This is to certify that the

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REGIOSPECIFIC SYNTHESIS OF VINYL

DIHYDROXYCHLORIN: MODEL COMPOUNDS FOR HEME d

presented by

EMMANUELLE GUERIN

has been accepted towards fulfillment
of the requirements for

Master degree in Chemistry

Chris K. Chang
Major professor

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REGIOSPECIFIC SYNTHESIS OF VINYL DIHYDROXYCHLORIN: MODEL COMPOUNDS FOR HEME d

By

Emmanuelle Guerin

A THESIS

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ABSTRACT

REGIOSPECIFIC SYNTHESIS OF VINYL DIHYDROXYCHLORIN: MODEL COMPOUNDS FOR HEME d

By

Emmanuelle Guerin

The structure of green-colored heme d prosthetic group of cytochrome bd oxidase originally deduced from spectral characterization was believed to be a vinyl dihydroxychlorin containing a γ-lactone ring derived from one hydroxy group and a geminal propionate side chain. Subsequent studies indicated that the lactone is not the true form of the heme d and probably arises as a consequence of the isolation procedures of the demetallated prosthetic group. Although the free base dihydroxychlorin of the heme d in the form of dimethyl ester can be obtained by synthesis, the iron complex of this chlorin has not be available synthetically because the facile lactonization occured during iron inertion. To satisfy the need for heme d as well as its model compounds in spectroscopic characterizations, a number of dihydroxychlorins were synthesized containing vinyl side chains but without a *gem*-propionic acid group in order to avoid the γ-lactone formation. The synthesis of two chlorins, 3-ethoxycarbonyl-13,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-12,13-dihydroxychlorin and 13,17-diethyl-2,7,12,18-tetramethyl-3,8-divinyl-12,13-dihydroxychlorin, using a selective formation of the diol is described in this thesis.

To my parents

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INTRODUCTION: FROM PORPHYRIN TO HEME d

I. STRUCTURE OF PORPHYRINS AND CHLORINS

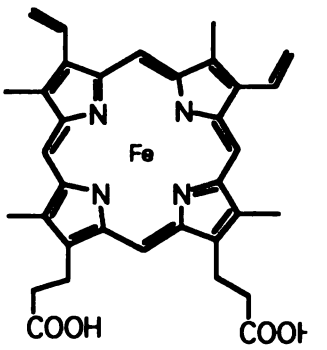
In nature, most heme-containing proteins and enzymes possess a protoheme prosthetic group made from an iron atom metal coordinated within a fully porphyrin macrocycle. Porphyrins[1] are macrocyclic structures which contain four pyrroles units connected together by four methene bridges. These planar aromatic structures are 18 Π–electron systems. They show an intense absorption, known as Soret band around 400 nm and four Q bands in the visible region between 490-640 nm. The intensity of these smaller bands varies with the nature of the substituents on the porphyrin ring. The porphyrin metal-complexes also exibit the Soret band but only two Q bands known as α and β bands.

Best known examples of hemoproteins include hemoglobin that play a vital role in transporting oxygen and myoglobin which is responsible for the storage of oxygen in muscle tissue. Other examples are cytochromes a,b and c that are responsible for the electron transfer in animals, plants and microorganisms. The prosthetic group present in cytochrome aa3 is different from the normal proteheme (figure1).

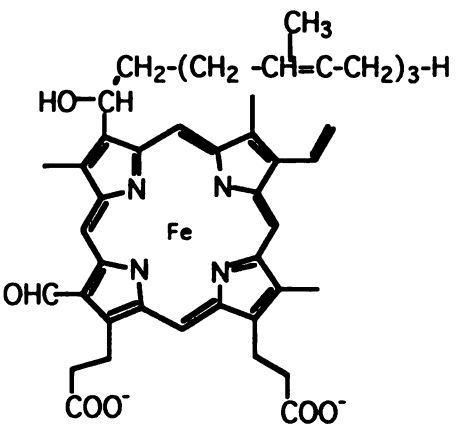
Porphyrins and their metal complexes can be reduce to give diverse isolable products[2]. They are dihydroporphyrins (chlorin) and tetrahydroporphyrins (bacteriochorins, isobacteriochlorins), all of them

resulting from the saturation of peripheral double bonds of the porphyrin ring (figure 2).

Unlike porphyrins, chlorins usually display a stong absorption for the most red-shifted Q band around 630 nm. A strong α band of the metal complexe is also observed.



Protoheme



Heme a

Figure 1: Structure of Protoheme and Heme a

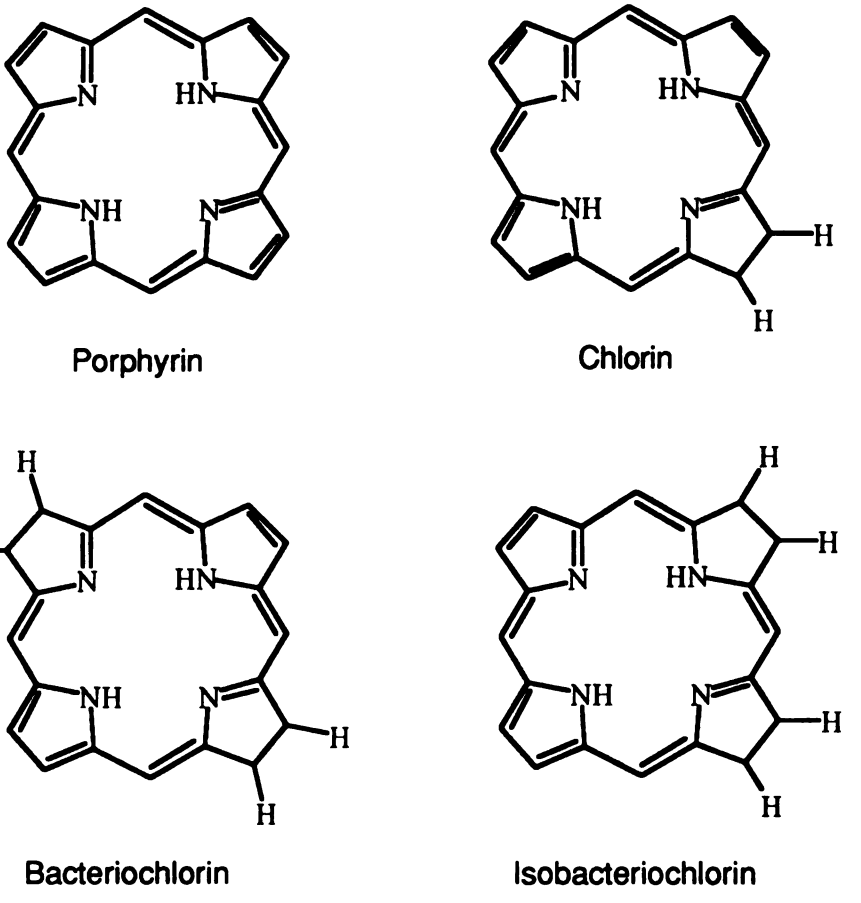


Figure 2: Structure of porphyrin and unsaturated porphyrin

The biological interest of these reduced porphyrins has centered mostly on the magnesium complexes of chlorins and bacteriochlorins that are the essential chromophoric units of algae and plant chlorophylls **1** and bacteriochlorophylls **2**.

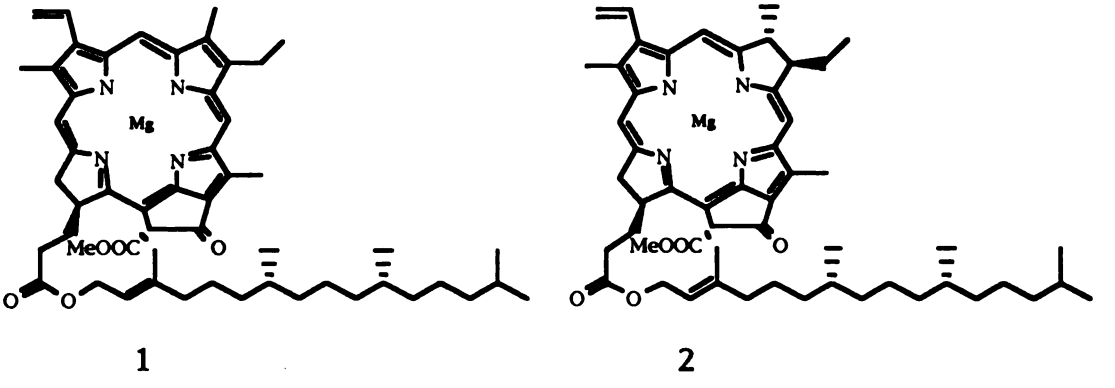
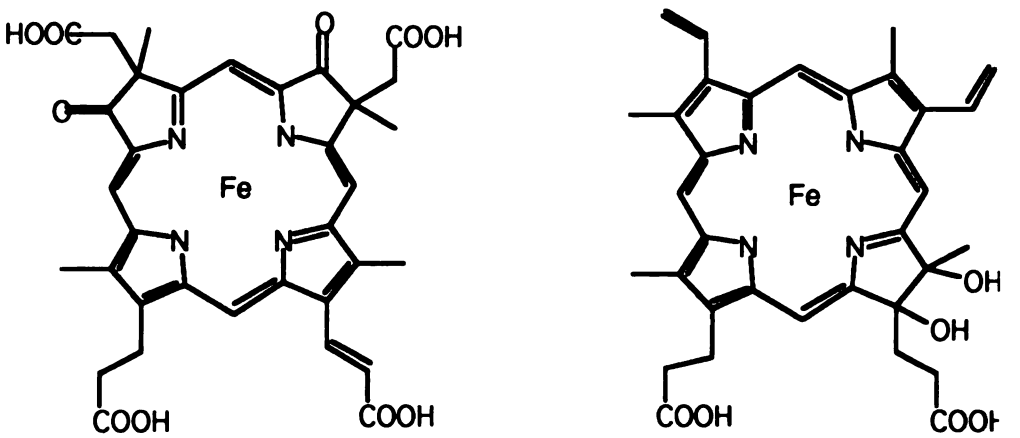


Figure 3: Structure of chlorophyll and bacteriochlorophyll

But more recently , it has been shown that a significant number of organisms contain C-substituted chlorins and isobacteriochlorins. Among these are Factor I from the B-12-producing *Clostridium tetanomorphum,*[4] heme d_1 from *Pseudomonas aeruginosa* [5]which is involved in a process known as denitrification[6] by reducing nitrite to nitrous oxide and heme d of *Escherichia coli*[7] which catalyzes the reduction of O_2 to H_2O.



heme d_1 3

Heme d 4

Figure 4: Structure of Heme d_1 and Heme d

The main difference between the substituted chlorins or bacteriochlorins and the unsubstituted chlorin is that the C or OH-substituted compounds can resist dehydrogenation. Therefore they are better suited for undertaking the redox processes with which they may be associated in vivo.

With the elucidation of the structure of these unique molecules, it has become timely to investigate their chemistry. Detailed accounts of heme d_1[5] and heme d[7,8] have been reported but an incertitude remains on the real structure of heme d[8].

II. PREVIOUS STUDY ON HEME d

In 1928 a green pigment was observed in cells of *Escherichia coli* and of *Shigella dysenteriae* grown aerobically. This appeared to be an unusual cytochrome with a pronounced red shift of the α band of the visible spectrum to the 630 nm region[9].

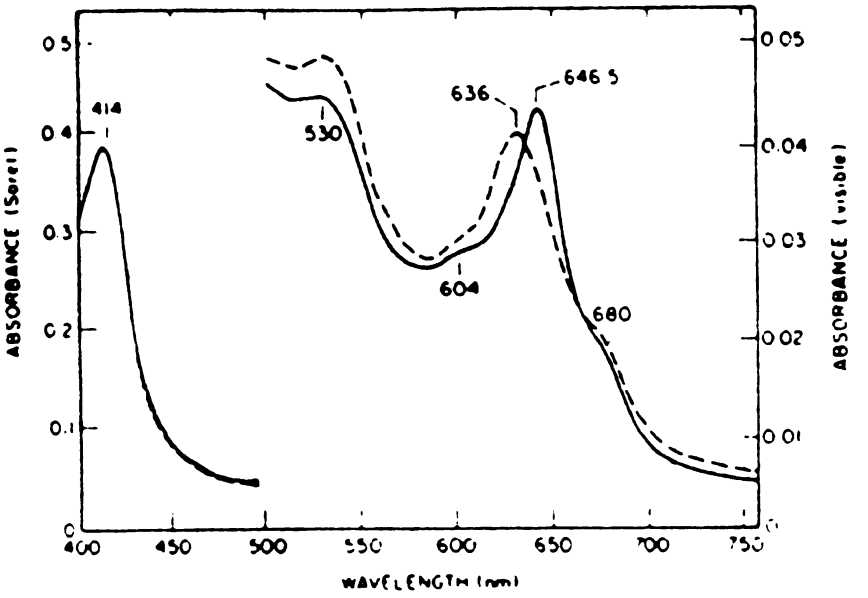


Figure 5: absorption spectra at 25°C of the air-oxidized form of the cytochrome d complex

Further work on the respiratory electron transport chain of certain bacteria confirmed the existence of this cytochrome which was originally called cytochrome a_2[10]. Since about 1970, this cytochrome and its prosthetic group heme have been termed respectively cytochrome d and heme d 4 to avoid confusion with cytochrome aa_3. This heme has also been detected in diverse bacteria including *Azotobacter, Proeus, Salmonella bacillus, Pseudomonas* and *Haemophilus.*

By in vivo experiments as well as isolation and characterization of purified enzymes, the function of cytochrome d was established. The aerobic respiratory chain of *Escherichia coli* like many other bacteria is branched[11].

Under conditions of high aeration, a respiratory chain containing only b-type cytochromes is predominant; the heme prosthetic group is referred to as cytochrome o.

When *Escherichia coli* is grown under conditions of limited oxygen, three new cytochromes are induced; cytochromes b_{558}, a_1 and d[9,12,13]. The K_m value for oxygen of cytochrome d is about eight times lower than that of cytochrome o[11](Figure 6). The more efficient utilization of oxygen by cytochrome d presumably allows the microbes to maintain efficient oxidative energy conservation over a wide range of oxygen pressures by changing the relative ratio of the two oxidases.

Dioxygen Reduction Catalyzed by Heme Enzymes of Bacteria Escherichia Coli

$$O_2 + 4\,e^- + 4\,H^+ \longrightarrow 2\,H_2O$$

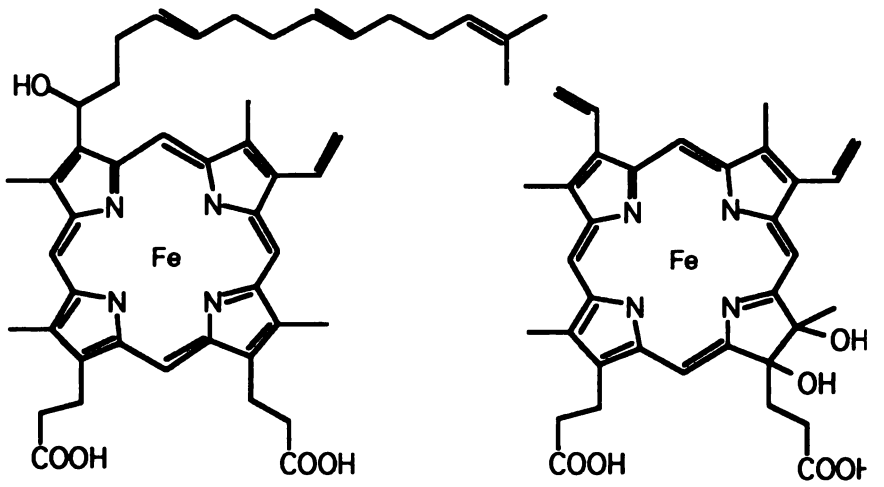


Figure 6: Structure of Heme o and Heme d, reponsible for the reduction of oxygen to water in cytochrome d.

The propose branched arrangement of the cytochromes in the respiratory chain of the cells in the late exponential phase of aerobic growth is shown in figure 7.

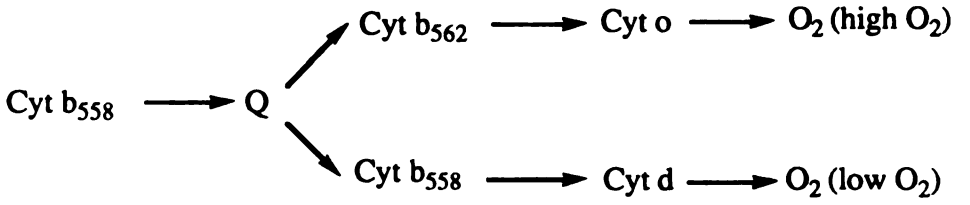
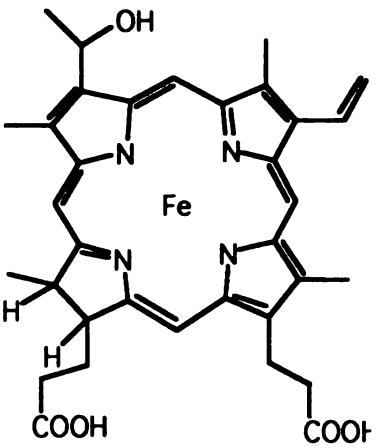


Figure 7: Arrangement of cytochromes in the respiratory chain of cells of Escherichia Coli in the late exponential phase of aerobic growth; Q; ubiquinone

In 1956 Barrett[14] proposed a structure for heme d as the iron chelate of 7,8-dihydroprotoporphyrin IX **5**. The site of saturation could not be determine at that time.

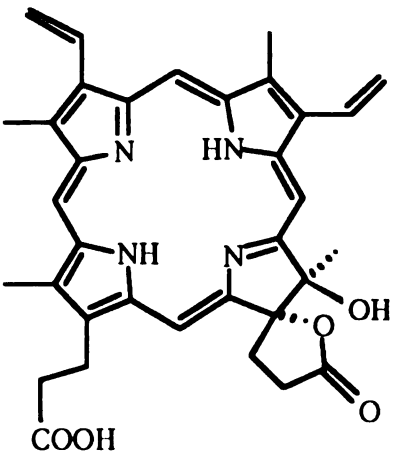


5

This proposal was based upon derivation chemistry as well as comparisons of chromatographic behavior and visible spectra with models. The main souce of material was cells of *Aerobacter aerogenes.*

The tentatively formulated structure of Barrett remains unchallenged in the literature for almost 30 years and has served as model for many other green hemes found in varions bacterial cytochromes.

In 1985, Timkovitch and collaborators[7] isolated sufficient amounts of the heme d prosthetic group from purified *Escherichia coli* oxidase and characterized the structure of the metal free, esterified chromophore by means of H NMR, IR, UV-visible, and mass spectrometry. The proposed structure comprises an unusual chlorin core with a spiro-γ-lactone group at the saturated pyrrole ring C 6.



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This "lactochlorin" structure is certainly unique but as the authors noted it is not clear whether the lactone ring found in the metal-free macrocycle is an authentic feature of the heme or whether it is an artifact formed during isolation. The evidence of the lactone in the metal free heme d was largely based on an intense IR absorption at 1782 cm^{-1}.

To verify the heme d structure, and to determine whether the lactone is an artifact or whether it is an authentic feature of the heme d, the Timkovitch's lactochlorin, its diastereomer and the nonlactonized forms have

been synthesized. The results supported that the lactone is not a feature of the heme d but an artifact of the isolated chromophore formed by prolonged contact with silica gel during purification.

Also the absence of IR peak at 1782 cm^{-1} in the extracted but unesterified heme is a strong argument against the lactone in the vivo heme[15]. The true ligand structure of heme d in the enzyme is more likely to be 5,6 dihydroxyprotochlorin IX **4**.

