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Synthesis of Carbon chain linked diporphyrins as a tumor localizing agent.

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Changwoo Park

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Chi K. Chang

Major professor

Date Aug. 5 88

SYNTHESIS OF CARBON CHAIN LINKED DIPORPHYRINS AS A TUMOR LOCALIZING AGENT

By

Changwoo Park

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ABSTRACT

SYNTHESIS OF CARBON CHAIN LINKED DIPORPHYRINS AS TUMOR LOCALIZING AGENTS.

By

Changwoo Park

Diporphyrins with six methylene carbon linkage were synthesized to elucidate the mechanism of the tumor localization by the active components of hematoporphyrin derivative (HPD). The all carbon linkage was designed to avoid cellular hydrolysis preventing the loss of localized porphyrin photosensitizer through the cell membrane as well as to decrease the ring-ring interactions between porphyrin units. The effects of solvent polarity on the structural conformations of these diporphyrins were examined by spectroscopic methods.

To my parents

ACKNOWLEMENT

First of all I would like to thank professor C.K. Chang for his patience, helpful guidance and financial support during the course of this work. Also I appreciate the chemistry department and Michigan State University for their financial support in the form of teaching assistantship.

I also express my gratitude to all our group members for their kind friendship. Especially, helpful discussions with Mr. Weish Wu is gratefully acknowledged. He was always friendly and his personality was as good as his chemistry. I also consider the friendship of Michael Tonaritis and Asaad Salehi truly valuable, as without their encouragement I might not have finished this work. My deep appreciation is extended to Mr. Jong-Shin Yoo, Younkyoo Kim and all other pKorean students in the chemistry department.

Finally, I would to thank my family for their consistant love, patience and encouragement.

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LIST OF ABBREVIATIONS

DHE.....Dihematoporphyrin Ester and Ether.

HDL.....High Density Lipoproteins.

HP.....Hematoporphyrin.

HPA.....Acetylated Hematoporphyrin.

HPD.....Hematoporphyrin Derivative

HVP.....Hydroxyethyl, vinyldeuteroporphyrin.

LDL.....Low Density Lipoproteins.

PP.....Protoporphyrin.

VHDL.....Very High Density Lipoproteins.

VLDL.....Very Low Density Lipoproteins.

INTRODUCTION

Introduction

Hematoporphyrin Derivative (HPD) : The chemical nature and clinical significance.

Photodynamic therapy (PDT) is a relatively new technique for treating neoplastic diseases in humans and animals.[1] This therapy is based on the selective localization of porphyrin photosensitizer on the tumor cell followed by light activated cytocidal effects. Illumination of porphyrin-loaded cells with visible light catalyzes the formation of singlet oxygen.[2,3] The potent oxidizing singlet oxygen interacts with cellular amino acids[4] and unsaturated fatty acids[5] leading to cross-linking to membrane proteins,[6-8] inhibition of transportation systems,[9] alteration of permeability barriers and finally loss of cell viability.[9-12] This photodynamic therapy is particularly valuable in treating lung cancer in older persons who frequently have poor pulmonary or cardiac function which precludes other therapies such as radio therapy and chemotherapy due to side effects.[13]

Since 1978, the use of porphyrin products derived from hematoporphyrin IX (Fig 1), so - called hematoporphyrin derivative, has been the most widely used porphyrin photosensitizer for PDT because of its high efficiency and

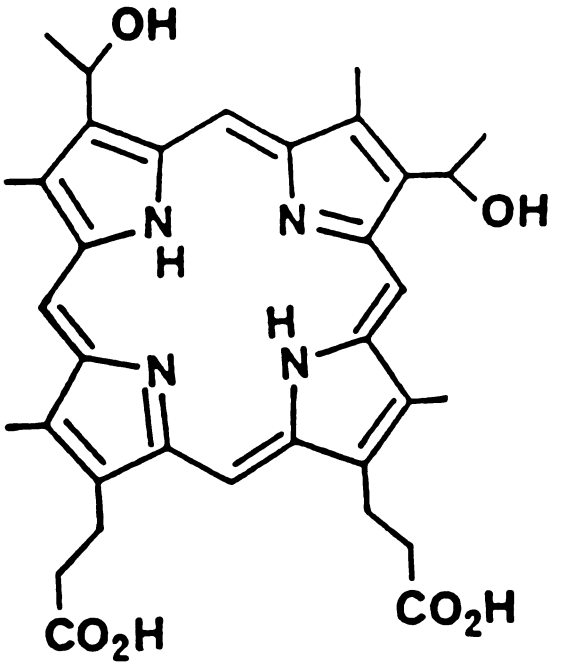

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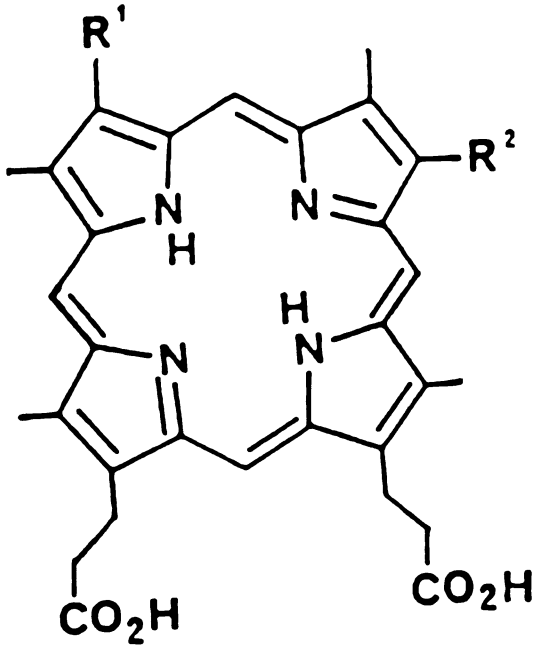
Figure 1. Structure of hematoporphyrin IX

selectivity.

"Hematoporphyrin Derivative" (HPD) was first prepared by Lipson et al. in 1960.[14] Hematoporphyrin (1) dihydrochloride was treated with 5% sulfuric acid in acetic acid to give a solid material. This was originally known as hematoporphyrin derivative. In order to avoid the solubility problem in aqueous media prior to injection to biological systems it was treated with base. It was later found that this treatment causes chemical changes in this solid material and the material prepared for injection became to be referred as real "HPD" while the original HPD was changed to HPA (acetylated hematoporphyrin). Intensive analysis of HPA and HPD have been completed in order to identify the active component in this drug.[15,16] The use of high-pressure liquid chromatography (HPLC) with a reverse-phase column and the comparison of retention time with authentic porphyrin dicarboxylic acid enable the separation and identification of the major components of HPA.

The composition of the HPA mixture is somewhat variable from one preparation to another, but the major components are O,O'-diacetyl hematoporphyrin **6**,O-acetyl hematoporphyrins **2,3**, 8(3)-(1-acetoxyethyl)-3(8)-vinyl deuteroporphyrin isomers **7,8**, and the corresponding alcohols **4,5** (Fig. 2). When the hematoporphyrin mono or diacetate dissolved in dimethyl sulfoxide was used alone no cell localizing ability was observed. But after base treatment, the acetoxy derivatives **2,3,6,7,8** all showed activity.[17]It



1 $R^1 = R^2 = CH(OH)CH_3$

2 $R^1 = CH(OH)CH_3$; $R^2 = CH(OAc)CH_3$

3 $R^1 = CH(OAc)CH_3$; $R^2 = CH(OH)CH_3$

4 $R^1 = CH = CH_2$; $R^2 = CH(OH)CH_3$

5 $R^1 = CH(OH)CH_3$; $R^2 = CH = CH_2$

6 $R^1 = R^2 = CH(OAc)CH_3$

7 $R^1 = CH = CH_2$; $R^2 = CH(OAc)CH_3$

8 $R^1 = CH(OAc)CH_3$; $R^2 = CH = CH_2$

9 $R^1 = R^2 = CH = CH_2$

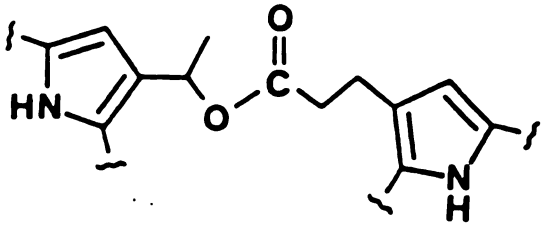
Figure 2. Components of HPA

was proposed that the base treatment changed the chemical nature of the acetate derivatives, and since the reactive hematoporphyrin acetates were clearly the precursors, active components of HPD could be covalently bonded dimer or oligomer. Recently, fast atom bombardment mass spectrometry (FAB-MS) has been utilized for the analysis of HPD and confirmed the presence of monomeric, dimeric, and up to pentameric hematoporphyrin.[18] The following three dimers linked by ester, ether, or carbon-carbon bonds were suggested (Fig. 3)[17,19]as the possible structures for the active component in HPD. There are several lines of evidence for the presence of either ether linkage or ester linkage. The presence of ether linked dimer is based on the fact that the ether bond is moderately stable toward base hydrolysis while the esteric dimer is not.[20] However the formation of esteric dimer is more likely to occur judging from a mechanistic point of view.[21] Recent studies involving use of the reducing agent, lithium aluminum hydride ($LiAlH_4$) for the reduction of the active component of HPD followed by HPLC separation and FAB - MS indicate the presence of ester linked dimer in the freshly prepared dimer.[22]

On standing at room temperature, the ester-linked porphyrins appear to undergo an ester to ether conversion. The formation of the more stable ether linked dimer possibly is caused from the reaction between unhydrolyzed acetate groups and free sec-OH groups in HPD.[22] Either ester or ether linked dimers seem to be effective in tumor

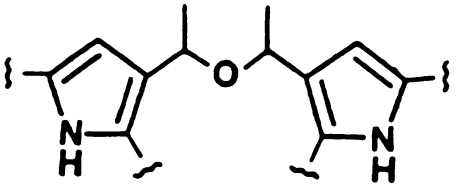
1. ESTER

Hydrolyzed fairly readily. Elimination possible.
Dimeric structure possible with four such bonds.



2. ETHER

More difficult to Hydrolyze. Elimination possible (to give vinyl and hydroxyethyl groups).



3. CARBON-CARBON BOND

Derived from electrophilic substitution of benzylic -type carbonium ion at meso position.
Likely to be quite stable.

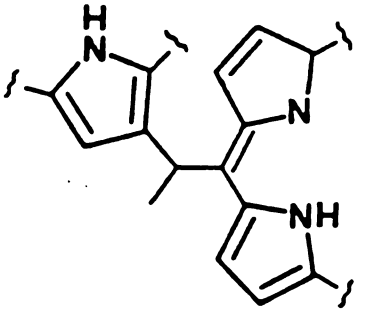


Figure 3. The possible types of linkages based on the condensation of hematoporphyrin acetates.

localization and the nature of the linkage between porphyrin units therefore is not a major determinant of the tumor localizing phenomenon.

Porphyrin Transport and Cellular Retention Mechanism.

The transport of porphyrins to tumor cells is very critical to the localization of porphyrins and subsequent photodynamic activity.[23] Binding of the various components of HPD to lipoproteins and serum proteins has been documented.[24] The affinity of several monoporphyrins with serum albumin has been measured through binding kinetic studies indicating that a major portion of bound porphyrin is delivered by low density lipoprotein (LDL).[25,26] The behavior of very low density lipoprotein (VLDL) associated with porphyrin uptake was less clear probably because VLDL is metabolically converted into other lipoprotein including LDL.

The differences in the behavior of porphyrin-high density lipoprotein (HDL),-LDL and -VLDL complexes can be explained on the basis of the two main modalities of lipoprotein internalization by cells. (i) non-specific fluid endocytosis. (ii) receptor-mediated endocytosis. The latter mechanism concerns LDL and becomes especially important for cells displaying hyperproliferative activity where the number of LDL-receptors on the cell surface drastically increase. Therefore the preferential accumulation and

retention of porphyrins by tumor cells does not seem to reflect an intrinsic property of the dye; rather it is a consequence of cell-interaction mechanism typical of the LDL. LDL has been proposed as a specific carrier of cytostatic drugs to tumors.[27]

Localization of porphyrin oligomers in tissue is well documented through numerous investigations. But the exact mechanism of porphyrin uptake and retention is still controversial. One possible mechanism of this accumulation is that the porphyrin oligomers enter the cell and undergo changes from a form which freely diffuses across the cell membrane to one which does not diffuse.[28,29] The change is probably due to the aggregative property of porphyrin species depending on pH and polarity of environment. However, other components of HPD, e.g. protoporphyrin (PP), hydroxyethyl vinyl deuteroporphyrin (HVP) also form aggregates under these conditions, but fail to localize in neoplastic tissues in vivo. The ability to aggregate in an aqueous environment is therefore not a sufficient condition to insure tumor localization. Dailey and Smith studied the interaction of some of the porphyrins present in HPD with ferrochelatase and suggested further modification of retained porphyrins in the tumor cell.[30]

Purpose of this work

While the mechanism of cellular retention of HPD is not yet clear, the major component of HPD retained in tumor cell during the PRT is now almost certain to be dihematoporphyrin derivatives linked by either ether or ester bonds (DHE). It is interesting to note that the nature of the linkage between porphyrin units is not a major factor of the localization phenomenon.

Space filling models of the DHE suggest that they can be folded over to form an internally H-bonded "clam shell" structure[31] and this self-association has been supported by absorption and fluorescence emission spectral studies.[32] Conformational changes of diporphyrin including this self association may offer a possible explanation for the mechanism of cellular retention of porphyrin, and for this reason it would be meaningful to synthesize a diporphyrin which has two porphyrin units connected by a long carbon chain and exhibits no apparent intramolecular interactions.

