SYNTHESIS OF PORPHYRINS, CHLORINS AND BACTERIOCHLORINS BY CHEMICAL MODIFICATIONS OF CHLOROPHYLL *a*

BY

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Date Sept. 29, 1995

ABSTRACT

The goal of this project was to synthesize new porphyrins, chlorins and bacteriochlorins via chemical modifications of chlorophyll *a* so that new chemistry and applications of chlorophyll *a* and its related derivatives could be developed. A magnesium-free yet stable derivative of chlorophyll *a*, **7**, isolated from the blue alga *Spirulina maxima*, was employed as the common intermediate in this work.

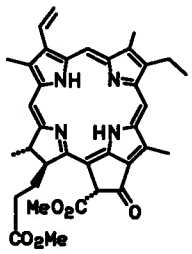
As the first objective, new methods of asymmetric hydroxylation and regioselective oxidation of chlorophyll derivatives were successfully developed. Stereoselective synthesis of natural antioxidative chlorins **81**, **82**(S), **82**(R), **83**(R), **84** and **102**, isolated from marine metabolites, was performed in a short and effective way. This biomimetic synthetic approach helped to elucidate the possible biogenetic evolution of these antioxidative chlorins from chlorophyll *a*.

The second objective of this work was to design a method to convert chlorin **112** into porphyrin **114**. A novel and effective acid-catalyzed tautomerization reaction was discovered and optimized, which has provided a new view on the migration of hydrogens in the saturated ring IV to the exocyclic ring V. The porphyrins so produced were used as intermediates for the further preparation of chlorophyll related petroporphyrins and regiochemically-pure benzoporphyrin derivatives (BPDs).

Making use of the aforementioned tetrapyrrolic materials, the third objective of this work was to develop new photosensitizers for photodynamic therapy of tumors. New monovinylporphyrins and an [A,C]-divinylporphyrin **147** were synthesized. Diels-Alder reaction of these (di)vinylporphyrins with dimethyl acetylenedicarboxylate (DMAD) afforded new regiochemically-pure BPDs **125** and **141** and dibenzoporphyrin derivative **165**. These new sensitizers have characteristics that meet or exceed the promising chemical features of benzoporphyrin derivative monoacid ring A (BPDMA), a second generation sensitizer in Phase-II clinical trials.

The final objective of this work was to exploit the nucleophilic behaviour of the bicyclic amidines, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1,5-diazabicyclo-

[4.3.0]non-5-ene (DBN). Two reactions were examined. Firstly, DBU acting as a difunctional nucleophile quantitatively reacted with DMAD to afford a fused tricyclic derivative **176**. Secondly, **7**, a weak electrophile which alone does not electrophilically react with DBU or DBN, has reacted, through catalytic activation by Lewis acids, with nucleophilic DBU and DBN to form chlorin e_6 amides **185** and **186**. These results have brought about further understanding of the nucleophilicity as well as the basicity of these common organic bases.



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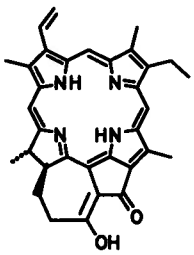

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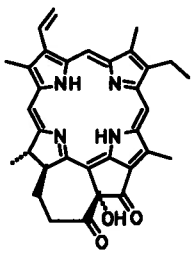

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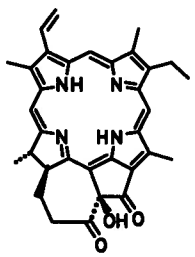

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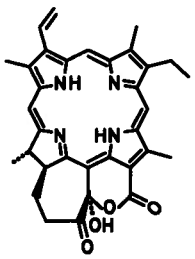

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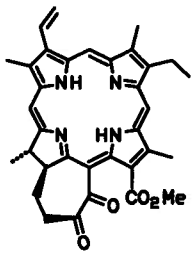

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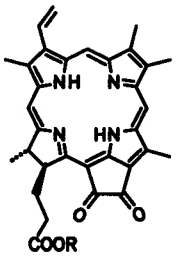

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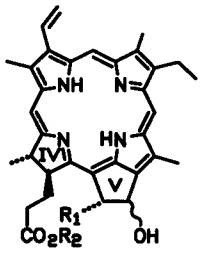

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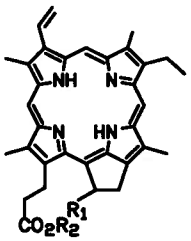

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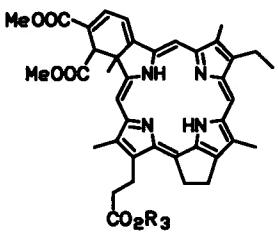

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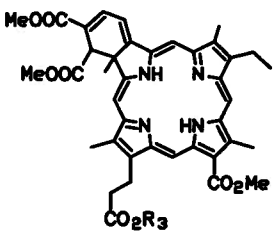

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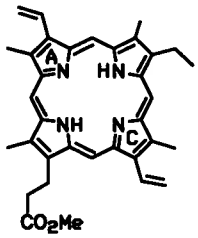

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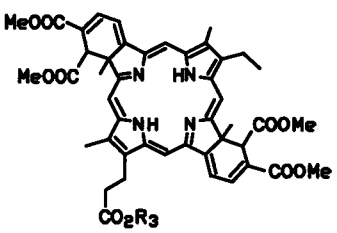

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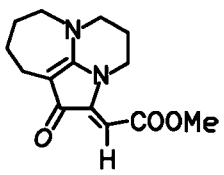

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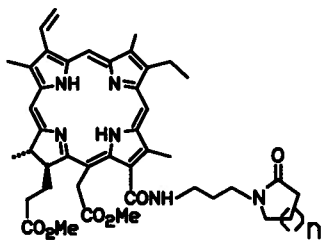

185. n=3

186. n=1

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LIST OF ABBREVIATIONS

Ac	acetyl
Anal.	microanalyses
APT	attached proton test
BChl	bacteriochlorophyll
BPD	benzoporphyrin derivative
BPDMA	benzoporphyrin derivative monoacid (ring A modified)
br	broad
Bu	butyl
calcd	calculated
CD	circular dichroism
Chl	Chlorophyll
COSY	two dimensional proton homonuclear correlation spectroscopy
d	doublet
d.e.	diastereomeric excess
(decomp.)	decomposes
DBN	1,5-diazabicyclo[4.3.0]non-5-ene
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMAD	dimethyl acetylenedicarboxylate
DMF	N,N-dimethylformamide
EI	electron impact
Et	ethyl
ether	diethyl ether
FAB	fast atom barbardment
HETCOR	two dimensional proton heteronuclear correlation spectroscopy
HOAc	acetic acid
HpD	hematoporphyrin derivative
HPLC	high performance liquid chromatography

HREIMS	high resolution electron impact spectroscopy
HRFABMS	high resolution fast atom bombardment spectroscopy
Hz	Hertz
isc	intersystem crossing
IUPAC	International Union of Pure and Applied Chemistry
J	coupling constant
L•	lipid radical
LDA	lithium diisopropylamide
LiBHT	lithium salt of 2,6-*t*-butyl-4-methylphenol
LREIMS	low resolution electron impact mass spectroscopy
LRFABMS	low resolution fast atom bombardment mass spectroscopy
m	multiplet (in NMR)
m	mass (in mass spectroscopy)
mCPBA	*m*-chloroperbenzoic acid
meq	milli-equivalent
Me	methyl
m.p.	melting point
NMR	nuclear magnetic resonance
nOe	nuclear Overhauser effect
obsd	observed
P	phytyl (in NMR assignments)
PDT	photodynamic therapy
Ph	phenyl
PhCOCl	benzoyl chloride
POV	peroxide value
ppm	parts per million
Pr	propyl
py	pyridine
q	quartet

R	R configuration at C-13^2 or C-15^1 or C-13^1 position where appropriate
re	face in which the groups in this sequence are clockwise
t	triplet
S	S configuration at C-13^2 or C-15^1 or C-13^1 position where appropriate
sh	shoulder
si	face in which the groups in this sequence are anti-clockwise
TBD	1,5,7-triazabicyclo[4.4.0]dec-5-ene
TBDMSOTf	*tert*-butyldimethylsilyl triflate
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
TMSOTf	trimethylsilyl triflate
t_R	retention time
UV	ultraviolet
UV/Vis	ultraviolet and visible
z	electron charge (in mass spectroscopy)

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Chapter 1

Introduction

1.1 Overview

Tetrapyrrolic macrocycles (**Fig. 1.1**), e.g. porphyrins, chlorins, bacteriochlorins, isobacteriochlorins, corphins and corrins, form the macrocyclic skeletons of important natural prosthetic groups of living systems on this planet.[1-4] Each of these porphinoids consists of four pyrrole-type rings linked together directly or more commonly through methine bridges. A striking feature of all these cofactors is that the substitution around each macrocycle is based on the same pattern derived from uroporphyrinogen III[5-7] (**Fig. 1.2**). This common intermediate is biosynthesized from 5-aminolevulinic acid by way of porphobilinogen and preuroporphyrinogen[5-7] (**Fig. 1.3**).

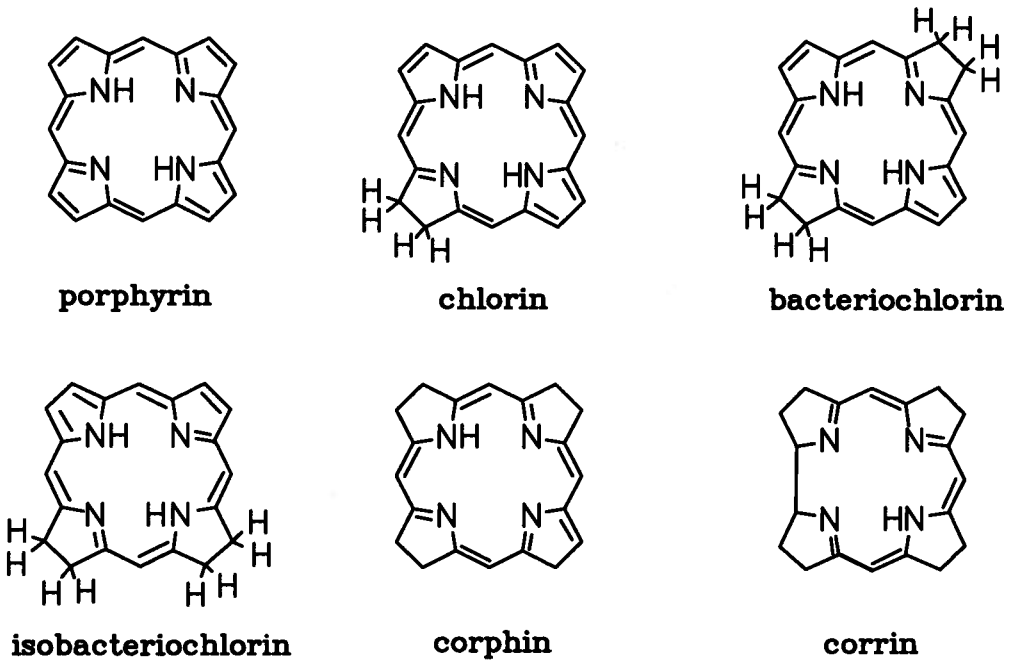


Fig. 1.1 Important Tetrapyrrolic Macrocycles

Fine tuning of these porphinoid ligands for optimal biological function is performed in nature by the appropriate choice of the oxidation levels of the macrocycle, the nature of the peripheral substituents and the coordinated metals[8-9] (**Fig. 1.2**). For example, porphyrins, the tetrapyrrolic macrocyles at the highest oxidation level, function

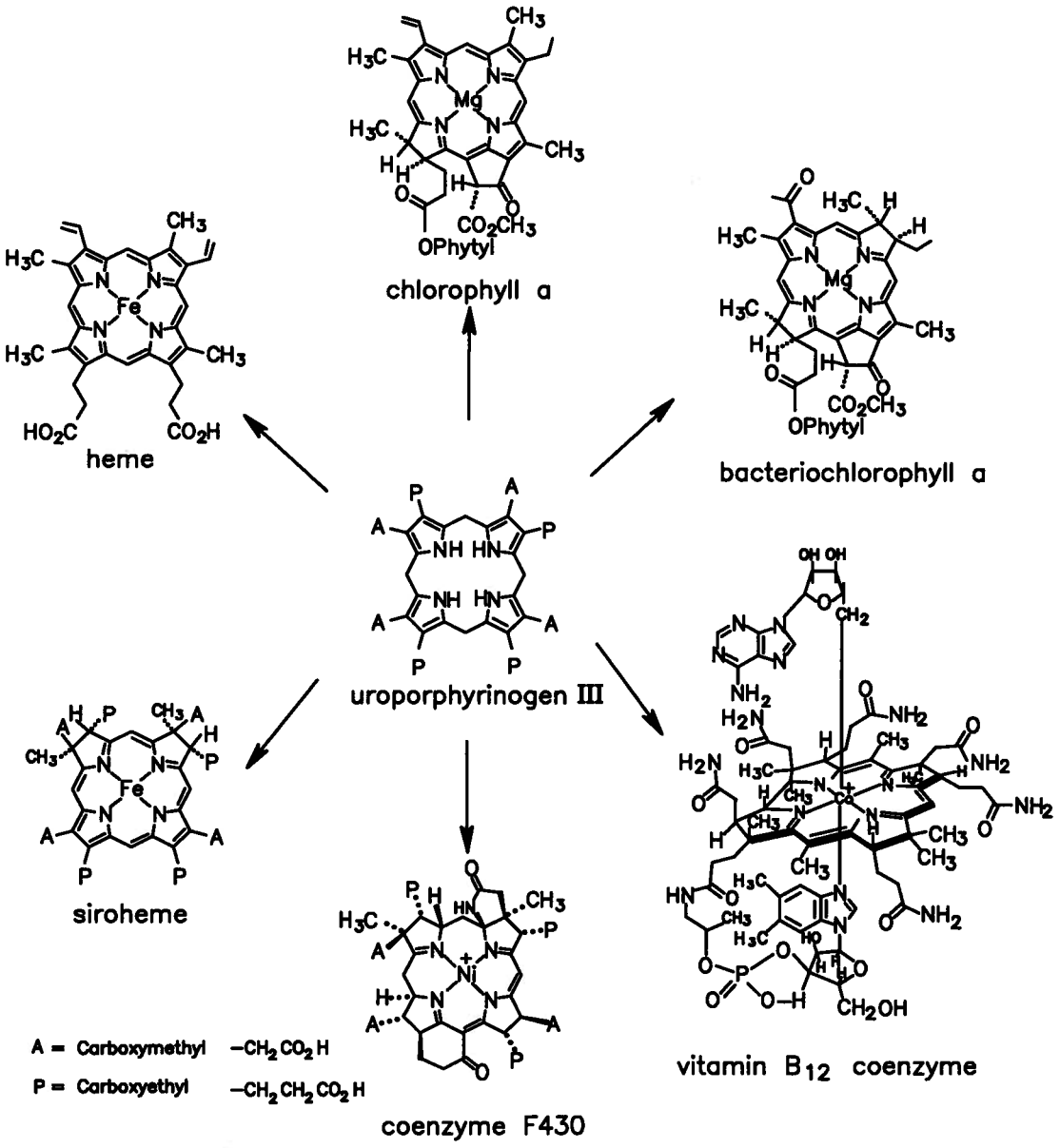


Fig. 1.2 The Cofactors Derived from Uroporphyrinogen III

as their ferrous complexes in the binding of dioxygen and electron transport (hemoglobin, myoglobin, cytochromes and cytochrome P-450) and ferric complexes for numerous oxidations utilizing hydrogen peroxide (catalases and peroxidases).[10] Chlorophyll *a*, a magnesium (II) complex of a dihydroporphyrin (a chlorin) is responsible for the light-harvesting and trapping activities in plants and algae.[11] A magnesium containing tetrahydroporphyrin, bacteriochlorophyll *a*, is the main component of the photosynthetic apparatus of purple and green bacteria. Recent advances in the study of the bacterial photosynthetic reaction center from the purple bacterium *Rhodopseudomonas viridis* illustrate the fundamental atomic structure of the basic machinery and the possible evolutionary sequence with which nature precisely manipulated the basic uroporphyrinogen molecule into an efficient photochemical device (for which Deisenhofer, Huber and Michel won the Nobel Prize in Chemistry in 1988).[12-13]

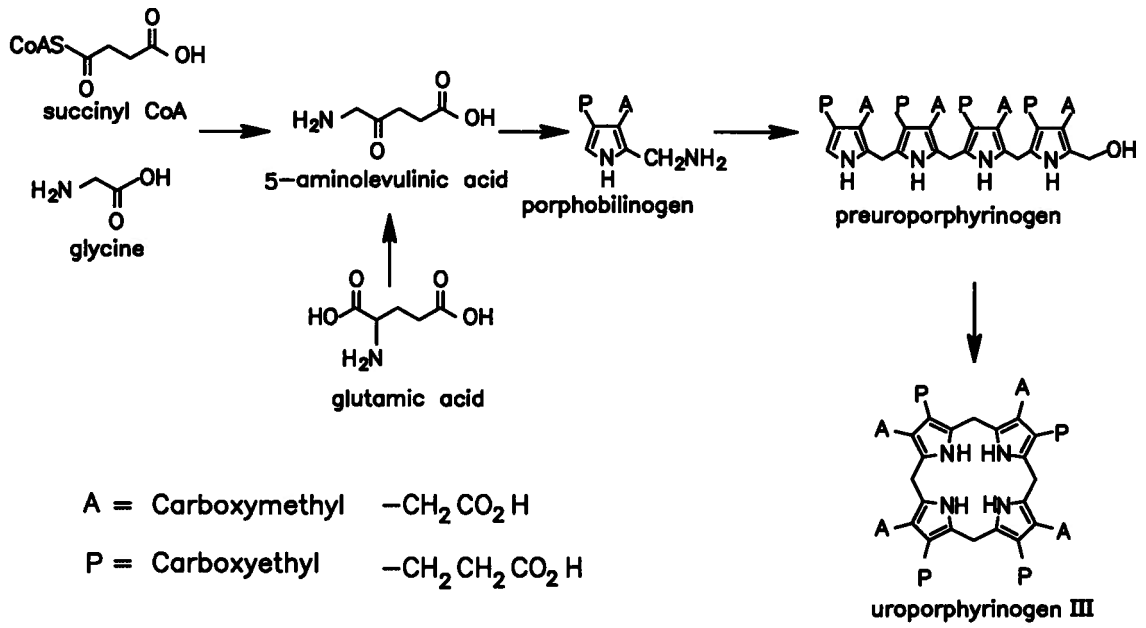


Fig. 1.3 Biosynthesis of Uroporphyrinogen III

The constitutional isomers of bacteriochlorins with two adjacent saturated pyrrolic rings are called isobacteriochlorins. They are widely distributed in bacteria and plants. One typical example is siroheme, the iron-coordinated sirohydrochlorin which is the prosthetic group of a number of sulfite and nitrite reductases.[14] These enzymes catalyze the six-electron reduction of sulfite to sulfide and nitrite to ammonia, respectively.[15] Further reduction of the tetrapyrrolic ring leads to the nickel hexahydrocorphin ring system of coenzyme F_{430}[9] (**Fig. 1.2**). Coenzyme F_{430} is a prosthetic group of methyl-coenzyme M reductase, an enzyme which catalyzes the reductive cleavage of S-methyl coenzyme M [2-(methylthio)ethanesulfonate] to methane.[16-17] A final example, the structurally most complicated member of such macrocycles is vitamin B_{12}, the cobalt complex of a corrin. Vitamin B_{12} is an essential vitamin for human health, a deficiency of which leads to pernicious anemia.[18] It is also the prosthetic group of numerous enzymes which carry out various rearrangement reactions and trans-methylations.[18] This unusual organic ligand surrounding the metal cobalt displays many stereogenic centers along its periphery carrying reactive functional groups. The saga around the complete pathway of its biosynthesis from 5-aminolevulinic acid has been successfully uncovered in 1995 after 25 years of research.[18]

The above examples illustrate the wide diversity of biological functions performed by the metallated tetrapyrroles in nature and account for the continued interest of researchers in the isolation, structural and biosynthetic elucidation, and total synthesis of such molecules. Interest in studies aimed at the isolation of new tetrapyrroles led to essentially two phases of development. The first phase, shortly after the discovery of

metalloporphyrin derivatives in petroleums and sendiments in the 1930's, was initiated by Treibs's hypothesis linking these fossil pigments with two classes of biological molecules, chlorophylls and hemes.[19] The development in this area has given birth to the field known today as "organic geochemistry" and these sendimental porphyrin pigments are appropriately named "petroporphyrins". Up to the time of writing this thesis, more than 80 petroporphyrins have been characterized, each of them either isolated as metal-free or complexed by 4 different metals: nickel, vanadium, copper and gallium.[20] The second phase, begun a half century later, resulted from the investigation of vitamin B_{12} biosynthesis and consisted of the search for the new porphinoid structures in marine metabolites, which has actually brought about the discovery of previously unknown porphinoid macrocycles (some of these new macrocyles even have unusual biological functions).[1] In both cases, isolation, identification, and structural elucidation of the novel structures, which often occur in trace amounts, had only just become possible due to the simultaneous development of new separation techniques and Fourier transform NMR spectroscopy.

Following the isolation and elucidation of these novel structures there are two additional fields of endeavor: total and (sometimes) partial chemical synthesis to demonstrate "the synthetic art of the chemical architect" and last, but not least, deeper understanding of the origin of these macrocyclic molecules. Usually the research between these two fields is closely related. The former research not only develops the new synthetic methodology (e.g. the outstanding synthesis of chlorophyll *a*[21]) and thus the ability to (in most cases) produce sufficient amounts of material for subsequent investigations, but also provides conceptual reasons and insights on the latter

(biosynthetic pathways). A good example of this is the synthetic approach of vitamin B_{12}, which uncovered the "dark" variant of the biosynthetic A/D-decocorrin → corrin cycloisomerization.[3]

A new practical application of the chemistry of porphinoid compounds is the burgeoning interest in photodynamic therapy that takes advantage of the tetrapyrrole-photosensitized generation of singlet oxygen to attack tumours.[22] Porphyrin derivatives incorporating boron clusters are also successfully used in boron neutron capture therapy to deliver radiation to tumors *in situ*.[23] Originally, hematoporphyrin derivatives were shown to have necrotic activity.[24] More recently, attention has shifted to chlorins and bacteriochlorins with their red-shifted absorption spectra since red light penetrates deeper into tissues than blue light.[25] Besides the medicinal application, there is a group of chemists fascinated by the possibilities of their practical application in a wide diversity of fields, such as solar energy conversion[26], catalysis[27], and the possibilities offered by the rapidly expanding area of materials with novel electrical and optical properties.[28]

In addition to medical and industrial applications, tetrapyrrolic macrocycles serve as important model compounds for the study of theoretical concepts such as aromaticity, electron transfer, quantum chemistry and diamagnetic ring currents. Interest in the systematic exploration of potentially aromatic porphinoid chromophores has led to the synthesis and study of the larger aromatic pyrrole-containing systems, the so-called "expanded porphyrins".[29] Some of examples are shown in **Fig. 1.4**. Such systems, by virtue of containing a greater number of π-electrons, a greater number of donating (e.g. pyrrolic) groups, or a larger central binding core have properties which differ

substantially from the far better studied porphyrin analogues. It has been demonstrated that the (4n+2) rule of Hückel is valid in these large systems as long as sufficient stablization of planar conformations is provided.[30]

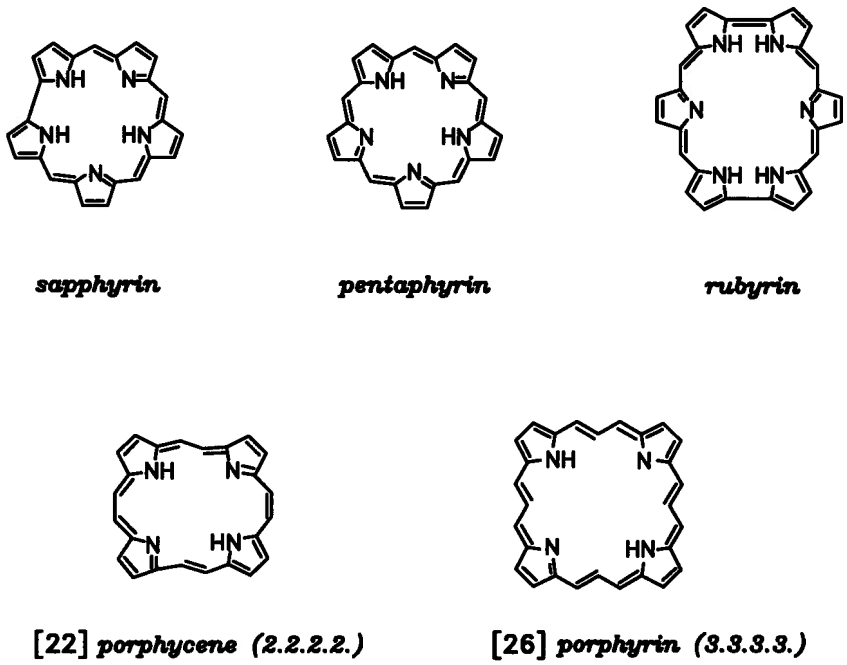
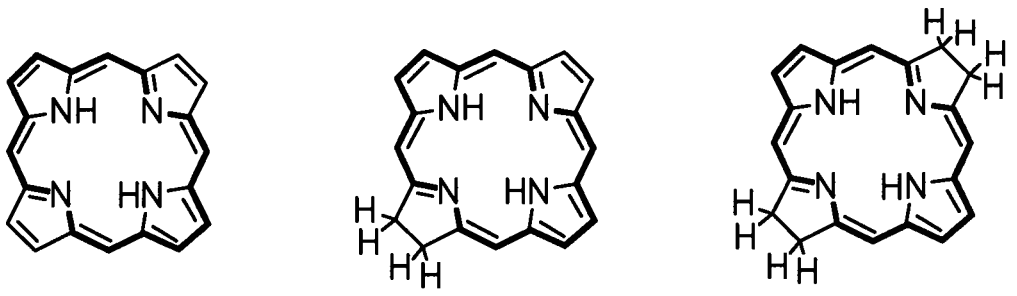


Fig. 1.4 Examples of Expanded Porphyrins

The continuing exploitation and further understanding of the seemingly inexhaustible treasury of porphinoids bear witness to the enigmatic inventiveness with which nature is able to manipulate molecular architecture. It is not unreasonably optimistic to believe that an effort with contributions from chemistry, physics, genetics and biochemistry will make progress toward the ultimate understanding of chemistry of these important molecules and, in turn, benefit mankind.

1.2 Structural Features

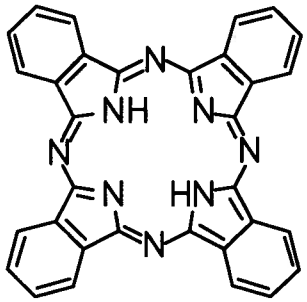
Porphinoids, e.g. porphyrins (fully-unsaturated porphinoids), chlorins (dihydro porphyrins) and bacteriochlorins (tetrahydroporphyrins), are tetrapyrrolic macrocycles containing four pyrrole units linked by methine bridges. They can be classed as polyene chromogens, as they contains no donor or acceptor groups, and as they are structurally similar to the annulenes.[31] Each of these species maintains aromaticity in the macrocycle through an 18 atom, 18 π-electron system, i.e.18-diazaannulene, inner-outer-inner-outer delocalization pathway (**Fig. 1.5**) in accordance with Hückel's 4n+2 rule (n=4). The macrocycle generally maintains the planarity demanded by the delocalized π-system although exceptions have been synthesized.[32] ^{1}H NMR spectroscopy shows clearly that this ring system is diatropic and the shielded inner NH protons appear at relatively high field (δ= 0 to -5 ppm). The outer methine protons, deshielded by the aromatic ring current, appear at 8 to 10 ppm. The remaining two double bonds in porphyrins are cross-conjugated and can be reduced to the corresponding chlorins and bacteriochlorins without markedly affecting the aromatic π-electron system despite the apparently large decrease in conjugation.



porphyrin **chlorin** **bacteriochlorin**

Fig. 1.5 Delocalized Electron Pathways of Tetrapyrrolic Macrocycles

Many structural modifications of the porphinoids are possible. Azaporphyrins are obtained by replacing the carbon atoms of the methine bridges [i.e. the α to δ positions of porphyrin in **Fig. 1.5**) by nitrogen, and the benzoporphyrins possess benzene rings fused to the 2-3, 7-8, 12-13, or 17-18 positions of macrocycle. Phthalocyanine is a commercially valuable blue pigment which embodies both modifications, and can be described as tetrazatetrabenzoporphyrin. The porphinoids that contain two hydrogen atoms at the center of the molecule, are called the *free-base porphinoids*. The central nitrogen atoms are basic, and can accept two more protons, to give the *porphinoid dications*, and in addition, the two N-H hydrogen atoms are acidic and can be removed by strong bases to give the *porphinoid dianions*. Various metal ions can replace the central hydrogen atoms of the free-bases, when coordination to all four nitrogen atoms is possible, as exemplified by the Mg^{2+} ion in the chlorophylls.



phthalocyanine

Usually the porphinoid nucleus is a highly thermal-stable macrocyclic system. It is even stable towards concentrated sulfuric acid and trifluoroacetic acid, both of which are often used to remove coordinated metals. Conversely, the non-fully conjugated porphyrinogens such as uroporphyrinogen III are considerably less stable and are randomized in acids. Solutions of porphinoids are relatively unstable to light, which may

result in photooxidation and/or photodegradation of the peripheral substituents and/or porphinoid macrocycles depending on the substrate and reaction conditions.[10]

1.3 Nomenclature of Porphinoids Related to Chlorophylls

Two systems of tetrapyrrole nomenclature are currently in wide use. The Fischer system for chlorophyll derivatives is shown in **Fig. 1.6**, and features eight peripheral positions, numbered 1-8, two additional positions (9,10) associated with the isocyclic ring E, and four interpyrrolic (or *meso*) positions, designated α, ß, γ and δ. Other carbons are numbered with primes (') inward, or with alphabetical letters outward from the central chelating core. The second nomenclature system, as shown in **Fig. 1.6** is recommended by the IUPAC-IUB and is based on the corrin (1-20) system of nomenclature. The four methine positions are numbered 5, 10, 15, 20, and the nitrogen atoms are numbered 21 through 24. Further, the IUPAC-IUB system provides a less ambiguous and more widely applicable way to number the extra exocyclic ring or rings fused to the porphinoid nucleus. Systematic names of substituted porphinoids are formed by the application of

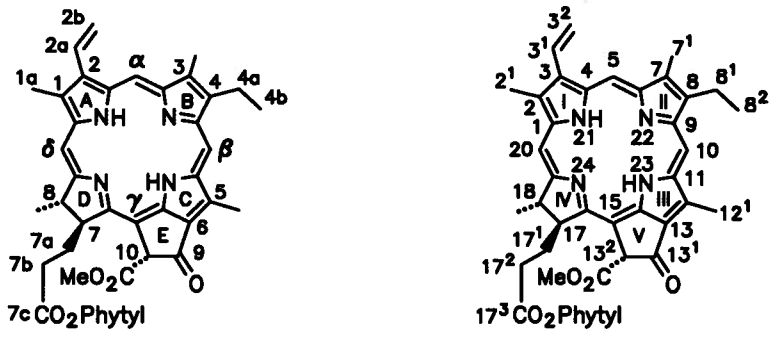
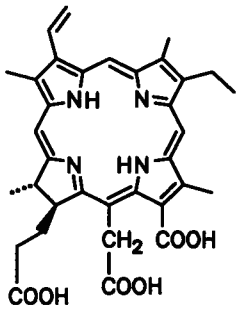


Fig. 1.6 Numbering System of Chlorins

the rules of systematic organic nomenclature. Despite the advantages of the IUPAC-IUB system, the systematic names of chlorins related to chlorophyll *a* become too long and impractical. Therefore, the Fischer system is widely used today and has the merit that it enables contemporary and historical work to be integrated and allows continued use of a large number of classical and indispensable trivial names.

Throughout this work, in order to take advantage of both nomenclature systems, the trivial names with the IUPAC-IUB numbering system, which are also widely adopted in the literature, will be used. For the chlorophyll derivatives, the chlorin (17,18-dihydroporphyrin) derived from direct demetallation of chlorophyll *a* is called pheophytin *a*. With the further loss of a phytyl group, the compound possessing a free propionic acid residue at position 17 is called pheophorbide *a*; its ester can be named in two alternate ways, e.g., methyl pheophorbide *a* or pheophorbide *a* methyl ester.



Fischer: Chlorin e_6

IUPAC: (2S, 3S)-18-Carboxy-20-(carboxymethyl)-13-ethyl-3,7,12,17-tetramethyl-8-vinylchlorin-2-propionic acid

Trivial nomenclature of chlorophylls often uses italic letters and subscript numbers; the latter indicate the number of oxygen atoms in the molecule, therefore, chlorin e_6 has six oxygen atoms and is a chlorin from the chlorophyll *a* series of

degradation products. Letters *a* and *e* are interchangeable, and are used in the chlorophyll *a* series only (e.g. in chlorin e_6), while *g* is used for chlorophyll *b* derivatives (e.g. in rhodin g_7). Much of the remaining chlorophyll nomenclature is difficult to interpret.

1.4 Optical Absorption Spectra

Porphinoids exhibit characteristic absorption and fluorescence properties in the visible region which make them useful as photosensitizers. The metal-free and metallated porphinoids have an intense absorption ($\varepsilon \sim 10^5$) around 400 nm, known as the Soret band.[33] This band is by far the most intense absorption found in all fully conjugated tetrapyrroles and can therefore be regarded as characteristic of this macrocyclic conjugation. The intensity is weaker in chlorins and metallochlorins and, as might be expected, it is totally absent in the non-conjugated tetrapyrroles such as porphyrinogens. The Soret band is also present in vitamin B_{12} and in the metal complexes of bile pigments; both of these types of compounds have interrupted conjugation in the ligand, but the pathway is maintained through the metal atom.[34]

Besides the Soret band, porphyrins also possess four accompanying less intense absorptions usually referred as "Q bands" which appear in the 450-650 nm region. The relative intensities of these four satellite bands, numbered I to IV as shown (**Fig. 1.7**), have been used to identify porphyrin spectra as four basic types: etio-type, rhodo-type, oxorhodo-type and phyllo-type.

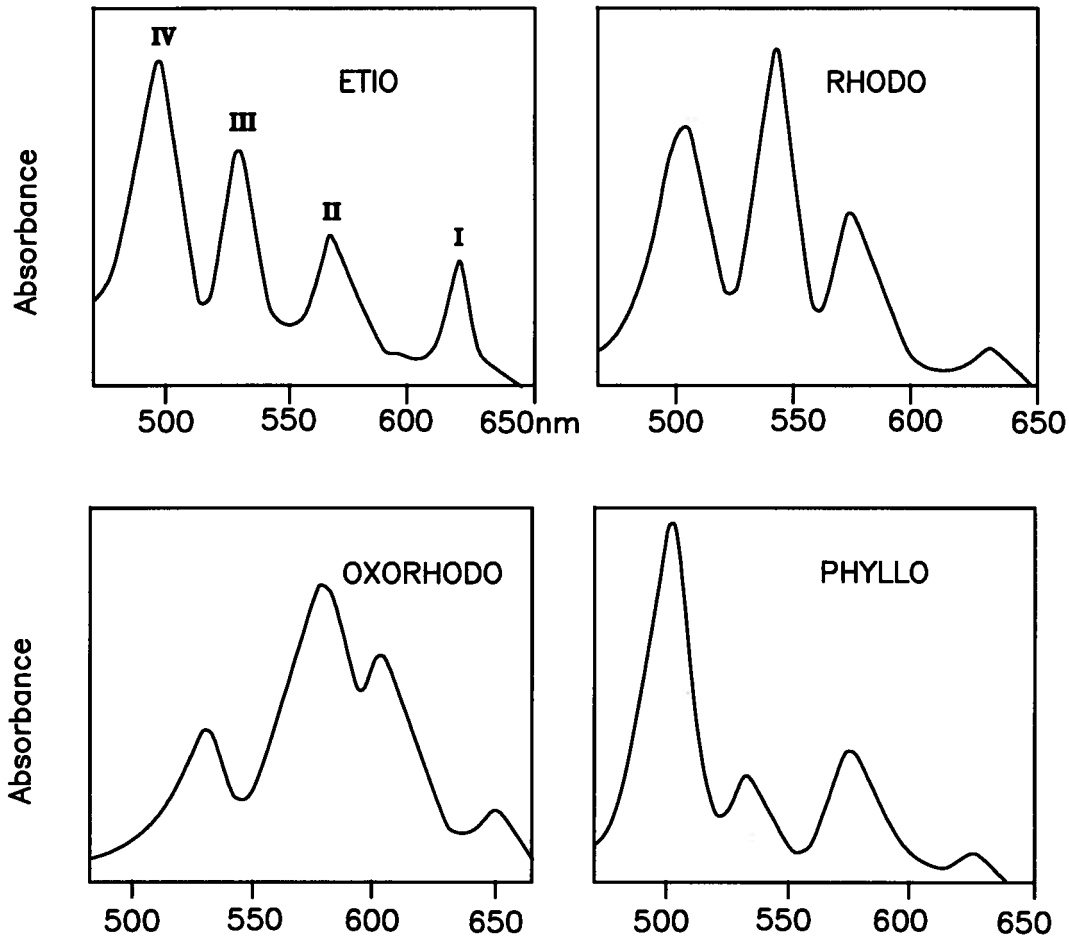


Fig. 1.7 The Four Types of Porphyrin Spectra

a) Etio-type spectrum:

The etio-type spectrum (**Fig. 1.7**) is characterized by a IV>III>II>I order of the band intensities and found in all naturally occurring porphyrins in which six or more peripheral positions carry side-chains such as methyl, ethyl, acetic, or propionic acid groups, the remaining positions being unsubstituted. Naturally occurring porphyrins such as copro-, hemato-, uro-, meso- and deutero-porphyrins all exhibit this type of spectrum.

b) Rhodo-type spectrum:

Porphyrins which display this type of spectra are those which have one strongly electron-withdrawing group (e.g. formyl, acetyl or carboxyl) conjugated with the porphyrin ring. This situation causes band III to be more intense than band IV resulting in the rhodo type spectrum (III>IV>II>I) as shown in **Fig. 1.7** (named after rhodoporphyrin, a degradation product of chlorophyll *a*). The extended conjugation resulting from the strongly electron-withdrawing group ("rhodofying" effect) produces a bathochromic shift of all the bands, which distinguishs this type of spectrum from the etio-type. Rhodo-type spectra are also exhibited by porphyrins which have a benzene ring fused to one pyrrolic ring (benzoporphyrin) and by those with vinyl group substituents. An interesting feature to note is that two rhodofying groups on adjacent "pyrrole" subunits cancel out each other's rhodofying effects and an etio-type spectrum results (e.g. 2,4-diformyl- and 3,8-diacetyl-deuteroporphyrins IX). However, the bathochromic shift of the absorption bands, being additive, still take place.

c) Oxorhodo-type spectrum:

Two rhodofying groups on diagonally opposite rings enhance each other's effect and the oxorhodo spectrum results (**Fig. 1.7**). This is characterized by a band intensity ratio of III>II>IV>I. The oxorhodo spectrum is considered to be the result of a further enhancement of the rhodofying effect.

d) Phyllo-type spectrum:

This spectral pattern (IV>II>III>I, **Fig. 1.7**), named after a chlorophyll *a* degradation product phylloporphyrin, is distinguished from the etio-type by less intense

bands III and I. Two substitution patterns on the periphery produce this spectrum: (i) a single meso-alkyl substitution and (ii) four or more unsubstituted ß-positions.

Metallation of the porphyrin (the dianion formed by the removal of the NH protons) which acts as a tetradentate ligand often changes the four band spectrum to one with two bands, designated α and ß, between 500 and 600 nm while retaining the "Soret" band absorption around 400 nm (**Fig. 1.8**). This is due to the increasing symmetry of the conjugated ring. The relative intensities and the absorption maxima of the α and the ß bands depend on the coordinated metal as well as on the nature of the porphyrin ligand.

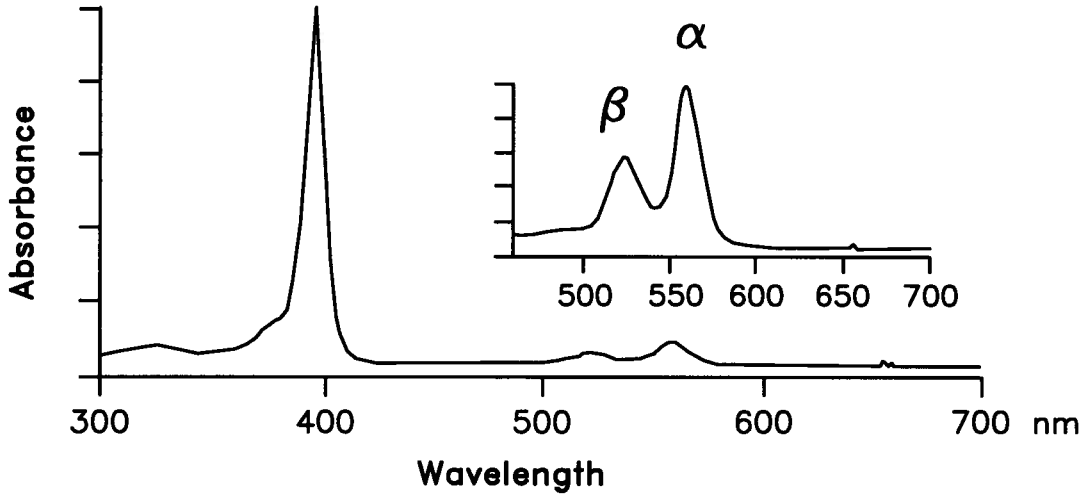


Fig. 1.8 Typical Visible Spectrum of Metallated Porphyrins

The intensity and the exact peak positions are dependent on the solvent as well as the concentration. More importantly, correlations have been shown to exist between the nature of the porphyrin side chains and the positions and the relative intensities of the absorption bands. Actually, the effects of the external substitution on optical spectra are not pronounced, and changes in the electronic structure and conformation of the molecules affect the porphinoid spectra significantly. The reduction of one and/or two

endocyclic double bonds respectively to dihydroporphyrins (chlorins) and tetrahydroporphyrins (bacteriochlorins), although not affecting the aromaticity of the molecule, produces visible spectra characterized by absorptions at 650-680 nm (chlorins) (**Fig. 1.9**) and 750-780 nm (bacteriochlorins) (**Fig. 1.10**).

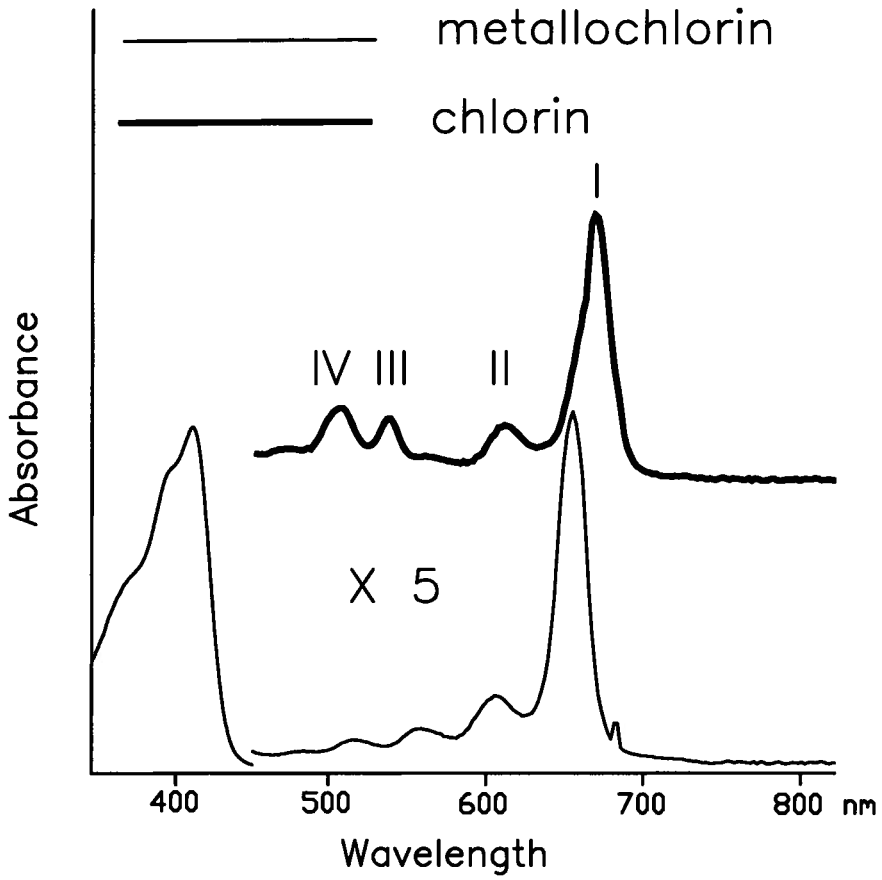


Fig. 1.9 Typical Spectra of Chlorins and Metallochlorins

In chlorins, band I in the visible region is very prominent (**Fig. 1.9**), and is about 25 nm longer wavelength than in porphyrins. The Soret to band I ratio is only about 5 (versus about 50 in porphyrins). The extinction coefficients of band IV and the Soret band, in neutral solvents, are comparable with those of the related bands in analogous porphyrins. Chlorin mono- and di-cations have spectra similar to those of the neutral

compounds, the band I and the Soret absorption being moved to shorter wavelengths in the dications (and in metallochlorins). The Soret band in chlorins has a tendency to be split, this being more noticeable where there is distortion of the resonance pathway, as in the chlorins described in Chapter 2 and Chapter 3.

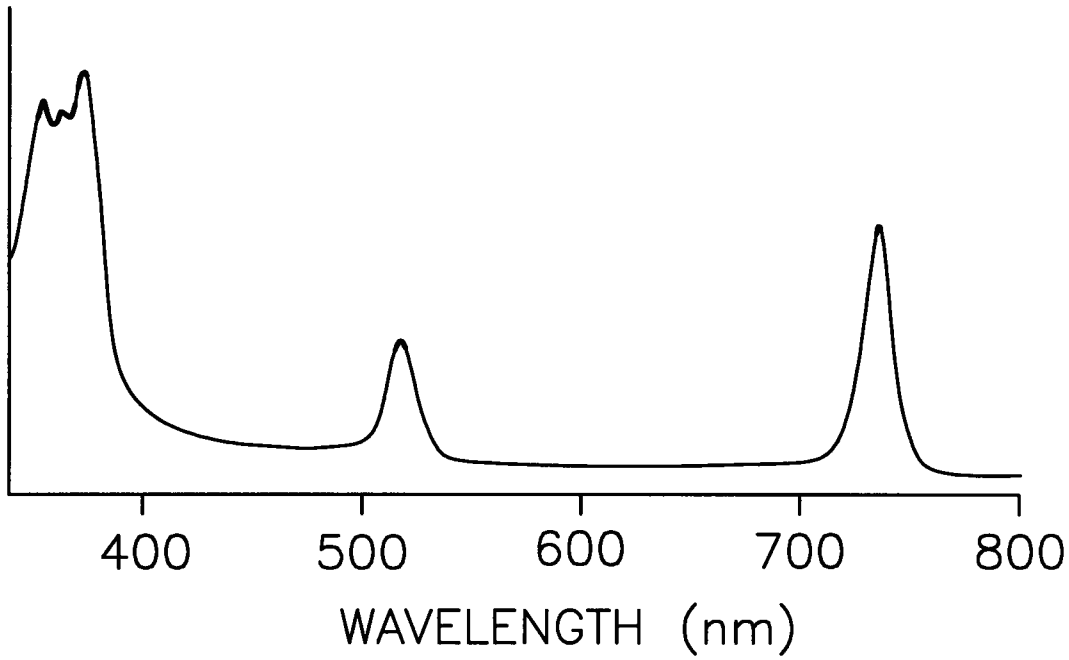


Fig. 1.10 Typical Spectrum of Bacteriochlorins

1.5 Overview of Chlorophyll Chemistry

Chlorophylls are the pigments of photosynthesis, and photosynthesis is the energetic basis of life on earth. The green colour of leaves has fascinated poets and scientists alike since the beginning of human civilization. The pioneering work by Willstätter and Stoll at the beginning of this century marked the establishment of methods for the isolation of the plant chlorophylls and some of their basic chemistry, which were highlighted in the first scientific book in this area.[35]

As the field was established, many famous names became associated with the chemistry of these fascinating compounds. The chemistry and structures of the most naturally occurring chlorophylls *a* and *b* were first elucidated by the efforts of Hans Fischer at a time when science contributed more to destruction than to the support of life. The wealth of these works, which was highlighted in one volume of his book "*Die Chemie Des Pyrroles*", has become as a classic in chlorophyll chemistry.[36] Woodward and his team achieved the enormous task of the total synthesis of chlorophyll *a* in 1960;[21,37,38] the final question of stereochemistry at the carbons 17 and 18 was settled by Fleming in 1967.[39]

The past 30 years also marked an enormous progress in chlorophyll research. Along with improvements in the separation, analytical techniques, coupled with better understanding of their chemistry, the structures of more than 50 chlorophylls and related derivatives from photosynthetic organisms have been established, and the number is still growing.[11] The biosynthesis has been elucidated in considerable detail; there is a beginning in understanding their biological breakdown; and the fossil record now provides good evidence for their fate in geological timescales.

1.5.1 Structure and Occurrence of Chlorophylls

Chlorophylls, named originally for Chl *a* and Chl *b*, now actually represent two large families, chlorophylls and bacteriochlorophylls. Bacteriochlorophylls are named after photosynthetic pigments present in bacteria and are different from chlorophylls present in plants and algae. They are a group of tetrapyrrolic pigments with common

structural elements and photosynthetic functions. In chemical terms, they are magnesium-metallated tetrapyrroles characteristic of a fifth, isocyclic ring that is biosynthetically derived from the C-13 propionic acid side chain of protoporphyrin. Under this definition, the cores of chlorophyll macrocycles can be any porphinoids. Only magnesium complexes of porphyrins, chlorins, or bacteriochlorins possess photosynthetic activity and have been found in nature. Conventionally, the magnesium-free structural analogs are excluded and are called chlorophyll related derivatives, although some of them do function in photosynthesis. Chlorophylls do not occur solitarily within the plant, alga and bacterium world. They are associated with one or two carotenes and several xanthophylls, and sometimes are accompanied by blue or red proteinaceous pigments. Although more than 15 different chlorophylls and bacteriochlorophylls have been isolated so far, only the following are widely distributed in nature.

Chlorophyll *a*

Chlorophyll *a* (Chl *a*) (**1**) (**Fig. 1.11**) is present in all organisms capable of oxygenic photosynthesis, where it occurs in both reaction centers (photosynthetic systems I and II) and in all light-harvesting complexes. It is the most abundant and most important chlorophyll. The molecular structure of Chl *a* has been established by total synthesis of the tetrapyrrole moiety and the 20 carbon terpenoid alcohol, phytol.[21] The stereochemistry at carbons 17 and 18 has been determined by relation to (–)α-santonin and dimethylpentane[39], that at C-13[2] and of phytol by a combination of synthetic and spectroscopic techniques.

Chl *a* has been used as a reference compound in the structural elucidation of many other chlorophylls and related pigments. Originally chlorophyll *a* was isolated from green vegetable spinach and alfalfa.[40] Now it can be readily available from cyanobacteria (blue-green algae) which do not contain Chl *b*.[41] Chl *a* provides a chiral and substituent pool from which a variety of reactions can produce modified structures and allows extensive modifications and correlations among the chlorophylls.

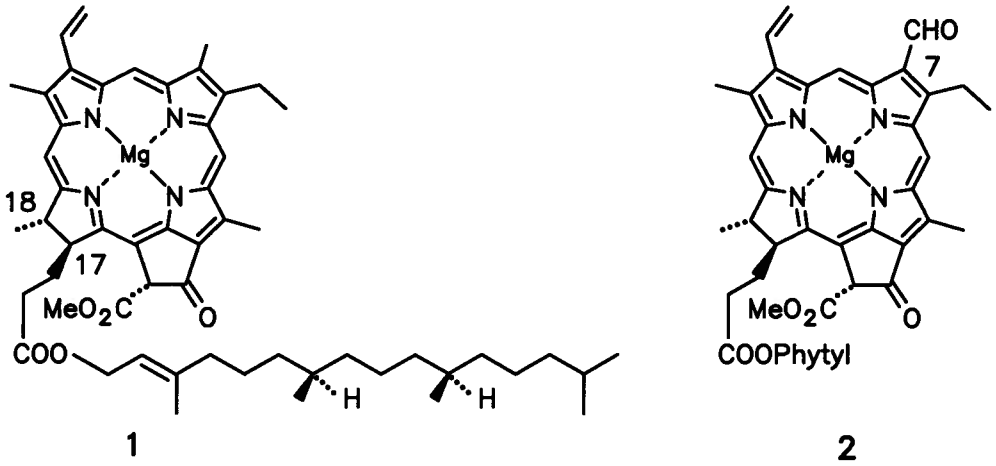


Fig. 1.11 Chlorophyll *a* (**1**) and Chlorophyll *b* (**2**)

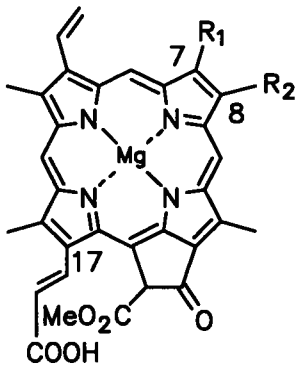
Chlorophyll *b*

Chlorophyll *b* (Chl *b*) (**2**) (**Fig. 1.11**) is distinguished from Chl *a* by a 7-formyl instead of the 7-methyl-substituent. Its structure has been established by chemical correlation with Chl *a*; the stereochemistry and esterifying alcohol (phytol) of both pigments are identical. Due to the electron-withdrawing effects of the carbonyl at C-7, the basicity of the central nitrogen is decreased[42], and the spectroscopic properties are markedly changed. In its electronic absorption spectrum, Chl *b* has a wider absorption of visible light since the Q band of Chl *b* is shifted to shorter wavelengths and the Soret band appears at longer wavelength than the corresponding absorptions of Chl *a*.

Chl *b* accompanies Chl *a* in the "green" series of oxygenic photosynthetic organisms and is generally present as a light-harvesting pigment in about a 1:3 ratio. It has also recently been identified together with Chlorophyll *c* in a few chromophytes, e.g. *Mantionella squamata.*

Chlorophyll *c*

Chlorophyll *c* (Chl *c*) (**3**) (**Fig. 1.12**) or chlorophyllide *c is* the common name for what were originally considered two, and now at least three chlorophylls, which are widely distributed and abundant in the chromophyte algae.[43] These pigments differ from all other chlorophylls in being fully unsaturated porphyrin macrocycles rather than chlorin derivatives. They generally do not carry a long-chain esterifying alcohol at the C-17 acrylic acid side chain and the stereochemistry at the only asymmetric C-13[2] position is unexplored. Three Chl *c* structures have been currently established and they all have an acrylic side chain at C-17. Chl c_1 and Chl c_2 differ by the respective presence of an 8-ethyl- and 8-vinyl-substituent[44,45] and Chl c_3 has a methoxycarbonyl group at the C-7 position.[46]



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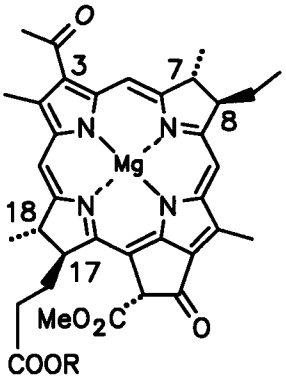

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Fig. 1.12 Chlorophylls of the *c*-type (**3**).Chl c_1: R_1=Me, R_2=Et; Chl c_2: R_1=Me, R_2=C_2H_3; Chl c_3: R_1=COOMe, R_2=C_2H_3; and Bacteriochlorophyll *a* (**4**)

Bacteriochlorophyll *a*

Bacteriochlorophyll *a* (BChl *a*) (**4**) (**Fig. 1.12**) is the most widely distributed bacteriochlorin pigment.[47] It occurs in most photosynthetic bacteria, and is the only bacteriochlorophyll in most *Rhodospirillales*. The stereochemistry at the reduced ring IV and the isocyclic ring V is identical to that of Chl *a* (17S, 18S, 13^2R).[48] The common asymmetric C-7 and C-8 at ring II are both R-configured. The crystal structure of a BChl *a* derivative and several BChl *a* proteins has confirmed this stereochemistry.[49]

Phytol is the most common esterfying alcohol of chlorophylls. Conversely, bacteriochlorophylls carrying alcohol other than phytol are most frequent and the esterifying alcohol varies in different bacteria. For example, BChl *a* from *Rhodospirillum rubrum* and *Rb sphaeroides* respectively, contains Δ2,6,10,14- and Δ2,4,10,14-(geranylgeraniol).[50] The most popular esterifying alcohol in bacteriochlorophylls is farnesol which is present in most bacteria containing BChl *c*, *d*, *e*.[51] The above mentioned specific esterification of bacteriochlorophylls points undoubtedly to the biological significance of these bacteria, but this is still poorly investigated on the molecular scale.

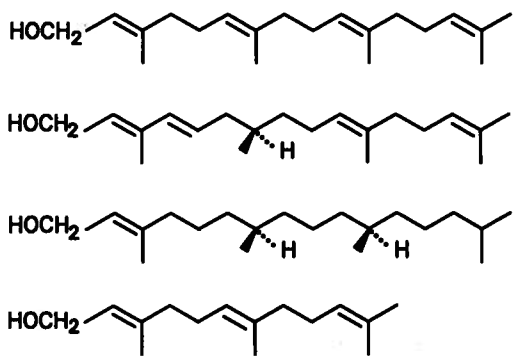
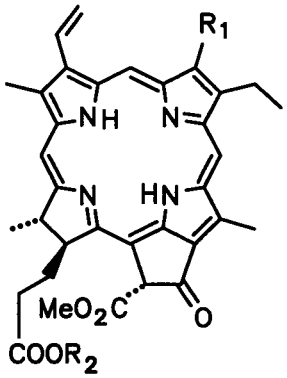


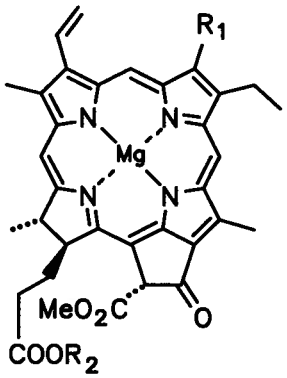
Fig. 1.13 Esterifying Alcohols of Chlorophylls and Bacteriochlorophylls. From top to bottom: geranylgeraniol (Δ2,6,10,14); geranylgeraniol (Δ2,4,10,14); phytol; farnesol

1.5.2 Chemical Modifications of Chlorophyll *a*

The isolation of intact chlorophylls from natural sources is known to be a difficult task because of their extreme susceptibility to various modification reactions, such as enolization, epimerization, allomerization, demethoxycarbonylation, solvolysis, demetallation, dephytylation, and photooxidation. Mild acid treatment of the chlorophylls affords the metal-free pheophytins and this is usually the form in which the pigments are stored prior to further degradation. The mixture of pheophytin *a* (**5**) and pheophytin *b* (**6**) is conveniently separated on a large scale by making use of the reaction of the formyl group in the '*b*' series with Girard's reagent T, followed by chromatographic separation.[52] Methanolysis of pheophytin *a* and *b* produces the corresponding methyl pheophorbides *a* (**7**) and *b* (**8**). It is possible to transesterify the phytyl residue without removal of the magnesium atom; with methanol, the methyl chlorophyllides (**9**) and (**10**) result.



5. R_1=Me, R_2=Phytyl
6. R_1=CHO, R_2=Phytyl
7. R_1=R_2=Me
8. R_1=CHO, R_2=Me



9. R_1=R_2=Me
10. R_1=CHO, R_2=Me

1.5.2.1 Reaction of the Isocyclic Ring

The most prominent group in chlorophylls is probably the enolizable ß-ketoester group at the isocyclic ring V, which (together with the central Mg) is responsible for the strong and specific self-aggregation of chlorophylls.[53] In addition, the C-13^2 is in a position comparable to a benzylic position in aromatic compounds. It is therefore subject to epimerization, enolization and other reactions under basic conditions.

1.5.2.1.1 Epimerization and Enolization

The extent to which ring V exists in the enol form has been much exploited in the past; it is ordinarily almost entirely in the keto form.[54] Closure of ring V introduces strain into the chlorin ring and any further strain that would be introduced by a double bond between C-13^1 and C-13^2 probably inhibits the presence of appreciable amounts of enol under nonbasic conditions. Due to the activation by the two carbonyl functions, the C-13^2 hydrogen atom is highly acidic (no pK_a values have been reported) and does exchange with protons of solvent methanol, even in neutral solution, and the rate constants have been measured by a magnetic resonance technique. Exchange is much accelerated in the presence of base, and in pyridine, even the C-13^2 hydrogens of pyrochlorophyll (demethoxycarbonylation product of chlorophyll) are exchanged at an appreciable rate.

The Chl enolate ions occur as intermediates in the formation of Chl C-13^2 epimers (**11**) (**Fig. 1.14**), which were named Chl *a*' and Chl *b*' by their discoverers, Strain and Manning, in 1942.[55] These chlorophylls and their closely related Mg-free derivatives

have recently been studied thoroughly by several investigators.[56] The epimerization rate was found to increase according to the polarity (Lewis basicity) of the organic solvent used. Thus, in pyridine or triethylamine the reaction occurred rapidly, whereas in benzene, it was very slow.

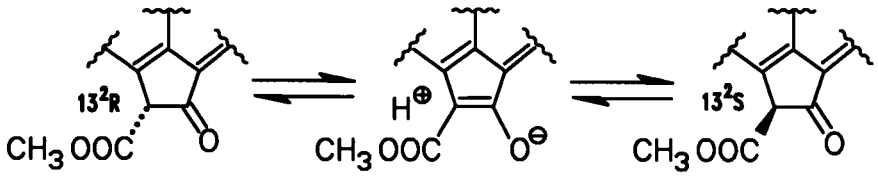


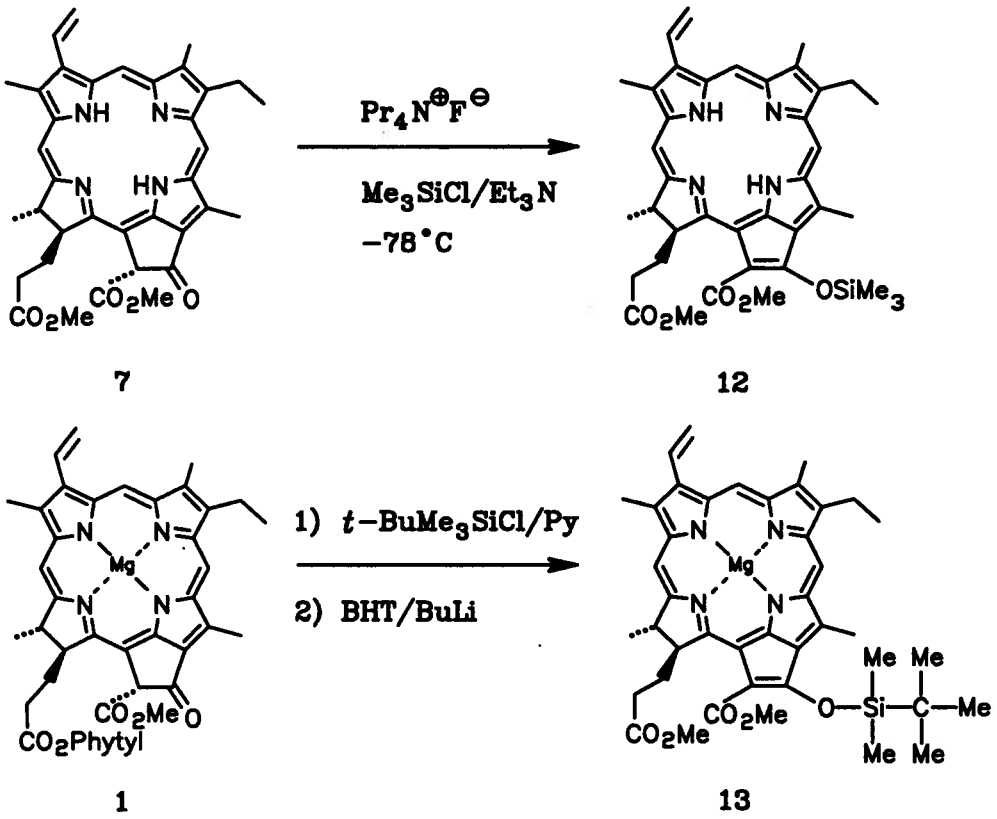
Fig. 1.14 Epimerization at C-13^2 of Chlorophylls

The higher thermodynamic instability of the 13^2 (S)-stereoisomers compared to the 13^2 (R)-isomers has been accounted for by the increase of steric crowding and strain in the periphery of the molecule when the epimerization occurs. The ratio of 13^2 (R)-isomers to 13^2 (S)-stereoisomers is about 83:17 in $CDCl_3$ at 25°C.

The existence of Chl *a*' in nature has been hypothesized, and it is thought to function as a kind of chain breaker in the chlorophyll aggregation *in vivo* or/and in the reaction center(s) of photosynthesis.[57] So far, no experimental evidence for Chl *a*' has been found.

Recently, the potassium enolate of Chl *a* has been prepared in adequate purity to give a well-defined ^{1}H NMR spectrum.[58] Methyl pheophorbide *a* trimethylsilyl enol ether[59] (**12**) (**Scheme 1**) and chlorophyll *a* *t*-butyldimethylsilyl enol ether[60] (**13**) (**Scheme 1**) have been obtained, respectively, by tetrapropylammonium fluoride and the lithium salt of 2,6-*t*-butyl-4-methylphenol (LiBHT) combining with the conventional silylating agents. The creation of a new double bond into ring V in the aforementioned pheophorbide and chlorophyll enol derivatives has a profound effect on the delocalized-

system. A considerable perturbation in the π-system can be experimentally observed (e.g. by ^{1}H NMR). The comparison of their absorption spectra is shown in **Fig. 1.15**.



Scheme 1 Syntheses of Silyl Enol Ethers **12** and **13**

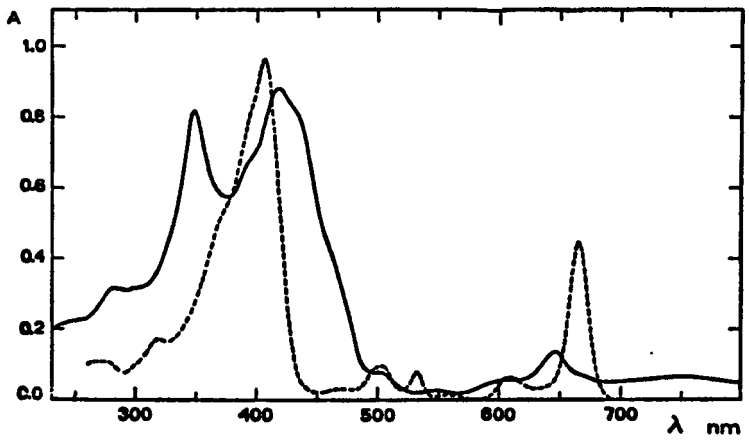


Fig. 1.15 Electronic Spectra of **7** (- - -) and the silylated enol ether **12** (——)

1.5.2.1.2 Ring Opening

The isocyclic ring can be opened by hydrolysis, methanolysis, or aminolysis without simultaneous oxidation at C-13[2]. The formed products usually are chlorin e_6 and its derivatives depending on the attacking nucleophiles. Alkaline hydrolysis (saponification) of Chl *a* or *b* under oxygen-free conditions yields a mixture of the sodium salts, which are water-soluble compounds and consist of more than 9 different chlorophyll degradation products with the major component being chlorin e_6 trisodium salt. By treatment with Cu(II)-acetate, they are converted to the corresponding Cu(II)-complexes, called "Cu chlorophyllin" in industry. Thousands of tons of Cu chlorophyllin are produced each year and it is widely used in the food industry, i.e., in oral hygiene such as chewing gums, toothpastes and in medicinal industry as a treatment for anemia and hypertension, etc..[61]

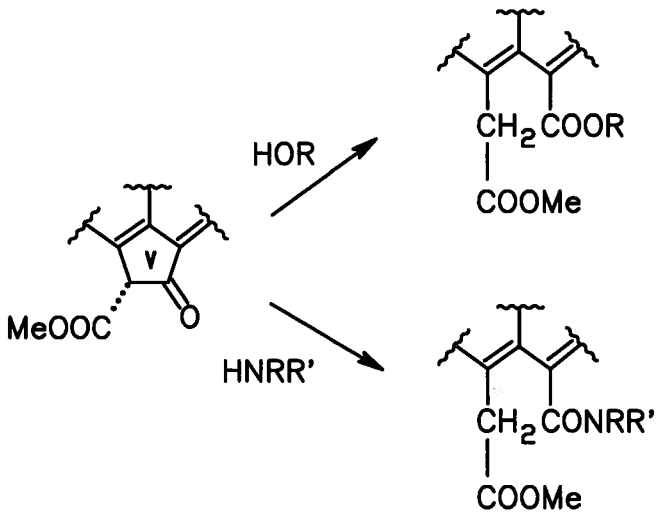


Fig. 1.16 The Cleavage of the Exocyclic Ring by Nucleophiles

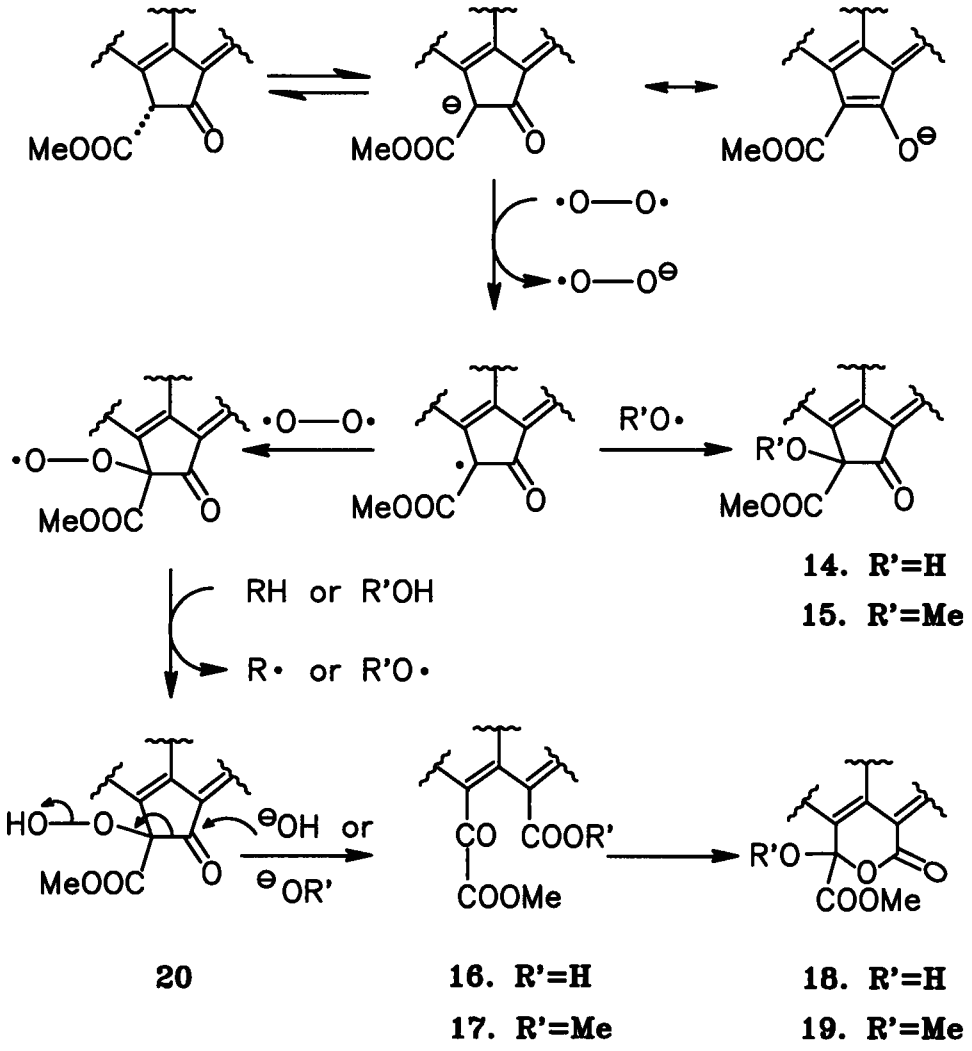
The reaction mechanism of ring cleavage was first proposed by us and will be discussed in Chapter 5.

Alkaline hydrolysis in the presence of oxygen leads to a variety of potentially useful degradation products by way of the "allomerization" reaction. The term "allomerization" was introduced to chlorophyll chemistry by Willstätter[35], who used it to describe the then unknown modification reactions of chlorophylls occurring on standing in alcohol solution in contact with air. Thus, allomerization is synonymous with the term "autooxidation", which implies oxidation by triplet oxygen.

The allomerization reaction is the main difficulty in the isolation of chlorophylls from natural sources and in the handling of chlorophylls to yield Chl-related chlorin and porphyrin derivatives, not easily accessible by total synthesis. When a pure sample of Chl *a* is permitted to stand in methanol for several days in the dark, a great number of allomers and other products can be separated from the mixture. Besides allomerization, solvolysis of the isocyclic ring, demetallation, dephytylation, and photooxidation, if light is present, may occur as side reaction. These reactions appear to be complex, and the precise nature of the products depends upon the conditions. Many of the allomers consist of two 13^2- or 15^1-stereoisomers, some of which have been separated by chromatography.[54]

Among the allomers (**Scheme 3**) that have been identified are 13^2 (R,S)-hydroxy- (**14**) and 13^2 (R,S)-methoxy-chlorophylls (**15**), the Mg-complexes of purpurin-7 trimethyl ester (**16**), 15^1(R,S)-hydroxypurpurin-7-lactone dimethyl ester (**17**), 15^1(R,S)-methoxy-

purpurin-7-lactone dimethyl ester (**18**), and purpurin-18 methyl ester (**19**) (instead of the 17^4-methyl, there can be a phytyl in these derivatives).[62]



Scheme 3 Allomerization and Its Proposed Mechanism

Allomerization has been attributed by Fischer and Pfeiffer to the formation of a 13^2-hydroperoxide[63] (**20**). The 13^2-hydroperoxide intermediate **20** subsequently undergoes a nucleophilic attack by hydroxide or methoxide ion with simultaneous heterolytic cleavage of the C-13^1—C-13^2 bond and the peroxide bond to result in a number of allomers (**Scheme 3**). The plausibility of this free-radical mechanism is that it

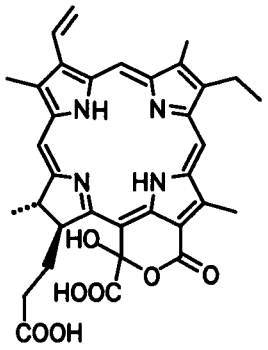
can account for the formation of most allomers, though no intermediates have been detected.

1.5.2.1.3 Pigments from Standard Chlorophyll Degradation Chemistry

Usually pheophytin *a* (**5**) and pheophorbide *a* methyl ester (**7**) are the staging points for literally dozens of subsequent degradation products. Thus, depending on the precise conditions, "unstable chlorin" (**21**) and a number of purpurins can be obtained along with rhodo-, phyllo-, and pyro-porphyrins, etc.. In view of the fact that chlorophyll degradation is a complicated process and usually yields many by-products, only those degradations which furnish chlorins and porphyrins in reasonable and useful quantities will be discussed.

Aerial oxidation of pheophytin *a* (**5**) in alkaline solution cleaves the C-13^1 to C-13^2 bond with concomitant loss of phytol, giving the 'unstable chlorin'[36] (**21**). Evaporation of the solution produces purpurin-18 (**22**), whereas esterification with diazomethane furnishes purpurin-7 trimethyl ester (**16**).[52] If the latter compound is heated in collidine, a 76% yield of 3-vinylrhodoporphyrin XV dimethyl ester (**23**) is obtained.[52] In an analogous fashion, the meso (dihydro, with the 3-vinyl reduced to ethyl) compound affords an 81% yield of rhodoporphyrin XV dimethyl ester (**24**).

If methyl pheophorbide *a* (**7**) is heated in collidine the corresponding methyl pyropheophorbide *a* (**25**) is produced in virtually quantitative yield;[41] heating in pyridine affords a lower yield. Similar reactions take place with the meso-series of compounds.



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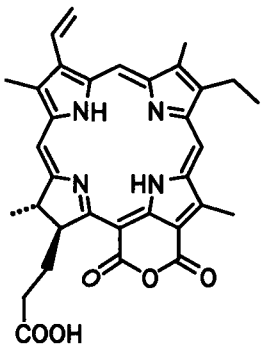

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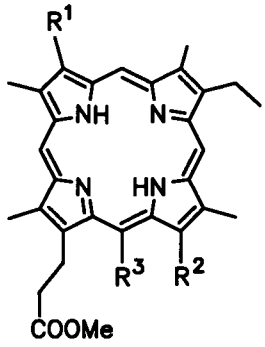
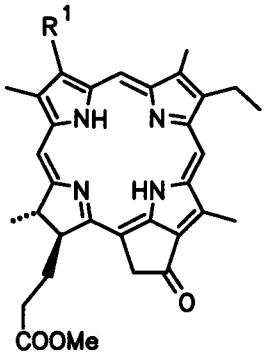


23 R^1=C_2H_3, R^2=COOMe, R^3=H

24 R^1=Et, R^2=COOMe, R^3=H

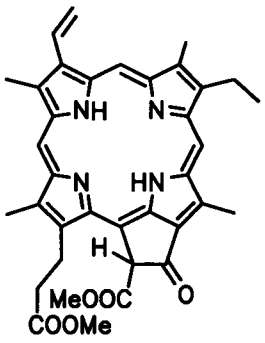
29 R^1=Et, R^2=H, R^3=Me

30 R^1=Et, R^2=R^3=H



25 R^1=C_2H_3

27 R^1=Et



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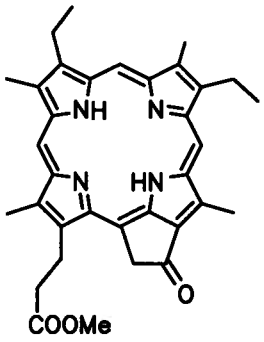

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Treatment of chlorins with high-potential quinones, such as 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) gives the corresponding porphyrins. The oxidation yields vary widely depending on the substrates. For example, methyl pheophorbide *a* (**7**) gives 3-vinylpheophorphyrin a_5 dimethyl ester (**26**) in 28% yield and methyl mesopyropheophorbide *a* (**27**) yields phylloerythrin methyl ester (**28**) in 35% yield.[34]

Various alkaline treatments of chlorophyll, followed by methanolysis, affords, in low yields, phylloporphyrin XV methyl ester (**29**) and pyroporphyrin XV methyl ester (**30**); pyroporphyrin XV(**31**) is also obtained from phylloporphyrin XV (**32**) by treatment with alkali.[36]

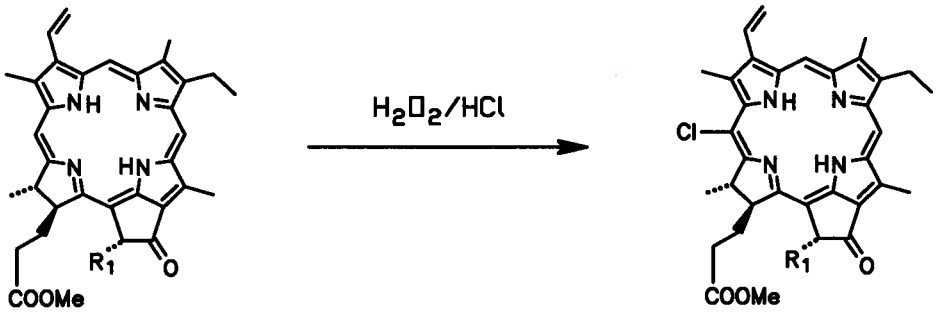
1.5.2.2 Peripheral Reactions

The chlorin macrocylic system, as compared to that of porphyrins, shows two distinct differences in reactivity. The methine positions next to the pyrroline ring have a higher electron density and are, thus, susceptible to electrophilic attack; and chlorins are more easily oxidized both by one- and two-electron oxidants. Both features have been linked to the reactivity of chlorophylls, which are, with the exception of chlorophyll *c*, either chlorins or bacteriochlorins. The easy proton exchange of the δ-H in chlorophyll *a* (**1**) is a direct result of the increased electron density at this position, and especially photooxidation to the cation radical is of eminent biological importance as the genuine light conversion step in photosystem I.[10]

1.5.2.2.1 Electrophilic Substitution

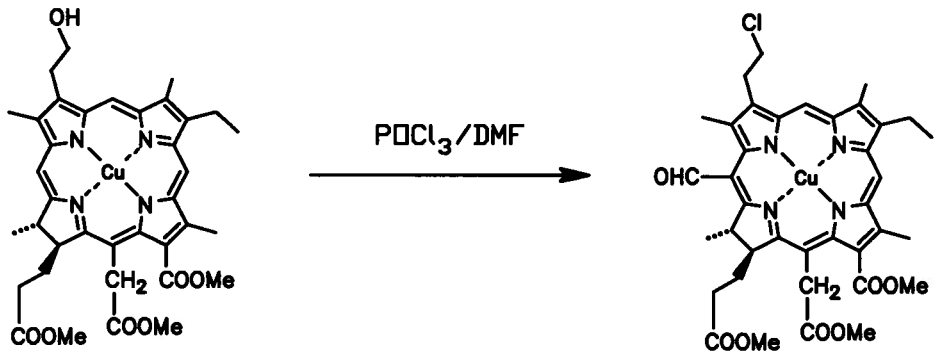
The higher susceptibility of the 15 and 20-positions to electrophiles in simple chlorins (20 position in pheophorbides) was qualitatively predicted and experimentally verified by Woodward.[64] These positions can therefore be selectively deuterated, halogenated, acylated, or nitrated by deuterium, halonium, acylium, or nitronium

electrophiles. In substituted chlorins, the reactivities of the free *meso* and ß-pyrrolic positions in electrophilic substitutions depend on the number and nature of substituents in occupied positions. In general, any substituent increasing the electron density (e.g., alkyl, amino) in the aromatic π-system will activate the *meso* positions and deactivate the ß-



7. R_1=COOMe
25. R_1=H

33. R_1=COOMe
34. R_1=H



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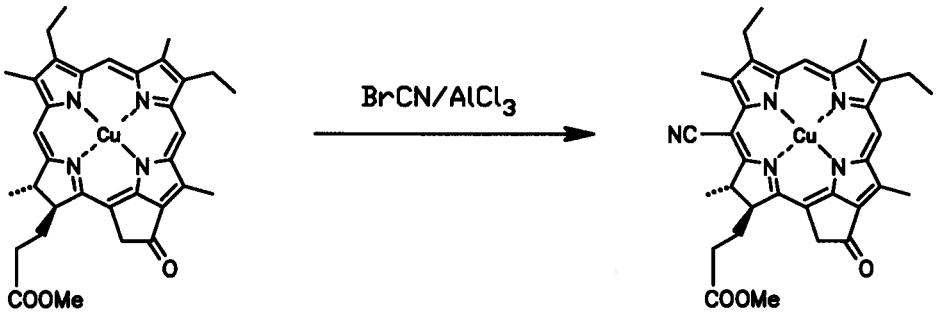

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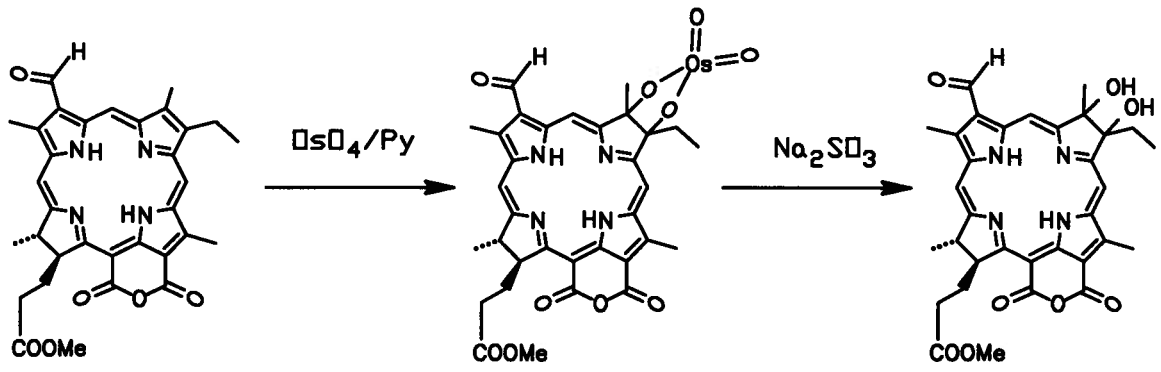
Scheme 4 Electrophilic Substitution Reactions of Chlorins

pyrrolic positions to electrophilic attack. In contrast, an electron-withdrawing substituent (e.g. formyl, acetyl) will deactivate the *meso* and activate the ß-pyrrolic positions in this respect. Also the central metal atom should be looked at as an electron-donating (e.g. Mg^{2+}, Cu^{2+}, Ni^{2+}) or electron-withdrawing (e.g. Sn^{4+}) substituent. Further, steric factors are also of importance. Bulky substituents exert steric hindrance toward electrophilic substitution at positions close to these substituents.

Some examples of electrophilic substitutions are showed in **Scheme 4.**

1.5.2.2.2 Oxidation

Chlorins and bacteriochlorins can be dehydrogenated by several oxidants, such as oxygen, quinones, $FeCl_3$, or $Fe(CN)_6^{3-}$. 3-Vinyl chlorins are dehydrogenated by O_2 or $Fe(CN)_6^{3-}$ in alkaline solution to 3-vinyl porphyrins.[65] These oxidation reactions are rarely controllable and usually lead to overoxidation and produce large amounts of by-products, and, as such, are not useful from a preparative viewpoint.

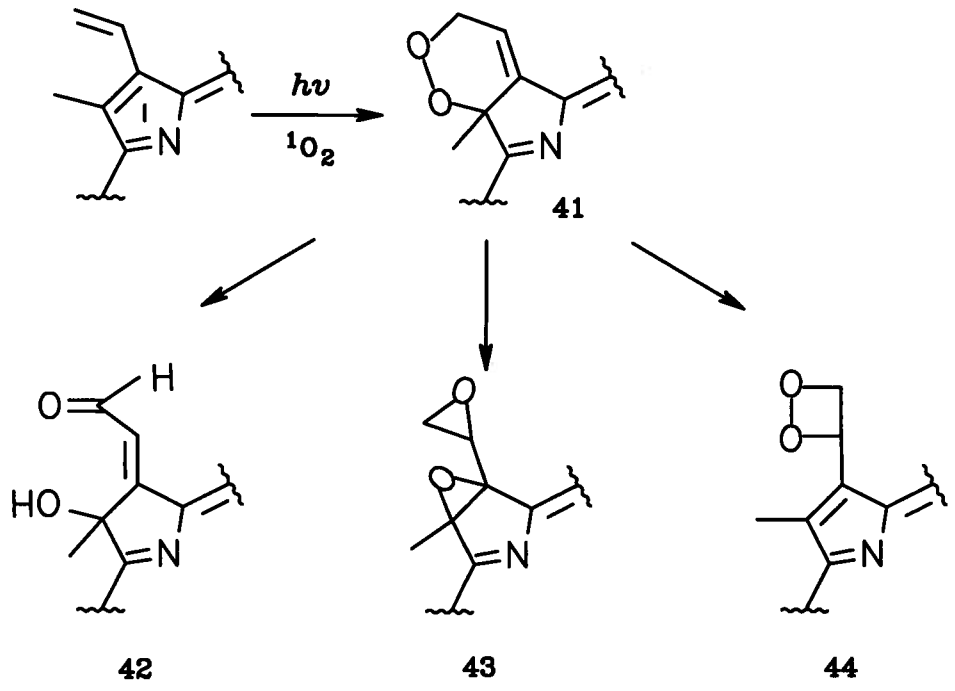


39 40

Scheme 5 Peripheral Oxidation of Chlorins by OsO_4

Chlorins can also form adducts (**39**) (**Scheme 5**) with osmium tetraoxide, which are hydrolyzed in sodium sulfite solution to dihydroxychlorins (**40**).[66] The 3-vinyl group, if present, is oxidized by OsO_4 to the 3-glycol group.

