This is to certify that the

dissertation entitled

SYNTHESIS AND MODIFICATION OF PORPHYRIN, PORPHYRINONE AND BENZOCHLORIN DERIVATIVES AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

presented by

Sangwan Lee

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Chemistry

Chi K. Chang
Major professor

Date 7 - 21 - 95

# SYNTHESIS AND MODIFICATION OF PORPHYRIN, PORPHYRINONE AND BENZOCHLORIN DERIVATIVES AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

By

Sangwan Lee

A DISSERTATION

Submitted to
Michigan State University
in Partial Fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

1995

# ABSTRACT

## SYNTHESIS AND MODIFICATION OF PORPHYRIN, PORPHYRINONE AND BENZOCHLORIN DERIVATIVES AS PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

By

Sangwan Lee

Photodynamic therapy (PDT) is a very promising therapy and a new modality for cancer control. This therapy is based on the selective accumulation of photosensitizing agents in tumor tissues, which activate oxygen molecule to become cytotoxic species upon irradiation. Especially PDT based on Photofrin II, a purified form of hematoporphyrin derivative (HPD), is currently in Phase III clinical trials, and the results of the work done so far with Photofrin II are very favorable and approval of its use for the treatment of neoplasms has been granted in Canada. However, it is not always clear which components of the product are responsible for cellular photosensitization *in vivo* or *in vitro* because of the complex nature of HPD. In addition, HPD has weak absorptions in the red region of the visible spectrum, a region with good tissue penetration. In recent years, using purer materials, attempts have been made to understand some of the important parameters for an effective *in vivo* photosensitizer.

To develop second generation photosensitizers for PDT, a series of structurally related porphyrins, chlorins and bacteriochlorins which have the

tunability of the absortpion maxima covering between 650 to 840 nm have been synthesized by introduction of oxo group and electron-withdrawing groups at the ring and by expanding the π-conjugation of the macrocycle.

As alternatives to Photofrin II, a dimeric porphyrin linked by 6-carbon chain has been prepared and then transformed to dimeric chlorin and dimeric benzochlorin to improve absorption in the longer wavelengths.

To study the localization and photodamage mechanism of cationic photosensitizer in tumor cell, several porphyrin and chlorin derivatives, which have ammonium group on β-position of pyrrole, and octaethylbenzochlorin derivatives, which have dimethyliminium group on *meso* position, have been prepared in high yields.

Successful biologic results of these compounds have been obtained and some biological studies with these compounds, especially the cationic photosensitizers, are still in progress.

*To my family*

# ACKNOWLEDGEMENTS

I would like to thank Professor C. K. Chang for his guidance, patience and financial support during the course of this work. I would also like to thank Professor G. T. Babcock and Professor D. Nocera for serving as members of my guidance committee. Particularly, I wish to thank Professor G. J. Karabatsos for serving as the second reader and for always being ready to help. Financial support from the chemistry department and Michigan State University is also gratefully acknowledged.

I would like to express my gratitude to the past and present members of Professor Chang's group - Dr. G. Aviles, Mr. Paul E. Luikart, Ms. Ying Liang, Mr. James P. kirby, Mr. Craig Shiner, Mr. Guo Baomin and Dr. Nilkamal Bag for their friendship. Especially, I wish to thank Dr. W. Wu for his helpful discussions and his kind friendship.

The deepest appreciate is due to my parents, Mr. Deugwoo Lee and Mrs. Sookjo Kim as well as my parents-in-law, Mr. Yongsub Son and Mrs. Kyesoon Park for their love and consistent faith.

Finally, I would like to express my thanks to my wife Myunghee for her sincere love, encouragement and patience. I am looking for a new life together with our lovely daughter Kyungmin and son Donghyun.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

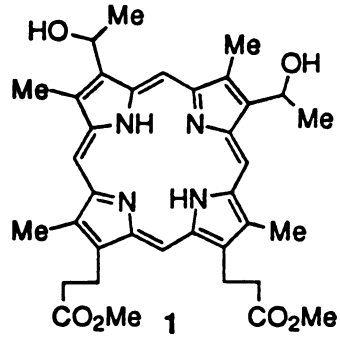
# CHAPTER 1

## INTRODUCTION: PORPHYRIN PHOTOSENSITIZATION AND PHOTODYNANIC THERAPY

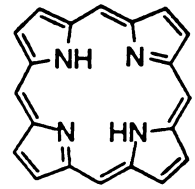
### I. GENERAL

Tetrapyrrolic macrocycles are probably the most ubiquitous of all naturally occuring pigments and include both heme and chlorophyll. The first porphyrin isolated was prepared from hemoglobin in 1867. Thudichum[1] first prepared this porphyrin by treatment of hemoglobin with concentrated acid. Four years later, Hoppe-Seyler[2] reported a similar preparation and obtained a purple substance which he called hematoporphyrin (1). About twenty years later, Nencki[3] prepared hematoporphyrin hydrochloride (from isolated hemin) as the first pure porphyrin. However, Schumm and co-workers[4] showed through spectroscopic investigation that hematoporphyrin was not the same porphyrin as that of the heme prosthetic group. Degradative studies by Küster[5] distinguished hematoporphyrin with its hydroxyethyl side chain from the prosthetic group porphyrin, which contained vinyl substituents. In 1912, Küster[6] proposed the correct ring structure (2) for porphyrins.

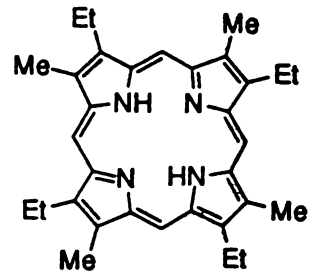
In 1926 Fischer[7] prepared etioporphyrin-I (3) by the first totally synthetic pathway and followed this shortly thereafter with the complete synthesis of octamethylporphyrin (4)[8] by two distinctly separate methods.




1

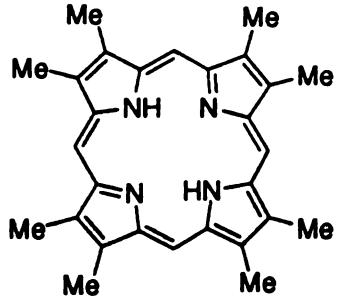



2

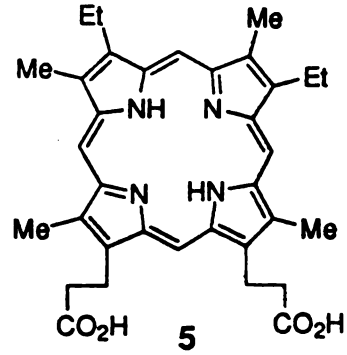



3

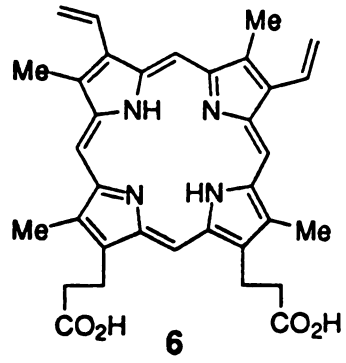
This led Fischer to adopt the porphyrin ring structure initially proposed by Küster[5] in 1912. The extensive porphyrin degradation work and the synthesis by his group of the four etioporphyrin isomers and twelve of the fifteen isomers of mesoporphyrin (5, type IX isomer) helped Fischer correlate the sequence of substituents about the natural porphyrin ring system as the least symmetrical of the possible fifteen substituent orientations; this was named as the naturally occurring type IX substituent array, which is related to the type-III arrangement in circumstances where only two, rather than three, types of substituent are present. In 1929, Fischer[9] synthesized protoporphyrin-IX (6), and the hemoglobin prosthetic group itself, hemin (7), the iron(III) chloride complex of protoporphyrin IX.



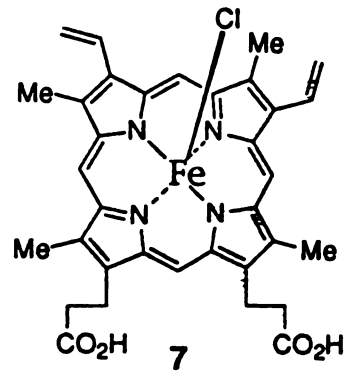

4




5




6




7

Porphyrins and other closely related tetrapyrrolic pigments occur widely in nature, and are implicated in a great variety of vital biological processes.

## II. SIGNIFICANCE AND BACKGROUND

Certain porphyrins are known to have a photodynamic effect in mammals; that is, they cause a sensitivity to light which can result in considerable damage to exposed tissue.[10] The reaction requires oxygen, and there is evidence for the view that singlet oxygen is involved, at least in certain circumstances.[11] The phenomenon was originally observed by Hausmann[12] in mice and by Meyer-Betz[13] in man (in himself, actually). Since the porphyrins are brilliantly fluorescent in UV light (365 nm), this offers a way of detecting tumors.[14,15] The porphyrin preparation in current clinical use is a product termed HPD (hematoporphyrin derivative) whose tumor-localizing properties were described over 20 years ago.[16] It was not until 1972 that the two fundamental ideas (localization and photodynamic effect) came together and the possibility that a porphyrin could be used to

photosensitize the preferential degradation of tumor tissue was demonstrated.[17] Subsequently hematoporphyrin derivative has been shown to be effective in causing photodegradation of tumor tissue both in experimental animals and in man.[18] The preferential uptake of the photosensitizers (porphyrins) by tumors and sensitivity to light of tumors that have taken up a photosensitizer has led to the development of a cancer therapy called photodynamic therapy (PDT).[19-25]

PDT is a highly promising therapy and a new modality for cancer control. This therapy is based on the selective accumulation of photosensitizing agents in tumor tissues,[26] which activate dissolved molecular oxygen to become cytotoxic species[27] upon irradiation. PDT with Photofrin, a photosensitizer made by Quadralogic Technologies, and its forerunners, hematoporphyrin derivative (HPD) and dihematoporphyrin ether/ester (DHE), has now been tested in well over 5000 patients world-wide with encouraging results against tumors in the lung, bladder, esophagus, skin, and other tissues.[28] Especially, PDT based on Photofrin II,[16] a purified form of HPD, is currently in Phase III clinical trials,[24] and the results of the work done so far with Photofrin II are very favorable and approval of its use for the treatment of neoplasms has been granted in Canada. For example, Kato *et al.*[29] have recently reported that 84% of early stage lung cancer patients are curable by PDT if the peripheral tumor is less than 2 cm in size and if metastasis has not yet occured. For early stage, central type lung cancer, curability increases to 100%.[29] In another report, Jocham *et al.*[30] reported that 9 of 15 patients with superficial bladder tumors were still tumor free some 24-54 months following PDT.

HPD is a complex mixture; although it has been referred to as "recrystallized",[15] it is prepared by precipitation, and proves to be a complex

mixture of porphyrins[31] with many investigators not even agreeing on the definition of the term HPD. Because of the complex nature of HPD, it is not always clear which components of the product are responsible for cellular photosensitization *in vivo* or *in vitro*. The most active component has been described as a dihematoporphyrin ether, or ester[15]; however, these were not available as pure chemicals until Pandey and Dougherty[32] synthesized an ether dimer which was shown to be an effective *in vivo* sensitizer. Red light, usually from an argon pumped dye laser tuned to 630 nm [corresponding approximately to the longest wavelength (but weakest) absorption peak of HPD], is most commonly used in PDT to permit the maximum of light penetration into mammalian tissue. However, porphyrins absorb poorly in this region. Thus, there is considerable interest in identifying effective photosensitizers for PDT that absorb more strongly in the red or near IR wavelengths to which tissues are highly transparent, and the development of low-cost reliable diode lasers, which emit in the 750-850 nm range, has accentuated the need for compounds absorbing around 750 nm (optimum wavelength for tissue penetration).[26]

PDT is based on the dye-sensitized photooxidation of biological matter in the target tissue. In the case of PDT, sensitizers are introduced into the organism as the first step of treatment. In the second step, the tissue-localized sensitizer is exposed to light of wavelength appropriate for absorption by the sensitizer. Through various photophysical pathways, also involving molecular oxygen, oxygenated products harmful to cell function arise and eventual tissue destruction results.

## III. SENSITIZER DELIVERY AND DISTRIBUTION IN CELLS AND TISSUES

The first step towards PDT tumor treatment is the delivery of the photosensitizer to the target tissue. The mechanism of delivery of the active PDT sensitizers to cells that will ultimately be effected is of great interest as it may provide insights into the uptake and retention of these compounds in tumor cells. It has been suggested that HPD may be delivered to tumor cells by low density lipoprotein (LDL) since HPD shows a high affinity for LDL and because tumor cells have increased levels of LDL receptors.[33]

Lipoproteins are well-known as a vector for cholesterol distribution, but their role in distribution[34] of drugs has only recently been appreciated. There are three classes of lipoproteins: very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL).[35] VLDLs contain a large percentage of lipids and low percentage of proteins. Therefore, these substances are very low in density because fats and oils are less dense than water. In contrast, low density and high density lipoproteins contain less lipid and more protein and, therefore, are more dense than water. VLDL, LDL and HDL can be separated by density-gradient ultracentrifugation.

LDL particles ultimately bind to cell-surface receptors. The predominant circulating lipoprotein species in several animals, notably dog, mouse rat and horse, is HDL. In contrast, in man, guinea pig and rabbit the predominant species is LDL. The latter two animals may therefore be good models for human distribition. In both mouse and man the presence of neoplastic disease lowers the level of circulating LDL, presumably because of the high level of LDL receptors in neoplastic tissues. Binding of the various components of HPD to lipoproteins and serum proteins has been

documented.[36] The affinity of several monoporphyrins with serum albumin has been measured through binding kinetic studies indicating that a major portion of bound porphyrin is delivered by LDL.[33,37] The behavior of VLDL associated with porphyrin uptake was less clear probably because VLDL is metabolically converted into other lipoproteins including LDL.

The differences in the behavior of porphyrin-HDL, -LDL and -VLDL complexes can be explained on the basis of the two main modalities of lipoprotein internalization by cells. (i) non-specific fluid endocytosis. (ii) receptor-mediated endocytosis. The latter mechanism concerns LDL and becomes especially important for cells displaying hyperproliferative activity where the number of LDL-receptors on the cell surface drastically increase. Therefore the preferential accumulation and retention of porphyrins by tumor cells does not seem to reflect an intrinsic property of the dye; rather it is a consequence of cell-interaction mechanism typical of the LDL. LDL has been proposed as a specific carrier of cytostatic drugs to tumors.[37]

Localization of porphyrins in tissue is well documented through numerous investigations. But the exact mechanism of porphyrin uptake and retention still remains obscure. The delivery of PDT sensitizers by LDL suggests a possible mechanism for retention of the active fraction of HPD in the cell. It is easy to imagine that once through the cell membrane, the porphyrin-protein complex could break up, during digestion of the lipoprotein, for example, and that the free porphyrin polymer would then have poor ability to diffuse out through the cell membrane. Unfortunately this elegant model has suffered recently because Korbelik and co-workers[38] reported that LDL actually inhibits the uptake of Photofrin-II by tumor cells both *in vitro* and *in vivo*.

An alternative mechanism for the retention of HPD involves the tendency of these species to aggregate in polar environments, such aggregates might adhere to the outside of the cell wall and be incorporated into the cell by pinocytosis or nonspecific fluid endocytosis. Once in the internal mileu of the cell such aggregates could break up and individual molecules could sequester themselves in lipophilic sites within the cell. Kessel[39] provides some support for this type of model with a study that suggests differing degrees of aggregation in the oligomeric fraction of HPD as a function of the polarity of the environment. The real mechanism for the photosensitizer delivery and distribution in cells and tissues is still obscure.

## IV. THE PHOTODYNAMIC EFFECT

The most basic requirement for any molecule to be considered for use in PDT is that it be a photosensitizer, i.e. capable of absorbing light and transferring some of the absorbed energy to acceptor molecules. Although either ultraviolet, visible or near infrared radiation could be possible energy sources, it is accepted that use of UV-radiation is disadvantageous, due to both its poor penetration of tissue[12] and the potential for initiating carcinogenesis.[13] Consequently, sensitizers currently being developed absorb visible light (400-760 nm) or near infrared radiation (760-900 nm).

Similar to ionizing radiation, the damage-initiating step of PDT occurs within a very small time frame. Photodynamic interactions take place wherever sensitizer, light of appropriate wavelength and oxygen are present simultaneously. As shown in Figure 1, once a photon of energy has been

absorbed, the sensitizer is transformed from its ground singlet state ($S_o$) to the excited singlet state ($S_1$), and a number of competing processes can occur. Energy can simply be lost by fluorescence which regenerates the ground singlet state ($S_o$), or by intersystem crossing (ISC) to generate the longer lived triplet state ($T_1$, lifetime $10^{-3}$-10 s) of the sensitizer.

The excited triplet state ($T_1$) can undergo two kinds of reactions and both processes may generate cytotoxic species.[40] It can react directly with either substrate or solvent by hydrogen atom or electron transfer to form radicals and radical ions, which after interaction with oxygen can produce oxygenated products (Type I reaction); or it can transfer its energy to oxygen directly to form mainly singlet oxygen ($^1O_2$, lifetime $4x10^{-6}$ s in water, 50-$100x10^{-6}$ s in lipid and most organic solvents, $0.6x10^{-6}$ s in cellular environment),[41,42,43] a highly reactive, oxidative species (type II reaction).[44,45] Electron transfer from sensitizer to oxygen molecule can also occur in some cases, giving oxidized sensitizer and superoxide ion.[46]

Strictly speaking, the term "Photodynamic" implies that these transfers require the involvement of molecular oxygen[47] although this may not be an absolute requirement for the generation of cytotoxic species in general. Where oxygen is the acceptor molecule, a number of reactive forms (singlet oxygen, superoxide anion radical, hydroxyl radical) can be generated. Among these, singlet oxygen appears to have the longest lifetime and would therefore seem to be the cytotoxic agent of choice, having the ability to diffuse farther through the cell and thus increasing the sphere of cell damage.

Since the generation of the triplet state depends on ISC from the excited singlet state ($S_1$), molecules with high fluorescence quantum yields will generate lower quantum yields of the triplet state ($T_1$) and consequently, low

conc

fluor

Fi

st

th

concentrations of cytotoxic species. Conversely, sensitizers having low fluorescence quantum yields will generate higher quantum yields of triplet

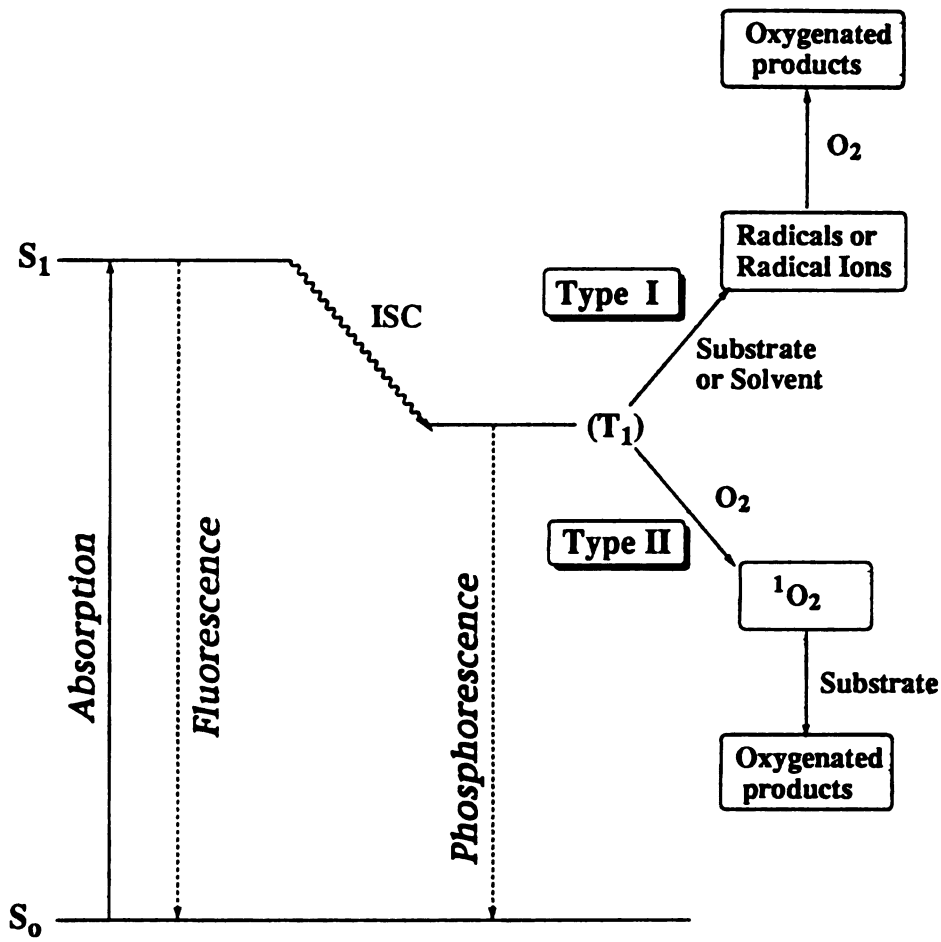



Figure 1 Radiative and collisional processes taking place following the optical excitation of a sensitizer in the presence of oxygen.

state ($T_1$) and therefore, higher concentrations of cytotoxic species. Clearly, this latter situation is more desirable for PDT while the former situation may be better suited to *in vivo* imaging of neoplastic tissues.

Several indications exist that singlet oxygen ($^1O_2$), formed by energy exchange with the triplet states ($T_1$) of photosensitizer, is of primary importance in initiating biological damage, although definitive conclusions are still lacking.[48] Certainly, there can be no doubt that the combination of oxygen, a photosensitizer, and light of the correct wavelength will generate singlet oxygen. When this reactive species is created in the vicinity of oxidizable biomolecules, oxidative damage will assuredly occur. However, it is not unequivocally shown that such damage will create a cell-killing lesion. Nevertheless, biological damage is the definitive result of light absorption by the chromophore, and investigations into the early physical and chemical events that succeed the photon absorption step are highly relevant within the context of PDT.

Most sensitizers showing promise in PDT are efficient generators of singlet oxygen. Since singlet oxygen is 1 eV (22.5 kcal/mol) higher in energy than the ground state oxygen, the tiplet state ($T_1$) of sensitizer should have at least this energy. Extrapolating this back to the energy of the excited singlet state ($S_1$) needed to generate the triplet state ($T_1$) and assuming some energy loss through other nonradiative processes, it has been suggested that, when porphyrin based compounds are used as sensitizers, the lowest energy capable of meeting these requirements translates to an absorption close to 800 nm.[49] Since tissue penetration of light increases with increasing wavelength,[50] sensitizers absorbing near 800 nm would be optimum for PDT.

Type I and type II reactions may occur simultaneously, and the ratio between the two processes is highly influenced by sensitizer, substrate and oxygen concentration, as well as the binding of sensitizer to substrate. There is much indirect evidence to suggest that singlet oxygen is the major damaging species in PDT, but direct measurement of singlet production in

cor

inc

int

inc

In

sor

*vit*

ab

me

ox

ex

see

for

pe

is

eff

lin

be

lin

co

ne

to

complex biological systems appears to be extremely difficult,[51] and most indirect methods such as the use of chemical quenchers of reactive intermediates or $D_2O$ (which prolongs the singlet oxygen lifetime and so can increase photosenitization) are not entirely specific for singlet oxygen.[44,52,53] In particular, indications are that superoxide ion ($O_2^-$) may be involved in some aspects of PDT damage.[54] For Photofrin-II photosensitization of cells *in vitro*, full effects are observed at about 5% $O_2$ levels, with a half-value at about 1% $O_2$. No photosensitization can be observed in the absence of measurable oxygen. From the above it is evident that PDT effects should be oxygen-dependent. This is indeed the case for most sensitizers,[55] with the exception of some cationic sensitizers such as the cyanine dye EDKC,[56] which seems to act by oxygen-independent mechanisms.

The measurement of light penetration through tissue is a difficult task for which mathematical models are still being developed and refined.[57] Light penetration is also dependent on sensitizer concentration. As sensitizer dose is increased, a limiting concentration is reached at which sensitizer can effectively screen out light and thus limit tissue penetration.[58] Since this limiting concentration depends on the effective capture of photons, it would be expected that sensitizers with larger extinction coefficients would reach a limiting concentration at lower dose than sensitizers with low extinction coefficients (assuming similar photodynamic efficiencies).

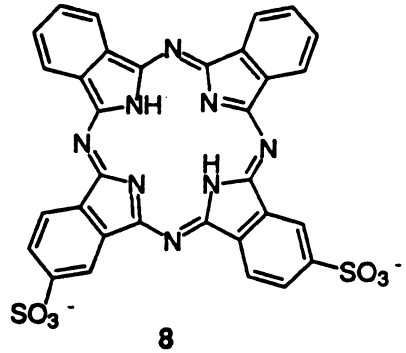
Progress with regard to drug development will undoubtedely involve new agents with strong absorbance in the longer wavelengths, with a view toward promoting eradication of larger tumors.

## V. ANIONIC AND NEUTRAL PDT SENSITIZERS

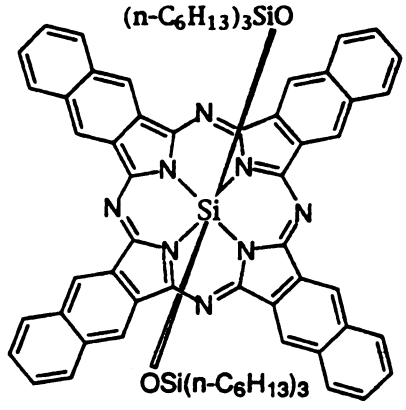
The products in Photofrin, and most sensitizers in clinical and pre-clinical trials (benzochlorin derivative, tin etiopurpurin, N-aspartyl chlorin e6, *m*-tetrahydroxyphenyl chlorin, zinc phthalocyanine) are anionic or neutral at physiologic pH, which showed oxygen-dependent PDT effects.

van Lier *et al.*[59] reported that in the disulfonated phthalocyanines (8), the isomer with the acid functions on the same side of the molecule is more effective than that with the groups on opposite sides of the molecule, which has been interpreted in terms of the amphiphilic character of the former.

Rodgers *et al.*[60] reported that bis(di-*i*-butyloctadenyl siloxy)silicon-2,3-naphthalocyanine (9, isoBoSiNc) is a reasonably efficient photosensitizer which can be activated with a diode laser at 774 nm. However, isoBoSiNc suffers from two drawbacks; relatively long skin retention and extreme water insolubility.



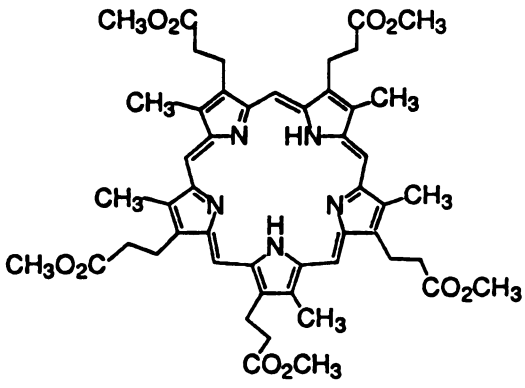

8



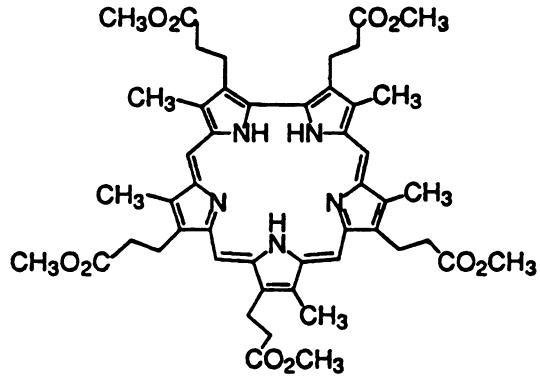

9

Cholorophyll derivatives are 7.8-dihydroporphyrins derived from photosynthetic plants and algae, which possess long wavelength absorption maxima between 600-700 nm. In plants, chlorophyll-a and chlorophyll-b are usually present in a ratio of about 3:1. This chemistry is particularly aided by the commercial availability of Spirulina maxima algae, which contains only chlorophyll-a. Large amounts of methyl pheopherbide-a can therefore be obtained,[61] which provides access to a large variety of potentially useful degradation products by way of the " allomerization" reaction. PDT studies with a series of chlorophyll derivatives are in progress.

Molecules possessing five pyrrole subunits possess interesting long wavelength absorption bands (22 π-electron macrocycles). Typical examples of such 22 π-electron macrocycles are the pentaphyrin (10)[62] and sapphyrin (11)[63] systems, whose longest wavelength absorptions are at 682 nm and 678 nm respectively.




10




11

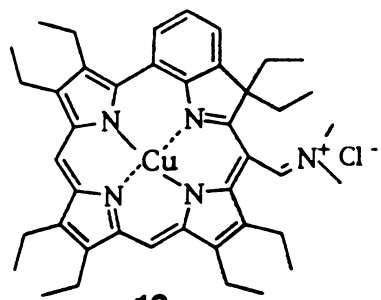
## VI. CATIONIC PDT SENSITIZERS

While singlet oxygen generating, anionic or neutral PDT sensitizers at physiologic pH are related to the direct cell kill, cationic sensitizers, non-singlet oxygen generator derivatives, showed the indirect cell kill *via* vascular effects.[56] Cationic sensitizers could have an advantage, since there have been reports of selective affinity of such agents for neoplastic cells.[64] The preferential mitochondrial accumulation of cationic sensitizers accounts for the predominance of mitochondrial damage induced by these dyes.[65] Oseroff and Cincotta[66,67] have developed cationic sensitizers, but these compounds tend to have low extinction coefficients at the longer wavelengths, or need to be transformed to active sensitizers by intracellular enzymes.

In our search for an effective cationic photosensitizer with strong absorbance in near-IR, Chang and co-workers[68] in studying reversible modification of formyl peripheral substituents, reported that protonated imines of "chlorin-type" compounds are characterized by an unusual red shift of the absorption maxima to the 800 nm region .

Most recently, Skalkos *et al.*[69] reported that copper benzochlorin iminium salts have unusual stability and an unusual red-shift in absorbance to wavelength as high as 800 nm. Initial photophysical experiments indicate that the triplet state lifetime of CuBI (copper benzochlorin iminium salt) (12) is extremely short (<20 nsec) and that singlet oxygen is not produced during photoactivation.[70,71] Hence, CuBI has no tendency to initiate type II photodynamic activity. Lipid peroxidation of erythrocyte membranes by photoactivation of CuBI indicate generation of superoxide/hydrogen peroxide and that involvement of singlet oxygen is not significant in this experimental

model.[72] It was known that singlet oxygen generating photosensitizers disrupt tumor blood flow when measured immediately after irradiation.[73] Using the radioactive microsphere technique, it was found that photoactivation of CuBI formulated in Cremophor EL resulted in decreased blood flow.[70,73]




**12**

## VII. OBJECTIVES OF THE PRESENT WORK

The principal objective of our study is to develop second generation photosensitizers for PDT. They should have attractive and chemical properties including strong absorption maxima at wavelengths where tissues provide optimal light transmissions, good capacity to generate singlet oxygen ($^1O_2$) and facile chemical accessibility. In particular, we planned the syntheses of porphyrins and chlorins which have the tunability of the absorption maxima covering almost anywhere between 650 to 840 nm, simply by selecting appropriate substituents at the appropriate positions on the macrocycle. It is known that porphyrin absorptions can be shifted to longer wavelengths by expanding the π-conjugation of the macrocycle and by

introducing electron-withdrawing groups at the ring. The following categories of compounds are our synthetic targets.

1. Anionic porphyrin derivatives.

To increase the tumor localization character, two amphiphilic porphyrins containing hydrophilic and hydrophobic groups were synthesized. To achieve strong absorption maxima in the red region of the visible spectrum where tissues provide optimal light transmissions (thus enabling treatment of larger tumors and enhancing capacity to generate singlet oxygen, cytotoxic agent), one or two oxo groups were introduced into the porphyrins and then transformed to sulfido, imino, and methide groups which are good auxochromes (bathochromic shift).

As alternatives to photofrin-II, a series of dimeric porphyrins were prepared for use in PDT. From previous results, a dimeric porphyrin linked by 6-carbon chain proved to be more effective *in vivo* than others. A dimeric porphyrin linked by 6-carbon chain was therefore designed and prepared and then transformed to dimeric chlorin and dimeric benzochlorin to improve absorption in the longer wavelengths.

2. Cationic photosensitizers.

To study the localization and retention mechanism of cationic lipophilic compound in tumor cell, several porphyrin derivatives, which have ammonium group on β-position of pyrrole, and octaethylbenzochlorin derivative, which has dimethyliminium group on *meso* position, were synthesized.

The structural and spectral properties of these compounds have been characterized by UV-vis, NMR, IR, and Mass spectroscopies. Throughout these studies, whenever necessary, comparative studies were carried out. For characterization of these compounds with regard to their stability, biophysical

and photophysical properties and pharmacokinetics, including modes of protein and lipoprotein-mediated distribution, the *in vitro* tests were done by Dr. D. Kessel's lab (Department of Pharmacology, Wayne State University School of Medicine, Detroit, MI 48201), and the *in vivo* tests by Dr. B. Henderson's lab (Department of Radiation Biology, Roswell Park Cancer Institute, Buffalo, NY 14263). In addition, the relative photooxygenation strengths were measured in our lab.

## VIII. RESULTS AND PRESENTATION

In this work, 9 kinds of anionic mono-porphyrin derivatives (total; 18) and a series of anionic dimeric porphyrin derivatives (total; 4) and 7 kinds of cationic porphyrin derivatives (total; 12) were prepared and sent to Dr. Kessel for biological tests. Among them, dioctylporphyrinone (15 in Scheme 4) showed very favorable biological result. Others were too toxic or too non-toxic to be used in PDT.

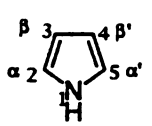
To determine the photodynamic efficiency (phototoxicity), the quantum yield of singlet oxygen molecule ($^1O_2$) production should be measured. Because the unavailability of some special instruments which are needed for measuring the $^1O_2$ quantum yield, the relative photooxygenation strengths were measured by using dansyl-L-methionine which was described in chapter 2 in detail.

In this thesis, chapter 2 describes the synthesis of anionic mono-porphyrin derivatives. To improve absorption maxima in the red region, some methodologies employed for introduction of "oxo" group and

transformation of "oxo" group to sulfido, imino, and methide group are described in detail. Chapter 3 describes the total synthesis of a dimeric porphyrin linked by 6-carbon chain and its transformations to vinyl, chlorin, benzochlorin analogues. In chapter 4, the total syntheses of two kinds of porphyrins and some structural modifications to obtain cationic analogues are described. The preparations of *meso*-dimethyliminium octaethylbenzochlorin and its sulfonated analogue from octaethylporphyrin are also described in detail.

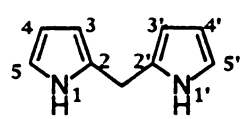
## IX. NOMENCLATURE

The parent monocyclic system, pyrrole, is numbered as shown (13). The Greek letter are sometimes used to distinguish the two types of carbon position. Two pyrrole rings linked at a α-position to a single carbon function were named by the Fischer system because the systematic names are tedious in common use. The macrocyclic system (14) is called porphyrin, a name originally used (in hematoporphyrin) by Hopper-Seyler.[74] The numbering of ring positions including nitrogen, and the use of letters to denote individual rings is shown in (14).

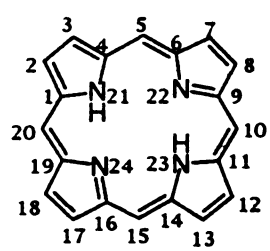



pyrrole

**13**




dipyrrylmethane




porphyrin

**14**

The nomenclature of porphyrinoids with keto group on the ring has not been standardized. The prefix "oxo", in fact, could be confused with "oxoporphyrin" or "oxophlorin", which denotes a porphyrin with an oxygen attached to the methine bridge. Furthermore, we now know that these ketone derivatives have very little chemical properties in common with those of the corresponding chlorins or bacteriochlorins. It is probably more appropriate to consider them "quinones" of porphyrins, hence we propose the use of "porphyrinone" and "porphyrindione". Sulfido derivatives were also named as "porphyrinthione" and "porphyrindithione". Other transformed (imino and methide) derivatives were named as "chlorins". For convenience sake, the trivial and systematic names were used whichever is simpler.

# CHAPTER 2

## SYNTHESES AND PROPERTIES OF ANIONIC MONO-PORPHYRIN DERIVATIVES

### I. INTRODUCTION

Although Photofrin II appears to be an effective photosensitizer, it shares with HPD the problem of contamination by various porphyrin species whose contribution to the total biological effect remains unknown. In addition, both HPD and Photofrin II have weak absorptions in red region of the visible spectrum, a region with good penetration. In recent years, using purer materials, attempts have been made to understand some of the important parameters for an effective *in vivo* photosensitizer. Chlorin derivatives typically having an intense visible absorption maxima near 640 nm appear to be an attractive system.

Saturation of β-β' pyrrole double bonds in a porphyrin ring can be brought about by either reduction or oxidation. Fischer *et al.*[75,76] pioneered the use of sodium in alcohol to reduced porphyrin to chlorin and this method has been extended by others[77,78] to obtain reduction levels beyond the chlorin stage, e.g., to bacteriochlorin and isobacteriochlorin. In the opposite direction, Fischer again was first to study the effect of oxidants on porphyrin. In the 1930s, he reacted porphyrin with hydrogen peroxide in concentrated sulfuric acid and obtained oxochlorin (porphyrinone)[79,80] whose

structure was characterized in the 1960s.[81,82,83] The hydrogen peroxide-sulfuric acid oxidation of β-substituted porphyrins result in a complex mixture of isomeric products containing one, two, and three oxo groups on the ring with uniformly poor yields.[83,84]

Recently our group devised a convenient porphyrinone synthesis with a significantly higher yield by an alternative 2-step reaction *via* osmium tetroxide oxidation and acid catalyzed pinacolic rearrangement.[85] C. Sotiriou of our group[86] completed a series of experiments aiming to elucidate the reactivity as well as the migratory aptitude of the biologically important porphyrin side chains. The results are cited as follows:

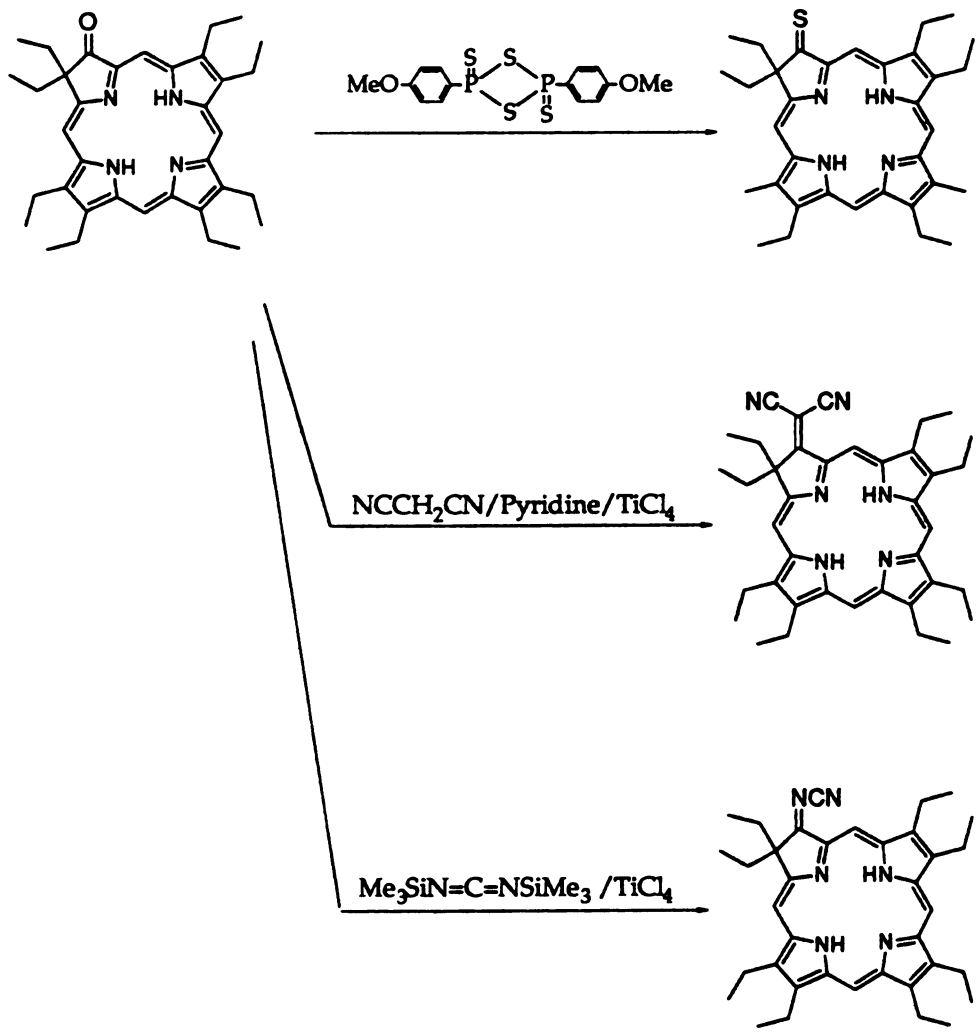
1). Relative reactivity of the pyrrole double bond towards dihydroxylation (*via* $OsO_4$ oxidation) is highly proportional to, barring electronic effect, the size of the substituents, the larger the substituent, the slower the rate. Thus, H = Methyl > Ethyl > $-CH_2CH_2CO_2R$ .

2). Migratory aptitude of the substituents is mainly related to their electronic effects: hydrogen, ethyl, alkyl groups including propionate side chain will migrate over methyl group and acetate side chain has a lower mobility than methyl group.

These general rules hold true in most porphyrins.

Most recently, our lab has synthesized another class of porphyrinoid macrocycles that have desirable absorption characters for use in PDT. These compounds comprise a porphyrin nucleus containing exocyclic double bond connecting a β-pyrroline carbon to an oxygen, sulfur, nitrogen, or electronegative carbon groups (see Scheme 1). They are structurally very similar to natural porphyrins, chemically stable, and synthetically uncomplicated to prepare. The greatest advantage of this system is the tunability of the absorption maxima covering between 650-840 nm, simply by

selecting appropriate substituents at the appropriate positions on the macrocycle. At present, the porphyrinone and the porphyrindione, along with their thione analogues have been proven to be photodynamically active *in vitro* .



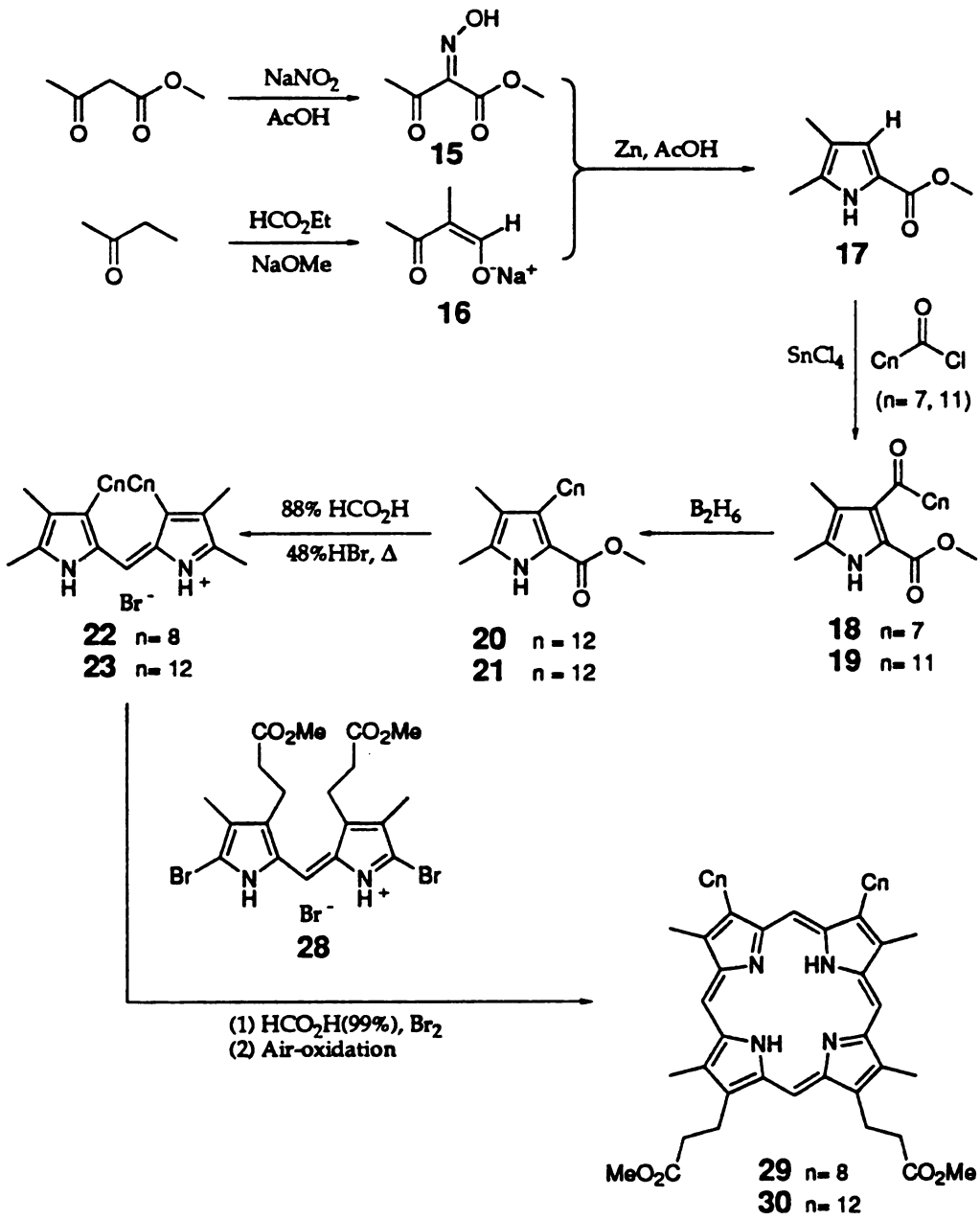

Scheme 1. Syntheses of Sulfido, Imino, and Methide Adducts

Since natural porphyrins are very difficult to be converted into specific structures, we designed an amphiphilic photosensitizer system (**29** and **30** in Scheme 2), primarily to investigate structure-activity relationship for PDT applications. The porphyrins (**29**) and (**30**) can be easily transformed to other derivatives containing many desirable functional groups which are good auxochromes (bathochromic shift) as shown in Scheme 4 and Scheme 5. In addition, the combination of hydrophilic and hydrophobic groups may increase the tumor localization character.

