THE DESIGN, SYNTHESIS, AND CHARACTERIZATION OF PORPHYRIN-BASED DENDRIMERS FOR THE DETECTION AND TREATMENT OF SOLID TUMORS

by

ANDREW M. PEARSON

(Under the Direction of THOMAS E. JOHNSON)

ABSTRACT

Porphyrins have demonstrated functionality as photodynamic therapy (PDT) photosensitizers and magnetic resonance (MRI) contrast agents. Many harmful side effects of current porphyrin-based drugs result from collection of the drug in healthy tissues. These unwanted side effects could be prevented by specific targeting of the drug to tumor tissue. Because of a porphyrin's unparallel ability to chelate many different metals, and efficiently sensitize the formation of singlet oxygen, there is a demand for research in the development of improved porphyrin-based drugs. The enhanced permeability and retention (EPR) effect states that macromolecules can actively target tumor tissue versus healthy tissue based on the different characteristics of each tissue. The most significant of these properties states that macromolecules cannot enter healthy tissue, but the deformed vasculature of tumor tissue allows entry of macromolecules. This research presents the design and synthesis of a porphyrinic dendrimer for the detection, destruction, and specific targeting of solid tumors. The triazine-based convergent synthesis is mild, efficient, and overcomes many common difficulties encountered in dendrimer synthesis. The exact dendrimer structure is fully characterized, which will allow for future modifications to optimize it's efficacy as a PDT and MRI drug candidate. Future work includes the incorporation of poly(ethylene)glycols (PEG)s, for added biocompatibility, water solubility, increased molecular size, and a longer circulation time in the plasma.

INDEX WORDS: Porphyrin, Dendrimer, Triazine, Photodynamic therapy (PDT), Magnetic resonance imaging (MRI), Enhanced permeability and retention effect (EPR), PEG, Solid tumors.

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Dean of the Graduate School
The University of Georgia
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DEDICATION

I would like to dedicate this dissertation to my wife Kirsten, my daughter Emelia, and my parents Phil and Carolyn Pearson. Both of my parents have always encouraged me to strive for what I wanted, and made me feel like anything I wanted was obtainable by hard work. My father has always been someone I have looked up to as a role model, and a parent. I would be very proud if, in my lifetime, I could achieve his success in business, and the respect and love his friends and family feel for him.

I would like to thank Kirsten for her undying love, and support in any endeavors I have attempted. I could not imagine having a better wife, and am grateful everyday that I will spend the rest of my life with her. Kirsten has always motivated me to work hard, and she assisted the writing of this dissertation with her, oftentimes, brutal editing. I admire and respect her accomplishments as a student, in her career, and as a wife. I am continually amazed by how naturally she has excelled as a new mother. My daughter Emelia, who was born about 10 weeks before the defense of this dissertation, has shown me love only a parent can feel for their child, and I will always work hard and strive to provide the best possible life, richest opportunities, and unconditional love and support for Kirsten and Emelia.

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other friends such as Brent Beck and Lindell Ward have also provided help and friendship to me. I thank them for this, and I will never forget how each of them have positively affected my life.

Last, I would like to thank my family: my wife Kirsten, my daughter Emelia, and both of our parents and sisters. Kirsten and I were married during graduate school, and her parents (Jim and Barbara Glassmoyer) and sister (Katie) have welcomed me into their family, and made me feel a part of it. I have always enjoyed our time with them, and look forward to many more visits. My parents (Philip and Carolyn Pearson) and my sister (Jeanne) have always been there for me with their love, advice, support, and encouragement my whole life. I could not imagine being raised in a better home, and thank each of you for being a big part of who I am today. To all of my family, I love you, and I look forward to our time together for many years.

TABLE OF CONTENTS

Page

CHAPTER 1

AN INTRODUCTION TO THE USE OF PORPHYRINS IN THE DETECTION AND TREATMENT OF SOLID TUMORS

1.1 Abstract

An introduction to the use of porphyrins in the detection and treatment of cancer is presented in this chapter. A brief literature review of current research within this area includes a discussion of photodynamic therapy (PDT) and magnetic resonance imaging (MRI). Although porphyrin-based drug candidates show great promise, the drawbacks of current treatments leave room for the design of a new generation of molecules.

1.2 Introduction

Cancer is the second leading cause of death in the United States. Three out of four American families, or more than 1.4 million people a year in the U.S. alone fall victim to cancer at some point. Statistically, half of all men and one third of all women will develop some type of cancer in their lifetimes, and over 80% of these cases occur in people over the age of 55. Using current technology, nearly 40% of all cancer patients can be cured, and these odds are improving each year. Sadly, the actual cure rate is not as good as it could be, largely because of delays in detection and treatment. While the preponderance of epidemiologic studies supports early detection as a major factor in the

survival rate, the actual diagnosis of cancer is often many years after the earliest stages of cancer development.

Although current research encompasses the search for the causes of cancer, cancer signatures, and preventative measures, finding an increasingly effective course of detection and treatment still remains a major objective of cancer research. Unfortunately, the majority of chemical agents used in chemotherapy have severe side effects. The cytotoxic action of these agents is not adequately selective, therefore, additional compounds must be developed to either deliver the active drug selectively to the tumor site or counter its side effects, for example, by boosting the immune system. In addition, because many tumor cells exhibit multi-drug resistance due to an overexpression of P-glycoprotein (Pgp), further drugs must be developed to prevent the tumor from clearing the therapeutic drug from its cells before it has a chance to function. Finally, because some drugs lose their activity over the course of a treatment cycle for reasons not related to Pgp, clinicians are often forced to switch from one drug regimen to another, or use a cocktail of two or more drugs.

To summarize, it is not unusual for a cancer patient to consume five different cytotoxic drugs during the course of treatment or up to three drugs at one time. This regimen does not even include the antibiotics to fight infection, stimulants to boost the immune system, antiemetics for vomiting, treatment for gout, pain killers, and medication for depression or anxiety. These complex drug regimens typically reduce the patient's quality of life over the course of treatment, and the resources required to develop each of these drugs are overwhelming. Even if a lead compound is discovered, less than 1 in 10,000 compounds make it to the drug market. Unfortunately, each drug takes about 10

years of research at a cost of 200-250 million dollars, and untold lives. Clearly, more rational approaches to lead drug discovery and optimization are needed[1].

1.3 Porphyrins in Medicine

1.3.1 Properties of Porphyrins

Monomeric porphyrins have been found in nature and serve as prosthetic groups of functional proteins such as: hemoglobin, cytochromes, catalases, peroxidases, photosynthetic reaction centers, and light harvesting complexes. Two common examples are shown below (**figure 1.1**).

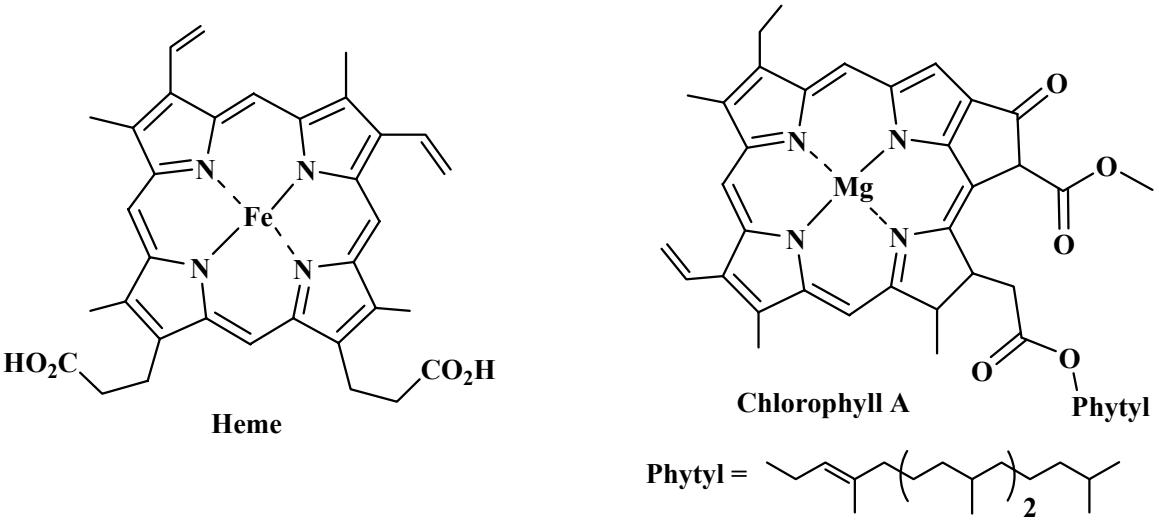
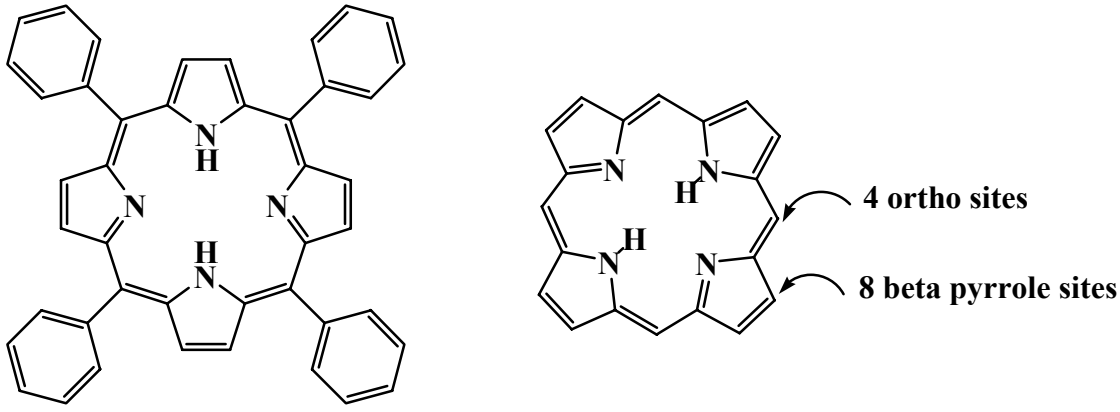


Figure 1.1 Structures of the naturally occurring porphyrins heme and chlorophyll.

They have also been explored for uses such as: catalysis[2], photodynamic therapy[3-6], synthetic receptors[7,8], and contrast enhanced magnetic resonance imaging[8-12].

Porphyrins have a cyclic tetrapyrrolic structure in which the pyrrole rings are linked by methine bridges (**figure 1.2**).



***meso*-tetraphenylporphyrin** **porphine**

Figure 1.2 Structure of a common synthetic porphyrin (*meso*-tetraphenylporphyrin), and the base structure of porphyrins (porphine) with the 12 available sites for substitution labeled.

The porphyrin macrocycle contains 12 sites available for substitution; 4 ortho and 8 beta pyrrole positions. The twenty six π-electron system of porphyrins gives them many interesting chemical and physical properties such as: intense color, large ultraviolet and visible absorption cross-sections, singlet and triplet excited states, and thermal stability. They also make an excellent ligand, complexing almost half of the elements on the periodic table (**figure 1.3**). These unique properties make the characteristics of porphyrins very tunable, thereby allowing for the easy optimization of porphyrin based therapeutics.

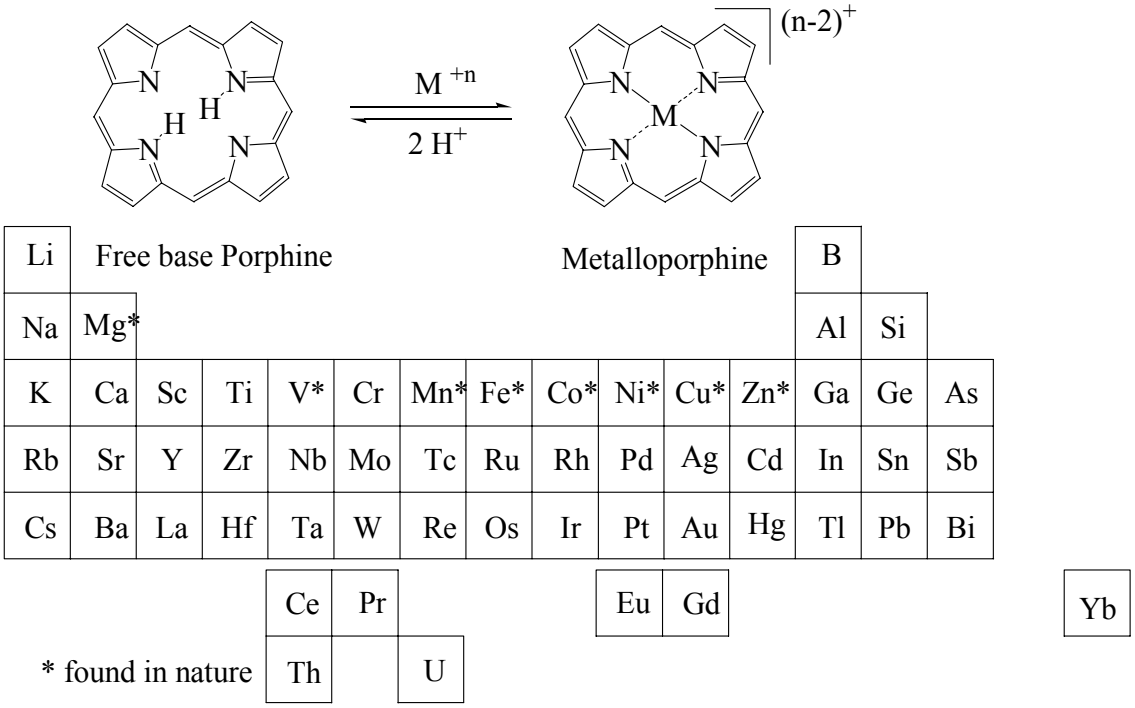
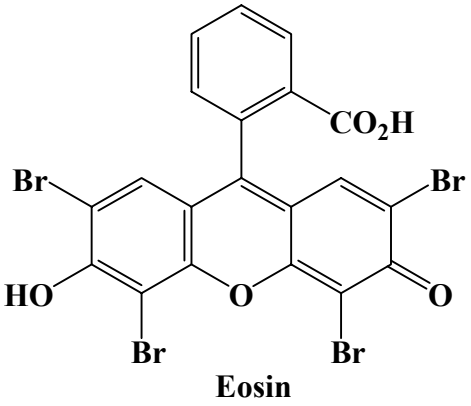


Figure 1.3 Appended periodic table of the elements known to insert into the core of porphyrins. The metals denoted by an asterisk are found in naturally occurring metalloporphyrins.

1.3.2 Photodynamic therapy and the treatment of tumors

1.3.2.1 Basics of photodynamic therapy

The use of light as a therapeutic agent can be traced back over thousands of years. It was used in ancient Egypt, India, and China to treat skin diseases such as: psoriasis, vitiligo, and cancer[13]. Centuries later, in the 1890's, Finsen used phototherapy to treat smallpox, and cutaneous tuberculosis[14]. Shortly after, in 1900, Raab[15] observed the first photochemical sensitization of tissue. His work was further investigated by Tappeiner[16], who administered a topical eosin treatment, combined with sunlight, to treat skin tumors (**figure 1.4**).



Eosin

Figure 1.4 Structure of eosin, a common dye, also explored for use as a photosensitizer.

The use of light and a photosensitizing agent as a therapeutic treatment in clinical medicine was termed "photochemotherapy" or "photodynamic therapy"[5]. These early observations led the way for future studies on the use of photosensitizers in the treatment of tumors.

Since the 1970's, PDT has been vigorously pursued as an attractive method for the treatment of cancer and other malignant conditions. Treatment involves the administration of a photosensitizing agent, followed by the carefully timed irradiation of the tumor with visible light (**figure 1.5**).

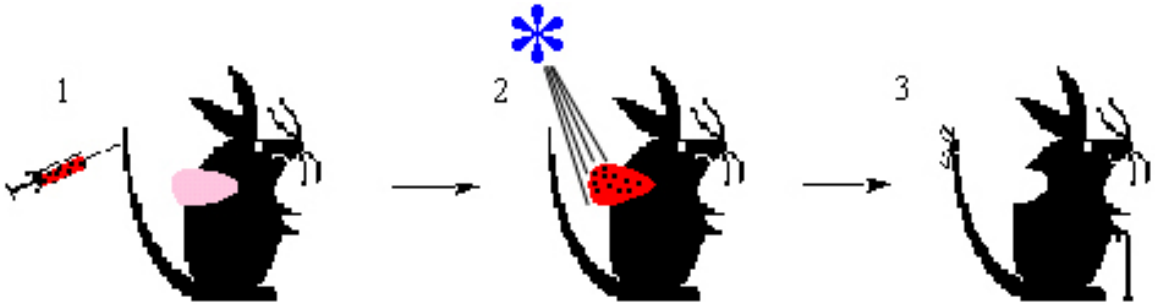


Figure 1.5 1) Injection of PDT sensitizer 2) Collection of sensitizer in tumor, followed by irradiation with light source 3) Destruction of tumor.

In practice, the photosensitizing agent must collect predominantly in the malignant tissue. When exposed to visible light (400-760 nm), the photosensitizer molecules, commonly porphyrins, are excited, resulting in a series of molecular energy transfers leading to the formation of singlet oxygen. This method is known as "indirect" PDT; singlet oxygen is a highly reactive and cytotoxic species, and is the actual therapeutic agent (**figure 1.6**).

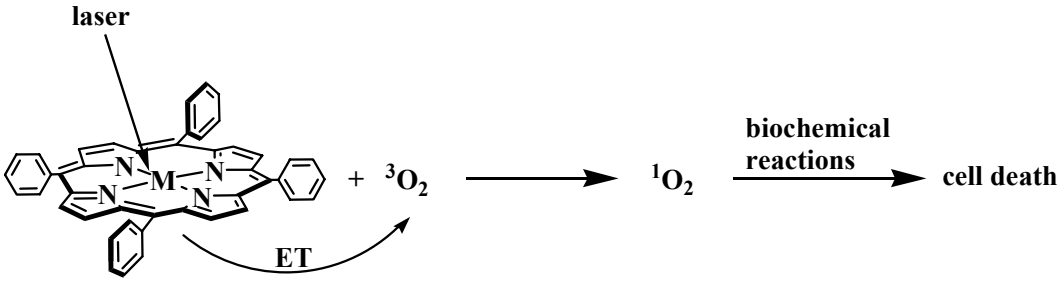


Figure 1.6 A porphyrin absorbs light, and sensitizes the formation of singlet oxygen, resulting in cell death.

Porphyrins absorb a quantum of light, generating a singlet state excited species which can then undergo intersystem crossing to its triplet excited state. This species can transfer its energy to a nearby dioxygen molecule, which is excited to its singlet state (**figure 1.7**).

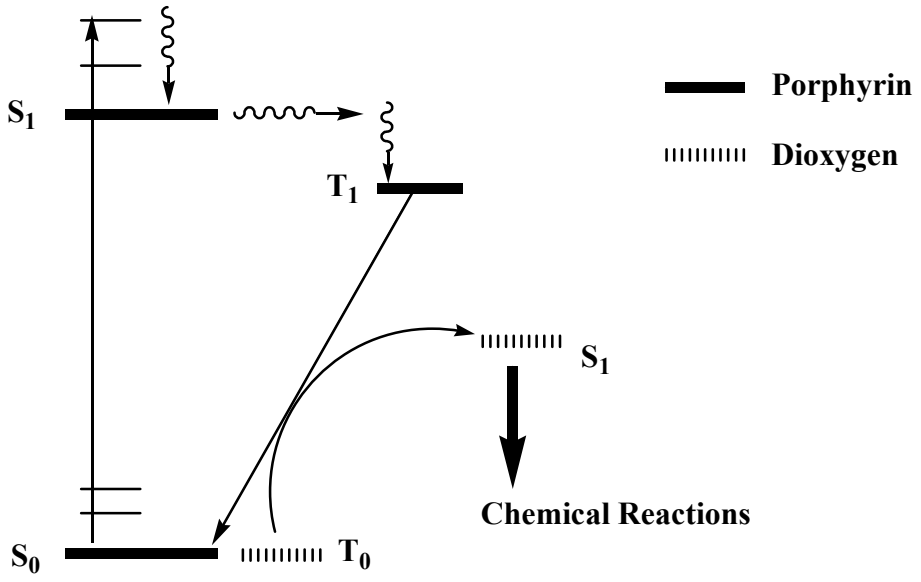
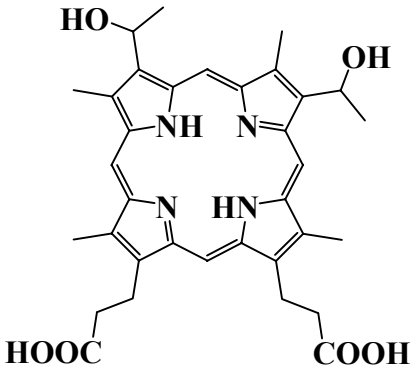


Figure 1.7 A Jablonski diagram of ground state triplet oxygen, which is converted to its singlet state upon transfer of energy from an excited porphyrin molecule.

Many current treatments for tumors such as surgery, radiation therapy, and chemotherapy are invasive, costly, and non-selective. An advantage of using PDT is that, in theory, it can selectively destroy diseased tissue without damaging the surrounding tissue. This method is much less invasive than most cancer regimes, and results in greater patient compliance and higher quality of life during treatment periods.

1.3.2.2 Porphyrins in photodynamic therapy

Some of the earliest studies using porphyrins in medicine were done with hematoporphyrin, a material isolated from blood (**figure 1.8**).



hematoporphyrin

Figure 1.8 Structure of hematoporphyrin.

The first studies of the biological effects of hematoporphyrin were done by Hausmann[17] in Vienna in 1911. He administered hematoporphyrin to mice and noted acute photosensitivity changes when the mice were exposed to light. The first human studies followed shortly thereafter when, in 1913, Friedrich Meyer-Betz injected himself with 200 mg of hematoporphyrin and noticed prolonged pain and swelling in light-exposed areas[18]. These studies, and others, demonstrated the potential role of porphyrins as useful photosensitizers in photodynamic therapy. However, the major disadvantage with these methods was that high concentrations of porphyrin had to be administered, which resulted in phototoxicity to healthy tissues.

Efforts to improve the efficacy of hematoporphyrin were undertaken as the medicinal properties of porphyrins were being discovered. Studies by Schwartz and coworkers[19] in 1955 demonstrated that after partial purification, hematoporphyrin was much less effective as a photosensitizing agent in PDT. The dosage had to be increased to achieve the same tumor tissue concentrations. However, the residue left behind after purification had a great affinity for tumor tissue. This residue became known as hematoporphyrin derivative (HpD) (**figure 1.9**), which was much more effective, and in

smaller amounts, than hematoporphyrin. The observation that porphyrins have some inherent targeting for tumor tissue further augmented the interest in exploring their therapeutic properties.

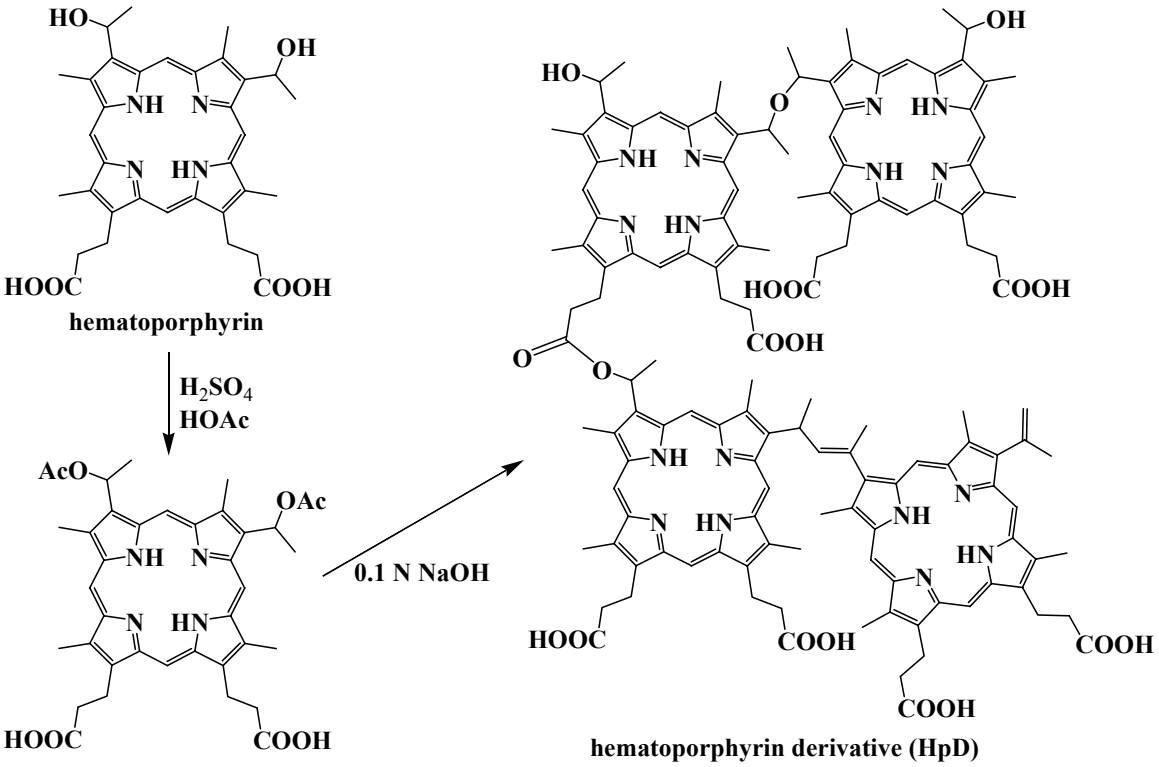


Figure 1.9 Synthesis of HpD. Figure adapted from reference[20].

These studies also demonstrated that in order to fully understand, design, and synthesize potent porphyrin based photosensitizers, the exact structure of the porphyrin, or mixture of porphyrins, must be completely characterized.

In 1973, a paper by Diamond and coworkers reinforced the concept that the localizing characteristics of some porphyrin mixtures, combined with their phototoxic properties, could potentially make them effective as a treatment for cancer[14,21]. They tested hematoporphyrin in both *in vitro* and *in vivo* studies on rat glioma. The cells, when

exposed to white light in the presence of hematoporphyrin for 50 min, underwent 100% cell death. The same cell line was used to induce tumors *in vivo*, and a positive result was obtained. The tumor size was appreciably decreased, with only the innermost regions of the tumor left untouched. As shown by Schwartz's earlier studies, the exact structures of these mixtures must be determined in order for more effective treatments to be developed. This would allow the design and synthesis of photosensitizers based solely on the active component of hematoporphyrin mixtures, and the evolution of a class of more potent, second generation photosensitizers.

