Title

I. Application of Organoselenium Chemistry in the Syntheses of Tetrapyrroles. II. Chemistry of Marine Natural Products from Xestospongia sp. and Dysidea herbacea

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I. Application of Organoselenium Chemistry in the Syntheses of Tetrapyrroles

II. Chemistry of Marine Natural Products from *Xestospongia* sp. and *Dysidea Herbacea*

By

SARAH ELIZABETH BRANTLEY

B.S. (Andrews University) 1992

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Chemistry

in the

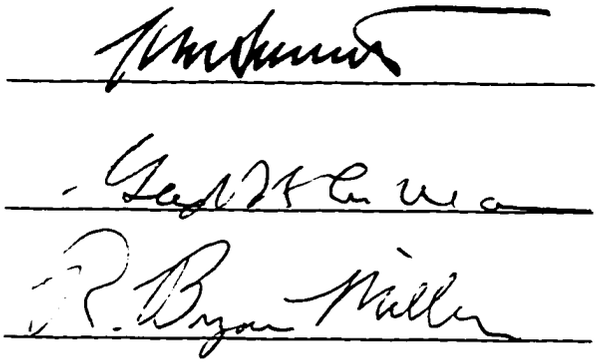
OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:



Committee in Charge

1997

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Dedicated to my parents
John and Elizabeth Brantley
and my brother Phillip

who taught me the importance
of education and perseverance

and to my husband Paul

Acknowledgments

My foremost acknowledgment must go to the person who has had the greatest impact on my scientific development. That person is my research advisor, Professor Kevin M. Smith. I am grateful to Dr. Smith for making my last years at UCDavis both an enjoyable and challenging time. His support and acceptance extended toward me when I joined his group will always be appreciated. He offered me an exciting project in porphyrin chemistry and helped me to stay focused on my research goals. He encouraged me to grow as a scientist by providing me the flexibility and independence to work out my research obstacles. His enthusiasm and confidence motivated me to work diligently even when the research was complex and tedious. Finally, I am thankful to Dr. Smith for our discussions on life and his guidance in my career decisions.

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While at UCDavis, I have had the privilege of working with a number of Smith group members. My lab mates, Dr. Benjamin Gerlach and Todd Clement deserve special recognition. Benni not only shared a hood with me, but he included me in his chlorin project. We designed a synthesis for 3,8-divinylchlorin-e_6 trimethyl ester using organoselenium chemistry. I learned a great deal from Benni and I am glad to have work with such a great scientist.

Todd was very helpful to me when I joined the Smith group. He always patiently answered my questions and took time to show me where to find laboratory equipment. He always turned down that awful music when I was around, and he even let me join in on the Andy Griffith lunches. From discussions about our projects to teasing me about MeOH washes, both Benni and Todd were great to work with.

Two past Smith group post docs, Dr. Isabella Meunier and Dr. Jay Singh, must be acknowledged for their pioneering work on the synthesis of 5-meso-methylhemin chloride. Their contribution described in Chapter 3 (section 4) was to synthesize 5, 10, and 20-meso-methylprotoporphyrin IX dimethyl ester using the 2-chloroethyl approach.

I would like to say thanks to two other Smith group members, Michelle Ressler and Cary Friedman, as well. Although they were not closely involved in my research, they impacted my life greatly. Michelle was always a good friend to me and encouraged me to join the Smith group. I hope a little of her courage and assertiveness has rubbed off on me. Cary is the most unique person I have ever met. He has expanded my horizons about life and people. Our many discussions ranging from spirituality to relationships to science have been appreciated.

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Finally, I would like to acknowledge the people dearest to me. They are my parents and my husband Paul. I thank them for their continual support and confidence in me.

Sarah Elizabeth Brantley
June 1997
Chemistry

I. Application of Organoselenium Chemistry in the Syntheses of Tetrapyrroles
II. Chemistry of Marine Natural Products from *Xestospongia* sp. and *Dysidea Herbacea*

Abstract

Part one of this Dissertation describes development of the *o*-nitrophenylselenium substituent for vinyl group protection and its application in the syntheses of tetrapyrrole derivatives. These derivatives, 5-meso-methylhemin chloride and 3,8-divinylchlorin-e_6 trimethyl ester, should be useful in the investigation of heme catabolism and chlorophyll *a* biosynthetic mechanisms.

Chapter one introduces two biologically important tetrapyrroles, heme and chlorophyll *a*. The theories of heme breakdown and the multiple biosynthetic routes of chlorophyll *a* are presented.

Chapter 2 describes the development of the 2-*o*-nitrophenylseleoethyl vinyl protecting group and demonstrates its use in the Vilsmeier formylation reaction which has been shown to adversely affect unmodified vinyl groups in tetrapyrrole systems. The X-ray crystal structure for 3-(*o*-nitrophenylselenoethyl)chlorin-e_6 trimethyl ester is illustrated.

The application of the 2-*o*-nitrophenylselenoethyl vinyl protecting group in the syntheses of 5-meso-methylhemin chloride and 3,8-divinylchlorin-e_6 trimethyl ester is discussed in Chapter three. Deprotection under non-basic conditions allows this organoselenium substituent to be a more successful protecting group than the 2-chloroethyl functionality in the synthesis of these base sensitive tetrapyrrole derivatives.

The second part of this Dissertation describes isolation and partial synthesis of secondary metabolites from the marine sponges, *Xestospongia* sp. and *Dysidea herbacea*. An introduction to marine natural products is discussed in Chapter four.

Chapter five describes the isolation of three new brominated fatty acids, (5*E*, 11*E*, 15*E*, 19*E*) 20-bromoeicosa-5,11,15,19-tetraene-9,17-diynoic acid, (5*Z*, 11*E*, 15*E*, 19*E*) 6,20-dibromoeicosa-5,11,15,19-tetraene-9,17-diynoic acid, and methyl (*Z*,*E*)-14,14-dibromo-4,6,13-tetradecatrienoate, from *Xestospongia* sp. These novel acids contain unencountered carbon numbers; C_{20} and C_{14}.

The partial synthesis of polychlorinated amino acid derivatives from the marine sponge *Dysidea herbacea* is examined in Chapter six. These unique polychlorinated metabolites are notable for their 5,5,5-trichloroleucine functionality. Four chlorinated amino nitrile diastereomers were synthesized from an asymmetric Strecker reaction and serve as key intermediates toward the synthesis of stereospecifically labeled leucine at carbon-5 with deuterium atoms.

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List of Abbreviations

Abbreviations

COSY	correlated spectroscopy
dibal-H	diisobutylaluminum hydride
DMF	dimethylforamide
EtOAc	ethyl acetate
HMBC	heteronuclear multiple bond coherence
HMQC	heteronuclear multiple quantum coherence
MeOH	methanol
NADPH	nicotinamide adenine dinucleotide
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser enhancement
SCUBA	self-contained underwater breathing apparatus
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TMS-Cl	chlorotrimethylsilane

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Part One

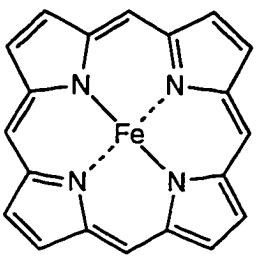
Application of Organoselenium Chemistry in the Syntheses of Tetrapyrroles

Chapter One

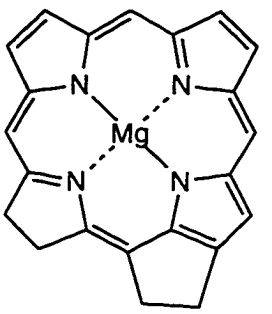
Heme Catabolism and Chlorophyll *a* Biosynthesis

1.1 Introduction

Tetrapyrroles are natural products essential to the life of both animals and plants. The most common tetrapyrroles known are hemes (**1.1**) and chlorophylls (**1.2**) (Figure 1.1). Hemes (iron containing porphyrins) are required for biological oxygen and electron transport. The Mg-containing chlorophylls are vital to the photosynthetic process in plants that mediates the transfer of energy from the sun into chemical energy.



1.1 Heme

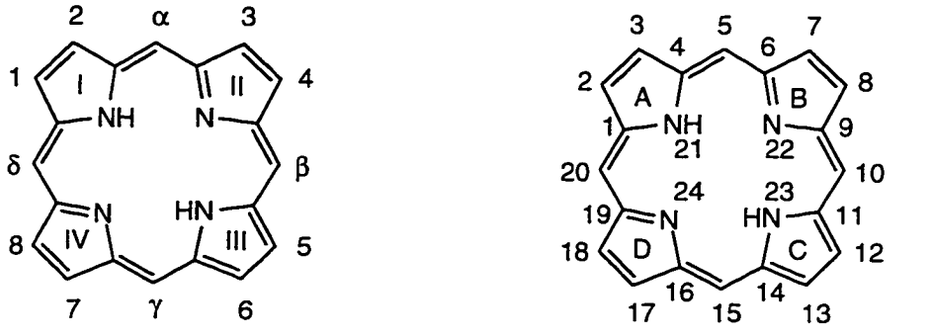


1.2 Chlorophyll

Figure 1.1 Basic structures of commonly known tetrapyrrole macrocycles.

These tetrapyrroles consist of an organic macrocycle chelating a metal atom. The nomenclature for the organic part was introduced by Fischer in the 1920s and 1930s and later changed by the International Union of Pure and Applied Chemistry and the International Union of Biochemistry (IUPAC-IUB) in 1980 (Figure 1.2).[1] For hemes, the macrocycle consists of four pyrrole rings linked together by methene bridges. The methene bridging carbons, known as the meso positions, are numbered 5, 10, 15, and 20 and the pyrrole rings are labeled A-D. A variety of side chains in the remaining eight positions (β

positions) help determine the physical characteristics of the porphyrin. The porphyrin is highly conjugated, and as a result is intensely colored. Chlorophylls result from differences in the conjugation pattern as well as in the presence of a fifth isocyclic ring.

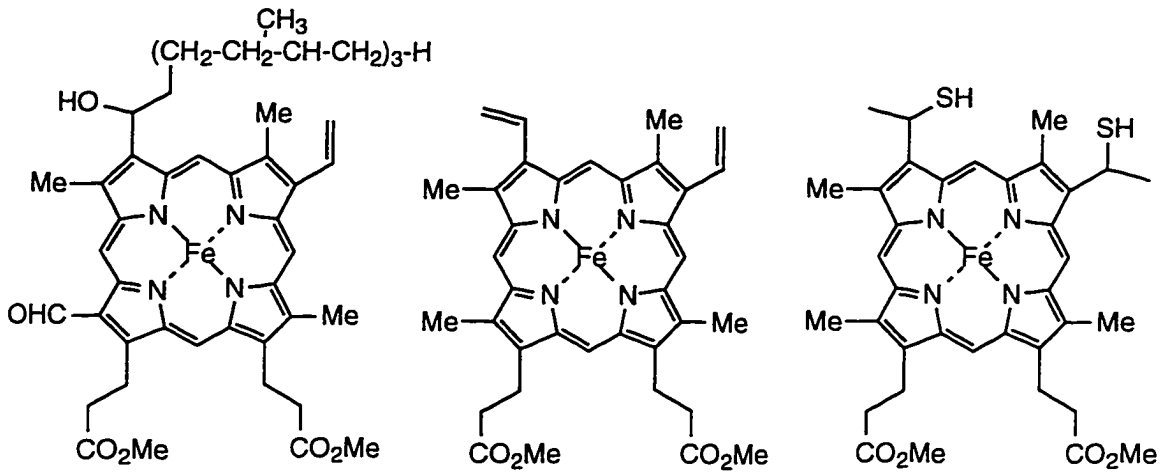


Porphin
Fisher Nomenclature

Porphyrin
IUPAC-IUB Nomenclature

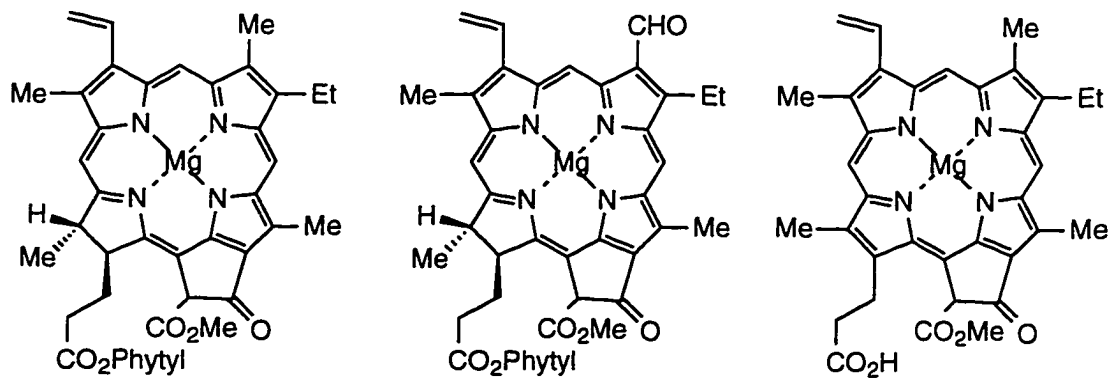
Figure 1.2 Nomenclature for the porphyrin macrocycle.

For over 150 years, the study of these biologically important tetrapyrroles has been pursued. Hemes found in mammalian systems are associated with proteins and called hemoproteins. Of the known hemes, most of the research has been performed on heme *b* (**1.4**) also known as protoheme, iron protoporphyrin-IX and heme (Figure 1.3). Heme is the prosthetic group found in the active sites of many hemoproteins. Hemoglobin contains around 95% of all the heme found in the body.[2] In plants, chlorophyll *a* (**1.6**) is the most abundant of the chlorophylls and likewise has been the most studied (Figure 1.4). Despite the amount of research done regarding these molecules, there are still many mysteries. This Chapter introduces the topics of heme catabolism and the biosynthesis of chlorophyll *a*. It concludes by relating these subjects to the Dissertation goals.



1.3 Heme *a* **1.4 Heme *b* = Heme** **1.5 Heme *c***

Figure 1.3 Different hemes found in mammalian systems.



1.6 Chlorophyll *a* **1.7 Chlorophyll *b*** **1.8 Chlorophyll c_1**

Figure 1.4 Some of the chlorophylls found in photosynthetic plants.

1.2 The History of Hemoglobin, Heme and Oxygen Transport

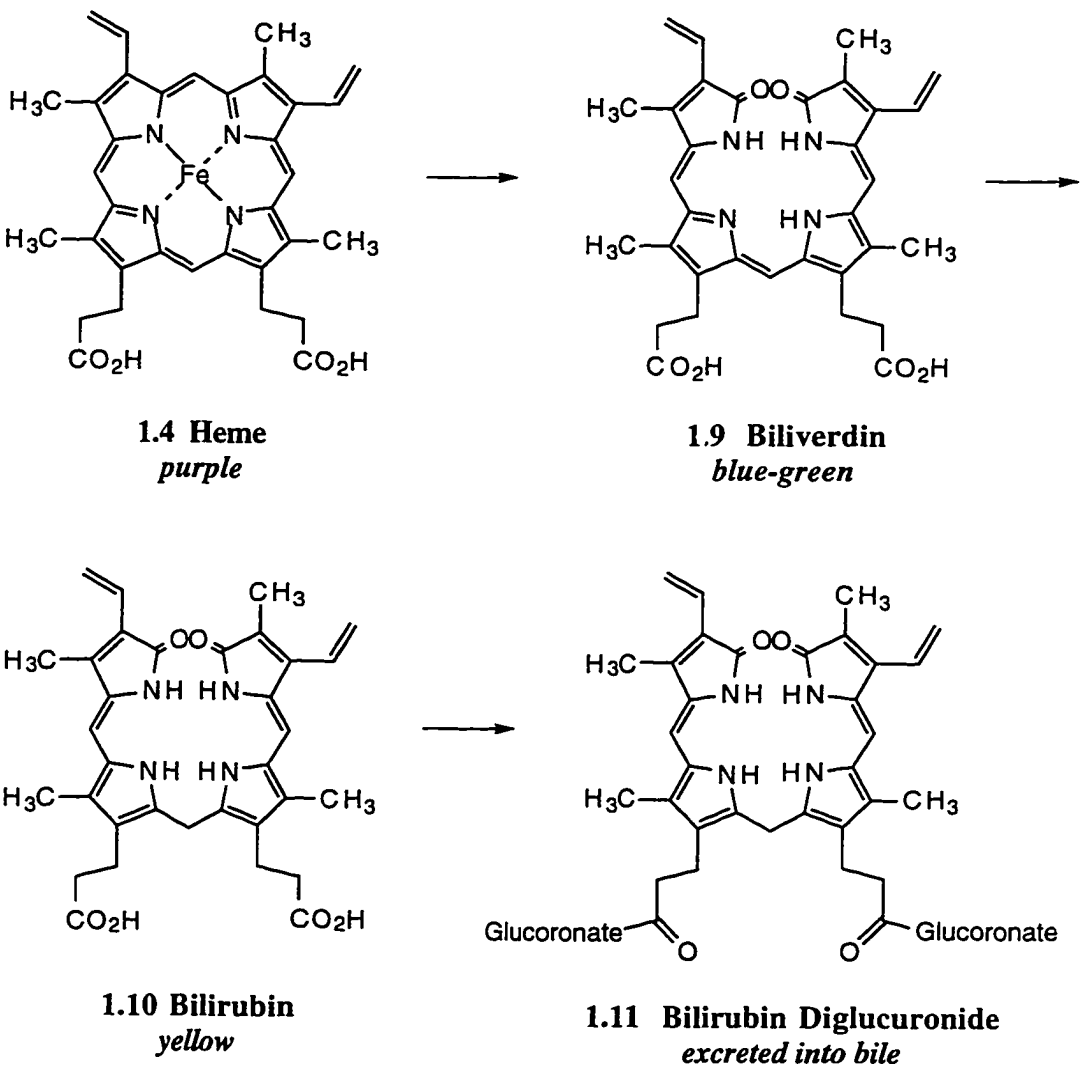
The study of oxygen (O_2) transport through the body has remained a topic of considerable interest since Priestly first noted the connection between O_2 and life in 1775.[3] The understanding that O_2 was transported through the blood was made cooperatively by several scientists, most notably Davy and Magnus in the early 1800s.[4] In 1862, Hoppe-Seyler was the first person to use the term hemoglobin to describe the red pigment of blood that bound O_2. Although heme was isolated in the mid-1800s (in both the metallated and non-metallated forms),[1,4] the role of heme as the prosthetic group of hemoglobin that binds O_2 was not confirmed until its synthesis by Fischer in the early 1900s.[5]

Hemoglobin is now understood to be the hemoprotein found in the red blood cells responsible for the O_2 carrying capacity of blood. The X-ray structure of hemoglobin, elucidated by Perutz in 1960, showed that the protein consists of four polypeptide chains (apoprotein) held together by noncovalent attractions, each associated with a heme prosthetic group.[6,7] Each heme group has a single oxygen-binding site, but the affinity of each site is dependent on the binding state of the other three hemes. This phenomenon is termed the cooperative binding of O_2.[8] Hemoglobin, while very stable in the circulating red blood cells (erythrocytes), degrades when the cells die. The average cell lifetime is approximately 125 days.[9]

The first step in the breakdown of hemoglobin is the disassociation of the prosthetic heme group and the apoprotein. The apoprotein is hydrolyzed into its constituent amino acids while heme is catabolized to bile pigments which after further degradation are excreted from the body in the urine and feces. This relationship between the formation of bile pigments such as bilirubin (**1.10**) and the catabolism of heme (hemoglobin) was noted as early as 1847,[10] but not confirmed until 1926.[11] Although concentrated research on the catabolism of heme has been done in the last three decades, the chemical details of the mechanism still remain a mystery. Reviews by Maines, Brown, and Beale describe in depth what is known about the breakdown process.[12-15]

1.3 Heme Catabolism

The mammalian breakdown of hydrophobic heme into hydrophilic bile pigments is necessary in order for excretion from the body to occur. Figure 1.5 illustrates the general enzymatic conversions of heme (**1.4**) to bilirubin diglucuronide (**1.11**). The changing color of a subcutaneous bruise is a highly graphic indication of these degradation reactions.[16] Heme, (**1.4**) which is a dark purple color, is oxidized to biliverdin (**1.9**), which has a blue-green color. Reduction of **1.9** to the yellow bilirubin (**1.10**) pigment followed by conjugation with glucoronic acid allows for excretion into the bile.



1.4 Heme
purple

1.9 Biliverdin
blue-green

1.10 Bilirubin
yellow

1.11 Bilirubin Diglucuronide
excreted into bile

Figure 1.5 A visible example of heme catabolism in the changing colors of a bruise.

Iron is recovered from this process and recycled. Iron is a biologically precious commodity since the metal is an integral part of the active sites of many proteins, and the iron compounds found in nature (Fe_3O_4, $FeCO_3$, and FeS_2)[17] are fairly water-insoluble. Therefore, the iron released in heme degradation is recycled and used again in heme biosynthesis.

Curiously, the poisonous gas, carbon monoxide (CO) is also formed in the breakdown of heme. Although virulent, CO does not present significant toxicity problems from this source since the oxygen concentration in the air is about 200,000 ppm and the level of exhaled CO gas is 1-2 ppm.[13] Recently, it has been suggested that CO may play a role as a neutral, second messenger akin to nitric oxide.[18-20]

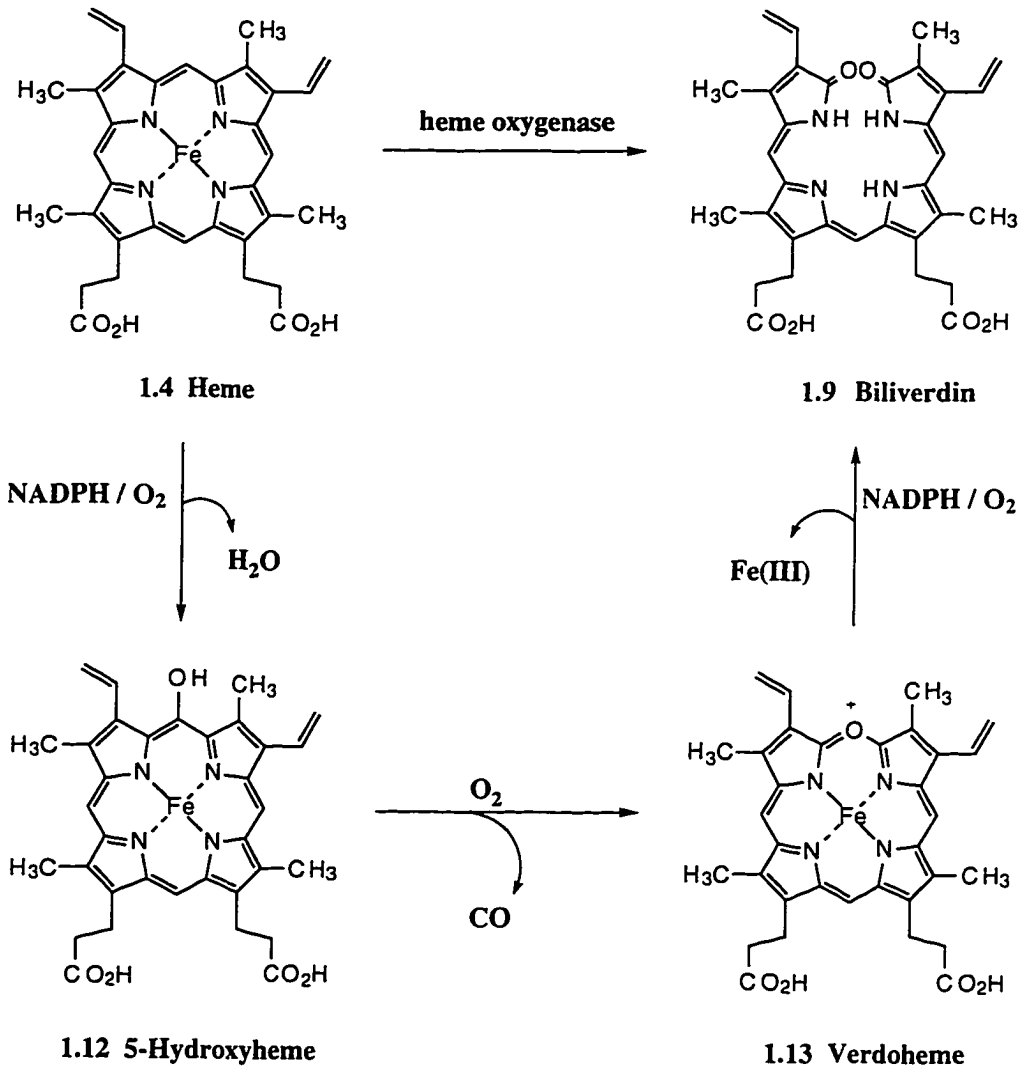
1.3.1 Oxidation of Heme to Biliverdin by Heme Oxygenase

Heme oxygenase, the first and rate-limiting enzyme in the heme breakdown pathway, regioselectively oxidizes heme (**1.4**) to biliverdin (**1.9**) with cleavage of the 5-meso carbon as CO and loss of an iron atom. This process was first described in 1964 by Wise and Drabkin,[21] but the name and characterization of this enzyme resulted from the research by Tenhunen and co-workers.[22-24] Their research also showed that 3 moles of molecular oxygen and 5 to 6 moles of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) were required for every mole of heme consumed.[25]

Through $^{18,18}O_2$ and $^{16,16}O_2$ labeling studies with no mixed species $^{16,18}O_2$, the pathway from heme to biliverdin has been shown to follow a "two-molecule" mechanism.[26-30] The term "two-molecule" refers to the fact that the two oxygen atoms of biliverdin are derived from separate O_2 molecules. Furthermore, the oxygen in CO is from a third molecule of O_2.

Deducing a mechanism that fits the observed experimental data is not trivial. Figure 1.6 illustrates the accepted mechanism proposed for the heme oxygenase reaction.[14,15,31] The first step begins with O_2 dependent oxidation of heme (**1.4**) to 5-meso-hydroxyheme

(**1.12**). The chief evidence for this intermediate is the finding that synthetic **1.12** is converted to biliverdin by heme oxygenase[32,33] as well as by a variety of model systems.[34-36] The second step in the reaction involves elimination of the 5-meso-carbon as CO and incorporation of one atom of oxygen (from O_2) to form verdoheme (**1.13**). The precise structural nature of this second intermediate (**1.13**), with a visible absorption maxima at 688 nm, has not been established.[37-39] The final step is the oxidation of **1.13** to biliverdin (**1.9**) with the loss of the iron atom. The weakness of the proposed mechanism is that the putative intermediates have not been well characterized.



1.4 Heme **1.9 Biliverdin**

1.12 5-Hydroxyheme **1.13 Verdoheme**

Figure 1.6 Proposed mechanism of heme to biliverdin by heme oxygenase.

1.3.2 Transformation from Biliverdin to Bilirubin Diglucuronide

Biliverdin (**1.9**) does not accumulate in the system, but is quickly reduced to the yellow bile pigment, bilirubin (**1.10**), by biliverdin reductase. This enzyme requires NADPH as a source of electrons. Conjugation of bilirubin with glucoronic acid by the enzyme bilirubin-UDP-glucuronyltransferase is necessary in order to excrete bilirubin into the bile. This step is important clinically since the build-up of bilirubin is potentially toxic.

1.3.3 Clinical Applications

The neurotoxic properties of bilirubin, a surprisingly lipophilic molecule, make its accumulation undesirable. This water-insolubility is caused by the molecule's ability to maximize intramolecular hydrogen bonding (Figure 1.7). One may wonder why bilirubin is formed since biliverdin is more water soluble. One possible answer is that bilirubin is an effective antioxidant.[12] A more imposing reason is that bilirubin, unlike biliverdin, can pass through the mother's placenta.[40] This facilitates the removal of heme catabolic products from the fetus.

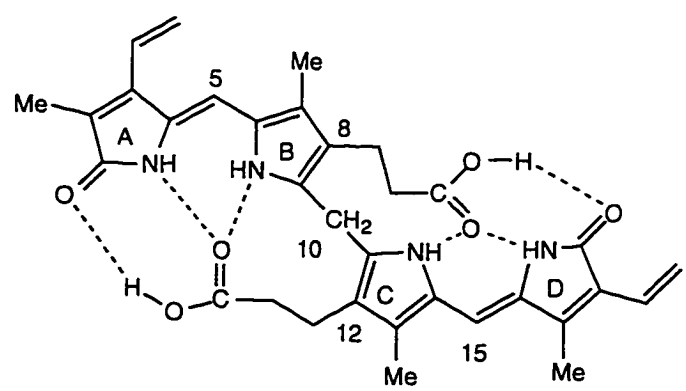


Figure 1.7 A planar representation of the intermolecular hydrogen bonding in bilirubin **1.10**.

The build-up of bilirubin can lead to jaundice and hyperbilirubinemia which is associated with the characteristic yellowing of the skin. The increased levels of bilirubin in newborns, known as neonatal jaundice, is especially serious because the infant can

experience brain damage (kernicterus) if the concentration of bilirubin is too high.[41] Jaundice, due to a malfunction of the liver, is also common.

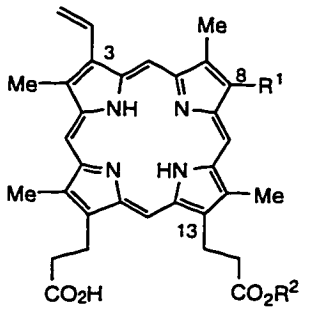
1.4 Chlorophyll *a* Biosynthesis

The biosynthesis of chlorophyll *a* begins with the carbon skeleton of glutamate which is converted into 5-aminolevulinic acid (**1.14**). In animals, the biosynthesis of heme also involves 5-aminolevulinic acid (**1.14**) which is made from glycine and succinyl-CoA. From 5-aminolevulinic acid (**1.14**), the biosynthesis of chlorophyll *a* follows the pathway for the biosynthesis of heme up to a branch point. This pathway diverges at protoporphyrin IX (**1.15b**) where magnesium insertion leads to chlorophyll *a* and chelation to iron results in the formation of heme. First proposed in 1950, the reaction sequence from **1.15b** to chlorophyll *a* was thought to proceed via a conventional linear pathway (Figure 1.8 & Figure 1.9).[42-44] For over 20 years, the scientific community accepted this biosynthetic route. This was a result of the main characterizing technique, chemical derivatization combined with electronic absorbance spectroscopy, which was inadequate to reveal the full chemical complexity of the biosynthetic pathway.[45]

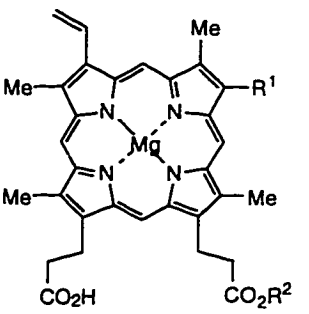
The development of finer instrumental techniques, such as low-temperature spectrofluorimetry and NMR spectroscopy, revealed experimental evidence that was contradictory to the linear model. Isolation of intermediates such as divinylchlorophyllide *a* (**1.18b**)[46] and the 13-methyl esters of both Mg-monoprotoporphyrin (**1.16c**) and Mg-protoporphyrin (**1.16d**)[47] suggested multiple and/or parallel biosynthetic routes. In 1983, Rebeiz et al. proposed a multi-branched model consisting mainly of two biosynthetic pathways for the formation of chlorophyll *a*. (Figure 1.10).[48] This biosynthetic heterogeneity involves both (3)-mono- and (3,8)-di-vinyl intermediates connected by 8-vinyl reductase enzymes.

Two 8-vinyl reductase enzymes, [8-vinyl] protochlorophyllide *a* reductase and [8-vinyl] chlorophyllide *a* reductase, have been identified.[49,50] Other putative 8-vinyl

reductases have not been found, but Mg-monovinylprotoporphyrin IX (**1.16a**) and divinyl chlorophyll *a* (**1.6b**) intermediates are known. Although it is not clear whether there is one enzyme of 8-vinyl specificity or several different 8-vinyl reductases with narrow specificity, evidence strongly implies the later.[45,51]

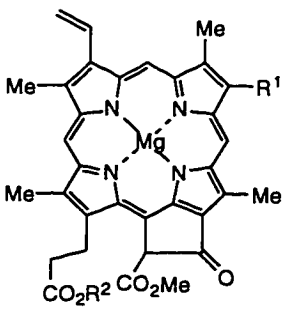


1.15

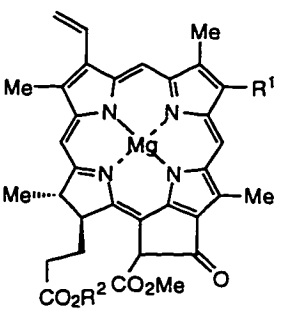


1.16

	R^1	R^2	trivial compound name
1.15a	Et	H	monovinylprotoporphyrin IX
1.15b	V	H	protoporphyrin IX
1.16a	Et	H	Mg-monovinyl protoporphyin IX
1.16b	V	H	Mg-protoporphyrin IX
1.16c	Et	Me	Mg-monovinyl protoporphyin IX methyl ester
1.16d	V	Me	Mg-protoporphyin IX methyl ester



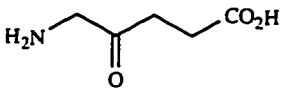
1.17



1.18

	R^1	R^2	trivial compound name
1.17a	Et	H	monovinylprotochlorophyllide *a*
1.17b	V	H	divinylprotochlorophyllide *a*
1.18a	Et	H	monovinyl chlorophyllide *a*
1.18b	V	H	divinyl chlorophyllide *a*
1.6a	Et	$C_{20}H_{39}$	monovinyl chlorophyll *a*
1.6b	V	$C_{20}H_{39}$	divinyl chlorophyll *a*

Figure 1.8 Chemical structures of tetrapyrroles involved in chlorophyll *a* biosynthesis.



1.14 aminolevulinic acid

↓

1.15b protoporphyrin IX ⟶ **1.4 Heme**

↓

1.16b Mg-protoporphyrin IX

↓

1.16d Mg-protoporphyrin IX methyl ester

↓

↓

1.17b divinylprotochlorophyllide *a*

↓

1.17a monovinylprotochlorophyllide *a*

↓

1.18a monovinylchlorophyllide *a*

↓

1.6a monovinylchlorophyll *a*

Figure 1.9 The linear chlorophyll *a* biosynthetic pathway.

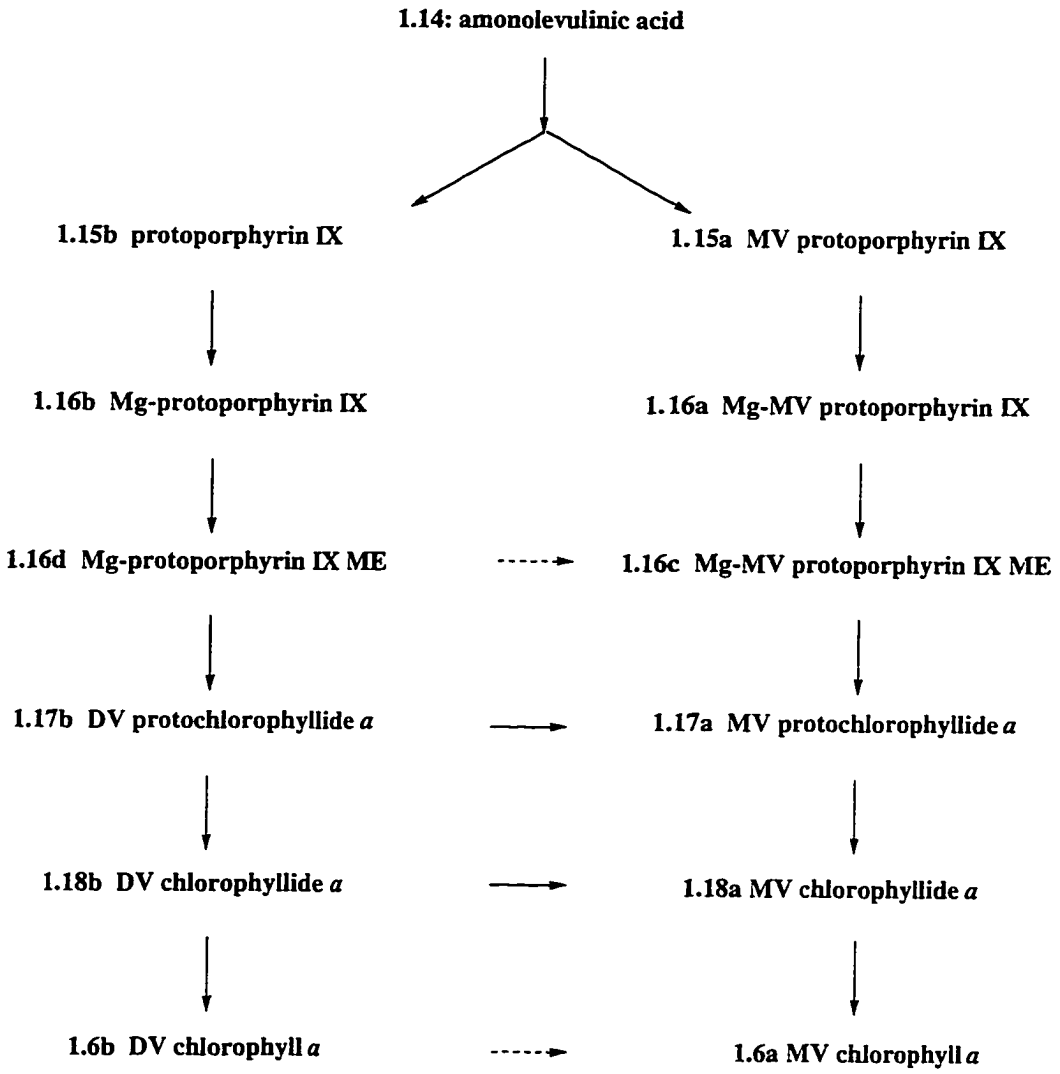
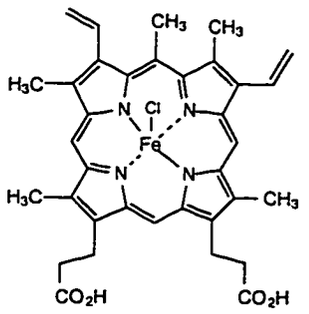


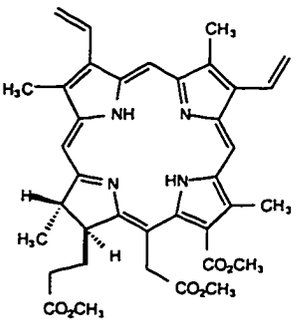
Figure 1.10: Partial model of multi-branched biosynthetic chlorophyll *a* pathway (MV=monovinyl, DV=divinyl, ME=methyl ester).

1.5 Dissertation Goals

The goal of this Dissertation is to demonstrate the application of organoselenium chemistry toward the synthesis of porphyrin compounds. This applicability is illustrated by the synthesis of two biological tetrapyrrole derivatives. Organoselenium chemistry is a useful way to convert alkyl halides or alcohols into vinyl groups. In Chapter 2, the organoselenium methodology is presented and in Chapter 3, the specific application of this chemistry as used to synthesize 5-meso-methylhemin chloride (**1.19**) and 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) is described.



1.19



1.20

The compounds are both of use in the study of macrocycle biochemistry. Synthesis of **1.19** will be useful in probing the first step in the heme catabolism mechanism. Our collaborator, Paul Ortiz de Montellano at the University of California, San Francisco, is interested in studing inhibition of heme oxygenase using our substrate. Blockage of the 5-meso position with a methyl group may inhibit the rate-limiting enzyme and prevent formation of 5-meso-hydroxy-heme (**1.12**).

The synthesis of the 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) is applicable to the study of chlorophyll biochemistry. As one of the first synthetic methods for making divinyl derivatives,[52,53] **1.20** will be useful in probing the biosynthetic heterogeneity seen with chlorophyll *a* formation. The ability to synthesize one of the metabolic intermediates

in large quantity should facilitate the assaying of the various putative 8-vinyl reductase enzyme specificities.

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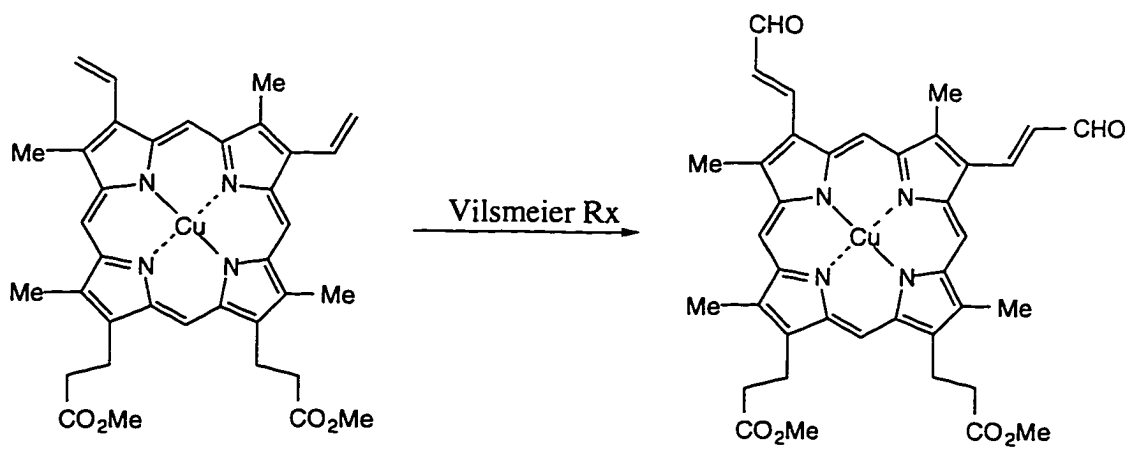
Chapter Two

Vinyl Group Protection In Porphyrins and Chlorins: Organoselenium Derivatives

2.1 Introduction

Substituent manipulations of naturally occurring tetrapyrrole derivatives such as those from heme and chlorophyll *a* produce a large number of chemically useful and biologically relevant compounds.[1] Many of these reactions affect the sensitive vinyl functionalities attached to the chromophore; consequently vinyl-protection is required. One such example is the standard Vilsmeier formylation reaction of copper(II) vinylporphyrin macrocycles (e.g. **2.1**).[2] Formylation takes place preferentially at the vinyl groups giving an acrolein derivative (**2.2**) rather than the desired meso-formyl product (Figure 2.1).[3]

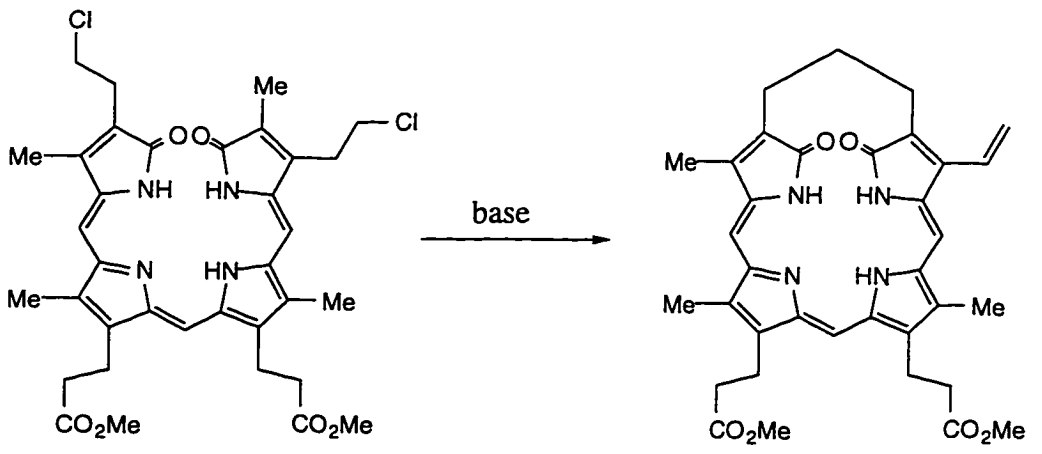


2.1 copper vinylporphyrin **2.2 acrolein derivative**

Figure 2.1 Under Vilsmeier reaction conditions, formylation takes place preferentially at the vinyl groups.

