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Synthesis of a Four Carbon Chain Linked Diporphyrin as a Tumor Localizing Agent

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Paul Eric Luikart

has been accepted towards fulfillment
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MS degree in **Chemistry**

Chi K. Chang
Major professor

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SYNTHESIS OF A FOUR CARBON CHAIN LINKED DIPORPHYRIN AS A TUMOR LOCALIZING AGENT

By

Paul E. Luikart

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ABSTRACT

SYNTHESIS OF A FOUR CARBON CHAIN LINKED DIPORPHYRIN AS A TUMOR LOCALIZING AGENT

By

Paul E. Luikart

A diporphyrin with a four methylene unit linkage was synthesized to help complete the existing sequence of related compounds and to elucidate the mechanisms of tumor localization and photoactivated cell toxicity exhibited by these compounds and by hematoporphyrin derivative (HpD).

This thesis is dedicated to the memory of my grandfather, Jan Schilt.

ACKNOWLEDGEMENTS

I would like to thank professor C. K. Chang for his patience and guidance and also for his financial support. I am also indebted to the chemistry department and Michigan State University for the teaching assistantships that I have received.

All of the various members of our group have earned my respect and gratitude for their help, friendship and glassware. Dr. W. Wu, in particular, has proven to be infinitely helpful and a joy to be around. Dr. G. Alviles was supportive and her sense of humor made the lab a more pleasant place. I also want to thank Ms. Grace Gibson, who was an unfailing source of support and inspiration.

Finally I want to thank my parents, Joan and Walter for their love and support, some of it financial, and my brother, Ernst Jan for being around.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF ABBREVIATIONS

HP . . . . . Hematoporphyrin.

HPA . . . . . Aceylated Hematoporphyrin.

HPD . . . . . Hematoporphyrin Derivative.

LDL . . . . . Low Density Lipoproteins.

P II . . . . . Photofrin II

Introduction

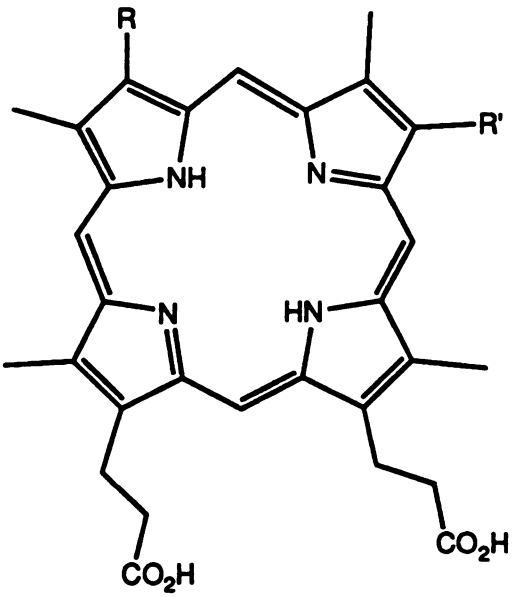
Photodynamic therapy (PDT) is a theraputic modality for the treatment of malignant tumors using a photosensitizing drug that localizes in cancerous tissues. Exposure of these tissues to visible light activates the drug which then exercises its cytotoxic effects. The requirements for such drugs are that they must be preferentially absorbed and retained by neoplastic cells; they must show good cytotoxic effects when exposed to tissue-penetrating light and they should be cleared quickly from the system following treatment (Pandey *et al.*, 1991).

Hematoporphyrin derivative (HpD) has been used successfully in clinical trials on a wide variety of tumors, including breast, colon, prostrate and skin cancers. No variety of tumor treated has proven unresponsive (Dougherty *et al.*, 1978). HpD shows good tumor localization and good photoactivated cytotoxicity. However, it takes a long time to clear from the patient's tissues, leaving the patient photosensitive for extended periods.

The utility of HpD in this type of therapy has generated extensive interest in the drug and its mode of action. Unfortunately HpD is far from chemically pure, and efforts to identify its active fraction have produced mixed results.

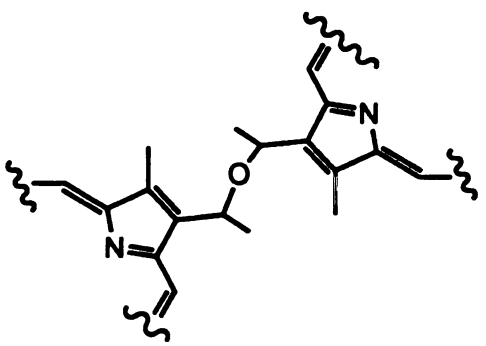
The preparation of HpD was first described by Lipson in 1960 (Lipson *et al.*,1960). HpD, as its name implies, is derived from hematoporphyrin, Figure 1, A (B-I are components of HpD), a porphyrin monomer with two secondary alcohol side chains and two propionic acid side chains. Lipson described HpD as a tumor locating

Figure 1. Hematoporphyrin and Components of Acylated Hematoporphyrin

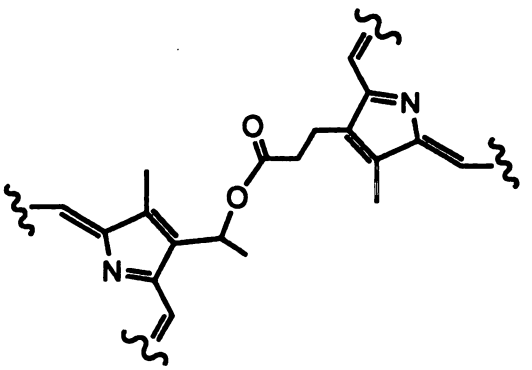


A. R = R' = $CH(OH)CH_3$
B. R = R' = $CH(OAc)CH_3$
C. R = R' = $CHCH_2$
D. R = $CH(OAc)CH_3$, R' = $CH(OH)CH_3$
E. R = $CH(OH)CH_3$, R' = $CH(OAc)CH_3$
F. R = $CH(OAc)CH_3$, R' = $CHCH_2$
G. R = $CHCH_2$, R' = $CH(OAc)CH_3$
H. R = $CH(OH)CH_3$, R' = $CHCH_2$
I. R = $CHCH_2$, R' = $CH(OH)CH_3$

Figure 2. Linkages in Hematoporphyrin Derivative



1. Ether Linkage



2. Ester Linkage

drug, useful because of its tendency to accumulate in neoplastic tissues and its intense fluorescence when irradiated with red light. He is also credited with being the first to recognize its potential to kill tumor cells and its possible usefulness in cancer therapy (Dougherty 1987).