The first human studies using HpD as a PDT agent were conducted in the 1970s. Kelly and Snell treated five patients with bladder cancer with HpD; fluorescence microscopy studies of the bladder tissue showed fluorescence confined to malignant lesions.[22] This demonstration reiterated some of the tumor localizing properties of porphyrin derivatives. In 1978, Dougherty conducted a larger study on 25 patients with 113 skin tumors. Subjects were treated with HpD, and then tumors were exposed to red light from a xenon arc lamp 24-168 hours later. Ninety-eight of the tumors completely regressed, 13 partially regressed, with only 2 showing no response to treatment. The treatment, while promising, suffered from side effects including edema, sunburn, and some cases of skin necrosis. However, this study showed that PDT was a viable option in the treatment of skin malignancies. The use of PDT to treat tumors was quickly applied to many areas such as: brain tumors, gynecological tumors, and cancers of the bladder, lung, and esophagus[23,24].

1.3.2.3 Current Treatments

Most modern-day photosensitizers are primarily derivatives of heme, and have not been fully characterized (**table 1.1, figure 1.10**)[25-27].

Table 1.1 Common photosensitizers used in PDT.

Photosensitizer	**Wavelength**	**Delivery vehicle**	**Duration of skin photosensitivity**
Hematoporphyrin-derivative	630	5% dextrose	2-3 months
Methylene blue	668	Water soluble	n/a
5-aminolaevulinic acid (protoporphyrin IX)	635	Water soluble	1-2 days
Verteporfin	690	Liposomal	3-5 days
Texaphyrins	732	Water soluble	Minimal
Phthalocyanines	670	Liposomal or water soluble	8-10 days

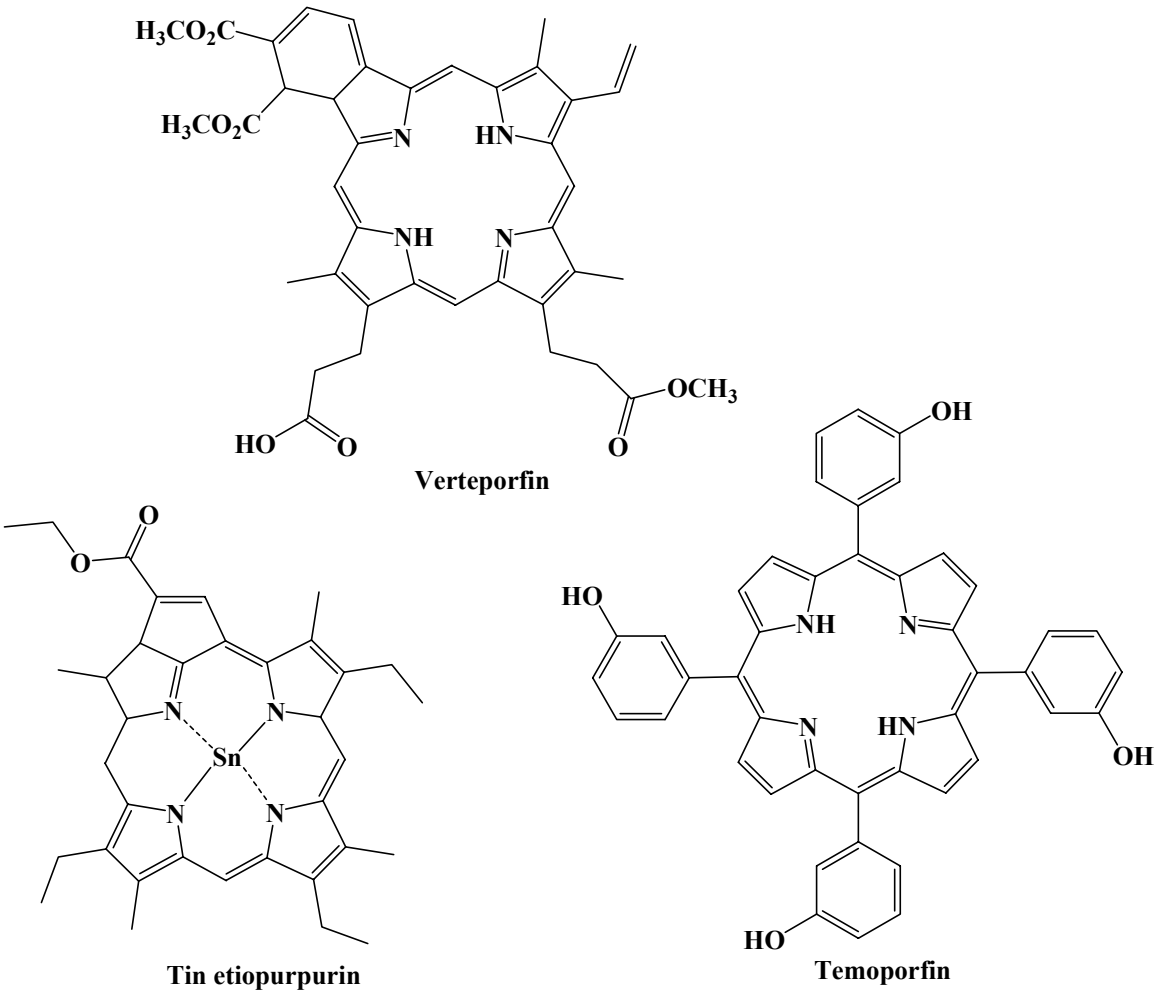


Figure 1.10 Common porphyrin-based photosensitizers: Tin etiopurpurin, Verteporfin, and Temoporfin.

The most frequently used porphyrin derivative was abbreviated DHE (dihematoporphyrin ether), and was thought to be composed of a range of different porphyrins and porphyrin polymers connected by ether and ester bonds. Kessel[28] and Dougherty[29] further characterized this mixture and proposed that the active component was an oligomer of porphyrins from five to eight monomers in length. This mixture is currently the most widely used photosensitizer, and is commonly known as Photofrin® (**figure 1.11a, 11.b**).

At present, Photofrin®, a mixture of hematoporphyrin derivatives, is the only photosensitizer being evaluated in phase-III clinical trials, specifically for the treatment of endobronchial and superficial bladder tumors.

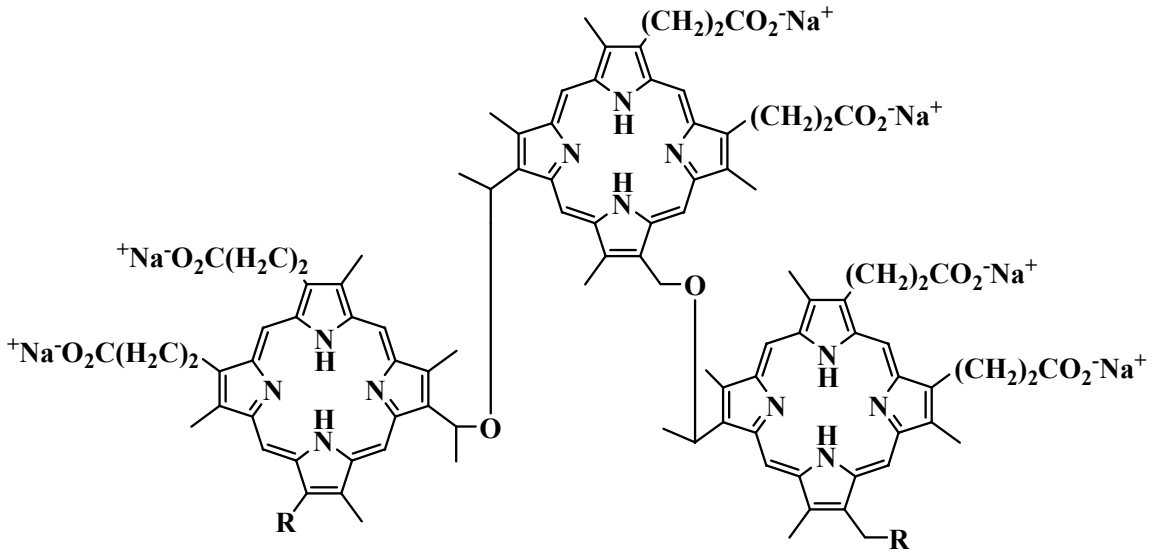


Figure 1.11a Photofrin is a complex mixture of dimers and oligomers ranging from two to nine porphyrin units.

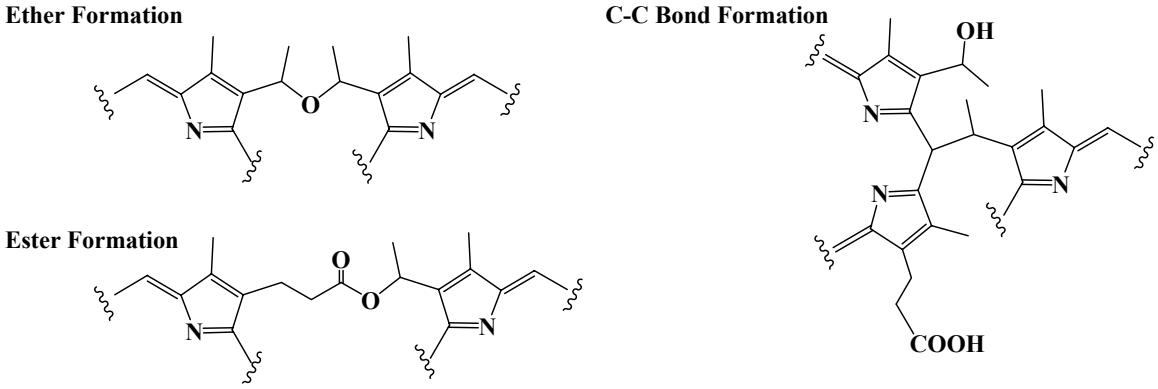


Figure 1.11b Photofrin bond ambiguities.

While Photofrin® has less harmful side effects than previous treatments, it has been shown that this hematoporphyrin derivative is still not highly specific for tumor tissue.

The efficacy of Photofrin® is dependant upon carefully timed irradiation, so that the PDT effect occurs when most of the photosensitizing agent is absent in healthy tissue but is still present in diseased tissue. This limitation results in rather severe cutaneous phototoxicity, requiring patients to remain protected from sunlight for periods of up to 4-6 weeks. More importantly, because of the low selectivity for tumor cells, large quantities of Photofrin® must be used in repetitive treatments, which normally leads to an immunosuppressive response. This problem often renders the drug ineffective, and the use of such compounds impractical, especially for the treatment of relatively large tumors[23]. In addition to these side effects, Photofrin® consists of a complex mixture of partially unidentified porphyrins, which makes its optimization difficult, and prevents researchers from gaining an understanding of what makes an effective photosensitizer.

PDT trials for the treatment of esophageal cancers have been reported in numerous studies. In 1995 Lightdale and coworkers reported a study comparing Photofrin-PDT versus Nd:YAG thermal ablation treatment of esophageal tumors. A total of 236 patients were treated, of which 32% showed response to PDT compared to 20% Nd:YAG. Nine PDT patients showed a complete response compared to two patients treated with Nd:YAG. Additionally, patients treated with PDT required fewer overall treatments.[30]

PDT has also been applied to the treatment of skin, bladder, and lung cancers. With the advancement of laser technologies, tumors once considered “unreachable” are now accessible, and PDT can be used for treatment.

1.3.2.4 Mechanism of Action

Porphyrins have been shown to catalyze or potentiate the transformation of molecular oxygen to singlet oxygen, and for this reason they are commonly used in PDT[31-33]. While the exact mechanism of how PDT results in cell death is controversial, it was shown in 1991 by Agarwal that PDT generates an apoptotic response in cells. Apoptosis, or programmed cell death, is a complex process that occurs when cells are injured by exposure to toxins, disruption by mechanical means, or are otherwise induced by the body to commit suicide. With the apoptotic response, cells undergo a series of changes that ultimately lead to death by the loss of membrane integrity and destruction of organelles such as mitochondria.

Singlet oxygen is widely thought to be the cause of cell destruction, by reaction with lipids (such as cholesterol), and α-amino-acid residues (**figure 1.12**). The exact method of action depends on the photosensitizers used, dose of light, and condition being treated. The mode of action determines which subcellular targets are attacked, and how cell death is achieved[34].

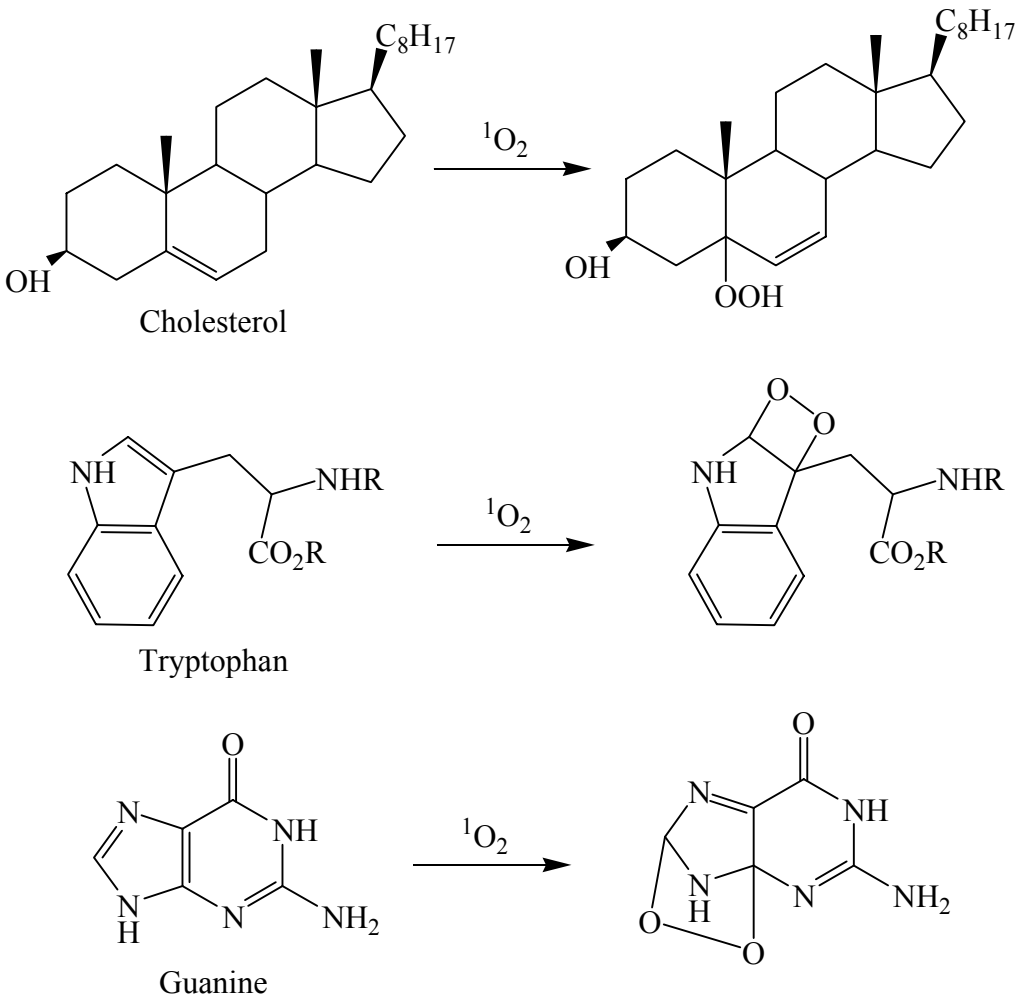


Figure 1.12 Mechanism of action of singlet oxygen in cell death. Figure adapted from reference[20].

Porphyrins and their derivatives compose the majority of common photosensitizers. They exhibit many of the ideal characteristics of a photosensitizer, including biological stability and efficiency in singlet oxygen sensitization. Since their medicinal value has been proven and is well documented, there is a demand for more selective and potent porphyrin-based photosensitizers with less harmful side effects.

1.3.2.5 Second Generation Photosensitizers

There are many limitations with the current generation of photosensitizers, leaving room for advancement, and the design of a second generation of porphyrin based drug regimens. A common problem with the administration of photosensitizers is that their solubility in water is very low, requiring the aid of liposomes, dispersions, or drug encapsulation. These systems are not only costly, but they also suffer from short circulation times due to removal by macrophage absorption. The most significant limitation concerns specifically targeting the photosensitizer to the diseased tissue.

Current research in photodynamic therapy is focused on the development of second generation photosensitizers. They must be pure single substances so interpretation of dose-response relationships can be more accurate. The photosensitizers need to be more soluble in aqueous medium; previously, hydrophobic sensitizers have been administered in phospholipid liposomes[5]. The molecules should be kinetically and thermodynamically stable, have adequate shelf-life, an efficient synthesis, and a low toxicity. An ideal photosensitizer would be able to have its photophysical properties tuned and optimized for the detection and/or treatment of diseased tissues. For optimal detection the fluorescence quantum yield should be high, while for therapeutic applications, the triplet yield, lifetime, energy, and singlet oxygen yield are most important[35]. Additionally, the second generation photosensitizers should have a higher target to healthy tissue ratio, and a fast elimination rate, decreasing harmful side effects to the body.

Another desired characteristic for photosensitizers is a higher wavelength absorbance; a number of synthetically modified porphyrins have been synthesized to achieve this (**figure 1.13**).

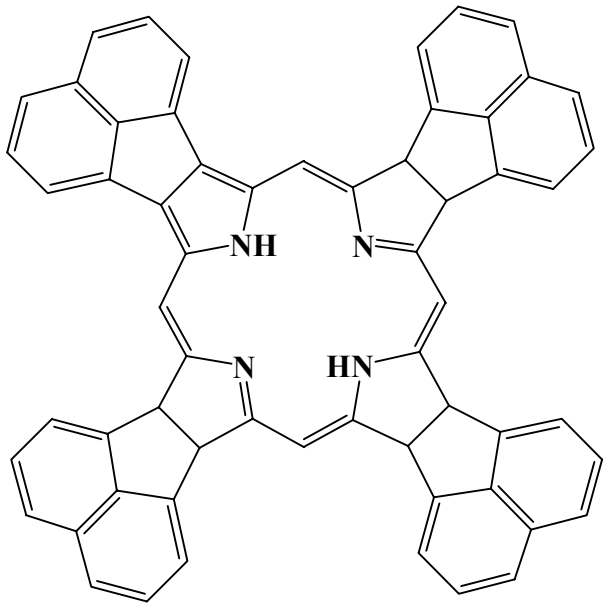


Figure 1.13 Extended chromophore synthesized by Lash and co-workers[36-39].
λ_{max}=701nm.

There is a direct relationship between the wavelength of absorbance of a porphyrin, and the amount of light scattered by tissues. The lower the wavelength of absorbance, the more light scattered in tissues increases (**figure 1.14**). This results in an increased number of doses to successfully treat larger tumors.

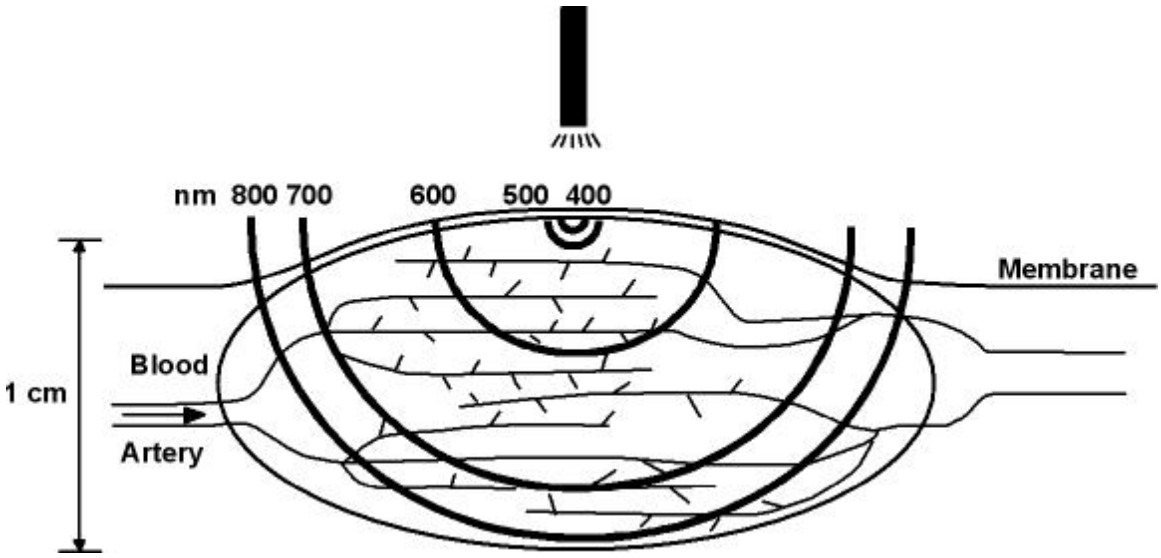


Figure 1.14 Light penetration into a tumor depends on wavelength (adapted from reference[20]).

However, the red shift cannot be too high, as this causes a decrease in efficiency of triplet energy transfer to ground state oxygen, and the photosensitizer becomes kinetically less stable and subject to photobleaching.

Many efforts to minimize the side effects of porphyrins, and increase their efficacy as photosensitizers, have centered around the synthesis of porphyrin derivatives such as phthalocyanines (**figure 1.15**)[40,41]. Phthalocyanines are similar to porphyrins, but instead of four pyrrole rings joined together by methine linkages, a phthalocyanine contains four isoindoles linked together by nitrogen atoms. These molecules have shown prolonged photosensitization (compared to porphyrin), but less toxicity upon exposure to ambient light[42,43].

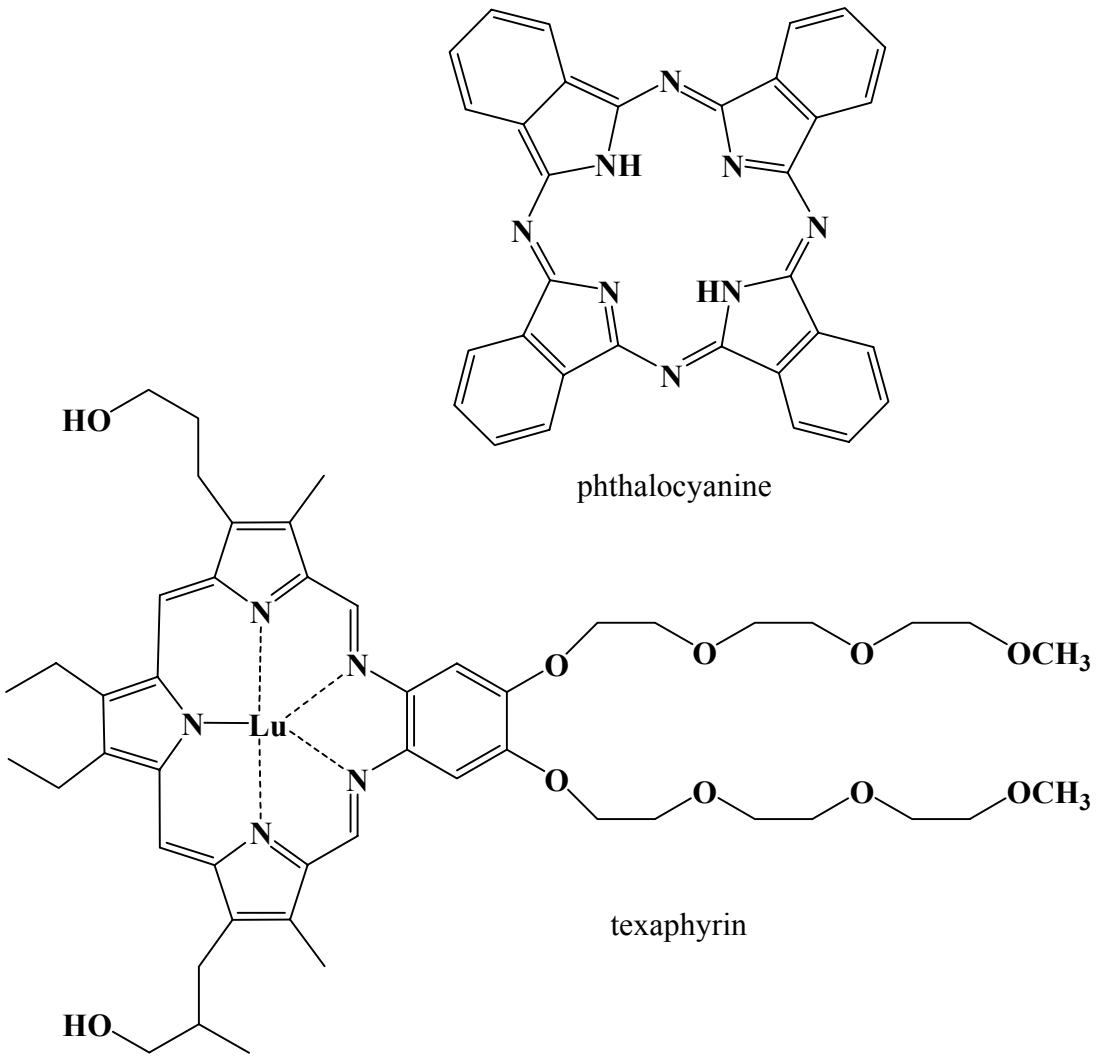


Figure 1.15 Phthalocyanine and texaphyrin (Lutetium texaphyrin)[44,45]: modified porphyrin backbones.

Chlorins[46,47] and bacteriochlorins are another class of structures with modified porphyrin backbones which are being studied for possible use in PDT (**figure 1.16**).

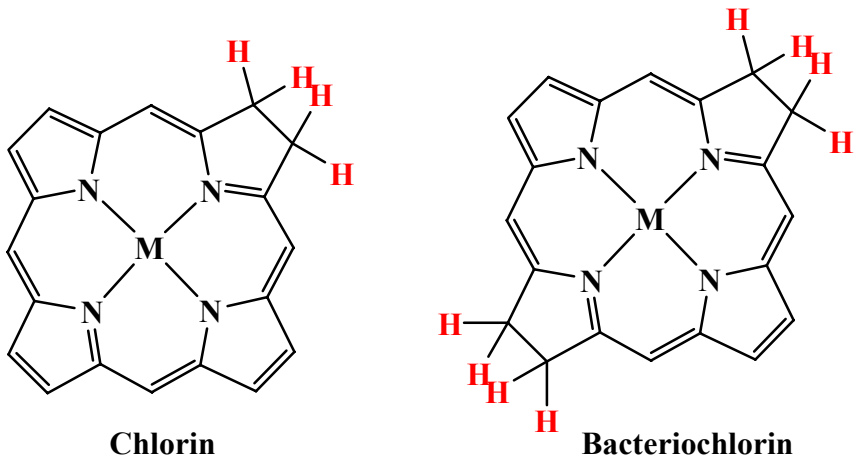


Figure 1.16 Chlorins and bacteriochlorins are two common variants of the porphyrin macrocycle.

Other advances in this area include the development of endogenous photosensitization methods; the administration of 5-aminovulinic acid (ALA) has been shown to produce similar effects as patients experiencing porphyria. This is a disease where the body's natural heme biosynthetic pathway does not occur properly, resulting in a buildup in porphyrin concentration. 5-aminovulinic acid is an intermediate in the heme biosynthetic pathway, and upon administering to patients, the formation of protoporphyrin IX (a known photosensitizer) has been observed (**figure 1.17**)[48].

