SYNTHESIS AND MODIFICATION OF BENZOCHLORIN PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY (PDT)

BY

LAU YAN KIN

B.Sc., Hong Kong University of Science and Technology, 1999

A Thesis Presented to
The Department of Chemistry in Partial Fulfillment
of the Requirements for the Degree of
Master of Philosophy in Chemistry

Hong Kong University of Science and Technology
August 2001
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Dedication

To

My Dearest Parents

And

My Love, Mandy
ACKNOWLEDGEMENTS

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# TABLE OF CONTENTS

- AUTHORIZATION ........................................................................................................... II
- ACKNOWLEDGEMENTS .................................................................................................... V
- TABLE OF CONTENTS ..................................................................................................... VI
- LIST OF TABLES ............................................................................................................... VIII
- LIST OF FIGURES ............................................................................................................ IX
- LIST OF ABBREVIATIONS ........................................................................................... X
- NOMENCLATURE ............................................................................................................ XI
- ABSTRACT ....................................................................................................................... XII

## CHAPTER 1  INTRODUCTION: ....................................................................................... 1

PORPHYRIN PHOTOSENSITIZATION AND PHOTODYNAMIC THERAPY 1

1) GENERAL ..................................................................................................................... 1
2) SIGNIFICANCE AND BACKGROUND ........................................................................ 3
3) SENSITIZER DELIVERY AND DISTRIBUTION IN CELLS AND TISSUES .............. 6
4) THE PHOTODYNAMIC EFFECT AND MECHANISM .................................................. 10
5) CATIONIC PDT SENSITIZERS ................................................................................... 15
6) OBJECTIVES OF THE PRESENT WORK ................................................................... 15

## CHAPTER 2  SYNTHESIS AND PROPERTIES OF CATIONIC BENZOCHLORIN SULFONAMIDE DERIVATIVES ................................................................. 17

1) INTRODUCTION .......................................................................................................... 17
LIST OF TABLES

Table 1  UV-vis absorption spectral data of (19-27), their precursors (15-18), (14), and (11) in DCM at room temperature .................................................. 27

Table 2  Photocytotoxicity of photosensitizers 17,18,22,23,25 ........................................ 32

Table 3  UV-vis spectrum of compound (30)-(38) and their precursor (11), (29) in DCM at room temperature ................................................................. 57
LIST OF FIGURES

Figure 1 Pathway of light-sensitive drug administration ................................................. 7
Figure 2 Modified Yablonski diagram .............................................................................. 11
Figure 3 Reactions of singlet oxygen with some biomolecules ........................................ 12
Figure 4 UV-vis absorption spectra of BC (11) and BCSO$_2$C$_6$ (18) in DCM at room temperature .................................................................................................................. 28
Figure 5 UV-vis absorption spectra of SO$_2$Cl (14) and cationic BCSO$_2$C$_6$ (23) in DCM at room temperature .................................................................................................................. 29
Figure 6 UV-vis absorption spectra of BCSO$_2$C$_6$ (18) (Upper) and cationic BCSO$_2$C$_6$ (23)(Lower) in DCM at room temperature .............................................................. 30
Figure 7 Fluorescence images of the NPC cells stained with photosensitizer (23) .......... 34
Figure 8 UV-vis absorption spectra of (29) and (33) in DCM at room temperature ......... 58
Figure 9 UV-vis absorption spectra of (29) and (33) in DCM at room temperature ......... 59
Figure 10 UV-vis absorption spectra of (29) and (51) in DCM at room temperature ....... 76
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Å</td>
<td>Angstrom ($10^{-10}$ m)</td>
</tr>
<tr>
<td>δ</td>
<td>Chemical shift (in ppm)</td>
</tr>
<tr>
<td>λ</td>
<td>Wavelength (nm)</td>
</tr>
<tr>
<td>J</td>
<td>Coupling constant (in Hertz, Hz)</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>MS</td>
<td>Mass Spectroscopy</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>Me</td>
<td>Methyl</td>
</tr>
<tr>
<td>Et</td>
<td>Ethyl</td>
</tr>
<tr>
<td>$t$-Bu</td>
<td>$t$-Butyl</td>
</tr>
<tr>
<td>PPh$_3$</td>
<td>Triphenylphosphine</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>Deuteriated Chloroform</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DME</td>
<td>1,2-dimethoxyethane</td>
</tr>
<tr>
<td>TiCl$_4$</td>
<td>Titanium (IV) tetrachloride</td>
</tr>
<tr>
<td>PDT</td>
<td>Photodynamic Therapy</td>
</tr>
<tr>
<td>BINAP</td>
<td>2,2’-bis(diphenylphosphino)-1,1’-binaphthyl</td>
</tr>
<tr>
<td>NPC</td>
<td>Nasopharyngeal carcinoma</td>
</tr>
</tbody>
</table>
The macrocyclic system (A) is called porphyrin, a name originally used (in hematoporphyrin) by Hopper-Seyler. The numberings of ring positions including nitrogen, and the use of letters to denote individual rings is shown in (A).

C) NiOEP [Ni(II) 2,3,7,8,12,13,17,18-octaethylporphyrin]

D) 2,3,8,8,12,13,17,18-octaethylbenzochlorin
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ABSTRACT

Photodynamic therapy (also called PDT, photoradiation therapy, phototherapy, or photochemotherapy) is a very promising therapy and a new modality for cancer control. It is based on the discovery that certain chemicals known as photosensitizing
agents (e.g. Photofrin® II, a hematoporphyrin derivative (HPD)) can selectively accumulate in tumor tissues, which activate oxygen molecule to become cytotoxic species upon irradiation. PDT destroys cancer cells through the use of a fixed-frequency laser light in combination with a photosensitizing agent. However, the first generation photosensitizer, hematoporphyrin derivatives (HPD), is a complex mixture and it has rather weak absorptivity in the red region of visible spectrum, a region with good tissue penetration. Recently, attempts have been made to improve the efficacy of in vivo photosensitizers by utilizing purer and well characterized materials.

To develop so-called second generation photosensitizers for PDT, different derivatives of chlorin and benzochlorin monomers which exhibit absorption maxima covering the region between 650 to 840 nm have been synthesized in high yields by introduction of electron-withdrawing and donating groups on the chromophore and by expanding the π-conjugation of the macrocycle.

In this thesis, monomeric and dimeric benzochlorin derivatives have been prepared to contain functional groups as well as to improve absorption in longer wavelengths. To study the localization and photodamage mechanism of photosensitizer in tumor cells, several cationic benzochlorin and chlorin imidinium salt have been prepared in high yields.

Photodynamic activities of these compounds have been screened on nasopharyngeal carcinoma (NPC) cells. The cationic photosensitizers particularly, gave promising results on in vitro NPC cells in terms of their photocytotoxicity.
CHAPTER 1 INTRODUCTION:
PORPHYRIN PHOTOSENSITIZATION AND PHOTODYNAMIC THERAPY

1) GENERAL

Tetapyrrolic macrocycles are probably the most ubiquitous of all naturally occurring pigments and include both heme and chlorophyll. The first porphyrin isolated was prepared from hemoglobin in 1867. Thudichum\(^2\) first prepared this porphyrin by treatment of hemoglobin with concentrated acid. Four years later, Hoppe-Seyler\(^3\) reported a similar preparation and obtained a purple substance that he called hematoporphyrin (1). About twenty years later, Nenchi\(^4\) prepared hematoporphyrin hydrochloride (from isolated hemin) as the first pure porphyrin. However, Schum and co-workers\(^5\) showed through spectroscopic investigation that hematoporphyrin was not the same porphyrin as that of the heme prosthetic group. Degradative studies by Küster\(^6\) distinguished hematoporphyrin with its hydroxyethyl side chain from the prosthetic group porphyrin which contains vinyl substances. In 1912, Küster\(^7\) proposed the correct ring structure (2) for porphyrins. In 1926 Fischer\(^8\) prepared etioporphyrin-I (3) by the first totally synthetic pathway and followed this shortly thereafter with the complete synthesis of
octamethylporphyrin (4) by two distinctly separate methods. This led Fischer to adopt the porphyrin ring structure originally proposed by Küster in 1912. The extensive porphyrin degradation work and the synthesis by his group of the four-etioporphyrin isomers and twelve of the fifteen isomers of mesoporphyrin (5, type IX isomer) helped Fischer correlate the sequence of substituents about the natural porphyrin ring system as the least symmetrical of the possible fifteen substituent patterns; this was named as the naturally occurring type IX substituents array, which is related to the type-III arrangement in circumstances where only two, rather than three, types of substituents are present. In 1929, Fischer synthesized protoporphyrin IX (6), and the hemoglobin prosthetic group itself, hemin (7), the iron (III) chloride complex of protoporphyrin IX.

Porphyrin and other closely related tetrapyrrlic pigments occur widely in nature, and are implicated in a great variety of vital biological process.
2) SIGNIFICANCE AND BACKGROUND

Photomedicine has been practiced since at least the time of the ancient Egyptians\textsuperscript{11}. Psoralins from orally ingested plants accumulated in the skin and when activated by sunlight brought about repigmentation of the skin. This 4000-year-old treatment for vitiligo is still used today with psoralins and UV exposure and represents the best-known treatment even though it has only limited success. Indeed, a number of skin diseases, including acne, eczema, herpes simplex, and psoriasis, have been treated in a similar manner.
Niels Finsen won the Nobel Prize in Medicine in 1903 for his treatment of cutaneous tuberculosis by UV radiation. However, the most promising areas of photomedicine are those that use a photosensitizer. Indeed, it was believed in the 19th century that all efficacious drugs were colored and while this belief led to the discovery of the sulfa drugs (Prontosil is an azo dye), it was not until the beginning of the 20th century that the medicine properties of photosensitizers were explored. Raab12 in 1900 showed that acridine dyes and light effectively killed paramecia. In 1925 Policard13 examined the ability of porphyrins, including hematoporphyrin (8), to produce a phototoxic effect. Mayer-Betz injected to himself with 200mg of hematoporphyrin14 and suffered no ill effects until exposed to sunlight, whereupon he suffered extreme swelling and remained photosensitized for several months. Similar accumulation of porphyrins in skin of porphyric patients may cause severe skin necrosis upon exposure to strong light15. Auler and Banzer in 1946 showed that hematoporphyrin accumulated in cancerous tissues and, since it exhibits strong fluorescence, its localization into neoplastic and rapidly dividing tissue could be quantitatively assessed16.

Hematoporphyrin derivative (HPD) was first prepared by Schwartz and co-workers and its potential as a radiosensitizer was examined17, but in 1964, Lipson et al. showed that HPD preferentially accumulated in cancerous tissue rather than in the surrounding healthy tissue181920. Interest in photomedicine and particularly photodynamic therapy (PDT) was rekindled when Dougherty21 showed that HpD could be purified by gel exclusion chromatography, which removed monomeric porphyrins. The remaining oligomeric material is called hematoporphyrin derivative
(HPD) or dihematoporphyrin ether/ester (DHE) \(^{22}\) and as Photofrin®, a photosensitizer now marketed commercially. This drug has now been tested on well over 5000 patients world-wide and is proven to be effective against tumors in the lung, bladder, esophagus, skin and other tissues\(^{23}\).

Red light, usually from an argon pumped dye laser tuned to 630 nm (corresponding approximately to the longest wavelength, but weakest, absorption peak of HpD), is most commonly used in PDT to permit the maximum of light penetration into mammalian tissue. However, porphyrins absorb poorly in this region. Thus, there is a considerable interest in identifying effective photosensitizers for PDT that absorb more strongly in the red or near IR wavelengths to which tissues are highly transparent, and the development of low-cost reliable diode lasers, which emit in the 750-850 nm range. The need for compounds absorbing around 750 nm (optimum wavelength for tissue penetration)\(^{24}\) should be obvious.

PDT is based on the dye-sensitized photooxidation of biological matter in the target tissue. In clinical PDT, sensitizers are introduced into the organism as the first step of treatment. In the second step, the tissue-localized sensitizer is exposed to light of wavelength appropriate for absorption by the sensitizer. Through various photophysical pathwats, also involving molecular oxygen, cytotoxic species harmful to cell function arise and eventual tissue destruction results.
3) SENSITIZER DELIVERY AND DISTRIBUTION IN CELLS AND TISSUES

The first step towards PDT tumor treatment is the delivery of photosensitizer to the target tissue. A light-sensitive drug is administered intravenously, within a period of two to three days, the drug selectively concentrates in diseased cells while largely clearing from normal tissue. The drug remains inactive until exposed to laser light. When applied, the laser energy, delivered to the cancer site through a fiberoptic device, chemically activates the drug and creates a toxic form of oxygen, which destroys the cancerous cells with minimal damage to healthy cells. Both the drug injection and the laser treatment can be performed, in many situations, on an outpatient basis.

Step by step, here's what happens (Figure 1):

1. Patient is injected intravenously with a light-sensitive drug.

2. The drug is retained by malignant tissue, remaining inactive until exposed to a specific wavelength of heatless laser light.

3. Laser energy is directed to the site (in this case, the lung) through a flexible fiberoptic device.
4. When activated by the laser's light energy, the drug creates a toxic form of oxygen (singlet oxygen) that destroys the cancerous cells with minimal damage to surrounding healthy cells.

Principal side effects of PHOTOFIN include a skin sensitivity to light for four to six weeks. When treating lung cancer, additional side effects following the procedure could include possible inflammation at the treatment site causing varying degrees of shortness of breath and coughing.

![Figure 1. Pathway of light-sensitive drug administration.](image)

In medical terminology, the pending late-stage lung cancer treatment is for the reduction of obstruction and palliation of symptoms in patients with completely or partially obstructing endobronchial nonsmall cell lung cancer. The U.S. Food and Drug Administration's approval of early stage lung cancer is for the treatment of microinvasive endobronchial non-small cell lung cancer in patients for whom surgery and radiotherapy are not indicated. The FDA's approval of esophageal
cancer is for the palliation of patients with completely obstructing esophageal cancer, or of patients with partially obstructing esophageal cancer who, in the opinion of their physician, cannot be treated satisfactorily with Nd:YAG laser therapy.