The carbon chain linked compounds may have another benefit that the previous DHE does not have. DHE may be hydrolyzed during application to give monomeric porphyrin that cannot be retained in the cell. In contrast, the diporphyrins with carbon linkage can not be hydrolyzed in the cell and could be retained more effectively.

With this aim the diporphyrin **34**, a dihematoporphyrin analogue, and diporphyrin **33** linked by six methylene carbons were synthesized. The comparison between the polar hydroxy and non-polar methyl substituents on the diporphyrin will

show the effect of polarity of porphyrin units on the cellular localization.

RESULTS AND DISCUSSION

Result And Discussion

The dimeric porphyrins were prepared from the corresponding bisdipyrromethenes **24,25** and 5,5′-dibromopyrromethene **26**. The synthesis of porphyrins is shown in Figure 4-8. Adipyl chloride **12** was used to connect two 2-ethoxycarbonylmethyl-3,5-dimethyl pyrrole **11** to form bispyrrole compound **13** with six carbon chain. The two carbonyl groups were reduced by employing diborane generated from etheral boron trifluoride and sodium borohydride giving six methylene bridge. Successful results were obtained by using excess diborane.[33] Incomplete drying of bispyrrole with two carbonyl groups often produced white precipitates with poor solubility in tetrahydrofuran (THF) solvent. NMR and mass spectra of this precipitate indicate the presence of incompletely reduced mono carbonyl compound. This compound may be further reduced in a large amount of THF with diborane.

The ethoxycarbonyl groups on the 4,4′-hexamethylenebis(2-ethoxycarbonyl-3,5-dimethylpyrrole) **14** was trans - esterified with benzyl alcohol catalyzed by sodium benzyloxide prepared from benzyl alcohol and sodium. The benzyl ester **15** was then hydrogenolyzed with 10% palladium on carbon catalyst to obtain the corresponding diacid **16** quantitatively. Diborane was also used to convert

benzyl- 4 - ethoxycarbonylmethyl - 3,5 - dimethylpyrrole - 2-carboxylate **17** into benzyl 4-(2-hydroxyethyl)-3,5-dimethylpyrrol-2-carboxylate **18**. Replacement of the hydroxy group with chlorine was carried out with thionyl chloride in benzene at reflux.[33] The addition of thionyl chloride was performed in room temperature and the solution was refluxed under argon to prevent air oxidation.

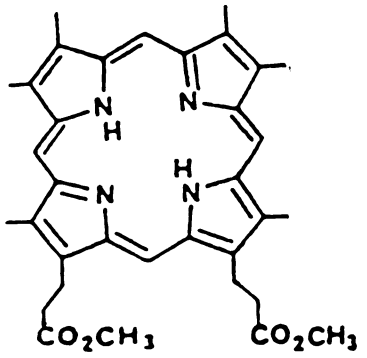
The benzyl ester group of benzyl 4-(2-chloroethyl)-3,5-dimethylpyrrole-2-carboxylate **19** was removed by hydrogenolysis accomplished under hydrogen atmosphere with 10% palladium charcoal mixture. Although the reduction reaction was very sluggish and sensitive to impurity, the reaction could be finished quantitatively. The acid **20** was converted into the formyl pyrrole **21** by decarboxylation in trifluoroacetic acid solution followed by formylation of the resultant α-free pyrrole using triethyl orthoformate.

Unlike the formylation of the chloroethyl pyrrole **21**, the procedure of Clezy et al. was directly used to convert the t-butyl pyrrole-2-carboxylate **22** into 2-formyl-3,4,5-methyl pyrrole **23**.[34] Trifluoacetic acid hydrolyzes the t-butyl ester and decarboxylates the resultant acid and then the α-free pyrrole is formylated by triethyl orthoformate. The formylated 3,4,5-trimethyl pyrrole **23** and chloroethyl pyrrole **21** were used for the coupling reaction with dipyrrole dicarboxylic acid **16** to form the corresponding bisdipyrromethenes **24,25** respectfully.

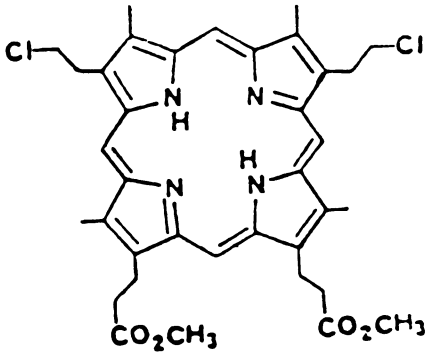
The formation of bisdipyrromethenes was performed in strong acidic condition using hydrobromic acid in methanol solvent where decarboxylation occurred followed by nucleophilic addition of the α-free pyrrole to the 2-formyl pyrroles producing bisdipyrromethenes. The coupling took place with evolution of carbon dioxide bubbles.

Dibenzyl 3,3′-bis-(2-methoxycarbonylethyl)-4,4′-dimethylpyrromethane-5,5′-dicarboxylate was converted into the 5,5′-dicarboxylic acid through hydrogenolysis and the resultant diacid was brominated to the 5,5′-dibromopyrromethene **26**. The dibromopyrromethene was used for the southern part of dimeric porphyrins. 5,5′-dibromopyrromethene was condensed with bisdipyrromethenes **24,25** in anhydrous formic acid in the presence of one equivalent of bromine to give the corresponding dimeric porphyrins **27,28**.[35]

During the separation of each of these porphyrin dimers on thin-layer chromatography (TLC), a fast moving reddish band was observed. NMR and mass spectral analysis of the fractions indicate the presence of monomeric dimethyl-3,7,8,12,13,17-hexamethyl-21H,23H-porphine-2,18-propionate. **29** and dimethyl-7,13-(2-chloroethyl)-3,8,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionate **30**. These fractions could be produced through the disconnection of the methylene carbon bridge.



29



30

The 2'-chloroethylporphyrin dimer **28** was readily converted into the corresponding vinylporphyrin dimer **31** by treatment with sodium hydroxide in refluxing aqueous pyridine as reported by Clezy.[36] Since the dehydrochlorination was carried out in very basic condition, hydrolysis of propionic methyl ester group occurred prior to the elimination reaction. This resultant tetraacid has a very poor solubility in pyridine and was immediately precipitated from the solvent. The use of more water than in normal dehydrochlorination procedure was necessary to accomplish the reaction in good yield. The tetrapropionic acid groups of vinylporphyrin dimer was changed back to the tetra propionic methyl ester by using diazomethane so that separation of the dimer could be performed easily. Diazomethane was produced from p-toluenesulfonylmethyl-nitrosoamide (diazald).

Treatment of vinylporphyrin dimethyl ester dimer **31** with saturated hydrogen bromide - acetic acid produced an

HBr adduct in the manner consistent with Markovnikov's rule. The adduct was then immediately hydrolyzed with water and neutralized with aqueous sodium hydroxide to give the desired 7-(1-hydroxyethyl)porphyrin dimer **34**. The resultant propionic acid groups which were formed on this hydrolysis process were methylated again with diazomethane to make facile separation.

Since the dimeric porphyrins will be used for pharmaceutical tests, the acid form is needed for the solubility reason. While the final hydrolysis of the propionic methyl ester group of hexamethylenebis(dimethyl pentamethyl porphyrin dipropionate) **27** was performed quantitatively in a mixture of formic acid and hydrochloric acid, the same condition could not be used for the hydrolysis of methyl propionate groups on the 7-(1-hydroxyethyl) substituted diporphyrin **32**, since the hydroxyl groups on the porphyrin might react with formic acid in the presence of acid catalyst. Basic hydrolysis in THF with aqueous sodium hydroxide solution was applied to convert methyl ester groups to the corresponding acids.

The effects of solvent polarity on the conformations of the dimers were examined by ^{1}H-NMR and absorption spectra. The space filling models of ether or ester linked HPD indicated the possibility of intramolecular ring-ring aggregation via internal hydrogen bonds,[31,37] and the effects of this aggregation on ^{1}H-NMR and absorption spectra have been documented with model studies of ester linked

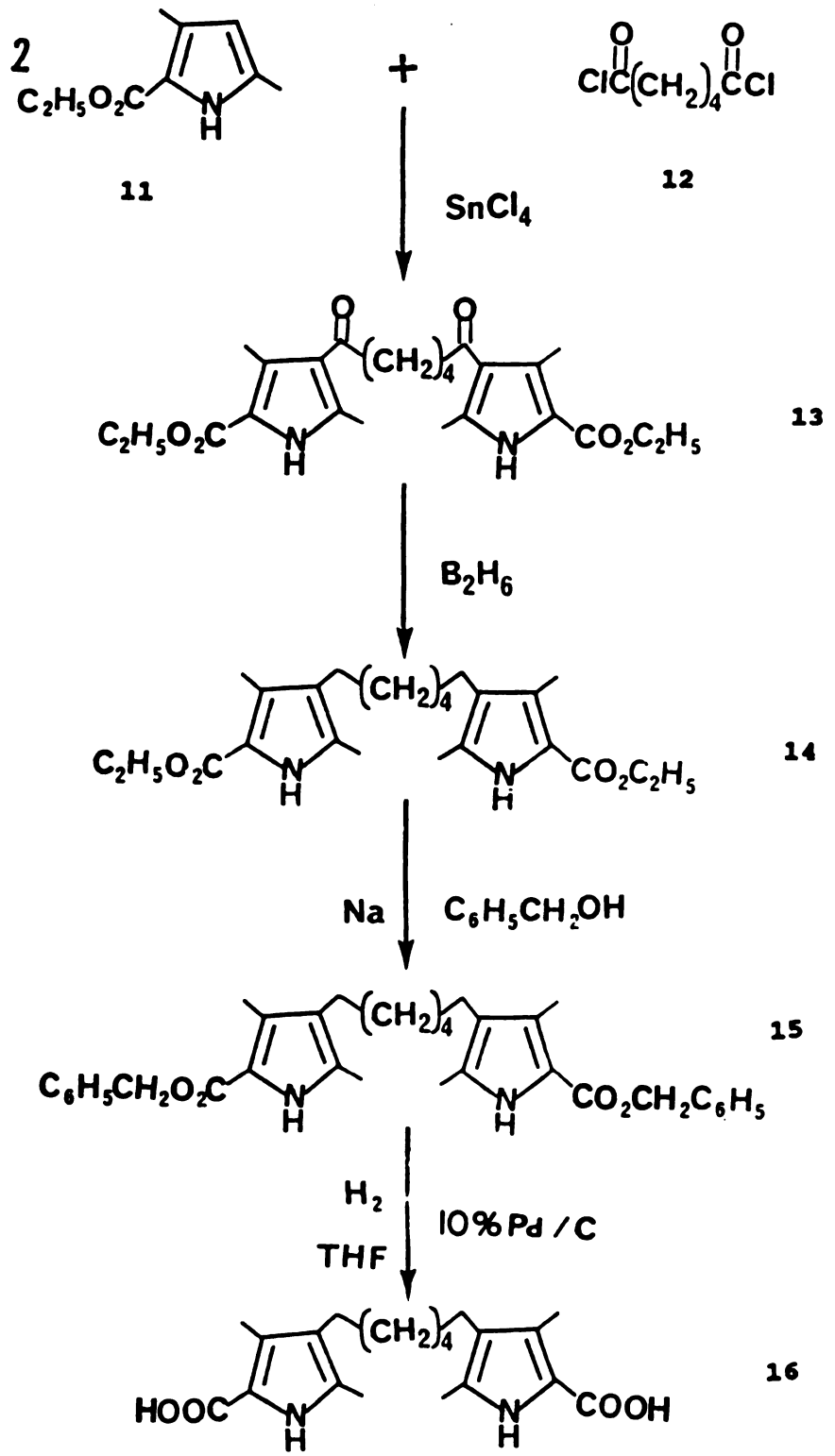


Figure 4 Synthesis of hexamethylenebispyrroles.