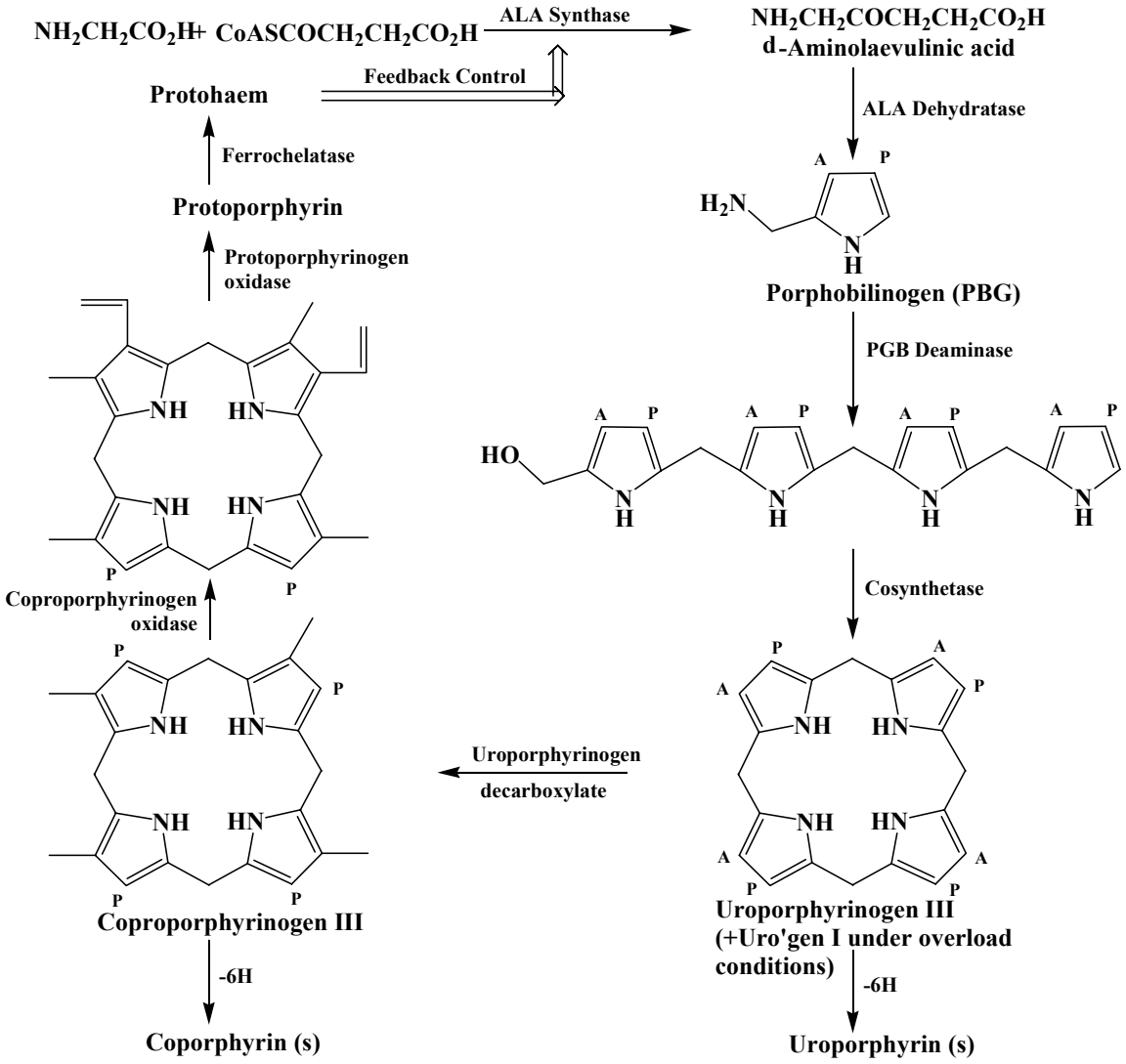


Figure 1.17 Heme Biosynthesis: porphyrin sensitizer formation under conditions of ALA overload. Protoporphyrin appears to be the main photosensitizer in the present context. (A = $-CH_2CO_2H$, P = $-CH_2CH_2CO_2H$) (altered from reference [49]).

Protoporphyrin IX has a rapid clearance rate, which leads to a shorter photosensitization of the skin, and has been shown to target the mucosa of hollow organs[50].

Another type of second generation sensitizer involves the use of a delivery device[51]. The carrier system must not alter the efficacy of the sensitizer, or increase harmful side effects. Ideally, the system should provide water solubility, increased

circulation time, targeting for tumor tissue, and anti-immunogenic properties. The device should also be biodegradable, non-toxic, inexpensive to synthesize, and not effect the ability of the sensitizer to absorb light[52,53].

One way this has been attempted was by the binding of monoclonal antibodies to the photosensitizer, or its carriers[54] (**figure 1.18**).

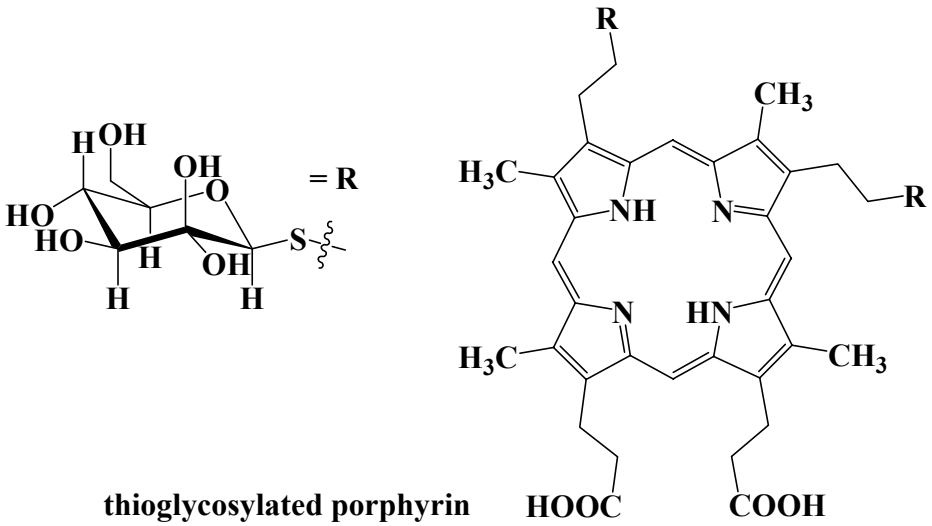


Figure 1.18 A porphyrin containing tumor targeting modifications.

1.3.3 Porphyrins in tumor detection

1.3.3.1 Fluorescence

The ability to detect cancer at early stages of development is crucial to a successful treatment. The observation that some porphyrin mixtures localized in tumors in high concentrations, combined with the knowledge of their fluorescent properties, lead researchers to believe these porphyrin-derived photosensitizers could be used as early detection devices, as well as curative agents. Lipson and coworkers studied the localization of HpD in tumors of patients undergoing bronchoscopy or esophagoscopy for

suspected malignant disease[55]. A total of 15 patients were studied, each treated with 2 mg HpD/kg body weight, 3 h before surgery. Of the 15 patients, 14 were found to have histologically proven malignancy of which 10 were detected by HpD fluorescence. The tumor tissue could be visualized under UV light due to the red fluorescence from porphyrin molecules. Other studies similar to this one showed that although this technique could be used in tumor detection, low sensitivity and specificity of the porphyrin-based photosensitizer limited its use.

1.3.3.2 Magnetic Resonance Imaging

Recently, the use of porphyrins for tumor detection with MRI has gained particular interest due to their ability to strongly chelate many different metals. MRI is a non-invasive method of *in vivo* tissue characterization which utilizes image contrast between normal and diseased tissue that derives from differences in ^{1}H nuclear spin relaxation rates[56]. Signal intensity in MRI results from the local value of the longitudinal relaxation rate of water protons, 1/T1, and the transverse rate, 1/T2. There is a direct relationship between 1/T1 and 1/T2; signal increases with increasing 1/T1 and decreases with increasing 1/T2.

It has been found that the administration of contrast agents during MRI greatly increases the resolution of healthy tissues compared to diseased tissues. Contrast agents work by coupling to the proton nucleus of coordinated water, which exchanges with the bulk water, increasing both 1/T1 and 1/T2. Upon localization of the contrast agent in a given tissue type, increased MRI sensitivity is achieved[49]. Complexes of gadolinium [Gd(III)], alter the relaxation of water protons bound to them, and thereby discriminate

them from bulk water. Present contrast agents commonly used include water soluble complexes of Mn(II), Gd(III), and Fe(III) (**figure 1.19)**. These complexes distinguish between different types of tissue and maintain many of the same characteristics as the paramagnetic metal itself [57].

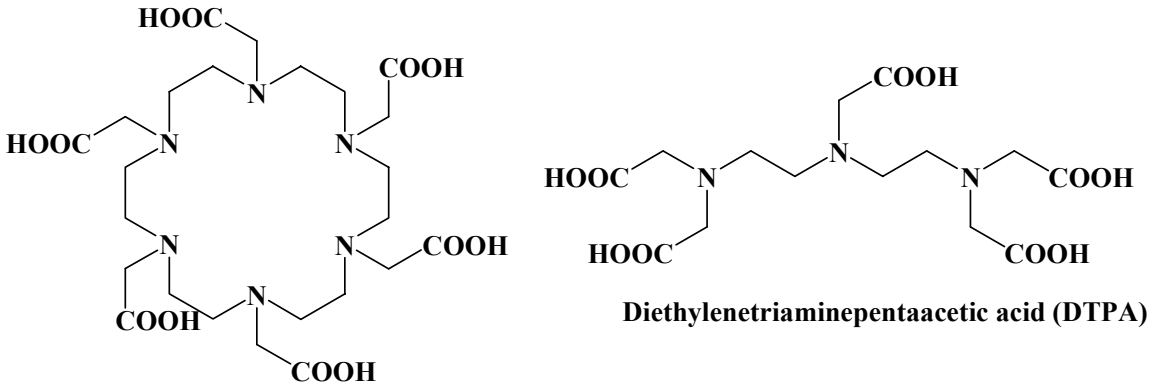


Figure 1.19 These common contrast agents chelate Gd(III).

It is clear that to increase the effectiveness of MRI to full potential, contrast agents are necessary. Characteristics of these agents should include: 1) selectively collecting in targeted tissue, 2) being non-toxic, and 3) enhancing the relaxation of protons in their environment. However, the majority of contrast agents are non-selective, and collect in any available extracellular spaces. In this regard, porphyrins have shown to be useful contrast agents for the characterization of excretory organs, and central nervous system diseases. Additionally, with the increasing use of magnetic resonance imaging in the detection of tumor tissues, there is a higher demand for the development of porphyrin-based contrast agents due to the passive localization of monomeric and

oligomeric porphyrins for tumor tissue, the affinity of porphyrins for paramagnetic metal ions, and the sensitivity of MRI for imaging of the soft tissues.

Bradshaw and coworkers have synthesized a polyhydroxylamide porphyrin, complexing Manganese, and studied it for use in MRI (**figure 1.20**).

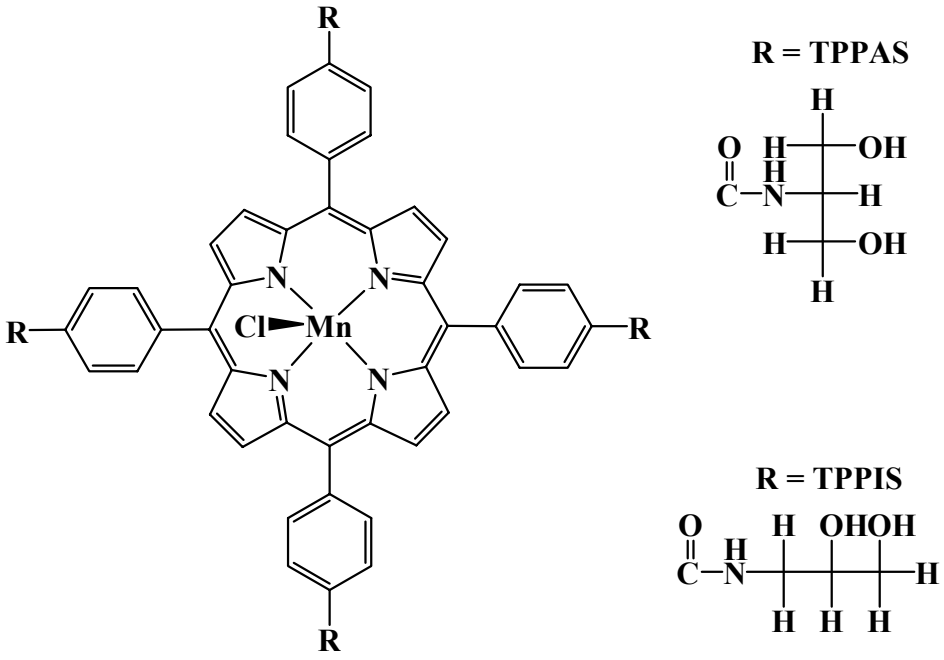


Figure 1.20 Polyhydroxylamide porphyrins synthesized by Bradshaw and coworkers[56]

Gadolinium derivatives have been studied, and shown to have high relaxation rates, however the practical use of these has suffered due to dissociation of the metal from the porphyrin because of the large size of the gadolinium atom[8]. More recent work makes use of expanded porphyrin systems such as texaphyrins in hopes of a more stable Gadolinium complex[58,59] (**figure 1.21**).

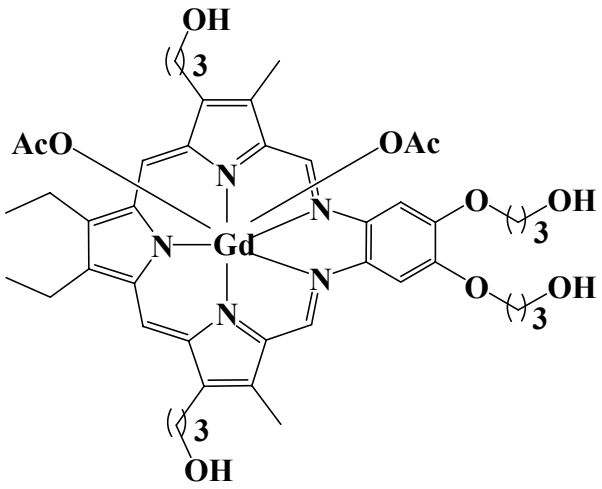


Figure 1.21 Sessler's Gd(III)-Texaphyrin, an expanded porphyrin.

Iron(III) complexes have been studied as well, but were shown to lose their paramagnetism upon spontaneous formation of dimers[8]. Perhaps the most promising porphyrin-metal complexes are with manganese(III), which have shown excellent potential as contrast agents[8]. The biggest drawback of these treatments has been the toxicity of the ionic complexes needed to form water soluble compounds.

1.4 Conclusions

Porphyrins have confirmed value as therapeutic agents in the detection and treatment of diseased tissues. They show some inherent targeting for tumor tissue, and once accumulated there, can destroy this diseased tissue by sensitizing the formation of singlet oxygen. Also, a porphyrin's ability to complex many different metals makes it an excellent candidate for the detection of solid tumors using MRI. With continuing advancements in the selectivity and efficacy of these molecules, their use as a mainstream drug regime will increase.

The largest obstacle in PDT is finding a drug that will selectively collect in tumor tissue, leaving surrounding healthy tissue unharmed. A solution to this tumor specificity problem would reduce many of the current harmful side effects, and be a significant step forward in battling skin cancers and other types of tumors. There is a growing demand for a new generation of highly selective PDT drugs which can be used for the detection and treatment of these tumors.

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CHAPTER 2

MOLECULAR DESIGN OF DENDRIMERS FOR DETECTION AND TREATMENT OF SOLID TUMORS

2.1 Abstract

The enhanced permeability and retention (EPR) effect describes properties of tumor tissue which differ from healthy tissue. The principles learned from these unique characteristics can be applied to develop compounds to selectively target tumors, thereby decreasing harmful side effects associated with most modern day drug regimens

In recent years, a significant amount of effort has been devoted toward the development of macromolecular drug carrier systems for small-molecule therapeutics[1-5]. Motivated, in part, to improve targeted biolocalization, protect drugs from premature biodegradation or excretion, and minimize side effects, researchers have prepared some stunning delivery devices. A class of macromolecules known as "dendrimers" has many properties which make them excellent candidates as drug carriers. The design of a porphyrin-based dendrimer, for applications in PDT and MRI, is presented in this chapter.

2.2 Enhanced Permeability and Retention Effect

In order to increase the efficacy of cancer drugs, the targeting of diseased tissues must be improved in order to limit side effects of current tumor treatments. There are

many methods used to target drugs to tumors; this chapter describes the design of a molecule based on the EPR effect[6] for lipid and macromolecular agents, which states that the physiological makeup of tumor tissue allows it to be accessed by macromolecular compounds which cannot collect in healthy tissues[7-11]. Most solid tumors possess unique pathophysiological characteristics that are not observed in normal tissues or organs such as: 1) extensive angiogenesis and hence hypervasculature[12-16], 2) defective vascular architecture[17], 3) an impaired lymphatic drainage-recovery system, and 4) greatly increased production of a number of permeability mediators[18-21] (**figure 2.1**).

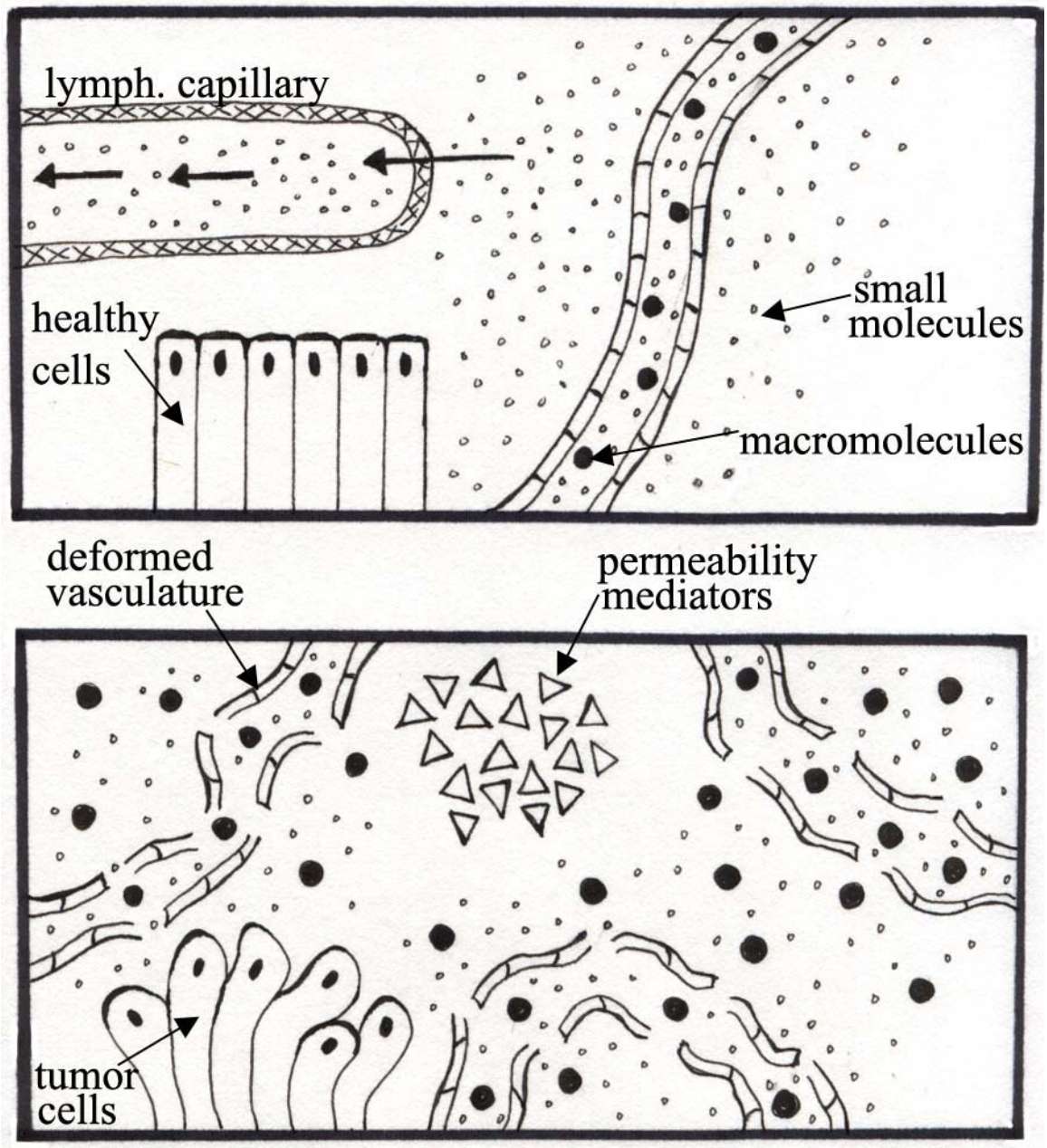

Figure 2.1 A cartoon representation of differences in the vascular anatomy of normal tissue (top) and tumor tissue (bottom). Tumor tissue contains an excessive network of deformed blood capillaries, an abundance of permeability mediators, and an impaired lymphatic drainage mechanism (Figure altered from Maeda).

The EPR effect has been observed in many experimental and human solid tumors, and is believed to be universal to all solid tumors (**table 2.1**).

Table 2.1 The EPR effect has been observed in these types of cancers.

Mice	**Rats**	**Rabbits**	**Humans**
S-180 sarcoma	Yoshida AH136B	VX-2	Hepatoma
Meth-A	Walker 256 carcinoma		renal cancer
melanoma B16	LY tumors		lung cancer
Ehrlich carcinoma			brain tumors
colon 38 adenocarcinoma			

Since the EPR effect also applies to most biocompatible synthetic polymers with molecular weights above the renal threshold,[7] approximately 40 kDa, it provides a tremendous opportunity for the development of tumor-selective delivery of macromolecular drugs without the need for a homing device (**figure 2.2**).

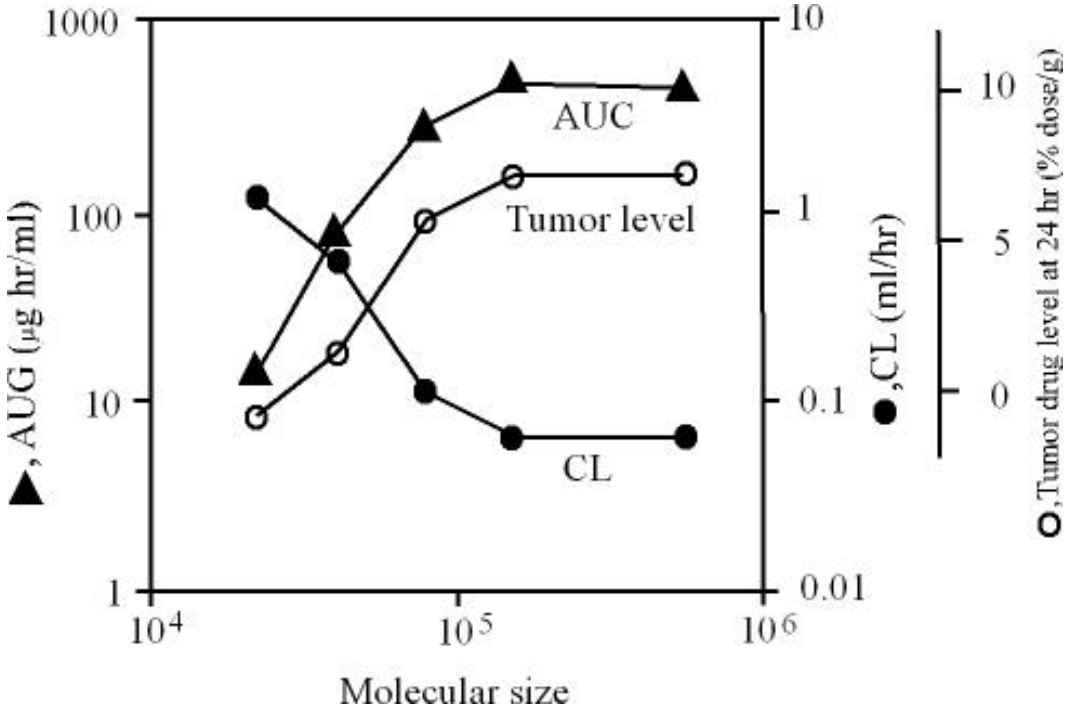

Figure 2.2 Relationships for molecular weight, tumor uptake and clearance of I-Tyr-HPMA-polymer drugs. Mice bearing S-180 solid tumor received about 1.8310 cpm per injection i.v.: d, CL, renal clearance rate; m, AUC, area under the concentration curve for plasma, both based on 72 h; s, tumor uptake based on 24 h (adapted from Maeda).

Tumors are composed of cells which are multiplying at a rate much greater than healthy cells. This increased growth rate is sustained by a higher number of blood vessels that supply a greater amount of nutrients. This also means that statistically, a molecule in the bloodstream should have a greater chance of encountering tumor tissue once administered. The leaky vascular structure of a tumor results in the retention of macromolecules once they enter the tumorous tissue. Healthy tissues, and even inflamed tissues, release macromolecules back into the bloodstream.

Most solid tumors have elevated levels of vascular permeability factors such as bradykinin, nitric oxide (NO), and, more recently discovered, peroxynitrite (ONOO).

These permeability mediators increase the vascular permeability of blood vessels, which are also passively dilated, with the endothelial intercellular junctions opened in the hypertensive state. It is also believed that blood vessels inside tumors lack both smooth muscle cells surrounding the endothelial cells and angiotensin II receptors (**table 2.2**). These characteristics provide unique opportunities for the design of macromolecular drug candidates with active targeting for tumors.

Table 2.2 Different characteristics solid tumors possess that are not usually observed in normal tissue.

1	Extensive angiogenesis and hence high vascular density.
2	Extensive extravasation (vascular permeability) induced by various vascular mediators such as: (a) bradykinin, which is produced via the activated kallikrein–kinin cascade involving various proteolytic (b) nitric oxide (NO) generated by the inducible form of nitric oxide synthase (iNOS) (c) VPF/VEGF and other cytokines (d) prostaglandins involving cyclooxygenases (e) matrix metalloproteinases (MMPs/ collagenases) (f) peroxynitrite
3	Defective vascular architecture: for example, lack of smooth muscle layer cells, lack of or reduced receptors for angiotensin II, large gap in endothelial cell–cell junctions, anomalous conformation (branching or stretching etc.).
4	Impaired lymphatic clearance of macromolecules and lipids from interstitial tissue (retention).