2.1.1 Vinyl-Protection Problem

Vinyl modified groups such as the chloroethyl substituent,[4] while useful, become problematic when alkaline deprotection from base sensitive tetrapyrroles is attempted. For example, attempted base-catalyzed deprotection of bis-(2-chloroethyl)biliverdin, **2.3**, resulted in dehydrohalogenation *and* cyclization, forming a novel bridged biliverdin derivative **2.4** (Figure 2.2).[5] Basic deprotection of 3-(2-chloroethyl)isochlorin-e_6 trimethyl ester derivatives also resulted in a mixture of undesired side products,[6,7] and dehydrohalogenation of 5-methyl-3,8-bis-(2-chloroethyl)porphyrin, **2.5**, led to the formation of porphyrin **2.6** bearing a vinyl and a novel six-membered ring (Figure 2.3).[8] The cyclization reaction presumably occurred by way of a mechanism similar to that proposed for the **2.3** → **2.4** transformation. See Chapter 3 for further details regarding the vinyl-protection problems of 5-methyl-3,8-bis(2-chloroethyl)porphyrin **2.5** and 3-(2-chloroethyl)isochlorin-e_6 trimethyl ester derivatives. The focus of this Chapter is the development of vinyl-protection during Vilsmeier meso-formylation of the macrocycle in porphyrins and chlorins.



2.3 **2.4**

Figure 2.2 Base-catalyzed deprotection of bis(2-chloroethyl) biliverdin **2.3** resulted in the formation of a bridged biliverdin derivative **2.4.**

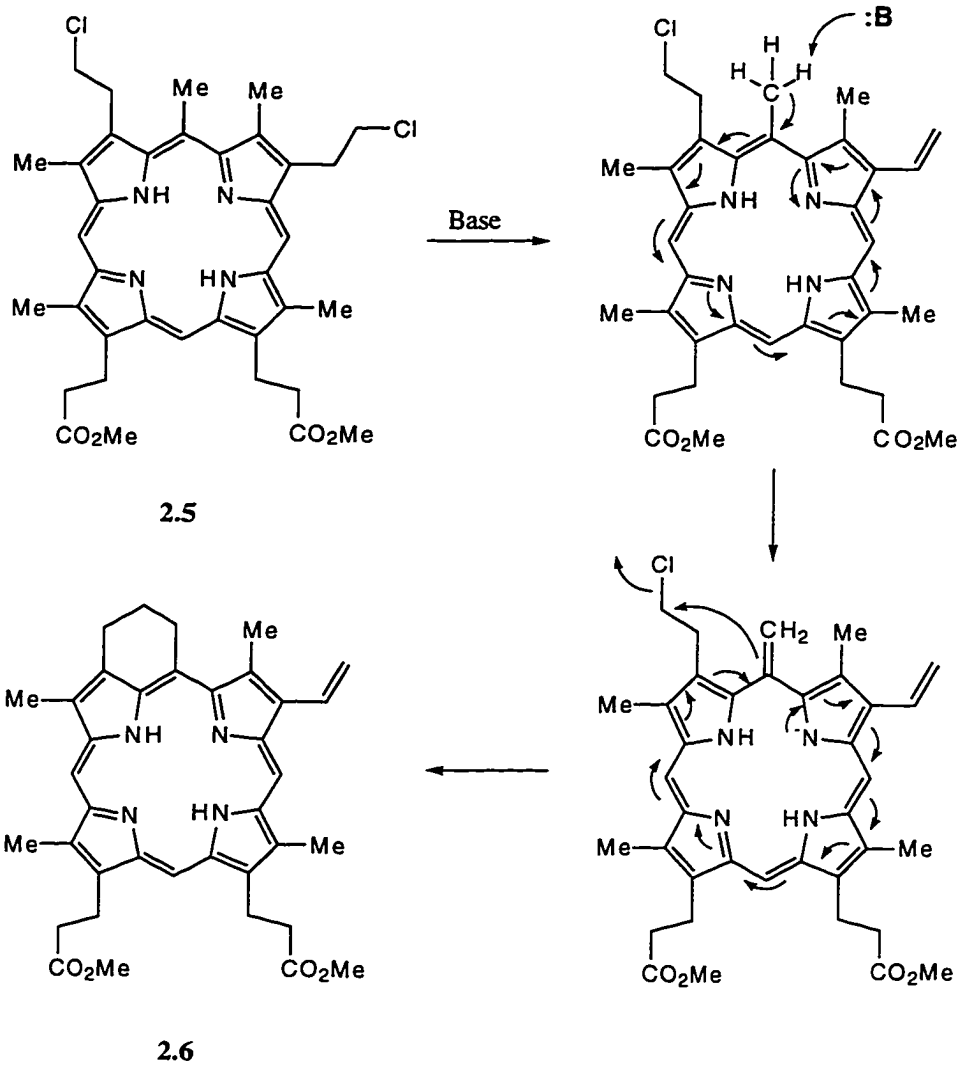


Figure 2.3 Basic deprotection of 5-methyl-3,8-bis(2-chloroethyl)porphyrin **2.5** resulted in the formation of porphyrin **2.6**.
Proposed mechanism for the cyclization is illustrated.

2.1.2 Organoselenium Chemistry

Organoselenium chemistry has been commonly used for conversion of alkyl halides or alcohols into olefins (Figure 2.4).[9-11] The selenide is formed with an arylselenium reagent which upon oxidation, eliminates to give the alkene. Gossauer et al. made use of selenium activation to produce vinyl-substituted bile pigments.[12-15] In our laboratories, we converted propionate side chains into acrylate substituents in porphyrin derivatives,[16] and Jacobi et al. recently used a pyrrole selenide derivative to synthesize a vinyl substituted

dipyrromethenone.[17] Work reported in this Chapter will now show that the *o*-nitrophenylselenoethyl substituent is a useful vinyl-protection group in porphyrins and chlorins, and that deprotection can be easily accomplished under mild, non-basic conditions.

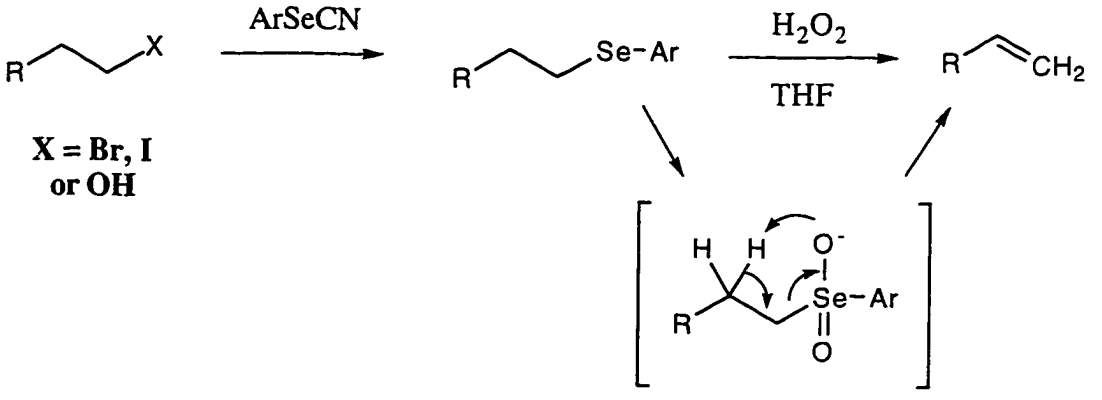
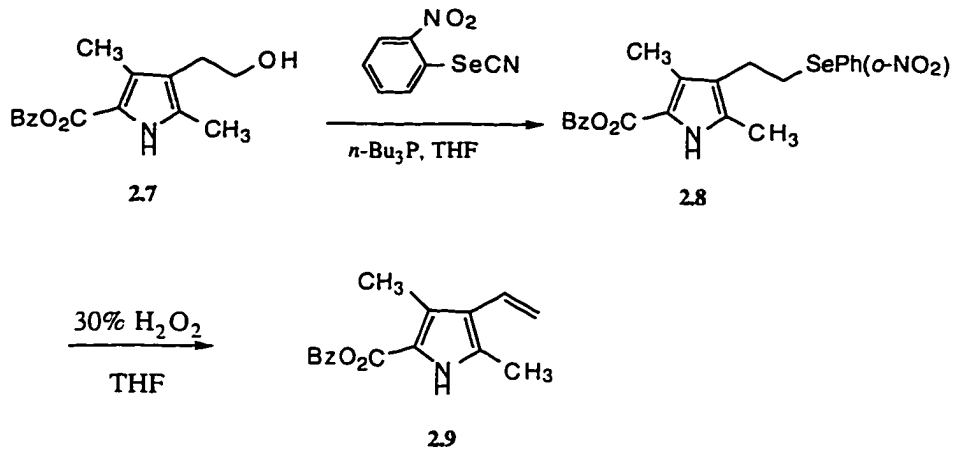


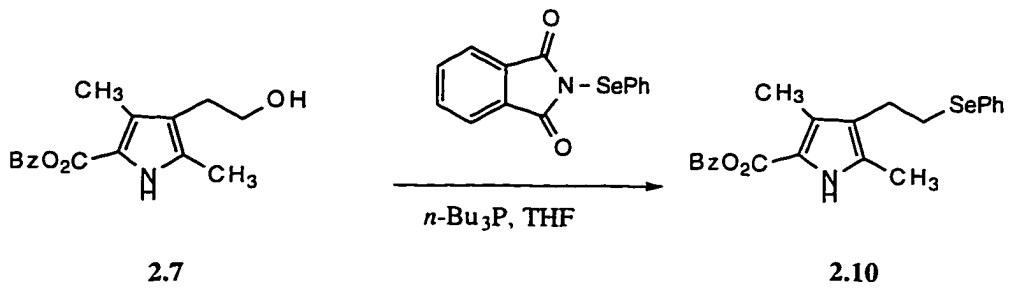
Figure 2.4 Conversion of an alkyl halide or alcohol to an alkene via organoselenium chemistry.

2.2 Development of Organoselenium Vinyl-Protection Methodology: The Pyrrole Model

The development of the organoselenium vinyl-protection methodology was first worked out on a pyrrole model. Following the method of Grieco et al.,[11,18] the 4-(2-hydroxyethyl)pyrrole **2.7**[19] was reacted with *o*-nitrophenylselenocyanate and tributylphosphine (*n*-Bu_3P) to give the selenide pyrrole **2.8** in quantitative yield. The selenide pyrrole **2.8** was oxidized with 30% aqueous H_2O_2 in THF to give the intermediate selenoxide which eliminated at room temperature to provided the vinyl pyrrole **2.9** in 45% yield (Scheme 2.1). A different selenium reagent, *N*-(phenylseleno)phthalimide (*N*-PSP) was also examined, and the selenide pyrrole **2.10** was prepared in 26% yield (Scheme 2.2).



Scheme 2.1 Synthesis of selenide pyrrole **2.8** and oxidation / elimination to vinyl pyrrole **2.9**.



Scheme 2.2 Synthesis of selenide pyrrole **2.10** with a different organoselenium reagent (*N*-PSP).

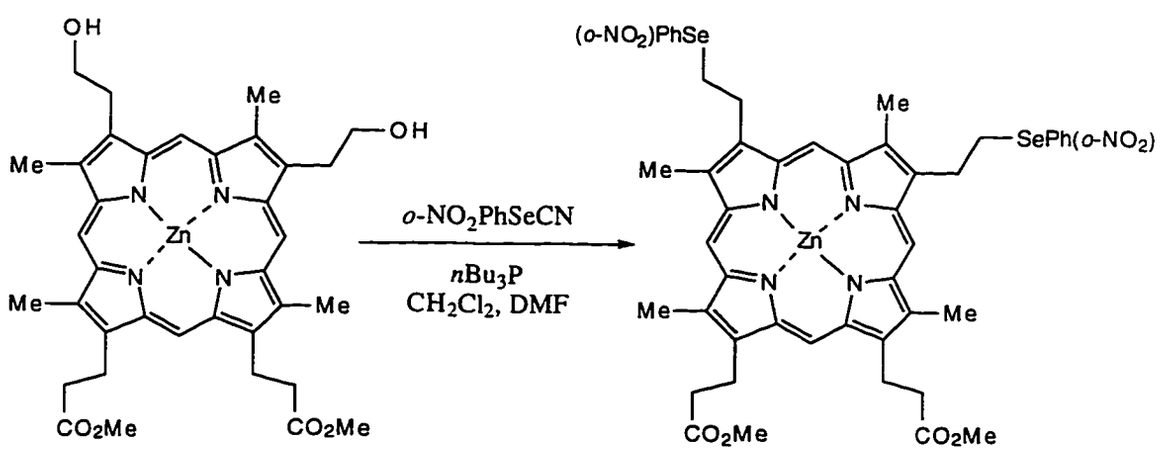
These selenide pyrroles, **2.8** and **2.10**, were tested in a catalytic (Pd/C) hydrogenation reaction to remove the benzyl protecting group and generate a carboxylic acid. The reactions resulted in unidentifiable complex mixtures. In the case of selenium pyrrole **2.8**, no reaction took place after 16 hours when the solvent was THF. A mixture of compounds formed when the solvent used was acetic acid, due in part to reduction of the nitro group under these conditions.[20] When selenium pyrrole **2.10** was dissolved in acetic acid and reacted in the same manner, unidentified mixtures also resulted.

2.3 Development of Organoselenium Vinyl-Protection Methodology: The Porphyrin Macrocycle

Three factors are important to the success of the organoselenium reaction on

porphyrin macrocycles. First, the reaction works best under concentrated conditions. Second, purification of the crude material on a silica gel column, followed by crystallization of the product, is crucial in order to remove contaminating tributylphosphate. Finally, the organoselenium reaction works well for alcohol porphyrin macrocycles [e.g. 3,8-bis(2-hydroxyethyl) protoporphyrin-IX dimethyl ester **2.16**[4]], but the reaction fails to produce the desired product when halide porphyrins [e.g. 3,8-bis(2-bromoethyl) protoporphyrin-IX dimethyl ester] are used; instead, starting material is recovered.

The organoselenium reaction also works well on metallated alcohol porphyrins [e.g. Zn-3,8-bis(2-hydroxyethyl)protoporphyrin-IX dimethyl ester **2.11**]. The zinc porphyrin **2.11** and *o*-nitrophenylselenocyanate were dissolved in a 2:1 CH_2Cl_2 : DMF solvent mixture followed by addition of *n*-Bu_3P. The zinc-selenide porphyrin **2.12** was obtained in 58% yield (Scheme 2.3). Oxidation of the Zn-porphyrin **2.12** did not provide the vinyl functionalities, but rather an unidentifiable mixture of decomposed compounds. Removal of the metal is required before oxidation and elimination of the selenium moiety is carried out. This was seen in the case of the Cu-selenium porphyrin **2.18** as well.

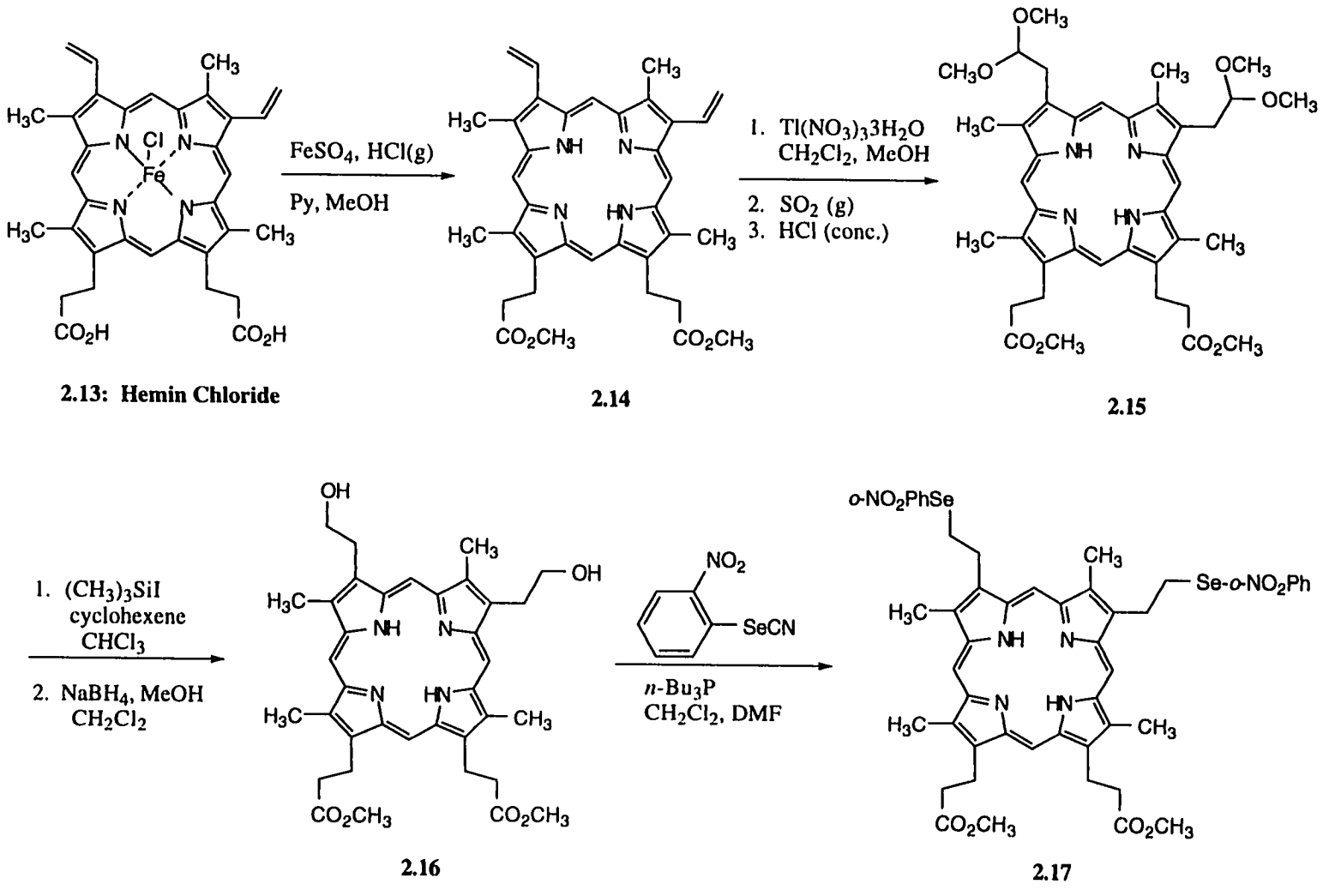


2.11 **2.12**

Scheme 2.3 Synthesis of Zn bis-3,8-(*o*-nitrophenylseleno)protoporphyrin IX dimethyl ester **2.12**. An example of the versatility of organoselenium chemistry.

2.4 Vinyl-Protection Under Vilsmeier Formylating Conditions: Syntheses of Meso-formylporphyrins 2.21a-d and Chlorin 2.27[21]

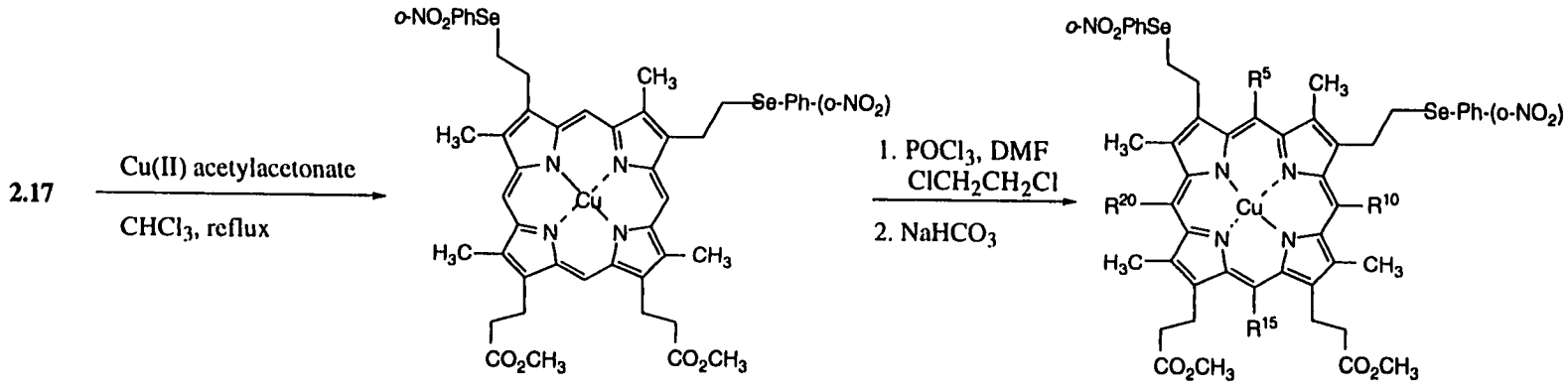
The synthetic approach to meso-formylporphyrins **2.21a-d** (Scheme 2.4) represents a typical example of the organoselenium vinyl-protection methodology, and Scheme 2.5 illustrates a new route to 20-formylchlorin-e_6 trimethyl ester[6] **2.27**. Beginning with commercially available hemin chloride **2.13**, protoporphyrin-IX dimethyl ester **2.14**, was prepared following the Grinstein method.[22] According to standard protocols using thallium chemistry,[4,23] the vinyl moieties in **2.14** were converted into acetal groups (**2.15**) followed by hydrolysis and reduction to afford the corresponding hydroxyethyl substituents,[23] giving **2.16** in 73% yield. Conversion of **2.16** into the selenium porphyrin **2.17** using *o*-nitrophenylselenocyanate and *n*-Bu_3P in CH_2Cl_2 was accomplished in 80% yield. With the vinyl groups protected for the model Vilsmeier reaction, insertion of copper (to give **2.18**) followed by formylation using $POCl_3$ and DMF provided the meso-formylporphyrin regioisomers **2.19a-d** in 70% yield. Demetallation with 5% H_2SO_4/TFA yielded **2.20a-d** in 85% yield. Finally, deprotection to the selenoxide by oxidation with 30% H_2O_2 in THF, followed by elimination, gave the four meso-formylprotoporphyrin-IX dimethyl ester isomers **2.21a-d** in 67% yield. In a similar synthesis starting with chlorin-e_6 trimethyl ester **2.22**,[6,24] 20-meso-formylchlorin **2.27** was the only formyl isomer formed because of enhanced nucleophilicity at the 20 position due to the saturation at ring-D.[25] See Figure 2.5 for ^1H NMR spectrum of 20-meso-formylchlorin-e_6 trimethyl ester **2.27**.

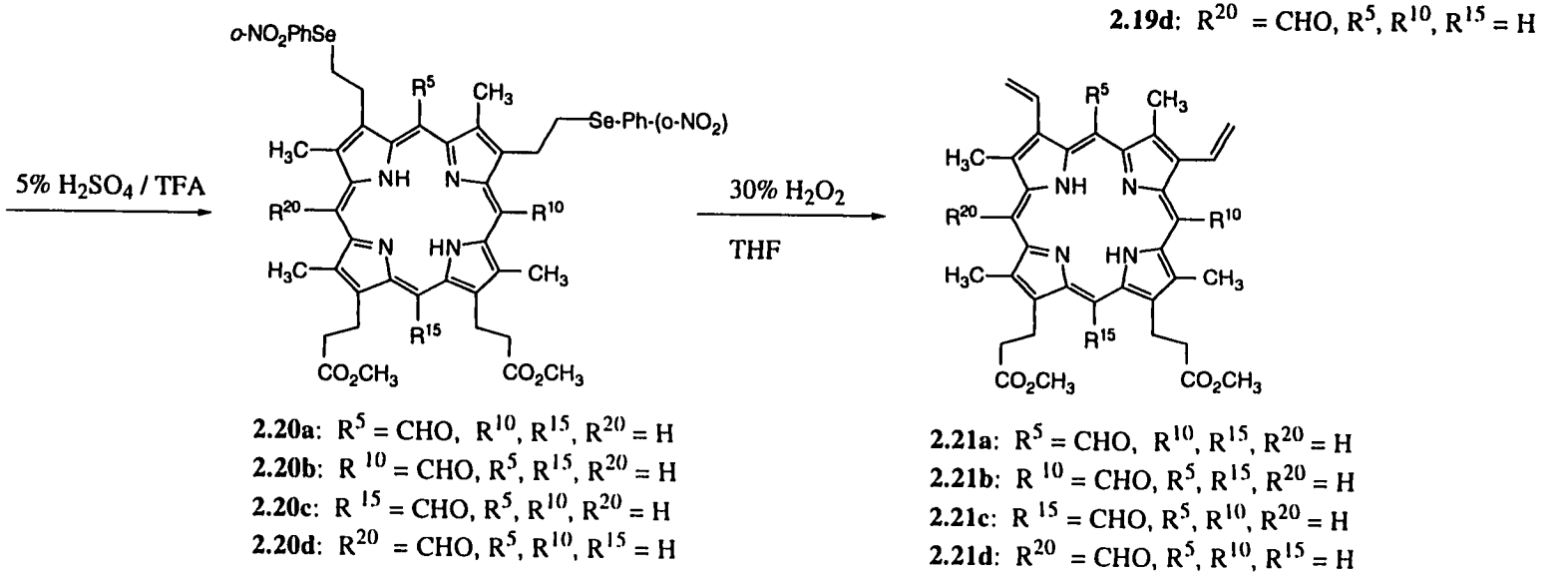


2.13: Hemin Chloride **2.14** **2.15**

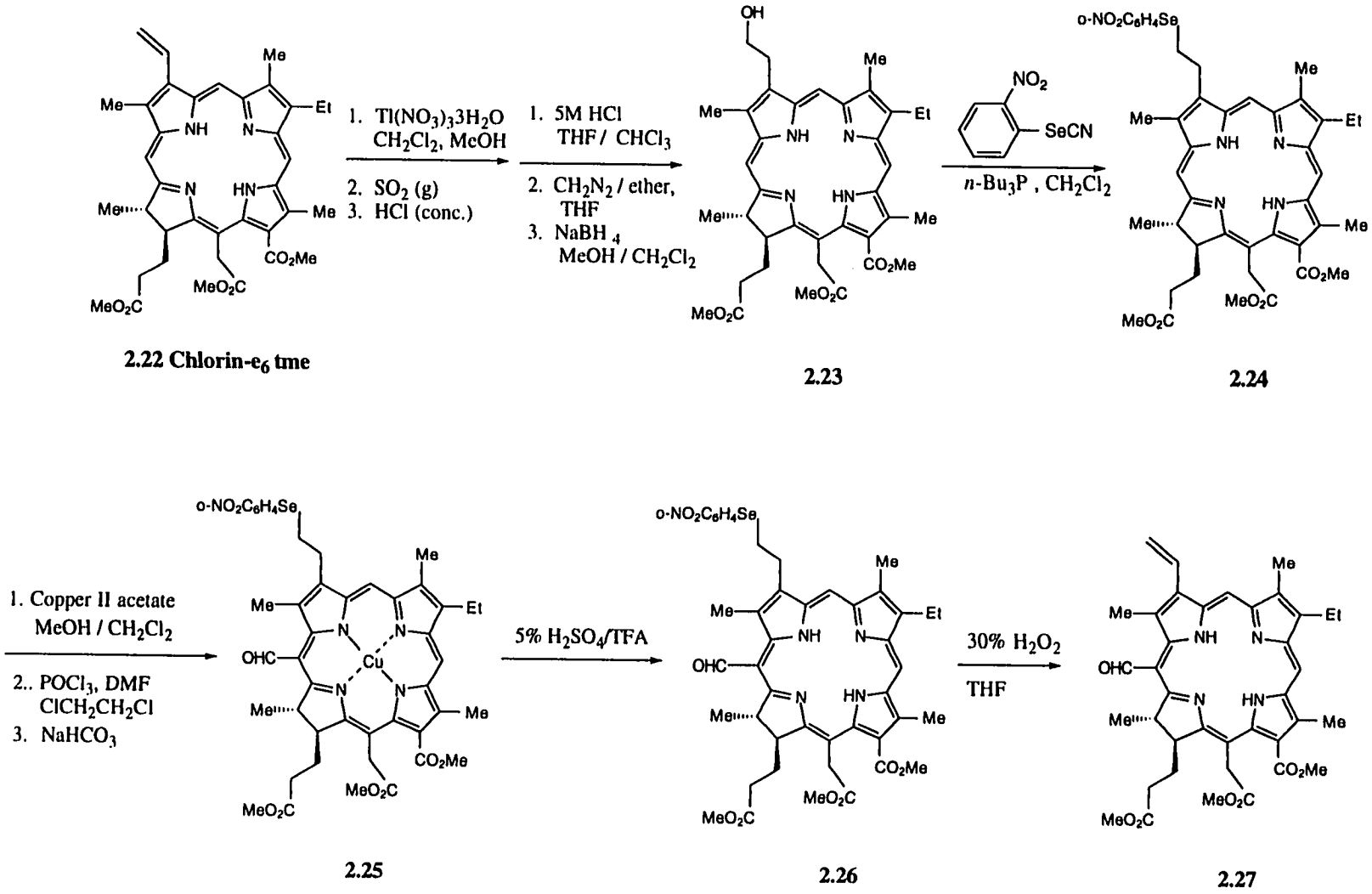
2.16 **2.17**

Scheme 2.4 Synthetic route to meso-formylprotoporphyrin IX dimethyl ester isomers **2.21a-d.**





Scheme 2.4 continued.



2.22 Chlorin-e_6 tme **2.23** **2.24**

2.25 **2.26** **2.27**

Scheme 2.5 Synthetic route toward 20-meso-formylchlorin-e_6 trimethyl ester **2.27.**

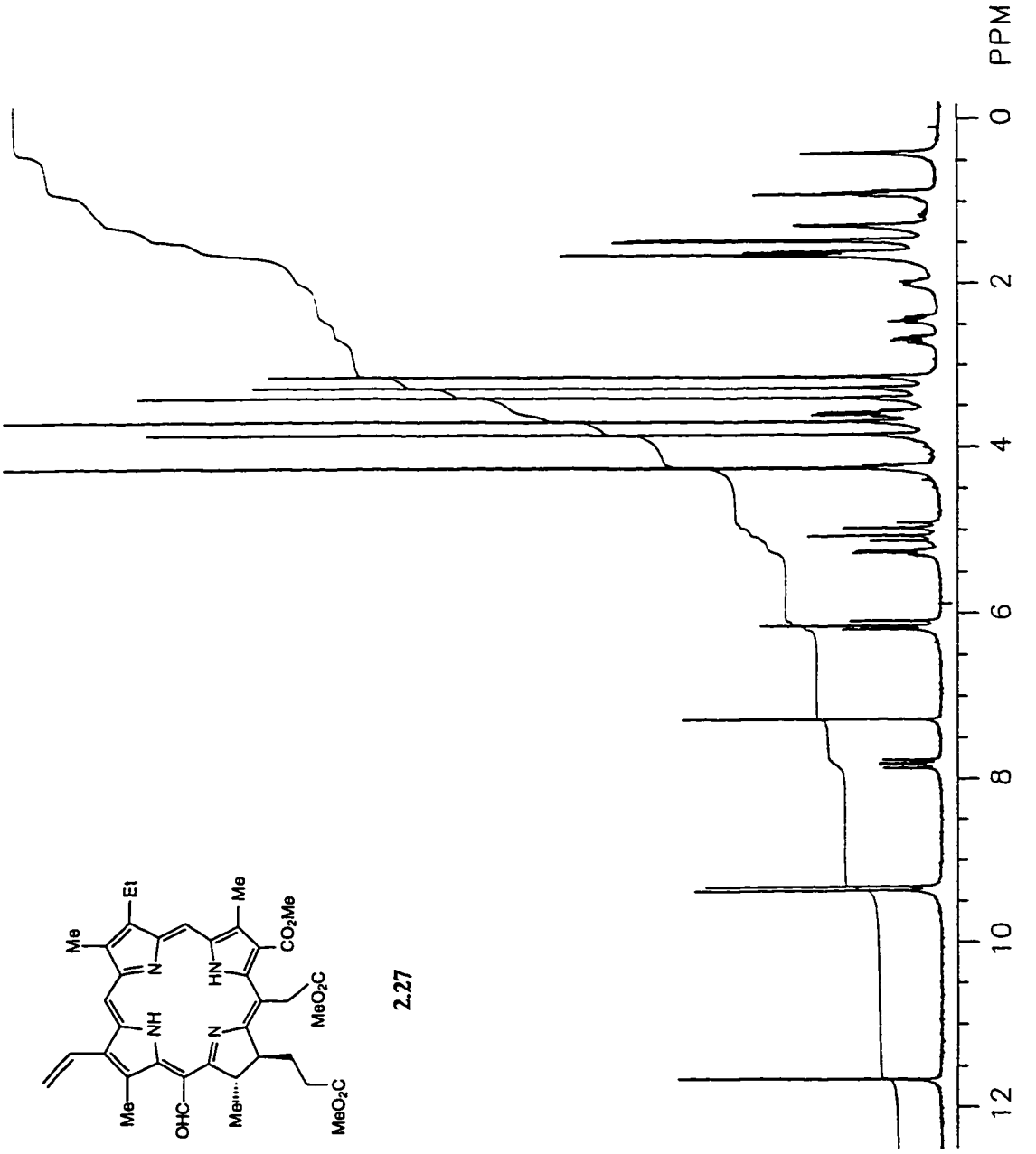


Figure 2.5 ^{1}H NMR spectrum of 20-meso-formylchlorin-e_6 trimethyl ester, **2.27**.

2.4.1 HPLC Separation and NOE-NMR Analysis of the Four Meso-Formyl Porphyrin Regioisomers

Separation of the four isomers **2.21a-d** using silica gel HPLC (0.5% THF/CH_2Cl_2) indicated a ratio of 5:8:14:72 respectively, and an elution order of **2.21a**:**2.21b**:**2.21d**:**2.21c**. Three of the regioisomers, **2.21a,c,d**, were pure and **2.21b** contained a minor amount of **2.21d.**

Regiochemistry of the three isomers **2.21a,c,d** was determined by analysis of spectra from two NOE-NMR experiments. Irradiation of the coincident 13^1-CH_2 and 17^1-CH_2 signals followed by irradiation of the formyl proton signal permitted unambiguous structural assignments (Figure 2.6). By exclusion, the regiochemistry of **2.21b** was deduced. Figure 2.7 compares the ^1H NMR spectra for the four isomers.

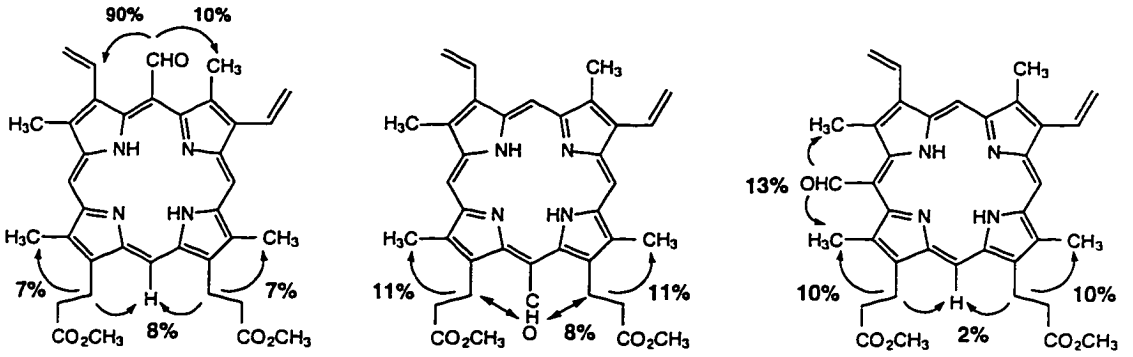


Figure 2.6 NOE connectivities for **2.21a,c,d.**

Due to the very similar structural environments of 5-meso-formyl regioisomer **2.21a** and 10-meso-formyl regioisomer **2.21b**, the assignment of the formyl group was not trivial. The two NOE NMR experiments described above allowed for the unambiguous assignment of **2.21a**. In the first experiment, irradiation of the coincident 13^1-CH_2 and

17^1-CH_2 signals of **2.21a** demonstrated an NOE to two methyl groups (12-CH_3 and 18-CH_3) and a meso-H. Irradiation of the formyl proton of **2.21a**, resulted in an NOE to a **different** methyl group (7-CH_3) than that of the first experiment. If the compound had been **2.21b**, irradiation of the formyl proton would have shown an NOE to the **same** methyl group (12-CH_3) as that of the first experiment.

2.4.2 Crystal Structure of Chlorin Selenide 2.24

The structure of the chlorin selenide **2.24** was confirmed by an X-ray crystal structure (Figure 2.8).[26] The chlorin core is essentially flat except for the pronounced twist out of the plane shown by the atoms responsible for the chlorin ring-D functionality. Hydrogen atoms of the central core are localized on N(1) of the pyrrole bearing the selenium substituent and on the opposite pyrrole nitrogen, N(3). The *o*-nitrophenyl group bonded to Se is coplanar with the core, enabling the molecules to stack in a head-to-tail fashion along the crystallographic **a** direction.

2.5 Conclusions

The *o*-nitrophenylselenoethyl substituent is a useful protected vinyl group in porphyrins and chlorins as demonstrated in the Vilsmeier formylation reaction. The advantage of the *o*-nitrophenylselenoethyl substituent is that it can be removed under mild non-basic conditions. The application of *o*-nitrophenylselenoethyl vinyl-protection in the syntheses of base sensitive porphyrin macrocycles such as 5-meso-methylhemin chloride (**1.19**) and 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) are described in the next Chapter. These compounds should be useful in studying heme catabolism and chlorophyll *a* biosynthesis.

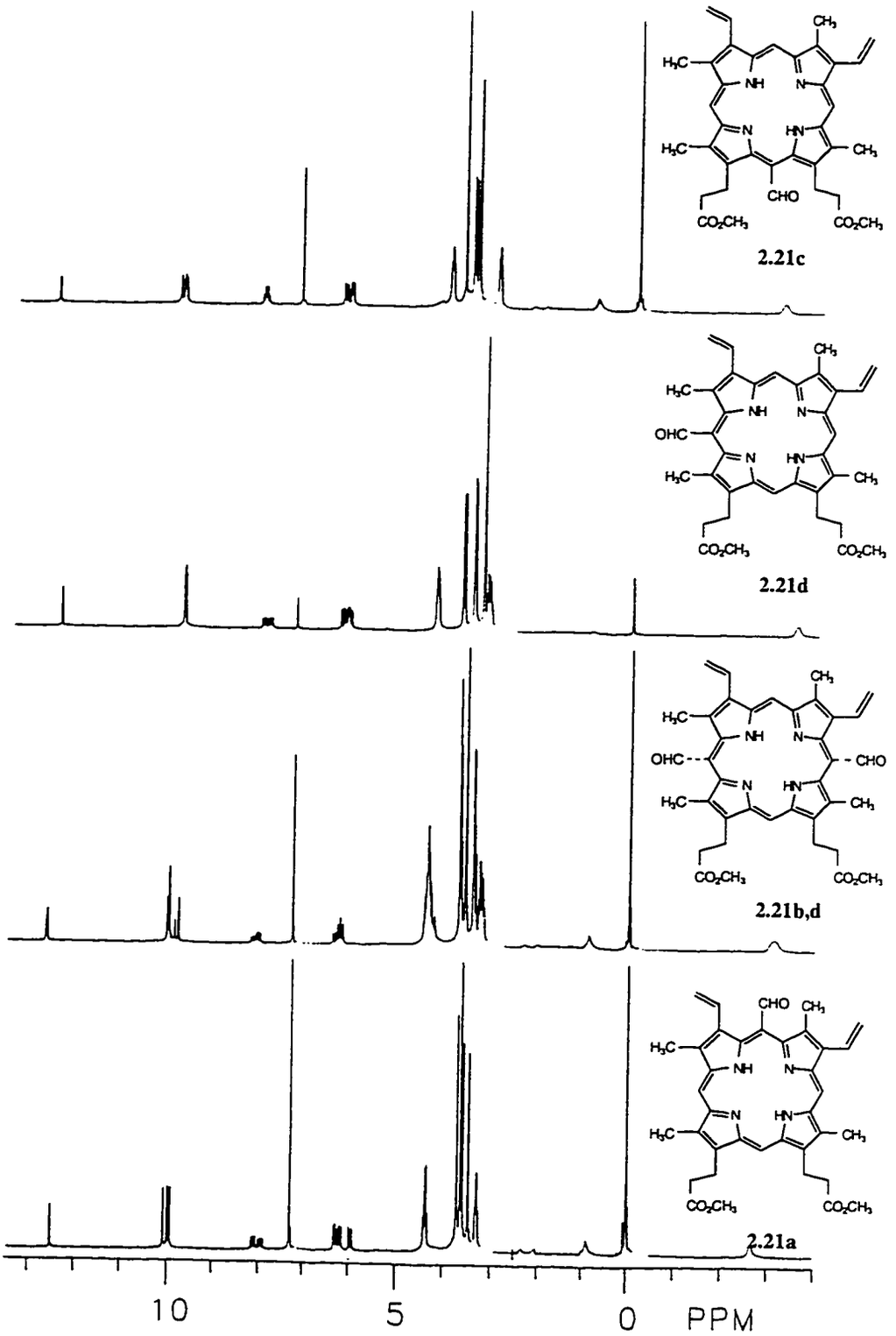


Figure 2.7 Comparison of ^{1}H NMR spectra for meso-formyl regioisomers, **2.21a-d**.

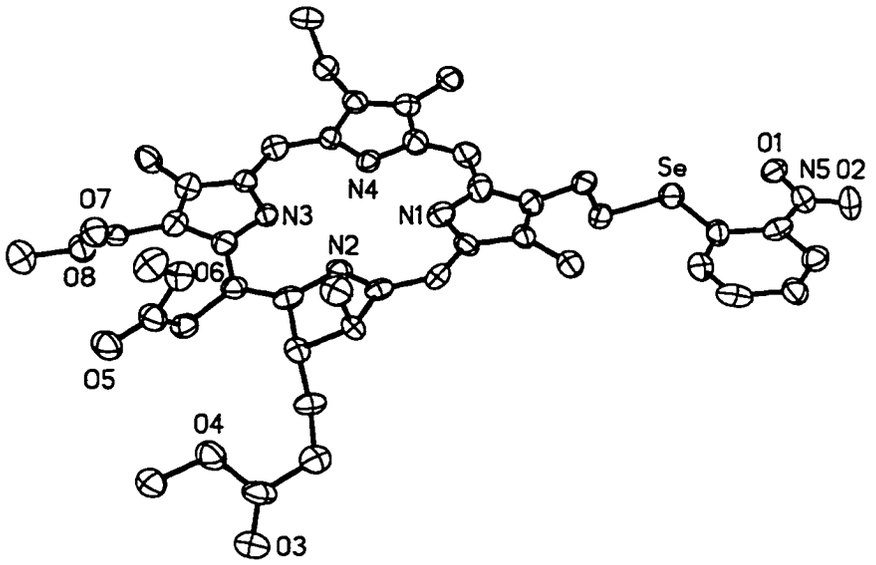


Figure 2.8 X-ray crystal structure of **2.24**.

2.6 References and Notes

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(26) *Crystal Data* : $C_{43}H_{47}N_5O_8Se$, **2.24**, M = 840.82, orthorhombic, a = 7.607(4), b = 14.671(11), c = 34.61(3) Å, U = 3863(5) Å^3 (by least-squares refinement on diffractometer angles for 46 centered reflections), λ = 1.54178 Å, space group $P2_12_12_1$; Z = 4, D_c = 1.446 g cm^{-3}, F(000) = 1752; green plates; crystal dimensions 0.004 x 0.12 x 0.50 mm, μCuKα = 1.83 mm^{-1}, Siemens P4 diffractometer and Cu rotating anode operating at 15 kW; scan type 2Θ-Θ, T = 130(2) K, $2\Theta_{max}$ = 112.3°; 2981 data, 2914 unique, R(int) = 0.025; 1824 > 2σ(I), XABS2 absorption correction, solution and refinement using Siemens SHELXTL

v. 5, refinement based on F^2, *wR* (all data) = 0.1252, *R* (obsd data) = 0.0696, hydrogens bonded to nitrogen located on difference map, remaining hydrogens added geometrically; all hydrogens refined using riding model; largest peak in final difference Fourier map = 0.35 $eÅ^{-3}$; absolute structure parameter (Flack) -0.02(7). Tables of atom positions, thermal parameters, and a complete listing of bond distances and angles have been deposited at the Cambridge Crystallographic Data Centre.