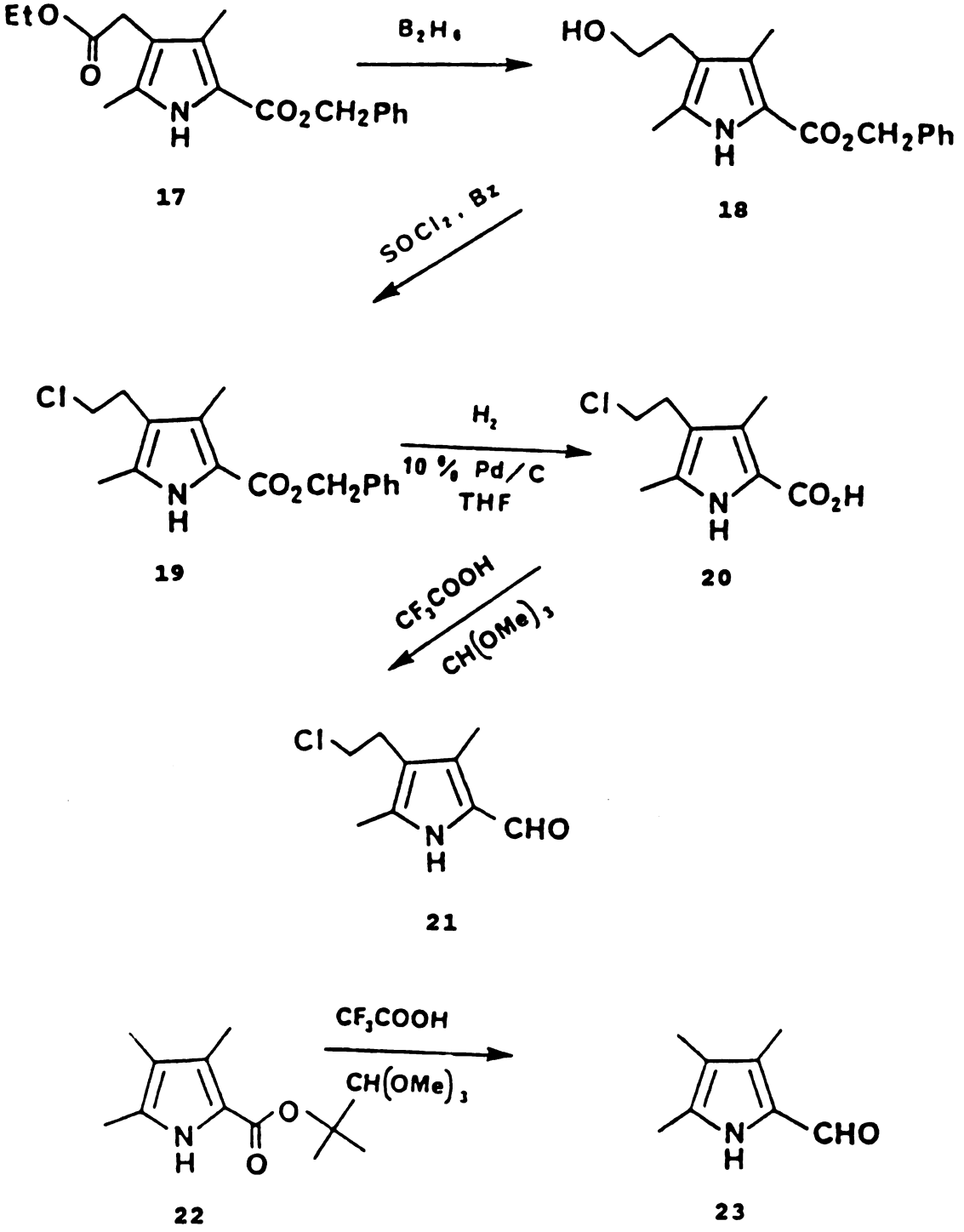


Figure 5 Synthesis of formyl pyrroles.

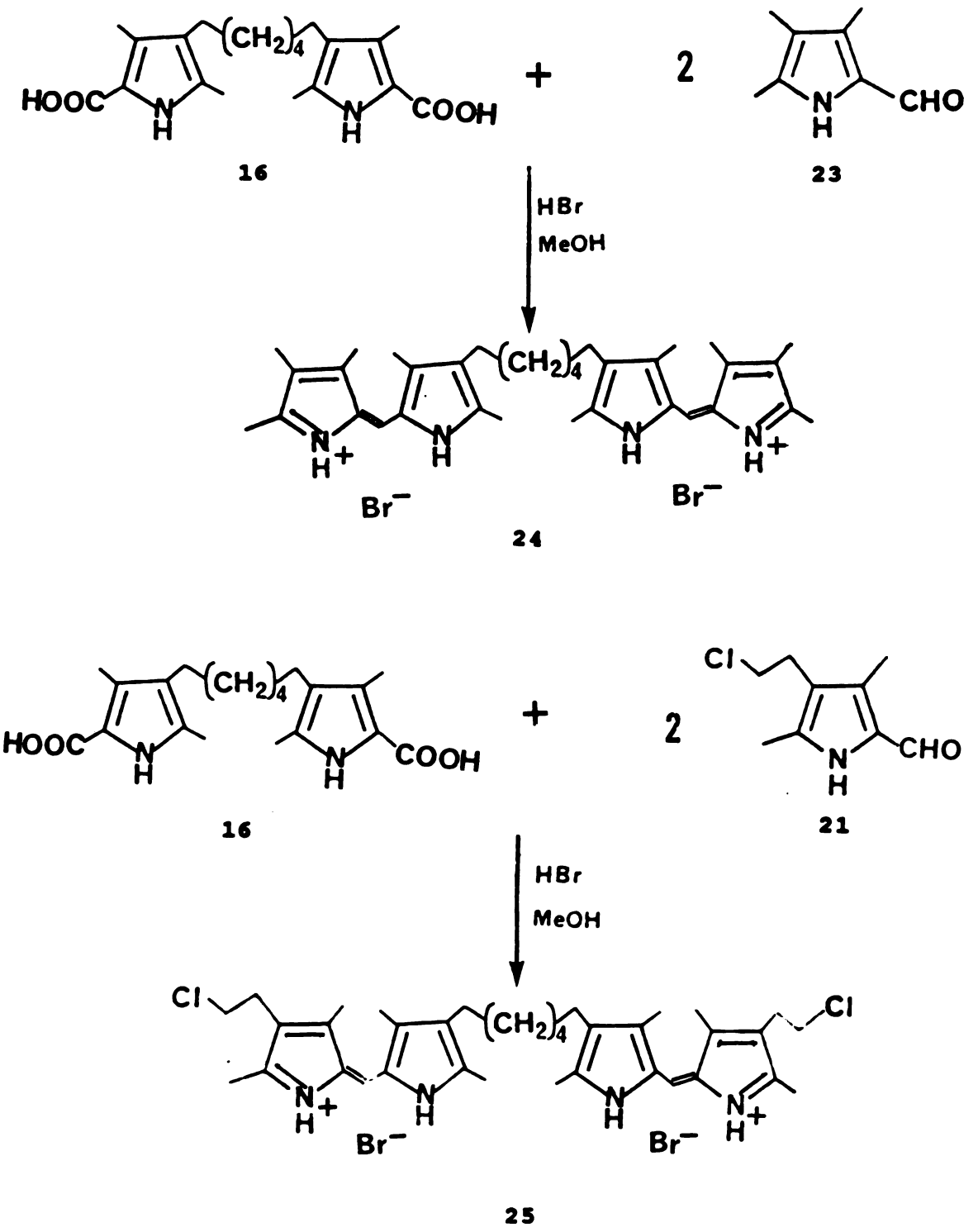
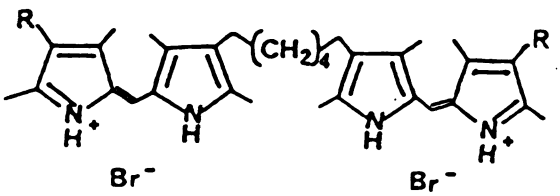


Figure 6 Synthesis of hexamethylenebisdipyrromethenes.

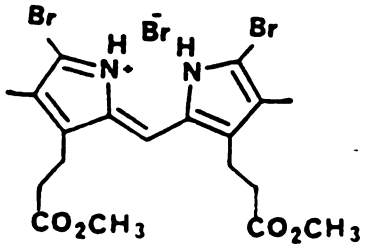


R = CH_3 24

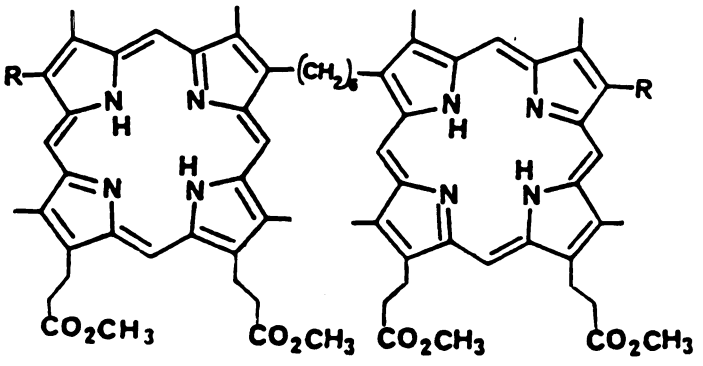
R = CH_2CH_2Cl 25

+

2



26



R = CH_3 27

R = CH_2CH_2Cl 28

Figure 7 Synthesis of hexamethylenebisporphyrins.

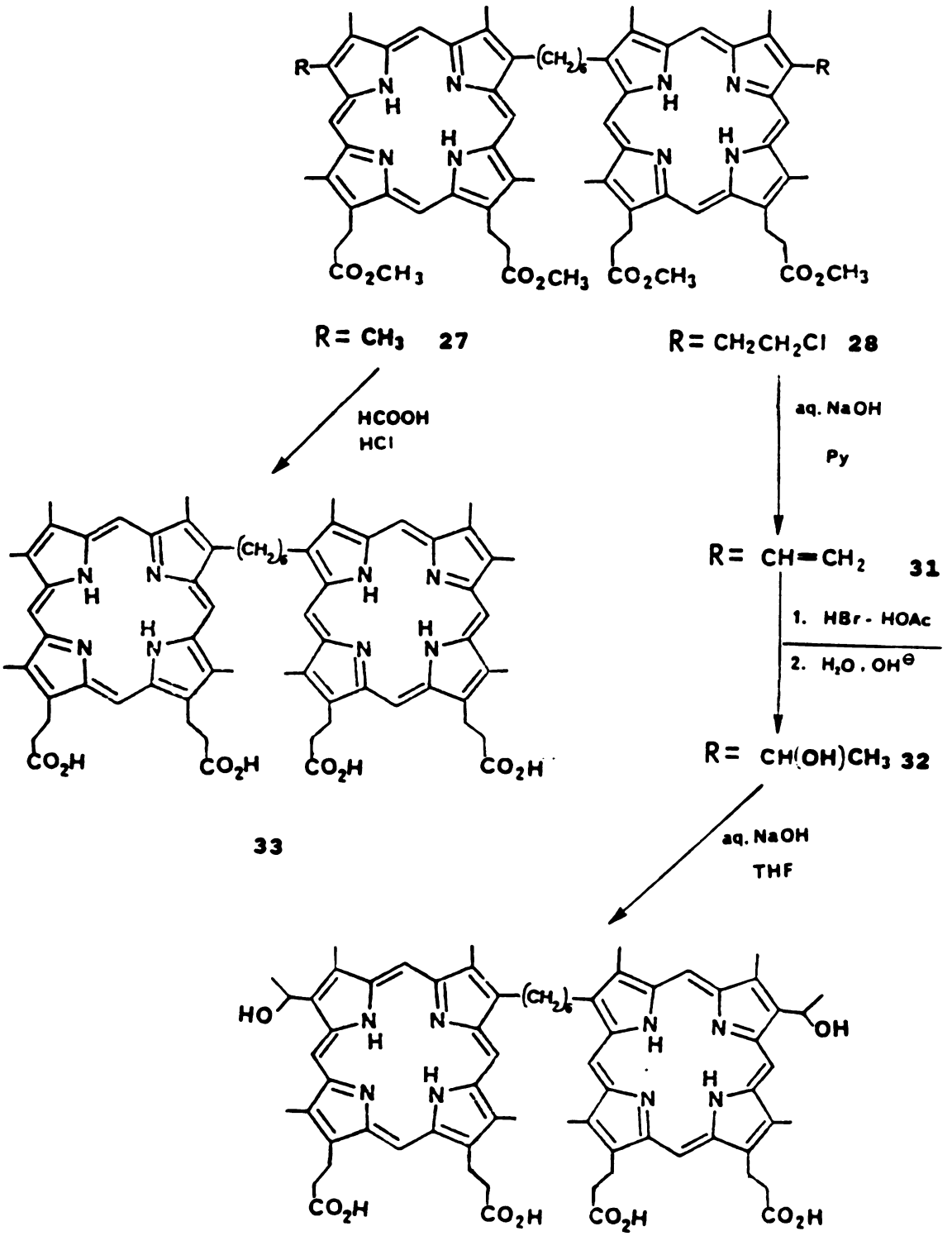


Figure 8 Modification of the substituents on the porphyrin ring

dihematoporphyrin derivative.[37] Through the model studies it was suggested that in polar solvent the ether linked dihematoporphyrin model compound adopts a close proximity between the two porphyrin rings (clam shell structure) which induces a significant downfield shift of the meso proton peaks on ^{1}H-NMR. Similar studies were carried out with our newly synthesized dimeric porphyrins **27,32** that have the haxamethylene linkage. The tetrapropionic acid groups on diporphyrins were esterified with diazomethane in the spectral analysis to avoid solubility problem.

The ^{1}H-NMR spectra of the diporphyrin tetramethyl esters were obtained in chloroform-D with or without a small amount of trifluoroacetic acid and aceton-D_6. The addition of trifluoroacetic acid was a well known technique for breaking the aggregation of porphyrins by protonation of the porphyrin nitrogens which cause the repulsion between charged rings.

Despite the changes of polarity, no significant chemical shift of meso hydrogens was observed for the hexamethylene bridged 7-(1-hydroxyethyl) substituted porphyrin dimeric **32** environment. When compared with the ^{1}H-NMR spectra of monomeric hematoporphyrin IX **1** in solvents with various polarity, the small shifts on the positions of meso hydrogens might be due to the change of intermolecular ring-ring interactions.