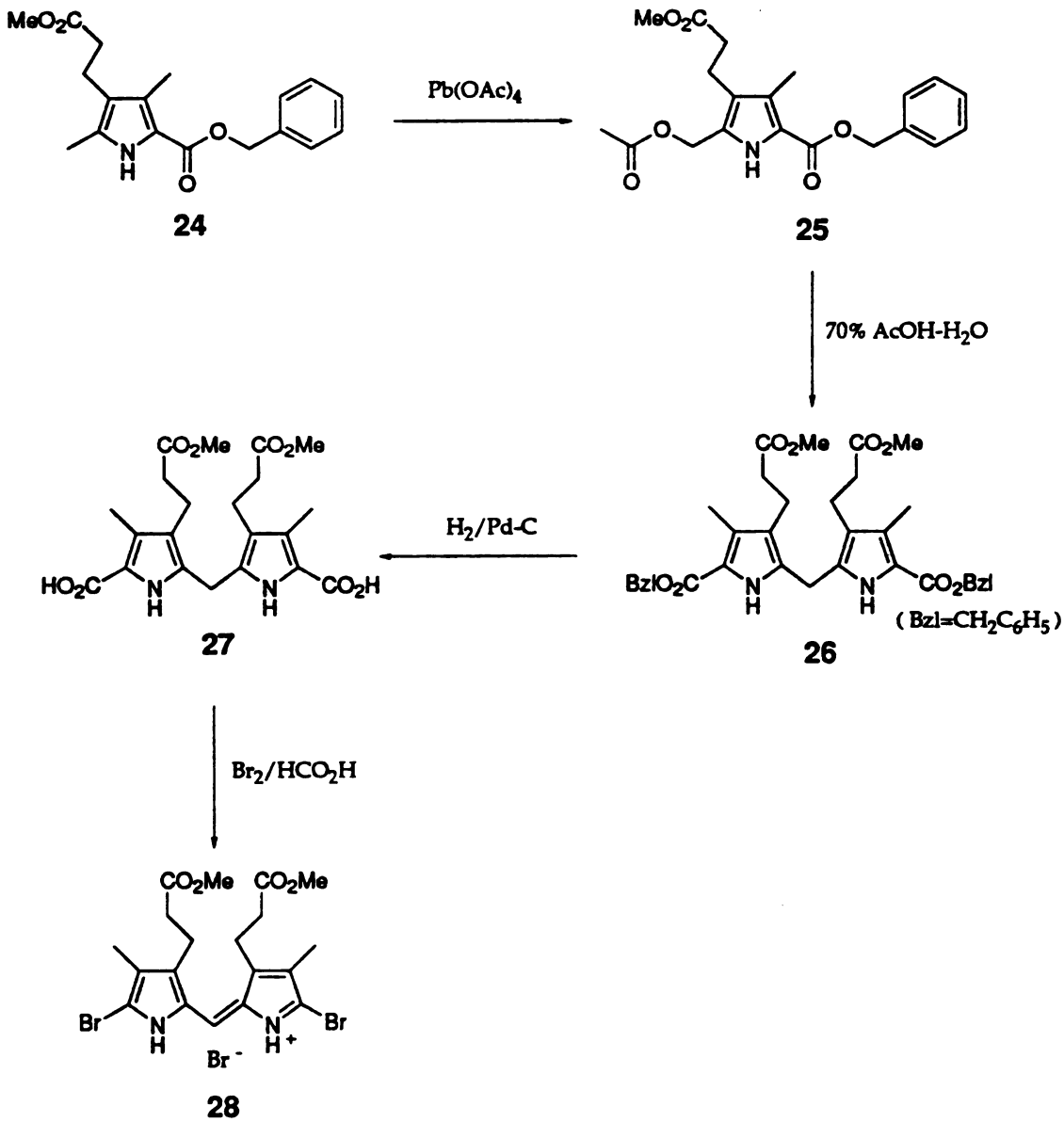
## II. SYNTHESES

As described in Scheme 2, octanoyl pyrrole (**18**) and dodecanoyl pyrrole (**19**) were prepared from β-free pyrrole (**17**) quantitatively by acylation with octanoyl chloride and lauroyl chloride respectively, and then reduced to octyl pyrrole (**20**) and dodecyl pyrrole (**21**) by employing diborane generated from boron trifluoride etherate and sodium borohydride. Successful results (> 97%) were obtained by using an excess of diborane. The reduced pyrrole (**20**) and (**21**) were separately condensed in 88% formic acid containing 48% hydrobromic acid to give dioctyl dipyrrylmethene (**22**) and didodecyl dipyrrylmethene (**23**) respectively, which provide the lipophilic character to porphyrins (**29**) and (**30**).

In Scheme 3, benzyl 4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate (**24**) was quantitatively transformed by oxidation with lead tetraacetate to the 5-acetoxymethyl analogue (**25**), which was condensed in hot 70% aqueous acetic acid to form the dibenzyloxycarbonyl dipyrrylmethane (**26**). The benzyl ester groups of the dipyrrylmethane (**26**) was hydrogenated


NaNO2
AcOH
15
HCO2Et
NaOMe
16
Zn, AcOH
17
SnCl4
(n= 7, 11)
18 n= 7
19 n= 11
B2H6
20 n = 12
21 n = 12
88% HCO2H
48%HBr, Δ
22 n= 8
23 n= 12
28
(1) HCO2H(99%), Br2
(2) Air-oxidation
29 n= 8
30 n= 12

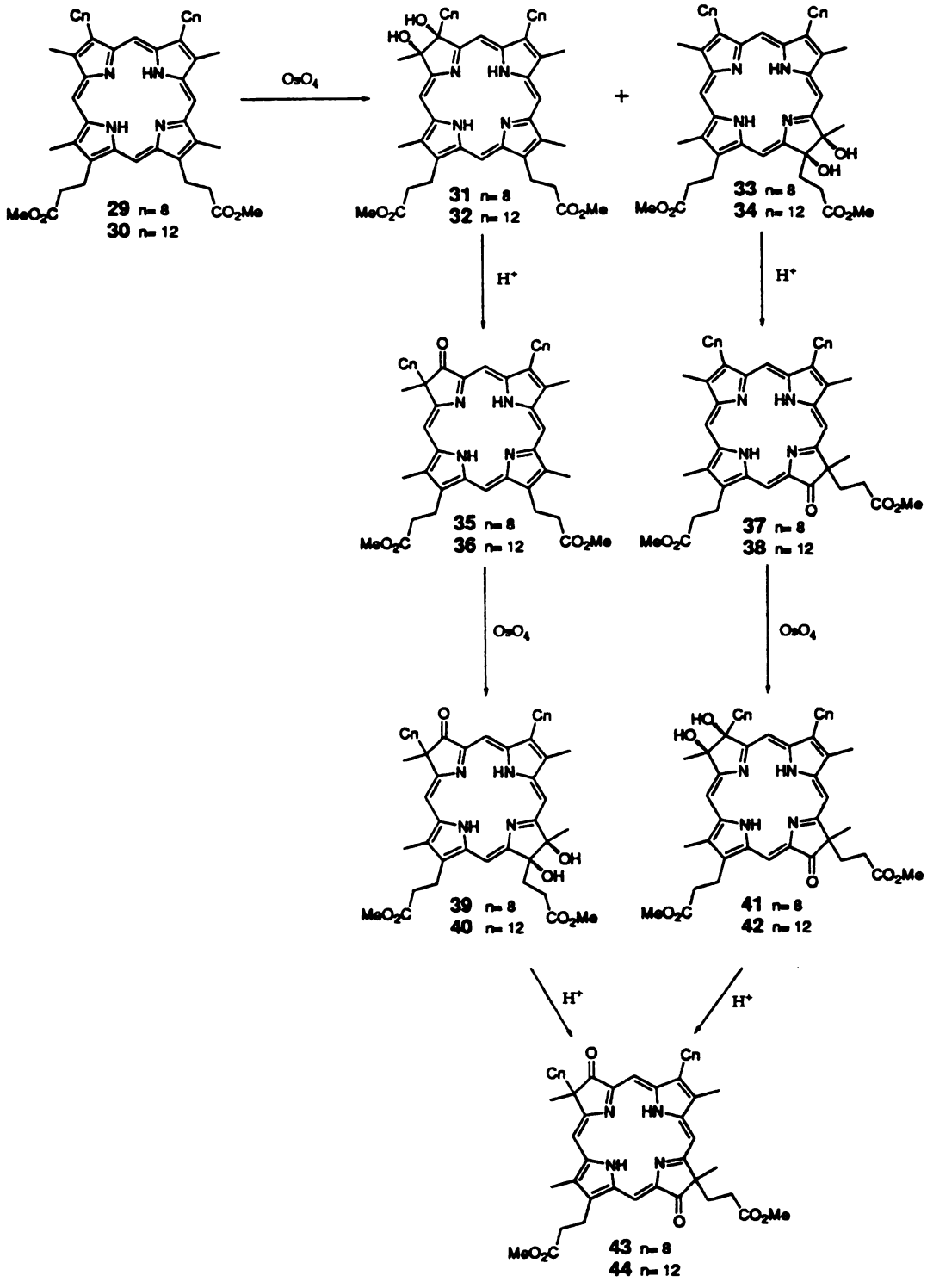
**Scheme 2**


MeO2C
Pb(OAc)4
24
25
70% AcOH-H2O
CO2Me CO2Me
H2/Pd-C
HO2C
CO2H
BzlO2C
CO2Bzl
(Bzl=CH2C6H5)
27
26
Br2/HCO2H
Br
Br -
28

**Scheme 3**

with 10% palladium/carbon to give the corresponding diacid (27) quantitatively. The 5,5'-dicarboxylic acid (27) was brominated to form the 5,5'-dibromodipyrrylmethene (28), which gives the hydrophilic character to porphyrins (29) and (30).

As shown in Scheme 2, the dioctyl dipyrrylmethene (22) and the didodecyl dipyrrylmethene (23) were respectively condensed with the 5,5'-dibromodipyrrylmethene (28) in anhydrous formic acid in the presence of one equivalent of bromine to give the dioctylporphyrin (29) and the didodecylporphyrin (30), which are stable and can be convered to the porphyrinones (35, 36, 37, 38) and the porphyrindiones (43, 44). In an effort to synthesize porphyrinones (35, 36, 37, 38) and porphyrindiones (43, 44), as shown in Scheme 4, we first made *vic*-dihydroxychlorins (31) and (33) which were obtained from oxidation of the dioctylporphyrin (29) by osmium tetroxide. The porphyrin (29) was allowed to react with 1.2 equivalent of osmium tetroxide in dichloromethane/pyridine and the reaction was quenched after 24 hours with hydrogen sulfide to decompose the osmium ester. In this reaction, the dihydroxylation of porphyrin occured predominantly at the "northern" pyrrole affording the 2,3-dihydroxychlorin (31) as the major product along with a small amount of the isomeric 12,13-dihydroxychlorin (33) which was cyclized to a γ-spirolactone derivative during separation on silica gel chromatography.[87] Thus, without isolation of them, the mixture of *vic*-dihydroxychlorin (31) and (33) were undergone the pinacolic rearrangement smothly in acidic medium (usually in concentrated sulfuric acid or in perchloric acid) to give the 2,7-dioctyl-3-porphyrinone (35) as the major product along with a small amount of the isomeric porphyrinon (37). The didodecylporphyrin (30) was also treated with osmium tetroxide

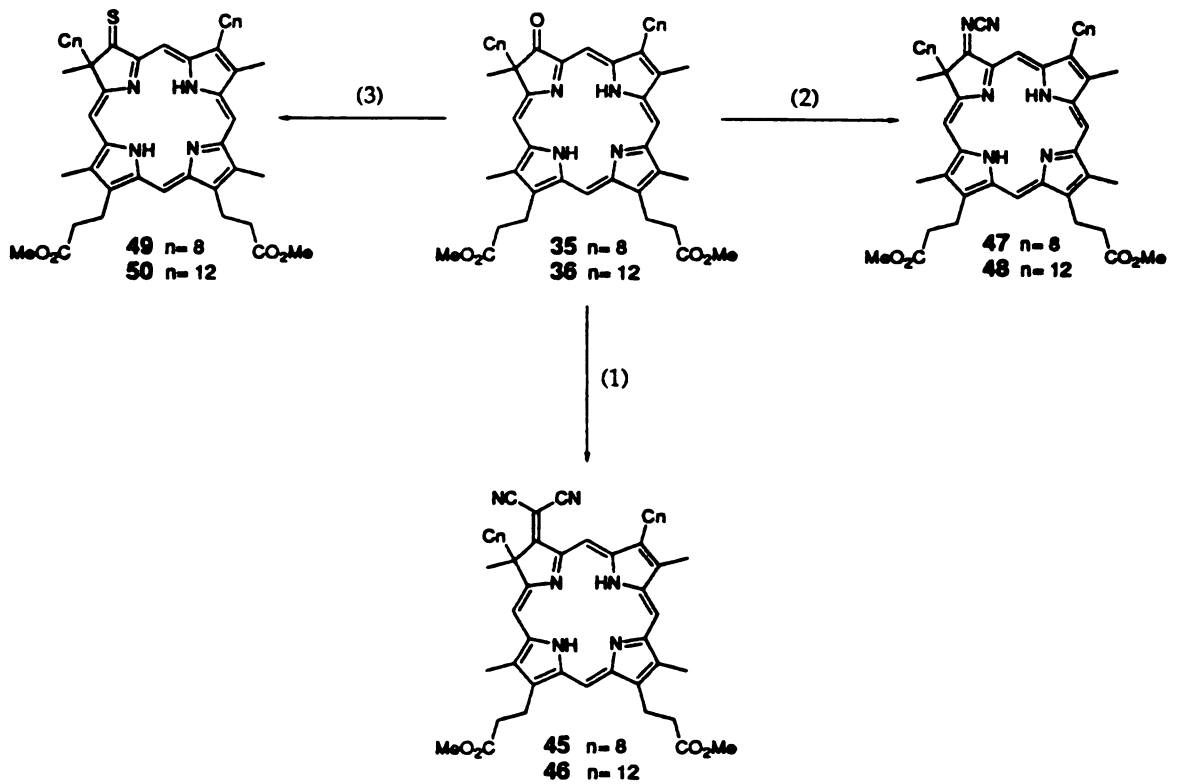

Cn
OsO4
HO
HN
NH
N
MeO2C
CO2Me
OH
H+
O
29 n= 8
30 n= 12
31 n= 8
32 n= 12
33 n= 8
34 n= 12
35 n= 8
36 n= 12
37 n= 8
38 n= 12
39 n= 8
40 n= 12
41 n= 8
42 n= 12
43 n= 8
44 n= 12

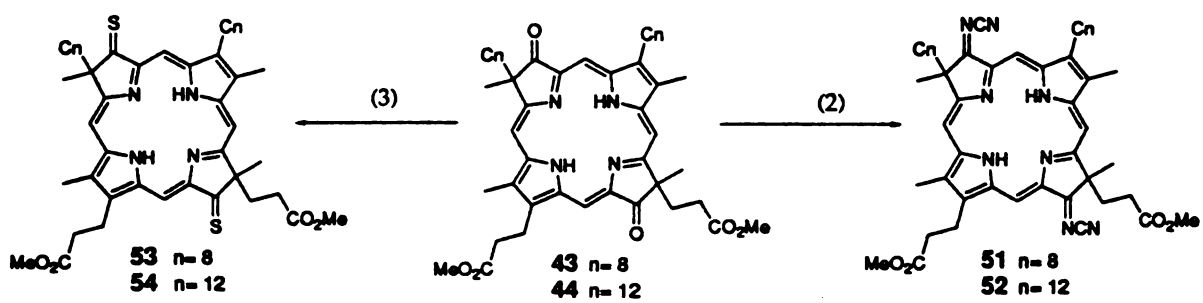
**Scheme 4**

and then with concentrated sulfuric acid in the same way as described above to yield the 2,7-didodecyl-3-porphyrinon (36) as the major product.

Reaction of the porphyrinone (35) and (36) with 1.2 equivalent of osmium tetroxide in dichloromethane/pyridine, followed by treatement with hydrogen sulfide effected dihydroxylation at the diagonal pyrrole β,β'-double bond of the oxopyrrole without any trace of isobacteriochlorin derivative, to give *vic*-dihydroxybacteriochlorins (39) and (40) respectively. This reaction pattern may be due to the prefered diagonal π-electron delocalization pathway present in all porphyrins, which prompts the saturation of the diagonal pyrrole β,β'-double bonds with minimum loss of π-resonance energy. Without isolation of the *vic*-diol derivatives (39) and (40), the reaction mixture was treated with concentrated sulfuric acid to yield the porphyrindiones (43) and (44) respectively in 40-50% yields. These porphyrinones (35 and 36) and porphyrindiones (43 and 44) were transformed to the corresponding dicyanomethylenyl (45 and 46), N-cyanoiminyl (47 and 48), bis(N-cyanoiminyl) (51 and 52), thionyl (49 and 50), dithionyl (53 and 54) derivatives as shown in Scheme 5, to shift their absorption maxima further to the red region.

In 1984 Aumüller and Hüing[88] devised a simple, one-step reaction to produce malonitrile adducts from the corresponding quinones by using malonitrile, pyridine and titanium tetrachloride. We have applied this method to the porphyrinones and the diones with good success. Copper(II) complexes of the porphyrinones (35) and (36) were separately treated with three equivalents of malonitrile in refluxing chloroform containing two equivalents of titanuim tetrachloride and an excess amount of pyridine for 30 minutes to yield the dicyanomethylenyl derivatives (45) and (46) respectively in 44-47% yields, after removal of the copper ion by treatment of the adduct




(1): i) $Cu(OAc)_2$; ii) $(CN)_2CH_2$, Py, $TiCl_4$; iii) TFA/$H_2S$, 20min
(2): i) $Me_3Si$-N=C=N-$SiMe_3$, $TiCl_4$, 4h, RT
(3): i) Lawesson's reagent, Toluene, reflux, 24h

**Scheme 5**

with hydrogen sulfide in trifluoroacetic acid. The bis-dicyanomethylenyl derivatives could not be otained because demetallation was impossible by any acid treatment after condensation with malonitrtle.

Aumüller and Hüing[89] also reported a convenient conversion of ketones and *p*-quinones to the corresponding N-cyanoimine and N,N'-dicyanoquinonediimine compounds by using bis(trimethylsilyl)-carbodiimide ($Me_3Si$-N=C=N-$SiMe_3$) with titanuim tetrachloride as auxiliary reagent. The N-cyanoiminyl (**47, 48**) and bis(N-cyanoiminyl) (**51** and **52**) compounds were easily prepared by treatment of the free base of the porphyrinones (**35** and **36**) and the porphyrindiones (**43** and **44**) respectively with titanium(IV) chloride and bis(trimethylsilyl)-carbodiimide in dichloromethane at room temperature. The reactions were completed within 4 hours in 41-59% yields.

In 1987 Chang and Sotiriou[90] reported the synthesis of octaethylporphyrin (OEP)-thione, dithiones and trithione from the oxo-analogues by using 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphosphetane-2,4-disulfide (Lawesson's Reagent). In 1988, independently, Balch *et al.*[91] also reported the synthesis of OEP-thione and a bacteriochlorin-type dithione. The porphyrinones (**35** and **36**) and the porphyrindiones (**43**and **44**) were treat with Lawesson's Reagent in refluxing toluene to give the corresponding thiones (**49**and **50**) and dithiones (**53** and **54**) respectively in 32-44% yields.

## III. RESULTS AND DISCUSSION

The starting porphyrins (**29** and **30**) were obtained in ~ 6% yield from condensation of the dipyrrymethenes (**22** or **23**) and (**28**). This low yield is

most likely due to the presence of bulky long aliphatic chains (octyl and dodecyl) which interrupt the cyclization of the two dipyrrylmethenes. The electronic obsorption spectra of the porphyrins (29 and 30) are exactly same as that of octaethylporphyrin in the Soret region as well as in the visible region.

The chlorin and bacteriochlorin derivatives have red shifted absorption spectra (compared to the unreduced porphyrin) as well as enhanced *in vivo* photosensitizing properties (measured by depth of tumor necrosis).[92] Enhancing long-wavelength absorption was achieved through oxo-substituted porphyrins which are the pinacol-type rearrangement products of the diols, and depending on the number and position of the oxo groups on a porphyrin ring, they can be considered structually derivatives of chlorin, isobacteriochlorin, or bacteriochlorin. The ketochlorin (35) was prepared according to Scheme 4. The intermediate porphyrin (29) was oxidized with osmium tetroxide to give predominantly the dihydroxychlorin (31) along with the isomeric chlorin (33). An acid-catalyzed pinacolic rearrangement was carried out,[93] and the principal product (35) was obtained. The isomeric chlorin (37) was also isolated in trace amounts but its PDT activities have not been evaluated yet. The didodecylporphyrin (30) showed the same reaction results as the dioctylporphyrin (29). The porphyrindione (43) was only obtained from the porphyrinone (35) by oxidation with osmium tetroxide and pinacolic rearrangement, and the porphyrindione (44) from the porphyrinone (36). This reaction pattern may be due to the prefered diagonal $\pi$-electron delocalization pathway, saturation of the diagonal pyrrole $\beta,\beta'$-double bonds, to minimize loss of $\pi$-resonance energy. The presence of the oxo group generally renders the ring more electronegative and consequently possessing red-shifted absorption peaks. It was realized that the red-shift can be greatly enhanced if the oxo group is to be modified into sulfido-, imino-,

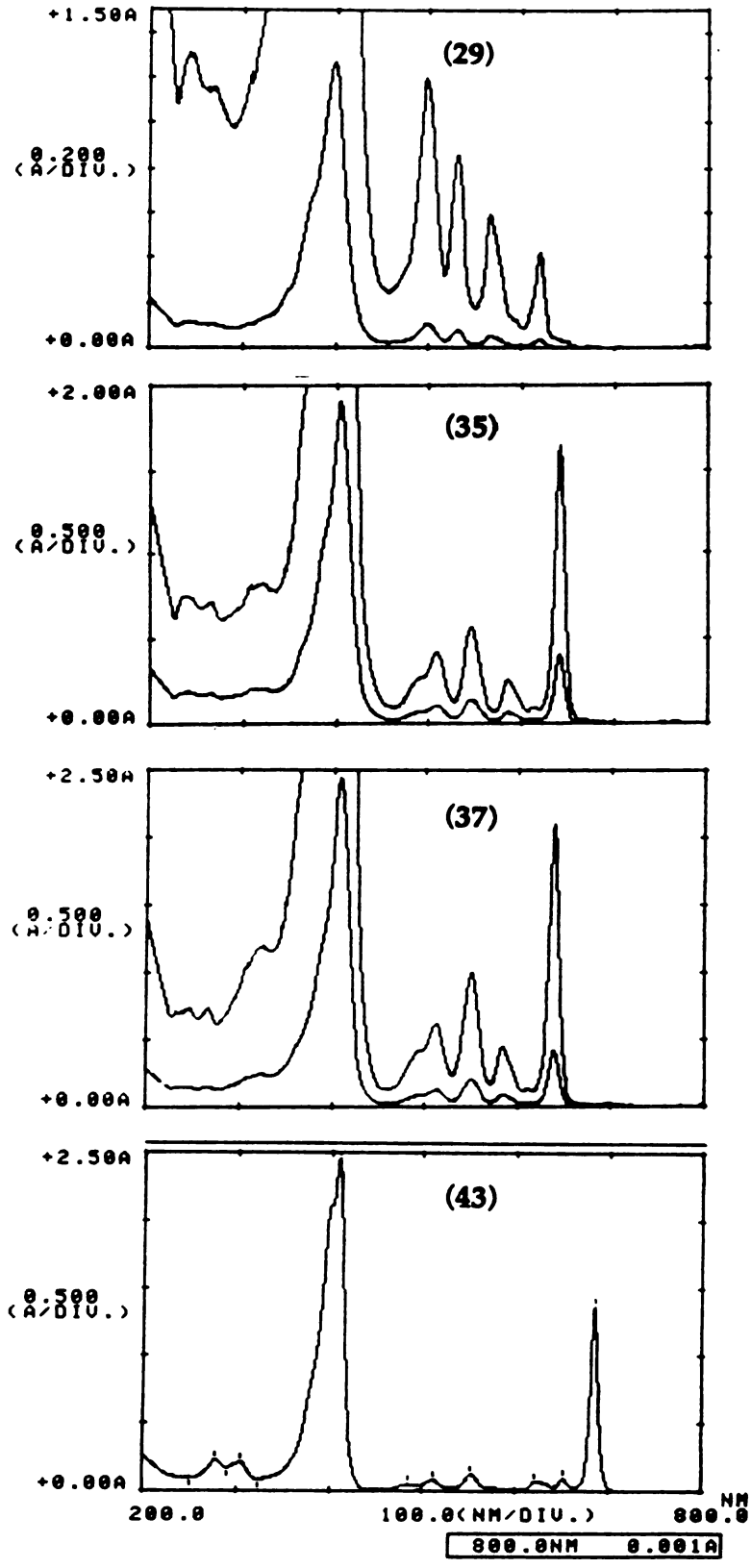



Figure 2. UV-vis absorption spectra of porphyrin (29), 3-porphyrinone (35), 13-porphyrinone (37), and 3,13-porphyrinone (43) in $CH_2Cl_2$.

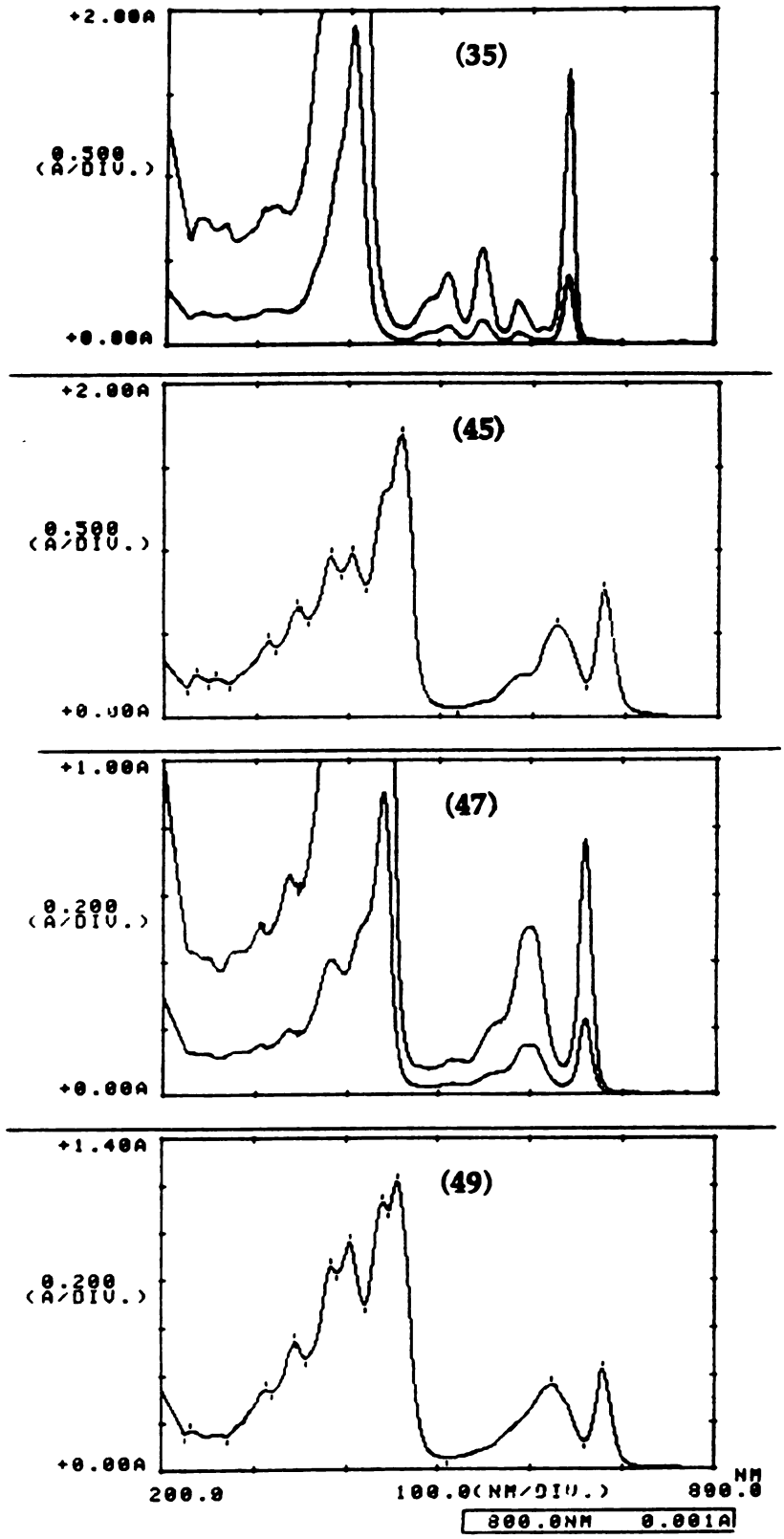



Figure 3. UV-vis absorption spectra of porphyrinone (35), 3-dicyanomethide (45), 3-imine (47), and 3-thione (49) in $CH_2Cl_2$.

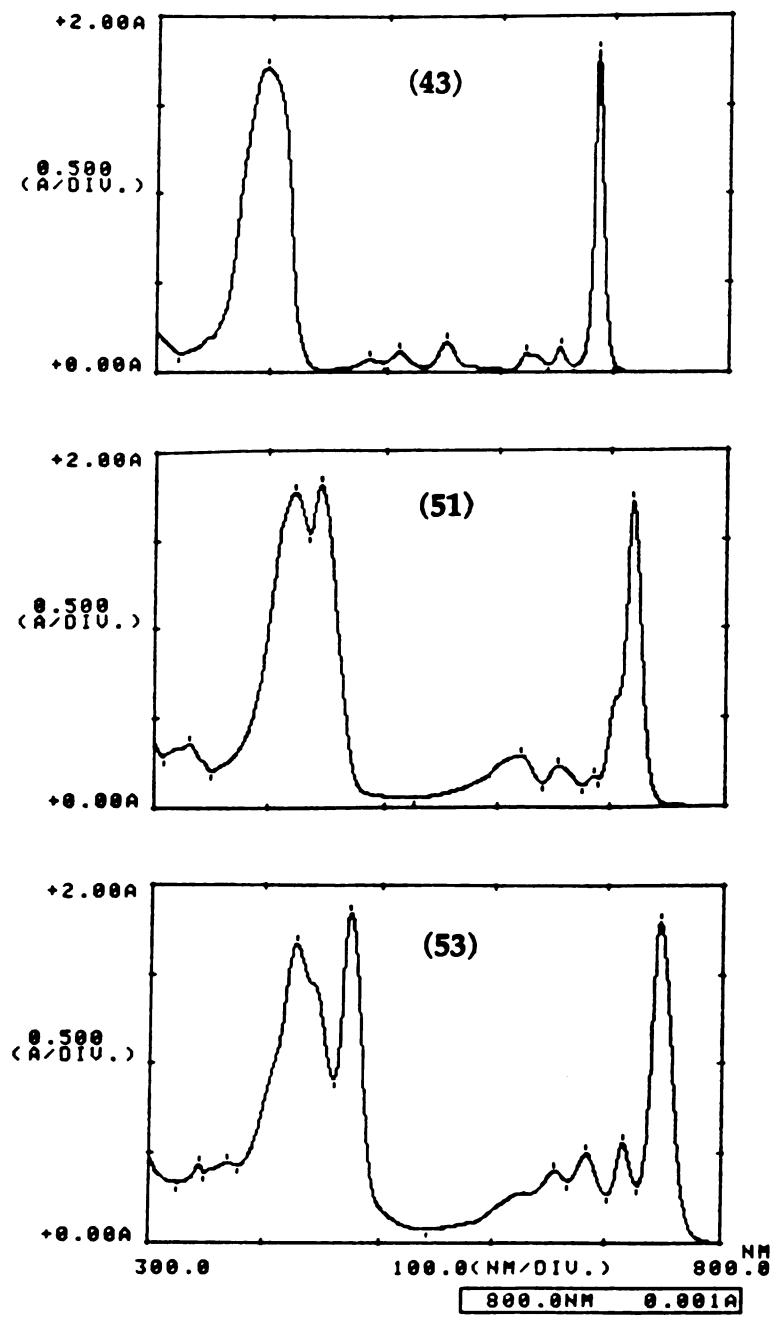



Figure 4. UV-vis absorption spectra of 3,13-dione (43), 3,13-diimine (51), and 3,13-dithione (53) in $CH_2Cl_2$.

and dicyanomethide adducts as shown in Figure 2~4. These transformations could be performed with satisfactory yields (35~60%).

The electronic absorption spectra of such adducts are always more complex than their oxo precursors, displaying multiple bands in the Soret region as well as in the visible region. While the theoretical interpretation of these spectral features is still incomplete, the extensive band shifts and splitting attest to the strong interactions existing between the porphyrin $\pi$-system and the exocyclic double bonds. Qualitatively, changing the number as well as the relative position of the oxo groups would affect the $\pi$ more than the $\pi^*$ orbitals (analogous to the trend: $\pi$ energy of porphyrin < chlorin < bacteriochlorin). However, addition of $\pi$-electronegative groups would perturb predominantly the $\pi^*$ orbitals. Therefore, with these compounds there is a great flexibility to modulate the long-wavelength absorption band which approximates the energy gap between $\pi$ and $\pi^*$. For the purpose of PDT applications, the simultaneous red-shift and the increase of the intensity in the red band(s) appear to be most remarkable

Visible spectroscopy can be a powerful tool in porphyrin chemistry for detecting changes in the chromophore of the macrocycle. One of the results of such a change is a shift to higher wavelength of the absorption of band I in the porphyrin visible spectrum. Because of both lower absorption and low scattering, tissue transmission is greater at the red end of the visible range than it is at the blue end, hence the advantage of having photosensitizers which absorb in the red with a higher molar extinction. Figure 5 illustrates the point schematically. The attractive absorption characteristics of these new porphyrinoidal chromophores seems to hold promise. The only potential complication may be that the sulfide-transforming Lawesson's reagent would simultaneously convert a carboxylic ester to a thionoester (-CSOR) because

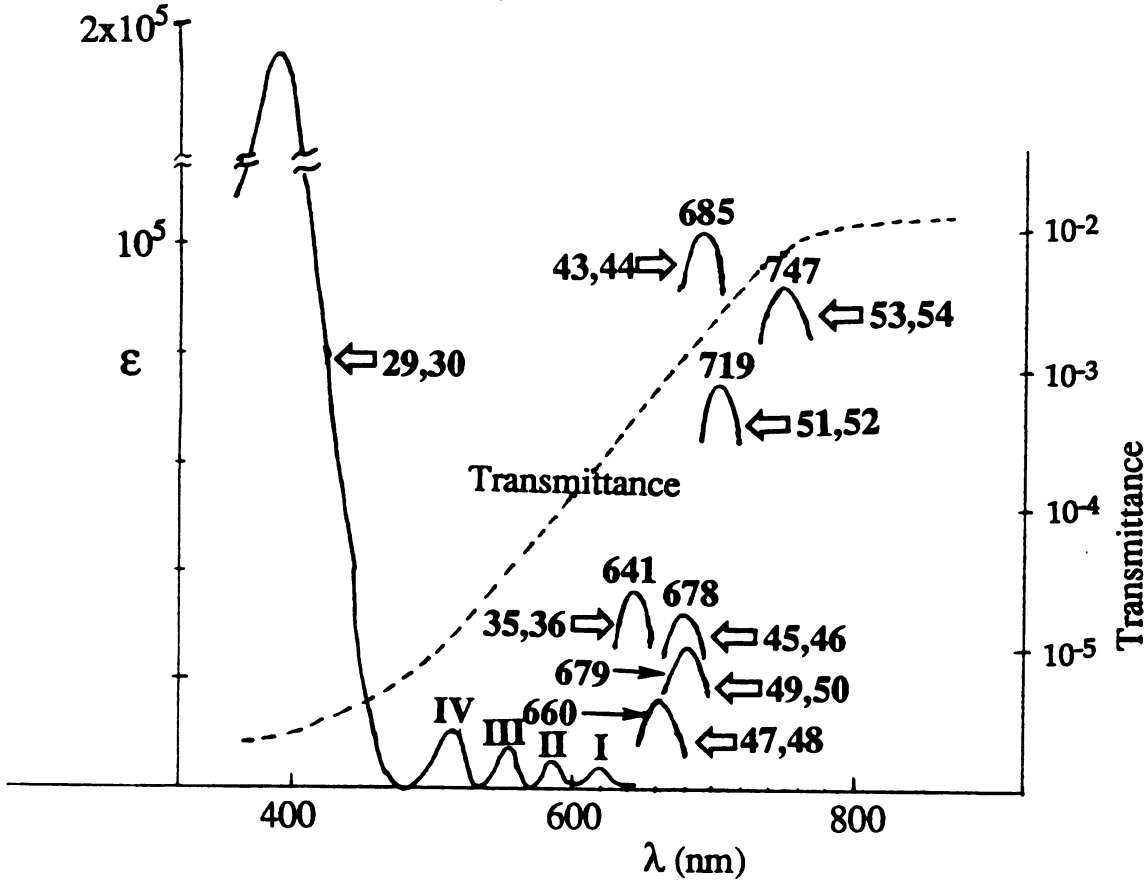



**Figure 5.** Tissue transmittance and photosensitizer absorbance (band I).

whi
sim
deli
and
bec
the
The
con
wit
turn
con
exa
Cre
ani
int
sen
bio
me
rea
mo
sub
me
the
pho
trip

which was detected by mass spectrometer. However, the thioacid is very similar and can be oxidized to an ordinary carboxylic acid. For drug delivering purposes, ester groups required prior hydrolysis. Under prolonged and strong base hydrolytic condition, the cyano group may be hydrolyzed because small portion of dicyanomethylenylchlorin (45) was turned back to the parent porphyrinone (35) after the hydrolysis in basic condition. Therefore the dicyanomethylenylchlorin (45) was hydrolyzed in acidic condition (in HCOOH containing 5% HCl, overnight, at room temperature) without any problem. The N-cyanoiminylchlorin (47) was unstable and turned back to the parent porphyrinone (35) in either acidic or basic hydrolytic condition. Fortunately ester compound could be solubilized in micells (for example, by using Tween 80; polyoxyethylene sorbitan monoleate or Cremophor EL; polyoxyethyleneglycol triricinoleate)[94] and injected directly to animals. As discussed in chapter 1, most of the phototoxicity may be divided into two major mechanisms[95]: first, the type I mechanism, in which the sensitizer molecules excited in the lowest triplet state ($T_1$) react directly with biological substrates to lead to cell damage, and second, the type II mechanism, in which the photogenerated triplet state ($T_1$) of the sensitizer reacts with the oxygen by an energy transfer process to produce singlet molecular oxygen ($^1O_2$), which in turn reacts with various biological substrates to injure the biological system. In either type I or type II mechanism, the photoreaction proceeds *via* the lowest excited triplet state of the sensitizer. Therefore, we can reasonably predict that the efficiency of the photosensitizing damage depends significantly on the lifetime of the lowest triplet state of the sensitizer.

When the type II mechanism predominates, the quantum yield of singlet molecular oxygen produced from the excited triplet state of the sensitizer, $\Phi_2$ is given to be [96~98]

$$\Phi_2 = k_{et}[^3O_2]/(k_{et}[^3O_2] + k_p)$$

where $k_{et}$ is the rate constant of the energy transfer from the sensitizer triplet to oxygen, $[^3O_2]$ is the effective concentration of oxygen dissolved in the medium concerned, and $k_p$ is the sum of the radiative and nonradiative deactivation rate constants for the sensitizer in the triplet state in the absence of oxygen, being the inverse of the triplet lifetime, $k_p=1/\tau_p$.

Accordingly, it is expected that the yield of singlet molecular oxygen is higher as the rate constant $k_p$ is smaller, that is, the triplet lifetime $\tau_p$ is longer. conversely, for compounds with short triplet lifetimes, it is suggested that the photooxygenation reaction is not apparently phototoxic. Thus, it is expected that compounds with a triplet lifetime longer than about 100$\mu$s are useful for PDT, while those with lifetime shorter than about 1$\mu$s are appropriate as diagnostic agents.[96] When heavy metal atom ions such as Fe, Co, Ni, Mn and Cu ions are introduced into the porphyrin, the decay rate constant, $k_p$, of the lowest triplet state ($T_1$) increases considerably and the rate constant in the intersystem crossing process ($S_1$-$T_1$) increases simultaneously because the spin-orbit interaction due to the heavy metal ion is exerted on both the $S_1$-$T_1$ and the $T_1$-$S_0$ processes.[99~101] Therefore, free-base (nonmetal) porphyrins have been used as photosensitizers for PDT.

The photooxygenation ability of the reagents synthesized in this study was examined as follows: A chloroform solution (2 mL) containing 100 $\mu$M of dansyl-L-methionine (d-Met) and 10 $\mu$M of the photosensitizer was irradiated

with a xenon lamp at room temperature with bubbling oxygen gas in the solution as shown in Figure 6. The reaction mixture was spotted on a thin-layer chromatography (TLC) plate (Kodak Chromagram Sheet 13181 silica gel) with micropipets (VWR 25 $\mu$L) every minute, and the TLC plate was developed in chloroform-methanol (7:3) and observed under a UV lamp ( 365 nm) after development. The reaction end point is represented by the time (min.) at which the spot of d-Met disappeared on the TLC plate.

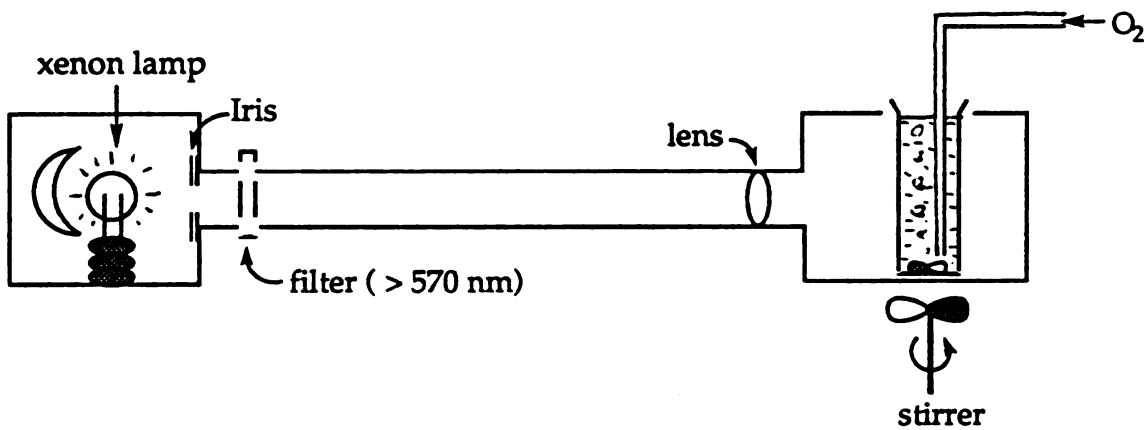



Figure 6 An outline of photolysis system

The photooxygenation strength (PS) of a compound is defined as

PS = 10 - (reaction end point)

A greater value of PS means that the photosensitizer possesses higher photooxygenation properties.[102,103] In Table 1, the absorption spectral peaks of Soret bands and the longest wavelengths and PS are summarized for all compounds in this study. The photoreactivities are represented by the relative rates of photooxygenation measured above. The highest rate is represented by 10 and the lowest by 0. For the sake of comparison, it should be noted that the rate of photooxygenation is zero for octaethylporphyrin.

Table 1 UV-vis absorption spectral data and relative photooxygenation strengh data

| | [ Absorbance (nm) ] | | | |
|---|---|---|---|---|
| Compound | $\lambda_{Soret}$[a] | $\lambda_{longest}$[a] | EP[b] | PS[c] |
| **29** | 398 | 620 | 8.33 | 1.67 |
| **35** | 405 | 641 | 3.33 | 6.67 |
| **45** | 459 | 678 | 0.83 | 9.17 |
| **47** | 439 | 660 | 1.25 | 8.75 |
| **49** | 457 | 679 | 1 | 9.00 |
| **43** | 409 | 685 | 1 | 9.00 |
| **51** | 448 | 719 | 0.42 | 9.58 |
| **53** | 477 | 747 | 0.42 | 9.58 |
| OEP[d] | 405 | 620 | 10 | 0 |

(a) $\lambda_{Soret}$ and $\lambda_{longest}$ represent $\lambda_{max}$ at Soret bands and at the longest wavelength in $CH_2Cl_2$.
(b) EP represents the standardized reaction end point. The highest rate is represented by 10 and the lowest by 0.
(c) PS represents relative photooxygenation strength. PS = 10 - EP
(d) OEP=Octaethylporphyrin.

The biological tests [94,104]of the porphyrin derivatives which have been carried out by Dr. Kessel and Dr Henderson can be summarized as follows: All experiments were performed on female C3H/HeJ mice which, for tumor response studies, carried the RIF (radiation-induced fibrosarcoma) tumor. All photosensitizers were dissolved with the aid of Tween 80 (TW80) or Cremophor EL (CRM) and injected into mice *via* tail vein. For light delivery, a 20 Watt argon dye laser system (Spectra Physics) pumping a dye laser using

DCM dye (Cooper Lasersonics, Inc., Palo Alto, CA), tuned to 642 nm (porphyrinone, **35**), 660 nm (N-cyanoiminylchlorin, **47**), 679 nm (porphyrinthione, **49**) and 685 nm (porphyrindione, **43**) by birefringent filter. In assessment of tumor response, mice were examined at 2-3 day intervals and the shortest and longest tumor diameters were measured with calipers. Tumor response was assessed through growth delay analysis as follows. Log relative tumor volumes (volume after treatment/volume before treatment) for the exponentially regrowing tumors were potted against time after treatment in days. Volumes were calculated from tumor diameters using the formula for a prolate ellipsoid $(LxW^2)/2$, where L is the longest diameter. The linear portion of the regrowth curve for each tumor was fitted with a regression line, and the time of tumor regrowth to 10 times its original (treatment) volume was determined. Tumors were considered cured if no tumor regrowth occurred by 30 days post treatment. The regrowth rate was therefore calculated only for animals in which a 30 day cure was not achieved. *In vivo/in vitro* (excision) cell survival assay, to assess photosensitization of RIF rumor cells after *in vivo* exposure to various sensitizers, the latter were injected and allowed to accumulate in the tumor for 3 or 24 hours. Tumors were then excised, finely minced and dispersed by an established enzyme procedure. Two mL aliquots of the single cell suspension were transferred to wells of a 24 well tissue culture plate, and exposed to light as described above for *in vivo* treatment. Following graded does of light, cells were transferred to 60 mm plastic culture plates for clonogenic assay.

Table 2 shows the tumor response data. Drug does (de)escalation was started at 4 $\mu$mole/kg, dropped to 0.4 $\mu$mole/kg if toxicity was observed, and in the case of porphyrinone(**35**) back-escalated to 0.8 and 1.6 $\mu$mole/kg. Light treatments were carried out generally at 3 and 24 hours after drug injection.

Table 2. Photodynamic therapy responses using the RIF tumor.

| Sensitizer | Drug dose (μmole /kg) | Light dose (J/cm$^2$) | Time[a] (hour) | n | Regrowth[b] (days) | 30 day cures | Death |
|---|---|---|---|---|---|---|---|
| none | – | – | – | 10 | 5.0 (0.3) | – | – |
| none | – | 135 | – | 4 | 7.8 (0.6) | – | – |
| Thione (49) | 4.0 | – | – | 3 | 6.8 (0.1) | – | – |
| | 4.0 | 135 | 3 | 4 | 12.2 (1.7) | – | – |
| | 4.0 | 135 | 24 | 4 | 9.6 (1.9) | – | – |
| Imine (47) | 0.4 | – | – | 5 | 6.7 (0.4) | – | – |
| | 0.4 | 135 | 3 | 12 | 17.4 (3.6) | 4 | 6 |
| | 0.4 | 135 | 24 | 10 | 11.4 (2.2) | – | – |
| Porphyrinone (35) in TW80 | 0.8 | – | – | 5 | 6.0 (0.3) | – | – |
| | 0.4 | 135 | 3 | 7 | 20.2 (4.3) | 1 | – |
| | 0.4 | 135 | 24 | 11 | 12.6 (1.7) | – | – |
| | 0.8 | 135 | 3 | 8 | 13.5[c] | 7 | – |
| | 0.8 | 135 | 24 | 5 | 11.2 (0.4) | | |
| Porphyrinone (35) in CRM | 0.4 | 135 | 3 | 10 | 15.0 (2.2) | 5 | – |
| | 0.4 | 135 | 24 | 9 | 11.0 (1.0) | 1 | – |
| Dione (43) | 0.2 | – | – | 5 | 6.5 (0.3) | – | – |
| | 0.2 | 135 | 3 | 5 | 13.7 (1.4) | – | – |
| | 0.2 | 135 | 24 | 4 | 10.1 (1.0) | – | – |

(a) Time in hours between injection of sensitizer and light treatment.
(b) Time (days) for tumor regrowth to 10 times the original tumor volume (average ±SE). This was calculated only for animals in which a 30 day cure was not achieved.
(c) Value from one animal only.