Chlorophylls can be photooxygenated by singlet oxygen to π-cation radicals. This is a "self-destruction" pathway of chlorophylls due to its photosensitizing capability. Photooxygenation of chlorins and bacteriochlorins is responsible for the cleavage of the macrocyclic ring system and subsequent degradation to smaller carbon- and/or nitrogen-containing fragments. This breakdown probably represents the fate of 10^9 tons of annual chlorophyll destruction occurring in nature.

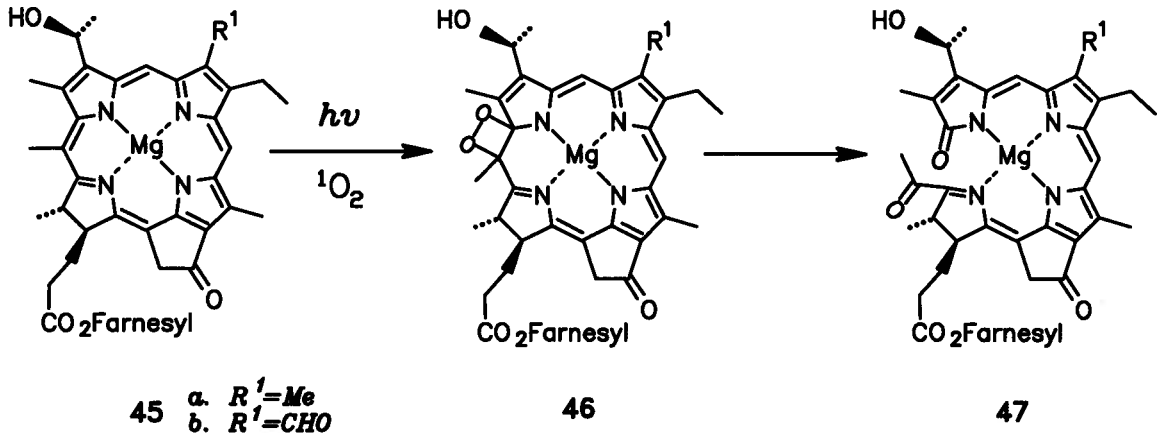


Scheme 6 Photooxygenation of 3-Vinylporphinoids

If a 3-vinyl group is present, as in chlorophylls, photoxidation happens via addition of singlet oxygen in a 1,4-fashion with ring I and 3-vinyl group to form a cyclic

peroxide **41** (**Scheme 6**). This compound can be transformed to the possible strucutures **42, 43**, or **44**. The presence of oxygen and EtOH or pyridine was found necessary for the reaction.[67] In addition, the reaction required a complexed Mg^{2+} or Zn^{2+}, but not an intact cyclopentanone ring. However, no detailed structural analysis of the photooxygenation products was presented to corroborate the proposed mechanism.

The photooxygenations of the non-vinyl chlorins such as bacteriochlorophylls *c* (**45**a) and *e* (**45**b) and their Mg-free derivatives have been studied by Troxler and co-workers[68] and Risch *et al.*.[69] The mechanism of these photooxygenations involves the [2+2] cycloaddition of singlet oxygen to positions 1 and 20 to yield the 1, 20-cycloperoxide intermediate (**46**) (**Scheme 7**), which then undergoes cleavage to afford a bilitriene (**47**). Because the reaction is typical of all Chl derivatives bearing a C-20 substituent, it may provide a route for the degradation of chlorophylls *in vivo*. Unlike the photooxygenation of Chl *a* , these photooxygenations do not require the presence of Mg^{2+} in the molecule.



Scheme 7 Photooxygenation of Bacteriochlorophylls *c* and *e*

1.6 Synthetic Aspects of Natural Chlorins

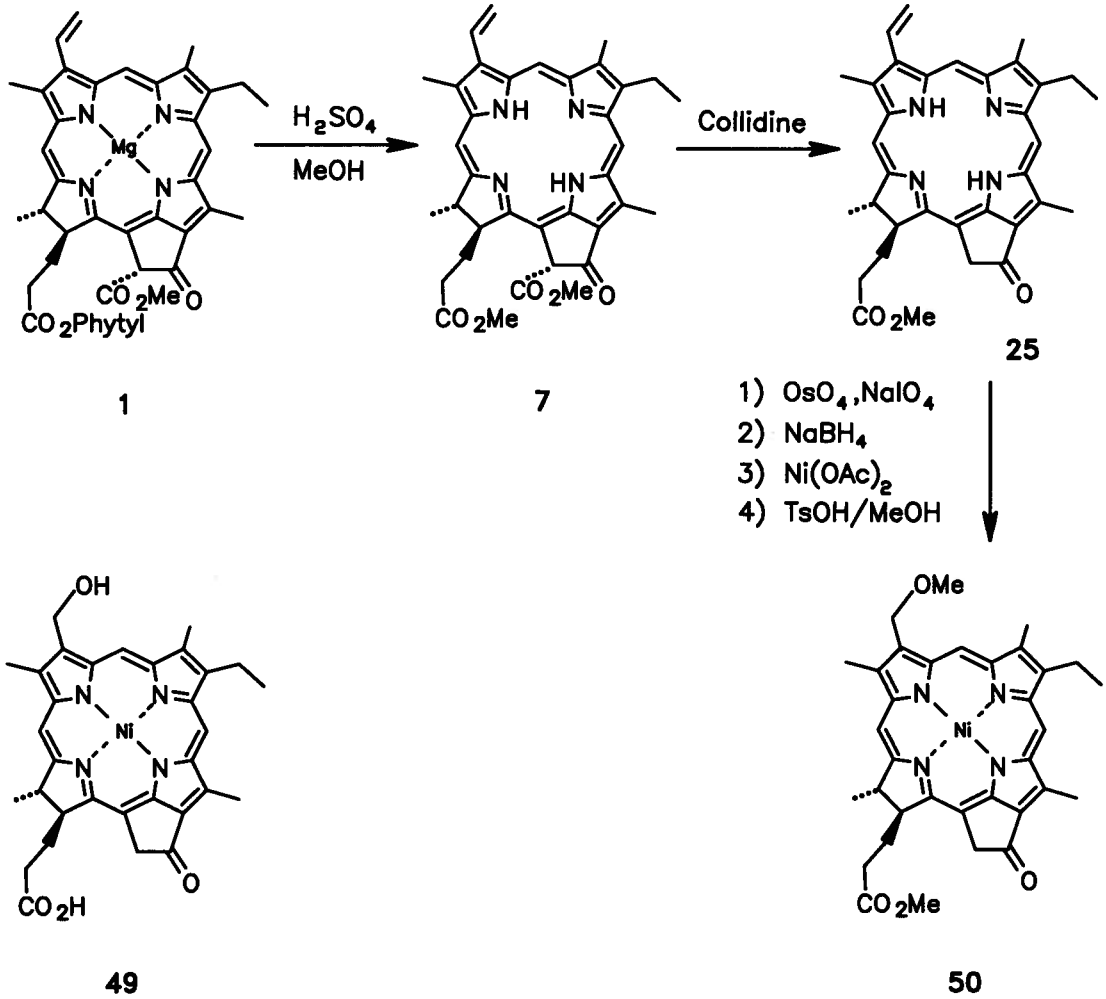
Although myriad synthetic routes to tetrapyrrolic macrocycles have been developed in the past century, most of them have been applied to the synthesis of porphyrins, which are planar with no stereogenic centers at the periphery. The progress in the synthesis of chlorins, bacteriochlorins and isobacteriochlorins has been very slow and in most cases these compounds have been proved to be elusive targets. The main reason for this lack of activity was probably the challenge posed by the presence of stereogenic centers in the molecule and the trans geometry in the reduced ring IV of natural chlorins and bacteriochlorins.

With the exceptions of Woodward's unique approach to the synthesis of chlorophyll *a*, up to the present time no synthetic methods have been devised in which the trans geometry of chlorin (17,18-dihydroporphyrin) ring systems is built-in in a rational stepwise fashion, although various "reduced" systems bearing geminally substituted (nonoxidizable) moieties have been synthesized.[70] Methods for preparation of *cis*-reduced chlorins[71] (e.g. catalytic hydrogenation, di-imide reduction, Raney nickel reduction, photoreduction) and *cis*-oxidized chlorins[76] (e.g. bishydroxylation with osmium tetraoxide) from porphyrins have also been developed. However, these methods are generally complicated by the possibility of stereoisomers, and, when unsymmetrically substituted porphyrins are to be modified, of structural isomers.

The following sections review the most recently synthetic work in the field of natural chlorins, most of which has been carried out by chemical modifications of pheophorbide *a*, the more stable and more readily accessible derivative of chlorophyll *a*.

1.6.1. Partial Synthesis of Dimethyl Tunichlorin From Chlorophyll *a*

The nickel-containing tunichlorin (**49**) was discovered by Rinehart *et al.*[72] in the tunicate *Tridemnum solidum* which occurs in the Caribbean Sea. Structural elucidation was carried out by UV-Vis, CD, MS and ^{1}H NMR spectroscopy on dimethyltunichlorin (**50**) (**Scheme 8**), which was prepared by etherification of the 3-hydroxymethyl group and esterification of 17-propionic acid side chain. Dimethyltunichlorin synthesized from tunichlorin (**49**) is in all respects identical to dimethyltunichlorin prepared from

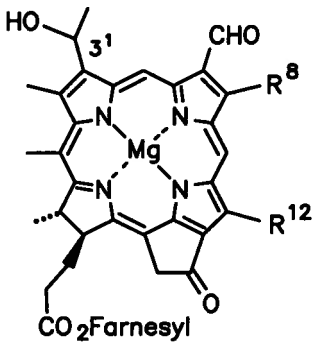


Scheme 8 Partial Synthesis of Dimethyl Tunichlorin

chlorophyll *a* (**1**) by partial synthesis[72] (**Scheme 8**). However, the biological function of tunichlorin (**49**) is still unknown. Its substitution pattern suggests biogenesis from chlorophyll *a* as being the most likely.

1.6.2. Partial Synthesis of Methyl Bacteriopheophorbide *c* [Et,Me] from Methyl Pyropheophorbide *a*

Bacteriochlorophylls *c* (**45**) (Bchls *c*) are found in bacterial strains such as *Prosthecochloris aestuarii*, and in the gliding filamentous bacterium *Chloroflexus aurantiacus*. The latter produces only one homologue (8-Et, 12-Me) of the Bchl *c* while the former occurs as a mixture of homologues (**45** a-f)[73] (**Fig.1.17**). Early structural work by Holt and coworkers[74] led to the derivation of the gross structures for the homologous mixtures of the Bchl *c*. The absolute stereochemistry in ring IV is the same as for Chl *a* and the chirality of the 3-(1-hydroxyethyl) was established on the basis of



45

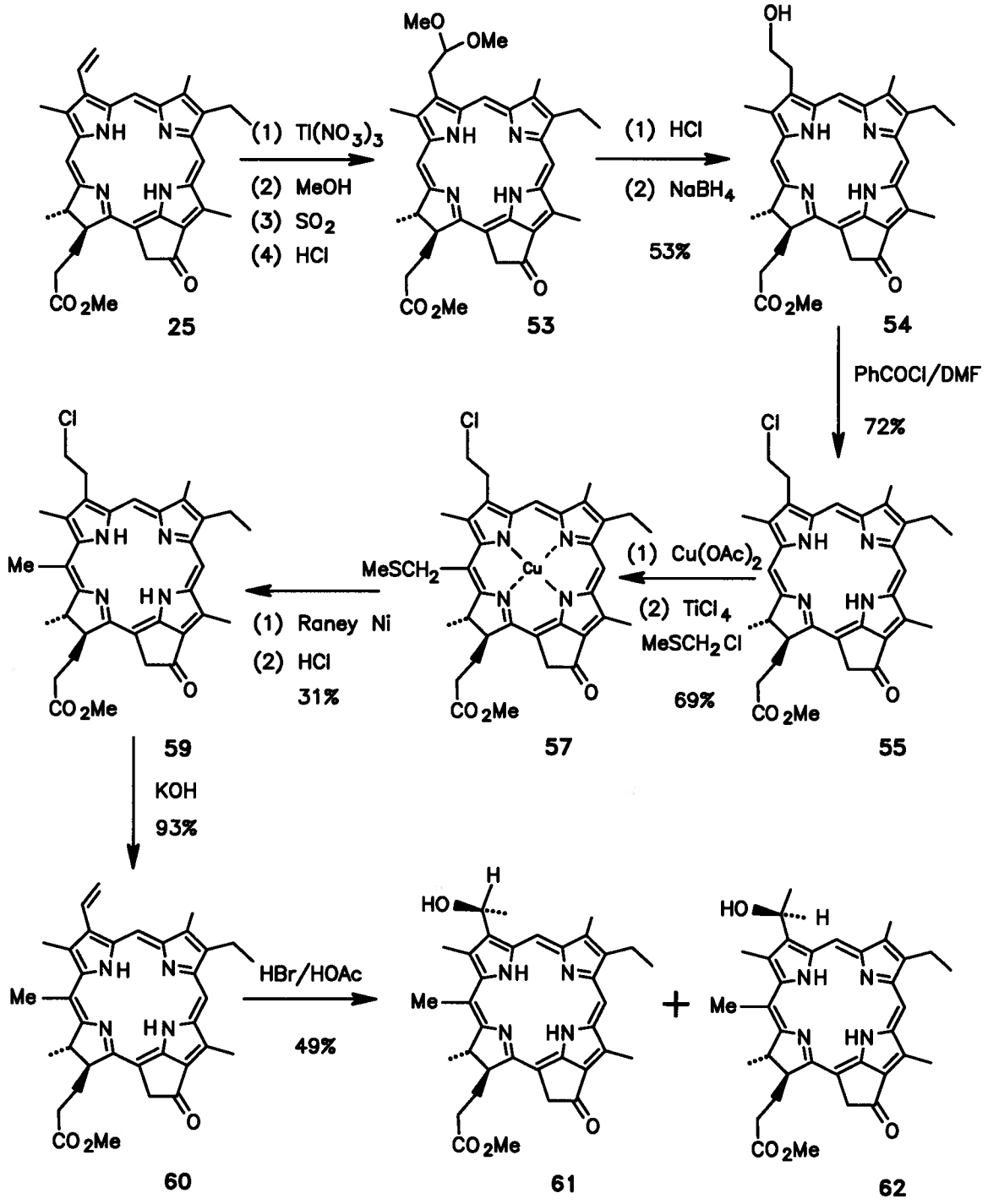
	R_1	R_2	Chirality at 3^1 position
a	Et	Me	R
b	Et	Et	R
c	*n*-Pr	Et	R
d	*n*-Pr	Et	S
e	*i*-Bu	Et	R
f	*i*-Bu	Et	S

Fig. 1.17 Structures of the Bacteriochlorophylls *c*

HPLC, NMR and X-ray crystallography.[73] The major structural difference between the Bchl *c* [Et, Me] and Chl *a* is in the δ-*meso*-methyl substituent found in the former.

The Bchls are most correctly designated as "pyro" compounds, because they are devoid of the 13^2-methoxycarbonyl group present in Chl *a*. Esterifying alcohols on the 17-side are mainly farnesol (instead of the phytol found in Chl *a*).

In 1985, Smith *et al.*[41] accomplished a partial synthesis of Bchl *c* [Et, Me] from pheophorbide *a* methyl ester (**5**) (**Scheme 9**). Treatment of **5** with hot collidine afforded methyl pyropheophorbide *a* (**25**). This compound when treated with two mole equivalents of thallium(III) nitrate in methanol gave the dimethoxyacetal **53** after removal of chelated thallium(III). Aqueous acid treatment followed by immediate reduction with sodium borohydride of compound **53** yielded the 3(2-hydroxyethyl) derivative **54**, which underwent halogenation with benzoyl chloride in dimethylformamide, to give the required vinyl-protected compound **55** in 72% yield. Insertion of copper(II) into compound **55**, followed by treatment with titanium tetrachloride and chloromethyl methyl sulfite provided the 20-methylthiomethyl derivative **57** in 69% yield. With Raney nickel in acetone, the thiomethyl derivative **57** gave the required 20-methylchlorin **58** in 59% yield, which was demetalated to give compound **59**. Vinylation, using KOH in pyridine, gave the 3-vinyl compound **60**, in 93% yield. The final step in the partial synthesis required Markovnikov hydration of the 3-vinyl group and this was accomplished with HBr/HOAc, in 49% yield, to give a mixture of diastereomers **61** and **62** in 3:2 ratio. Reversed-phase HPLC separated the diastereomers, and the 3^1-(R) configuration was shown to be identical with that of 8-ethyl-12-methyl natural product.

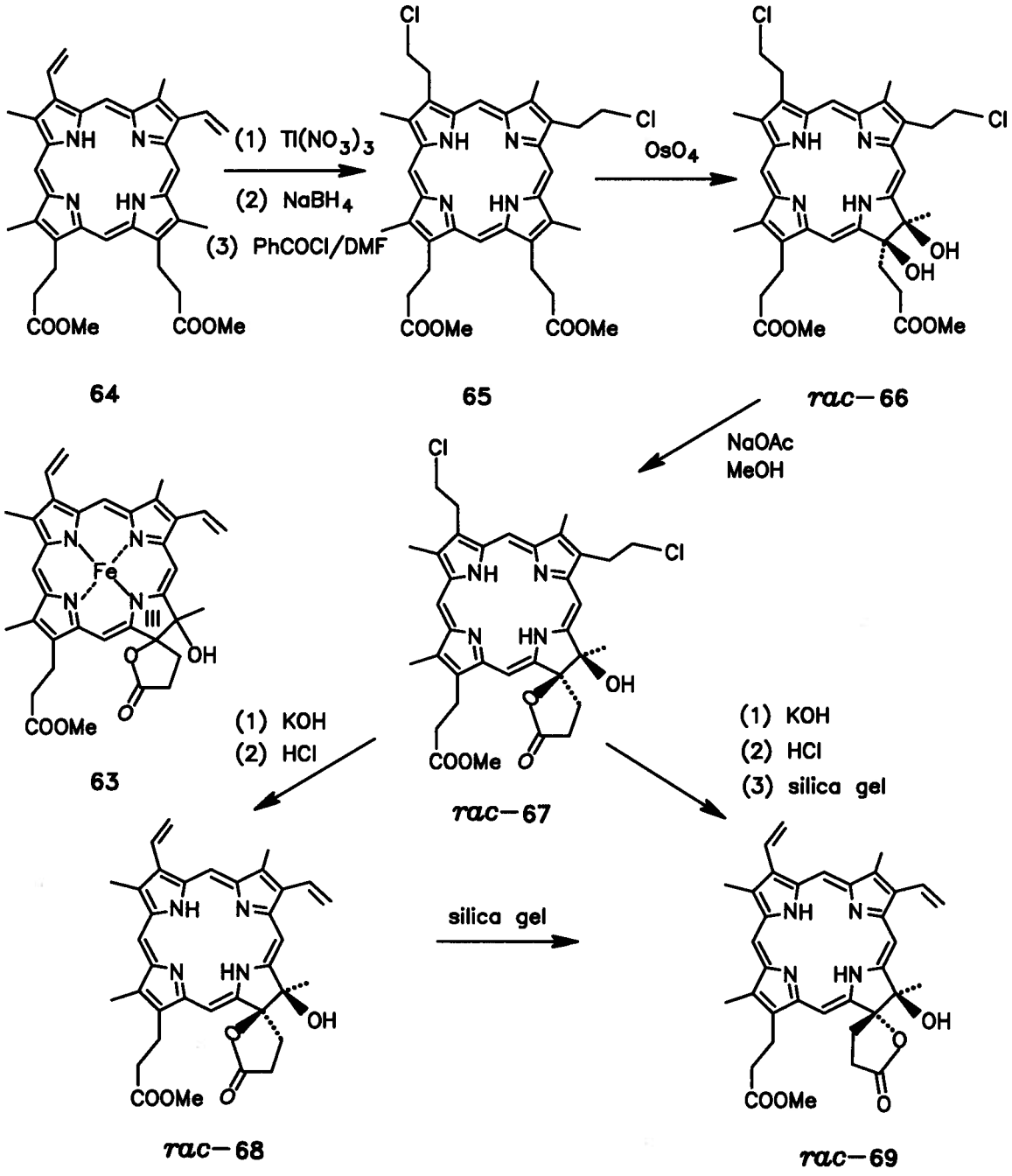


Scheme 9 Partial Synthesis of Bacteriopheophorbide *c*

1.6.3. Partial Synthesis of Heme *d*

Heme *d* (**63**) is the prosthetic group of the terminal oxidase cytochrome *d*, one of the two terminal oxidases found in many bacteria. Heme *d* (**63**) is bishydroxylated in the periphery of ring III, which gives it the characteristic geminally disubstituted structure and the typical chlorin chromophore. The absolute and relative configurations of heme *d* (**63**) have still not been determined.[75] The correct configurational formula of heme *d* may therefore be a stereoisomer of formula **63**. In addition, there is no certainty as to whether the spirocyclic lactone structure is the natural structural element or whether it is generated during isolation.

Porphyrin *d*, the metal-free ligand system of heme *d*, was synthesized from protoporphyrin IX (**64**, **Scheme 10**) by Sotiriou and Chang.[76] Before bishydroxylation of ring III of compound **64** is carried out, the vinyl groups have to be protected. This is achieved by converting them into chloroethyl residues. Subsequent bishydroxylation with osmium tetraoxide yields the four possible constitutional isomers as expected. The desired isomer *rac*-**66** is present in the mixture in 22% yield along with 26% ring IV isomer, 6.8% ring I isomer and 6.8% ring II isomer. In the presence of sodium acetate the bis-hydroxyporphyrin *rac*-**66** forms the spirolactone structure *rac*-**67**, which can be transformed into either of the stereoisomeric porphyrins *rac*-**68** or *rac*-**69** depending on the reaction conditions chosen. Since the stereochemistry of natural heme *d* is unknown, both stereoisomers formed may be of interest.



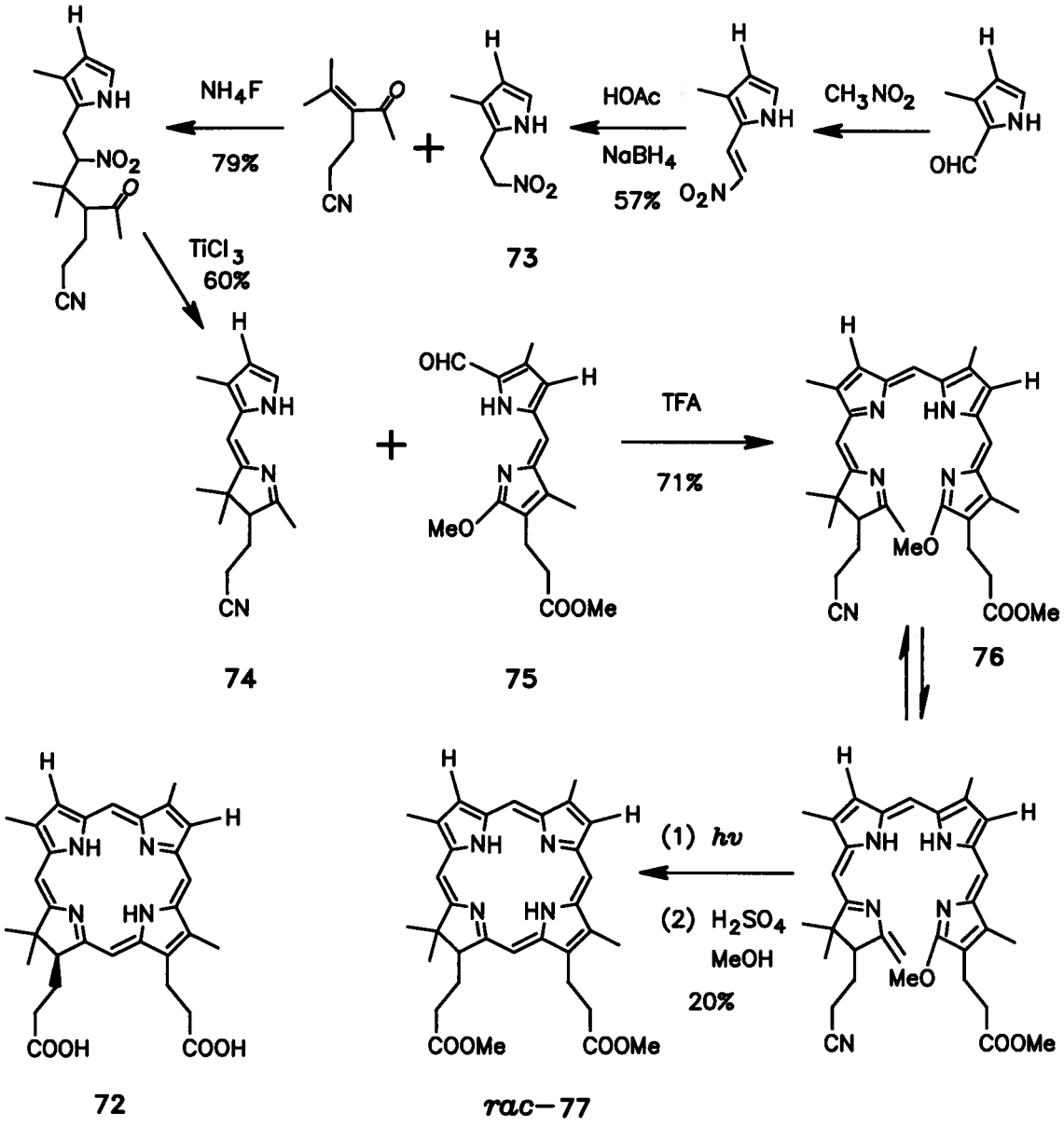
Scheme 10 Partial Synthesis of (±) Porphyrin *d*

1.6.4. Total Synthesis of Bonellin Dimethyl Ester

Bonellin (**72**) is the green sex-differentiating pigment of *Bonellia viridis*, a marine animal found throughout the Mediterranean and belonging to the *Echiuroida* class of animals. *Bonellia viridis* possesses a remarkable sex dimorphism, which is induced by bonellin. Any of the initially asexual larvae that come into contact with the body wall of the female, which contains the green bonellin, develop into males of about 1-3 mm in size. After contact with bonellin the males live inside the body cavity of the larger female (15 cm). The female of the species develop from those larvae which have had no contact with bonellin.

Although pure crystalline bonellin (**72**) was first isolated by Lederer *et al.*[77] in 1939, it was not until 1976 that Pelter *et al.*[78] determined the constitutional formula of bonellin by modern spectroscopic methods. The ring IV degradation product of bonellin (**72**) was found to be constitutionally and configurationally identical to the already familiar ring III degradation product of vitamin B_{12}.

The photochemical cyclization opened the way to the synthesis of bonellin dimethyl ester.[79,80] The bonellin macrocycle was divided along the north-south line giving rise to a western and an eastern block. The western half (**74, Scheme 11**) with a reduced pyrrolic ring (IV) was prepared from the nitropyrrole **73**. The eastern half (a dipyrromethene, **75**) was prepared separately from readily available pyrroles. Condensation of the western block **74** with the eastern block **75** under acidic conditions generated a seco-system **76** which gave the recemate mixture of bonellin dimethyl ester **77** after methanolysis of the nitrile with methanolic sulphuric acid.



Scheme 11 Synthesis of (±) Bonellin Dimethyl Ester

Chapter 2

Stereoselective Synthesis of Natural Antioxidative Chlorins

2.1 Synthetic Targets and Research Objective

The metallated complexes of tetrapyrrolic macrocycles constitute the basic macrocyclic pigments of living systems on this planet (Chapter 1, **Fig. 1.2**). The functions of these cofactors are determined by the incorporation of different metal ions into the centers of their tetrapyrrolic macrocycles. As mentioned in the introduction, complexation with a central metal ion by tetrapyrroles allows fine tuning of its electronic and redox properties. In this way, these coordination compounds have developed unique reactivity and biological interaction with their various molecular environments in cells.

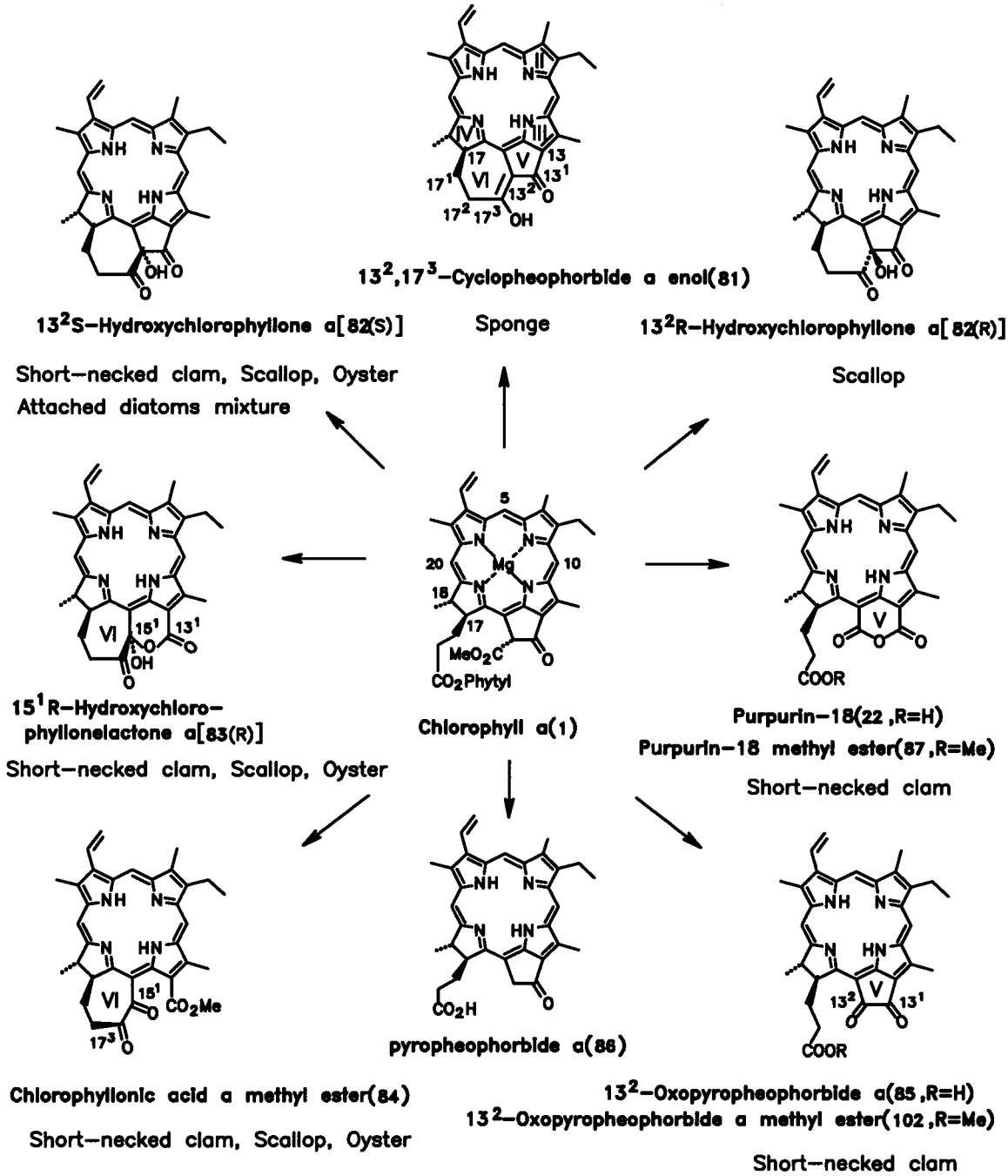
However, other than the metal-containing cofactors, metal-free natural tetrapyrroles having special biological functions may be not the exception rather than the rule, since the number of related compounds isolated in metal-free form from marine metabolites is still increasing. In addition to the well-known pigment bonellin[78] (Chapter 1), which controls the sex of the larvae of the mediterranean sea worm, *Bonnellia viridis*, another new class of chlorins (called "new chlorophyll *a* related chlorins") with strong antioxidative activity has also been recently discovered.

The term "antioxidative activity" can be defined as a function which inhibits oxygen-mediated oxidations. In nature, antioxidative activity is inherent in a class of simple compounds and/or complex bio-macromolecules which have evolved in many organisms and microorganisms as a defense against the detrimental effects of oxygen.[81] Oxidative by-products (most commonly in the form of highly reactive and potentially harmful free-radicals such as HO•, $O_2^{\bullet -}$, L•)[82] of normal cellular metabolism cause extensive damage to DNA, proteins, and lipids.[83] This damage (the same as that

produced by radiation) appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction.[84] Antioxidative defenses against this damage include two types of natural antioxidants that are highly effective in neutralizing free-radicals or/and in inhibiting the bio-reactions responsible for free radical production.[85] The first type is low-molecular-weight compounds such as vitamin C (ascorbic acid), vitamin E (α-tocopherol), glutathione, and uric acid. These hydroxylated compounds can donate hydrogen atoms to other free radicals, resulting in the formation of relatively stable, often oxygen-centered antioxidative radicals. The long life-spans (usually seconds for antioxidative radicals in contrast to 10^{-6} seconds for most lipid radicals)[86] of these antioxidative radicals allow them to slow down the progression of the radical-chain propagations and to finally react with other free-radicals to terminate the radical chains. Therefore, these compounds are free radical scavengers and are thus able to inhibit lipid peroxidation (lipid damage). The second type of natural antioxidants is antioxidative enzymes, e.g. copper-, zinc- or manganese-containing superoxide dismutases, iron-containing catalase and selenium-containing glutathione peroxidase, and metal-binding proteins (such as transferrin, ferritin, and ceruloplasmin).[85] Instead of scavenging free radicals, these bio-macromolecules inactivate reactive electrophilic mutagens and inhibit the reaction systems responsible for the radical production.[85]

The discovery of antioxidative activity for the new chlorophyll *a* related chlorins dates back to 1986, when Karuso *et al.*[87] reported the structure of a marine chlorin, 13^2, 17^3-cyclopheophorbide *a* enol (**81**) (**Scheme 12**). 13^2, 17^3-Cyclopheophorbide *a* enol, as

revealed by single-crystal X-ray crystallography, exists only in the enolic form and is the only tetrapyrrole isolated from the sponge *Darwinella oxeata*. Its biological function



Scheme 12 Natural Antioxidants and Their Origins

remained unknown until the closely related chlorin, 13^2S-hydroxychlorophyllone *a* [**82**(S)] ("S" or "R" denotes the absolute configuration at C-13^2 or C-15^1 position where appropriate) was found as an antioxidant in the short-necked clam, *Ruditapes philippinarum* in 1990.[88] The antioxidative biological activity of these chlorins was discovered as a result of studies on the peroxide value (POV, which is an index of free radical-mediated oxidative damage in lipids) and mutagenicity among the extracts of various marine species that contain high content of highly unsaturated fatty acids (the most readily oxidized lipids), e.g. various kinds of marine fish, bivalves, and attached and wafting diatoms. However, instead of expected high POV (larger than 100 meq/per kg extracts) found in their extracts, much lower POV (less than 30 meq/per kg extracts) and mutagenicity were identified, suggesting the existence of strong antioxidants in these organisms.[89] Further screening of these extracts has revealed the presence of new strong antioxidative chlorins, 13^2S-hydroxychlorophyllone *a* [**82**(S)], 13^2R-hydroxy-chlorophyllone *a* [**82**(R)], 15^1R-chlorophyllonelactone *a* [**83**(R)], chlorophyllonic acid *a* methyl ester (**84**)[90], 13^2-oxopyropheophorbide *a* (**85**) as well as the known chlorins, pyropheophorbide *a* (**86**), purpurin-18 (**22**) and purpurin-18 methyl ester (**87**).[91] A detailed description of their structures and their sources is shown in **Scheme 12**.

The finding of antioxidative activity in these marine metabolites not only suggests a new biogenetic pathway but also provides new insights into the evolution of marine chlorophylls. These novel antioxidative chlorins share a similar structural framework and molecular substitution pattern to chlorophyll *a* (**1**). For example, the structural difference between 13^2, 17^3-cyclopheophorbide *a* enol (**81**) and chlorophyll *a* is in the presence of

an additional exocyclic ring VI in **81**. It is, therefore, most likely that such natural antioxidants are evolved from chlorophyll *a*. In nature, antioxidants have evolved in many organisms and microorganisms as a defense against the detrimental effects of oxygen since the time photosynthetic organisms began releasing oxygen into the primitive atmosphere about 3.5 billion years ago.[92] It is interesting to note that instead of employing the common antioxidants, hydroxylated and polyhydroxylated aromatic and heterocyclic compounds such as vitamin A (retinol), vitamin C (ascorbic acid) and vitamin E (a-tocopherol),[93,94] nature chose to modify the very molecule (chlorophyll) that was producing oxygen to protect the unwanted oxidation processes. As can be deduced from the occurrence in animals, these pigments have no photosynthetic activity.

The rich stereostructural diversity of these antioxidants has been a challenge to the synthetic chemist and will continue to be so as more skeletal types are found. The useful biological properties, coupled with their interesting and challenging structures, make them targets for extensive synthetic studies. The objective of our research has been two-fold: The first was to develop new and efficient synthetic routes to these unusual compounds and thus be able to provide sufficient amounts of materials for subsequent antioxidative and biomedical investigations. Antioxidants as therapeutic agents have shown potential in the treatment of inflammation, cancer, aging and neurodegenerative diseases.[95] Secondly, we have striven to design synthetic routes that are both simple and biomimetic. This requires the synthetic routes to be as close as possible to their possibly enzymatic pathways.

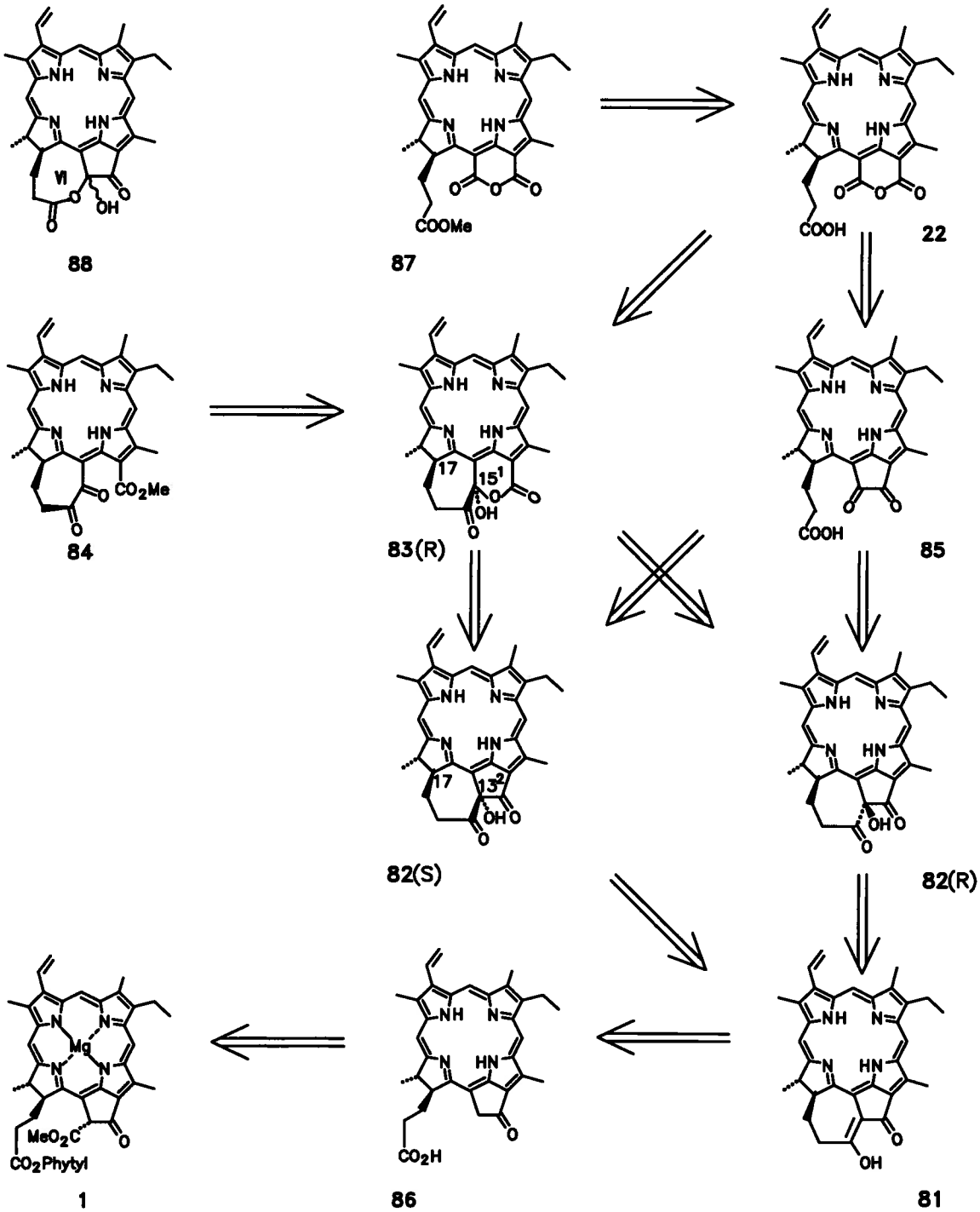
The following sections describe our synthetic strategy and detail the synthetic routes that are required to achieve the transformations of these unusual structures.

2.2 Synthetic Approach

The exocyclic rings of these unusual chlorins, as a class of naturally occurring antioxidants, display different oxidation states. Based on the number of the oxygen atoms in these natural antioxidative molecules, purpurin-18 (**22**) and its methyl ester (**87**) both possess 5 oxygen atoms and are in the highest oxidation state. The latter can be produced from the former upon treatment with diazomethane. Purpurin-18 is the fully oxidized product from the four-oxygen compounds, 15^1R-hydroxychlorophyllonelactone *a* [**83**(R)] and/or 13^2-oxopyropheophorbide *a* (**85**). Chlorophyllonic acid *a* methyl ester (**84**) is the methylation product of 15^1R-hydroxy-chlorophyllonelactone *a* [**83**(R)].

The regioselective mono-oxidation of the seven- and five-membered exocyclic rings in the three-oxygen compounds, 13^2S- and/or 13^2R-hydroxychlorophyllone *a*, **82**(S) and/or **82**(R), should bring about the generation of the four-oxygen compounds **85** and **83**(R), respectively (**Scheme 13**). The exact way in which the regioselective oxidation might be performed was not known at the start of the research. However, the naturally-occurring hydroxylactone **83**(R) has an α 13^2-OH moiety, a stereochemistry resulting from the cisoid geometry between positions 15^1 and 17, which minimises the exocyclic ring strain of the peripheral substituents associated with the chiral centers (C-15^1, C-17). Thus, it could be envisioned that the thermodynamically-favoured epimer **83**(R) should be obtainable under equilibrating conditions. Further, the diketone **85**, is also present in

the thermodynamically-favoured form. Its corresponding hydroxylactone isomer **88**, which should possess an eight-membered exocyclic ring, is thermodynamically-unfavoured and has not been found (**Scheme 13**). Consequently, the goal was simplified



Scheme 13 General Strategy for the Synthesis of the Antioxidative Chlorins.

to devise a new regioselective oxidation to the seven- and five-membered exocyclic rings, which should ensure high yields and avoid the appreciable formation of the di-oxidized product, purpurin-18 (**22**).

Development of a successful asymmetric hydroxylation of 13^2, 17^3-cyclopheophorbide *a* enol (**81**) to produce compound **82**(R) or/and **82**(S) was thus thought to be central to the entire effort. These compounds could then be used in the oxidative formation of hydroxylactone **83**(R) and diketone **85**.

As was described in Chapter 1, the partial synthesis of compound **81** from pyropheophorbide *a* (**86**), a degradation product of chlorophyll *a*, had been completed by Eschenmoser and coworkers[96,97] some years before it was found in nature. The strategy for the cyclization between the two peripheral substituents to form the additional exocyclic ring VI involved a Claisen-type intramolecular condensation.

Based on molecular modelling studies (InsightII, Biosym), formation of the enolic exocyclic ring in compound **81** should result in steric compression on its chlorin macrocycle. Further oxidation to form 13^2S- and 13^2R-hydroxychlorophyllone, **82**(S) and **82**(R), should relieve the strain to a great extent. The cisoid (C-17, C-13^2) geometric isomer **82**(S) should be more stable than the transoid epimer **82**(R) if one considers the ring-strain of the exocyclic rings discussed above. A retrosynthetic analysis linking all the antioxidative chlorins to chlorophyll *a* (**1**) is summarized in the antithetic format of **Scheme 13**. Once effective routes to these antioxidative chlorins were devised, next the starting material was required to achieve these chemical transformations.

2.3 Starting Materials : Natural Pheophorbides

Because chlorophylls are extremely light-sensitive and labile pigments, it is virtually impossible to extract these plant materials without the formation of various alteration products (even when samples are handled and chromatographed in the dark). The nature of these undesirable transformations and the yield of the secondary products vary with the plant material, its treatment, and the conditions to which the extracts are exposed.[98]

The usual chlorophyll extraction consists of the following steps: a) primary extraction, b) separation of contaminating substances, c) conversion to stable derivatives.[99] The last two steps should immediately follow the first, as a variety of artifacts have been reported from stored samples.[100]

Chlorophylls are highly insoluble in water and are always accompanied by other lipophilic compounds within living cells. Therefore, isolation of chlorophylls requires, firstly, their detachment from the chlorophyll-binding proteins. This is achieved by extraction from photosynthetic tissues into polar organic solvents after mechanical disruption of cells. The solvents most suitable for the extraction of the chlorophylls from fresh plant material are those miscible with water, such as acetone and methanol.[101]

The isolated coloured matter of plants and algae is a mixture, with yellow pigments tending to dominate the extracts, coupled with a very small amount of the green pigments. The ratio of the total green to total yellow pigments is approximately 1:3 in higher plants. In blue algae, the ratio of the green to yellow pigments can be as low as 1:300.[102] Purification and separation of the mixture of pigments leads to the two principal green pigments (chlorophylls *a* and *b*), the orange to red carotenoids and the yellow to red

xanthophylls. The ratio of chlorophyll *a* to chlorophyll *b* obtained is usually 3:1; that of carotene to xanthophyll, 1:2.

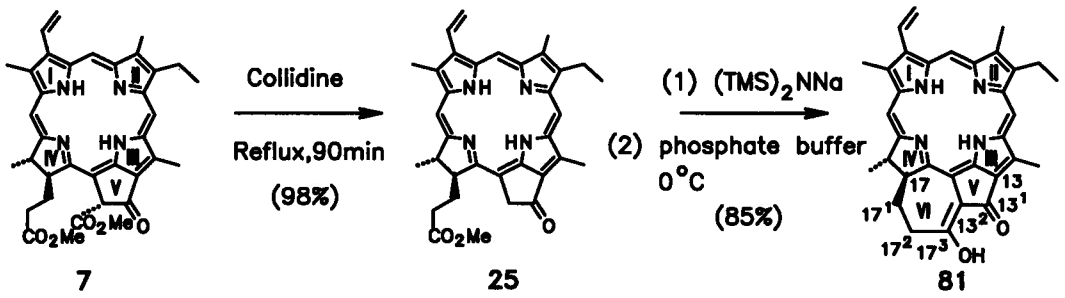
As mentioned in the introduction (Chapter 1), the blue alga *Spirulina maxima* is commercially available and contains no chlorophyll *b*. This advantage makes it a good source of chlorophyll *a* and its closely-related derivatives. Thus, we have employed the blue alga *Spirulina maxima* as the source of chlorophyll *a* in our work. In 1985, Smith *et al.*[41] reported an isolation procedure for pheophytin *a* (**5**) from *Spirulina maxima* using acetone as the extracting solvent, followed by chromatography on alumina. No chlorophyll *a* could be obtained directly from this procedure due to repeated chromatography on alumina. However, the demetallated derivative of chlorophyll *a*, pheophytin *a* was obtained as the major product. According to their report, approximately 2.4-3.6 g pheophorbide *a* methyl ester (**7**) was obtained from 1 kg of *Spirulina maxima* alga after the final methanol/sulfuric acid step was used to transesterify the phytyl ester to give the methyl ester.[103]

However, a lower isolated yield (~1.0-1.5 g of compound **7** from 1 kg of alga), was observed (by other researchers[104] and also in our hands), along with a relatively large amount of alteration products when this procedure was repeated. The main problem associated with this method is the presence of large quantity of carotenoids and xanthophylls in the original acetone extract. Separation of these yellow-red pigments from the acetone extracts to afford the desired green chlorophyll required exhaustive chromatography which led to oxidation and alteration of the material. To overcome this problem, an improved method was developed in this work by introducing a two-phase

extraction in the purification step (to remove large amounts of yellow-red pigments) before the extracts were subjected to chromatography. Furthermore, pheophytin *a* (**5**), the magnesium-free derivative of chlorophyll *a*, is less sensitive, can better be handled in chromatographic systems, and is also the initial intermediate for chemical synthesis of the antioxidative chlorins in the present work. Therefore, demetallation of chlorophyll before chromatography was performed in our isolation in order to minimize the alteration reaction in the chromatographical purification.

In our method, the acetone extract was dissolved in petroleum ether. Successive washing of the petroleum ether solution with 30% methanol in water removed most of the yellow-red pigments while the chlorophyll remained in the petroleum ether layer. When the aqueous phase became colorless, the organic layer was collected, dried over sodium sulphate, filtered and evaporated *in vacuo*. The residue obtained was redissolved in diethyl ether and conc. HCl was added to remove the magnesium ion in chlorophyll. The ether layer was immediately washed with water, dried over sodium sulphate, filtered and evaporated *in vacuo*, affording crude pheophytin *a* (**5**). The remaining yellow-pigments in the crude pheophytin *a* were readily removed by flash chromatography on neutral alumina (Brockman Grade III). About 6.5 g of pheophytin *a* (**5**) was obtained from approximately 1 kg of *Spirulina* alga. Treatment of pheophytin *a* (**5**) with 5% sulfuric acid in methanol, followed by recrystallization from methylene chloride/methanol, gave 4.2 g of pheophorbide *a* methyl ester (**7**).

The conversion of pheophorbide *a* methyl ester into 13^2,17^3-cyclopheophorbide *a* enol (**81**) was based on the modification of a method reported by Eschenmoser and co-



Scheme 14 Synthesis of Enol **81**

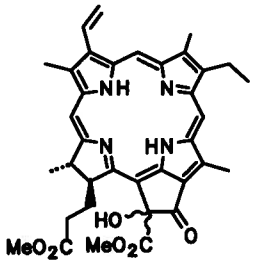
workers[96] (**Scheme 14**). Thus, decarbomethoxylation of pheophorbide *a* methyl ester (**7**) in collidine afforded a 98% yield of pyropheophorbide *a* methyl ester (**25**) which was then treated with seven equivalents of $(TMS)_2NNa$ in THF for 3 min to bring about the Claisen-type intramolecular condensation to produce, after flash chromatography on deactivated silica, 13^2,17^3-cyclopheophorbide *a* enol (**81**) in 85% yield. Enol **81** is the common intermediate to new chlorophyll *a* related chlorins and exists only in the enolic form in solution and in the crystalline state[87].

After the key intermediate 13^2, 17^3-cyclopheophorbide *a* enol (**81**) was made available, attention was then focused on its asymmetric hydroxylation.

2.4 Hydroxylation Studies Leading to Asymmetric Hydroxylation

To bring about the conversion of enol **81** to hydroxychlorophyllone *a* **82**, a number of methods were investigated, which included aerial and iodine oxidation. None of them were successful since decomposition and overoxidation readily occurred during these procedures. It is important to note that the aerial oxidation of pheophorbide *a* methyl ester (**7**) occurs during chromatography on silica, especially on a TLC plate, to

give a diastereomeric mixture of 13^2-hydroxypheophorbide *a* methyl ester (**95**) (in ~20% yield).[105] Unfortunately, enol **81** is so unstable that it decomposes when applied to silica gel plates. Attempts at aerial oxidation of enol **81** in an alcoholic solution of zinc acetate also failed in our hands. Although this method of aerial oxidation was used in the preparation of 13^2-hydroxychlorin **95** from pheophorbide *a* methyl ester (**7**) in 30% yield[106], it did not work in the case of compound **81**, which gave back neither **82** nor zinc-metallated **81**, but an overoxidized green mixture which was not identified. Hydroxylation of enol **81** using molecular iodine[107,108], a mild oxidizing agent, in the presence of sodium acetate in aqueous THF solution was only partially successful, giving a mixture (19% yield) of 13^2-hydroxychlorophyllone *a* (**82**) and 13^2-acetoxychlorophyllone *a* (**90**). However, in both cases (**82** and **90**) no epimeric enrichment was noted and no attempts have been made to separate the optically-pure **82**.



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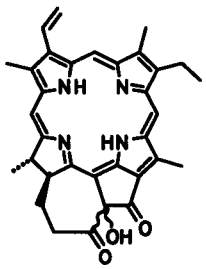
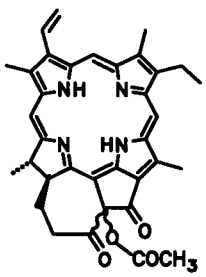


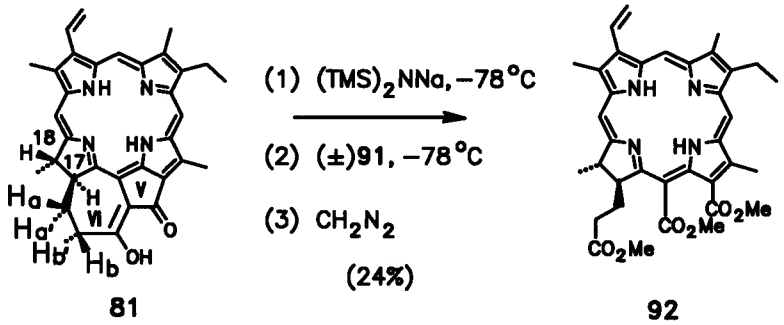
82



90

Due to the unacceptably low yield and stereoselectivity of the above method, alternative reactions for introduction of a 13^2-hydroxy group were sought. Thus, we turned our attention to the oxidation of enolates with *N*-sulfonyloxaziridines, a method introduced by Davis and coworkers.[109,110] Following the standard procedure[110], treatment of enol **81** with $(TMS)_2NNa$ or LDA followed by oxidation with 1-phenyl-*N*-

(phenylsulfonyl)oxaziridine[111] (**91**) at –78°C for 15 min, failed to give the desired product **82** after the standard workup, but resulted in a very polar yellowish-green mixture. The visible spectrum (λ_{max} 672 nm, 404 nm) of this mixture indicated cleavage of both exocyclic rings. Treatment of this mixture with diazomethane gave chlorin p_6 trimethyl ester (**92**) (24% yield from **81**) (**Scheme 15**). The amount of base and/or oxaziridine as well as reaction conditions were varied in order to avoid the cleavage of the exocyclic rings, but all were largely unsuccessful. These results suggested that the two exocyclic rings of enol **81** could not withstand strong ionic bases such as $(TMS)_2NNa$ and LDA.



Scheme 15 Oxidative Cleavage of Exocyclic Rings

Therefore, the matter of choosing a suitable base which would not ring-open enol **81** had to be attended to before attempting the asymmetric hydroxylation. Towards this end, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), a strong organic base, was used as the base which was found to be very efficient at promoting this reaction. Upon addition of DBU (0.4 mL) to a THF solution (10 mL) of enol **81** (20 mg) at 0°C under N_2, the green solution changed instantly to the reddish-brown enolate (a typical color of the Molish phase-test intermediate[112]). After 10 minutes, 1.2 equivalents of (±)-1-phenyl-*N*-(phenyl-sulfonyl)oxaziridine (**91**) was injected and allowed to react for 2 hours at 0°C (the

reaction was monitored by UV/Vis and TLC). Following a standard workup and flash chromatography on deactivated silica, a dark green solid was collected (entry 1 in **Table 2.1**). The ^{1}H NMR of the material obtained showed that it was a diastereomeric mixture. The most significant changes were that the 17^3-OH peak (δ 13.24 ppm) of **81** disappeared and two new broad OH peaks at δ 4.56 and 4.14 ppm (exchangeable with CD_3OD) appeared. The absorption spectrum Q band showed a blue shift of 20 nm to 670 nm.

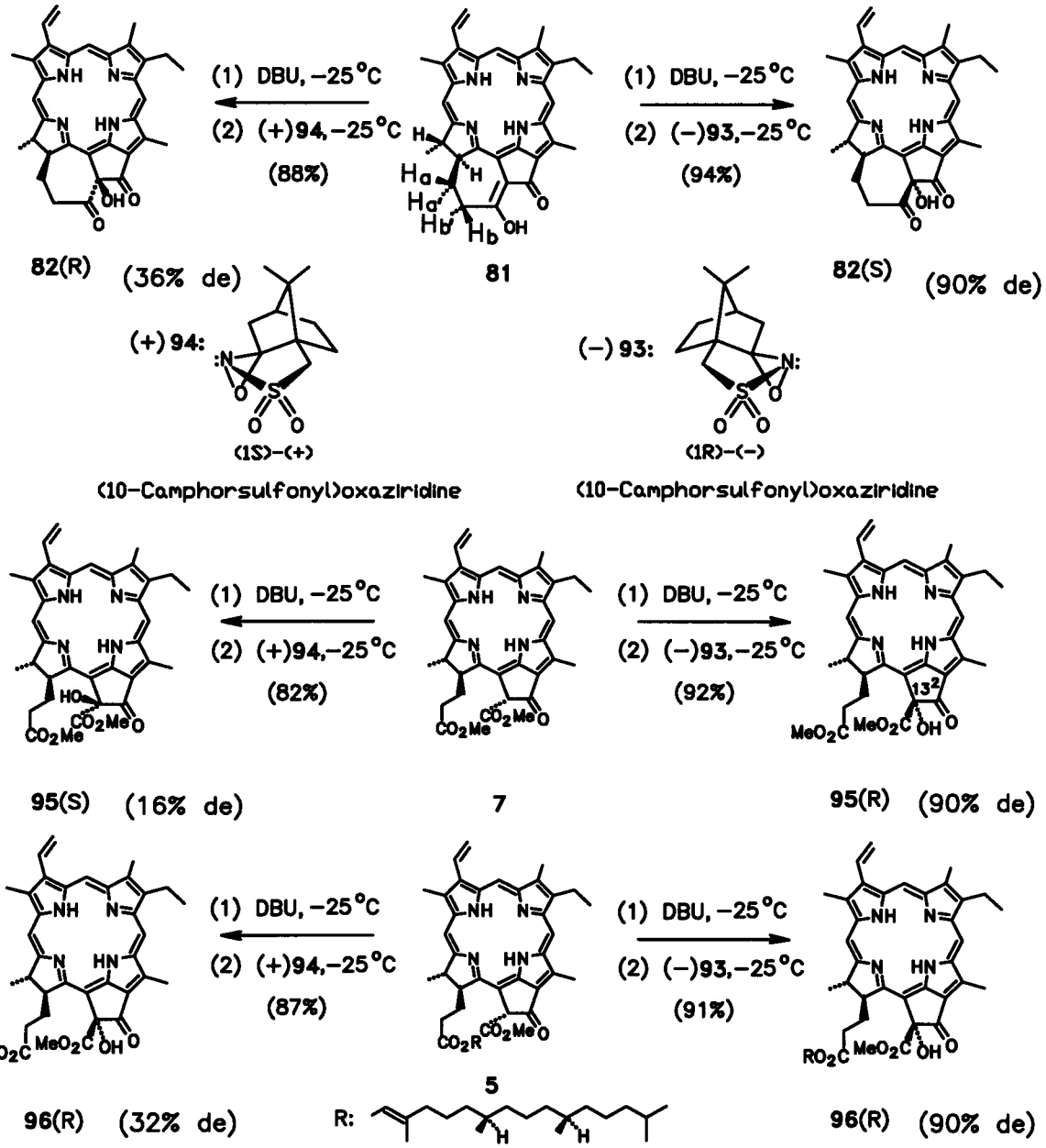
A FABMS analysis showed a molecular mass increase of 17 units to 533 (M+1) and reversed-phase HPLC analysis confirmed a 68:32 mixture of diastereomers (close to the intensity ratio shown in the ^{1}H NMR). The two diastereomers were separated by preparative HPLC. The more mobile band (t_R = 15.37 min) (**Fig. 2.1**) was 13^{2}S-hydroxychlorophyllone *a* [**82**(S)] and the less mobile band (t_R = 17.14 min) was 13^{2}R-

Table 2.1 Asymmetric Hydroxylation of **5**, **7** and **81** with Oxaziridines **91**, **93** and **94**

entry	oxa-ziridine	sub-strate	reaction temp. and time [h]	product	d.e.%[a]	13^{2}R : 13^{2}S	isolated yield(%)
1	(±)-**91**	**81**	0°C [2]	**82**	24	38 : 62	75
2	(±)-**91**	**81**	–25°C [12]	**82**	78	11 : 89	98
3	(–)-**93**	**81**	0°C [2]	**82**	90	5 : 95	93
4	(–)-**93**	**81**	–25°C [12]	**82**	90	95 : 5	94
5	(+)-**94**	**81**	0°C [2]	**82**	14	43 : 57	77
6	(+)-**94**	**81**	–25°C [12]	**82**	36	68 : 32	88
7	(±)-**91**	**7**	0°C [2]	**95**	38	69 : 31	85
8	(–)-**93**	**7**	0°C [2]	**95**	88	94 : 6	88
9	(–)-**93**	**7**	–25°C [12]	**95**	90	95 : 5	92
10	(+)-**94**	**7**	0°C [2]	**95**	14	57 : 43	49
11	(+)-**94**	**7**	–25°C [12]	**95**	16	42 : 58	82
12	(±)-**91**	**5**	–25°C [12]	**96**	60[b]	80 : 20	55
13	(–)-**93**	**5**	–25°C [12]	**96**	100[b]	100 : 0	91
14	(+)-**94**	**5**	–25°C [12]	**96**	32[b]	66 : 34	87

a: The % d.e.'s determined by reverse-phase HPLC. b: The % d.e.'s determined by ^{1}H NMR.

hydroxychlorophyllone *a* [**82**(R)]. The newly introduced 13^2-OH group caused downfield shifts in the ^{1}H NMR of nearby protons [H-17, H_a'-17^1, H_b'-17^2, in **82**(S); H-18, H_a-17^1, H_b-17^2, in **82**(R)] comparison to those in the starting material **81** (**Table 2.2**). The synthetic chlorins **82**(S) and **82**(R) exhibit the same spectral data as their natural counterparts.[89]



Scheme 16 Asymmetric Hydroxylation of Chlorophyll Derivatives

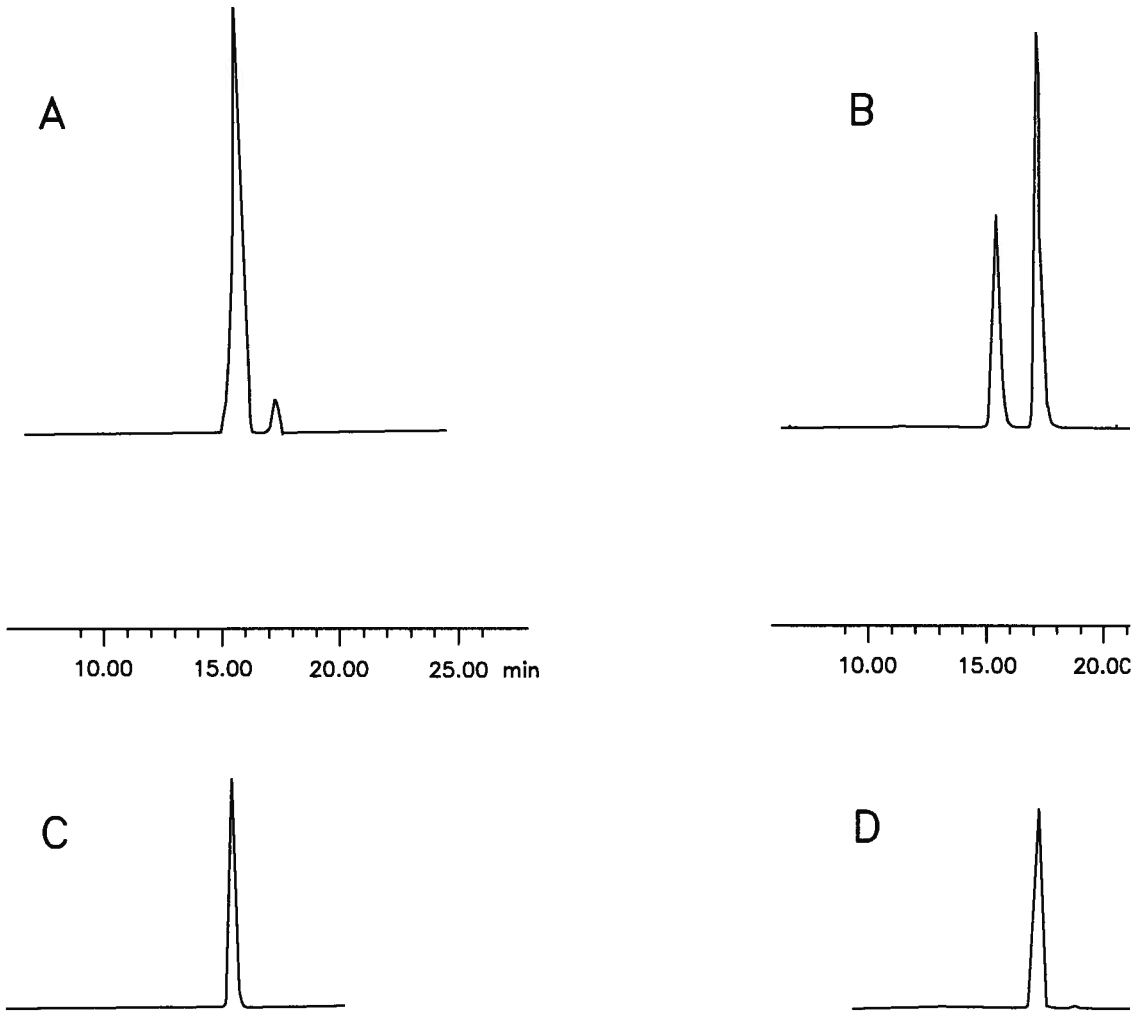


Fig. 2.1 HPLC Chromatography: (A) The epimeric mixture of 95% **82**(S) and 5% [**82**(R)]. (B) The epimeric mixture of 32% **82**(S) and 68% **82**(R). (C) Epimerically-pure **82**(S) from the semipreparative HPLC separation of (A). (D) Epimerically-pure **82**(R) from the semipreparative HPLC separation of (B). HPLC conditions: Waters 600E HPLC system using a Waters C_{18} 4μ 60 Å (3.9 mm×15 cm) column; solvent system was 25% (0.1% TFA in H_2O) and 75% (0.1%TFA in CH_3CN) with flow rate 1 mL/min and ~1300 psi back pressure.

Table 2.2 Selected ^{1}H-NMR Spectral Data ($CDCl_3$, 400 MHz)

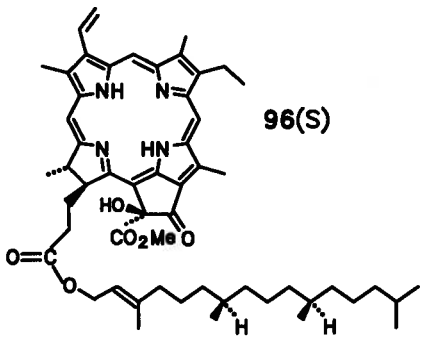
	proton						
Compound	13^2-OH/H	H-17	H-18	H_a-17^1	$H_{a'}$-17^1	H_b-17^2	$H_{b'}$-17^2
7[a]	6.24(s)	4.22(ddd)	4.52(dq)	2.60(dddd)	2.58(dddd)	2.20(ddd)	2.18(ddd)
81[a]	13.24(s)	2.58(m)	2.93(q)	1.71(m)	2.58(m)	2.45(t)	2.45(t)
82(S)[a]	4.56(br s)	4.90(ddd)	4.33(dq)	2.23(dddd)	2.88(dddd)	2.78(ddd)	4.31(ddd)
82(R)[b]	4.14(br s)	3.82(ddd)	4.75(dq)	3.71(dddd)	2.65(dddd)	3.83(ddd)	2.95(ddd)
95(R)[b]	5.32(s)	4.69(dd)	4.49(q)	2.13(m)	2.46(m)	2.09(m)	2.29(m)
95(S)[a]	5.43(s)	4.15(dd)	4.49(q)	2.92(m)	2.28(m)	2.55(m)	2.26(m)
96(R)[a]	5.32(s)	4.68(d)	4.47(dq)	2.09(m)	2.42(m)	1.99(m)	2.24(m)
96(S)[c]	5.49(s)	4.15(d)	4.55(dq)	2.91(m)	2.25(m)	2.53(m)	2.25(m)

a: Concentration: 1.5 mg / 0.6 mL, b: 1.0 mg / 0.6 mL, c: 2.0 mg / 0.6 mL 38%d.e., d: overlap peaks

To achieve direct diastereoselective hydroxylation, two commerically-available enantiomerically-pure oxidants have been used. Reaction of **81** with (–)-(1R)-(10-camphorsulfonyl)oxaziridine **93** (98%, Aldrich) using the above procedure, gave a 93% yield of **82**(S) (90% d.e.) (entry 3 in **Table 2.1**). Higher selectivity was obtained at lower temperature where reaction of **81** with (±)-**91** and (–)-**93** at –25°C for 12 hours (the reaction was monitored by UV/Vis and TLC analyses) respectively, gave a 98% (78% d.e.) and 94% yield (90% d.e.) of **82**(S) (**Scheme 16**). Reaction with (+)-(1S)-(10-camphorsulfonyl)oxaziridine [(+)-**94**] (98%, Aldrich), the enantiomer of (–)-**93**, gave slightly lower yields and diastereomeric selectivity (entry 5 and 6 in **Table 2.1**). These observations can be rationalized when one considers the steric bulk of the 17-propionic group which hinders the approach from the *re* face of the chlorin enolate.

In a similar fashion, treatment of pheophorbide *a* methyl ester (**7**) and pheophytin *a* (**5**) with the oxaziridines gave excellent yields and diastereoselectivity. As described above, the 17-propionic group affects the stereoselectivity. With the increased size of the 17-propionic ester chain from methyl ester **7** to phytyl ester **5**, the d.e.% of the products

after reaction with (±)-**91** and (–)-**93** increased (entry 9 and 13 in **Table 2.1**), in the latter case, a 100% d.e. was observed with *re* face recognition of the nucleophilic oxaziridine **93**. This is in contrast to a 90% d.e. in the reaction with the smaller ester where only a 32% d.e. was observed in the reaction of **5** with (+)-**94** (entry 14 in **Table 2.1**) along with reversed stereoselectivity such that **96**(R) and **96**(S) were produced in a ratio of 66:34 (entry 14 in **Table 2.1**) (**Scheme 16**). The bulky phytyl ester (methyl ester) and 13^2-methoxycarbonyl moieties are on opposite sides of the molecular plane in **96**(S) [**95**(S)] while they are at the same side in **96**(R) [**95**(R)]. 13^2S configuration (which gave lower d.e. and yields) is the thermodynamically more stable form than the 13^2R configuration showing that these reactions are under kinetic control. Diastereomerically-pure **95**(R) and **95**(S) were prepared by HPLC separation of the products from entries 9 and 11 in **Table 2.1**. Attempts to purify 13^2S-hydroxypheophytin *a* [96(S)] from entry 14 (in **Table 2.1**) were partially successful. A sample of 32% d.e.'s **96**(S) was obtained after preparative normal-phase HPLC separation. Further purification by HPLC (reversed and normal phases) and preparative TLC was attempted and was largely unsuccessful due to the decomposition of the material.



96(S)

In these hydroxychlorins, the downfield shifts (of the nearby protons of 13^2-OH moiety) in the ^{1}H NMR were less pronounced due to the greater distance between the 17-alkyl protons and the newly introduced OH group (**Table 2.2**).

Additional hydroxylation methods were also tested to better understand the different reactivities between ionic bases [$(TMS)_2NNa$ and LDA] and the organic base (DBU). For example, reaction of the enolate from pheophorbide *a* methyl ester (**7**) formed by DBU, with mCPBA at room temperature for 10 hours, gave less than 5% hydroxylation product. Bubbling molecular oxygen into the enolate of **7** in the presence of DBU and triethyl phosphite[113] at room temperature for five hours gave no reaction and the starting material was recovered. Furthermore, reaction the enolate of **7**, formed by ionic bases, with mCPBA[114] (30 min at room temperature) or O_2 (5 min at 0°C) led to exocyclic ring cleavage.

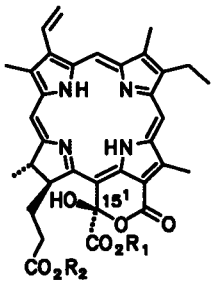
To our knowledge, this method is the first to use DBU as a base for α-hydroxylation and the first successful diastereoselective oxidation of chlorophyll derivatives. The mild conditions, high yields and d.e.% make it an efficient method to introduce a hydroxy group into ß-dicarbonyl chlorins and other similar systems. These hydroxychlorins are not available from other methods and will serve as models for an understanding of chlorophyll allomerization mechanisms.

13^2S-hydroxypheophytin *a* [**96**(S)], recently isolated from *Silkworm excreta*, is reported to possess a high quantum efficiency (50%) for the photosensitized production of singlet oxygen[115] suggesting that the hydroxychlorins produced here have a potential as drugs in photodynamic therapy.

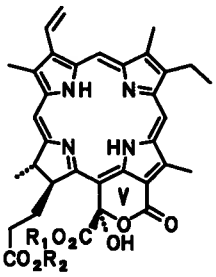
2.5 Model Studies for Hydroxylactonization

As mentioned earlier, 15^1R-hydroxychlorophyllone *a* [**83**(R)] and diketone **85** are the mono-oxidized products at different exocyclic rings of 13^2-hydroxychlorophyllone *a* (**82**). The purpurin-18 (**22**) results from their further oxidation (di-oxidized product from **82**). Therefore, with sufficient amounts of hydroxychlorin **82** having been produced, our next objective was to generate these further oxidized products. Initially, because 13^2-hydroxychlorophyllone *a* (**82**) possesses two similar carbonyl groups which are both reactive (though the carbonyl at the 17^3-position appeared to be less sterically encumbered and less conjugated), the monoketone 13^2-hydroxypheophorbide *a* methyl ester (**95**) was used as a model for the hydroxylactonization.

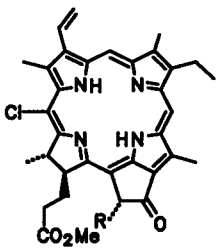
The first hydroxylactonechlorin **21**, a diastereomeric mixture of hydroxylactone **21** (S) and **21**(R), was prepared by Fischer[116] via aerial oxidation of pheophorbide *a* methyl ester (**7**) in the presence of pyridine and alkali in the 1930s. This mixture, called "unstable chlorin" due to its extremely instability, is the precursor to many chlorophyll *a* degradative products (detailed discussion appears in Chapter 1). The "unstable chlorin"



21 (S). R_1=R_2=H
97 (S). R_1=Me, R_2=H
101 (S). R_1=R_2=Me



21 (R). R_1=R_2=H
97 (R). R_1=Me, R_2=H
101 (R). R_1=R_2=Me



99. R=COOMe
100. R=H

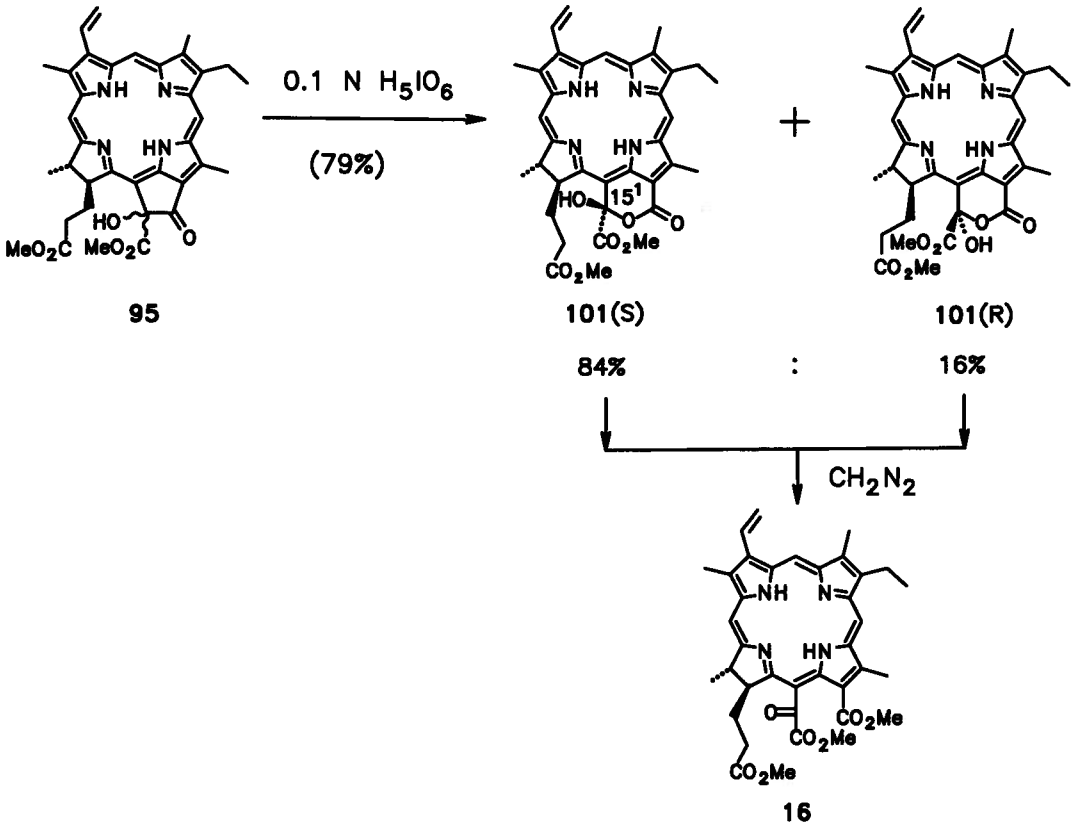
monomethylester **97** is more stable and was obtained by Fischer and Kahr from $KMnO_4$ oxidation of pheophorbide *a* followed by acid fractionation.[117]

Treatment of the (95%R, 5%S) 13^2-hydroxypheophorbide *a* methyl ester (**95**) with methanolic alkali at room temperature for 12 h under N_2, resulted in the hydrolytic cleavage of the ring V generating "unstable chlorin" **21**, a result similar to the alkaline aerial oxidation of pheophorbide *a* methyl ester (**7**). Attempts to purify "unstable chlorin" **21** were unsuccessful due to its ready decomposition. However, purpurin-7 trimethyl ester (**16**) (total 45% yield from **82**) was obtained after treating the "unstable chlorin" (**21**) with diazomethane. Attempts to prepare "unstable chlorin" dimethyl ester (**101**) by partial esterification (via Et_3N/MeOH) of **21** were also unsuccessful since methylation of the hydroxylactone is fast and readily proceeds without selective differentiation from the esterification of the 17^3-carboxylic acid. Rather than expend further efforts on optimizing this process, it was decided to establish whether this route was suitable to the oxidation of 13^2S-hydroxychlorophyllone *a* [**82**(S)]. Treatment of the (95%S, 5%R) 13^2-hydroxychlorophyllone *a* (**82**) with KOH/MeOH under N_2 for 10 hours led to the cleavage of both the exocyclic rings and gave chlorin p_6 trimethyl ester (**92**) after methylation with diazomethane.

Chemically, the transformation of hydroxyketones **82** to hydroxylactone **83**(R) is oxygen atom insertion and Sakata *et al.*[89] have suggested that this conversion is biogenetically via a Baeyer-Villiger type oxidation. With this in mind, we also investigated oxidation reaction of chlorins with peroxycarboxylic acids. Unfortunately, reaction of **95** (95%R, 5%S) or **82** (95%S, 5%R) with mCPBA or CF_3COOOH in the

dark resulted in overoxidation and no products could be identified. Conversely, treatment of the non-hydroxylated chlorins, pheophorbide *a* methyl ester (**7**) and pyropheophorbide *a* methyl ester (**25**), with the above oxidants gave no reactions if electrophiles were not present in solution. Instead of oxidation, electrophilic substitution (detailed description of electrophilic substitution reaction appears in Chapter 1) at the H-20 position of **7** and **25** occurred when only a trace amount of electrophile was present. For example, the trace amount of HCl present in solvent chloroform brought about the formation of 20-chloropheophobide *a* methyl ester (**99**) and 20-chloropyropheophobide *a* methyl ester (**100**).

The only method which worked in our hands was found to be periodate oxidation[118], which occurred only in acidic conditions. Periodic acid (0.1 N) in aqueous



Scheme 17 Model Study for Periodate Oxidation

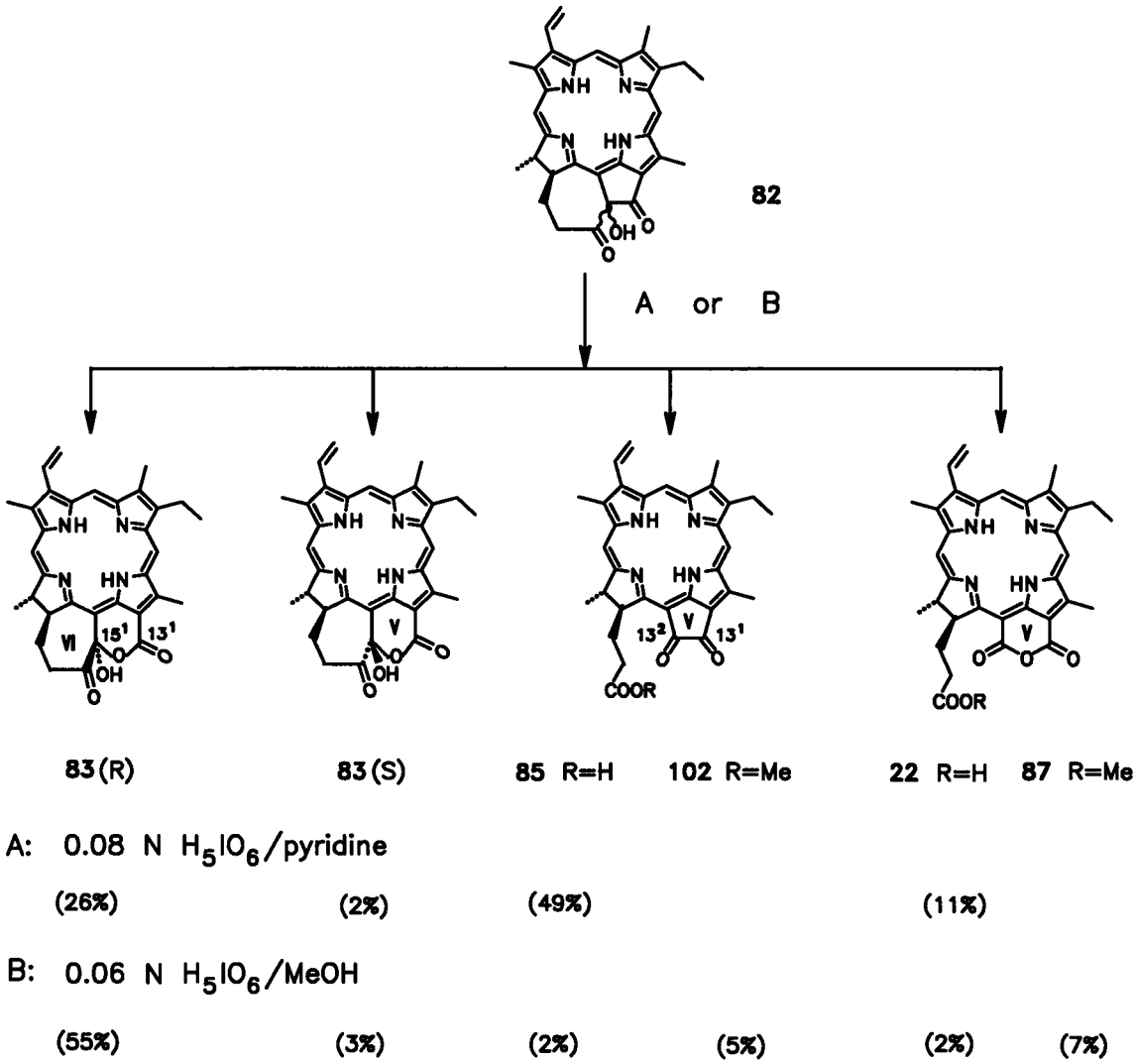
dioxane was found to be the most satisfactory and 15^1-hydroxypurpurin-7-lactone dimethyl ester (**101**) was isolated in 79% yield (the reaction was monitored by UV/Vis spectroscopy and completed in 16 h at room temperature). Under these conditions **101** was obtained as a diastereomeric mixture of 84% **101**(S) and 16% **101**(R) (from signal integration of the 400 MHz ^{1}H NMR spectrum). Both epimers of **95** were found to give the same diastereomeric mixture of **101** under the above oxidation conditions. The reason for this is that hydroxylactone **101** is very sensitive to both acid and base which give rise to epimerization via the reversible opening of the hydroxylactone ring. The equilibration of epimerization under acidic conditions predominantly favours formation of the thermodynamically more stable epimer **101**(S) in which the methoxycarbonyl moiety at position 15^1 is on the opposite side to that of the bulky 17-propionic group. Therefore, in subsequent reactions, we used the (95%S, 5%R) **82** instead of using the epimerically-pure compounds. Reaction of hydroxylactone **101** with an excess of ethereal diazomethane caused almost quantitative conversion to purpurin-7 trimethyl ester (**16**).

2.6 Regioselective Oxidation

When the initial experiment utilizing the above periodate oxidation was used to make hydroxylactone **83** and 1,2-diketone **85** from hydroxychlorin **82**, only a low yield (total <10%) of the desired products were obtained, the major product (>50%) being the di-oxidized product, purpurin-18 (**22**). This result indicated that the mono-oxidized products **83** and **85** were more reactive than the starting material **82**. This was further

confirmed by following oxidations of **83** and **85**, with periodic acid, where both more rapidly gave purpurin-18 (**22**).