Lipson's procedure for the preparation of HpD as clarified by Dougherty (Dougherty, 1979) involves two steps. The first is the treatment of HP with a mixture of acetic and sulfuric acids, resulting in the acylation of some of the HP's alcohols and the dehydration of others. The product of this step, HPA, hematoporphyrin acetate, is already a complex mixture, containing diacetate and a variety of monoacetates (Bonnet, 1981). The structures of the nine possible products of this step are shown in Figure 1, A-I. The next step of the procedure calls for the treatment of the HPA with dilute alkali, which promotes the cleavage of the acetates and the formation of dimers and higher oligomers of the porphyrins in the mixture.

The presence of these dimers and oligomers has been demonstrated by fast atom bombardment mass spectrometry. Oligomers composed of up to 5 porphyrin rings were revealed as well as dimers and monomers (Musselman *et al.*, 1988). It is these dimers and oligomers that are responsible for the tumor localizing and photodynamic activity of HpD (Kessel *et al.*, 1987). Much debate has occured concerning the chemical nature of the connections between the porphyrin rings in this material. Evidence of ester linkages was found by some researchers (Kessel 1986), while others favored ether type connections (Swincer *et al.*, 1985) Figure 2. One

line of evidence for ester linkages was provided by the cleavage of HpD polymers with lithium aluminum hydride, a reagent that selectively reduces esters but not ethers (Kessel *et al.*, 1987). The confusion about the nature of the linkage is understandable in hindsight as it turns out that HpD contains both kinds of connectivity, that the relative ratios of each are dependant on the conditions used to prepare the HpD (Byrne *et al.*, 1987, Kessel *et al.*, 1987) and that ester linkages are slowly converted into ether linkages in this system (Kessel *et al.*, 1987). Both ether- (Pandey *et al.*, 1988) and ester- (Pandey *et al.*, 1989) linked diporphyrins have been synthesized and tested for photosensitizing ability. The ether-linked dimer showed more activity than the ester-linked dimer, however both were effective. Photophrin II (PII), a commercially available form of HpD that is enriched in the oligomeric fraction, has been shown to contain a relatively higher proportion of ether linkages to ester linkages than freshly prepared HpD, with about 50% of each, while fresh HpD contains almost all esters (Dougherty 1987). Photophrin II is also quite effective in PDT, implying that the exact chemical nature of the link between porphyrin rings is not critical in tumor localization and photosensitization.

Mechanisms of Delivery, Retention and Mode of Activity

Singlet oxygen, a high energy form of oxygen, a potent oxidizer, has been identified as the probable mediating agent in the photodynamic activity of HpD (Wieshaupt *et al.*, 1976). Wieshaupt

and co-workers treated mouse carcinoma cells *in vitro* with an efficient singlet-oxygen trapper, 1,3-diphenylisobenzofuran, and observed inhibition of the photodynamic effect, as well as the formation of *o*-dibenzoylbenzene, the singlet oxygen trapping product. Furthermore, they report a similar protection *in vivo* by the same singlet oxygen trap, of tumors in mice treated with HpD and red light.

Apart from the role of singlet oxygen as an oxidizer, the subcellular mechanisms of HpD photodynamic cytotoxicity are poorly understood; *in vitro* experiments have implicated a variety of cellular sites as being important in PDT, including the cell membrane (Kessel 1977), the nucleus (Gomer *et al.*, 1983), lysosomes (Torinuki *et al.*, 1980) and the mitochondria (Hilf *et al.*, 1987; Singh *et al.*, 1987). Hilf and co-workers (1987) also reported a significant and rapid decline in ATP levels, *in vivo* , in tumors following treatment with HpD and light, reinforcing the suggestion of damage to mitochondria. Recent work on the subcellular localization of HpD indicates that following absorbance into the cell, HpD concentrates in all the lipophillic sites, including the cytomembrane of the mitochondria (Shulok *et al.*, 1990). Clearly, attack at such a sensitive and important site as the mitochondria, the cell's source of energy in the form of ATP, could result in profound effects for the cell. Even if HpD levels in other subcellular organelles proved to be higher than that in mitochondria, this could still be the most important location of damage.

The mechanism of delivery of the active fraction of HpD and PII, 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. Unfortunately these mechanisms remain obscure. 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 (Jori *et al.*, 1984). This mode of delivery 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 of work by Korbelik and co-workers (1990) that indicates that LDL actually inhibits the uptake of PII by tumor cells both *in vitro* and *in vivo*.

An alternative mechanism for the retention of the oligomeric fraction 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 non-specific 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 (1989) 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. However, Kessel and co-workers, studying a related group of photodynamic porphyrin dimers, reported that there is no relationship between *in vivo* photodynamic efficiency and hydrophobicity. The aggregation model is also somewhat unsatisfactory in that it fails to provide any mechanism that explains the tendency of these compounds to selectively localize in neoplastic tissues.

Purpose of this Work

The development of synthetic analogs of the active fraction of HpD has two main driving forces: first, the hope that better photoactive drugs may be found serendipitously somewhere in the family of related compounds; and second, that the development of chemically pure synthetic models of HpD's active compounds will provide investigators on all levels with more clearly defined tools for probing the mechanisms of PDT.

When the tumor-localizing fraction of HpD was recognized to contain porphyrin dimers, and when it was evident that the exact nature of the bridge between the rings was not vital, the synthesis of porphyrin dimers linked by stable methylene bridges suggested itself. The initial success of this approach led to the development of a family of porphyrin dimers, with three propionic acid side chains per ring, joined by all carbon bridges. Dimers linked by 3-, 5-, 6- and 13-carbon chains was achieved. These compounds have been tested *in vivo* for effectiveness in PDT (Kessel *et al.*, 1988, Kessel *et*

al., 1991). The dimer linked with a six-carbon chain proved to be the most effective, followed by the five-carbon and then the three-carbon compound. The compound linked by the thirteen carbon-chain showed no activity. The C-6 dimer was at least as efficient as PII, and all showed less persistence than HpD.

For this work the synthesis of the four-carbon-linked-dimer, Figure 5, **14**, of the above family of compounds was achieved, in an effort to help complete the sequence of the methylene linked dimers. It is hoped that through the study of a complete sequence of these compounds some patterns will emerge that may lead to elucidation of the mechanisms and processes involved in tumor localization and photodynamic action.

Results and Discussion

The tetramethylene linked porphyrin dimer was prepared from the bisdipyrromethene **11** and the 5,5'-dibromopyrromethene **12**.

