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## SYNTHESES AND PROPERTIES OF ISOPORPHYRINS AND RELATED DERIVATIVES FOR APPLICATION IN PHOTODYNAMIC THERAPY

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy

in

The Department of Chemistry

by Sandra Celinah Mwakwari B.S., University of Nairobi, 1999 May 2007

# **DEDICATION**

This dissertation is dedicated to my parents, Emily and Hamilton M. Mwakwari, for their endless love, support and sacrifice throughout my life, and in memory of my late mother Mrs. Emily Mwakwari, who departed this earth before completion of this Ph.D program.

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## **GLOSSARY OF ABBREVIATIONS**

<sup>t</sup>**Bu** = tertiary butyl  $-C(CH_3)_3$ 

**DCM** = Dichloromethane  $CH_2Cl_2$ 

**DDQ** = Dichlorodicyanoquinone

**DEPT** = Distortionless Electron Polarization Transfer

**DMAP** = Dimethylaminopyridine

**DMF** = Dimethylformamide  $(CH_3)_2NCHO$ 

**DMSO** = Dimethylsulfoxide  $(CH_3)_2SO_2$ 

**EPR** = Electron Paramagnetic Resonance

**ER** = Endoplasmic Reticulum

 $Et = Ethyl - CH_2CH_3$ 

 $\mathbf{G} = \text{Gauss}$ 

**GHz** = Giga Hertz

**HR ESI** = High Resolution (mass spectrometry) Electron Spray Ionization

HR MALDI = High Resolution (mass spectrometry) Matrix Assisted Laser Desorption

Ionisation

 $IC_{50} = 50\%$  cell survival

 $\mathbf{K} = \text{Kelvin}$ 

 $\mathbf{KHz} = \mathrm{Kilo} \mathrm{Hertz}$ 

 $Me = Methyl - CH_3$ 

**MeOH** = Methanol

**MS** = Mass Spectrophotometry

**mW** = Milli Watts

**NMR** = Nuclear Magnetic Resonance

- **OMP** = Octamethylporphyrin
- **PDT** = Photodynamic Therapy
- $\mathbf{Ph} = \text{Phenyl} C_6 H_6$
- **TEA** = Triethylamine  $(CH_3CH_2)_3N$
- **TFA** = Trifluoroacetic acid ( $CF_3CO_2H$ )
- **THF** = Tetrahydrofuran
- **TLC** = Thin Layer Chromatography
- *p*-**T**sOH = para-Toluenesulfonic acid

#### ABSTRACT

Isoporphyrins are tautomers of porphyrins with an interrupted macrocyclic conjugation owing to the presence of a sp<sup>3</sup> hybridized *meso*-carbon. Due to their absorption at long wavelengths (~800nm), isoporphyrins are potential candidates as photosensitizers in photodynamic therapy (PDT), a ternary modality of cancer treatment. They are also of biological interest due to their unique redox properties among porphyrin derivatives and could be a new class of near-IR dyes.

Isoporphyrins are known to be unstable with respect to transformation back to the fully conjugated porphyrin chromophore except when the *meso*-carbon is geminally substituted and for this reason a detailed study of this macrocycle has previously been prevented. However, a better synthetic route (the main objective of this research project) by way of open-chain tetrapyrrole precursors has been developed to afford the target compound, zinc(II) isoporphyrin in better yields (28%) than previously reported (6%). No other metal isoporphyrin is known and a novel copper(II) isoporphyrin was synthesized and characterized. This was achieved by utilizing cuprous chloride as the metal ion donor. Stability studies have also been done to obtain stable metal-free isoporphyrins which have not previously been isolated and characterized. A detailed <sup>1</sup>H and <sup>13</sup>C NMR study allows their complete structural elucidation. Substitution of one of the *meso*-dimethyl groups for an ester using  $\alpha,\beta$ -ketoesters as carbon-linking units in the cyclization reaction of b-bilenes, affords novel zinc isoporphyrins, whose preliminary chemical properties show that they can be used as intermediates to *meso*-monosubstituted porphyrins. However, during the synthesis, various intermediates were observed and studied. An interesting transformation - b-bilene, to a,b-biladine, to a,c-biladiene - was observed; this is the first example of such a transformation that we are aware of so far. This route has also been applied to synthesize *meso*-monosubstituted porphyrins in higher yields than previously reported.

These compounds have also been studied for their biological properties applicable to photodynamic therapy, including dark- and phototoxicity, intracellular localization, and cell uptake. The preliminary results indicate that the compounds exhibit low dark toxicity and are phototoxic, they localize both in the mitochondria (major) and the lysosomes, and thus are very good candidates for tumor destruction in PDT.

#### **CHAPTER 1**

### **GENERAL INTRODUCTION**

The porphyrin macrocycle (1), as illustrated in **Figure 1.1**, consists of four pyrrole units joined by four methine bridges. This macrocycle is highly conjugated and highly colored. It posses 22  $\pi$ -electrons, but only 18 of these are included in any delocalization pathway, thus it is aromatic on the basis of Huckel's rule (4n + 2). The main absorption band, an intense Soret band with very high extinction coefficient (about 10<sup>5</sup>), is found around 400 nm and is characteristic of this macrocyclic conjugation. An interruption to this conjugation results in the disappearance of this band.<sup>1</sup> The four pyrrole nitrogens in the porphyrin core form a cavity into which metal ions can bind by coordinate or covalent bonds, between the nitrogens and the metal, thus giving metalloporphyrins.

The tetrapyrrole macrocycle forms the core of many important biological molecules that occur in nature. For example heme (**2**), the prosthetic group in hemoglobins (blood) and myoglobins, (and many other hemoproteins), is responsible for oxygen transport and storage in living tissue, respectively. Other hemoproteins like peroxidases, cytochrome c oxidase, are enzymes involved in chemical catalysis, and cytochrome, cytochrome c, are responsible for active membrane transport and electron transport. Chlorophylls (**3**), found in green plants, plays important roles in the process of photosynthesis. Other examples include bacteriochlorophylls (found in photosynthetic bacteria), and vitamin  $B_{12}$ .



Figure 1.1: Examples of naturally occurring porphyrins

Porphyrins can be obtained using several synthetic approaches, the most common ones being tetramerization of pyrroles and an aldehyde;<sup>2</sup> condensation of dipyrrolic intermediates, for example dipyrromethanes (4) (utilizing the MacDonald<sup>3</sup> [2 + 2] synthesis), and dipyrromethenes (5) (a synthetic approach developed by Fischer<sup>4</sup>); and

cyclization of open chain tetrapyrroles like bilanes (6), b-bilenes (11), a,c-biladienes (12), just to name a few.<sup>5</sup>



Figure 1.2: Porphyrin syntheses precursors

The carbon skeletons of these macrocycles contain only  $sp^2$  - hybridized *meso* bridging carbons. Introduction of an  $sp^3$  – hybridized *meso* carbon to this macrocycle alters every chemical and physical property associated with it. A new class of compounds emerges with different features and characteristics. Herein, we shall discuss closely related tetrapyrrolic compounds featuring a mixture of  $sp^2$  - and  $sp^3$  – hybridized *meso* bridging carbons. These include isoporphyrins (7) (which are the main objective of this dissertation), phlorins (8), porphodimethenes (9), porphomethenes (10) and open-chain tetrapyrroles (bilenes (11) and a,c-biladienes (12)).





Figure 1.3: Structures of tetrapyrroles with mixed sp<sup>2</sup>- and sp<sup>3</sup>- bridging carbons

## 1.1 Isoporphyrins

Isoporphyrins (7) contain three  $sp^2$  – and one  $sp^3$  – hybridized *meso* carbons and one NH hydrogen atom. The existence of this type of compound was first suggested by Woodward<sup>6</sup> in 1961 after the discovery of a similar class of compounds called phlorins (8) and the recognition of their stability. His prediction was confirmed about a decade later when Dolphin et al.<sup>7</sup> reported the first synthesis of metalloisoporphyrin (13) by way of electrochemical oxidation of zinc(II) *meso*-tetraphenylporphyrin (ZnTPP) (14).



Figure 1.4: Zinc TPP (14) and Zinc Isoporphyrin (13)

Isoporphyrins are tautomers of porphyrins in which hydrogen is transferred to a meso-carbon from its normal position on a nitrogen atom. The presence of a saturated bridging meso-carbon interrupts the macrocyclic conjugation pathway, thus making isoporphyrins non-aromatic in the normal tetrapyrrole sense. The interruption of conjugation is confirmed in their absorption spectrum due to the disappearance of the Soret band (around 400 nm) that is characteristic of porphyrin macrocycle conjugation. The absorption spectra of the metalloisoporphyrins show striking differences (**Figure 1.5**) from those of porphyrins with the Q-band being much more intense (extinction coefficient  $=10^4$ ) and red shifted (800nm) compared to 500-600nm in porphyrins. A bathochromic shift of the Soret band to 420-430nm is also observed. This shift to longer wavelengths is characteristic of partial conjugation as seen in chlorins and chlorophylls.<sup>8</sup>



Figure 1.5: Illustration of the Q-band absorption spectra of zinc porphyrin and zinc isoporphyrin

Normally, the cutting down of the chromophore of an organic molecule, for example by one conjugated double bond, leads to a hypsochromic (blue) shift of the absorption band. Yet when a porphyrin is reduced, in cases like chlorins and bacteriochlorins, the lowest energy transition undergoes a bathochromic (red) shift and intensifies. This phenomenon can be explained using the four-orbital model originally proposed by Martin Gouterman in 1961.<sup>9</sup> The four  $\pi$  molecular orbitals that are principally involved are the two highest occupied molecular orbitals, which are similar but of distinct energies [a<sub>1u</sub>(HOMO) and a<sub>2u</sub>(HOMO-1)], and the two lowest, nearly degenerate, unoccupied molecular orbitals [e<sub>gy</sub>(LUMO) and e<sub>gx</sub> (LUMO+1)], which are considered to have equal energies. The two lowest energy transitions (HOMO to LUMO, HOMO to LUMO + 1) are called the Q-bands, while the two higher energy ones (HOMO

- 1 to LUMO, HOMO – 1 to LUMO + 1) are called the B-bands or Soret bands. These transitions correspond to  $Q_y$ ,  $Q_x$ ,  $B_x$ , and  $B_y$ , in increasing order of energy.

The diagram in **Figure 1.6**, illustrates an oversimplification of what is a complex relationship between electronic states and orbital energies.<sup>10</sup> It indicates the relative changes in orbital energies and transition energies as one goes from the more symmetric porphyrin to the more asymmetric bacteriochlorin. Perturbations of the electronic structure of porphyrin chromophores that can arise from altering the symmetries and/ or energies of the porphyrin frontier orbitals, are very sensitive to the Q-bands. Reduction of the *meso*- (as in isoporphyrins) and  $\beta$ -positions (as in chlorins and bacteriochlorins) provides such asymmetry. The HOMO energy of the Q-band transition is raised, thus reduced energy gap between HOMO and LUMO, which results in a red shift of the Q-band and an increase of its intensity.

Their intense absorption at long wavelengths is crucial for application in photodynamic therapy (PDT) of cancer treatment. Several porphyrin analogs (**refer to section 1.5**) have been applied in this modality of cancer treatment due to their absorption of red light. However, most of these candidates absorb in the range of 600-700nm, and at this wavelength, light can not penetrate deeply through tissue. This is a major drawback in PDT. Researchers are investigating photosensitizers that can treat deep tumors, and while isoporphyrins absorb at wavelengths greater than 800nm, this surely makes them potential candidates for deep-seated tumors.

Since the initial prediction regarding isoporphyrins<sup>6</sup> and their transient synthesis,<sup>7</sup> other methods of generating isoporphyrins particularly from tetraphenylporphyrins include chemical oxidation,<sup>11,12</sup> photo-oxidation,<sup>13,14</sup> and electrochemical oxidation.<sup>15,16</sup>

7



**Figure 1.6:**<sup>10</sup> Molecular orbital energy level diagram of porphyrin, chlorin and bacteriochlorin. A very simplified representation of the different electronic transitions is indicated

These isoporphyrins however, were unstable in the sense that they were easily converted into the corresponding porphyrins. For this reason, the field of isoporphyrins has been handicapped. This problem was overcome by Xie and Smith<sup>17</sup> when they reported the first stable metalloisoporphyrin. The availability of these stable species paved way to crystallography,<sup>18</sup> photophysical studies<sup>19</sup> and electrochemical studies.<sup>20</sup>

The structural consequences<sup>18</sup> of porphyrin tautomerization that disrupts the  $\pi$  system of isoporphyrin have been determined by crystallography for zinc (II) isoporphyrin perchlorate. The presence of a perchlorate counterion and the two methyl groups at the *meso* C5 confirm the cationic nature and the tetrahedral *meso* carbon features characteristic of a metalloisoporphyrin. The isoporphyrin also displays distinctive bond distance values and bond angles when compared to zinc octaethylporphyrin (ZnOEP), with the overall pattern of bond distances being consistent with the resonance forms of the interrupted  $\pi$  system as illustrated in **Figure 1.7.** The zinc atom is displaced from the plane of the four nitrogens and from the average plane of the macrocycle. In general, the macrocyclic structure is slightly ruffled, with the meso carbons moved above and below the macrocycle plane, and the pyrrole rings slightly twisted above and below the plane.

The ground-state optical absorption<sup>19</sup> of the perchlorate salt of zinc isoporphyrin in acetonitrile has major bands at ~320, 420 and 790nm, with the corresponding fluorescence emission of the low energy transition occurring at 830nm. In comparison to (ZnOEP) whose Stoke's shift is 50 cm<sup>-1</sup>, the zinc isoporphyrin exhibits a large Stoke's shift of ~600 cm<sup>-1</sup> and an unusually short singlet excited state lifetime of  $130 \pm 15$  ps at room temperature.



Figure 1.7: Resonance structures of zinc (II) isoporphyrin perchlorate based on its crystal structure

Its fluorescence quantum yield,  $\Phi_{\rm F}$  is 0.004 ± 0.002 at 295 K compared to  $\Phi_{\rm F}$  0.2 of bactereochlorophyll *a*. The persistence of the fluorescence at lower temperatures confirms that the emission is primarily from the lowest excited state rather than the triplet excited state.

The effect of the interruption of the conjugation in the isoporphyrin ring on the kinetics of electron transfer has also been determined.<sup>20</sup> The study shows that the redox properties of ZnOEP and zinc isoporphyrin differ markedly, particularly the reduction potentials, and the stability of the species formed on electron transfer is solvent dependant; stabilized in polar solvent than less polar solvent. Also, the electron transfer for the zinc isoporphyrin system involves the surrounding ring and not the central metal ion. With the oxidation and reduction potential of 0.63 and -1.61 V for ZnOEP, compared to 1.09 and -0.29 V for zinc isoporphyrin, respectively, it is clear that zinc isoporphyrin is

substantially easier to reduce and harder to oxidize than ZnOEP, and the potential difference,  $\Delta E$ , for the oxidation and reduction potentials of zinc isoporphyrin is only 1.38 V compared with 2.25 V for ZnOEP. These redox potentials of porphyrin derivatives correspond approximately to the energy difference between HOMO and LUMO, and have been shown to track their relative energies. As a HOMO is stabilized, its energy level is lowered and the molecule becomes harder to oxidize, while on the other hand, if the LUMO is lowered, the molecule becomes easier to reduce. Extrapolation of these trends to zinc isoporphyrin and ZnOEP clearly suggests that the LUMO of the isoporphyrin has shifted down significantly more than the HOMO relative to ZnOEP and it is this resulting smaller gap between the HOMO and LUMO that causes the observed red shift in the optical spectrum.

Due to the low yields reported, a better synthetic pathway needed to be developed. **Chapter 2** of this dissertation discusses in detail the historical background of these macrocycles, and the main objective of this research; to develop a better synthetic pathway to isoporphyrins.

#### 1.2 Phlorins

Phlorins (8) are dihydroporphyrins. They contain three  $sp^2$ - and one  $sp^3$ hybridized meso carbon atoms and three-NH. They are similar to isoporphyrins (7) with the exception that phlorins contain three-NH while isoporphyrins have one-NH. Phlorins are therefore dihydroporphyrins in which one of the hydrogen is added to nitrogen and the second to a *meso*-carbon. They are tautomeric structures of chlorins (**refer to figure 1.4**). This class of compounds was discovered and characterized by Woodward<sup>6</sup> during his total synthesis of chlorophyll *a*. The chromophore was also detected by Mauzerall<sup>21</sup> as

the first intermediate in the photo- or chemical reduction of uroporphyrins with absorption maxima at 440 and 735 nm. However, this reduced product was unstable in light and air, and was oxidized back to the corresponding porphyrins.<sup>22</sup> Phlorins bearing a hydrogen atom at the sp<sup>3</sup> hybridized meso carbon atom are easily oxidized, and for those which the sp<sup>3</sup> meso position is incorporated two substituents, are subject to oxidation to ring opened biladienone species.<sup>23</sup> Borohydride reduction of N-alkyl/arylporphyrins (15), vielded stable phlorins  $(16)^{24}$  (Scheme 1.1). The stability of this compound was related to the bulky substituent on the core nitrogen. In comparison to isoporphyrins (7), phlorins are formed during nucleophillic attack on a meso position of porphyrin (through  $\pi$ dianions by electrochemical- or photo-reduction) while isoporphyrins have been directly synthesized by electrophillic addition to the porphyrin ring through  $\pi$ -dication intermediates.<sup>7</sup> However, direct nucleophillic attack on the porphyrins had not been achieved and was ascribed to the poor electrophillicity of the conventional metalloporphyrins used, but with the use of Au (III) porphyrins, which are strong electrophiles, direct nucleophilic attack was achieved to obtain novel hydroxyphlorin products.<sup>25</sup> Senge<sup>26</sup> has suggested phlorin intermediates during nucleophilic reaction of porphyrins and metaloporphyrins with organolithium reagents. Addition of nbutyllithium base *meso*-tetraphenylporphyrin 5-butyl-mesoto free gave tetraphenylphlorin<sup>24</sup> whose stability was moderate in solution in the presence of oxygen but was stable enough for characterization as crystals in room temperature. Phlorin stability has further been enhanced by incorporation of mesityl substituents<sup>23</sup> at the two *meso* postions adjacent to the  $sp^3$ -hybridized carbon or at all the three *meso* positions of the phlorin.



Scheme 1.1: Synthesis of stable phlorin

Due to the presence of a saturated bridging carbon atom (as with isoporphyrins), phlorins absorb at longer wavelengths than porphyrins. Protonated phlorins absorb at 440 and 737 nm, while the metal-free neutral phlorins absorb at 387 and 620 nm. Their metal complexes absorb at 440 nm with a red shifted Q-band at 800 nm. Although the Q-band absorption of both metallo-phlorins and metallo-isoporphyrins are in the same near-IR region, the two can be differentiated by the fact that phlorins have only one broad peak, while isoporphyrins have two strong absorption peaks.

#### **1.3** Porphomethenes and Porphodimethenes

Porphomethenes (10) contain one  $sp^2$ - and three  $sp^3$ - hybridized meso carbons, while porphodimethenes (9) contain two  $sp^2$ - and two  $sp^3$ - hybridized meso carbons arranged in either 'cis' or 'trans' manner. These saturated compounds are suggested as intermediates (Figure 1.8) in the porphyrin synthesis via condensation of pyrrole and aldehyde, followed by oxidation of the resulting porphyrinogen by six electrons and six protons, to porphyrin.<sup>27,28</sup> However, these intermediates were not isolated since they were easily dehydrogenated to porphyrins by oxygen. The first air-stable porphomethenes were reported by Buchler and Puppe in 1970.<sup>29</sup> They were prepared by reductive methylation of octaethylporphyrinato zinc(II) bearing ethyl protected  $\beta$ -positions, sterically discouraging the alkylation of these carbons. The scope of this approach was later expanded to produce other metalloporphodimethenes bearing various metals and other alkyl substituents on the meso positions.<sup>30</sup> This was the most general procedure for the synthesis of porphodimethenes; reductive alkylation of porphyrins at the meso position. Unfortunately, this approach proved limited to the synthesis of symmetric metalated porphodimethenes.<sup>29</sup> Further, this reductive alkylation strategy did not prove effective on all types of metalated porphyrins or compatible with all types of pyrrolic substituents.