Subsequent work showed that regioselective oxidation of *one of the two carbonyl groups* can be modestly achieved by using different reaction media. Addition of pyridine (15 mL) to the periodic acid solution (40 mL) resulted in the formation of 13^2-oxopyropheophorbide *a* (**85**) (49%) and two minor products, an 11% yield of purpurin-18



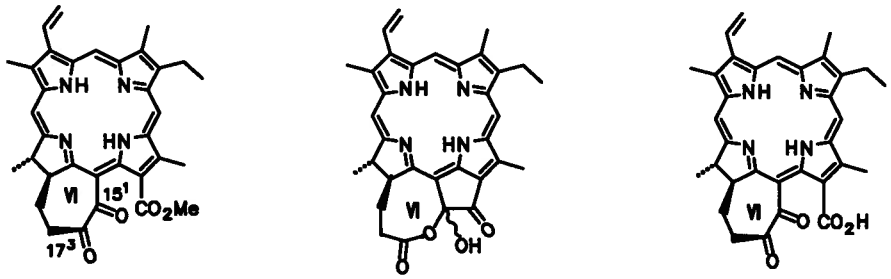
Scheme 18 Regioselective Periodate Oxidation of Hydroxychlorin **82**

(**22**) and a 28% yield of 15^1-hydroxychlorophyllonelactone *a* (**83**) after preparative TLC separation on deactivated silica (**Scheme 18**, Condition **A**). Structural assignments are based on visible spectra and ^1H and ^{13}C NMR spectroscopy (see the following spectral discussion). This procedure which preferably oxidizes the seven-membered ring (exocyclic ring VI) allowed for the preparation of 13^2-oxopyropheophorbide *a* (**85**) in 4 steps from pheophorbide *a* methyl ester (**7**) in ~40% overall yield.

Another procedure which oxidizes the exocyclic ring V over ring VI results from replacement of pyridine with methanol (45 mL) as a component in the reaction medium (144 mL) (with periodic acid). Under these conditions, the (95%S, 5%R) **82** was predominantly converted into 15^1-hydroxychlorophyllonelactone *a* (**83**) (57%), together with four minor products, purpurin-18 methyl ester (**87**) (7.2%), purpurin-18 (**22**) (2.7%), 13^2-oxopyropheophorbide *a* methyl ester (**102**) (5.4%) and 13^2-oxopyropheophorbide *a* (**85**) (1.8%) (**Scheme 18**, Condition **B**). Separation was achieved by preparative TLC on deactivated silica gel. The two purple-red bands, **22** (the second least mobile band) and its methyl ester **87** (most mobile band), could be readily identified from their different R_f values and their identical visible spectra (700 nm, 404 nm) while the two yellow bands, **85** (the least mobile band) and its methyl ester **102** (the second mobile band) displayed the same visible spectra (678 nm, 420 nm, 390 nm). The gray-green major band (**83**) was analyzed by 400 MHz ^1H NMR to be a (R,S) diastereomeric mixture of 94% 15^1R-hydroxychlorophyllonelactone *a* [**83**(R)] and 6% 15^1S-hydroxychlorophyllonelactone *a* [**83**(S)]. Hydroxylactone **83** was found, like the model hydroxylactone **101**, to be very sensitive to both acid and base which give rise to epimerization via the reversible opening

of the hydroxylactone ring. Nevertheless, the principal epimer **83**(R) has an α (downward) 15^1-OH group rather than a β-OH (upward) as in the model compound **101**(S). The reversed orientation of 15^1-OH group in **83**(R) is due to the conformation at C-15^1 position which reduces the major steric congestion with the carbonyl group at C-17^3. Optically-pure **83**(R) was obtained by subjecting the above diastereomeric mixture to preparative TLC separation on deactivated silica gel. The synthetic **83**(R) exhibits a spectrum identical to that of the natural product (see the following spectral discussion). This procedure provided an efficient synthesis of 15^1R-hydroxychlorophyllonelactone *a* [**83**(R)] in 4 steps from pheophorbide *a* methyl ester (**7**) in ~45% overall yield.

As expected, direct reaction of the (94%R, 6%S) 15^1-hydroxychlorophyllone-lactone *a* (**83**) with an excess of ethereal diazomethane gave a good yield (82%) of chlorophyllonic acid *a* methyl ester (**84**) (**Scheme 19**). Attempts to isolate **88**, the lactonized isomer of 13^2-oxopyropheophorbide *a* (**85**) by using milder oxidation conditions failed. In addition, attempts to obtain a pure sample of chlorophyllonic acid *a* (**104**), a non-lactonized isomer of 15^1-hydroxychlorophyllonelactone *a* (**17**), were also unsuccessful. These observations, and a consideration of the peripheral overcrowding,



84 **88** **104**

Scheme 19 Chlorin **84** and Its Unfavored Non-Esterified Isomers

indicate that the formation of the six-membered hydroxylactone ring (as in chlorins **22**, **83**, and **101**) is predominant and cyclization to the eight-membered hydroxylactone ring (as in **88**) is unfavoured under our reaction conditions. Although chlorins **84** and **102** are 1,2-diketones, their oxidation by periodic acid was found to be even faster than the α-hydroxy-1,3-diketone **82**, a difference perhaps mainly attributed to the ring strain of the 1,2-diketone twisted conformation in **84** and **102** and the obviously more encumbered conformation of **82**.

2.7 Structure and Spectral Characterization

All the synthetic chlorins were subjected to various spectral analyses, including mass, UV-Vis absorption, ^{1}H and ^{13}C NMR spectroscopies. All compounds were homogeneous and diastereomerically-pure materials, as ascertained by reversed-phase HPLC analyses (except **81** and **83**(R) due to their instability). Further, their ^{1}H and ^{13}C spectral assignments were carefully compared with the reported spectral data[89] of the corresponding natural compounds including their ^{1}H-^{1}H coupling constants.

The conformational changes of the chlorin macrocycles resulting from variations of the peripheral substituents and the resulting shift on the frontier orbitals of these chlorins are reflected in their electronic absorption spectra. We have divided these chlorins into 4 different types as a result of their optical absorption spectra. Each type represents special exocyclic structures and the conjugation effects of the exocyclic rings with the chlorin framework.

Type I (**Fig. 2.2**), including hydroxychlorins **82**(R), **82**(S), **95**(R) and **95**(S), is characterized by a strong Soret band, in chloroform, at ~412 nm and a relatively strong Q

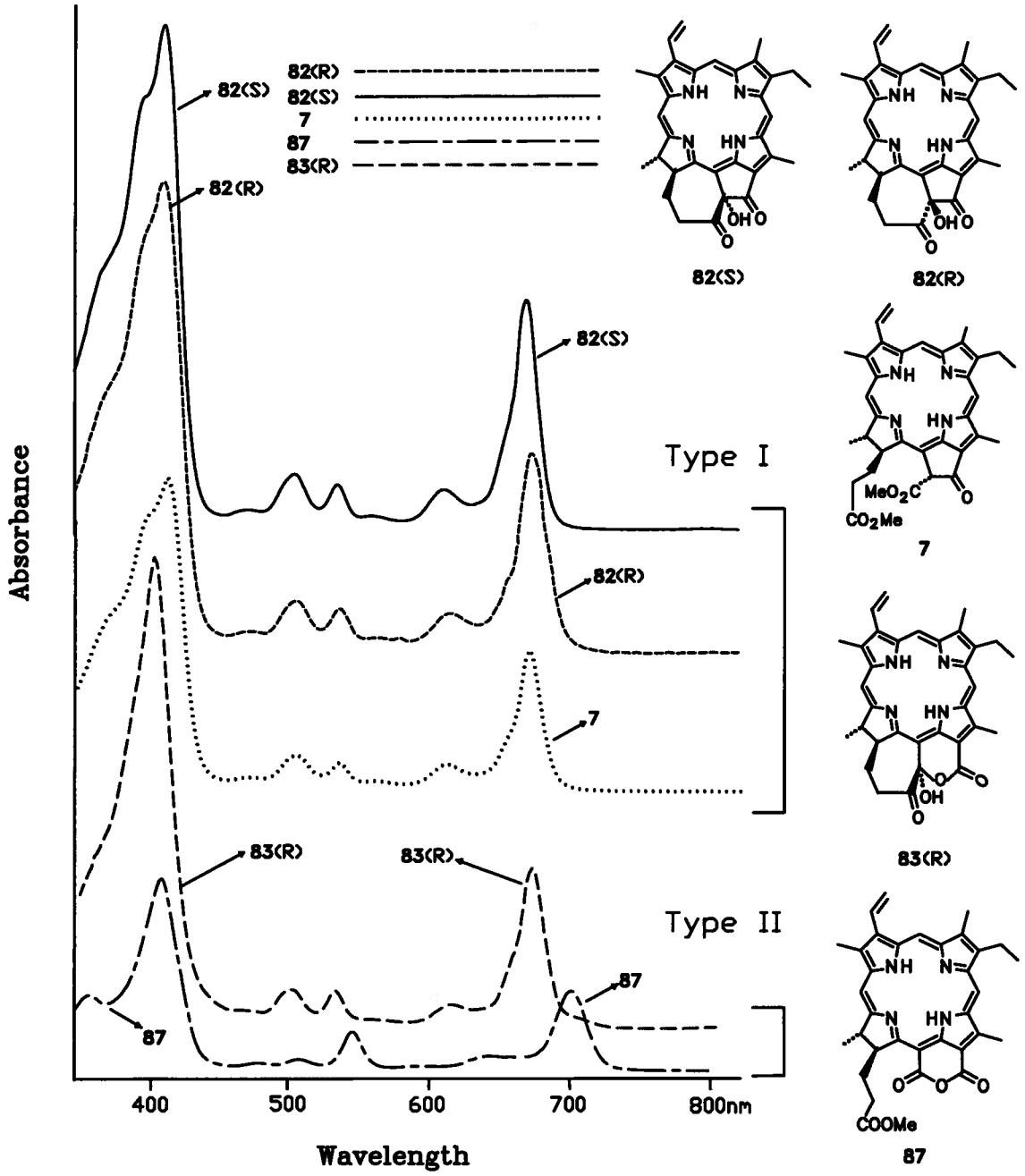


Fig. 2.2 Structures and UV/Vis Spectra ($CHCl_3$) of Pheophorbide *a* Methyl Ester (**7**), 13^2S-Hydroxychlorophyllone *a* [**82**(S)], 13^2R-Hydroxychlorophyllone *a* [**82**(R)], 15^1R-Hydroxychlorophyllonelactone *a* [**83**(R)] and Purpurin-18 Methyl Ester(**87**)

band at ~670 nm. They exhibit electronic spectra similar to that of pheophorbide *a* methyl ester (**7**). The only difference among them is that conformational twisting of the ring VI in **82**(R), due to the trans-orientation of 17-17^1 and 13^2-17^3 bond, make its Q band shift 4 nm to the red.

Type II (**Fig. 2.2**), including chlorins **22**, **83**(R), **87**, **92** and **101**, is another type of chlorin spectra. These chlorins (except **92**) contain the exocyclic rings V in the form of six-membered lactone. With the insertion of oxygen into the five-membered exocyclic ring V of type I, the exocyclic twisting is relieved which results in blue-shifted (~8 nm) Soret band and disappearance of the Soret shoulders. The pronounced red shifts (~30 nm) of the Q bands in purpurin-18 (**22**) and its ester **87** result from conjugation with the 15^1 carbonyl group which markedly extends the conjugation of the aromatic macrocycle.

When there are two conjugated sp^2 group (two carbonyl or one carbonyl and one C=C double bond) directly connected in the five-membered exocyclic ring V, the conformational changes of the chlorin macrocycles resulting from the effects of the twisted and electron-withdrawing exocyclic rings become so significant that the Soret bands of Type III, enol **81**, chlorins **85** and **102**, are split, as shown in **Fig. 2.3**. In addition to the split Soret bands, their Q bands are also significantly red-shifted 20 nm (**81**) and 8 nm (**85** and **102**) in contrast to that of chlorin **7**.

The Q bands of Type IV, including chlorins **16** and **84**, are somewhat resolved and slightly split into two peaks as shown in **Fig. 2.3**. Two electron-withdrawing groups (conjugated sp^2 groups) at C-15^1 and C-15^2 significantly extend the conjugation of the chlorin macrocycle which results in more than 10 nm bathochromic shifts of the Q bands

although no shift of their Soret bands was observed. It should be noted that the splitting of these Soret bands is solvent-dependent and has only been observed in chlorinated

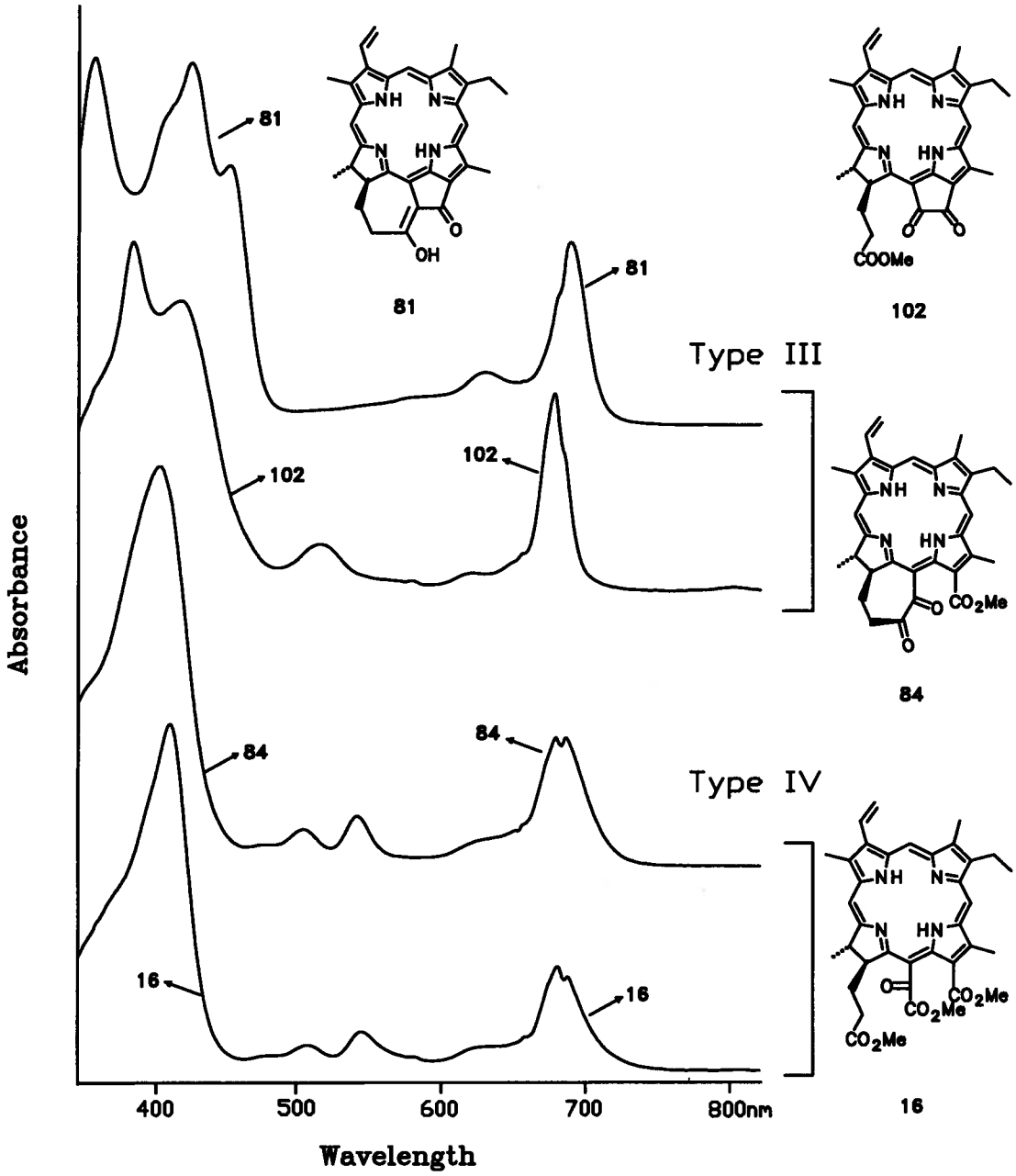


Fig. 2.3 Structures and UV/Vis Spectra ($CHCl_3$) of 13^2,17^3-Cyclopheophorbide *a* Enol(**81**), 13^2-Oxopyropheophorbide *a* Methyl Ester(**102**), Chlorophyllonic Acid *a* Methyl Ester(**84**) and Purpurin-7 Trimethyl Ester(**16**)

solvents such as methylene chloride and chloroform. In non-chlorinated solvents the two peaks of the split Q band become a peak with a shoulder on the long-wavelength side.

Consequently, the formation of ring VI has less effects on the macrocyclic conformation if the electron-withdrawing carbonyl (C-17^3) is not conjugated with the chlorin ring. The sp^2 conjugate groups (electron-withdrawing) of the exocyclic rings deform the conformations of exocyclic rings and thereby the macrocycles, which in turn affects the absorption spectra.

The *meso* hydrogens in the chlorins of Type I and Type II do not show any shifts in their ^{1}H NMR spectra since the macrocyclic conformation remains almost identical. The hydroxy groups at C-13^2 [**82**(R), **82**(S), **95**(R) and **95**(S)] and C-15^1 [**83**(R) and

Table 2.3 Selected ^{1}H NMR Spectral Data ($CDCl_3$, 400 MHz)

	Compound									
Proton	**7**[a]	**81**[a]	**82**(S)[a]	**82**(R)[b]	**95**(R)[b]	**95**(S)[a]	**101**(S)[a]	**83**(R)[b]	**102**[b]	**84**[b]
H-10	9.52	8.64	9.40	9.47	9.53	9.62	9.77	9.68	9.90	9.70
H-5	9.39	8.43	9.35	9.35	9.47	9.48	9.55	9.50	9.86	9.49
H-20	8.57	7.38	8.70	8.52	8.61	8.63	8.80	8.78	9.00	8.60
H-18	4.52	2.93	4.33	4.75	4.49	4.49	4.43	4.38	4.68	4.40
H-17	4.22	2.58	4.90	3.82	4.69	4.15	4.05	4.42	5.16	4.53
H_a-17^1	2.60	1.71	2.23	3.71	2.13	2.92	2.46	2.19	2.78	2.38
$H_{a'}$-17^1	2.58	2.58	2.88	2.65	2.46	2.28	2.18	2.85	2.36	2.90
H_b-17^2	2.20	2.45	2.78	3.83	2.09	2.55	2.45	3.49	2.67	3.83
$H_{b'}$-17^2	2.18	2.45	4.31	2.95	2.29	2.26	1.80	3.01	2.32	3.05
13^2-OH/H	6.24		4.56	4.14	5.32	5.43				
15^1/17^3-OH		13.24					6.05	5.86		

[a]Concentration 1.5 mg/0.6 mL [b]Concentration 1.0 mg/0.6 mL

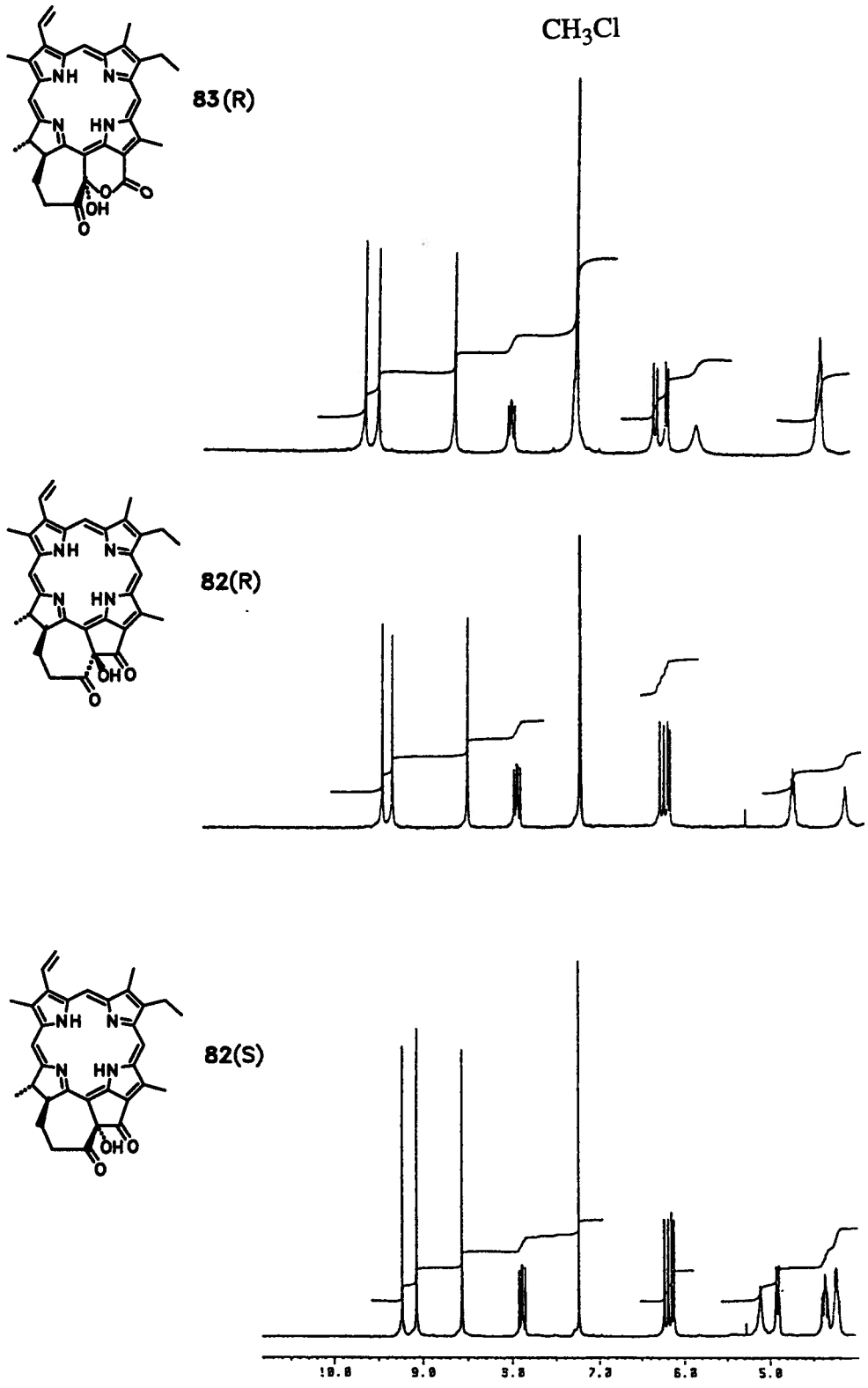


Fig. 2.4 ^{1}H NMR Spectral Comparison (the Low Field Region) of Natural Hydroxychlorins **82**(S), **82**(R) and **83**(R) ($CDCl_3$, 400 MHz)

101(S)] cause pronounced anisotropical effects on their nearby protons, which results in downfield shifts for H-17, $H_{a'}$-17^1 and $H_{b'}$-17^2 in **82**(S), **95**(R), **83**(R) and for H-18, H_a-17^1 and H_b-17^2 in **82**(R), **95**(S), **101**(S) (**Table 2.3**).

For chlorins with Type III and Type IV absorption spectra, the effects of the π electrons at C-15^1 and/or exocyclic rings on their chemical shifts in ^{1}H NMR spectroscopy are less pronounced than on their electron absorption spectra. However, two peak shifts are observed for chlorins **81** and **102**, aromatic rings with split Soret bands (Type III). For enol **81**, the $\Delta\delta$ for H-10 resonance was –0.88 ppm, for H-5 –0.96 ppm, and for H-20 –1.19 ppm together with general up-fielded shifts more than 0.40 ppm in comparison with the corresponding protons in a normal chlorin such as **7**. These shifts suggest an apparently increased local aromatic ring current in **81** due to the strain on the

Table 2.4 Selected ^{1}H -^{1}H Coupling Constants J (Hz, $CDCl_3$)

	Compound									
Protons	**7**	**81**	**82**(S)	**82**(R)	**95**(R)	**95**(S)	**101**(S)	**83**(R)	**102**	**84**
3^1,3^2(E)	17.1	18.0	18.1	18.1	18.2	18.3	17.6	17.6	18.5	17.4
3^1,3^2(Z)	11.6	11.6	11.4	11.6	11.9		11.5	11.2	12.4	12.1
3^2(E),3^2(Z)		1.6	1.2	1.0	1.0	0.8	1.2	1.0	1.3	1.0
8^1,8^2	7.8	7.9	8.1	7.2	7.7	7.6	8.0	7.9	7.7	7.7
18^1,18	7.1	7.2	7.4	7.0	7.0	7.3	6.8	7.5	7.6	7.3
18,17	1.7		3.8	8.3				1.6	1.3	1.7
17,17^1_a	3.1		13.3	11.0	1.7	2.2	2.4	11.5	2.9	12.3
17,$17^1_{a'}$	9.3		3.5	1.6	8.5	10.2	10.4	5.3	8.8	6.6
17^1_a, $17^1_{a'}$	13.3		12.4	13.1				12.8		12.3
17^1_a, 17^2_b	7.1		2.1	6.2				8.3		10.0
17^1_a, $17^2_{b'}$	6.2		14.0	12.8				2.5		1.4
$17^1_{a'}$, 17^2_b	9.3		4.4	1.5				9.0		10.0
$17^1_{a'}$, $17^2_{b'}$	5.3		3.5	5.2				9.6		8.3
17^2_b, $17^2_{b'}$	15.1		11.7	15.0				11.5		12.7

chlorin nucleus resulting from the conjugated enol conformation. This conformation is energetically-favoured through formation of an intramolecular hydrogen bond between the 17^3-OH (enol) and the 13^2-carbonyl group. Compound **102**, another chlorin of Type III, has showed pronounced down-fielded shifts [$\Delta\delta$ for H-10 (0.38 ppm), H-5 (0.47 ppm) and H-20 (0.43 ppm)] in contrast to the corresponding hydrogens in **7**.

The large coupling constants between H-17 and H_a-17^1 in **82**(S) (J = 13.3 Hz), **82**(R) (J = 11.0 Hz), and **83**(R) (J = 11.5 Hz) indicate their 1,2-trans diaxial relationships (**Table 2.4**). The relative stereochemistry around rings IV, V and VI was deduced by a 2D COSY spectrum (**Fig. 2.13**) and decoupling experiments. These assignments shown in **Table 2.3** and **2.4** are consistent with their assigned structures.

The ^{13}C NMR spectra of epimeric **82**(R) and **82**(S) showed upper field shifts of C-17^1 ($\Delta\delta$ = −15.28 ppm) and C-18^1 ($\Delta\delta$ = −5.38 ppm) in **82**(R) from **82**(S), which result from the deformation of the seven-membered ring VI in **82**(R) where the 17-17^1 and 13^2-17^3 bonds are trans-oriented (**Table 2.5**). This deformation does not exist in the absence of exocyclic ring VI, such as in epimers **95**(R) and **95**(S). The ^{13}C NMR spectra of diketonic isomers **84** and **102**, shows a different effect of exocyclic rings VI and V on the macrocyclic conformations. The downfield shift of the carbonyl group bonded to C-15 position, C-15^1 (δ = 192.38 ppm) of **84** (with the exocyclic ring VI) from that of the C-13^2 (δ = 185.19 ppm) in **102** (with the exocyclic ring V) is attributed to a change in hybridization modified by the more twisted ring V (five-membered ring) than ring VI (seven-membered ring).

Table 2.5 Selected ^{13}C NMR Spectral Data ($CDCl_3$)

	Compound								
Carbon	**7**	**81**	**82**(S)	**82**(R)	**95**(R)	**95**(S)	**83**(R)	**102**	**84**
C-17^3	173.36	167.35	208.00	206.21	173.46	172.83	203.62	174.87	196.90
C-13^1	189.63	191.78	195.44	193.39	191.93	192.00	162.57	192.79	166.90
C-13^2	64.71	116.83	93.43	92.66	89.09	88.94		185.19	52.31
C-17	52.88	52.47	51.91	53.71	50.75	51.75	49.84	52.67	50.16
C-18	51.70	49.33	51.51	50.31	50.16	50.29	51.18	51.63	51.20
C-17^2	29.86	34.00	40.12	43.17	30.99	31.40	34.17	29.70	36.25
C-17^1	31.06	25.03	37.99	22.71	30.18	31.11	31.37	31.50	29.47
C-15^1							104.68		192.38
C-18^1	23.10	19.06	22.37	16.99	22.69	22.65	23.56	23.85	23.62

Detailed structural assignments of these antioxidative chlorins appear in the experimental section (section **6.2**). Selected spectra for these unusual chlorins are shown at the end of this chapter (from pp89 to pp107). These spectra are: ^1H NMR spectra: **7** (**Fig. 2.5**); **81** (**Fig. 2.7**); **82**(S) (**Fig. 2.9**); **82**(R) (**Fig. 2.11**); **83**(R) (**Fig. 2.12**); **84** (**Fig. 2.14**); **87** (**Fig. 2.16**); **92** (**Fig. 2.17**); **95**(R) (**Fig. 2.18**); **95**(S) (**Fig. 2.20**); **96**(R)(**Fig. 2.21**); **102** (**Fig. 2.23**). ^{13}C NMR spectra: **7** (**Fig. 2.6**); **81** (**Fig. 2.8**); **82**(S) (**Fig. 2.10**); **84** (**Fig. 2.15**); **95**(R) (**Fig. 2.19**); **96**(S) (**Fig. 2.22**). ^1H-^1H COSY spectrum: **83**(R) (**Fig. 2.13**).

2.8 Biogenetic Rationalization

The concern about the origin of these antioxidative chlorins takes the form of two basic questions: "Where are they from ?" and "How are they formed?". The first question is not difficult to answer. Although these compounds have been identified in diverse marine organisms such as sponge, fish, bivalves, attached and wafting diatoms, these species are all plankton feeders.[119] These marine animals themselves do not contain any

chlorophylls, but the food chain of these herbivorous species provides them a dietary source of chlorophyll *a*.

The second question should be answered with discussion. The key point is how to determine with certainty whether these chlorins were created from chlorophyll *a* by digestion of these herbivorous species or whether they were biosynthesized by these marine species to serve a special biological function. The former (degradation) view appears to suggest that these antioxidative chlorins are yet another example of chlorophyll breakdown, which happens everyday on this planet. Many degradation products of chlorophyll *a* have been reported, which includes simple oxidation and complicated breakdown to colourless products.[120] For instance, the acidic environment of the stomach alone would bring about the loss of Mg to produce pheophytin.[7,121] The removal of the phytol group from chlorophyll to form pheophorbide and the oxidation (ring IV) and decarboxylation (methoxycarbonyl moiety in ring V) steps to form phytoporphyrin (porphyrin derived from chlorophylls) have been discovered to be produced in the gut of microflora.[122]

However, the degradation view of these compounds does not explain their biological (antioxidative) function since degradation usually occurs randomly or stochastically without any biotic evolution and no chlorophyll degradation products have been reported to have any biological function. Further, it also can not explain the fact that chlorins **81**, **82**(S), **83**(R), **84** and **82**(R), **22**, **87**, **85** have been isolated and that many complicated chemical-specific steps (based on our synthetic research) are involved in their pathways from chlorophyll *a*. The epimers at C-13^2, hydroxychlorin **82**(S) (found in

short-necked clam and diatoms)[89] and hydroxychlorin **82**(R) (found in the scallop), occur in different marine species, strongly suggesting that both **82**(S) and **82**(R) are enzymatically produced in each bivalve or diatom and that new biosynthetic pathways of chlorophyll *a* to these antioxidative chlorins are present. Compound **82**(S) was also isolated from the mixture of attached diatoms[91], indicating that **82**(S) was produced by the attached diatoms themselves. Thus, these antioxidative chlorins are not serendipitous chlorophyll degradation products. Rather, it appears reasonable that they are biosynthesized from chlorophyll *a*. They are a class of compounds having a specific metabolic function (antioxidative activity). The biogenetic evolution may result from biotic regulation of these marine organisms in order to develop antioxidative function as a defence of the detrimental effects of oxygen. It is not clear at this stage why these species choose to modify chlorophyll from their food chain rather than other simpler molecules.

Many enzymes, which mimic the synthetic transformations of these antioxidative chlorins from chlorophyll *a*, have been found in nature. Enzymatic transformation of chlorophyll *a* to pyropheophorbide *a* has been confirmed in a mutant strain of the microalga, *Chlorella fusca*.[123] The assumed biosynthetic origin of 13^2, 17^3-cyclopheophorbide *a* enol (**81**), via an intramolecular Claisen-type condensation of pyropheophorbide *a* methyl ester (**25**), is firmly supported by the facile chemical transformation. Actually, only pyropheophorbide *a* and its esters (not their metallated compounds) can participate in this cyclization reaction. Our attempts to extend this reaction to chlorophyll *a* and its closely related derivatives, pheophytin *a* (**5**) and pheophorbide *a* methyl ester (**7**) were unsuccessful.

Enzymatic hydroxylation of chlorophyll *a* to 13^2-hydroxychlorophyll and 13^2S-hydroxypheophytin *a* has been found in alga[124] and in silkworm[115], respectively. The chemical possibility [oxidation of 13^2,17^3-cyclopheophorbide *a* enol (**81**) to give **82**(S) and **82**(R)] has been confirmed by our asymmetric hydroxylation. Periodate oxidation of either epimer of **82**, **82**(R) or **82**(S), was found to give the similar products, i.e., a mixture of mono- and di-oxidized products **22**, **83** and **85**, which coincidently parallels all the antioxidative chlorins isolated from the short-necked clam, *Ruditapes philippinarum*. This observation suggests that **22**, **83**(R) and **85** were probably biosynthesized by "periodate type" oxidation of **82**(S). In fact, flavin-dependent monooxygenases have been identified which catalyse oxidation of acyclic and alicyclic hydroxyketones to hydroxylactones in soil bacteria.[125] However, the natural oxidation of hydroxychlorin **82**(R) has found to be highly regioselective in the scallop, *Pactinopecten yessoensis*, which only metabolizes the mono-oxidized products of the exocyclic ring V, hydroxylactone **83**(R) and its methyl ester **84**.

Esterification is a common reaction in marine metabolism. Chlorophyllonic acid *a* methyl ester (**84**) and purpurin-18 methyl ester (**87**) presumably arise from the corresponding methylation of hydroxylactone **83**(R) by ring V opening and direct methylation of purpurin-18 (**22**). Thus, the biosynthetic pathway to these compounds from chlorophyll *a* has been predicted to be parallel to their chemical synthetic routes, which has met our initial objective that the chemical synthetic routes to these unusual structures should be developed in a simple and biomimetic way.

2.9 Summary

Short and efficient stereoselective synthesis of new chlorophyll *a* related chlorins from chlorophyll *a* has been accomplished in a way that parallels their probable biogenesis. The key stereochemical issues were addressed via DBU-promoted asymmetric hydroxylation and via the anticipation that the rigid exocyclic ring VI (in hydroxylactone **83**) would provide an exploitable diastereofacial bias for ensuing hydroxylactonization to the desired epimer **83**(R).

Effective improvements on chlorophyll extraction from the blue alga *Spirulina maxima* have been achieved. The improved method affords isolation of pheophytin *a* easily and in high yield. DBU as a base for promoting the hydroxylation reactions is certain to be applicable to other 1,3-dicarbonyl systems, particularly those sensitive to ionic bases. Model studies for hydroxylactonization have shown that periodate oxidation of hydroxyketone **95** stereoselectively and predominantly forms hydroxylactone **101**(S). Periodate oxidation of α-hydroxy-1,3-diketone **82**(S) and/or **82**(R) to furnish hydroxy-lactone **83**(R) and diketone **102** was found out to be regioselective and the site of reaction depended on the appropriate choice of reaction media.

The effects of the formation of an additional exocyclic ring (ring VI) on the macrocyclic conformation and electronic absorption spectra were also discussed. The biogenesis of these antioxidative chlorins has been rationalized and a new biosynthetic

route has been proposed. These new chlorophyll *a* related chlorins will also provide strong structural evidence to support the hypothesis that the antioxidative chlorins synthesized in this work are the precursors to the so-called *disturbing petroporphyrins* characterized by exocyclic rings, the molecular fossils from chlorophyll *a* derivatives in the marine sediment.

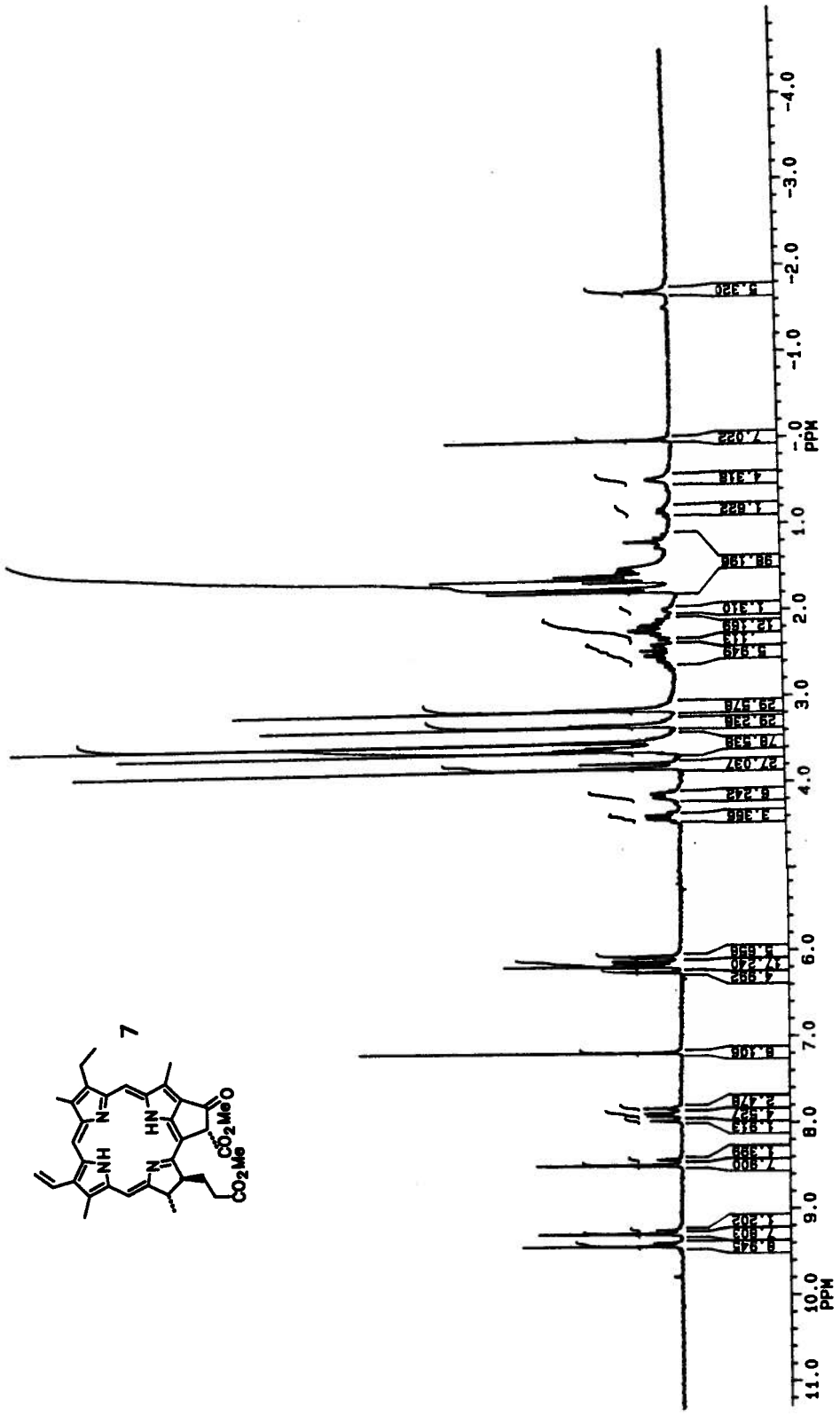


Fig. 2.5 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of Pheophorbide *a* Methyl Ester (**7**)

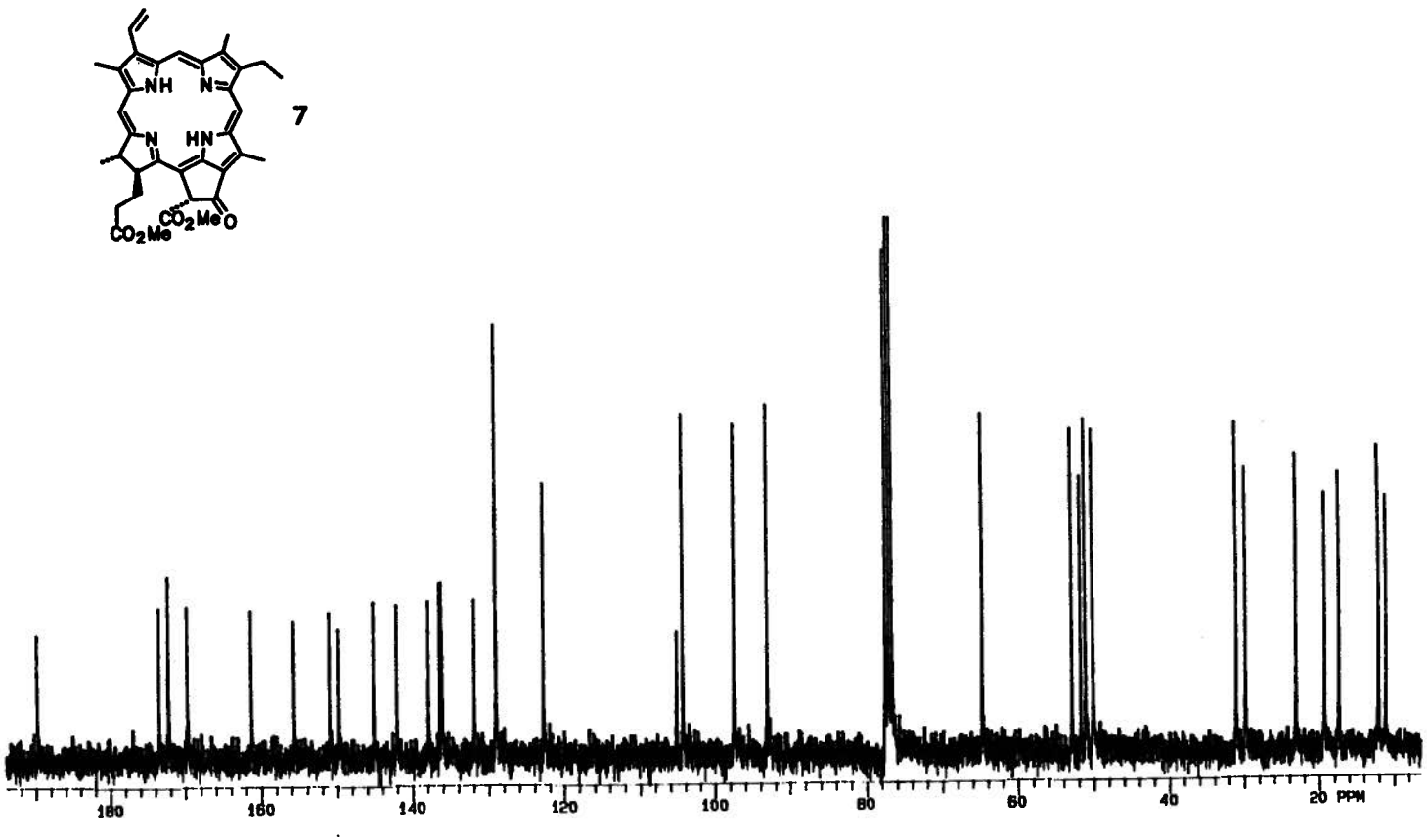


Fig. 2.6 Structure and ^{13}C NMR Spectrum ($CDCl_3$) of Pheophorbide *a* Methyl Ester (**7**)

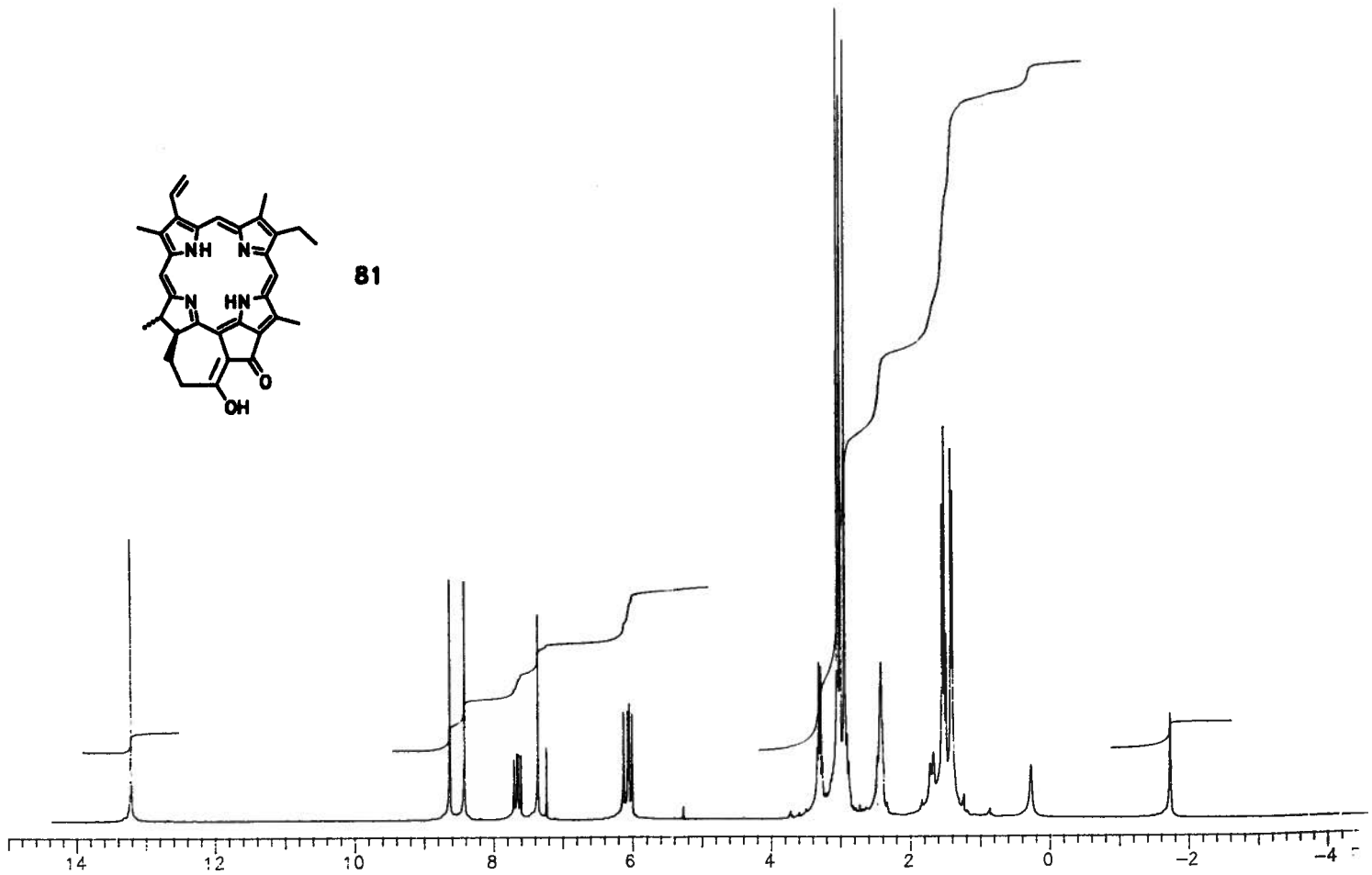


Fig. 2.7 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of 13^2,17^3-Cyclopheophorbide *a* enol (**81**)

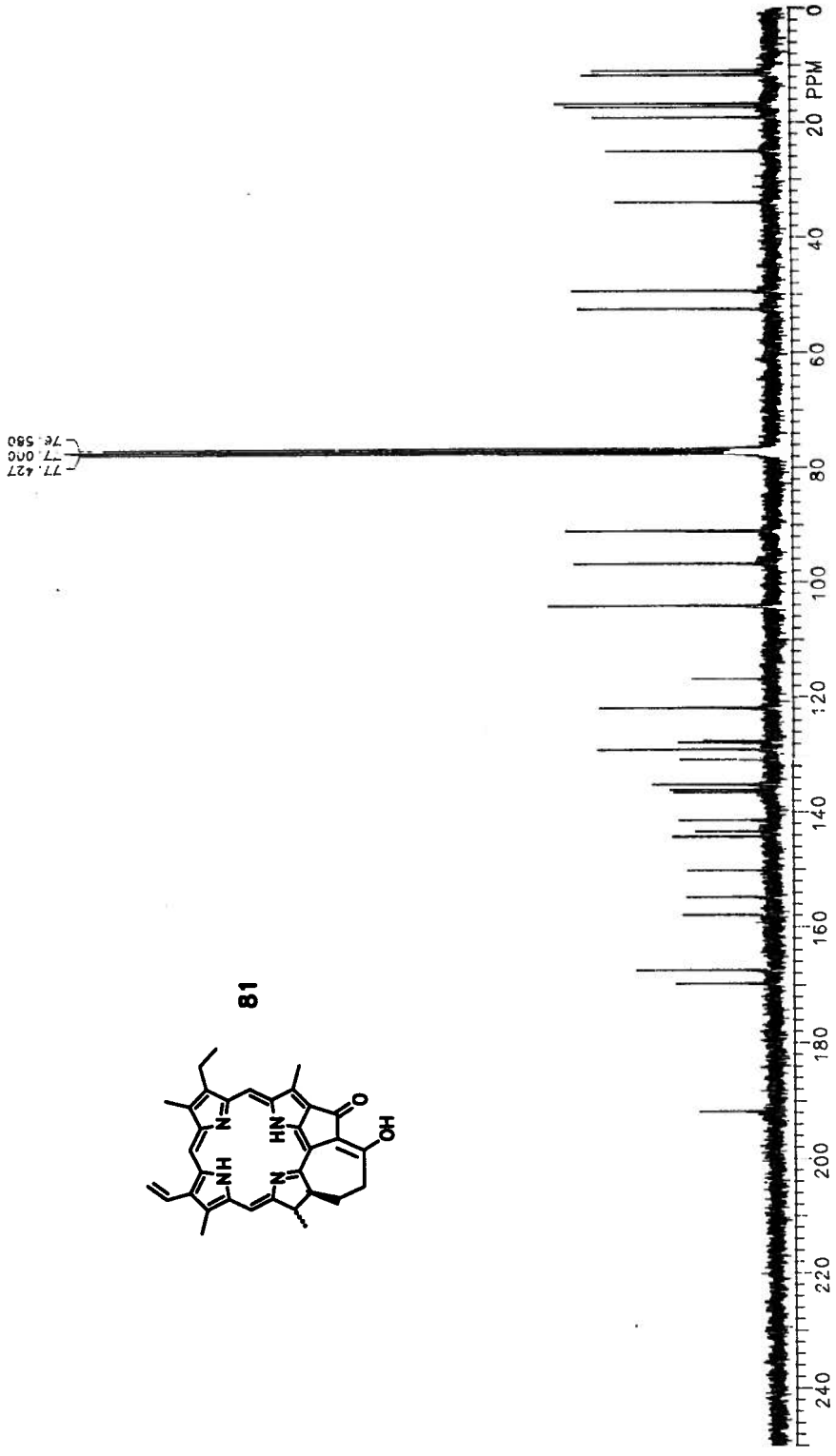


Fig. 2.8 Structure and ^{13}C NMR Spectrum ($CDCl_3$) of $13^2,17^3$-Cyclopheophorbide *a* enol (**81**)

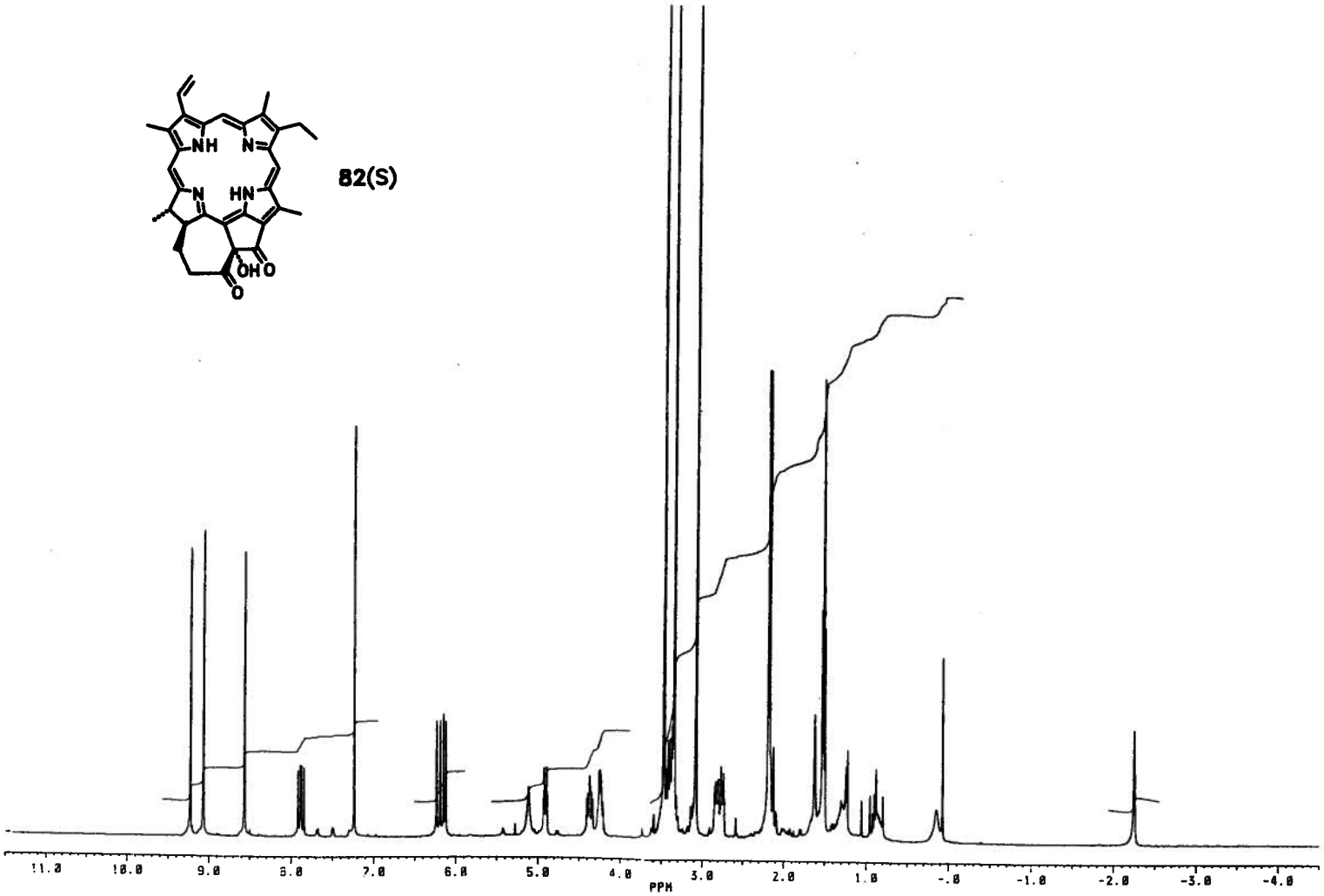


Fig. 2.9 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of 13^{2}S-Hydroxychlorophyllone *a* **[82**(S)]

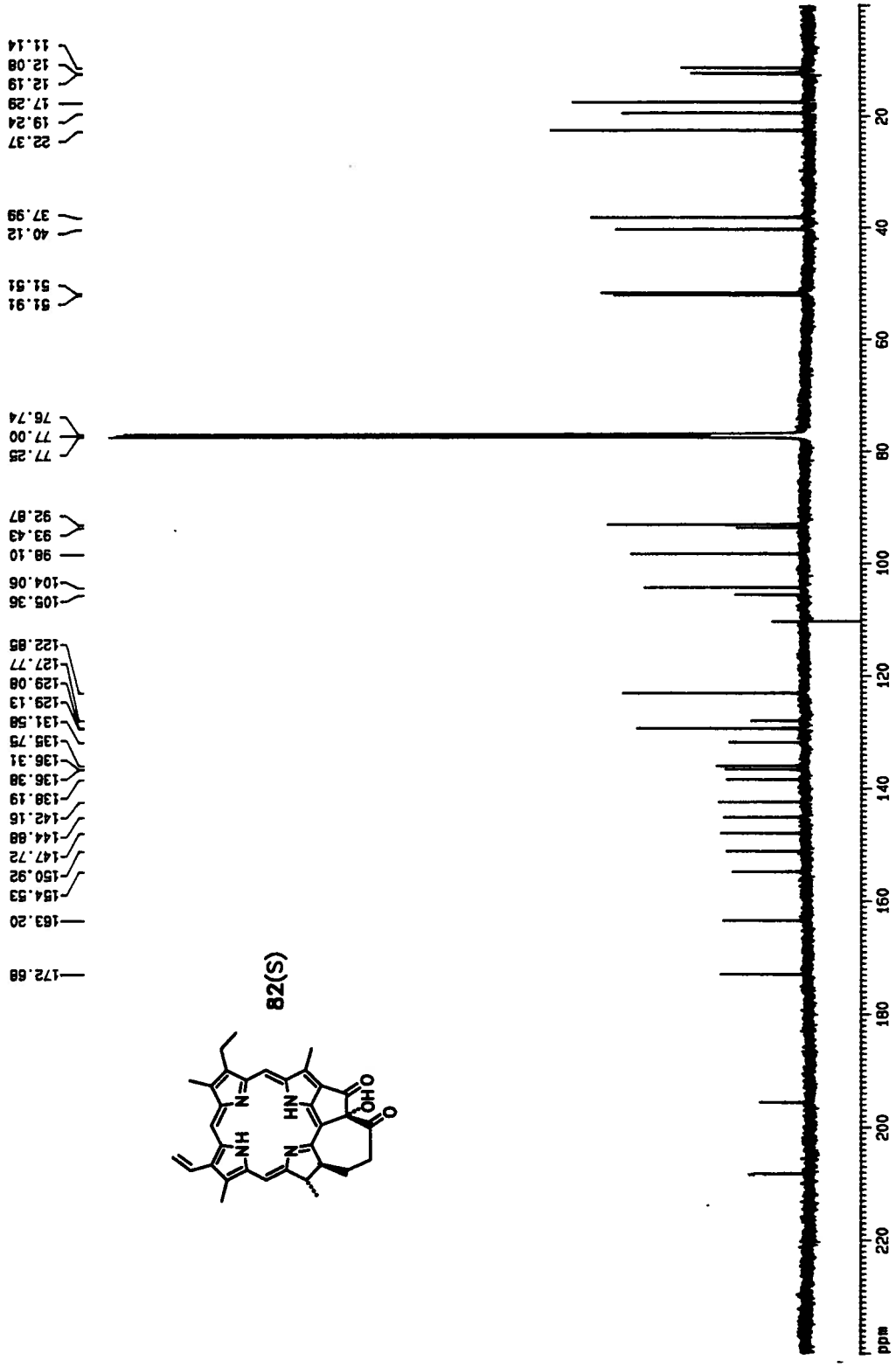


Fig. 2.10 Structure and ^{13}C NMR Spectrum ($CDCl_3$) of 13^2S-Hydroxychlorophyllone *a* [**82(S)**]

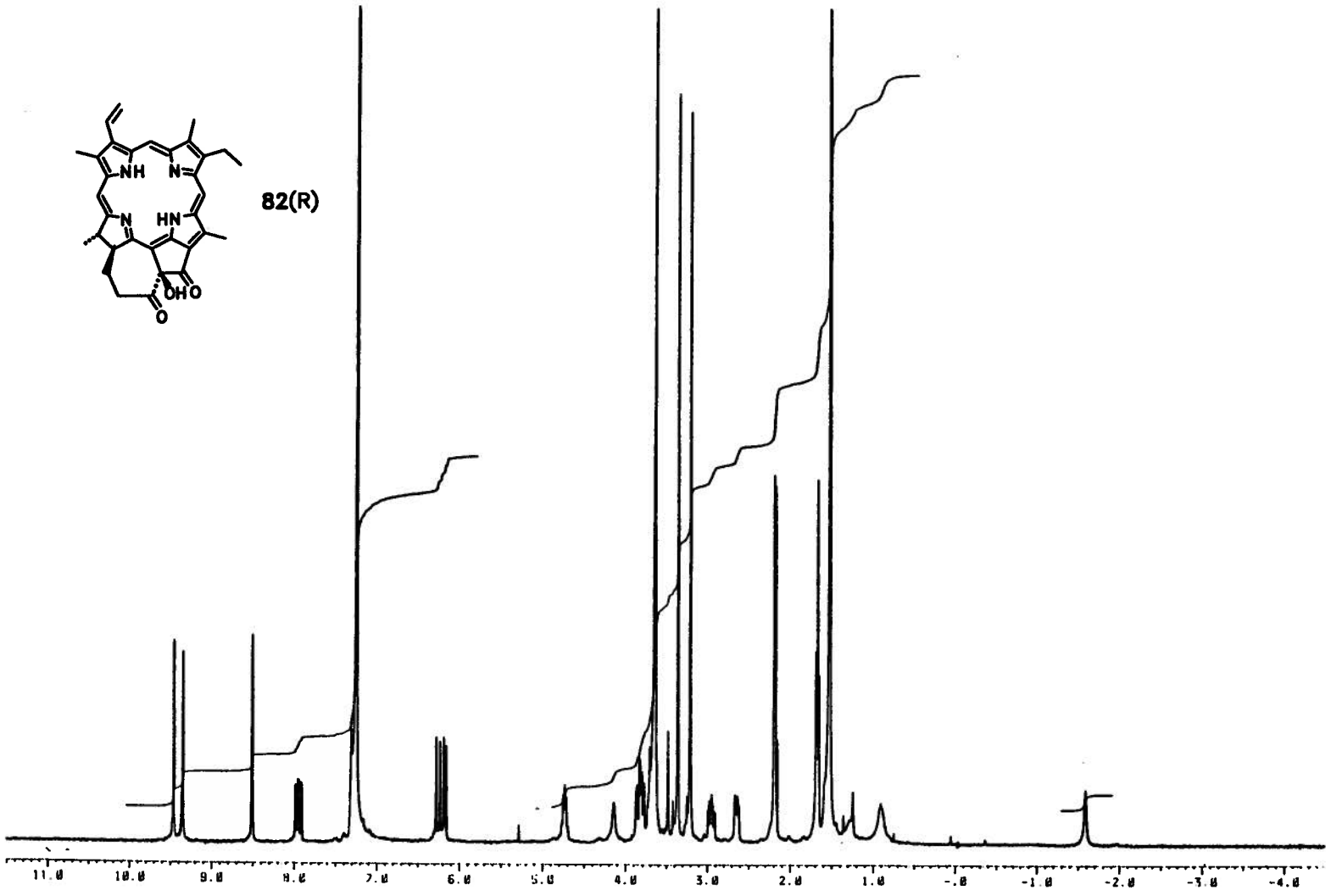


Fig. 2.11 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of 13^2R-Hydroxychlorophyllone *a* [**82**(R)]

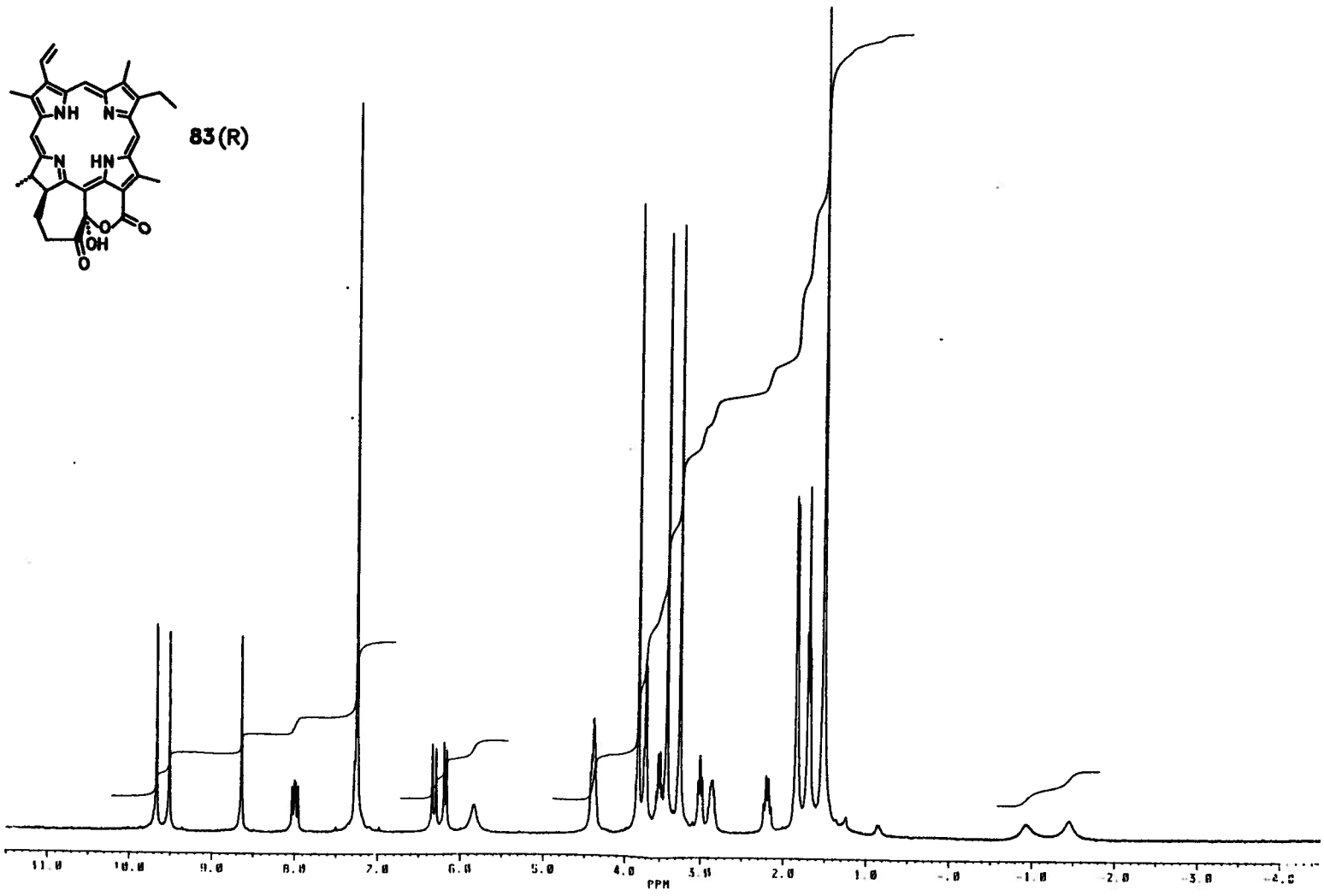


Fig. 2.12 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of 15^{1}R-Hydroxychlorophyllonelactone *a* [**83**(R)]

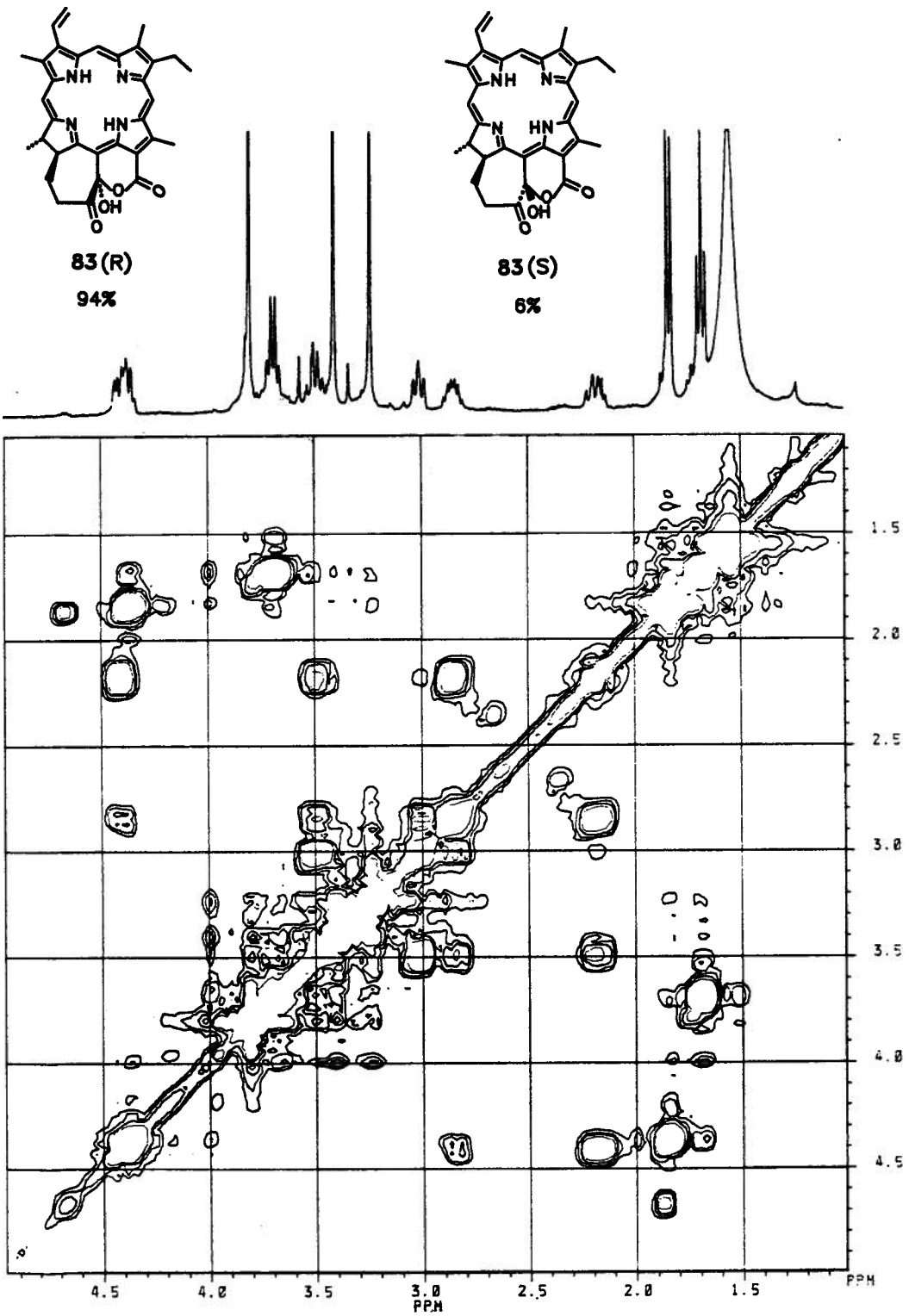


Fig. 2.13 Structure and ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of 15^{1}R-Hydroxychlorophyllonelactone *a* [**83**(R)] (88% d.e.)

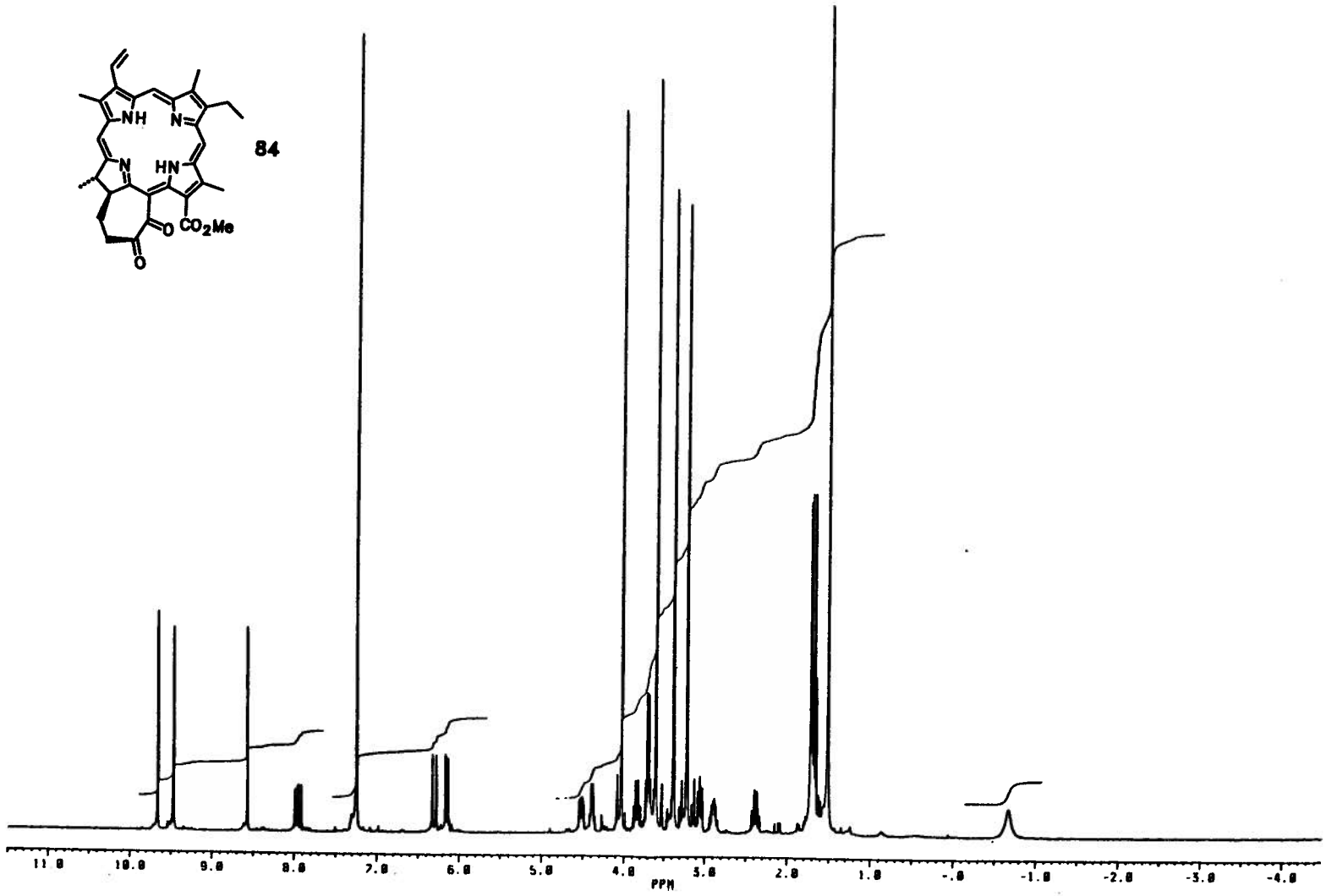


Fig. 2.14 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of Chlorophyllonic Acid *a* Methyl Ester (**84**)

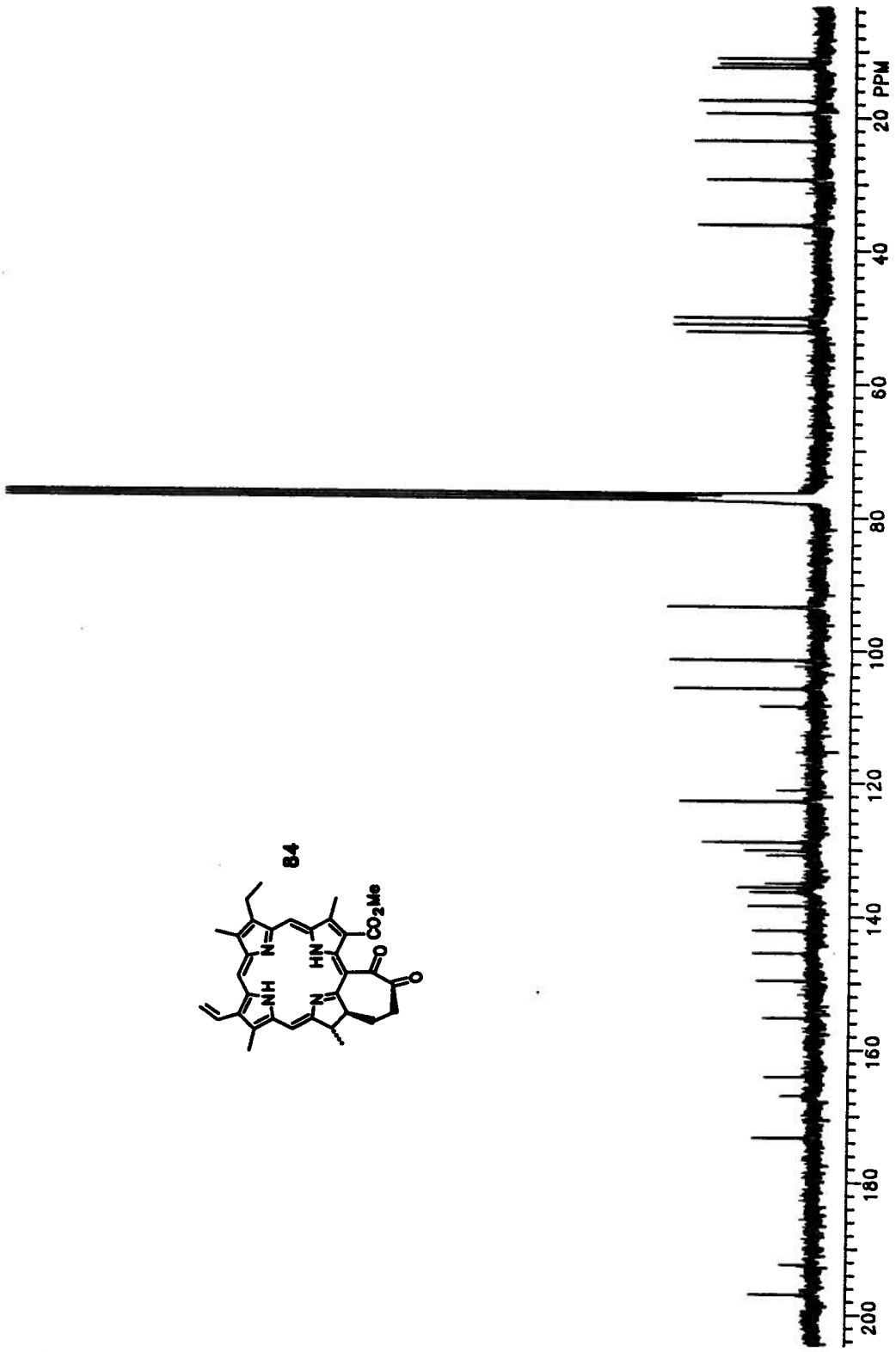


Fig. 2.15 Structure and ^{13}C NMR Spectrum ($CDCl_3$) of Chlorophyllonic Acid *a* Methyl Ester (**84**)

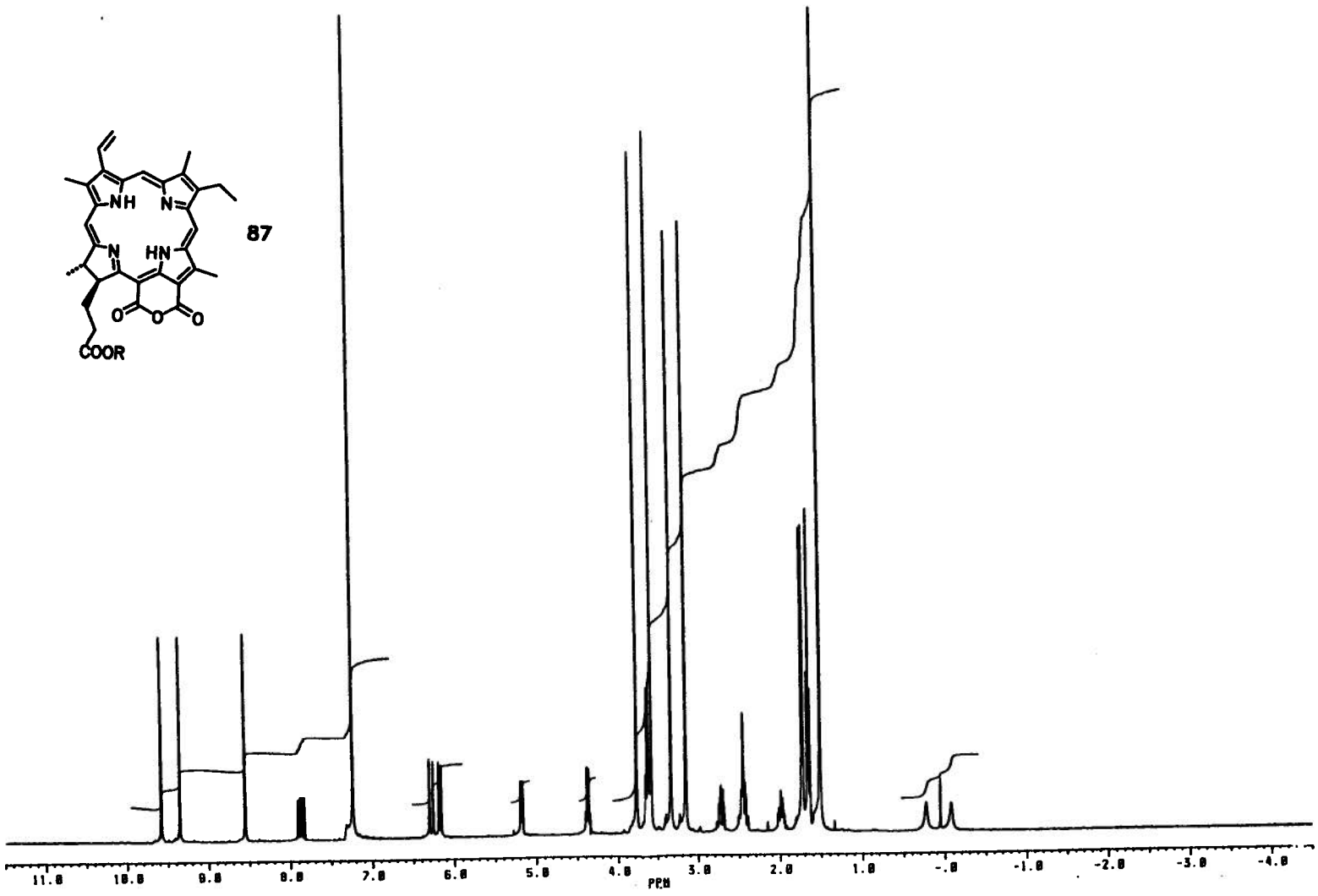


Fig. 2.16 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of Purpurin-18 Methyl Ester (**87**)

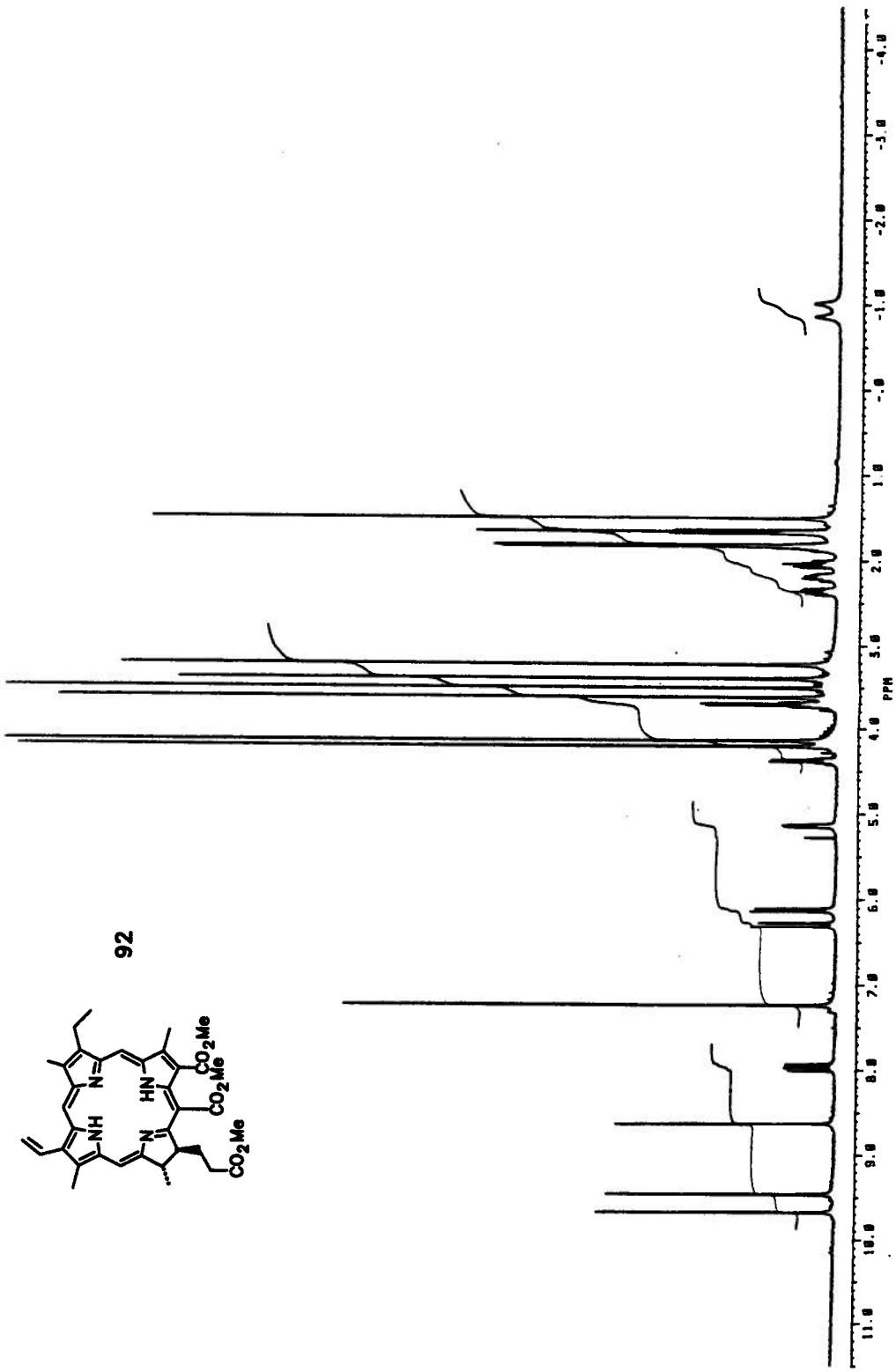


Fig. 2.17 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of Chlorin p_6 Trimethyl Ester (**92**)