The nucleophilic aromatic substitution reaction of the four carbon acid chloride **2** and the beta-free position of ethoxycarbonylmethyl-3,5-dimethylpyrrole **1** resulted in the formation of pyrrole **3**. The ketone functionality of the keto ester side chain was selectively reduced with diborane generated *in situ* by the dropwise addition of boron trifluoride etherate to a solution of the pyrrole and sodium borohydride in a 60/40 solution of tetrahydrofuran and ethyl acetate. The ethyl acetate provides a measure of protection for the ester of the four carbon side chain, preventing over reduction of the product. The methyl ester of the resulting pyrrolylbutanoate **4** was then saponified by aqueous potassium hydroxide in tetrahydrofuran, to give, upon work up with dilute acid, the corresponding carboxylic acid **5**. This acid was converted into an acid chloride **6** with oxalyl chloride. Condensation of this acid chloride with another equivalent of the beta-free pyrrole **1**, used initially, gave a bispyrrole linked with a four carbon chain **7**. Once again the carbonyl of this chain was reduced to a methylene by diborane generated *in situ*. The ethyl esters of the 4,4'-tetramethylenebis(2-ethoxycarbonyl-3,5-dimethylpyrrole) **8** were trans esterified to the benzyl esters by treatment of a benzyl alcohol solution of the bispyrrole with sodium metal at reflux temperature.

Figure 3. Synthesis of Ethyl-3,5-dimethyl-4-(3-hydroxycarbonylpropyl)pyrrole-2-carboxylate