In some instances they have tried other intervals, but since no great improvement of effects were observed, these data are not listed for clarity.

The thione (49) was the least effective. The cyanoiminylchlorin (47) was potent but resulted in a high number of death after treatment (animals apparently dying of some shock syndrome). The porphyrindione (43) was also toxic at drug doses higher than 0.2 μmole/kg (only after treatment, not in the dark), and not very effective at that dose. The porphyrinone (35) was the best compound of this group, both in TW 80 and CRM, giving high numbers of cures. The difference between TW 80 and CRM is not significant with this numbers of animals tested, but CRM may be slightly better. All drugs were relatively ineffective when light was delivered 24 h after drug injection.

Like the dimeric ketochlorins described in Kessel *et al.*,[105] the porphyrinone (35) was a very effective short-acting sensitizer against the RIF tumor *in vivo* , with a significant number of animals tumor-free after 30 days. Because the porphyrinone (35) must be solubilized for injection, the role of carrier systems in drug biodistribution becomes an important consideration.

The PDT efficacy of the porphyrinone (35) was correlated with plasma rather than with tumor concentration of the porphyrinone (35) (Table 2 and Table 3). Such a result was previously reported for the chlorin photosensitizer NPe6[106] and this was attributed to vascular shut-down, rather than a direct tumor cell kill during PDT. While the porphyrinone (35) was an efficacious photosensitizing agent using either vehicle, the number of 30 day survivors was substantially greater when a 0.4 μmol/kg drug does was formulated with CRM than with TW 80. This result was associated with a longer persistence of the sensitizer in plasma and tissues, suggesting that binding of the sensitizer to a CRM-induced lipoprotein degradation product plays an important role in the promotion of vascular photosensitization.

Table 3. Distribution of porphyrinone (35) in tumor-bearing mice*

| | CRM | | TW80 | |
|---|---|---|---|---|
| Tissuue | 3 h | 24 h | 3 h | 24 h |
| Plasma | 46.94 ± 2.39 | 9.97 ± 2.14 | 27.73 ± 2.87 | 4.72 ± 0.60 |
| Tumor | 3.25 ± 0.15 | 6.69 ± 1.25 | 2.34 ± 0.05 | 3.50 ± 0.43 |
| Skin | 1.37 ± 0.29 | 2.27 ± 0.12 | 1.28 ± 0.20 | 1.46 ± 0.18 |
| Liver | 19.39 ± 0.87 | 22.45 ± 2.51 | 34.84 ± 1.27 | 24.77 ± 0.40 |
| Muscle | 0.91 ± 0.07 | 1.67 ± 0.16 | 0.84 ± 0.19 | 0.84 ± 0.05 |

* Levels of (35) in RIF tumor and plasma 3 and 24 h after administration as a function of the drug-delivery vehicle. The compound (35) concentrations are expressed as mg/g tissue (wet weight) or mg/mL of plasma. These values represent the mean ± SD of three determinations.

It is important to note that blood from many animals used in preclinical studies, e.g. mouse, rat and dog, exhibit very low levels of LDL, with the major lipoprotein species represented by HDL.[107] The effects of different drug-delivery vehicles on drug biodistribution in man, where LDL> HDL, remains to be determined. They interpreted the data presented here to indicate that choice of delivery vehicle may be as important as the choice of photosensitizer with regard to the long-term efficacy of PDT.

# IV. EXPERIMENTAL

## General

Proton NMR spectra were obtained in $CDCl_3$ at 300 MHz (Varian Gemini 300 FT NMR spectrometer) using TMS or $CHCl_3$ (7.24 ppm) as internal standards. Spectra were mostly recorded in $CDCl_3$; the residue $CHCl_3$ was used as the internal standard set at 7.24 ppm. Melting points were measured on an electrothermal melting apparatus and are uncorrected. Visible absorption spectra were obtained on a Shimatzu UV-160 spectrophotometer using solution in $CH_2Cl_2$. Mass spectral data were obtained on a Fisons VG TRIO-1 GC-MS mass spectrometer purchased under a NIH Shared Instrumentation Grant (S10RR06506-01) or a JEOL HX 110-HF mass spectrometer equipped with a fast atom bombardment (FAB) gun which purchased under a NIH grant (DRR-00480), for high resolution mass spectra. Porphyrin-modification reactions were usually carried out in the dark (aluminium foil) under argon and were monitored using TLC on commercially available Eastman Kodak 13181 (100 $\mu$m thick) silica gel sheets. Preparative TLC was carried out on 20x20 cm glass plates coated with Analtech silica gel GF (1000 or 1500 $\mu$m thick), and in column chromatography 200-400 mesh silica gel was used.

### Methyl α-oximinoacetoacetate (15)

Methyl acetotacetate (209 g, 1.8 mol) was dissolved in acetic acid (360 mL) and the solution was cooled in an ice bath. A saturated aqueous solution of sodium nitrite (138 g, 2 mol) was added dropwise with stirring and the temperature was controlled below 20 °C. The reaction was continued for

another one hour after the addition. The orange oxime solution was kept at low temperature or used immediately.

Sodium 2-methyl-3-oxobutyraldehyde (16)

Sodium methoxide (140 g) suspended in dry diethyl ether (2 L) was cooled in an ice bath. 2-Butanone (144.3 g, 2 mol) and ethyl formate (148.2 g, 2 mol) were added dropwise for 2-3 hours with stirring and the temperature was maintained below 20 °C. The reaction was continued for one hour at room temperature after the addition. After distillation of diethyl ether, the product was further dried under vaccum. The resulting product was directly used to prepare (17).

Methyl 4,5-dimethylpyrrole-2-carboxylate (17)

The above oxime solution (1.8 mol) was added dropwise to the well stirred solution of sodium 2-methyl-3-oxobutyraldehyde (16) (220 g, 1.8 mol) in acetic acid (650 mL) along with addition of zinc dust in small portions. During addition of zinc powder (400 g, 6 mol), the reaction temperature was maintained 85-90 °C. After the addition, the reaction mixture was refluxed for an additional one hour and then poured into ice/water (2 L). The crude product was precipitated as a yellow solid which was colleted by filtration and wash with water. The solid was dissolved in dichloromethane (800 mL), filtered again to remove zinc powder and dried over sodium sulfate. Evaporation of solvent gave the pyrrole which was crystallized from methanol to give the title compound (104.5 g, 38%): m.p. 138-139 °C; $^1$H NMR ($CDCl_3$) δ 1.98(3H, s, 4-Me), 2.18 (3H, s, 5-Me), 3.79 (3H, s, OMe), 6.65 (1H, s, 3-H), 8.95 (1H, br s, NH); MS for $C_8H_{11}NO_2$, found m/e 153 ($M^+$).

Methyl 4,5-dimethyl-3-octanoylpyrrole-2-carboxylate (18)

Methyl 4,5-dimethylpyrrole-2-carboxylate (17) (30.6 g, 0.2 mol) was dissolved in dry dichloromethane (160 mL) with heating. The solution was cooled to 10 °C in an ice bath and octanoyl chloride (38 mL, 0.22 mol) was added. To this solution, tin(IV) chloride (35 mL, 0.3 mol) was slowly added through a pressure-equalized dropping funnel (temperature < 20 °C). After the addition, the reaction mixture was stirred for another one hour in the ice bath and then poured into ice/water (200 mL). The organic phase was separated, washed twice with aqueous sodium carbonate, then with water, and dried over anhydrous sodium sulfate. The solvent was evaporated under vacuum to yield a white mass of octanoyl pyrrole (54.2 g, 97%). The product was essentially pure and no further purification was needed.

For the title compound: m.p. 73-75 °C; $^1$H NMR ($CDCl_3$) δ 0.85 (3H, t, octanoyl Me), 1.27 (8H, m, $CH_2$), 1.63 (2H, q, $COCH_2\underline{CH_2}$), 1.93 (3H, s, 4-Me), 2.17 (3H, s, 5-Me), 2.82 (2H, t, $CO\underline{CH_2}$), 3.79 (3H, s, OMe), 8.95 (1H, br s, NH); MS for $C_{16}H_{25}NO_3$, found m/e 279 ($M^+$).

Methyl 4,5-dimethyl-3-dodecanoylpyrrole-2-carboxylate (19)

Methyl 4,5-dimethylpyrrole-2-carboxylate (17) (30.6 g, 0.2 mol) was treated with lauroyl chloride (50.9 mL, 0.22 mol), following the method described for the octanoylpyrrole (4) to give the title compound (65.3 g, 97.6%). The product was essentially pure and no further purification was needed.

For the title compound: m.p. 71-72 °C; $^1$H NMR ($CDCl_3$) δ 0.84 (3H, t, dodecanoyl Me), 1.30 (16H, m, $CH_2$), 1.64 (2H, q, $COCH_2\underline{CH_2}$), 1.92 (3H, s, 4-Me), 2.18 (3H, s, 5-Me), 2.82 (2H, t, $CO\underline{CH_2}$), 3.80 (3H, s, OMe), 8.95 (1H, br s, NH); MS for $C_{20}H_{33}NO_3$, found m/e 335 ($M^+$).

Methyl 4,5-dimethyl-3-octylpyrrole-2-carboxylate (20)

Sodium borohydride (7.5 g, 0.2 mol) was added to the essentially pure methyl 4,5-dimethyl-3-octanoylpyrrole-2-carboxylate (18) (27.9 g 0.1 mol) dissolved in dry tetrahydrofurane (100 mL). The reaction mixture was cooled to 10 °C with stirring in an ice bath and boron trifluoride etherate (37 mL 0.3 mol) was slowly added so that the reaction temperature was maintained below 20 °C. After the addition of boron trifluoride etherate, the mixture was stirred for an additional one hour in the ice bath and then poured into a mixture of ice (200 mL), hydrochloric acid (1N, 50 mL) and dichlormethane (200 mL). The organic phase was separated and washed with hydrochloric acid (0.5N, 200 mL). Methanol (50 mL) was added and the solvent was evaporated to dryness. The white product was crystallized from methanol-water (85:15) to yield the title compound (26.2 g, 98%): m.p. 77-80 °C; $^1$H NMR ($CDCl_3$) δ 0.85 (3H, t, octyl Me), 1.25-1.45 (12H, m, $CH_2$), 1.90 (3H, s, 4-Me), 2.18 (3H, s, 5-Me), 2.66 (2H, t, $CH_2$), 3.79 (3H, s, OMe), 8.55 (1H, br s, NH); MS for $C_{16}H_{27}NO_2$, found m/e 265.20, calcd m/e 265 ($M^+$).

Methyl 4,5-dimethyl-3-dodecylpyrrole-2-carboxylate (21)

Methyl 4,5-dimethyl-3-dodecanoylpyrrole-2-carboxylate (19) (33.5 g, 0.1 mol) was treated with sodium borohydride (7.5 g, 0.2 mol) and boron trifluoride etherate (37 mL, 0.3 mol) following the method described for the octylpyrrole (5) to give the title compound (31.5 g, 98%), after crystallization from methanol-water (85:15): m.p. 75-78 °C; $^1$H NMR ($CDCl_3$) δ 0.86 (3H, t, octyl Me), 1.25-1.53 (20H, m, $CH_2$), 1.91 (3H, s, 4-Me), 2.18 (3H, s, 5-Me), 2.67 (2H, t, $CH_2$), 3.80 (3H, s, OMe), 8.55 (1H, br s, NH); MS for $C_{20}H_{35}NO_2$, found m/e 321 ($M^+$).

4,4',5,5'-Tetramethyl-3,3'-dioctyl-2,2'-dipyrrylmethenium bromide (22)

A mixture of methyl 4,5-dimethyl-3-octylpyrrole-2-carboxylate (20) (5.3 g, 0.02 mol), 48% hydrobromic acid (5 mL) and 88% formic acid (50 mL) was heated on a steam bath for 80 minutes until effervescense subsided. After standing overnight, chunky solids formed were collected by filtration, and recrystallized from methanol/hexane to give the title compound as red-orange sparkling crystals (4.4 g, 87%): $^1$H NMR ($CDCl_3$) δ 0.85 (6H, t, octyl Me), 1.30 (20H, m, $CH_2$), 1.48 (4H, q, $CH_2$), 1.95 (6H, s, 4,4'-Me), 2.58 (4H, t, $CH_2$), 2.62 (6H, s, 5, 5'-Me), 6.99 (1H, s, methine), 12.90 (2H, br s, NH); MS for $C_{29}H_{49}N_2Br$, found m/e 426 ($M^+$).

4,4',5,5'-Tetramethyl-3,3'-didodectyl-2,2'-dipyrrylmethenium bromide (23)

Methyl 4,5-dimethyl-3-dodecylpyrrole-2-carboxylate (21) (6.5 g, 0.02 mol) was treated with 48% hydobromic acid (5 mL) and 88% formic acid (50 mL) following the procedure described for the 3,3'-dioctyl-2,2'-dipyrrylmethenium bromide (22) to afford the title compound (10.8 g, 87.5%), after crystallization from methanol/hexane: $^1$H NMR ($CDCl_3$) δ 0.86 (6H, t, octyl Me), 1.32 (36H, m, $CH_2$), 1.46 (4H, q, $CH_2$), 1.96 (6H, s, 4, 4'-Me), 2.58 (4H, t, $CH_2$), 2.62 (6H, s, 5, 5'-Me), 7.00 (1H, s, methine), 12.90 (2H, br s, NH); MS for $C_{37}H_{65}N_2Br$, found m/e 538 ($M^+$).

Benzyl 5-acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (25)

Lead tetraacetate (48.7 g, 0.11 mol) was added to a solution of (24) (32.9 g, 0.1 mol) in glacial acetic acid (150 mL) with stirring at room temperature. The reacton was accomplished by heating on a steam bath for one hour and then the reaction mixture was poured into a large amount of water ( > 1 L). The

precipitated solid was separated by filtration, rinsed with water, and crystallized from aqueous acetone to give the title compound as ivory-white needles (34.4 g, 95%): m.p. 111-112 °C; $^1$H NMR ($CDCl_3$) δ 2.04 (3H, s, 3-Me), 2.26 (3H, s, $CH_3CO$), 2.43 (2H, t, $\underline{CH_2}CH_2CO$), 2.76 (2H, t, $CH_2\underline{CH_2}CO$), 3.64 (3H, s, OMe), 5.02 (2H, s, $CH_3CO_2\underline{CH_2}$), 5.28 (2H, s, $C_6H_5\underline{CH_2}O$), 7.38 (5H, m, phenyl H), 9.09 (1H, br s, NH); MS for $C_{20}H_{23}NO_6$, found m/e 373 ($M^+$).

3,3'-Bis(2-methoxycarbonylethyl)-5,5'-dibenzyloxycarbonyl-4,4'-dimethyl-2,2'-dipyrrylmethane (26)

Benzyl 5-acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (25) (280 g, 0.75 mol) was dissolved in 70% acetic acid-water (200 mL) and heated to reflux for one hour. After reaction was accomplished, the hot reaction mixture was poured into a large amount of water and allowed to cool down slowly to precipitate solid product. The precipitated solid was collected by filtration, washed with water, and crystallized from ethanol to give the title compound as ivory-white needles (188.7 g, 82%): m.p. 96-97 °C; $^1$H NMR ($CDCl_3$) δ 2.28 (6H, s, 4, 4'-Me), 2.50 (4H, t, $\underline{CH_2}CH_2CO$), 2.76 (4H, t, $CH_2\underline{CH_2}CO$), 3.58 6H, s, OMe), 3.96 (2H, s, 2, 2'-methane), 5.24 (4H, s, $O\underline{CH_2}C_6H_5$), 7.33 (10H, m, phenyl protons), 9.29 (2H, br s, NH); MS for $C_{35}H_{38}N_2O_8$, found 614 ($M^+$).

5,5'-Dibromo-3,3'-bis(2-methoxycarbonylethyl)-4,4'-dimethyl-2,2'-dipyrrylmethenium bromide (28)

3,3'-Bis(2-methoxycarbonylethyl)-5,5'-dibenzyloxycarbonyl-4,4'-dimethyl-2,2'-dipyrrylmethane (26) (16 g, 0.026 mol) was dissolved in freshly distilled tetrahydrofuran (300 mL) containing a few drops of triethylamine. 10% Palladium/carbon (0.5 g) was added and the reaction mixture was hydrogenated under hydrogen (1 atm, room temperature) until hydrogen uptake ceased. The solvent was evaporated and dried under vacuum without removing the catalyst by filtration because the resultant 2,2'-dipyrrylmethane-5,5'-dicarboxylic acid (27) is only slightly soluble in tetrahydrofuran. The dried reaction mixture, which contains the compound (27) and catalyst carbon, was added in a mixture of 98-100% formic acid (85 mL) and bromine (8.5 mL) in small portions. The reaction was accomplished by stirring at room temperature for an additional one hour after the addition, and then carbon was filtered off and washed with formic acid. Most of formic acid was removed under reduced pressure and then diethyl ether (50 mL) was added to precipitate the purple solid product. The purple solid was collected by filtration and rinsed with cyclohexene and hexane to give the title compound (12.20 g, 80.5%): m.p. 180-182 °C; $^1$H NMR ($CDCl_3$) δ 2.05 (6H, s, 4, 4'-Me), 2.59 (4H, t, $\underline{CH_2}CH_2CO$), 3.07 (4H, t, $CH_2\underline{CH_2}CO$), 3.60 (6H, s, OMe), 7.59 (1H, s, methine), 14.12 (2H, br s, NH); MS for $C_{19}H_{23}N_2O_4Br_3$, found m/e 503 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-3,7-dioctylporphyrin-13,17-dipropionate (29)

4,4',5,5'-Tetramethyl-3,3'-dioctyl-2,2'-dipyrrylmethenium bromide (22) (5.05 g, 0.01 mol) and 5,5'-dibromo-3,3'-bis(2-methoxycarbonylethyl)-4,4'-dimethyl-2,2'-dipyrrylmethenium bromide (28) (5.83 g, 0.01 mol) were dissolved in anhydrous formic acid (50 mL). To this reaction mixture ,

bromine (0.52 mL, 0.01 mol) was added and the mixture was heated to reflux in an oil bath for 2 hours. The solvent was allowed to boil off over 4 hours with a stream of air or until completely dried. Methanol (100 mL) and concentrated sulfuric acid (2 mL) were added to the dried reaction residue, followed by addition of trimethyl orthoformate (5 mL). After standing overnight, protected from moisture, the reaction mixture was diluted with dichloromethane (100 mL) and then neutralized with saturated aqeous sodium acetate (100 mL). The organic layer was separated, washed once again with saturated aqueous sodium acetate(60 mL) and then three times with water (100 mL). After evaporation of the solvent, the residue was chromatographed on silica gel column (50 to 250 mesh) with 1% methanol in dichloromethane as eluent. A dark non-fluorescent forerun was discarded and the moving porphyrin band on chromatography column can be monitored by using UV-lamp to ensure a complete collection. The fractions containing porphyrin were combined, evaporated to dryness under vacuum, and crystallized from dichloromethane and methanol to give the title compound as sparkling crystals (0.46 g, 6%): $^1$H NMR ($CDCl_3$) δ -3.80 (2H, br s, NH), 0.82 (6H, t, octyl Me), 1.28 (12H, m, $CH_2$) 1.49, 1.72, 2.27 (4H each, q, $CH_2$), 3.28 (4H, t, $CH_2\underline{CH_2}CO$), 3.60, 3.63, 3.64 (6H each, s, 2, 8, 12, 18-Me and OMe), 4.03 (4H, t, $CH_2$), 4.41 (4H, t, $\underline{CH_2}CH_2CO_2$), 10.07, 10.08 (1H each, s, Meso H), 10.09 (2H, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 620 nm (5,100), 566 (7,000), 531 (10,200), 498 (14,300), 398 (178,100); MS for $C_{48}H_{66}N_4O_4$, found m/e 763 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-3,7-didodecylporphyrin-13,17-dipropionate (30)

4,4',5,5'-Tetramethyl-3,3'-didodectyl-2,2'-dipyrrylmethenium bromide (23) (6.17 g, 0.01 mol) and 5,5'-dibromo-3,3'-bis(2-methoxycarbonylethyl)-4,4'-dimethyl-2,2'-dipyrrylmethenium bromide (28) (5.83 g, 0.01 mol) was treated with one equivalent of bromine in anhydrous formic acid (50 mL) as described before for the dioctylporphyrin (29) to give the title compound (0.49 g, 5.6%), after crystallization from dichloromethane and methanol: $^1$H NMR ($CDCl_3$) δ -3.90 (2H, br s, NH), 0.84 (6H, t, dodecyl Me), 1.21 (32H, m, $CH_2$), 1.49, 1.71, 2.27 (4H each, q, $CH_2$), 3.29 (4H, t, $CH_2\underline{CH_2}CO$), 3.57, 3.60, 3.69 (6H each, s, ring Me, OMe), 3.99 (4H, t, $CH_2$), 4.39 (4H, t, $\underline{CH_2}CH_2CO$), 9.97, 9.99, 9.99, 10.00 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 620 nm (5,200), 566 (7,100), 531 (10,100), 497 (14,400), 398 (177,900); MS for $C_{56}H_{82}N_4O_4$, found m/e 875 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3-porphyrinone-13,17-dipropionate (35), Dimethyl 2,8,12,18-tetramethyl-3,7-dioctyl-13-porphyrinone-12,17-dipropionate (37)

Osmium tetroxide (310 mg, 1.2 mmol) and pyridine (1 mL) were added to dimethyl 2,8,12,18-tetramethyl-3,7-dioctylporphyrin-13,17-dipropionate (29) (763 mg, 1.0 mmol) dissolved in dichloromethane (100 mL). After the reaction mixture was stirred for 24 hours at room temperature under nitrogen in the dark for 24 hours, it was quenched by adding methanol (50 mL), then bubbled with hydrogen sulfide through the reaction solution for 20 minutes to decompose the osmate adducts and allowed to stand for 1-2 hours. The precipitated black osmium sulfide was removed by filtration through a bed of Celite. Evaporation of the filtrate gave crude intermediates (*vic*-

dihydroxychlorins (31) and (33)) which were subsequently treated with concentrated sulfuric acid (10 ml) for 30 minutes at room temperature without further purification. The reaction mixture was cooled in an ice bath and diluted with methanol (50 mL). The reaction solution was then allowed to stand overnight at room temperature, protected from moisture, to ensure re-esterification. The solution was diluted with dichloromethane (100 mL) and the organic layer was washed twice with saturated aqueous sodium acetate (100 mL) and twice with water (100 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under vacuum and the residue was chromatographed on silica gel with 1% methanol in dichloromethane as eluent. The porphyrinone (35) (250 mg, 46% yield based on reacted (29))was isolated as the major product along with the unreacted porphyrin (29) (230 mg). A small amount of the isomeric porphyrinone (37) (10 mg, 1.3%) was also obtained.

For dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3-porphyrinone-13,17-dipropionate (35): $^1$H NMR ($CDCl_3$) δ -3.05 (2H, br s, NH), 0.65 (3H each, t, octyl Me), 0.90 (12H, m, $CH_2$), 1.30 (6H, m, $CH_2$), 1.45, 1.69, 2.19 (2H each,q, $CH_2$), 2.04 (3H, s, 2-Me), 2.70 (2H, t, 7-$CH_2$), 3.19, 3.26 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.48, 3.57, 3.57, 3.65, 3.66 (3H each, s, 8, 12, 18-Me, OMe), 3.90 (2H, dt, 2-$CH_2$), 4.24 (2H, t, 13-$\underline{CH_2}CH_2CO_2$), 4.40 (2H, t, 17-$\underline{CH_2}CH_2CO_2$), 9.14, 9.82, 9.89, 9.93 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 641 nm (36,500), 614 (2,000), 586 (5,700), 547 (13,200), 508 (9,300), 405 (162,300); MS for $C_{48}H_{66}N_4O_5$, found m/e 779 ($M^+$).

For dimethyl 2,8,12,18-tetramethyl-3,7-dioctyl-13-porphyrinone-12,17-dipropionate (37): $^1$H NMR ($CDCl_3$) δ -2.95 (2H, br s, NH), 0.65 (3H each, t, octyl Me), 0.90 (12H, m, $CH_2$), 1.30 (6H, m, $CH_2$), 1.47, 1.70, 2.20 (2H each, q, $CH_2$), 2.05 (3H, s, 12-Me), 2.72 (2H, t, 3-$CH_2$), 3.25 (4H, dt, $CH_2\underline{CH_2}CO_2$), 3.58,

3.58, 3.66, 3.67, 3.73 (3H, each, s, 2, 8, 18-Me, OMe), 3.98 (2H, dt, 12-$\underline{CH_2}CH_2CO_2$), 4.34 (2H, t, 7-$CH_2$), 4.40 (2H, t, 17-$\underline{CH_2}CH_2CO_2$), 9.13, 9.80, 9.95, 10.13 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 639 nm (37,000), 583 (2,300), 551 (6,400), 511 (12,800), 408 (9,700); MS for $C_{48}H_{66}N_4O_5$, found m/e 779 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3-porphyrinone-13,17-dipropionate (36). Dimethyl 2,8,12,18-tetramethyl-3,7-didodecyl-13-porphyrinone-12,17-dipropionate (38)

Dimethyl 2,8,12,18-tetramethyl-3,7-didodecylporphyrin-13,17-dipropionate (30) (875 mg, 1.0 mmol) was treated with some excess of osmium tetroxide and then with concentrated sulfuric acid by following the methode described before for the dioctylporphyrinones (35) and (37) to afford the didodecylporphyrinone (36) (249 mg, 43% yield based on reacted (30)) as the major product along with the unreacted porphyrin (30) (307 mg). A small amount of the isomeric porphyrinone (38) (17 mg, 1.9%) was also obtained.

For dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3-porphyrinone-13,17-dipropionate (36): $^1H$ NMR ($CDCl_3$) δ -2.95 (2H, s, NH), 0.80, 0.85 (3H each, s, dodecyl Me), 0.98-1.35 (34H, m, $CH_2$), 1.45, 1.70 (2H each, q, $CH_2$), 2.05 (3H, s, 2-Me), 2.20 (2H, q, $CH_2$), 2.70 (2H, t, 7-$CH_2$), 3.20, 3.27 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.47, 3.57, 3.58, 3.67, 3.68 (3H each, s, 8, 12, 18,-Me, OMe), 4.00 (2H, dt, 2-$CH_2$), 4.24, 4.40 (2H each, t, $\underline{CH_2}CH_2CO_2$), 9.14, 9.82, 9.89, 9.93 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 641 (36,000), 586 (5,500), 546 (13,100), 509 (9,500), 405 (162,000); MS for $C_{56}H_{82}N_4O_5$ found m/e 891.3 ($M^+$).

For dimethyl 2,8,12,18-tetramethyl-3,7-didodecyl-13-porphyrinone-12,17-dipropionate (38): $^1H$ NMR ($CDCl_3$) δ -2.90 (2H, s, NH), 0.80, 0.90 (3H, t, 0ctyl Me), 1.00-1.20 (34H, m, $CH_2$), 1.50, 1.70 (2H each, q, $CH_2$), 2.10 (3H, s, 12-Me), 2.70 (2H, t, 3-$CH_2$), 3.20 (4H, t, $CH_2\underline{CH_2}CO_2$), 3.58, 3.57, 3.65, 3.65, 3.72 (3H

each, s, 2, 8, 18, -M, OMe), 4.02 (2H, dt, 12-$\underline{CH_2}CH_2CO_2$), 4.30 (2H, t, 7-$CH_2$), 4.40 (2H, t, 17-$\underline{CH_2}CH_2CO_2$), 9.12, 9.80, 9.95, 10.14 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 639 nm (37,500), 583 (2,400), 551 (6,500), 511 (12,800), 409 (9,900); MS for $C_{56}H_{82}N_4O_5$, found m/e 891 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3,13-porphyrindione-12,17-dipropionate (43)

Osmium tetroxide (207 mg, 0.8 mmol) and pyridine (0.5 mL) were added to (35) (390 mg, 0.5 mmol) dissolved in dry dichloromethane (50 mL). The reaction was allowed to proceed at room temperature in the dark for 24 hours and worked up in the same manner as described before. After osmium tetroxide oxidation, sulfuric acid catalyzed rearrangement was accomplished as described previously. The porphyrindione (43), was isolated as the major product (95 mg, 30% yield based on reacted (35) along with the unreacted porphyrinone (35) (74 mg), the former moved slower on the silica gel column than the latter.

For the title compound: $^1H$ NMR ($CDCl_3$) $\delta$ -2.81, -2.76 (1H each, s, NH), 0.64, 0.84 (3H each, t, octyl Me), 0.90 (14H, m, $CH_2$), 1.27 (6H, m, $CH_2$), 1.46, 1.66 (2H each, dq, $CH_2$), 1.97, 2.02 (3H each, s, 2, 12-Me), 2.16 (2H, dt, 12-$CH_2\underline{CH_2}CO_2$), 2.65 (2H, t, 17-$CH_2\underline{CH_2}CO_2$), 3.01 (2H, dt, 2-$CH_2$), 3.20 (2H, t, 7-$CH_2$), 3.32, 3.47, 3.51, 3.75 (3H each, s, 8, 18-Me, OMe), 3.92, 4.29 (2H each, t, $\underline{CH_2}CH_2CO_2$), 9.04, 9.05, 9.66, 9.68 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 685 nm (101,800), 651 (7,000), 621 (5,300), 552 (9,000), 511 (6,000), 485 (3,700), 409 (182,300); MS for $C_{48}H_{66}N_4O_6$, found m/e 795 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3,13-porphyrinedione-12,17-dipropionate (44)

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3-porphyrinone-13,17-dipropionate (36) (446 mg, 0.5 mmol) was treated with some excess of osmium tetroxide and then with concentrated sulfuric acid following the procedure described before for the dioctylporphyrindione (43) to give the didodecylporphyrindione (44) (99.8 mg, 27% yield based on reacted (36)) as the major product along with the unreacted porphyrin (36) (83 mg).

For the title compound: $^1$H NMR ($CDCL_3$) δ -2.80, -2.74 (1H each, s, NH), 0.64, 0.84 (3H each, t, dodecyl Me), 1.00 (30H, m, $CH_2$), 1.30 (6H, m, $CH_2$), 1.46, 1.68 (2H each, q, $CH_2$), 1.95, 2.00 (3H each, s, 2, 12-Me), 2.14 (2H, dt, 12-$CH_2\underline{CH_2}CO_2$), 2.65 (2H, t, 17-$CH_2\underline{CH_2}CO_2$), 3.00 (2H, dt, 2-$CH_2$), 3.22 (2H, t, 7-$CH_2$), 3.30, 3.46, 3.52, 3.75 (3H each, s, 8, 18-Me, OMe), 3.92 (2H, dt, 12-$\underline{CH_2}CH_2CO_2$), 4.28 (2H, t, 17-$\underline{CH_2}CH_2CO_2$), 9.05, 9.06, 9.66, 9.69 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 685 nm (101,000), 651 (6,900), 621 (5,100), 552 (9,000), 511 (5,900), 486 (3,700), 409 (182,100); MS for $C_{56}H_{82}N_4O_6$, found m/e 907 ($M^+$).

Dimethyl 3-dicyanomethylenyl-2,8,12,18-tetramethyl-2,7-dioctylchlorin-13,17-dipropionate (45)

Dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3-porphyrinone-13,17-dipropionate (35) (93.4 g, 0.12 mmol) was dissolved in 100 mL of the mixture of chloroform-methanol (3:1) and a solution of copper(II) acetate monohydrate (72 mg, 0.36 mmol) in methanol (7 mL) was added. The reaction mixture was refluxed for 30 minutes and then cooled to room temperature. Excess copper(II) acetate and methanol were removed by washing with a large amount of water. Copper(II) porphyrinone derivative

(35), resulted from evaporation of solvent, was dissolved in dry chloroform (50 mL) and treated with titanium(IV) chloride (0.22 mL, 0.18 mmol) and with a solution of malonitrile (31.7 mg, 0.48 mmol) and pyridine (0.08 mL, 1 mmol) in dry chloroform (10 mL), which was prepared one hour before use. After the reaction was accomplished by refluxing for 30 minutes, the reaction mixture was cooled to room temperature, diluted with chloroform (50 mL), washed with water (2x100 mL), and dried over anhydrous sodium sulfate. The solvent was removed under vacuum. The resulting residue, copper(II) complex of dicyanomethylenylchlorin, was dissolved in trifluoroacetic acid (15 mL), bubbled with hydrogen sulfide through the reaction solution for 20 minutes to remove copper from the dicyanomethylenylchlorin, and allowed to stand for 1-2 hours. The precipitated black copper(II) sulfide was removed by filtration through a bed of Celite which was rinsed with chloroform (20 ml), the filtrates were combined and washed twice with saturated aqueous sodium acetate (20 mL) and twice with water (20 mL). The solvent was removed under vacuum and the residue was chromatographed on silica gel with 0.5-1% methanol in dichloromethane to give the title compound(46.6 mg, 47%): $^1$H NMR ($CDCl_3$) $\delta$ -2.63, -2.47 (1H each, br s, NH), 0.65, 0.85 (3H each, t, octyl Me), 0.90-1.04 (12H, m, $CH_2$), 1.26 (6H, m, $CH_2$), 1.44, 1.69, 2.20 (2H each, q, $CH_2$), 2.35 (3H, s, 2-Me), 2.89 (2H, t, 7-$CH_2$), 3.16, 3.24 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.39, 3.49, 3.53, 3.66, 3.68 (3H each, s, 8, 12, 18-Me, OMe), 3.95 (2H, dt, 2-$CH_2$), 4.14, 4.32 (2H each, t, $\underline{CH_2}CH_2CO_2$), 9.97, 9.68, 9.75, 10.70 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 678 nm (32,000), 627 (23,000), 459 (70,000), 405 (41,000), 381 (40,400), 345 (27,800); MS for $C_{51}H_{66}N_6O_4$, found m/e 827 ($M^+$).

Dimethyl 3-dicyanomethylenyl-2,8,12,18-tetramethyl-2,7-didodecylchlorin-13,17-dipropionate (46)

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3-porphyrinone-13,17-dipropionate (36) (89.2 mg, 0.1 mmol) was treated with copper(II) acetate and then with a solution of malonitrile (26 mg, 0.4 mmol) and pyridine (0.07 mL, 0.87 mmol) in dry chloroform (9 mL) in the presence of titanium(IV) chloride (0.19 mL, 0.15 mmol) following the method described before for the dicyanomethylenyl dioctylchlorin (45) to afford the title compound (46) (43.2 mg, 46%): $^1$H NMR ($CDCl_3$) δ -2.62, -2.47 (1H each br s, NH), 0.65, 0.86 (3H each, t, dodecyl Me), 0.92-1.08 (20H, m, $CH_2$), 1.26 (14H, m, $CH_2$), 1.45, 1.70, 2.19 (2H each, q, $CH_2$), 2.36 (3H, s, 2-Me), 2.90 (2H, t, 7-$CH_2$), 3.16, 3.25 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.40, 3.49, 3.54, 3.66, 3.68 (3H each, s, ring Me, OMe), 3.96 (2H, dt, 2-$CH_2$), 4.14, 4.32 (2H each, t, $\underline{CH_2}CH_2CO_2$), 9.97, 9.69, 9.76, 10.71 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 678 nm (31,500), 627 (22,700), 459 (69,000) 405 (40,800), 381 (40,200), 345 (27,400); MS for $C_{59}H_{82}N_6O_4$, found 939 ($M^+$).

Bis(trimethylsilyl)-carbodiimide

To a solution of chlorotrimethylsilane (25.7 mL, 0.2 mol) and triethylamine (27.9 mL, 0.2 mol) in dry diethyl ether (50 mL), a solution of cyanamide (4.2 g, 0.1 mol) in dry diethyl ether (50 mL) was added with stirring within one hour. The triethylamine hydrochloride, byproduct, was removed by filtration and the filtrate was distilled under reduced pressure to give the title compound as colorless liquid (32 g, 86%): b.p. 67-72 °C/water aspirator; $^1$H NMR ($CDCl_3$) δ 1.21 (18H, s, Me); MS for $C_7H_{18}N_2Si_2$, found 186.4 ($M^+$).

Dimethyl 3-(N-cyanoiminyl)-2,8,12,18-tetramethyl-2,7-dioctylchlorin-13,17-dipropionate (47)

To a solution of dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3-porphyrinone-13,17-dipropionate (35) (31 mg, 0.04 mmol) in dichloromethane (30 mL), titanium(IV) chloride (0.013 mL, 0.12 mmol) and bis(trimethylsilyl)-carbodiimide (0.03 mL, 0.12 mmol) was consecutively added under argon. The reaction mixture was allowed to stir at room temperature for 4 hours. After the reaction was accomplished which was monitored by TLC, the reaction mixture was filtered through a bed of Celite and the filtrate was washed twice with water (30 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under vacuum and the residue was chromatographed on silica gel with 0.5% methanol in dichloromethane to give the title compound as major procduct (18.9 mg, 59% ): $^1$H NMR ($CDCl_3$) $\delta$ -2.78, -2.71 (1H each, br s, NH), 0.66, 0.88 (3H each, t, octyl Me), 0.99-1.05 (12H, m, $CH_2$), 1.30 (6H, m, $CH_2$), 1.50, 1.69, 2.20 (2H each, q, $CH_2$), 2.40 (3H, s, 2-Me), 2.98 (2H, dt, 7-$CH_2$), 3.19, 3.28 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.40, 3.48, 3.57, 3.67, 3.69 (3H each, s, 8, 12, 18-Me, OMe), 3.92 (2H,dt, 2-$CH_2$), 4.18, 4.47 (2H each, t, $\underline{CH_2}CH_2CO_2$), 9.06, 9.78, 10.00, 10.94 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 660 nm (16,500), 599 (10,700), 439 (64,500), 384 (29,200), 337 (14,000); MS for $C_{49}H_{66}N_6O_4$, found m/e 803 ($M^+$).

Dimethyl 3-(N-cyanoiminyl)-2,8,12,18-tetramethyl-2,7-didodecylchlorin-13,17-dipropionate (48)

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3-porphyrinone-13,17-dipropionate (36) (35.7 mg, 0.04 mmol) was treated with an excess of bis(trimethylsilyl)-carbodiimide in the presence of titanium(IV) chloride (0.013 mL, 0.12 mmol) following the method described before for the dimethyl

N-cyanoiminyl dioctylchlorin (47) to give the title compound as major product (22.5 mg, 60%): $^1$H NMR ($CDCl_3$) δ -2.77, -2.71 (1H each, br s, NH) 0.67, 0.89 (3H each, t, dodecyl Me), 0.96-1.12 (20H, m, $CH_2$), 1.31 (14H, m, $CH_2$), 1.50, 1.70, 2.21 (2H each, q, $CH_2$), 2.40 (3H, s, 2-Me), 2.99 (2H, dt, 7-$CH_2$), 3.19, 3.29 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3,40, 3.48, 3.58, 3.67, 3.70 (3H each, s, 8.12, 18-Me, OMe), 3.93 (2H, dt, 2-$CH_2$), 4.18, 4.47 (2H each, t, $\underline{CH_2}CH_2CO_2$), 9.06, 9.78, 10,00, 10.94 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 660 nm (16,500), 599 (10,700), 439 (64,500), 384 (29,200), 337 (14,000); MS for $C_{57}H_{82}N_6O_4$, found m/e 915 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3-porphyrinthione-13,17-dipropionate (49)

Lawesson's reagent (48 mg, 0.12 mmol) was added to a solution of dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3-porphyrinone-13,17-dipropionate (35) (77.8 mg, 0.1 mmol) in dry toluene (50 mL) under nitrogen. After the reaction mixture was refluxed for 24 hours under nitrogen, the solvent was removed under high vacuum. The resulting residue was chromatographed on silica gel with 30% haxane in dichloromethane to isolate the porphyrinthione as the major product which was further purified by recrystallization from dichloromethane and methanol to give the title compound (34.7 mg, 44%): $^1$H NMR ($CDCl_3$) δ -2.50 (2H, br s, NH), 0.66, 0.88 (3H each, t, octyl Me), 0.90-1.05 (12H, m, $CH_2$), 1.60 (6H, m, $CH_2$), 1.50, 1.68, 2.20 (2H each, q, $CH_2$), 2.08 (3H, s, 2-Me), 2.75, 2.95 (1H each, dt, 7-$CH_2$), 3.19, 3.27 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.40, 3.48, 3.56, 3.67, 3.69 (3H each, s, 8, 12, 18-Me, OMe), 3.95 (2H, dt, 2-$CH_2$), 4.18, 4.36 (2H each, t, $\underline{CH_2}CH_2CO_2$), 9.13, 9.74, 9.74, 10.32 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 679 nm (26,000), 623

(22,000), 457 (75,000), 441 (69, 600) 405 (59,200), 384 (52,600), 345 (33,700), 313 (21,000); MS for $C_{48}H_{66}N_4O_4S$, found 795 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3-porphyrinthione-13,17-dipropionate (50)

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3-porphyrinone-13,17-dipropionate (36) (89.2 mg, 0.1 mmol) was treated with an excess of Lawesson's reagent following the method described for the dioctyl porphyrinthione (49) to give the title compound as a major product (39 mg, 43%): $^1H$ NMR ($CDCl_3$) δ -2.52, -2.49 (1H each, br s, NH), 0.77, 0.84 (3H each, t, dodecyl Me), 0.90-1.12 (20H, m, $CH_2$), 1.23 (14H, m, $CH_2$), 1.49, 1.67, 2.20 (2H each, q, $CH_2$), 2.08 (3H, s, 2-Me), 2.72, 2.92 (1H each, dt, 7-$CH_2$), 3.18, 3.25 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.42, 3.52, 3.54, 3.66, 3.67 (3H each, s, 8, 12, 18-Me, OMe), 3.97 (2H, dt, 2-$CH_2$), 4.19, 4.35 (2H each, t, $\underline{CH_2}CH_2CO_2$), 9.12, 9.73, 9.77, 10.30 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 679 nm (25,700), 623 (21,900), 457 (74,800), 441 (69,400), 405 (59,000), 384 (52,400), 345 (33,500), 313 (20,800); MS for $C_{56}H_{82}N_4O_4S$, found m/e 907 ($M^+$).

Dimethyl 3,13-bis(N-cyanoiminyl)-2,8,12,18-tetramethyl-2,7-dioctylbacteriochlorin-12,17-dipropionate (51)

Dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3,13-porphyrindione-12,17-dipropionate (43) (39.7 mg, 0.05 mmol) was dissolved in dry dichloromethane (30 mL) and bis(trimethylsilyl)-carbodiimide (0.04 mL, 0.15 mmol) was added followed by addition of titanium(IV) chloride (0.03 mL, 0.15 mmol) under nitrogen. After the reaction was completed by stirring at room temperature in the dark for 4 hours, which was monitored by TLC eluted with hexane-dichloromethane (1:3), the reaction mixture was filtered through a bed of

Celite and the filtrate was washed twice with water (40 mL). The organic layer was separated and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue which was purified by preparative thick layer plates (silica gel) eluted with hexane-dichloromethane (1:3). The product was extracted from the silica gel to afford the title compound (17.7 mg, 42%): $^1$H NMR ($CDCl_3$) δ -2.41 (2H, br s, NH), 0.62, 0.83 (3H each, t, octyl Me), 0.80-1.02 (12H, m, $CH_2$), 1.30 (6H, m, $CH_2$), 1.43, 1.55, 1.64 (3H each, m, $CH_2$), 2.16, 2.63 (2H, t, $CH_2\underline{CH_2}CO_2$), 2.28, 2.32 (3H each, s, 2.12-Me), 2.85 (2H, dt, 2-$CH_2$), 3.20 (2H, t, 7-$CH_2$), 3.46, 3.66, 3.68, 3.71 (3H each, s, ring Me, OMe), 3.87, 4.23 (2H each, t, $\underline{CH_2}CH_2CO_2$), 8,92, 9.77 (2H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 719 nm (74,000), 654 (9,500), 619 (12,200), 448 (109,800), 426 (95,600); MS for $C_{50}H_{66}N_8O_4$, found m/e 843 ($M^+$).

Dimethyl 3,13-bis(N-cyanoiminyl)-2,8,12,18-tetramethyl-2,7-didodecylbacteriochlorin-12,17-dipropionate (52)

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3,13-porphyrindione-12,17-dipropionate (44) (54.5 mg, 0.06 mmol) was treated with an excess of bis(trimethylsilyl)-carbodiimide in the presence of titanium(IV) chloride (0.036 mL, 0.18 mmol) following the method described for the bis(N-cyanoiminyl)-dioctylbacteriochlorin (51) to give the title compound as a major product (22.8 mg, 39.8%): $^1$H NMR ($CDCl_3$) δ -2.42 (2H, br s, NH), 0.63, 0.83 (3H each, t, dodecyl Me), 0.80-1.07 (20H, m, $CH_2$), 1.30 (14H, m, $CH_2$), 1.44, 1.55, 1.65 (3H each, m, $CH_2$), 2.17, 2.65 (2H, t, $CH_2\underline{CH_2}CO_2$), 2.28, 2.34 (3H each, s, ring Me), 2.86 (2H, dt, 2-$CH_2$), 3.21 (2H, t, 7-$CH_2$), 3.45, 3.66, 3.68, 3.72 (3H each, s, ring Me, OMe), 3.88, 4.24 (2H each, t, $\underline{CH_2}CH_2CO_2$), 8,92, 9,78 (2H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 719 nm (74,000), 654 (9,500), 619 (12,200), 448 (109,800), 426 (95,600); MS for $C_{58}H_{82}N_8O_4$, found 955 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3,13-porphyrindithione-12,17-dipropionate (53)

Lawesson's reagent (80 mg, 0.2 mmol) was added to a solution of dimethyl 2,8,12,18-tetramethyl-2,7-dioctyl-3,13-porphyrindione-12,17-dipropionate (43) (55.6 mg, 0.07 mmol) in dry toluene (55 mL). After the reaction mixture was refluxed under nitrogen in the dark for 24 hours, the reaction was completed which was monitored by TLC using hexane-dichloromethane (1:3). The solvent was removed under vacuum, and the residue was purified by preparative thick layer plates (silica gel) eluted with 30% hexane in dichloromethane. The product was extracted from the silica gel and then crystallized from dichloromethane and methanol to afford the title compound as green fluffy crystals (20.2 mg, 35%): $^1$H NMR ($CDCl_3$) δ -1.78, -1.74 (1H each, br s, NH), 0.66, 0.86 (3H each, t, octyl Me), 0.80-1.08 (12H, m, $CH_2$), 1.30 (8H, m, $CH_2$), 1.49, 1.66 (2H each, m, $CH_2$), 1.96, 2.02 (3H each, s, 2.12-Me), 2.16, 2.66 (2H each, t, $CH_2\underline{CH_2}CO_2$), 2.83, 2.98 (1H each, t, 2-$CH_2$), 3.21 (2H, t, 7-$CH_2$), 3.27, 3.40, 3.44, 3.72 (3H each, s, ring Me, OMe), 3.86, 4.24 (2H each, t, $\underline{CH_2}CH_2CO_2$), 8.89, 8.90, 9.97, 10.00 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 747 nm (92,200), 714 (12,800), 682 (21,900), 655 (17,600), 477 (102,700), 446 (64,500), 431 (79,800); MS for $C_{48}H_{66}N_4O_4S_2$, found 827 ($M^+$).

