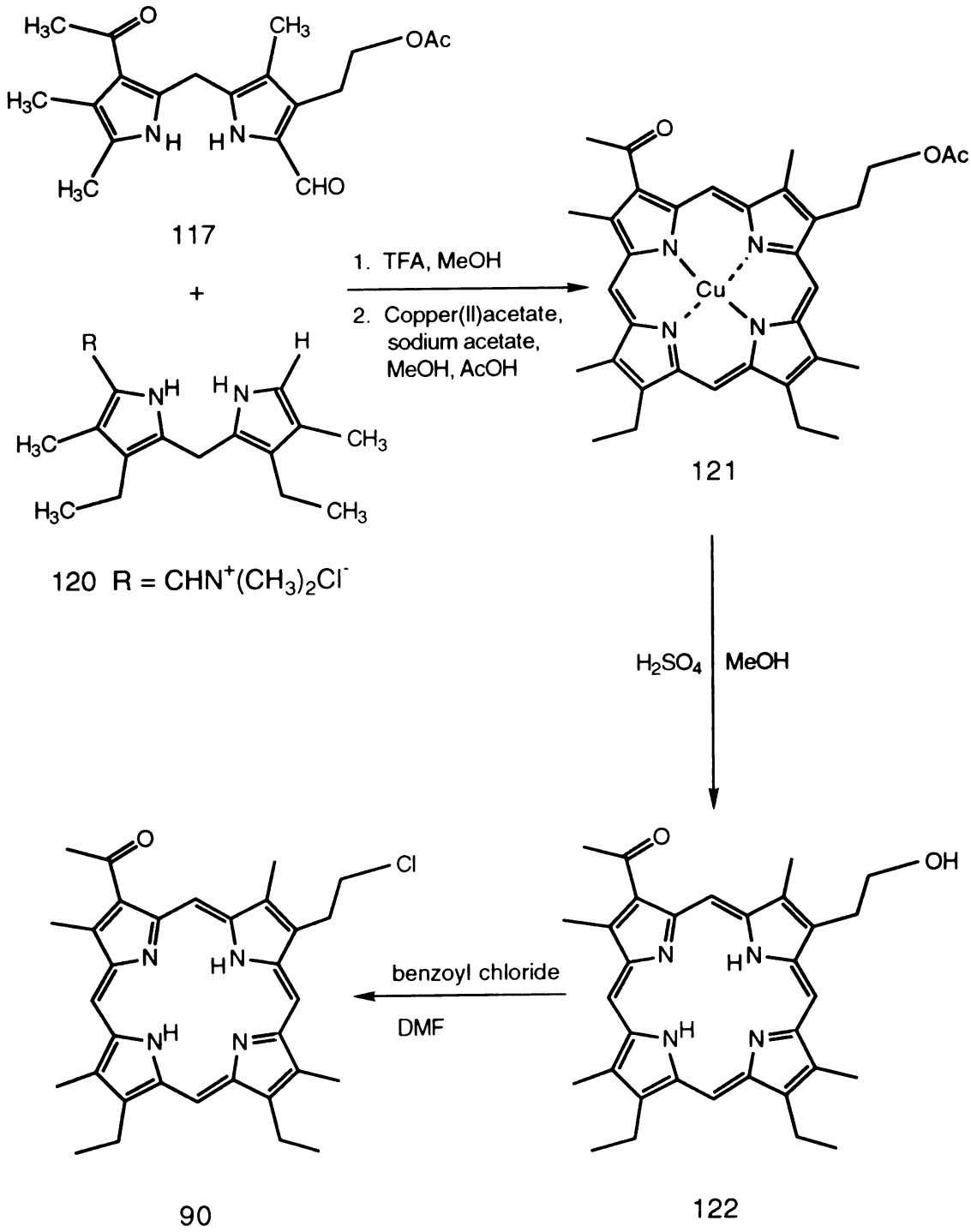
Scheme 24. Synthesis of Dipyrrole (120)

In the previous study, the reduction and chloroethyl elimination steps were carried out in the reverse order.[105] This reaction sequence was also investigated and found to give comparable results. However, we found that it was more convenient to carry out the reduction of the acetyl group before the elimination of the chloroethyl group.

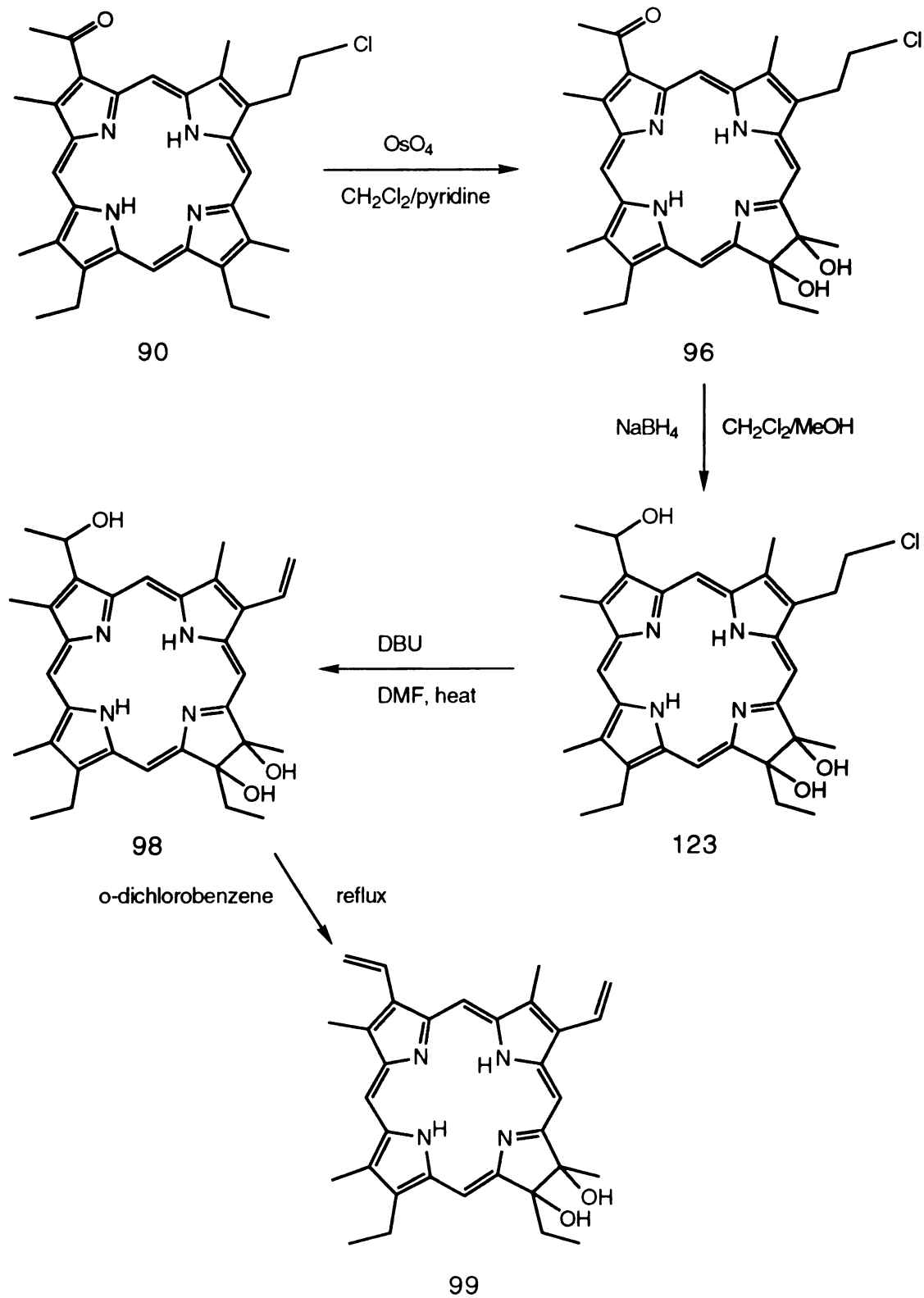
It was envisioned that compound (98) could be heated in DMF to give divinyl chlorin (99). Initial transformation attempts at 110°C, did not result in elimination of the secondary alcohol. Refluxing compound (98) in DMF (bp 154°C) for up to one hour, also failed to facilitate the elimination. The solvent was then replaced with N,N-dimethylacetamide (DMA) (bp 165°C). As before, elimination did not occur. We then decided to try o-dichlorobenzene (bp 179°C). Fortunately, this proved to be a suitable solvent for the elimination of the secondary alcohol. Refluxing chlorin (98) in o-dichlorobenzene for ten

minutes, finally afforded the desired divinyl chlorin (99) in a 3 step (96->123 ->98->99) yield of 12.0%. We found that it was important to carefully monitor the reflux time, in order to minimize decomposition products and maximize the yield of (99). Also, the overall yields were higher if only modest purifications were carried out at the reduction and chloroethyl elimination steps.

The discrepancy between these elimination conditions and those initially reported, were most likely due to the limited amount of material synthesized in the previous study. As stated earlier, only small amounts of compound were available for the last several steps of the synthetic sequence. Also, reliable $^1H$ NMR data was not obtained for the the final three compounds of the reaction sequence. Our $^1H$ NMR data revealed that even after treatment with hot DMA, compound (98) still possessed the the secondary alcohol and only one set of vinyl protons. In the original study, mass spectrometry was mainly used to characterize the compounds in the latter stages of the synthetic sequence. It is possible that the mass spectrometer could have been facilitating the elimination of the secondary alcohol during the analysis. This would produce a peak consistent with divinyl chlorin (99), even though the actual compound was (98). A similar phenomena was observed in the improved synthesis. The mass spectrum of (98) displayed major *m/z* peaks consistent with (99), even though the NMR data confirmed the presence of the alcohol.



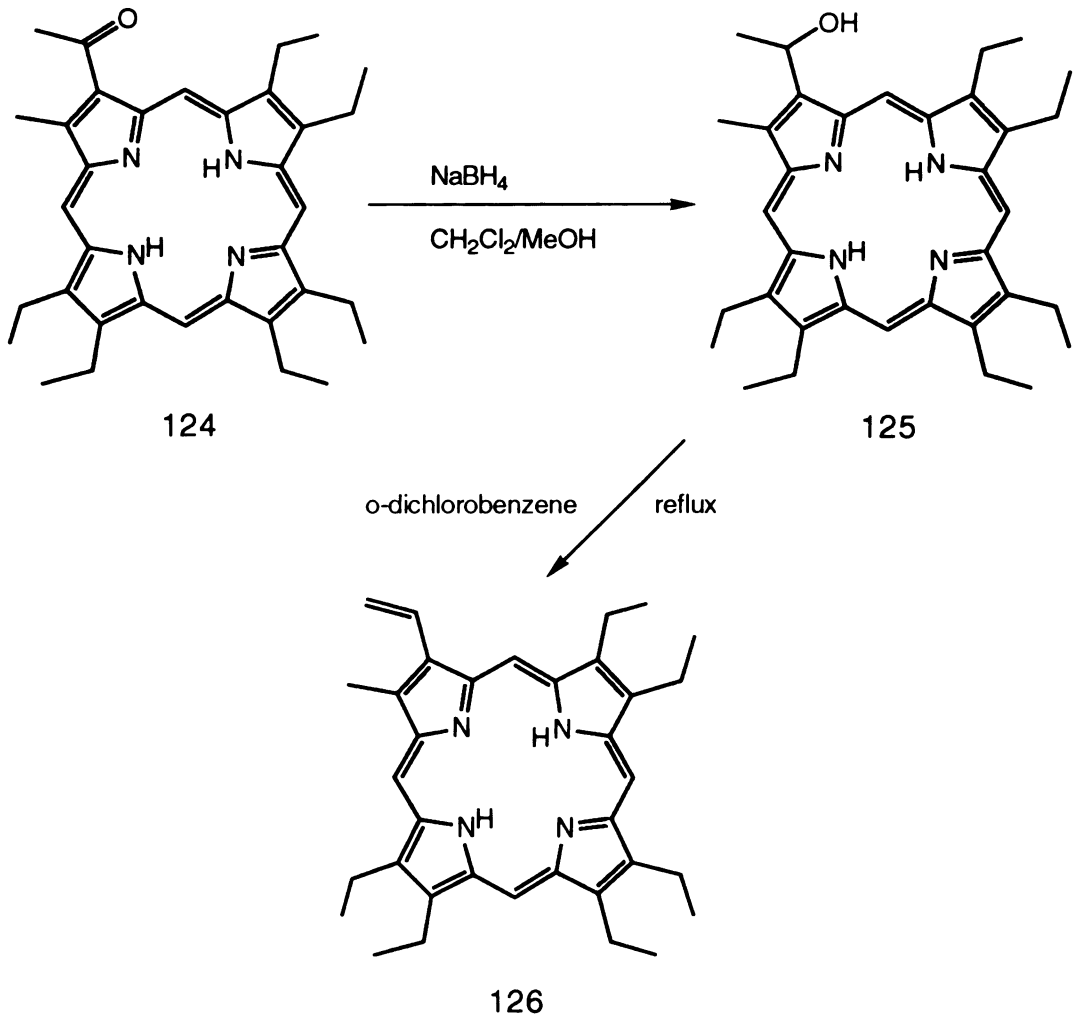

Scheme 25. Synthesis of Porphyrin (90)




Scheme 26. Improved Synthesis of Chlorin (99)

We were then curious to determine whether or not these high reaction temperatures would be required to eliminate the secondary alcohol from a porphyrin. Porphyrin (124) was reduced cleanly with sodium borohydride to give hydroxyethylporphyrin (125). Compound (125) was then refluxed in DMF. As in the case of chlorin (98), the elimination did not take place. DMA was also unsuccessful in eliminating the secondary alcohol. However, the elimination did take place in o-dichlorobenzene to give (126) (Scheme 27). It is interesting to note that the reflux time required for elimination (>20 min), was actually longer than that of chlorin (98) (~10 min). The reduction and elimination steps did however, appear to be more efficient in the case of the porphyrins. These types of hydroxyethyl groups are usually eliminated from porphyrin, by treatment with benzoyl chloride and DMF.[111] Acidic conditions had to be avoided in the case of chlorin (98) however, in order to avoid possible rearrangement (e.g. pinnacolic) or elimination of the pyrrolic diol substituents.

We have demonstrated that our improved procedure allows for the preparation of useful quantities of chlorin (99). Spectroscopic investigations involving various metal complexes of (99) are underway. For example, resonance Raman spectroscopy has shown potential as a tool for distinguishing between chlorin diols and spirolactones.[112] The copper complex of chlorin (99) is currently being studied by this method, which should give further insights into heme *d*.




124 125 126

Scheme 27. Synthesis of Porphyrin (126)

## III. Experimental

### General

Melting points were determined on a Thomas Hoover melting point apparatus or a Mel-Temp apparatus and are uncorrected. Infrared spectra were measured on a Nicolet IR/42 spectrometer with absorptions reported in $cm^{-1}$. Nuclear magnetic resonance spectra were obtained on a Varian Gemini 300, VXR 300, or VXR 500 spectrometer. In most instances, spectra were recorded in $CDCl_3$ with chemical shifts expressed in parts per million (ppm) relative to residual chloroform (7.24 ppm). Electronic absorption spectra were measured in dichloromethane solutions, using a Shimadzu UV-160 spectrophotometer. Mass spectra were obtained on a Fisons VG Trio-1 mass spectrometer (EI) or a JEOL HX 110 110-HF mass spectrometer (FAB). Porphyrin and chlorin manipulations were usually carried out in the absence of light. Reactions were monitored using Eastman Kodak 13181 silica gel sheets. In general, column chromatography was performed using silica gel (70-230 mesh) or grade III alumina (6 ml of water to 100 g of alumina). Preparative thin layer chromatography was carried out on 20 x 20 $cm^2$ glass plates, coated with Analtech silica gel (1.0 or 1.5 mm thick).

Benzyl-4,5-dimethylpyrrole-2-carboxylate (108)

Sodium (58 g) was added portionwise to a vigorously stirred solution of methyl ethyl ketone (104) (190 g; 236 ml) and ethyl formate (105) (197g; 215 ml), in petroleum ether (1 L), over a three hour period. At the end of this time, the precipitated sodium salt of 2-methyl-3-oxo-butanal (106) (210 g) was collected, washed well with ether, dried under vacuum and used without further purification.

Benzylacetoacetate (192 g) was dissolved in acetic acid (200 ml) and cooled to 5°C in a ice/salt bath. A solution of sodium nitrite (69 g) in water (150 ml) was added dropwise to the stirred solution, while maintaining the temperature below 10°C. After the addition was complete, the resulting oxime solution (107) was allowed to stir at room temperature for one hour. This solution and a mixture of zinc dust (210 g) and sodium acetate (100 g) were added with stirring to a solution of the sodium salt of 2-methyl-3-oxo-butanal in acetic acid (300 ml), while maintaining the reaction temperature between 65-75°C. The solution was then warmed to 95°C and stirred for an additional hour at this temperature. The solution was cooled to 70°C and poured into ice water (12 L). The resulting precipitate was filtered, washed thoroughly with water and recrystallized from methanol to give the title compound as a light tan solid (43.2 g; 21.6%); mp 109-111°C (lit. mp 112°C[113]). $^1$H NMR ($CDCl_3$): δ 2.03 (3H, s), 2.20 (3H, s) (2 x pyrrole-$CH_3$), 5.30 (2H, s, $OCH_2$), 6.75 (1H, s, β-H), 7.27-7.45 (5H, m, Ph), 9.08 (1H, br, NH).

Benzyl 3-acetyl-4,5-dimethylpyrrole-2-carboxylate (109)

A solution of benzyl-4,5-dimethylpyrrole-2-carboxylate (108) (32.0 g) , acetic anhydride (110 ml) and dichloromethane (225 ml) was cooled to 0°C in a ice/salt bath. Tin(IV) chloride (20 ml) was added dropwise to the stirred mixture while maintaining the temperature below 5°C. After the addition was complete, the solution was allowed to stir at room temperature for one hour. Water (300 ml) was then slowly added and mixture stirred for three hours. A saturated aqueous sodium bicarbonate solution (300 ml) was added and the solution stirred for an additional two hours. The organic layer was separated and the aqueous layer was extracted with additional dichloromethane (200 ml). The organic layers were combined, washed with water (2 x 400 ml), and dried over

anhydrous sodium sulfate. The dichloromethane was removed under reduced pressure to give the required pyrrole as a light tan solid which was used without further purification (33.5 g; 94.6%). A sample was recrystallized from ethanol to give a off-white solid; mp 108-110°C (lit. mp 108-109°C[114]). $^1$H NMR ($CDCl_3$): δ 1.98 (3H,S), 2.17 (3H,S) (2 x pyrrole-$CH_3$), 2.48 ($COCH_3$), 5.27 ($OCH_2$), 7.32-7.37 (5H, m, Ph), 9.16 (1H, br, NH).

Benzyl-3-methoxycarbonylmethyl-4,5-dimethyl-2-carboxylate (110)

Benzyl-3-acetyl-4,5-dimethylpyrrole-2-carboxylate (109) (10.5 g) was added to a solution of thallium(III) nitrate trihydrate (18.9 g) in methanol (350 ml), aqueous perchloric acid (70%; 18.3 ml) and water (2.1 ml). The solution was allowed to stir at room temperature for three hours and then placed in the freezer overnight. The thallium(I) nitrate precipitate was removed by filtration and washed with methanol (100 ml). The filtrate was diluted with water (1.25 L) and then extracted with chloroform (4 x 100 ml). The organic layers were combined and washed with water (400 ml). The chloroform layer was then passed through an alumina column, and elution continued with ethanol-free chloroform. The solvent was removed under reduced pressure to give the desired pyrrole as a light yellow solid, which was sufficiently pure for use in the next step (9.95 g; 85.3%). A sample was recrystallized from hexane to give off-white solid mp; 88-90°C (lit. mp 78-80°C, resolidification and then 91-92°C[109]). $^1$H NMR ($CDCl_3$): δ 1.87 (3H, s), 2.15 (3H, s) (2 x pyrrole-$CH_3$), 3.55 (3H, s, $CO_2CH_3$), 3.80 (2H, s, $C\underline{H}_2CO_2CH_3$), 5.25 (2H, s, $OCH_2$), 7.25-7.40 (5H, m, Ph), 9.03 (1H,br,NH).

Benzyl 3-(2-acetoxyethyl)-4,5-dimethylpyrrole-2-carboxylate (111)

Diborane (prepared by the addition of boron trifluoride etherate (25 ml) into a stirred solution of sodium borohydride (4.9 g) and diglyme (10 ml), over one hour and 15 minutes) was transferred in a stream of nitrogen through a solution of benzyl-3-methoxycarbonylmethyl-4-5-dimethylpyrrole-2-carboxylate (110) (8.6 g) in dry tetrahydrofuran (50 ml). The diborane generator was then warmed to 70°C and the reaction mixture stirred for an additional hour. Methanol was then added until the evolution of hydrogen ceased. Aqueous sodium hydroxide (0.1M; 300 ml) was added and the mixture extracted with chloroform (150 ml). The organic layer was separated and the aqueous layer extracted with fresh chloroform (150 ml). The organic layers were combined, dried over anhydrous sodium sulfate, and the solvent removed under reduced pressure. The residue was dissolved in pyridine (50 ml) and acetic anhydride (10 ml) was added. The mixture was then stirred overnight at room temperature. Water (300 ml) was added and the solution extracted with chloroform (150 ml). The organic layer was washed well with water and dried over anhydrous sodium sulfate. The solvent was then removed to give pyrrole (111) as a yellow solid, which was used without further purification (7.7 g; 85.6%); mp 77.5-80°C (lit. mp 87-88°C[109], 79-80°C[115]). $^1$H NMR ($CDCl_3$): δ 1.92 (3H,s), 1.97 (3H,s) (2 x pyrrole-$CH_3$), 2.15 (3H, s, $OCOCH_3$), 3.05 (2H, t, $C\underline{H_2}CH_2OCOCH_3$), 4.15 (2H, t, $CH_2C\underline{H_2}OCOCH_3$), 5.25(2H, s, $OC\underline{H_2}Ph$), 7.28-7.44 (5H, m, Ph), 8.87 (1H, br, NH).

Benzyl 3-(2-acetoxyethyl)-5-acetoxymethyl-4-methylpyrrole-2-carboxylate (112)

Lead tetraacetate (12.26 g) was added in one portion to a stirred solution of benzyl 3-(2-acetoxyethyl)-4,5-dimethylpyrrole-2-carboxylate (111) (6.71 g) in acetic acid (150 ml) and acetic anhydride (5 ml). The mixture was stirred for

three hours at room temperature and then poured into ice water (500 ml). The precipitate was washed well with water and then dissolved in dichloromethane. The organic layer was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure to give the desired pyrrole as an off-white solid (6.92 g; 87.1%); mp 131-133°C (lit. mp 138-140°C[109], 133-134°C[115]). $^1$H NMR ($CDCl_3$): δ 1.94 (3H, s, pyrrole-$CH_3$), 2.01 (3H, s), 2.03 (3H, s) (2 x $OCOCH_3$), 3.02 ( 2H, t, $C\underline{H_2}CH_2OCOCH_3$), 4.10 (2H, t, $CH_2C\underline{H_2}OCOCH_3$), 4.96 (2H, s, $C\underline{H_2}OCOCH_3$), 5.25 (2H, s, $OC\underline{H_2}Ph$), 7.25-7.40 (5H, m, Ph), 9.05 (1H, br, NH).

4-Acetyl-2,3-dimethylpyrrole (114)

Benzyl 3-acetyl-4,5-dimethylpyrrole-2-carboxylate (109) (20.0 g) was dissolved in methanol (400 ml) and placed in a one liter round bottomed flask. The vessel was purged with nitrogen and palladium-charcoal (10%; 800 mg) was added. The mixture was then stirred under an atmosphere of hydrogen for one hour. The solution was filtered to remove the catalyst and the solvent evaporated, under reduced pressure, to give (42). This compound was suspended in a mixture of ethanol (150 ml), water (30 ml) and hydrochloric acid (10 M; 30 ml) and heated on a steam bath until carbon dioxide evolution ceased (20 min). The reaction mixture was cooled to room temperature, an aqueous potassium carbonate solution (50 g in 300 ml) added, and the resulting mixture placed in the refrigerator overnight. The next day the precipitate was filtered and vacuumed dried to give the title pyrrole as a light brown solid (5.2 g; 51.0%); mp 131-133°C (lit. mp 135-137°C[114], 137°C[116]). $^1$H NMR ($CDCl_3$): δ 2.14 (3H, s), 2.21 (3H, s) (2 x pyrrole-$CH_3$), 2.35 ($COCH_3$), 7.25 (1H, α-H), 8.57 (1H, br, NH).

Benzyl 4-(2-acetoxyethyl)-3'-acetyl-3,4',5'-trimethyl-2,2'-dipyrrylmethane-5-carboxylate (115)

Benzyl 3-(2-acetoxyethyl)-5-acetoxymethyl-4-methylpyrrole-2-carboxylate (112) (7.22 g) and 4-acetyl-2,3-dimethylpyrrole (114) (2.71 g) were dissolved in pyridine and refluxed under nitrogen, for twenty four hours. The pyridine was then evaporated and the residue chromatographed on alumina, eluting with chloroform. The removal of the solvent afforded the desired dipyrrole as a golden foam, which was used without further purification (6.05 g; 69.5%); mp 139-142°C (lit. mp 144-146°C[109]). $^1$H NMR ($CDCl_3$): δ 1.93 (3H, s, $OCOCH_3$), 1.98 (3H,s), 2.06 (3H, s), 2.13 (3H, s) (3 x pyrrole-$CH_3$), 2.42 (3H, s, $COCH_3$), 3.05 ( 2H, t, $C\underline{H}_2CH_2OCOCH_3$), 4.07 (2H, s, methane-$CH_2$), 4.15 (2H, t, $CH_2C\underline{H}_2OCOCH_3$), 5.23 (2H, s, $OC\underline{H}_2Ph$), 7.26-7.40 (5H, m, Ph), 8.07 (1H, br), 9.77 (1H, br) (2 x NH).

4-(2-Acetoxyethyl)-3'-acetyl-3,4',5'-trimethyl-2,2'-dipyrrylmethane-5-carboxaldehyde (117)

Benzyl-(2-acetoxy)-3'-acetyl-3,4',5'-trimethyl-2,2'-dipyrrylmethane-5-carboxylate (115) (6.53 g) was dissolved in a mixture of ethanol (100 ml) and triethylamine (20 drops) and placed in a 250 ml round bottom flask. The system was purged with nitrogen, palladium-charcoal (10%, 250 mg) added and the solution stirred under a atmosphere of hydrogen for one hour. The catalyst was removed by filtration and the solvent evaporated under reduced pressure. The resulting residue was vacuum dried to give carboxylic acid (116) as a light brown foam (4.91 g).

The above carboxylic acid was dissolved in trifluoroacetic acid (25 ml) and stirred for ten minutes, while cooling in a ice/salt bath. Triethyl orthoformate (8 ml) was added, the solution stirred for five minutes in a ice/salt bath and then

for an additional ten minutes at room temperature. The mixture was diluted with ice water (200 ml) and stirred until the oil solidified. The resulting precipitate was collected, washed well with water and then dissolved in ethanol. Ammonium hydroxide (4 ml) was added to the stirred solution, followed by water (200 ml). The precipitate was collected, washed well with water and vacuumed dried to give a light brown solid (3.91 g; 83.4%); mp 168-171°C (lit. mp 174-175°C[109]). $^1$H NMR ($CDCl_3$): δ 1.97 (3H, s, $OCOCH_3$), 2.05 (3H, s), 2.08 (3H, s), 2.14 (3H, s) (3 x pyrrole-$CH_3$), 2.43 (3H, s, $COCH_3$), 2.97 (2H, t, $C\underline{H}_2CH_2OCOCH_3$), 4.16 (2H, t, $CH_2C\underline{H}_2OCOCH_3$), 4.22 (2H, s, methane-$CH_2$), 9.15 (1H, br, NH), 9.42 (1H, s, CHO), 10.50 (1H, br, NH).

3,3'-Diethyl-4,4'-dimethyl-5-(N,N'-dimethyliminomethyl)-2,2'-dipyrrylmethane chloride (120)

Diethyl 3,3'-diethyl-4,4'-dimethyldipyrromethane-5,5'-dicarboxylate (118) (8.5 g) and sodium hydroxide (9.5 g) were suspended in ethlyene glycol (90 ml). The mixture was refluxed under nitrogen for one hour and then cooled to room temperature. Hexane (100 ml) and water (200 ml) were then added. The organic layer was separated and the aqueous layer was extracted with fresh hexane (100 ml). The hexane layers were combined, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure to give dipyrrole (112). The light brown oil was dissolved in N,N-dimethylformamide (9.5 ml) and the solution diluted with benzene (35 ml). The mixture was cooled to 10°C in a ice/salt bath and benzoyl chloride (3.5 ml) added dropwise, while maintaining the temperature below 15°C. After the addition was complete, the solution was allowed to stir at room temperature for one hour. The imine salt was collected and washed with benzene to give the title compound as a light-cream colored solid (3.95 g; 54.0%); mp 177-180°C. $^1$H NMR ($CDCl_3$): δ 0.87-

0.97 (6H, overlapping triplets, 2 X $CH_2C\underline{H}_3$), 1.85 (3H, s), 2.05 (3H, s) (2 x pyrrole-$CH_3$), 2.27-2.45 (4H, overlapping quartets, 2 x $C\underline{H}_2CH_3$), 3.32 (3H, s), 3.70 (3H, s) (2 x imminium-$CH_3$), 4.05 (2H, s, methane-$CH_2$), 6.32 (1H, br, α-H), 7.27 (1H, s, $C\underline{H}$=$N(CH_3)_2$), 10.57 (1H, br), 12.65 (1H, br) (2 x NH).

3-Acetyl-8-(2-hydoxyethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin (122)

A solution of 3,3'-diethyl-4,4'-dimethyl-5-(N,N'-dimethyliminomethyl)-2,2'-dipyrrylmethane chloride (120) (3.01 g) in methanol (55 ml) was added to a stirred and cooled solution of 4-(2-acetoxyethyl)-3'-acetyl-3',4',5'-trimethyl-2,2'-dipyrrylmethane-5-carboxaldehyde (117) (2.70 g) in trifluoroacetic acid (18 ml). The mixture was stirred for thirty minutes and then added to a boiling solution of copper(II) acetate (17.85 g), sodium acetate (8.92 g), methanol (800 ml) and acetic acid (800 ml). The reaction mixture was then allowed to reflux in the dark for eighteen hours. At the end of this time, the solution was cooled and chloroform (400 ml) and water (400 ml) were added. The mixture was shaken, the organic layer was separated and the aqueous layer extracted with fresh chloroform (4 x 100 ml). The combined chloroform extracts were combined, washed with water (2 x 400 ml) and the solvent removed under reduced pressure. The residue was chromatographed on silica gel, eluting with chloroform. The resulting copper porphyrin (121) was then dissolved in mixture of trifluoroacetic/sulfuric acid (10 ml/75 ml). The solution was stirred for ten minutes, diluted with ice cold methanol (1.5 L) and allowed to stand in the refrigerator overnight. Water (1.5 L) was added and the product extracted into chloroform (5 x 200 ml). The combined organic layers were washed with water, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was purified by silica gel chromatography, eluting with dichloromethane, and recrystallized from dichloromethane/methanol to afforded

the title porphyrin as a purple solid (1.28 g, 32.1%); mp >300°C. $^1$H NMR ($CDCl_3$): δ -3.65 (2H, br, 2 x NH), 1.72-1.92 (6H, two overlapping triplets, 2 x $CH_2C\underline{H}_3$), 3.05 (1H, s, $CH_2CH_2O\underline{H}$), 3.28 (3H, s, $COCH_3$), 3.52 (3H, s), 3.62 (3H, s), 3.66 (3H, s), 3.84 (3H, s) ( 4 x $CH_3$), 3.98 (2H, q), 4.09 (2H, q) (2 x $C\underline{H}_2CH_3$), 4.27 (2H, t, $C\underline{H}_2CH_2OH$), 4.50 ( 2H, t, $CH_2C\underline{H}_2OH$), 9.83 (1H, s), 9.97 (1H, s), 10.08 (1H, s), 10.78 (1H, s) (4 x meso-H). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (log ε) 407.5 ( 5.15), 508.5 (3.97), 548.0 (4.05), 575.0 (3.87), 633.0 (3.19) nm. EI MS: m/e (Relative Intensity) 508. 5 (100.0), 490.1 (60.4).

3-Acetyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethyl porphyrin (90)

A solution of 3-acetyl-8-(2-hydoxyethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin (122) (680 mg) in N,N-dimethylformamide (90 ml), was purged with nitrogen and cooled to 10°C in a ice bath. Benzoyl chloride (9.0 ml) was added dropwise while maintaining the temperature below 15°C. Once the addition was complete the mixture was allowed to warm to room temperature. The solution was then heated at 80°C, for one hour. The mixture was cooled to room temperature and poured into a solution of water (200 ml) and triethylamine (20 ml). The purple film was collected on a celite bed and washed well with water. The film was then dissolved in dichloromethane (150 ml) and again washed with water ( 2 x 150 ml). The organic layer was dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was then purified on silica, eluting with dichloromethane. Recrystallization from dichloromethane/hexane gave the desired porphyrin as a purple solid (580 mg; 82.3%); mp >300°C. $^1$H NMR ($CDCl_3$): δ -3.67 (2H, br, 2 x NH), 1.78-1.90 (6H, two overlapping triplets, 2 x $CH_2C\underline{H}_3$), 3.28 (3H, s, $COCH_3$), 3.52 (3H, s,), 3.61 (3H, s), 3.66 (3H, s) 3.85 (3H, s) ( 4 x $CH_3$), 3.97 (2H, q), 4.08 (2H, q) (2 x $CH_2CH_3$), 4.28 (2H, t, $C\underline{H}_2CH_2Cl$), 4.50 ( 2H, t, $CH_2C\underline{H}_2Cl$), 9.85

(1H, s), 9.96 (1H, s), 10.10 (1H, s), 10.78 (1H, s) (4 x meso-H). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (log $\varepsilon$) 408.0 (5.21), 509.0 (4.01), 547.5 (4.07), 575.0 (3.89), 631.0 (3.21) nm. EI MS: m/e (Relative Intensity) 526.3 (100.0), 490.2 (38.79).

3-Acetyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethyl-12,13-dihydroxychlorin (96)

A dichloromethane solution of osmium tetroxide (229 mg in 2 ml) was added to a solution of 3-acetyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin (90) (317 mg) and pyridine, (1.5 ml) in dichloromethane (200 ml). The solution was allowed to stir under nitrogen, for eighteen hours. Methanol (10 ml) was then added and the reaction mixture quenched by hydrogen sulfide for thirty minutes. The osmium sulfide precipitate was removed by filtration through celite and the solvent removed under reduced pressure. The residue was chromatographed on silica, eluting with chloroform, to initially remove the starting material (69 mg). Continued elution with chloroform/methanol (95/5), afforded the desired chlorin. The title compound was recrystallized from dichloromethane/hexane to give a dark green solid (229 mg; 85.2% based upon starting material consumed (248 mg) ); decomposes ~150°C. $^1$H NMR ($CDCl_3$): $\delta$ -2.28(1H, br), -2.42 (1H, br) (2 x NH), 0.75 (3H, t, $CH_2C\underline{H}_3$), 1.72 (3H, t, $CH_2C\underline{H}_3$), 2.27 (3H, s, $CH_3$), 2.20-2.33, 2.40-2.52 ( 2H, m, $C\underline{H}_2CH_3$), 2.96 (3H, s, $COCH_3$), 3.04 (3H, s), 3.41 (3H, s), 3.44 (3H, s) (3 x $CH_3$), 3.70-4.07 (6H, m, $C\underline{H}_2C\underline{H}_2Cl$, $C\underline{H}_2CH_3$), 8.74 (1H, s), 8.98 (1H, s), 9.52 (1H, s), (9.97) (1H, s) (4 x meso-H). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (log $\varepsilon$) 412.5 (5.13), 510.0 (4.03), 545.5 (4.02), 583.5 (3.90), 635.0 (4.40) nm. EI MS: m/e (Relative Intensity) 560.3 (11.98), 542.3 (100.00), 513.2 (25.52).

13,17-Diethyl-2,8-divinyl-2,7,12,18-tetramethyl-12,13-dihydroxychlorin (99)

3-Acetyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethyl-12,13-dihydroxychlorin (96) (53.2 mg) was dissolved in dichloromethane (30 ml) and cooled in a ice bath. The solution was purged with nitrogen and a solution of sodium borohydride (175 mg) in cold methanol (2.5 ml) was added. The mixture was stirred for 10 minutes and then acetic acid was carefully added to quench the excess sodium borohydride. The solution was washed with water, the organic layer dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was placed on a preparative TLC plate and developed using a mixture of dichloromethane/methanol (95/5). The major green bands were removed and combined to give 8-(2-chloroethyl)-3-(1-hydroxyethyl)-13,17-diethyl-2,7,12,18-tetramethyl-12,13-dihydroxychlorin (123). EI MS: m/e (Relative Intensity) 526.0 (70.81, molecular ion -36).

The above alcohol was dissolved in N,N-dimethylformamide (7 ml) and DBU (0.7 ml) was added. The system was purged with nitrogen and the solution stirred for two hours between 80-90°C. The mixture was cooled and ethyl acetate (30 ml) was added. The solution was washed well with water, the organic layer dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The resulting film was purified on a preparative TLC plate using a mixture of dichloromethane/ethyl acetate (90/10). The major green bands were collected and combined to give 3-(1-hydroxyethyl)-13,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-12,13-dihydroxychlorin (98). EI MS: m/e (Relative Intensity) 508.0 (15.38, molecular ion -18), 490.1 (66.43, molecular ion -36).

o-Dichlorobenzene (10 ml) was placed in a round bottom flask equipped with a condenser and brought to reflux under nitrogen. Chlorin (98) was dissolved in o-dichlorobenzene (2 ml) and added to the refluxing solvent. The

solution was then refluxed under nitrogen for 10 minutes. The solvent was removed under reduced pressure and the residue purified on a preparative TLC plate, using a mixture of dichloromethane/ethyl acetate (90/10). The major light green band was collected to give the desired divinyl chlorin (99) as a greenish-brown film (5.8 mg; 12.0%). $^1$H NMR ($CDCl_3$): δ -2.32 (2H, br, 2 x NH), 0.67 (3H, t, $CH_2C\underline{H}_3$), 1.72 (3H, t, $CH_2C\underline{H}_3$), 2.27 (3H, s, $CH_3$), 2.18-2.30, 2.38-2.50 (2H, m, $C\underline{H}_2CH_3$), 3.47 (3H, s), 3.51 (3H, s), 3.57 (3H, s) (3 x $CH_3$), 3.80-3.94 (2H, m, $C\underline{H}_2CH_3$), 5.97-6.32 (4H, m, 2 x CH=$C\underline{H}_2$), 8.04-8.16 (2H, m, 2 x $C\underline{H}$=$CH_2$), 9.07 (1H, s), 9.13 (1H, s), 9.72 (1H, s), 9.88 (1H, s) (4 x meso-H). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (log ε) 401.5 (5.21 ), 501.0 (4.09), 533 (3.63), 595.5 (3.61 ), 650.0 (4.61) nm. EI MS: m/e (Relative Intensity) 490.0 (37, molecular ion -18), 305.1 (100).

# CHAPTER 3

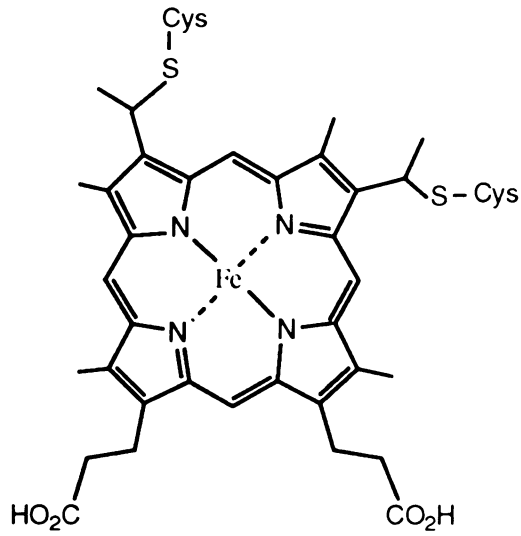
# HEME MODELS OF CYTOCHROME P460

## I. Introduction

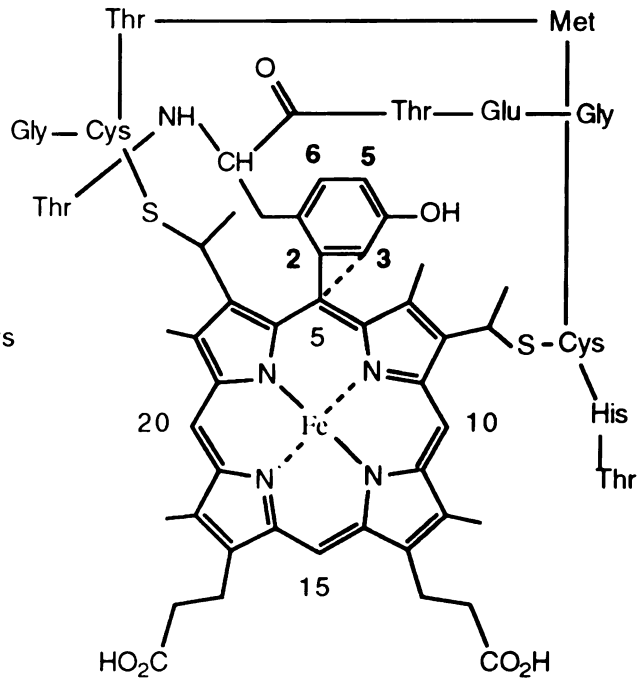
The study of porphyrins derived from natural sources is often a tedious process. Many times these compounds can only be isolated in minute quantities. This unfortunately makes purification, characterization and reactivity studies extremely difficult. Therefore, it is often desirable to synthesize model compounds to help answer the questions associated with the naturally occurring structures. The preparation of these model compounds often results in the development of new methodologies and techniques, which have value in other areas of porphyrin chemistry.