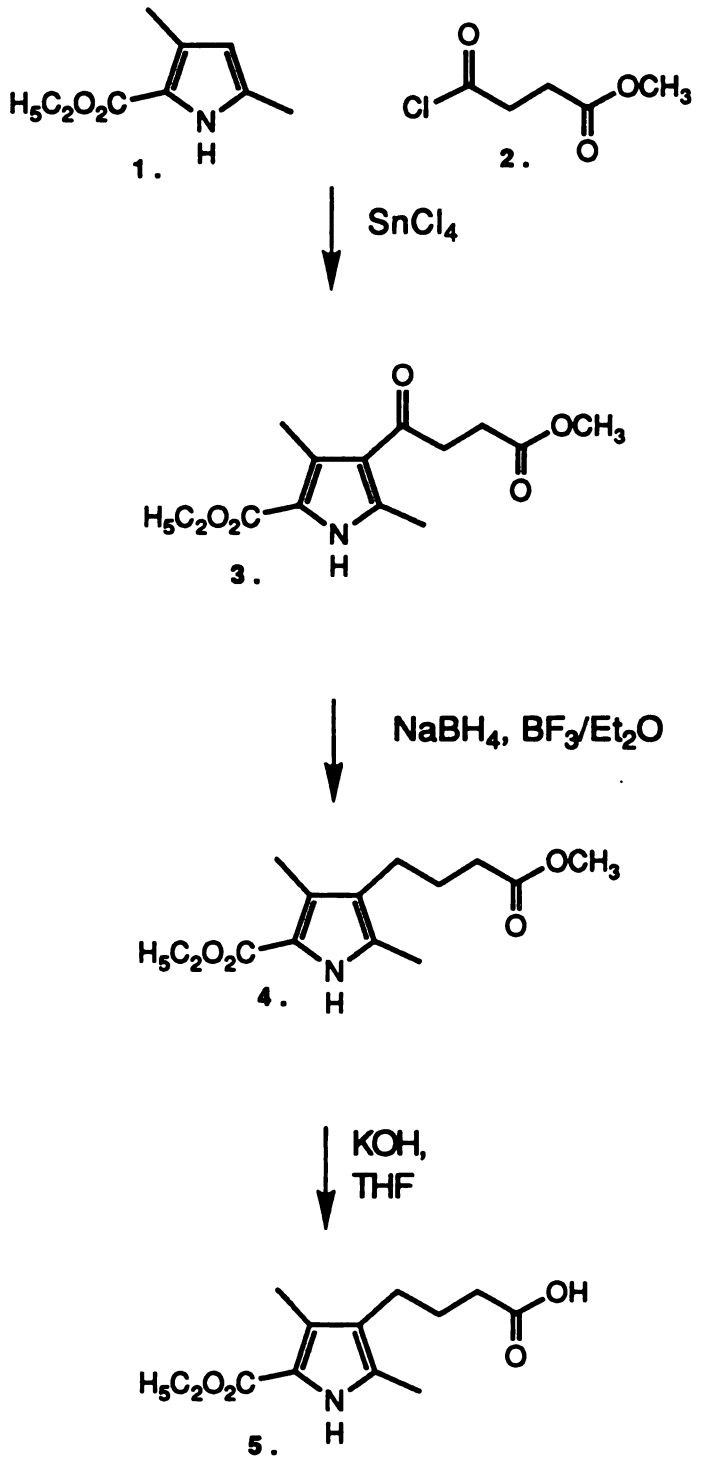


Figure 4. Synthesis of tetramethylenebispyrrole

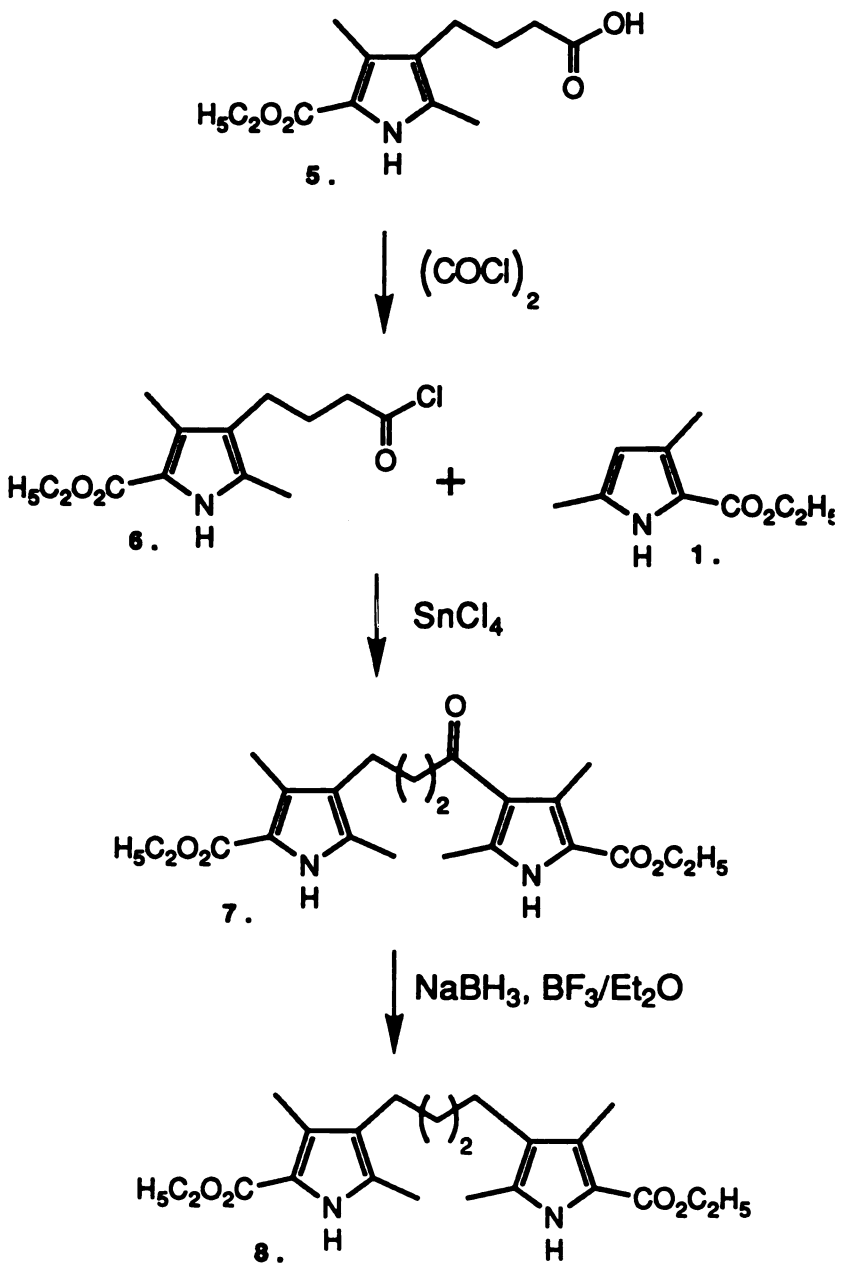


Figure 5. Synthesis of tetramethylenebis(dipyrromethene).

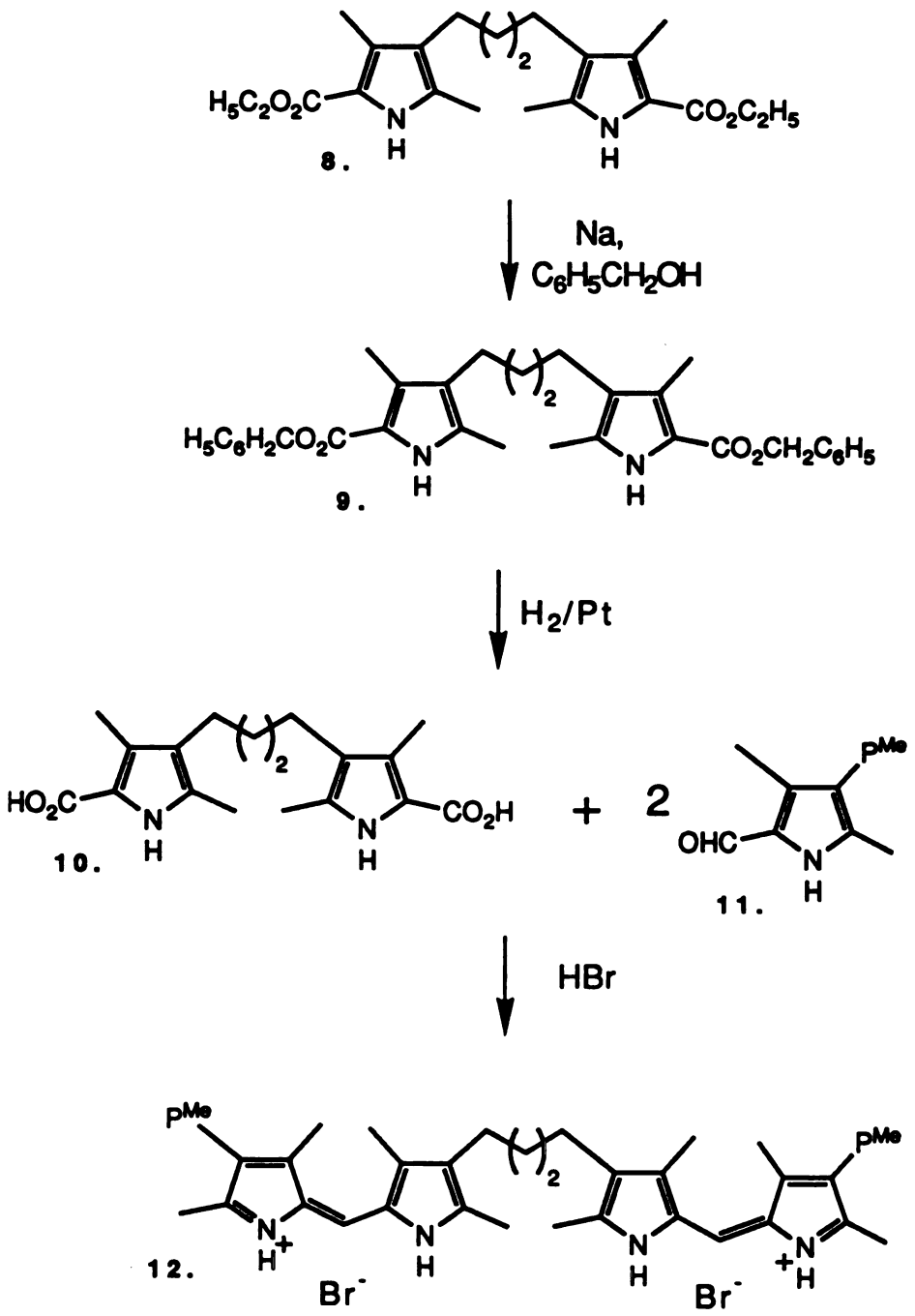
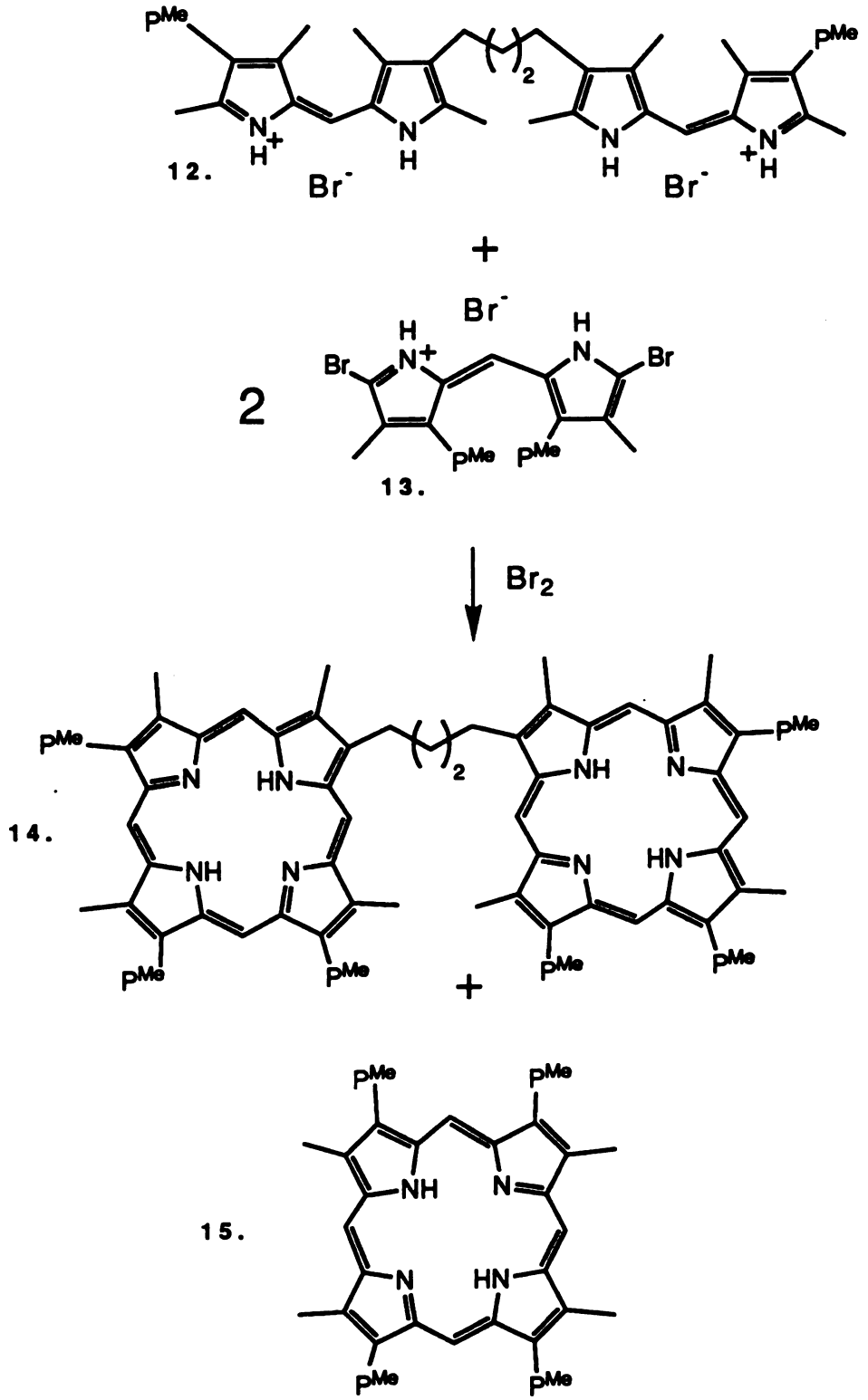