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3,13-porphyrindithione-12,17-dipropionate (54)

Dimethyl 2,8,12,18-tetramethyl-2,7-didodecyl-3,13-porphyrindione-12,17-dipropionate (44) (63.5 mg, 0.07 mmol) was treated with an excess of Lawesson's reagent following the method described for the dioctyl-porphyrindithione (53) to give the title compound as a major product (23.6

mg, 36%): $^1$H NMR ($CDCl_3$) δ -1.77, -1.74 (1H each, br s, NH), 0.67, 0.87 (3H each, t, dodecyl Me), 0.80-1.12 (20H, m, $CH_2$), 1.30 (16H, m, $CH_2$), 1.50, 1.68 (2H each, m, $CH_2$), 1.97, 2.04 (3H each, s, 2.12-Me), 2.16, 2.67 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.84, 2.98 (1H each, t, 2-$CH_2$), 3.22 (2H, t, 7-$CH_2$), 3.27, 3.40, 3.43, 3.72 (3H each, s, ring Me, OMe), 3.86, 4.25 (2H each, t, $\underline{CH_2}CH_2CO_2$), 8.89, 8.90, 9.97, 10.01 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 747 nm (92,200), 714 (12,800), 682 (21,900), 655 (17,600), 477 (102,700), 446 (64,500) 431 (79,800); MS for $C_{56}H_{82}N_4O_4S_2$, found 939 ($M^+$).

# CHAPTER 3

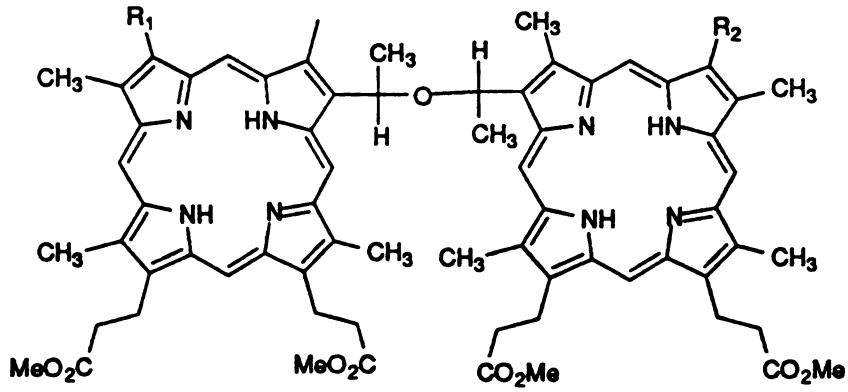
## SYNTHESES AND PROPERTIES OF ANIONIC DI-PORPHYRIN DERIVATIVES

## I. INTRODUCTION

Photofrin-II suffers from the lack of a defined structure because of its complex chemical nature. Dougherty *et al.*[108] first described the compound as dihematoporphyrin ether (55, DHE, and isomers), but it is now generally accepted to be a mixture of dimers and higher oligomers linked by ether, ester, and even carbon-carbon bonds.[109] Interconversion between ester and ether links has also been noted.[110] Recently, using purer materials, attempts have been made to understand some of the parameters important for an effective *in vivo* photosensitizer. An HP dimer joined by ester linkages was synthesized by Pandey and Dougherty,[111] but it was biologically inactive; an ether linked HP oligomers was synthesized by Scourides *et al.*,[112] but though it was shown to be as active as Photofrin II, it was also shown to be a complex mixture.

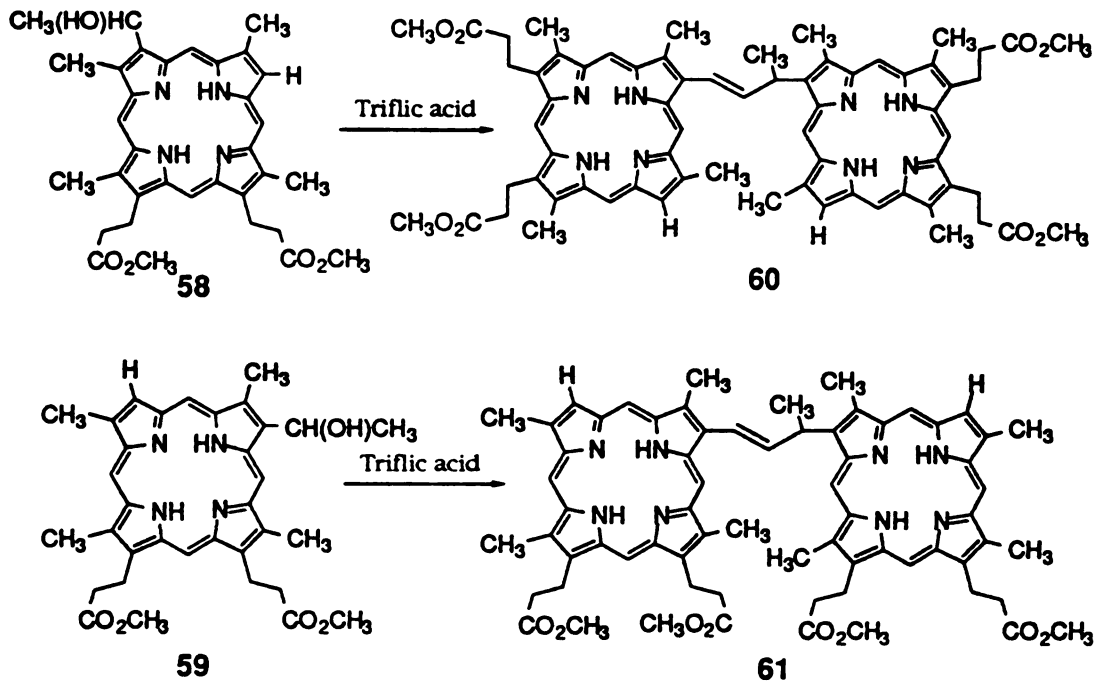
In 1988, synthesis of a simple ether dimer was first reported by Pandey and Dougherty.[113] It was shown to be an effective *in vivo* sensitizer. Morris and Ward[114] used similar chemistry to obtain DHE tetramethyl ester. Mild hydrolysis gave DHE (55) but it was inactive. The DHE variants possessing

one (56) or two vinyls (57) were also synthesized, and the latter was just as active as Photofrin II.



(55) $R_1$=$R_2$= $CH(OH)CH_3$
(56) $R_1$=$CH(OH)CH_3$; $R_2$=$CH=CH_2$
(57) $R_1$=$R_2$= $CH=CH_2$
( all as mixtures of regioisomers)

Recently, Pandey *et al.*[115] have prepared the carbon-carbon linked dimers related to HP. Treatment of hydroxyethylporphyrin (58) and (59) with trifluoromethanesulfonic acid (triflic acid) affords > 90% yield of the corresponding carbon-carbon dimer (60) and (61) respectively as shown in Scheme 6. In preliminary biological testing for tumorcidal activity, the tetracarboxylic acid dimer from the dimeric porphyrin (61) was found to be more active than that from the dimeric porphyrin (60).




58 60

59 61

**Scheme 6**

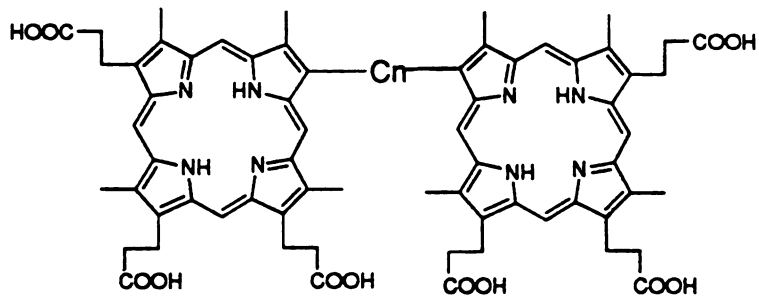
A series of dimeric porphyrins linked by 3-, 5-, 6-, and 13-carbon chains have been prepared in our laboratory. These compounds have been tested *in vivo* for effectiveness in PDT. The dimers linked with 6-carbon chain proved to be the most effective against mouse tumor system *in vivo*. The compounds linked by 3-carbon chain showed no activity *in vivo*.[116,117] The dimers linked with 5- and 6-carbon chain were at least as efficient as Photofrin II, and all showed less skin persistence than Photofrin-II. Furthermore, dimeric tetrahydroxychlorin (63), and dimeric porphyrinone (65) were more potent than Photofrin II *in vivo*.[117] Unfortunately, the methylene-linked dichlorin derivatives suffered from a lack of regio-specific structure; for

example, it was very difficult to make dimeric porphyrin (64) containing one oxo group with the oxo position attached at the specific pyrrole.

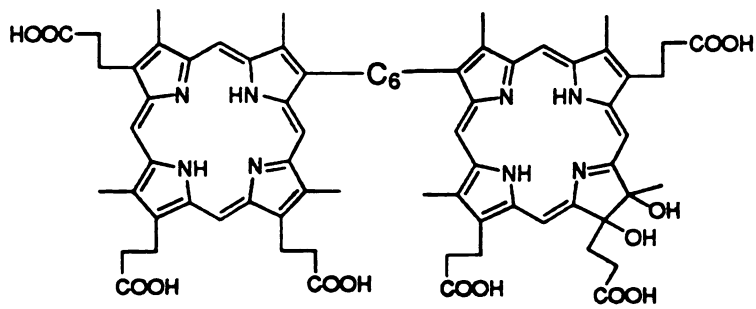
To control the regio-selectivity and to prepare effective PDT photosensitizers, we designed a dimeric porphyrin linked by 6-carbon chains (83, in Scheme 12) which contains both lipophilic character at the "northern" part and hydrophilic character at the "southern" part in the molecule, which are called amphiphilic character, to improve intracellular localization.

Although dimeric porphyrins linked by space chains proved to have higher affinity for tumor tissue than mono-porphyrins, porphyrin dimers absorb in the same region as mono-porphyrins. Thus, some structural modifications of porphyrin dimers are needed to increase extinction coefficients of absorption bands at the longer wavelengths by adding desirable functional group which are good auxochromes (bathochromic shift).

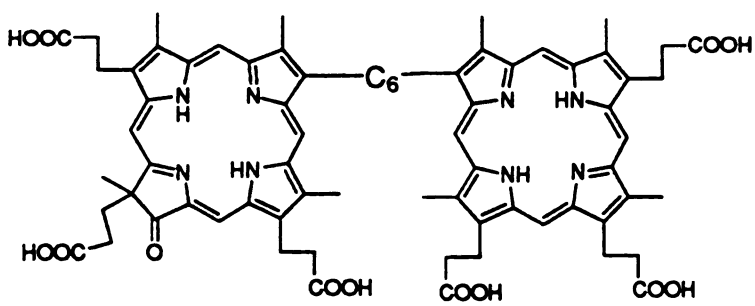
To prepare dimeric chlorin derivative (85) and dimeric benzochlorin derivative (86), the porphyrin dimer (83) was first converted to the vinyl analogue (84) as shown in Scheme 13. Chlorins typically abosrb strongly in the red region of the visible light spectrum.[118] These compounds have large extinction coefficients at wavelengths above 650 nm, suggesting improved light transmission through tissue as well as greater photon efficiency when compared to porphyrins. The dimeric chlorin derivative (85) was easily obtained from the reaction of the dimeric vinylporphyrin (84) with singlet oxygen ($^1O_2$).


HOOC
COOH
Cn
HN
NH
N
COOH
COOH
COOH
COOH

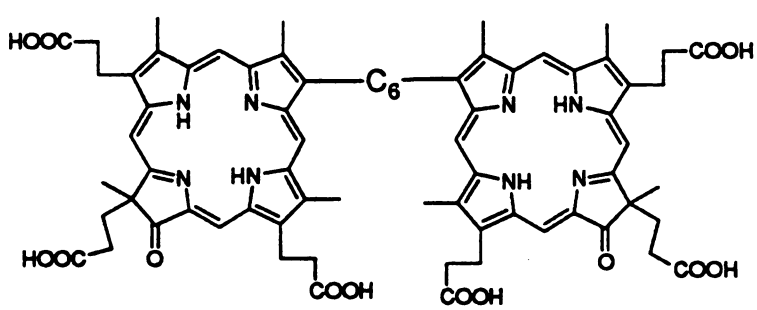
62


HOOC
COOH
C6
HN
NH
N
OH
OH
COOH
COOH
COOH
COOH

63

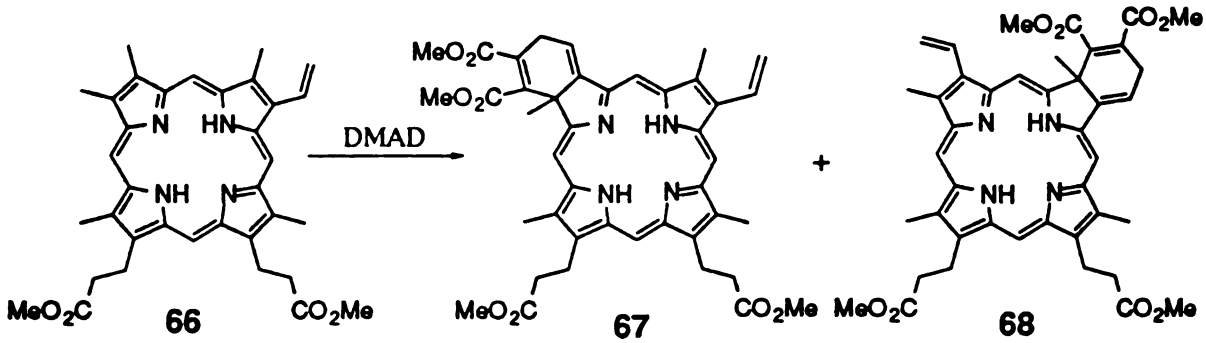

HOOC
COOH
C6
N
H
HN
NH
O
HOOC
COOH
COOH
COOH

64


HOOC
COOH
C6
N
H
HN
NH
O
HOOC
O
COOH
COOH
COOH

65

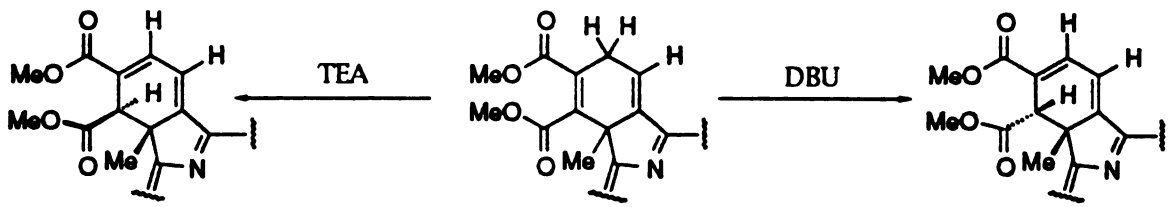
Benzochlorin derivatives have significant extinction coefficients at around 680 nm and appear to have low density lipoprotein (LDL) mediated localization parameters similar to those observed with HPD.[119]




Scheme 7

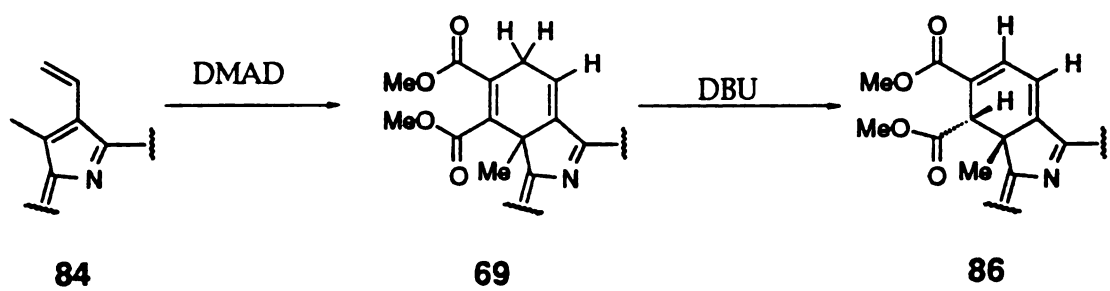
A number of years ago, Johnson and co-workers[120] reported a facile synthesis of compounds possessing the chlorin chromophore by using the Diels-Alder reaction on vinylporphyrins. Dolphin *et al.*[121] extended this methodology and showed that the reaction of the vinyl groups of protoporphyrin IX dimethyl ester (66) with dimethyl acetylenedicarboxylate (DMAD) gave initially the Diels-Alder adducts (67) and (68), as shown in Scheme 7, which could be rearranged by treatment with base. The reaction with triethylamine (TEA) or 1,5-diazabicyclo[5.4.0]undec-5-ene (DBU) gave, in every case, two diastereomers, where the former rearrangement led to the kinetically controlled product and the latter the thermodynamically controlled one as shown in Scheme 8. Recently Smith *et al.*[122] eliminated the problem of isomer formation in the Diels-Alder reaction of protoporphyrin

IX dimethyl ester (66) due to its asymmetry by using symmetrically substituted divinylporphyrins.




Scheme 8

We achieved significant simplification in the regioselectivity of the Diels-Alder reaction of the symmetrically substituted dimeric vinylporphyrin (84) with electron-deficient DMAD as follows (see Scheme 9). Diels-Alder reaction of the dimeric vinylporphyrin (84) with DMAD gave initially the Diels-Alder adduct (69) which was rearranged by DBU to give the thermodynamically controlled isomer (86) as mentioned before.

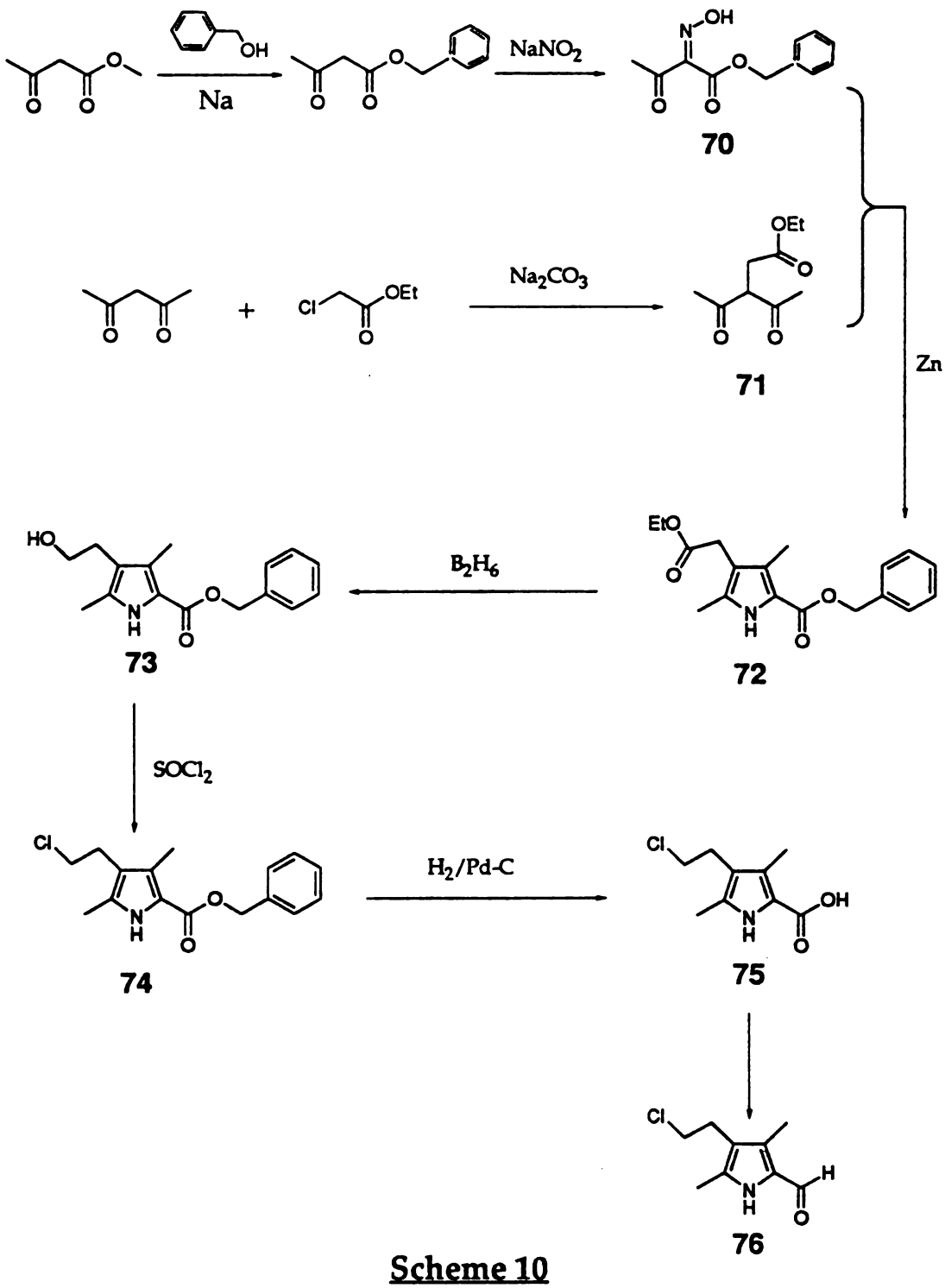



Scheme 9

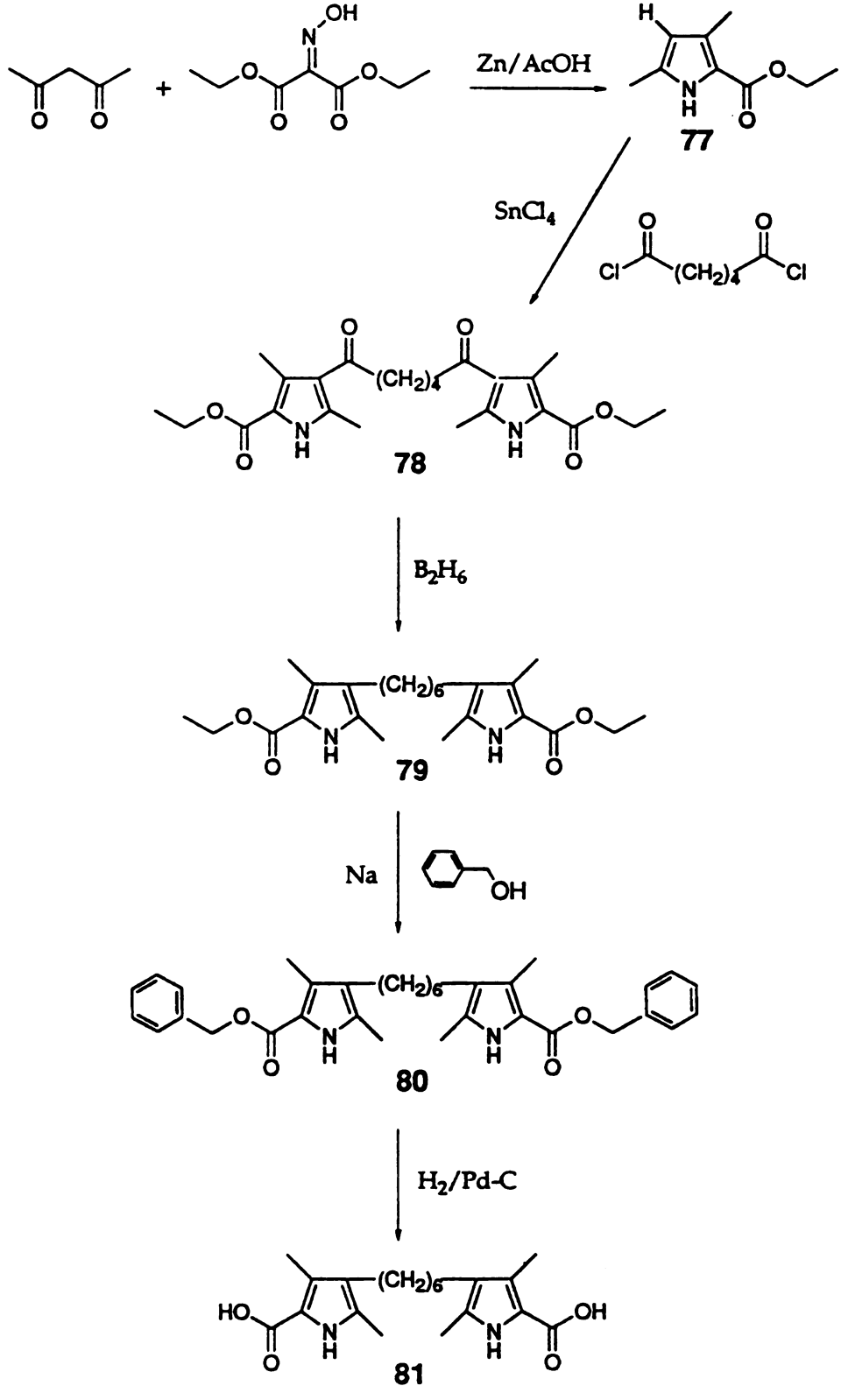
## II. SYNTHESES

As shown in Scheme 12, to obtain the dimeric porphyrin (83) we first prepared 4-(2-chloroethyl)-2-formyl-3,5-dimethylpyrrole (76) as follows (Scheme 10). Benzyl 4-(ethoxycarbonylmethyl)-3,5-dimethylpyrrole-2-carboxylate (72) was prepared by reaction of ethyl 3-acetyl-4-oxopentanoate (71) and the a-oximino derivative of benzyl acetoacetate (70) with zinc dust in 45% yield. The ester group of the pyrrole (72) was easily reduced to the hydroxyethylpyrrole (73) by diborane in 94% yield. The chlorinated pyrrole (74) was smoothly obtained in almost quantitative yield by treatment of the hydroxylpyrrole (73) in benzene with some excess of thionyl chloride. The reaction was completed within 3 hours at room temperature without any base as an acid scavenger. Traditionally base was added to remove the acid which was formed as a by-product in the chlorination by thionyl chloride. But base is not necessary in this reaction because the reaction with base was not smooth and the product was obtained in poor yield (<30%). The chlorinated pyrrole (74) was hydrogenated with 10% palladium/carbon to give the corresponding acid pyrrole (75) quantitatively, which was transformed to the corresponding formyl pyrrole (76) by treating with trifluoroacetic acid and triethyl orthoformate in 84% yield.

In Scheme 11, the 1,6-hexanedione dipyrrole (78) was prepared by treating the β-free pyrrole (77)[122] with adipoyl chloride in the presence of tin(IV) chloride in 86% yield. The hexamethylene bispyrrole (79) was obtained by reduction of the hexanedionedipyrrole (78) with an excess of diborane in 87% yield. Transesterification of the ethyl ester of the dipyrrole (79) in an excess of hot benzyl alcohol with sodium benzyloxide gave the


OH
Na
NaNO2
N-OH
70
OEt
Cl
OEt
Na2CO3
71
Zn
EtO
72
B2H6
HO
73
SOCl2
Cl
74
H2/Pd-C
Cl
OH
75
Cl
H
76

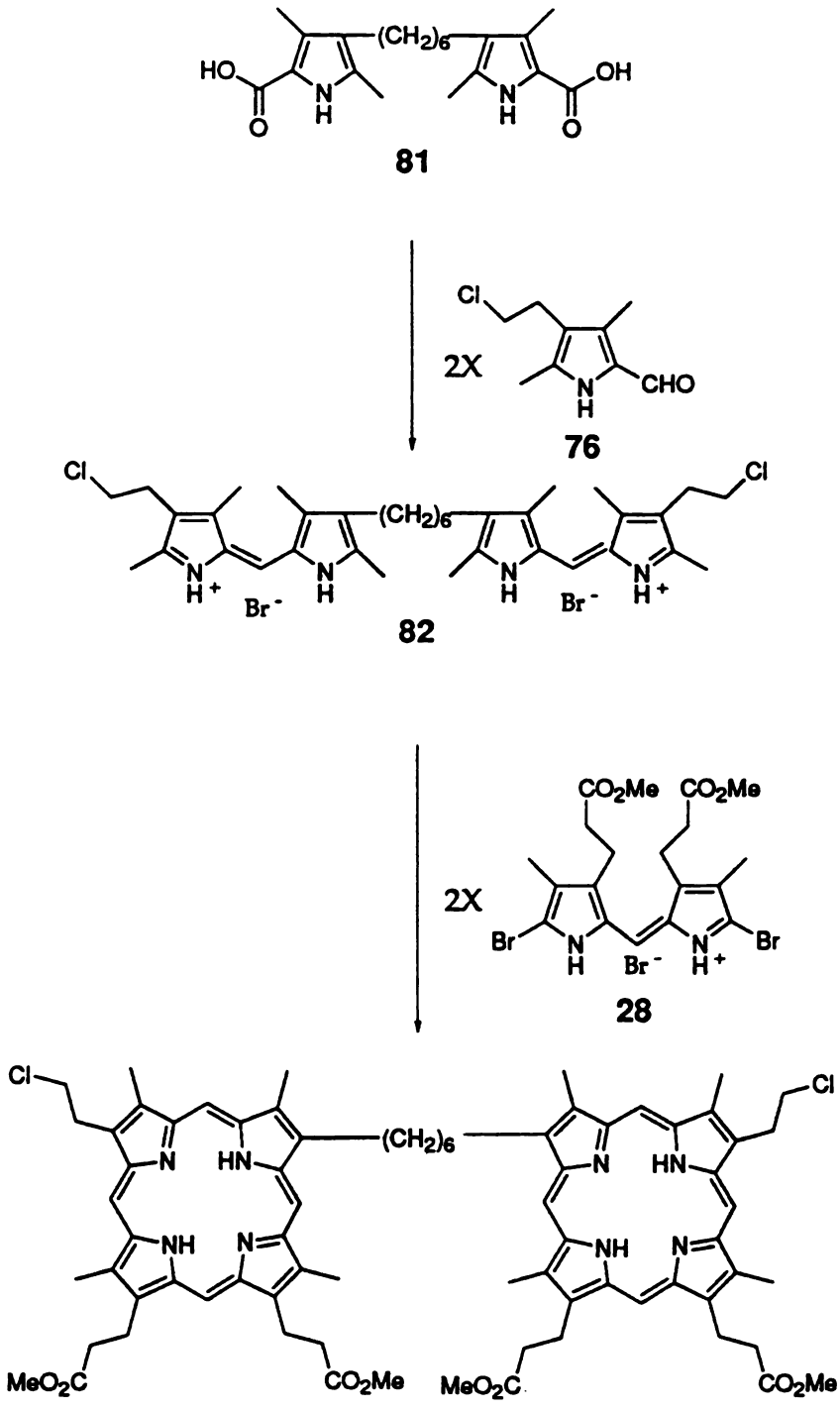
Scheme 10



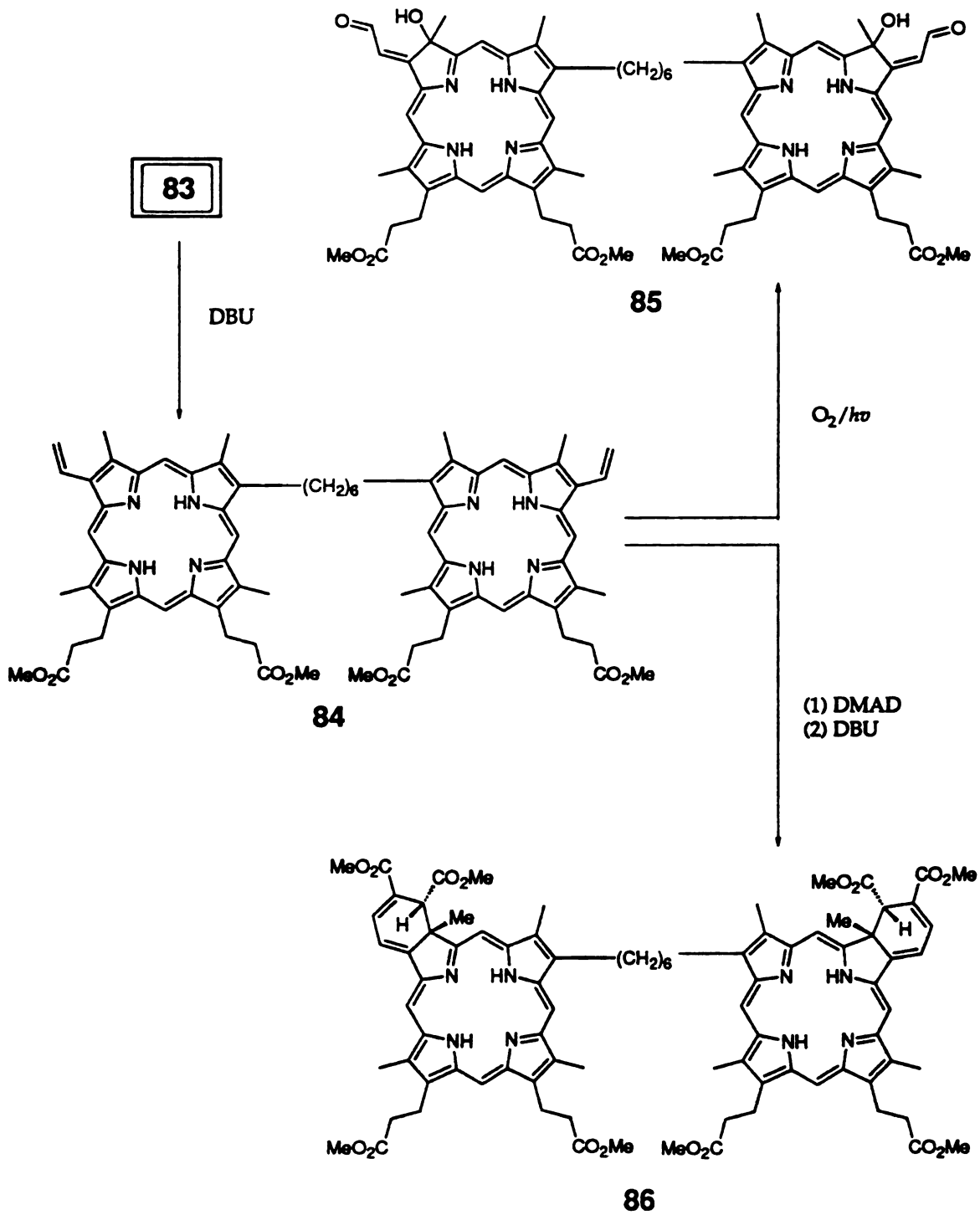

**Scheme 11**

correspond benzyl ester compound (80) (>94% yield), which was hydrogenated with 10% palladium/carbon to give the corresponding acid dipyrrole (81) in 97% yield. As shown in Scheme 12, the tetrapyrrole (82) was obtained by condensation of the acid dipyrrole (81) with two equivalents of the formyl pyrrole (76) in 88% yield. The reaction was accomplished within 30 minutes in boiling methanol in the presence of 48% hydrobromic acid. The dimeric porphyrin (83) was synthesized through condensation of the tetrapyrrole (82) with two equivalents of the dibromopyrrylmethene (65) in anhydrous formic acid in the presence of one equivalent of bromine (9% yield). According to Scheme 13, the dimeric porphyrin (83) was transformed to the dimeric vinylporphyrin (84) to prepare the formyl analogue (85) and the benzochlorin analogue (86) which have strong absorption bands in the red region of visible light spectrum. The dimeric vinylporphyrin (84) was easily obtained by treatement of the dimeric porphyrin (83) with DBU in 75% yield. The reaction was completed in N,N-dimethylformamide (DMF) within 2 hours at 80 °C. The dimeric chlorin (85) was prepared from the reaction of the dimeric vinylporphyrin (84) with singlet oxygen ($^1O_2$) which was generated by treatment of bubbling oxygen gas in the reaction solution with irradiation of xenon lamp. The reaction was stopped when a tenth part of the vinylporphyrin (84) was remained which was monitored by TLC and UV-vis spectrum because longer irradiation of light gave more by-products and chewed up the vinylporphyrin (84) by photobleaching.

Diels-Alder adduct (69 in Scheme 9), initially formed from reaction of the dimeric vinylporphyrin (84) with DMAD, was rearranged by DBU to give the thermodynamically controlled dimeric benzochlorin (86 in Scheme 9 and Scheme 13). Thus, when the dimeric vinylporphyrin (84) was allowed to react with an excess of DMAD in refluxing toluene for 5 days, followed by


HO
O
N
H
(CH2)6
OH
O
81
2X
Cl
CHO
76
Br-
82
CO2Me
Br
28
MeO2C
CO2Me
HN
NH
83

**Scheme 12**




Scheme 13

treatment with DBU, the desired dimeric benzochlorin (86) was obtained (27% yield) as the major product along with a small amount of by-product in which only one of the two vinyl groups of the dimeric porphyrin (84) was transformed (identified by spectrophotometry).

## III. RESULTS AND DISCUSSION

The sensitizers, designed, prepared and tested in this study, consist of two carboxylic porphyrin or chlorin molecules linked by 6-carbon chain. The synthesis of the dimeric porphyrin (83) was quite straightforward and which was easily transformed to the dimeric vinylporphyrin (84). In proton NMR spectrum, the newly formed vinylic structure shows an ABX spectrum. Proton A (δ~6.29) is deshielded about 52.4 Hz compared with proton B, because of its relative proximity to the porphyrin ring. Proton X (δ~8.21) is strongly deshielded by the porphyrin ring and is split by proton A (*J*~17.8 Hz) and by proton B (*J*~11.4 Hz). The A proton signal is split by the X proton (*J*~17.8 Hz) and by the B proton (*J*~1.6 Hz). The B proton signal is also split by the X proton (*J*~11.4 Hz) and by the A proton (*J*~1.6 Hz). The coupling constants show the characteristic of a vinyl system; the *trans* coupling is larger than the *cis*, and the geminal coupling is very small. The 8 *meso* protons absorb in a characteristically narrow range, δ~9.946 to δ~10.138, and are strongly deshielded by the strong aromatic ring current of porphyrin. The 4 NH protons absorb at δ~-3.83 which are strongly shielded by the porphyrin ring. The mass spectrum of the dimeric vinylporphyrin (84) shows the strong molecular ion peak at m/e=1210.2. The dimeric formylmethylenylchlorin (85) was easily prepared by treatment of the dimeric vinylporphyrin (84) with

singlet oxygen ($^1O_2$). In proton NMR spectrum, two formyl protons absorb at δ~9.38-δ~9.54. Two hydroxyl protons are seen as a broad peak at δ~6.36, downfield compared with the alcoholic proton (δ~2.0-δ~4.0). The four NH protons are split into two peaks (each 2H) at δ~-4.17 and δ~-3.88.

Diels-Alder reaction of the dimeric vinylporphyrin (84) with DMAD was tried in several reaction conditions. The best condition was to react the vinyl conpound (84) with a large excess of DMAD in refluxing toluene for 5 days. When the chlorin (69 in Scheme 9) formed by Diels-Alder addition was treated with DBU, the isolated double bond tautomerized to the fully conjugated system (86), accompanied by a red shift in the visible spectrum of band I (from 657 to 681 nm). The proton NMR spectrum of the dimeric benzoporphyrin (86) shows the 8 *meso* protons absorb in characteristically low field, δ~8.97 to δ~9.67. The vicinal exocyclic protons (H-$7^3$, H-$7^4$) at δ~7.42 and δ~7.81 are split by each other ($J$~5.6 Hz) and the other exocyclic proton (H-$7^1$) is shown a singlet peak at δ~5.03. The splitting pattern and coupling constant show the characteristic of the exocyclic system in benzochlorin. The 4 NH protons are split into two peaks (each 2H) at δ~-2.43 and δ~-2.51.

Absorbance spectrum of the dimeric porphyrin (83) was not unusual: a Soret band in the vicinity of 400 nm, and 4 peaks of decreasing intensity at 498, 533, 568 and 621 nm (in $CH_2Cl_2$; Figure 7). The dimeric vinylporphyrin (84) showed only a small red-shift from the peaks obtained with the dimeric porphyrin (83) as shown in Figure 7. The absorbance spectrum of the dimeric formylmethylenylchlorin (85) showed red-shifted absorption of band I and the remarkable increase of the intensity in the red bands as shown in Figure 8 and Table 4. The absorption spectrum of the dimeric benzoporphyrin (86) showed more (20 nm) red-shift than that of the dimeric formylmethylenylchlorin (85) with almost same intensity in the red region.

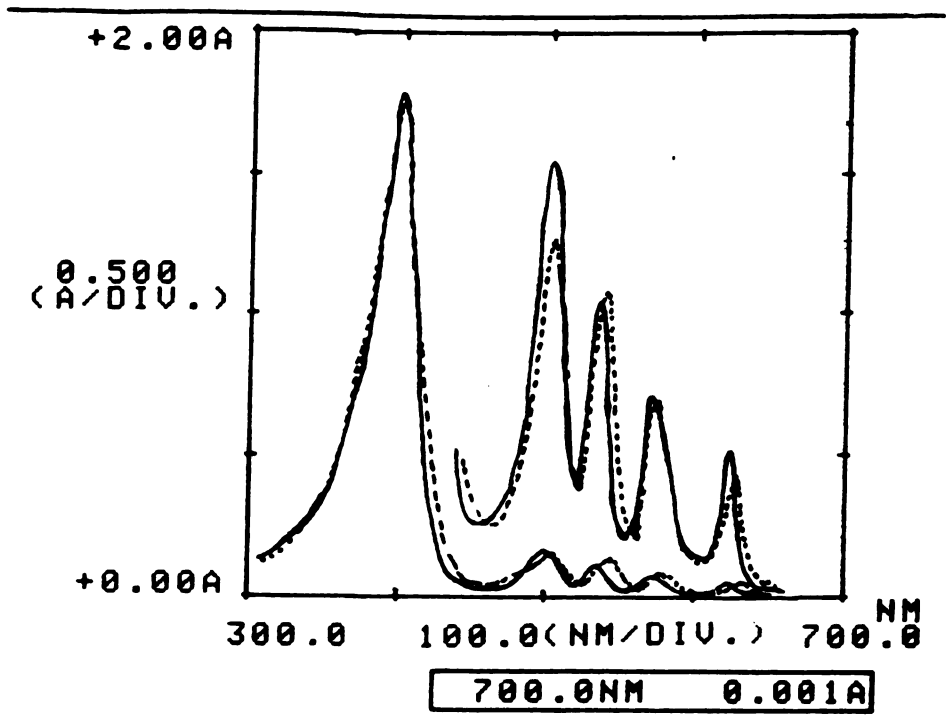



Figure 7. UV-vis absorption spectra of dimeric chloroethylporphyrin (83; —) and dimeric vinylporphyrin (84; ·····) in $CH_2Cl_2$.

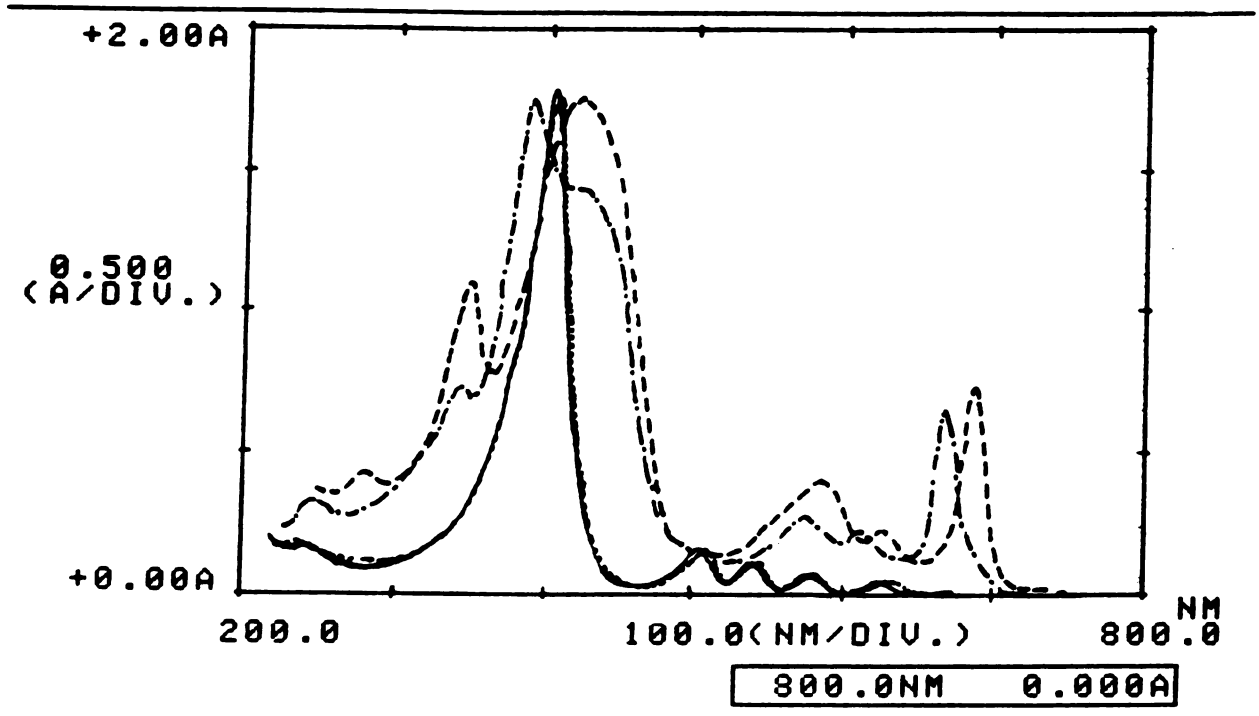



Figure 8. UV-vis absorption spectra of dimeric chloroethylporphyrin (83; —), dimeric vinylporphyrin (84; ·····), dimeric formylmethylenylchlorin (85; - . -), and dimeric benzoporphyrin (86; ——) in $CH_2Cl_2$.

Table 4. UV-vis absorption spectral data of dimeric chloroethylporphyrin (83), vinylporphyrin (84), formylmethylenylchlorin (85) and benzoporphyrin (86) in $CH_2Cl_2$.

| | Absorbance (nm) in $CH_2Cl_2$ | | | | |
|---|---|---|---|---|---|
| Compound | Soret | IV | III | II | I |
| 83 | 400 | 498 | 533 | 568 | 621 |
| 84 | 402 | 502 | 539 | 570 | 625 |
| 85 | 387 | – | 570 | 670 | 662 |
| 86 | 417 | – | 580 | 620 | 681 |

This strong absorbance in the longer wavelengths may be advantageous in eradication of larger tumors and utilization of inexpensive laser sources. Fluorescence lifetimes of dimeric porphyrins were approximately 15±1 ns, a result also obtained with porphyrin monomers, e. g., hematoporphyrin. In contrast, the ether dimers found in HPD showed a shorter lifetime, 7 ns. These results demonstrate the absence of fluorescence-quenching ring-ring interactions in the dimeric porphyrins linked by a hexamethylene bridge. The dimeric porphyrins were short term sensitizers *in vivo*. Optimal timing between injection and irradiation *in vivo* (mouse tumor system) was 3 hr for the active products. In contrast, Photofrin-II allows a 24 hr interval. Persistence in plasma of the dimeric porphyrins was substantially less than that of HPD. The determinants of persistence of dyes in neoplastic cells are not yet fully elucidated. Biological studies of the dimeric porphyrins *in vitro* and *in vivo* were not entirely carried out.