New synthetic routes have been reported, including, 2 + 2 MacDonald-like condensation approach,<sup>31</sup> and use of sterically hindered aldehydes in mixed pyrrole condensation resulting in an oxidation resistant conformation.<sup>32</sup>

Porphodimethenes bearing alkyl groups at the sp<sup>3</sup>-hybridized *meso* centres are stable since, in contrast to most porphyrinogens, they are able to resist oxidative dehydrogenation leading to porphyrins.<sup>33</sup> Typical hydroporphyrins exhibit a very broad absorption band between 400-500 nm, while porphyrins mostly have sharp Soret absorption bands at 400 nm. Neutral porphomethenes (7), which are yellow in color, absorb at 425 nm, while their protonated form at 500 nm, and the orange-red porphodimethene absorb at 471 and 686 nm.<sup>22,32</sup>



**Figure 1.8:** Illustration of the intermediates in the oxidation pathway of porphyrinogen to porphyrin

#### 1.4 *b*-Bilenes and *a*,*c*-Biladienes

*b*-Bilenes (**11**) and *a*,*c*-biladienes (**12**) are open chain tetrapyrrole salts that contain one sp<sup>2</sup>- and two sp<sup>3</sup>- or two sp<sup>2</sup>- and one sp<sup>3</sup>-hybridized bridging carbon atoms, respectively. Both of these open chain tetrapyrroles have been employed widely in the synthesis of porphyrins.<sup>5</sup> Oxidative cyclization of *b*-bilenes has provided the most successful avenue to porphyrins substituted with electron withdrawing groups, since the procedure works best when the terminal rings of the *b*-bilene are substituted with electron withdrawing groups.<sup>34,35</sup> The b-bilene oxidative cyclization has also been investigated as an approach to meso-monosubstituted porphyrins. Although in most cases the required porphyrin was formed, the yield was generally low and the sequence complicated by the presence of by-products, therefore limiting this approach.<sup>36</sup> Other porphyrin derivatives like biliverdins have been afforded by oxidation of *b*-bilenes (**Figure 1.9**).

*a,c*-Biladienes, on the other hand, are the most commoly used open chain tetrapyrroles for preparation of porphyrins and its derivatives. Oxidative cyclization of these biladienes to give porphyrins using the copper(II) salt method, or using chromium(III) or electrochemical oxidation,<sup>5</sup> have been extensively investigated, occasionally in parallel with the corresponding *b*-bilenes. 1,19-Dimethyl-*a,c*-biladiene salts have been mostly employed and are undoubtedly highly effective intermediates for porphyrin synthesis. High yields of porphyrins are often obtained after the removal of the chelating copper atom, but it has been found that the use of chromium(III) for the oxidative cylization results in the isolation of the metal-free porphyrin product; and there is no obvious symmetry or substituent difficulties associated with these intermediates.<sup>5</sup>

diunsubstituted-a,c-biladiene salts have been employed to afford sapphyrins (expanded porphyrin),<sup>37</sup> and in alcoholic solutions for preparation of corroles in one pot<sup>38</sup> (**Figure 1.9**).



Figure 1.9: Some of the products obtained from b-bilene and a,c-biladiene

a,c-Biladiene dihydrobromide salts show two peaks of similar intensity in their optical spectra, one at 450 and the other at 530 nm. On the other hand, b-bilene chloride salts show a strong absorption at around 500 nm (**Figure 1.10**).

These open chain tetrapyrroles are best employed for porphyrins with a complex array of substituents possessing no symmetry characteristics. Simple porphyrins like TPP or OEP are best prepared by polymerization of monopyrrole.<sup>1</sup>



Figure 1.10: Electronic absorption of b-bilene salt verses a,c-biladiene salt

#### **1.5 Photodynamic Therapy (PDT)**

Photodynamic therapy is a ternary modality for cancer treatment and is one of the most promising anticancer therapies still under investigation.<sup>39</sup> Matured as a feasible medical technology in the 1980's at several institutions throughout the world, PDT combines three key components: light source, a photosensitizer (a drug that is activated by light), and tissue oxygen, to destroy cancer cells.<sup>40,41</sup> The most coherent light sources are lasers and since the maximum of skin permeability occurs in the range of approximately 620-850 nm, light of this spectral range, called the "phototherapeutic window" is predominantly used in phototherapy.<sup>39</sup> Hence, photosensitizers with a strong absorption band in this region can be activated to penetrate deeper into the tissues.<sup>42</sup> Porphyrins and other porphyrin-based compounds are the most explored photosensitizers

and consequently their syntheses, chemistry and biological properties continue to be the subject of intense investigation.<sup>43</sup>

The photophysical processes of PDT are illustrated on a simplified Jablonski diagram shown in **Figure 1.11**. Upon light absorption, the photosensitizer, in this case porphyrin ( $^{0}$ P in its ground state) is excited to a short-lived first excited singlet state ( $^{1}$ P\*) which can undergo radiative decay back to the ground state with release of energy in the form of fluorescence – enabling identification of tumor tissue. A good photosensitizer will at this stage undergo a spin forbidden inter system crossing (ISC), converting the photosensitizer into the triplet excited state ( $^{3}$ P\*). The triplet state relaxes back to ground state via phosphorescence or by internal conversion (transfer of energy to another triplet state). One of the very few molecules with a triplet ground state is dioxygen, which is found in most living cells. Energy transfer therefore takes place between excited triplet state of photosensitizer ( $^{3}$ P\*) and stable triplet oxygen ( $^{3}$ O<sub>2</sub>) producing short lived and highly reactive excited singlet oxygen ( $^{1}$ O<sub>2</sub>), a cytotoxic species that causes irreversible destruction of tumor cells.<sup>42,44</sup>

A typical PDT session (Figure 1.12) would involve the following steps:

1. The photosensitizer is administered into the body, either by injection into the vein for cancers inside the body or topical application onto the skin for cancers of the skin.

2. Permit time for the chemical to be cleared from normal tissues and be preferentially retained by the tumor

3. Application of light onto the tumor to provide the catalyst for chemical reactions.

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**Figure 1.11:** Simplified Jablonski diagram<sup>44</sup> illustrating the photophysical and photochemical processes of PDT.



Figure 1.12: A typical PDT session. Taken from reference <sup>45</sup>

4. Generation of toxic oxygen species thus tumor destruction.

Administration of the photosensitizer depends on its hydrophillicity or hydrophobicity properties. Water-soluble compounds require no formulation whilst all others require formulation in lyposomes or other solubilizing agents.

Porphyrins have been known to have the ability to selectively localize in tumors<sup>46</sup> and possess low-dark toxicities thus leading to their initial choice as the most promising PDT photosensitizers compared with other aromatic macrocycles. Porphyrin-based compounds possess a number of key photochemical, photophysical and biological properties that make them highly desirable for medical applications: they absorb strongly in the visible region of the optical spectrum, are fluorescent, are non-toxic in the dark, have high chemical stability, have high affinity for serum proteins, have favorable pharmacokinetic properties, and form very stable complexes with a variety of metal ions while retaining their *in vivo* tumor-localization properties. For these reasons, several porphyrin-type compounds are currently in various stages of preclinical or clinical development as phototherapeutic agents.<sup>43</sup>

For the last two decades, an FDA approved Photofrin<sup>®</sup> (a purified hematoporphyrin derivative, HPD, **Figure 1.13**) has been used for treating various forms of cancer in many countries for example melanoma, early and advanced stage cancer of the lung, digestive tract, bladder cancer, and early stage cervical cancer.<sup>42,43</sup> This first generation porphyrin-based drug; even though proven efficacious in the treatment of a wide variety of cancers, fulfilling certain criterion for ideal photosensitizers, it suffers from several drawbacks. It consists of a complex mixture of porphyrins with various monomeric and oligomeric forms which has not been characterized to even the most

minimal levels expected by organic chemists. Its long wavelength is a weak absorption band, which falls at 630 nm, well below the wavelength suggested for maximum tissue penetration and treatment of deep-seated tumors, and its prolonged skin photosensitivity as a result of long retention times, adds to the disadvantages.<sup>42-44</sup> Nevertheless, new and improved second generation photosensitizers which are chemically pure, have high fluorescence detection and quantum yields, absorb at longer wavelengths, and induce significantly less skin photosensitivity, are being developed. Examples of these porphyrin-type photosensitizing drugs include mono-L-aspartyl chlorines e<sub>6</sub> (MACE, **17**), benzoporphyrin derivative mono-carboxylic acid (BPD-MA, Visudyne<sup>TM</sup>, **18**), zinc(II) phthalocyanine (**19**) and texaphyrins (**20**) (tripyrrole expanded macrocycles), (**Figure 1.14**) all absorbing strongly in the 650-750 nm spectral region.<sup>43</sup>



Figure 1.13: Hematoporphyrin

Due to the limitations encountered by first-generation photosensitizers, extensive research is underway to develop modern photosensitizers that meet the following criteria for an ideal photosensitizer:<sup>42</sup>

- It should be chemically pure and of known specific composition with a reproducible synthesis.
- ii) It should have high quantum yield for singlet oxygen production for effective destruction of tumor cells.
- iii) It should have strong absorption with high extinction coefficient (ε) at longer wavelength (between 700-800 nm) where scattering of light is minimal and tissue penetration is at its maximum.
- iv) It should have excellent photochemical reactivity, with high triplet state yields and long triplet state life times and be able to effectively produce singlet oxygen  $({}^{1}O_{2})$  and other reactive species.
- V) It should possess minimal dark toxicity and only be cytotoxic in presence of light.
- vi) It should have preferential retention by target tissue (tumor cells)
- vii) It should be rapidly excreted from the body, thus inducing a low systemic toxicity.
- viii) Finally it should be synthesizable from easily available precursors and should be stable and easy to dissolve in the body's tissue fluids and be capable of formulation (dissolution of photsensitizer in injectable solvents).





 $R^1 = Me (H), R^2 = H (Me)$ 

17









20

Figure 1.14: Examples of second-generation PDT photosensitizers
PDT has obvious advantages over other conventional cancer treatments such as surgery, chemotherapy and radiotherapy, in that it can provide local control of the disease (selectively removing or destroying diseased tissue and sparing normal healthy cells) with minimal side effects. Unlike the other modalities, it can be applied repeatedly many times at the same site without risking the integrity of surrounding tissues. Furthermore, PDT is a cold photochemical process, which can be applied before, or after chemotherapy, ionizing radiation or surgery, without compromising these treatments or being compromised itself.<sup>42,43</sup>

#### **CHAPTER 2**

## SYNTHESIS OF METALLO-ISOPORPHYRINS

# 2.1 Introduction

Isoporphyrins (1) are tautomers of porphyrins (2) in which hydrogen is transferred to a *meso*-carbon from its normal position on a nitrogen atom. Isoporphyrins exhibit an interrupted macrocyclic conjugation due to the presence of a saturated bridging carbon atom. This lack of a continuous ring of overlapping p orbitals makes the system nonaromatic. The existence of this type of compound was first suggested by Woodward<sup>6</sup> in 1961 after the discovery of a related class of compounds called phlorins (3) and the recognition of their stability. Phlorins are dihydroisoporphyrins in which two hydrogen atoms are added to the nitrogens.



Woodward's prediction was confirmed about a decade later when Dolphin et al. reported the first synthesis of metalloisoporphyrin (**4**) by way of electrochemical oxidation of zinc(II) *meso*-tetraphenylporphyrin (ZnTPP) (**5**), followed by nucleophilic attack of methanol on the oxidized dication of ZnTPP (**Scheme 2.1**).<sup>7</sup> Metalloisoporphyrin was a dark green solid which was stable in glacial acetic acid.



Scheme 2.1: Electrochemical oxidation of ZnTPP (5) to afford zinc isoporphyrin (4)

Addition of potassium iodide to such a solution brought about a rapid and quantitative reduction and demetalation to give tetraphenylporphyrin dication (6).

Metalloisoporphyrins such as (4) can also be generated by peroxide oxidation of metalloporphyrins. For example, reaction of iron(III) porphyrin (7) and *t*-butyl hydroperoxide gave the isoporphyrin cation (8) (Scheme 2.2),<sup>11</sup> and zinc(II) tetraphenylporphyrin (5) upon reaction with benzoyl peroxide afforded the zinctetraphenylbenzoyloxyisoporphyrin benzoate (9), which could easily be converted back to porphyrin either by photolysis or treatment with amines (Scheme 2.3).<sup>12</sup> Metalloisoporphyrins can also be generated by photooxidation of the corresponding metalloporphyrins<sup>13,47-49</sup> in the presence of nucleophiles.

The interruption in the macrocyclic conjugation of metalloisoporphyrins is evident in their <sup>1</sup>H NMR spectra. The signals for the pyrrolic protons of metalloisoporphyrin (**4**) undergo a 2.5 ppm upfield shift with respect to tetraphenylporphyrin (**5**), appearing at about 6.5 ppm. This upfield shift is caused by the loss of the aromatic ring current leading to a decrease in anisotropic effect. Porphyrins exhibit strong absorptions around 400 nm (Soret band) and at 500-600 nm (Q bands); however, partial saturation of the conjugated ring system as seen in chlorins and chlorophylls extends the absorption to longer wavelengths.<sup>8</sup> With respect to their electronic spectrum, metalloisoporphyrins show strong absorptions at about 400 and 800 nm. Their characteristic absorptions at long wavelengths make metalloisoporphyrins potential candidates as photosensitizers for photodynamic therapy. Also, they could not only be a new class of infra-red dyes of moderate photosensitivity, but also of biological interest due to their unique redox behavior among porphyrin derivatives.<sup>12,50-54</sup>



Scheme 2.2: Peroxide oxidation of metalloporphyrin (7)



Scheme 2.3: Oxidation of metalloporphyrin and reduction of metallo-isoporphyrin

Unlike porphyrins, isoporphyrins are not very sensitive to near-infrared and long wavelength visible light and therefore can be handled under room light.

Previously reported isoporphyrins were all derived from tetraphenylporphyrins and they are usually not stable in the sense that they are easily converted into the corresponding porphyrins.<sup>7,12</sup> Except in unusual circumstances, [such as when a *meso*carbon is geminally substituted]<sup>6,7</sup> isoporphyrins are usually unstable with respect to transformation back to the fully conjugated porphyrin chromophore. This problem was overcome when Xie and Smith<sup>17</sup> successfully reported the total synthesis of the first thermodynamically stable zinc isoporphyrin (10). The 5,5-dimethyl group on zinc isoporphyrin (10) prevents the compound from going through the thermodynamically favored isomerization into the corresponding porphyrin. The synthesis of zinc isoporphyrin was achieved by a variation of the MacDonald<sup>3</sup> '2+2' method of porphyrin synthesis involving the condensation of dipyrromethane dicarboxylic acid (11) and diformydipyrromethane (12) in the presence of zinc(II) acetate (Scheme 2.4). The insertion of zinc during the reaction helps stabilize the isoporphyrin. Zinc isoporphyrin (10) was isolated as dark green solid in very low yields. The availability of this stable zinc isoporphyrin immediately initiated a series of studies on this compound including the electrochemistry,<sup>20</sup> crystollography,<sup>18</sup> and the photophysical properties<sup>19</sup> of the isoporphyrin.

Unlike previously reported isoporphyrins, zinc isoporphyrin (10) was found to be stable with respect to transformation to porphyrins.<sup>17,55</sup> Zinc isoporphyrin (10) can be stored in solid form at room temperature in daylight for months without decomposition. It





Scheme 2.4: Synthesis of stable isoporphyrin via MacDonald 2+2 approach

is stable in water and methanol, even at temperatures as high as 250 <sup>o</sup>C without either melting or decomposing. It is also stable in glacial acetic acid and 10% hydrochloric acid, however when treated with trifluoroacetic acid (TFA) at room temperature, it decomposes within 30 minutes with concomitant demetalation.

# 2.2 Research Objective

Due to their instability, the investigation of isoporphyrins has been hampered. The product yields reported were low between 2-23%. Methods for synthesis of 5,5-dimethyl dipyrromethanes<sup>55</sup> (such as **11**) utilized in the MacDonald 2+2 synthesis also gave moderate yields (40-50%). This led us to our project of finding a better synthetic pathway of isoporphyrins and that is by way of open-chain tetrapyrrole precursors with ring closure as the final step. It was reported<sup>56</sup> that the a,c-biladiene synthetic approach failed to yield isoporphyrin and we opted to explore the b-bilene approach. The b-bilene route was chosen because it is simple, direct, and utilizes crystalline intermediates at all stages. However, its disadvantages can concern symmetry, substituent limitations associated with the bilene intermediates, and difficulty in purification of the b-bilenes if they do not happen to crystallize readily.<sup>5</sup> This method for regular porphyrin synthesis works best when electron-withdrawing substituents are present on the b-bilene.<sup>34</sup>

## 2.3 Synthesis

Retrosynthetically, our target molecule, zinc(II) -2,3,5,5,7,8,12,13,17,18decamethylisoporphyrin chloride (**13**), was synthesized by cyclisation of 1,19-di-tertbutoxycarbonyl-2,3,7,8,12,13,17,18-octamethyl-b-bilene (**14**) which utilizes the condensation of 1-(*t*-butyloxycarbonyl)dipyrromethane-9-carboxylic acid (**15**) with tbutyl 9-formyldipyrromethane-1-carboxylate (**16**) (**Scheme 2.5**).

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The synthesis of the first dipyrromethane precursors, pyrrole **20**, was achieved as outlined in **Scheme 2.6**. Treatment of *t*-butyl acetoacetate with sodium nitrite, acetic acid and water yielded tert-butyl oximinoacetate<sup>57</sup> (**17**) which was condensed with 3-methyl-2,4pentanedione (**18**) in acetic acid under standard Johnson conditions<sup>57,58</sup> in the presence of zinc dust and sodium acetate at 100-115  $^{0}$  C to afford *t*-butyl-3,4,5-trimethylpyrrole-2carboxylate (**19**) in 40% yield. This pyrrole was then treated with lead tetra-acetate<sup>59</sup> (LTA) at room temperature to undergo a radical reaction, which afforded 5acetoxymethylpyrrole (**20**) in 61% yield. This yield was achieved after using excess of LTA. Low amounts or same equivalent of LTA gave reduced yields of the pyrrole.

The other precursor pyrrole, the 5-unsubstituted pyrrole (**24**) was prepared via the Barton-Zard method,<sup>60</sup> **Scheme 2.7**. These kind of pyrroles with substituents on the 3,4-positions, a benzyl ester on 2-position, and unsubstituted in the 5-position, are important and widely used precursors to the dipyrromethanes utilized in the stepwise synthesis of porphyrins, because the benzyl esters can be easily (and quantitatively) removed or reduced to carbinols under neutral conditions.<sup>1</sup> 2-Acetoxy-3-nitrobutane (**21**), which was obtained by base catalyzed addition of nitroethane to acetaldehyde (the Henry Reaction) followed by acetylation,<sup>61</sup> was condensed with commercially available ethyl isocyanoacetate (**22**) to give ethyl ester pyrrole (**23**) in 80% yield. Due to the difficulty in handling and storing small, very base sensitive nitroolefins, β-acetoxy nitroalkanes are employed. Under basic conditions, β-elimination of the acetate group generates the requisite nitroolefin in situ.<sup>60</sup>



Scheme 2.5: Retrosynthesis of (13)



Scheme 2.6: Synthesis of pyrrole (20)

Transesterification of the ethyl ester pyrrole (23) with PhCH<sub>2</sub>ONa in refluxing benzyl  $alcohol^{62}$  gave a very high yield of the pyrrole (24) bearing the benzyl ester moiety. When equal amounts of ethyl ester pyrrole (23) and sodium were used, the yields were reduced. Attempts to synthesize benzyl ester pyrrole (24) in one step using benzyl isocyanoacetate<sup>63</sup> (thus avoiding the transesterification step) failed, after synthesis of

benzyl isocyanoacetate seemed unproductive. **Figure 2.1** shows the crystal structure of pyrrole (**24**). The crystal was obtained by dissolving the pyrrole in dichloromethane and letting it stand at room temperature, allowing the solvent to evaporate off.



Scheme 2.7: Synthesis of pyrrole (24) via Barton-Zard's method



Figure 2.1: Crystal structure of benzylester pyrrole (24)

Two synthetic pathways were attempted for the construction of dipyrromethane unit by condensation of pyrroles (**20**) and (**24**). First, the 5-unsubstituted pyrrole (**24**) was coupled with 5-acetoxymethylpyrrole (**20**) under acidic conditions<sup>64</sup> to afford the symmetrical dipyrromethane (**25**) in low yields (**Scheme 2.8**). This was not the expected product and it is postulated that acidic conditions initiated the formation of benzylic carbocation (**27**) (very stable), which further reacted with the 5-unsubstituted pyrrole (**24**) to yield the symmetrical dipyrromethane (**25**) (**Scheme 2.9**). The second pathway utilized a catalyst, Montmorillonite clay K-10<sup>65</sup> in methylene chloride at room temperature, and the expected dipyrromethane, *t*-butyl 9-((benzyloxy) carbonyl)-3, 4, 7, 8-tetramethyldipyrromethane-1-carboxylate (26) was produced in 96% yield after column chromatography (Scheme 2.8). The crystal structure of (26) is as shown in figure 2.2 and was obtained by slow evaporation (at room temperature) of solvent from a solution of dipyrromethane (26) and dichloromethane.