Absorption spectra of the hexamethylenebis[dimethyl 7-(1-hydroxyethyl)-3,8,12,17-tetramethyl-21H,23H-porphyrin-

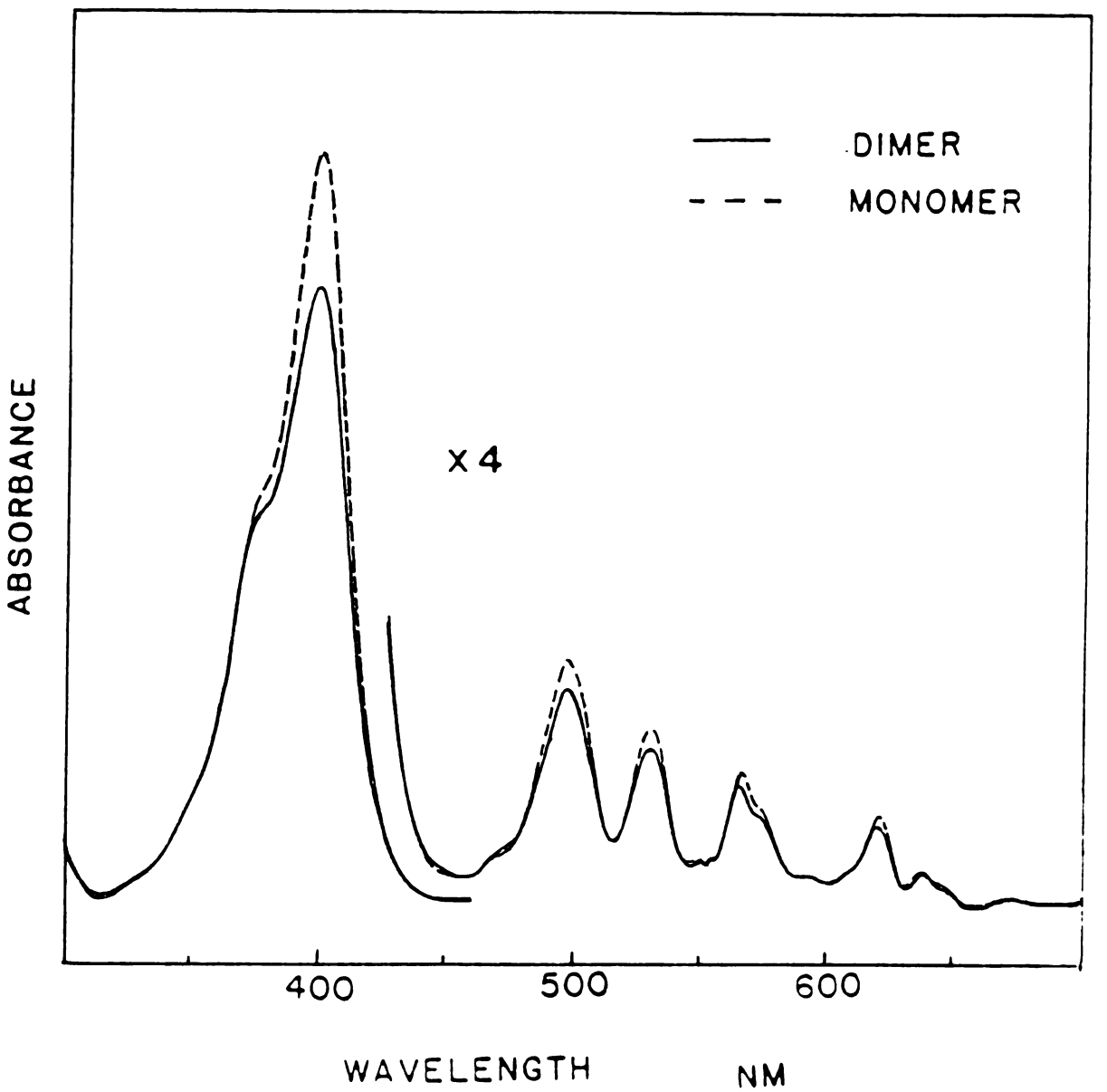


Figure 9 **Visible absorption spectra of hexamethylenebis-(dimethyl pentamethylporphyrindipropionate) 27 (solid line) versus hexamethylporphyrin dimethyl dipropionate 29 (broken line) in CH_2Cl_2.**

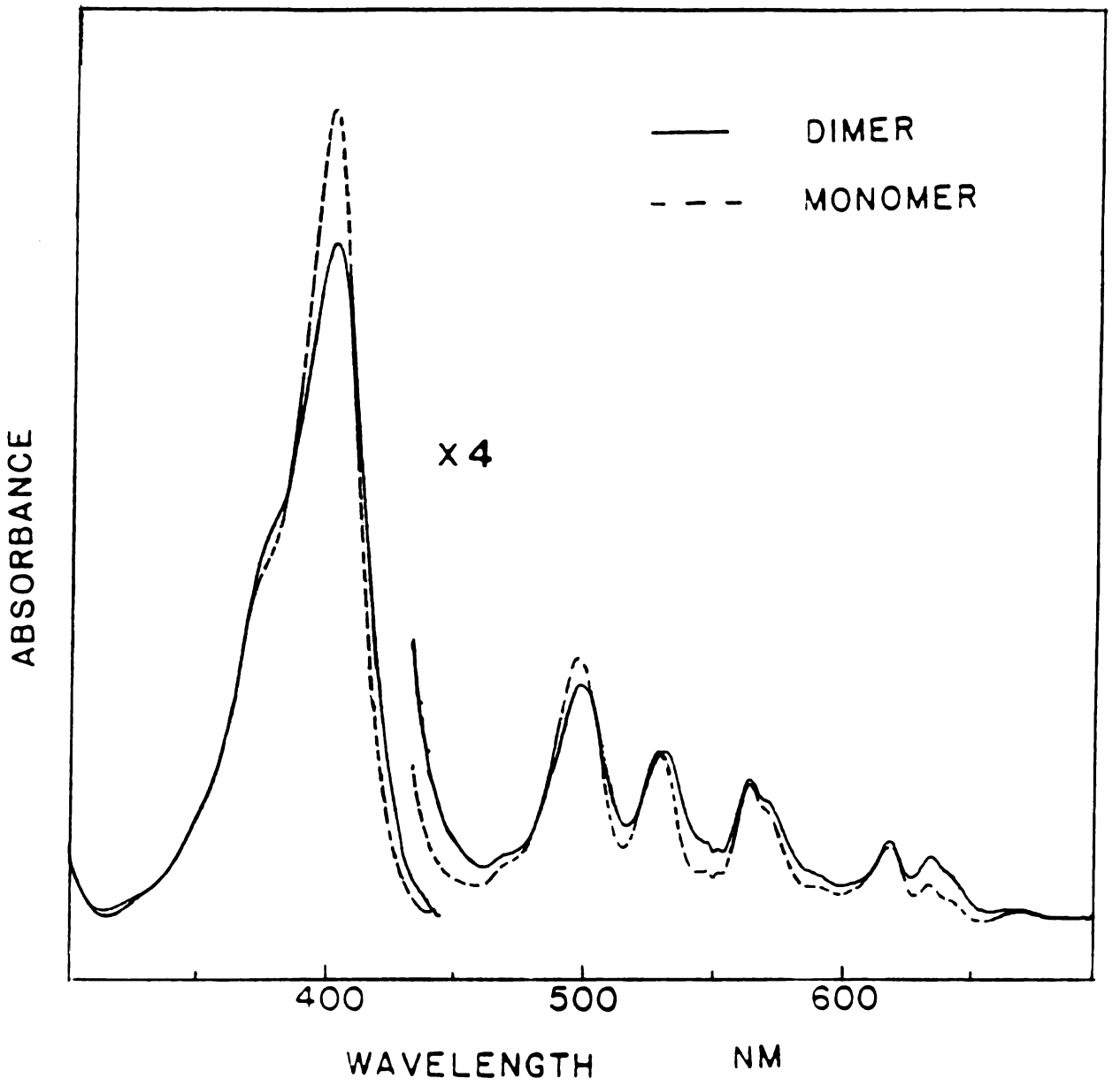


Figure 10 **Visible absorption spectra of hexamethylenebis-[dimethyl 7-(1-hydroxyethyl)porphyrindiproionate]21 (solid line) versus hematoporphyrin IX dimethyl ester 1 (broken line) in CH_2Cl_2.**

2,18-dipropionate] **32** and monomeric hematoporphyrin IX **1** dimethyl ester were shown in Figure 9. One of the characteristic features of absorption spectra of diporphyrins that have internal ring-ring interactions is a significant blue-shift of Soret band accompanied with small bathochromic shifts in the visible bands.[38,39] Unlike dimers with strong ring-ring interactions, the absorption spectrum of the hexamethylene linked 7-(1-hydroxyethyl)diporphyrin **32** does not show such properties, but rather maintains the absorption features of monomeric hematoporphyrin IX dimethyl ester. In addition to the ^{1}H-NMR spectral analysis, this absorption spectrum suggests that there are no significant ring-ring interactions through clam shell type folding in our dihematoporphyrin.

Similar results on ^{1}H-NMR and absorption spectra were obtained for hexamethylenebis(dimethyl-3,7,8,12,17-pentamethyl-21H,23H-porphine-2,18-dipropionate) **27**. (Fig. 10)

The results presented in this thesis describe the synthesis and physical properties of diporphyrins interconnected with a six methylene chain. The all carbon linkage was designed to avoid cellular hydrolysis preventing the loss of localized porphyrin photosensitizer through membrane as well as to decrease the ring-ring interaction between porphyrin units in the cell. ^{1}H-NMR and absorption spectra indicate the lack of tight ring-ring interactions in our diporphyrins. The absence of these interactions can be

explained on the basis of increased length and degree of freedom between the two porphyrin units, as compared with the ester or ether linked diporphyrin - the active component of HPD.

The effects concerning the polarity of substituents on the porphyrin ring or the lack of intra ring-ring aggregation on the mechanism of tumor localization may be understood with further studies involving fluorescence microscopy, pharmacokinetics, and cellular distribution.

EXPERIMENTAL

Experimental

General

^1H Nuclear magnetic resonance spectra were obtained on a Bruker WM-250 or Varian T-60 instrument in $CDCl_3$ with tetramethylsilane (TMS) as the internal standard set at 0.00 ppm. Mass spectra were obtained on a Finnigan 4021 GC-MS, or a JOEL HX 110-HF spectrometer equipped with a fast atom bombardment (FAB) gun. Solvents for FAB matrix were made up of thioglycerol, dithiothretol and dithioerythretol (2:1:1) with addition of 0.1 M trifluoacetic acid to facilitate the ionization of porphyrins. UV-visible spectra were measured on a Varian Cary 219 or Schimadzu 160 spectrophotometer. Melting points were obtained on an electrothermal melting point apparatus and are uncorrected. Preparative TLC plates were purchased from Analtech (silicagel GF, 1000 or 1500 um).

1,6-[4,4'-Bis(2-ethoxycarbonyl-3,4-dimethylpyrroyl)]-1,6-hexandione **13**

2-Ethoxycarbonyl-3,5-dimethylpyrrole **11** (16.7 g, 0.1 mol) and adipyl chloride **12** (14.5 ml, 0.1 mol) were dissolved in dry methylene chloride (200 ml) and stannic

chloride (15 ml) solution in dry methylene chloride (15 ml) was added dropwise during a period of 30 minutes with stirring. Stirring was continued for an additional 1 hr and at the end of the reaction, water (200 ml) was added precipitating the bis-pyrrole product. The product was filtered and washed with water followed by methylene chloride and dried in vacuo overnight. ; yield 35 g (79%) ; m.p. 144-146 ; NMR (DMSO-d_6) δ 1.35 (t, 6H), 1.62 (br.m, 4H), 2.47 (s, 6H), 2.52 (s, 6H), 2.74 (br.t, 4H), 4.27 (q, 4H) ; mass spectrum, m/e (rel. inten.) 148 (100), 194 (59), 295 (5), 444 (2, molecular ion).

4,4'-Hexamethylenebis(2-ethoxycarbonyl-3,5-dimethyl pyrrole) **14**

Boron trifluoride-ether complex (240 ml) was added dropwise to sodium borohydride (50 g, 1.32 mol) in bis-(2-methoxy-ethyl) ether (100 ml).The diborane generated was passed with a slow stream of argon into a solution of 1,6-[4,4'-bis(2-ethoxycarbonyl-3,4-dimethylpyrroyl]-1,6-hexandione **13** (28 g, 0.063 mol) in tetrahydrofuran (200ml) for 45 min and the solution was heated up to 80^{o}C for 1 hr with the continuous argon stream. Methanol was then carefully added until effervescence ceased. The solvent was evaporated under reduced pressure and the product was purified by passing through a short silica column. The white product was crystallized from methylene chloride-methanol;

yield ; 17.3 g (66%) ; m.p. 188-190°C ; NMR ($CDCl_3$), δ 1.38 (br, 14H), 2.17 (s, 6H, CH_3), 2.25 (s, 6H, CH_3), 2.32 (t, 4H, CH_2), 4.30 (q, 4H, CH_2), 8,61 (br.s, 2H, NH) ; mass spectrum, m/e (rel. inten.) 180 (100), 370 (15), 416 (18.5, molecular ion).

4,4'-Hexamethylenebis(2-benzyloxycarbonyl-3,5-dimethyl pyrrole) **15**

4,4'-Hexamethylenebis(2-ethoxycarbonyl-3,5-dimethyl pyrrole) **14** (8.32 g, 0.02 mol) was suspended in benzyl alcohol and the suspension was heated up to 209°C under argon. To this homogeneous solution the saturated solution of sodium in benzyl alcohol (6 ml) was added in portion resulting in the vigorous evolution of ethanol and lowering of the reaction temperature. Heating was continued for 10 min after evolution of ethanol ceased. The hot solution was poured carefully into a stirred mixture of acetic acid (25 ml) and methanol (400 ml). Water (700 ml) was added and the benzyl ester product was precipitated and collected by suction filtration and dried in vacuo overnight ; yield, 9.29 g (86%) ; m.p. 158-160°C ; NMR ($CDCl_3$), δ 1.35 (br S, 8H, bridge-CH_2), 2.14 (S, 6H, CH_3), 2.29 (S, 6H, CH_3), 2.34 (t, 4H, CH_2 on pyrrole ring), 5.30 (S, 4H, benzyl), 7.36 (m, 10H, benzene), 8.62 (br S, 2H, NH). ; mass spectrum, m/e (rel. inten.) 91 (100), 297 (3), 405 (6), 540 (5, molecular ion).