## IV. EXPERIMENTAL

Ethyl 3-acetyl-4-oxopentanoate (71)

Anhydrous potassium carbonate (415 g, 3 mol) and potassium iodide (99.6 g, 0.6 mol) were added to a solution of 2,4-pentanedione (308 mL, 3 mol) and ethyl chloroacetate (320 mL, 3 mol) in 2-butanon (1 L). The reaction mixture was carefully heated to reflux for 5 hours because of its exothermic character, and then diluted with acetone to precipitate salt. The solid was filtered off and washed with acetone. The filtrates were concentrated under vacuum and the residual oil was distilled under reduced pressure to yield the title compound as colorless liquid (260 g, 50.4%): b.p. 128-131 °C/8-10 mm; $^1$H-NMR ($CDCl_3$) δ 1.09 (3H, t, -$OCH_2\underline{C}H_3$), 2.11 (6H, s, -$CO\underline{C}H_3$), 2.72 (2H, s, -$CO\underline{C}H_2$), 3.10 (1H, t, -CH), 3.96 (2H, q, -$O\underline{C}H_2CH_3$) ; MS for $C_9H_{14}O_4$, found m/e 186 (M+).

Benzyl acetoacetate (70)

Ethyl acetoacetate (1280 mL, 10 mol) and benzyl alcohol (1040 mL, 10 mol) were mixed and heated to boil away formed ethyl alcohol. The residual oil was distilled under reduced pressure to give the title compound as colorless liquid (1693 g, 88.2%): b.p. 156-159 °C/10 mm; $^1$H NMR ($CDCl_3$) δ 2.26 (3H, s, $\underline{C}H_3CO$), 3.50 (2H, s, $CO\underline{C}H_2CO$), 5.19 (2H, s, $\underline{C}H_2C_6H_5$), 7.37 (5H, m, phenyl protons); MS for $C_{11}H_{12}O_3$, found m/e 192 ($M^+$).

Benzyl 4-(ethoxycarbonylmethyl)-3,5-dimethylpyrrole-2-carboxylate (72)

Benzyl acetoacetate (70) (192 g, 1 mol) dissolved in acetic acid (200 mL) was cooled in an ice bath and an ice cold solution of sodium nitrite (76 g, 1.1 mol) in water (100 mL) was added dropwise over 2 hours. The resulting cold solution of benzyl α-oximinoacetoacetate was added dropwise, along with zinc dust (200 g) in small portions, to a stirred solution of ethyl 3-acetyl-4-oxopentanoate (71) (186 g, 1 mol) in acetic acid (350 mL). Near the end of the addition, the temperature of reaction mixture reached above 80 °C and then the reaction mixture was refluxed for an additional one hour. After the reaction was completed, the mixture was poured into a large amount of water to result solid product. The resulting solid was collected by filtration and washed repeatedly with water. The filtered cake was dissolved in dichloromethane and zinc was filtered off and rinsed with dichloromethane. The filtrate was evaporated to give the solid pyrrole which was crystallized from methanol and water to give the title compound as a white sparkling power (141.8 g, 45%): m.p. 79-80 °C; $^1$H NMR ($CDCl_3$) δ 1.23 (3H, t, -$OCH_2\underline{CH_3}$), 2.21 (3H, s, 3-Me), 2.29 (3H,s, 5-Me), 3.38 (2H, s, $\underline{CH_2}CO_2C_2H_5$), 4.11 (2H, q, -$O\underline{CH_2}CH_3$), 5.38 (2H, s, -$O\underline{CH_2}Ph$), 7.39 (5H, m, phenyl protons), 8.93 (1H, br s, NH); MS for $C_{18}H_{21}NO_4$, found m/e 315 ($M^+$).

Benzyl 4-(2-hydroxyethyl)-3,5-dimethylpyrrole-2-carboxylate (73)

Sodium borohydride (8 g, 0.2 mol) was added to a solution of benzyl 4-(ethoxycarbonylmethyl)-3,5-dimethylpyrrole-2-carboxylate (72) (31.5 g, 0.1 mol) in dry tetrahydrofuran (150 mL) and cooled to 10 °C with stirring in an ice bath. To the reaction mixture, boron trifluoride etherate (35 mL, 0.28 mol) was added dropwise under nitrogen such that the temperature of the reaction mixture was maintained 15±3 °C. After the addition, the reaction mixture was

stirred for an additional 30 minutes in the ice bath and 2 hours at room temperature or until the reaction was completed, which was monitored by TLC using chloroform. Methanol (50 mL) was carefully added to the reaction mixture until the vigorous effervescense ceased and the reaction mixture was diluted with dichloromethane (150 mL) and then with water (300 mL). The organic layer was separated, washed with hydrochloric acid (0.5N, 100 mL) and finally twice with water (100 mL). The solvents were evaporated under reduced pressure after methanol (50 mL) was added. The resulting white solid was crystallized from dichloromethane and hexane to give the title compound as pale bluffy needles (25.7 g, 94%): m.p. 115-116 °C ; $^1$H NMR ($CDCl_3$) δ 1.92 (1H, br s, O<u>H</u>), 2.21 (3H, s, 3-Me), 2.31 (3H, S, 5-Me), 2.65 (2H, t, -<u>C</u>$H_2$$CH_2OH$), 3.65 (2H, t, -$CH_2$<u>C</u>$H_2$OH), 5.30 (2H, S, -O<u>C</u>$H_2$Ph), 7.39 (5H, m, phenyl protons), 9.19(1H, br s, NH) ; MS for $C_{16}H_{19}NO_3$, found m/e 273 ($M^+$).

<u>Benzyl 4-(2-chloroethyl)-3,5-dimethylpyrrole-2-carboxylate (74)</u>

Benzyl 4-(2-hydroxyethyl)-3,5-dimethylpyrrole-2-carboxylate (73) (54.6 g, 0.2 mol) was dissolved in dry benzene (1 L) with warming. The homogeneous solution was cooled down to room temperature and fresh thionyl chloride (29.2 mL, 0.4 mol) was added under nitrogen. The reaction was accomplished by stirring for 3 hours at room temperature without any base as an acid scavenger. The solvent was evaporated to dryness under vacuum, and the residue was chromatographed through a short flash column of silica gel with dichloromethane. A trace amount of unreacted starting material (73) and some impurities remained in the silica gel and only the title compound (74) was eluted by dichloromethane. The eluates were combined and evaporated under reduced pressure. The resulting residue was

crystallized from dichloromethane and hexane to give the title compound as ivory-white fluffy crystals (57.2 g, >99%): m.p. 119-120 °C ;$^1$H NMR ($CDCl_3$) δ 2.20 (3H, s, 3-Me), 2.28 (3H, s, 5-Me), 2.83 (2H, t, -$\underline{CH_2}CH_2Cl$), 3.51 (2H, t, -$CH_2\underline{CH_2}Cl$), 5.29 (2H, s, -O$\underline{CH_2}$Ph), 7.39 (5H, m, phenyl protons), 8.88 (1H, br s, NH); MS for $C_{16}H_{18}NO_2Cl$, found m/e 291 ($M^+$).

4-(2-Chloroethyl)-3,5-dimethylpyrrole-2-carboxylic acid (75)

Benzyl 4-(2-chloroethyl)-3,5-dimethylpyrrole-2-carboxylate (74) (29.2 g, 0.1 mol) was dissolved in freshly distilled tetrahydrofuran (400 mL), and then 10% palladium/carbon (1 g) and triethylamine (10 drops) were added. The reaction mixture was stirred under hydrogen (1 atm., room tempersture) until hydrogen uptake ceased. After hydrogenation, palladium/carbon was filtered off and washed further with tetrahydrofuran. The filtrates were combined and evaporated under vacuum to give the title compound as a white-gray powder (20.0 g, >99%): m.p. 114-116 °C; $^1$H NMR ($CDCl_3$) δ 2.23 (3H, s, 3-Me), 2.29 (3H, s, 5-Me), 2.85 (2H, t, -$\underline{CH_2}CH_2Cl$), 3.51 (2H, t, -$CH_2\underline{CH_2}Cl$), 8.88 (1H, br s, NH), 11.20 (1H, br s, -COOH); MS for $C_9H_{12}NO_2Cl$, found m/e 201 ($M^+$).

4-(2-Chloroethyl)-2-formyl-3,5-dimethylpyrrole (76)

4-(2-Chloroethyl)-3,5-dimethylpyrrole-2-carboxylic acid (75) (10.1 g, 0.05 mol) was dissolved in trifluoroacetic acid (50 mL) at 40 °C and triethyl orthoformate (16.7 mL, 0.1 mol) was added in one portion. The reaction mixture was stirred further for 5 minutes at 40 °C and quenched by adding water slowly with gentle stirring to give precipitated oil which soon solidified. The resulting solid was collected by filtration and dissolved in ethanol (100 mL). To the ethanolic solution, ammonium hydroxide (2N, 70

mL) was slowly added with stirring and after 10 minutes, water (150 mL) was added to precipitate product. The resulting solid was collected by filtration and chromatographed on silica gel with 0.5% methanol in dichloromethane and then crystallized from ethanol and water to give the title formyl pyrrole (7.8 g, 84%): m.p. 144-148 °C ; $^1$H NMR ($CDCl_3$) δ 2.26, 2.27 (3H each, s, 3, 5-Me), 2.83 (2H, t, -$\underline{CH_2}CH_2Cl$), 3.51 (2H, t, -$CH_2\underline{CH_2}Cl$), 9.46 (1H, s, -$\underline{CH}O$), 9.56 (1H, br s, NH); MS for $C_9H_{12}NOCl$, found m/e 185 and 187 ($M^+$).

Diethyl oximinomalonate.

Diethyl malonate (160.7 g, 1.0 mol) was dissolved in glacial acetic acid (200 mL) and then cooled in an ice bath. A saturated aqueous solution of sodium nitrite (207 g, 3.0 mol) was added dropwise with stirring in an ice bath and the temperature was controlled below 20 °C. After addition the reaction mixture was stirred in an ice bath for another one hour. The light orange oxime solution was kept at low temperature and used immediately.

Ethyl 3.5-dimethylpyrrole-2-carboxylate. (77)

A solution of 2,4-pentanedione (100.1 g, 1.0 mol,)in glacial acetic acid (200 mL) was heated, and at 80 °C anhydrous sodium acetate (246 g, 3.0 mol) and zinc dust (197 g, 3.0 mol) were added with vigorous stirring. At 95 °C the diethyl oximinomalonate, prepared before, was added dropwise with vigorous stirring and the temperature was maintained between 95 and 105 °C. After heating to 100-105 °C for an additional 30 minutes, the reaction mixture was poured with stirring into ice-water mixture (3 L). The crude product was collected by filtration, washed with water, and then dissolved in hot 95% ethanol (1 L). After filtration of the hot mixture to remove the zinc dust, the filtrate was concentrated to 300 mL, poured into ice-water mixture (1.5 L) to

precipitate product. The solid product was collected by filtration and recrystallized from 95% ethanol to afford the title compound (108.6 g, 65% yield): m.p. 124-125 °C; $^1$H NMR ($CDCl_3$) δ 1.33 (3H, t, -$OCH_2\underline{CH_3}$), 2.23, 2.29 (3H each, s, 3, 5-Me), 4.27 (2H, q, -$O\underline{CH_2}CH_3$), 5.77 (1H, s, 4-H), 8.85 (1H, br s, NH); MS for $C_9H_{13}NO_2$, found m/e 167 ($M^+$).

1,6-Bis[5-(ethoxycarbonyl)-2,4-dimethylpyrrole-3-yl]-1,6-hexanedione (78)

Ethyl 3,5-dimethylpyrrole-2-carboxylate (77) (16.7 g, 0.1 mol) was dissolved in a mixture of dry dichloromethane (150 mL) and dry nitromethane (100 mL) with heating, and adipoyl chloride (7.3 mL, 0.05 mol) was added in an ice bath under nitrogen. To the cold reaction mixture, tin(IV) chloride (17.5 mL, 0.15 mol) was added dropwise in the ice bath over 30minutes with stirring. The reaction mixture was stirred for an additional 4 hours at room temperature, poured into ice/water (200 mL), acidified with concentrated hydrochloric acid, and stirred for 15 minutes to complete the hydrolysis of the tin(IV) complex. The precipitated mass of product was collected by filtration, washed alternately with water and dichloromethane, and finally with methanol (collected separately). After drying, the solid weighted 37.8 g (73.5% yield). Evaporation in of the organic phases from the filtrates afforded a second crop of 6.3 g (12.3%). Total 44.1 g (85.8%).

For analysis, a sample was recrystallized from tetrahydrofuran and methanol: $^1$H NMR ($CDCl_3$) δ 1.27 (6H, t, -$OCH_2\underline{CH_3}$), 1.57 (4H, m, $CH_2$), 2.42, 2.47 (6H each, s, 2, 4-Me), 2.70 (4H, t, -$CO\underline{CH_2}$), 4.22 (4H, q, -$O\underline{CH_2}CH_3$), 11.73 (2H, br s, NH); MS for $C_{24}H_{32}N_2O_6$, found m/e 444 ($M^+$).

1,6-Bis[5-(ethoxycarbonyl)-2,4-dimethylpyrrole-3-yl]-1,6-hexane (79)

Sodium borohydride (8 g, 0.2 mol) was added to 1,6-bis[5-(ethoxycarbonyl)-2,4-dimethylpyrrole-3-yl]-1,6-hexanedione (78) (22.2 g, 0.05 mol) dissolved in dry tetrahydrofuran (100 mL). The reaction mixture was cooled to 10 °C with stirring in an ice bath and treated with dropwise boron trifluoride etherate (35 mL, mol) under nitrogen such that the temperature of the reaction mixture was maintained 15±3 °C. The reaction mixture was kept stirring for additional 30 minutes in the ice bath and 3-4 hours at room temperature until the reaction was completed which was monitored by TLC using chloroform. The reaction mixture was poured into a mixture of ice (200 mL), hydrochloric acid (IN, 50 mL) and chloroform (200 mL). The organic phase was washed with hydrochloric acid (0.5N, 100 mL) and then with water and dried over anhydrous sodium sulfate. Methanol (50 mL) was added to the organic solution and then the solvents were removed under reduced pressure until the product crystallized out. The solids were collected by filtration, washed with methanol, and dried to give the title compound (25.1 g, 82.9%). Evaporation of the organic filtrates gave a further 1.2 g (4.0%).

For analysis, a sample was recrystallized from dichloromethane and methanol: $^1$H NMR ($CDCl_3$) δ 1.31, 1.38 (4H each, m, $CH_2$), 1.32 (6H, t, -$OCH_2\underline{C}H_3$), 2.15, 2.23 (6H each, s, 2, 4-Me), 2.31 (4H, t, $CH_2$), 4.27 (4H, q, -$O\underline{C}H_2CH_3$), 8.66 (2H, br s, NH); MS for $C_{24}H_{36}N_2O_4$, found m/e 416 ($M^+$).

1,6-Bis[5-(benzyloxycarbonyl)-2,4-dimethylpyrrole-3-yl]-1,6-hexane (80)

After benzyl alcohol (150 mL) was heated up to 180 °C to remove moisture, 1,6-bis[5-(ethoxycarbonyl)-2,4-dimethylpyrrole-3-yl]-1,6-hexane (79) (41.6 g, 0.1 mol) was added. The reaction mixture was heated up to boiling for 10 minutes to drive off water. To this hot homogeneous solution, a fresh

saturated solution of sodium in dry benzyl alcohol (10 mL) was cautiously added in small (1 mL) portions under nitrogen until the evolution of ethanol ceased. Further portions were added every several minutes until the exchange was completed when the boiling point had again risen above 200 °C. Heating was continued to reflux for 10 minutes after the effervescence subsided. The hot reaction mixture was poured cautiously into a magnetically stirred solution of acetic acid (20 mL) in methanol (500 mL), and water was then added until crystallization was completed. The light pinkish solid was collected by filtration, washed with 50% aqueous methanol and dried to give the title compound (50.9 g, 94.3%).

For analysis a sample was recrystallized from tetrahydrofuran and methanol: m.p. 95-97 °C; $^1$H NMR ($CDCl_3$) δ 1.30, 1.39 (4H each, m, $CH_2$),2.13, 2.24 (6H each, s, 2, 4-Me), 2.30 (4H, t, $CH_2$), 5.27 (4H, s, -$\underline{CH_2}C_6H_5$), 1.36 (10H, m, phenyl protons), 8.56 (2H, br s, NH); MS for $C_{34}H_{40}N_2O_4$, found m/e 540 ($M^+$).

1,6-Bis[5-(hydroxycarbonyl)-2,4-dimethylpyrrole-3-yl]-1,6-hexane (81)

1,6-Bis[5-(Benzyloxycarbonyl)-2,4-dimethylpyrrole-3-yl]-1,6-hexane (80) (22.5 g, 0.05 mol) was dissolved in freshly distilled tetrahydrofuran (400 mL) containing 10 drops of triethylamine. 10% Palladium/carbon (1 g) was added and the reaction mixture stirred under hydrogen (1 atm., at room temperature) until hydrogen uptake ceased. The catalyst carbon was filtered off and rinsed with tetrahydrofuran. Evaporation of filtrates gave the title compound as a light gray power (17.5 g, 97%): m.p. 89-90 °C; $^1$H NMR ($CDCl_3$) δ 1.32, 3.42 (4H each, m, $CH_2$), 2.20, 2.31 (6H each, s, 2, 4-Me), 2.38 (4H, t, $CH_2$),

8.73 (2H, br s, NH), 11.21 (2H, br s, -COOH); MS for $C_{20}H_{28}N_2O_4$, found m/e 360 ($M^+$).

1,6-Bis[4'-(2-chloroethyl)-3,3',5,5'-tetramethyl-2,2'-dipyrrylmethenium-4-yl]hexane bromide (82)

1,6-Bis[5-(Hydroxycarbonyl)-2,4-dimethylpyrrole-3-yl]-1,6-hexane (81) (7.20 g, 0.02 mol) and 4-(2-chloroethyl)-2-formyl-3,5-dimethylpyrrole (76) (7.42 g, 0.04 mol) were suspended in methanol (100 mL) and heated up to boiling. Hydrobromic acid (48%, 16 mL) was carefully added in one portion (vigorous forming), and the red-orange product was immediately formed. After the reaction was accomplished by heating for 30 minutes, the reaction mixture was transfered into a beaker and then allowed to stand for 2 hours at room temperature. The resulting orange fluffy solid was collected by filtration, rinsed with slightly acidified water containing small amount of hydrobromic acid, and dried in air to give the title compound (13.1 g, 88%).

For analysis, a sample was recrystallied from dichloromethane and hexane : $^1$H NMR ($CDCl_3$) δ 1.28, 1.40 (4H each, br s, $CH_2$), 2.23, 2.28 (3H each, s, $-CH_3$), 2.39 (4H, t, $CH_2$), 2.63, 2.65 (6H each, s, $CH_3$), 2.86 (4H, t, $-\underline{CH_2}CH_2Cl$), 3.53 (4H, t, $-CH_2\underline{CH_2}Cl$), 7.04 (2H, s, methine), 13.03, 13.10 (2H each, br s, NH).

1,6-Bis[8-(2-chloroethyl)-13,17-bis(2-methoxycarbonylethyl)-3,7,12,18-tetramethylporphyrin-2-yl]hexane (83)

1,6-Bis[4'-(2-chloroethyl)-3,3',5,5'-tetramethyl-2,2'-dipyrrylmethenium-4-yl]hexane bromide (82) (3.73 g, 5 mmol) and 5,5'-dibromo-3,3'-bis(2-methoxycarbonylethyl)-4,4'-dimethyl-2,2'-dipyrrylmethenium bromide (65) (5.83 g, 10 mmol) were suspended in formic acid (98-100%, 90 mL) and the mixture was heated up to 80 °C in an oil bath. At this temperature, bromine

(0.52 mL, 10 mmol) was added in one portion and the reaction mixture was refluxed for 2 hours. The solvent was then allowed to boil off and the residue was dried completely with further heating in the oil bath up to 130-140 °C during which time the porphyrin was formed. Methanol (100 mL) and concentrated sulfuric acid (5 mL) were added to the residue, and after 10 minutes triethyl orthoformate (12 mL) was added. After standing overnight, protected from moisture, the solution was diluted with dichloromethane and the tar residue was filtered off by passing the solution through glass wool. The solution was neutralized with saturated aqueous sodium bicarbonate. The organic layer was separated, washed twice with water and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to dryness and the resulting crude product was chromatographed on silica gel column with 1% methanol in dichloromethane as eluent. A dark nonfluorescent forerun was discarded. The major reddish fractions containing dimeric porphyrin were collected and crystallized from dichloromethane and methanol to give the title compound as red-brown sparkling crystals (0.58 g, 9%): m.p. > 248 °C (decomp.) ; $^1$H NMR ($CDCl_3$) δ -3.86 (4H, br s, NH), 1.90, 2.32 (4H, br s, $CH_2$), 3.14, 3.26 (4H each, t, $CH_2\underline{CH_2}CO_2$), 3.42, 3.52, 3.56, 3.58, 3.63, 3.65 (6H each, s, ring Me, OMe), 4.03 (4H, t, 2-$CH_2$), 4.12 (4H, t, $CH_2\underline{CH_2}Cl$), 4.22 (4H, t, $\underline{CH_2}CH_2Cl$), 4.42, 4.54 (4H each, t, $\underline{CH_2}CH_2CO_2$), 9.95, 9.99, 10.01, 10.01 (2H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 621 nm (5,000), 568 (7,000), 533 (10.000), 498 (14,000), 400 (178.000); MS for $C_{74}H_{84}N_8O_8Cl_2$, found m/e 1283 ($M^+$).

1,6-Bis[13,17-bis(2-methoxycarbonylethyl)-3,7,12,18-tetramethyl-8-vinyl-porphyrin-2-yl]hexane (84)

1,8-Diazabicyclo[5.4.0]undec-7-ene (1 mL) was added to 1,6-bis[8-(2-chloroethyl)-13,17-bis(2-methoxycarbonylethyl)-3,7,12,18-tetramethylporphyrin-2-yl]hexane (83) (154 mg, 0.12 mmol) dissolved in dry N,N-dimethylformamide (30 mL). The reaction mixture was heated in an oil bath at 80 °C for 2 hours and then cooled to room temperature. To the reaction solution, water was added with stirring until the product precipitated. The precipitated solid was collected by filtration on a bed of Celite and rinsed with excess water, finally with a small amount of methanol. The product on Celite was extracted with dichloromethane and the solvent was removed under vacuum. The resulting residue was chromatographed on silica gel column with 0.5% methanol in dichloromethane as eluent [the title compound (84) moves slower than the starting compound (83) on the silica gel column], and crystallized from dichloromethane and methanol to give the title compound (109 mg, 75%): m.p. > 248 °C (decomp.); $^1$H NMR ($CDCl_3$) δ -3.83 (4H, br s, NH), 1.90, 2.33 (4H each, m, $CH_2$), 3.14, 3.22 (4H each, t, $CH_2\underline{CH_2}CO_2$), 3.41, 3.49, 3.58, 3.61, 3.63, 3.63 (6H each, s, ring Me, OMe), 4.00 (4H, t, 2-$CH_2$), 4.20, 4.40 (4H each, t, $\underline{CH_2}CH_2CO_2$), 6.10, 6.30 (2H each, d, -CH=$\underline{CH_2}$), 8.22 (2H, dd, -$\underline{CH}$=$CH_2$), 9.94, 9.96, 9.98, 10.14 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 625 nm (4,000), 570 (8,800), 539 (12,000), 502 (14,000), 402 (170,000); MS for $C_{74}H_{82}N_8O_8$, found m/e 1211 ($M^+$).

1,6-Bis[8-formylmethylenyl-7-hydroxy-13,17-bis(2-methoxycarbonylethyl)-3,7,12,18-tetramethylchlorin-2-yl]hexane (85)

1,6-bis[13,17-bis(2-Methoxycarbonylethyl)-3,7,12,18-tetramethyl-8-vinyl-porphyrin-2-yl]hexane (84) (121 mg, 0.1 mmol) was dissolved in

dichloromethane (100 mL) with pyridine (0.5 mL) and oxygen gas was bubbled in the reaction solution with irradiation of xenon lamp (200 W). The irradiation was stopped when a tenth part of the starting vinyl porphyrin (84) was remained which was monitored by TLC. After the reaction, the reaction mixture was neutralized with diluted acetic acid. The organic layer containing the product was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness under vacuum. The resulting residue was chromatographed on silica gel with 1-1.5% methanol in dichloromethane to give the title compound (54 mg), 47% yield based on reacted the compound (84): $^1$H NMR ($CDCl_3$) δ -4.17, -3.88 (2H each, br s, NH), 1.14 (6H, s, 7-Me), 1.81, 2.20 (4H each, m, $CH_2$), 3.01, 3.11 (4H each, t, $CH_2\underline{CH_2}CO_2$), 2.92, 2.94, 3.17, 3.17, 3.18, 3.18, 3.57, 3.58, 3.61, 3.61 (3H each, s, ring Me, OMe), 3.70 (4H, t, 2-$CH_2$), 3.94, 4.16 (4H each, t, $\underline{CH_2}CH_2CO_2$), 6.36 (2H, br s, OH), 8.23 (2H, br s, =$\underline{CH}$-CHO), 9.38, 9.39, 9.51, 9.54 (1H each, s, meso H), 9.83 (2H, br s, -CHO); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 662 nm (71.000), 605 (22.900), 570 (30,100), 387 (193,000); MS for $C_{74}H_{82}N_8O_{12}$, found m/e 1275 ($M^+$).

1,6-Bis{7$^1$,7$^2$-bis(methoxycarbonyl)-13,17-bis(2-methoxycarbonylethyl)-3,7,12,18-tetramethyl-7,7$^1$-dihydrobenzo[g]porphyrin-2-yl}hexane (86)

Dimethyl acetylenedicarboxylate (DMAD) (5 mL, 40 mmol) was added to 1,6-bis[13,17-bis(2-methoxycarbonylethyl)-3,7,12,18-tetramethyl-8-vinylporphyrin-2-yl]hexane (84) (84.7 mg, 0.07 mmol) dissolved in dry toluene (40 mL). The reaction mixture was refluxed under nitrogen in the dark for 5 days. After the Diels-Alder reaction was completed, the solvent was evaporated and the residue was further dried under high vacuum to remove

toluene and the remaining trace of DMAD. A few drops of 1,8-diazabicyclo[5.4.0]undec-7-ene were added to the Diels-Alder adduct (**69**) dissolved in dichloromethane (35 mL), which is isolable but not isolated. The rearrangement reaction occured immediately. The reaction mixture was poured into 2N hydrochloric acid and the product was extracted with dichloromethane. The organic layers were combined, washed with water and brine, and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue which was purified by preparaive thick layer plates (silica gel) eluted with 1% methanol in dichloromethane. The product was extracted from silica gel and crystallized from dichloromethane and petroleum ether to give the title compound (28.2 mg, 27%): $^1$H NMR ($CDCl_3$) $\delta$ -2.51, -2.43 (2H each, br s, NH), 1.78 (6H, s, exocyclic ring Me), 1.88, 2.24 (4H each, m, $CH_2$), 2.88, 3.33, 3.43, 3.47, 3.61, 3.62, 3.97 (6H each, s, ring Me, OMe), 3.09, 3.18 (4H each, t, $CH_2\underline{CH_2}CO_2$, 4.12, 4.31 (4H each, t, $\underline{CH_2}CH_2CO_2$), 5.03 (2H, s, H-7'), 7.42, 7.81 (2H each, d, H-$7^3$,H-$7^4$), 8.97, 9.38, 9.67, 9.67 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 681 nm (27,000) 620 (8,200), 580 (15,000), 417 (70,000), 353 (42,300); MS for $C_{86}H_{94}N_8O_{16}$, found m/e 1495 ($M^+$).

# CHAPTER 4

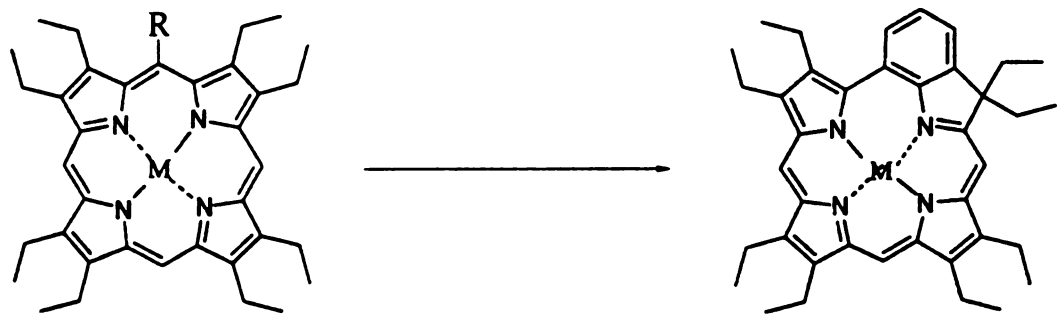
## SYNTHESES AND PROPERTIES OF CATIONIC PORPHYRIN AND BENZOCHLORIN DERIVATIVES

## I. INTRODUCTION

In preliminary studies, a cationic photosensitizer has shown itself to be a non-traditional photodynamic sensitizer in that it exhibits an extremely short triplet state formation in solution (approx. 20 nsec).[50,51] It seems unlikely that its PDT effect is initiated by type II reactions (*via* singlet oxygen, $^1O_2$). To study the mechanisms of action of cationic photosensitizers, we designed and prepared several cationic photosensitizer derivatives.

To prepare porphyrins containing ammonium group, we first synthesized some porphyrins containing ester group (**87, 97, 124, 125**) which were hydrolyzed, chlorinated with oxalyl chloride and reacted with N,N-dimethylethylenediamine to give the corresponding derivatives containing dimethylamine group (**90, 100, 130, 131**), which were treated with iodomethane to yield the corresponding trimethylammonium group (**91, 101, 132, 133**). Also we introduced "oxo" group on porphyrin ring to prepare some porphyrinones (**104, 136**) containing trimethylammonium group which possess better absorption character and 20 nm red-shift in the visible spectrum than the corresponding porphyrins (**101, 132, 133**).

In 1978, Johnson *et al.*[124] reported the synthesis of the first benzochlorin (G), after cyclization of nickel *meso*-(2-formylvinyl) octaethylporphyrin (B), obtained from nickel(II) complex of octaethylporphyrin (A) *via* a three step, low yield procedure. Successive formylation and Wittig reaction of the nickel octaethylporphyrin (A) gave the *meso*-vinyl derivative (E) (see Scheme 14). A second formylation gave compound (B) which was treated with mineral acid for cyclization to give nickel(II) octaethylbenzochlorin (G) in 22% yield. Recently, Smith and co-workers[125,126] improved this pathway, utilizing a modified Vilsmeier reagent [3-(dimethylamino)acrolein and phosphoryl chloride; (3-DMA/$POCl_3$)] which gave access to the formyl derivative (B) in one step, starting from (A). Cyclization generated, once again, the nickel benzochlorin (G).



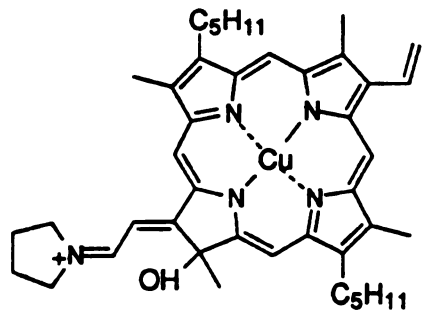

**A** M=Ni, R=H
**B** M=Ni, R=CHCHCHO
**C** M=2H, R=CHCHCH$_2$OH
**D** M=2H, R=CHCHCO$_2$Et
**E** M=Ni, R=CHCH$_2$

**F** M=2H
**G** M=Ni

**Scheme 14**

Alternatively, Morgan *et al.*[129] showed that cyclization of the *meso*-(3-hydroxypropenyl)octaethylporphyrin (C), obtained after reduction of *meso*-[β-(ethoxycarbonyl)vinyl]octaethylporphyrin (D), gives also the target benzochlorin (F) in 36% yield (Scheme 14). Benzochlorins possess an intense absorption at 658 nm, whereas addition of a metal, into the aromatic cavity, is characterized by a 15 nm red shift in the visible spectrum.[125,126,129]

In 1984, our group, in studying reversible modification of formyl peripheral substituents, reported that protonated Schiff bases (imines) of "chlorin-type" compounds (i.e. pyrrolidinium salt (H) are characterized by an unusual red shift of the absorption maxima in the visible region (up to 800 nm).[68]




H

Recently, Skalkos *et al.*[69] reported the synthesis of the iminium salts from the reaction of metallo benzochlorins with the Vilsmeier reagent ($DMF/POCl_3$). The iminium salts are very stable cationic adducts, characterized by a strong red shift (around 100 nm) of the absorption maxima in the visible region of the electromagnetic spectrum.

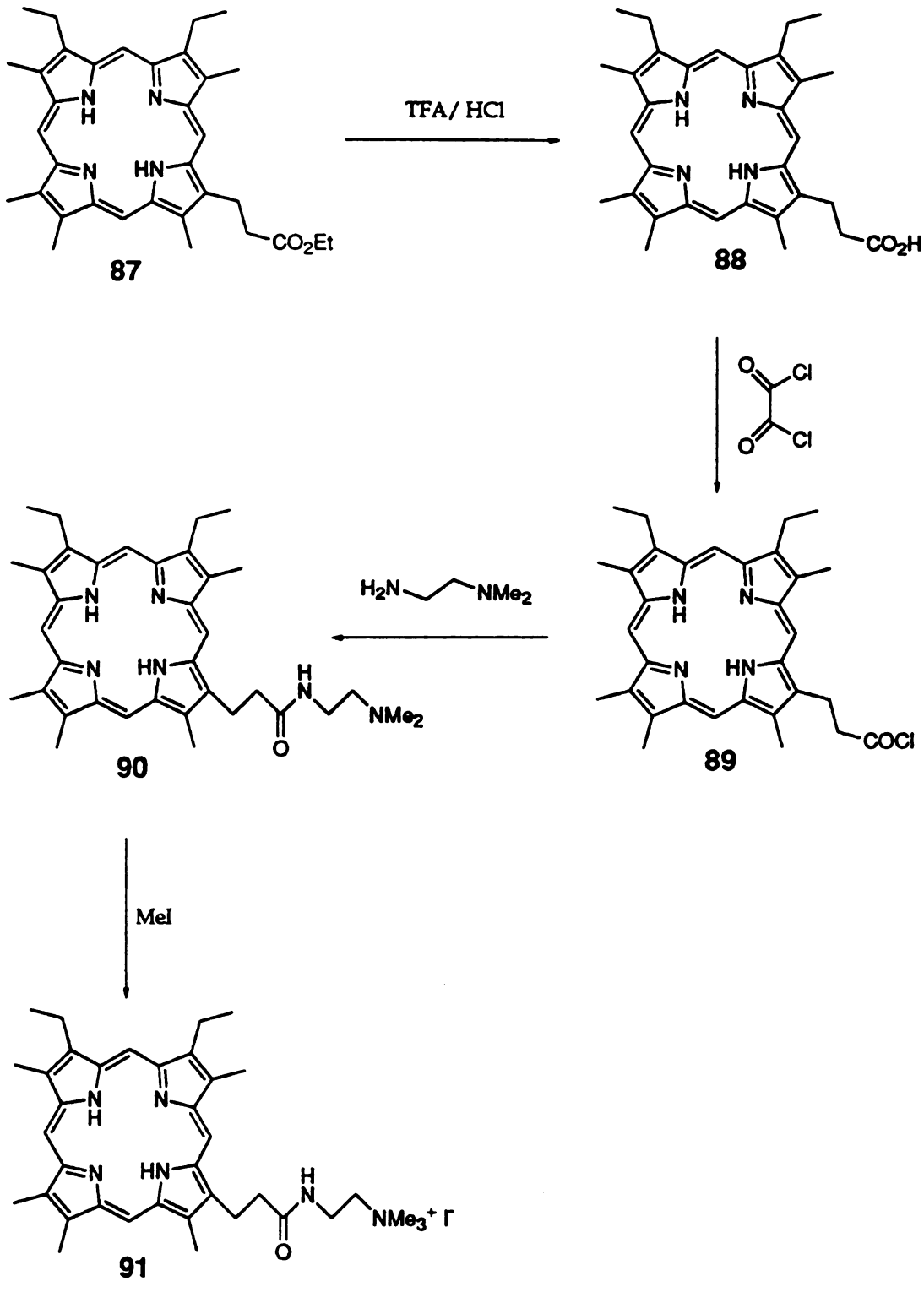
The previous observations prompted us to investigate the syntheses of cationic porphyrin derivatives by introduction of trimethylammonium group on porphyrins or by reactions of benzochlorins with the Vilsmeier reagent.

## II. SYNTHESES

Several rational approaches toward the preparation of cationic porphyrin derivatives were empoyed by total syntheses of target materials or modifications of precusor porphyrins. Five porphyrins (87, 97, 124, 125, 137) were chosen for investigation.

As shown in Scheme 15, ethyl 3,7-diethyl-2,8,13,17,18-pentamethylporphyrin-12-propionate (87), prepared before by our group, was hydrolyzed by heating on a steam bath for 6 hours in trifluoroacetic in the presence of hydrochloric acid, and then chlorinated by oxalyl chloride in dichloromethane for one hour at room temperature, and then condensed with N,N-dimethylethylenediamine in dichloromethane for one hour at room temperature to give aminoporphyrin (90) in 80% yield which was purified on silica gel column, using methanol-dichloromethane (15:85) as eluant. A cationic porphyrin (91) containing trimethylammonium group was prepared by treatment of the aminoporphyrin (90) in methanol-dichloromethane (10:90) with an excess of iodomethane for 5 hours at room temperature.

To prepare other two cationic porphyrin derivatives (101) in Scheme 17) and(104 in Scheme 18), the porphyrin (97) was synthesized as shown in Scheme 16. Two dipyrrylmethenes, "north" dipyrrylmethene (93) and



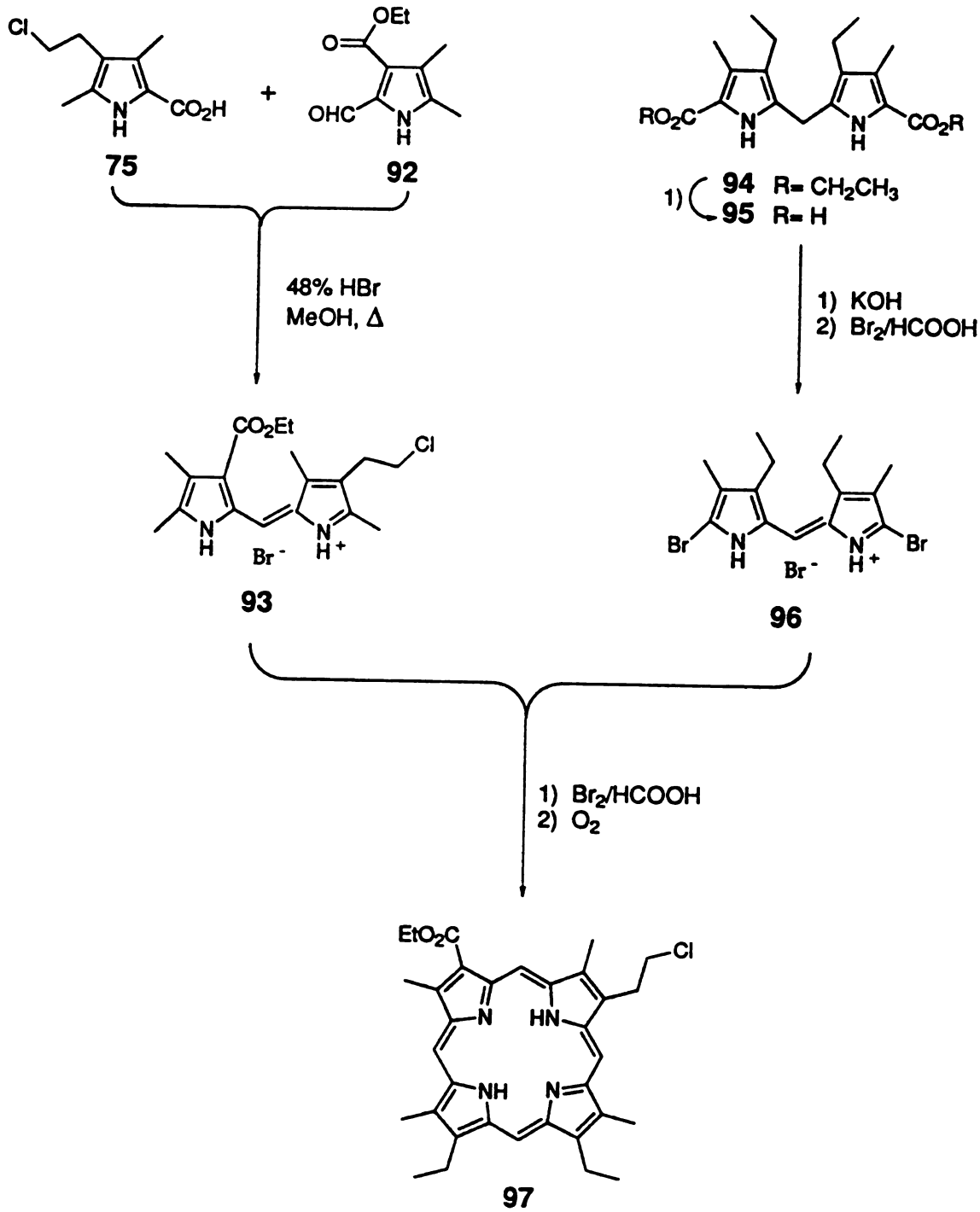

**Scheme 15**

"south" dipyrrylmethene (96), were refluxed together in anhydrous formic acid in the presence of one equivalent of bromine to form the porphyrin (97) in 8.5% yield, after purification on silica gel column. This low yield is most likely due to the presence of a withdrawing group which deactivates the cyclization.

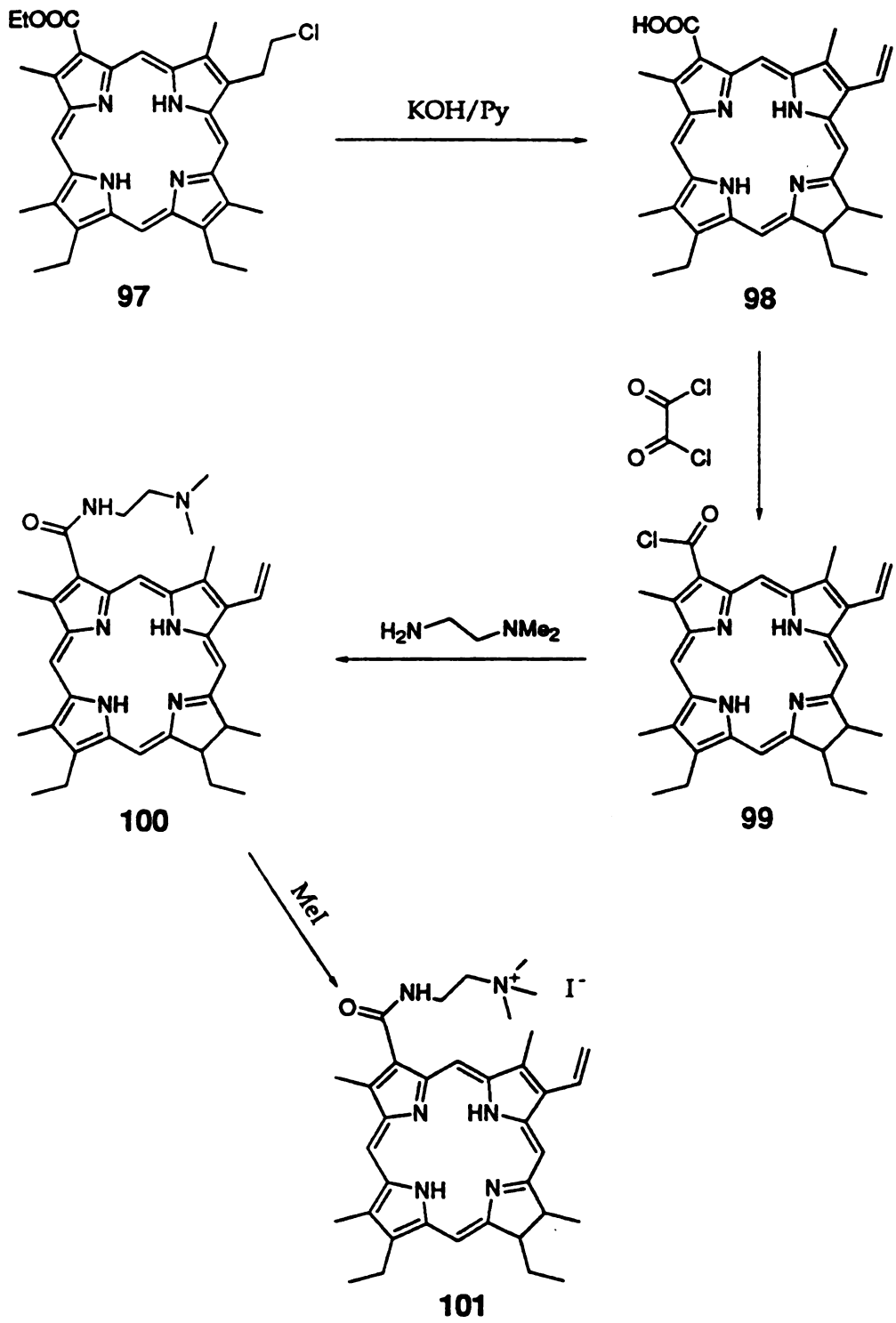
Dipyrrylmethene (96) was easily prepared from ethoxycarbonyl dipyrrylmethane (94) as follows. The ethoxycarbonyl dipyrrylmethane (94) was refluxed for one hour in ethanol with 40% aqueous potassium hydroxide for hydrolysis. The obtained acid dipyrrole (95) was treated in anhydrous formic acid with an excess of bromine for decarboxylation and bromination to give the dipyrrylmethenium bromide (96) as shown in Scheme 16.

The other dipyrrylmethene (93) was obtained by condensation of the acid pyrrole (75) and the formyl pyrrole (92) in hot methanol with hydrobromic acid in 92% yield. The two pyrroles (75 and 92) were respectively prepared as shown in Scheme 10 and Scheme 21.