The mechanism of delivery of the active PDT sensitizers to cells that will ultimately be affected is of great interest as it may provide insights into the uptake and retention of these compounds in tumor cells. It has been suggested that HpD may be delivered to tumor cells by low-density lipoprotein (LDL) since HpD shows a high affinity for LDL and because tumor cells, like all fast-growing cells, have increased levels of LDL receptors\textsuperscript{25}.

Lipoproteins are well-known as a vector for cholesterol distribution, but their role in distribution\textsuperscript{26} of drugs has only recently been appreciated. There are three classes of lipoproteins: very low density lipoprotein (VLDL), low density lipoprotein (LDL), and high density lipoprotein (HDL)\textsuperscript{27}. VLDLs contain a large percentage of lipids and low percentage of proteins. Therefore, these substances are very low in density because fats and oils are less dense than water. In contrast, low density and high density lipoproteins contain less lipid and more protein and, therefore, are dense than water. VLDL, LDL and HDL can be separated by density-gradient ultracentrifugation.

LDL particles ultimately bind to cell-surface receptors. The predominant circulating lipoprotein species in several animals, notably dog, mouse, rat and horse, is HDL. In contrast, in man, guinea pig and rabbit the predominant species is LDL.
The latter two animals may therefore be good models for human distribution. In both mouse and man the presence of neoplastic disease lowers the level of circulating LDL, presumably because of the high level of LDL receptors in neoplastic tissues. Binding of the various components of HpD to lipoproteins and serum proteins has been documented\textsuperscript{28}. The affinity of several monoporphyrins with serum albumin has been measured, and the results indicated that a major portion of bound porphyrin is delivered by LDL\textsuperscript{25,29}. The behavior of VLDL associated with porphyrin uptake was less clear probably because VLDL is metabolically converted into other lipoprotein including LDL.

The differences in the behavior of porphyrin-HDL, -LDL and -VLDL complexes can be explained on the basis of the two main modalities of lipoprotein internalization by cells. (i) non-specific fluid endocytosis. (ii) receptor-mediated endocytosis. The latter mechanism concerns LDL and becomes especially important for cells displaying hyperproliferative activity where the number of LDL-receptors on the cell surface drastically increases. Therefore the preferential accumulation and retention of porphyrins by tumor cells does not seem to reflect an intrinsic property of the dye; rather it is a consequence of cell-interaction mechanism typical of the LDL. LDL has been proposed as a specific carrier of cytostatic drugs to tumors\textsuperscript{29}.

Localization of porphyrins in tissue is well documented through numerous investigations. But the exact mechanism of porphyrin uptake and retention still remains obscure. The delivery of PDT sensitizers by LDL suggests a possible mechanism for retention of the active fraction of HpD in the cell. It is easy to
imagine that once through the cell membrane, the porphyrin-protein complex could break up, during digestion of the lipoprotein, for example, and that the free porphyrin polymer would then have poor ability to diffuse out through the cell membrane. Unfortunately this elegant model has suffered recently because Korbelik and co-workers\textsuperscript{30} reported that LDL actually inhibits the uptake of Photofrin\textsuperscript{®} II by tumor cells both \textit{in vitro} and \textit{in vivo}.

An alternative mechanism for the retention of HpD involves the tendency of these species to aggregate in polar environments, such aggregates might adhere to the outside of the cell membrane and be incorporated into the cell by pinocytosis or nonspecific fluid endocytosis. Once in the internal milieu of the cell such aggregates could break up and individual molecules could sequester themselves in lipophilic sites within the cell. Kessel\textsuperscript{31} provides some support for this type of model with a study that suggests differing degrees of aggregation in the oligomeric fraction of HpD as a function of the polarity of the environment. The precise mechanisms for the photosensitizers delivery and distribution in cells and tissues remain unclear.

4) THE PHOTODYNAMIC EFFECT AND MECHANISM

Photodynamic therapy (PDT) requires that the photoactive agent first absorb a photon of a specific wavelength. As can be seen from the modified Yablonski diagram in Figure 3, the first excited singlet state ($S_1$) can fluoresce or this excited state can participate in an electron transfer process with a biological substrate,
resulting in the photobleaching of the photosensitizer and modification (destruction/inactivation) of the substrate. This is known as Type I photoprocess.\textsuperscript{32} Photosynthesis using reduced porphyrins (chlorophylls, bacteriochlorophylls) makes use of a Type I process but the initially oxidized "porphyrin", at the reaction center, is reduced by another porphyrin (cytochrome) such that no net destruction of the photoactive species occurs.

A second, and far more interesting, photochemical process known as a Type II photoprocess results in the conversion of stable triplet oxygen ($^3O_2$) to the short-lived but highly reactive (toxic) singlet oxygen ($^1O_2$). This reaction occurs, as shown in Figure 3, when the $S_1$ state of the photosensitizer undergoes intersystem crossing to its first excited triplet state ($T_1$) followed by a triplet-triplet reaction with ($^3O_2$) to regenerate the photosensitizer in its ground state and singlet oxygen.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{modified_yablonski_diagram.png}
\caption{Modified Yablonski diagram}
\end{figure}

1. Absorption - Depends upon extinction coefficient and wavelength
2. Fluorescence - Lifetime depends on molecular interactions
3. Intersystem crossing
4. Phosphorescence
5. Conversion of triplet oxygen $^3O_2$ to singlet oxygen $^1O_2$
Singlet oxygen has a lifetime of ~6 μs in water and a little longer in lipid and cell membranes, which means that it cannot diffuse more than a single cell length. Singlet oxygen is a powerful but fairly indiscriminant, oxidant that reacts with a variety of biological molecules and assemblies. As shown in Figure 3, oxygen atom transfer can result in the oxidation of both carbon and sulfur and in the formation of hydroperoxides from a variety of substrates including cholesterol.

**Figure 3. Reactions of singlet oxygen with some biomolecules.**

Singlet oxygen (¹O₂), is considered to be most important in initiating biological damage, although definitive conclusions are still lacking. Certainly,
there can be no doubt that combination of oxygen, a photosensitizer, and light of the
correct wavelength will generate singlet oxygen. When this reactive species is
created in the vicinity of oxidizable biomolecules, oxidative damage will assuredly
occur. However, it has not been unequivocally shown that such damage will create a
cell-killing lesion. Recently, Oleinick and colleagues\textsuperscript{34} have shown that cells can die
by apoptosis triggered by PDT.

Most sensitizers showing promise in PDT are efficient generators of singlet
oxygen. Since singlet oxygen is 1 eV (22.5 kcal/mol) higher in energy than the
ground state oxygen, the triplet state ($T_1$) of sensitizer should have at least this
energy. Extrapolating this back to the energy of the excited singlet state ($S_1$) needed
to generate the triplet state ($T_1$) and assuming some energy loss through other
nonradiative processes, it has been suggested that, when porphyrin based
compounds are used as sensitizers, the lowest energy capable of meeting these
requirements translates to an absorption close to 800 nm\textsuperscript{35}. Since tissue penetration
of light increases with increasing wavelength\textsuperscript{36}, sensitizers absorption near 800 nm
would be optimum for PDT.

Type I and type II reactions may occur simultaneously, and the ratio between
the two processes is highly influenced by sensitizer, substrate and oxygen
concentration, as well as the binding of sensitizer to substrate. There is much
indirect evidence to suggest that singlet oxygen is the polar damaging species in
PDT, but direct measurement of singlet production in complex biological systems
appears to be extremely difficult\textsuperscript{37}, and most indirect methods such as the use of
chemical quenchers of reactive intermediates or D$_2$O (which prolongs the singlet oxygen lifetime and so can increase photosensitization) are not entirely specific for singlet oxygen.$^{38,39,40}$ In particular, indications are that superoxide ion (O$_2^-$) may be involved in some aspects of PDT damages. For Photofrin-II photosensitization of cells in vitro, full effects are observed at about 5% O$_2$ levels, with a half-value at about 1% O$_2$. No photosensitization can be observed in the absence of measurable oxygen. From the above it is evident that PDT effects should be oxygen-dependent. This is indeed the case for most sensitizers$^{41}$, with the exception of some cationic sensitizers such as the cyanine dye EDKC, which seems to act by oxygen-independent mechanisms.

The measurement of light penetration through tissue is a difficult task for which mathematical models are still being developed and refined.$^{42}$ Light penetration is also dependent on sensitzer concentration. As sensitzer dose is increased, a limiting concentration is reached at which sensitzer can effectively screen out light and thus limit tissue penetration.$^{43}$ Since this limiting concentration depends on the effective capture of photons, it would be expected that sensitizers with larger extinction coefficients would reach a limiting concentration at lower dose than sensitizers with low extinction coefficients (assuming similar photodynamic efficiencies).

Progress with regard to drug development will undoubtedly involve new agents with strong absorbance in the longer wavelengths, with a view toward promoting eradication of larger tumors.
5) CATIONIC PDT SENSITIZERS

Cationic sensitizers could have an advantage, since there have been reports of selective affinity of such agents for neoplastic cells. The preferential mitochondrial accumulation of cationic sensitizers accounts for the predominance of mitochondrial damage induced by these dyes. Oseroff and Cincotta have developed cationic sensitizers, but these compounds tend to have low extinction coefficients at the longer wavelengths, or need to be transformed to active sensitizers by intracellular enzymes.

6) OBJECTIVES OF THE PRESENT WORK

The principal objective of our study is to develop second generation photosensitizers for PDT. They should have suitable chemical properties including strong absorption maxima at wavelengths where tissues provide optimal light transmissions, good capacity to generate singlet oxygen \((^1\text{O}_2)\) and facile chemical accessibility. In particular, we planned the syntheses of benzochlorin derivatives which have absorption maxima covering almost anywhere between 650 to 800 nm, simply by selecting appropriate substituents at the appropriate positions on the macrocycle. It is known that porphyrin absorptions can be shifted to longer wavelengths by expanding the \(\pi\)-conjugation of the macrocycle and by introducing electron-withdrawing groups at the benzo-ring. The following categories of compounds and mechanistic studies are the goals of this study.
1) Synthesis of cationic benzochlorin sulfonamide derivatives by treating benzochlorin with chlorosulfonic acid and followed by various amines and methyl iodide.

2) Synthesis of cationic benzochlorin amide derivatives by reacting brominated benzochlorin with different amines through palladium catalyzed reaction; the resultant side chain may be further methylated to give quaternary ammonium salts. Cationic imidinium salt of benzochlorin derivative was also obtained.

3) Functionalization of the exocyclic benzo ring of benzochlorin by introducing different groups to the acrolein or allyl alcohol precursor before the cyclization of the 6-memberd ring.

4) Exploring the effects of the side chains and polar groups of benzochlorin derivatives on the photocytotoxicities and subcelllar localization on the NPC cells.
CHAPTER 2 SYNTHESSES AND PROPERTIES OF CATIONIC BENZOCHLORIN SULFONAMIDE DERIVATIVES

1) INTRODUCTION

Although Photofrin II appears to be an effective photosensitizer, it shares with HPD the problem of contamination by various porphyrin species whose contribution to the total biological effect remains unknown. In addition, both HPD and Photofrin II have weak absorptions in red region of the visible spectrum, a region with deep tissue penetration. In recent years, using purer materials, attempts have been made to understand some of the important parameters for an effective \textit{in vivo} photosensitizer. Benzochlorin derivatives typically having an intense visible absorption maximum near 660nm appear to be an attractive system.

In preliminary studies, cationic photosensitizers have shown it to be a photodynamic sensitizer that specifically localize on mitochondria and causing mitochondrial damages.\textsuperscript{44} To study the mechanisms of action of cationic photosensitizers, we designed and prepared several cationic photosensitizer derivatives.

To prepare cationic benzochlorin sulfonamide containing ammonium group, we first synthesized benzochlorin sulfonamide by treating benzochlorin with chlorosulfonic acid, followed by different amines, good yield of sulfonamides could
be obtained. Cationic derivatives were obtained by treating the sulfonamides with methyl iodide. Cationic benzochlorin sulfonamides possess better absorption characteristics and a 20 nm red-shift in the visible spectrum with reference to that of benzochlorin.