In the nitrifying bacteria *nitrosomonas europae*, hydroxylamine oxidoreductase (HAO) is responsible for oxidizing hydroxylamine to nitrite ($NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$). Many structural,[117] spectroscopic,[118] and catalytic[119] properties of this enzyme have been studied. HAO has been found to contain a total of eight covalently bound hemes. Seven of the hemes are believed to be C-type hemes (127). The eight heme, which has been termed P460, can be differentiated from the others on the basis of visible, EPR and resonance Raman data. This heme appears to be unique to ammonia oxidizing bacteria and is believed to be the active site in HAO. Timkovich and coworkers have proposed structure (128) for P460.[120] This conclusion was based upon mass spectral and NMR analyses of the fragments resulting from the enzymatic digestion of HAO. A variety of NMR techniques were utilized in the structural determination of P460. These included two dimensional

homonuclear, rotating frame, and one and two dimensional NOE spectroscopy. Their findings suggested that there were at least four possible ways that the tyrosyl fragment could be attached to the meso position of the porphyrin. The most likely possibilities were thought to be Tyr C2 to 5-meso (128) and Tyr C3 to 5-meso (129). In the simple tyrosine molecule, the C2 and C6 resonances are at higher frequencies than the C3 and C5 resonances. The 2D NMR of P460, revealed two resonances at relatively low frequencies. This led the researchers to assign compound (128) as the most probable structure of P460. However, this assignment was tentative and based upon speculative assumptions.



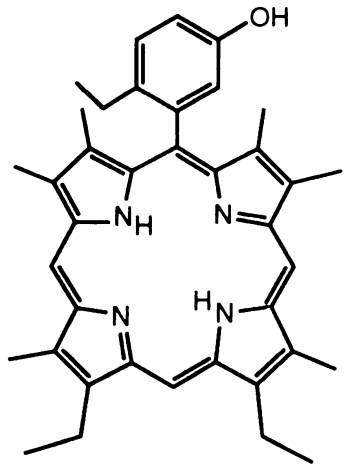

127



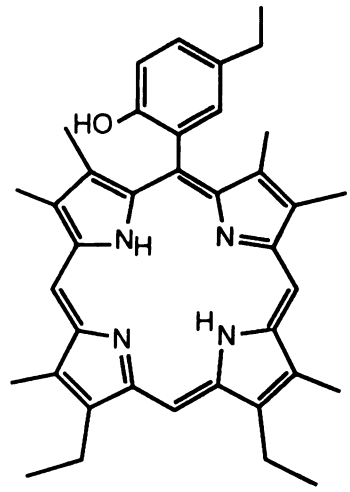

128 Tyr C**2** to 5-meso ——
129 Tyr C**3** to 5-meso ----

We decided that it would be beneficial to prepare several model compounds, which could be used to further elucidate the structure of P460. It was believed that by looking at the aromatic substitution patterns of our model compounds and those of P460, we could assign the substitution pattern of the

tyrosyl ring in relation to the porphyrin macrocycle. The model compounds of interest were porphyrins (145a) and (146a). Compound (145a) was designed to model (128), while compound (146a) was design to model (129).



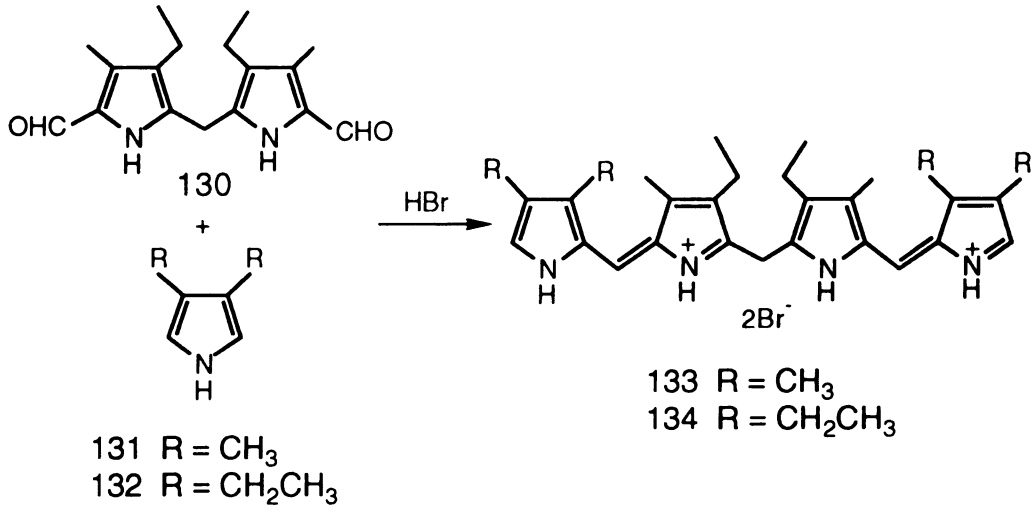

145a.




146a.

## II. Results and Discussion

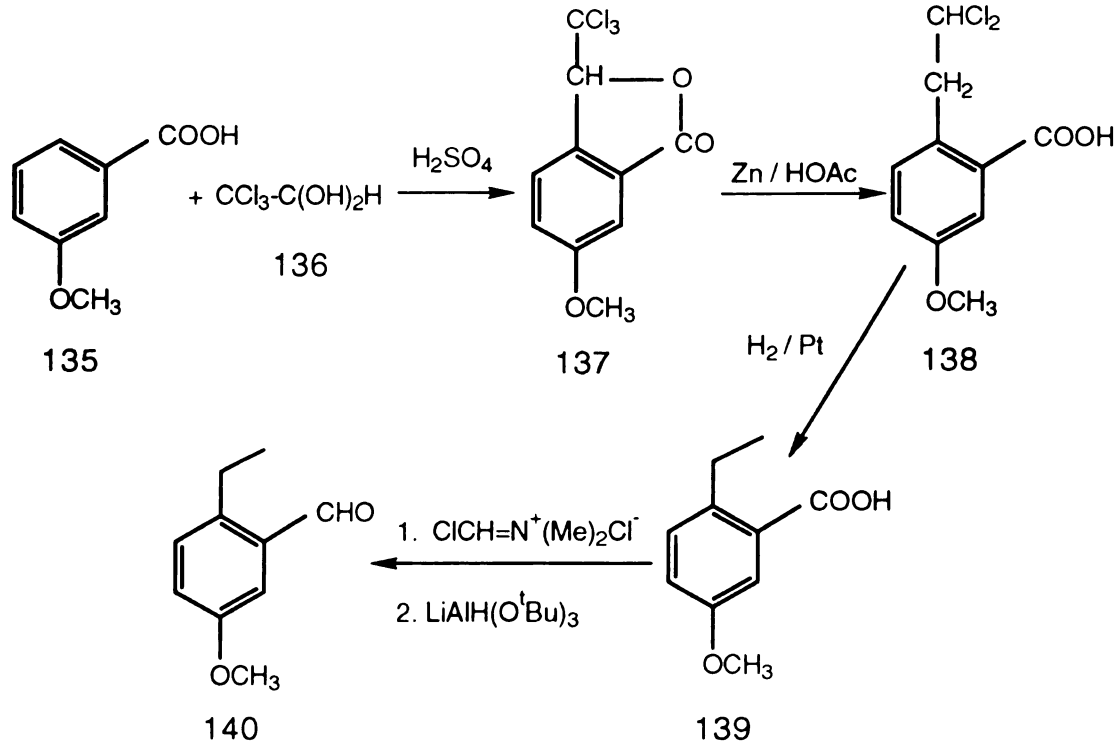
It was determined that the most efficient route to the model compounds, would be through the condensation of tetrapyrrolic salts and appropriately functionalized aldehydes. The first step in the synthesis of porphyrins (145a) and (146a), was the preparation of (133). The condensation of dialdehyde (130) and pyrrole (131), in the presence of HBr, afforded tetrapyrrole (133) in 80.3% yield. Compound (134) was also prepared in 77.4% yield, by the condensation of dialdehyde (130) and pyrrole (132) (Scheme 28).




Scheme 28. Synthesis of Tetrapyrroles (133) and (134)

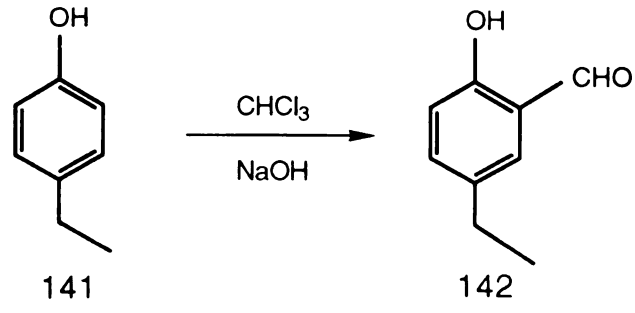
Aldehyde (140) was required for the preparation of porphyrin (145a). Compound (135) and chloral hydrate (136) were condensed with concentrated sulfuric acid to give compound (137). The subsequent treatment of (137) with zinc and refluxing acetic acid produced (138). The dehalogenation of compound (138) with hydrogen, in the presence of platinum oxide, afforded (139) in an overall yield of 29.1%. Compound (139) was then reduced with

N,N-dimethylmethyleneammonium chloride and $LiAl(OBu^t)_3$,[121] to give aldehyde (140) in 50.5% yield (Scheme 29).




Scheme 29. Synthesis of Aldehyde (140)

The preparation of porphyrin (146a) required benzaldehyde (142). Fortunately, the synthesis of this compound proved to be straightforward. Phenol (141) was treated with chloroform and sodium hydroxide to give benzaldehyde (142) in 34% yield (Scheme 30).




Scheme 30. Synthesis of Aldehyde (142)

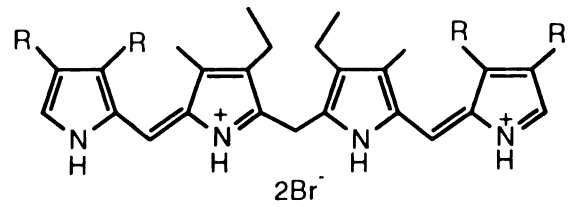
Once the various building blocks were obtained, it was then possible to synthesize the required model compounds. Tetrapyrrole (133) was condensed with an 10 fold excess of aldehyde (140), in the presence of HBr, to give porphyrin (143) in 46.9% yield. The subsequent treatment of (143) with $BBr_3$, gave compound (145a) in 34.1% yield. An alternate route to (145a), involved the demethylation of the benzaldehyde before condensation. Compound (140) was treated with $BBr_3$ to afford (144) in 42.4% yield. The condensation of compounds (133) and (144), led to (145a) in 40.9% yield (Scheme 31). Porphyrin (146a) was prepared in 63.1% yield, through condensation of compounds (133) and (142). Iron complexes (145b) and (146b) were also prepared in yields of 72.1 and 78.1%, respectively.

We were also interested in determining substituent effects in these porphyrin cyclizations, through the utilization of tetrapyrrole (134). The condensation of compounds (134) and (140), afforded (147) in 29.8% yield. Porphyrin (148) was also prepared in 53.4% yield, by the condensation of compounds (134) and (142) (Scheme 31). Overall, the incorporation of the additional ethyl groups decreased the yields in these condensations by approximately 10%. However, the reactions did reveal that these types of meso-substituted porphyrins can still be efficiently prepared even when steric interactions are increased.

The above techniques were found to afford the desired model porphyrins in good yields. Unfortunately, we discovered that a resolved $^1H$ NMR spectra of P460 does not exist at this time. This is due to the fact that the acquisition of a pure sample of P460 has proven difficult. Therefore, a direct comparison of our model compounds and P460 cannot be carried out at this time.

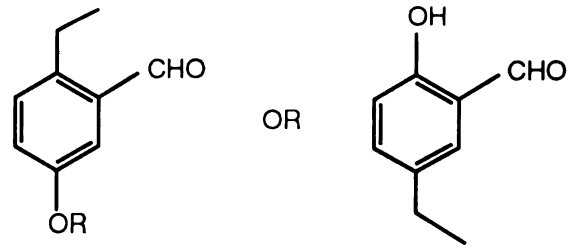
As mentioned above however, Timkovich and coworkers utilized various NMR techniques to elucidate the structure of P460. One of the NMR methods

used was Homonuclear Hartman-Hahn (HOHAHA) spectroscopy, which is also known as total correlation spectroscopy (tocsy).[122] Tocsy spectroscopy is a two-dimensional technique that utilizes magnetization transfer between homonuclear coupled spins, to determine connectivities in molecules. It was envisioned that by running a similar set of two dimensional experiments with our model compounds, we could make comparisons with the tocsy data of the natural heme to help confirm the structure. The tocsy aromatic regions of compounds (145a) and (146a) are shown in Figures 2 and 3. The aromatic region of Timkovich's tocsy spectrum contained resonances at 7.42, 7.54 and 7.73 ppm. Their data revealed that the resonance at 7.42 ppm was coupled to the resonances at 7.54 and 7.73 ppm. Also, the resonance at 7.54 ppm was coupled to the resonance and 7.73 ppm. Our experiments revealed that the tocsy spectrum of (145a) most closely resembled their results. The limited solubility of porphyrin (145a) in $CD_2Cl_2$, required the use of DMSO-$d_6$ as a co-solvent in the two-dimensional experiment. This slightly effected the position of the aromatic resonances, but did not effect the coupling patterns. In straight $CD_2Cl_2$, the aromatic resonances of (145a) were observed at 7.26, 7.39 and 7.55 ppm. When $CD_2Cl_2$/DMSO-$d_6$ was used as the solvent combination, the aromatic resonances were found at 7.19, 7.30 and 7.43 ppm. The low field resonance at 7.19 ppm, was found to couple with the resonances at 7.30 and 7.43 ppm. Also, coupling was observed between the resonances at 7.30 and 7.43 ppm. As mentioned above, these results were consistent with Timkovich's findings. The aromatic resonances of porphyrin (146a) were observed at 7.24, 7.56 and 7.59 ppm in $CD_2Cl_2$. The only observed coupling in the tocsy spectrum of (146a), was between the aromatic resonances at 7.24 and 7.56 ppm.



133 R = $CH_2$
134 R = $CH_2CH_3$

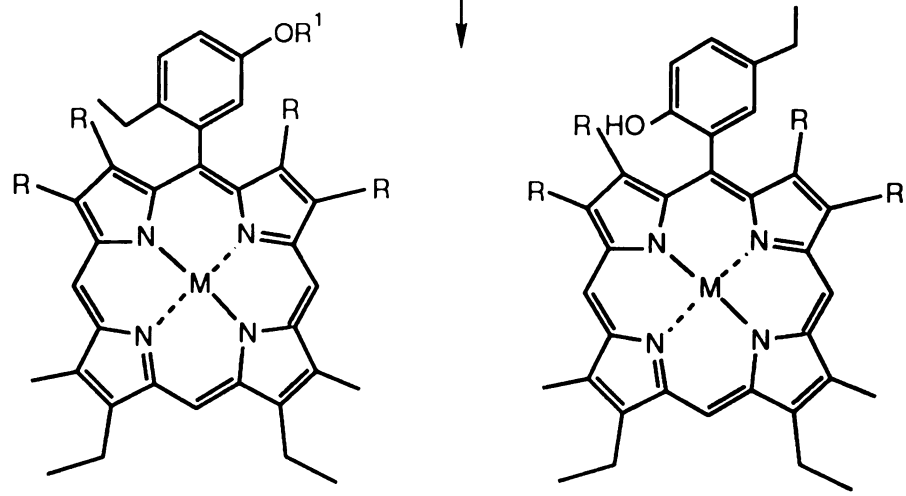
+



140 R = $CH_3$
144 R = H

142

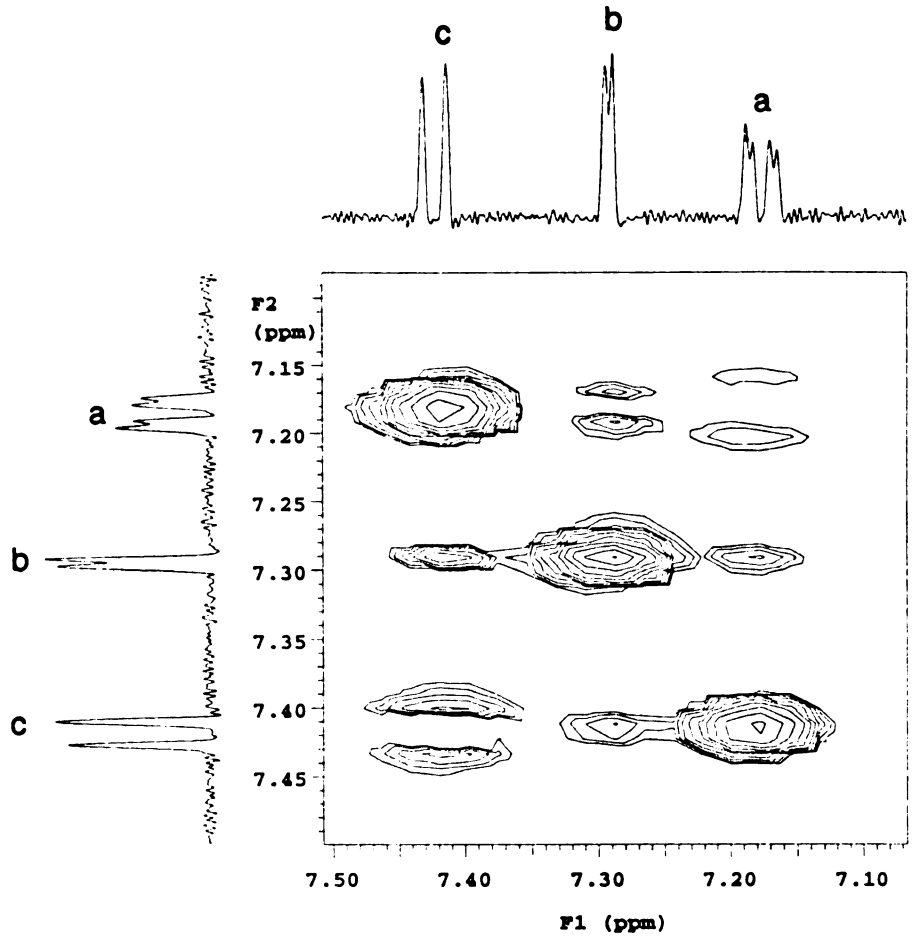
HBr | MeOH

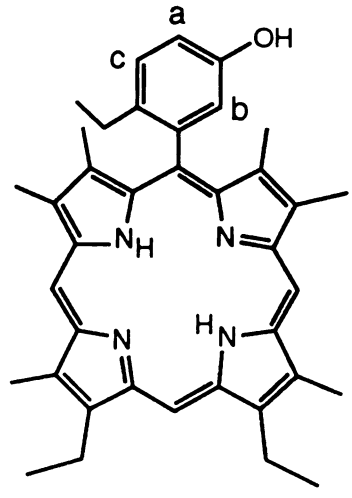


143 R = $CH_3$, $R^1$ = $CH_3$, M = 2H
145a. R = $CH_3$, $R^1$ = H, M = 2H
b. R = $CH_3$, $R^1$ = H, M = Fe•Cl
147 R = $CH_2CH_3$, $R^1$ = $CH_3$, M = 2H

146a. R = $CH_3$, M = 2H
b. R = $CH_3$, M = Fe•Cl
148 R = $CH_2CH_3$, M = 2H

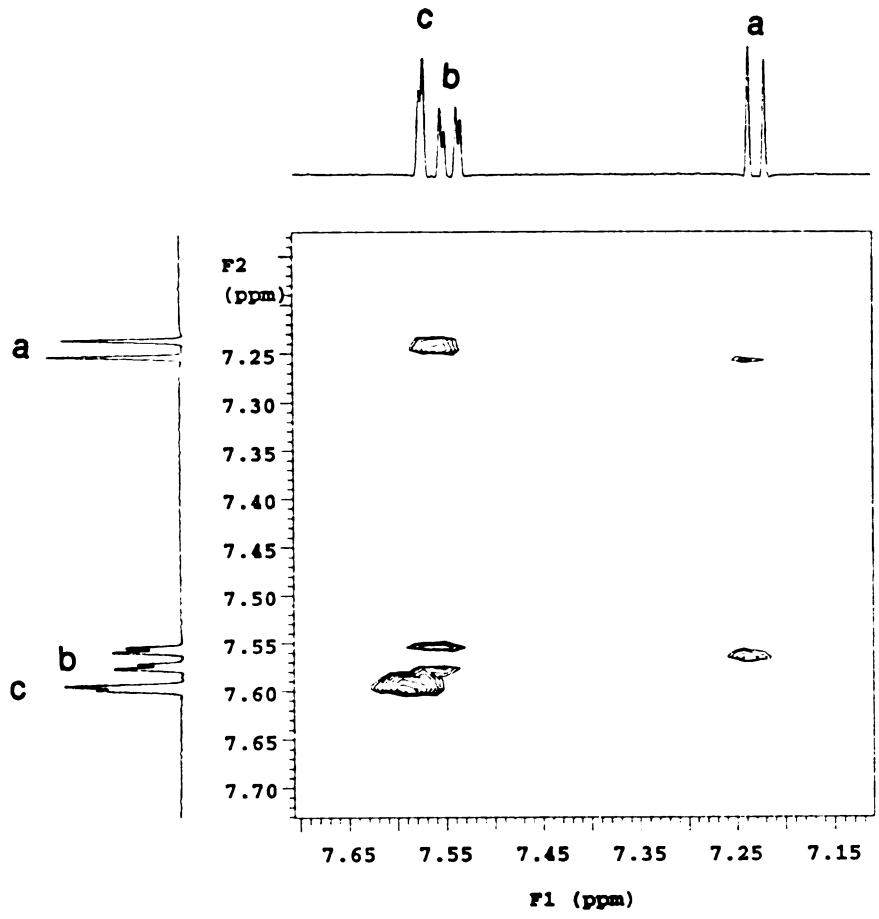
Scheme 31. Synthesis of Porphyrins (143), (145) and (146-148)

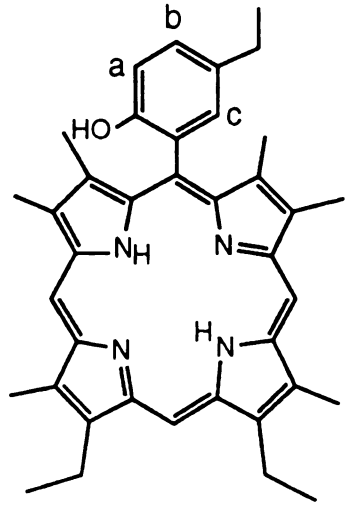



145a.

Figure 2. The Tocsy Aromatic Region of Porphyrin (145a)




146a.

Figure 3. The Tocsy Aromatic Region of Porphyrin (146a)

It should be noted that the results obtained in tocsy spectroscopy are dependent upon the duration of the pulse sequence chosen. When short pulse sequences are utilized (<20 ms), magnetization transfer is usually limited to directly coupled protons. Relay connectivities become observable when longer pulse sequences are chosen. Our two-dimensional experiments appear to support compound (128), as the most probable structure of P460. However, further studies of P460 are required for a definitive answer on the structure.

## III. Experimental

1,19-Dideoxy-7,13-ethyl-2,3,8,12,17,18-hexamethyl-biladiene-ac-dihydrobromide (133)

Hydrobromic acid (3.5 ml, 48%) was added to a solution of 3,3'-diethyl-5,5'-diformyl-4,4'-dimethyl-2,2'-dipyrrylmethane[123] (130) (653 mg) and 3,4-dimethylpyrrole[124] (131) (433 mg) in methanol (35 ml). The mixture was heated on a steam bath for five minutes, cooled to room temperature and then placed in the refrigerator for 1 hour. The salt was collected, washed with methanol containing a little hydrobromic acid, then with ether. The resulting product was vacuumed dried to give the title compound as a reddish-brown powder (1.110 g; 80.3%); mp 270-272°C with decomposition (lit. mp >300°C[24]). $^1$H NMR ($CDCl_3$): δ 0.64 (6H, t, 2 x $CH_2C\underline{H}_3$), 2.06 (6H, s), 2.24 (6H, s), 2.28 (6H, s) (6 x $CH_3$), 2.55 (4H, q, 2 x $C\underline{H}_2CH_3$), 5.25 (2H, s, 2 x 10-H), 7.22 (2H, s, 5 and 15-H), 7.62 (2H, d, 1 and 19-H), 13.27 (2H, s), 13.50 (2H, s) (4 x NH).

1,19-Dideoxy-7,13-dimethyl-2,2,8,12,17,18-hexaethyl-biladiene-a,c-dihydrobromide (134)

Compound (134) was prepared from 3,3'-diethyl-5,5'-diformyl-4,4'-dimethy-2,2'-dipyrrylmethane (130) (308 mg) and 3,4-diethylpyrrole[125] (132) (265 mg), by the method described above, to give the desired product as a red powder (548 mg; 77.4%); mp 241-243°C with decomposition (lit. mp darkens >160°C without melting[24]). $^1$H NMR ($CDCl_3$): δ 0.67 (6H, t, 2 x $CH_2C\underline{H}_3$), 1.18 (12H, t, 4 x $CH_2C\underline{H}_3$), 2.23 (6H, s, $CH_3$), 2.37-2.76 (12H, 6 overlapping quartets, 6 x $CH_2CH_3$ ), 5.24 (2H, s, 2 x 10-H), 7.18 (2H, s, 5 and 15-H), 7.65 (2H, d, 1 and 19-H), 13.28 (s, 2H), 13.51 (s, 2H) (4 x NH).

2-Ethyl-5-methoxybenzoic acid (139)

3-Methoxybenzoic acid (135) (10.0 g) and chloral hydrate (136) (10.0 g) were dissolved in concentrated sulfuric acid (50 ml) and stirred at room temperature for 24 hours. The mixture was diluted with ice-water, and the resulting crystalline mass (137) filtered. Compound (137) was recrystallized from ethanol and the first crop (12.16 g; mp 135-136.5°C) placed in acetic acid (120 ml). The resulting mixture was then refluxed for 1.5 hours in the presence of zinc dust (44.5 g). Water (75 ml) was added and the resulting mixture refluxed for an additional 30 minutes. The solution was decanted from the excess zinc and the mixture cooled. The resulting solid (138) was filtered and vacuum dried. This solid was dissolved in methanol, the solution purged with nitrogen, and platinum oxide (500 mg) added. The solution was then stirred under an atmosphere of hydrogen for two hours. The mixture was filtered and the solvent removed under reduced pressure to give the title compound (139) as a light yellow solid (3.45 g; 29.1%); mp 67-68°C (lit. mp 65°C[126]). $^1$H NMR ($CDCl_3$): $\delta$ 1.27 (3H, t, $CH_2C\underline{H}_3$), 3.03 (2H, q, $C\underline{H}_2CH_3$), 3.87 (3H, s, $OCH_3$), 7.09 (1H, dd), 7.28 (1H, d), 7.60 (1H, d) (3 x phenyl-H), 12.29 (1H, br, COOH). EI MS: m/e (Relative Intensity) 180 (43.5), 165 (100.00), 1.38 (45.0).

2-Ethyl-5-methoxybenzaldehyde (140)

A solution of (chloromethylene)dimethylammonium chloride (746 mg; 5.55 x $10^{-3}$ moles), in acetonitrile (9 ml) and tetrahydrofuran (14 ml), was cooled to -45°C in a dry ice/isopropanol bath. The system was purged with nitrogen and a solution of 2-ethyl-5-methoxybenzoic acid (139) (1.00 g; 5.55 x $10^{-3}$ moles) and pyridine (439 mg; 5.55 x $10^{-3}$ moles), in tetrahydrofuran (9 ml), was added dropwise at -45°C. The solution was stirred at this temperature for 1 hour and then cooled to -78°C in a dry-ice/acetone bath. A suspension of

copper(I)iodide (106 mg; 5.56 x $10^{-4}$ moles), in tetrahydrofuran (5 ml), and a diglyme solution of $LiAlH(OBu^t)_3$ (0.5 M; 22.2 ml; 1.11 x $10^{-2}$ moles) were then slowly added to the cooled solution. After the addition was complete, the mixture was stirred for 10 minutes and then quenched by the addition of a 5% aqueous hydrochloric acid solution (30 ml). After stirring for 15 minutes, the solution was extracted with ether (2 x 30 ml) and the organic layer washed with a 5% aqueous sodium bicarbonate solution (30 ml). The solution was dried over anhydrous sodium sulfate and the solution removed under vacuum. The residue was chromatographed over silica, eluting with dichloromethane, to give the desired aldehyde as a pale yellow oil (460 mg; 50.5%). $^1H$ NMR ($CDCl_3$): $\delta$ 1.22 (3H, t, $CH_2C\underline{H}_3$), 2.96 (2H, q, $C\underline{H}_2CH_3$), 3.81 (3H, s, $OCH_3$), 7.05 (1H, dd), 7.18 (1H, d), 7.33 (1H, d) (3 x phenyl-H) 10.26 (1H, s, CHO). EI MS: m/e (Relative Intensity) 163 (100.00), 135 (14.63), 121 (32.50), 105 (22), 91 (24.88), 77 (21.88).

5-Ethyl-2-hydroxybenzaldehyde (142)

Sodium hydroxide (73.7 g; 1.84 moles) was suspended in water (75 ml) and a solution of 4-ethylphenol (141) (50g; 4.09 x $10^{-1}$ moles) in methanol (150 ml), was added. The temperature was raised to 65°C and chloroform (195.8 g; 1.64 moles) was added dropwise while maintaining the temperature between 65-75°C. After the addition was complete the solution was allowed to stir for an additional two hours at 70°C. After cooling, the mixture was acidified to pH 5 and water (164 ml) was added. The organic layer was washed with water and the solvent removed under reduced pressure. The crude product was then steam distilled. The resulting oil was then vacuumed distilled to yield a pale yellow oil (25.5g; bp 40-45°C at 0.15-0.18 mm Hg, approximately 65% pure by NMR) (lit. bp, 236°C at 760 mm Hg[127]). A portion (2.8 g) of this material was

columned over silica, eluting with dichloromethane, to give the title compound as a colorless oil (0.92 g). $^1$H NMR ($CDCl_3$): δ 1.13 (3H, t, $CH_2C\underline{H}_3$), 2.52 (2H, q, $C\underline{H}_2CH_3$), 6.80 (1H, d), 7.23-7.28 (2H, m) (3 x phenyl-H), 9.75 (1H, s, CH0), 10.75 (1H, s, OH). EI MS: m/e (Relative Intensity) 150.1 (50.70), 135.0 (100.00).

5-(6-Ethyl-3-methoxyphenyl)-13,17-diethyl-2,3,7,8,12,18-hexamethylporphyrin (143)

1,19-Dideoxy-8,12-diethyl-2,3,7,13,17,18-hexanethylbiladiene-a,c-dihydrobromide (133) (281 mg; 4.66 x $10^{-4}$ moles) and 2-ethyl-5-methoxybenzaldehyde (140) (766 mg; 466 x $10^{-3}$ moles) were suspended in methanol (70 ml). A mixture of hydrobromic acid/acetic acid (50/50, 12 drops) was then added and the solution heated under reflux for 24 hours. After cooling to room temperature dichloromethane (75 ml) was added and the solution washed with water (75 ml), saturated aqueous sodium bicarbonate solution (75 ml) and water (75 ml). The solution was dried over anhydrous sodium sulfate, the solvent removed under reduced pressure and the residue chromatographed on silica, eluting with dichloromethane. The fractions containing porphyrin were combined and recrystallized from dichloromethane/methanol to give the desired porphyrin as purple crystals (128 mg; 46.9%); mp >300°C. $^1$H NMR ($CDCl_3$): δ -3.13 (2H, br, 2 x NH) 0.80 (3H, t, phenyl-$CH_2C\underline{H}_3$), 1.86 (6H, t, 2 x $CH_2C\underline{H}_3$), 2.21 (2H, q, phenyl-$C\underline{H}_2CH_3$), 2.52 (6H, s, 3,7-$CH_3$), 3.54 (6H, s), 3.62 (6H, s) (6 x $CH_3$), 3.90 (3H, s, $OCH_3$), 4.06 ( 4H, q, 2 x $C\underline{H}_2CH_3$), 7.31 (1H, dd), 7.53 (1H, s), 7.56 (1H, s) (3 x phenyl-H), 9.92 (1H, s), 10.15 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 402.0 (5.25), 501.5 (4.18), 533.5 (3.74), 571.0 (3.72), 623.0 (3.38). EI MS: m/e (Relative Intensity) 584 (100.00), 569 (17.42), 292 (23.16), 285 (27.27), 277 (29.44).

5-(6-Ethyl-3-hydroxyphenyl)-13,17-diethyl-2,3,7,8,12,18-hexamethylporphyrin (145a)

a. A 1M dichloromethane solution of $BBr_3$ (0.21ml; 2.1 x $10^{-3}$ moles, 4.1 eq) was added under nitrogen, to a round bottom flask containing dichloromethane (2.5 ml). The mixture was cooled to -78°C in a dry ice/acetone bath and a solution of 5-(6-ethyl-3-methoxypheny)-13,17-diethyl-2,3,7,8,12,18-hexamethylporphyrin (143) (30 mg; 5.13 x $10^{-4}$ moles) in dichloromethane (5 ml), was added by syringe. The solution was allowed to warm to room temperature and stir for 6 hours. The mixture was treated with water (15 ml), stirred for 30 minutes, and then diluted with dichloromethane (25 ml). The organic layer was washed with water (25 ml) and the solvent removed under reduced pressure. Initial purification was carried out by preparative thin layer chromatography with a mixture of chloroform/methanol (90/10). The residue was then columned over alumina (grade III) with chloroform, to afford the title compound as a purple film (10 mg; 34.1%) mp >300°C. $^1H$ NMR ($CDCl_2/CCl_4$/DMSO-$d_6$): δ -3.37 (1H, br), -3.22 (1H, br) (2 x NH), 0.82 (3H, t, phenyl-$CH_2C\underline{H}_3$), 1.90 (6H, t, 2 x $CH_2C\underline{H}_3$), 2.15-2.25 (2H, m, phenyl-$C\underline{H}_2CH_3$), 2.55 (6H, s, 3,7-$CH_3$), 3.55 (6H, s), 3.65 (6H,s) (2,8,12,18-$CH_3$), 4.07 (4H, q, 2 x $C\underline{H}_2CH_3$), 7.17 (1H, dd), 7.32 (1H, d), 7.44 (1H, d) (3 x phenyl-H), 8.65 (1H, s, OH), 9.87 (1H, s), 10.08 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 402.5 (5.23), 502.0 (4.16), 534.5 (3.83), 570.0 (3.80), 624.0 (3.39). EI MS: m/e (Relative Intensity) 570 (100.00), 555 (12.50), 270 (24.07).

b. Boron tribromide (3.23 g; 1.22 ml; 1.29 x $10^{-2}$ moles) was added under nitrogen, to a round bottom flask containing dichloromethane (15 ml). The mixture was cooled to -78°C in a dry ice/acetone bath and a solution of 5-ethyl-2-methoxy benzaldehyde (140) (1.01g; 6.15 x $10^{-3}$ moles) in dichloromethane (10 ml), was added by syringe. The solution was then allowed to warm to room

temperature and stir overnight. The mixture was treated with water (25 ml) and allowed to stir for 30 minutes. The layers were separated and the aqueous layer extracted with dichloromethane (10 ml). The combined organic layers were washed with water (25 ml), dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was columned over silica, eluting with a mixture of dichloromethane/ethyl acetate (75/25), to give 5-ethyl-2-hydroxybenzaldehyde (144) as a pale yellow oil (392 mg; 42.4%). $^1H$ NMR ($CDCl_3$): $\delta$ 1.22 (3H, t, $CH_2C\underline{H}_3$), 2.95 (2H, q, $C\underline{H}_2CH_3$), 5.67 (OH), 7.02 (1H, dd), 7.15 (1H, d), 7.29 (1H, d) (3 x phenyl-H), 10.23 (1H, s, CHO). EI MS: m/e (Relative Intensity) 150.1 (100.00), 135.0 (92.73), 120.9 (70.30), 107.0 (91.52).

1,19-Dideoxy-8,12-diethyl-2,3,7,13,17,18-hexamethylbiladiene-ac-dihydrobromide (133) (124 mg; 2.06 x $10^{-4}$ moles) and 5-ethyl-2-hydroxybenzaldehyde (144) (324 mg; 2.16 x $10^{-3}$ moles) were suspended in methanol (30 ml). A mixture of hydrobromic acid/acetic acid (1/1, 7 drops) was then added and the solution heated under reflux for 24 hours. The solution was cooled to room temperature and dichloromethane (40 ml) was added. The mixture was washed with water (40 ml), saturated aqueous sodium bicarbonate solution (40 ml) and water (40 ml). The solution was dried over sodium sulfate and the solvent removed under reduced pressure. The residue was columned over alumina (grade III), eluting with dichloromethane, to give the title porphyrin as a purple solid (48 mg; 40.9%).

Iron(III) 5-(6-ethyl-3-hydroxyphenyl)-13,17-diethyl-2,3,7,8,12,18-hexamethylporphyrin chloride (145b)

5-(6-ethyl-3-hydroxyphenyl)-13,17-diethyl-2,3,7,8,12,18-hexamethylporphyrin (145a) (7.8 mg) was dissolved in a mixture of

pyridine/acetic acid (1/20, 8 ml) and placed in a 25 ml pear-shaped flask equipped with a gas inlet tube. A slow stream of nitrogen was passed into the solution while the flask was placed in a preheated oil bath (80°C). A saturated aqueous solution of iron(II) sulfate (0.5 ml) was added through the gas outlet side arm, and the temperature of the bath was raised to 90°C. The solution was then allowed to stir for 30 minutes while maintaining the temperature between 90-95°C. The flask was removed from the heating bath and the mixture allowed to cool to room temperature. A stream of air was passed through the solution for 10 minutes and the mixture partitioned between water (30 ml) and chloroform (30 ml). The organic phase was separated and washed first with a 5% HCl solution (50 ml) and then with water (2 x 50 ml). The solvent was removed under reduced pressure and the resulting solid vacuum dried to give the title porphyrin as a bluish-brown film (6.5 mg, 72.1%). UV/visible ($CH_2Cl_2$): $\lambda$ max 388.0, 505.5, 621.5. Porphyrin as $\upsilon$-oxo dimer ($CH_2Cl_2$): $\lambda$ max (log $\varepsilon$) 387.0 (3.99). EI MS: Calc. for $C_{38}H_{40}N_4OFeCl$: 660.0675. Found: 624.3 (molecular ion -Cl).

5-(3-Ethyl-6-hydroxyphenyl)-13-17-diethyl-2,3,7,8,12,18-hexamethylporphyrin (146a)

Compound (146a) was prepared from 1,19-dideoxy-8,12-diethyl-2,3,7,13,17,18-hexamethylbiladiene-a,c-dihydrobromide (133) (281 mg; 466 x $10^{-4}$ moles) and 5-ethyl-2-hydroxybenzaldehyde (142) (700 mg; 4.66 x $10^{-3}$ moles), by the procedure described for (145a). The crude product was columned over silica, eluting with dichloromethane, and recrystallized from dichloromethane/methanol to give the desired porphyrin as deep purple crystals (168 mg, 63.1%); mp >300°C. $^1$HNMR ($CDCl_3$): $\delta$ -3.20 (2H, br, 2 x NH), 1.35 (3H, t, phenyl-$CH_2C\underline{H}_3$), 1.87 (6H, t, 2 x $CH_2C\underline{H}_3$), 2.62 (6H, s, 3, 7-

$CH_3$), 2.80 (2H, q, phenyl-C$\underline{H_2}CH_3$), 3.31 (1H, s, phenyl-0H), 3.53 (6H, s), 3.62 (6H, s) (2, 8, 12, 18-$CH_3$), 4.05 (4H, q, 2 x C$\underline{H_2}CH_3$), 7.26 (1H, d), 7.53 (1H, dd), 7.58 (1H, d) (3 x phenyl-H), 9.98 (1H, s), 10.16 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 401.5 (5.21), 500.5 (4.16), 534.5 (3.87), 571.0 (3.80), 623.0 (3.49). EI MS: m/e (Relative Intensity) 570 (56.68), 556 (26.04), 555 (71.89), 554 (36.41), 553 (100.0), 285 (68.20), 278 (27.65), 277 (25.92), 270 (56.22).