2.7 Experimental

General. The solvents were dried and/or distilled under the following conditions. THF was distilled from Na^o and benzophenone. CH_2Cl_2 and 1,2-dichloroethane were distilled from CaH_2. MeOH was distilled from Mg^o. $CHCl_3$ was distilled from P_2O_5 and stored over oven-dried K_2CO_3. DMF was stored over molecular sieves (4 Å) to remove any water. All reactions were run under an atmosphere of argon unless otherwise noted.

For column chromatography, either silica gel 60 (230-400 mesh) or alumina (70-230 mesh; deactivated by water to grade III or V) were used. Grade of alumina is measured by adding water (30 mL for grade III and 75 mL for grade V) to alumina (500 g). Pressure was supplied by house compressed air.

Instrumentation used for characterization of compounds are as follows. Melting points were measured on a Thomas/Bristoline microscopic hot stage apparatus and were uncorrected. Electronic absorption spectra (UV/VIS) were measured on Hewlett-Packard 8450A spectrophotometer in CH_2Cl_2 unless otherwise stated. Mass spectra were obtained at the Mass Spectrometry Facility at University of California, San Francisco, University of California, Berkeley or University of Minnesota. ^1H NMR spectra were recorded at 300 MHz and ^{13}C NMR spectra were recorded at 75 MHz. ^{13}C multiplicities were determined by DEPT experiments. Signals were referenced to $CHCl_3$, δ = 7.26 ppm or TMS, δ = 0.00 ppm. Elemental Analyses were performed by the Midwest Microlab in Indianapolis,

IN., or Zerchemie Laboratories, in Milpitas, CA.

Benzyl 3,5-Dimethyl-4-(2-*o*-nitrophenylselenoethyl)pyrrole-2-carboxylate 2.8. To a stirred solution of benzyl 4-(2-hydroxyethyl)-3,5-dimethyl pyrrole-2-carboxylate **2.7**[19] (105.0 mg) dissolved in dry THF (2 mL) was added o-nitrophenylselenocyanate (129.5 mg). Dropwise, nBu$_3$P (0.140 mL) was added and the reaction mixture was stirred under N_2 at room temp for 30 min. Solvent was removed in vacuo to give a light brown solid. The selenium pyrrole **2.8** (183.1 mg) was isolated by silica gel flash chromatography (eluted with CH_2Cl_2; R_f = 0.26) in quantitative yield as a yellow solid: mp 154.2-154.8 ^oC; ^1H NMR ($CDCl_3$, ref. to TMS): δ 8.63 (s, 1 H), 8.23 (d, J = 8.35 Hz, 1 H), 7.41 (m, 8 H), 5.29 (s, 2 H), 3.01 (t, J = 7.6 Hz, 2 H), 2.83 (t, J = 7.6 Hz, 2 H), 2.32 (s, 3 H), 2.21 (s, 3 H); HREIMS calcd for $C_{22}H_{22}N_2O_4Se$ m/z 458.0744, found m/z 458.0705. Anal. Calcd for $C_{22}H_{22}N_2O_4Se$: C, 57.77; H, 4.85; N, 6.12. Found: C, 57.61; H, 4.90; N, 6.01.

Benzyl 3,5-Dimethyl-4-vinylpyrrole-2-carboxylate 2.9. Benzyl 3,5-dimethyl-4-(2-*o*-nitrophenylselenoethyl)pyrrole-2-carboxylate **2.8** (255.5 mg) was dissolved in distilled, but not necessarily dry, THF (10 mL). The solution was cooled in an ice bath and 30% H_2O_2 (0.7 mL) was added dropwise. The reaction was stirred under N_2 at room temp for 5 h. The reaction mixture was diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (3X40 mL). The organic extract was filtered through neutral alumina (activity 1) and the solvent was evaporated in vacuo. The residue was purified by silica gel chromatography (25% cylcohexane / CH_2Cl_2) to give the vinylpyrrole **2.9** (64.1 mg) as light yellow feather like needles in 45% yield (R_f = 0.43 in CH_2Cl_2): mp 95 ^oC, decom.; ^1H NMR ($CDCl_3$, ref. to TMS): δ 8.98 (bs, 1 H), 7.39 (m, 5 H), 6.59 (dd, J = 17.9, 11.6 Hz, 1 H), 5.30 (s, 2 H), 5.26 (dd, J = 17.9, 1.5 Hz, 1 H), 5.15 (dd, J = 11.6, 1.5 Hz, 1 H), 2.40 (s, 3 H), 2.29 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 161.5 (s), 136.4 (s),

131.3 (s), 128.8 (d), 128.5 (d, 2 C), 128.0 (d), 127.9 (d, 2 C), 127.4 (s), 120.3 (s), 116.8 (s), 112.7 (t), 65.6 (t), 12.7 (q), 11.4 (q). HREIMS calcd for $C_{16}H_{17}NO_2$ *m/z* 255.1259, found *m/z* 255.1251. Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.27; H, 6.71; N, 5.49. Found: C, 75.27; H, 6.83; N, 5.48.

Benzyl 3,5-Methyl-4-(2-phenylselenoethyl)pyrrole-2-carboxylate 2.10. To a stirred solution of benzyl 4-(2-hydroxyethyl)-3,5-dimethylpyrrole-2-carboxylate **2.7**[19] (100.0 mg) dissolved in dry THF (5 mL) was added *N*-(phenylseleno)phthalimide (221 mg). Dropwise, nBu$_3$P (0.2 mL) was added and the reaction mixture was stirred at room temp for 20 h. Solvent was removed in vacuo to give a light brown solid. The crude material was purified by silica gel flash chromatography using a gradient of 15% petroleum ether / CH_2Cl_2 to 1% acetone / CH_2Cl_2 (R_f = 0.7 in 2% MeOH / CH_2Cl_2). Crystallization from CH_2Cl_2 / *n*-hexane gave the title pyrrole **2.10** (183.1 mg) in 26% yield as a white solid: mp 98-99 °C; ^{1}H NMR ($CDCl_3$, ref. to TMS): δ 8.45 (bs, 1 H), 7.27 (m, 10 H), 5.23 (s, 2 H), 2.88 (t, J = 7.3 Hz, 2 H), 2.68 (t, J = 7.3 Hz, 2 H), 2.20 (s, 3 H), 2.05 (s, 3 H); HREIMS calcd for $C_{22}H_{23}NO_2Se$ *m/z* 413.0893, found *m/z* 413.0882. Anal. Calcd for $C_{22}H_{23}NO_2Se$: C, 64.08; H, 5.62; N, 3.40. Found: C, 63.75; H, 5.59; N, 3.36.

Zinc Bis-3,8-(2-*o*-nitrophenylselenoethyl)protoporphyrin IX Dimethyl Ester 2.12. To the refluxing solution of the corresponding free-base bis-3,8-(2-hydroxyethyl)protoporphyrin IX dimethyl ester **2.16**[4,23] (323.9 mg) in $CHCl_3$ (100 mL) was added a sat. solution of Zn(II) acetate in MeOH (2 mL). The reaction mixture immediately turned a brighter pink and was monitored by spectrophotometry. After 15 min the reaction mixture was cooled, poured into H_2O and extracted with CH_2Cl_2. The organic extract was filtered through Na_2SO_4 and the solvent was evaporated to give the zinc porphyrin **2.11** (250.0 mg) in quantitative yield. Vis: λ_{max} 402, 532, 568 nm. To an

oven dried flask, Zn-bis-3,8-(hydroxyethyl) protoprophyrin IX dimethyl ester **2.11** (250.0 mg) and *o*-nitrophenylselenocyanate (823.0 mg) were dissolved in dry DMF (2.5 mL) and dry, distilled CH_2Cl_2 (4.0 mL). Dropwise, nBu$_3$P (0.9 mL) was added to the reaction mixture. After 4 h at room temp, the solvent was evaporated. The crude material was purified by silica gel chromatography and eluted with a gradient from 100% CH_2Cl_2 to 5% acetone / CH_2Cl_2. The major red fraction (after checking ^{1}H NMR spectrum) was crystallized from CH_2Cl_2 / MeOH to remove excess tributylphosphate. The desired product **2.12** was obtained in 58% yield (218.4 mg); mp 244-248°C dec.; Vis: λ_{max} 404 nm (ε 147 300), 532 (16 800), 570 (23 500); ^{1}H NMR ($CDCl_3$, ref. to TMS): δ 9.50 (s, 1 H, meso-H), 9.28 (s, 1 H, meso-H), 9.20 (s, 1 H, meso-H), 8.83 (s, 1 H, meso-H), 7.97 (overlapping d, 2 H, phenyl-H), 7.30 (d, 1 H, phenyl-H), 7.24 (d, 1 H, phenyl-H), 6.94 (m, 4 H, phenyl-H), 4.18 (m, 4 H, -CH_2C**H**$_2$CO_2CH_3), 3.98 (bt, 2 H, -CH_2C**H**$_2$Se) 3.78 (bt, 2 H, -CH_2C**H**$_2$Se), 3.69 (s, 3 H, -CO_2C**H**$_3$), 3.68 (s, 3 H, -CO_2C**H**$_3$), 3.40 (s, 3 H, ring-CH_3), 3.36 (s, 3 H, ring-CH_3), 3.16 (s, 3 H, ring-CH_3), 3.07 (s, 3 H, ring-CH_3), 3.32 (m, 4 H, -C**H**$_2$CH_2CO_2CH_3), 3.13 (m, 4 H, -C**H**$_2$CH_2Se); LRSIMS calcd for $C_{48}H_{46}N_6O_8Se_2Zn$ *m/z* 1058.1, found *m/z* 1058.2. Anal. Calcd for $C_{48}H_{46}N_6O_8Se_2Zn$·CH_3OH: C, 53.98; H, 4.62; N, 7.71. Found: C, 53.88; H, 4.47; N, 7.75.

Bis-3,8-(2-*o*-nitrophenylselenoethyl)protoporphyrin IX Dimethyl Ester 2.17. Bis-3,8-(hydroxyethyl)protoporphyrin-IX dimethyl ester[4,23] (**2.16**, 50 mg) was dissolved in a minimum amount of dry DMF (0.5 mL) and added to a solution of *o*-nitrophenylselenocyanate (91 mg) in dry, distilled CH_2Cl_2 (9.0 mL). Dropwise, nBu$_3$P (82 mg) was added and the reaction mixture was stirred at room temp. The reaction appeared to be complete after 20 min, however, a mixture of the mono and di-selenated products resulted. After 4 h the reaction was complete, so the solvent was evaporated in vacuo and the crude mixture was purified by silica gel chromatography using a gradient of

25% cyclohexane / CH_2Cl_2 to 5% acetone / CH_2Cl_2. The red fraction was further purified by crystallization from CH_2Cl_2 / *n*-hexane to give **2.17** in 80% yield (63.2 mg); mp 209 °C; Vis: λ_{max} 402 nm (ε 153 200), 498 (9 600), 534 (6 200), 568 (3 800), 622 (2 300), 664 (1 600); ^{1}H NMR ($CDCl_3$, ref. to TMS): δ 9.91 (s, 1 H, meso-H), 9.67 (s, 1 H, meso-H), 9.43 (s, 1 H, meso-H), 8.29 (s, 1 H, meso-H), 8.07 (overlapping d, 2 H, phenyl-H), 7.23 (overlapping d, 2 H, phenyl-H), 6.97 (m, 4 H, phenyl-H), 4.26 (m, 4 H, -$CH_2CH_2CO_2CH_3$), 3.78 (m, 4 H, -CH_2CH_2Se), 3.67 (s, 3 H, -CO_2CH_3), 3.66 (s, 3 H, -CO_2CH_3), 3.45 (s, 3 H, ring-CH_3), 3.34 (s, 3 H, ring-CH_3), 3.08 (s, 3 H, ring-CH_3), 3.06 (s, 3 H, ring-CH_3), 3.26 (m, 8 H, -$CH_2CH_2CO_2CH_3$ and -CH_2CH_2Se), -4.23 (bs, 2 H, NH); LRSIMS calcd for $C_{48}H_{48}N_6O_8Se_2$ *m/z* 994.9, found *m/z* 995.3. Anal. Calcd for $C_{48}H_{48}N_6O_8Se_2$: C, 57.95; H, 4.86; N, 8.45. Found: C, 57.70; H, 4.88; N, 8.34.

Copper Bis-3,8-(2-*o*-nitrophenylselenoethyl)protoporphyrin IX Dimethyl Ester 2.18. To a solution of bis-3,8-(*o*-nitrophenylselenoethyl) protoporphyrin IX dimethyl ester, (**2.17**, 218.1 mg) dissolved in reagent grade $CHCl_3$ (25 mL) was added a solution of Cu(II) acetylacetonate (1.15 g) dissolved in $CHCl_3$ (25 mL). The reaction mixture was stirred at reflux for 1 h. The mixture was cooled, poured over a column of dry alumina (grade V) and eluted with $CHCl_3$. The crude material was crystallized from $CHCl_3$ / *n*-hexane to give **2.18** in 90% yield (209.3 mg); mp 174-176 °C; Vis: λ_{max} 400 nm (ε 350 000), 526 (16 800), 564 (28 000); LRSIMS calcd for $C_{48}CuH_{46}N_6O_8Se_2$ *m/z* 1056.4, found *m/z* 1057.1. Anal. Calcd for $C_{48}CuH_{46}N_6O_8Se_2$: C, 54.57; H, 4.39; N, 7.96. Found: C, 54.60; H, 4.40; N, 7.86.

Copper Meso-formyl-bis-3,8-(2-*o*-nitrophenylselenoethyl) protoporphyrin IX Dimethyl Ester isomers 2.19a-d. The Vilsmeier reagent was prepared by adding together distilled 1,2-dichloroethane (2 mL), dry DMF (5 mL) and

$POCl_3$ (0.4 mL) to a dry flask cooled to 0 °C. The solution was stirred for 10 min before a solution of copper-bis-3,8-(*o*-nitrophenylselenoethyl)protoporphyrin IX dimethyl ester (**2.18**, 300 mg), dissolved in distilled 1,2-dichloroethane (200 mL) and DMF (15 mL), was added (may need to warm solvent to completely dissolve **2.18**). The reaction mixture was warmed to 70 °C. After stirring for 5 h, the reaction mixture was cooled in an ice bath and sat. $NaHCO_3$ (100 mL) was added. The reaction mixture was heated to 50 °C for approximately 3 h. After hydrolysis, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic material was filtered through Na_2SO_4. After evaporation of solvent, the crude material was chromatographed on a silica gel flash column using a gradient of 1-5 % acetone / CH_2Cl_2. The reddish brown fraction was collected and crystallized using CH_2Cl_2 / *n*-hexane to provide the four isomers (**2.19a-d**) in 77% yield (302 mg). If recovered starting material is considered, **2.19a-d** was produced in 90% yield; Vis: λ_{max} 404 nm (ε 463 100), 532 (20 700), 568 (32 300); LRSIMS calcd for $C_{49}H_{46}CuN_6O_9Se_2$ *m/z* 1084.4, found *m/z* 1085.0. Anal. Calcd for $C_{49}H_{46}CuN_6O_9Se_2$: C, 54.27; H, 4.18; N, 7.75. Found: C, 54.58; H, 4.18; N, 7.80.

Meso-formyl-bis-3,8-(2-*o*-nitrophenylselenoethyl)protoporphyrin IX Dimethyl Ester isomers 2.20a-d. The regioisomers, **2.19a-d** (112 mg) were added to degassed 5% H_2SO_4 / TFA (10 mL) and the reaction mixture was degassed again. After 20 min the reaction mixture was poured into ice water and extracted with 10% THF / CH_2Cl_2 until most of the green material was out of the aqueous phase. The organic extract was washed 2X with sat. $NaHCO_3$ (color changes to reddish-brown) and once with sat. NaCl. The combined organic material was filtered through Na_2SO_4. After evaporation of solvent, the crude material was chromatographed on a silica gel flash column using a gradient of 100% CH_2Cl_2 to 5% acetone / CH_2Cl_2. The product eluted with 1% acetone / CH_2Cl_2 and was crystallized with from CH_2Cl_2 / MeOH to give **2.20a-d** (90 mg) in 85% yield; Vis: λ_{max} 408 nm (ε 157 800), 506 (20 600), 540 (17 500), 578 (17 400), 628 (15

300); ^{1}H NMR ($CDCl_3$ ref. to TMS): consistent with structure; see four formyl-Hs at δ 12.52, 12.50, 12.27, 12.21. LRSIMS calcd for $C_{49}H_{48}N_6O_9Se_2$ *m/z* 1022.9, found *m/z* 1023.2. Anal. Calcd for $C_{49}H_{48}N_6O_9Se_2 \cdot H_2O$: C, 56.54; H, 4.84; N, 8.07. Found: C. 56.41; H, 4.55; N, 8.03.

Meso-formylprotoporphyrin IX Dimethyl Ester isomers 2.21a-d. Meso-formyl-bis-3,8-(*o*-nitrophenylselenoethyl)protoporphyrin IX dimethyl ester isomers **2.20a-d** (70 mg) were dissolved in distilled THF (35-40 mL). The reaction flask was covered to protect the reaction from light. The solution was cooled in an ice bath and 30% H_2O_2 (0.1 mL) was added. The reaction mixture stirred for 30 min before warming to room temp. After 5-6 h, the reaction mixture was poured into H_2O and extracted with CH_2Cl_2. The organic extract was filtered through Na_2SO_4 and the solvent evaporated *in vacuo*. The crude material was chromatographed on a silica gel column using a gradient of 100% CH_2Cl_2 to 5% acetone / CH_2Cl_2. The main fraction was eluted with 1% acetone / CH_2Cl_2 and was crystallized from CH_2Cl_2 / *n*-hexane. The four regioisomers **2.21a-d** (28.1 mg) were obtained in 67% yield; Vis: λ_{max} 410 nm (ε 141 800), 510 (13 700), 546 (11 800), 580 (11 000), 636 (8600), 664 (7200); LRSIMS calcd for $C_{49}H_{38}N_4O_5$ *m/z* 618.7, found *m/z* 619.3.

Further purification by normal phase HPLC (column; μ-Porasil radial-pak cartridge 8X10 #85720; solvent: 0.5% THF / CH_2Cl_2, flow rate: 1.5 mL/min.) indicated a ratio of 5:8:14:72 respectively, and an elution order of **2.21a**:**2.21b**:**2.21d**:**2.21c**. Three of the regioisomers, **2.21a,c,d**, were pure and **2.21b** co-eluted with a minor amount of **2.21d** (integration of formyl signals in ^{1}H NMR spectrum provided for correction in ratios).

5-Meso-formylprotoporphyrin IX dimethyl ester, 2.21a: retention time: 18.3 min; mp >300 °C; ^{1}H NMR ($CDCl_3$ ref. to TMS): δ 12.54 (s, 1 H, formyl-H), 10.09 (s, 1 H, meso-H), 10.00 (s, 1 H, meso-H), 9.94 (s, 1 H, meso-H), 8.09 (dd, 1 H, *J* = 17.6, 11.5 Hz, -C**H**=CH_2), 7.95 (dd, 1 H, *J* = 17.6, 11.5 Hz, -C**H**=CH_2), 6.24

(overlapping dd, 2 H, -CH=C**H**$_2$), 6.12 (dd, 1 H, J = 11.5, 1.5 Hz, -CH=C**H**$_2$), 5.91 (dd, 1 H, J = 17.6, 1.5 Hz, -CH=C**H**$_2$), 4.31 (overlapping t, 4 H, J = 7.7 Hz, -C**H**$_2$CH$_2$CO$_2$CH$_3$), 3.66 (s, 3 H, -CO$_2$C**H**$_3$), 3.65 (s, 3 H, -CO$_2$C**H**$_3$), 3.57 (s, 6 H, ring-CH$_3$), 3.53 (s, 3 H, ring-CH$_3$), 3.42 (s, 3 H, ring-CH$_3$), 3.24 (overlapping t, 4 H, -CH$_2$C**H**$_2$CO$_2$CH$_3$), -2.68 (bs, 2 H, NH).

15-Meso-formylprotoporphyrin IX dimethyl ester, 2.21c: retention time: 61.9 min; mp >300 °C; ^{1}H NMR (CDCl$_3$ ref. to TMS): δ 12.58 (s, 1H, formyl-H), 9.98 (s, 1 H, meso-H), 9.90 (s, 1 H, meso-H), 9.87 (s, 1 H, meso-H), 8.11 (m, 2 H, -C**H**=CH$_2$), 6.30 (overlapping bdd, 2 H, J = 17.7 Hz, -CH=C**H**$_2$), 6.16 (bdd, 2 H, J = 11.5 Hz, -CH=C**H**$_2$), 4.03 (bt, 4 H, J = 7.9 Hz, -C**H**$_2$CH$_2$CO$_2$CH$_3$), 3.75 (s, 6 H, -CO$_2$C**H**$_3$), 3.58 (s, 3 H, ring-CH$_3$), 3.54 (s, 3 H, ring-CH$_3$), 3.47 (s, 6 H, ring-CH$_3$), 3.06 (bt, 4 H, J = 7.9 Hz, -CH$_2$C**H**$_2$CO$_2$CH$_3$), -3.13 (bs, 2 H, NH).

20-Meso-formylprotoporphyrin IX dimethyl ester, 2.21d: retention time: 46.4 min; mp 202-203 °C; ^{1}H NMR (CDCl$_3$ ref. to TMS): δ 12.41 (s, 1 H, formyl-H), 9.78 (s, 1 H, meso-H), 9.77 (s, 1 H, meso-H), 9.75 (s, 1 H, meso-H), 8.02 (dd, 1 H, J = 17.6, 11.5 Hz, -C**H**=CH$_2$), 7.87 (dd, 1 H, J = 17.6, 11.5 Hz, -C**H**=CH$_2$), 6.16 (four overlapping dd, 4 H, J_{trans} = 17.6, J_{cis} = 11.5 Hz, -CH=C**H**$_2$), 4.21 (m, 4 H, -C**H**$_2$CH$_2$CO$_2$CH$_3$), 3.67 (s, 3 H, -CO$_2$C**H**$_3$), 3.64 (s, 3 H, -CO$_2$C**H**$_3$), 3.44 (s, 3 H, ring-CH$_3$), 3.41 (s, 3 H, ring-CH$_3$), 3.24 (s, 6 H, ring-CH$_3$), 3.14 (overlapping t, 4 H, J = 7.7 Hz, -CH$_2$C**H**$_2$CO$_2$CH$_3$), -3.54 (bs, 2 H, NH).

3-(2-*o*-Nitrophenylselenoethyl)chlorin-e$_6$ Trimethyl Ester 2.24. To a solution of 3-(hydroxyethyl)chlorin-e$_6$ trimethyl ester **2.23**[6] (50 mg) dissolved in dry, distilled CH$_2$Cl$_2$ (9.0 mL) was added *o*-nitrophenylselenocyanate (175 mg) and *n*Bu$_3$P (155 mg). The reaction mixture was stirred at room temp for 1 h at which time the solvent was evaporated *in vacuo*. The crude material was chromatographed on a silica gel column using a gradient of 25% cyclohexane / CH$_2$Cl$_2$ to 5% acetone / CH$_2$Cl$_2$. The main green

fraction was crystallized from CH_2Cl_2 / *n*-hexane to provide **2.24** in 40% yield (25.7 mg); mp 192-195 °C; Vis: λ_{max} 400 nm (ε 155 500), 496 (12 500), 524 (2 700), 554 (1000), 600 (4 400), 654 (46 600); ^1H NMR ($CDCl_3$, ref. to TMS): δ 9.67 (s, 1 H, meso-H), 9.36 (s, 1 H, meso-H), 8.67 (s, 1 H, meso-H), 8.13 (dd, 1 H, phenyl-H), 7.45 (dd, 1 H, phenyl-H), 7.12 (ddd, 1 H, phenyl-H), 7.02 (ddd, 1 H, phenyl-H), 5.29 ($2d_{AB}$, 2 H, J = 19 Hz, -$CH_2CO_2CH_3$), 4.41 (m, 2 H, -CH_2CH_2Se), 4.25 (s, 3 H, ring-CO_2CH_3), 4.13 (bt, 2 H, ring-D H), 3.78 (s, 3 H, ester-CH_3), 3.73 (m, 2 H, -CH_2CH_2Se or -CH_2CH_3), 3.63 (s, 3 H, ester-CH_3), 3.56 (s, 3 H, ring-CH_3), 3.53 (m, 2 H, -CH_2CH_2Se or -CH_2CH_3), 3.28 (s, 3 H, ring-CH_3), 3.24 (s, 3 H, ring-CH_3), 2.56 (m, 2 H, -$CH_2CH_2CO_2CH_3$), 2.20 (m, 2 H, -$CH_2CH_2CO_2CH_3$), 1.70 (m, 6 H, ring-D CH_3 and -CH_2CH_3), -1.40 (bs, 1 H, NH), -1.52 (bs, 1 H, NH); LRSIMS calcd for $C_{43}H_{47}N_5O_8Se$ *m/z* 840.8, found *m/z* 841.2. Anal. Calcd for $C_{43}H_{47}N_5O_8Se$: C, 61.42; H, 5.63; N, 8.33. Found: C, 61.21; H, 5.72; N, 8.36.

Copper 20-Meso-formyl-3-(2-*o*-nitrophenylselenoethyl)chlorin-e_6 Trimethyl Ester 2.25. To a refluxing solution of 3-(*o*-nitrophenylselenoethyl)chlorin-e_6 trimethyl ester, (**2.24**, 73.5 mg) dissolved in CH_2Cl_2 (30 mL), was added a sat. solution of Cu(II) acetate in MeOH (5 mL). By spectrophotometry, the disappearance of one peak at 654 nm was monitored. After 20 min, the reaction mixture was poured into H_2O and extracted with CH_2Cl_2. The organic extract was filtered through Na_2SO_4 and the solvent was evaporated. Crystallization from CH_2Cl_2 / MeOH gave the metallated chlorin in 92% yield (72.5 mg); Vis: λ_{max} 408 nm, 500, 542, 628. The turquoise colored Cu 3-(*o*-nitrophenylselenoethyl)chlorin-e_6 trimethyl ester was then subjected to a Vilsmeier formylation reaction. The Vilsmeier reagent was prepared by adding dry DMF (1 mL) and $POCl_3$ (1 mL) to freshly distilled 1,2-dichloroethane (10 mL) cooled in an ice bath. The reagent was heated to 50 °C and then a solution of Cu 3-(*o*-nitrophenylselenoethyl)-chlorin-e_6 trimethyl ester (72.5 mg), dissolved in 1,2-dichloroethane (45 mL), was added

to the Vilsmeier reagent. After 1.25 h, the reaction mixture was cooled and sat. $NaHCO_3$ was added. The reaction mixture was heated again to 60 °C for 3 h. After hydrolysis, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2. The combined organic material was filtered through Na_2SO_4. After evaporation of solvent, the crude material was chromatographed on a silica gel flash column using 2% MeOAc / CH_2Cl_2, to remove starting material, followed by 5% acetone / CH_2Cl_2 to elute **2.25** (65.2 mg) in 86% yield; mp 189-193 °C; Vis: λ_{max} 418 nm (ε 105 000), 508 (18 100), 548 (17 800) 620 (25 600), 664 (47 900); LRSIMS calcd for $C_{44}H_{45}CuN_5O_9Se$ *m/z* 930.4, found *m/z* 930.1. Anal. Calcd for $C_{44}H_{45}CuN_5O_9Se$: C, 56.8; H, 4.88; N, 7.53. Found: C, 56.6; H, 4.81; N, 7.49.

20-Meso-formyl-3-(2-*o*-nitrophenylselenoethyl)chlorin-e_6 trimethyl ester 2.26. Copper 20-meso-formyl-3-(2-*o*-nitrophenylselenoethyl)chlorin-e_6 trimethyl ester **2.25** (19 mg) was added to degassed 5% H_2SO_4 / TFA (5 mL) and the flask was degassed again. After 15 min the reaction mixture was poured into ice water and extracted with CH_2Cl_2. The brownish-green organic extract was washed 2X with sat. $NaHCO_3$ and once with sat. NaCl and the color turned to a purple-brown. The combined organic material was filtered through Na_2SO_4. After evaporation of solvent, the crude material was chromatographed on a silica gel flash column using 4% MeOAc / CH_2Cl_2 to elute fronting impurities and 2.5% to 5% acetone / CH_2Cl_2 to elute the product. Crystallization from CH_2Cl_2 / *n*-hexane provided **2.26** (15.8 mg) in 93% yield; mp 174-184 °C; Vis: λ_{max} 412 nm (ε 143 600), 508 (27 800), 546 (29 600), 638 (25 700), 692 (55 100); ^1H NMR ($CDCl_3$): δ 11.64 (s, 1 H, formyl-H), 9.33 (s, 1 H, meso-H), 9.20 (s, 1 H, meso-H), 8.10 (d, 1 H, phenyl-H), 7.48 (d, 1 H, phenyl-H), 7.20 (dd, 1 H, phenyl-H), 7.04 (dd, 1 H, phenyl-H), 5.22 (m, 1 H, ring-D H), 5.01 ($2d_{AB}$, 2 H, J = 19 Hz, -C**H**$_2$CO$_2$CH$_3$), 4.23 (s, 3 H, ring-CO$_2$C**H**$_3$), 4.07 (m, 2 H, -CH$_2$C**H**$_2$Se), 3.83 (s, 3 H, ester-CH$_3$), 3.67 (s, 3 H, ester-CH$_3$), 3.56 (q, 2 H, -C**H**$_2$CH$_3$), 3.50 (t, 2 H, -C**H**$_2$CH$_2$Se), 3.39 (s,

3 H, ring-CH_3), 3.18 (s, 3 H, ring-CH_3), 3.10 (s, 3 H, ring-CH_3), 2.70 (m, 2 H, -CH_2**C**$H_2CO_2CH_3$), 2.42 (m, 2 H, -**C**$H_2CH_2CO_2CH_3$), 1.98 (m, 1 H, ring-D H), 1.64 (t, 3 H, -CH_2**C**H_3), 1.48 (d, 3 H, ring-D CH_3), 0.21 (bs, 2 H, NH); LRSIMS calcd for $C_{44}H_{47}N_5O_9Se$ *m/z* 868.9, found *m/z* 869.2. Anal. Calcd for $C_{44}H_{47}N_5O_9Se{\cdot}2H_2O$: C, 58.40; H, 5.68; N, 7.74. Found: C, 58.58; H, 5.35; N, 7.32.

20-Meso-formylchlorin-e_6 Trimethyl Ester 2.27. 20-Meso-formyl-3-(*o*-nitrophenylselenoethyl)chlorin-e_6 trimethyl ester **2.26** (10 mg) was dissolved in distilled THF (2.0 mL). The reaction flask was protected from light and cooled in an ice bath. 30% H_2O_2 (2 drops from Pasteur pipet) was added and the reaction mixture was stirred for 30 min before warming to room temp. After 4 h, the reaction mixture was poured into H_2O and extracted with CH_2Cl_2. The organic extract was filtered through $NaSO_4$ and the solvent was evaporated. The crude material was chromatographed on an alumina (grade III) column using 1% acetone / CH_2Cl_2. The product was crystallized from CH_2Cl_2 / *n*-hexane to afford **2.27** (7.3 mg) in 73% yield; mp 145-146 °C; Vis: λ_{max} 416 nm (ε 125 600), 514 (15 300), 550 (17 700), 654 (14 200), 700 (44 000); ^{1}H NMR ($CDCl_3$): δ 11.65 (s, 1 H, formyl-H), 9.35 (s, 1 H, meso-H), 9.31 (s, 1 H, meso-H), 7.80 (dd, 1 H, J = 17.7, 11.5 Hz, -C**H**=CH_2), 6.14 (overlapping dd, 2 H, J = 17.7, 1.3 Hz and J = 11.5, 1.3 Hz, -CH=C**H**$_2$), 5.25 (m, 1 H, ring-D H), 5.01 ($2d_{AB}$, 2 H, J = 19 Hz, -**C**$H_2CO_2CH_3$), 4.23 (s, 3 H, ring-CO_2**C**H_3), 3.83 (s, 3 H, ester-CH_3), 3.67 (s, 3 H, ester-CH_3), 3.58 (q, 2 H, -**C**H_2CH_3), 3.38 (s, 3 H, ring-CH_3), 3.26 (s, 3 H, ring-CH_3), 3.12 (s, 3 H, ring-CH_3), 2.70 (m, 2 H, -CH_2**C**$H_2CO_2CH_3$), 2.42 (m, 2 H, -**C**$H_2CH_2CO_2CH_3$), 1.98 (m, 1 H, ring-D H), 1.63 (t, 3 H, -CH_2**C**H_3), 1.46 (d, 3 H, ring-D CH_3), 0.35 (bs, 2 H, NH); LRSIMS calcd for $C_{38}H_{42}N_4O_7$ *m/z* 666.3053, found *m/z* 666.3067. Anal. Calcd for $C_{38}H_{42}N_4O_7{\cdot}0.5H_2O$: C, 67.54; H, 6.41; N, 8.29. Found: C, 67.45; H, 6.16; N, 8.24.

Chapter Three

Syntheses of 5-Meso-methylhemin Chloride and 3,8-Divinylchlorin-e_6 Trimethyl Ester

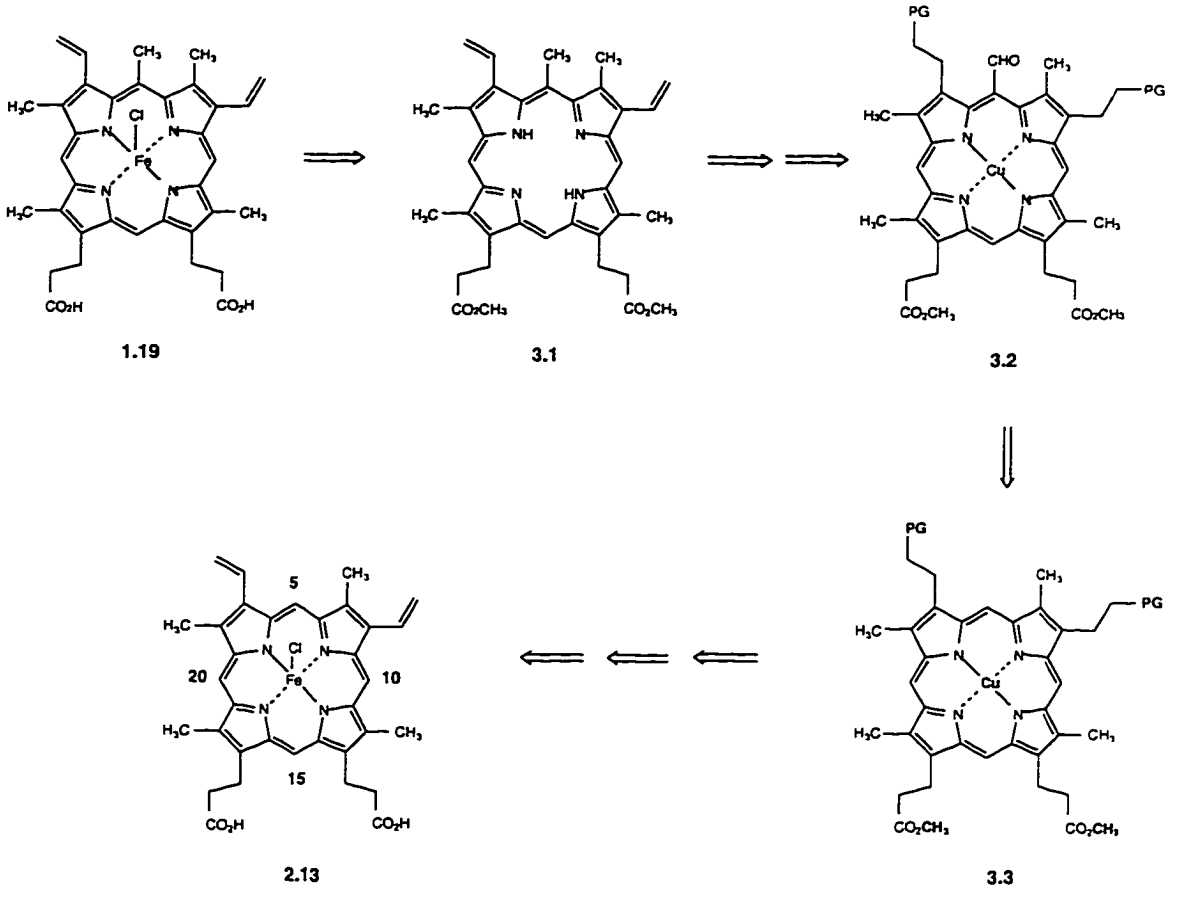
3.1 Introduction

In the preceding Chapter, the *o*-nitrophenylselenium substituent was shown to be a valuable vinyl protecting group for porphyrins and chlorins that can be removed under mild non-basic conditions. In this Chapter, applications of this key protecting group to the syntheses of 5-meso-methylhemin chloride (**1.19**) and 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) will be discussed. These compounds are useful in probing the mechanisms of heme catabolism and chlorophyll *a* biosynthesis, respectively.

3.2 Retrosynthetic Analysis of 5-Meso-methylhemin Chloride

Scheme 3.1 illustrates the retrosynthetic analysis of 5-meso-methylhemin chloride (**1.19**). The target compound can be prepared from 5-meso-methylprotoporphyrin IX dimethyl ester (**3.1**) by insertion of iron and hydrolysis of the ester functionalities. The meso-methyl moiety can be formed by reduction of the meso-formyl group of porphyrin **3.2**. This formyl porphyrin **3.2** can be synthesized in a Vilsmeier formylation reaction involving the appropriate protected porphyrin **3.3**.

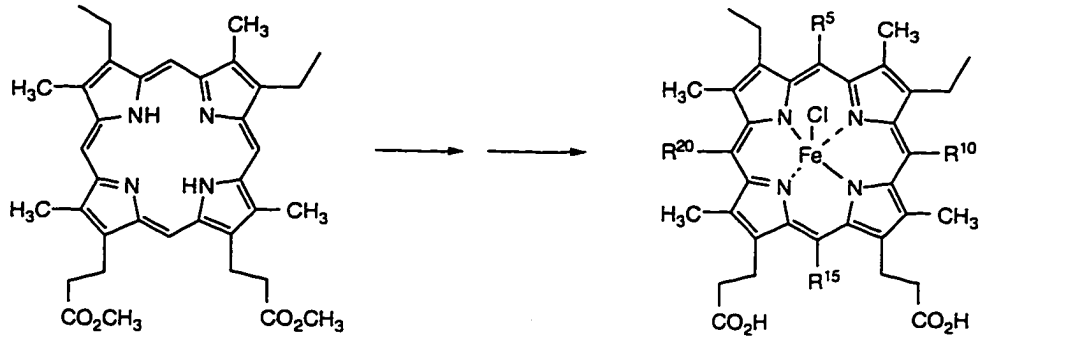
Porphyrin **3.3** is protected in three ways. First, a metal guards the macrocycle from protonation by the acidic Vilsmeier reagent. Second, vinyl-protection (PG) permits the use of the Vilsmeier reagent which reacts preferentially with vinyl groups (Figure 2.1). Third, protection of the carboxylic acids by esterification allows the porphyrins to dissolve in organic solvents. Protected porphyrin **3.3** easily can be synthesized from hemin chloride (**2.13**).



Scheme 3.1 Retrosynthetic analysis of 5-meso-methylhemin chloride **1.19**.

3.3 Synthesis of Meso-methylmesohemin Chloride Regioisomers

Torpey and Ortiz de Montellano avoided the protoporphyrin IX dimethyl ester vinyl group reactivity problem associated with the Vilsmeier reaction by irreversibly reducing the vinyl moieties to their corresponding ethyl groups. They synthesized the four meso-methylmesohemin chloride regioisomers (**3.4a-d**) from the corresponding mesoporphyrin IX dimethyl ester (**3.5**) (Figure 3.1).[1] The disadvantage of this method is that the product is not a meso-methyl derivative of the natural heme. However, studies of heme catabolism with these compounds elucidate some interesting findings.



3.5

3.4a: $R^5 = CH_3$, R^{10}, R^{15}, $R^{20} = H$
3.4b: $R^{10} = CH_3$, R^5, R^{15}, $R^{20} = H$
3.4c: $R^{15} = CH_3$, R^5, R^{10}, $R^{20} = H$
3.4d: $R^{20} = CH_3$, R^5, R^{10}, $R^{15} = H$

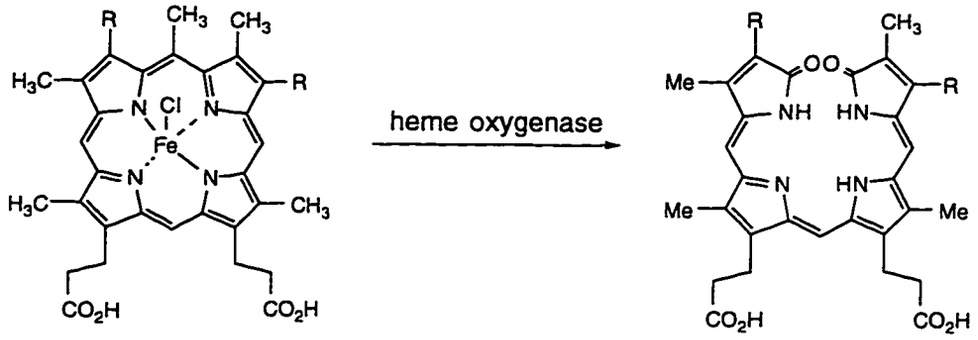
Figure 3.1 Synthesis of meso-methylmesohemin chloride regioisomers **3.4a-d** from mesoporphyrin IX dimethyl ester **3.5**.[1]

3.3.1 Results with Heme Oxygenase

Two very unexpected results were obtained when 5-meso-methylmesohemin chloride (**3.4a**) or 5-meso-methylhexamethylhemin chloride **3.6**,[2,3] was reacted with heme oxygenase, NADPH and P450 reductase (Figure 3.2).[4,5] First, the product of this reaction was surprisingly the corresponding biliverdin derivative **3.7** or **3.8**. As discussed in Chapter 1, the first step in the conversion of heme to biliverdin is regioselective oxidation to the 5-meso-hydroxyheme (**1.12**). Thus, this result was unanticipated because it was thought that the meso-methyl group would block the 5-meso-hydroxylation step.

The reaction was monitored for production of CO to determine if the 5-meso-methyl group was catalytically removed from the hemin derivative **3.4a** or **3.6**, permitting normal 5-hydroxylation and CO cleavage. This led to the second surprising result. The reaction proceeded without formation of CO, a by-product in the normal conversion of heme to biliverdin. The above results imply that an alternative mechanism occurred; one that does not involve 5-meso-hydroxyheme (**1.12**). Synthesis of 5-meso-methylhemin chloride, a

derivative more similar to the natural heme, should greatly help in further probing the heme catabolism mechanism.



3.4a R = Et
3.6 R = Me

3.7 R = Et
3.8 R = Me

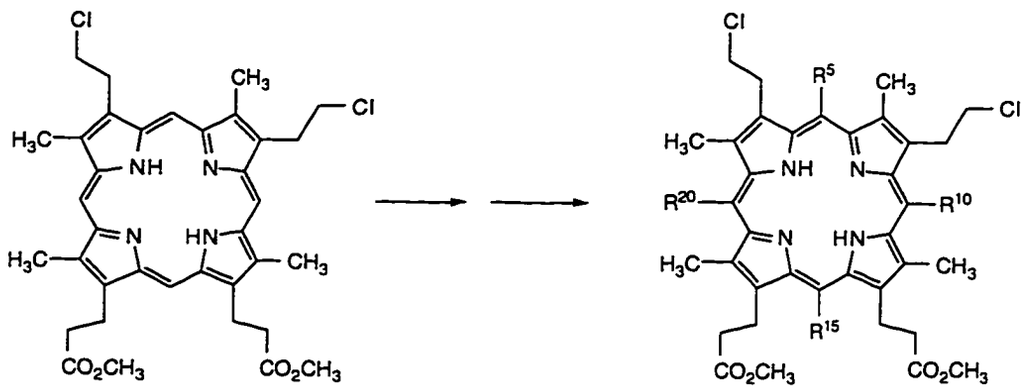
Figure 3.2: Reaction of 5-meso-methylhemin derivative **3.4a** or **3.6** with heme oxygenase resulted in formation of the biliverdin derivative **3.7** or **3.8**, respectively.