4,4'-Hexamethylenebis(3,5-dimethylpyrrole-2-carboxylic acid) 16

4,4'-Hexamethylenebis(benzyloxy-3,5-dimethylpyrrole-2-carboxylate) **15** (7.6 g, 0.014 mol) was dissolved in tetrahydrofuran (200 ml) and 5 drops of triethylamine was added followed by 10 % palladium-charcoal powder (1 g). The reaction mixture was stirred under hydrogen (1 atm, room temperature) until the hydrogen uptake ceased. The mixture was filtered and the solvent was evaporated to dryness under reduced pressure resulting in a white product ; yield, 5.0 g (99%) ; m.p. 89-90 ; NMR($CDCl_3$) δ 1.27 (br.s, 8H, CH_2), 2.12 (s, 6H, CH_3), 2.23 (t, 4H, CH_2), 10.85 (s, 2H, COOH), 11.66 (br.s, 2H, NH) ; mass spectrum, m/e (rel. inten.) 108 (100), 272 (82), 316 (0.1), 344 (0.4).

Benzyl 4-(2-hydroxyethy)-3,5-dimethylpyrrole-2-carboxylate 18

Diborane generated externally by adding boron trifluoride-ether complex (120 ml) dropwise to sodium borohydride (25 g, 0.66 mole) in bis-(2-methoxy-ethyl) ether (50 ml) was passed in a slow stream of argon through a solution of benzyl 4-ethoxycarbonylmethyl-3,5-dimethylpyrrole-2-carboxylate **17** (20 g, 0.063 mole) in tetrahydrofuran (100 ml) for 45 minutes. Then methanol was carefully added until the vigorous effervescence ceased. The

solvents were removed on a rotary evaporator, and the hydroxyethyl pyrrole was crystallized from methylene chloride-hexane as pale buff needles ; yield 16.17g (94%) ; m.p. 115-116°C ; NMR ($CDCl_3$), δ 1.58 (br S, 1H), 2.18 (S, 3H), 2.28 (S, 3H), 2.62 (t, 2H), 363 (t, 2H), 5.29 (S, 2H), 7.36 (S, 5H), 9.0 (br S, NH) ; mass spectrum, m/e, (%) 91 (100), 134 (15), 242 (19), 273 (9, molecular ion).

Benzyl 4-(2-Chloroethyl)-3,5-dimethylpyrrole-2-carboxylate **19**

Benzyl 4-(2-hydroxyethyl)-3,5-dimethylpyrrole-2-carboxylate **18** (10.0 g, 0.037 mol) was dissolved in benzene (200 ml) and the solution was warmed for 30 min. The homogeneous solution was cooled down to room temperature and anhydrous potassium carbonate (20 g, 0.145 mol) was added, followed by thionyl chloride (6 ml, 0.082 mol). The mixture was refluxed under argon for 3 hours. The solution was filtered and evaporated to dryness under reduced pressure, and the residue was chromatographed on a silica gel column eluted with 50% hexane / methylene chloride solution. The major fraction was collected and solvent was evaporate under reduced pressure. The product was recrystallized from methylene chloride-hexane ; yield 9.37 (87%) ; m.p. 119-120°C ; NMR ($CDCl_3$), δ 2.20 (S, 3H), 2.28 (S,3H), 2.81 (t, 2H), 3.52 (t, 2H), 5.31 (S ,2H), 7.40 (S, 5H), 8.97 (br S,

NH) ; mass spectrum, m/e, (%) 91 (100), 242 (11), 291 (6, molecular ion).

4-(2-Chloroethyl)-3,5-dimethylpyrrole-2-carboxylic acid **20**

Benzyl 4-(2-chloroethyl)-3,5-dimethyl-2-carboxylate **19** (4.0 g, 0.014 mol) and 10% palladium on charcoal (0.7g) was added in tetrahydrofuran (125 ml). A few drops of triethylamine were added in the solution and the reaction mixture was stirred under hydrogen (1 atm, room temperature). The reaction was stopped when hydrogen uptake ceased. Charcoal was filtered off and the filtrate was evaporated under reduced pressure to give a white product; yield 2.68 g (97 %) ; m.p. 112-113^{o}C ; NMR ($CDCl_3$), δ2.23 (S, 3H), 2.30 (S, 3H), 2.83 (t, 2H), 3.51 (t, 2H), 8.77 (br S, NH) ; mass spectrum, m/e, (%) 134 (100), 152 (50), 201 (13, molecular ion).

4-(2-Chloroethyl)-2-formyl-3,5-dimethyl pyrrole **21**

Finely ground 4-(2-chloroethyl)-3,5-dimethyl pyrrole-2-carboxylic acid **20** (2 g, 0.01 mol) was added in portions (0.2 g) in 10 ml of trifluoroacetic acid and the reaction mixture was heated to 40^{o}C for 5 min followed by addition of triethyl orthoformate (3 ml, 0.017 mol) in one portion. After heating for further 5 min, water was added and the precipitated oil, which soon solidified, was collected and

dissolved in ethanol (20 ml). Aqueous ammonia (2 N, 20 ml) was added slowly with stirring followed by water (20 ml) 10 minutes later. The product was collected and purified by chromatography on silica gel to give the formylpyrrole as pale yellow needles ; yield 1.55 g (84%) ; m.p. 143-144 ^{o}C ;NMR ($CDCl_3$), δ 2.27 (s, 6H), 2.82 (t, 2H), 3.50 (t, 2H), 9.45 (s, 2H),9.90 (br s, NH) ; mass spectrum, m/e (rel. inten.) 136 (100), 150 (14), 185 (16, molecular ion).

2-Formyl-3,4,5-trimethyl pyrrole **23**

Finely ground 2-t-butoxycarbonyl-3,4,5-trimethylpyrrole **22** (2 g, 0.01 mol) was added in portions (0.2 g) to stirred trifluoroacetic acid (10 ml) and the solution was warmed to 40^{o}C. After 5 min triethyl orthoformate (3.0 ml, 0.017 mol) was added in one portion and stirring was continued at 40^{o}C for further 5 minutes. Water was slowly added and the precipitated oil, which soon solidified was collected, and dissolved in ethanol (20 ml). Aqueous ammonia (2N, 10 ml) was added slowly with stirring followed by water (20ml) 10 min later. The product was collected and purified by chromatography on silica gel to give the formylpyrrole. Solvent was evaporated and the product was recrystallized with aqueous ethanol ; yield 0.62 g (48%) ; m.p. 144-148^{o}C ; NMR ($CDCl_3$), δ 1.91 (S, 3H), 2.23 (S, 6H), 9.44 (1H CHO), 10.75 (br S, NH) ; mass

spectrum, m/e (rel. inten.) 108 (8.2), 122 (20), 136 (100, molecular ion).

4,4'-Hexamethylenebis(3,3',4,5,5'-pentamethylpyrromethene) dihydrobromide **24**

A suspension containing the diacid **16** (1.5 g, 4.2 mmol) and 2- formyl-3,4,5-trimethyl pyrrole **23** (1.14, 8.4 mmol) in methanol (30 ml) was heated up to boiling. Bromic acid (48%, 4 ml) was added in one portion. The orange-red product was immediately precipitated with evolution of CO_2 gas. Heating was continued for 1/2 hr and the reaction was allowed to stand at room temperature for 3 hrs. The solid product was collected by suction filtration and the product was recrystallized from methylene chloride/hexane ; yield 2.31 g (85%) ; m.p. 263-265 ^{o}C ; NMR ($CDCl_3$), δ 1.30, 1.42 (2 br.s, 8H, CH_2), 1.99 (s, 6H, CH_3), 2.25 (s, 12H, CH_3), 2.38 (t, 4H, CH_2), 2.65 (s, 12H, CH_3), 7.28 (s, 2H, CH) 12.20 (br.s, 4H, NH).

4,4'-Hexamethylenebis[4-(2-chloroethyl)-3,3'5,5'-tetramethyl pyrromethene] dihydrobromide **25**

A suspension containing the diacid **16** (1.80 g, 5.0 mmol) and 3-(2-chloroethyl)-5-formyl-2,4-dimethyl pyrrole **21** (1.85 g, 10 mmol) in methanol (30 ml) was heated up to boiling. Bromic acid (48%, 4 ml) was added in one portion.

The orange-red product was immediately formed. Heating was continued for 1/2 hr and the reaction mixture was allowed to stand at room temperature for 3 hrs. The solid product was collected by suction filtration and the product was recrystallized from methylene chloride hexane ; yield 3.28 g (88%) ; NMR ($CDCl_3$) δ 1.79, 1.94 (2br.s, 8H, CH_2), 2.23, 2.27 (2s, 6H, CH_3), 2.29 (t, 4H, CH_2), 2.62, 2.65 (2s, 6H, CH_3), 2.86, 3.53 (2t, 4H, CH_2), 7.04 (s, 2H, CH), 13.03 (br. m, 4H, NH).

Dimethyl-5,5'-dibromo-4,4'-dimethylpyrromethene-3,3'-dipropionate hydrobromide **26**

Dimethyl-5,5'-bis-(benzyloxycarbonyl)-4,4'-dimethylpyrromethene-3,3'-dipropionate hydrobromide (16 g, 0.026 mol) was dissolved in tetrahydrofuran and triethyl amine (5 drops) was added followed by 10% palladium on charcoal (1 g). The reaction mixture was stirred under hydrogen (1 atm, room temperature) until hydrogen uptake ceased. The mixture was filtered and the filtered charcoal product mixture was washed with small amounts of tetrahydrofuran and dried. The mixture was suspended in formic acid (85 ml) and bromine (8.5 ml) was added in one portion. After most of formic acid was removed with vacuum distillation ether (50 ml) was added precipitating the purple solid and the product was collected by the suction filtration ; yield 12.20 g (80.5%) ; m.p 180-182 ^{o}C ; NMR

($CDCl_3$), δ 2.02 (s, 6H, CH_3), 2.90, 3.06 (t, 4H, CH_2), 3.59 (s, 6H, CH_3), 7.60 (s, 1H, CH), 11.25(br.s, 1H, NH) ;mass spectrum, m/e (rel. inten.)275 (53), 334 (100), 355 (45), 502 (15).

13,13'-Hexamethylenebis(dimethyl-3,7,8,12,17-pentamethyl-21H,23H-porphine-2,18-dipropionate) **27**

4,4'-Hexamethylenebis(3,3',4,5,5'-pentamethyl pyrromethene) **24** (1.60g, 0.0025 mol) and dimethyl-5,5'-dibromo-4,4'-dimethylpyrromethene-3,3'-dipropionate hydrobromide **26** (3.15 g, 0.005 mol) were suspended in formic acid (25 ml, 98-100%) and the mixture was heated to 80°C in an oil bath. At this temperature Br_2 (0.27 ml, 0.0054 mol) was added in one portion and the temperature of reaction mixture was kept constant for 2.5 hrs. The solvent was then allowed to boil off over a period of 30 min with further heating in the bath up to 130°C during which time the porphyrin was formed. To the residue was added methanol (24 ml) and concentrated sulfuric acid (2.61 ml), followed after 10 min by triethyl orthoformate (3.8 ml). After standing overnight, protected from moisture, the tar residue was filtered off by passing the solution through glass wool. The solution was neutralized with saturated aqueous sodium bicarbonate solution. The organic phase was separated, washed with water several times and dried with anhydrous

sodium sulfate. The solvent was removed under reduced pressure to dryness and the crude product was chromatographed on silica gel using 50% methylene chloride / hexane as an eluent. The fast moving dark non-fluorescent fraction was discarded. The monomeric porphyrin was followed by a yellowish fraction containing dipyrromethene and was identified as dimethyl-3,7,8,12,13,17-hexamethyl-21H,23H-porphine-2,18-propionate **29** ; yield 70 mg (5%) ; m.p.,337-339 ; NMR ($CDCl_3$), δ -3.89 (br. s, 2H, NH), 3.24 (t, 4H, CH_2), 3.55, (s, 6H, CH_3), 3.60, 3.67 (2s, 6H, CH_3),9.89 (s, 1H, meso). 9.97 (s, 2H, meso), 10.02 (s, 1H, meso) ; mass spectrum, m/e, 566 (molecular ion) ; λ_{max} (CH_2Cl_2) nm 397 (Soret), 497, 531, 568, 620.

The fraction containing dimeric porphyrin was eluted behind the monomeric porphyrin and was collected. The crude product was further purified by preparative TLC plate developed with methylene chloride containing 1% methanol as eluent ; yield 0.237 g (8 %) ; m.p. 243 ^{o}C < decomp. ; NMR ($CDCl_3$ with CF_3COOH inside), δ -3.78(br.s, 4H, NH), 1.95, 2.36 (2br.s, 8H, CH_2), 3.16, 3.27 (2t, 8H, CH_2), 3.47,3.54,3.55,3.59,3.65 (5s, 36H, CH_3), 4.05, 4.27,4.41 (t, 12H, CH_2) 10.03 (m, 8H, meso) ; mass spectrum, m/e 1187.59 (M+1) ; λmax (CH_2Cl_2) nm 397 (Soret), 497, 531, 564, 619, 637.