Scheme 2.8: Synthesis of dipyrromethane



Figure 2.2: Crystal structure of dipyrromethane (26)



Scheme 2.9: Postulated mechanism for generation of symmetrical dipyrromethane (25)

Construction of b-bilene (14) was more difficult. The mixed ester dipyrromethane (26) was de-benzylated by hydrogenolysis over 10% Pd/C to afford the corresponding dipyrromethane monocarboxyllic acid (15) in 100% yield. Decarboxylation with 2 equivalents of *p*-toluenesulfonic acid followed by formylation using the modified Vilsmeier-Haack<sup>66</sup> procedure (PhCOCl/ DMF) gave the required formyldipyrromethane in a relatively good yield (16) (Scheme 2.10). Precipitation of the imine salt after addition of PhCOCl/DMF complex, as expected for diformylation (bisimine salt), did not occur for our case because it is a mono-imine salt. Vilsmeier



Scheme 2.10: Synthesis of formyl dipyrromethane (16)

conditions were chosen over the standard triethyl orthoformate/TFA conditions to prevent decomposition of the t-butyl ester groups on the starting material. Some difficulty, however, was encountered in the formylation reaction with the original Vilsmeier-Haack reagent (POCl<sub>3</sub>/DMF), which gave non-crystalline product. The yield of the product was extremely low in cases when crystallization occurred. PhCOCl/DMF forms the reactive

intermediate chloromethyleneiminium salt **29** (Scheme 2.11), which is similar to the species suggested as being involved in POCl<sub>3</sub>/DMF formylations. It occurs that the use of benzoyl chloride in place of phosphorous oxychloride might have some advantages in formylation procedures, particularly in systems such as the dipyrromethanes, which are often sensitive to acidic reagents.<sup>66</sup> It is predicted that, the modified Vilsmeier conditions are mild compared with the harsh conditions of POCl<sub>3</sub>/DMF which generates hydrochloric acid. Phosphorous, being an 'oxygen lover', would easily release chloride ions in exchange of oxygen, which inturn generate hydrochloric acid in the presence of hydrate conditions.



Scheme 2.11: Generation of chloroethyliminium salt intermediate

Condensation of formyldipyrromethane (16) with dipyrromethane monocarboxylic acid (15) using 2 equivalents of *p*-toluenesulfonic acid in dichloromethane afforded the corresponding b-bilene (14) which showed a strong absorption at around 500 nm.<sup>64</sup> This was converted into the hydrochloride salt by brief treatment with HCl gas and there was a noticeable color change from yellow-orange to dark red. Recrystalization of the species yielded orange-red prisms of 14 as the crystalline hydrochloride in 70-80% yield (Scheme 2.12). The UV/Visible spectrum of (14) in dichloromethane is as shown in figure 2.3 at 502 nm with an extinction coefficient of  $1.11 \times 10^5 \text{ M}^{-1} \text{cm}^{-1}$ . Figure 2.4 shows the x-ray structure of (14) whose crystals were obtained by slow diffusion of petroleum ether into a concentrated solution of (14) in dichloromethane, and figure 2.5 shows its proton NMR spectrum in CDCl<sub>3</sub>.

Cyclization of b-bilene (Scheme 2.13) was attempted by first cleaving the *t*-butyl ester units with TFA to give the  $\alpha$ -unsubstituted open chain tetrapyrrole which was then treated with excess cyclohexanone as the isoporphyrin carbon-linking unit under the templating effect of zinc (II) acetate in methanol in the presence of *p*-TsOH in air. Cyclohexanone was used since earlier attempts<sup>56</sup> to cyclize a,c-biladiene with acetone failed to yield isoporphyrin. The reaction was monitored using UV/Visible spectrophotometer for 6 days, which showed no absorption peak around 800nm, but at around 400 nm and Q-like-bands around 500-600 nm, implying that no zinc isoporphyrin (13) was produced. Attempts to facilitate oxidation by addition of *p*-chloranil failed to yield the product. The UV/Visible spectra suggested that a very stable compound was being formed because there were no changes in the absorption even after addition of *p*-chloranil or bubbling of oxygen to the reaction solution.



Scheme 2.12: Synthesis of b-bilene salt (14)



Figure 2.3: UV-Visible spectrum of b-bilene hydrochloride (14) in CH<sub>2</sub>Cl<sub>2</sub>



Figure 2.4: Crystal structure of b-bilene hydrochloride (14)



Figure 3: <sup>1</sup>H-NMR spectrum of b-bilene hydrochloride (14)



Scheme 2.13: Attempted cyclization of b-bilene with cyclohexanone

The absorption bands suggested the formation of a metal-porphyrin, zinc octamethylporphyrin as was evident from the mass spectrum. The product implies that cyclohexanone did not react as a carbon-linking unit in the cyclization step. Instead, in the presence of acid and lack of a carbon-linking unit, the open-chain precursor underwent cleavage and recombination of the dipyromethane links to yield the porphyrin.<sup>5</sup> Studies by Ghosh and Lightner<sup>67</sup> in their synthesis of 10,10-gem substituted bilirubin analogs (scheme 2.14) indicate that ketones are very unreactive in these kinds of reactions. These workers attempted activation of the ketone to its reactive ketal by treatment with trimethyl orthoformate and condensation of the ketals in acidic conditions with suitable substrates,  $\alpha$ -unsubstituted dipyrrinones, to give excellent yields of the

product within 5minutes of reaction time (Scheme 2.14). We attempted to adapt the above method for the cyclization step. Commercially available cyclohexanone dimethyl ketal was reacted with b-bilene applying the same synthetic procedure as before and with TFA as acid catalyst but did not yield the product. Different reaction conditions were employed, only to yield ZnOMP or OMP as the major products as shown by mass spectrometry, (Scheme 2.15).



Scheme 2.14: Condensation reactions utilizing dimethyl ketals



Scheme 2.15: Cyclization of b-bilene with cyclohexanone under various reaction conditions

This initiated a study of steric factors verses electronic factors for the carbon-linking unit.

#### • Electronic Factors

As a starter, aldehyde, which is more reactive than a ketone, was used in the cyclization step and within 1 h, reaction was complete to yield *meso*-monosubstituted porphyrin in good yield. Various benzaldehydes with different substituents were employed as carbon linking units to generate the corresponding porphyrins. (See Chapter 4b)

### • Steric Factors

2,2-Dimethoxypropane, the smallest of the ketals, was used as the reference. Using the same procedure as above, after 28 hours, UV/Visible spectroscopy suggested formation of the metal-free isoporphyrin (green) with a characteristic absorption at around 440 and 690 nm. Due to previous reports on unstability of metal-free isoporphyrins in the presence of bases,<sup>56</sup> work-up was done by washing the reaction mixture several times with water to remove the last traces of TFA. The <sup>1</sup>H NMR spectrum of the crude product showed singlets for the *meso* protons at 8.20 and 7.33 ppm. To confirm that the <sup>1</sup>H NMR and electronic absorption properties described above were actually for metal-free isoporphyrin, insertion of zinc ions by treating the crude product with zinc(II) acetate caused a red shift in the absorption spectrum to 810 nm, the characteristic absorption of zinc isoporphyrin (**Scheme 2.16**). Purification on a silica gel column and recrystallization gave zinc isoporphyrin cation (greenish) in 28% yield compared to 6% yield previously reported.<sup>56</sup>

The unsuccessful cyclization of b-bilene with cyclohexanone dimethyl ketal as the carbon linking unit is most likely associated with steric hindrance. Molecular modeling

51





+



Abs 448nm and 700nm







30

Scheme 2.16: Synthesis of zinc isoporphyrin (30)



Total E: 101.07Kcal/mol



Figure 2.6: Molecular modeling structure of 5,5-spirocyclohexylisoporphyrin







Figure 2.7: Molecular modeling structure of 5,5-dimethylisoporphyrin



Figure 2.8: UV-Visible spectrum of zinc isoporphyrin (30) in CH<sub>2</sub>Cl<sub>2</sub>



**Figure 2.9:** Emission spectrum of zinc isoporphyrin in CH<sub>2</sub>Cl<sub>2</sub>, Excitation  $\lambda = 410$ nm



**Figure 2.10:** <sup>1</sup>H-NMR spectrum of zinc isoporphyrin (30)

technique using energy models by calculating the minimal energy of the molecular structure of 5,5-dimethylisoporphyrin and 5,5-spirocyclohexylisoporphyrin (Figure 2.6 & 2.7) were employed using Sybyl® software to study the concept of sterics on the macrocycle. It was expected that 5,5-spirocyclohexylisoporphyrin would give a higher energy than 5,5-dimethylisoporphyrin, but that was not the case. The low-energy models indicate similar energy for both compounds and we believe that the transition state for the cyclization process with cyclohexanone dimethyl ketal may have a higher energy, thus unstable and unfavorable to yield 5,5-spirocyclohexylisoporphyrin.

**Figure 2.8** shows the optical spectrum of zinc (II) isoporphyrin chloride (**30**) in dichloromethane, with characteristic absorptions at 416nm and 804nm. Its fluorescence spetrum in dichloromethane at 295k is shown in **Figure 2.9**. The fluorescence maximum occurs at 820nm with the excitation wavelength of 410nm, exhibiting a Stoke's shift of 243 cm<sup>-1</sup> which is larger than 50 cm<sup>-1</sup> observed for zinc octaethylporphyrin, but smaller than 600 cm<sup>-1</sup> reported for zinc isoporphyrins in a different solvent, acetonitrile. **Figure 2.10** shows the proton NMR spectrum of (**30**) in deuterated chloroform.

### 2.4 Synthesis of a Novel Copper Isoporphyrin

The same synthetic procedure of zinc isoporphyrin discussed above was followed. Metalation of the metal free isoporphyrin with cuprous chloride yielded a novel copper isoporphyrin (**Scheme 2.17**) with an observed bathochromic shift of both the Soret and the Q band in its optical spectrum (**Figure 2.11**) to 428 and 842 nm (compare with zinc isoporphyrin – 416 and 804 nm). Since the copper(I) salt was used, we speculated formation of a neutral copper(I) isoporphyrin complex. However, qualitative EPR studies (**Figure 2.12**) performed to detect the presence or absence of paramagnetism in the copper isoporphyrin macrocycle, confirmed the presence of the paramagnetic copper(II) species. This yielded a paramagnetic copper cationic isoporphyrin complex with a chloride counterion. <sup>1</sup>H-NMR spectroscopy of this compound in CDCl<sub>3</sub> showed no signals for the macrocycle. Characterization was therefore done using UV/Visible, low resolution and high resolution mass spectrophotometry (**Figure 2.13**).

Since the analysis proved that the metal complex was copper(II), we attempted to synthesize the copper isoporphyrin using copper(II) salts but this was unsuccessful. Probably, copper (I) is small enough to fit well into the cavity of isoporphyrin, but due to its unstable nature, it is easily oxidized to the more stable copper (II) species.



Scheme 2.17: Synthesis of copper isoporphyrin (31)



Figure 2.11: UV-Visible spectrum of copper isoporphyrin (31) in CH<sub>2</sub>Cl<sub>2</sub>



**Figure 2.12:** WinEPR spectrum of copper(II) isoporphyrin in dichloromethane. Frequency: 9.634 GHz, Mod. Frequency: 100.00 kHz, Power: 20.170 mW, Mod. Amplitude: 4.00G, Temprature: 295K



**Figure 2.13:** High Resolution Mass Spectrum of copper isoporphyrin (**31**), showing an overlay of theoretical (dashed line) vs. actual (solid line) spectra

While porphyrin complexes with diamagnetic metals exhibit fluorescence, those with paramagnetic metals are non-fluorescent due to quenching of the fluorescence. Apparently, this was not the case with the copper(II) isoporphyrin (**31**). Figure 2.14 shows the emission spectrum of (**31**) in chloroform/methanol (4:1) upon excitation at wavelength 420 nm. The emission properties of the copper complex were confirmed in the fluorescence microscopy studies for PDT application (see chapter 5). The copper isoporphyrin therefore is not as paramagnetic as copper porphyrin, probably because the coordination geometry of copper in copper isoporphyrin is not square planar as in porphyrin. Probably, it is more pyramidal, thus changing the energy of the orbitals. Due to the observed abnormalities, further physicochemical properties, geometries..., etc, on this complex need to be investigated.

This complex is stable in solid form and in various solvents.



**Figure 2.14:** Emission spectrum of copper (II) isoporphyrin; Ex  $\lambda$  = 420nm
# 2.5 Conclusion

b-Bilene route proved to be a successful pathway for the synthesis of metalloisoporphyrin in higher yields (28% vs. 6%) and in a much shorter reaction time (24 h vs. 6 days) than previously reported. Through this route, a novel copper isoporphyrin was obtained. Sterically hindered ketones or their ketals are unfavorable carbon-linking units for this kind of cyclization reaction to obtain isoporphyrins.

Since there is not much known about isoporphyrins, more studies is at stake for these compounds; synthesis of other metal complexes and studying their physicochemical properties, geometries, stabilities, ligand properties of isoporphyrins, etc.

# 2.6 Experimental

## • Characterization of Compounds

UV/Visible: Electronic absorption spectra were measured on PerkinElmer Lambda 35 UV/VIS Spectrometer

**Mass Spectrometry:** Low and high resolution mass spectra were obtained at the Mass Spectrometry Facility at Louisiana State University, Baton Rouge, LA, on a Bruker ProFlex III MALDI-TOF and Hitachi M8000 ESI mass spectrometer. The compounds were dissolved in dichloromethane or chloroform using dithranol as the matrix and in acetonitrile for HR-ESI.

**EPR:** The EPR data were acquired on Bruker WinEPR.

**NMR:** Proton NMR spectra were obtained on a Bruker DPX-250, Bruker ARX-300, Bruker DPX-400 and a Varian INOVA-500 MHz spectrometers.

Deuterated solvents:  $CDCl_3 - 7.26$  ppm,  $(CD_3)_2SO - 2.54$  ppm,  $(CD_3)_2CO - 2.05$  ppm, Chemical shifts ( $\delta$ ) are given in parts per million, multiplicities are indicated as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet) and m (multiplet). **Fluorescence:** The emission spectra were measured on Fluorolog-3 spectrofluorometer.

### • Chromatographic Methods

Analytical thin-layer chromatography: Sorbent Technologies 200  $\mu$ m silica gel or alumina neutral plates with UV 254 were used to monitor all reactions.

**Column chromatography:** Two types of packing material were used: (i) E. Merck neutral alumina (70-230 mesh) either deactivated with 6% water (grade III) or non-deactivated (grade 0); (ii) Merck silica gel 60 (70-230 mesh).

# • Purification of Solvents and Reagents

Unless otherwise indicated, all commercially available starting materials were used directly without further purification.

Dimethylformamide (DMF): stored over 4A molecular sieves

Methanol: distilled over CaCl<sub>2</sub>

Dry dichloromethane (DCM), toluene, tetrahydrofuran (THF), and hexane were obtained from a specially designed solvent purification system from Innnovative Technology, Inc.

# *t*-Butyl 3,4,5-Trimethylpyrrole-2-carboxylate (19)

*t*-Butyl oximinoacetate (17), which was prepared by addition of *t*-butyl acetoacetate (13.0 g, 0.08 mol), acetic acid (27 ml), sodium nitrite (5.75 g, 0.08 mol) and water, was added dropwise to a solution of 3-methyl-2,4-pentanedione (18, 12.5 g, 0.11mol) in acetic acid during portion wise addition of an intimate mixture of zinc dust (12.5 g) and sodium acetate (12.5 g). The rate of addition was controlled so that the

mixture was maintained at 65 °C. After addition was complete the mixture was heat under reflux for 1 h after which TLC showed product. The solution was poured into ice water, filtered, washed with water, dissolved in dichloromethane, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and then evaporated to dryness. The solid was recrystallized in dichloromethane/ethanol and put in the freezer to further crystallization, to yield yellow crystals (6.32 g, 38%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  8.45 (s, br, 1H), 2.23 (s, 3H), 2.18 (s, 3H), 1.91 (s, 3H), 1.56 (s, 9H).

# t-Butyl 5-Acetoxymethyl-3,4-dimethylpyrrole-2-carboxylate (20)

In a round-bottomed flask, trimethylpyrrole, (**19**, 1.74 g, 8.3 mmol) was stirred in acetic acid (39.0 ml) and acetic anhydride (1.0 ml) for 1.5 h with portion wise addition of lead tetra-acetate (4.05 g, 9.1 mmol) under argon. The stirring was continued overnight, after which TLC showed completion of reaction. The reddish-brown solution was treated drop wise with 40 ml ice water and a flaky precipitate formed. The crude pyrrole was filtered, washed with water, dissolved in dichloromethane, dried over anhydrous MgSO<sub>4</sub> and evaporated to dryness. The solid was then recrystallized from DCM/ hexane and put in the freezer to further crystallization, to give a fluffy off-white solid (1.357 g, 61%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  8.85 (s, br, 1H), 5.02 (s, 2H, -CH<sub>2</sub>-O), 2.23 (s, 3H, COCH<sub>3</sub>), 2.07 (s, 3H), 2.01 (s, 3H), 1.56 (s, 9H).

#### 2-Acetoxy-3-nitrobutane (21)

In a three-necked round-bottomed flask equipped with a magnetic stirrer and chilled in ice-salt bath, acetaldehyde (13.0 ml, 0.465 mol), 2-propanol (8.5 ml) and potassium fluoride (0.67 g, 0.023 mol, 0.05 mol equiv.) were added. To the mixture, nitroethane (16.5 ml, 0.46 mol) was added drop wise at 0 °C over a period of 1hr. The

mixture was slowly warmed up to room temperature and kept for 10 h under argon with continuous stirring before removing all solvent under vacuum. The resulting oily crude product was filtered to remove solid inorganic waste and washed with dichloromethane. After removing all solvent under vacuum, colorless oily product, 2-nitro-butanol-3, was obtained (33.78 g, 62%) which was immediately used for the next step synthesis.

2-Nitro-butanol-3 (7.5 g, 63 mmol) was added drop wise over 10 minutes period to a solution of dichloromethane (5 ml), acetic anhydride (9.60 g, 94 mmol, 1.5 equiv.), and 4-dimethylaminopyridine (DMAP, 0.1 g). The reaction is exothermic. The mixture was allowed to stir for 4 h at room temperature under argon. Methanol (30 ml) was added to destroy excess acetic anhydride and allowed to stir for further 30 minutes. The mixture was then poured into dilute sodium bicarbonate (9 g in 50 ml water) and extracted with dichlomethane (3 x 20 ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and filtered through a short column of silica. Evaporation of the solvent gave the desired product **21** as a yellowish liquid (11.86 g, 58%).

**2-Nitro-butanol-3** <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz) δ 4.43-4.46 (m, 1H), 4.39-4.40 (m, 0.5H), 4.04-4.09 (m, 0.5H), 3.27 (s, br, OH), 1.44-1.51 (m, 3H), 1.16-1.21 (m, 3H) (it is a mixture of two isomers)

**21** <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz) δ 5.19-5.34 (m, 1H), 4.55-4.65 (m, 1H), 2.05 (d, 3H, COCH<sub>3</sub>), 1.52-1.56 (m, 3H), 1.27-1.31 (m, 3H) (it is a mixture of isomers)

# Ethyl 3, 4-Dimethylpyrrole-2-carboxylate (23)

In a multi-neck round-bottomed flask, equipped with a magnetic stirrer, ethyl isocyanoacetate (**22**, 2.51 g, 19 mmol, 1.05 equiv.), tetramethylguanidine (4.41 g, 38 mmol, 2. 05 equiv.) and a 1/4 mixture of dry THF (3 ml) and isopropanol (3 ml) were

added and the flask cooled in ice water bath. To the mixture, at 0 °C, a solution of 2acetoxy-3-nitrobutane (**21**, 3.0 g, 18.6 mmol) in the rest of 3/4 mixture of dry THF and isopropanol was added drop wise over a period of 30 minutes. The mixture was allowed to stir at room temperature for another 20 h under argon after the addition was complete. The resulting mixture was concentrated under vacuum to dryness. The oily residue was taken up by dichloromethane (22 ml) and washed successively with water (3 x 6 ml), 5% aqueous HCL (93 x 6 ml), water (3 x 6 ml), aqueous saturated sodium bicarbonate (6 ml) and brine (6 ml). After drying over anhydrous Na<sub>2</sub>SO<sub>4</sub>, solvent was removed under vacuum to yield the product **23** (2.5 g, 83%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  8.79 (s, br, 1H), 6.59 (d, 1H), 4.32 (q, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.27 (s, 3H), 2.02 (s, 3H), 1.36 (t, 3H, CH<sub>2</sub>-CH<sub>3</sub>)

# Benzyl 3, 4-Dimethylpyrrole-2-carboxylate (24)

Sodium (0.168 g, 7.3 mmol) was added to benzyl alcohol (60 ml) in a roundbottomed flask. Once all the sodium had reacted, ethyl ester pyrrole (**23**, 2.32 g, 14 mmol) was added and the resultant mixture heated in an oil bath for 6 h. The mixture was allowed to stand at room temperature overnight and acetic acid (0.438 g, 7.3 mmol) was added to neutralize the sodium benzyloxide. Benzyl alcohol was evaporated under reduced pressure to dryness and the residue which solidified on standing was dissolved in dichloromethane, washed with water and treated with decolorizing carbon. After filtration through a celite cake, the solution was dried over MgSO<sub>4</sub> and evaporated the solvent under vacuum to yield a liquid, which was further distilled off under vacuum to give a thick liquid. The residue was dried under vacuum to give brown solid (1.5 g, 90%). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz) δ 8.70 (s, br, 1H), 7.35-7.41 (m, 5H), 6.62 (d, 1H), 5.31 (s, 2H), 2.29 (s, 3H), 2.02 (s, 3H)

*t*-Butyl 9-(Benzyloxy carbonyl)-3,4,7,8-tetramethyldipyrromethane-1carboxylate.