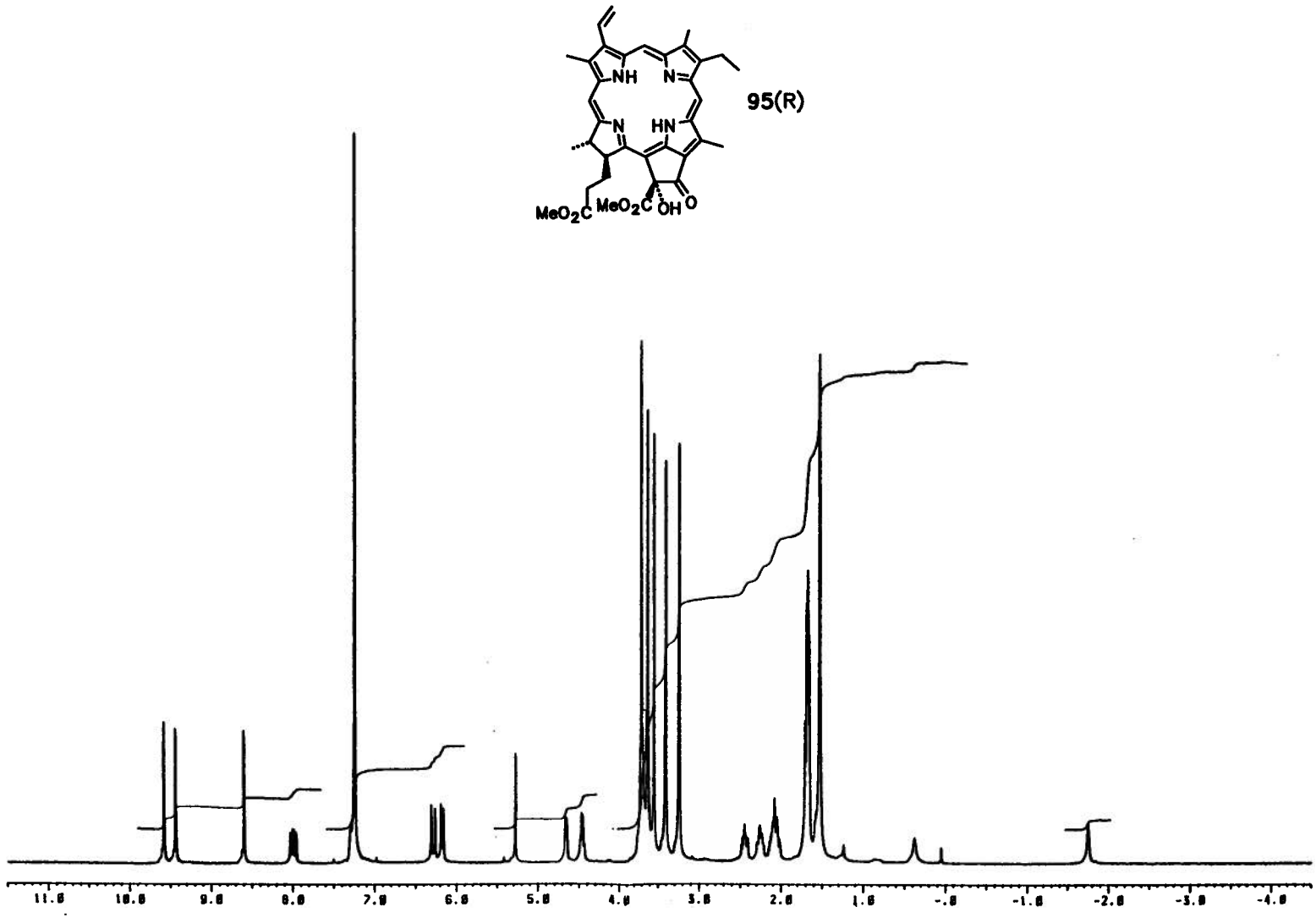


Fig. 2.18 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of 13^{2}R-Hydroxypheophorbide *a* Methyl Ester [**95**(R)]

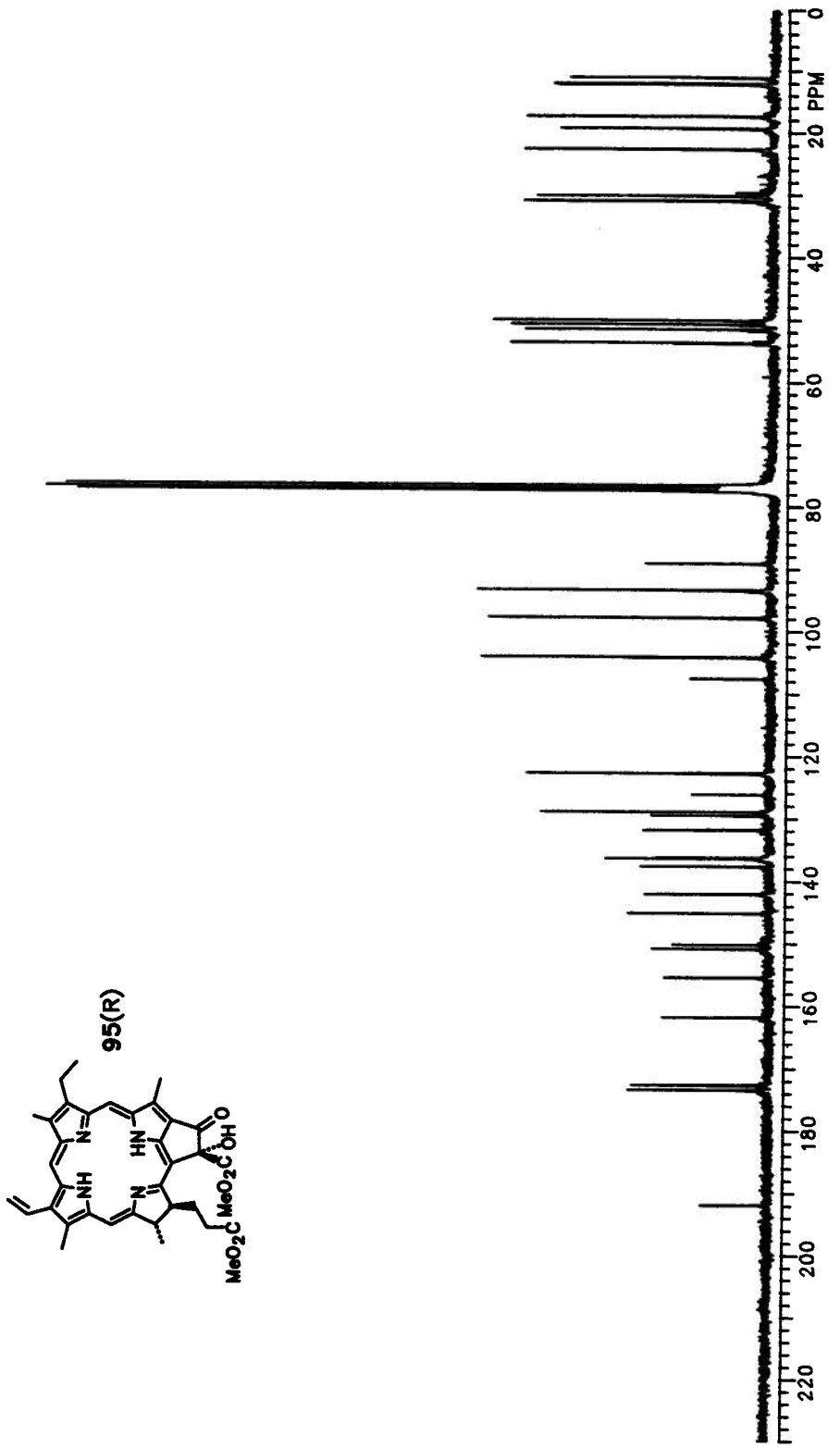


Fig. 2.19 Structure and ^{13}C NMR Spectrum ($CDCl_3$) of 13^2R-Hydroxypheophorbide *a* Methyl Ester [**95**(R)]

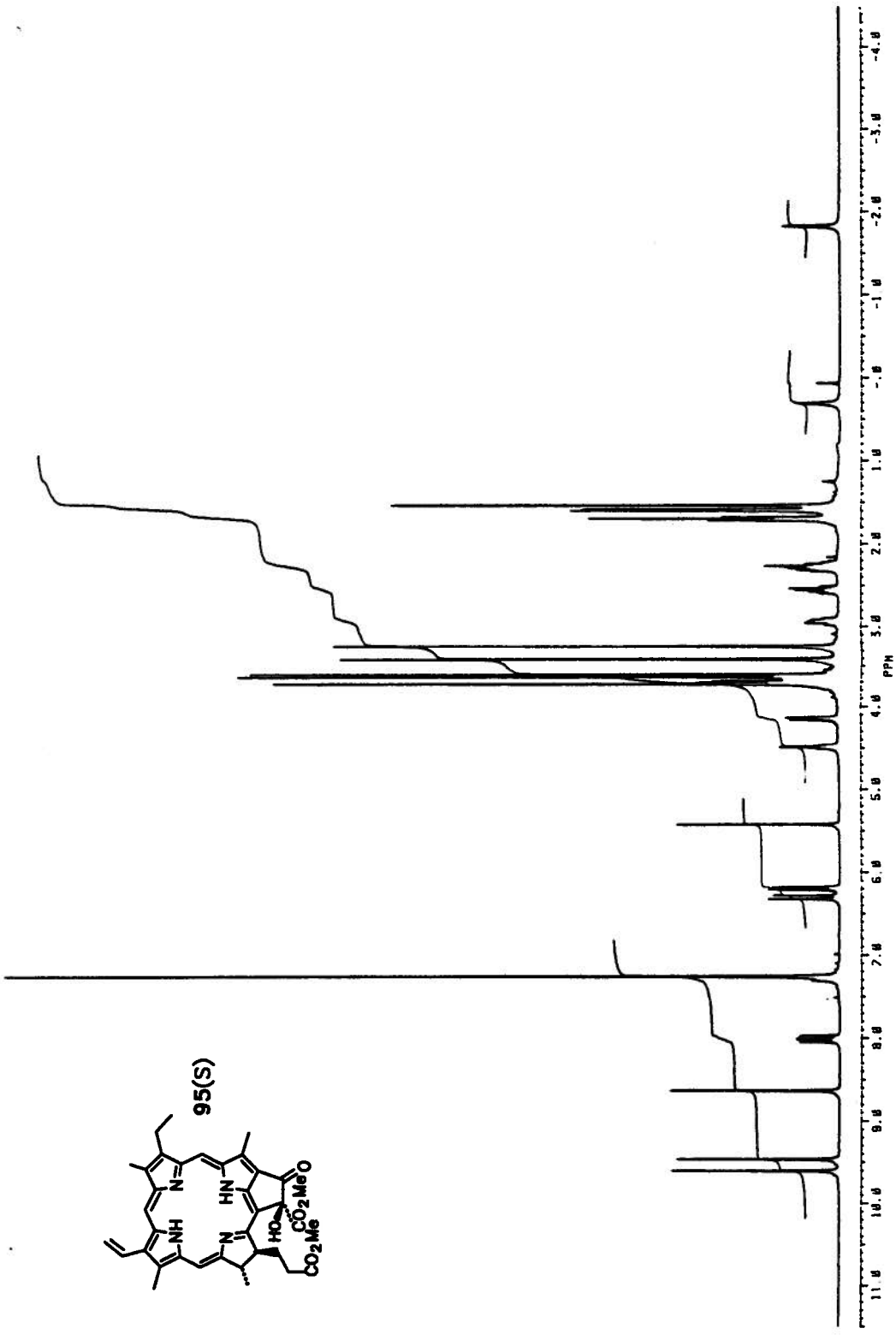


Fig. 2.20 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of 13^{2}S-Hydroxypheophorbide *a* Methyl Ester [**95**(S)]

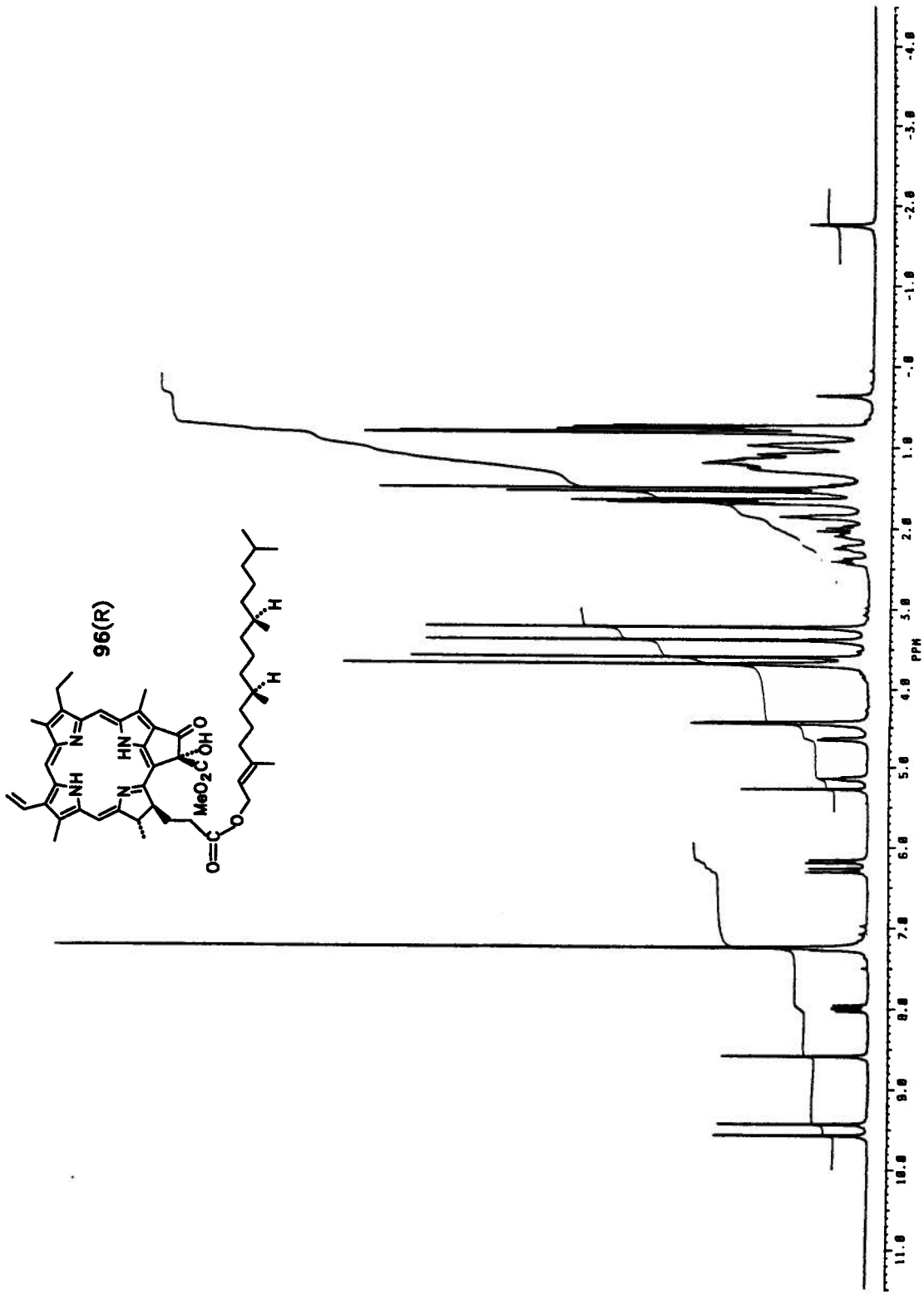


Fig. 2.21 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of 13^2R-Hydroxypheophytin *a* [**96**(R)]

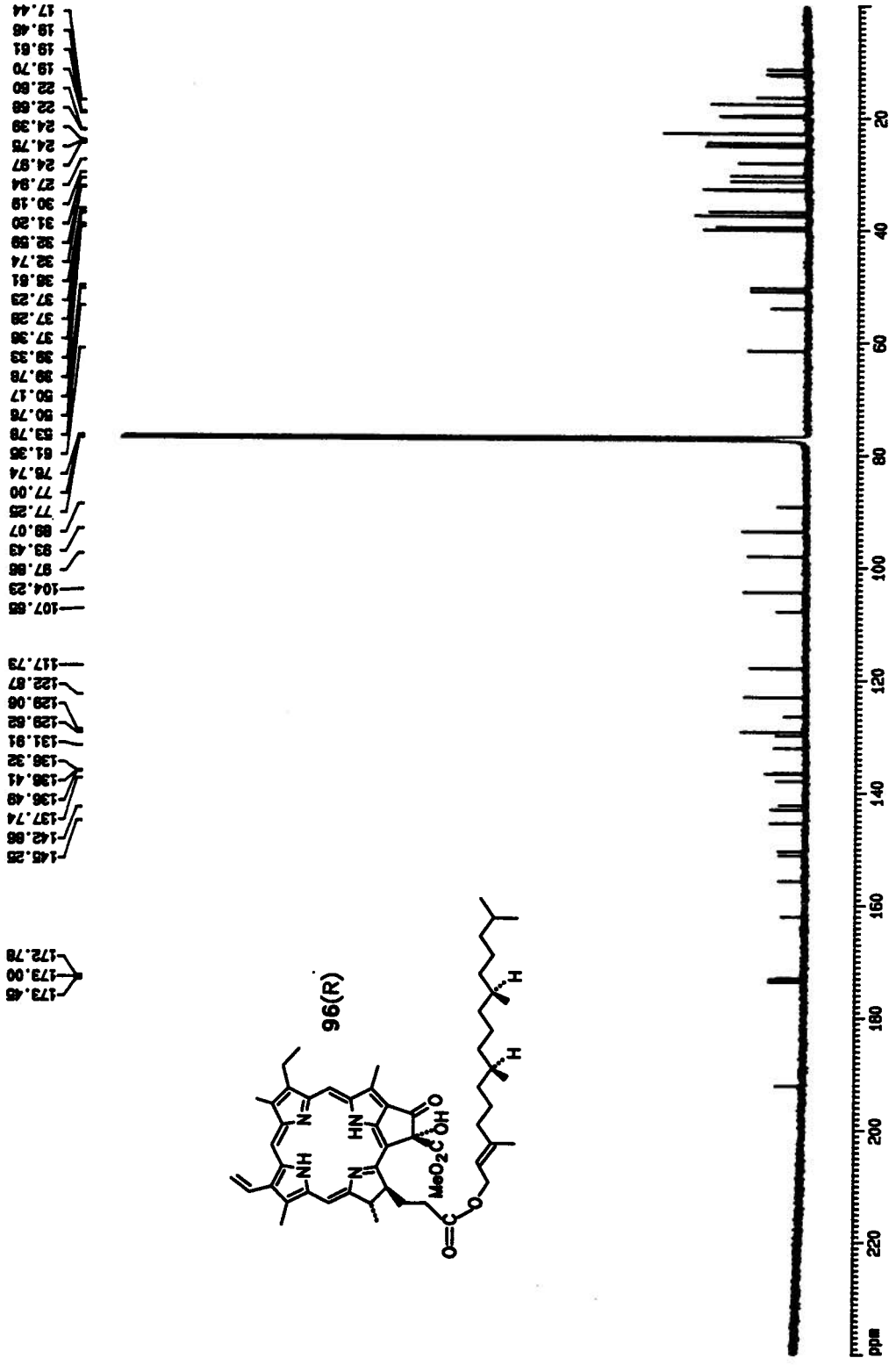


Fig. 2.22 Structure and ^{13}C NMR Spectrum ($CDCl_3$) of 13^2R-Hydroxypheophytin *a* [**96**(R)]

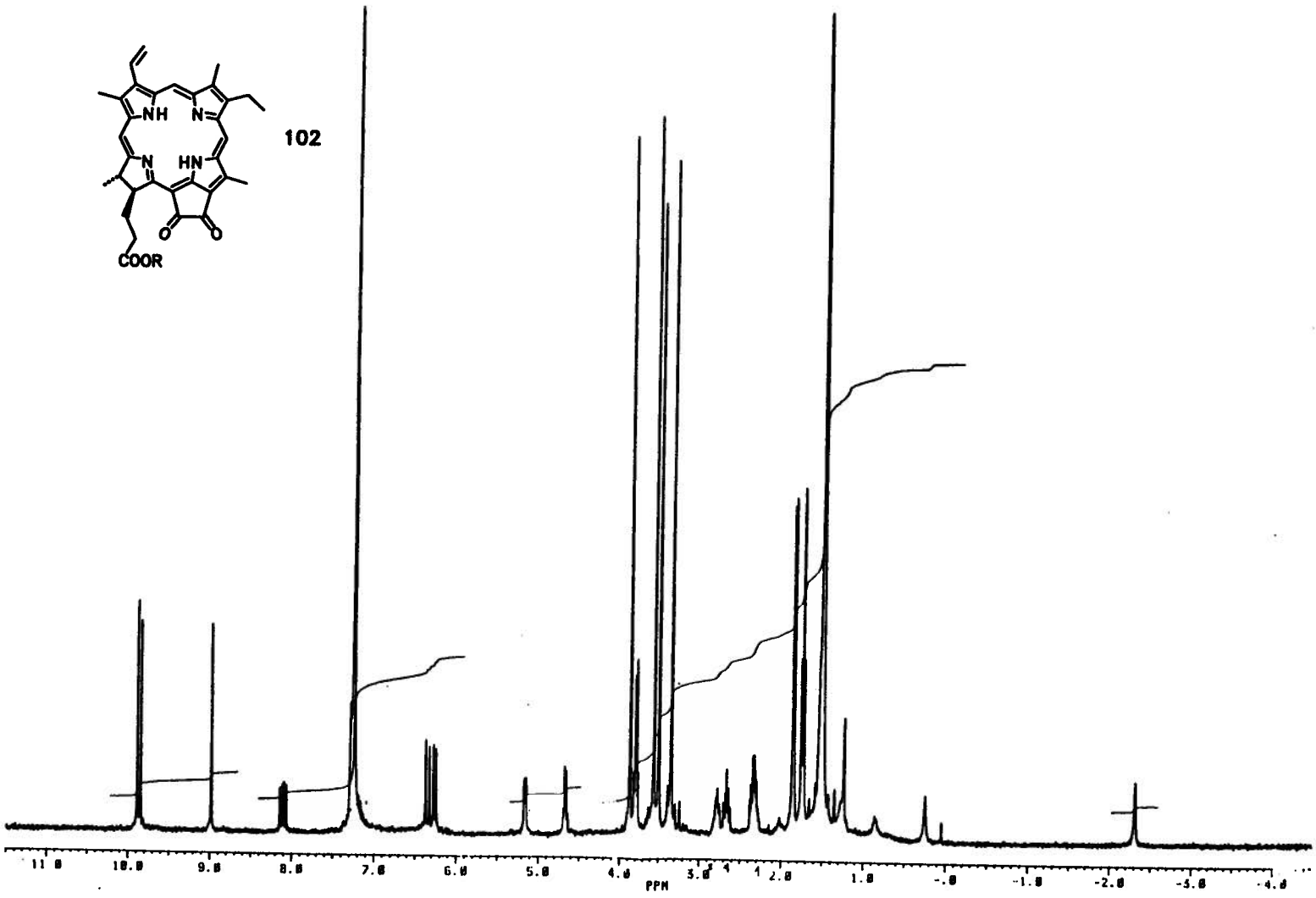


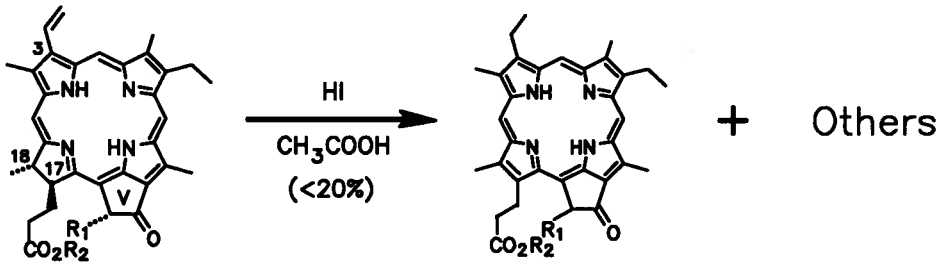
Fig. 2.23 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of 13^2-Oxopyropheophorbide *a* Methyl Ester (**102**)

Chapter 3

Phytoporphyrins: Novel Synthesis and Applications

3.1 Research Objective

Phytoporphyrins are porphyrins directly derived from chlorophylls and their closely related derivatives.[65] Chemically, they are defined as porphyrins characterized by a five-membered exocyclic ring that is typical of chlorophylls. Research on phytoporphyrins can be dated back to the 1930's, when Fischer and Bäumler[126] used hydrogen iodide in glacial acetic acid to convert 3-vinylchlorins into 3-ethylporphyrin for the structural elucidation of chlorophylls (**Scheme 20**). Based on this transformation, chlorophyll and its derivatives were believed to be vinyldihydroporphyrins (vinylchlorins). This ready transformation from vinylchlorins to simple porphyrins was easily understood as a process simply involving the transference of two hydrogens from the dihydroporphyrin nucleus to the peripheral vinyl group, which is thus converted into ethyl. This reaction, later termed as "HI reduction" or "HI isomerization", was a useful method for the preparation of chlorophyll related porphyrins (i.e. phytoporphyrins) although a large amount of by-products was always observed and the major product separated from the complex mixture was usually obtained in less than 20% yield.[65] However, the reaction mechanism, involving isomerization and migration of hydrogens at



Scheme 20 "HI Isomerization" of Chlorophyll Derivatives

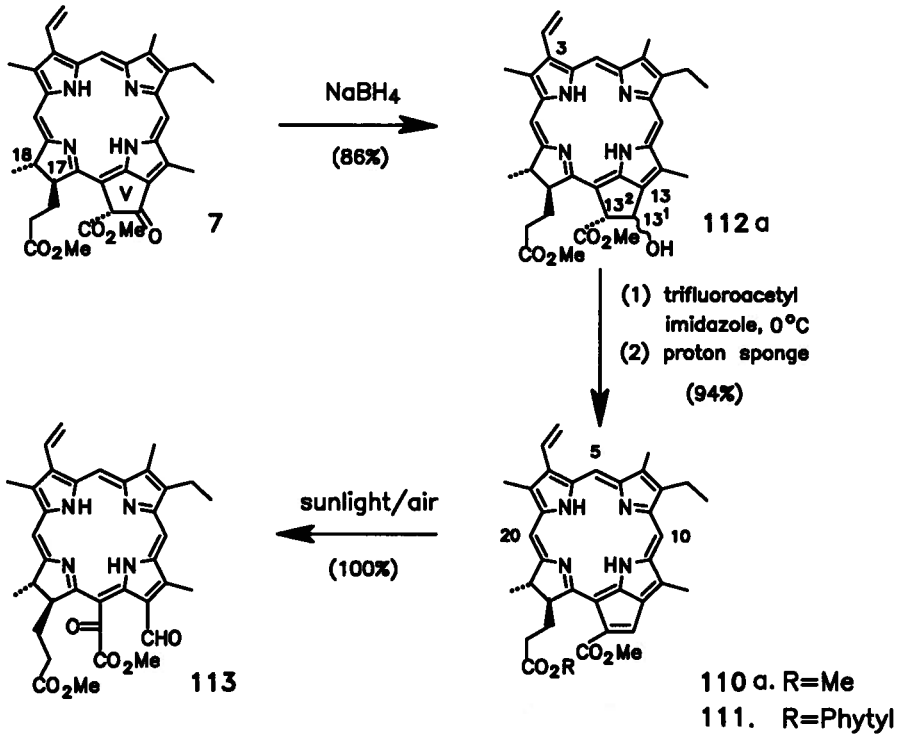
positions 17, 18 to the exocyclic 3-vinyl group, remained obscure. Although a carbocation rearrangement mechanism has been recently suggested by Hynninen[127], the harsh acidic condition and complex product distribution have made mechanistic research difficult.

The objective of our research was to develop a new method for the conversion of chlorins into phytoporphyrins which may help to elucidate this reaction mechanism, especially the acid-catalysed migration of H atoms at postions 17, 18. With this in mind, we focused our attention on the active position (13^1 carbonyl group in the exocyclic ring V) of chlorophyll, where most chemical reactions related to chlorophylls are known. It was assumed that if another vinyl group is introduced into chlorophyll derivatives, especially into the exocyclic ring V, the acid-catalysed migration of protons at H-17, 18 may compete for the two peripheral vinyl groups. Comparison of hydrogen migration capabilities to these two groups should provide some new insight on the reaction mechanism. In an effort to verify this assumption, synthesis of a chlorin with two vinyl groups was envisioned.

The following discussions detail the synthetic studies toward 13^2-deoxo-13^1,13^2-dehydropheophorbide *a* methyl ester (**110**a) (a chlorin with two exocyclic vinyl groups) and its various reactivities. During the course of our studies, we discovered a novel method for the isomerization of chlorins to phytoporphyrins which is addressed in the following sections.

3.2 Synthesis of Divinylchlorin and its Photooxygenation

The reaction sequence used for the synthesis of 13^2-deoxo-13^1,13^2-dehydropheophytin *a* (**111**)[128], a chlorin with two vinyl groups, was previously elaborated in studies related to the enolic tautomer of the β-keto ester in ring V of chlorophyll *a*. This enolic tautomer was once considered to be a possible active intermediate in the photosystem I reaction center ($P700^+$).[129] Thus, following the procedure[128] previously described for the transformation of pheophytin *a* (**5**) into 13^2-deoxo-13^1,13^2-dehydropheophytin *a*, 13^1-deoxo-13^1,13^2-dehydropheophorbide *a* methyl ester (**110**a) was prepared (**Scheme 21**). In this synthetic approach, the first step was $NaBH_4$ reduction[130] of pheophorbide *a* methyl ester (**7**) to give a diastereomeric mixture of 13^1-deoxo-13^1-hydroxypheophorbide *a* methyl ester (**112**a). Conversion of alcohol **112**a into a trifluoroacetyl ester, followed by elimination of a molecule of trifluoroacetic acid (via proton sponge), provided the desired compound **110**a in 80 % overall yield.



Scheme 21 Synthesis of Divinylchlorin and Its Photoreaction

The proton chemical shifts in the NMR spectrum (**Fig. 3.1**) of **110**a differ substantially from those of pheophorbide *a* methyl ester (**7**) (**Table 3.1**). For example, the H-5, H-10 and H-20 methine protons of pheophorbide *a* methyl ester (**7**) exhibit large downfield shifts characteristic of an effective 18π electron macrocycle. However, the resonance of the corresponding protons in **110**a are shifted upfield by nearly 1 ppm relative to those in **7**. Therefore, compound **110**a is somewhat less diatropic than chlorin **7**. This indicates that the formation of a 13^1-13^2 double bond in the exocyclic ring of **110**a significantly perturbs the π electronic structure of the chlorin macrocycle.

Table 3.1 Comparison of Proton Chemical Shifts ($CDCl_3$, 400 MHz)[a]

	Proton								
	H-10	H-5	H-20	H-3^1	H-3^2(E)	H-3^2(Z)	H-13^1	H-17	H-18
7	9.52(s)	9.39(s)	8.57(s)	8.01(dd)	6.30(dd)	6.19(dd)	—	4.20(dd)	4.47(q)
110a	8.41(s)	8.33(s)	7.42(s)	7.42(dd)	6.01(dd)	5.94(dd)	7.19(s)	4.77(d)	3.77(q)

[a]Concentration 1.5 mg/0.6 mL

This view is further supported by a comparison between the electronic spectra of **110**a and pheophorbide *a* methyl ester (**7**) (**Fig. 3.2**). The Soret band of **110**a is split into two distinct maxima at 434 nm and 354 nm. This is quite different from the single broad Soret band of **7** at 416 nm. The 630 nm absorption band of **110**a is substantially blue shifted from the corresponding 670 nm band in **7**. In addition, the strength of this band is only 10% that of the band in chlorin **7**. This loss in strength is consistent with a decrease in the dipole moment of **110**a along the ring I → ring III axis due to the absence of the 13^1-keto group. The most interesting feature in the spectrum of compound **110**a (**Fig. 3.2**) is the broad, low energy absorption centered at 800 nm.

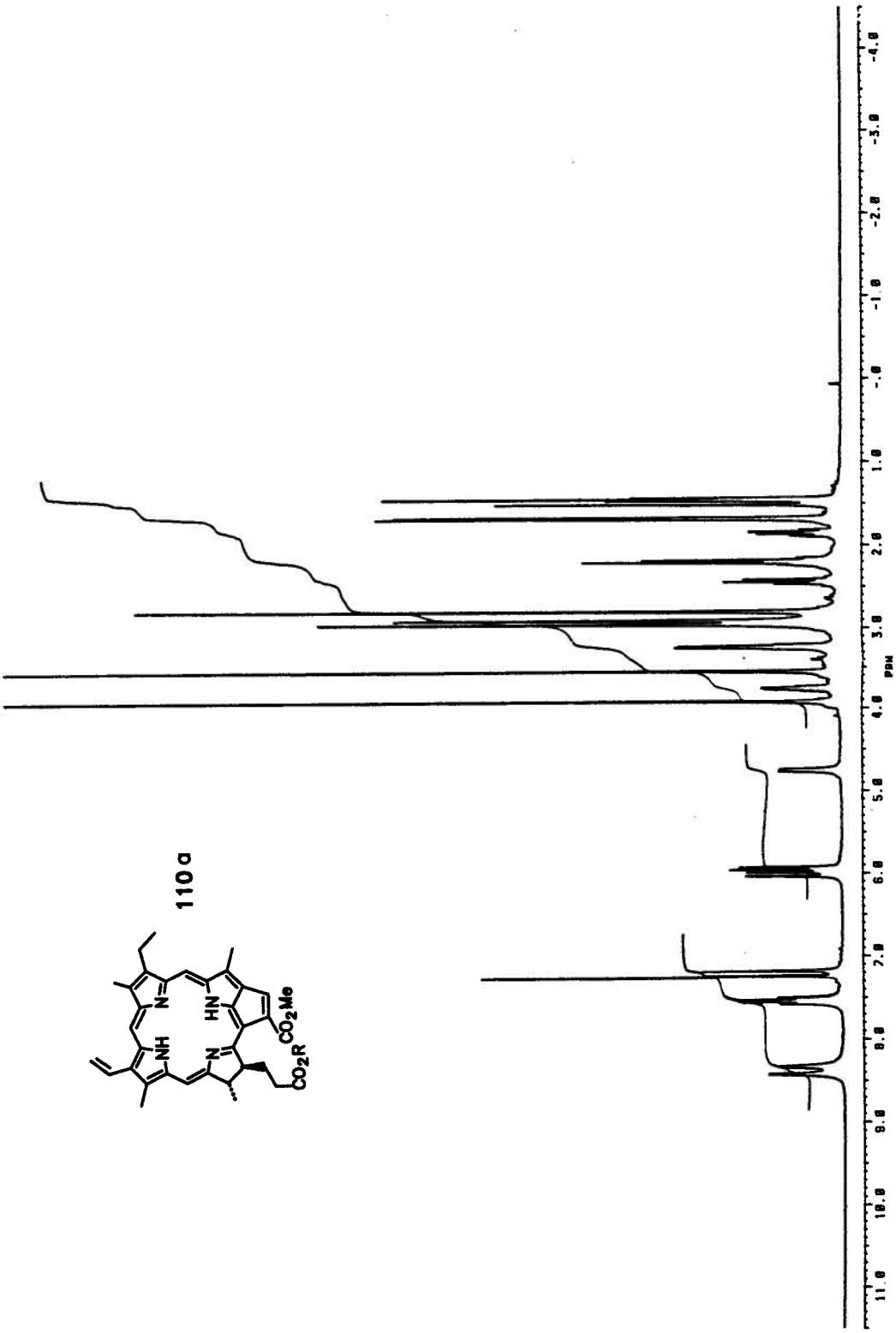


Fig. 3.1 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of Divinylchlorin **110a**

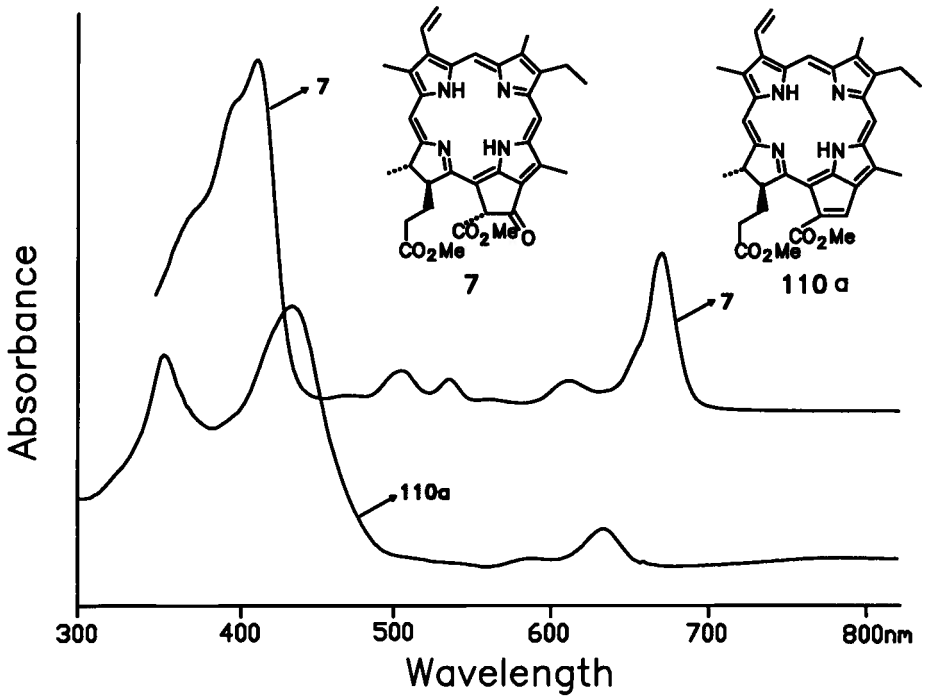


Fig. 3.2 Structures and Comparison of UV/Vis Spectra (CH_2Cl_2) of **110**a and **7**

Although **110**a (m.p.>300°C) has high thermal stability, we found that it is extremely photosensitive, and reaction (in dichloromethane solution) in air with Vancouver sunlight (20 min) oxidatively cleaved the 13^1-13^2 double bond to quantitatively give vinylpurpurin **113** (**Scheme 21**), while the 3-vinyl group remained unchanged.

The ^{1}H NMR and UV/Vis spectra of purpurin **113** appear in **Fig. 3.3**. The main differences in the NMR spectrum of purpurin **113** from divinylchlorin **110**a are the loss of the 13^1 vinyl hydrogen located at 7.19 ppm and the addition of the -CHO proton at 10.93 ppm. An interesting feature is the downfield shift of the all proton resonances in purpurin **113** relative to those in compound **110**a. For example, the H-5 and H-10 *meso* protons of **113** are appeared at 9.50 and 9.20 ppm, with the H-20 *meso* proton next to the reduced

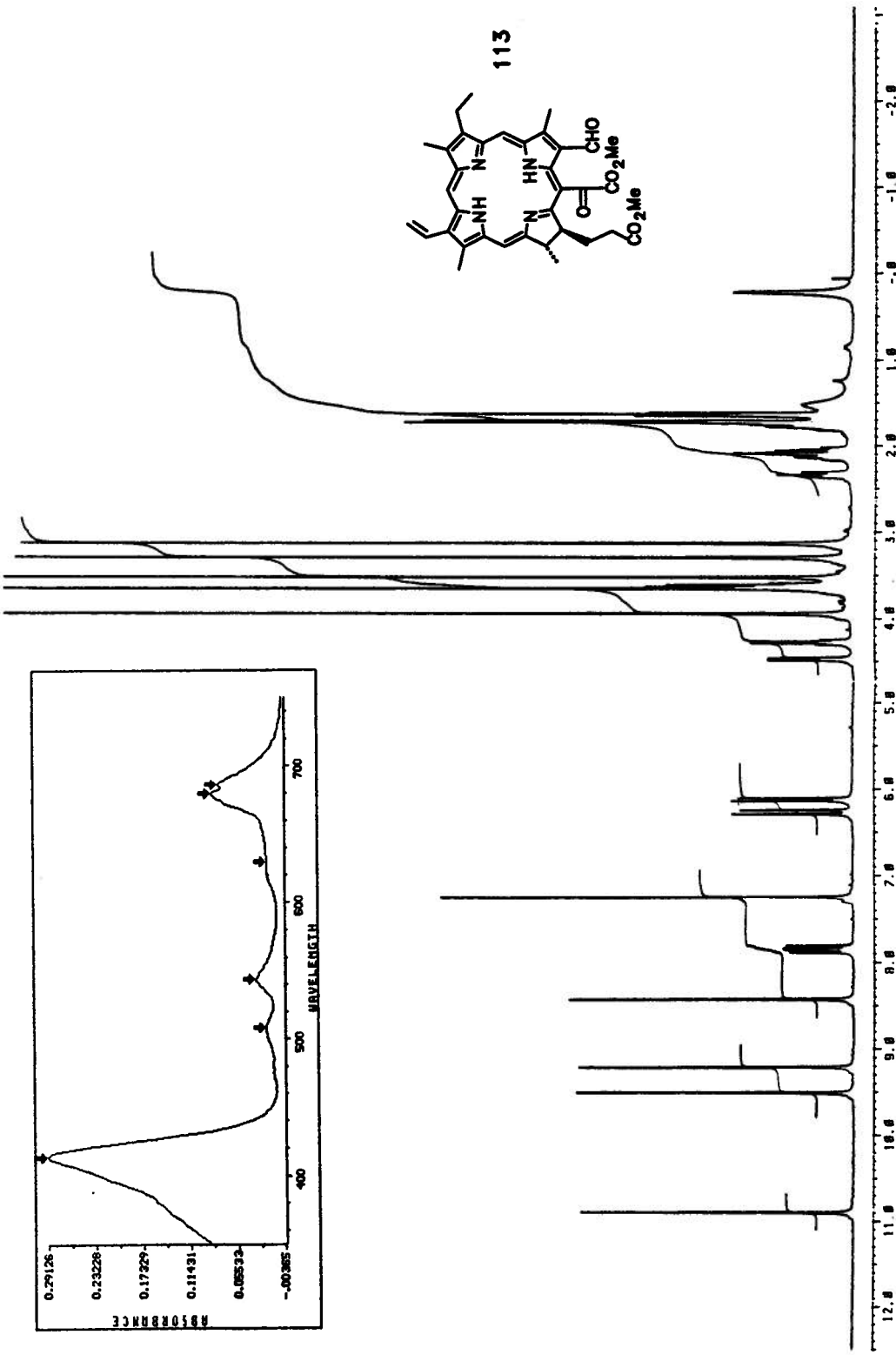


Fig. 3.3 ^1H NMR ($CDCl_3$) and UV/Vis (CH_2Cl_2) Spectra of Purpurin **113**

ring, at higher field (8.45 ppm) suggesting the expected reappearance of the common 18 π electronic ring current. With a formyl group at C-13, compound **113** has the characteristic color (dark brown) and visible spectrum of a purpurin. In addition, the Q band of purpurin **113** had shifted to longer wavelength (686 nm), an observation similar to what was reported by Woodward and coworkers during the corresponding oxidation of purpurin[21] in the synthesis of chlorophyll *a*.

The photoreaction of compound **110a** is regarded as a photooxygenation in which singlet oxygen is generated from ground state oxygen by triplet purpurin[21], as follows:

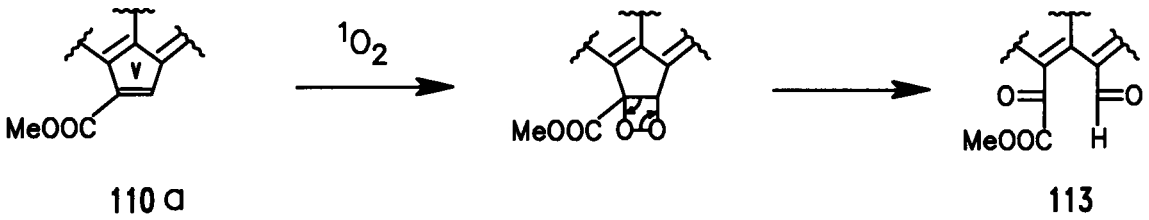
$$\text{Purpurin (}\mathbf{110a}\text{) } S_0 \xrightarrow{h\nu} \text{Purpurin (}\mathbf{110a}\text{) } S_1$$

$$\text{Purpurin (}\mathbf{110a}\text{) } S_1 \xrightarrow{\text{isc}} \text{Purpurin (}\mathbf{110a}\text{) } T_1$$

$$\text{Purpurin (}\mathbf{110a}\text{) } T_1 + {}^3O_2 \longrightarrow \text{Purpurin (}\mathbf{110a}\text{) } S_0 + {}^1O_2$$

$$\text{Purpurin (}\mathbf{110a}\text{) } S_0 + {}^1O_2 \longrightarrow \text{Purpurin (}\mathbf{113}\text{)}$$

The detailed mechanism is not known, but a dioxetane intermediate as shown in **Scheme 22** is regarded as plausible. That the reaction should have left the 3-vinyl group untouched is remarkable but not exceptional.[21]



110 a 113

Scheme 22 A Possible Mechanism for the Photooxygenation

3.3 Novel Synthesis of Phytoporphyrins

The high reactivity of the isocyclic double bond in the ring V reflects strong perturbation in the chlorin chromophore of **110**a. An acid-catalysed isomerization of this double bond should, we felt, bring about the conversion of **110**a into the more stable porphyrin product **114**a.

Benzoyl chloride (PhCOCl) in DMF, which significantly reduces side-reactions, was found to be a much milder reagent than HI in acetic acid (which gave less than 65% yield for the same conversion) to achieve this transformation. The reaction of **110**a with 1 equiv benzoyl chloride in DMF at 100°C, under an atmosphere of nitrogen for 20 min (reaction was monitored by TLC and UV/Vis spectroscopy), gave phytoporphyrin **114**a (**Scheme 23**) in quantitative yield after recrystallization from dichloromethane/hexane.

This compound was characterized by ^{1}H NMR, high resolution mass and UV/Vis spectroscopies. Its ^{1}H-^{1}H COSY spectrum is shown in **Fig. 3.4**. The main differences in the NMR spectrum of phytoporphyrin **114**a from divinylchlorin **110**a are the loss of the signals: H-13^1 (s, 7.19 ppm), H-17 (d, 4.77 ppm), H-18 (q, 3.77 ppm) and the appearance of a new signal at 6.69 ppm (1H, dd, J_{trans}=17.5 and J_{cis}=7.7 Hz) denoting the H-13^2. The characteristic signals of 3-vinyl protons remained largely unchanged and located at 8.27, 6.27 and 6.12 ppm. The peaks at 3-4.5 ppm region are well-resolved and were somewhat difficult to assign to the corresponding hydrogens because of their complex multi-couplings. Thus, an ^{1}H-^{1}H COSY spectrum was performed, which revealed their connectivity and enabled assignments of all the hydrogens in the molecule. The interesting features of the ^{1}H-^{1}H COSY spectrum are that six hydrogens of H-17^1, H-17^2

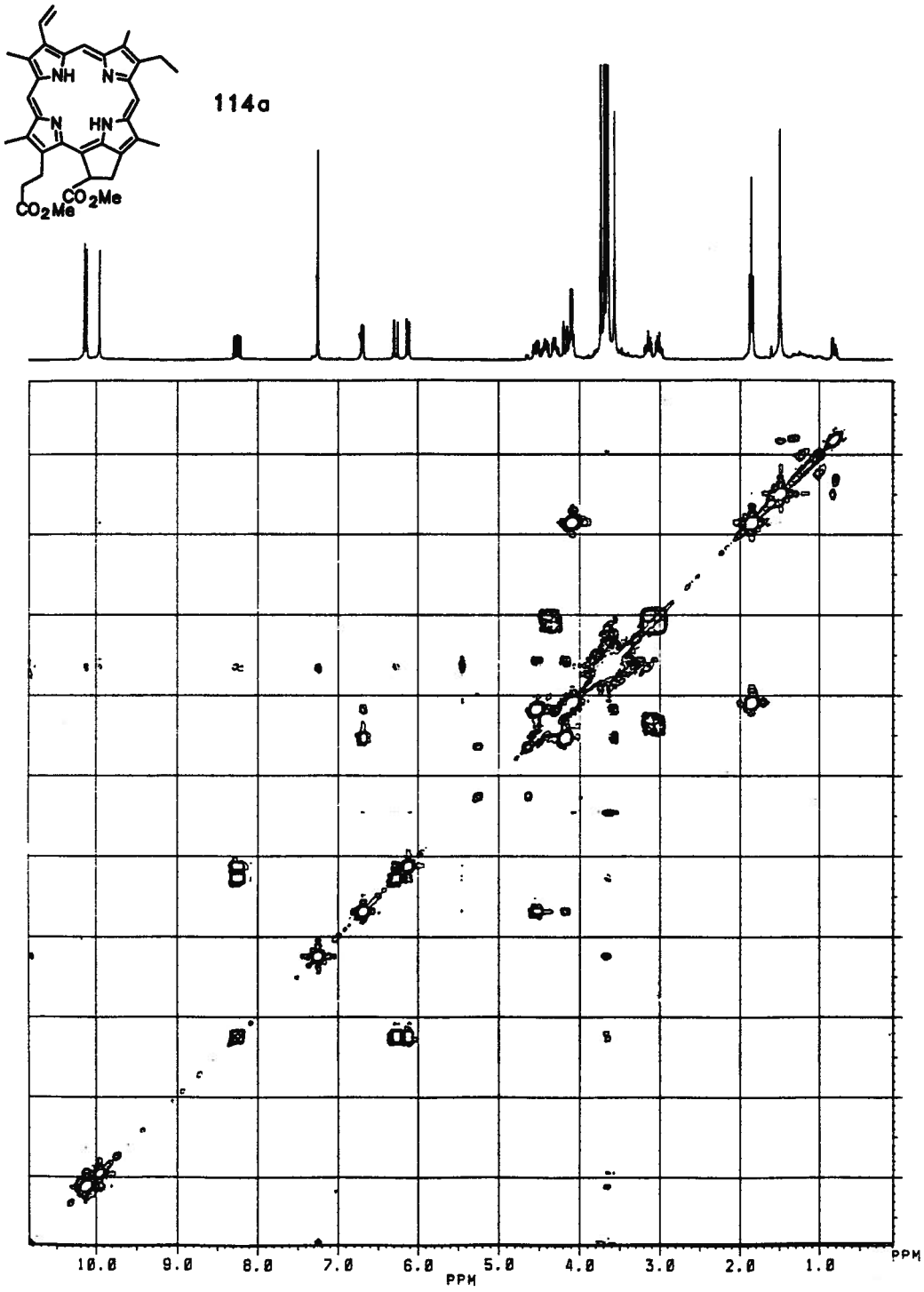


Fig. 3.4 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Phytoporphyrin **114a**

and H-13^1 (2H each) are well-resolved and are assigned as H-17^1 [4.42 ppm (ddd), 4.31 (ddd)]; H-17^2 [(3.12 (dt), 3.00 (dt)] and H-13^1 [(4.52 (dd), 4.18 (dd)]. The fine resolution of four protons at 17^1 and 17^2 was exceptional and not observed in other porphyrins. The electronic spectrum of **114a** as shown in **Fig. 3.5** is characteristic of a phyllo-type porphyrin (IV>II>III>I, $\lambda_{max}(CH_2Cl_2)$ = 404 nm) suggesting a single *meso* alkyl substitution in the molecule. Therefore, the tautomerization of the two protons at positions 17, 18 is regioselective with respect to the exocyclic double bond in ring V without generating any migration to the 3-vinyl group.

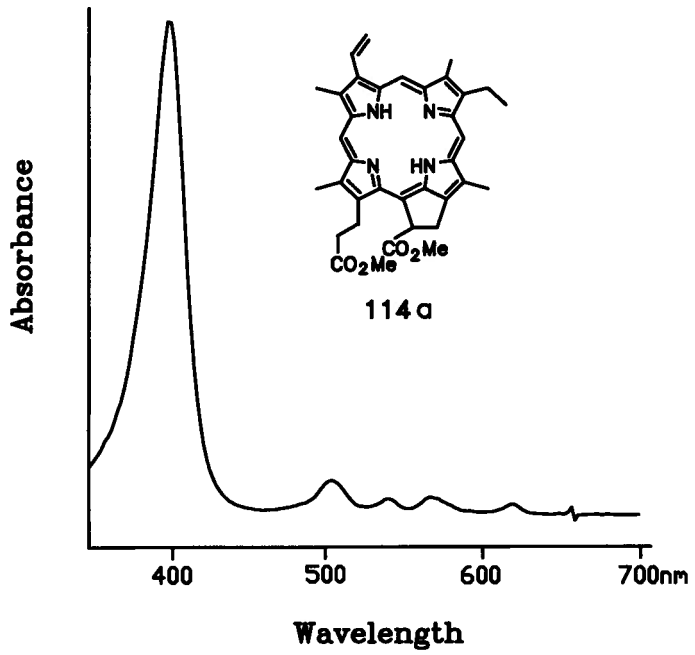
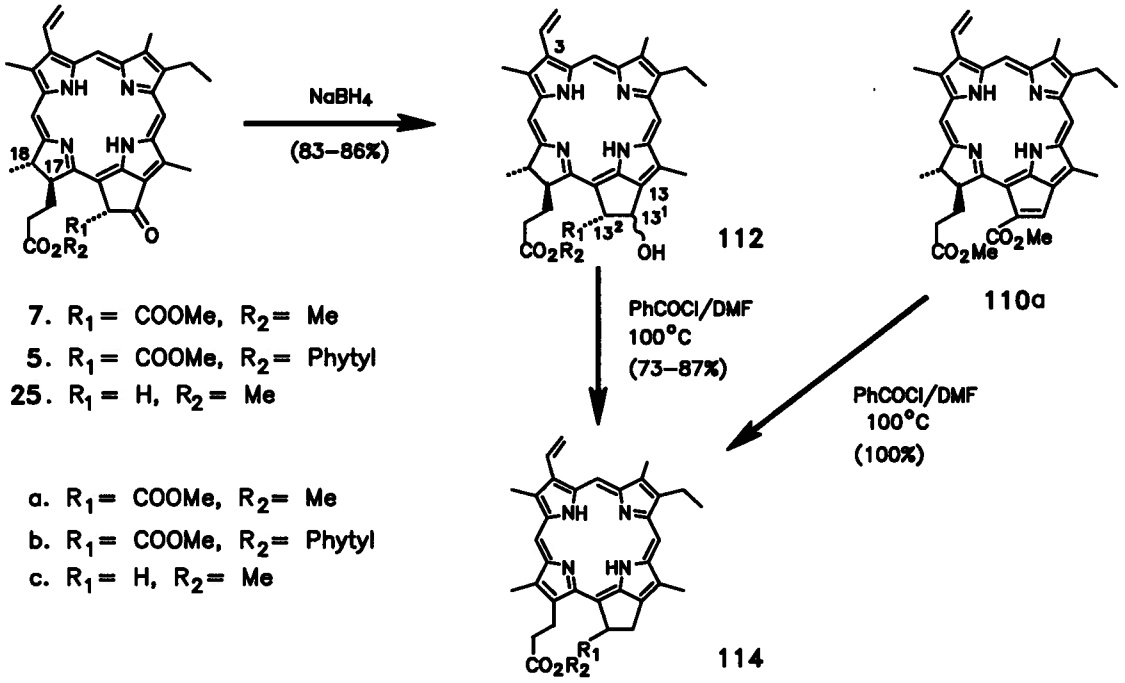


Fig. 3.5 Structure and UV/Vis Spectrum (CH_2Cl_2) of Phytoporphyrin **114a**

Additionally, it was also found that a weaker acid such as acetic acid is not strong enough to promote the tautomerization of the exocyclic double bond. For instance,

treatment of **110**a with glacial acetic acid in DMF at 100°C for 2 h gave no products and the starting material was recovered.

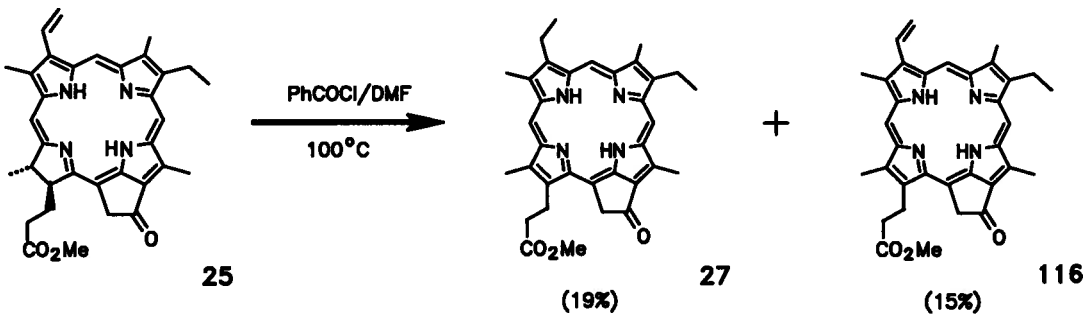
Encouraged by this exciting finding, we decided to expand the same reaction conditions to hydroxychlorin **112**a; after reaction under the above conditions for 45 min, it gave the same product **114**a in 87% yield. Similarly, the conversion of another two hydroxychlorins **112**b and **112**c gave **114**b and **114**c in 81% and 73% yield, respectively (**Scheme 23**).



Scheme 23 Direct Conversion of Chlorins to Phytoporphyrins

When the above procedure was applied directly to monovinylchlorin (3-vinylchlorin) pyropheophorbide *a* methyl ester **25**, a mixture (**Scheme 24**) of phytoporphyrin methyl ester **27** (19% yield) and 3-vinylphytoporphyrin methyl ester **116** (15% yield) was obtained after the preparative TLC separation, along with recovery of

the starting material **25** (40% yield) (the reaction was monitored by UV/Vis and TLC analyses). The formation of the non-isomerized (oxidized) product **116** is probably due to direct autooxidation under the acidic reaction conditions.



Scheme 24 Reaction of 3-Vinylchlorin (**25**) Using Benzoyl Chloride

The products **27** and **116** were characterized by ^{1}H NMR, UV/Vis, and high resolution mass spectroscopies. The most important difference between ^{1}H NMR spectra of the two products is the presence of 3-vinyl protons in phytoporphyrin **116**. Further structural confirmation was derived from the 2 unit difference of the molecular ions (m/z) in their mass spectra. Their UV/Vis spectra are shown in **Fig. 3.6**. Compound **116** has an oxorhodo-type spectrum (III>II>IV>I) characteristic of two rhodofying groups (3-vinyl and 13^1-carbonyl) on diagonally opposite rings. Compound **27** has a rhodo-type spectrum (III>IV>II>I) suggesting only one electron-withdrawing group (13^1-carbonyl) in the molecule. The Soret of the former compound (420 nm) is 4 nm to the red in contrast to that of the latter (416 nm) (**Fig. 3.6**).

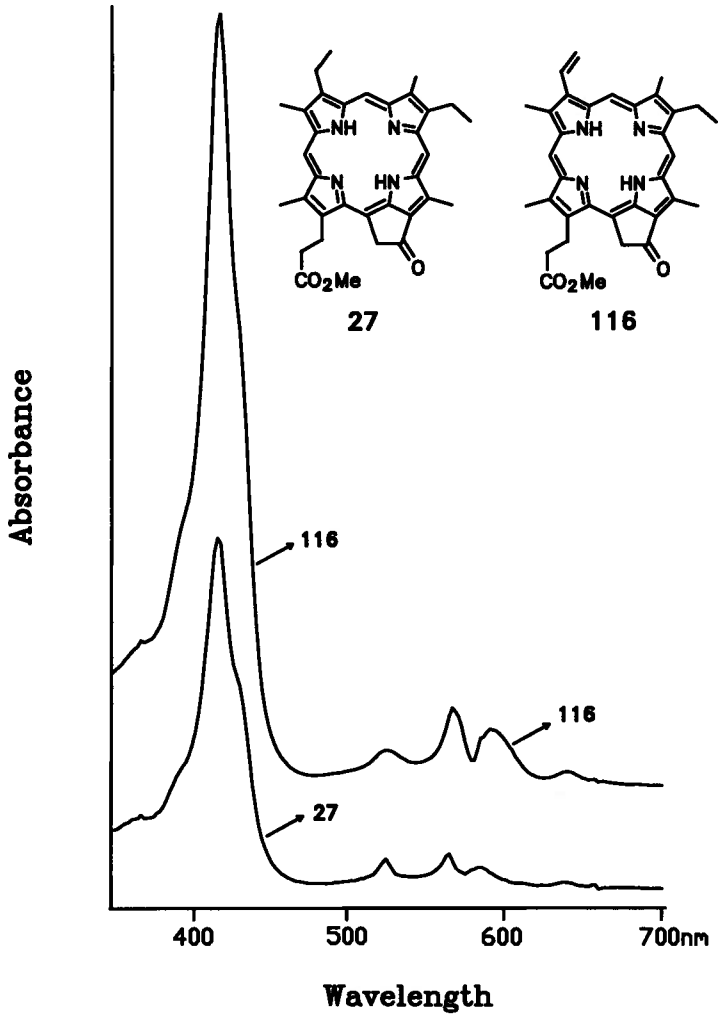
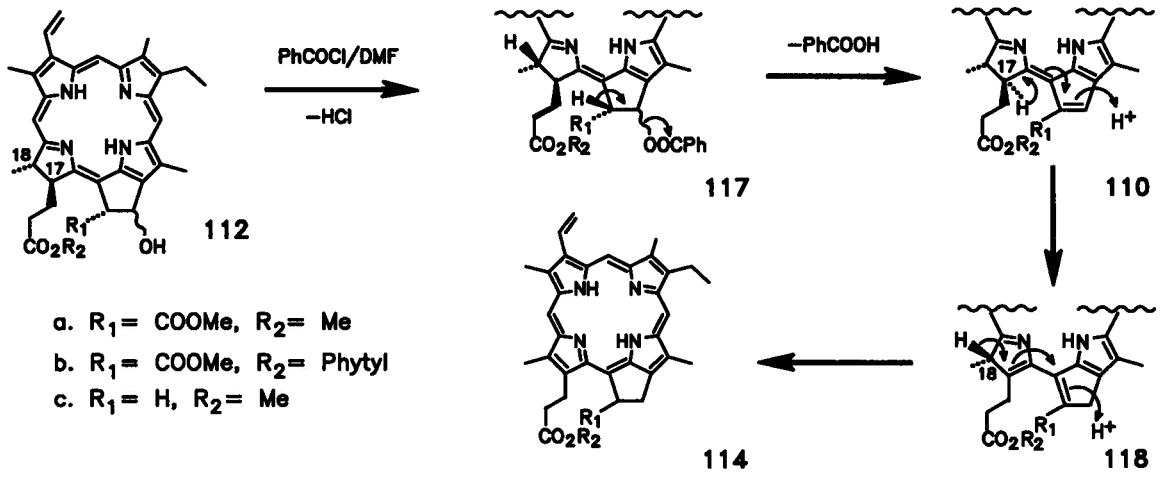


Fig. 3.6 Structures and Comparison of UV/Vis Spectra (CH_2Cl_2) of **27** and **116**

3.4 Tautomerization Mechanism

A possible tautomerization mechanism is presented in **Scheme 25**. The formation of phytoporphyrin **114** from hydroxychlorin **112** can be regarded as acid-catalyzed double isomerization of the intermediate **110**, which is formed by elimination of a molecule of

benzoic acid from the initial intermediate **117**. Migration of the proton at position 17, with the relief of the steric strain in the intermediate **110**, gives the intermediate **118** which subsequently tautomerizes, with the loss of a hydrogen at position 18, to give the fully conjugated product **114**.

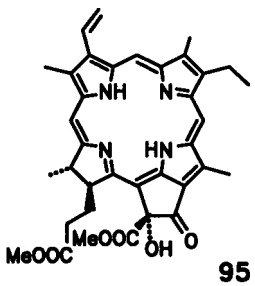


Scheme 25 Proposed Tautomerization Mechanism of Chlorins to Phytoporphyrins

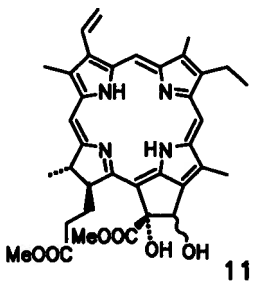
Although there is no direct evidence implicating the formation of the intermediate **118**, support for the formation of intermediate **110** and the fate of stereogenic center (C-13^2) involved in the reaction have been found experimentally. For instance, phytoporphyrin **114**a, directly derived from hydroxychlorin **112**a (13^2R), was found to be a racemic mixture (at asymmetric C-13^2 position) by ^{1}H NMR determination using a shift reagent, tris-[3-(trifluoromethylhydroxymethylene)-(+)-camphorato]praseodymium (III) derivative [Pr(tfc)$_3$]. Furthermore, the yield (87%, **114**a) of the PhCOCl-induced isomerization of **112**a was greater than that (73%, **114**c) of **112**c. This observation can be

related to the electron-withdrawing methoxycarbonyl group at C-13^2, which increases acidity of the H-13^2 and thereby facilitates the elimination of a molecule of benzoic acid from the exocyclic ring V to furnish the intermediate divinylpurpurin **110** as shown in **Scheme 25**. Several pathways can be written for the further transformation of **110** to the final products and the most straightforward appears the one via the intermediate **118** as shown in **Scheme 25**.

Water present in the reaction medium will quench the carbocation intermediates and generate side products or cause decomposition. Similar results were also observed in the transformation of polyhydroxychlorins. For example, reaction of benzoyl chloride (2 equiv) with the diastereomeric diol **119** (**Scheme 26**), obtained (89% yield) from the $NaBH_4$ reduction of 13^2R-hydroxypheophorbide *a* methyl ester (**95**) (90% d.e., see Chapter 2), gave 2% yield pheophorbide *a* methyl ester (**7**) and an unidentified mixture of porphyrins. Similarly, the diastereomeric triol **120** (**Scheme 26**), prepared (72% yield) from the $LiAlH_4$ reduction of pheophorbide *a* methyl ester (**7**), also afforded a complicated porphyrin mixture.



95



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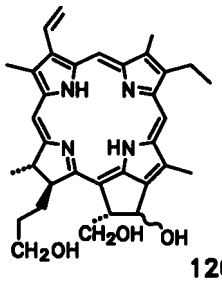


120

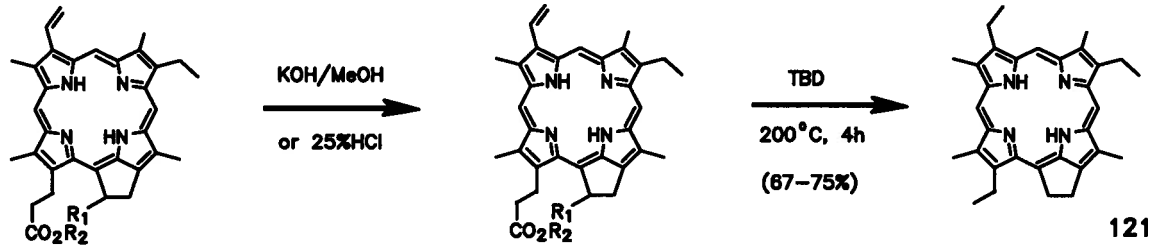
Scheme 26 Structures of Hydroxychlorin **95** and Polyhydroxychlorins **119** and **120**

3.5 Applications

Mechanistic research aside, the synthesis of these phytoporphyrins has opened the way to a variety of porphyrins and their reduced derivatives, especially petroporphyrins[19]. For example, we have employed phytoporphyrins as intermediates to synthesize deoxophylloerythroetioporphyrin[131](**121**), one of the most abundant porphyrin derivatives on earth. Compound **121**, occurring largely in oil shales and related deposits, is the major pigment in most samples of petroporphyrins. Its isolation from natural sources is made difficult by the complexity of the porphyrin mixtures, this fact explaining why the total synthesis[131] of **121** has been carried out. Although it has been long believed that compound **121** is derived by degradation of chlorophyll over the course of time, its partial synthesis[132] from pheophytin *a* resulted in very low yield. However, we sought out to develop an efficient way to obtain this unique reference compound for identification and chemical reactivity studies.

Making use the ready availability of phytoporphyrins **114**a, **114**b and **114**c from our method, petroporphyrin **121** was synthesized from chlorophyll *a* in a short and efficient way (**Scheme 27**). Thus, alkaline (10% KOH/MeOH) or acidic (25% HCl) hydrolysis of either of the phytoporphyrins, **114**a, **114**b or **114**c, gave the corresponding porphyrin diacid or monoacid, which could be decarboxylated and reduced to give deoxophylloerythroetioporphyrin (**121**) (**Scheme 27**). It appeared likely that both these steps could be carried out by a one-pot procedure described by Kämpfen and Eschenmoser[133], where protoporphyrin was transformed into etio-porphyrin III (reduction of vinyl to ethyl groups and decarboxylation of side-chains) in one step by heating in

1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD). When the phytoporphyrin mono- or di-acid subjected to these conditions (large excess TBD; tube sealed under vacuum; 200°C; 4 h) a reaction mixture largely containing compound **121** was obtained. Purification of product by flash chromatography (elution with dichloromethane) gave petroporphyrin **121** (**Scheme 27**) in 67-75% yield after recrystallization from dichloromethane/hexane.

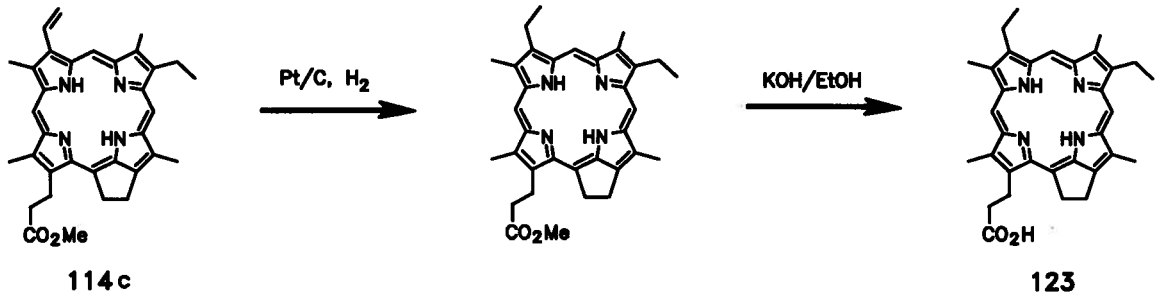


a. R_1= COOMe, R_2= Me
b. R_1= COOMe, R_2= Phytyl
c. R_1= H, R_2= Me

R_1= COOH, H; R_2= H

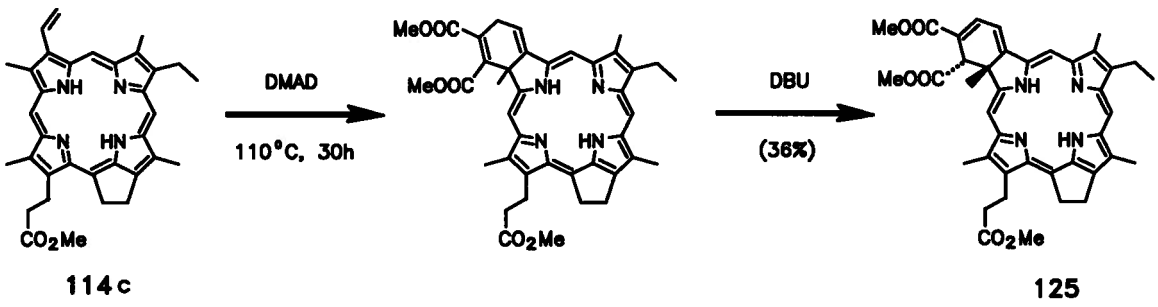
Scheme 27 Synthesis of Petroporphyrin **121**

Another petroporphyrin acid[134] **123** (**Scheme 28**), which is the major portion in Messel oil shale, can also be prepared from catalytic hydrogenation of phytoporphyrin **114**c (prepared according to our method) followed by saponification with KOH in EtOH.



Scheme 28 Synthesis of Petroporphyrin **123**

Another application of phytoporphyrins was to develop new photosensitizers for photodynamic therapy of tumors. For example, Diels-Alder reaction of monovinylporphyrin **114c** with dimethyl acetylenedicarboxylate, followed by DBU-promoted rearrangement of the cycloadduct, gave 36% yield of **125** (**Scheme 29**), a new class of regiochemically-pure benzoporphyrin derivative, which can act as photosensitizer in the photodynamic therapy. The work on this topic had brought about the research of another new field ("the third generation photosensitizers"), which is described in Chapter 4.



Scheme 29 Synthesis of Benzoporphyrin Derivative **125**
(Only One Enantiomer of **125** Is Shown)

3.6 Summary

Chlorophyll derivatives **110**, **112a**, **112b** and **112c** have been readily converted to their corresponding phytoporphyrins **114a**, **114b** and **114c** in excellent yields using benzoyl chloride in DMF. This new approach has added more scope to the acid-catalysed migration of hydrogens at the saturated ring IV into the exocyclic ring V. This method also affords a novel and efficient route to prepare monovinyl porphyrins which can be

used as the important intermediates for the further preparation of chlorophyll related petroporphyrins and regiochemically-pure benzoporphyrin derivatives for use in the photodynamic therapy.

Chapter 4

The Third Generation Photosensitizers

4.1 Background and Research Objective

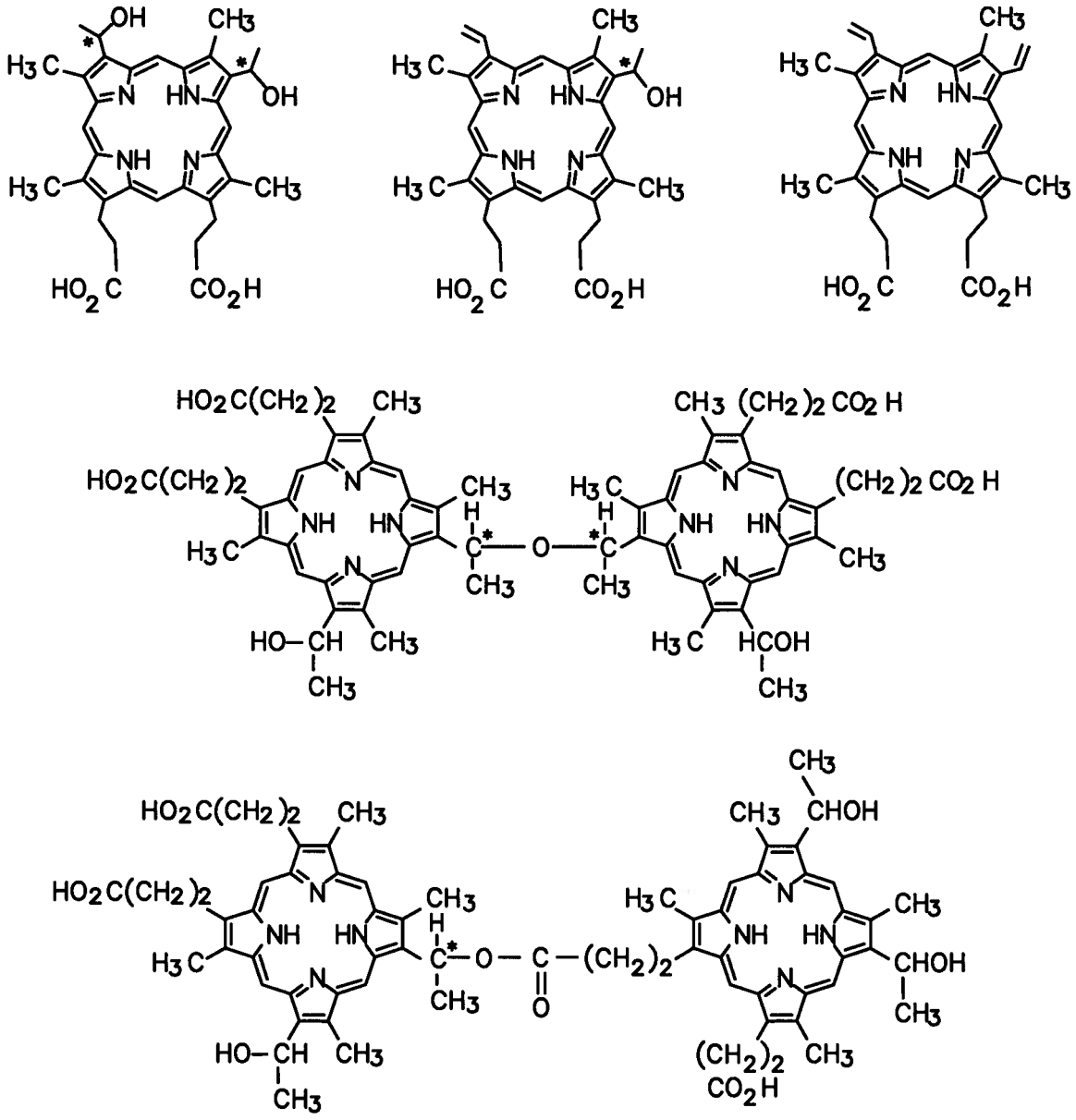
Photodynamic therapy (PDT), known also as photochemotherapy of cancer, is a medical treatment which employs a combination of light and drug to create cytotoxic ('cell-lethal') forms of oxygen (singlet-oxygen and superoxide radical), as well as other reactive species, to bring about the destruction of cancerous or unwanted tissue.[135]

The present treatment schedules which are used in the clinic are based on the retention of a photosensitizer such as Photofrin™ in tumor tissue in concentrations which are higher than in surrounding nonmalignant tissues. Subsequent photoactivation (usually by a laser source) of the sensitizer evokes tumor destruction. The exact mechanisms of action for effective PDT in cancer treatment are unclear. While studies *in vitro* have indicated that generation of singlet oxygen is the main mechanism for PDT cytotoxicity,[136,137] studies *in vivo* with animal models have suggested that photodynamic damage to the blood vessels of the tumor may be the major cause of tumor destruction.[138,139]

Contemporary PDT began in the late 19th century when Finsen[140] discovered that the skin condition *Lupus vulgaris* could be treated using UV light. Alexandra, the future queen of Edward VII, brought the discovery back to the London Hospital.[141] In 1913, Meyer-Betz[142] demonstrated the phototoxicity of porphyrins by injecting himself with 200 mg of hematoporphyrin, and in 1924 Policard[143] discovered that the certain malignant tumors were selective accumulators of porphyrins.

Although the potential for photochemotherapy with porphyrins was exposed by these pioneers, it was not until 1972, when the first sustained series of tests on animals

and humans were begun by Dougherty *et al.*[144] and others using hematoporphyrin derivative (HpD), that the field of clinical PDT truly began. By 1975, there was ample proof that HpD and light could be used to destroy cancers in animals,[145] and in 1976 the first successful trials in humans were initiated.[146]

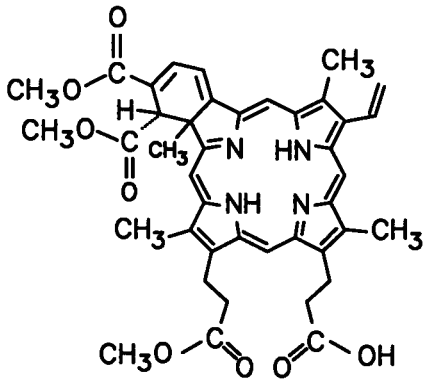


Scheme 31 Various Components of Hematoporphyrin Derivative

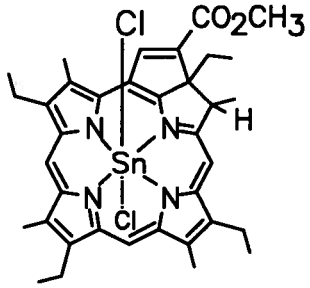
HpD (the first generation photosensitizer), together with its commercial variants Photofrin™, Photosan, Photogem, and Photocarcinorin, has been used to treat a variety of lung and bladder cancers, breast cancer, and certain occular cancers. However, because it is a mixture of compounds (the major components of HpD are listed in **Scheme 31**), some of which are PDT-inactive, HpD is not the ideal sensitizer. It also localises in healthy as well as cancerous tissues, where it persists, leading to generalised photosensitivity. HpD also locates in the liver and brain, and its clearance rate from human body requires 4-6 weeks, post-injection, for systemic concentrations to fall to acceptable levels.[147] During this time the patient must remain in subdued light to prevent skin photosensitivity. Another disadvantage is that the excitation wavelength (630 nm, $\varepsilon \approx 1\ 170$) is not the most efficient for producing photodamage or for penetrating the skin (less than 4 mm depth for HpD) from an external light source. This lack of efficiency, resulting from poor light absorption ($\varepsilon \approx 1\ 170$) of HpD, coupled with light loss from optical absorption by endogenous tissue chromophores (mainly hemoglobin) and light scattering, disables the treatment of large tumors or tumors which are deeply seated within the body.

In an effort to improve on the first generation drug, new "second generation photosensitizers" [148] were sought out. During the last few years, a number of dyes which strongly absorb in the range of 650-700 nm, such as a benzoporphyrin derivative (BPD), tin etiopurpurin, zinc (II) phthalocyanine, m-THPC [5,10,15,20-tetrakis(*m*-hydroxyphenyl)-chlorin], and monoaspartyl chlorin e_6 ('MACE') (**Scheme 32**) have been patented as potential clinical photosensitizers.

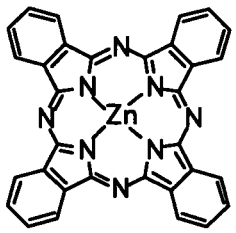
In the chlorin series, benzoporphyrin derivative monoacid ring A (BPDMA), developed in our laboratory, has generated interest due to low skin phototoxicity compared with the first generation drug Photofrin™, the industry standard used in the clinic. As a result, BPDMA is one of the long wavelength photosensitizers (λ_{max} = 690 nm, ε = 33 000) which are currently in Phase-II clinical trials. To synthesize BPDMA[149], protoporphyrin IX dimethyl ester (**131**) is reacted with dimethyl acetylenecarboxylate



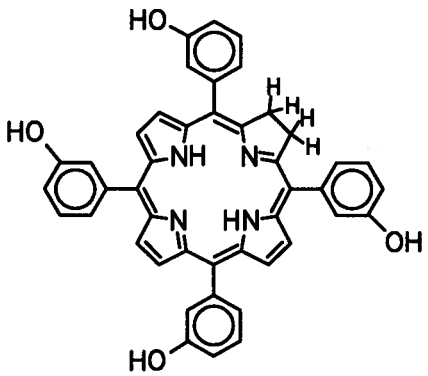
Benzoporphyrin derivative mono-acid (BPDMA)



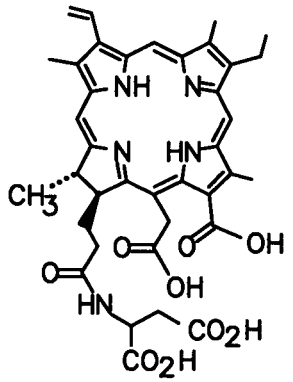
Tin Etiopurpurin



Zinc Phthalocyanine



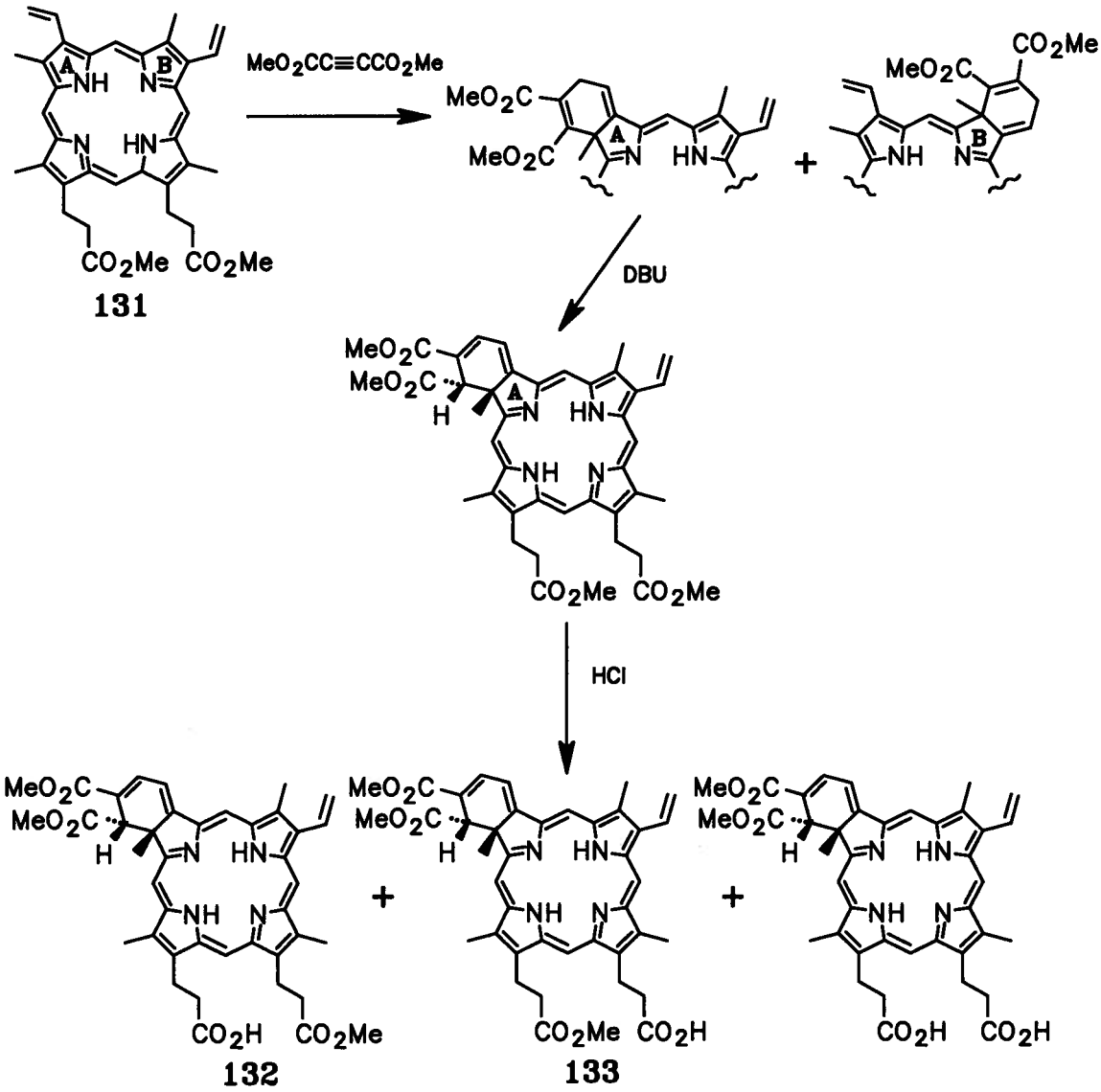
***meso*-Tetra-(*m*-hydroxy-phenyl)chlorin**



Mono-aspartyl chlorin-e_6

Scheme 32 Major Second Generation Photosensitizers

(DMAD) in refluxing toluene (**Scheme 33**). The ring A Diels-Alder adduct (i.e. 1,4-cyclohexadiene derivative) is then separated by crystallization from the ring B adduct and is subsequently rearranged with base (DBU). The rearranged ring "A" 1,3-cyclohexadiene derivative is then partially hydrolyzed to afford, after purification, the two regioisomers, $BPDMA_{ad}$ (**132**) and $BPDMA_{ac}$ (**133**), which are used for biological studies.[150] From the biological data reported so far, photodynamic activity is maximised



Scheme 33 Synthesis of the Biologically Active BPD Monoacids (BPDMA) (Only One Enantiomer of Each Molecule is Shown)

for BPD when: (a) "ring A" of the porphyrin nucleus is modified by the Diels-Alder cycloaddition/base rearrangement procedure, (b) only one of the two methyl propionate groups is hydrolysed to the free acid.

Due to the troublesome separation involved with BPDMA, porphyrins with one monovinyl group (in ring A) and only one propionic side chain in the molecules immediately stand out as target molecules. Further, various chlorophyll derivatives, such as pheophorbide *a*, pyropheophorbide *a* and chlorin e_6 have been reported as photosensitizers for the photodynamic therapy of tumors.[151] Preliminary *in vivo* results led us to conclude that the five-membered exocyclic ring plays an important role in the photosensitizing ability of these compounds. To our knowledge, further research, aimed at comparing the structure/activity relationships among chlorins, pheophorbides and BPDs and at understanding the basic requirements for an effective long wavelength photosensitizer, has not been performed.

The objectives of this section of research (the third generation photosensitizers) were two-fold. The first was to eliminate the problem of isomer formation in the Diels-Alder reaction of protoporphyrin and to synthesize regiochemically-pure benzoporphyrin derivatives (BPDs) characteristic of the successful and promising chemical features of BPDMA. These regiochemically-pure BPDs should be more readily characterized and purified and would subsequently lead to less ambiguity at the biological screening stage. Synthesis of porphyrins with a single exocyclic vinyl group and one propionic side chain, therefore, became the initial goal of this project.

Secondly, because depth of penetration through tissue varies with the wavelength of light, the ideal photosensitizer for treatment of more deep-seated tumors should absorb

at wavelengths >700 nm.[150] There is therefore a need to synthesize new macrocycles which will absorb in the far red or the near infrared region of the electromagnetic spectrum. It was reasoned that since natural bacteriochlorins (tetrahydroporphyrins with two opposite reduced rings) absorb at 750-800 nm, they could be good candidates for photodynamic therapy.