Iron(III) 5-(3-ethyl-6-hydroxyphenyl)-13,17-diethyl-2,3,7,8,12,18-hexamethylporphyrin chloride (146b)

Compound (146b) was prepared from 5-(3-Ethyl-6-hydroxyphenyl)-13,17-diethyl-2,3,7,8,12,18-hexamethylporphyrin (146a) (30 mg), by the procedure detailed for (145b). The resulting solid was columned over silica, eluting with a mixture of chloroform/methanol (90/10). The solvent was removed under reduced pressure and the porphyrin redissolved in chloroform (50 ml). The solution was washed with a 5% HCl solution (3 x 50 ml) and with water (2 x 50 ml). The organic layer was passed through several pieces of filter paper and the solvent removed under reduced pressure. The solid was dried under vacuum overnight to give the desired porphyrin as a bluish-brown film (27.1 mg, 78.1%). UV/visible ($CH_2Cl_2$): λ max (log ε) 383.5 (4.85), 507.5 (3.83), 534.0 (3.78), 640.5 (3.48). EI MS: Calc. for $C_{38}H_{40}N_4OFeCl$: 660.0675. Found: 624.4 (molecular ion -Cl).

5-(6-Ethyl-3-methoxyphenyl)-12,18-dimethyl-2,3,7,8,13,17-hexaethylporphyrin (147)

Compound (147) was prepared from 1,19-dideoxy-7,13-dimethyl-2,3,8,12,17,18-hexaethylbiladiene-ac-dihydrobromide (134) (200 mg; 3.04 x

$10^{-4}$ moles) and 2-ethyl-5-methoxybenzaldehyde (140) (300 mg; 1.83 x $10^{-3}$ moles), by the procedure described for (146a). The crude product was columned twice over silica gel, eluting with dichloromethane, and recrystallized from dichloromethane/methanol to give deep purple crystals (58 mg; 29.8%); mp 245-246°C. $^1$H NMR ($CDCl_3$): δ-3.08 (1H, br), 2.95 (1H, br) (2 x NH), 0.70 (3H, t, phenyl-$CH_2C\underline{H}_3$), 1.24 (6H, t), 1.87 (12H, t) (6 x $CH_2C\underline{H}_3$), 1.99 (2H, q, phenyl-$C\underline{H}_2CH_3$), 2.64 (2H, q), 3.07 (2H, q), 3.99-4.09 (8H, overlapping q) (6 x $C\underline{H}_2CH_3$), 3.64 (6H, s, 2 x $CH_3$), 3.97 (3H, s, $OCH_3$), 7.34 (1H, dd), 7.48 (1H, d), 7.89 (1H, d) (3 x phenyl-H), 9.90 (1H, s), 10.17 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 404.5 (5.21), 503.0 (4.13), 536.5 (3.80), 571.0 (3.78), 623.0 (3.33). EI MS: m/e (Relative Intensity) 640.0 (100.00), 305.8 (24.52), 291.3 (41.94).

5-(3-Ethyl-6-hydroxyphenyl)-12,18-dimethyl-2,3,7,8,13,17-hexaethylporphyrin (148)

Compound (148) was prepared from 1,19-dideoxy-7,13-dimethyl-2,3,7,8,12,17,18-hexaethylbiladiene-ac-dihydrobromide (134) (200 mg; 3.04 x $10^{-4}$ moles) and 5-ethyl-2-hydroxybenzaldehyde (142) (456 mg; 3.04 x $10^{-3}$ moles), by the procedure detailed above. The crude product was columned on alumina (grade III), eluting with dichloromethane. The fractions containing porphyrin were combined and the solvent removed under reduced pressure. The resulting solid was recrystallized from a minimal amount of dichloromethane/methanol to give the title porphyrin as a dark purple crystals (102 mg, 53.4%); mp 195-196°C. $^1$H NMR ($CDCl_3$): δ -3.19 (1H, br), -3.05 (1H, br) (2 x NH), 1.23 (6H, t, 2 x $CH_2C\underline{H}_3$), 1.39 (3H, t, phenyl-$CH_2C\underline{H}_3$), 1.82-1.93 (12H, m, 4 x $C\underline{H}_2CH_3$), 2.84 (2H, q, phenyl-$C\underline{H}_2CH_3$), 2.88-3.08 (4H, m, 2 x $C\underline{H}_2CH_3$), 3.64 (6H, s, 2 x $CH_3$), 3.97-4.12 (8H, m, 4 x $C\underline{H}_2CH_3$), 4.75 (1H, br,

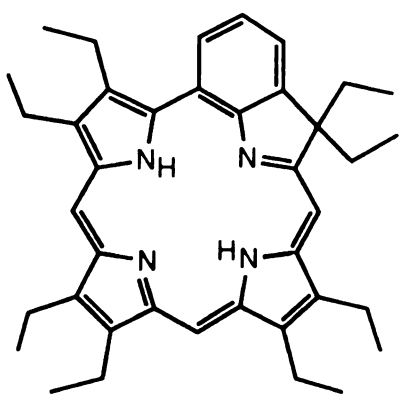
OH), 7.21 (1H, d), 7.56 (1H, dd), 7.89 (1H, d) (3 x phenyl-H), 9.95 (1H, s), 10.19 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 404.0 (5.25), 503.5 (4.19), 537.0 (3.91), 571.0 (3.84), 624.5 (3.51). EI MS: m/e (Relative Intensity) 626 (25.35), 610 (30.70), 599 (36.74), 598 (100.00), 314 (24.88), 269 (24.77).

# CHAPTER 4

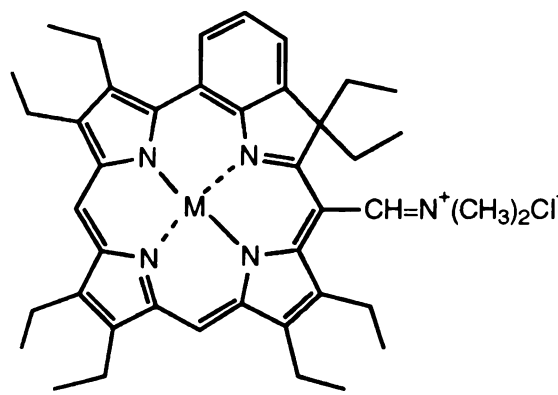
# THE STUDY OF BENZOCHLORIN DYES FOR PDT

## I. Hydrolysis Studies of Imminium Chlorin Compounds

As a long wavelength absorbing dye, benzochlorin (72a) is an interesting and versatile compound.[128-130] The imminium derivatives (73a-d), are an intriguing set of cationic structures that have received considerable attention as potential PDT photosensitizers. Preliminary *in vitro* studies, revealed that the efficacy of these compounds followed the order (73a)>(73b)>(73c)>(73d).[130] As mentioned in Chapter 1, benzochlorin imminium salts have also been found to possess *in vivo* PDT effectiveness and are unusually stable towards hydrolysis. Interestingly, the PDT efficacy was also found to be dependent upon the presence of the imminium group.[131]



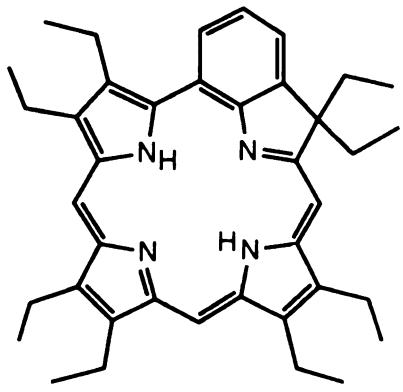

72a.



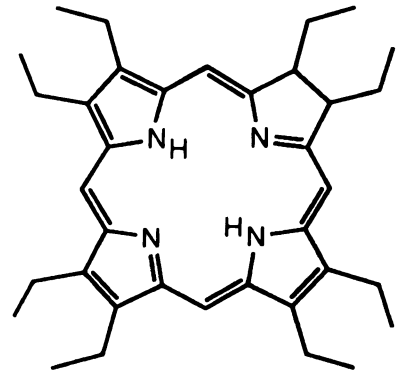

73a. M = 2H
b. M = Zn
c. M = Cu
d. M = Ni

In an attempt to explain the stability of these cationic benzochlorins, we decided to investigate the hydrolysis properties of a series of chlorin imminium compounds. It was envisioned that a possible trend may be revealed, which would give insights into the synthesis of stable chlorin structures that are suitable for PDT studies. The compounds utilized are shown in Figure 4, and were synthesized by known literature procedures.[130,132-134] These chlorins were initially metallated with copper acetate to give compounds (72c) and (153-156). The copper complexes were then treated with dimethyl formamide and phosphorus oxychloride to give imminium salts (73c) and (157-160) (Scheme 32). Compounds (73c), (157) and (158) were sufficiently stable to be isolated and purified by column chromatography. Chlorins (159) and (160) were unstable towards purification techniques. In these instances, the resulting residue was vacuum dried for several hours and then used without further purification.

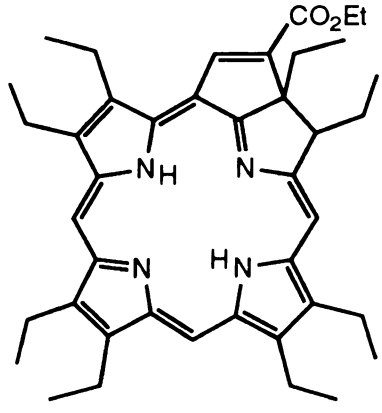
Each imminium salt was dissolved in 10 to 15 ml of dry dichloromethane (approximately $10^{-5}$ M) and an initial UV/visible spectrum taken. An equal volume of water or pH 10 buffer solution (sodium hydroxide/sodium borate) was then added, the starting time recorded, and the mixture stirred. Aliquots of the dichloromethane were then removed at various time intervals and the UV/visible spectrum taken. The hydrolysis was continued until the imminium salt was completely converted into the corresponding aldehyde (Scheme 32). Figure 5 displays an overlay of the UV/visible spectrum of imminium salt (73c) and the resulting hydrolysis product, aldehyde (161). Figure 6 is an example overlay of the UV/visible spectrum of imminium salt (157) and the corresponding aldehyde (162).



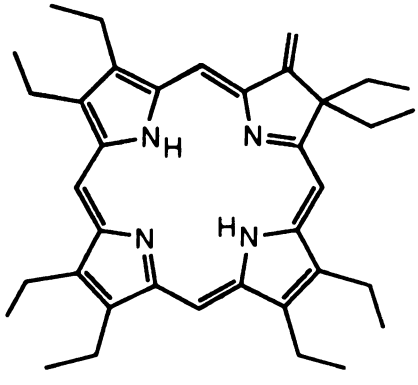
72a. benzochlorin



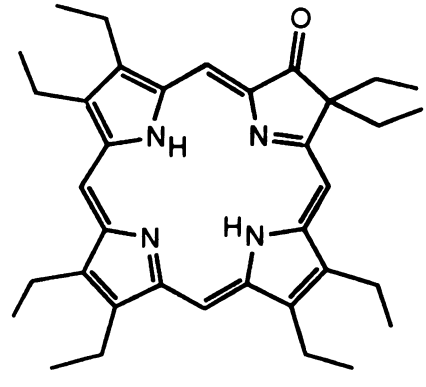
149 octaethylchlorin



150 purpurin

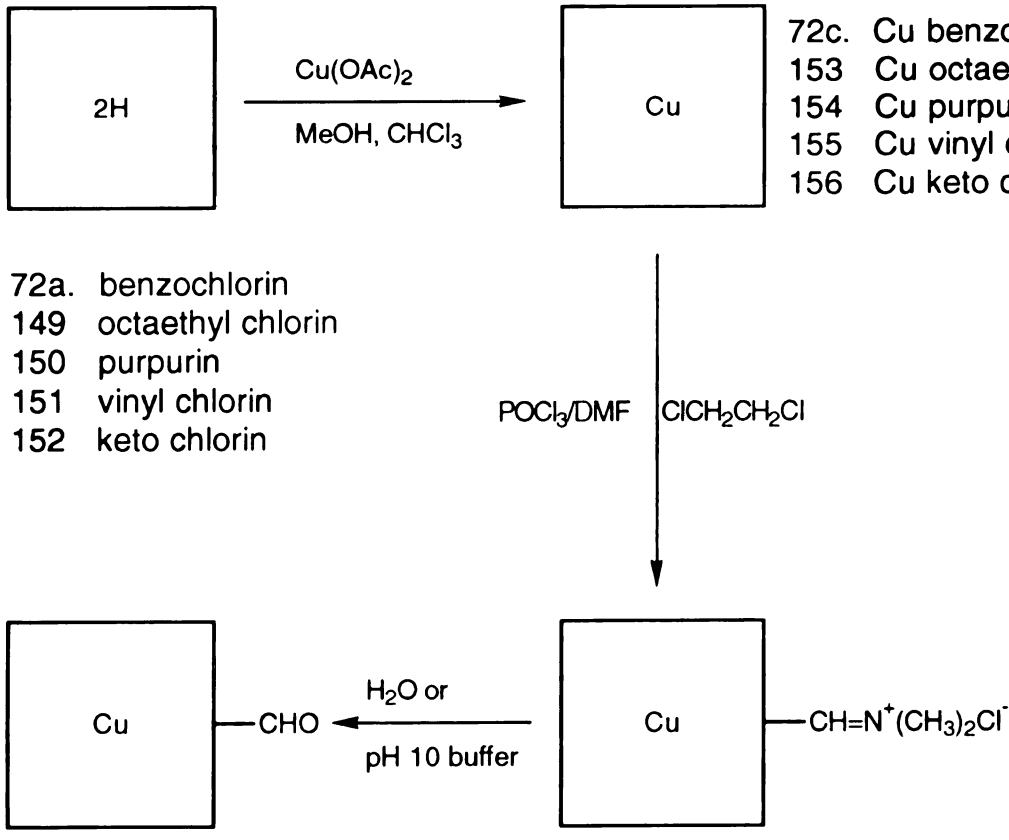


151 vinyl chlorin



152 keto chlorin

Figure 4. Chlorins Utilized in the Hydrolysis Study




| | | | |
|---|---|---|---|
| 161 | Cu benzochlorin aldehyde | 73c. | Cu benzochlorin imminium salt |
| 162 | Cu octaethyl chlorin aldehyde | 157 | Cu octaethyl chlorin imminium salt |
| 163 | Cu purpurin aldehyde | 158 | Cu purpurin imminium salt |
| 164 | Cu vinyl chlorin aldehyde | 159 | Cu vinyl chlorin imminium salt |
| 165 | Cu keto chlorin aldehyde | 160 | Cu keto chlorin imminium salt |

Scheme 32. Synthesis of Chlorin Aldehydes (161-165)

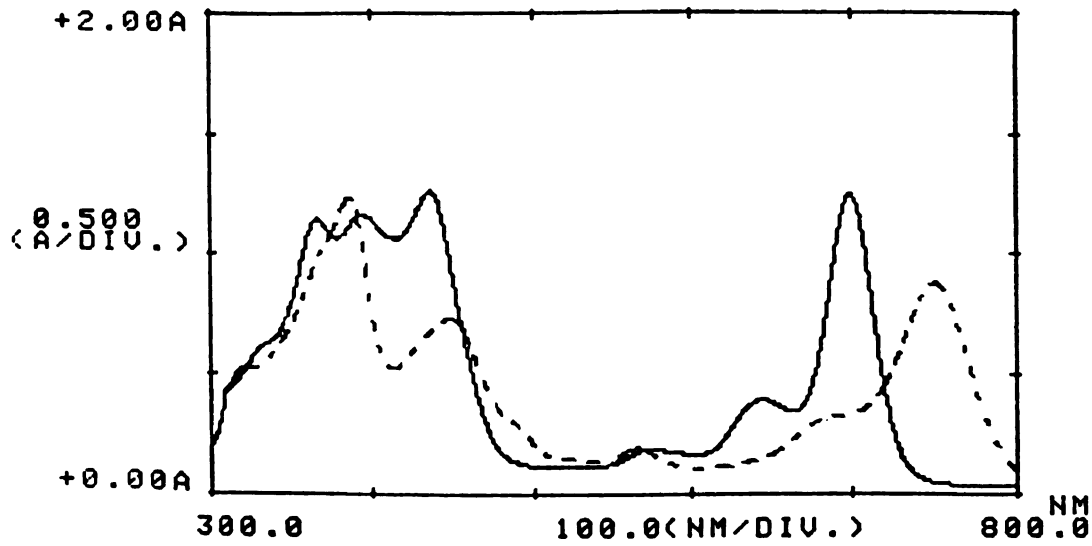



Figure 5. UV/Visible Absorption Spectra of Imminium Benzochlorin (73c) (dashed line) and Hydrolysis Product (161) (solid line) in $CH_2Cl_2$

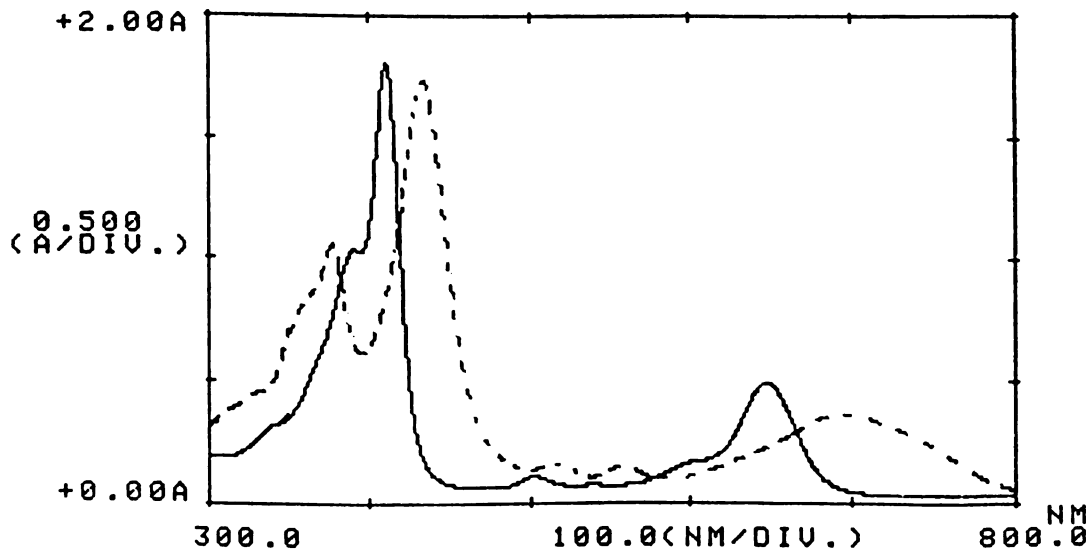



Figure 6. UV/Visible Absorption Spectra of Imminium Chlorin (157) (dashed line) and Hydrolysis Product (162) (solid line) in $CH_2Cl_2$

By measuring absorption changes in the UV/visible spectra and plotting those values against the corresponding reaction time (logarithmic plot), we were able to estimate the half-life of each imminium salt. Where possible, both an increasing and decreasing intensity absorption was followed and the half-life determined in each case. The two values were then averaged. Water hydrolysis plots typical of compound (157), are shown in Figures 7 and 8. Figure 7 displays a hydrolysis plot resulting from the observation of absorption changes at 647 nm (increasing intensity), while Figure 8 displays a hydrolysis plot resulting from the observation of absorption changes at 697 nm (decreasing intensity). In most instances, multiple runs were also recorded for each compound and these results averaged as well. Each chlorin was treated in a similar manner, and the results of the various hydrolysis experiments are shown in Table 1.

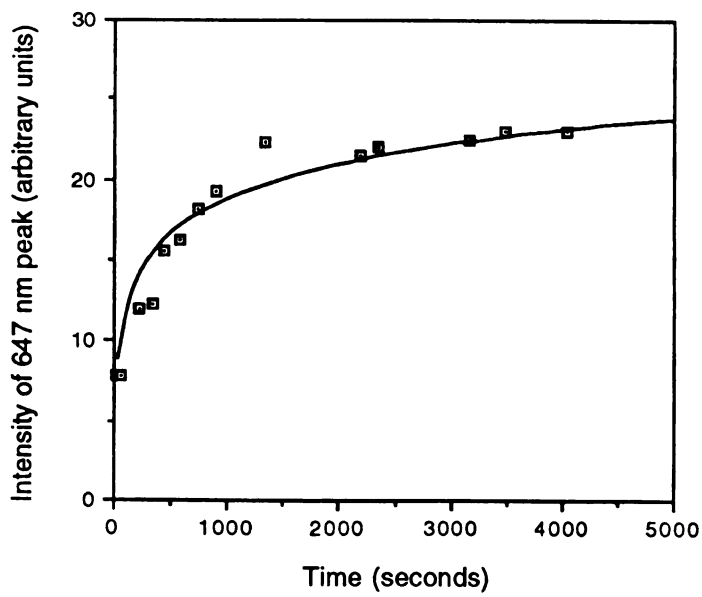



Figure 7. A Water Hydrolysis Plot of (157), Resulting from the Observation of Absorption Changes at 647 nm

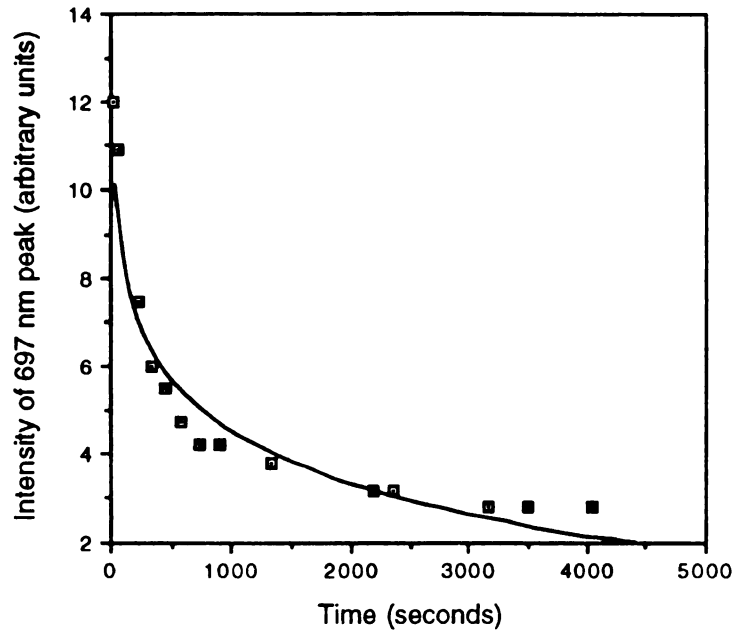



Figure 8. A Water Hydrolysis Plot of (157), Resulting from the Observation of Absorption Changes at 697 nm

Table 1. Estimated Half-Lives of Imminium Chlorins (73c) and (157-160)

| | Half-lives | |
|---|---|---|
| Compound | pH 10 | Water |
| 73c | 70 minutes | >1 week |
| 157 | 3 minutes | 140 minutes |
| 158 | 10 seconds | 10 minutes |
| 159 | ---------- | <5 seconds |
| 160 | ---------- | <5 seconds |

The results of the hydrolysis study revealed that the stability of the imminium salts followed the order (73c)>>(157)>(158)>(159), (160). Examination of these results and the structural features of the chlorin imminium salts, led to certain conclusions. It appeared unlikely that the stability of compound (73c) was due to structural and/or steric effects alone. Initially, the geminal ethyl substituents of (73c) were thought to the shield the imminium group against hydrolysis. The experimental data revealed however, that both compounds (159) and (160) hydrolyzed rapidly even though they also contain a similar set of geminal ethyl groups. These results suggested that there were also electronic factors influencing the hydrolysis rates.

Each chlorin undoubtedly contains a somewhat different distribution of electron density, due to the presence of various types of functional groups. This was evident during the preparation of the imminium compounds. Benzochlorin and octaethylchlorin appeared to possess a greater amount of electron density on the meso position adjacent to the site of saturation, as compared to the other chlorin structures. Immium salt (73c) was readily prepared at room temperature, in less than 1 hour. Compound (157) was also prepared in less than one hour. However, chlorins (158-160) required longer reaction times and elevated temperatures to facilitate the transformations. These electron density differences may also explain the greater stability of chlorins (73c) and (157), as compared to compounds (158-160). The more electron density available at the meso position containing the imminium group, the less susceptible that substituent should be to attack by water or hydroxide ions. Consequently, the hydrolysis rate should also be slower.

The experimental results suggested that ethyl substituents common to each chlorin, were not a major factor in the stability of these compounds. Therefore, the unique substituents were more likely responsible for the

observed results. The benzo group of chlorin (73c) may have been electron donating, and/or causing distortion of the macrocycle in such a manner as to alter the ring electronics and shift electron density towards the meso position. Chlorins (158-160) possessed electron withdrawing substituents (e.g. keto, vinyl, and ester), which could have resulted in a relative deficiency of electron density at the meso position. This would be expected to result in faster hydrolysis of the imminium substituent, as compared to chlorin (73c). Chlorin (157) did not contain any unique, electron donating or electron withdrawing substituents. Therefore, it is not surprising that the stability of this compound was found to be between chlorins (73c) and (158-160).

Since compound (73c) was found to be the most stable imminium salt, further research into cationic compounds will likely focus on structures of this type. As mentioned in Chapter 1, cationic structures tend accumulate in diseased tissue to a greater extent than their neutral counterparts and are usually more water soluble. These characteristics are highly desirable in photodynamic therapy. However, new cationic structures also need to be stable enough to resist chemical changes that might result in the loss of PDT efficacy.

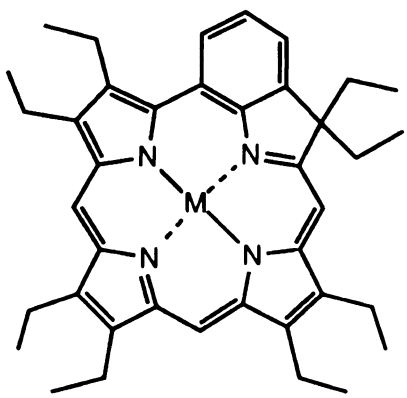
## II. The Synthesis of Benzochlorin Derivatives

The interesting properties associated with the reported derivatives of benzochlorins (72a-c), have sparked our interest in the synthesis of other analogues of these compounds. We have subjected benzochlorins (72b-d) to various reactions conditions, in order to further define the chemistry of these compounds. It was expected that the information gathered on the scope and limitations of these reactions would provide insights into the synthesis of other potential, chlorin-type PDT compounds.

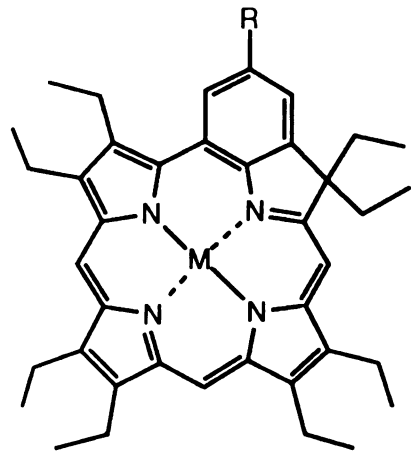
Acetylation and formylation reactions were initially carried out on benzochlorins (72b-d), in an attempt to synthesize compounds (166a,b) and (167a,b). The rationale behind these electrophilic reactions was based upon the fact that benzochlorins (72a) and (72b) afford the sulfonated derivative (168), when treated with concentrated sulfuric acid (Scheme 33).[94] It was envisioned that the acetyl and formyl groups of compounds (166a,b) and (167a,b), could then be further functionalized. We were also interested in the derivatization of benzochlorins (166a) and (167a), through the use of Vilsmeyer reagent (N,N-dimethymethyleneammonium chloride), to afford imminium compounds (169) and (170).

Compound (72b) was treated with acetic anhydride and $SnCl_4$, in an attempt to synthesize chlorin (166a) (Scheme 34). However, only starting material was recovered along with decomposition products. Stirring the reaction mixture at 40°C overnight, also gave similar results. Increasing the temperature to 85°C, led to total consumption of the starting material and no actetylated product. Trace amounts of chorin (166a) were observed, when (72b) was treated with acetic anhydride and a combination of $AlCl_3$ and $SnCl_4$. However, the majority of the reaction mixture again contained starting material

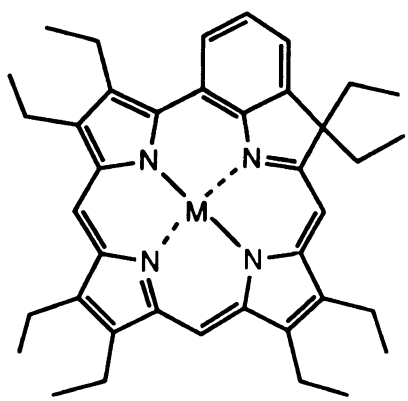
and decomposition products. The utilization of the copper complex, resulted in similar findings. The treatment of compound (72c) with acetic anhydride and $SnCl_4$, only afforded chlorin (166b) in trace amounts. The use of acetyl chloride was also unsuccessful in facilitating the above transformations.



72a. M = 2H
b. M = Ni
c. M = Cu
d. M = Fe•Cl

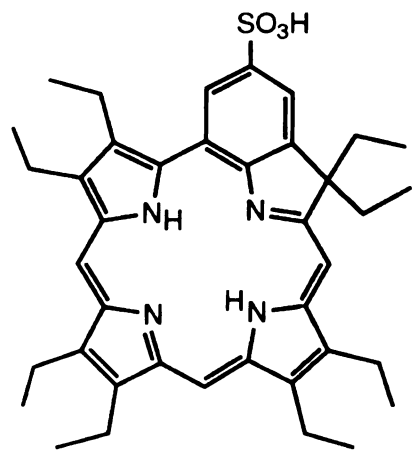


166a. R = $COCH_3$, M = Ni
b. R = $COCH_3$, M = Cu
167a. R = CHO, M = Ni
b. R = CHO, M = Fe•Cl



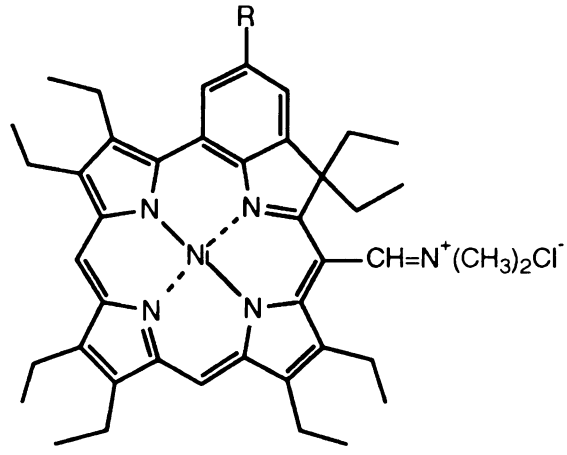
72a. M = 2H
b. M = Ni

$H_2SO_4$

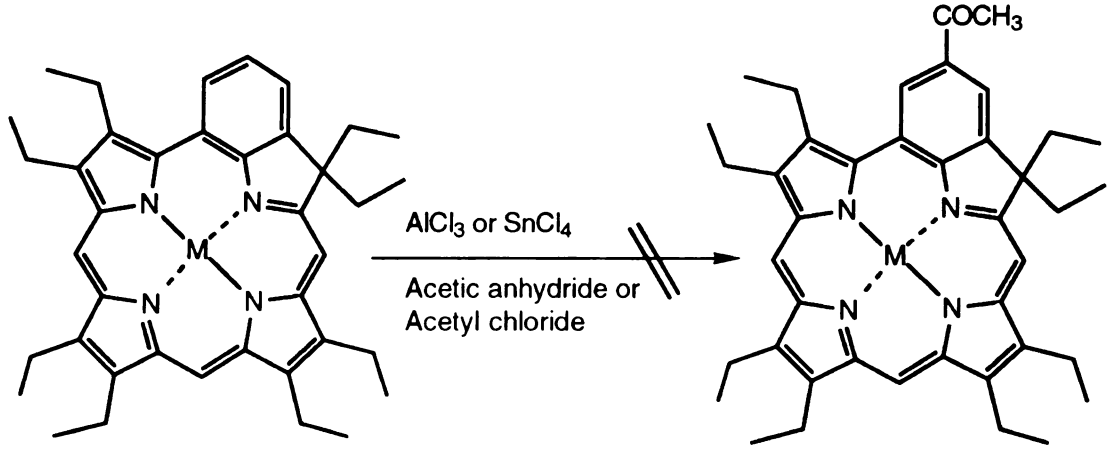


168

Scheme 33. Synthesis of Sulfonated Benzochlorin (168)




169 R = $COCH_3$
170 R = CHO



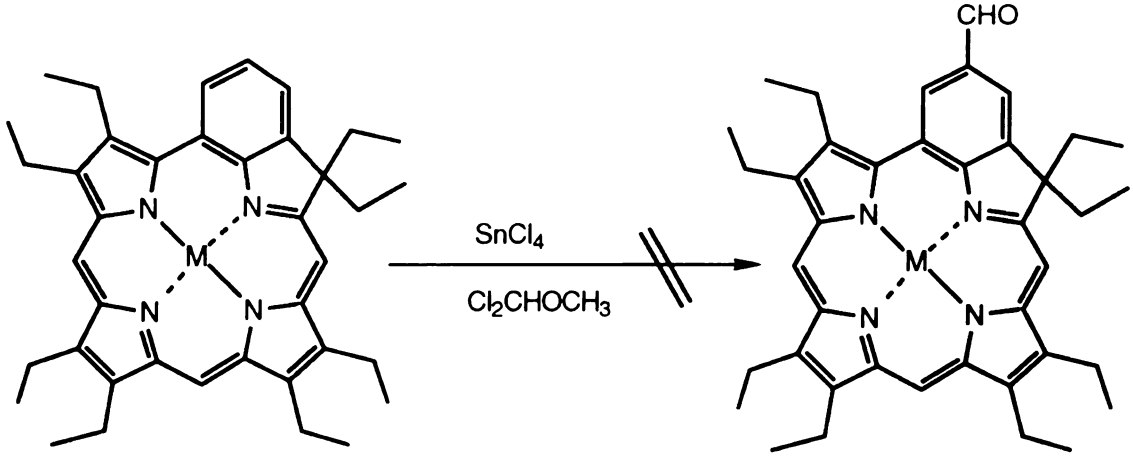

72b. M = Ni
c. M = Cu

166a. M = Ni
b. M = Cu

Scheme 34. Attempted Synthesis of Acetylated Benzochlorins (166a,b)

We then attempted to formylate the benzo group of chlorin (72b). The treatment of compound (72b) with dichloromethyl methyl ether and $SnCl_4$, failed to give compound (167a) (Scheme 35). As before, the major constituent of the reaction mixture was recovered starting material. Iron benzochlorin (72d) was then utilized. The treatment of compound (72d) with dichloromethyl methyl ether and $SnCl_4$, also failed to produce (167b) and only resulted in the decomposition of the starting material. These acetylation and formylation

reactions were typically run by dissolving the benzochlorin (~30 mg) in a mixture of dichloromethane (10-15 ml), and acetic anhydride or dichloromethyl methyl ether (0.1-0.2 ml). These solutions were then cooled in a ice bath and the $SnCl_4$ added (0.1-0.2 ml). The mixtures were subsequently allowed to warm to room temperature and stir anywhere from 1 to 24 hours. After several unsuccessful formylation attempts using these conditions, we decided to increase the amount of $SnCl_4$ and carry out the formylations in neat dichloromethyl methyl ether. Benzochlorin (72d) (~30 mg) in dichloromethyl methyl ether (15 ml), was treated with $SnCl_4$ (1 ml) and sampled at various time intervals from 1 hour to 2 days. Unfortunately, only a trace amount of compound (167b) was observed by mass spectral analysis. Also, the starting material was totally consumed in this reaction. It became apparent that electrophilic addition under the conditions described above, would not afford useful quantities of compounds (166a,b) or (167a,b).




72b. M = Ni
d. M = Fe•Cl

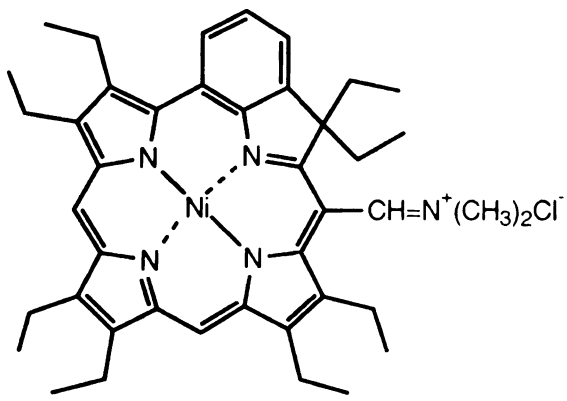
167a. M = Ni
b. M = Fe•Cl

Scheme 35. Attempted Synthesis of Formylated Benzochlorins (167a,b)

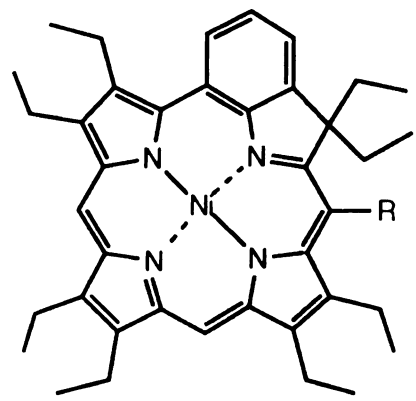
Imminium chlorin (73d) has also been shown to undergo sulfonation at the benzo group, when treated with sulfuric acid.[130,135] In theory then, the

acetylation and formylation of (73d) should directly lead to compounds (169) and (170). A comparison between the sulfonation reaction times of compounds (72a,b) and (73d) however, suggested that the imminium derivative was even less reactive in electrophilic reactions. Chlorins (72a) and (72b) were found to sulfonate within several hours, while chlorin (73d) required a reaction time of several days. Therefore, compound (73d) was not subjected to acetylation or formylation experiments.

We then decided to investigate meso-substituted benzochlorins. The treatment of benzochlorin (72b) with a mixture of dimethylaminoacrolein and phosphorus oxychloride has been shown to afford compound (171), as evident by the red-shifted absorbance band at 816 nm.[129,135] Unfortunately, attempts at isolating and purifying this compound have resulted in rapid hydrolysis of the imminium group to afford chlorin aldehyde (172).[135] Cationic structures with absorbance properties similar to (171), may be of interest in photodynamic therapy. However, these benzochlorin derivatives would need to be fairly stable for use in PDT applications.



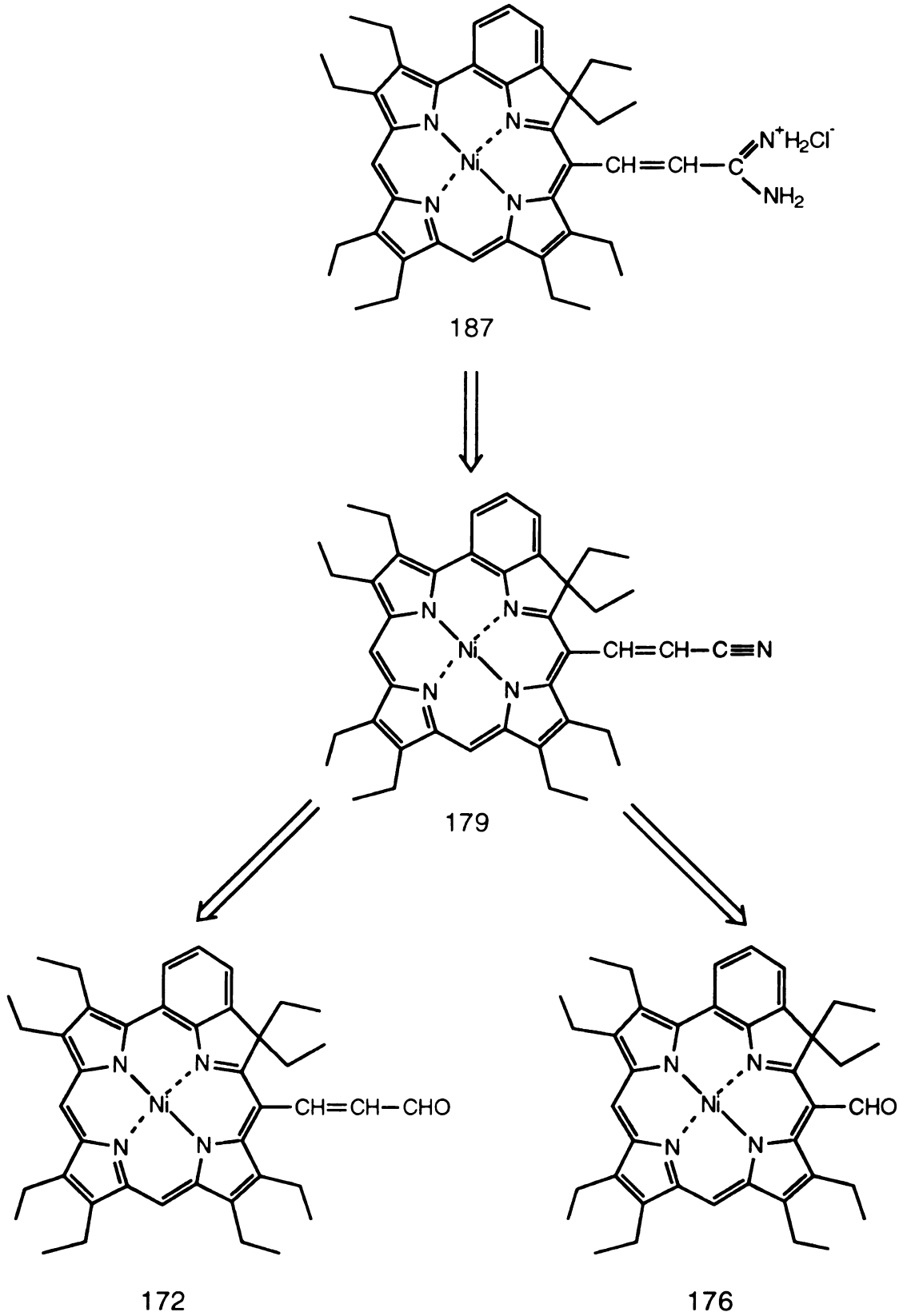

73d.