As shown in Scheme 17, the porphyrin (97) was transformed to the aminoporphyrin (100) as follows. The porphyrin (97) was refluxed for one hour in pyridin with 5% aqueous potassium hydroxide for hydrolysis (in this case, the ester group directly attached on porphyrin ring can be hydrolyzed only in severer condition due to stabilization by aromatic porphyrin ring.) which was monitored by TLC, to give the acid vinylporphyrin (98). Without purification, the obtained acid porphyrin (98) was treated for one hour at room temperature with oxalyl chloride in dry dichloromethane for chlorination. The dried chlorinated porphyrin (99) was directly treated for one hour at room temperature with an excess amount of N,N-dimethylethylenediamine in dry dichloromethane to give aminoporphyrin


Cl
CO2H
N
H
75
+
OEt
O
OHC
92
RO2C
CO2R
1)
94 R= CH2CH3
95 R= H
48% HBr
MeOH, Δ
1) KOH
2) Br2/HCOOH
CO2Et
Cl
Br -
93
Br
96
1) Br2/HCOOH
2) O2
EtO2C
HN
NH
97

**Scheme 16**


EtOOC
Cl
N
HN
NH
N
97
KOH/Py
HOOC
98
O
Cl
O
Cl
Cl
O
99
$H_2N$
$NMe_2$
NH
N
O
100
MeI
NH
$N^+$
$I^-$
101

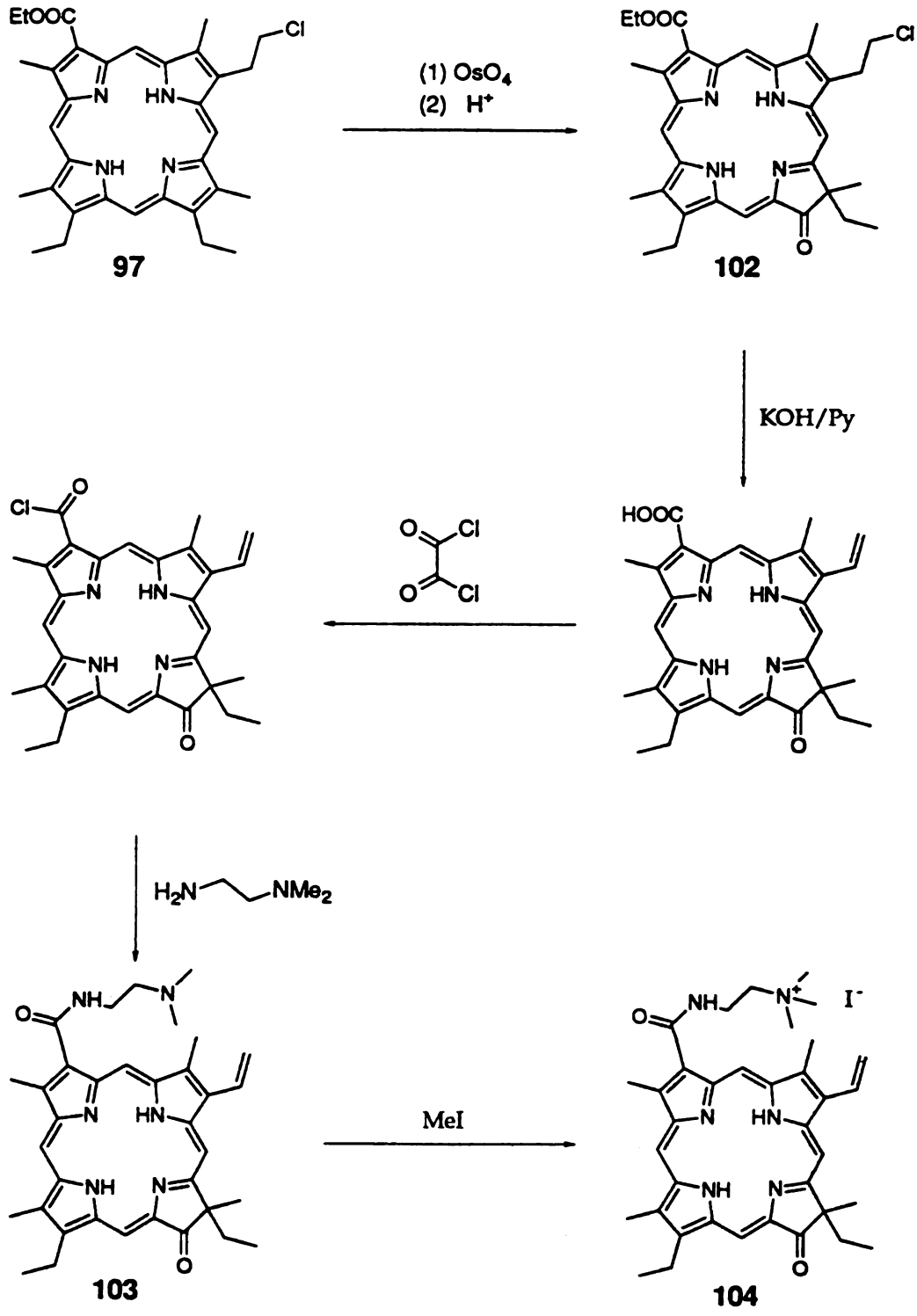
**Scheme 17**

(100). The cationic porphyrin (101) was then prepared by treatment of the aminoporphyrin (100) with iodomethane as described before.

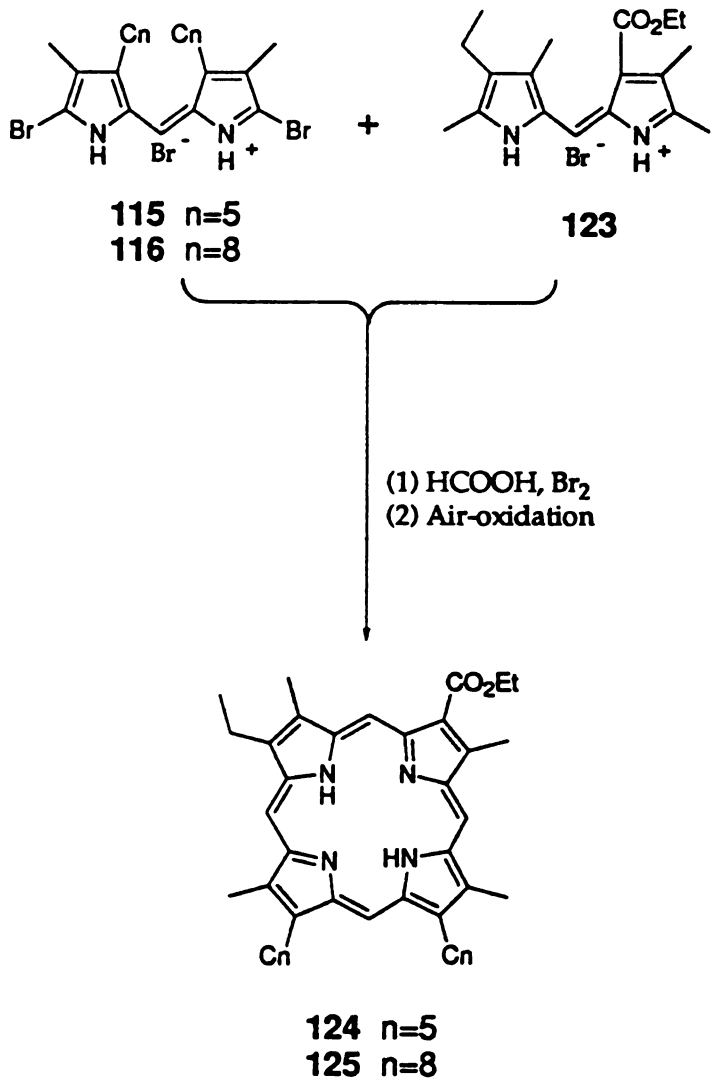
The cationic porphyrinone (104) was prepared as described in Scheme 18. The intermediate porphyrinone (102), made by oxidation of the porphyrin (97) with osmium tetroxide and followed by acid-catalyzed pinacolic rearrangement, was hydrolyzed with potassium hydroxide in pyridine, chlorinated with oxalyl chloride and condensed with N,N-dimethylethylenediamine to give the aminoporphyrinone (103) which was treated with an excess amount of iodomethane as described before to give the cationic porphyrinone (104).

In order to investigate relative PDT efficacy of cationic sensitizers with lipophilic character, two porphyrins (124) and (125) were synthesized as shown in Scheme 19. Dipyrrylmethenes (115) and (116), which provide the lipophilic character to the porphyrins (124) and (125), were respectively condensed with dipyrromethene (123), to form the porphyrins (124) and (125) in 12-14% yields, after purified by chromatography on silica gel with dichloromethane-hexane (2:1).

As shown in Scheme 20, the dipyrromethanes (115) and (116) were prepared as follows. β-Free pyrrole (77) was treated for 2 hours at around 10 °C with octanoyl chloride in the presence of tin(IV) chloride to obtain octanoylpyrrole (105) in almost quantitative yield. The octanoylpyrrole (105) was reduced to octylpyrrole (106) in 94% yield with an excess of diborane, which was transesterificated to the corresponding benzyl ester pyrrole (107) in an excess amount of benzyl alcohol in the presence of sodium benzyloxide in 85% yield. The pyrroles (107) and (108) were treated for one hour in hot acetic acid with lead tetraacetate to give acetoxymethylpyrroles (109) and (110) respectively in 95-96% yields, which were separately treated for one hour in




**Scheme 18**

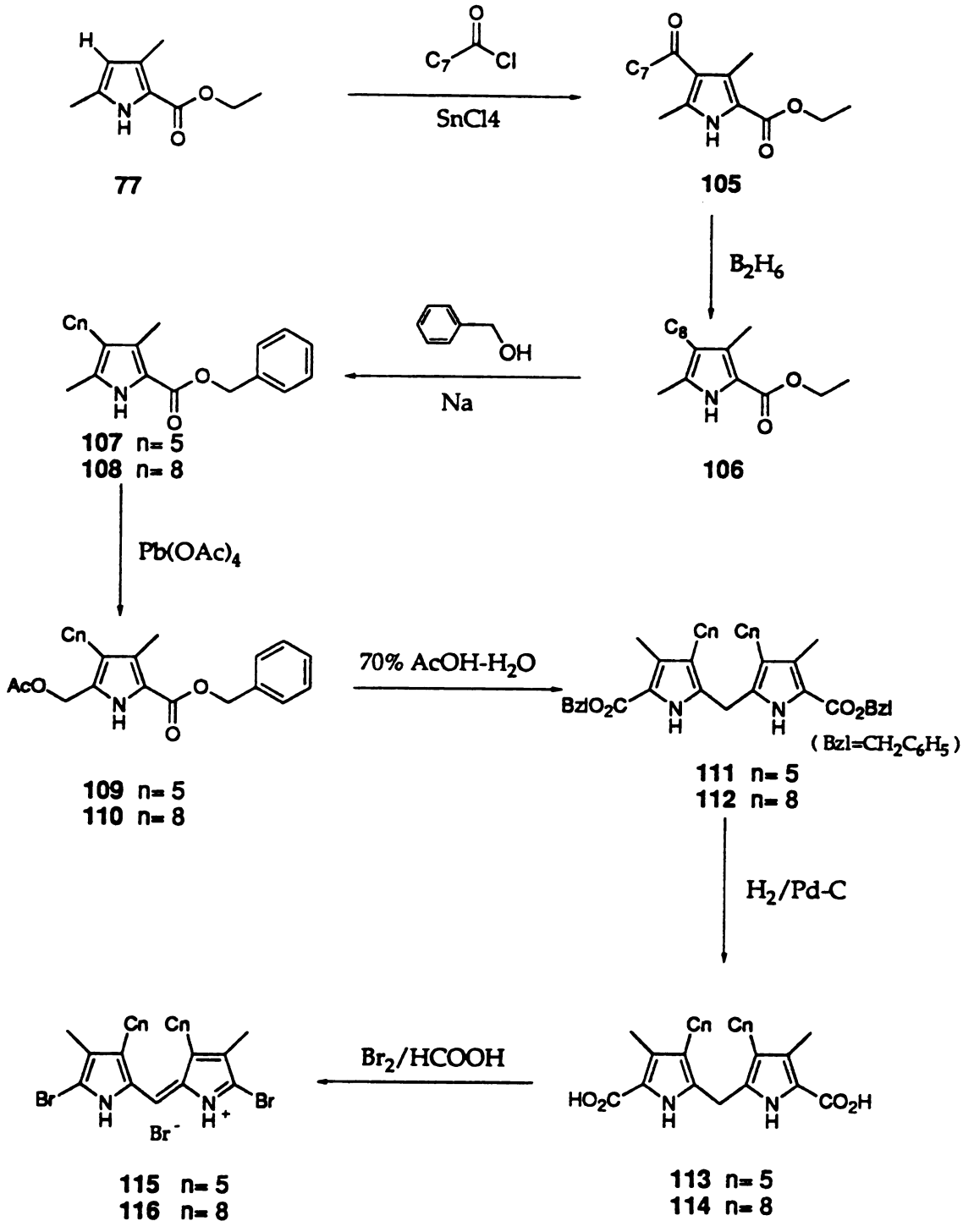



**115** n=5
**116** n=8

**123**

**124** n=5
**125** n=8

**Scheme 19**


77
SnCl4
105
B2H6
106
Na
107 n= 5
108 n= 8
Pb(OAc)4
109 n= 5
110 n= 8
70% AcOH-H2O
111 n= 5
112 n= 8
(Bzl=CH2C6H5)
H2/Pd-C
Br2/HCOOH
113 n= 5
114 n= 8
115 n= 5
116 n= 8

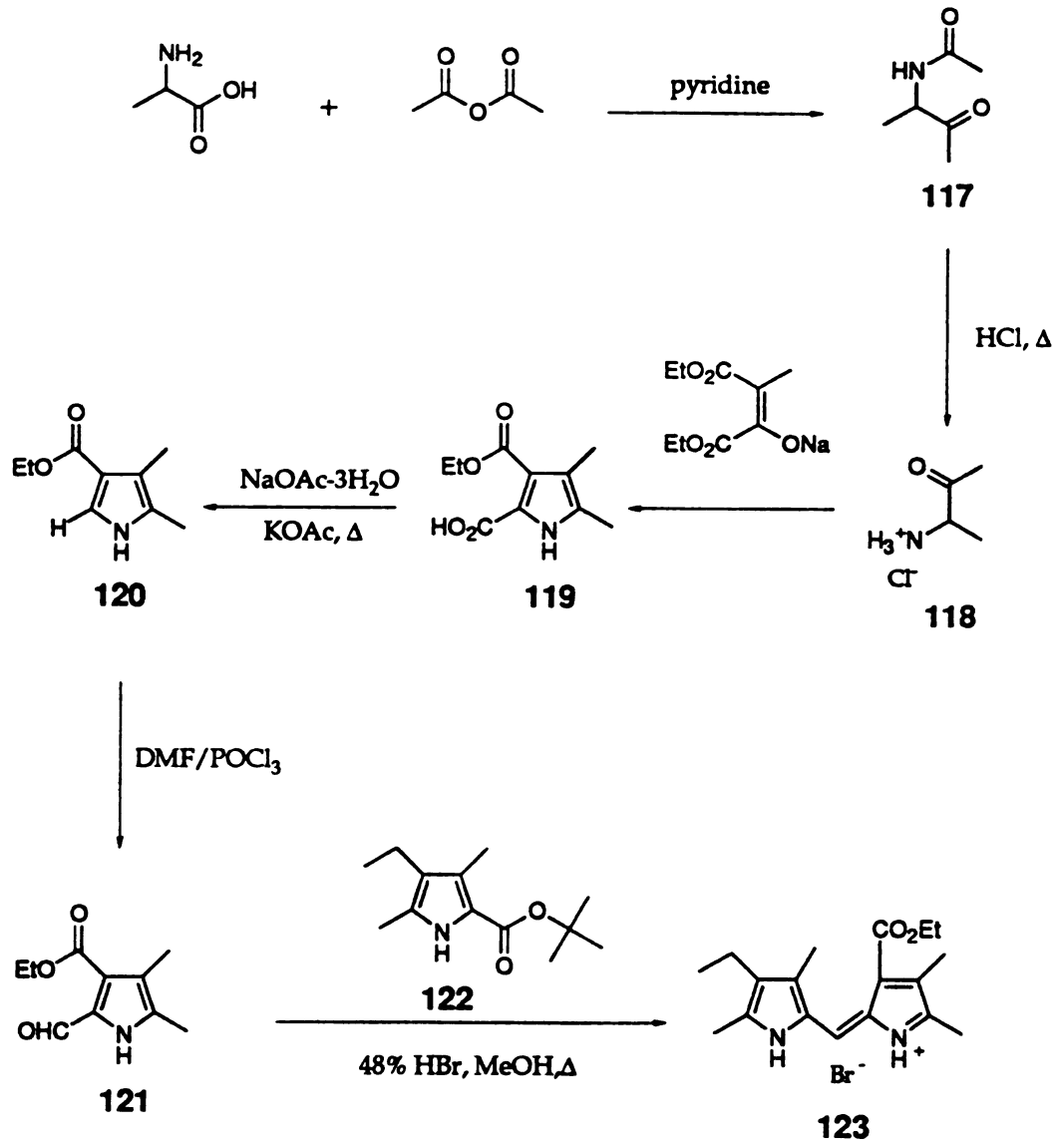
**Scheme 20**

refluxing acetic acid-water (70:30) for self-condensation to yield dipyrrylmethanes (**111**) and (**112**) in 78% yield, after recrystallization from ethanol-water. The dipyrrylmethanes (**111**) and (**112**) were hydrogenated in THF with 10% palladium/carbon to give the acid analogues (**113**) and (**114**) which were directly treated respectively for one hour at room temperature with anhydrous formic acid in the presence of an excess amount of bromine without isolation of them because of their poor solubility in THF. After reaction, catalyst carbon was removed by filtration and the desired dipyrrylmethenes (**115**) and (**116**) were obtained as violet crystals in 82% yield.

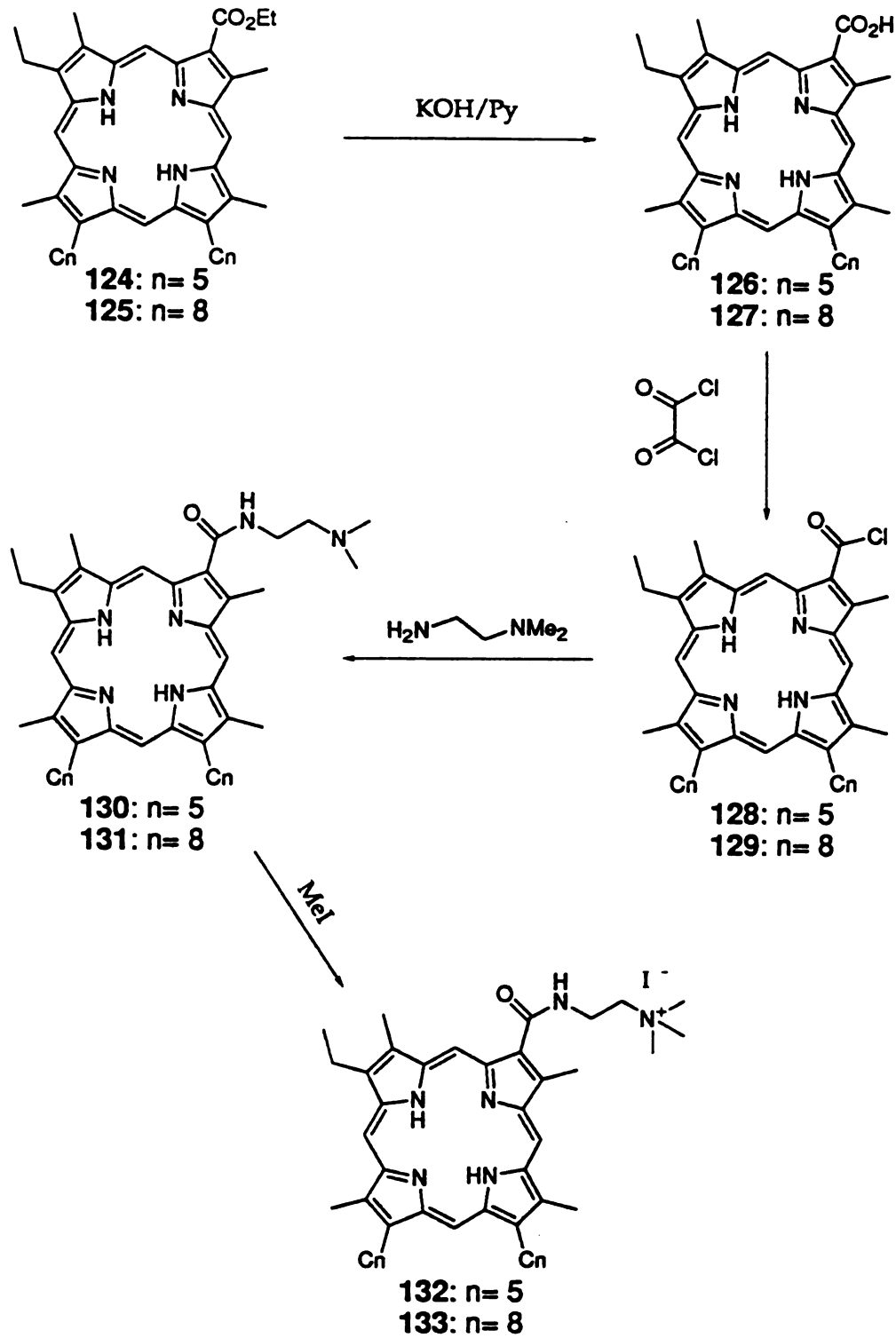
The other dipyrrylmethene (**123**) was synthesized according to Scheme 21. The dipyrrylmethene (**123**) was prepared by the condensation of two pyrroles (**121**) and (**122**) in methanol in the presence of an excess amount of 48% hydrobromic acid. The reaction was accomplished within 30 minutes on a steam bath.

Alanine was heated in pyridine with an excess of acetic anhydride to give 3-acetamido-2-butanone (**117**) as a light yellow liq. (b.p. 110-125 °C/3 mm) in 88% yield, which was treated in refluxing hydrochloric acid for 8 hours to give 3-ammonio-2-butanone chloride (**118**) as a white powder in 88% yield. The ammonium salt (**118**) was allowed to react with diethyl oxalacetate sodium salt in water in the presence of sodium hydroxide to yield the acid pyrrole (**119**) in 37% yield, which was decarboxylated by heating in molten mixture of $NaOAc{\cdot}3H_2O$-KOAc (1:1) to give the α-free pyrrole (**120**) in 94% yield. The formylpyrrole (**121**) was then obtained by the Vilsmeier reaction of the α-free pyrrole (**120**) with $DMF/POCl_3$ in 56% yield.

As shown in Scheme 22, the porphyrin ammonium salts (**132**) and (**133**) were synthesized as follows. The ester group of the porphyrins (**124**) and (**125**) was hydrolyzed with potassium hydroxide in pyridine, chlorinated with


pyridine
117
HCl, Δ
118
119
NaOAc-3H2O
KOAc, Δ
120
DMF/POCl3
121
122
48% HBr, MeOH,Δ
123

**Scheme 21**

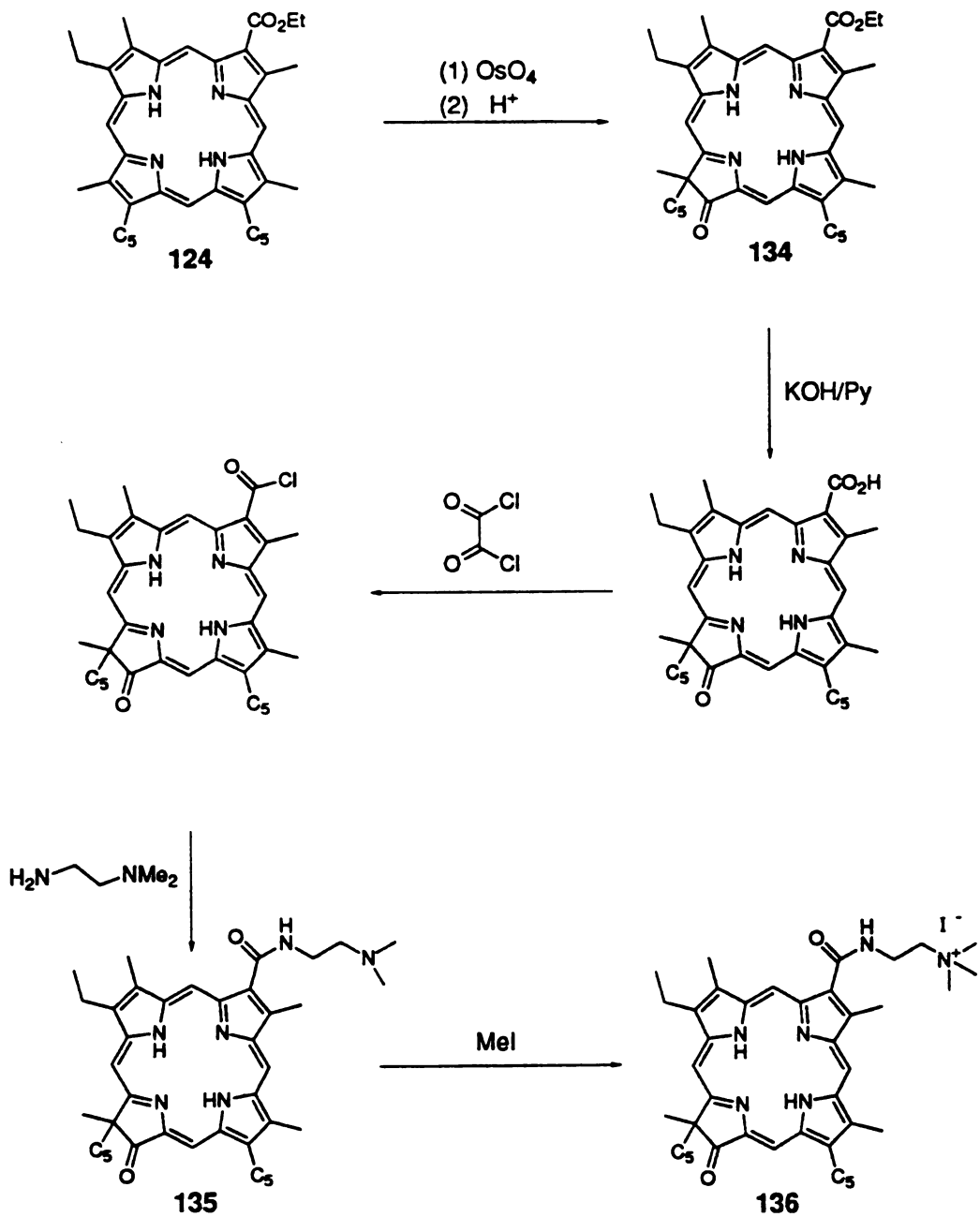

CO2Et
KOH/Py
CO2H
124: n= 5
125: n= 8
126: n= 5
127: n= 8
Cl
Cl
O
O
H2N
NMe2
128: n= 5
129: n= 8
130: n= 5
131: n= 8
MeI
I -
132: n= 5
133: n= 8

**Scheme 22**

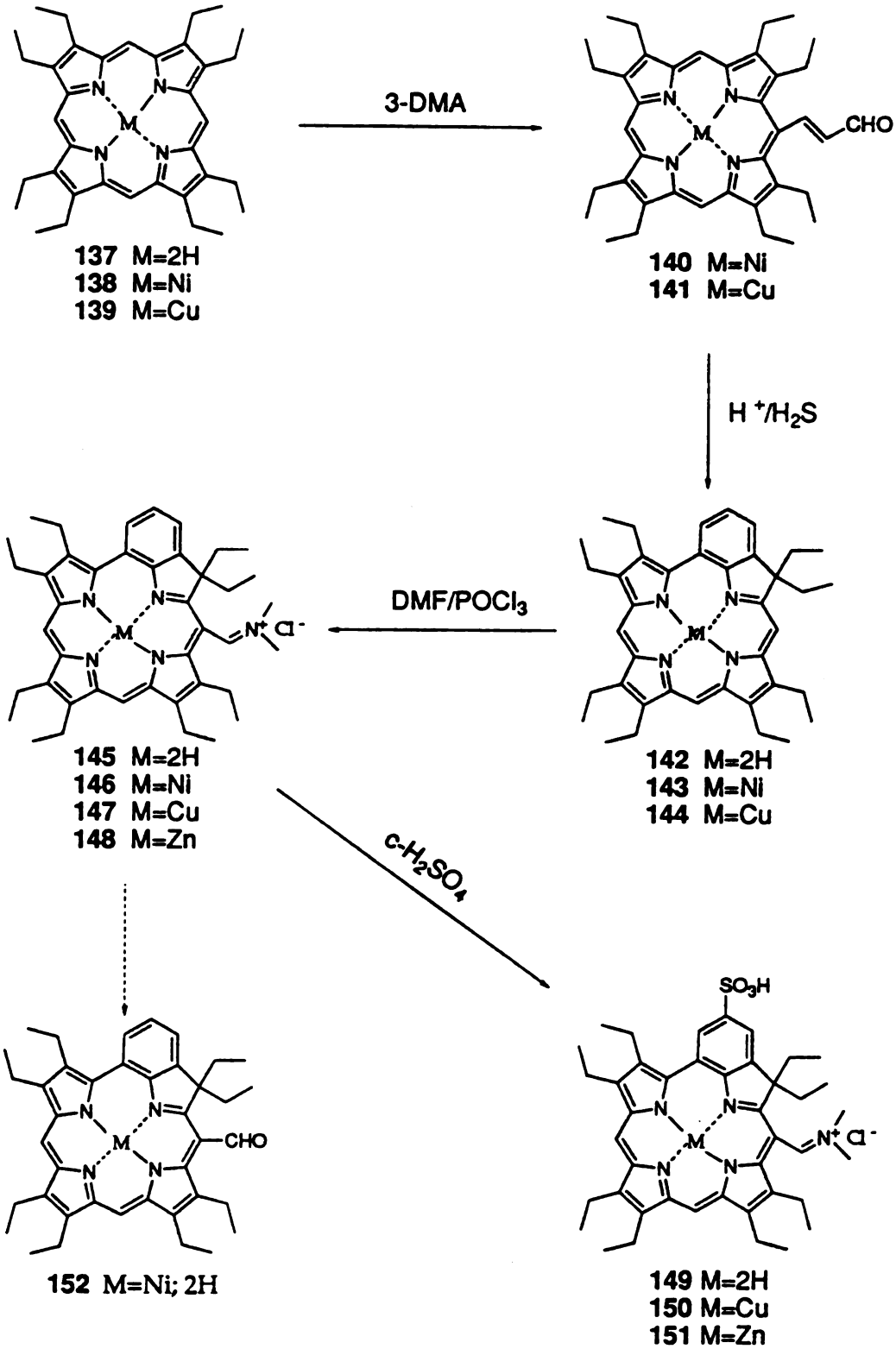
oxalyl chloride, and reacted with N,N-dimethylethylenediamine to give aminoporphyrins (**130**) and (**131**) respectively in around 80% yields which were separately treated with an excess amount of iodomethane to give the final cationic porphyrins (**132**) and (**133**) respectively as described before.

Another cationic porphyrinone (**136**) was prepared from porphyrinone (**134**) as described in Scheme 23. The porphyrinone (**134**) was prepared by oxidation of the porphyrin (**124**) with osmium tetroxide in dichloromethane and pinacolic rearrangent with perchloric acid in 35% yield as described before. The obtained porphyrinone (**134**) was treated with potassium hydroxide in pyridine, oxalyl chloride and then N,N-dimethylethylenediamine to give aminoporphyrinone (**135**) which was further treated with an excess amount of iodomethane to give the desired cationic porphyrinone (**136**) as described before.

In order to prepare the *meso*-dimethyliminium chloride derivatives (**145, 146, 147, 148, 149, 150,** and **151**) in Scheme 24, nickel(II) octaethylporphyrin (**138**) was treated with 3-DMA/$POCl_3$ for 10 hours at room temperature to give *meso*-acrolein derivative (**140**) in 87% yield after the normal hydrolysis of the imine salt intermediate with saturated aqueous sodium carbonate. The cyclization of the acrolein group onto the adjacent pyrrole subunit β-position occured by treatment of the compound (**140**) in 18% sulfuric acid in trifluoroacetic acid with hydrogen sulfide for one hour at room temperature to produce the metal-free benzochlorin (**142**) in 82% yield. The compound (**142**) was previously prepared by Smith and Vicente[125,126] by two-step process, consisting of cyclization with concentrated sulfuric acid followed by demetallation with trifluoroacetic acid and 1,3-propanedithiol in 4.7% overall yield, along with a 70% recovery of the nickel(II) complex (**143**). The nickel(II) complex (**143**) was prepared from metallalation of the


CO2Et
(1) OsO4
(2) H+
124
134
KOH/Py
CO2H
Cl
O
Cl
O
Cl
H2N
NMe2
MeI
I -
135
136

Scheme 23




**137** M=2H
**138** M=Ni
**139** M=Cu

**140** M=Ni
**141** M=Cu

**142** M=2H
**143** M=Ni
**144** M=Cu

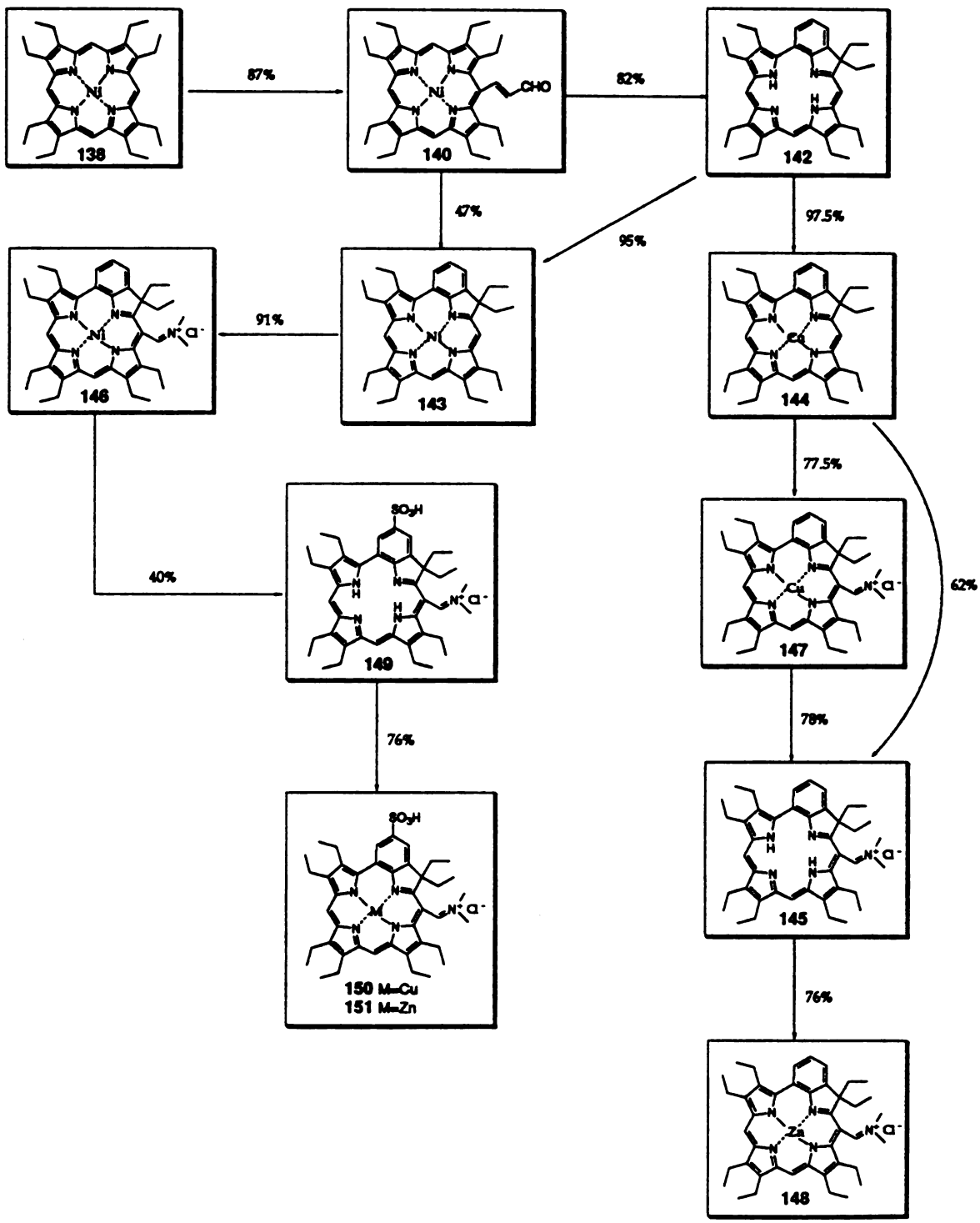
**145** M=2H
**146** M=Ni
**147** M=Cu
**148** M=Zn

**149** M=2H
**150** M=Cu
**151** M=Zn

**152** M=Ni; 2H

**Scheme 24**

octaethylbenzochlorin (**142**) with nickel(II) chloride in 95% yield, or from cyclization of the nickel(II) compound (**140**) with concentrated sulfuric acid in 47% yield. Treatment of the nickel complex of octaethylbenzochlorin (**143**) with DMF/$POCl_3$ (overnight at room temperature) gave the nickel *meso*-dimethyliminium chloride derivative (**146**) in 91% yield. Removal of the robust central nickel(II) ion from the compound (**146**) was accomplished by using concentrated sulfuric acid for 4 days at room temperature, and a 40% yield of the metal-free iminium chloride benzochlorin (**145**), along with a 40% yield of the metal-free sulfonated benzochlorin (**149**). A better way to prepare the metal-free iminium chloride benzochlorin (**145**) is the treatment of copper(II) complex of octaethylbenzochlorin (**144**) with DMF/$POCl_3$, which gave copper(II) *meso*-dimethyliminium octaethylbenzochlorin (**147**), followed by the treatment of copper(II) complex (**147**) with concentrated sulfuric acid for 4 hours at room temperature to afford the metal-free iminium benzochlorin (**145**) in 78% yield. The copper(II) complex of octaethylbenzochlorin (**144**) was obtained by reflux of octaethylbenzochlorin (**142**) with copper acetate in dichlormethane-methanol (3:1), for 20 minutes, in 97.5% yield. According to our experience, in order to prepare the iminium chloride benzochlorin (**145**) in better overall yield, without isolation, the crude nickel(II) compound (**140**), obtained by the treatment of the nickel(II) octaethylporphyrin (**138**) with 3-DMA/$POCl_3$, was first treated with hydrogen sulfide in 18% sulfuric acid/trifluoroacetic acid for one hour at room temperature to give directly the metal-free benzochlorin (**142**) which was treated with copper acetate in refluxing dichloromethane-methanol (3:1) for 20 minutes to form the copper(II) octaethylbenzochlorin (**144**). The compound (**144**) was treated with DMF/$POCl_3$ to produce the copper(II) *meso*-dimethyliminium octaethylbenzochlorin (**147**) which was treated with


87%
82%
47%
95%
97.5%
91%
40%
77.5%
62%
76%
78%
76%
138
140
142
143
144
146
147
148
149
145
150 M=Cu
151 M=Zn

Scheme-25

concentrated sulfuric acid for 4 hours at room temperature to afford the metal-free iminium benzochlorin (**145**) in 48% overall yield from the nickel octaethylporphyrin (**138**) (in 4 steps). The sulfonated derivative (**149**) was prepared by the treatment of the nickel(II) compound (**146**) with concentrated sulfuric acid for 4 days as mentioned before. An outline of overall reaction for effective preparation of benzochlorin derivatives and iminium derivatives was summarized in Scheme 25.

## III. RESULTS AND DISCUSSION

Broadly cationic porphyrin derivatives, prepared and characterized in this study, can structually fall into two categories: cationic groups linked with the β-position of pyrrole and with the *meso*-position of octaethylbenzochlorin. In the former category, the porphyrins (**87, 97, 124,** and **125**) containing ester group were easily transformed into the N-[2-(dimethylamino)ethyl]carbamido derivatives (**90, 100, 130,** and **131**) which were purified and analyzed by UV-vis spectrophotometer, $^1$H NMR spectrometer, and mass spectrometer. All the amino compounds (**90, 100, 130,** and **131**) were purified before being treated with iodomethane to afford the desired cationic compounds (**91, 101, 132,** and **133**). All their spectral results are shown in Table 5. The cationic compounds have the same absorbance spectra as their amino compounds which are also similar to that of the parent porphyrins. Enhancing long-wavelength absorption was achieved by introduction of oxo group on porphyrin ring. The ketochlorins (**104, 136**) were easily prepared by oxidation with osmium tetroxide and by acid-catalyzed pinacolic rearrangement as mentioned before. The oxidation of the

Table 5 UV-vis absorption spectral data of cationic porphyrin derivatives containing trimethylammonium group ( **91, 101, 104, 132, 133** and **136**) and their precursors (**87, 90. 97, 100, 102, 103, 124, 125, 130, 131, 134** and **135**).

| Compound | Soret | IV | III | II | I |
|---|---|---|---|---|---|
| **87** | 397 | 497 | 531 | 565 | 618 |
| **90** | 397 | 497 | 531 | 565 | 618 |
| **91** | 396 | 497 | 531 | 565 | 618 |
| **97** | 406 | 506 | 545 | 573 | 628 |
| **100** | 408 | 506 | 542 | 574 | 628 |
| **101** | 408 | 506 | 542 | 574 | 628 |
| **102** | 319 | 412 | 537 | 568 | 643 |
| **103** | 404 | 417 | 541 | 573 | 647 |
| **104** | 404 | 417 | 542 | 573 | 648 |
| **124,125** | 406 | 509 | 547 | 573 | 630 |
| **130, 131** | 403 | 504 | 541 | 568 | 622 |
| **132, 133** | 403 | 505 | 542 | 569 | 623 |
| **134** | 412 | 524 | 516 | 578 | 634 |
| **135** | 412 | 524 | 516 | 578 | 634 |
| **136** | 413 | 525 | 561 | 579 | 635 |

porphyrin (**97, 124**) mainly effected dihydroxylation at the diagonal pyrrole double bond of the pyrrole containing ester group as would be expected by electronic effects. The cationic ketochlorins (**104, 136**) showed 12~20 nm red shift and an increase of intensity in the visible region as compared with the corresponding cationic porphyrins (**101, 132**) as shown in Table 5.

In the first category monocationic porphyrin (MCP) derivatives, 12-{N-[2-(trimethylammonio)ethyl]carbamido}-3,7-diethyl-2,8,13,17,18-pentamethyl porphyrin iodide (**91**) was fully examined by biologic study. It has a typical porphyrin absorbance spectrum, with maxima at 396 >> 497 > 531 > 565 > 618 nm. MCP (**91**) has similar extinction coefficients to protoporphyrin IX (PP) at these wavelengths, although the spectrum of MCP (**91**) is slightly red-shifted. The octanol:water partition ratio (P) for MCP (**91**) was 6, indicating a much less hydrophobic structure than that of PP, P=180.

In MCP (**91**) vs. PP accumulation *in vitro*, after a 30 minutes incubation at 37 °C, the distribution ratios of MCP and PP were 15 and 130, respectively, indicating preferential uptake of the former sensitizer. In *vivo* PDT studies and drug biodistribution in human plasma have been carried out.[123]

As mentioned before, our discovery that protonated Schiff bases (imines) of "chlorin-type" compound show an unusual red shift of the absorption maxima in the visible region[50,51] prompted us to investigate the synthesis of another type of cationic PDT photosensitizers by introduction of dimethyliminium group on *meso* position of octaethylbenzochlorin. The nickel(II) octaethylbenzochlorin (NiOEBC; **143**) was easily prepared by treatment of the nickel(II) formylethenylporphyrin (**140**), obtained by treatment of the nickel(II) octaethylporphyrin (NiOEP; **138**) with 3-DMA/$POCl_3$, with concentrated sulfuric acid, which was developed by

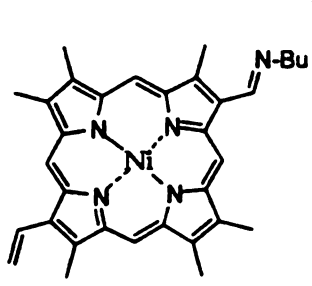
Smith.[125] However the metal-free octaethylbenzochlorin (OEBC; **142**) was obtained in poor yield (~10%) by treatment of NiOEBC (**143**) with trifluoroacetic acid and 1,3-propanedithiol. In our study, OEBC was easily prepared through one-step reaction: when the nickel(II) formylethenylporphyrin (**140**) was treated with hydrogen sulfide in 18% sulfuric acid/trifluoroacetic acid, both cyclization and demetallation took place to afford directly the metal-free OEBC in 82% yield. Evidence supporting the formulation of OEBC (**142**) was given both by mass spectrometry (molecular ion at m/e=572) and by $^1$H NMR spectroscopy. In the latter spectrum, the resonances of two ethyl groups were upfield of the remaining ethyl groups as expected for moieties attached to the $sp^3$ hybridized carbon of a reduced pyrrole ring. The three resonances attributable to the protons of the newly formed aromatic ring (doublets at δ=8.02; 9.53; triplet at 8.10 ppm). The three *meso* protons absorb at δ=8.01, 8.56, and 9.22 ppm.

The Vilsmeier formylation reaction has been used as an efficient method for introduction of substituents into the *meso* positions of numerous copper(II) and nickel(II) porphyrins and chlorins. In a typical experiment, the iminium salt that is formed after the addition of the Vilsmeier complex, is an unstable intermediate and is therefore hydrolyzed to the formyl group, using sodium acetate aqueous solution, immediately after is formed, and without further characterization.

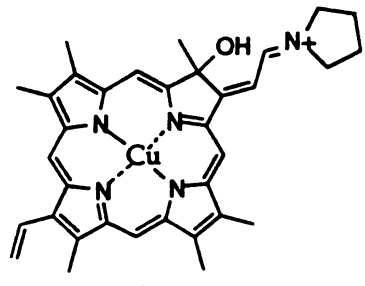
Copper ion was inserted into OEBC (**142**) before it was converted into copper(II) dimethyliminium octaethylbenzochlorin (CuImBC; **147**) in 75% yield by the Vilsmeier reaction (DMF/$POCl_3$). Surprisingly CuImBC is so stable that the iminium group survives in conc. $H_2SO_4$ over extended time. CuImBC was converted into the free base dimethyliminium octaethylbenzochlorin (ImBC; **145**), after 5 hours reaction without yielding

any side products. The structure of ImBC was supported both by mass spectrometry (molecular ion at m/e=628.4) and by $^1$H NMR spectroscopy. In $^1$H NMR spectrum, the resonances of two ethyl groups were upfield of the remaining ethyl groups as those of OEBC. The three resonances attributable to the protons of the formed aromatic ring absorb at δ=7.81, 8.78 ppm (two doublets) and 7.81 ppm (triplet). The newly formed dimethyliminium group was proven both by the two resonances attributable to the methyl protons (singlets at δ=2.77; 4.32 ppm) and by the resonance of the methine proton (singlet at δ=10.31 ppm).

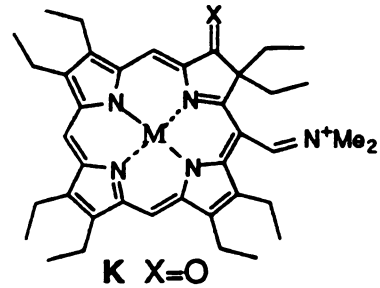
ImBC (**145**) is unique among iminium salts in that its iminium group is extremely resistant to hydrolysis. To examine this stability issue in perspective several related compounds were compared. The imine or iminium derivatives (I and J) hydrolyzed easily in wet organic solvents with half-live < 3 minutes at room temperature. The chlorin derivatives (K) and (L) also hydrolyzed easily (half-life < 10 minutes). In contrast, nickel(II) complex of ImBC, NiImBC (**146**), required at least 12 hours treating with aqueous sodium acetate solution before it changed to its formyl derivative.