With the goals of isomeric purity and long wavelength absorption in mind, therefore, the syntheses of regiochemically-pure benzoporphyrin derivatives (BPDs) and [A,C]-dibenzoporphyrin derivatives (bacteriochlorins) were attempted. The following sections describe the detailed syntheses and characterization of these compounds.

4.2 Syntheses of Regiochemically-pure Benzoporphyrin Derivatives

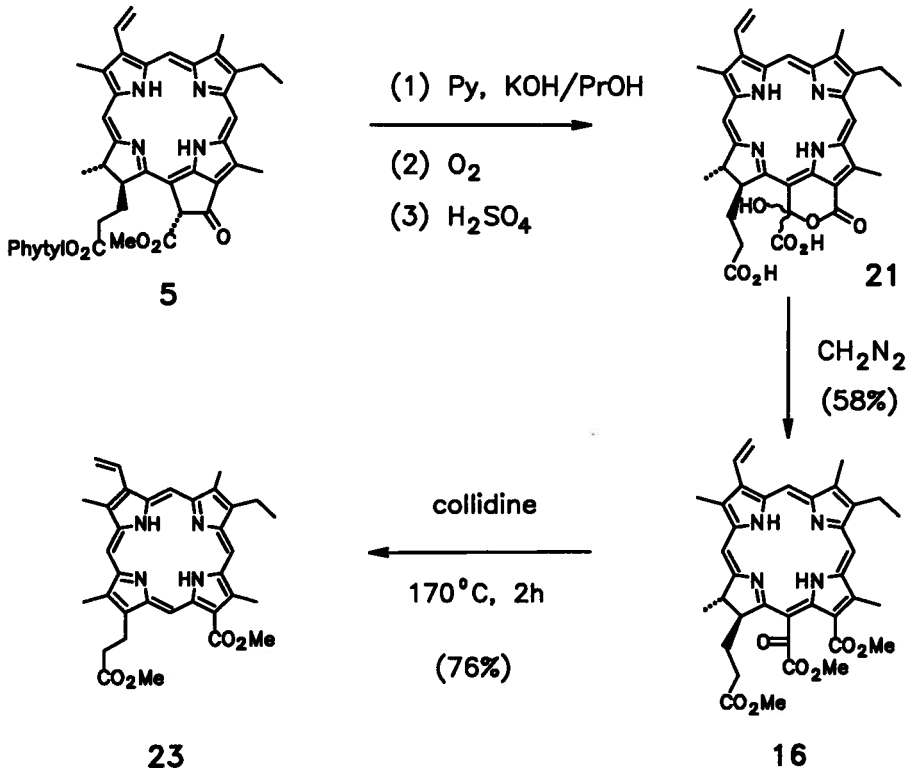
4.2.1 Rationale

The brief introduction to photodynamic therapy (PDT) and benzoporphyrin derivatives (BPDs) above described the relative cytotoxicities of the ring A and ring B BPD compounds. It was shown that the monoacid derivatives of ring A had the best initial biological results. However, in the industrial production of BPDMA, an equal amount of the ring B material is produced as an "unwanted" by-product. A solution to this problem would be to use monovinylporphyrins to prepare regiochemically-pure benzoporphyrin derivatives (BPDs). As a continuation of our research into natural antioxidative chlorins and phytoporphrins, two series of photosensitizers related to BPD were synthesized by using 3-vinylrhodoporphyrin XV (**23**) and 3-vinylphytoporphyrin

(**114c**) as starting materials. These are ideal substrates for the synthesis of compounds to be used in probing structure/activity relationships of BPDs because both systems have a vinyl group at position 3 (i.e. in ring "A", and thus will produce only ring "A" chlorin after the Diels-Alder reaction), and also have only one propionic ester side chain, which can be hydrolyzed to the corresponding monocarboxylic acid at the final step of the synthesis.

4.2.2 Via 3-Vinylrhodoporphrin XV Dimethyl Ester

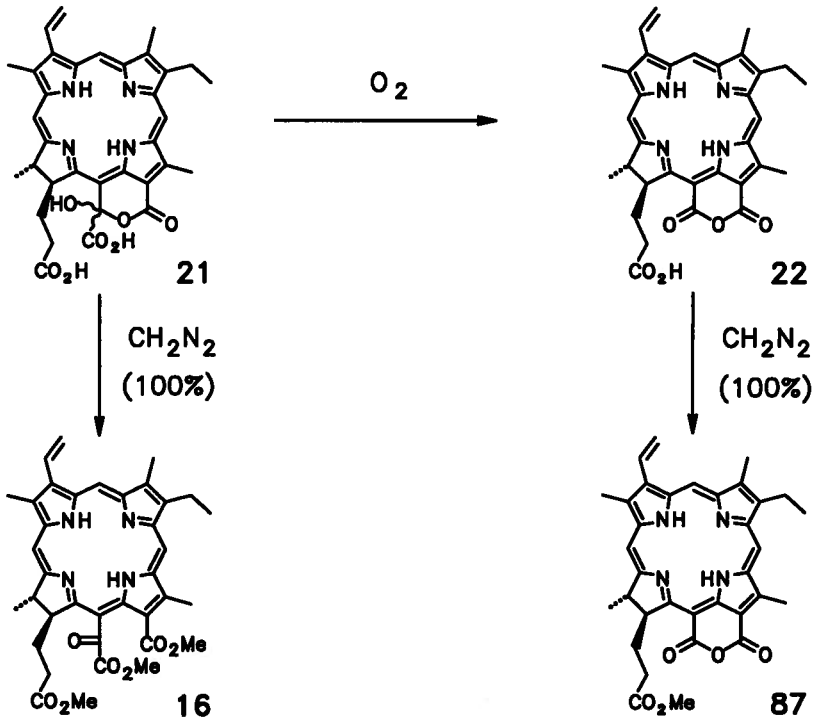
As described in the Introduction (Chapter 1), 3-vinylrhodoporphyrin XV could be prepared from the stardard degradation of chlorophyll *a*.[52] Thus, pheophytin *a* (**5**) (from the blue alga *Spirulina maxima*) was transformed into purpurin-7 trimethyl ester (**16**) by



Scheme 34 Synthesis of 3-Vinylporphyrin **23** via Basic Aeration of Pheophytin *a* (**5**)

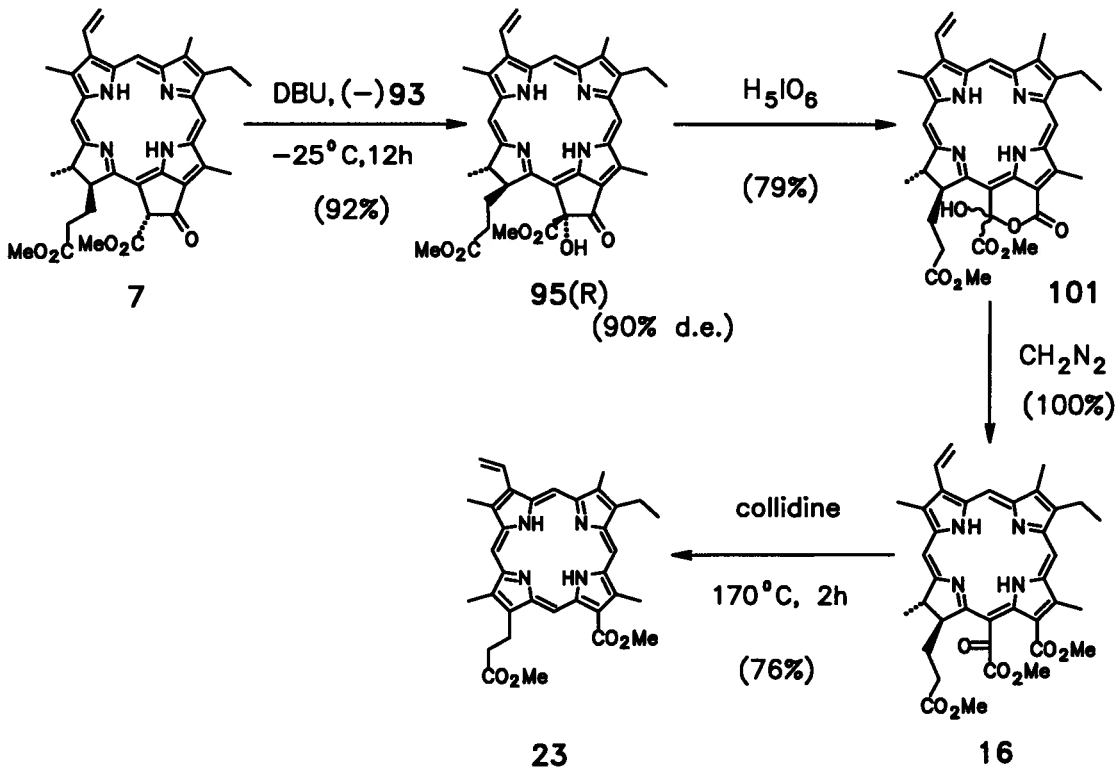
dissolution in pyridine, dilution with ether and addition of KOH in n-propanol. After aeration for 30 min Fischer's 'unstable chlorin' was obtained.[52] Esterification of 'unstable chlorin' (**21**) with diazomethane gave purpurin-7 trimethyl ester (**16**). The overall yield from pheophytin *a* was 58%. Deglyoxylation, and concomitant oxidation of purpurin-7 trimethyl ester in collidine, furnished a 76% yield (44% overall yield from pheophytin *a*) of 3-vinylrhodoporphyrin XV dimethyl ester (**23**) (**Scheme 34**).

One important characteristic of the above method is that **21** was somewhat unstable and had a tendency to oxidize further in the aerial oxidation step. The esterification with diazomethane therefore produced both purpurin-18 methyl ester (**87**) in addition to the desired purpurin-7 trimethyl ester (**16**) (**Scheme 35**). Separation of purpurin-18 methyl ester from purpurin-7 trimethyl ester by chromatography is difficult due to their very close structures and polarities.



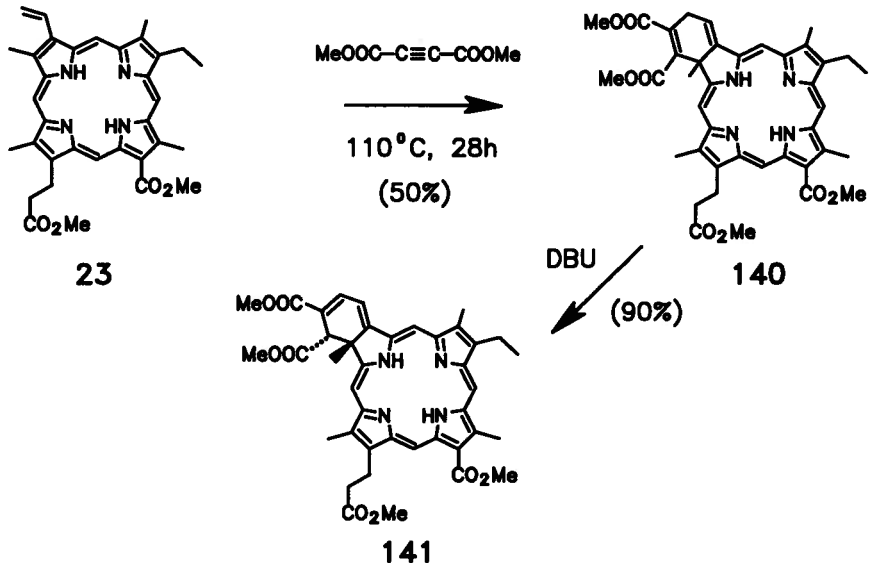
Scheme 35 Purpurin-18 Methyl Ester (**87**) and Purpurin-7 Trimethyl Ester (**16**)

Based on our synthetic methods developed in the research of antioxidative chlorins (Chapter 2), an alternate route to synthesize purpurin-7 trimethyl ester (**16**) was via 15[1]-hydroxypurpurin-7-lactone methyl ester (**101**). As described in Chapter 2, asymmetric hydroxylation of pheophorbide *a* methyl ester (**7**) using (–)-(1R)-(10-camphorsulfonyl)oxaziridine [(-)**93**] and DBU gave 13[2]R-hydroxypheophorbide *a* methyl ester (**95**) (90% d.e.) in 94% yield. Periodate oxidation of 13[2]R-hydroxypheophorbide *a* methyl ester followed by esterification with diazomethane gave purpurin-7 trimethyl ester (**16**) in 79% yield (73% overall yield from pheophytin *a*). Final deglyoxylation and concomitant oxidation of purpurin-7 trimethyl ester gave 3-vinylrhodoporphyrin XV methyl ester (**23**) in 76% yield (55% overall yield from pheophytin *a*) (**Scheme 36**). This



Scheme 36 Synthesis of 3-Vinylporphyrin **23** via Hydroxychlorin **95**

route has the advantages of higher yield and facile separations although it necessitates two extra steps compared with the original aerial oxidation of pheophytin *a*. Both methods have been used in the preparation of 3-vinylrhodoporphyrin XV (**23**) from pheophytin *a* (**5**) in this work.



Scheme 37 Synthesis of Regiochemically-pure BPD **141**
(Only One Enantiomer of **141** is Shown)

For the preparation of new regiochemically-pure benzoporphyrin derivatives, the porphyrin **23** was heated in degassed toluene solution at 110°C with a 50 fold molar excess of dimethyl acetylenedicarboxylate (DMAD). The desired Diels-Alder reaction was complete in 28 hours. Isolation and purification of the product by chromatography afforded the cycloadduct **140** in 50% yield (**Scheme 37**). The ring A 1,4-cyclohexadiene derivative **140** was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene in dichloromethane to provide the chlorin-like BPD 1,3-cyclohexadiene dimethyl ester **141** in 90% yield after chromatography (45% overall yield from **23**). The reaction progress was monitored by

UV/Vis spectroscopy which showed a new absorbance at 672 nm (**Fig. 4.1**). This rearrangement gave the thermodynamically more stable product, i.e., the carbomethoxy substituent attached at 2^1 in relation to the angular methyl group located at C-2 is in a transoid orientation. This transoid orientation was confirmed by a positive nOe effect observed for the C-2^1 proton (δ 5.04 ppm) and the methyl group at C-2 (δ 1.79 ppm) (**Fig. 4.2**).

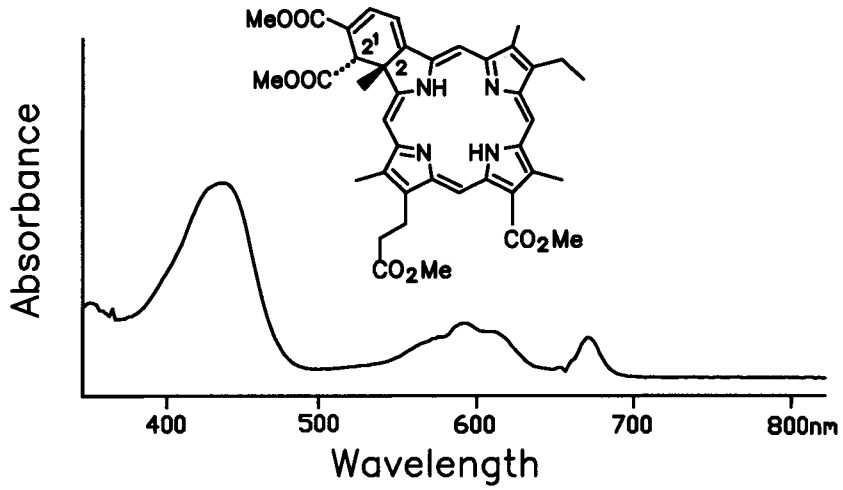


Fig. 4.1 Structure and UV/Vis Spectrum (CH_2Cl_2) of BPD **141**
(Only One Enantiomer of **141** is Shown)

The absorption spectrum of BPD **141** is shown in **Fig. 4.1**. It is characteristic of a chlorin-like chromophore but it has an unusually broad Soret band and the lowest energy absorption (Q band) at 672 nm (ε 14 600) is blue-shifted some 18 nm in comparison with that (690 nm) of the BPDMA. The difference is due to the presence of an 8-vinyl group in BPDMA, which extends the conjugation of the molecule.

We have also used olefinic dienophiles to synthesize photosensitizers related to the BPD system, which absorb at shorter wavelengths than the parent BPD **141**, but were prepared in much higher yields due to the higher reactivity of these dienophiles. The

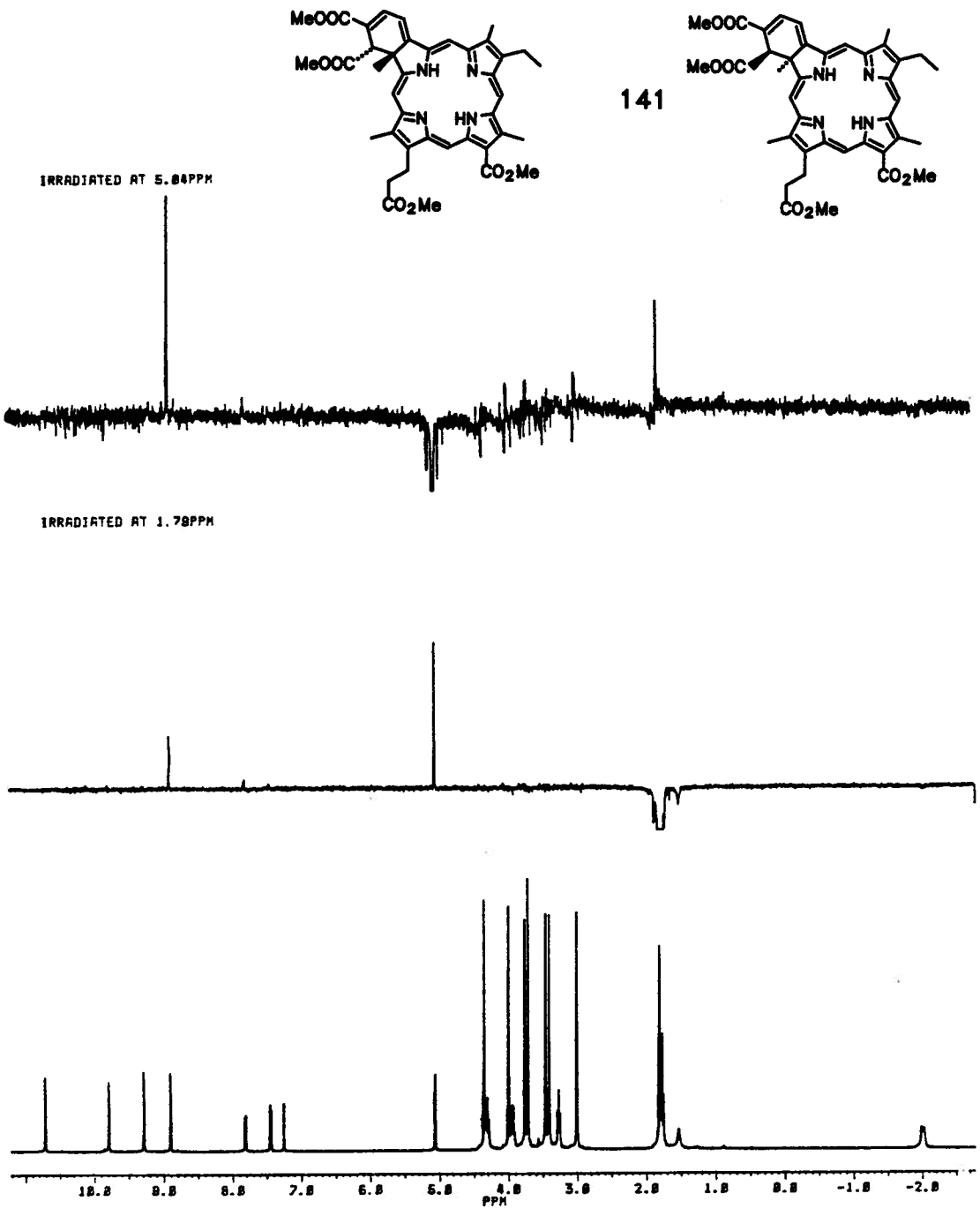
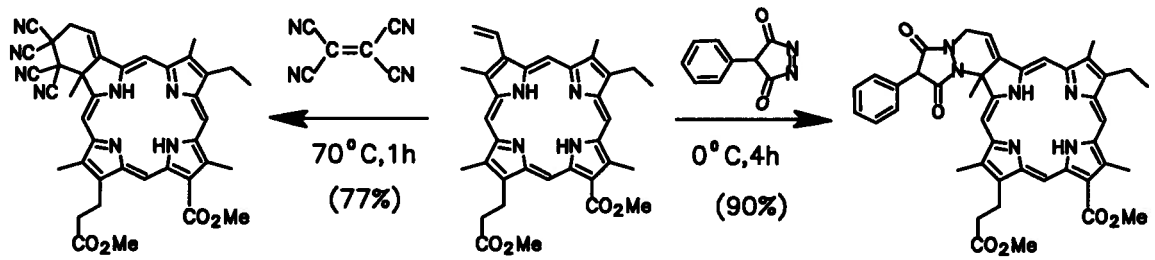


Fig. 4.2 The Difference nOe Spectra on Regiochemically-pure BPD **141**

Diels-Alder adduct **142** (77% yield) obtained from 3-vinylrhodoporphyrin XV (**23**) with tetracyanoethylene (TCNE) exhibits an absorption maximum at 642 nm (ε 20 500). Using a similar approach, reaction of the powerful heteroatomic dienophile, 4-phenyl-1,2,4-triazoline-3,5-dione, with 3-vinylrhodoporphyrin XV (**23**) gave an adduct **143** with maximum absorption at 640 nm (ε 19 500) in 90% yield (**Scheme 38**). The blue shift in the absorption maximum of adducts **142** and **143** is explained by lack of conjugation in these Diels-Alder adducts when compared with the Diels-Alder product **141**.



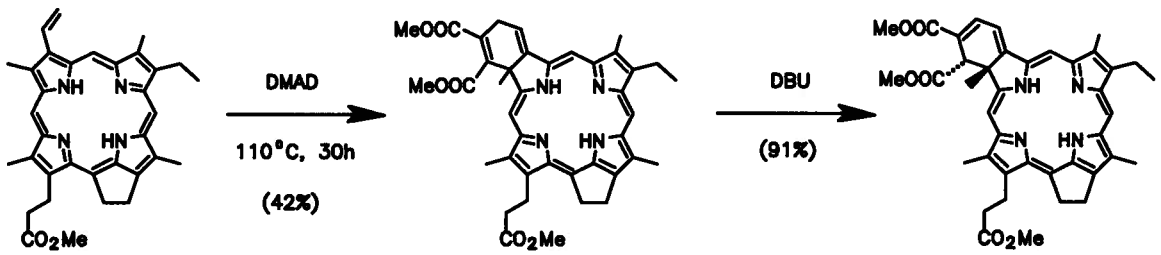
142 23 143

Scheme 38 Regiochemically-pure BPDs **142** and **143**

In addition, Pandey *et al.*[152] have very recently published a communication in which they exploited chemistry similar to that which we have described above, and obtained a similar Diels-Alder adduct **141** from 3-vinylrhodoporphyrin XV and DMAD. However, these authors reported a 23% overall yield (**141** from **23**) in contrast to a 45% total yield for the same reaction sequence in our hands.

4.2.3 Synthesis of Regiochemically-pure Benzoporphyrin Derivative Via 3-Vinyl-13^1-Deoxophytoporphyrin Methyl Ester (114c)

As has been described in Chapter 3, phytoporphyrins with a vinyl group in the position 3 can be used to synthesize the new regiochemically-pure benzoporphyrin derivatives bearing an isocyclic ring. Thus, for the preparation of BPD **125**, reaction of phytoporphyrin **114c** with dimethyl acetylenedicarboxylate in refluxing toluene under nitrogen gave the desired chlorin **134** in 42% yield. Compound **134** was then treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dichloromethane to provide the fully-conjugated chlorin **125** in 91% yield (36% overall yield) after chromatography (**Scheme 39**). The reaction progress was monitored by UV/Vis spectroscopy which showed a new absorbance at 686 nm (ε 37 000) (**Fig. 4.3**) and the disappreance of the peak at 648 nm of compound **134**.



Scheme 39 Synthesis of Regiochemically-pure BPD **125**
(Only One Enantiomer of **125** is Shown)

The FAB mass spectrum of BPD **125** showed a molecular ion at m/z 675 ($[MH]^+$). High resolution mass spectroscopy gave an accurate molecular mass at 675.3176 (calculated value for $C_{40}H_{43}N_4O_6$ = 675.3182). Compound **125** exhibited

almost the same electronic spectrum as that of the BPD **141**. The difference is that the Q band of compound **125** shifts about 14 nm to the red in comparison with BPD **141**. This is due to the "rhodofying effect" of the five-membered isocyclic ring. The ^{1}H NMR spectrum (**Fig. 4.4**) showed the three *meso* protons as singlets at δ 9.59, 9.30 and 8.95 ppm, each corresponding to one proton. The H-13^2 and H-13^1 appeared at δ 5.20 (triplet) and 3.93 ppm (multiplet) respectively corresponding to two protons. The two aromatic protons on the exocyclic ring characteristic of the benzoporphyrins were seen as doublets at δ 7.83 and 7.45 ppm (J = 5.8 Hz) as expected. Another hydrogen located at 2^1 in this exocyclic ring was observed as singlet at δ 5.05 ppm. This observation indicated an isomerization of the double bond in the six-membered ring thus extending the conjugation in the molecule.

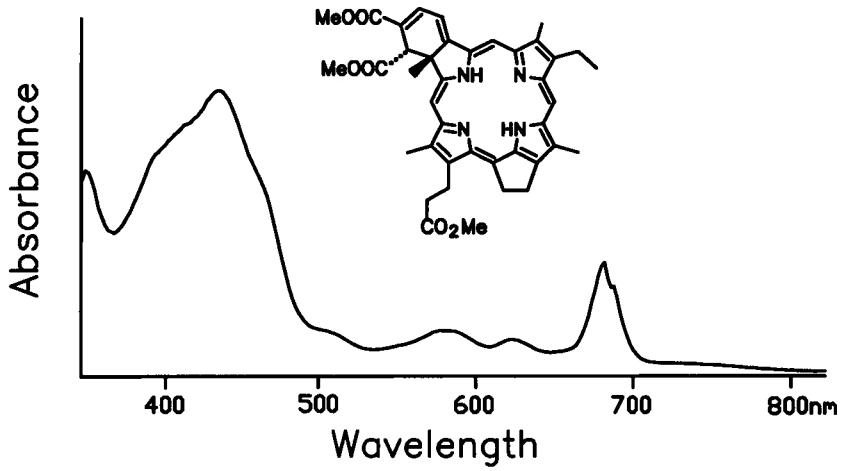


Fig.4.3 Structure and UV/Vis Spectrum (CH_2Cl_2) of BPD **125**
(Only One Enantiomer of **125** is Shown)

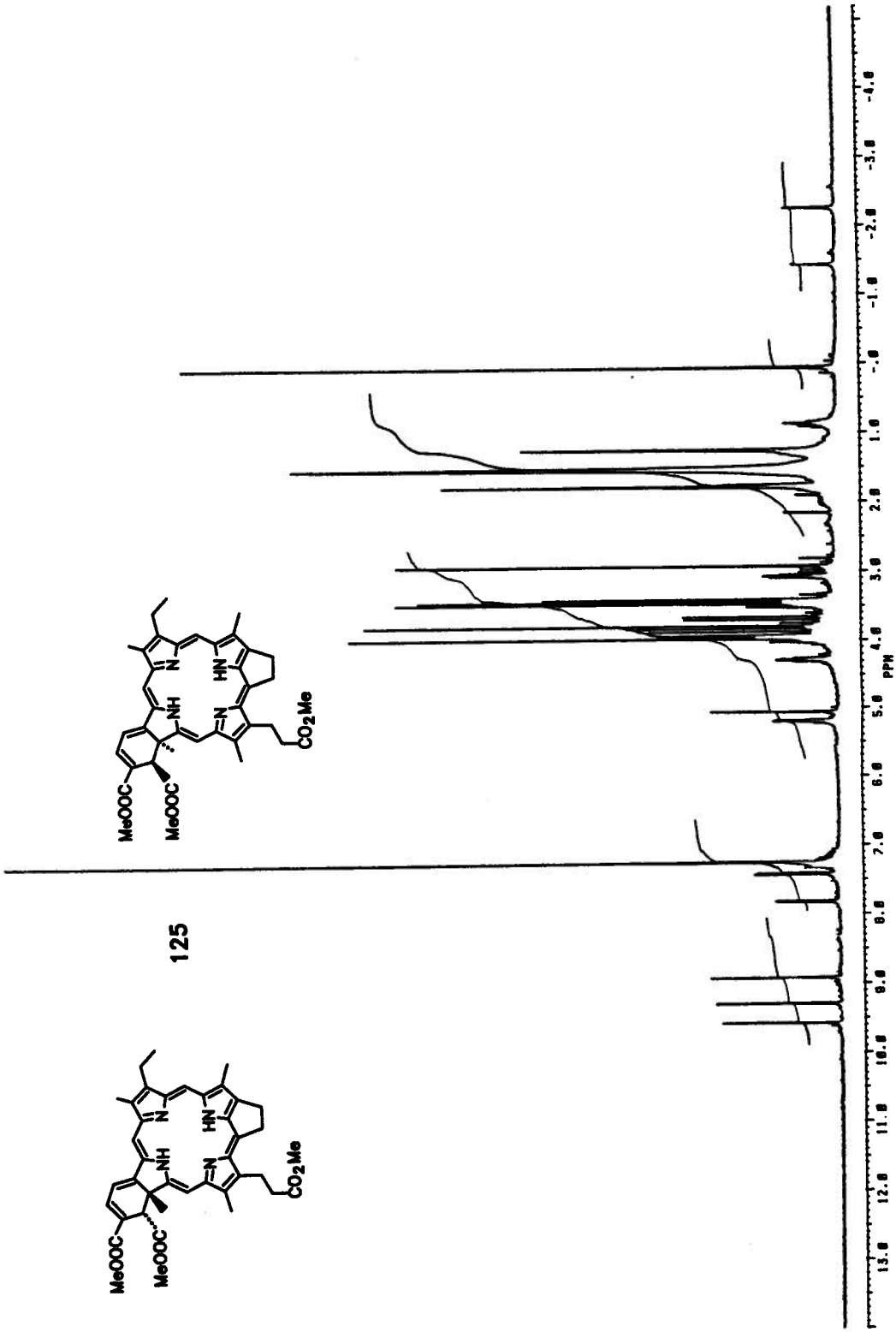


Fig. 4.4 Structure and ^1H NMR Spectrum ($CDCl_3$) of BPD **125**

4.3 Synthesis of [A,C]-Dibenzoporphyrin Derivatives (Bacteriochlorins)

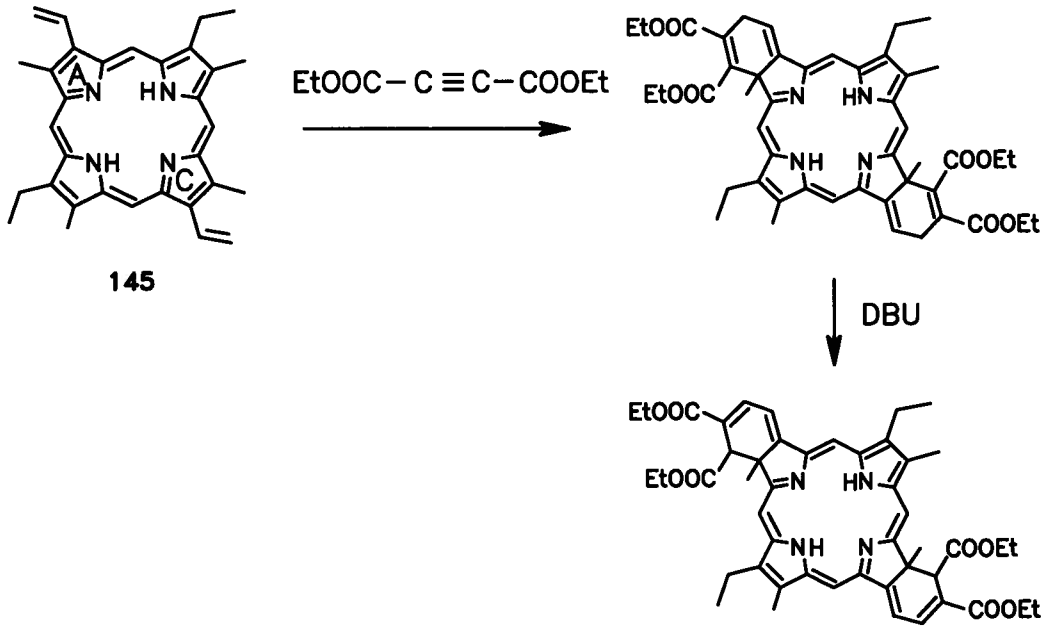
4.3.1 Rationale

The penetration of light through tissue is attenuated by a number of factors but absorption and scattering are the most important in limiting the penetration depth for effective treatment.[153] Heme proteins account for most of the absorption of light in the visible region. Since this drops off rapidly beyond 550 nm, the effective depth of penetration doubles in going from 550 nm to 630 nm and doubles again in going to 700 nm. The difference of light penetration is the main reason that BPDMA (a chlorin, λ_{max} = 690 nm, ε = 33 000) is about 70-80 times more effective than is HpD (a porphyrin mixture, λ_{max} = 630 nm, ε = 1 170) in terms of tumor photonecrosis. Biological effectiveness could be further enhanced by going to the corresponding bacteriochlorin systems. The light absorption properties of bacteriochlorins (750-800 nm) have thus caused them to be regarded as prospective candidates as photosensitizers for photodynamic therapy. In recent years, the search has begun for a third generation photosensitizer which absorbs strongly in the far visible red or near infra red region, thereby conferring a therapeutic advantage of greater tissue penetration and thus enabling treatment of large tumors. The advent of low-cost, reliable diode lasers operating at 790 nm to 850 nm has spurred in the development of several new compounds having absorption maxima in the 750-800 nm range.

Naturally-occurring bacteriochlorins, which absorb in the red region, have been reported to show both *in vitro* and *in vivo* photosensitizing activity; however by virtue of

their tetrahydro reduction states, they can be readily oxidized back to the parent (dihydro)porphyrins with an accompanying loss of their long-wavelength absorption bands (thus reducing the photodynamic efficiency).[150] This potential lack of stability has led researchers to examine other ways of producing stable bacteriochlorin-like chromophores.

In 1991, Dolphin *et al.*[153] extended the Diels-Alder cycloaddition reaction of vinylporphyrins to include several methods for synthesis of stable bacteriochlorins with absorption maxima up to 786 nm. Their synthetic strategy was based on two successive Diels-Alder cycloadditions on a symmetric [A,C]-divinylporphyrin (**145**) (**Scheme 40**). The [A,C]-divinylporphyrin **145** was further totally synthesized via a stepwise route from pyrrolic precursors by following the MacDonald dipyrromethane approach. In 1992, another research group led by Smith[154] at UC, Davis also published their work in this



145

Scheme 40 Dolphin's Synthesis of [A,C]-Dibenzoporphyrin Derivative

area in which they exploited chemistry similar to that described by Dolphin *et al.*, and obtained a similar bacteriochlorin (a dibenzoporphyrin) derivative.

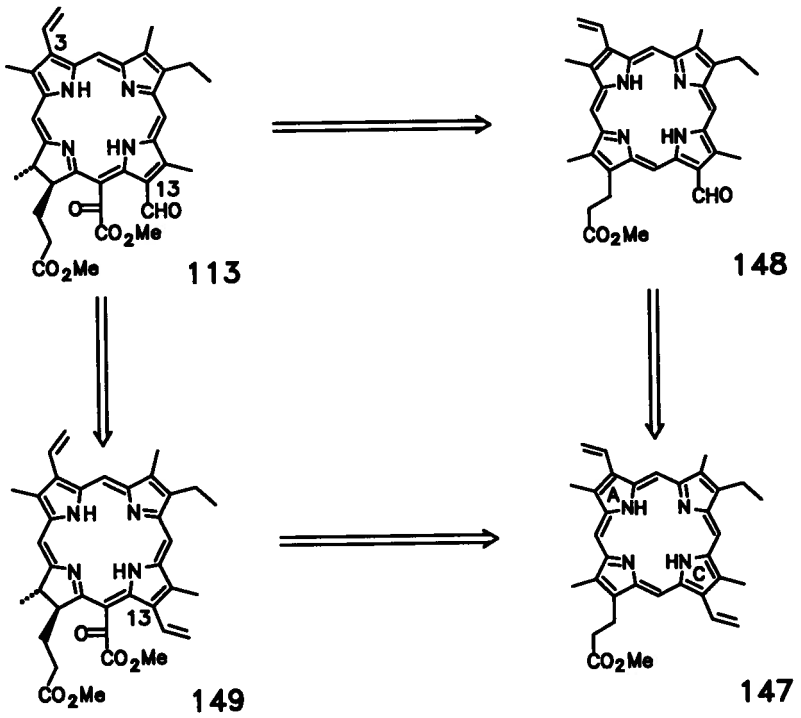
Preliminary biological results by the above two research groups indicated that the synthetic dibenzoporphyrin (bacteriochlorin) derivatives were highly photocytotoxic.[155] However, further biological investigation of these compounds as potential phototoxins in PDT was delayed by problems associated with the availability of the [A,C]-divinylporphyrin **145**. The total synthesis of [A,C]-divinylporphyrin **145** described by these groups requires a large number of steps and therefore requires a huge amount of effort in order to obtain the quantities required for clinical investigations. It was envisioned, however, that performing a partial synthesis, from a readily-available natural tetrapyrrole, might facilitate and greatly simplify the synthetic sequence of an [A,C]-divinylporphyrin. Continuing our work on Diels-Alder reactions of vinylporphyrins and on chemical modifications and applications of chlorophyll derivatives, it was thought that an [A,C]-divinylporphyrin could be synthesized by chemical modification of a chlorophyll related 3-vinyl-porphyrin or chlorin. So the partial synthesis of an [A,C]-divinylporphyrin described below was undertaken.

4.3.2 Synthesis of [A, C]-Divinylporphyrins

4.3.2.1 Via 3-Vinylpurpurin 113

Attention was focused then on the development of a new and efficient route to obtain an [A,C]-divinylporphyrin from a chlorophyll derivative. To keep the number of

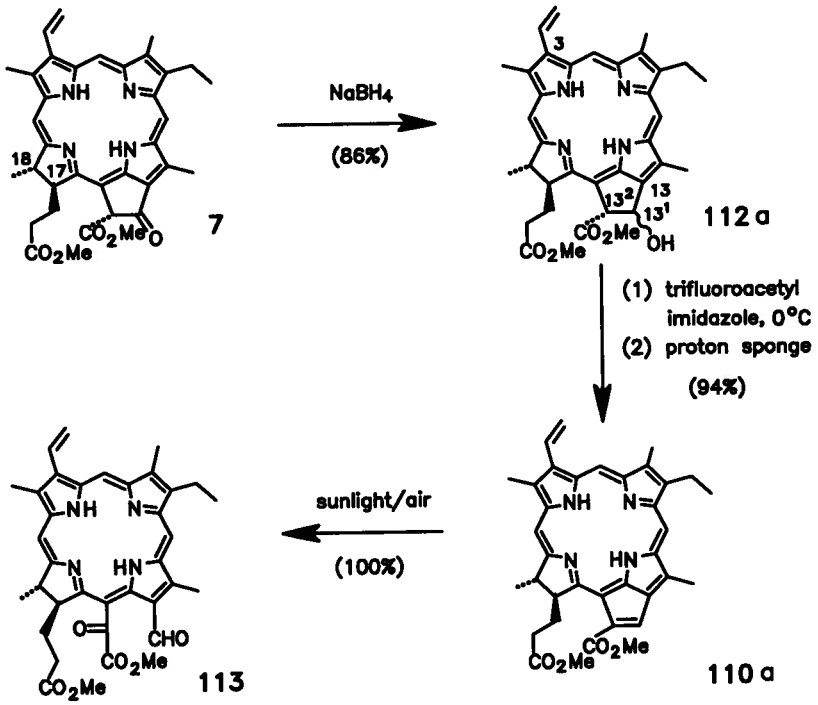
chemical modifications involved in this program to a minimum, the porphinoids synthesized in the study of natural antioxidative chlorins and of phytoporphyrins were screened and 3-vinylpurpurin **113** (Chapter 3) was chosen as the required precusor to an [A,C]-divinylporphyrin. With a 3-vinyl group at ring A and a 13-formyl group at ring C, purpurin **113** was susceptible to a Wittig olefination reaction on the 13-formyl group to form a second vinyl group at ring C and, after further decarboxylation and oxidation, to give the desired target [A,C]-divinylporphyrin **147** (**Scheme 41**). It was reasoned that the glyoxylic ester group at position 15 of the purpurin **113** could be lost with concomitant oxidation of the chlorin macrocycle into the porphyrin **148** in a one pot procedure as was used in the transformation of purpurin-7 trimethyl ester (**16**) into 3-vinylrhodoporphyrin XV (**23**) in collidine at 170°C. Further, Wittig olefination of the resulting **148** with



Scheme 41 Retrosynthetic Analysis of [A,C]-Divinylporphyrin **147**

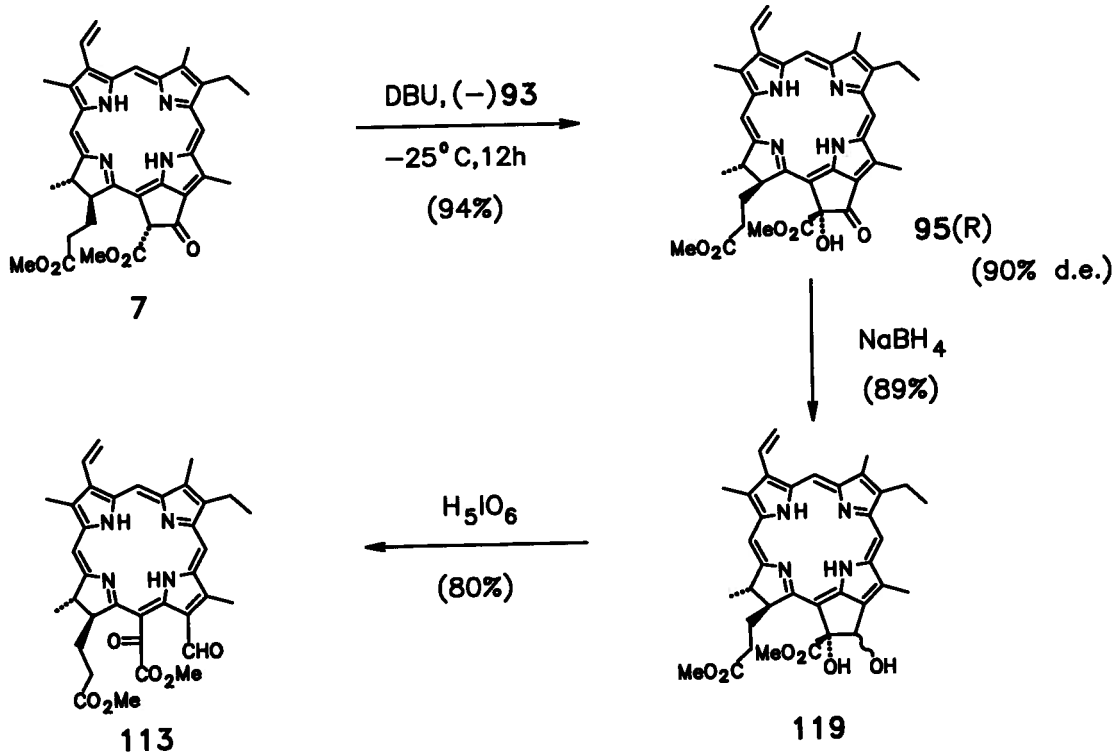
methylene triphenylphosphorane would furnish the desired [A,C]-divinylporphyrin **147**. Alternatively the [A,C]-divinylporphyrin **147** may be formed firstly by a Wittig olefination of the purpurin **113**, to furnish the [A,C]-divinylpurpurin **149**, followed by deglyoxylation of the glyoxylic ester and oxidation *in situ* in refluxing collidine. Both pathways appeared plausible because of the known transformation procedures and the ready-availability of the starting material **113** from the synthetic method developed in Chapter 3.

For the preparation of 3-vinylpurpurin **113**, two synthetic methods were investigated and the one giving the higher overall yield was subsequently used to make **113** on a 400 mg scale. In the first method, as described in Chapter 3, 3-vinylpurpurin



Scheme 42 Synthesis of Purpurin **113** via Photooxidation

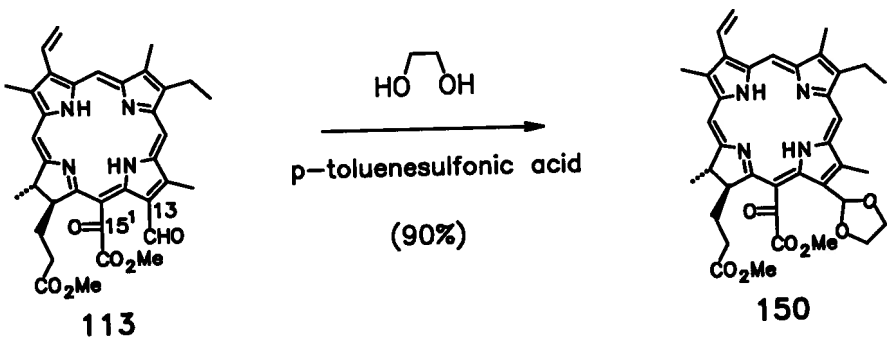
113 was derived from the photooxidation of 13^1-deoxo-13^1,13^2-dehydropheophorbide *a* methyl ester (**110**a), which was prepared from pheophorbide *a* methyl ester (**7**) in 80% overall yield (**Scheme 42**). In the second method, 3-vinylpurpurin **113** was derived from the periodate oxidation (**Scheme 43**) of the diastereomerical mixture, 13^2-deoxo-13^1-hydroxy-13^2R-hydroxypheophorbide *a* methyl ester (**119**), which was in turn obtained from the $NaBH_4$ reduction of 13^2R-hydroxypheophorbide *a* methyl ester [**95**(R)] as described in chapter 3. With the second method, purpurin **113** has been obtained in 67% overall yield from pheophorbide *a* methyl ester (**7**) in contrast to 80% overall yield from **7** in the first method by means of photooxidation.



Scheme 43 Synthesis of Purpurin **113** via Periodate Oxidation of Dihydroxychlorin **119**

When purpurin **113** was heated in collidine at 170°C for 60 minutes, 13-decarboxyl-13-formyl-3-vinylrhodoporphyrin XV methyl ester (**148**) (**Scheme 41**) was obtained only in 15% yield; the remainder of the starting material was decomposed or overoxidized under these conditions. Attempts to avoid these side-reactions by reducing reflux time and temperature (using pyridine instead of collidine) were all unsuccessful. The problem seemed to be due to the susceptibility of the 13-formyl group which led to overoxidation when the purpurin-into-porphyrin conversion occurred.

Protection of the 13-formyl function to circumvent this unwanted side reaction was therefore considered next. The ethylene acetal **150** was readily formed in 90% yield by heating purpurin **113** with ethylene glycol in THF with *p*-toluenesulfonic acid as the catalyst (**Scheme 44**). In this case, the 15^1 carbonyl function was found to be unaffected. The difference in reactivities between the 13-formyl group and the 15^1-ketone group, coupled with the steric hindrance of the 15^1-ketone moiety, is responsible for this.



113 **150**

Scheme 44 Protection of Purpurin **113** via the Formation of Ethylene Acetal **150**

The salient features of the electronic spectrum (**Fig. 4.5**) of acetal **150** (λ_{max} = 660 nm), which looks like a hydroxychlorin described in Chapter 3, are the hypsochromic shift of its Q band in comparison with that (λ_{max} = 686 nm) of the purpurin **113** and the appearance of large absorbance band at 496 nm, which suggested that the electron-withdrawing group at C-13 was removed.

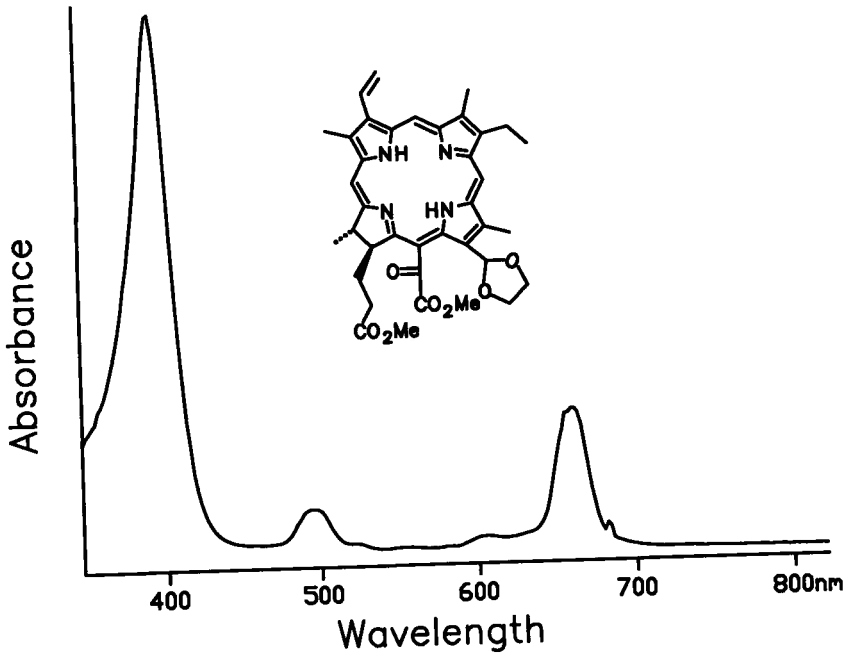


Fig. 4.5 Structure and UV/Vis Spectrum (CH_2Cl_2) of Purpurin **150**

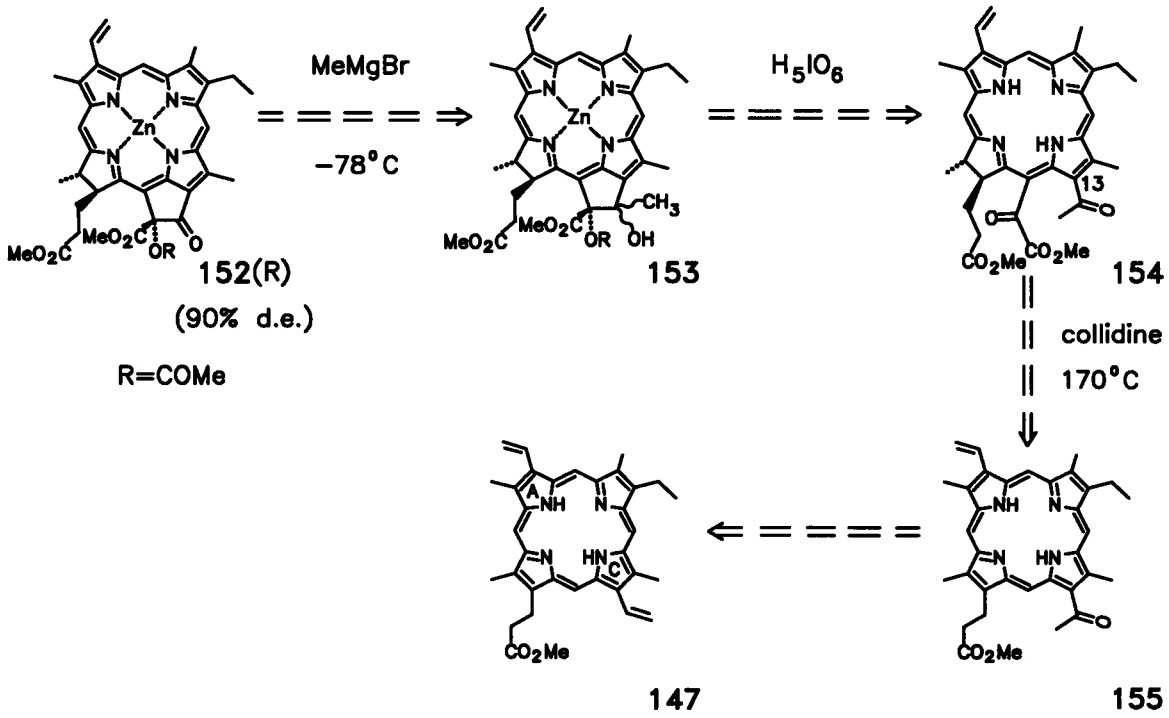
Unfortunately, when the ethylene acetal **150** was refluxed in collidine even for 4 hours, no reactions occurred and the starting material was recovered. Various attempts to achieve the purpurin-into-porphyrin conversion for the ethylene acetal **150** were all unsuccessful. It appears that a electron-withdrawing group at position 13 is required for the loss of the 15-glyoxylic ester group and concomitant oxidation of a purpurin macrocycle to a porphyrin as observed in purpurin-7 trimethyl ester (**16**) and in purpurin

113. The key reason appears to be the dramatic change in the oxidation potential of the ethylene acetal **150** with the protection of the 13-formyl group. The presence of a electron-withdrawing group at position 13 in these purpurins seems to destablize the π-system of the molecule and render the molecule easier to oxidize than the corresponding protected derivative.

The conversion of purpurin-7 trimethyl ester (**16**) into 3-vinylrhodoporphyrin XV (**23**) was originally investigated by Fischer[36], who found the loss of the glyoxylic ester residue from purpurin-7 trimethyl ester (**16**) occurred upon refluxing in pyridine. Later, Kenner *et al.*[52] reported that the use of refluxing collidine in place of pyridine results in higher yield for the purpurin-into-porphyrin conversion, because prolonged reflux in pyridine often gives rise to decomposition products. Interestingly, we found that the presence of oxygen in the reaction medium has a beneficial effect on the conversion, but is not required, since the conversion was also observed under oxygen-free condition, suggesting the facile transformation ability of purpurin **16**.

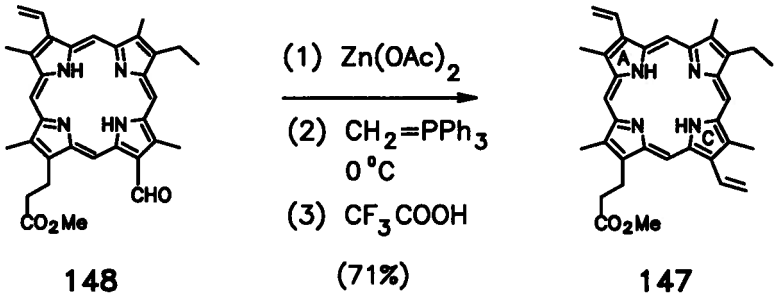
Given the anticipated difficulty in the purpurin-into-porphyrin conversion for the [A,C]-divinylpurpurin **149** (**Scheme 41**), a purpurin with a non-electron-withdrawing group (i.e. vinyl) at position 13, the planned synthetic route to [A,C]-divinylporphyrin **147** via the [A,C]-divinylpurpurin **149** was abandoned. Another attempt to synthesis [A,C]-divinylporphyrin **147** was via purpurin **154** (**Scheme 45**), a purpurin with a 13-acetyl (electron-withdrawing) group, which should undergo the purpurin-into-porphyrin conversion. The planned synthetic route to purpurin **154** was via Grignard reaction with Zn(II) 13^2R-acetoxypheophorbide *a* methyl ester [**152**(R)] to afford 13^2R-acetoxy-13^1-

deoxo-13^1-hydroxy-13^1-methylpheophorbide *a* methyl ester (**153**), which could be further oxidized by periodic acid to give 13^1-decarboxy-13^1-acetylpurpurin-7 dimethyl ester (**154**) as shown in **Scheme 45**. Unfortunately, the Grignard reaction at –78°C with Zn(II) 13^{2}R-acetoxypheophorbide *a* methyl ester [**152**(R)] {prepared from acetoxylation of 13^{2}R-hydroxypheophorbide *a* methyl ester [**95**(R)] (90% d.e.) followed by metallation with zinc acetate} was unsuccessful and gave none of the desired product **153**, and led to decomposition of the material under these conditions.



Scheme 45 Unsuccessful Transformation of Zn(II) Chlorin **152**

Finally, we came back to the direct conversion of purpurin **113** and prepared sufficient 13-decarboxy-13-formyl-3-vinylrhodoporphyrin XV methyl ester (**148**) by



Scheme 46 Synthesis of [A,C]-Divinylporphyrin **147** via Porphyrin **148**

refluxing purpurin **113** in collidine. Treatment of 13-formyl-3-vinylporphyrin **148** with zinc(II) acetate in methanol/dichloromethane gave the zinc complex **156** which was subjected to a Wittig reaction to give, after removal of the zinc by treatment with trifluoroacetic acid, a 71% yield of the desired [A,C]-divinylporphyrin **147** (**Scheme 46**). With this synthetic approach the [A,C]-divinylporphyrin **147** was obtained in 6 steps from pheophorbide *a* methyl ester (**7**) in 9% overall yield. The structure of **147** was confirmed by its ^{1}H NMR (**Fig. 4.6**) and UV/Vis spectra (**Fig. 4.7**). In its ^{1}H NMR, both vinyl groups in this non-symmetrical porphyrin were magnetically equivalent and they were analyzed as an ABX system as before. The doublet of doublets at δ 6.15 ppm was assigned to H-3^2(Z) and H-13^2(Z). The doublet of doublets centered at δ 6.33 ppm was assigned to H-3^2(E) and H-13^2(E). The doublet of doublets centered at δ 8.27 ppm was assigned to H-3^1 and H-13^1. The coupling data found here agreed with the information already gained for the other vinylporphyrins. Its electronic spectrum as shown in **Fig. 4.7** exhibited a rhodo-type pattern (λ_{max} = 404, 506, 544, 572, 630 nm, III>IV>II>I) and this observation corresponded to that reported by Dolphin *et al.*.[153]

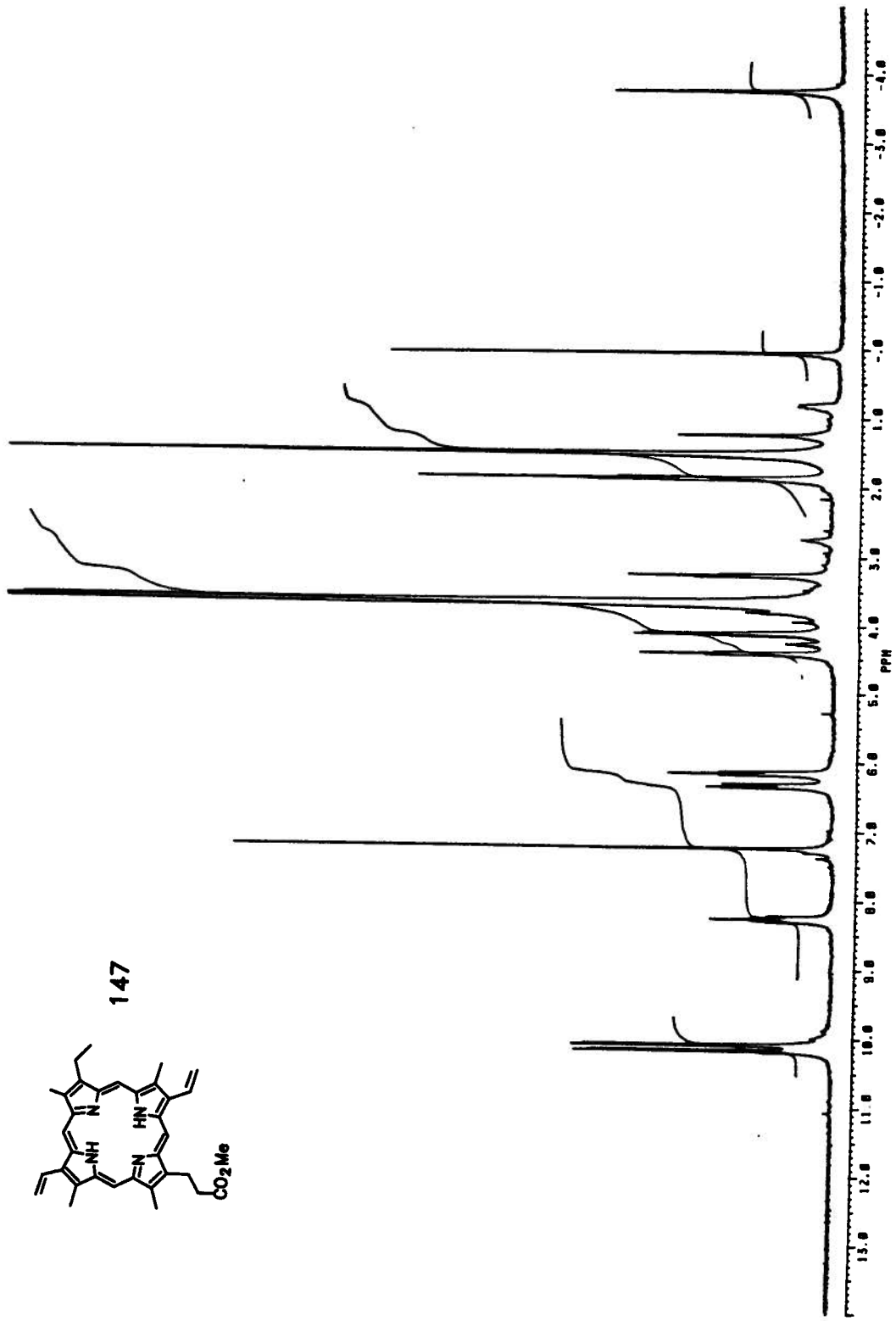


Fig. 4.6 Structure and ^{1}H NMR Spectrum ($CDCl_3$) of [A,C]-Divinylporphyrin **147**

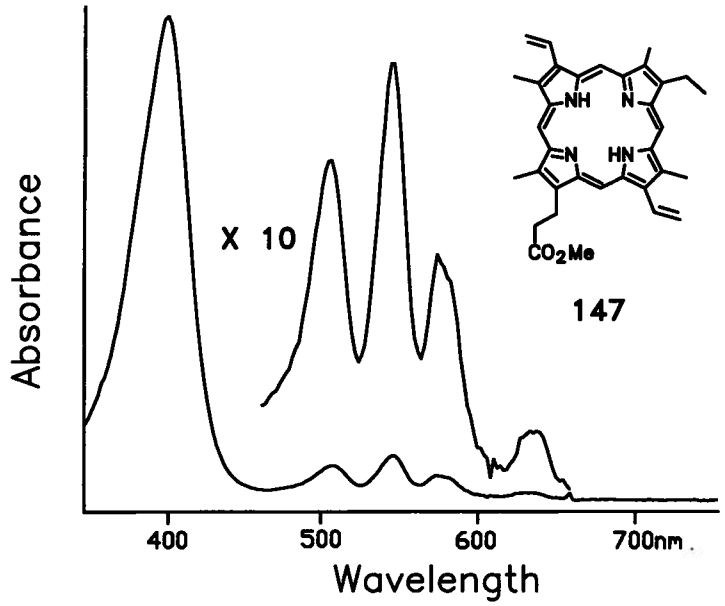


Fig. 4.7 Structure and UV/Vis Spectrum (CH_2Cl_2) of [A,C]-Divinylporphyrin **147**

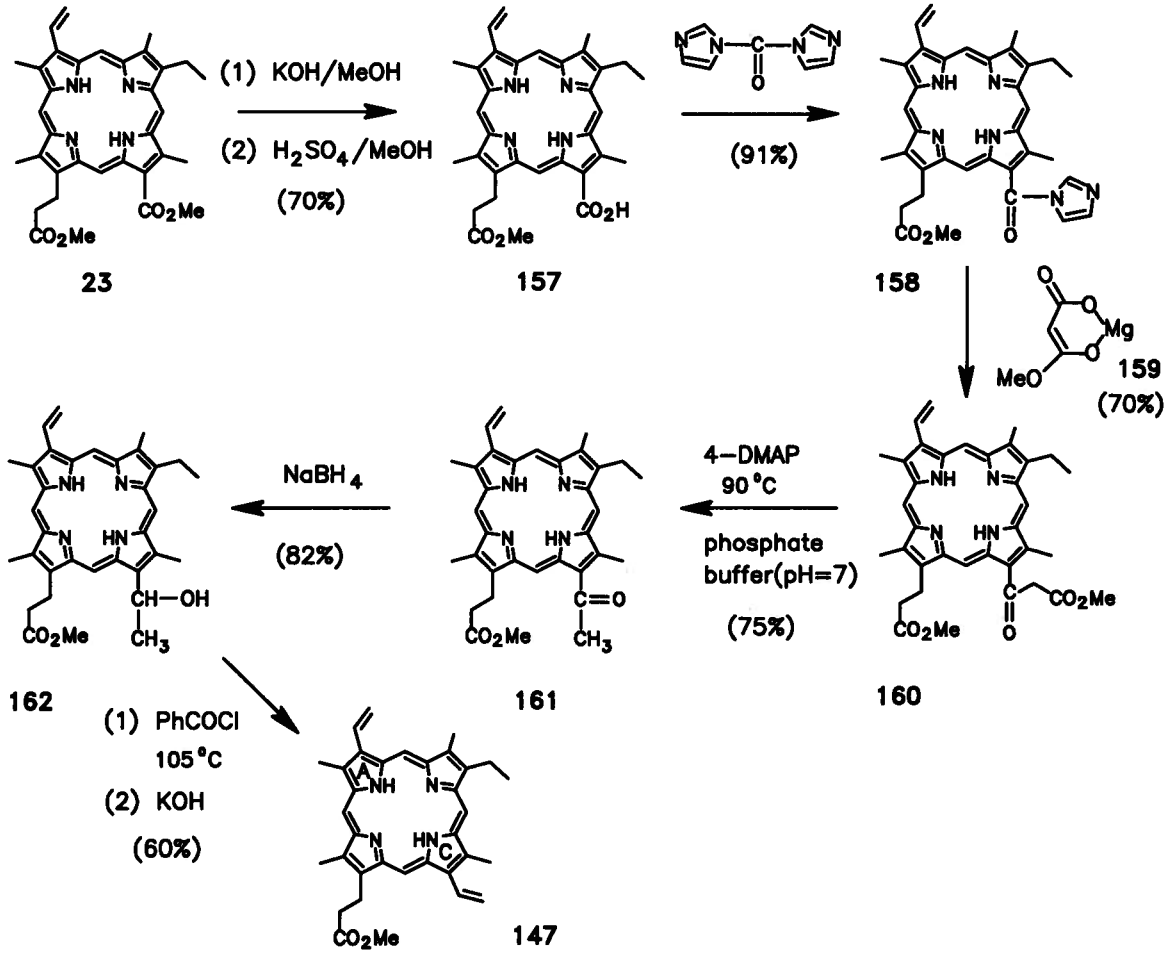
4.3.2.2 Synthesis of [A,C]-Divinylporphyrin Via Porphyrin 23

At this point, it was felt that the 15% yield of the formyl-rhodoporphyrin XV (**148**) obtained by the oxidation and deglyoxylation of purpurin **113** in collidine was unsatisfactory and should be improved upon by pursuing an alternative approach.

Previous studies led us to conclude that the 13-methoxycarbonyl group and other non-formyl groups are advantageous in this regard. 3-Vinylrhodoporphyrin XV dimethyl ester (**23**) was chosen as the starting material since it is readily available from the oxidation of purpurin-7 trimethyl ester (**16**) as described before.

In this approach (**Scheme 47**), 3-vinylrhodoporphyrin XV dimethyl ester (**23**) was hydrolyzed to its di-acid analog and was partially remethylated on the propionic side chain using 5% H_2SO_4 in methanol to give the 3-vinylrhodoporphyrin XV monomethyl

ester (**157**) in 70% overall yield. The reesterification step was based on the reactivity difference of the two carboxy groups which was found by Kenner and coworkers[156]. It was predicted that protonation of the nuclear carboxy-group was inhibited by the double positive charge on the macrocycle, whereas the side-chain carboxy-group retained aliphatic character and therefore a greater disposition to esterification. A monoester was indeed obtained without difficulty, and its structure **157** was confirmed by the lack of the low-field methoxy-resonance (ring current effect) in the ^{1}H NMR spectrum (**Fig. 4.8**) (in deuterotrifluoacetic acid) compared to the presence of the singlet signal located at δ 4.45 ppm (i.e. 13-$COOCH_3$ moiety) in the porphyrin dimethyl ester **23**.



Scheme 47 Synthesis of [A,C]-Divinylporphyrin **147** via Porphyrin **23**

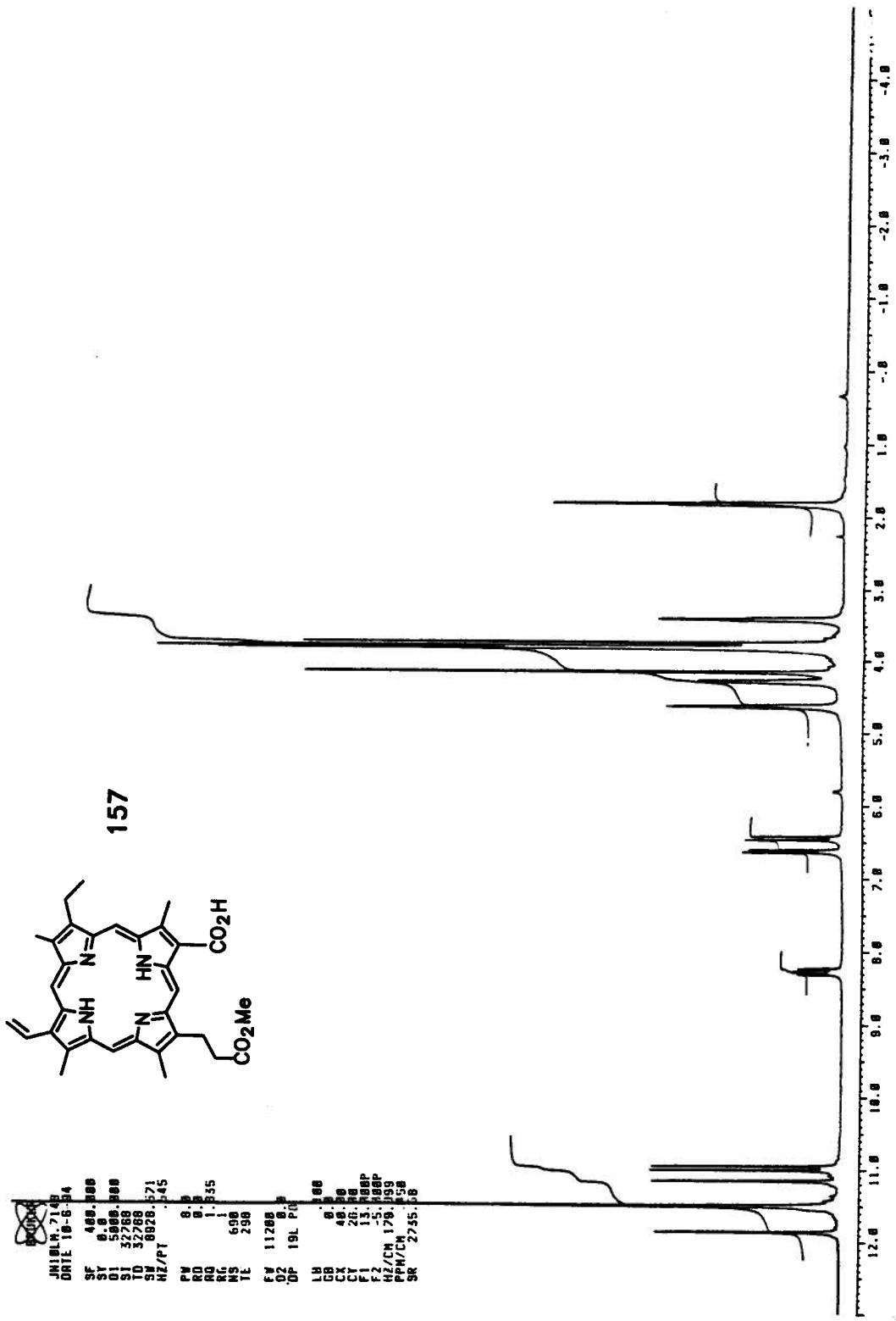


Fig. 4.8 Structure and ^1H NMR Spectrum (CF_3COOD) of Porphyrin Monoacid **157**

The resulting monomethyl ester (**157**) was added to a solution of *N,N'*-carbonyl-di-imidazole in THF and heated to reflux for 30 min.[157] The desired imidazolide **158** was obtained in 91% yield after flash chromatography on silica gel. The FAB mass spectrum of **158** showed the molecular ion ([MH]/z 601) and a fragment derived from the loss of the imidazole moiety (M/z 533). The ^{1}H NMR spectrum (**Fig. 4.9**) of the compound was in agreement with the structure of the expected porphyrin.

The nucleophilic displacement[156] of the imidazolide **158** with the magnesium salt of methyl hydrogen malonate (**159**)[158] gave the expected ß-keto-ester **160** in 70% yield. This step was based on a method which was introduced by Bram and Vilkas[158] and later improved by Kenner and coworkers[156]. The magnesium salt of methyl hydrogen malonate (**159**) was prepared by treatment of methyl hydrogen malonate with 2 equiv of isopropylmagnesium bromide in THF. The advantage of this procudure was its selectivity which allowed the reaction to proceed without deprotonation of the porphyrin macrocycle to the green dianion.

With the enolizable ß-keto-ester **160** in hand, we were able to attempt the decarboxylation step. A high yield of the desired acetylporphyrin **161** was obtained by modifying the decarboxylation method described by Taber and coworkers[159]. In this reaction, the ß-keto-ester **160** was stirred in a solution of 4-(dimethylamino)pyridine (4-DMAP) in phosphate buffer (pH = 7) at 90°C for 12 hours under nitrogen to effect the demethoxycarbonylation (75% yield). The progress of the reaction was monitored by TLC analysis and its completion was indicated by the disappearance of the less mobile enolizable ß-keto-ester **160**.

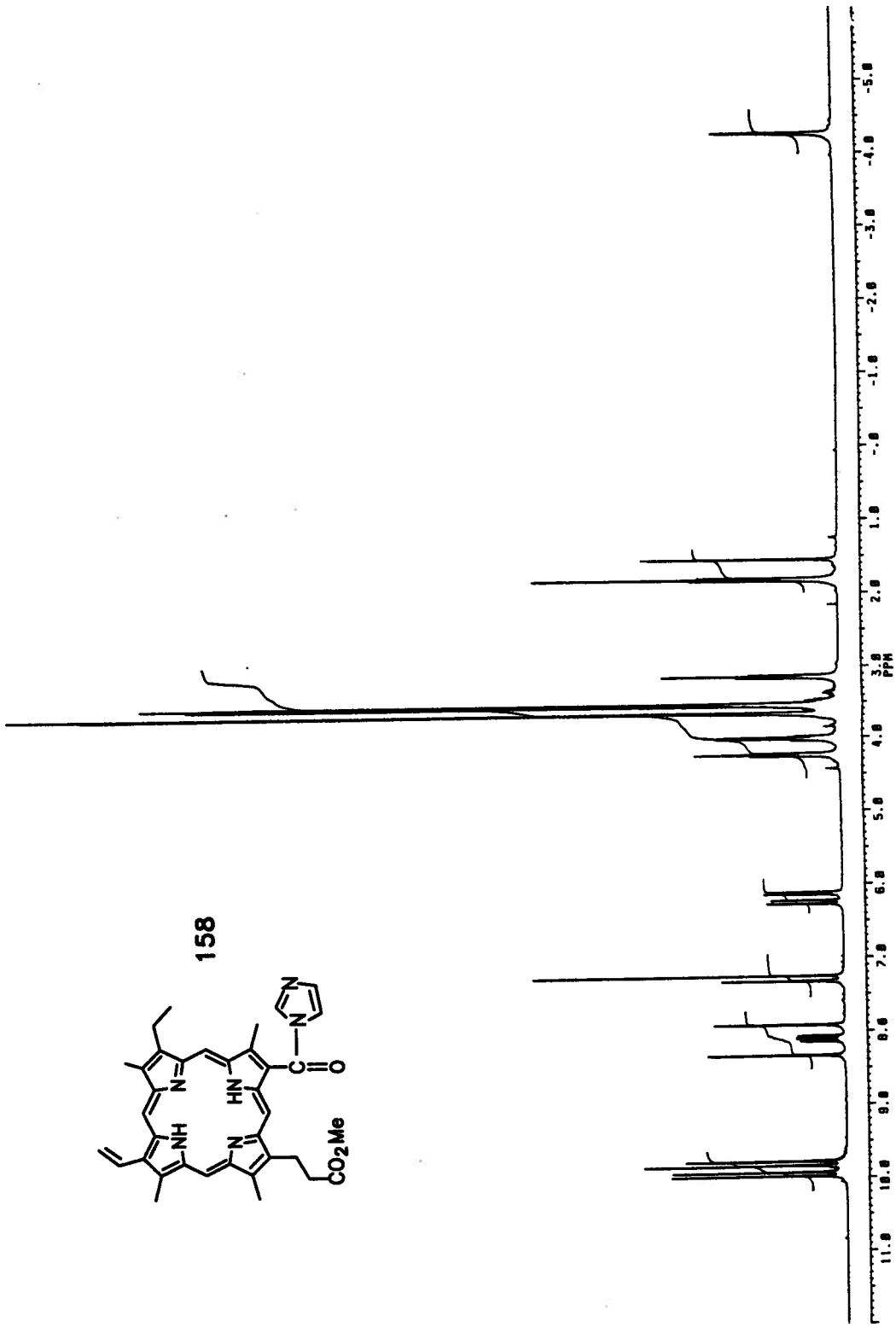


Fig. 4.9 Structure and ^1H NMR Spectrum ($CDCl_3$) of Imidazoyl Porphyrin **158**

The FAB mass spectrum of acetylporphyrin **161** showed a molecular ion at m/z 549 ($[MH]^+$). High resolution mass spectroscopy gave an accurate molecular mass at 549.2861 (calculated value for $C_{34}H_{37}N_4O_3$ = 549.2865). The electronic spectrum of this compound was almost the same as that of the imidazoylporphyrin **158** (oxorhodo-type spectrum). The ^1H NMR spectrum of porphyrin **161** (**Fig. 4.10**) showed the four *meso* protons as singlets at δ 10.75, 10.10, 10.07, and 9.98 ppm, each corresponding to one proton. The H-13^2 (i.e. 13-$COCH_3$) appeared as singlet at δ 3.32 ppm. This observation indicated the presence of the desired 13-$COCH_3$ moiety.

The acetylporphyrin **161** was reduced with sodium borohydride in THF to give the expected hydroxyporphyrin **162**. With the absence of the electron-withdrawing group at position 13, hydroxyporphyrin **162** exhibited a rhodo-type electronic spectrum in contrast to an oxorhodo-type spectrum in the starting material **161**, suggesting only one rhodofying group (i.e the 3-vinyl moiety) present in the molecule.

The last step in the present synthetic sequence involves the generation of the 13-vinyl group. This transformation was achieved by subjecting the hydroxyporphyrin **162** to benzoyl chloride in DMF at 105°C for 2 hours.[160] Under these conditions, the hydroxyporphyrin **162** was dehydrated to give the desired [A,C]-divinylporphyrin **147** in 60% yield after chromatography. This material was found identical to be the divinylporphyrin prepared from the Wittig reaction of 13-formylporphyrin **148**. With this synthetic approach, the [A,C]-divinylporphyrin **147** was prepared in 9 steps from pheophorbide *a* methyl ester (**7**) in 12% overall yield.

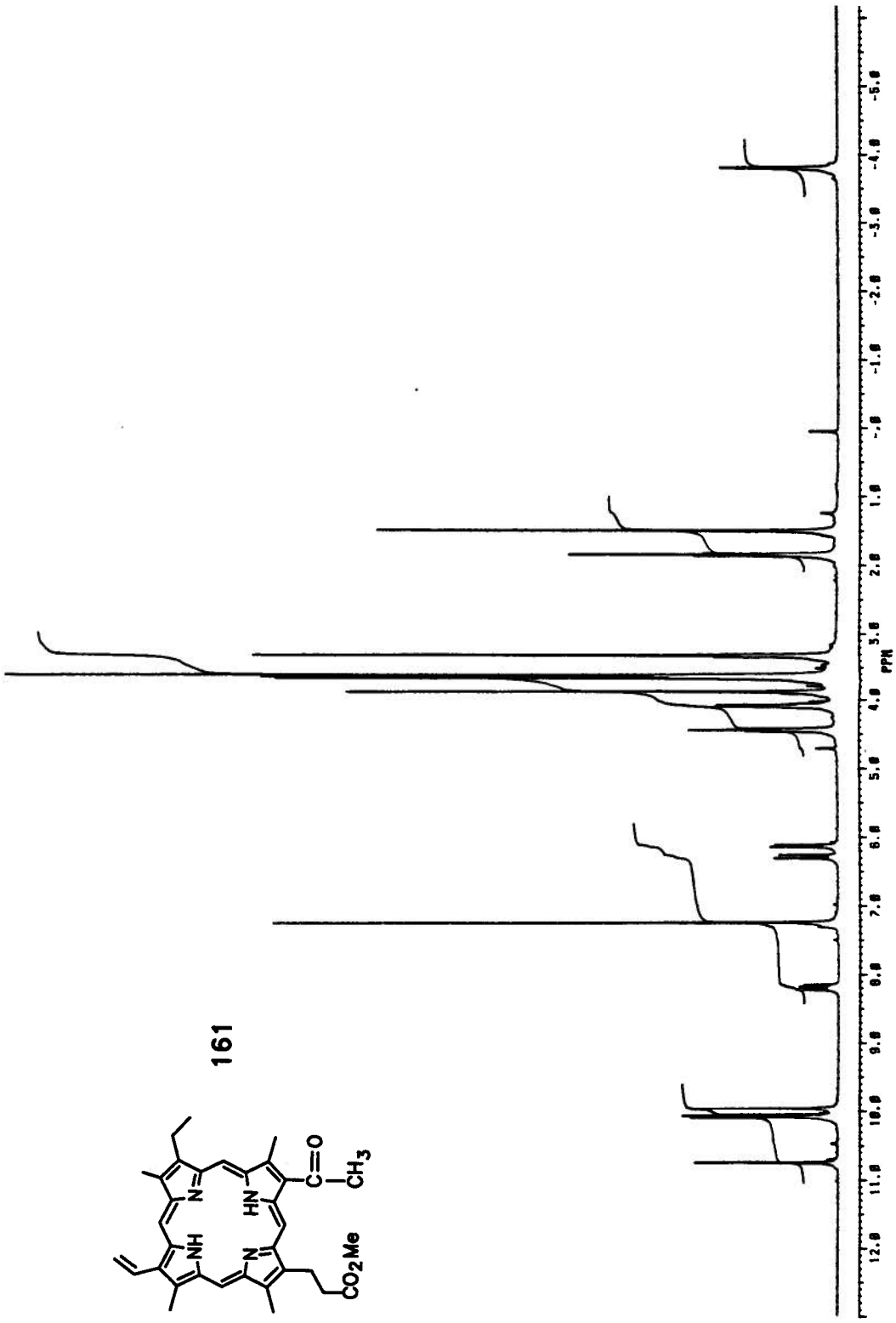
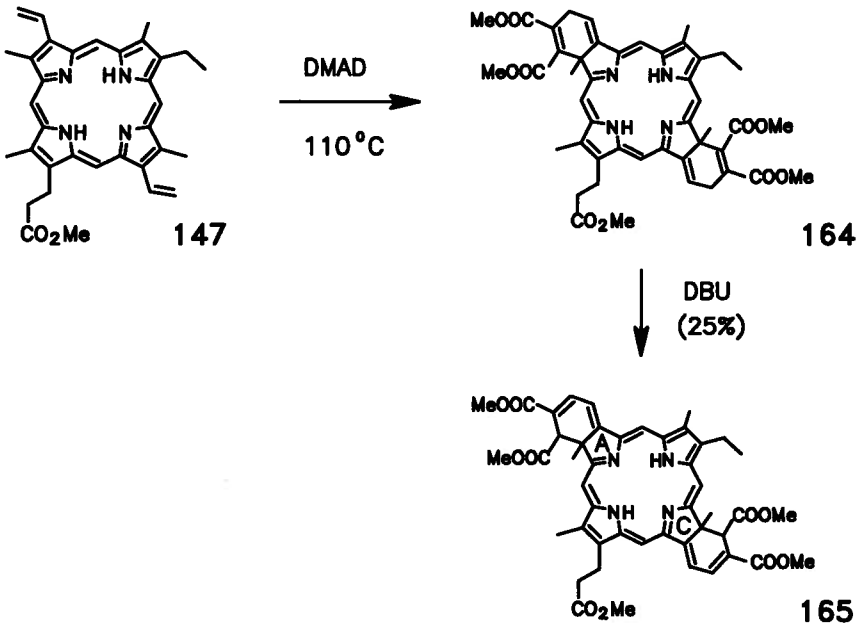


Fig. 4.10 Structure and ^1H NMR Spectrum ($CDCl_3$) of Acetylporphyrin **161**

4.3.3 Synthesis of [A,C]-Dibenzoporphyrin Derivatives

As has already been mentioned, the synthetic strategy for the [A,C]-dibenzoporphyrin derivative relied on two cycloaddition reactions on an [A,C]-divinylporphyrin system. The first step in this program was to prepare an [A,C]-divinylporphyrin by the chemical modification of chlorophyll derivatives. Two routes were investigated and both gave the desired [A,C]-divinylporphyrin **147** in moderate yields (described in section **4.3.2**). Having solved the problem of preparing the precusor, we next turned to the ultimate goal of this program, the synthesis of a bacteriochlorin chromophore.



Scheme 48 Synthesis of [A,C]-Dibenzoporphyrin Derivative **165**

Refluxing the [A,C]-divinylporphyrin **147** with 100-fold molar excess of dimethyl acetylendicarboxylate in toluene for three days gave a mixture with a strong absorption at 720 nm. The desired bis-adduct **164** (**Scheme 48**) was obtained as the major product

along with a small amount of chlorin in which only one of the vinyl groups (either ring A and ring C) was transformed (identified by spectrophotometry). The best reaction time was found to be 80 hours with a yield of ~30%, while prolonged reflux resulted in decomposition and thus gave the product **164** in a lower yield. Flash chromatography on a silica gel column followed by further purification by preparative TLC plate gave the bis-adduct **164** in 25% yield. The electronic spectrum of the bis-adduct exhibited the characteristic features of a bacteriochlorin (λ_{max} = 720 nm). The FAB mass spectrum of this adduct showed the molecular ion as the base peak at m/z 817 ($[MH]^+$). High resolution mass spectroscopy gave an accurate mass at 817.3455 (calculated value for $C_{46}H_{49}N_4O_{10}$ = 817.3449).