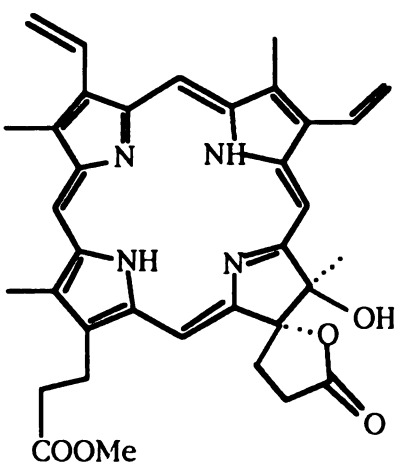
Despite the previous success in the synthesis of the dimethyl ester of 5,6-dihydroxyprotochlorin IX, the iron complex of which was not synthesized because of the facile lactonization occured during a variety of iron insertion procedures. Due to the need for heme d or its model compounds in spectroscopic characterization of the cytochrome d, we decided to synthesize a number of dihydroxychlorins with conjugated vinyl side chains but without the propionic acid group so as to avoid the γ-lactone formation. The synthesis of two chlorins, 3-ethoxycarbonyl-13,17-diethyl-2,7,12,18-tetramethyl-12,13-dihydroxy-8-vinyl-chlorin and 13,17-diethyl-2,7,12,18-tetramethyl-12,13-dihydroxy-8,13-vinyl-chlorin, using a selective formation of the diol is described in this thesis.

CHAPTER 2

RESULTS AND DISCUSSION: SYNTHESIS OF A MODEL FOR HEME d

I. BACKGROUND

As we said in the introduction, if a diol chlorin bears a geminal propionate ester it seems that it leads to the formation of a lactone **7** during the purification on t.l.c. plates or with the presence of a base[16].



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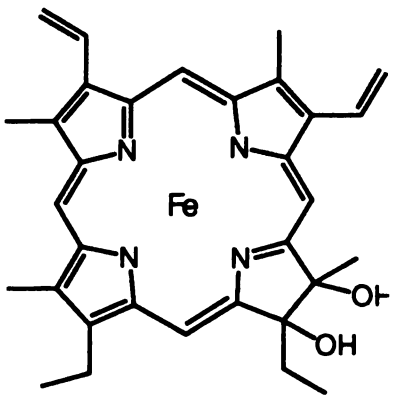
To avoid the formation of this lactone and then be able to ensure that lactone is only an artifact and not a feature of nascent heme d, the object of this work is the synthesis of a possible model having ethyl groups instead of propionic ester groups (structure **8**).



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In general, dihydroxylation of the porphyrin can be accomplished by osmium tetraoxide addition to double bonds followed by quenching with hydrogen disulfide. However such a reaction on porphyrin would lead to a mixture of four different products resulting from the attack of the double bond at any of the four pyrrole ring (see figure 9). This would give a very poor yield of the desired chlorin. Furthermore, the mixture of the chlorins would be very difficult to separate.

Recently K. M. Smith[17] reported that introduction of an electron-withdrawing group directly linked to the macrocycle of the porphyrin may direct selectively the attack of the osmium tetraoxide at the subunit in the quadrant opposite to the electronegative group (figure 8).

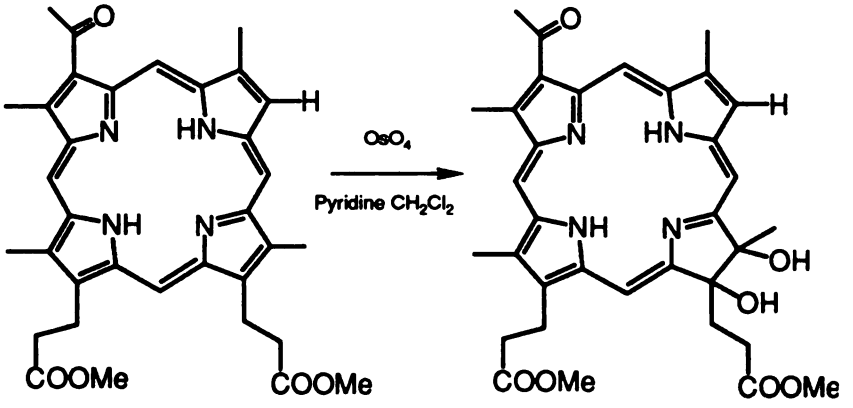


Figure 8: Selective hydroxylation of porphyrin in the quadrant opposite to the electronegative group

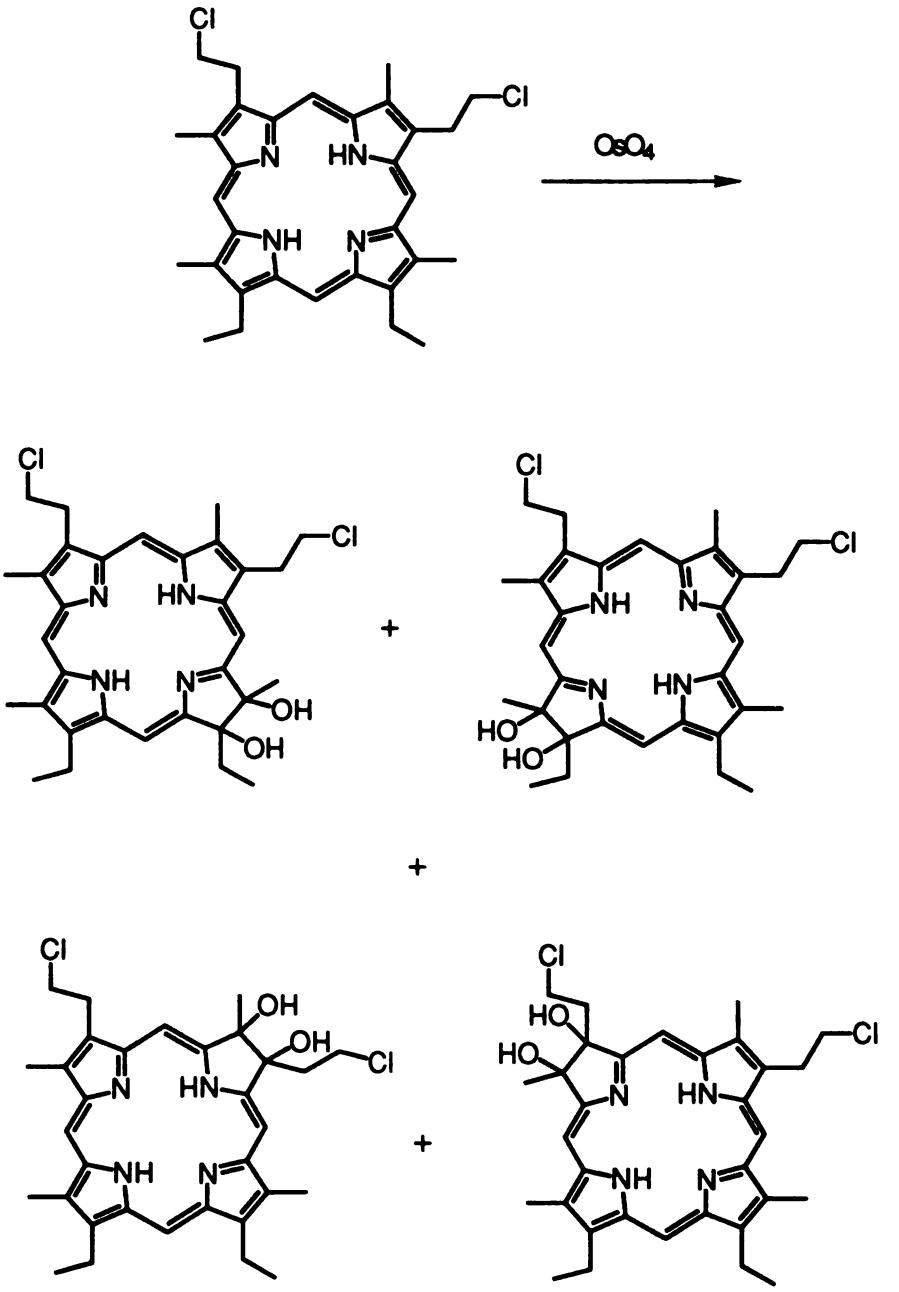
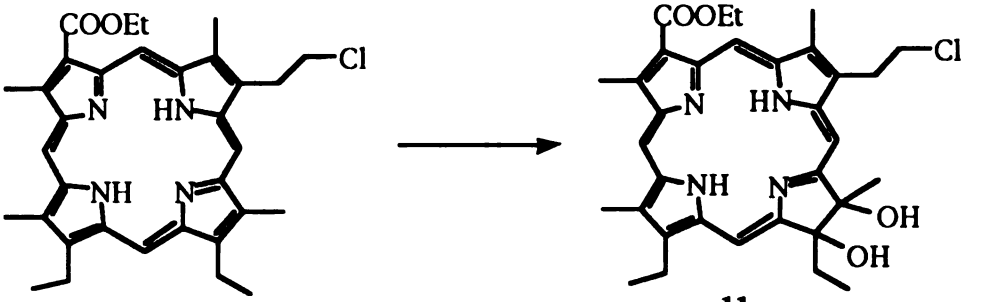
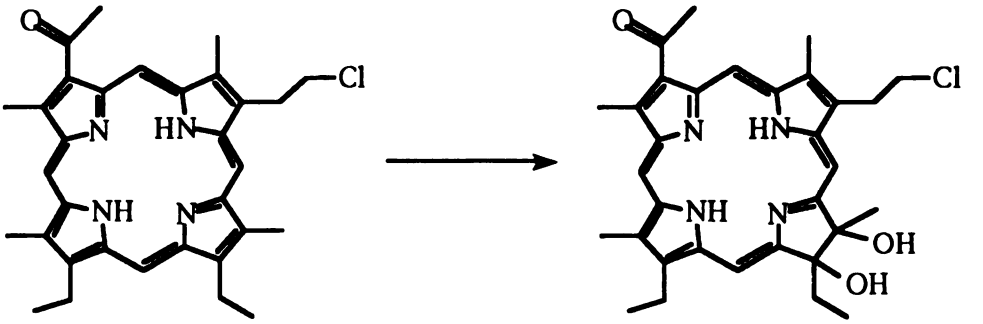


Figure 9: Non-regioselective hydroxylation with the abscence of electron-withdrawing group

Thus we describe first the synthesis of two porphyrins: 3-ethoxycarbonyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethyl-porphyrin **9** and 3-acetyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethyl-porphyrin **10** with a withdrawing group placed at a key point on the macrocycle to lead to the selective formation of the desired chlorins **11** and **13**, respectively.



9 **11**



10 **12**

Initially we did the synthesis of the ester porphyrin **9** to verify the account of K. M. Smith, a synthesis that required less steps than the formation of the acetyl porphyrin. We were hoping to be able to transform the chlorin **11** into our target molecule **8**, which unfortunately proved not to be the case.

II. PREPARATION OF THE ESTER CHLORIN

The synthetic strategy is shown in figure 10 in which the two dipyrrylmethenes, "north" **15** and "south" **17** were refluxing together in a mixture of formic acid and one equivalent of bromine to form the expected ester porphyrin with a limited yield of 6 to 8%[18]. This low yield is most likely due to the presence of a withdrawing group which deactivates the cyclization. This porphyrin was obtained after chromatography on silica gel column. It was the first pink-purple band eluted by dichloromethane.

Dipyrryl methene **17** was best prepared via the decarboxylation of 5,5'-bis(ethoxycarbonyl)-4,4'diethyl-3,3'-dimethyl-2,2'dipyrrylmethane **16** and its bromination with an excess of bromine.

On an other hand the synthesis of 4,3',5,5'-dimethyl-3-ethoxycarbonyl-4'-(2-chloroethyl)-2,2'-dipyrrylmethene **15** required much more step for its synthesis. It was prepared by the condensation of ethyl-2-formyl-4,5-dimethylpyrrole-3-carboxylate **13** and 4-(2-chloroethyl)-3,5-dimethyl pyrrole-2-carboxylic acid **14** in methanol and hydrobromic acid. Therefore we began by the synthesis of these two pyrroles that is summarized in figure 11 and figure 12.

Starting from alanine heated with acetic anhydride in pyridine[19], 3-acetamido-2-butanone **19** was formed and was allowed to be acidified to give 3-ammonium-2-butanone chloride salt **20**. By reaction of this salt with diethyl oxalacetate sodium, the pyrrole 3-ethoxycarbonyl-4,5-dimethylpyrrole-2-carboxylic acid **21** is formed, which after decarboxylation in molten sodium acetate and potassium acetate gave the α free pyrrole **22**. The formyl pyrrole **23** was then obtained by a classic Vielsmeyer reaction.

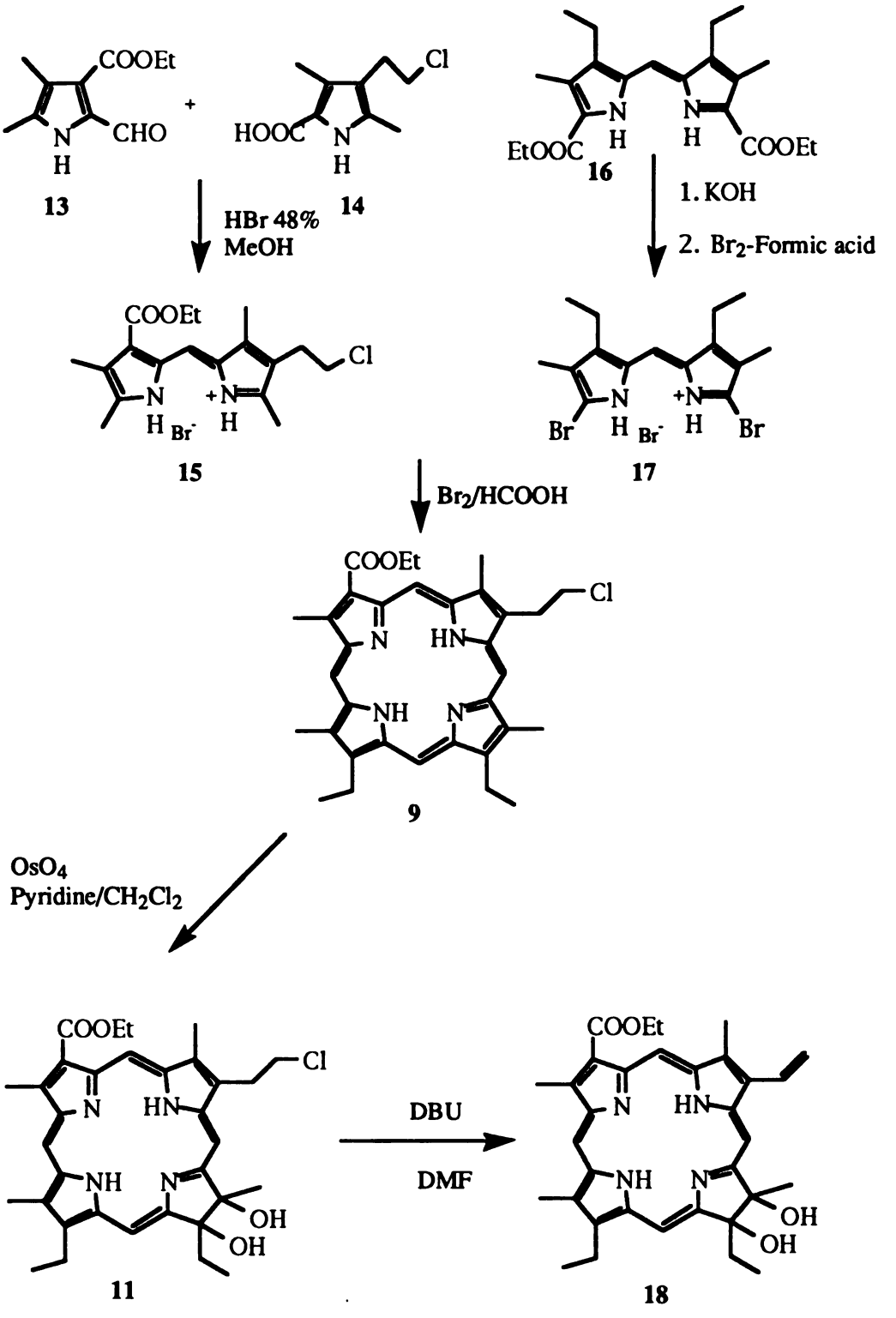


Figure 10: Synthesis of 3-ethoxycarbonyl-13,17-diethyl-2,7,12,18-tetramethyl-12,13-dihydroxy-8-vinylchlorin

The pyrrole **25** was formed in two steps: (1) the reaction of 2,4-pentanedione and ethyl-2-chloro-acetate **23** and (2) the reaction of **23** in the presence of zinc dust with the oxime **24** formed by the reaction of benzyl acetoacetate with sodium nitrite. Diborane was then used to reduce **25** into benzyl-4-(2-hydroxyethyl)-3,5-dimethylpyrrole-2-carboxylate[21]. Substitution of the hydroxy group by chlorine was carried out with thionyl chloride in benzene at reflux. The reaction was run under atmosphere of argon to prevent air oxidation. The benzyl ester group of benzyl-4-(-2-chloroethyl)-3,5-dimethylpyrrole-2-carboxylate **27** was removed by hydrogenolysis accomplished under hydrogen atmosphere with 10% palladium on charcoal catalist. The hydrogenolysis with this pyrrole is very sensitive and failed several times, but the reaction could be carried out to gived **14** with quantitative yield.

Once the porphyrin **9** was isolated and characterized, the oxidation of the porphyrin to prepare the hydroxychlorin could be realized. Reaction of porphyrin **9** with 0.9 equivalent of OsO_4 in dichloromethane followed by H_2S give 60% of one major chlorin product with 15 to 20% of remaining starting porphyrin[8,16,22,23]. Thus we observed the fact that having a withdrawing group to the porphyrin lead to a more regiospecific reaction.

The products were, isolated by column followed by t.l.c. plates. The major product was of purple color, contrary to the green color of other dihydroxychlorin compound without withdrawing group linked to the macrocycle porphyrin. The dihydroxychlorin was identified by UV-visible spectra and NMR. NOE study was carried out to ascertain that the oxidation of the porphyrin takes place at the subunit in the quadrant opposite to the electron-withdrawing group. More details on the NOE study and the determination of the chlorin structure are given in the next chapter.