I




J




K X=O
L X=$CH_2$

Subsequent hydrolysis of CuImBC to Cu(II) formylbenzochlorin was completed in 15 hours. The unusual resistant to hydrolysis is what makes it possible to carry out demetallation of the copper in CuImBC as well as re-metallation of the resultant free-base ImBC to afford other metal derivatives for PDT studies.

The exceptional stability of the iminium group in ImBC is interesting and may arise from structural and/or electronic effects. Structurally the *meso*-substituted dimethyliminium group is adjacent to the geminal diethyl groups on the neighboring pyrrole which may provide steric shielding to hinder hydrolysis of the iminium group. However, comparisons with a recently synthesized ketochlorin derivatives (K and L) suggest that shielding, if any, by the geminal ethyl groups may be insufficient to impart such unusual stability because they (K and L) are still very labile toward hydrolysis.

Electronically, the benzochlorin ring may alter the rate of nucleophilic attack on the conjugated iminium carbon leading to hydrolysis. Currently there is little known about the electronic properties of these compounds. The iminium salt seemed stable enough to withstand electronic attack because sulfonated ImBC (SImBC; **149**) has been made by trteatment of NiImBC or CuImBC with conc. $H_2SO_4$ for 4 days. This SImBC also possesses good stability and can be converted quantitatively into its Ni-, Cu- and Zn-complex. Evidence supporting the structure of SImBC was given both by mass spectrometry (molecular ion at m/e=708) and by $^1H$ NMR spectroscopy. In the latter spectrum, the resonances of two ethyl groups were upfield of the remaining ethyl groups as those of ImBC (**145**). The resonances of three protons of the aromatic ring of ImBC (doublets at $\delta$=7.81; 8.78; triplet at 7.81 ppm) were replaced by two singlets (at $\delta$=8.25; 9.33 ppm). The absence of the triplet resonance and the change in chemical shifts of the remaining singlets

are all consistent with sulfonation *alpha* to both protons. The resonances of the methyl protons (singlets at δ=2.71; 3.79 ppm) as well as the methine proton (singlet at δ=9.37 ppm) of the dimethyliminium group still exist.

Both ImBC and SImBC exhibit a strong absorbance in the vicinity of 800 nm, while insertion of a nickel, copper or zinc ion into ImBC (or SImBC) results in a blue-shift of the absorption of band I approximately 25, 45 and 60 nm respectively (Figure 9 and Figure 10).

Initial studies involved comparison of Ni(II) and Cu(II) benzochlorin iminium salts. Both are paramagnetic metals, but the Ni atom has more low-lying energy states[125,126] which could result in inhibition of photoprocesses. Biologic studies, involving a clonogenic assay and FANFT tumor, indicated that NiImBC formulated in Cremophor EL (CRM) was inactive *in vitro* at levels as high as 1.4 μM.[127] It was also found no effect of a 7 mg/kg (10 μmoles/kg) dose of NiImBC against the FANFT tumor *in vivo*. To assess the need for iminium group, we prepared an analog of NiImBC with an aldehyde group replacing the iminium group. This product had no phototoxic effect on the FANFT cells in culture or in the rat.[128] The iminium group therefore represents an essential element in the production of photodamage.

Initial photophysical experiments indicate that the triplet state lifetime of CuImBC extremely short ( < 20 nsec) and that singlet oxygen is not produced during photoactivation.[68,129] Hence, CuImBC has no tendency to initiate type II photodynamic activity. It was found that photoactivation of CuImBC formulated in CRM resulted in decreased blood flow. It have been demonstrated that singlet oxygen generated by photosensitizers disrupted tumor blood flow when measured immediately after irradiation.[69] Further studies have been proposed to examine the mechanism of vascular shutdown, specially endothelial cell integrity. Using a suspension of

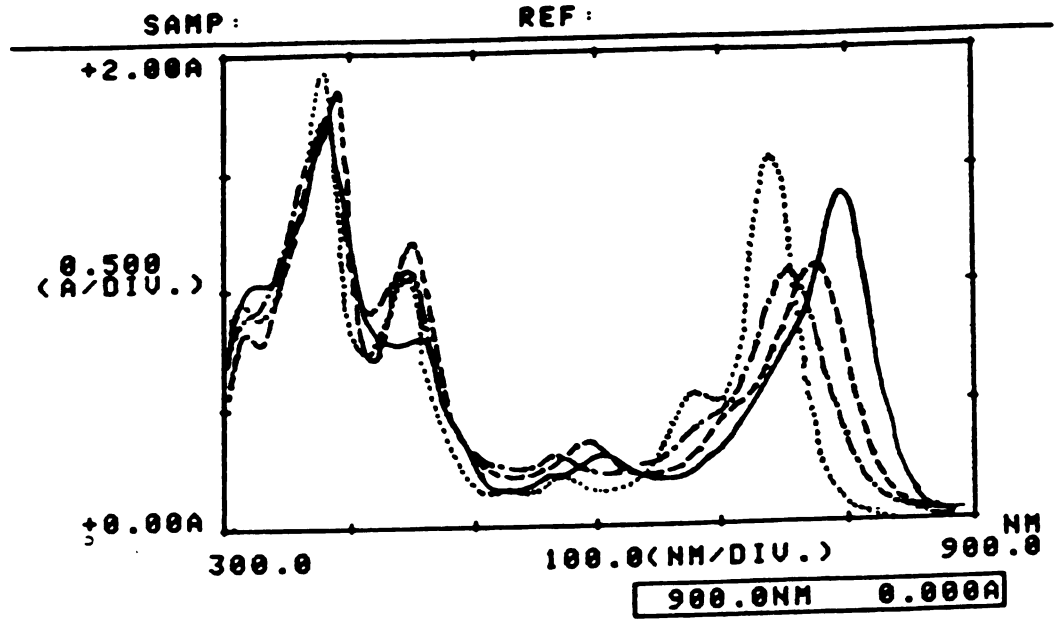



Figure 9. UV-vis absorption spectra of ImBC (145; —), NiImBC (146; - - -), CuImBC (147; _ . _) and ZnImBC (148; ·····) in $CH_2Cl_2$.

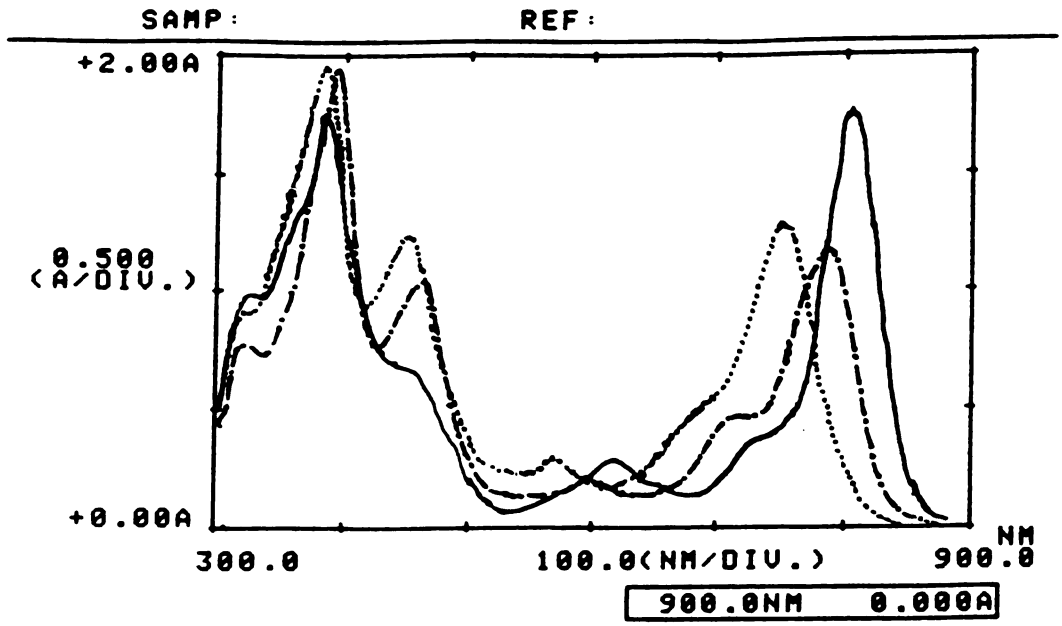



Figure 10. UV-vis absorption spectra of SImBC (149; —), CuSImBC (150; ·····) and ZnSImBC (151; _ . _) in $CH_2Cl_2$.

erythrocyte ghosts, it was found that addition of either superoxide dismutase (a superoxide quencher) or catalase (a $H_2O_2$ quencher) resulted in a substantial inhibition of erythrocyte ghost lipid peroxidation produced by CuImBC (in DMSO) and exposed to broad band red light (590~850 nm) from a xenon arc light source. In contrast, neither enzyme was effective in a system containing type II photosensitizer. These results indicate the involvement of oxygen

Table 6. *In vitro* phototoxicity of iminium salts (**145, 146, 147 and 148**)

| Drug | DR[a] | Light dose[b] ($J/cm^2$) | Absorbance[c] | | $IC_{50}$ [d] | Efficacy[e] |
|---|---|---|---|---|---|---|
| | | | λ (nm) | OD | | |
| **145** | 2.8 | 0.53 | 758 | 0.21 | 84 | 28 |
| **148** | 2.6 | 1.92 | 723 | 0.47 | 104 | 6.3 |
| **147** | 11 | 3.84 | 733 | 0.38 | 330 | 1.0 |
| **146** | 13 | >20 | 735 | 0.5 | N/A | 0 |

a) Distribution ratio of intracellular to extracellular sensitizer concentration at steady-state conditions.
b) For 50% cell kill with a 30 μM extracellular concentration.
c) Absorbance (optima and OD) of a 10 μM sensitizer solution in ethanol.
d) Intracellular sensitizer concentration required decrease in cell viability to 50%.
e) Based on light dose and intracellular dye level.

*L1210 cells were incubated with a 30 μM sensitizer concentration formulated in Cremophor EL for 30 min at 37 °C, then irradiated at 610-800 nm with a sufficient light dose to lethally photodamage 50% of the cell population. The resulting intracellular sensitizer levels are shown, along with the required light dose. Efficacy is estimated in terms of cells killed/light dose [IC level]. For the purpose of this comparison, the efficacy of CuBI is defined as 1.

radicals other than singlet oxygen in the phototoxicity of CuImBC. Since both ZnImBC and ImBC fluoresce and possess a significantly longer triplet lifetime when compared to CuImBC, it was predicted that both these iminium salts are singlet oxygen generators.

ImBC and ZnImBC were carried out with L1210 cells to assess the efficacy of photodamage *in vitro*. The comparisons shown in Table 6 indicate that the metal free iminium salt is the most potent sensitizer, while Cu analogue is much less effective; the Ni analogue shows no activity. These results may reflect the capacity of ZnImBC or ImBC to catalyze both type I and type II reactions. It should be noted that these results reflect only efficacy in cell culture, and that the results in an experimental animal tumor system may be quite different.

## IV. EXPERIMENTAL

12-{N-[2-(Dimethylamino)ethyl]carbamido}-3,7-diethyl-2,8,13,17,18-pentamethylporphyrin (90)

Ethyl 3,7-diethyl-2,8,13,17,18-pentamethylporphyrin-12-propionate (87) (102 mg, 0.19 mmol) was dissolved in trifluoroacetic acid (25 mL) and mixed with concentrated hydrochloric acid (2-3 mL). This solution was heated on a steam bath for 6 hours or until hydrolysis was completed which was monitored by TLC. After hydrolysis, the solvent was evaporated and dried under vacuum to yield the acid porphyrin (88). Without purification, this acid porphyrin was suspended in dry dichloromethane (50 mL) and then oxalyl chloride (0.17 mL, 1.9 mmol) was slowly added in an ice bath. After the

chlorination by stirring for one hour at room temperature, the solvent was removed under vacuum to give the carbonyl chloride porphyrin (89). Without isolation, this product was directly dissoved in dry dichloromethane (50 mL) and then N,N-dimethylethylenediamine (0.03 mL, 0.27 mmol) was slowly added in an ice bath. After stirring for one hour at room temperature, the solvent was evaporated to dryness under high vacuum to remove excess N,N-dimethylethylenediamine. The resulting residue was chromatographed on silica gel with 15% methanol in dichloromethane. The desired compound was collected to give the title compound (88 mg, 80%): $^1$H NMR ($CDCl_3$) δ -3.99 (2H, s, NH), 1.59 (6H, s, $N\underline{Me_2}$), 1.79 (2H, t, -$NHCH_2\underline{CH_2}N$), 1.87 1.88 (3H each, t, peripheral -$CH_2\underline{CH_3}$), 2.96 (2H, t, $CH_2\underline{CH_2}CO$), 3.07 (2H, q, -$NH\underline{CH_2}CH_2N$), 3.43, 3.44, 3.47, 3.54, 3.61 (3H each, s, 2, 8, 13, 17, 18-$CH_3$), 4.06, 4.07 (2H each, q, peripheral -$\underline{CH_2}CH_3$), 4.29 (2H, t, $\underline{CH_2}CH_2CO$) 5.65 (1H, brs, peripheral NH), 9.82, 9.89, 9.98, 10.05 (1H each, s, meso H); UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 618 nm (4, 800), 565 (6, 600), 531 (9, 900) 497 (13, 700), 397 (153, 600); MS for $C_{36}H_{46}N_6O$, found m/e 578.3 ($M^+$).

<u>12-{N-[2-(Trimethylammonio)ethyl]carbamido}-3,7-diethyl-2,8,13,17,18-pentamethylporphyrin iodide (91)</u>

12-{N-[2-(Dimethylamino)ethyl]carbamido}-3,7-diethyl-2,8,13,17,18-pentamethylporphyrin (90) (40.5 mg, 0.07 mmol) was dissolved in dichloromethane (35 mL) containing 10% methanol and then iodomethane (0.044 mL, 0.7 mmol) was added. The reaction mixture was stirred for 5 hours at room temperature and the solvent was evaporated to dryness under vacuum to give the title compound in almost quantitative yield: UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 618 nm (2, 300), 565 (4, 650), 531 (5, 850), 497 (7, 470), 396 (88, 350); MS for $C_{37}H_{49}N_6OI$, found m/e 593.7 ($M^+$).

4'-(2-Chloroethyl)-3-(ethoxycarbonyl)-3',4,5,5'-tetramethyl-2,2'-dipyrryl-methenium bromide. (93)

4-(2-Chloroethyl)-3,5-dimethylpyrrole-2-carboxylic acid (75) (6.05 g, 30 mmol) and 3-(ethoxycarbonyl)-2-formyl-4,5-dimethylpyrrole (92) (5.85 g, 30 mmol) were dissolved in methanol (80 mL). 48% hydrobromic acid (8 mL) was added dropwise to the stirred solution and the reaction mixture was heated on a steam bath for 10 minutes. Some red purple crystals formed immediately. After standing over night at room temperature, the precipitated solid was collected by filtration, washed with methanol containing small amount of hydrobromic acid, and finally rinsed with a small amount of ether to give the title compound as a red-purple powder (9.27 g, 92% yield): m.p. 201-203 °C; $^1$H NMR($CDCl_3$) δ 1.44 (3H, t, -$OCH_2\underline{CH_3}$), 2.27, 2.37, 2.70, 2.79 (3H each, s, 3', 4, 5, 5'-Me), 2.94 (2H, t, -$\underline{CH_2}CH_2Cl$), 3.60 (2H, t, -$CH_2\underline{CH_2}Cl$), 4.41 (2H, q, -$O\underline{CH_2}CH_3$), 8.44 (1H, s, methine), 13.32, 13.50 (1H each, br s, NH); MS for $C_{17}H_{24}N_2O_2BrCl$, found m/e 335.3 ($M^+$).

3,3'-Diethyl-4,4'-dimethyl-2,2'-dipyrrylmethane (95)

5,5'-Bis(ethoxycarbonyl)-3,3'-diethyl-4,4'-dimethyl-2,2'-dipyrrylmethane (94) (29.96 g, 80 mmol) was dissolved in hot ethanol (100 mL) and 40% aqueous potassium hydroxide (25 mL) was added. The hydrolysis was accomplished by refluxing for 2 hours and then the solvent was evaporated to one third by heating without condenser. The condensed reaction mixture was diluted with water (40 mL) containing a few drops of hydrazine and refluxed 24 hours. At the end of this period, a layer of brown oil was separated which was solidified at room temperature. The solidified material was collected by filtration and washed with water to give the title compound

as a dark-brown shinny solid (18.09 g, >98%): m.p. 51-53 °C ; $^1$H NMR ($CDCl_3$) δ 1.12 (6H, t, -$CH_2\underline{CH_3}$), 2.03 (6H, s, 4, 4'-Me), 2.47 (4H, q, -$\underline{CH_2}CH_3$), 3.79 (2H, s, methylene), 6.35 (2H, m, 5, 5'-H), 7.26 (2H, br s, NH); MS for

5,5'-Dibromo-3,3'-diethyl-4,4'-dimethyl-2,2'-dipyrrylmethenium bromide (96)

3,3'-Diethyl-4,4'-dimethyl-2,2'-dipyrrylmethane (95) (9.21 g, 40 mL) was added in small portions into a mixture of anhydrous formic acid (60 mL) and bromine (2.08 mL, 40 mmol) with stirring at room temperature. The reaction mixture was stirred for an additional 30 minutes during which some solids precipitated. Most of formic acid was evaporated under reduced pressure to give solid product which was dried in vacuo and triturated in cyclohexane containing small amount of cyclohexene to remove excess bromine. The solid product was collected by filtration and dried in air to give the title compound (18.12 g, 97%): MS for $C_{15}H_{19}N_2Br_3$, found m/e 386 ($M^+$).

Ethyl 8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin-3-carboxylate (97)

4'-(2-Chloroethyl)-3-(ethoxycarbonyl)-3',4,5,5'-tetramethyl-2,2'-dipyrryl-methenium bromide. (93) (6.72 g, 20 mmol) and 5,5'-dibromo-3,3'-diethyl-4,4'-dimethyl-2,2'-dipyrrylmethenium bromide (96) (9.34 g, 20 mmol) were dissolved in anhydrous formic acid (70 mL) and treated with bromine (1.04 mL, 20 mmol). The reaction mixture was heated to reflux in an oil bath for 2 hours. The solvent was then allowed to boil off over 4 hours with a stream of air (until dryness). The residue was redissolved in ethanol containing a few drops of concentrated sulfuric acid and triethyl orthoformate (5 mL). After standing overnight , protected from moisture, the reaction mixture was diluted with dichloromethane (100 mL) and then meutralized with saturated

aqueous sodium acetate (50 mL). The organic layer was separated, washed once again with saturated sodium acetate (30 mL) and then twice with water (50 mL). After evaporation of the solvent, the residue was chromatographed on a silica gel column with 0.5% of methanol in dichloromethane as eluent. The fractions containing the expected porphyrin were combined and recrystallized from dichloromethane and methanol to give the title compound (957 mg, 8.6%): m.p. 245-247 °C; $^1$H NMR ($CDCl_3$) δ -3.70 (2H, br s, NH), 1.92 (9H, overlapping t, $-CH_2CH_3$, $-OCH_2CH_3$), 3.52, 3.64, 3.66, 3.94 (14H, s, Me), 3.98, 4.08 (4H, q, $-CH_2CH_3$), 4.28, 4.50 (2H each, t, $CH_2CH_2Cl$), 4.90 (2H, q, $-OCH_2CH_3$), 9.85, 9.95, 10.15, 11.10 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 628 (3,300), 573 (7,300), 545 (10,400), 506 (12,900), 406 (172,000); MS for $C_{33}H_{37}N_4O_2Cl$, found m/e 556.4 ($M^+$).

3-[N-[2-(Dimethylamino)ethyl]carbamido]-13,17-diethyl-2,7,12,18-tetramethyl-8-vinylporphyrin (100)

Ethyl 8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin-3-carboxylate (97) (55.7 mg, 0.1 mmol) was dissolved in pyridine (20 mL) with heating and aqueous potassium hydroxide (5%, 4 mL) was added. The reaction mixture was heated under reflux for 1 hour or until the hydrolysis was completed which was monitored by TLC. After hydrolysis, the reaction mixture was diluted with water (100 mL) and neutralized with acetic acid until the acid product precipitated and then filtered through a bed of celite. The product was extracted from the celite with formic acid and the filtrate was evaporated to dryness under vacuum to give the acid vinylporphyrin (98). Without purification, this acid product was suspended in dry dichloromethane (30 mL) and then oxalyl chloride (0.1 mL, 1.1 mmol) was slowly added in an ice bath. After the chlorination by stirring for 1 hour at

room temperature, the solvent was removed under vacuum to give the carbonyl chloride porphyrin (**99**). Without isolation, this product was also dissoved in dry dichloromethane (30 mL) and then N,N-dimethylethylenediamine (0.02 mL, 0.18 mmol) was slowly added in an ice bath. After stirring for 1 hour at room temperature, the solvent was evaporated to dryness under high vacuum to remove excess N,N-dimethylethylenediamine. The resulting residue was chromatographed over silica gel with 15% methanol in dichloromethane. The desired compound was collected to give the title compound (43.9 mg, 78%): $^1$H NMR ($CDCl_3$) δ -4.28 (2H, br s, NH),1.74, 1.76 (3H each, t, $CH_2\underline{CH_3}$), 2.49 (6H, s, $N\underline{Me_2}$), 2.94 (2H, t, $CONHCH_2\underline{CH_2}N$), 3.35, 3.41, 3.66, 3.68 (3H each, s, ring Me), 3.90 (4H, q, $\underline{CH_2}CH_3$), 4.05 (2H, q, $CONH\underline{CH_2}CH_2N$), 6.15, 6.35 (1H each, dd, $-CH=\underline{CH_2}$), 7.64 (1H, br s, CON<u>H</u>), 8.17 (1H, dd, -<u>CH</u>=CH2), 9.65, 9.70, 9.87, 10.40 (1H each, s, meso H); UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 628 nm (3,000), 574 (6,900), 542 (10,000), 506 (12,500), 408 (166,000); MS for $C_{35}H_{42}N_60$, found m/e 562.3 ($M^+$).

<u>3-{N-[2-(Trimethylammonio)ethyl]carbamido}-13,17-diethyl-2,7,12,18-tetramethyl-8-vinylporphyrin iodide (**101**)</u>

3-{N-[2-(Dimethylamino)ethyl]carbamido}-13,17-diethyl-2,7,12,18-tetramethyl-8-vinylporphyrin (**100**) (39.4 mg, 0.07 mmol) was treated with iodomethane (0.044 mL, 0.7 mmol) following the method described for the ammonium compound (**91**) to give the title compound (48.3 mg, 98%): UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 628 nm (3,000), 574 (6,900), 542 (10,000), 506 (12,500), 408 (166,000); MS for $C_{36}H_{45}N_6OI$, found m/e 577.6 ($M^+$).

Ethyl 8-(2-chloroethyl)-12,17-diethyl-2,7,12,18-tetramethyl-13-porphyrinone-3-carboxylate (102)

Osmium tetroxide (88 mg, 1.5 mmol) and pyridine (0.05 mL) were added to ethyl 8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin-3-carboxylate (97) (195 mg, 0.35 mmol) was dissolved in dichloromethane (45 mL). After the reaction mixture was stirred for 24 hours at room temperature under nitrogen in the dark for 24 hours, it was quenched by adding methanol (10 mL), then bubbled with hydrogen sulfide through the reaction solution for 30 minutes to decompose the osmate adduct and allowed to stand for 2 hours. The precipitated black osmium sulfide was removed by filtration through a bed of Celite and the filtrate was evaporated. The resulting residue was dissolved in dichloromethane (50 mL) and perchloric acid (70%, 0.8 mL) was added and then stirred for 30 minutes at room temperature. The reaction mixture was diluted with dichloromethane (50 mL) and the organic layer was washed twice with saturated aqueous sodium acetate (50 mL) and twice with water (50 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under vacuum and the residue was chromatographed on silica gel with dichloromethane as eluent to give the title compound (64 mg, 32%): $^1$H NMR ($CDCl_3$) δ -3.66 (2H, br s, NH), 1.74 (3H, t, 12-$CH_2\underline{CH_3}$), 1.92, 1.94 (3H each, t, 17-$CH_2\underline{CH_3}$, 3-$OCH_2\underline{CH_3}$), 2.07 (3H, s, 12-Me), 2.98 (2H, q, 12-$\underline{CH_2}CH_3$), 3.52, 3.64, 3.66 (3H each, s, ring Me), 4.08 (2H, q, 17-$\underline{CH_2}CH_3$), 4.28 (2H, t, -$CH_2\underline{CH_2}Cl$), 4.50 (2H, t, -$\underline{CH_2}CH_2Cl$), 4.90 (2H, q, -$O\underline{CH_2}CH_3$), 9.12, 9.67, 9.75, 10.35 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 643 nm (15,600), 568 (18,000), 537 (8,400) 412 (182,000), 319 (17,100); MS for $C_{33}H_{37}N_4O_3Cl$, found m/e 572.5 ($M^+$).

3-[N-[2-(Dimethylamino)ethyl]carbamido]-12,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-13-porphyrinone (103)

Ethyl 8-(2-chloroethyl)-12,17-diethyl-2,7,12,18-tetramethyl-13-porphyrinone-3-carboxylate (102) (57.3 mg, 0.1 mmol) was treated with excess potassium hydroxide, with oxalyl chloride (0.1 mL, 1.1 mmol) and with N,N-dimethylethylenediamine (0.02 mL, 0.18 mmol) following the method described for the porphyrin amide (100) to afford the title compound (43.7 mg, 75.6%): $^1$H NMR ($CDCl_3$) δ -3.72 (2H, br s, NH), 1.76, 1.95 (3H each, t, -$CH_2\underline{CH_3}$), 2.09 (3H, s, 12-Me), 2.96 (2H, t, NHCH$_2\underline{CH_2}$N), 3.02 (2H, q, 12-$\underline{CH_2}CH_3$), 3.98 (2H, q, NH$\underline{CH_2}CH_2$N), 4.06 (2H, t, 17-$\underline{CH_2}CH_3$), 6.18, 6.40 (1H each, dd, -CH=$\underline{CH_2}$), 7.77 (1H, br s, CO$\underline{NH}$), 8.27 (1H, dd, -$\underline{CH}$=$CH_2$), 9.14, 9.68, 9.78, 10.42 (1H each, s, meso H); UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 647 nm (14,500), 573 (17,800), 541 (8,300), 417 (17,900), 404 (16,800); MS for $C_{35}H_{42}N_6O_2$, found m/e 578.2 ($M^+$).

3-[N-[2-(Trimethylammonio)ethyl]carbamido]-12,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-13-porphyrinone iodide (104)

3-[N-[2-(Dimethylamino)ethyl]carbamido]-12,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-13-porphyrinone (103) (34.7 mg, 0.06 mmol) was treated with iodomethane (0.38 mL, 0.6 mmol) following the method described for the ammonium compound (91) to give the title compound (41.7 mg, 96.5%): UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 648 (14,500), 573 (17,800), 542 (8,300), 417 (17,900), 404 (16,800); MS for $C_{36}H_{45}N_6O_2I$, found m/e 593.5 ($M^+$).

Ethyl 3,5-dimethyl-4-octanoylpyrrole-2-carboxylate (105)

Ethyl 3,5-dimethylpyrrole-2-carboxylate (77) (83.5 g, 0.5 mol) was dissolved in dry dichloromethane (400 mL) with heating. The solution was

cooled to 10 °C in an ice bath and octanoyl chloride (94 mL, 0.55 mol) was added. To this solution, tin(IV) chloride (82 mL, 0.7 mol) was slowly added through a pressure-equalized dropping funnel (temperature < 20 °C). The reaction is exothermic only during the period when the first mole equivalent of tin(IV) chloride was added. The reaction mixture was stirred for another 2 hours in the ice bath after the addition and then poured into ice/water (1 L). The organic phase was separated, washed twice with aqueous sodium carbonate, then with water, and dried over anhydrous sodium sulfate. The solvent was evaporated under vacuum to yield a white mass of octanoyl pyrrole (143 g, 98%). The product was essentially pure and no further purification was needed.

For the title compound: $^1$H NMR ($CDCl_3$) δ 0.85 (3H, t, octanoyl Me), 1.26 (8H, m, $CH_2$), 1.42 (3H, t, $OCH_2\underline{CH_3}$), 1.65 (2H, m, $CH_2$), 2.18, 2.29 (3H each, s, 3,5-Me), 2.83 (2H, t, 4-$CO\underline{CH_2}$), 4.27 (2H, q, $O\underline{CH_2}CH_3$), 8.96 (1H, br s, NH); MS for $C_{17}H_{27}NO_3$, found m/e 293.3 ($M^+$).

Ethyl 3,5-dimethyl-4-octylpyrrole-2-carboxylate (106)

Sodium borohydride (7.6 g, 0.2 mol) was added to the essentially pure ethyl 3,5-dimethyl-4-octanoylpyrrole-2-carboxylate (105) (29.3 g, 0.1 mol) dissolved in dry tetrahydrofuran (100 mL). The reaction mixture was cooled to 10 °C with stirring in an ice bath and boron trifluoride etherate (37 mL, 0.3 mol) was slowly added so that the reaction temperature was maintained below 20 °C. After the addition of boron trifluoride etherate, the mixture was stirred for an additional one hour in the ice bath and then poured into a mixture of ice (200 mL), hydrochloric acid (1N, 50 mL) and dichlormethane (200 mL). The organic phase was separated and washed with hydrochloric acid (0.5N, 200 mL). To the neutralized organic phase, methanol (50 mL) was

added and the solvent was evaporated to dryness. The resulting white product was crystallized from methanol-water (85:15) to yield the title compound (26.2 g, 94%): m.p. 74-77 °C; $^1$H NMR ($CDCl_3$) δ 0.84 (3H, t, octyl Me), 1.25 (10H, m, $CH_2$), 1.36 (2H, m, $CH_2$), 1.40 (3H, t, -$OCH_2\underline{CH_3}$), 2.15, 2.24 (3H each, s, 3,5-Me), 2.32 (2H, t, 4-$CH_2$), 4.25 (2H, q, -$O\underline{CH_2}CH_3$), 8.60 (1H, br s, NH); MS for $C_{17}H_{29}NO_2$, found m/e 279.4 ($M^+$).

Benzyl 3,5-dimethyl-4-octylpyrrole-2-carboxylate (108)

After benzyl alcohol (150 mL) was heated up to 180 °C to remove moisture, ethyl 3,5-dimethyl-4-octylpyrrole-2-carboxylate (106) (55.8 g, 0.2 mol) was added. The reaction mixture was heated up to boiling for 10 minutes to drive off water. To this hot homogeneous solution, a fresh saturated solution of sodium in dry benzyl alcohol (10 mL) was cautiously added in small (1 mL) portions under nitrogen until the evolution of ethanol ceased. Further portions were added every several minutes until the exchange was completed when the boiling point had again risen above 200 °C. Heating was continued to reflux for 10 minutes after the effervescense subsided. The hot reaction mixture was poured cautiously into a stirred solution of acetic acid (20 mL) in methanol (400 mL), and water was then added until crystallization was completed. The light pinkish solid was collected by filtration, washed with 50% aqueous methanol and dried to give the title compound (58 g, 85%).

For analysis a sample was recrystallized from tetrahydrofuran and methanol: m.p. 78-81 °C; $^1$H NMR ($CDCl_3$) δ 0.86 (3H, t, octyl Me), 1.25 (10H, m, $CH_2$), 1.38 (2H, m, $CH_2$), 2.16, 2.26 (3H each, s, 3,5-Me), 2.32 (2H, t, 4-$CH_2$), 5.27 (2H, s, $O\underline{CH_2}C_6H_5$), 7.35 (5H, m, phenyl protons), 8.61 (1H, br s, NH); MS for $C_{22}H_{31}NO_2$, found m/e 341.3 ($M^+$).

Benzyl 5-acetoxymethyl-3-methyl-4-pentylpyrrole-2-carboxylate (109)

Lead tetraacetate (48.7 g, 0.11 mol) was added to a solution of benzyl 3,5-dimethyl-4-pentylpyrrole-2-carboxylate (107) (31.3 g, 0.1 mol) in glacial acetic acid (150 mL) with stirring at room temperature. The reacton was accomplished by heating on a steam bath for one hour, when the color of the reaction mixture was changed from yellow-brown to dark-brown, and the reaction mixture was poured into a large amount of water ( > 1 L). The precipitated solid was separated by filtration, rinsed with water and crystallized from aqueous acetone to give the title compound as ivory-white needles (35.1 g, 95%): m.p. 83-86 °C; $^1$H NMR ($CDCl_3$) δ 0.92 (3H, t, pentyl Me), 1.28 (10H, m, $CH_2$), 1.43 (2H, m, $CH_2$), 2.04, 2.27 (3H each, s, 3,5-Me), 2.42 (2H, t, 4-$CH_2$), 5.00 (2H, s, 5-$\underline{CH_2}$OCO), 5.30 (2H, s, -O$\underline{CH_2}C_6H_5$), 7.38 (5H, m, phenyl protons), 9.16 (1H, br s, NH); MS for $C_{21}H_{27}NO_4$, found m/e 357.4 ($M^+$).

Benzyl 5-acetoxymethyl-3-methyl-4-octylpyrrole-2-carboxylate (110)

Benzyl 3,5-dimethyl-4-octylpyrrole-2-carboxylate (108) (34.1 g, 0.1 mol) was treated with lead tetraacetate (48.7 g, 0.11 mol) following the method described for the acetoxymethyl pyrrole (109) to give the title compound (38.2 g, 96%): m.p. 80-85 °C; $^1$H NMR ($CDCL_3$) δ 0.87 (3H, t, octyl Me), 1.27 (10H, m, $CH_2$), 1.42 (2H, m, $CH_2$), 2.04, 2.27 (3H each, s, 3.5-Me), 2.41 (2H, t, 4-$CH_2$), 5.00 (2H, s, 5-$\underline{CH_2}$OCO), 5.30 (2H, s, -O$\underline{CH_2}C_6H_5$), 7.37 (5H, m, phenyl protons), 9.15 (1H, br s, NH); MS for $C_{24}H_{33}NO_4$, found m/e 399.2 ($M^+$).

5,5'-Dibenzyloxycarbonyl-4,4'-dimethyl-3,3'-dipentyl-2,2'-dipyrrylmethane (111)

Benzyl 5-acetoxymethyl-3-methyl-4-pentylpyrrole-2-carboxylate (109) (37.1 g, 0.1 mol) was dissolved in 70% aqueous acetic acid (200 mL) and heated under reflux for one hour. After reaction was accomplished, the hot reaction mixture was poured into a large amount of water and allowed to cool down slowly to precipitate solid product. The precipitated solid was collected by filtration, washed with water and crystallized from ethanol to give the title compound as ivory-white needles (47.5 g, 78%): m.p. 98-101 °C; $^1$H NMR ($CDCl_3$) δ 0.88 (6H, t, pentyl Me), 1.28 (8H, m, $CH_2$), 1.38 (4H, m, $CH_2$), 2.26 (6H, s, 4,4'-Me), 2.34 (4H, t, 3,3'-$CH_2$), 3.79 (2H, t, 2,2'-methylene), 5.21 (4H, s, -O$\underline{C}H_2C_6H_5$), 7.30 (10H, m, phenyl protons), 9.05 (2H, br s, NH); MS for $C_{37}H_{46}N_2O_4$, found m/e 582.7 ($M^+$).

5,5'-Dibenzyloxycarbonyl-4,4'-dimethyl-3,3'-dioctyl-2,2'-dipyrrylmethane (112)

Benzyl 5-acetoxymethyl-3-methyl-4-octylpyrrole-2-carboxylate (110) (39.9 g, 0.1 mol) was treated with 70% aqueous acetic acid as described for the dipyrrylmethane (111) to give the title compound (51.7 g, 78%): m.p. 96-97 °C; $^1$H NMR ($CDCl_3$) δ 0.87 (6H, t, octyl Me), 1.28 (20H, m, $CH_2$), 1.37 (4H, m, $CH_2$), 2.26 (6H, s, 4,4'-Me), 2.35 (4H, t, 3,3'-$CH_2$), 3.79 (2H, t, 2,2'-methylene), 5.21 (4H, s, O$\underline{C}H_2C_6H_5$), 7.30 (10H, m, phenyl protons), 9.07 (2H, br s, NH); MS for $C_{43}H_{58}N_2O_4$, found m/e 666.3 ($M^+$).

5,5'-Dibromo-4,4'-dimethyl-3,3'-dipentyl-2,2'-dipyrrylmethenium bromide (115)

5,5'-Dibenzyloxycarbonyl-4,4'-dimethyl-3,3'-dipentyl-2,2'-dipyrrylmethane (111) (29.1 g, 0.05 mol) was dissolved in freshly distilled tetrahydrofuran

(350 mL) containing a few drops of triethylamine. 10% Palladium/carbon (0.5 g) was added and the reaction mixture was hydrogenated under hydrogen (1 atm., at room temperature) until hydrogen uptake ceased. The solvent was evaporated and dried under vacuum without removing carbon by filtration because 5,5'-dicarboxylic acid analogue (**114**) is partially soluble in tetrahydrofuran. The dried reaction mixture, which contains the dicarboxylic acid analogue (**114**) and catalyst carbon, was added in a mixture of 98-100% formic acid (150 mL) and bromine (10 mL) in small portions. The reaction was accomplished by stirring at room temperature for addition an additional one hour after the addition, and then carbon was filtered off and washed with formic acid. Most of formic acid was removed under reduced pressure and the resulting solid was rinsed with cyclohexene and hexane to give the title compound as violet crystals (23.7 g, 82% yield): m.p. 179-182 °C; MS for $C_{21}H_{31}N_2Br_3$, found m/e 471.2 ($M^+$).

5,5'-Dibromo-4,4'-dimethyl-3,3'-dioctyl-2,2'-dipyrrylmethenium bromide (**116**)

5,5'-Dibenzyloxycarbonyl-4,4'-dimethyl-3,3'-dioctyl-2,2'-dipyrrylmethane (**112**) (33.3 g, 0.05 mol) was hydrogenated and then treated with bromine by following the method described before for the dipyrromethenium bromide (**115**) to give the title compound (26.1 g, 82%): m.p. 180-182 °C; MS for $C_{27}H_{43}N_2Br_3$, found m/e 555.3 ($M^+$).

3-Acetamido-2-butanone (**117**)

DL-Alanine (35.1 g, 0.39 mol), pyridine (159 mL, 1.98 mol) and acetic anhydride (224 mL, 2.35 mol) were mixed and heated on a steam bath with stirring until a clear solution was formed. This orange-brown solution was

then heated for additional six hours. After the reaction was completed, excess pyridine, excess acetic anhydride and formed acetic acid were removed under vacuum. The dark residue (about 50 mL) was distilled through a fractional column under reduced pressure to yield the title compound as a light yellow liquid (41 g, 88%): b.p. 110-125 °C/3 mm.

3-Ammonio-2-butanone chloride (118)

3-Acetamido-2-butanone (117) (41 g, 0.31 mol) dissolved in 35% hydrochloric acid (400 mL) was heated to reflux for 8 hours, cooled to room temperature and then filtered. The filtrate was evaporated to dryness under vacuum to yield a yellow-brown residue which was washed with plenty of acetone to give the title compound as a white powder (34.5 g, 88%): MS of $C_4H_9NOCl$, found m/e 8.71 ($M^+$).

3-(Ethoxycarbonyl)-4,5-dimethylpyrrole-2-carboxylic acid (119)

Hydrochloric acid (10%, 11.2 mL) was added to 3-ammonio-2-butanone chloride (118) (16 g, 0.13 mol) dissolved in water (22.4 mL). This solution was slowly added to a stirred solution of diethyl oxalacetate sodium salt (10 g) in water (15 mL) and aqueous sodium hydroxide (10%, 15 mL) with heating on a steam bath. During the addition, the title compound precipitated out. After the reaction was completed, the reaction mixture was diluted with water (50 mL) and acidified with 10% hydrochloric acid until all the pyrrole precipitated out. The white solid was collected by filtration, washed intensively with water and dried in air to yield the title compound (10.2 g, 37%): m.p. 203-205 °C ; $^1H$ NMR ($CDCl_3$) δ 1.42 (3H, t, $-CO_2CH_2\underline{CH_3}$), 2.18, 2.26 (3H, s, 4,5-Me), 4.44 (2H, q, $-CO_2\underline{CH_2}CH_3$), 10.22 (1H, br s, NH), 13.25 (1H, br s, $-CO_2H$); MS for $C_{10}H_{13}NO_4$, found m/e 211.2 ($M^+$).

3-(Ethoxycarbonyl)-4,5-dimethylpyrrole (120)

3-(Ethoxycarbonyl)-4,5-dimethylpyrrole-2-carboxylic acid (**119**) (21.1 g, 0.1 mol) was mixed with sodium acetate trihydrate (50 g) and potassium acetate (50 g). This powdery mixture was heated in an oil bath between 140 °C and 160 °C until it was completely melted, cooled and stirred with water (200 mL) to precipitate product. The solid product was collected by filtration and washed intensively with water to give the title compound as a pinkish brown powder (15.7 g, 94%): m.p. 102-104 °C;$^1$H-NMR ($CDCl_3$) δ 1.32 (3H, t, -$OCH_2\underline{CH_3}$), 2.17, 2.20 (3H each, s, 4,5-Me), 4.26 (2H, q, -$O\underline{CH_2}CH_3$), 8.23 (1H, s, a proton), 8.86 (1H, br s, NH); MS for $C_9H_{13}NO_2$, found m/e 167 ($M^+$).

3-(Ethoxycarbonyl)-2-formyl-4,5-dimethylpyrrole (121)

Phosphorus oxychloride (14 mL, 0.15 mol) was added dropwise to 3-(ethoxycarbonyl)-4,5-dimethylpyrrole (**120**) (16.7 g, 0.1 mol) dissolved in dry N,N-dimethylformamide (130 mL) in an ice bath under nitrogen. During the addition, the reaction mixture was kept below 10 °C and then further stirred for 2 hours at room temperature. The reaction mixture was diluted with benzene (130 mL), stirred for 30 minutes and allowed to stand for a while. The product was precipitated out by addition of water and a small amount of aqueous sodium hydroxide (10%). The white solid was collected by filtration, washed with water and dried in air to yield the title compound as white crystals(10.9 g, 56%): m.p. 125-127 °C ;$^1$H NMR ($CDCl_3$) δ 1.38 (3H, t, -$CH_2\underline{CH_3}$), 2.22 2.28 (3H each, s, 4,5-Me), 4.37 (2H, q, -$\underline{CH_2}CH_3$), 10.04 (1H, s, -CHO), 10.46 (1H, br s, NH) ; MS for $C_{10}H_{13}NO_3$, found m/e 195 ($M^+$).

3'-Ethoxycarbonyl-4-ethyl-3,4',5,5'-tetramethyl-2,2'-dipyrrylmethenium bromide (123)

3-(Ethoxycarbonyl)-2-formyl-4,5-dimethylpyrrole (121) (7.80 g, 40 mmol) and *t*-butyl 4-ethyl-3,5-dimethylpyrrole-2-carboxylate (122) (8.92 g, 40 mmol) were dissolved in methanol (100 mL) with warming. Hydrobromic acid (48%, 16 mL) was added dropwise to the stirred solution and the reaction mixture was heated for 30 minutes on the steam bath during which time the solvent was partially evaporated and some red crystals precipitated. It was then left stand for 3 hours at room temperature. The precipitated solid was collected by filtration, washed with methanol containing a few drops of hydrobromic acid and dried in air. This crude product was purified by recrystallization from dichloromethane and hexane to give the title compound as violet crystals (14.1 g, 93%): m.p. 205-208 °C; $^1$H-NMR ($CDCN_3$) δ 1.07 (3H, t, 4-$CH_2\underline{CH_3}$), 1.40 (3H, t, -$OCH_2\underline{CH_3}$), 2.21, 2.28, 2.58, 2.69 (3H each, s, 3, 4', 5, 5'-Me), 2.47 (2H, q, 4-$\underline{CH_2}CH_3$), 4.37 (2H, q, -$O\underline{CH_2}CH_3$), 8.23 (1H, s, methine), 13.37, 13.64 (1H each, br s, NH); MS for $C_{18}H_{25}N_2O_2Br$, found m/e 301 ($M^+$).

Ethyl 2-ethyl-3,8,12,18-tetramethyl-13,17-dipentylporphyrin-7-carboxylate (124)

5,5'-Dibromo-4,4'-dimethyl-3,3'-dipentyl-2,2'-dipyrrylmethenium bromide (115) (5.51 g, 0.01 mol) and 3'-ethoxycarbonyl-4-ethyl-3,4',5,5'-tetramethyl-2,2'-dipyrrylmethenium bromide (123) (3.81 g, 0.01 mol) were suspended in anhydrous formic acid (60 mL). To this reaction mixture , bromine (0.52 mL, 0.01 mol) was added and the mixture was heated to reflux for 2 hours in an oil bath. The solvent was allowed to boil off over 4 hours with a stream of air. Ethanol (100 mL) and concentrated sulfuric acid (2 mL) were added to the dried reaction residue, followed by addition of triethyl orthoformate (10 mL). After standing overnight, protected from moisture,

the reaction mixture was diluted with dichloromethane (150 mL) and then neutralized with saturated aqeous sodium acetate (100 mL). The organic layer was separated, washed once again with saturated aqueous sodium acetate(60 mL) and then three times with water (100 mL). After evaporation of the solvent, the residue was chromatographed on silica gel column with dichloromethane-hexane (2:1) as eluent. A dark non-fluorescent forerun was discarded and the moving porphyrin band on chromatography column can be monitored by using an UV-lamp to ensure a complete collection. The fractions containing porphyrin were combined, evaporated to dryness under vacuum, and then crystallized from dichloromethane and methanol to give the title compound as sparkling purple crystals (0.84 g, 14%): $^1$H NMR ($CDCl_3$) δ -3.98 (2H, br s, NH), 0.84, 0.85 (3H each, t, pentyl Me), 1.47 (8H, m, $CH_2$), 1.68 (4H, m, $CH_2$), 1.84 (3H, t, 2-$CH_2\underline{CH_3}$), 2.21, 2.24 (2H each, t, 13.17-$CH_2$), 3.47, 3.47, 3.60, 3.82 (3H each, s, ring Me), 3.88 (3H, t, $CO_2CH_2\underline{CH_3}$), 4.04 (2H, q, 2-$\underline{CH_2}CH_3$), 4.88 (2H, q, $CO_2\underline{CH_2}CH_3$), 9.78, 9.85, 9.94, 11.01 (1H each, s, meso H); UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 630 nm (1,600), 573 (8,100), 547 (13,400), 509 (9,100), 406 (168,000); MS for $C_{39}H_{50}N_4O_2$, found m/e 606.3 ($M^+$).