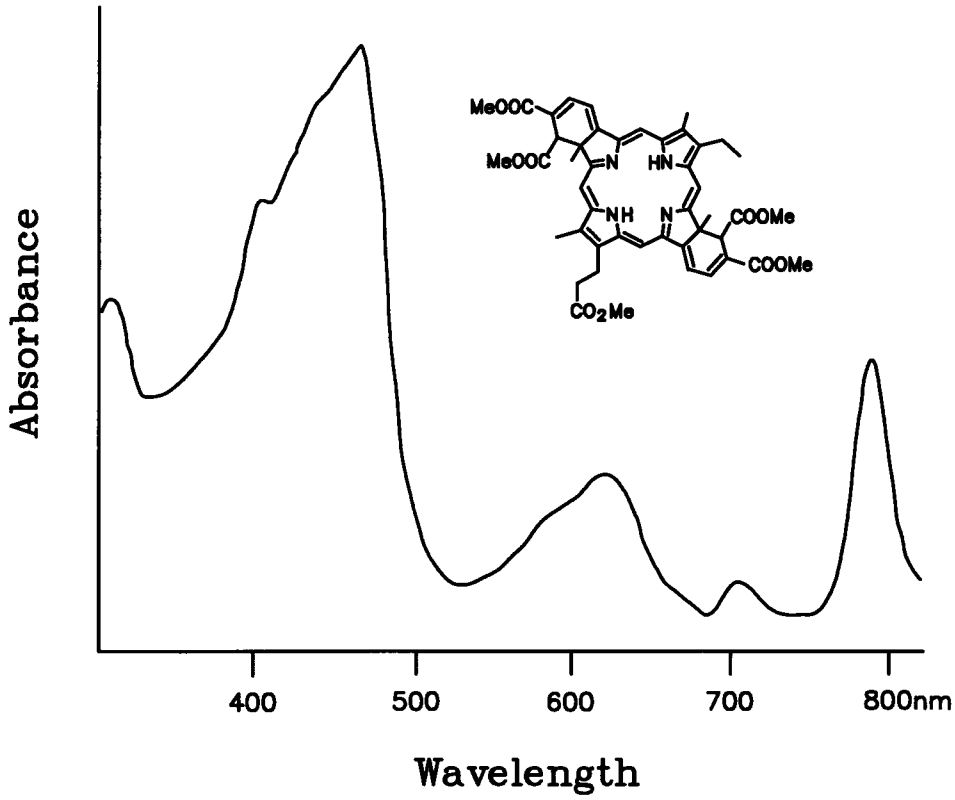


Fig. 4.11 UV/Vis Spectrum (CH_2Cl_2) of [A,C]-Dibenzoporphyrin Derivative **165**

When the above bis-adduct was stirred overnight with 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) at room temperature, a dramatic bathochromic shift was observed (λ_{max} = 784 nm, ε = 38 000) (**Fig. 4.11**). This [A,C]-dibenzoporphyrin derivative **165** showed almost the same electronic spectrum as the bacteriochlorin reported by Dolphin *et al.*[153]. The most significant features in the ^{1}H NMR spectrum of the new adduct **165** were the appearance of a signal at δ 4.89 ppm for the new sp^3 center generated at C-2^1 and C-12^1 and two doublets at δ 7.30 ppm and δ 7.81 ppm for the new sp^2 centers. This observation indicated an isomerization of the double bond in both 1,4-cyclohexadienes thus extending the conjugation in the molecule as described before.

4.4 Monoacid Analogues of New Regiochemically-pure BPDs and [A,C]-Dibenzoporphyrin Derivatives

As described earlier, the monocarboxylic acid analogue of benzoporphyrin derivative BPDMA shows better photosensitizing efficacy than its corresponding diester. Therefore, to parallel the structural requirements for this biologically active benzoporphyrin derivative and to satisfy our originally planned goals, the new regiochemically-pure benzoporphyrin derivatives **141** and **125**, and the [A,C]-dibenzoporphyrin derivative **165** were partially hydrolyzed with 25% HCl at the propionic ester side chain to afford the corresponding monocarboxylic acids (i.e. propionic acid analogues) (**Fig. 4.12**). These monocarboxylic acids together with their corresponding esters described herein are being evaluated for photodynamic ability.

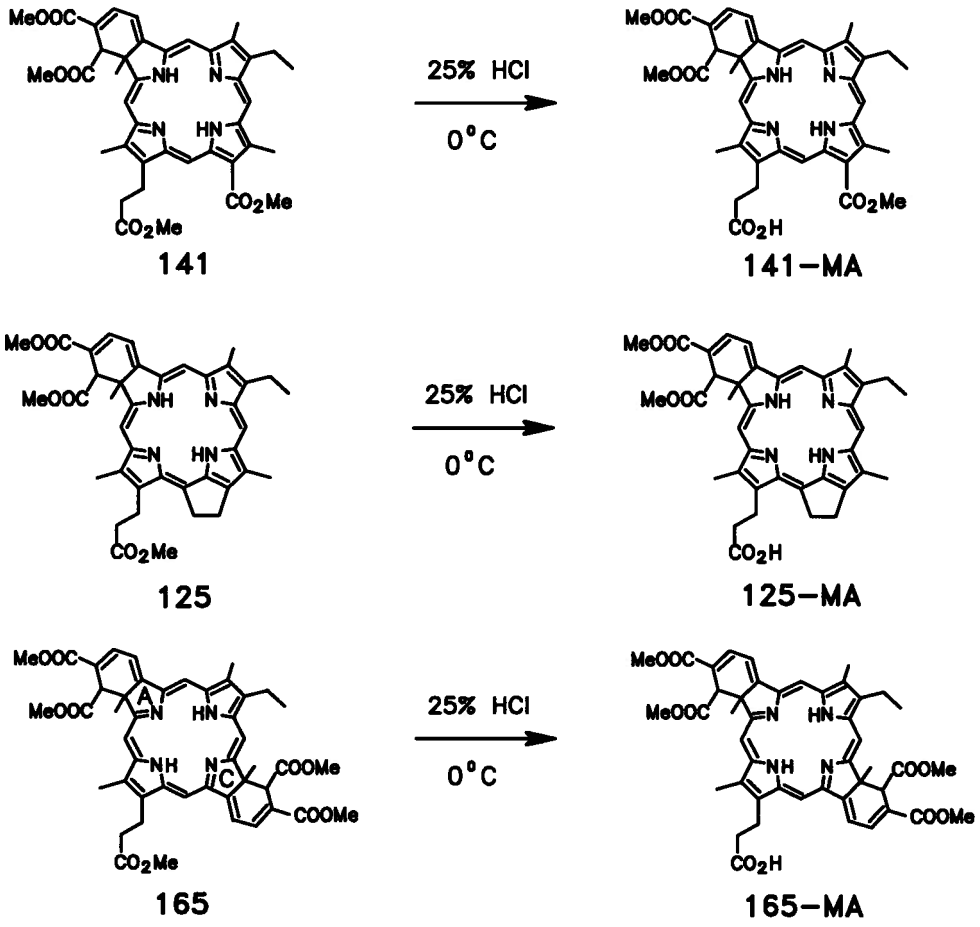


Fig. 4.12 Synthesis of Monoacid Analogues of BPDs and Dibenzoporphyrin Derivative

4.5 Summary

Two series of new regiochemically-pure benzoporphyrin derivatives **141** and **125** have been synthesized from Diels-Alder reactions on 3-vinylrhodoporphyrin XV (**23**) and 3-vinylphytoporphyrin (**114c**) with dimethyl acetylenedicarboxylate. The corresponding monocarboxylic analogues of these new regiochemically-pure BPDs have been prepared by partial hydrolysis. These new photosensitizers have characteristics which meet or

exceed the promising chemical features of BPDMA, a second generation photosensitizer in Phase-II clinical trials.

The [A,C]-divinylporphyrin **147** was synthesized via two routes and its Diels-Alder reaction with dimethyl acetylenedicarboxylate was studied. The resulting bis-adduct **165**, being a stable bacteriochlorin derivative, absorbs strongly at 784 nm, a fact which, according to current thinking, renders it eminently desirable as a photosensitizer for treatment of large tumors or tumors which are deeply-seated within the body.

In conclusion, the present work has led to the synthesis of a class of compounds, via chemical modifications of chlorophyll *a*, with potential as future drugs in the field of photodynamic therapy. All these new photosensitizers are of high purity and fully characterized, and thus are readily accessible for subsequently biological investigations.

Chapter 5

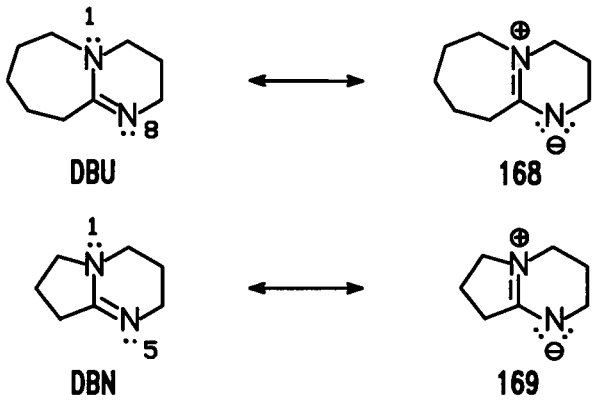
DBU and DBN: Nucleophilicity *vs.* Basicity

5.1 Background and Research Objective

The effectiveness of the bicyclic amidines, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) as "non-nucleophilic strong bases" in organic and inorganic chemistry has been widely demonstrated.[161] These compounds initially became known because of their activity in dehydrohalogenations, which was first noted in the synthesis of vitamin A, and was rapidly exploited in other organic syntheses. The applications of DBU and DBN have rapidly increased because of their favorable "non-nucleophilic", yet strongly basic, properties. They can, therefore, be applied to the preparation of even relatively sensitive molecules. It was also found beneficial to use these compounds in condensation[162], substitution[163], addition[164], isomerization and rearrangement[165], cyclization and cyclocondensation[166], and oxidation and reduction [167] reactions; and as catalysts in the syntheses of macromolecules [161].

The base strength of DBU has been determined by several groups.[168,169] The pK_a value of the corresponding conjugate acid of DBU is in the range of 11.5-13.4 depending on the determination medium in contrast to that of 10.7 for triethylamine (TEA)[170]. Therefore, DBU and DBN are slightly stronger bases than triethylamine. A plausible explanation is the resonance contribution of their bicyclic amidine structures. The unshared pair of electrons at N-1 position in DBU and DBN can be delocalized over two nitrogen atoms, as represented by the corresponding resonance structures **168** and **169** (**Scheme 49**). Since the actual molecule is a hybrid of these resonance forms, the electron density of the nonbridged nitrogen atoms (i.e., N-8 in DBU and N-5 in DBN) is "increased" and the unshared pair of electrons in these positions is more available for

attack by an acid (a proton) or an electrophile. In other words, DBU and DBN can act as either base or nucleophile. It is, therefore, of great interest to be able to predict when DBU and DBN will act as nucleophiles and when they will act as bases.



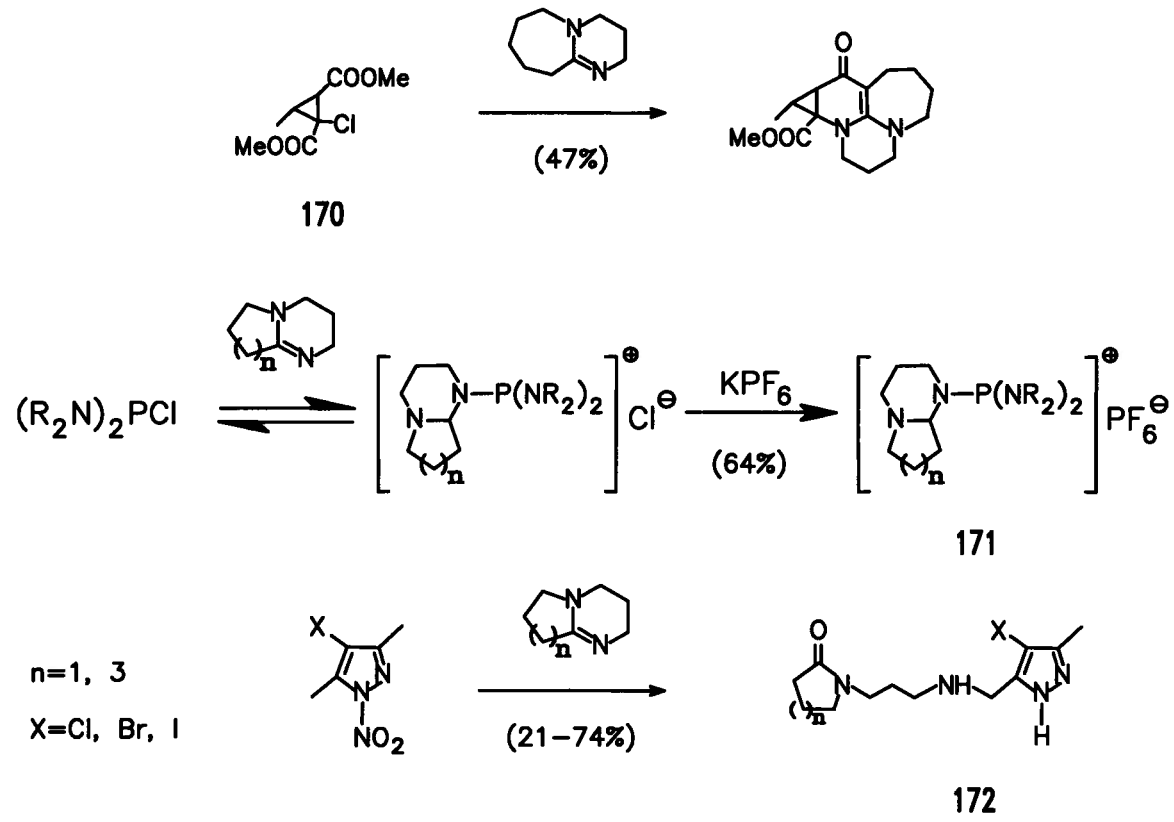
Scheme 49 Resonance Structures of DBU and DBN

Based on Lewis acid-base theory, any nucleophile is also a Lewis base. However, there is a clearly conceptual relationship between the properties called basicity and nucleophilicity. The term *basicity* is defined in terms of the position of an equilibrium reaction with a proton or some other acid, while *nucleophilicity* is generally accepted to refer to the effect of a Lewis base on the *rate* of a nucleophilic substitution reaction. Therefore, *basicity* is the so-called *thermodynamic basicity* and *nucleophilicity* is the so-called *kinetic basicity*. [170]

Nucleophilicity is used to describe trends in the kinetics of reactions. The competition between nucleophilicity and basicity has been observed in many organic reactions, e.g., S_N1 Substitution Reaction *versus* E_1 Elimination; and Nucleophilic Addition at a Carbonyl Carbon *versus* Enolate Formation. In some cases, nucleophilicity

is correlated directly with basicity, although the relative nucleophilicity as well as the relative basicity of a given species may differ from substrate to substrate. It has not been possible to devise an absolute scale of nucleophilicity or basicity. Steric influences often play a major part in nucleophilicity. For example, NH_3 (conjugate acid has $pK_a = 9.2$)[170] is a much weaker base than Et_3N, DBU and DBN, but a much stronger nucleophile because its molecular size facilitates close approach to a substrate (electrophile).

From the above discussion, DBU and DBN should also be capable of acting as nucleophiles under a certain set of circumstances, although the steric hindrance of the molecules usually blocks the approaching of an electrophile. In 1981, McCoy and Mal[171] became the first to provide chemical evidence for the nucleophilic nature of DBU. In a serendipitious discovery, they found DBU acting as a difunctional nucleophile during the reaction of DBU with methyl cyclopropene-1,2-dicarboxylate derivatives **170** (**Scheme 50**). In 1993, Bertrand *et al.*[172] found that DBU and DBN showed remarkable nucleophilicity in their reactions with halogenated phosphanes, a reaction which provided ion-pair products **171** (**Scheme 50**). In 1994, Lammers and his coworkers[173] also observed another reaction in which DBU and DBN acted as nucleophiles when reacted with 4-halo-3,5-dimethyl-1-nitro-1H-pyrazoles to form lactam-type products **172** (**Scheme 50**). In the products **172**, one of the bicyclic rings in DBU and DBN was opened by water.



Scheme 50 Examples of DBU and DBN Acting as Nucleophiles

Therefore, DBU and DBN can act as nucleophiles (via the N-8 atom in DBU and the N-5 atom in DBN) to form a nitrogen-phosphorus bond, a nitrogen-carbon bond and act as difunctional nucleophiles. The objective of this portion of research, which was also initiated by a serendipitious discovery from a side-reaction product in the research on "the third generation photosensitizers" (Chapter 4), was to further exploit the nucleophilic behaviour of DBU and DBN; and thus be able to bring about further understanding of the nucleophilicity as well as the basicity of these common organic bases. In the first reaction, DBU acting as a difunctional nucleophile quantitatively reacted with a strong electrophile (DMAD) to afford a fused tricyclic derivative **176** through concomitant formation of new nitrogen-carbon and carbon-carbon bonds. In the second reaction,

pheophorbide *a* methyl ester (**7**), a weak electrophile which alone does not electrophilically react with DBU or DBN, has reacted, through catalytic activation by Lewis acids, with nucleophilic DBU and DBN to form chlorin e_6 lactams **185** and **186**. The nucleophilic reaction mechanisms will also be discussed.

5.2 DBU as a Difunctional Nucleophile

In studies related to the photosensitizers (BPD series) for the photodynamic therapy of tumors (detailed discussion appears in Chapter 4), we have used DBU as a base to tautomerize the isolated double bond formed in the Diels-Alder reaction of vinyl porphyrins with dimethyl acetylenedicarboxylate (DMAD), to the fully conjugated chromophores. A yellow material is occasionally observed in the chromatographic purification of the rearranged benzoprophyrin derivatives (green). Since this material shows no electronic absorptions in visible region (all porphyrins have visible absorptions), we considered that it was not a porphyrin related by-product. When DBU was added to DMAD (1:1 ratio), in chloroform at room temperature, a golden yellow product was instantly formed with a strong exothermicity. After recrystallization (CH_2Cl_2/hexane), red crystals (mp 158°C) were obtained (96% yield) and, surprisingly, mass spectrometry (m/z 262) and microanalysis indicated this product to be an adduct of DBU and DMAD minus a molecule of methanol. The ^1H NMR spectrum is clean and distinct at 200 MHz (**Fig. 5.1**), and exhibits two singlets at δ 5.94 (1H) and δ 3.33 (3H) together with seven completely resolved methylene groups. The connectivity of these

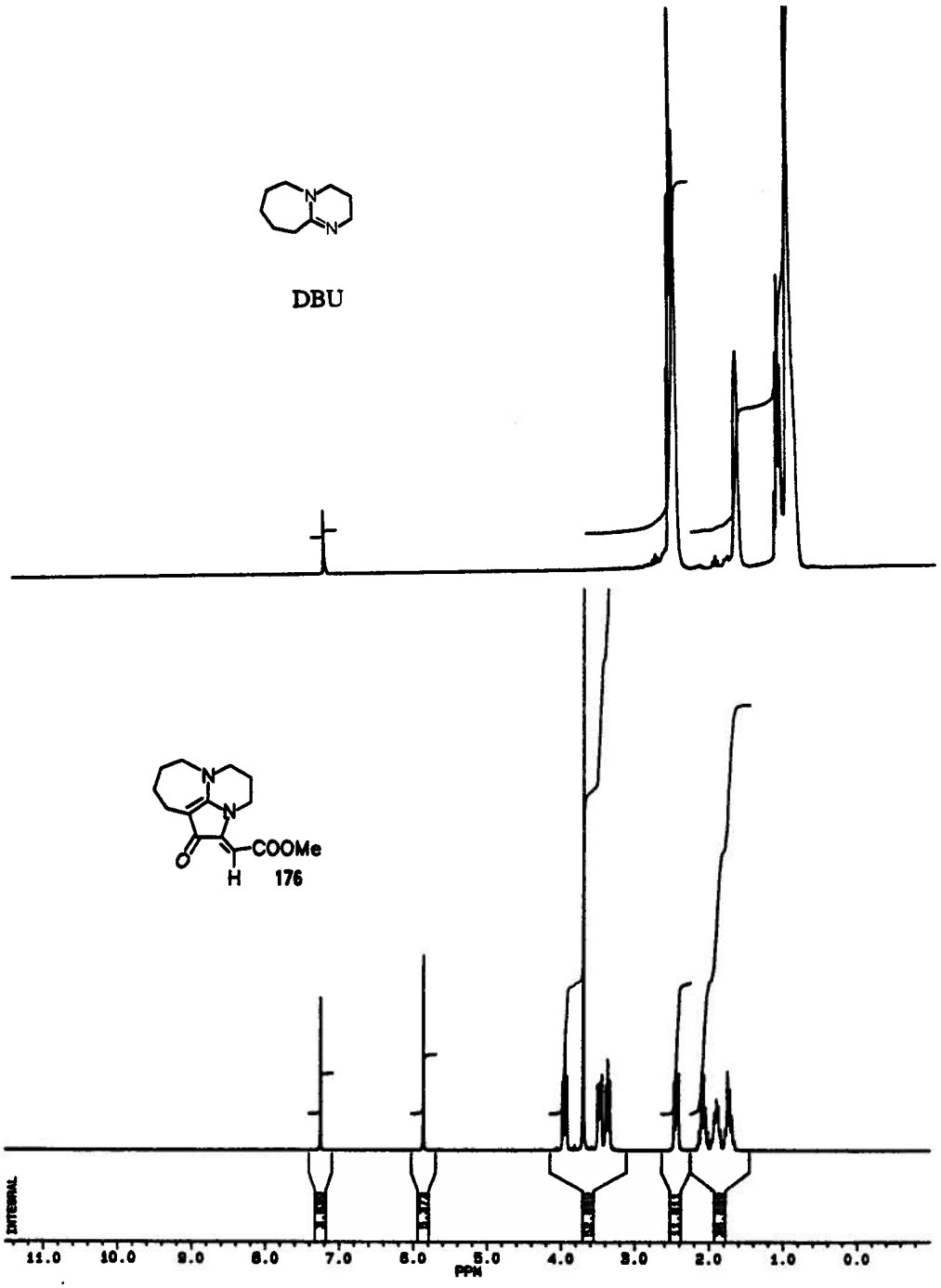
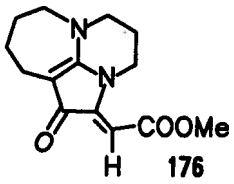


Fig. 5.1 ^{1}H NMR Spectra ($CDCl_3$) of DBU and Tricyclic Derivative **176**



176

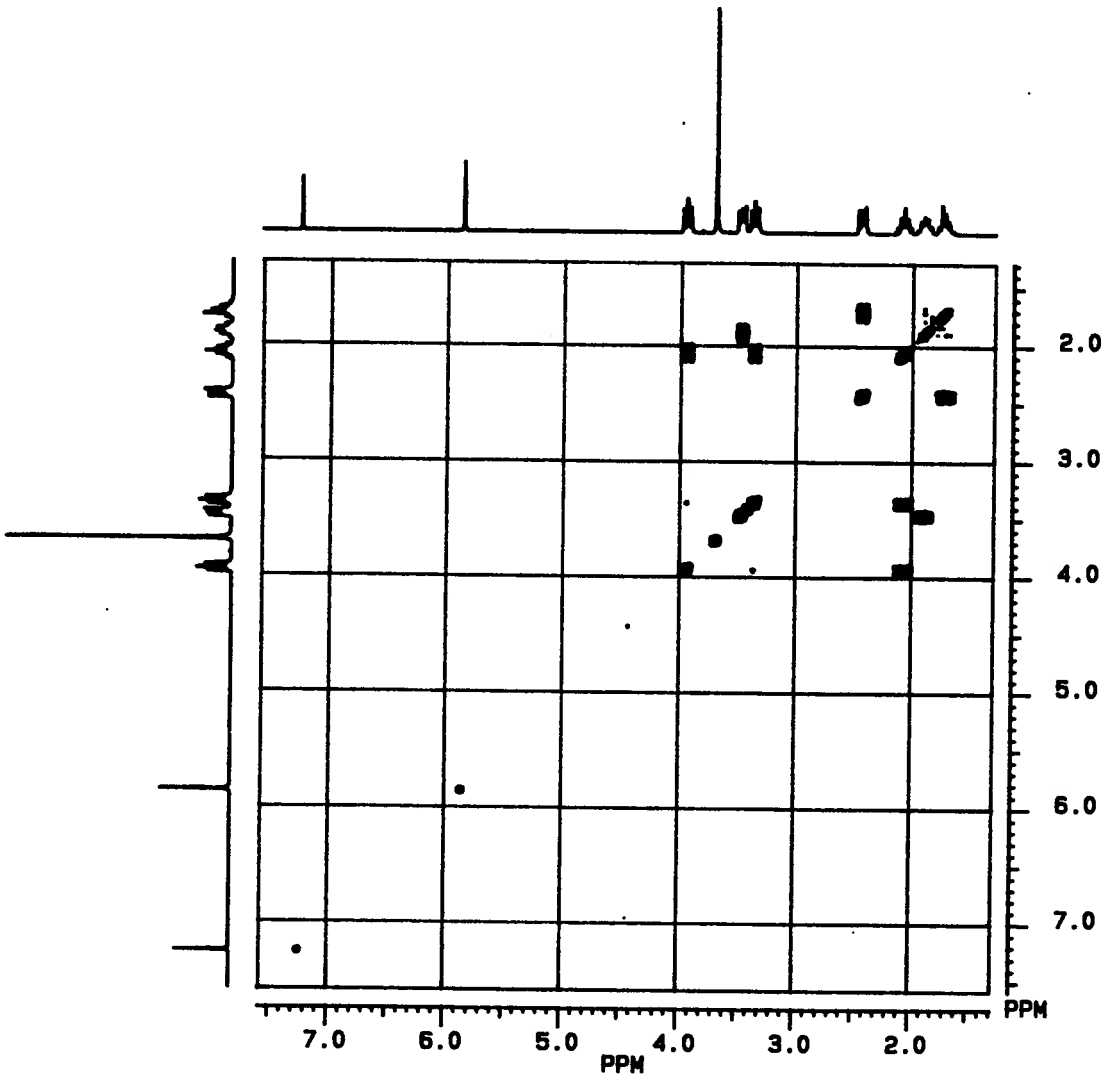


Fig. 5.2 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Tricyclic Derivative **176**

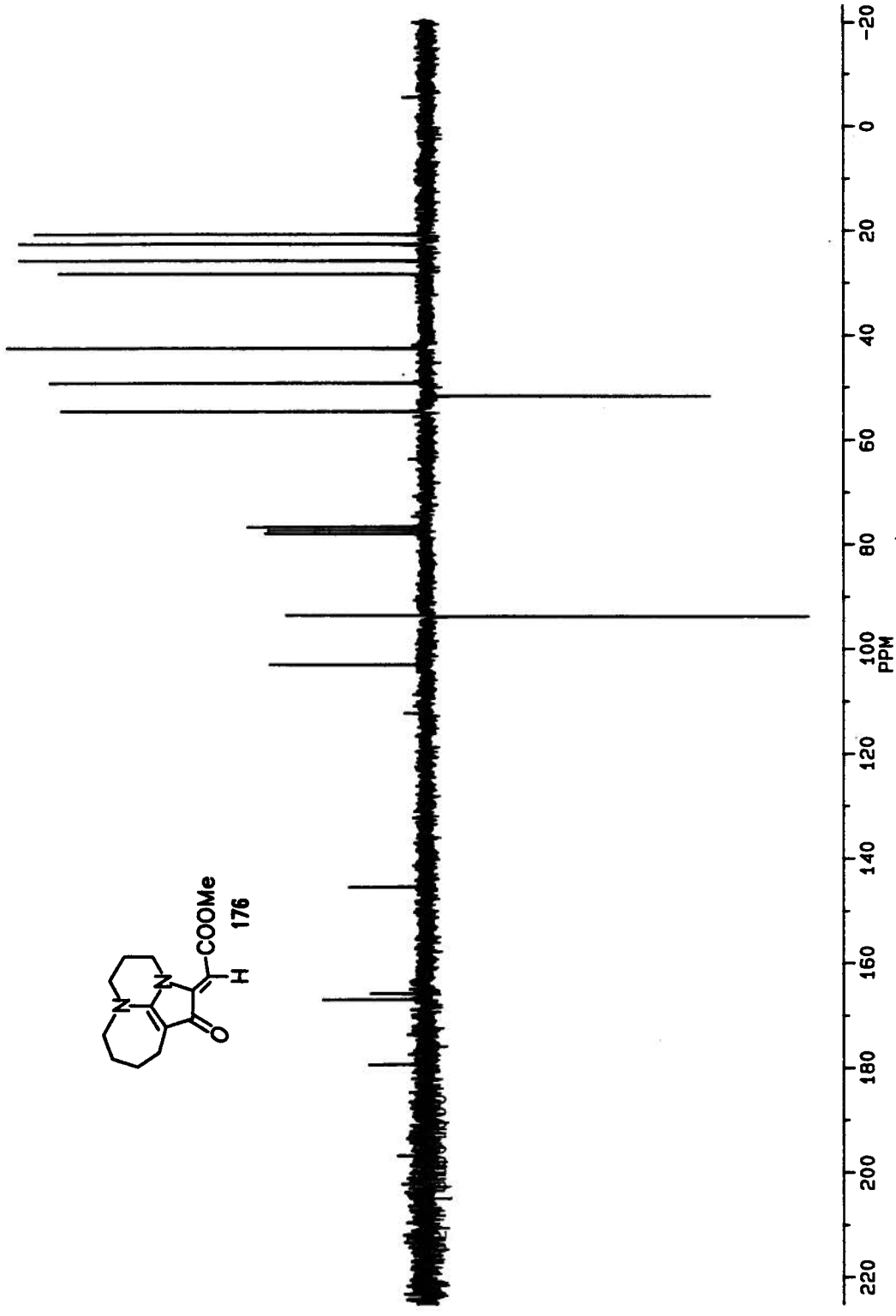


Fig.5.3 ^{13}C NMR and APT Spectra ($CDCl_3$) of Tricyclic Derivative **176**

methylene signals was correlated by a ^{1}H-^{1}H COSY spectrum (**Fig. 5.2**) as two isolated spin systems with three and four methylene groups, respectively. In combination with its ^{13}C NMR (**Fig. 5.3**) and attached proton experiments (APT, **Fig. 5.3**), all fourteen carbons and eighteen hydrogens were accounted for and structures **176** and **178** were proposed. Since the spectral analysis was unable to discriminate between these two structures, a single crystal suitable for X-ray analysis was prepared and we have now shown this product to have the unusual structure **176**, as shown in **Fig. 5.4**.

Formation of **176** was initiated by Michael addition of DBU with DMAD via the N-8 nitrogen atom (**Scheme 51**), affording the quanternary ammonium salt[174] **173**. Abstraction of the H-6 proton in **173** leads to an intermediate **174** which could react with

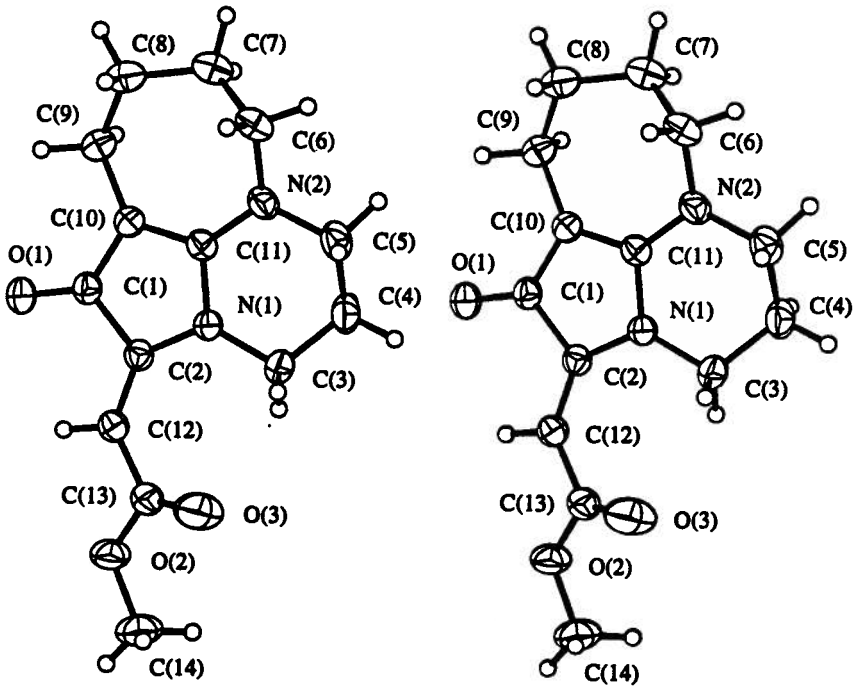
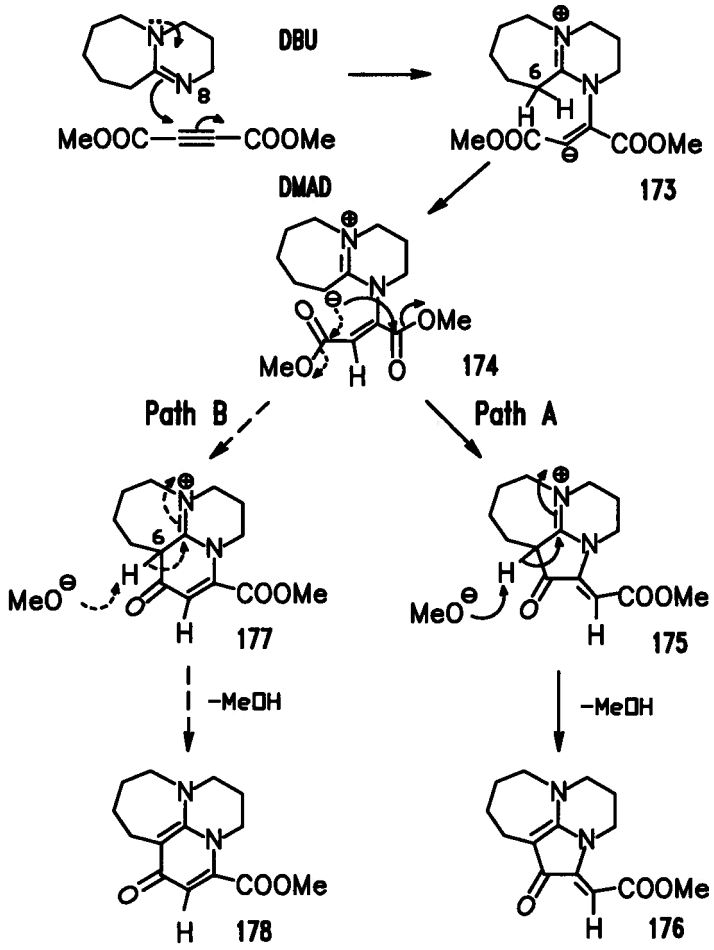


Fig. 5.4. X-Ray Structure of **176**. Five-membered ring is planar, six-membered ring has an envelope-like conformation, and seven-membered ring adopts a distorted chair conformation. Important bond lengths(Å): C(2)-C(12) 1.340(2), C(10)-C(11) 1.389(2).

either ester functionality to give either a five (Path A) or a six (Path B) membered ring (**175** or **177**). Loss of the H-6 proton from the cycloadduct (**175** or **177**) generates the incipient double bond conjugated with the carbonyl to produce the neutral tricyclic product **176** or **178**. Although the formation of a six-membered ring (Path B) seems to be thermodynamically more favourable, the reaction is highly selective and produces only isomer **176**, suggesting that this reaction is similar to typical Michael reactions and is under kinetic control.



Scheme 51 Michael-type Addition of DBU to DMAD

We have also found that DBU reacts with other activated triple bonds, such as, methyl propiolate and methyl cyanoformate, but, so far, no products have been characterized from these complex reaction mixtures. The reaction of DBN with DMAD is also strongly exothermal and gives an, as yet, intractable unidentified red mixture. The conformational rigidity of the five membered ring undoubtedly hinders a transformation similar to that of **174** to **175**.

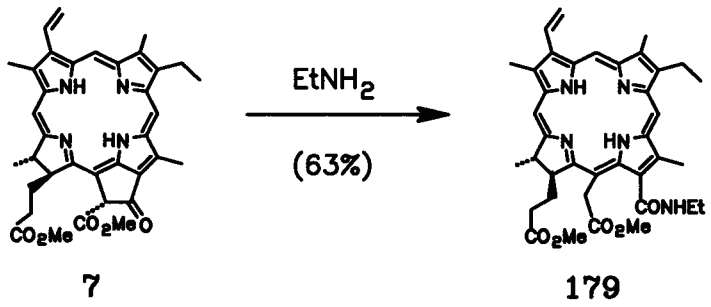
5.3 Nucleophilic Reaction with Pheophorbide *a* Methyl Ester

5.3.1 Rationale

At this point, the remarkable Michael-type addition of DBU to dimethyl acetylenedicarboxylate (DMAD) had been discovered, where DBU acts as a difunctional nucleophile to form the unusual tricyclic derivative **176**. Unfortunately, the nucleophilic behaviour of DBN had been brought into question by the unidentified products from the reaction of DBN and DMAD. It was envisioned that designing a reaction, in which both DBU and DBN should act as nucleophiles to form identifiable products, would provide a better example to understand the nucleophilic behaviour of these bicyclic amidines. Continuing our research into the chemical modifications of chlorophyll *a*, it is desirable that this reaction should make use of a chlorophyll derivative as the electrophilic substrate.

Previous studies led us to conclude that the five-membered exocyclic ring in chlorophyll *a* and its non-metallated derivatives is advantageous in this regard. As

described in Chapter 1, this exocyclic ring (ring V) can be nucleophilically cleaved by primary and secondary amines. For example, reaction of pheophorbide *a* methyl ester (**7**) with an excess amount of ethylamine at room temperature for 25 hours gave chlorin e_6 13-(2-*N*-ethyl)amide-15,17-dimethyl ester (**179**) in 63% yield (**Scheme 52**). The reaction mechanism was not clear at that stage. It was reasoned that DBU and DBN are stronger bases than $EtNH_2$ but are poorer nucleophiles for steric reasons. As was described in Chapter 2, DBU is effective at promoting the asymmetric hydroxylation of pheophorbide *a* methyl ester (**7**) and its derivatives at position 13^2 by protonating the ß-ketoester in the exocyclic ring V without cleavage of the exocyclic ring. Therefore, DBU and DBN themselves can not act as nucleophiles to react with the ß-ketoester in the exocyclic ring V because of the poor reactivity activity of DBU and the substrate.



Scheme 52 Nucleophilic Reaction of $EtNH_2$ with Pheophorbide *a* Methyl Ester (**7**)

The facility with which an electrophile-nucleophile reaction takes place depends of course on the strengths of the electrophile and the nucleophile. Low nucleophilic activity of DBU and DBN presents the reaction with the weak electrophilic substrate pheophorbide *a* methyl ester (**7**). Therefore, increasing either the nucleophilic strength of DBU and DBN or the electrophilic strength of pheophorbide *a* methyl ester (**7**) was

required for bringing about the generation of the reaction. Since we were trying to demonstrate the nucleophilicity of DBU and DBN, improving the electrophilic activity of pheophorbide *a* methyl ester (**7**) was attempted.

An electrophile with a positive charge is always a more powerful electrophile than its neutral counterpart (assuming the latter is also an electrophile). Therefore, the easiest method to increase the electrophilic activity of a given substrate is by increasing its "acid strength". However, common protic acids such as H_2SO_4, CH_3COOH or $ArSO_3H$ are totally ineffective in this special case because these acids protonate the substrate and subsequent addition of DBU or DBN will deprotonate the substrate back to its original state, i.e., the procedure virtually becomes an acid-base reaction, without any effects on the desired reaction. After a number of experiments, strong Lewis acids, the trialkylsilyl triflates, were found to be the best at promoting the nucleophilic reaction of DBU and DBN with pheophorbide *a* methyl ester (**7**). The following sections will present this reaction, the characterization of the reaction products and the mechanistic rationalization.

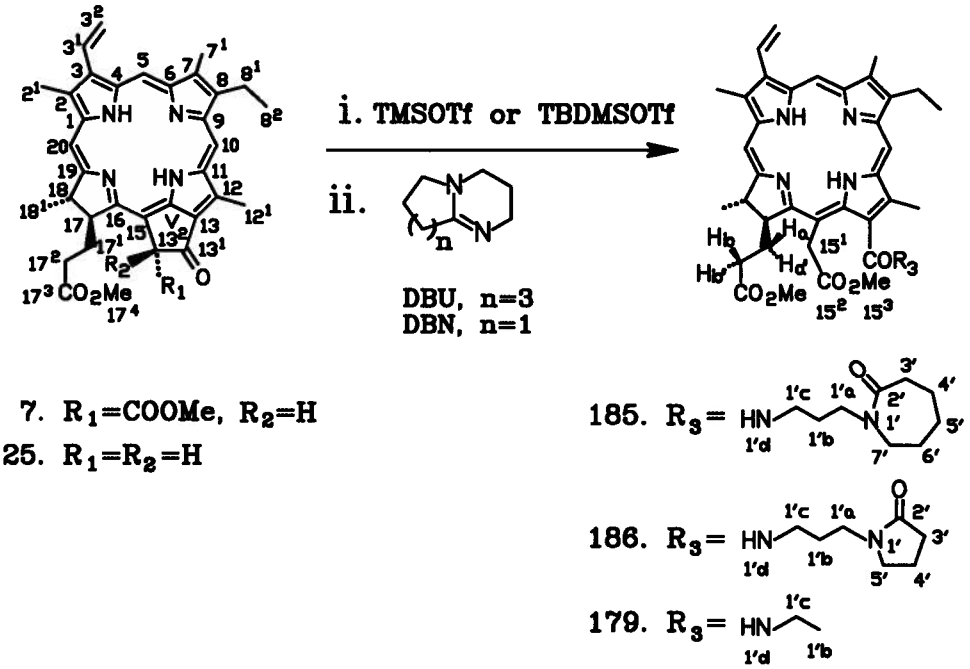
5.3.2 The Generation and Fate of Nucleophilic DBU and DBN

Trimethylsilyl triflate (TMSOTf) and *tert*-butyldimethylsilyl triflate (TBDMSOTf), super-reagents bearing a highly electron-withdrawing triflate moiety, can activate various oxygen-containing organic compounds through a one-center (not multi-center) interaction at the electron-deficient silicon atom and, in some cases, generate reactive ion-pair intermediates even in aprotic solvents.[175] Many reports have shown that they can act as catalysts to accelerate a variety of nucleophilic reactions in aprotic

media.[176] For example, reactions of enol silyl ethers with acetals or related compounds have been reported to be catalyzed efficiently by trialkylsilyl triflates, TMSOTf and TBDMSOTf, leading to the aldol-type products in a directed manner.[177] They are also powerful silylating agents and a wide range of active hydrogen containing compounds are silylated in the presence of amines (including DBU and DBN).[178] Their combination with hindered amidine DBU has also been used in the selective ring-opening reactions of oxiranes.[179]

Instead of simple protonation observed in the protic acids such as H_2SO_4, CH_3COOH or $ArSO_3H$, trialkylsilyl triflates catalyze a substrate by converting it to a relatively stable ion in which the positive charge on the carbon is greatly increased, thus making it more susceptible to nucleophilic attack.[180] This was found to be the case in the reaction of pheophorbide *a* methyl ester (7) with DBU and DBN. Upon activation for ~10 min by trialkysilyl triflates, trimethylsilyl triflate or *tert*-butyldimethylsilyl triflate, pheophorbide *a* methyl ester (**7**) behaved as a powerful electrophile and reacted with DBU giving a green product **185** (>50% yield). The product **185** was characterized surprisingly as the chlorin e_6 13-[1-(3-*N*-propyl)-2-azacycloheptane]amide-15,17-dimethyl ester, where the exocyclic ring V of pheophorbide *a* methyl ester and one of the bicyclic rings in DBU were opened. An analogue {found to be chlorin e_6 13-[1-(3-*N*-propyl)-2-pyrrolidinone]amide-15,17-dimethyl ester, **186**} was also obtained on the reaction of compound **7** with DBN. Their exhaustive characterization is fully described in the following section.

The reaction can run in either dry THF or dry DMF. In THF, DBU and DBN reacted with pheophorbide *a* methyl ester (**7**) giving yields greater than 50% along with ~20% starting material **7** (no side reaction was observed). In DMF the reactions proceeded faster, reaching completion in ~3 h, but only ~45% yields were obtained along with ~20% side- (degradative) products. The reactions promoted by TMSOTf and TBDMSOTf were both efficient, and no difference between them was observed.



Scheme 53 Nucleophilic Reaction of DBU and DBN with Methyl Pheophorbide *a* (**7**)

Therefore, the "planned nucleophilic reaction" of DBU and DBN with pheophorbide *a* methyl ester (**7**) has been successfully achieved through catalytic promotion by trialkylsilyl triflates, TMSOTf and TBDMSOTf. This present work has demonstrated that DBU and DBN are not only strong bases that can deprotonate the β-ketoester of the exocyclic ring V, but also can act as nucleophiles to cleave the ring V

under certain conditions. These observations could help explain the previously observed and anomalous products in the enolate-trapping reaction of chlorophyll *a* and related compounds.[181,182]

5.3.3 Structural Elaboration of the Products

Unambiguous structure assignments of **185** and **186** were carried out using mass spectroscopy, ^{1}H NMR, proton decoupling, ^{13}C NMR, attached proton test (APT), ^{1}H homonuclear correlation spectra (COSY) and ^{1}H-^{13}C heteronuclear correlation spectra (HETCOR). Furthermore, the structure of **185** was confirmed by nuclear Overhauser effect (nOe) while the structure of **186** was confirmed by its direct preparation through reaction of commercially available 1-(3-aminopropyl)-2-pyrrolidinone with pheophorbide *a* methyl ester (**7**). The assignments are described as follows.

HRMS and elemental analysis showed **185** to be an adduct of DBU and methyl pheophorbide *a* methyl ester (**7**) plus the elements of a water molecule (formula: $C_{45}H_{56}N_6O_6$). ^{13}C NMR showed that **185** consists of 45 carbons (36 carbons in **7**) and an attached proton test (APT) confirmed that eight of the additional nine carbon atoms are methylene carbons in the high field and only one sp^2 carbon at low field. The ^{13}C signals of the eight additional methylene carbons are matched with related chemical shifts in DBU. In its ^{1}H-^{1}H homonuclear correlation spectrum (COSY) (**Fig. 5.5**), chlorin **185**, like the starting material **7**, only has three downfield methine peaks (1H each) suggesting that one of the compound's four bridging carbons (i.e. C-15) is still functionalized. Five sharp singlets (3H each) between 3.0 and 4.0 ppm suggest three aryl-substituted methyl

groups as well as two methoxyl groups, which showed these groups were unchanged after the reaction. A doublet of quartets at 4.46 ppm (1H) was coupled to one proton (ddd) at 4.39 ppm (1H), suggesting the former (4.46 ppm) is the H-18 and the latter is H-17. H-17 is also coupled to another two multiplet hydrogens at 2.23 and 1.83 ppm, and these two protons were found to be coupled with the corresponding carbon at 29.66 ppm (see **Table 5.2** for ^{13}C NMR data) as shown by a HETCOR spectrum (**Fig. 5.6**). This methylene is assigned to 17^1-CH_2 and the two hydrogens are assigned as Ha-17^1 (2.23 ppm) and Ha'-17^1 (1.83 ppm). Consequently, Hb-17^2 (2.52 ppm) and Hb'-17^2 (2.12 ppm) were identified based on their coupling to Ha-17^1 and Ha'-17^1. There are two other obvious coupling pairs at low field. (i). A triplet (1H, J = 6.0 Hz) at 7.55 ppm was exchangeable with CD_3OD and coupled to the multiplets at 3.68 and 3.64 ppm (1H each), suggesting a possible $CONHCH_2$ unit; (ii) an asymmetrically substituted methylene was suggested by a split AB quartet at 5.54 and 5.27 ppm (1H each) with coupling constant J = 19.1 Hz, whose corresponding carbon is at 38.02 ppm. Assignment of this methylene unit as C-15^1 was based on the nOe enhancement (**Scheme 54**) of the H-17 (4.39 ppm). These assignments confirmed that the exocyclic ring V was the only part of the molecule to have reacted and also suggested that **185** has a chlorin-e_6-type structure. Since it contains a $CONHCH_2$ moiety, it was believed to be a chlorin e_6 amide derivative. This conclusion is firmly supported by its absorption spectrum, a typical chlorin-e_6-type spectrum with the Soret band at 404 nm (ε 171 900) and Q band at 666 nm (48 700).[34]

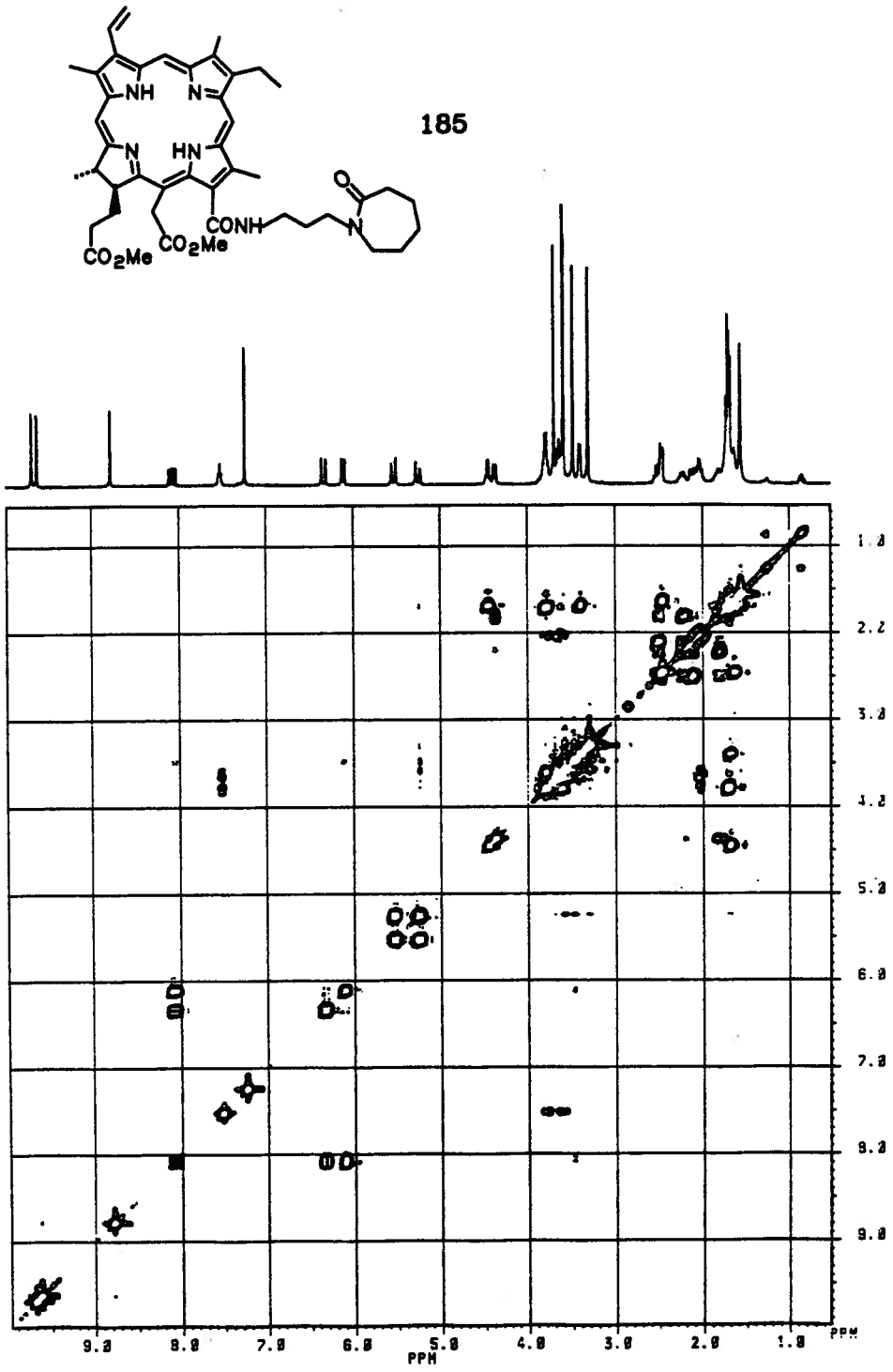


Fig. 5.5 ^{1}H-^{1}H COSY Spectrum ($CDCl_3$) of Chlorin e_6 Amide **185** (between δ_H 0-10 ppm for Proton Resonances)

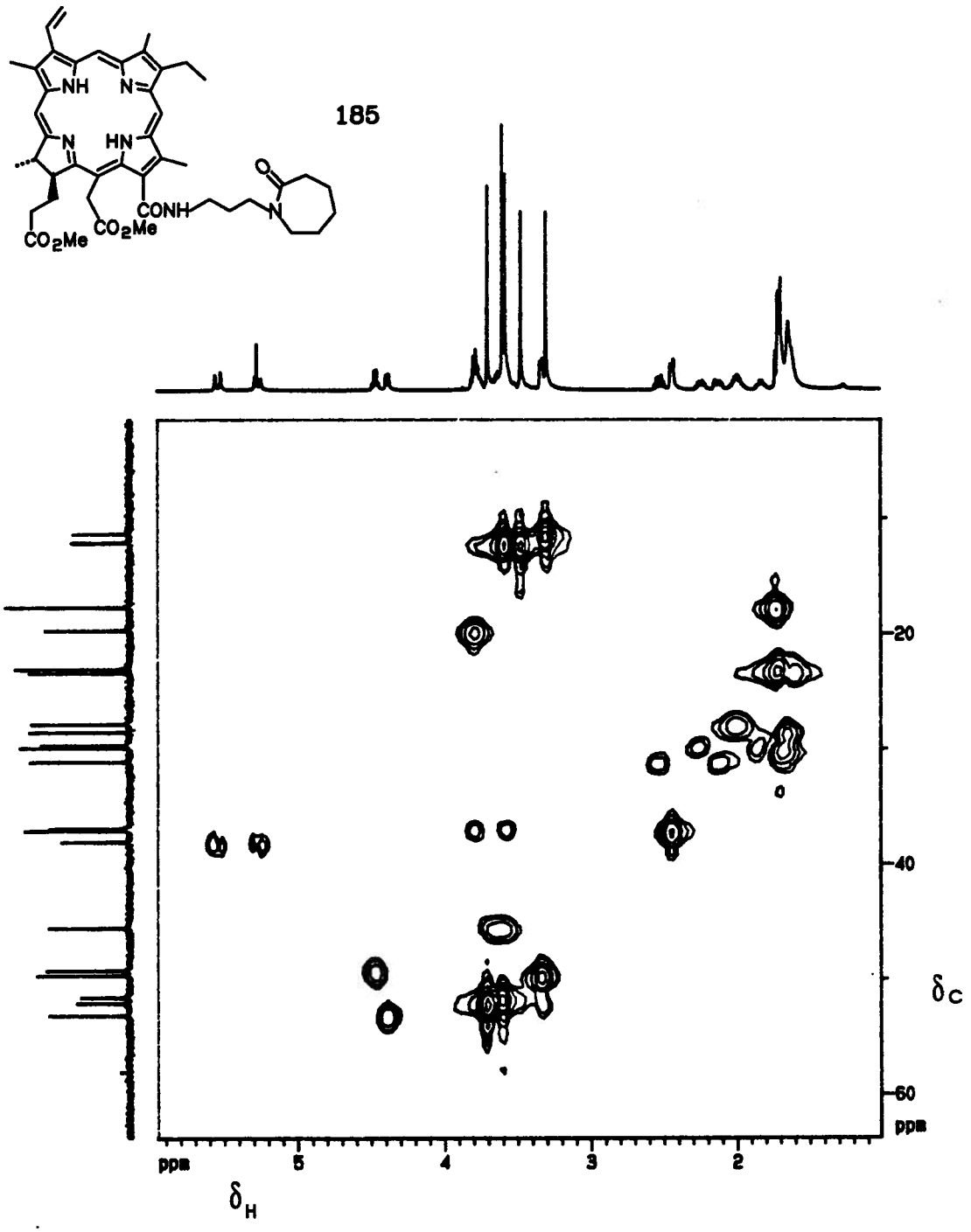


Fig. 5.6. ^{1}H-^{13}C Heteronuclear Correlation Spectrum of Chlorin e_6 Amide **185** (12.0 mg in 1.0 mL $CDCl_3$) between δ_H 0.0-6.0 ppm for proton resonances, and between δ_C 0-60 ppm for the carbon resonances

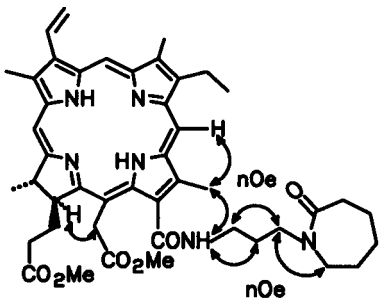
Table 5.1. ^{1}H NMR Spectral Data ($CDCl_3$, 400 MHz)

Proton	Compound			
	185[a]	**186**[b]	**179**[b]	**7**[a]
H-10	9.69(s)	9.70(s)	9.70(s)	9.52(s)
H-5	9.62(s)	9.63(s)	9.62(s)	9.39(s)
H-20	8.89(s)	8.80(s)	8.80(s)	8.57(s)
H-3^1	8.07(dd)	8.08(dd)	8.05(s)	8.01(s)
H-3^2(E)	6.32(dd)	6.34(dd)	6.38(dd)	6.30(dd)
H-3^2(Z)	6.10(dd)	6.12(dd)	6.15(dd)	6.19(dd)
H-8^1	3.78(q)	3.79(q)	3.79(q)	3.67(q)
H-12^1	3.57(s)	3.57(s)	3.55(s)	3.68(s)
H-2^1	3.47(s)	3.48(s)	3.48(s)	3.39(s)
H-7^1	3.30(s)	3.30(s)	3.32(s)	3.21(s)
H-18^1	1.71(d)	1.68(d)	1.70(d)	1.80(d)
H-8^2	1.70(t)	1.70(t)	1.70(t)	1.68(t)
H-18	4.46(dq)	4.44(q)	4.46(dq)	4.47(q)
H-17	4.39(ddd)	4.36(dd)	4.35(dd)	4.20(dd)
Ha-17^1	2.23(m)	2.21(m)	2.15(m)	2.62(dt)
Ha'-17^1	1.83(m)	1.80(m)	1.78(m)	2.31(dt)
Hb-17^2	2.52(ddd)	2.52(ddd)	2.50(ddd)	2.50(dt)
Hb'-17^2	2.12(ddd)	2.13(ddd)	2.15(m)	2.24(t)
NH	-1.60(br s)	-1.60(br s)	-1.87(br s)	0.53(br s)
NH	-1.82(br s)	-1.82(br s)	-1.96(br s)	-1.62(br s)
H-15^1/13^2	5.54(d)	5.51(d)	5.56(d)	6.27(s)
	5.27(d)	5.23(d)	5.25(d)	
H-17^4	3.60(s)	3.58(s)	3.59(s)	3.57(s)
H-15^3/13^4	3.69(s)	3.71(s)	3.80(s)	3.88(s)
H-D-1'd	7.55(t)	7.31(t)	6.40(t)	
H-D-1'c	3.68(m)	3.81(m)	3.80(q)	
	3.64(m)	3.60(m)		
H-D-1'b	2.08(m)	2.08(m)	1.41(t)	
H-D-1'a	3.64(m)	3.51(m)		
H-D-3'	2.48(dd)	2.37(t)		
H-D-4'	1.64(m)	2.08(m)		
H-D-5'	1.66(m)	3.50dd)		
H-D-6'	1.70(m)			
H-D-7'	3.41(dd)			

[a]Concentration 1.5 mg/0.6 mL [b]Concentration 1.0 mg/0.6 mL

Reaction of ethylamine with pheophorbide *a* methyl ester (**7**) gave chlorin e_6 ethylamide **179** (**Scheme 52**), an analog of **185**. Compound **179** exhibited a relatively simple and easily-assigned NMR spectrum. Comparing the spectral data of **185** and **179**, the assignment of **185** as a chlorin e_6 amide with 13-substitution was confirmed.

For the 13 side-chain protons in **185**, the methylene (δ 3.68 ppm, 3.64 ppm) of the $CONHCH_2$ unit was assigned as H-D-1'c and the corresponding carbon (C-D-1'c) is at 36.87 ppm. A multiplet at 2.04 ppm (2H) was believed to be H-D-1'b based on their coupling with H-D-1'c. Two protons at 3.64 ppm (m) were assigned as H-D-1'a since they are coupled with H-D-1'b (2.04 ppm). A doublet of doublet (2H) at 3.41 ppm, coupled with 2 protons at 1.70 ppm, were assigned as H-D-7' (NCH_2) because its corresponding carbon is at 49.64 ppm and the three other carbons are all in the higher field. With the same technique, the remaining three methylenes, whose proton chemical shifts overlap with H-18[1] and H-8[2] at 1.64-1.70 ppm, were assigned as H-D-4', H-D-5' and H-D-6' based on the relative cross couplings.



185

Scheme 54 Observed nOe Enhancement for Chlorin e_6 Amide **185**

Further details of the NMR assignments are given in **Table 5.1** and **Table 5.2**. In addition, nOe experiments (**Scheme 54**) have confirmed the above assignments. Compound **185** is thus chlorin e_6 13-[1-(3-*N*-propyl)-2-azacycloheptane]amide-15,17-dimethyl ester.

A similar analysis of spectral data has shown that product **186** is chlorin e_6 13-[1-(3-*N*-propyl)-2-pyrrolidinone]amide-15,17-dimethyl ester. This was further confirmed by comparison with the product from direct nucleophilic reaction of commercially-available 1-(3-aminopropyl)2-pyrrolidinone with methyl pheophorbide *a* (**7**). The products prepared by these two methods were identical.

Table 5.2 ^{13}C NMR Spectral Data ($CDCl_3$, 125 MHz)

	Compound					Compound			
Carbon	**185**[a]	**186**[b]	**179**[c]	**7**[d]	Carbon	**185**[a]	**186**[b]	**179**[c]	**7**[d]
C-17^3	173.49	173.57	173.54	173.36	C-15^1/13^2	38.02	38.01	37.80	64.71
C-13^1	173.55	173.57	174.18	189.63	C-17	53.12	53.11	53.08	52.88
C-15^2/13^3	169.25	169.36	169.39	169.60	C-18	49.20	49.23	49.23	51.70
C-19	168.53	168.64	168.73	172.14	C-17^2	31.09	31.10	31.12	31.06
C-16	166.65	166.67	166.62	161.16	C-17^1	29.66	29.66	29.65	29.86
C-6	153.93	154.04	154.16	155.50	C-18^1	23.03	23.03	23.05	23.10
C-9	149.07	149.08	149.08	150.85	C-8^1	19.67	19.68	19.70	19.29
C-14	144.62	144.68	144.74	149.61	C-8^2	17.68	17.69	17.75	17.36
C-8	138.65	138.76	138.83	145.05	C-12^1	12.11	12.15	12.20	12.09
C-1	135.96	136.76	136.11	141.99	C-2^1	12.05	12.08	11.92	12.06
C-11	135.32	135.23	134.95	137.85	C-7^1	11.30	11.31	11.37	11.08
C-3	134.70	134.76	134.87	136.39	C-17^4	51.54	51.55	51.63	50.10
C-4	134.58	134.69	134.77	136.12	C-15^3/13^4	52.06	52.08	52.13	51.10
C-7	134.34	134.43	134.02	136.02	C-D-1'c	36.87	37.17	35.52	
C-2	130.07	130.00	130.13	131.76	C-D-1'b	27.82	17.93	14.79	
C-12	129.89	130.00	130.13	128.87	C-D-1'a	45.50	40.00		
C-3^1	129.54	129.53	129.49	128.94	C-D-2'	176.64	175.66		
C-13	128.76	128.48	128.34	128.94	C-D-3'	37.05	30.80		
C-3^2	121.39	121.48	121.63	122.66	C-D-4'	23.36	27.16		
C-15	102.42	102.32	102.12	105.13	C-D-5'	28.52	47.32		
C-10	101.34	101.39	101.36	104.28	C-D-6'	29.90			
C-5	98.78	98.81	98.84	97.39	C-D-7'	49.64			
C-20	93.53	93.57	93.66	93.07					

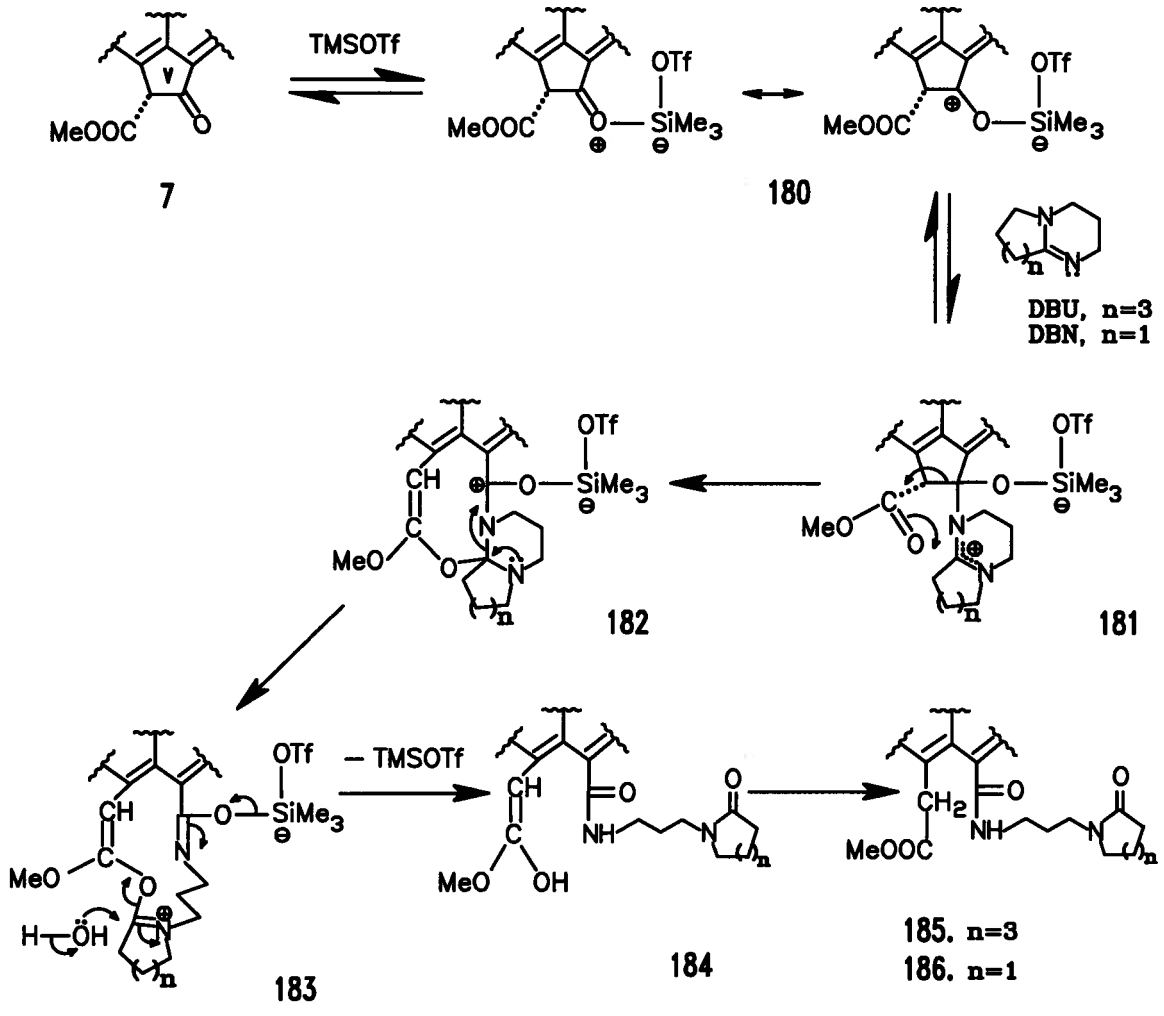
[a]Concentration 12.0 mg /1.0mL [b]Concentration 9.0 mg /1.0 mL
[c]Concentration 18.0 mg /1.0mL [d]Concentration 25.0 mg /1.0mL

5.3.4 Reaction Mechanism

This reaction requires the β-ketoester system of pheophorbide *a* methyl ester (**7**). Reaction with pyropheophorbide *a* methyl ester (**25**), a decarboxylated product of pheophorbide *a* methyl ester (**7**), failed. Because of the similarity of methyl 2-oxocyclopentanecarboxylate to the ring V in pheophorbide *a* methyl ester (**7**), we also investigated its reaction with the amidine bases but found that ring opening did not occur. The initial reaction between methyl 2-oxocyclopentanecarboxylate and DBU (DBN) resulted in the formation of a trace amount of 1:1 "adduct", which could be only identified by mass spectra and chromatographical analysis.

Since direct mixing of TMSOTf with DBU resulted in no new products and the enolate from deprotonation of pheophorbide *a* methyl ester (**7**) by DBU (or DBN) is relative stable in the absence of oxygen in the dark, the nucleophilic behaviour of DBU and DBN must be initiated from the activation of compound **7** by Lewis acids (TMSOTf and TBDMSOTf). In **Scheme 55** a mechanism is proposed for the formation of **185** and **186**. The first step is coordination of the 13^1 carbonyl group of **7** by TMSOTf or TBDMSOTf to generate a reactive ion-pair intermediate **180**. This kind of ion-pair intermediate is common and found to form easily in the TMSOTf (or TBDMSOTf) catalyzed aldol-type reaction of silyl enol ethers and acetals.[177] The pentacoordinate silicon species[183] are so electron-deficient that they can react with the nonbonding electrons in DBU and/or DBN nitrogen to give **181**. The further cleavage of bond (C-13^1 –C-13^2) of ring V, step **181** to **182**, drives the reaction towards completion. Indeed, the formation of **179** from the nucleophilic reaction of ethylamine with **7** is also through this

type of bond cleavage. The intramolecular rearrangement to generate **183** is facilitated thermodynamically by the formation of the amide (with higher bond energy). For 2-oxocyclopentanecarboxylate, it can also form an activated ion-pair of this kind, but due to the lack of delocalization which should stabilize the charges, no ring cleavage occurred. The intramolecular rearrangement step arises from the generation of **183**, no doubt facilitated by both the adjacent nonbonding nitrogen electrons and by the breaking of the



Scheme 55 Proposed Mechanism for the Formation of Chlorin e_6 amides **185** and **186**

C-N bond to relieve ring strain. Similar rearrangements have been extensively studied through the hydrolysis of bicyclic imidates by Deslongchamps *et al.*.[184] Compound **183** is trapped by water in the NH_4Cl workup to release TMSOTf (or TBDMSOTf) to give the more stable amide **184**. Consequently, **184** tautomerizes to afford products **185** and **186**.

5.4 Summary

In the first part of this work, the remarkable Michael-type addition of DBU (as a difunctional nucleophile) to dimethyl acetylenedicarboxylate was discovered. The unusual tricyclic structure of the reaction product **176** (kinetically-controlled product) has been confirmed by X-ray diffraction. A possible mechanism was proposed.

In the second part of this work, unusual nucleophilic reactions of DBU and DBN with pheophorbide *a* methyl ester (**7**) were successfully achieved. The catalytic promotion of these reactions by Lewis acids, the trialkylsilyl triflates, as well as the reaction mechanism was discussed.

In conclusion, the present work demonstrates the nucleophilic behaviour of the bicyclic amidines DBU and DBN and serves to remind researchers of possible nucelophilicity when they choose DBU and/or DBN as a base.

Chapter 6

Experimental

6.1 General Methods

This general section covers the techniques and instruments used for the analysis and the purification of the products.

Melting Point Determinations

Melting points were performed on a 6548-J17 microscope equipped with a Thomas model 40 hot stage melting apparatus; the values are uncorrected.

Nuclear Magnetic Resonance Spectroscopy

Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained from samples in deuteriochlorform ($CDCl_3$) on a Bruker AC-200 (200 MHz), a Varian XL-300 (300 MHz), a Bruker WH-400 (400 MHz) or a Bruker AMX-500 (500 MHz) spectrometer. The chemical shifts are expressed in parts per million (ppm) on the δ scale with residual chloroform (δ = 7.24 ppm) as internal standard. Signal multilicities, coupling constants, integration ratios and assignments appear in parentheses. Selective decouplings were performed on the same instruments.

Carbon-13 NMR (^{13}C NMR) spectra were obtained in $CDCl_3$ with a Varian XL-300 (75 MHz) or a Bruker AMX-500 (125 MHz) spectrometer. The chemical shifts are reported on the δ scale with residual chloroform (δ = 77.0 ppm) as internal standard. Signal assignments appear in parentheses.

Attached proton test (APT) spectra were obtained in $CDCl_3$ with a Bruker AC-200 (50 MHz) or a Varian XL-300 (75 MHz) spectrometer. The chemical shifts are reported on the δ scale with residual chloroform (δ = 77.0 ppm) as internal standard.

Two dimensional proton homonuclear correlation spectra (^1H-^1H COSY) were performed in deuteriochlorform ($CDCl_3$) on a Bruker WH-400 (400 MHz) spectrometer to help assign the signals.

Two dimensional proton heteronuclear correlation spectra (HETCOR) were performed in $CDCl_3$ on a Bruker AMX-500 (500 MHz) spectrometer to help assign the signals.

Nuclear Overhauser effect difference (nOe) spectra were performed in deuteriochlorform ($CDCl_3$) on a Bruker WH-400 (400 MHz) spectrometer.

Exchange test experiments of active hydrogens (OH and NH) in hydroxychlorins and chlorin e_6 amides were performed in a $CDCl_3$ + 10%CD_3OD on a Bruker WH-400 (400 MHz) spectrometer to help assign the OH and NH signals by comparison with the spectra obtained in $CDCl_3$.

Elemental Analysis

Microanalyses were carried out in the microanalytical laboratory at the University of British Columbia by Mr. Peter Borda using a Carlo Erba Elemental Analyzer 1106.

Mass Spectroscopy

Low and high resolution fast atom bombardment (FAB) mass spectra were measured on a Varian Mat CH 4-B spectrometer and 1-thioglycerol or 3-

nitrobenzylalcohol was used as a matrix. Low and high resolution electron impact (EI) mass spectra were recorded on a Kratos/AEI MS-902 spectrometer.

Electronic Spectroscopy

Electronic spectra were measured in chloroform or dichloromethane or methanol using a Hewlett Packard Model 8452A Diode Array spectrophotometer.

Chromatography

Flash column chromatography was performed on silica gel 60 (70-230 mesh, supplied by E. Merck Co; usually silica III, i.e deactivated with 6% water or silica V, i.e. deactivated with 15% water) or neutral alumina (usually Brockman Grade III, i.e, deactivated with 6% water or Brockman Grade V, i.e., deactivated with 15% water).

Analytical thin layer chromatography (TLC) was performed using commercially-available Merck 60 F_{254} silica gel (precoated sheets, 0.2 mm thick).

Preparative TLC was prepared on pre-coated 20 × 20 cm 0.5, 1 or 2 cm thick Whatman or Merck silica gel plates. The deactivated silica gel plates, in some cases when necessary, were prepared by blank development of the commercially available plates with 10% methanol in dichloromethane followed by air-drying before use.

Analytical high performance liquid chromatography (HPLC) was obtained using a Waters Novapak C_{18} 4μ 60 Å (3.9 mm × 15 cm) column with a flow rate of 1 mL min^{-1} and detection at 410 nm using a Waters 994 photodiode array detector. Semi-preparative HPLC separations were performed on a Waters Novapak C_{18} 10μ 125 Å (7.8 mm × 30

cm) column with a flow rate of 3 mL min^{-1} and detection at 410 nm using a Waters 994 photodiode array detector. Solvent systems used are specified where appropriate.

Extraction and Reaction Conditions

Due to the inherent light sensitivity of these compounds, in particular the isolated chlorophyll and antioxidative chlorins, all extractions, separations and reactions were performed in the dark. Reactions were monitored by TLC and spectrophotometry and were carried out under a atomsphere of nitrogen.

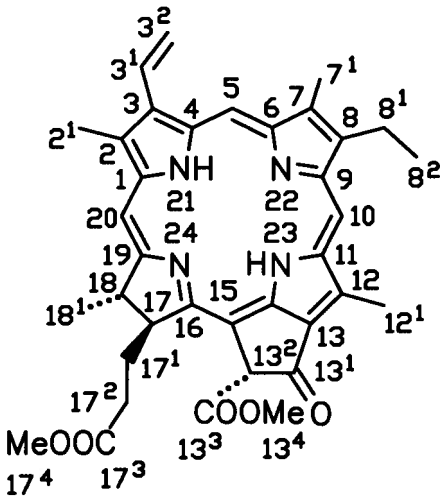
Reagents and Solvents

All chemicals and solvents were reagent grade. When necessary, the solvents were purified according to procedures given in the literature. *Spirulina maxima* alga (food quality) was purchased from Sosa Texcoco S. A., Mexico. (–)-(1R)-(10-camphorsulfonyl)oxaziridine and (+)-(1S)-(10-camphorsulfonyl)oxaziridine were purchased from Aldrich. 1-Phenyl-*N*(phenylsulfonyl)oxaziridine[111] and monomethyl malonate[158] were prepared by following the literature procedure.

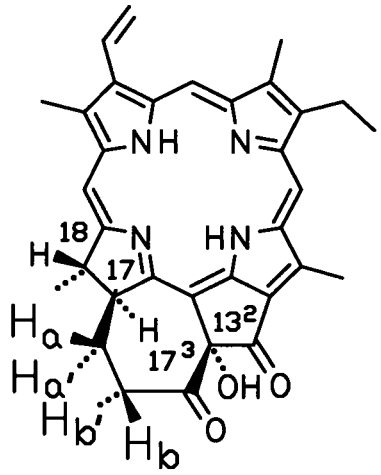
Nomenclature and Numbering System Used for the Synthesized Compounds

The trivial names with IUPAC-IUB numbering system, which is exemplified below, will be used in this work. When no corresponding trivial names are available, the compounds will be named in the IUPAC-IUB nomenclature. These compounds include the regiochemically-pure benzoporphyrin derivatives (BPDs) and the dibenzoporphyrin

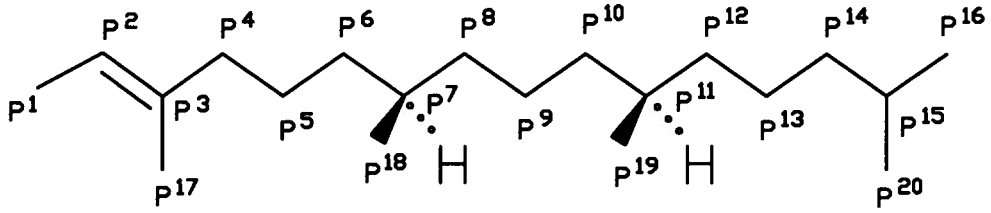
derivatives. "S" or "R" (labelled after a compound number) denotes the absolute configuration at C-13^2 or C-15^1 or C-13^1 position where appropriate.



Pheophorbide *a* methyl ester (**7**)



13^2S-Hydroxychlorophyllone *a* [**82**(S)]

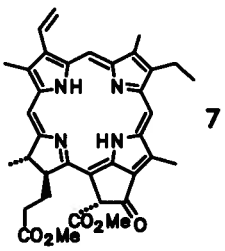


Numbering of Phytyl Group

6.2 Stereoselective Synthesis of Natural Antioxidative Chlorins

6.2.1 Starting Materials : Natural Pheophorbides

Pheophorbide *a* methyl ester (7) from *Spirulina maxima*



7

Approximately 1 kg of dried *Spirulina maxima* alga was slurried in 3 L acetone in a 5 L three-neck round-bottom flask and liquid nitrogen was added to rupture the cells. After 1 h the frozen slush was refluxed under nitrogen with continuous stirring for 2 h. The supernatant was then filtered through Whatman filter paper on a Buchner funnel and was washed with more acetone (~2 L) (even though the solid remains dark blue, the yield of pigment obtained from further extraction was low). The green filtrate was evaporated and the viscous oil so obtained was dissolved in 1.5 L petroleum ether (b.p. 35-60°C). The petroleum ether layer was successively washed with water (3 times), to remove the residual supernatant and water solubles, and 30% aqueous methanol until the aqueous phase was colorless. Petroleum ether was evaporated and the dark green residue was further dissolved in diethyl ether and treated with conc. HCl (~80 mL) for 1 min (color changed from green to black), immediately washed with water (2 × 800 mL) and 20% aqueous methanol (3 × 800 mL). The organic layer was dried over anhydrous sodium sulphate, filtered and evaporated *in vacuo*. The extract was purified by flash

chromatography on neutral alumina (Brockmann Grade III), first eluting with methanol to remove most of the yellow-red band (carotenoids), further eluting with dichloromethane to remove the black pheophytin *a* band. Flash chromatography was repeated, first eluting with hexane (or petraleum ether) to remove the residual yellow band, and further eluting with 30% dichloromethane in tetrachloromethane to remove the pheophytin *a*. Removal of solvent, followed by recrystallization from dichloromethane/methanol, gave pheophytin *a* (6.5 g) as a black solid. The pheophytin *a* was treated with 5% sulphuric acid in methanol (v/v) (900 mL) (degassed by bubbling with nitrogen) for 13 h at room temperature in the dark, followed by dilution with dichloromethane, successively washed twice with water, once with saturated sodium bicarbonate and finally three times with water. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. Recrystallization of the product from dichloromethane/methanol gave the title compound (4.2 g) as blue prisms.

M.p.: 241°C (lit.[41] 228°C, lit.[36] 206°C)

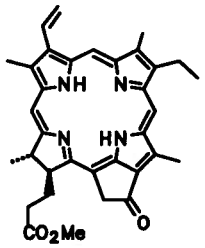
^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) (the major epimer) δ: 9.58 (s, 1H, H-10), 9.40 (s, 1H, H-5), 8.59 (s, 1H, H-20), 8.01 (dd, 1H, H-3^1, J = 17.1 and 11.6 Hz), 6.38 [d, 1H, H-3^2(E), J = 17.1 Hz], 6.22 [d, 1H, H-3^2(Z), J = 11.6 Hz)], 6.24 (s, 1H, H-13^2), 4.52 (dq, 1H, H-18, J = 9.3 and 1.7 Hz), 4.22 (ddd, 1H, H-17, J = 9.3, 3.1 and 1.7 Hz), 3.90 (s, 3H, H-13^4), 3.64 (q, 2H, H-8^1, J = 7.8 Hz), 3.62 (s, 3H, H-12^1), 3.59 (s, 3H, H-17^4), 3.40 (s, 3H, H-2^1), 3.21 (s, 3H, H-7^1), 2.60 (dddd, 1H, H_a-17^1, J = 13,3, 7.1, 6.2 and 3.1 Hz), 2.58 (dddd, 1H, $H_{a'}$-17^1, J = 13,3, 9.3, 9.3 and 5.3 Hz), 2.20 (ddd, 1H, H_b-17^1, J = 15.1,

9.3 and 7.1 Hz), 2.18 (ddd, 1H, $H_{b'}$-17^1, J = 15.1, 6.2 and 5.3 Hz), 1.82 (d, 3H, H-18^1, J = 7.1 Hz), 1.70 (t, 3H, H-8^2, J = 7.8 Hz), 0.39 (br s, 1H, NH), −1.60 (br s, 1H, NH)

^{13}C NMR (75 MHz, 25 mg/1.0 mL $CDCl_3$) δ: 189.63 (C-13^1), 173.36 (C-17^3), 172.14 (C-13^3), 169.60 (C-19), 161.16 (C-16), 155.50 (C-6), 150.85 (C-9), 149.61 (C-14), 145.05 (C-8), 141.99 (C-1), 137.85 (C-11), 136.39 (C-3), 136.12 (C-4), 136.02 (C-7), 131.76 (C-2), 128.87 (C-12), 128.94 (C-13), 128.94 (C-3^1), 122.66 (C-3^2), 105.13 (C-15), 104.28 (C-10), 97.39 (C-5), 93.07 (C-20), 62.71 (C-13^2), 52.88 (C-17), 51.70 (C-18), 51.10 (C-13^4), 50.10 (C-17^4), 31.06 (C-17^2), 29.86 (C-17^1), 23.10 (C-18^1), 19.29 (C-8^1), 17.36 (C-8^2), 12.09 (C-12^1), 12.06 (C-2^1), 11.08 (C-7^1)

UV/Vis λ_{max} ($CHCl_3$) 412 nm (ε 104 800), 506(15 800), 536(14 000), 610(13 100), 668 (45 300) [lit.[41] λ_{max} (CH_2Cl_2) 412 nm (ε 106 000), 506 (10 800), 538 (9 710), 610 (8 620), 668 (44 600)]

Pyropheophorbide *a* methyl ester (25)



25

Pheophorbide *a* methyl ester (7) (930 mg, 1.53 mmol) was dissolved in collidine (150 mL) and stirred at reflux for 1.5 h under nitrogen in the dark. Removal of solvent by distillation at reduced pressure (2.0 mm Hg) gave a dark blue residue, which was

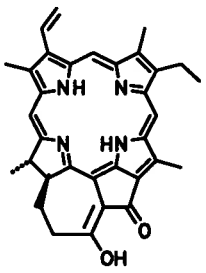
crystallized from CH_2Cl_2/CH_3OH to give the title compound (840 mg, 98%) as tiny blue needles.

M.p.: 233°C [lit.[41] 217-219°C]

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 9.50 (s, 1H, H-10), 9.39 (s, 1H, H-5), 8.59 (s,1H, H-20), 8.00 (dd, 1H, H-3^1, *J* = 17.4 and 11.4 Hz), 6.30 [dd, 1H, H-3^2(E), *J* = 17.4 and 1.1 Hz], 6.18 [dd, 1H, H-3^2(Z), *J* = 11.4 and 1.1 Hz], 5.29, 5.10 (ABq, 2H, H-13^2), 4.49 (dq, 1H, H-18, *J* = 7.3 and 2.2 Hz), 4.27 (ddd, H-17, *J* = 7.9, 3.0 and 2.2 Hz), 3.68 (q, 1H, H-8^1, *J* = 7.6 Hz), 3.66 (s, 3H, H-12^1), 3.60 (s, 3H, H-17^4), 3.39 (s, 3H, H-2^1), 3.22 (s, 3H, H-7^1), 2.68 (m, 1H, H_a-17^1, *J* = 7.9 Hz), 2.55 (m, 1H, $H_{b'}$-17^2), 2.30 (m, 1H, $H_{a'}$-17^1, *J* = 3.0 Hz), 2.28 (m, 1H, H_b-17^2), 1.79 (d, 1H, H-18^1, *J* = 7.3 Hz), 1.68 (t, 3H, H-8^2, *J* = 7.6 Hz), 0.45 (br s, 1H, NH), –1.70 (br s, 1H, NH)

UV/Vis λ_{max} ($CHCl_3$) 418 nm (ε 147 700), 510 (13 500), 538 (9 800), 608 (11 200), 668 (53 500) [lit.[41] λ_{max} (CH_2Cl_2), 410 (ε 113 000), 508 (11 500), 538 (9 800), 610 (8 500), 668 (47 100)

13^2, 17^3-Cyclopheophorbide *a* enol (81)



81

To a solution of pyropheophorbide *a* methyl ester (**25**) (546 mg, 1 mmol) in dry THF (60 mL) under an atmosphere of nitrogen was added, $NaN[Si(CH_3)_3]_2$ (7.0 mL, 7.0

mmol, 1.0 M in THF). The resultant yellow solution was stirred at room temperature for 3 min then poured into a deoxygenated (N_2) mixture of dichloromethane (800 mL), saturated NaH_2PO_4 (200 mL) and ice (200 g). The mixture was strongly shaken until the yellow color turned to bright-green. After separation of the aqueous phase, the organic layer was dried over sodium sulphate, filtered and evaporated *in vacuo*. The residue was purified by chromatography on silica V, eluting with dichloromethane (2 L). The product was crystallized from methylene chloride/hexane under nitrogen, giving the title product (438 mg, 85%) as lustrous dark green needles.