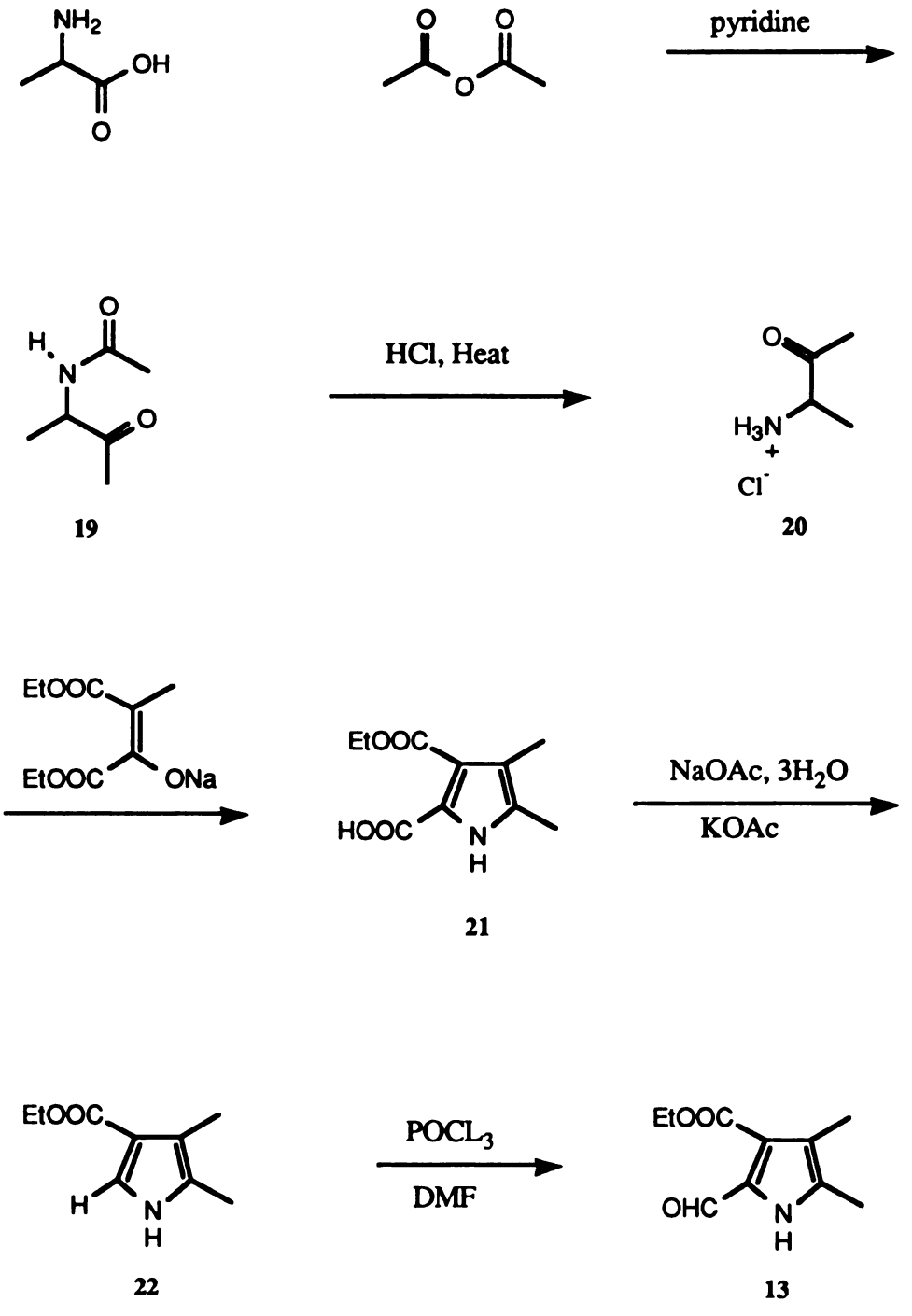


Figure 11: Synthesis of 3-ethoxycarbonyl-2-formyl-4,5-dimethylpyrrole

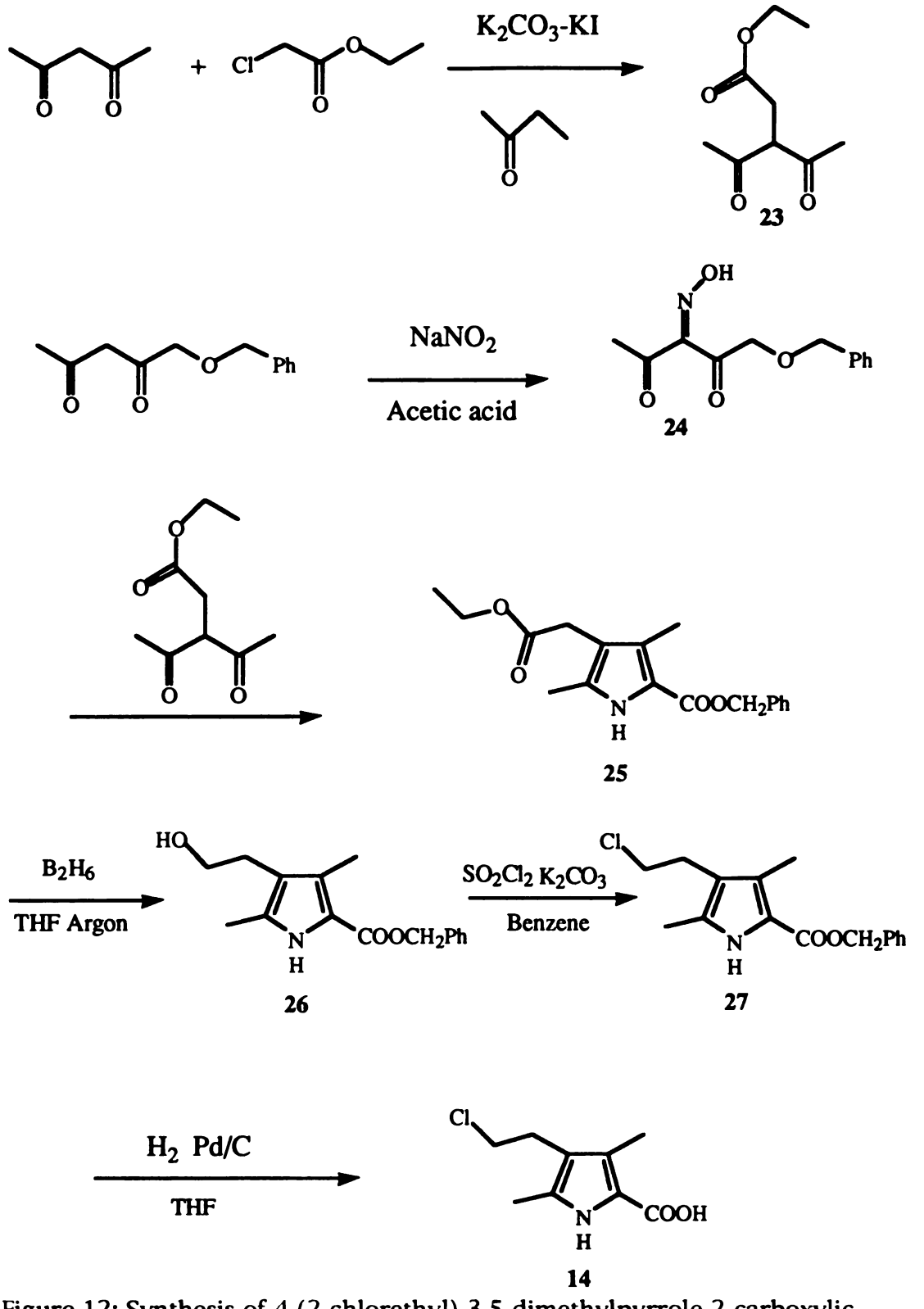


Figure 12: Synthesis of 4-(2-chlorethyl)-3,5-dimethylpyrrole-2-carboxylic acid

The chloroethylchlorin was then converted to the vinylchlorin by elimination of the chlorin with DBU heated in dimethylformamide for two hours. The structure of this compound was confirmed by NMR and mass spectra.

The synthesis of this dihydroxychlorin was a success. The same pathway using an electron-withdrawing group as directing group for the oxidation can then be utilized in the synthesis of a model for heme d.

III. NOE STUDY ON THE ESTER CHLORIN

By "irradiating" the four different meso peaks a, b, c, and d appeared in the region of 9 to 10 ppm we can determine their environment and deduce the structure of the dihydroxychlorin. Thus we can prove that our hydroxy groups occupy the position 12 and 13.(see figure 13 and figure 19 p. 47)

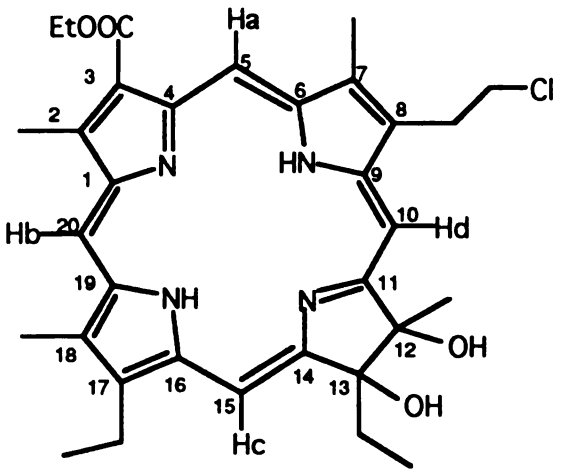


Figure 13: Structure of ester chlorin in NOE study

We can already assume before the measurements that proton a is the most downfield shifted proton due to its location near the electron-withdrawing group. Also the peak corresponding to the methyl group geminal

to the hydroxy group has to be upfield compared to the three other methyl peaks. We expect to find it at 2.24 ppm due to the lost of the conjugaison in this area. Thus by irradiating Ha we can isolated which methyl is in the position 7.

We operated the same way with the meso proton b. This proton enhances two methyl groups. Hb has to be between the two methyl groups of the positions 2 and 18. For the peak c, no real signals could be observed. This suggest that this proton is between the two ethyl groups. Peaks of the ethyl groups are small so their enhancement by NOE is difficult to see due to the strong baseline.

From those data we can deduce that the hydroxy groups have to be in the quadrant opposite to the ester group, since none of the three meso peaks has NOE effect on the upfield shifted methyl group. This is confirmed by the irradiation of the last meso peak d. The 2.24 ppm peak is enhanced like expected.

Thus like predicted by K.M. Smith's article[17] the oxidation took place effectively in the quadrant opposite to the withdrawing group.

IV. PREPARATION OF 13-17-diethyl-2,3,12,18-tetramethyl-12,13-dihydroxy-3,8-divinylchlorin: MODEL FOR HEME d

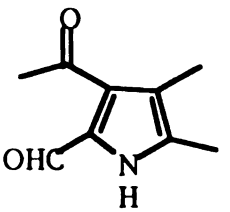
We first thought that we could reduce or decarboxylate the ester porphyrin to obtain a unsubstituted position which can then be acetylated and transformed later into a vinyl group togive the desired chlorin. But unfortunately the carboxylester group proved to be very difficult to remove. The ester group resisted acid hydrolisis as well as alkaline hydrolysis under conditions that did not eliminate the chlorine on the side chain. Heating the ester porphyrin in resorcinol melt gave us only products from substitution of

the chlorine by resorcinol and other non identified side products; the ester group remained intact

We then envisaged the synthesis of a new porphyrin starting from pyrroles, having an acetyl group that can be further reduced to a vinyl group.

The first route attempted to obtain this porphyrin was based on a strategy similar to a recent publication[24] (shown in figure 14). Unfortunatly the dipyrrylmethene, condensation between the acetyl pyrrole and the chloro ethyl pyrrole failed possibly for two reasons: the carboxylic acid group of the chloro compound could not be remove without destruction of the pyrrole and secondly, the electron-withdrawing acetyl pyrrole hindered such a condensation.Several alternative methods were tried unsuccessful.

Another way to get this porphyrin was to follow the same pathway as in the ester porphyrin **9**, using an acetyl pyrrole instead of the starting ethoxycarbonyl pyrrole **13**.



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But unlike the formylation of the pyrrole **22**, 3-acetyl-2-formyl-4,5-dimethyl pyrrole **28** could not be obtained by a Vielsmeyer reaction on 3-acetyl-4,5-dimethyl pyrrole. We were forced then to prepare the acetylporphyrin in a stepwise manner. The strategy is shown in figure15 and 16.

As we did for the preparation of the "north" dipyrrole of the ester porphyrin, we reacted the two pyrroles **14** and **29** with hydrobromic acid in

acetic acid. We chose acetic acid as solvent instead of methanol to improve solubility and also in order to avoid ester formation.

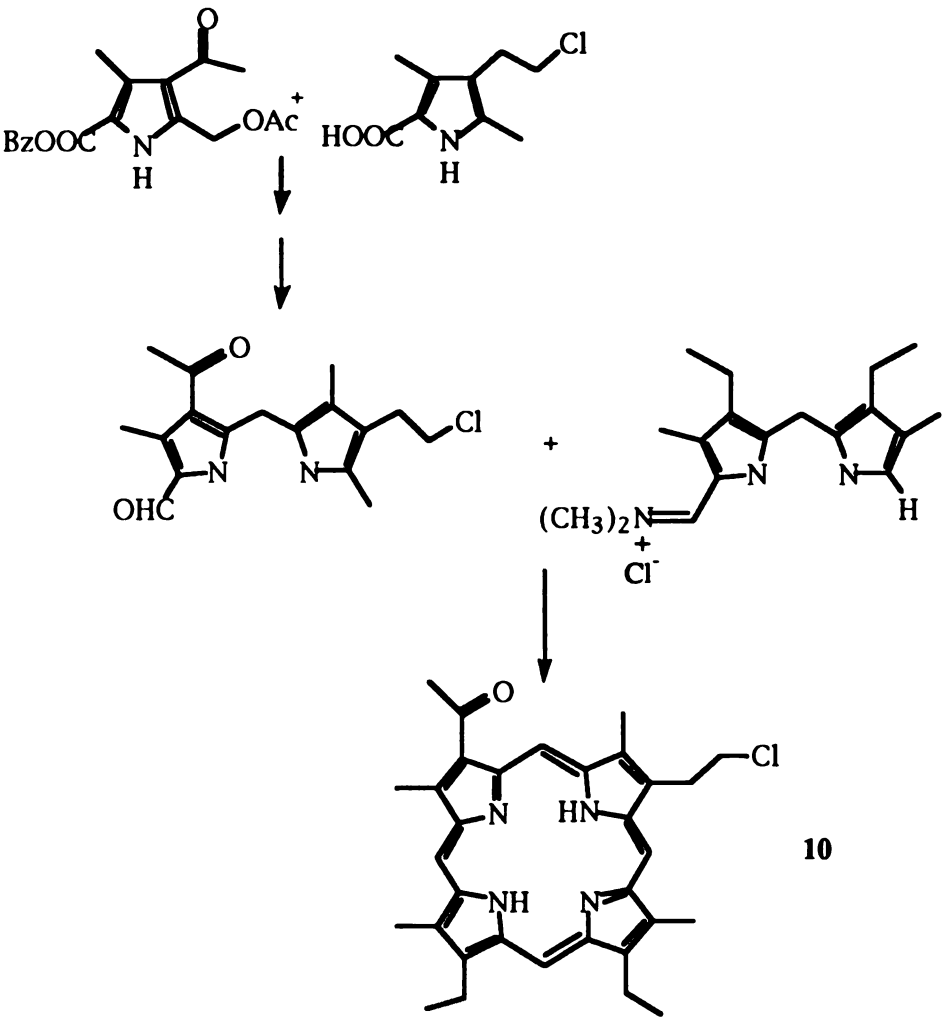
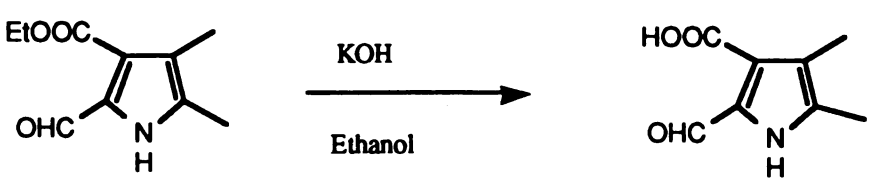


Figure 14: Attempted route for synthesis of 3-acetyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethyl porphyrin

Pyrrole **29** was obtained from **14** after saponification of the ester with potassium hydroxide in ethanol.



29

The dipyrrylmethene **30** was then refluxed with **17** in a mixture of formic acid and bromine to form the porphyrin. During the reaction the porphyrin was decarboxylated and the carboxylic group was replaced by bromine (major product) or hydrogen. The total yield was about 6%, poor yield due to the withdrawing group[18].

Since the major product of this reaction is the bromoporphyrin **31** we reduced this porphyrin by hydrogenation under one atmosphere of hydrogen with palladium on charcoal. Some porphynogen was formed during the hydrogenation, which must be oxidized by DDQ, In addition, byproducts were present and the debromination was never complete. The hydrogen porphyrin **32** after purification on silica gel column gave a yield of 49%.

Iron was inserted into the porphyrin[26] with ferric bromide and metal complex was acetylated by a Friedel and Craft reaction with acetic anhydride and stannic chloride[27]. The iron porphyrin was then demetallated and the acetyl porphyrin **10** was obtained with a yield of 51%. After recrystallization in dichloromethane and methanol, the porphyrin was analyzed by H-NMR, mass spectroscopy, and UV visble spectroscopy. Like in the case of the ester porphyrin we observed, due to the acetyl group, a rhodo-type visible spectrum with the second Q band more intense than the other peaks.

The formation of the dihydroxychlorin **12** was carried out in the same condition as the oxidation of the ester porphyrin. Reaction of 1.5 eqivalent of osmium tetraoxide in dichloromethane followed by quenching with hydrogen sulfide gave the expected chlorin. The reaction was as selective as with the ester and gave only one major product of purple color also. A larger amount of osmium tetraoxide had to be used, the acetyl group being more withdrawing, thus more ring deactivating for the porphyrin.

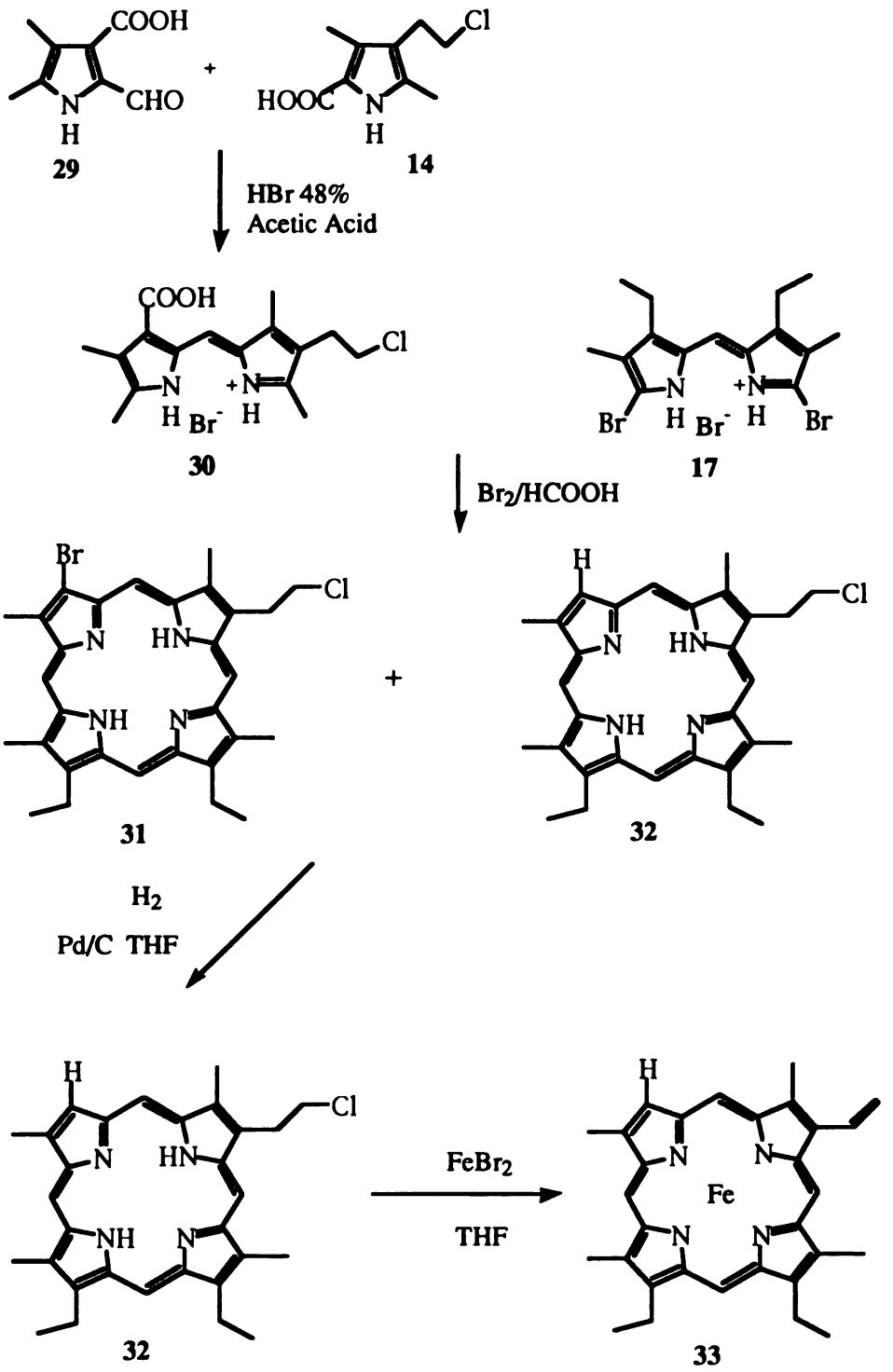


Figure15: Synthesis of iron chelate 13,18-diethyl-2,7,12,18-tetramethyl-3,8-divinylchlorin (part 1)