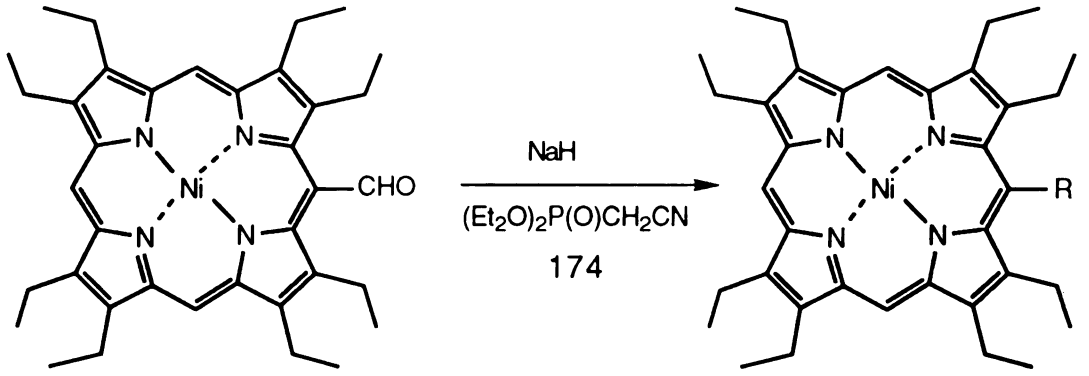

171 R = CH=CH-CH=$N^+(CH_3)_2Cl^-$

172 R = CH=CH-CHO

Compound (187) was chosen as a target molecule, due to its cationic nature and resemblance to chlorins (73d) and (171). It was envisioned that compounds (172) or (176) could be converted to nitrile (179), which would then lead to amidinium (187) (Scheme 36). Compound (176) was initially chosen in the synthesis of nitrile (179). Porphyrin (173) can be readily converted into nitrile (175a), by treatment with diethylcyanomethylphosphonate (174) and sodium hydride (Scheme 37).[136] We thought that benzochlorin (179) could be obtained by using a similar set of reaction conditions. Benzochlorin (72b) was formylated with dimethyl formamide and phosphorus oxychloride to give (176) in 73.4% yield. Benzochlorin (176), diethylcyanomethylphosphonate (174) and sodium hydride were then refluxed in toluene for 1 hour. However, the expected nitrile (179) was only obtained in trace amounts (Scheme 38). This transformation was then attempted using a number of different solvents (tetrahydrofuran, toluene, dimethoxyethane) and bases (sodium hydride, n-butylithium). Unfortunately, none of the various reaction conditions led to appreciable quantities of nitrile (179). A number of different Wittig-type reagents were investigate as well. Triphenylphosphoranylidenacetronitrile (177)[137] and chlorin (176) and were refluxed in toluene, as well as xylene, for 24 hours. In both cases, the only identifiable compound was recovered starting material. The final reagent utilized was trimethylsilylacetonitrile (178).[138,139] The treatment of chlorin (176) with (178) and lithium diisopropylamine, also failed to produce the required nitrile and resulted in almost complete destruction of the starting material. It became apparent that Wittig-type reactions were not the route to chlorin (179), and other methods would be required.



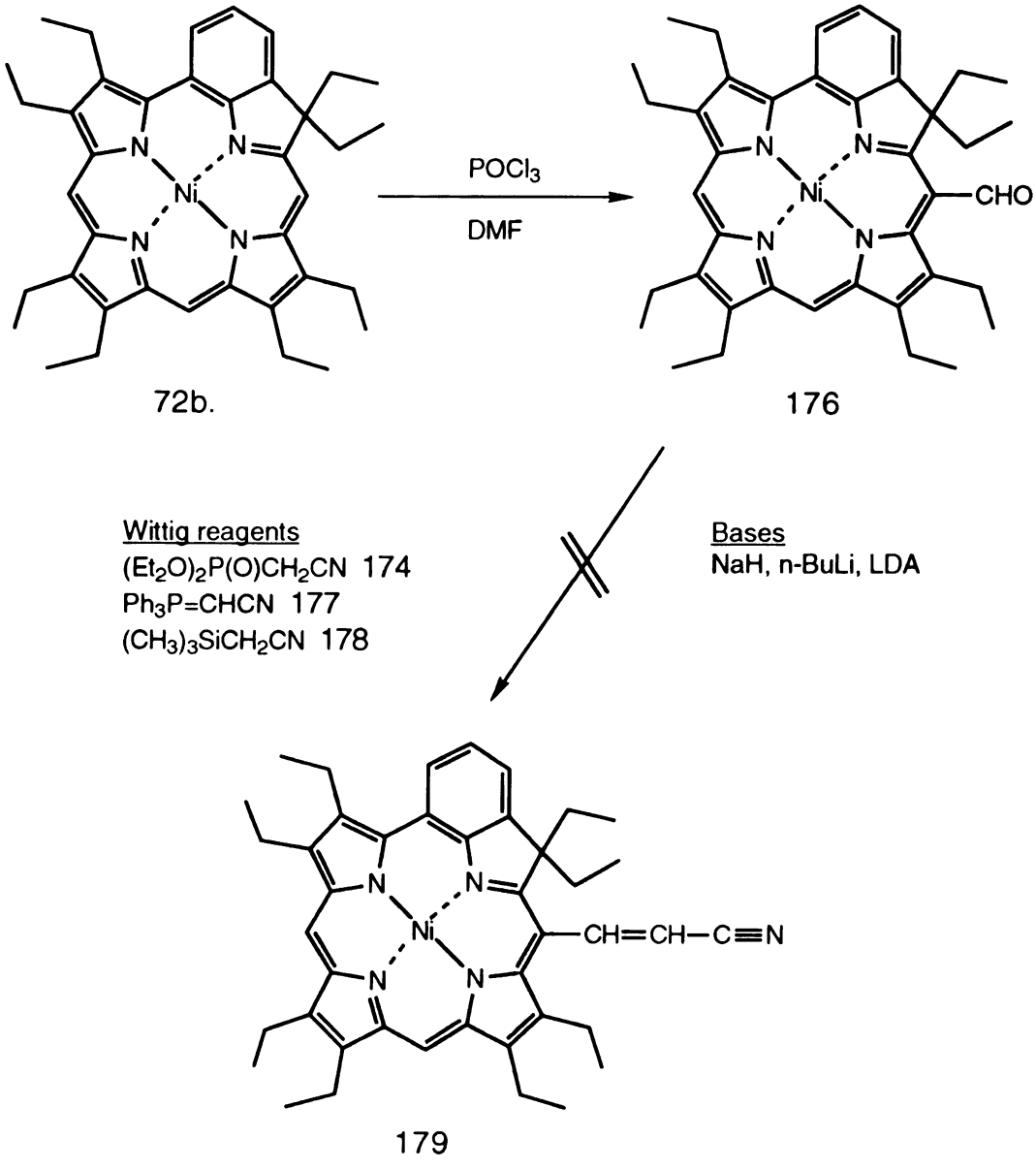

Scheme 36. Retrosynthetic Analysis of Benzochlorin Amidinium (187)



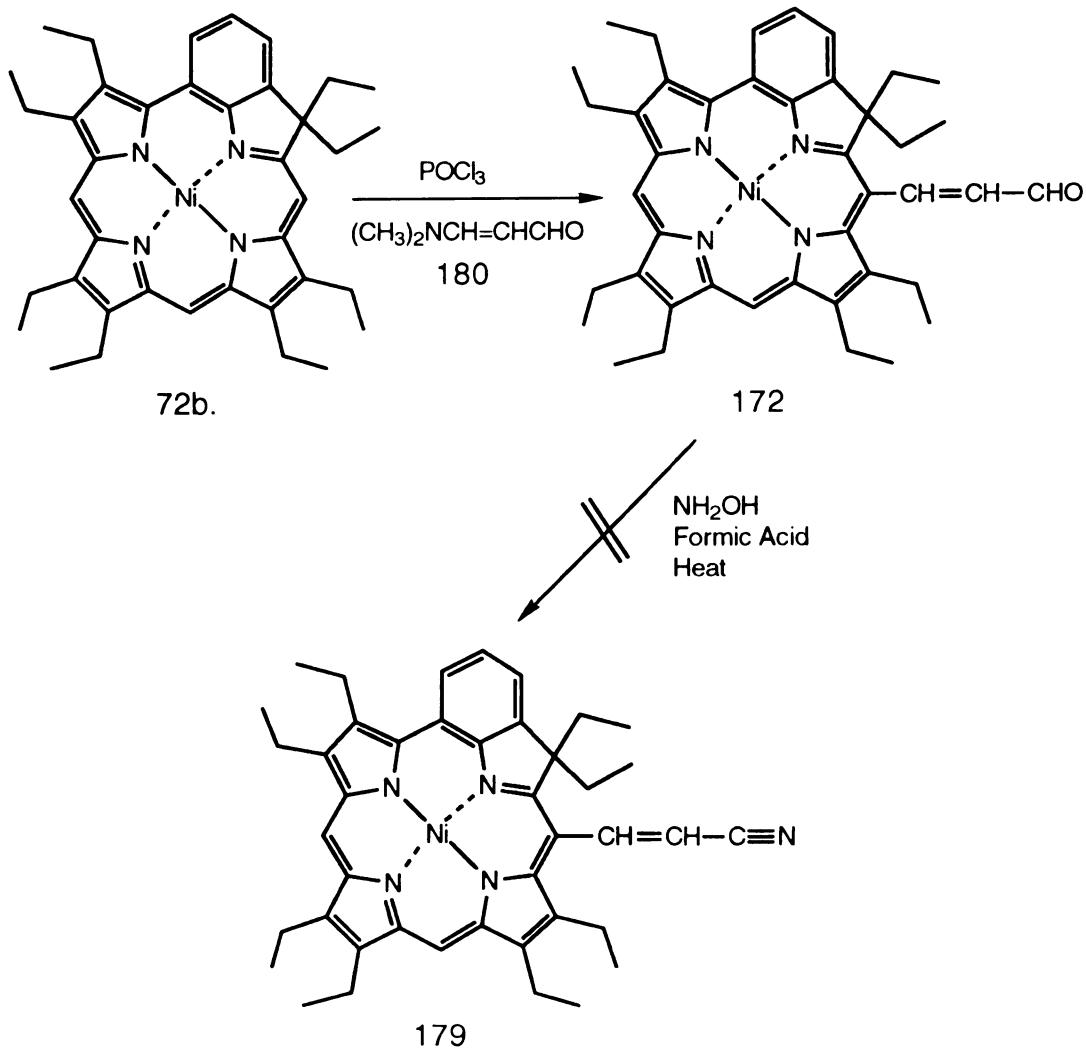

173 175a. R = CH=CH—C≡N

Scheme 37. Synthesis of Porphyrin (175a)

Olah and coworkers have reported that aldehydes can be readily converted to nitriles by treatment with hydroxylamine and formic acid.[140] This methodology has recently been applied to the synthesis of porphyrins bearing nitrile substituents.[141] It was envisioned that chlorin (172) could be reacted under these conditions to give (179). Benzochlorin (72b) was treated with dimethylaminoacrolein (180) and phosphorus oxychloride to give (172) in 78.4% yield. Compound (172) and hydroxylamine were then refluxed in formic acid. However, this reaction afforded an unidentified green compound instead of the expected nitrile (179) (Scheme 39). The paramagnetic nature of this compound, made it necessary to perform several side reactions to help in the identification. Porphyrin (181) was treated with dimethylaminoacrolein (180) and phosphorus oxychloride to afford compound (182a) in 84.7% yield. When porphyrin (182a) and hydroxylamine were refluxed in formic acid, the major product was benzochlorin (72b) instead of porphyrin (175a) (Scheme 40). These results suggested that the unidentified compound obtained from (172), could have been dibenzochlorin (183) (Scheme 41). Fortunately, this chlorin had been previously reported.[129] The subsequent comparison of the property data, revealed that our compound was in fact (183).

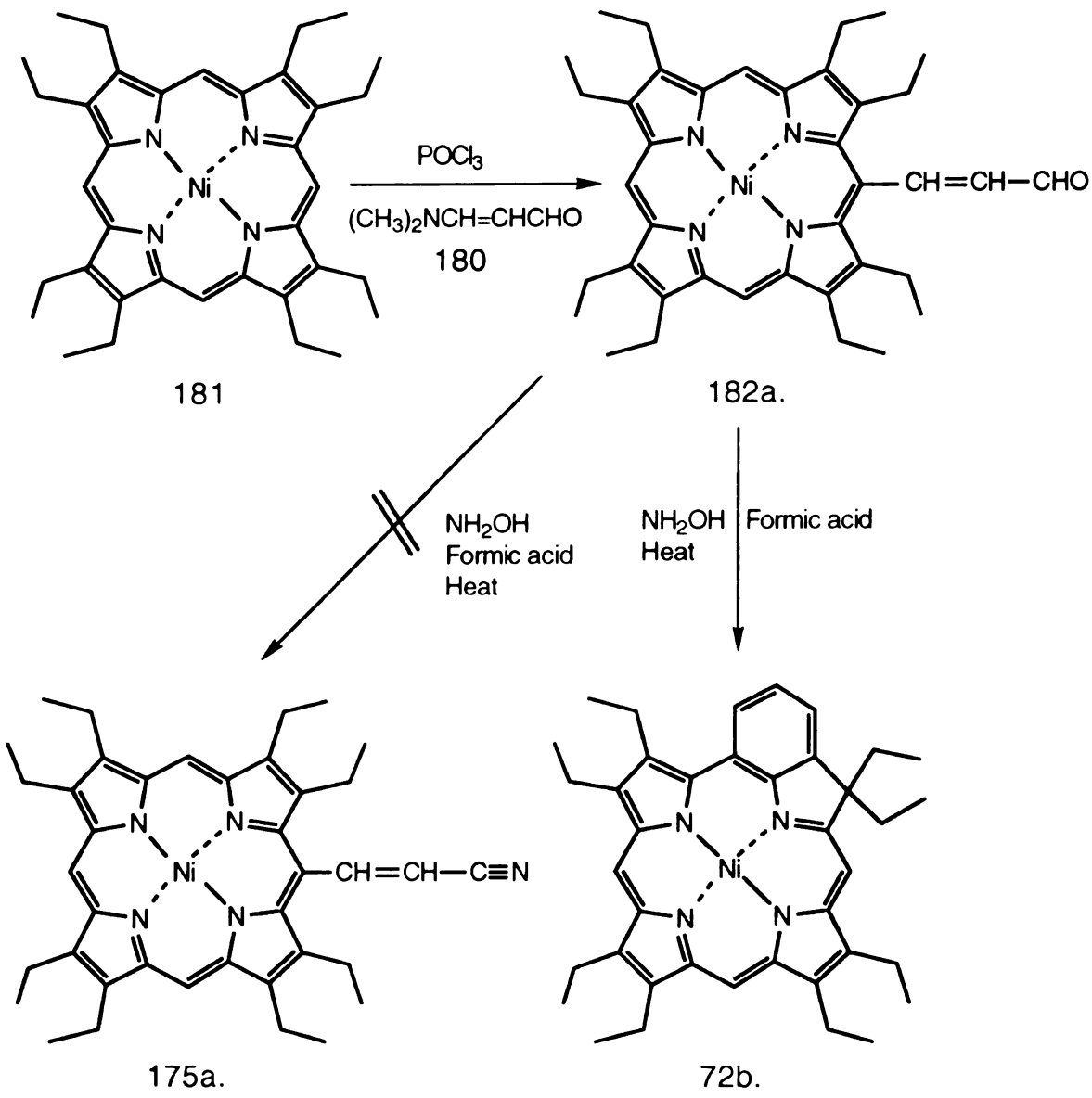



Scheme 38. Attempted Synthesis of (179) Through Wittig Chemistry

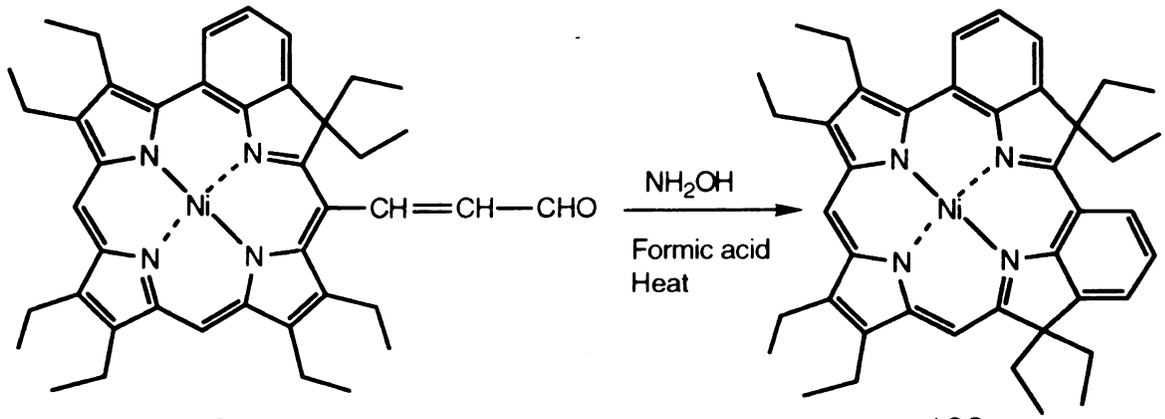



72b. 172 179

Scheme 39. Attempted Synthesis of (179) Through a Hydroxylamine Adduct




Scheme 40. Synthesis of (72b) Through the Utilization of Formic Acid

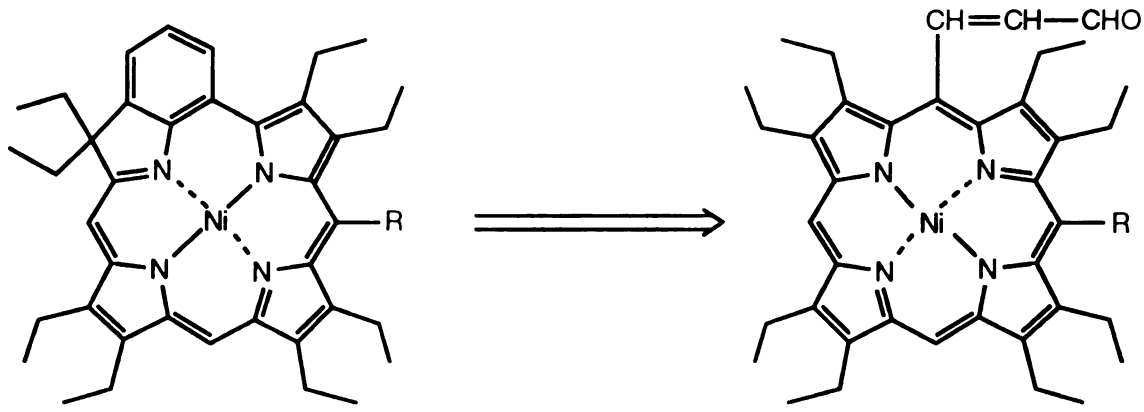



172 183

Scheme 41. Synthesis of Dibenzochlorin (183)

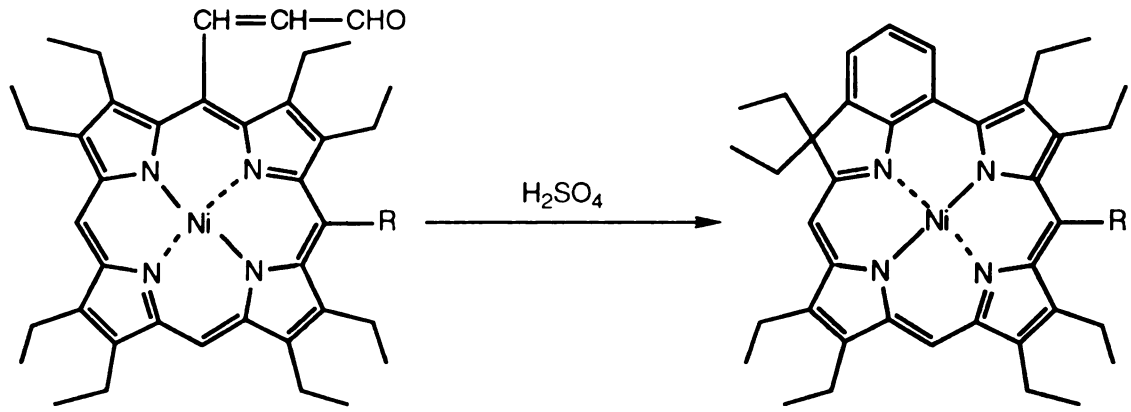
The benzochlorin structures described above, appear to be unreactive towards the common transformation techniques used to synthesize nitrile compounds. We then decided to investigate the possible synthesis of other chlorins closely related to (179). It was envisioned that nitrile (185a) could be synthesized by the approach outlined in Scheme 42. The rationale for this reaction was based upon the fact that the cyclization of compound (186) in sulfuric acid, afforded chlorin (187) as the major product (Scheme 43).[129]

Before attempting the synthesis of chlorin (185a), we decided to further investigate formic acid as a cyclization technique. Compound (172) was refluxed in formic acid, without hydroxylamine, to give dibenzochlorin (183) in 42.2% yield. The cyclization of compound (182a) under the same conditions, afforded benzochlorin (72b) in 58.8% yield. The original yields reported for chlorins (183) and (72b) were 13% and 47%, respectively. These results were obtained by treating compounds (172) and (182a), with concentrated sulfuric acid.[129] Our results suggested that formic acid may be more efficient than sulfuric acid in these types of cyclizations. We then decided to utilize this cyclization technique in the synthesis of (185a).




185a. R = CH=CH—C≡N 184a.

Scheme 42. Retrosynthetic Analysis of Chlorin (185a)



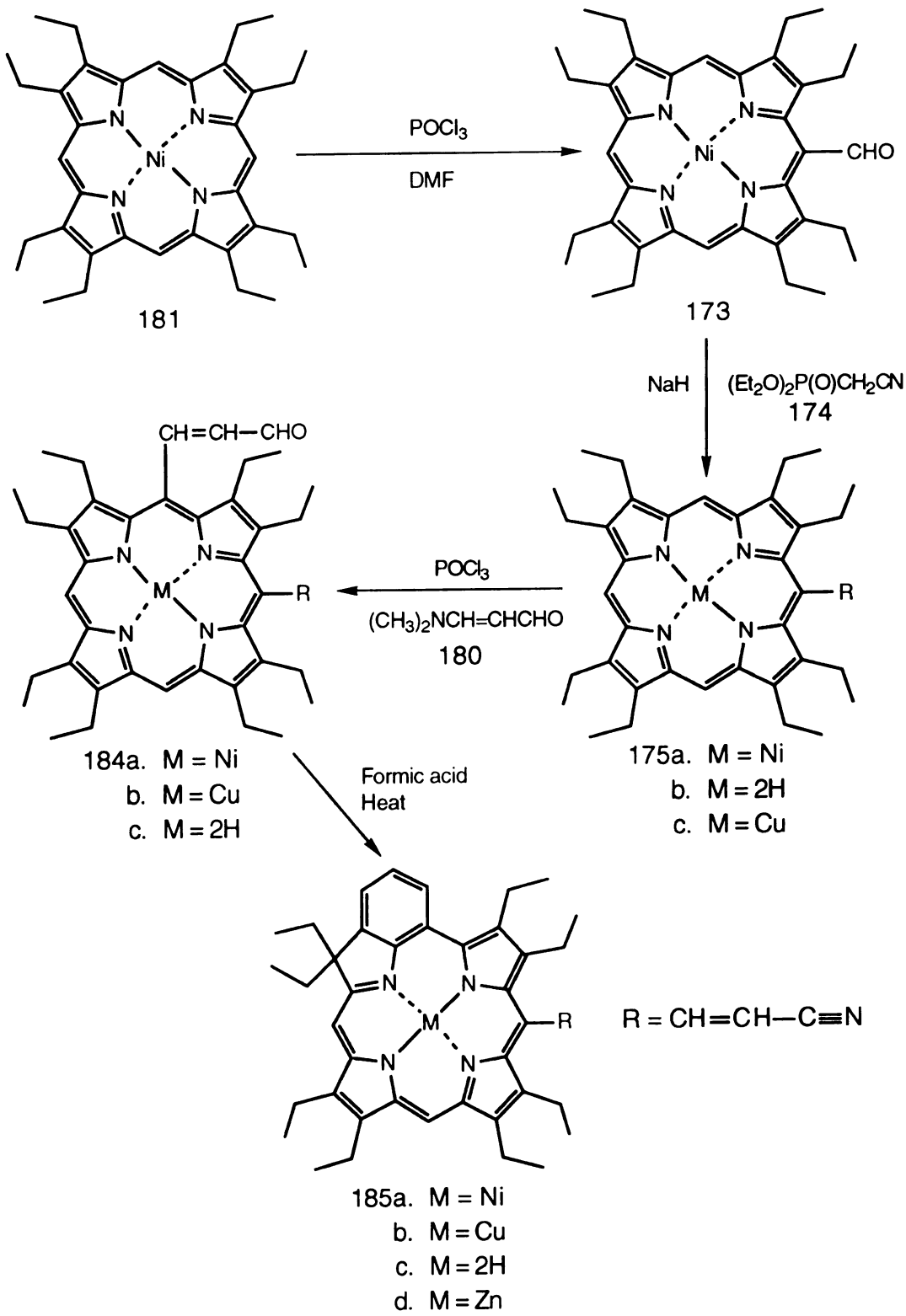

186 R = CH=CH—CHO 187

Scheme 43. Synthesis of Chlorin (187)

The reaction sequence used to synthesize (185a) is outlined in Scheme 44. Porphyrin (181) was treated with phosphorus oxychloride and DMF to give aldehyde (173) in 82.8% yield. Compound (173) was then reacted with diethylcyanophosphonate (174) and sodium hydride to afford (175a) in 83.3% yield. The treatment of (175a) with dimethylaminoacrolein (180) and phosphorus oxychloride, produced compound (184a) in yields of 50-77.8%. Porphyrin (184a) was then refluxed in formic acid for fifteen minutes, to give

nitrile (185a) in 52.3% yield. The UV/visible spectrum of (185a) is shown in Figure 9.

We were then interested in obtaining free base chlorin (185c), through the demetallation of (185a). Unfortunately, various acidic demetallation techniques only afforded chlorin (185c) in low yields (~10%). The best results were obtained when compound (185a) was dissolved in concentrated sulfuric acid, cooled, and treated with hydrogen sulfide for 30 minutes. The robust nature of nickel benzochlorins and their resistance to demetallation has been previously noted.[129] It was determined that a different metal such as copper would have to be introduced earlier in the reaction sequence. Copper was chosen because these complexes generally behave similar to their nickel counterparts and are usually easier to demetallate. We initially thought that the demetallation of compound (184a) would lead to (184c). However, the attempted demetallation of this compound also proved unsatisfactory. Compound (175a) was then demetalled with concentrated sulfuric acid to afford (175b) in 89.4% yield. Porphyrin (175b) was subsequently metallated with copper acetate to give (175c) in 96.8% yield. Compound (175c) was then treated with dimethylaminoacrolein (180) and phosphorus oxychloride to afford compound (184b) in 29.9% yield. The yield of (184b) was noticeably lower than that of compound (184a). However, this was somewhat expected. It has been previously reported that copper complexes often give lower yields in Vilsmeyer-type reactions, as compared to their nickel counterparts.[129-130] Porphyrin (184b) was then refluxed in formic acid to give benzochlorin (185b) in 33.3% yield (Scheme 44). The demetallation of compound (185b) also proved to be troublesome. The best yields of (185c) (~20%) were obtained when compound (185b) was stirred in cold sulfuric acid for 1 hour.




Scheme 44. Synthesis of Chlorins (185a-d)

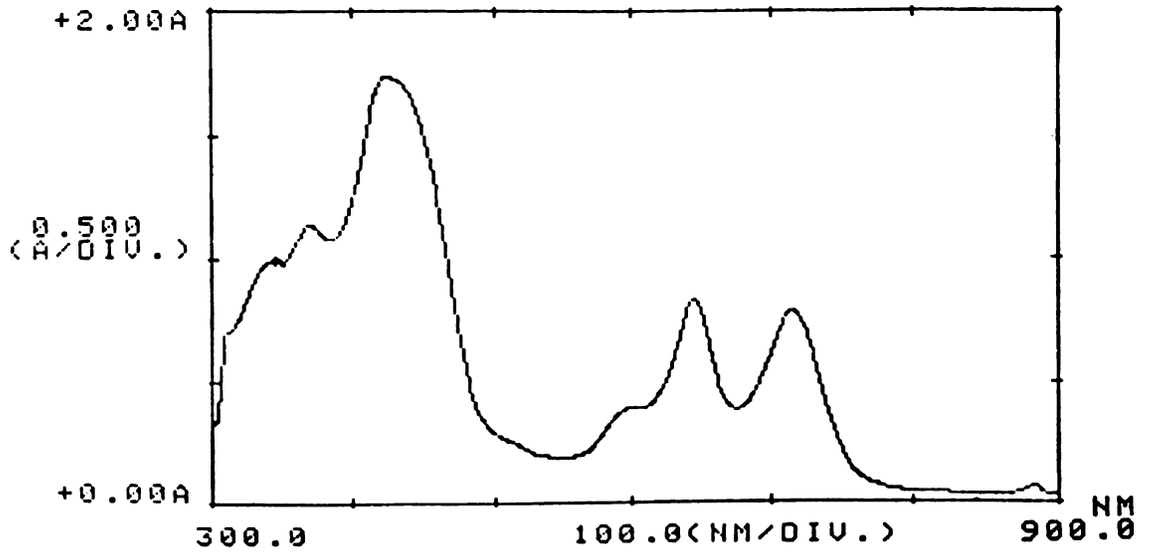
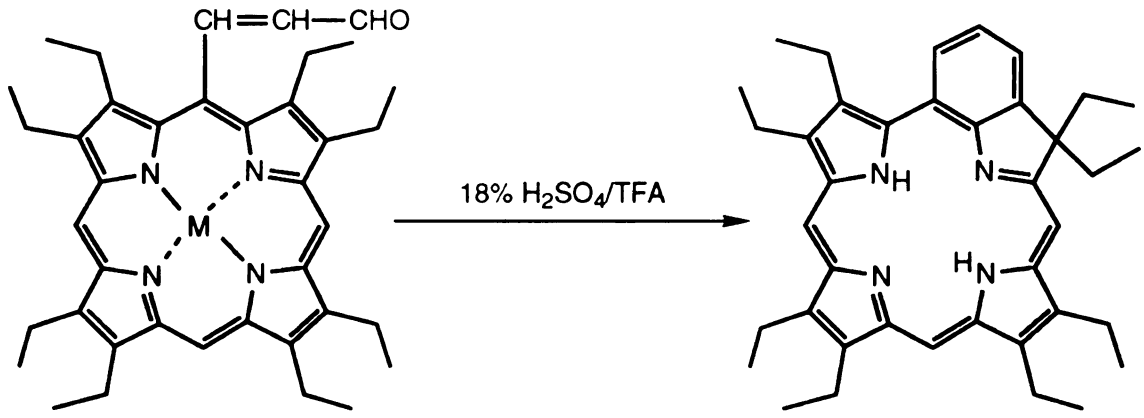



Figure 9. UV/Visible Absorption Spectrum of Chlorin (185a) in $CH_2Cl_2$

Chlorin (185c) was found to readily react with zinc acetate to afford (185d). Preliminary studies revealed that the removal of zinc was much less difficult than nickel or copper, and could be accomplished by treating compound (185d) with a 10% hydrochloric acid solution.

Benzochlorin (72a) is usually prepared by bubbling hydrogen sulfide through a 18% sulfuric acid/TFA solution of (182a). These reaction conditions facilitate cyclization and demetallation in a one-pot procedure, and afford benzochlorin (72a) in 82% yield.[129] When compound (182b) is utilized and hydrogen sulfide eliminated, chlorin (72a) can be obtained in 70% yield.[130] (Scheme 45). These conditions did not work well for porphyrins (184a,b) and led to decomposition products, recovered starting material, trace amounts of chlorins (185a-c) and other unidentified compounds. No attempt was made to characterize all the various products of these cyclization reactions. However, the results did confirm that formic acid was superior to sulfuric acid in the synthesis of compounds (185a) and (185b).



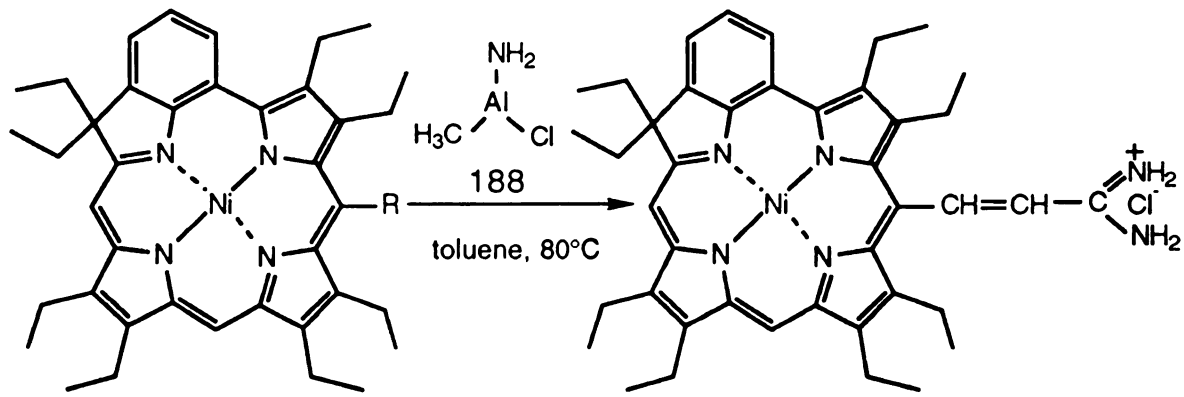

182a. M = Ni
b. M = Cu

72a.

Scheme 45. Traditional Synthesis of Chlorin (72a)

Garigipati[142] has reported that nitriles can be converted into amidiniums by treatment with chloromethylaluminum(III) amide (188).[143] Recently, this reaction was utilized in the preparation of a number of porphyrin amidiniums.[144] By analogy, we envisioned that nitrile (185a) would lead to amidinium (189). Compound (188) (30 equivalents) was added under argon, to a solution of chlorin (185a) in toluene. The mixture was then heated at 80°C for four days (Scheme 46). The solution was treated with a slurry of silica gel/chloroform and subsequently columned over silica, to afford amidinium (189) in 35.5% yield. The UV/visible spectrum of (189) is shown in Figure 10.

Unfortunately, time constraints precluded detailed studies of amidinium (189). However, we have shown that chlorins (185a-d) and amidinium (189) can be prepared. Currently, the above findings are being utilized in the continued study of benzochlorins (185a-d).[145] Particular areas of interest include yield optimizations and improved demetallation techniques. The information gathered in these studies should also be of value in the preparation of other derivatized benzochlorins.




185a. R = CH=CH—C≡N 189

Scheme 46. Synthesis of Benzochlorin Amidinium (189)

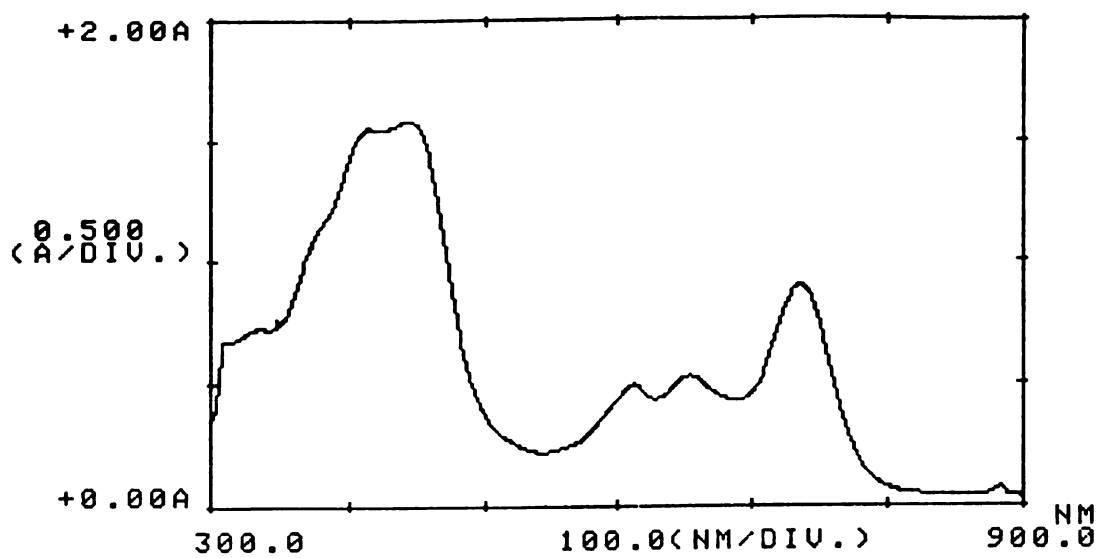



Figure 10. UV/Visible Absorption Spectrum of Amidinium (189) in $CH_2Cl_2$

## III. Experimental

Copper(II) 10-formyl-2,3,8,8,12,13,17,18-octaethylbenzochlorin (161)

N,N-dimethylformamide (35 drops) was added to a solution of copper benzochlorin (72c) (31.4 mg) in dichloroethane (15 ml). The stirred solution was cooled to below 0°C in a ice/salt bath and phosphorus oxychloride (30 drops) was added dropwise, while maintaining the temperature below 5°C. The mixture was then allowed to stir for one hour at room temperature. Dichloromethane (20 ml) was added and the solution washed with a saturated aqueous sodium bicarbonate solution (2 x 30 ml), water (2 x 30 ml) and dried over anhydrous sodium sulfate. The residue was chromatographed over silica, eluting with a mixture of dichloromethane/methanol (90/10), to give imminium salt (73c) (17.8 mg). UV/visible ($CH_2Cl_2$): $\lambda$ max 386.0, 448.5, 565.5, 749.5.

The above imminium salt was then divided and utilized in a number of hydrolysis experiments. In the hydrolysis experiments, the imminium salt was dissolved in dry dichloromethane to afford solutions with a maximum UV/visible absorbance of <2 (~0.2-0.5 mg in 10-15 ml of $CH_2Cl_2$; approximately $10^{-5}$ M). An equal volume of pH 10 buffer solution (sodium hydroxide/sodium borate) was then added to the stirred solution. After the required hydrolysis data was obtained, the solution was stirred overnight. The organic layer was removed and the aqueous layer extracted with fresh dichloromethane. The organic layers were combined, washed with water and the solvent removed under reduced pressure. The crude products from the various hydrolysis reactions were then combined. This residue was chromatographed over silica, eluting with dichloromethane, to afford (161) as a green film. The combined hydrolysis experiments produced 12 mg of (161), from 15.1 mg of (73c). UV/visible

($CH_2Cl_2$): $\lambda_{max}$ 366.0, 394.5, 436.0, 525.5, 573.0, 643.5, 698.5. EI MS: Calc. for $C_{40}H_{46}N_4OCu$: 661.2967. Found 661.3.

Copper(II) 5-formyl-2,3,7,8,12,13,17,18-octaethylchlorin (162)

The initial imminium salt was prepared from (153) (30.6 mg), N,N-dimethylformamide (35 drops) and phosphorus oxychloride (30 drops), by the procedure detailed above. After the addition of the phosphorus oxychloride was complete, the mixture was allowed to stir for one hour at room temperature. Dichloromethane (30 ml) was added and the solution was washed with a saturated aqueous sodium bicarbonate solution (2 x 30 ml), water (2 x 30 ml) and dried over anhydrous sodium sulfate. The residue was chromatographed over silica, eluting with a mixture of dichloromethane/methanol (90/10), to give imminium salt (157) (31 mg). UV/visible ($CH_2Cl_2$): $\lambda$ max 376.0, 433.0, 516.0, 557.0, 698.0.

Imminium salt (157) was divided into portions as above, and dissolved in $CH_2Cl_2$. The hydrolysis experiments were then carried out using water and pH 10 buffer solutions. In both cases the mixture was allowed to stir overnight after the appropriate data had been collected. The crude products from the various hydrolysis reactions were then combined. This residue was chromatographed over silica, eluting with dichloromethane, to afford (162) as a green film. The combined hydrolysis experiments produced 18.7 mg of (162), from 24.0 mg of (157). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 411.0, 502.0, 540.0, 648.0. EI MS: Calc. for $C_{37}H_{46}N_4OCu$: 625.2967. Found 625.3.