M.p.: > 300°C [lit.[96] >300°C, lit.[87] >360°C]

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 13.24 (s, 1H, OH), 8.64 (s, 1H, H-10), 8.43 (s, 1H, H-5), 7.38 (s, 1H, H-20), 7.70 (dd, 1H, H-3^1, J = 18.0 and 11.6 Hz), 6.12 [dd, 1H, H-3^2(E), J = 18.0 and 1.6 Hz], 6.04 [dd, 1H, H-3^2(Z), J = 11.6 and 1.6 Hz], 3.31 (q, 2H, H-8^1, J = 7.9 Hz), 3.08 (s, 3H, H-12^1), 3.02 (s, 3H, H-2^1), 2.94 (s, 3H, H-7^1), 2.93 (q, 1H, H-18, J = 7.2 Hz), 2.58 (m, 1H, H-17), 2.45 (t, 2H, H-17^2), 1.71 (m, 2H, H-17^1), 1.80 (d, 3H, H-18^1, J = 7.1 Hz), 1.52 (t, 3H, H-8^2, J = 7.9 Hz), 0.30 (br s, 1H, NH), −1.72 (br s, 1H, NH)

^{13}C NMR (75 MHz, 18.0 mg/0.6 mL $CDCl_3$) δ: 191.78 (C-13^1), 169.63 (C-19), 167.35 (C-17^3), 157.77 (C-16), 154.65 (C-6), 150.04 (C-9), 144.15 (C-14), 143.21 (C-8), 141.27 (C-1), 136.35 (C-11), 135.95 (C-3), 135.04 (C-4), 134.99 (C-7), 130.80 (C-2), 128.93 (C-12), 127.78 (C-3^1), 127.52 (C-13), 121.80 (C-3^2), 116.83 (C-13^2), 104.10 (C-15), 104.02 (C-10), 96.74 (C-5), 91.02 (C-20), 52.47 (C-17), 49.33 (C-18), 34.00 (C-17^2), 25.03 (C-

17^1), 19.06 (C-18^1), 17.24 (C-8^1), 16.69 (C-8^2), 11.69 (C-12^1), 11.58 (C-2^1), 10.90 (C-7^1)

UV/Vis λ_{max} ($CHCl_3$) 364 nm (ε 67 200), 430 (66 000), 456 (47 200), 592 (5 200), 630 (4 800), 690 (33 400) [lit.[96] λ_{max}(CH_2Cl_2) 361 nm (ε 65 500), 429 (64 000), 455 (44 400), 629 (9 000), 688 (33 000); lit.[87] λ_{max} 359 nm (ε 63 000), 426 (63 000), 452 (50 000), 626 (10 000), 686 (32 000)]

LREIMS (m/z): 516 (M^+, 100%), 501 (M^+–CH_3, 28)

HREIMS: $C_{33}H_{32}N_4O_2$ (M^+): calcd 516.2525

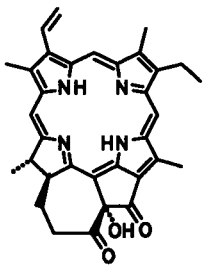
obsd 516.2534

Anal. calcd for $C_{33}H_{32}N_4O_2$: C, 76.72; H, 6.24; N, 10.84 %

found: C, 76.91; H, 6.19; N, 10.37 %

6.2.2 Asymmetric Hydroxylation

13^2S-Hydroxychlorophyllone *a* [82(S)]



82(S)

A cold (–25°C) solution of 13^2, 17^3-cyclopheophorbide *a* enol (**81**) (103 mg, 0.2 mmol) in dry THF (60 mL) was blanketed with N_2 and stirred vigorously while DBU (1.0 mL) was injected dropwise via a syringe. The mixture was kept at this temperature for 15

min while a solution of (1R)-(−)-(10-camphorsulfonyl)oxaziridine (50 mg, 0.22 mmol) in cold (−25°C), dry THF (12 mL) was transferred into the reaction vessel via a cannula. This mixture was stirred at −25°C for 12 h and quenched with saturated NH_4Cl. The aqueous phase was extracted with dichloromethane (2 × 100 mL) and the combined organic phases were dried over sodium sulphate, filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on silica V, eluting with dichloromethane. The product was recrystallized from methanol, giving 100 mg (94%) of a blue powder, which was analyzed by reversed-phase HPLC as a diastereomeric mixture of 95% 13^2S-hydroxychlorophyllone *a* [**82**(S)] and 5% 13^2R-hydroxychlorophyllone *a* [**82**(R)].

M.p.: >300°C

Anal. calcd for $C_{33}H_{32}N_4O_3$: C, 74.41; H, 6.06; N, 10.52%

found: C, 74.65; H, 6.10; N, 10.19%

After HPLC separation (12 mg) using a Waters C_{18} 10μ 125Å (7.8 mm × 30 cm) column with a flow rate of 3 mL min^{-1} and detection at 410 nm [the mobile phase: 75% (0.1% TFA in CH_3CN)/25% (0.1% TFA in water)], 13^2S-hydroxychlorophyllone *a* [**82**(S)] (10 mg) was obtained. After treatment with methanol, the optically-pure title compound (9.1 mg) was collected as a dark green solid.

M.p.: >300°C

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 9.40 (s, 1H, H-10), 9.35 (s, 1H, H-5), 8.70 (s, 1H, H-20), 7.96 (dd, 1H, H-3^1, J = 18.1 and 11.4 Hz), 6.28 [dd, 1H, H-3^2(E), J = 18.1 and 1.2 Hz], 6.18 [dd, 1H, H-3^2(Z), J = 11.4 and 1.2 Hz], 4.90 (ddd, 1H, H-17, J = 13.3, 3.8 and 3.5 Hz), 4.56 (br s, 1H, OH), 4.33 (dq, 1H, H-18, J = 7.4 and 3.8 Hz), 4.31

(ddd, 1H, H_b,-17^2, J = 14.0, 11.7 and 3.5 Hz), 3.60 (s, 3H, H-12^1), 3.59 (q, 2H, H-8^1, J = 8.1 Hz), 3.39 (s, 3H, H-2^1), 3.20 (s, 3H, H-7^1), 2.88 (dddd, 1H, H_a,-17^1, J = 12.4, 4.4, 3.5 and 3.5 Hz), 2.78 (ddd, 1H, H_b-17^2, J = 14.0, 11.7 and 2.1 Hz), 2.23 (dddd, 1H, H_a-17^1, J = 14.0, 13.3, 12.4 and 2.1 Hz), 2.19 (d, 3H, H-18^1, J = 7.4 Hz), 1.64 (t, 3H, H-8^2, J = 8.1 Hz), 0.41 (br s, 1H, NH), –2.05 (br s, 1H, NH)

^{13}C NMR (125 MHz, 7.0 mg/0.6 ml $CDCl_3$) δ: 208.00 (C-17^3), 195.44 (C-13^1), 172.68 (C-19), 163.20 (C-16), 154.53 (C-6), 150.92 (C-9), 147.72 (C-14), 144.88 (C-8), 142.16 (C-1), 138.19 (C-11), 136.38 (C-3), 136.31 (C-4), 135.75 (C-7), 131.58 (C-2), 129.13 (C-12), 129.08 (C-3^1), 127.77 (C-13), 122.85 (C-3^2), 105.36 (C-15), 104.06 (C-10), 98.10 (C-5) , 93.43 (C-13^2), 92.87 (C-20), 51.92 (C-17), 51.51 (C-18), 40.12 (C-17^2), 37.99 (C-17^1), 22.37 (C-18^1), 19.24 (C-8^1), 17.29 (C-8^2), 12.20 (C-12^1), 12.08 (C-2^1), 11.14 (C-7^1)

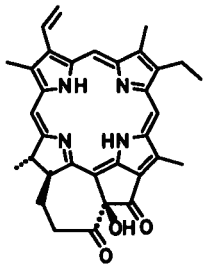
UV-Vis λ_{max} ($CHCl_3$) 416 nm (ε 111 000), 506 (12 500), 536 (10 200), 612 (9 200), 670 (50 900); λ_{max} (CH_3OH) 408 nm (ε 103 000), 504 (11 600), 534 (9 000), 608 (8 300), 666 (47 600) [lit.[89,91] λ_{max}(CH_3OH) 408 nm, 503, 534, 608, 665]

LRFABMS (m/z): 533 ($[MH]^+$, 100%)

HRFABMS: $C_{33}H_{33}N_4O_3$ ($[MH]^+$): calcd 533.2552

obsd 533.2540

13^{2}R-Hydroxychlorophyllone *a* [82(R)]



82(R)

The same hydroxylation procedure as for **82**(S) was employed by reaction of 13^2, 17^3-cyclopheophorbide *a* enol (**81**) (52 mg, 0.1 mmol) with (1S)-(+)-(10-camphor-sulfonyl)oxaziridine (27 mg, 0.118 mmol). The product was purified as described above and gave a blue solid (45.9 mg, 88%) after trituration with methanol. A reversed-phase HPLC analysis showed the product to be a diastereomeric mixture of 68% 13^{2}R-hydroxychlorophyllone *a* [**82**(R)] and 32% 13^{2}S-hydroxychlorophyllone *a* [**82**(S)].

M.p.: >300°C

Anal. calcd for $C_{33}H_{32}N_4O_3$: C, 74.41; H, 6.06; N, 10.52%

found: C, 74.13; H, 6.14; N, 10.44%

After HPLC separation (9 mg) using a Waters C_{18} 10μ 125Å (7.8 mm × 30 cm) column with a flow rate of 3 mL min^{-1} and detection at 410 nm [the mobile phase: 75% (0.1% TFA in CH_3CN)/25% (0.1% TFA in water), 13^{2}R-hydroxychlorophyllone *a* [**82**(R)] (4.5 mg) was obtained. After treatment with methanol, the optically-pure title compound (4.2 mg) was collected as a dark green solid.

M.p.: >300°C

^{1}H NMR (400 MHz, 1.0 mg/0.6 mL $CDCl_3$) δ: 9.47 (s, 1H, H-10), 9.35 (s, 1H, H-5), 8.52 (s, 1H, H-20), 7.96 (dd, 1H, H-3^1, *J* = 17.6 and 11.2 Hz), 6.29 [dd, 1H, H-3^2(E), *J* =

17.6 and 1.0 Hz], 6.18 [dd, 1H, H-3^2(Z), J = 11.2 and 1.0 Hz], 4.75 (dq, 1H, H-18, J = 8.3 and 7.0 Hz), 4.14 (br s, 1H, OH), 3.83 (ddd, 1H, H_b-17^2, J = 15.0, 6.2 and 1.5 Hz), 3.82 (ddd, 1H, H-17, J = 11.0, 8.3 and 1.6 Hz), 3.71 (dddd, 1H, H_a-17^1, J = 13.1, 12.8, 11.0 and 6.2 Hz), 3.68 (s, 3H, H-12^1), 3.68 (q, 2H, H-8^1, J = 7.2 Hz), 3.35(s, 3H, H-2^1), 3.20 (s, 3H, H-7^1), 2.95 (ddd, 1H, H_b'-17^2, J = 15.0, 12.8 and 5.2 Hz), 2.65 (dddd, 1H, H_a'-17^1, J = 13.1, 5.2, 1.6 and 1.5 Hz), 2.20 (d, 3H, H-18^1, J = 7.0 Hz), 1.68 (t, 3H, H-8^2, J = 7.2 Hz), 0.90 (br s, 1H, NH), −1.56 (br s, 1H, NH)

^{13}C NMR (75 MHz, 4.0 mg/0.6 mL $CDCl_3$) δ: 206.21 (C-17^3), 193.39 (C-13^1), 172.36 (C-19), 162.82 (C-16), 154.68 (C-6), 150.80 (C-9), 149.43 (C-14), 144.95 (C-8), 142.44 (C-1), 138.18 (C-11), 136.32 (C-3), 136.26 (C-4), 135.89 (C-7), 131.60 (C-2), 129.55 (C-12), 128.93 (C-3^1), 127.11 (C-13), 122.83 (C-3^2), 105.73 (C-15), 104.73 (C-10), 98.29 (C-5), 92.66 (C-13^2), 91.73 (C-20), 53.71 (C-17), 50.31 (C-18), 43.17 (C-17^2), 22.71 (C-17^1), 19.36 (C-8^1), 17.39 (C-8^2), 16.99 (C-18^1), 12.25 (C-12^1), 12.01 (C-2^1), 11.16 (C-7^1)

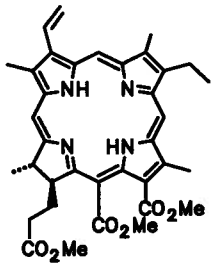
UV-Vis λ_{max} ($CHCl_3$) 416 nm (ε 113 000), 508 (15 500), 538 (11 100), 616 (10 000), 674 (48 300); λ_{max} (CH_3OH) 408 nm (ε 119 000), 504 (13 500), 534 (11 000), 612 (10 500), 670 (50 000) [lit.[89,91] λ_{max} (CH_3OH) 408 nm, 505, 535, 612, 670]

LREIMS (m/z): 532 (M^+, 100%), 501 (M^+–OCH_3, 21)

HREIMS: $C_{33}H_{32}N_4O_3$ ([M]$^+$): calcd 532.2474

obsd 532.2474

Chlorin p_6 trimethyl ester (92)



92

Method A A cold (–78°C) solution of $13^2,17^3$-cyclopheophorbide *a* enol (**81**) (30 mg, 58 μmol) in dry THF (15 mL) was blanketed with N_2 and stirred vigorously while a similarly cold solution of sodium hexamethyldisilazide in dry THF (0.18 mL of 1.0 M, 0.18 mmol) was introduced dropwise via a syringe. The mixture was kept at this temperature for 15 min while a solution of 1-phenyl-*N*-(phenylsulfonyl)oxaziridine[111] (20.8 mg, 79 μmol) in cold (–78°C), dry THF (1 mL) was transferred into the reaction vessel via a cannula. This mixture was stirred at –78°C for 30 min before it was quenched with saturated NH_4Cl. The aqueous phase was extracted with dilchloromethane (3 × 30 mL) and the combined organic phases were dried over sodium sulphate and evaporated *in vacuo*. TLC analysis showed that the product did not move on a TLC plate even with development by 5% methanol in dichloromethane. The residue was dissolved in THF and acidified with 1M HCl. The aqueous layer was re-extracted with dichloromethane before the organic layer was treated with an excess of ethereal diazomethane. The evaporated residue was purified by chromatography on silica gel, eluting with dichloromethane (100 mL). The product was crystallized from dichloromethane/methanol, giving the title compound (8.8 mg, 24%) as small dark green needles.

Method B A solution of the foregoing (95%S, 5%R) mixture of 13^2-hydroxychlorophyllone a (**82**) (15 mg, 0.0282 mmol) in dry THF (15 mL) was blanketed with N_2 and stirred vigorously while KOH (0.5 g) in CH_3OH (5 mL) was added. This mixture was stirred at room temperature in the dark for 10 h before the mixture was acidified to pH 3 by 2N HCl and extracted with dichloromethane. The organic layer was washed with water 3 times before being subjected to an excess of ethereal CH_2N_2. The material was purified as described in (a) to give chlorin p_6 trimethyl ester (**92**) (8.0 mg, 46%).

M.p.: 237°C [lit.[185] 235-236°C, lit.[186] 236°C]

^{1}H NMR (400 MHz, 1.5 mg/0.6 ml $CDCl_3$) δ: 9.70 (s, 1H, H-10), 9.49 (s, 1H, H-5), 8.77 (s, 1H, H-20), 8.00 (dd, 1H, H-3^1, J = 16.8, 12.0 Hz), 6.31 [dd, 1H, H-3^2(E), J = 16.8 and 1.2 Hz], 6.15 [dd, 1H, H-3^2(Z), J = 12.0 and 1.2 Hz], 5.15 (dd, 1H, H-17, J = 9.2 and 2.8 Hz), 4.38 (q, 1H, H-18, J = 7.6 Hz), 4.22 (s, 3H, H-15^2), 4.14 (s, 3H, H-13^2), 3.72 (q, 2H, H-8^1, J = 7.7 Hz), 3.63 (s, 3H, H-17^4), 3.52 (s, 3H, H-12^1), 3.40 (s, 3H, H-2^1), 3.23 (s, 3H, H-7^1), 2.38 (m, 1H, H_b'-17^2), 2.20 (m, 1H, H_a'-17^1), 2.05 (m, 1H, H_b-17^2), 1.87 (m, 1H, H_a-17^1), 1.84 (d, 3H, H-18^1, J = 7.6 Hz), 1.69 (t, 3H, H-8^2, J = 7.7 Hz), –0.82 (br s, 1H, NH), –1.00 (br s, 1H, NH)

^{13}C NMR (75 MHz, 7.5 mg/0.6 ml $CDCl_3$) δ: 173.54 (C-17^3), 172.84 (C-19), 170.72 (C-15^1), 167.23 (C-13^1), 167.02 (C-16), 154.93 (C-6), 148.89 (C-9), 145.24 (C-14), 141.19 (C-8), 137.75 (C-1), 135.98 (C-11), 135.80 (C-3), 135.71 (C-4), 135.46 (C-7), 130.87 (C-2), 129.53 (C-12), 129.06 (C-3^1), 122.44 (C-13), 122.34 (C-3^2), 104.64 (C-15), 103.08 (C-10), 100.33 (C-5), 93.60 (C-20), 52.67 (C-17), 52.56 (C-15^2), 52.14 (C-13^2), 51.48

(C-17^4), 49.39 (C-18), 31.43 (C-17^2), 31.25 (C-17^1), 23.56 (C-18^1), 19.56 (C-8^1), 17.64 (C-8^2), 12.54 (C-12^1), 12.04 (C-2^1), 11.20 (C-7^1)

UV-Vis λ_{max} ($CHCl_3$) 404 nm (ε 158 500), 500 (12 000), 532 (7 300), 616 (6 200), 672 (44 900) [lit.[185] λ_{max} (CH_2Cl_2) 402 nm (ε 137 000), 498 (9 900), 532 (5 500), 614 (4 900), 668 (40 900)]

LRFABMS (m/z): 625 ($[MH]^+$,100%)

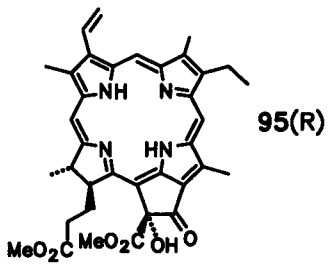
HRFABMS: $C_{36}H_{41}N_4O_6$ ($[M+H]^+$): calcd 625.3026

obsd 625.3043

Anal. calcd for $C_{36}H_{40}N_4O_6$:: C, 69.21; H, 6.45; N, 8.97 %

found: C, 68.75; H, 6.38; N, 8.80 %

13^{2}R-Hydroxypheophorbide *a* Methyl Ester [95(R)]



95(R)

The same hydroxylation procedure as for **82**(S) was employed by reaction of pheophorbide *a* methyl ester (**7**) (61 mg, 0.1 mmol) with (1R)-(−)-(10-camphorsulfonyl)-oxaziridine (27 mg, 0.118 mmol). The product was purified as described for hydroxychlorin **82**(S) and, after treatment with methanol, gave a blue powder (57 mg, 92%) which was analyzed by reversed-phase HPLC as a diastereomeric mixture of 95% 13^{2}R-hydroxypheophorbide *a* methyl ester [**95**(R)] and 5% 13^{2}S-hydroxypheophorbide *a* methyl ester [**95**(S)].

M.p.: >300°C

Anal. calcd for $C_{36}H_{38}N_4O_6$: C, 69.44; H, 6.15; N, 9.00 %

found: C, 69.10; H, 6.16; N, 8.70 %

After HPLC separation (10 mg) using a Waters C_{18} 10μ 125Å (7.8 mm × 30 cm) column with a flow rate of 3 mL min^{-1} and detection at 410 nm [the mobile phase: 85% (0.1% TFA in CH_3CN)/15% (0.1% TFA in water)], 13^2R-hydroxypheophorbide *a* methyl ester **95**(R) (7.5 mg) was obtained. After treatment with methanol, the optically-pure title compound (7.0 mg) was collected as shiny blue plates.

M.p.: >300°C

^{1}H NMR (400 MHz, 1.0 mg/0.6 mL $CDCl_3$) δ: 9.53 (s, 1H, H-10), 9.47 (s, 1H, H-5), 8.61 (s, 1H, H-20), 7.96 (dd, 1H, H-3^1, J = 18.2 and 11.9 Hz), 6.29 [dd, 1H, H-3^2(E), J = 18.2 and 1.0 Hz], 6.17 [dd, 1H, H-3^2(Z), J = 11.9 and 1.0 Hz], 5.32 (s, 1H, OH), 4.69 (dd, 1H, H-17, J = 8.5 and 1.7 Hz), 4.49 (q, 1H, H-18, J = 7.0 Hz), 3.70 (q, 2H, H-8^1, J = 7.7 Hz), 3.69 (s, 3H, H-13^4), 3.66 (s, 3H, H-12^1), 3.56 (s, 3H, H-17^4), 3.39 (s, 3H, H-2^1), 3.18 (s, 3H, H-7^1), 2.46 (m, 1H, H_a'-17^1, J = 8.5 Hz), 2.29 (m, 1H, H_b'-17^2), 2.13 (m, 1H, H_a-17^1, J = 1.7 Hz), 2.09 (m, 1H, H_b-17^2), 1.68 (d, 3H, H-18^1, J = 7.0 Hz), 1.65 (t, 3H, H-8^2, J = 7.7 Hz), 0.39 (br s, 1H, NH), −1.74 (br s, 1H, NH)

^{13}C NMR (75 MHz, 7.0 mg/0.6 mL $CDCl_3$) δ: 191.93 (C-13^1), 173.46 (C-17^3), 173.42 (C-15^2), 172.71 (C-19), 161.80 (C-16), 155.46 (C-6), 150.88 (C-9), 150.19 (C-14), 145.15 (C-8), 142.09 (C-1), 137.68 (C-11), 136.40 (C-3), 136.35 (C-4), 136.24 (C-7), 131.87 (C-2), 129.56 (C-12), 128.96 (C-3^1), 126.22 (C-13), 122.31 (C-3^2), 107.58 (C-15), 104.15 (C-10), 97.79 (C-5), 93.40 (C-20), 89.09 (C-13^2), 53.77 (C-13^4), 51.33 (C-

17^4), 50.75 (C-17), 50.16 (C-18), 30.99 (C-17^2), 30.18 (C-17^1), 22.69 (C-18^1), 19.35 (C-8^1), 17.41 (C-8^2), 12.27 (C-12^1), 12.08 (C-2^1), 11.15 (C-7^1)

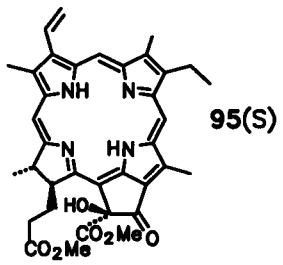
UV-Vis λ_{max} ($CHCl_3$) 416 nm (ε 136 600), 506 (15 500), 538 (11 600), 560 (4 200), 612 (11 500), 670 (62 100)

LRFABMS (m/z): 623 ($[MH]^+$, 100%)

HRFABMS: $C_{36}H_{39}N_4O_6$ ($[MH]^+$): calcd 623.2869

obsd 623.2874

13^2S-Hydroxypheophorbide *a* Methyl Ester [95(S)]



95(S)

The same hydroxylation procedure as for **95**(R) was employed by reaction of pheophorbide *a* methyl ester (**7**) (61 mg, 0.1 mmol) with (1S)-(+)-(10-camphorsulfonyl)-oxaziridine (27 mg, 0.118 mmol). The product was purified as above and, after treatment with methanol, gave a blue solid (51 mg, 82%) which was analyzed by reversed-phase HPLC as a diastereomeric mixture of 58% 13^2S-hydroxypheophorbide *a* methyl ester [**95**(S)] and 42% 13^2R-hydroxypheophorbide *a* methyl ester [**95**(R)].

M.p.: >300°C

After HPLC separation (10 mg) using a Waters C_{18} 10μ 125Å (7.8 mm × 30 cm) column with a flow rate of 3 mL min^{-1} and detection at 410 nm [the mobile phase: 85%

(0.1% TFA in CH_3CN)/15% (0.1% TFA in water)], 13^2S-hydroxy pheophorbide *a* methyl ester [**95**(S)] (4.1 mg) was separated and was treated with methanol, giving the optically-pure title product (3.9 mg) as a dark blue solid.

M.p.: >300°C

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 9.62 (s, 1H, H-10), 9.48 (s, 1H, H-5), 8.63 (s, 1H, H-20), 8.01 (dd, 1H, H-3^1, J = 18.3 and 11.7 Hz), 6.30 [dd, 1H, H-3^2(E), J = 18.3 and 0.8 Hz], 6.20 [dd, 1H, H-3^2(Z), J = 11.7 and 0.8 Hz], 5.43 (s, 1H, OH), 4.49 (q, 1H, H-18, J = 7.3 Hz), 4.15 (dd, 1H, H-17, J = 2.2 and 10.2 Hz), 3.72(s, 3H, H-13^4), 3.69 (q, 2H, H-8^1, J = 7.6 Hz), 3.64 (s, 3H, H-12^1), 3.59(s, 3H, H-17^4), 3.41(s, 3H, H-2^1), 3.23 (s, 3H, H-7^1), 2.92 (m, 1H, H_a-17^1, J = 2.2 Hz), 2.55 (m, 1H, H_b-17^2), 2.28 (m, 1H, H_a'-17^1, J = 10.2 Hz), 2.26 (m, 1H, H_b'-17^2), 1.68 (t, 3H, H-8^2, J = 7.6 Hz), 1.58 (d, 3H, H-18^1, J = 7.3 Hz), 0.31 (br s, 1H, NH), −1.83 (br s, 1H, NH)

^{13}C NMR (75 MHz, 3.5 mg/0.6 mL $CDCl_3$) δ: 192.00 (C-13^1), 173.96 (C-19), 172.83 (C-17^3), 172.37 (C-15^1), 162.37 (C-16), 155.35 (C-6), 151.03 (C-9), 149.84 (C-14), 145.22 (C-8), 142.03 (C-1), 137.80 (C-11), 136.52 (C-3), 136.29 (C-4), 136.21 (C-7), 131.76 (C-2), 129.41 (C-12), 129.04 (C-3^1), 122.89 (C-13), 122.88 (C-3^2), 107.59 (C-15), 104.26 (C-10), 97.97 (C-5), 93.62 (C-20), 88.94 (C-13^2), 53.44 (C-13^4), 51.78 (C-17^4), 51.75 (C-17), 50.29 (C-18), 31.40 (C-17^2), 31.11 (C-17^1), 22.65 (C-18^1), 19.47 (C-8^1), 17.45 (C-8^2), 12.30 (C-12^1), 12.11 (C-2^1), 11.26 (C-7^1)

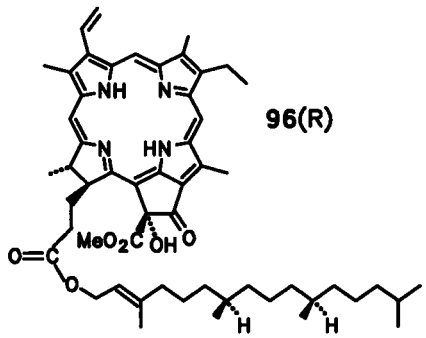
UV-Vis λ_{max} ($CHCl_3$) 414 nm (ε 141 200), 506 (9 800), 536 (15 800), 612 (11 300), 670 (63 500)

LREIMS (m/z): 622 (M^+, 56%), 563 (M^+–$COOCH_3$, 100)

HREIMS: $C_{36}H_{38}N_4O_6$ (M^+): calcd 622.2791

obsd 622.2802

13²R-Hydroxypheophytin *a* [96(R)]



96(R)

A cold (–25°C) solution of pheophytin *a* (**5**) (50 mg, 0.0575 mmol) in dry THF (30 mL) was blanketed with N_2 and was stirred vigorously while DBU (0.3 mL) was injected dropwise via a syringe. The mixture was kept at this temperature for 15 min while a solution of (1R)-(–)-(10-camphorsulfonyl)oxaziridine (15.8 mg, 0.069 mmol) in cold (–25°C), dry THF (6 mL) was transferred into the reaction vessel via a cannula. This mixture was stirred at –25°C for 12 hours before the reaction was quenched with saturated NH_4Cl. The aqueous phase was extracted with CH_2Cl_2 and the resulting organic layer was washed with water, dried, filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on silica V, eluting with CH_2Cl_2. The product was recrystallized from MeOH, giving 46.2 mg (91% yield) of a black solid, which was analyzed by ^1H NMR to be an 100% d.e. of 13²R-hydroxypheophytin *a* [**96**(R)].

M.p.: 199-200°C

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 9.70 (s, 1H, H-10), 9.45 (s, 1H, H-5), 8.60 (s, 1H, H-20), 8.00 (dd, 1H, H-3^1, *J* = 17.6 and 11.2 Hz), 6.31 [dd, 1H, H-3^2(E), *J* = 17.6 and 1.0 Hz], 6.21 [dd, 1H, H-3^2(Z), *J* = 11.2 and 1.0 Hz], 5.32 (s, 1H, OH-13^2), 5.15 (t, 1H, H-P^2, *J* = 8.3 Hz), 4.68 (d, 1H, H-17, *J* = 9.6 Hz), 4.47 (dq, 1H, H-18), 4.47 (d, 2H, H-P^1, *J* = 8.0 Hz), 3.75 (q, 2H, H-8^1, *J* = 7.1 Hz), 3.74 (s, 3H, H-12^1), 3.61 (s, 3H, H-13^4), 3.41 (s, 3H, H-2^1), 3.25 (s, 3H, H-7^1), 2.42 (m, 1H, H-17^1), 2.24 (m, 1H, H-17^2), 2.09 (m, 1H, H-17^1), 1.99 (m, 1H, H-17^2), 1.86 (t, H-P^4), 1.68 (t, 3H, H-8^2, *J* = 7.1 Hz), 1.65 (d, 3H, H-18^1), 1.55 (s, 3H, H-P^{17}), 1.52 (s, 3H, H-P^{15}), 1.19 [m, 16H, H-P$(CH_2)_8$], 0.82 (m, 8H, H-P^{20}, H-P^{16}, H-P^7, and H-P^{11}), 0.39 (br s, 1H, NH), –1.73 (br s, 1H, NH)

^{13}C NMR (125 MHz, 14 mg/1.0 mL $CDCl_3$) δ: 191.96 (C-13^1), 173.45 (C-17^3), 173.00 (C-13^3), 172.78 (C-19), 161.90 (C-16), 155.54 (C-6), 150.96 (C-9), 150.23 (C-14), 145.25 (C-8), 142.86 (C-P^3), 142.10 (C-1), 137.74 (C-11), 136.49 (C-3), 136.41 (C-4), 136.32 (C-7), 131.91 (C-2), 129.62 (C-12), 129.06 (C-3^1), 126.29 (C-13), 122.87 (C-3^2), 117.73 (C-P^2), 107.65 (C-15), 104.23 (C-10), 97.86 (C-5), 93.43 (C-20), 89.07 (C-13^2), 53.78 (C-13^4), 50.76 (C-17), 50.17 (C-18), 39.78 (C-P^4), 39.33 (C-P^{14}), 37.36 (C-P^8), 37.28 (C-P^{10}), 37.23 (C-P^{12}), 36.61 (C-P^6), 32.74 (C-P^{11}), 32.59 (C-P^7), 31.20 (C-17^2), 30.19 (C-17^1), 27.94 (C-P^{15}), 24.97 (C-P^5), 24.75 (C-P^{13}), 24.39 (C-P^9), 22.68 (C-P^{16} and C-P^{20}), 22.60 (C-18^1), 19.70 (C-P^{18}), 19.61 (C-P^{19}), 19.46 (C-8^1), 17.44 (C-8^2), 16.25 (C-P^{17}), 12.29 (C-12^1), 12.09 (C-2^1), 11.25 (C-7^1)

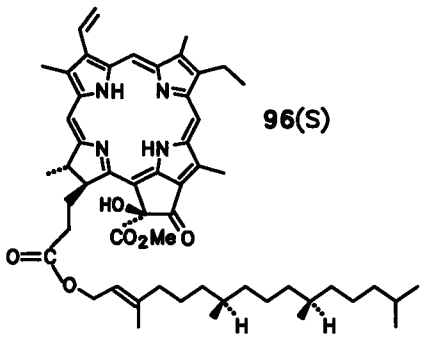
UV/Vis λ_{max} ($CHCl_3$) 416 nm (ε 101 000), 506 (11 800), 536 (9 500), 612 (9 000), 670 (45 600)

LREIMS (m/z): 886 (M^+, 41%), 607 (M^+– Phytyl, 62%)

HREIMS: $C_{55}H_{74}N_4O_6$ (M^+): calcd 886.5608

obsd 886.5616

13²S-Hydroxypheophytin *a* [96(S)]



96(S)

A cold (–25°C) solution of pheophytin *a* (**5**) (60 mg, 0.069 mmol) in dry THF (35 mL) was blanketed with N_2 and was stirred vigorously while DBU (0.35 mL) was injected dropwise via a syringe. The mixture was kept at this temperature for 15 min while a solution of (1S)-(+)-(10-camphorsulfonyl)oxaziridine (19.0 mg, 0.083 mmol) in cold (–25°C), dry THF (6 mL) was transferred into the reaction vessel via a cannula. This mixture was stirred at –25°C for 12 hours before the reaction was quenched with saturated NH_4Cl. The aqueous phase was extracted with CH_2Cl_2 and the resulting organic layer was washed with water, dried, filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on silica V, eluting with CH_2Cl_2. The product was recrystallized from MeOH, giving 52.9 mg (87% yield) of a black solid, which was analyzed by ^1H NMR to be a diastereomeric mixture of 66% 13²R-hydroxypheophytin *a* [**96**(R)] and 34% 13²S-hydroxypheophytin *a* [**96**(S)]. After normal phase HPLC separation (30 mg), using a Rainin silica 10μ 125 Å (7.8 mm × 25 cm) column with a flow rate of 4 mL min^{-1} and detection at 414 nm using a Waters 994

photodiode array detector (the mobile phase: hexane : isopropanol : ethyl acetate = 92 : 2 : 6), a sample of 38% d.e. of 13^2S-hydroxypheophytin *a* [**96**(S)] was obtained. Further purification by HPLC (normal-phase) and preparative TLC was largely unsuccessful due to decomposition of the material.

M.p.: 187°C

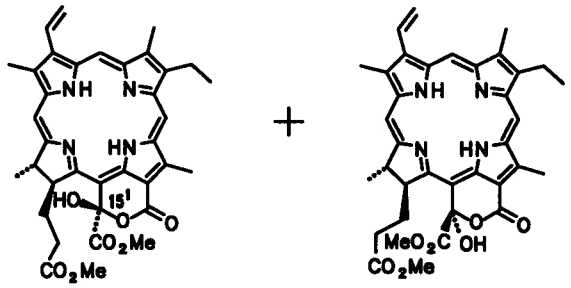
^{1}H NMR (400 MHz, 2.0 mg/0.6 mL $CDCl_3$) [**96**(S)] δ: 9.73 (s, 1H, H-10), 9.49 (s, 1H, H-5), 8.65 (s, 1H, H-20), 8.01 (dd, 1H, H-3^1, *J* = 17.9 and 11.9 Hz), 6.30 [d, 1H, H-3^2(E), *J* = 17.9 Hz], 6.21 [dd, 1H, H-3^2(Z), *J* = 11.9 Hz], 5.49 (s, 1H, OH-13^2), 5.20 (t, 1H, H-P^2, *J* = 7.5 Hz), 4.55 (dq, 1H, H-18, *J* = 7.6 Hz), 4.47 (d, 2H, H-P^1, *J* = 5.5 Hz), 4.15 (d, 1H, H-17, *J* = 10.0 and 1.0 Hz), 3.71 (s, 3H, H-12^1), 3.69 (q, 2H, H-8^1, *J* = 7.3 Hz), 3.60 (s, 3H, H-13^4), 3.40 (s, 3H, H-2^1), 3.24 (s, 3H, H-7^1), 2.91 (m, 1H, H-17^1), 2.53 (m, 1H, H-17^2), 2.25 (m, 1H, H-17^1), 2.25 (m, 1H, H-17^2), 1.90 (t, H-P^4), 1.71 (t, 3H, H-8^2, *J* = 7.3 Hz), 1.67 (d, 3H, H-18^1, *J* = 7.6 Hz), 1.59 (s, 3H, H-P^{17}), 1.54 (s, 3H, H-P^{15}), 1.21 [m, 16H, H-P$(CH_2)_8$], 0.84 (m, 8H, H-P^{20}, H-P^{16}, H-P^7 and H-P^{11}), 0.29 (br s, 1H, NH), –1.83 (br s, 1H, NH)

^{13}C NMR (125 MHz, 18 mg/1.0 mL $CDCl_3$) [**96**(S)] δ: 192.01 (C-13^1), 173.58 (C-17^3), 172.99 (C-13^3), 172.39 (C-19), 162.45 (C-16), 155.33 (C-6), 151.03 (C-9), 149.82 (C-14), 145.22 (C-8), 142.77 (C-P^3), 142.01 (C-1), 137.80 (C-11), 136.52 (C-3), 136.27 (C-4), 136.20 (C-7), 131.74 (C-2), 129.41 (C-12), 129.06 (C-3^1), 122.95 (C-13), 122.88 (C-3^2), 117.84 (C-P^2), 107.65 (C-15), 104.25 (C-10), 97.97 (C-5), 93.64 (C-20), 88.95 (C-13^2), 61.54 (C-P^1), 53.40 (C-13^4), 51.80 (C-17), 50.31 (C-18), 39.82 (C-P^4), 39.33 (C-P^{14}), 37.37 (C-P^8), 37.30 (C-P^{10}), 37.24 (C-P^{12}), 36.64 (C-P^6), 32.74 (C-P^{11}), 32.61 (C-

P^{7}), 31.56 (C-17^{2}), 31.12 (C-17^{1}), 27.95 (C-P^{15}), 25.00 (C-P^{5}), 24.76 (C-P^{13}), 24.41 (C-P^{9}), 22.70 (C-P^{16} and C-P^{20}), 22.61 (C-18^{1}), 19.71 (C-P^{18}), 19.64 (C-P^{19}), 19.49 (C-8^{1}), 17.46 (C-8^{2}), 16.32 (C-P^{17}), 12.31 (C-12^{1}), 12.09 (C-2^{1}), 11.25 (C-7^{1})

6.2.3 Model Studies for Hydroxylactonization

15^{1}-Hydroxypurpurin-7-lactone dimethyl ester (101)



101(S) **101(R)**

84% : **16%**

A solution of the foregoing (95%R, 5%S) mixture of 13^{2}-hydroxypheophorbide *a* methyl ester (**95**) (30 mg, 0.05 mmol) in dioxane (20 mL) was stirred with an aqueous solution (20 mL) of periodic acid dihydrate (900 mg, 3.95 mmol) at room temperature for 20 h before the mixture was extracted with dichloromethane (2 × 40 mL). The organic layer was dried over sodium sulphate, filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on silica III, eluting with 2% methanol in dichloromethane. The product was crystallized from dichloromethane/hexane, giving a black solid (25.2 mg, 79%), which was analyzed by ^{1}H NMR as a diastereomeric mixture of 84% 15^{1}S-hydroxypurpurin-7-lactone dimethyl ester [**101**(S)] and 16% 15^{1}R-hydroxypurpurin-7-lactone dimethyl ester [**101**(R)].

M.p.: 217°C

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) [**101**(S)] δ: 9.77 (s, 1H, H-10), 9.55 (s, 1H, H-5), 8.80 (s, 1H, H-20), 8.00 (dd, 1H, H-3^1, J = 17.6 and 11.5 Hz), 6.34 [dd, 1H, H-3^2(E), J = 17.6 and 1.2 Hz], 6.19 [dd, 1H, H-3^2(Z), J = 11.5 and 1.2 Hz], 6.05 (s, 1H, OH), 4.43 (q, 1H, H-18, J = 6.8 Hz), 4.05 (dd, 1H, H-17, J = 10.4 and 2.4 Hz), 3.89(s, 3H, H-15^3), 3.76 (s, 3H, H-12^1), 3.75 (q, 2H, H-8^1, J = 8.0 Hz), 3.51 (s, 3H, H-17^4), 3.40 (s, 3H, H-2^1), 3.26 (s, 3H, H-7^1), 2.46 (m, 1H, H_a-17^1, J = 2.4 Hz), 2.45 (m, 1H, H_b-17^2), 2.18 (m, 1H, H_a'-17^1, J = 10.4 Hz), 1.80 (m, 1H, H_b'-17^2), 1.70 (t, 3H, H-8^2, J = 8.0 Hz), 1.59 (d, 3H, H-18^1, J = 6.8 Hz), −1.10 (br s, 1H, NH), −1.41 (br s, 1H, NH)

UV-Vis λ_{max} ($CHCl_3$) 404 nm (ε 189 000), 502 (17 300), 530 (13 600), 562 (4 200), 614 (9 300), 672 (61 200)

LREIMS (m/z): 638 (M^+, 20%), 622 (M^+–O, 80)

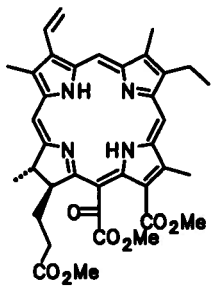
HREIMS: $C_{36}H_{38}N_4O_7$ (M^+): calcd 638.2740

obsd 638.2745

Anal. calcd for $C_{36}H_{38}N_4O_7$: C, 67.70; H, 6.00; N, 8.77 %

found: C, 68.00; H, 6.14; N, 8.95 %

Purpurin-7 trimethyl ester (16)



16

A solution of the foregoing (84%S, 16%R) mixture of 15^1-hydroxypurpurin-7-lactone dimethyl ester (**101**) (15 mg, 0.05 mmol) in dichloromethane (20 mL) was treated with an excess of ethereal diazomethane and then washed with water 3 times before the organic layer was dried over sodium sulphate, filtered and evaporated *in vacuo*. The product was crystallized from dichloromethane/hexane, giving the title compound (14.8mg, 97%) of a purple solid.

M.p.: 232°C [lit.[186] 227-230°C]

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 9.56 (s, 1H, H-10), 9.27 (s, 1H, H-5), 8.47 (s, 1H, H-20), 7.87 (dd, 1H, H-3^1, J = 17.9 and 11.7 Hz), 6.28 [dd, 1H, H-3^2(E), J = 17.9 and 1.1 Hz], 6.11 [dd, 1H, H-3^2(Z), J = 11.7 and 1.1 Hz], 4.66 (d, 1H, H-17, J = 7.6 Hz), 4.29 (q, 1H, H-18, J = 7.2 Hz), 4.12 (s, 3H, H-15^2), 3.86 (s, 3H, H-13^2), 3.63 (q, 2H, H-8^1, J = 7.6 Hz), 3.58 (s, 3H, H-12^1), 3.51 (s, 3H, H-17^4), 3.31 (s, 3H, H-2^1), 3.14 (s, 3H, H-7^1), 2.35 (t, 1H, H_b-17^2), 2.08 (m, 2H, H-17^1), 1.77 (d, 3H, H-18^1, J = 7.2 Hz), 1.75 (t, 1H, $H_{b'}$-17^2), 1.64 (t, 3H, H-8^2, J = 7.6 Hz), –0.01 (br s, 1H, NH), –0.09 (br s, 1H, NH)

UV-Vis λ_{max} ($CHCl_3$) 410 nm (ε 99 500), 506 (7 500), 548 (10 000), 680 (25 300), 688 (24 800) [lit.[186] λ_{max} 408 nm, 504, 544, 682 (ε 23 900)]

LREIMS (m/z): 652 (M^+, 11%), 565 (M^+–$COCOOCH_3$, 100)

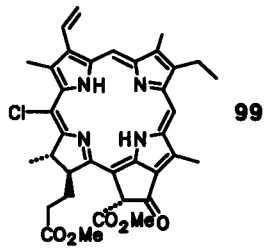
HREIMS: $C_{37}H_{40}N_4O_7$ (M^+): calcd 652.2897

obsd 652.2898

Anal. calcd for $C_{37}H_{40}N_4O_7$: C, 68.08; H, 6.18; N, 8.58 %

found: C, 67.72; H, 5.92; N, 8.36 %

20-Chloropheophorbide *a* methyl ester (99)



99

A mixture of pheophorbide *a* methyl ester (**7**) (20 mg, 0.033 mmol), *meta*-chloroperbenzoic acid (8.7 mg, 0.05 mmol), and $CHCl_3$ (15 mL) was magnetically stirred at room temperature in the dark for 12 hours. The mixture was diluted with CH_2Cl_2, washed once with water, once with 2N $Na_2S_2O_3$, twice with water, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on Brockman III, eluting with CH_2Cl_2, first to recycle 5.4 mg (27% yield) of the starting material, pheophorbide *a* methyl ester (**7**), and further eluting to furnish a brwonish-red band. The main band was collected, evaporated and recrystalized from CH_2Cl_2/MeOH to give the title compound (11.4 mg, 54% yield) as a brown-red solid.

M.p.: 217°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.60 (s, 2H, H-10 and H-5), 7.95 (dd, 1H, H-3^1, J = 18.8 and 11.8 Hz), 6.29 [dd, 1H, H-3^2(Z), J = 11.7 and 0.5 Hz], 6.23 (s, 1H, H-13^2), 6.15 [s, 1H, H-3^2(E), J = 18.8 and 0.5 Hz], 4.78 (q, 1H, H-18, J = 8.0 Hz), 4.12 (dd, 1H, H-17, J = 8.1 and 1.0 Hz), 3.90 (s, 3H, H-2^1), 3.68 (s, 3H, H-17^4), 3.68 (q, 2H, H-8^1, J = 7.5 Hz), 3.59 (s, 3H, H-7^1), 3.52 (s, 3H, H-13^4), 3.22 (s, 3H, H-12^1), 2.45 (m, 2H, H-17^1), 2.17 (m, 2H, H-17^2), 1.69 (t, 3H, H-8^2, J = 7.5 Hz), 1.60 (d, 3H, H-18^1, J = 8.0 Hz), –1.87 (br s, 2H, NH)

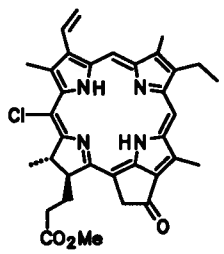
UV/Vis λ_{max} (CH_2Cl_2) 416 nm (ε 109 000), 518 (10 500), 550 (15 000), 616 (7 500), 678 (45 000)

LREIMS (m/z): 640 (M^+, 16%), 608 ($M^+ - OCH_4$, 12%),

HREIMS: $C_{36}H_{37}N_4O_5Cl$ (M^+): calcd 640.2452

obsd 640.2449

20-Chloropyropheophorbide *a* methyl ester (100)



100

A mixture of pyropheophorbide *a* methyl ester (**25**) (20 mg, 0.036 mmol), *meso*-chloroperbenzoic acid (8.3 mg, 0.048 mmol), and $CHCl_3$ (15 mL) was magnetically stirred at room temperature in the dark for 12 hours. The mixture was diluted with CH_2Cl_2, washed once with water, once with 2N $Na_2S_2O_3$, twice with water, dried over Na_2SO_4, filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on Brockman III, first eluting with CH_2Cl_2, to recover 10.2 mg (50% yield) of the starting material, pyropheophorbide *a* methyl ester (**25**), and further eluting to furnish a brwonish-red band. The appropriate band was collected, evaporated and recrystallized from CH_2Cl_2/MeOH to give the title compound (8.4 mg, 40% yield) as tiny dark needles.

M.p.: 234°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.56 (s, 1H, H-10), 9.50 (s, 1H, H-5), 7.92 (dd, 1H, H-3^1, J = 18.2 and 11.7 Hz), 6.26 [dd, 1H, H-3^2(Z), J = 11.7 and 1.9 Hz], 6.14[s, 1H, H-3^2(E), J = 18.2 and 1.9 Hz], 5.23 (s, 1H, H-13^2), 5.20 (s, 1H, H-13^2), 4.80 (q, 1H, H-18, J = 7.3 Hz), 4.22 (dd, 1H, H-17, J = 9.3 and 2.0 Hz), 3.68(s, 6H, H-2^1 and H-17^4), 3.59 (s, 3H, H-7^1), 3.59 (q, 2H, H-8^1, J = 7.8 Hz), 3.23 (s, 3H, H-12^1), 2.55 (m, 2H, H-17^1), 2.19 (m, 2H, H-17^2), 1.62 (d, 3H, H-18^1, J = 7.3 Hz), 1.68 (t, 3H, H-8^2, J = 7.8 Hz), –1.95 (br s, 2H, NH)

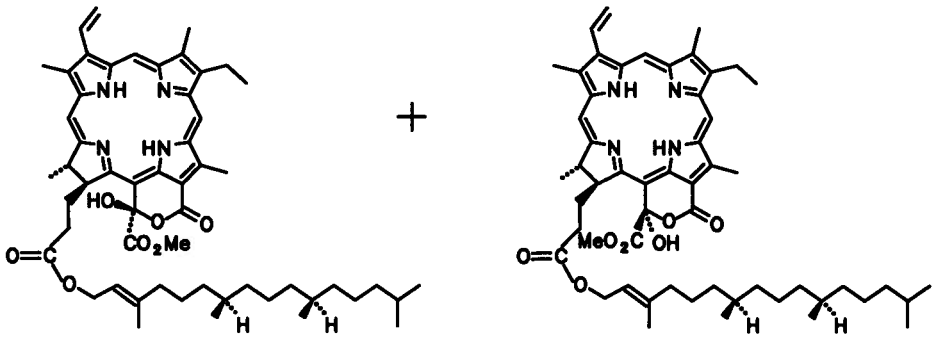
UV/Vis λ_{max} (CH_2Cl_2) 420 nm (ε 111 000), 520 (18 000), 552 (20 000), 614 (15 000), 676 (39 000)

LREIMS (m/z): 582 (M^+, 100%)

HREIMS: $C_{34}H_{35}N_4O_3Cl$ (M^+): calcd 582.2398

obsd 582.2406

15^1-Hydroxypurpurin-7-lactone methyl phytyl ester



81% : 19%

A solution of the foregoing 13^{2}R-hydroxypheophytin *a* [**96**(R)] (25 mg, 0.029 mmol) in dioxane (35 mL) was stirred magnetically at room temperature. To this was

added an aqueous solution (20 mL) of periodic acid dihydrate (400 mg, 1.76 mmol) and the resulting mixture was stirred in the dark for 25 hours before the mixture was extracted with CH_2Cl_2. The organic layer was washed with water 3 times, dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatography on silica III, eluting with 2% MeOH in CH_2Cl_2. The product was recrystallized from CH_2Cl_2/hexane, giving a black solid (19.6 mg, 75% yield), which was analyzed by ^1H NMR as a diastereomeric mixture of 81% 15^{1}S-hydroxypurpurin-7-lactone methyl phytyl ester and 19% 15^{1}R-hydroxypurpurin-7-lactone methyl phytyl ester.

M.p.: 183°C

^{1}H NMR (400 MHz, 1.1 mg/0.6 mL $CDCl_3$) δ: (the major epimer) 9.76 (s, 1H, H-10), 9.55 (s, 1H, H-5), 8.71 (s, 1H, H-20), 8.00 (dd, 1H, H-3^1, J = 18.4 and 12.1 Hz), 6.51 [d, 1H, H-3^2(E), J = 18.4 Hz], 6.19 [d, 1H, H-3^2(Z), J = 12.1 Hz], 6.10 (s, 1H, OH-15^1), 5.15 (t, 1H, H-P^2, J = 7.1 Hz), 4.07 (dd, 1H, H-17, J = 10.6 and 1.2 Hz), 4.43 (q, 1H, H-18, J = 6.7 Hz), 4.43 (d, 2H, H-P^1, J = 8.0 Hz), 3.73 (q, 2H, H-8^1, J = 7.6 Hz), 3.76 (s, 3H, H-12^1), 3.88 (s, 3H, H-15^3), 3.41 (s, 3H, H-2^1), 3.25 (s, 3H, H-7^1), 2.58 (m, 1H, H-17^1), 2.44 (m, 1H, H-17^2), 2.19 (m, 1H, H-17^1), 1.89 (m, 1H, H-17^2), 1.86 (t, H-P^4), 1.70 (t, 3H, H-8^2, J = 7.6 Hz), 1.59 (d, 3H, H-18^1), 1.58 (s, 3H, H-P^{17}), 1.52 (s, 3H, H-P^{15}), 1.19 [m, 16H, H-P$(CH_2)_8$], 0.82 (m, 8H, H-P^{20}, H-P^{16}, H-P^7 and H-P^{11}), –1.10 (br s, 1H, NH), –1.43 (br s, 1H, NH)

UV/Vis λ_{max} ($CHCl_3$) 404 nm (ε 112 000), 500 (9 300), 532 (7 200), 564 (1 600), 614 (4 800), 672 (36 300)

LRFABMS (m/z): 904 ([M+ H]$^+$, 56%), 625 ([M+ H]$^+$– Phytyl, 20%), 565 ([M+ H]$^+$– Phytyl – COOMe – H, 100%), 503 ([M+ H]$^+$– Phytyl – COOMe – COOH – H_2O, 87%)

HRFABMS: $C_{55}H_{74}N_4O_7$ ([M+ 2H]$^+$): calcd 904.5714

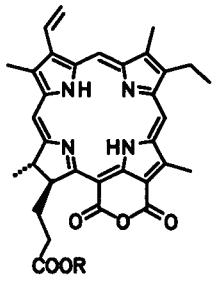
obsd 904.5714

6.2.4 Regioselective Oxidation

Oxidation of 13^2-Hydroxychlorophyllone *a* (82) by H_5IO_6 in Methanol

A solution of the foregoing (95%S, 5%R) mixture of 13^2-hydroxychlorophyllone *a* (**82**) (53 mg, 0.1 mmol) in dioxane (54 mL) and methanol (45 mL) was mixed with an aqueous solution (45 mL) of periodic acid dihydrate (2.1 g, 9.17 mmol) and stired at room temperature for 14 h before the mixture was extracted with dichloromethane (80 mL). The organic layer was washed with water 3 times before it was dried over sodium sulphate, filtered and evaporated *in vacuo*. The residue was separated by preparative TLC on deactivated silica gel (developed twice by 5% acetone, 1% methanol in dichloromethane), giving 5 distinct bands: the most mobile band (purple-red, **87**), the second mobile band (yellow, **102**), the major band (gray-green, **83**), the second least mobile band (purple-red, **22**) and the least mobile band (yellow, **85**). Unambiguous structure assignments of these compounds were accomplished by electronic absorption and ^{1}H and ^{13}C NMR spectroscopy.

Purpurin-18 methyl ester (87)



87

4.2 mg (7.2% yield) of a purple red shiny small flakes, recrystallized from methylene chloride/methanol.

M.p.: 267°C [lit.[185] >270°C; lit.[186] >260°C (decomp.)]

^{1}H NMR (400 MHz, 1.0 mg/0.6 mL $CDCl_3$) δ: 9.60 (s, 1H, H-10), 9.39 (s, 1H, H-5), 8.58 (s, 1H, H-20), 7.90 (dd, 1H, H-3^1, J = 18.3 and 11.3 Hz), 6.30 [dd, 1H, H-3^2(E), J = 18.3 and 1.6 Hz], 6.20 [d, 1H, H-3^2(Z), J = 11.3 and 1.6 Hz], 5.20 (dd, 1H, H-17, J = 9.9 and 2.5 Hz), 4.38 (dq, 1H, H-18, J = 9.9 and 7.7 Hz), 3.77 (s, 3H, H-12^1), 3.63 (q, 2H, H-8^2, J = 8.3 Hz), 3.32 (s, 3H, H-2^1), 3.15 (s, 3H, H-7^1), 2.73 (m, 1H, H_b'-17^2), 2.45 (m, 1H, H_a'-17^1), 2.43 (m,1H, H_b-17^2), 1.99 (m, 1H, H_a-17^1), 1.73 (d, 3H, H-18^1, J = 7.7 Hz), 1.65 (t, 3H, H-8^2, J = 8.3 Hz), 0.25 (br s, 1H, NH), –0.08 (br s, 1H, NH)

^{13}C NMR (75 MHz, 12.0 mg/0.6 mL $CDCl_3$) δ: 177.52 (C-17^3), 176.61 (C-13^1), 176.61 (C-15^1), 173.66 (C-19), 164.20 (C-16), 156.35 (C-6), 150.17 (C-9), 146.05 (C-8), 144.15 (C-1), 140.04 (C-140), 139.15 (C-11), 137.82 (C-7), 136.70 (C-3), 136.64 (C-4), 131.87 (C-2), 131.84 (C-12), 131.61 (C-13), 128.42 (C-3^1), 123.75 (C-3^2), 111.56 (C-15), 107.73 (C-10), 103.14 (C-5), 95.01 (C-20), 55.01 (C-17), 51.63 (C-17^4), 49.26 (C-18), 32.55 (C-17^2), 31.27 (C-17^1), 23.85 (C-18^1), 19.38 (C-8^1), 17.43 (C-8^2), 12.41 (C-12^1), 11.96 (C-2^1), 11.09 (C-7^1)

UV-Vis λ_{max} ($CHCl_3$) 362 nm (ε 48 900), 412 (126 100), 480 (4 800), 508 (7 200), 548 (25 500), 644 (9 400), 702 (52 100) [lit.[89] λ_{max} (CH_3OH) 359 nm, 407, 478, 508, 642, 697; lit.[186] λ_{max} ($CHCl_3$) 411 nm, 478, 507, 545, 593, 64, 700 (ε 52 500); lit.[185] λ_{max}(CH_2Cl_2) 410 nm (ε 123 000), 478 (5 100), 508 (7 500), 546 (24 600), 642 (9 800), 698 (49 800)]

LREIMS (m/z): 578 (M^+, 52%), 491 (M^+–$CH_2CH_2COOCH_3$, 100)

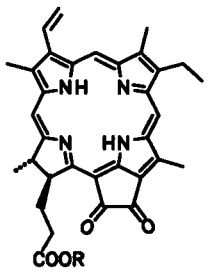
HREIMS: $C_{34}H_{34}N_4O_5$ (M^+): calcd 578.2529

obsd 578.2527.

Anal. calcd for $C_{34}H_{34}N_4O_5$: C, 70.57; H, 5.92; N, 9.68 %

found: C, 70.95; H, 6.03; N, 9.70 %

13^2-Oxopyropheophorbide *a* methyl ester (102)



102

3.0 mg (5.4% yield) of a yellow solid, recrystallized from dichloromethane/hexane.

M.p.: 245°C

^{1}H NMR (400 MHz, 1.0 mg/0.6 mL $CDCl_3$) δ: 9.90 (s, 1H, H-10), 9.86 (s, 1H, H-5), 9.00 (s, 1H, H-20), 8.10 (dd, 1H, H-3^1, *J* = 18.5 and 12.4 Hz), 6.34 [dd, 1H, H-3^2(E), *J* = 18.5 and 1.3 Hz], 6.26 [d, 1H, H-3^2(Z), *J* = 12.4 and 1.3 Hz], 5.16 (ddd, 1H, H-17, *J* =

8.8, 2.9 and 1.3 Hz), 4.68 (dq, 1H, H-18, J = 7.6 and 1.3 Hz), 3.72 (s, 3H, H-12^1), 3.70 (q, 2H, H-8^2, J = 7.7 Hz), 3.56(s, 3H, H-17^4), 3.42 (s, 3H, H-2^1), 3.35 (s, 3H, H-7^1), 2.78 (m, 1H, H_a-17^1, J = 2.9 Hz), 2.67 (m, 1H, H_b-17^2), 2.36 (m, 1H, H_a'-17^1, J = 8.8 Hz), 2.32 (m, 1H, H_b'-17^2), 1.87 (d, 3H, H-18^1, J = 7.6 Hz), 1.75 (t, 3H, H-8^2, J = 7.7 Hz), 0.26 (br s, 1H, NH), –2.32 (br s, 1H, NH)

^{13}C NMR (75 MHz, 6.2 mg/0.6 mL $CDCl_3$) δ: 192.79 (C-13^1), 185.19 (C-13^2), 174.87 (C-17^3), 173.67 (C-19), 166.86 (C-16), 153.87 (C-6), 152.58 (C-9), 151.30 (C-14), 144.71 (C-8), 142.33 (C-1), 137.77 (C-11), 137.40 (C-3), 136.32 (C-4), 134.51 (C-7), 131.29 (C-2), 130.34 (C-13), 128.95 (C-3^1), 126.42 (C-12), 123.65 (C-3^2), 105.05 (C-10), 104.41 (C-15), 101.50 (C-5), 95.59 (C-20), 52.67 (C-17), 52.67 (C-17^4), 51.63 (C-18), 31.50 (C-17^1), 29.70 (C-17^2), 23.85 (C-18^1), 19.48 (C-8^1), 17.51 (C-8^2), 12.60 (C-12^1), 12.22 (C-2^1), 11.32 (C-7^1)

UV-Vis λ_{max} ($CHCl_3$) 390 nm (ε 49 500), 420 (42 900), 518 (6 900), 622 (3 300), 678 (29 000); λ_{max} (CH_3OH) 386 nm (ε 40 000), 514 (7 200), 622 (4 700), 676 (20 800) [lit.[89] λ_{max} (CH_3OH) 386 nm, 514, 618, 676]

LREIMS (m/z): 562 (M^+, 100%), 475 (M^+–$CH_2CH_2COOCH_3$, 96)

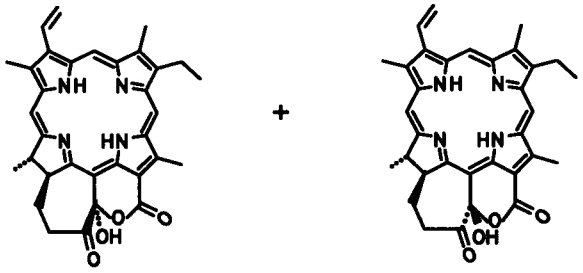
HREIMS: $C_{34}H_{34}N_4O_4$ (M^+): calcd 562.2580

obsd 562.2589

Anal. calcd for $C_{34}H_{34}N_4O_4$: C, 72.58; H, 6.09; N, 9.96 %

found: C, 72.09; H, 6.01; N, 9.80 %

15[1]-Hydroxychlorophyllonelactone *a* (83)



83(R) + **83(S)**

94% : **6%**

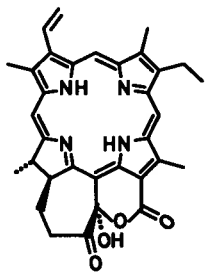
31 mg (57% yield) of a gray-green solid, recrystallized from dichloromethane/hexane.

M.p.: >300°C

Anal. calcd for $C_{33}H_{32}N_4O_4$: C, 72.24; H, 5.88; N, 10.21 %

found: C, 71.89; H, 6.01; N, 10.35 %

^{1}H NMR analysis showed that it is a diastereomeric mixture of 94% 15[1]R-hydroxychlorophyllonelactone *a* [**83**(R)] and 6% 15[1]S-hydroxychlorophyllonelactone *a* [**83**(S)]. Further purification of this diastereomeric mixture (14 mg) by preparative TLC on deactivated silica gel (developed three times by 5%acetone, 1%methanol in dichloromethane) gave the optically-pure 15[1]R-hydroxychlorophyllonelactone *a* [**83**(R)] (11.5 mg, 100% d.e. from ^{1}H NMR) of a dark green powder after recrystallization from dichloromethane/hexane.



83(R)

M.p.: >300°C

^{1}H NMR (400 MHz, 1.0 mg/0.6 mL $CDCl_3$) δ: 9.68 (s, 1H, H-10), 9.50 (s, 1H, H-5), 8.78 (s, 1H, H-20), 8.00 (dd, 1H, H-3^1, *J* = 17.6 and 11.2 Hz), 6.32 [dd, 1H, H-3^2(E), *J* = 17.6 and 1.0 Hz], 6.20 [d, 1H, H-3^2(Z), *J* = 11.2 and 1.0 Hz], 5.86 (br s, 1H, OH), 4.42 (ddd, 1H, H-17, *J* = 11.5, 5.3 and 1.6 Hz), 4.38 (dq, 1H, H-18, *J* = 7.5 and 1.6 Hz), 3.79 (s, 3H, H-12^1), 3.69 (q, 2H, H-8^1, *J* = 7.9 Hz), 3.49 (ddd, 1H, H_b-17^2, *J* = 11.5, 9.0 and 8.3 Hz), 3.42 (s, 3H, H-2^1), 3.33 (s, 3H, H-7^1), 3.01 (ddd, 1H, H_b'-17^2, *J* = 11.5, 9.6 and 2.5 Hz), 2.85 (dddd, 1H, H_a'-17^1, *J* = 12.8, 9.3, 9.0 and 5.3 Hz), 2.19 (dddd, 1H, H_a-17^1, *J* = 12.8, 11.5, 8.3 and 2.5 Hz), 1.84 (d, 3H, H-18^1, *J* = 7.5 Hz), 1.69 (t, 3H, H-8^2, *J* = 7.9 Hz), –0.93 (br s, 1H, NH), –1.45 (br s, 1H, NH)

^{13}C NMR (75 MHz, 8.5 mg/0.6 mL $CDCl_3$) δ: 203.62 (C-17^3), 173.31 (C-19), 163.85 (C-16), 162.57 (C-13^1), 155.84 (C-6), 150.35 (C-9), 145.68 (C-8), 141.89 (C-1), 138.89 (C-12), 136.59 (C-7), 136.39 (C-3), 136.06 (C-4), 134.07 (C-14), 131.62 (C-11), 131.45 (C-2), 128.96 (C-3^1), 123.01 (C-3^2), 111.69 (C-13), 104.68 (C-15^1), 104.61 (C-10), 100.24 (C-15), 99.82 (C-5), 93.44 (C-20), 51.18 (C-18), 49.84 (C-17), 34.17 (C-17^2), 31.37 (C-17^1), 23.56 (C-18^1), 19.54 (C-8^1), 17.57 (C-8^2), 12.44 (C-12^1), 12.15 (C-2^1), 11.33 (C-7^1)

UV-Vis λ_{max} ($CHCl_3$) 404 nm (ε 147 000), 500 (12 400), 532 (11 900), 614 (7 700), 670 (50 200); λ_{max} (CH_3OH) 400 nm (ε 164 000), 498 (14 200), 528 (12 500), 610 (8 200), 666 (53 000) [lit.[89] λ_{max} (CH_3OH) 400 nm, 498, 529, 610, 667]

LREIMS (m/z): 549 ($[MH]^+$, 50%)

HREIMS: $C_{33}H_{32}N_4O_4$ ($[MH]^+$): calcd 549.2502
obsd 549.2506

Purpurin-18 (22)

1.5 mg (2.7% yield). Treatment of this product with excess of ethereal diazomethane gave purpurin-18 methyl ester (**87**). After crystallization from dichloromethane/methanol, the product was found identical to **87** as described before.

13^2-Oxopyropheophorbide *a* (85)

1.0 mg (1.8% yield). Treatment of this product with excess of ethereal diazomethane gave 13^2-oxopyropheophorbide *a* methyl ester (**102**). After crystallization from dichloromethane/hexane, this material was found identical to **102** as described before.

Oxidation of 13^2-Hydroxychlorophyllone *a* (82) by H_5IO_6 in Pyridine

A solution of the foregoing (95%S, 5%R) mixture of 13^2-hydroxychlorophyllone *a* (**82**) (27 mg, 0.05 mmol) in dioxane (20 mL) and pyridine (15 mL) was stirred with an aqueous solution (20 mL) of periodic acid dihydrate (1 g, 4.38 mmol) at room temperature for 18 h before the mixture was extracted with dichloromethane (60 mL). The organic layer was washed with water 3 times before it was dried over sodium sulphate, filtered and evaporated *in vacuo*. The residue was separated by preparative TLC on deactivated silica gel (developed twice by 5% acetone, 1% methanol in dichloromethane), giving 3 distinct bands: the most mobile band (gray-green, **83**); the second mobile band (purple-red, **22**) and the least mobile band (yellow, **85**).

15^1-Hydroxychlorophyllonelactone *a* (83)

7.7 mg (28% yield) as a gray-green solid, recrystallized from dichloromethane/hexane.

M.p.: >300°C

This material was analyzed by ^{1}H NMR as a diastereomeric mixture of 92% 15^{1}R-hydroxychlorophyllonelactone *a* [**83**(R)] and 8% 15^{1}S-hydroxychlorophyllone-lactone *a* [**83**(S)].

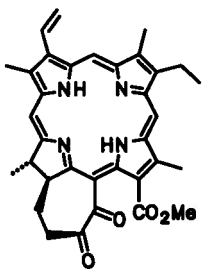
Purpurin-18 (22)

3.2 mg (11.3% yield). Treatment of this product with excess of ethereal diazomethane gave purpurin-18 methyl ester (**87**). After crystallization from dichloromethane/methanol, the product was found identical to **87** as described previously.

13^2-Oxopyropheophorbide *a* (85)

13.4 mg (49% yield). Treatment of this product with an excess of ethereal diazomethane, followed by recrystallization from dichloromethane/hexane gave 13^2-oxopyropheophorbide *a* methyl ester (**102**) (12.7 mg). This material was found identical to the product **102** as described previously.

Chlorophyllonic acid *a* methyl ester (84)



84

A solution of the foregoing (94%R, 6%S) mixture of 15^1-hydroxychlorophyllonelactone *a* (**83**) (8 mg, 0.0146 mmol) in dichloromethane (20 mL) was treated with an excess of ethereal diazomethane and then washed with water 3 times, dried over sodium sulphate, filtered and evaporated *in vacuo*. The product was crystallized from dichloromethane/hexane, giving the title compound (6.7 mg, 82%) of a gray-brown solid.

M.p.: 219°C

^{1}H NMR (400 MHz, 1.0 mg/0.6 mL $CDCl_3$) δ: 9.70 (s, 1H, H-10), 9.49 (s, 1H, H-5), 8.60 (s, 1H, H-20), 7.96 [dd, 1H, H-3^1, J = 17.4 and 1.0 Hz], 6.30 [dd, 1H, H-3^2(E), J = 17.4 and 1.0 Hz], 6.15 (dd, 1H, H-3^2(Z), J = 12.1 and 1.0 Hz), 4.53 (ddd, 1H, H-17, J = 12.3, 6.6 and 1.7 Hz), 4.40 (dq, 1H, H-18, J = 7.3 and 1.0 Hz), 4.03(s, 3H, H-13^2), 3.83 (ddd, 1H, H_b-17^2, J = 12.7, 10.0 and 10.0 Hz), 3.70 (q, 2H, H-8^1, J = 7.7 Hz), 3.60 (s, 3H, H-12^1), 3.38 (s, 3H, H-2^1), 3.21 (s, 3H, H-7^1), 3.05 (ddd, 1H, H_b'-17^2, J = 12.7, 8.3 and 1.4 Hz), 2.90 (dddd, 1H, H_a'-17^1, J = 12.3, 10.0, 8.3 and 6.6 Hz), 2.38 (dddd, 1H, H_a-17^1, J = 12.3, 12.3, 10.0 and 1.4 Hz), 1.73 (d, 3H, H-18^1, J = 7.3 Hz), 1.67 (t, 3H, H-8^2, J = 7.7 Hz), –0.68 (br s, 2H, NH)

^{13}C NMR (75 MHz, 6.5 mg/0.6 mL $CDCl_3$) δ: 196.90 (C-17^3), 192.38 (C-15^1), 173.24 (C-19), 166.90 (C-13^1), 164.07 (C-16), 155.22 (C-6), 149.61 (C-9), 145.48 (C-8), 142.09 (C-1), 138.42 (C-12), 136.38 (C-3), 136.31 (C-4), 135.61 (C-7), 135.07 (C-14), 130.82 (C-2), 130.12 (C-11), 128.85 (C-3^1), 122.77 (C-3^2), 121.12 (C-13), 108.52 (C-15), 105.87 (C-10), 101.48 (C-5), 93.41 (C-20), 52.31 (C-13^2), 51.20 (C-18), 50.16 (C-17),

36.25 (C-17^2), 29.47 (C-17^1), 23.62 (C-18^1), 19.49 (C-8^1), 17.56 (C-8^2), 12.57 (C-12^1), 12.00 (C-2^1), 11.17 (C-7^1)

UV-Vis λ_{max} ($CHCl_3$) 408 nm (ε 163 000), 504 (14 500), 544 (20 000), 628 (10 500), 680 (53 000), 688 (53 000); λ_{max} (CH_3OH) 400 nm (ε 170 000), 504 (15 400), 540 (20 000), 628 (11 000), 678 (52 400), 686 (sh 46 300) [lit.[89] λ_{max} (CH_3OH) 400 nm, 504, 540, 610, 677]

LRFABMS (m/z): 563 ([MH]$^+$, 55%)

HRFABMS: $C_{34}H_{35}N_4O_4$ ([MH]$^+$): calcd 563.2658

obsd 563.2665

Anal. calcd for $C_{34}H_{34}N_4O_4$ C, 72.58; H, 6.09; N, 9.96 %

found: C, 72.19; H, 5.92; N, 9.85 %

Oxidation of 15^1-Hydroxychlorophyllonelactone *a* (83) by H_5IO_6

A solution of the foregoing (94%R, 6%S) mixture of 15^1-hydroxychlorophyllonelactone *a* (**83**) (5.5 mg, 0.01 mmol) in dioxane (10 mL) was stirred with an aqueous solution (10 mL) of periodic acid dihydrate (50 mg, 0.22 mmol) at room temperature for 8 h before the mixture was extracted with dichloromethane (30 mL). The organic layer was washed with water 3 times before it was dried over sodium sulphate, filtered and evaporated *in vacuo*. The residue was redissolved in dichloromethane and treated with an excess of ethereal diazomethane. The product was purified by chromatography on silica gel, eluting with dichloromethane. After crystallized from dichloromethane/methanol, purpurin-18 methyl ester (**87**) (4.5 mg,

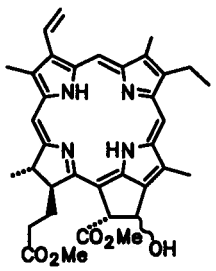
78%) was obtained as a purple-red shiny flakes, which was identical to the material prepared from previous methods.

Oxidation of 13^2-Oxopyropheophorbide *a* Methyl Ester (84) by H_5IO_6

A solution of the foregoing 13^2-oxopyropheophorbide *a* methyl ester (**102**) (3.5 mg, 6.2 μmol) in dioxane (5 mL) was stirred with an aqueous solution (4 mL) of periodic acid dihydrate (25 mg, 0.11 mmol) at room temperature for 6 h before the mixture was extracted with dichloromethane (20 mL). The organic layer was washed with water 3 times before it was dried over sodium sulphate, filtered and evaporated *in vacuo*. The residue was purified by chromatography on silica gel, eluting with dichloromethane. The product was crystallized from dichloromethane/methanol, giving purpurin-18 methyl ester (**87**) (3.0 mg, 84%) of a purple-red shiny flakes, which was found identical to the material prepared from previous methods.

6.3 Synthesis of Phytoporphyrins

13^1-Deoxo-13^1-hydroxypheophorbide *a* methyl ester (112a)



112a

Pheophorbide *a* methyl ester (**7**) (100 mg, 0.16 mmol) was dissolved in 50 mL of pyridine/methanol solution (pyridine : methanol = 1:1) and the solution stirred at room temperature. $NaBH_4$ (90 mg) was added and the reaction mixture was stirred for 2 h at which time the UV/Vis spectrum indicated no more starting material. The reaction mixture (bright green) was poured into cooled 2N HCl and the chlorin was extracted with CH_2Cl_2. The green organic layer was carefully washed once with water, twice with saturated $NaHCO_3$, three times with water, and then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed on a alumina III column eluting with 1%MeOH in CH_2Cl_2. The main band was collected, evaporated and recrystallized from $CHCl_3$/hexane to give the required compound (83.7 mg, 86% yield) as a dark-green powder.

M.p.: 135-136°C

^{1}H NMR (200 MHz, $CDCl_3$) δ: (the major epimer) 9.84 (s, 1H, H-10), 9.60 (s, 1H, H-5), 8.88 (s, 1H, H-20), 8.20 (dd, 1H, H-3^1), 6.63 (dd, 1H, H-13^1), 6.34 [dd, 1H, H-3^2(E)], 6.18[s, 1H, H-3^2(Z)], 5.90 (s, 1H, H-13^2), 4.60 (m, 1H, H-18), 4.45 (m, 1H, H-17), 3.81, 3.55, 3.55, 3.39, 3.22 (5s, 15H, H-2^1, H-7^1, H-12^1, H-13^4 and H-17^4), 3.80 (q, 2H, H-8^1), 2-2.70 (m, 4H, H-17^1 and H-17^2), 1.90 (d, 3H, H-18^1), 1.75 (t, 3H, H-8^2), –1.39 (br s, 1H, NH), –3.05 (br s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 400 nm (ε 124 400), 498 (12 000), 656 (35 800)

LREIMS (m/z): 608 (M^+, 100%)

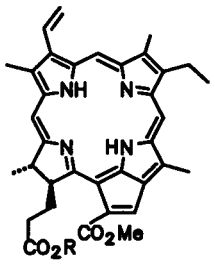
HREIMS: $C_{36}H_{40}N_4O_5$ (M^+): calcd 608.2999

obsd 608.2993

Anal. calcd for $C_{36}H_{40}N_4O_5$: C, 71.03; H, 6.62; N, 9.20 %

found: C, 70.81; H, 6.79; N, 8.97 %

13^1-Deoxo-13^1, 13^2-dehydropheophorbide *a* methyl ester (110a)



110 a

13^1-Deoxo-13^1-hydroxypheophorbide *a* methyl ester (**112**a) (50 mg, 0.0822 mmol) was dissolved in 20 mL of pyridine and the solution stirred at 0°C under N_2. Excess trifluoroacetylimidazole (150 mg) was added via a syringe and the reaction mixture was stirred at this temperature for 5 minutes. After addition of proton sponge (1.81 mmol), the reaction mixture was kept at 0°C for 30 minutes and 25°C for 3 hours at which time the UV/Vis spectrum indicated no more starting material. The reaction mixture (bright green) was poured into water and the chlorin was extracted with CH_2Cl_2. The green organic layer was washed three times with water, and then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was chromatographed on a silica column eluting with CH_2Cl_2. The main band was collected, evaporated and the residue was recrystallized from $CHCl_3$/MeOH to give the title compound (45.6 mg, 94% yield) as a dark-green powder.

M.p.: >300°C

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 8.41(s, 1H, H-10), 8.33 (s, 1H, H-5), 7.42 (s, 1H, H-20), 7.42 (dd, 1H, H-3^1, J = 18.5 and 11.8 Hz), 7.19 (s, 1H, H-13^1), 6.01

[d, 1H, H-3^2(E), J = 18.5 Hz], 5.94 [d, 1H, H-3^2(Z), J = 11.8 Hz], 4.77 (d, 1H, H-17, J = 8.6 Hz), 3.93 (s, 3H, H-13^4), 3.77 (q, 1H, H-18, J = 8.5 Hz), 3.57 (s, 3H, H-17^4), 3.25 (q, 2H, H-8^1, J = 7.1 Hz), 2.96 (s, 3H, H-12^1), 2.91 (s, 3H, H-2^1), 2.80 (s, 3H, H-7^1), 2.44 (dd, 1H, H-17^1, J = 8.6 Hz), 2.20 (dd, 2H, H-17^2), 1.85 (dd, 1H, H-17^1), 1.69 (d, 3H, H-18^1, J = 8.5 Hz), 1.45 (t, 3H, H-8^2, J = 7.1 Hz)

UV/Vis λ_{max} ($CHCl_3$) 354 nm (ε 55 600), 434 (67 900), 586 (4 100), 630 (11 600), 760 (4 100), 820 (4 100)

LREIMS (m/z): 590 (M^+, 100%)

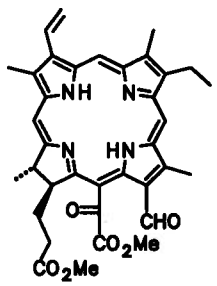
HREIMS: $C_{36}H_{38}N_4O_4$ (M^+): calcd 590.2893

obsd 590.2895

Anal. calcd for $C_{36}H_{38}N_4O_4$: C, 73.21; H, 6.48; N, 9.48 %

found: C, 72.73; H, 6.53; N, 9.21 %

13-Decarboxy-13-formyl-purpurin-7 dimethyl ester (113)



113

A solution of 13^1-deoxo-13^1,13^2-dehydropheophorbide *a* methyl ester (**110**a) (20 mg, 0.0339 mmol) in CH_2Cl_2 (100 mL) was stirred in Vancouver sunlight for 20 minutes. Removal of solvent *in vacuo* and crystallization from CH_2Cl_2/hexane gave a brown powder of the aldehyde **113** (21 mg, 100% yield).

M.p.: 216-217°C

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 10.93 (s, 1H, H-13^1), 9.50 (s, 1H, H-10), 9.20 (s, 1H, H-5), 8.45 (s, 1H, H-20), 7.90 (dd, 1H, H-3^1, *J* = 18.2 and 11.9 Hz), 6.30 [d, 1H, H-3^2(E), *J* = 18.2 Hz], 6.11 [d, 1H, H-3^2(Z), *J* = 11.9 Hz], 4.48 (dd, 1H, H-17, *J* = 9.4 Hz), 4.28 (q, 1H, H-18, *J* = 9.0 Hz), 3.93 (s, 3H, H-12^1), 3.66 (s, 3H, H-17^4), 3.61 (q, 2H, H-8^1, *J* = 7.5 Hz), 3.52 (s, 3H, H-15^3), 3.30 (s, 3H, H-2^1), 3.13 (s, 3H, H-7^1), 2.35 (m, 1H, H-17^1), 2.10 (m, 2H, H-17^2), 1.80 (m, 1H, H-17^1), 1.73 (d, 3H, H-18^1, *J* = 9.0 Hz), 1.65 (t, 3H, H-8^2, *J* = 7.5 Hz), 0.2 (br s, 2H, NH)

^{13}C NMR (75 MHz, 15 mg/1.0 mL $CDCl_3$) δ: 186.83 (C-13^1), 184.92 (C-15^1), 174.85 (C-15^2), 173.30 (C-17^3), 168.22 (C-19), 163.28 (C-16), 157.05 (C-6), 149.12 (C-9), 145.98 (C-14), 143.24 (C-8), 140.40 (C-1), 137.34 (C-11), 136.62 (C-3), 135.97 (C-4), 134.20 (C-7), 131.90 (C-2), 129.56 (C-12), 124.36 (C-13), 128.57 (C-3^1), 122.89 (C-3^2), 106.90 (C-5), 105.80 (C-15), 100.45 (C-10), 93.74 (C-20), 53.27 (C-17), 52.63 (C-15^3), 51.53 (C-17^4), 49.71 (C-18), 31.44 (C-17^2), 30.93 (C-17^1), 22.78 (C-18^1), 19.40 (C-8^1), 17.47 (C-8^2), 11.90 (C-12^1), 11.39 (C-2^1), 11.06 (C-7^1)

UV/Vis λ_{max} (CH_2Cl_2) 414 nm (ε 113 200), 508 (7 400), 544 (10 600), 630 (7 000), 680 (35 400), 686 (31 700)

LREIMS (m/z): 622 (M^+, 15%), 563 (M^+ – COOMe, 27%), 535 (M^+ – COOMe – CO, 27%)

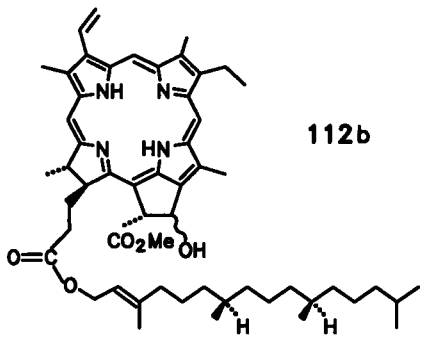
HREIMS: $C_{36}H_{38}N_4O_6$ (M^+): calcd 622.2791

obsd 622.2786

Anal. calcd for $C_{36}H_{38}N_4O_6$: C, 69.44; H, 6.15; N, 9.00 %

found: C, 69.11; H, 5.93; N, 8.92 %

13^1-Deoxo-13^1-hydroxypheophytin *a* (112b)



112b

Pheophytin *a* (**5**) (50 mg, 0.057 mmol) was dissolved in 30 mL of pyridine/methanol solution (pyridine: methanol = 1:1) and the solution stirred at room temperature. $NaBH_4$ (50 mg) was added and the reaction mixture was stirred for 2 h at which time the UV/Vis spectrum indicated no more starting material. The green reaction mixture was poured into ice-cooled saturated NH_4Cl and the chlorin was extracted with CH_2Cl_2. The organic layer was carefully washed three times with water, and then dried over anhydrous $NaSO_4$, filtered and evaporated. The residue was purified by flash chromatography on silica III eluting with 1%MeOH in CH_2Cl_2. The main band, after evaporation, gave the title compound (41.2 mg, 83% yield) as a black solid.

M.p.: 124-126°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: (the major epimer) 9.89 (s, 1H, H-10), 9.68 (s, 1H, H-5), 8.89 (s, 1H, H-20), 8.20 (dd, 1H, H-3^1), 6.70 (dd, 1H, H-13^1), 6.38 [dd, 1H, H-3^2(E)], 6.20 [s, 1H, H-3^2(Z)], 5.95 (s, 1H, H-13^2), 5.20 (t, 1H, H-P^2), 4.60 (m, 2H, H-18 and H-P^1), 4.50 (m, 1H, H-17), 3.83, 3.72, 3.65, 3.55, 3.40, 3.15 (5s, 15H, H-2^1, H-7^1, H-12^1, H-13^4 and H-17^4), 3.80 (q, 2H, H-8^1), 2-2.70 (m, 4H, H-17^1 and H-17^2), 1.90 (d, 3H, H-

18^1), 1.75 (t, 3H, H-8^2), 0.82-1.90 (m, 38H, H-Phytyl), –1.35 (br s, 1H, NH), –3.12 (br s, 1H, NH)

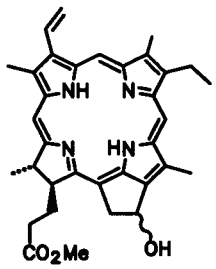
UV/Vis λ_{max} (CH_2Cl_2) 400 nm (ε 156 600), 498 (16 000), 656 (46 500)

LRFABMS (m/z): 874 ($[M+ 2H]^+$, 100%)

HRFABMS: $C_{55}H_{79}N_4O_5$ ($[M+ 2H]^+$): calcd 874.5972

obsd 874.5972

13^1-Deoxo-13^1-hydroxypyropheophorbide *a* methyl ester (112c)



112c

Pyropheophorbide *a* methyl ester (**25**) (150 mg, 0.274 mmol) was dissolved in 100 mL of pyridine/methanol solution (pyridine:methanol = 1:1) and the solution stirred at room temperature. $NaBH_4$ (200 mg) was added and the reaction mixture was stirred for 2 h at which time the UV/Vis spectrum indicated no more starting material. The green reaction mixture was poured into cooled 2N HCl and the chlorin was extracted with CH_2Cl_2. The organic layer was carefully washed once with water, twice with saturated $NaHCO_3$, three times with water, and then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatography on silica III eluting with 1% MeOH in CH_2Cl_2. The main band was collected, evaporated and recrystallized from CH_2Cl_2/hexane to give the title compound (129 mg, 86% yield) as a dark-green powder.