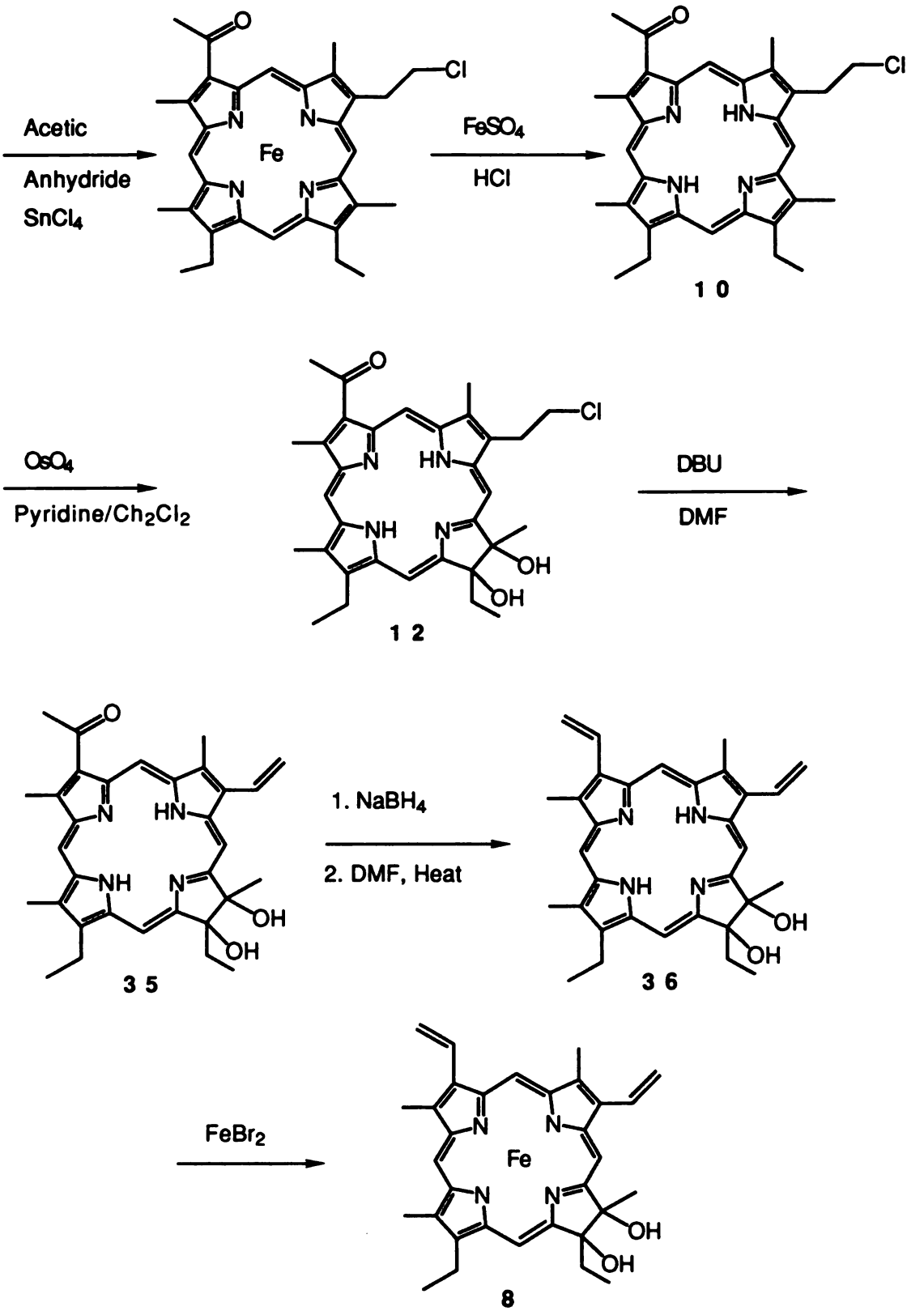


Figure16: Synthesis of iron chelate 13,18-diethyl-3,8-divinyl-2,7,12,18-tetramethylchlorin (part 2)

Elimination of the chlorine with DBU in dimethylformamide gave the acetyl vinyl chlorin **35**. The structure of this chlorin was verified by the presence of vinyl peaks near 6 and 8 ppm in the H-NMR spectrum.

To obtain the divinyl chlorin **36**, the acetyl group was reduced in alcohol using sodium borohydride in a mixture of tetrahydrofuran and methanol. With the reduction of the acetyl group to eliminate the electron withdrawing group, the chlorin color turned from red-puple to green (characteristic color of chlorin and heme d). This hydroxyethyl side chain could be converted into the second vinyl group by heating the chlorine for 10 minutes under argon in dimethylformamide. Mass spectrum of the product clearly indicated the formation of the divinyl dihydroxychlorin **36.**

At this point, our overall yield was so low that there was not enough material on hand to carry out the iron insertion to obtain the final target chlorin-heme. Worse, the small amount of **36** appeared to be unstable in solution to preclude a complete analysis of this compound.

In conclusion, this work demonstrated a regiospecific synthesis of the dihydroxychlorin as well as the general validity of the protection-deprotection strategy to incorporate the vinyl groups on the chlorin core structure.

CHAPTER 3
EXPERIMENTAL

GENERAL

^1H Nuclear Magnetic Resonance spectra were obtained on a Varian Gemini 300 or VRX 300 instrument in $CDCl_3$ or solvents otherwise indicated. UV-visible spectra were measured on a Schimadzu 160 spectrophometer. Mass spectra were obtained on a VG trio-1 spectrometer. Melting points were obtained on a capillary melting point apparatus Unimelt and are uncorrected. Preparative TLC plates were purchased from analtech (silicagel GF, 1000 or 1500 mm).

3-Acetamido-2-butanone **19**

35.1 g (0.39 mol) of D,L-alanine, 159 ml (1.98 mol) of pyridine and 224 ml (2.35 mol) of acetic anhydride were mixed together and heated over a steam bath with stirring until a clear solution was obtained. This orange-brown solution was then heated for six more hours. After this period of time, the excess of solvents, pyridine, acetic anhydride and formed acetic acid were removed under reduced pressure and low heating. The dark residue (about 50 ml) was distillled through a packed column with helices under vaccum to give the crude product boiling at 110-125°C/3mm Hg. Redistillation gave 41g of a light yellow liquid; yield 88%.

3- Ammonium-2-butanone chloride **20**

A mixture of 41g (0.31 mol) of 3-acetamido-2-butanone **19** and 410 ml of a 35% solution of HCl was refluxed for 8 hours. After it was cooled down at 20°C, it was filtered and the filtrate was evaporated under vaccum with low heating until dryness. The brown yellow residue was filterd off and washed with plenty of acetone to give 34.5g of a white powder; yield 88%; mass spectrum m/e (rel. intens.) 123 (35%) (molecular ion), 97 (44%), 82 (59%), 44 (100%).

3-Ethoxycarbonyl-4,5-dimethylpyrrole-2-carboxylic acid **21**

16g (0.13 mol) of the above 3- ammonium-2-butanone chloride **20** were dissolved in 22.4 ml of water and 11.2 ml of a 10 % solution of HCl was added to it. This solution was slowly added (4 to 5 hours) to a stirred solution of 10g of diethyl oxalacetate sodium salt in 9 ml of water and 15 ml of 10% aqueous sodium hydroxide (this solution could be obtained only under heating on a steam bath). During the addition, 3-ethoxycarbonyl-4,5-dimethylpyrrole-2-carboxylic acid precipitated out. The solution was further diluted with 50 ml of water and acidified with a 10% HCl solution until all the pyrrole precipitated out. The white solid was collected by filtration, washed with plenty of water and dried in air.; yield 37%; m.p. 203-205°C; NMR(in $CDCL_3$), δ 1.42 (t, 3H, $\underline{CH_3}CH_2CO$-), 2.18 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.44 (q, 2H, $CH_3\underline{CH_2}CO$-), 10.22 (s, 1H, NH); mass spectrum, m/e (rel. inten.) 211 (35.5%) (molecular ion), 165 (100%), 147 (62%), 121 (58%)

3-Ethoxycarbonyl-4,5-dimethylpyrrole **22**

2g of 3-ethoxycarbonyl-4,5-dimethylpyrrole-2-carboxylic acid **21** were mixed with 5g of sodium acetate trihydrate and 5g of potassium acetate in a round bottom flask. This powdery mixture is heated with an oil bath between 120 and 160°C until it was completed melted. It was cooled down, stirred with water (20 ml) filtered and washed with plenty of water to give 1.46g of a pink-brownish powder.; yield 93% ; m.p. 102-104°C; NMR (in $CDCL_3$) δ 1.32 (t, 3H, $-OCH_2\underline{CH_3}$), 2.17, 2.20 (s, 3H, $-CH_3$), 4.26 (q, 2H, $-O\underline{CH_2}CH_3$), 8.23 (s, 1H, a proton), 8.86 (s, 1H, NH); mass spectrum, m/e (rel. inten.) 167(62%) (molecular ion), 138(67%), 122(100%).

2-Formyl-3-ethoxycarbonyl-4,5-dimethylpyrrole **13**

4g of 3-ethoxycarbonyl-4,5-dimethylpyrrole was dissolved in 35 ml of dimethylformamide **22**. The solution was cooled in an ice bath while argon gas was running through the solution. 3.5 ml of phosphoryl chloride were added dropwise to the stirred cooled solution over a period of 15 minutes. The internal temperature was kept below 10°C and then further stirred for two hours at room temperature. 35 ml of benzene were added and stirred for 30 minutes and the mixture was left standing for a while. Addition of water and a small amount of 10% sodium hydroxide made the product precipitate out. The white precipitates were filtered, washed with water and dried in air; yield 56%, m.p. 125-127°C; NMR (in $CDCl_3$), δ 1,38 (t, 3H, $\underline{CH_3}CH_2$-), 2.22 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 4.37 (q, 2H, $CH_3\underline{CH_2}$-), 10.04 (s, 1H, -CHO), 10.46 (s, 1H, -NH); mass spectrum, m/e (rel. inten.) 195(71%) (molecular ion), 148(81%), 121(100%).

Ethyl-3-acetyl-4-oxopentanoate **23**

To 500 ml of 2-butanone were added with stirring 150 g of 2-4-pentadione (1.5mol), 184 g of ethyl chloroacetate (1.5 mol), 107 g of anhydrous potassium carbonate (1.5 mol) and 45.15 g of potassium iodide (0.27 mol). The reaction was brought to reflux. The reaction became violent because of its exothermic character and the heating mantle had to be removed. When the reaction slowed down, it was refluxed for several hours with a hot water bath until a yellow liquid mixed with white solids were resulted. The reaction mixture was diluted with acetone and the salts were filtered off and washed further with acetone. The filtrates were concentrated in vacuo and the residual oil was distilled under reduced pressure.; yield 51%; b.p. 126°C (8-9 mm Hg) to 139°C (13 mm Hg); NMR (in $CDCl_3$) δ keto form 1.09 (t, 3H, $-OCH_2\underline{CH_3}$), 2.11 (s, 6H, $-CO\underline{CH_3}$), 2.72 (s, 2H, $-CO\underline{CH_2}$), 3.10 (s, 1H, -CH), 3.96 (q, 2H, $-O\underline{CH_2}CH_3$); mass spectrum m/e (rel. intens.) 186 (10%) (molecular ion), 185 (100%), 141 (61%), 43 (97%).

Benzyl-4-ethoxycarbonylmethyl-3,5-dimethylpyrrole-2-carboxylate **25**

A stirred solution of 288 g of benzyl acetoacetate (1.5 mol) in 300 ml of glacial acetic acid was cooled in an ice bath and a solution of 105 g of sodium nitrite diluted in 150 ml of water was added dropwise over a period of 2 hours. The orange solution was let stand overnight.

The resulting solution of benzyl α-oxoaminoacetate **24** was added dropwise, along with small portions of zinc dust (301 g, 4.6 mol), to a stirred solution of 279 g of ethyl-3-acetyl-4-oxopentanoate **23** (1.5 mol) in 500 ml of acetic acid over a period of about 45 minutes. The temperature was kept below 80°C. After

the completion of the addition the mixture was refluxed for an hour. It was cooled to room temperature, poured into 2 liters of water and stood for a while to grow crystals. The solid was then filtered off and washed with plenty of water. The product was recrystallized in methanol/water; yield 45% ; m.p. 79-80°C ; NMR (in $CDCl_3$) δ 1.23 (t, 3H, $-OCH_2\underline{CH_3}$), 2.21 (s, 3H, $-\underline{CH_3}$), 2.29 (s, 3H, CH_3), 4.11 (q, 2H, $-O\underline{CH_2}CH_3$), 5.38 (s, 2H, $-O\underline{CH_2}Ph$), 7.39 (m, 5H, phenyl protons), 8.68 (s, 1H, NH); mass spectrum m/e (rel. intens.) 315 (39%) (molecular ion), 242 (58%), 91 (100%).

Benzyl-4-(2-hydroxyethyl)-3,5-dimethyl pyrrole-2 carboxylate **26**

63 g of benzyl-4-ethoxycarbonylmethyl-3,5-dimethylpyrrole-2-carboxylate **25** and 9.45 g of sodium borohydride were dissolved in 150 ml of dry tetrahydrofuran under argon in a round bottom flask with stirring. Trifluoride-ether complex (42.5 ml) was added dropwise to the solution over a period of 25 minutes. The mixture reached reflux spontaneously and had to be cooled externally with an ice bath. After further stirring for one hour 25 ml of glacial acetic acid were caustiously added dropwise to the mixture followed by water until gas evolution ceased, The mixture was diluted with 200 ml of dichlomethane and washed with 500 ml of water. The organic phase is filtered off to removed the boric acid and evaporated in vacuo. Acetic acid still present in the organic layer was removed by addition of toluene that was evaporated and itself removed by methanol. The residue was recrystallized in 125 ml of methanol and in 125 ml of water. The crystals were filtered off the next day; yield 92%; m.p. 115-116°C ;NMR (in $CDCl_3$) δ 2.21 (s, 3H, $-CH_3$), 2.29 (s, 3H, $-CH_3$), 2.65 (t, 2H, $-\underline{CH_2}CH_2OH$), 3.66 (t, 2H, $-CH_2\underline{CH_2}OH$), 5.29 (s, 2H, $-O\underline{CH_2}Ph$), 7.39 (m,

5H, phenyl protons), 8.80(s, 1H, NH); mass spectrum m/e (rel. intens.) 273 (80%) (molecular ion), 242 (100%), 134 (49%), 91 (100%).

Benzyl-4-(2-chloroethyl)-3,5-dimethylpyrrole-2 carboxylate **27**

45 g (0.16 mol) of benzyl-4-(2-hydroxyethyl)-3,5-dimethylpyrrole-2 carboxylate **26** were mixed with 90 g (0.65 mol) of anhydrous potassium carbonate and dissolved in 900 ml of benzene and 28 ml (0.38 mol) of thionyl chloride. This solution was refluxed for 3 hours under argon. It was cooled and the solid was filtered off. The filtrate was evaporated to dryness to obtain a brown crude product which was purified on silica gel column. The product was eluted by dichloromethane. The pure pyrrole was the light yellow fraction that was washed out after the dark yellow-orange impurities. The solvent was removed and the product was recrystallized from a mixture of hexane and CH_2Cl_2.; yield 86%; m.p. 119-120°C; NMR (in $CDCl_3$) δ 2.21 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 2.84 (t, 2H, -CH_2CH_2Cl), 3.51 (t, 2H, -CH_2CH_2Cl), 5.29 (s, 2H, -OCH_2Ph), 7.39 (m, 5H, phenyl protons), 8.75 (s, 1H, NH); mass spectrum m/e (rel. intens.) 291 (12%) (molecular ion), 242 (21%),91 (100%).

4-(2-Chloroethyl)-3,5-dimethylpyrrole-2 carboxylic acid **14**

22 g (0,075%) of benzyl-4-(2-chloroethyl)-3,5-dimethylpyrrole-2 carboxylate **27** were dissolved in 250 ml of tetrahydrofuran in a 1-L round bottom flask of and stirred with a magnetic stirrer. To this solution 2.3 g of 10% palladium on charcoal was added under argon as well as 10 drops of triethylamine. The mixture was then stirred under hydrogen (1 atm., room temperature) until the expected volume of hydrogen (1.66 liter) necessary to

the reduction was absorbed by the mixture. After complete hydrogenation, argon was bubbled for half an hour and palladium charcoal was filtered off and washed further with dichloromethane. The solvent was removed. The pink product became hard and very dark with time and light. [Note: the reaction is often unreproducible and can either failed to give back 100% of the starting material or succeed to give a quantitative yield] m.p. 114-116°C; NMR (in $CDCl_3$) δ 1.13 (s, 1H, -COOH), 2.23 (s, 3H, $-CH_3$), 2.29 (s, 3H, $-CH_3$), 2.73 (t, 2H, $-\underline{CH_2}CH_2Cl$), 3.51 (t, 2H, $CH_2\underline{CH_2}Cl$), 8.70 (s, 1H, NH); mass spectrum m/e (rel. intens.) 201 (18%) (molecular ion), 152 (51%), 134 (100%).

2,3',5,5'-Dimethyl-3-ethoxycarbonyl-2'-chloroethyl-2,2'-dipyrrylmetheniumbromide **15**

6 g (29.8 mmol) of 4-chloroethyl-3,5-dimethylpyrrole carboxylic acid **14** and 5.81 g (29.8mmol) of 2-formyl-3-ethoxycarbonyl-4,5-dimethylpyrrole **13** were dissolved in 80 ml of methanol. 8 ml of 48% hydrobromic acid were added dropwise to the stirred solution and the mixture was heated up for 10 minutes on the steam bath. Some red crystals formed immediately. It was then left stand for two hours at room temperature. The precipitate was filtered, washed with methanol containing few drops of hydrobromic acid and rinsed with a little bit of ether. 11.35g of nice red powder were obtained; yield 92%; m.p. 199-201°C; NMR (in $CDCl_3$) δ -1.50 (s, 1H, -NH), -1,32 (s, 1H, -NH), 1.44 (t, 3H, $-CH_2\underline{CH_3}$), 2.27 (s, 3H, $-CH_3$), 2.37 (s, 3H, $-CH_3$), 2.70 (s, 3H, $-CH_3$), 2.79 (s, 3H, $-CH_3$), 2.94 (t, 2H, $-\underline{CH_2}CH_2Cl$), 3.60 (t,2H, $-CH_2\underline{CH_2}Cl$), 4.41 (q, 2H, $-\underline{CH_2}CH_3$), 8.44 (s, 1H, bridge proton); mass spectrum, m/e (rel. inten.) 80 (100%), 198 (61.72%), 261 (72.66%), 334 (24%) (molecular ion -HBr).

4,4'-Diethyl-3,3'-dimethyl-2,2'-dipyrrylmethane **15 b**

30 g of 5,5'-bis(ethoxycarbonyl)-4,4'-diethyl-3,3'-dimethyl-2,2'-dipyrrylmethane **16** were dissolved in 100 ml of hot ethanol and brought to a gently reflux. A solution of 15 g of potassium hydroxide dissolved in 25 ml of water was added through the condenser and refluxed for two hours. After this period of time the condenser was removed and the solution was evaporated to one third. 38 ml of water were added with few drops of hydrazine. The mixture was refluxed for twelve hours without interruption. At the end of this period, a layer of brown oil was separated. The mixture was allowed to cool at room temperature and the solidified material was filtered off and washed with water. The fresh product had a dark brown shiny color and can be storred indefinitely in a refrigerator; quantitative yield; m.p. 49-50°C; NMR (in $CDCl_3$) δ 1.12(t, 6H, $-CH_2CH_3$), 2.03 (s, 6H, CH_3), 2.47 (q, 4H,$-CH_2CH_3$), 3.79 (s, 2H,$-CH_2$), 6.35 (m, 2H, 5-H), 7.26 (s, 2H, NH); mass spectrum m/e (rel. intens.) 230 (68%) (molecular ion), 201 (21%), 121 (100%).

