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Synthesis and Properties of Heme d-, d_1-

And Sulfur-Containing Green Hemes

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Chariklia Sotiriou

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Ph.D. degree in Chemistry

Clark C. Champ
Major professor

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SYNTHESIS AND PROPERTIES OF HEME d-, d_1- AND SULFUR-CONTAINING GREEN HEMES

By

Chariklia Sotiriou

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ABSTRACT

SYNTHESIS AND PROPERTIES OF HEME d-, d_1- AND SULFUR-CONTAINING GREEN HEMES

By

Chariklia Sotiriou

The discovery of the presence of chlorin and isobacteriochlorin green heme prosthetic groups in a significant number of proteins and enzymes has generated much interest. In this work, syntheses of C-hydroxy, alkyl, alkenyl, and oxo chlorins are described. The conditions and migratory aptitudes for the pinacol-pinacolone type rearrangements involved in the formation of oxo and dioxo compounds have been studied. These results have laid the foundation from which porphyrindiones such as heme d_1 can be synthesized. The vic-dihydroxychlorins in dilute acids can also undergo non-pinacolic rearrangement by which new functional groups can be introduced at the porphyrin side chain. This route has also been applied successfully to creating the acrylic acid group in the d_1-type porphyrindiones.

vic-Dihydroxychlorins substituted with a geminal propionic ester group have a propensity to lactonize. Mild bases such as NaOAc cyclize the geminal groups without inversion of configuration while prolonged contact with silica gel, invariably gives the trans diastereomer. ^1H

NMR spectra have provided important information on the conformation of the cis and trans spirolactones. This work supports the argument that the proposed γ-spirolactone structure of heme d in E. coli is most likely 12,13-dihydroxyprotochlorin IX, the cis and trans isomers and the lactone forms of which have been synthesized.

Sulfur-containing porphyrin thiones have been synthesized from the corresponding oxo-analogues and Lawesson's reagent. These compounds exhibited "hyper" type absorption spectra. Moreover, their redox potentials obtained by cyclic voltammetry revealed a reduction of the HOMO-LUMO energy gap.

Finally, the kinetic and equillibrium constants of CO and O_2 binding to myoglobin reconstituted with several synthetic green hemes were measured by using flash photolysis and spectrophotometric titrations. In comparison with the native myoglobin, the generally faster association rates observed in the green hemes might be attributed to their larger core size which facilitates the spin state change during ligand binding.

To My Mother

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Professor C. K. Chang for his encouragement and support throughout the course of this "colorful" work. I would also like to thank Dr. K. Hallenga who taught me the "secrets" of the NOE experiments. Special thanks are also extended to the members of our group, particularly to Weishih Wu and Gladys Avilés for their friendship.

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TABLE OF CONTENTS

LIST OF TABLES

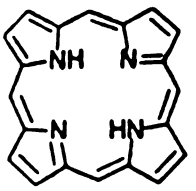
LIST OF FIGURES

LIST OF APPENDIX FIGURES

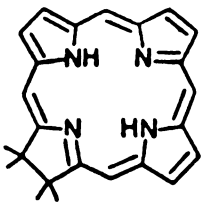
GENERAL INTRODUCTION

The majority of heme-containing proteins and enzymes found in nature possess prosthetic groups in which an iron atom is bound to a fully unsaturated porphyrin macrocycle 1 and is further coordinated by one or two axial ligands from protein side chains. Familiar examples include myoglobin and hemoglobin (oxygen transport and storage), cytochromes b and c (electron transfer), cytochrome P_{450} (substrate hydroxylation), and peroxidases (substrate halogenation and peroxidation).[1]

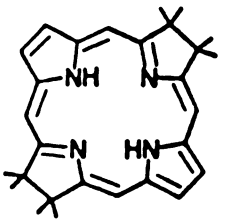
Porphyrins and certain of their metal complexes are subject to reduction leading to a variety of isolable products.[2] Among these are dihydroporphyrins (chlorins, 2) and tetrahydroporphyrins (bacteriochlorins, 3; isobacteriochlorins, 4) resulting from saturation of peripheral double bonds of the parent porphyrin. Biological interest in



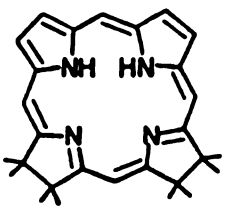
porphyrin
1



chlorin
2



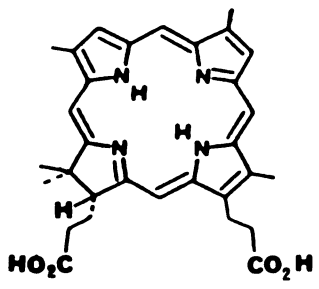
bacteriochlorin
3



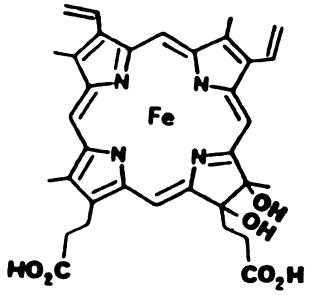
isobacteriochlorin
4

reduced porphyrins has centered principally on chlorins and bacteriochlorins inasmuch as the magnesium complexes of these macrocycles are the essential chromophoric units of algae and plant chlorophylls and bacteriochlorophylls, respectively.[3]

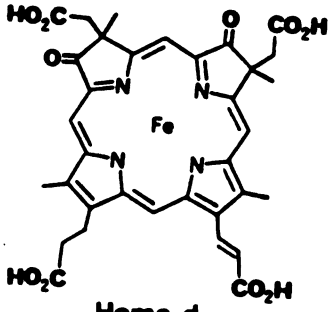
However, a significant number of organisms have now been shown to contain pyrrolic macrocycles based on C-substituted chlorins and isobacteriochlorins. Examples include bonellin (5), the sex-differentiating pheromone isolated from the echurian worm Bonellia viridis[4], Factor I from the B_{12}-producing Clostridium tetanomorphum[5], heme d (6) of Escherichia coli[6] which catalyzes the reduction of O_2 to H_2O, heme d_1 (7) from Pseudomonas aeruginosa[7], which is involved in a



Bonellin
5



Heme d
6



Heme d_1
7

process known as "denitrification"[8] by reducing nitrite to nitrous oxide, and sirohydrochlorin of nitrite and sulfite reductases as well as a B_{12} intermediate.[9] The principal difference between these macrocycles and the unsubstituted hydroporphyrins is that the C-substituted compounds can resist dehydrogenation (back to porphyrins) and therefore, are better suited for undertaking the redox processes with which they may be associated in vivo. As the structures of these unique molecules are being elucidated, it has become timely to investigate the chemistry

and to identify their functional roles in their respective host systems. To realize such goals, however, requires workable quantities of materials which are often difficult to obtain from natural sources.

In this thesis, Chapter 1 is devoted to the development of short and reliable syntheses for functionalized C-substituted chlorins and isobacteriochlorins for general reactivity and biomimetic studies. The work initially focused on vicinal dihyroxychlorins and progressed into other substituted ring systems as a consequence of the lability of the vicinal diol. Chapter 1 also describes a novel method by which alkyl groups of porphyrins can be functionalized. Chapter 2 then gives a detailed account of the total synthesis of heme d and the effort to deduce its true structure. In chapter 3 the synthesis and properties of several sulfur containing saturated porphyrins, derived from their oxo-analogues are reported for the first time. Finally, the kinetic study of CO and O_2 binding to myoglobin reconstituted with some of the above synthetic green hemes is presented in chapter 4, in an effort to cast some light on their structure-function relationship.

CHAPTER 1

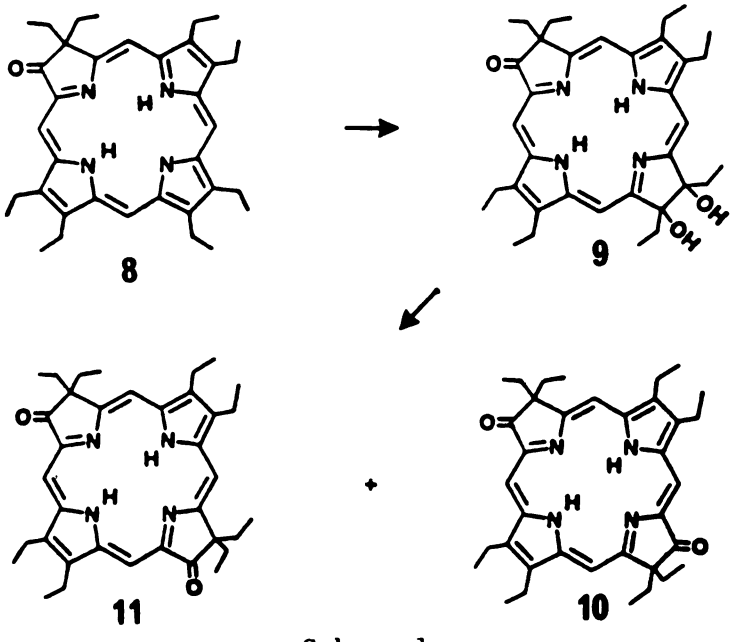
SYNTHETIC METHODOLOGY FOR C-SUBSTITUTED CHLORINS AND OTHER PORPHYRINOIDS

I. INTRODUCTION

Saturation of β-β′ pyrrole double bonds in a porphyrin ring can be brought about by either reduction or oxidation. Fischer and his group pioneered the use of sodium in alcohol to reduce porphyrin to chlorin[10] and this method has been extented by others[11] to obtain reduction levels beyond the chlorin stage, e.g., to bacteriochlorin and isobacteriochlorin. In the opposite direction, Fischer again was first to study the effect of oxidants on porphyrin. In the 1930s, he reacted porphyrin with hydrogen peroxide in concentrated sulfuric acid and obtained what he thought at first was the vic-dihydroxy adduct.[12] This product was later determined to contain only one oxygen.[13] It was not until the 1960s that the keto-gem-dialkylporphyrin (oxochlorin) structure was characterized.[14-16] The acidic hydrogen peroxide oxidation, which yields not only oxochlorins but diketo-(dioxoisobacteriochlorins and dioxobacteriochlorins) and triketoporphyrins arising from pinacolic rearrangements, has prevented the isolation of the expected dihydroxy intermediate. Fischer, however, demonstrated that hydroxylation of type IX porphyrins can be achieved with osmium tetraoxide although the resultant trimeric dihydroxy chlorins were not individually identified.[13,17]

The passage from a vic-dihydroxy or epoxy chlorin to the keto porphyrin by means of pinacolic rearrangements could be a useful method to provide C-substituted derivatives. Chang has reported the synthesis of methyl octaethylchlorin as well as dimethyloctaethylisobacteriochlorins via the keto porphyrins.[18] However, precedence of this rearrangement in porphyrin so far has been limited to octaethylporphyrin (OEP) and etioporphyrin I with simple alkyl groups. A central question in the pinacolic rearrangement is the migratory aptitude of the side chains. While this question has been addressed amply in alicyclic systems,[19] the outcome when applied to porphyrin rings is not readily predictable. This information would be absolutely necessary if this method is to be used for the synthesis of biologically relevant molecules whose side chain substituents often determine the function.

Recently, the keto porphyrins themselves also became the center of interest because of the discovery made in our laboratory that the green colored d_1 heme prosthetic group present in cytochrome cd_1 has a dioxoisobacteriochlorin structure.[7b] The only method known to date for the synthesis of such compounds is the hydrogen peroxide–sulfuric acid oxidation of β-substituted porphyrins resulting in a complex mixture of isomeric products containing one, two, and three oxo groups on the ring with uniformly poor yields.[16,18] The oxochlorins (porphyrinones), such as **8** can be prepared with a significantly higher yield by an alternative 2-step reaction via OsO_4 oxidation and acid catalyzed pinacolic rearrangement.[14,20] Unfortunately, further oxidation of **8** by OsO_4 invariably leads to the bacteriochlorin **9**,[16] which upon rearrangement gives two isomeric dioxobacteriochlorins, **10** and **11** (Scheme 1). The



Scheme 1

preference of attacking the opposite pyrrole double bond may be prompted by the diagonal electron delocalization pathway in chlorin that bypass the outer β-β′ bond of the pyrroline ring and its opposite partner, leading to the bacteriochlorin formation with minimum loss of π-energy. If this is the case, we reasoned, any disruption of such a locked-in tautomeric form should decrease the bacteriochlorin formation and at the

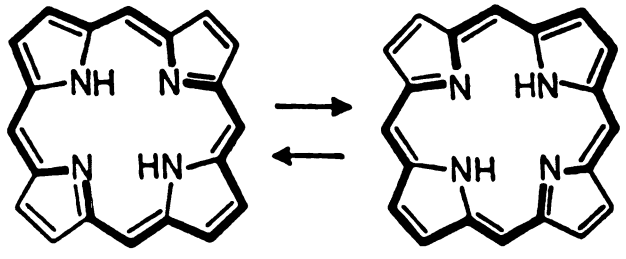
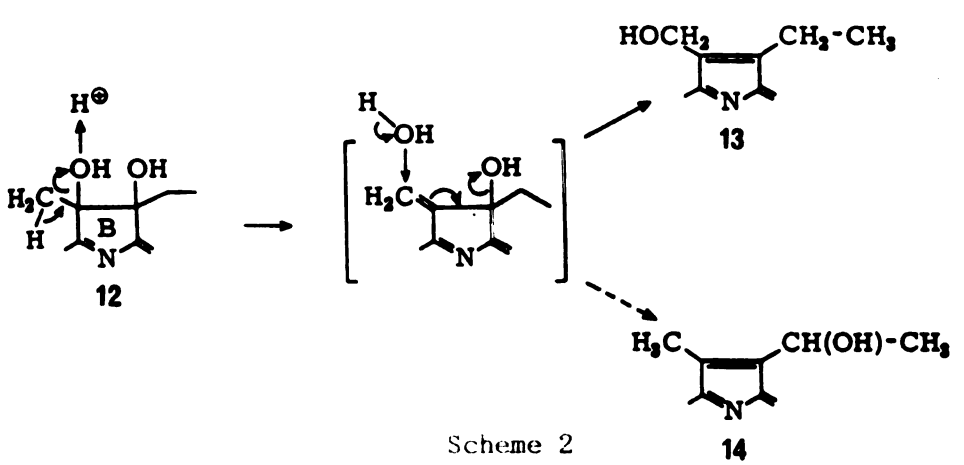


Figure 1. Tautomeric forms of porphyrin.

same time, promote the isobacteriochlorin formation. One simple way to accomplish this feat would be the use of metal ion in the ring so that the two diagonal NH protons would be removed and the overall D_4 symmetry

is enhanced. There is precedence that the reduction site of porphyrin can be altered by metalation. Whitlock and Oester observed that the diimide reduction of free base tetraphenylchlorin (TPC) produces only tetraphenylbacteriochlorin whereas Zn^{II}TPC gives exclusively Zn^{II} tetraphenylisobacteriochlorin.[21] Similarly, reduction of the Ni^{II} pheophorbide family of chlorins by Raney nickel promotes the formation of isobacteriochlorins.[22] However, the osmate reaction with metalloporphyrins has never been studied before. As it turned out, this hypothesis was a complete success and this method now becomes the foundation for the total synthesis of d_1 heme and its analogues.[7e,23]

In the course of our study of the vic-dihydroxy chlorins, it was frequently observed that acid treatment of certain diols also gave porphyrins that are very different from the expected oxo products. This phenomenon is particularly common if less concentrated acid was used to promote the rearrangement. The product is usually a mixture comprising red porphyrins and some purple porphyrinone derived from pinacolic rearrangement of the diol.[16,24] With OEP-diol, the major porphyrin component is OEP-alcohol, presumably derived via hydration of an ethenylhydroxychlorin intermediate. An analogous reaction has been observed previously in a vic-dihydroxybacteriochlorin (scheme 2).[2]



Scheme 2

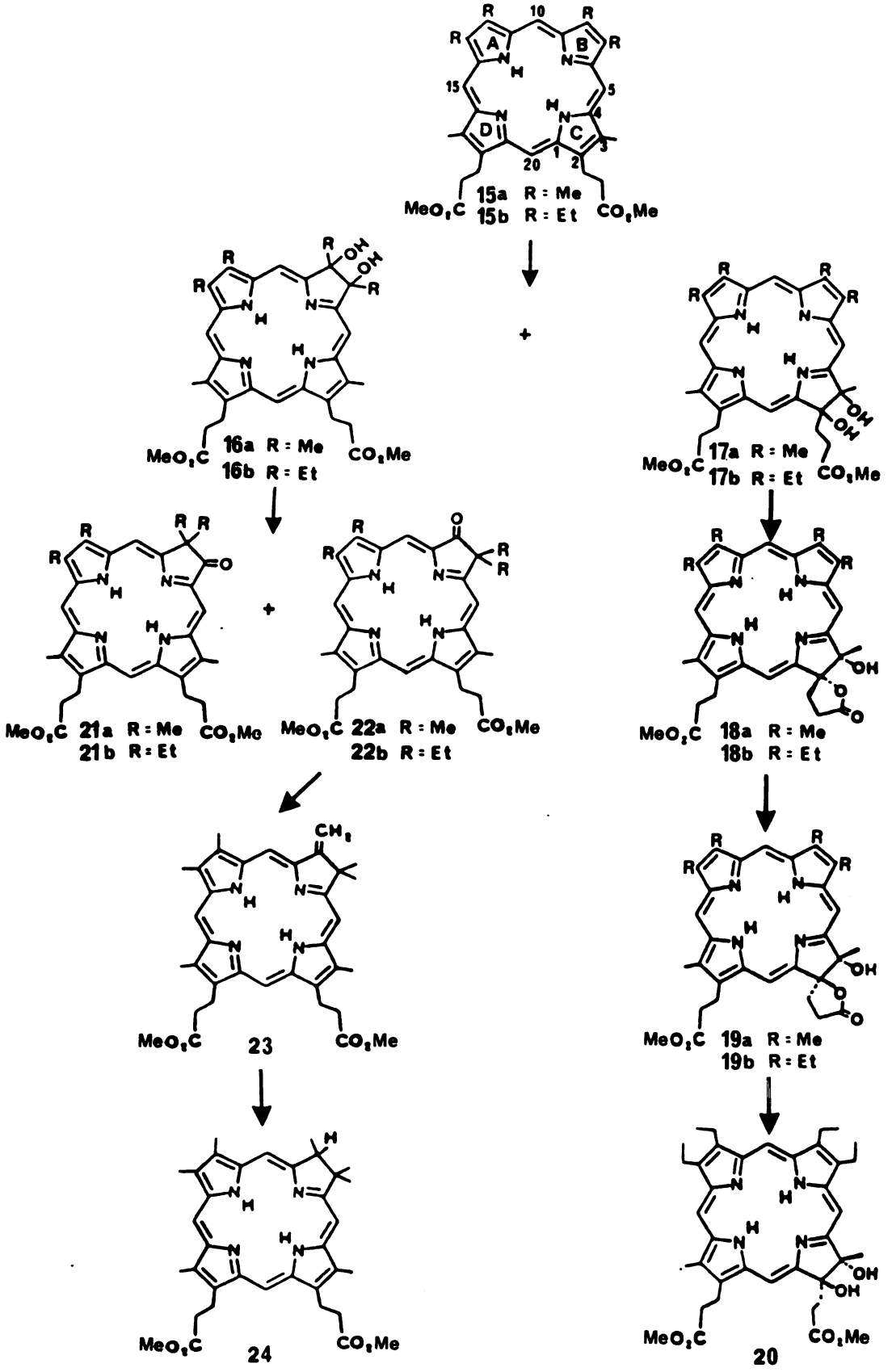
This reaction appeared to us as a potentially attractive method to introduce functional groups to the side chain of alkylporphyrins. For example, OEP has been a tremendously useful compound in porphyrin chemistry but the lack of functional groups can hinder its application in studies wherein some manipulation of side chains would be required. In such cases, it is often a choice between total synthesis, which is usually lengthy and of low yield, and the natural protoporphyrin or its derivatives, which on the other hand may have too many functional groups all at once. The mild elimination-hydration reaction obtainable from OEP-diol gives a simple method to functionalize the ethyl chain of OEP so that the broad range of chemical transformations[26] ascribed for the vinyl group of protoporphyrin would become accessible to OEP.

In the following sections the synthesis and reactivities of vic-dihydroxychlorins will be described first, followed by the pinacolic rearrangement migratory aptitudes and site specificity. Examples of C-alkylated chlorins derived from the keto porphyrins are given and finally the functionalization of OEP and other porphyrin macrocycles are discussed.

II. RESULTS AND DISCUSSION

A. *vic*-Dihydroxychlorins and Derivatives

Osmium tetroxide was added to dimethyl 3,7,8,12,13,17-hexamethylporphine-2,18-dipropionate (15a)[27] in CH_2Cl_2. The reaction was quenched after 20 h to yield the two dihydroxychlorins 16a (37%) and 17a (8%) plus the unreacted porphyrin (30%). Increasing the amount of OsO_4 and lengthening the reaction time invariably led to the formation of tetrahydroxybacteriochlorin and intractable pigments at the expense of the dihydroxy product. A similar reaction was tested on dimethyl


15a R = Me
15b R = Et
16a R = Me
16b R = Et
17a R = Me
17b R = Et
21a R = Me
21b R = Et
22a R = Me
22b R = Et
18a R = Me
18b R = Et
19a R = Me
19b R = Et
23
24
20

SCHEME 3

7,8,12,13-tetraethyl-3,17-dimethylporphine-2,18-dipropionate (**15b**).[28] With this porphyrin apparently for steric reasons, the dihydroxylation occurred more favorably at the "southern" pyrroles (ring C or D reduced) affording nearly 1:1 ratio of **16a** and **17a**. During the separation of **16a** and **17a** on TLC plates with CH_2Cl_2/CH_3OH (pair **16a,17a** requires multiple developments, whereas pair **16b,17b** requires only one development for separation) we observed the gradual development of a third, fastest-moving green spot. The IR spectrum of this new pigment showed a strong new band at 1780 cm^{-1} that was characteristic of a γ-spirolactone[29] (Figure 2). Mass spectral analyses confirmed that, the south diol **17a** had lost a methanol and had become a spirolactone. Further study revealed that this intramolecular lactonization is a general base-catalyzed reaction that can be brought about by sodium acetate, pyridines, basic alumina, as well as silica gel.

When a small amount of the above lactone chlorin (**18a**) was extensively chromatographed on TLC plate (silica gel, CH_2Cl_2/CH_3OH), it was converted to an even faster moving green spot. IR and mass spectral analyses still showed the presence of a γ-spirolactone. This lactone chlorin model compound, **19a** exhibits a ^1H NMR spectrum almost indistinguishable from that of Timkovich's lactochlorin (from heme d)[6] for those structural elements that are directly comparable to one another. Particularly interesting is the region between 2.3 and 3.4 ppm where the methylene protons of the rigid spirolactone should appear. On the basis of the absence of a measurable NOE between the 3-Me group (2.0 ppm) and any lactone ring protons, as well as on analyses of the lactone methylene peaks, Timkovich et. al. concluded that the two oxygen substituents have a trans configuration.[6] In our model chlorin lactone,

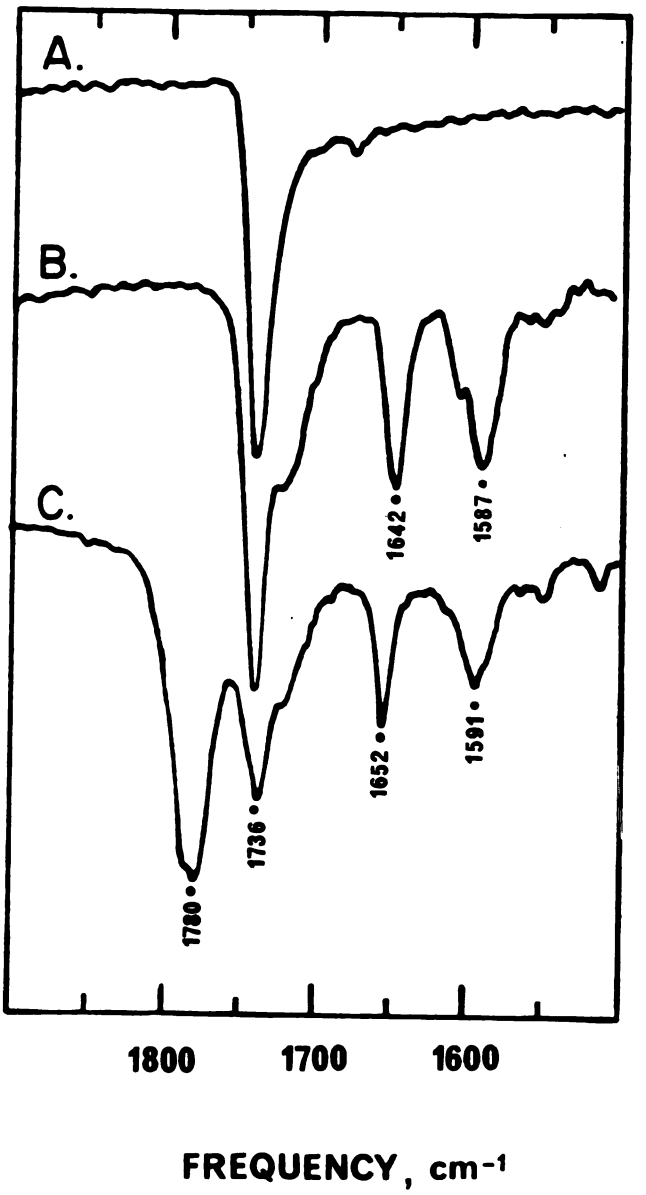


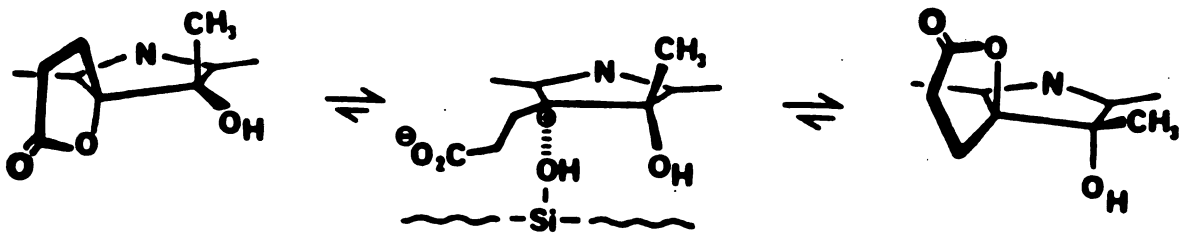
Figure 2 1900-1500 cm^{-1} IR spectra of (A) porphyrin; (B) dihydroxychlorin; (C) γ-spirolactone chlorin.

19a we also could not detect any NOE between the 3-Me peak (1.92 ppm) and the lactone protons. The remarkable similarity of the overall pattern between the model complex and the heme d derived lactones would thus argue that the model should likewise have a trans configuration about the pyrroline substituents. If this is true, it means that an inversion of the diol configuration has taken place, because the osmium tetroxide oxidation only affords cis diols.

In an effort to elucidate this possible inversion process, we have examined the lactonization of a number of south diol chlorins. The tetraethylchlorin complex 17b, by virtue of its superior chromatographic mobility on silica gel, proves to be a more informative system for delineating the reactions involved. When diol 17b is heated briefly in MeOH with sodium acetate on a steam bath, TLC (silica gel, 10% ethyl acetate in CH_2Cl_2) indicates that the slow-moving diol (Rf 0.3) is cleanly converted into a fast-moving spot (Rf 0.8). Both mass spectral (diol 17b minus 32) and IR (1780 cm^{-1}) analyses suggest that this compound is a lactone, but ^1H NMR shows the lactone methylene protons merged together between 3.3 and 3.8 ppm, distinctively different from that of 11a or the lactochlorin methyl ester.[6] If this green compound is rechromatographed on TLC, an even faster moving spot (Rf 0.88) emerges when the major spot is halfway up on the plate. If the plate is sufficiently long or the chromatography is repeated, the new pigment will eventually replace the original one and become the major spot. This new compound, as shown by mass spectroscopy and IR after isolation is still a lactone and has ^1H NMR features very similar to those of the trans lactones observed earlier. This compound can also be shown to be

identical with the lactone prepared by repeated chromatography of the diol **17b**.

On the basis of these observations, we have assigned the NaOAc-cyclized product to be the cis lactone, while the silica gel induced lactone is trans (the NMR assignments for the cis and trans chlorin lactones are discussed in detail in Chapter 2). The cis-trans isomerization is illustrated in Scheme 4, with the key step being a unimolecular alkyl-oxygen fission process.

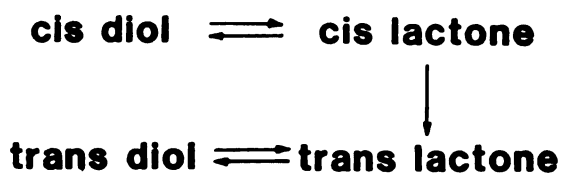


Scheme 4

The north diol chlorins (**16a** and **16b**), lacking the propensity to lactonize, apparently can resist inversion upon repeated chromatography on silica gel; we have not observed another isomeric diol during chromatography or base-catalyzed conditions. A possible indirect way then, for the cis-trans diol conversion might be through silica gel-promoted lactone opening. Indeed, heating the cis lactone **18b** and trans lactone **19b** under reflux in pyridine-30% aq. KOH for prolonged periods of time, resulted in their hydrolysis giving back the diols. The product from the cis lactone **18b** was identical in all respects to the cis diol **17b**, whereas the product from the trans lactone **19b** was identified by spectral analyses (see Chapter 2) as being the trans diol

20. If repeatedly chromatographed on TLC plate, both diols would eventually convert to the trans lactone 19b.

From all the above, the following general Scheme 5 for the cis-trans diol and lactone transformations can be written.



Scheme 5

Treating the dihydroxychlorin 16a in CH_2Cl_2 with 70% $HClO_4$ cleanly produced the rearranged ketones 21b and 22b in equal amount. The two isomers were separated by chromatography and their structures were determined by nuclear Overhauser enhancements (NOE) on the proton resonances. Selective irradiation of the methyl substituents resulted in NOEs (>5%) at the adjacent positions; by determining the nearest meso protons it is possible to assign the structures unambiguously (see Figure 3). Similar reaction and characterization were applied successfully for the tetramethyl homologues 21a and 22a. It is interesting to note that the NH protons of 21a and 21b should appear as two peaks while they remain as singlet in 22a and 22b. It is not evident whether an alteration of the tautomeric patterns or structural distortions is a possible cause for the splitting of the NH resonance.

The oxochlorin (porphyrinone) 22a reacted sluggishly with methylenetriphenylphosphorane. The excess Wittig reagent present in the reaction invariably converted the ester group into the β-keto methylphosphonium salt.[30] Thus the methyl ester 22a was first hydrolized in aqueous KOH, and the carboxyl groups were protected as the

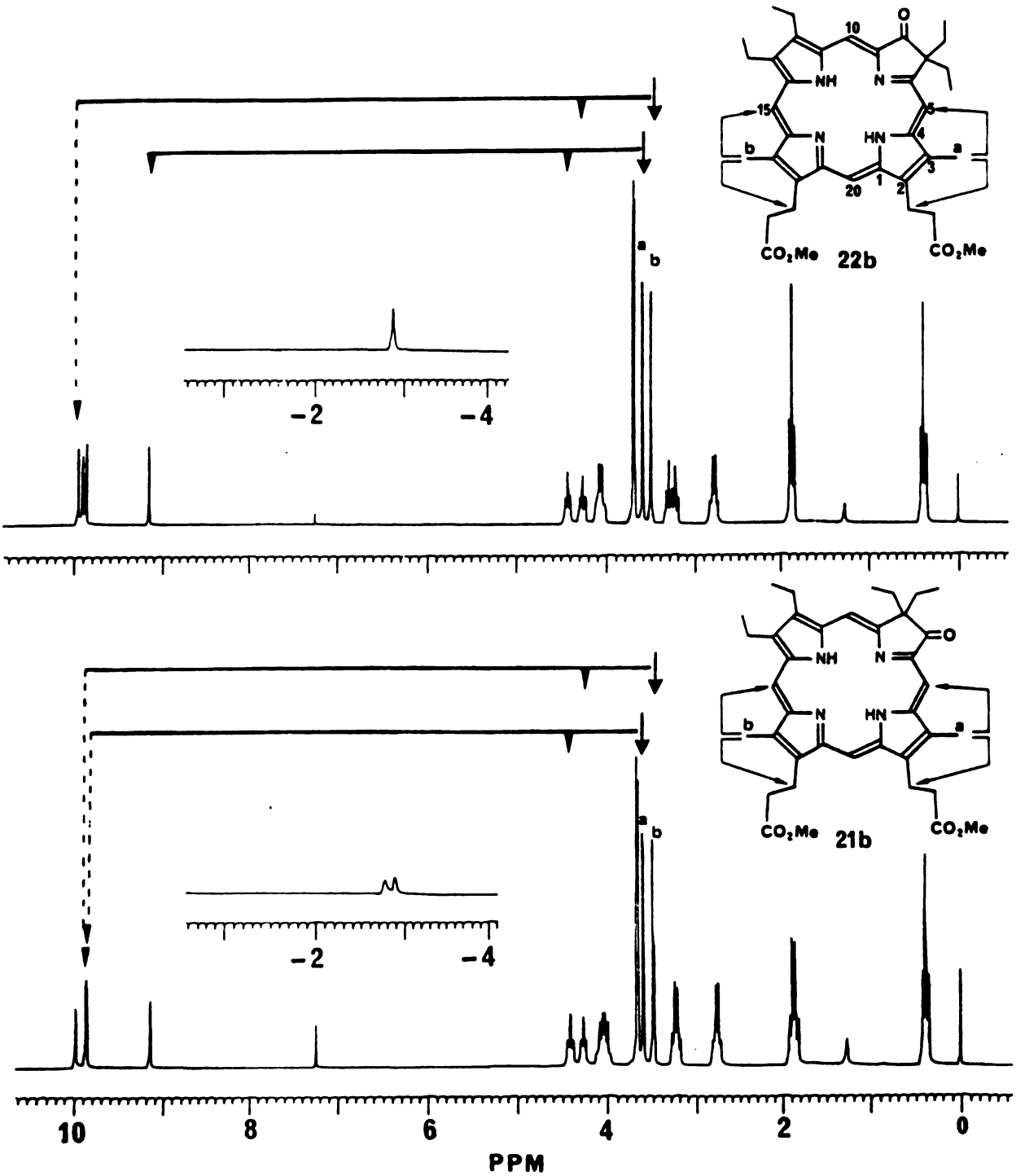
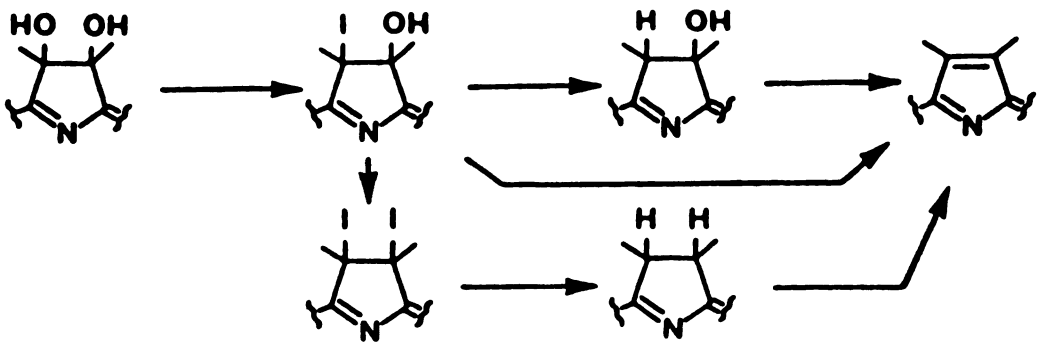


Figure 3 250 MHz ^{1}H NMR spectra of 21b and 22b. Irradiations of the methyl resonances as indicated resulted in NOE observable at the neighboring groups whose chemical shifts are marked by the pointers.

carboxylate ion during the Wittig reaction.[31] The resultant methylenechlorin was esterified and then hydrogenated quantitatively to the methylchlorin 24 with PtO_2 in formic acid.

The dihydroxychlorin isomers 16a/17a and 16b/17b have almost identical visible absorption spectra whose overall features are indistinguishable from that of the common dihydroporphyrins or the methylchlorin 24. The dihydroxychlorins are inert toward quinone oxidation; at room temperature they are relatively stable in most acids (including concentrated HCl) and undergo the pinacolic rearrangement only with >60% sulfuric acid or perchloric acid. When left in concentrated HI/HOAc, the dihydroxychlorin slowly changes into porphyrin presumably via one of the sequences in Scheme 6. This conversion is

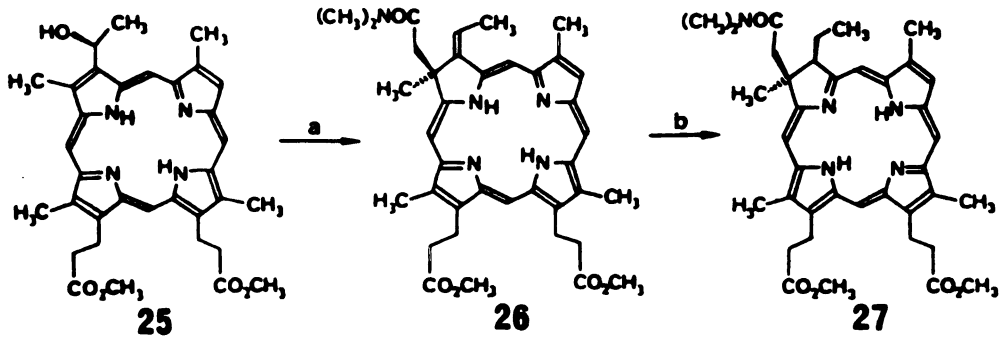


Scheme 6

probably responsible for Barrett[32] to propose that porphyrin d from Aerobacter aerogenes is a dihydroporphyrin. Reductive removal of OH group by HI was also used previously by Chang to prepare symmetric alkylated chlorins and isobacteriochlorins.[18] The present series of C-methyl- and C-hydroxychlorindipropionic acids is particularly useful for hemoprotein reconstitution studies.

Several rational approaches toward the synthesis of C-alkyl chlorins, starting with alicyclic precursors, have been described very recently.[33-35] Unfortunately these lengthy and demanding syntheses do not lend themselves as a more serviceable route than harvesting organisms for providing the compound. Our approach from the vic-diols would seem to be a highly attractive route at providing the C-substituted chlorins.

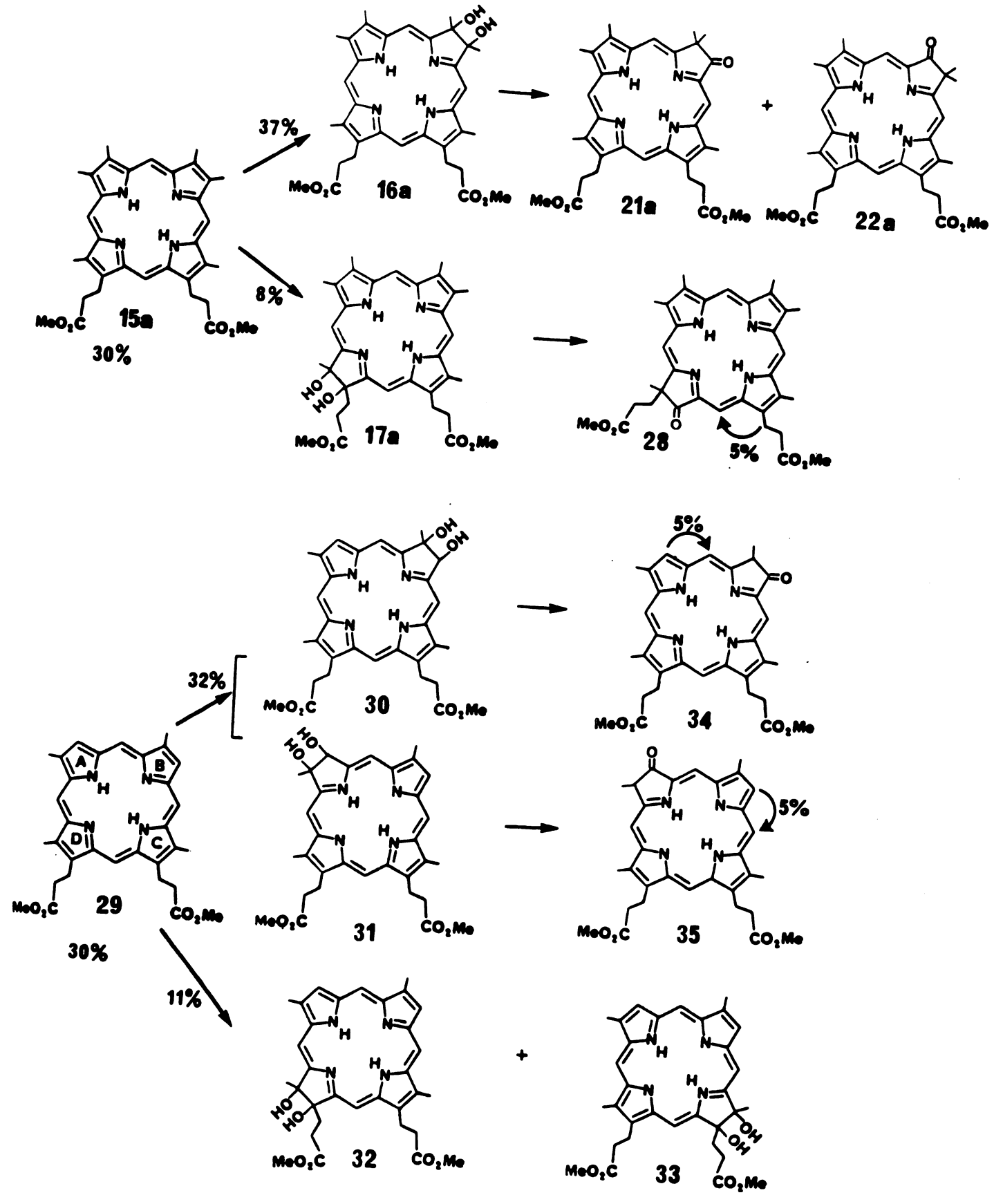
There is one recent report[36] that a C-alkyl chlorin (27) can be obtained from a hydroxy porphyrin (25) by a Claisen rearrangement followed by hydrogenation (Scheme 7). The generality of this approach, however, seems to be limited.



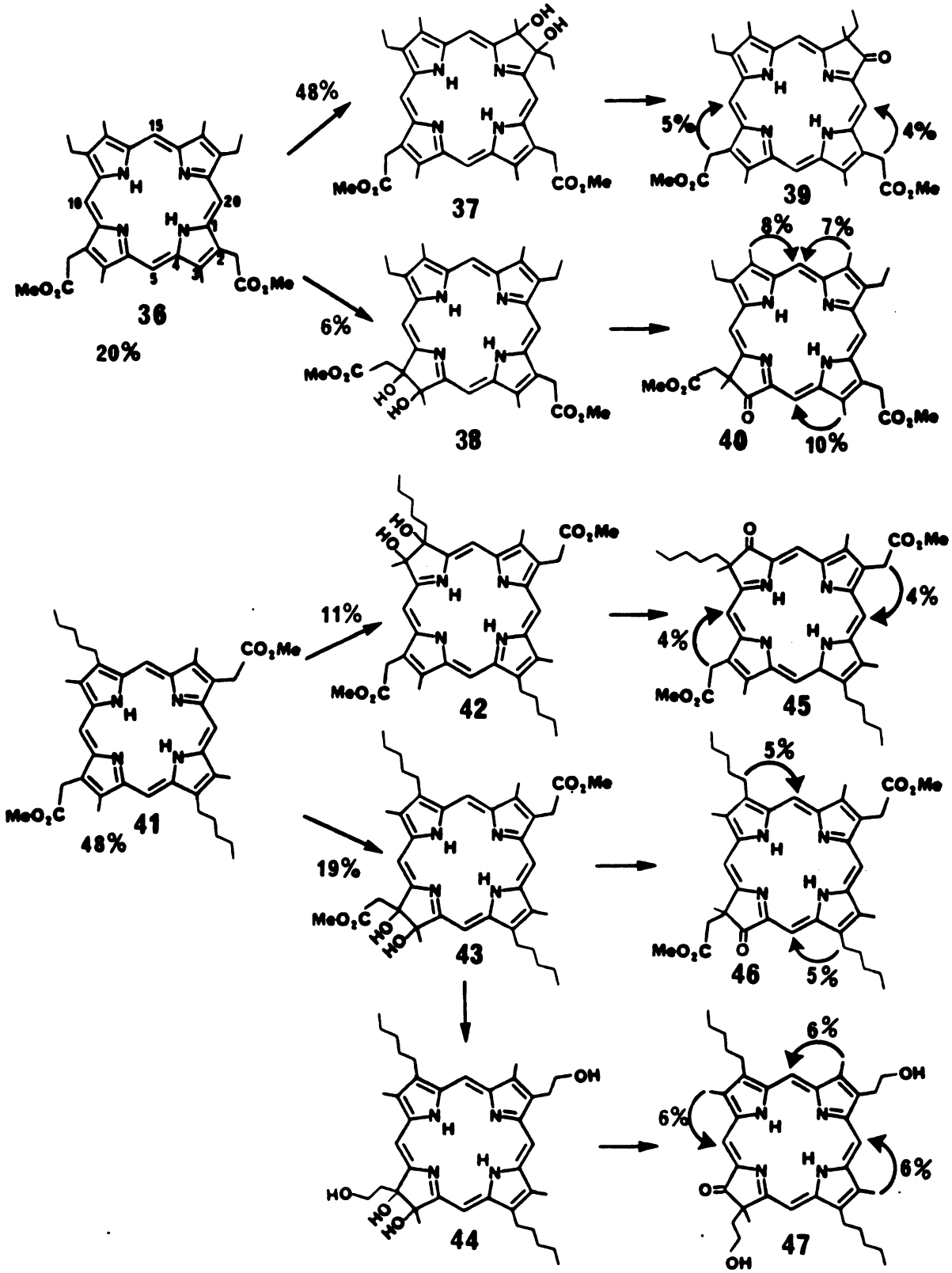
Scheme 7. Reagents: (a) $CH_3C(OCH_3)_2N(CH_3)_2$; (b) H_2, Pd/C

B. Migratory Aptitudes in Pinacol Rearrangement of *vic*-Dihyroxychlorins.

Four porphyrins 15a, 29, 36, 41 were chosen for investigation.[37] These porphyrins were converted into the dihydroxychlorins using osmium tetroxide as reported.[38] The porphyrins were allowed to react with 1.2 equivalents of osmium tetroxide in methylene chloride and the reaction was quenched after 20 hours with the osmium esters being decomposed by hydrogen sulfide. Analyses (tlc, silica gel, methylene chloride-



SCHEME 8a



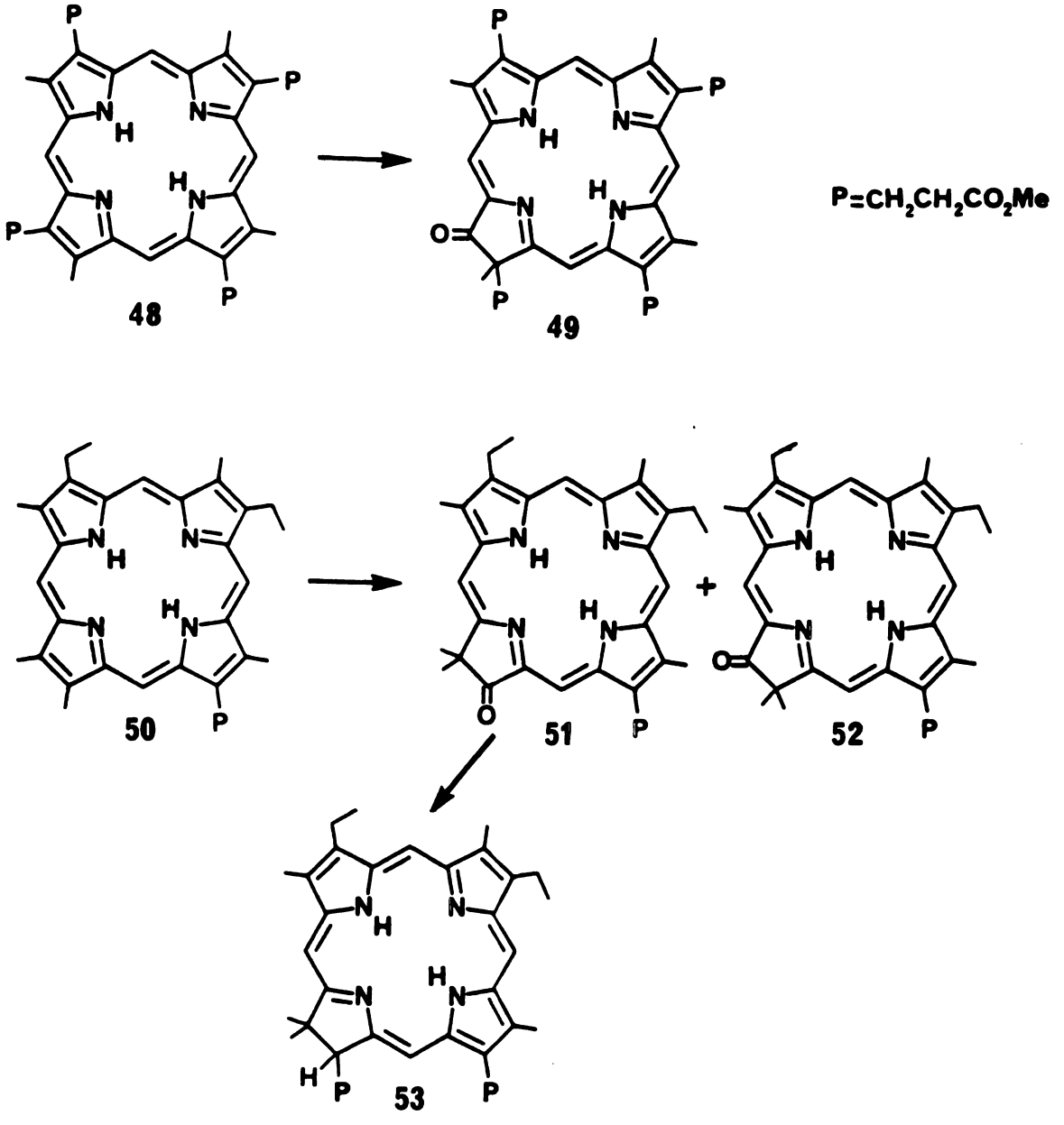
SCHEME 8b

methanol) indicated that the reaction mixtures contained variable amounts of chlorins plus the unreacted porphyrin. Isolation of individual components by chromatography and crystallization afforded the chlorin isomers as well as the starting porphyrin with yields shown in Scheme 8. The formation of the osmium esters is highly dependent on the porphyrin substituents. The relative yields of the chlorins thus produced a crude reactivity scale for the osmium tetroxide addition to porphyrin β-β′ double bonds: as would be expected, barring electronic effects, the larger the side chain, the slower the rate. The chlorin structures were determined by ^{1}H NMR and by mass spectra. In the case of deuteroporphyrin dimethyl ester 29, the separation of A-ring and B-ring chlorins was difficult; the mixture was employed for the subsequent rearrangement study.

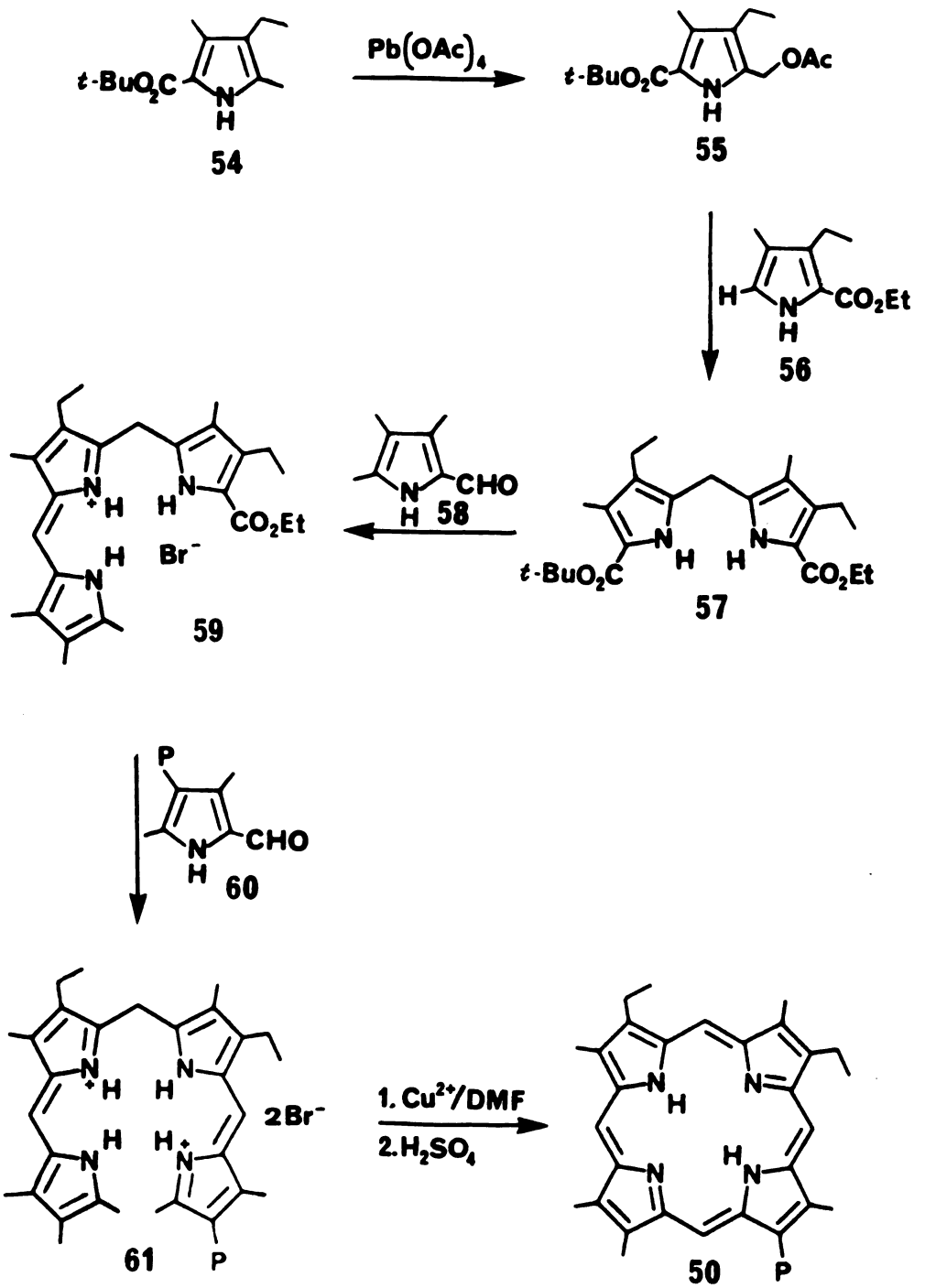
The acid-catalyzed pinacol-pinacolone rearrangement required different acid strength depending on whether the substituents are electron releasing or withdrawing. For example, while diol 16a was converted smoothly into equal amounts of the two ketones by one drop of 70% perchloric acid in methylene chloride, the rearrangement of 38 required dissolution in 98% sulfuric acid for 2 hours. Except for 15a, each diol gave only one oxochlorin (porphyrinone) with a yield generally greater than 80% (only 40 and 46 were obtained with ~30% yield). The structure of the porphyrinones was established by nuclear Overhauser enhancements (NOE). Selective irradiation of methyl or methylene protons resulted in enhancement (≥4%) at the nearest meso protons (see Scheme 8). Since the meso proton adjacent to the reduced ring, but not next to the keto group, invariably appears as a singlet near 9.0-9.1 ppm and the other three meso protons are around 9.5-9.9 ppm, it is possible

to assign the structures unambiguously. In the case of the mixture of **34** and **35**, irradiation of the 3 and 8-pyrrole protons resulted in a strong enhancement of both the 9.12 and 9.50 ppm meso protons; had the rearrangement gone the other direction, NOE should occur only at the two downfield meso protons, not at the 9.12 ppm peak. These experiments thus established the migratory aptitudes of the substituents; hydrogen, ethyl, alkyl groups including propionate side chains will migrate over methyl group. The only group that has a lower mobility than methyl is acetate; this is confirmed in two compounds **40** and **46**. It seems that the electron-withdrawing nature of the acetate plays a determinant role. If the acetate group is first reduced with $LiAlH_4$, the pinacol rearrangement of the 2-hydroxyethyl group has been found to migrate over the methyl group.

Applications. The knowledge of the migratory aptitudes, besides being useful in discerning possible biosynthetic precursors, is immediately applicable in planning new chlorin syntheses. For example, starting with coproporphyrin I (**48**), the above hydroxylation-rearrangement sequence gave a type III coprochlorin **49**. Alkylation of the keto groups by Wittig reagents as described before[20] would afford all-alkyl chlorins (Scheme 9). Similarly, porphyrin **50**, prepared by stepwise assembling of the a,c-biladiene dihydrobromide **61** followed by Cu(II)-catalyzed cyclization[38] (Scheme 10), produced two easily separable gem-dimethyl porphyrinones, one of which, **51**, after coupling with the appropriate Wittig reagent and hydrogenation, would provide and easy entry into the family of the exotic echurian pigment bonellin **53**. Previously, (±) bonellin has been made by a rather long synthesis.[34b]



SCHEME 9


t-BuO2C
Pb(OAc)4
OAc
54
55
H
CO2Et
56
CHO
58
Br-
59
57
2Br-
1. Cu2+/DMF
2. H2SO4
P
60
61
50

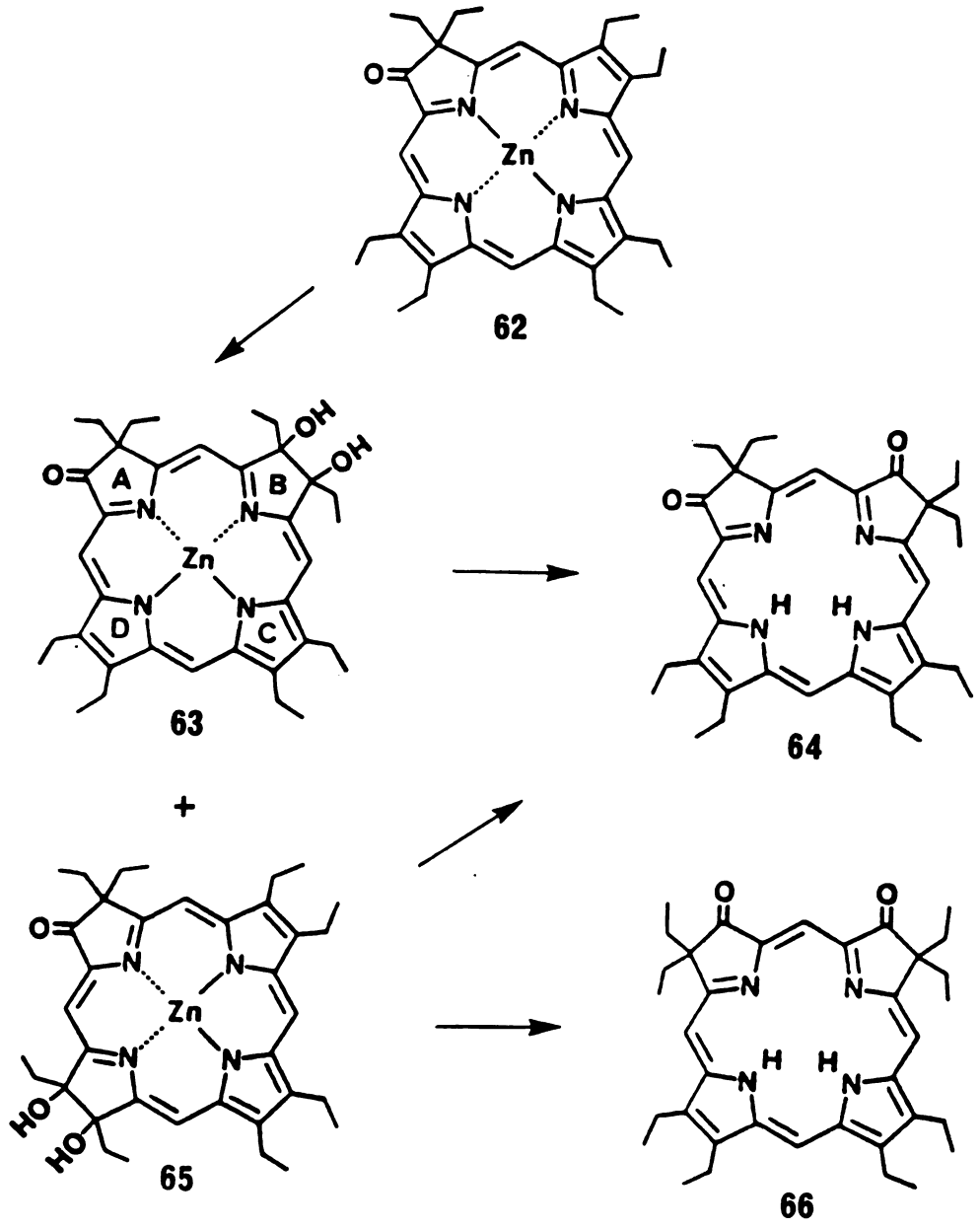
SCHEME 10

Finally, the total synthesis of the green heme prosthetic group in cytochrome cd_1 achieved recently,[7e] was based on the knowledge of the migratory aptitudes.

C. Differentiation of Bacteriochlorin and Isobacteriochlorin Formation by Metalation.

The OsO_4 addition preference can be altered dramatically in favor of the isobacteriochlorin formation simply by metalation of the ring. The zinc complex 62 was found to react with OsO_4 (1.5 equiv.) in CH_2Cl_2 containing 1% pyridine to give predominantly 63 (>60% yield) which can be treated with sulphuric acid to give 64. A small amount of the ring D diol 65 was also obtained which rearranged to yield about equally 64 and 66. If the synthetic goal is 64, the crude dihydroxylation product can be used directly in the pinacol rearrangement as the ratio of 64:66 is usually greater than 30. That the osmate addition mainly occurred at ring B (63) is possibly due to the electron withdrawing effect of the carbonyl group rendering the adjacent ring D double bond less reactive. It is also noteworthy that during the pinacol rearrangement of 63 or its free base, none of the possible porphyrin-2,8-dione was observed. Insertion of other metal ions such as Cu^{II} and Ni^{II} had the same effect of switching the osmate addition pattern but the yields of osmate esters were less satisfactory.[39] The remarkable alteration of site of attack by metalation in the chlorin system appears to be a general phenomenon.