• Method A: Using acid (25)

A suspension of 5-acetoxymethyl pyrrole (**20**, 268 mg, 1.0 mmol) and 5unsubstituted pyrrole (**24**, 230 mg, 1.0 mmol) in acetic acid or methanol (5 ml) was treated with *p*-toluenesulphonic and then heated at 40-42 °C with stirring under argon. After 4 h, TLC analysis showed completion of reaction. Dichloromethane was added and the solution washed with aqueous sodium acetate, aqueous sodium hydrogen carbonate and finally water. The organic phase was dried over MgSO<sub>4</sub> and evaporated to dryness. The residue was recrystallized from dichloromethane/hexane and put in freezer to further crystallization. After filtration, product **25** was obtained as pink fluffy solid in a very low yield (80 mg). <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz with a relaxation delay of 60 seconds)  $\delta$  8.75 (s, br, 2H), 7.28-7.36 (m, 10H), 5.26 (s, 4H, CH<sub>2</sub>-Ph), 3.80 (s, 2H), 2.26(s, 6H), 1.94 (s, 6H)

# • Method B: Using catalyst K-10 (26)

5-Acetoxymethylpyrrole (**20**, 1.36 g, 5.1 mmol) and 5-unsubstituted pyrrole (**24**, 2.29 g, 10 mmol), under argon in a round-bottomed flask, were stirred with distilled dichloromethane (100 ml) and Montmorillonite clay K-10 (1.92 g) for 24 h at room temperature. The clay was filtered off and rinsed copiously with dichloromethane (600 ml). Evaporation of solvent gave brownish oil, which was then flashed-chromatographed on silica gel, eluting with 3% methanol/dichloromethane, to give an oily residue, which

under high vacuum yielded a yellowish-brownish solid (2.136 g, 96%). Some 5unsubstituted pyrrole (**24**) was recovered. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz) δ 8.65, 8.50 (s, br, 1H each), 7.36-7.39 (m, 5H), 5.28 (s, 2H, CH<sub>2</sub>-Ph), 3.82 (s, 2H), 2.27 (s, 3H), 2.22 (s, 3H), 1.95 (s, 3H), 1.93 (s, 3H), 1.54 (s, 9H)

1-(*t*-Butyloxycarbonyl)-2,3,7,8-tetramethyldipyrromethane-9-carboxylic acid (15)

Mixed ester dipyrromethane (**26**, 2.0 g, 46 mmol) was dissolved in approximately 80 ml of freshly distilled THF and degassed with argon for approximately 15 minutes. 10% Pd/C (0.13 g) was added to the solution and the flask evacuated of air, sealed and filled with hydrogen gas. (Hydrogen gas was filled in a balloon and connected to the reaction flask.) The reaction mixture was left to stir for 16 h after which TLC showed reaction was complete. The reaction mixture was then filtered through a bed of celite cake to remove the catalyst, which was washed with THF. The collected filtrate was evaporated at which point yellowish foamy solid formed. Drying under vacuum gave the product in 100% yield. (1.57 g) <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  11.45, 10.80 (s, br, 1H each), 3.85 (s, 2H), 2.29, 2.19, 2.08, 2.01 (s, 3H each), 1.55 (s, 9H)

# *t*-Butyl 9-Formyl-2,3,7,8-tetramethyldipyrromethane-1-carboxylate (16)

Dipyrromethane monocarboxylic acid (**15**) (300 mg, 0.86 mmol) was dissolved in 40 ml of distilled dichloromethane under argon. *p*-Toluenesulphonic acid (328 mg, 1.7 mmol) was added in three portions at 2 minute interval. Stirring was continued for 1.5 h afterwhich TLC showed completion of reaction. Saturated sodium bicarbonate was added to neutralize the solution, then brine, and dried over MgSO<sub>4</sub>. Evaporation of the solvent yielded a brown-reddish oil which was dried under high vacuum to remove any solvents. Dry DMF (1.0 ml) was added to the crude material and the solution cooled to 0 °C. Meanwhile, Vilsmeier complex was prepared by adding benzoyl chloride (0.6 ml, 5.1 mmol) dropwise to dry DMF (0.8 ml, 10.3 mmol) at 0 °C, with stirring for 30 minutes. The Vilsmeier complex was added dropwise to the decarboxylated mixture with stirring and after 15 minutes, stirring was continued at room temperature for further 50 minutes. TLC showed formation of the iminium salt. Toluene (10 ml) was added and little grains of precipitate were formed. Toluene was evaporated and ethanol plus 1.5 g of sodium bicarbonate in 25 ml was added. Stirring was continued overnight at room temperature, followed by extraction of the aldehyde with dichloromethane. Drying of the solution over MgSO<sub>4</sub> and evaporation of the solvent yielded a brown semi-solid, which was chromatographed on silica gel column eluting with 3% methanol/dichloromethane to afford 148 mg (52%) of the title product. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  10.05(s, br,1H), 9.50 (s, 1H), 9.22 (s, br, 1H), 3.88 (s, 2H), 2.26, 2.24, 1.99, 1.96 (s, CH<sub>3</sub> each), 1.52 (s, 9H)

Di-*t*-butyl 2,3,7,8,12,13,17,18-Octamethyl-b-bilene-1,19-dicarboxylate hydrochloride (14)

50.0 mg (0.14 mmol) of dipyrromethane monocarboxylic acid (15) and formyldipyrromethane (16) (40 mg, 0.12 mmol) were dissolved in 10 ml of dry dichloromethane and stirred under argon. *p*-Toluenesulfonic acid (53.0 mg, 2 equiv.) was added in two portions to the solution and stirring was continued for 2 h afterwhich TLC showed no starting material and the UV/Visible spectrum showed a strong absorption at 502 nm. The dark red solution was washed with 5% sodium carbonate solution and water and dried over magnesium sulphate. Evaporation of solvent under reduced pressure

afforded the tetrapyrrolic intermediate **30** (b-bilene). The dark residue was then dissolved in 5 ml dichloromethane and hydrogen chloride gas was bubbled through the yellowishorange solution for 30 seconds and the color changed to dark red, forming the hydrochloride salt. Immediately, the solvent was evaporated and the residue taken up twice in dry toluene and evaporated in order to remove any traces of water and HCl. The residue was recrystallized in DCM/hexane and left in the freezer overnight. Filtration of solvent yielded orange-red prisms of the title compound (54 mg, 70%). UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm ( $\epsilon$  x 10<sup>5</sup>, M<sup>-1</sup>.cm<sup>-1</sup>): 502 (1.11); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 250 MHz)  $\delta$  13.8 (br, NH<sup>+</sup>, 2H), 10.4 (br, NH, 2H), 7.08 (1H), 4.2 (CH<sub>2</sub>, 4H), 2.23, 2.18, 2.04, 2.02, (each CH<sub>3</sub>, 6H), 1.55 (*t*-butyl, 18H).

# Zinc(II) 5-Spirocyclohexyl-2,3,7,8,12,13,17,18-octamethylisoporphyrin chloride (13)

b-Bilene (14) (22 mg, 0.03 mmol) was dissolved in 3 ml cold TFA and stirred for 10 minutes under argon. TFA was evaporated off and 8 ml of DCM added. Cylohexanone (35  $\mu$ l, 0.3 mmol, 10 equiv.), *p*-TsOH (38 mg, 0.2 mmol, 6 equiv.), and Zn(OAc)<sub>2</sub> (20 mg, 0.09 mmol, 3 equiv.) in 1 ml methanol were added to the stirring solution in air. The reaction was monitored by UV/Visible spectroscopy, which showed no absorption peak around 800 nm but at 400 and 500-600 nm. The reaction mixture was left to stir in air for 6days and there was no change in absorption peaks. Several attempts to facilitate fast oxidation by addition of *p*-chloranil and bubbling of oxygen seemed unsuccessful. The compound formed seemed very stable, because there was no change in the absorption peaks. It was assumed that cyclohexanone was not reactive enough and to activate it, more amounts of *p*-TsOH were added, but still cyclization was not complete. The

absorption spectra at 400 nm (Soret band) and Q-like bands between 500-600 nm suggest the formation porphyrin but in very small amounts as shown also by TLC.

# Zinc(II) 2,3,5,5,7,8,12,13,17,18-Decamethylisoporphyrin chloride (30)

Bilene (14) (50 mg, 0.08 mmol) was dissolved in 0.2 ml of cold TFA in a round bottomed flask and stirred for 10 min under argon. The mixture was diluted with dry dichloromethane followed by addition of zinc(II) acetate (50 mg) dissolved in dry methanol (2 ml), which acted as a template, and excess 2,2-dimethoxy propane. The mixture was left to stir in air for 28 h. TLC and UV/Visible showed formation of product. The electronic absorption spectrum showed peaks at around 440 and 690 nm characteristic of metal-free isoporphyrin. Work-up was done by washing with water twice, drying over Na<sub>2</sub>SO<sub>4</sub> and evaporating off the solvent. The residue was immediately dissolved in dichloromethane and zinc(II) acetate in methanol were added. After 15 minutes, the absorption spectrum showed successful insertion of zinc ions with absorption red shifting to 810nm, characteristic of zinc isoporphyrins.

The product was chromatographed on a silica gel column, eluting with 1-3% methanol/dichloromethane. The appropriate fractions were collected and the solvent was removed. The product was dissolved in dichloromethane, washed with saturated sodium chloride solution and dried over Na<sub>2</sub>SO<sub>4</sub>. Recrystalization using dichloromethane/petroleum ether afforded the product as green solid (12 mg, 28%). UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm ( $\epsilon \times 10^3$ , M<sup>-1</sup>.cm<sup>-1</sup>): 420.5 (48.9), 807.1 (47.5); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz)  $\delta$ , ppm 7.70 (s, *meso*-H, 1H), 7.60 (s, *meso*-H, 2H), 2.47, 2.43 (s,  $\beta$ -CH<sub>3</sub>, 24H), 1.96 (s, 5-CH<sub>3</sub>, 6H); HRMS MALDI-TOF calcd. for C<sub>30</sub>H<sub>33</sub>N<sub>4</sub>Zn 513.1996,

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found m/z 513.1990 (M<sup>+</sup>); MS MALDI-TOF calc. 514.999, found m/z 514.868 (M<sup>+</sup>) (dithranol).

# Copper (II) 2,3,5,5,7,8,12,13,17,18-Decamethylisoporphyrin chloride (31)

In a round bottomed flask equipped with a stirrer under argon, was added 50 mg (0.08 mmol) of b-bilene and 0.2 ml of cold TFA. The mixture was left to stir for 10 minutes, afterwhich dry dichloromethane (20 ml) was added followed by zinc (II) acetate dissolved in dry methanol, then excess 2,2-dimethoxypropane. The reaction mixture was left to stir in air for 24 h. UV-Visible (abs at 700 nm) and TLC on alumina showed completion of reaction. Excess TFA and dichloromethane were evaporated off and the reaction mixture was purified on neutral alumina using acidic solution of chloroform as eluant (2 drops of TFA were added to 200 ml of chloroform) to collect a fraction of mixed isoporphyrin and porphyrin. A second column using silica gel and chloroform/ethylacetate 4:1 and a few drops of TFA was done to purify the isoporphyrin from the porphyrin. The pure isoporphyrin was metalated using cuprous chloride in chloroform at room temperature for 2 h. The mixture was filtered through a celite cake, and recrystallized from chloroform/petroleum ether to yield the product in 23% (10 mg). UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$  nm ( $\epsilon \times 10^3$ , M<sup>-1</sup>.cm<sup>-1</sup>): 428.02 (10.98), 842.05 (9.75); HRMS MALDI-TOF calcd. for  $C_{30}H_{33}N_4Cu$  512.1995, found m/z 512.2006 (M<sup>+</sup>); MS MALDI-TOF calc. 513.155, found m/z 513.339 (M<sup>+</sup>) (dithranol).

### **CHAPTER 3**

# SYNTHESIS AND CHARACTERIZATION OF METAL-FREE ISOPORPHYRINS

# 3.1 Introduction

Metal-free isoporphyrins have not been previously isolated and characterized. Since Xie and her group were already aware that the metal-free derivative could not be obtained directly by ring synthesis, studies to synthesize metal-free isoporphyrins by demetalation of the stable zinc isoporphyrin were initiated. The studies revealed that treatment of zinc(II) isoporphyrin with TFA usually resulted in decomposition of the product after work-up. Although demetalation was successful, following several trials, the product was unstable and decomposed readily. As a result it was not possible to fully purify or fully characterize the metal-free isoporphyrin.<sup>56</sup> The other approach that can be taken to obtain a metal-free isoporphyrin is to synthesize it directly without inserting a metal. This approach was explored by Leung using a variation of the MacDonald procedure condensing a dipyrromethane dicarboxylic acid by (1) and diformyldipyromethane (2) in the presence of *p*-toluenesulfonic acid but without any metal salts, followed by treatment of DDQ. However the expected metal-free isoporphyrin (3) did not form<sup>68</sup> (Scheme 3.1). With the successful development of the bbilene route as a pathway to the synthesis of metal isoporphyrins, we decided to explore the synthesis of metal-free isoporphyrins using the direct synthesis approach thus eliminating the difficulties of work-up encountered by Xie, or so we thought.



Scheme 3.1: Direct synthesis of metal-free isoporphyrin via a MacDonald 2 + 2 approach



Scheme 3.2: Synthesis of metal-free isoporphyrin via a b-bilene approach

# 3.2 Results and Discussion

The synthetic procedure is as shown in Scheme 3.2 where b-bilene (4) is cyclized in the presence of zinc as a template with a ketal 2.2-dimethoxypropane as the the carbon-linking unit at room temperature in the presence of air. Attempts to isolate and characterize the metal-free isoporphyrin (5) was challenging due to instability of the product. Due to previous reports<sup>56</sup> of product decomposition during work-up with sodium bicarbonate solution, we avoided that problem by evaporating off excess TFA and solvent before purification by column chromatography. The reaction mixture (which was exposed to light and air) showed the formation of the metal-free product by UV-visible absorption spectroscopy, with the characteristic absorption at 700 nm, but the compound decomposed during separation/purification by column chromatography. This was also witnessed on TLC as the green spot eventually changed color to purple. Even after a successful separation on a short column, the compound decomposed on exposure to air and light by once again turning purple. Storage of the product in the freezer saved it from decomposing immediately. Insertion of zinc stabilizes it and there are no signs of decomposition even when left in excess acetate ions or exposed to air. So a study on stability of metal-free isoporphyrins was initiated.

#### • Stability studies

It was predicted that since the reaction mixture was stable upon exposure to air and light but unstable or decomposed after alumina column chromatography, then the presence of acid in the reaction mixture may have been playing a major role on the stability of the compound. So after the alumina column, the green fraction was trapped in an acidic solvent (dichloromethane with a few drops of TFA) and successfully, the UV

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showed the expected spectrum. However, after a few minutes of exposure to light and air, the compound (dissolved in acidic dichloromethane) changed color from green to purple. All factors supposedly thought to cause decomposition were eliminated; running of the column under argon, storage of the compound under argon and in the dark or at 0 °C, but still there was decomposition after several hours under these conditions. Finally, it was discovered that the activated alumina (alumina grade III) that was used to purify the metal-free complex played a major role on the stability of this compound. It was speculated that the basic alumina was deprotonating the product while on the column, and although it was trapped in acidic conditions to restore the protonation, it was not sufficient enough to stabilize the product. Enough damage to the product was done while on the column. Therefore, neutral alumina was used and prepared using the acidic eluant to prepare a short column of approximately 2 inches in length. It was also observed that porphyrin formation was a competing reaction, in high yields too, which could have led to reduced yields of isoporphyrin. Isolation and purification of the product (green) was successful under the acidic conditions, to give protonated metal-free isoporphyrin in 23% yield; this showed absorptions at around 430 and 695 nm (Figure 3.1). Addition of base or basic solvents (e.g. pyridine, DMF, THF) to this sample destroyed the absorption pattern characteristic of metal-free isoporphyrins and finally led to decomposition (color changed to blue and finally to purple). The same behavior was observed when the product was purified under non acidic conditions. Also, when base was added to the reaction mixture (that contained some acid), the same results were observed as shown in Figure 3.2. This may explain why the compound obtained after demetalation of zinc isoporphyrin (by Xie and Leung) decomposed during work-up with saturated sodium bicarbonate solution; metal-free isoporphyrin is unstable under basic conditions. We investigated the stability of this compound in different solvents bearing lone pairs of electrons. The electronic absorption spectra, **Figure 3.2**, illustrates a trend where the more basic solvents deprotonate the metal-free isoporphyrin, changing their electronic absorption whereas the less basic tend to have less effect on the compound; that is acetone, methanol, dichloromethane and chloroform.



**Figure 3.1:** UV/Visible spectrum of protonated metal-free isoporphyrin in dichloromethane at room temperature







Figure 3.2: UV-Visible spectra of metal-free isoporphyrin dissolved in different solvents/ reagents at room temperature



Figure 3.3: <sup>1</sup>H NMR spectrum of metal-free isoporphyrin(5) in chloroform-d at room temperature

These results proved that the absorption spectrum (Figure 3.1) was characteristic of protonated metal-free isoporphyrins, which were stable enough to allow full characterization. Mass spectroscopy proved the formation of the product but NMR spectroscopy showed all the signals except for the 5,5-dimethyl protons (expected to show between 1-2 ppm) plus a strong signal at 1.26 ppm which did not integrate to the expected 6-methyl protons (Figure 3.3). This same problem was encountered by Xie and Leung who assumed that the signal was obscured beneath the impurity peak at 1.26ppm. They also associated the missing signal with some unusual dynamic processes which they ruled out after performing a variable-temperature <sup>1</sup>H-NMR at 10 °C and 0 °C with no successful identification. To investigate the phenomenon of the missing NMR signal, we undertook a deuterium labeling strategy of the 5,5-dimethyl substituents by chemical synthesis. This was achieved by cyclizing a b-bilene with a deuterated reagent molecule, in this case acetone-d<sub>6</sub> as the carbon-linking unit to introduce deuterium in the macrocycle (Scheme 3.3). It was necessary to establish that the reaction conditions did not allow the reverse D/H isotopic exchange by carrying out the synthesis using TFA-d and acetone-d<sub>6</sub>. The reaction was followed by UV-Visible spectroscopy and after 24 h, the absorption peak at 700 nm was at its maximum. Purification on neutral alumina and silica gel isolated the target deuterium labeled compound, green in color. Figure 3.4 shows the electronic spectrum in dichloromethane. Low resolution and high resolution mass spectrum were used to further characterize the macrocycle. <sup>1</sup>H-NMR spectroscopy of this sample in chloroform-d further confirmed successful deuterium labeling due to the missing signal between 1.5-2.0 ppm corresponding to 5,5-dimethyl substituent.



Scheme 3.3: Synthesis of deuterium labeled metal-free isoporphyrin



Figure 3.4: UV-Visible spectrum of deuterium labeled metal-free isoporphyrin (6) in dichloromethane at room temperature

Since we had speculated that the missing signal was obscured beneath the strong singlet of impurities at 1.26 ppm, we expected this signal to be less intense in the deuterium labeled sample. However, the signal showed the same intensity as the spectrum of nondeuterated sample leading to inconclusive results on the missing signal.

Following the solvent studies performed on this macrocycle and the stability demonstrated in acetone and methanol, we attempted to carry out the NMR studies in a different solvent hoping to obtain better solubility and resolution. Our solvent choice was acetone- $d_6$  and a drop of TFA-d to stabilize the metal-free isoporhyrin (**5**). To our amazement, we observed a signal at ~1.7 ppm which integrated to 6-H, corresponding to the 5,5-dimethyl protons (Figure 3.6). To confirm the identity of this signal, the deuterium labeled metal-free isoporphyrin (**6**) was analyzed under the same conditions by <sup>1</sup>H-NMR in acetone- $d_6$  and the signal was absent (Figure 3.7). Another observation made was the reduced intensity of the signal at 1.26ppm in acetone- $d_6$  compared with chloroform-d, which has no clear explanation, but is probably due to better solubility/reduced aggregation in acetone/TFA mixture and stability of the metal-free macrocycle in this solvent mixture leading to reduced rate of decomposition.

In addition, we adopted a more sensitive NMR technique, <sup>13</sup>C DEPT (Distortionless Enhancement by Polarization Transfer), an example of a carbon-editing pulse sequence via polarization transfer, for further structure verification of the metal-free isoporphyrin and to confirm the identity of the 5,5-dimethyl signal. Systematic changes in the internal delays in the complex pulse program make different carbons respond in different fashions, based upon the number of protons attached.

DEPT 45: This experiment yields a positive peak for every carbon with attached protons.