In 1978, Johnson et al.\textsuperscript{48} reported the synthesis of the first benzochlorin (G), after cyclization of nickel meso-(2-formylvinyl) octaethylporphyrin (B), obtained from nickel (II) complex of octaethylporphyrin (A) via a three-steps, low yield procedure. Successive formylation and Wittig reaction of the nickel octaethylporphyrin (A) gave the meso-vinyl derivative (E) (see Scheme 1). A second formylation gave compound (B), which was treated with mineral acid for cyclization to give nickel (II) octaethylbenzochlorin (G) in 22% yield. Recently, Smith and co-workers\textsuperscript{49,50} improved this pathway, utilizing a modified Vilsmeier reagent [3-(dimethylamino)acrolein and phosphoryl chloride; (3-DMA / POCl₃)] which gave access to the formyl derivative (B) in one step, starting from (A). Cyclization generated, once again, the nickel benzochlorin (G).

\begin{center}
\textbf{Scheme 1}
\end{center}
Alternatively, Morgan et al.\textsuperscript{51} showed that cyclization of the meso-(3-hydroxypropenyl)octamethylporphyrin (C), obtained after reduction of meso-[β-(ethoxycarbonyl)vinyl]octamethylporphyrin (D), gives also the target benzochlorin (F) in 36% yield (Scheme 2). Benzochlorins possess an intense absorption at 659 nm; metallobenzochlorins, on the other hand, are characterized by a 15 nm red shift in the visible spectrum.\textsuperscript{49,50,51}

2) SYNTHESSES

In order to prepare the benzochlorin sulfonamide derivatives in Scheme 2, benzochlorin itself was first synthesized. Nickel (II) octaethylporphyrin (9) was treated with 3-DMA/POCl\textsubscript{3} for 10 hours at room temperature to give meso-acrolein derivative (10) in 87% yield after the normal hydrolysis of the imine salt intermediate with saturated aqueous sodium carbonate. The cyclization of the acrolein group onto the adjacent pyrrole subunit β-position occurred by treatment of the compound in 18% sulfuric acid in trifluoroacetic acid with hydrogen sulfide for one hour at room temperature to produce the metal-free benzochlorin (11) in 82% yield. The compound was previously prepared by Smith and Vicente\textsuperscript{49,50} by a two-step process, consisting of cyclization with concentrated sulfuric acid followed by demetallation with trifluoroacetic acid and 1,3-propanedithiol in 47% overall yield, along with a 70% recovery of the nickel (II) benzochlorin (12). The nickel (II) benzochlorin (12) can be prepared from metallation of the octaethylbenzochlorin with nickel (II) chloride in 95% yield, or from cyclization of the nickel (II) meso-
acrolein with concentrated sulfuric acid in 47% yield. Treatment of the metal-free octaethylbenzochlorin with chlorosulfonic acid (in dichloromethane for 2 hours at room temperature) gave the benzochlorin sulfonyl chloride, which can be isolated out (79% yield). Treatment of the metal-free octaethylbenzochlorin sulfonyl chloride (14) with different amines gave the monomeric octaethylbenzochlorin sulfonamide derivatives in 48-82% yield. Metal-free octaethylbenzochlorin sulfonyl chloride reacted with $N,N,N'$-trimethylene-diamine in dichloromethane at room temperature for two hours to give (17) in 82% yield, which is the best yield among all amines. Similarly, metal-free octaethylbenzochlorin sulfonyl chloride reacted with $N$-methylpiperazine, $N,N'$-dimethyl 1,6-hexanediamine, 1,9-nonanediamine and 1,12-dodecanediamine to give (15), (18), (19), (20) in 72%, 64%, 55%, 48%, respectively. Reactions of octaethylbenzochlorin sulfonyl chloride with various amines were summarized in Scheme 2.

Treatment of octaethylbenzochlorin sulfonamides (15-20) with methyl iodide in dichloromethane at room temperature for 2 hours gave cationic octaethylbenzochlorin sulfonamides (Scheme 3). Quaternary ammonium salt is much more polar than the neutral benzochlorin sulfonamides and required the use of 15 : 85 methanol/dichloromethane as an eluent for column chromatography of cationic compound. Methyl iodide reacted with $5^2$-sulfonyl($N,N,N'$-trimethylene-diamine)-2,3,8,8,12,13,17,18 octaethylbenzochlorin (17) and $5^2$-sulfonyl($N$-methylpiperazine)-2,3,8,8,12,13,17,18 octaethylbenzochlorin (15) to give $5^2$- sulfonyl($N,N,N,N'$-tetramethylene-diamine)-2,3,8,8,12,13,17,18
Scheme 2
octaethylbenzochlorin iodide (22) and 5\(^2\)-sulfonyl(N,N-dimethylpiperazine)-2,3,8,8,12,13,17,18 octaethylbenzochlorin iodide (21) in 80% and 73% yield, respectively. During the methylation, all amino hydrogens in the side chain of (18), (19), (20), (23), (24) and (25) had been methylated and with 82%, 63%, and 55% yield respectively.

In order to compare the monomeric and dimeric effect of octaethylbenzochlorin sulfonamides on PDT, octaethylbenzochlorin sulfonamide dimer derivatives were prepared. This dimer could be formed by reacting metal-free octaethylbenzochlorin sulfonyl chloride with 0.5 equivalent of piperazine in dichloromethane at room temperature until all the starting materials reacted and a more non-polar spot shown on the TLC (Scheme 4). Following the procedures in Scheme 4, octaethylbenzochlorin sulfonamide dimers (26) and (27) were obtained in 42% and 40% yield respectively.
Scheme 3
3) RESULTS AND DISCUSSIONS

3.1) UV-VIS Spectra

Benzochlorin sulfonamide derivatives (15, 16, 17, 18, 19, 20, 26, 27) and cationic compounds (21, 22, 23, 24, 25) were purified and analyzed by UV-vis
spectrophotometer, $^1$H NMR spectrometer and mass spectrometer. The absorption spectral data are shown in Table 1. The cationic compounds have nearly the same absorption spectra as their parent neutral sulfonamide derivatives. A comparison of octaethylbenzochlorin (11) and the sulfonamide derivatives shows that sulfonamide derivatives exhibit a 8-10 nm bathochromic shift in the long wavelength maxima (Figure 5) which is smaller than the shift produced by octaethylbenzochlorin sulfonyl chloride (14) (~20 nm). It also shows that the conversion of amides to quaternary ammonium salts did not alter the absorption characteristics much as the electronic effect is remote from the chromophore. Apart from bathochromic shift in the long wavelength maxima (Figure 6), the spectral features of the benzochlorin sulfonamides and their precursors seem to be very similar. (Figure 7)
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</table>

*Table 1. UV-vis absorption spectral data of (19-27), their precursors (15-18), (14), and (11) in DCM at room temperature.*
Figure 4. UV-vis absorption spectra of BC (11) and BCSO$_2$C$_6$ (18) in DCM at room temperature
Figure 5. UV-vis absorption spectra of SO₂Cl (14) and cationic BCSO₂C₄ (23) in DCM at room temperature
Figure 6  UV-vis absorption spectra of BCSO$_2$C$_6$ (18) (Upper) and cationic BCSO$_2$C$_6$ (23) (Lower) in DCM at room temperature
3.2) Photocytotoxicity on NPC cells
   (This part of study was carried out by Mak, N.K.; Lee, Y.L. and Wong. R.N.S at HK
   Baptist University)\textsuperscript{52}

Photosensitizers 17, 18, 22, 23, 25 were selected for photocytotoxicity studies
using Nasopharyngeal carcinoma (NPC) cells. Two parameters were selected to
examine for the effectiveness of the photosensitizers mentioned above. The level of
photocytotoxicity was used to measure the potency of the drugs, and the frequency
of proliferative cells after PDT was used to measure the possible recovery of the
NPC cells after PDT. The results obtained from the two NPC cells lines are shown
in Table 2. Basically, drugs with cationic ammonium salts on the side chain
exhibited more potent photocytotoxicity. Among the five selected photosensitizers,
compound (23) with cationic side chain were found to be most effective in killing
both HK1 (NPC cells from a China patient) and CNE2 (NPC cells from a HK
patient) cells. The LC\textsubscript{50} of (23) on the NPC cells is below 1 \mu g/ml. Further increase
in the length of the side chain like photosensitizer (25) did not increase the
photocytotoxicity. Apart from photocytotoxicity studies on the selected
photosensitizers, subcellular localization in CNE-2 cells is being evaluated.

3.2.1) Cytotoxicity Assay

The cytotoxicity of neutral and cationic benzochlorin sulfonamides on the
NPC cells was assessed with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-
tetrazolium bromide] reduction assay\textsuperscript{53}. Briefly, 0.2 ml of NPC cells (1x10\textsuperscript{4}
cells/well) in 96-well flat bottom tissue culture plates were incubated with various
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<th>Photosensitizer</th>
<th>NPC/CNE2</th>
<th>NPC/HK1</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>LC50 (µg/ml)*</td>
<td>Freq. Of proliferative cells (Control/DC / PDT)*</td>
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<tr>
<td>17</td>
<td>NP</td>
<td>ND (lack of photocytotoxicity)</td>
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<td>18</td>
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<td>1 in 1.8 / 1 in 1.6 / 1 in 502.6</td>
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<tr>
<td>22</td>
<td>0.830</td>
<td>1 in 1.6 / 1 in 1.73 / 1 in 54.7</td>
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<tr>
<td>23</td>
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<td>1 in 1.28 / 1 in 1.3 / 1 in 80.95</td>
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<tr>
<td>25</td>
<td>8.17</td>
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</tbody>
</table>

*Table 2. Photocytotoxicity of photosensitizers 17, 18, 22, 23, 25*

* : Light dosage = 80 kJ/m², wavelength (500-900 nm)
$ : Frequency of proliferative cells in control, dark control (DC), and PDT (µg/ml, 80 kJ/m²)
ND : Not done
NP : No photocytotoxicity

Concentrations of sensitizers for 24 hours at 37°C. The cells were centrifuge-washed, and exposed to light emitted from a 400 W tungsten lamp with heat-isolation filter and 500 nm long-pass filter at an intensity of 7 mW/cm², as measured with a power meter (OPHIR). The cells were incubated at 37°C in CO₂ incubator for 24 hours. Viability was then assessed with the MTT reduction assay. The optical density of dissolved formazan crystal was measured using the iEMS Analyzer (Lab-system, Type 1401) at 570 nm and 690 nm wavelength.
3.2.2) Subcellular localization

Confocal microscopy was used to examine the subcellular localization of photosensitizer (23). **Figure 5** shows the fluorescence images of the NPC cells stained with (23) and the organelle specific fluorescence probes. Photosensitizer (23) was found to localize, in a distinct and isolated pattern, in the cytoplasm of the NPC cells (**Figure 5A**). Significant localization of (23) on the plasma membrane and the nuclear membrane was not observed. **Figure 5B** shows the staining of mitochondria by mitochondrial probe M-7514. To reveal the subcellular localization of (23), the fluorescence images of (23) and M-7514 were combined. The degree of overlapping of the fluorescence images of (23) and M-7514 was analyzed. The representative combined fluorescence images and the intensity profiles of (23) and M-7514 were shown in **Figure 5C**. The yellow dots in Figure 5C indicate the overlapping of (23) and M-7514. The results clearly show that some of the photosensitizer (23) was localized in the mitochondria of the NPC cells.
Figure 7. Fluorescence images of the NPC cells stained with photosensitizer (23)
4) EXPERIMENTAL

INSTRUMENTATION

UV/VIS Spectra were obtained on Milton Roy Spectronic 3000 diode array spectrophotometer. Infrared spectra (Nujol) were obtained on a Perkin Elmer 10 PC FT-IR spectrophotometer. Mass spectra were recorded by a Finngan TSQ-7000 mass spectrometer. NMR spectra were recorded using a Bruker ARX 300 MHz spectrometer. Chemical shift (δ, ppm) were reported with respect to SiMe₄.

Nickel (II) meso-formylethenyl octaethylporphyrin (10)

3-(Dimethylamino)acrolein (3.0 ml) was added to a solution of nickel (II) octaethylporphyrin (1.78 g) in dry dichloromethane (200 ml) at room temperature. Phosphorus oxychloride (2.83ml) was added dropwise to the reaction mixture with continuous stirring, at 0 °C. The final mixture was stirred at room temperature for 10 hours and then treated with saturated aqueous sodium carbonate (300 ml) overnight. The mixture was extracted with dichloromethane and the combined organic layers were washed three times with water (300 ml). The solution was dried over anhydrous sodium sulfate and the solvent was removed under vacuum. The resulting residue was chromatographed on silica gel with hexane-dichloromethane (1:2) as eluent and the desired compound was collected and recrystallised from dichloromethane and methanol to give the title compound (1.68 g, 87 %): UV-vis:
\( \lambda \) 406, 536, 564, 582 nm; \(^1\)H NMR (300MHz, CDCl\(_3\)) \( \delta \) 1.67-1.82 (24H, overlapping t, CH\(_3\) of peripheral methyl), 3.74-3.89 (16H, overlapping q, CH\(_2\) of peripheral ethyl), 5.51-5.59 (1H, dd, -CH=CH-CHO), 9.38 (3H, s, meso H), 9.84 (1H, d, -CH=CH-CHO), 10.10 (1H, d, CHO); MS found m/e 644.2; cald 645.5 for C\(_{39}\)H\(_{46}\)N\(_4\)NiO.

**2,3,8,12,13,17,18-octaethylbenzochlorin (11)**

Nickel (II) meso-formylethenyl octaethylporphyrin (1.29 g) was dissolved in 18 (v/v) % sulfuric acid in trifluoroacetic acid (35 ml). The reaction mixture was bubbled with hydrogen sulfide for one hour at room temperature before being poured into ice/water (150 ml). The mixture was extracted with dichloromethane, and the combined organic layer was washed with saturated aqueous sodium carbonate and finally with water. The organic solution was dried over anhydrous sodium sulfate, the solvent was removed under vacuum, and the resulting residue was chromatographed on silica gel with hexane-dichloromethane (3:2) as eluent. The desired green band was collected and crystallized from dichloromethane and methanol to give the title compound (0.94 g, 82 %). UV-vis: \( \lambda \) 411, 532, 564, 605, 658 nm; \(^1\)H NMR (300MHz, CDCl\(_3\)) \( \delta \) 0.05 (6H, t, gem CH3), 1.29-1.74 (18H, overlapping t, CH\(_3\) of peripheral methyl), 2.63 (4H, overlapping q, gem CH\(_2\)), 3.52-3.93 (12H, overlapping q, CH\(_2\) of peripheral ethyl), 8.02, 8.58, 9.24 (1H each, s, meso H 8.03 (1H, d, H of benzene ring), 8.10 (1H, t, para-H of benzene ring), 9.54 (1H, d, H of benzene ring); MS found m/e 572.5; cald 572.8 for C\(_{39}\)H\(_{46}\)N\(_4\).
2,3,8,8,12,13,17,18-octaethylbenzochlorin-5\(^2\)-sulfonyl chloride (14)

Dried chloroform (5 mL) was added into a 10mL round bottom flask with 2,3,8,8,12,13,17,18-octaethylbenzochlorin (80 mg). Chlorosulfonic acid (300 \(\mu\)L) was injected into the flask by a syringe and the system was stirred under nitrogen. After 2 hours, the reaction mixture was poured into a separation funnel and dichloromethane (30 mL) followed by water (30 mL) was added. The organic layer was separated and dried by anhydrous magnesium sulfate. The solution was removed under reduced pressure and chromatographed by silica gel with hexane-dichloromethane (1:1) as eluent to obtain the titled compound (69 mg, 73.5 %): UV-vis: \(\lambda\) 428, 534, 562, 625, 677 nm; \(^1\)H NMR (300MHz, CDCl\(_3\)) \(\delta\) 0.05-0.10 (6H, t, gem CH\(_3\)), 1.63-1.76 (18H, overlapping t, CH\(_3\) of peripheral methyl), 2.70-2.77 (4H, overlapping q, gem CH\(_2\)), 3.52-4.00 (12H, overlapping q, CH\(_2\) of peripheral ethyl), \(\delta\) 8.16, 8.78, 9.37 (1H each, s, meso H), 8.58, \(\delta\) 10.45 (1H each, s, H of benzene ring); MS found m/e 642.1; cald 643.3 for C\(_{39}\)H\(_{47}\)ClN\(_4\)O\(_2\)S.