3.4 Synthesis of 5, 10, and 20-Meso-methylprotoporphyrin IX Dimethyl Ester: The (2-Chloroethyl) Approach

Synthesis of 5-meso-methylhemin chloride (**1.19**) requires vinyl-protection. The 2-chloroethyl substituent has been used successfully for vinyl-protection in a number of Smith group syntheses.[6-8] Thus, the vinyl groups of protoporphyrin IX dimethyl ester (**2.14**) were converted into the 2-chloroethyl substituents.[9] Under Vilsmeier reaction conditions, this bis-(2-chloroethyl)porphyrin, **3.9**, was further elaborated into the meso-formyl protoporphyrin IX dimethyl ester regioisomers (**3.10a-d**) (Figure 3.3). Separation of the four regioisomers **3.10a-d** was achieved using silica gel HPLC (1% THF / CH_2Cl_2).[10] The regioisomers **3.10a,b,d** were characterized by Isabelle Meunier.[11] Regioisomer **3.10c** was not characterized.

Direct reduction of the individual meso-formyl regioisomers **3.10a,b,d** to 5,10, and 20-meso-methylprotoporphyrin IX dimethyl ester (**3.11a,b,d**) was accomplished with $NaBH_4$ and TFA in about 46% yield (Scheme 3.2). A second product was formed as well

and was identified by ^1H NMR spectroscopy to be 5, 10, or 20-meso-hydroxymethyl protoporphyrin IX dimethyl ester (**3.12a,b,d**). Isolation of the meso-hydroxymethyl compounds, **3.12a,b,d**, indicates that the reaction did not go to completion. A higher yield of the meso-methyl product may have been obtained if reduction had been performed on the copper porphyrin.[6,12]

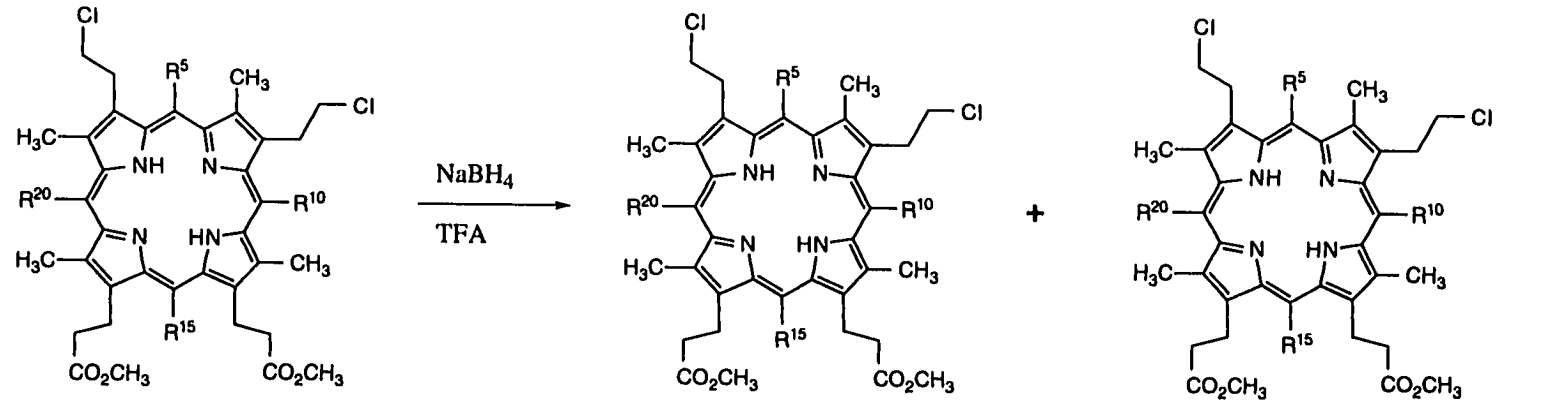


3.9

3.10a: R^5 = CHO, R^{10}, R^{15}, R^{20} = H
3.10b: R^{10} =CHO, R^5, R^{15}, R^{20} = H
3.10c: R^{15} = CHO, R^5, R^{10}, R^{20} = H
3.10d: R^{20} = CHO, R^5, R^{10}, R^{15} = H

Figure 3.3 Synthesis of the four meso-formylprotoporphyrin IX dimethyl ester regioisomers **3.10a-d** from bis-3,8-(2-chloroethyl) protoporphyrin IX dimethyl ester **3.9**.[10]

Deprotection of the 2-chloroethyl substituents of the 5- and 10-meso-methylporphyrins, **3.11a** and **3.11b**, proved to be problematic. Treatment of these porphyrins with base yielded unexpected cyclized porphyrins **3.13a** and **3.13b** respectively (Scheme 3.3). These cyclized porphyrins are reminiscent of the bridged biliverdin derivative (**2.4**, see Chapter 2 and Figure 2.3). A minor compound was produced and identified as the desired meso-methylprotoporphyrin IX dimethyl ester **3.14a**, **3.14b**, respectively, by ^1H NMR spectroscopy. Deprotection of 20-meso-methylporphyrin **3.11d** with pyridine and 10% NaOH was accomplished in 56% yield to provide the 20-meso-methylprotoporphyrin IX dimethyl ester **3.14d** (Scheme 3.3).

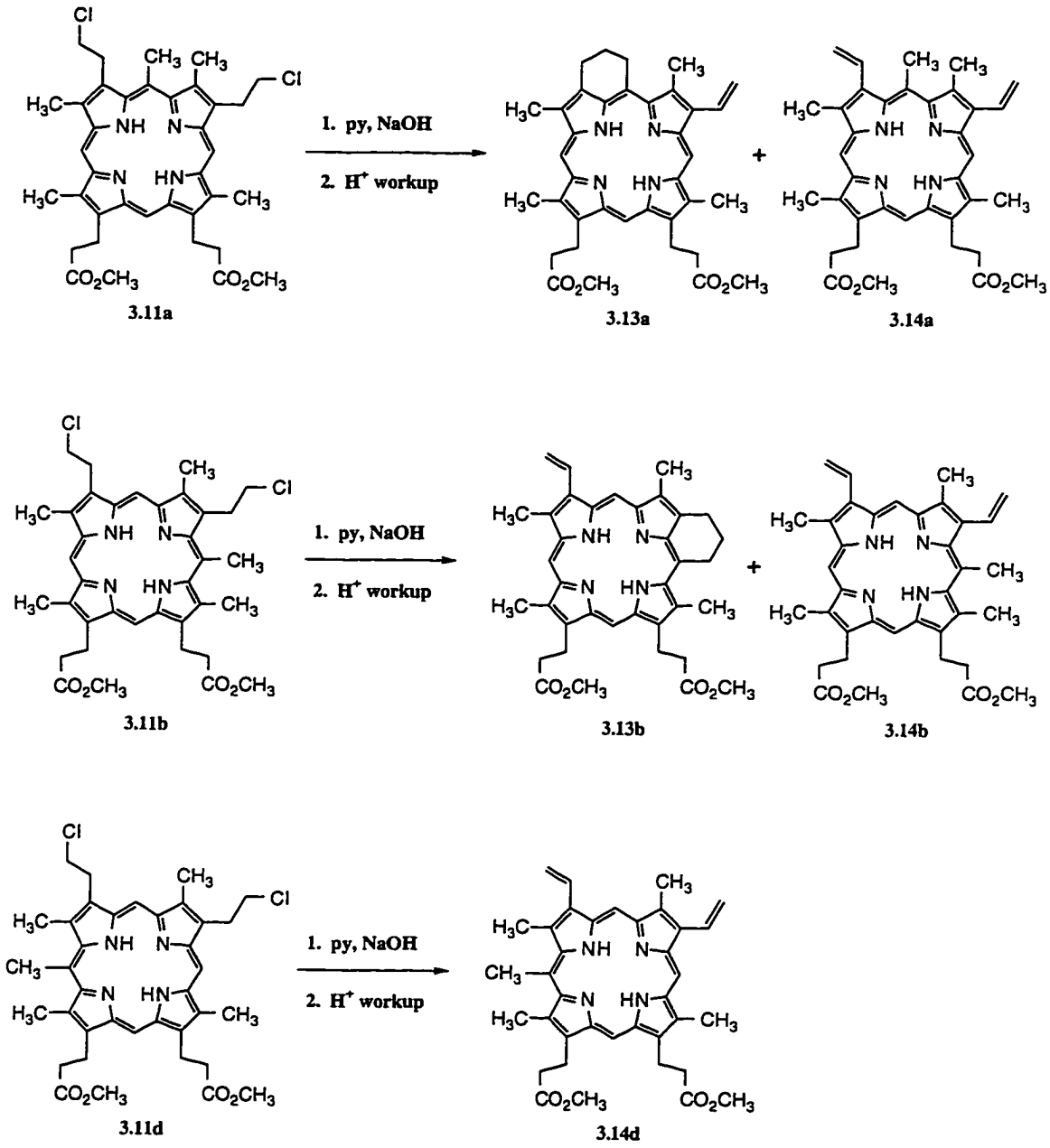


3.10a: R^5 = CHO, R^{10}, R^{15}, R^{20} = H
3.10b: R^{10} =CHO, R^5, R^{15}, R^{20} = H
3.10d: R^{20} = CHO, R^5, R^{10}, R^{15} = H

3.11a: R^5 = CH_3, R^{10}, R^{15}, R^{20} = H
3.11b: R^{10} =CH_3, R^5, R^{15}, R^{20} = H
3.11d: R^{20} = CH_3, R^5, R^{10}, R^{15} = H

3.12a: R^5 = CH_2OH, R^{10}, R^{15}, R^{20} = H
3.12b: R^{10} =CH_2OH, R^5, R^{15}, R^{20} = H
3.12d: R^{20} = CH_2OH, R^5, R^{10}, R^{15} = H

Scheme 3.2 Reduction of the individual meso-formyl group of prophyrin **3.10a,b,d** resulted in production of the meso-methyl moiety **3.11a,b,d** along with the meso-hydroxymethyl by-product **3.12a,b,d**.



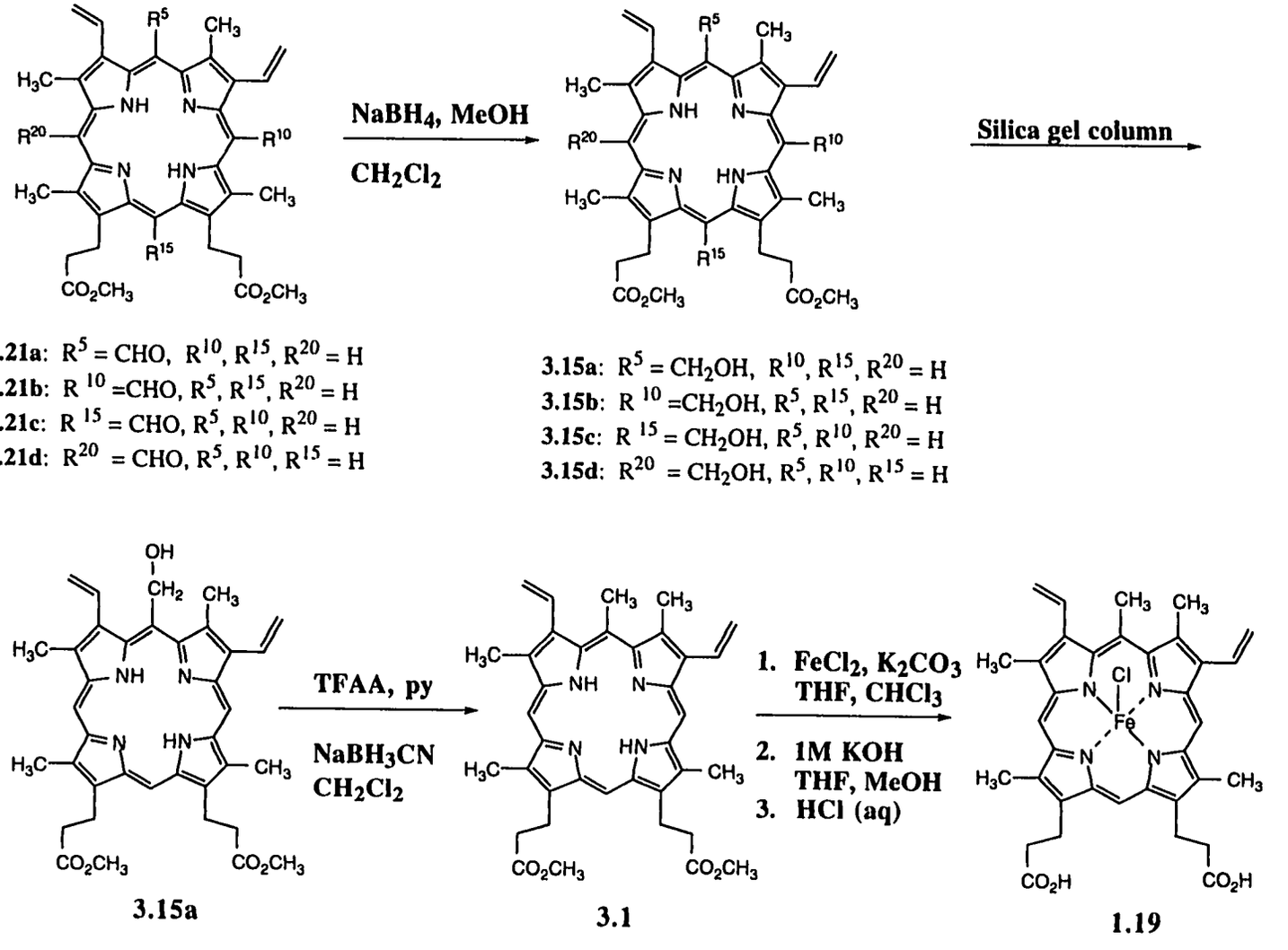
Scheme 3.3 Deprotection of 2-chloroethyl substituents from porphyrins **3.11a,b,d** resulted in surprising cyclization products **3.13a,b** and the meso-methyl products **3.14a,b,d**.

3.5 Synthesis of 5-Meso-methylhemin Chloride using 2-*o*-Nitrophenylselenoethyl Vinyl-Protection

The application of the newly developed 2-*o*-nitrophenylselenoethyl vinyl-protection group, as described in Chapter 2, in the synthesis of 5-meso-methylhemin chloride (**1.19**) was ideal due to the unfortunate base sensitivity of the meso-methyl-bis-(2-chloroethyl)porphyrins, **3.11a** and **3.11b**. Before conversion of the starting material (5-meso-formyl Vilsmeier product **2.19a**), into 5-meso-methylhemin chloride, two problems had to be solved.

The first problem to overcome was separation of the meso-formyl regioisomers. Separation of the copper meso-formyl regioisomers **2.19a-d**, and the bis-(2-*o*-nitrophenylselenoethyl) regioisomers **2.20a-d** was not successful using normal and reversed phase HPLC. Separation of meso-methylprotoporphyrin IX dimethyl ester regioisomers (i.e. **3.1**) was also unsuccessful. The best separation of the regioisomers found was after the selenium moieties were removed (Chapter 2, section 2.3.1). Unfortunately, separation of these regioisomers **2.21a-d** was very time consuming with very little product resulting (i.e. over two days, less than 1 mg of pure isomer **2.21a** was achieved) which led to the second problem.

The second challenge faced was how to prepare an adequate amount of compound to complete the synthesis. Following the synthetic scheme of Torpey and Ortiz de Montellano[1] the meso-formyl regioisomers **2.21a-d** were reduced to the meso-hydroxymethylporphyrins **3.15a-d** with $NaBH_4$ in 86% yield. Separation of regioisomers **3.15a-d** by silica gel column chromatography serendipitously provided the desired isomer, **3.15a**, where as the other three regioisomers eluted together (Scheme 3.4).



2.21a: R^5 = CHO, R^{10}, R^{15}, R^{20} = H
2.21b: R^{10} =CHO, R^5, R^{15}, R^{20} = H
2.21c: R^{15} = CHO, R^5, R^{10}, R^{20} = H
2.21d: R^{20} = CHO, R^5, R^{10}, R^{15} = H

3.15a: R^5 = CH_2OH, R^{10}, R^{15}, R^{20} = H
3.15b: R^{10} =CH_2OH, R^5, R^{15}, R^{20} = H
3.15c: R^{15} = CH_2OH, R^5, R^{10}, R^{20} = H
3.15d: R^{20} = CH_2OH, R^5, R^{10}, R^{15} = H

3.15a **3.1** **1.19**

Scheme 3.4 Second half of the synthetic route toward 5-mesomethylhemin chloride (**1.19**). See Scheme 2.4 for first half of synthetic scheme.

It is interesting to note that direct reduction of the meso-formyl group to the meso-methyl group of copper porphyrin regioisomers **2.21a-d** with $NaBH_4$ and TFA resulted in a mixture of products in which the major product was deformylated, yielding protoporphyrin IX dimethyl ester **2.14**. This result was confirmed by isolation of the major product using normal phase HPLC (0.5% THF / CH_2Cl_2) and analysis by mass and ^1H NMR spectroscopy.

To complete the synthesis of 5-meso-methylhemin chloride (**1.19**), the 5-meso-hydroxymethyl moiety **3.15a** was converted into the trifluoroacetoxy group with trifluoroacetic anhydride (TFAA) followed by immediate reduction with $NaBH_3CN$ to provide 5-meso-methylprotoporphyin IX dimethyl ester (**3.1**) in 45% yield. Insertion of iron with $FeCl_2$ under inert conditions, followed by base hydrolysis, provided 5-meso-methylhemin chloride (**1.19**) in 62% yield. Scheme 3.4 illustrates the synthesis of 5-meso-methylhemin chloride (**1.19**) from the meso-formyl isomers **2.21a-d**. Figure 3.4 compares the 5-meso-methyl, 5-meso-hydroxymethyl, and protoporphyrin IX dimethyl ester compounds (**3.1**, **3.15a**, **2.14**, respectively).

3.5.1 Assignment of 5-Meso-hydroxymethylprotoporphyrin IX Dimethyl Ester Regiochemistry

The regiochemistry of 5-meso-hydroxymethylprotoporphyrin IX dimethyl ester (**3.15a**) was determined by analysis of spectra from two NOE-NMR experiments. Following similar experiments performed for 5-meso-formylprotoporphyin IX dimethyl ester (**2.21a**, see Chapter 2.3.1), irradiation of the coincident 13^1-CH_2 and 17^1-CH_2 signals followed by irradiation of the hydroxymethyl-CH_2 signal defined the structural assignment for **3.15a** as the 5-hydroxymethyl derivative (Figure 3.5).

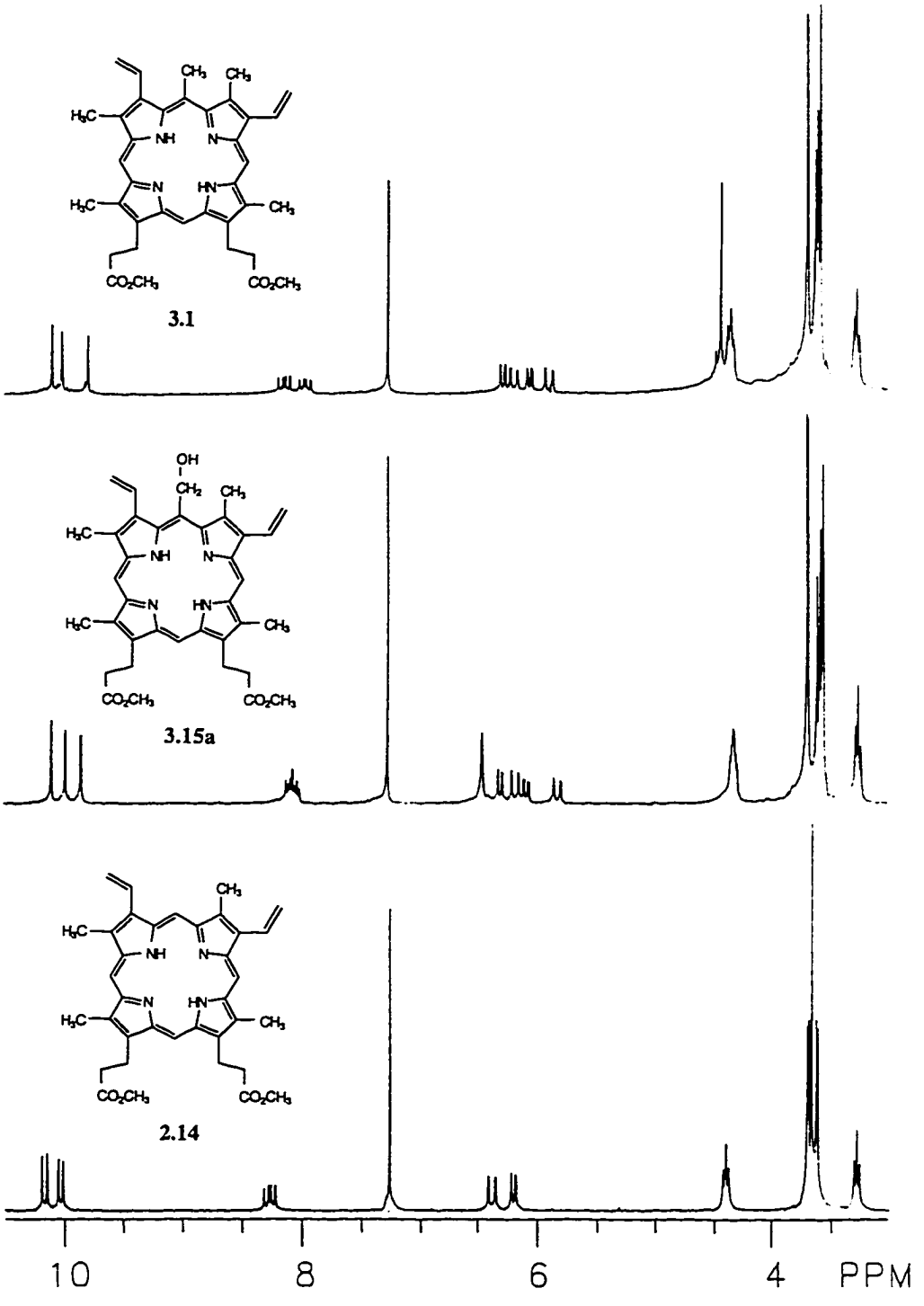
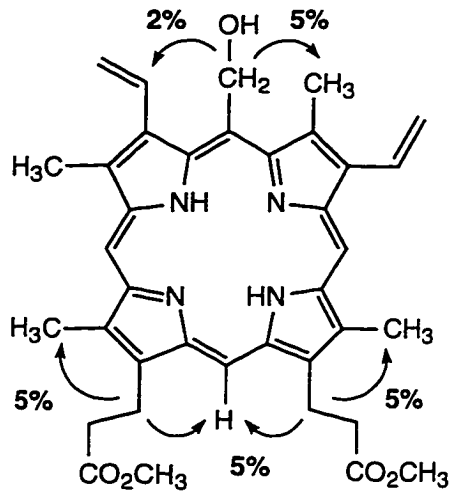


Figure 3.4 Comparison of ^{1}H NMR spectra of 5-meso-methyl, 5-meso-hydroxymethyl, and protoporphyrin IX dimethyl ester compounds (**3.1**, **3.15a**, **2.14**, respectively).



3.15a

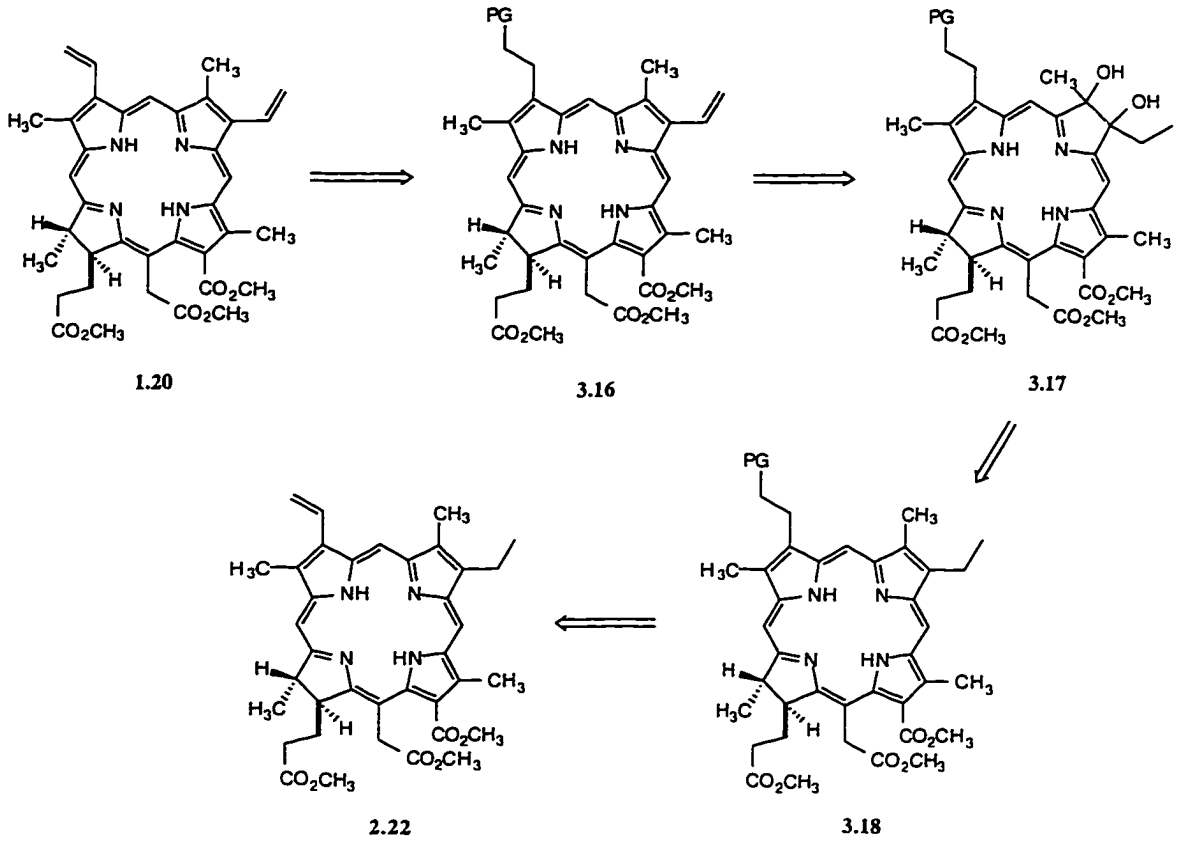
Figure 3.5 NOE connectivities for porphyrin **3.15a**.

3.6 Retrosynthetic Analysis of 3,8-Divinylchlorin-e_6 Trimethyl Ester

The synthesis of 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) is another example where vinyl-protection is essential. Retrosynthetically, the target compound can be prepared by dehydration of the 7,8-diol **3.17** followed by deprotection of the vinyl protecting group from isochlorin **3.16**. Before the 7,8-diol **3.17** was prepared by regioselective osmylation of ring-B, the more reactive 3-vinyl group was protected. The starting material, chlorin-e_6 trimethyl ester (**2.22**) can be easily prepared from chlorophyll *a* (Scheme 3.5).[6,13]

3.7 Deprotection Difficulties with 2-Chloroethyl Vinyl-Protection

Deprotection of the 2-chloroethyl substituent proved to be problematic due to the base-sensitive nature of chlorin-e_6 trimethyl ester derivatives. Reaction of chlorin **3.16** (PG = 2-chloroethyl) with base favored the cyclization of ring-E leading to complex product mixtures and poor yields.[14] An alternative vinyl protecting group was required for the synthetic success of the target compound **1.20**.



Scheme 3.5 Retrosynthetic analysis for 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**).

3.8 Synthesis of 3,8-Divinylchlorin-e_6 Trimethyl Ester

The first convenient synthesis of 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) was accomplished by Gerlach and Smith.[15] Their synthetic route avoided the complication of vinyl-protection by converting the 3-vinyl group into a formyl moiety. To regenerate the 3-vinyl group, a Wittig reaction was used. The advantage of this synthetic pathway was that it allowed for the synthesis of labeled analogs at the 3-vinyl position [e.g. 3-(2-^{13}C-vinyl)-8-vinylchlorin-e_6 trimethyl ester].[14] The disadvantage of this synthetic route was the large number of reaction steps resulting in a low yield of product.

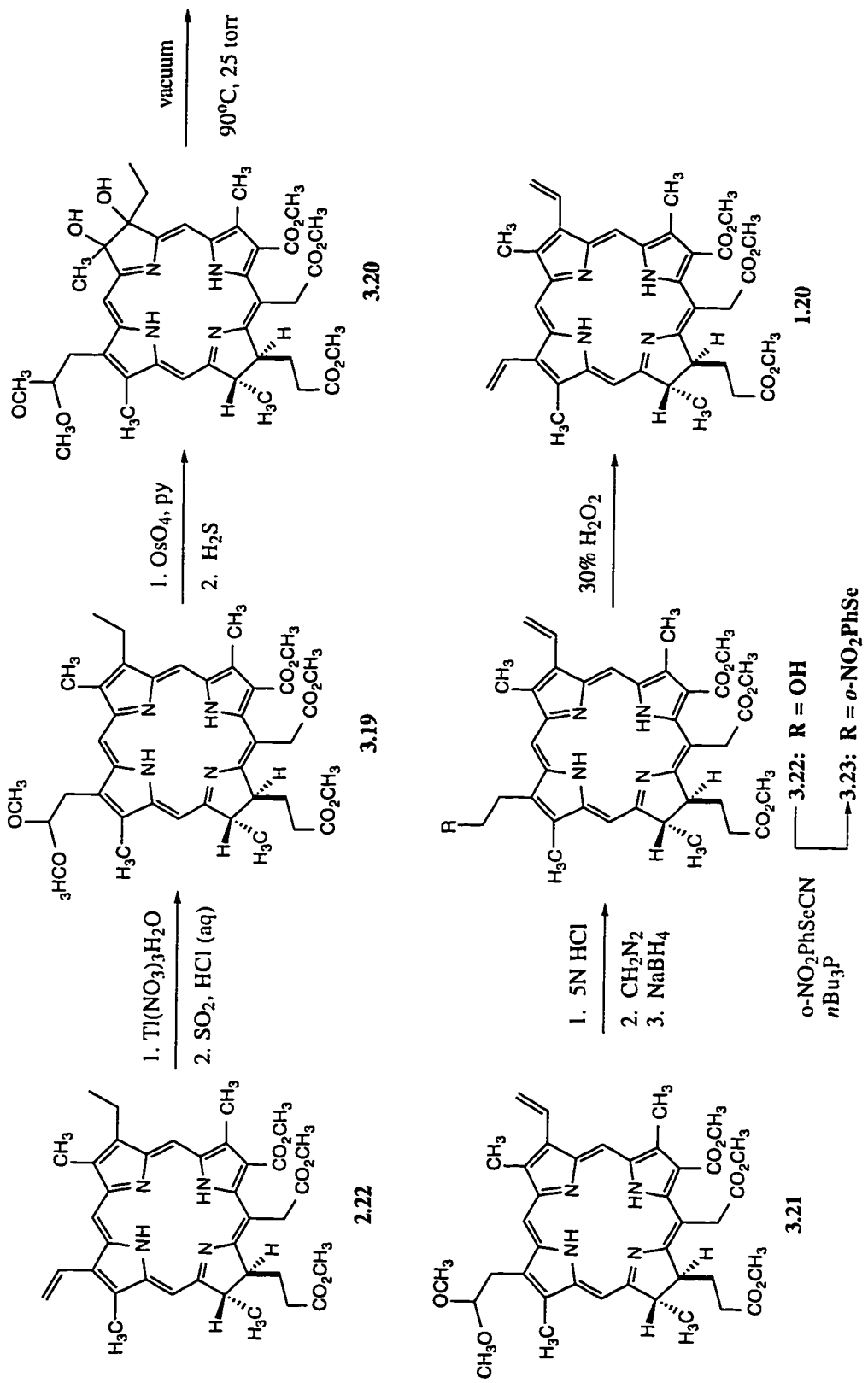
3.8.1 Synthesis of 3,8-Divinylchlorin-e_6 Trimethyl Ester using Organoselenium Chemistry: A Scale-up Approach

The synthetic scale-up of 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) to a 10 mmol range was possible with the use of organoselenium chemistry.[14] Oxidation of the 3-vinyl group of chlorin-e_6 trimethyl ester (**2.22**) with thallium chemistry to the corresponding dimethyl acetal (**3.19**)[16] was first accomplished. Regioselective[17,18] osmylation[19-23] of ring-B with OsO_4 / pyridine, followed by subsequent reduction with H_2S gas, gave the 7,8-diol **3.20** in 62% yield. Dehydration[24-26] of the 7,8-diol **3.20** by heating in vacuum provided the 8-vinyl group of the isochlorin derivative **3.21** in 50% yield (Scheme 3.6).

Conversion of the 3-(2,2-dimethoxyethyl) substituent into the 3-vinyl group required organoselenium chemistry. Under standard protocol,[6] the acetal group was converted into the 2-hydroxyethyl moiety to give isochlorin **3.22** in 82% yield. Isochlorin **3.22** was allowed to react with *o*-nitrophenylselenocyanate and nBu$_3$P to provide the organoselenium derivative **3.23** in 70% yield. Oxidation of the 2-*o*-nitrophenylselenoethyl substituent with 30% H_2O_2 / THF followed by elimination at room temperature provided 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) in 73% yield (Scheme 3.6).

3.9 Potential Use of 3,8-Divinylchlorin-e_6 Trimethyl Ester

Based on Woodward's synthesis,[27] 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) should be a good starting material for the synthesis of divinyl compounds such as **1.17b**, **1.18b** and **6b**. These compounds are known to be intermediates in one of the less understood multi-branched biosynthetic pathways of chlorophyll *a* (Figure 1.10).[28] The biosynthesis of chlorophyll *a* has attracted considerable interest due to its remarkable biosynthetic heterogeneity where both the 8-ethyl and the 8-vinyl intermediates exist in any single transformation step and are linked by 8-vinyl reductases. Hence, 3,8-divinylchlorin-e_6 trimethyl ester (**1.20**) may prove useful in further understanding the biosynthesis of chlorophyll *a*.



Scheme 3.6 Large scale synthetic route toward 3,8-divinylchlorin-e_6 **1.20**.

3.10 References

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3.11 Experimental

General. General procedures are described in Chapter 2 (Experimental section).

Meso-formyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester Regioisomers 3.10a-d.[10] Separation of the regioisomers using silica gel HPLC (column: μ-Porasil; solvent: 1.0% THF / CH_2Cl_2, flow rate: 2 mL/min.) was achieved. Only three of the four regioisomers were characterized[11] (**3.10c** retention time: 8.0 min.).

5-Meso-formyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester, 3.10a; retention time: 4.5 min.; mp 204-205 °C; Vis: λ_{max} 406 nm (ε 153 300), 506 (15 400), 538 (11 800), 576 (11 400), 628 (8700); ^{1}H NMR ($CDCl_3$): δ 12.31 (s, 1 H, formyl-H), 9.90, 9.85, 9.74 (each s, 1 H, meso-H), 4.29-3.87 (m, 12 H, -CH_2CH_2Cl, and -$CH_2CH_2CO_2CH_3$), 3.67, 3.66, 3.52, 3.50, 3.36, 3.16 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.23 (m, 4 H, -$CH_2CH_2CO_2CH_3$), -3.30, -3.60 (each bs, 1 H, NH); Anal. Calcd for $C_{37}H_{40}Cl_2N_4O_5$: C, 64.33; H, 5.84; N, 8.11. Found: C, 64.37; H, 5.89; N, 7.99.

10-Meso-formyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester, 3.10b; retention time: 5.8 min.; mp 184-185 °C; Vis: λ_{max} 406 nm (ε 148 500), 504 (12 100), 538 (8800), 576 (8400), 628 (5400); ^{1}H NMR ($CDCl_3$): δ 12.61 (s, 1 H, formyl-H), 10.03, 9.92, 9.90 (s, 1 H, meso-H), 4.52-4.31 (m, 12 H, -CH_2CH_2Cl,and -$CH_2CH_2CO_2CH_3$), 4.14, 3.67, 3.63, 3.54, 3.53, 3.38 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.23, 3.20 (each t, 2 H, -$CH_2CH_2CO_2CH_3$), -3.20 (bs, 2 H, NH); Anal. Calcd for $C_{37}H_{40}Cl_2N_4O_5$: C, 64.33; H, 5.84; N, 8.11. Found: C, 64.47; H, 5.90; N, 8.20.

20-Meso-formyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester, 3.10d; retention time: 6.9 min.; mp 219-220 °C; Vis: λ_{max} 404 nm (ε 164 800), 506 (21 500), 538 (17 500), 574 (16 300), 628 (13 000); ^{1}H NMR ($CDCl_3$): δ 12.60 (s, 1 H, formyl-H), 10.04, 9.85, 9.83 (each s, 1 H, meso-H), 4.36-4.16 (m, 12 H, -CH_2CH_2Cl,and -$CH_2CH_2CO_2CH_3$), 3.67, 3.65, 3.55, 3.54, 3.31, 3.25, (each s, 3 H, ester-CH_3 and ring-CH_3), 3.17 (m, 4 H, -$CH_2CH_2CO_2CH_3$), -3.25, -3.40 (each bs, 1 H, NH); Anal. Calcd for $C_{37}H_{40}Cl_2N_4O_5$: C, 64.33; H, 5.84; N, 8.11. Found: C, 64.43; H, 5.85; N, 8.01.

5, 10, and 20-Meso-methyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester 3.11a, 3.11b, and 3.11d. A solution of 5, 10, or 20-formyl-bis-3,8-(2-chloroethyl)protoporphyrin IX dimethyl ester **3.10a**, **b**, or **d** (20 mg) in dry

CH_2Cl_2 (20 mL) was added to TFA (7 mL) previously cooled to 0 °C in an ice bath. After stirring for 20 min, 2 pellets of $NaBH_4$ (ca. 100 mg) were added for **3.10b** and **3.10d** isomers, where as 4 pellets of $NaBH_4$ (ca. 200 mg) were added for **3.10a** isomer. The mixture was stirred under the following conditions: 1.25 h at 0 °C and 4.5 h at room temp for **3.10a** and 1 h at 0 °C and 5 h at room temp for **3.10b** and **3.10d**. The reaction mixture was then poured into ice and the excess $NaBH_4$ was slowly destroyed. After extraction with CH_2Cl_2 and $NaHCO_3$ work-up, the organic layer was dried over Na_2SO_4 and the solvent evaporated to dryness. The crude material was purified by silica gel preparative plates eluted with 2.5% MeOH / CH_2Cl_2. The faster running band was isolated and crystallized from CH_2Cl_2 / n-hexane to provide the desired compounds in 46% yield for **3.11a** and **3.11d** and 35% yield for **3.11b**. The slower running band was found to be an intermediate reaction product, meso-hydroxymethyl-bis-3,8-(2-chloroethyl)protoporphyrin IX dimethyl ester **3.12a,b,d**.

5-Meso-methyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester, 3.11a; mp 177-178 °C; Vis: λ_{max} 408 nm (ε 173 700), 506 (17 000), 540 (9000), 578 (8800), 630 (4400); ^1H NMR ($CDCl_3$): δ 10.04, 9.95, 9.84 (each s, 1 H, meso-H), 4.51 (s, 3 H, meso-CH_3), 4.50 (m, 4 H, -CH_2**CH_2**Cl), 4.32 (m, 4 H, -**CH_2**CH_2Cl), 4.24 (t, 2 H, -**CH_2**$CH_2CO_2CH_3$), 4.14 (t, 2 H, -**CH_2**$CH_2CO_2CH_3$), 3.67, 3.66, 3.63, 3.62, 3.61, 3.60 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.26 (t, 4 H, -CH_2**CH_2**CO_2CH_3), -2.85, -3.01 (each bs, 1 H, NH); HRMS calcd for $C_{37}H_{42}Cl_2N_4O_4$ *m/z* 676.2583, found *m/z* 676.2572; Anal. Calcd for $C_{37}H_{42}Cl_2N_4O_4 \cdot 0.5H_2O$; C, 64.71; H, 6.31; N, 8.16. Found: C, 64.57; H, 6.20; N, 7.99.

10-Meso-methyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester, 3.11b: mp 220-221 °C; Vis: λ_{max} 408 nm (ε 164 100), 506 (15 500), 540 (7800), 578 (5800), 630 (3500); ^1H NMR ($CDCl_3$): δ 10.06, 9.97, 9.86 (each s, 1 H, meso-H), 4.52 (s, 3 H, meso-CH_3), 4.51 (m, 4 H, -CH_2**CH_2**Cl), 4.39 (m, 4 H, -**CH_2**CH_2Cl), 4.30, 4.18 (each t, 2 H, -**CH_2**$CH_2CO_2CH_3$), 3.69, 3.65, 3.59, 3.58 (each s, 3 H, ester-CH_3

and ring-CH_3), 3.27, 3.21 (each t, 2 H, -$CH_2CH_2CO_2CH_3$), -3.00 (bs, 2 H, NH); HRMS calcd for $C_{37}H_{42}Cl_2N_4O_4$ *m/z* 676.2583, found *m/z* 676.2580; Anal. Calcd for $C_{37}H_{42}Cl_2N_4O_4$; C, 65.66; H, 6.26; N, 8.28. Found: C, 65.12; H, 6.37; N, 8.16.

20-Meso-methyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester, 3.11d: mp 166-167 °C; Vis: λ_{max} 406 nm (ε 188 600), 506 (22 400), 540 (13 700), 576 (13 600), 628 (9000); ^1H NMR ($CDCl_3$): δ 10.06, 9.98, 9.78 (each s, 1 H, meso-H), 4.47 (s, 3 H, meso-CH_3), 4.51 (m, 4 H, -CH_2CH_2Cl), 4.41 (m, 4 H, -CH_2CH_2Cl), 4.30, 4.25 (each t, 2 H, -$CH_2CH_2CO_2CH_3$), 3.68, 3.66, 3.65, 3.64, 3.58, 3.57 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.27, 3.20 (each t, 2 H, -$CH_2CH_2CO_2CH_3$), NH not seen; HRMS calcd for $C_{37}H_{42}Cl_2N_4O_4$ *m/z* 676.2583, found *m/z* 676.2564; Anal. Calcd for $C_{37}H_{42}Cl_2N_4O_4$; C, 65.66; H, 6.26; N, 8.28. Found: C, 65.35; H, 6.19; N, 8.14.

5-Meso-hydroxymethyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester 3.12a; ^1H NMR ($CDCl_3$): δ 10.10, 10.02, 9.95 (each s, 1 H, meso-H), 6.69 (s, 2 H, meso-CH_2OH), 4.62-4.15 (6t, 12 H, -CH_2CH_2Cl, and -$CH_2CH_2CO_2CH_3$), 3.71, 3.67, 3.66, 3.63, 3.60 (each s, 3 H, ester-CH_3, and ring-CH_3), 3.27 (overlapping t, 4 H, -$CH_2CH_2CO_2CH_3$), -3.25 (bs, 2 H, NH).

10-Meso-hydroxymethyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester 3.12b; ^1H NMR ($CDCl_3$): δ 9.96, 9.80, 9.79 (each s, 1 H, meso-H), 6.40 (s, 2 H, meso-CH_2OH), 4.41-4.01 (m, 12 H, -CH_2CH_2Cl, and -$CH_2CH_2CO_2CH_3$), 3.69, 3.65, 3.49, 3.48, 3.44 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.21, 3.14 (each t, 2 H, -$CH_2CH_2CO_2CH_3$), -3.25 (bs, 2 H, NH).