DEPT 90: In this variant of the DEPT experiment, only CH yields peaks; CH<sub>0</sub>, CH<sub>2</sub>, and CH<sub>3</sub> are invisible.

DEPT 135: In this variant of the DEPT experiment, CH<sub>2</sub> yields negative peaks, whereas CH and CH<sub>3</sub> are positive.

We opted to use DEPT 90 and DEPT 135 since the metal-free isoporphyrin macrocycle contained CH and CH<sub>3</sub> carbons. Note in this experiment that carbon in the deuterated solvents (in our case CDCl<sub>3</sub> solvent) used to dissolve the samples for NMR, does not give a signal, since it has no attached protons.

Theoretically, since the metal-free macrocycle has symmetry through the 5,15-*meso* positions, we expect to observe positive signals corresponding to 2-CH carbons in DEPT 90, while positive 2-CH carbons and 5-CH<sub>3</sub> carbons in DEPT 135, for non-deuterated metal-free isoporphyrin. On the other hand, deuterium labeled metal-free isoporphyrin is expected to show positive signals for 2-CH carbons in DEPT 90, and positive 2-CH carbons and 4-CH<sub>3</sub> carbons in DEPT 135, due to deuterium labeling of 5,5-dimethyl substituent. Both samples were dissolved in CDCl<sub>3</sub> and four different experiments were run overnight to obtain results as indicated in **Figures 3.10 - 3.13** which comply with the theoretical speculations/predictions. These results confirmed both structures and the identity of the 5,5-dimethyl signal.

We also utilized <sup>2</sup>H-NMR spectroscopy for characterization of the two deuterium labeled methyl groups of metal-free isoporphyrin. The typical solvent used in deuterium-NMR experiment is non-deuterated solvent, in this case, chloroform. For a given chemical species and its deuterium isotopomer, their chemical shifts in H-NMR and D-NMR are very similar, with a minor isotope effect, allowing easy spectral interpretation

extrapolated from the knowledge of <sup>1</sup>H NMR. The deuterium resonance was observed as a broad signal at 1.8 ppm (**Figure 3.13**) which is 0.2 ppm downfield shifted compared to that of the corresponding proton resonance ( $\delta$  1.6 ppm). This was in agreement with <sup>2</sup>H NMR studies of porphyrins reported in the literature; broadening due to quadrupolar relaxation since <sup>2</sup>H is a quadrupolar nucleus, fairly sharp linewidths and essentially similar chemical shifts in <sup>2</sup>H spectra as those seen in <sup>1</sup>H.<sup>5</sup> The triplet observed in the spectrum (**Figure 3.13**) can be associated with impurities or noise, taking into consideration the limitations of deuterium-NMR; (1) due to low magnetogyric ratio of deuteron, D-NMR is better when applied to compounds that are highly deuterated, (2) it takes a longer time to achieve the desired signal/noise (S/N) ratio, and (3) with 100% natural abundance solvent, D-NMR has to be run in the unlocked mode, and shimming has to be done manually.<sup>69</sup>

# **3.3 Decomposition Studies**

Metal-free isoporphyrins are very unstable and decompose readily in presence of air and light (while dissolved in solvents or dry), under basic conditions or solvents, and under alumina chromatography. The green product gradually changes color to purple, which is not identifiable by its UV/Visible spectrum. TLC of the purple fraction indicates several spots. A sample of protonated metal-free isoporphyrin, which is relatively stable compared with free-base metal-free isoporphyrin, was left to stand in air and light. Progressively, the color turned from green to purple and separation on a neutral alumina column to isolate the purple fraction was attempted. A first green fraction was collected which corresponded to metal-free isoporphyrin, while the major fraction (purple) was



**Figure 3.5:** <sup>1</sup>H-NMR spectrum of deuterium labeled metal-free isoporphyrin (6) in CDCl<sub>3</sub> at room temperature



Figure 3.6: <sup>1</sup>H-NMR spectrum of metal-free isoporphyrin (5) in (CD<sub>3</sub>)<sub>2</sub>CO at room temperature



**Figure 3.7:** <sup>1</sup>H-NMR spectrum of deuterium labeled metal-free isoporphyrin (6) in (CD<sub>3</sub>)<sub>2</sub>CO at room temperature



**Figure 3.8:** Comparison of <sup>1</sup>H-NMR spectra of metal-free isoporphyrin in CDCl<sub>3</sub> and (CD<sub>3</sub>)<sub>2</sub>CO



Figure 3.9: DEPT 90 spectrum of metal-free isoporphyrin (5) in CDCl<sub>3</sub>, overnight at room temperature



Figure 3.10: DEPT 135 spectrum of metal-free isoporphyrin (5) in CDCl<sub>3</sub>, overnight at room temperature



Figure 3.11: DEPT 90 spectrum of deuterium labeled metal-free isoporphyrin (6), overnight at room temperature



Figure 3.12: DEPT 135 spectrum of deuterium labeled metal-free isoporphyrin (6), overnight at room temperature



Figure 3.13: <sup>2</sup>H-NMR spectrum of compound (6) in CHCl<sub>3</sub>

retained on the column and was eluted with methanol. The UV/Visible spectrum of this fraction did not identify the compound. TLC showed three different spots which were separated by silica gel column and could not be identified.

# **3.4** Experimental

# • Characterization of Compounds, Chromatographic methods and Purification of Solvents

As for Chapter 2, section 2.6

# • Experimental Procedures

**b-Bilene (4):** This compound was synthesized following the synthetic procedure described in Chapter 2 for compound (14).

# 2,3,5,5,7,8,12,13,17,18-Decamethylisoporphyrin (5)

Bilene (4) (50 mg, 0.08 mmols) was dissolved in 0.2ml of cold TFA in a round bottomed flask and stirred for 10 min under argon. The mixture was diluted with dry DCM followed by addition of zinc(II) acetate (20 mg) dissolved in dry methanol (0.3 ml) and 0.1 ml of 2,2-dimethoxy propane (excess). The mixture was left to stir in air for 24h. TLC and UV Visible spectrophotometer showed formation of the product. The electronic absorption showed peaks at around 440 and 690 nm characteristic of metal-free isoporphyrin. Excess TFA and solvent were evaporated to dryness. The product was chromatographed on a neutral (grade 0) alumina column (approximately 2 inches long), prepared and eluted with slightly acidified chloroform (CHCl<sub>3</sub>/TFA: pH= 4-5) to collect the major green fraction. The solvent was evaporated to dryness and dried under vacuum to yield 28% (10 mg) of the target compound. UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm: 432, 700; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>CO, 300MHz)  $\delta$ , ppm 8.43 (s, *meso*-H, 2H), 7.50 (s, *meso*-H, 1H), 2.86, 2.77, 2.61, 2.49 (s,  $\beta$ -CH<sub>3</sub>, 24H), 1.64 (s, 5-CH<sub>3</sub>, 6H); HRMS MALDI-TOF calcd. for  $C_{30}H_{36}N_4$  452.2929, found *m/z* 452.2875 (M<sup>+</sup>) (dithranol); MS MALDI-TOF calc. 452.61, found *m/z* 452.59 (M<sup>+</sup>) (dithranol).

<sup>13</sup>C DEPT 90 (CDCl<sub>3</sub>, 300MHz) δ, ppm 106.383, 84.204

<sup>13</sup>C DEPT 135 (CDCl<sub>3</sub>, 300MHz) δ, ppm 106.383, 84.204, 28.295, 13.059, 11.255, 11.143, 10.786.

# 5,5-Dideuteromethyl-2,3,7,8,12,13,17,18-octamethylisoporphyrin (6)

Bilene (4) (50 mg, 0.08mmols) was dissolved in 0.2 ml of cold TFA-d in a round bottomed flask and stirred for 10 min under argon. The mixture was diluted with dry DCM (20 ml) followed by addition of zinc (II) acetate (20 mg) dissolved in dry methanol (0.3 ml) and 0.07 ml of acetone-d<sub>6</sub> (excess). The rest of the procedure is similar to that described for compound (5).The solvent was evaporated to dryness and dried under vacuum to yield 22% (8 mg) of the target compound. UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm: 429, 692 ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz)  $\delta$ , ppm 8.19 (s, *meso*-H, 2H), 7.33 (s, *meso*-H, 1H), 2.80, 2.72, 2.59, 2.47 (s,  $\beta$ -CH<sub>3</sub>, 24H); HRMS MALDI-TOF calcd. for C<sub>30</sub>H<sub>30</sub>D<sub>6</sub>N<sub>4</sub> 458.3299, found *m/z* 458.3262 (M<sup>+</sup>) (dithranol); MS MALDI-TOF calc. 458.6, found *m/z* 456.9 (M-D<sup>+</sup>) (dithranol).

<sup>2</sup>H NMR (CHCl<sub>3</sub>, 300MHz) δ, ppm 1.85

<sup>13</sup>C DEPT 90 (CDCl<sub>3</sub>, 300MHz) δ, ppm 106.383, 84.204

<sup>13</sup>C DEPT 135 (CDCl<sub>3</sub>, 300MHz) δ, ppm 106.383, 84.204, 13.059, 11.255, 11.143, 10.786.

#### **CHAPTER 4**

#### SYNTHESIS OF MESO-MONOSUBSTITUTED PORPHYRINS

# A: SYNTHESIS OF MESO-MONOSUBSTITUTED ALKYL PORPHYRINS VIA ISOPORPHYRINS: CHEMICAL PROPERTIES OF NOVEL ISOPORPHYRIN MACROCYCLES

#### 4.1 Introduction

Isoporphyrins have been reported to be possible intermediates in the biosynthesis of chlorophylls,<sup>18</sup> and in peroxidase heme meso-alkylations.<sup>51</sup> Smith<sup>70</sup> also proposed that isoporphyrins are intermediates in electrophilic substitution reactions of porphyrins that involve attack at the meso positions. Treatment of zinc octamethylporphyrin (1) with thallium(III) trifluoroacetate (TTFA) yielded zinc *meso*-trifluoroacetoxyoctaethylporphyrin. The proposed mechanism for this transformation involves formation of  $\pi$ -cation radicals of metallo-porphyrin (2) which readily lose an electron to give the  $\pi$ -dication (3), a very strong electrophile, which is then attacked by trifluoroacetate anion to yield metallo-isoporphyrin (4) intermediate and finally proton loss to afford metallo-meso-trifluoroacetoxyporphyrin (5) Scheme 4.1.

Isoporphyrins are known to be unstable in that they are easily converted by tautomerization into the corresponding porphyrins. Substitution at the 5-meso position with a gem-5,5-dialkyl substituent yields a stable isoporphyrin that cannot undergo tautomerization (**Chapter 1**).<sup>55</sup> Herein, we explore the chemical properties of stable metallo-isoporphyrins, investigating their potential for use as intermediates for synthesis of *meso*-substituted porphyrins. The idea was to be accomplished by synthesis of 5-methyl ester 2,3,7,8,12,13,17,18-octamethyl zinc isoporphyrin (**7a**), followed by cleavage of the ester group to afford *meso*-monosubstituted porphyrin.



**Scheme 4.1:** Trifluoroacetoxylation reaction of metalloporphyrins (1) to afford metallo*meso*-trifluoroacetoxyporphyrins
# 4.2 Results and Discussion

During the synthesis of isoporphyrin derivatives using  $\alpha$ -ketoesters and  $\alpha$ diketones as the carbon-linking units for cyclization of b-bilene (6), using the same synthetic methodology as described with simple ketone/acetone (refer to Chapter 2), no isoporphyrin (7) was produced (Scheme 4.2). Instead, various interesting intermediates were observed which were worth identifying. After cleavage of the BOC-protecting groups on the b-bilene using TFA, the reaction mixture was diluted with dry dichloromethane followed by addition of methyl pyruvate to cyclize the b-bilene into the expected isoporphyrin. However, after 10 min-1 h reaction time under argon (or when left to stir in air for 24 h, in the presence or absence of zinc ions), the electronic absorption spectrum of the red colored product did not indicate either the starting material or an isoporphyrin, but a structure with absorptions at 450 and 520 nm characteristic of an open chain or non-conjugated tetrapyrrole (spectrum (I), Figure 4.1). <sup>1</sup>H NMR spectrum of this compound confirmed the UV/Visible results with the chemical shift of NH protons in the  $\delta$  12 – 13 ppm ranges, instead of the usual upfield region of the shielded NH protons in porphyrins (Figure 4.2). We also observed a set of three signals integrating to one proton each in the same chemical environment ( $\delta$  7.0-7.5 ppm), probably -CH- protons and another singlet at 4.4 ppm integrating to two-protons, suggesting -CH<sub>2</sub>- protons.

When the red fraction was washed with water or aqueous sodium carbonate to remove excess TFA, or a few drops of base were added, or diluted in any basic solvent for example THF, pyridine, or purified on alumina column, the color changed from red to



Scheme 4.2: Attempted synthesis of zinc (II) isoporphyrin (7) using  $\alpha$ -ketoester as the carbon-linking unit following the same reaction conditions as with simple ketone.

green. The absorption spectrum taken in dichloromethane also changed to 430 and 790 nm corresponding to spectrum (II) which is quite close to the characteristic optical absorption of metal-free isoporphyrins (430 and 700 nm, **Chapter 3**). The <sup>1</sup>H NMR spectrum in CDCl<sub>3</sub> indicated a slight upfield shift of the 4NHs to  $\delta$  11 ppm (still appearing in the downfield region, suggesting an open-chain or non-conjugated tetrapyrrole) and the set of 3-Hs. This may be due to the neutral nature of the compound

compared to compound (I) which was in acidic conditions. We also observed a new signal at 5.2 ppm integrating to 1-H, and the disappearance of the signal at 4.4 ppm which had integrated to 2-Hs in the first fraction. This was interpreted to be dehydrogenation at the meso sp<sup>3</sup> center. Such kinds of species with hydrogens on the bridging atoms are unable to resist oxidative dehydrogenation.<sup>71</sup> In basic conditions, deprotonation at the -CH<sub>2</sub>- bridging carbon occurs, leading to the formation of a conjugated tetrapyrrole – an *a,b,c*-bilatriene the driving force for this reaction. The rest of the signals appeared at their original chemical shift as the reaction mixture (I) above. However, another important observation was made when the UV/Visible spectrum was taken in various different solvents. It was noted that spectrum (II) was obtained when the green species was dissolved in chloroform, dichloromethane and ethyl acetate. When dissolved in THF, acetone, acetonitrile, pyridine, DMSO, the color changed further to green-blue, to give broad absorption bands at 400 and around 700 nm of their optical spectrum, suggesting formation of another species. A series of trials on different solvents and bases led to one conclusion that the green compound was unstable or rather reacted further in donor solvents/bases.

When acid was added to the green fraction (II), the color changed back to red and so did the optical spectrum, with absorptions similar to those of the reaction mixture (I) at 450 and 520 nm.

Addition of zinc acetate to fraction (II) had a similar effect as with addition of acid. The color changed from green to red with the optical spectrum showing absorption bands at 470 and 540 nm, spectrum (III), slightly red shifted to that of (I).



Figure 4.1: Optical spectrum (in CH<sub>2</sub>Cl<sub>2</sub>) of various intermediates during the synthesis of zinc (II) isoporphyrin



Figure 4.2: <sup>1</sup>H-NMR spectrum of the reaction mixture (fraction I), in CDCl<sub>3</sub>



Figure 4.3: <sup>1</sup>H-NMR spectrum of fraction II after alumina column, in CDCl<sub>3.</sub>

A similar intermediate trend in both the optical spectra and <sup>1</sup>H NMR was observed when an  $\alpha$ -diketone (2,3-butanedione) was used as a carbon linking unit in the cyclization.

To help understand the mechanistics of the intermediate formation, the same reaction procedure with methyl pyruvate was carried out using 2-equivalents of  $\alpha$ -free pyrrole (8) as a model simulating b-bilene,. After stirring for 24 h at room temperature, <sup>1</sup>H NMR spectroscopy indicated that a condensation reaction occurred at the carbonyl carbon, not methoxy-carbonyl, to yield dipyrromethane (9) (Scheme 4.3). These results were also confirmed by mass spectroscopy. This reaction eliminated our earlier speculations that both carbonyls reacted to yield the macrocycle. With these results in mind, we were confident that the b-bilene should react or was reacting in the same way. After several trials, it was concluded that the reactivity of the b-bilene with  $\alpha$ -ketoester or  $\alpha$ -diketone (10 min) was different from that of pyrrole (24 h).



Scheme 4.3: Reaction of methyl pyruvate with  $\alpha$ -free pyrrole (8) to yield dipyrromethane (9)

In a desperate attempt to isolate and identify the mystery product, fraction (III) was left to stir overnight under argon, in dry conditions, and was purified on an alumina column to isolate an orange colored major fraction that absorbed at 470 and 511 nm (spectrum (**V**), **Figure 4.2**). The x-ray structure (**Figure 4.3**) of this fraction identified the product as a zinc dimer of open chain tetrapyrrole (a,c-biladiene), indicating that reaction with methyl pyruvate had occurred on one of the  $\alpha$ -free positions of the b-bilene. The crystals were grown by slow diffusion of petroleum ether into a concentrated solution of (**V**) in dichloromethane. When TFA was added to the zinc dimer complex, demetalation occurred producing an optical spectrum identical with (**I**).

However, in another attempt, fraction (III) was left to stir in DDQ and cyclization occurred to give the expected product, zinc isoporphyrin (spectrum IV). When DDQ was added to the zinc dimer complex (V), no cylization was observed, only decomposition of the compound. We also investigated cyclization of fraction (II) in the presence of DDQ with no success, bringing to conclusion that zinc ions are necessary to effect ring closure.

Based on the supporting data from NMR spectroscopy, low resolution MS, UV/Visible spectroscopy, and the crystal structure, we postulate a reaction scheme for the intermediate products as outlined in **Scheme 4.4.** Treatment of (**6**) with TFA to cleave the t-butyl esters followed by addition of methyl pyruvate rapidly gave intermediate (**I**), through a b-bilene, a-b-biladiene to a,c-biladiene transformation; the first example of such a transformation that we are aware of so far. The initially formed b-bilene (**a**) and a,b-biladiene dication (**b**) must have undergone acid-base equilibria to give the a,c-biladiene (**I**) in which the two cationic charges are separated. Indeed, measurement of



Figure 4.2: Optical spectrum in  $CH_2Cl_2$  of the zinc dimer complex (V) and its transformation to fraction (I)



**Figure 4.3:** Crystal structure of intermediate (V) showing two acyclic tetrapyrrole units forming a dimer with zinc (zinc *a*,*c*-biladiene dimer)

proton NMR spectra in CDCl<sub>3</sub> and D<sub>2</sub>O show that the b- ( $\delta$  4.2ppm) and c- ( $\delta$  7.3ppm) protons in (**I**) readily undergo exchange with deuterium during this process. Washing with aqueous sodium bicarbonate gave intermediate (**II**), a more conjugated chromophore – a,b,c-bilatriene, which was converted to (**III**) after addition of zinc(**II**) acetate. Treatment of this zinc(**II**) complex (**III**) with DDQ gave a high yield of the corresponding isoporphyrin chloride (**IV**) after anion exchange, possibly via the tautomer (**c**). Refer to **Figure 4.1** for the optical spectra of fractions (**I** – **IV**). <sup>1</sup>H-NMR chemical shifts of the protons evaluated for structure elucidation of the intermediates are as listed in **Table 4.1**.

With this achievement at hand, we were able to cyclize the b-bilene with various  $\alpha$ -ketoesters (entries **a-g**) and  $\alpha$ -diketone (entry **h**) as shown in **Table 4.2** to yield the corresponding zinc isoporphyrin cation complexes as novel compounds (**Figure 4.4**) in very good yields, and higher than previously reported (**Scheme 4.5**). The reactions were carried out at room temperature and were complete in about 1 h depending on the carbonyl substrate. The high electrophilicity of the carbonyl on these substrates ( $\alpha$ -ketoesters and  $\alpha$ -diketone) compared to that of simple ketones (e.g., acetone, refer to **Chapter 2**), facilitated the enhanced rate of the reaction. A general reactivity profile summarized from the table relates to sterics where the more bulky the substrate, the lower the yields and vice versa.

To our surprise, as seen in entry (c) of the table, no cyclization occurred with methyl 3,3,3-trifluoromethylpyruvate to give zinc isoporphyrin, despite the high electrophilic character of the carbonyl due to the presence of very electronegative fluorine atoms. In fact, we observed that the reaction was very slow (24 h) and required an excess of trifluoromethylpyruvate to yield the intermediate that corresponds to



Scheme 4.4: Postulated mechanism and structures of intermediates/fractions I, II, III, IV

Table 4.1: <sup>1</sup> H Chemical shifts	(in CDCl <sub>3</sub> ) for intermediate	fractions (I) and (II)
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	Compound/Intermediate	NH	H-1, 5, 10	H-15	H-20	Me-21
Reacted with	Reaction mixture (I)	12.74 (s,1H)	7.61 (d, 1H)	4.36 (s, 2H)	4.26 (q, 1H)	1.62 (d, 3H)
methyl		12.64 (s, 1H)	7.33 (s, 1H)			
pyruvate / 1,2-		12.39 (s, 1H)	7.27 (s, 1H)			
diketone		12.04 (s, 1H)				
	Washed with water	11.5 (s, 1H)	6.88 (s, 1H)	5.41 (s, 1H)	3.94 (q, 1H)	1.47 (d, 3H)
			6.42 (s, 1H)			
			6.40 (s, 1H)			
	After alumina column (II)	11.95 (s, 2H)	7.00 (s, 1H)	5.44 (s, 1H)	4.33 (q, 1H)	1.62 (d, 3H)
		11.34 (s, 1H)	6.43 (s, 1H)			
		11.15 (s, 1H)	6.41 (s, 1H)			
	Added Zinc or D <sub>2</sub> O	Absent				
Reacted with	Reaction mixture (I)				5.8 (s, 1H)	Absent
phenyl pyruv.						