\(N\)-methylpiperazine sulfonamide benzochlorin (15)

2,3,8,8,12,13,17,18-octaethylbenzochlorinato-5\(^2\)-sulfonyl chloride (30 mg) was dissolved in dichloromethane (10 mL). \(N\)-methylpiperazine (400 \(\mu\)L) was added by micro-syringe. The reaction mixture was stirred under nitrogen for 2 hours and
then dichloromethane (20 mL) and water (30 mL) were added. The organic layer was extracted and washed by water (30 mL). The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with methanol-dichloromethane (1:9) as eluent to give the desired compound (72%): UV-vis: \( \lambda \ 414, 529, 562, 613, 665 \text{ nm}; \)

\(^1\text{H NMR (300MHz, CDCl}_3\) \( \delta \ 0.04-0.09 \text{ (6H, t, gem CH}_3\text{), 1.64-1.76 (18H, overlapping t, CH}_3\text{ of peripheral methyl), 2.32 (3H, s, CH}_3\text{ N-), 2.61-2.79 (8H, multi, 4H of gem CH}_2\text{ and 4H of piperazine), 3.54-3.97 (16H, multi, 12H of peripheral ethyl and 4H of piperazine), 8.11, 8.71, 9.33 (1H each, s, meso H), 8.32, 10.14 (1H each, s, H of benzene ring); MS found m/e 734.5; cald 735.0 for C\(_{44}\)H\(_{58}\)N\(_6\)O\(_2\)S.\)

**Piperazine sulfonamide octaethylbenzochlorin (16)**

2,3,8,8,12,13,17,18-octaethylbenzochlorinato-\(\text{SO}_4\)- sulfonyl chloride (30 mg) was dissolved in dichloromethane (10 mL), piperazine (300 \(\mu\)L) was added by micro-syringe. The reaction mixture was stirred under nitrogen for 2 hours and then dichloromethane (20 mL) and water (30 mL) was added. The resulting organic layer was extracted and washed by water (30 mL). The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with methanol-dichloromethane (1:9) as eluent to give the desired compound (21 mg, 84.2%): UV-vis: \( \lambda \ 412, 530, 560, 615, 664 \text{ nm}; \)

\(^1\text{H NMR (300MHz, CDCl}_3\) \( \delta \ 0.04-0.09 \text{ (6H, t, gem CH}_3\text{), 1.64-1.76 (18H,}\)
overlapping t, CH₃ of peripheral methyl), 2.61-2.79 (8H, multi, 4H of gem CH₂ and
4H of piperazine), 3.54-3.97 (16H, multi, 12H of peripheral ethyl and 4H of
piperazine), 8.11, 8.71, 9.33 (1H each, s, meso H), 8.32, 10.14 (1H each, s, H of
benzene ring); MS found m/e 720.5; cald 721.04 for C₄₃H₅₆N₆O₂S.

**N,N,N’-trimethylethylene-diamine sulfonamide benzochlorin (17)**

2,3,8,8,12,13,17,18-octaethylbenzochlorinato-5²-sulfonyl chloride (40 mg)
was dissolved in dichloromethane (10 mL), N,N,N’-trimethylethylene-diamine (400
µL) was added by micro-syringe. The reaction mixture was stirred under nitrogen
for 2 hours and then dichloromethane (20 mL) and water (30 mL) were added. The
organic layer was extracted and washed by water (30 mL). The resulting organic
layer was dried by anhydrous magnesium sulfate and solvent was removed under
reduced pressure. Purification was done by chromatography with methanol-
dichloromethane (1:9) as eluent to give the desired compound (82 %): UV-vis: λ
413, 528, 562, 610, 665 nm; ¹H NMR (300MHz, CDCl₃) δ 0.04-0.09 (6H, t, gem
CH₃), 1.66-1.96 (18H, overlapping t, CH₃ of peripheral methyl), 2.35 (6H, s, CH₃ of
terminal amine), 2.68-2.75 (6H, overlapping q, 4H of gem CH₂ and 2H of
CH₂NMe₂), 3.04 (3H, s, CH₃ of bridging amine), 3.41-3.45 (2H, t, CH₂CH₂NMe₂),
δ 3.53-4.03 (12H, overlapping q, CH₂ of peripheral ethyl), 8.13, 8.73, 9.40 (1H
each, s, meso H), 8.40, 10.18 (1H each, s, H of benzene ring); MS found m/e 737.5;
cald 737.1 for C₄₄H₆₀N₆O₂S.
**N,N’-dimethyl 1,6-hexanediamine sulfonamide benzochlorin (18)**

2,3,8,8,12,13,17,18-octaethylbenzochlorinato-5\(^2\)-sulfonyl chloride (30 mg) was dissolved in dichloromethane (10 mL), N,N’-dimethyl 1,6-hexanediamine (400 μL) was added by micro-syringe. The reaction mixture was stirred under nitrogen for 2 hours and then dichloromethane (20 mL) and water (30 mL) were added. The organic layer was extracted and washed by water (30 mL). The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with methanol-dichloromethane (1:9) as eluent to give the desired compound (64 %): UV-vis: λ 413, 529, 560, 610, 665 nm; \(^1\)H NMR (300MHz, CDCl\(_3\)) δ 0.02-0.06 (6H, t, gem CH\(_3\)), 1.36-1.53 (6H, multi, CH\(_2\) of amine), 1.59-1.75 (18H, overlapping t, CH\(_3\) of peripheral methyl), 2.41 (3H, s, CH\(_3\) N-), 2.54-2.59 (2H, t, -NCH\(_2\)(CH\(_2\))\(_4\)CH\(_2\)N), 2.63-2.74 (4H, overlapping q, gem CH\(_2\)), 2.93 (3H, s, CH\(_3\) of amine), 3.22-3.26 (2H, t, -NCH\(_2\)(CH\(_2\))\(_4\)CH\(_2\)N), 3.51-4.00 (12H, overlapping q, peripheral ethyl), 8.10, 8.70, 9.31 (1H each, s, meso H), 8.36, 10.13 (1H each, s, H of benzene ring); MS found m/e 779.5; cald 779.1 for C\(_{47}\)H\(_{68}\)N\(_6\)O\(_2\)S.

**1,9-nonanediamine sulfonamide benzochlorin (19)**

2,3,8,8,12,13,17,18-octaethylbenzochlorinato-5\(^2\)-sulfonyl chloride (30 mg) was dissolved in dichloromethane (10 mL), 1,9-nonanediamine (400 μL) was added
by micro-syringe. The reaction mixture was stirred under nitrogen for 2 hours and then dichloromethane (20 mL) and water (30 mL) were added. The organic layer was extracted and washed by water (30 mL). The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with methanol-dichloromethane (1:9) as eluent to give the desired compound (55 %): UV-vis: \( \lambda \) 414, 526, 562, 614, 664 nm;

\(^1\)H NMR (300MHz, CDCl\(_3\) \( \delta \)) -0.03-0.02 (6H, t, gem CH\(_3\)), 1.06-1.46 (14H, m, CH\(_2\) of carbon chain), 1.62-1.73 (18H, overlapping t, CH\(_3\) of peripheral methyl), 2.60-2.72 (4H, overlapping q, gem CH\(_2\)), 2.88-2.94 (2H, t, NCH\(_2\)(CH\(_2\))\(_2\)CH\(_2\)N), 3.10-3.14 (2H, t, NCH\(_2\)(CH\(_2\))\(_2\)CH\(_2\)N), 3.47-3.99 (12H, overlapping q, peripheral ethyl), 8.08, 8.70, 9.30 (1H each, s, meso H), 8.46, 10.24 (1H each, s, H of benzene ring); MS found m/e 792.5; cald 793.2 for C\(_{48}\)H\(_{68}\)N\(_6\)O\(_2\)S.

**1,12-dodecanediamine sulfonamide benzochlorin (20)**

2,3,8,8,12,13,17,18-octaethylbenzochlorinato-5\(^{-}\)-sulfonyl chloride (30 mg) was dissolved in dichloromethane (10 mL), 1,12-dodecanediamine (400 \( \mu \)L) was added by micro-syringe. The reaction mixture was stirred under nitrogen for 2 hours and then dichloromethane (20 mL) and water (30 mL) were added. The organic layer was extracted and washed by water (30 mL). The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with methanol-dichloromethane.
(1:9) as eluent to give the desired compound (45 %); UV-vis: \( \lambda \) 413, 522, 560, 614, 645 nm; \(^1\)H NMR (300MHz, CDCl\(_3\)) \( \delta \) 0.03-0.02 (6H, t, \textit{gem CH}_3), 1.06-1.46 (20H, m, CH\(_2\) of carbon chain), 1.62-1.73 (18H, overlapping t, CH\(_3\) of peripheral methyl), 2.60-2.72 (4H, overlapping q, \textit{gem CH}_2), 2.88-2.94 (2H, t, N\textsubscript{CH}(CH\(_2\))\(_4\)CH\(_2\)N), 3.10-3.14 (2H, t, N\textsubscript{CH}(CH\(_2\))\(_4\)CH\(_2\)N), 3.47-3.99 (12H, overlapping q, peripheral ethyl), 8.08, 8.70, 9.30 (1H each, s, \textit{meso} H), 8.46, 10.24 (1H each, s, H of benzene ring); MS found m/e 834.5; cald 835.2 for C\(_{51}\)H\(_{74}\)N\(_6\)O\(_2\)S.

\textbf{5\(^2\)-sulfonyl(N,N-dimethylpiperazine)-2,3,8,12,13,17,18-octaethylbenzochlorin iodide (21)}

The \( N \)-methylpiperazine sulfonamide (15) (25 mg) was dissolved in dichloromethane (10 mL) and then methyl iodide (500 \( \mu \)L) was introduced by syringe. The mixture was stirred for 1 hour and then solvent was removed by reduced pressure. The residue was purified by column chromatography with methanol-dichloromethane (15:85) as eluent to give the methylated product (73 %). UV-vis: \( \lambda \) 417, 530, 558, 612, 667 nm; \(^1\)H NMR (300MHz, CDCl\(_3\)) \( \delta \) 0.04-0.09 (6H, t, \textit{gem CH}_3), 1.22 (3H, s, CH\(_3\) N-), 1.74-1.92 (18H, overlapping t, CH\(_3\) of peripheral methyl), 2.32 (3H, s, CH\(_3\) N-), 2.43-2.79 (8H, multi, 4H of \textit{gem CH}_2 and 4H of piperazine), 3.44-3.97 (16H, multi, 12H of peripheral ethyl and 4H of piperazine), 8.11, 8.71, 9.33 (1H each, s, \textit{meso} H), 8.32, 10.14 (1H each, s, H of benzene ring); MS found m/e 749.5; cald 750.1 for C\(_{45}\)H\(_{61}\)N\(_6\)O\(_2\)S.
**N,N,N',N'-tetramethylethylene-diamine sulfonamide benzochlorin iodide (22)**

The \(N,N,N'\)-trimethylethylene-diamine sulfonamide (17) (31 mg) was dissolved in dichloromethane (10 mL) and then methyl iodide (500 \(\mu\)L) was introduced by syringe. The mixture was stirred for 1 hour and then solvent was removed by reduced pressure. The residue was purified by column chromatography with methanol-dichloromethane (15:85) as eluent to give the methylated product (80%). UV-vis: \(\lambda\) 416, 529, 559, 611, 666 nm; \(^1\)H NMR (300MHz, CDCl\(_3\)) \(\delta\) 0.03-0.02 (6H, t, \textit{gem CH}_3), 1.61-1.73 (18H, overlapping t, \textit{CH}_3 of peripheral methyl), 2.53-2.76 (4H, overlapping q, \textit{CH}_2 of peripheral ethyl), 3.13 (3H, s, \textit{CH}_3 of internal amine), 2.35 (6H, s, \textit{CH}_3 of terminal amine), 3.49-3.82 (8H of overlapping q, \textit{CH}_2 of peripheral ethyl and 9H, s, \textit{CH}_3 of terminal amine), 3.85-3.90 (2H, t, \textit{CH}_2NMe_3), 4.23 (2H, t, CH\(_2\)CH\(_2\)NMe\(_2\)), 8.08, 8.29, 9.28 (1H each, s, \textit{meso H}), 8.08, 10.10 (1H each, s, H of benzene ring); MS found m/e 751.5; cald 752.1 for \(C_{45}H_{63}N_6O_2S\).