M.p.: 178-180°C

^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.98 (s, 1H, H-10), 9.82 (s, 1H, H-5), 9.05 (s, 1H, H-20), 8.30 (dd, 1H, H-3^1), 6.45 [dd, 1H, H-3^2(E)], 6.32 [s, 1H, H-3^2(Z)], 6.27 (dd, 1H, H-13^1), 5.30 (m, 1H, H-13^1), 4.48 (m, 2H, 13^2), 4.20 (m, 1H, H-18), 4.10 (m, 1H, H-17), 3.90 (q, 2H, H-8^1), 3.83, 3.72, 3.65, 3.40, 3.15 (5s, 12H, H-2^1, H-7^1, H-12^1 and H-17^4), 2.70 (m, 2H, H-17^1), 2.30 (m, 2H, H-17^2), 1.95 (d, 3H, H-18^1), 1.82 (t, 3H, H-8^2), –1.40 (br s, 1H, NH), –3.10 (br s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 402 nm (ε 117 600), 502 (9 100), 654 (32 900)

LREIMS (m/z): 550 (M^+, 5%), 532 ($M^+ - H_2O$, 100%)

LRFABMS (m/z): 551 ($[M+H]^+$, 100%)

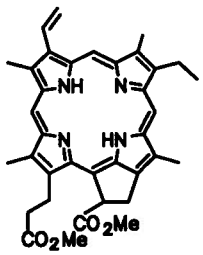
HREIMS: $C_{34}H_{36}N_4O_3$ ($[M-H_2O]^+$): calcd 532.2838

obsd 532.2828

HRFABMS: $C_{34}H_{39}N_4O_4$ ($[M+H]^+$): calcd 551.3021

obsd 551.3019

3-Vinyl-13^1-deoxo-13^2-methoxycarbonyl-phytoporphyrin methyl ester (114a)



114a

Method A 13^1-Deoxo-13^1,13^2-dehydropheophorbide *a* methyl ester (**110**a) (20 mg, 0.0339 mmol) was dissolved in dry DMF (15 mL) and the solution stirred at

100°C under N_2. Benzoyl chloride (0.041 mmol) was injected via a syringe and the reaction mixture (which turned red immediately) was stirred for 20 minutes at which time the UV/Vis spectrum indicated no more starting material. The reaction mixture was poured into ice-cooled 10% NaOH and the porphyrin was extracted with CH_2Cl_2. The organic layer was carefully washed three times with water, dried over anhydrous Na_2SO_4, filtered and evaporated. Recrystallization of the residue from CH_2Cl_2/hexane gave the title compound (19.2 mg, 100% yield) as violet prisms.

Method B 13^1-Deoxo-13^1-hydroxypheophorbide *a* methyl ester (**112**a) (50 mg, 0.0822 mmol) was dissolved in dry DMF (35 mL) and the solution stirred at 100°C under N_2. Benzoyl chloride (0.106 mmol) was added via a syringe and the reaction mixture (which turned red in 15 minutes) was stirred at this temperature for 45 minutes at which time the UV/Vis spectrum indicated no more starting material. The reaction mixture was poured into ice-cooled 10% NaOH and the porphyrin was extracted with CH_2Cl_2. The organic layer was washed three times with water, and then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatograph on silica eluting with CH_2Cl_2. The main band was collected, evaporated and the residue was recrystallized from CH_2Cl_2/hexane to give the title compound (42.1 mg, 87% yield) as violet prisms.

M.p.: 250°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.19 (s, 1H, H-10), 10.15 (s, 1H, H-5), 10.00 (s, 1H, H-20), 8.27 (dd, 1H, H-3^1, J = 17.5 and 11.8 Hz), 6.69 (dd, 1H, H-13^2, J = 7.7 Hz), 6.27 [dd, 1H, H-3^2(E), J = 17.5 and 1.5 Hz], 6.12 [s, 1H, H-3^2(Z), J = 11.8 and 1.5 Hz], 4.54

[dd, 1H, H-13^1(E), J = 17.5 and 7.7 Hz], 4.18 [dd, 1H, H-13^1(Z), J = 17.5], 4.42 (ddd, 1H, H-17^1), 4.31 (ddd, 1H, H-17^1), 4.10 (q, 2H, H-8^1, J = 7.7 Hz), 3.72 (s, 3H, H-12^1), 3.69 (s, 3H, H-13^4), 3.66 (s, 3H, H-17^4), 3.64 (s, 3H, H-7^1), 3.63 (s, 3H, H-18^1), 3.56 (s, 3H, H-2^1), 3.12 (dt, 1H, H-17^2), 3.00 (dt, 1H, H-17^2), 1.87 (t, 3H, H-8^2, J = 7.7 Hz), –3.00 (s, 1H, NH), –3.73 (s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 404 nm (ε 179 800), 504 (13 600), 540 (6 900), 568 (7 300), 618 (4 800)

LREIMS (m/z): 590 (M^+, 100%), 531 (M^+– COOMe, 88%)

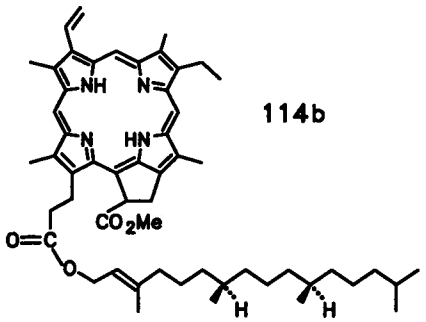
HREIMS: $C_{36}H_{38}N_4O_4$ (M^+): calcd 590.2893

obsd 590.2886

Anal. calcd for $C_{36}H_{38}N_4O_4$: C, 73.20; H, 6.48; N, 9.48 %

found: C, 73.51; H, 6.62; N, 9.68 %

3-Vinyl-13^1-deoxo-13^2-methoxycarbonylphytoporphyrin phytyl ester (114b)



114b

13^1-Deoxo-13^1-hydroxypheophytin *a* (**112**b) (35 mg, 0.040 mmol) was dissolved in dry DMF (20 mL) and the solution stirred at 100°C under N_2. Benzoyl chloride (0.052 mmol) was added via a syringe and the reaction mixture (which turned red in 15 minutes)

was stirred at this temperature for 60 minutes at which time the UV/Vis spectrum indicated no more starting material. The reaction mixture was poured into ice-cooled saturated $NaHCO_3$ and the porphyrin was extracted with CH_2Cl_2. The organic layer was washed three times with water, and then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatograph on silica eluting with CH_2Cl_2. The main band was collected, evaporated and the residue was recrystallized from CH_2Cl_2/hexane to give the title compound (27.5 mg, 81% yield) as a black-purple solid.

M.p.: 137-138°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.58 (s, 1H, H-10), 9.50 (s, 1H, H-5), 9.40 (s, 1H, H-20), 8.14 (dd, 1H, H-3^1, J = 18.2 and 12.8 Hz), 6.35 (d, 1H, H-13^2, J = 7.3 Hz), 6.25 [dd, 1H, H-3^2(E), J = 18.2 and 1.5 Hz], 6.10 [s, 1H, H-3^2(Z), J = 12.8 and 1.5 Hz], 5.40 (t, 1H, H-P^2, J = 7.3 Hz), 4.74 (d, 2H, H-P^1, J = 7.3 Hz), 4.47 [dd, 1H, H-13^1(E), J = 17.8 and 8.4 Hz], 4.10 [dd, 1H, H-13^1(Z), J = 17.8 and 7.3 Hz], 3.98 (m, 2H, H-17^1), 3.82 (q, 2H, H-8^1, J = 7.8 Hz), 3.67 (s, 3H, H-12^1), 3.64 (s, 3H, H-13^4), 3.52 (s, 3H, H-7^1), 3.46 (s, 3H, H-18^1), 3.34 (s, 3H, H-2^1), 2.96 (m, 1H, H-17^2), 2.73 (m, 1H, H-17^2), 1.72 (t, 3H, H-8^2, J = 7.8 Hz), 0.8-2.0 (m, 33H, H-phytyl), –3.20 (br s, 1H, NH), –3.95 (br s, 1H, NH)

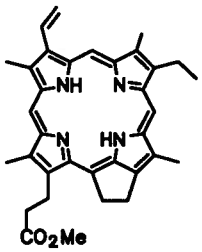
UV/Vis λ_{max} (CH_2Cl_2) 408 nm (ε 165 500), 506 (9 400), 538 (14 000), 568 (12 600), 580 (11 200)

LRFABMS (m/z): 856 ([M+ 2H]$^+$, 100%), 577 ([M+ 2H]$^+$– phytyl, 69%)

HRFABMS: $C_{55}H_{77}N_4O_4$ (M^+): calcd 856.5866

obsd 856.5850

3-Vinyl-13^1-deoxo-phytoporphyrin methyl ester (114c)



114 c

13^1-Deoxo-13^1-hydroxypyropheophorbide *a* methyl ester (**112c**) (105 mg, 0.191 mmol) was dissolved in dry DMF (50 mL) and the solution stirred at 100°C under N_2. Benzoyl chloride (0.250 mmol) was added via a syringe and the reaction mixture (which turned red in 40 minutes) was stirred at this temperature for 120 minutes at which time the UV/Vis spectrum indicated no more starting material. The reaction mixture was poured into ice-cooled 10% NaOH and the porphyrin was extracted with CH_2Cl_2. The organic layer was washed three times with water, and then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatograph on silica eluting with CH_2Cl_2. The main band was collected, evaporated and the residue was recrystallized from CH_2Cl_2/hexane to give the title compound (74.0 mg, 73% yield) as a purple solid.

M.p.: > 300°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.10 (s, 1H, H-10), 9.99 (s, 1H, H-5), 9.93 (s, 1H, H-20), 8.26 (dd, 1H, H-3^1, *J* = 17.9 and 11.6 Hz), 6.27 [dd, 1H, H-3^2(E), *J* = 17.9 and 1.7 Hz], 6.11 [dd, 1H, H-3^2(Z), *J* = 11.6 and 1.7 Hz], 5.20 (br s, 2H, H-13^2), 4.25 (t, 2H, H-17^1, *J* = 8.3 Hz), 4.10 (q, 2H, H-8^1, *J* = 7.1 Hz), 3.96 (br s, 2H, H-13^1), 3.78 (s, 3H, H-12^1), 3.65 (s, 6H, H-17^4 and H-7^1), 3.59 (s, 3H, H-18^1), 3.52 (s, 3H, H-2^1), 3.03 (t, 2H,

H-17^2, J = 8.3 Hz), 1.86 (t, 3H, H-8^2, J = 7.1 Hz), –3.02 (br s, 1H, NH), –3.89 (br s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 404 nm (ε 161 300), 504 (15 900), 540 (7 800), 566 (8 300), 620 (5 800)

LREIMS (m/z): 532 (M^+, 100%), 459 (M^+– CH_2COOMe, 47%)

HREIMS: $C_{34}H_{36}N_4O_2$ (M^+): calcd 532.2838

obsd 532.2835

Anal. calcd for $C_{34}H_{36}N_4O_2$: C, 76.66; H, 6.81; N, 10.52 %

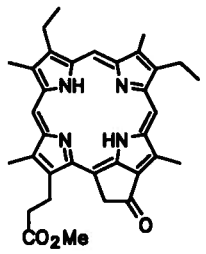
found: C, 76.50; H, 6.85; N, 10.58 %

Phytoporphyrin methyl ester (27) and 3-vinylphytoporphyrin methyl ester (116)

Pyropheophorbide *a* methyl ester (**25**) (45 mg, 0.0821 mmol) was dissolved in dry DMF (20 mL) and the solution stirred at 100°C under N_2. Benzoyl chloride (0.107 mmol) was added via a syringe and the reaction mixture was stirred at this temperature for 120 minutes after which time TLC analyses indicated no more formation of the products. The reaction mixture was poured into ice-cooled 10% NaOH and the porphyrin was extracted with CH_2Cl_2. The organic layer was washed three times with water, and then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatograph on silica eluting with CH_2Cl_2 to give the unreacted starting material **25** (20.0 mg, 44% yield), further eluting with 1% MeOH in CH_2Cl_2 to give the porphyrin band. The porphyrin band was then subjected to preparative TLC separation on silica

(0.5 cm thick) eluting with 2% MeOH/CH_2Cl_2 to give two bands. The separated bands containing the porphyrins were carefully dissolved in THF, filtered, evaporated and the residue was recrystallized from CH_2Cl_2/hexane to give 8.5 mg (19% yield of a black solid) of the phytoporphyrin **27** as the first product, followed by 6.7 mg (15% yield of a black solid) of the phytoporphyrin **116** as the other product.

Phytoporphyrin methyl ester (27)



27

M.p.: 234-236°C

^{1}H NMR (200MHz, $CDCl_3$) δ: 9.79 (s, 1H, H-10), 9.71 (s, 1H, H-5), 9.40 (s, 1H, H-20), 5.10 (br s, 2H, H-13^2), 3.68 (s, 3H, H-12^1), 3.64 (s, 3H, H-17^4), 3.60 (s, 3H, H-7^1), 3.58 (m, 2H, H-17^1), 3.50 (s, 3H, H-18^1), 3.40 (q, 4H, H-3^1 and H-8^1, J = 8 Hz), 3.25 (s, 3H, H-2^1), 2.78 (t, 2H, H-17^2), 1.90 (t, 6H, H-3^2 and H-8^2,J = 8 Hz), –3.35 (br s, 1H, NH), –4.21 (br s, 1H, NH)

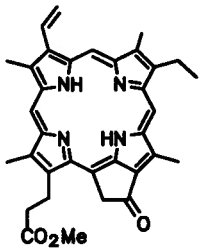
UV/Vis λ_{max} (CH_2Cl_2) 416 nm (ε 179 300), 524 (15 300), 564 (17 800), 586 (12 800), 636 (3 600)

LREIMS (m/z): 548 (M^+, 100%), 475 (M^+– CH_2COOMe, 26%)

HREIMS: $C_{34}H_{36}N_4O_3$ (M^+): calcd 548.2787

obsd 548.2796

3-Vinylphytoporphyrin methyl ester (116)



116

M.p.: 206-208°C

^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.80 (s, 1H, H-10), 9.60 (s, 1H, H-5), 9.30 (s, 1H, H-20), 8.18 (dd, 1H, H-3^1, J = 18.1 and 12.0 Hz), 6.30 [dd, 1H, H-3^2(E), J = 18.1 and 1.8 Hz), 6.19 [dd, 1H, H-3^2(Z), J =12.0 and 1.8 Hz], 5.01 (br s, 2H, H-13^2), 3.98 (q, 2H, H-8^1, J = 7.5 Hz), 3.70 (s, 3H, H-12^1), 3.64 (s, 3H, H-17^4), 3.59 (s, 3H, H-7^1), 3.59 (m, 1H, H-17^1), 3.50 (s, 3H, H-18^1), 3.22 (br s, 2H, H-17^2), 3.20 (s, 3H, H-2^1), 2.72 (s, 1H, H-17^1), 1.80 (t, 3H, H-8^2, J = 7.5 Hz), –3.70 (br s, 1H, NH), –3.80 (br s, 1H, NH)

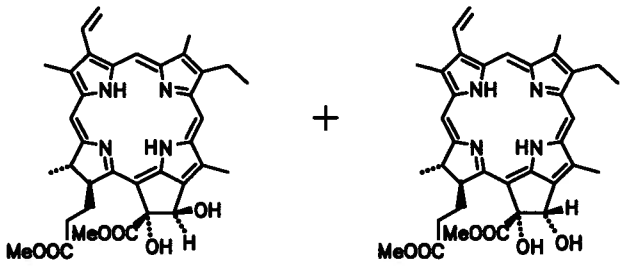
UV/Vis λ_{max} (CH_2Cl_2) 420 nm (ε 148 000), 526 (7 100), 568 (14 500), 590 (11 200), 640 (3 000)

LREIMS (m/z): 546 (M^+, 58%)

HREIMS: $C_{34}H_{34}N_4O_3$ (M^+): calcd 546.2631

obsd 546.2628

13^1-Deoxo-13^1-hydroxy-13^2R-hydroxypheophorbide *a* methyl ester (119)



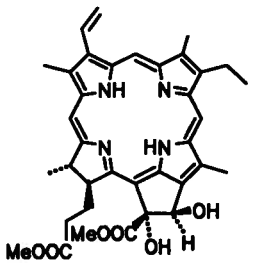
119RR : **119SR**

56% : **44%**

13^2R-Hydroxypheophorbide *a* methyl ester (**95**) (90% d.e.) (50 mg, 0.0803 mmol) was dissolved in 25 mL of pyridine/methanol solution (pyridine:methanol = 1:1) and the solution stirred at room temperature. $NaBH_4$ (70 mg) was added and the reaction mixture was stirred for 3 h at which time the UV/Vis spectrum indicated no more starting material. The reaction mixture was poured into ice-cooled 2N HCl and the chlorin was extracted with CH_2Cl_2. The organic layer was carefully washed once with water, twice with saturated $NaHCO_3$, three times with water, and then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatography on silica III eluting with 1% MeOH in CH_2Cl_2. The main band was collected (44.5 mg, 89% yield) and was analyzed by ^{1}H NMR to be a diastereomeric mixture of 56% 13^1-deoxo-13^1R-hydroxy-13^2R-hydroxypheophorbide *a* methyl ester (**119**RR) and 44% 13^1-deoxo-13^1S-hydroxy-13^2R-hydroxypheophorbide *a* methyl ester (**119**SR). Further purification of this diastereomeric mixture (20 mg) by preparative TLC on silica (developed by 2% MeOH, 5% acetone in dichloromethane) gave the optically-pure 13^1-Deoxo-13^1R-hydroxy-13^2R-

hydroxypheophorbide *a* methyl ester (**119**RR) (100% d.e. from ^{1}H NMR) (8.5 mg) as a green solid after recrystallization from CH_2Cl_2/hexane.

13^1-Deoxo-13^{1}R-hydroxy-13^{2}R-hydroxypheophorbide *a* methyl ester (119RR)



119RR

M.p.: 180°C

^{1}H NMR (400 MHz, 1.4 mg/0.6 mL $CDCl_3$) δ: 9.80 (s, 1H, H-10), 9.65 (s, 1H, H-5), 8.80 (s, 1H, H-20), 8.20 (dd, 1H, H-3^1, *J* = 18.3 and 12.0 Hz), 6.42 (br s, 1H, OH-13^2), 6.33 [dd, 1H, H-3^2(E), *J* =18.3 and 1.4 Hz], 6.16 [dd, 1H, H-3^2(Z), *J* = 12.0 and 1.4 Hz], 5.60 (s, 1H, H-13^1), 4.93 (d, 1H, H-17, *J* = 8.2 Hz), 4.59 (q, 1H, H-18, *J* = 7.5 Hz), 4.48 (m, 2H, 13^2), 3.81 (q, 2H, H-8^1, *J* = 7.6 Hz), 3.57 (s, 3H, H-17^4), 3.55 (s, 3H, H-13^4), 3.54 (s, 3H, H-12^1), 3.53 (s, 3H, H-7^1), 3.38 (s, 3H, H-2^1), 2.45 (ddd, 1H, H-17^1), 2.32 (ddd, 1H, H-17^2), 2.10 (ddd, 1H, H-17^1), 1.96 (ddd, 1H, H-17^2), 1.74 (d, 3H, H-18^1, *J* = 7.5 Hz), 1.71 (t, 3H, H-8^2, *J* = 7.6 Hz), –1.40 (br s, 1H, NH), –3.10 (br s, 1H, NH)

^{13}C NMR (75 MHz, 12 mg/1.0 mL $CDCl_3$) δ: 176.03 (C-13^3), 173.81 (C-17^3), 167.27 (C-19), 163.05 (C-16), 150.59 (C-6), 150.48 (C-9), 143.18 (C-14), 142.49 (C-8), 139.81 (C-1), 138.18 (C-11), 136.49 (C-3), 135.63 (C-4), 134.48 (C-7), 132.21 (C-2), 129.88 (C-3^1), 128.83 (C-12), 128.41 (C-13), 121.56 (C-3^2), 108.87 (C-15), 99.75 (C-10), 99.29 (C-5), 95.00 (C-13^2), 93.46 (C-20), 82.41 (C-13^1), 53.32 (C-13^4), 51.57 (C-17^4), 50.98 (C-17), 49.95 (C-18), 30.66 (C-17^1), 29.33 (C-17^2), 23.54 (C-18^1), 19.64 (C-8^1), 17.71 (C-8^2), 12.31 (C-12^1), 11.43 (C-2^1), 11.43 (C-7^1)

UV/Vis λ_{max} (CH_2Cl_2) 400 nm (ε 114 100), 498 (10 700), 656 (33 700), 682 (6 200)

LREIMS (m/z): 624 (M^+, 38%)

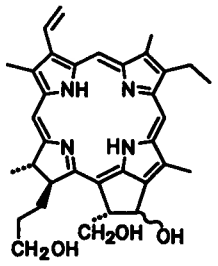
HREIMS: $C_{36}H_{40}N_4O_6$ (M^+): calcd 624.2948

obsd 624.2938

Anal. calcd for $C_{36}H_{40}N_4O_6$: C, 69.20; H, 6.46; N, 8.97 %

found: C, 68.97; H, 6.44; N, 8.70 %

13^1-Deoxo-13^1-hydroxy-13^2R-hydroxymethyl-17-depropionate-17-(γ–hydroxypropyl)pheophorbide *a* (120)



120

Pheophorbide *a* methyl ester (7) (50 mg, 0.0825 mmol) was dissolved in dried THF (50 mL) and the solution stirred at 0°C under N_2. $LiAlH_4$ (30 mg) was quickly added and the reaction mixture was stirred for 5h at which time the UV/Vis spectrum indicated no more starting material. The reaction was quenched by addition of ethyl acetate to destroy excess $LiAlH_4$; aqueous ammonium chloride was then introduced and the solution was extracted with CH_2Cl_2. The organic layer was carefully washed once with 2N HCl, once with water, twice with saturated $NaHCO_3$, three times with water, and then dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified by flash chromatography on silica III eluting with 2%MeOH in CH_2Cl_2. The main band was

collected and was subjected to further purification by preparative TLC on silica (developed by 2%MeOH, 5% acetone in dichloromethane) to give the title compound (32.1 mg, 72% yield) as a green solid after recrystallization from CH_2Cl_2/hexane.

M.p.: >300°C

^{1}H NMR (200 MHz, $CDCl_3$) δ: (the major epimer) 9.82 (s, 1H, H-10), 9.59 (s, 1H, H-5), 8.90 (s, 1H, H-20), 8.20 (dd, 1H, H-3^1), 6.32 [dd, 1H, H-3^2(E)], 6.16 [s, 1H, H-3^2(Z)], 5.18 (s, 1H, H-13^1), 6.00 (br s, 1H, OH-13^2), 4.58 (d, 1H, H-17), 4.38 (br s, 2H, H-17^3), 4.10 (m, 1H, H-18), 3.20-3.60 (4s, 12H, H-8^1, H-12^1, H-7^1 and H-2^1), 2.25 (m, 1H, H-17^1), 2.02 (m, 1H, H-17^1), 1.82 (d, 3H, H-18^1), 1.79 (m, 5H, H-17^2 and H-8^2), –3.35 (br s, 2H, NH)

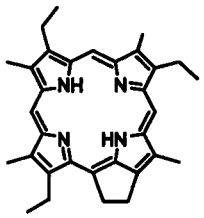
UV/Vis λ_{max} (CH_2Cl_2) 402 nm (ε 163 000), 500 (17 400), 654 (41 700)

LRFABMS (m/z): 553 ([M+ H]$^+$, 100%)

HRFABMS: $C_{34}H_{41}N_4O_3$ (M^+): calcd 553.3178

obsd 553.3165

Deoxophylloerythroetioporphyrin (121)



121

3-Vinyl-13^1-deoxo-phytoporphyrin methyl ester (**114**c) (10 mg, 0.0188 mmol) or 3-vinyl-13^1-deoxo-13^2-methoxycarbonyl-phytoporphyrin phytyl ester (**114**b) (16 mg, 0.0188 mmol) or 3-vinyl-13^1-deoxo-13^2-methoxycarbonyl-phytoporphyrin methyl ester

(**114**a) (11 mg, 0.0188 mmol) was saponified in the following conditions: **114**c, 10% KOH/MeOH (10 mL) at room temperature for 10 hours, **114**a or **114**b, 25% HCl (10 mL) at room temperature for 100 minutes. After that time TLC analyses showed good conversion to the desired mono-acid and/or diacids. The porphyrin acid (the product from the basic saponification was acidified with HCl to pH 3 before it was transferred) was transferred to a separatory funnel and diluted to 100 mL with ice. An equal portion of $CHCl_3$ was added to extract the aqueous layer. After the extraction was repeated 6 times, the combined organic layers were pooled, washed with water (100 mL). The organic layer was quickly dried with Na_2SO_4, filtered and evaporated *in vacuo*. The residue was dissolved in THF and tranferred to a small vial. The vial was kept at room temperature until the solvent had evaporated. The vial was then sealed with a small rubber septum and was dried (via a needle) under high vacuum (0.5 mm Hg) at 120°C for 8 hours. After that, 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) (0.6 g) was quickly squeezed into the vial and dried under high vacuum at 120°C for another 16 hours. The vial was then flame-sealed under high vacuum, was heated at 200°C for 4 hours, and, after cooling down, the vial was broken and the porphyrin mixture was immediately purified by flash chromatography on silica eluting with 2% MeOH in CH_2Cl_2. The main band was collected, evaporated and recrystallized from CH_2Cl_2/MeOH to give the title compound (5.9-6.7 mg, 67-75% yield) as a red solid.

M.p.: >300°C (decomp.)

^{1}H NMR (200 MHz, $CDCl_3$) δ: 9.96 (s, 1H, H-10), 9.90 (s, 1H, H-5), 9.89 (s, 1H, H-20), 5.32 (br s, 2H, H-13^2), 4.05 (br s, 2H, H-13^1), 4.10, 4.08, 4.00 (3q, 6H, H-3^1, H-8^1 and

H-17^1), 3.50-3.70 (4s, 12H, H-18^1, H-12^1, H-7^1 and H-2^1), 1.70-1.90 (3t, 9H, H-3^2, H-8^2 and H-17^2), –3.00 (br s, 1H, NH), –3.90 (br s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 402 nm (ε 195 000), 500 (15 000), 536 (3 000), 566 (5 200), 620 (6 100)

LREIMS (m/z): 476 (M^+, 100%)

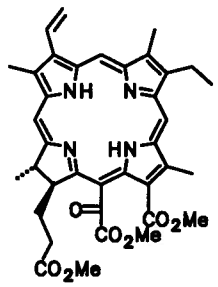
HRFABMS: $C_{32}H_{36}N_4$ (M^+): calcd 476.2940

obsd 476.2951

6.4 Synthesis of Benzo- and Dibenzo-porphyrin Derivatives

6.4.1 Synthesis of Regiochemically-pure Benzoporphyrin Derivatives

Purpurin-7 trimethyl ester (16)



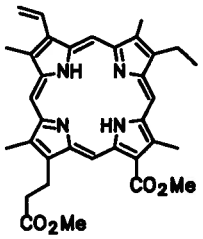
16

Method A Described in section **6.2.3**.

Method B Pheophytin *a* (**5**) (1.52 g, 1.75 mmol) was dissolved in warm pyridine (25 mL) and the solution was diluted with ether (700 mL). The solution was stirred with a stream of air passing through it, and a solution of potassium hydroxide (11.5 g) in *n*-propanol (40 mL) was added. The bright green mixture (containing precipitated KOH) was stirred and aerated for 30 min and then extracted with water (2 ×

300 mL). The ethereal solution was discarded; the aqueous extracts were combined, acidified with concentrated H_2SO_4 (11.5 mL) in water (60 mL), and then extracted with methylene chloride (2 × 350 mL). The extracts were washed with water (350 mL) and immediately treated with an excess of ethereal diazomethane. The brownish-purple soluion was left at room temperature for 10 min, and then evaporated *in vacuo*. Chromatography (alumina V) of the residue (elution with methylene chloride) gave a major band which was collected, evaporated and subjected to preparative TLC separation on deactivated silica plates (elution with 0.5% MeOH in CH_2Cl_2) to give the desired product (660 mg, 58% yield) after crystallization from CH_2Cl_2/hexane, along with 35 mg of a unseparated mixture of purpurin-18 methyl ester (**87**) and purpurin-7 trimethyl ester (**16**) as the other products. The corresponding physical data of this compound appear in section **6.2.3**.

3-Vinylrhodoporphyrin XV dimethyl ester (23)



23

Purpurin-7 trimethyl ester (**16**) (450 mg, 0.69 mmol) was heated in an oil-bath at 180-185°C in 2,4,6-collidine (60 mL) during 2 h. After cooling, the solvent was evaporated off at 0.5 mm Hg and the residue was recrystallized from $CHCl_3/CH_3OH$, giving the title porphyrin (295 mg, 76%) as purple needles.

M.p.: 266-267°C (lit.[52] 271-272.5°C)

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.81, 9.89, 9.82, 9.68 (4s, 4H, H-5, H-10, H-15 and H-20), 8.12 (dd, 1H, H-3^1, J = 18.4 and 11.8 Hz), 6.23 [dd, 1H, H-3^2(E), J = 18.4 and 1.6 Hz), 6.10 [dd, 1H, H-3^2(Z), J = 11.8 and 1.6 Hz], 4.42 (s, 3H, H-13^2), 4.30 (t, 2H, H-17^1, J = 8.0 Hz), 3.93 (q, 2H, H-8, J = 8.0 Hz), 3.84, 3.67, 3.52, 3.50, 3.48 (5s, 15H, H-2^1, H-7^1, H-12^1, H-18^1 and H-17^4), 3.23 (t, 2H, H-17^2, J = 8.0 Hz), 1.79 (t, 3H, H-8^2, J = 8.0 Hz), –4.50 (br s, 2H, NH)

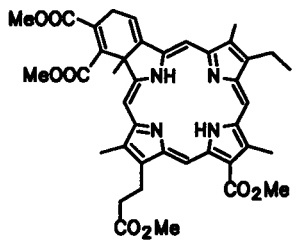
UV/Vis λ_{max} (CH_2Cl_2) 408 nm (ε 187 000), 514 (10 000), 552 (20 000), 576 (11 000), 636 (1 600)

LREIMS (m/z): 564 (M^+, 100%)

Anal. calcd for $C_{34}H_{36}N_4O_4$: C, 72.32; H, 6.43; N, 9.92 %

found: C, 71.98; H, 6.45; N, 9.65 %

2^1,2^2-Bis(methoxycarbonyl)-8-ethyl-13-methoxycarbonyl-17-methoxycarbonylethyl-2,7,12,18-tetramethyl-2^3-hydrobenzo[b]porphyrin (140)



140

3-Vinylrhodoporphyrin XV dimethyl ester (**23**) (57 mg, 0.1 mmol) and dimethyl acetylenedicarboxylate (710 mg, 5 mmol) were suspended in degassed toluene (N_2) (20 mL) and the mixture heated at 110°C for 28 hours. After removal of the toluene *in vacuo*, the residue was chromatographed on silica (methylene chloride eluant). After elution of the excess dienophile, the solvent polarity was increased to 2% methanol in methylene

chloride to elute the major reaction product. After evaporation to dryness, the residue was recrystallized from CH_2Cl_2/hexane to afford the 1,4-cyclohexadiene adduct **140** as a green solid (35 mg, 50% yield).

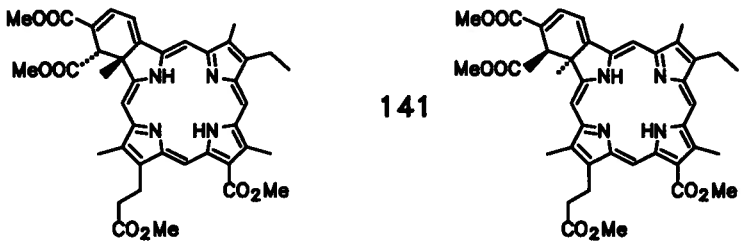
M.p.: 244-245°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.75, 9.80, 9.25, 9.01 (4s, 4H, H-5, H-10, H-15 and H-20), 7.40 (dd, 1H, H-3^1, J = 7.4 and 2.1 Hz), 4.31 (t, 2H, H-17^1, J = 8.3 Hz), 3.95 (dd, 1H, H-2^3, J = 3.0 and 0.5), 3.94 (q, 2H, H-8^1, J = 7.5 Hz), 3.62 (s, 1H, H-2^3, J =3.3 and 0.5 Hz), 3.25 (t, 2H, H-17^2, J = 8.3 Hz), 4.33, 4.02, 3.89, 3.75, 3.69, 3.45, 3.41 (7s, 21H, H-7^1, H-12^1, H-18^1, H-17^4, 2^1-$COOCH_3$ and 2^2-$COOCH_3$), 2.07 (s, 3H, 2-CH_3), 1.78 (t, 3H, H-8^2, J = 7.5 Hz), –2.26 (br s, 1H, NH), –2.30 (br s, 1H, NH)

UV/Vis λ_{max} (CH_3COCH_3) 410 nm (peak ratio, 1.40), 516 (0.119), 646 (0.138)

LREIMS (m/z): 706 (M^+, 100%), 690 ($M^+ - CH_4$, 60%)

2^1,2^2-Bis(methoxycarbonyl)-8-ethyl-13-methoxycarbonyl-17-methoxycarbonylethyl-2,7,12,18-tetramethyl-2^1-hydrobenzo[b]porphyrin (141)



The adduct **140** (32 mg, 0.045 mmol) was dissolved in methylene chloride and a few drops of DBU were added. The reaction mixture was stirred in the dark and monitored by visible spectroscopy (complete in 4 hours). The mixture was poured into 1M HCl and extracted with methylene chloride. The organic layer was washed twice

with brine, once with water and dried over anhydrous magnesium sulfate. The solvent was evaporated *in vacuo* and the compound was chromatographed on silica gel (1% MeOH in CH_2Cl_2 eluent). The pure 1,3-cyclohexadiene fraction was evaporated *in vacuo* yielding 29 mg (90% yield) of a dark green tiny needles after recrystallization from CH_2Cl_2/hexane.

M.p.: 248-249°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.70, 9.80, 9.30, 8.90 (4s, 4H, H-5, H-10, H-15 and H-20), 7.80 (d, 1H, H-2^4, J = 6.0 Hz), 7.42 (d, 1H, H-2^3, J = 6.0 Hz), 5.40 (s, 1H, H-2^1), 4.29 (t, 2H, H-17^1, J = 7.7 Hz), 3.92 (q, 2H, H-8^1, J = 7.9 Hz), 3.25 (t, 2H, H-17^2, J = 7.7 Hz), 4.34, 3.98, 3.75, 3.70, 3.43, 3.40, 2.98 (7s, 21H, H-7^1, H-12^1, H-18^1, H-17^4, 2^1-$COOCH_3$ and 2^2-$COOCH_3$), 1.79 (s, 3H, 2-CH_3), 1.76 (t, 3H, H-8^2, J = 7.9 Hz), –1.95 (br s, 1H, NH), –2.00 (br s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 356 nm (ε, 27 000), 440 (70 400), 592 (19 900), 672 (14 600)

LREIMS (m/z): 706 (M^+, 100%)

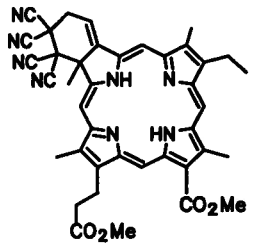
HREIMS: $C_{40}H_{42}N_4O_8$ (M^+): calcd 706.3002

obsd 706.3010

Anal. calcd for $C_{40}H_{42}N_4O_8$: C, 67.97; H, 5.99; N, 7.93 %

found: C, 68.03; H, 5.96; N, 7.92 %

$2^1,2^2$-Tetracyano-ethyl-13-methoxycarbonyl-17-methoxycarbonyl-ethyl-2,7,12,18-tetramethyl-2^3-hydrobenzo[b]porphyrin (142)



142

TCNE (50 mg) was added to 3-vinylrhodoporphyrin XV dimethyl ester (**23**) (57 mg, 0.1 mmol) dissolved in dry chloroform (20 mL) and the reaction mixture was refluxed for 1 hours (monitored by spectrophotometry). The solvent was evaporated and the residue was purified on an alumina column (Brockman Grade III; elution with methylene chloride). The eluates were collected and evaporated to give a residue which was crystallized from CH_2Cl_2/hexane to afford the title compound as a green powder (53 mg, 77% yield).

M.p.: 252-253°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.90, 9.90, 9.28, 9.19 (4s, 4H, H-5, H-10, H-15 and H-20), 7.03 (dd, 1H, H-2^4, J = 8.0 and 3.6 Hz), 4.34 (s, 3H, H-13^2), 4.32 (t, 2H, H-17^1, J = 7.6 Hz), 4.02 (d, 1H, H-2^3, J = 8.0 Hz), 3.98 (q, 2H, H-8^1, J = 7.3 Hz), 3.95 (d, 1H, H-2^3, J = 3.6 Hz), 3.29 (t, 2H, H-17^2, J = 7.6 Hz), 3.76, 3.69, 3.50, 3.42 (4s, 12H, H-7^1, H-12^1, H-18^1 and H-17^4), 2.37 (s, 3H, 2-CH_3), 1.80 (t, 3H, H-8^2, J = 7.3 Hz), –2.26 (br s, 1H, NH), –2.30 (br s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 414 nm (ε, 172 000), 518 (8 500), 552 (15 700), 588 (5 900), 642 (20 500)

LRFABMS (m/z): 692 (M^+, 13%)

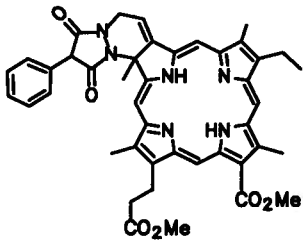
HRFABMS: $C_{40}H_{36}N_4O_8$ (M^+): calcd 692.2859

obsd 692.2853

Anal. calcd for $C_{40}H_{36}N_4O_8$: C, 69.35; H, 5.24; N, 16.17 %

found: C, 68.67; H, 5.44; N, 15.83 %

2^1,2^2-Diazo(*N*-phenylmaleimide)-ethyl-13-methoxycarbonyl-17-methoxycarbonylethyl-2,7,12,18-tetramethyl-2^3-hydrobenzo[b]-porphyrin (143)



143

4-Phenyl-1,2,4-triazoline-3,5-dione (25 mg) was added to 3-vinylrhodoporphyrin XV dimethyl ester (**23**) (29 mg, 0.05 mmol) dissolved in dry methylene chloride (10 mL) and the reaction mixture was stirred at 0°C for 4 hours (monitored by spectrophotometry). The solvent was evaporated and the residue was purified on an alumina column (Brockman Grade V; elution with methylene chloride). The eluates were collected and evaporated to give a residue which was crystallized from CH_2Cl_2/hexane to afford the title compound as a green powder (37 mg, 90% yield).

M.p.: 242°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.90, 10.80, 9.80, 9.10 (4s, 4H, H-5, H-10, H-15 and H-20), 7.80 (d, 2H, Ph-H, J = 7.2 Hz), 7.62 (dd, 2H, Ph-H, J = 8.1 and 7.2 Hz), 7.50 (t, 1H, J =8.1 Hz), 6.93 (t, 1H, H-2^4, J = 3.6 Hz), 5.10 (dd, 1H, H-2^3, J = 17 and 3.6 Hz), 4.59

(dd, 1H, H-2^3, J = 17 and 3.6 Hz), 4.33 (s, 3H, H-13^2), 4.30 (t, 2H, H-17^1, J = 7.3 Hz), 3.95 (q, 2H, H-8^1, J = 7.0 Hz), 3.22 (t, 2H, H-17^2, J = 7.3 Hz), 3.73, 3.69, 3.48, 3.41 (4s, 12H, H-7^1, H-12^1, H-18^1 and H-17^4), 2.08 (s, 3H, 2-CH_3), 1.79 (t, 3H, H-8^2, J = 7.0 Hz), –2.57 (br s, 1H, NH), –2.65 (br s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 414 nm (ε, 180 000), 518 (8 400), 552 (13 900), 584 (6 700), 640 (19 500)

LRFABMS (m/z): 739 (M^+, 10%)

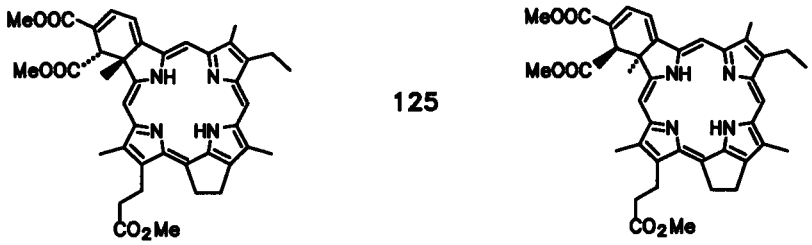
HRFABMS: $C_{42}H_{41}N_7O_6$ (M^+): calcd 739.3118

obsd 739.3105

Anal. calcd for $C_{40}H_{36}N_4O_8$: C, 68.19; H, 5.59; N, 13.25 %

found: C, 67.84; H, 5.52; N, 13.52 %

2^1,2^2-Bis(methoxycarbonyl)-13,15-ethano-8-ethyl-17-methoxycarbonyl-ethyl-2,7,12,18-tetramethyl-2^1-hydrobenzo[b]porphyrin (125)



125

Phytoporphyrin **114c** (27 mg, 0.05 mmol) and dimethyl acetylenedicarboxylate (360 mg, 25 mmol) were suspended in degassed toluene (N_2) (20 mL) and the mixture heated at 110°C for 30 hours. After removal of the toluene *in vacuo*, the residue was chromatographed on silica (methylene chloride eluant). After elution of the excess

dienophile, the solvent polarity was increased to 2% methanol in methylene chloride to elute the major reaction product. After evaporation to dryness, 14 mg (42% yield) of the 1,4-cyclohexadiene adduct **134** (λ_{max} = 404, 550, 650 nm) was obtained.

The adduct **134** (14 mg, 0.02 mmol) was dissolved in methylene chloride and a few drops of DBU were added. The reaction mixture was stirred in the dark and monitored by visible spectroscopy (completed in 6 hours). The mixture was poured into 1M HCl and extracted with methylene chloride. The organic layer was washed twice with brine, once with water and dried over anhydrous magnesium sulfate. The solvent was evaporated *in vacuo* and the compound was chromatographed on silica gel (1% MeOH in CH_2Cl_2 eluent). The pure 1,3-cyclohexadiene fraction was evaporated *in vacuo* yielding 12.7 mg (91% yield) of a dark green tiny needles after recrystallization from CH_2Cl_2/hexane.

M.p.: 258°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.59 (s, 1H, H-10), 9.30 (s, 1H, H-5), 8.95 (s, 1H, H-20), 7.83 (d, 1H, H-2^4, J = 5.8 Hz), 7.45 (d, 1H, H-2^3, J = 5.8 Hz), 5.20 (t, 2H, H-13^2), 5.05 (s, 1H, H-2^1), 4.30 (m, 2H, H-17^1), 3.93 (m, 2H, H-13^1), 3.93 (q, 2H, H-8^1, J = 7.9 Hz), 3.10 (t, 2H, H-17^2), 3.97, 3.78, 3.46, 3.42, 3.39, 2.92 (6s, 18H, H-7^1, H-12^1, H-18^1, H-17^4, 2^1-$COOCH_3$ and 2^2-$COOCH_3$), 1.78 (t, 3H, H-8^2, J = 7.9 Hz), 1.77 (s, 3H, 2-CH_3), –1.42 (br s, 1H, NH), –2.22 (br s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 354 nm (ε, 86 000), 438 (120 000), 580 (18 000), 620 (14 000), 680 (47 000), 686 (37 000)

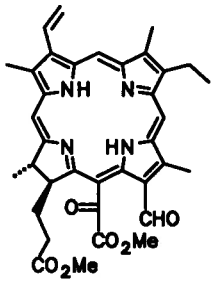
LRFABMS (m/z): 675 ($[M+H]^+$, 100%)

HRFABMS: $C_{40}H_{43}N_4O_6$ ([M+H]$^+$): calcd 675.3182

obsd 675.3176

6.4.2 Synthesis of [A,C]-Divinylporphyrin 147 via Purpurin 113

13-Decarboxy-13-formyl-purpurin-7 dimethyl ester (113)



113

Method A A solution of 13^1-Deoxo-13^1,13^2-dehydropheophorbide *a* methyl ester (**110**a) (20 mg, 0.0339 mmol) in CH_2Cl_2 (100 mL) was stirred in Vancouver sunlight for 20 minutes. Removal of solvent *in vacuo* and crystallization from CH_2Cl_2/hexane gave a brown powder of the aldehyde **113** (21 mg, 100% yield).

Method B A solution of 13^2-deoxo-13^1-hydroxy-13^2R-hydroxypheophorbide *a* methyl ester (**119**) (31 mg, 0.05 mmol) in dioxane (20 mL) was stirred with an aqueous solution (20 mL) of periodic acid dihydrate (900 mg, 3.95 mmol) at room temperature for 20 h before the mixture was extracted with dichloromethane (2 × 40 mL). The organic layer was dried over sodium sulphate, filtered and evaporated *in vacuo*. The residue was purified by flash chromatography on silica III, eluting with dichloromethane. Recrystallization from dichloromethane/hexane gave the title compound as a brown powder (24.8 mg, 80%).

M.p.: 216-217°C

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 10.93 (s, 1H, H-13^1), 9.50 (s, 1H, H-10), 9.20 (s, 1H, H-5), 8.45 (s, 1H, H-20), 7.90 (dd, 1H, H-3^1, J = 18.2 and 11.9 Hz), 6.30 [d, 1H, H-3^2(E), J = 18.2 Hz], 6.11 [d, 1H, H-3^2(Z), J = 11.9 Hz], 4.48 (dd, 1H, H-17, J = 9.4 Hz), 4.28 (q, 1H, H-18, J = 9.0 Hz), 3.93 (s, 3H, H-12^1), 3.66 (s, 3H, H-17^4), 3.61 (q, 2H, H-8^1, J = 7.5 Hz), 3.52 (s, 3H, H-15^3), 3.30 (s, 3H, H-2^1), 3.13 (s, 3H, H-7^1), 2.35 (m, 1H, H-17^1), 2.10 (m, 2H, H-17^2), 1.80 (m, 1H, H-17^1), 1.73 (d, 3H, H-18^1, J = 9.0 Hz), 1.65 (t, 3H, H-8^2, J = 7.5 Hz), 0.2 (br s, 2H, NH)

^{13}C NMR (75 MHz, 15 mg/1.0 mL $CDCl_3$) δ: 186.83 (C-13^1), 184.92 (C-15^1), 174.85 (C-15^2), 173.30 (C-17^3), 168.22 (C-19), 163.28 (C-16), 157.05 (C-6), 149.12 (C-9), 145.98 (C-14), 143.24 (C-8), 140.40 (C-1), 137.34 (C-11), 136.62 (C-3), 135.97 (C-4), 134.20 (C-7), 131.90 (C-2), 129.56 (C-12), 124.36 (C-13), 128.57 (C-3^1), 122.89 (C-3^2), 106.90 (C-5), 105.80 (C-15), 100.45 (C-10), 93.74 (C-20), 53.27 (C-17), 52.63 (C-15^3), 51.53 (C-17^4), 49.71 (C-18), 31.44 (C-17^2), 30.93 (C-17^1), 22.78 (C-18^1), 19.40 (C-8^1), 17.47 (C-8^2), 11.90 (C-12^1), 11.39 (C-2^1), 11.06 (C-7^1)

UV/Vis λ_{max} (CH_2Cl_2) 414 nm (ε 113 200), 508 (7 400), 544 (10 600), 630 (7 000), 680 (35 400), 686 (31 700)

LREIMS (m/z): 622 (M^+, 15%), 563 (M^+ – COOMe, 27%), 535 (M^+ – COOMe – CO, 27%)

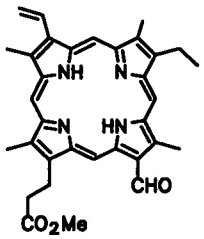
HREIMS: $C_{36}H_{38}N_4O_6$ (M^+): calcd 622.2791

obsd 622.2786

Anal. calcd for $C_{36}H_{38}N_4O_6$: C, 69.44; H, 6.15; N, 9.00 %

found: C, 69.11; H, 5.93; N, 8.92 %

13-Decarboxyl-13-formyl-3-vinylrhodoporphyrin XV methyl ester (148)



148

Purpurin **113** (150 mg, 0.24 mmol) was heated in an oil-bath at 180-185°C in 2,4,6-collidine (30 mL) during 60 minutes. After cooling, the solvent was evaporated off at 0.5 mm Hg and the residue was recrystallized from $CHCl_3/CH_3OH$, giving the title porphyrin (19.3 mg, 15% yield) as purple needles.

M.p.: 245-246°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 11.40 (s, 1H, 13-CHO), 10.70, 9.92, 9.88, 9.76 (4s, 4H, H-5, H-10, H-15 and H-20), 8.15 (dd, 1H, H-3^1, J = 17.5 and 11.0 Hz), 6.29 [dd, 1H, H-3^2(E), J = 17.5 and 1.0 Hz], 6.15 [dd, 1H, H-3^2(Z), J = 11.0 and 1.0 Hz], 4.37 (t, 2H, H-17^1, J = 7.5 Hz), 4.00 (q, 2H, H-8^1, J = 8.3 Hz), 3.80, 3.69, 3.58, 3.58, 3.58 (5s, 15H, H-2^1, H-7^1, H-12^1, H-18^1 and H-17^4), 3.23 (t, 2H, H-17^2, J = 7.5 Hz), 1.80 (t, 3H, H-8^2, J = 8.3 Hz), –4.50 (br s, 2H, NH)

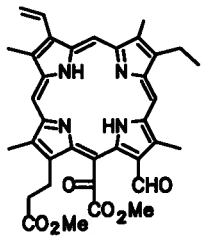
UV/Vis λ_{max} (CH_2Cl_2) 414 nm (ε 189 000), 518 (9 000), 560 (21 400), 582 (15 300), 636 (2 500)

LREIMS (m/z): 534 (M^+, 100%)

HREIMS: $C_{33}H_{34}N_4O_3$ (M^+): calcd 534.2631

obsd 534.2634

13-Decarboxyl-13-formyl-15-methoxyglyoxylic-3-vinylrhodoporphyrin XV methyl ester



A mixture of 15^1-hydroxypurpurin-7-lactone methyl ester (**101**) (60 mg, 0.094 mmol) and *N,N'*-carbonyldi-imidazole was refluxed in THF (30 mL) for 35 minutes. The solution was evaporated and the residue was chromatographed on alumina V (elution with methylene chloride). The appropriate band was collected and the elute was evaporated. The residue was recrystallized from $CHCl_3$/hexane, giving the title porphyrin (4.6 mg, 7.9%) as a red solid.

M.p.: >300°C

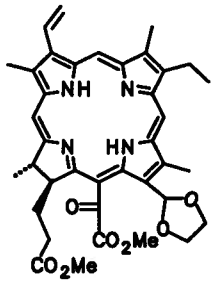
^{1}H NMR (400 MHz, $CDCl_3$) δ 10.30 (s, 1H, 13-CHO), 10.19, 10.15, 8.40 (3s, 3H, H-5, H-10 and H-20), 8.19 (dd, 1H, H-3^1, *J* = 18.2 and 11.5 Hz), 6.30 [dd, 1H, H-3^2(E), *J* = 18.2 and 1.0 Hz], 6.15 [dd, 1H, H-3^2(Z), *J* = 11.5 and 1.0 Hz], 4.81 (m, 1H, H-17^1), 4.48 (m, 1H, H-17^1), 4.15 (q, 2H, H-8^1, *J* = 8.0 Hz), 4.10, 3.78, 3.78, 3.65, 3.65, 3.30 (6s, 18H, H-2^1, H-7^1, H-12^1, H-18^1, H-17^4 and 15^1-$COOCH_3$), 3.39 (m, 1H, H-17^2), 3.23 (m, 1H, H-17^2), 1.90 (t, 3H, H-8^2, *J* = 8.3 Hz), –3.18 (br s, 1H, NH), –3.40 (br s, 1H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 414 nm (ε 142 000), 522 (6 400), 564 (11 300), 582 (9 300), 634 (2 600)

LRFABMS (m/z): 621 ($[M+H]^+$, 73%)

HREIMS: $C_{36}H_{36}N_4O_6$ (M^+): calcd 621.2713
obsd 621.2714

13-Decarboxy-13-(ethyleneacetal)-purpurin-7 dimethyl ester (150)



150

The foregoing purpurin **113** (20 mg, 0.032 mmol) in dried THF (50 mL) under nitrogen was stirred in a 3-necked round-bottom flask equiped with a Soxhlet apparatus and 4Å molecular sieve inside. Ethylene glycol (0.9 mL) and *p*-toluenesulfonic acid (2 mg) were added to the solution and the reaction mixture was brought to reflux. Progress of the reaction was followed by spectrophotometry, using the change in absorption spectrum from λ_{max} = 686 nm to λ_{max} = 660 nm; the reaction was complete after 1 hour. The mixture was diluted with methylene chloride (50 mL), washed with water (3 × 50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was chromatographed on alumina V (elution with methylene chloride) to give the principal green band. Removal of solvent *in vacuo* and recrystallization from CH_2Cl_2/hexane gave a dark green powder of the title acetal **150** (19.2 mg, 90% yield).

M.p.: 265°C

^{1}H NMR (400 MHz, 1.5 mg/0.6 mL $CDCl_3$) δ: 10.93 (s, 1H, H-13^1), 9.75 (s, 1H, H-10), 9.68 (s, 1H, H-5), 8.80 (s, 1H, H-20), 8.12 (dd, 1H, H-3^1, *J* = 18.3 and 11.7 Hz), 6.33 [d, 1H, H-3^2(E), *J* = 18.3 Hz), 6.15 [d, 1H, H-3^2(Z), *J* = 11.7 Hz), 4.70 (m, 1H, 13^1-OCH_2OCH_2), 4.40 (q, 1H, H-18, *J* = 7.3 Hz), 4.18 (dd, 1H, H-17, *J* = 13.3 and 3.6 Hz), 4.06 (s, 3H, 15^1-$COOCH_3$), 4.01 (m, 3H, 13^1-OCH_2OCH_2), 3.79 (q, 2H, H-8^1, *J* =

7.56Hz), 3.59 (s, 3H, H-17^4), 3.50 (s, 3H, H-12^1), 3.44 (s, 3H, H-2^1), 3.33 (s, 3H, H-7^1), 2.38 (m, 1H, H-17^1), 2.20 (m, 1H, H-17^2), 1.89 (m, 1H, H-17^1), 1.82 (d, 3H, H-18^1, J = 7.3 Hz), 1.80 (m, 1H, H-17^2), 1.73 (t, 3H, H-8^2, J = 7.6 Hz), –1.85 (br s, 1H, NH), –2.03 (br s, 1H, NH)

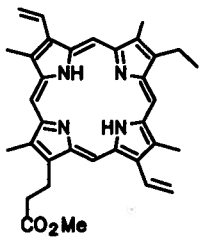
UV/Vis λ_{max} (CH_2Cl_2) 400 nm (ε 149 000), 496 (13 000), 606 (5 000), 660 (39 000)

LREIMS (m/z): 666 (M^+, 100%)

HREIMS: $C_{38}H_{42}N_4O_7$ (M^+): calcd 666.3053

obsd 666.3061

13-Decarboxyl-3,13-divinylrhodoporphyrin XV methyl ester (147)



147

13-Decarboxy-13-formyl-3-vinylrhodoporphyrin XV methyl ester (**148**) (18 mg, 0.0337 mmol) was dissolved in methylene chloride (10 mL), and a saturated solution of $Zn(OAc)_2$ in methanol (0.5 mL) was added. The resulting solution was stirred at room temperature for 3 hours at which time TLC and UV/Vis spectroscopy showed full conversion of the desired compound. The solvent was removed by evaporation *in vacuo* and the product was redissolved in methylene chloride, filtered through a short cake of alumina V, and evaporated to dryness. The product [i.e. Zn(II) porphyrin **148**] was further dried by an oil pump at 0.5 mm Hg for 4 hours.

Dry methyltriphenylphosphonium bromide (30 mg, 0.084 mmol) in dry THF (25 mL) and diisopropylamine (0.5 mL) were treated with *n*-butyllithium in *n*-hexane (50 μL, 1.6 M). The resulting ylide was stirred at 0°C under nitrogen gas and a THF solution (10 mL) of the above Zn(II) porphyrin **148** was added via a cannule. After 30 min the solution was evaporated to dryness to give a residue which was taken into methylene chloride (30 mL), filtered through anhydrous sodium sulfate, and then evaporated again to dryness. The residue was dissolved in trifluoroacetic acid (3 mL) and washed twice with water (30 mL). The organic phase was evaporated to dryness and the residue was chromatographed on amulina III (elution with methylene chloride). The red eluates were evaporated to dryness to furnish a residue which was recrystallized from $CHCl_3$/hexane to give the title porphyrin (12.6 mg, 71%) as a purple-red powder.

M.p.: 227°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.16, 10.15, 10.08, 10.07 (4s, 4H, H-5, H-10, H-15 and H-20), 8.27 (dd, 2H, H-3^1 and H-13^1), 6.33 [dd, 2H, H-3^2(E) and H-13^2(E)], 6.15 [dd, 2H, H-3^2(Z) and H-13^2(Z)], 4.42 (t, 2H, H-17^1, J = 7.6 Hz), 4.10 (q, 2H, H-8^1, J = 7.5 Hz), 3.69, 3.67, 3.66, 3.65, 3.63 (5s, 15H, H-2^1, H-7^1, H-12^1, H-18^1 and H-17^4), 3.26 (t, 2H, H-17^2, J = 7.6 Hz), 1.86 (t, 3H, H-8^2, J = 7.5 Hz), –3.75 (br s, 2H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 404 nm (ε 108 000), 506 (7 500), 544 (9 800), 572 (5 600), 630 (1 500)

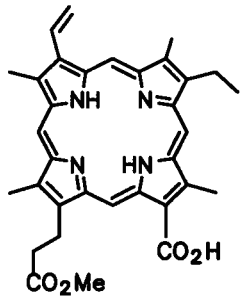
LRFABMS (m/z): 533 (M^+, 100%)

HRFABMS: $C_{34}H_{37}N_4O_2$ (M^+): calcd 533.2916

obsd 533.2916

6.4.3 Synthesis of [A,C]-Divinylporphyrin 147 via Porphyrin 23

3-Vinylrhodoporphyrin XV methyl ester (157)[156]



157

3-Vinylrhodoporphyrin XV dimethyl ester (**23**) (850 mg) in warm pyridine (175 mL) was refluxed during 4 hours with a solution of potassium hydroxide (20 g) in water (25 mL) and methanol (150 mL). The solution was cooled, diluted with iced water (1 L), acidified with concentrated sulphuric acid (15 mL) in water (100 mL), and then stirred for 10 min. The precipitated porphyrin diacid was filtered off on Celite, and washed with warm water followed by dry methanol. A mixture of dry methanol (650 mL) and concentrated sulphuric acid (20 mL) was passed slowly through the bed of Celite, thereby dissolving the porphyrin, and the resultant solution was kept at room temperature in the dark for 16 hours before addtion to water (1.5 L). The porphyrin was extracted with methylene chloride (3 × 500 mL) and the extracts were washed with water (1 L), dried over anhydrous $MgSO_4$, and evaporated. The residue was recrystallized from THF/benzene to give the title porphyrin monocarboxylic acid (605 mg, 70%) as a dark red powder.

M.p.: >300°C [lit.[156] >300°C]

^{1}H NMR (400 MHz, CF_3COOD) δ: 11.90, 11.20, 11.00, 10.95 (4s, 4H, H-5, H-10, H-15 and H-20), 8.28 (dd, 1H, H-3^1, *J* = 18.3 and 11.0 Hz), 6.65 [d, 1H, H-3^2(Z), *J* = 11.0 Hz], 6.40 [d, 1H, H-3^2(E), *J* = 18.3 Hz], 4.62 (t, 2H, H-17^1), 4.25 (q, 2H, H-8^1), 4.19, 3.80, 3.78, 3.76, 3.72 (5s, 15H, H-2^1, H-7^1, H-12^1, H-18^1 and H-17^4), 3.40 (t, 2H, H-17^2), 1.82 (t, 3H, H-8^2)

UV/Vis λ_{max} (CH_2Cl_2) 408 nm (ε 154 000), 514 (7 900), 554 (16 300), 578 (9 500) [lit.[156] λ_{max} (pyridine) 409 nm (ε 189 000), 511(10 000), 552 (17 200), 579 (9 200); λ_{max} ($CHCl_3$-HCl) 427 nm (ε 206 000), 569 (12 000), 620 (12 900); λ_{max} (0.1 M-NaOMe-MeOH) 399 nm (ε 146 000), 503 (10 500), 550 (12 000), 572 (6 700), 624 (1 900)]

LREIMS (m/z): 550 (M^+, 100%), 506 (M^+-CO_2, 30%)

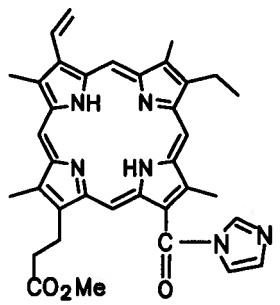
HREIMS: $C_{33}H_{34}N_4O_4$ (M^+): calcd 550.2580

obsd 550.2580

Anal. calcd for $C_{33}H_{34}N_4O_4$: C, 71.98; H, 6.22; N, 10.17 %

found: C, 71.93; H, 6.16; N, 9.86 %

13-Imidazoyl-3-vinylrhodoporphyrin XV methyl ester (158)



158

A mixture of 3-vinylrhodoporphyrin XV methyl ester (**157**) (600 mg, 1.09 mmol) and *N,N'*-carbonyldi-imidazole (600 mg) was refluxed during 30 min in THF (125 mL). The solution was evaporated to about 60 mL and then applied to a short alumina column (Brockman Grade V; elution with methylene chloride). The appropriate eluates were evaporated and the residue was recrystallized from CH_2Cl_2/benzene to give the title porphyrin (595 mg, 91%) as a dark red powder.

M.p.: 239°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.01, 9.97, 9.88, 9.70 (4s, 4H, H-5, H-10, H-15 and H-20), 8.38 (s, 1H, H-imidazole), 8.12 (dd, 1H, H-3^1, *J* = 17.5 and 12.0 Hz), 7.95 (s, 1H, H-imidazole), 7.35 (s, 1H, H-imidazole), 6.29 [dd, 1H, H-3^2(E), *J* = 17.5 and 1.5 Hz], 6.15 [dd, 1H, H-3^2(Z), *J* = 17.5 and 1.5 Hz], 4.25 (t, 2H, H-17^1, *J* =8.1 Hz), 4.00 (q, 2H, H-8^1, *J* =8.0 Hz), 3.67, 3.65, 3.55, 3.53, 3.51 (5s, 15H, H-2^1, H-7^1, H-12^1, H-18^1 and H-17^4), 3.18 (t, 2H, H-17^2, *J* = 8.1 Hz), 1.80 (t, 3H, H-8^2, *J* = 8.0 Hz), –4.30 (br s, 2H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 408 nm (ε 160 000), 514 (6 900), 554 (15 300), 574 (9 900)

LRFABMS (m/z): 601 ([M+H]$^+$, 73%), 533 (M$^+$-$C_3H_3N_2$, 100%)

LREIMS (m/z): 533 (M$^+$-$C_3H_3N_2$, 100%)

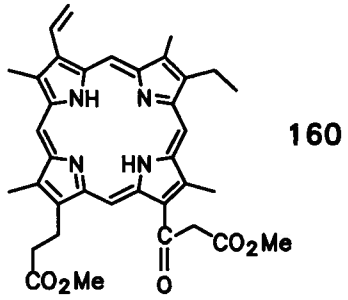
HRFABMS: $C_{36}H_{37}N_6O_4$ ([M+H]$^+$): calcd 601.2927

obsd 601.2929

HREIMS: $C_{33}H_{33}N_4O_4$ (M$^+$-$C_3H_3N_2$): calcd 601.2927

obsd 601.2929

13-Decarboxy-13-methoxycarbonylacetyl-3-vinylrhodoporphyrin XV methyl ester (160)



160

A solution of 2-PrMgBr was prepared by refluxing under dry nitrogen a mixture of Mg turnings (1.2 g) and 2-PrBr (4 g) in freshly distilled THF (150 mL). When the metal had dissolved the solution was cooled to 0°C and redistilled methyl hydrogen malonate[158] (3 g) in dry THF (30 mL) was added via a cannula. The mixture (i.e. **159**) was warmed to 65°C and stirred for 10 min before introduction of a solution of 13-imidazoyl-3-vinylrhodoporphyrin XV methyl ester (**158**) (400 mg) in dry THF (200 mL). Stirring was maintained for 2.5 hours while heating under reflux, and glacial AcOH (15 mL) was added. Heating and stirring were continued for a further 15 min after which the mixture was diluted with chloroform (1400 mL), washed with 0.1 M HCl (1100 mL) and water (2 × 800 mL), and then dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was chromatographed on alumina V (elution with 5% acetone in methylene chloride) and after evaporation of the appropriate eluates the product was recrystallized from CH_2Cl_2/hexane to give the required porphyrin-ß-ketoester **160** (280 mg, 70%) as a dark red solid.

M.p.: 270-274°C [lit.[156] 250-254°C]

^{1}H NMR (400 MHz, $CDCl_3$) δ: (the enol tautomer) 13.30 (s, 1H, OH), 10.62, 10.62, 10.42, 9.75 (4s, 4H, H-5, H-10, H-15 and H-20), 6.10 (s, 1H, H-13^2); δ: (the ketone tautomer) 9.98, 9.97, 9.86, 9.85 (4s, 4H, H-5, H-10, H-15 and H-20), 4.70 (s, 2H, H-13^2); δ: (their mixture) 8.10 (m, 1H, H-3^1), 6.25 [m, 1H, H-3^2(E)], 6.12 [m, 1H, H-3^2(Z)], 4.41 (t, 2H, H-17^1), 3.98 (q, 2H, H-8^1), 4.02-3.45 (6s, 18H, H-2^1, H-7^1, H-12^1, H-18^1, H-17^4 and 13^2-$COOCH_3$), 3.25 (t, 2H, H-17^2), 1.83 (t, 3H, H-8^2), –4.26 (br s, 1H, NH), –4.30 (br s, 1H, NH)

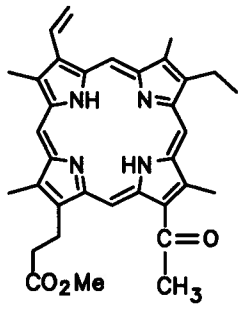
UV/Vis λ_{max} (CH_2Cl_2) 410 nm (ε 140 000), 512 (7 400), 552 (14 000), 576 (9 200) [lit.[156] λ_{max} (CH_2Cl_2) 409 nm (ε 176 000), 512 (7 200), 553 (15 400), 574 (9 900), 635 (1 300); λ_{max} (CH_2Cl_2-CF_3COOH) 412 nm (ε 267 000), 559 (12 200), 608 (8 600); λ_{max} (0.1 M-NaOMe-MeOH) 401 nm (ε 154 000), 504 (10 500), 542 (12 400), 572 (6 900), 625 (2 200)]

LRFABMS (m/z): 607 ($[M+H]^+$, 16%)

HRFABMS: $C_{36}H_{39}N_4O_5$ ($[M+H]^+$): calcd 607.2920

obsd 607.2919

13-Acetyl-13-decarboxy-3-vinylrhodoporphyrin XV methyl ester (161)



161

A mixture of porphyrin-ß-ketoester **160** (180 mg, 0.297 mmol), 4-(dimethylamino)pyridine (36 mg, 2.92 mmol), 1.0 M phosphate buffer (pH = 7) (40 mL), and toluene (150 mL) was stirred at 90°C for 12 hours at which time TLC analysis showed the full disapperance of the porphyrin-ß-ketoester band and apperance of a more mobile red band. The reaction mixture was extracted with ethyl acetate (250 mL) and the organic layer was washed once with saturated NH_4Cl, twice with water (200 mL), dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on silica III (elution with methylene chloride) to give a red major band. The appropriate eluates were evapoarted and the residue was recrystallized from CH_2Cl_2/hexane to give the title acetylporphyrin (122 mg, 75%) as purple-red small leaves.

M.p.: 284°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.75, 10.10, 10.07, 9.98 (4s, 4H, H-5, H-10, H-15 and H-20), 8.20 (dd, 1H, H-3^1, J = 17.4 and 12.5 Hz), 6.30 [d, 1H, H-3^2(E), J = 17.4 Hz], 6.12 [d, 1H, H-3^2(Z), J = 12.5], 4.43 (t, 2H, H-17^1, J = 7.5 Hz), 4.09 (q, 2H, H-8^1, J =8.8 Hz), 3.88, 3.67, 3.65, 3.61, 3.61 (5s, 15H, H-2^1, H-7^1, H-12^1, H-18^1 and H-17^4), 3.33 (t, 2H, H-17^2, J = 7.5 Hz), 3.32 (s, 3H, H-13^2), 1.85 (t, 3H, H-8^2, J = 8.8 Hz), –3.80 (br s, 2H, NH)

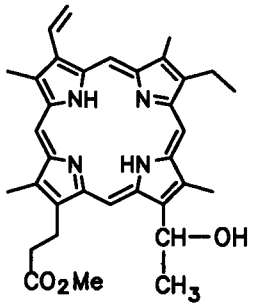
UV/Vis λ_{max} (CH_2Cl_2) 410 nm (ε 147 000), 514 (7 400), 554 (13 800), 576 (8 600), 608 (1 300)

LRFABMS (m/z): 549 ($[M+H]^+$, 100%)

HRFABMS: $C_{34}H_{36}N_4O_3$ ($[M+H]^+$): calcd 549.2865

obsd 549.2861

13-Decarboxy-13-(α-hydroxy)ethyl--3-vinylrhodoporphyrin XV methyl ester (162)



162

To a mixture of the foregoing porphyrin **161** (100 mg, 0.182 mmol) in methylene chloride (80 mL) and methanol (15 mL) was added sodium borohydride (80 mg). The mixture was stirred for 90 min at room temperature, after which time TLC monitoring indicated reduction of the acetyl group to be complete. The reaction was quenched by slow addition of ice-cooled 1 M HCl. The organic layer was separated, washed twice with water, dried over $MgSO_4$, filtered and evaporated. The residue was chromatographed on silica V (elution with 0.5% methanol in methylene chloride) to give a red major band. The appropriate eluates were evaporated and the residue was recrystallized from CH_2Cl_2/hexane to give the title hydroxyporphyrin (82 mg, 82%) as a purple solid.

M.p.: 219°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 10.18, 10.00, 9.88, 9.88 (4s, 4H, H-5, H-10, H-15 and H-20), 8.20 (dd, 1H, H-3^1, *J* = 17.1 and 11.4 Hz), 6.31 [dd, 1H, H-3^2(E), *J* = 17.1 and 1.9 Hz], 6.15 [dd, 1H, H-3^2(Z), *J* = 12.5 and 1.9 Hz], 4.27 (t, 2H, H-17^1, *J* = 8.5 Hz), 4.00 (q, 2H, H-8^1, *J* =8.2 Hz), 3.58, 3.58, 3.54, 3.51, 3.49 (5s, 15H, H-2^1, H-7^1, H-12^1, H-18^1 and H-17^4), 3.45 (q, 1H, H-13^1, *J* = 7.0 Hz), 3.16 (t, 2H, H-17^2, *J* = 8.5 Hz), 2.72 (br s, 1H, OH-13^1), 2.14 (d, 3H, H-13^2, *J* = 7.0 Hz), 1.81 (t, 3H, H-8^2, *J* = 8.2 Hz), –4.30 (br s, 2H, NH)

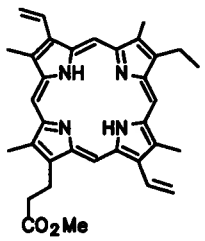
UV/Vis λ_{max} (CH_2Cl_2) 404 nm (ε 178 000), 502 (10 600), 540 (12 000), 572 (9 300), 624 (2 700)

LRFABMS (m/z): 551 ($[M+H]^+$, 100%)

HRFABMS: $C_{34}H_{38}N_4O_3$ ($[M+H]^+$): calcd 551.3022

obsd 551.3011

13-Decarboxy-3,13-divinylrhodoporphyrin XV methyl ester (147)



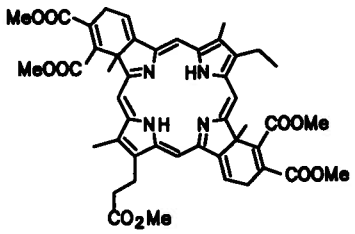
147

The foregoing hydroxyporphyrin **162** (55 mg, 0.1 mmol) was dissolved in dry DMF (20 mL) and stirred at 105°C under nitrogen. Benzoyl chloride (1 mL) was added to this solution via a syringe and the mixture was kept at this temperature for 2 hours.

The solution was then diluted with methylene chloride (100 mL), and successively washed with 2M sodium hydroxide (75 mL) and water (2 × 100 mL). The organic layer was dried over $MgSO_4$, filtered and evaporated *in vacuo*. The residue was chromatographed on silica III (elution with methylene chloride) to give a major red band. The appropriate fractions were collected, evaporated and recrystallized from CH_2Cl_2/hexane to give the title divinylporphyrin **147** (31.9 mg, 60%) as a purple-red solid. This material was found identical to the compound prepared from the Wittig reaction of 13-formylporphyrin **148**. The corresponding physical data of this compound are shown in section **6.4.2**.

6.4.4 Synthesis of [A,C]-Dibenzoporphyrin Derivative 165

2^1,2^2,12^1,12^2-Tetrakis(methoxycarbonyl)-8-ethyl-17-methoxycarbonylethyl-2,7,12,18-tetramethyl-2^3,12^3-dihydrobenzo[b,l]porphyrin (164)



164

The foregoing 3,13-divinylporphyrin **147** (30 mg, 0.0564 mmol) and dimethyl acetylenedicarboxylate (800 mg, 5.6 mmol) were suspended in degassed toluene (N_2) (20 mL) and the mixture heated at 110°C for 80 hours. After removal of the toluene *in vacuo*, the residue was chromatographed on silica (methylene chloride eluant). After elution of the excess dienophile, the solvent polarity was increased to 2% methanol in methylene

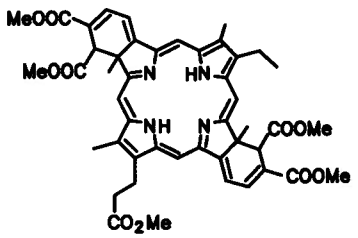
chloride to elute the major reaction product. After evaporation to dryness, the resulting residue was subjected to preparative TLC on silica (1 mm thick plate; elution with 2% methanol in methylene chloride). The more mobile band was found to be the mono-adduct (showing a band at 656 nm) in negligible amount; the major band was found to be the bis-adduct which was recrystallized from CH_2Cl_2/hexane to afford the bis-adduct **164** as a green solid (10.8 mg, 25% yield).

M.p.: 198°C

UV/Vis λ_{max} (CH_3COCH_3) 406 nm (peak ratio, 1.10), 484 (0.21), 538 (0.15), 666 (0.13), 720 (0.338)

LREIMS (m/z): 817 (M^+, 100%)

2^1,2^2,12^1,12^2-Tetrakis(methoxycarbonyl)-8-ethyl-17-methoxycarbonyl-ethyl-2,7,12,18-tetramethyl-2^1,12^1-dihydrobenzo[b,l]porphyrin (165)



165

The bis-adduct **164** (9 mg, 0.011 mmol) was dissolved in methylene chloride and a few drops of DBU were added. The reaction mixture was stirred in the dark and monitored by visible spectroscopy (completed in 16 hours). The mixture was poured into 1M HCl and extracted with methylene chloride. The organic layer was washed twice with brine, once with water and dried over anhydrous magnesium sulfate. The solvent

was evaporated *in vacuo* and the compound was chromatographed on silica gel (1% MeOH in CH_2Cl_2 eluent). The pure 1,3-cyclohexadiene fraction was evaporated *in vacuo* yielding 8.0 mg (90% yield) of a dark green solid after recrystallization from CH_2Cl_2/hexane.

M.p.: 289-291°C

^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.15, 9.15, 8.74, 8.74 (4s, 4H, H-5, H-10, H-15 and H-20), 7.82 (d, 2H, H-2^4 and H-12^4, J = 7.8 Hz), 7.35 (d, 2H, H-2^3 and H-12^3, J = 7.8 Hz), 4.95 (s, 2H, H-2^1 and H-12^1), 4.10 (t, 2H, H-17^1, J = 8.1 Hz), 3.98 (q, 2H, H-8^1, J = 7.7 Hz), 3.25 (t, 2H, H-17^2, J = 7.7 Hz), 3.90, 3.86, 3.63, 3.60, 3.32, 3.01 (6s, 18H, H-7^1, H-18^1, H-17^4, 2^1-$COOCH_3$, 2^2-$COOCH_3$, 12^1-$COOCH_3$ and 12^2-$COOCH_3$), 1.81 (s, 6H, 2-CH_3), 1.74 (t, 3H, H-8^2, J = 7.7 Hz), –1.90 (br s, 2H, NH)

UV/Vis λ_{max} (CH_2Cl_2) 468 nm (ε, 81 000), 620 (20 900), 700 (4 700), 784 (38 000)

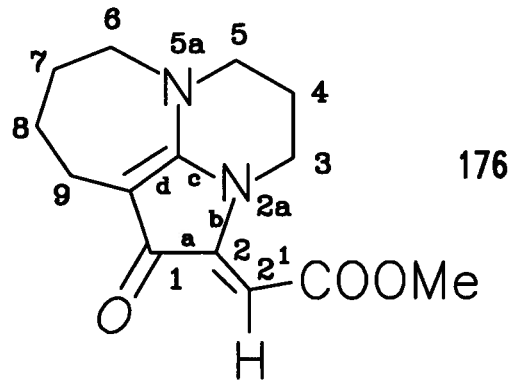
LRFABMS (m/z): 817 ($[M+H]^+$, 100%)

HRFABMS: $C_{40}H_{42}N_4O_8$ (M^+): calcd 817.3455

obsd 817.3449

6.5 Nucleophilic Reactions of DBU and DBN

3,4,5,6,7,8,9-Heptahydro-1-oxo-2a,5a-diazacyclohepta[cd]indan-2[2^1 (Z)-methoxycarbonyl]-methylene (176)



176

To a solution of dimethyl acetylenedicarboxylate (1.42 g, 0.01 mol) in chloroform (10 mL) at room temperature was added, a solution of DBU (1.52 g, 0.01 mol) in chloroform (10 mL). The golden-red solution was stirred at room temperature for 5 min. Removal of solvent, followed by recrystallization from CH_2Cl_2/hexane, gave the title compound (2.51 g, 96% yield) as red prisms.

M.p.: 158°C

^{1}H NMR (200 MHz, $CDCl_3$) δ: 5.94 (s, 1H, H-2^1), 3.98 (t, 2H, H-3), 3.33 (s, 3H, OCH_3), 3.30 (t, 2H, H-5), 3.23 (dd, 2H, H-6), 2.22 (dd, 2H, H-9), 2.05 (m, 2H, H-4), 1.95 (m, 2H, H-7), 1.86 (m, 2H, H-8)

^{13}C NMR (50 MHz, $CDCl_3$) δ: 179.21, 166.84, 165.64, 145.25, 93.57, 93.31, 54.39, 51.44, 48.96, 42.32, 28.06, 25.52, 22.32, 20.47

LRFABMS (m/z): 263 ($[MH]^+$, 100%)

LREIMS (m/z): 262(M^+, 100%)

HREIMS: $C_{14}H_{18}N_2O_3$ ([M]$^+$): calcd 262.1317

obsd 262.1309

Anal. calcd for $C_{14}H_{18}N_2O_3$: C, 64.09; H, 6.92; N, 10.50 %

found: C, 64.04; H, 6.86; N, 10.50 %

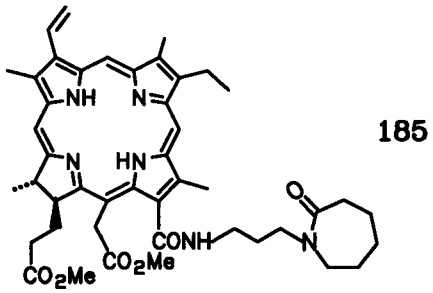
A single crystal suitable for X-ray diffraction was obtained by slow diffusion of *n*-hexane into a concentrated $CHCl_3$ solution of compound **176**.

Crystal data for **176**: Red prism, $C_{14}H_{18}N_2O_3$ M = 262.31, triclinic, space group $P\bar{1}$ (No. 2), a = 12.066(1), b = 12.946(2), c = 9.0900(8) Å, α = 103.695(9), β = 103.828(8), γ = 101.68(1)°, V = 1288.1(3) Å^3, Z = 4 (two molecules in the asymmetric unit), D_C = 1.190 g cm^{-3}. The final unit-cell parameters were obtained by least-squares analysis on the setting angles for 25 reflections with 2θ = 110.5-114.6°. The intensities of three standard reflections were measured every 200 reflections throughout the data collection: no decay correction was necessary. Data were corrected for Lp and absorption (azimuthal scans for three reflections, relative transmission factors 0.86-1.00). The structure was solved by direct methods. Non-hydrogen atoms were refined with anisotropic thermal parameters and hydrogen atoms were refined with isotropic thermal parameters. An isotropic Zachariasen type I secondary extinction correction was applied, the final value of the extinction coefficient being 5.58(6) × 10^{-5}. The refinement converged at R = 0.042 and R_W = 0.047 for 4303 independent reflections with I ³ 3$\sigma(I)$. Calculations were performed using the *teXsan* structure analysis package (Molecular Structure Corporation, 1985 & 1992).

Typical Procedure of Nucleophilic Reaction of DBU and DBN with Pheophorbide *a* Methyl Ester (7)

To a solution of pheophorbide *a* methyl ester (**7**) (60.7 mg, 0.1 mmol) and imidazole (10 mg) in dry THF (15 mL) under nitrogen, TMSOTf or TBDMSOTf (0.3 mmol) was injected via a syringe. After 15 min at room temperature, DBU (~1.0ml, 6.7 mmol) or DBN (~0.83ml, 6.7 mmol) was added slowly to this blue-gray solution. The brown-red mixture was then stirred in the dark for 5 h and was poured into an ice-cooled mixture of saturated NH_4Cl (30 mL) and dichloromethane (50 mL). The organic layer was washed with water (2 × 30 mL), saturated aqueous sodium bicarbonate (30 mL) and water (2 × 30 mL), dried over anhydrous sodium sulfate, filtered and evaporated *in vacuo*. The product was purified by flash chromatography on silica III, first eluting with dichloromethane to give unreacted starting material **7** (20-25% yield) and further eluting with 1.5% methanol in dichloromethane to give the products. The products were recrystallized from dichloromethane/hexane.

Chlorin e_6 13-[1-(3-*N*-propyl)2-azacycloheptanone]amide-15,17-dimethyl ester (185)



185

45.3 mg (58% yield) as a green powder.

M.p.: 124°C

^{1}H NMR see **Table 5.1.**

^{13}C NMR see **Table 5.2.**

UV-Vis $\lambda_{max}(CHCl_3)$ 404 nm (ε 171 900), 502 (15 100), 528 (5 000), 610 (5 700), 666 (48 700)

LRFABMS (m/z): 777 ($[MH]^+$, 100%), 579 ($[MH]^+$–$C_{10}H_{18}N_2O_2$, 76)

LREIMS (m/z): 776 (M^+, 73%), 744 (M^+–CH_3OH, 20), 717 (M^+–$COOCH_3$, 37), 606 (M^+–$C_9H_{18}N_2O$, 51)

HRFABMS: $C_{45}H_{57}N_6O_6([MH]^+)$: calcd 777.4339

obsd 777.4365

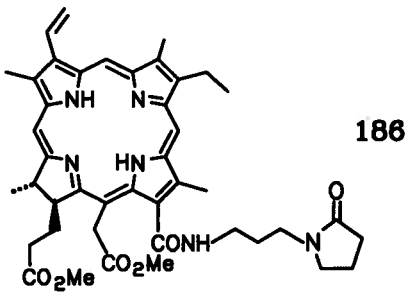
HREIMS: $C_{45}H_{56}N_6O_6(M^+)$: calcd 776.4261

obsd 776.4267

Anal. calcd for $C_{45}H_{56}N_6O_6$: C, 69.55; H, 7.27; N, 10.82 %

found: C, 69.77; H, 7.60; N, 10.52 %

Chlorin e_6 13-[1-(3-*N*-propyl)2-pyrrolidinone]amide-15,17-dimethyl ester (186)



186

38.7 mg (52% yield) as a green powder.

M.p.: 127°C

^{1}H NMR see **Table 5.1.**

^{13}C NMR see **Table 5.2.**

UV-Vis $\lambda_{max}(CHCl_3)$ 404 nm (ε 162 000), 502 (12 500), 528 (2 800), 610 (3 600), 666 (45 000)

LRFABMS (m/z): 749($[MH]^+$, 100%), 579($[MH]^+$–$C_8H_{14}N_2O_2$, 43)

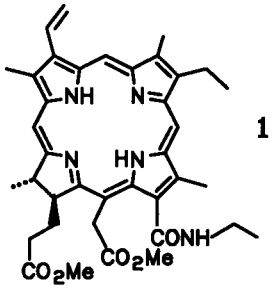
HRFABMS: $C_{43}H_{53}N_6O_6$ ($[MH]^+$): calcd 749.4026

obsd 749.4017

Anal. calcd for $C_{43}H_{52}N_6O_6$: C, 68.95; H, 7.00; N, 11.23 %

found: C, 68.80; H, 7.11; N, 11.04 %

Chlorin e_6 13-(2-*N*-ethyl)amide-15,17-dimethyl ester (179)



179

To a solution of pheophorbide *a* methyl ester (**7**) (30 mg, 0.05 mmol) in THF (10 mL) at room temperature under nitrogen, ethylamine (20 mL) was added and the mixture was allowed to react in the dark for 25 h before the solvents were evaporated *in vacuo*. The product was purified by preparative TLC on silica gel (developed by 2% methanol in dichloromethane). Recrystallization of the product from dichlomethane/hexane gave the title compound (20.5 mg, 63%) as a green solid.

M.p.: 134°C

^{1}H NMR see **Table 5.1.**

^{13}C NMR see **Table 5.2.**

UV-Vis $\lambda_{max}(CH_2Cl_2)$ 402 nm (ε 194 000), 500 (16 900), 528 (5 000), 608 (5 600), 664 (52 000)

LRFABMS (m/z): 652($[MH]^+$,100%), 580($[MH]^+$–CONHEt, 19)

HRFABMS: $C_{38}H_{46}N_5O_5$ ($[MH]^+$): calcd 652.3499

obsd 652.3475

Anal. calcd for $C_{38}H_{45}N_5O_5$ C, 69.90; H, 7.11; N, 10.73 %

found: C, 69.55; H, 6.85; N, 10.76 %

Nucleophilic reaction of 1-(3-aminopropyl)2-pyrrolidinone with pheophorbide *a* methyl ester (7)

To a solution of pheophorbide *a* methyl ester (**7**) (15 mg, 0.025 mmol) in THF (5 mL) at room temperature under nitrogen was added, 1-(3-aminopropyl)2-pyrrolidinone (tech., Aldrich) (2 g) in THF (10 mL). The mixture was allowed to react in the dark for 30 h and poured into a mixture of saturated sodium chloride and dichloromethane. The organic layer was washed with water (3 × 30 mL), dried over anhydrous sodium sulphate, filtered and evaporated. The product was purified first by flash chromatography on alumina (Brockman V) to remove unreacted starting material **7** (2 mg, 13%) and excess 1-(3-aminopropyl)2-pyrrolidinone, and further by preparative TLC on silica gel (developed by 5% acetone,1% methanol in dichloromethane). Recrystallization of the

product from dichloromethane/hexane gave chlorin e_6 13-[1-(3-*N*-propyl)2-pyrrolidinone]amide-15,17-dimethyl ester (**186**) (8.5 mg, 45%) as a green powder, which was found identical to the material prepared from the reaction of pheophorbide *a* methyl ester (**7**) with DBN.

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