Copper(II) purpurin aldehyde (163)

The initial imminium salt was prepared from compound (154) (33.8 mg), N,N-dimethylformamide (60 drops), and phosphorus oxychloride (50 drops), by

the procedure detailed above. After the addition of the phosphorus oxychloride was complete, the mixture was allowed to stir at 40°C for six hours. Dichloromethane (30 ml) was added, the solution washed with water (2 x 30 ml) and then dried over anhydrous sodium sulfate. The residue was chromatographed over silica, eluting with a mixture of dichloromethane/methanol (90/10), to give imminium salt (158) (15.5 mg). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 378.0, 416.0, 438.0, 554.0, 599.0, 765.0.

Imminium salt (158) was divided into portions as above, and dissolved in $CH_2Cl_2$. The hydrolysis experiments were then carried out using water and pH 10 buffer solutions. These mixtures were then allowed to stir for an additional three hours (pH 10), or overnight (water), after the appropriate data had been collected. The crude products from the various hydrolysis reactions were then combined. This residue was chromatographed over silica, eluting with a mixture of dichloromethane/methanol (95/5), to afford (163) as a green film. The combined hydrolysis experiments produced 6.0 mg of (163), from 15.5 mg of (158). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 416.0, 669.0. EI MS: Calc. for $C_{42}H_{50}N_4O_3Cu$: 721.3179. Found 721.2.

Copper(II) vinyl chlorin aldehyde (164)

The initial imminium salt was prepared from compound (155) (11.3mg), N,N-dimethylformamide (20 drops) and phosphorus oxychloride (15 drops), by the procedure detailed above. After the addition of the phosphorus oxychloride was complete, the mixture was allowed to stir for two hours at 40°C. The solvent was then removed under reduced pressure and the residue vacuum dried, to give crude imminium salt (159). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 371.0, 432.0, 470.0, 501.0, 579.0, 766.0.

Imminium salt (159) was divided into portions as above, and dissolved in $CH_2Cl_2$. The hydrolysis experiments were then carried out using water. The mixture was allowed to stir overnight, after the appropriate data had been collected. The crude products from the various hydrolysis reactions were then combined. This residue was chromatographed over silica, eluting with dichloromethane, to give (164) as a green film. The combined hydrolysis experiments afforded 3.1 mg of (164), from 11.3 mg of chlorin (155). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 350.0, 397.0, 414.0, 435.5, 615.0, 645.5. EI MS: Calc. for $C_{38}H_{46}N_4OCu$: 637.2967. Found 637.2.

Copper(II) keto chlorin aldehyde (165)

The initial imminium salt was prepared from compound (156) (19.2 mg), N,N-dimethylformamide (25 drops) and phosphorus oxychloride (20 drops), by the procedure detailed above. After the addition of the phosphorus oxychloride was complete, the mixture was allowed to stir for two hours at 40°C. The solvent was then removed under reduced pressure and the residue vacuum dried, to give imminium salt (160). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 414.0, 545.0, 573.5, 620.5.

Imminium salt (160) was divided into portions as above, and dissolved in $CH_2Cl_2$. The hydrolysis experiments were then carried out using water. The mixture was allowed to stir overnight, after the appropriate data had been collected. The crude products from the various hydrolysis reactions were then combined. This residue was chromatographed over silica, eluting with dichloromethane, to give (165) as a green film. The combined hydrolysis experiments afforded 5.2 mg of (165), from 19.2 mg of chlorin (156). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 321.5, 417.0, 573.5, 618.5. EI MS: Calc. for $C_{37}H_{44}N_4O_2Cu$: 639.2760. Found 639.2.

Nickel(II) 5-formyl-2,3,7,8,12,13,17,18-octaethylporphyrin (173)

Nickel(II) 2,3,7,8,12,13,17,18-octaethylporphyrin (181) (850 mg; 1.44 x $10^{-3}$ moles) was dissolved in a mixture of 1,2-dichloroethane (500 ml) and N,N-dimethylformamide (6.0 ml). The solution was purged with nitrogen and cooled to 5°C in a ice bath. Phosphorus oxychloride (8.0 ml) was then added to the cooled, stirred solution over a period of thirty minutes. The mixture was allowed to warm to room temperature and then heated at 50°C for two hours. A saturated aqueous solution of sodium acetate (500 ml) was added and the mixture stirred for an additional two hours. The organic layer was removed and the aqueous layer extracted with dichloromethane (2 x 200 ml). The organic layers were combined, washed well with water, dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was chromatographed over silica, eluting with dichloromethane. The resulting solid recrystallized from dichloromethane/methanol to give the title porphyrin as purple crystals (737 mg; 82.8%); mp 262-265°C. $^1$H NMR ($CDCl_3$): $\delta$ 1.62-1.78 (24 H, overlapping triplets, 8 x $CH_2\underline{C}H_3$), 3.63-3.82 (16 H, overlapping quartets, 8 x $C\underline{H}_2CH_3$), 9.28 (2H, s), 9.33 (1H,s) (3 x meso-H), 11.87 (1H, s, CHO). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 402.0, 530.5, 561.0, 642.0.

Nickel(II) 5-(2-cyanoethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (175a)

Diethylcyanomethylphosphonate (174) (1.1 g; 6.22 x $10^{-3}$ moles) was added dropwise to a cooled (ice bath) solution of sodium hydride (250 mg; 60% dispersion in mineral oil) in tetrahydrofuran (10 ml). Nickel(II) 5-formyl -2,3,7,8,12,13,17,18-octaethylporphyrin (173) (700 mg; 1.13 x $10^{-3}$ moles) in tetrahydrofuran (20 ml) was added to the stirred solution and the mixture refluxed under nitrogen, for one hour. At the end of this time the solution was cooled to room temperature and dichloromethane was added (50 ml). The

mixture was then washed with water (2 x 50 ml), the organic layer dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The residue was chromatographed on silica, eluting with a mixture of dichloromethane/hexane (50/50). The solvent was removed under vacuum and the resulting solid recrystallized from dichloromethane/methanol to give the desired porphyrin as purple crystals (605 mg; 83.3%); mp 234-235°C (lit mp 228-230°C[141]). $^1$H NMR ($CDCl_3$): δ 1.62-1.78 (24 H, overlapping triplets, 8 x $CH_2C\underline{H}_3$), 3.70-3.84 (16 H, overlapping quartets, 8 x $C\underline{H}_2CH_3$), 4.55 (1H, d, α-H of acrylic nitrile), 9.41 (3H, s, 3 x meso-H), 9.71 (1H, d, β-H of acrylic nitrile). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 405.0, 530.5, 565.5.

5-(2-Cyanoethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (175b)

Nickel(II) 5-(2-cyanoethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (175a) (988 mg; 1.54 x $10^{-3}$ moles) was dissolved in concentrated sulfuric acid (50 ml) and stirred in a ice bath for fifteen minutes. The solution was poured over ice and dichloromethane (100 ml) was added. The mixture was shaken, the organic layer removed and the aqueous layer extracted with additional dichloromethane (2 x 50 ml). The organic layers were combined, dried over anhydrous sodium sulfate and the solvent removed under vacuum. The resulting residue was recrystallized from dichloromethane/methanol to give a reddish-brown solid (805 mg; 89.4%); mp 215-217°C (lit mp 215-216°C[141]). $^1$H NMR ($CDCl_3$): δ 1.64-1.94 (24 H, overlapping triplets, 8 x $CH_2C\underline{H}_3$), 3.84-4.12 (16 H, overlapping quartets, 8 x $C\underline{H}_2CH_3$), 5.57 (1H, d, α-H of acrylic nitrile), 9.93 (1H, s), 10.08 (2H,s) (3 x meso-H), 10.15 (1H, d, β-H of acrylic nitrile). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 407.0, 505.5, 541.5, 574.5, 627.0.

Copper(II) 5-(2-cyanoethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (175c)

Copper(II) acetate monohydrate (1.00 g) in methanol (5 ml) was added to a solution of 5-(2-cyanoethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (175b) (650 mg; 1.11 x $10^{-3}$ moles) in dichloromethane (125 ml). The solution was then refluxed for one hour, washed well with water and solvent removed under reduced pressure. The recrystallization of the residue from dichloromethane/methanol, afforded the title porphyrin as a red solid (695 mg; 96.8%); mp 258-260°C with decomposition. UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 329.5, 405.0, 531.0, 569.5.

Nickel(II) 5-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (182a)

3-(Dimethylamino)acrolein (180) (3.2 ml; 3.2 x $10^{-2}$ moles) was added to a solution of nickel(II) 2,3,7,8,12,13,17,18-octaethylporphyrin (181) (1.58 g; 3.13 x $10^{-3}$ moles) in dry dichloromethane (500 ml). The stirred solution was cooled to below 0°C in a ice/salt bath and phosphorous oxychloride (3.0 ml; 3.2 x $10^{-2}$ moles) was added dropwise, while maintaining the temperature below 5°C. The solution was then allowed to warm to room temperature and stir for an additional seven hours. A saturated aqueous solution of sodium carbonate (250 ml) was added and the solution stirred for two hours. The organic layer was separated and the aqueous layer extracted with dichloromethane (3 x 100 ml). The organic layers were combined, washed with water (2 x 250 ml) and the solvent removed under reduced pressure. The resulting residue was chromatographed on silica, eluting with a mixture of dichloromethane/hexane (2/1). The solvent was removed under reduced pressure and the product recrystallized from dichloromethane/hexane to give the title compound as a bluish-green solid (1.46 g; 84.7%); mp 246.5-247.5°C (lit mp 245-246°C[129]). $^1H$ NMR ($CDCl_3$): $\delta$ 1.62-1.76 (24 H, overlapping triplets, 8 x $CH_2C\underline{H}_3$), 3.70-

3.85 (16 H, overlapping quartets, 8 x $C\underline{H}_2CH_3$), 5.52 (1H, dd, α-H of acrylic aldehyde), 9.35 (3H, s, 3 x meso-H), 9.67 (1H, d, β-H of acrylic aldehyde). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 336.0, 407.5, 531.5, 567.0.

2,3,8,8,12,13,17,18-Octaethylbenzochlorin (72a)

Nickel(II) 5-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (182a) (650 mg; 1.01 x $10^{-3}$ moles) was dissolved in a 18% sulfuric acid/trifluoroacetic acid mixture (25 ml) and stirred for five minutes. Hydrogen sulfide gas was then bubbled through the stirred solution for one hour. The mixture was poured into ice/water (150 ml) and then extracted with dichloromethane (3 x 100 ml). The organic layers were combined, washed with aqueous sodium carbonate solution (3 x 100 ml), and then with water (3 x 100 ml). The dichloromethane layer was dried over anhydrous sulfate and the solvent removed under vacuum. The resulting solid was chromatographed over silica, eluting with a mixture of hexane/dichloromethane (60/40), and recrystallized from dichloromethane/methanol to give the desired chlorin as a green solid (446 mg; 77.3%); mp 242.5-244.5°C (lit mp 241-243°C[129]). $^1H$ NMR ($CDCl_3$): δ 0.08 (6H, t, 2 x gem-$CH_2C\underline{H}_3$), 1.57-1.71, 1.76-1.86 (18H, overlapping triplets, 6 x peripheral-$CH_2C\underline{H}_3$), 2.50-2.69 (4H, overlapping quartets, 2 x gem-$C\underline{H}_2CH_3$), 3.43-3.63, 3.71-3.92 (12H, overlapping quartets, 6 x peripheral-$C\underline{H}_2CH_3$), 8.01 (1H,d), 8.08 (1H, t), 9.51 (1H, s) (3 x benzo-H), 7.97 (1H,s), 8.53 (1H,s), 9.51 (1H,s) (3 x meso-H). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 410.5, 528.5, 564.0, 603.0, 658.5.

Nickel(II) 2,3,8,8,12,13,17,18-octaethylbenzochlorin (72b)

a. Nickel(II) acetate ( 500 mg) was added to a solution of 2,3,8,8,12,13,17,18-octaethylbenzochlorin (72a) (530 mg; 8.21 x $10^{-4}$ moles) in

N,N-dimethylformamide (40 ml). The mixture was heated under reflux for one hour, the solvent removed under reduced pressure, and the residue dissolved in dichloromethane (100 ml). The solution was washed well with water (2 x 100 ml), the organic layer dried over anhydrous sodium sulfate and the solvent removed under reduced pressure. The resulting solid was chromatographed over silica, eluting with a mixture of dichloromethane/hexane (50/50), and recrystallized from dichloromethane/methanol to give the title compound as a dark green solid (544 mg; 93.5%); mp 225-227°C (lit mp 224-225°C[129]). $^1$H NMR ($CDCl_3$): $\delta$ 0.16 (6H, t, 2 x gem-$CH_2C\underline{H}_3$), 1.49-1.68 (18H, overlapping triplets, 6 x peripheral-$CH_2C\underline{H}_3$), 2.33-2.48 (4H, overlapping quartets, 2 x gem-$C\underline{H}_2CH_3$), 3.31-3.69 (12H, overlapping quartets, 6 x peripheral-$C\underline{H}_2CH_3$), 7.40-7.83 (2H, m), 8.96 (1H, dd) (3 x benzo-H), 7.81 (1H, s), 8.52 (1H, s), 8.87 (1H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 415.5, 567.5, 621.0, 670.5.

b. A solution of nickel(II) 5-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (182a) (43.1 mg; 6.68 x $10^{-5}$ moles) in formic acid (20 ml), was refluxed under nitrogen for fifteen minutes. The solution was cooled to room temperature and poured into water (50 ml). Dichloromethane (50 ml) was added and the mixture shaken. The organic layer was removed and washed with a saturated aqueous sodium bicarbonate solution (50 ml) and water (50 ml). The solvent was removed under reduced pressure and the resulting solid columned on silica, eluting with a mixture of hexane/dichloromethane (60/40). Recrystallization from dichloromethane/ hexane afforded the title chlorin as a dark green solid (24.7 mg; 58.8%).

Nickel(II) 10-(2-formylethenyl)-2,3,8,8,12,13,17,18-octaethylbenzochlorin (172)

The title chlorin was prepared from compound (72b) (79 mg; 1.25 x $10^{-4}$ moles), 3-(dimethylamino)acrolein (180) (0.5 ml; 5.0 x $10^{-3}$ moles) and

phosphorous oxychloride (0.47 ml; 5.0 x $10^{-3}$ moles), by the procedure detailed for (182a). The crude product was columned over silica, eluting with dichloromethane, and recrystallized from dichloromethane/hexane to give the desired chlorin as a dark green solid (67 mg; 78.4%); mp >300°C (lit mp 298-299°C[129]). $^1$H NMR ($CDCl_3$): δ 0.08 (6H, t, 2 x gem-$CH_2C\underline{H}_3$), 1.32 (3H, t), 1.41-1.57 (15H, overlapping triplets) (6 x peripheral-$CH_2C\underline{H}_3$), 2.44 (2H, sext), 2.73 (2H, sext) (2 x gem-$C\underline{H}_2CH_3$), 3.13-3.44 (12H, overlapping quartets, 6 x peripheral-$C\underline{H}_2CH_3$), 5.62(1H, dd, α-H of acrylic aldehyde) 7.57-7.68 (2H, m), 8.57 (1H, d) (3 x benzo-H), 8.13 (1H, s), 8.41 (1H, s) (2 x meso-H), 8.63 (1H, d, β-H of acrylic aldehyde), 9.59 (1H, CHO). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 375.5, 445.5, 618.5, 727.0.

Nickel(II) 10-formyl-2,3,8,8,12,13,17,18-octaethylbenzochlorin (176)

N,N-dimethylformamide (2.0 ml; 2.58 x $10^{-2}$ moles) was added to a solution of nickel(II) 2,3,8,8,12,17,18-octaethylbenzochlorin (72b) (335 mg; 5.32 x $10^{-4}$ moles) in dichloromethane (200 ml). The solution was cooled to below 0°C in a ice/salt bath and phosphorous oxychloride (2.4 ml; 2.58 x $10^{-2}$ moles) was added dropwise to the stirred solution while maintaining the temperature below 5°C. The mixture was allowed to warm to room temperature and stir overnight. A saturated aqueous solution of sodium carbonate (200 ml) was added and the solution stirred overnight. The organic layer was separated and the aqueous extracted with dichloromethane (2 x 100 ml). The organic layers were combined, washed with water (2 x 200 ml) and the solvent removed under reduced pressure. The residue was chromatographed on silica, eluting with dichloromethane, to give the title porphyrin as a green film (257 mg; 73.4%). $^1$H NMR ($CDCl_3$): δ 0.16 (6H, t, 2 x gem-$CH_2C\underline{H}_3$), 1.32-1.53 (18H, overlapping triplets, 6 x peripheral-$CH_2C\underline{H}_3$), 2.23-2.40 (2H, m), 2.74-2.92 (2H, m) (2 x gem-

$C\underline{H_2}CH_3$), 3.07-3.36 (12H, overlapping quartets, 6 x peripheral-$C\underline{H_2}CH_3$), 7.52-7.64 (2H, m), 8.42 (1H, d) (3 x benzo-H), 7.93 (1H, s), 8.24 (1H, s) (2 x meso-H). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 370.5, 440.0, 585.5, 656.5, 709.0.

Nickel(II) 2,2,7,7,12,13,17,18-octaethyldibenzoisobacteriochlorin (183)

The title compound was prepared from nickel(II) 10-(2-formylethenyl)-2,3,8,8,12,13,17,18-octaethylbenzochlorin (172) (59.9 mg; 8.76 x $10^{-5}$ moles), using the procedure for detailed for nickel benzochlorin (72b) (method b). The residue was columned over silica, eluting with a mixture of dichloromethane/hexane (50/50), and recrystallized from dichloromethane/methanol to give the desired compound as bluish-gray crystals (24.7 mg; 42.2%); mp 292-294°C (lit mp 292-302°C[129]). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ 325.5, 365.0, 449.0, 627.0. EI MS: m/e (Relative Intensity) 666.5 (46.78), 637.4 (46.78), 593.3 (25.88).

Nickel(II) 5-(2-cyanoethenyl)-10-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (184a)

3-(Dimethylamino)acrolein (180) (0.25 ml; 2.5 x $10^{-3}$ moles) was added to a solution of nickel(II) 5-(2-cyanoethenyl)-2,3,7,8,12,17,18-octaethylporphyrin (175a) (50 mg; 7.78 x $10^{-5}$ moles) in dichloroethane (25 ml). The solution was cooled to below 0°C in a ice/salt bath and phosphorous oxychloride (0.23 ml; 2.5 x $10^{-3}$ moles) was added dropwise to the stirred solution, while maintaining the temperature below 5°C. The mixture was allowed to warm to room temperature and stir overnight. A saturated aqueous solution of sodium carbonate (25 ml) was added and the solution stirred for one hour. The organic layer was separated and the aqueous extracted with dichloromethane (75 ml). The organic layers were combined, washed with water (2 x 50 ml) and the

solvent removed under reduced pressure. The residue was chromatographed on silica, eluting with a mixture of dichloromethane/hexane (50/50), to give the title porphyrin as a bluish-purple film (42 mg; 77.8%). Increasing the scale to 500 mg of starting porphyrin, resulted in a 50% yield of the title compound. $^1$H NMR ($CDCl_3$): $\delta$ 1.55-1.77 (24H, 8 overlapping triplets, 8 x $CH_2C\underline{H}_3$), 3.58-3.78 (16H, 8 overlapping quartets, 8 x $C\underline{H}_2CH_3$), 4.60 (1H, d, $\alpha$-H of acrylic nitrile), 5.49 (1H, dd, $\alpha$-H of acrylic aldehyde), 9.24 (1H, s), 9.27 (1H, s) (2 x meso-H), 9.50 (1H, d), 9.55 (1H, d) ($\beta$-H of acrylic nitrile and $\beta$-H of acrylic aldehyde), 9.83 (1H, d, CHO). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (log $\varepsilon$) 335.5 (4.28), 449.5 (4.82), 589.5 (3.90). EI MS: m/e (Relative Intensity) 695.1 (44.78), 637.2 (20.33).

Copper(II) 5-(2-cyanoethenyl)-10-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (184b)

A solution of phosphorous oxychloride (3.0 ml; 3.2 x $10^{-2}$ moles) in dichloromethane (10 ml) was added dropwise to a stirred solution of 3-(dimethylamino)acrolein (180) (3.2 ml; 3.2 x $10^{-2}$ moles) in dichloromethane (30 ml), while maintaining the temperature at 0°C. This mixture was stirred at 0°C for fifteen minutes and then added to a stirred solution of copper(II) 5-(2-cyanoethenyl)-2,3,7,8,12,17,18-octaethylporphyrin (175c) (303 mg; 4.68 x $10^{-4}$ moles) in dichloromethane (200 ml), while maintaining the temperature below 0°C. The mixture was then allowed to warm to room temperature and stir overnight. A saturated aqueous solution of sodium carbonate (250 ml) was added and the solution stirred for two hours. The organic layer was separated and the aqueous layer extracted with dichloromethane (2 x 125 ml). The organic layers were combined, washed with water (2 x 200 ml) and the solvent removed under reduced pressure. The residue was initially purified by chromatography over silica, eluting with a mixture of dichloromethane/hexane

(75/25). The resulting solid was subsequently purified by preparative TLC chromatography, utilizing the same solvent combination. The title porphyrin was obtained as a greenish-purple film (98 mg; 29.9%). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (log $\varepsilon$) 336.0 (4.23), 421.0 (4.89) 556.5 (3.81), 583.5 (3.86). EI MS: m/e (Relative Intensity) 700.4 (44.50), 671.3 (14.98), 642.3 (22.64), 614.3 (22.07).

Nickel(II) 10-(2-cyanoethenyl)-2,2,7,8,12,13,17,18-octaethylbenzochlorin (185a)

A solution of nickel(II) 5-(cyanoethenyl)-10-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (184a) (90 mg) in formic acid (30 ml), was refluxed under nitrogen for fifteen minutes. The mixture was cooled to room temperature and dichloromethane (75 ml) was added. The solution was washed with water (50 ml), saturated aqueous sodium bicarbonate solution (50 ml), water (50 ml), and the organic layer dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the residue purified on silica gel, eluting with a mixture of dichloromethane/hexane (50/50), to give the desired chlorin as a dark green film (46 mg; 52.3 %). IR (Neat): $\gamma$ 2201 (nitrile stretch). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (log $\varepsilon$) 372.0 (4.40), 424.5 (4.48), 601.0 (3.90), 644.5 (4.26), 714.5 (4.23). EI MS: m/e (Relative Intensity) 679.3 (100.0), 677.2 (81.7), 650 (58.6), 648.1 (53.8). FAB MS: Calc. for $C_{42}H_{47}N_5Ni$: 679.3185. Found: 679.2

Copper(II) 10-(2-cyanoethenyl)-2,2,7,8,12,13,17,18-octaethylbenzochlorin (185b)

The title compound was prepared from copper(II) 5-(cyanoethenyl)-10-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (184b) (46 mg), by the procedure detailed above. However, the reaction time was ten minutes. The

residue was initially purified by chromatography over silica, eluting with a mixture of dichloromethane/hexane (75/25). The resulting solid was subsequently purified by preparative TLC chromatography, utilizing the same solvent combination. The desired chlorin was obtained as a green film (15 mg; 33.3%). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (Relative Intensity) 337.0 (0.930), 411.5 (1.763), 449.0 (1.309), 644.0 (1.045), 718.0 (0.182). EI MS: Calc. for $C_{42}H_{47}N_4Cu$: 684.3127. Found: 684.2.

Zinc(II) 10-(2-cyanoethenyl)-2,2,7,8,12,13,17,18-octaethylbenzochlorin (185d)

Copper(II) 10-(2-cyanoethenyl)-2,3,8,8,12,13,17,18-octaethylbenzochlorin (185b) (15 mg) was dissolved in concentrated sulfuric acic (5 ml). The mixture was then stirred under nitrogen in a ice bath, for one hour. The acid was poured over ice and dichloromethane (20 ml) was added. The organic layer was separated and the aqueous layer extracted with additional dichloromethane (20 ml). The organic layers were combined, washed with water (2 x 25 ml) and dried over anhydrous sodium sulfate. The residue was purified on a preparative TLC plate, using a mixture of dichloromethane and ethyl acetate (95/5), to give 10-(2-cyanoethenyl)-2,3,8,8,12,13,17,18-octaethylbenzochlorin (185c) (2.5 mg). UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (Relative Intensity) 395.0 (1.211), 493.5 (0.131), 525.5 (0.097), 564.0 (0.117), 615.5 (0.128), 683.0 (0.148). EI MS: Calc. for $C_{42}H_{49}N_4$: 623.3988. Found: 623.3.

Chlorin (185c) was dissolved in dichloromethane (5 ml). A saturated solution of zinc acetate in methanol (0.5 ml) was then added and the mixture allowed to stir for one hour. Water (15 ml) was added, the solution shaken, and the organic layer removed. The aqueous layer was extracted with additional dichloromethane (2 x 10 ml) and the organic layers were combined. The combined organic layers were washed with water (2 x 20 ml), dried over

anhydrous sodium sulfate and the solvent removed under vacuum. The resulting film was vacuumed dried to give the title chlorin as a green film. UV/visible ($CH_2Cl_2$): $\lambda_{max}$ (Relative Intensity) 355.0 (0.365), 415.5 (0.866), 450.0 (0.532), 510.5 (0.154), 631.0 (0.377), 649.0 (0.412). EI MS: Calc. for $C_{42}H_{47}N_4Zn$: 685.3123. Found: 685.3.

Nickel(II) 10-(2-amidiniumethenyl)-2,2,7,8,12,13,17,18-octaethylbenzochlorin chloride (189)

Nickel(II) 10-(2-cyanoethenyl)-2,3,8,8,12,13,17,18-octaethylbenzochlorin (185a) (13.8 mg; 2.03 x $10^{-5}$ moles) and dry toluene (15 ml) were placed in a Schlenk tube. The system was purged with argon three times and a toluene solution of chloromethylaluminum(III) amide (188) (1 M; 0.6 ml; 6 x $10^{-4}$ moles) was added by syringe. The reaction mixture was then stirred at 80°C for four days, under an inert atmosphere. At the end of this time, the solution was cooled to room temperature and added to a slurry of silica gel (5 g) and chloroform (20 ml). The mixture was stirred for fifteen minutes and then filtered. The filter cake then washed with a mixture of dichloromethane/methanol (80/20). The organic layers were combined and the solvent removed under reduced pressure. The resulting film was then chromatographed on a short silica column. Initial elution with dichloromethane, produced an unidentified green compound (6 mg). IR (Neat): $\upsilon$ 2199 (nitrile stretch). UV/visible ($CH_2Cl_2$): $\lambda$ max (Relative Intensity) 338.0 (1.205), 415.0 (1.565), 460.0 (1.113), 644.0 (1.450), 711.0 (0.159). FAB MS: Found: 677.0. Continued elution with a mixture of dichoromethane/methanol (80/20), afforded the title compound as a green film (5.0 mg; 35.5%). IR (Neat): $\upsilon$ 3125 (NH, stretch), no nitrile stretch. UV/visible ($CH_2Cl_2$): $\lambda$ max (Relative Intensity) 336.0 (0.515), 444.0 (1.089),

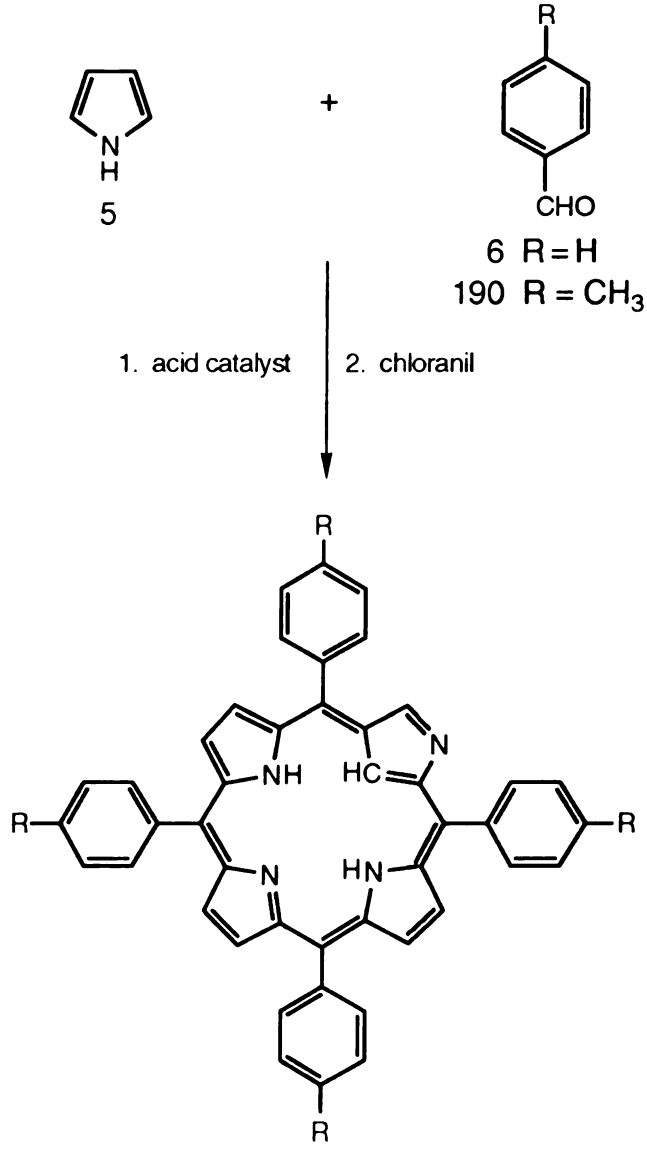
612 (0.326), 653.0 (0.397), 735.5 (0.620). FAB MS: Calc. for $C_{42}H_{50}N_6Ni$: 696.3450. Found: 697.2 ($MH^+$).

# CHAPTER 5

# SYNTHETIC STUDIES OF PORPHYRIN ISOMERS

## I. Introduction

With the advent of photodynamic therapy, there has been an increased interest in porphyrin isomers. As mentioned in Chapter 1, porphycene is the best known and most widely studied example. In 1994 however, two independent research groups reported new porphyrin isomers that contained inverted pyrrole subunits. Furuta and coworkers discovered that the condensation of pyrrole (5) and benzaldehyde (6) under modified Adler and Longo tetraphenylporphyrin (TPP) cyclization conditions, afforded compound (191) in 5-7% yield.[146] TPP was also obtained in 20% yield (Scheme 47). Latos-Grazynski determined that the condensation of pyrrole (5) and tolualdehyde (190) under modified Lindsey TPP cyclization conditions, gave compound (192) in 4% yield.[147] The major product, tetra-p-tolylporphyrin (TTP), was also produced in 16% yield. It is interesting to note that compounds (191) and (192) displayed broadened and red-shifted UV/visible absorption bands (Soret ~440 nm, $Q_1$ ~730 nm), as compared to TPP (7) (Soret = 419 nm, $Q_1$ = 647 nm). Unfortunately, these condensations only resulted in a relatively small amount of "N-confused" porphyrin in comparison to the typical reaction products (TPP and TTP). These papers sparked our interest in the synthesis of "N-confused" porphyrins, and possible yield improvements, through the use of "2 + 2" or "3 + 1" methodologies. We believed that the utilization of more systematic methods would also lead to porphyrin isomers possessing greater synthetic flexibility, than those derived from modified TPP syntheses.

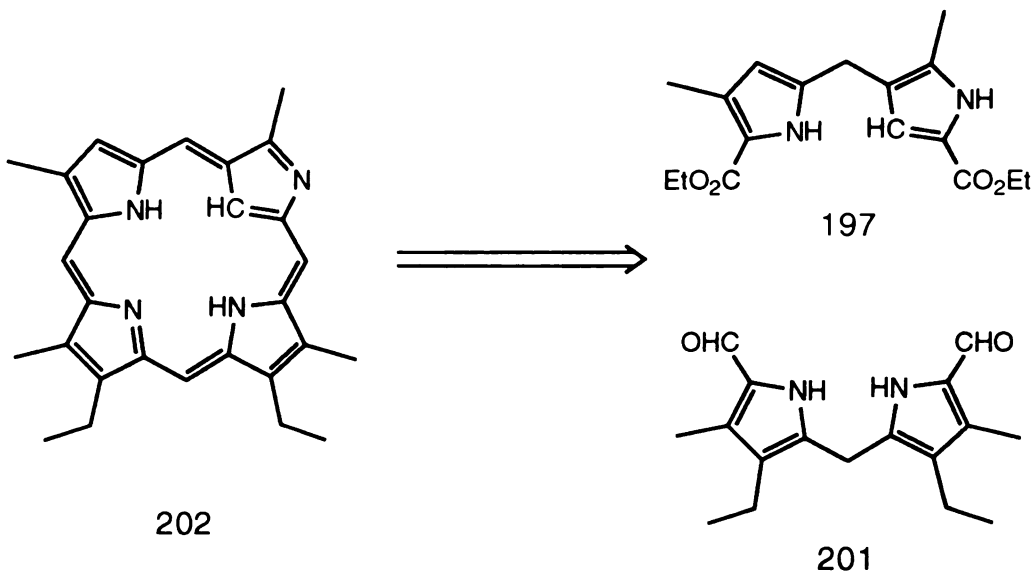



191 R = H (5-7% yield) + TPP (20% yield)
192 R = $CH_3$ (4% yield) + TTP (16% yield)

Scheme 47. Synthesis of Porphyrin Isomers (191) and (192)

## II. Results and Discussion

Our initial attempts at producing these types of compounds focused on the synthesis of porphyrin isomer (202). We envisioned that (202) could be prepared through the utilization of the compounds shown in Scheme 48. Dipyrrole (197) was required for the condensation, and the reaction sequence used to synthesize this novel compound is shown in Scheme 49. Pyrrole (193) was treated with bromine to give compound (194). The acid-catalyzed condensation of pyrroles (194) and (195), led to dipyrrole (196) in 74.4% yield. Compound (196) was then treated with hydrogen, in the presence of 10% palladium/charcoal, to give (197) in 85% yield.

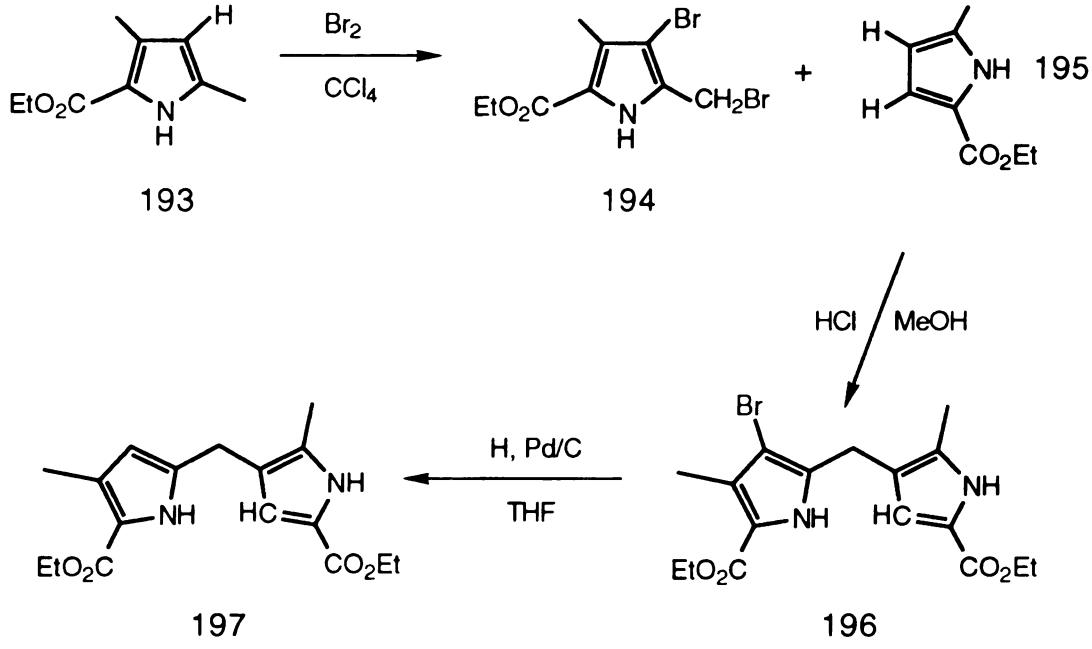



202 197 201

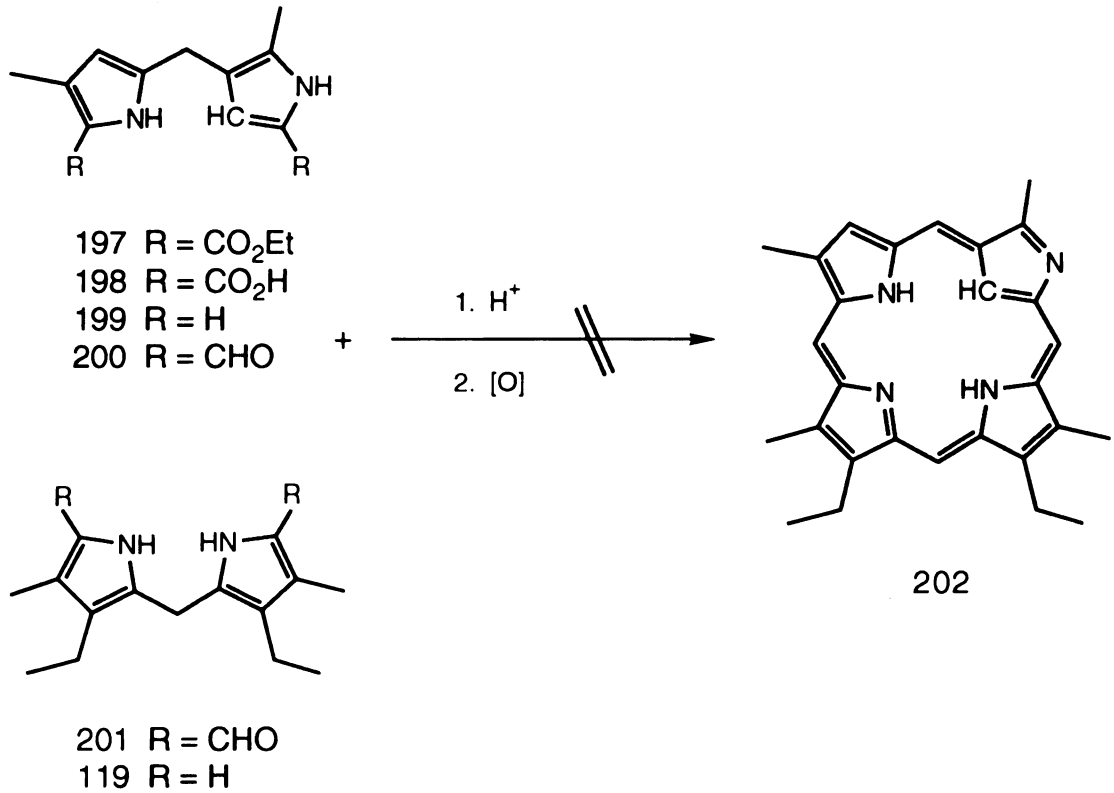
Scheme 48. Retrosynthetic Analysis of Porphyrin Isomer (202)

The initial cyclization approaches are outlined in Scheme 50. The treatment of compound (197) with NaOH, afforded carboxylic acid (198). The condensation of compound (198) and diformyldipyrrylmethane (201), in the presence of p-toluenesulfonic acid, failed to give porphyrin (202) and only

resulted in polymeric material. Compound (197) was then decarboxylated with NaOH and ethylene glycol to give dipyrrole (199). The acid-catalyzed condensation of (199) and diformyldipyrrylmethane (201), also failed to give porphyrin isomer (202). We then decided to utilize dipyrrylmethanes (200) and (119) instead. Dipyrrole (199) was initially treated with benzoyl chloride and dimethylformamide to give dialdehyde (200). The subsequent condensation of compounds (200) and (119) also failed to give (202). Experiments utilizing various concentrations, acids (p-toluenesulfonic, acetic), and oxidizing agents (air, DDQ, chloranil) all failed to give the desired porphyrin isomer. In most cases, the condensations led to a complex mixture of unidentifiable reaction products. Under certain conditions, small amounts of etio-type porphyrin were observed.

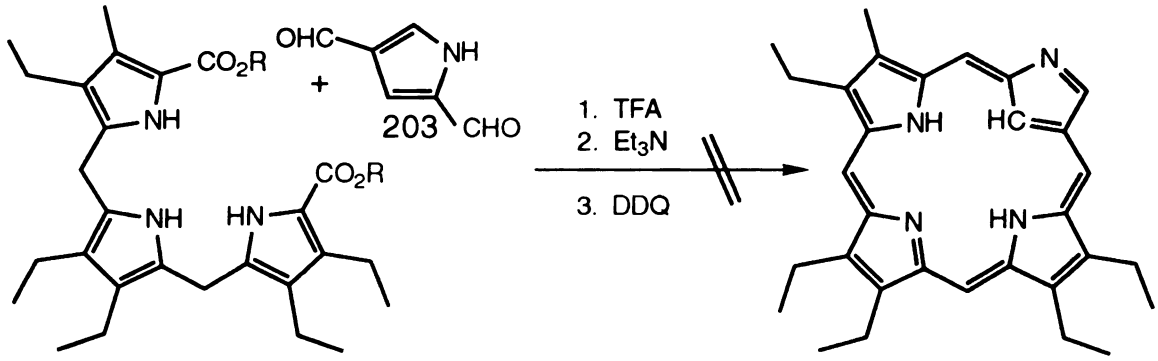



Scheme 49. Synthesis of Dipyrrole (197)




Scheme 50. Attempted Synthesis of Porphyrin Isomer (202)

We then thought that it may be possible to synthesize porphyrin isomer (206), through the "3 + 1" approach outlined in Scheme 51. The hydrogenolysis of tripyrrane (204)[148] over 10% palladium/charcoal, afforded carboxylic acid (205). Compound (205) was the dissolved in trifluoroacetic acid and stirred under nitrogen for ten minutes. A solution of pyrrole (203)[149] in dichloromethane was then added. The mixture was stirred under nitrogen for two hours, neutralized with triethylamine and oxidized with DDQ (Scheme 51). Unfortunately, this reaction sequence failed to produce compound (206). The utilization of various reaction concentrations and attempted oxidation before neutralization, also proved unsuccessful in the synthesis of (206).