2.3 Design of macromolecular delivery agents

2.3.1 General characteristics

Synthetic polymers, glycoproteins, lipoproteins, lectins, hormones, albumin, liposomes, DNA, dextran, and antibodies have been explored as potential drug carriers[22-24]. Because the absorption and distribution of the drug depend on the physiochemical

properties of the macromolecular carrier, not the drug, these parameters can be altered by manipulation of the properties of the carrier. The ideal properties of a macromolecular drug carrying system are shown below[4].

- Protect the drug until it is at the site of action
- Localize the drug at the site of action
- Allow for release of the drug
- Minimize toxicity to the host
- System must be biodegradable, biochemically inert, and nonimmunogenic
- System must be easily and inexpensively prepared
- System must be chemically and biochemically stable in its dosage form

Although a few macromolecular drug carrier systems exert their effects while the drug is still attached to the carrier, the majority are designed so that the drugs are released at the site of action by diffusion, chemical reaction, or solvent activation. In this regard, drug delivery systems unshackle drug actions from the limitations imposed by their pharmacokinetics.

Ringsdorf described a general scheme for the design of a site-specific macromolecular drug delivery system.[25] A drug is attached to a polymer backbone, usually through a spacer so that it can be cleaved hydrolytically or enzymatically without steric hinderance. The desired solubility of the drug-polymer conjugate can then be adjusted by the attachment of appropriate ligands. Finally, site specificity can be manipulated by attachment of a "homing device" such as an antibody (f**igure 2.3**).

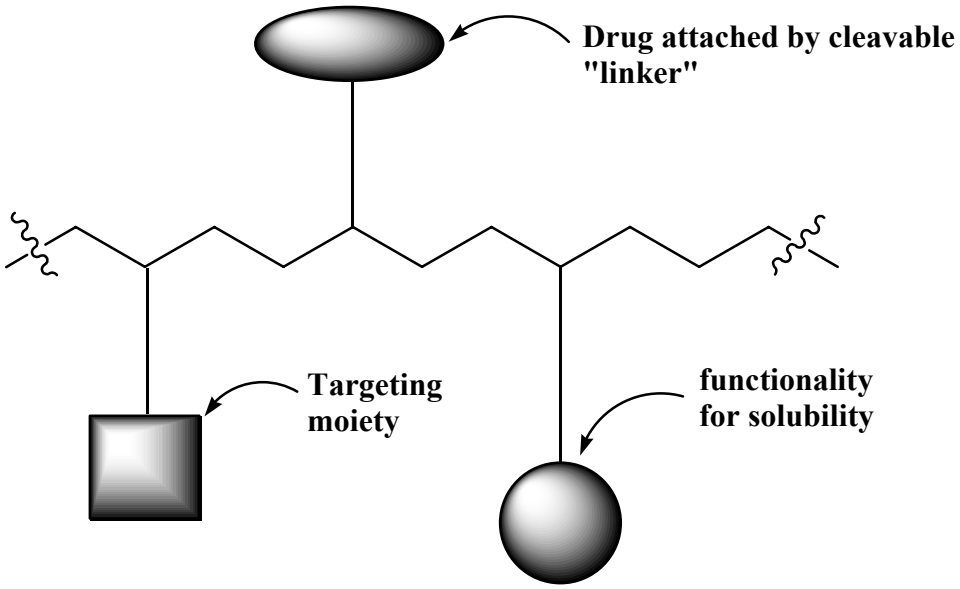

Figure 2.3 Cartoon drawing of drug-polymer conjugate.

Macromolecular drug carrier systems have found widespread use in controlled drug release, and offer numerous advantages compared to conventional dosage forms. These advantages include improved efficacy, reduced toxicity, and improved patient compliance. Although considerable resources must be spent to design and synthesize these carrier systems, simpler macromolecular systems can be envisioned for the delivery of antitumor agents.

A number of polymer-drug conjugates (**figure 2.4**) have been synthesized and studied in hopes of implementing a macromolecular targeting mechanism to small molecules with known functionality[26].

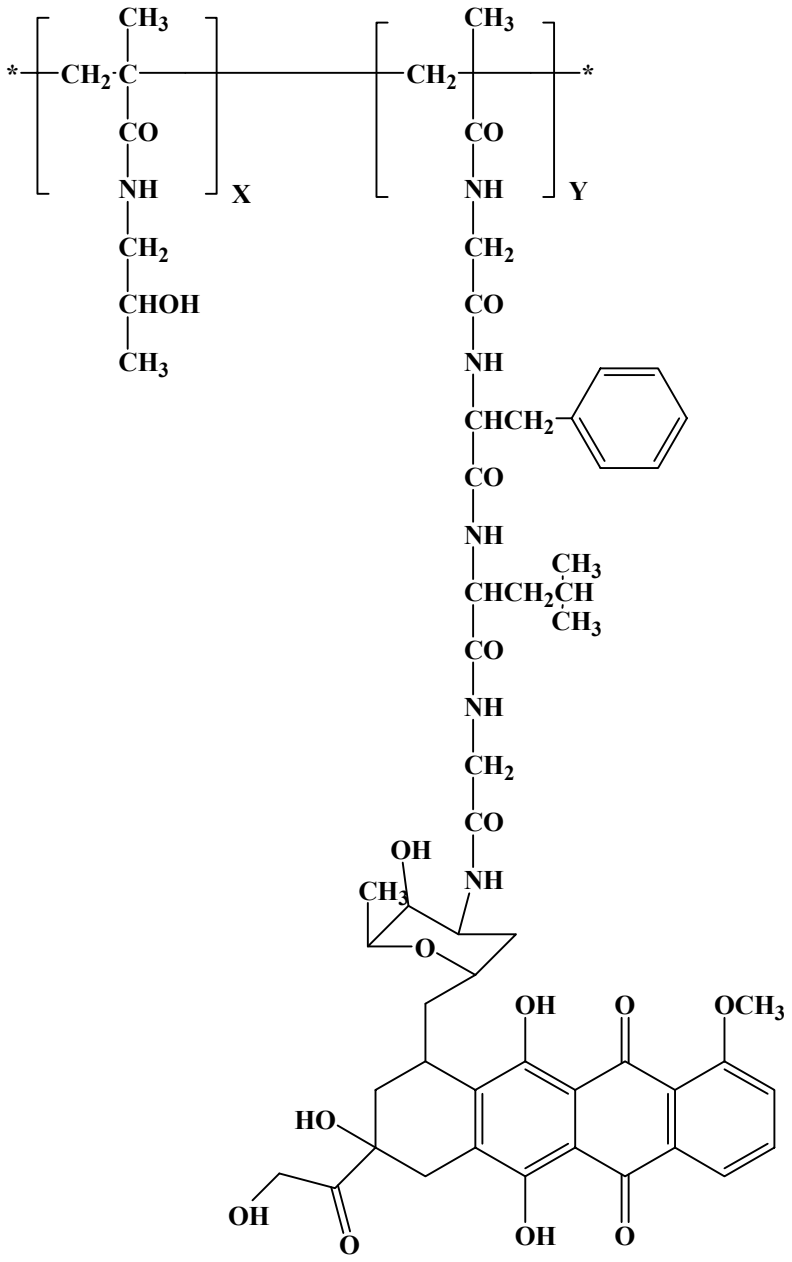

Figure 2.4 Structure of PK1 (HPMA copolymer doxorubicin). The molecular weight of the compound is 28,000 (figure altered from Vasey[27]).

This polymer-drug conjugate, synthesized by Vasey, was designed to selectively deliver doxorubicin to tumor tissue.

2.3.2 Molecular Size

The first step towards creating a seamless interface between early detection and targeted destruction of solid tumors is the selective delivery of a functional agent to the target tissue. Since most chemotherapeutic agents studied to date are relatively low molecular weight materials (< 10,000 Da), they are distributed to and retained by a variety of different tissue types.

The goal of this project is to design a carrier molecule or "scaffolding" for light-absorbing compounds (porphyrins) that will make them more specific for tumor tissue. One major aspect of the enhanced permeability and retention phenomenon is that the deformed vasculature of tumor tissue allows macromolecules to permeate from the blood capillaries into the tissue itself. This only occurs for small molecules in healthy tissue (**figure 2.5**) [7,9].

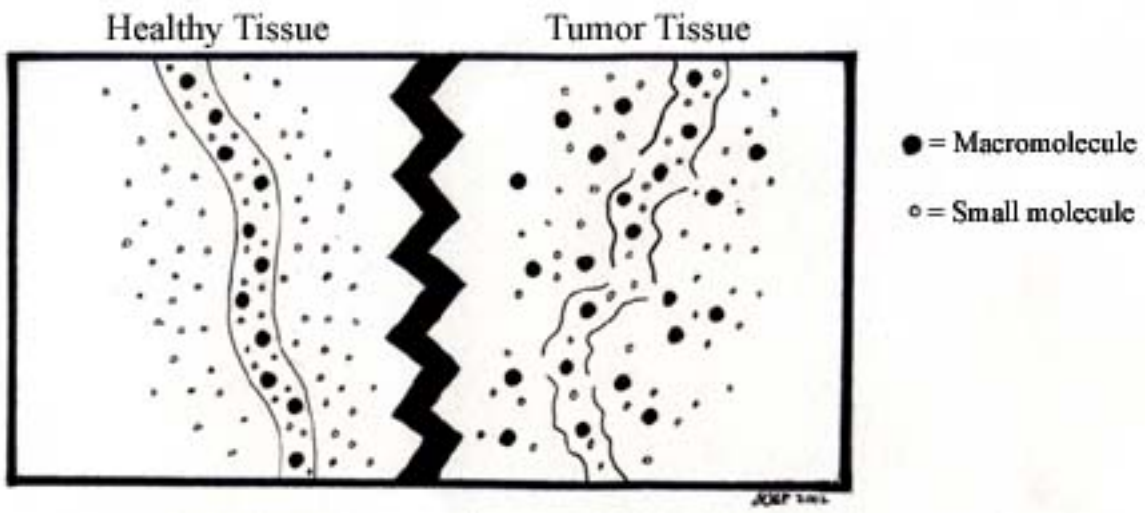

Figure 2.5 The deformed vasculature of tumor tissue allows for uptake of macromolecules, whereas in healthy tissue only small molecules can permeate the blood capillary membranes.

This means if the carrier molecule for a PDT agent has a high molecular weight, it cannot enter healthy tissues; additionally, as long as the molecule is less than 40,000 amu, the kidneys can still excrete it. Also, tumor tissue demands an increased flow of blood, which heightens the probability that the PDT drug will preferentially collect there.

2.3.3 Functionality of macromolecule

Given the rich and diverse properties of monomeric porphyrins, it is not surprising that the synthesis of porphyrin-based therapeutics has been pursued in recent years[28,29]. Porphyrins are being intensively studied for a wide range of medical applications as relatively low molecular weight monomers and oligomers.

The use of porphyrin derivatives as the functional components for the diagnosis or treatment of solid tumors has been one of the most promising research areas in this field. Interest in photosensitizers for photodynamic therapy and contrast agents for magnetic resonance imaging has lead to the synthesis of monomeric and oligomeric porphyrin, metalloporphyrin therapeutics, and their derivatives, including expanded porphyrins, chlorins, porphycenes, and phthalocyanines[28,30-35] (**figure 2.6**).

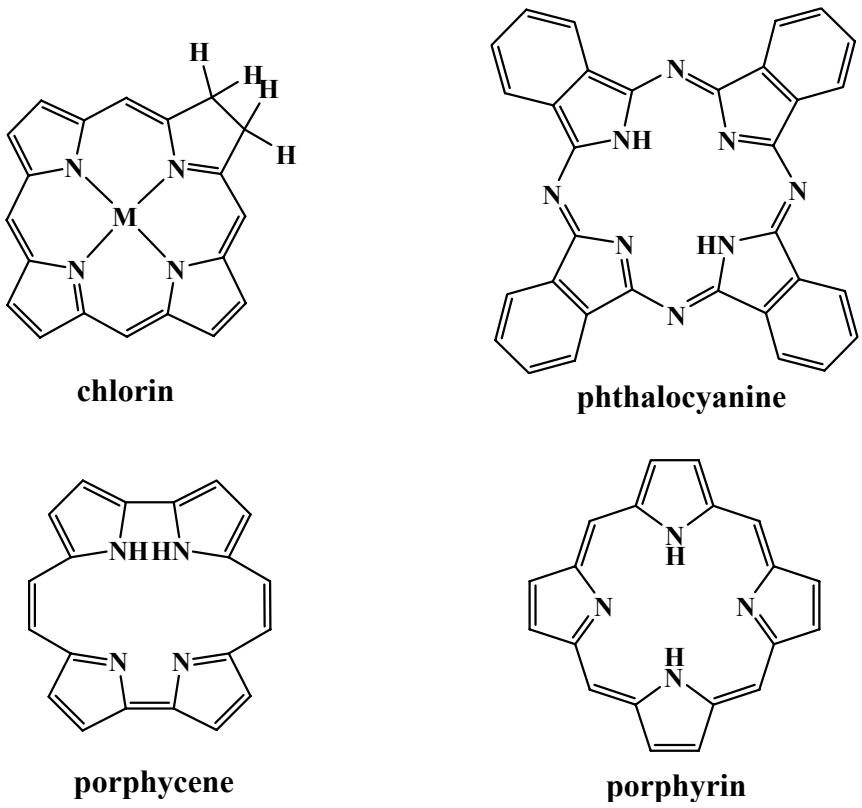

Figure 2.6 Porphyrin derivatives explored for use in therapeutics.

Interestingly, low molecular weight porphyrins[36-39] and their derivatives[40] have demonstrated tumor-localizing characteristics. Presumably their selectivity stems from the impaired lymphatic drainage of tumor cells rather than a selective uptake mechanism. However, this limitation requires the use of high doses, and side effects predictably linger for up to eight weeks as excess drug seeps from normal tissues.

We decided to incorporate the known functionality of porphyrins into a macromolecule which, based on the EPR effect, could be used for targeting tumors (**figure 2.7**).

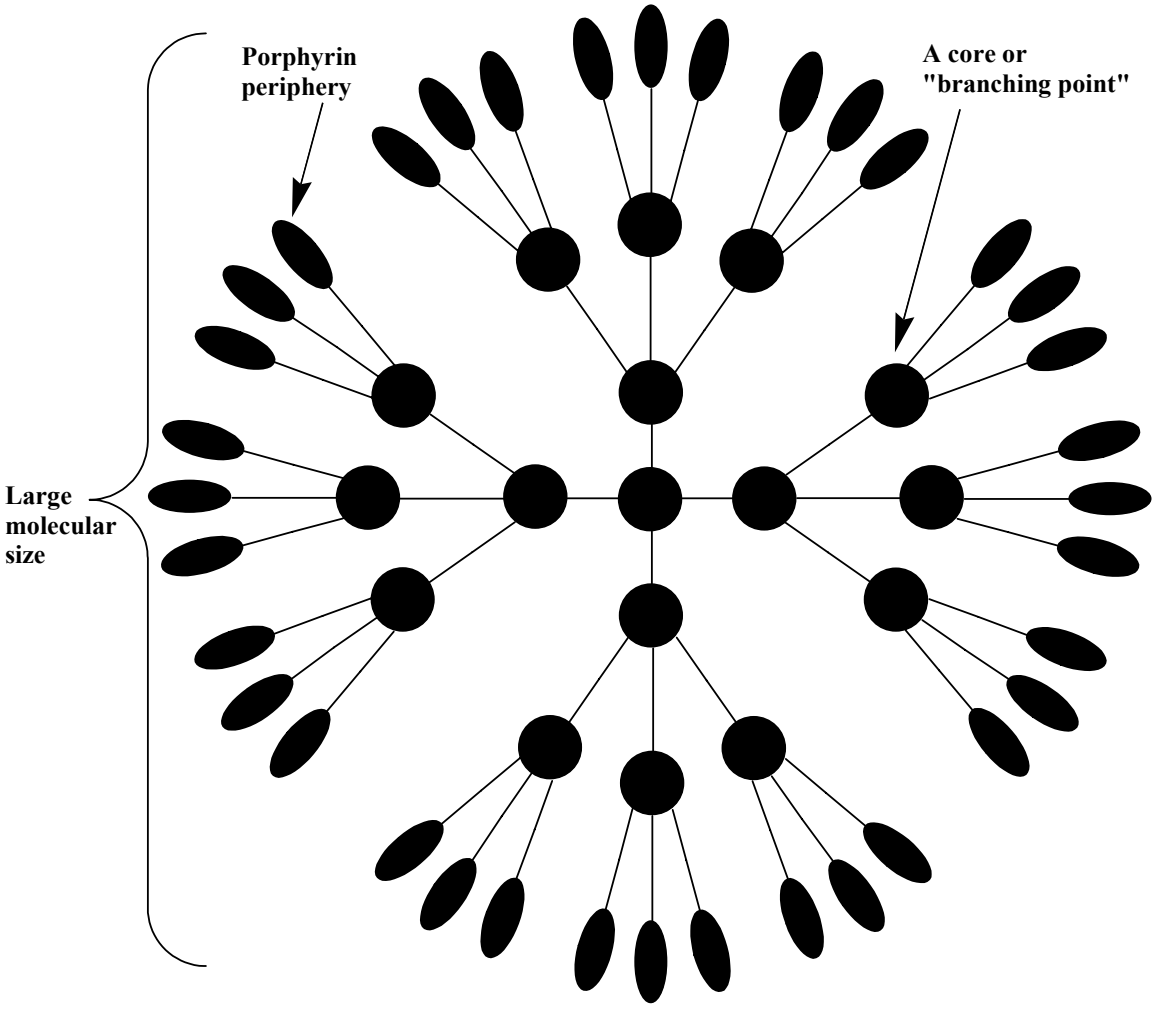

Figure 2.7 Theoretical representation of macromolecule containing porphyrins. The size of the macromolecule imparts a targeting for tumor tissue, and the porphyrins can be used for detection and destruction.

A macromolecule composed of multiple porphyrins should have a higher chance of success in the detection and destruction of tumors compared to a monomeric porphyrin. In addition, by inserting one of fifty-five different metals into an existing organic backbone, fifty-five drug candidates with different chemical, redox, photophysical and ligating properties could be created. Even beyond the fifty-five candidates, mixed metal porphyrin macromolecules would greatly expand the number of new compounds that can

be obtained using a single organic scaffold. This flexibility could translate into improved therapeutic properties from fewer synthetic compounds.

2.4 Dendrimer synthesis

2.4.1 A review of dendrimer synthesis

A class of compounds known as "dendrimers" was chosen to be used as the macromolecular drug delivery device because dendrimers have many desirable properties which have, in recent years, increased their popularity as useful drug delivery agents[22,41-43]. One of the most important characteristics of a dendrimer, is that its molecular weight can be controlled with high precision, compared to the molecular weight distribution of a polymer[44]. The molecular weight of a dendrimer can be controlled by creating a specific number of generations or "layers". There has been much work on dendrimers since they were first discovered approximately 20 years ago[45]. The word 'dendrimer' is a combination of the Greek 'dendron' (tree, branch) and 'meros' (part). Beginning with a core molecule or atom, the dendrimer grows outward in generations of 'branches' until a globular shape with a dense surface is achieved[46].

The first well-publicized dendrimers were synthesized in the 1980's by Newkome and Tomalia, who are known for their divergent synthesis of polyamidoamine (PAMAM) dendrimers (**figure 2.8**). PAMAM dendrimers have since been explored for a wide variety of applications.[47-51]

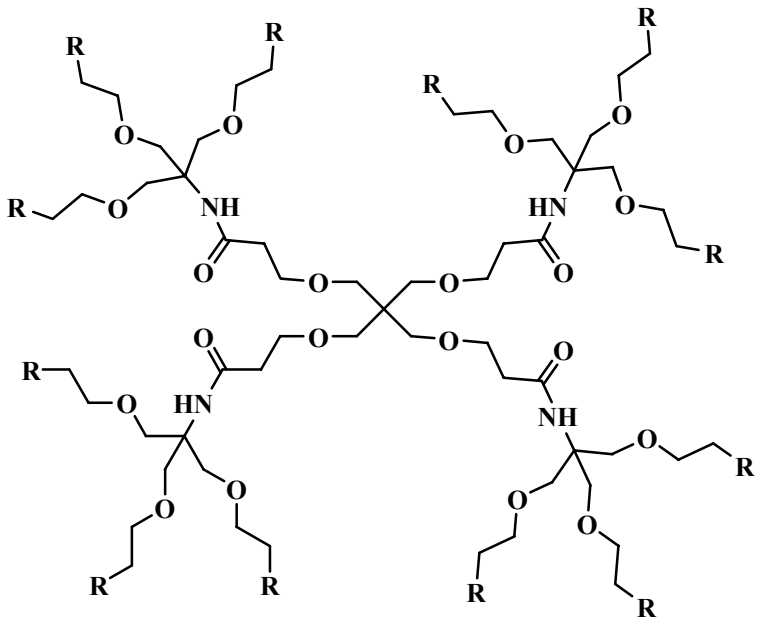

Figure 2.8 PAMAM dendrimer by Newkome and Tomalia, synthesized by a divergent approach [52].

The approach to be used for this particular dendrimer synthesis is based on a *convergent* strategy pioneered by Frechet (**figure 2.9**). The 'branches' are synthesized first, then coupled to a core[44]. This approach is preferred, because when dendrimers are built convergently from the outside toward the core the number of deletion errors are fewer than what would occur during a divergent approach.

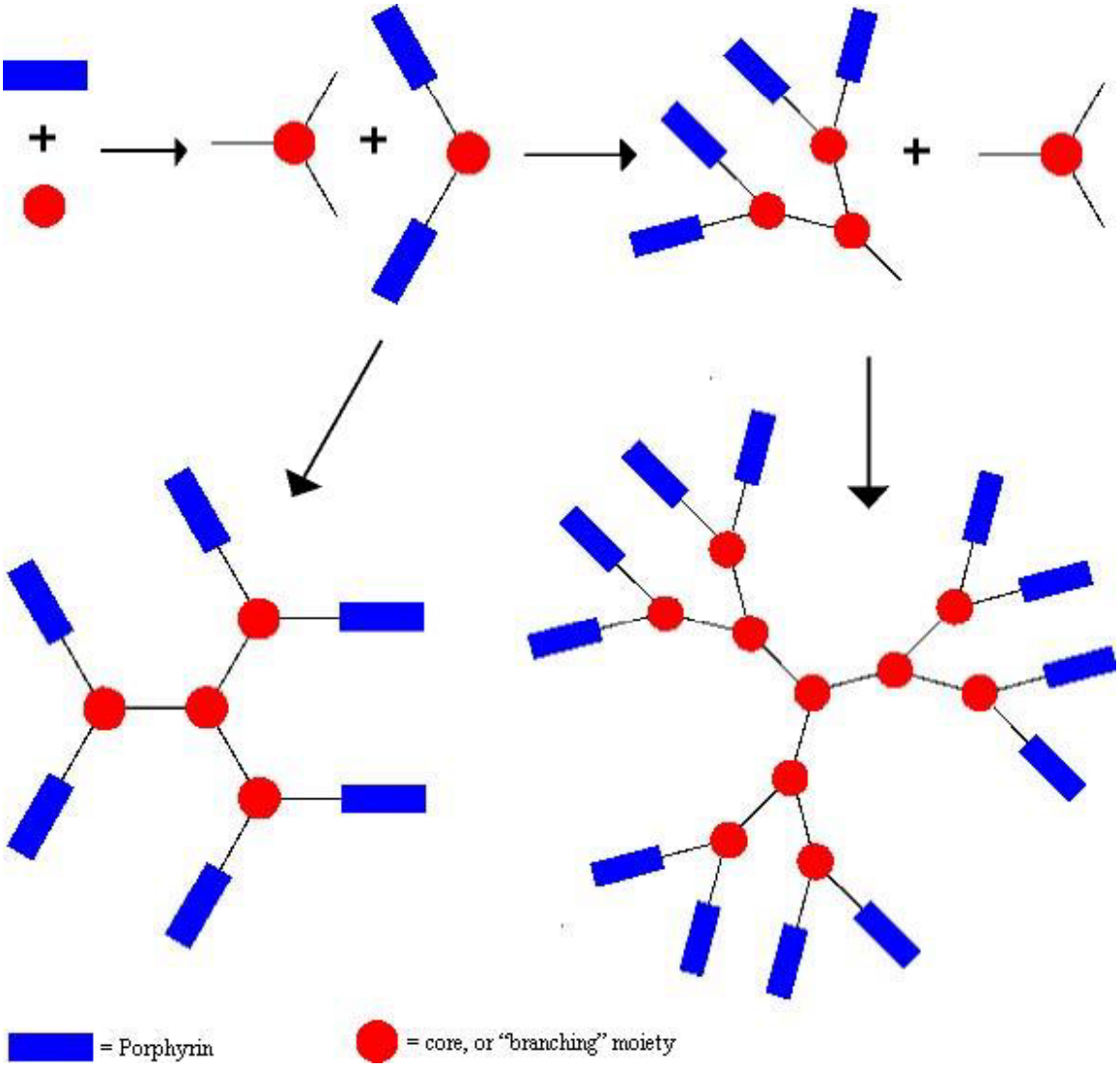

Figure 2.9 Convergent dendrimer synthesis.

Based on this convergent strategy, in the early 1990's Frechet and coworkers synthesized dendrimers using poly aryl ethers as their building blocks (**figure 2.10**).