Figure 6. Synthesis of tetramethylenebisporphyrin



The benzyl esters **9** were then converted to carboxylic acids by the action of palladium on carbon catalyst and hydrogen. The dicarboxylic acid bispyrrole **10** was reacted with two equivalents of 3,5-dimethyl-2-formyl-4-(2-methoxycarbonyl)-ethylpyrrole **11**, in the presence of HBr, to form the bisdipyrromethene **12**. Two equivalents of 3,3'-bis-(2-methoxycarbonylethyl)-4,4'-dimethyl-5,5'-dibromodipyrromethene **13** provided the southern halves of the porphyrin dimer and were condensed with the bisdipyrromethenes in formic acid with bromine to give the dimeric porphyrin **14**.

Purification of the product on TLC revealed a small reddish brown band followed closely by a larger reddish band that proved to be the dimeric porphyrin. The faster moving band was shown to be the symmetric porphyrin monomer resulting from the combination of two units of the dibromopyrromethene, coproporphyrin II **15**.

In order to investigate the extent, if any, of ring-ring interactions in the dimer, the UV/visible absorbance spectrum, 700nm - 300nm, of the bisporphyrin product was taken in neutral methylene chloride Figure 6 and compared to that taken in methylene chloride containing trifluoroacetic acid Figure 7. Paine and co-workers (1978) report an investigation of this phenomenon in a family of porphyrin dimers linked with methylene bridges. Their work suggests that for the diprotonated forms of these dimers, ring-ring interactions are revealed by splitting of the soret band. They investigated dimers connected by chains of zero, one, and eight methylene units and found this splitting in the first two but not in the eight carbon dimer. The UV/vis absorbance spectra of the