204 R = $CH_2Ph$
205 R = H

206

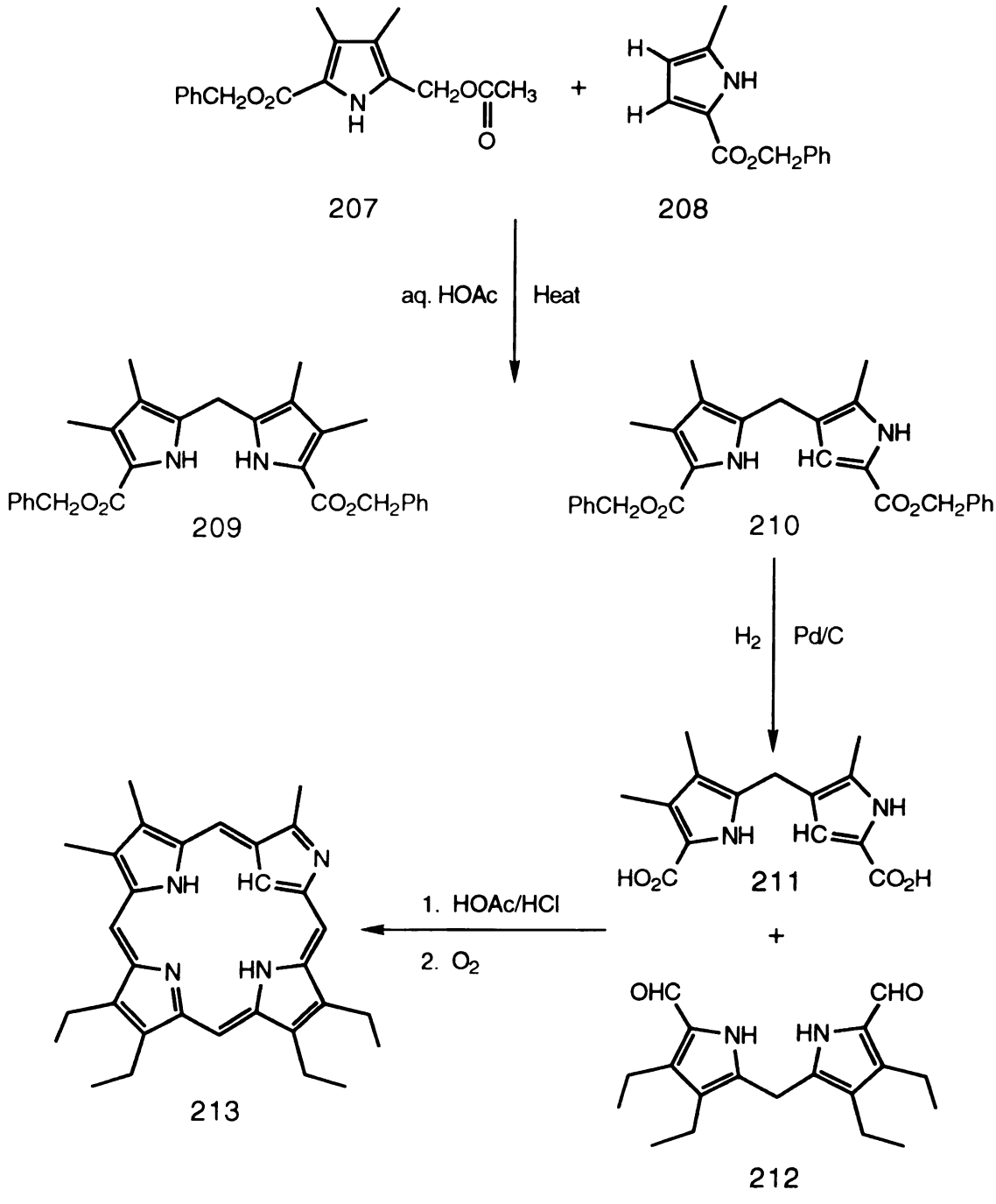
Scheme 51. Attempted Synthesis of Porphyrin Isomer (206)

After our initial investigations into these porphyrin isomers, Dolphin and coworkers reported the synthesis of "N-confused" porphyrin (213).[150] The synthetic route they used is shown in Scheme 52. Pyrroles (207) and (208) were heated with aqueous acetic acid to give a mixture of dipyrroles (209) and (210), which were subsequently separated by column chromatography. The debenzylation of (210), in the presence of hydrogen and palladium/charcoal, gave carboxylic acid (211). Dipyrroles (211) and (212) were then condensed in a mixture of acetic/hydrochloric acid and air oxidized, to give compound (213) in 25% yield.

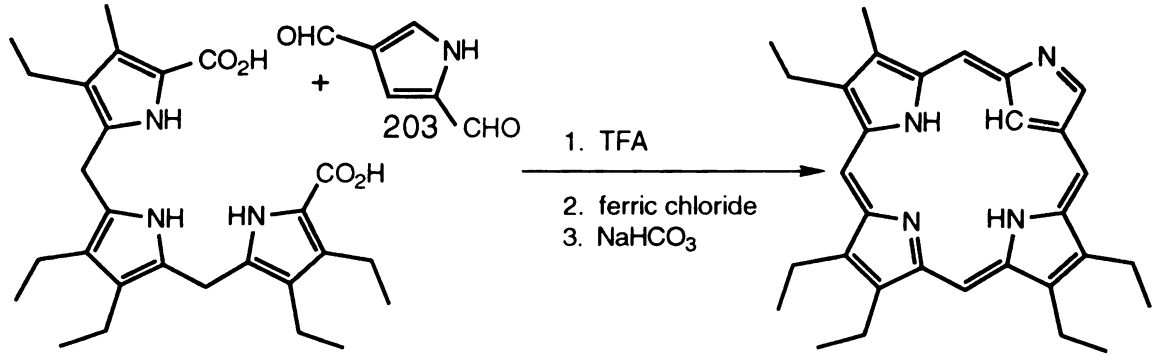
We then decided to reinvestigate our synthetic routes to "N-confused" porphyrins, due to the similarity between the proposed compound (202) and porphyrin isomer (213). The exact experimental procedures were not given in Dolphin's paper, which led to difficulties in the replication of their results. The condensation of compounds (207) and (208) did in fact give a mixture of dipyrroles (209) and (210). However, this mixture proved difficult to separate. The ethyl ester versions of dipyrroles (209) and (210) were also synthesized, but again difficulties were encountered in the purification. Even though the

synthesis of compound (213) was not repeated, we decided to apply Dolphin's cyclization conditions to our dipyrroles. The condensation of dipyrrole (199) and dialdehyde (201) under these conditions, also failed to give porphyrin (202) and only resulted in small amounts of etio-type porphyrin.

Recently however, Lash and coworkers discovered that ferric chloride is a useful oxidant in the synthesis of various porphyrinoid compounds.[151] This oxidation method has also been utilized in the synthesis of corrphycenes and hemiporphycenes.[152] We then decided to attempt the condensation of porphyrin (206), using ferric chloride as the oxidizing agent (Scheme 53). Tripyrrane (205) was dissolved in trifluoroacetic acid and stirred under nitrogen for ten minutes. The mixture was diluted with dichloromethane, and a solution of pyrrole (203) in dichloromethane was added. The solution was then allowed to stir under nitrogen overnight. The mixture was treated with a dilute aqueous ferric chloride solution (~2% w/v), and neutralized with an aqueous sodium bicarbonate solution. The crude residue was columned over alumina and then further purified by preparative TLC, to give compound (206) in 15.5% yield. The structure of (206) was confirmed by MS, UV/visible and NMR spectroscopy. The UV/visible spectrum of (206) showed a Soret-type band at 419 nm, and a series of typical Q bands between 500 and 700 nm (Figure 11). The $^{1}H$ NMR spectrum of this compound was also porphyrin-like, and displayed the internal CH proton at -6.16 ppm.




Scheme 52. Dolphin's Synthesis of Porphyrin Isomer (212)



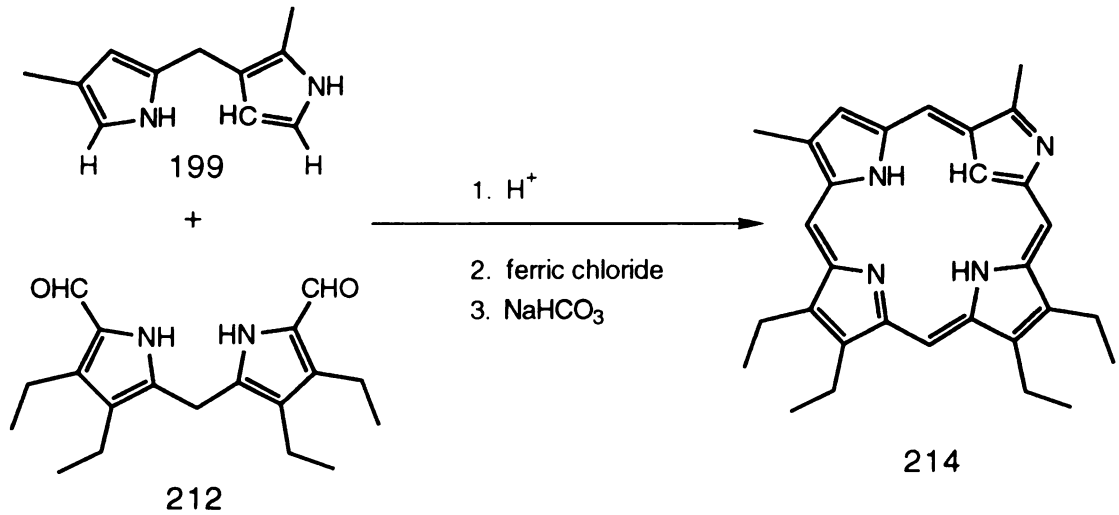

205 206

Scheme 53. Synthesis of Porphyrin Isomer (206)

Encouraged by the results obtained with ferric chloride in the "3 + 1" synthesis, we decided to reinvestigate our "2 + 2" approach to "N-confused" porphyrins. Dipyrroles (199) and (212) were dissolved in dichloromethane. A solution of p-toluenesulfonic acid in methanol was added, and the mixture allowed to stir under nitrogen overnight. The solution was then treated with an aqueous ferric chloride solution and subsequently neutralized with a saturated aqueous sodium bicarbonate solution. The purification of the resulting residue over basic alumina, afforded compound (214) in 7.6% yield (Scheme 54). The UV/visible spectrum of (214) showed a Soret-type band at 422 nm, and a series of Q bands between 500 and 700 nm (Figure 12). In the $^1H$ NMR spectrum, the internal CH proton was observed at -6.29 ppm.

These results led to the question of whether or not the conditions reported by Dolphin would actually afford compound (214) if carried out precisely. Eventually, we were able to contact one of the co-authors of the Dolphin paper.[153] Even though exact reaction conditions were still unavailable, several pieces of useful information were obtained. The major discovery was that the reported acid ratio was incorrect. It was also determined that their cyclizations sequences were carried out using approximately 30 mg of

total starting material, which produced around 5 mg of (213) per run. The purification of compound (213) was also found to be tedious, which probably accounted for the small reaction scales.




Scheme 54. Synthesis of Porphyrin Isomer (214)

We then decided carry out the synthesis of (214) using this new information, so that yield comparisons could be obtained between the various acid catalysts. However, to be of practical value and allow closer comparison to our results, the quantities utilized in the Dolphin reaction conditions were increased approximately 20 fold. Also, air as well as ferric chloride were investigated in oxidation tests. Dipyrroles (199) and (212) were initially dissolved in acetic acid. Hydrochloric acid was added (total acid ratio; 40/3; acetic/hydrochloric) and the solution stirred for two hours under nitrogen. A portion of the reaction mixture was then removed, diluted with dichloromethane, treated with an aqueous ferric chloride solution and neutralized with an aqueous sodium bicarbonate solution. The UV/visible spectrum of this solution, revealed the presence of (214). The rest of the reaction mixture was allowed to stir under nitrogen for 18 hours. At the end of this time, the remaining solution

was split into two separate fractions. The first fraction was treated as above with ferric chloride, which produced results similar to those obtained from the two hour reaction. The second fraction was allowed to stir open for 36 hours, before evaporation of the solvent by a slow stream of air. This oxidation method appeared inferior to ferric chloride, as judged by UV/visible spectroscopy. The best overall results were obtained when the reaction mixture was stirred under nitrogen for 18 hours, and then oxidized with ferric chloride. The treatment of dipyrroles (199) and (212) under these conditions, led to "N-confused" porphyrin (214) in 6.2% yield (Scheme 54). Condensations involving the quantities originally utilized by Dolphin, were subsequently determined to give similar results. Trifluoroacetic acid was also investigated in the "2 + 2" condensation. However, this proved to be an ineffective acid catalyst and only afforded (214) in approximately 2% yield.

Ferric chloride appears to be a general method for the oxidation of these "N-confused" porphyrins. It is interesting to note that other oxidation procedures proved to be ineffective, regardless of their application before or after neutralization. This may be partially related to the stability of the porphyrinogen-type intermediates in the presence of the various oxidants. The yield of compound (214) was also found to be lower than that reported for (213). It is possible that hepta-substituted porphyrin isomers (e.g. 213) inherently give better yields in "2 + 2" cyclization techniques, as compared to hexa-substituted isomers (e.g. 214). This trend also seems to be followed in the case of "3 + 1" condensations. Confused porphyrin (215) has been recently prepared in 50% yield, using "3 + 1" techniques.[151] The use of similar reaction conditions afforded compound (206) in 15.5% yield. During the condensations, the extra functional group may increase steric interactions that enhance the helical geometry required for the cyclization of the tetrapyrrolic intermediates.

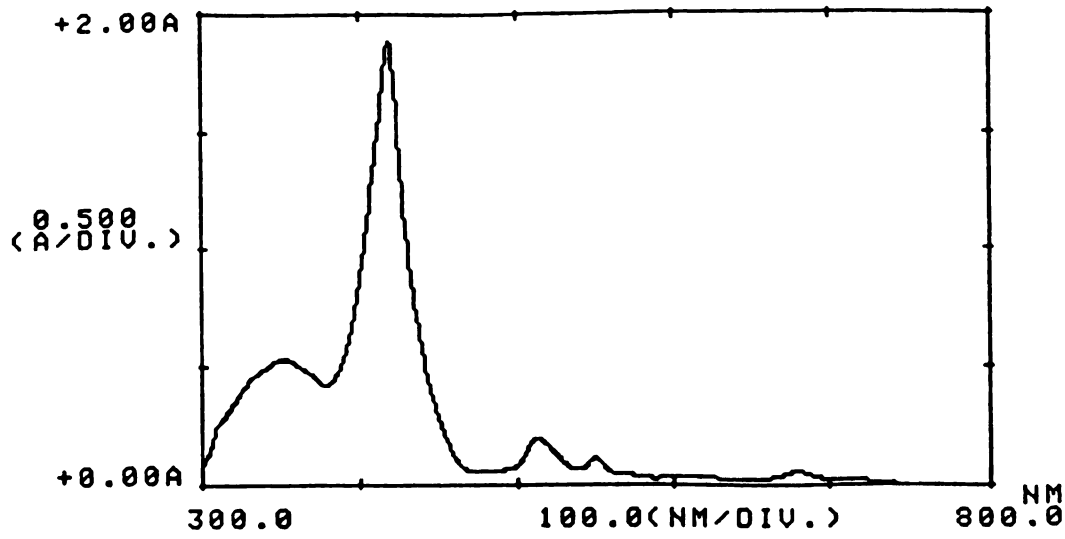



Figure 11. UV/Visible Absorption Spectrum of (206) in $CH_2Cl_2$

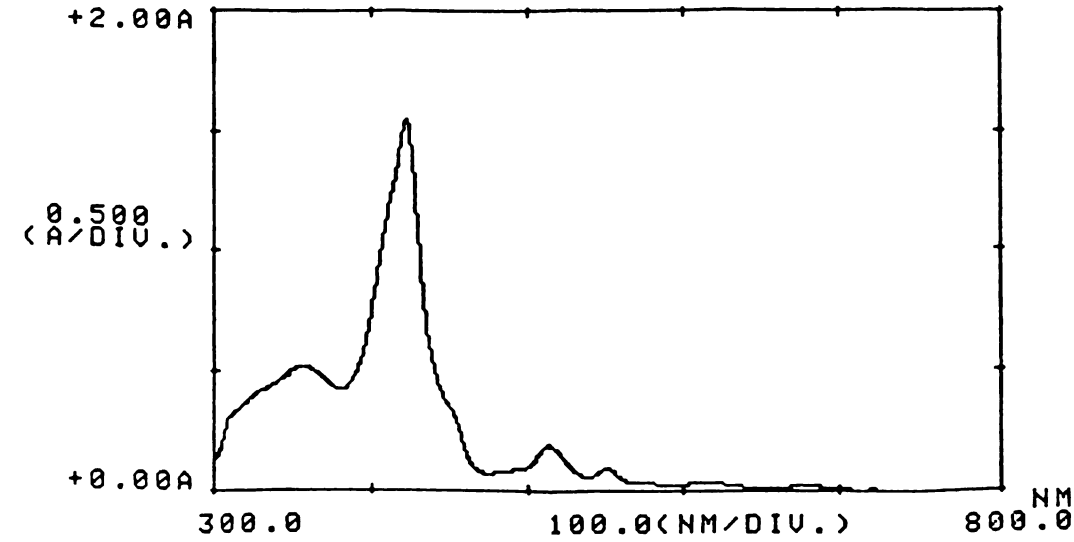
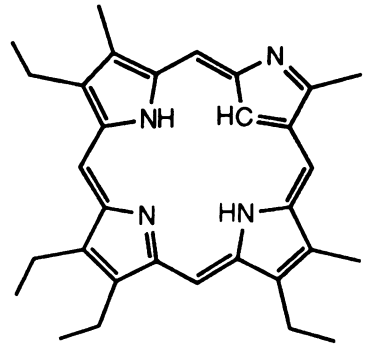
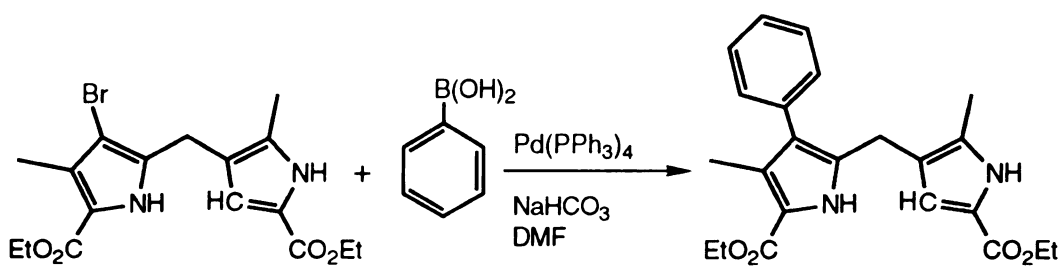



Figure 12. UV/Visible Absorption Spectrum of (214) in $CH_2Cl_2$



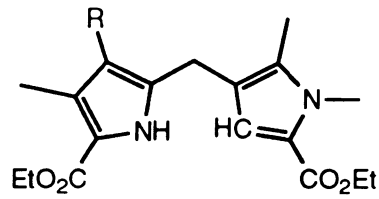
215

Our intermediates and confused porphyrins may offer a number of advantages over compounds (210) and (213). For example, dipyrrole (196) can be readily prepared in good yields and multi-gram quantities. Tedious chromatographic techniques are required to obtain pure samples of dipyrrole (210). The bromine of (196) can also be removed by treatment with hydrogen and palladium/charcoal to give (197), or it can be utilized in further functionalizations. Preliminary investigations revealed that the Suzuki[154] coupling of compound (196) and phenylboronic acid (216), afforded dipyrrole (217) in 50% yield (unoptimized) (Scheme 55). The α-free version of (217) has been synthesized as well. The N-methylated dipyrroles (218) and (219) have also been prepared and await further testing.



196 216 217

Scheme 55 Synthesis of Dipyrrole (217)

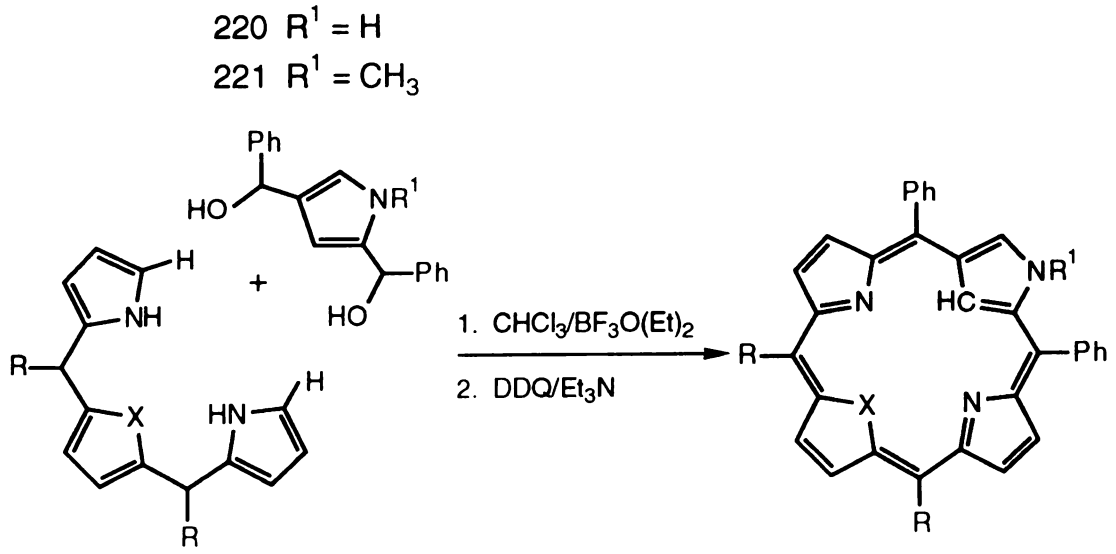



218 R = Br
219 R = H

Compounds (206) and (214) may also have an advantage over (213), because they each contain a site available for β-functionalization. Furthermore, the ring position of this functionalization site is different in each porphyrin isomer. This should be of value when designing new compounds, since it provides a choice in substituent placement. The future derivatization of these porphyrin isomers may involve the incorporation of functional groups such as CHO, $CH_2OH$, $CH_3$, $COCH_3$, $CHOH\text{-}CH_3$, $CH{=}CH_2$, and $CH_2CH_3$.

Interestingly, a series of "N-confused" porphyrin heteroanalogues have been recently prepared. The condensation of diols (220, 221) and tripyrranes (222-224), afforded compounds (225-228) in yields of 5.5-30% (Scheme 56).[155-157] However, these condensations only afforded product in a limited number of instances and did not work well for the synthesis of "N-confused" porphyrins. Our procedures may provide alternate routes to similar "N-confused" heteroanalogues.

We have demonstrated that "N-confused" porphyrins can be prepared using "2 + 2" and "3 + 1" condensation techniques. It should be noted that our results are preliminary findings. Detailed investigations would most likely lead to improved yields in both condensation techniques. Eventual work in this area should lead to compounds that are suitable for PDT studies.




222 R = H, X = O
223 R = Ph, X = O
224 R = Ph, X = S

225 R = H, $R^1$ = H, X = O
226 R = Ph, $R^1$ = $CH_3$, X = O
227 R = Ph, $R^1$ = H, X = S
228 R = Ph, $R^1$ = $CH_3$, X =S

Scheme 56. Synthesis of "N-confused" Porphyrin Heteroanalogues (225-228)

## III. Experimental

Ethyl 4-bromo-5-bromomethyl-3-methylpyrrole-2-carboxylate (194)

Ethyl 3,5-dimethylpyrrole-2-carboxylate (193)[158] (25.08 g; 0.15 moles) was dissolved in carbon tetrachloride (330 ml) and warmed to 50°C. Bromine (50.34 g; 16.2 ml; 0.315 moles) was dissolved in carbon tetrachloride (80 ml) and added dropwise to the stirred solution, while maintaining the reaction temperature at 50°C. After the addition was complete, the solution was allowed to stir at room temperature for three hours and then placed in the refrigerator overnight. The precipitate was filtered and the solid recrystallized twice from chloroform/hexane to give a light pink solid (26.2 g; 53.7%): mp 165-167°C (lit. mp 168°C[159]). $^1$H NMR ($CDCl_3$): δ 1.37 (3H, t, $CH_2C\underline{H}_3$), 2.27 (3H, s, $CH_3$), 4.35 (2H, q, $C\underline{H}_2CH_3$), 4.51 (2H, s, $CH_2$-Br), 9.56 (1H, br, NH). EI MS: m/e (Relative Intensity) 327.0 (4.16), 325.0 (8.25), 323.0 (4.34), 246.1 (93.60), 244.0 (100.00), 200.0 (91.13), 198 (90.64).

Diethyl 3-bromo-2',4-dimethyl-2,3'-dipyrrylmethane-5,5'-dicarboxylate (196)

Ethyl 5-methylpyrrole-2-carboxylate (195)[160] (9.90 g; 6.46 x $10^{-2}$ moles) was dissolved in methanol (100 ml) and heated to 60°C. Ethyl 4-bromo-5-bromomethyl-3-methylpyrrole-2-carboxylate (194) (21.0 g; 6.46 x $10^{-2}$ moles) was dissolved in methanol (200 ml) and added dropwise to the stirred solution, while maintaining the temperature at 60°C. After the addition was complete (twenty minutes) the solution was refluxed for fifteen minutes. The mixture was then allowed to stir for three hours and then placed in the refrigerator for two hours. The precipitate was filtered and washed with cold methanol to give the title compound as an off-white solid (17.7 g). The filtrate was evaporated and the residue recrystallized with methanol to give a second crop of the desired

dipyrrole (1.4 g; 19.1 g total; 74.4%): mp 203-205°C. $^1$H NMR ($CDCl_3$/DMSO-$d_6$), 1.05-1.15 (6H, overlapping triplets, 2 x $CH_2C\underline{H}_3$), 2.02 (3H, s), 2.05 (3H, s) (2 x $CH_3$), 3.47 (2H, s, methane-$CH_2$), 3.96-4.11 (4H, overlapping quartets, 2 x $C\underline{H}_2CH_3$), 6.45 (1H, d, β-H), 9.72 (1H, br), 10.22 (1H, br) (2 x NH). EI MS: m/e (Relative Intensity) 398.0 (44.10), 396.0 (44.66), 317.1 (37.36), 271.1 (100.00), 225.0 (54.49), 198.0 (32.58), 197.0 (45.51).

Diethyl 2',4-dimethyl-2,3'-dipyrrylmethane-5,5'-dicarboxylate (197)

Diethyl 3-bromo-2',4-dimethyl-2,3'-dipyrrylmethane-5,5'-dicarboxylate (196) (10.0 g; 2.52 x $10^{-2}$ moles) was dissolved in tetrahydrofuran (300 ml) and triethylamine (1 ml). The solution was purged with nitrogen and 10% palladium charcoal (500 mg) was added. The mixture was then stirred under an atmosphere of hydrogen overnight. The solution was filtered to remove the catalyst and the solvent removed under reduced pressure to give the desired compound as a off-white solid (6.83 g; 85.2%): mp 163-165°C. $^1$H NMR ($CDCl_3$): δ 1.27-1.37 (6H, overlapping triplets, 2 x $CH_2C\underline{H}_3$), 2.16 (3H,S), 2.27 (3H,S) (2 x $CH_3$), 3.68 (2H, s, methane-$CH_2$), 4.21-4.32 (4H, overlapping quartets, 2 x $C\underline{H}_2CH_3$), 5.79 (1H, d) 6.69 (1H, d) (2 x β-H), 8.65 (1H ,br), 9.23 (1H ,br) (2 x NH). EI MS: m/e (Relative Intensity) 318.2 (36.10), 199.0 (31.22), 165.0 (31.71).

2',4-Dimethyl-2,3'-dipyrrylmethane (199)

Diethyl 2',4-dimethyl-2,3'-dipyrrylmethane-5,5'-dicarboxylate (197) (1.50 g; 4.71 x $10^{-3}$ moles) was suspended in ethylene gylcol (20 ml) and sodium hydroxide (1.75 g) added. The mixture was then refluxed under nitrogen, for one hour and forty-five minutes. Water (5 ml) was added and the solution heated for an additional fifteen minutes. The mixture was cooled to room

temperature, water (25 ml) added and the solution extracted with methylene chloride (2 x 25 ml). The organic layer was then washed with water (2 x 25 ml) and dried over anhydrous sodium sulfate. The removal of the solvent afforded the title dipyrrole as a light brown oil, which was used without further purification (656 mg, 79.9%). $^1$H NMR ($CDCl_3$) δ 2.19 (3H, S), 2.22 (3H, S) (2 x $CH_3$), 3.82 (3H, S, methane-$CH_2$), 5.90 (1H, br), 6.43 (1H, br) (2 x α-H), 6.08-6.13 (1H, m), 6.62-6.67 (1H, m) (2 x β-H), 7.63 (1H, br), 7.82 (1H, br) (2 x NH). EI MS: m/e (Relative Intensity) 174.3 (100.00), 173.3 (51.25), 159.2 (36.67), 94.1 (52.92), 93.1 (87.92).

7,18-Dimethyl-8,12,13,17-tetraethyl-2-aza-21-carbaporphyrin (206)

2,5-Bis[[5-(benzyloxycarbonyl)-3-ethyl-4-methylpyrrol-2-yl]methyl]-3,4-diethylpyrrole (204)[148] (1.20 g; 1.90 x $10^{-3}$ moles) was dissolved in tetrahydrofuran (150 ml) and triethylamine (5 drops). The system was purged with nitrogen and 10% palladium/charcoal (100 mg) was added. The mixture was then stirred under an atmosphere of hydrogen overnight. The solution was filtered to remove the catalyst and the solvent removed under reduced pressure. The residue was vacuum dried to give (205) as light pink solid, which was used without further purification (771 mg; 89.7%).

Tripyrrane (205) (165 mg; 3.64 x $10^{-4}$ moles) was dissolved in trifluoroacetic acid (1.7 ml) and stirred under nitrogen for ten minutes. Dichloromethane (100 ml) was added, followed by 2,4-diformylpyrrole (19)[149] (45 mg; 3.64 x $10^{-4}$ moles) dissolved in dichloromethane (70 ml). The solution was then stirred under nitrogen overnight. The mixture was treated with an aqueous ferric chloride solution (~2% w/v; 200 ml), and then washed with a saturated aqueous sodium bicarbonate solution (200 ml) and water (200 ml). The solvent was removed and the residue chromatographed over neutral

alumina (grade III), eluting with chloroform. The resulting solid was further purified on a preparative TLC plate, with a mixture of dichloromethane/methanol (93/7), to give the title compound as a purple film (25.4 mg; 15.5%). $^1$H NMR ($CDCl_3$): δ -6.16 (1H, s, internal CH), -3.83 (2H, br, 2 x NH), 1.73-1.82 (12H, overlapping triplets, 4 x $CH_2C\underline{H}_3$), 3.48 (3H, s), 3.53 (3H, s) (2 x $CH_3$), 3.80-4.02 (8H, overlapping quartets, 4 x $C\underline{H}_2CH_3$), 9.54 (1H, s), 9.56 (1H, s) 9.61 (1H, s), 9.77 (1H, s), 10.13 (1H, s), (β-H, 4 x meso-H). UV/visible ($CH_2Cl_2$): λ max (Relative intensity) 350.0 (0.539), 418.5 (1.880), 513.5 (0.196), 550.5 (0.111), 616.5 (0.039), 680.0 (0.055). EI MS: m/e (Relative Intensity) 450.3 (100.00), 435.2 (24.47), 421.2 (17.55).

3,8-Dimethyl-12,13,17,18-tetraethyl-2-aza-21-carbaporphyrin (214)

a. 2',4-Dimethyl-2,3',-dipyrrylmethane (199) (168.3 mg; 9.66 x $10^{-4}$ moles) and 5,5'-diformyl-3,3',4,4'-tetraethyl-2,2'-dipyrrylmethane[161] (212) (303.7 mg; 9.66 x $10^{-4}$ moles) were dissolved in dichloromethane (150 ml) and the system purged with nitrogen. A methanolic solution of p-toluenesulfonic acid (250 mg in 5 ml) was then and added and the mixture allowed to stir under nitrogen overnight. The solution was treated with an aqueous ferric chloride solution (~2% w/v; 150 ml), and then washed with a saturated aqueous sodium bicarbonate solution (150 ml) and water (150 ml). The solvent was removed and the residue chromatographed over neutral alumina (grade III), eluting with chloroform. The resulting solid was further purified over basic alumina, eluting with dichloromethane, to give the title compound as a greenish-purple film (33.1 mg; 7.6%). $^1$H NMR ($CDCl_3$): δ -6.29 (1H, s, internal CH), -3.69 (2H, br, 2 x NH), 1.72-1.83 (12H, overlapping triplets, 4 x $CH_2C\underline{H}_3$), 3.57 (3H, s), 3.59 (3H, s) (2 x $CH_3$), 3.76-3.88 (4H, m), 3.92-4.04 (4H, m) (4 x $C\underline{H}_2CH_3$), 8.92 (1H, s), 9.51 (1H, s) 9.62 (1H, s), 9.68 (1H, s), 10.06 (1H, s), (β-H, 4 x meso-H).

UV/visible ($CH_2Cl_2$): λ max (Relative intensity) 356.5 (0.518), 421.5 (1.542), 513.5 (0.189), 551.0 (0.093), 614.0 (0.039), 678 (0.026). EI MS: m/e (Relative Intensity) 450.2 (94.19), 435.2 (19.81).

b. 2',4-Dimethyl-2,3'-dipyrrylmethane (199) (122 mg; 7.00 x $10^{-4}$ moles) and 5,5'-diformyl-3,3',4,4'-tetraethyl-2,2'-dipyrrylmethane (212) (220 mg; 7.00 x $10^{-4}$ moles) were dissolved in acetic acid (65 ml) and the system purged with nitrogen. Hydrochloric acid (5 ml) was added and the mixture stirred under nitrogen overnight. Dichloromethane (125 ml) was added and the solution treated with an aqueous ferric chloride solution (~2% w/v; 150 ml). The organic layer was then washed with aqueous saturated sodium bicarbonate solution (150 ml) and finally with water (150 ml). The removal of the solvent and purification of the residue as above, afforded (214) as a greenish-purple film (19.6 mg; 6.2%).

# CHAPTER 6

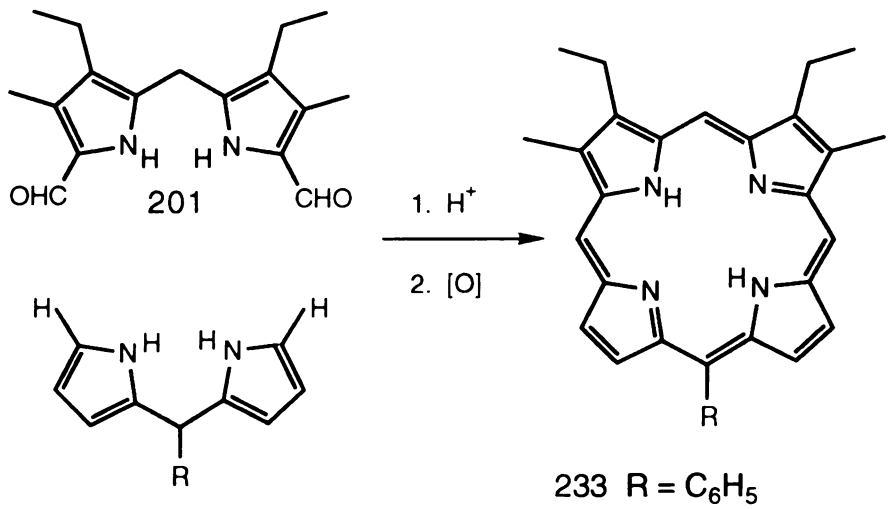
# SYNTHETIC STUDIES ON THE CYCLIZATION OF A,C-BILADIENES AND ALDEHYDES

## I. Introduction

A major factor to consider when designing a synthetic strategy, is the availability of the intermediates required to prepare the target molecule. In porphyrin chemistry, two of the most widely utilized precursory compounds are octaethyl and tetraphenylporphyrin. As shown in Chapter 4, the meso positions of octaethylporphyrin can often provide convenient sites for functionalization. In tetraphenylporphyrin however, the meso positions are already substituted and unavailable for further derivatization. Therefore, the transformation of this compound usually involves functionalization at one the β-unsubstituted positions. Precursors containing several different functionalization sites are often desirable, because they can be applied to the synthesis of various target molecules. Porphyrins such as (233-236), contain both meso and β-unsubstituted positions that could be utilized in further transformations. Unfortunately, there are relatively few literature examples of these types of potentially useful compounds.

Theoretically, "2 + 2" condensation techniques could be used to synthesize porphyrins (233-236) (Scheme 57). There are various literature reports that describe the preparation of the β-unsubstituted dipyrrylmethanes required for such condensations. Lindsey and coworkers reported that the acid-catalyzed condensation of various aldehydes and excess pyrrole, resulted in meso-substituted dipyrrylmethanes.[162] This synthetic method is relatively

straightforward and has afforded dipyrroles (229) and (230) in yields of 49 and 76%, respectively. However, dipyrroles such as (231) and (232) are somewhat troublesome to prepare. Compound (231) can be prepared from pyrrole in an overall yield of 30%.[163] The main disadvantage of this synthesis, is the required use of the toxic compounds phosgene or thiophosgene. Compound (232) has been synthesized in 55% yield.[164] Drawbacks of this synthesis include dry box manipulations and derivatization of the dipyrrole as the methyl pyridinium salt, before isolation as a stable solid.



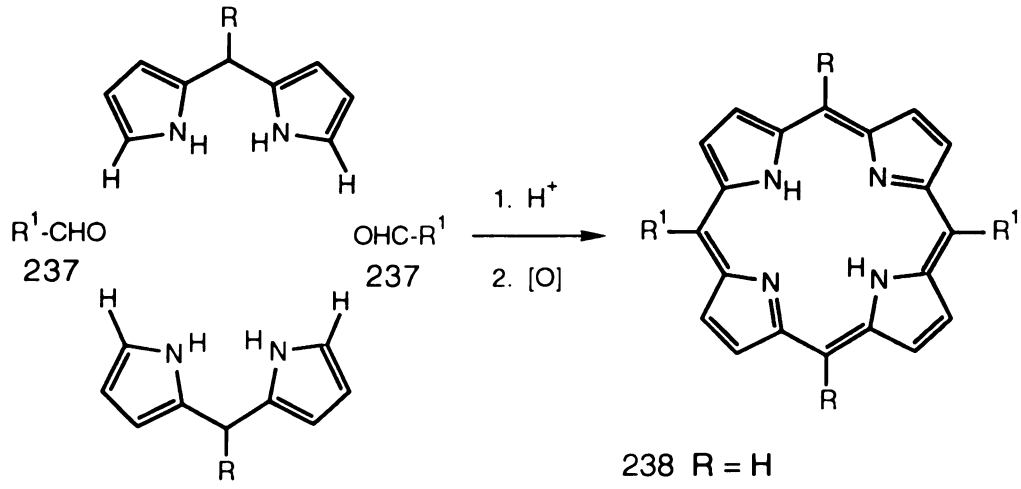

229 R = $C_6H_5$
230 R = p-$CH_3C_6H_4$
231 R = H
232 R =(4)-$C_4H_4N^+I^-$

233 R = $C_6H_5$
234 R = p-$CH_3C_6H_4$
235 R = H
236 R =(4)-$C_4H_4N^+I^-$

Scheme 57. Possible Synthetic Routes to Porphyrins (233-236)

In the majority of instances, β-unsubstituted dipyrroles are utilized in the synthesis of porphyrins bearing multiple aryl substituents. Porphyrin (238) was obtained in 83% yield, by the acid-catalyzed condensation of dipyrrole (231) and benzaldehyde (237) (Scheme 58).[165] Unfortunately, results become complicated when meso-substituted dipyrroles are utilized in these reactions.