20-Meso-hydroxymethyl-bis-3,8-(2-chloroethyl)protoporphyrin IX Dimethyl Ester 3.12d; ^1H NMR ($CDCl_3$): δ 9.97, 9.78, 9.77 (each s, 1 H, meso-H), 6.34 (s, 2 H, meso-CH_2OH), 4.36-4.13 (m, 12 H, -CH_2CH_2Cl, and -$CH_2CH_2CO_2CH_3$), 3.70, 3.68, 3.56, 3.54, 3.47, 3.37 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.25, 3.14 (each t, 2 H, -$CH_2CH_2CO_2CH_3$), -3.50 (bs, 2 H, NH).

Bis-13,17-(2-methoxycarbonylethyl)-3:5-(propano)-2,7,12,18-tetramethyl-8-vinylporphyrin 3.13a. 5-Meso-methyl-bis-3,8-(2-chloroethyl) protoporphyrin IX dimethyl ester **3.11a** (30 mg) was dissolved in pyridine (13.5 mL). After 10 min of refluxing, water (2.6 mL) and a 10% solution of NaOH (3 mL) were added quickly. After refluxing for 2.5 h, the stirring reaction was cooled, and over 30 min, 20% acetic acid (3 mL) was added. The solvent was then evaporated and toluene was added as a chaser and evaporated under high vacuum. The brown solid was taken up in 5% H_2SO_4 / MeOH (30 mL) and stirred overnight at room temp. The reaction mixture was poured into ice and then extracted with $CHCl_3$. The combined organic extract was washed with water then sat. $NaHCO_3$ and dried over Na_2SO_4. The crude material was purified on a silica gel plate eluted several times with 0.5% MeOH / CH_2Cl_2. The slower running band was isolated by extraction of the silica with TFA in CH_2Cl_2 to provide the cyclized porphyrin **3.13a** in 23% yield; mp 202-203 °C; Vis: λ_{max} 406 nm (ε 173 300), 504 (47 500), 538 (41 000), 574 (39 400), 628 (15 700); ^{1}H NMR ($CDCl_3$): δ 10.18, 10.00, 9.87 (each s, 1 H, meso-H), 8.20 (dd, 1 H, C**H**=CH_2), 6.26, 6.20 (each dd, 1 H, CH=C**H**$_2$), 5.04 (t, 1 H, meso-C**H**$_2$$CH_2CH_2$), 4.37 (t, 4 H, -C**H**$_2$$CH_2CO_2CH_3$), 3.86 (t, 2 H, -meso-$CH_2CH_2$C**H**$_2$), 3.67, 3.65, 3.62, 3.61, 3.57 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.27 (t, 4 H, -CH_2C**H**$_2$$CO_2CH_3$), 2.85 (q, 2 H, meso-$CH_2$C**H**$_2$$CH_2$), -3.00 (bs, 2 H, NH); HRMS calcd for $C_{37}H_{40}N_4O_4$ *m/z* 604.3049, found *m/z* 604.3061. The faster running band provided 5-meso-methylprotoporphyrin IX dimethyl ester **3.14a** in 7% yield; ^{1}H NMR ($CDCl_3$): δ 10.07, 9.97, 9.77 (each s, 1 H, meso-H), 8.10, 7.91 (each dd, 1 H, C**H**=CH_2), 6.18, 6.16, 6.02, 5.83 (d, 1 H, CH=C**H**$_2$), 4.38 (s, 3 H, meso-CH_3), 4.32 (t, 4 H, -C**H**$_2$$CH_2CO_2CH_3$), 3.68, 3.59, 3.53 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.27 (t, 4 H, -CH_2C**H**$_2$$CO_2CH_3$), -3.00 (bs, 2 H, NH).

Bis-13,17-(2-methoxycarbonylethyl)-8:10-(propano)-2,7,12,18-tetramethyl-3-vinylporphyrin 3.13b. The procedure followed was

exactly as above to give **3.13b** in 23% yield; mp 247-248 °C; Vis: λ_{max} 408 nm (ε 181 600), 506 (27 300), 542 (20 400), 576 (19 500), 630 (15 700); ^{1}H NMR ($CDCl_3$): δ 10.12, 10.05, 9.92 (each s, 1 H, meso-H), 8.28 (dd, 1 H, C**H**=CH_2), 6.37, 6.16 (each dd, 1 H, CH=C**H**$_2$), 4.98 (t, 2 H, meso-C**H**$_2$C H$_2$C H$_2$), 4.40 (t, 4 H, -C**H**$_2$$CH_2CO_2CH_3$), 3.81 (t, 2 H, -meso-$CH_2CH_2$C**H**$_2$), 3.69, 3.68, 3.67, 3.59, 3.56, 3.54 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.27, 3.21 (each t, 2 H, -CH_2C**H**$_2$$CO_2CH_3$), 2.85 (q, 2 H, meso-$CH_2$C**H**$_2$$CH_2$), -3.40 (bs, 2 H, NH); HRMS calcd for $C_{37}H_{40}N_4O_4$ *m/z* 604.3049, found *m/z* 604.3037. The minor compound, 10-meso-methylprotoporphyrin IX dimethyl ester **3.14b** was also produced in 7% yield; ^{1}H NMR ($CDCl_3$): δ 10.19, 10.01, 9.88 (each s, 1 H, meso-H), 8.27, 8.00 (each dd, 1 H, C**H**=CH_2), 6.27, 6.18, 6.05, 5.91 (each d, 1 H, CH=C**H**$_2$), 4.48 (s, 3 H, meso-CH_3), 4.39 (t, 4 H, -C**H**$_2$$CH_2CO_2CH_3$), 3.69, 3.66, 3.63, 3.57, 3.56 (each s, 3 H, ester-CH_3 and ring-CH_3), 3.27, 3.20 (each t, 2 H, -CH_2C**H**$_2$$CO_2CH_3$), -3.20 (bs, 2 H, NH).

20-Meso-methylprotoporphyrin IX Dimethyl Ester 3.14d. The reaction conditions were the same as described above for porphyrin **3.13a** except that the purification was different. The crude materal was purified on a silica gel plate eluted with 2% MeOH / CH_2Cl_2 to provide the 20-meso-methylprotoporphyrin IX dimethyl ester **3.14d** in 56% yield; mp 215-216 °C; Vis: λ_{max} 412 nm (ε 168 200), 510 (17 500), 546 (10 300), 582 (9500), 636 (5200); ^{1}H NMR ($CDCl_3$): δ 10.18, 10.01, 9.96 (each s, 1 H, meso-H), 8.20 (overlapping dd, 2 H, C**H**=CH_2), 6.37, 6.19 (each dd, 1 H, *J* = 17.9, 1.5 Hz, CH=C**H**$_2$), 6.27, 6.13 (each dd, 1 H, *J* = 11.5, 1.5 Hz, CH=C**H**$_2$), 4.48 (s, 3 H, meso-CH_3), 4.40 (m, 4 H, -C**H**$_2$$CH_2CO_2CH_3$), 3.69, 3.68, 3.63, 3.59, 3.56, (each s, 3 H, ester-CH_3 and ring-CH_3), 3.24, 3.21 (each t, 2 H, -CH_2C**H**$_2$$CO_2CH_3$), -2.80 (bs, 2 H, NH); HRMS calcd for $C_{37}H_{40}N_4O_4$ *m/z* 604.3049, found *m/z* 604.3035; Anal. Calcd for $C_{37}H_{40}N_4O_4 \cdot 0.5H_2O$; C, 72.40; H, 6.73; N, 9.12. Found: C, 72.63; H, 6.67; N, 8.94.

5-Meso-hydroxymethylprotoporphyrin IX Dimethyl Ester, 3.15a. A solution of meso-formylprotoporphyin IX dimethyl ester regioisomers **2.21a-d** (88 mg) dissolved in CH_2Cl_2 (100 mL) was added to a solution of $NaBH_4$ (350 mg) in MeOH (25 mL), cooled in an ice bath. After the reaction mixture was stirred for 15 min at room temp, it was quenched with 1N HCl to destroy excess $NaBH_4$. The aqueous layer was extracted with CH_2Cl_2 and the combined organic extract was washed with sat. $NaHCO_3$ and NaCl and filtered through Na_2SO_4. After evaporation of the solvent, the crude material was slowly purified on a silica gel column (40 mL silica gel, 35 mm wide X 4 inches long). Taking test tube fractions (approx. 10 mL) the desired isomer eluted with 0.5% acetone / CH_2Cl_2. The other three isomers did not elute separately and were combined. Crystallization from CH_2Cl_2 / *n*-hexane provided 5-meso-hydroxymethylprotoporphyrin IX dimethyl ester, **3.15a** (10 mg) in 11% yield; mp 173-175°C; Vis: λ_{max} 410 nm (ε 172 700), 510 (23 000), 546 (18 700), 582 (15 700), 634 (13 400); ^{1}H NMR ($CDCl_3$): δ 10.09, 9.98, 9.85 (each s, 1 H, meso-H), 8.04 (overlapping dd, 2 H, C**H**=CH_2), 6.44 (s, 3 H, meso-CH_2OH), 6.28, 6.07 (each dd, 1 H, J = 11.5, 2.0 Hz, CH=C**H**$_2$), 6.16, 5.81 (each dd, 1 H, J = 17.7, 2.0 Hz, CH=C**H**$_2$), 4.31 (m, 4 H, -C**H**$_2$$CH_2CO_2CH_3$), 3.69, 3.68, 3.60, 3.57, 3.55, (each s, 3 H, ester-CH_3 and ring-CH_3), 3.25 (t, 4 H, -CH_2C**H**$_2$$CO_2CH_3$), -3.49 (bs, 2 H, NH); HRMS calcd for $C_{37}H_{40}N_4O_5$ *m/z* 620.2999, found *m/z* 620.3011; Anal. Calcd for $C_{37}H_{40}N_4O_5$; C, 71.59; H, 6.50; N, 9.03. Found: C, 71.34; H, 6.61; N, 8.94.

5-Meso-methylprotoporphyrin IX Dimethyl Ester, 3.1. To a solution of 5-meso-hydroxymethylprotoporphyrin IX dimethyl ester **3.15a** (26.4 mg) in CH_2Cl_2 (20 mL) cooled to 0 °C in an ice bath, was added NEt_3 (0.6 mL) and TFAA (0.4 mL). The reaction mixture stirred for 10 min and $NaBH_3CN$ (spatula tip, ca. 50 mg) was added and the reaction mixture was stirred for an additional 15 min. The reaction mixture was poured into sat. $NaHCO_3$ to quench the excess $NaBH_3CN$ and extracted with CH_2Cl_2. The

organic extract was washed 2X with 0.01N HCl and once with NaCl then filtered through Na_2SO_4. After solvent evaporation, the crude material was purified by silica gel chromatography and the product eluted with 0.5% acetone / CH_2Cl_2. Crystallization from CH_2Cl_2 / *n*-hexane provided 5-meso-methylprotoporphyrin IX dimethyl ester **3.1** (12.0 mg) in 45% yield; mp >300 °C; Vis: λ_{max} 412 nm (ε 130 000), 512 (14 000), 546 (8500), 584 (8000), 638 (5000); ^1H NMR ($CDCl_3$): δ 10.09, 10.00, 9.79 (each s, 1 H, meso-H), 8.14, 7.97 (each dd, 2 H, J = 17.7, 11.5 Hz, -C**H**=CH_2), 6.26, 6.04 (each dd, 1 H, J = 11.5, 1.7 Hz, CH=C**H**$_2$), 6.17, 5.95 (each dd, 1 H, J = 17.7, 2.0 Hz, CH=C**H**$_2$), 4.43 (s, 3 H, meso-CH_3), 4.35 (m, 4 H, -**H**$_2$$CH_2CO_2CH_3$), 3.69, 3.68, 3.60, 3.59, 3.57, (each s, 3 H, ester-CH_3 and ring-CH_3), 3.26 (t, 4 H, -CH_2C**H**$_2$$CO_2CH_3$), -2.99 (bs, 2 H, NH); HRMS calcd for $C_{37}H_{40}N_4O_4$ *m/z* 604.4039, found *m/z* 604.3045; Anal. Calcd for $C_{37}H_{40}N_4O_4$·0.5H_2O; C, 72.41; H, 6.73; N, 9.13. Found: C, 72.16; H, 6.68; N, 9.15.

5-Meso-methylhemin Chloride, 1.19. A solution of 5-meso-methylprotoporphyrin IX dimethyl ester **3.1** (13.0 mg) dissolved in $CHCl_3$ (5 mL, freshly filtered through basic alumina) was degassed by the freeze/pump/thaw method 3X. Oven-dried K_2CO_3 (ca. 50 mg) and $FeCl_2$ (585 mg) were added and the flask was degassed one more time. The reaction mixture was stirred under reflux for 2 h after which it was poured into water and extracted with CH_2Cl_2. The combined organic extract was washed once with H_2O and NaCl and filtered through Na_2SO_4. A visible spectrum was taken after solvent evaporation Vis: λ_{max} 396 nm, 399, 512, 546, 644. The crude Fe porphyrin was dissolved in THF (10 mL) and MeOH (5 mL). 1N KOH (2 mL) was added and the reaction mixture was stirred for 24 h in the dark at room temp. The reaction mixture was poured into water and washed once with $CHCl_3$ to remove any starting material. The aqueous layer as acidified with HCl to a pH of 2 and then was extracted 3X with 30% THF / ether (CH_2Cl_2 rather than ether would have been a better choice since not so much water

extracts with the solvent). The combined organic extract was washed with sat. NaCl and filtered through Na_2SO_4 and the solvent was evaporated. Crystallization from THF / *n*-hexane afforded 5-meso-methylhemin chloride **1.19** (8.8 mg) in 62% yield; HRMS calcd for $C_{35}H_{34}N_4O_4$·FeCl *m/z* 665.1687, found (MH^+) *m/z* 666.1696 and (M-Cl) *m/z* 630.1929. A ^1H NMR spectrum was taken, but the signals were not easily assignable. When KCN was added the compound decomposed.

3-(2,2-Dimethoxyethyl)isochlorin-e_6 Trimethyl Ester (3.21). A solution of osmium(VIII) oxide (OsO_4, 2.5 g) in THF (500 mL) was added, at 0°C, to 3-(2,2-dimethoxyethyl)chlorin-e_6 trimethyl ester **3.19**[6] (7.0 g) suspended in pyridine (7.0 mL). The mixture was stirred for 30 min in the dark before the ice bath was removed allowing the mixture to stir at room temp. After 10 d, the visible spectrum showed 79% of the starting material **3.19** had reacted. The reaction was quenched by bubbling H_2S gas for 3 min through the mixture. After evaporation of the solvents, the residue was redissolved in CH_2Cl_2 and passed through cotton wool to filter off the black Os_2S_3 precipitate. Chromatography on silica gel with 1% MeOH / CH_2Cl_2 eluted first the starting material **3.19** (1.45 g, 21%) followed by the 7,8-diol product **3.20** (3.62 g, 62%). The crude product **3.20** was redissolved in MeOAc and, after raising the surface area by addition of glass balls (100 g, 4-5 mm diameter) and evaporation of the solvent, heated in a vacuum (90°C, 25 Torr) for 7 d. Chromatography on silica gel with 3% MeOAc / CH_2Cl_2 afforded the title compound **3.21** (1.55 g) in 45% yield. Crystallization from acetone / water gave green plates; mp 95-97 °C; Vis: λ_{max} 404 nm (ε 133,000), 502 (11,900), 598 (5000), 652 (34,800); ^1H-NMR ($CDCl_3$): δ 9.84, 9.52, 8.73 (each s, 1 H, meso-H), 8.03 (dd, 1 H, J = 17.8, 11.5 Hz, 8-C**H**=CH_2), 6.15, 6.08 (each dd, 1 H, J = 17.8, 11.5, 0.5 Hz, 8-CH=C**H**$_2$), 5.27 ($2d_{AB}$, 2 H, J_{AB} = 21 Hz, -C**H**$_2CO_2CH_3$), 5.04 (t, 1 H, J = 5 Hz, 3-CH_2C**H**$(OCH_3)_2$), 4.43 (m, 2 H, ring-D H), 4.28 (s, 3 H, -CO_2CH_3), 4.15 (d, 2 H, J = 5 Hz, -C**H**$_2$CH$(OCH_3)_2$), 3.80, 3.79 (each s, 3 H, ester-CH_3), 3.58, 3.40,

3.37 (each s, 3 H, ring-CH_3), 3.43, 3.42 (each s, 6 H, acetal-CH_3), 2.58, 2.19 (each m, 4 H, -$CH_2CH_2CO_2CH_3$), 1.75 (d, 3 H, ring-D CH_3), -1.32, -1.43 (bs, 2 H, NH): LSIMS calcd. for $C_{39}H_{46}N_4O_8$ *m/z* 698.8, found *m/z* 699; Anal. Calcd for $C_{39}H_{46}N_4O_8$ x 0.5 H_2O: C, 66.18; H, 6.69; N, 7.92. Found: C, 66.52; H, 7.00; N, 7.80.

3-(2-Hydroxyethyl)isochlorin-e_6 Trimethyl Ester 3.22. To a refluxing solution of 3-(2,2-dimethoxyethyl)isochlorin-e_6 trimethyl ester **3.21** (1.5 g) dissolved in $CHCl_3$ (25 mL) was added THF (100 mL) and 5 M HCl (5 mL). The reaction mixture was refluxed 30 min and was then extracted with CH_2Cl_2 (100 mL). The organic extract was washed with water and 0.01 N HCl then filtered through Na_2SO_4 and the solvent was evaporated. The dry residue was redissolved in THF (10 mL) and treated with excess diazomethane in ether. Before evaporating the solvents and redissolving the residue in CH_2Cl_2 (50 mL), the unreacted diazomethane was destroyed with AcOH. To the crude material, a solution of $NaBH_4$ (810 mg) in MeOH (50 mL) was added, allowing the solution to stir for 5 min at room temp. Unreacted $NaBH_4$ was quenched with 1 M HCl before the organic layer was washed with sat. $NaHCO_3$ and water, filtered through Na_2SO_4, and evaporated. Chromatography on silica gel with 1% MeOH / CH_2Cl_2 followed by crystallization from CH_2Cl_2 / *n*-hexane afforded the title compound **3.22** as green needles (1.15 g) in 82% yield; mp 176-178 °C; Vis: λ_{max} 404 nm (ε 138,000), 500 (10,500), 598 (5000), 652 (35,400); ^{1}H-NMR ($CDCl_3$): δ 9.80, 9.26, 8.70 (each s, 1 H, meso-H), 7.95 (dd, 1 H, J = 17.8, 11.4, Hz, 8-C**H**=CH_2), 6.08, 5.67 (each dd, 2 H, J = 17.8, 11.4, 0.5 Hz, 8-CH=C**H**$_2$), 5.31 ($2d_{AB}$, 2 H, J_{AB} = 20 Hz, -$CH_2CO_2CH_3$), 4.94 (m, 2 H, ring-D H), 4.51, 3.90 (each m, 1 H, -CH_2CH_2OH), 4.29 (s, 3 H, -CO_2CH_3), 4.10 (m, 1H, CH_2CH_2O**H**), 3.82, 3.65 (each s, 3 H, ester-CH_3), 3.56, 3.29, 3.28 (each s, 3 H, ring-CH_3), 3.51, 3.40 (each m, 1 H, 3-CH_2CH_2OH), 2.60 and 2.23 (each m, 4 H, -$CH_2CH_2CO_2CH_3$), 1.75 (d, 3 H, ring-D CH_3), -1.40, -1.46 (bs, 2 H, NH); LSIMS

calcd. for $C_{37}H_{42}N_4O_7$ *m/z* 654.8, found *m/z* 654; Anal. Calcd for $C_{37}H_{42}N_4O_7$: C, 67.87; H, 6.47; N, 8.56. Found: C, 67.66; H, 6.52; N, 8.38.

3-(2-*o*-Nitrophenylselenoethyl)isochlorin-e_6 Trimethyl Ester 3.23. *n*Bu_3P (1.9 mL) was added dropwise to a solution of 3-(2-hydroxyethyl)isochlorin-e_6 trimethyl ester **3.22** (1.00 g) and *o*-nitrophenylselenocyanate (1.73 g) in CH_2Cl_2 (125 mL). The mixture was stirred for 1 h at room temp before the solvent was evaporated. Chromatography on silica gel with 1% acetone / CH_2Cl_2 yielded the title compound **3.23** (881 mg) in 70% yield. Crystallization from CH_2Cl_2 /*n*-hexane gave green needles: mp 200-202 °C; Vis: λ_{max} 406 nm (ε 180,000), 502 (23,700), 600 (15,800), 654 (52,300); ^{1}H-NMR ($CDCl_3$): δ 9.74, 9.05, 8.62 (each s, 1 H, meso-H), 8.07 (dd, 1 H, phenyl-H), 7.93 (dd, 1 H, J = 17.8, 11.4, Hz, 8-C**H**=CH_2), 7.28, 6.99 (each m, 2 H, phenyl-H), 6.08, 5.90 (each dd, 1 H, J = 17.8, 11.4, 0.5 Hz, 8-CH=C**H**$_2$), 5.31 ($2d_{AB}$, 2 H, J_{AB} = 20 Hz, -C**H**$_2CO_2CH_3$), 4.41 (m, 2 H, ring-D H), 4.29 (s, 3 H, ring-CO_2C**H**$_3$), 3.85 (m, 2 H, -CH_2C**H**$_2$SePh), 3.80, 3.62 (each s, 3 H, ester-CH_3), 3.51, 3.20, 3.16 (each s, 3 H, ring-CH_3), 3.32 (m, 2 H, -C**H**$_2$C**H**$_2$SePh), 2.60, 2.20 (each m, 4 H, -C**H**$_2$C**H**$_2CO_2CH_3$), 1.75 (d, 3 H, ring-D CH_3), -1.50, (bs, 2 H, NH); LSIMS calcd. for $C_{43}H_{45}N_5O_8Se$ *m/z* 838.8, found 839.2; Anal. Calcd for $C_{43}H_{45}N_5O_8Se$: C, 61.57; H, 5.41; N, 8.35. Found: C, 61.55; H, 5.41; N, 8.34.

3,8-Divinylchlorin-e_6 Trimethyl Ester 1.20.

3-(2-*o*-Nitrophenylselenoethyl)isochlorin-e_6 trimethyl ester **3.23** (500 mg) was dissolved in THF (50 mL). The solution was cooled in an ice bath and an excess of 30% H_2O_2 (2.5 mL) was added. The reaction mixture was stirred at room temp for 3 h before being washed with H_2O. The organic extract was passed through Na_2SO_4 and after evaporation of the solvent, the crude material was chromatographed on silica gel and the product was eluted with 1% acetone / CH_2Cl_2. Crystallization from CH_2Cl_2 / *n*-hexane afforded

1.20[15,29] (278 mg) in 73%; mp 201-203 °C; λ_{max} 410 nm (ε 149,000), 506 (11,000), 608 (3000), 662 (40,400); ^{1}H-NMR ($CDCl_3$): δ 9.84, 9.60, 8.74 (each s, 1 H, meso-H), 8.06 (m, 2 H, -C**H**=CH_2), 6.15, 6.36 (each dd, 1 H, J = 17.8, 11.5, 1.4 Hz, 3-CH=C**H**$_2$), 6.01, 6.13 (each dd, 1 H, J = 17.5, 11.5, 1.7 Hz, 8-CH=C**H**$_2$), 5.28 ($2d_{AB}$, 2 H, J_{AB} = 19 Hz, -C**H**$_2$$CO_2CH_3$), 4.45 (m, 2 H, ring-D H), 4.26 (s, 3 H, ring-CO_2C**H**$_3$), 3.78, 3.67 (each s, 3 H, ester-CH_3), 3.59, 3.50, 3.44 (each s, 3 H, ring-CH_3), 2.57, 2.20 (each m, 4 H, -C**H**$_2$C**H**$_2$$CO_2CH_3$), 1.75 (d, 3 H, ring-D CH_3), -1.32, -1.46 (each bs, 2 H, NH); LSIMS calcd. for $C_{37}H_{40}N_4O_6$ *m/z* 636.5, found *m/z* 636.3; Anal. Calcd for : C, 69.70; H, 6.33; N, 8.80. Found: C, 69.56; H, 6.54; N, 8.72.

Part Two

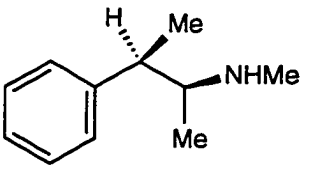
Chemistry of Marine Natural Products from *Xestospongia* sp. and *Dysidea herbacea.*

Chapter Four

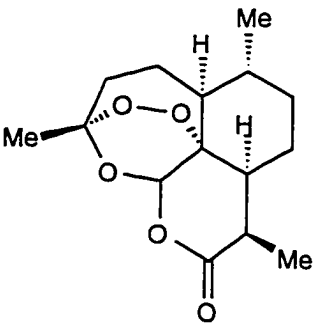
Introduction to Marine Natural Product Chemistry

4.1 General History

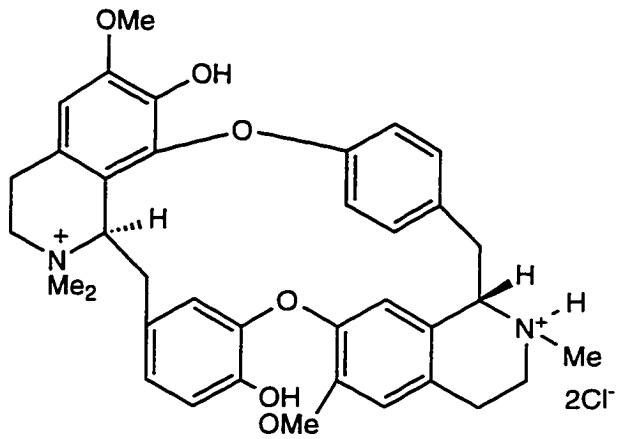
Natural products have been utilized by humans for centuries as necessities as well as luxuries. Medicines, poisons, perfumes, stimulants and dyes are just a few of the many uses to which natural products have contributed. Modern medicine has successfully applied the purified active ingredients of many ancient herbal cures. For instance, the ancient Chinese used an extract from *Ephedra* species known as 'Ma Huang' to treat respiratory conditions like bronchitis and asthma. Today the active substance, ephedrine (**4.1**), is used in medicines as a decongestant. Over 2000 years ago, the Chinese also discovered an extract from the plant *Artemisia annua* that prevents and treats malaria. Not until 1972 was the active ingredient, artimisinin (**4.2**, known by the Chinese as qinghaosu), isolated, and in 1979 the structure was elucidated. The South American natives used a plant extract commonly known as curare to poison their arrows. The neuromuscular blocking agent, tubocurarine (**4.3**), is now used as a muscle relaxant in surgery. Figure 1.1 illustrates a few of the many drugs that ancient cultures have used as crude extracts that are now commonly applied as pure substances.[1,2]



4.1 Ephedrine

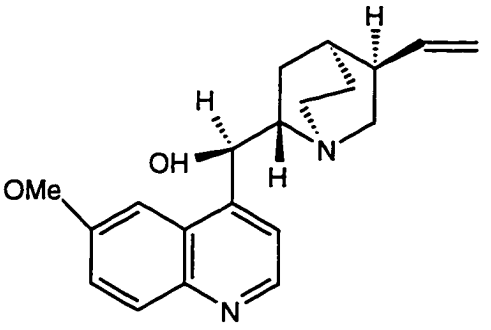


4.2 Artemisinin

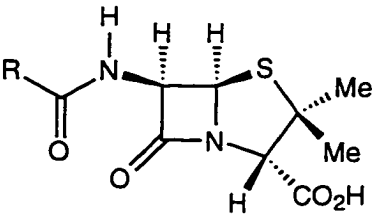


4.3 Tubocurarine chloride

In the early nineteenth century, chemists started to concentrate their efforts on isolating and characterizing medicinally useful compounds from plant or animal extracts. The characterization of the pure natural product was very difficult. At best, the molecular formula, melting or boiling point and a few chemical transformations were all that described these substances. Structure elucidation was extremely complex, but through total synthesis along with the biosynthetic hypothesis, some structures were described with amazing accuracy. With the advances of modern technology, such as nuclear magnetic resonance (NMR), mass spectrometry, and X-ray crystallography in the middle 1900s, structure elucidation has become much easier.



4.4 Quinine



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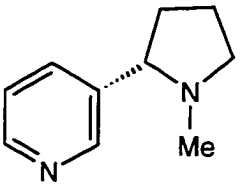

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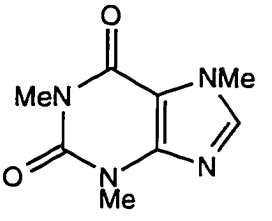

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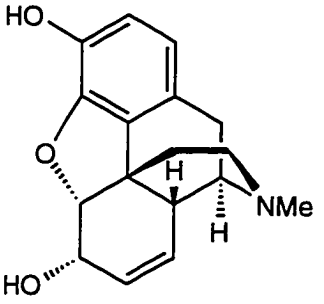

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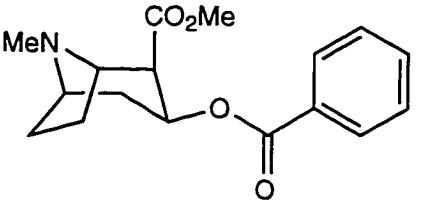

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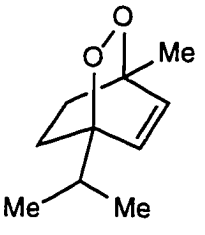

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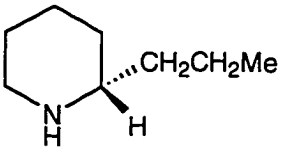
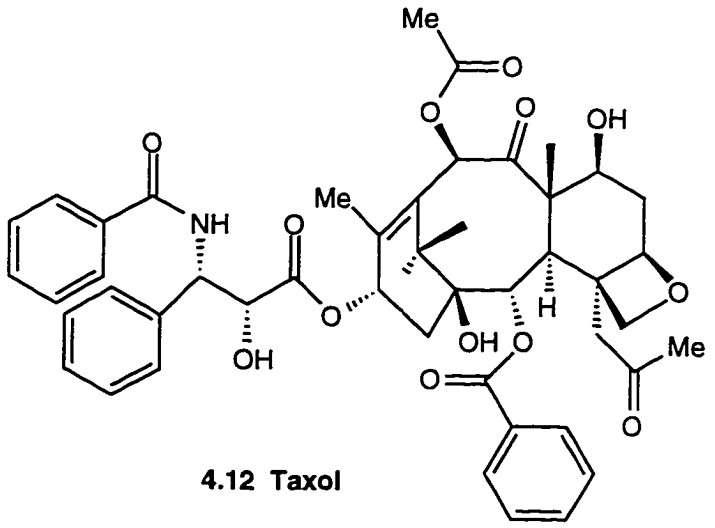


4.11 Coniine (hemlock)

Figure 4.1 Common drugs that were ancient crude extracts.

Natural products chemistry has remained an important area of research. New pharmaceuticals continue to be discovered. For example, taxol, isolated from the bark of the Pacific yew, *Taxus brevifolia*, recently has been approved by the U. S. Food and Drug Administration to treat ovarian cancer.[3] Discovery of novel compounds is not limited to terrestrial plants and animals. In the past 25-30 years, marine natural product chemistry has flourished.[4]

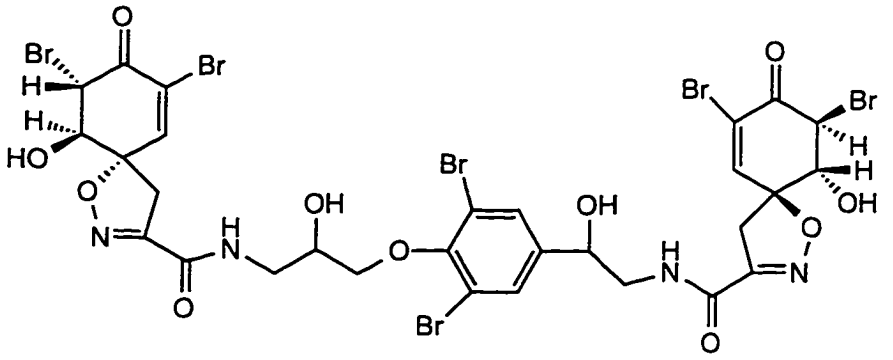


4.12 Taxol

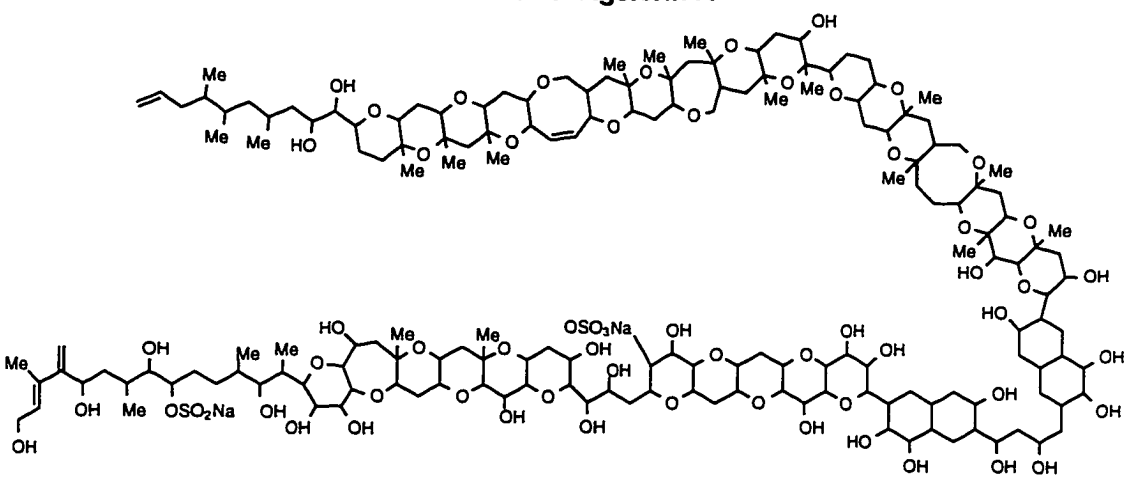
4.2 Marine Natural Products

The study of marine natural products has proven to be an important source for new chemistry that provides novel compounds that are both scientifically and medicinally interesting. The different environmental influences in the marine world have generated many natural products that rarely have terrestrial counterparts. For example, the antibacterial agelorin A (**4.13**)[5] illustrates one of many unique compounds that incorporate a high degree of halogenation. The extremely toxic maitotoxin (**4.14**, LD_{50} is ca. 50 ng/kg, ip. in mice), isolated from the dinoflagellate *Gambierdiscus toxicus*,[6] represents perhaps the most complex natural product to date, with a weight of 3422 Da, with the exception of biopolymers. These examples illustrate the structural complexity that marine secondary metabolites can have as well as the tremendous biological activity. For detailed

information on novel marine natural products with interesting biological and pharmaceutical properties refer to Faulkner's yearly reviews.[7]



4.13 Agelorin A

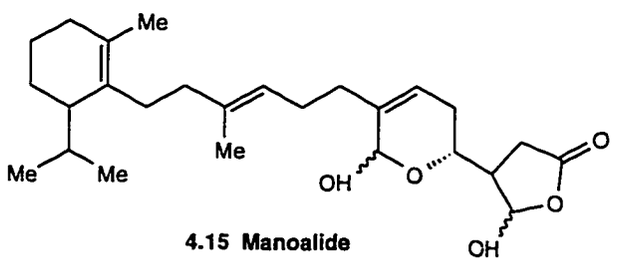


4.14 Maitotoxin

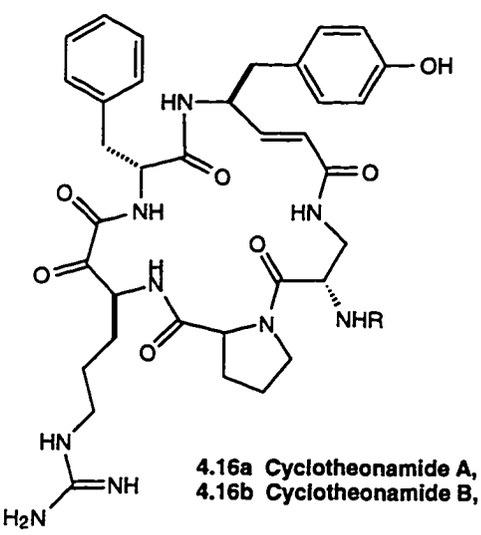
Despite the interesting medicinal characteristics that marine natural products possess, a commercially successful "drug from the sea" has not been found, although there are encouraging possibilities. Three potent antitumor alkaloids, bryostatin A from the bryozoan *Bugula neritina*,[8] didemnin B from the ascidian *Didemnun solidum*,[9] and halichondrin B from the sponge *Lissodendoryx*[10] are currently in clinical or late preclinical phase trials administered by the National Cancer Institute (NCI). The NCI tests around 2500 new marine natural products against 60 types of tumor cells each year.[10] Most of the potent bioactive compounds discovered come from soft-bodied animals, particularly sponges.

4.3 Marine Sponge Metabolites

Sponges are perhaps the richest source of highly biologically active marine metabolites. Research on these animals continues to grow, surpassing that of other marine organisms.[7] Polypeptides,[11] polyether macrolides,[12-14] like that of maitotoxin (**4.14**), steroids,[15-17] and many other classes of compounds possess powerful pharmaceutical properties. The sesterterpene manoalide (**4.15**) is one example of a medicinally potential compound that has received intense research. Isolated from the sponge *Luffariella variabilis*,[18] manoalide possesses potent anti-inflammatory properties and irreversibly inhibits phospholipase A_2.[19,20] The nonsteroidal treatment of inflammation such as arthritis, without the undesirable side effects of usual steroid therapy, is a potential value of this natural product. The cyclic pentapeptides, cyclotheonamide A and B (**4.16a,b**), from the sponge *Theonella swinhoei* [21] are also a subject of concentrated research for their potent ability to inhibit thrombin, the enzyme responsible for blood clotting.



4.15 Manoalide



4.16a Cyclotheonamide A, R = CHO
4.16b Cyclotheonamide B, R = $COCH_3$

4.3.1 Theories for Bioactivity

Why are sponges so rich in biologically active secondary metabolites? One hypothesis is that the animals use these compounds as a defense mechanism.[22] Sponges are simple multicellular animals of the phylum porifera. They lack a skeleton, but their shape comes from spicules, glass-like silica or limestone, or from a fibrous protein material called spongin. These soft-bodied animals are usually bright colored and sessile. In fact, most sponges root themselves to coral or the ground. Therefore, a sponge's best defense against predators is through toxins. "Chemical weaponry" is only one defense mechanism that sponges use. Other defense functions include prevention of fouling, inhibition of overgrowth, and protection from ultraviolet radiation.

Another theory for the production of bioactive metabolites is that they come from symbionts associated with the sponge.[11] Common symbionts include brittle stars, annelid worms, dinofagellates, and blue-green algae. One reason the filter feeding sponge may live in symbiosis is because the ocean waters do not offer enough nourishment. Sponges that contain blue-green algae use the bacteria's photosynthetic abilities to provide nutrients. A sponge can chemically force the algae to leak as much as 80% of their photosynthetic products into the sponge cells.[23] Some sponges are known to have symbiotic associations with bacteria that may be up to half their cellular mass. So, it is reasonable to believe that some compounds are produced by symbionts. There is little experimental evidence, however, to support or refute this theory.[24,25]

4.4 Goals of Marine Natural Product Chemists

The goals of marine natural product chemists are threefold. First, the discovery of new secondary metabolites, compounds that are not essential to life, that either possess bioactivity or are scientifically interesting in structure, is of constant interest. However, isolation of compounds is often limited to a milligram scale. Since it is not environmentally sound to deplete the ocean of marine life, alternative methods for procuring large amounts

of secondary metabolites are needed. This leads to the second goal; total or partial synthesis of interesting compounds. Structural activity relationship experiments with synthetic derivatives may lead to the discovery of more potent medicinal compounds as well as provide for a better understanding of the biological activity. Finally, the newest area of research involves investigation into the origin and biosynthesis of secondary metabolites.[26] From the knowledge gained, mass production of pharmaceutically new compounds may be initiated using fermentation or recombinant DNA technologies.

4.5 Dissertation Goals

The two Dissertation goals accomplished in the following two Chapters involve the isolation of new secondary metabolites and the partial synthesis of a marine natural product derivative. First, three new brominated fatty acids (**5.37-5.39**) from the marine sponge, *Xestospongia* sp. were isolated and characterized. These acids belong to a class of compounds unique to marine sponge metabolites. Second, the synthesis of chlorinated amino nitrile diastereomers (**6.29a-d**) was achieved. These diastereomers are synthetic intermediates toward deuterium or tritium labeled leucine, the synthesis of which would be useful in examining the biosynthesis of unique polychlorinated metabolites from the marine sponge, *Dysidea herbacea.*

4.6 References

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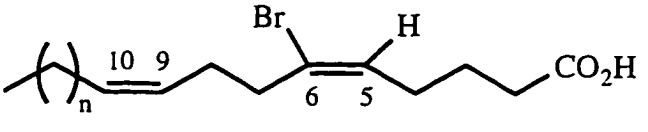
Chapter Five

Brominated Acetylenic Fatty Acids from the Marine Sponge *Xestospongia* sp.

5.1 Introduction: Brominated Fatty Acids

Brominated fatty acids are rare in nature and have been discovered only in marine sponges.[1-13] There are a total of 36 brominated acids that have been isolated from sponges of the families Nepheliospongidae (order Nepheliospongida), Hymeniacidonidae (order Halichondrida) and Agelasidae (order Axinellida). Nine of the fatty acids **5.1-5.9** were isolated from the phospholipids of the sponge and range in carbon lengths between C_{24} and C_{28}.[1-3,12,13] All nine phospholipid acids have structural similarities in that the bromine atom is always located on the vinylic carbon-6 of the 5,9-diene system, regardless of carbon chain length or methyl branching pattern. The other twenty-seven unsaturated acids were isolated from cell free extracts. The unsaturation includes both olefinic and acetylenic functionalities with the exception of two acids (**5.34**, **5.36**).[4,9] The pattern of unsaturation, the number and position of bromine, and the chain length, makes each acid unique. Nineteen of these acids have a chain length of C_{18} where as seven are C_{16} acids and one is a C_9 acid (**5.10-5.36**).