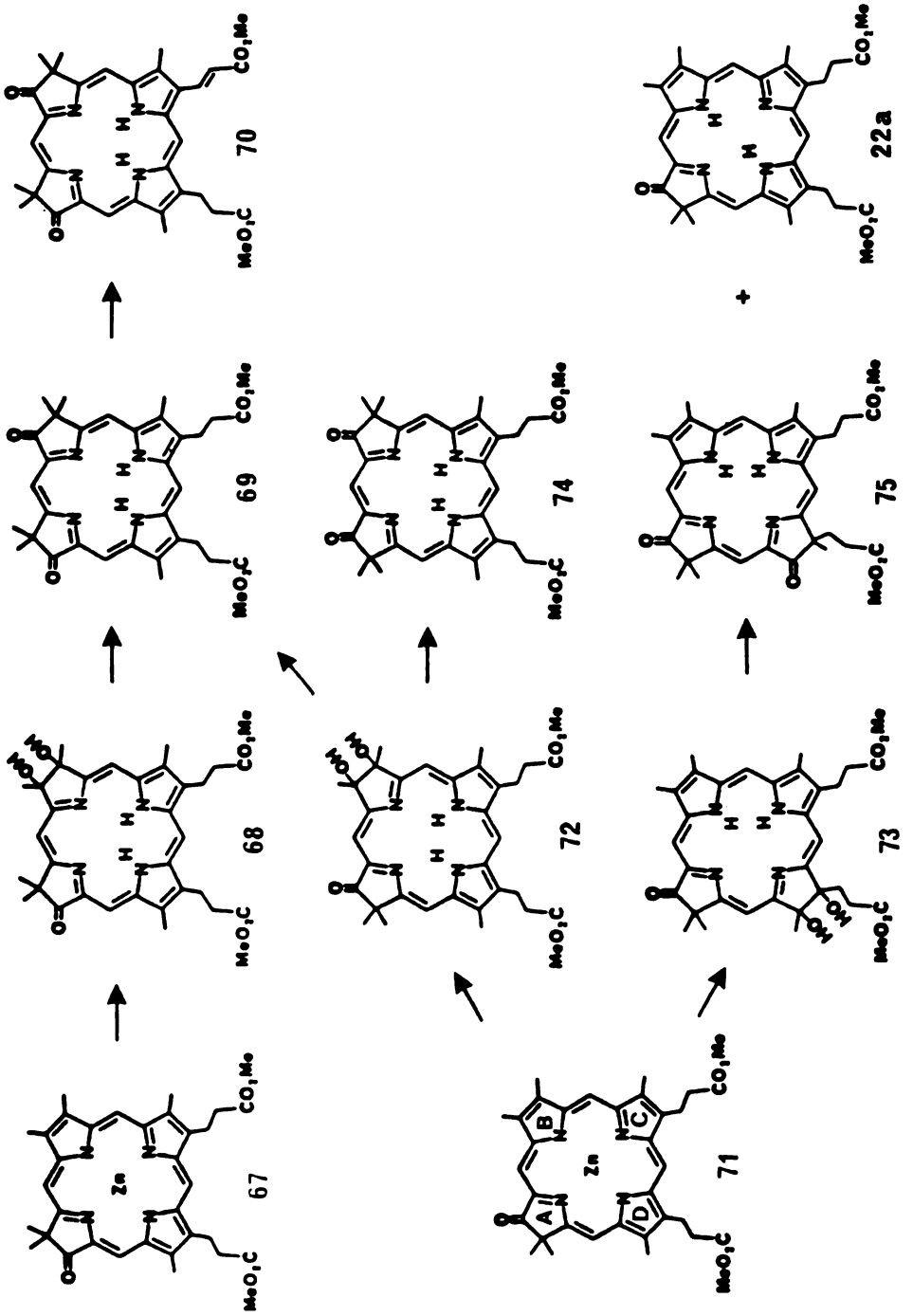
The previously observed diimide reduction of $Zn^{II}TPP$[21] as well as the reduction of Ni^{II} pheophorbides[22] all serve to attest the significance of the metalation effect. The tautomerization patterns were thought to be more equalizing in a metal chlorin than in a free base chlorin to promote isobacteriochlorin formation. This hypothesis



SCHEME II

did not explain why the double bond saturation occurs exclusively at the adjacent ring in the metal complex since one would expect that the absence of a preferred π-delocalizing pattern only favors a more random attack. Neither did previous MO calculations of ZnTPC show a significant difference in π-electron density between the opposite and the adjacent β-β′ double bonds.[40] Presently, extended Huckel calculations are being undertaken on the metalloporphyrinone system using the newly acquired crystal structure parameters of Ni^{II} OEP-porphinone.[41] It is hoped that the refined calculations may uncover clues to explain this phenomenon.

The selective saturation of the porphyrinone double bonds has made possible the synthesis of a variety of porphyrin-2,7-diones with side chains at specific positions. For example, the stereochemically uncomplicated dione 69 and its acrylic derivative 70, either in solution or in a reconstituted protein environment, proved to be accurate model compounds and spectral probes for heme d_1. The dione 69 could be prepared by the H_2O_2-H_2SO_4 oxidation of 15a, with a <2% yield after tedious separations from a mixture of no less than 10 oxo products.[42] With the zinc method, 69 was prepared from 15a[20] cleanly with a high yield (Scheme 12), and the unreacted starting material in the OsO_4 oxidation of 15a and 67 could always be recovered for recycling. The intermediacy of the porphyrinone 67 seems necessary. Attempts to react the Zn complex of 15a or Zn^{II} octaethylporphyrin directly with an excess of OsO_4 have only resulted in intractable pigments. The two-stage oxidation via isolated porphyrinone has also imparted a higher degree of regioselectivity for the isobacteriochlorin-type porphyrindione formation. In the present study, if the isomeric 71[20] is used, the



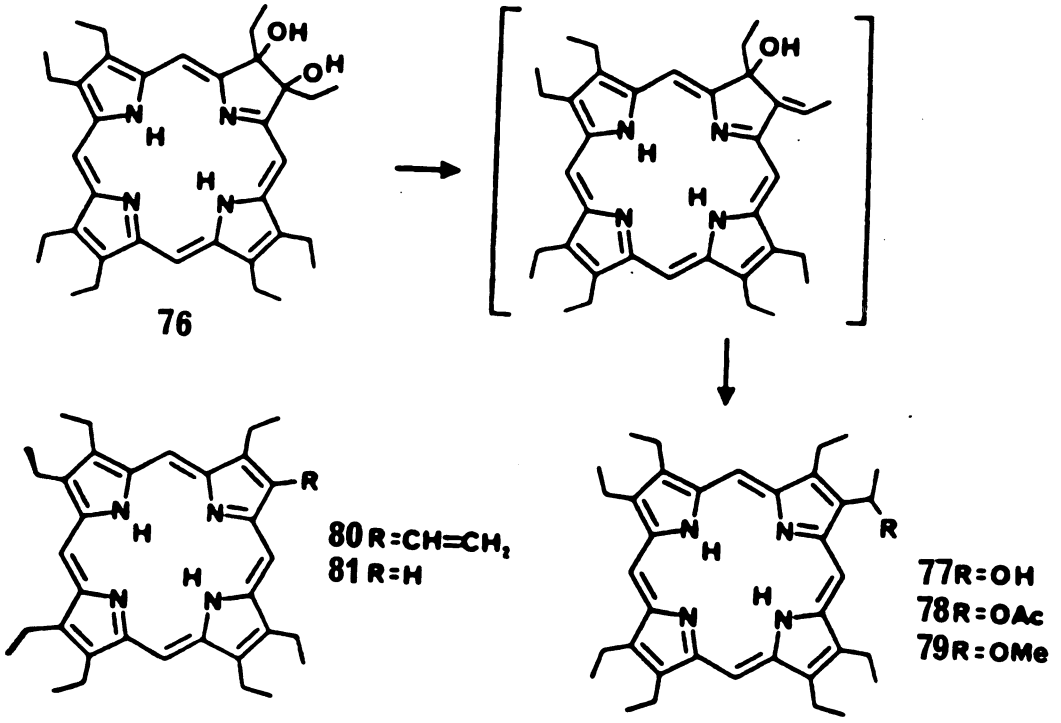
SCHEME 12

major product is 73 under reaction times > 36 h, with osmate selectivity of ring D vs. B 4:3. However, for shorter reaction times (24 h) the osmate selectivity is not obvious (ring B vs. ring D 1.2:1). In any case, the attack of ring D is much more favorable for porphyrinone 71 than for porphyrin 15a. The pinacolic rearrangement of 72 gave 69 and 74 in equal amounts. On the other had, the porphyrindione derived from the pinacolic rearrangement of 73 is exclusively 75, apparently reversing the migratory aptitude of methyl < propionate observed in simple vic-dihydroxychlorins[43] but fully agreeing with the above observation of porphyrinone diols.

Further reaction of free base 69 with 1.8 equiv. of OsO_4 resulted in exclusively a ring C-diol, presumably for the same reason cited above for 63: to avoid the carbonyl group next to ring D. Heating the diol in HCl-dioxane-H_2O or HCl-benzene, triggered an elimination of H_2O and yielded a β-hydroxy propionate which eliminates further to give the acrylate 70 in 80% yield using the first solvent system or quantitatively using the second one.

D. A Novel Method of Functionalizing the Ethyl Chain of Octaethylporphyrin.

When OEP-diol 76, is heated in aqueous HCl/dioxane, the major porphyrin component is OEP-alcohol 77 with yields never greater than 50%. This reaction seemed highly sensitive to the acid concentration: dilute acid gave insufficient reaction while too strong an acid only let to pinacol rearrangement. To improve the reaction, other nucleophilic media were tested. When 76 was dissolved in glacial acetic acid and heated at 90°C, the acetoxy 78 was obtained within 10 min in 85% yield. Likewise, if 76 in methanol was heated in the presence of HCl, the

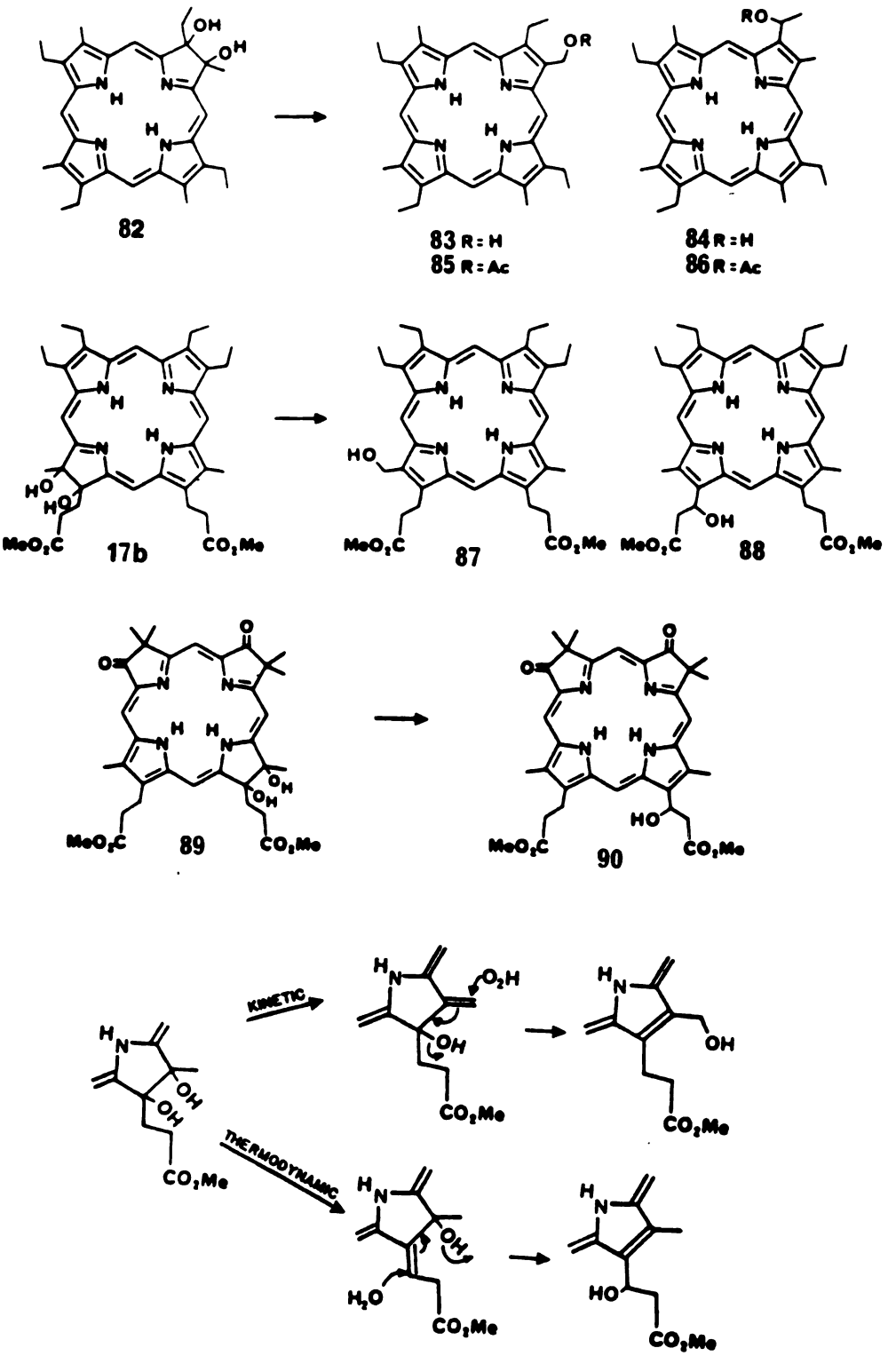


SCHEME 13

methoxy **79** can be isolated in >75% yields. Both **78** and **79**, of course, can serve as starting points for further derivatizations. Vinylation may also be achieved in a single step (90-95% yield) by heating **76** in benzene containing HCl. As **76** can be prepared readily from OEP by OsO_4 oxidation, the simple preparation of **80** therefore offers expeditious synthetic routes to a wide range of monosubstituted heptaethylporphyrins having for instance, Br,[44] CN,[45] CHO,[26,46] $COCH_3$,[26] $CH{=}CHCO_2R$,[45,47] CH_2OH,[48] CH_2CH_2OH,[49] $CH_2CH_2CO_2R$,[49] plus other moieties attached via these groups. The Experimental Section includes a procedure for making **81** by devinylating **80**. It was also observed that the preparation of **77**-**81** can be scaled up without lowering the yield.

When the above procedures are applied to other vic-diols with dissimilar alkyl chains, two products are expected. Indeed, dihydroxyetiochlorin **82** has been found to yield the two alcohols **83** and **84** or the two acetoxyetioporphyrins **85** and **86** under appropriate conditions. The same is true with porphyrins bearing methyl and propionate substituents (e.g., **87** and **88**). While the ratio of the two possible products, varies from case to case, the methyl group appears to be the more favorable site of attack, at least with common dihydroxychlorins. Such a result would suggest that the reaction is subject more to kinetic control.

With porphyrinones, the course of the reaction can be dependent upon the symmetry of the molecule as well as the reaction conditions employed.[39] However, in our recently reported synthesis of heme d_1 analogue, **89** gave exclusively **90** before it was dehydrated to the acrylate.[23] In this case, the stability of the exocyclic alkene intermediate seems to be the determinant factor. The specificity associated with these molecules further suggests that the mild


82
83 R = H
85 R = Ac
84 R = H
86 R = Ac
17b
87
88
89
90
KINETIC
THERMODYNAMIC

SCHEME 14

elimination-hydration of vic-diol (or an epoxide precursor) may have some biosynthetic significance. We speculate that the acrylate group of heme d_1 is indeed produced biosynthetically from a propionate side chain by this route. The demonstrated conversion[25] from chlorophyll a to chlorophyll b via a vic-diol could be a viable biosynthetic pathway. As for the heme a moiety of cytochrome oxidase,[50] the 18-carboxaldehyde group could come from a CH_3, not by harsh direct oxidation but by way of CH_2OH resulted from the vic-diol. These hypotheses possibly can be tested by future experiments.

III. EXPERIMENTAL

General

NMR spectra were obtained at 250 MHz on a Bruker WM-250 instrument. Spectra were recorded in $CDCl_3$; the residue $CHCl_3$ was used as the internal standard set at 7.24 ppm. Nuclear Overhauser enhancements (NOE) were measured by difference between a spectrum with preirradiation on a target peak minus a spectrum with equivalent preirradiation at a dummy position. Typical parameters: D_1 (relaxation delay) $\approx 5T_1$=1.5 sec, D_2 (NOE generation time) = 0.03 or 0.095 sec, D_3 (pulse interval) = 0.1-0.3 sec, DP (decoupling power) = 26 or 36 L (depending on D_2), PW = 90-degree pulse. Magnitudes of NOEs were calculated as the area of the enhanced resonance in difference spectra divided by the area in the control spectrum with no enhancement. Mass spectra were obtained using a Finnigan 4021 GC-MS (direct insertion probe, 70ev, 200-300°C), or a JEOL HX 110-HF spectrometer equipped with a fast atom bombardment (FAB) gun. A matrix of thioglycerol-dithioerythreitol-dithiothreitol (2:1:1) containing 0.1% trifluoroacetic

acid was used for the FAB-MS. Elementary analyses were performed by MicAnal. Visible absorption spectra (in CH_2Cl_2) were measured with a Cary 219 or a Shimadzu 160 spectrophotometer. IR spectra were obtained from KBr pellets on a Perkin-Elmer 283B spectrophotometer. Melting points were obtained on an electrothermal melting point apparatus and are uncorrected. Preparative TLC plates were from Analtech (silica gel G, 1000 or 1500 μm). Methylene chloride and pyridine were distilled from CaH_2, THF from $LiAlH_4$, and methanol from sodium before use.

Dimethyl cis-7,8-dihydroxy-3,7,8,12,13,17-hexamethylchlorin-2,18-dipropionate (16a)

Osmium tetroxide (300 mg, 1.2 mmol) in anhydrous ether (3 ml) was added to a methylene chloride (200 ml) solution of dimethyl 3,7,8,12,13,17-hexamethylporphine-2,18-dipropionate (15a)[27] (566 mg, 1 mmol). Dry pyridine (0.2 ml) was added subsequently, and the mixture was allowed to stir at room temperature, under nitrogen, in the dark for 20 h. It was then diluted with methanol (100 ml) and bubbled with H_2S for 10 min in order to decompose the osmium ester. The precipitated osmium sulfide was removed by filtration, and the crude product in the filtrate was chromatographed on a silica gel column. Unreacted porphyrin was eluted first with CH_2Cl_2/3% MeOH. The slower moving major isomer which turned out to be 16a was crystallized from CH_2Cl_2-hexane. The mother liquor combined with the faster moving component was chromatographed once again by preparative TLC (CH_2Cl_2/2% MeOH) to give pure 16a yields: unreacted porphyrin, 164 mg, 30%; 16a, 220 mg, 37%; 17a, 50 mg, 8%.

16a: NMR δ 2.10, 2.12 (3H each, s, 7,8-Me), 3.11, 3.13 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.40 (6H, s, 2xMe), 3.43, 3.45 (3H each, s, Me), 3.63,

3.66 (3H each, s, CO_2Me), 4.15, 4.22 (2H each, t, $CH_2CH_2CO_2$), 9.07, 9.09 (1H each, s, 5,10-H), 9.68 (2H, s, 15,20- H), -2.78 (2H, br s, NH); UV-vis λ_{max} (ε_M) 642 nm (44 700), 614 (3 800), 588 (4 700), 522 (3 400), 495 (13 300), 490 (13 300), 392 (179 000); MS, m/e 600.2936 (calcd for $C_{34}H_{40}N_4O_6$ 600.2950).

Dimethyl cis-2,3-dihydroxy-3,7,8,12,13,17-hexamethylchlorin-2,18-dipropionate (17a)

NMR δ 2.12 (3H, 2, 3-Me), 2.47-2.83 (4H, m, 2-$CH_2CH_2CO_2$), 3.16, 3.18 (1H each, t, 18-$CH_2CH_2CO_2$), 3.36, 3.37, 3.39, 3.45, 3.46 (3H each, s, ring Me), 3.50 (3H, s, 2-$CCCO_2Me$), 3.68 (3H, s, 18-$CCCO_2Me$), 4.16, 4.20 (1H each, t, 18-$CH_2CH_2CO_2$), 9.08, 9.10 (1H each, s, 5,20-H), 9.58, 9.67 (1H each, s, 10,15-H), -2.91 (2H, br s, NH); UV-vis λ_{max} (ε_M) 643 nm (43 400), 614 (3 400), 589 (3 900), 521 (2 500), 495 (13 400), 490 (13 400), 392 (188 000).

Methyl cis-3-hydroxy-3,7,8,12,13,17-hexamethyl-2,2-γ-spirolactone-chlorin-18-propionate (18a)

Prepared either by heating the dihydroxychlorin, 17a, in methanol with sodium acetate under reflux for 20 min, or by repeated chromatography on preparative TLC plates. NMR δ 1.80 (3H, s, 3-Me), 3.05 (2H, t, 18-$CH_2CH_2CO_2$), 3.21-3.83 (4H, m, 2-$CH_2CH_2CO_2$), 3.30 (6H, s, 2xMe), 3.34, 3.40, 3.43 (3H each, s, ring Me), 3.48 (3H, s, CO_2Me), 3.87 (br s, OH), 4.17 (2H, m, 18-$CH_2CH_2CO_2$), 9.02, 9.08 (1H each, s, 5,20-H), 9.59, 9.71 (1H each, s, 10,15-H), -2.65 (2H, br s, NH); UV-vis λ_{max} (ε_M) 641 nm (41 600), 614 (3 500), 588 (4 000), 520 (3 100), 493 (12 900), 488 (13 100), 389 (176 000); MS, found: m/e 569.2753 for $(M+H)^+$, $C_{33}H_{37}N_4O_5$ requires m/e 569.2766.

Methyl trans-3-hydroxy-3,7,8,12,13,17-hexamethyl-2,2-γ-spirolactone-chlorin-18-propionate (19a)

Obtained from the cis-lactone **18a** by repetitive chromatography. NMR δ 1.92 (3H, s, 3-Me), 2.38 (1H, sext, $2a_4$), 2.98 (1H, oct, $2b_2$), 3.16 (2H, t, 18-$CH_2CH_2CO_2$), 3.21 (1H, $2b_3$), 3.35 (6H, s, 2xMe), 3.39, 3.40, 3.50 (3H each, s, Me), 3.47 (1H, $2a_1$), 3.66 (3H, s, CO_2Me), 4.18 (2H, t, 18-$CH_2CH_2CO_2$), 8.86, 8.91 (1H each, s, 5,20-H), 9.63, 9.71 (1H each, s, 10,15-H), -2.58 (2H, br s, NH); UV-vis λ_{max} (ε_M) 643 nm (34 800), 590 (3 400), 520 (2 500), 492 (11 400), 488 (11 100), 388 (150 000); MS, found: m/e 569.2761 for $(M+H)^+$, $C_{33}H_{37}N_4O_5$ requires m/e 569.2766.

Dimethyl cis-7,8,12,13-tetraethyl-7,8-dihydroxy-3,17-dimethylchlorin-2,18-dipropionate (16b)

Osmium tetroxide (90 mg, 0.35 mmol) in ether (1 ml) was added to dimethyl 7,8,12,13-tetraethyl-3,17-dimethylporphine-2,18-dipropionate **15b**[28] (170 mg, 0.27 mmol) in CH_2Cl_2 (50 ml), followed by dry pyridine (0.1 ml). The reaction was allowed to proceed in the dark for 20 h and worked up in the same manner as described above. The products isolated according to their elution pattern from the silica gel column were the unreacted porphyrin (20 mg, 11.8%), isomer **16b** (50 mg, 28%), and isomer **17b** (47 mg, 28%). (Notice that the isomer **16b** is the faster moving chlorin in this case).

16b: NMR δ 0.90, 0.98 (3H each, t, 7,8-CH_2CH_3), 1.74, 1.76 (3H each, t, 12,13-CH_2CH_3), 2.50, 2.58 (2H each, q, 7,8-CH_2CH_3), 2.93, 3.06 (2H each, t, $CH_2CH_2CO_2$), 3.27, 3.37 (3H each, s, Me), 3.59, 3.66 (3H each, s, CO_2Me), 3.85 (4H, q, 12,13-CH_2CH_3), 3.94, 4.06 (2H each, t, $CH_2CH_2CO_2$), 8.92, 9.00 (1H each, s, 5,10-H), 9.46, 9.67 (1H each, s, 15,20-H), -2.62 (2H, br s, NH); UV-vis λ_{max} (ε_M) 643 nm (46 900), 614 (4

000), 590 (4 200), 522 (2 900), 494 (14 200), 490 (14 200), 392 (198 000).

Dimethyl cis-7,8,12,13-tetraethyl-2,3-dihydroxy-3,17-dimethylchlorin-2,18-dipropionate (17b)

NMR δ 1.79 (12H, m, 4xCH_2CH_3), 2.18 (3H, s, 3-Me), 2.52-2.92 (4H, m, 2-$CH_2CH_2CO_2$), 3.15 (2H, t, 18-$CH_2CH_2CO_2$), 3.48 (3H, s, 17-Me), 3.54 (3H, s, 2-$CCCO_2Me$), 3.68 (3H, s, 18-$CCCO_2Me$), 3.86, 3.90, 3.92, 3.96 (2H each, q, CH_2CH_3), 4.19 (2H, t, 18-$CH_2CH_2CO_2$), 9.04, 9.08 (1H each, s, 5,20-H), 9.71, 9.73 (1H each, s, 10,15-H), -2.57 (2H, br s, NH); UV-vis λ_{max} (ε_M) 643 nm (44 000), 615 (3 600), 590 (3 900), 521 (2 600), 494 (13 700), 490 (13 700), 392 (193 000).

Methyl cis-7,8,12,13-tetraethyl-3-hydroxy-3,17-dimethyl-2,2-γ-spirolactone-chlorin-18-propionate (18b)

Prepared the same way as 18a. NMR δ 1.79, 1.80, 1.81, 1.84 (3H each, t, CH_2CH_3), 1.86 (3H, s, 3-Me), 3.10, 3.11 (1H each, t, 18-$CH_2CH_2CO_2$), 3.26-3.85 (4H, m, 2-$CH_2CH_2CO_2$), 3.46 (3H, s, 17-Me), 3.52 (3H, s, CO_2Me), 3.87 (4H, q, 2xCH_2CH_3), 3.93, 4.00 (2H each, q, CH_2CH_3), 4.17, 4.20 (1H each, t, 18-$CH_2CH_2CO_2$), 9.10, 9.21 (1H each, s, 5,20-H), 9.76 (2H, s, 10,15-H), -2.62 (2H, br s, NH). IR 1780 cm^{-1}, 1736; UV-vis λ_{max} (ε_M) 640 nm (42 600), 614 (3 600), 586 (3 600), 521 (2 700), 492 (12 300), 488 (12 400), 389 (179 000); MS, found: m/e 625.3401 for $(M+H)^+$, $C_{37}H_{45}N_4O_5$ requires m/e 625.3392.

Methyl trans-7,8,12,13-tetraethyl-3-hydroxy-3,17-dimethyl-2,2-γ-spirolactone-chlorin-18-propionate (19b)

Prepared the same way as 19a. NMR δ 1.79 (12H, m, 4xCH_2CH_3), 1.96 (3H, s, 3-Me), 2.43 (1H, sext, $2a_4$), 3.04 (1H, oct, $2b_2$), 3.22 (1H, quint, $2b_3$), 3.50 (1H, sept, $2a_1$) [$J(2a_1,2b_2$ = 4.1 Hz, $J(2a_1,2b_3)$ = 9.6,

J($2a_1$,$2a_4$) = -12.8, J($2b_2$,$2b_3$) = -17.9, J ($2b_2$,$2a_4$ = 9.6, J($2b_3$,$2a_4$) = 9.6], 3.15 (2H, t, 18-$CH_2CH_2CO_2$), 3.52 (3H, s, 17-Me), 3.66 (3H, s, CO_2Me), 3.85 (2H, q, 2xCH_2CH_3), 3.88, 3.98 (2H each, q, CH_2CH_3), 4.20 (2H, t, 18-$CH_2CH_2CO_2$), 8.89, 9.04 (1H each, s, 5,20-H), 9.75 (2H, s, 10,15-H), -2.54 (2H, br s, NH). IR 1780 cm^{-1}, 1734, 1714; UV-vis λ_{max} (ε_M) 643 nm (34 800), 591 (3 300), 521 (2 800), 489 (10 900), 484 (10 800), 391 (162 000). MS, found: m/e 625.3387 for $(M+H)^+$, $C_{37}H_{45}N_4O_5$ requires m/e 625.3392.

Dimethyl trans-7,8,12,13-tetraethyl-2,3-dihydroxy-13,17-dimethylchlorin-2,18-dipropionate (20)

To a refluxing solution of trans-lactone 19b (22 mg, 0.035 mmol) in pyridine (20 ml) under argon, KOH (0.48 g) in water (1.6 ml) was added and the heating was continued for 7 h before the mixture was evaporated to dryness under reduced pressure. The residue was redissolved in cold dry methanol saturated with HCl gas. The mixture was allowed to stir in an ice-water bath for 10 min before being partitioned between CH_2Cl_2 and water. The organic layer was separated, washed three times with water, dried (Na_2SO_4) and evaporated to dryness. The product was chromatographed rapidly on TLC (10% EtOAc/CH_2Cl_2) to separate the slower moving trans diol 20 (8 mg, 36.4%) from the faster moving trans lactone 19b (10 mg, 43.5%).

20: NMR δ 1.76, 1.78, 1.79, 1.81 (3H each, t, CH_2CH_3), 1.86 (3H, s, 3-Me), 1.88, 2.06, 2.47, 2.66 (1H each, quint, 2-$CH_2CH_2CO_2$) [J($2a_1$, $2b_2$ = 7.3Hz, J($2a_1$,$2b_3$) = 7.3, J($2a_1$,$2a_4$) = -14.6, J($2b_2$,$2b_3$) = -14.6, J($2b_2$,$2a_4$) = 7.3, J($2b_3$,$2a_4$) = 7.3], 3.17 (2H, t, 18-$CH_2CH_2CO_2$), 3.34 (3H, s, 17-Me), 3.54 (3H, s, 2-$CCCO_2Me$), 3.67 (3H, s, 18-$CCCO_2Me$), 3.84 (6H, q, 3xCH_2CH_3), 3.97 (2H, q, CH_2CH_3), 4.22 (2H, t, 18-$CH_2CH_2CO_2$),

8.97, 9.03 (1H each, s, 5,20-H), 9.70, 9.72 (1H each, s, 10,15-H), -2.34 (2H, br s, NH); UV-vis λ_{max} (ε_M) 647 nm (35 900), 592 (3 800), 522 (2 700), 495 (10 900), 490 (11 100), 391 (150 000); MS, found: m/e 657.3649 for $(M+H)^+$, $C_{38}H_{49}N_4O_6$ requires m/e 657.3655.

Dimethyl 3,8,8,12,13,17-hexamethyl-7-porphinone-2,18-dipropionate (21a) and Dimethyl 3,7,7,12,13,17-hexamethyl-8-porphinone-2,18-dipropionate (22a)

Perchloric acid (70%, 1 ml) was added to a methylene chloride solution (60 ml) of 16a (150 mg, 0.25 mmol). The mixture was allowed to stir at room temperature for one-half hour before being extracted with water (3x, 60 ml each). The CH_2Cl_2 layer contained the mixture of 21a and 22a, which were separated on preparative TLC plates (silica gel, CH_2Cl_2/1% MeOH), yielding 60 mg (42%) each of the porphyrinones.

21a (slower component on TLC): NMR δ 2.09 (6H, s, 8,8-Me), 3.12, 3.15 (2H each, t, $CH_2CH_2CO_2$), 3.37, 3.43, 3.46, 3.49 (3H each, s, ring Me), 3.57, 3.59 (3H each, s, CO_2Me), 4.15, 4.30 (2H each, t, $CH_2CH_2CO_2$), 9.02, (1H, s, 10-H), 9.70 (1H, s, 5-H), 9.75 (1H, s, 15-H), 9.84 (1H, s, 20-H), -3.06, -2.86 (1H each, br s, NH); MS, m/e 582.2838 (calcd for $C_{34}H_{38}N_4O_5$); m.p. 265-266°C; UV-vis λ_{max} (ε_M) 642 nm (32 400), 585 (6 000), 546 (12 000), 508 (9 500), 490 (6 200), 404 (169 000).

22a (faster component on TLC): NMR δ 2.00 (6H, s, 7,7-Me), 3.13, 3.18 (2H each, t, $CH_2CH_2CO_2$), 3.39, 3.44, 3.46, 3.50 (3H each, s, ring Me), 3.59 (6H, s, 2xCO_2Me), 4.16 (2H, t, 18-$CH_2CH_2CO_2$), 4.32 (2H, t, 2-$CH_2CH_2CO_2$), 9.07 (1H, s, 5-H), 9.74 (1H, s, 10-H), 9.79 (1H, s, 20-H), 9.80 (1H, s, 15-H), -3.12 (2H, br s, NH). Irradiating the triplet at δ 4.32 caused the singlets at δ 9.79 and 3.50 to increase in intensity. Moreover, irradiating the singlet at δ 3.50 caused the singlet at δ 9.07 (most upfield meso proton) to increase in intensity. MS, m/e 582 (M^+);

m.p. 266-268°C; UV-vis λ_{max} (ε_M) 642 nm (32 300), 585 (5 500), 546 (11 300), 508 (8 500), 490 (5 600), 404 (151 000). Anal. calcd: C, 70.07; H, 6.58; N, 9.62. Found C, 70.18; H, 6.66; N, 9.57.

Dimethyl 8,8,12,13-tetraethyl-3,17-dimethyl-7-porphinone-2,18-dipropionate (21b) and Dimethyl 7,7,12,13-tetraethyl-3,17-dimethyl-8-porphinone-2,18-dipropionate (22b)

The dihydroxychlorin **16b** (50 mg, 0.076 mmol) in CH_2Cl_2 was treated with perchloric acid (70%, 1 ml), and the reaction was worked up in the same manner as described above to afford 19 mg of each (40%) of the isomeric porphyrinones. The structure assignment for the slower moving **21b** and the faster moving **22b** was achieved via NOE measurements (see Figure 3).

21b: NMR δ 0.38 (6H, t, 8,8-CH_2CH_3), 1.86 (6H, t, 12,13-CH_2CH_3), 2.75 (4H, q, 8,8-CH_2CH_3), 3.20, 3.24 (3H each, t, $CH_2CH_2CO_2$), 3.48, 3.59 (3H each, s, Me), 3.65, 3.66 (3H each, s, CO_2Me), 4.00, 4.06 (2H each, q, 12,13-CH_2CH_3), 4.25 (2H, t, 18-$CH_2CH_2CO_2$), 4.40 (2H, t, 2-$CH_2CH_2CO_2$), 9.13 (1H, s, 10-H), 9.84 (1H, s, 5-H), 9.85 (1H, s, 15-H), 9.95 (1H, s, 20-H), -2.91, -2.78 (1H each, br s, NH); UV-vis λ_{max} (ε_M) 642 nm (34 700), 586 (5 900), 546 (11 800), 508 (9 600), 490 (6 300), 406 (173 000).

22b: NMR δ 0.38 (6H, t, 7,7-CH_2CH_3), 1.85 (6H, t, 12,13-CH_2CH_3), 2.76 (4H, q, 7,7-CH_2CH_3), 3.21, 3.28 (3H each, t, $CH_2CH_2CO_2$), 3.48, 3.58 (3H, s, Me), 3.67, 3.68 (3H each, s, CO_2Me), 4.06 (4H, q, 12,13-CH_2CH_3), 4.25 (2H, t, 18-$CH_2CH_2CO_2$), 4.41 (2H, t, 2-$CH_2CH_2CO_2$), 9.13 (1H, s, 5-H), 9.84 (1H, s, 10-H), 9.88 (1H, s, 20-H), 9.93 (1H, s, 15-H), -2.90 (2H, br s, NH); UV-vis λ_{max} (ε_M) 642 nm (35 000), 586 (5 700), 546 (11 800), 508 (9 000), 490 (6 000), 406 (162 000).

Dimethyl 3,7,7,12,13,17-hexamethyl-8-methylenechlorin-2,18-dipropionate (23)

The methyl ester groups of porphyrinone **22a** (100 mg, 0.18 mmol) were hydrolyzed in a mixture of equal volume of THF and 2N aqueous KOH. The mixture was stirred for 12 h before the THF solvent was removed in a rotorvap. The remainder of the aqueous solution was acidified with HCl, and the precipitated porphyrinone diacid was collected by filtration, washed with water and dried.

To a suspension of Ph_3PCH_3Br (614 mg, 1.72 mmol) in dry THF (20 ml) was added an equivalent amount of n-butyllithium (1.6M solution in hexane) under nitrogen. The resultant orange suspension was allowed to stir at room temperature for 30 min before being added to a solution of the porphyrinone diacid (95 mg, 0.172 mmol) in dry THF (25 ml) at 0°C. The mixture was allowed to stir at room temperature for 12 h, after which time the reaction was quenched with water. The solvent was evaporated, and the residue was esterified in dry methanol (50 ml), saturated with HCl gas, and left overnight. The solvent was again evaporated, and the residue was taken in CH_2Cl_2, washed with water, and chromatographed on silica gel (CH_2Cl_2). The methylenechlorin **23** (68 mg, 71% yield), migrating in front of the unreacted **22a** (20 mg), was further purified by crystalization from CH_2Cl_2/hexane: m.p. 229-231°C; NMR δ 2.03 (6H, s, gem-Me), 3.17, 3.20 (2H each, t, $CH_2CH_2CO_2$), 3.41 (6H, s, 2xMe), 3.45, 3.49 (3H each, s, Me), 3.66, 3.67 (3H each, s, CO_2Me), 4.19, 4.33 (2H each, t, $CH_2CH_2CO_2$), 5.81, 6.78 (1H each, s, $=CH_2$), 8.86, 9.38 (1H each, s, 5,10-H), 9.65, 9.71 (1H each, s, 15,20-H), -2.54 (2H br s, NH); MS, m/e 580.3049 (calcd for $C_{35}H_{40}N_4O_4$ 580.3052); UV-vis λ_{max} (ε_M) 656 nm (36 000), 600 (4 400), 534 (13 000), 506 (9 600), 498 (9 600), 400 (136 000).

Dimethyl 3,7,7,8,12,13,17-heptamethylchlorin-2,18-dipropionate (24)

The above chlorin 23 (10 mg) was dissolved in formic acid (88%, 8 ml), to which a small amount of Adams catalyst (PtO_2, 5 mg) was added. A gentle stream of hydrogen was passed into the mixture for 5 min. A distinct color change was observed. The hydrogenated product was obtained almost quantitatively by evaporating the formic acid and purified by passing through a short silica gel pad with CH_2Cl_2: m.p. 215-218°C; NMR δ 1.83, 2.01 (3H each, s, gem-Me), 1.98 (3H, d, tertiary Me), 3.17, 3.20 (2H each, t, $CH_2CH_2CO_2$), 3.41, 3.42, 3.47, 3.50 (3H each, s, ring Me), 3.67 (6H, s, $2xCO_2Me$), 4.20, 4.33 (2H, t, $CH_2CH_2CO_2$), 4.55 (1H, q, tertiary H), 8.81, 8.85 (1H each, s, 5,10-H), 9.68, 9.70 (1H each, s, 15,20-H), -2.42 (2H, br s, NH); MS, m/e 582.3200 (calcd for $C_{35}H_{42}N_4O_4$ 582.3208; UV-vis λ_{max} (ε_M) 643 nm (36 900), 614 (3 700), 589 (4 200), 524 (4 000), 497 (9 900), 490 (9 800), 392 (141 000).

General Procedure of Oxidation and Rearrangement of 15a, 29, 36 and 41

Osmium tetroxide (1.2 mmol) in ether (3 ml) was added to a dichloromethane solution (200 ml) of porphyrin (1 mmol) containing pyridine (0.2 ml). The mixture was allowed to stir at room temperature under nitrogen for 20 hours. The reaction was quenched by addition of methanol (100 ml) and followed by bubbling hydrogen sulfide into the solution. The precipitated osmium sulfide was filtered, the filtrate was evaporated, and the residue was chromatographed on silica gel using dichloromethane/ 1 3% methanol as eluent.

The pinacolic rearrangement of the dihydroxychlorins was brought about by three different acid treatments: (1) dichloromethane with a couple drops of 70% perchloric acid (16a, 37, 42); (2) chlorin in

dichloromethane, shaking with concentrated sulfuric acid (17a, 30, 31, 44); (3) neat concentrated sulfuric acid for several hours, followed by esterification (38, 43).

Dimethyl 3,7,8,12,13,17-hexamethyl-18-porphinone-2,17-dipropionate (28)

Yield (from 17a): 85%; m.p. 191-193°C, MS (direct probe, 70 ev) m/e 582 (M^+). NMR δ 1.51 (2H, m, 17-$CH_2CH_2CO_2$), 2.08 (3H, s, 17-Me), 3.06 (2H, t, 17-$CH_2CH_2CO_2$), 3.23 (2H, t, 2-$CH_2CH_2CO_2$), ring Me: 3.28 (3H, s), 3.40 (6H, s), 3.51 (3H, s), 3.55 (3H, s), 3.60 (3H, s, 17-$CCCO_2Me$), 3.74 (3H, s, 2-$CCCO_2Me$), 4.35 (2H, t, 2-$CH_2CH_2CO_2$), 9.08 (1H, s, 15-H), 9.77 (2H, s, 10,20-H), 9.87 (1H, s, 5-H), -2.94, -2.81 (1H each, br s, NH); UV-vis λ_{max} (ε_M) 643 nm (35 400), 586 (5 500), 548 (10 800), 508 (8 500), 490 (6 300), 406 (160 000).

Dimethyl 12,18-diethyl-3,7,13,17-tetramethylporphine-2,8-diacetate (36)

4,4′-Dimethoxycarbonylmethyl-3,3′,5,5′-tetramethyl-2,2′-dipyrromethenium bromide[37] (4.25 g, 10 mmol) and 5,5′-dibromo-4,4′-diethyl-3,3′-dimethyl-2,2′-dipyrromethenium bromide[51] (4.67 g, 10 mmol) were suspended in formic acid (50 ml, 98-100%) and treated with bromine (0.5 ml). The mixture, protected from moisture, was refluxed in an oil bath maintained at 130-135°C for 2.5 h. The condenser was then removed and the solvent was boiled off under air. The black residue was dissolved in methanol (100 ml), the solvent was boiled off again, and the residue redissolved in 100 ml of methanol. Trimethyl orthoformate (20 ml) and sulfuric acid (concentrated, 2 ml) were added and the mixture was allowed to stand at room temperature, protected from moisture in the dark, for a day. Crystalline porphyrin often separated from the liquid

by this time. If so the crystals were collected by filtration, the filtrate was evaporated and the residue was loaded onto a silica gel column. A black nonfluorescent band was eluted first by using dichloromethane and discarded. The porphyrin methyl ester came off with 1% CH_3OH/CH_2Cl_2 but often required 2% CH_3OH/CH_2Cl_2 to be eluted completely. The main porphyrin fractions were combined and evaporated while the heavily contaminated fractions required a second column. The porphyrin **36** was further purified by recrystallization from CH_2Cl_2/CH_3OH. Yield (1.8 g, 32 %); m.p. 310-312°C; MS found: m/e 567.2887 for $(M+H)^+$, $C_{34}H_{38}N_4O_4$ requires m/e 567.2895; NMR δ (6H, t, 2xCH_2CH_3), 3.58, 3.61 (6H, s, ring Me), 3.74 (6H, s, 2xCCO_2Me), 4.05 (4H, q, 2xCH_2CH_3), 5.01 (4H, s, 2xCH_2CO_2), 10.01, 10.02 (1H each, s, 5,15-H), 10.06 (2H, s, 10,20-H), -3.82 (2H, br s, NH); UV-vis λ_{max} (ε_M) 620.5 nm (4 300), 567 (6 600), 531.5 (9 400), 498.5 (14 000), 399 (169 000).

Dimethyl 12,18-diethyl-12,13-dihydroxy-3,7,13,17-tetramethyl-chlorin-2,8-diacetate (37)

Slower moving component on TLC: yield 48%; MS found: m/e 601.3018 for $(M+H)^+$, $C_{34}H_{41}N_4O_6$ requires m/e 601.3028; NMR δ 0.67 (3H, t, 12-CH_2CH_3), 1.75 (3H, t, 18-CH_2CH_3), 2.12 (3H, s, 13-Me), 2.20, 2.37 (1H each, m, 12-CH_2CH_3), 3.22, 3.33, 3.37 (3H each, s, ring Me), 3.66, 3.68 (3H each, s, CO_2Me), 3.89 (2H, q, CH_2CH_3), 4.55, 4.65 (1H each, AB, J_{AB} = 15.8 Hz, 9-CH_2CO_2), 4.72 (2H, s, 1-CH_2CO_2), 8.88, 8.89 (1H each, s, 10,15-H), 9.50, 9.66 (1H each, s, 5,20-H), -2.61 (2H, br s, NH); UV-vis λ_{max} (ε_M) 642 nm (41 000), 612 (3 200), 588 (3 900), 526 (3 000), 498 (12 800), 494 (12 500), 394 (173 000).

Dimethyl 12,18-diethyl-2,3-dihydroxy-3,7,13,17-tetramethylchlorin-2,8-diacetate (38)

Faster moving component on TLC: yield 6%; MS found: m/e 601.3022 for $(M+H)^+$, $C_{34}H_{41}N_4O_6$ requires m/e 601.3028; NMR δ 1.74, 1.75 (3H each, t, CH_2CH_3), 1.93 (3H, s, 3-Me), 3.33, 3.38, 3.47 (3H each, s, ring Me), 3.67 (3H, s, 2-CCO_2Me), 3.62, 4.07 (1H each, AB, J_{AB} = 16.5 Hz, 2-CH_2CO_2), 3.85 (4H, m, CH_2CH_3), 3.92 (3H, s, 8-CCO_2Me), 4.72, 4.82 (1H each, AB, J_{AB} = 15.3 Hz, 8-CH_2CO_2), 9.02, 9.14 (1H each, s, 5,20-H), 9.67, 9.73 (1H each, s, 10,15-H), -2.80 (2H, br s, NH); UV-vis λ_{max} (ε_M) 642 nm (47 400), 614 (3 500), 590 (3 800), 538 (1 600), 520 (2 200), 492 (13 600), 488 (13 300), 389 (186 000).

Dimethyl 13,18-diethyl-3,7,13-17-tetramethyl-12-porphinone-2,8-diacetate (39)

Yield (from 37): 90%; MS found: m/e 583.2925 for $(M+H)^+$, $C_{34}H_{39}N_4O_5$ requires m/e 583.2923; NMR δ 0.44 (3H, t, 13-CH_2CH_3), 1.82 (3H, t, 18-CH_2CH_3), 2.06 (3H, s, 13-CH_3), 2.75 (2H, q, 13-CH_2CH_3), 3.40 3.47, 3.51 (3H each, s, ring Me), 3.71, 3.80 (3H each, s, 2xCCO_2Me), 4.00 (2H, q, 18-CH_2CH_3), 4.78, 4.94 (2H each, s, CH_2CO_2), 9.08 (1H, s, 15-H), 9.74, 9.80 (1H each, s, 10,20-H), 9.79 (1H, s, 5-H), -2.88, -2.73 (1H each, br s, NH); UV-vis λ_{max} (ε_M) 638 nm (26 400), 582 (5 000), 546 (10 600), 508 (8 200), 490 (5 500), 406 (151 000).

Dimethyl 12,18-diethyl-2,7,13,17-tetramethyl-3-porphinone-2,8-diacetate (40)

Yield (from 38): 30%; MS found: m/e 583.2930 for $(M+H)^+$, $C_{34}H_{39}N_4O_5$ requires m/e 583.2923; NMR δ 1.79 (6H, t, 2xCH_2CH_3), 1.96 (3H, s, 2-Me), 2.90 (3H, s, 2-CCO_2Me), 3.44, 3.58 (3H each, s, 13,17-Me), 3.62 (3H, s, 7-Me), 3.74 (3H, s, 8-CCO_2Me), 3.94 (6H, m, 2xCH_2CH_3 and 2-CH_2CO_2), 5.06 (2H, s, 8-CH_2CO_2), 9.07 (1H, s, 20-H), 9.82 (1H, s,

15-H), 9.93 (1H, s, 5-H), 9.95 (1H, s, 10-H), -2.96, -2.73 (1H each, br s, NH); UV-vis λ_{max} (ε_M) 642 nm (35 300), 586 (5 400), 541 (9 700), 504 (9 900), 490 (6 400), 402 (162 000).

Dimethyl 7,8-dihydroxy-3,8,13,18-tetramethyl-7,17-dipentylchlorin-2,12-diacetate (42)

Slower moving component on TLC: yield 11%; MS found: m/e 685.3961 for $(M+H)^+$, $C_{40}H_{53}N_4O_6$ requires m/e 685.3968; NMR δ 0.64 (3H, t, 7-$C_4H_8CH_3$), 0.95 (3H, t, 17-$C_4H_8CH_3$), 1.03 (4H, m, 7-$C_2H_4CH_2CH_2CH_3$), 1.52 (4H, m, 17-$C_3H_6CH_2CH_3$) and 7-$CH_2CH_2C_3H_7$), 1.66 (2H, quint, 17-$C_2H_4CH_2C_2H_5$), 2.16 (2H, m, $CH_2CH_2C_3H_7$), 2.24 (3H, s, 8-Me), 2.38 (2H, m, 7-$CH_2C_4H_9$), 3.38, 3.41, 3.49 (3H each, s, ring Me), 3.73, 3.75 (3H each, s, CO_2Me), 3.81 (2H, t, 17-$CH_2C_4H_9$), 4.84 (4H, s, 2xCH_2CO_2), 9.01, 9.06 (1H each, s, 5,10-H), 9.68, 9.70 (1H each, s, 15,20-H), -2.50 (2H, br s, NH); UV-vis λ_{max} (ε_M) 648 nm (46 800), 622 (3 700), 594 (3 700), 546 (1 700), 520 (2 700), 494 (13 400), 490 (13 400), 392 (178 000).

Dimethyl 2,3-dihydroxy-3,8,13,18-tetramethyl-7,17-dipentylchlorin-2,12-diacetate (43)

Faster moving component on TLC: yield 19%; MS found: m/e 685.3959 for $(M+H)^+$, $C_{40}H_{53}N_4O_6$ requires m/e 685.3968; NMR δ 0.94, 0.95 (3H each, t, $C_4H_8CH_3$), 1.53 (4H, sext, 2x$C_3H_6CH_2CH_3$), 1.64 (4H, quint, 2x$C_2H_4CH_2C_2H_5$), 1.95 (3H, s, 3-Me), 2.19 (4H, quint, 2x$CH_2CH_2C_3H_7$), 3.44, 3.47, 3.52 (3H each, s, ring Me), 3.68, 4.17 (1H each, AB, J_{AB} = 16.5 Hz 2-CH_2CO_2), 3.73 (3H, s, 2-CCO_2Me), 3.89, 3.96 (2H each, t, $CH_2C_4H_9$), 3.98 (3H, s, 12-CCO_2Me), 4.86 (2H, s, 12-CH_2CO_2), 9.01, 9.18 (1H each, s, 5,20-H), 9.78, 9.80 (1H each, s, 10,15-H), -2.67, -2.59 (1H each, br s, NH); UV-vis λ_{max} (ε_M) 638 nm (39 900), 610 (2 800), 584 (3 700), 526 (3 600), 498 (12 700), 494 (12 300), 394 (170 000).

2,3-Dihydroxy-2,12-bis(2-hydroxyethyl)-3,8,13,18-tetramethyl-7,17-dipentylchlorin (44)

The dihydroxychlorin **43** (75 mg, 0.11 mmol) was dissolved in dry THF (50 ml) and $LiAlH_4$ (21 mg, 0.55 mmol) was added carefully. The mixture was stirred at room temperature under argon for 1 h (the progress of the reaction can be monitored by TLC as the product's Rf value is much smaller than that of the starting material), the reaction was then quenched by addition of 5 ml EtOAc, followed by addition of 5 ml of water after 5 min. The solvent was removed in vacuo and the residue was redissolved in CH_2Cl_2, washed with 5% NaOH, then water and dried (anhydrous Na_2SO_4). The tetrahydroxychlorin **44** was purified by passing through a short silica gel pad (CH_2Cl_2/3% MeOH). Yield (62 mg, 90%); MS found: m/e 629.4059 for $(M+H)^+$, $C_{38}H_{53}N_4O_4$ requires m/e 629.4070; NMR δ 0.97, 0.98 (3H each, t, $C_4H_8CH_3$), 1.56 (4H, m, $2xC_3H_6CH_2CH_3$), 1.68 (4H, m, $2xC_2H_4CH_2C_2H_5$), 1.71 (3H, s, 3-Me), 2.23 (4H, m, $2xCH_2CH_2C_3H_7$), 2.28, 2.38 (H each, m, $2-CH_2CH_2O$), 3.12, 3.18 (1H each, m, $2-CH_2CH_2O$), 2.79, 3.36, 3.43 (3H each, s, ring Me), 3.70, 3.85 (2H each, t, $12-CH_2CH_2O$), 3.95 (4H, t, $2xCH_2C_4H_9$), 8.47, 8.93 (1H each, s, 5,20-H), 9.13, 9.45 (1H each, s, 10,15-H), -2.86 (2H, br s, NH); UV-vis λ_{max} (ε_M) 640 nm (34 600), 614 (3 100), 588 (3 200), 524 (2 700), 496 (10 600), 492 (10 400), 392 (151 000).

Dimethyl 3,9,13,18-tetramethyl-8,17-dipentyl-7-porphinone-2,12-diacetate (45)

Yield (from 42) 90%; MS found: m/e 667.3871 for $(M+H)^+$, $C_{40}H_{51}N_4O_5$ requires m/e 667.3862; NMR δ 0.50 (3H, t, $8-C_4H_8CH_3$), 0.86 (2H, m, $8-C_3H_6CH_2CH_3$), 0.95 (3H, t, $17-C_4H_8CH_3$), 1.67 (4H, m, $17-C_3H_6CH_2CH_3$ and $8-CH_2CH_2C_3H_7$), 1.70 (2H, quint, $17-C_2H_4CH_2C_2H_5$), 2.05

(3H, s, 8-Me), 2.18 (2H, quint, 17-$CH_2CH_2C_3H_7$), 2.71 (2H, t, 8-$CH_2C_4H_9$), 3.41, 3.56, 3.62 (3H each, s, ring Me), 3.75 (6H, s, 2xCO_2Me), 3.82 (2H, t, 17-$CH_2C_4H_9$), 4.98, 5.00 (2H each, s, CH_2CO_2), 9.18 (1H, s, 10-H), 9.80, 9.84 (1H each, s, 5,15-H), 9.89 (1H, s, 20-H), -2.79, -1.84 (1H each, br s, NH); UV-vis λ_{max} (ε_M) 646 nm (39 000), 588 (5 100), 540 (9 700), 504 (10 200), 486 (6 100), 402 (172 000).

Dimethyl 2,8,13,18-tetramethyl-7,17-dipentyl-3-porphinone-2,12-diacetate (46)

Yield (from 43) 31%; MS found: m/e 667.3867 for $(M+H)^+$, $C_{40}H_{51}N_4O_5$ requires m/e 667.3862; NMR δ 0.97 (6H, t, 2x$C_4H_8CH_3$), 1.53 (4H, m, 2x$C_3H_6CH_2CH_3$), 1.70 (4H, m, 2x$C_2H_4CH_2C_2H_5$), 1.96 (3H, s, 2-Me), 2.23 (4H, m 2x$CH_2CH_2C_3H_7$), 2.93 (3H, s, 2-CCO_2Me), 3.51, 3.52, 3.59 (3H each, s, ring Me), 3.75 (3H, s, 12-CCO_2Me), 4.01 (4H, t, 2x$CH_2C_4H_9$), 3.89, 3.98 (1H each, AB, J_{AB} = 16.8 Hz, 2-CH_2CO_2), 4.92 (2H, s, 12-CH_2CO_2), 9.08 (1H, s, 20-H), 9.87 (2H, s, 5,15-H), 9.98 (1H, s, 10-H), -3.00, -2.92 (1H each, br s, NH); UV-vis λ_{max} (ε_M) 640 nm (30 500), 584 (5 500), 548 (10 800), 510 (8 400), 490 (5 400), 406 (160 000).

3,12-Bis(2-hydroxyethyl)-3,8,13,18-tetramethyl-7,17-dipentyl-2-porphinone (47)

Yield (from 44) 80%; MS found: m/e 611.3973 for $(M+H)^+$, $C_{38}H_{51}N_4O_3$ requires m/e 611.3964; NMR δ 0.93, 0.95 (3H each, t, $C_4H_8CH_3$), 1.56 (4H, m, 2x$C_3H_6CH_2CH_3$), 1.65 (4H, m, 2x$C_2H_4CH_2C_2H_5$), 2.09 (3H, s, 3-Me), 2.22 (4H, m, 2x$CH_2CH_2C_3H_7$), 2.98, 3.10, 3.32, 3.48 (1H each, m, 3-CH_2CH_2O), 3.51, 3.56, 3.59 (3H each, s, ring Me), 3.97, 4.03, 4.19, 4.42 (2H each, t, 2x$CH_2C_4H_9$ and 12-CH_2CH_2O), -2.98, -2.89 (1H each, br s, NH); UV-vis λ_{max} (ε_M) 640 nm (30 900), 584 (5 600), 548 (9 900), 508 (8 200), 490 (6 000), 406 (152 000).

Tetramethyl 2,7,12,17-tetramethyl-3-porphinone-2,8,13,18-tetrapropionate (49)

Osmium tetroxide (52 mg, 0.21 mmol) in ether (0.5 ml) was added to coproporphyrin I tetramethyl ester, 48 (100 mg, 0.14 mmol) in dry CH_2Cl_2 (50 ml) followed by dry pyridine 0.1 ml. The reaction was allowed to proceed in the dark for 26 h and worked up in the same manner as described before. Yield: unreacted porphyrin (31 mg, 31%), vic-dihydroxycoprochlorin I tetramethylester (42 mg, 40%). The latter was dissolved in 20 ml CH_2Cl_2 containing 0.5 ml of concentrated sulfuric acid. The mixture was allowed to stir at room temperature for 30 min before being extracted with water (3 x 20 ml each). The CH_2Cl_2 layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure. Recrystallization from CH_2Cl_2/CH_3OH gave pure 49 (35 mg, 85%) as purple crystals. MS found: m/e 727.3338 for $(M+H)^+$, $C_{40}H_{47}N_4O_9$ requires m/e 727.3345; NMR δ 1.50 (2H, m, 2-$CH_2CH_2CO_2$), 2.09 (3H, s, 2-Me), 3.10 (2H, m, 2-$CH_2CH_2CO_2$), 3.22 (6H, m, 3x$CH_2CH_2CO_2$), 3.25, 3.49, 3.58 (3H each, s, ring Me), 3.63, 3.65, 3.67, 3.69 (3H each, s, CO_2Me), 4.23, 4.34, 4.38 (2H each, t, $CH_2CH_2CO_2$), 9.18 (1H, s, 20-H), 9.83 (1H, s, 5-H), 9.85, 9.94 (1H each, s, 10,15-H), -2.88, -2.98 (1H each, br s, NH). Irradiation at δ 4.23 caused the signal at δ 9.85 to increase in intensity by 5%, while irradiation at δ 4.36 caused the signals at δ 9.18 and δ 9.94 to increase in intensity by 8%; had the rearrangement gone the other direction NOE should never occur at δ 9.18. UV-vis λ_{max} (ε_M) 642 nm (31 900), 614 (2 200), 584 (5 800), 544 (11 800), 508 (9 700), 490 (6 400), 406 (165 000).

t-Butyl 2-acetoxymethyl-3-ethyl-4-methyl-5-pyrrole-carboxylate (55)

t-Butyl 3-ethyl-2,4-dimethyl-5-pyrrole-carboxylate[52] (54) (20 g, 0.09 mol) was dissolved in glacial acetic acid (100 ml) and acetic anhydride (10 ml). This solution was added to lead tetraacetate (47.68 g, 0.11 mol) and heated with stirring under nitrogen at 70°C for 15 min. The solution was then diluted with water until a precipitate formed. The product was isolated by filtration and purified by recrystallization from methanol. Yield (23 g, 91%); m.p. 96-98°C; MS m/e 281 (M^+); NMR δ 1.1 (3H, t, $CH_2\underline{CH_3}$), 1.6 (9H, s, t-Bu), 2.0, 2.2 (3H each, s, arom. Me and OAc), 2.4 (2H, q, $\underline{CH_2}CH_3$), 5.0 (2H, s, CH_2O), 8.9 (1H, br s, NH).

Ethyl 3′,4-diethyl-3,4′-dimethyl-5′-t-butoxycarbonyl-dipyrromethane-5-carboxylate (57)

A suspension of ethyl 3-ethyl-4-methyl-2-pyrrole-carboxylate[53] (56) (7.73 g, 43 mmol) and the foregoing pyrrole (55) (12 g, 43 mmol) in methanol (300 ml) was treated with toluene-p-sulfonic acid hydrate (350 mg) and heated with stirring under argon at 40°C for 5 h. The homogenized mixture was then partitioned between water (300 ml) and methylene chloride (400 ml) and the organic layer was dried (Na_2SO_4) and evaporated to dryness. The product was used in the next steps without further purification; MS m/e 403 (M^+); NMR δ 1.0, 1.1 (3H each, t, $CH_2\underline{CH_3}$), 1.3 (3H, t, $CO_2CH_2\underline{CH_3}$), 1.6 (9H, s, t-Bu), 1.9, 2.2 (3H each, s, Me), 2.4, 2.7 (2H each, q, $\underline{CH_2}CH_3$), 3.8 (2H, s, methane CH_2), 4.3 (2H, q, $CO_2\underline{CH_2}CH_3$), 8.5 (2H, br s, NH).

Methyl 7,12-diethyl-3,8,13,17,18-pentamethylporphine-2-propionate (50)

Dipyrromethane 57 (3g, 7.5 mmol) was treated with trifluoro-acetic acid (20 ml) under argon atmosphere at ambient temperature for 5 min. A solution of formyl pyrrole 58[54] (1.03 g, 7.5 mmol) in methanol (100 ml)

was then added all at once and the dark red solution was stirred for an additional 90 min, followed by addition of a 30% HBr-CH_3COOH solution (1 ml) and ether (200 ml). Continued stirring for 15 min resulted in the formation of reddish-orange crystals which were collected by filtration and washed thoroughly with ether (1.5 g). The mother liquor was concentrated to approximately 100 ml, and ether (200 ml) was added to give a second crop of the product (0.5 g). Overall yield 53%; MS m/e 421 (M^+ - HBr). This tripyrrin 59 (2 g, 4 mmol) was stirred in a mixture of 48% HBr (30 ml), acetic acid (20 ml) and trifluoroacetic acid (30 ml) under N_2, at 65-70°C for 6 h. A solution of formylpyrrole 50[54] (0.84 g, 4 mmol) in methanol (100 ml) was then added all at once and stirring was continued for 1 h at room temperature. The reaction mixture was evaporated under reduced pressure and redissolved in dry DMF (100 ml) containing copper (II) chloride (10 g). The solution was stirred for 8 h at room temperature under argon. The reaction mixture was then poured into water and extracted with methylene chloride. The organic layer was then dried (Na_2SO_4) and evaporated to dryness, followed by column chromatography (silica gel - CH_2Cl_2). The red eluants were evaporated to dryness and the residue was treated with concentrated H_2SO_4 (30 ml) in order to demetalate the copper-porphyrin. The mixture was then partitioned carefully between CH_2Cl_2 and water. The organic layer was washed with water (2 x 50 ml), saturated $NaHCO_3$ (2 x 50 ml), dried (Na_2SO_4) and evaporated to dryness. This crude product was further purified by column chromatography (silica gel-1% CH_3OH/CH_2Cl_2) and recrystallized from CH_2Cl_2/CH_3OH to give purple crystals. Overall yield (from dipyrromethane 57): 324 mg, 8%; m.p. 234-236°C; MS found m/e 523.3085 for $(M+H)^+$ $C_{33}H_{39}N_4O_2$ requires m/e

523.2076; NMR δ 1.84 (6H, t, 2xCH_2CH_3), 3.24 (2H, t, $CH_2CH_2CO_2$), ring Me: 3.55 (3H, s), 3.56 (6H, s), 3.57 (3H, s), 3.60 (3H, s), 3.67 (3H, s, CO_2Me), 4.03, 4.06 (2H each, q, CH_2CH_3), 4.35 (2H, t, $CH_2CH_2CO_2$), 9.96, 9.98 (1H each, s, meso), 10.03 (2H, s, meso), -3.85 (2H, br s, NH); UV-vis λ_{max} (ε_M) 619.5 nm (5 600), 566 (7 700), 530.5 (11 000), 497 (14 700), 396.5 (166 000).