The acid-catalyzed condensation of dipyrrole (230) and benzaldehyde (237), afforded porphyrin (239) and a least three other compounds (Scheme 58). These results were attributed to the acidic decomposition and recombination of the dipyrrolic pieces.[166]



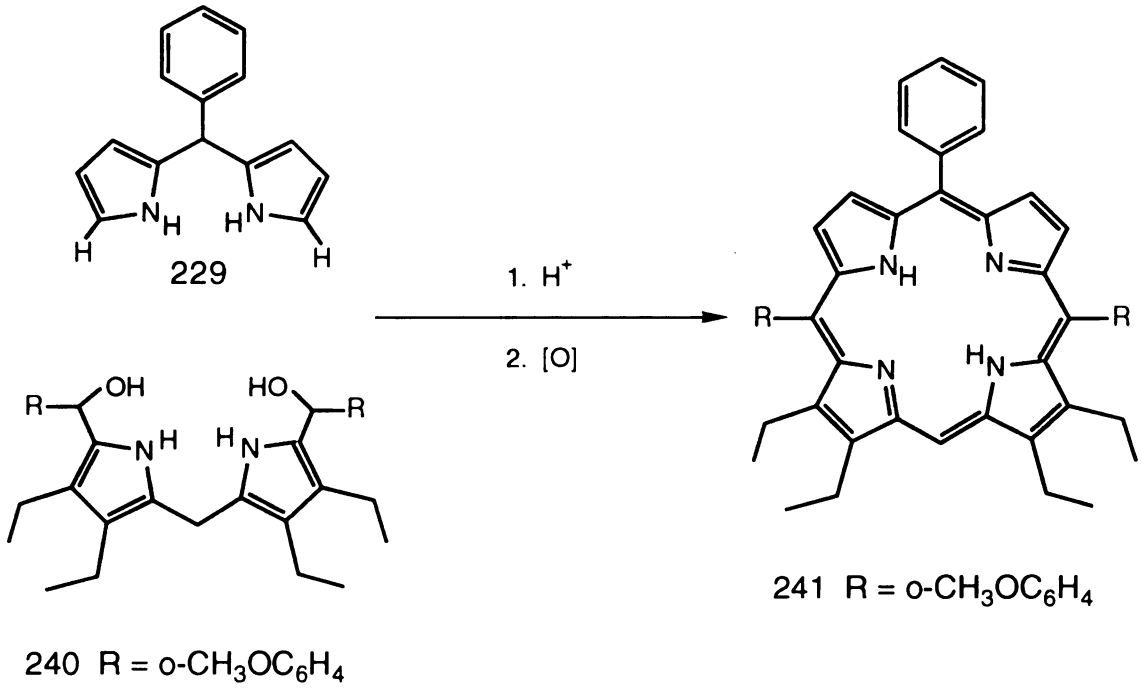

230 R = p-$CH_3C_6H_4$
231 R = H
237 $R^1$ = p-$CH_3OC_6H_4$

238 R = H
$R^1$ = p-$CH_3OC_6H_4$

239 R = $R^1$ = p-$CH_3C_6H_4$
and 3 other mixed
porphyrins where
R and $R^1$ = p-$CH_3C_6H_4$,
p-$CH_3OC_6H_4$

Scheme 58. Synthesis of Porphyrins (238) and (239)

In "2 + 2" type condensations, β-unsubstitued dipyrroles are usually cyclized with dipyrrylmethane-diols. Porphyrin (241) was obtained in 8% yield, by the acid-catalyzed condensation of compound (229) and diol (240) (Scheme 59).[167] Recently however, Boyle and coworkers utilized β-unsubstituted dipyrroles in a series of MacDonald condensations.[168] The cyclization of dipyrrole (242) and dipyrroles (243a-e), afforded porphyrins (244a-e) in yields of 8.9-28.1% (Scheme 60).

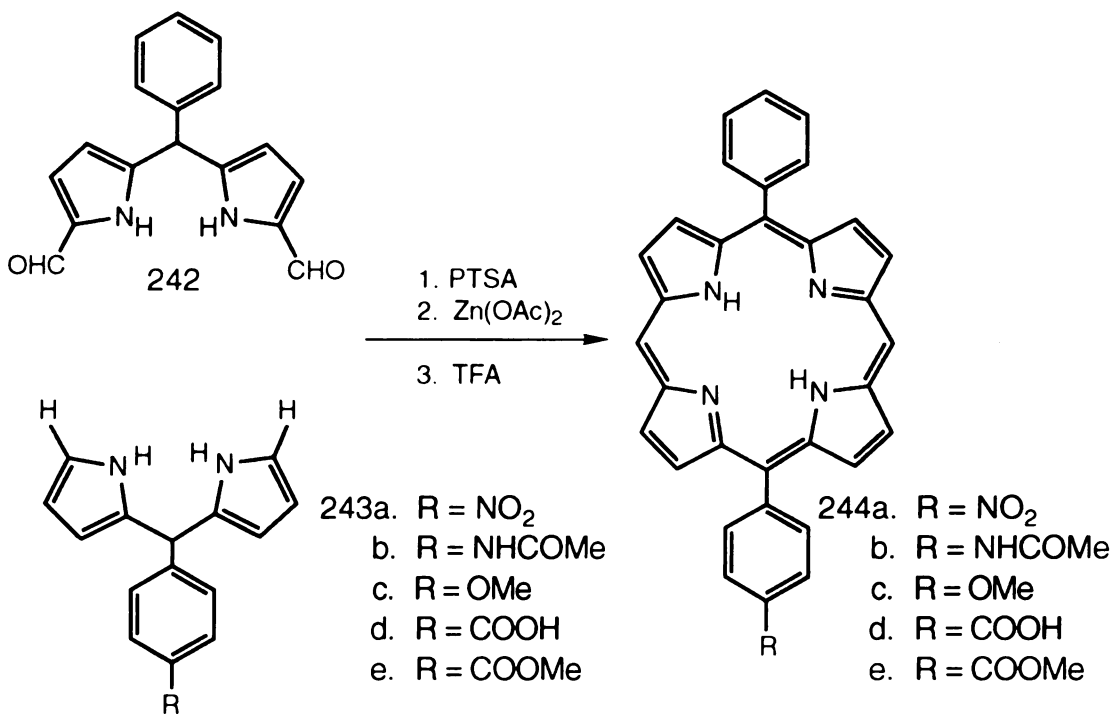



Scheme 59. Synthesis of Porphyrin (241)

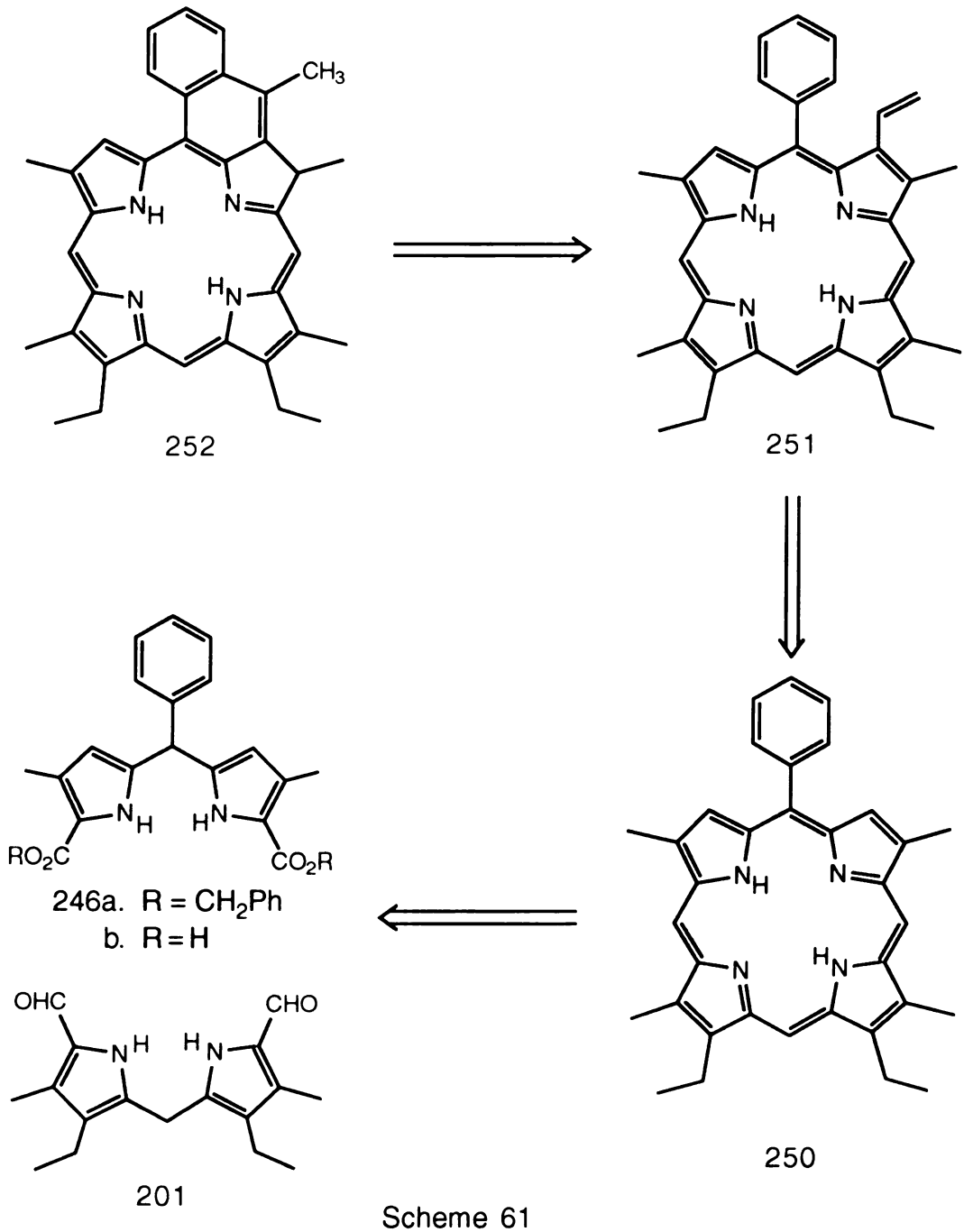
The above examples provide a fairly inclusive list of the different types of porphyrins that have been synthesized from β-unsubstituted dipyrroles. Unfortunately, questions still remain about the possible use of dipyrroles (229-232) in the preparation of porphyrins (233-236). To the best of our knowledge, dipyrrole (232) has not been utilized in the synthesis of any porphyrins.

There is even less information available on the synthesis or use of partially alkylated dipyrroles such as (246a), in the preparation of porphyrins. One example of the attempted utilization of dipyrrole (246a) comes from our own research. We have been interested in synthesis of naphthochlorins[169] such as (252), for possible use in PDT therapy. It was initially envisioned that chlorin (252) could be synthesized utilizing the precursors shown in Scheme 61. The major step in the sequence was thought to be the preparation of porphyrin (250), through the condensation of compounds (246b) and (201).

Primary attention was given to the synthesis of dipyrrole (246a), since it was the unreported component required for the preparation of (250). It was discovered that the condensation of pyrrole (245)[170] and benzaldehyde (6), afforded a combination of dipyrroles (246a) and (247) (Scheme 62). The investigation of several acid catalysts all resulted in a mixture of compounds, which were difficult to separate. We then decided to utilize pyrrole (248),[171] as a protected form of (245) (Scheme 63). It was thought that the bromines of the resulting dipyrrole (249), could then be removed during the hydrogenolysis of the benzyl esters to give (246b). Unfortunately, the condensation of pyrrole (248) and benzaldehyde (6) failed to give dipyrrole (249). This was most likely due to steric interactions resulting from the bulky bromine groups.

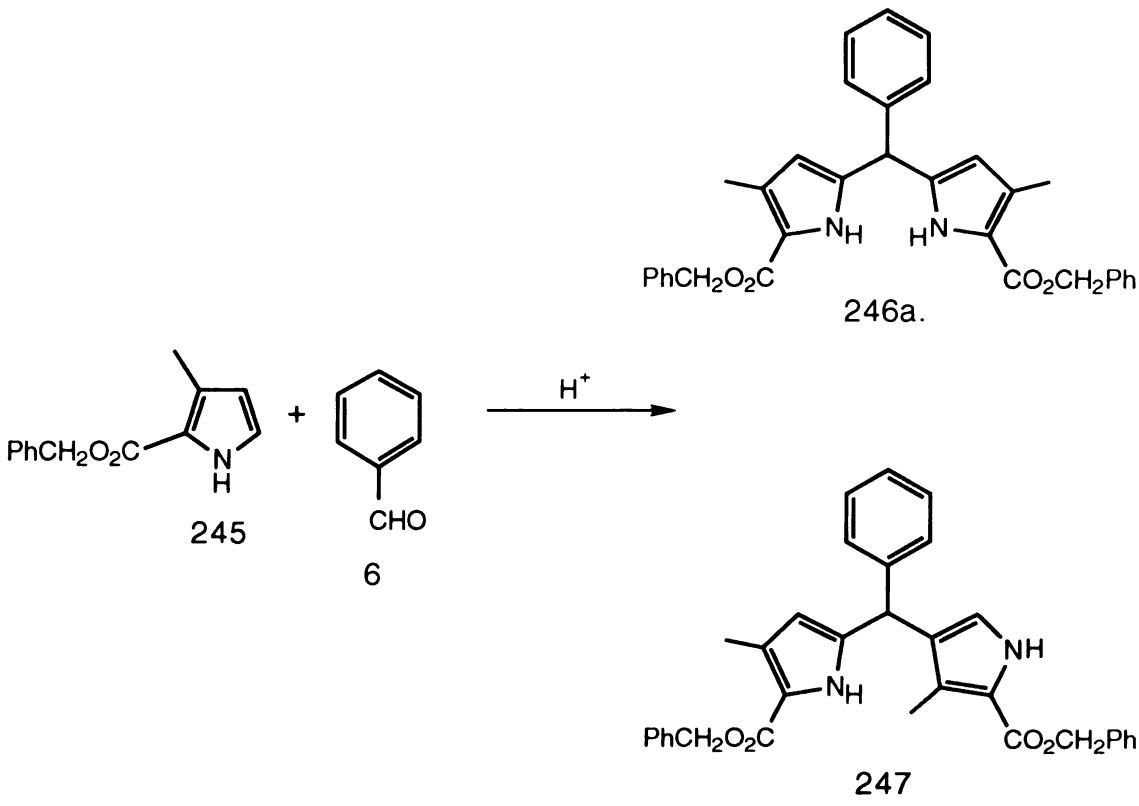



Scheme 60. Synthesis of Porphyrins (244a-e)

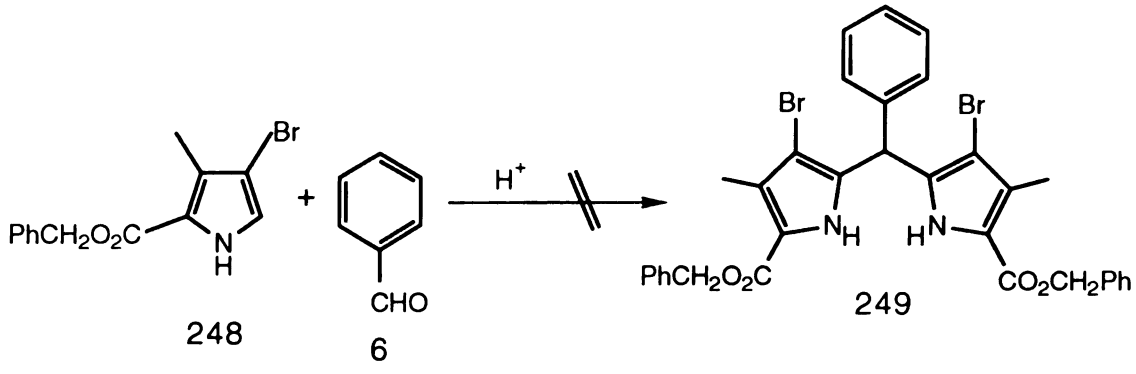



Scheme 61

Scheme 61. Retrosynthetic Analysis of Chlorin (252)

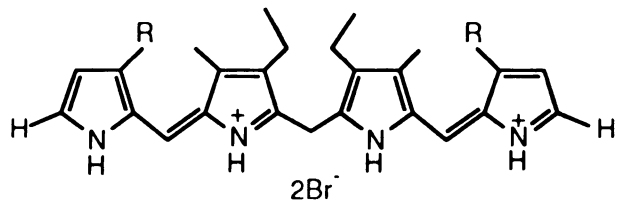



Scheme 62. Synthesis of Dipyrroles (246a) and (247)




Scheme 63. Attempted Synthesis of Dipyrrole (249)

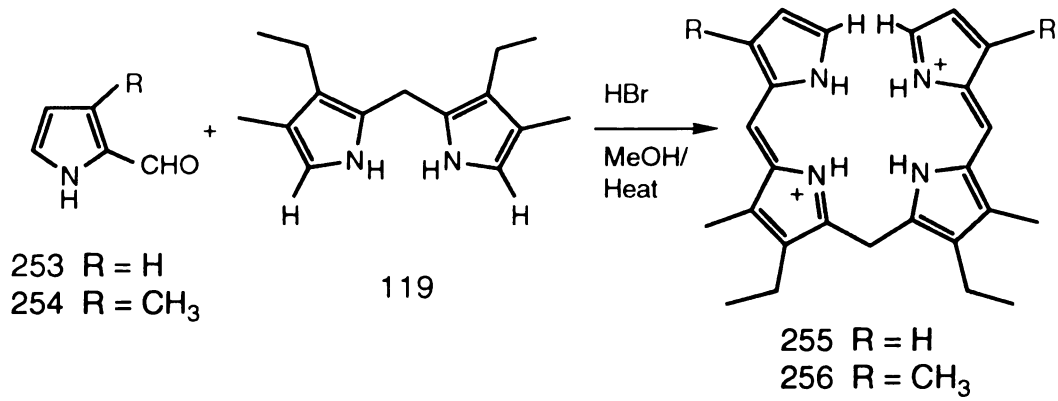
It became evident that other methods were required to synthesize compound (250). In the preparation of our P460 models, the condensation of a,c-biladienes and aromatic aldehydes led to the desired meso-substituted compounds. It was envisioned that it should be possible to synthesize porphyrin (250) and compounds similar to porphyrins (233-236), by the application of this methodology. Tetrapyrroles (255) and (256) appeared to be likely precursors to porphyrins of this type. We then decided to synthesize a series of simple porphyrins that would test the versatility of compounds (255) and (256). The information gathered from these experiments was expected to be applicable to the preparation of a wide range of β-unsubstituted porphyrins.




255 R = H
256 R = $CH_3$

## II. Results and Discussion

Tetrapyrroles (255) and (256) can be synthesized from readily accessible precursors. The condensation of formyl pyrrole (253) and dipyrrole (119), in the presence of hydrobromic acid, afforded tetrapyrrole (255) in 78.3% yield. Compound (256) was similarly prepared in 86.0% yield, by the condensation of formyl pyrrole (254) and dipyrrole (119) (Scheme 64).




253 R = H
254 R = $CH_3$

119

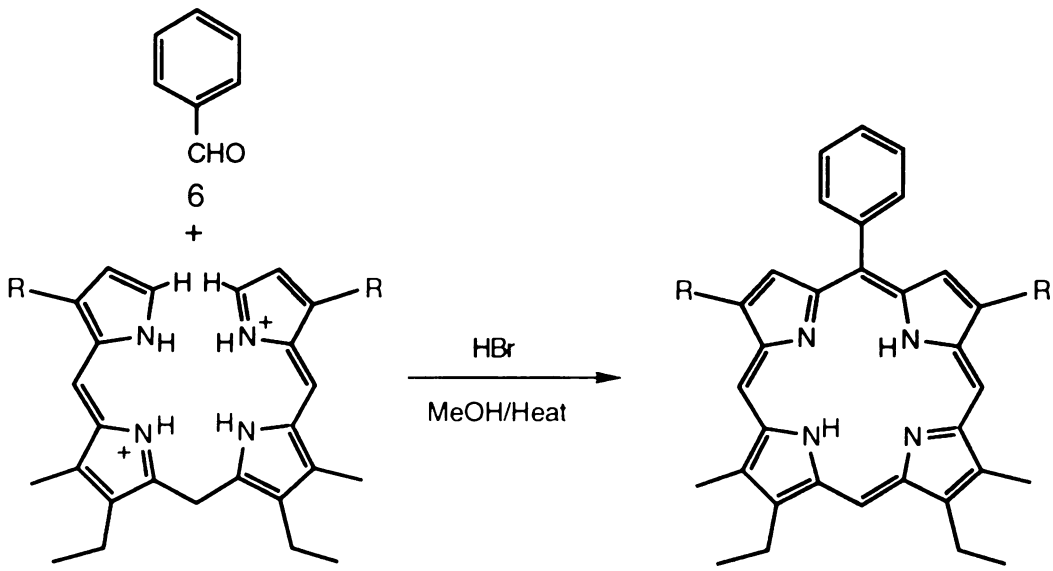
255 R = H
256 R = $CH_3$

Scheme 64. Synthesis of Tetrapyrroles (255) and (256)

Tetrapyrrole (255) was condensed with benzaldehyde (6), in the presence of hydrogen bromide, to give porphyrin (233) in 23.2% yield. The cyclization of tetrapyrrole (256) and benzaldehyde (6), afforded porphyrin (250) in 38.0% yield (Scheme 65). For comparison, porphyrin (258) was previously prepared by this procedure in 40% yield.[172]

The condensation of tetrapyrrole (255) and formaldehyde (258), produced porphyrin (235) in 5.0% yield. The replacement of formaldehyde with trimethyl orthoformate, also afforded porphyrin (235) in similar yield. The cyclization of tetrapyrrole (256) with formaldehyde (258), gave porphyrin (259) in 26.4% yield (Scheme 66). Similar condensation procedures have afforded

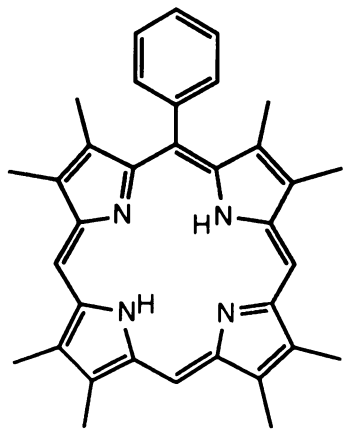
porphyrin (260) in 57% yield.[173] It appears that the lack of alkyl substituents substantially decreases the yield of porphyrin when formaldehyde is used as the linking fragment. These β-free tetrapyrroles may lead to reaction intermediates that possess greater conformational freedom than those produced in the synthesis of (260). This could facilitate polymerization over cyclization, which would result in lower yields of porphyrin.



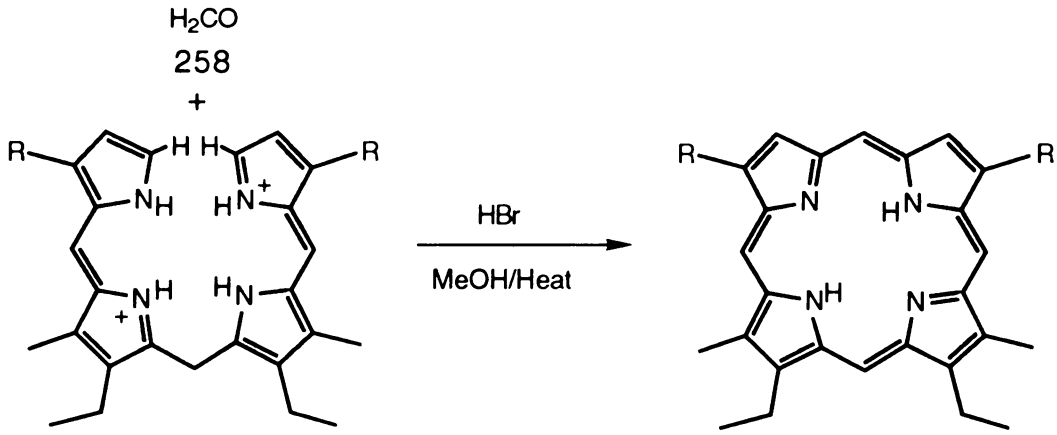

255 R = H
256 R = $CH_3$

233 R = H
250 R = $CH_3$

Scheme 65. Synthesis of Porphyrins (233) and (250)



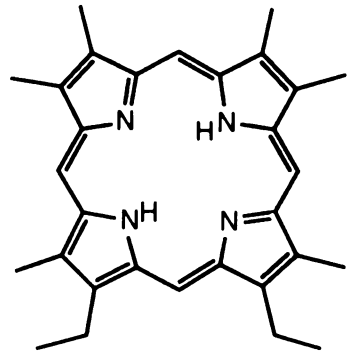
257




255 R = H
256 R = $CH_3$

235 R = H
259 R = $CH_3$

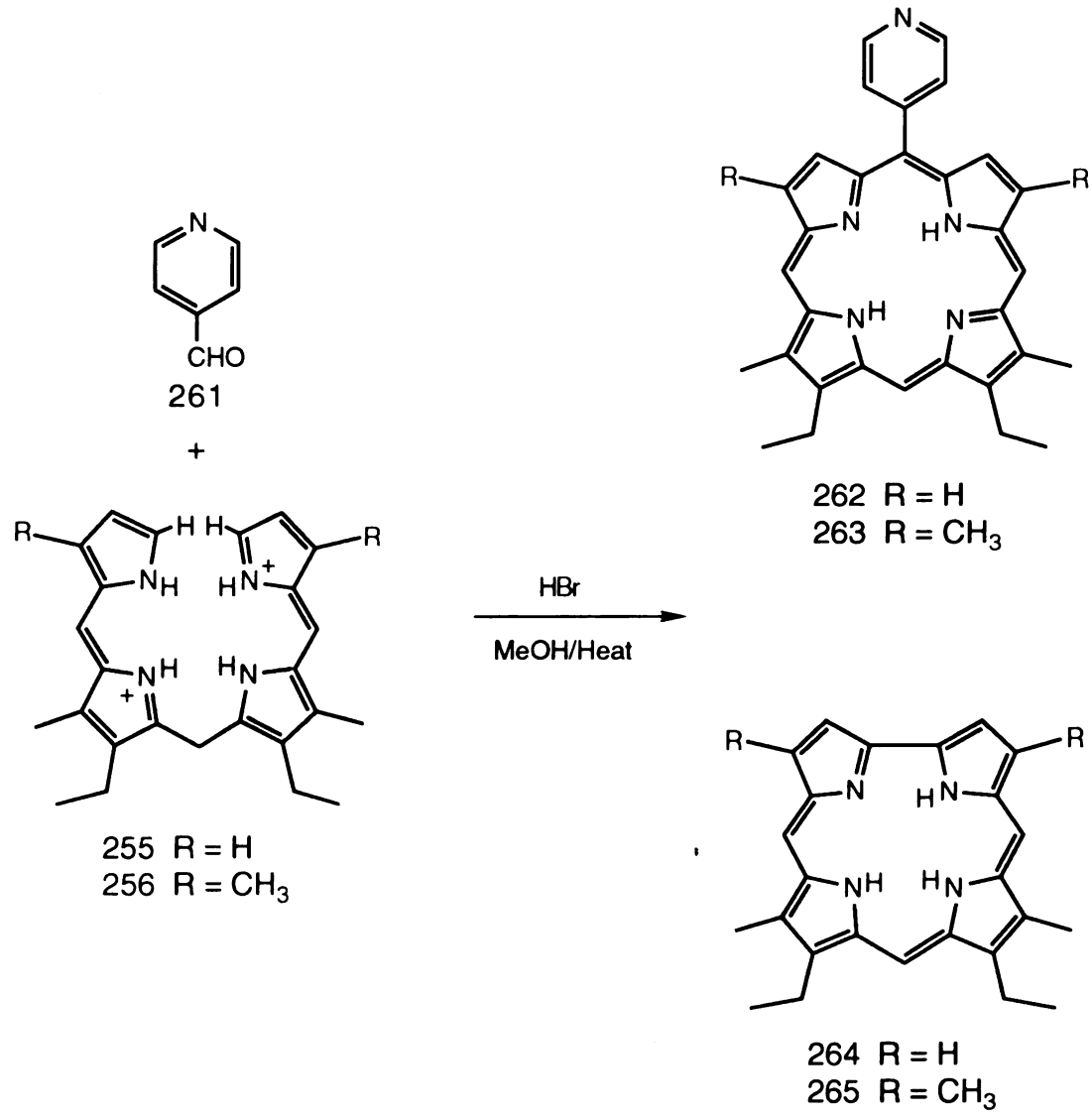
Scheme 66. Synthesis of Porphyrins (235) and (259)



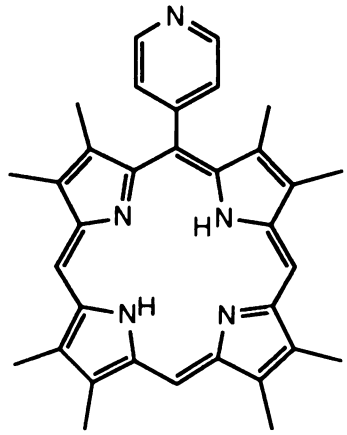
260

The reaction of 4-pyridinecarboxaldehyde (261) with tetrapyrroles (255) and (256), led to several interesting results. The acid-catalyzed condensation of compounds (255) and (261), afforded pyridyl porphyrin (262) in 9.4% yield. Corrole (264) was also obtained in ~1% yield. The cyclization of (256) and (261) however, produced corrole (265) as the major product in 22.2% yield. The expected porphyrin (263), was obtained in 5.3% yield (Scheme 67). For comparison, porphyrin (266) has been prepared by this procedure in 33% yield.[174] Unfortunately, the authors did not comment on whether or not corrole was also formed in this condensation. In our other porphryrin syntheses, only

trace amounts of corrole were observed. It is well known however, that biladienes cyclize with base to give corroles.[173] Therefore, we thought that basic nature of compound (261) may have been partially responsible for the substantial amount of corrole formed during the cyclization of (265). The higher yields obtained with tetrapyrrole (256) also suggested that during the cyclizations, this compound adopted a helical conformation more readily than tetrapyrrole (255). This could potentially result in greater amounts of corrole being formed in the case of (256). The lower yields generally obtained in the pyridyl porphryin syntheses, also suggested that (261) was not as reactive as aldehydes (6) or (258). This may allow greater opportunity for corrole formation to occur as well. It is also plausible that a combination of these situations is leading to the observed results. We then decided to carry out the reactions using excess hydrogen bromide, as compared to (261), in an attempt to suppress the corrole formation. The cyclization of compounds (255) and (261), in the presence of excess hydrogen bromide (~1.2 equiv), afforded porphyrin (262) in 2.5% yield. Corrole (264) was also obtained in 1.5-2% yield. The condensation of compounds (256) and (261) under the same conditions, led to porphyrin (263) in 3.2% yield and corrole (265) in 9.1% yield. The cyclization of (256) and (261) under nitrogen, in the presence of excess hydrogen bromide, afforded trace amounts of porphyrin (263) and corrole (265) in 12.1% yield. Overall, the additional acid was detrimental to the production of the pyridyl porphyrins and only slightly increased the yield of corrole (264). The results of the condensation reactions are summarized in Table 2. We did not attempt to prepare the corroles through the use of standard, base-catalyzed cyclization techniques.[173] However, these conditions would likely increase the yields of corrole. It is interesting to note that fair yields of corrole were recently obtained, when fully substituted biladienes were cyclized under acidic conditions.[175]




Scheme 67. Synthesis of Porphyrins (262), (263) and Corroles (264), (265)

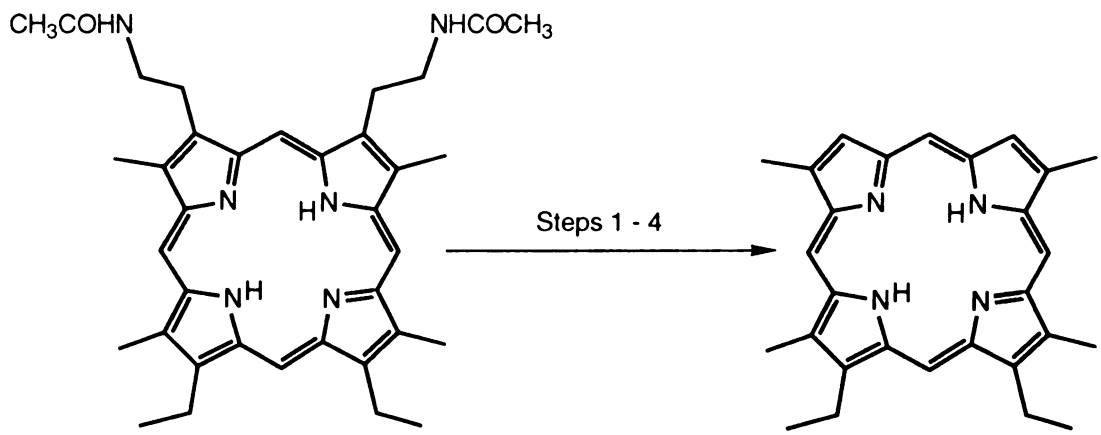


266

We have demonstrated that it was possible to synthesize of variety of β-unsubstituted porphyrins, through utilization of tetrapyrroles (255) and (256). Of the compounds described above, only porphyrin (259) was found to be reported in the literature. Porphyrin (267) was transformed in four steps, to porphyrin (259) (Scheme 68). The overall yield for this sequence was 22%.[176] We were able to obtain porphyrin (259) in 26.4% yield, using a one-step procedure. Also, the successful synthesis of porphyrin (250) should allow reinvestigations into the preparation of novel chlorin compounds such as (252). It may also be possible to synthesize chlorin (268), through the utilization of porphyrin (263). Even though the yields of porphyrin are low in some instances, the availability of the starting materials makes this a viable method for the synthesis of these compounds. Alternate approaches to these compounds would likely be more difficult, and could also lead to porphyrin in low yield. The information gathered in this study should be useful in the preparation of other β-unsubstituted porphyrins and interesting porphyrinoid structures.

Table 2. Yield Comparisons Between Compounds (233), (235), (260) and (262-265)

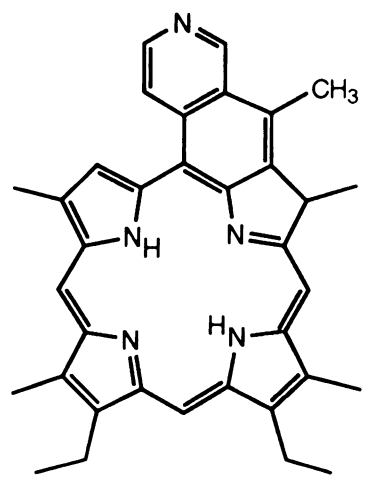
| Compound | Acid Amount (relative to ald.) | Reaction Atmosphere | Yield (%) |
|---|---|---|---|
| 233 | trace | Air | 23.2 |
| 250 | trace | Air | 38.0 |
| 235 | trace | Air | 5.0 |
| 260 | trace | Air | 26.4 |
| 262 | trace | Air | 9.4 |
| 262 | excess | Air | 2.5 |
| 263 | trace | Air | 5.3 |
| 263 | excess | Air | 3.2 |
| 263 | excess | Nitrogen | trace |
| 264 | trace | Air | 1.1 |
| 264 | excess | Air | 1.5-2.0 |
| 265 | trace | Air | 22.2 |
| 265 | excess | Air | 9.1 |
| 265 | excess | Nitrogen | 12.1 |




267 259

1. HCl, sealed tube, 100°C; 80%
2a. NaOH, dimethyl sulfate
b. Methanolic potash; 68%
3. Pyridine, ferrous sulfate solution (aqueous), acetic acid, 80°C; 65%
4a. Resorcinol melt, 190-210°C
b. Pyridine, ferrous sulfate solution (HCl), acetic acid, 80°C; 67%

Scheme 68. Alternate Literature Preparation of Porphyrin (259)




268

## III. Experimental

3-Methylpyrrole-2-carboxaldehyde (254)

4-Methylpyridine-N-oxide (2.0 g; 1.83 x $10^{-2}$ moles) and copper(II) sulfate pentahydrate (45.8 g; 1.83 x $10^{-1}$ moles) were dissolved in water (800 ml; HPLC grade) and placed in an irradiation vessel. The solution was purged with nitrogen for fifteen minutes and the vessel sealed. The mixture was then irradiated for 24 hours using a medium pressure mercury vapor lamp (450 W). At the end of this time the solution was saturated with sodium chloride and extracted with diethyl ether (6 x 100 ml). The ether layers were combined and the solvent removed under reduced pressure. The residue was chromatographed over silica, eluting with dichloromethane, to give the title compound as a pale yellow solid (456 mg; 22.8%); mp 90-91.5°C (lit mp 90-92°C[177]). $^1$H NMR ($CDCl_3$): $\delta$ 2.36 (3H, s, $CH_3$), 6.10 (1H, m, 4-H), 7.03 (1H, m, 5-H), 9.58 (1H, d, CHO), 10.32 (1H, br, NH). EI MS: m/e (Relative Intensity) 190.0 (97.06), 108.0 (100.00), 79.8 (96.22), 53.0 (78.57).

1,19-Dideoxy-8,12-diethyl-7,13-dimethylbiladiene-a,c-dihydrobromide (255)

Hydrobromic acid (3.0 ml, 48%) was added to a solution of 3,3'-diethyl-4,4'-dimethyl-2,2'-dipyrrylmethane (119) (508 mg; 2.20 x $10^{-3}$ moles) and 2-formylpyrrole (253)[178] (419 mg; 4.41 x $10^{-3}$ moles), in methanol (40 ml). The mixture was heated on a steam bath for five minutes, cooled to room temperature and then placed in the refrigerator for two hours. The salt was collected, washed with methanol containing a little hydrobromic acid and then with ether. The resulting product was vacuumed dried to give the title compound as a green metallic solid (943 mg; 78.3%); mp >300°C. $^1$H NMR ($CDCl_3$/ DMSO-$d_6$): $\delta$ 0.90 (6H, t, 2 x $CH_2C\underline{H_3}$), 1.93 (6H, s, 2 x $CH_3$), 2.30 (4H, q, 2 x

$C\underline{H_2}CH_3$), 5.30 (2H, s, 2 x 10-H), 6.14-6.17 (2H, m, 3 and 17-H) 6.39 (2H, s, 5 and 15-H), 6.49-6.52 (2H, m, 2 and 18-H), 6.88-6.91 (2H, m, 1 and 19-H) 10.26 (2H, s), 11.24 (2H, s) (4 x NH).

1,19-Dideoxy-8,12-diethyl-3,7,13,17,-tetramethyl-biladiene-a,c-dihydrobromide (256)

Compound (256) was prepared from 3-methylpyrrole-2-carboxaldehyde (254) (400 mg; 3.67 x $10^{-3}$ moles) and 3,3'-diethyl-4,4'-dimethy-2,2'-dipyrrylmethane (119) (422 mg; 1.83 x $10^{-3}$ moles), by the method described above. The title compound was obtained as a red powder (905 mg, 86.0%); mp 273-274°C with decomposition. $^1$H NMR ($CDCl_3$/DMSO-$d_6$): δ 0.57 (6H, t, 2 x $CH_2C\underline{H_3}$), 1.64 (6H, s), 1.68 (6H, s) (4 x $CH_3$), 1.98 (4H, q, 2 x $C\underline{H_2}CH_3$), 4.97 (2H, s, 2 x 10-H), 5.62-5.66 (2H, m, 2 and 18-H), 5.96 (2H, s, 5 and 15-H), 6.54-6.58 (2H, m, 1 and 19-H), 9.99 (2H, s), 10.67 (2H, s) (4 x NH).

5-(Phenyl)-13,17-diethyl-12,18-dimethylporphyrin (233)

1,19-Dideoxy-8,12-diethyl-7,13-dimethylbiladiene-a,c-dihydrobromide (255) (205 mg; 3.75 x $10^{-4}$ moles) and benzaldehyde (6) (398 mg; 3.75 x $10^{-3}$ moles) were suspended in methanol (70 ml). A solution of hydrogen bromide in acetic acid (30%; 8 drops) was added and the mixture refluxed for 22 hours. The solution was allowed to cool to room temperature and dichloromethane (100 ml) was added. The mixture was washed with water (100 ml), saturated aqueous sodium bicarbonate solution (100 ml) and water (100 ml). The organic layer was dried over sodium sulfate and the solvent removed under reduced pressure. The residue was chromatographed over silica, eluting with dichloromethane, and recrystallized from dichloromethane/hexane to give the title porphyrin as a purple solid (41 mg; 23.2%); mp >300°C. $^1$H NMR ($CDCl_3$):

δ -3.40 (2H, br, 2 x NH), 1.87 (6H, t, 2 x $CH_2C\underline{H}_3$), 3.61 (6H, s, 2 x $CH_3$), 4.06 (4H, q, 2 x $C\underline{H}_2CH_3$) 7.74-7.80 (3H, m, 3,4,5 phenyl-H) 8.22-8.28 (2H, m, 2,6 phenyl-H), 9.00 (2H, d), 9.30 (2H, d) (4 x pyrrolic-H), 10.01 (1H s), 10.15 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 401.5 (5.34), 499.0 (4.20), 531 (3.59), 569.0 (3.77), 622.0 (2.96). EI MS: m/e (Relative Intensity) 470.3 (100.00), 455.3 (27.1), 235.3 (28.55).