**Scheme 4.5:** Synthesis of zinc(II) isoporphyrin cation (7) employing various 1,2-dicarbonyl substrates

spectrum (**I**). We speculated that the reagent was very reactive and required no acid for the addition step. To optimize the reaction conditions, after cleavage of the BOC protecting groups, TFA was neutralized with base by washing with bicarbonate and the reaction was carried out in the absence of acid. No change on reactivity was noted; a similar trend as with the presence of acid was observed. We concluded that the intermediate formed in this case was unstable and thus favored no product formation. For entry (**f**), steric factors may have played a role in the lack of cyclization.

Entry	R1	R2	Reaction	% yield	% yield
			time*(min)	(7)	(18)
a	Me	OMe	10	55	30
b	Me	OEt	10	54	31
c	CF <sub>3</sub>	OMe	-	0	0
d		OEt	30	35	32
	Û,				
e	>-	OEt	60	33	26
f	>-	OEt	-	0	0
g <sup>a</sup>	Me	OEt	10	54	63
h	Me	Me	10	56	-

**Table 4.2:** Cyclization of b-bilene (6) with various 1,2-diketones to yield (7) and conversion to (18)

<sup>*a*</sup>Different substituents (propionate side chains) on macrocycle . Refer to **Scheme 4.6** \*Reaction of b-bilene with substrate, before addition of zinc and DDQ









Figure 4.4: Structures of zinc isoporphyrin complexes (7)

The optical spectra of some of the zinc (II) isoporphyrin complexes (7) are shown in **Figure 4.5**, and a summary of the absorption peaks and molar extinction coefficients for **7a-h** in **Table 4.3**. The absorption is characteristic of metal isoporphyrins with absorption maxima at 420 and 800 nm<sup>56</sup> (**Chapter 1**). However, substitution of an ester group for one of the methyl groups at the sp<sup>3</sup>-hybridized meso-carbon affects the spectrum. There is an observed red shift on the Soret and Q-bands of the metal complex. Substitution of the other methyl group for a phenyl group further red shifts the absorption to 842 nm. Similar changes were observed with peripheral substitution (**7g**).

The emission spectra of **7a**, **b**, **d** and **g** are as shown in **Figure 4.6**, taken in dichloromethane, with a summary of the experimental conditions and fluorescence emission maxima in **Table 4.4**. These compounds exhibit large Stoke's shift  $(144 - 411 \text{ cm}^{-1})$  as reported (600 cm<sup>-1</sup>)<sup>19</sup> compared to zinc octaethylporphyrin (50 cm<sup>-1</sup>).

So far, we had been utilizing a methyl substituted b-bilene that yielded an octamethyl-substituted zinc isoporphyrin. In order to study in depth the properties of this library of zinc isoporphyrins, we attempted to vary the peripheral substituents on the macrocycle. This was achieved by synthesizing a 2,8-methylpropionate substituted zinc isoporphyrin (entry (g)) by cyclization of b-bilene (12), obtained in 82% yield by condensation of formyldipyrromethane (10) and acid dipyrromethane (11) (Scheme 4.6a). The precursor dipyrromethane (13) was derived in 51% yield from condensing 2-acetoxymethyl pyrrole bearing 3-pMe substituent (14) with 2-unsubstituted pyrrole (15) using montorillonite K-10 clay as the acid catalyst (procedure described in Chapter 2) Scheme 4.6b.



Figure 4.5: Optical spectrum of zinc (II) isoporphyrin complexes (7)

Table 4.3: Summary	of the absorption	peaks for compo	ounds (7 <b>a-h</b> ) in	dichloromethane
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	Soret band $\lambda_{max}$ , (nm)	Q-band $\lambda_{max}$ , (nm)		
	$(\varepsilon \times 10^4, M^{-1}.cm^{-1})$	$(\varepsilon \times 10^4, M^{-1}.cm^{-1})$		
7a	430 (3.37)	830 (2.63)		
7b	429 (3.58)	826 (2.89)		
7d	439 (4.84)	842 (4.27)		
7e	431 (2.75)	822 (2.28)		
7g	435 (4.78)	841 (3.65)		
7h	430 (4.23)	812 (3.87)		



Figure 4.6: Fluorescence spectra bands in the  $Q'_y$  region of (7) derivatives at room temperature in dichloromethane

Table 4.4: Fluorescence emission data of (7) in dichlorometha
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Entry	Concentration	Excitation $\lambda$ , nm	Emission $\lambda_{max}$ ,	Stoke's shift
	[moles/L]		nm	(nm)
7a	3.36 x 10 <sup>-6</sup>	420	840	$10 (144 \text{ cm}^{-1})$
7b	3.29 x 10 <sup>-6</sup>	420	840	14
7d	3.0 x 10 <sup>-6</sup>	430	860	18
7e	2.94 x 10 <sup>-6</sup>	425	850	26
7g	2.84 x 10 <sup>-6</sup>	430	860	19
7h	3.46 x 10 <sup>-6</sup>	420	840	28 (411 cm <sup>-1</sup> )

Although the cyclization reactivity was similar to the rest of the library (similar yields), it was observed that complex (7g) had better solubility due to the presence of the ester groups and it absorbed and emitted at longer wavelengths compared to the model (7a). The <sup>1</sup>H NMR spectra of b-bilene 12 and isoporphyrin 7g are as shown in Figures 4.7 and 4.8, respectively.

For comparison purposes, we also examined the MacDonald 2+2 synthesis of isoporphyrin 7a\*, utilizing the available 5,5-disubstituted dipyrromethane obtained in **Scheme 4.3** which was debenzylated to give dipyrromethane-1,9-dicarboxylic acid (16) followed by condensation with 1,9-diformyldipyrromethane (17) (Scheme 4.7). However, the yields were extremely low (14%) and the reaction time was much longer (24 h) compared with the b-bilene route which achieves approximately four-fold this yield, and can be carried through from start to finish in less than 1 h.

Having successfully synthesized a library of our target compound zinc(II) isoporphyrin (2), the next hurdle to jump was to examine their potential as intermediates in the synthesis of *meso*-substituted porphyrins. Saponification of the 5-alkyl ester substituent to give carboxylic acid followed by base-catalyzed decarboxylation on the sp<sup>3</sup>-hybridized meso carbon led to a rapid rearrangement and reduction of the cationic complex into the corresponding neutral *meso*-monosubstituted porphyrin, (18), (Scheme 4.8). 10% KOH dissolved in dry methanol was used. It was discovered that when KOH was dissolved in water, the reaction did not proceed as expected. The reaction occurred at room temperature and formation of the product was monitored by UV/Visible spectroscopy for the Soret band around 400 nm to reach its maximum intensity.



Scheme 4.6a: Synthesis of 2,8-bis(methylpropionate) substituted zinc isoporphyrin (7g)



Scheme 4.6b: Synthesis of precursor dipyrromethane (13)



Scheme 4.7: Synthesis of 2a through MacDonald 2 + 2 cyclization (\*Notice the ethyl substituents at position 13 & 17)



Figure 4.7: <sup>1</sup>H NMR of b-bilene (12) in CDCl<sub>3</sub> at room temperature



Figure 4.8: <sup>1</sup>H NMR spectrum of zinc isoporphyrin 7g in CDCl<sub>3</sub> at room temperature

Purification was done on alumina (grade III) column with the yields indicated in **Table 4.2.** Note that for entry **7g**, the methyl-ester peripheral substituents were hydrolyzed but not decarboxylated under similar conditions indicating regioselective decarboxylation (**Scheme 4.9**). The driving force for the decarboxylation reaction is the considerable thermodynamic stabilization gained upon the formation of a fully conjugated isomer (porphyrin **18**) in comparison to zinc isoporphyrin (**7**) that exhibits an interrupted macrocyclic conjugation owing to the presence of a sp<sup>3</sup>-hybridized meso carbon. **Figure 4.9** shows the electronic absorption spectra of the transformation from zinc isoporphyrin (**7**) to zinc porphyrin (**18**).



Scheme 4.8: Transformation of zinc (II) isoporphyrin (7) into *meso*-monosubstituted porphyrin (18)



Scheme 4.9: Transformation of (7g) to (18g) showing partial decarboxylation



**Figure 4.9:** Electronic absorption spectra of zinc isoporphyrin (7) before (-----) and after (---) transformation to zinc *meso*-monosubstituted porphyrin (18), and after demetalation (.....) in  $CH_2Cl_2$ .

Owing to the success of this methodology whereby zinc metal was incorporated into the intermediate before cyclization and oxidation to zinc isoporphyrin, it was speculated that incorporation of other transition metals may allow for the isolation of metalloisoporphyrins that may otherwise be inaccessible by direct metallation of metalfree macrocycles. Several transition metal salts including CuCl, Cu (Cl)<sub>2</sub>, Ni (acac)<sub>2</sub>, Fe, Co, Ag, Cd, were incorporated into the intermediate followed by addition of DDQ at room temperature with no successful cyclization to the corresponding metalisoporphyrin. Mostly, decomposed products were obtained (by UV/Vis spectroscopy).

# B: SYNTHESIS OF *MESO*-MONOSUBSTITUTED PORPHYRINS THROUGH B-BILENES

#### 4.3 Introduction

During the synthesis of metallo-isoporphyrins by cyclization of b-bilenes, the scope of the reaction was expanded by investigating the kinetic factors verses the steric factors of the carbon-linking units (**Chapter 2**) and discovered that when the carbon-linking unit was an aldehyde (kinetic factors), cyclization occurred to yield a *meso*-monosubstituted porphyrin.

Previously, *meso*-monosubstituted porphyrins have been reported to be synthesized by condensation of *a*,*c*-biladiene with an aldehyde by refluxing in acidified solvents for several hours to several days,<sup>5</sup> monofuctionalization of porphyrins,<sup>72,73</sup> or they are described as byproducts,<sup>74</sup> but in very low yields. The b-bilene oxidative cyclization has also been investigated as an approach to meso-monosubstituted porphyrins. Although in most cases the required porphyrin was formed, the yield was generally low and the sequence complicated by the presence of by-products, therefore limiting this approach.<sup>36</sup> Recently, Senge et.al.<sup>75</sup> reported a practical synthesis of these

porphyrins prepared by condensation of dipyrromethane, pyrrole-2-carbaldehyde and the desired aromatic or aliphatic aldehyde with yields between 2 and 12% and in most cases, the 5,15-disubstituted porphyrin was obtained as a second product.

### 4.4 Results and Discussion

Treatment of b-bilene (6) with TFA to cleave the *tert*-butyl esters, followed by cyclization with benzaldehyde as the carbon-linking unit in the presence of metal ions, then oxidation with DDQ, yielded *meso*-monosubstituted porphyrin (19) as shown in Scheme 4.9. TFA was used for deprotection as well the acid catalyst for the condensation reaction. The metal salt, in this case Ni (acac)<sub>2</sub> was used as a templating metal, whereby in the absence of it, no cyclization or ring closure occurred. Rather, nucleophillic addition



Scheme 4.9: Cyclization of b-bilene (6) to meso-monosubstituted porphyrin (19)

of the aldehyde occurred at both 1 and 19 positions of the b-bilene. The reaction takes place at room temperature in about an hour and purification is very easy on an alumina (grade III) column. No other *meso*-monosubstituted porphyrin side products are isolated; exclusively the target compound is obtained as the major product.

Various benzaldehydes with different functional groups were used (**Table 4.5**) and it was demonstrated from the product percentage yields (range between 10 and 30%) that the reaction efficiency varied considerably depending on the steric and electronic properties of the aldehyde.



Figure 4.10: Optical spectrum of *meso*-monosubstituted porphyrin (19c)

	Benzaldehyde	Metal ions	Product (19) %
			yield
a	СНО	Zn (II)	11.5
b	CHO	Ni (II)	28
c	Me Me	Ni(II)	25
d	MeO OMe	Ni (II)	10
e	CHO CO <sub>2</sub> Me	Ni (II)	30

 Table 4.5: Various benzaldehydes condensed with b-bilene (6) to yield (19)



Figure 4.11: <sup>1</sup>H NMR spectrum of *meso*-monosubstituted porphyrin (19c)



Figure 4.12: Crystal structure of zinc meso-monosubstituted porphyrin (19c)

The yields increased with substitution of electron-withdrawing groups on the benzaldehyde while electron-donating groups induced deactivation and thus reduced product yield. When zinc was used as a templating metal, both metal-free porphyrin and zinc porphyrin (in small amounts) were obtained, accounting for the low yields of entry **19a**. The yields obtained through this method are much higher than previously reported. **Figures 4.10** and **4.11** show the electronic absorption spectrum and <sup>1</sup>H NMR of *meso*-monosubstituted porphyrin (**19c**), respectively. The x-ray structure of its zinc-complex is as shown in **Figure 4.12**. The crystals were grown by slow diffusion of petroleum ether into a concentrated solution of the porphyrin in DCM.

### 4.5 Experimental

#### General procedure for cyclization of b-bilene with dicarbonyl compounds

Cold TFA (0.2 ml) was added to 50 mg (0.08 mmol) of b-bilene hydrochloride in a 50 ml RBF and left to stir under argon for 10 min. The mixture was diluted with dry dichloromethane (20 ml) followed by addition of  $\alpha$ -ketoester (1 equiv. of methyl, ethyl pyruvate and 1,2-diketone, and excess of phenyl and *i*-butyl pyruvates). The reaction was left to stir under argon for 1 h after which the UV/Visible spectrum showed no starting material but a new product absorbing at 450 and 520 nm. Excess TFA was removed by washing with aqueous Na<sub>2</sub>CO<sub>3</sub>, changing the product color from reddish to green. The UV/Visible absorption for the green product was 430 and 790 nm. Zn(OAc)<sub>2</sub> (20 mg) dissolved in 1 ml of dry methanol was added to the green product in dry dichloromethane and stirred under argon. The reaction mixture immediately changed color to reddish, and after stirring for 5 min the UV/Visible spectrum indicated a new absorption at around 470 and 540 nm. 50 mg (0.22 mmol) of DDQ dissolved in dry dichloromethane (0.3 ml) was added to oxidize the product. After 15 min, the UV-Visible spectrum of the mixture absorbed at 430 and 840 nm suggesting formation of a zinc isoporphyrin. The mixture was washed with water, then brine, and then dried over  $Na_2SO_4$ .

Purification on an alumina column (III) eluting with DCM separated the main fraction which absorbed at 430 and 830 nm, similar to a zinc isoporphyrin. Further purification was done on silica using DCM/ethyl acetate 7:3 to yield a pure product which was further recrystallized using DCM/petroleum ether.

# Zinc(II) 2,3,5,7,8,12,13,17,18-Nonamethyl-5-methoxycarbonylisoporphyrin chloride (7a):

25 mg, 55% yield. UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm ( $\epsilon \times 10^4$ , M<sup>-1</sup>.cm<sup>-1</sup>): 430 (3.37), 830 (2.63) ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz)  $\delta$ , ppm 7.69 (s, *meso*-H, 1H), 7.62 (s, *meso*-H, 2H), 3.73 (s, 5-OCH<sub>3</sub>, 3H), 2.58, 2.47, 2.45, 2.42 (s,  $\beta$ -CH<sub>3</sub>, 24H), 2.01 (s, 5-CH<sub>3</sub>, 3H); HR ESI calcd. for C<sub>31</sub>H<sub>33</sub>N<sub>4</sub>O<sub>2</sub>Zn 557.1889, found *m/z* 557.1895 (M<sup>+</sup>); MS MALDI-TOF calc. 559.01, found *m/z* 559.80 (M<sup>+</sup>) (dithranol).

# Zinc(II) 5-Methyl, 5-ethylester, 2,3,7,8,12,13,17,18-octamethyl zinc isoporphyrin (7b):

25 mg, 54% yield. UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm (ε x 10<sup>4</sup>, M<sup>-1</sup>.cm<sup>-1</sup>): 429 (3.58), 826 (2.89) ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ, ppm 7.68 (s, *meso*-H, 1H), 7.61 (s, *meso*-H, 2H), 4.10-4.15 (q, OCH<sub>2</sub>CH<sub>3</sub>, 2H), 2.45, 2.43, 2.40, 2.31 (s, β-CH<sub>3</sub>, 24H), 1.96 (s, 5-CH<sub>3</sub>, 3H), 1.07-1.04 (t, OCH<sub>2</sub>CH<sub>3</sub>, 3H); HR ESI calcd. for C<sub>32</sub>H<sub>35</sub>N<sub>4</sub>O<sub>2</sub>Zn 571.2051, found *m/z* 571.2041 (M<sup>+</sup>); MS MALDI-TOF calc. 573.04, found *m/z* 573.70 (M<sup>+</sup>) (dithranol).

# Zinc(II) 5-Ethoxycarbonyl-2,3,7,8,12,13,17,18-octamethyl-5-phenylisoporphyrin chloride (7d):

18 mg, 35% yield. UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm (ε x 10<sup>4</sup>, M<sup>-1</sup>.cm<sup>-1</sup>): 439 (4.84), 842 (4.27) ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 8.35, 7.70 (m, 5H, Ph), 7.77 (s, *meso*-H, 1H), 7.66 (s, *meso*-H, 2H), 4.27-4.24 (q, OCH<sub>2</sub>CH<sub>3</sub>, 2H,), 2.50, 2.44, 2.34, 1.94 (s, β-CH<sub>3</sub>, 24H), 1.14 (t, OCH<sub>2</sub>CH<sub>3</sub>, 3H); HR ESI calcd. for C<sub>37</sub>H<sub>37</sub>N<sub>4</sub>O<sub>2</sub>Zn 633.2207, found *m/z* 633.2202 (M<sup>+</sup>); MS MALDI-TOF calc. 635.10, found *m/z* 635.61 (M<sup>+</sup>) (dithranol).

# Zinc(II) 13,17-Diethyl-5-ethoxycarbonyl-5-isobutyl-2,3,7,8,12,18hexamethylisoporphyrin chloride (7e):

16 mg, 33% yield. UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm (ε x 10<sup>4</sup>, M<sup>-1</sup>.cm<sup>-1</sup>): 431 (2.75), 822 (2.28) ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ, ppm 7.89 (s, *meso*-H, 1H), 7.80 (s, *meso*-H, 2H), 4.01-3.89 (q, OCH<sub>2</sub>CH<sub>3</sub>, 2H), 3.04-2.96 (q, -CH<sub>2</sub>CH<sub>3</sub>, 4H), 2.55, 2.51, 2.43 (s, β-CH<sub>3</sub>, 18H), 1.96 (s, 5-CH<sub>3</sub>, 3H), 1.32-1.20 (m, -CH<sub>2</sub>-CH-, 3H), 1.01-0.96 (t, OCH<sub>2</sub>CH<sub>3</sub>, 3H), 0.94-0.86 (t, -CH<sub>2</sub>CH<sub>3</sub>, 6H), 0.44 (d, -CH (CH<sub>3</sub>)<sub>2</sub>, 6H); HR ESI calcd. for C<sub>37</sub>H<sub>45</sub>N<sub>4</sub>O<sub>2</sub>Zn 641.2833, found *m/z* 641.2832 (M<sup>+</sup>); MS MALDI-TOF calc. 643.17, found *m/z* 643.56 (M<sup>+</sup>) (dithranol).

# Zinc(II) 5-Methoxycarbonyl-2,8-bis(2-methoxycarbonylethyl)-3,5,7,12,13,17,18heptamethylisoporphyrin chloride (7g):

27 mg, 56% yield. UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm ( $\epsilon \times 10^4$ , M<sup>-1</sup>.cm<sup>-1</sup>): 435 (4.78), 841 (3.65) ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz)  $\delta$ , ppm 7.68 (s, *meso*-H, 1H), 7.65 (s, *meso*-H, 2H), 3.67, 3.65 (s, OCH<sub>3</sub>, 9H), 3.20-3.16, 2.66-2.62 (t, -CH<sub>2</sub>CH<sub>2</sub>-, 8H), 2.43, 2.41, 2.22 (s,  $\beta$ -CH<sub>3</sub>, 18H), 1.96 (s, 5-CH<sub>3</sub>, 3H); HR ESI calcd. for C<sub>37</sub>H<sub>41</sub>N<sub>4</sub>O<sub>6</sub>Zn 701.2312, found *m/z* 701.2310 (M<sup>+</sup>); MS MALDI-TOF calc. 703.13, found *m/z* 702.48 (M<sup>+</sup>) (dithranol).