**N,N',N',N'-tetramethyl 1,6-hexanediame sulfonamide benzochlorin iodide (23)**

The \(N,N'\)-dimethyl 1,6-hexanediame sulfonamide (18) (30 mg) was dissolved in dichloromethane (10 mL) and then methyl iodide (500 \(\mu\)L) was introduced by syringe. The mixture was stirred for 1 hour and then solvent was removed by reduced pressure. The residue was purified by column chromatography with methanol-dichloromethane (15:85) as eluent to give the methylated product (82%...
%): UV-vis: λ 413, 530, 561, 610, 664 nm; $^1$H NMR (300MHz, CDCl$_3$) δ -0.01-0.04 (6H, t, gem CH$_3$), 1.25-1.51 (8H, m, CH$_2$ of amine), 1.61-1.74 (18H, overlapping t, CH$_3$ of peripheral methyl), 2.66-2.71 (4H, overlapping q, gem CH$_2$), δ 2.92 (3H, s, CH$_3$ N-), 2.98 (9H, s, CH$_3$ N-), 3.22-3.25 (2H, t, -NCH$_2$(CH$_2$)$_4$CH$_2$N), 3.48-3.92 (14H, m, 2H of -NCH$_2$(CH$_2$)$_4$CH$_2$N, 12H of peripheral ethyl), 8.08, 8.68, 9.28 (1H each, s, meso H), 8.31, 10.10 (1H each, s, H of benzene ring); MS found m/e 807.6; cald 808.2 for C$_{49}$H$_{71}$N$_6$O$_2$S.

$N'$-$N'$-$N'$-trimethyl 1,9-nonanediamine sulfonamide benzochlorin iodide (24)

The 1,9-nonanediamine sulfonamide (20) (20 mg) was dissolved in dichloromethane (10 mL) and then methyl iodide (500 µL) was introduced by syringe. The mixture was stirred for 10 hours and then solvent was removed by reduced pressure. The residue was purified by column chromatography with methanol-dichloromethane (15:85) as eluent to give the methylated product (63 %): UV-vis: λ 414, 529, 561, 612, 665 nm; $^1$H NMR (300MHz, CDCl$_3$) δ -0.03-0.02 (6H, t, gem CH$_3$), 1.09-1.35 (8H, m, CH$_2$ of amine), 1.61-1.73 (18H, overlapping t, CH$_3$ of peripheral methyl), 2.81-2.95 (8H, m, CH$_2$ of amine), 2.61-2.78 (6H, m, gem CH$_2$ and 2H of -NCH$_2$(CH$_2$)$_7$CH$_2$N), 2.83 (9H, s, CH$_3$ N-), 3.11-3.19 (2H, t, -NCH$_2$(CH$_2$)$_7$CH$_2$N), 3.37 (3H, s, CH$_3$ N-), 3.48-3.96 (12H, m, H of peripheral ethyl), 8.10, 8.68, 9.29 (1H each, s, meso H), 8.52, 10.20 (1H each, s, H of benzene ring); MS found m/e 849.6; cald 850.3 for C$_{52}$H$_{77}$N$_6$O$_2$S.
N',N',N'-trimethyl 1,12-dodecanediamine sulfonyamide benzochlorin iodide (25)

The 1,12-dodecanediamine sulfonyamide (20) (20 mg) was dissolved in dichloromethane (10 mL) and then methyl iodide (500 μL) was introduced by syringe. The mixture was stirred for 15 hours and then solvent was removed by reduced pressure. The residue was purified by column chromatography with methanol-dichloromethane (15:85) as eluent to give the methylated product (55 %): UV-vis: λ 413, 529, 562, 611, 665 nm; 1H NMR (300MHz, CDCl₃) δ -0.01-0.04 (6H, t, gem CH₃), 1.06-1.25 (20H, m, CH₂ of amine), 1.61-1.73 (18H, overlapping t, CH₃ of peripheral methyl), 2.67-2.70 (4H, overlapping q, gem CH₂), 3.10-3.12 (2H, t, -NCH₃(CH₂)₄CH₂N), 3.21 (12H, s, CH₃ N-), 3.32-3.38 (2H, t, -NCH₃(CH₂)₄CH₂N), 3.57-3.96 (12H, peripheral ethyl), 8.09, 8.68, 9.29 (1H each, s, meso H), 8.50, 10.20 (1H each, s, H of benzene ring); MS found m/e 892.7; calcd 892.4. for C₅₅H₇₁N₁₆O₂S.

Piperazine sulfonyamide octaethylbenzochlorin dimer (26)

2,3,8,8,12,13,17,18-octaethylbenzochlorinato-5²-sulfonyl chloride (100 mg) was dissolved in dichloromethane (50 mL), piperazine (6.3 mg) was dissolved in 5 mL dichloromethane and added dropwise via a micro-syringe. The reaction mixture was stirred under nitrogen for 2 hours and then dichloromethane (20 mL) and water (30 mL) were added. The organic layer was extracted and washed by water (30 mL). The resulting organic layer was dried by anhydrous magnesium sulfate and solvent
was removed under reduced pressure. Purification was done by chromatography with dichloromethane-hexane (7:3) as eluent to give the desired compound in 42 % yield: UV-vis: \( \lambda \) 413, 531, 560, 616, 667 nm; MS found m/e 1356.7; cald 1355.9 for \( \text{C}_{92}\text{H}_{102}\text{N}_{10}\text{O}_{4}\text{S}_{2} \).

**1,12-dodecanediamine sulfonamide benzochlorin dimer (27)**

2,3,8,8,12,13,17,18-octaethylbenzochlorinato-5\(^2\)-sulfonyl chloride (100 mg) was dissolved in dichloromethane (50 mL), 1,12-dodecanediamine (14.6 mg) was dissolved in 5 mL dichloromethane and added dropwise via a micro-syringe. The reaction mixture was stirred under nitrogen for 2 hours and then dichloromethane (20 mL) and water (30 mL) were added. The organic layer was extracted and washed by water (30 mL). The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with dichloromethane-hexane (8:2) as eluent to give the desired compound in 40 % yield: UV-vis: \( \lambda \) 412, 528, 559, 618, 665 nm; MS found m/e 1471.2; cald 1470.1 for \( \text{C}_{98}\text{H}_{120}\text{N}_{10}\text{O}_{4}\text{S}_{2} \).
CHAPTER 3 SYNTHESSES AND PROPERTIES OF CATIONIC BENZOCHLORIN AMIDE DERIVATIVES VIA COUPLING REACTION USING PALLADIUM CATALYST AND SUZUKI COUPLING

1) INTRODUCTION

The previous chapter described the methodology to functionalize benzochlorin by connecting amine to the exocyclic benzo-ring via a sulfonyl group. In this chapter, we describe the reactions by which alkyl groups and amines can be directly added to the exocyclic ring of benzochlorin using palladium catalyzed coupling reactions with nickel (II) 5²-bromo-2,3,8,12,13,17,18-octaethylbenzochlorin (29). It was found that the reaction of benzochlorin towards electrophilic brominating reagents such as NBS, bromine water and resin results in meso-substituted bromo-derivative. In our research, we found that when octaethylbenzochlorin is treated with neat thionyl bromide, bromination occurs only at the exocyclic benzo-ring providing a unique and convenient method to brominate the benzochlorin without having any meso-benzochlorin positions being attacked. Alkyl groups can then be introduced onto the benzochlorin via Suzuki coupling reaction. Carbon-nitrogen bond formation that connects benzochlorin and amine is demonstrated by the palladium-catalyzed coupling reaction utilizing BINAP (2,2'-bis(diphenylphosphino)-1,1'-binaphthyl) as a supporting ligand.

47
2) SYNTHESSES

In order to perform palladium catalyzed reaction on the benzo-ring of octaethylbenzochlorin, the target position on the benzochlorin should first be brominated. In this chapter, thionyl bromide was used as a brominating reagent to brominate the benzo position of (11). Neat thionyl bromide was added to the 100mg (11) in a 1 ml reaction vial. After 2 hours stirring at room temperature and quenching by sodium hydrogen carbonate, the yield was about 60-65%.

Scheme 5
This bromination process could not be carried out in large scale, as meso-substitution would occur and total yield was lowered to 20-30 %. After the bromination in Scheme 5, nickel was inserted to the benzochlorin chromophore center by hydrated Ni (II) acetate and DMF. After purification by column chromatography the yield of metal insertion was usually up to 95 % or even higher. After the insertion of the Ni (II) 29 was used as a starting material for the palladium catalyzed coupling reaction.

3.1) Suzuki Coupling

Alkyl groups can be introduced to the benzo ring by Suzuki coupling. Following a Suzuki’s coupling procedure as shown in Scheme 6, 2 mol % tetrakis(triphenylphosphine)palladium (0) [Pd(PPh₃)₄] was used as catalyst and hydrated barium hydroxide was used as a base. For this series of Suzuki coupling several boronic acids were tried. Nickel (II) 5²⁻-phenyl-2,3,8,8,12,13,17,18-octaethylbenzochlorin (30) was prepared by reaction of phenyl boronic acid and the bromo-octaethylbenzochlorin with Pd(PPh₃)₄ and Ba(OH)₂ 8H₂O in DME (1,2-dimethoxyethane) in toluene under nitrogen at 80 °C. Reaction was completed after 20 hours with 62 % yield. Similarly, nickel (II) 5²⁻-(4-t-butylbenzene)-2,3,8,8,12,13,17,18-octaethylbenzochlorin (31) and nickel (II) 5²⁻-(4-fluorobenzene)-2,3,8,8,12,13,17,18-octaethylbenzochlorin (32) were prepared by coupling with 4-t-butylbenzene boronic acid and 4-fluorobenzene boronic acid respectively with 68 % and 59 % yield.
Scheme 6
3.2) Formation of benzochlorin amides

For the same reasons stated in chapter 2, various second-generation photosensitizers (benzochlorin amides in this chapter) with different side chains containing polar and/or charged groups were synthesized. The anti-cancer activities of these photosensitizers are now being investigated. In Scheme 7, Pd$_2$(dba)$_3$ [tris-(dibenzylideneacetone)dipalladium (0)] was used as a catalyst and BINAP [2,2'-bis(diphenylphosphino)-1,1'binaphthyl] was used as a supporting ligand. Nickel(II) 5$_2^2$-($N,N,N'$-trimethylene-diamine)-2,3,8,8,12,13,17,18 octaethylbenzochlorin (33) was prepared by reaction between nickel (II) bromo-benzochlorin (29) and $N,N,N'$-trimethylene-diamine to form nickel (II) 5$_2^2$-( $N,N,N'$-trimethylene-diamino)-2,3,8,8,12,13,17,18 octaethylbenzochlorin (33) with Pd$_2$(dba)$_3$ / BINAP as catalyst and sodium tert-butoxide as base.

![Scheme 7](image)
The yield so obtained was 62%. Similarly, nickel (II) 5\(^2\)-(N-methylpiperazine)-2,3,8,8,12,13,17,18 octaethylbenzochlorin (34) was also prepared using N-methylpiperazine in 51% yield. Methylation of compound (33) and (34) with methyl iodide produced the corresponding quaternary ammonium salts (35) and (36) in excellent yields. It is intriguing that this cross coupling reaction can be utilized to link two benzochlorin units with appropriate cyclic diamine such as piperazine in **Scheme 8.** With the same reaction condition, dimeric benzochlorin (37) was prepared in 51% yield. Methylation of (37) with methyl iodide produced cationic dimeric benzochlorin (38) in 57% yield. An attempt to isolate products of double methylation by using methyl iodide was unsuccessful.
Scheme 8
3) RESULTS AND DISCUSSIONS

The key starting material, nickel (II) bromo-octaethylbenzochlorin (29), was prepared by reaction with thionyl bromide in solvent free condition. Various conventional brominating reagents such as bromine water, NBS (N-bromosuccinimide), and bromine have been employed to react with benzochlorin. We found only meso-positions are attacked to produce mono, di, and tri bromo derivatives while the benzo-ring remains intact. Surprisingly, thionyl bromide selectively introduces bromo group onto the 5^2- position of the benzo-ring without formation of any other positional isomer. These results reflected that the bromination of para-benzo-ring might not be caused by nucleophilic or radical reactions.

However, bromination of nickel octaethylbenzochlorin with thionyl bromide was unsuccessful. The ligated of metal center can act as an electron withdrawing effect. As electron density of the aromatic macrocycle is reduced, the reactivity of octaethylbenzochlorin at benzo-position towards thionyl bromide diminishes. It is speculated that electrophilic nature of thionyl bromide is of great importance in the brominating process. The double bond of the benzo-ring may be regarded as an isolated alkene, which to certain extent accounts for the preferential attack of thionyl bromide towards the benzo-ring.

Suzuki coupling of phenyl, 4-t-butylphenyl and 4-fluoro-phenyl boronic acids with bromo-benzochlorin in the presence of Pd(PPh₃)₄ as catalyst produced 5^2-
aryl octaethylbenzochlorin in good yields. However, there were no reactions between boronic acids bearing electron-withdrawing substituents such as 4-carboxy and 4-acetyl on the phenyl ring and bromo-benzochlorin. This is rationalized by the decrease in nucleophilicity of carbanion derived from the combination of electron-deficient boronic acids and Pd(PPh$_3$)$_4$. Solvent was also the main concern for this reaction. If DMF or benzene was used instead of DME, no coupling reaction occurred.

Primary and secondary amines were successfully incorporated onto the benzo-ring of benzochlorin via palladium catalyzed cross-coupling reaction. With BINAP as supporting ligand, the yield of the coupling reaction ranges from 50 to 70%. The yield of these reactions might be further improved by employing other phosphine ligand and palladium catalyst. It is noteworthy that free base of benzochlorin is incapable of undergoing this coupling reaction. The Ni(II) and Zn(II) complexes are necessary to obtain the aforementioned reaction yields.