Methyl 7,12-diethyl-3,8,13,17,17-pentamethyl-18-porphinone-2-propionate (51) and Methyl 7,12-diethyl-3,8,13,18,18-pentamethyl-17-porphinone-2-propionate (52)

Osmium tetroxide (90 mg, 0.35 mmol) in ether (0.9 ml) was added to porphyrin 50 (150 mg, 0.29 mmol) in dry CH_2Cl_2 (50 ml) followed by dry pyridine 0.1 ml. The reaction was allowed to proceed in the dark for 18 h and worked up in the same manner as described before. The reaction mixture was chromatographed on TLC plates, developed with CH_2Cl_2/1% CH_3OH. There were four distinct bands: the recovered red porphyrin 50 moving at the front (30 mg, 20%), followed by ring C dihydroxychlorin (6 mg, 4%), mixture of ring A and B dihydroxychlorins (46 mg, 29%) and another green band containing ring D dihydroxychlorin (21 mg, 13%). The structure assignments were based on ^1H NMR data: the mixture of A and B dihydroxychlorins had δ 0.61 (3H, t, pyrroline Et), ring C dihydroxychlorin had δ 2.05-2.58 (4H, m, pyrroline $CH_2CH_2CO_2$) and ring D dihydroxychlorin had 2.13 (6H, s, 2 x pyrroline Me). The latter (20 mg, 36 mmol) in CH_2Cl_2 was treated with perchloric acid (70%, 0.5 ml) and the reaction was worked up in the same manner as described for 21a, 22a to afford 8 mg of each (41%) of the isomeric porphyrinones.

51 (most polar band on TLC): NMR δ 1.78, 1.82 (3H each, t, CH_2CH_3), 2.08 (6H, s, 17,17-Me), 3.23 (2H, t, $CH_2CH_2CO_2$), 3.44, 3.54, 3.60 (3H each, s, ring Me), 3.74 (3H, s, CO_2Me), 3.88, 4.04 (2H each, q,

CH_2CH_3), 4.35 (2H, t, $CH_2CH_2CO_2$), 9.12 (1H, s, 15-H), 9.78 (1H, s, 10-H), 9.81 (1H, s, 20-H), 9.87 (1H, s, 5-H); -2.81, -2.96 (1H each, br s, NH). Irradiating the triplet at δ 4.35 caused the singlet at δ 9.81 to increase in intensity by 12%.

52 (faster moving component on TLC): NMR δ 1.78, 1.81 (3H each, t, CH_2CH_3), 2.09 (6H, s, 18,18-Me), 3.21 (2H, t, $CH_2CH_2CO_2$), 3.44, 3.55, 3.61 (3H each, s, ring Me), 3.65 (3H, s, CO_2Me), 3.88, 4.03 (2H each, q, CH_2CH_3), 4.34 (2H, t, $CH_2CH_2CO_2$), 9.15 (1H, s, 20-H), 9.81, 9.84, 9.89 (1H, s, 5,10,15-H), -2.93, -3.00 (1H each, br s, NH). Irradiating the triplet at δ 4.34 caused the singlet at δ 9.15 to increase in intensity by 5%.

Dimethyl 12,13-dihydroxy-3,8,8,12,13,17-hexamethyl-7-porphinone-2,18-dipropionate (68)

To the porphyrinone 21a (500 mg, 0.86 mmol) in $CHCl_3$ (200 ml) is added a saturated solution of zinc acetate in methanol (3 ml). After 15 min refluxing (the reaction can be monitored by TLC as the Rf value of the green-colored product is smaller than that of the purple-colored starting material), the mixture was concentrated, diluted with a little methanol, and after cooling the zinc complex is filtered off in virtually quantitative yield.

To a solution of 68 (500 mg, 0.77 mmol) in CH_2Cl_2 (250 ml), osmium tetroxide (295 mg, 1.16 mmol) and pyridine (0.3 ml) were added and the reaction was allowed to proceed in the dark, under argon for 36 h at 23°C. The reaction mixture was worked up in the same manner as described for 16a to give after separation on silica gel column (CH_2Cl_2/1% MeOH, then CH_2Cl_2/2% MeOH): unreacted starting material, 200 mg, 40% and a green zinc dihydroxy-porphyrinone, 250 mg, 47%. A

methylene chloride solution of the latter was completely demetalated, by shaking with 10% HCl in a separtory funnel, to give the violet 68 as the exclusive isomer. M.p. 216-219°C; NMR δ 1.68, 1.71, 1.76, 1.99 (3H each, s, pyrroline Me), 2.84, 2.93 (3H each, s, ring Me), 2.87 (4H, t, 2x$CH_2\underline{CH_2}CO_2$), 3.59, 3.62 (3H each, s, CO_2Me), 3.67, 3.73 (2H each, t, $\underline{CH_2}CH_2CO_2$), 7.42 (1H, s, 10-H), 7.78 (1H, s, 15-H), 8.56 (1H, s, 5-H), 8.66 (1H, s, 20-H); UV-vis λ_{max} (ε_M) 642 nm (7 500), 589 (13 100), 583 (10 000), 547 (10 000), 512 (6 600), 429 (18 100), 386 (57 800).

Dimethyl 3,8,8,13,13,17-hexamethyl-7,12-porphinedione-2,18-dipropionate (69)

The foregoing dihydroxyporphyrinone 68 (200 mg, 0.32 mmol) was dissolved in 5 ml of concentrated H_2SO_4 and the reaction mixture was allowed to stir at room temperature for 5 min before the careful addition of CH_3OH (20 ml). The solution was then partitioned between CH_2Cl_2 (100 ml) and water (100 ml). The organic layer was separated, washed with saturated $NaHCO_3$ (100 ml), water (100 ml), dried (Na_2SO_4) and evaporated to dryness, to give after recrystallization from CH_2Cl_2/CH_3OH the green colored 69 in almost quantitative yield. M.p. 293-295°C; NMR δ 1.93, 1.97 (6H each, s, gem-Me), 3.12, 3.13 (2 H each, t, $CH_2\underline{CH_2}CO_2$), 3.28, 3.33, (3H each, s, ring Me), 3.59, 3.62 (3H each, s, CO_2Me), 4.17, 4.18 (2H each, t, $\underline{CH_2}CH_2CO_2$), 8.51 (1H, s, 15-H), 8.74 (1H, s, 10-H), 9.33 (1H, s, 5-H), 9.58 (1H, s, 20-H), -0.32 (2H, br s, NH); UV-vis λ_{max} (ε_M) 638 nm (15 500), 592 (14 400), 584 (14 800), 540 (9 700), 436 (91 300), 415 (88 600), 400 (74 900); MS found: m/e 599.2865 for $(M+H)^+$, $C_{34}H_{39}N_4O_6$ requires m/e 599.2872.

Dimethyl 17,18-dihydroxy-3,8,8,13,13,17-hexamethyl-7,12-porphinedione-2,18-dipropionate (89)

Osmium tetroxide (115 mg, 0.45 mmol) and pyridine (0.4 ml) were added to a dichloromethane solution of 69 (150 mg, 0.25 mmol) and the reaction was allowed to proceed under argon at room temperature for 36 h. The reaction mixture was worked up in the same manner as described for 16a. The products isolated from the silica gel column were the unreacted 69 (65 mg, 43%) followed by the gray 89 (55 mg, 35%). NMR δ 1.57 (6H, s, pyrroline Me), 1.59, 1.71, 1.72 (3H each, s, pyrroline Me), 2.52 - 3.01 (4H, m, 18-$CH_2CH_2CO_2$), 2.80 (3H, s, ring Me), 3.52 (2H, t, 2-$CH_2CH_2CO_2$), 3.62 (3H, s, 18-$CCCO_2Me$), 3.74 (3H, s, 2-$CCCO_2Me$), 3.88 (2H, t, 2-$CH_2CH_2CO_2$), 7.12 (1H, s, 15-H), 7.60 (1H, s, 10-H), 7.66 (1H, s, 20-H), 8.42 (1H, s, 5-H). Irradiating the singlet at δ 2.80 caused the singlet at δ 8.42 to increase in intensity by 4.4%. UV-vis λ_{max} (ε_M) 684 nm (7 200), 656 (8 700), 602 (6 600), 539 (8 100), 505 (6 300), 410 (39 700), 382 (47 800).

Methyl 18-[2-(methoxycarbonyl) ethenyl]-3,8,8,13,13,17-hexamethyl-7,12-porphinedione-2-propionate (70)

Compound 89 (50 mg, 0.08 mmol) was heated to reflux in benzene (25 ml), followed by the gradual addition of 5 drops of concentrated HCl. After 20 min, water (20 ml) was added, the organic layer was separated, washed two more times with water (20 ml each), dried (Na_2SO_4), evaporated to dryness and recrystallized from CH_2Cl_2/MeOH to give the green 70 in quantitative yield. M.p. 337-340°C, NMR δ 1.87, 1.94 (6H each, s, gem-Me), 3.04 (2H, t, $CH_2CH_2CO_2$), 3.22, 3.32 (3H each, s, ring Me), 3.62, 4.00 (3H each, s, CO_2Me), 4.03 (3H, t, $CH_2CH_2CO_2$), 6.88, 8.94 (1H each, d, acrylic, J_{AB} = 16.1 Hz), 8.31 (1H, s, 15-H), 8.48 (1H, s, 10-H), 9.15 (1H, s, 5-H), 9.39 (1H, s, 20-H), 0.67 (2H, br s, NH); UV-

vis ($CHCl_3$) λ_{max} (ε_M) 661 nm (15 400), 611 (25 000), 568 (14 000), 446 (66 000), 423 (93 000); MS found: m/e 597.2721 for $(M+H)^+$ $C_{34}H_{37}N_4O_6$ requires m/e 597.2715.

Dimethyl 12,13-dihydroxy-3,7,7,12,13,17-hexamethyl-8-porphinone-2,18-dipropionate (72) and Dimethyl 2,3-dihydroxy-3,7,7,12,13,17-hexamethyl-8-porphinone-2,18-dipropionate (73)

Prepared in the same way as described for 68 yield: unreacted starting material 71 (40%), green 72 (15%), and violet 73 (20%).

72 (slower moving component on TLC): NMR δ 1.86, 1.90, 2.02, 2.05 (3H each, s, pyrroline Me), 2.94, 3.03 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.13, 3.23 (3H each, s, ring Me), 3.57, 3.58 (3H each, s, CO_2Me), 3.93, 4.04 (2H each, t, $\underline{CH_2}CH_2CO_2$), 8.45 (1H, s, 5-H), 8.60 (1H, s, 15-H), 8.74 (1H, s, 10-H), 9.28 (1H, s, 20-H); UV-vis λ_{max} (ε_M) 634 nm (15 700), 593 (14 000), 520 (6 200), 431 (92 000), 406 (104 000).

64 (Faster moving component on TLC): NMR δ 1.70, 1.71, 1.86 (3H each, s, pyrroline Me), 1.78 (2H, t, 2-$CH_2\underline{CH_2}CO_2$), 2.64 (2H, t, 2-$\underline{CH_2}CH_2CO_2$), 2.86 (2H, t, 18-$CH_2\underline{CH_2}CO_2$), 2.92, 2.94, 2.98 (3H each, s, ring Me), 3.59 (2H, t, 18-$\underline{CH_2}CH_2CO_2$), 3.64, 3.68 (3H each, s, CO_2Me), 7.43 (1H, s, 5-H), 7.78 (1H, s, 20-H), 8.59 (1H, s, 10-H), 8.67 (1H, s, 15-H); UV-vis λ_{max} (ε_M) 640 nm (8 800), 589 (21 000), 546 (13 200), 412 (31 300), 385 (71 000), 376 (73 000).

Dimethyl 3,7,7,13,13,17-hexamethyl-8,12-porphinedione-2,18-dipropionate (74)

Obtained from the pinacolic rearrangement of 72, the same way as described for 69, and separated from the faster moving isomer 69 by TLC plates using 1-2% CH_3OH/CH_2Cl_2. The yield of each isomer was 45%. The yellow-green porphyrindione 74 was further purified by recrystallization from CH_2Cl_2/MeOH. M.p. 296-297.5°C; NMR δ 1.99 (12H, s, gem-Me), 3.19

(4H, t, 2x$CH_2CH_2CO_2$), 3.45 (6H, s, ring Me), 3.59 (6H, s, 2xCO_2Me), 4.33 (4H, t, 2x$CH_2CH_2CO_2$), 8.91 (1H each, s, 5,15-H), 9.65 (1H, s, 10-H), 9.89 (1H, s, 20-H), -1.73 (2H, br s, NH). Irradiating the triplet at δ 4.33 caused the singlet at δ 9.89 to increase in intensity by 9.4%. UV-vis λ_{max} (ε_M) 634 nm (15 300), 620 (21 400), 590 (9 400), 577 (8 200), 432 (116 000), 411 (136 000); MS found: m/e 599.2881 for $(M+H)^+$, $C_{34}H_{39}N_4O_6$ requires m/e 599.2872.

Dimethyl 2,7,7,12,13,17-hexamethyl-3,8-porphinedione-2,18-dipropionate (75)

Obtained from the pinacolic rearrangement of 73 in the same manner as described for 69, except that the reaction time was 30 min. The products isolated from the TLC plates (CH_2Cl_2/1-2% MeOH), were the faster moving porphyrinone 22a (42%) and the slower moving green porphyrindione 75 (42%). [Note: if the spirolactone of 73 is employed for the pinacolic rearrangement, porphyrinone 22a is the only product obtained.] NMR δ 1.95 (3H, s, 2-Me), 1.96 (6H, s, 7,7-Me), 1.74, 2.15 (1H each, m, 2-$CH_2CH_2CO_2$), 2.94 (2H, t, 2-$CH_2CH_2CO_2$), 3.08 (2H, t, 18-$CH_2CH_2CO_2$), 3.32, 3.37, 3.40 (3H each, s, ring Me), 3.44 (3H, s, 2-$CCCO_2Me$), 3.62 (3H, s, 18-$CCCO_2Me$), 4.09 (2H, t, 18-$CH_2CH_2CO_2$), 8.64 (1H, s, 20-H), 8.83 (1H, s, 5-H), 9.40 (1H, s, 10-H), 9.55 (1H, s, 15-H), -0.69 (2H, br s, NH); UV-vis λ_{max} (ε_M) 634 nm (15 600), 591 (13 000), 582 (12 200), 542 (7 800), 435 (76 500), 413 (83 200), 400 (72 500); MS found: m/e 599.2863 for $(M+H)^+$, $C_{34}H_{39}N_4O_6$ requires m/e 599.2872.

vic-Dihydroxyoctaethylchlorin (76)

To a solution of OEP[55] (1.168 g, 2.2 mmol) in CH_2Cl_2 (250 ml) and pyridine (1 ml) was added osmium tetroxide (1.0 g, 3.9 mmol) in diethyl ether (10 ml). The mixture was allowed to stir at room temperature in the dark for two days. This mixture was diluted with methanol (50 ml) and was bubbled with H_2S for 15 min. The precipitated osmium sulfide was recovered by filtration, and the solvent was evaporated. The residue was triturated with methanol, which dissolved most of the diol chlorin from unreacted OEP. The solution was filtered, and the product was further purified on a silica gel column, eluting with CH_2Cl_2 containing 0.5% of methanol: yield 827 mg (66.6%), plus unreacted OEP [201 mg (17.1%)]; m.p. 213-214°C; NMR δ 0.96 (6H, t, pyrroline Et), 1.74 (18H, t, Et), 2.55 (4H, q, pyrroline Et), 3.38 (2H, s, OH), 3.79, 3.82, 3.91 (12H, q, Et), 9.00 (2H, s, 5,20-H), 9.68 (2H, s, 10,15-H), -2.68 (2H, br s, NH); UV-vis λ_{max} (ε_M) 643 nm (54 000), 590.5 (9 700), 523.5 (8 700), 496 (19 900), 392 (206 000); MS, found m/e 569.3887 for $(M+H)^+$, $C_{36}H_{49}N_4O_2$ requires m/e 569.3858. The dihydroxylation of OEP with OsO_4 was found to proceed poorly under catalytic conditions, e.g., using N-methylmorpholine oxide.[56]

3,7,12,13,17,18-Heptaethyl-2-(1-hydroxyethyl)-porphine (77)

Diol 76 (20 mg, 0.035 mmol) was heated in a mixture consisting of dioxane (6 ml), water (3.5 ml), and concentrated HCl (0.5 ml) on a steam bath for 30 min. The mixture was partitioned in CH_2Cl_2 and water; the organic layer was evaporated and the residue was separated on TLC plate with CH_2Cl_2 as solvent. The major product 77 was further crystallized from CH_2Cl_2/hexane: yield 9.7 mg (49%); NMR δ 1.90, 1.91 (21H, t, Et), 2.34 (3H, d, $CH(OH)CH_3$), 2.78 (1H, br s, OH), 4.06, 4.12 (14H, q, Et),

6.56 (1H, q, CH(OH)CH_3), 10.08 (2H, s, meso), 10.10, 10.62 (1H each, s, meso), -3.73 (2H, br s, NH); UV-vis λ_{max} (ε_M) 620.5 nm, 566.5, 533, 500, 400; MS, found m/e 551.3726 for $(M+H)^+$, $C_{36}H_{47}N_4O$ requires m/e 551.3753.

3,7,8,12,13,17,18-Heptaethyl-2-(1-acetoxyethyl)-porphine (78)

Diol 76 (20 mg, 0.035 mmol) was heated in glacial acetic acid (5 ml) at 90°C for 10 min. The reaction mixture was partitioned in CH_2Cl_2 and water; the organic layer was separated and evaporated. The residue was recrystallized from CH_2Cl_2/MeOH: yield 17.8 mg (85%) [note: upon purification on TLC plates using CH_2Cl_2/MeOH, 78 was largely converted to the methoxide 79. Silica gel promoted solvolysis is a facile reaction for acetylated hematoporphyrins.[57]]; NMR δ 1.95 (21H, t, Et), 2.30 (3H, s, acetyl), 2.41 (3H, d, $CHCH_3$), 4.09, 4.16, 4.20 (14H, q, Et), 7.53 (1H, q, $CHCH_3$), 10.12 (2H, s, meso), 10.16, 10.52 (1H each, s, meso), -3.70 (2H, br s, NH); UV-vis λ_{max} (ε_M) 620 nm (9 400), 567 (12 000), 536 (15 300), 500 (17 300), 401 (156 000); MS, found m/e 593.3873 for $(M+H)^+$, $C_{38}H_{49}N_4O_2$ requires m/e 593.3858.

3,7,8,12,13,17,18-Heptaethyl-2-(1-methoxyethyl)-porphine (79)

Diol 76 (20 mg) was heated to reflux for 1 h in methanol (20 ml) with one drop of concentrated HCl. The solvent was then evaporated, and the residue was crystallized from CH_2Cl_2/MeOH: yield 15.4 mg (77.5%); m.p. 243-245°C; NMR δ 1.93 (21H, t, Et), 2.32 (3H, d, $CHCH_3$), 3.62 (3H, s, OMe), 4.08, 4.14 (14H, q, Et), 5.96 (1H, q, $CHCH_3$), 10.10 (2H, s, meso), 10.12, 10.66 (1H each, s, meso), -3.70 (2H, br s, NH); UV-vis λ_{max} (ε_M) 620.5 nm (9 200), 566.5 (11 500), 533 (14 700), 499 (18 300), 399.5 (172 000); MS, found m/e 565.3888 for $(M+H)^+$, $C_{37}H_{49}N_4O$ requires 565.3909.

3,7,8,12,13,17,18-Heptaethyl-2-vinylporphine (80)

Diol **76** (100 mg, 0.175 mmol) was heated to reflux in benzene (25 ml) containing five drops of concentrated HCl for 3 h. The solvent was evaporated, and the residue was crystallized from CH_2Cl_2/MeOH: yield 85 mg (90%); m.p. > 300°C; NMR δ 1.91, 1.93 (21H, t, Et), 4.09, 4.16, 4.22 (14H, q, Et), 6.16, 6.38 (2H, dd, CH=CH_2), 8.25, 8.31 (1H, dd, CH=CH_2), 10.12 (2H, s, meso), 10.16, 10.28 (1H each, s, meso), -3.68 (2H, br s, NH); UV-vis λ_{max} (ε_M) 623.5 nm (10 000), 569.5 (14 700), 539 (19 300), 503 (20 000), 402.5 (179 000); MS, found m/e 533.3641 for $(M+H)^+$, $C_{36}H_{45}N_4$ requires m/e 533.3647.

2,3,7,8,12,13,17-Heptaethylporphine (81)

Solid vinylporphyrin **80** (60 mg, 0.113 mmol) was mixed and ground with resorcinol (240 mg, 2.18 mmol) in a mortar. The powder placed in a test tube was swirled over a flame until boiling. The mixture was cooled momentarily and then heated again to boiling. This cycle was repeated three times, and the residue, after cooling, was extracted with CH_2Cl_2 and water. The porphyrin in the organic phase was purified by crystallization from CH_2Cl_2/MeOH: yield 51 mg (89%); m.p. 236-239°C; NMR δ 1.98 (15H, t, Et), 2.10 (6H, t, Et), 4.16 (10H, q, Et), 4.30 (4H, q, Et), 9.16 (1H, s, 8-H), 10.11, 10.16, 10.18, 10.20 (1H each, s, meso); UV-vis λ_{max} (ε_M) 618.5 nm (9 700), 565 (13 000), 531.5 (16 300), 498 (21 000), 398.5 (215 000); MS, found m/e 507.3457 for $(M+H)^+$, $C_{34}H_{43}N_4$ requires m/e 507.3491.

Reactions of *vic*-Dihydroxyetiochlorin I (82)

Diol **82** was heated in dioxane/aqueous HCl in the same manner as described above. The overall yield for the two etioporphyrin alcohols

was about 50%, with the ratio of **83** to **84** being near 2:1. If the diol **82** was heated in acetic acid, the acetoxy porphyrin **85** was obtained in 67% yield and **86** in 11% yield (6:1 ratio). These ratios can be observed directly by using NMR peaks of CH_2OR vs. CH(OR)Me: δ 6.10 (s, **83**):6.52 (q, **84**) = 4:1; 6.61 (s, **85**):7.55 (q, **86**) = 12:1.

Dimethyl 7,8,12,13-tetraethyl-3-(hydroxymethyl)-17-methyl-2,18-porphinedipropionate (87)

Diol **17b**[20] (40 mg) was heated in dioxane (10 ml)/aqueous HCl (10%, 4 ml) on a steam bath overnight. The solvent was evaporated, and the residue was esterified in $MeOH/H_2SO_4$. The principal product isolated from TLC plates, was **87**: 9.8 mg (~25% yield); m.p. 196-198°C; NMR δ 1.90 (12H, t, Et), 3.26 (4H, t, $CH_2\underline{CH_2}CO_2$), 3.49 (3H, s, CH_3), 3.61, 3.66 (3H each, s, CO_2Me), 4.05, 4.11 (8H, q, Et), 4.42, 4.45 (2H each, t, $\underline{CH_2}CH_2CO_2$), 6.11 (2H, s, CH_2OH), 10.06 (2H, s, meso), 10.08, 10.28 (1H each, s, meso), -3.78 (2H, br s, NH); UV-vis λ_{max} (ε_M) 621.5 nm, 567, 535.5, 499.5, 402; MS, found m/e 639.3521 for $(M+H)^+$, $C_{38}H_{47}N_4O_5$ requires m/e 639.3549. The alternative alcohol **88** was not detected, but a small amount of acrylic porphyrin [~5% yield; NMR δ 7.05, 7.11, 9.28, 9.36, acrylic] was isolated, which can only result from **88**.

CHAPTER 2

SYNTHESIS OF THE HEME d PROSTHETIC GROUP OF BACTERIAL TERMINAL OXIDASE

I. INTRODUCTION

Escherichia coli, like many other microbes, have a branched respiratory system with two terminal oxidases, cytochrome d and cytochrome o, which catalyze the reduction of O_2 to H_2O.[59] Both cytochrome d and o complexes contain two polypeptides. Moreover, the cytochrome d complex contains two cytochrome d centers (heme d), one cytochrome b_{595} center (high-spin protoheme IX), and one cytochrome b_{558} center (low-spin protoheme IX); whereas the cytochrome o complex contains one cytochrome b_{562} and one cytochrome o, which is again a b-type cytochrome. Cytochrome o prevails in the early exponential phase during aerobic growth of culture while cytochrome d becomes important in the late exponential phase, or when cells are grown under limiting oxygen supply.[60,61] The K_m value for oxygen of cytochrome d is about eight times lower than that of cytochrome o.[59] 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 (Figure 4).

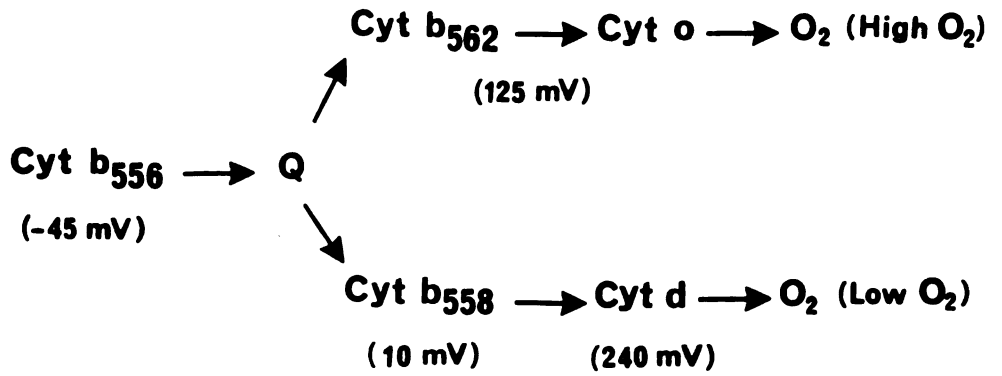
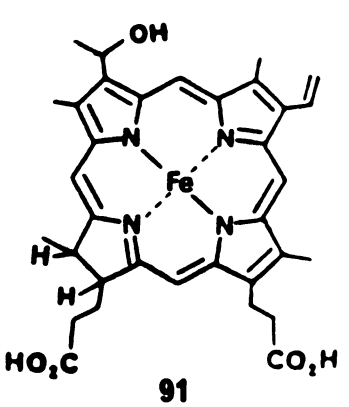


Figure 4. Arrangement of cytochromes in the respiratory chain of cells of E. coli in the late exponential phase of aerobic growth. The Em′ values are indicated in parentheses. Cyt, cytochrome; Q, ubiquinone-8.

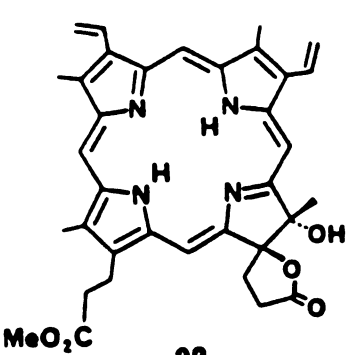
The oxygen binding site in the membrane-bound cytochrome d complex is a green-colored heme prosthetic group displaying a prominent α-band near 630 nm. Keilin[62] originally designated the name "a_2" for this cytochrome absorbing in the red region; a_2 was later changed to d to avoid confusion with aa_3 hemes of mammalian cytochrome oxidase. It should be made clear that cytochrome d does not reduce nitrite; it is different from the soluble cd_1-type oxidases[7,63] from Pseudomonas aeruginosa and Paracoccus denitrificans, which primarily function as nitrite reductase and are not components of the aerobic respiratory chain.

The green heme moiety of cytochrome d was first studied by Barrett (1956) who extracted cells of Aerobacter aerogenes and identified an iron chlorin core structure 91.[32] The site of saturation and the nature of the side chains on the chlorin macrocycle could not be determined at that time. Barrett suggested the possible presence of vinyl,

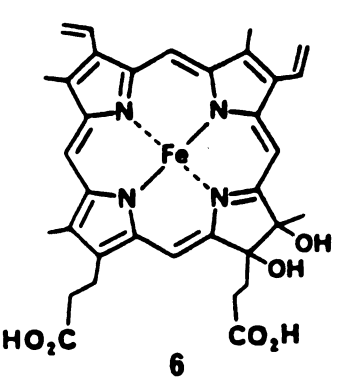
hydroxyethyl, and propanoic acid substituents in an arrangement similar to a hydrated protoporphyrin IX. The tentatively formulated structure of Barrett remained unchallenged in the literature for almost 30 years and has served as the de facto model for many other green hemes[64] subsequently found in various bacterial cytochromes. Recently, Timkovich and collaborators[6] isolated sufficient amounts of the heme d prosthetic group from purified E. coli oxidase and characterized the structure of the metal-free, esterified chromophore by means of ^{1}H NMR, IR, UV-vis, and mass spectroscopy. The proposed structure comprises an unusual chlorin core with a spiro-γ-lactone group at the saturated pyrrole ring C (92). 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 γ-lactone in the metal-free heme d was largely based on an intense IR absorption at ~1782 cm^{-1}. In a recent paper, however, the Timkovich group reported that this IR peak was not present in the extracted heme d,[65] thus supporting structure 6.



91



92



6

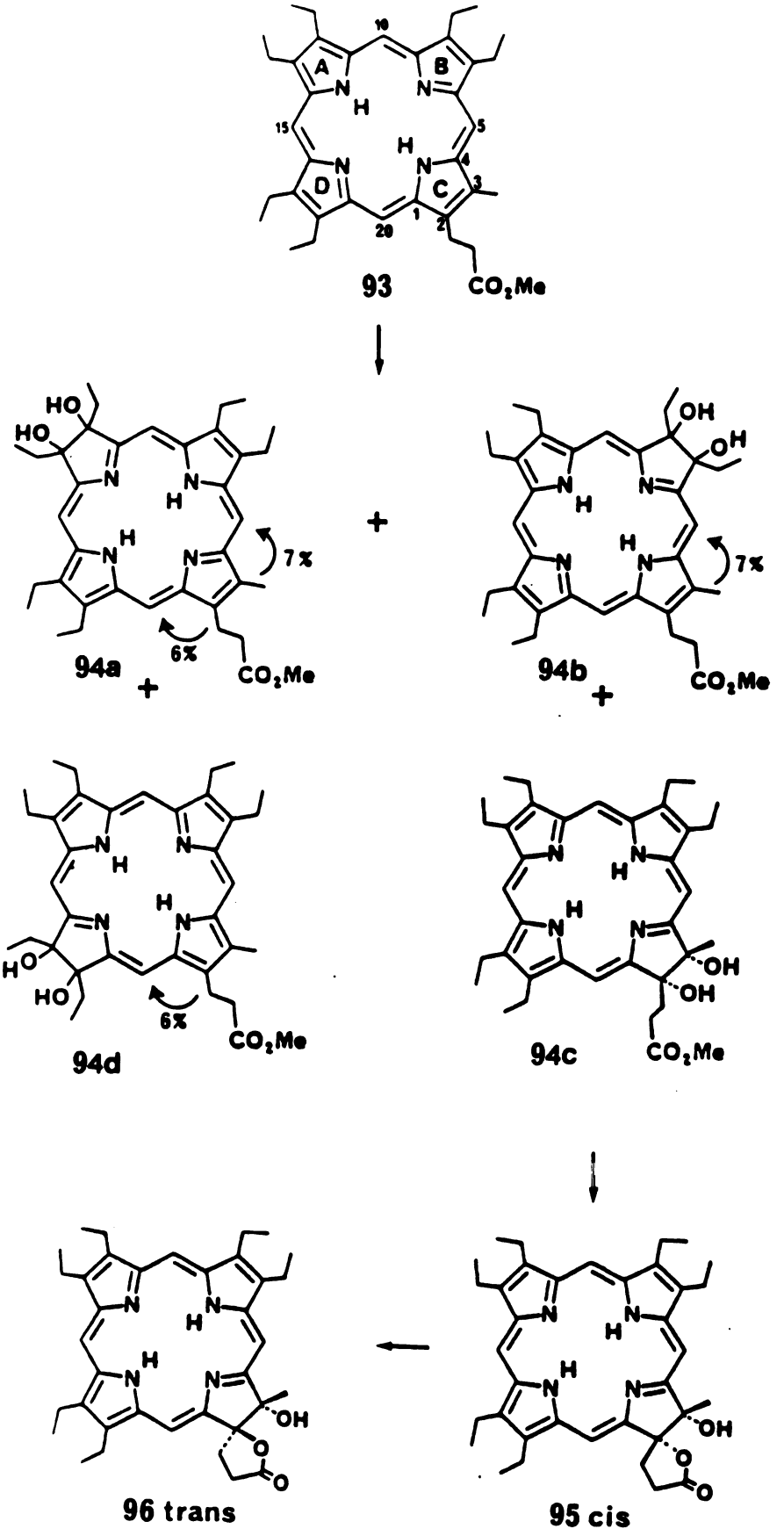
Considering the limitation imposed by the scarcity of natural material, we believe that a full-scale study of heme d as well as the confirmation of the proposed structure must rely upon organic syntheses. In this chapter, we report the total synthesis of Timkovich's "lactochlorin", its diastereomer, and the non-lactonized forms. The chemical reactivities inherent in these structures are also addressed.

II. RESULTS AND DISCUSSION

A. Model Studies

In the first chapter, we examined several vic-dihydroxychlorins and the principal results can be summarized here.[20,43,66,67] Dihydroxylation of the porphyrin β-β′ double bond can be routinely accomplished by using osmium tetroxide. If the diol chlorin bears a geminal propionate ester side chain, It is unstable during chromatography, especially on TLC plate. Given enough time, the initially slow moving diol can completely change into a fast moving γ-spirolactone as evidenced by the characteristic strong IR band near 1780 cm^{-1}. It turns out that hydroxychlorins with an angular propionate ester always have a tendency to lactonize into the 5-membered ring under the influence of general base catalysis.[67] A variety of bases including silica gel and sodium acetate are effective to bring about this cyclization. However, preliminary results indicate that lactones resulting from different reagents may not be spectroscopically identical, a difference perhaps attributable to diastereomers.[67]

The above description can be exemplified by the reaction of a model compound 93 in Scheme 15; the reason for choosing this particular porphyrin will become evident in later discussion. Treatment of 93 with OsO_4 - H_2S yielded a mixture of four isomeric chlorins, 94a - 94d, which

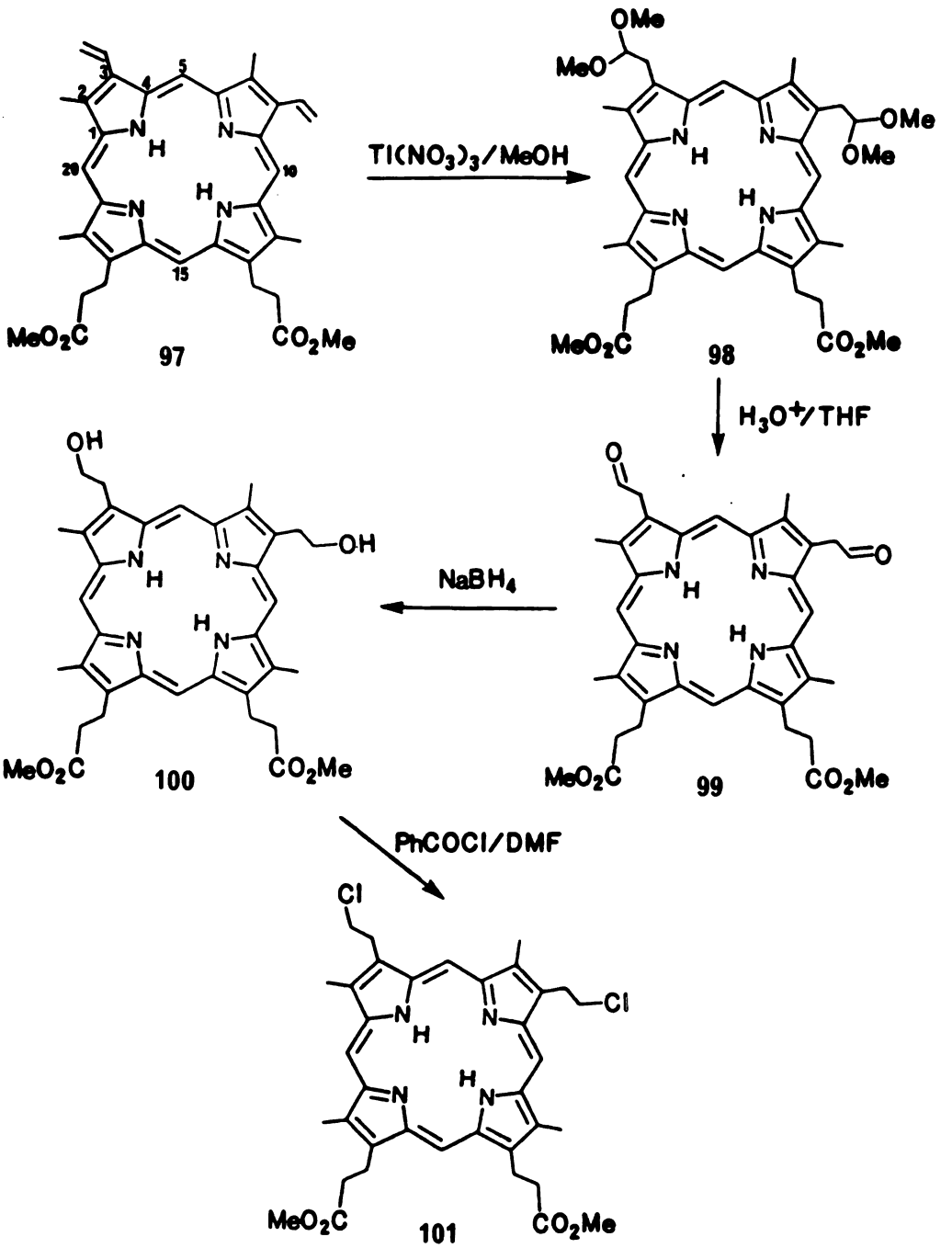


SCHEME 15

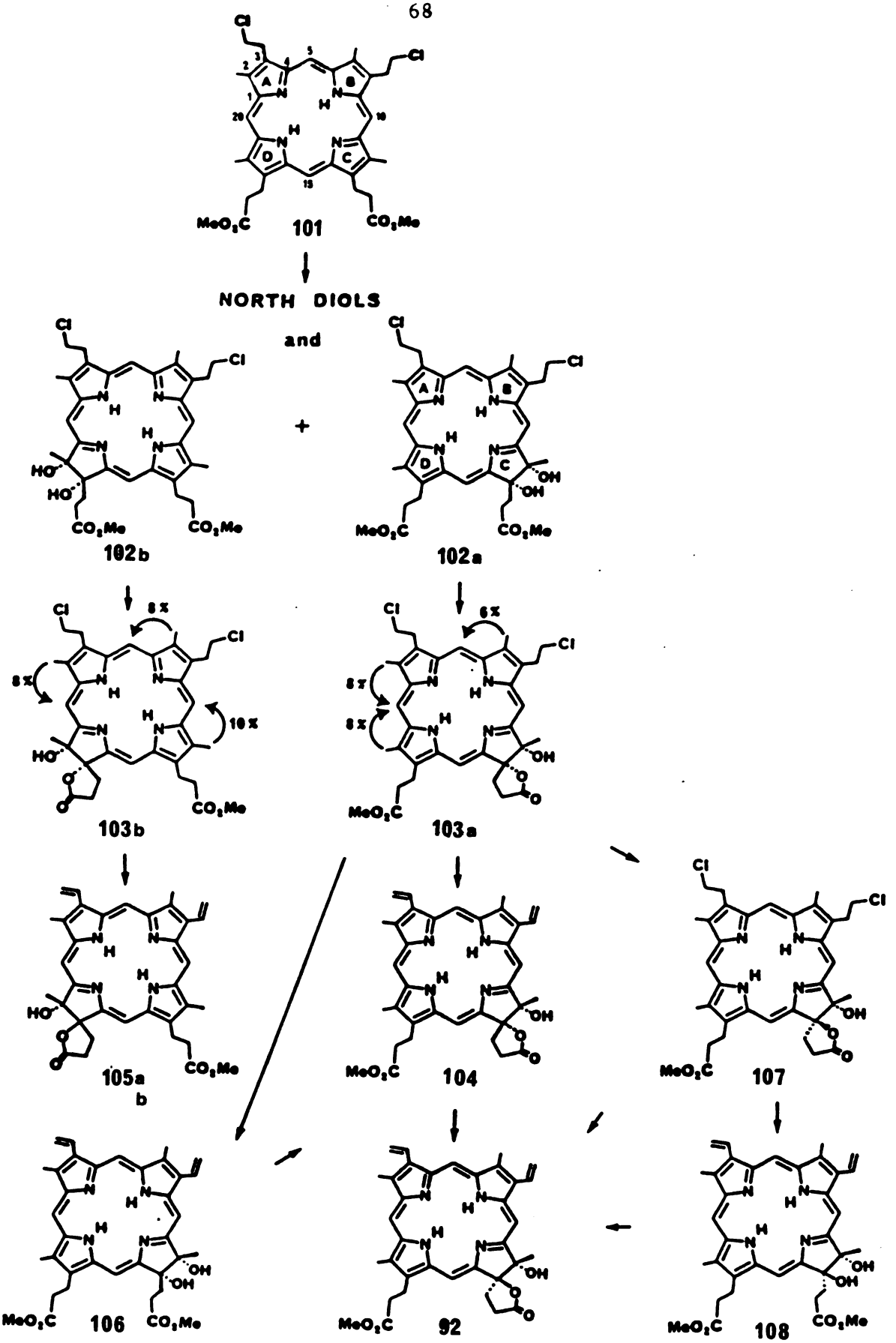
can be separated by preparative TLC into three green bands. The two faster moving chlorins were characterized by ^{1}H NMR to be the two north diols, **94a** and **94b**; the site of saturation was unambiguously identified by nuclear Overhauser enhancements (NOE's), as indicated in Scheme 15. The two south diols, contained in the slow moving TLC band, was difficult to separate directly. Consequently, we recovered the mixture and heated it in CH_2Cl_2/MeOH with sodium acetate, and the cyclized **95** was then separated easily from **94d**. Lactone **95**, which has a strong IR peak at 1780 cm^{-1}, was found to undergo further changes during developing on TLC plates; it slowly converted to another green compound possessing unchanged mass spectral and IR peaks. However, ^{1}H NMR of the two (**95** and **96**) were different, which eventually allowed us to deduce the configuration of the two lactone forms (vide infra). The more stable **96** could also be obtained directly by lactonizing diol **94c** under prolonged contact with silica gel.

B. Synthesis of "Lactochlorin"

The synthetic strategy, which was guided by the results of model compounds, is shown in Schemes 16 and 17. We began with protoporphyrin (**97**), a probable precursor also in the biosynthesis of heme d. The reactive vinyl groups were protected as the chloroethyl side chains by oxidation with $Tl(NO_3)_3$ to the aldehyde **99**,[49] followed by reduction to **100** with $NaBH_4$, and then by chlorination with PhCOCl/DMF[68] to **101**, each step giving essentially quantitative yield (literature procedures have been modified). Porphyrin **101** was treated with 1.3 equivalents of OsO_4 for a day before being quenched with H_2S to effect dihydroxylation at the four possible sites: pyrrole ring A (6.8%), ring B (6.8%), ring C (22%), and ring D (26%). The separation of the north *vic*-diols from the



SCHEME 16


101
NORTH DIOLS
and
+
102b
102a
8%
8%
10%
6%
8%
8%
103b
103a
105a
b
104
107
106
92
108

SCHEME 17

south was easily accomplished by chromatography. The two south regioisomers were separated by TLC via the lactones. Structural assignments of **103a** and **103b** were based on NOE connectivities (key measurements are indicated by structures in Scheme 17). Regeneration of the vinyl side chains was brought about by heating **103a** in pyridine-30% KOH,[69] during which most of the lactone chlorin was hydrolized but some survived. The hydrolyzed chlorin was methylated with diazomethane to give the cis-diol **106**. The dehydrated lactone **104** has all the structural elements of Timkovich's "lactochlorin" yet it exhibited a ^{1}H NMR spectrum showing discrepancies in the lactone proton region as compared with that of the natural compound. An obvious explanation is that this compound does not have the correct configuration. Indeed, when this lactone was chromatographed on silica gel repeatedly, the expected isomeric chlorin emerged which was shown by IR and mass spectra to be a γ-lactone; more significant, it exhibited ^{1}H NMR features basically indistinguishable from that of "lactochlorin." Furthermore, the synthetic lactone and the natural compound have identical retention time in HPLC analyses. Based on their NMR spectra discussed below, the lactones **103** and **104** were assigned a cis configuration (with respect to the two oxygen position) while the "lactochlorin" should have a trans configuration, as suggested by Timkovich. When **107**, obtained from **103a** by repetitive chromatography on silica gel, was subjected to hydrolysis under the same reaction conditions as **103a**, a different diol form was obtained. This diol, **108**, assigned as trans (based on ^{1}H NMR data) can be lactonized to give once again the trans "lactochlorin" **92**.

When the regioisomer **103b** was subjected to the same reaction conditions described above, the cis and trans lactones, **105a** and **105b**,

were obtained. These unnatural compounds not only have provided us more variety of this class of chromophores, but may be used for probing the structure-function relationship in the heme protein.

C. ^{1}H NMR Spectra and Structure of the Chlorins

The two synthetic lactones **104** and **92** have distinctive ^{1}H NMR signatures, particularly in the δ 2.4-3.4 ppm region where the methylene protons of the γ-lactone appear. As shown in Figure 5A, at 250 MHz **92** has a spectrum almost identical to the published spectrum of "lactochlorin."[6] Timkovich concluded the trans configuration based on the assumption that the single proton peaks around 2.4 ppm are from H4 (refer to Figure 7) which points away from the OH group and should have a chemical shift similar to those of α-methylene protons of normal pyrroline alkyl substituents. The peaks of H1, because of the large deshielding imparted by the nearby OH oxygen, should occur relatively downfield. The four protons were thus labelled as the following: H1 3.169, H2 3.032, H3 3.231, and H4 2.431. However, these peak assignments and the reported simulation had a large degree of uncertainty due to the presence of overlapping peaks in this region. In fact, on close examination of Figure 5A and the spectra of the natural molecule, as well as several other synthetic analogues,[67] it becomes evident that there are peaks near 3.5 ppm (at the shoulder of the dominant 18-methyl singlet) which can be nothing but part of the lactone methylenes. This is illustrated by the spectrum of the model lactone **96** whose 4-spin system can be readily assigned and simulated (Figure 5B). Going back to the "lactochlorin" spectrum, we now assign H1 at 3.490 ppm and other parameters as tabulated on Table 1, which fit the natural spectrum more closely in terms of relative heights of some of the

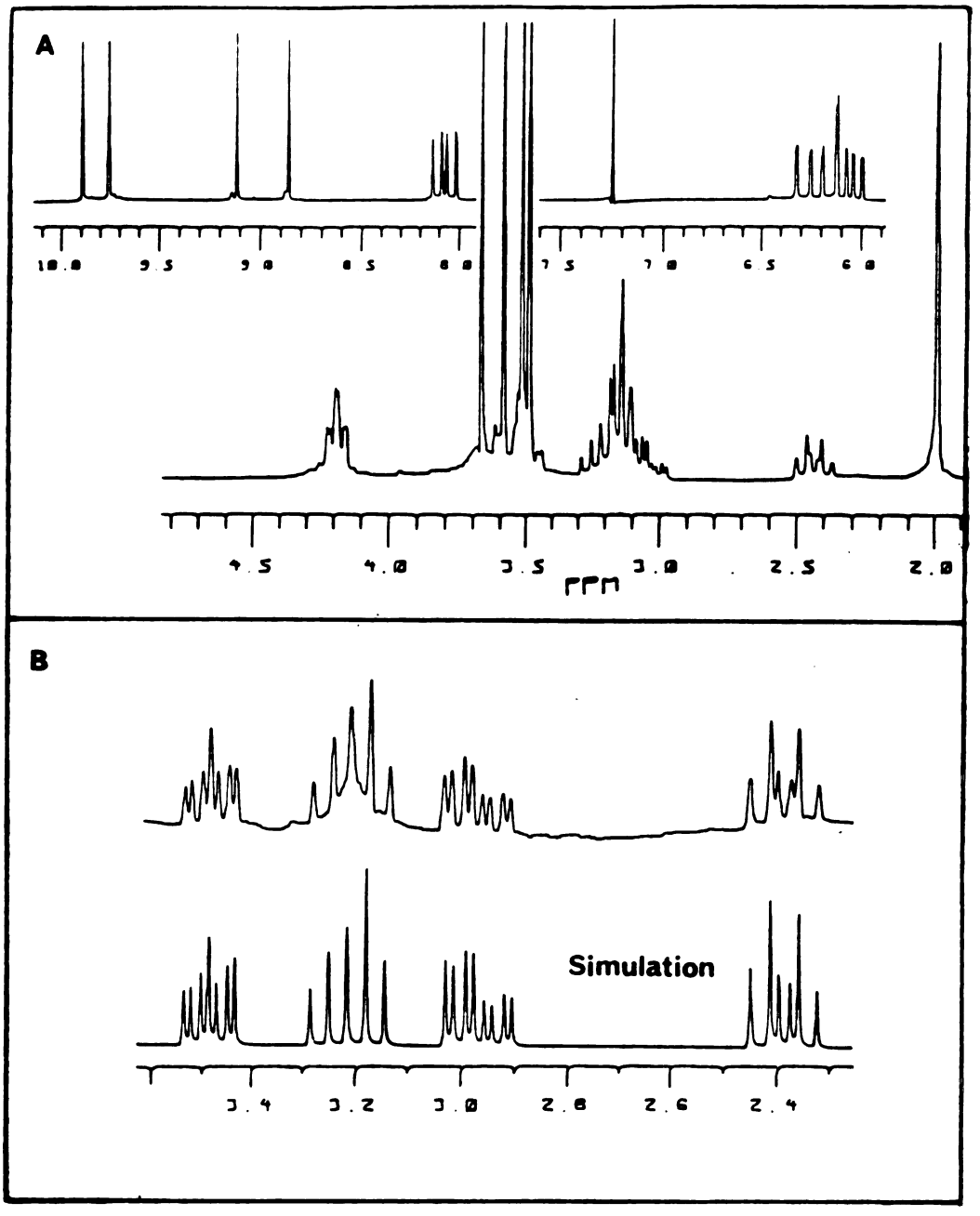


Figure 5 ^{1}H NMR spectra (in $CDCl_3$, 250 MHz) of trans-lactone **83** (A); of the hexaethyl trans-lactone **87** with simulation (B).

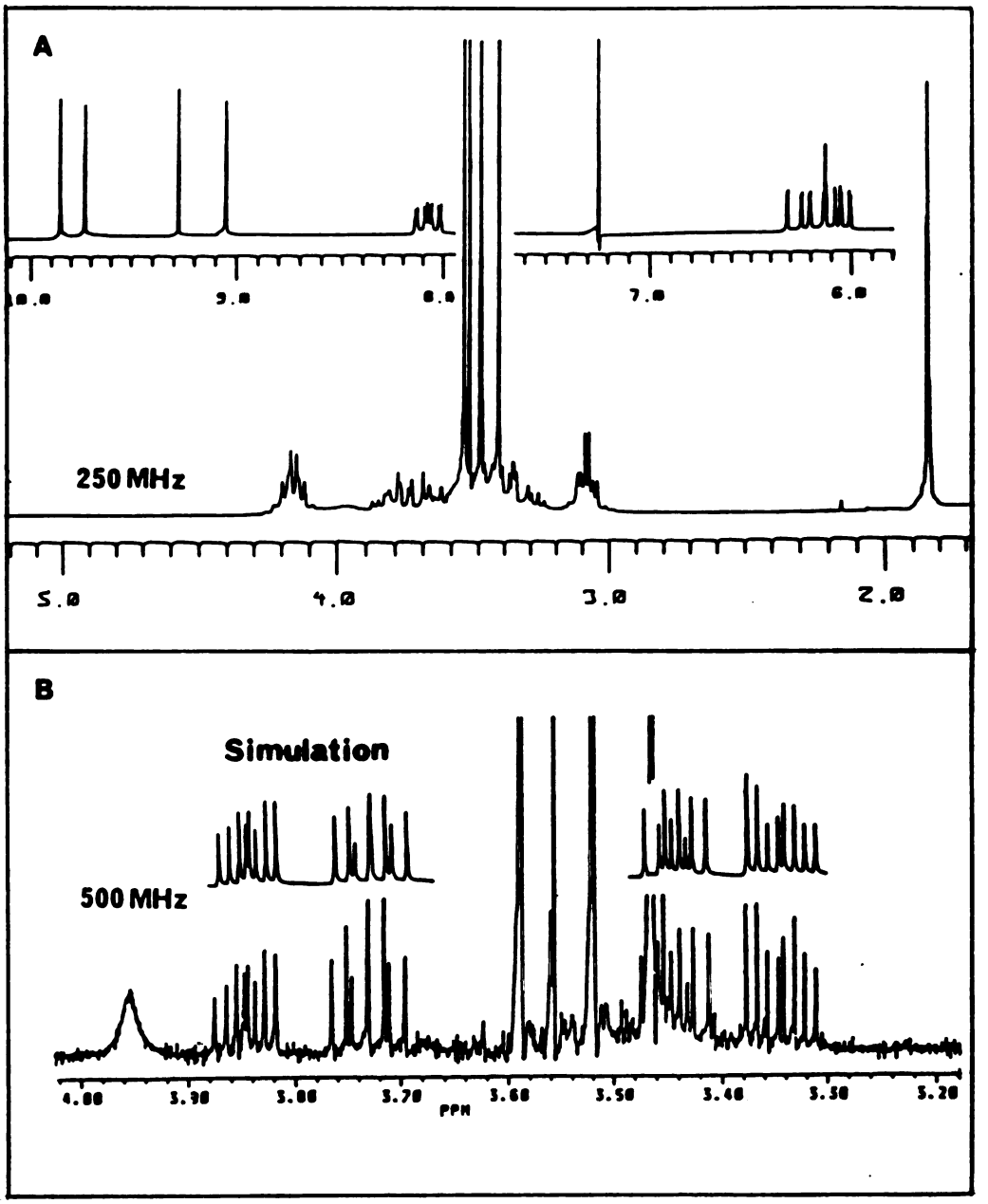


Figure 6 ^1H NMR spectra (in $CDCl_3$) of cis lactone 95 at 250 MHz (A); at 500 MHz with simulation (B).

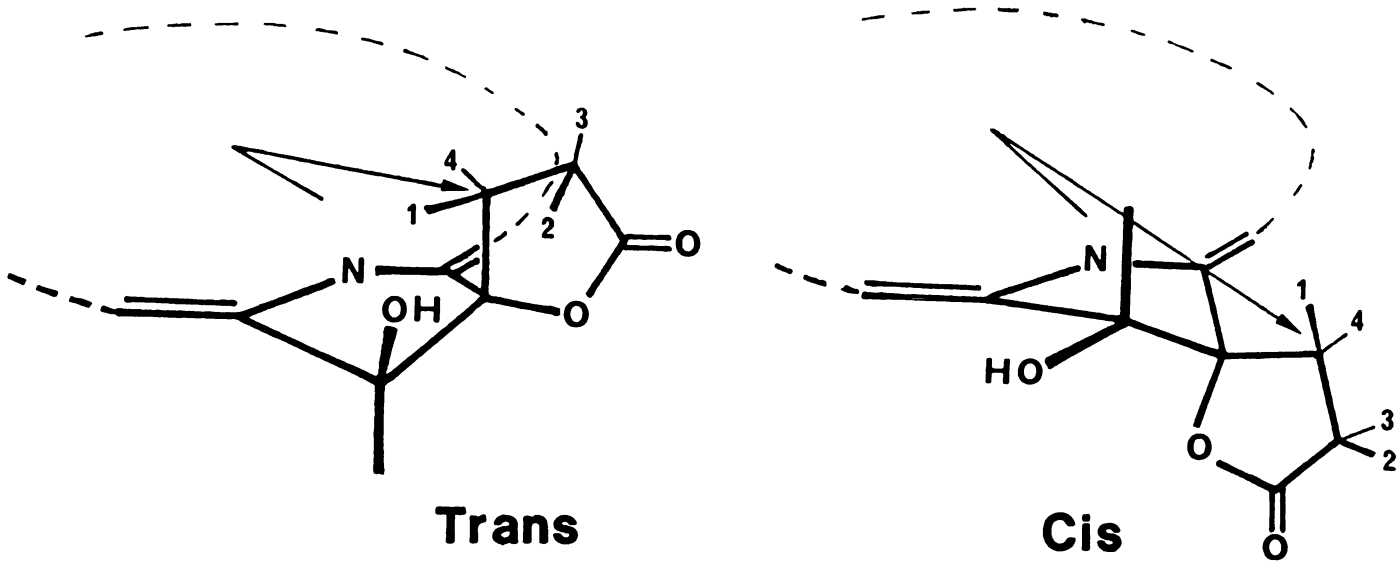


Figure 7 Suggested conformation of the isomeric spirolactones. The slope estimated for the trans isomer is 35° while for the cis isomer is less than 10°.

resolved transitions and in explaining the weak signals overlapping with the 18-methyl.

The spectrum of the lactone isomer cyclized by NaOAc has very different features in the spectral region discussed above (Figure 6A). The most evident difference is that there are no lactone peaks lower than 3.3 ppm. The complex splitting pattern of the methylene protons is not discernible at 250 MHz but reduced to first-order at 500 MHz (Figure 6B). The chemical shifts and parameters are listed in Table 1. The lack of large differentiation between H1 and H4 or, for that matter, any two protons in this group suggests that the shielding and deshielding effect of the OH group is absent or diminished. The fact that the chemical shifts are all located at a much downfield region indicates that the four methylene protons must be experiencing uniformly a greater deshielding than in the trans isomer. Using the known patter of isoshielding lines of porphyrin ring current,[70] our NMR data can best be fit into a cis-lactone in which the methylene protons are near the horizontal plane of the macrocycle (Figure 7). In the trans isomer, the relatively small deshielding effect experienced by the methylene protons, particularly H4, is an indication that they are located higher above the plane, near the "blank region" interfacing the opposite isotropic and anisotropic ring current effects. The presence of the adjacent OH oxygen to H1 could add up to 0.8 ppm deshielding to H1 but the shielding effect on H4 should not exceed 0.2 ppm. These arguments can be applied to the 12-methyl equally well. In the cis isomer (1.823 ppm), it is axial and has no nearby deshielding oxygen whereas in the trans form (1.992 ppm) it is more equatorial and also closer to the ester oxygen.

The broad peak at 3.924 ppm in Figure 6B is the 12-OH proton. NOE experiments revealed that irradiation of the 12-Me singlet enhances the lactone H1 peaks as well as the 10-H proton (9.272 ppm), and irradiation of the 12-OH peak enhances the same meso proton. This observation ruled out the possibility that the NaOAc-cyclized product may be a 6-membered lactone fused across the 12,13-position.

The NMR spectra of the diols **106** and **108** are shown in Figures 8 and 9. The methylene protons of the angular propionate side chain were anaylzed at 500 and 250 MHz, respectively (Figure 8B and 9B). The 4-spin systems were simulated to give the chemical shifts and J values tabulated in Table 1. Their configuration was deduced from several considerations. Firstly, cis-diols were frequently obtained in model compounds following the osmate cleavage. In these cis-diols,[20] the NMR peaks of the methylene protons of the pyrroline propionic acid side chain usually gave a densely packed pattern which resembled the ABCM pattern of the diol **106**. Second, when either the cis or trans diol was lactonized in the presence of NaOAc, which should not epimerize the lactone, each gave only the corresponding cis or trans lactone. The symmetric pattern of diol **108** suggests there is a greater rotational freedom of the pyrroline methylene protons in the trans configuration. The lack of a large differentiation between H1 and H4, in contrast to the case of the trans lactone **92** (Figure 7), is again a consequence of this freedom of rotation. In both diols, no NOE could be detected between the 12-Me or any of the methylene protons.

The vicinal couplings obtained from the cis-diol **106** can be used to provide information about the conformation of the pyrroline propionic group. These measured numbers are average values of the component

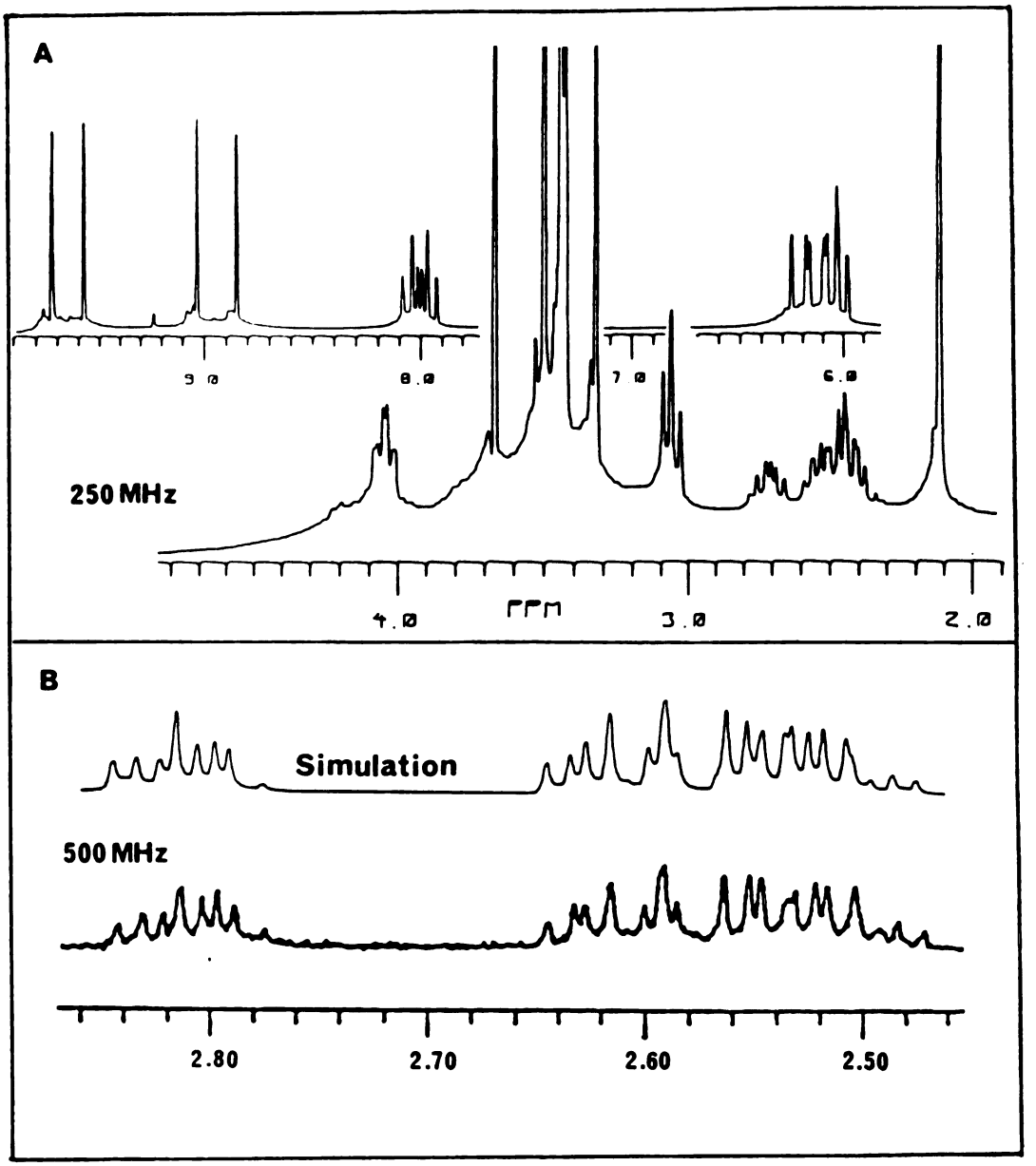


Figure 9 ^{1}H NMR spectra (in $CDCl_3$) of cis-diol **97** at 250 MHz (A); at 500 MHz with simulation (B).