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#### Zinc(II) 5-Acetyl-2,3,5,7,8,12,13,17,18-nonamethylisoporphyrin chloride (7h):

24 mg, 54% yield. UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm (ε x 10<sup>4</sup>, M<sup>-1</sup>.cm<sup>-1</sup>): 430 (4.23), 812 (3.87) ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400MHz) δ, ppm 8.10 (s, *meso*-H, 1H), 8.07 (s, *meso*-H, 2H), 2.60, 2.57(s, β-CH<sub>3</sub>, 24H), 2.30 (s, COMe, 3H), 1.91 (s, 5-CH<sub>3</sub>, 3H); HR ESI calcd. for C<sub>31</sub>H<sub>33</sub>N<sub>4</sub>OZn 541.1940, found *m/z* 541.1943 (M<sup>+</sup>); MS MALDI-TOF calc. 543.01, found *m/z* 542.80 (M<sup>+</sup>) (dithranol).

## Fraction (I) Intermediate- Reaction Mixture (Orange-red)

Excess TFA and DCM from the reaction mixture were evaporated off and dried under vacuum before obtaining the <sup>1</sup>H NMR of the intermediates.

**A.** Reaction of b-bilene with methyl pyruvate UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm: 454, 524 ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 12.74, 12.64, 12.39, 12.04 (s, NH, 4H) 7.61 (d, 1-H, 1H), 7.33 (s, 5-H, 1H), 7.27 (s, 15-H, 1H), 4.36 (s, 10-H, 2H), 4.27-4.24 (q, CH<sub>3</sub>-CH-CO<sub>2</sub>CH<sub>3</sub>, 1H), 3.70(s, OCH<sub>3</sub>, 3H), 2.31-.2.28, 2.08-1.97 (s, CH<sub>3</sub>, 24H), 1.64 (d, -CH<sub>3</sub>-CH-, 3H); MS MALDI-TOF calc. 500.67, found *m/z* 500.47 (M<sup>+</sup>).

**B.** Reaction of b-bilene with 1,2-diketone UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$ ,nm: 454, 524 ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz)  $\delta$ , ppm 12.81, 12.71, 12.43, 12.16 (s, NH, 4H) 7.60 (s, 1-H, 1H), 7.32 (s, 5-H, 1H), 7.27 (s, 15-H, 1H), 4.42 (10-H, 2H), 4.23 (q, CH<sub>3</sub>-CH-CO<sub>2</sub>CH<sub>3</sub>, 1H), 2.31-.2.28, 2.17, 2.08, 2.03, 1.97, 1.96 (s, CH<sub>3</sub>, 27H), 1.54 (d, -CH<sub>3</sub>-CH-, 3H); MS MALDI-TOF calc. 484.67, found *m/z* 484.34 (M<sup>+</sup>).

Fraction (II) Intermediate – After washing fraction (I) with base, or separation on an alumina (grade III) column (Green)

UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>): λ<sub>max</sub>,nm: 431, 789 ; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 11.95 (s, NH, 2H), 11.34, 11.15 (s, NH, 2H) 7.00 (s, 1-H, 1H), 6.43 (s, 5-H, 1H), 6.41 (s, 15-H, 1H),
5.44 (s, 10-H, 1H), 4.33 (q, CH<sub>3</sub>-CH-CO<sub>2</sub>CH<sub>3</sub>, 1H), 3.69 (s, OCH<sub>3</sub>, 3H), 2.18-2.15, 2.08-1.95 (s, CH<sub>3</sub>, 24H), 1.62 (d, -CH<sub>3</sub>-CH-, 3H)

# Fraction (III) Intermediate – After addition of zinc acetate to the green fraction II (Reddish)

MS MALDI-TOF calc. 560.04, found m/z 560.07 (M<sup>+</sup>).