In summary, palladium catalyzed cross-coupling reactions have been shown to be very useful in that various aryl boronic acids and secondary amines can be utilized to substitute the bromo group of the benzo-ring. Nickel (II) $^{52}$-($N,N,N'$-trimethylethylenediamino)-2,3,8,8,12,13,17,18 octaethylbenzochlorin (33) and nickel (II) $^{52}$-($N$-methylpiperazine)-2,3,8,8,12,13,17,18 octaethylbenzochlorin (34) were obtained in good yields. Also, a novel dimeric benzochlorin (37) was successfully prepared with piperazine as bridge. Cationic monomeric (35), (36) and
dimeric (38) benzochlorin amides were also synthesized by methylating the amine group with methyl iodide.

Table 3 lists the UV-vis absorption maxima of the free base benzochlorin (11), its nickel (II) complex (29) and the coupling products (30)-(32). There appears to be a significant shift to longer wavelength by 12-13 nm for the coupling products. The absorption spectra of compounds (33)-(36) showed bathochromic shift of about 13-15 nm in Band I with a remarkable increase of the intensity in the red bands as shown in Figure 8 and Table 3. The absorption spectra of the dimeric benzochlorin amide (37) and the cationic dimmer (38) showed similar red-shift when compared to monomeric benzochlorin amides (Figure 9) and with almost same intensity in the red region. This strong absorbance in the longer wavelengths may be advantageous for eradication of large tumors and utilization of inexpensive laser sources. Biological studies of the above photosensitizers in vitro and in vivo are still under investigation.
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Table 3: UV-vis spectrum of compound (30)-(38) and their precursor (11), (29) in DCM at room temperature
Figure 8. UV-vis absorption spectra of (29) and (33) in DCM at room temperature.
4) EXPERIMENTAL

$5^{2}\text{bromo-2,3,8,8,12,13,17,18-octaethylbenzochlorin (28)}$

2,3,8,8,12,13,17,18-octaethylbenzochlorin (100 mg) was added into a V-vial (1mL) as a reaction tube and purged with nitrogen. Thionyl bromide (750 µL) was added to
the vial by micro-syringe. The resulting solution was stirred for 2 hours under nitrogen. The solution was then poured into a separatory funnel and dichloromethane (20 mL) was added, followed by saturated solution of sodium bicarbonate (20 mL). The organic layer was separated and the aqueous layer was extracted three times with dichloromethane (10 mL). The combined organic layer was washed by water (20 mL) and then dried by anhydrous magnesium sulfate. The solvent was removed under vacuum, and the resulting residue was chromatographed on silica gel with hexane-dichloromethane (1:1) as eluent. The desired green band was collected and crystallized from dichloromethane and methanol to give the title compound (73 mg, 64.1%). UV-vis: $\lambda$ 411, 532, 564, 605, 655 nm; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.14-0.18 (6H, t, gem CH$_3$), 1.29-1.74 (18H, overlapping t, CH$_3$ of peripheral methyl), 2.57 (4H, overlapping q, gem CH$_2$), 3.52-3.93 (12H, overlapping q, CH$_2$ of peripheral ethyl), 7.99, 8.59, 9.23 (1H each, s, meso H 8.08, 9.65 (1H each, s, H of benzene ring); MS found m/e 652.4; cald 651.7 for C$_{39}$H$_{47}$N$_2$Br.

**Nickel (II) 5$^2$bromo-2,3,8,12,13,17,18-octaethylbenzochlorin (29)**

200 mg of 5$^2$bromo-2,3,8,12,13,17,18-octaethylbenzochlorin (11) was mixed with 50 mg of Ni (II) acetate and both were dissolved in 30 mL DMF. The reaction mixture was refluxed for 20 minutes and water was used to wash the reaction mixture followed by extraction of 30 mL dichloromethane. The organic layer was dried by anhydrous magnesium sulfate. The solvent was removed under
vacuum, and the resulting residue was chromatographed on silica gel with hexane-
dichloromethane (5:3) as eluent. The desired green band was collected and
crystallized from dichloromethane and methanol to give the title compound (95 %).
UV-vis: $\lambda$ 411, 565, 605, 655 nm; MS found m/e 707.2; cald 708.4 for
C$_{39}$H$_{45}$N$_{4}$NiBr.

**Nickel (II) 5$^2$-phenyl-2,3,8,8,12,13,17,18-octaethylbenzochlorin (30)**

Dimethoxyethane (15 mL) was added to a mixture of nickel (II) 2,3,8,8,12,13,17,18-octaethylbenzochlorin (34 mg), phenyl boronic acid (34 mg),
Pd(PPh$_3$)$_4$ (10mg) and barium hydroxide octahydrate (50 mg). After the addition of
water (0.5 mL), the reaction mixture was heated to about 80 °C under nitrogen
atmosphere for 24 hours. Solvent was evaporated and dichloromethane was added.
The organic layer was washed by water for three times and purified by column
chromatography with dichloromethane/hexane (3:2) as eluent to give the title
compound (26 mg, 76.8 %): UV-vis: $\lambda$ 418, 570, 620, 672 nm; $^1$H NMR (300MHz,
CDCl$_3$) $\delta$ 0.22-0.27 (6H, t, gem CH$_3$), 1.54-1.69 (18H, overlapping t, CH$_3$ of
peripheral methyl), 2.50-2.52 (4H, overlapping q, gem CH$_2$), 3.41-3.79 (12H,
overlapping q, CH$_2$ of peripheral ethyl), 7.61-7.66 (3H, t, CH of phenyl group),
8.02-8.05 (2H, overlapping d, H of phenyl group), 7.89, 8.59, 8.94 (1H each, s,
meso H), 8.09, 9.31 (1H each, s, H of benzene ring); MS found m/e 704.3; cald
705.6 for C$_{45}$H$_{50}$N$_{4}$Ni.
Nickel (II) $\text{C}_{52}^2$-(4-tert-butylphenyl)-2,3,8,12,13,17,18-octaethylbenzochlorin (31)

Dimethylformamide (10 mL) was added to the mixture of nickel (II) $\text{C}_{52}^2$-bromo-2,3,8,12,13,17,18-octaethylbenzochlorin (30 mg), 4-t-butylbenzene boronic acid (3 mg), Pd(PPh$_3)_4$ (8 mg) and potassium carbonate (100 mg). The reaction mixture was purged with nitrogen and stirred at room temperature for 3 days. Dichloromethane (30 mL) was added and the organic layer was washed by water (30 mL) for three times. The organic layer was dried by anhydrous magnesium sulfate and the solvent was removed. Purification was done by chromatography on silica gel with dichloromethane/hexane (3:7) as eluent to give the coupling product (21 mg, 64.6 %): UV-vis: $\lambda_{max}$ 418, 574, 619, 671 nm; $^1$H NMR (300MHz, CDCl$_3$) $\delta$ 0.05-0.10 (6H, t, gem CH$_3$), 1.49 (9H, s, CH$_3$ of t-butyl), 1.61-1.73 (18H, overlapping t, CH$_3$ of peripheral methyl), 2.59-2.69 (4H, overlapping q, gem CH$_2$), $\delta$ 3.48-3.99 (12H, overlapping q, CH$_2$ of peripheral ethyl), 7.68-7.70 (2H, d, H of t-butyl phenyl group), 8.08-8.11 (2H, d, H of t-butyl phenyl group), 7.99, 8.56, 9.21 (1H each, s, meso H), 8.29, 9.81 (1H each, s, H of benzene ring); MS found m/e 760.5; calcd 761.7 for C$_{46}$H$_{68}$N$_4$Ni.

Nickel (II) $\text{C}_{52}^2$-(4-fluorophenyl)-2,3,8,12,13,17,18-octaethylbenzochlorin (32)

Dimethoxyethane (15 mL) was added to a mixture of Nickel (II) 2,3,8,12,13,17,18-octaethylbenzochlorin (32 mg), 4-fluorobenzene boronic acid (36 mg), Pd(PPh$_3)_4$ (10 mg) and barium hydroxide octahydrate (50 mg). After the
addition of water (0.5 mL), the reaction mixture was heated to about 80 °C under nitrogen atmosphere for 48 hours. Solvent was evaporated and dichloromethane was added. The organic layer was washed by water for three times and purified by column chromatography with dichloromethane/hexane (3:2) as eluent to give the title compound (26 mg, 76.8 %): UV-vis: λ 419, 572, 618, 672 nm; ¹H NMR (300MHz, CDCl₃) δ 0.21-0.26 (6H, t, gem CH₃), 1.54-1.68 (18H, overlapping t, CH₃ of peripheral methyl), 2.49-2.52 (4H, overlapping q, gem CH₂), 3.40-3.74 (12H, overlapping q, CH₂ of peripheral ethyl), 7.28-7.36 (2H, overlapping d, H of 4-fluoro-phenyl group), 7.93-7.99 (2H, overlapping d, H of 4-fluoro-phenyl group), δ 7.88, 8.59, 8.93 (1H each, s, meso H), 8.01, 9.23 (1H each, s, H of benzene ring); MS found m/e 722.4; cald 723.6 for C₄₅H₄₉FN₄Ni.

Nickel (II) 5²-(N,N,N’-trimethylene-diamino)-2,3,8,8,12,13,17,18 octaethylbenzochlorin (33)

Nickel (II) 5²-bromo-2,3,8,8,12,13,17,18-octaethylbenzochlorin (50 mg) was added to a mixture of sodium tert-butoxide (11mg), 2,2'-bis(diphenylphosphino)-1,1’binaphthyl (1.3 mg) and tris-(dibenzylideneacetone)dipalladium (0) (1.3 mg). To this mixture, toluene (8 mL) was added, followed by N,N,N’-trimethylene-diamine (20 µL) were added to the reaction mixture. The whole system was purged with nitrogen and heated to 80 °C for 6 hours. Dichloromethane (30 mL) was added and the organic layer was washed by water (30 mL) for three times. The organic
layer was dried by anhydrous magnesium sulfate and the solvent was removed. Purification was done by chromatography on silica gel with dichloromethane/methanol (9:1) as eluent to give the title coupling product (62 %):

UV-vis: $\lambda$ 437, 620, 670 nm; $^1$H NMR (300MHz, CDCl$_3$) $\delta$ 0.14-0.19 (6H, t, gem CH$_3$), 1.55-1.74 (18H, overlapping t, CH$_3$ of peripheral methyl), 2.44 (6H, s, CH$_3$ of terminal amine), 2.37-2.53 (4H, overlapping q, gem CH$_2$), 3.41-3.45 (2H, t, CH$_2$CH$_2$NMe$_2$), 3.27 (3H, s, CH$_3$ of amine), 3.37-3.75 (12H, overlapping q, CH$_2$ of peripheral ethyl), 3.81-3.90 (2H, t, H of CH$_2$CH$_2$NMe$_2$), 7.86, 8.12, 8.57 (1H each, s, meso H), 7.49, 8.90 (1H each, s, H of benzene ring); MS found m/e 728.3; cald 729.7 for C$_{44}$H$_{58}$N$_6$Ni.

**Nickel (II) $^{52}$-(N-methylpiperazinyl)-2,3,8,12,13,17,18 octaethylbenzochlorin (34)**

Nickel (II) $^{52}$bromo-2,3,8,12,13,17,18-octaethylbenzochlorin (50 mg) was added to a mixture of sodium tert-butoxide (11mg), 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (1.3 mg) and tris-(dibenzylideneacetone)dipalladium (0) (1.3 mg). Then toluene (8 mL) was added, followed by N-methylpiperazine (20 $\mu$L) using a micro-syringe, to the reaction mixture. The reaction mixture was stirred under nitrogen for 2 hours and then dichloromethane (20 mL) and water (30 mL) were added. The organic layer was extracted and washed by water (30 mL). The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with methanol-dichloromethane (1:9) as eluent to give the desired compound (51 %): UV-vis: $\lambda$
435, 618, 668 nm; \[^1\text{H} \text{NMR (300MHz, CDCl}_3\]^\delta\ 0.13-0.18 (6H, t, \text{gem CH}_3), 1.55-1.72 (18H, overlapping t, CH\textsubscript{3} of peripheral methyl), 2.38-2.48 (4H, multi, CH\textsubscript{2} of piperazine), 2.54 (3H, s, CH\textsubscript{3} of amine), 2.95 (4H, overlapping d, \text{gem CH}_2), 3.54-3.97 (16H, m, 12H of peripheral ethyl and 4H of piperazine), 7.55, 8.39, 8.59 (1H each, s, \textit{meso} H), 7.55, 8.92 (1H each, s, H of benzene ring); MS found m/e 728.2; cald 727.6 for C\textsubscript{44}H\textsubscript{56}N\textsubscript{6}Ni.

\textbf{Nickel (II) \textit{5}^2\textit{-(N,N,N',N'-tetramethylethylenediamino)-2,3,8,12,13,17,18 octaethylbenzochlorinato iodide (35)}}

Nickel (II) \textit{5}^2\textit{-(N,N,N',N'-trimethylethylenediamino)-2,3,8,12,13,17,18 octaethylbenzochlorinato (26 mg) was dissolved in dichloromethane (10 mL) and then methyl iodide (500 \mu L) was introduced by syringe. The mixture was stirred for 1 hour and then solvent was removed under reduced pressure. The residue was purified by column chromatography with methanol-dichloromethane (15:85) as eluent to give the methylated product (79\%\,). UV-vis: \lambda\ 424, 614, 667 nm; \[^1\text{H} \text{NMR (300MHz, CDCl}_3\]^\delta\ -0.20-0.25 (6H, t, \text{gem CH}_3), 1.71-1.86 (18H, overlapping t, CH\textsubscript{3} of peripheral methyl), 2.75-2.78 (4H, q, \text{gem CH}_2), 2.99, 3.56, 3.74, 3.92 (3H each, s, CH\textsubscript{3} of amine), 3.60-3.85 (12H of overlapping q, CH\textsubscript{2} of peripheral ethyl), 4.27-4.30 (2H, t, CH\textsubscript{2}NMe\textsubscript{3}), 4.57-4.59 (2H, t, CH\textsubscript{2}CH\textsubscript{2}NMe\textsubscript{3}), 8.28, 8.88, 9.19 (1H each, s, \textit{meso} H), 8.15, 8.37 (1H each, s, H of benzene ring); MS found m/e 743.4; cald 744.7 for C\textsubscript{45}H\textsubscript{61}N\textsubscript{6}Ni.
Nickel (II) $5^2$-(N,N-methylpiperazinyl)-2,3,8,8,12,13,17,18 octaethylbenzochlorin iodide (36)

26 mg of nickel (II) $5^2$-(N-methylpiperazinyl)-2,3,8,8,12,13,17,18 octaethylbenzochlorin (34) was dissolved in dichloromethane (10 mL) and then methyl iodide (500 μL) was introduced by syringe. The mixture was stirred for 1 hour and then solvent was removed by reduced pressure. The residue was purified by column chromatography with methanol-dichloromethane (15:85) as eluent to give the methylated product (82%). UV-vis: $\lambda$ 424, 614, 667 nm; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0-0.08 (14H, overlapping t of gem CH$_3$ and CH$_2$ of piperazine), 1.56, (6H, s, CH$_3$ of amine), 1.62-1.88 (18H, overlapping t, CH$_3$ of peripheral methyl), , 2.60-2.70 (4H, q, gem CH$_2$), 3.49-3.93 (12H of overlapping q, CH$_2$ of peripheral ethyl), 8.06, 8.68, 9.29 (1H each, s, meso H), 8.19, 9.92 (1H each, s, H of benzene ring); MS found m/e 741.4; cald 742.7 for C$_{45}$H$_{59}$N$_{8}$Ni.