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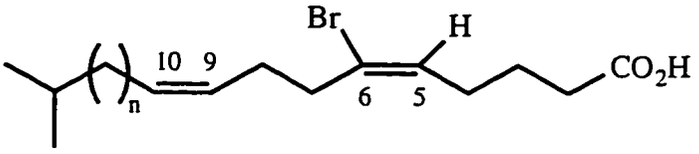

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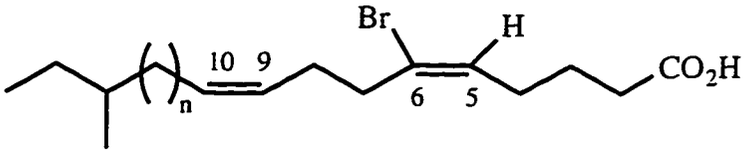
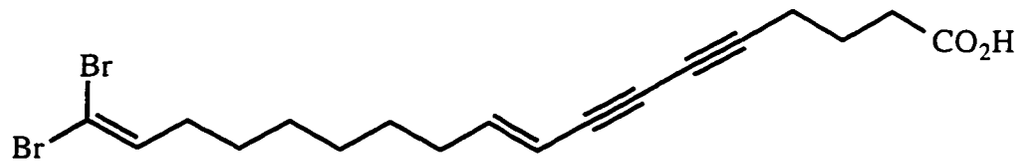


5.9: n = 13

5.1.1 Biological Activity of Brominated Acetylenic Acids

Many of the brominated acetylenic acids possess a variety of mild biological activity. Acid **5.33** showed cytotoxicity toward L1210, lymphoid leukemia cells *in vitro*, with an effective dose for 50% inhibition (ED_{50}) greater than 20 μg/mL[11] where a crude mixture of **5.33**, **5.34**, and **5.35** showed cytotoxicity against KB cells, human epidermoid carcinoma in the mouth (2+ at 10 μg/mL).[4] Six acids (**5.10-5.14, 5.36**), from the sponge *Xestospongia* sp., were found to have antibacterial activity against *Bacillus subtilis*.[9] Patil and co-workers discovered that acids **5.16**, **5.18-5.20**, and **5.22** showed inhibition against HIV-1 protease, a crucial enzyme in the replication of the virus.[6] The 50% inhibition of cells (IC_{50}) ranged from 6 μg/mL to 12 μg/mL, but the mode of action for the inhibition was not specific. Acids **5.17** and **5.35** possessed weak antimicrobial activity against *Staphylococcus aureus*[7] and Gram-positive bacteria,[4] respectively. Acids **5.16-5.17**, **5.23-5.32** were antifungal against *Mortierella ramannianus*.[5]



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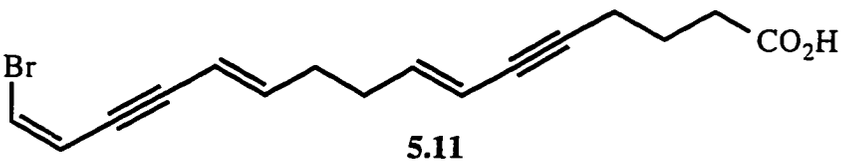

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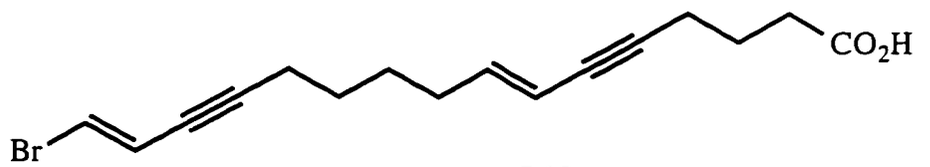

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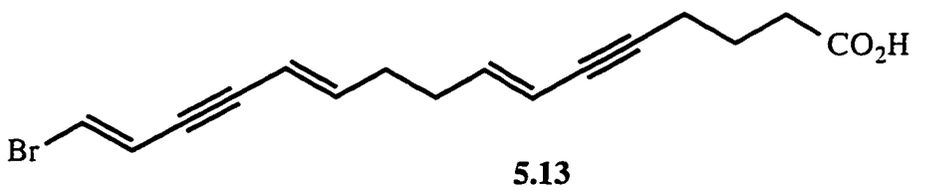

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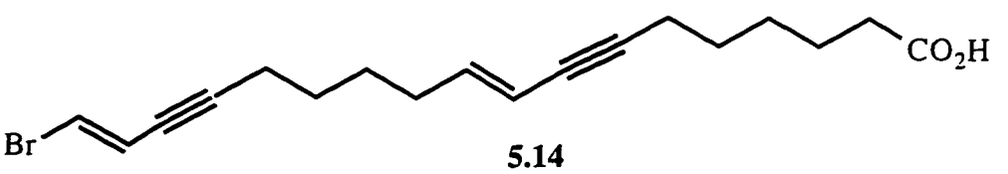

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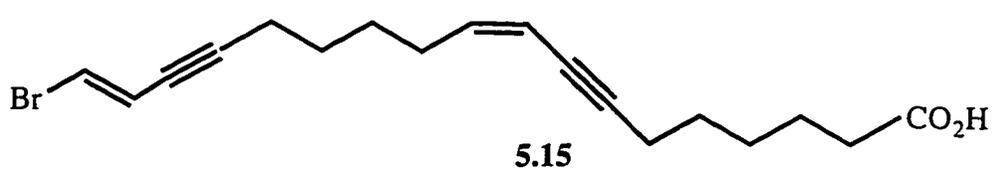

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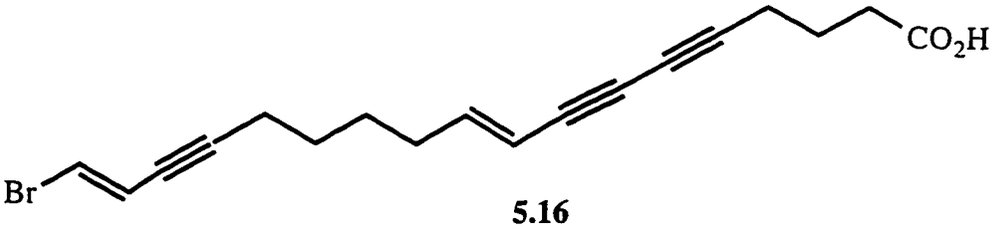

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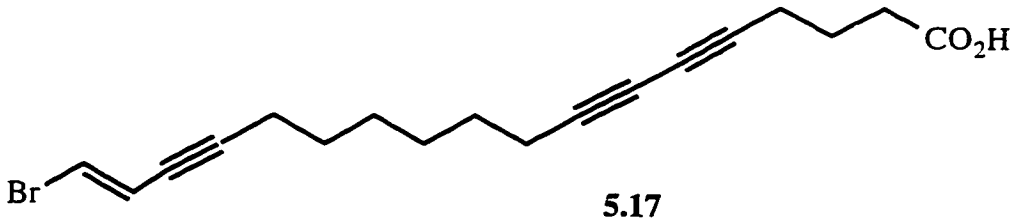

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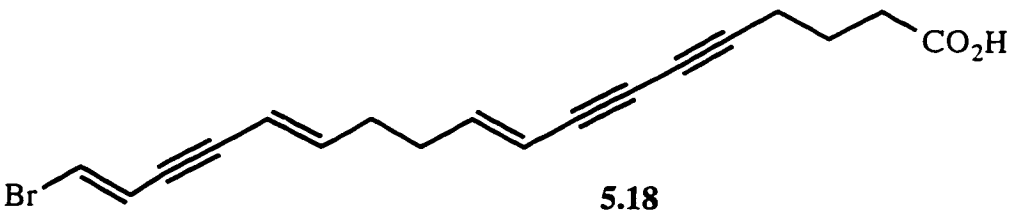

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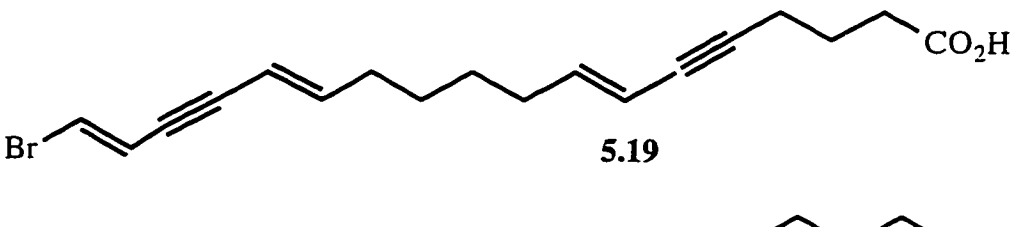

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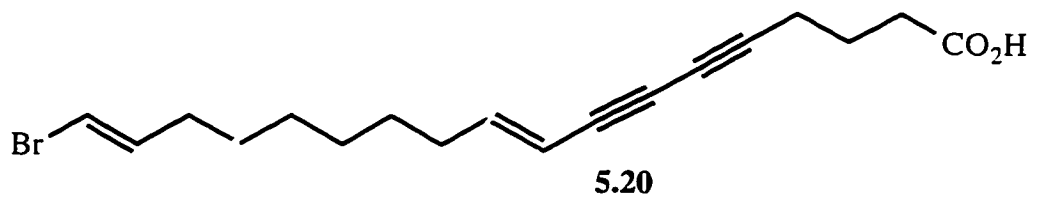

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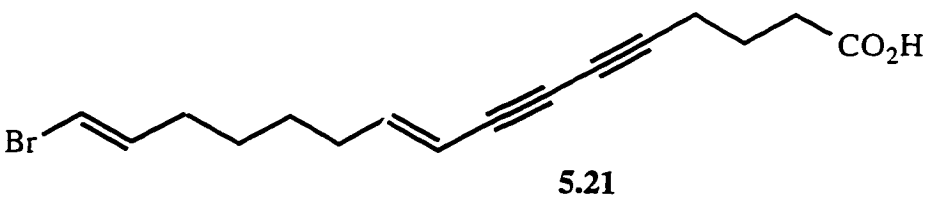

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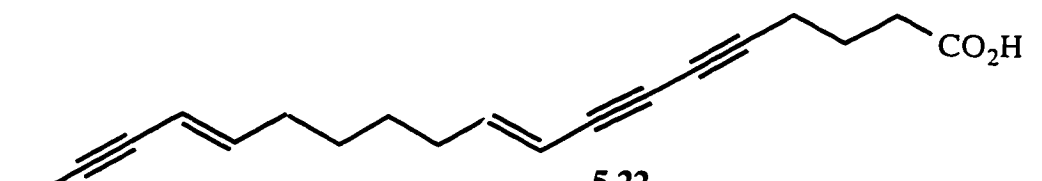

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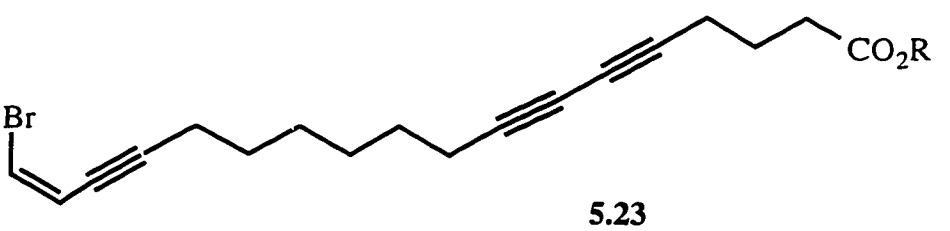

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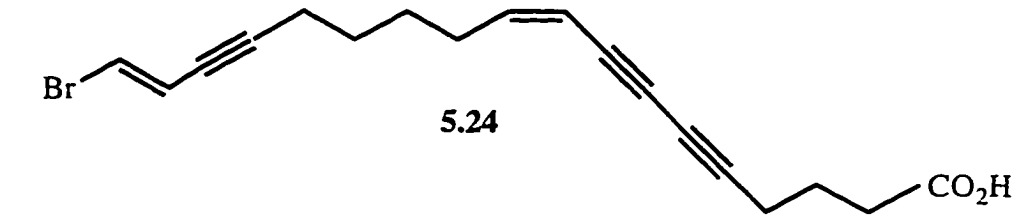

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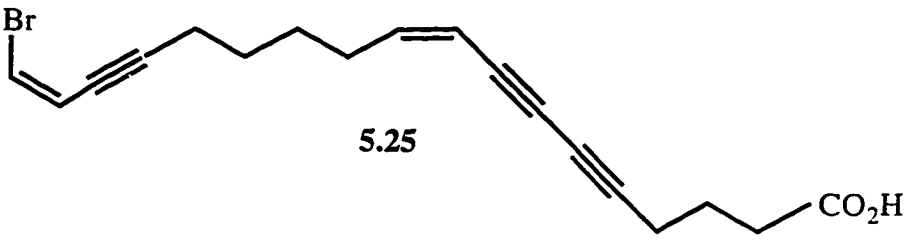

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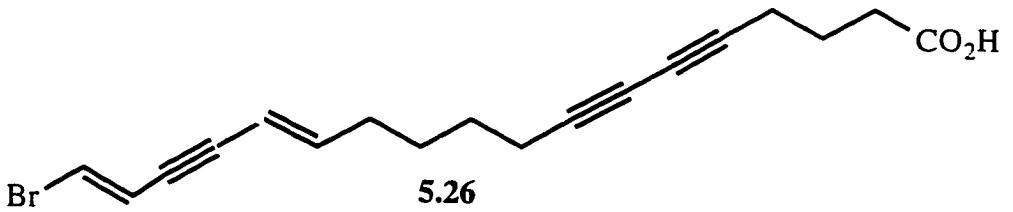

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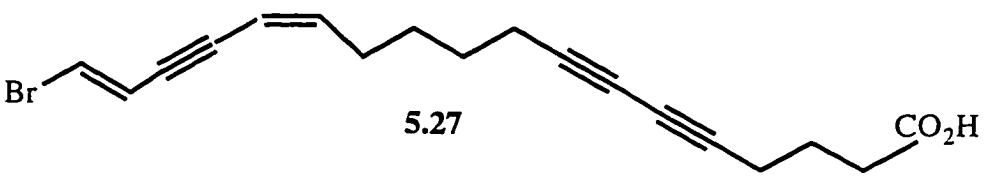

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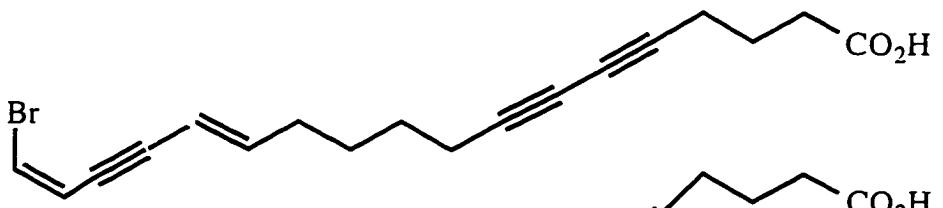

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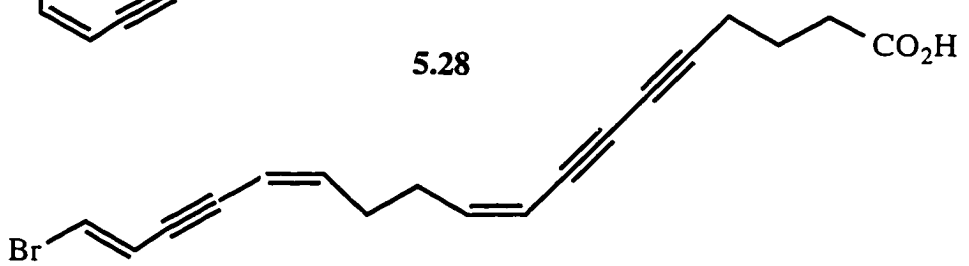

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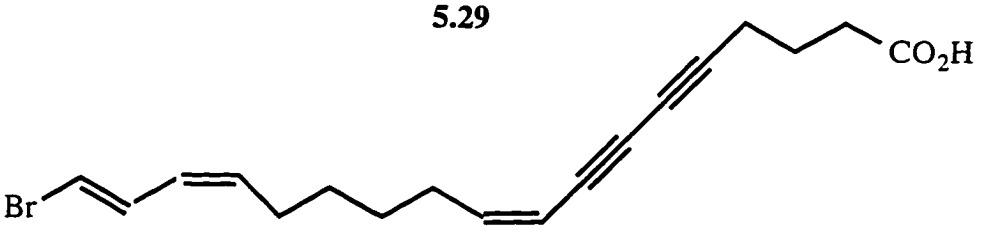

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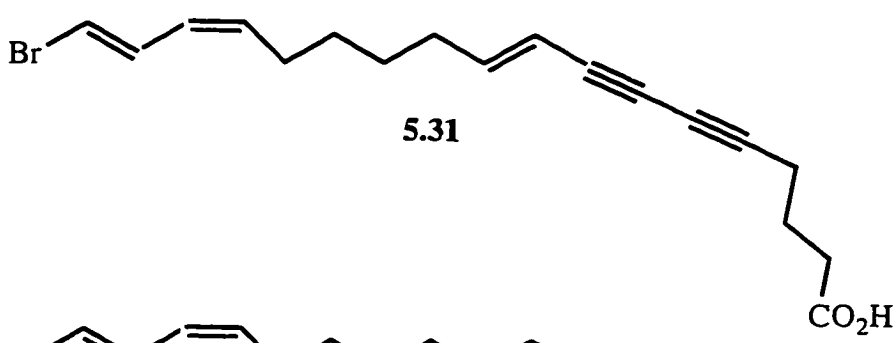

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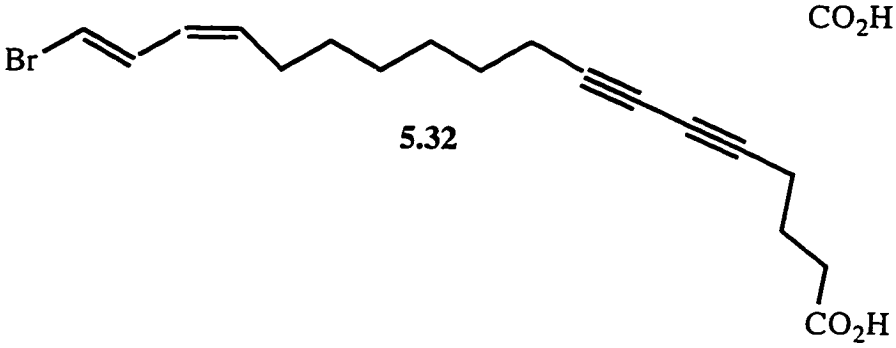

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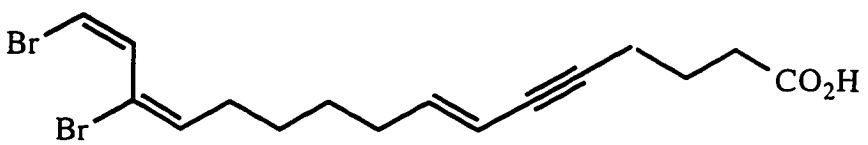

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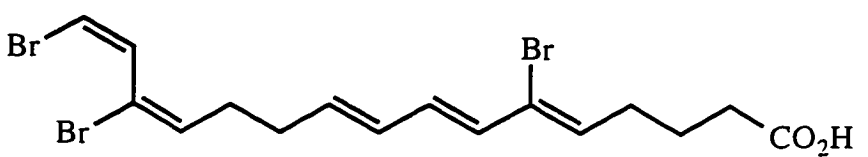

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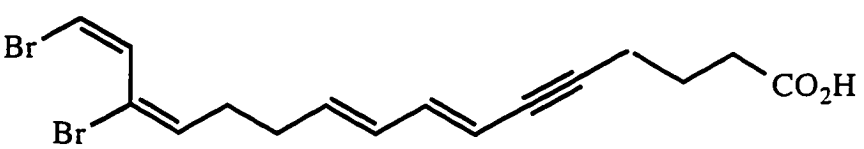

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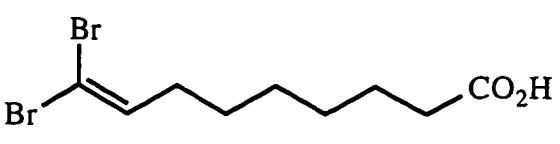
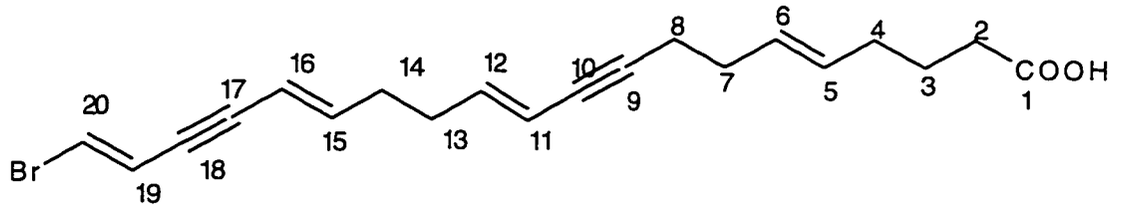


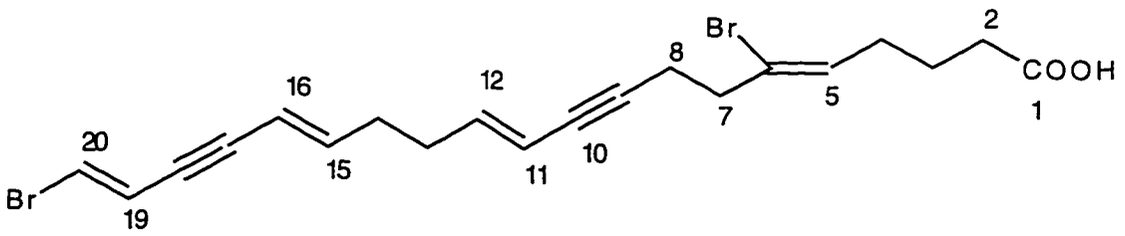
5.36

5.1.2 Three New Brominated Fatty Acids

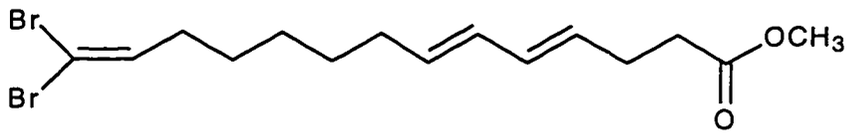
The work described in this Chapter shows how three new brominated fatty acids, **5.37**, **5.38**, and **5.39** from the marine sponge, *Xestospongia*, sp. (family Nepheliospongidae) were isolated and characterized.[14] The acids are unique in their unusual carbon chain lengths. Compounds **5.37** and **5.38** are C_{20} polyacetylenic acids where as **5.39** is a ω,ω-dibromovinylidene C_{14} fatty acid. Free C_{20} acids are rarely found as marine natural products while myristic acid (C_{14}), more commonly seen in bacteria, has been found only in the esterified form in the sponge-derived alkaloids bengazole A and bengamide A.[15,16]



5.37



5.38: R = H
5.38a: R = CH_3



5.39

5.2 Collection and Identification of the Sponge, *Xestospongia* sp.

Three sponges of *Xestospongia* sp. were collected by hand using SCUBA from Bennett Shoal at Exmouth Gulf in Western Australia, and were immediately frozen. Their shape resembles a volcano (conical) with ridges on the side. Their texture is crumbly and they are colored tan-brown with pink highlights. Samples from each of the sponges were sent to M. K. Harper at Scripps Institution of Oceanography for identification. She identified all three morphologically identical sponges as *Xestospongia* sp. She was not able to unambiguously identify the species, however.

5.3 Isolation and Characterization of C_{20} Brominated Acids

A sample of the freeze-dried sponge was extracted with MeOH and the extract was solvent partitioned into *n*-hexane, CCl_4, $CHCl_3$ and *n*-BuOH following a modified Kupchan partition.[17] From ^1H NMR spectra of the crude extracts, the acids were found to be in the chlorinated solvent extracts.

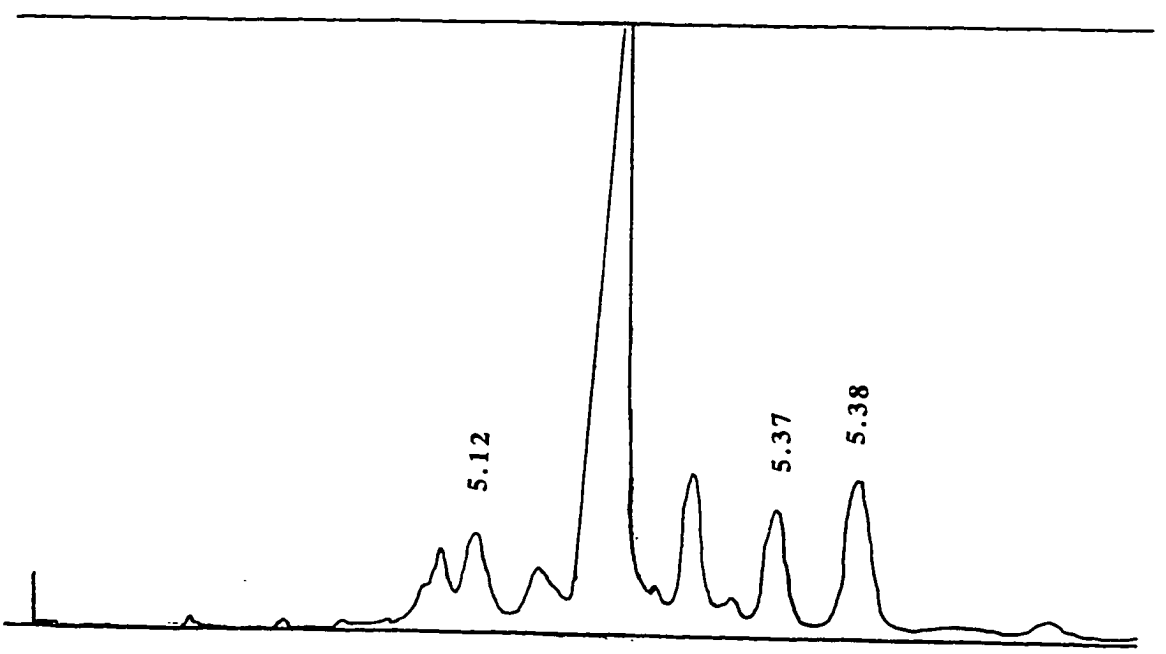


Figure 5.1 HPLC trace for acids **5.12**, **5.37** and **5.38**.

The combined chlorinated solvent extracts were separated by vacuum flash chromatography using TLC grade silica gel with a gradient of 10% to 100% EtOAc/*n*-hexane. The fifth fraction (93-146-2-E) was further separated by reversed phase HPLC (85% MeOH/H_20) to give two novel C_{20} acids, **5.37** (0.0015% dry weight), **5.38** (0.0027%) and a known C_{16} acid **5.12** (0.0013%).[9] Figure 5.1 shows the reversed phase HPLC trace and Figure 5.2 illustrates the isolation procedure in a flowchart.

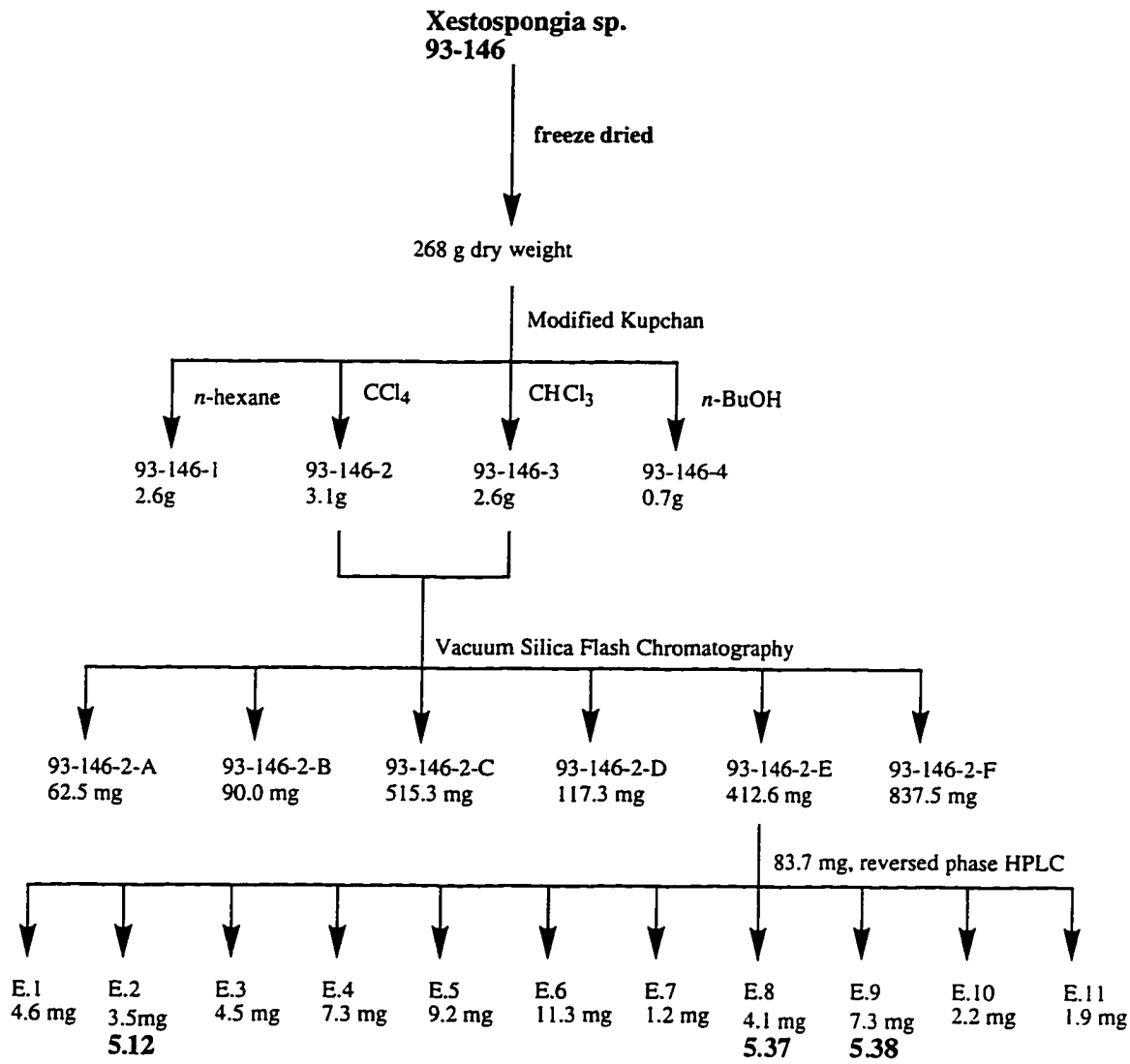


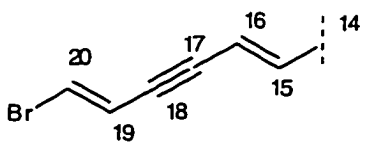
Figure 5.2 Isolation Flowchart of C_{20} acids **5.37**, **5.38** and C_{16} acid **5.12**.

5.3.1 Characterization of C_{20} Acid 5.37

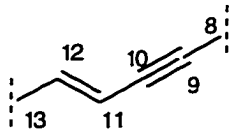
Initial evaluation of **5.37** began with analysis of the ^{1}H and ^{13}C NMR, infrared (IR), and ultraviolet absorption (UV) spectra. From the ^{1}H NMR spectrum, six signals ranging between 5.2-6.7 ppm indicated a large degree of unsaturation, while the upfield region of the spectrum contained mostly signals in the region 2.0-2.5 ppm associated with allylic protons. Integration was difficult due to overlapping signals. The carbon-13 spectrum indicated a total of 20 carbons: a carbonyl carbon (177 ppm), eight sp^2 carbons (110-145), four quaternary acetylenic carbons (79-90 ppm) and seven sp^3 carbons (20-35 ppm). IR spectra confirmed the presence of a carbon-carbon triple bond (ν 2214) and a carboxylic acid, while the UV spectrum revealed chromophores due to conjugated unsaturated bonds.

Two-dimensional NMR experiments, COSY (correlated spectroscopy), HMQC (heteronuclear multiple quantum coherence), and HMBC (heteronuclear multiple bond coherence) are commonly used to piece together a structure. From these experiments, cross peaks on a topographical spectrum reveal coupling between two nuclei. For instance, the COSY experiment reveals ^{1}H, ^{1}H coupling ($^{1}J_{HH}$). The HMQC experiment allows for ^{1}H and ^{13}C connections one bond away ($^{1}J_{HC}$), where as the HMBC experiment provides ^{1}H and ^{13}C long-range coupling two or three bonds away ($^{2}J_{HC}$, $^{3}J_{HC}$).

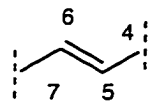
Interpretation of the COSY spectrum for **5.37** (Fig. 5.3) along with analysis of the ^{1}H and ^{13}C NMR spectra identified three separate unsaturated systems; an ene-yne-ene moiety, an ene-yne group and an isolated double bond. The double bonds all have *E* geometry as shown by the magnitudes of vinylic vicinal coupling constants, which ranged from J = 14-15.9 Hz. The experiments also revealed homo-propargylic couplings (J = 2.2 Hz) and sequential vicinal and allylic correlations. Figure 5.3 shows the four partial structures (**5.37a-d**) that the NMR experiments provided. Due to overlapping signals in the region between 2.2 to 2.4 ppm, the methylene connections that would complete the structure were difficult to determine.



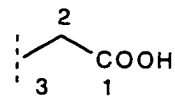
5.37a



5.37b



5.37c



5.37d

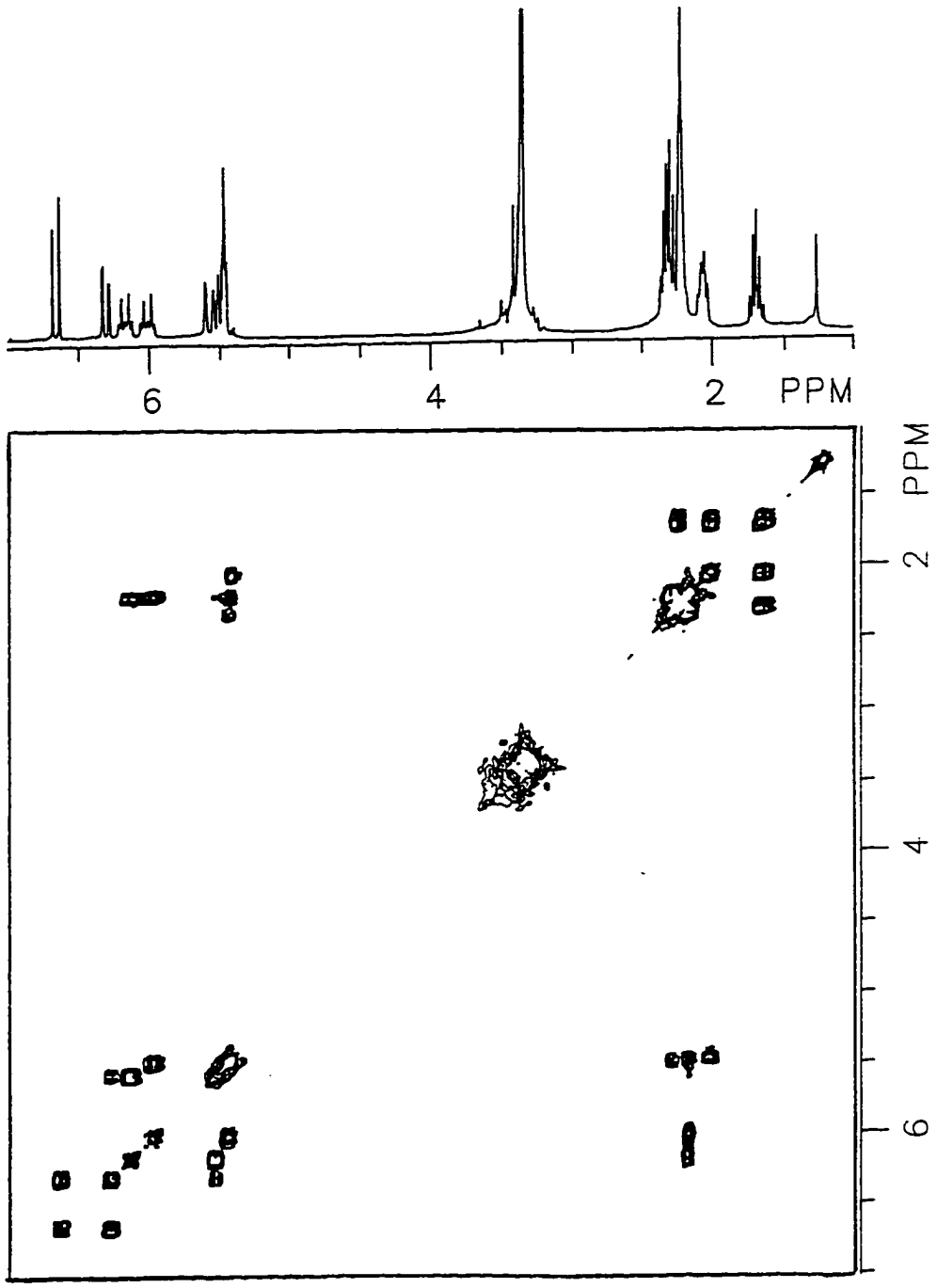


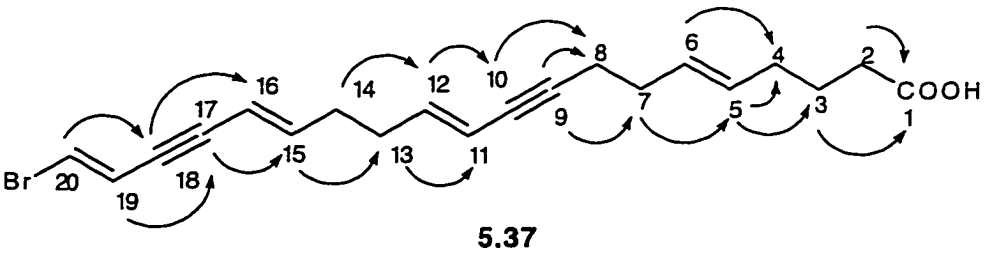
Figure 5.3 Elucidation of partial structures **5.37a-d** from ^{1}H and COSY NMR spectra.

Interpretation of the HMBC experiment yielded the final correlations required to piece the structure together. From Figure 5.4, the three correlations between C10, H8 and C9, H8 and C9, H7 showed that there are two methylene groups attaching partial structures **5.37b** and **5.37c**. Also, the two correlations between C1, H2 and C1, H3 confirm that there are three methylenes attaching partial structures **5.37c** and **5.37d**. Finally, there were two methylenes left which connected partial structures **5.37a** and **5.37b**.

The final experiment necessary to complete the characterization of acid **5.37** is the HRMS. Mass spectrometric measurements of the C_{20} acids using FABMS and EIMS were unsucessful, however. The University of Minnesota Mass Spectroscopy Service Facility was able to provide the chemical ionization HRMS for **5.37** (HRCIMS; *m/z* $M+NH_4^+$ 392.1218). The MH^+ ions (*m/z* 375/377) observed were of equal intensity indicating that a bromine was present, this being consistent with a molecular formula of $C_{20}H_{23}BrO_2$.

5.3.2 Characterization of C_{20} Acid 5.38

Acid **5.38** differs from **5.37** by the substitution of bromine for hydrogen at C6. The free acid failed to provide a pseudomolecular ion in the FAB, EI or CI MS. Treatment of **5.38** with diazomethane yielded the methyl ester **5.38a** which was able to provide a LRCIMS. The $M+NH_4^+$ ions (*m/z* 484/486/488) observed had an intensity ratio of 1:2:1 indicating the presence of two bromines. Therefore, the molecular formula for **5.38** is consistent with $C_{20}H_{22}Br_2O_2$. The ^1H and ^{13}C NMR spectra were very similiar to that of acid **5.37**. The main difference in the ^1H spectrum is the presence of a triplet vinyl proton signal, H5 (5.93 ppm), suggesting that the additional bromine was present at C6. The calculated chemical shift of H5 for both the *E*-isomer (5.36 ppm) and *Z*-isomer (5.98 ppm) is more consistent with the 5,6-*Z* configuration for **5.38** (5.93 ppm), as shown.[18] Comparison with the data to **5.37** and other brominated acids along with interpretation of the COSY spectrum (Fig. 5.5) allowed elucidation of the structure for **5.38**.



5.37

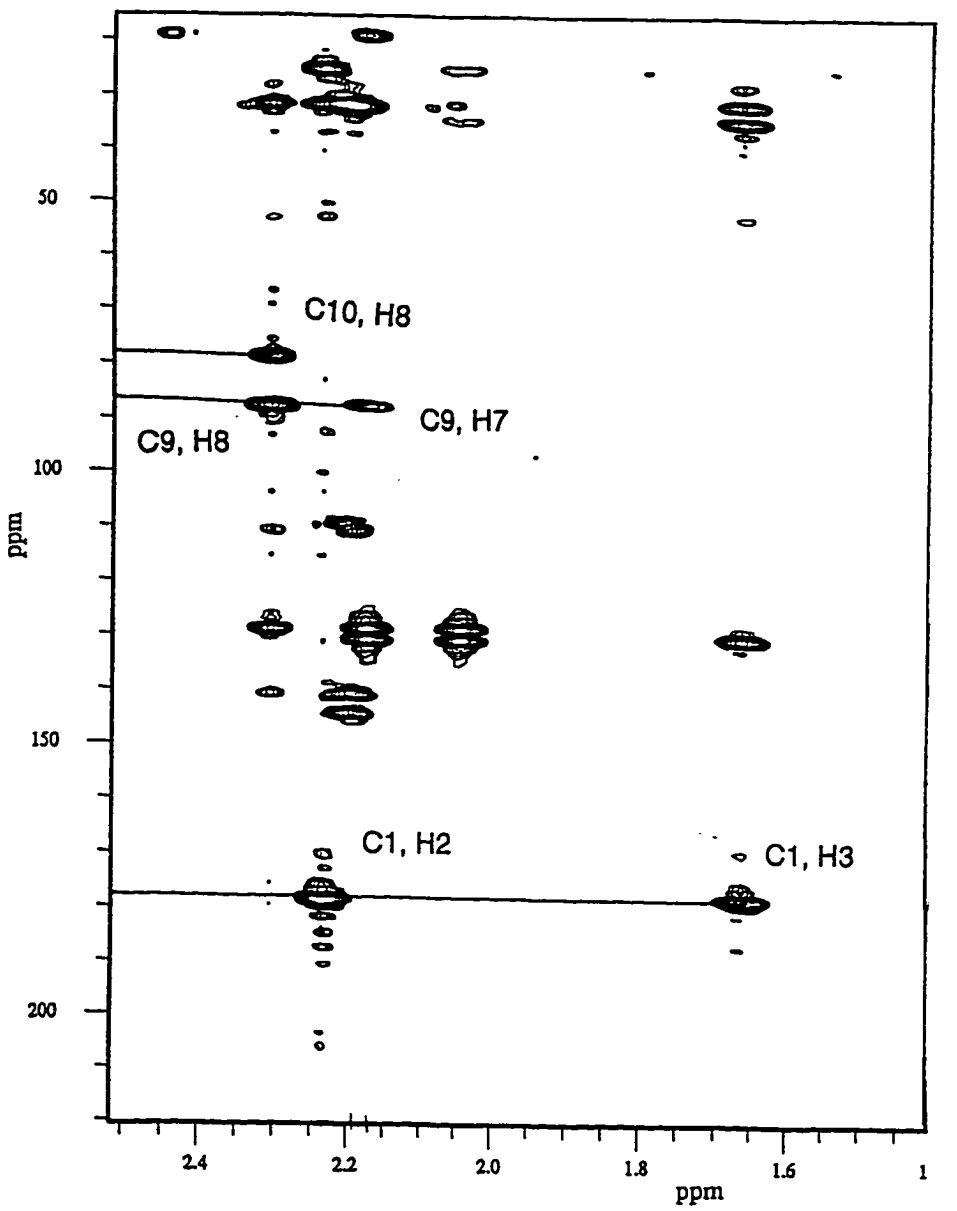
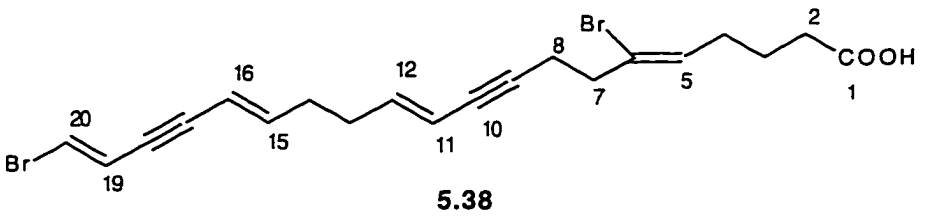


Figure 5.4 Partial HMBC spectrum for acid **5.37** along with HMBC correlations.