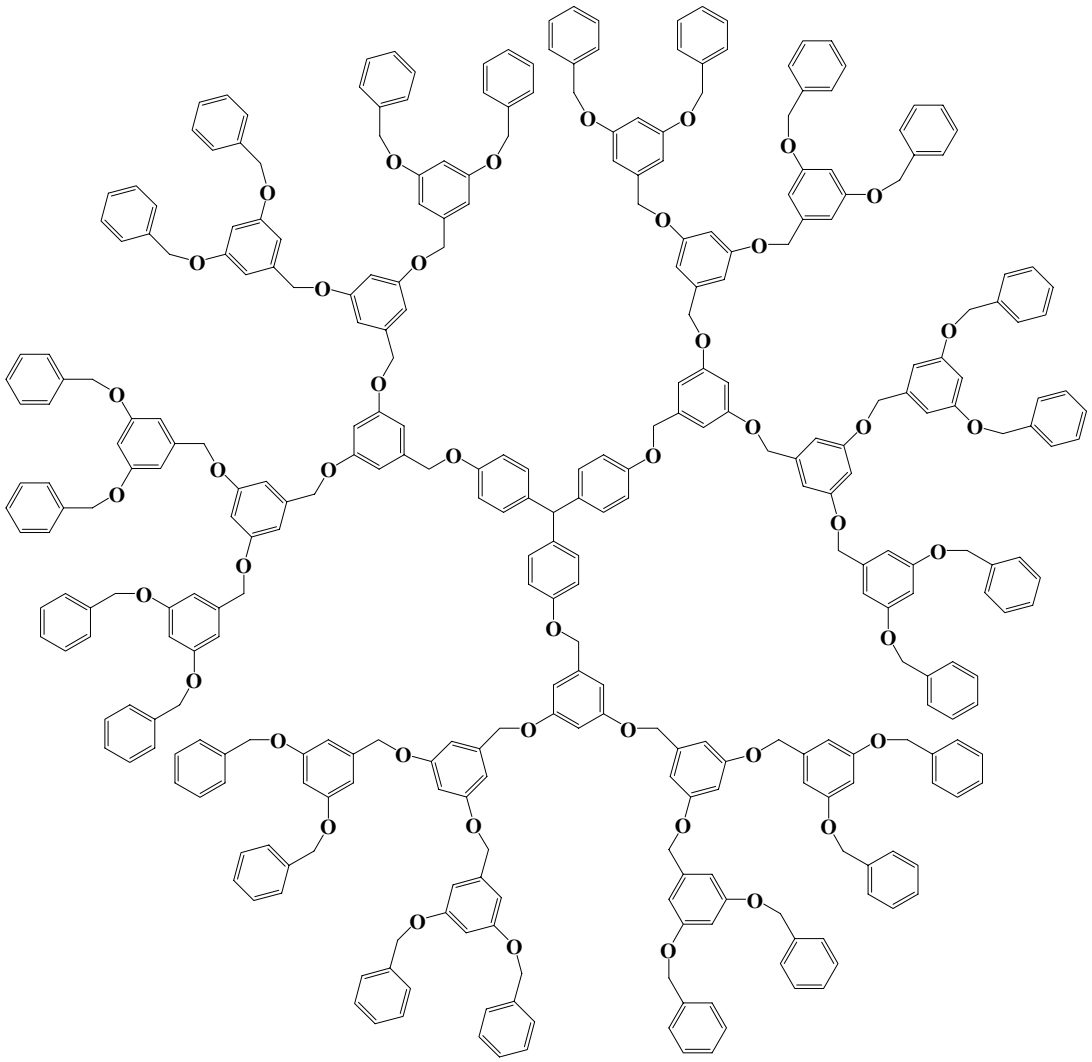

Figure 2.10 Frechet pioneered the convergent synthesis of dendrimers such as this poly aryl ether dendrimer[44].

In the last decade, a multitude of different dendrimers have been synthesized for a wide variety of applications. Past research has focused heavily on chemistry, firmly establishing the synthetic feasibility of dendritic materials. The research emphasis in dendrimer chemistry has recently switched from the development of synthetic methodologies to an exploration of the practical uses of functional dendritic molecules (**figure 2.11**).

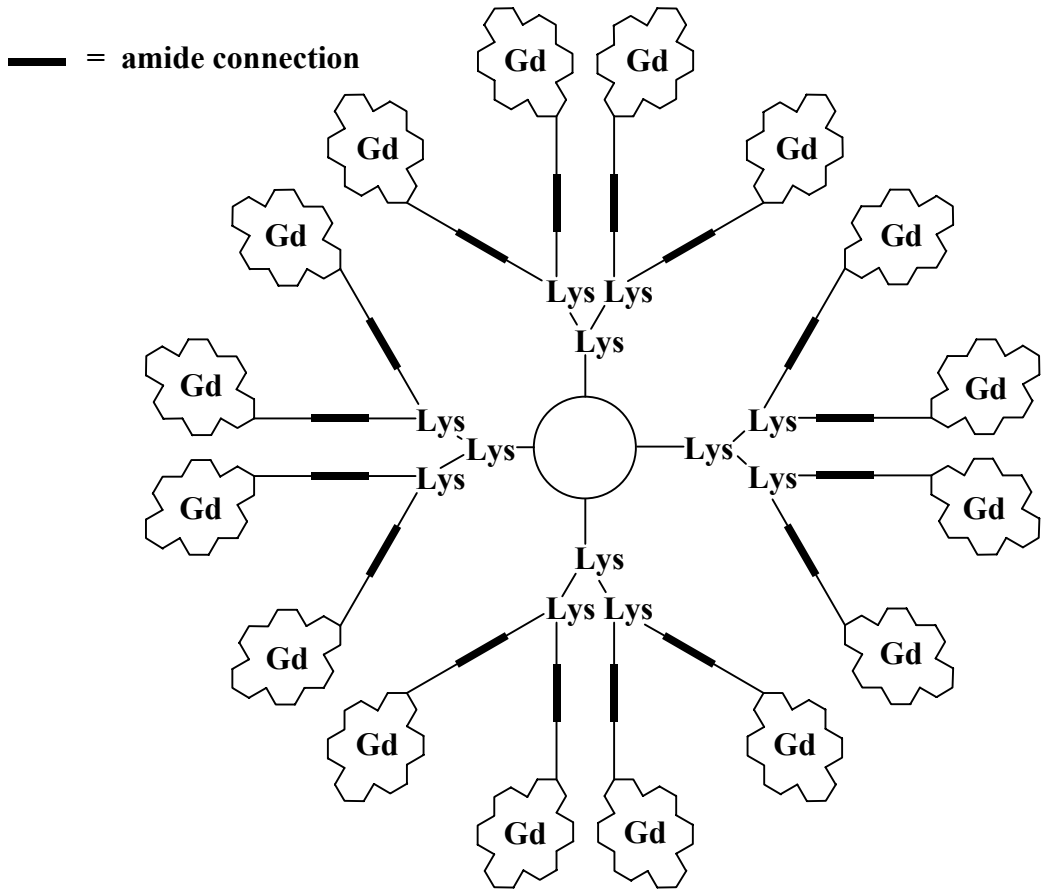

Figure 2.11 A cartoon representation of a dendrimer with 24 complexed gadolinium ions used as an MRI contrast agent (adapted from reference[53]).

A team from Schering-AG developed this gadolinium complexing dendrimer, which is composed of a trimesinic acid core, and 2nd generation lysine dendrons. Animal tests have shown this compound exhibits a high circulation time, and a quantitative renal elimination rate, and therefore is a very promising new contrast medium.

An azobenzene containing dendrimer synthesized by Mekelburger and Vogtle performs macroscopic conformational changes when irradiated with light. These changes, caused by the cis-trans isomerization of azobenzene, could enable the use of this type of molecule as a molecular switch (**figure 2.12**).

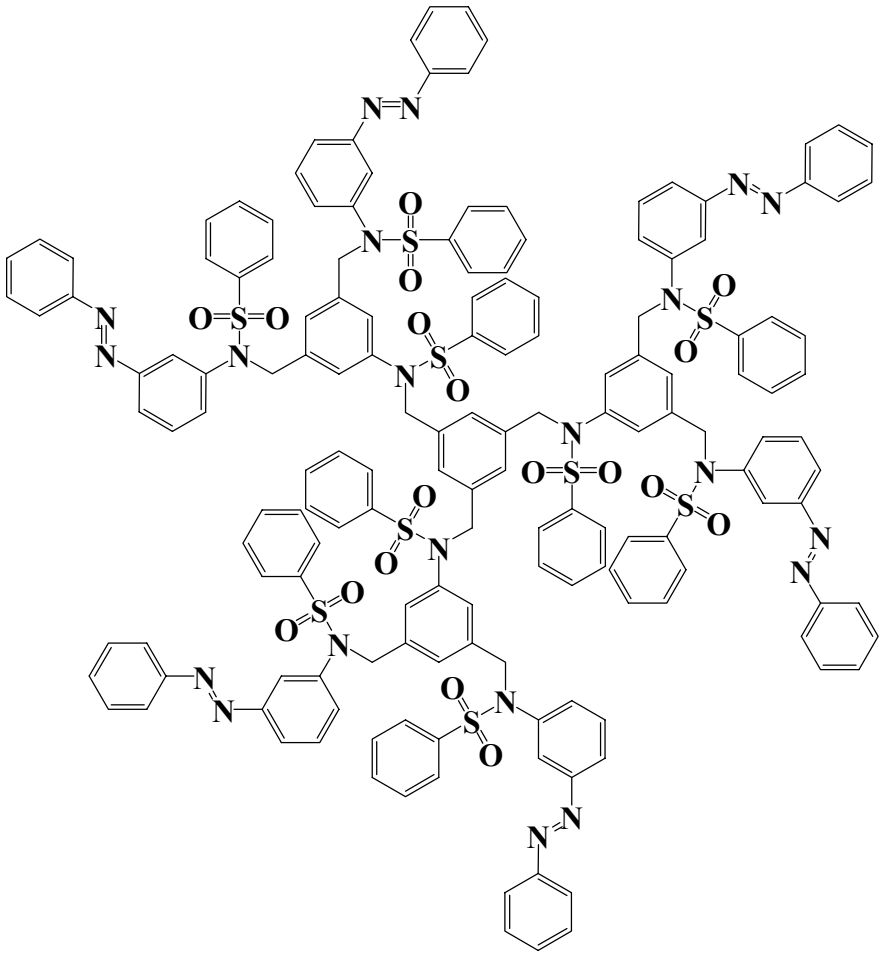

Figure 2.12 The first photo-responsive dendrimer synthesized by Mekelburger and Vogtle[54].

Shinkai synthesized a 2nd generation PAMAM dendrimer containing eight boronic acid periphery functional groups which act as saccharide complexing units. The eight anthracene units serve as fluorescence indicators (**figure 2.13**).

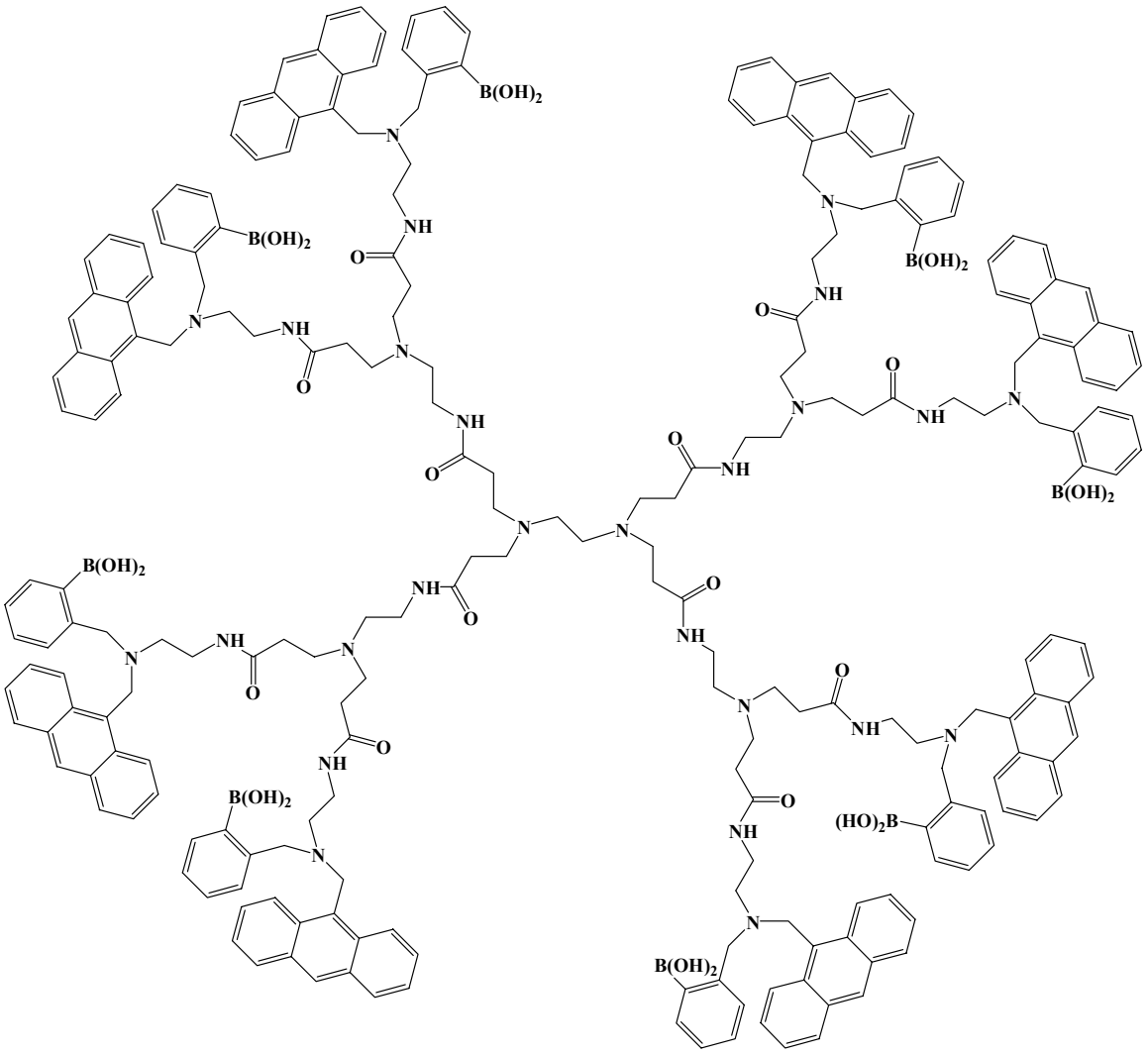

Figure 2.13 Dendritic saccharide sensor by Shinkai[55].

Meijer and co-workers synthesized this "dendritic box", which was shown to host small molecules such as eosin. This type of molecule could be used to release a drug upon a chemical or enzymatic cleavage of a periphery group **(figure 2.14)**.

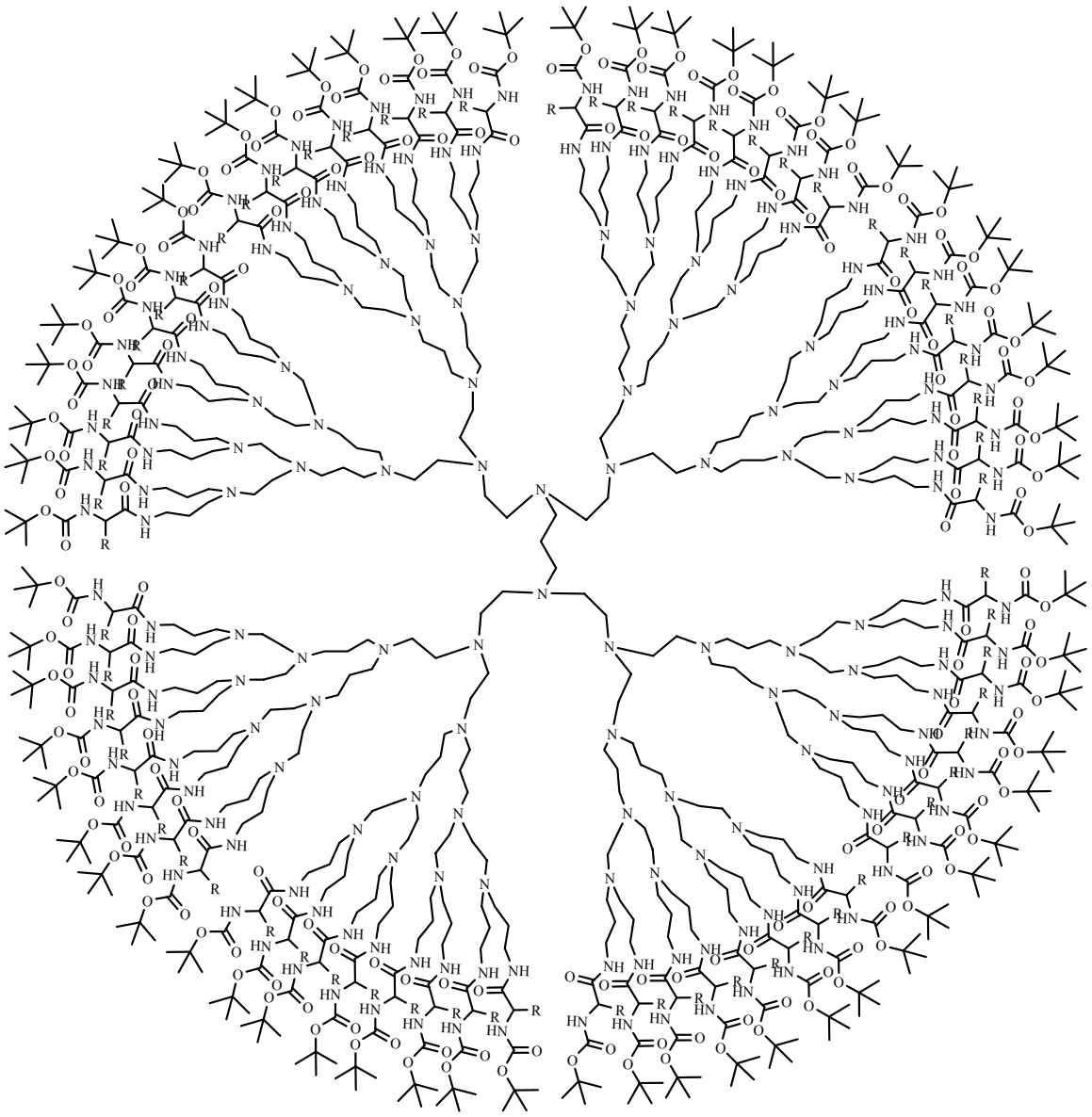

Figure 2.14 Meijer's "dendritic box"[56].

There are number of reasons why a dendrimer makes a desirable therapeutic delivery device. Dendrimers may contain many surface functional groups, which can be used for the delivery of covalently attached drugs, increased water solubility, and targeting; there are many excellent reviews on these subjects[57-64]. Also, dendrimers have controllable nanoscale dimensions, predetermined molecular shape, precise molecular

mass and tunable interior or surface features. This structural homogeneity and regularity should allow unique drug candidates to be synthesized, and a more precise rationalization of the structure-activity relationships of the targets. As demonstrated by the previous figures, functionalization of dendritic systems is now the area of dendrimer research receiving the most attention. Although dendrimer chemistry was initially a spin-off from the conventional polymer arena in the early 1940s, it is now commencing to play a unique role not only in the polymer and materials industries, but also in various medical and pharmaceutical areas. Though synthetically more challenging than their polymer counterparts, dendritic molecules can be tailor made to contain discrete functional domains having unique physical and chemical characteristics.

2.4.2 Design of porphyrin based dendrimer

Based on the impact of EPR theory, and on our understanding of tumor biology, a new class of high molecular weight (≈40,000 Da) dendritic metalloporphyrins have been designed to overcome many limitations of current chemotherapy regimens. This design is based upon a modular approach whereby important physical properties including molecular weight, shape, effective size, relaxivity, chelate stability, solubility and biocompatibility can be tuned independently, thereby quickly optimizing prospective drug candidates for *in vivo* studies. By using porphyrins as functional units we take advantage of the porphyrin's demonstrated ability to serve as both a contrast agent for magnetic resonance imaging, and as a type-II photosensitizer for photodynamic therapy.

There are several different categories of functional dendrimers. One example is to encapsulate a functional moiety into the central core of a dendrimer. This design

allows for monitoring of the influence of a dendritic periphery on the properties of the core unit. However, it is anticipated that since triplet energy transfer processes occur within 40 A, diffusion of singlet oxygen through the dendrimer may destroy the dendrimer along with the tumor tissue. Alternatively, the functional groups may be appended on the surface of the dendrimer. At the outset, this seems to be a more appealing approach given the aforementioned complication. This also allows for studies on cooperativity and allosteric interactions of the porphyrin units.

With this synthetic design, structure-activity relationships could be readily studied. In addition, multiple porphyrins combined in a single macromolecule may improve sensitivity for both MRI and PDT by an order of magnitude or more, given that these macromolecular agents contain upwards of 10 times the number of functional units for a small-molecule therapeutic. These developments alone may translate into even earlier detection or more effective treatment since fewer molecules will be required to elicit a comparable response.

A convergent synthesis generates large molecules with less deletion errors. This enables a more accurate characterization, resulting in the development of more optimized dendrimer delivery devices. Porphyrin placement around the periphery of the dendrimer provides the photodynamic functionality (**figure 2.15**).

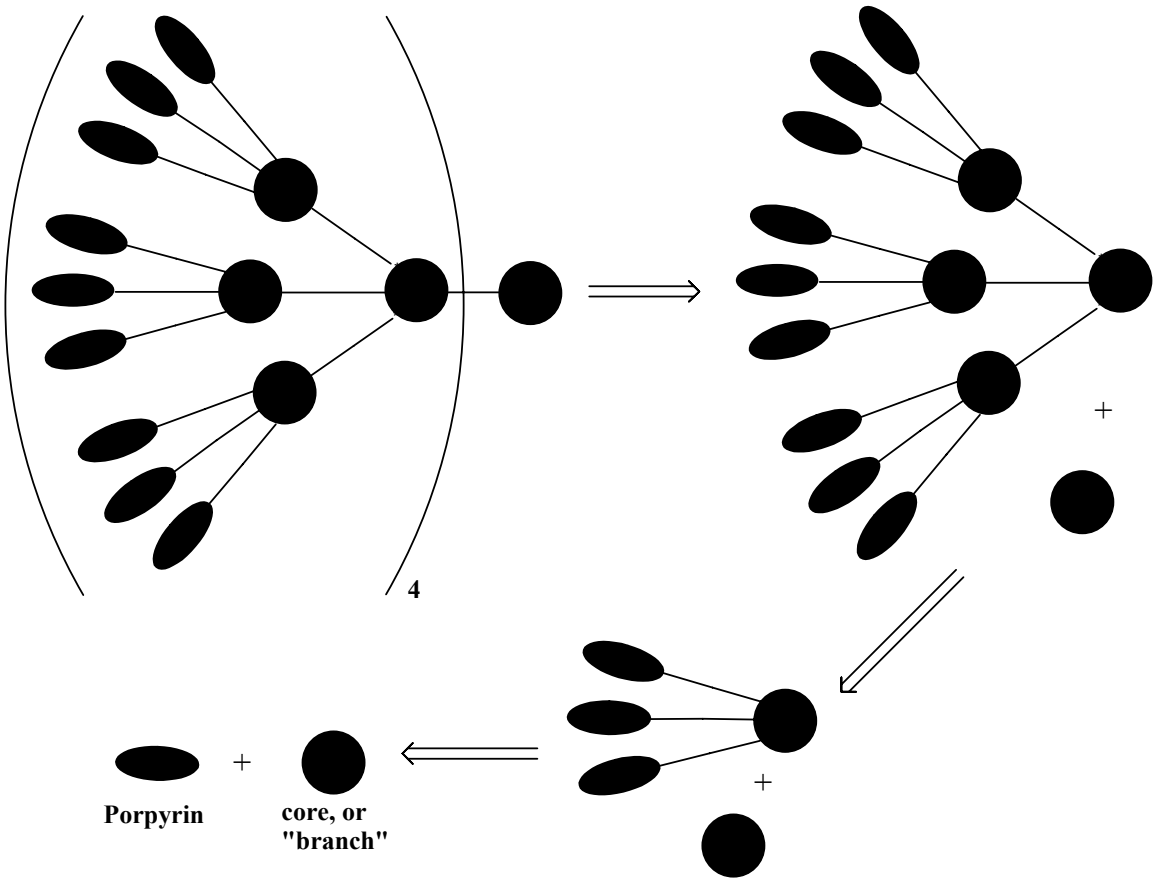

Figure 2.15 Convergent retrosynthesis of a porphyrin based dendrimer.

In this convergent retrosynthesis, porphyrins are coupled to a “branching point” to form a dendron. This dendron can either be used to form a dendrimer, or further coupled to a “branching point” to form a larger dendron.

2.5 Conclusions

There are many theories on how to target drug molecules to specific sites in the body, and the search for better, more accurate methods to do this is never ending. The EPR effect is a theory which states that cancerous tissue has many different physiological characteristics compared to healthy tissue. One of these characteristics in particular, the

fact that macromolecules are collected and retained selectively in solid tumor tissue, has far reaching implications in the design of selective drugs. Many current drugs, which have harmful side effects, could possibly be improved by their containment in macromolecules. By combining their known functionality with this passive targeting mechanism, a new generation of therapeutics could be developed.

Porphyrins have been shown to be excellent singlet oxygen sensitizers, and are also known to complex numerous metals. By taking advantage of this functionality, and based on the EPR effect, their containment in a macromolecule could result in a new class of delivery devices which may contribute greatly to the fields of MRI and PDT, and ultimately save countless lives.

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CHAPTER 3

DESIGN AND SYNTHESIS OF TRIAZINE BASED DENDRIMERS

3.1 Abstract

The goal of this project was to develop a mild and efficient synthesis for porphyrin dendrimers based on a triazine core. The porphyrins act as the PDT sensitizer, or MRI contrast agent, and the dendrimer scaffolding imparts an active targeting for tumor tissue. One advantage of using a dendrimer is that the molecular weight can be specifically controlled, allowing for both tumor specificity and renal excretion. A second advantage to using a dendrimer as a delivery agent is that the precise structure may be being fully characterized, resulting in a better understanding of what makes a potent photosensitizing agent. The modular dendrimer synthesis is also easy to modify, simplifying further optimization of properties for future studies.

Although a number of porphyrinic dendrimers have been synthesized[1-3], their synthesis, and the synthesis of dendrimers in general, is usually difficult. The approach to this triazine based porphyrinic dendrimer uses optimized biphasic reaction conditions to achieve a high yielding, efficient coupling of the phenolic porphyrin to triazine. Further adaptation of these conditions results in control over the degree of substitution of triazine, allowing different substituents to be added to the triazine ring. By taking advantage of the difference in reactivity between a phenol and an aniline, the use of 4-

aminophenol as a "linker" eliminates the need for functional group transformations or deprotections.

3.2 Introduction to triazine chemistry

2,4,6-Trichlorotriazine, or "cyanuric chloride" has been widely used in such areas as materials[4], polymers[5,6], dyes[7], and pharmaceuticals[8-10]. It reacts primarily by aromatic nucleophilic substitution. The chlorine substituted aromatic ring is activated for nucleophilic attack, and the resulting negative charge is stabilized by resonance around the aromatic ring. Aromaticity is restored when a carbon-chlorine bond is broken, and the chlorine ion leaves (**figure 3.1**).