Nickel (II) $5^2$-(piperazinyl)-2,3,8,8,12,13,17,18 octaethylbenzochlorin dimer (37)

Nickel (II) $5^2$bromo-2,3,8,8,12,13,17,18-octaethylbenzochlorin (50 mg) was added to a mixture of sodium tert-butoxide (11 mg), 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (1.3 mg) and tris-(dibenzylideneacetone)dipalladium (0) (1.3 mg). Toluene (8 mL) was added, followed by piperazine (12 μL) using a micro-syringe, to the reaction mixture. The reaction mixture was stirred under nitrogen for 12 hours
and then dichloromethane (20 mL) and water (30 mL) was added. The organic layer was extracted and washed by water (30 mL). The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with dichloromethane-hexane (3:7) as eluent to give the desired compound (26 mg, 50.5 %): UV-vis: λ 424, 516, 668 nm; 1H NMR (300MHz, CDCl3) δ 0.17-0.25 (12H, t, gem CH3), 0.88-0.90 (8H, multi, CH2 of piperazine), 1.55-1.69 (36H, overlapping t, CH3 of peripheral methyl), 2.38-2.58 (8H, multi, q, gem CH2), 3.43-3.78 (24H, overlapping q, peripheral ethyl), 7.92, 8.56, 8.57, 8.62, 8.88, 8.96 (1H each, s, meso H), 7.75, 7.75, 7.81, 7.92 (1H, s, H of benzene ring); MS found m/e 1340; cald 1341.1 for C42H98N10Ni2.

Nickel (II) S2-\(-(N\text{-methylpiperazinyl})\)-2,3,8,12,13,17,18 octaethylbenzochlorinato iodide dimmer (38)

Nickel (II) S2-\-(piperazinyl)-2,3,8,12,13,17,18 octaethylbenzochlorin dimmer (26 mg) was dissolved in dichloromethane (10 mL) and then methyl iodide (500 µL) was introduced by syringe. The mixture was stirred for 1 hour and then solvent was removed under reduced pressure. The residue was purified by column chromatography with methanol-dichloromethane (15:85) as eluent to give the methylated product (15 mg, 57.0 %). UV-vis: λ 418, 616, 667 nm; 1H NMR (300MHz, CDCl3) δ 0.07-0.11 (12H, t, gem CH3), 0.83-0.89 (8H, multi, CH2 of piperazine), 1.50-1.62 (36H, overlapping t, CH3 of peripheral methyl), 2.33-2.91 (8H, multi, q, gem CH2), 3.39-3.48 (24H, overlapping q, peripheral ethyl), 4.25 (3H,
s, CH₃ of amine), 7.94, 8.52, 8.57, 8.85, 8.93, 9.08 (1H each, s, meso H), 7.40, 7.55, 7.86, 8.23 (1H, s, H of benzene ring); MS found m/e 1356.1; cald 1356.1 for C₈₃H₁₀₁N₁₀Ni₂.
CHAPTER 4 ALTERNATIVE SYNTHETIC METHODOLOGY TO DERIVATIZE OCTAETHYLBENZOCHLORIN

1) INTRODUCTION

In Chapters 2 and 3, methodologies of functionalization of the octaethylbenzochlorin core structure were developed utilizing octaethylbenzochlorin itself as a starting material. However, introducing functional groups specifically at the benzo-ring of benzochlorin is difficult to achieve due to the low aromatic character of the exocyclic double bonds. Frequently reactions occurring at the benzo-ring are not specific due to the competitive attacks at the meso-positions under nucleophilic, electrophilic and radical reaction conditions. Consequently, alternative synthetic methodologies to derivatize octaethylbenchlorin have been studied, which is described in this chapter. Basically, we took an approach in which the precursor of benzochlorin, nickel (II) meso-formylethenyl octaethylporphyrin (10), was modified before the cyclization took place.

2) SYNTHESES

Our first method used ethyl cyanoacetate or mono-ethyl malonate to condense with meso-formyl octaethylbenzochlorin (39) which was prepared by the
Vilsmeier reaction of NiOEP with POCl₃/DMF. In the condensation reaction of ethyl cyanoacetate and (39) in Scheme 8, a soxhlet extractor packed with dried molecular sieve was used to remove water (a co-product) during the reaction. This set-up helped shifting the equilibrium and increased the reaction rate. Compound (40) was obtained in high yield (91 %). For the malonate condensation, titanium tetrachloride was used as a catalyst to speed up the reaction. Mono-ethyl malonate was prepared by partial hydrolysis of diethyl malonate. The condensation of meso-formyl octaethylbenzochlorin (39) and mono-ethyl malonate was achieved by adding titanium tetrachloride in the presence of pyridine to achieve high yield (72 %). The ester group of (40) and (41) was reduced by LiBH₄ at 0 °C before cyclization of the acrolein group. If reduction were done at room temperature, Michael addition of hydride to the nearby double bond would occur. Among several acids (formic acid, conc. sulfuric acid and trifluoroacetic acid) that had been tried for the cyclization, conc. sulfuric acid showed the best cyclization yield of 88 % for (44) and 72 % for (45) (Scheme 9).

Previously, it has been shown that an iminium type benzochlorin gives significantly red-shift in visible spectrum, an amidinium salt was also synthesized in this present study, tin (IV) complex was chosen for its good stability and high polarity. Tin (IV) complex of 5'-cyano-2,3,8,8,12,13,17,18-octaethylbenzochlorin (50) was obtained by heating 5'-cyano-2,3,8,8,12,13,17,18-octaethylbenzochlorin (44) and tin (II) chloride in acetic acid, for 2 hours, in 84 % yield. The tin (IV) dichloride complex (50) was then treated with 1 M methyl aluminium (III) chloroamide in toluene in a Schlenck tube for 48 hours under nitrogen atmosphere.
to gave the tin benzochlorin amidinium salt (51) in 57 % yield. The above reactions were summarized in Scheme 10.

Scheme 8
Scheme 10

Due to the difficulty in scaling up the synthesis of bromo-octaethylbenzochlorin using the thionyl bromide method (Chapter 3), bromo-octaethylbenzochlorin was obtained by a different method in our study. We first introduced a bromo substituent to the acrolein side chain of nickel (II) meso-formylethenyl octaethylporphyrin (10) before cyclization of the 5-membered ring. In Scheme 11, nickel (II) meso-α-bromoformylethenyl 2,3,8,8,12,13,17,18-octaethylbenzochlorin (46), which has two position isomers, was obtained by reacting NBS with compound (10) in dichloromethane at −78 °C for 8 hours to give 42 % yield, along with 39 % recovery of the starting material 10. Similarly, nickel (II) meso-α-iodoformylethenyl 2,3,8,8,12,13,17,18-octaethylbenzochlorin (47) could be obtained by replacing NBS with NIS to give 56 % yield, along with 23 % recovery of the starting material (10). Cyclization of the acrolein groups in (46) and (47) by concentrated sulfuric acid gave the desired product 5²-bromo-2,3,8,8,12,13,17,18-octaethylbenzochlorin (29) and 5²-iodo-2,3,8,8,12,13,17,18-octaethylbenzochlorin (49) in 82 % and 88 % yield, respectively.
3) RESULTS AND DISCUSSIONS

As discussed in Chapter 2 and Chapter 3, functional groups such as cyano, carboxy, hydroxyl and iodo groups were very difficult to add onto the benzo-ring of octaethylbenzochlorin. These functional groups have now been successfully introduced to the benzo-ring prior to the ring cyclization. Condensation reaction using TiCl₄ as catalyst provides a convenient and high yield methodology to
derivatize the acrolein group before cyclization to benzochlorin. Previously, an attempt to introduce a carboxy group by Suzuki coupling of 4-carboxyphenyl boronic acid and bromo-benzochlorin was unsuccessful. However, by using the substituted arylate (43), the desired carboxyl product can be obtained. As an electron withdrawing group, the carboxyl group produces a remarkable red-shifted (about 20 nm) absorption band near 672 nm.

In Scheme 10, tin complex of the amidinium salt (51) was successfully obtained. The UV-vis spectrum in Figure 11 shows a significant red shift to 700 nm and absorption intensity was greatly increased after tin metal insertion. These characteristics together with the stability of (51) make it a potentially useful photosensitizer for PDT.

5²-iodo-2,3,8,8,12,13,17,18-octaethylbenzochlorin (49), another benzo-ring substituted with halogen groups, was synthesized successfully by introducing iodine onto the double bond of acrolein group before cyclization of (47). Previous attempts by reacting benzochlorin directly with ICl, PhIO and iodine all failed to yield the desired product. The alternative method of synthesis described in this Chapter is specific and convenient.
Figure 10. UV-vis absorption spectra of (29) and (51) in DCM at room temperature
4) EXPERIMENTAL

Nickel(II) meso-formyloctaethylporphyrin (39)

Phosphorus oxychloride (5.48 ml) was added dropwise to dry DMF (4 ml) cooled in an ice-bath, and the solution was kept at room temperature for 30 minutes. The mixture was then warmed up on hot water bath to 50 °C and a solution of Ni-OEP (1 g) dissolved in dry 1,2-dichloroethane (600 ml) was added dropwise with vigorous stirring, maintaining the temperature at 50 °C-55 °C over a period of 15 minutes. The solution was then warmed for another hour and a saturated solution of sodium acetate (200 ml) was added. Further stirring and heating were continued for 2 hours. The organic phase was separated and the water phase extracted with DCM twice, which was then added to the organic layer. The organic layer was dried by anhydrous magnesium sulphate and then was filtered. The solvent was removed under reduced pressure and the residue was dissolved in DCM and chromatographed on silica gel with CH$_2$Cl$_2$ as eluent. The title compound was obtained in 86 % yield. UV-vis: λ 402, 561, 643 nm; $^1$H NMR (300MHz, CDCl$_3$) δ 1.82 (24H, m, CH$_3$ of ethyl), 3.79 (16H, m, CH$_2$ of ethyl), 9.38 (2H, s, meso H), 9.40 (1H, s, meso H), 12.00 (1H, s, H of aldehyde); MS found m/e 618.0; cald 619.5 for C$_{37}$H$_{44}$N$_4$NiO.
**mono-Ethyl malonate**

Potassium hydroxide (85 %, 182.8 g, 3 mol) in ethanol (750 mL) was added rapidly dropwise to dimethyl malonate (97.5 g) in ethanol (750 mL) over an hour at room temperature. Overnight, the precipitate was filtered off and rinsed with ether (100 mL). A 2nd crop was obtained by adding more ether to the filtrate. The residue was dried and then dissolved in water (200 mL). Concentrated hydrochloric acid was added dropwise to the aqueous solution until pH 3.4 was reached, diethyl ether (50 mL) was added and the organic layer was separated. The aqueous phase was extracted three times with 30 mL diethyl ether and then was dried by anhydrous magnesium sulphate. Solvent of the dried organic layer was evaporated and the residue was purified by vacuum distillation. The title compound was obtained in 87 % yield. $^1$H NMR (300MHz, CDCl$_3$) $\delta$ 1.50 (3H, t, CO$_2$CH$_2$CH$_3$), 3.50 (2H, s, CH$_2$ CO$_2$CH$_2$CH$_3$), 4.20 (2H, q, CO$_2$CH$_2$CH$_3$); b.p. 106.5 °C; MS found m/e 132.6; cald 132.12 for C$_5$H$_6$O$_4$.

**meso-[(1-cyano-1-ethoxycarbonyl)ethenyl]octaethylporphyrin (40)**

A setup of soxhlet extractor with dried molecular sieve was assembled. 1 mL of ethyl cyanoacetate was added to 0.4 g of nickel (II) meso-formyloctaethylporphyrin (39) in 30 mL of triethylamine and reflux for 48 hours under nitrogen atmosphere. During the 48 hours, molecular sieve in the chamber was replaced with a freshly dried batch after 24 hours refluxing until the reaction
was complete (monitored by TLC). Excess triethylamine was distilled out and the residue was washed by 20 mL water twice and then extracted by dichloromethane. The organic layer was dried by magnesium sulphate and solvent was removed under vacuum. Purification by column chromatography using hexane/dichloromethane (1:1) as eluent gave 91 % yield. UV-vis: λ 403, 529, 567 nm; $^1$H NMR (300MHz, CDCl₃) δ 1.36-1.40 (3H, t, COOCH₂CH₃), 1.65-1.76 (24H, m, CH₃ of ethyl), 3.67-3.80 (16H, m, CH₂ of ethyl), 4.31-4.44 (2H, q, COOCH₂CH₃), 9.35-9.37 (3H, s, meso H), 10.56 (1H, s, CH(CN)(COOEt)); MS found m/e 713.3; cald 714.6 for C₄₂H₄₈N₃NiO₂.