13,17-Diethyl-12,18-dimethylporphyrin (235)

Compound (235) was prepared from 1,19-dideoxy-8,12-diethyl-7,13-dimethylbiladiene-a,c-dihydrobromide (255) (210 mg; 3.84 x $10^{-4}$ moles) and aqueous formaldehyde (258) (37%, 10 ml), by the procedure detailed above. The crude product was columned on silica, eluting with dichloromethane. The fractions containing porphyrin were combined and further purified by preparative TLC, using dichloromethane. The resulting solid was recrystallized from dichloromethane/hexane to give the title compound as a purple solid (7.6 mg; 5.0%); mp 232-235°C (rapid heating). Replacement of aqueous formaldehyde with trimethyl orthoformate produced similar results. $^1$H NMR ($CDCl_3$): δ -3.83 (2H, br, 2 x NH), 1.87 (6H, t, 2 x $CH_2C\underline{H}_3$), 3.62 (6H, s, 2 x $CH_3$), 4.09 (4H, q, 2 x $C\underline{H}_2CH_3$), 9.37-9.48 (4H, m, 4 x pyrrolic-H), 10.09 (1H, s), 10.17 (2H, s), 10.25 (1H, s), (4 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 394.5 (5.20), 493.5 (4.07), 524.0 (3.68), 562.5 (3.68), 614.0 (3.21). EI MS: m/e (Relative Intensity) 394.1 (100.00), 379.1 (40.42).

5-(Phenyl)-13,17-diethyl-2,8,12,18-tetramethylporphyrin (250)

1,19-Dideoxy-8,12-diethyl-3,7,13,17-tetramethylbiladiene-a,c-dihydrobromide (256) (233.5 mg; 4.06 x $10^{-4}$ moles) and benzaldehyde (6) (431 mg; 4.06 x $10^{-3}$ moles) were suspended in methanol (250 ml). A solution

of hydrogen bromide in acetic acid (30%; 8 drops), was added and the mixture refluxed for 22 hours. The solution was allowed to cool to room temperature and dichloromethane (150 ml) was added. The mixture was washed with water (150 ml), saturated aqueous sodium bicarbonate solution (150 ml) and water (150 ml). A saturated methanolic solution of zinc acetate (15 ml) was added to the organic layer, the resulting mixture shaken for five minutes, and the solvent removed. The crude product was then columned on silica, eluting with dichloromethane, to give the purified zinc complex of the title compound. The zinc porphyrin was dissolved in dichloromethane (200 ml) and washed with a 5% hydrochloric acid solution (200 ml), water (200 ml), a saturated sodium bicarbonate solution (200 ml) and water (200 ml). The solvent was removed and the resulting solid recrystallized from dichloromethane/methanol to give the desired compound as a purple metallic solid (77 mg; 38.0%); mp >300°C. $^1$H NMR ($CDCl_3$): δ -3.48 (2H, br, 2 x NH), 1.87 (6H, t, 2 x $CH_2\underline{CH_3}$), 3.62 (6H, s), 3.64 (6H, s), (4 x $CH_3$) 4.07 (4H, q, 2 x $C\underline{H_2}CH_3$), 7.71-7.79 (3H, m, 3,4,5 phenyl-H), 8.13-8.23 (2H, m, 2,6 phenyl-H), 8.59-8.66 (2H, m, 2 x pyrrolic-H), 10.02 (1H, s), 10.11 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 404.0 (5.33), 501.5 (4.22), 533.0 (3.76), 571.0 (3.83), 625.0 (3.34). EI MS: m/e (Relative Intensity) 498.3 (100.00), 483.2 (24.36).

13,17-Diethyl-2,8,12,18-tetramethylporphyrin (259)

Porphyrin (259) was prepared from 1,19-dideoxy-8,12-diethyl-3,7,13,17-tetramethylbiladiene-a,c-dihydrobromide (256) (154.2 mg; 2.68 x $10^{-4}$ moles) and aqueous formaldehyde (258) (37%, 7 ml), by the procedure detailed above. The crude product was columned on silica, eluting with dichloromethane. The resulting solid was recrystallized from dichloromethane/hexane to give the title compound as a purple solid (29.8 mg; 26.4%); mp >300°C (lit mp 318-

322°C[176]). $^1$H NMR ($CDCl_3$): δ -3.85 (2H, s, 2 x NH), 1.86 (6H, t, 2 x $CH_2C\underline{H}_3$), 3.62 (6H, s, 12,18-$CH_3$), 3.74 (6H, d, 2,8-$CH_3$), 4.09 (4H, q, 2 x $C\underline{H}_2CH_3$), 9.04-9.08 (2H, m, 2 x pyrrolic-H), 9.96 (1H, s), 10.10 (1H, s), 10.11 (2H, s) (4 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 396.5 (5.24), 495.0 (4.15), 528.5 (3.94), 565.0 (3.80), 617.5 (3.60). EI MS: m/e (Relative Intensity) 422.0 (46.12), 407.1 (14.56).

5-(4'-Pyridyl)-13,17-diethyl-12,18-dimethylporphyrin (262) and 8,12-diethyl 13,17-dimethylcorrole (264)

The title compounds were prepared from 1,19-dideoxy-8,12-diethyl-7,13-dimethylbiladiene-a,c-dihydrobromide (255) (197 mg; 3.61 x $10^{-4}$ moles) and 4-pyridinecarboxaldehyde (261) (386 mg; 3.61 x $10^{-3}$ moles), by the procedure detailed above. The crude product was columned over silica, eluting with dichloromethane. A small amount of 8,12-diethyl-13,17-dimethylcorrole (264) (~1%) eluted first. $^1$H NMR ($CDCl_3$): δ -2.50 (3H, br, 3 x NH), 1.72 (6H, t, 2 x $CH_2C\underline{H}_3$), 3.37 (6H, s, 2 x $CH_3$), 3.85 (4H, q, 2 x $C\underline{H}_2CH_3$), 8.68 (2H, d), 8.82 (2H, d) (4 x pyrrolic-H) 9.13 (1H, s), 9.37 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 385.0 (4.73), 401.0 (4.78), 527.5 (3.96), 542.0 (4.01), 565.0 (3.82), 583.0 (3.96). EI MS: m/e (Relative Intensity) 382.1 (100.00), 367.0 (32.83). Elution was then continued using a mixture of dichloromethane/ethyl acetate (90/10). The fractions containing porphyrin were combined and further purified by preparative TLC, using a mixture of chloroform/ethyl acetate (90/10). The resulting solid was recrystallized from dichloromethane/hexane to give the desired compound (15) as a purple solid (16 mg; 9.4%); mp >300°C. $^1$H NMR ($CDCl_3$): δ -3.50 (2H, br, 2 x NH), 1.88 (6H, t, 2 x $CH_2C\underline{H}_3$), 3.61 (6H, s, 2 x $CH_3$),4.07 (4H, q, 2 x $C\underline{H}_2CH_3$), 8.19 (2H, d), 9.04 (2H, d) (4 x pyridyl-H), 8.96 (2H, d), 9.34 (2H, d) (4 x pyrrolic-H), 10.05 (1H, s), 10.18 (2H, s) (3 x meso-H).

UV/visible ($CH_2Cl_2$): λ max ($log_{10}\varepsilon$) 401.5 (5.32), 499.5 (4.18), 531.0 (3.66), 569.0 (3.77), 621.5 (3.18). EI MS: m/e (Relative Intensity) 471.3 (100.00), 456.3 (29.29), 235.8 (28.70). Increasing the acid concentration (from 8-10 drops to 1.5 ml) decreased the amount of pyridyl porphyrin (262) (~2.5%) and slightly increased the amount of corrole (264) (~1.5-2%).

5-(4'-Pyridyl)-13,17-diethyl-2,8,12,18-tetramethylporphyrin (263) and 8,12-diethyl-3,7,13,17-tetramethylcorrole (265)

The title compounds were prepared from 1,19-dideoxy-8,12-diethyl-3,7,13,17-tetramethylbiladiene-a,c-dihydrobromide (256) and 4-pyridinecarboxaldehyde (261), by the procedure detailed above. Two separate runs (116.6 mg of (256), 217 mg of (261) and 8 drops of HBr solution; 166.2 mg of (256), 308 mg of (261) and 10 drops of HBr solution) were combined and subjected to chromatography over silica. The faster moving corrole was eluted using dichloromethane. The resulting solid was recrystallized from dichloromethane/hexane to give compound (265) as a fluffy purple solid (44.7 mg; 22.2%); mp 249-251°C. $^1H$ NMR ($CDCl_3$): δ -3.05 (3H, br, 3 x NH), 1.74 (6H, t, 2 x $CH_2C\underline{H}_3$), 3.42 (6H, s), 3.47 (6H, s) (4 x $CH_3$), 3.89 (4H, q, 2 x $C\underline{H}_2CH_3$), 8.63 (2H, s, 2 x pyrrolic-H), 9.24 (1H, s), 9.37 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): λ max (log ε) 391.5 (5.09), 404.5 (5.10), 532.0 (4.28), 589.5 (4.17). EI MS: m/e (Relative Intensity) 410.0 (100.00), 395.0 (22.03), 205.2 (28.70). Elution was then continued with a mixture of dichloromethane/ethyl acetate (90/10). The resulting product was recrystallized from dichloromethane/hexane to give pyridyl porphyrin (263) as a metallic purple solid (12.9 mg; 5.3%); mp >300°C. $^1H$ NMR ($CDCl_3$): δ -3.54 (2H, br, 2 x NH), 1.87 (6H, t, 2 x $CH_2C\underline{H}_3$), 3.62 (6H, s), 3.65 (6H, m) (4 x $CH_3$), 4.07 (4H, q, 2 x $C\underline{H}_2CH_3$), 8.14 (2H, d), 9.01 (2H, d) (4 x pyridyl-H), 8.59 (2H, d, 2 x pyrrolic-

H), 10.06 (1H, s), 10.14 (2H, s) (3 x meso-H). UV/visible ($CH_2Cl_2$): $\lambda$ max (log $\varepsilon$) 404.0 (5.25), 501.5 (4.16), 534.5 (3.76), 572.0 (3.77), 625.0 (3.41). EI MS: m/e (Relative Intensity) 499.0 (100.00), 484.0 (21.77), 249.8 (22.14). Increasing the acid concentration (from 8-10 drops to 1.5 ml) decreased the amount of pyridyl porphyrin (262) (3.2%) and corrole (265) (9.1%).

## REFERENCES


1. Rothemund, P. *J. Am. Chem. Soc.* **1935**, 57, 2010.
2. Adler, A.D.; Longo, F.R.; Finarelli, J.D.; Goldmacher, J.; Assour, J.; Korsakoff, L. *J. Org. Chem.* **1967**, 32, 476.
3. Lindsey, J.S.; Schreiman, I.C.; Hsu, H.C.; Kearney, P.C.; Marguerettaz, A.M. *J. Org. Chem.* **1987**, *52*, 827.
4. Lindsey, J.S.; Wagner, R.W. *J. Org. Chem.* **1989**, *54*, 828.
5. Smith, K.M. *Porphyrins and Metalloporphyrins*, K.M. Smith, Ed.; Elsevier: Amsterdam, 1975, p *33*.
6. Fischer, H.; Friedrich, H.; Lamatsch, W.; Morgenroth, K. *Ann.* **1927**, *459*, 53.
7. Fischer, H.; Orth, H. *Die Chemie des Pyrrols*, Akadamische Verlag: Leipzig, 1937, Vol II.
8. Arsenault, G.P.; Bullock, E.; MacDonald, S.F. *J. Am. Chem. Soc.* **1960**, *82*, 4384.
9. Ogashi, H.; Sugimoto, H.; Nishiguchi, T.; Watanabe, T.; Matsuda, Y.; Yoshida, Z. *Chem. Lett.* **1978**, 29.
10. Gunter, M.J.; Mander, L.N. *J. Org. Chem.* **1981**, *46*, 4792.
11. Manka, J.S.; Lawrence, D.S. *Tetrahedron Lett.* **1989**, *30*, 6989.
12. Osuka, A.; Nagata, T.; Kobayashi, F.; Maruyama, K. *J. Heterocyclic Chem.* **1990**, *27*, 1657.
13. Furusho, Y.; Kimura, T.; Mizuno, Y.; Aida, T. *J. Am. Chem. Soc.* **1997**, *119*, 5267.
14. Broadhurst, M.J.; Grig, R.; Johnson, A.W. *J. Chem. Soc. (C)* **1971**, 3681.
15. Boudif, A.; Momenteau, M. *J. Chem. Soc. Chem. Commun.* **1994**, 2069.
16. Chandrasekar, P.; Lash, T.D. *Tetrahedron Lett.* **1996**, *37*, 4873.
17. Berlin, K.; Steinbeck, C.; Breitmaier, E. *Synthesis* **1996**, 336.
18. Berlin, K.; Breitmaier, E. *Agnew. Chem. Int. Ed. Engl.* **1994**, *33*, 1246.
19. Lash, T.D. *Agnew. Chem. Int. Ed. Engl.* **1995**, *34*, 2533. Lash, T.D.; Chaney, S.T. *Chem. Eur. J.* **1996**, *2*, 944.

20. Nguyen, L.T.; Senge, M.O.; Smith, K.M. *J. Org. Chem.* **1996**, *61*, 998.

21. Kai, S.; Suzuki, M.; Masaki, Y. *Tetrahedron Lett.* **1998**, *39*, 4063.

22. Jackson, A.H.; Kenner, G.W.; Smith, K.M. *J. Chem. Soc.* **1971**, 502.

23. Baptista de Almeida, J.A.P.; Kenner, G.W.; Rimmer, R.; Smith, K.M. *Tetrahedron* **1976**, *32*, 1793.

24. Johnson, A.W.; Kay, I.T. *J. Chem. Soc.* **1965**, 1620.

25. Harris, D.; Johnson, A.W.; Gaete-Holmes, R. *Bioorganic Chemistry* **1980**, 63.

26. Collman, J.P.; Zhang, X.M.; Wong, K.; Brauman, J.I. *J. Am. Chem. Soc.* **1994**, *116*, 6245. Johnson, M.R.; Seok, W.K.; Ibers, J.A. *J. Am. Chem. Soc.* **1991**, *113*, 3998. Momenteau, M.; Loock, B.; Tetreau, C.; Lavalette, D.; Croisy, A.; Schaeffer, C.; Huel, C.; LHoste, J.M. *J. Chem. Soc., Perkin Trans. 2* **1987**, 249.

27. Kirby, J.P.; Roberts, J.A.; Nocera, D.G. *J. Am. Chem. Soc.* **1997**, *119*, 9230. Seth, J.; Palaniappan, V.; Johnson, T.E.; Prathapan, S.; Lindsey, J.S.; Bocian, D.F. *J. Am. Chem. Soc.* **1994**, *116*, 10578. Lindsey, J.S.; Maccrum, K.A.; Tyhonas, J.S.; Chuang, Y.Y. *J. Org. Chem.* **1994**, *59*, 579. Wasieleski, M.R. *Chem. Rev.* **1992**, *92*, 435.

28. Mitzutani, T.; Murakami, T.; Kurahashi, T.; Ogoshi, H.; *J. Org. Chem.* **1996**, *61*, 539. BonarLaw, R.P.; *J. Am. Chem. Soc.* **1995**, *117*, 12397. BonarLaw, R.P.; Sanders, J.K.M. *J. Am. Chem. Soc.* **1995**, *117*, 259. Konishi, K.; Yahara, K.; Toshishige, H.; Aidia, T.; Inove, S. *J. Am. Chem. Soc.* **1994**, *116*, 1337.

29. Liang, Y.; Chang, C.K.; Peng, S.M. *J. Mol. Recognit.* **1996**, *9*,149. Kuroda, Y.; Kato, Y.; Higashioji, T.; Hasegawa, J.; Kawanami, S. Takachashi, M.; Shiraishi, N.; Tanabe, K.; Ogoshi, H. *J. Am. Chem. Soc.* **1995**, *117*, 10950. Bonarlaw, R.P.; Mackay, L.G.; Walter, L.J.; Maravaud, V.; Sanders, J.K.M. *Pure Appl. Chem.* **1994**, *66*, 803. Mizutani, T.; Ema, T.; Yoshida, T.; Kuroda, Y.; Ogoshi, H. *Inorg. Chem.* **1993**, *32*, 2072.

30. Philp, D.; Stoddart, J.F. *Agnew. Chem., Int. Ed. Engl.* **1996**, *35*, 1155. Drain, C.M.; Russell, K.C.; Lehn, J.M. *Chem. Comm.* **1996**, 337. McCallien, D.W.J.; Sanders, J.K.M. *J. Am. Chem. Soc.* **1995**, *117*, 6611. Lipskier, J.F.; Tranthi, T.H. *Inorg. Chem.* **1993**, *32*, 722.

31. Pandey, R.K.; Leung, S.H.; Smith. K.M. *Tetrahedron Lett.* **1994**, *35*. 8093. Clezy, P.S. *Aust. J. Chem.* **1991**, *44*, 1163. Clezy, P.S.; Vanphuc, L.; Prashar, J.K. *Aust. J. Chem.* **1991**, *44*, 1061.

32. Spikes, J. *The Science of Photobiology*; K.C. Smith, Ed.; Plenum Press: New York, 1989, p 79.

33. Henderson, B.W.; Dougherty, T.J. *Photochem. Photobiol.* **1992**, *55*, 145.

34. Henderson, W.W.; Fingar, V.H. *Cancer Res.* **1987**, *47*, 3110.

35. Van Lier, J.E. *Photodynamic Therapy of Neoplastic Diseases*; D. Kessel, Ed.; CRC Press: Boca Raton, 1990, Vol. 1; pp 279-291.

36. Hamblin, M.R.; Newman, E.L. *J. Photochem. Photobiol, B: Biol.* **1994**, *23*, 3.

37. Jori, G.; Reddi,E.; Salvalto, B.; Pagnam, A.J.; Ziron, L. *Cancer Lett.* **1984**, *24*, 291. Norato, G.; Conti, G.; Ricci, L.; Nicolin, A.; Catapano, A.L. *Cancer Lett.* **1984**, *25*, 203.

38. Lipson, R.L.; Baldes, E.J.; Olsen, A.M. *J. Natl. Cancer Inst.* **1961**, *26*, 1.

39. Simons, I.; McDonagh, D.; Wilson , D.S.; Granelli, L.; Nielson, S.; Jaenicke, R. Lancett, ii, 1972, 1175. Dougherty, T.J. *J. Natl. Cancer Inst.* **1974**, *51*, 1333. Daugherty, T.J.; Lawrence, G.; Kaufman, J.H.; Boyle, D.; Weishaupt,K.R.; Goldfarb, A.J. *J. Natl. Cancer Inst.* **1979**, *62*, 231.

40. Bellineier, D.A.; Ho, Y.-H.; Pandey, R.K.; Missert, J.R.; Daugherty, T.J. *Photochem. Photobiol.* **1989**, *50*, 221.

41. Mironov, A.; Nizhnik, A.; Nochel, A. *Photochem. Photobiol.* **1990**, *4*, 297.

42. Morris, I.K.; Ward, A.D. *Tetrahedron Lett.* **1988**, *29*, 2501.

43. Pandey, R.K.; Dougherty, T.J. *Cancer Res.* **1989**, *49*, 2042.

44. Byrne, C.J.; Marshallsay, L.V.; Ward, A.D. *J. Photochem. Photobiol, B: Biol.* **1990**, *6*, 13.

45. Hueger, B.F.; Lawter, J.R.; Waringrekar, V.H.; Cucolo, M.C. United States Patent No. 5059619, 1991.

46. Cincotta, L.; Foley, J.W.; Cincotta, A.H. *Photochem. Photobiol.* **1987**, *46*, 751.

47. Potter, W.R.; In *Proceedings of SPIE* **1989**, *1065*, 88.

48. Pandey, R.K.; Dougherty, T.J. *Photochem. Photobiol.* **1988**, *47*, 769.

49. Kessel, D.; Dougherty, T.J.; Truscott, T.G. *Photochem. Photobiol.* **1988**, *47*, 741.

50. Kessel, D.; Chang, C.K.; Henderson, B.W.; *J. Photochem. Photobiol. B: Biol.* **1993**, *18*, 177.

51. Olenick, N.L.; Antunez, A.R.; Clay, M.E.; Rihter, B.D.; Kenney, M.E. *Photochem. Photobiol.* **1993**, *57*, 242.

52. Daziano, J.P.; Humeau, L.; Henry, M.; Mannoni, P.; Chanon, M.; Chabannon, C.; Julliard, M. *J. Photochem. Photobiol. B: Biol.* **1998**, *43*, 128.

53. Firey, P.A.; Rodgers, M.A.J. *Photochem. Photobiol.* **1987**, *45*, 535.

54. Dougherty, T.G. *Advances in Photochemistry*, John Wiley and Sons: New York, 1992, Vol. 17; p 275.

55. Wohrle, D.; Shopova, M.; Muller, S.; Mileu, A.D.; Mantareva, V.N.; Krasteu, K.K.; *J. Photochem. Photobiol. B: Biol.* **1993**, *21*, 155. Shopova, M.; Wohrle, D.; Stoichkova, N.; Mileu, A.; Mantareva, V.; Muller, S.; Kassabov, K.; Georgieu, K. *J. Photochem. Photobiol. B: Biol.* **1994**, *23*, 35.

56. Kostenich, G.A.; Zhuravkin, I.N.; Zhavrid, E.A. *J. Photochem. Photobiol. B: Biol.* **1994**, *22*, 211.

57. Nelson, J.S.; Roberts, W.G.; Berns, M.W. *Cancer Res.* **1987**, *47*, 4681. Roberts, W.G.; Smith, K.M.; McCullough, J.L.; Berns, M.W. *Photochem. Photobiol.* **1989**, *49*, 431. Gomer, C.J.; Ferrario, A. *Cancer Res.* **1990**, 50, 3985. Orenstein, A.; Kostenich, G.; Roitman, L.; Shechtman, Y.; Kopolovic, Y.; Ehrenberg, B.; Malik, Z. *Br. J. Cancer* **1996**, *73*, 937.

58. Song, L.M.W.K.; Wang, K.K.; Zinsmeister, A.R. *Cancer* **1993**, *82*, 421.

59. Richter, A.M.; Waterfield, E.; Jain, A.K.; Sternberg, E.D.; Dolphin, D. *Photochem. Photobiol.* **1990**, *52*, 495.

60. Richter, A.M.; Waterfield, E.; Jain, A.K.; Allison, B.; Sternberg, E.D.; Dolphin, D.; Levy, J.G. *Br. J. Cancer* **1991**, *63*, 87.

61. Trauner, K.B.; Gandour-Edwards, R.; Bomberg, M.; Shortkroff, S.; Sledge, C.; Hansan, T. *Photochem. Photobiol* **1998**, *67*, 133.

62. Bonnett, R.; White, R.D.; Winfield, U.J.; Berenbaum, M.C. *Biochem. J.* **1989**, *261*, 277.

63. Berenbaum, M.C.; Bonnett, R.; Chevretton, E.B.; Akande-Adebakin, S.L.; Ruston, M. *Lasers Med. Sci.* **1993**, *8*, 235.

64. Ris, H.-B.; Altermatt, H.J.; Nachbur, B.; Stewart, J.C.M.; Wang, Q.; Lim, C.K.; Bonnett, R.; Althaus, U. *Int. J. Cancer.* **1993**, *53*, 141.

65. Ris, H.-B.; Altermatt, H.J.; Nachbur, B.; Stewart, J.C.M.; Wang, Q.; Lim, C.K.; Bonnett, R.; Althaus, U. *Lasers Surg. Med.* **1996**, *18*, 39.

66. Dilkes, M.G.; DeJode, M.L.; Rowntree-Taylor, A.; McGilligan, J.A.; Kenyon, G.S.; McKelvie, P. *Lasers Med. Sci.* **1997**, *11*, 23.

67. Fon, K.F.M.; Hopper, C.; Speight, P.M.; Buonaccorsi, G.A.; Brown, S.G. *Int. J. Cancer.* **1997**, *73*, 25.

68. Morgan, A.R.; Garbo, G.M.; Keck, R.W.; Eriksen, L.D.; Selman, S.H. *Photochem. Photobiol.* **1990**, *51*, 1990.

69. Kaplan, M.J.; Somers, R.G.; Greenberg, R.H.; Ackler, J. *J. Surg. Oncol.* **1998**, *67*, 121.

70. Allison, R.R.; Mang, T.S.; Wilson, B.D.; Vongtama, V. *Curr. Ther. Res. Clin. E.* **1998**, *59*, 23.

71. Kessel, D.; Morgan, A. *Photochem. Photobiol.* **1993**, *58*, 521.

72. Sessler, J.L.; Cyr, M.; Burrel, A.K. *Tetrahedron* **1992**, *48*, 9661.

73. Sessler, J.L.; Hemmi, G.; Mody, T.D.; Murai, T.; Burrell, A.; Young, S.W. *Acc. Chem. Res.* **1994**, *27*, 43.

74. Young, S.W.; Sidhu, M.K.; Fan, Q.; Muller, H.H.; Neuder, M.; Znassi, G.; Mody, T.D.; Hemmi, G.; Dow, W.; Mutch, J.D.; Sessler, J.L.; Miller, R.A. *Invest. Radiol.* **1994**, *29*, 330.

75. Young, S.W.; Woodburn, K.W.; Wright, M.; Mody, T.D; Fan, Q.; Sessler, J.L.; Dow, W.C.; Miller, R.A. *Photochem. Photobiol.* **1996**, *63*, 892.

76. Kostenich, G.; Orenstein, A.; Roitman, L.; Malik, Z.; Ehremberg, B. *J. Photochem. Photobiol, B: Biol.* **1997**, *39*, 36.

77. Wieman, T.J.; Fingar, V.; Tober, S.; Panjehpour, M.; Julius, C.; Panella, T.J.; Yuen, A.; Horning, S.; Woodburn, K.; Engel, J.; Miller, R.A.; Renschler, M.F.; Young; S.W. *Photochem. Photobiol.* **1996**, 80S.

78. Vogel, E.; Kocher, M.; Schmickler, H.; Lex, J. *Agnew. Chem. Int. Ed. Engl.* **1986**, *25*, 257.

79. Callot,.J.; Rohrer, A.; Tschamber, T.; Metz, B. *New J. Chem.* **1995**, *19*, 155.

80. Sessler, J.L.; Bruckner, E.A.; Weghorn, S.J.; Kisters, M.; Schafer, M.; Lex, J.; Vogel, E. *Agnew. Chem, Int. Ed. Engl.* **1994**, *33*, 2308.

81. Wu, Y.-D.; Chan, K.W.K.; Yip, C.-P.; Vogel, E.; Plattner, D.A.; Houk, K.N. *J. Org. Chem.* **1997**, *62*, 9240.

82. Braslausky, S.E.; Muller, Martire, D.O.; Porting, S.; Bertolotti, S.G.; Chakravorti, S.; Koc-Weier, G.; Knipp, B.; Schaffer, K. *J. Photochem. Photobiol., B: Biol.* **1997**, *40*, 191.

83. Schaffer, K.; Vogel, E.; Jori, G. Porphycenes as photodynamic therapy agents, in *Biological Effects of Light*, deGruyler: Berlin, 1994, pp 312-321. Segalla, A.; Fedelli, F.; Reddi, E.; Jori, G.; Cross, A. *Int. J. Cancer.* **1997**, *72*, 329.

84. Szeimies, R.-M.; Karrer, S.; Abels, C.; Steinbach, P.; Fickweiler, S.; Messmann, H.; Baumler, W.; Landthales, M. *J. Photochem. Photobiol., B: Biol.* **1996**, *34*, 67.

85. Richert, C.; Wessels, J.M.; Muller, M.; Kisters, M.; Benninghaus, T.; Goetz, A.E. *J. Med. Chem.* **1994**, *37*, 2792.

86. Abels, C.; Szeimies, R.-M.; Steinbach, P.; Richert, C.; Goetz, A.E. *J. Photochem. Photobiol., B: Biol.* **1997**, *40*, 305.

87. Nonell, S.; Bou, N.; Borrell, J.I.; Teindo, J.; Villanveva, A.; Juarranz, A.; Canete, M. *Tetrahedron Lett.* **1995**, *36*, 3405.

88. Cincotta, L.; Foley, J.W.; Cincotta, A.H. *Photochem. Photobiol.* **1987**, *46*, 751.

89. Becker, R.S.; Charkravorti, S.; Das, S. *Photochem. Photobiol.* **1990**, *51*, 533.

90. Fiedorowicz, M.; Galidino, J.R.; Julliard, M.; Mannoni, P.; Chanan, M. *Photochem. Photobiol.* **1993**, *58*, 356.

91. Ososoff, A.R.; Ohuoha, D.; Ara, G.; McAuliffe, D.; Foley, J.; Cincotta, L. *Proc. Natl. Acad. Sci. USA.* **1986**, *83*, 9729.

92. Kessel, D.; Woodburn, K.; Henderson, B.W.; Chang, C.K. *Photochem. Photobiol.* **1995**, *62*, 875.

93. Villanueva, A.; Caggiari, L.; Jori, G.; Milanesi, C. *J. Photochem Photobiol., B: Biol.* **1994**, *23*, 49.

94. Morgan, A.R.; Skalkos, D.; Maguire, G; Rampersaud, A.; Garbo, G.; Keck; Selman, S.H. *Photochem. Photobiol.* **1992**, 55, 136. Selman, S.H.; Hampton, J.jA.; Morgan, A.R.; Keck, R.W.; Balkony, D; Skalkos, D. *Photochem. Photobiol.* **1993**, *57*, 681.

95. Hampton, J.A.; Skalkos, D.; Taylor, P.M; Selman, S.H. *Photochem. Photobiol.* **1993**, *58*, 100.

96. Agius, L.; Ballantine, J.A.; Ferrito, V.; Jaccarini, V.; Murry-Rust, P.; Pelter, A.; Psailia, A.F.; Schembri, P.J. *J. Pure Appl. Chem.* **1979**, *51*, 1847.

97. Timkovich, R.; Cork, M.S.; Taylor, P.V. *J. Biol. Chem.* **1984**, *259*, 15089. Chang, C.K. *J. Biol. Chem.* **1985**, *260*, 9520.

98. Deeg, R.; Kriemler, H.-P.; Bergman, K.-H.; Muller, G. *Z. Physiol. Chem.* **1977**, *358*, 339. Imfeld, M.; Arigoni, D. Deeg, R.; Muller, G. *Viamin $B_{12}$*, Zagalak, B.J.; Friedrich, W., Eds.; deGruyter: Berlin, 1979, p 315.

99. Timkovitch, R.; Cork, M.S.; Gennis, R.B.; Johnson, P.Y. *J. Am. Chem. Soc.* **1985**, *107*, 6069.

100. Lemberg, R. ; Barret, J.; *Cytochromes* Academic Press: New York, 1973, pp 8-14 and 233-240.

101. Barret, J. *Biochem. J.* **1956**, *64*, 626.

102. Varva, M.R.; Timkovich, R. Yap, F.; Gennis, R.B. *Arch. Biochem. Biophys.* **1986**, *250*, 461.

103. Sotiriou, C.; Chang, C.K. *J. Am. Chem. Soc.* **1988**, *110*, 2264.

104. Guerin, E. M.S. thesis, Michigan State University, 1993.

105. Pandey, K.D.; Shiau, F.-Y.; Isaac, M; Ramaprasad, S.; Dougherty, T.J.; Smith, K.M. *Tetrahedron Lett.* **1992**, *33*, 7815.

106. Paine, J.B. III; Chang, C.K.; Dolphin, D. *Heterocycles* **1977**, *7*, 813.

107. Murshudov, G.N.; Grebenko, A.I.; Barynin, V.; Dauter, Z.; Wilson, K.S.; Vainshtein, B.K.; Melik-Adamyan, W.; Bravo, J.; Ferran, J.M.; Ferrer, J.C.; Switala, J.; Loewen, P.C.; Fita, I. *J. Biol. Chem.* **1996**, *271*, 8868.

108. Chiu, J.T.; Loewen, P.C.; Switala, J.; Gennis, R.B.; Timkovich, R. *J. Am. Chem. Soc* . **1989**, *111*, 7046. Bravo, J.; Verdaquer, N.; Tormo,J.; Betzel, C.; Switala, J.; Loeweu, P.C.; Fita, I. *Structure* **1995**, *3*, 491.

109. Clezy, P.S.; Fookes, C.J.R.; Hai, T.T. *Aust. J. Chem.* **1978**, *31*, 365.

110. Clezy, P.S.; Fookes, C.J.R. *J. Chem Soc. Chem. Commun.* **1975**, 707.

111. Clezy, P.S.; Liiepa, A.J. *Aust J. Chem* **1970**, *23*, 2477.

112. Anderson, L.A.; Sotiriou, C.; Chang C.K.; Loehr, T.M. *J. Am. Chem. Soc.* **1987**, *109*, 258.

113. Budzikiewics, H.; Djerassi, C.; Jackson, A.H.; Kenner, G.W.; Newman, D.J.; Wilson, J.M. *J. Chem. Soc.* **1964**, 1949.

114. Clezy, P.S.; Diakiw, V. *Aust. J. Chem.* **1973**, *26*, 2697.

115. Al-Hazimi, H.M.G.; Jackson, A.H.; Knight, D.W.; Lash, T.D. *J. Chem. Soc., Perkin Trans.1* **1978**, 26.

116. Piloty, O.; Blomer, A. *Chem. Ber.* **1912**, *45*, 3749.

117. Arciero, D.M.; Hooper, A.B. *J. Biol. Chem.* **1993**, *286*, 14645.

118. Prince, R.C.; Hooper, A.B. *Biochemistry* **1987**, *26*, 1970.

119. Hooper, A.B.; Terry, K.R. *Biochemistry* **1977**, *16*, 455.

120. Arciero, D.M.; Hooper, A.B.; Cai, M.; Timkovich, R. *Biochemistry* **1993**, *32*, 9370.

121. Fujisawa, T.; Mori, T.; Tsuge, S.; Sato, T. *Tetrahedron Lett.* **1983**, *24*, 1543.

122. Bax, A.; Davis, D.G. *J. Magn. Reson.* **1985**, *65*, 355.

123. Jackson, A.H.; Kenner, G.W.; Warburton, D. *J. Chem. Soc.* **1965**, 1328.

124. Booth, H.; Johnson, A.W.; Langdale-Smith, R.A. *J. Chem. Soc.* **1963**, 650.

125. Eisner, V.; Lichtarowicz, A.; Linstead, R.P. *J. Chem. Soc.* **1957**, 733.

126. Berner, E. *Acta Chem. Scand.* **1956**, *10*, 1208.

127. Stephaniak-Biniakiewicz, D. *Polish J. Chem.* **1980**, *54*, 1567.

128. Arnold, D.P.; Gaete-Holmes, R.; Johnson, A.W.; Smith, A.R.P.; Williams, G. *J. Chem. Soc., Perkin Trans. 1* **1978**, 1660.

129. Graca, M.; Vincente, H.; Smith, K.M. *J. Org. Chem.* **1991**, *56*, 4407.

130. Lee, S.W. Ph.D. Dissertation, Michigan State University, 1995.

131. Skalkos, D.; Hampton, J.A.; Heck, R.W.; Wagoner, M.; Selman, S.H. *Photochem. Photobiol.* **1994**, *59*, 175.

132. Whitlock, H.W.; Hanauer, R.; Oester, M.Y.; Bower, B.K. *J. Am. Chem. Soc.* **1969**, *91*, 7485.

133. Morgan, A.R.; Tertel, N.C. *J. Org. Chem.* **1986**, *51*, 1347.

134. Chang, C.K.; Sotiriou, C. *J. Org. Chem.* **1985**, *50*, 4989.

135. Morgan, A.R.; Gupta, S. *Tetrahedron Lett.* **1994**, *35*, 5347.

136. Kirby, J.P. Ph.D. Dissertation, Michigan State University, 1997.

137. Trippet, S.; Walker, D.M. *J. Chem. Soc* **1959**, 3874.

138. Matsuda, I.; Murata, S.; Ishii, Y. *J. Chem. Soc. Perkin Trans.1* **1979**, 26.

139. Ojima, I.; Kumagai, M.; Nagai, Y. *Tetrahedron Lett.* **1974**, 4005.

140. Olah, G.A.; Keumi, T. *Synthesis* **1979**, 112.

141. Deng, Y. Ph.D. Dissertation, Michigan State University, 1997.

142. Garigipati, R.A. *Tetrahedron Lett.* **1990**, *31*, 1969.

143. Levin, J.I.; Turos, Weinreb, S.M. *Syn. Commun.* **1982**, *12*, 989.

144. Kirby, J.P.; Van Dantzig, N.A.; Chang, C.K.; Nocera, D.G. *Tetrahedron Lett* **1995**, *36*, 3471.

145. Professor C.K. Chang research group, Hong Kong Universty of Science and Technology.

146. Furuta, H.; Asano, T.; Ogawa, T. *J. Am. Chem. Soc.* **1994**, *116*, 767.

147. Chmielewski, P.J.; Latos-Grazynski, L.; Rachelwicz, K.; Glowiak, T. *Agnew Chem. Int. Ed. Engl.* **1994**, *33*, 779.

148. Sessler, J.L.; Johnson, M.R.; Lynch, V. *J. Org. Chem.* **1987**, *52*, 4394.

149. Anderson, H.J.; Loader, C.E.; Foster, A. *Can. J. Chem.* **1980**, *58*, 2527.

150. Liu, B.Y.; Bruckner, C.; Dolphin, D. *J. Chem. Soc. Chem. Comm.* **1996**, 2141.

151. Professor Timothy D. Lash, Department of Chemistry, Illinois State University, personal communication.

152. Vogel, E. *Pure and Appl. Chem.* **1996**, *68*, 1355.

153. Professor Christian Bruckner, Department of Chemistry, University of Conneticut, personal communication.

154. Miyaura, N.; Ishiyama, T.; Sasaki, H.; Ishikaua, M.; Satoh, M.; Suzuki, A. *J. Am. Chem. Soc.* **1989**, *111*, 314.

155. Heo, P.Y.; Shin, K.; Lee, C.H. *Tetrahedron Lett.* **1996**, *37*, 197.

156. Heo, P.Y.; Lee, C.H. *Bull. Korean Chem. Soc.* **1996**, *17*, 778.

157. Lee, C.H.; Kim, H.J. *Tetrahedron Lett.* **1997**, *38*, 3935.

158. Kleinspehn, G. *J. Am. Chem. Soc.* **1955**, *77*, 1546.

159. Fischer, H.; Weiss, B.; Schubert, M. *Chem. Ber.* **1923**, *56*, 1194.

160. Motekaitis, R.J.; Heinert, D.H.; Martell, A.E. *J. Org. Chem.* **1970**, *35*, 2504.

161. Paine, J.B. III, Woodward, R., B.; Dolphin, D. *J. Org. Chem.* **1976**, *41*, 2826.

162. Lee, C.-H.; Lindsey, J.S. *Tetrahedron* **1994**, *50*, 11427.

163. Clezy, P.S.; Symthe, G.A. *Aust. J. Chem.* **1969**, *22*, 239.

164. Nagarkatti, J.P.; Ashley, K.R. *Synthesis* **1974**, 186.

165. Manka, J.S.; Lawrence, D.S. *Tetrahedron Lett.* **1989**, *30*, 6989.

166. Wallace, D.M.; Leung, S.H.; Senge, M.O.; Smith, K.M. *J. Org. Chem.* **1993**,*58*, 7245.

167. Mizutani, T.; Ema, T.; Tomita, T.; Kuroda, Y.; Ogoshi, H. *J. Am. Chem. Soc.* **1994**, *116*, 4240.

168. Clarke, O.J.; Boyle, R.W. *Tetrahedron Lett.* **1998**, *39*, 7176.

169. Faustino, M.A.; Neves, M.G.P.M.S.; Vicente, M.G.H.; Silva, A.M.S.; Calvaleiro, J.A.S. *Tetrahedron Lett.* **1995**, *36*, 5977.

170. Lash, T.D.; Hoehner, M.C. *J. Heterocycl. Chem.* **1991**, *28*, 1671.

171. Lash, T.D.; Balasubramaniam, R.P. *Tetrahedron Lett.* **1990**, *31*, 7545.

172. Osuka, A.; Maruyama, K. *Chem. Lett.* **1987**, 825.

173. Dolphin, D.; Harris, R.L.N.; Huppatz, J.L.; Johnson, A.W.; Kay, I.T. *J. Chem.Soc. (C)* **1966**, 30. Dolphin, D.; Johnson, A.W.; Leng, L.; van den Broek, P. *J. Chem. Soc.(C)* **1966**, 880.

174. Ming, L.; Yao, Y.-M.; Zou, J.-Z.; Xu, Z.; You, X.-Z. *Chinese J. Chem.* **1994**, *12*, 237.

175. Licoccia, S.; Di Vona, M.-C.; Paolesse, R. *J. Org. Chem.* **1998**, *63*, 3190.

176. Collier, G.C.; Jackson, A.H.; Kenner, G.W. *J. Chem. Soc.(C)* **1967**, 66. Burbidge, P.A.; Collier, G.C.; Jackson, A.H.; Kenner, G.W. *J. Chem. Soc.(C)* **1967**, 930.

177. Fischer, F.; Wiedemann, O. Z. *Physiol. Chem.* **1926**, *155*, 90.

178. Silverstein, R.M.; Ryskiewicz, E.E.; Willard, C. *Org. Synth. Coll. Vol IV*, John Wiley & Sons: New York, 1963, p 831.