5,5'-Dibromo-4,4'-diethyl-3,3'-dimethyl-2,2'-dipyrrylmetheniumbromide **16**

9.45 g of bromine were added to 60 ml of a stirred solution of formic acid. To this solution under stirring 4,4'-diethyl-3,3'-dimethyl-2,2'-dipyrrylmethane **15 b** was added in small portions. The mixture was stirred for an additional 20 minutes during which precipitation occured. The mixture was then evaporated in vacuo and the residue was triturated in 10 ml of ether/methanol. The product was collected by filtration and dried in air; mass spectrum m/e (rel. intens.) 386 (23%) (molecular ion -HBr), 276 (100%), 198 (81%), 80 (68%)

3-Ethoxycarbonyl-8-chloroethyl-13,17-diethyl-2,7,12,18-tetramethylporphyrin **11**

11 g (0.26 mmol) of 4,3',5,5'-dimethyl-3-ethoxycarbonyl-4'-chloroethyl-2,2'-dipyrrylmetheniumbromide **15** and 12.38 g (0.26 mmol) of 5,5'-dibromo-3,3'-diethyl-4,4'-dimethyl-2,2'-dipyrrylmetheniumbromide **16** were dissolved in 125 ml of formic acid and treated with 1.5 ml (0.26 mmol) of bromine. The mixture was refluxed in an oil bath for two and half hours . The solvent was then allowed to boil off over a period of an hour (until dryness). The residue was redissolved in dichloromethane and chromatographed on a silica gel column. The first purple band was the expected porphyrin mixed with a little bit of bromoethylporphyrin. It was then recrystallized from dichloromethane and methanol to give 950 mg of porphyrin.; yield 6.5%; m.p. 245-247°C; NMR (in $CDCL_3$) δ -3.70 (s, 2H, -NH), 1.92 (t, 9H, $-CH_2\underline{CH_3}$ and $-OCH_2\underline{CH_3}$), 3.52, 3.64, 3.66, 3.94 (4s, 12H, $-CH_3$), 3.98, 4.08 (2q, 4H, $-\underline{CH_2}CH_3$), 4.28, 4.50 (2t, 4H, $\underline{CH_2}\underline{CH_2}Cl$), 4.90 (q, 2H, $-O\underline{CH_2}CH_3$), 11.10, 10.15, 9.95 and 9.85 (s, 4H, meso protons); UV-vis l(e) 630 (5700), 573 (11300), 545 (17000), 506 (15000), 405.5 (182200); mass spectrum, m/e (rel.inten.) 600 (10.9%) (bromoporphyrin), 556 (19.4%) (molecular ion), 520 (40.5%), 108 (100%).

3-Ethoxycarbonyl-8-chloroethyl-13,17-diethyl-2,7,12,18-tetramethyl-12,13-dihydroxy-chlorin **11**

To a solution of 60 mg (0.107 mmol) of 3-ethoxycarbonyl-8-chloroethyl-13,17-diethyl-2,7,12,18-tetramethyl-porphyrin **9** in 15ml of dichloromethane and one drop of pyridine, 25 mg (0.0963 mmol, 0.9 eq.) of osmium tetraoxide were added. The stirred solution was left under argon at room temperature in the dark and monitored by thin layer chromatography. After 30 hours the

reaction was diluted with 2ml of methanol and quenched by H_2S for twenty minutes. Osmium was removed by filtration and the solvent was evaporated under reduced pressure. The mixture of starting material and the product were chromatographed on t.l.c. plates with CH_2Cl_2/3%MeOH. The porphyrin was eluted first while the major chlorin product of purple color was the second band.; yield 60%; m.p.>260°C; NMR(in CDCL3) δ -2.26 (s, 2H, -NH), 1.70 (t, 6H, $CH_2\underline{CH_3}$ and $O\underline{CH_2}CH_3$), 2.24 (s 3H, -CH_3), 2.44 (q, 2H, $\underline{CH_2}CH_3$), 3.20 (s, 3H, CH_3), 3.40 (s, 3H, -CH_3), 3.51 (s, 3H, -CH_3), 3.80-4.16 (m, 6H, $\underline{CH_2}CH_2Cl$, $CH_2\underline{CH_2}Cl$ and $\underline{CH_2}CH_3$), 4.68 (q, 2H, -$O\underline{CH_2}CH_3$), 8.78, 8.98, 9.57 and 10.38 (s, 4H, meso protons); UV-vis l(e) 640 (18700), 587 (6200), 545 (8100), 510 (9300), 413 (92000); mass spectrum, m/e (rel. intens.) 634 (11%) (molecular ion, bromoethylchlorin), 616 (63%), 590 (13%) (molecular ion), 572 (87%), 536 (92%), 450, (100).

3-ethoxycarbonyl-13,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-12,13-dihydroxy-chlorin **18**

To a stirred solution of 100 mg of 3-ethoxycarbonyl-8-chloroethyl-13,17-diethyl-2,7,12,18-tetramethyl-12,13-dihydroxychlorin **11** dissolved in 20 ml of dimethyl formamide, 0.2 ml of DBU was added. (DBU was opened under argon to avoid moisture but the reaction does not require either argon or nitrogen). This solution was heated on the steam bath at 80°C for two hours. After cooling to room temperature, 25 ml of water were added and the mixture was stirred for a further hour. It was then filtered on celite, rinsed with water and few drops of methanol. The product was extracted from celite with dichloromethane. Solvent was removed; yield 90%; m.p. 210-212°C; NMR (in $CDCl_3$) δ -2.70 (-NH), 1.58, 1.72 (t, 6H, -CH_2CH_3, -OCH_2CH_3), 2.22 (s, 3H, -CH_3), 3.08 (s, 6H, -CH_3), 3.28 (s, 3H, -CH_3), 3.70-4.10 (m. 6H, -CH_2CH_2Cl, -CH_2CH_3), 4.57 (q, 2H, -OCH_2CH_3), 6.15 (dd,

2H,-CH=CH$_2$), 7.90 (dd, 1H, -CH=CH$_2$), 8.96, 9.15, 9.65 (s, 4H, meso protons); UV-vis l(e) 641 (19800), 587 (6500), 545 (8700), 510 (9800), 413 (95500); mass spectrum, m/e (rel. intens.) 554 (85%) (molecular ion), 536 (98%), 44 (100%)

2-Formyl-4,5-dimethyl-3-carboxylic acid pyrrole **29**

9 g of 2-formyl-3-ethoxycarbonyl-4,5-dimethylpyrrole **13** were dissolved in 180 ml of a solution of 15% sodium hydroxide and 90 ml of ethanol heated on a steam bath. It was heated for two and half hours and then cooled in a ice bath. The solution was acidified by addition of a solution of 15% hydrochlorhydric acid until pink fine crystals precipitated out. They were filtered, washed with a lot of water and dried in air.; yield 93%; m.p. >260°C; NMR (in C_5D_5N) δ 2.22 (s, 3H, -CH3), 2.45 (s, 3H, -CH3), 10.80 (s, 1H, -CHO), 11.15 (s, 1H, -NH) ; mass spectrum m/e (rel. intens.) 167 (96.5%) (molecular ion), 149 (93%), 138 (87%), 121 (100%).

2,3',5,5'-Dimethyl-2'-chloroethyl-2,2'-dipyrrylmetheniumbromide-3-carboxylic acid **30**

6 g (0.036 mol) of 2-formyl-4,5-dimethyl-3-carboxylic acid pyrrole **29** and 7.22 g (0.036 mol) of 4-chloroethyl-3,5-dimethylpyrrole carboxylic acid **14** were dissolved in 200 ml of acetic acid by heating on the steam bath. 20 ml of 48 % hydrobromic acid to this warm solution and heating on the steam bath was continued for 10 more minutes. Dark red crystals formed during this time. The mixture was allowed to stand for an hour and the precipitates were filtered off and washed with a little bit of ether.; yield 90%; m.p.208-210°C; NMR (in $CDCl_3$) δ 2.31 (s, 3H, -CH3), 2.36 (s, 3H, -CH3), 2.68 (s, 3H, -CH3), 2.77 (s, 3H, -CH3), 2.92 (t, 2H, -CH$_2$CH$_2$Cl), 3.60 (t, 2H, -CH$_2$CH$_2$Cl), 8.40 (s, 1H, bridge proton); mass

spectrum m/e (rel. intens.) 306 (44%) (molecular ion -HBr), 261 (100%), 198 (82%).

3-Bromo-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin **31**

10 g of 2,3',5,5'-dimethyl-2'-chloroethyl-2,2'-dipyrrylmetheniumbromide-3-carboxylic acid **30** and 12 g of 5,5'-dibromo-3,3'-diethyl-4,4'-dimethyl-2,2'-dipyrrylmetheniumbromide **15** were dissolved in 120 ml of formic acid and treated with 1.5 ml of bromine. The mixture was refluxed in an oil bath for two and half hours . The solvent was then allowed to boil off over a period of an hour (drynees) during which the porphyrin was formed. The residue was redissolved in dichloromethane and was chromatographed on a silica gel column. The first purple band was the expected porphyrin mixed with a little bit of the bromoethylporphyrin. It was then recrystallized from dichloromethane and methanol to give 850 mg of porphyrin.; yield 6%; NMR (in $CDCL_3$) δ -4.05 (s, 2H, -NH), 1.85 (t, 9H, -$CH_2\underline{CH_3}$ and -$OCH_2\underline{CH_3}$), 3.52 to 3.64 (4s, 12H, -CH_3), 4.02, 4.12 (2q, 4H, -$\underline{CH_2}CH_3$), 4.26, 4.50 (2t, 4H, $\underline{CH_2}\underline{CH_2}Cl$), 9.88, 9.96, 10.03 and 10.15 (s, 4H, meso protons); UV-vis l(e) 620 (5300), 566 (8000), 535 (10692), 500 (12150), 400 (109000); mass spectrum, m/e (rel.inten.) 608 (35%) (bromoethylporphyrin), 562 (78%) (molecular ion), 484 (100%).

8-(2-Chloroethyl)-13,17-diethyl-2,7,12,18-tetramethyl-porphyrin **32**

600 mg of 3-bromo-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin **31** were dissolved in tetrahydrofuran and 100 mg of 10% palladium on charcoal were added to the solution under argon with few drops of triethylamine. The mixture was stirred under hydrogen (1 atm, room

temperature) for 4 hours. After this time argon was bubbled into the solution for half an hour and the catalist was filtered off. Some of the porphyrin was over hydrogenated to become porphyrinogen (monitored by t.l.c.). Therefore DDQ was added to the solution until most of the porphyrinogen was reoxidized. The reaction was not totally complete and the product had to be chromatographed on silica gel column. Bromoporphyrin and hydroporphyrin could be separated with a 40/60 mixture of hexane/dichloromethane; yield 49%; NMR (in $CDCl_3$) δ -3.80 (s, 2H, NH), 1.82 (t, 6H, $-CH_2CH_3$), 3.59 (s, 9H, 3 CH_3), 3.72 (s, 3H, CH_3), 4.06 (q, 4H, $-CH_2CH_3$), 4.29 (t, 2H, $-CH_2CH_2Cl$), 4.48 (t, 2H, $-CH_2CH_2Cl$), 9.06 (s, 1H, 3-H), 9.95, 10.01, 10.07, 10,10 (4s, 4H, meso protons); UV-vis l(e) 618 (6400), 566 (8300), 530 (10500), 497 (14550), 397 (140000) ; mass spectrum m/e (rel. intens.) 528 (38%) (bromoethylporphyrin), 484 (84%) (molecular ion), 448 (100%), 108 (95%).

3-Acetyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin **10**

Iron insertion

A solution of 200 mg of 8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin **32** in 60 ml of benzene, 100 ml of tetrahydrofuran and 20 drops of collidine was aerated by bubbling argon in it for 20 minutes. Then ferric bromide (400 mg) was quickly added and argon was bubbled for 10 more minutes. The mixture was kept under of argon and brougth to a gentle reflux until the fluorescence disappeared totally (about 15 minutes). The solution was evaporated and washed with water and 10% HCl. The metallated porphyrin was collected after evaporation of the solvent.; quantitative yield; UV-vis. λ 637, 503, 381; mass spectrum, m/e (rel. intens.) 538 (52%) (molecular ion), 504 (100%), 490 (65%).

Acetylation

250 mg of the iron porphyrin were dissolved in 25 ml of acetic anhydride and cooled down in an ice bath. To this solution 2 ml of stannic chloride was added under stirring and left for 5 minutes in an ice bath. Then the ice bath was removed and the solution was stirred for an additional ten minutes. The reaction was quenched with ice-water added dropwise at first. The mixture was filtered and the solid was redissolved in dichloromethane, dried with magnesium sulfate and evaporated. The presence of iron acetyl porphyrin was verified by t.l.c. and mass spectrum; mass spectrum m/e (rel. intens.) 580 (67%) (molecular ion), 504 (47%), 207 (100%).

Demetallation

The iron porphyrin was dissolved in 70 ml of acetic acid and was aerated by bubbling argon for 20 minutes at room temperature. 10 ml of HCl and 100 mg of ferric sulfate was added and the resulting mixture was left under argon with stirring for 30 minutes. The solution was then diluted with 100 ml of dichloromethane and washed twice with water and once with a solution of 30% of sodium acetate. The solvent was evaporated and the products were chromatographed on a silica gel column with dichloromethane as eluent; yield 51%; m.p. >260 C; UV-vis λ(ε) 634 (5000), 575 (11000), 547 (15300), 509 (13694), 407.5 (165000); NMR (in $CDCl_3$) δ -3.65 (s, 2H, NH), 1.92 (2t, 6H, -$\underline{CH_2}CH_3$), 3.28 (s,3H, -CO$\underline{CH_3}$), 3.52 (s, 3H, -CH_3), 3.62 (s, 3H, -CH_3), 3.66 (s, 3H, -CH_3), 3.84 (s, 3H, -CH_3), 3.98 (t, 2H, -$\underline{CH_2}CH_3$), 4.09 (t,2H, -$\underline{CH_2}CH_3$), 4.27 (t, 2H, -$\underline{CH_2}CH_2Cl$), 4.50 (t,2H, -$CH_2\underline{CH_2}Cl$), 9.83, 9.97, 10.08, 10.78 (4s, 4H, meso protons), mass spectrum m/e (rel. intens.) 570 (24%) (bromoethyl porphyrin), 526 (100%) (molecular ion), 490 (42%), 44 (81%).

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

To a solution of 60 mg (0.11 mmol) of 3-acetyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethylporphyrin **10** in 15ml of dichloromethane, 45 mg (0.17 mmol, 1.5 eq.) of osmium tetraoxide were added. The stirred solution was left under argon at room temperature in the dark and monitored by thin layer chromatography. After 30 hours the reaction was diluted with 2ml of methanol and quenched by H_2S for twenty minutes. Osmium sulfide was removed by filtration and the solvent was evaporated under reduced pressure. The mixture of starting material and the product was chromatographed on t.l.c. plates with CH_2Cl_2/3% MeOH. The porphyrin was eluted first while the major chlorin product of purple color was the second band.; yield 55%; NMR(in CDCL3) δ -2.21 (s, 2H, -NH), 0.78 (t, 3H, -CH_2CH_3), 1.75 (t, 6H, -CH_2CH_3), 2.24 (s 3H, -CH_3), 2.43 (q, 2H, -CH_2CH_3), 3.14 (s, 3H, -$COCH_3$), 3.39 (s, 3H, CH_3), 3.48 (s, 3H, -CH_3), 3.61 (s, 3H, -CH_3), 3.82 (q, 2H, -CH_2CH_3), 3.99 (t, 2H, -CH_2CH_2Cl), 4.16 (t, 2H, CH_2CH_2Cl), 8.88, 9.11, 9.76 and 10.38 (s, 4H, meso protons); UV-vis λ(ε) 641 (16700), 587 (5900), 546 (7900), 511 (8900), 413 (89000); mass spectrum, m/e (rel. intens.) 586 (19%) (bromoethyl chlorin, loss of water), 560 (15%) (molecular ion), 542 (95%), 108 (100%).

3-acetyl-13,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-12,13-dihydroxychlorin **35**

To a stirred solution of 30 mg of 3-acetyl-8-(2-chloroethyl)-13,17-diethyl-2,7,12,18-tetramethyl-12,13-dihydroxychlorin **12** dissolved in 7ml of dimethyl formamide,0.7ml of DBU was added. This solution was heated on the steam bath at 80°C for two hours. After it was cooled to room temperature, 9 ml

of water were added and the mixture was stirred for another hour. It was then filtered on celite, rinsed with water and few drops of methanol. The product was extracted from celite with dichloromethane. Solvent was then removed.; yield 90%; UV-vis λ(ε) 641 (19000), 587 (6500), 545 (8900), 510 (9500), 413 (99500); mass spectrum, m/e (rel. intens.) 524 (48%) (molecular ion), 506 (100%), 490 (40%), 44 (100%).