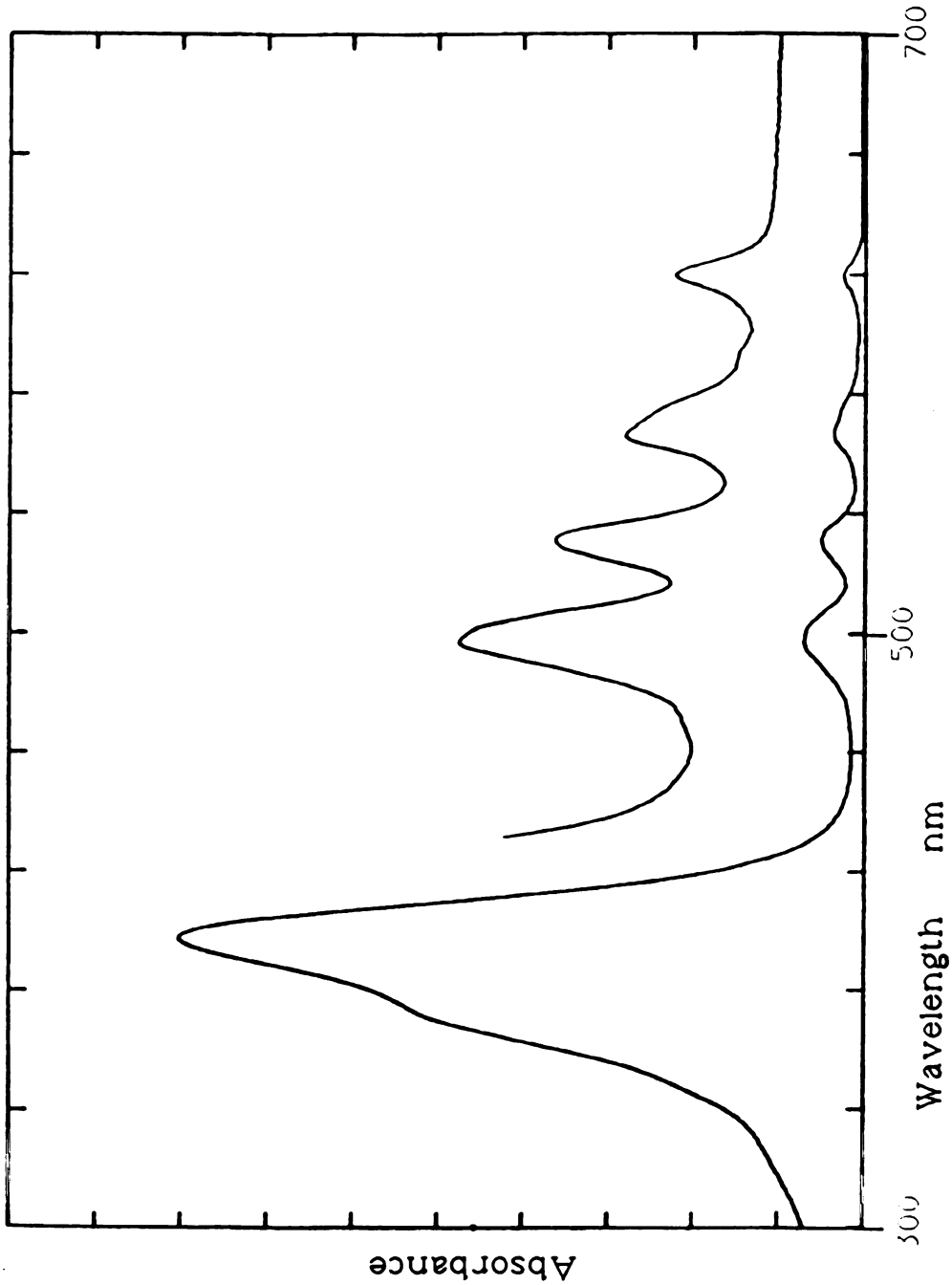


Figure 7. Visible absorbtion spectra, in neutral methylene chloride, of the tetramethylenebisporphyrin 14.

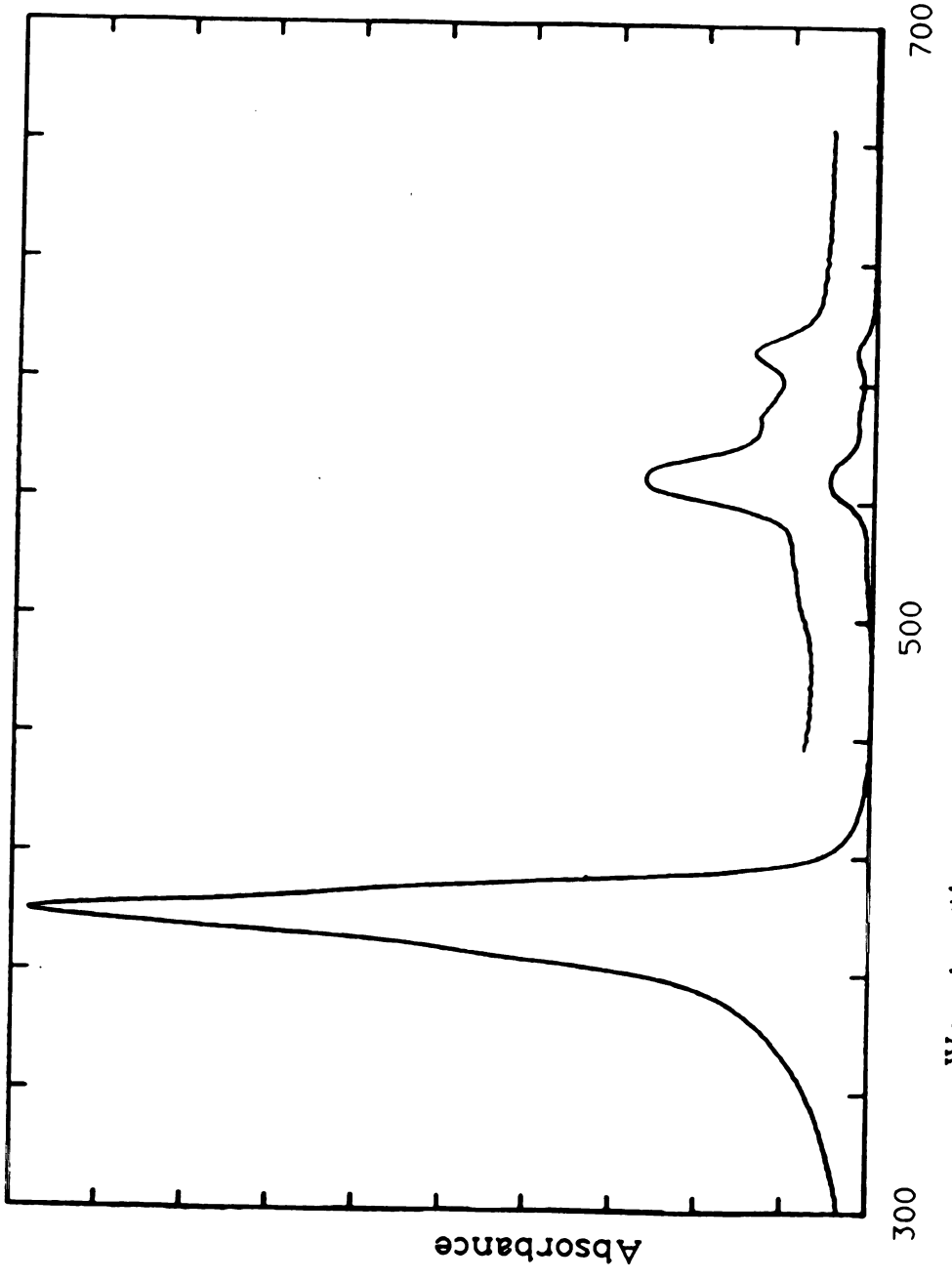


Figure 8. Visible absorbtion spectra, in methylene chloride with trifluoroacetic acid, of the tetramethylenebisporphyrin 14.