5.38

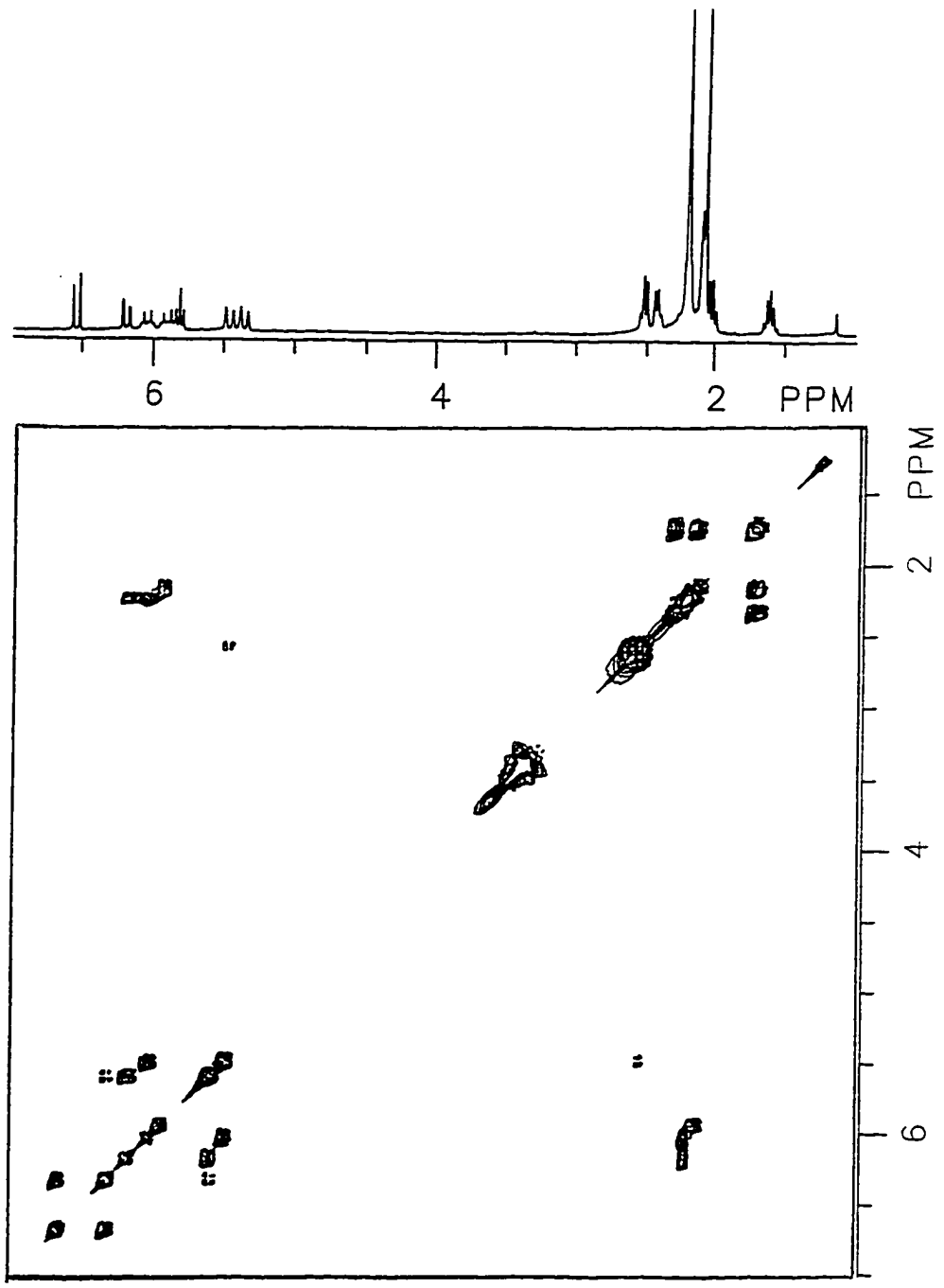


Figure 5.5 ^{1}H and COSY spectra for acid **5.38**.

5.3.3 Antifungal Test on Acids 5.37, 5.38, and 5.12

The three acids **5.37**, **5.38** and the known compound **5.12** were tested for antifungal activity against *Candida albicans*. An acid concentration of 0.1 mg/mL was placed on an agar plate treated with *C. albicans*. After incubating in an oven at 37 ^{o}C for 16 hours, no antifungal activity was detected. More material of the C_{20} acids (**5.37**, **5.38**) was desired in order to further test for other biological activity.

5.3.4 Attempts to Isolate more C_{20} Acids 5.37,5.38

Extraction and purification of the other two specimens from the collection of *Xestospongia* sp. failed to provide the C_{20} acids **5.37** and **5.38**. Analysis of the crude chlorinated solvent extracts by ^{1}H NMR spectroscopy shows that the C_{20} acids were absent (Fig. 5.6). The absence of the terminal ene-yne-ene system was indicated by the missing ^{1}H NMR signals at 6.66 ppm and 6.31 ppm. Also, analysis by analytical HPLC of the C_{20} acids with fractions from the other two sponges showed no corresponding signals. However, there is strong evidence from the ^{1}H NMR spectrum that there are other fatty acids present and upon further purification a new brominated C_{14} fatty acid was isolated as its methyl ester **5.39**.

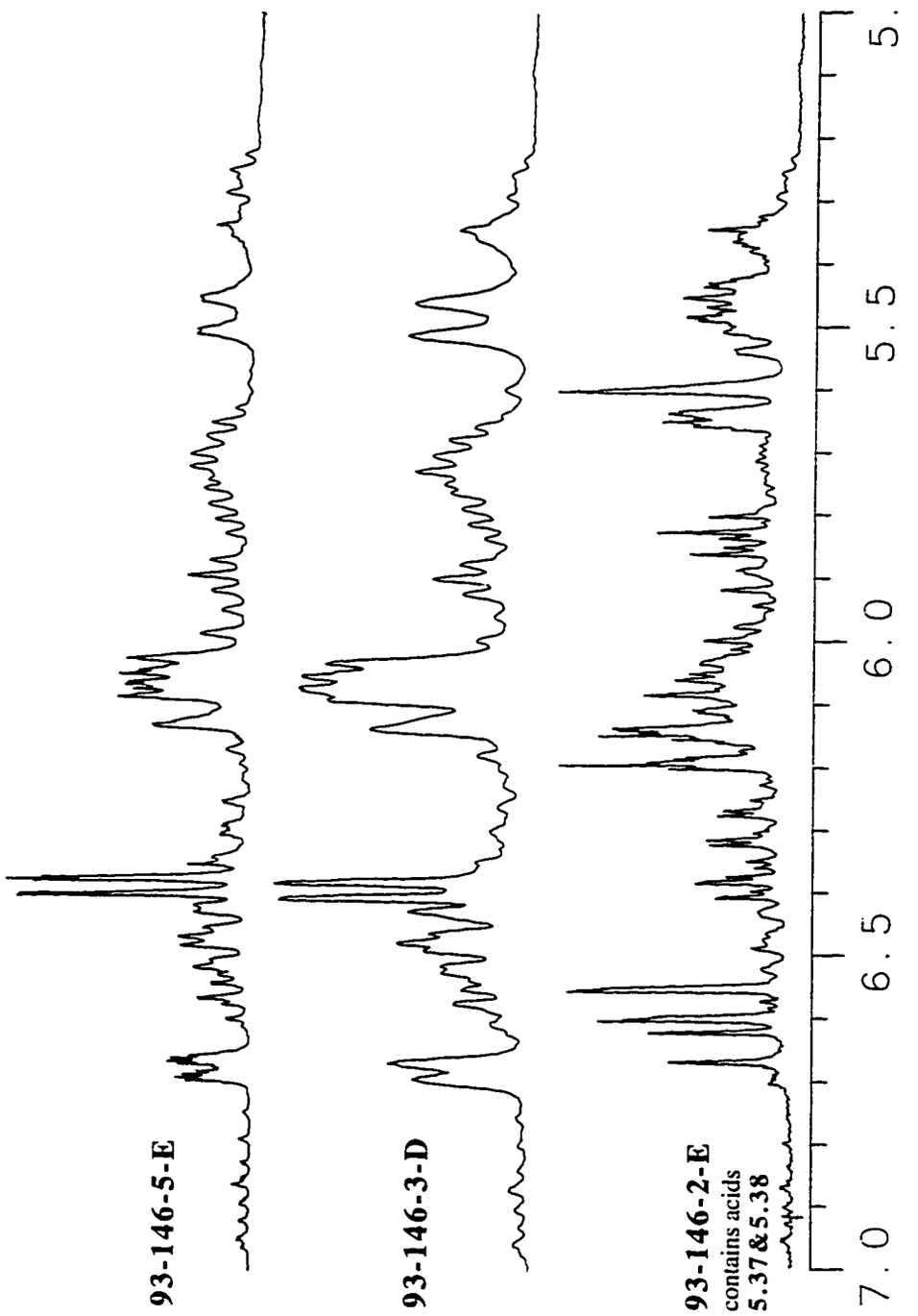


Figure 5.6 Comparison of three ^{1}H NMR spectra of crude extracts from the three morphologically identical sponges, *Xestospongia* sp.

5.4 Isolation and Characterization of C_{14} Acid 5.39

Following a similiar extraction procedure, the crude chlorinated solvent extract was obtained. Analysis of the ^1H NMR spectrum showed a mixture of fatty acids. The extract was chromatographed on silica gel with a gradient of 5% to 100% EtOAc/*n*-hexane to provide six fractions. The fifth fraction (93-146-5-E) was passed through a short C18 reversed phase cartridge with a gradient of 60% to 100% MeOH/H_2O. For better separation, the major fraction was treated with diazomethane to convert the mixture of acids to their methyl esters. This material was purified by reversed phase HPLC (87% MeOH/H_2O) to provide the new C_{14} acid **5.39** (0.0099% dry weight) as its methyl ester (Fig. 5.7).

The HRCIMS provided a molecular formula of $C_{15}H_{22}Br_2O_2$ ($M + NH_4^+$ at *m/z* 410.0310) for **5.39**. Initial analysis of the ^1H NMR spectrum showed a downfield triplet (6.40 ppm) suggesting a terminal dibromovinylidene group. The ^{13}C NMR, UV, and COSY experiments indicated a conjugated diene. No carbon-carbon triple bonds were seen in either the ^{13}C NMR or IR spectra. An HMQC experiment correlated the protons with their respective carbons and the COSY experiment provided three partial structures **5.39a-c** (Fig. 5.8). Due to overlapping upfield signals in the ^1H NMR spectrum, an HMBC experiment was necessary in order to assemble the partial structures. Four cross peaks, C1, H2 and C1, H3 and C4, H3 and C5, H3 pieced partial structures **5.39b** and **5.39c** together with three methylenes (Fig. 5.9). The remaining five methylenes attached partial structures **5.39a** to **5.39b**.

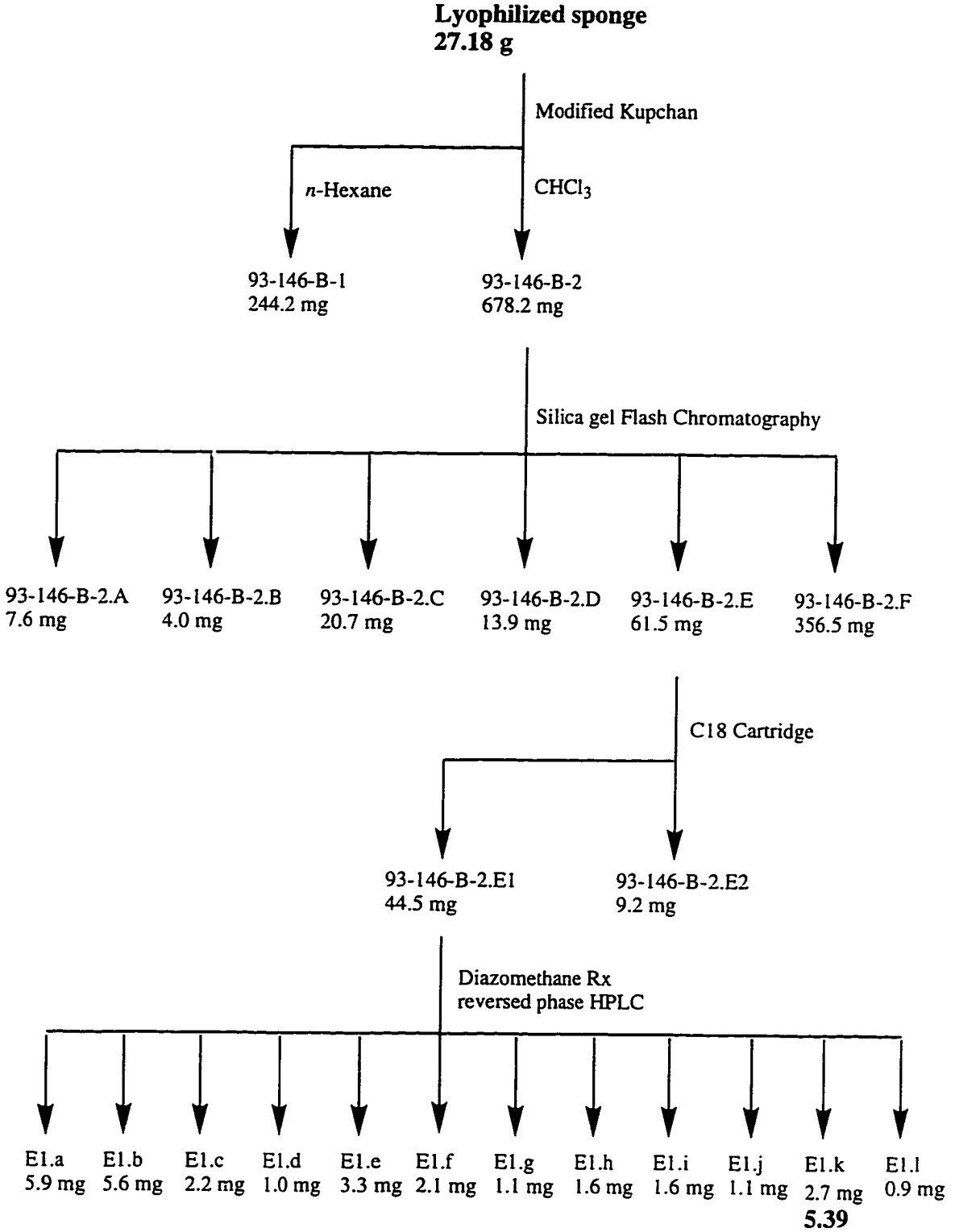
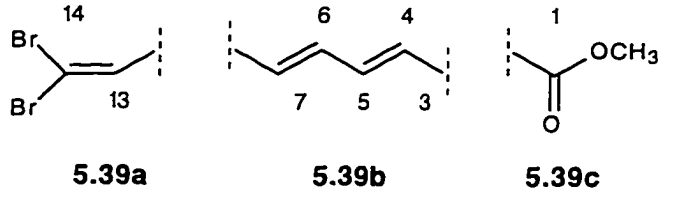


Figure 5.7 Isolation Flowchart of C_{14} methyl ester **5.39.**



5.39a **5.39b** **5.39c**

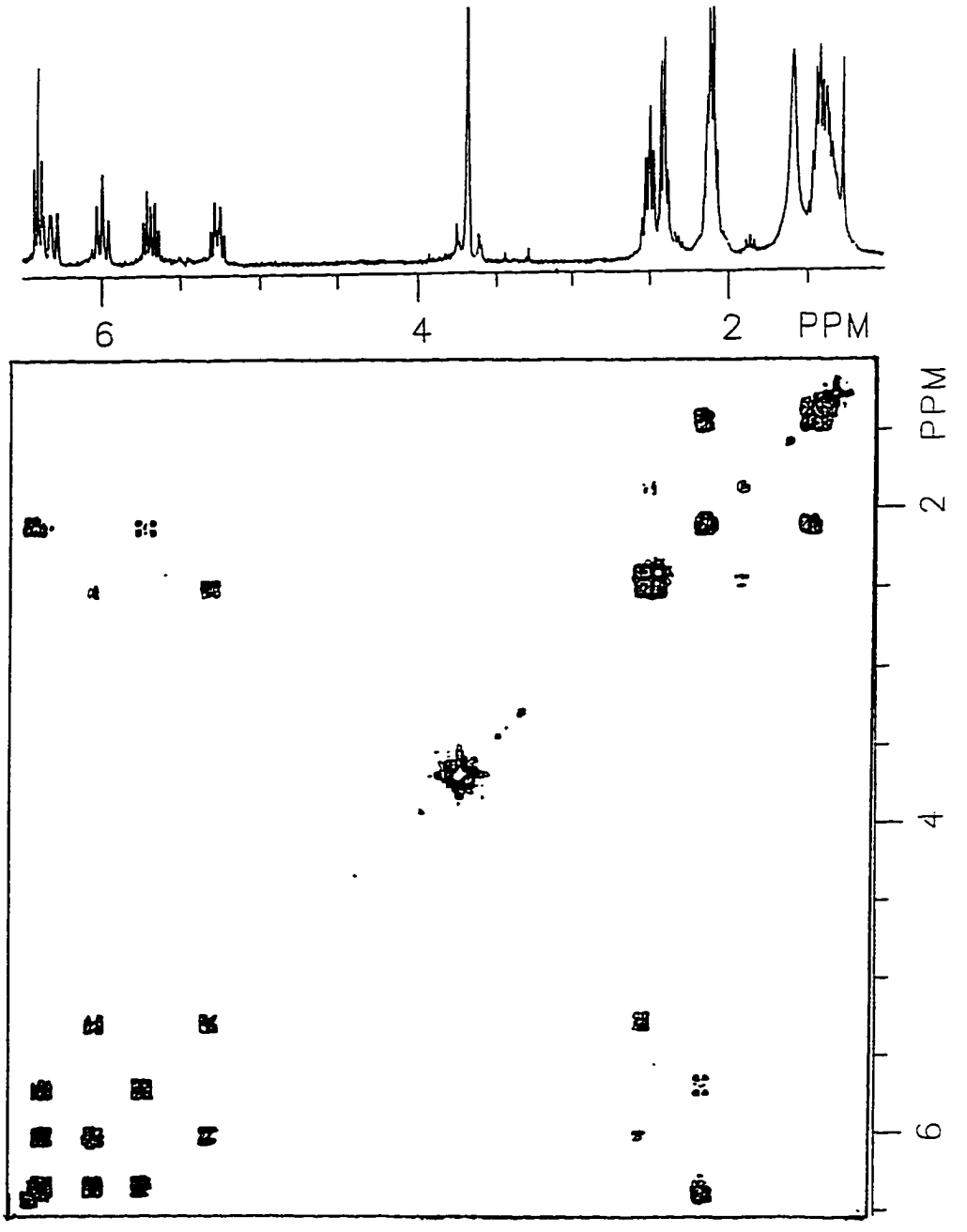
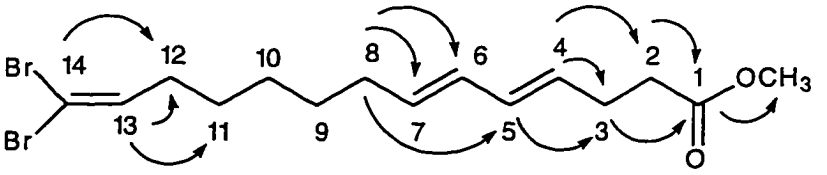


Figure 5.8 Elucidation of partial structures **5.39a-c** from ^{1}H and COSY NMR spectra.



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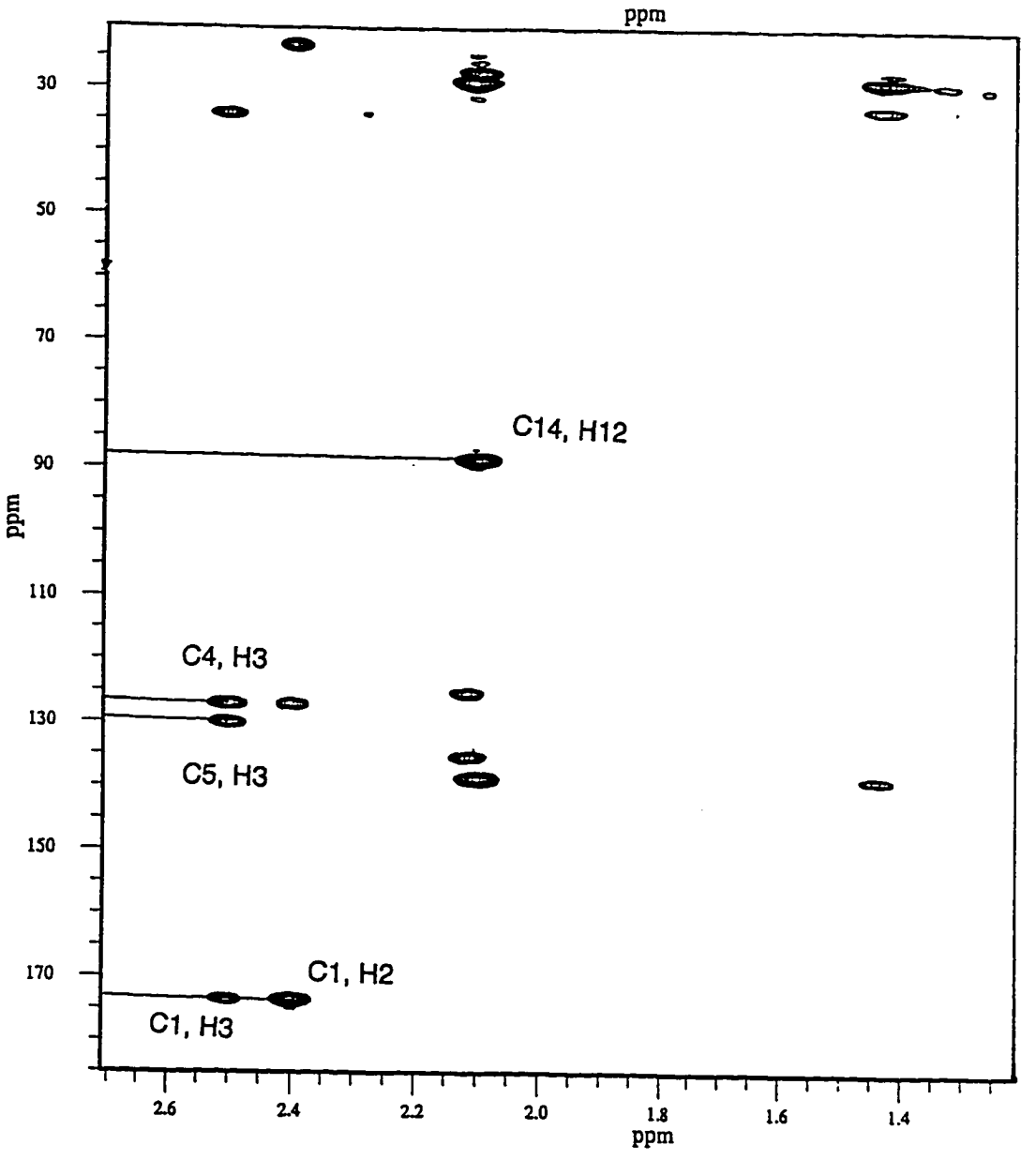
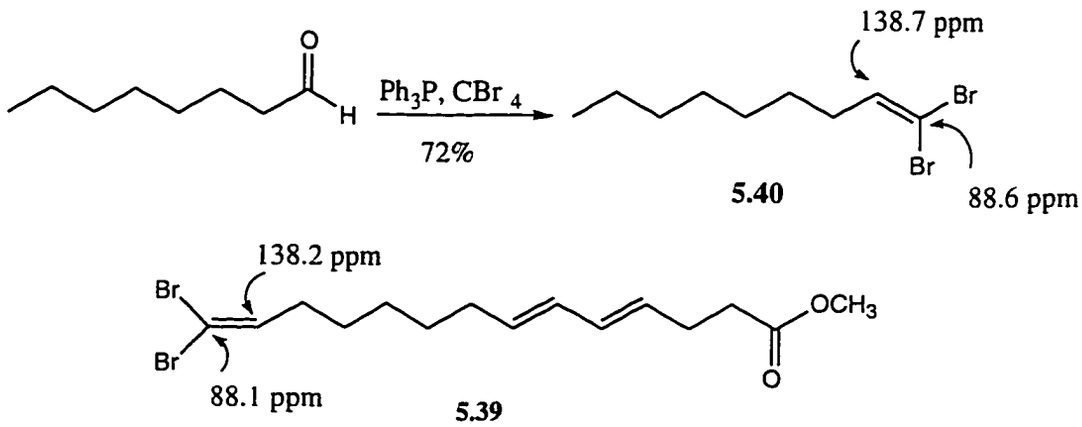


Figure 5.9 Partial HMBC spectrum and for acid **5.39** with HMBC correlations.

5.4.1 Synthesis of 1,1-Dibromo-1-nonene 5.40

The exceptionally high field ^{13}C signal observed for C14 (88.1 ppm) is in disagreement with the C9 assignment (118.8 ppm) for the known 9,9-dibromo-8-nonenoic acid (**5.36**).[9] 1,1-Dibromo-1-nonene, **5.40**, was synthesized in a Wittig-type reaction from *n*-octanal with Ph_3P and CBr_4 in 72% yield (Scheme 5.1).[19-22] The ^{13}C NMR data of the sp^2 carbons in **5.40** (C1 88.6 ppm, C2 138.7ppm) were in agreement with the assignments for **5.39** (C14 88.1 ppm, C13 138.2). The high field shift for C14 is due to the 'heavy atom' effect caused by substitution with two bromine atoms.[23]



Scheme 5.1 Synthesis of 1,1-dibromo-1-nonene and comparison of ^{13}C NMR data to **5.39.**

5.5 Bacteria Association

The three samples of *Xestospongia* sp. were analyzed by Preston and DeLong, collaborators from the University of California Santa Barbara, for bacterial content. The relative proportion of eubacterial and eukaryotic ribosomal ribonucleic acid (rRNA) in the sponge was determined by measuring the proportion of each radiolabeled group-specific oligonucleotide bound to total rRNA extracted from the sponge.[24-26] The eubacterial rRNA accounted for an average of 46% of the total sponge rRNA. Nucleic acid sequence analysis of the sponge-associated eubacterial rRNAs indicated the presence of several bacterial phyla.[14,27]

5.6 Variability in Chemical Composition

Why did only one of the sponges contain the C_{20} acids, where as the other two sponges did not? Garson and co-workers looked into the distribution of brominated phospholipid fatty acids from the sponge *Amphimedon terpenensis* and reported that factors such as seasons, geography, and environment may influence the observed fatty acid composition.[28] Symbiotic associations may also affect the acid profile.

It is reasonable to hypothesize that the bacterial symbionts associated with the three samples of *Xestopongia* sp. sponges are reponsible for the variability in chemical composition. This is particularly valid since the animals are morphologically identical and were collected at the same site during the same day. However, experiments identifing the source for the production of the brominated acids **5.37-5.39**, i.e. the bacteria or sponge cells, is necessary in order to substantiate this hypothesis.

5.7 References

(1) Garson, M. J.; Zimmermann, M. P.; Hoberg, M.; Larsen, R. M.; Battershill, C. N.; Murphy, P. T. *Lipids* **1993**, *28*, 1011.

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5.8 Experimental

General. ^{1}H NMR spectra were recorded at 300 MHz or 500 MHz and ^{13}C NMR were recorded at 75 MHz or 125 MHz. Signals were referenced to TMS, $\delta = 0.00$ ppm (^{1}H) or solvent signal (^{13}C; CD_3OD, $\delta = 49.00$ ppm, $CDCl_3$, $\delta = 77.00$ ppm). ^{13}C multiplicities were determined by DEPT or HMQC experiments. Other general procedures can be found in Chapter 2.

Collection and Extraction of Xestospongia bergquistia; Isolation of 5.37, 5.38, and 5.12. The sponge was collected from Bennett Shoal, Exmouth Gulf, Western Australia (-5m; 22° 06' S 114° 08' E), in January 1993 and frozen immediately. The sponge has an erect conical form (ca. 20 cm diameter) with a tan-pink ectoderm. A portion of the sponge (268 g) was freeze-dried, covered with methanol (2 L), and stored overnight. The solvent was filtered and the sponge was extracted one more time with methanol in the same manner. The combined extracts were concentrated to 1 liter and separated using a modified Kupchan partition[17] as follows. The water content (% v/v) of the MeOH extract was adjusted prior to sequential partitioning against *n*-hexane (10% v/v), CCl_4 (20% v/v), and $CHCl_3$ (40% v/v). The CCl_4 and $CHCl_3$ extracts were combined (3.94 g) and separated by vacuum flash chromatography using TLC grade silica gel H (5-40 μm) and 5-100% EtOAc / *n*-hexane solvent system. The acids eluted with 50% - 100% EtOAc / n-hexane. Further purification by reversed phase HPLC (Dynamax -60A C18; 85% MeOH / H_2O) gave two new C_{20} acids **5.37** (0.0015% dry weight) and **5.38** (0.0027% dry weight), in addition to the known C_{16} acid **5.12** (0.0013% dry weight).

(5*E*, 11*E*, 15*E*, 19*E*) 20-Bromoeicosa-5,11,15,19-tetraene-9,17-diynoic acid (5.37): $C_{20}H_{23}O_2Br$; amorphous powder, UV (MeOH) 270 (27500),

288 nm (ε 22200); IR (NaCl) 2214 (w), 2190 (w), 1697 cm^{-1}; ^1H NMR (10:1 $CDCl_3$: CD_3OD, .01% TMS) δ 6.66 (d, 1 H, *J* = 14 Hz, H20), 6.31 (dd, 1 H, *J* = 14, 2.24 Hz, H19), 6.17 (dt, 1 H, *J* = 15.9, 6.7 Hz, H15), 6.02 (dt, 1 H, *J* = 15.8, 6.6 Hz, H12), 5.58 (dd, 1 H, *J* = 15.9, 1.7 Hz, H16), 5.48 (m, 3 H, H5, 6, 11), 2.31 (m, 2 H, H8), 2.22 (m, 8 H, H2, 7, 13, 14), 2.06 (m, 2 H, H4), 1.68 (quin., 2 H, *J* = 7.4 Hz, H3); ^{13}C NMR (10:1 $CDCl_3$: CD_3OD, .01% TMS) δ 177.2 (s, C1), 144.5 (d, C15), 141.4 (d, C12), 130.8 (d, C5), 129.5 (d, C6), 117.8 (d, C19, 20), 111.1 (d, C11), 110.1 (d, C16), 90.5 (s, C17), 89.0 (s, C9), 85.0 (s, C18), 79.4 (s, C10), 33.9 (t, C2), 32.6 (t, C7 or 4), 32.1(t, C13, 14, 4 or 7), 25.0 (t, C3), 19.9 (t, C8); HRCIMS (NH_3) obsd *m / z* 392.1218 ($M+NH_4$), $C_{20}H_{27}BrNO_2$ requires 392.1221.

(5*Z*, 11*E*, 15*E*, 19*E*) 6,20-Dibromoeicosa-5,11,15,19-tetraene-9,17-diynoic acid (5.38): $C_{20}H_{22}O_2Br_2$; amorphous powder; UV (MeOH) 271 (1670), 286 nm (ε 1399); IR (NaCl) 2214 (w), 2187 (w), 1716 cm^{-1}; ^1H NMR (10:1 $CDCl_3$: CD_3OD, .01% TMS) δ 6.67 (d, 1 H, *J* = 14.0 Hz, H20), 6.31 (dd, 1 H, *J* = 14.0, 2.3 Hz, H19), 6.16 (dt, 1 H, *J* = 15.9, 6.8 Hz, H15), 6.01 (dt, 1 H, *J* = 15.8, 6.6 Hz, H12), 5.93 (t, 1 H, *J* = 7.7 Hz, H5), 5.58 (dd, 1 H, *J* = 15.9, 1.9 Hz, H16), 5.47 (br d, 1 H, *J* = 15.8 Hz, H11), 2.64 (m, 2H, H7), 2.48 (td, 2 H, *J* = 7.3, 2.0 Hz, H8), 2.30 (t, 2 H, *J* = 7.5 Hz, H2), 2.16 (m, 4H, H13, 14), 2.13 (q, 2 H, *J* = 7.5 Hz, H4), 1.71 (quin., 2 H, *J* = 7.5 Hz, H3); ^{13}C NMR (10:1 $CDCl_3$: CD_3OD, .01% TMS) δ 176.5 (s), 144.4 (d), 141.4 (d), 133.2(d), 124.3 (s), 117.9 (d), 117.8 (d), 110.9 (d), 110.2 (d), 90.5 (s), 87.4 (s), 85.0 (s), 79.8 (s), 35.1 (t), 33.8 (t), 32.5 (t), 32.0 (t), 29.2 (t), 24.7 (t), 18.8 (t); LRCIMS (NH_3) obsd *m / z* for the methyl ester 484/486/488 ($M + NH_4^+$). $C_{21}H_{28}Br_2NO_2$.

16-Bromo-(7*E*, 15*E*)-hexadeca-7,15-dien-5,13-diynoic acid (5.12)[9]: $C_{16}H_{19}O_2Br$; amorphous powder; IR (NaCl) 2215 (w), 1700 cm^{-1}; ^1H NMR (10:1 $CDCl_3$: CD_3OD, .01% TMS) δ 6.58 (d, 1 H, *J* = 14.0 Hz), 6.18 (dt, 1 H, *J* = 14.0, 2.3 Hz), 6.04 (dt, 1 H, *J* = 15.8, 7.0 Hz), 5.46 (dt, 1 H, J = 15.8, 1.7 Hz), 2.43 (t, 2H, *J* =

7.5 Hz), 2.38 (td, 2H, J = 7.0, 1.7 Hz), 2.27 (td, 2H, J = 6.7, 2.3 Hz), 2.11 (qd, 2H, J = 7.0, 1.0 Hz), 1.83 (sex., 2H, J = 7.5 Hz), 1.52 (m, 4H); ^{13}C NMR (10:1 $CDCl_3$: CD_3OD, .01% TMS) δ 143.0 (d), 118.1 (d), 117.2 (d), 110.4 (d), 92.2 (s), 87.8 (s), 80.0 (s), 33.6 (t), 32.5 (t), 28.1 (t), 27.9 (t), 24.4 (t), 19.4 (t), 19.0 (t). C1 signal below level of detection. C14 obscured by $CDCl_3$.

Methyl (*Z*,*E*)-14,14-dibromo-4,6,13-tetradecatrienoate (5.39): A second specimen of *Xestospongia* sp. was extracted as follows. The sponge (27.18 g) was lyophilized and extracted twice with methanol (2 X 500 ml; first for 4 hours and secondly, 16 hours). The combined extracts were evaporated to one third volume and solvent partitioned as described above. The $CHCl_3$ soluble extract (672.8 mg) was flash chromatographed over silica with a gradient solvent system of 5-100% EtOAc/*n*-hexane. The fifth fraction (61.5 mg) was placed through a short C_{18} cartridge (Varian Mega Bond Elute, 60-100% MeOH/ H_2O.) and the eluate (44.5 mg) was treated with diazomethane to give a mixture of methyl esters. This material was separated by reversed phase HPLC (Dynamax C_{18}, 87% MeOH/H_2O) to give pure **5.39** (2.7 mg, 0.0099% of dry weight) as a colorless oil. **5.39**: $C_{15}H_{22}O_2Br_2$; oil; UV (MeOH) 231 nm (ε 7915); IR (NaCl) 1739, 1437, 1169 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.40 (t, 1H, J = 7 Hz, H13), 6.33 (dd, 1H, J = 14.3, 11.5 Hz, H6), 6.02 (t, 1H, J = 10.8 Hz, H5), 5.70 (dt, 1H, J = 15.4, 7.4 Hz, H7), 5.26 (dt, 1H, J = 11.5, 7.4 Hz, H4), 3.72 (s, 3H, OCH_3), 2.51 (bt, 2H, J = 7.6 Hz, H3), 2.45 (bt, 2H, H2), 2.15 (m, 4H, H8, H12), 1.4 (m, 3H, H9-11); ^{13}C NMR ($CDCl_3$) δ 173.8 (C1), 138.2 (C13), 134.9 (C7), 129.4 (C5), 126.6 (C4), 124.9 (C6), 88.1, (C14), 51.0 (OCH_3), 33.6 (C2), 32..2-32.4 (C8, C12), 28.1-28.5 (C9, C11), 27.1 (C10), 22.7 (C3); HRCIMS (NH_3) obsd m/z 410.0310 ($M+NH_4$), $C_{15}H_{25}Br_2NO_2$ requires 410.0330.

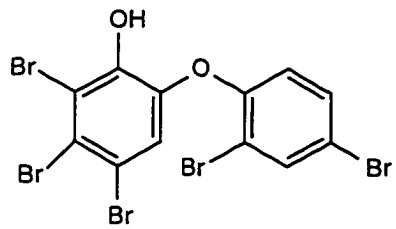
1,1-Dibromo-1-nonene (5.40): n-Octanal (2.16g, 17 mmol) was added to a stirred mixture of triphenylphosphine (9.8 g, 37 mmol) and carbon tetrabromide (6.4 g, 19 mmol) in CH_2Cl_2 (55 mL) at 0° according to Seebach *et al.*[22] and allowed to warm to room temperature with stirring (30 h). The crude yellow oil obtained after work-up was purified by silica flash chromatography (1:1 hexane/EtOAc) to give pure *1,1-Dibromo-1-nonene* (2.16 g, 72%). ^1H NMR spectrum identical to that in literature.[22] ^{13}C NMR ($CDCl_3$) 138.7 (d, C2), 88.6 (s, C1), 33.0 (t), 31.8 (t), 29.0 (t), 27.8 (t), 22.7 (t), 14.1 (q, C9).

Chapter Six

Synthesis of Chlorinated Amino Nitrile Diastereomers: A Strategy Toward the Synthesis of Labeled Leucine

6.1 Introduction: The Marine Sponge *Dysidea herbacea*

Dysidea herbacea, from the order Dictyoceratida and family Dysideidae, is a thinly-encrusting, blue to green marine sponge found primarily in shallow tropical waters of the Indo-Pacific. Chemical investigations of this sponge have shown the presence of diverse secondary metabolites belonging to three unrelated structrual classes. The first series of compounds, polybrominated diphenyl ethers,[1-3] are exemplified by the pentabromophenol (**6.1**)[4] and the dimethoxy derivative (**6.2**).[5] Sesquiterpene derivatives,[6-9] such as spirodysin (**6.3**)[10] and the furanosesquiterpene acetate (**6.4**),[11] represent the second group of compounds. The third class of secondary metabolites isolated from *Dysidea herbacea* are unique amino acid derivatives (**6.5-6.24**) notable for their trichloromethyl functionality, which has not been found elsewhere in nature.[12-21] Due to this unusual trichloromethyl moiety, these metabolites have received considerable attention, including a preliminary study of their biosynthesis.



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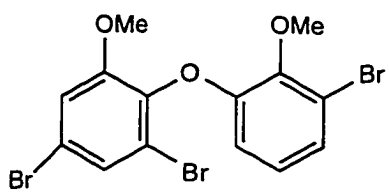
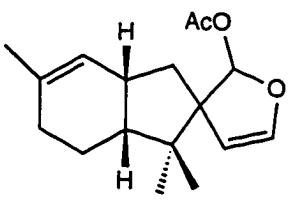


6.2



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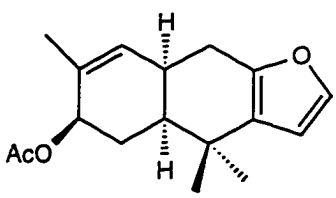

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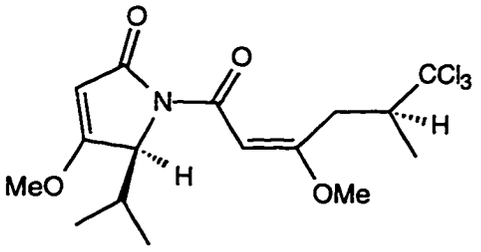
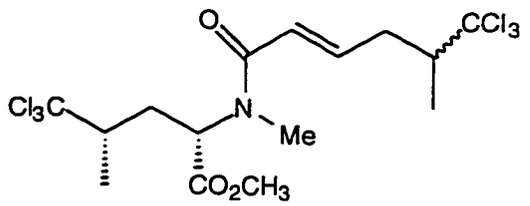


6.5



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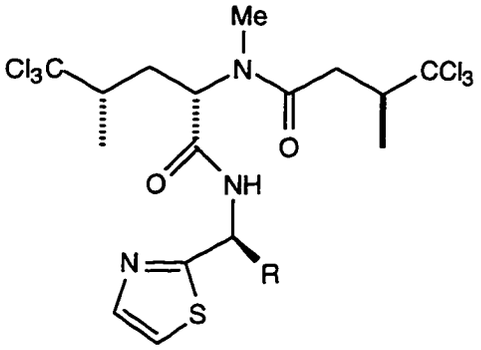
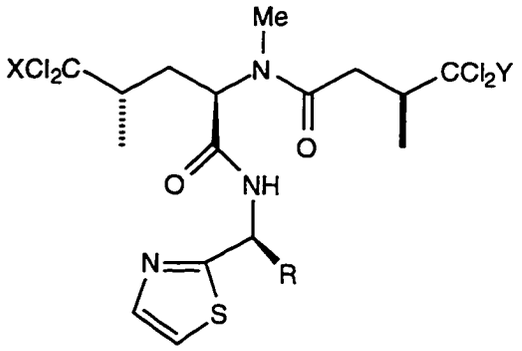
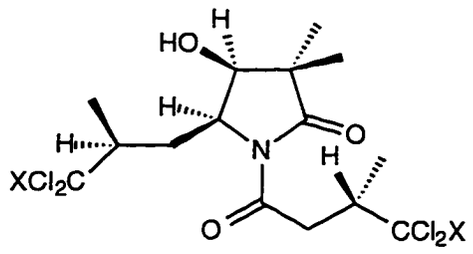


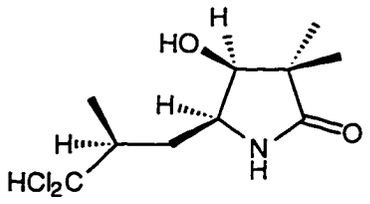
6.7: R = Me
6.8: R = H



	R	X	Y
6. 9:	**Me**	**Cl**	**Cl**
6.10:	**H**	**Cl**	**Cl**
6.11:	**H**	**Cl**	**H**
6.12:	**H**	**H**	**Cl**



6.13: X = Cl
6.14: X = H



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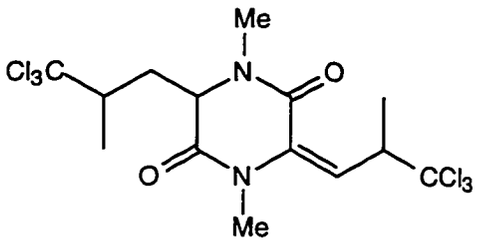

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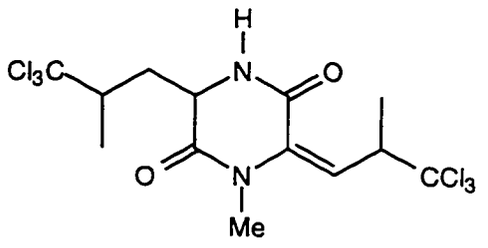

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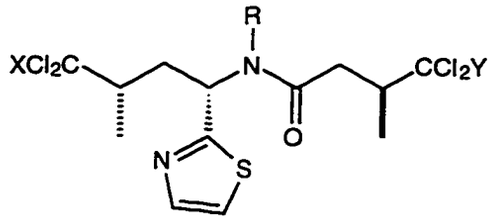
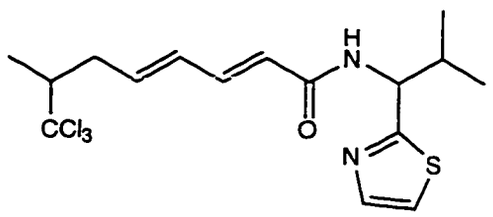
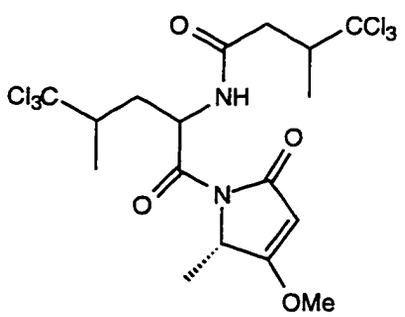


	R	X	Y
6.18:	H	Cl	Cl
6.19:	Me	Cl	Cl
6.20:	Me	Cl	H
6.21:	H	Cl	H
6.22:	Me	H	H



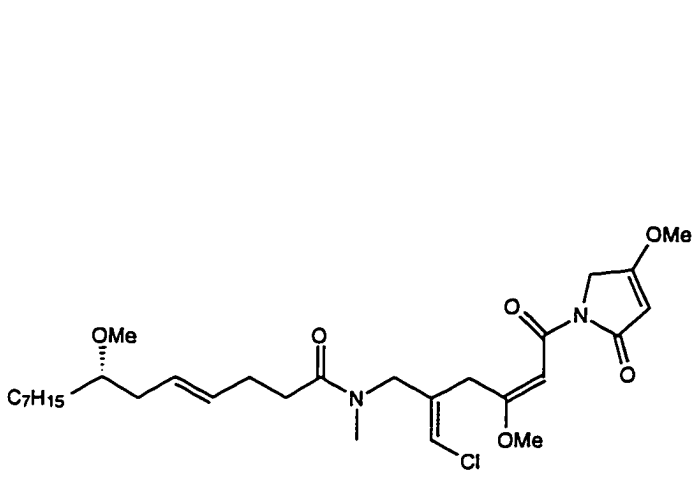
6.23



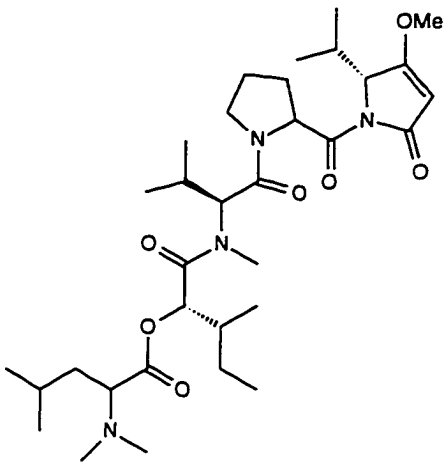
6.24

6.1.1 Biosynthetic Studies of Polychlorinated Amino Acid Metabolites

Dysidea herbacea has been shown to contain the symbiotic filamentous cyanobacterium *Oscillatoria spongeliae*.[22] This blue-green algae occurs extracellularly within the sponge's mesohyl and can represent up to 50% of the tissue volume. It has generally been accepted that the terpenoid derivatives were the true metabolites from *Dysidea herbacea* since other terpenes have been isolated from species of the genus *Dysidea*.[23] The polychlorinated metabolites, on the other hand, were thought to be produced by the cyanobacteria symbiont because of the structural similarity of **6.5** with malyngamide A (**6.25**) from the free-living cyanobacterium *Lyngbya majuscula* and mirabimide A (**6.26**) from a terrestrial bacterium *Scytonema mirabile*.[19] Unson and Faulkner have experimentally proven these assumptions to be accurate by analysis of derivatives from flow-cytometric separated symbiont and sponge cells.[19,24] The polybrominated diphenyl ethers have also been shown to be produced by the cyanobacteria symbiot. [25]

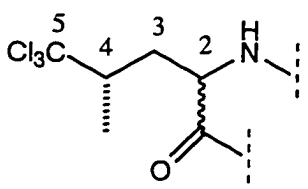


6.26



6.25

With the source of the polychlorinated metabolites established, the biosynthetic precursors were studied. Incorporation of radiolabeled leucine, valine, and alanine into intact sponge tissue confirmed the amino acid origin.[25] It is not surprising that leucine is a precursor since most of the polychlorinated compounds contain a trichloroleucine moiety (**6.27**). The absolute stereochemistry of the trichloroleucine functionality (**6.27**) is always 4S and either 2R or 2S at the α carbon.