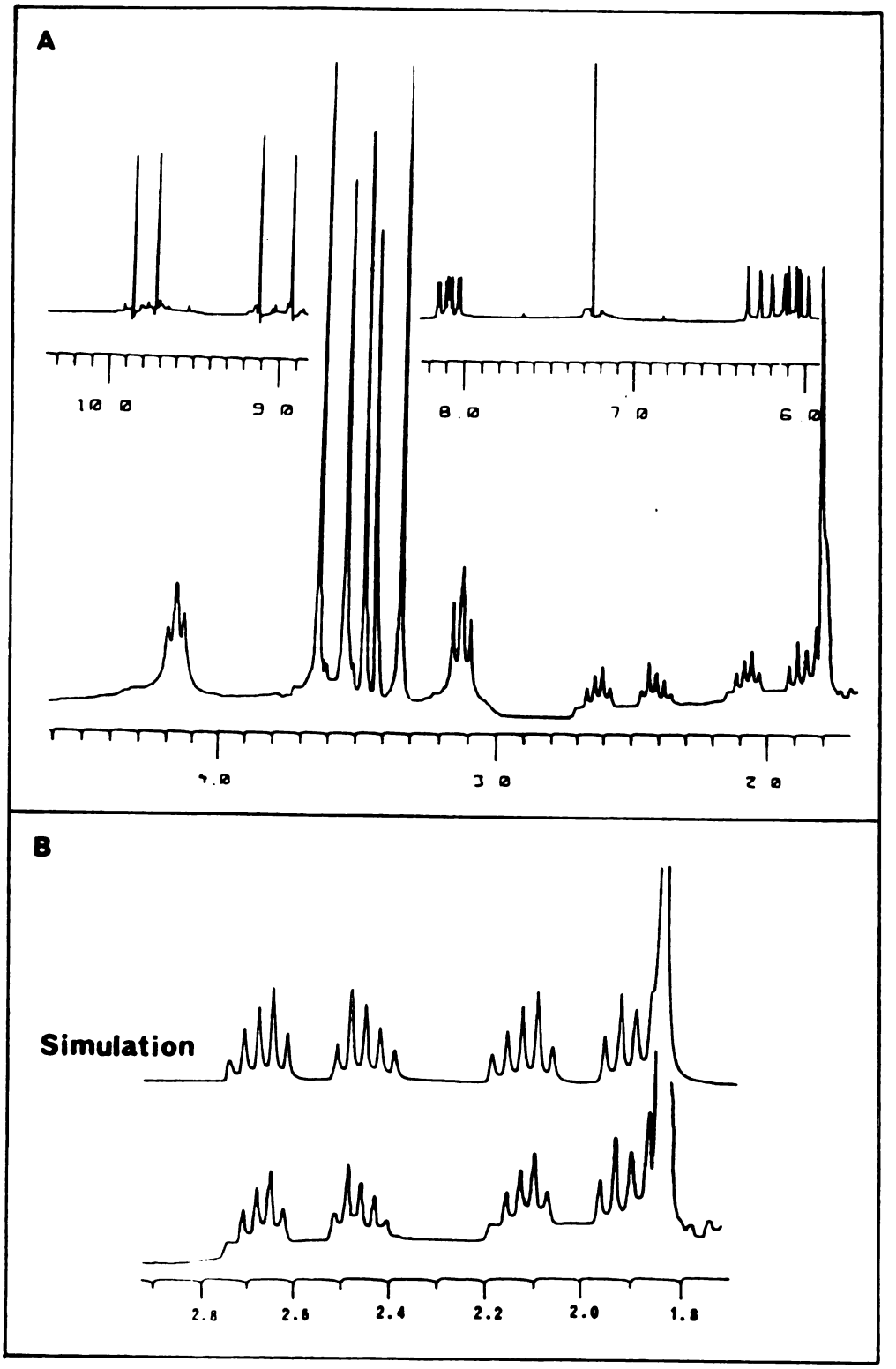


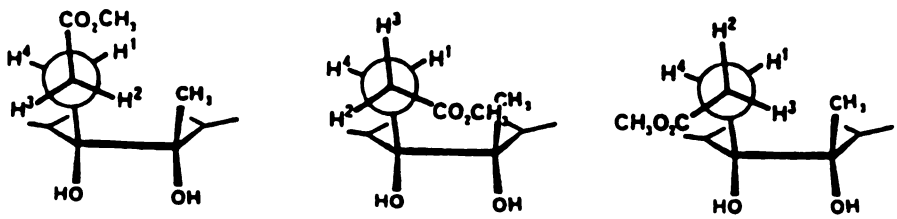
Figure 9 ^{1}H NMR spectra (in $CDCl_3$) of trans-diol **99** at 250 MHz (A); at 250 MHz with simulation (B).

Table 1. ^1H NMR Assignments for the Pyrroline Substituents of the Four Forms of 12,13-Dihydroxyprotochlorin.

Form	H	δ(ppm)	Coupling Constant (Hz)
Lactone			
trans	1	3.490	$J_{1,2}$ = 4.1, $J_{2,4}$ = 9.6
	3	3.213	$J_{1,3}$ = 9.6, $J_{3,4}$ = 9.6
	2	3.032	$J_{1,4}$ = -13.3
	4	2.430	$J_{2,3}$ = -17.9
	12-Me	1.992	
cis	4	3.846	$J_{1,2}$ = 7.4, $J_{2,4}$ = 9.8
	2	3.731	$J_{1,3}$ = 9.8, $J_{3,4}$ = 5.2
	1	3.443	$J_{1,4}$ = -13.3
	3	3.345	$J_{2,3}$ = -17.8
	12-Me	1.823	
Diol			
cis	1	2.816	$J_{1,2}$ = 5.0, $J_{2,4}$ = 9.4
	4	2.614	$J_{1,3}$ = 9.2, $J_{3,4}$ = 5.0
	2	2.562	$J_{1,4}$ = -14.4
	3	2.508	$J_{2,3}$ = -16.2
	12-Me	2.110	
trans			
	2,3	2.660 2.417	$J_{1,2}$ = 7.3, $J_{2,4}$ = 7.3 $J_{1,3}$ = 7.3, $J_{3,4}$ = 7.3
	1,4	2.116 1.882	$J_{1,4}$ = -14.6 $J_{2,3}$ = -14.6
	12-Me	1.847	

coupling constants in rotamers I to III weighted by their fractional populations.[71] The component coupling constants of I - III have previously been estimated for chlorophyll derivatives[72] and can be applied directly in this case. These J values and our calculated populations for **106** are given in Table 2. While this anaylsis may have substantial error margins (±10%), the results are entirely consistent with what a molecular model would qualitatively predict: the anti conformer I is most favorable while the sterically congested rotamer II may be neglected. In the trans-diol **108**, because of the greater rotational freedom, there should be no significant difference in the population of the rotamers.

Table 2. Couplings (Hz)[a] and Rotamer Populations of the cis-Diol **106**.



	I	II	III
$J_{1,2}$	4.4	13.2	2.8
$J_{1,3}$	13.2	3.6	3.6
$J_{2,4}$	13.2	3.6	3.6
$J_{3,4}$	4.4	2.8	13.2
Population	0.60	0.12	0.28

[a] From reference 72.

D. Structure of Heme d: Lactone versus Diol?

Before commenting on this quesiton, we would like to recapitulate the experimental observation. cis,*vic*-Dihydroxychlorins carrying a pyrroline propionate ester chain would readily cyclize into either a cis or trans lactone by reagents encountered in common heme extraction protocols. Mild bases such as sodium acetate, sodium bicarbonate, and pyridine cleanly lactonize the ester without inversion of configuration. Silica gel acts upon the diol in two steps: first it lactonizes the geminal groups and then promotes the inversion of lactone to give the more stable trans diastereomer. In an ideal case, all three compounds can be seen on TLC plate during developing, arising from a pure diol. Under alkaline hydroytic conditions, the cis or trans lactone, if not hydrolyzed, apparently do not epimerize, and the hydrolysis of which yields the diol with complete retention of configuration, undoubtedly the result of a common $B_{Ac}2$ mechanism.[73]

Given these intrinsic labilities, we may quite safely conjecture that the lactone ring found in "lactochlorin" is produced during the chromatographic purification of the demetalated and esterified chromophore. The observed trans configuration is also irrelevant as far as the true structure of the in vivo heme d is concerned. That issue, unfortunately, remains unanswered. From a biosynthetic point of view, saturation of the protoporphyrin ring is most likely brought about by an epoxidation. There have been efforts to prepare epoxychlorin but thus far such a compound has not been observed, presumably it is too labile to have a stable existence. If the epoxy ring is opened in aqueous medium, most likely a trans diol will result. However, one cannot be certain about the configuration as the propionate group may participate

in epoxide opening even though the resulting lactone may not be the final form of heme d. To date, the strongest argument against the lactone being present in the in vivo heme d is the absence of IR peak in the extracted, but unesterified, heme d.[65] We noticed that the lactones are uniformly resistant to acid or base hydrolysis and the extraction procedure employed by Timkovich et.al. is too mild to open the lactone ring. In any case, the true structure of heme d awaits further confirmation. Our finding that the various forms of dihydroxychlorin have slightly different absorption and resonance Raman (RR) spectra[67] indicates that this is a problem solvable by RR studies of the heme enzyme.

III. EXPERIMENTAL

NMR spectra were routinely obtained at 250 MHz on a Bruker WM-250 instrument. Occasionally we managed to obtain spectra recorded at 360, 400, or 500 MHz on spectrometers (all Bruker make) located at other institutions. Spectra were recorded in $CDCl_3$; the residue $CHCl_3$ was used as the internal standard set at 7.240 ppm. The concentration of chlorin samples were maintained at 2-3 mM to avoid concentration effects. All NOE's were positive and are expressed as the area of the enhanced resonance in difference spectra divided by the area in the control spectrum. Simulations were carried out first on an IBM-PC with the PMR software and then on Aspect 2000 which outputs to the NMR plotter.

Dihydroxychlorins 94a, 94b, and 94d

Methyl 7,8,12,13,17,18-hexaethyl-4-methylporphine-2-propionate (93) was synthesized by condensing 5,5′-dibromo-3,3′,4′,4′-tetraethyl-

2,2′-dipyrrylmethene hydrobromide[74] and 4′-(2-carboxyethyl)-3,4-diethyl-3′,5,5′-trimethyl-2,2′-dipyrrylmethene hydrobromide[74] in formic acid and worked up in the usual manner;[51] yield: 32%; m.p. 193-195°C; ^{1}H NMR: 1.94 (9H each, t, Et), 3.29 (2H, t, $CH_2\underline{CH_2}CO_2$), 3.65, 3.68 (3H each, s, ring Me and CO_2Me), 4.09, 4.12 (6H each, q, Et), 4.42 (2H, t, $\underline{CH_2}CH_2CO_2$), 10.09, 10.10 (1H each, s, 5,20-H), 10.12 (2H, s, 10,15-H), -3.73 (2H, br s, NH); UV-vis λ_{max} (ε_M) 619 nm (8 800), 566.5 (10 400), 533 (13 100), 499 (15 900), 399 (142 000). To a solution of this porphyrin (200 mg, 0.35 mmol) in CH_2Cl_2 (60 ml) and pyridine (0.25 ml) was added osmium tetraoxide (114 mg. 0.45 mmol). The mixture was stirred at room temperature in the dark for 36 h; it was then diluted with methanol (20 ml) and bubbled with H_2S for 10 min. The precipitated osmium sulfide was removed by filtration and the filtrate was concentrated and chromatographed on preparative TLC plates, developed with CH_2Cl_2/5% EtOAc. There were four distinct bands: the red porphyrin moving at the front followed by **94a**, **94b**, and another green band containing **94c** and **94d**. Unambiguous structure assignments were achieved by NOE (see Scheme 15). The two south diols were dissolved in a mixture of methanol (20 ml) and CH_2Cl_2 (5 ml) and was brought to reflux in the presence of anhydrous NaOAc (1 g). The reflux was continued for 20 min before the mixture was washed with water and evaporated. The residue was chromatographed on TLC to separate the lactonized **95** (higher Rf) and **94d**; overall yields: unchanged **93**, 22 mg (11%); **94a**, 34 mg (16%); **94b**, 29 mg (13.7%); **94d**, 32 mg (15%); and **95**, 28 mg (14%).

Methyl 7,8,12,13,17,18-hexaethyl-12,13-dihydroxy-4-methyl-chlorin-2-propionate (**94a**). M.p. 138°C (dec); NMR δ 1.00 (6H, t, pyrroline Et),

1.79, 1.81, 1.82, 1.85 (3H each, t, Et), 2.59, 2.61 (2H each, q, pyrroline Et), 3.11 (2H, t, $CH_2CH_2CO_2$), 3.40 (3H, S, Me), 3.68 (3H, S, CO_2Me), 3.91 (8H, q, Et), 4.14 (2H, t, $CH_2CH_2CO_2$), 9.01, 9.02 (1H each, s, 10,15-H), 9.66 (1H, s, 20-H), 9.69 (H, s, 5-H), -2.53 (2H, br s, NH): UV-vis λ_{max} (ε_M) 642 nm, 590.5, 525, 496, 393; MS, found m/e 613.4152 for $(M+H)^+$, $C_{37}H_{49}N_4O_4$ requires m/e 613.4189.

Methyl 7,8,12,13,17,18-hexaethyl-7,8-dihydroxy-3-methyl-chlorin-2-propionate (94b). M.p. 180-182°C (dec); NMR δ 0.90, 1.00 (3H each, t, pyrroline Et), 1.80, 1.83 (6H each, t, Et), 2.54, 2.64 (2H each, q, pyrroline Et), 3.08 (2H, t, $CH_2CH_2CO_2$), 3.36 (3H, s, Me), 3.64 (3H, s, CO_2Me), 3.88, 3.89 (4H each, q, Et), 4.15 (2H, t, $CH_2CH_2CO_2$), 8.96 (1H, s, 5-H), 9.02 (1H, s, 10-H), 9.66, 9.73 (1H each, s, 15,20-H), -2.57 (2H, br s, NH): UV-vis λ_{max} (ε_M) 644 nm, 590.5, 523, 494, 392.

Methyl 7,8,12,13,17,18-hexaethyl-17,18-dihydroxy-3-methyl-chlorin-2-propionate (94d). NMR δ 0.92, 1.02 (3H each, t, pyrroline Et), 1.80, 1.83 (6H each, t, Et), 2.58, 2.64 (2H each, q, pyrroline Et), 3.10 (2H, t, $CH_2CH_2CO_2$), 3.41 (3H, s, Me), 3.65 (3H, s, CO_2Me), 3.86, 3.92 (4H each, q, Et), 4.11 (2H, t, $CH_2CH_2CO_2$), 8.94 (1H, s, 20-H), 9.04 (1H, s, 15-H), 9.65, 9.72 (1H each, s, 5,10-H): UV-vis λ_{max} (ε_M) 643.5 nm, 591, 523, 497, 391.5.

cis-7,8,12,13,17,18-Hexaethyl-3-hydroxy-3-methyl-2,2-γ-spirolactone-chlorin (95)

M.p. 239-241°C; NMR δ 1.82, 1.85 (9H each, t, Et), 1.88 (3H, s, Me), 3.29-3.54 (2H, m $2a_2$ and $2b_4$), 3.75-4.14 (14H, m, 6xEt, $2a_1$,$2b_3$), 9.02, 9.20 (1H each, s, 5,20-H), 9.78, 9.85 (1H each, s, 10,15-H), -2.65, -2.57 (1H each, br s, NH); UV-vis λ_{max} (ε_M) 640.5 nm (42 000),

588 (5 900), 522 (5 000), 492 (14 000), 390 (161 000); MS, found m/e 581.3480 for $(M+H)^+$, $C_{36}H_{45}N_4O_3$ requires m/e 581.3494.

trans-7,8,12,13,17,18-Hexaethyl-3-hydroxy-3-methyl-2,2-γ-spirolactone-chlorin (96)

The cis-lactone 95 (20 mg, 0.034 mmol) was loaded on a 1500 μm TLC plate and left in the dark overnight. The plate was developed with CH_2Cl_2/3% EtOAc to give the faster moving 96 in about 20% and the unchanged 95 (75%). If the plate was developed repetitively, 95 was completely converted to 96; however, some minor degradation was also observed. M.p. 253-255°C; NMR δ 1.79, 1.83 (9H each, t, Et), 1.97 (3H, s, Me), 2.38 (1H, sext, 2a4), 2.97 (1H, oct, 2b2), 3.21 (1H, quint, 2b3), 3.48 (1H, sept, 2a1) [J(2a1,2b2) = 3.7 Hz, J(2a1,2b3) = 9.6, J(2a1,2a4) = -13.3, J(2b2,2b3) = -17.9, J(2b2,2a4) = 9.6, J(2b3,2a4 = 9.6], 3.87, 3.90, 4.00 (4H each, q, Et), 8.87, 9.00 (1H each, s, 5,20-H), 9.76, 9.77 (1H each, s, 10,15-H), -2.51 (2H, br s, NH); UV-vis λ_{max} (ε_M) 643 nm (31 500), 590 (2 600), 522 (4 800), 493 (11 000), 390 (153 000); MS, found m/e 581.3473 for $(M+H)^+$, $C_{36}H_{45}N_4O_3$ requires m/e 581.3494.

3,8-Bis(2,2-dimethoxyethyl)-deuteroporphyrin IX dimethyl ester (98)

To a solution of 1 lt of dichloromethane and 170 ml of methanol containing 4 g of protoporphyrin IX dimethyl ester[75] was added 10.5 g (3.3 mol equiv) of thallium (III) trinitrate trihydrate dissolved in 340 ml of methanol. The mixture was stirred under argon for 10 min at room temperature. Hydrogen sulfide was then bubbled through the mixture for 10 min, followed by the addition of 17 ml of concentrated hydrochloric acid. The mixture was stirred for 5 min before the supernatant was decanted and the precipitated thallium (I) salts were washed with

methylene chloride. The combined organic solutions were washed three times with 1 lt of water and evaporated under vacuum, to give porphyrin **98** in quantitative yield. NMR δ 3.29, 3.32 (2H each, t, $CH_2CH_2CO_2$), ring methyl: 3.45 (3H, s), 3.53 (6H, s), 3.60 (3H, s), 3.46, 3.51 (6H, each, s, OCH_3), 4.09, 4.20 (2H each, d, $CH_2CH(OCH_3)_2$), 4.34, 4.39 (2H each, t, $CH_2CH_2CO_2$), 5.08, 5.14 (1H each, t, $CH_2CH(OCH_3)_2$), 9.84, 9.87, 9.95, 9.97 (1H each, s, meso), -4.05 (2H, br s, NH).

3,8-Bis(2-hydroxyethyl)-deuteroporphyrin IX dimethyl ester (100)

The foregoing porphyrin **98** (2 g, 2.8 mmol) was dissolved in 500 ml of tetrahydrofuran and the solution was brought to reflux, followed by the addition of 5 ml conc. HCl in 15 ml of H_2O. After 5 min, the solution was cooled immediately, diluted with 500 ml CH_2Cl_2, and washed three times with water. The solvent was evaporated under vacuum and the residue was dissolved in an arbitrary mixture of THF/MeOH. This solution (mixture of dialdehyde diester **99** and dialdehyde monoesters) was treated at 0°C with 6 g of $NaBH_4$ in ice-cold methanol. It was stirred for 10 min before ~20 ml of acetic acid was carefully added to quench excess borohydride. The solvent was evaporated under vacuum and the residue was stirred for 12 h in 500 ml of dry methanol containing 25 ml of concentrated sulfuric acid. The acidic solution was then diluted with 500 ml CH_2Cl_2 and washed with 3 x 500 ml of water. The product was chromatographed on neutral alumina (Brochmann Grade III) to separate a small amount of the faster moving mixture of 3-(2-hydroxyethyl)-8-(2,2′-dimethoxyethyl)-deuteroporphyrin IX dimethyl ester and 8-(2-hydroxyethyl)-3-(2,2′-dimethoxyethyl)-deuteroporphyrin IX dimethyl ester (elution with $CHCl_3$) from the desired compound **100** (elution with $CHCl_3$/2% CH_3OH). Yield 71%; NMR ($CDCl_3$ at 315°K) δ 3.25 (4H, t,

$CH_2\underline{CH_2}CO_2$), 3.57 (12H, s, ring Me), 3.65 (6H, s, CO_2Me), 4.23 (4H, t, $\underline{CH_2}CH_2CO_2$), 4.37 (8H, m, CH_2CH_2O), 9.98, 10.00 (2H each, s, meso), -3.80 (2H, br s, NH).

3,8-Bis(2-chloroethyl)-deuteroporphyrin IX dimethyl ester (101)

To a solution of the foregoing porphyrin 100 (1 g, 1.59 mmol) in dry DMF (200 ml) was added benzoyl chloride (20 ml). The mixture was heated as 98°C for 1 h under nitrogen and allowed to cool. Water (500 ml) and triethyl amine (30 ml) were then added and the precipitated compound was filtered, washed with water and purified by passing through a short silica gel column using CH_2Cl_2/1% CH_3OH as eluant. The product was further purified by recrystallization from CH_2Cl_2/CH_3OH. Yield 91%; m.p. 216-217°C (lit.[68a] m.p. 216-217°C); NMR δ 3.24, 3.26 (2H each, t, $CH_2\underline{CH_2}CO_2$), 3.46, 3.50, 3.53, 3.56 (3H each, s, ring Me), 3.66, 3.67 (3H each, s, CO_2Me), 4.19-4.38 (12H, m, CH_2CH_2Cl and $\underline{CH_2}CH_2CO_2$), 9.75, 9.82, 9.87, 9.96 (1H each, s, meso), -4.06 (2H, br s, NH); MS, m/e 663.1049 (calcd for $C_{36}H_{41}N_4O_4Cl_2$ 663.2508).

Spirolactones 103a and 103b

To a solution of 3,8-bis(2-chloroethyl)deuteroporphyrin IX dimethyl ester 101 (850 mg, 1.28 mmol) in CH_2Cl_2, osmium tetraoxide (423 mg, 1.66 mmol) was added, followed by addition of pyridine (0.2 ml). The reaction was stirred in the dark for 26 h before being diluted with methanol (50 ml) and quenched by H_2S. The chlorin products were chromatographed on a silica gel column. Porphyrin 101 (180 mg, 21% recovered) was eluted first with CH_2Cl_2 while the mixture of the four dihydroxychlorins was washed out with CH_2Cl_2/2% MeOH. This mixture was then chromatographed on TLC plates (CH_2Cl_2/10% EtOAc) to separate the

two faster moving north diols (120 mg, 1:1 ratio) from the two south diols **102a** and **102b** (430 mg, 1.2:1 ratio). The distinction of the north and south diols was based on NMR of the methyl ester singlets: δ 3.64, 3.63, 3.60, 3.59 (north diols) versus 3.67, 3.65, 3.55, 3.52 (south diols).

The mixture of 102a and 102b in MeOH (100 ml) was refluxed with anhydrous NaOAc (5 g) for 30 min. the solution was evaporated and the residue was dissolved in CH_2Cl_2 and chromatographed on TLC (CH_2Cl_2/10% EtOAc) without interruption, in a single path, to yield the faster moving 103a (160 mg) and the slower moving 103b (180 mg), the structure assignment of which had been based on NOE (see Scheme 17).

cis-3,8-Bis(2-chloroethyl)-12-hydroxy-13,13-γ-spirolactone-deuterochlorin IX dimethyl ester (**103a**). M.p. 226-228°C; NMR δ 1.84 (3H, s, 12-Me), 3.06 (2H, t, 17- $CH_2CH_2CO_2$), 3.38, 3.41 (3H each, s, 2,18-Me), 3.51 (3H, s, 7-Me), 3.52 (3H, s, CO_2Me), 3.26-3.90 (4H, m, 13-CH_2CH_2), 4.06 (1H, br s, OH), 4.12, 4.20, 4.28 (10H, t, 2xCH_2CH_2Cl and 17-$CH_2CH_2CO_2$) 9.08, 9.12 (1H each, s, 10,15-H), 9.64 (1H, s, 20-H), 9.71 (1H, s, 5-H), -2.67 (2H, br s, NH): UV-vis λ_{max} (ε_M) 641 nm (57 000), 588.5 (10 000), 522.5 (9 000), 497 (21 900), 392 (226 000); MS, found m/e 665.2345 for $(M+H)^+$, $C_{35}H_{39}N_4O_5Cl_2$ requires m/e 665.2300.

cis-3,8-Bis(2-chloroethyl)-18-hydroxy-17,17-γ-spirolactone-deuterochlorin IX dimethyl ester (**103b**). M.p. 239-240°C; NMR δ 1.85 (3H, s, 18-Me), 3.12 (2H, t, 13- $CH_2CH_2CO_2$), 3.41, 3.49 (3H each, s, 7,12-Me), 3.44 (3H, s, 2-Me), 3.52 (3H, s, CO_2Me), 3.27-3.86 (4H, m, 17-CH_2CH_2), 3.98 (1H, br s, OH), 4.19, 4.22, 4.30 (10H, t, 2xCH_2CH_2Cl and 13-$CH_2CH_2CO_2$), 9.14 (1H, s, 15-H), 9.19 (1H, s, 20-H), 9.66, 9.70 (1H

each, s, 5,10-H), -2.70 (2H, br s, NH); UV-vis λ_{max} (ε_M) 641 nm (55 000), 588 (11 900), 524.5 (12 800), 494 (22 000), 395.5 (255 000).

cis-12-Hydroxy-13,13-γ-spirolactone-protochlorin IX methyl ester (104)

To a refluxing solution of cis-lactone **103a** (100 mg, 0.15 mmol) in pyridine (50 ml) under argon, KOH (1.2 g) in water (4 ml) was added and the heating was continued for 6 h before the mixture was evaporated to dryness under reduced pressure. The residue dissolved in ice-water was treated with 10% HCl whereupon the product precipitated. The solid was filtered, washed with water and esterified in MeOH with diazomethane. The product was chromatographed rapidly on TLC to separate the faster moving lactone **104** (8 mg) and the slower moving diol **106** (12 mg). The non-mobile material, after eluting with CH_2Cl_2/2% MeOH was believed to be degradation products due to the prolonged heating with strong base. However, if more dilute solution of KOH or less time (<3 h) was allowed for the reaction, the mono-vinyl compound: 3-(2-chloroethyl)-12,13-dihydroxy-8-vinyldeuterochlorin IX dimethyl ester was mainly resulted (structure determined by NOE). The condition for this elimination has not been optimized. NMR of **104**: ring methyl (3H each, s): 3.433 (18-Me), 3.490 (2), 3.527 (7); propionate: 4.263 (2H, t, 17a), 3.087 (2H, t, 17b) [J(17a,17b) = 7.4 Hz], 3.558 (3H, s, CO_2Me); vinyl (1H each): 8.073 (X_i), 8.073 (X_j), 6.281 (A_i), 6.101 (B_i), 6.165 (A_j), 6.022 (B_j) [$J(X_i,A_i)$ = 17.8, $J(X_i,B_i)$ = 11.5, $J(A_i,B_i)$ = 1.3, $J(A_j,B_j)$ = 1.7]; meso (1H each, s): 9.856 (5), 9.272 (10), 9.048 (15), 9.737 (20); NH: -2.618 (2H, br s); pyrroline substituents: see Table 1. IR 1780 cm^{-1}, 1735; UV-vis λ_{max} (ε_M) 650 nm (37 200), 594 (7 500), 530 (8 000), 500

(14 200), 401 (147 000). MS, found m/e 593.2742 for $(M+H)^+$, $C_{35}H_{37}N_4O_5$ requires m/e 593.2766.

cis-12,13-dihydroxyprotochlorin IX dimethyl ester (106)

NMR δ 2.11 (3H, s, 12-Me), 3.06 (2H, t, 17b), 4.04 (2H, t, 17a) [J(17a,17b) = 7.4], 3.32, 3.34, 3.44 (3H each, s, ring Me), 3.49 (3H, s, 13-$CCCO_2Me$), 3.66 (3H, s, 17-$CCCO_2Me$), 5.99, 6.05, 6.13, 6.22 (1H each, dd, vinyl), 8.01 (2H, m, vinyl), 8.84 (1H, s, 15-H), 9.03 (1H, s, 10-H), 9.57, 9.72 (1H each, s, 5,20-H), -2.41 (2H, br s, NH), 13-$CH_2CH_2CO_2$: see Table 1; UV-vis λ_{max} (ε_M) 650 nm (45 000), 594 (4 600), 532 (4 400), 498 (13 800), 401 (179 000). MS found m/e 625.3039 for $(M+H)^+$, $C_{36}H_{41}N_4O_6$ requires m/e 625.3028.

trans-12-Hydroxy-13,13-γ-spirolactone-protochlorin IX methyl ester (92)

The cis lactone 104 (20 mg) was loaded on a TLC plate and was developed with CH_2Cl_2/3% EtOAc) in the dark for at least 4 times to convert 104 into the slightly faster moving 92. NMR δ ring methyl (3H each, s): 3.484 (18-Me), 3.514 (2), 3.584 (7); propionate: 3.132 (2H, t, 17b), 4.189 (2H, t, 17b) [J(17a,17b) = 7.4 Hz]; 3.662 (3H, s, CO_2Me), vinyl (1H each): 8.069 (X_i), 8.069 (X_j), 6.286 (A_i), 6.091 (B_i), 6.157 (A_j), 6.010 (B_j) [$J(X_i,A_i)$ = 17.8, $J(X_i,B_i)$ = 11.5, $J(A_i,B_i)$ = 1.3, $J(A_j,B_j)$ = 1.7]; meso (1H each, s): 9.889 (5), 9.113 (10), 8.856 (15), 9.752 (20); NH: -2.254 (2H, br s); pyrroline substituents: see Table 1. IR 1780 cm^{-1}, 1738, 1718; UV-vis λ_{max} (ε_M) 653 nm (40 500), 596 (6 300), 532 (6 600), 501 (14 500), 401 (156 000). MS, found m/e 593.2736 for $(M+H)^+$, $C_{35}H_{37}N_4O_5$ requires m/e 593.2766.

cis-18-Hydroxy-17,17-γ-spirolactone-protochlorin IX methyl ester (105a)

The chlorin-bearing lactone **103b** was treated with KOH in refluxing pyridine and worked up in the same manner as described for **104**. NMR δ 1.84 (3H, s, 18-Me), 3.06 (2H, t, 13b), 3.27-3.88 (4H, ABMN, lactone), 3.32, 3.39, 3.43 (3H each, s, ring Me), 3.54, (3H, s, CO_2Me), 4.05 (1H, br s, OH), 4.10 (2H, t, 13a), 5.99, 6.07, 6.13 6.25 (1H each, dd, vinyl), 8.00 (2H, m, vinyl), 9.04, 9.15 (1H each, s, 15,20-H), 9.71, 9.72 (1H each, s, 5,10-H), -2.70 (2H, br s, NH); UV-vis λ_{max} (ε_M) 650 nm (40 000), 596 (7 300), 533 (7 400), 501 (15 800), 402 (164 000).

trans-18-Hydroxy-17,17-γ-spirolactone-protochlorin IX methyl ester (105b)

Obtained from the cis-lactone **105a** by a repetitive chromatography. NMR δ 2.01 (2H, s, 18-Me), 2.43 (1H, sext, 17a4), 3.04 (1H, oct, 17b2), 3.22 (1H, quint, 17b3), 3.48 (1H, sept, 17a1) [J(17a1,17b2) = 3.7 Hz, J(17a1,17b3) = 9.6, J(17a1,17a4) = -13.3, J(17b2,17b3) = -17.9, J(17b2,17a4) = 9.6, J(17b3,17a4) = 9.6], 3.13 (2H, t, 13b), 3.66 (3H, s, CO_2Me), 4.19 (2H, t, 13a), 6.02, 6.13, 6.19, 6.35 (1H each, dd, vinyl), 8.11 (2H, m, vinyl), 8.88, 9.05 (1H each, s, 15,20-H), 9.86, 9.93 (1H each, s, 5,10-H), -2.34 (2H, br s, NH); UV-vis λ_{max} (ε_M) 652 nm (41 800), 597 (8 200), 533 (9 000), 500 (16 800), 401 (166 000); MS, found m/e 593.2730 for $(M+H)^+$, $C_{35}H_{37}N_4O_5$ requires m/e 593.2766.

cis-17,18-Dihydroxy-protochlorin IX dimethyl ester

Obtained by pyridine/KOH treatment of **105a**. NMR δ 2.09 (3H, s, 12-Me), 2.32-2.78 (4H, ABCM, 17-$CH_2CH_2CO_2$), 3.01 (2H, t, 13b), 3.24, 3.40, 3.41 (3H each, s, ring Me), 3.50 (3H, s, 17-$CCCO_2Me$), 3.65 (3H, s, 13-$CCCO_2Me$), 3.97 (2H, t, 13a), 5.99, 6.07, 6.11, 6.24 (1H each, dd, vinyl), 7.98 (2H, m, vinyl), 8.82, 8.91 (1H each, s, 15,20-H), 9.62,

9.71 (1H each, s, 5,10-H), -2.45 (2H, br s, NH); UV-vis λ_{max} (ε_M) 651 nm (41 000), 597 (8 000), 533 (8 600), 501 (15 800), 402 (159 000).

trans-3,8-Bis(2-chloroethyl)-12-hydroxy-13,13-γ-spirolactone-deuterochlorin IX dimethyl ester (107)

Obtained from the cis-lactone **103a** by repetitive chromatography. NMR δ 1.99 (3H, s, 12-Me), 2.41 (1H, sext, $13a_4$), 3.03 (1H, oct, $13b_2$), 3.23 (1H, quint, $13b_3$), 3.45 (1H, sept, $13a_1$) [$J(13a_1,13b_2$ = 4.1 Hz, $J(13a_1,13b_3)$ = 9.6, $J(13a_1,13a_4)$ = -13.3, $J(13b_2,13b_3)$ = -17.9, $J(13b_2,13a_4)$ = 9.6, $J(13b_3,13a_4)$ = 9.6], 3.14 (2H, t, 17b), 3.42, 3.52, 3.54 (3H each, s, ring Me), 3.66 (3H, s, CO_2Me), 4.16, 4.20, 4.21, 4.24, 4.30 (2H each, t, 2xCH_2CH_2Cl and 17a), 8.98, 8.90 (1H each, s, 10,15-H), 9.75, 9.66 (1H each, s, 5,20-H), -2.46 (2H, br s, NH); UV-vis λ_{max} (ε_M) 641 nm (42 200), 588 (6 300), 520 (4 600), 495.5 (18 700), 392 (184 000); MS, found m/e 665.2335 for $(M+H)^+$, $C_{35}H_{39}N_4O_5Cl_2$ requires m/e 665.2300.

trans-12,13-Dihydroxyprotochlorin IX dimethyl ester (108)

Obtained by pyridine/KOH treatment of **107**. NMR δ 1.85 (3H, s, 12-Me), 3.15 (2H, t, 17b), 3.39, 3.49, 3.52 (3H each, s, ring Me), 3.59 (3H, s, 13-$CCCO_2Me$), 3.66 (3H, s, 17-$CCCO_2Me$), 4.19 (2H, t, 17a), 6.00, 6.07, 6.15, 6.29 (1H each, dd, vinyl), 8.08 (2H, m, vinyl), 8.93, 9.11 (1H each, s, 10,15-H), 9.72, 9.86 (1H each, s, 5,20-H), -2.08 (2H, br s, NH), 13-$CH_2CH_2CO_2$: see Table 1; UV-vis λ_{max} (ε_M) 653 nm (37 200), 598 (4 600), 533 (4 800), 499.5 (12 800), 401 (154 000); MS found m/e 625.3014 for $(M+H)^+$, $C_{36}H_{41}N_4O_6$ requires m/e 625.3028.

CHAPTER 3

SYNTHESIS AND PROPERTIES OF SULFUR-CONTAINING SATURATED OCTAETHYLPORPHYRINS

I. INTRODUCTION

Our interest in sulfur-containing saturated porphyrins stems from sulfmyoglobin (SMb) and sulfhemoglobin (SHb). These sulfglobins are unusual green derivatives of either myoglobin or hemoglobin produced *in vitro* according to the following scheme:

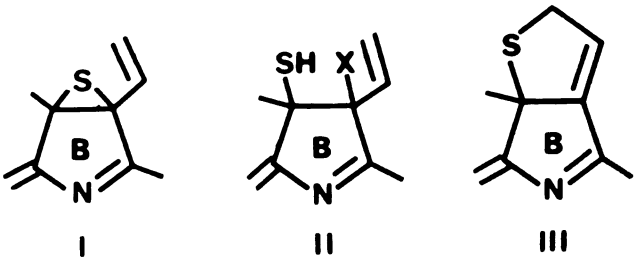
$$Fe(III)Mb + H_2O_2 \rightarrow Fe(IV)Mb \cdot Peroxo$$

$$Fe(IV)Mb \cdot Peroxo + H_2S \rightarrow Fe(III)SMb$$

The existence of sulfglobins is more than a laboratory curiosity. SHb has been reported to be formed *in vivo* under certain pathological conditions or in the presence of high dosages of some drugs related to common analgesics such as phenacetin.[76] Increased levels of Shb have been correlated with exposure to chemical pollutants.[77]

Over the years, several groups have made contributions to the available structural knowledge of sulfglobins.[76,78] Today, it is generally accepted that these abnormal globins contain a chlorin prosthetic group with a sulfur moiety on the pyrroline ring, however, both the final sulfur modification and the site of ring reduction are still questions under investigation by a number of laboratories. Recently, La Mar[79] and Timkovich[80] have independently shown that it is possible to isolate a stable sulfchlorin from SMb and suggested a

thiophene-like cyclic structure (III) based on ^1H NMR data. They further proposed than an episulfide (I) or a thio-substituted chlorin (II) is most likely to be the initial product during sulfheme formation (Figure 10).



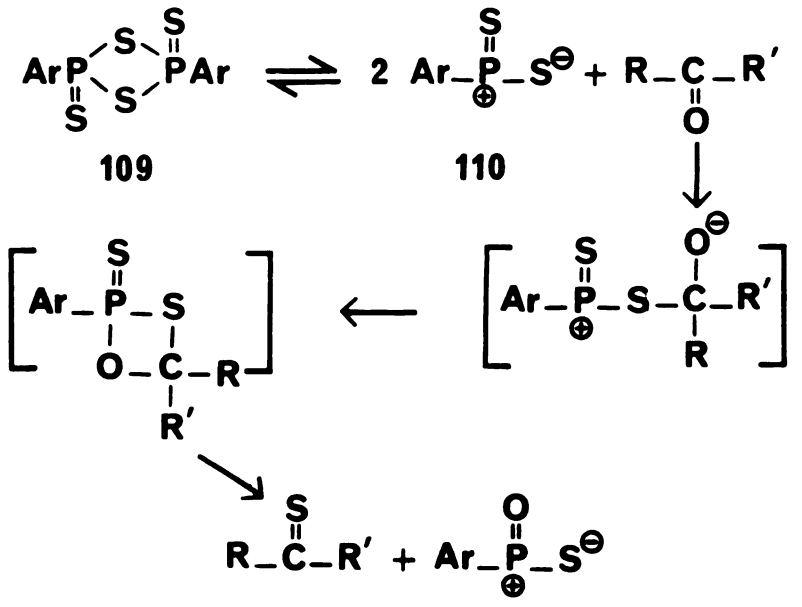
I II III

Figure 10. Sulfheme Models

Since such a sulfchlorin structure has not been know in the literature, we decided to develop the synthesis and to study the properties of sulfur-containing chlorins. Moreover, our curiosity let us proceed further and explore the chemistry of even higher saturated porphyrin systems containing up to three sulfur atoms.

II. RESULTS AND DISCUSSION

Our approach towards the synthesis of sulfur-containing saturated porphyrins starts with the transformation of a carbonyl into a thiocarbonyl group, using 2,4-bis(p-methoxy-1,3-dithiadiphosphetane-2,4-disulfide) **109** now popularly referred to as Lawesson's reagent. This reagent has been used in the literature for the high yield thionation of a variety of aliphatic and aromatic carbonyls.[5b,81] It has been suggested that a highly reactive dithiophosphine ylide **110**, rather than Lawesson's reagent itself, might be the active thionating agent[82] and two possible mechanisms might be envisioned, both involving Wittig-type intermediates[83] (Scheme 18).

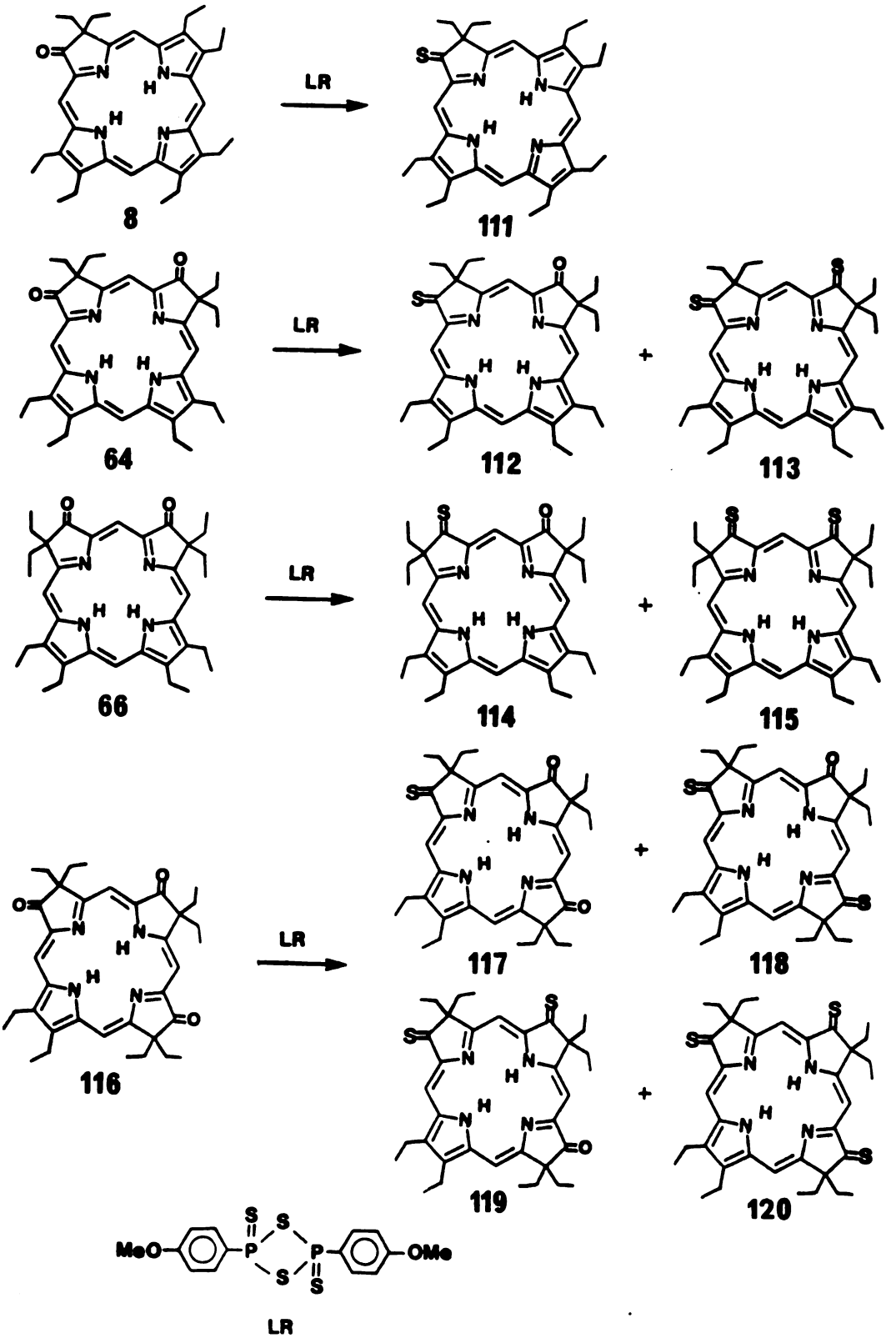


Scheme 18

In an effort to synthesize OEP-thione (111), we reacted OEP-monoketone[18] (8) with two equivalents of Lawesson's reagent in refluxing THF for 24 h, to yield the thione 111 (60%), plus the unreacted starting material (35%). Lengthening the reaction time had no effect on the product's yield. On the other hand, increasing the amount of Lawesson's reagent led to the formation of polymeric material at the expense of the desired product.

Thione 111 exhibited a characteristic IR band at 1230 cm^{-1} [84] (Figure 11) and a ^1H NMR spectrum shown in Figure 12 in comparison with its oxo-analogue. Notable features of this spectrum are: (1) the deshielding effect of the sulfur on the adjacent meso proton; (2) the multiplication of the geminal ethyl groups, suggesting that these groups have less freedom of rotation in comparison with the oxo-analogue 8; and (3) the slight downfield shift of the N-H protons.

The thione structure is stable in acids and on silica gel (during purification), however, in the presence of oxidizing agents, e.g., OsO_4,



SCHEME 19

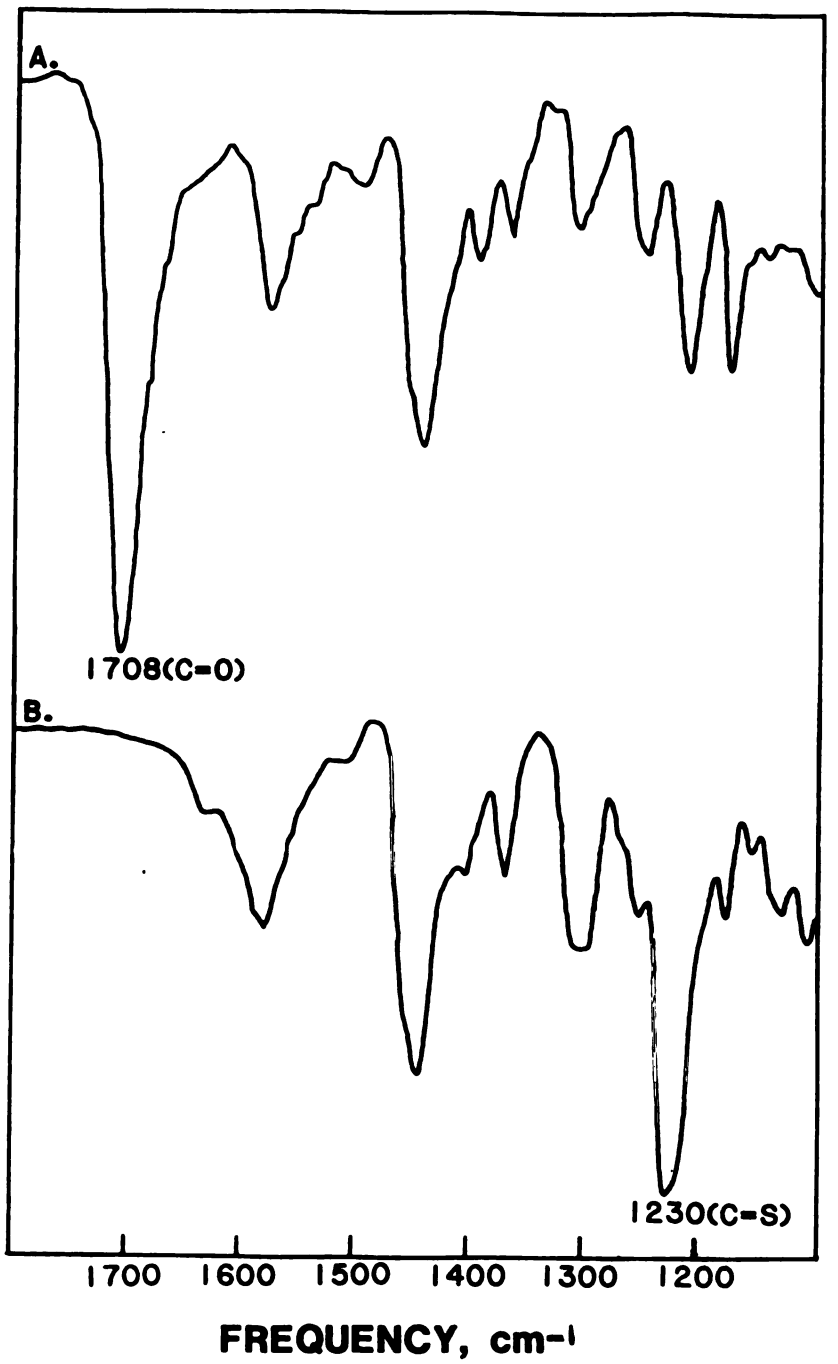


Figure 11 1800-1300 cm^{-1} IR spectra of: (A) porphyrinone 8; (B) porphyrinthione **111.**

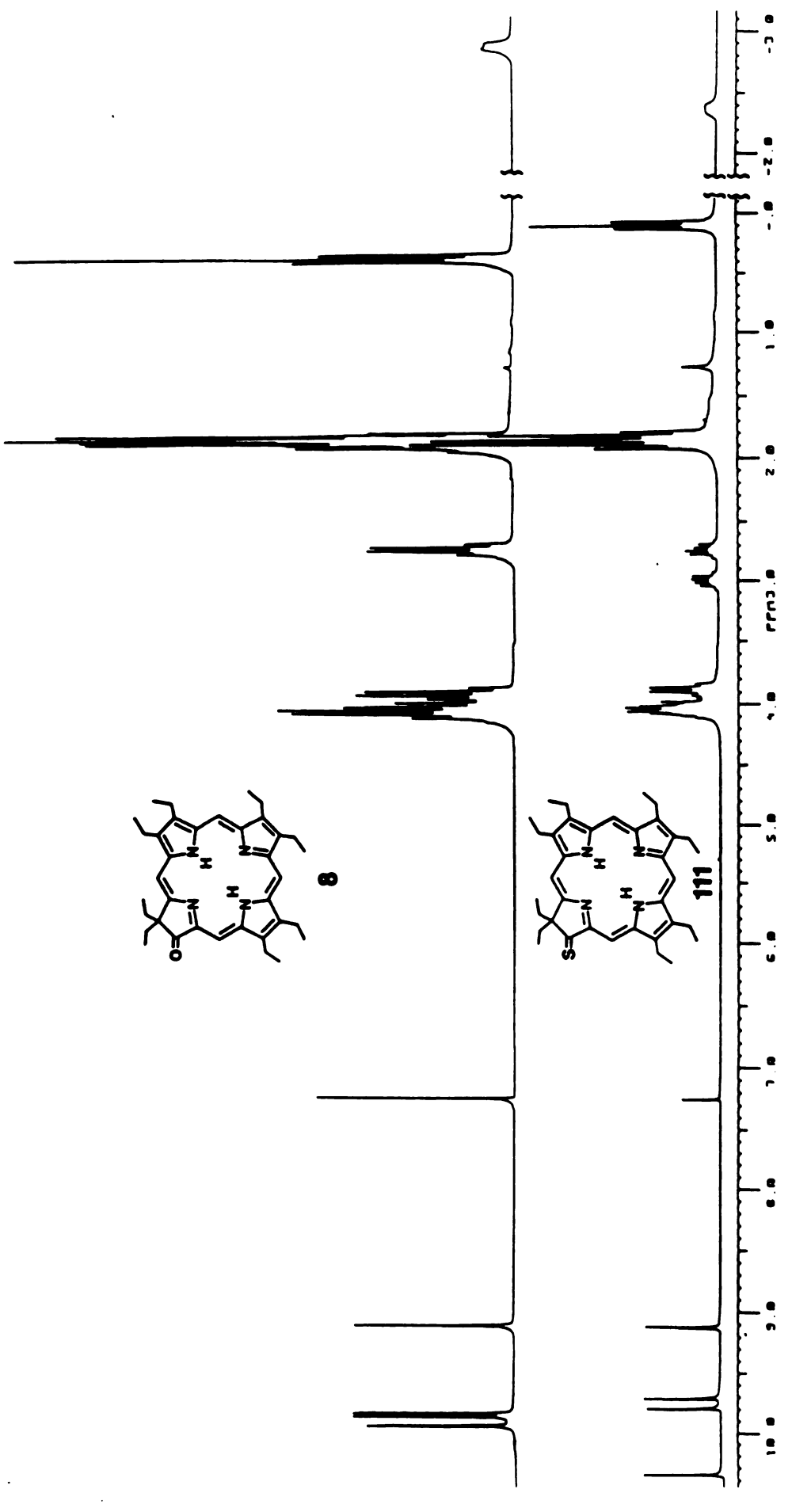
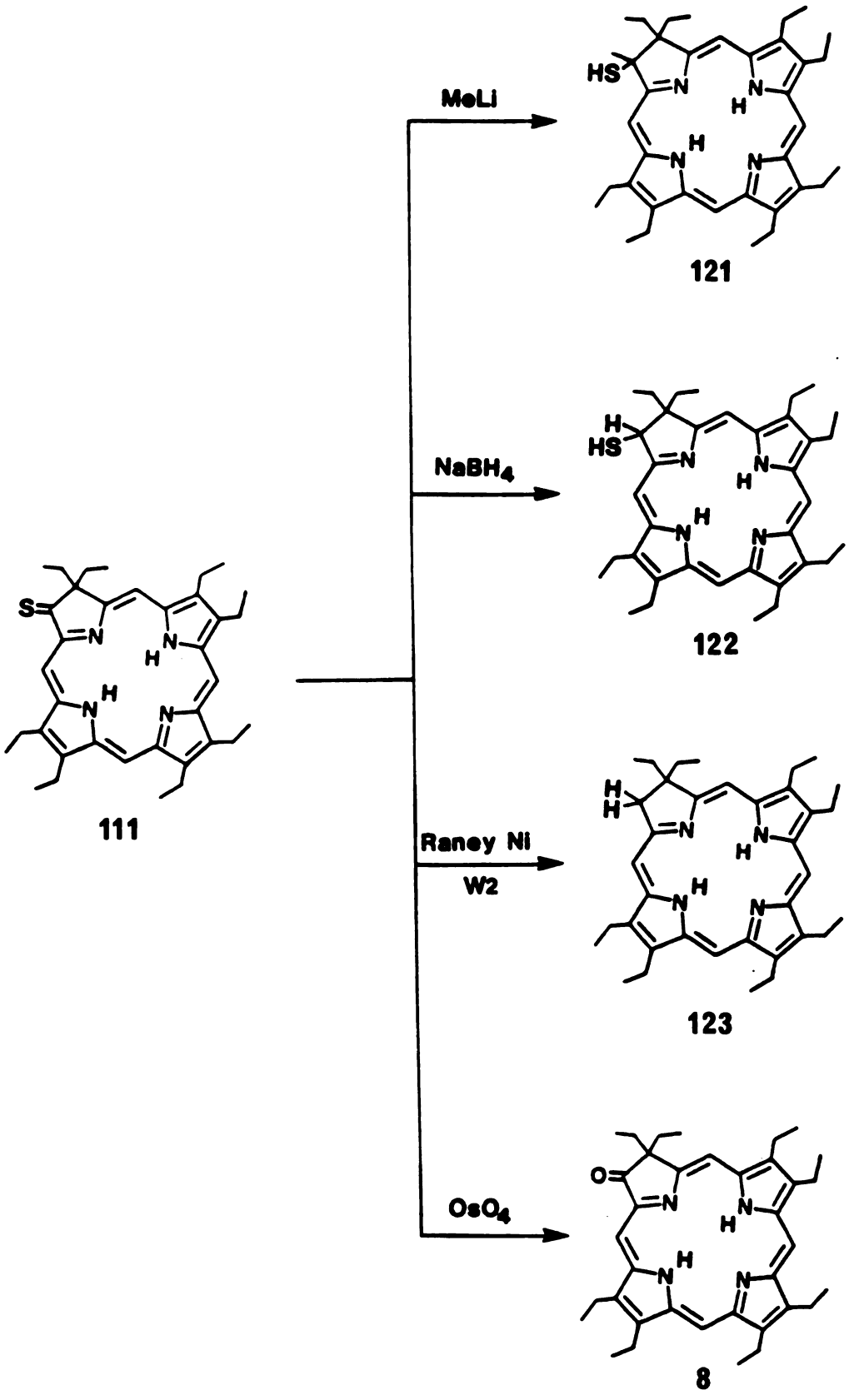


Figure 12 250 MHz ^{1}H NMR spectra of porphyrinone **8** and porphyrinthione **111**.



SCHEME 20

Table 3. ^{1}H NMR Data of Sulfur-Containing Saturated Octaethylporphyrins in Comparison with Their Oxo-Analogues.

Compound	Meso Protons, δ			
	5-H	10-H	15-H	20-H
Ketone (8)	9.13	9.86	9.94	9.84
Thione (111)	9.10	9.69	9.78	10.32
Methyl-thiol (121)	8.81	(9.75)	(9.77)	9.32
Thiol (122)	8.79	9.76	9.76	9.35
2,7-dione (64)	8.61	8.42	9.41	9.26
2-thiol-7-dione (112)	8.65	8.37	9.25	9.72
2,7-dithione (113)	9.04	8.37	9.19	9.69
3,7-dione (66)	9.58	8.87	9.77	8.87
3-thio-7-dione (114)	10.01	(8.87)	9.61	(8.80)
3,7-dithione (115)	10.40	8.74	9.40	8.74
2,7,12-trione (116)	8.05	7.81	8.12	8.92
2-thio-7,12-trione (117)	8.03	7.70	7.98	9.38
2,12-dithio-7-trione (118)	8.04	8.26	8.10	9.34
2,7-dithio-12-trione (119)	8.59	7.92	8.08	9.53
2,7,12-trithione (120)	8.60	8.46	8.19	9.48

Note: The chemical shifts in parentheses are tentative assignments.

it reverts to the starting ketone. Moreover, thione 111 reacts readily with alkyllithium reagents, e.g., CH_3Li in a manner similar to porphyrinone 8[18], giving rise to methylthiol 121. In contrast to porphyrinone 8 which requires $LiAlH_4$ for complete reduction,[85] thione 111 can be reduced cleanly to 122 with $NaBH_4$. Finally, desulfuration of 111 with Raney Nickel (W-2)[86] affords gem-OEC (octaethylchlorin) (123), quantitatively.

Dithiones 113, 115 and trithione 120 can also be obtained from the reaction of their corresponding oxo-analogues[16,18] with 4eq and 6eq of Lawesson's reagent, respectively (Scheme 19). The low yields (<12%) associated with these molecules can be attributed to both steric and electronic reasons. The ^1H NMR data for the meso protons of all the above sulfur-containing saturated octaethylporphyrins are listed in Table 3 for a general comparison.

Desulfurations by Ra-Ni have also been carried out with dithione 113 and trithione 120, providing an easy access to the tetrahydro-isobacteriochlorins[11b] and hexahydro-pyrrocorphins[87], respectively.

Absorption Spectra. The visible spectra of thione 111, thiol 121 and their Cu-complexes as well as those of dithiones 113, 115 and trithione 120 are shown in Figures 13 and 14. Thione 111 gives rise to a "hyper" type spectrum, presumably as a consequence of the mixing of $n-\pi^*$ and $\pi-\pi^*$ transitions, where n are the nonbonding sulfur orbitals, π are the highest occupied molecular orbitals (HOMO) and π^* are the lowest unoccupied molecular orbitals (LUMO) of the porphyrin ring. Moreover, the absorption bands are shifted to longer wavelengths due to the conjugation of the thione with the ring.[88] On the other hand, thiol 121

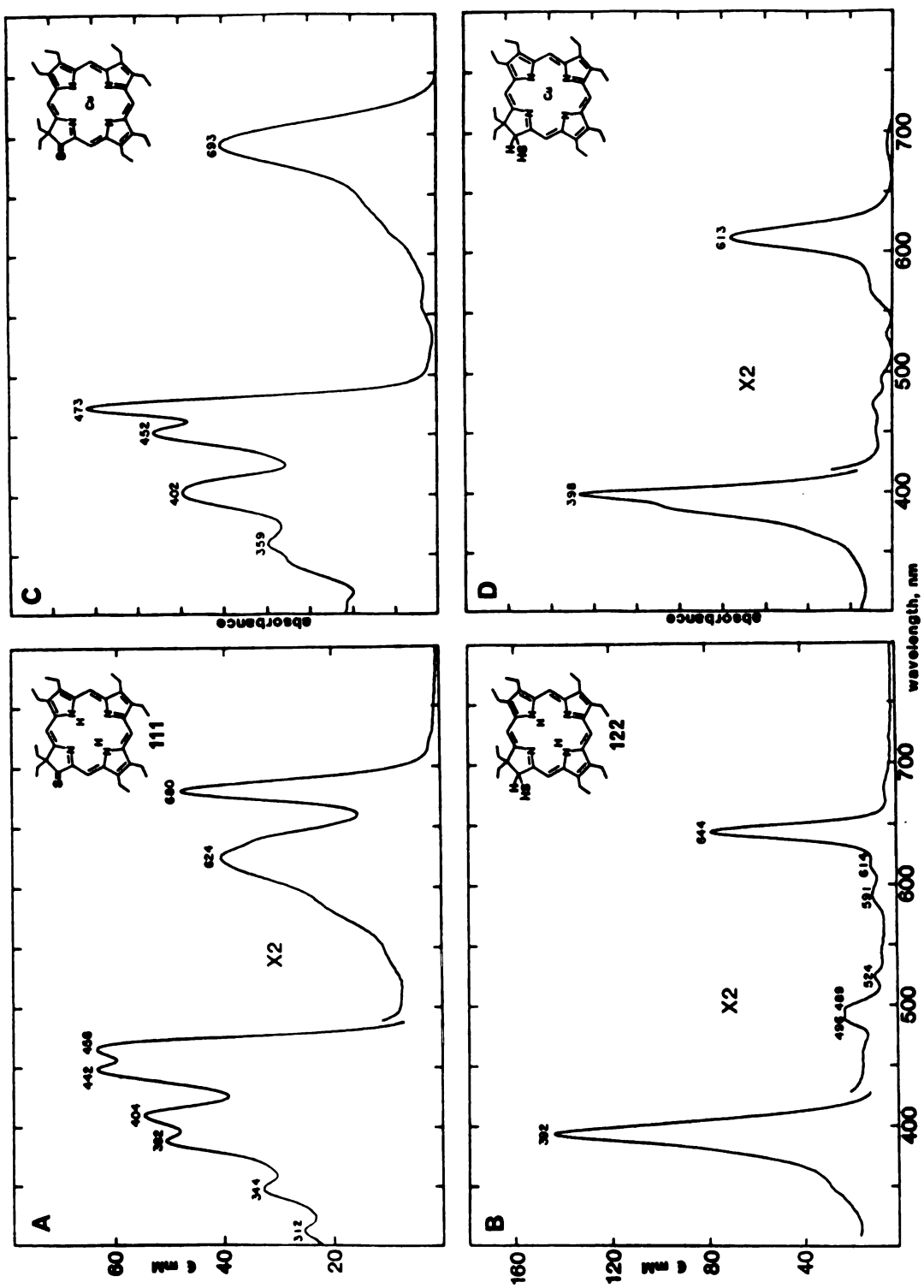


Figure 13 Visible spectra (in CH_2Cl_2) of thione **111** (A); thiol **122** (B); Cu-thione (C); Cu thiol (D).