3-(1-hydroxyethyl)-13,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-12,13-dihydroxychlorin **35 b**

30 mg of 3-acetyl-13,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-12,13-dihydroxychlorin **35** were dissolved in a mixture of methanol/tetrahydrofuran. At 0°C this solution was treated with 150 mg of sodium borohydride dissolved in cold methanol. It was stirred for 10 minutes in an ice bath before 0.3 ml of acetic acid was carefully added to quench the excess of borohydride. The acidic solution was then diluted with 30 ml of dichloromethane and washed 3 times with water and once with an ammonium hydroxide solution. Organic layer was dried and the solvent was removed; yield; mass spectrum m/e (rel. intens.) 508 (10%) (molecular ion), 490 (80%), 44 (100%)

13,17-Diethyl-3,8-divinyl-2,7,12,18-tetramethyl-12,13-dihydroxychlorin **36**

3-(1-hydroxyethyl)-13,17-diethyl-2,7,12,18-tetramethyl-8-vinyl-12,13-dihydroxychlorin **35 b** was dissolved in dimethylformamide and was brought to 110°C in an oil bath under argon. It was left for 5 minutes then cooled to room temperature and quenched with water. The product was extracted with

dichloromethane and the solvent was evaporated. The major green product was purified by chromatography on plate; mass spectrum m/e (rel. intens.) 490 (82%) (molcular ion), 461 (45%), 44 (100%)