6.27

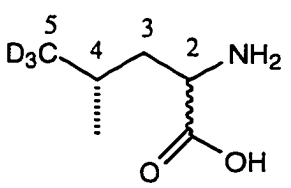
The trichloromethyl functionality in the polychlorinated metabolites is unique in halogenated natural products. The mechanism of this structural enigma does not follow the typical mechanism known for halogenation involving the enzyme haloperoxidase. Haloperoxidase produces an electrophilic halogen which is attacked by a nucleophile in an

electrophilic substitution mechanism.[26,27] In the case of the polychlorinated compounds, there is no apparent way for the corresponding methyl group to be nucleophilic.

Several assumptions can be made about the chlorination step in the biosynthesis of the polychlorinated amino acids. From analysis of compounds **6.9-6.14** and **6.18-6.22**, the mechanism for chlorination seems to be stepwise, adding one chlorine at a time to the appropriate methyl group. This mechanism is likely to be enzyme directed since all the polychlorinated amino acids have the same stereochemistry about the trichloromethyl stereocenters (S stereoconfiguration, see above). Addition of chlorines also appears to be one of the final steps in the biosynthesis sequence.

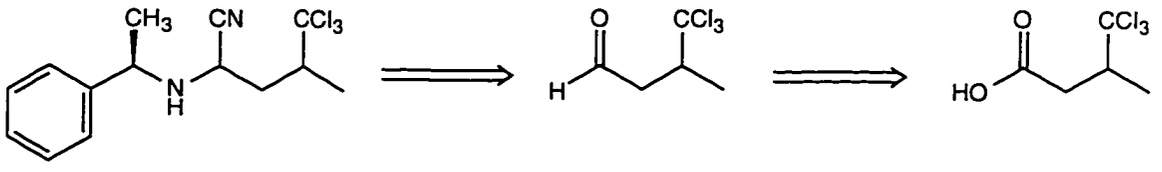
6.1.2 Dissertation Goal: Syntheses of Chlorinated Amino Nitrile Diastereomers

In order to investigate the biosynthetic chlorination step, specifically labeled amino acids are necessary. The stereoselective synthesis of labeled leucine such as (2R,4S)-5,5,5-trideuteroleucine (**6.28**) is particularly difficult. The work described in this Chapter involves syntheses of chlorinated amino nitrile diastereomers (**6.29a-d**), key intermediates toward the synthesis of labeled leucine. These diastereomers solve the synthetic problem of stereospecific labeling of carbon-5 with deuterium atoms. From these chlorinated amino nitriles, labeled leucine derivatives such as **6.28** could theoretically be made following literature procedures.



6.28

Retrosynthetically, the amino nitriles **6.29a-d** were to be approached from an asymmetric Strecker synthesis involving the appropriate aldehyde (**6.30**) and a chiral amine. The aldehyde was readily available from 3-methyl-4,4,4-trichlorobutanoic acid (**6.31**) (Scheme 6.1). Although the syntheses of racemic, (+)- and (-)-**6.31** have been achieved previously,[28-31] this work represents novel syntheses of (+)- and (-)-**6.31**.

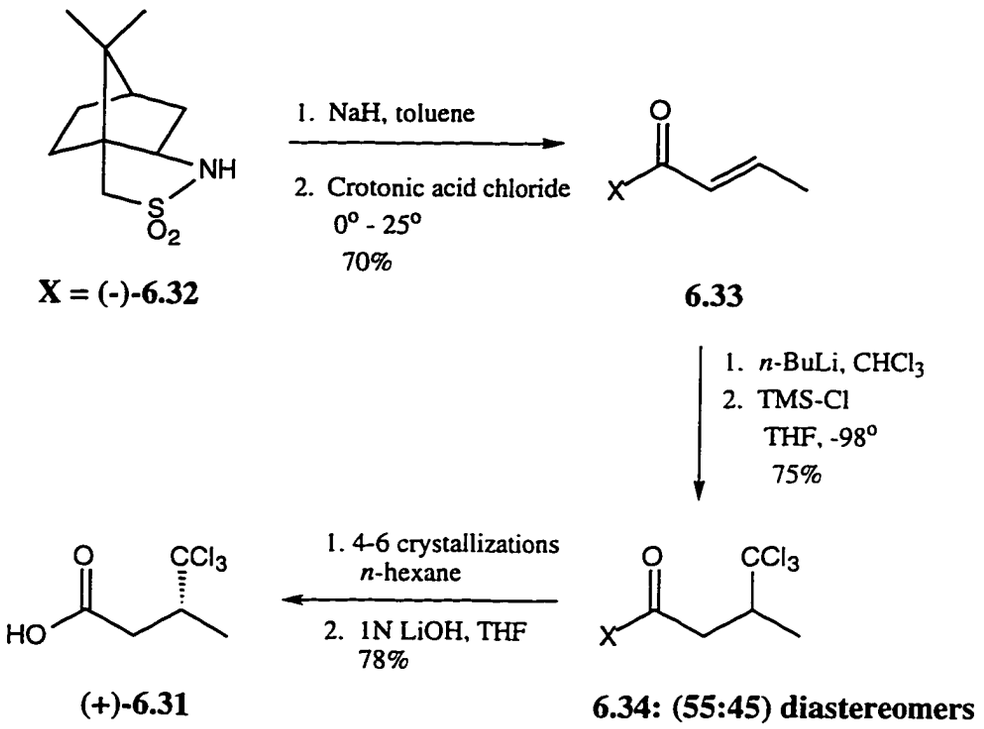


6.29 **6.30** **6.31**

Scheme 6.1 Retrosynthesis of chlorinated amino nitrile diastereomers.

6.2 Synthesis of (+)-(3R)-Methyl-4,4,4-Trichlorobutanoic Acid

The novel synthesis of (+)-**6.31** was achieved using Oppolzer's camphor sultam (**6.32**)[32] as a chiral auxillary in a Michael addition reaction with the trichloromethyl anion (CCl_3^-). *N*-Acylation of Oppolzer's camphor sultam, (-)-(2R)-borane-10,2-sultam (**6.32**), by successive treatment with NaH and crotonic acid chloride provided *N*-crotonoyl sultam (**6.33**)[33 34]in 70% yield. The Michael addition reaction between **6.33** and the nucleophile CCl_3^-, formed *in situ* with $CHCl_3$ and *n*-BuLi, yielded the Michael product **6.34** (Scheme 6.2).



Scheme 6.2 Synthesis of **(+)-6.31** from Oppolzer's camphor sultam **(-)-6.32**.

Table 6.1 summarizes the optimization of the Michael addition reaction. First, in order to prepare the CCl_3^-, the order of addition of $CHCl_3$ to *n*-BuLi is important (entry 3). Second, evidence (entries 1, 3, 4, 6) suggests that the reaction is an equilibrium, involving **6.33** and the corresponding enolate, which disfavors formation of product **6.34**. Regardless of the number of mole equiv. used of $CHCl_3$ and *n*-BuLi, the yield of **6.34** remained approximately the same and starting material was always recovered. Trapping the incipient enolate with TMS-Cl drove the reaction forward, resulting in a yield of 75% **6.34** (entry 7). Despite the good yield, the reaction provided poor diastereoselectivity in a ratio of 55:45. The poor diastereoselectivity was suprising since it was assumed that the camphor auxillary (**6.32**) would direct the attack of the CCl_3^- by providing steric hinderence from its methyl group. Several Lewis acids were used in an attempt to increase the diastereoselectivity, but either a minor increase was observed or the reaction did not proceed (entries 2, 5 and 8).

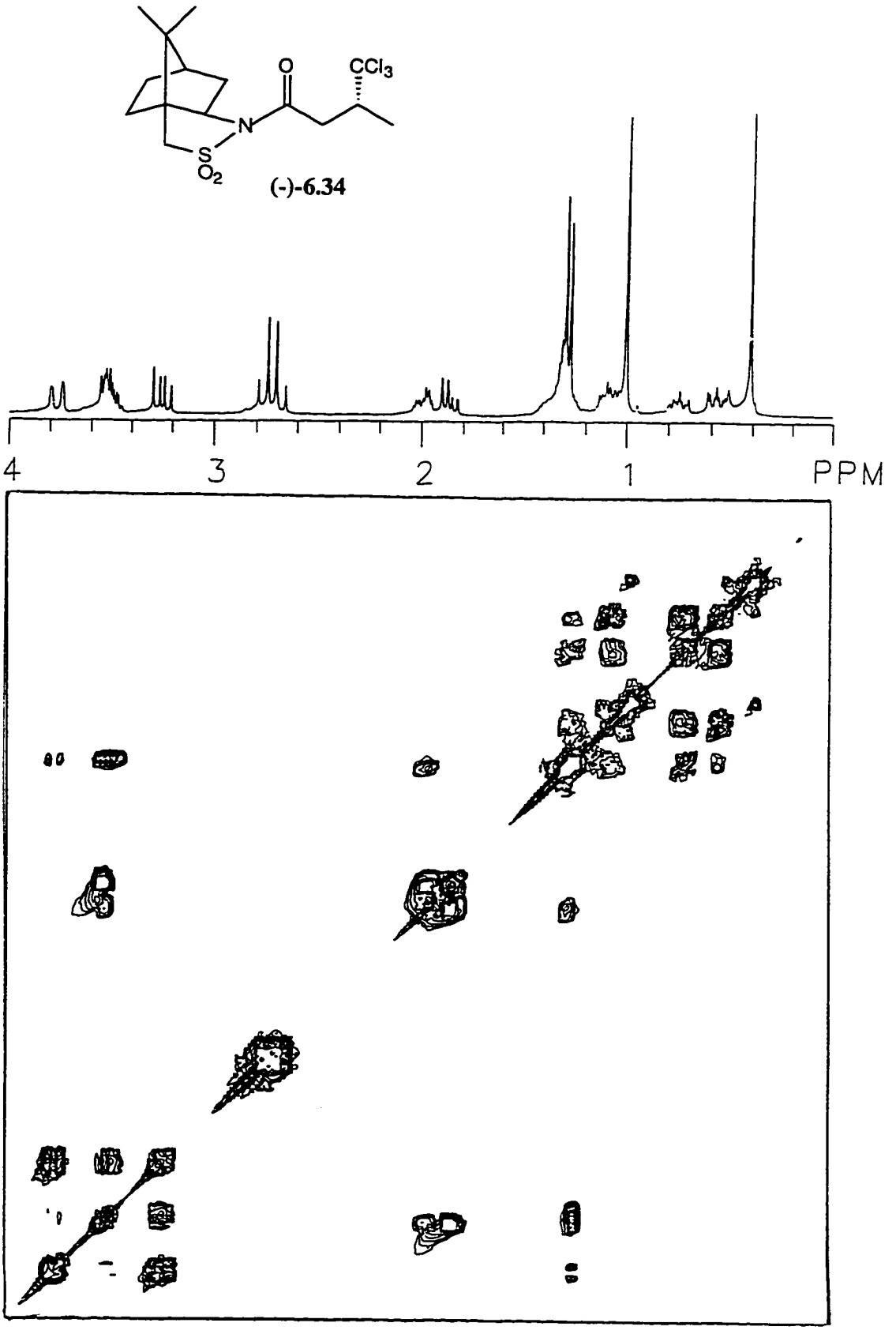
Table 6.1 Michael addition of CCl_3^- to s.ultam **6.33**.*

Entry	$CHCl_3$ (equiv.)	*n*-BuLi (equiv.)	Lewis Acid & additive	% Yield **6.34**	Ratio of Diastereomers
1	1.2	1.1		27%	62:38
†2	1.2	1.1 *sec*-BuLi	$MgCl_2$	20%	70:30
††3	5.0	5.0		< 5%	-------
4	5.0	5.0		22%	55:45
5	5.0	5.0	$ZnCl_2$	no rx	
6	20.0	20.0		25%	-------
7	5.0	5.0	TMS-Cl	75%	55:45
8	5.0	5.0	$TiCl_4$ & TMS-Cl	no rx	-------

* Sultam **6.33** was added to a solution of CCl_3^- at -98°C, prepared by adding $CHCl_3$ to n-BuLi at -98°C in THF.
† *sec*-BuLi was added to a solution of $CHCl_3$ and sultam **6.33** at -98°C in THF.
†† *n*-BuLi was added to $CHCl_3$ (order of addition reversed).

Several recrystallizations of **6.34** with *n*-hexane provided pure diastereomer (-)-**6.34**. The ratio of the diastereomers was monitored by integration of the camphor auxillary (**6.32**) methyl groups in a ^1H NMR (C_6D_6) spectrum. The structure of (-)-**6.34** was confirmed by analysis of ^1H, ^{13}C, DEPT 90 and 135, and COSY NMR experiments and optical rotation $[\alpha]_D$ -40.96° (c = 1.14 $CHCl_3$). See Figure 6.1 for ^1H and COSY NMR spectra.

Hydrolysis of (-)-**6.34** with 1N LiOH provided (+)-(3R)-methyl-4,4,4-trichlorobutanoic acid ((+)-**6.31**) in 78% yield (Scheme 6.2). Comparison with literature values provided the stereochemistry of (+)-**6.31** since the (+)-acid is the R configuration.[17,29,35,36]

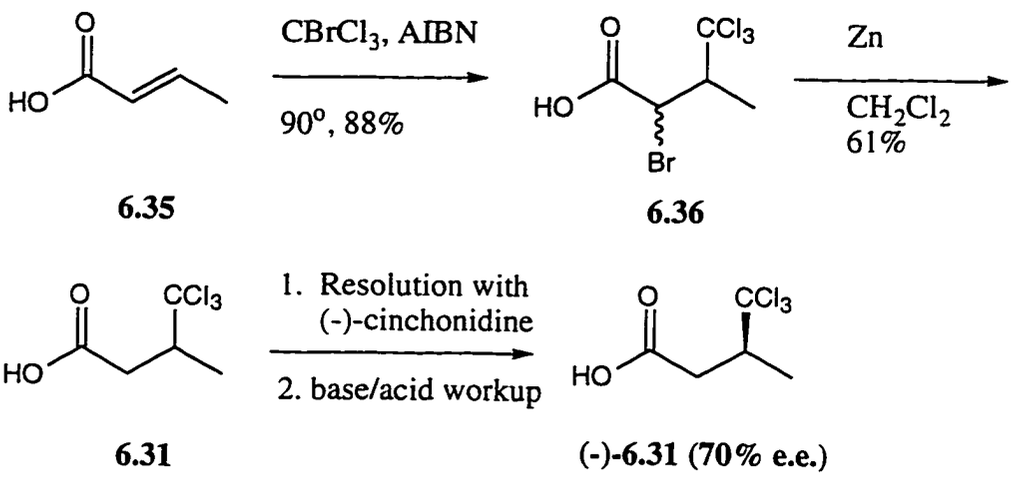


(-)-6.34

Figure 6.1 ^{1}H and COSY NMR spectra for (-)-**6.34**.

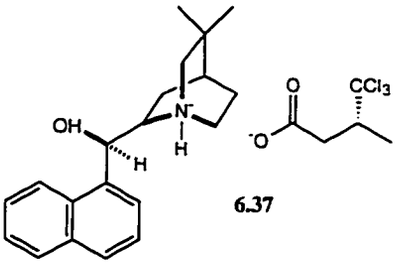
6.2.1 Synthesis of (-)-(3S)-Methyl-4,4,4-Trichlorobutanoic Acid

The synthesis of racemic **6.31** followed the procedure by Williard and deLaszlo.[28] Radical chain addition of bromotrichloromethane ($CBrCl_3$) to crotonic acid (**35**) followed by treatment with activated zinc provided **6.31** in 54% overall yield (Scheme 6.3). The acid, **6.31**, was resolved with the basic amine, (-)-cinchonidine, after several recrystallizations of the mother liquor with $MeOH/H_2O$ to give (-)-**6.31** in 70% enantiomeric excess (e.e.). Resolution of the acid was followed by ^1H NMR spectroscopy of the cinchonidine salt (**6.37**) in 11:2 $CDCl_3/C_6D_6$ (Figure 6.2).



6.35 **6.36** **6.31** **(-)-6.31 (70% e.e.)**

Scheme 6.3 Synthesis of **(-)-6.31** by resolution of racemic acid.



6.37

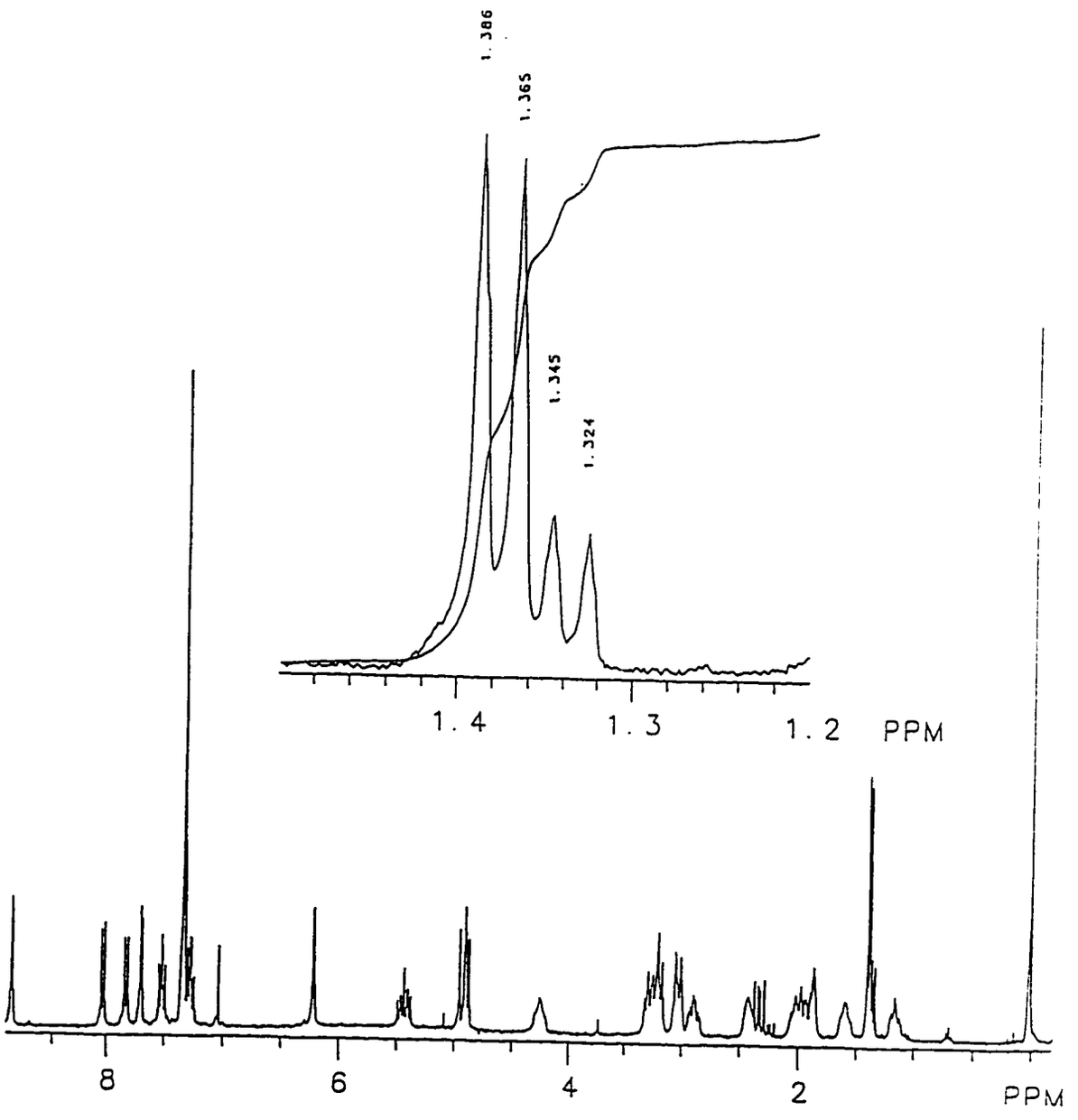
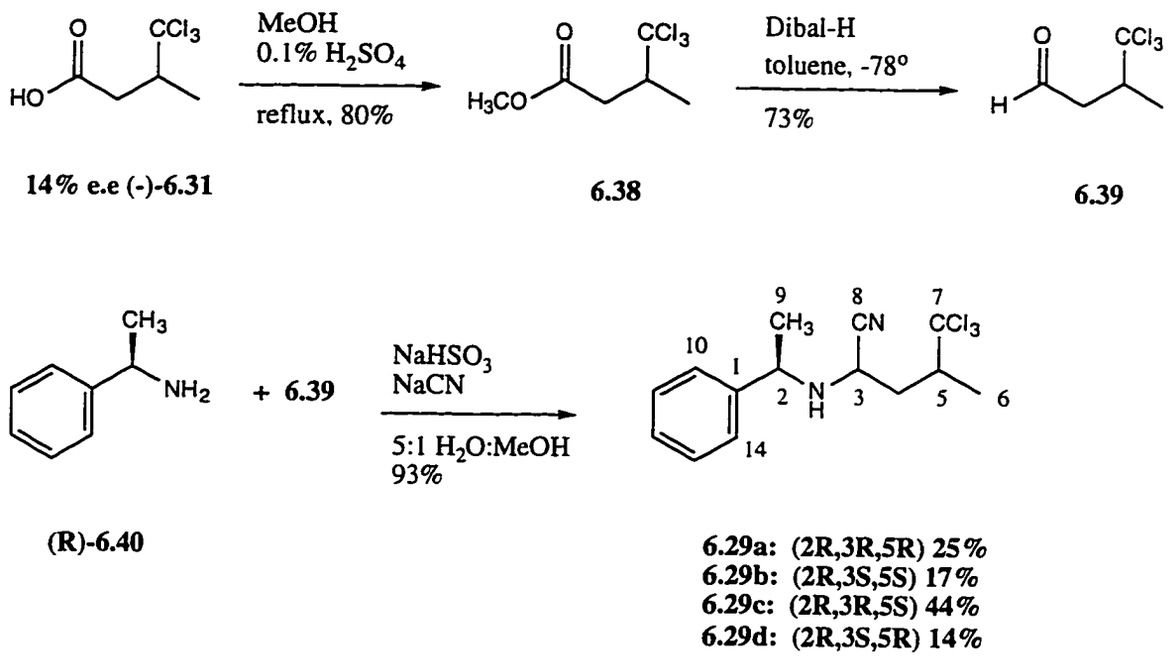


Figure 6.2 ^{1}H NMR spectrum of cinchonidine salt **6.37**. The method used to monitor resolution.

6.3 Synthesis of Chlorinated Amino Nitrile Diastereomers

The synthesis of amino nitrile diastereomers, **6.29a-d**, was performed by the one-pot asymmetric Strecker reaction between an aldehyde, chiral amine and NaCN.[37] The aldehyde (**6.38**) necessary for this reaction was produced by esterification of 14% e.e. (-)-**6.31** followed by reduction with dibal-H (Scheme 6.4). The Strecker reaction of (R)-methylbenzylamine (**6.40**) with **6.38** provided the four chlorinated amino nitrile diastereomers in 93% yield. Separation by HPLC (10 % EtOAc / *n*-hexane) provided a ratio of (25:17:44:14) % for **6.29a-d**, respectively. Since the diastereomers are separable, enantiomerically pure **6.39** is not required.



Scheme 6.4 Synthesis of chlorinated amino nitriles **6.29a-d.**

Each diastereomer was fully characterized. The stereochemical assignments were elucidated through chemical means in conjunction with literature precedent. It is well known that the (R)-methylbenzylamine provides the (R)-amino nitrile and vice versa as the major diastereomer.[38] It is also documented that the αH to the nitrile for the (R)-amino nitrile has a chemical shift further upfield than the corresponding αH in the (S)-amino

nitrile, by approximately 0.5 ppm.[37] From comparison of the proton chemical shifts of 3-H and the percent yields in compounds **6.29a-d**, the amino nitrile pairs, (3R)-**6.29a&c** and (3S)-**6.29b&d** can be deduced (Table 6.2). Table 6.3 compares the ^{13}C NMR data and Figure 6.3 shows the 1H NMR spectra for **6.29a-d**.

Table 6.2 ^{1}H NMR chemical shifts of 3H for **6.29a-d**.

amino nitrile (% yield)	δ H-3	Stereochemistry at 3
6.29a (25%)	3.18	R
6.29b (17%)	3.73	S
6.29c (44%)	3.29	R
6.29d (14%)	3.81	S

Table 6.3 Comparison of ^{13}C NMR data for **6.29a-d**.

C#	**6.29a**	**6.29b**	**6.29c**	**6.29d**
1	142.74	144.19	142.71	144.02
2	56.52	56.04	56.54	56.24
3	45.49	45.67	46.91	46.84
4	36.89	37.00	37.16	37.09
5	50.53	50.83	51.77	51.85
6	15.38	15.87	16.91	16.93
7	105.17	105.06	104.69	104.78
8	119.98	119.73	119.31	119.07
9	24.62	21.79	24.79	22.13
10-14	126.90 127.89 128.74	126.64 127.77 1287.72	126.64 127.70 128.73	126.63 127.78 128.72

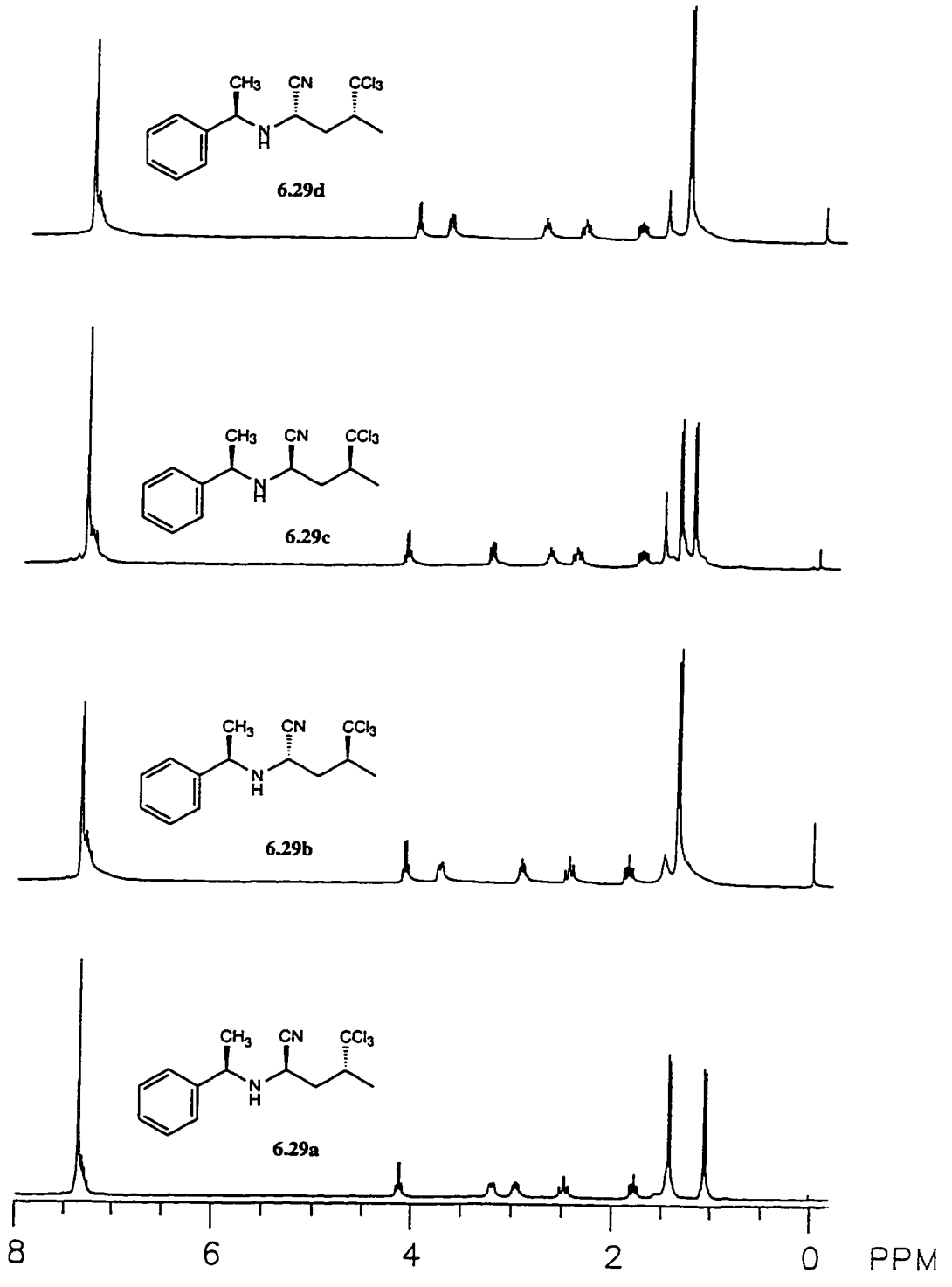
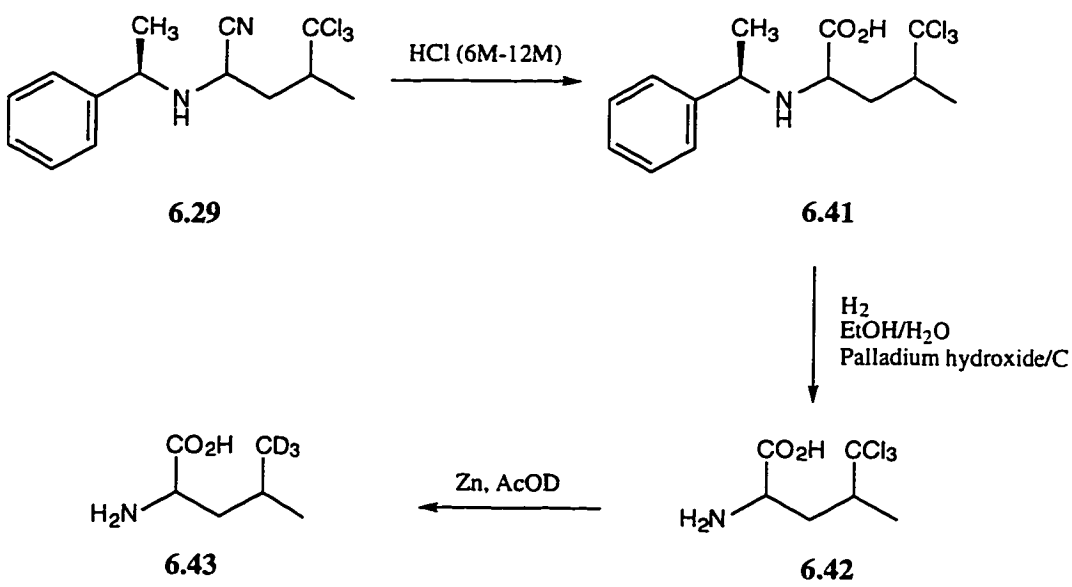


Figure 6.3 Comparison of ^{1}H NMR spectra for **6.29a-d**.

The stereochemistry at the 5 position was determined by asymmetric Strecker reaction with 85% e.e (+)-(R)-**6.31** which produced two diastereomers in a ratio of (1.7:1.0)%. The major diastereomer produced should have the stereochemistry of 2R,3R,5R since the stereocenters at carbon-2 and carbon-5 are fixed. The minor diastereomer, on the other hand, should possess the stereochemistry of 2R,3S,5R. Comparison of ^{1}H and ^{13}C NMR data showed that **6.29a** was the major diastereomer indicating the stereochemistry (**2R,3R,5R**). The minor compound was **6.29d** with the stereochemistry (**2R,3S,5R**). By exclusion, the stereochemisty for the other two diastereomers was deduced to be (**2R,3S,5S**)-**6.29b** and (**2R,3R,5S**)-**6.29c**.

6.4 Literature Precedent for Synthesis of Labeled Leucine

Conversion of the chlorinated amino nitrile diastereomers **6.29a-d** into the corresponding labeled 5,5,5-trideuteroleucine can in principle be accomplished following literature procedures. Scheme 6.5 illustrates this conversion with the acid hydrolysis of the nitrile to the carboxylic acid,[39] removal of the benzyl moiety by hydrogenolysis,[40] followed by reduction of the trichloromethyl group with deuterated acetic acid.[18,29]



Scheme 6.5 Theoretical conversion of **6.29** to labeled leucine **6.43**.

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6.6 Experimental

General. General procedures can be found in Chapter 2 (Experimental section).

Trichloro sultam (6.34). To an oven dried two neck flask, cooled to -98° C, was added dry THF (0.5 mL) and n-BuLi (0.94 mL, 5.0 equiv.). To this solution, dry, distilled $CHCl_3$ (0.165 mL, 5.0 equiv.) was added dropwise maintaining an internal temperature below -80° C. A whitish precipitate first formed and then the solution turned brown. To the trichloromethyl anion, *N*-crotonoyl sultam **6.33** (0.1140 g), dissolved in THF (1mL), was slowly added. After 50 min., when TLC showed no further formation of product, TMS-Cl (0.26 mL, distilled and passed through basic alumina) was added. After 1.5 h the reaction was stopped by quenching with saturated NH_4Cl at -90 °C.

The reaction mixture was transferred to a separatory funnel and the aqueous layer was extracted with ether (3X). The combined organic layers were washed with H_2O followed by saturated NaCl and dried with $MgSO_4$. Evaporation in vacuo was followed by flash chromatography with 10% *n*-hexane / CH_2Cl_2 to obtain **6.34** (55 : 45 diastereomers) in 87% yield based on recovered starting material. Several crystallizations from *n*-hexane provided diastereomerically pure (-)-**6.34**; m.p. 134-135 °C; $[\alpha]_D$ -40.96° (c = 1.14, $CHCl_3$); UV (MeOH) 205 (6358), 207 (ε 6049) nm; IR (NaCl) 1693.19 cm^{-1}; ^1H NMR (C_6D_6) δ 3.77 (dd, 1H, *J* = 16.4, 1.8 Hz), 3.50 (m, 2H), 3.29 (dd, 1H, *J* = 16.4, 9.7 Hz), 2.73 (q, 2H, *J* = 13.8 Hz), 2.00 (ddd, 1H, *J* = 12.0, 8,0, 3.6 Hz), 1.87 (dd, 1H, *J* = 14.0, 8.0 Hz), 1.30 (bd, 3H, *J* = 6.5 Hz), 1.28 (m, 2H), 1.10 (m, 1H), 1.01 (s, 3H), 0.75 (m, 1H), 0.57 (ddd, 1H, *J* = 12.0, 9.4, 3.1 Hz), 0.41, (s, 3H); ^{13}C NMR (C_6D_6) δ 169.5 (C=O), 105.6 (CCl_3), 65.7 (d), 52.9 (t), 52.4 (d), 48.7 (s), 48.0 (s), 45.3 (d), 40.2 (t), 39.2 (t), 32.9 (t), 26.8 (t), 21.4 (q), 20.0 (q), 17.4 (q); HRMS (EI) found 401.0357, $C_{15}H_{22}Cl_3NO_3S$ requires 401.0386.

Trichloro aminonitriles (6.29a-d). Literature procedure by Inaba[37] was followed except 1:1 mol equivalent of (R)-methylbenzylamine (**6.40**) and 3-methyl-4,4,4-trichlorobutanal (**6.39**) was used. Purification of the crude material by silica flash chromatography (15% EtOAc/ hexane) followed by HPLC (Microsorb Si-80-120-C5, 10% EtOAc / hexane, flow rate = 13 mL/min.) provided the four diastereomers **6.29a-d** in 93% overall yield in the ratio 25:17:44:14 % respectively. HPLC retention times were 9.6, 10.8, 11.0, 14.0 minutes.

(**6.29a**): $C_{14}H_{17}Cl_3N_2$; 25% yield; retention time = 9.6 minutes; solid; m.p. 80-81 °C; $[\alpha]_D$ + 135.9° (c = .75 in $CHCl_3$); UV (MeOH) 209 (10570), 251 (556), 257 (ε 590) nm; IR (NaCl) 3301, 2229 cm^{-1}; ^1H NMR ($CDCl_3$ ref. to TMS) δ 7.38 (m, 5H), 4.11 (q, 1H, *J* = 6.5 Hz), 3.18 (dd, 1H, *J* = 11.6, 3.9 Hz), 2.94 (dqd, 1H, *J* = 10.1, 6.5, 2.1 Hz), 2.46 (ddd, 1H, *J* = 13.9, 11.6, 2.2 Hz), 1.78 (ddd, 1H, *J* = 14.0, 10.1, 3.9 Hz), 1.42 (d, 3H, *J* = 6.4 Hz), 1.06 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 142.7 (C1), 128.7 (2C, d), 127.9 (d), 126.9 (2C, d), 120.0 (C8), 105.2 (C7), 56.5 (C2), 50.5 (C5), 45.5 (C3), 36.9 (C4), 24.6 (C9), 15.4 (C6); HRCIMS obsd *m / z* 319.0541 ($M+H^+$), $C_{14}H_{17}Cl_3N_2H$ requires 319.0536.

(**6.29b**): $C_{14}H_{17}Cl_3N_2$; 17% yield; retention time = 10.8 minutes; solid; m.p. 70-71 °C; $[\alpha]_D$ -34.6° (c = 0.41 in $CHCl_3$); UV (Hexane) 205 (5000), 252 (ε 2000) nm; IR (NaCl) 3301, 2218 cm^{-1}; ^1H NMR ($CDCl_3$ ref. to TMS) δ 7.38 (m, 5H), 4.09 (q, 1H, *J* = 6.4 Hz), 3.73 (dd, 1H, *J* = 11.0, 4.6 Hz), 2.94 (dqd, 1H, *J* = 9.7, 6.5, 2.7 Hz), 2.46 (ddd, 1H, *J* = 13.8, 11.0, 2.7 Hz), 1.89 (ddd, 1H, *J* = 14.2, 9.7, 4.5 Hz), 1.38 (d, 6H, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 144.2 (s, C1), 128.7 (2C, d), 127.8 (d), 126.6 (2C, d), 119.7 (s, C8), 105.1 (s, C7), 56.0 (d, C2), 50.9 (d, C5), 45.7 (d, C3), 37.0 (t, C4), 21.8 (q, C9), 15.9 (q, C6); HRCIMS obsd *m / z* 319.0538 ($M+H^+$), $C_{14}H_{18}Cl_3N_2$ requires 319.0536.

(**6.29c**): $C_{14}H_{17}Cl_3N_2$; 44% yield; retention time = 11.0 minutes; solid; m.p. 90-91 °C; $[\alpha]_D$ +87.3° (c = 0.65 in $CHCl_3$); UV (Hexane) 209 (11282), 251 (818), 257

(ε 813) nm; IR (NaCl) 3321, 2225 cm^{-1}; ^1H NMR ($CDCl_3$ ref. to TMS) δ 7.38 (m, 5H), 4.13 (q, 1H, *J* = 6.5 Hz), 3.29 (dd, 1H, *J* = 9.6, 6.2 Hz), 2.72 (dqd, 1H, *J* = 9.4, 6.5, 2.5 Hz), 2.45 (ddd, 1H, *J* = 13.7, 9.6, 2.5 Hz), 1.80 (ddd, 1H, *J* = 13.7, 9.3, 6.2 Hz), 1.40 (d, 3H, *J* = 6.5 Hz), 1.27 (d, 3H, *J* = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 142.7 (s, C1), 128.7 (2C, d), 127.7 (d), 126.6 (2C, d), 119.3 (s, C8), 104.7 (s, C7), 56.5 (d, C2), 51.8 (d, C5), 46.9 (d, C3), 37.2 (t, C4), 24.8 (q, C9), 16.9 (q, C6); HRCIMS obsd *m / z* 319.0543 ($M+H^+$), $C_{14}H_{18}Cl_3N_2$ requires 319.0536.

(**6.29d**): $C_{14}H_{17}Cl_3N_2$; 14% yield; retention time = 14.0 minutes; solid; m.p. 101-102 °C; $[\alpha]_D$ +14.6° (c = 0.55 in $CHCl_3$); UV (Hexane) 211 (23200), 252 (511), 257 (ε 539) nm; IR (NaCl) 3309, 2214 cm^{-1}; ^1H NMR ($CDCl_3$ ref. to TMS) δ 7.38 (m, 5H), 4.14 (q, 1H, *J* = 6.5 Hz), 3.81 (dd, 1H, *J* = 9.7, 6.1 Hz), 2.83 (dqd, 1H, *J* = 9.4, 6.5, 2.7 Hz), 2.44 (ddd, 1H, *J* = 13.7, 9.7, 2.7 Hz), 1.82 (ddd, 1H, *J* = 13.7, 9.4, 6.1 Hz), 1.38 (d, 6H, *J* = 7.0 Hz); ^{13}C NMR ($CDCl_3$) δ 144.0 (s, C1), 128.7 (2C, d), 127.8 (d), 126.6 (2C, d), 119.0 (s, C8), 104.8 (s, C7), 56.2 (d, C2), 51.9 (d, C5), 46.8 (d, C3), 37.1 (t, C4), 22.1 (q, C9), 16.9 (q, C6); HRCIMS obsd *m / z* 319.0549 ($M+H^+$), $C_{14}H_{18}Cl_3N_2$ requires 319.0536.