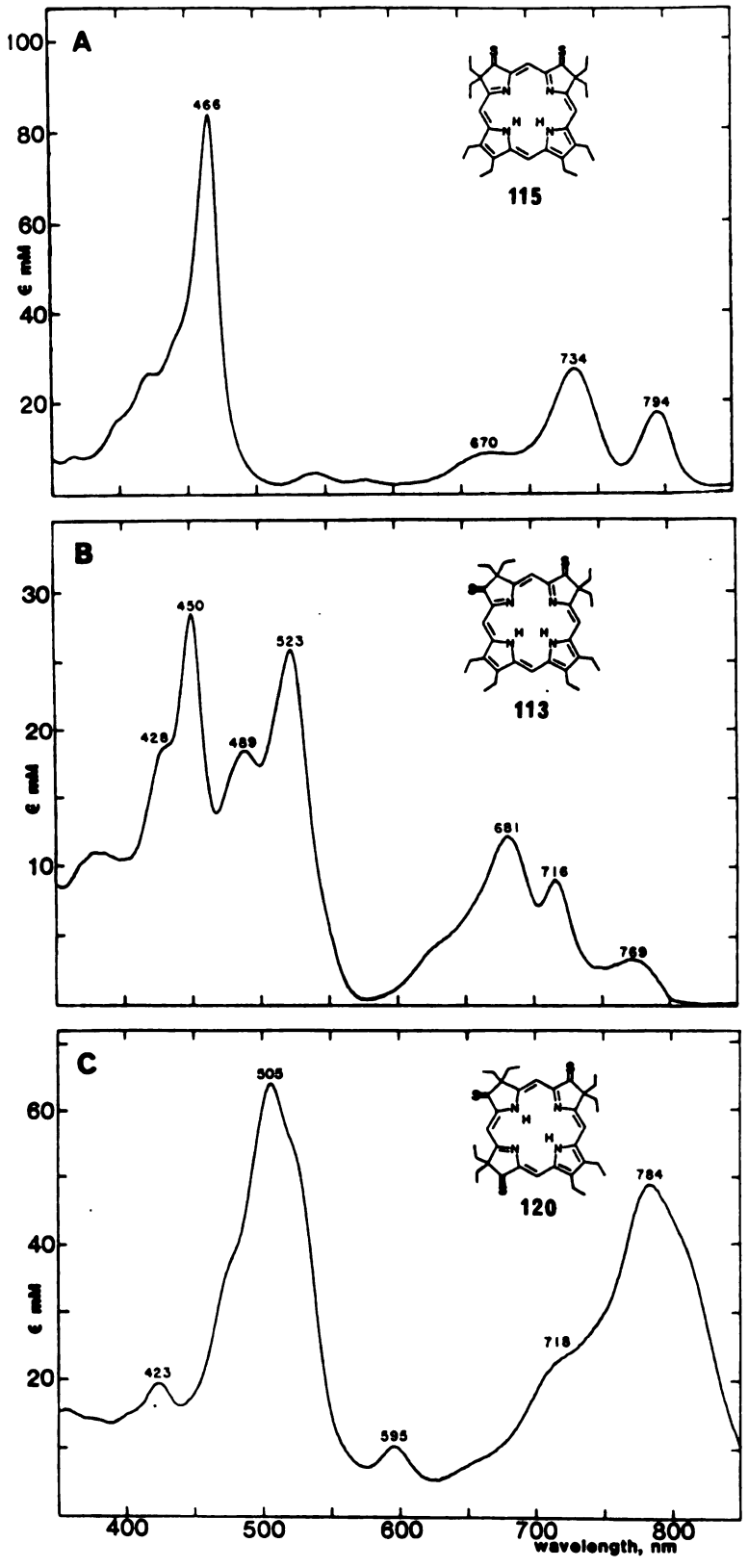


Figure 14 Visible spectra (in CH_2Cl_2) of 3,7-dithione 115(A); 2-7-dithione 113 (B); 2,7,12-trithione 120 (C).

exhibits a typical chlorin spectrum since the sulfur atom is no longer in conjucation with the aromatic ring.

Introduction of a second and third sulfur atom into the porphyrin ring (dithiones 113, 115 and trithione 120) shifts the absorption bands further to the red and the overall pattern depends on the relative position of the sulfur atoms, as well as the saturation degree of the porphyrin ring.

Cyclic Voltammetry. The redox properties of free-base and metal complexes of porphyrins,[89,90] hydroporphyrins[90-97] and oxo-porphyrins[41,98] have been extensively investigated and the principal resutls, for the OEP series, can be summarized here. Porphyrinones exhibit ring oxidation potentials very similar to those of the parent porphyrin, in sharp contrast to those of chlorins, isobacteriochlorins and bacteriochlorins, which are significantly easier to oxidieze than the porphyrin (by as much as 0.6 V). Again, in contrast to the behavior of the isobacteriochlorin derivatives, which are harder to reduce than porphyrins by ~0.3 V, the dioxo-isobacteriochlorins are easier to reduce by ~0.2 V for a net change of ~0.5 V in reduction potential upon introduction of the dioxo-functions onto the isobacteriochlorin skeleton.

The redox properties of sulfur containing saturated octaethyl-porphyrins are being examined here, for the first time. Potentials are listed in Table 4 in comparison with those of oxoporphyrins. These measurements reveal that substitution of O with S renders the porphyrin ring easier to reduce (by as much as 0.53 V in trithione 120, see Figure 15) and easier to oxidize (by as much as 0.3 V in Zn-3,7-dithione 115. The potentials of the redox processes of the Cu and Zn complexes of

Table 4. Redox Potentials[a] of Sulfur-Containing Saturated Octaethylporphyrins and Oxo-Octaethylporphyrins. The latter are Indicated in Parenthesis.

Compound	Ring Oxidation	Ring Reduction		
	0/1+	0/1-	1-/2-	2-/3-
Thione (111)				
H_2	0.76(0.84)	-1.03(-1.36)		
Cu	0.61(0.68)	-1.08(-1.37)		
Zn	0.50(0.56)	-1.10(-1.46)		
Thiol (122)				
H_2	0.68	-1.49		
Cu	0.47	-1.49		
Zn	0.52	-1.51		
2-thio-7-dione (112)				
H_2	0.72[b]	-1.01		
Zn	0.40	-1.20		
2,7-dithione (113)				
H_2	0.70[b](0.82)	-0.85(-1.29)	-1.20	
Zn	0.44(0.70)	-0.97	-1.40	
3-thio-7-dione (114)				
H_2	0.70[b]	-0.86	-1.41	
Zn	0.38	-0.94	-1.50	
3,7-dithione (115)				
H_2	0.70[b](0.79)	-0.77(-1.14)	-1.24	
Zn	0.40(0.70)	-0.85	-1.38	
2,7,12-trithione (120)				
H_2	0.70(0.79)	-0.71(-1.24)	-1.02 (-1.50)	-1.43

[a] $E_{1/2}$ vs. SCE obtained by cyclic voltammetry at a Pt electrode in CH_2Cl_2 containing 0.1 M tetrabutylammonium perchlorate. Scan rate: 20 mv/s.
[b] Quasi-reversible.

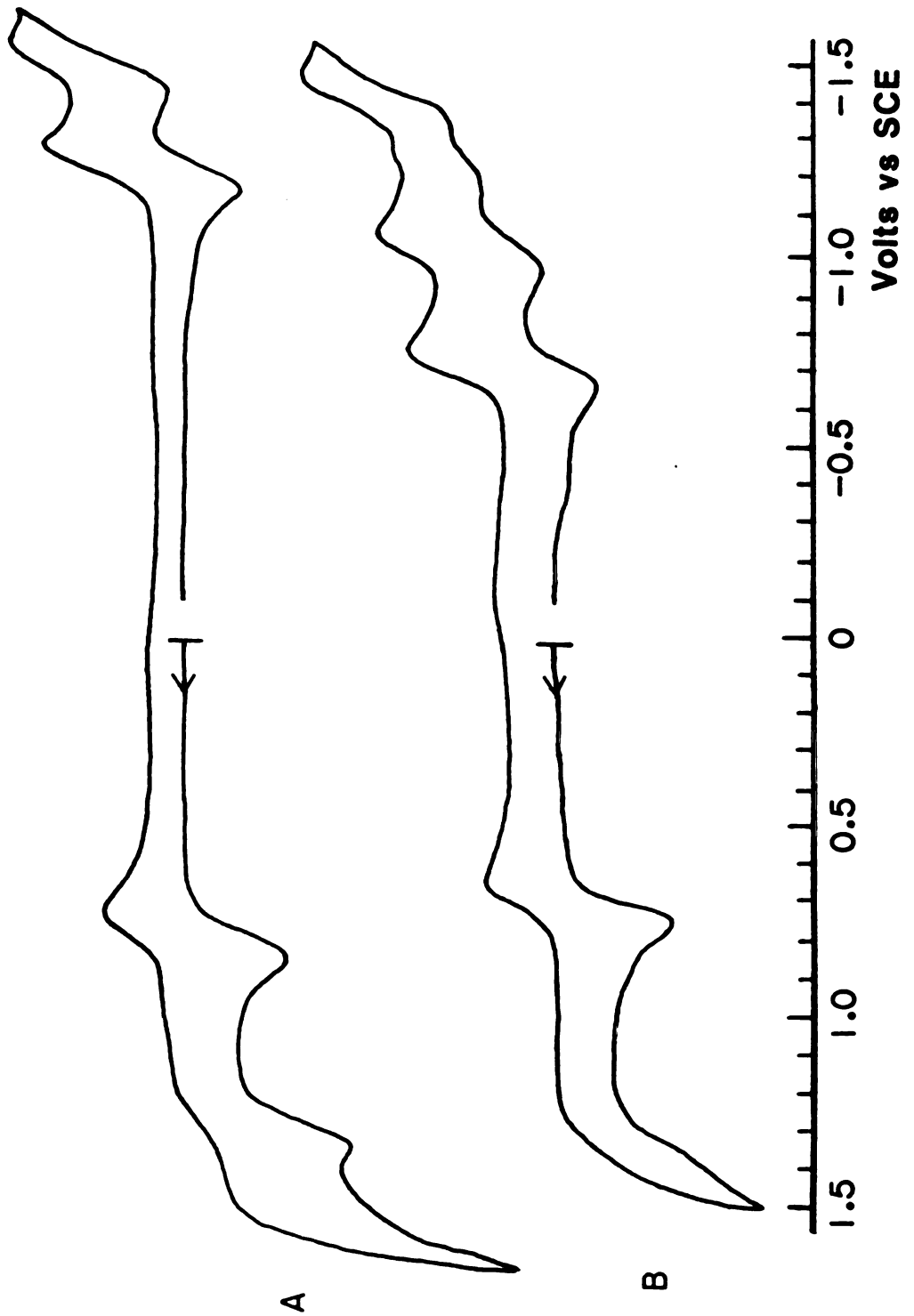


Figure 15 Cyclic voltammograms of 2,7,12-trione 116 (A); 2,7,12-trithione 120 (B).

these sulfur-containing saturated porphyrins are shifted negatively relative to the potentials of their free bases in a fashion parallel to the shifts observed upon metalation of oxoporphyrins.[41,97] Thus, the metal exerts an inductive influence (via the sigma framework) on the porphyrin π-levels.

To a first approximation, the redox span for formation of the monocation and monoanion radicals corresponds to the HOMO-LUMO energy gap which can be related further to the wavelength of the first absorption band.[90-99] Since the inductive substituent effect is expected to shift the whole stack of π and π* orbital energies up or down without altering the HOMO-LUMO gap while the resonance effect should narrow the gap with increasing delocalization, it is obvious from the electrochemical data that sulfur atoms conjugated to the porphyrinoid ring have strong π-interactions with it. The redox potentials of thiol 122 which is not in conjugation with the ring, are very similar to those of any typical chlorin.

An intriguing point is that the redox potentials are also sensitive to the position of the sulfur atoms at the prophyrinoid ring, e.g., 114 and 115 are easier to reduce than 112 and 113, respectively.

Closing Remarks. The above work presented the synthesis and properties of the very first model sulfchlorins. In addition, the sulfur effects were examined in model compounds with different number and position of thio groups.

Further efforts will be aimed at the synthesis of episulfides and vicinal -SH and -OH (ring opened products) which are closer mimics of the alleged biological sulfhemes.

The chemical events that lead to the sulfheme formation in proteins remain to be the most intriguing question. Judging from the obligatory presence of ferryl (Fe^{IV}) species in the proteins prior to the sulfheme formation, it may be reasonable to consider the involvement of certain electron-deficient forms of sulfur (RS^+ or Fe(IV)-OS) as the reactive species. This hypothesis awaits to be tested by future model experiments (for example, reactions of Fe(IV)OEP with $(Bu_4)_2S$ or other appropriate sulfur reagent).

III. EXPERIMENTAL

Visible spectra were recorded on a Cary 219 spectrophotometer interfaced to a Bascom-Turner recorder. Spectra shown here were plotted direclty from data stored on floppy diskettes. Cyclic voltammetry was performed using a Bioanalytical System CV-IA unit. All measurements were carried out in CH_2Cl_2 containing 0.1 M tetrabutylammonium perchlorate at a scan rate of 200 mV/sec.

3,3,7,8,12,13,17,18-Octaethyl-2-porphinethione (111)

Lawesson's reagent (40 mg, 0.1 mmol) was added to a solution of porphinone 8 (55 mg, 0.1 mmol) in dry THF (50 ml) and the reaction mixture was refluxed under N_2 for 24 h or until no more product was formed (monitored by TLC). The solvent was evaporated in vacuo and the residue was chromatographed on preparative TLC plate (silica gel, CH_2Cl_2/hexane) to separate the faster moving yellow-green thione 111 (34 mg, 60%) from the purple starting material 8 (20 mg, 35%). Thione 111 was further purified by recrystallization from CH_2Cl_2/CH_3OH. M.p. 234-236°C; NMR δ 0.07 (6H, t, $CH_2\underline{CH_3}$ sat), 1.79, 1.83, 1.86 (6H each, t, $CH_2\underline{CH_3}$), 2.70, 2.97 (2H each, m, $\underline{CH_2}CH_3$ sat), 3.86, 4.00, 4.03 (4H each,

q, CH_2CH_3), 9.10 (1H, s, 5-H), 9.69 (1H, s, 10-H), 9.78 (1H, s, 15-H), 10.32 (1H, s, 20-H), -2.38, -2.42 (1H each, br s, NH); IR 1230 cm^{-1}; UV-vis λ_{max} (ε_M) 680 nm (24 200), 624 (20 500), 458 (63 700), 442 (63 700), 404 (54 800), 382 (48 400), 344 (32 200), 312 (24 200); MS (direct probe, 70 eV) m/e 566(M^+). Anal. Calcd for $C_{36}H_{46}N_4S$: C, 76.28; H, 8.18; N, 9.88. Found: C, 76.17; H, 8.28; N, 9.79.

3,3,8,8,12,13,17,18-Octaethyl-2-thio-7-prophinedione (112) and 3,3,8,8,12,13,17,18-Octaethyl-2,7-porphinedithione (113)

56 mg (0.1 mmol) of dione 64 and 81 mg (0.2 mmol) of Lawesson's reagent were reacted as described for 111. Separation on TLC plate gave the fastest moving brown-orange dithione 113 (5.6 mg, 9.4%) followed by the green 112 (31.7 mg, 53%) and the dark-green starting material 64 (10.1 mg, 17.8%).

112: NMR δ 0.17, 0.58 (6H each, t, CH_2CH_3 sat), 1.69, 1.70, 1.72, 1.73 (3H each, t, CH_2CH_3), 2.60 (6H, m, CH_2CH_3 sat), 2.87 (2H, m, CH_2CH_3 sat), 3.73 (8H, m, CH_2CH_3), 8.37 (1H, s, 10-H), 8.65 (1H, s, 5-H), 9.25 (1H, s, 15-H), 9.72 (1H, s, 20-H), 0.47 (2H, br s, NH). Irradiating the multiplets at δ 2.60 and 2.80 caused the respective singlets at δ 8.37 and 8.65 to increase in intensity. IR 1716 cm^{-1}, 1248, 1216; UV-vis λ_{max} (ε_M) 681 nm (24 000), 641 (16 900), 612 (12 900), 476.5 (34 900), 453 (37 400), 419.5 (59 800). MS (direct probe, 70 eV), m/e 582 (M^+).

113: NMR δ 0.15, 0.26 (6H each, t, CH_2CH_3 sat), 1.66, 1.70 (6H each, t, CH_2CH_3), 2.56, 2.79 (4H each, m, CH_2CH_3 sat), 3.63 (2H, q, CH_2CH_3), 3.73 (6H, m, CH_2CH_3), 8.37 (1H, s, 10-H), 9.04 (1H, s, 5-H), 9.19 (1H, s, 15-H), 9.69 (1H, s, 20-H). Irradiating the multiplets at δ 2.56 and 2.79 caused the respective singlets at δ 8.37 and 9.04 to increase in intensity. IR 1073 cm^{-1}; UV-vis λ_{max} (ε_M) 769 nm (5 100),

716 (8 400), 681 (12 700), 523 (25 800), 489 (18 200), 450 (28 500), 428 (19 600); MS (direct probe, 70 eV) m/e 598 (M^+).

2,2,8,8,12,13,17,18-Octaethyl-3-thio-7-porphinedione (114) and 2,2,8,8,12,13,17,18-Octaethyl-3,7-porphinedithione (115)

Prepared as above. Separation on TLC plate gave three green bands. The least polar band 115 (4.1%) followed by 114 (22.3%) and the recovered starting material 66 (50.2%).

114: NMR δ 0.19, 0.52 (6H each, t, CH_2CH_3 sat), 1.74, 1.76, 1.78, 1.81 (3H each, t, CH_2CH_3), 2.62, 2.85 (6H,2H each, m, CH_2CH_3 sat), 3.83, 3.86, 3.94, 3.95 (2H each, q, CH_2CH_3), 8.80, 8.87 (1H each, s, 10,20-H), 9.61 (1H, s, 15-H), 10.01 (1H, s, 5-H), -1.06 (2H, br s, NH). Irradiating at δ 3.84 caused the singlets at δ 8.80 and 8.87 to increase in intensity. Moreover, irradiating at δ 3.94 caused the singlet at δ 9.61 to increase in intensity. IR 1700 cm^{-1}, 1108, 1200; UV-vis $\lambda_{max}(\varepsilon_M)$ 701 nm (34 100), 631 (11 200), 455 (124 000), 429 (68 500); MS (direct probe, 70 eV) m/e 582 (M^+).

115: NMR δ 0.20 (12H, t, CH_2CH_3 sat), 1.71, 1.76 (6H each, t, CH_2CH_3), 2.60, 2.81 (4H each, m, CH_2CH_3 sat), 3.76, 3.85(4H each, q, CH_2CH_3), 8.74 (2H, s, 10,20-H), 9.40 (1H, s, 15-H), 10.40 (1H, s, 5-H), -0.45 (2H, br s, NH). Irradiating at δ 3.76 and 3.85 caused the respective singlets at δ 8.74 and 9.40 to increase in intensity. IR 1072 cm^{-1}, UV-vis $\lambda_{max}(\varepsilon_M)$ 794 nm (17 300), 734 (27 900), 670 (9 900), 466 (82 900); MS (direct probe, 70 eV) m/e 598 (M^+).

3,3,8,8,13,13,17,18-Octaethyl-2-thio-7,12-porphinetrione (117), 3,3,8,8,13,13,17,18-Octaethyl-2,12-dithio-7-porphinetrione (118), 3,3,8,8,13,13,17,18-Octaethyl-2,7,dithio-12-porphinetrione (119), and 3,3,8,8,13,13,17,18-Octaethyl-2,7,12-porphinetrithione (120)

58 mg (0.1 mmol) of trione 116 and 121 mg (0.3 mmol) of Lawesson's reagent were reacted as described for 111. Separation on TLC plate gave the fastest moving rosy trithione 120 (7.1 mg, 11.3%) followed by the brown 119 (16.2 mg, 26.5%), the green 118 (7.1 mg, 11.6%) and the yellow-green 117 (21 mg, 35.2%).

117: NMR δ 0.17, 0.54, 0.57 (6H each, t, $CH_2\underline{CH_3}$ sat), 1.54, 1.61 (3H each, t, $CH_2\underline{CH_3}$), 2.27-2.60 (12H, m, $CH_2\underline{CH_3}$ sat), 3.48, 3.56 (2H each, q, $\underline{CH_2}CH_3$), 7.70 (1H, s, 10-H), 7.98 (1H, s, 15-H), 8.03 (1H, s, 5-H), 9.38 (1H, s, 20-H). Irradiating at δ 3.48 and 3.56 caused the respective singlets at δ 7.98 and 9.38 to increase in intensity. UV-vis $\lambda_{max}(\varepsilon_M)$ 692 nm (30 100), 670 (35 200), 636 (24 300), 448 (65 400), 430 (67 400). MS found: m/e 599.3411 for $(M+H)^+$, $C_{36}H_{47}N_4O_2S$ requires m/e 599.3423.

118: NMR δ 0.18, 0.26, 0.60 (6H each, t, $CH_2\underline{CH_3}$ sat), 1.57, 1.63 (6H, t, $CH_2\underline{CH_3}$), 2.30 (1H, br s, NH), 2.34-2.64 (12H, m, $CH_2\underline{CH_3}$ sat), 3.51, 3.58 (2H each, q, $\underline{CH_2}CH_3$), 8.04 (1H, s, 5-H), 8.10 (1H, s, 15-H), 8.26 (1H, s, 10-H), 9.34 (1H, s, 20-H). Irradiating at δ 3.51 and 3.58 caused the respective singlets at δ 8.10 and 9.34 to increase in intensity. UV-vis $\lambda_{max}(\varepsilon_M)$ 767 nm (50 400), 698 (26 300), 652 (9 400), 486 (67 600), 454 (47 300), 445 (45 500), 422 (37 900), 388 (21 700), 376 (20 400); MS found: m/e 615.3200 for $(M+H)^+$, $C_{36}H_{47}N_4OS_2$ requires m/e 615.3195.

119: NMR δ 0.18, 0.28, 0.54 (6H each, t, $CH_2\underline{CH_3}$ sat), 1.57, 1.64 (3H each, t, $CH_2\underline{CH_3}$), 1.92, 2.22 (1H each, br s, NH), 2.32-2.81 (12H, m, $CH_2\underline{CH_3}$ sat), 3.53, 3.61 (2H each, q, $\underline{CH_2}CH_3$), 7.92 (1H, s, 10-H), 8.08

(1H, s, 15-H), 8.59 (1H, s, 5-H), 9.53 (1H, s, 20-H). Irradiating at δ 3.53 and 3.61 caused the respective singlets at δ 8.08 and 9.53 to increase in intensity. UV-vis λ_{max} (ε_M) 794 nm (22 700), 692 (32 700), 646 (15 500), 556 (9 100), 498 (31 700), 468 (75 700), 443 (34 400), 396 (21 000); MS found: m/e 615.3180 for $(M+H)^+$, $C_{36}H_{47}N_4OS_2$ requires m/e 615.3195.

120: NMR δ 0.19, 0.26, 0.30 (6H each, t, CH_2CH_3 sat), 1.60, 1.65, (3H each, t, CH_2CH_3 sat), 3.56, 3.62 (2H each, q, CH_2CH_3), 8.19 (1H, s, 15-H), 8.46 (1H, s, 10-H), 8.60 (1H, s, 5-H), 9.48 (1H, s, 20-H). Irradiating at δ 3.56 and 3.62 caused the respective singlets at δ 8.19 and 9.48 to increase in intensity. UV-vis λ_{max} (ε_M) 784 nm (50 500), 718 (26 100), 595 (10 600), 505 (65 800), 423 (20 000); MS found: m/e 631.2975 for $(M+H)^+$, $C_{36}H_{47}N_4S_3$ requires m/e 631.2967.

3,3,7,8,12,13,17,18-Octaethyl-2-methyl-2-mercaptochlorin (121)

Thione 111 (10 mg, 0.018 mmol) was dissolved in dry THF (10 ml) and treated with a two-fold excess of CH_3Li (1.4 M in ether) at R.T. under argon. After a few minutes the reaction was quenched with water (2 ml). The organic layer was separated, dried and evaporated to dryness. The residue was redissolved in CH_2Cl_2 and passed through a short silica gel pad (CH_2Cl_2 as eluant) to afford 121 in almost quantitative yield. NMR δ 0.73, 0.99 (3H each, t, CH_2CH_3 sat), 1.62 (3H, s, Me), 1.81, 1.86 (12H, 6H each, t, CH_2CH_3), 2.15, 2.49, 2.89 (1H, 2H,1H each, m, CH_2CH_3 sat), 3.90, 3.93, 4.03 (2H each, q, CH_2CH_3), 5.65 (1H, s, SH), 8.81 (1H, s, 5-H), 9.32 (1H, s, 20-H), 9.75, 9.77 (1H each, 10,15-H), -2.53 (2H, br s, NH). Irradiating the singlet at δ 1.62 caused the singlet at δ 9.32 to increase in intensity. UV-vis λ_{max} 644

nm, 615.5, 590.5, 524.5, 496, 393; MS (direct probe, 70 eV) m/e 582 (M^+).

3,3,7,8,12,13,17,18-Octaethyl-2-mercaptochlorin (122)

Thione 111 (10 mg, 0.018 mmol) was dissolved in dry THF (10 ml) and a three-fold excess of $NaBH_4$ in CH_3OH was added. After 10 min at R.T., the reaction was worked up in the same manner as described for 121 to give (122), quantitatively. NMR δ 0.51, 0.94 (3H each, t, CH_2CH_3 sat), 1.79, 1.84 (9H each, t, CH_2CH_3), 2.34 (1H, d, SH), 2.34, 2.58, 2.70 (2H,1H,1H each, m, CH_2CH_3 sat), 3.88, 3.95, 4.01 (4H each, q, CH_2CH_3), 5.92 (1H, d, 2-H), 8.79 (1H, s, 5-H), 9.35 (1H, s, 20-H), 9.76 (2H, s, 10,15-H), -2.55 (2H, br s, NH). Irradiating the doublet at δ 5.92 caused the singlet at δ 9.35 to increase in intensity. UV-vis $\lambda_{max}(\varepsilon_M)$ 644 nm (38 600), 614 (4 300), 591 (4 000), 524 (3 600), 496 (11 700), 489 (11 700), 392 (143 000); MS (direct probe, 70 eV) m/e 568 (M^+).

2,2,7,8,12,13,17,18-Octaethylchlorin (123)

Thione 111 (10 mg, 0.018 mmol) was dissolved in dioxane (10 ml) and an excess of freshly prepared Raney Nickel-W2[86] was added under argon. The reaction mixture was heated in an oil bath at 60°C for 10 min, followed by filtration through a glass wool. The filtrate was evaporated to dryness to give 123 quantitatively. NMR δ 0.68 (6H, t, CH_2CH_3 sat), 1.80 (18H, m, CH_2CH_3), 2.41 (4H, q, CH_2CH_3 sat), 3.87, 3.90, 4.00 (4H each, q, CH_2CH_3), 4.60 (2H, s, 3,3-H), 8.71 (1H, s, 5-H), 8.96 (1H, s, 20-H), 9.71, 9.74 (1H each, 10,15-H), -2.53 (1H, br s, NH). Irradiating the singlet at δ 4.60 caused the singlet at δ 8.96 to

increase in intensity. UV-vis λ_{max} 643 nm, 613, 589, 521, 495, 488, 391; MS (direct probe, 70 eV) m/e 536 (M^+).

General Procedure for Zinc and Copper Insertion

To a solution of the free-base porphyrinoid (10 mg) in boiling chloroform (10 ml) was added a saturated solution (1 ml) of the metal (II) acetate in methanol, followed by the addition of sodium acetate (~2 mg). After ~30 min refluxing and checking by TLC (metal-complex moves slower than the corresponding free-base), the solvents were evaporated in vacuo and the residue was redissolved in CH_2Cl_2/H_2O (1:1). The organic phase was separated, washed twice with water, dried (Na_2SO_4) and evaporated to dryness to give the metal complex in quantitative yield. The ^1H NMR peaks of the zinc complexes are generally shifted upfield in relation to their free bases.

CHAPTER 4

KINETIC AND EQUILIBRIUM STUDIES OF CO AND O_2 BINDING TO HORSE HEART MYOGLOBIN RECONSTITUTED WITH SYNTHETIC GREEN HEMES

I. INTRODUCTION

Reconstitution of heme proteins is often used as a method of probing the prosthetic group structure-protein function relationship. To date, heme proteins that have been successfully reconstituted with foreign hemes or recombined with the native prosthetic group include myoglobin, hemoglobin, peroxidases, catalase, cytochrome P-450, b-type, and c-type cytochromes as well as cytochrome cd$_1$.[100] Among these, myoglobin is the simplest system for monitoring perturbations brought by heme structure changes.

Myoglobin (Mb) is a monomeric protein of 160 amino acid residues (MW 17,800) and one molecule of heme (Protoheme IX). Mb is found in the skeletal muscles and stores diogygen, transported to it by hemoglobin (Hb) for use in the mitochondria.[101] A generic structure for Mb is shown in Figure 16A. A closer examination of the region near the heme in the deoxy form reveals the iron atom in a square pyramidal structure, the equator defined by the porphyrin plane and an axial position occupied by the imidazole of histidine F8 which is called the "proximal histidine" (Figure 16B). The iron atom is approximately 0.5A out of the plane of the porphyrin and the Fe-N (imidazole) bond vector is

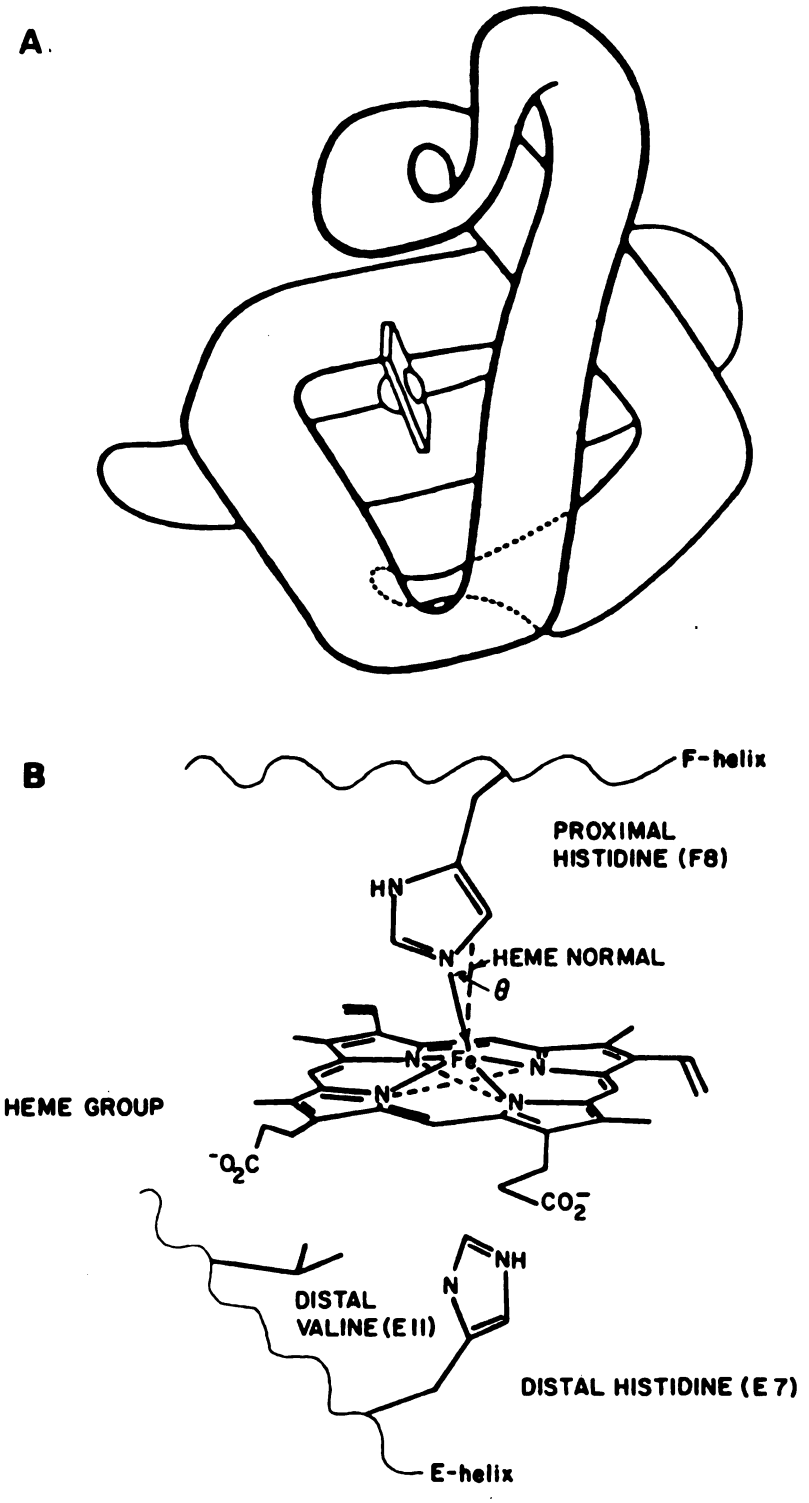
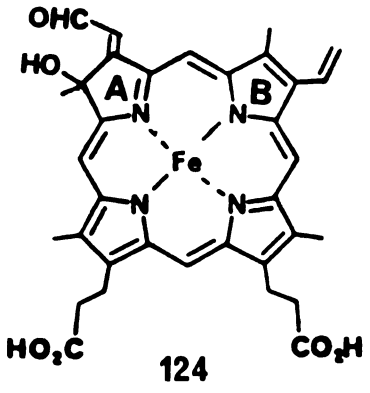


Figure 16 The low-resolution structure of Mb (A); the O_2-binding site in Mb (B).

approximately 8° off the heme normal.[102] The iron atom is high-spin (S=2) Fe(II). Upon binding a sixth ligand (CO or O_2), Mb undergoes a change in the tertiary structure that places the iron within 0.2Å of the porphyrin plane, resulting in a diamagnetic, low-spin (S=0) Fe(II) for carbon-monoxymyoglobin (MbCO).[103] MbO_2 has been discussed as various spin-coupled system.[104]

The ligand binding properties of myoglobin as well as myoglobin reconstituted with a variety of porphyrin hemes modified at the side chains, have been extensively investigated.[105-115] Studies on myoglobin containing saturated macrocycles are limited to only few systems which include sulfmyoglobin[78e,f,79,116] chlorophyllide-Mb[117] as well as iron and cobalt complexes of pheophorbide-a-Mb, pyro-, meso-, and mesopyropheophorbide-a-Mb.[118] In each of these cases however, greater structural perturbation than simple pyrrole ring reduction has been involved.

As part of an effort to probe structure-function relationships of green heme enzymes, we report here the oxygen and carbon monoxide binding behavior of horse heart myoglobin reconstituted with several synthetic green heme (124-130) shown in Figure 17. We believe that these derivatives are more useful approximations of the iron-prosthetic groups found in naturally occuring chlorin and isobacteriochlorin containing enzymes[6,64,65] than the previously reported cases. Moreover, the dehydrogenation (oxidation of chlorin to porphyrin) problem often encountered with regular chlorin hemes[119] is not present in our stable systems. Finally, the static and kinetic parameters measured from reconstituted myoglobins may be closer to the primary systems than those obtained with isolated hemes in organic solution.[119,120]



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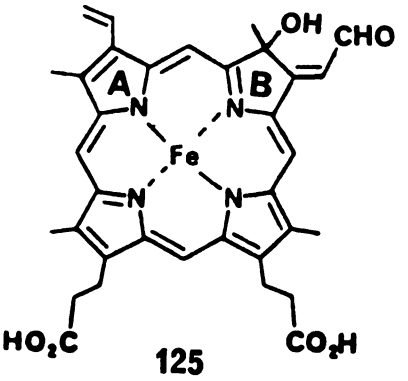
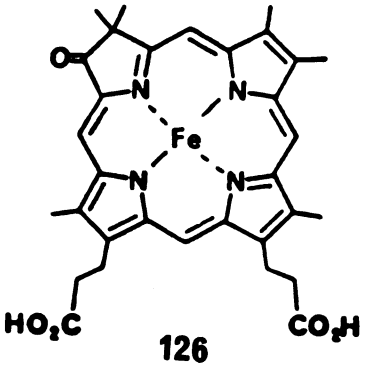
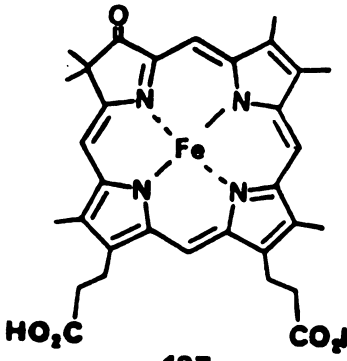


125



126



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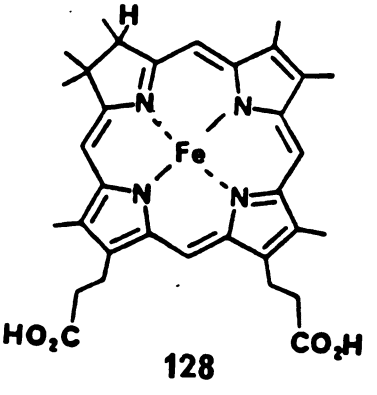

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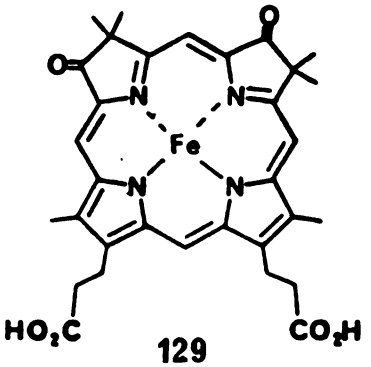

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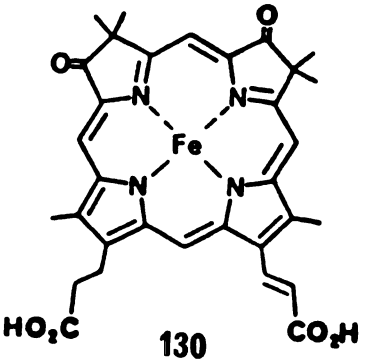

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Figure 17 Structures of synthetic green hemes used for myoglobin reconstitutions.

II. RESULTS AND DISCUSSION

The kinetic and equilibrium constants for CO and O_2 binding to the reconstituted Mbs of this study are summarized in Table 5. The results indicate that substitution of the protoheme with saturated hemes, particularly 126-130 affects the ligand binding constants.

Focusing on methylchlorin heme-Mb 128, it can be seen that the carbon monoxide association rate k' underwent an almost seven-fold increase in comparison with the natural protoheme-Mb. This can arise from the fact that chlorins have intrinsically larger cores than porphyrins[120b] and can more easily accommodate the low-spin six-coordinate Fe(II) atom which moves towards the plane of the macrocycle. Clearly, other explanations may be offered. It could be argued that chlorins are weaker bases than porphyrins causing an Fe(II) chlorin to be a stronger Lewis acid than an Fe(II) porphyrin towards the substrate ligand such as CO. In fact, it is well known that free-base chlorins are weaker Bronsted bases than free-base porphyrins.[2,121] However, metal-ligand affinities could be governed not by the basicity of the free-bases, but by the total basicity of the macrocycle dianions, of which nothing quantitative has been reported.

The oxygen association rate, k', for the same myoglobin has experienced only a two-fold increase, in comparison with the native Mb. Perhaps, triplet oxygen can induce the S=0 state of iron even before the heme becomes planar, consequently, diminishing the core-size effect. In the case of CO, this singlet molecule must combine with a high spin Fe(II) giving a singlet product. To pair these spins, the necessity for spin inversion could retard the rate of reaction.[122]

Table 5. Kinetic and Equilibrium Constants for CO and O_2 Binding.[a]

	Reaction With CO			Reaction With O_2		
Myoglobins	$P_{1/2}$(torr)	$k'(M^{-1}s^{-1})$	$k(s^{-1})$	$P_{1/2}$(torr)	$k'(M^{-1}s^{-1})$	$k(s^{-1})$
Protoheme	0.025	$6.4x10^5$	0.022	0.40	$2.4x10^7$	15
Photoprotoheme A (124)	0.028	$1.3x10^6$	0.050	0.66	$1.8x10^7$	21.4
Photoprotoheme B (125)	0.062	$8.0x10^5$	0.067	0.43	$1.7x10^7$	13.1
7-Keto-heme (126)	0.049	$3.4x10^6$	0.227	0.71	$4.0x10^7$	51.3
8-keto-heme (127)	0.024	$6.5x10^6$	0.210	1.59	$5.8x10^7$	166
Methylchlorin-heme (128)	0.016	$4.2x10^6$	0.089	0.64	$4.9x10^7$	56.3
Dione-heme (129)	0.018	$3.5x10^6$	0.085	1.59	$3.9x10^7$	111.4
Acrylo-dione-heme (130)	0.018	$3.2x10^6$	0.076	2.31	$4.2x10^7$	175

[a] In 10 mM phosphate buffer, pH 7.4, 20-22°C.

Focusing on a different chlorin heme, 8-keto heme-Mb 127 the electronic effect can be monitored on the oxygen dissociation rate constant, k. The presence of the e^- withdrawing oxygen atom in conjugation with the ring causes k to increase while k′ remains fairly constant. Therefore, the $P_{1/2}O_2$ is larger. This indicates that decreasing e^- density in the iron d orbitals could lessen the Fe-O_2 π-backbonding and effectively weaken the Fe to O_2 bonding. For the CO ligand, the same trend in λ value can be seen, but to a lesser extent. This suggests the predominant role of σ bond formation in determining the iron-carbon monoxide binding while π-bond break-up may be predominant for iron-oxygen binding.[111]

To probe the protein effect, 7-keto heme-Mb 126 possessing the oxo group on the same pyrrole ring but on the other β-pyrrole position has been examined in comparison with 8-keto heme-Mb 127. These two Mbs show differences in their electronic spectra, particularly in their met (ferric) forms. Moreover, the absorption maxima of 7-keto heme-Mb 126 are shifted slightly, bathochromically relative to 8-keto heme-Mb 127 (Figure 18). In contrast, visible absorption peaks of pyridine hemochrome of both hemes are essentially identical (see Table 6). In addition, their ligand binding constants are quite different (see Table 5). This can only be attributed to modulations by local environment.

To verify these results, P_3A-Mb 124 and P_3B-Mb 125 (photoprotochlorin Mbs saturated at either ring A or B) were tested and indeed showed differences in electronic spectra (Table 6) and binding properties (Table 5). Although these chlorin Mbs behave differently from the previously discussed chlorin Mbs (126-128), due to the conjugation of a formyl group with the macrocycle, they offer another

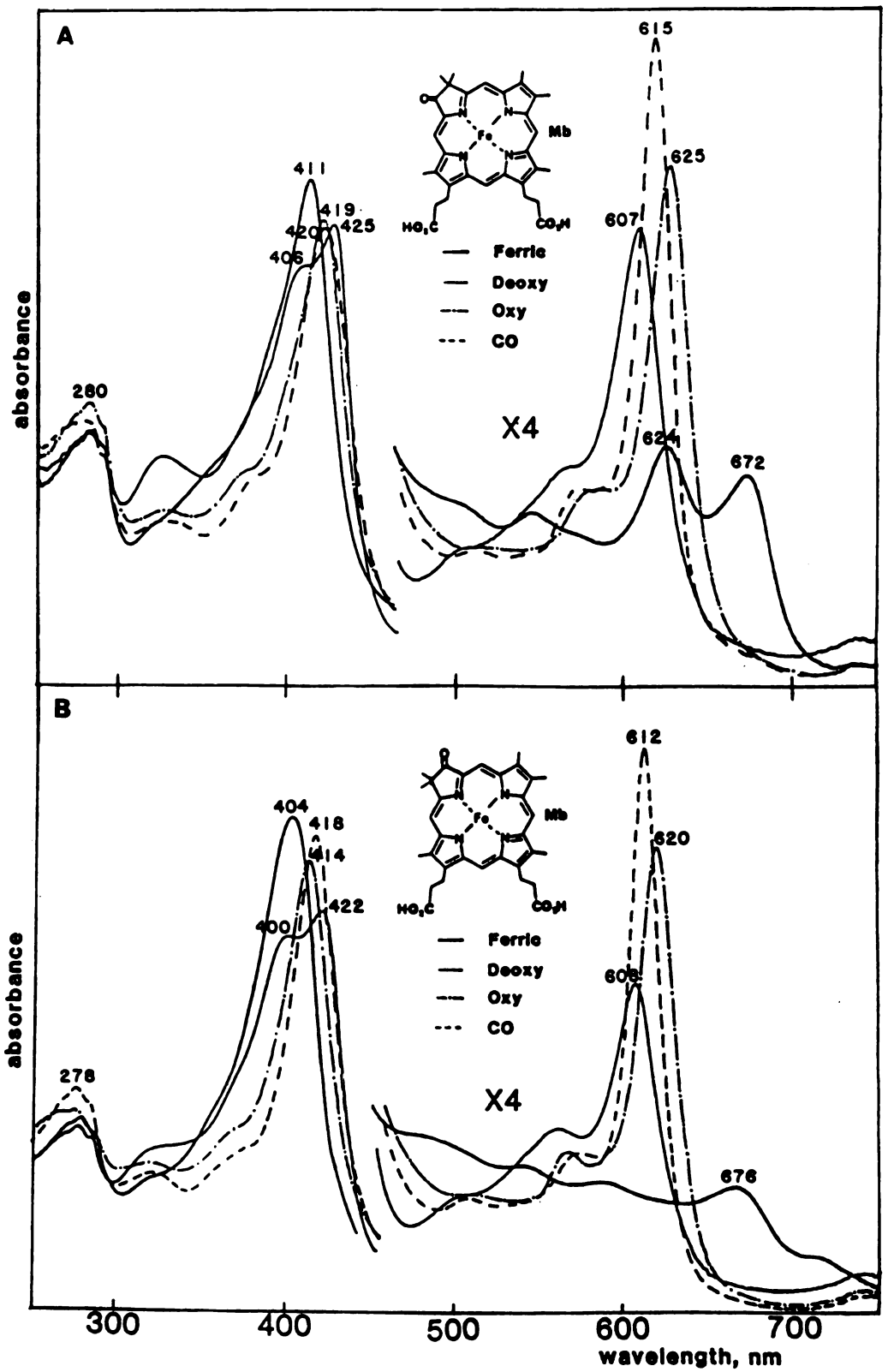

Figure 18 Optical spectra of 7-keto-heme myoglobin (A); 8-keto-heme myoglobin (B); Ferric (——), Deoxy (——), Oxy (— · —), CO (---), in 10 mM (pH 7.4) potassium phosphate buffer.

Table 6. Absorption Spectral Maxima of Synthetic Hemes and Myoglobins.[a]

Mb/Heme	Soret (nm)	Visible Bands (nm)
Photoprotoheme A (124)		
met Mb	408.5	500, 602
deoxy Mb	431	562
O_2 Mb	414	542, 578, 598
CO Mb	421	542, 576, 594
Pyridine hemochrome[b]	419.5	558
Photoprotoheme B (125)		
met Mb	406	599, 500
deoxy Mb	428	593, 556
O_2 Mb	409	594, 544
CO Mb	415	593, 538
Pyridine hemochrome[b]	418.5	556.5
7-keto heme (126)		
met Mb	411	624, 672
deoxy Mb	406, 425	607
O_2 Mb	419	625
CO Mb	420	615
Pyridine hemochrome[b]	414	589
8-keto heme (127)		
met Mb	404	676
deoxy Mb	400, 422	608
O_2 Mb	414	620
CO Mb	418	612
Pyridine hemochrome[b]	415	590
methylchlorin heme (128)		
met Mb	390	602
deoxy Mb	408	616
O_2 Mb	400	624
CO Mb	401	614
Pyridine hemochrome[b]	415	598.5
Dione heme (129)		
met Mb	388, 424	634, 638
deoxy Mb	398, 436	616
CO Mb	394, 439	628
Pyridine hemochrome[b]	395, 319, 445	605
Acrylo-dione heme (130)		
met Mb	411	643
deoxy Mb	424	632
CO Mb	410, 434	644
Pyridine hemochrome[b]	376, 404, 450	626

[a] In phosphate buffer, pH 7.4; [b] In pyridine-1% NH_2NH_2.

example in which the Mb reaction parameters can be affected upon changing the position of the "northern" saturated pyrrole ring. A similar case reported by Sono et al.[105] noticed that Mbs reconstituted with spirographis and isospirographis hemes exhibit different absorption maxima even though the two hemes have the same spectrum outside the protein.

The stability of the various chlorin-containing oxymyoglobins was examined and their autoxidation kinetics are presented in Figure 19 in comparison with the native myoglobin. As is apparent, isomers P_3A-MbO_2/ P_3B-MbO_2 and 7-keto heme-MbO_2/8-keto heme-MbO_2 exhibit different autoxidation rates. P_3B-MbO_2 and 7-keto heme-MbO_2 are about two times slower towards autoxidation than their corresponding isomers. In view of the fact that P_3B-Mb and 7-keto heme-Mb have slower CO association rate constants (ℓ') when compared with their corresponding isomers, it would appear that the heme pocket is tighter around the ligand binding site that stabilizes the oxyheme. This stabilization is also reflected in the k′ values which are smaller in the P_3B-Mb and 7-keto heme-Mb cases. On the other hand, the autoxidation rate of native myoglobin and 125, 126, 128, Mbs are approximately within experimental error with one another. This suggests that the saturation of a porphyrin ring alone, does not perturb the protein native structure and produces stable myoglobins. However, both the position of the saturated pyrrole ring and the side chains are crucial in determining myoglobin's stability.

Myoglobin has also been reconstituted with two porphinedione-hemes (heme d_1 analogues). However, the soret/A_{280} ratios (1.1 for dione heme-Mb 129 and 0.6 for acrylo-dione heme-Mb 130 indicate that these hemes do not fit well in the myoglobin pocket, particularly heme 130

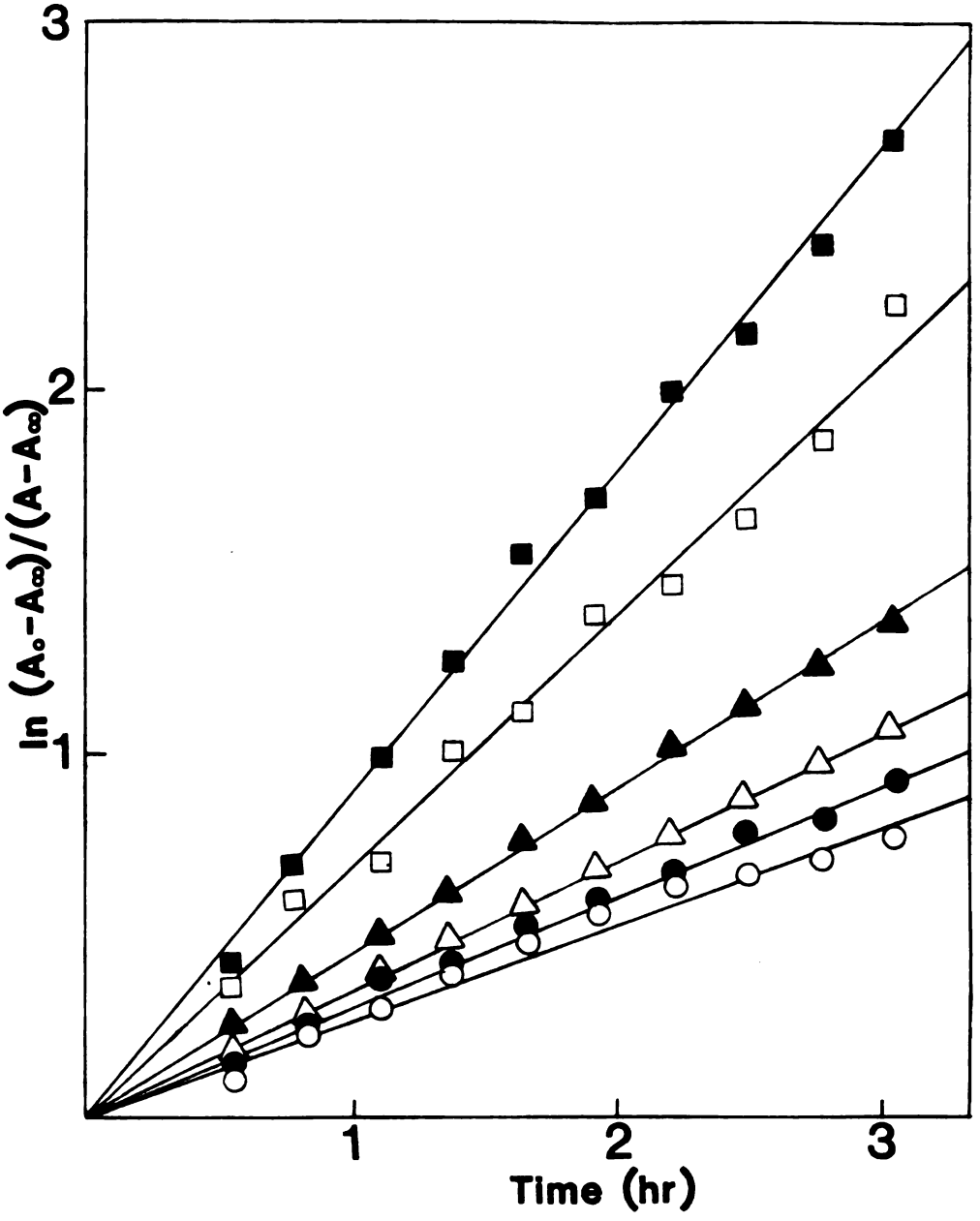

Figure 19 Autoxidation of reconstituted myglobins at 22°C in 10mM (pH 7.4) potassium phosphate buffer, saturated with O_2. Protoheme (native)○, slope = 8.6×10^{-5}, r = 0.989; P_3B●, slope = 9.0×10^{-5}, r = 0.984; methylchlorin-heme△, slope = 9.7×10^{-5}, r = 0.999; 7-keto-heme▲, slope = 1.3×10^{-4}, r = 0.999; P_3A□, slope = 1.9×10^{-4}, r = 0.989; 8-keto-heme ■, slope = 2.5×10^{-4}, r = 0.996.

that possesses one acrylic propionic acid chain. The significance of the two propionic acid groups of the heme at positions 2 and 18, which constitute hydrogen bonds with Arg CD3 and His FG2, respectively, of the apoprotein, has been addressed by Ogoshi.[113] 129-Mb and 130-Mb retained the ability to bind CO in the ferrous state but bleached rather quickly upon exposure to oxygen. Apparently the macrocyclic ring destruction is faster than iron autoxidation in these cases. It is quite possible that the quinone porphyrin ring structure of these heme d_1 analogues reduces O_2 and generates its own hydrogen peroxide equivalent, which, in turn, might attack the macrocycle ring, resulting in its oppening.[122] The fact that the protein conformation around these hemes is significantly altered due to the introduction of a second pair of angular methyl groups should contribute to the instability of the oxy-form. In any case, this is a point that merits further investigation.

III. SUMMARY

The present studies establish that apomyoglobin can be reconstituted with chlorin hemes to produce stable forms of protein that retain the ability to bind dioxygen and carbon monoxide in the reduced state. In comparison with the native Mb, the generally faster association rates observed in the green hemes might be attributed to their larger core size which facilitates the spin state change during ligand binding. In an attempt to probe the effect of the degree of ring saturation on the protein function, we reconstituted Mb with two isobacteriochlorins, having the core structure of heme d_1. Although these synthetic isobacteriochlorin-hemes didn't provide us with any further clue for possible relationship between protein function and the degree of ring saturation, an interesting phenomenon was observed:

their ferrous forms bleached upon exposure to oxygen. Further studies of this phenomenon are necessary. For example, it would be interesting to see if the heme destruction was due to the protein environment or to an intrinsic property of this heme.

IV. MATERIALS AND METHODS

Preparation of Hemins. Photoprotoporphyrin IX dimethyl ester A(P_3A) and B(P_3B) were synthesized according to a literature procedure.[124]

Dimethyl 3,8,8,12,13,17-hexamethyl-7-porphinone-2,18-dipropionate (21a), dimethyl 3,7,7,12,13,17-hexamethyl-8-porphinone-2,18-dipropionate (22a), dimethyl 3,7,7,8,12,13,17-heptamethylchlorin-2,18-dipropionate (24), dimethyl 3,8,8,13,13,17-hexamethyl-7,12-porphinedione-2,18-dipropionate (69) and methyl 18-[2-(methoxycarbonyl)ethenyl]-3,8,8,13,13,17-hexamethyl-7,12-porphinedione-2-propionate (70) were synthesized as described in Chapter 1 of this thesis. Iron insertions were accomplished by the ferrous bromide method[125] (P_3A, P_3B and methylchlorin 24) and by the ferrous sulfate method[126] (porphyrinones 21a, 22a and porphyrindiones 69, 70). The ester groups of these hemins were hydrolyzed in 1:1 THF/2N KOH at R.T. as described for 22a (Chapter 1) to afford the diacid hemins shown in Figure 17.

Preparation of Myoglobins. Horse heart myoglobin (Sigma, Type III) was used as received. Apomyoglobin was prepared from myoglobin by the acidified butanone procedure.[100,127,128] The concentration of apoprotein was determined on the basis of its absorbance at 280 nm (ε_{280} = 15.4 $mM^{-1}cm^{-1}$).[129]

For reconstitution, a 1.2-fold excess of green heme to apoprotein was dissolved in a minimal volume of 1% KOH/MeOH and added to the

apoprotein solution (0.2-0.5 mM) at 0°C. The resultant solution was gently stirred for 20 min at 0°C (frothing should be avoided as it denatures the protein). The mixture was then adjusted to pH 8.0 with 0.2 M Tris-HCl buffer (PH 5.0) and dialyzed (Spectrapor: 25 or 45 mm x 100′ membrane tubing) against 10 mM phosphate buffer (pH 7.0) and against distilled water twice. The deionized Mb solution was centrifuged (15 KG at 4°C), and loaded onto a DE 23 column that was equilibrated with 10 mM phosphate buffer (pH 7.4). The column was developed with the equilibrating buffer at 4°C. Excess heme was adsorbed on the top of the column, while the reconstituted myoglobin was easily eluted from the column (reconstituted myoglobins have bathochromically shifted absorption maxima relative to their free hemes and possess a new absorption peak at ~280 nm). Reconstituted Mbs had A_{soret}/A_{280} ratios: ~3.0 (P_3A, P_3B, porphyrinones 126, 127 and methylchlorin 128 Mbs), 1.1 (dione 129 Mb) and 0.6 (acrylo-dione 130 Mb). In the last two cased the A_{soret}/A_{280} ratio was increased upon storage (-70°C freezer).

For kinetic and equilibrium measurements, solutions of met Mbs (~10^{-5}M) in pH 7.4, 10 mM potassium phosphate buffer were degassed in a 120 ml tonometer by freeze-pump-thraw cycles at 10^{-5} Torr and were reduced with a minimum amount of aqueous sodium dithionite in an argon atmosphere.

Kinetic and Equilibrium Measurements. Kinetic rates were measured at 22°C by flash photolysis[122a,130] according to:

$$Fe + CO \underset{(h\nu)}{\overset{k'}{\rightleftharpoons}} FeCO$$

$$Fe + O_2 \underset{k}{\overset{k'}{\rightleftharpoons}} FeO_2$$

$$FeO_2 \xrightarrow{R} FeCO$$

Flash photolysis was carried out with either a Xenon photographic flash gun (Braun 2000) or a flash lamp pumped dye laser (Phase-R DL 2100) with rhodamine 6G dye. With carbon monoxide present, ℓ' was measured directly as a pseudo-first order decay, $\ell'[CO] = \ell'_{obs}$ which included concentration of CO (Figure 20a). However, when oxygen was added, two decays were observed (Figure 20b). The fast decay to a certain absorbance A_{FeO2} is $k'[O_2] = k'_{obs}$ followed by a decay R from this absorbance to A_{FeCO}. CO association rates (ℓ') were calculated from plots of the observed pseudo-first order rate constants vs. CO concentration and O_2 association rates (k') were calculated from similar plots. $P_{1/2}{}^{O2}$ (oxygen pressure at half saturation) were obtained from direct titration or calculated according to the Gibson equation:[130b]

$$1/R = 1/k + K[O_2]/\ell'[CO]$$

where $\ell'[CO]$ is the pseudo-first order rate constant measured before introducing O_2, R is the displacement rate of oxyMb by CO, k is the O_2 dissociation rate constant and K is the O_2 equilibrium constant. Carbon monoxide affinities (L) were determined by direct titration of each Mb with a gas mixture containing 1% CO in nitrogen, at R.T., using a standard spectrophotometric procedure developed by Halpern and coworkers[131] (Figures 21, 22). The dissociation rate constants ℓ and k were calculated from $L = \ell'/\ell$ and $k = k'/k$, respectively; where $L = (P_{1/2}{}^{CO} \times 1.35\times10^{-6}\ M/torr)^{-1}$ and $K = (P_{1/2}{}^{O_2} \times 1.80\times10^{-6}\ M/torr)^{-1}$. Optical spectra were recorded on a Cary 219 spectrophotometer.

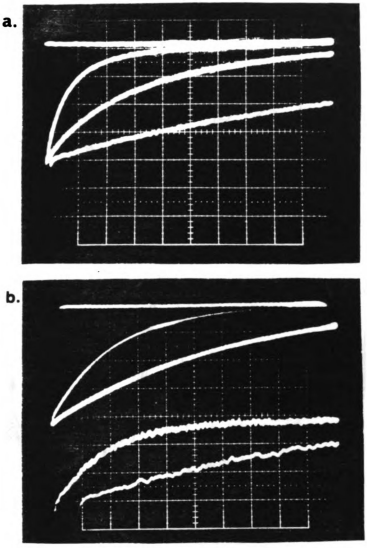

Figure 20 (a) Oscilloscope trace of absorbance vs. time for the recombination of P_3A-Mb and CO after flash photolysis at 22°C in 10 mM (pH 7.4) potassium phosphate buffer. [CO] = 5.25×10^{-5}M; sweeptime = 1,5,20 msec/div.; wavelength = 420 nm. (b) The recombination of P_3A-Mb with O_2 and CO at 422 nm. [CO] = 5.25×10^{-5}, [O_2] = 3.49×10^{-5}M. Upper trace; sweeptime = 0.1, 0.2 sec/div.; lower trace; sweeptime = 0.2, 1 msec/div.