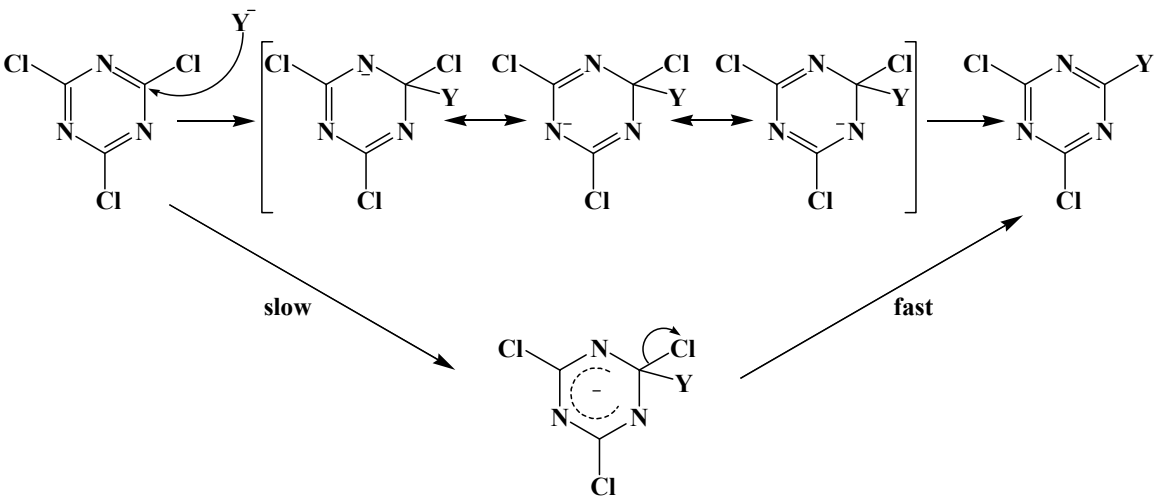
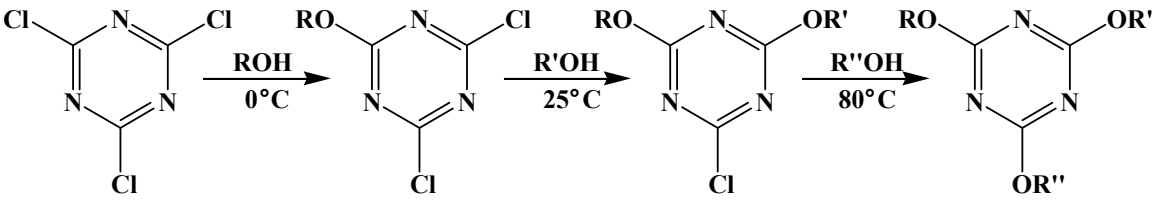


Figure 3.1 Nucleophilic aromatic substitution of trichlorotriazine.

In theory, after each nucleophilic substitution the aromatic ring becomes less activated because the new substituent does not stabilize a negative charge as well as chlorine. This means that a triazine could be substituted with three different nucleophiles by using a "thermo-control" method (**scheme 3.1**). Each substitution would require a

higher temperature than the previous in order to overcome the energy barrier needed for nucleophilic attack.



Scheme 3.1 Thermo-controlled substitution of triazine.

The kinetics of triazine substitution have been thoroughly studied by Marchukov; a variety of nucleophiles such as amines and alcohols were reacted with triazine, using aqueous hydroxide as the base (**figure 3.2**) [11].

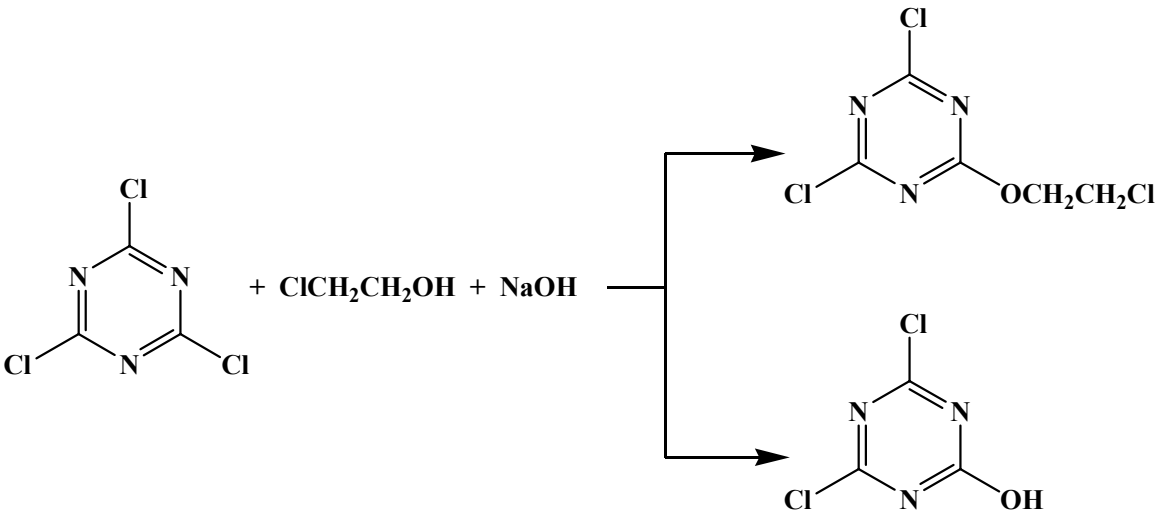
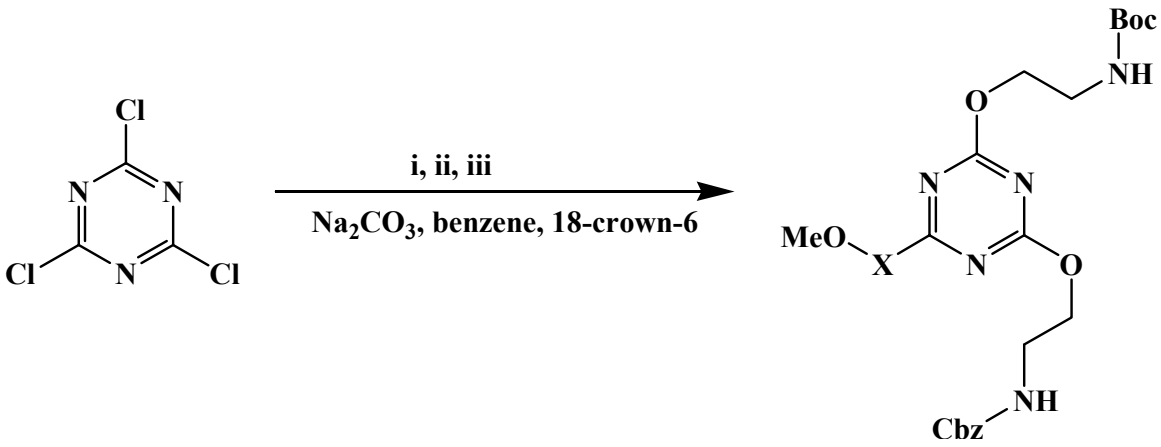


Figure 3.2 Kinetic studies of triazine substitution by Marchukov[12,13].

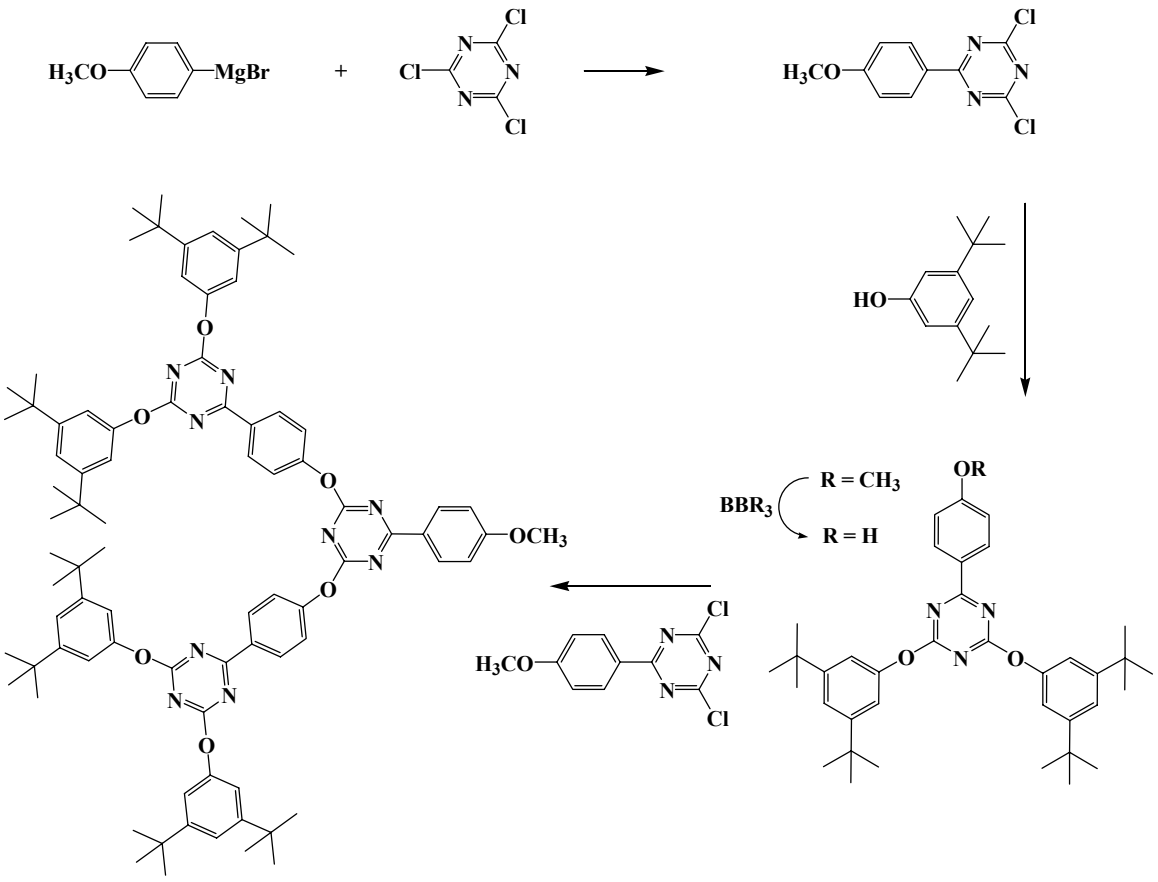
Triazine derivatives have also been used as templates for the homogeneous phase synthesis of chemical libraries as shown by Falorni and co-workers[14] (**scheme 3.2**).



Scheme 3.2 i) N-Boc-ethanolamine, 24 h, 80 C, ii) N-Cbz-ethanolamine, 24 h, 80 C, iii) HCl-HVal-OMe or HCl-H-Phe-OMe or HCl-H-Pro-OMe, 4 days, rt.

As demonstrated by the above reaction conditions, the substitution of triazine usually requires high temperatures, and long reaction times.

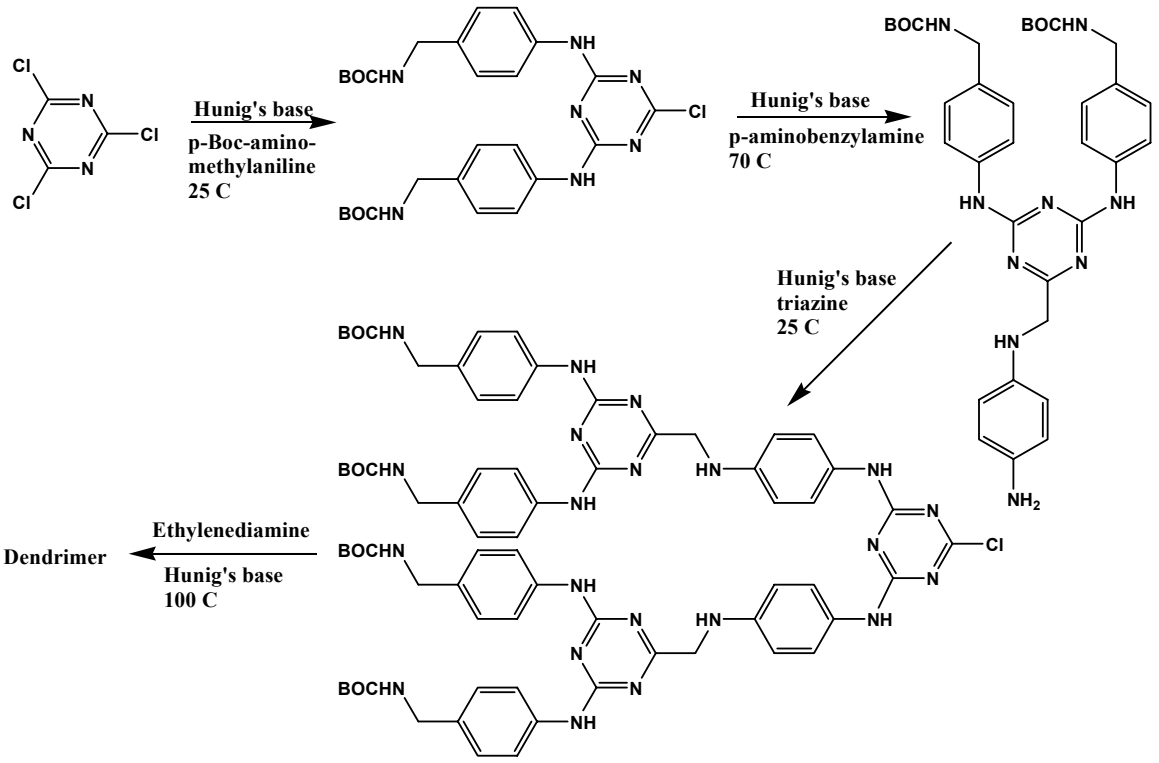
Triazine has also been used in dendrimer chemistry; Dehaen has prepared second generation dendrimers using a Grignard as the nucleophile (**scheme 3.3**)[15].



Scheme 3.3 Triazine based dendron formation. The dendron can be reacted with a "core" to form a dendrimer, or be used to form a larger dendron[16].

Like most dendrimer synthesis, the design above requires a functional group transformation (the conversion of methoxy to hydroxyl) in order to continue building larger dendrons.

There are a few cases of dendrimer synthesis strategies which do not involve functional group transformations or deprotections. Simanek coupled the benzylamine functionality of p-aminobenzylamine to triazine selectively, by taking advantage of the higher nucleophilicity of alkyl amines compared to anilines (**scheme 3.4**)[17-19].



Scheme 3.4 Convergent synthesis of melamine based dendrimer.

This is an efficient strategy, however the reaction conditions required high temperatures, and reaction times typically required several days to complete.

3.3 Development of reaction conditions, methodology, and reaction control

To develop a dendrimer based on triazine, the degree of substitution on the triazine ring must be controllable, reaction times should be short, and reaction conditions must be mild and high yielding. Salunkhe was able to efficiently tri-substitute triazine with a variety of phenols using mild biphasic reactions conditions (**figure 3.3**)[20,21].

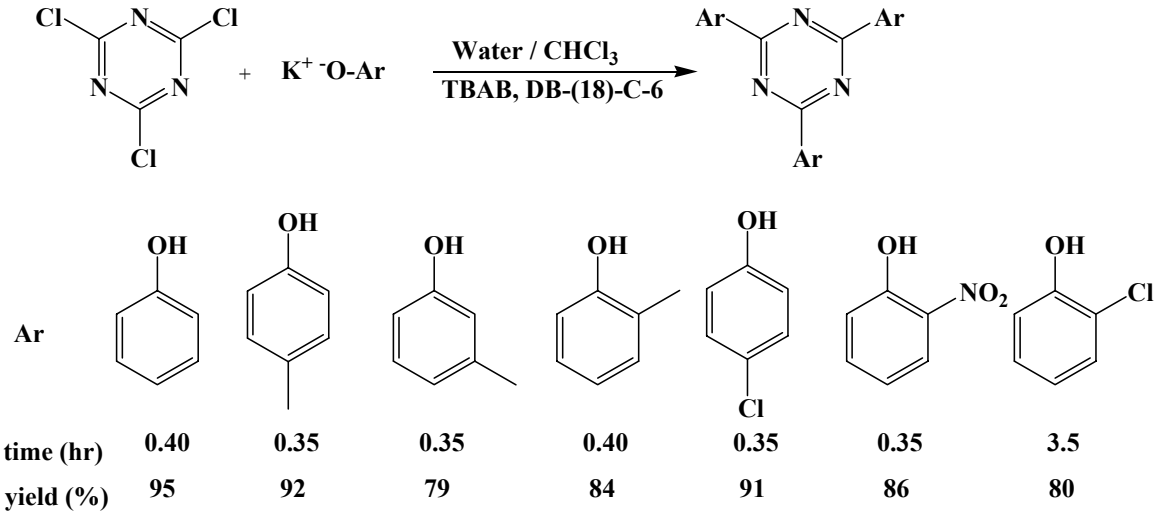


Figure 3.3 Mild and efficient synthesis of triaryl cyanurates using biphasic conditions.

Salunkhe's reaction conditions provided the basis on which the triazine dendrimer was synthesized. A retrosynthetic analysis of the 1st generation triazine dendrimer is shown in **figure 3.4**. The porphyrin contains a phenolic moiety, which can substitute for each of the three chlorides on triazine to form the dendrimer.

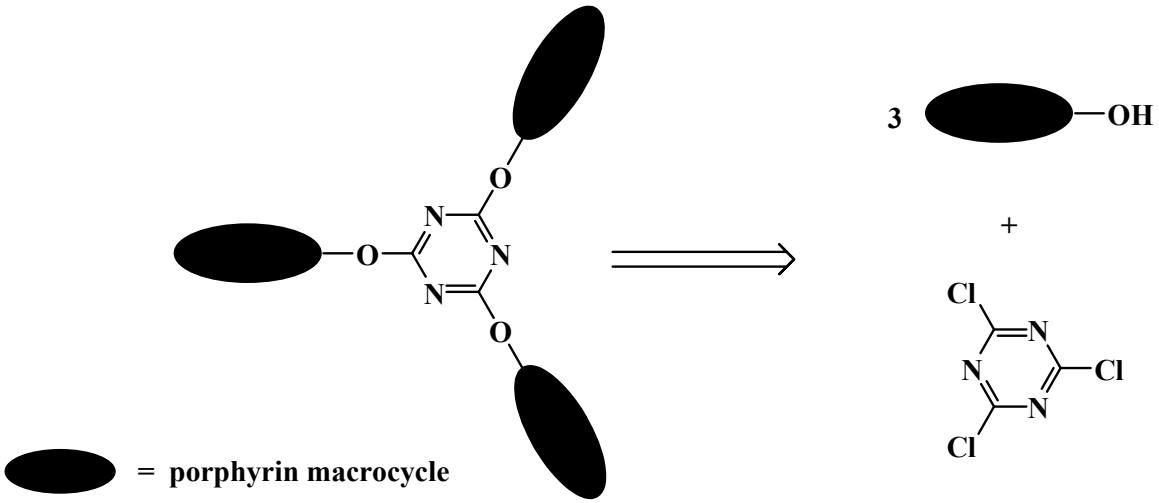


Figure 3.4 Retrosynthesis of 1st generation dendrimer.

A retrosynthetic analysis of the 2nd generation dendrimer is shown in **figure 3.5** Reaction conditions must be such that triazine can be selectively di-substituted, and a different substituent (4-aminophenol) attached to the 3rd position on triazine. The resulting "dendron" contains phenol functionality, which can tri-substitute triazine using the same biphasic reaction conditions as the 1st generation dendrimer formation.

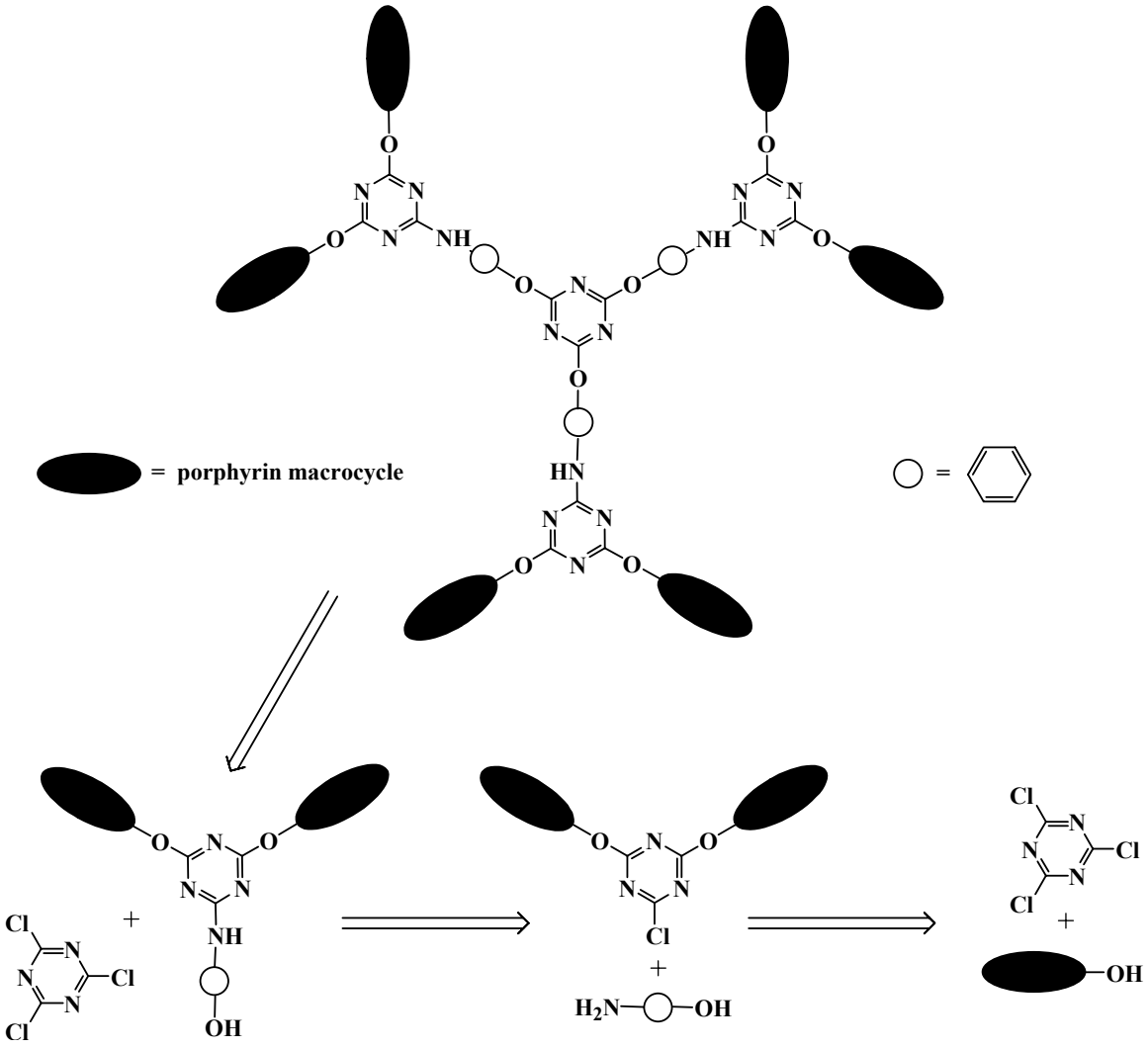
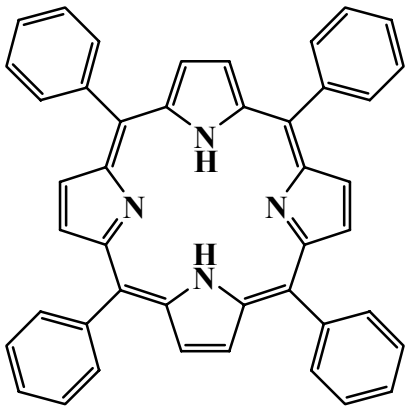


Figure 3.5 Retrosynthesis of 2nd generation dendrimer.

This synthesis of porphyrin dendrimers overcomes a limitation of many dendrimer synthesis, in that it requires no functional group transformations, or protecting group strategies. The synthesis is based on control of the nucleophilicities of functional groups by varying reaction conditions, and the selective use of catalysts. The reaction conditions are high yielding, and it is possible for large scale preparation of dendrimeric drug candidates.

3.4 Synthesis of porphyrin monomers and dendrons

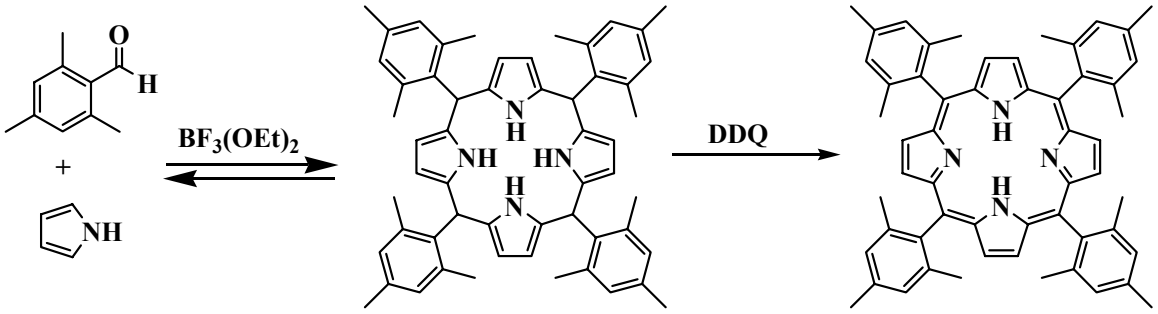
The first porphyrin synthesis was by Rothemund[22] in 1936, and later improved upon by Adler and Longo[23] in 1967. These procedures involved reacting pyrrole and benzaldehyde for 30 min. in refluxing propionic acid. This method is good for generating large quantities of porphyrins, but can only be used on non-acid sensitive functional groups (**figure 3.6**). Also, if the porphyrin does not crystallize or precipitate out of solution, it can be difficult to purify due to the formation of tar(s) during the reaction.



***meso*-tetraphenylporphyrin**

Figure 3.6 Structure of *meso*-tetraphenylporphyrin.

Since these early porphyrin-forming methodologies were discovered, there have been a number of new synthetic methods for the development of porphyrins. Currently, the most commonly used method for synthesizing porphyrins was developed by Lindsey[3] (**scheme 3.5**). Because of milder reaction conditions, this method is useful for preparing porphyrins from aldehydes with more sensitive functionality, in addition to aldeyhydes with ortho-substituents. Pyrrole and an aldehyde, with trace acid catalyst, react reversibly at room temperature to form the porphyrinogen, which can then be converted to the porphyrin by addition of an oxidant such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The main drawback of this method is that it is only useful for preparation of small quantities of porphyrins.



Scheme 3.5 Pyrrole and mesitaldehyde react to form a porphyrinogen, which is then oxidized to the porphyrin by DDQ. Lindsey conditions allow for the reaction of ortho-substituted, and acid sensitive functional groups.

Porphyrins with different substituents can also be synthesized by using two different aldehydes. In these cases, a mixture of six possible porphyrins is obtained (**figure 3.7**).