**meso-[(1-carboxyl-1-ethyloxy carbonyl)ethenylloctaethylporphyrin (41)**

TiCl₄ (11 mL) in CCl₄ (25 ml) was added dropwise at 0 °C to anhydrous tetrahydrofuran (200 mL); a yellow precipitate was formed. A solution of nickel(II) meso-formylloctaethylporphyrin (100 mg) and mono-ethyl malonate (5 g) in tetrahydrofuran (25 mL) was then added to the mixture. This was followed by dropwise addition of a solution of anhydrous pyridine (16 ml) in tetrahydrofuran (30 mL) within 3-4 hours at 0 °C. More rapid addition of pyridine leads to precipitation of tarry products. After further 10 hours stirring at room temperature water was added. The organic phase was separated and was dried by anhydrous magnesium sulphate and was filtered. The solvent was removed under reduced pressure and the residue was dissolved in DCM and chromatographed on silica gel with methanol-
dichloromethane (1:9) as eluent. The title compound was obtained in 72 % yield. 
UV-vis: λ 406, 530, 565 nm; $^1$H NMR (300MHz, CDCl$_3$) δ -1.41 (3H, t, CO$_2$CH$_2$CH$_3$), 1.30 (24H, overlapping t, CH$_3$ of peripheral methyl), 2.38 (2H, q, CO$_2$CH$_2$CH$_3$), 3.45 (16H, overlapping q, CH$_2$ of peripheral ethyl), 9.40 (1H, board, COOH), 9.46 (2H, s, meso H), 9.49 (1H, s, meso H), 10.87 (1H, s, H of -CH=C(CO$_2$CH$_2$CH$_3$)(COOH)); MS found m/e 732.5; cald 733.6 for C$_{42}$H$_{50}$N$_4$NiO$_4$.

meso-[(1-cyano-1-hydroxymethyl)ethenyl]octaethylporphyrin (42)

100 mg of (40) was dissolved in THF and cooled 0 °C under nitrogen atmosphere. 100 μL of 1M LiBH$_4$ in THF was introduced by micro-syringe and the reaction mixture was stirred at 0 °C for 10 hours then warmed up to room temperature. The solution was then stirred for 30 minutes and the solvent was removed in vacuo. Column chromatography was done by using methanol/dichloromethane (1:19) as eluent to give the yield 68 % of the title compound. MS found m/e 673.3; cald 671.3 for C$_{40}$H$_{47}$N$_3$NiO. UV-vis: λ 406, 531, 569 nm; $^1$H NMR (300MHz, CDCl$_3$) δ 1.56-1.81 (24H, m, CH$_3$ of ethyl), 2.17-2.21 (1H, t, OH), 3.72-3.88 (16H, m, CH$_2$ of ethyl), 4.58-4.60 (2H, dd, CH$_2$OH), 9.48 (3H, s, meso H), 9.80 (1H, s, allylic H); MS found m/e 671.3; cald 672.5 for C$_{46}$H$_{47}$N$_3$NiO.
meso-[(1-carboxyl-1-hydroxymethyl)ethenyl]octaethylporphyrin (43)

100 mg of (41) was dissolved in THF and 0 °C under nitrogen atmosphere. 100 μL of 1M LiBH₄ in THF was introduced by micro-syringe and the reaction mixture and stirred for 10 hours. Solvent was removed. Column chromatography was done by using methanol/dichloromethane (1:19) as eluent to give the yield 68% of the title compound. MS found m/e 690.3; cald 691.5 for C₄₀H₄₆N₄NiO₃.

5²-cyano-2,3,8,8,12,13,17,18-octaethylbenzochlorin (44)

5 mL of concentrated sulfuric acid was added to a solution of 50 mg of nickel (II) complex (42) in 20 mL dichloromethane and stirred until all the green color concentrated into the acid layer (c.a. 20 mins). Ice and saturated sodium hydrogen carbonate solution was added to neutralize the acid. The cyclized product was extracted by dichloromethane. The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with hexane-dichloromethane (6:4) as eluent to give the desired compound in 88% yield. UV-vis: λ 413, 529, 562, 615, 657 nm; ¹H NMR (300MHz, CDCl₃) δ 0.01-0.06 (6H, t, gem CH₃), 1.62-1.74 (18H, overlapping t, CH₃ of peripheral methyl), 2.56-2.73 (4H, overlapping q, gem CH₂), 3.49-3.93 (12H, overlapping q, CH₂ of peripheral ethyl), 8.06, 8.68, 9.29 (1H each, s,
meso H), 8.19, 9.93 (1H each, s, H of benzene ring); MS found m/e 597.4; cald 597.8 for C₄₀H₄₇N₅.

5²-carboxy-2,3,8,12,13,17,18-octaethylbenzochlorin (45)

5 mL of concentrated sulfuric acid was added to a solution of 50 mg of nickel (II) complex (43) in 20 mL dichloromethane and stirred until all the green color transferred into the acid layer (c.a. 20 mins). Ice and saturated sodium hydrogen carbonate solution was added until neutralize the acid. The cyclized product was extracted by dichloromethane. The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with hexane-dichloromethane (3:7) as eluent to give the desired compound in 72 % yield. UV-vis: λ 411, 533, 569, 620, 672 nm; ¹H NMR (300MHz, CDCl₃) δ 0.01-0.06 (6H, t, gem CH₃), 1.55-1.73 (18H, overlapping t, CH₃ of peripheral methyl), 2.57-2.65 (4H, overlapping q, gem CH₂), 3.50-3.89 (12H, overlapping q, CH₂ of peripheral ethyl), 7.99, 8.59, 9.29 (1H each, s, meso H), 8.08, δ 9.65 (1H each, s, H of benzene ring); MS found m/e 617.0; cald 616.8 for C₄₀H₄₈N₄O₂.
meso-[(1-bromo-1-formyl)ethenyl]octaethylporphyrin (46)

100 mg of nickel (II) meso-formylethenyl octaethylporphyrin (10) was dissolved in 20 mL dichloromethane and cooled to −78 °C by mixing liquefied nitrogen and acetone. 5 mg of NBS (N-bromosuccinimide) was added into the mixture and stirred under nitrogen for 8 hours until the reaction temperature rose to room temperature. After the removal of solvent, the solid residue was chromatographed on silica gel using hexane/dichloromethane (1:1) as eluent to give 42 % yield of the brominated compound. UV-vis: λ 405, 533, 568nm; \(^1\)H NMR (300MHz, CDCl\(_3\)) \(\delta\) 1.57-1.83 (24H, overlapping t, CH\(_3\) of peripheral methyl), 3.74-3.91 (16H, overlapping q, CH\(_2\) of peripheral ethyl) 6.96 (1H, s, \(\text{CHC(Br)(CHO)}\)), 9.53 (3H, s, meso H), 10.55 (1H, s, H of aldehyde); MS found m/e 724.5; cald 724.4 for C\(_{39}\)H\(_{45}\)N\(_4\)NiOBr.

meso-[(1-iodo-1-formyl)ethenyl]octaethylporphyrin (47)

100 mg of nickel (II) meso-formylethenyl octaethylporphyrin (10) was dissolved in 20 mL dichloromethane and cooled to −78 °C by mixing liquefied nitrogen and acetone. 5 mg of NIS (N-iodosuccimide) was added into the mixture and stirred under nitrogen for 8 hours until the reaction temperature rose to room temperature. After removal of solvent, The solid residue was chromatographed on silica gel using hexane/dichloromethane (1:1) as eluent to give 56 % yield of the
iodinated product. UV-vis: λ 404, 531, 569nm; \(^1\)H NMR (300MHz, CDCl\(_3\)) δ 1.69-
1.82 (24H, overlapping t, CH\(_3\) of peripheral methyl), 3.69-3.91 (16H, overlapping q,
CH\(_2\) of peripheral ethyl) 6.31 (1H, s, CH(C(I)(CHO))), 9.51 (3H, s, meso H), 10.83
(1H, s, H of aldehyde); MS found m/e 770.4; cald 771.4 for C\(_{39}\)H\(_{45}\)N\(_4\)NiO\(_I\).

5\(^b\)bromo-2,3,8,8,12,13,17,18-octaethylbenzochlorin (48)

5 mL of concentrated sulfuric acid was added to a solution of nickel (II)
complex (46) in 20 mL dichloromethane and stirred until all the green color
concentrated into the acid layer (c.a. 20 mins). Ice and saturated sodium hydrogen
carbonate solution was added to neutralize the acid. The cyclized product was
extracted by dichloromethane. The resulting organic layer was dried by anhydrous
magnesium sulfate and solvent was removed under reduced pressure. Purification
was done by chromatography with hexane-dichloromethane (6:4) as eluent to give
the desired compound in 88 % yield. UV-vis: λ 411, 532, 564, 605, 655 nm; \(^1\)H
NMR (300MHz, CDCl\(_3\)) δ 0.14 (6H, t, gem CH\(_3\)), 1.29-1.74 (18H, overlapping t,
CH\(_3\) of peripheral methyl), 2.57 (4H, overlapping q, gem CH\(_2\)), 3.52-3.93 (12H,
overlapping q, CH\(_2\) of peripheral ethyl), 7.99, 8.59, 9.23 (1H each, s, meso H) 8.08,
9.65 (1H each, s, H of benzene ring); MS found m/e 652.4; cald 651.7 for
C\(_{39}\)H\(_{47}\)N\(_4\)Br.
$5^2$ido-2,3,8,8,12,13,17,18-octaethylbenzochlorin (49)

5 mL of concentrated sulfuric acid was added to a solution of nickel (II) complex (47) in 20 mL dichloromethane and stirred until all the green color contracted into the acid layer (c.a. 20 mins). Ice and saturated sodium hydrogen carbonate solution was added to neutralize the acid. The cyclized product was extracted by dichloromethane. The resulting organic layer was dried by anhydrous magnesium sulfate and solvent was removed under reduced pressure. Purification was done by chromatography with hexane-dichloromethane (6:4) as eluent to give the desired compound in 88 % yield. UV-vis: $\lambda$ 412, 537, 559, 604, 659 nm; $^1$H NMR (300MHz, CDCl$_3$) $\delta$ 0.04-0.09 (6H, t, gem CH$_3$), 1.62-1.74 (18H, overlapping t, CH$_3$ of peripheral methyl), 2.58-2.68 (4H, overlapping q, gem CH$_2$), 3.52-3.89 (12H, overlapping q, CH$_2$ of peripheral ethyl), 8.01, 8.62, 9.26 (1H each, s, meso H), 8.01, 9.88 (1H each, s, H of benzene ring); MS found m/e 699.4; cald 698.7 for C$_{39}$H$_{47}$N$_4$I.

Tin (IV) $5^2$cyano-2,3,8,8,12,13,17,18-octaethylbenzochlorin (50)

Tin (II) chloride (70 mg) and anhydrous sodium acetate (40 mg) were added to a solution of 50 mg of $5^2$cyano-2,3,8,8,12,13,17,18-octaethylbenzochlorin (44) in 15 mL acetic acid. The mixture was refluxed until chelation was complete (monitored by both UV-vis and TLC, ca. 4 hours). The mixture was added to water and followed by dichloromethane. The organic layer was washed with water, saturated
sodium hydrogen carbonate solution and then water. After removal of solvent, the solid residue was chromatographed on silica gel using methanol/dichloromethane (1:9) as eluent to give the product in 84 % yield. UV-vis: $\lambda$ 427, 523, 565, 623, 677 nm; $^1$H NMR (300MHz, CDCl$_3$) $\delta$ 0.06-0.11 (6H, t, gem CH$_3$), 1.65-1.73 (18H, overlapping t, CH$_3$ of peripheral methyl), 2.55-2.68 (4H, overlapping q, gem CH$_2$), 3.52-3.89 (12H, overlapping q, CH$_2$ of peripheral ethyl), 8.07, 8.89, 9.33 (1H each, s, meso H), $\delta$ 8.16, $\delta$ 10.02 (1H each, s, H of benzene ring); MS found m/c 714.4; cald 714.5 for C$_{40}$H$_{45}$N$_5$Sn.

**Tin (IV) 2,3,8,8,12,13,17,18-octaethylbenzochlorin amidinium salt (51)**

To a Schlenk tube, 35 mg of tin (IV) $^{52}$cyano-2,3,8,8,12,13,17,18-octaethylbenzochlorin (50) and 20 mL dried toluene was added. After the system was purged with nitrogen three times, 1 mL of 1 M methyl aluminium (III) chloroamide was introduced by syringe. The reaction mixture was stirred at 80°C for 48 hours under nitrogen. Dichloromethane (30 mL) was added after the mixture had been stirred in the air for 2 more hours. Precipitates in the mixture were filtered off and the filtrate was dried under reduced pressure. The solid residue was chromatographed on silica gel using methanol/dichloromethane (15:85) as eluent to give 57 % yield. UV-vis: $\lambda$ 452, 550, 603, 642, 700 nm; $^1$H NMR (300MHz, CDCl$_3$) $\delta$ -0.14--0.10 (6H, t, gem CH$_3$), 1.65-1.77 (18H, overlapping t, CH$_3$ of peripheral methyl), 2.43 (4H, overlapping q, gem CH$_2$), 3.49-3.71 (12H, overlapping q, CH$_2$ of
peripheral ethyl), 7.91, 8.84, 9.39 (1H each, s, meso H), 8.28, 10.02 (1H each, s, H of benzene ring); 8.70 (NH, board) MS found m/e 730.1; cald 732.6 for C_{40}H_{49}N_{6}Sn.
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