APPENDIX

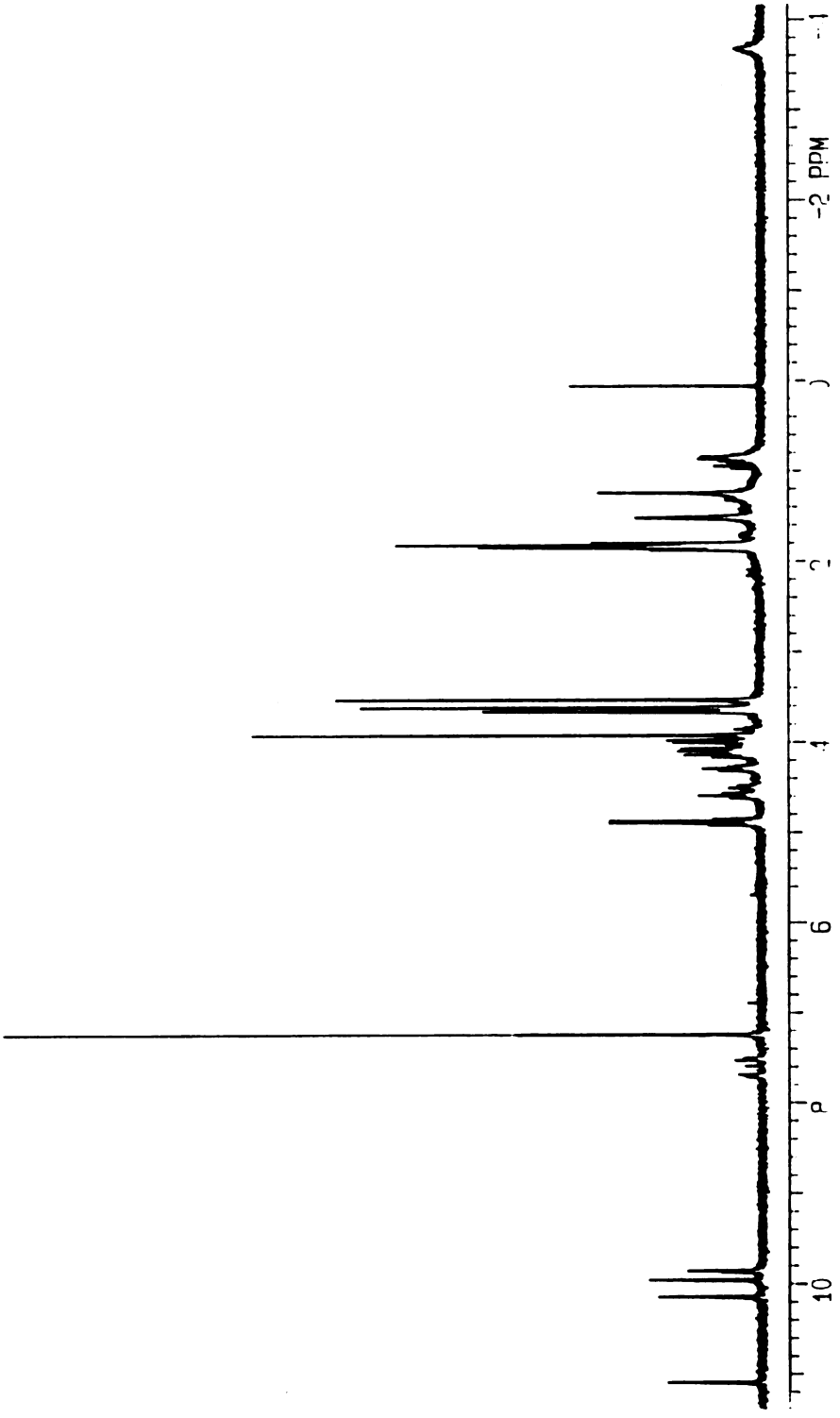


Figure 17. 300 MHz ^{1}H-NMR spectrum of porphyrin **9**

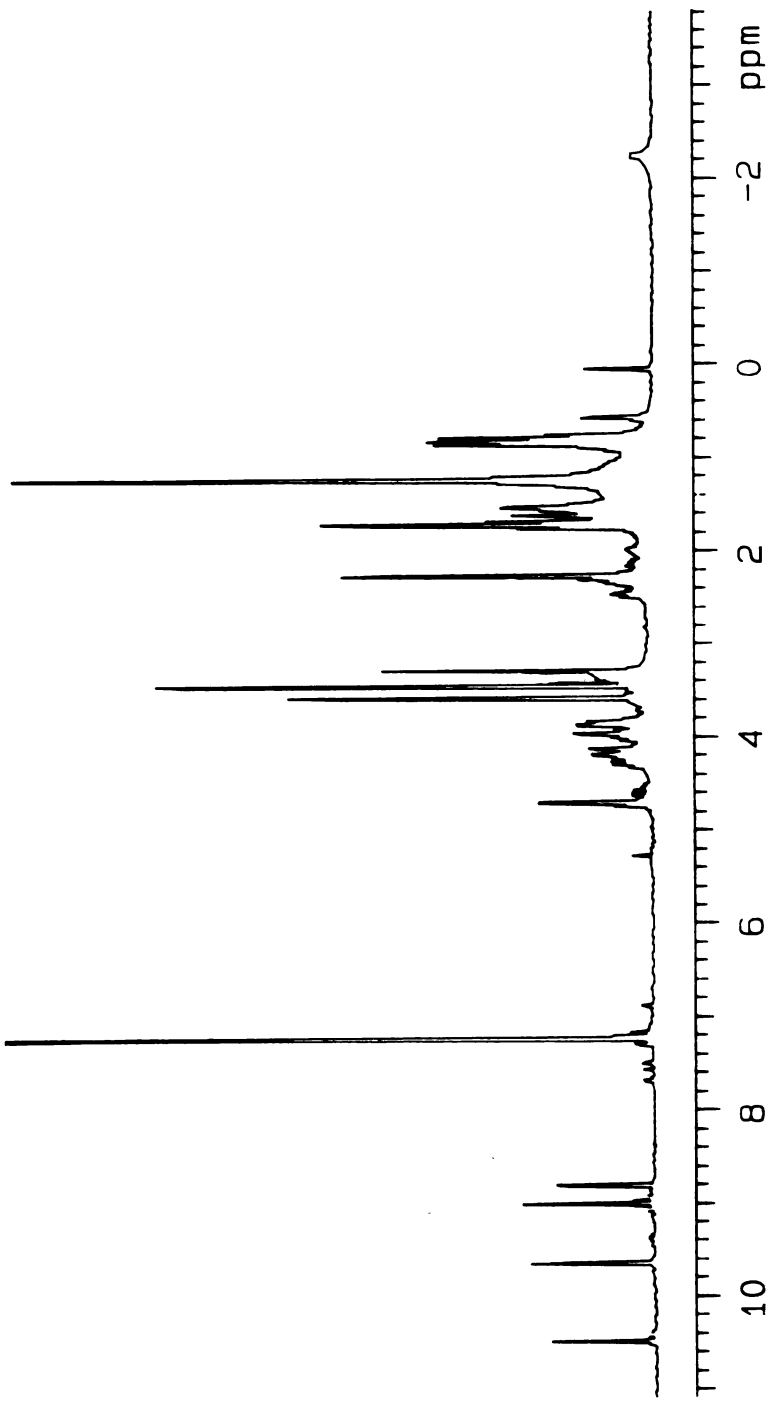


Figure 18. 300 MHz ^{1}H-NMR spectrum of chlorin 11

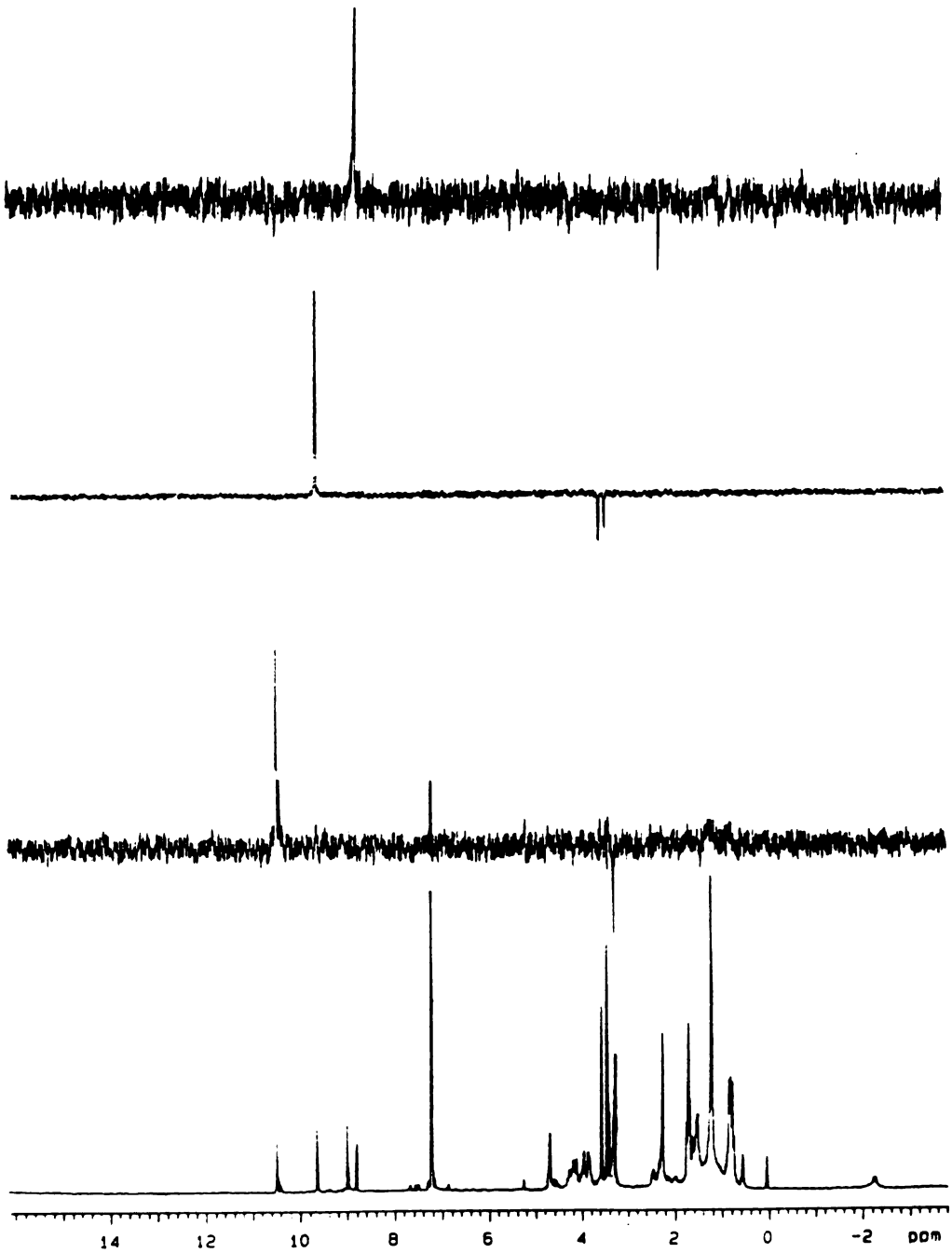


Figure 19. 300 MHz ^{1}H-NMR spectrum of chlorin **11** and NOE study

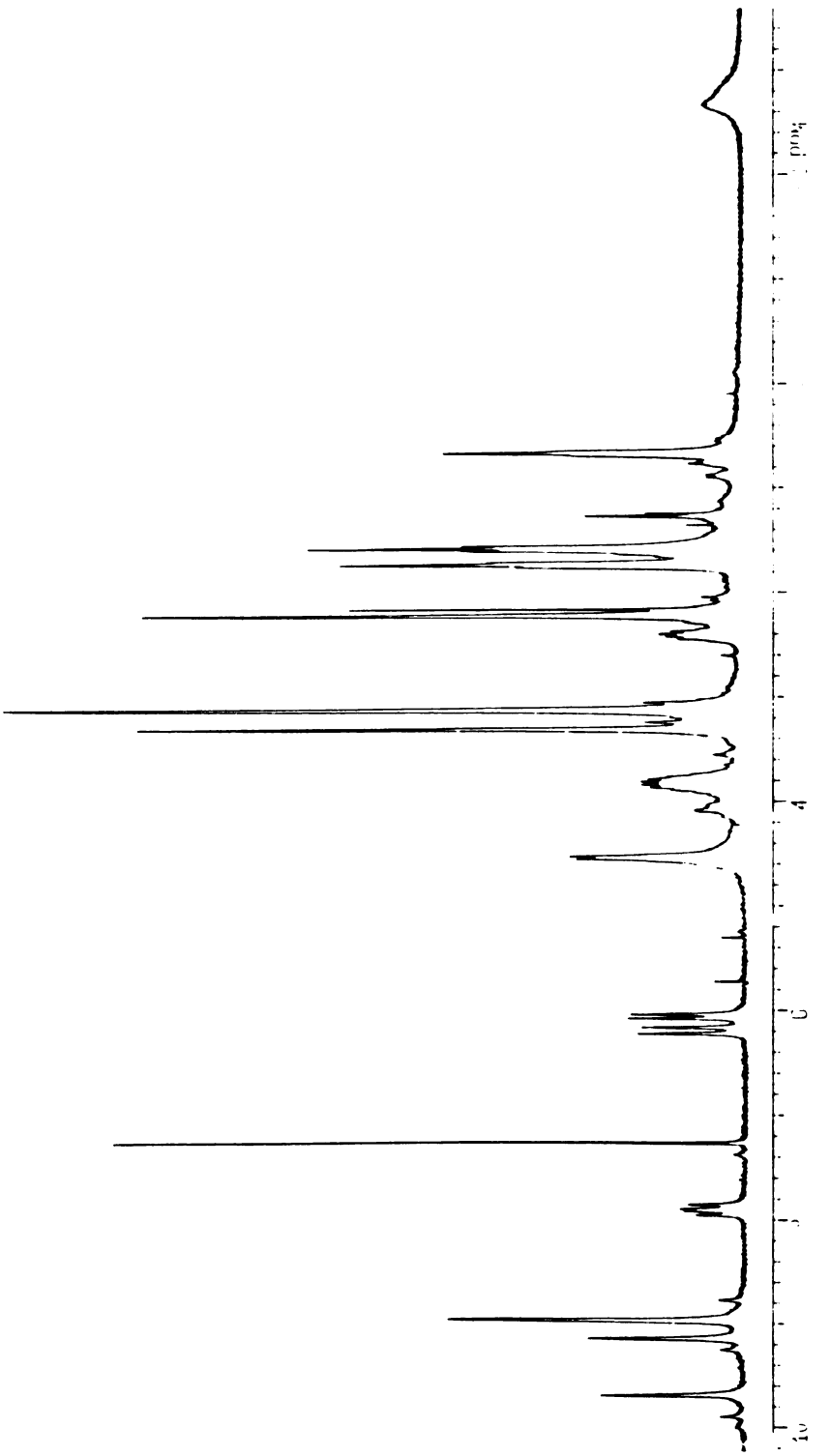


Figure 20. 300 MHz ^{1}H-NMR spectrum of chlorin **18**

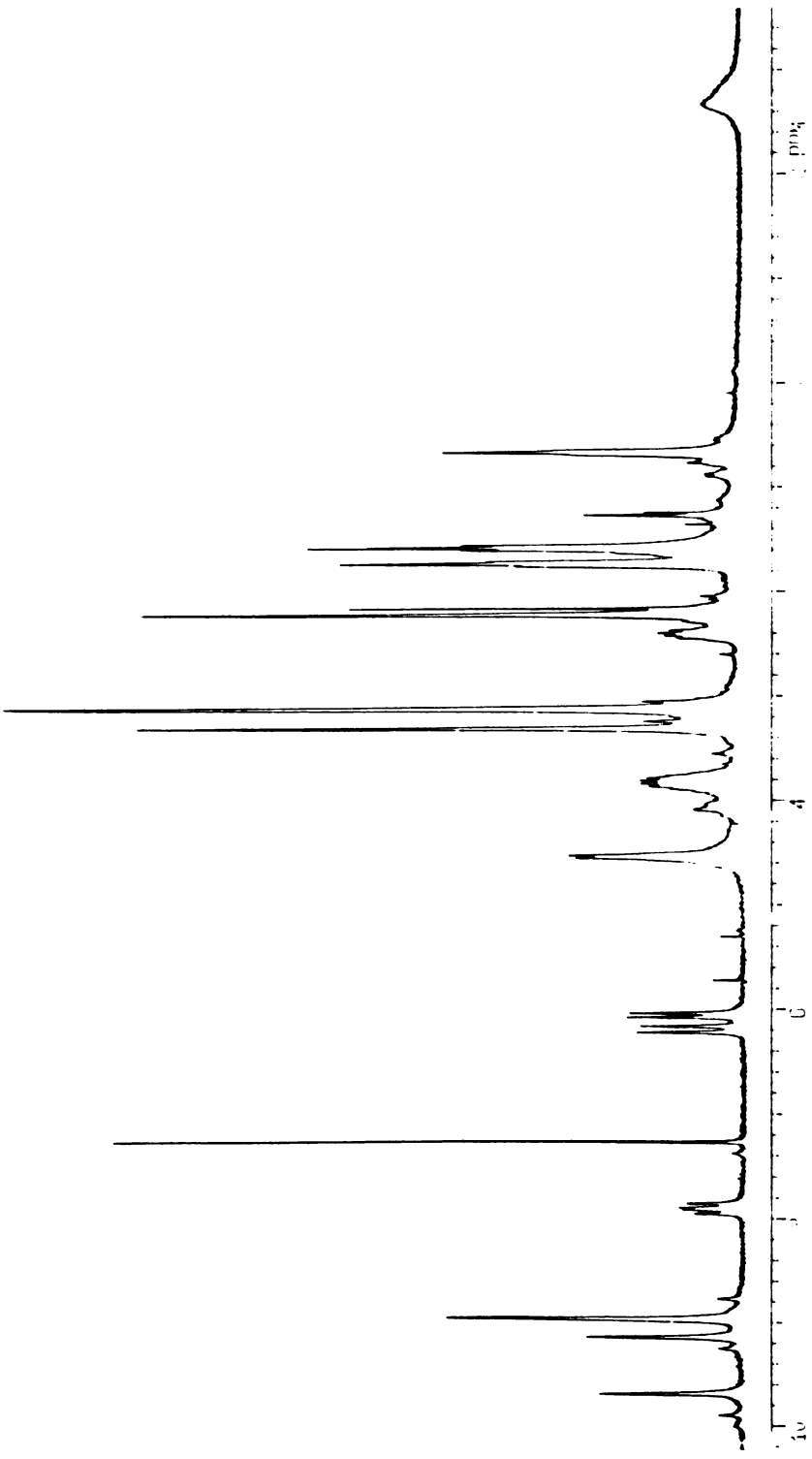


Figure 20. 300 MHz ^{1}H-NMR spectrum of chlorin **18**

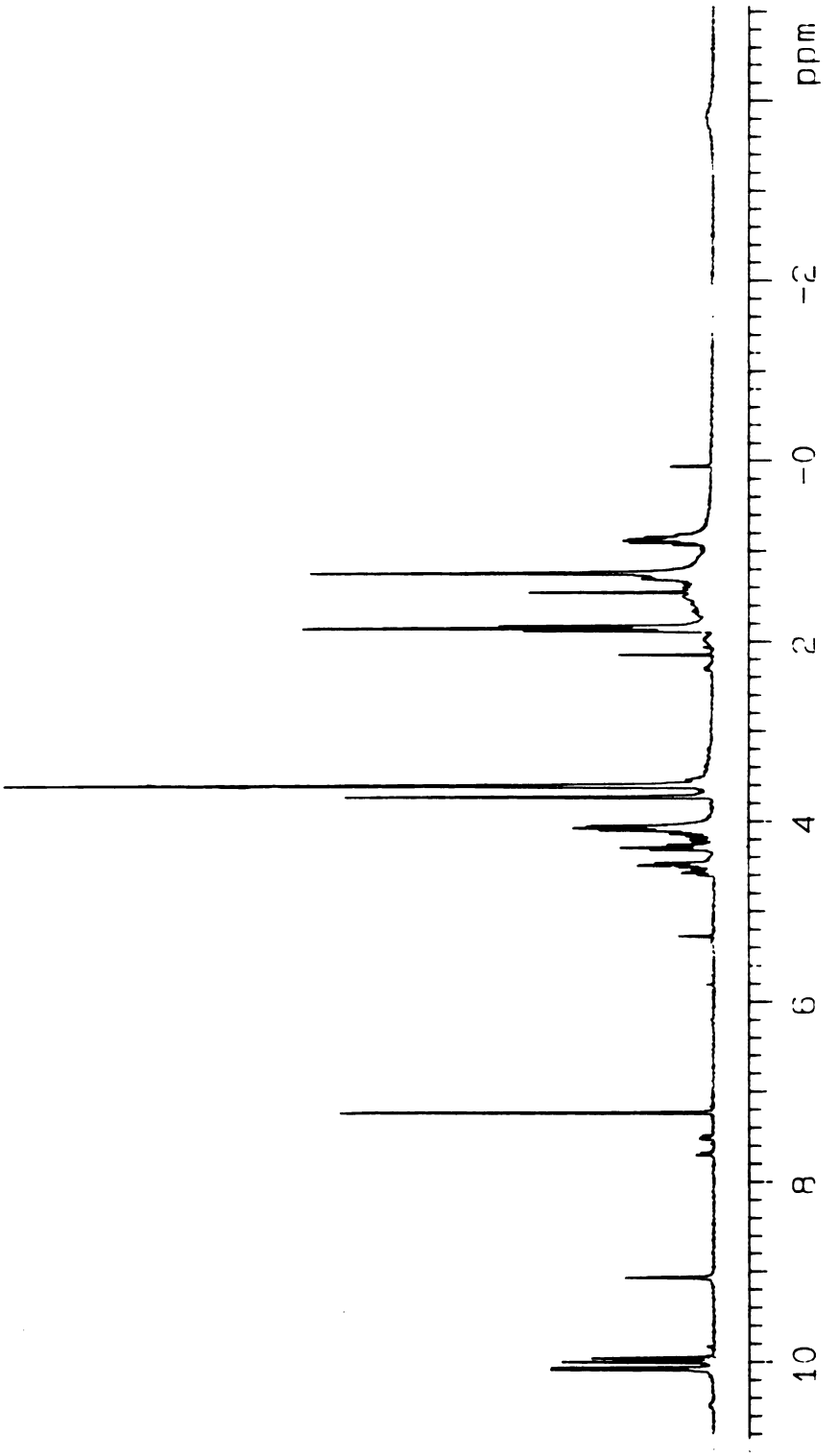


Figure 21. 300 MHz ^{1}H-NMR spectrum of porphyrin 32

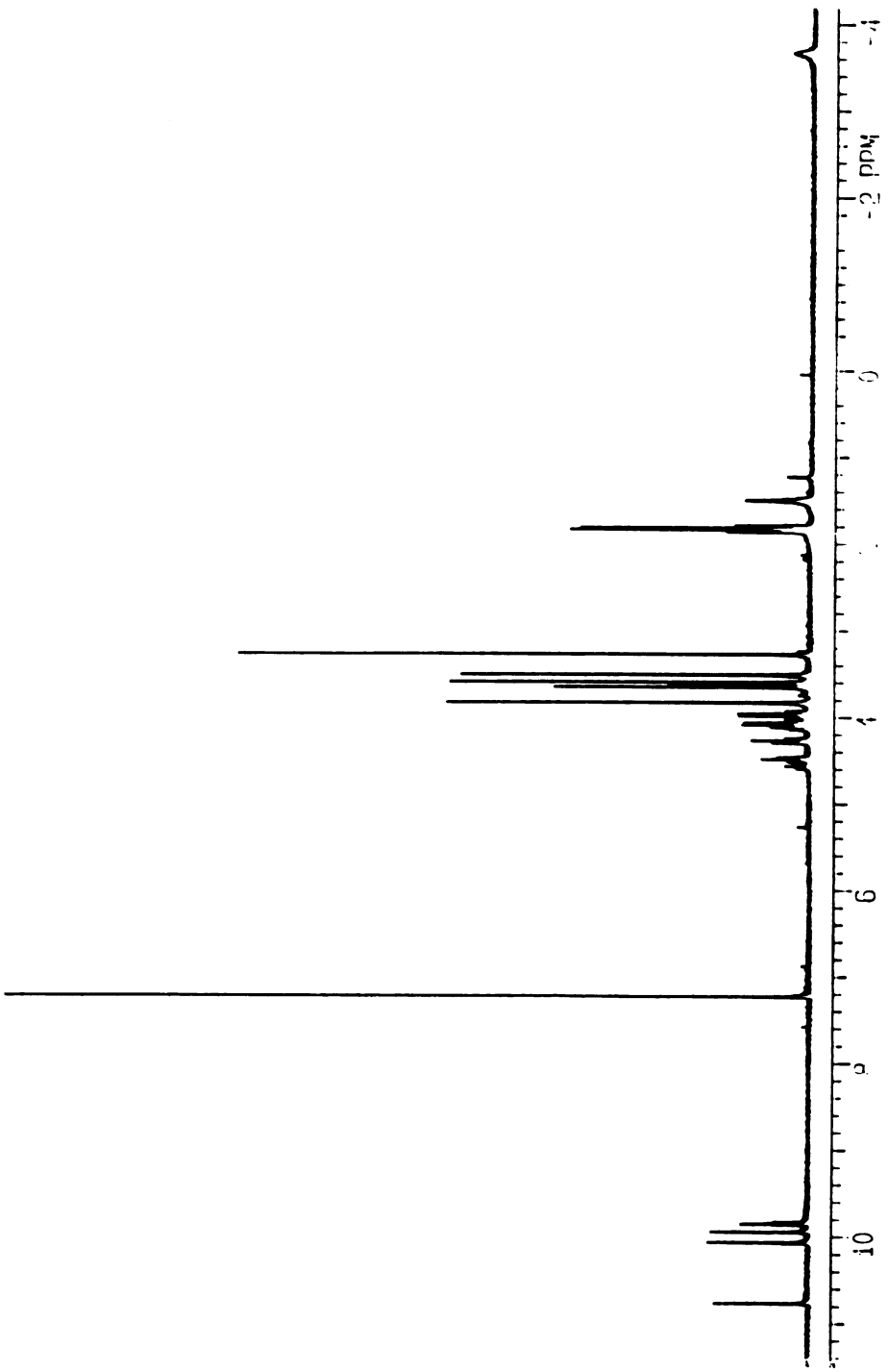


Figure 22. 300 MHz ^{1}H-NMR spectrum of porphyrin 10

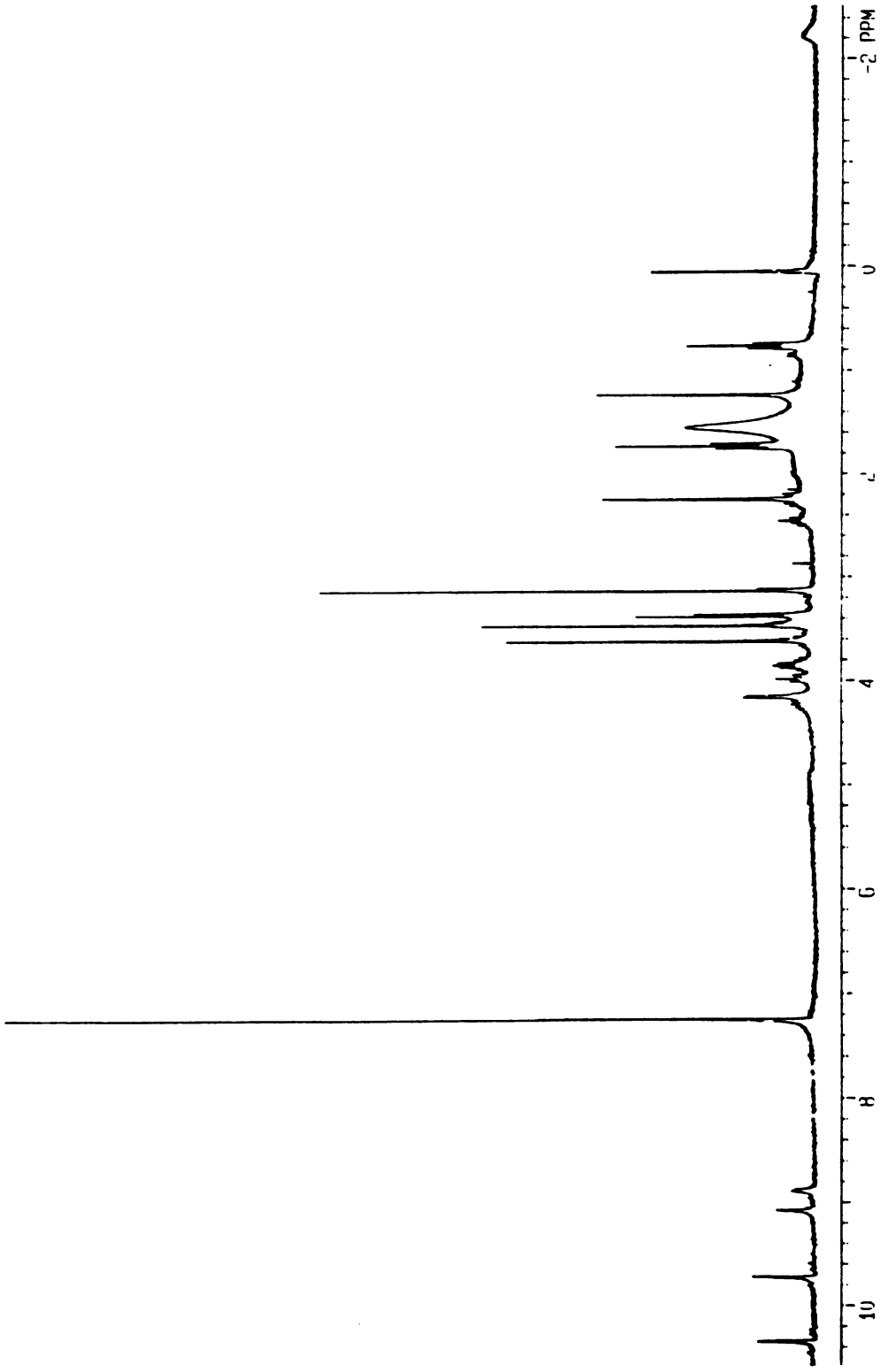


Figure 23. 300 MHz ^{1}H-NMR spectrum of chlorin 12

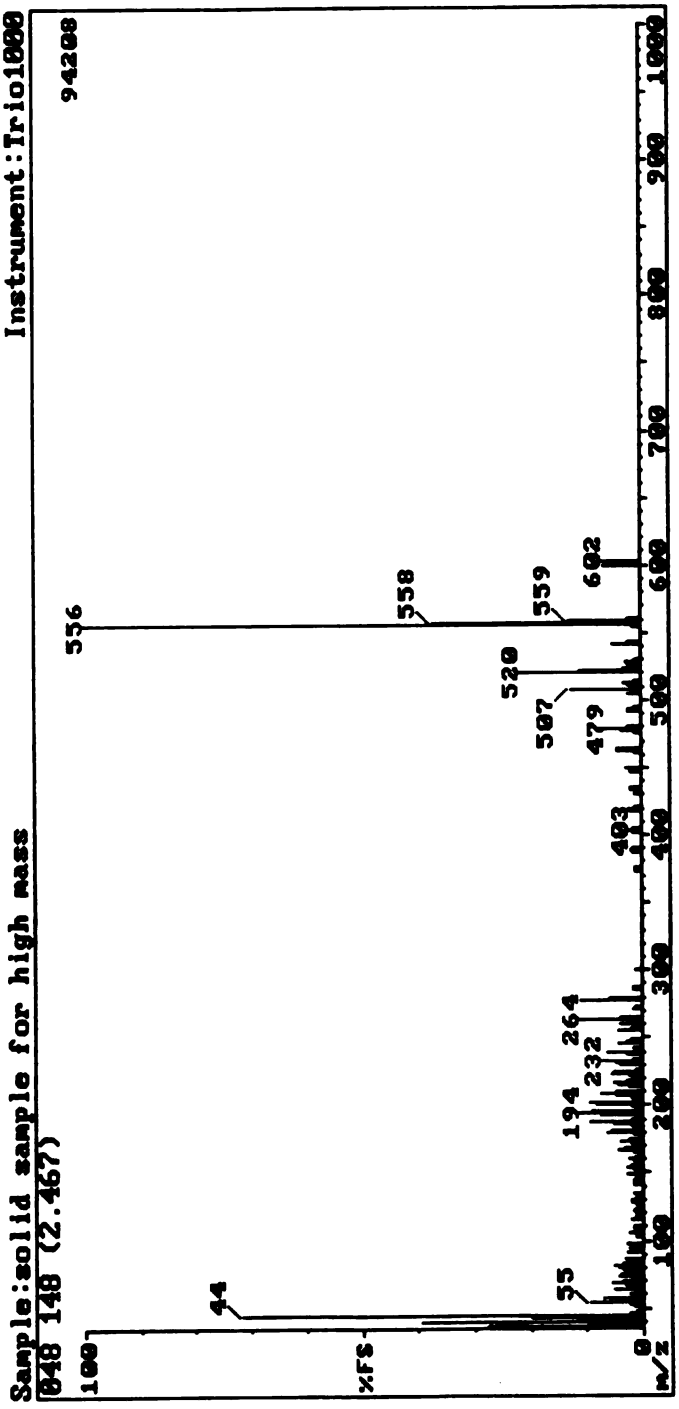


Figure 24. Mass spectrum of porphyrin 9

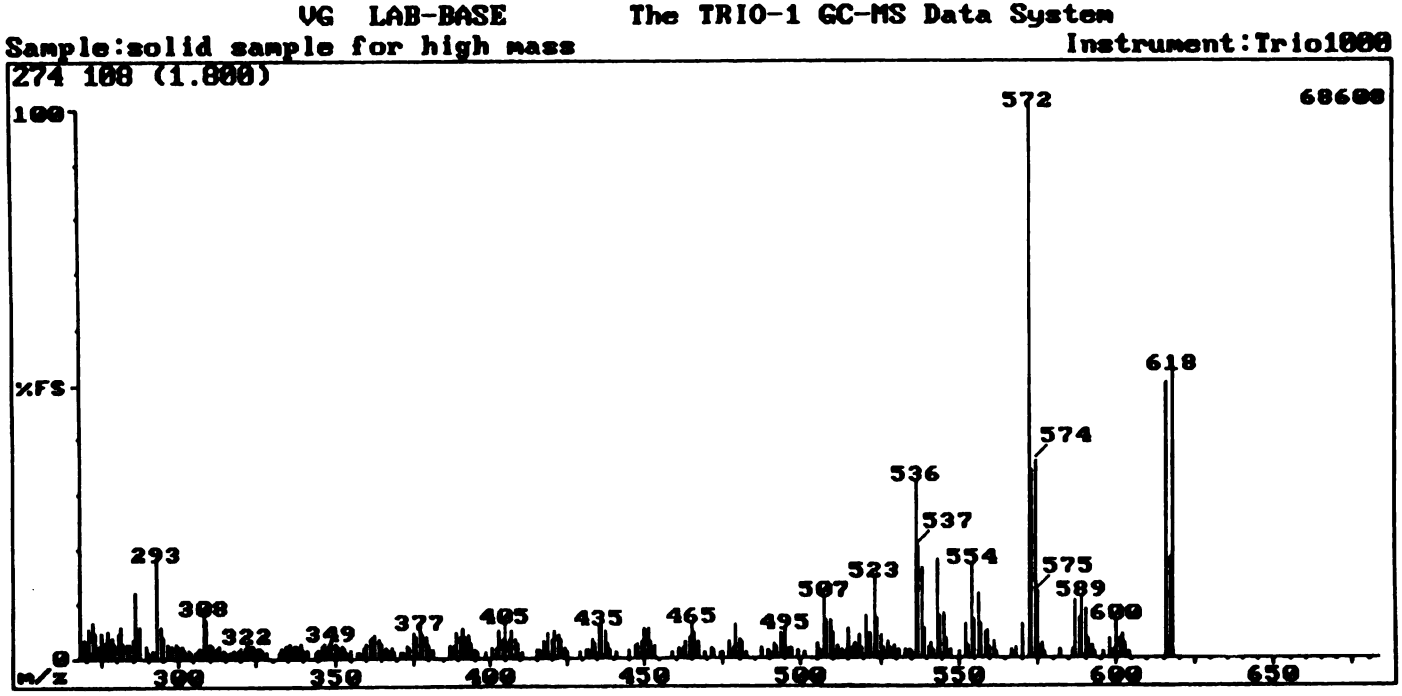