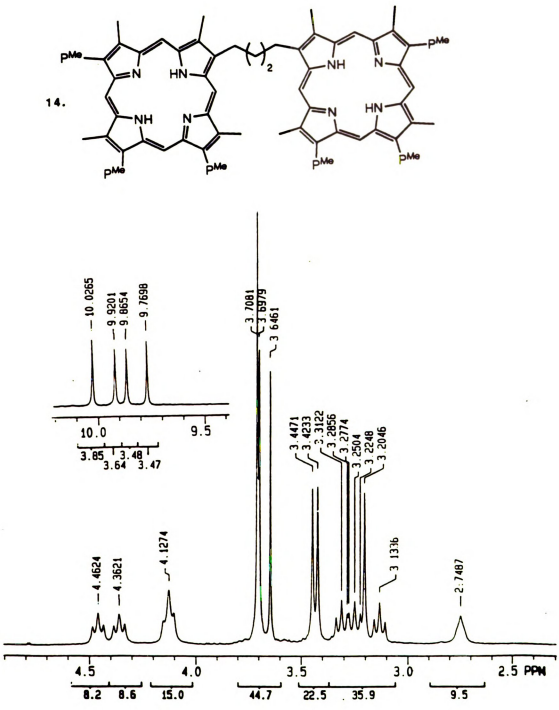


Figure 9. 300 MHz 'H NMR spectra of tetra methylenebisporphyrin **14**, in $CDCL_3$.

tetramethylene bisporphyrin investigated here shows no splitting of the soret band in the diprotonated form, indicating that there are no ring-ring interactions under the conditions used.

The effects of conformation on the efficacy of porphyrin dimers for PDT is not clearly understood, although a role in the uptake and retention of these compounds by tumor cells can be imagined.

The synthesis of the tetramethylene bisporphyrin described here was accomplished in an effort to fill out the sequence of methylene linked porphyrin dimers which are being used to unravel the mysteries of tumor localization and photodynamic activity. Once the mechanisms of these processes are better understood, investigators will be able to take a more informed approach to the design of compounds for PDT. It is hoped that this process will lead to the creation of powerful therapeutic modalities for the treatment of cancer.

Experimental

General

Proton nuclear magnetic reconance spectra were obtained on a Gemini 300 or VXR 500 in either deuterated chloroform with tetramethylsilane as the internal standard, set at 0.00 ppm., or in acetone-d_6, with the 2.04 ppm peak as the internal standard. UV-visible spectra were taken with a Varian Cary 219. Melting points were measured with an electrothermal melting point apparatus and are uncorrected. Preparative thin layer chromotography plates from Analtech were used (silicagel GF, 1000 or 1500 um.)

2-Ethoxycarbonyl-3,5-dimethyl-4-(1-oxo-3-methoxycarbonyl)propylpyrrole. 3

2-Ethoxycarbonyl-3,5-dimethylpyrrole **1** (16.7 g., 0.1 mol.) and methylchloro-1-oxobutanoate **2** (15.0 g., 0.1 mol.) were stirred in dry methylene chloride (200 ml.) and cooled with an ice bath while a stannous chloride solution (0.13 mol.) in methylene chloride (20 ml.) was added dropwise. When the addition was complete the solution was allowed to warm to room temperature and was stirred overnight. After 15 hours the flask contained a gummy brown solid; water (500 ml.) and methylene chloride (100 ml.) were stirred with

the reaction mixture. The solid completely dissolved, the organic layer was separated and dried with magnesium sulfate, and the solvent was removed under reduced pressure. The resulting solid was recrystallized from methanol and water. Yield 24.0 g. (96%), m.p. 119-123°C; NMR ($CDCL_3$) δ; 1.35 (t. 3H), 2.55 (s. 3H), 2.60 (s. 3H), 2.70 (t. 2H), 3.05 (t. 2H), 3.70 (s. 3H), 4.35 (q. 2H); mass spectrum, m/e (rel. inten.) 148 (92), 194 (100), 282 (75, molecular ion).

2-Ethoxycarbonyl-3,5-dimethyl-4-(3-methoxycarbonyl)propylpyrrole 4

2-Ethoxy-3,5-dimethyl-4-(1-oxo-3-methoxycarbonylpropyl)pyrrole (0.071 mol., 20 g.) was dissolved in tetrahydrofuran (100 ml.) and ethyl acetate (67 ml.) under nitrogen and cooled with an ice bath. Sodium borohydride (3.92 g.) was added followed by the dropwise addition of boron trifluoride etherate (19 ml.) over 15 mins. The reaction was quenched by the dropwise addition of water (50 ml.) the resulting solution was filtered, and the organic solvents were removed under reduced pressure. The resulting solid was collected by filtration and recrystallized from ethanol and water. Yeild 18.22 g. (96%), m.p. 90-91°C; NMR ($CDCL_3$) δ 1.40 (t. 3H), 1.80 (m. 2H), 2.25 (s. 3H), 2.30 (s. 3H), 2.40 (b.m. 4H), 3.65 (s. 3H), 4.30 (q. 2H); mass spectrum, m/e (rel. inten.) 134 (68), 222 (90), 267 (100), 268 (100 molecular ion).

2-Ethoxycarbonyl-3,5-dimethyl-4-(3-hydroxycarbonyl)propylpyrrole 5

2-Ethoxy-3,5-dimethyl-4-(3-methoxycarbonylpropyl)pyrrole (10 g., 0.037 mol.) was stirred in tetrahydrofuran (300 ml.) to which potasium hydroxide (2.2 g.) in water (30 ml.) was added. The mixture was stirred at room temperature for 48 hours, nuetralized with acetic acid and the organic solvent removed under reduced pressure. The solid was collected by filtration and recrystallized from methanol and water. Yield 7.5 g. (80%), m.p. 146-150°C NMR ($CDCL_3$) δ 1.40 (t. 3H), 1.80 (m. 2H), 2.20 (s. 3H), 2.30 (s. 3H), 2.4 (m. 4H), 4.30 (q. 2H); mass spectrum, m/e (rel. inten.) 180 (90), 219 (60), 253 (100 molecular ion).

1,4-Bis(2-ethoxycarbonyl-3,5-dimethyl-4-pyrrolyl)-1-butanone 7

2-Ethoxy-3,5-dimethyl-4-(3-hydroxycarbonyl)propylpyrrole 5 (2g., 0.0079 mol.) was slurried in dry methylene chloride (50 ml.) under nitrogen atmosphere and oxalyl chloride (0.8 ml.) was added all at once. The solution was heated to reflux, effervescence not due to the solvent boiling was observed and the solid dissolved completely. The solution was refluxed for one hour, then evaporated under reduced pressure to give a dark oil which solidified upon standing. This solid was redissolved in dry methylene chloride (50 ml.), returned to a nitrogen atmosphere and 2-ethoxycarbonyl-3,5-dimethylpyrrole 1 (1.32 g., 0.0079 mol.) was added. This solution

was cooled on an ice bath while stannous chloride (1.2 ml.) in dry methylene chloride (12 ml.) was added dropwise. When addition was complete the solution is allowed to warm to room temperature and is stirred for a further 3 hours. Addition of water (100 ml.) then caused the formation of a pinkish-white precipitate which was collected by filtration and washed with water. Yield 2.77 g. (87.2 %); m.p. 203-204°C; NMR (acetone-d_6) δ 1.26 (t. 3H), 1.28 (t. 3H), 1.56 (p. 4H), 2.28 (s. 3H), 2.32 (s. 3H), 2.42 (t. 2H), 2.47 (s. 3H), 2.52 (s. 3H), 2.72 (t. 2H), 4.18 (q. 2H), 4.23 (q. 2H); mass spectrum, m/e (rel. reten.) 236 (55), 357 (100), 403 (99 molecular ion).