<u>13,13'-Hexamethylenebis[dimethyl-7-(2-chloroethyl)-3,8,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionate **28**</u>

4,4'-Hexamethylenebis[4-(2-chloroethyl)-3,3'5,5'-tetramethylpyrromethene] **25** (1.70 g, 0.0023 mol) and dimethyl-5,5'-dibromo-4,4'-dimethylpyrromethene-3,3'-dipropionate hydrobromide **26** (2.66 g, 0.0046 mol) was suspended in formic acid (22 ml, 98-100%) and the mixture was heated up to 80°C in an oil bath. At this temperature Br_2 (0.24 ml, 0.0046 mol) was added in one portion and the temperature of the reaction mixture was kept constant for 2.5 hrs. The solvent was then allowed to boil off over a period of 30 min with further heating in the bath up to 130°C during which time the porphyrin was formed. To the residue was added methanol (23 ml) and concentrated sulfuric acid (2.22 ml), followed after 10 min by triethyl orthoformate (3.2 ml). After standing overnight, protected from moisture, the solution was diluted with methylene chloride and the tar residue was filtered off by passing the solution through glass wool. The solution was neutralized with saturated aqueous sodium bicarbonate solution. The organic layer was separated, washed with water several times and dried with anhydrous sodium sulfate. The solvent was removed under reduced pressure to dryness and the crude product was chromatographed over silica gel using 50% methylene chloride / hexane as an eluent. A dark non-fluorescent forerun was discarded. The monomeric porphyrin was followed by yellowish fraction containing unreacted dipyrromethene and was identified as dimethyl-7,13-(2-

chloroethyl)-3,8,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionate **30**. ; yield 0.152 g (5%) ; m.p. 204-205 ; NMR ($CDCl_3$), δ 3.20 (t, 4H, CH_2), 3.48, 3.57, 3,81 (3s, 18H, CH_3), 9.80 (s, 2H, meso) 9.97, 10.00 (2s, 2H, meso) ; mass spectrum, m/e 664 (molecular ion) ; λ_{max} (CH_2Cl_2), nm 399 (soret),498, 532, 567, 621.

The reddish fraction containing dimeric porphyrin was eluted after the monomeric porphyrin and the crude product was further purified by preparative TLC plate developed with methylene chloride containing 1% methanol as eluent and crystallized from methylene chloride-methanol ; yield 0.265 g (9%) ; m.p. 248 ^{o} C > decomp. ; NMR ($CDCl_3$), δ -3.86 (br s, 4H, NH), 1.90 (br s, 4H, CH_2), 2.32 (br s, 4H, CH_2), 3.13 , 3.26 (2t, 8H, CH_2), 3.41, 3.51, 3.58, 3.63, 3.64 (6s, 36H, CH_3), 4.03 (t, 4H, CH_2), 4.26 (m, 6H, CH_2), 4.41 (m, 6H, CH_2), 9.95 (s, 2H, meso), 10.82 (s, 6H, meso) ;mass spectrum, m/e 1285.53 (M+1) ; λ_{max} (CH_2Cl_2) nm 399.0 (Soret), 498.5, 533,0, 567.0, 620.5.

<u>13,13′-Hexamethylenebis(dimethyl-7-vinyl-3,8,12,17-tetramethyl-21H,32H-porphine-2,18-dipropionate) **31**</u>

A solution of 13,13′-hexamethylenebis[dimethyl-7-(2-chloroethyl)-3,8,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionate **28** (200 mg, 0.15 mmol) in pyridine (200 ml) was refluxed under argon for five minutes, and then sodium hydroxide (2.0 g, 0.05 mol) in water (20 ml) was carefully

added and heating was continued for 30 minutes. After the period, more water (120 ml) was added leading to a homogeneous solution. The solution was kept refluxing under argon for further 1.5 hrs. At the end of time period, aqueous acetic acid (6M) was added to neutralize the excess sodium hydroxide, and the solution was dried under reduced pressure. Water (60 ml) was added and The pH of the solution was adjusted to 2 with dilute hydrochloric acid. The precipitate was collected and dissolved in THF followed by treatment with diazomethane. After stirring 2 hrs, THF was evaporated and the product was separated on preparative TLC plate developed with methylene chloride containing 1% methanol and crystallized from methylene chloride-methanol; yield 102 mg (54 %); m.p. 248 ^{o}C > decom. ; NMR($CDCl_3$), δ - 4.18 (br s, 4H, NH), 1.91 (br s, 4H, CH_2), 2.33 (br s, 4H, CH_2), 3.14 , 3.25 (2t, 8H, CH_2), 3.42, 3.52, 3.58, 3.63 (4s, 36H, CH_3), 4.04, 4.22, 4.41 (3t, 12H, CH_2), 6.14, 6.31 (2d, 4H, vinyl-CH_2), 8.25 (q, 2H, CH), 9.98(s, 4H, meso), 10.02, 10.17 (2s, 2H, meso); mass spectrum ,m/e 1211.55 (M+1) ; λ_{max} (CH_2Cl_2) nm 401.5 (Soret), 501.5, 538, 570, 624.5.

<u>13,13'-Hexamethylenebis[dimethyl-7-(1-hydroxyethyl)-3,8,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionate] **32**</u>

13,13'-Hexamethylenebis(dimethyl-7-vinyl-3,8,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionate) **31** (100 mg, 0.08 mmol) was stirred with the saturated hydrogen bromide-

acetic acid solution (4 ml) in subdued light for 24 hrs at room temperature (ca. 20°C). The mixture was poured into excess water (15 ml), and after allowing 10 minutes for hydrolysis of the HBr-adduct, it was neutralized with aquous NaOH. The precipitate was collected and dissolved in THF followed by treatment with diazomethane. After 2 hrs stirring THF was evaporated and the product was separated on preparative TLC plate with methylene chloride containing 3% methanol as eluent and crystallized from methylene chloride-methanol. ; yield 46 mg (46%) ; m.p. 253 °C > decomp. ;NMR ($CDCl_3$ with CF_3COOH inside), δ -3.90 (br s, 4H, NH), 2.21 (d, 6H, CH_3), 2.46 (br s, 4H, CH_2), 3.01,3.30 (2t,8H,CH_2), 3.21, 3.49, 3.57, 3.62, 3.69, 3.70 (6s, 36H, CH_3), 3.98, 4.10, 4.42 (3t, 12H, CH_2), 6.59 (q, 2H, CH) 10.02, 10.09, 10.12, 10.71 (4s, 8H, meso) ; mass spectrum, m/e 1247.46 (M+1) ; λ_{max} (CH_2Cl_2) nm 400 (Soret), 498, 534, 567, 622, 637.

<u>13,13'-Hexamethylenebis(3,7,8,12,17-pentamethyl-21H,23H-porphine-2,18-dipropionic acid) **33**</u>

13,13'-Hexamethylenebis(dimethyl-3,7,8,12,17-pentamethyl-21H,23H-porphine-2,18-dipropionate) **27** (50 mg, 0.04 ,mmol) was dissolved in formic acid (98 %, 30 ml) and hydrochloric acid (35 %,3 ml) was added. The solution was heated to boiling on the steam bath for 1/2 hr and formic acid was removed under vacuum. The product was collected,

washed with methylene chloride, and dried in vacuo.;yield 48 mg (98 %); mass spectrum, m/e 1131.59 (M+1)

13,13'-Hexamethylenebis[7-(1-hydroxyethyl)-3,8,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionic acid] **34**

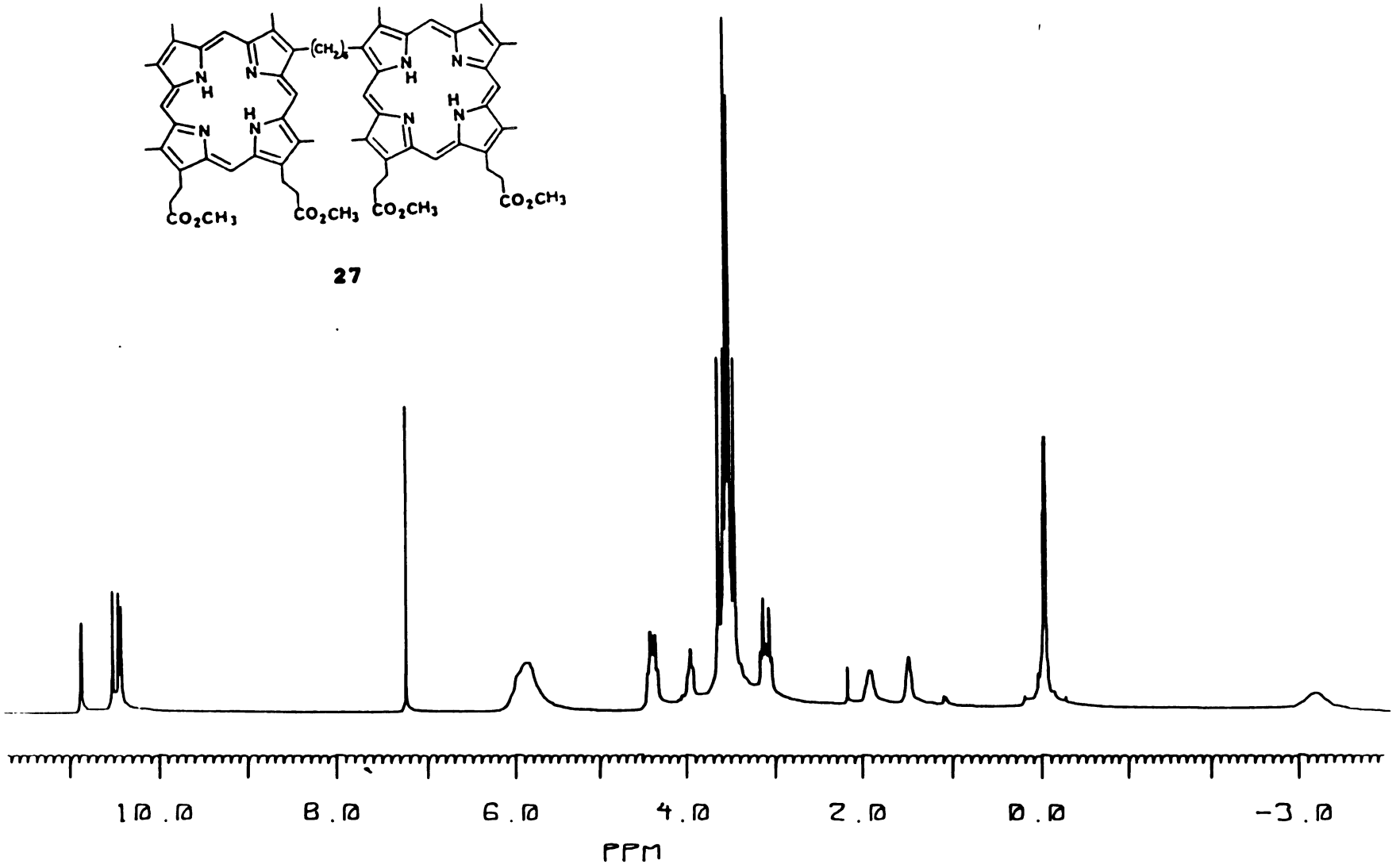
13,13'-Hexamethylenebis[dimethyl-7-(1-hydroxyethyl)-3,8,12,17-tetramethyl-21H,23H-porphine-2,18-dipropionate] **32** (25 mg, 0.02 mmol) was dissolved in THF (30 ml) and sodium hydroxide (0,3 g, 7.5 mmol) in water (5 ml)was added. The solution was refluxed for 1.5 hrs and at the end of the period the pH of the solution was adjusted to 2 with diluted hydrochloric acid. The solvent was removed under reduced pressure and the precipitates were collected by suction filtration followed by washing with water and methylene chloride. ; yield, 22 mg (93 %); mass spectrum, m/e, 1191.67 (M+1)

Preparation of diazomethane from p-toluenesulfonylmethyl nitrosoamide (diazald)

2-(2-Ethoxyethyl)-ethanol (6.0 ml)and ether (3.5 ml) were added to a solution of potassium hydroxide (1.0 g, 0.0178 mol) in water (1.8 ml). The solution was placed in a 50 ml three-necked (one was stopped) distilling flask fitted with a dropping funnel and an efficient condenser in an oil