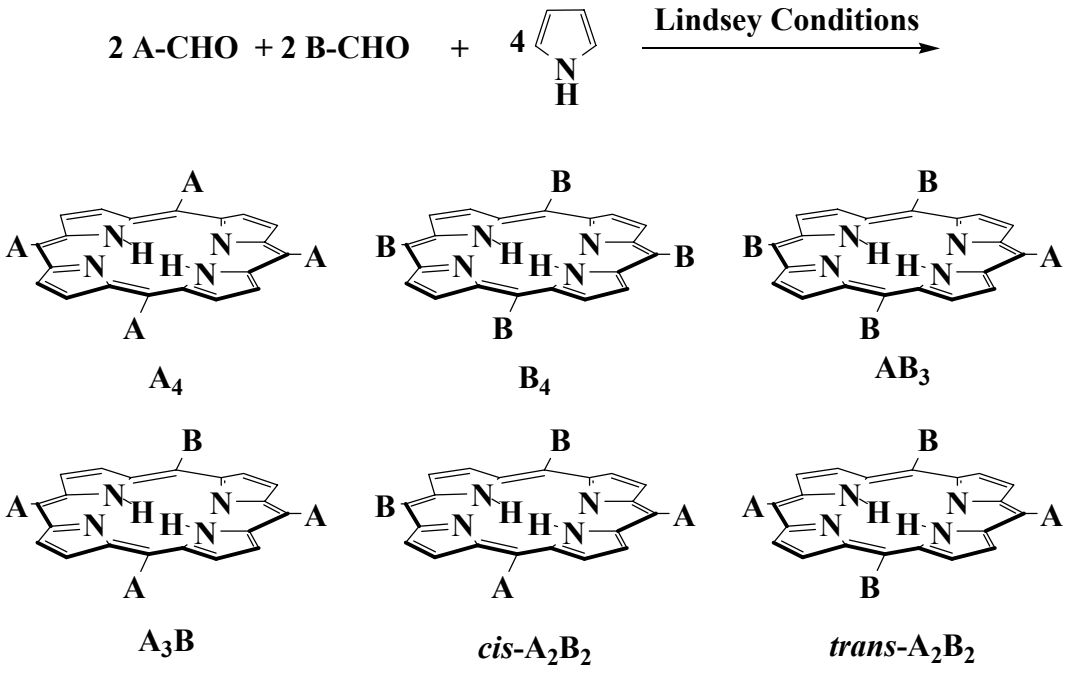


Figure 3.7 The traditional procedure for porphyrin synthesis produces a mixture of 6 compounds when two different aldehydes are used in the reaction.

To use a porphyrin as a functional molecule it is often desirable to be able to synthesize a specific substitution pattern. In these instances, reaction conditions can be tailored to give an excess of the desired porphyrin. According to the probability calculated by the binomial distribution equation shown below, the theoretical yield of A_3B porphyrins (using a 1:1 ratio of aldehydes) is only 25% (**Figure 3.8**), where **a** and **b** are the mole ratios of A and B aldehyde respectively, and **x** is the number of "A" groups.

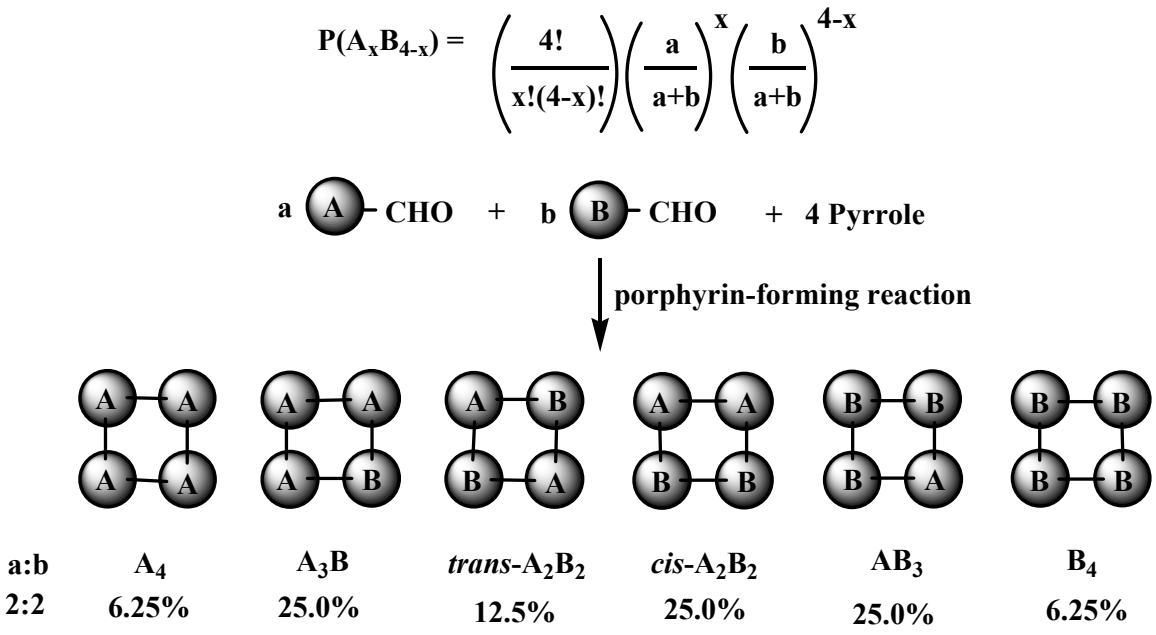


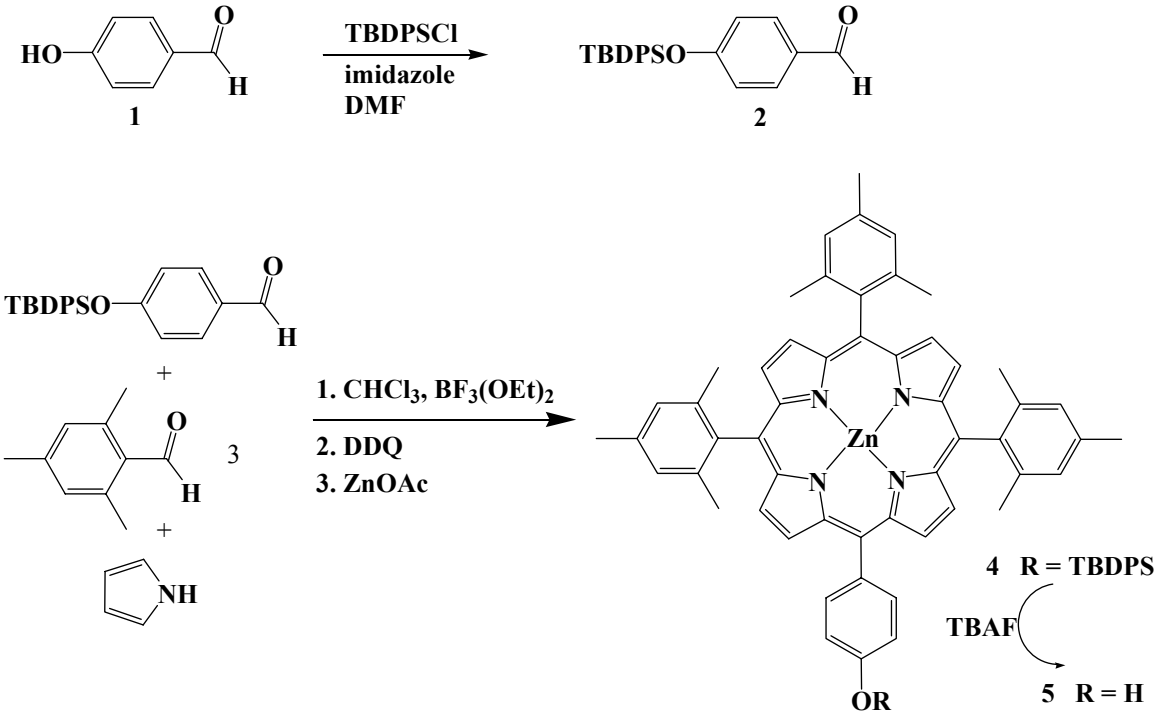
Figure 3.8 Probabilities of generating each type of porphyrin substitution pattern resulting from standard porphyrin reaction conditions.

However, if the A:B aldehyde ratio used is 3:1, the theoretical yield of A_3B porphyrin is 42%. Likewise, higher percent yields of any desired substitution pattern can be obtained by adjusting the molar ratio of the aldehyde components.

3.4.1 A_3B porphyrin synthesis

In order to synthesize the A_3B porphyrin **5**, a "B" aldehyde (4-hydroxybenzaldehyde **1**) was protected to be compatible with the porphyrin-forming reaction conditions. *tert*-Butyldiphenylsilylchloride (TBDPSCl) was chosen as the protecting group for the phenol because it can easily be cleaved with fluoride, but it is also more stable to acid than other silicon protecting groups such as trimethylsilylchloride (TMSCl). Mesitaldehyde **3** was chosen as the "A" aldehyde because the ortho methyl groups impart an increased solubility to the porphyrin by projecting over the plane of the

macrocycle and preventing π-π interactions. The A_3B porphyrin was then synthesized from commercially available mesitaldehyde, pyrrole, and the TBDPS protected 4-hydroxybenzaldehyde **2** (**scheme 3.6**). The reaction mixture was only partially purified before metallation and silyl deprotection, which allowed for easier purification due to the higher polarity of the final porphyrin. The TBDPS aldehyde **2** was characterized by ^{1}NMR spectroscopy (**appendix A**), and the porphyrin **5** was characterized by mass spectrometry (**figure 3.9**), UV/VIS spectroscopy (**figure 3.10**), size exclusion chromatography (SEC) (**figure 3.11**), and ^{1}H NMR (**appendix A**), and ^{13}C NMR (**appendix A**) spectroscopies.



Scheme 3.6 Synthesis "B" aldehyde **2**, and A_3B porphyrin **5**.

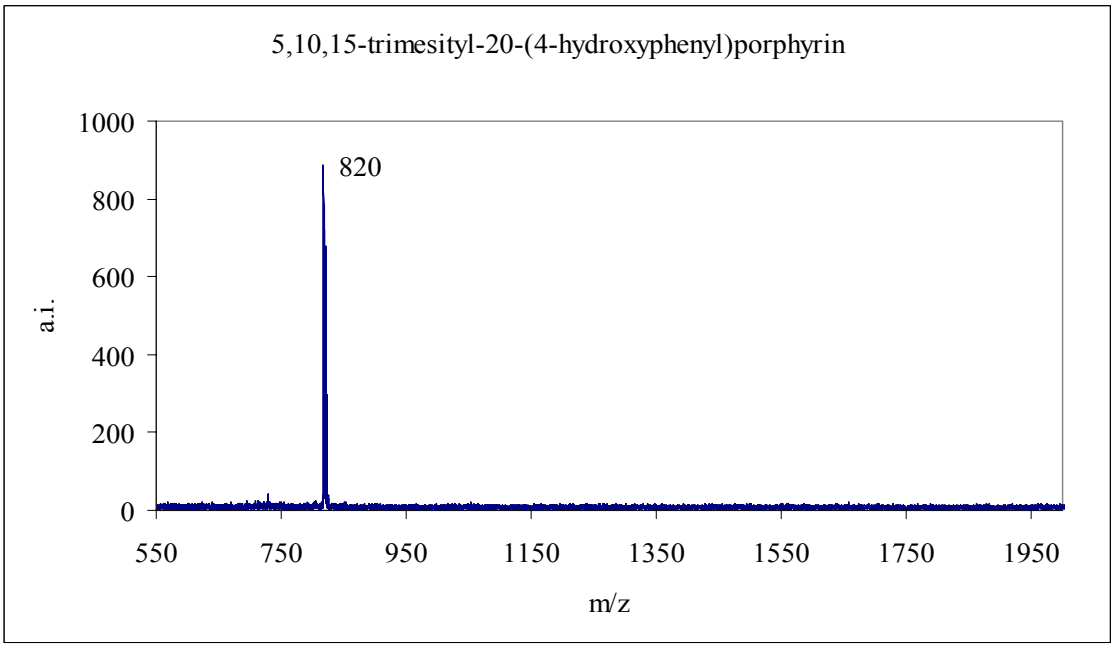


Figure 3.9 Laser desorption mass spectrum of A_3B porphyrin **5** (theoretical mass is 820.35).

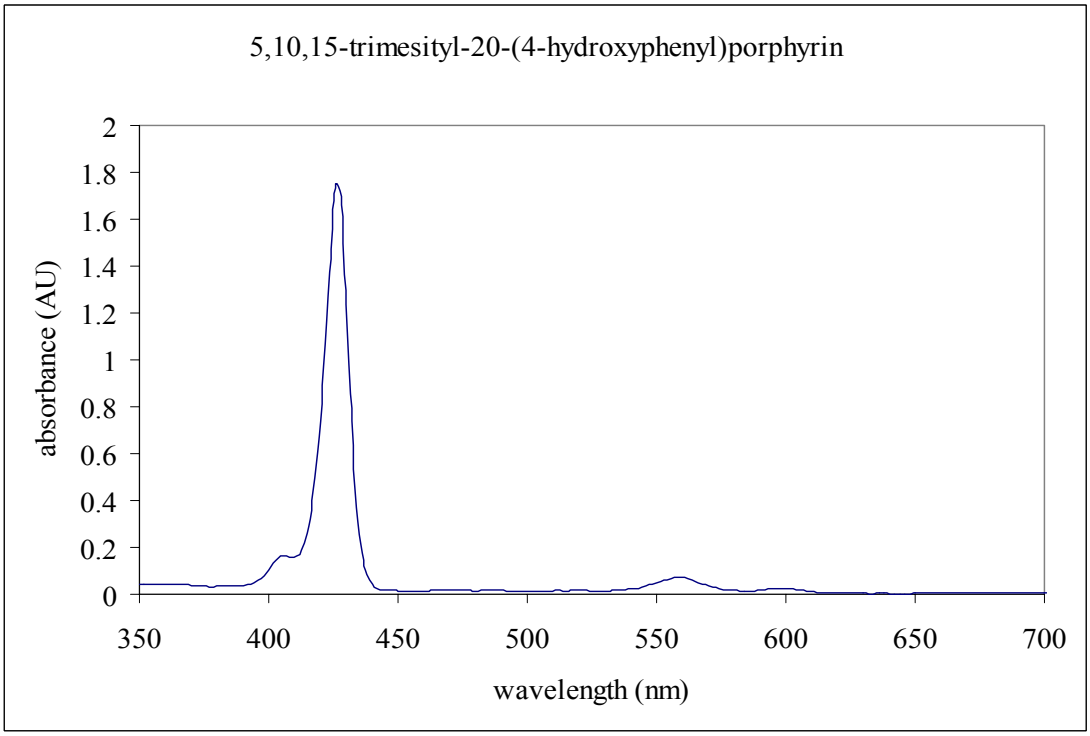


Figure 3.10 UV/VIS spectrum of A_3B porphyrin **5**.

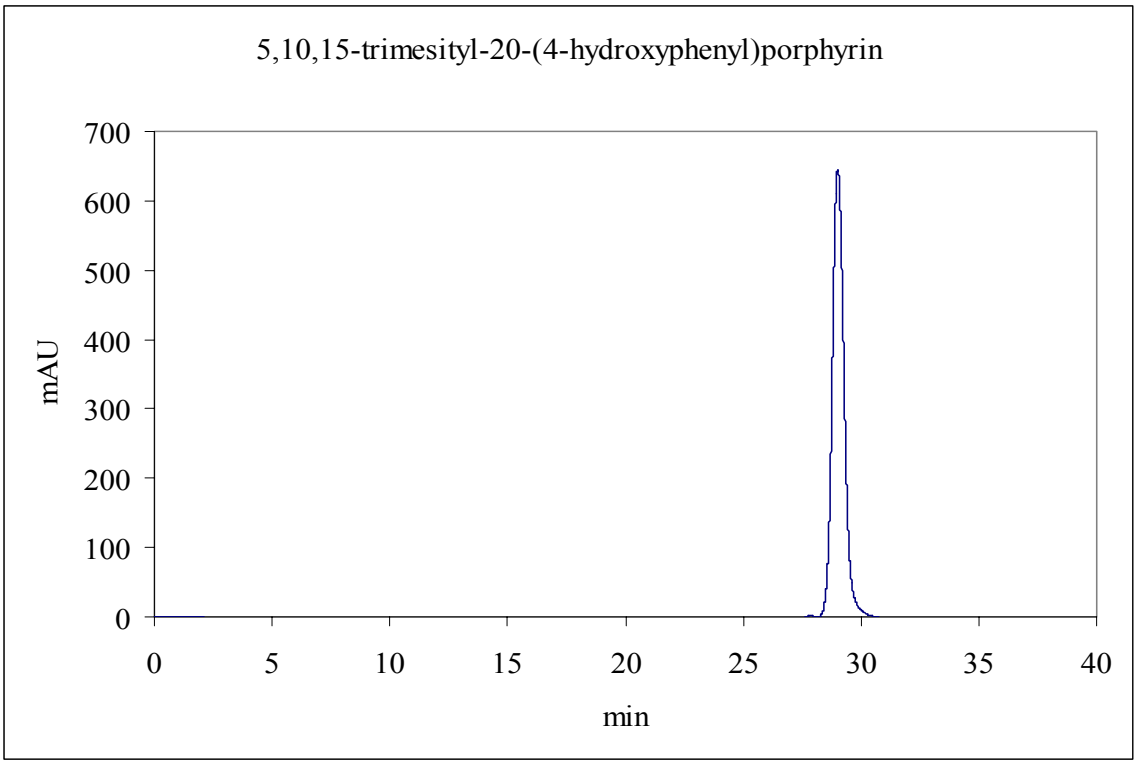
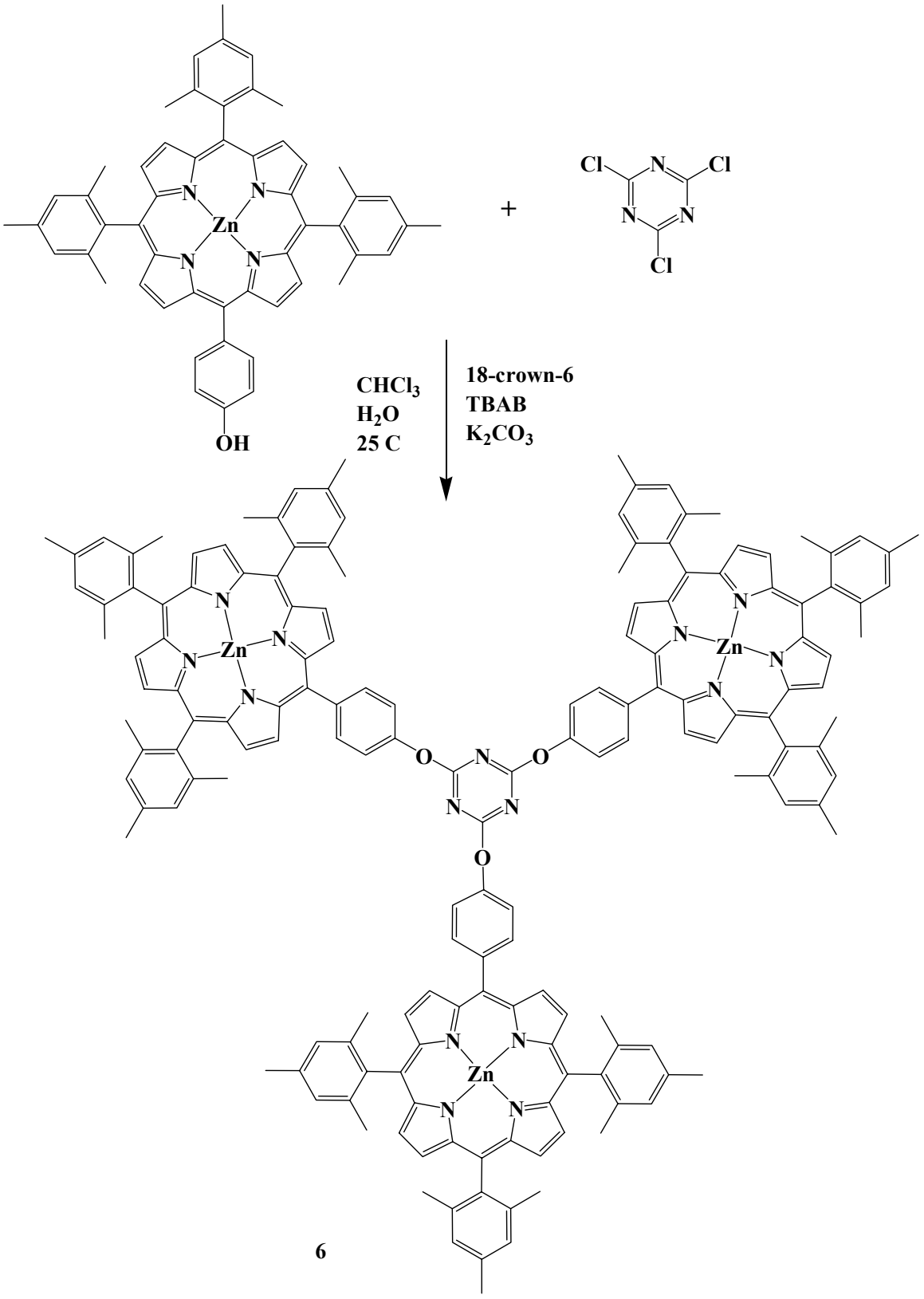


Figure 3.11 SEC of A_3B porphyrin **5**, retention time is 29.02 min.

3.4.2 Synthesis of 1st generation dendrimer

With purification and characterization of the phenolic A_3B porphyrin **5** complete, the 1st generation dendrimer **6** was assembled using mild phase transfer reaction conditions adapted from Salunkhe[24] (**scheme 3.7**). The porphyrin **5** was dissolved in minimal chloroform, and an aqueous solution of K_2CO_3 was added resulting in a two phase mixture. 18-crown-6 was added, with the purpose of complexing the potassium ion and making carbonate a stronger base. Then, a phase transfer catalyst (tetrabutylammonium bromide) was added to this biphasic reaction mixture, which increases the nucleophilicity of the porphyrin phenoxide ion by "stripping" away its solvent shell. At this stage, a solution of trichlorotriazine in chloroform was added to the

biphasic reaction, and it was stirred at room temperature. After purification, the dendrimer **6** was characterized by mass spectrometry (**figure 3.12)**, UV/VIS spectroscopy (**figure 3.13**), SEC (**figure 3.14**), and ^{1}H NMR (**appendix A**), and ^{13}C NMR (**appendix A**) spectroscopies.



Scheme 3.7 Synthesis of 1st generation dendrimer **6**.

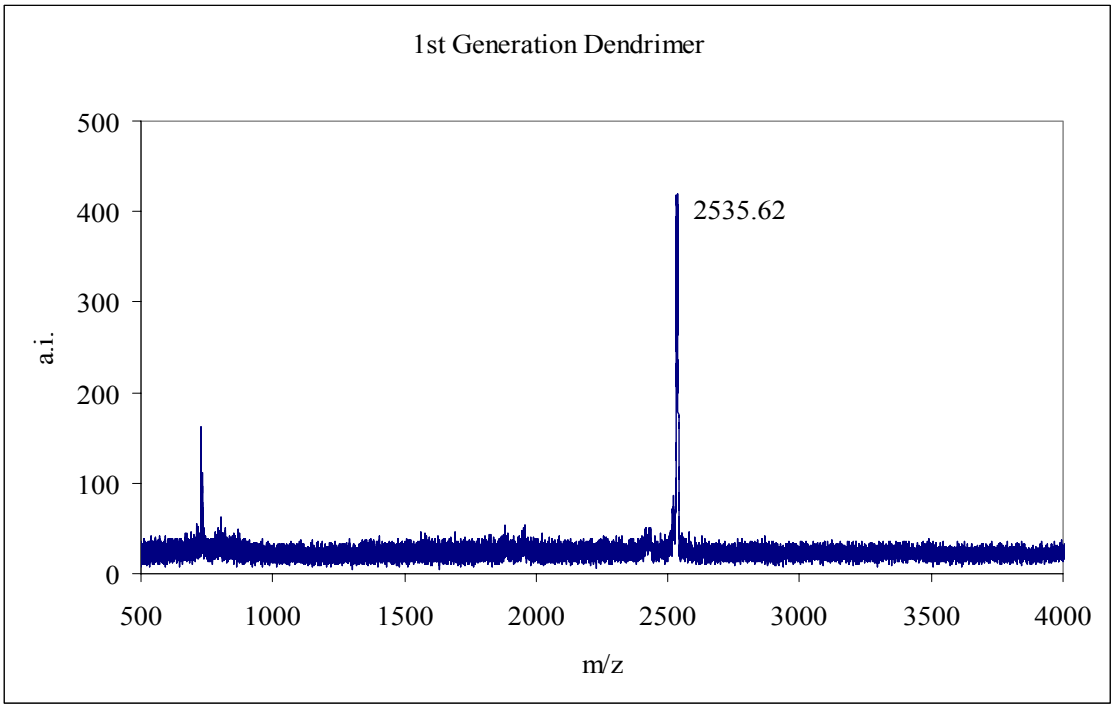


Figure 3.12 Laser desorption mass spectrum of 1st generation dendrimer **6** (theoretical mass is 2536.07).

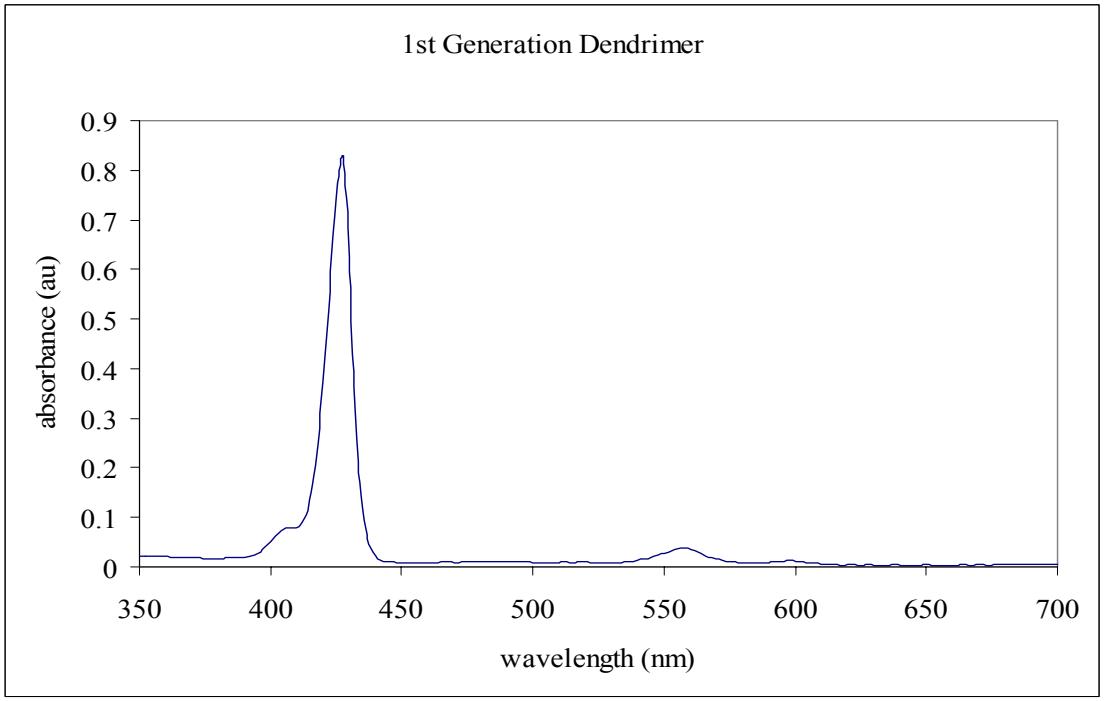


Figure 3.13 UV/VIS spectrum of 1st generation dendrimer **6**.