4,4'-Tetramethylenebis(2-ethoxycarbonyl-3,5-dimethylpyrrole) **8**

1,4-Bis(2-ethoxycarbonyl-3,5-dimethyl-4-pyrrolyl)-1-butanone **7** (1 g., 0.00248 mol.) was dissolved dry tetrahydrofuran (20 ml.), under nitrogen, and sodium borohydride (0.475 g.) was added. This solution was cooled in an ice bath while borontrifluoride etherate (2.3 ml.) in dry tetrahydrofuran (5 ml.) was added dropwise. After addition was complete, the mixture was allowed to warm to room temperature and was stirred for a further 3 hours. Methanol was added cautiously until the effervescence subsided, at which point the mixture was diluted with four volumes of water. The solid was collected by filtration, dried and hot filtered in chloroform with decolorizing charcoal and celite. Yield 0.67 g. (70%); m.p 215-216°C; NMR (acetone-d_6) 1.30 (t. 6H), 1.50 (br. m. 4H), 2.15 (s. 6H),

2.20 (s. 6H), 2.45 (t. 4H), 4.20 (q. 4H); mass spectrum m/e (rel. inten.) 343 (80), 371 (10), 388 (80 molecular ion).

4,4'-tetramethylenebis(2-benzyloxycarbonyl-3,5-dimethylpyrrole) **9**

4,4'-Tetramethylenebis(2-ethoxycarbonyl-3,5-dimethylpyrrole) **8** (1 g., 0.00257 mol.) was dissolved in benzyl alcohol (25 ml.) and heated to 200°C, under nitrogen. Sodium metal (0.3 g.) was added in small fragments while maintaining a good flow of nitrogen. When the addition was complete, the reaction mixture was allowed to cool and poured into methanol (40 ml.) containing acetic acid (3.5 ml.). Water (70 ml.) was added and after stirring for one hour, the precipitate was collected, dried and purified on a silica gel column by elution with a mixture of methylene chloride, hexane, and ether (1:1:1). Yield 0.98 g. (75%); m.p. 195-200°C; NMR (acetone-d_6) δ 2.14 (s. 6H), 2.30 (s. 6H), 2.34 (t. 4H), 5.30 (s. 4H), 7.35 (m. 10H); mass spectrum m/e (rel. Inten.) 91 (100), 135 (90), 306 (30), 405 (23), 511 (20 molecular ion).

4,4'-Tetramethylenebis[3,3',5,5'-tetramethyl-4-(2-methoxycarbonylethyl)dipyrrylmethene]dihydrobromide **12**

4,4'- Tetramethyenebis(2-benzyloxycarbonyl-3,5-dimethylpyrrole) **9** (.4 g., 0.78 mmol.) was dissolved in dry tetrahydrofuran (10 ml.) with 10% palladium on carbon powder (.04

g.) and 2 drops of triethylamine. This mixture was stirred under hydrogen (1 atm, room temperature) overnight. Then filtered through a layer of celite and followed by tetrahydrofuran (10 ml.). The filtrate was evaporated under reduced pressure at room temperature and the solid was allowed to dry. Methanol (10 ml.) was added followed by 2-formyl-3,5-dimethyl-4-(2-methoxycarbonyl)ethylpyrrole **11** (0.17 g., 0.8 mmol.). This solution was brought to reflux and hydrobromic acid (48%, 1 ml.) was added. An orange precipitate formed immediately. The mixture was refluxed for a further 1/2 hour and then allowed to sit open tq the air for 3-4 hours at which point the product was collected by suction filtration. Yield 0.43 g. (70 %); NMR ($CDCL_3$) δ 1.45 (m. 4H), 2.25 (s. 3H), 2.30 (s. 3h), 2.45 (m. 4H), 2.65 (s. 3H), 2.68 (s. 3H), 2.74 (t. 2H), 7.1 (s. 2H).

<u>13,13'-Tetramethylenebis[3,8,12,17-tetramethyl-2,7,18-tri(2-methoxycarbonylethyl)porphine]</u>**14**

4,4'-Tetramethylenebis[3,3',5,5'-tetramethyl-4-(2-methoxycarbonylethyl)pyrrylmethene]dihydrobromide **12** (211 mg., 2.268 mmol.) was suspended in formic acid (8 ml., 98-100%) with dimethyl-5,5'-dibromo-4,4'-dimethylpyrromethene-3,3'-dipropionate hydrobromide **13** (312 mg., 0.535 mmol.) and the solution was brought to reflux. Bromine (2 drops) was added and the evolution of HBr fumes were observed. The mixture was allowed to

reflux for 3 hours; the condenser was then removed, and the mixture was allowed to boil to dryness. At the final stages of drying, a gentle stream of compressed air was used to bring the mixture to total dryness. A black metallic looking, crispy solid resulted, to which was added methanol (10 ml.) and sulfuric acid (2 drops), followed by triethyl orthoformate (2 drops). After standing overnight the solution was neutralized with saturated aqueous sodium acetate. Methylene chloride (50 ml.) was added and the whole mixture was filtered through glass wool. The organic phase was separated, washed with water, and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure and the crude product was partially purified on a silica gel column, using a 1% solution of methanol and methylene chloride to remove a dark-fast moving, non-fluorescent fraction. Increasing the solvent strength to 5% methanol, eluted a fluorescent fraction, which was further purified on preparitive TLC plates, developed with a 2% methanol/methylene chloride solution. This allowed the separation of a small faster-moving reddish band composed of coproporphyrin II **15**, and a larger, slower-moving reddish band containing the dimeric porphyrin product **14**. Yield 37 mg. (10.6 %); NMR ($CDCL_3$) 2.75 (br. s. 4H), 3.13 (t. 4H), 3.25 (br. m. 16H), 3.64 (s. 6H), 3.69 (s. 6H), 3.71 (s. 12H), 4.12 (t. 8H), 4.36 (t. 4H), 4.46 (t. 4H), 9.77 (s. 2H), 9.86 (s. 2H), 9.92 (s. 2H), 10.02 (s. 2H); mass spectrum m/e (rel. inten.), 307 (100), 711 (80), 625 (30), 1304 (95 molecular ion).

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