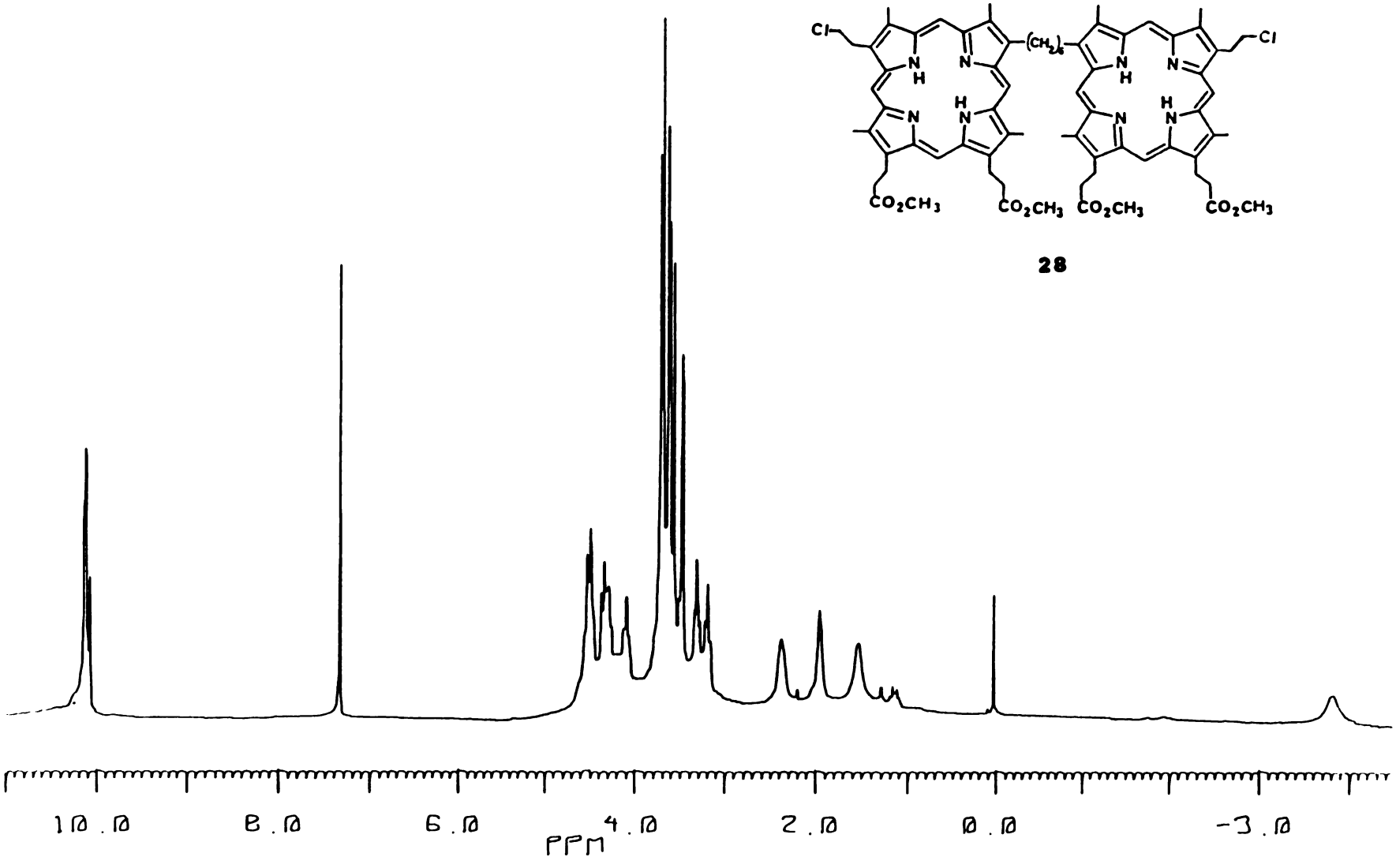
bath at 70^{o}C . As the distillation of the ether started, a solution of p-toluenesulfonylmethylnitrosoamide (3.5 g, 0.0146 mol) in ether (30 ml) was added through the dropping funnel slowly. During the distillation, the solution was stirred efficiently, and the yellow ethereal diazomethane was distilled out. When the dropping funnel was empty, another portion of ether (10 ml) was added slowly, and distillation was continued until the distilling ether was colorless.

APPENDIX



27

Figure 11. 250 MHz ^{1}H-NMR spectrum of diporphyrin. 27



28

Figure 12. 250 MHz ^{1}H-NMR spectrum of diporphyrin 28

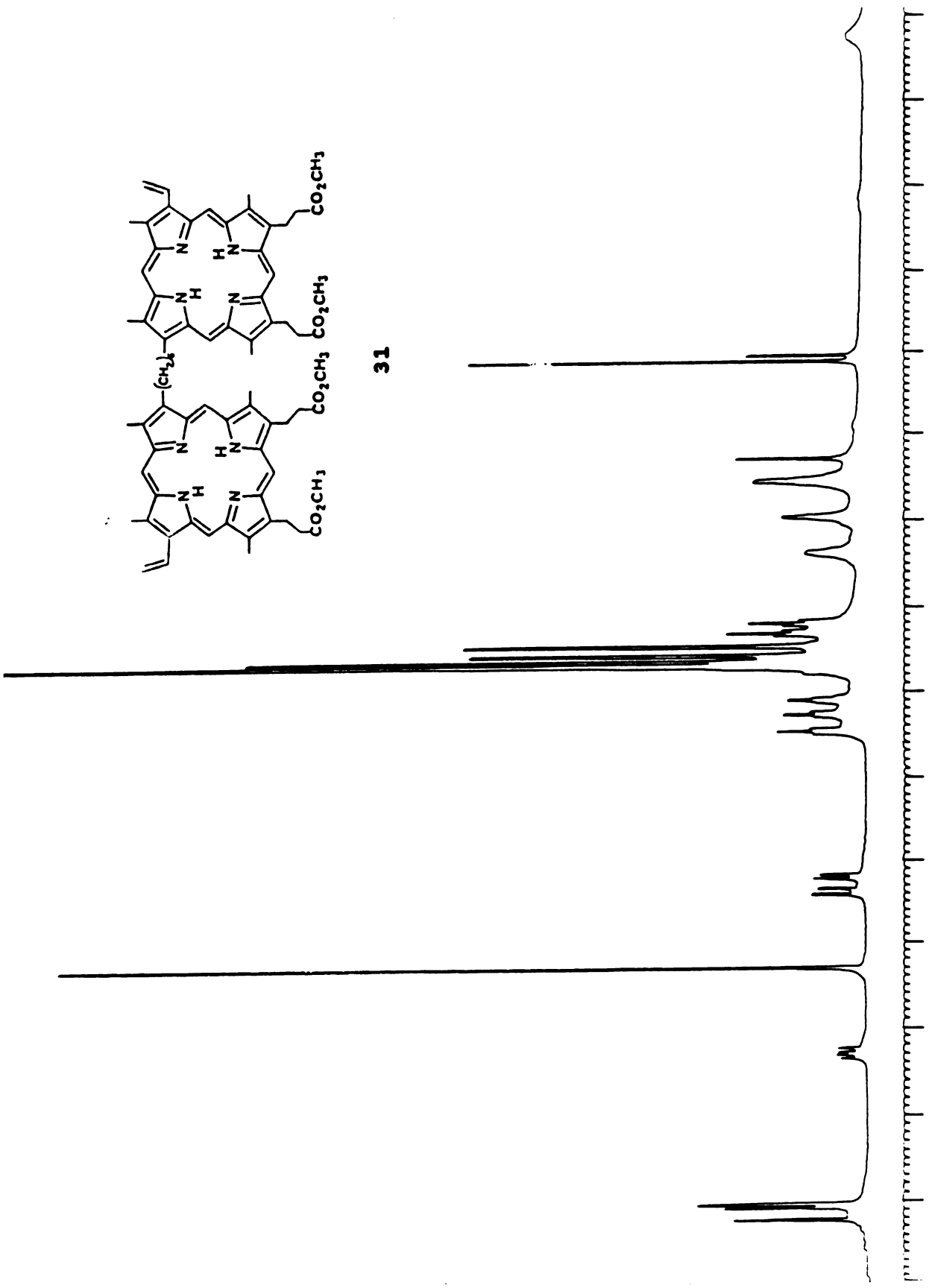
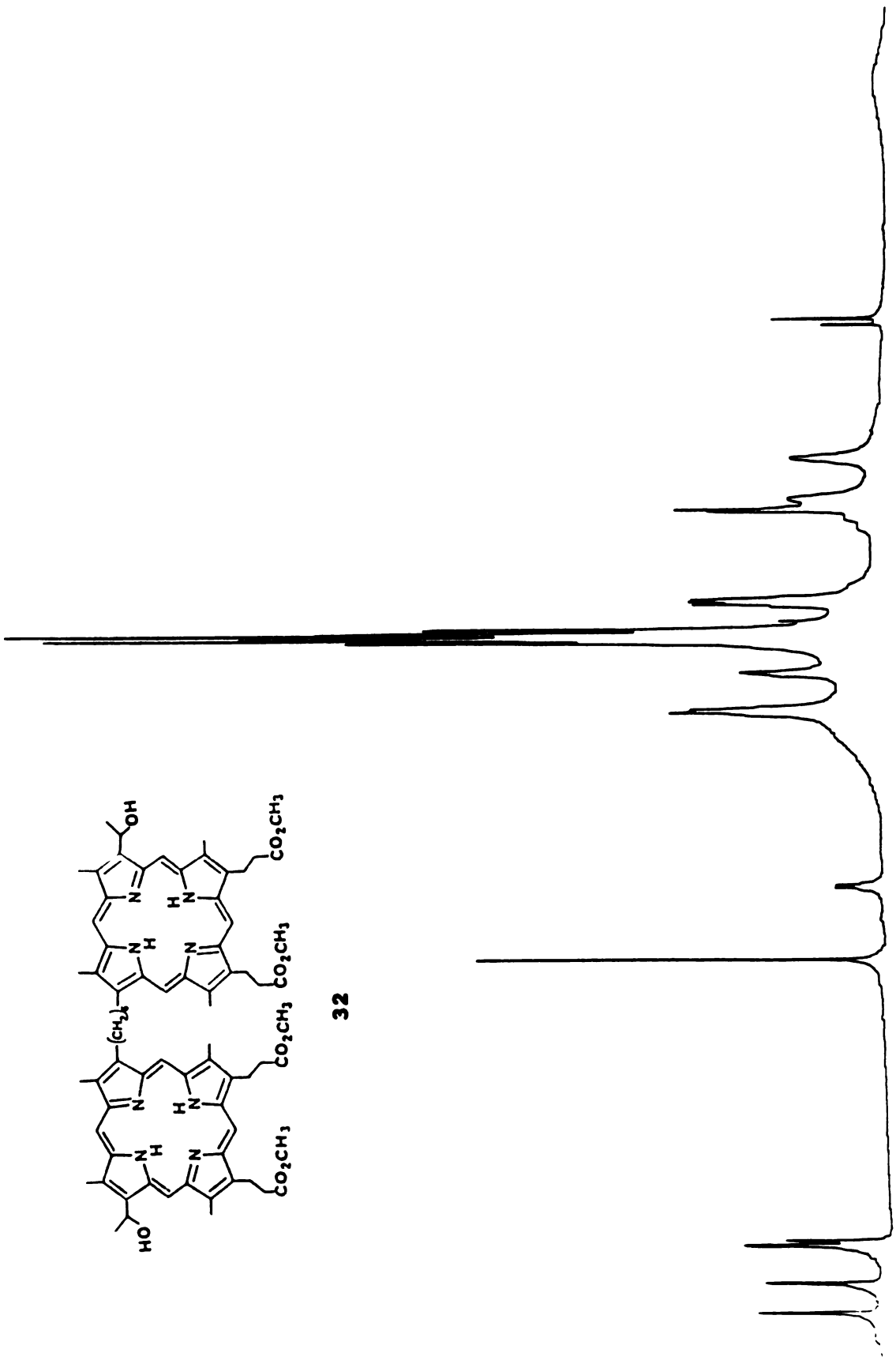
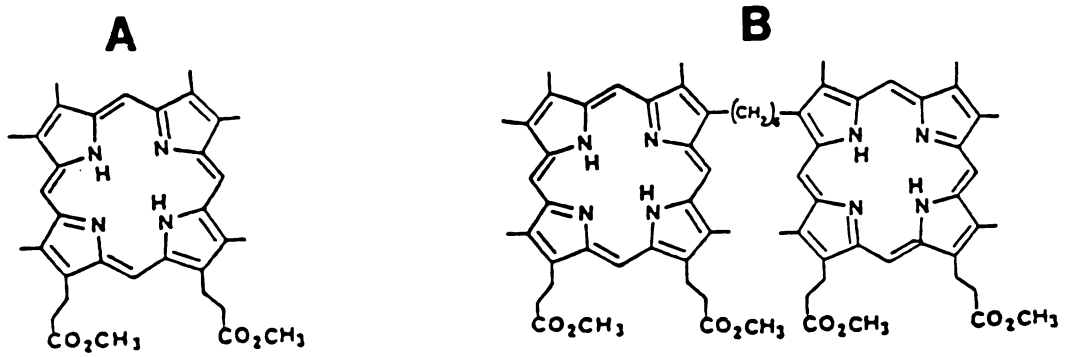


Figure 13. 250 MHz ^{1}H-NMR spectrum of diporphyrin 31



32

Figure 14. 250 MHz ^{1}H-NMR spectrum of diporphyrin. 32



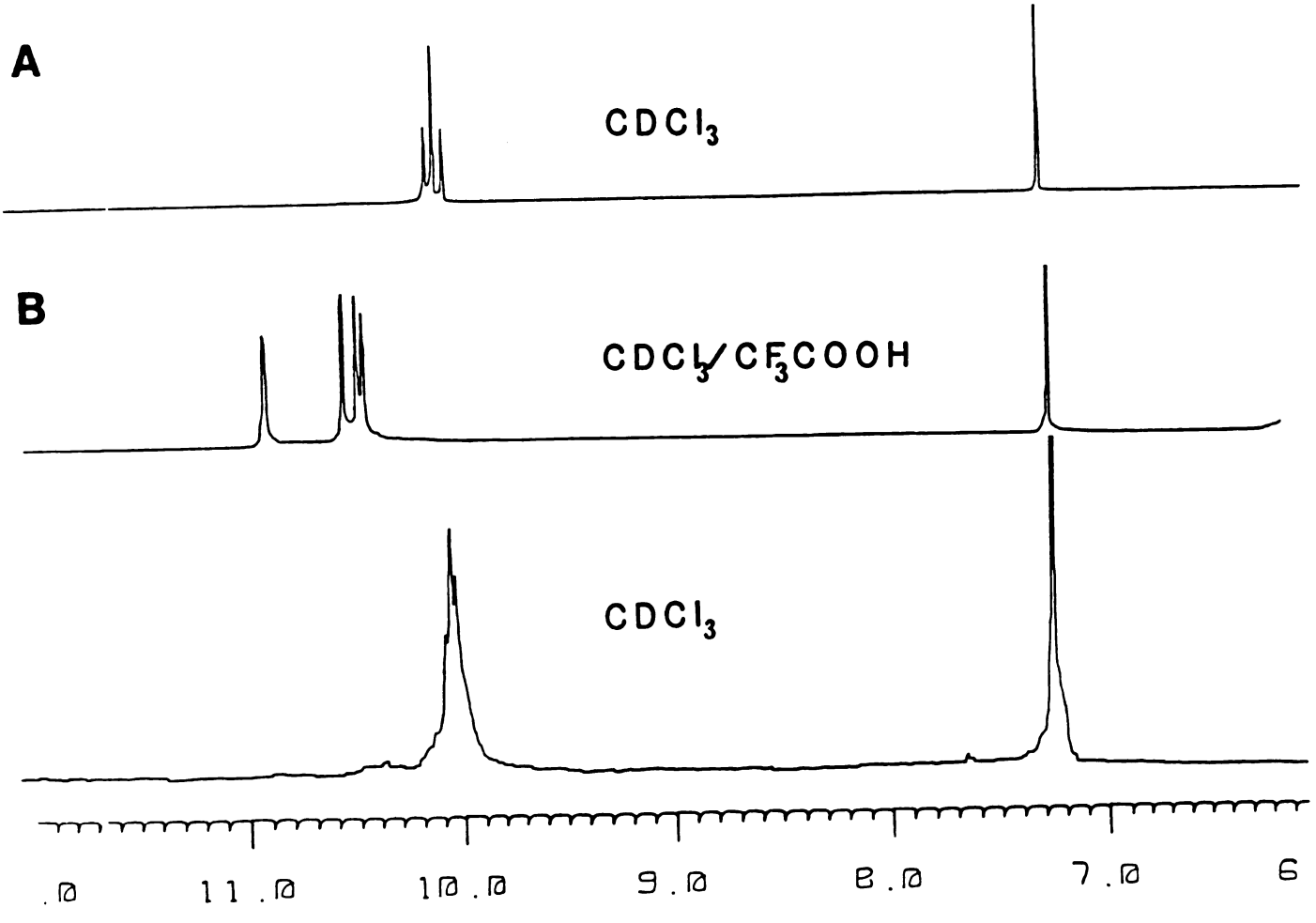


Figure 15. Effects of solvents on the 250 MHz ^{1}H-NMR spectrum of diporphyrin **27**