Ethyl 2-ethyl-3,8,12,18-tetramethyl-13,17-dioctylporphyrin-7-carboxylate (125)

5,5'-Dibromo-4,4'-dimethyl-3,3'-dioctyl-2,2'-dipyrrylmethenium bromide (**116**) (6.35 g, 0.01 mol) and 3'-ethoxycarbonyl-4-ethyl-3,4',5,5'-tetramethyl-2,2'-dipyrrylmethenium bromide (**123**) (3.81 g, 0.01 mol) were treated with bromine and then oxidized in air following the method described for the porphyrin (**124**) to give the title compound as sparkling purple crystals (0.83 g, 12%): $^1$H NMR ($CDCl_3$) δ -3.99 (2H, br s, NH), 0.86, 0.87 (3H each, t, octyl Me), 1.29 (16H, m, $CH_2$), 1.49, 1.70 (4H each, m, $CH_2$), 1.83 (3H,

t, 2-$CH_2\underline{C}H_3$), 2.20, 2.22 (2H each, t, 13,17-$CH_2$), 3.48, 3.48, 3.60, 3.83 (3H each, s, ring Me), 3.88 (3H, t, $CO_2CH_2\underline{C}H_3$), 4.03 (2H, q, 2-$\underline{C}H_2CH_3$), 4.88 (2H, q, $CO_2\underline{C}H_2CH_3$), 9.77, 9.84, 9.92, 10.99 (1H each, s, meso H); UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 630 nm (1,600), 573 (8,100), 547 (13,400), 509 (9,100), 406 (168,000); MS for $C_{45}H_{62}N_4O_2$, found m/e 690.3 ($M^+$).

7-{N-[2-(Dimethylamino)ethyl]carbamido}-2-ethyl-3,8,12,18-tetramethyl-13,17-dipentylporphyrin (130)

Ethyl 2-ethyl-3,8,12,18-tetramethyl-13,17-dipentylporphyrin-7-carboxylate (124) (61 mg, 0.1 mmol) was dissolved in pyridine (20 mL) with heating and aqueous potassium hydroxide (5%, 4 mL) was then added. The reaction mixture was heated under reflux for one hour or until the hydrolysis was completed which was monitored by TLC. After hydrolysis, the reaction mixture was diluted with water (100 mL) and neutralized with acetic acid until the acid product precipitated and then filtered through a bed of Celite. The product was extracted from the Celite with formic acid and the filtrate was evaporated to dryness under vacuum to give the acid porphyrin (126). Without purification, this acid product was suspended in dry dichloromethane (30 mL) and then oxalyl chloride (0.1 mL, 1.1 mmol) was slowly added in an ice bath. After the chlorination by stirring for one hour at room temperature, the solvent was removed under vacuum to give the carbonyl chloride porphyrin (128). Without isolation, this product (128) was dissolved in dry dichloromethane (30 mL) and then N,N-dimethylethylenediamine (0.02 mL, 0.18 mmol) was slowly added in an ice bath. After stirring for one hour at room temperature, the solvent was evaporated to dryness under high vacuum to remove excess N,N-dimethylethylenediamine. The resulting residue was chromatographed on

silica gel with 15% methanol in dichloromethane. The desired compound was collected to give the title compound (51.8 mg, 80%): $^1$H NMR ($CDCl_3$) δ 0.93, 0.94 (3H each, t, pentyl Me), 1.48, 1.67 (6H each, m, $CH_2$), 1.81 (3H, t, 2-$CH_2\underline{CH_3}$), 2.18, 2.21 (2H each, t, 13.17-$CH_2$), 2.41 (6H, s, $NMe_2$), 2.81 (2H, t, $NHCH_2\underline{CH_2}N$), 3.37, 3.41, 3.46, 3.54 (3H each, s, ring Me), 3.86 (2H, m, $NH\underline{CH_2}CH_2N$), 3.99 (2H, q, 2-$\underline{CH_2}CH_3$), 7.39 (1H, t, $CO\underline{NH}CH_2$), 9.73, 9.79, 9.85, 10.32 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 622 nm (1,600), 568 (6,800), 541 (11,200), 504 (10,200), 403 (166,000); MS for $C_{41}H_{56}N_6O$, found m/e 648.7 ($M^+$).

<u>7-[N-[2-(Dimethylamino)ethyl]carbamido]-2-ethyl-3,8,12,18-tetramethyl-13,17-dioctylporphyrin (131)</u>

Ethyl 2-ethyl-3,8,12,18-tetramethyl-13,17-dioctylporphyrin-7-carboxylate (125) (69 mg, 0.1 mmol) was hydrolized with potassium hydroxide in pyridine and then chlorinated with oxalyl chloride and treated with N,N-dimethylethylenediamine following the method described for the porphyrin amide (130) to give the title compound (59.3 mg, 81%): $^1$H-NMR ($CDCl_3$) δ 0.93, 0.95 (3H each, t, octyl Me), 1.27 (16H, m, $CH_2$), 1.47, 1.68 (4H each, m, $CH_2$), 1.82 (3H, t, 2-$CH_2\underline{CH_3}$), 2.19, 2.22 (2H each, t, 13, 17-$CH_2$), 2.42 (6H, s, $NMe_2$), 2.82 (2H, t, $NHCH_2\underline{CH_2}N$), 3.38, 3.42, 3.47, 3.55 (3H each, s, ring Me), 3.87 (2H, m, $NH\underline{CH_2}CH_2N$), 4.00 (2H, q, 2-$\underline{CH_2}CH_3$), 7.40 (1H, t, $CO\underline{NH}$), 9.72, 9.79, 9.85, 10.31 (1H each, s, meso H); UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 622 nm (1,600), 568 (6,800), 541 (11,200), 504 (10,200), 403 (166,000); MS for $C_{47}H_{68}N_6O$, found m/e 733.1 ($M^+$).

7-{N-[2-(Trimethylammonio)ethyl]carbamido}-2-ethyl-3,8,12,18-tetramethyl-13,17-dipentylporphyrin iodide (132)

7-{N-[2-(Dimethylamino)ethyl]carbamido}-2-ethyl-3,8,12,18-tetramethyl-13,17-dipentylporphyrin (130) (45.4 mg, 0.07 mmol) was dissolved in dichloromethane (35 mL) containing 10% methanol and iodomethane (0.044 mL, 0.7 mmol) was then added. The reaction mixture was stirred for 5 hours at room temperature and the solvent was evaporated to dryness under vacuum to give the title compound in almost quantitative yield: UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 623 nm (1,600), 569 (7,000), 542 (11,400), 505 (9,400), 403 (164,000); MS for $C_{42}H_{59}N_6OI$, found m/e 663.7 ($M^+$).

7-{N-[2-(Trimethylammonio)ethyl]carbamido}-2-ethyl-3,8,12,18-tetramethyl-13,17-dioctylporphyrin iodide (133)

7-{N-[2-(Dimethylamino)ethyl]carbamido}-2-ethyl-3,8,12,18-tetramethyl-13,17-dioctylporphyrin (131) (51.3 mg, 0.07 mmol) was treated with excess iodomethane following the procedure described for the ammonium derivative (132) to give the title compound in almost quantitative yield: UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 623 nm (1,600), 569 (7,000), 542 (11,400), 505 (9,400), 403 (164,000); MS for $C_{48}H_{71}N_6OI$, found m/e 748.1 ($M^+$).

Ethyl 2-ethyl-3,8,12,18-tetramethyl-13,18-dipentyl-17-porphyrinone-7-carboxylate (134)

Osmium tetroxide (115 mg, 0.45 mmol) and pyridine (0.05 mL) were added to ethyl 2-ethyl-3,8,12,18-tetramethyl-13,17-dipentylporphyrin-7-carboxylate (124) (182 mg, 0.3 mmol) dissolved in dichloromethane (40 mL). After the reaction mixture was stirred for 24 hours at room temperature

under nitrogen in the dark, it was quenched by adding methanol (10 mL), then bubbled with hydrogen sulfide through the reaction solution for 30 minutes to decompose the osmate adduct, and allowed to stand for 2 hours. The precipitated black osmium sulfide was removed by filtration through a bed of Celite and the filtrate was evaporated. The resulting residue was dissolved in dichloromethane (50 mL) and perchloric acid (70%, 0.8 mL) was added and then stirred for 30 minutes at room temperature. The reaction mixture was diluted with dichloromethane (50 mL) and the organic layer was washed twice with saturated aqueous sodium acetate (50 mL) and twice with water (50 mL). The organic layer was dried over anhydrous sodium sulfate and the solvent was removed under vacuum and the residue was chromatographed on silica gel with dichloromethane as eluent. The porphyrinone [84] (65.3 mg, 35%) was isolated as the major product: $^1$H NMR ($CDCl_3$) δ -2.77 (2H, br s, NH), 0.58, 0.98 (3H each, t, pentyl Me), 0.99, 1.13, 1.13, 1.52, 1.65, 2.14 (2H each, m, $CH_2$), 1.75 (3H, t, 2-$CH_2\underline{CH_3}$), 1.83 (3H, t, $CO_2CH_2\underline{CH_3}$), 2.13 (3H, s, 18-Me), 2.74 (2H, t, 18-$CH_2$), 3.26, 3.54, 3.54 (3H each, s, ring Me), 3.80 (2H, t, 13-$CH_2$), 3.98 (2H, q, 2-$\underline{CH_2}CH_3$), 4.78 (2H, q, $CO_2\underline{CH_2}CH_3$), 9.07, 9.59, 9.68, 10.76 (1H each, s, meso H); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 634 nm (14,400), 561 (16,600), 524 (7,700), 412 (168,000), 319 (15,800); MS for $C_{39}H_{50}N_4O_3$, found m/e 622.7 ($M^+$).

<u>7-[N-[2-(Dimethylamino)ethyl]carbamido]-2-ethyl-3,8,12,18-tetramethyl-13,18-dipentyl-17-porphyrinone (135)</u>

Ethyl 2-ethyl-3,8,12,18-tetramethyl-13,18-dipentyl-17-porphyrinone-7-carboxylate (134) (62.2 mg, 0.1 mmol) was treated with potassium hydroxide, with oxalyl chloride and with N,N-dimethylethylenediamine following the method described for the porphyrin amide (130) to afford the title compound

(51.6 mg, 77.7%): $^1$H NMR ($CDCl_3$) δ -2.89 (2H, br s, NH), 0.53, 0.93 (3H each, t, pentyl Me), 0.94, 1.09, 1.09, 1.50, 1.66, 2.58 (2H each, m, $CH_2$), 1.79 (3H, t, 2-$CH_2\underline{CH_3}$), 2.07 (3H, s, 18-Me), 2.17 (2H, q, $NHCH_2CH_2N$), 2.48 (6H, s, $NMe_2$), 2.72 (2H, t, $NHCH_2\underline{CH_2}N$), 2.93 (2H, t, 18-$CH_2$), 3.35, 3.52, 3.55 (3H each, s, ring Me), 3.88 (2H, t, 13-$CH_2$), 4.00 (2H, q, 2-$\underline{CH_2}CH_3$), 7.46 (1H, br s, CONH), 9.08, 9.66, 9.73, 10.29 (1H each, s, meso H); UV-vis (in 10% $CH_2Cl_2/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 634 nm (13,900), 578 (9,300), 561 (16,100), 524 (7,300), 412 (165,000); MS for $C_{41}H_{56}N_6O_2$, found m/e 664.7 ($M^+$).

7-[N-[2-(Trimethylammonio)ethyl]carbamido]-2-ethyl-3,8,12,18-tetramethyl-13,18-dipentyl-17-porphyrinone iodide (136)

7-[N-[2-(Dimethylamino)ethyl]carbamido]-2-ethyl-3,8,12,18-tetramethyl-13,18-dipentyl-17-porphyrinone (135) (46.5 mg, 0.07 mmol) was treated with excess iodomethane following the method described for the ammonium derivative (132) to give the title compound in almost quantitative yield: UV-vis (in 10% $CH_3OH/CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 635 nm (13,900), 579 (9,300), 561 (16,100), 525 (7,300), 413 (165,000); MS for $C_{42}H_{59}N_6O_2I$, found m/e 679.6 ($M^+$).

Nickel(II) 2,3,7,8,12,13,17,18-octaethylporphyrin (138)

some excess nickel (II) hexahydrate was added to a solution of octaethylporphyrin (137) (1.07 g, 2.00 mmol) in N,N-dimethylformamide (50 mL). The mixture was heated to reflux for one hour and then allowed to evaporate two thirds of N,N-dimethylformamide without condenser. After standing one hour, crystallized product was collected by filtration and washed with methanol to give the title compound as red-purple sparkling crystal (1.17 g, 98.7%): $^1$H NMR ($CDCl_3$) δ 1.81 (24H, t, -$CH_2\underline{CH_3}$), 3.92 (16H, q,

-$\underline{CH_2}CH_3$),9.77 (4H, s, meso H); UV-vis (in $CH_2Cl_2$) λmax (rel. int.) 551 nm (38.0), 516 (12.7), 391 (221.8), 329.5 (16.0); MS for $C_{36}H_{44}N_4Ni$, found m/e 591.3 ($M^+$).

Copper(II) 2,3,7,8,12,13,17,18-octaethylporphyrin (139)

Octaethylporphyrin (**137**) (1.07 g, 2.0 mmol) was dissolved in mixture of dichloromethane (100 mL) and methanol (25 mL) and then a solution of copper (II) acetate monohydrate (0.60 g, 3.0 mmol) was added. After the mixture was refluxed for 20-30 minutes, it was washed several times with water. The organic solution was dried over anhydrous sodium sulfate and the solvent was evaporated under vacuum to give the title compound (1.18 g, 98.9%): UV-vis (in $CH_2Cl_2$) λmax (rel. int.) 560 nm (10.3), 522 (5.3), 397 (147.5), 326 (8.0); MS for $C_{36}H_{44}N_4Cu$, found m/e 596.2 ($M^+$).

Nickel(II) 10-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (140)

3-(Dimethylamino)acrolein (3.0 mL, 30 mol) was added to a solution of nickel(II) complex of 2,3,7,8,12,13,17,18-octaethylporphyrin (**138**) (1.78 g, 3.0 mol) in dry dichloromethane (200 mL) at room temperature. Phosphorus oxychloride (2.83 mL, 30 mol) was added dropwise to the reaction mixture with continuous stirring, at 0 °C. The final mixture was stirred at room temperature for 10 hours and then treated with saturated aqueous sodium carbonate (300 mL) overnight. The mixture was extracted with dichloromethane and the combined organic layers were washed three times with water (300 mL). The solution was dried over anhydrous sodium sulfate and the solvent was removed under vacuum. The resulting residue was chromatographed on silica gel with hexane-dichloromethane (1:2) as eluent and the desired compound was collected and recrystallized from

dichloromethane and methanol to give the title compound (1.68 g, 87%): m.p. 245-246 °C; $^1$H NMR ($CDCl_3$) δ 1.65-1.77 (24H, overlapping t, peripheral $CH_2\underline{CH_3}$), 3.70-3.80 (16H, overlapping q, peripheral $\underline{CH_2}CH_3$), 5.50 (1H, dd, -CH=$\underline{CH}$-CHO), 9.34 (3H, s, meso H), 9.67 (1H, d, -CH=$\underline{CH}$-CHO), 9.82 (1H, d, -CHO); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 582 nm (17,200), 564 (17,900), 536 (15,850), 406 (70,800); MS for $C_{39}H_{46}N_4NiO$, found m/e 644 ($M^+$).

Copper(II) 10-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (141)

3-(Dimethylamino)acrolein (0.17 mL, 1.7 mmol) was added to copper (II) octaethylporphyrin (139) (100 mg, 0.17 mmol) dissolved in dry dichloromethane (20 mL) at room temperature and then treated with phosphorus oxychloride (0.16 mL, 1.7 mmol), following the method described for compound (140) before, to give the title compound (64 mg, 58%): m.p. 230-232 °C; UV-vis (in $CH_2Cl_2$) λmax ($\varepsilon_M$) 568 nm (14,200), 534 (12,100), 408 (139,500), 331 (22,100); MS for $C_{39}H_{44}CuN_4O$, found m/e 648.3 ($M^+$).

2,3,8,8,12,13,17,18-octaethylbenzochlorin (142)

Nickel(II) complex of 10-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (140) (1.29 g, 2.0 mmol) was dissolved in 18 (v/v)% sulfuric acid in trifluoroacetic acid (35 mL). The reaction solution was bubbled with hydrogen sulfide for one hour at room temperature before being poured into ice/water (150 mL). The mixture was extracted with dichloromethane, and the combined organic layer was washed with saturated aqueous sodium carbonate and finally with water. The organic solution was dried over anhydrous sodium sulfate, the solvent was removed under vacuum, and the resulting residue was chromatographed on silica gel with hexane-dichloromethane (3:2) as eluent. The desired green band was

collected and crystallized from dichloeomethane and methanol to give the title compound (0.94 g, 82%). This compound can be obtained from crude nickel(II) 10-(2-formylethenyl)-octaethylporphyrin (**140**), without purification, by the same procedure described above. The overall yield of reaction from the nickel(II) octaethylporphyrin (**138**) to the free base octaethylbenzochlorin (**142**) was 73%: m.p. 241-243 °C; $^1$H NMR ($CDCl_3$) δ 0.02 (6H, t, *gem* $CH_3$), 1.55-1.87 (18H, overlapping t, peripheral $CH_3$), 2.61 (4H, overlapping q, *gem* $CH_2$), 3.45-3.93 (12H, overlapping q, peripheral $CH_2$), 8.03 (1H, d, H of benzene ring), 8.10 (1H, t, H of benzene ring), 8.01, 8.56, 9.22 (1H each, s, meso H), 9.51 (1H, d, H of benzene ring); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 658 (35,000), 605 (17,000), 564 (15,400), 532 (13,700), 411 (107,500); MS for $C_{39}H_{48}N_4$, found m/e 572 ($M^+$).

Nickel(II) 2,3,8,8,12,13,17,18-octaethylbenzochlorin (**143**)

(i) From 2,3,8,8,12,13,17,18-octaethylbenzochlorin (**142**). Some excess nickel(II) chloride hexahydrate was added to a solution of octaethylbenzochlorin (**142**) (0.58 g, 1.0 mmol) in N,N-dimethylformamide (25 mL) and heated to reflux. Reaction progress was monitored by spectrophotometry. The reaction mixture was then diluted with dichloromethane (40 mL), washed with water to remove excess nicked chloride, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was chromatographed on silica gel with dichloromethane/hexane (1:1). The desired green band was collected and crystallized from dichloromethane and methanol to yield the title compound as shining crystals (0.60 g, 95%)

(ii) From nickel(II) 10-(2-formylethenyl)-2,3,7,8,12,13,17,18-octaethylporphyrin (**140**). Nickel(II) formylethenylporphyrin (**140**) (200 mg, 0.31 mmol) was treated with concentrated sulfuric acid (15 mL) at room

temperature for 1.5 hours. The reaction was poured into ice/water (300 mL) and extracted with dichloromethane. The organic layers were combined, washed with saturated aqueous sodium bicarbonate and with water, dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The residue was purified by the method described above to yield the title compound (92 mg, 47%).

For the title compound: m.p. 224-225 °C ; $^1$H NMR (CDCl3) δ 0.01 (6H, t, *gem* $CH_3$), 1.60-1.87 (18H, overlapping t, peripheral $CH_3$), 2.62 (4H, overlapping q, *gem* $CH_2$), 3.48-3.93 (12H, overlapping q, peripheral $CH_2$), 8.02 (1H, d, H of benzene ring), 8.10 (1H, t, H of benzene ring), 8.01, 8.56, 9.22 (1H each, s, meso H), 9.53 (1H, d, H of benzene ring); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 670 nm (20,000), 618 (6,800), 564 (4,200), 415 (37, 100); MS for $C_{39}H_{46}N_4Ni$, found m/e 628 ($M^+$).

Copper(II) 2,3,8,8,12,13,17,18-octaethylbenzochlorin (144)

A solution of copper(II) acetate monohydrate (0.40 g, 2.0 mol) in methanol (7 mL) was added to a solution of 2,3,8,8,12,13,17,18-octaethylbenzochlorin (142) (0.63 g, 1.1 mmol) in dichloromethane (60 mL) and methanol (20 mL). After the reaction mixture was refluxed for 20 minutes, it was washed several times with water. The solution was dried over anhydrous soduim sulfate and the solvent was removed under vacuum. The resulting residue was chromatographed on silica gel with hexane-dichloromethane (1:1). The desired product was collected and recrystallized from dichloromethane/methanol to give the title compound (0.68 g, 97.5%): UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 670 nm (18,500), 620 (5,100), 563 (2,100), 519 (2,400), 418 (37,100); MS for $C_{39}H_{46}N_4Cu$, found m/e 633 ($M^+$).

Copper(II) 10-(N,N-dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin chloride (147)

Copper(II) 2,3,8,8,12,13,17,18-octaethylbenzochlorin (144) (0.61 g, 0.96 mmol) was dissolved in a mixture of dry dichloromethane (100 mL) and N,N-dimethylformamide (1.2 mL, 15 mmol) was added. Phosphorus oxychloride (1.1 mL, 11 mmol) was added dropwise to the reaxtion mixture with continuous stirring at room temperature. The final mixture was stirred for 18 hours at room temperature and washed with diluted sodium bicarbonate. The organic layer was washed with a large amount of water and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The resulting residue was chromatographed on silica gel with 10% methanol in dichloromethane as eluent to give the title compound (0.54 g, 77.5%): UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 752 nm (35,000), 690 (12,000), 570 (7,000), 448 (30,000), 386 (51,000); MS for $C_{42}H_{52}N_5CuCl$, found m/e 689.3 ($M^+$).

10-(N,N-Dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin chloride (145)

Copper(II) 10-(N,N-dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin chloride (147) (0.73 g, 1.0 mmol) was stirred in concentrated sulfuric acid (15 mL) for 5 hours. The mixture was poured into ice/water (100 mL), neutralized with aqueous sodium bicarbonate, and then extracted with dichloromethane. The organic layer was washed three times with water, dried over anhydrous sodium sulfate, and evaporated under vacuum. The resulting residue was chromatographed on silica gel with 10% metanol in dichloromethane, and the desired green band was collected to give the title compound (0.49 mg, 78%). If the same proudure as above was used to treat

the crude copper(II) dimethyliminium benzochlorin (**147**), the yield from the copper(II) octaethylbenzochlorin (**144**) to the title compound (**145**) was 62%.

For the title compound: $^1$H NMR ($CDCl_3$) δ -0.09, 0.11 (3H each, t, *gem* $CH_3$), 1.30-1.60 (18H, overlapping t, peripheral $CH_3$), 2.53-2.75 (4H, m, *gem* $CH_2$), 2.77, 4.32 (3H each, s, $NMe_2$), 3.16-3.47 (12H, overlapping q, peripheral $CH_2$), 5.78 (2H, br s, NH), 7.81 (1H, d, H of benzene ring), 7.81 (1H, t, H of benzene ring), 8.26, 8.53 (1H each, s, meso H), 8.78 (1H, d, H of benzene ring), 10.31 (1H, br s, -CH=$NMe_2$); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 797 nm (49,000), 606 (9,670) 387 (52,300); MS for $C_{42}H_{54}N_5Cl$, found m/e 628.4($M^+$).

Nickel(II) 10-(N,N-dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin chloride (**146**)

(i) From 10-(N,N-dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin chloride (**145**). Some excess nickel (II) acetate was added to a solution of dimethyliminium octaethylbenzochlorin (**145**) (66.4 mg, 0.1 mmol) in acetic acid (35 mL) and refuxed. Reaction progress was monitored by spectrophotometry. After 6 hours, the reaction mixture was diluted with dichloromethane (80 mL), washed with water and brine to remove excess nickel(II) chloride, and dried over anhydrous sodium sulfate. After removal of the solvent, the crude product was chromatographed on silica gel with 10% methanol in dichloromethane to yield the title compound (63.6 mg, 93%).

(ii) From nickel(II) 2,3,8,8,12,13,17,18-octaethylbenzochlorin (**143**). N.N-Dimethylformamide (1.2 mL, 15 mmol) was added to nickel(II) octaethylbenzochlorin (**143**) (62.9 mg, 1.0 mmol) dissolved in dry dichloromethane (100 mL). Phosphorus oxychloride (1.1 mL, 11 mmol) was added dropwise to the reaction mixture with continuous stirring at room temperature. The reaction mixture was stirred overnight at room

temperature, diluted with dichloromethane, and treated with diluted aqueous sodium bicarbonate. The organic layer was washed with water and brine, and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue which was purified as above to yield the title compound (62.3 mg, 91%).

For the title compound ; $^1$H NMR ($CDCl_3$) δ 0.01, 0.08 (3H each, t, *gem* $CH_3$), 1.22-1.45 (18H, overlapping t, peripheral $CH_3$), 2.35-2.60 (4H, m, *gem* $CH_2$), 2.69, 4.13 (3H each, s, $NMe_2$), 2.93-3.24 (12H, overlapping q, peripheral $CH_2$), 7.61 (1H, d, H of benzene ring), 7.61 (1H, t, H of benzene ring), 7.75, 7.97 (1H each, s, meso H), 8.33 (1H, d, H of benzene ring), 9.86 (1H, s, -CH=$NMe_2$); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 773 nm (46,200), 588 (13,600), 452 (47,400), 393 (80,600), 321 (34,500); MS for $C_{42}H_{52}N_5NiCl$, found m/e 684.4 ($M^+$).

Zinc(II) 10-(N,N-dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin chloride (148)

A solution of zinc acetate dihydrate (30 mg, 0.13 mmol) in methanol (10 mL) was added to a solution of 10-(N,N-dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin chloride (145) (30 mg, 0.045 mmol) in chloroform (40 mL). The reaction mixture was heated to reflux until zinc insertion was completed which was monitored by spectrophotometry. The reaction mixture was diluted with chloroform (30 mL), washed with water to remove excess zinc acetate, dried over anhydrous sodium sulfate, and evaporated to dryness under vacuum. The residue was purified by preparative thick layer plates (silica gel) eluted with 8% methanol in dichloromethane. The product, extracted from the silica gel, was crystallized from dichloromethane-hexane to give the title compound (25 mg, 76%): UV-

vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 739 nm (51,300), 679 (17,800), 574 (6,200), 448 (35,500), 383 (65,000), 321 (31,700); MS for $C_{42}H_{52}N_5ZnCl$, found m/e 692.2 ($M^+$).

10-(N,N-Dimethyliminium)-2,3,8,8,12,13,17,18 octaethylbenzochlorin-5$^2$-sulfonic acid chloride (149)

Nickel(II) 10-(N,N-dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin chloride (146) (68.5 mg, 0.1 mmol) was stirred in concentrated sulfuric acid (3 mL) for 4 days at room temperature. The resulting solution was then poured into an ice-cold saturated aqueous sodium bicarbonate and extracted with dichloromethane. The organic layer was separated, washed with water, dried over anhydrous sodium sulfate, and evaporated. The residue was purified by column chromatography using 8% methanol in dichloromethane. The free base octaethylbenzochlorin iminium salt (145) was isolated from the first green band in 40% yield (26.5 mg). A second band which was the free base octaethyl sulfonated benzochlorin iminium salt derivative (149) was isolated with 12% methanol in dichloromethane. It was crystallized from dichloromethane-hexane to afford the title compound (29.6 mg, 40%): $^1H$ NMR ($CD_3CN$) $\delta$ -0.08, 0.13 (3H each, t, *gem* $CH_3$), 1.22-1.62 (18H, overlapping t, peripheral $CH_3$), 2.42-2.81 (4H, m, *gem* $CH_2$), 2.71 (3H, s, N-$CH_3$), 3.19-3.49 (12H, overlapping g, peripheral $CH_2$), 3.79 (3H, s, N-$CH_3$), 5.75 (2H, br s, NH), 8.25 (1H, s, H of benzene ring), 8.40, 8.63 (1H each, s, meso H), 9.33 (1H, s, H of benzene ring), 9.37 (1H, s, -$\underline{CH}$=$NMe_2$); UV-vis (in 10% MeOH/$CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 803 nm (33,800), 615 (9,800), 389 (55,400); MS for $C_{42}H_{54}N_5SO_3Cl$, found m/e 708 ($M^+$).

Zinc(II) 10-(N,N-dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin-5²-sulfonic acid chloride (151)

The sulfonic acid iminium chloride (149) was treated with zinc acetate dihydrate following the method described before for the zinc (II) iminium derivative (148) to give the title compound in 76% yield after crystallization from dichloromethane-hexane: $^1$H NMR ($CD_3CN$) δ 0.10, 0.20 (3H each, t, *gem* $CH_3$), 1.10-1.52 (18H, overlapping t, peripheral $CH_3$), 2.30-2.80 (4H, m, *gem* $CH_2$), 2.80 (3H, s, N-$CH_3$), 3.00-3.20 (12H, overlapping q, peripheral $CH_2$), 3.65 (3H, s, N-$CH_3$), 7.95, 8.11, 8.15, 8.85 (1H each, s, meso H and H of benzene ring), 9.35 (1H, s, -CH=$NMe_2$); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ ($\varepsilon_M$) 744 nm (36,400), 682 (14,100) 570 (5,600), 449 (31,900), 384 (60,100); MS for $C_{42}H_{52}N_5SO_3ZnCl$, found m/e 772 ($M^+$).

Nickel(II) 10-formyl-2,3,8,8,12,13,17,18-octaethylbenzochlorin (152)

Nickel(II) 10-(N,N-dimethyliminium)-2,3,8,8,12,13,17,18-octaethylbenzochlorin chloride (146) was dissolved in dichloromethane and stirred with saturated aqueous sodium carbonate for one day to give the title compound in more than 90% yield: $^1$H NMR ($CDCl_3$) δ 0.12 (6H, t, *gem* $CH_3$), 1.36-1.50 (18H, overlapping t, peripheral $CH_3$), 2.32, 2.82 (2H each, m, *gem* $CH_2$), 3.06-3.32 (12H, overlapping q, peripheral $CH_2$), 7.56 (1H, d, H of benzene ring ), 7.89, 8.19 (1H each, s, meso H), 8.40 (1H, d, H of benzene ring), 10.64 (1H, br s, -CHO); UV-vis (in $CH_2Cl_2$) $\lambda_{max}$ (rel. int) 710 nm (66.5), 439 (96.1), 406 (100), 371 (85.7); MS for $C_{40}H_{46}N_4NiO$, found m/e 656.4 ($M^+$).

# REFERENCES


1. Thudichum, J.L.W. *Report Med. Off. Privy. Council Appendix 7* **1867,** *10,* 152.
2. Hoppe-Seyler, F. *Medizin-chem. Untersuchungen., Heif* **1971,** 1.
3. a) Nencki, M. *Z.Physiol. Chem.* **1900,** *30,* 423.
   b) Nencki, M.; Zaieski, J. *Z. Physiol. Chem.* **1901,** *34,* 997.
4. Lemberg, R; Legge, J.W. *Hematin Compounds and Bile Pigments,* Interscience Publishers, Inc., New York **1949,** p.61.
5. Küster, W.*Ber.* **1912,** *45,* 1935.
6. Küster, W.*Z. Physiol. Chem.* **1912,** *82,* 463.
7. Fisher, H.; Klarer, J. *Justus Libigs Ann. Chem.* **1926,** *450,* 181.
8. Fisher, H.; Wallach, B. *Justus Libigs Ann. Chem.* **1926,** *450,* 164.
9. Fischer, H.; Zeile, K. *Justus Libigs Ann. Chem.* **1929,** *468,* 98.
10 Blum, H.F. *Photodynamic Action and Disease Caused by Light, ACS Monograph,* Washington D.C. **1941**
11. a) Lamola, A.A.; Yamane,T. ; Trozzolo, A.M. *Science* **1973,***179,* 1131.
    b) Weishaupt, K.R: Gomer, C.J.; Dougherty, T.J. *Cancer Res.* **1976,** *36,* 2326.
12. Hausmann, W. *Biochem. Z.* **1911,** *30,* 276.
13. Meyer-Betz, F. *Dtsch. Arch. Klin. Med.* **1913,** *112,* 476.
14. a) Figge, F.H.J.; Weiland, G.S.; Manfanielle, L.O. *Proc. Soc. Exp. Biol. Med.* **1948,** *68,* 640.
    b) Schwartz, S.; Absolon,K.; Vermund, H. *Univ. Minnostoa Med. Bull.* **1955,** *27,* 7.
15. Sanderson, D.R.; Fontana, R.S.; Lipson, R.L.; Baldes, E.J. *Cancer* **1970,** *30,* 1368.
16. Lipson, R.L.; Baldes, E.J.; Olsen, A.M. *J. Natl. Cancer Inst.* **1961,** *26,* 1.
17. Diamond, I.; McDonagh, A.F.; Willson, C.B.; Granelli, S.G.; Nielsen, S.; Jaenicke, R. *Lancet.* **1972,** *2,* 1175.
18. Dougherty, T.J.; Grindey, G.B.; Fiel, R.; Wishaupt, K.R.; Boyle, D.G. *J. Nat. Cancer Inst.* **1975,** *55,* 115.
19. Kessel, D. *Photochem. Photobiol.* **1984,** *39,* 851.

21. Henderson, B.W.; Dougherty, T.J. *Photochem. Photobiol.* **1992**, *55*, 145.
22. Moan, J. *Photochem. Photobiol*, **1986**, *43*, 681.
23. Spikes, J.D. *Photochem.Photobiol*, **1986**, *43*, 691.
24. Dougherty, T.J. *Photochem.Photobiol*, **1987**, *45*, 879.
26. Dougherty, T.J. *Semin. Surg. Oncol.* **1989**, *5*, 6.
27. Laustriat, G. *Biochimie* **1986**, *68*, 771.
28. Spikes, J.D. "*Photosensitisation*" in *The Science of Photobiology*, Smith, K.C. Ed., Plenum, New York, **1977**, p87.
29. Kato, H.; Kawate, N.; Kinoshita, K.; Yamamoto, H.; Furukawa, K.; Hayata, Y. *Biolog and Clinical Use-CIBA Foundation Symposium*, Wiely, **1989**, *146*, 183.
30. Jocham, D.; Beer, M.; Baumgartner, R.; Staehler, G.; Unsold, E. *ibid.* **1989**, *146*, 198.
31. Bonnett, R.; Charalambides, A.A.; Jones, K.; Magnus, I.A.; Ride, R.J. *Biochem.J* . **1978**, *173*, 693.
32. Pandey, R.K.; Dougherty, T.J. *Photochem. Photobiol.* **1988**, *47*, 769.
33. Jori, G.; Reddi, E.: Salvato, B.; Pagnam, A.J.; Ziron, L. *Cancer Lett.* **1984**, *24*, 291.
34. Bonnett, R.; Nizhnik, A.N.; Berenbaum, M.C. *J. Chem. Soc. Chem. Commun.* **1989**, 1822.
35. Scheve, L.G. *Elements of Biochemistry*, Allyn and Bacon, Inc., Boston, **1984**, 253.
36. Grossweiner, L.I. ; Goyal, G.C. *Photochem. Photobiol.* **1984**, *40*, 721.
37. Norata, G.; Conti, G.; Ricci, L.; Nicolin, A.; Catapano, A.L. *Cancer Lett.* **1984**, *25*, 203.
38. Korbelik, M.; Hung, J.; Lam, S.; Palcic, B. *Photochem. Photobiol.* **1990**, *51*, 191.
39. Kessel, D. *Photochem. Photobiol.* **1989**, *50*, 345.
40. Laustiat, G. *Biochimie* **1986**, *68*, 771.
41. Wiknson, F.; Brummer, J.G. *J. Phys. Chem. Ref. Date.* **1981**, *10*, 809.
42. Rodgers, M.A.J. *Photochem. Photobiol.* **1983**, *37*, 99.
43. Schmidt, R.; Brauer, H.D. *J. Am. Chem. Soc.* **1987**, *109*, 6976.
44. a) Foote, C.S. *Proc. SPIE Institute IS6* **1990**, 115.
    b) Foote, C.S. *Photochem.Photobiol.* **1991**, *54*, 659.
45. Moan, J. *J. Photochem. Photobiol.*, B: *Biol.* **1990**, *6*, 343.

56. Lee, P.C.C.;Rodger, M.A.J. *Photochem. Photobiol.* **1987**, *45*, 79.
47. Spikes, J.D. *The Science of Photobiology*, 2nd Edn., Smith, K.C. Ed., Plenum Press, **1989**, p79.
48. Weishaupt, K.R.; Gomer, C.J.; Dougherty, T.J. *Cancer Res.* **1976**, *36*, 2326.
49. Morgan, A,R.; Skalkos, D. *SPIE Institute Series* vol.IS6, **1990**, 87.
50. Wan, S.; Parrish, J.A.; Anderson, R.R.; Madden, M. *Photochem. Photobiol.* **1981**, *34*, 679.
51. Patterson, M.S.; Madsen, S.J.; Wilson, B.C. *J.Photochem. Photobiol.* B: *Biol.* **1990**, *5*, 69.
52. Henderson, B.W.; Miller, A.C. *Radiat. Res.* **1986**, *108*, 196.
53. Truscott, T.G.; McLean, A.J.; Phillips, A.M.R.; Foulds, W.S. *Cancer Lett.* **1988**, *41*, 31.
54. Athar, M.; Elmets, C.A.;Bickers, D.R.; Mukhtar, H. *J. Clin. Invest.* **1989**, *83*, 1137.
55. a) Moan, J. ; Sommer, S. *Cancer Res.* **1985**, *45*, 1608.
    b) Brasseur, N.; Ali, H.; Autenrieth, D.; Langlois, R.; van Lier, J.E. *J. Photochem. Photobiol.* **1985**, *42*, 515.
    c) Henderson, B.W.; Fingar, V.H. *Cancer Res.* **1987**, *47*, 3110.
56. Oseroff, A.R.; Ohuoha, D.; Ara, G.; McAuliffe, D.; Foley, J.; Cincotta, L. *Proc. Natl. Acad. S i. USA* **1986**, *83*, 9729.
57. Nossal, R.; Kiefer, J.; Weiss, G.H.; Bonner, R.; Taitebaum, H.; Havlin, S. *Appl. Opt.* **1988**, *27*, 3382.
58. Patterson, M.S.;Wilson, B.C.; Graff, R. *NATO ASI Series H: Cell Biology*, vol.15, Moreno, R.H. and Truscott, T.G.Ed., Springer-Verlag, **1987**, p21.
59. Brasseur, N.; Ali, H.; Langlois, R.; van Lier, J.E. *Photochem. Photobiol.* **1988**, *47*, 705.
60. Firey, P.A.; Rodgers, M.A.J. *Photochem.Photobiol.* **1987**, *45*, 535.
61. Smith, K.M.; Goff.D.A.; Simpson, D.J. *J. Am. Chem. Soc.* **1985**, *107*, 4946.
62. Rexhausen, H.; Gossauer, A. *J. Chem. Soc., Chem. Commun.* **1983**, 275.
63. a) Broadhurst, M.J.; Grigg, R.; Johnson, A.W. *J. Chem. Soc., Perkin Trans., 1*, **1972**, 2111.

b) Bauer, V.J.; Clive, D.L.J.; Dolphin, D.; Pain III, J.B.; Harris, F.L.; King, M.M.; Loder, J.; Wang, S.W.C.; Woodward, R.B. *J. Am. Chem. Soc.* **1983**, *105*, 6429.

64. Chen, L.B. *Ann. Rev. Cell Biol.* **1988**, *4*, 151.
65. Salet, C.; Moreno, G. *J. Photochem. Photobiol., B: Biol.* **1990**, *5*, 133.
66. Oseroff, A.R.; Ohuoha, D.; Ara, G.; McAuliffe, D.; Foley, J.; Cincotta, L. *Proc. Natl. Acad. Sci.* **1986**, *83*, 9729.
67. Cincotta, L.; Foley, J.W.; Cincotta, A.H. *Cancer Res.* **1993**, *53*, 1571.
68. Ward, B.; Chang, C.K.; Young, R. *J. Am. Chem. Soc.* **1984**, *106*, 3943.
69. Skalkos, D.; Hampton, J.A. *Med. Chem. Res.* **1922**, *2*, 276.
70. Selman, S.H.; Hampton, J.A.; Morgan, A.R.; Keck, R.W.; Balkany, A.D.; Skalkos, D. *Photochem. Photobiol.* **1993**, *57*, 681.
71. Kessel, D. *Photochem. Photobiol.* **1993**, *58*, 623.
72. Hampton, J.A.; Skalkos, D.; Tayler, P.M.; Selman, S.H. *Photochem. Photobiol.* **1993**, *58*, 100.
73. Selman, S.H.; Birnbaum, M.K.; Klaunig, J.E.; Goldblatt, P.J.; Keck, R.W.; Britton, S.L. *Cancer Res.* **1984**, *44*, 1924.
74. Hopper-Seyler, F. *Z. Physiol. Chem.* **1877-1878**, *1*, 121.
75. Fischer, H.; Helberger, H. *Liebigs Ann.Chem.* **1929**, *471*, 285.
76. Fischer, H.; Gibian, A. *Liebigs Ann.Chem*, **1942**, *550*, 208.
77. Eisner, U. *J. Chem. Soc.* **1957**, *1957*, 3461.
78. Stolzenberg, A.M.; Spreer, L.Q.; Holm, R.H. *J. Am. Chem. Soc.* **1980**, *102*, 364.
79. Fischer, H.; Orth, H. *"Die Chemie des Pyrrols"*; Akademische Verlagsgesselschaft, Leipzig, **1937**, vol.II, Part I, p269.
80. Fischer, H.; Pfeiffer, H. *Liebigs Ann.Chem.* **1944**, *556*, 131.
81. Bonnett, R.; Dolphin, D.; Johnson, A.W.; Oldfield, D.; Stephenson, G.F. *Proc. Chem. Soc. (London)* **1964**, 371.
82. Bonnett, R.; Dimsdale, M.J.; Stephenson, G.F. *J. Chem. Soc (C)* **1969**, 564.
83. Inhoffen, H.H.; Nolte, W. *Liebigs Ann.Chem.* **1969**, *725*, 167.
84. Chang, C.K. *Biochemistry* **1980**, *19*, 1971.
85. Chang, C.K.; Sotiriou, C. *J. Org. Chem.* **1985**, *50*, 4989.
86. Sotiriou, C. *Ph. D. Dissertation*, Michigan State University, E. Lansing, MI **1987**.

87. Sotiriou, C. *Ph. D. Dissertation,* Michigan State University, E. Lansing, MI **1987**, p66.
88. Aumüller, A.; Hüing, S. *Liebigs Ann. Chem.* **1984**, 618.
89. Aumüller, A.; Hüing,S. *Angew. Chem. Int. Ed. Engl.* **1984**, *23*, 447.
90. Sotiriou, C. *Ph. D. Dissertation,* Michigan State University, E. Lansing, MI **1987**, p92.
91. Arasasingham, R.D.; ;Balch, A.L.; Olmstead, M.M. *Heterocycles* **1988**, *27*, 2111.
92. Gomer, C.J. *Photochem. Photobiol.* **1991**, *54*, 1093.
93. Chang, C.K.; Sotiriou, C. *J. Org. Chem.* **1985**, *50*, 4989.
94. Woodburn, K.; Chang, C.K.; Lee, S.; Henderson, B.; Kessel, D. *Photochem. Photobiol.* **1994**, *60*, 154.
95. Yosizawa, Z.; Sato, T. *J.Biochem.* **1951**, *51*, 155.
96. Takemura, T.; Ohta, N.; Nakajima, S.; Sakata, I. *Photochem. Photobiol.* **1989**, *50*, 339.
97. Takemura, T,; Ohta, N.; Nakajima, S.; Sakata, I. *Photochem. Photobiol.* **1991**, *54*, 683.
98. Takemura, T.; Ohta, N.; Nakajima, S.; Sakata I. *Photochem. Photobiol.* **1992**, *55*, 137.
99. Takamura T.; Aikawa, M.A.; Baba, H.; Shindo, Y. *J. Am. Chem. Soc.* **1976**, *98*, 2205.
100. Takamura, T.; Yamada, Y.; Sugawara, M.;Baba, H. *J. Phys. Chem.* **1986**, *90*, 2324.
101. Yamamoto, K.; Takemura, T.; Baba, H. *J. Luminesc.* **1977**, *15*, 445.
102. Takemura, T.; Nakajima, S.; Sakata, I. *Photochem. Photobiol.* **1994**, *59*, 366.
103. Sakata, I.; Maruyama, S.; Takada, H.; Ikeuchi, M.; Inui, H.; Nakajima, T.; Takemura, T.; Kishimiz, K. *Porphyrins* **1992**, *1*, 235.
104. Henderson, B.W. *Unpublished progress report* Sept.9, **1993**.
105. Kessel, D.; Dougherty, T.J.; Chang, C.K. *Photochem. Photobiol.* **1991**, *53*, 475.
106. Ferrario, A.; Kessel, D.; Gomer, C.J. *Cancer Res.* **1992**, *52*, 2890.
107. Chapman, M.J. *Methods Enzymol.* **1986**, *128*, 70.
108. Dougherty, T.J.; Potter, W.R.; Weishaupt, K.R. *Porphyrin Localization and Treatment of Tumors;* Doiron, D. Gomer, C.J. Eds. NewYork, **1984**, 301.

109. Bonnett, R.; Berenbaum, M.C. *Adv. Exp. Med. Biol.* **1983**, *160*, 241.
110. Byne, C.J.; Marshallsay, L.V.; Ward, A.D. *Photochem. Photobiol.* **1987**, *46*, 575.
111. Pandy, R.K.; Dougherty, T.J. *Cancer Res.* **1989**, *49*, 2042.
112. Scourides, P.A.; Bohmer, R.M.; Kaye, A.H.; Morstyn, G. *Cancer Res.* **1987**, *47*, 3439.
113. Pandey, R.K.; Dougherty, T.J. *Photochem. Photobiol.* **1988**, *47*, 769.
114. Morris, I.K.; Ward, A.D. *Tetrahedron Lett.* **1988**, *29*, 2501.
115. Pandey, R.K.; Shiau, F.Y.; Medforth, C.J.; Dougherty, T.J.; Smith, K.M. *Tetrahedron Letters* **1990**, *31*, 789.
116. Kessel, D.; Dougherty, T.J.; Truscott, T.G. *Photochem. Photobiol.* **1988**, *48*, 741.
117. Kessel, D.; Dougherty, T.J.; Chang, C.K. *Photochem. Photobiol.* **1991**, *53*, 475.
118. Spikes, J.D. *J. Photochem. Photobiol.* **1990**, *6*, 259.
119. Spikes, J.D.; Kessel, D. *Photochem. Photobiol* **1989**, *49*, 579.
120. Callot, H.J.; Johnson, A.W.; Sweeney, A. *J. Chem. Soc., Perkin Trans.1* **1973**, 1424.
121. Dolphin, D. *J. Org. Chem.* **1986**, *51*, 1094.
122. Pandey, R.K.; Shiau, F.Y.; Ramachandran, K.; Dougherty.T.J.; Smith, K.M. *J. Chem. Soc., Perkin Trans.1* **1992**, 1377.
123. Kessel, D.; Woodburn, K.; Henderson, B. W.; Chang, C. K. *Unpublished progress report.* July 28, **1994**.
124. Arnold, D.P.; Holmes, R.G.; Johnson, A.W.; Smith, A.R.P.; Williams, G.A. *J. Chem. Soc., Perkin Trans.1* **1978**, 1660.
125. Vicente, M.G.H.; Rezzano, I.N.; Smith, K.M. *Tetrahedron Lett.* **1990**,*31*, 1365.
126. Graça, H.; Vincente, H.; Smith, K. M. *J. Org. Chem.* **1991**, *56*, 4407.
127. Inhoffen, H.H.; Fuhrhop, J.H.; Voigt, H.; Brockmann, H.,Jr. *Justus Liebigs Ann. Chem.* **1966**, *695*, 133.
128. Gosmann, M.; Frank, B. *Angew. Chem.,Int. Ed. Engl.* **1986**, *25*, 1100.
129. Morgan, A.R.; Skalkos, D.; Maguire, G.; Rampersaud, A.; Garbo, G.; Keck, R.; Selman, S.H. *Photochem. Photobiol.* **1992**, *55*, 133.