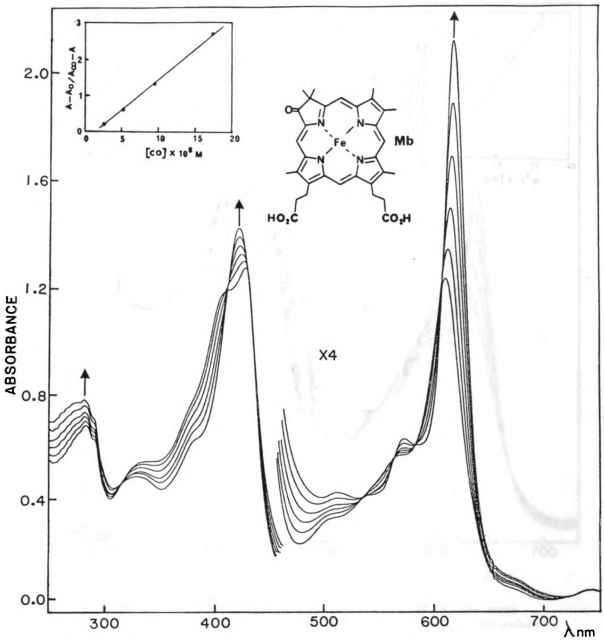

Figure 21 Spectrophotometric titration of 7-keto-heme myoglobin in 10 mM (pH 7.4) potassium phosphate buffer, with CO at R.T.; [CO]x10^{8}M = 0.0, 2.63, 5.26, 9.65, 17.55 and 1760. Inset: plot of $A-A_o/A_\infty-A$ vs. [CO] at 615 nm.

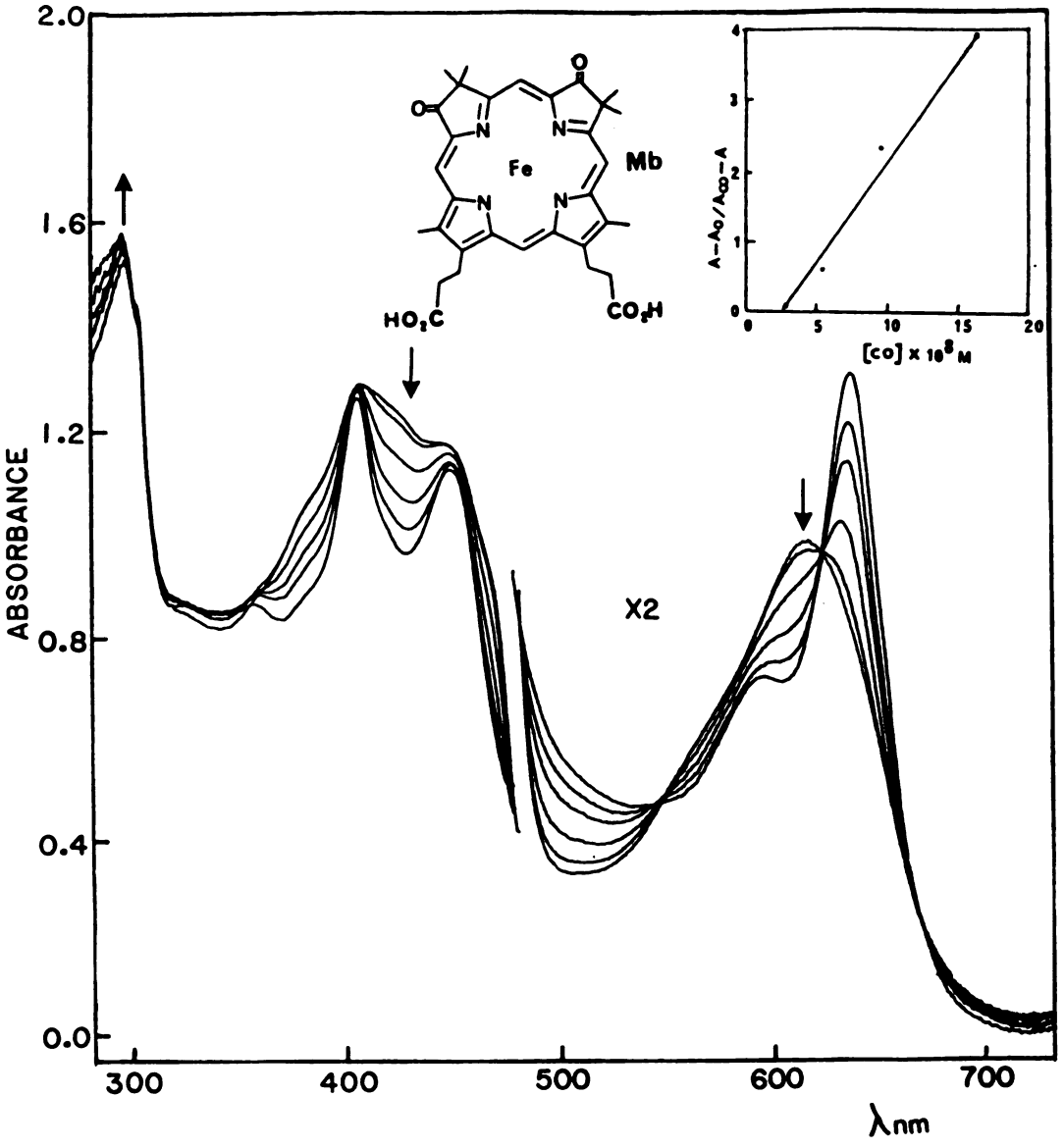

Figure 22 Spectrophotometric titration of dione-heme myglobin in 10 mM (pH 7.4) potassium phosphate buffer with CO at R.T.; [CO]x10^{8}M = 0.0, 2.66, 5.32, 9.31, 15.97, 1346. Inset: plot of A-A_o/A_∞-A vs. [CO] at 628 nm.

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APPENDIX

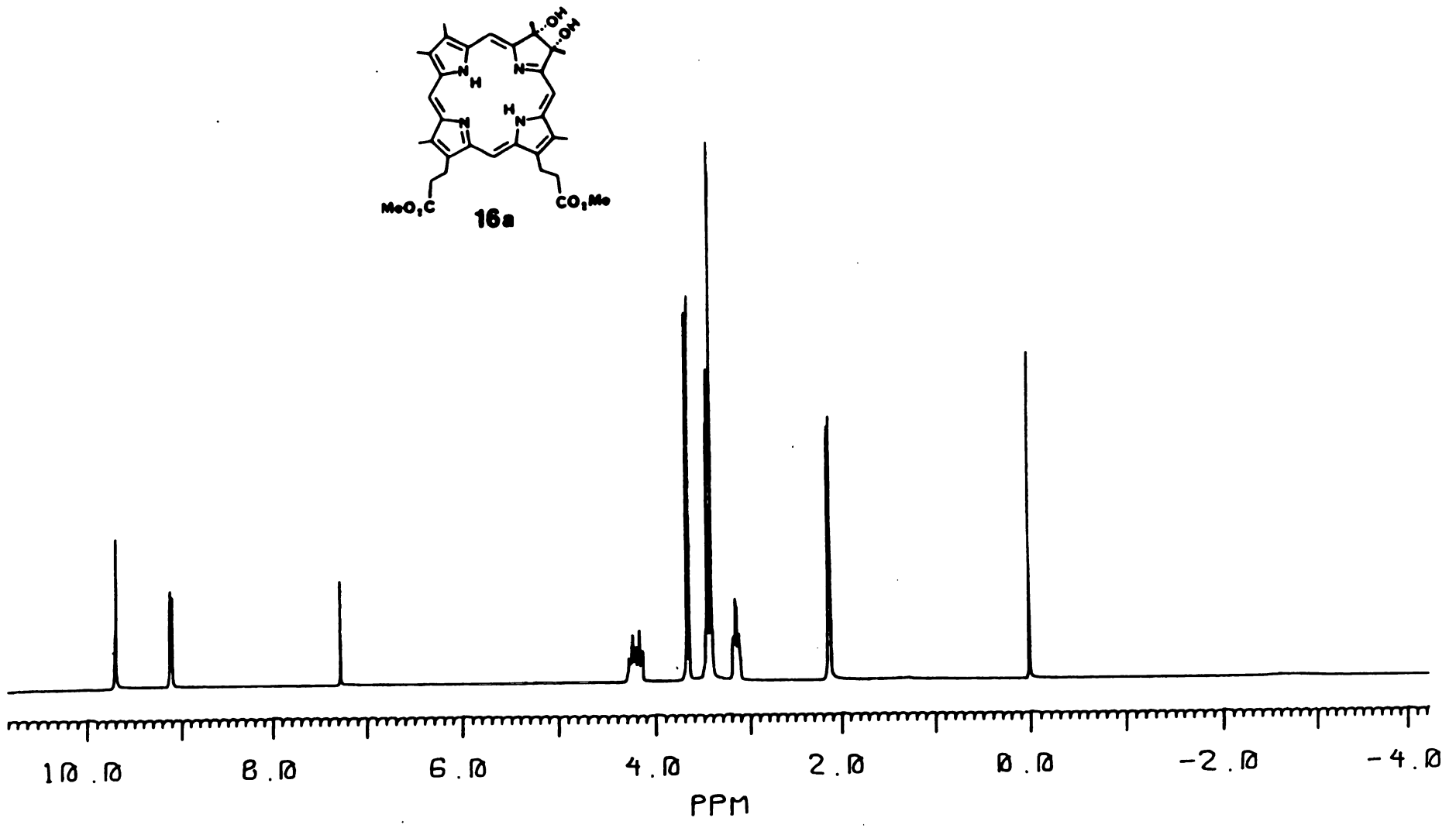


Figure A1 250 MHz ^{1}H NMR spectrum of dimethyl cis-7,8-dihydroxy-3,7,8,12,13,17-hexamethylchlorin-2,18-dipropionate (16a).

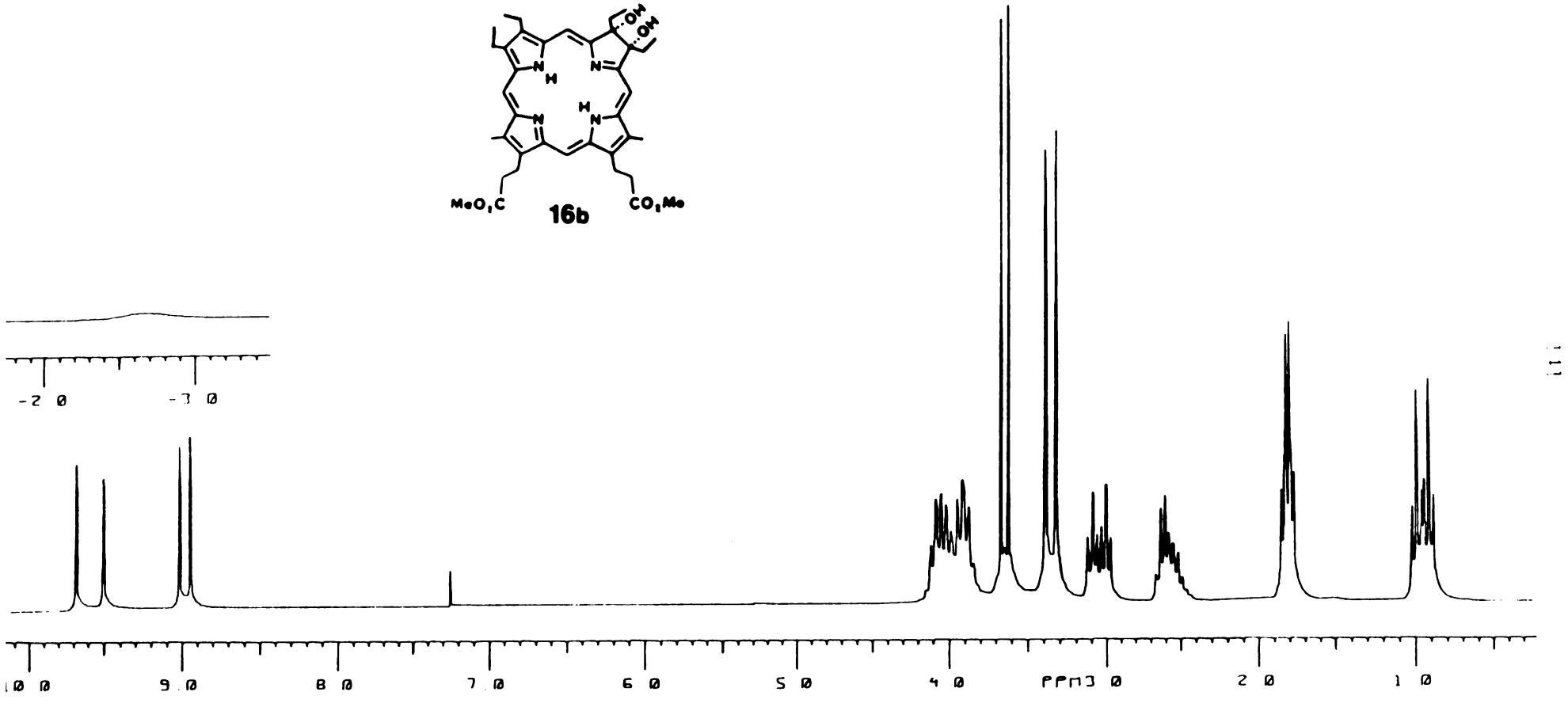


Figure A2 250 MHz ^{1}H NMR spectrum of dimethyl cis-7,8,12,13-tetraethyl-7,8-dihydroxy-3,17-dimethylchlorin-2,18-dipropionate (16b).

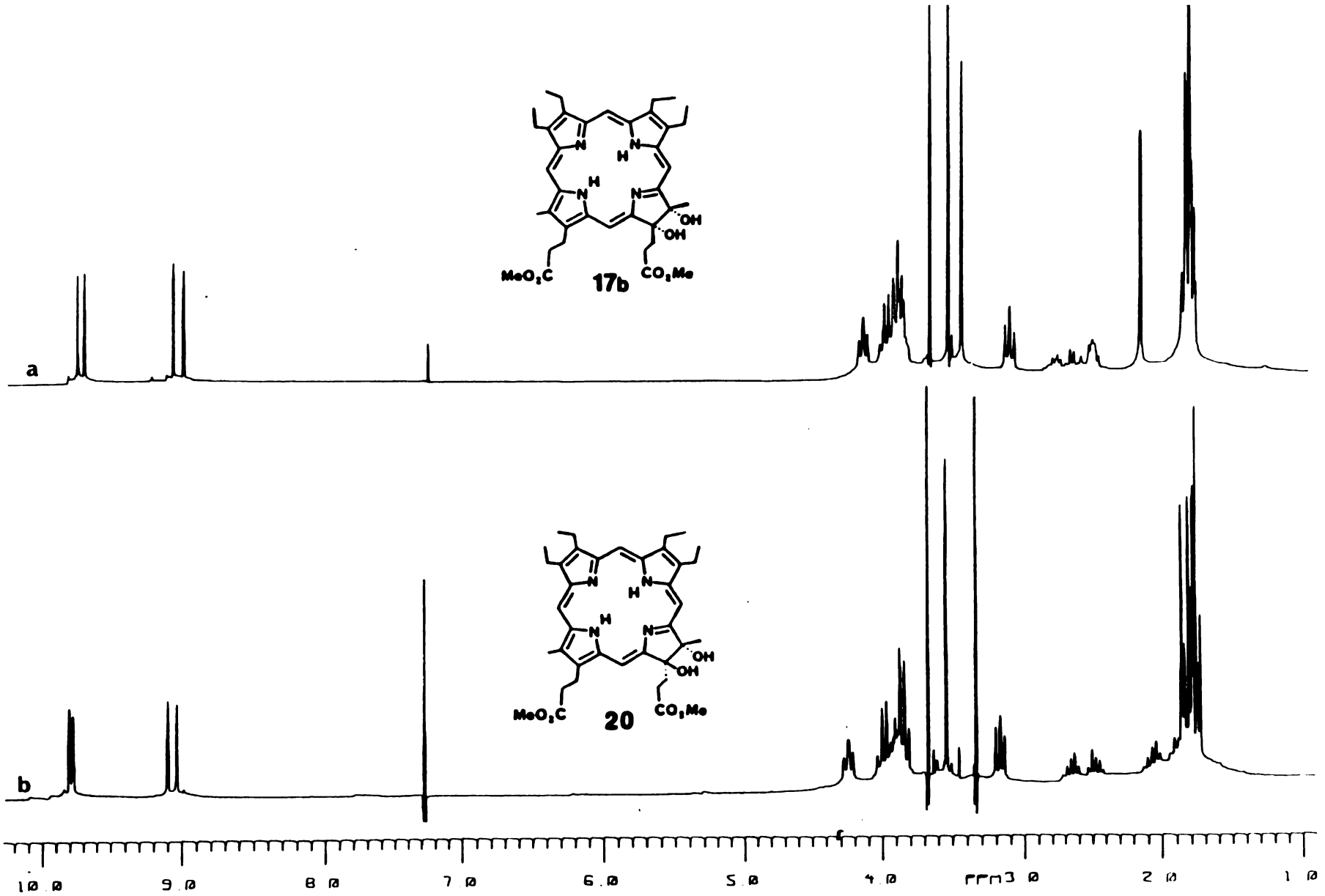


Figure A3 250 MHz ^{1}H NMR spectra of (a) dimethyl cis-7,8,12,13-tetraethyl-2,3-dihydroxy-3,17-dimethylchlorin-2,18-dipropionate (**17b**); (b) dimethyl trans-7,8,12,13-tetraethyl-2,3-dihydroxy-13,17-dimethylchlorin-2,18-dipropionate (**20**).

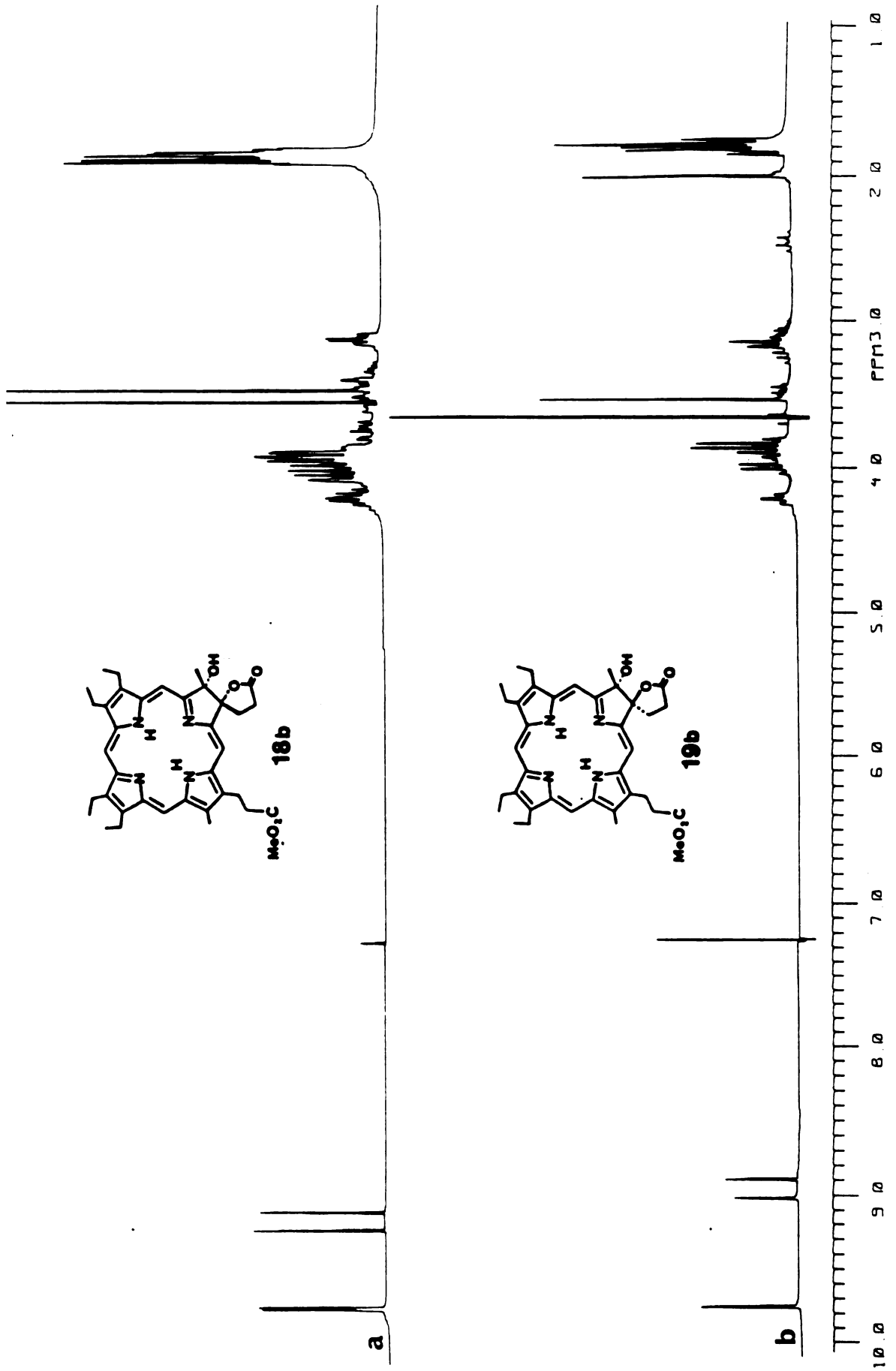
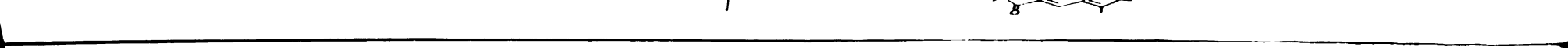


Figure A4 250 MHz ^{1}H NMR spectra of (a) methyl cis-7,8,12,13-tetraethyl-3-hydroxy-3,17-dimethyl-2,2-γ-spirolactone-chlorin-18-propionate (**18b**); (b) methyl trans-7,8,12,13-tetraethyl-3-hydroxy-3,17-dimethyl-2,2-γ-spirolactone-chlorin-18-propionate (**19b**).



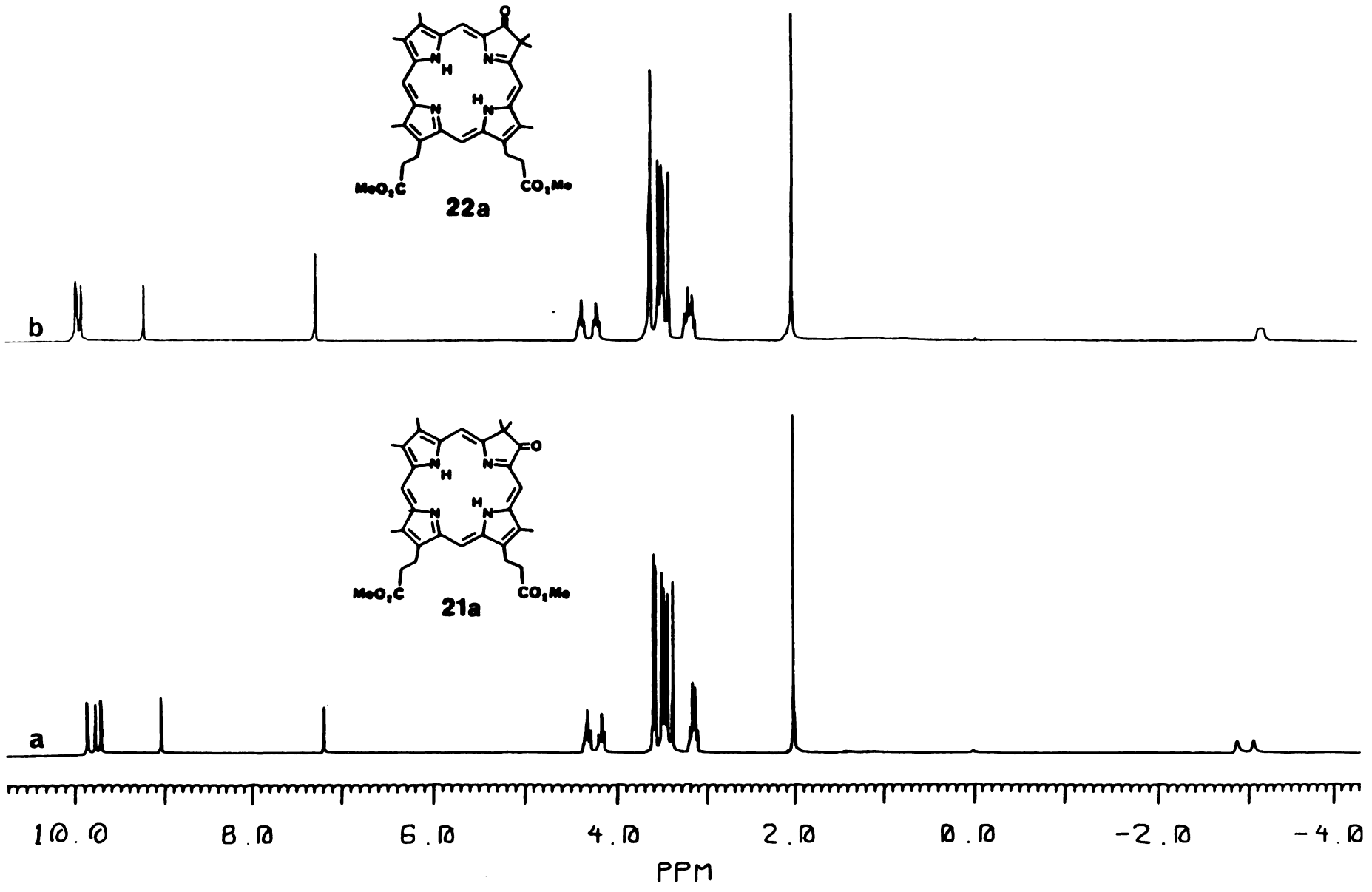


Figure A5 250 MHz ^1H NMR spectra of (a) dimethyl 3,8,8,12,13,17-hexamethyl-7-porphinone-2,18-dipropionate (21a); (b) dimethyl 3,7,7,12,13,17-hexamethyl-8-porphinone-2,18-dipropionate (22a).

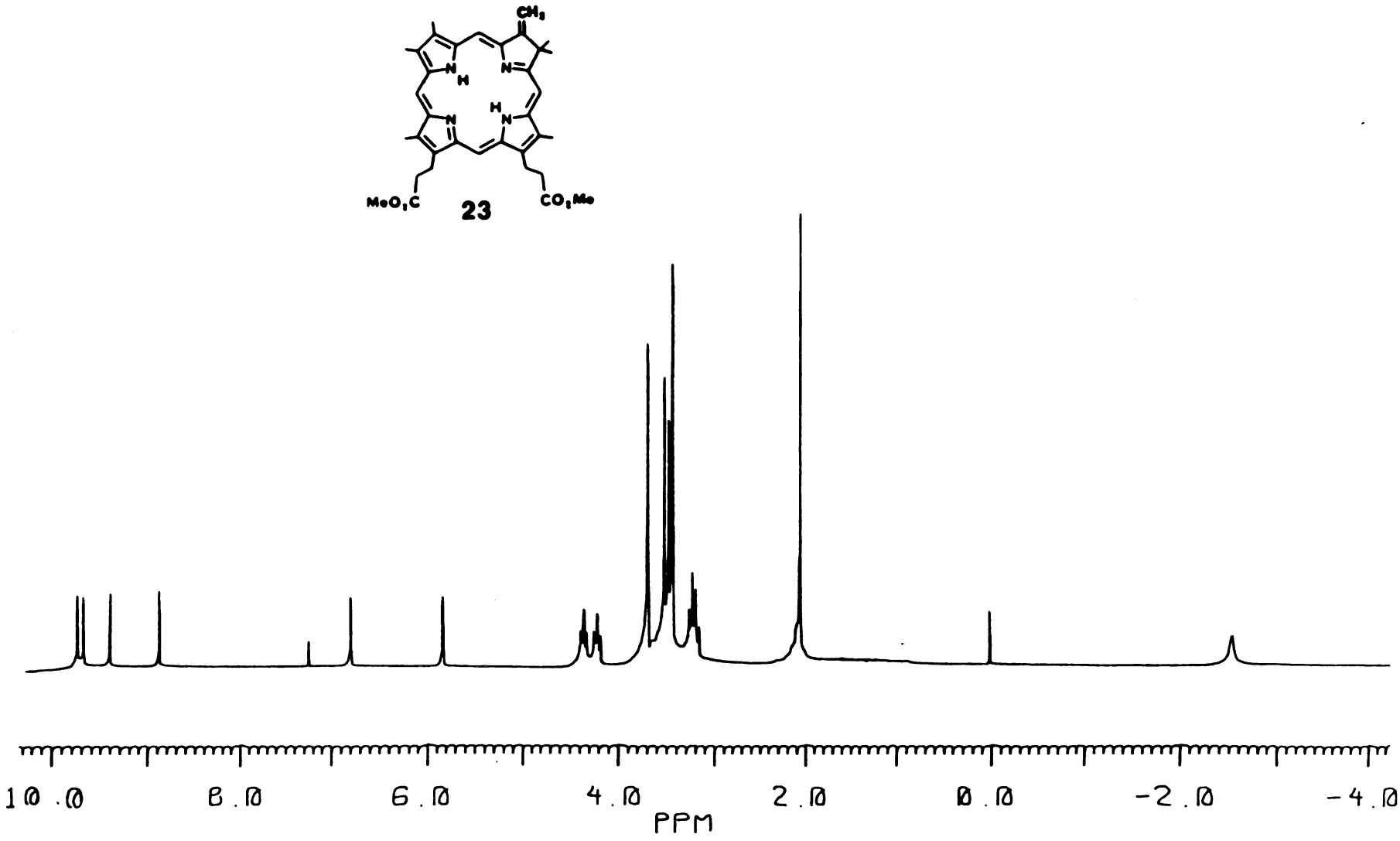


Figure A6 250 MHz ^{1}H NMR spectrum of dimethyl 3,7,7,12,13,17-hexamethyl-8-methylenechlorin-2,18-dipropionate (23).

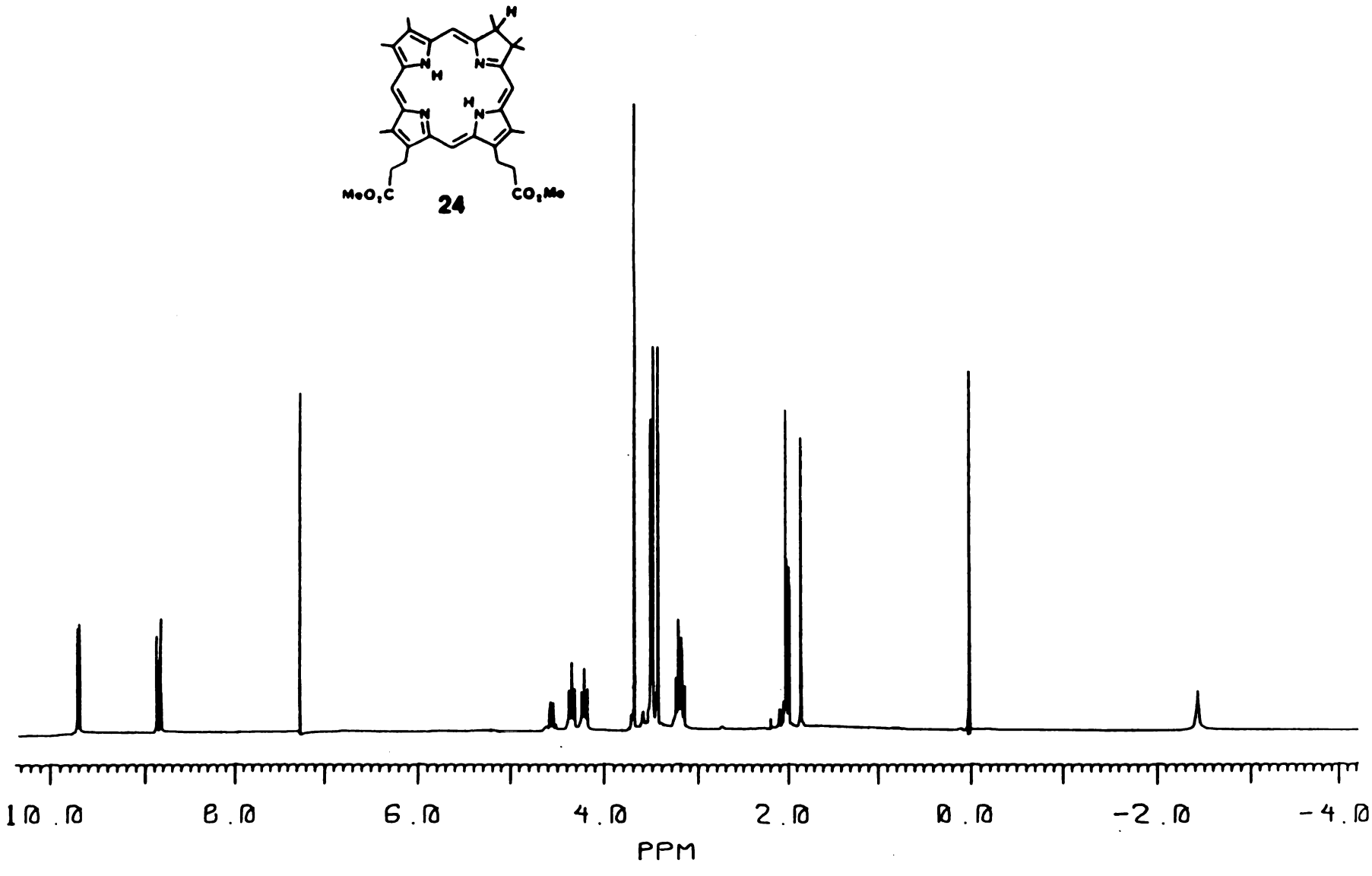


Figure A7 250 MHz ^{1}H NMR spectrum of dimethyl 3,7,7,8,12,13,17-heptamethylchlorin-2,18-dipropionate (24).

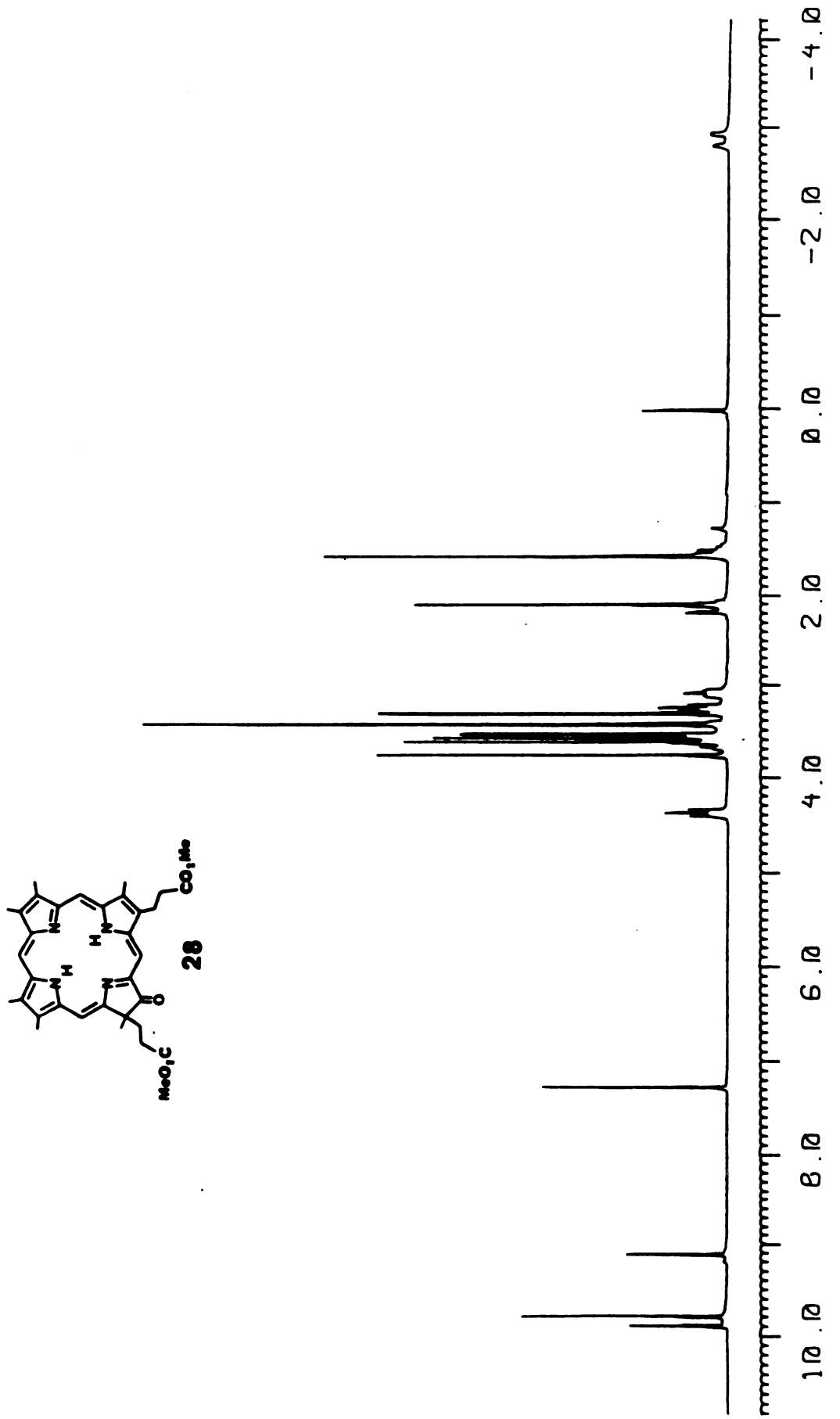


Figure A8 250 MHz ^{1}H NMR spectrum of dimethyl 3,7,8,12,13,17-hexamethyl 18-porphinone-2,17-dipropionate (28).

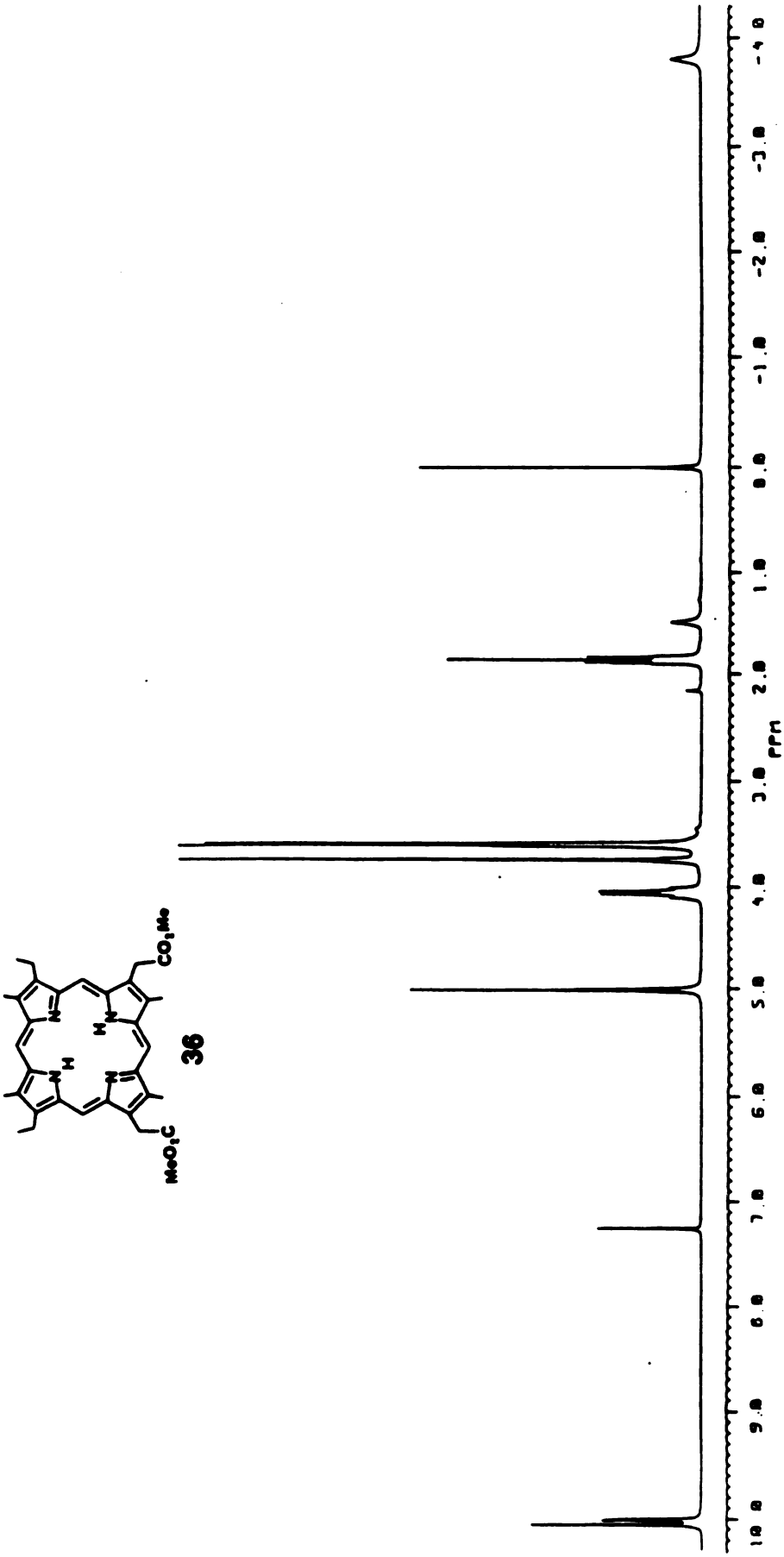


Figure A9 250 MHz ^{1}H NMR spectrum of dimethyl 12,18-diethyl-3,7,13,17-tetramethylporphine-2,8-diacetate (**36**).

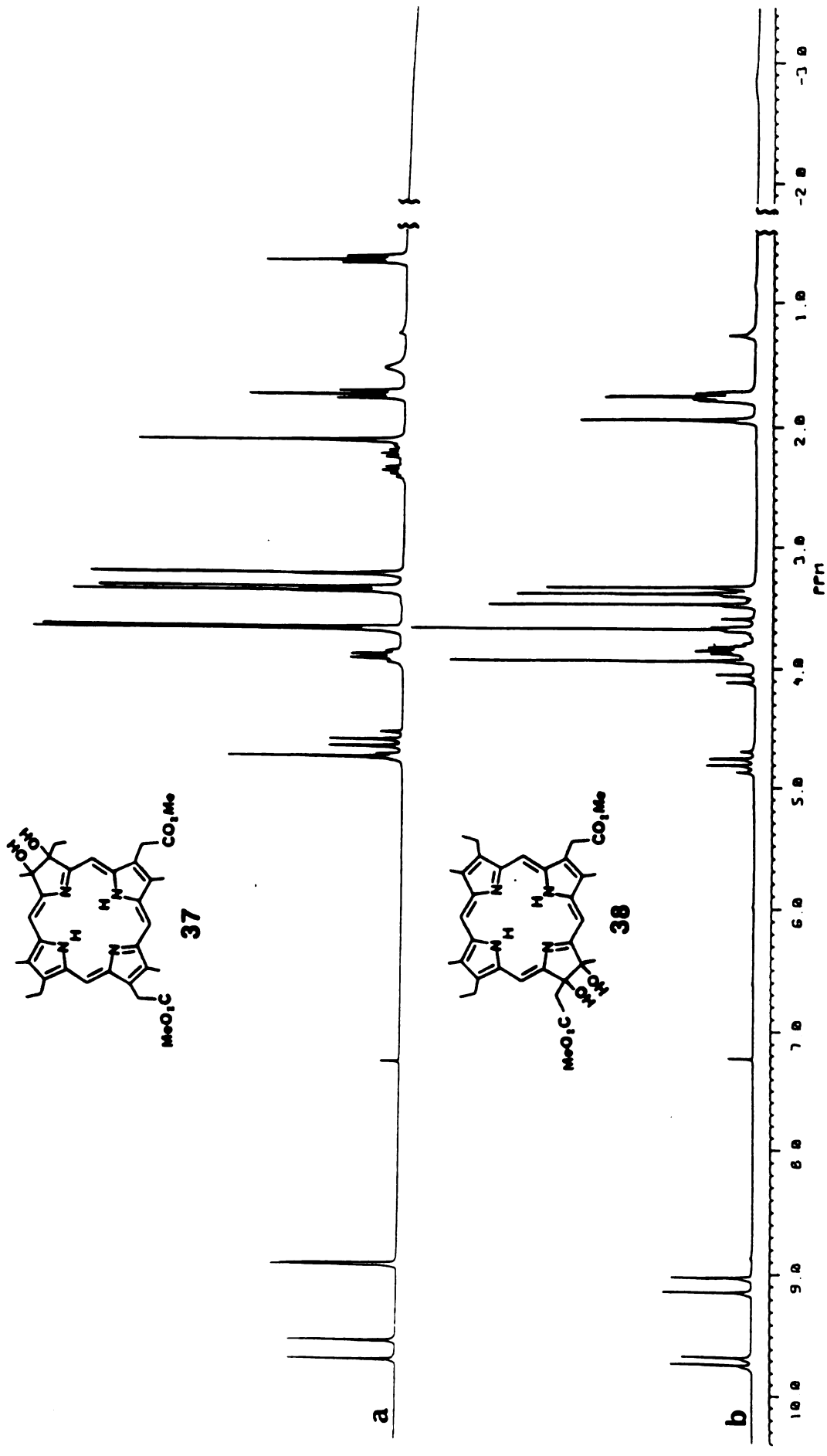


Figure A10 250 MHz ^{1}H NMR spectra of (a) dimethyl 12,18-diethyl-12,13-dihydroxy-3,7,13,17-tetramethylchlorin-2,8-diacetate (**37**); (b) dimethyl 12,18-diethyl-2,3-dihydroxy-3,7,13,17-tetramethylchlorin-2,8-diacetate (**38**).

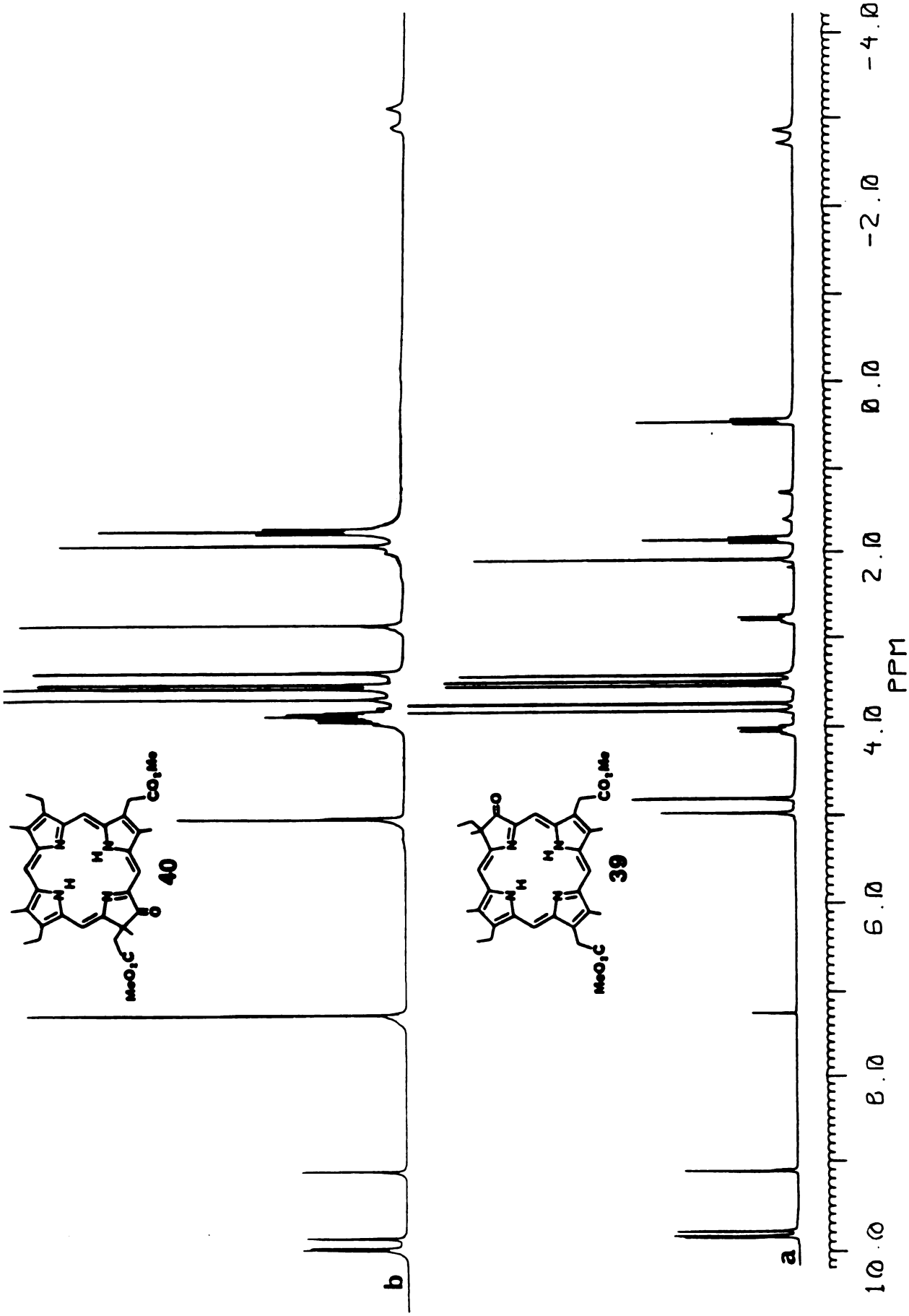


Figure All 250 MHz ^{1}H NMR spectra of (a) dimethyl 13,18-diethyl-3,7,13,17-tetramethyl-12-porphinone-2,8-diacetate (**39**); (b) dimethyl 12,18-diethyl-2,7,13,17-tetramethyl-3-porphinone-2,8-diacetate (**40**).

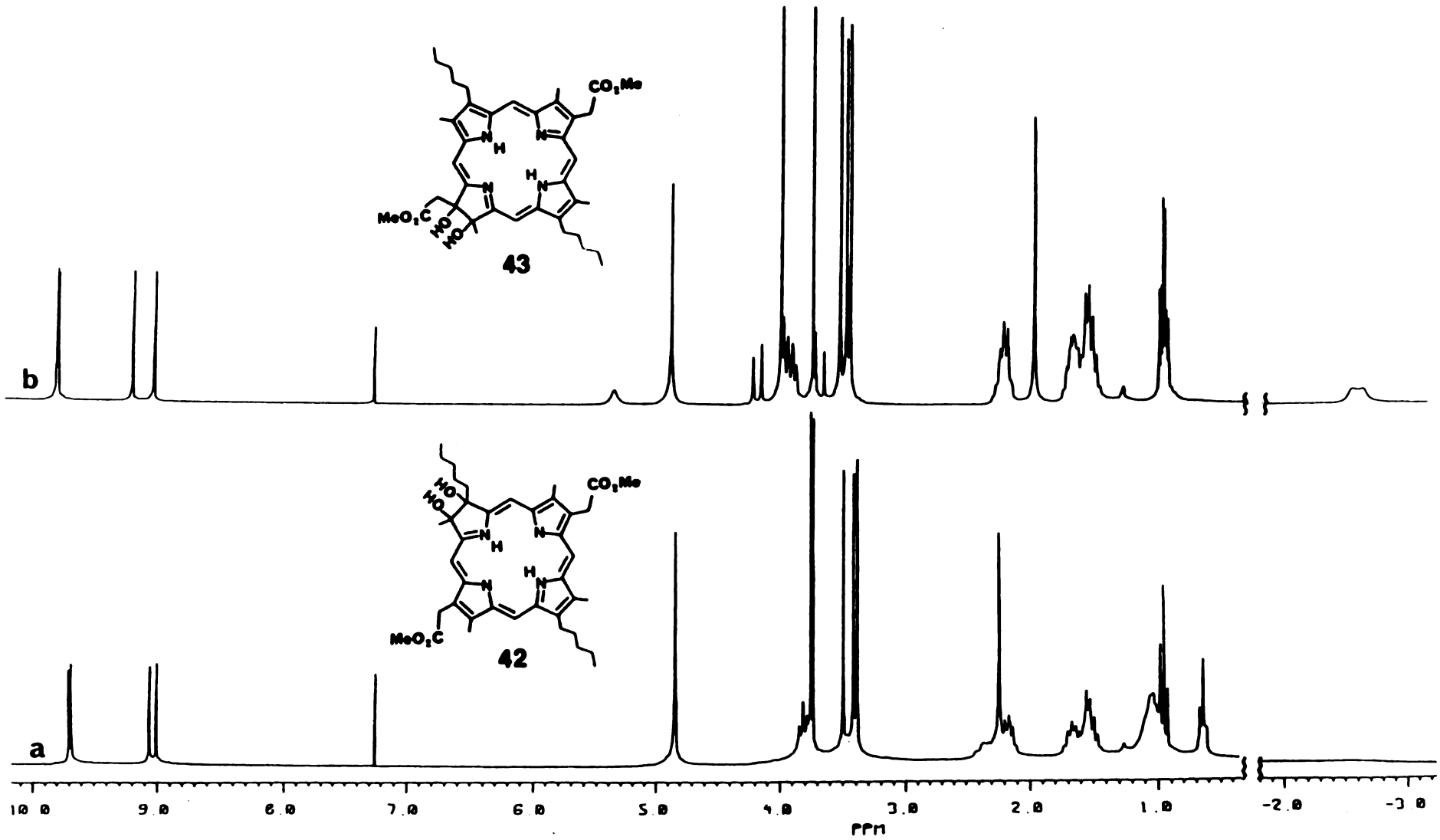


Figure A12 250 MHz ^{1}H NMR spectra of (a) dimethyl 7,8-dihydroxy-3,8,13,18-tetramethyl-7,17-dipentylchlorin-2,12-diacetate (**42**); (b) dimethyl 2,3-dihydroxy-3,8,13,18-tetramethyl-7,17-dipentylchlorin-2,12-diacetate (**43**).

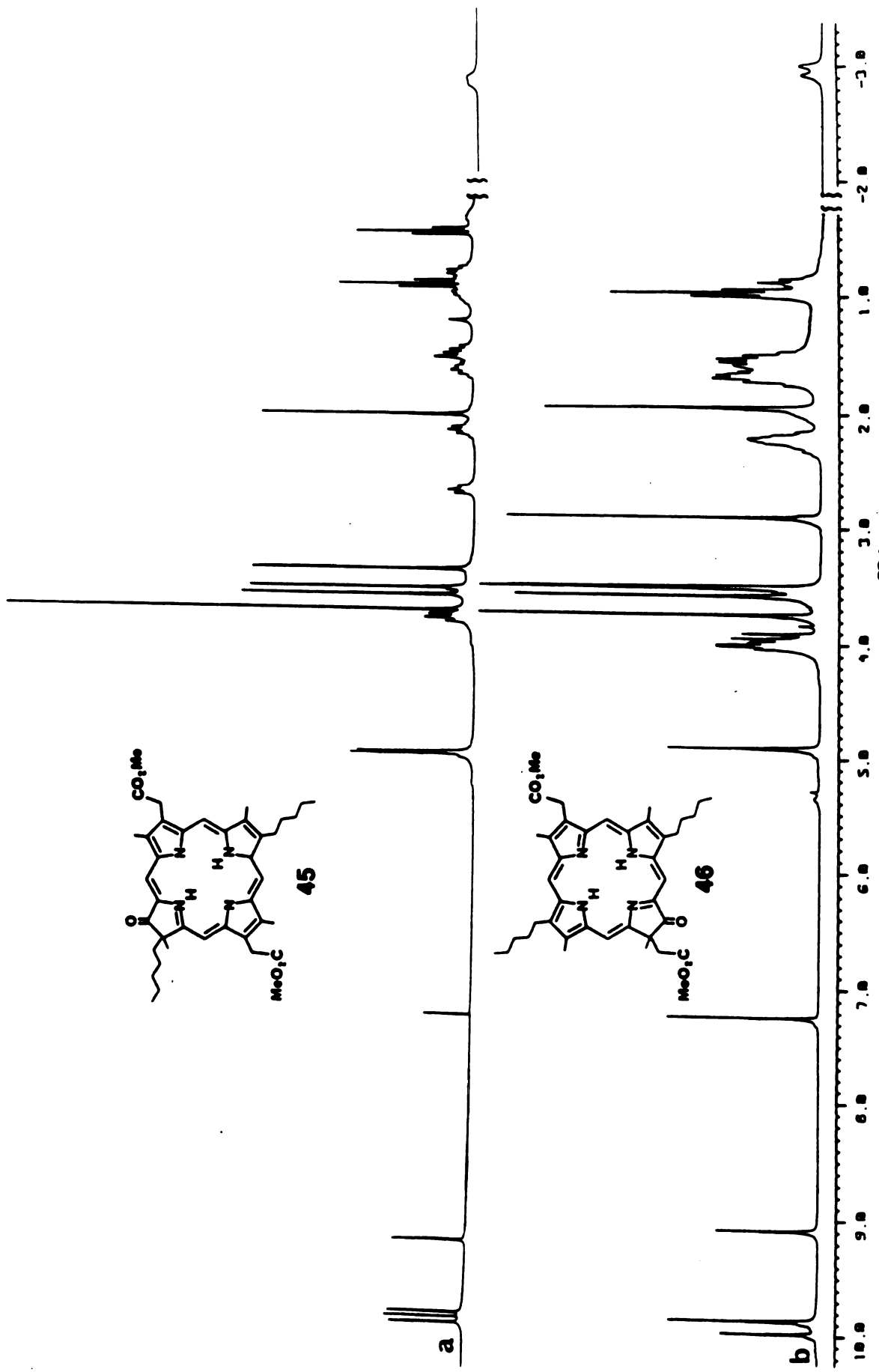


Figure A13 250 MHz ^1H NMR spectra of (a) dimethyl 3,9,13,18-tetramethyl-8,17-dipentyl-7-porphinone-2,12-diacetate (45); (b) dimethyl 2,8,13,18-tetramethyl-7,17-dipentyl-3-porphinone-2,12-diacetate (46).

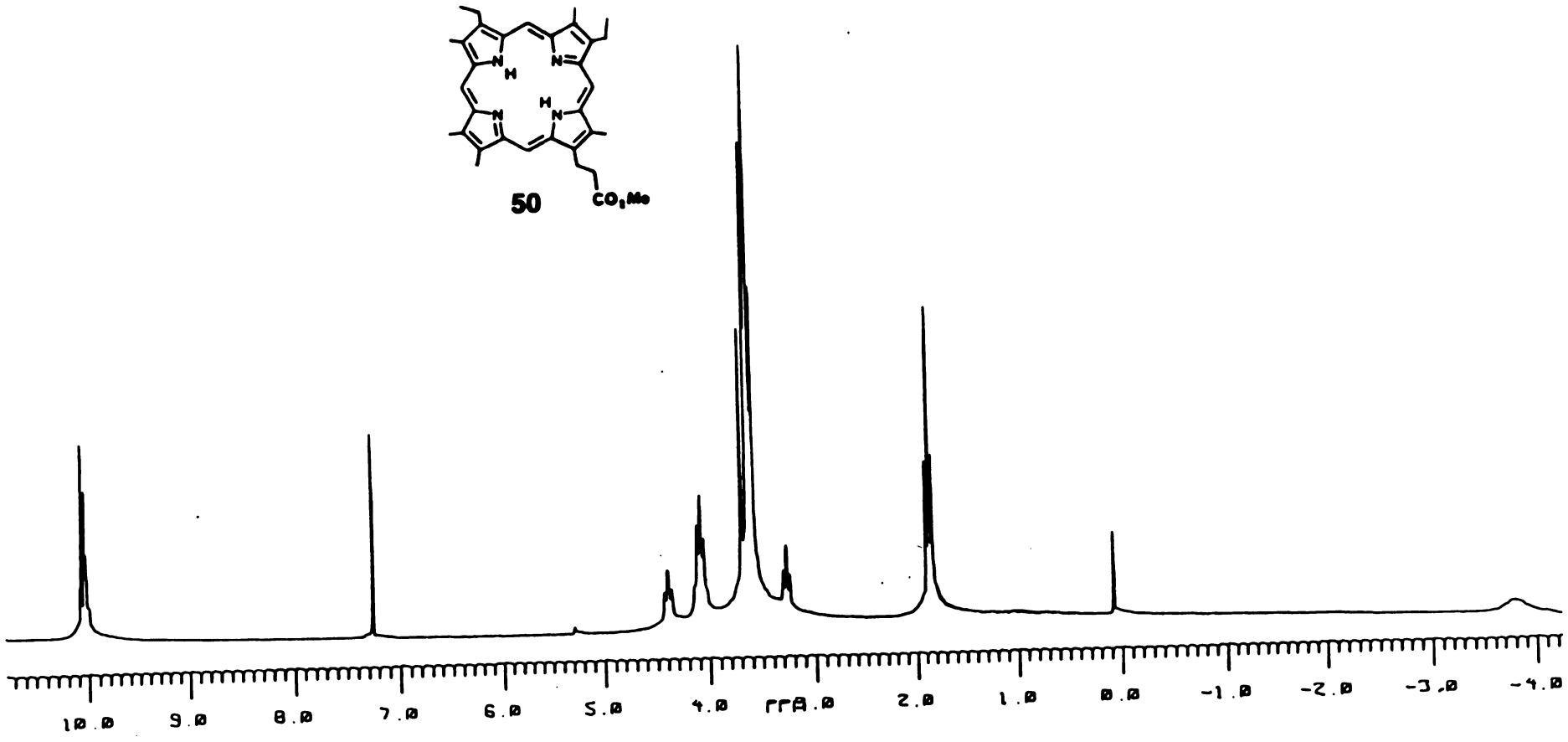


Figure A14 250 MHz ^{1}H NMR spectrum of methyl 7,12-diethyl-3,8,13,17,18-pentamethyl-porphine-2-propionate (**50**).