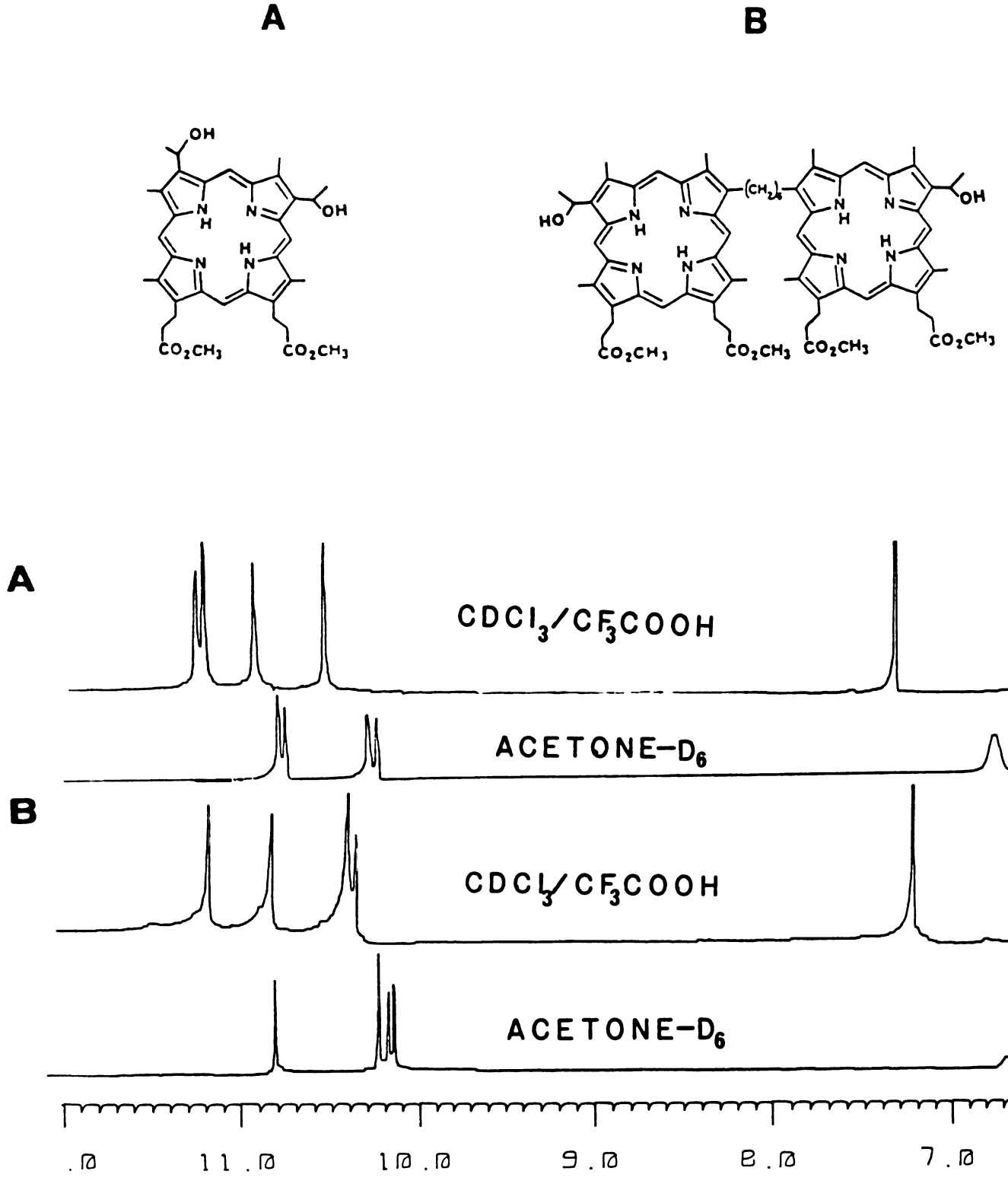


Figure 16. Effects of solvents on the 250 MHz ^{1}H-NMR spectrum of diporphyrin **32**

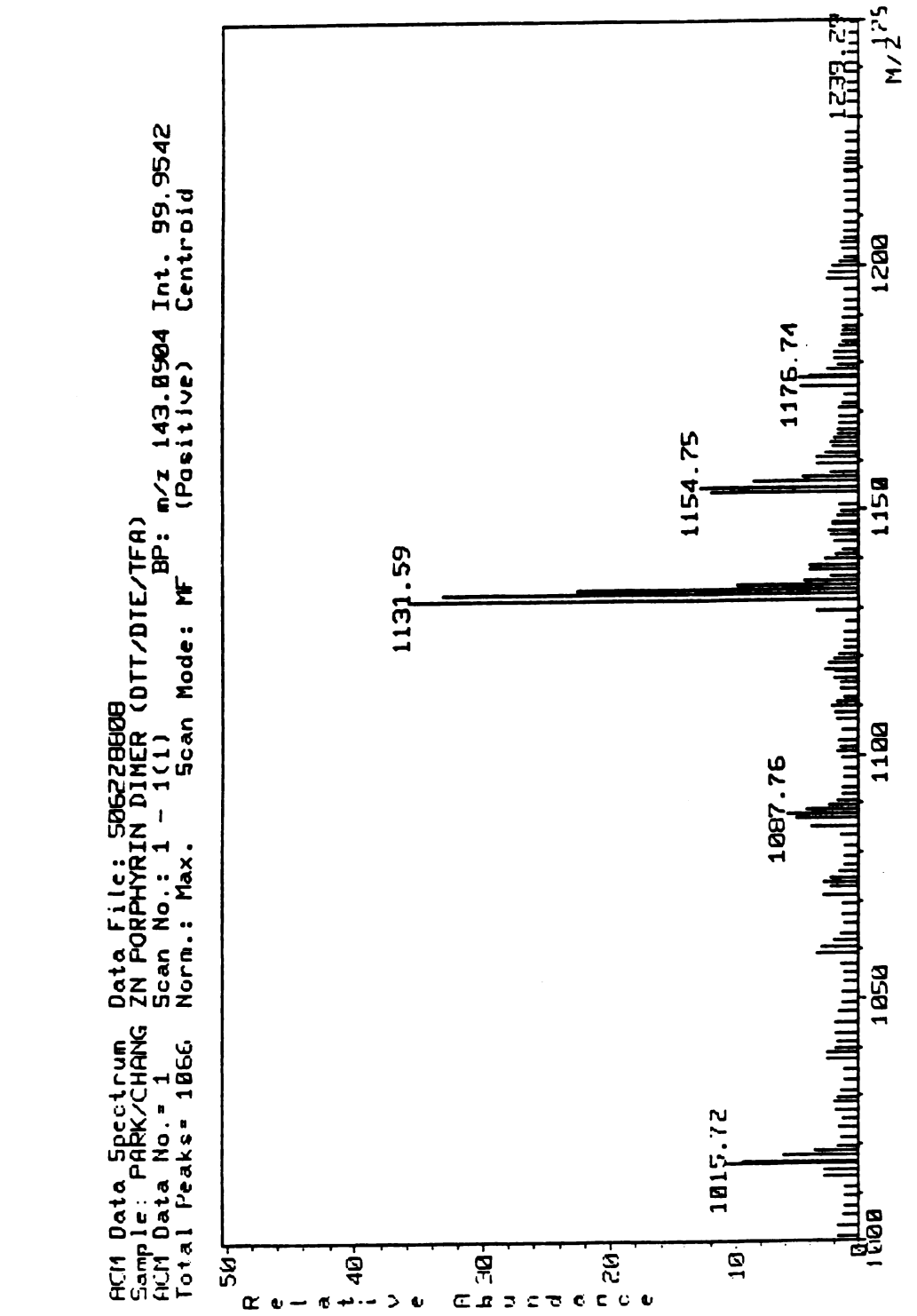


Figure 15. Fast atom bomardment mass spectrum (FAB-MS) of diporphyrin. 33

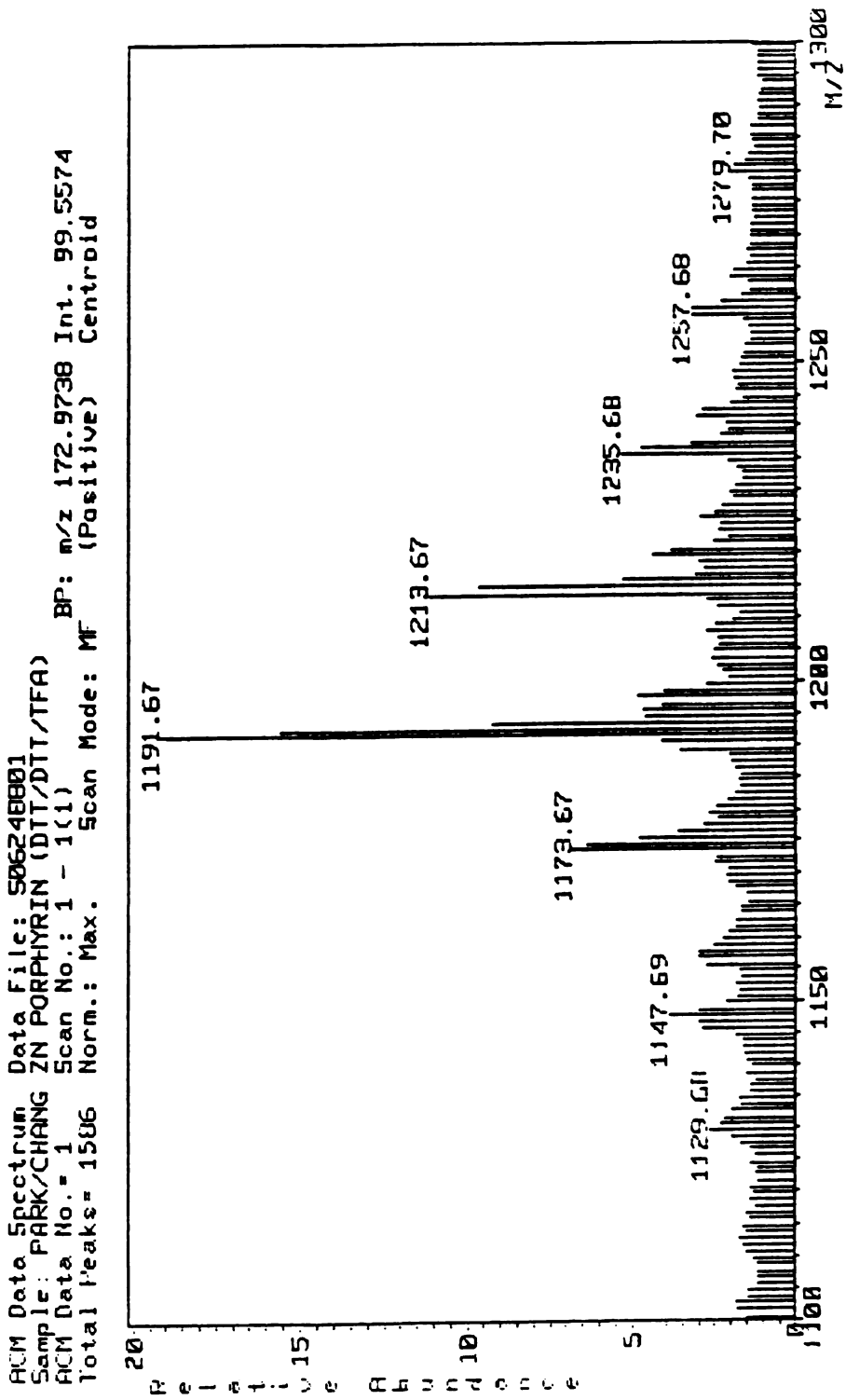


Figure 16. Fast atom bomardment mass spectrum (FAB-MS) of diporphyrin. 34

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