#### Synthesis of (7) using MacDonald 2 + 2 route

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Dibenzyl 5-(methoxycarbonyl)-2,3,5,7,8-pentamethyldipyrromethane-1,9-
dicarboxylate (9)
```

600 mg (0.0025 mol) of α-free pyrrole (**8**) were dissolved in 30 ml dry DCM, followed by excess TFA (100 equiv.), and 0.125 g (0.0012 mol) of methyl pyruvate. The reaction was left to stir under argon at room temperature for 12 h afterwhich TLC confirmed completion of reaction. The mixture was washed with water, then aqueous Na<sub>2</sub>CO<sub>3</sub>, then water again, before being purified on silica gel column using DCM as eluant. A yellow fraction was collected first, followed by a slow moving fraction (product). Evaporation of the solvent yielded a yellowish liquid product, which was dried under vacuum to give 70% (1.0 g) of (**9**). <sup>1</sup>**H NMR** (CDCl<sub>3</sub>, 300MHz) δ, ppm 9.3 (s, NH, 2H), 7.48-7.34 (m, Ph-H, 10H), 5.36 (s, -CH<sub>2</sub>-Ph, 4H), 3.77(s, OMe, 3H), 2.30, 1.65 (s, β-Me, 12H), 1.98 (s, 5-Me, 3H)

**5-Methoxycarbonyl-2,3,5,7,8-pentamethyldipyrromethane-1,9-dicarboxylic acid (16)** The procedure for debenzylation is as described in chapter 1 (500 mg, 0.88 mmol) to yield a off-white solid after recrystalization from THF/petroleum ether in 84% (254 mg) yield. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 250MHz) δ, ppm 10.33 (s, NH, 2H), 3.64(s, OMe, 3H), 2.11, 1.39 (s, β-Me, 12H), 1.89 (s, 5-Me, 3H)

# Zinc(II) 13,17-Diethyl-2,3,5,7,8,12,18-heptamethyl-5-methoxycarbonylisoporphyrin chloride (7)

193 mg (0.53 mmol) of diacid dipyrromethane (16) was suspended in 30 ml of dry dichloromethane, then added p-TsOH (406 mg) dissolved in 4 ml dry methanol. The suspension cleared and 153 mg (0.53 mmol) of diformyl dipyrromethane (17) was added to the mixture, followed by zinc acetate (40 mg) in dry methanol (2 ml). The reaction mixture was left to stirr under argon overnight, then opened to air for 3 more days. It was then washed with water, aqueous NaHCO<sub>3</sub>, then brine and dried over Na<sub>2</sub>SO<sub>3</sub>. Purification was done on a silica column using DCM as eluant to separate a fast moving porphyrin fraction (purple), then gradually increasing the solvent polarity (1% MeOH/DCM) to separate a second fraction (yellowish), then a third fraction which appeared red on the column (major product - isoporphyrin) to give 7 in 14% yield (46 mg). UV/Vis (CH<sub>2</sub>Cl<sub>2</sub>):  $\lambda_{max}$  nm ( $\epsilon \times 10^4$ , M<sup>-1</sup>.cm<sup>-1</sup>): 433 (3.22), 839 (2.45); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 7.68 (s, meso-H, 1H), 7.63 (s, meso-H, 2H), 3.67 (s, 5-OCH<sub>3</sub>, 3H), 2.92-2.90 (q, -CH<sub>2</sub>-CH<sub>3</sub>, 4H), 2.49, 2.45, 2.27, 2.23 (s, β-CH<sub>3</sub>, 18H), 1.90 (s, 5-CH<sub>3</sub>, 3H), 0.92-0.87 (t, -CH<sub>2</sub>-CH<sub>3</sub>, 6H); MS MALDI-TOF calc. for C<sub>33</sub>H<sub>37</sub>N<sub>4</sub>O<sub>2</sub>Zn 587.06, found m/z 587.68 (M<sup>+</sup>) (dithranol).

# General procedure for synthesis of meso-monosubstituted porphyrins (18) from (7)

Zinc(II) isoporphyrin (7) was dissolved in dry dichloromethane followed by addition of 5% KOH dissolved in dry methanol. The reaction was left to stir under argon for 1 h after which the color of the reaction mixture turned purple. The UV/Visible

spectrum indicated no starting material. Excess KOH was neutralized by washing with acetic acid (pH=5), then with water several times, it was dried over Na<sub>2</sub>SO<sub>3</sub>, and purified on alumina grade III using DCM as eluant, to isolate the target compound.

# General procedure for synthesis of *meso*-monosusbtituted porphyrins (19) from bbilene (6)

0.2ml of TFA was added to b-bilene (35 mg, 0.054 mmol) in a RBF and stirred for 10 min under argon. Dry dichloromethane (15 ml) was added to the mixture followed by Ni(Acac)<sub>2</sub> (18 mg, 0.07 mmol) in 0.5 ml of methanol, then 1.0 equivalents of benzaldehyde. The mixture was left to stir at room temperature under argon, in the dark, for 1 h. Spectrophotometry showed absorption peaks at 453 and 490 nm. TEA (0.1 ml) was added to neutralize excess TFA, and immediately DDQ was added and stirring was continued for 30 min after which the reaction was stopped (UV/Visible spectrometry showed a strong absorption at 416 nm). After washing several times with water, the product was isolated by alumina column grade III, using dichloromethane as eluant. A second column on silica gel column using 2% MeOH/DCM was done to separate excess benzaldehyde from the product (8 mg).

### 19b

8 mg, 28% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 10.17 (s, *meso*-H, 2H), 9.96(s, *meso*-H, 1H), 8.10, 7.79 (m, Ph, 5H), 3.65, 3.62, 3.56 (s, β-CH<sub>3</sub>, 24H), -3.15 (br, NH, 2H); MS MALDI-TOF calc. for C<sub>34</sub>H<sub>34</sub>N<sub>4</sub>498.66, found *m/z* 498.68 (M<sup>+</sup>).

#### 19c

7 mg, 25% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 10.16 (s, *meso*-H, 2H), 9.93(s, *meso*-H, 1H), 7.71 (s, Ph, 2H), 7.47 (s, Ph, 1H), 3.63, 3.60, 3.57, 3.50 (s, β-CH<sub>3</sub>, 24H),

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2.62, 2.55 (s, Ph-CH<sub>3</sub>, 6H), -3.17 (br, NH, 2H); MS MALDI-TOF calc. for  $C_{36}H_{38}N_4$ 526.71, found *m/z* 526.94 (M<sup>+</sup>).

# 19d

3 mg, 10% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 10.18 (s, *meso*-H, 2H), 9.95(s, *meso*-H, 1H), 7.50 (s, Ph, 2H), 7.05 (s, Ph, 1H), 3.98 (s, Ph-OMe, 6H), 3.64, 3.61, 3.58, 3.56 (s, β-CH<sub>3</sub>, 24H), -3.22 (br, NH, 2H); MS MALDI-TOF calc. for C<sub>36</sub>H<sub>38</sub>N<sub>4</sub> 558.71, found *m/z* 559.20 (M<sup>+</sup>).

# 19e

9 mg, 30% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 10.18 (s, *meso*-H, 2H), 9.97(s, *meso*-H, 1H), 8.48 (d, Ph, 2H), 8.21 (d, Ph, 2H), 4.04 (s, Ph-CO<sub>2</sub>Me, 3H), 3.65, 3.62, 3.56 (s, β-CH<sub>3</sub>, 24H), -3.16 (br, NH, 2H)

#### CHAPTER 5

# BIOLOGICAL EVALUATIONS OF METALLO-ISOPORPHYRINS FOR APPLICATION IN PHOTODYNAMIC THERAPY

#### 5.1 Introduction

One of the limitations of first-generation photodynamic therapy (PDT) photosensitizers is that light needed to activate them cannot pass through more than about one-third of an inch of tissue (1 <u>centimeter</u>). For this reason, PDT is usually used to treat tumors on or just under the skin or on the lining of internal organs or cavities.<sup>76</sup> PDT is also less effective in treating large tumors, because the light cannot pass far into these tumors.<sup>76-78</sup> Optimal human tissue penetration by light apparently occurs between 650-800 nm and hence photosensitizers with a strong absorption band in this region (the phototherapeutic window is approximately 620-850 nm) can be activated to penetrate deeper into the tissues.<sup>39,42,44</sup> Focus on the development of long-wavelength absorbing photosensitisers is crucial.

Due to their absorptions at long wavelengths, around 800 nm, metalloisoporphyrins are potential candidates for PDT. They are also chemically pure, of known specific composition, and they are fluorescent. This last property enables the detection of tumors by fluorescence imaging. The use of a fluorescent sensitizer in PDT allows the combination of diagnosis and therapy, as well as effective treatment planning.<sup>43</sup>

Other factors for an ideal photosensitizer are minimal toxicity in the dark but they must be phototoxic and it should be selectively accumulated into malignant tissues. These biological properties are evaluated by conducting experiments that will reflect dark- and phototoxicity, intracellular localization and cell-uptake of the photosensitizer. The photosensitizers in question are: zinc isoporphyrin (1), copper isoporphyrin (2), 'Me-

















**Figure 5.1:** Metallo-isoporphyrin compounds evaluated for their biological properties applicable in PDT.

pyruvate' derived zinc isoporphyrin (**3**), 'Et-pyruvate' derived zinc isoporphyrin (**4**), 'Phpyruvate' derived zinc isoporphyrin (**5**), 'i-Bu-pyruvate' derived zinc isoporphyrin (**6**) and 'PMe' zinc isoporphyrin (**7**) as shown in **Figure 5.1.** These compounds were synthesized by varying the central metal ion, and the substituents on the 5-*meso* position and the periphery of the isoporphyrin (see **Chapter 4**).

### 5.2 Results and Discussion

#### 5.2.1 Dark- and Photo-toxicity

It is necessary for an ideal photosensitizer to have low dark-toxicity and only be cytotoxic in the presence of light. Dark-toxicity assays for both zinc (1) and copper (2) isoporphyrins were performed in vitro using human HEp2 cells. The cells were incubated for a period of 20-24 h with various concentrations of metallo-isoporphyrin of up to 100  $\mu$ M in medium and viable/survival cells were measured fluorescently. Both compounds showed low dark-toxicity as shown in **Figures 5.2 and 5.3**, black curve, especially copper isoporphyrin. Concentrations of up to 60  $\mu$ M for zinc isoporphyrin reflected low dark-toxicity while a significant decrease in cell survival is observed at higher concentrations. The IC<sub>50</sub>, value which defines 50% cell viability, for the dark-toxicity is ~95  $\mu$ M for copper isoporphyrin and 85  $\mu$ M for zinc isoporphyrin.

When activated by light (low dose, 1 J/cm<sup>2</sup>), both isoporphyrins were found to be phototoxic especially zinc isoporphyrin with IC<sub>50</sub> of ~35  $\mu$ M; copper isoporphyrin was moderately phototoxic with IC<sub>50</sub> of ~85  $\mu$ M. At concentrations of 100  $\mu$ M, we observe almost 100% cell kill for zinc isoporphyrins.

Copper isoporphyrin reflects very low dark-toxicity with increased concentration compared to zinc isoporphyrin, while a significant phototoxic activity is observed with

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Figure 5.2: Dark-(black) and photo- (red) toxicity of zinc isoporphyrin towards human HEp2 cells at concentrations of up to  $100 \ \mu M$ 



**Figure 5.3:** Dark- (black) and photo- (red) toxicity of copper isoporphyrin towards human HEp2 cells at concentrations of up to  $100 \mu$ M.

zinc isoporphyrin compared to copper isoporphyrin. These results reflect the profound effect due to difference in metal ions on the macrocycle, and indicate that very low concentrations of both compounds are sufficient to induce photo-activity.

Figure 5.4 shows the dark toxicity of 3, 4, 5, 6, and 7 at concentrations of up to 20  $\mu$ M. At concentrations higher than 20  $\mu$ M, no results were obtained due to poisoning of the cells, predominantly for compounds 6 and 7, while compound 5 precipitated out of the solution. The compounds show low dark toxicity (above 80% cell survival) except for compounds 6 and 7 which allow only  $\sim 20\%$  cell survival at the same concentration. This demonstrates the effects of substitution pattern/functional groups on the macrocycle. Compared to zinc isoporphyrin (1), varying the substituents at the 5-meso position (for 3, 4, and 5) has little effect on dark toxicity at the same concentration, 20  $\mu$ M (~ between 70-90% cell survivals vs. 87% cell survival for compound 1). However, compound 6 which exhibit a similar substitution pattern, shows a high dark toxicity (at 20  $\mu$ M, there is  $\sim 20\%$  cell survival). Compound 7, which has the same substitution pattern (as the rest of the library) at the *meso* position but different peripheral substitution (methyl groups compared with propionic ester substituents), behave similarly to compound 6. The only similarity between these two compounds, 6 and 7, is that they posses a 3-carbon alkyl substituent, which is flexible and can orient to different angles/positions within the macrocycle. This may interpret the similar behavior observed for both the compounds. The other compounds (3, 4, and 5) posses rigid functional groups (methyl, phenyl), thus classifying their reactivity together.



Figure 5.4: Dark toxicity of 'pyruvate' zinc isoporphyrin library (3, 4, 5, 6, and 7) towards human HEp2 cells at concentrations of up to  $20 \mu$ M.



Figure 5.5: Photo toxicity of 'pyruvate' zinc isoporphyrin library (3, 4, 5, 6, and 7) towards human HEp2 cells at concentrations of up to  $20 \mu$ M.

As demonstrated by zinc isoporphyrin (1), metallo-isoporphyrins **3**, **4**, **5**, **6** and **7** are also phototoxic. However, change in functional groups at the 5-*meso* position of the zinc isoporphyrin reflects increased phototoxic activity for **3**, **4**, **6** and **7**. The IC<sub>50</sub> of these compounds is ~17, 19, 11, and 15  $\mu$ M, respectively compared to ~35  $\mu$ M for compound **1**. Infact, for compound **6** and **7**, we observe 100% cell death at 20  $\mu$ M. This may be in part due to the partial contribution from dark toxicity effect. These concentrations are relatively low compared to compounds **1** and **2** above, and even much lower compared to porphyrin derivatives synthesized in our group. Compound **5** shows low or almost no phototoxicity at this concentration. There is no clear explanation to the results, but this compound has been identified to precipitate out of the medium, probably due to aggregation, and this may have quenched the fluorescence. Also, since the concentrations used for this experiment were low (20  $\mu$ M), increased concentration on this compound may reflect phototoxic activity.

#### 5.2.2 Cellular Uptake

The time dependent cellular uptake of compounds 1, 3, 4, 5, 6 and 7 was investigated in HEp2 cells at a concentration of 20  $\mu$ M over a time period of 24 h. The compound concentration was read using FLUOstar plate reader with fluorescence filter range of 410 nm (excitation filter) and 840 ± 40 nm (emission filter). Figure 5.6 shows the uptake results. All the compounds exhibit a rapid uptake within the first few hours. Compound 1 reaches a plateau after 4 h of accumulation, whereas compounds 3, 4, and 7 continue to accumulate slowly until the accumulation levels out. These compounds show the same amount of accumulation within 24 h. Compounds 5 and 6 showed continued increase in accumulation with respect to time, especially compound 7 which demonstrated higher levels of uptake among all the compounds. The significantly higher extent of uptake by compound 7 may be correlated to its high phototoxicity. Compound 5 precipitated, so we might be measuring settling rates with it.

### 5.2.3 Fluorescence Microscopy

The intracellular localization of porphyrins is an important factor in determining the efficiency of tumor-cell destruction. Porphyrin compounds preferentially accumulate in certain organelles, such as the lysosomes, the mitochondria, the endoplasmic reticulum, the Golgi apparatus, and to a certain extent, in the nuclei of tumor cells.

Human HEp2 cells were allowed to grow for 48 h and incubated overnight with metallo-isoporphyrin at 10  $\mu$ M. Organelle tracers (mito-tracker green, lyso-sensor green, ER-tracker green, and BODIPY ceramide) were added concurrently with compound and distribution of compound determined using Zeiss AxioVert 200M fluorescence microscope fitted with standard filter sets (Texas Red, FITC, DAPI and Cy5LP). **Figures 5.7** – **5.13** show the experimental results of sites of localization of zinc and copper isoporphyrin complexes, **1-7**. Slide (a) shows the phase contrast, (b) shows the overlay of phase contrast with fluorescence of zinc isoporhyrin, (c) shows the fluorescence of BODIPY ceramide, (d) shows the overlay of the fluorescences of zinc isoporphyrin and BODIPY ceramide, (e) shows the fluorescence of mitotracker green, (f) shows the fluorescence of lysotracker green, (h) shows the overlay of the fluorescences of zinc isoporphyrin and lysotracker green, (i) shows the fluorescence of ER-tracker green, and (j) shows the overlay of the fluorescence of zinc isoporphyrin and ER-tracker green. The



Figure 5.6: Time-dependant cell uptake for compounds 1, 3, 4, 5, 6 and 7, in HEp2 cells at 20  $\mu$ M, for 24 h.

BODIPY ceramide, mitotracker green, lysotracker green and ER-tracker green are fluorescence probes that specifically label the golgi, mitochondria, lysosomes and endoplasmic reticulum, respectively. The overlay (h) (Figure 5.7) of the fluorescence signals from mitotracker green and zinc isoporphyrin, indicated preferential localization of zinc isoporphyrin in the mitochondria. We also observed a signal from the lysosomes (f) and the ER (j), showing that some compound accumulated in the lysosomes and endoplasmic reticulum (ER). Zinc(II) isoporphyrin is hydrophobic and cationic. Cationic compounds are reported to accumulate preferentially in the mitochondria<sup>79</sup> in part due to the highly negative electrochemical potential of the inner mitochondrial potential. Mitochondria localization is vital because porphyrin-induced apoptosis in tumors is primarily correlated with mitochondrial photodamage, and usually occurs rapidly, probably as a result of cascade-like cell killing process, leading to a rapid loss of treated tissue.<sup>43</sup> In addition to localizing in this critically important organelle, zinc isoporphyrin also localizes in lysosomes, displaying multilple localization sites within the cell, a general trend for porphyrin type compounds, which might account for their effectiveness in tumor cell-destruction.<sup>43</sup> Copper isoporphyrin (2) predominantly localizes in the lysosomes (f) with some signal being observed from the mitochondria (h) and ER (j) (Figure 5.8). A different stain (blue) was used to label ER (i) in this experiment with copper isoporphyrin instead of green that was used for all the other compounds. A whitish staining on the overlay (j), indicates localization. This similar localization pattern to the mitochondria, lysosomes and ER is also observed for compounds **3** and **4**, Figure 5.9 and 5.10 respectively. Compound 5 predominantly accumulates in the lysosomes and ER (Figure 5.11). This compound had a tendency to precipitate out of the medium.



Figure 5.7: Intracellular localization of zinc isoporphyrin (1) at 10  $\mu$ M in HEp2 cells



Figure 5.8: Intracellular localization of copper isoporphyrin (2) at 10 $\mu$ M, overnight, in HEp2 human cells



Figure 5.9: Fluorescence microscopy of 3 (Me) at 10  $\mu M,$  overnight, in HEp2 cells



Figure 5.10: Fluorescence microscopy of 4 (Et) at 10 µM, overnight, in HEp2 cells



Figure 5.8: Fluorescence microscopy of 5 (Ph) at 10 µM, overnight, in HEp2 cells



Figure 5.9: Fluorescence microscopy of 6 (iBu) at 2.5  $\mu M$  , overnight, in HEp2 cells



Figure 5.10: Fluorescence microscopy of 7 (pMe) at 2.5  $\mu$ M , overnight, in HEp2 cells

Due to the observed high toxicity of compounds **6** and **7**, the fluorescence microscopy studies were performed at lower concentrations of 2.5  $\mu$ M. For compound **6**, the effect of high toxicity is observed on the cell morphology - the cells are 'not happy', but all the same, localization of this compound is in the lysosomes and mitochondria (**Figure 5.12**). Compound **7** predominantly localizes in the endoplasmic reticulum (ER), and some compound accumulates into the lysosomes, Golgi and mitochondria (**Figure 5.13**).

### 5.3 Conclusion

All the compounds were found to show low dark toxicity with an exception of **6** and **7** which exhibited a relatively high dark toxicity. They are also phototoxic and their accumulation in cells is time dependant. A unique observation about compounds **3-7** is that small concentrations are required for efficacy. Also, these cationic compounds preferentially localize in the mitochondria (the most crucial organelle) and the lysosomes, and even the ER and Golgi, displaying multiple localization sites within a cell.

Further work needs to be done on introducing water soluble functional groups to the macrocyle to generate both amphiphilic and water soluble compounds for easy administration into the human body.

#### **CHAPTER 6**

# SYNTHESIS OF PROTOPORPHYRIN IX DERIVATIVES FOR MECHANISTIC STUDIES OF PHOTODYNAMIC THERAPY; COMPLETION OF THE FINAL SYNTHETIC STEPS

#### 6.1 Introduction

X-Ray and NMR studies have shown that the hydrophobic vinyl-bearing rings of protoheme IX (1) are usually the most deeply embedded in the protein pockets of, for example, myoglobins and hamoglobins, and that the carboxylate groups are consequently pointing to the outside of the protein cleft, interacting with the polar outside of the protein.<sup>5,80</sup> It seems likely that the length of the carboxylate side chains might affect physiological action by displacing the heme within the protein pocket, thereby inducing tension at the iron-histidine bond; the apoprotein is, of course, exquisitely designed to accommodate the protoheme IX prosthetic group. NMR studies have shown that if the carboxylates are best situated at the polar edge of the heme pocket then modifying and positioning of the propionates around the heme periphery will affect the depth and protein contacts of the heme within the cleft.<sup>5,81-83</sup> In connection with studies of the mechanistics of photodynamic therapy (PDT) in membranes,<sup>84,85</sup> it has correspondingly been shown that the depth of a sensitizing porphyrin within a membrane can be controlled by the length of the carboxylate-containing side chain; it has also been shown that the depth of the membrane penetration by a porphyrin affects strongly the effectiveness of PDT sensitization.

To probe this phenomenon of PDT sensitization at greater depths, protoporphyrin IX (PPIX) derivatives, each with a different carboxylic acid chain length were prepared for study. Total and partial syntheses of PPIX with 13,17-di-acetic (**2**) and 13,17-di-

butyric (**3**) side chains have been previously reported; compound (**2**) was prepared by total synthesis using a,c-biladiene intermediate,<sup>86</sup> whereas compound (**3**) was prepared by total synthesis using the a,c-biladiene<sup>87</sup> and MacDonald<sup>87</sup> routes, as well as by bis-(one-carbon) homologation of intact protoporphyrin IX dimethyl ester (**4**).<sup>86</sup> In comparison to the PDT sensitization allowed by bis-propionic protoporphyrin IX (**5**) within a membrane,<sup>85</sup> two additional PPIX derivatives, bis-pentanoic (**6**) and bis-heptanoic (**7**) were targeted as illustrated in **Figure 6.1**.







Figure 6.1: PPIX analogues

# 6.2 Results and Discussion

This project involves the completion of Dr. Jianmin Lu's project in Smith group, who synthesized porphyrins **11a**, **b**, and **c**, through a,c-biladiene cyclization (**Scheme 6.1**).

Retrosynthetically, the PPIX (6) and (7) can be envisioned as being prepared via the a.c-biladiene route.<sup>5</sup> Since the top half of the molecule remains constant, only one dipyrromethane (8) need be prepared, and methods for its synthesis were available in the literature.<sup>88</sup> The future vinyl groups are best masked against side-reactions by use of 2chloroethyl substituents.<sup>88</sup> To provide the lower half of the PPIX analogues, new formylpyrroles (9) were required. These were prepared by Dr. Lu, followed by condensation with dipyrromethane to afford the corresponding a,c-biladienes (10), then copper induced cylization to yield all three desired porphyrins (**11a.b.c**).<sup>89</sup> My own part in this project involved the conversion of porphyrins (11) to (14). Dehydrohalogenation of the porphyrins (11) in the presence of base led to the required divinyl products 12 (Scheme 6.1). During this step, hydrolysis of the esters also occurred. Although this certainly was the desired product, purification was difficult without first re-esterifying the porphyrin in the presence of acid and MeOH, and then purifying the crude material on an alumina (grade III) column. Once isolated, the pure esters were hydrolyzed at room temperature in the presence of KOH and THF to yield the diacid PPIX derivatives (13), which were isolated and purified by recrystalization. These were converted into hematoporphyrin IX derivatives (14) by hydrohalogenation of the vinyl groups followed by S<sub>N</sub>1 hydrolysis reaction of the HBr-adduct (Scheme 6.2). PPIX acid derivative (PP3, 4), which is commercially available, was used as a model for this reaction. Its <sup>1</sup>H NMR



Scheme 6.1: Synthesis of PPIX derivatives



Scheme 6.2: Synthesis of hematoporphyrin IX derivatives (14)

**Table 6.1:** Yields of PPIX and hematoporphyrin IX derivatives

	Protoporphyrin% yield		Hematoporphyrin % yield
	Diester (12)	Diacid (13)	(14)
PP2 (a)	49	45	57
PP5 ( <b>b</b> )	47	50	51
PP7 (c)	56	54	58

spectrum in DMSO-d<sub>6</sub> is as shown in **Figure 6.2**; surprisingly all the signals (including the carboxylic acid protons) were evident in the spectrum. Addition of  $D_2O$  to this sample helped to identify the exchangeable protons – COOH, OH, and NH- **Figure 6.3**. The yields of the protoporphyrin IX and hematoporphyrin IX derivatives are as indicated in **table 6.1**, with bis-heptanoic PPIX derivative (**c**) obtaining the highest yields.

### 6.3 Conclusion

These compounds were sent out to Professor B. Ehrenberg, Bar-Ilan, Israel, for membrane incorporation studies. The results from this will be published in due course.

#### 6.4 Experimental

# 13, 17-Bis (methoxycarbonylmethyl)-2,7,12,18-tetramethyl-3,8-divinylporphyrin(12a)

To100 mg (0.157 mmol)of bischloroethylporphyrin (**11**) in a 100ml RBF was added 10 ml of pyridine and 5 ml aqueous 3% KOH and the mixture was refluxed in the dark for 2.5 h. TLC indicated that the ester groups were hydrolyzed to acids. Excess pyridine was reduced under high vacuum then washed with acetic acid (pH=4), extracting with DCM/THF mixture. The organic layers were combined, dried over Na<sub>2</sub>SO<sub>4</sub> and evaporated. This crude product was re-esterified using 5% H<sub>2</sub>SO<sub>4</sub>/MeOH solution at room temperature, overnight. Work-up was done by washing the mixture with aqueous sodium bicarbonate, then water, extracting with dichloromethane. Purification of the product on an alumina (grade III) column using dichloromethane as eluant, then recrystalization from dichloromethane/petroleum ether, yielded the product **12**.

(12a)

43mg, 49% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 10.02 (ss, *meso*-H, 1H), 9.95 (ss, *meso*-H, 2H), 9.89 (ss, *meso*-H, 1H), 8.4 (m, -CH=CH<sub>2</sub>, 2H), 6.54, 6.32 (dd, -CH=CH<sub>2</sub>, 4H), 5.02 (s, -CH<sub>2</sub>CO<sub>2</sub>Me, 4H), 3.78 (s, -OCH<sub>3</sub>, 6H), 3.62, 3.60, 3.57, 3.53 (s, β-CH<sub>3</sub>, 12H), -3.97 (s, NH, 2H)

# 13, 17-Bis (4-methoxycarbonylbutyl)-2,7,12,18-tetramethyl-3,8-divinylporphyrin(12b)

42 mg, 47% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 10.25 (s, *meso*-H, 1H), 10.20 (s, *meso*-H, 1H), 10.11 (s, *meso*-H, 1H), 9.98 (s, *meso*-H, 1H), 8.34 (m, -CH=CH<sub>2</sub>, 2H), 6.45, 6.39 (dd, -CH=CH<sub>2</sub>, 4H), 4.13 (t, -CH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>CO<sub>2</sub>Me, 4H), 3.75 (s, -OCH<sub>3</sub>, 6H), 3.68, 3.67, 3.64, 3.63 (s, β-CH<sub>3</sub>, 12H), 2.55 (t, -(CH<sub>2</sub>)<sub>3</sub>CH<sub>2</sub>CO<sub>2</sub>Me, 4H), 2.38, (m, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Me, 4H), 2.13 (m, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Me, 4H), -3.75 (s, NH, 2H); MS-MALDI cald. 646.82, found 646.50

# 13, 17-Bis (6-methoxycarbonylhexyl)-2,7,12,18-tetramethyl-3,8-divinylporphyrin (12c)

51 mg, 56% yield. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300MHz) δ, ppm 10.08 (s, *meso*-H, 2H), 9.95 (s, *meso*-H, 1H), 9.88 (s, *meso*-H, 1H), 8.38 (m, -CH=CH<sub>2</sub>, 2H), 6.39, 6.19 (dd, -CH=CH<sub>2</sub>, 4H), 4.01 (t, -CH<sub>2</sub>(CH<sub>2</sub>)<sub>5</sub>CO<sub>2</sub>Me, 4H), 3.64 (s, -OCH<sub>3</sub>, 6H), 3.63, 3.55 (s, β-CH<sub>3</sub>, 12H), 2.35 (t, -(CH<sub>2</sub>)<sub>5</sub>CH<sub>2</sub>CO<sub>2</sub>Me, 4H), 2.29, 1.71, 1.56 (m, -CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>Me, 16H), -3.73 (s, NH, 2H); MS-MALDI cald. 702.92, found 702.93

# 2,7,12,18-Tetramethyl-3,8-divinylporphyrin-13,17-bis-acetic Acid (13a)

The pure diester porphyrin (**12**), (36mg, 0.064 mmol), was dissolved in 5ml THF followed by addition of 3% KOH (3ml) and stirred at room temperature in the dark. After 3h, the reaction was complete. It was then worked up with dilute acetic acid, and then

evaporated off THF. The product crashed out of the aqueous layer and was washed with water (X3) extracting with ethyl acetate. The organic layer was dried over sodium sulphate, evaporated and recrystalized (put in the freezer to further crystallization) using THF/petroleum ether, to afford **13**.

# (13a)

16 mg, 45% yield. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300MHz) δ, ppm 10.29 (s, *meso*-H, 1H), 10.24 (s, *meso*-H, 1H), 10.20 (s, *meso*-H, 1H), 10.15 (s, *meso*-H, 1H), 8.53 (m, -CH=CH<sub>2</sub>, 2H), 6.48,6.25 (dd, -CH=CH<sub>2</sub>, 4H), 5.15 (s, -CH<sub>2</sub>CO<sub>2</sub>H, 4H), 3.70, 3.65, 3.60, 3.56 (s, β-CH<sub>3</sub>, 12H), -4.12 (s, NH, 2H)

# 2,7,12,18-Tetramethyl-3,8-divinylporphyrin-13,17-bis-butyric Acid (13b)

17 mg, 50% yield. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300MHz) δ, ppm 10.27 (s, *meso*-H, 1H), 10.23 (s, *meso*-H, 1H), 10.17 (s, *meso*-H, 1H), 10.10 (s, *meso*-H, 1H), 8.50 (m, -CH=CH<sub>2</sub>, 2H), 6.44,6.23 (dd, -CH=CH<sub>2</sub>, 4H), 4.13 (t, -CH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>CO<sub>2</sub>H, 4H), 3.67, 3.65, 3.63, 3.62 (s, β-CH<sub>3</sub>, 12H), 2.50 (t, -CH<sub>2</sub>COOH, 4H), 2.37-2.09(m, -CH<sub>2</sub>(CH<sub>2</sub>)<sub>3</sub>CO<sub>2</sub>H, 20H), -3.60 (s, NH, 2H)

# 2,7,12,18-Tetramethyl-3,8-divinylporphyrin-13,17-bis-hexanoic Acid (13c)

19 mg, 54% yield. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 300MHz) δ, ppm 10.25 (s, *meso*-H, 1H), 10.21 (s, *meso*-H, 1H), 10.13 (s, *meso*-H, 1H), 10.04 (s, *meso*-H, 1H), 8.42 (m, -CH=CH<sub>2</sub>, 2H), 6.45,6.20 (dd, -CH=CH<sub>2</sub>, 4H), 4.11 (t, -CH<sub>2</sub>(CH<sub>2</sub>)<sub>5</sub>CO<sub>2</sub>H, 4H), 3.67, 3.63 (s, β-CH<sub>3</sub>, 12H), 2.4-2.28, 1.74-1.62, 0.94 (m, -CH<sub>2</sub>(CH<sub>2</sub>)<sub>5</sub>CO<sub>2</sub>H, 20H), -3.58 (s, NH, 2H); MS-MALDI calc. 674.87, found 674.72

### 2,7,12,18-Tetramethyl-3,8-bis(1-hydroxyethyl)porphyrin-13,17-bis-acetic Acid (14a)

PPIX derivative (13) (30 mg, 0.056 mmol), was reacted with 5 ml of HBr-acetic acid for 1.5 h. The mixture was poured into excess water, and further allowing a few minutes for hydrolysis of the HBr-adduct. It was then neutralized with NaOH pellets, portion wise and carefully, until neutral or slightly basic. At this pH, the product crushed out of the aqueous mixture, but increased basicity dissolved the product. It was then extracted with THF/Chloroform mixture and the organic layers were combined, dried over sodium sulphate, evaporated and recrystalized from THF/petroleum ether to yield 57% (18 mg) of the title compound 14.

### **PP3 Model (4):**

### 2,7,12,18-Tetramethyl-3,8-bis(1-hydroxyethyl)porphyrin-13,17-bis-propanoic Acid

<sup>1</sup>**H NMR** (DMSO-d<sub>6</sub>, 250MHz) δ, ppm 12.35 (br, -COOH, 2H), 10.73 (s, *meso*-H, 1H), 10.69 (s, *meso*-H, 1H), 10.29 (s, *meso*-H, 1H), 10.22 (s, *meso*-H, 1H), 6.52(q, -CH-(OH)CH<sub>3</sub>, 2H), 6.18 (s, CH-(OH)CH<sub>3</sub>, 2H), 4.35 (t, -CH<sub>2</sub>-CH<sub>2</sub>-COOH, 4H), 3.70, 3.68, 3.61, 3.58 (s, β-CH<sub>3</sub>, 12H), 3.19 (t, -CH<sub>2</sub>-CH<sub>2</sub>-COOH, 4H), 2.15, 2.12 (d, -CH(OH)-CH<sub>3</sub>, 6H), -3.98 (s, NH, 2H)

<sup>1</sup>**H NMR** (DMSO-d<sub>6</sub> + D<sub>2</sub>O, 250MHz) δ, ppm 10.71 (s, *meso*-H, 1H), 10.68 (s, *meso*-H, 1H), 10.27 (s, *meso*-H, 1H), 10.21 (s, *meso*-H, 1H), 6.49(q, -CH-(OH)CH<sub>3</sub>, 2H), 4.34 (t, -CH<sub>2</sub>-CH<sub>2</sub>-COOH, 4H), 3.68, 3.67, 3.61, 3.58 (s, β-CH<sub>3</sub>, 12H), 3.19 (t, -CH<sub>2</sub>-CH<sub>2</sub>-COOH, 4H), 2.14, 2.11 (d, -CH(OH)-CH<sub>3</sub>, 6H)

### (14a)

<sup>1</sup>**H NMR** (DMSO-d<sub>6</sub>, 250MHz) δ, ppm 11.96 (br, -COOH, 2H), 10.26 (s, *meso*-H, 1H), 10.24 (s, *meso*-H, 2H), 10.12 (s, *meso*-H, 1H), 6.51(q, -CH-(OH)CH<sub>3</sub>, 2H), 6.17 (s, CH-(OH)CH<sub>3</sub>, 2H), 4.50 (s, -CH<sub>2</sub>-COOH, 4H), 3.68, 3.63(s, β-CH<sub>3</sub>, 12H), -3.97 (s, NH, 2H)

# 2,7,12,18-Tetramethyl-3,8-bis(1-hydroxyethyl)porphyrin-13,17-bis-butyric Acid (14b)

16 mg, 51% yield. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>, 250MHz) δ, ppm 11.99 (br, -COOH, 2H), 10.71 (s, *meso*-H, 1H), 10.68 (s, *meso*-H, 1H), 10.30 (s, *meso*-H, 1H), 10.13 (s, *meso*-H, 1H), 6.50(q, -CH-(OH)CH<sub>3</sub>, 2H), 6.16 (s, CH-(OH)CH<sub>3</sub>, 2H), 4.10 (t, -CH<sub>2</sub>-(CH<sub>2</sub>)<sub>3</sub>COOH, 4H), 3.70, 3.67, 3.62, 3.59 (s, β-CH<sub>3</sub>, 12H), 2.24 (t, -CH<sub>2</sub>COOH, 4H), 2.14, 1.92 (m, CH<sub>2</sub>-(CH<sub>2</sub>)<sub>2</sub>-CH<sub>2</sub>COOH, 8H), -3.99 (s, NH, 2H)

# 2,7,12,18-Tetramethyl-3,8-bis(1-hydroxyethyl)porphyrin-13,17-bis-hexanoic Acid (14c)

18 mg, 58% yield. <sup>1</sup>**H NMR** (DMSO-d<sub>6</sub>, 250MHz) δ, ppm 11.94 (br, -COOH, 2H), 10.34 (s, *meso*-H, 1H), 10.31 (s, *meso*-H, 1H), 10.22 (s, *meso*-H, 1H), 10.14 (s, *meso*-H, 1H), 6.52(q, -CH-(OH)CH<sub>3</sub>, 2H), 6.21 (s, CH-(OH)CH<sub>3</sub>, 2H), 4.06 (t, -CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>COOH, 4H), 3.72, 3.67, 3.60, 3.58 (s, β-CH<sub>3</sub>, 12H), 2.19-1.51 (m, CH<sub>2</sub>-(CH<sub>2</sub>)<sub>5</sub>-COOH, 20H), - 3.99 (s, NH, 2H)

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