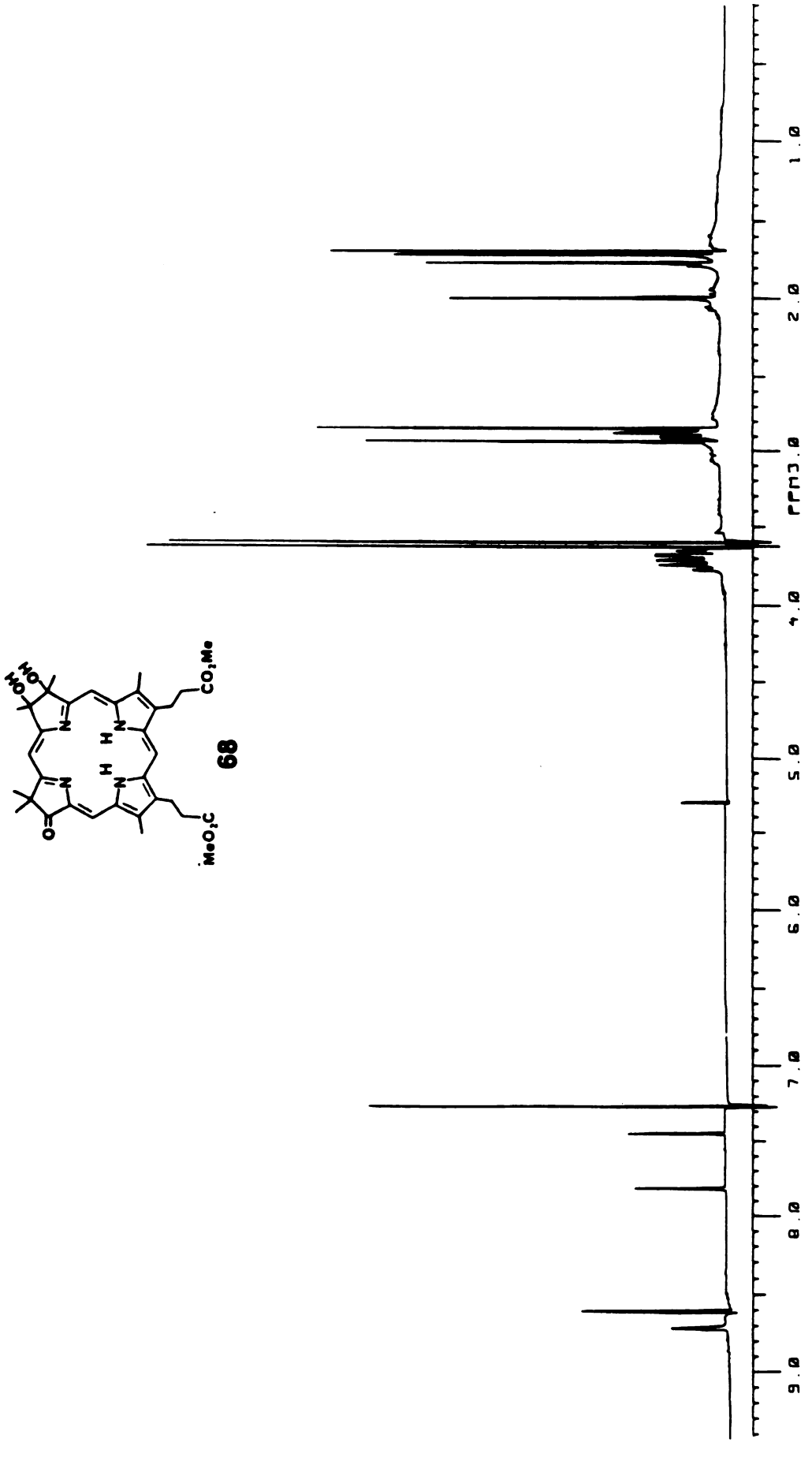


Figure A15 250 MHz ^{1}H NMR spectrum of dimethyl 12,13-dihydroxy-3,8,8,12,13,17-hexamethyl-7-porphinone-2,18-dipropionate (68).

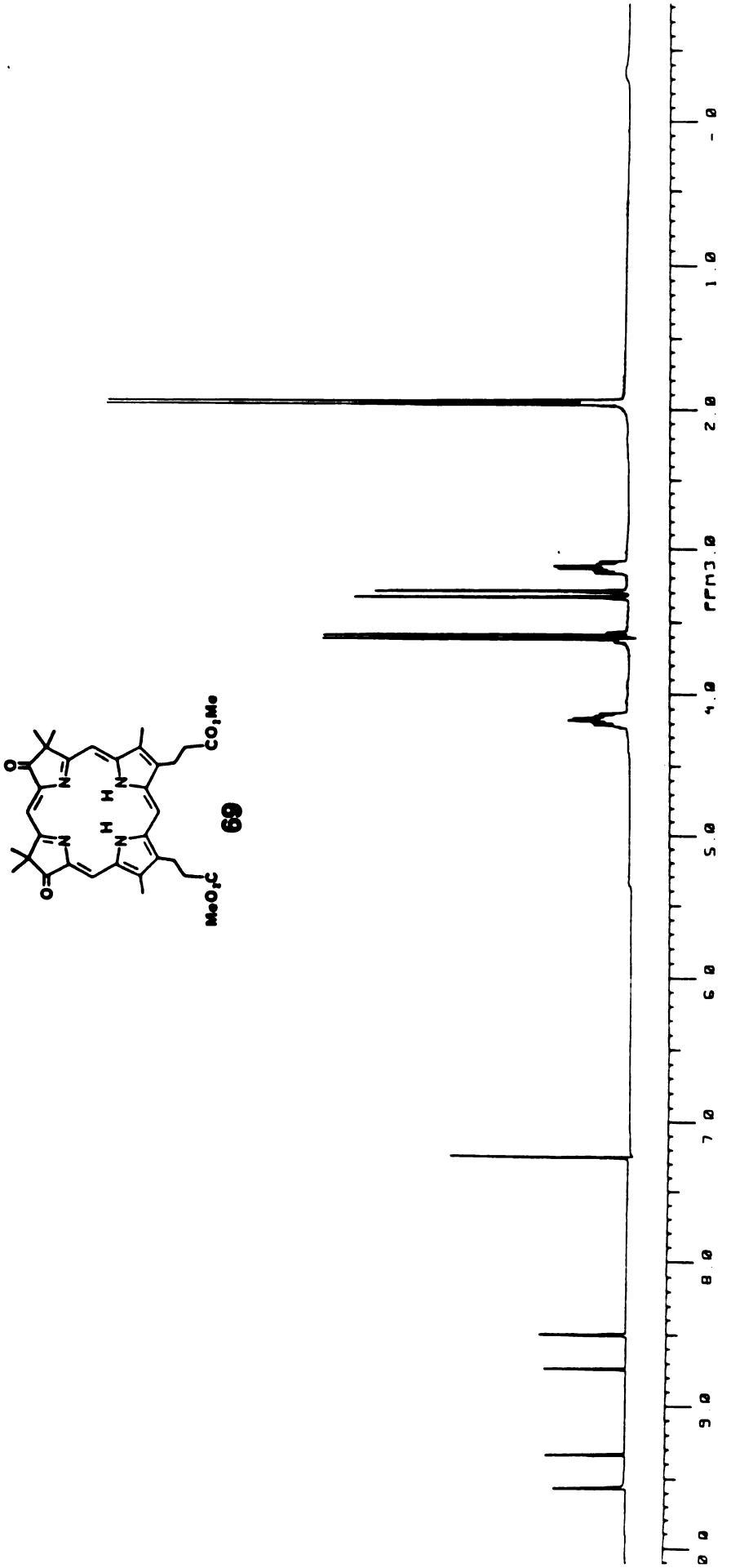


Figure A16 250 MHz ^{1}H NMR spectrum of dimethyl 3,8,8,13,13,17-hexamethyl-7,12-porphinedione-2,18-dipropionate (69).

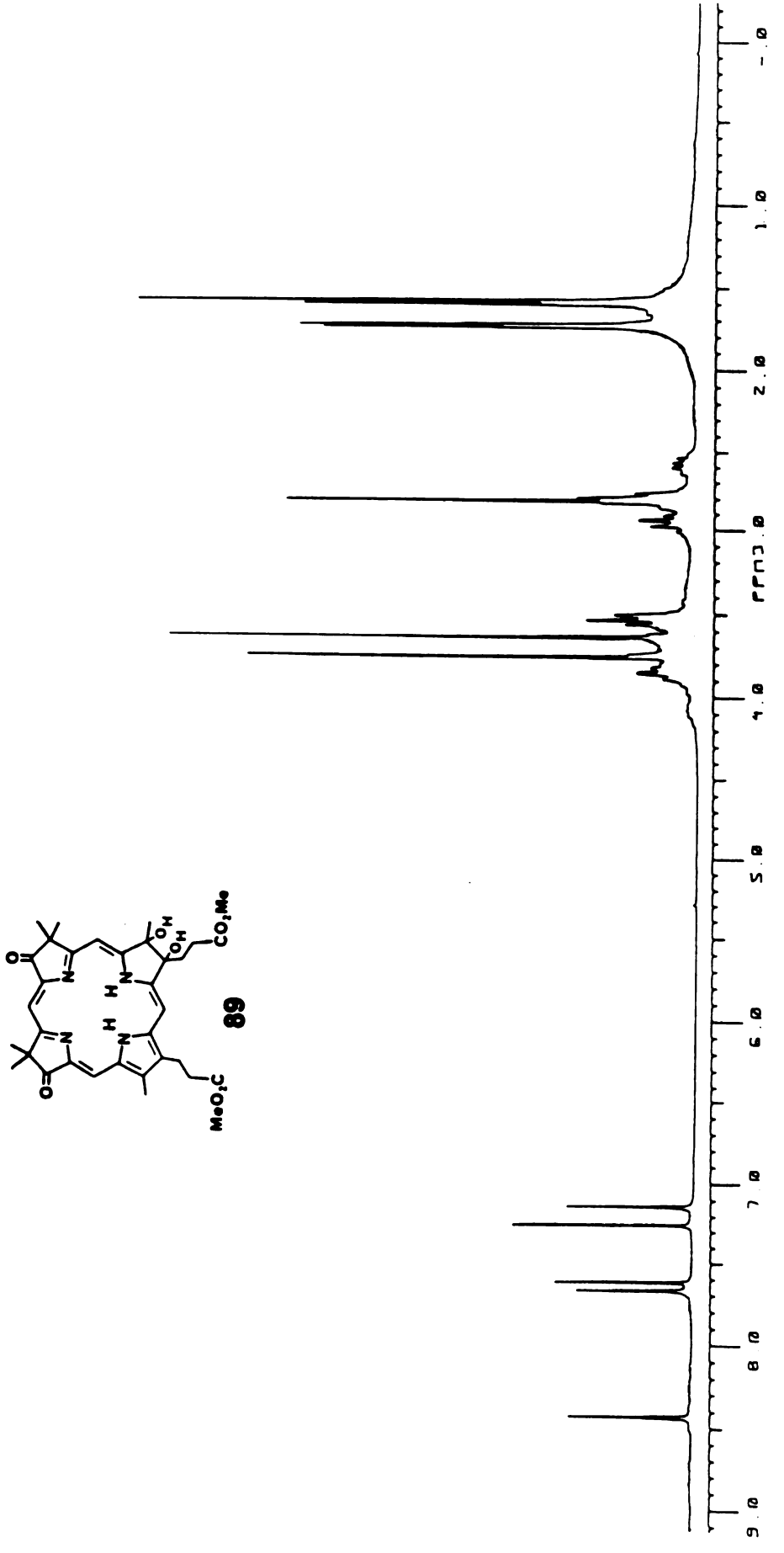


Figure A17 250 MHz ^{1}H NMR spectrum of dimethyl 17,18-dihydroxy-3,8,8,13,13,17-hexamethyl-7,12-porphinedione-2,18-dipropionate (89).

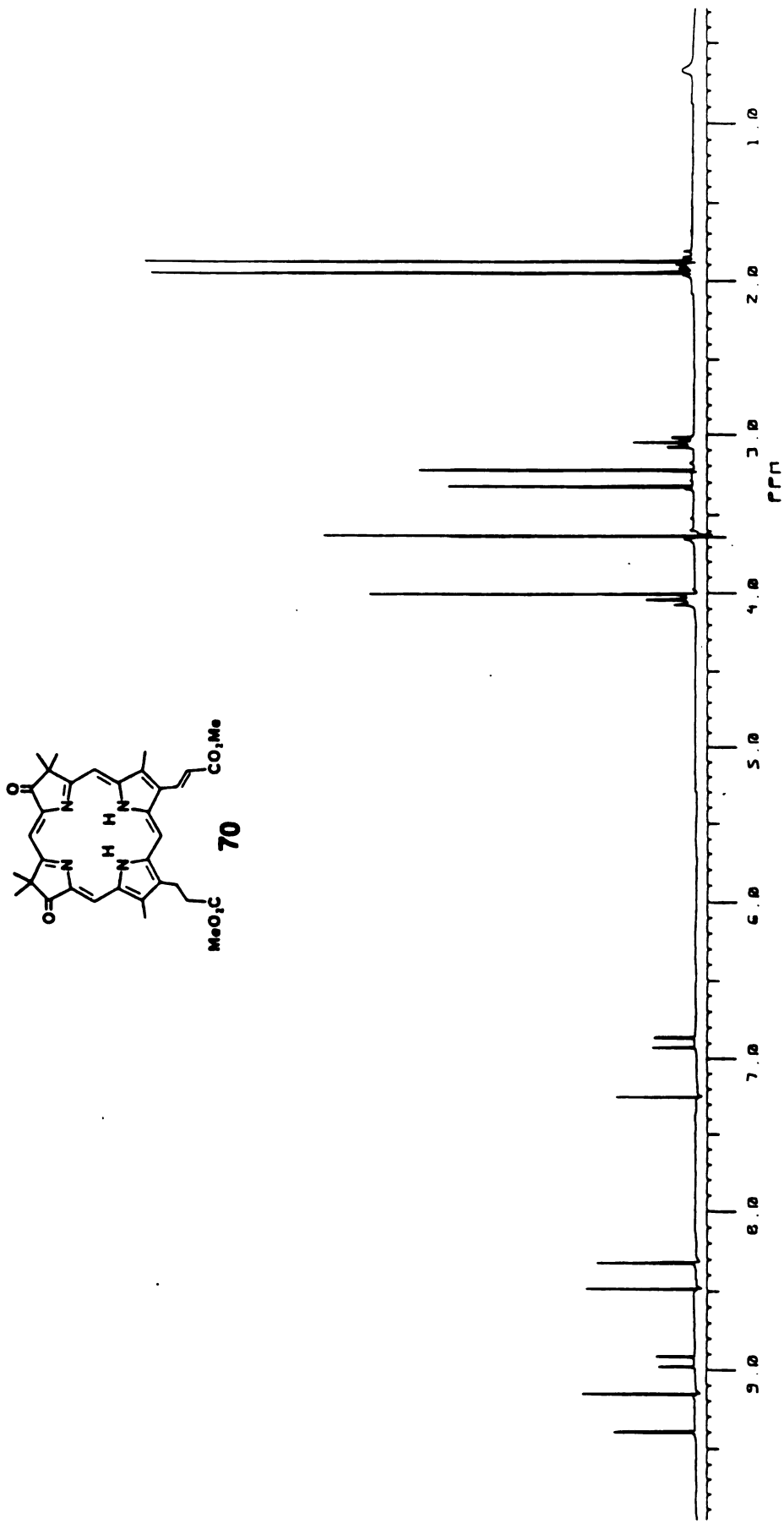


Figure A18 250 MHz ^{1}H NMR spectrum of methyl 18-[2-(methoxycarbonyl)ethenyl]-3,8,8,13,13,17-hexamethyl-7,12-porphinedione-2-propionate (**70**).

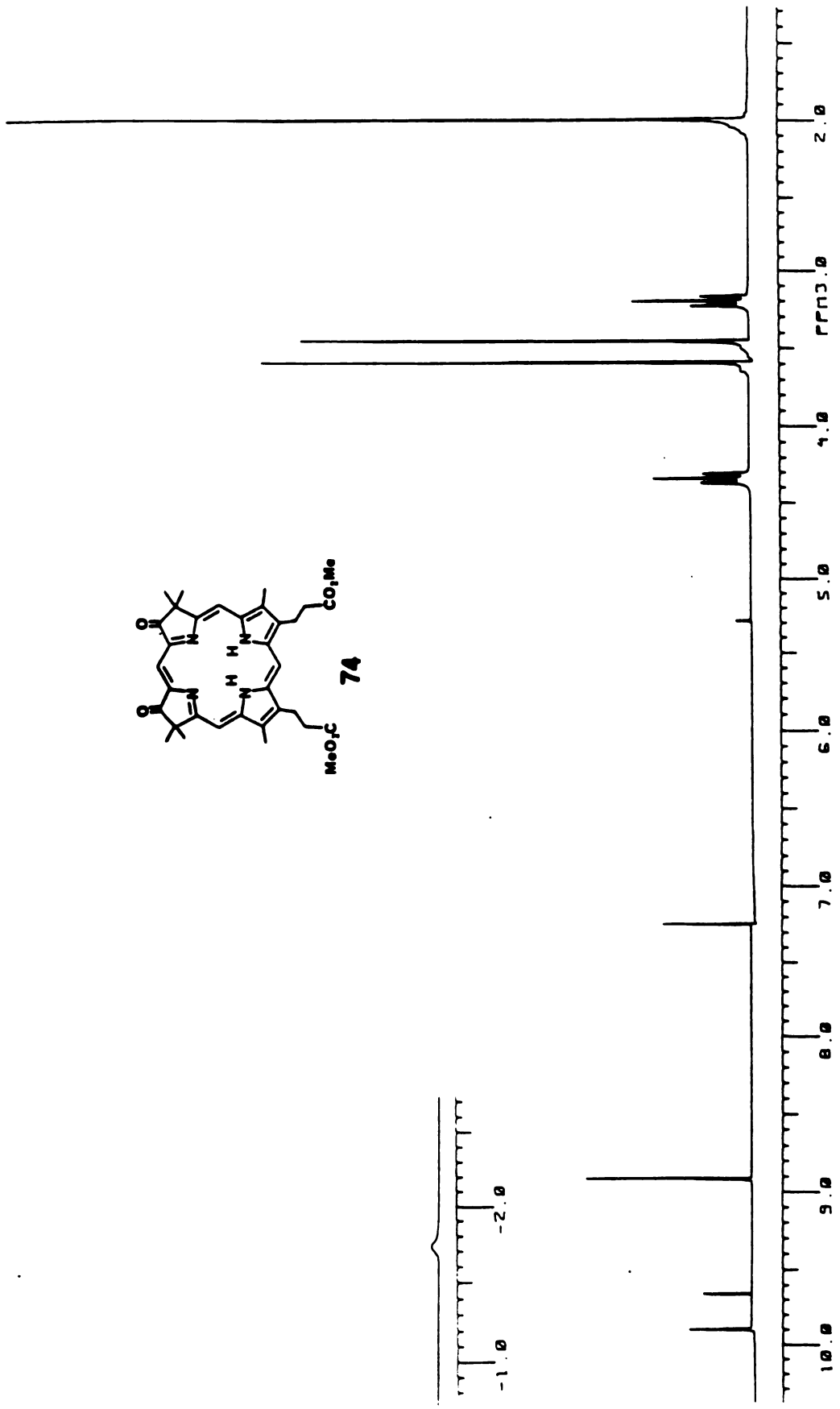


Figure A19 250 MHz ^{1}H NMR spectrum of dimethyl 3,7,7,13,13,17-hexamethyl-8,12-porphinedione-2,18-dipropionate (74).

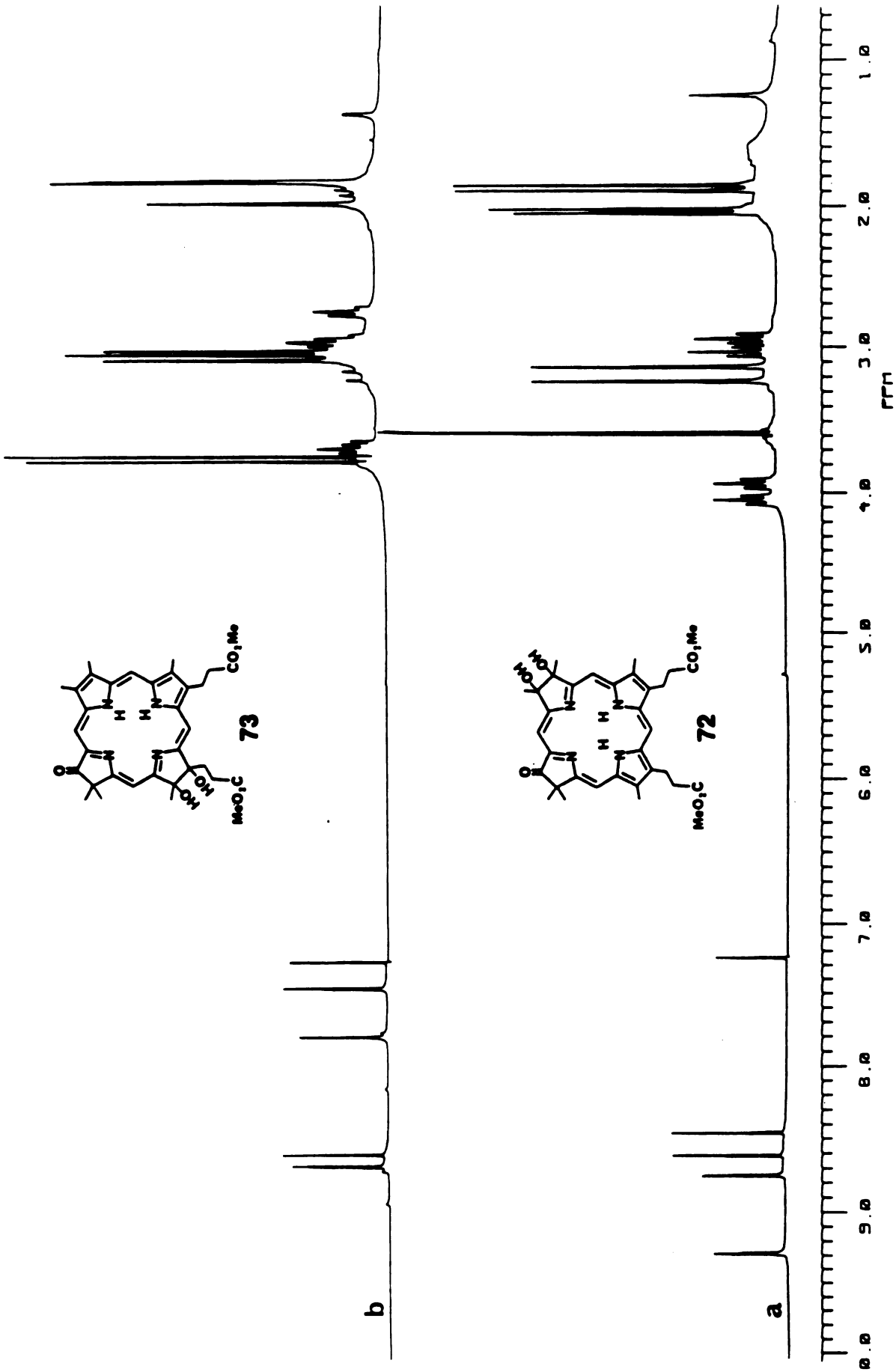


Figure A20 250 MHz ^{1}H NMR spectra of (a) dimethyl 12,13-dihydroxy-3,7,7,12,13,17-hexamethyl-8-porphinone-2,18-dipropionate (72); (b) dimethyl 2,3-dihydroxy-3,7,7,12,13,17-hexamethyl-8-porphinone-2,18-dipropionate (73).

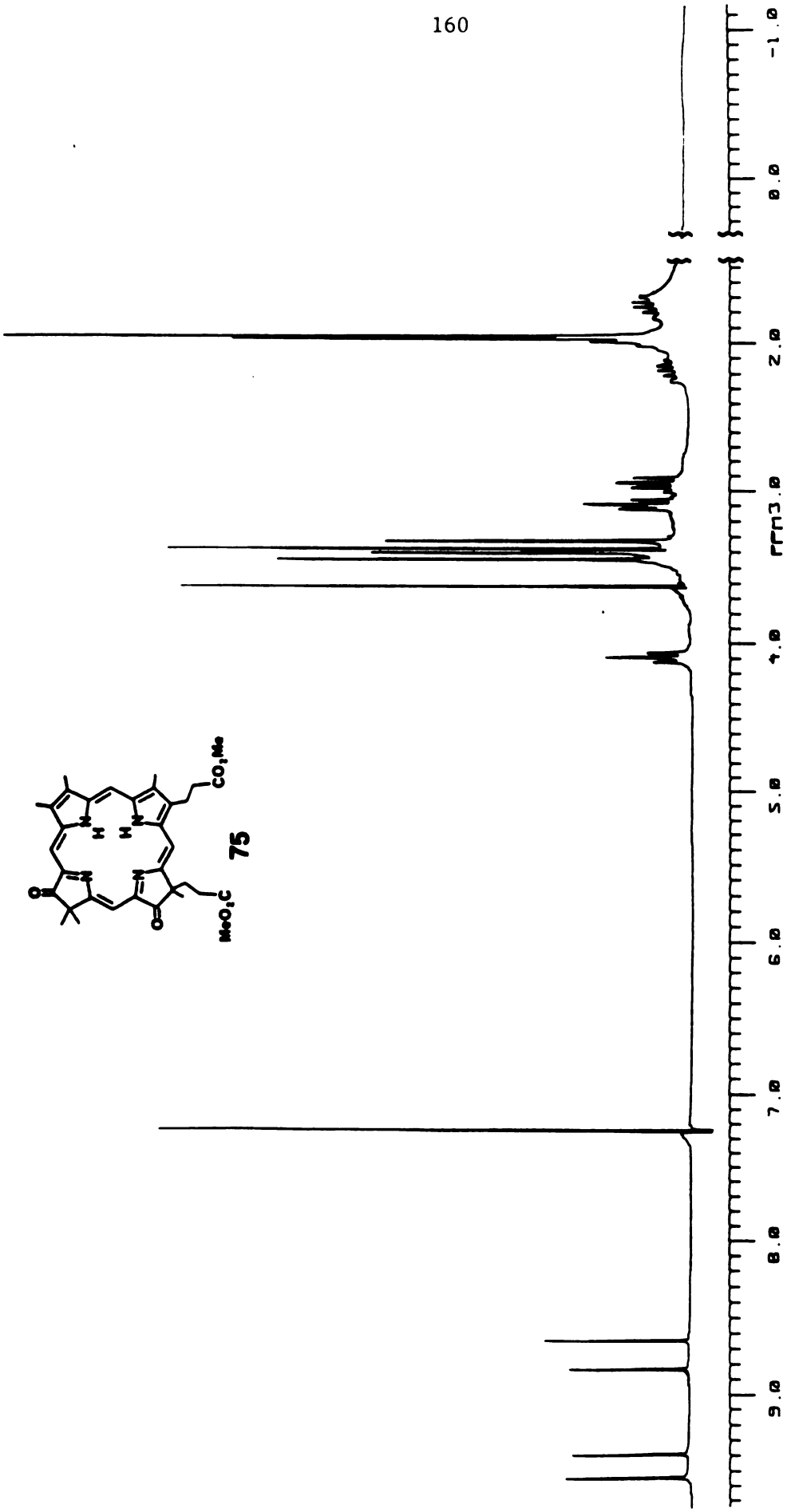


Figure A21 250 MHz ^{1}H NMR spectrum of dimethyl 2,7,7,12,13,17-hexamethyl-3,8-porphinedione-2,18-dipropionate (75).

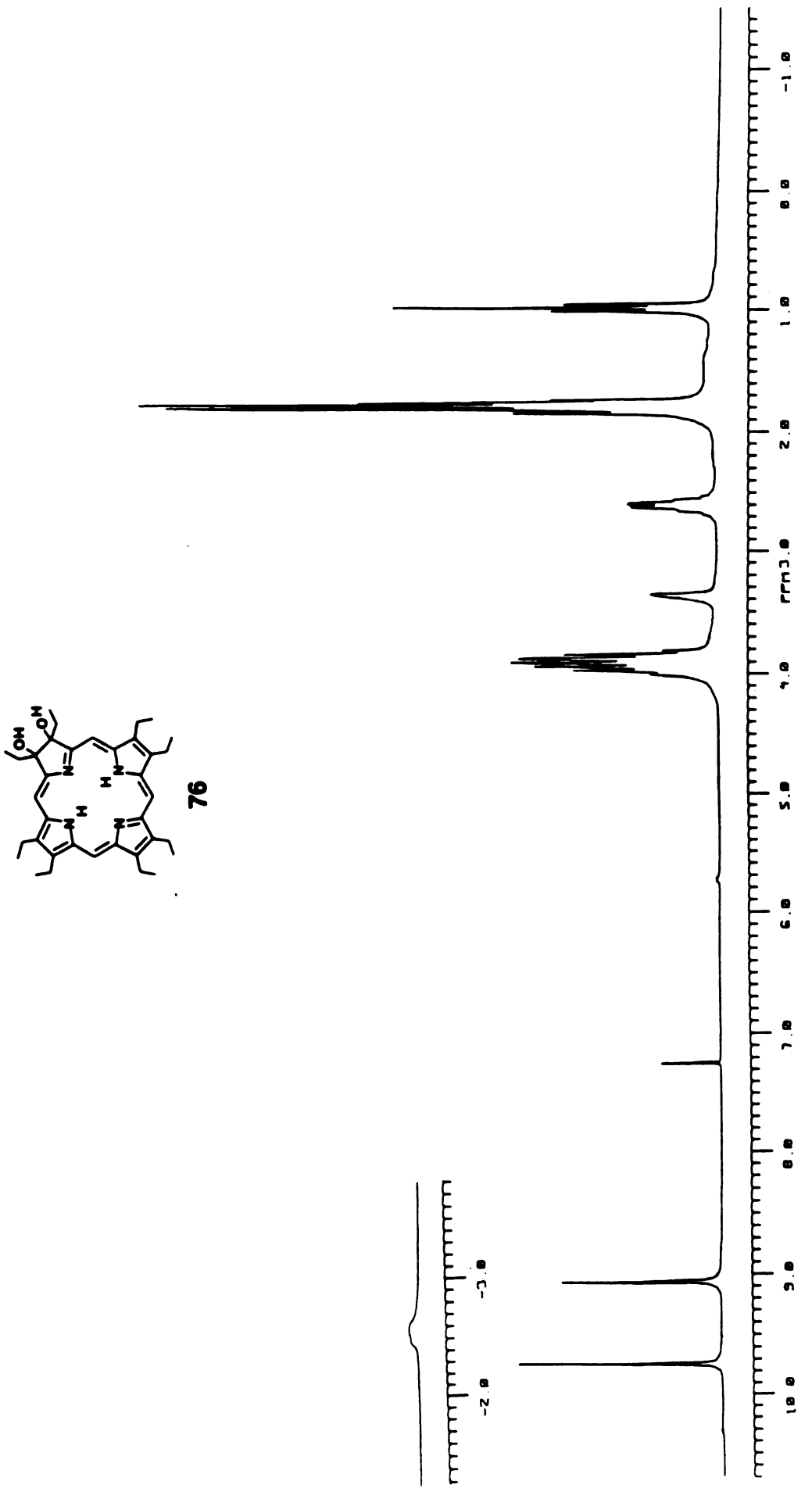


Figure A22 250 MHz ^{1}H NMR spectrum of *vic*-dihydroxyoctaethylchlorin (76).

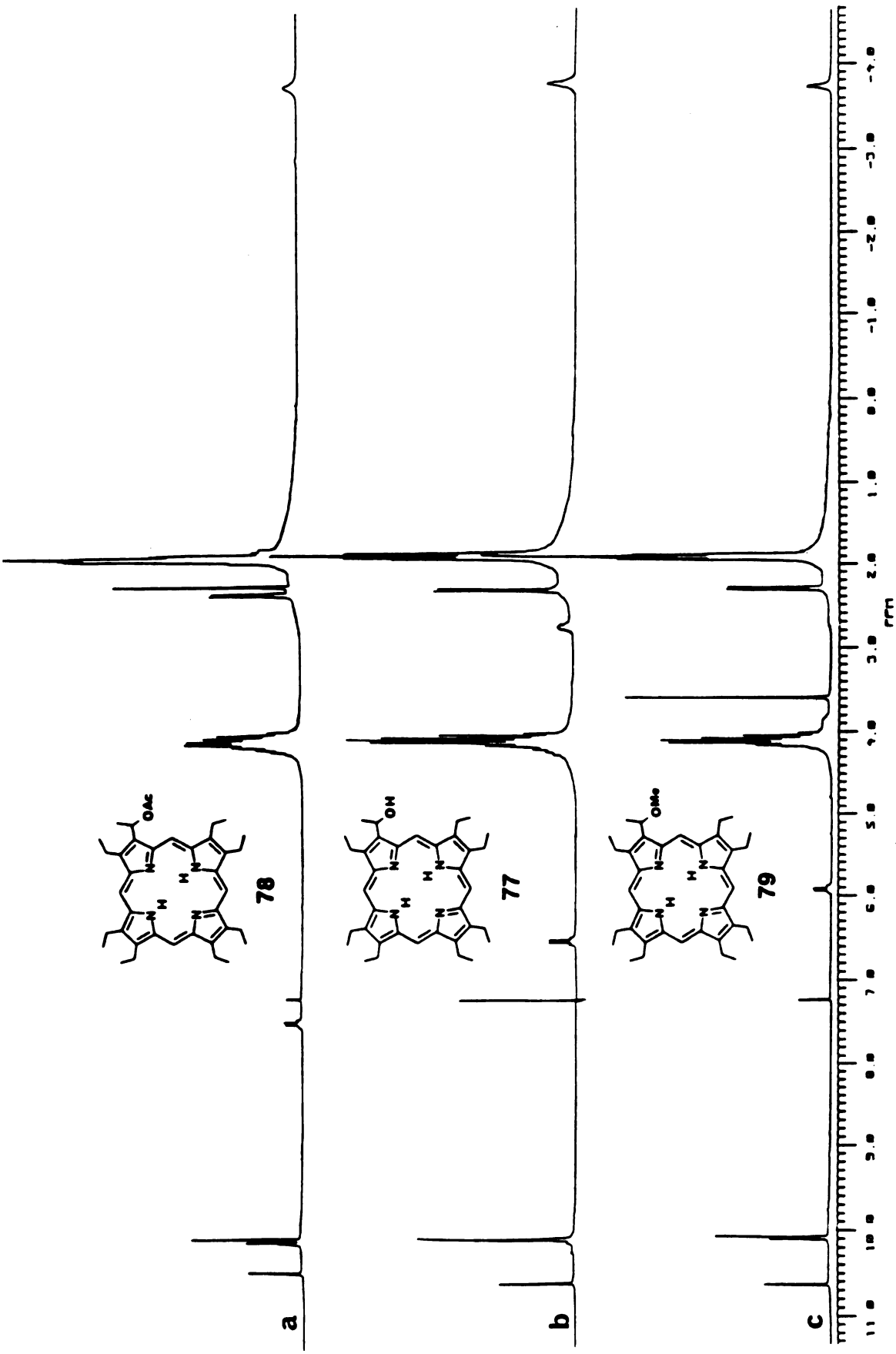


Figure A23 250 MHz ^{1}H NMR spectra of (a) 3,7,8,12,13,17,18-heptaethyl-2-(1-acetoxyethyl)-porphine (**78**); (b) 3,7,12,13,17,18-heptaethyl-2-(1-hydroxyethyl)-porphine (**77**); (c) 3,7,8,12,13,17,18-heptaethyl-2-(1-methoxyethyl)-porphine (**79**).

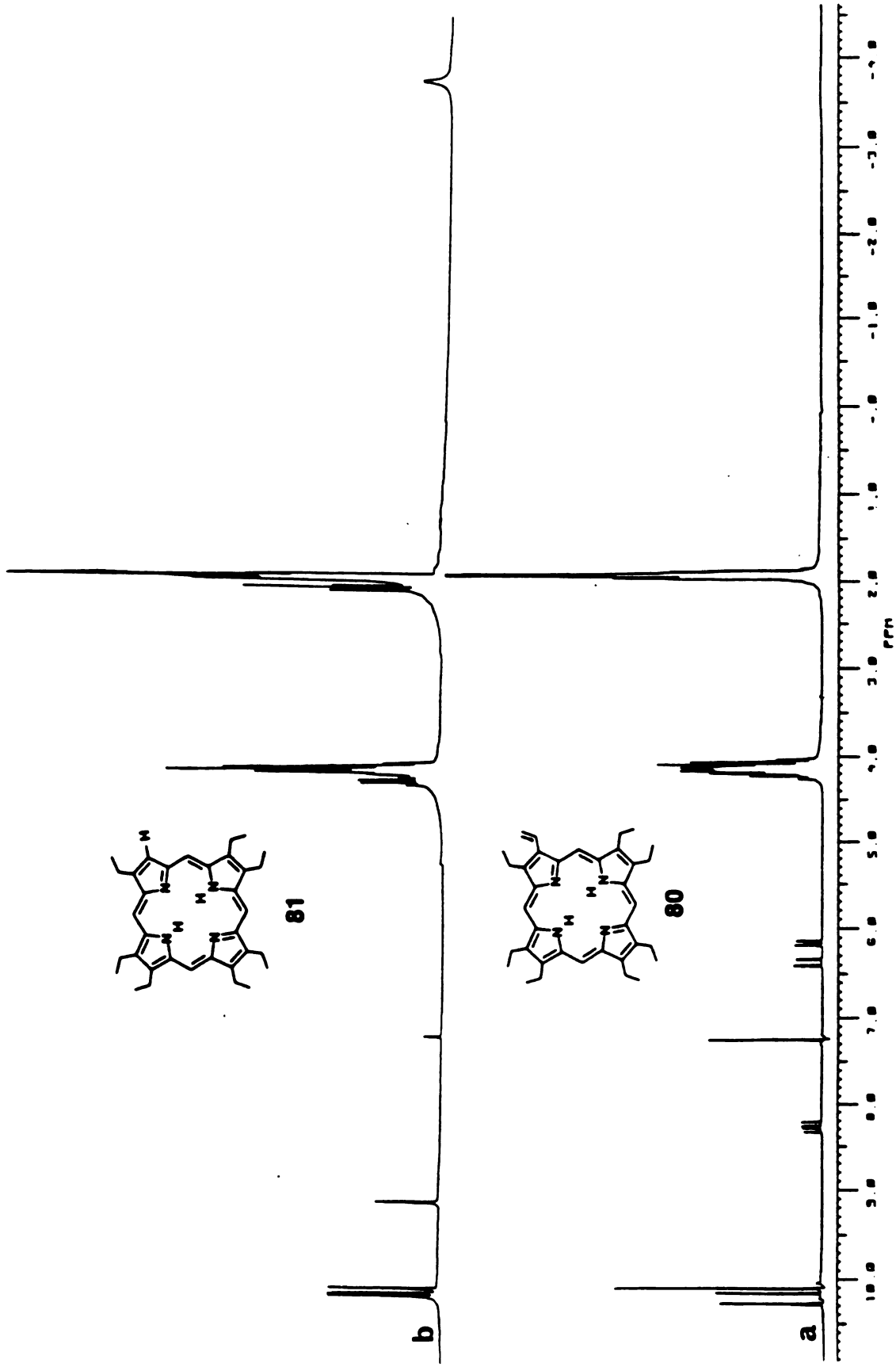


Figure A24 250 MHz ^{1}H NMR spectra of (a) 3,7,8,12,13,17,18-heptaethyl-2-vinylporphine (80); (b) 2,3,7,8,13,17-heptaethylporphine (81).

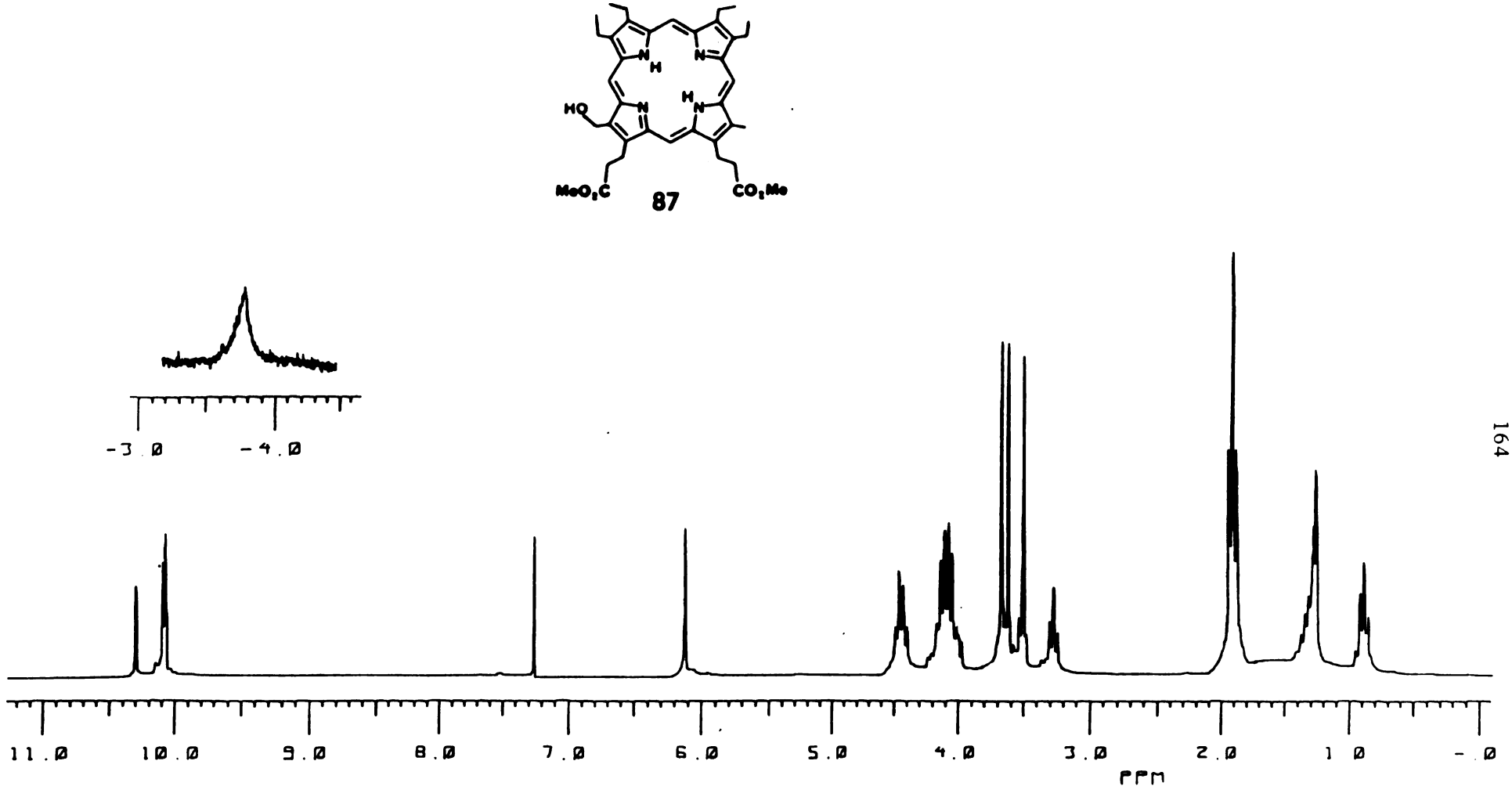


Figure A25 250 MHz ^{1}H NMR spectrum of dimethyl 7,8,12,13-tetraethyl-3-(hydroxymethyl)-17-methyl-2,18-porphinedipropionate (87).

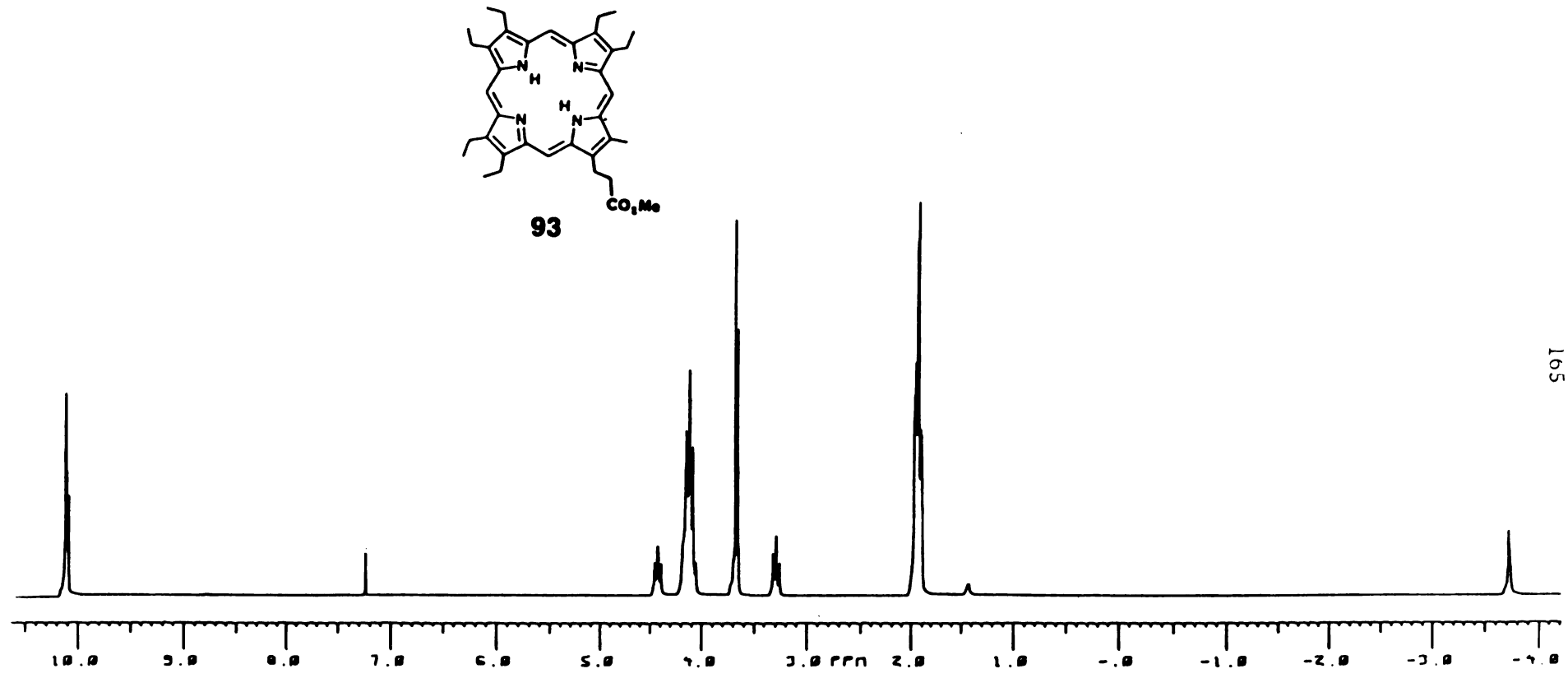


Figure A26 250 MHz ^{1}H NMR spectrum of methyl 7,8,12,13,17,18-hexaethyl-4-methyl-porphine-2-propionate (93).

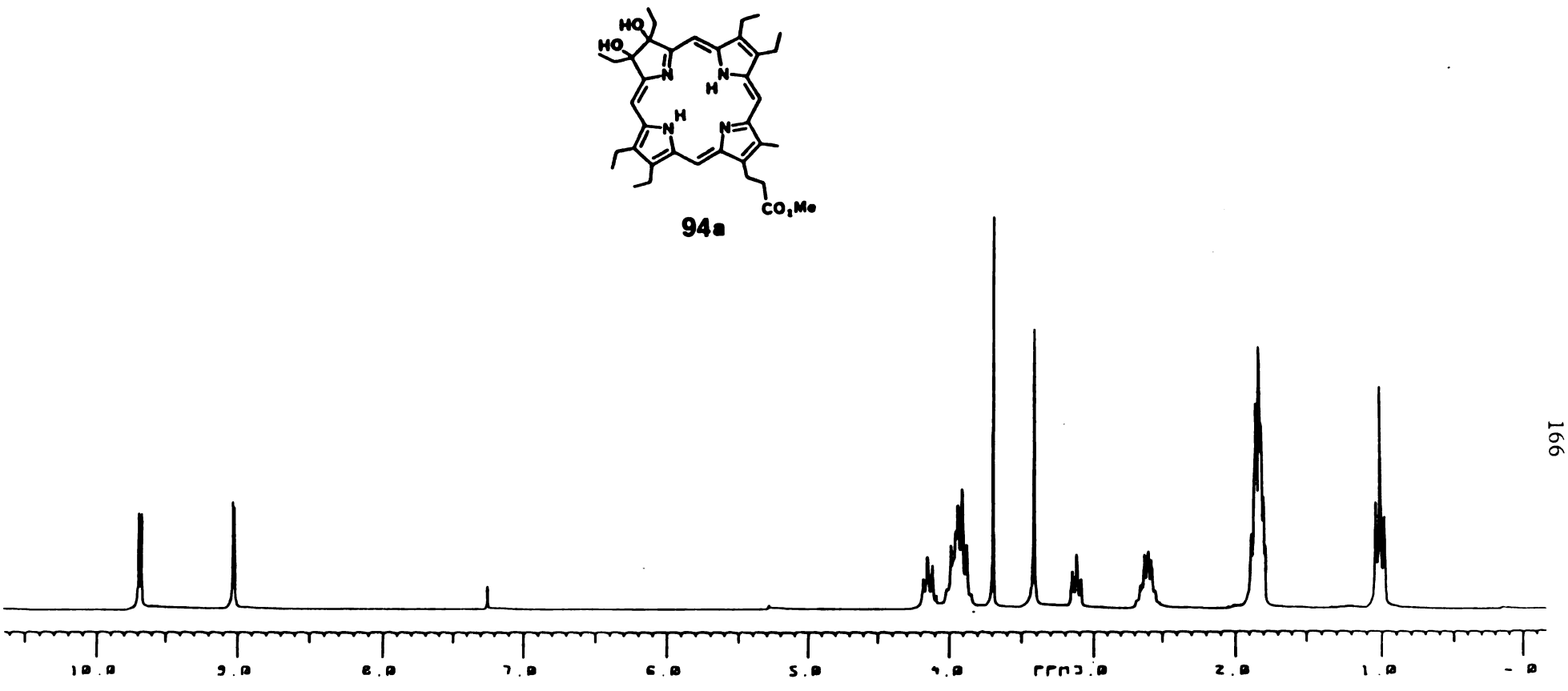


Figure A27 250 MHz ^{1}H NMR spectrum of methyl 7,8,12,13,17,18-hexaethyl-12,13-dihydroxy-4-methylchlorin-2-propionate (94a).

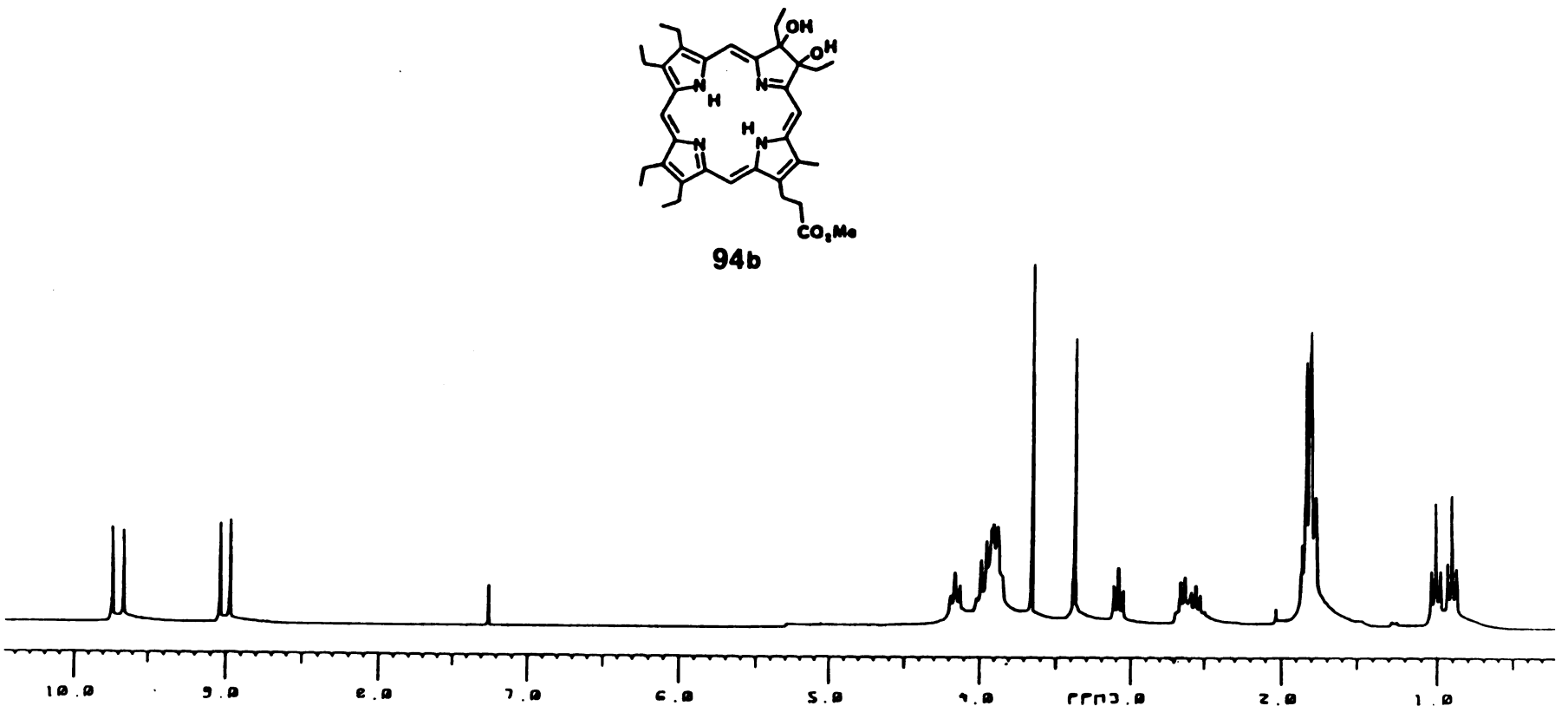


Figure A28 250 MHz ^{1}H NMR spectrum of methyl 7,8,12,13,17,18-hexaethyl-7,8 dihydroxy-3-methylchlorin-2-propionate (**94b**).

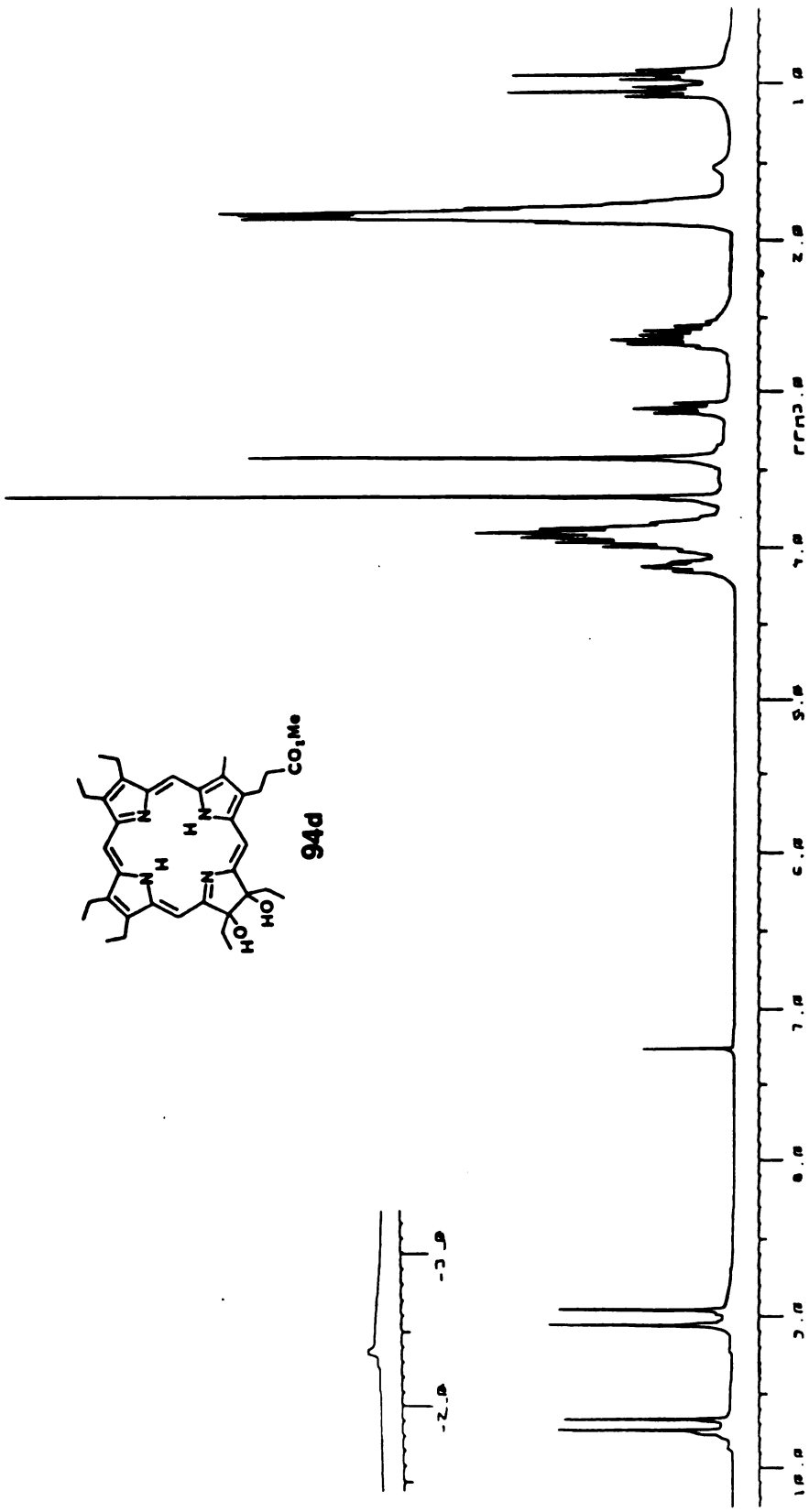


Figure A29 250 MHz ^{1}H NMR spectrum of methyl 7,8,12,13,17,18-hexaethyl-17,18-dihydroxy-3-methylchlorin-2-propionate (**94d**).

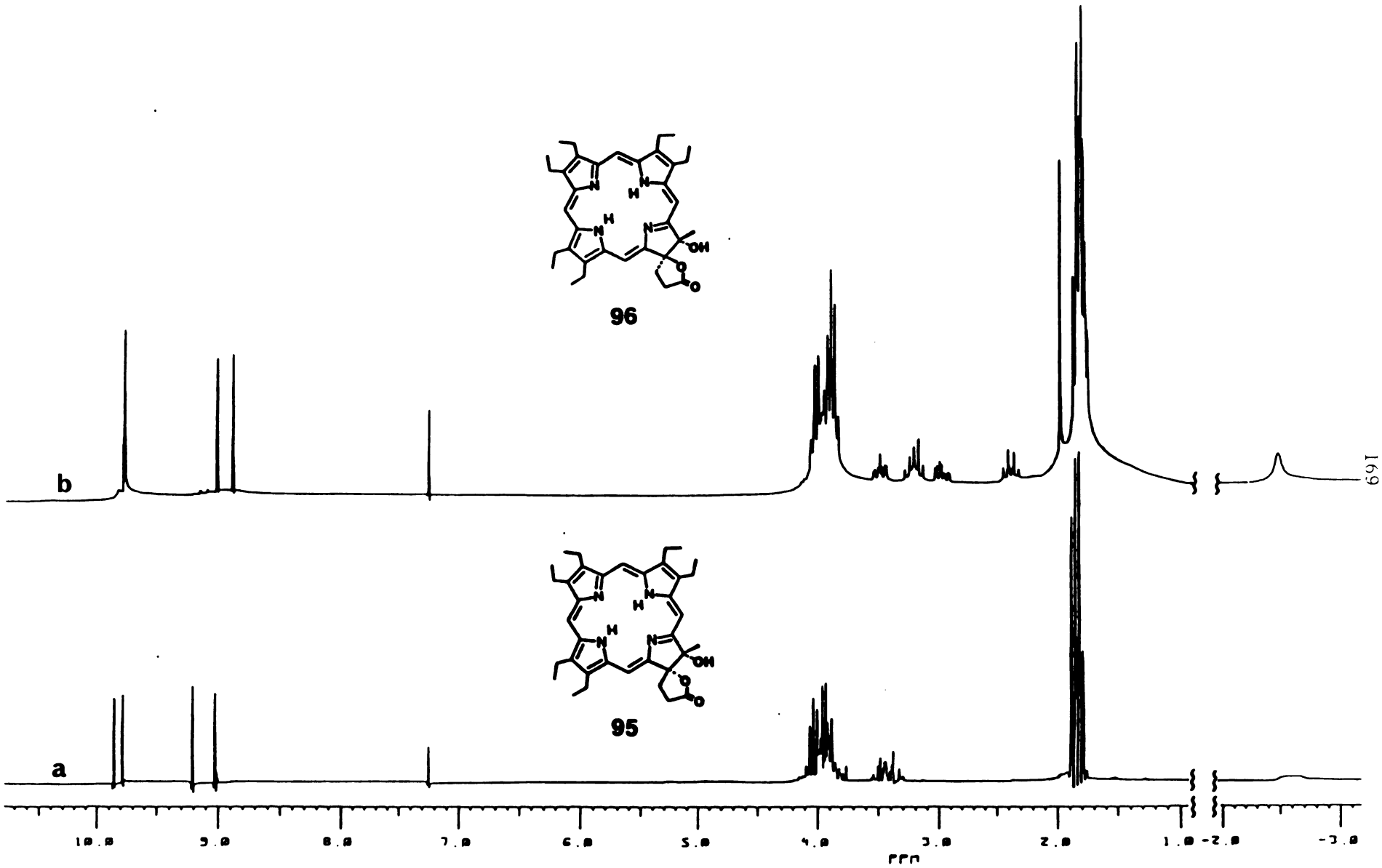


Figure A30 250 MHz ^{1}H NMR spectra of (a) cis-7,8,12,13,17,18-hexaethyl-3-hydroxy-3-methyl-2,2-γ-spirolactone-chlorine (**95**); (b) trans-7,8,12,13,17,18-hexaethyl-3-hydroxy-3-methyl-2,2-γ-spirolactone chlorin (**96**).

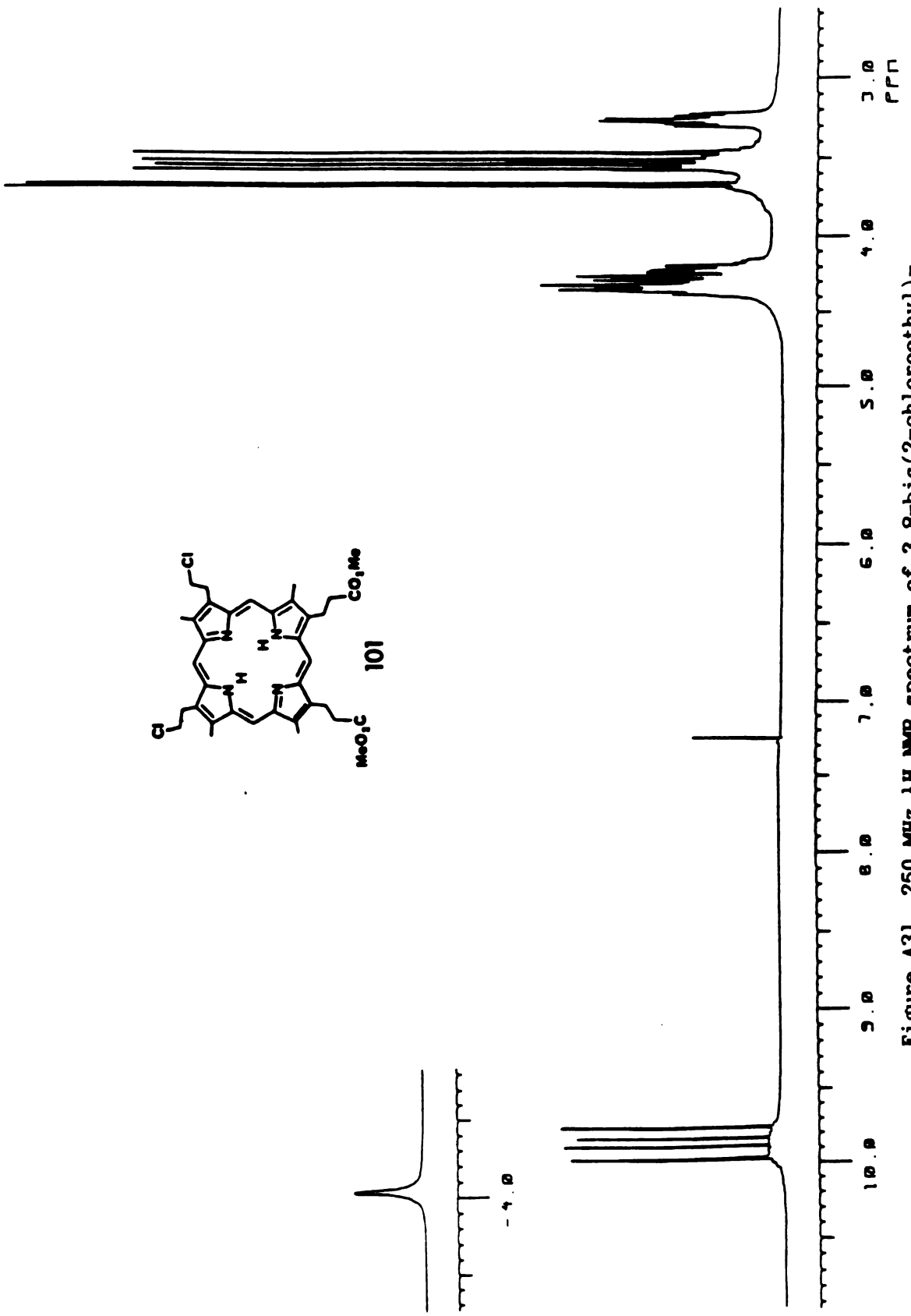


Figure A31 250 MHz ^{1}H NMR spectrum of 3,8-bis(2-chloroethyl)-deuteroporphyrin IX dimethyl ester (101).