Figure 25. Mass spectrum of chlorin 11

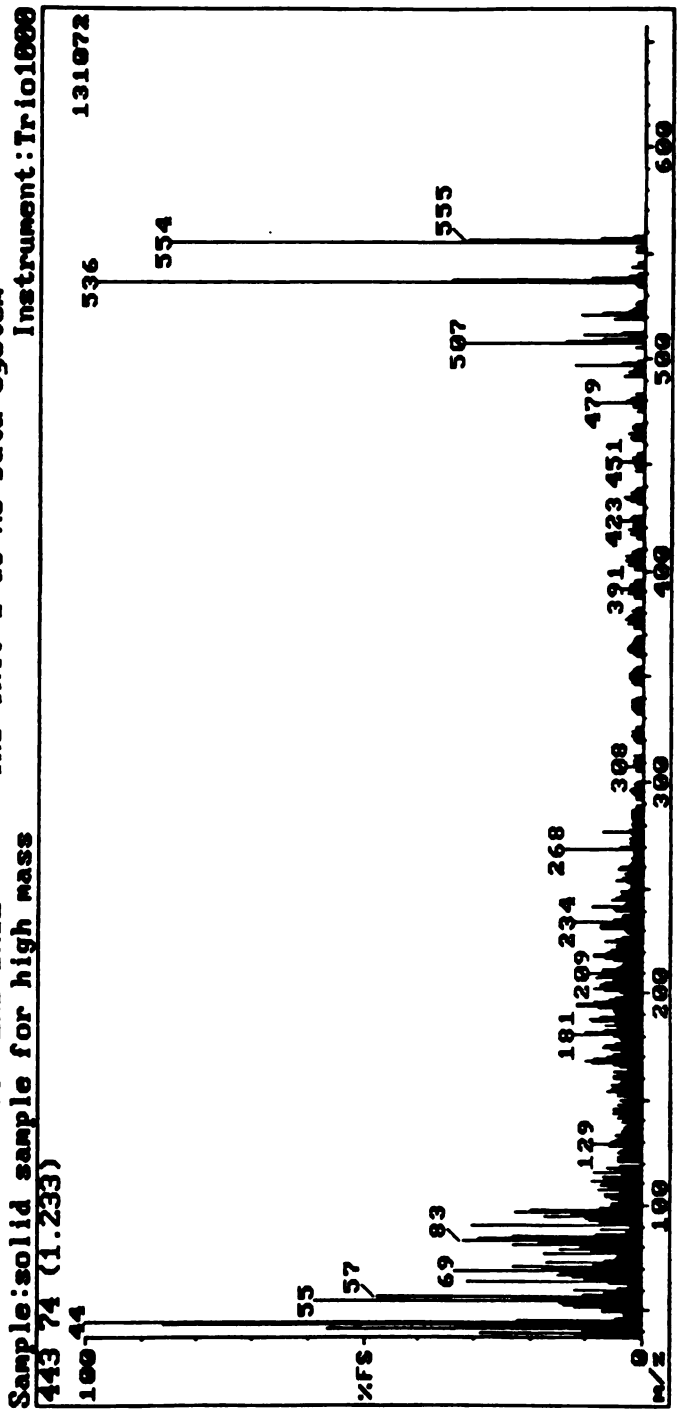


Figure 26. Mass spectrum of chlorin 18

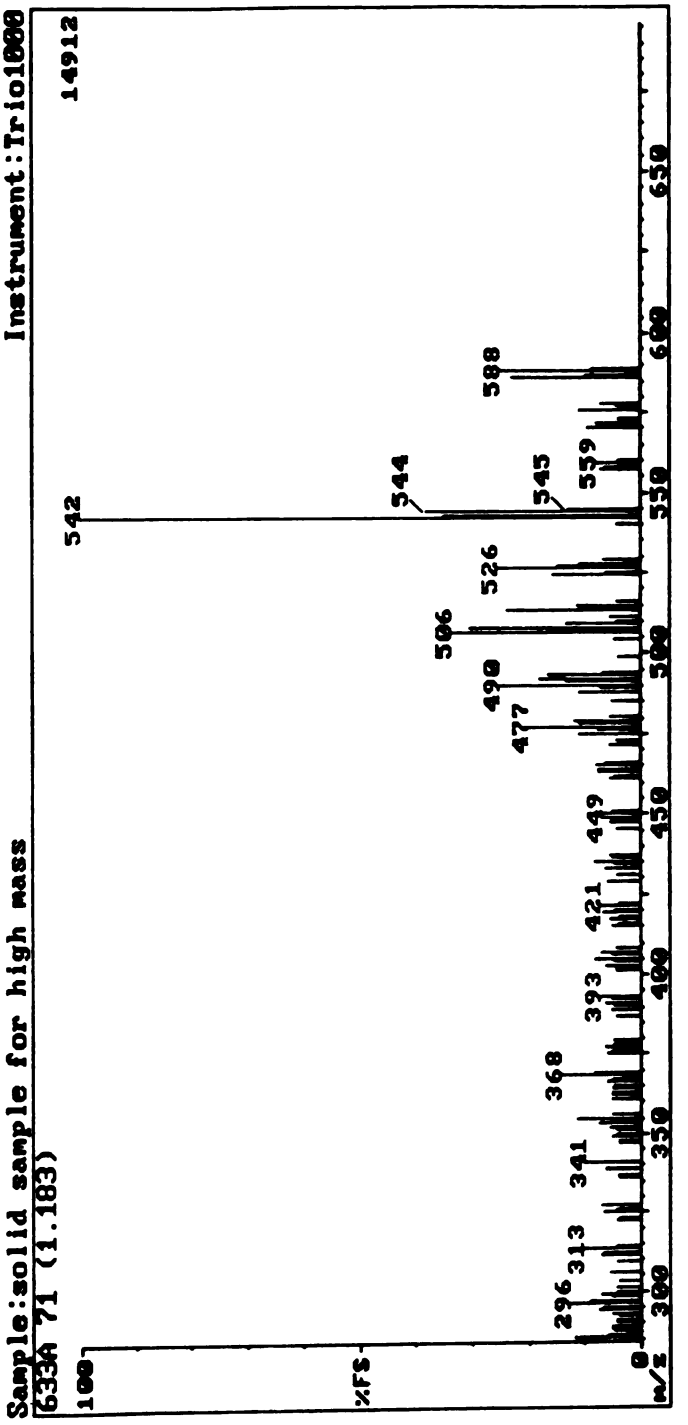


Figure 27. Mass spectrum of chlorin 12

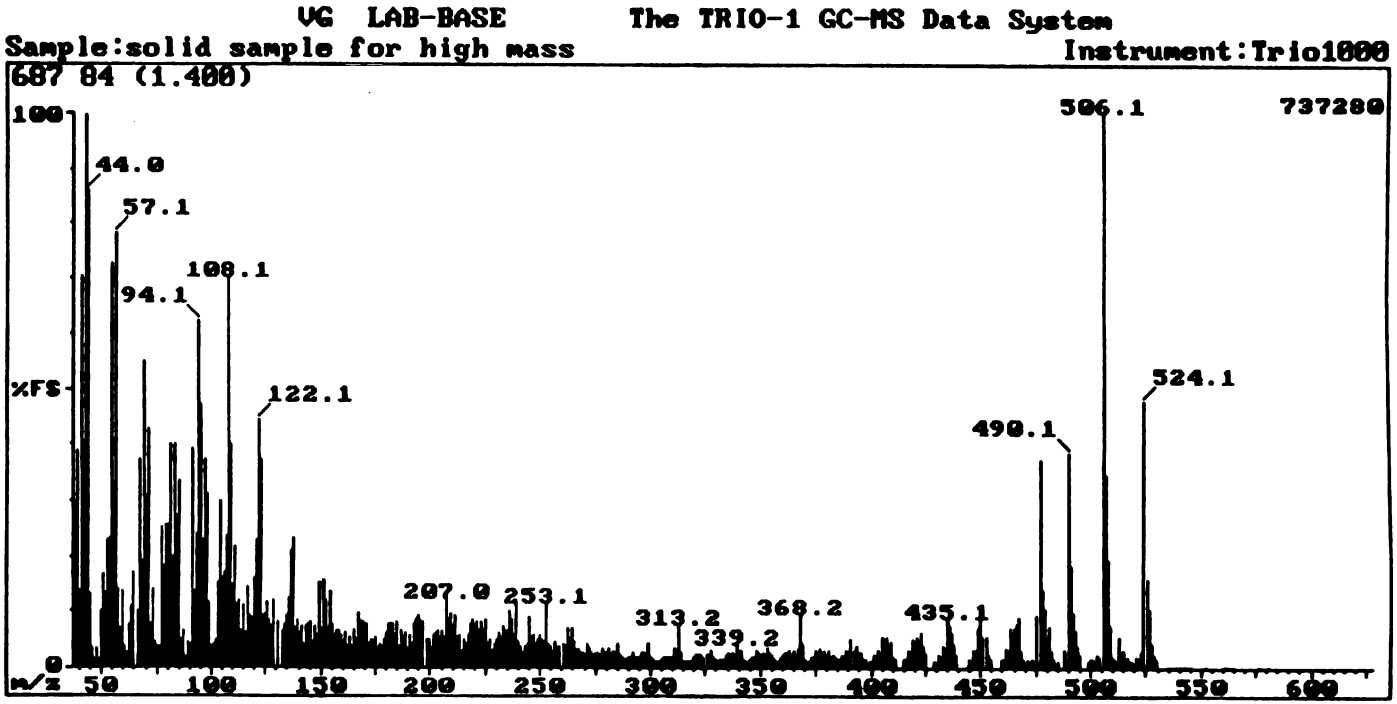


Figure 28. Mass spectrum of chlorin 35

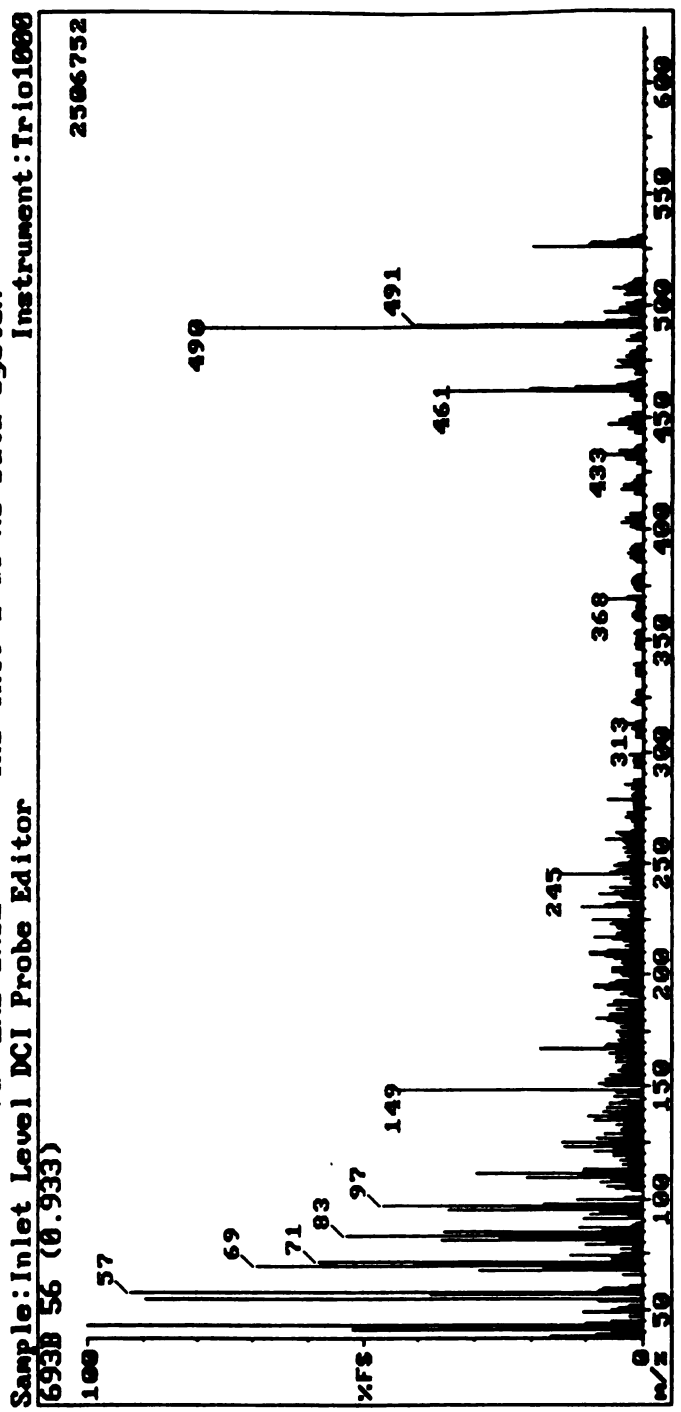


Figure 29. Mass spectrum of chlorin 35 bis

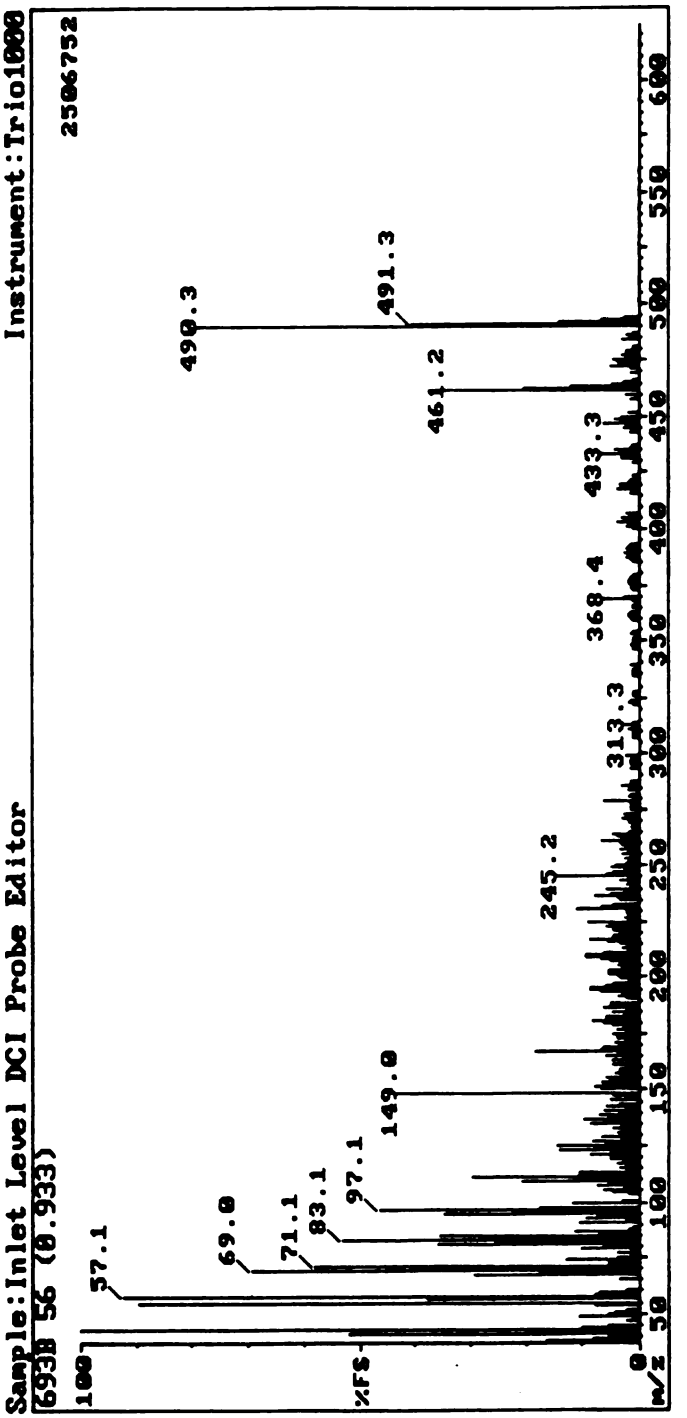


Figure 30. Mass spectrum of chlorin 36

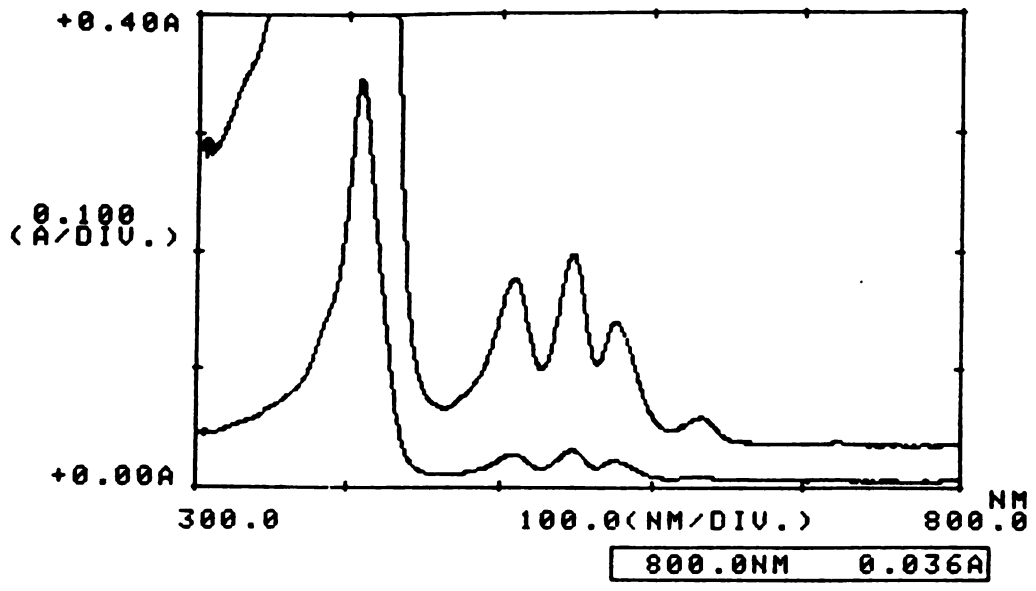


Figure 31. Visible absorption spectrum of porphyrin 9

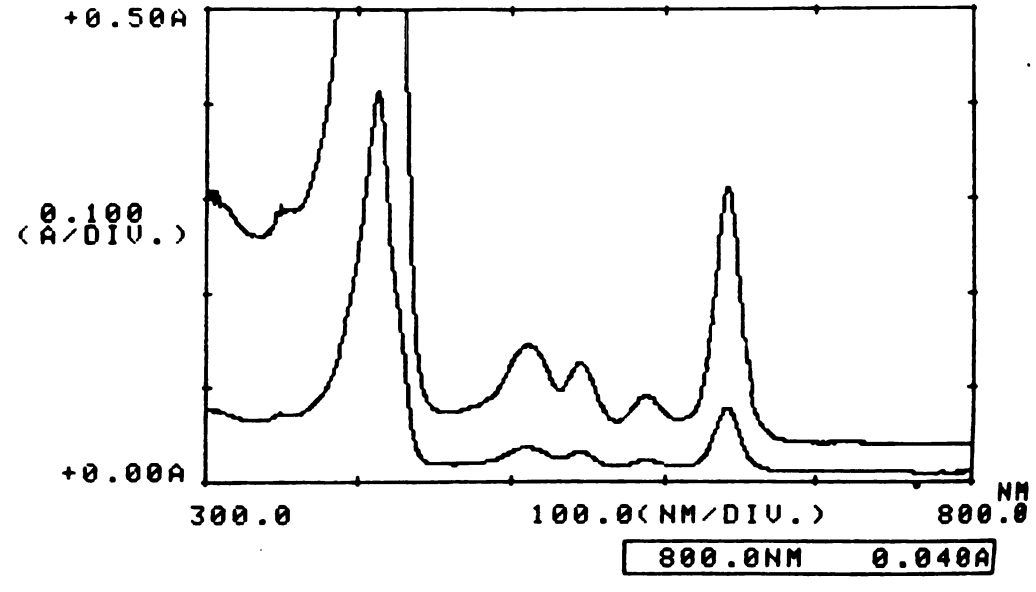


Figure 32. Visible absorption spectrum of chlorin 11

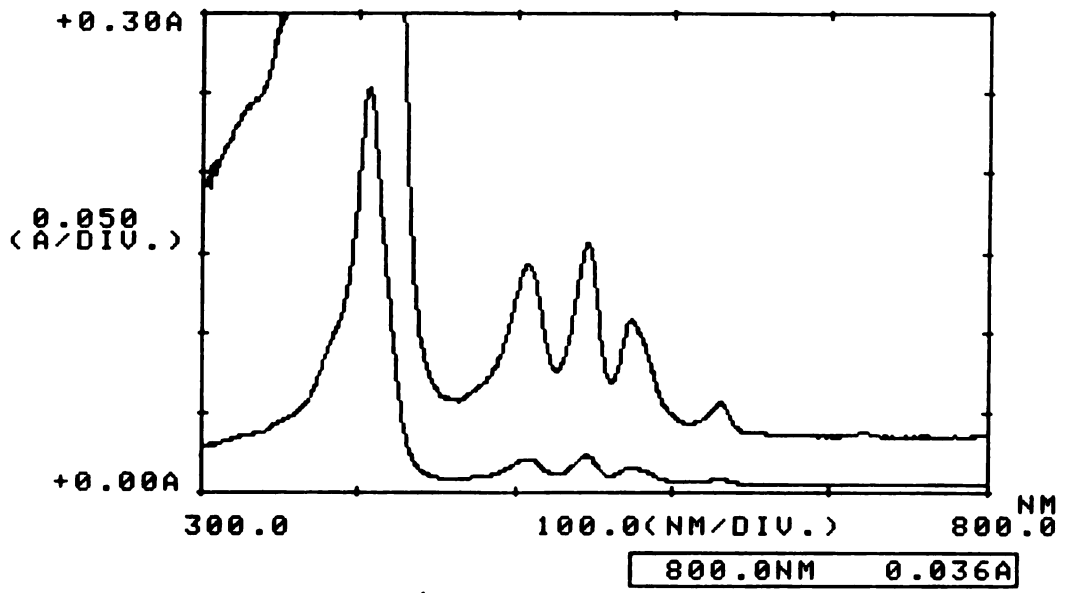


Figure 33. Visible absorption spectrum of porphyrin 10

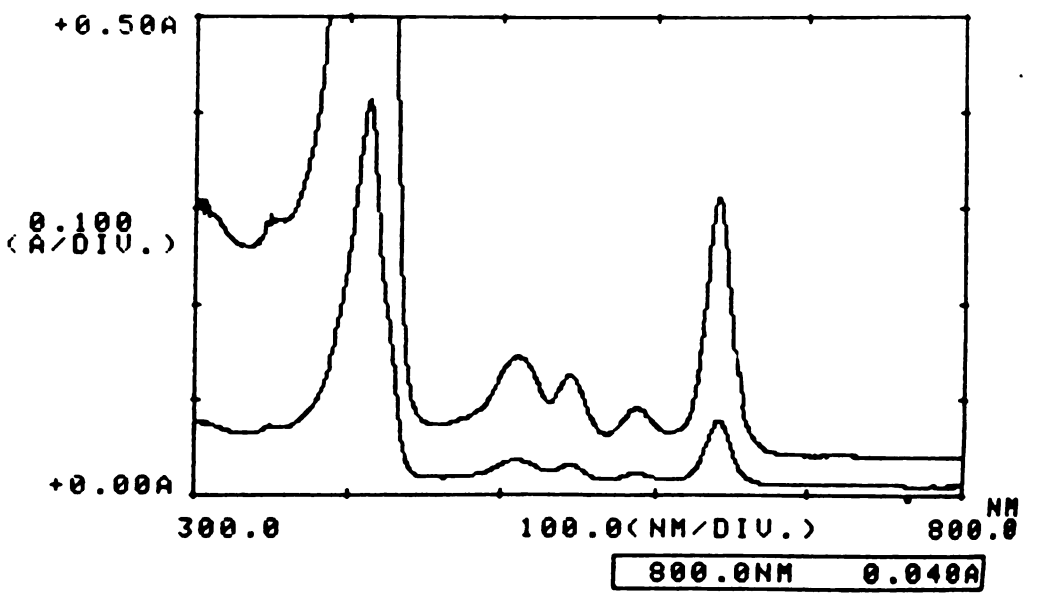


Figure 34. Visible absorption spectrum of chlorin 12

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