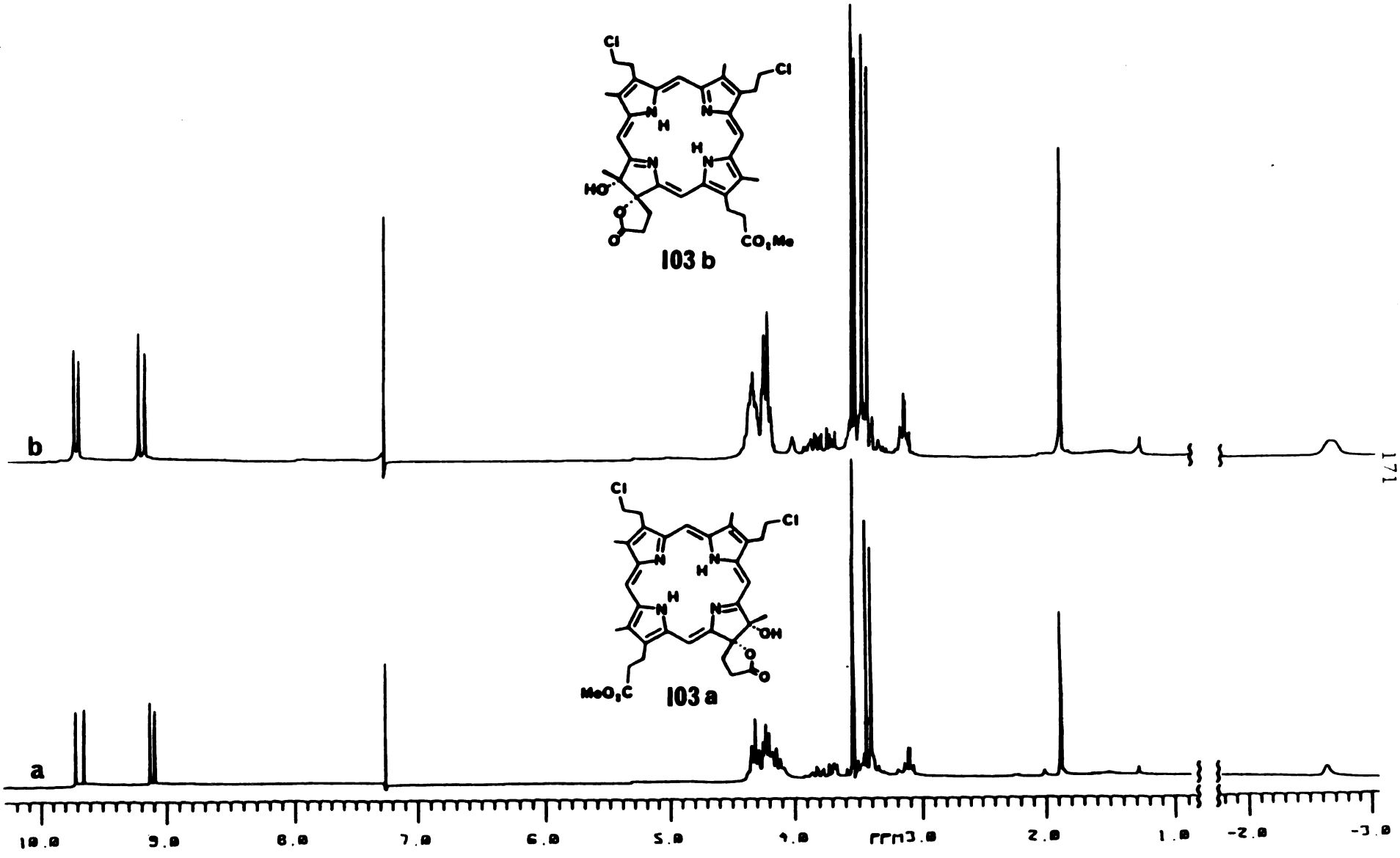


Figure A32 250 MHz ^{1}H NMR spectra of (a) cis-3,8-bis(2-chloroethyl)-12-hydroxy-13,13-γ-spirolactone-deuterochlorin IX dimethyl ester (**103a**); (b) cis-3,8-bis(2-chloroethyl)-18-hydroxy-17,17-γ-spirolactone-deuterochlorin IX dimethyl ester (**103b**).

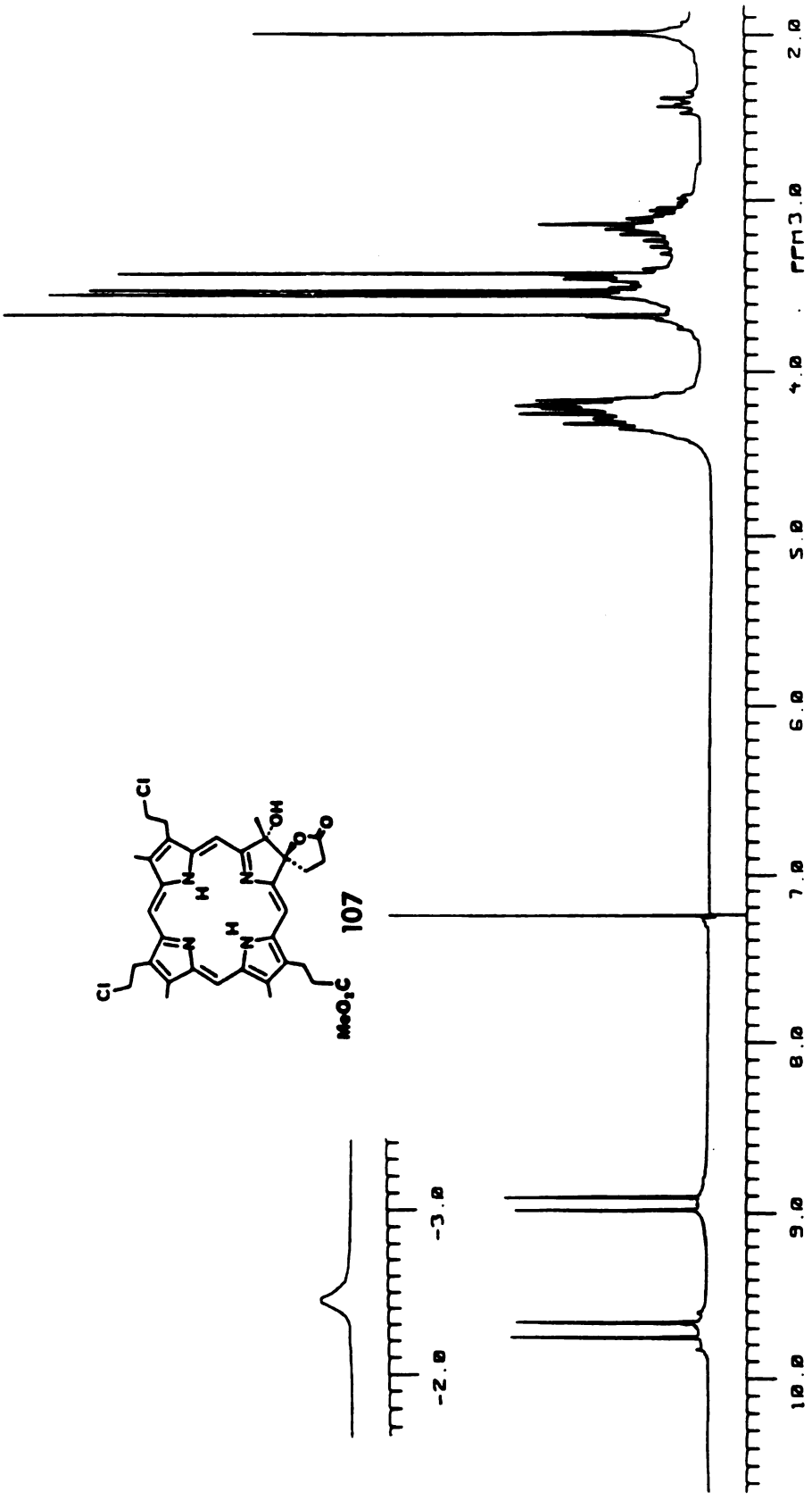


Figure A33 250 MHz ^{1}H NMR spectrum of trans-3,8-bis(2-chloroethyl)-12-hydroxy-13,13-γ-spirolactone-deuterochlorin IX dimethyl ester (107).

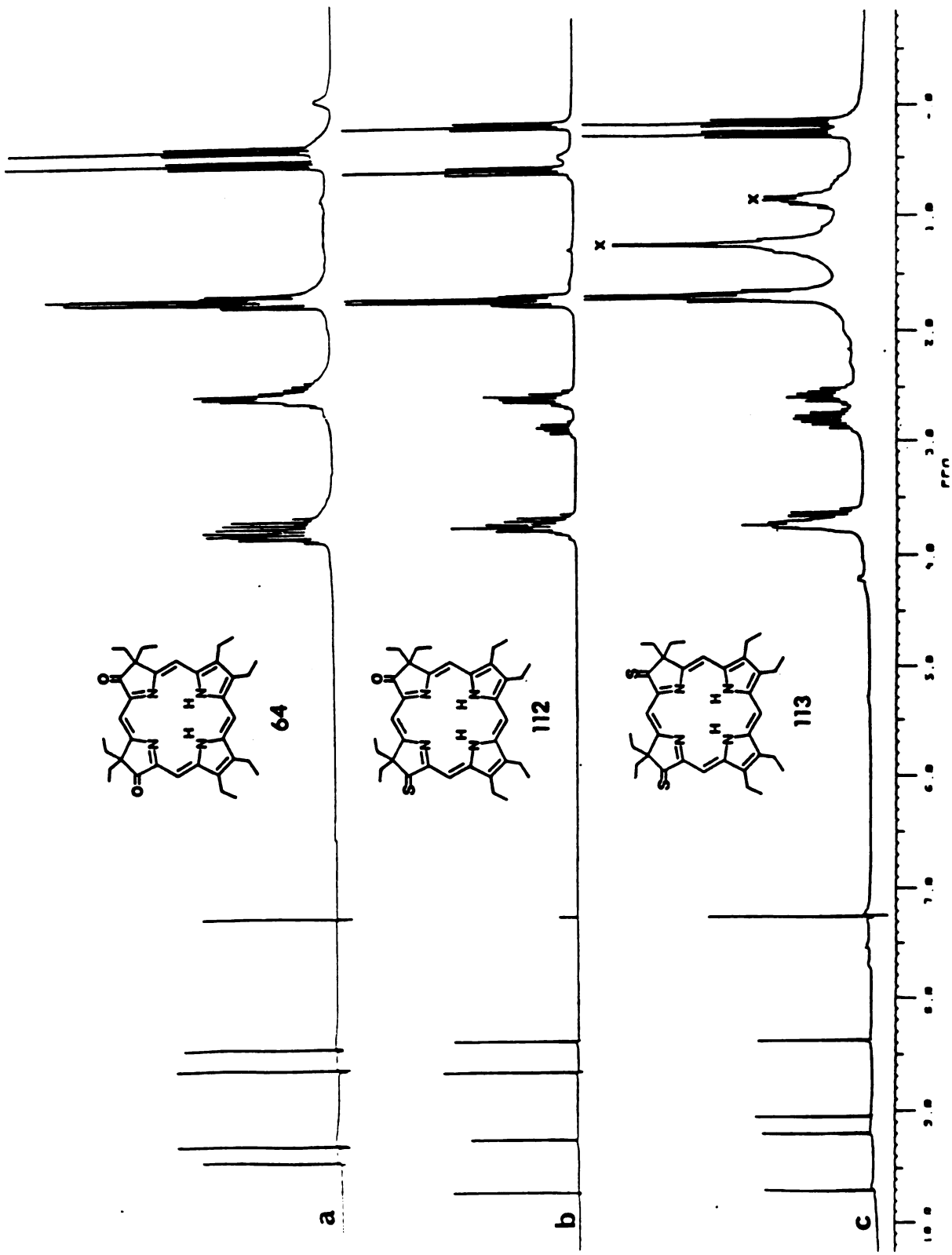


Figure A34 250 MHz ^{1}H NMR spectra of (a) 3,3,8,8,12,13,17,18-octaethyl-2,7-porphinedione (**64**); (b) 3,3,8,8,12,13,17,18-octaethyl-2-thio-7-porphinedione (**112**); (c) 3,3,8,8,12,13,17,18-octaethyl-2,7-porphinedithione (**113**).

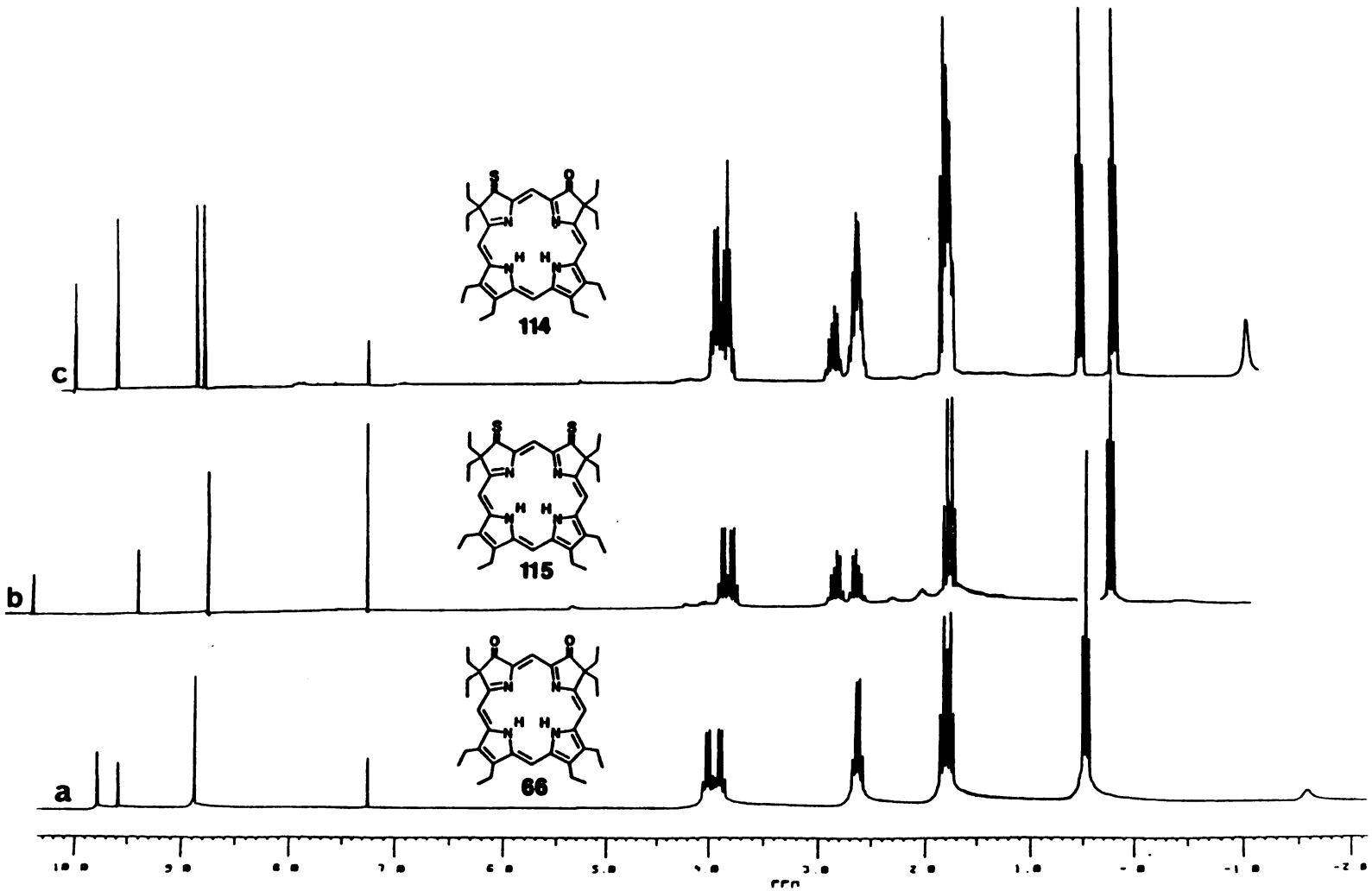


Figure A35 250 MHz ^{1}H NMR spectra of (a) 2,2,8,8,12,13,17,18-octaethyl-3,7-porphinedione (**66**); (b) 2,2,8,8,12,13,17,18-octaethyl-3,7-porphinedithione (**115**); (c) 2,2,8,8,12,13,17,18-octaethyl-3-thio-7-porphinedione (**114**).

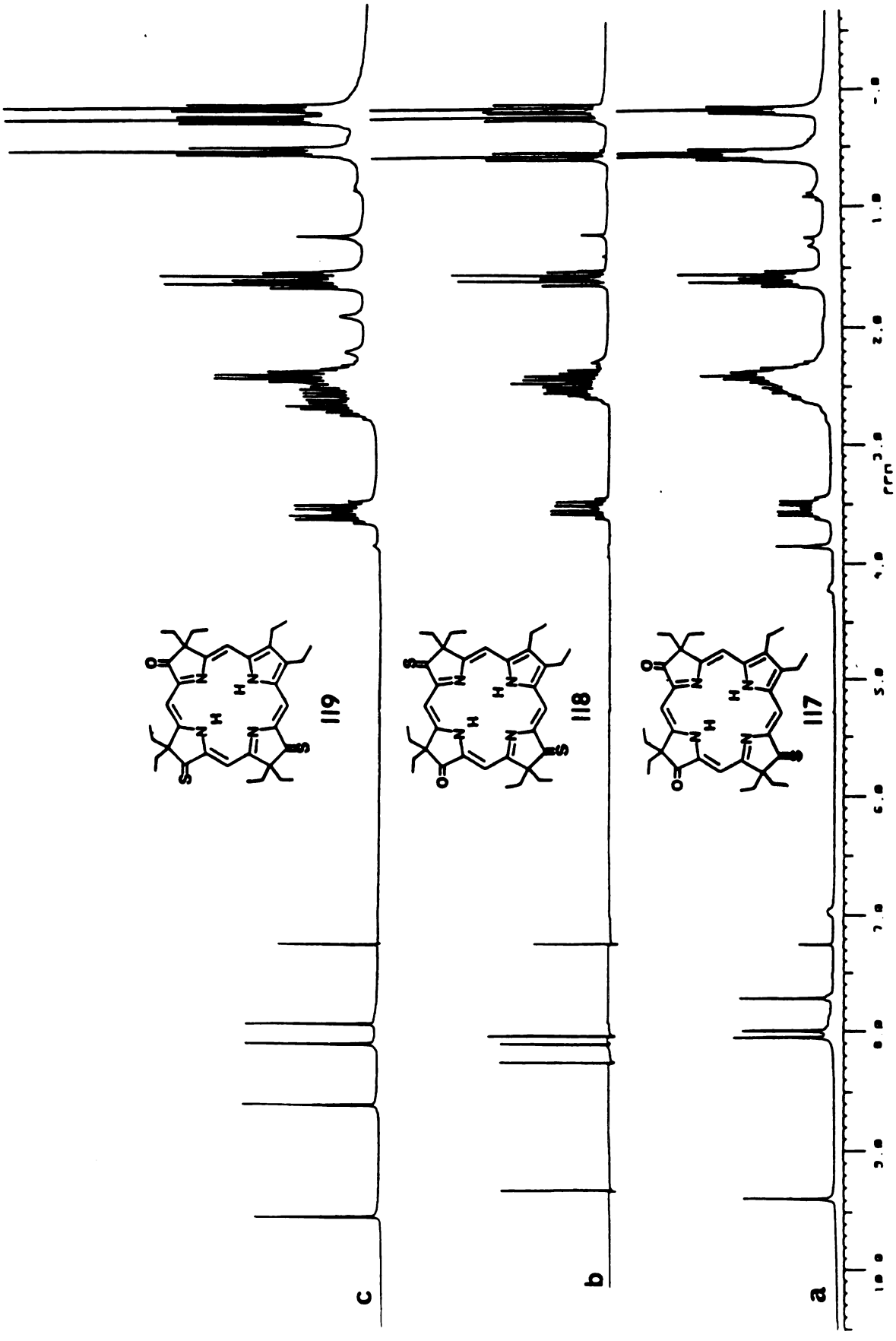


Figure A36 250 MHz ^{1}H NMR spectra of (a) 3,3,8,8,13,13,17,18-octaethyl-2-thio-7,12-porphinetrione (117); (b) 3,3,8,8,13,13,17,18-octaethyl-2,12-dithio-7-porphinetrione (118); (c) 3,3,8,8,13,13,17,18-octaethyl-2,7,dithio-12-porphinetrione (119).

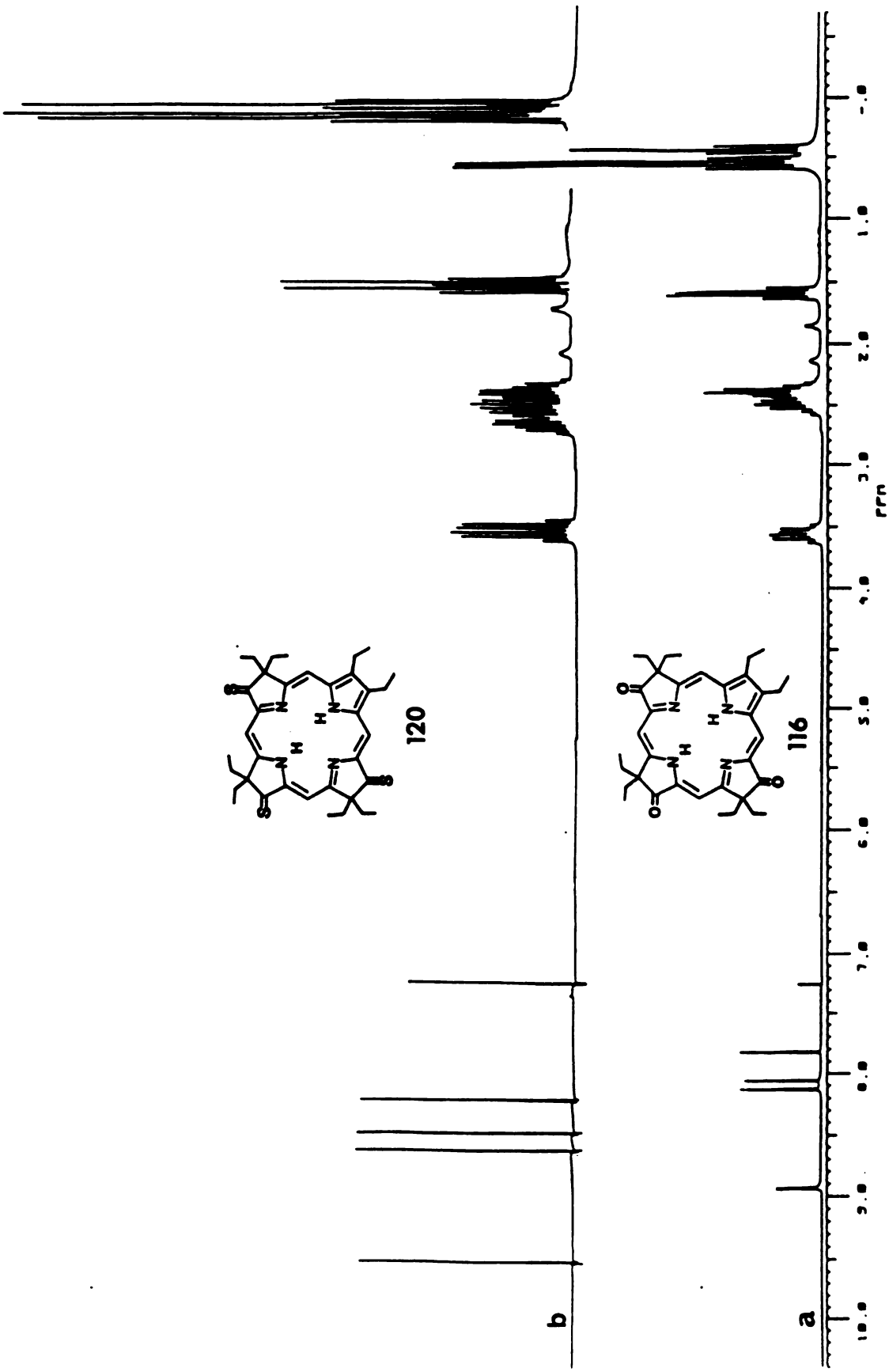


Figure A37 250 MHz ^{1}H NMR spectra of (a) 3,3,8,8,13,13,17,18-octaethyl-2,7,12-porphinetrione (116); (b) 3,3,8,8,13,13,17,18-octaethyl-2,7,12-porphinetrithione (120).

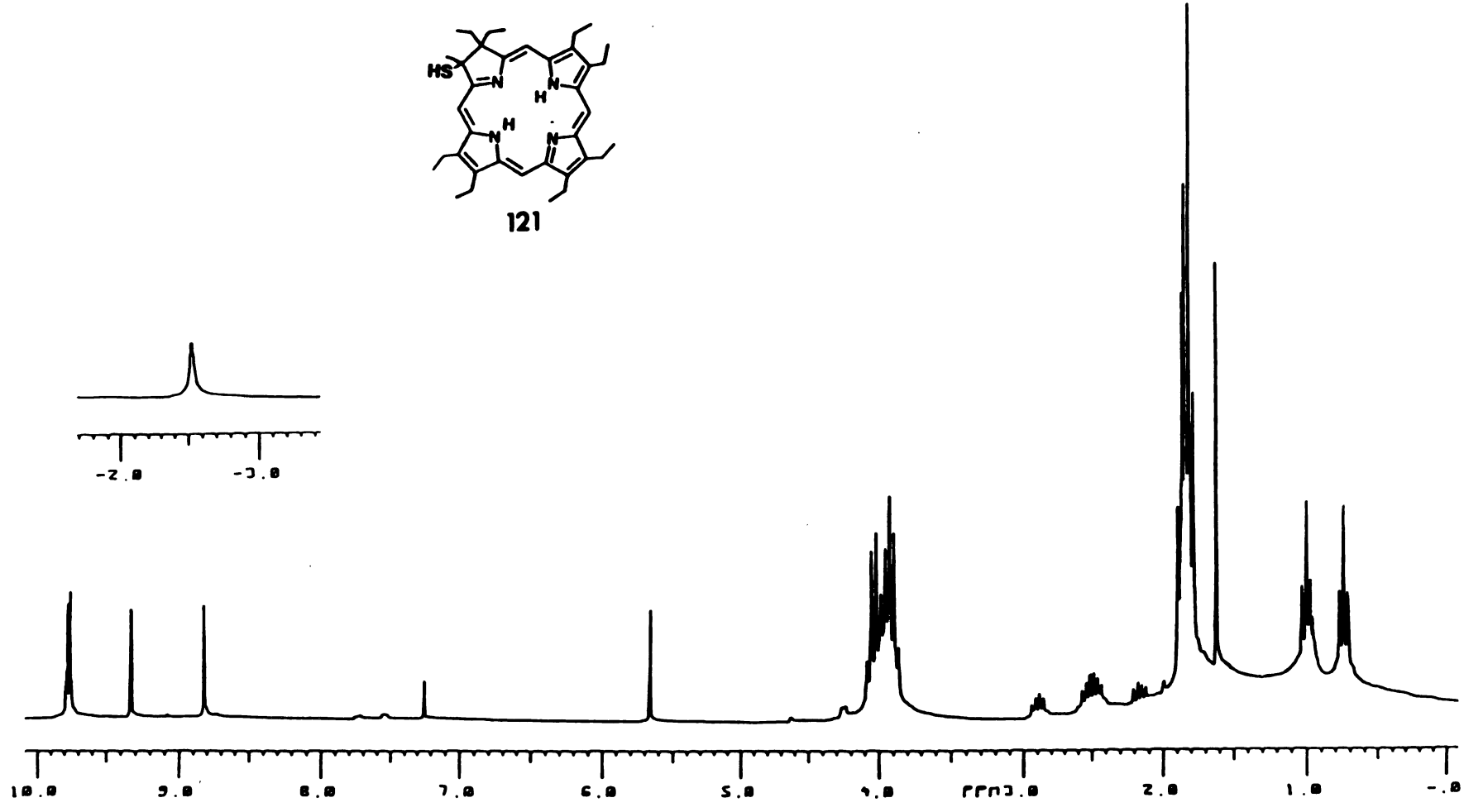


Figure A38 250 MHz ^{1}H NMR spectrum of 3,3,7,8,12,13,17,18-octaethyl-2-methyl-2-mercaptochlorin (121).

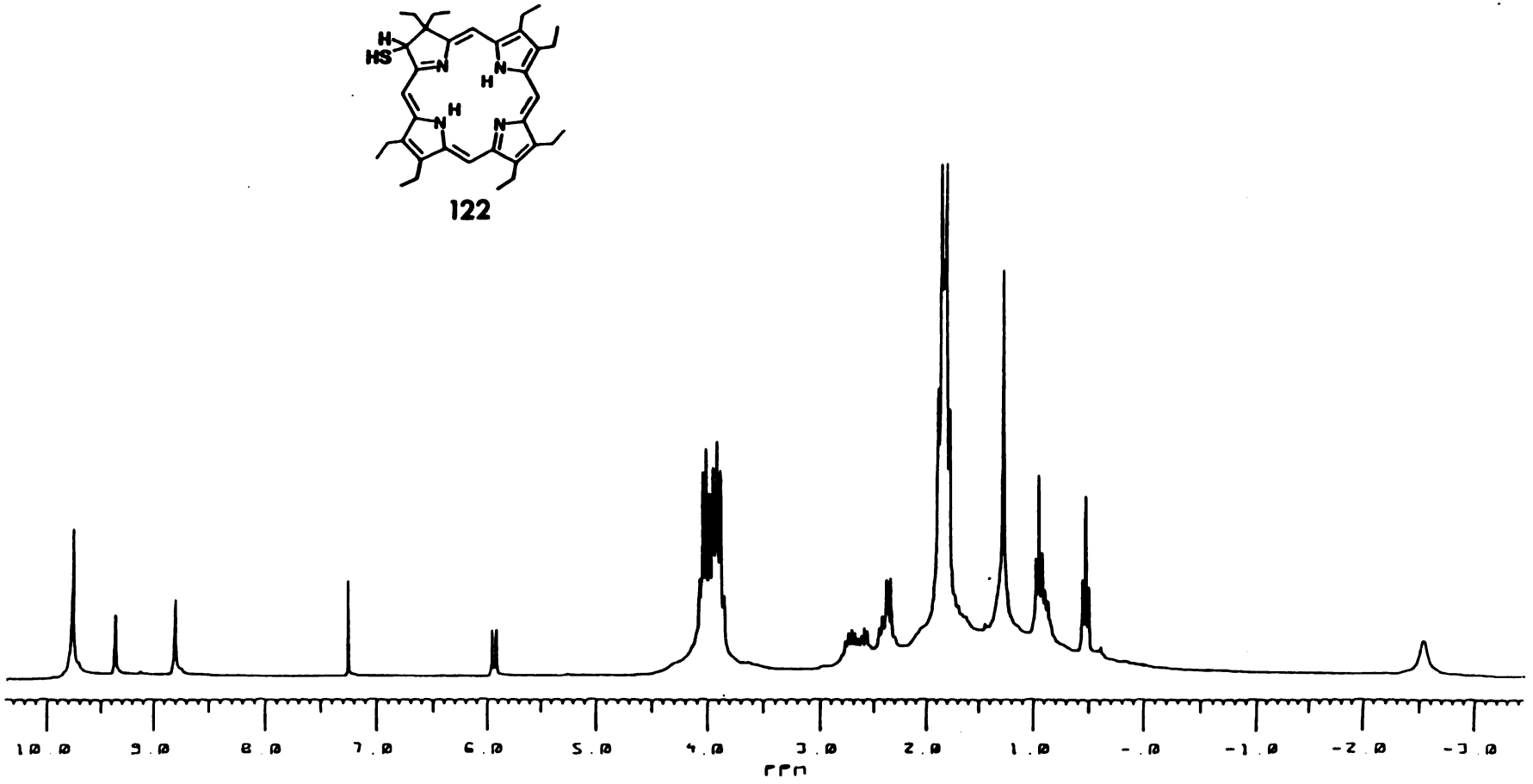
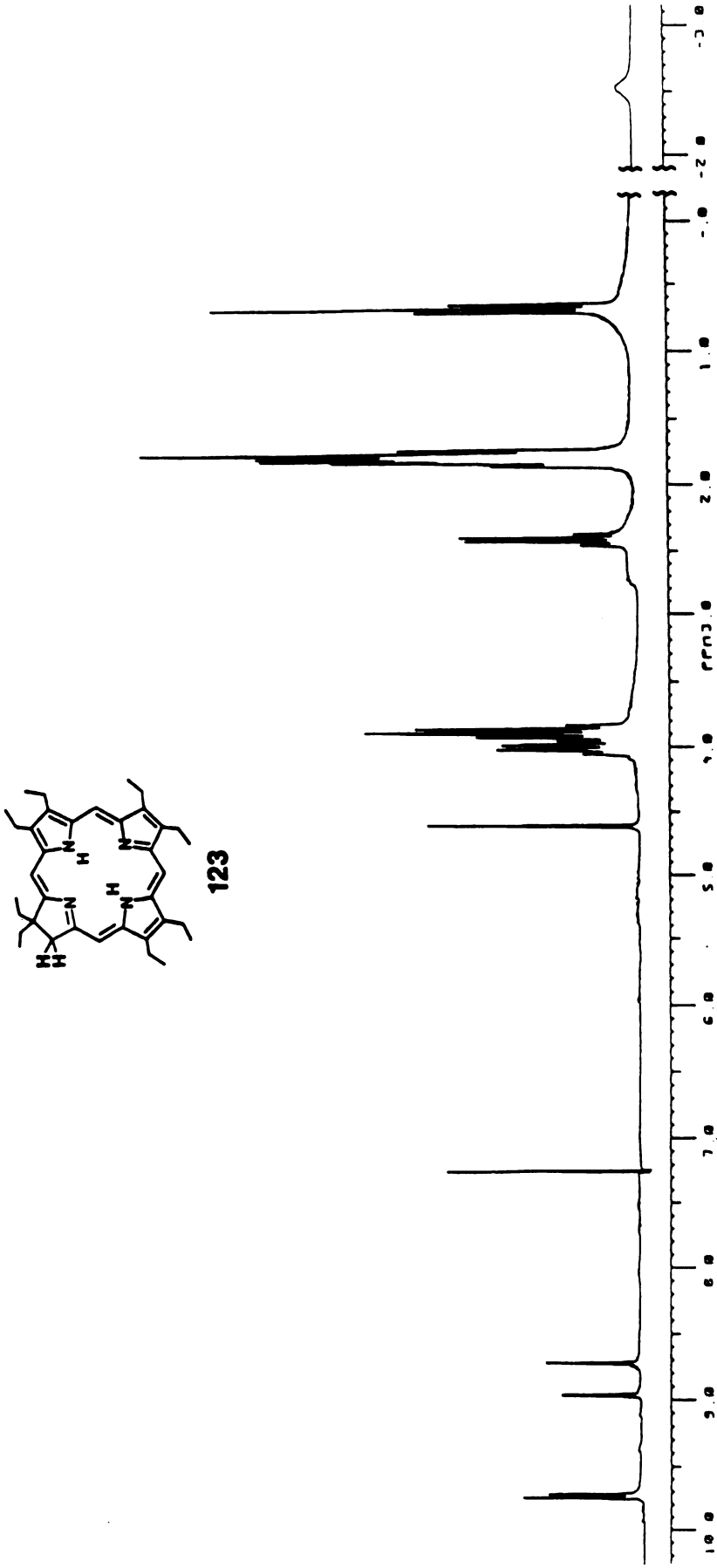


Figure A39 250 MHz ^{1}H NMR spectrum of 3,3,7,8,12,13,17,18-octaethyl-2-mercaptochlorin (122).



123

Figure A40 250 MHz ^{1}H NMR spectrum of 2,2,7,8,12,13,17,18-octaethylchlorin (123).

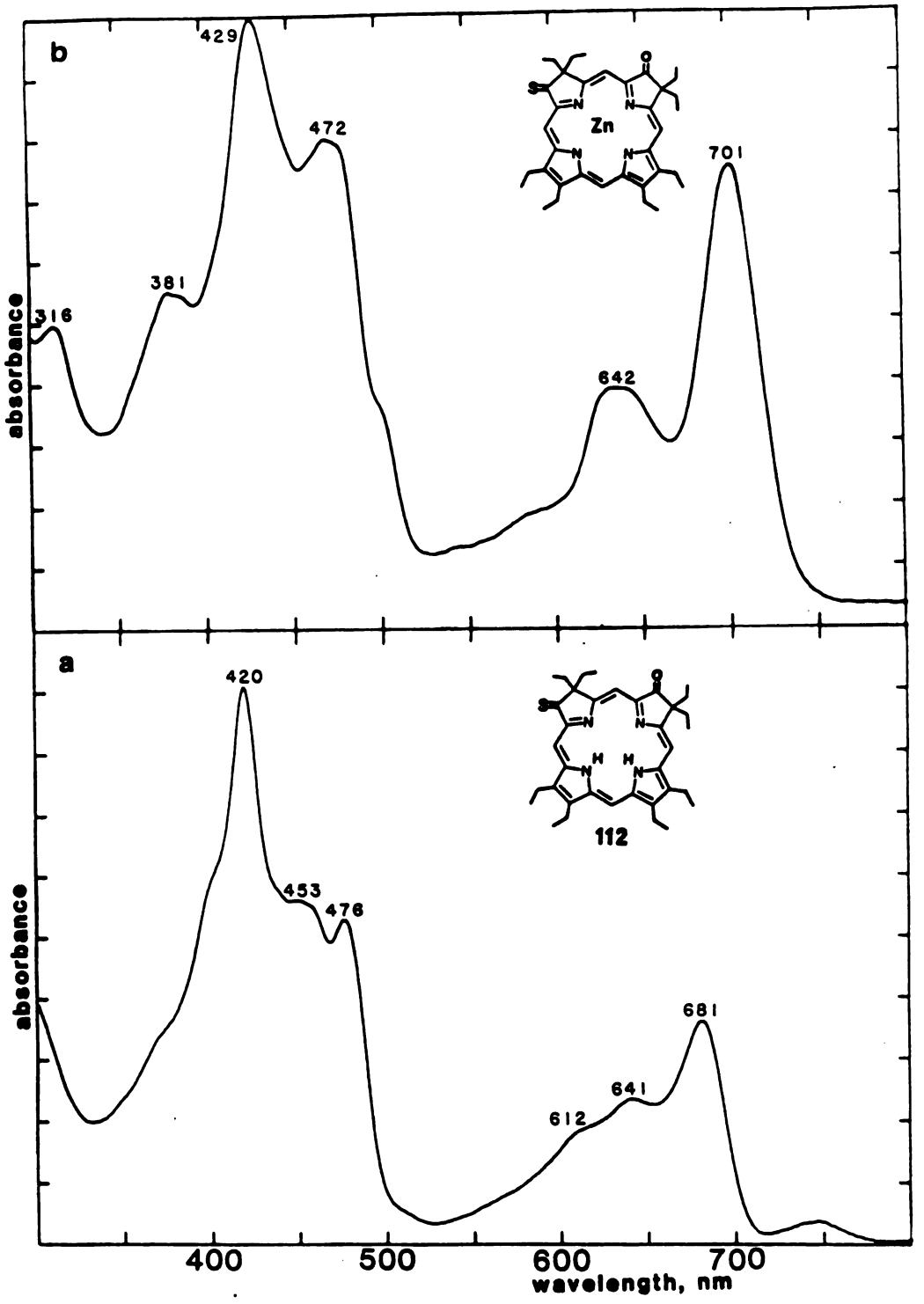


Figure A41 Visible spectra (in CH_2Cl_2) of (a) 3,3,8,8,12,13,17,18-octaethyl-2-thio-7-porphinedione (112); (b) its Zn-complex.

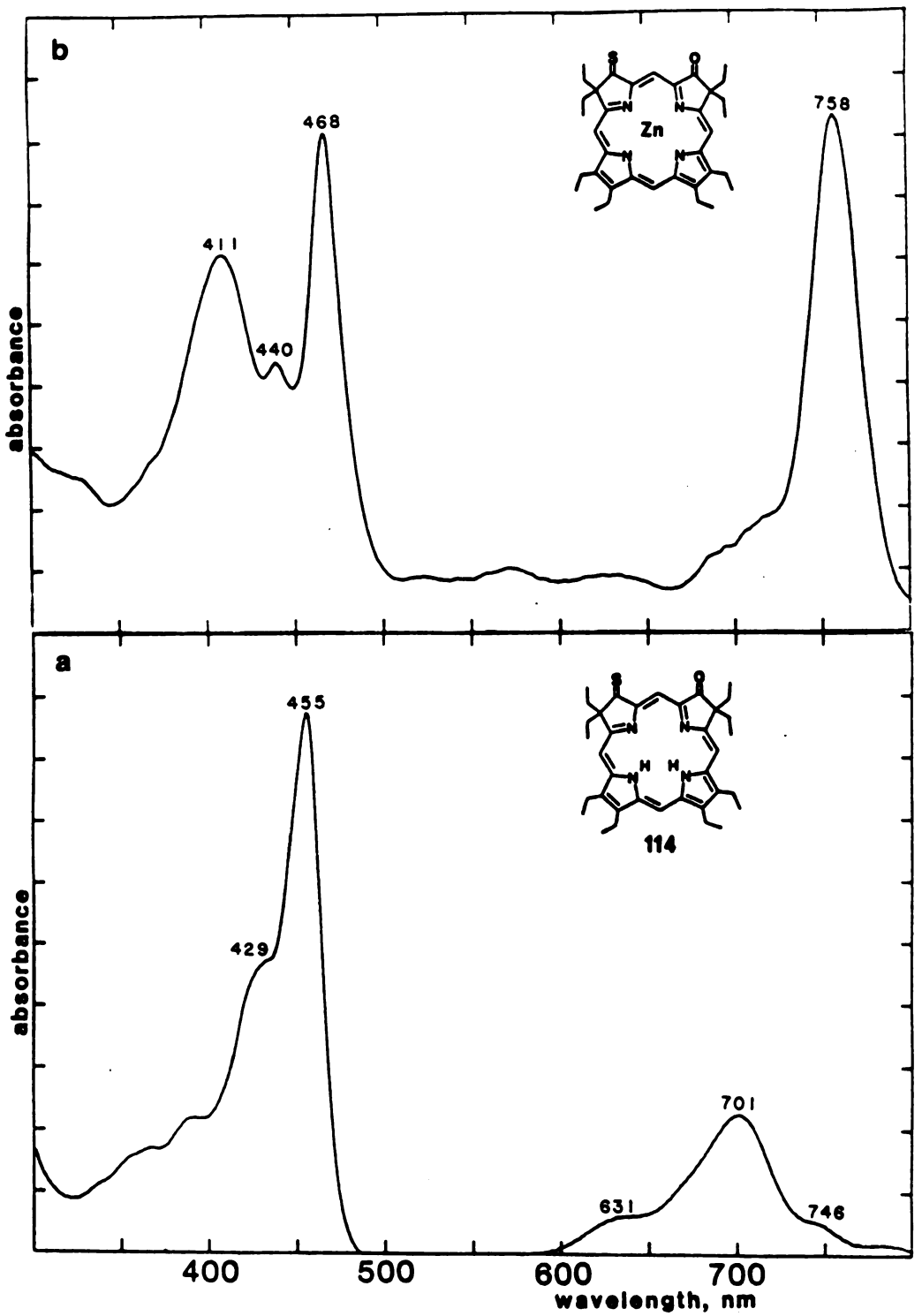


Figure A42 Visible spectra (in CH_2Cl_2) of (a) 2,2,8,8,12,13,17,18-octaethyl-3-thio-7-porphinedione (114); (b) its Zn-complex.

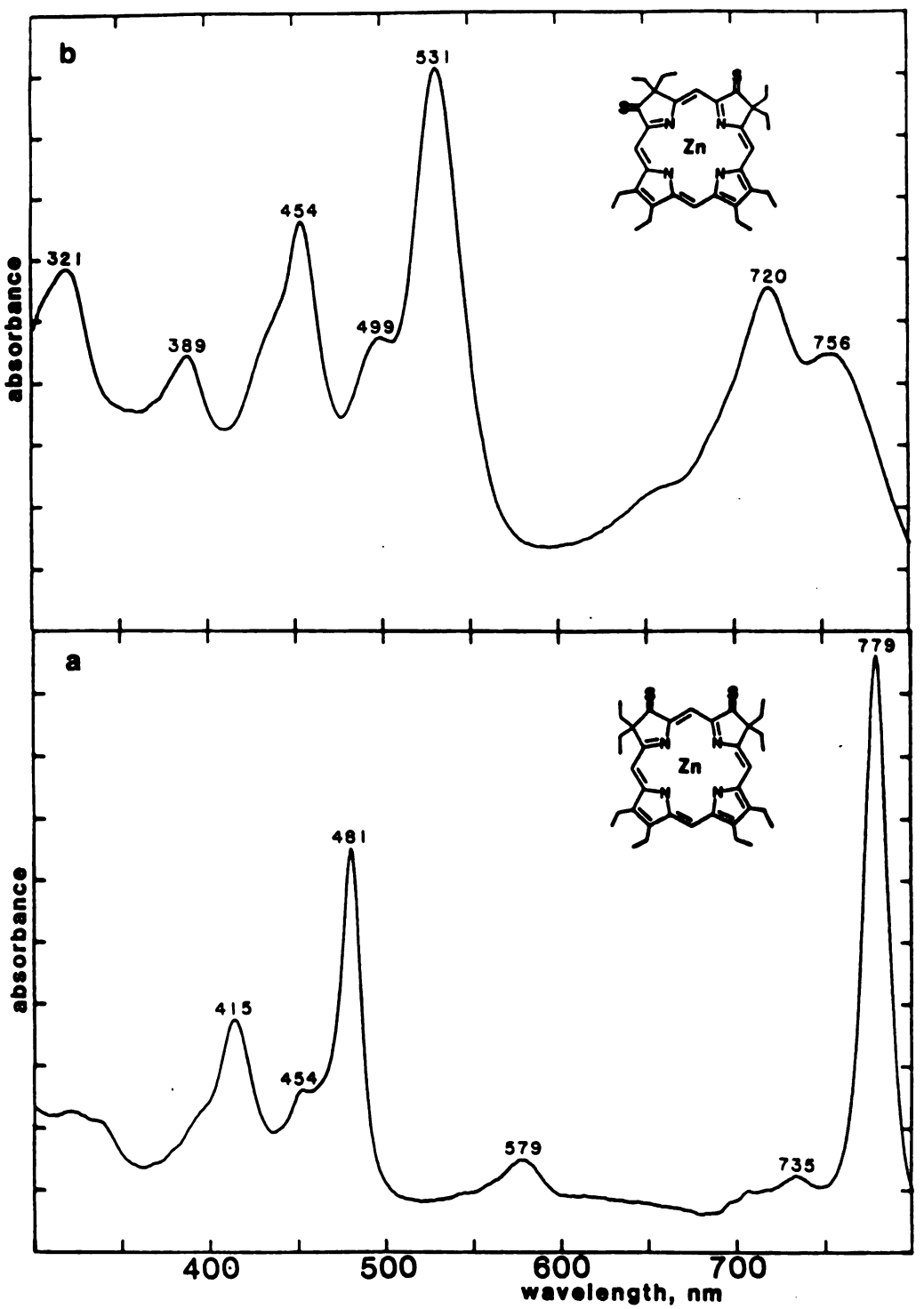


Figure A43 Visible spectra (in CH_2Cl_2) of Zn-complexes of (a) 3,3,8,8,12,13,17,18-octaethyl-2,7-porphinedithione (113); (b) 2,2,8,8,12,13,17,18-octaethyl-3,7-porphinedithione (115).

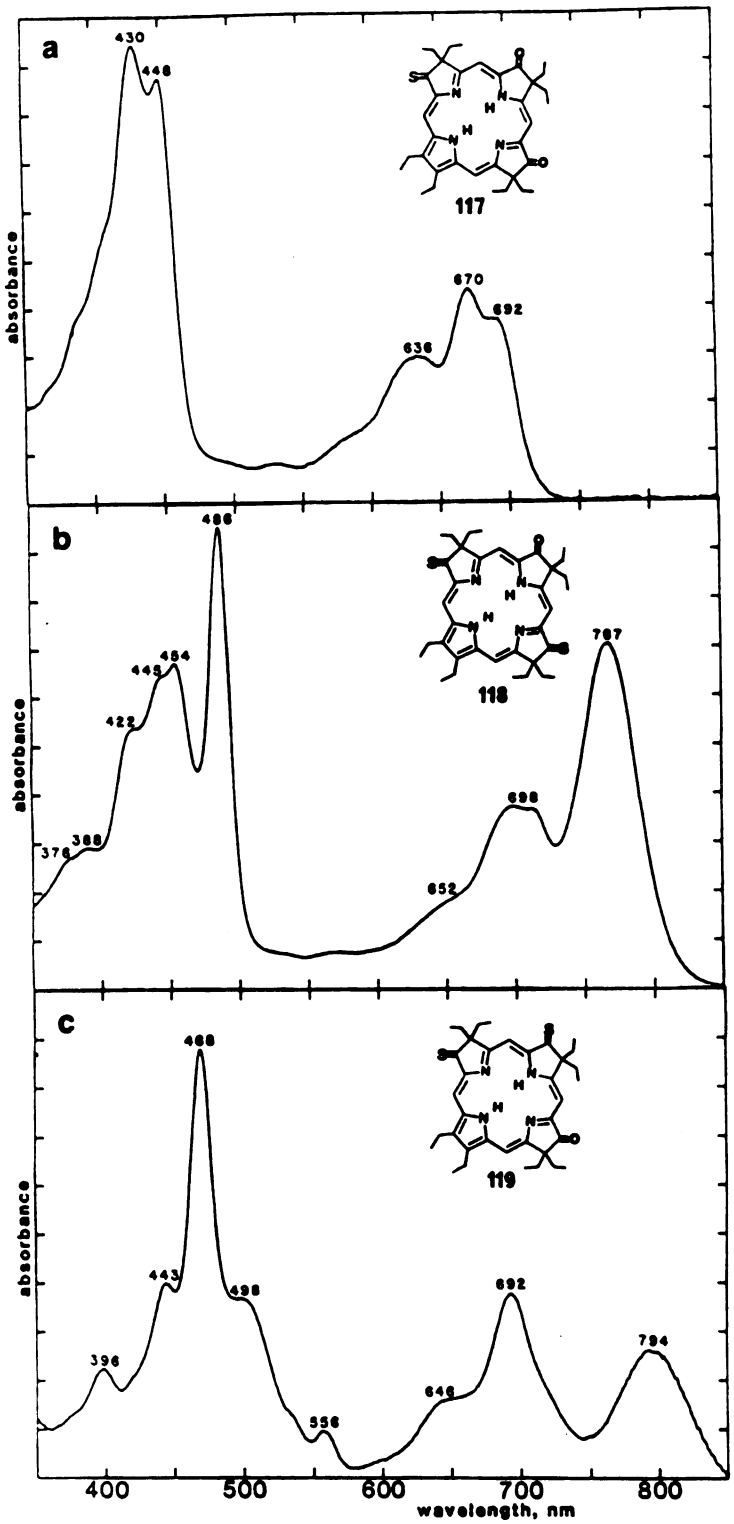


Figure A44 Visible spectra (in CH_2Cl_2) of (a) 3,3,8,8,13,13,17,18-octaethyl-2-thio-7,12-porphinetrione (117); (b) 3,3,8,8,13,13,17,18-octaethyl-2,12-dithio-7-porphinetrione (118); (c) 3,3,8,8,13,13,17,18-octaethyl-2,7-dithio-12-porphinetrione (119).

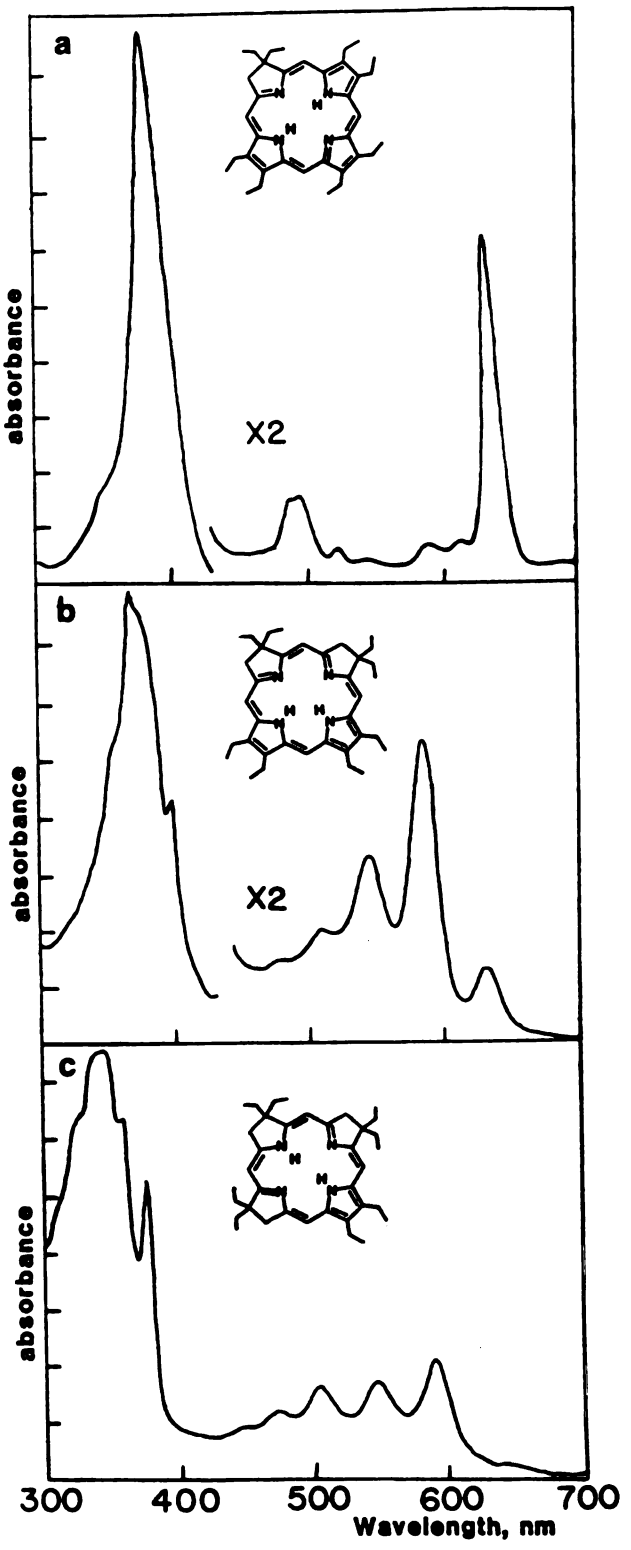


Figure A45 Visible spectra (in CH_2Cl_2) of (a) dihydro-OEP; (b) tetrahydro-OEP; (c) hexahydro-OEP (under argon).

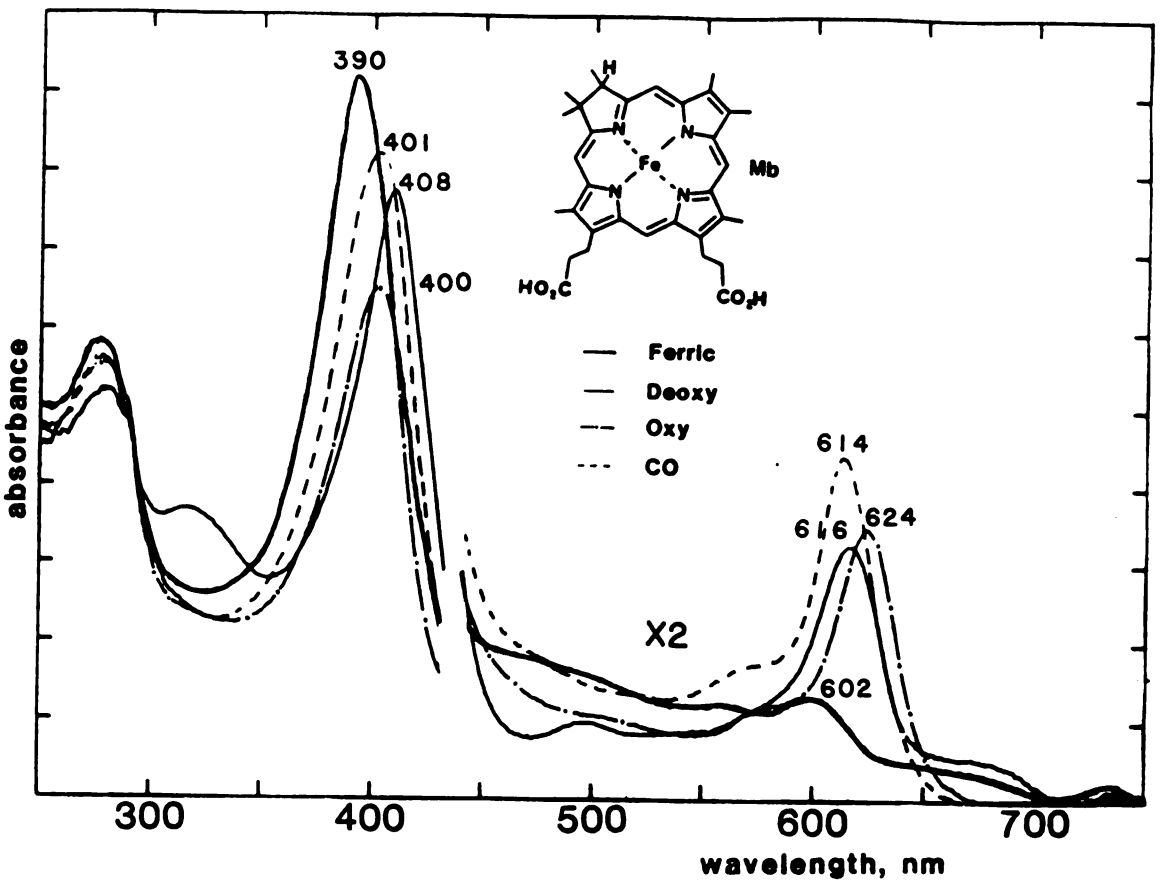


Figure A46 Optical spectra of methylchlorin-heme myoglobin; Ferric (——), Deoxy (——), Oxy (——·——), CO (----) in 10 mM (pH 7.4) potassium posphate buffer.

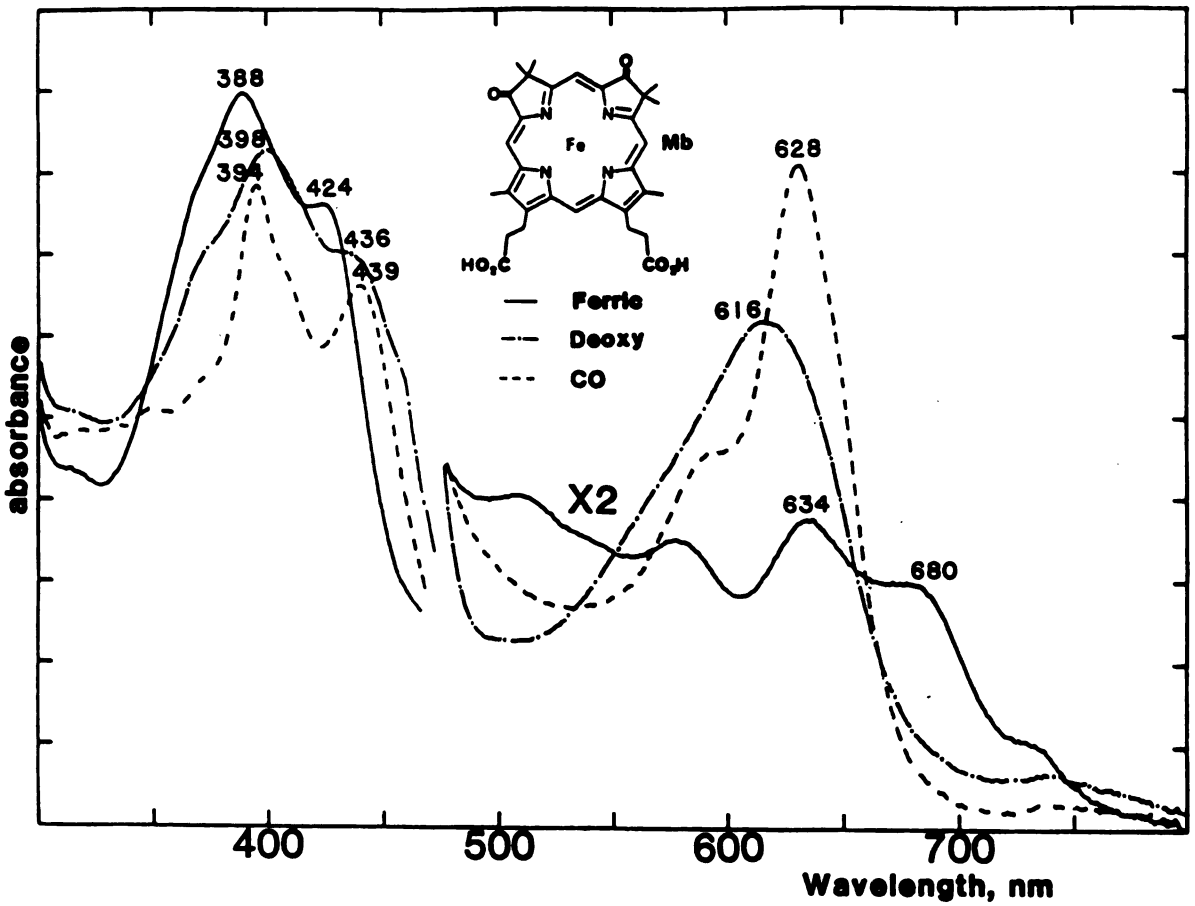


Figure A47 Optical spectra of dione-heme myoglobin; Ferric (——), Deoxy (—·—), CO (---), in 10 mM (pH 7.4) potassium posphate buffer.