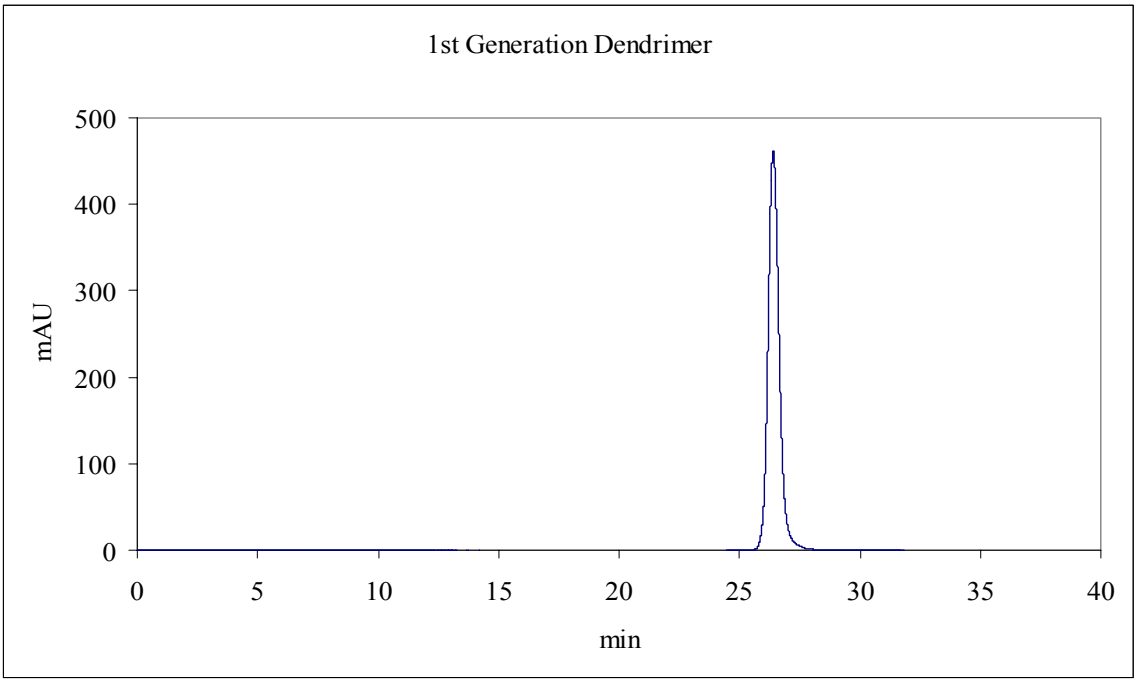


Figure 3.14 SEC of 1st generation dendrimer **6**, retention time is 26.40 min.

It was found that with these conditions, the reaction proceeded quickly, and in high yields. Monitoring the reaction by SEC proved to be very effective (**figure 3.15**).

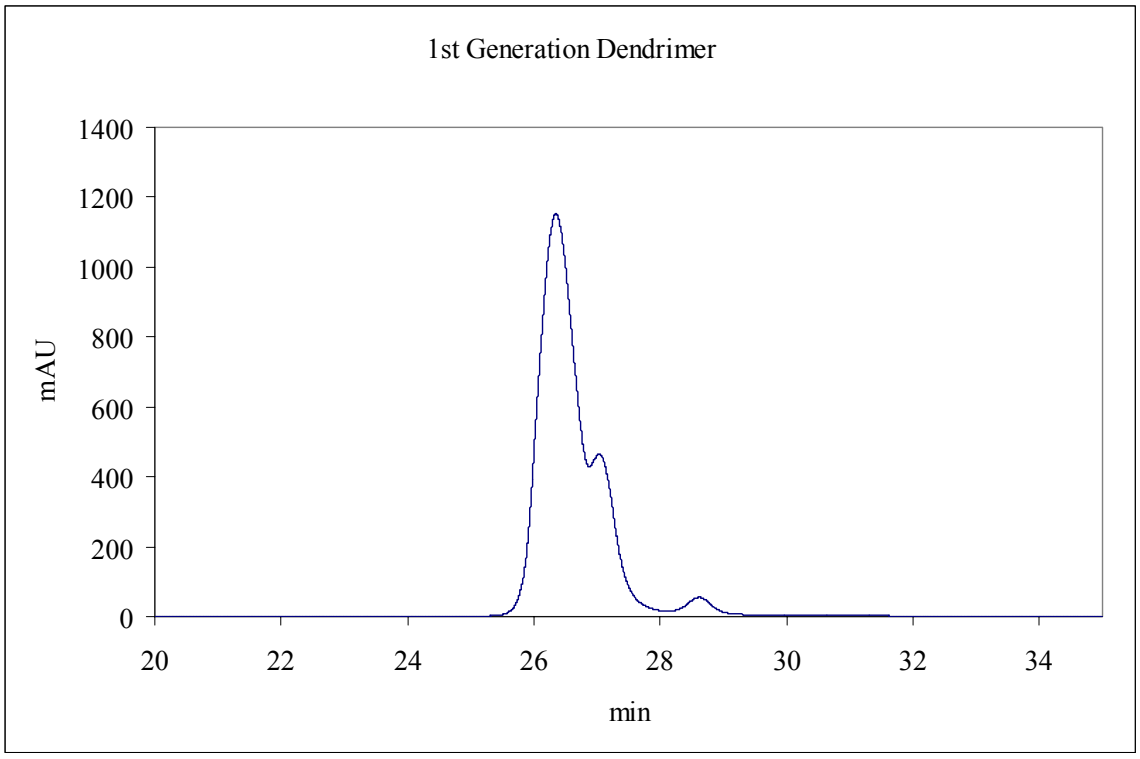


Figure 3.15 Reaction time at 1 hour shows almost complete disappearance of the porphyrin **5** peak, and a small dimer-cl **7** peak; the major peak is 1st generation dendrimer **6**.

A small amount of porphyrin **5** was added to the reaction to react with the remaining dimer-cl **7**. Upon disappearance of the A_3B porphyrin peak in SEC, the reaction was stopped, and the organic layer (containing dendrimer **6**) was purified by size exclusion and adsorption (silica gel) chromatographies (**figure 3.16**).

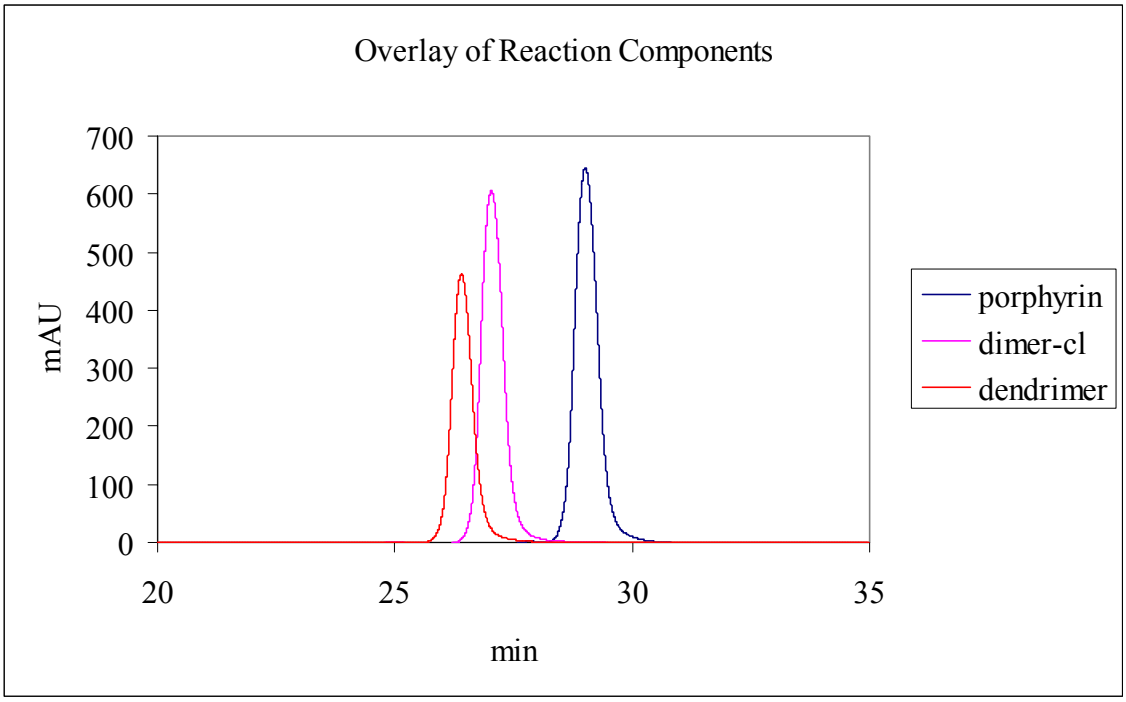


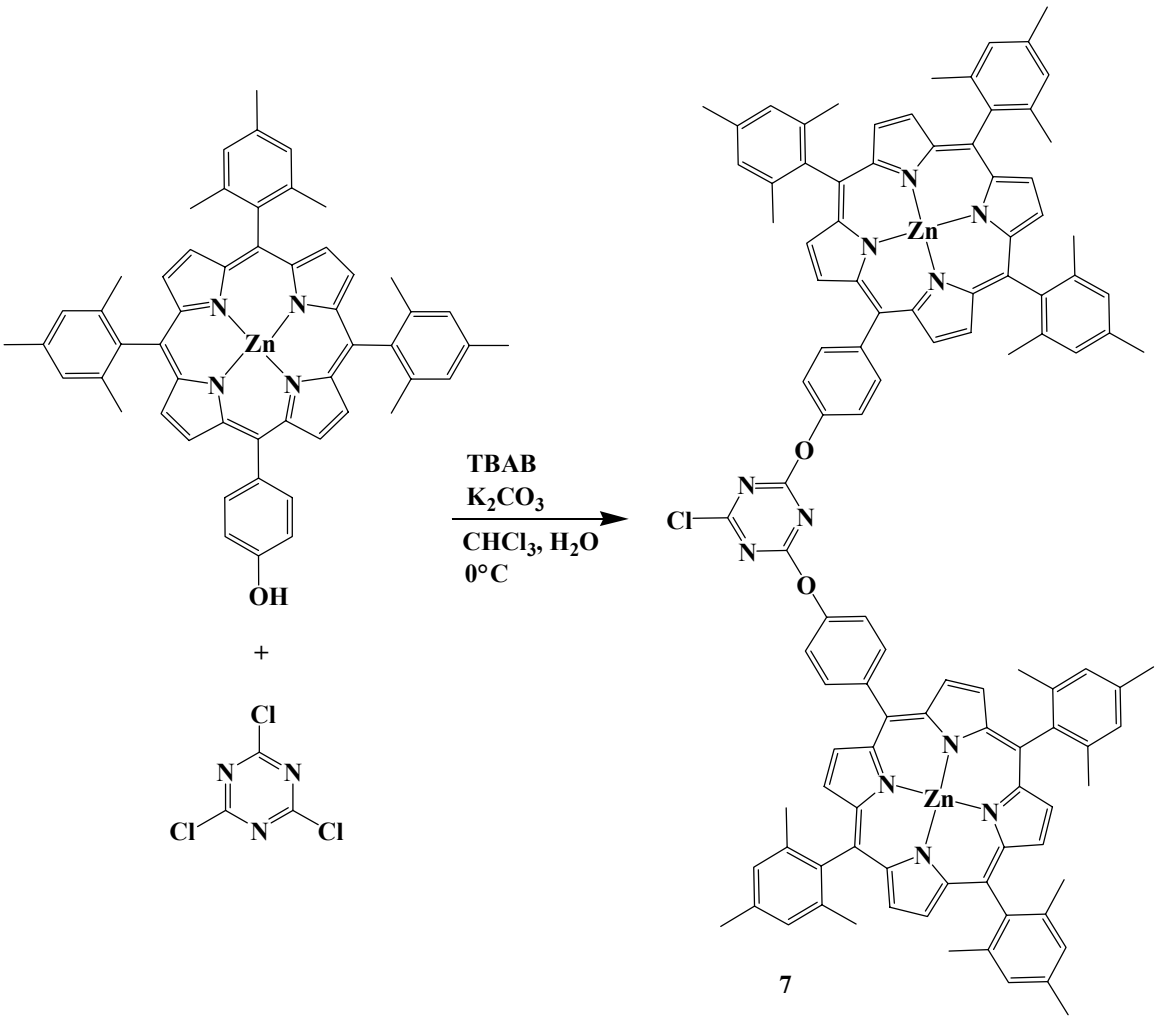
Figure 3.16 The starting A_3B porphyrin **5**, dimer-cl **7**, and 1st generation dendrimer **6** after purification.

The mild reaction conditions for this dendrimer formation are noteworthy because most other triazine-based reactions require high temperatures, long reaction times, and result in only moderate yields. These biphasic conditions could allow the use of more sensitive functionalized porphyrins.

3.4.3 Dimer-Cl Synthesis

In order to synthesize higher generation dendrimers, the coupling of porphyrin to triazine must be controlled, and the reaction stopped when disubstituted triazine is obtained. The dimer-Cl 7 was synthesized using reaction conditions adapted from the 1st generation dendrimer coupling. Two equivalents of the A_3B porphyrin 5 were selectively coupled to cyanuric chloride under mild and high yielding conditions. The

nucleophilicity of the phenol moiety was carefully controlled with temperature (0º C), and by tailoring the use of catalysts. By leaving out 18-crown-6, the di-substituted cyanuric chloride **7** was readily obtained with no tri-substituted product. The reaction mixture was easily purified by size exclusion chromatography (**scheme 3.9**). The dimer-Cl **7** was characterized by mass spectrometry (**figure 3.17**), UV/VIS spectrometry (**figure 3.18**), SEC (**figure 3.19**), and ^{1}H NMR (**appendix A**) and ^{13}C NMR (**appendix A**) spectroscopies.



Scheme 3.9 Synthesis of dimer-Cl **7**.

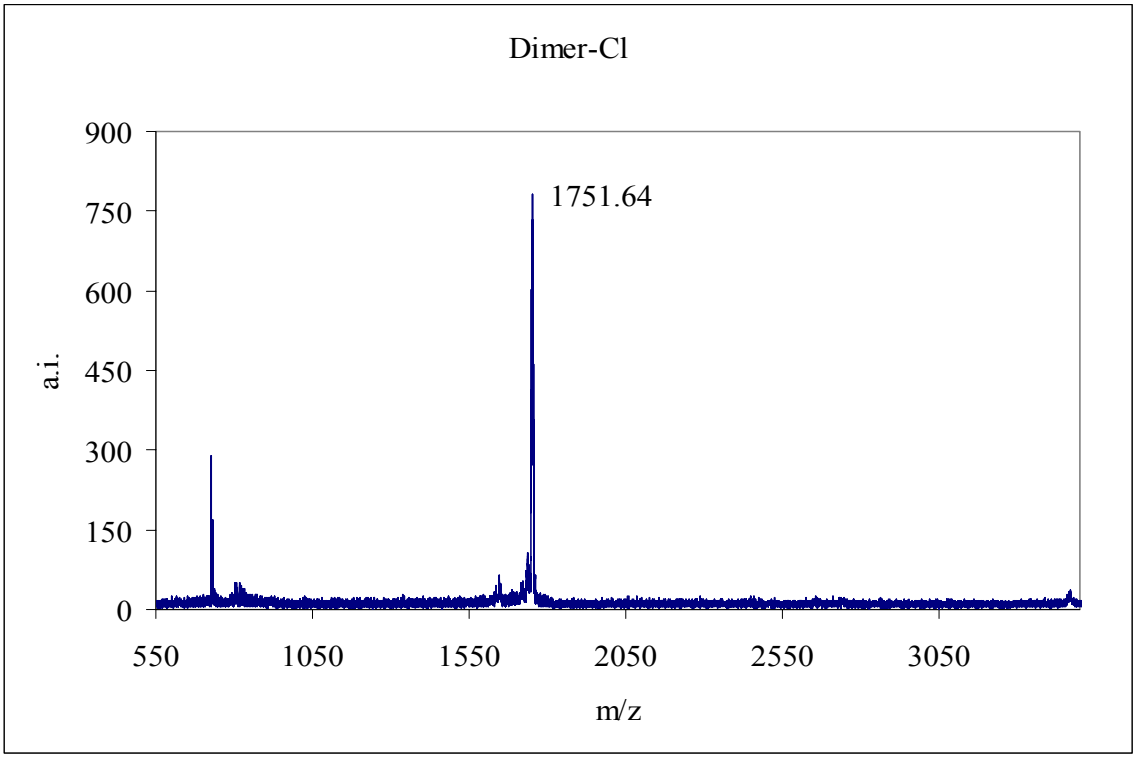


Figure 3.17 Laser desorption mass spectrum of dimer-Cl **7** (theoretical mass is 1752.19).

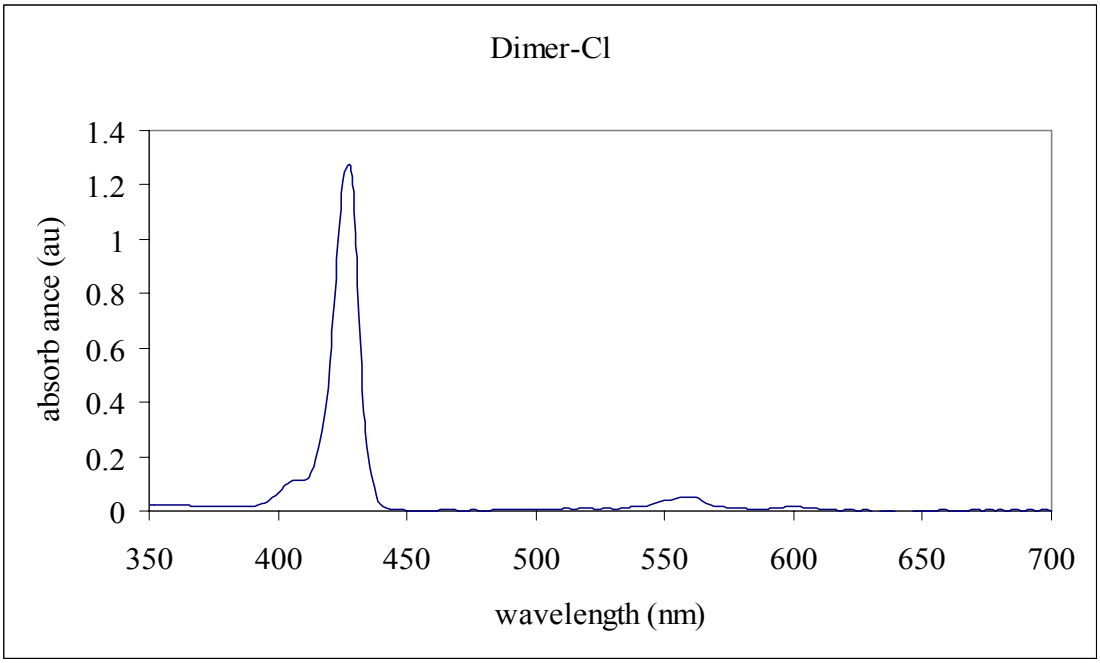


Figure 3.18 UV/VIS spectrum of dimer-Cl **7**.

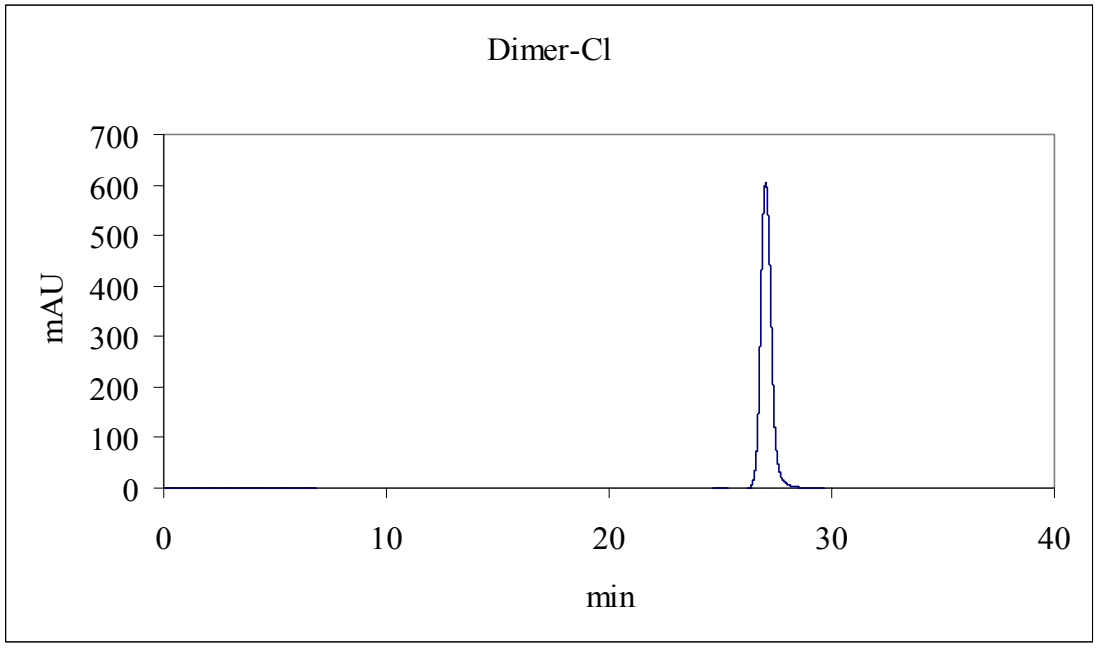


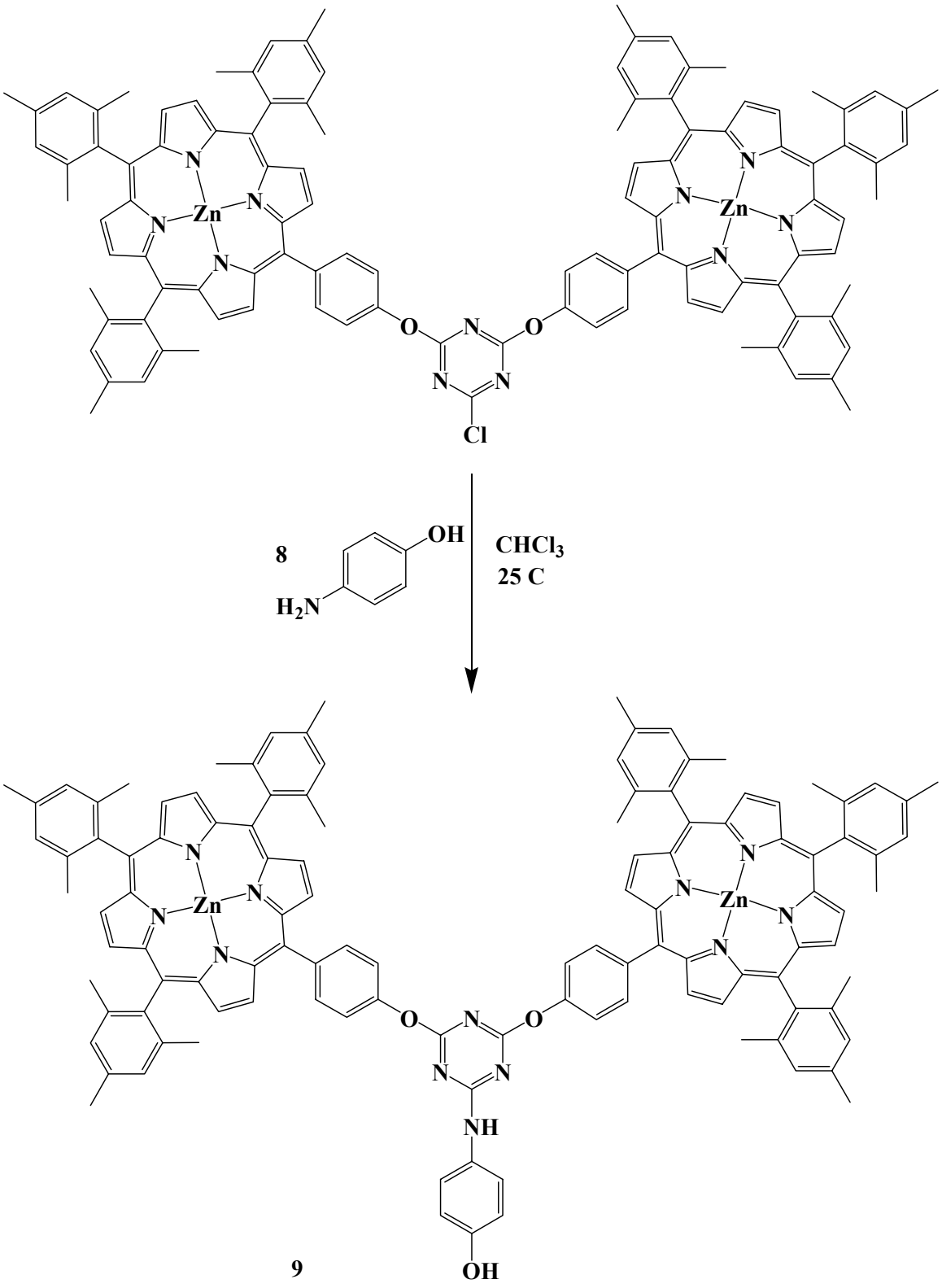
Figure 3.19 SEC of dimer-Cl **7**, retention time is 27.03 min.

When the reaction was performed at higher concentrations, the rate of reaction was much higher, however, the porphyrin solubility was exceeded and it precipitated out of solution. It was discovered that if the reaction was warmed to room temperature, the porphyrin would dissolve and react to form monosubstituted triazine, which was more soluble. The mixture could then be cooled back to 0ºC and the remaining porphyrin would remain in solution.

3.4.4 Dendron-OH Synthesis

In order to couple the dimer-Cl to triazine, the remaining chloride on triazine must be substituted with a "linker" which has additional functionality for further couplings. Typically, this is a functional group which requires deprotection, or transformation to a another reactive functional group. It was found that by using p-aminophenol **8**, the

difference in nucleophilicity between the phenol and the amine was large enough so that a selective coupling of the amine to triazine could be accomplished (**scheme 3.10**). The dimer-Cl **7** was reacted with an excess of p-aminophenol **8** in dry chloroform. This resulted in a phenol **9** as the available reactive moiety, which was already shown to couple in high yields to triazine using biphasic reaction conditions. The reaction was followed by TLC and when complete, the dendron **9** was purified by size exclusion chromatography, and characterized by mass spectrometry (**figure 3.20**), UV/VIS spectrometry (**figure 3.21**), SEC (**figure 3.22**), and ^{1}H NMR (**appendix A**), and ^{13}C NMR (**appendix A**) spectroscopies.



Scheme 3.10 Synthesis of dendron **9**.

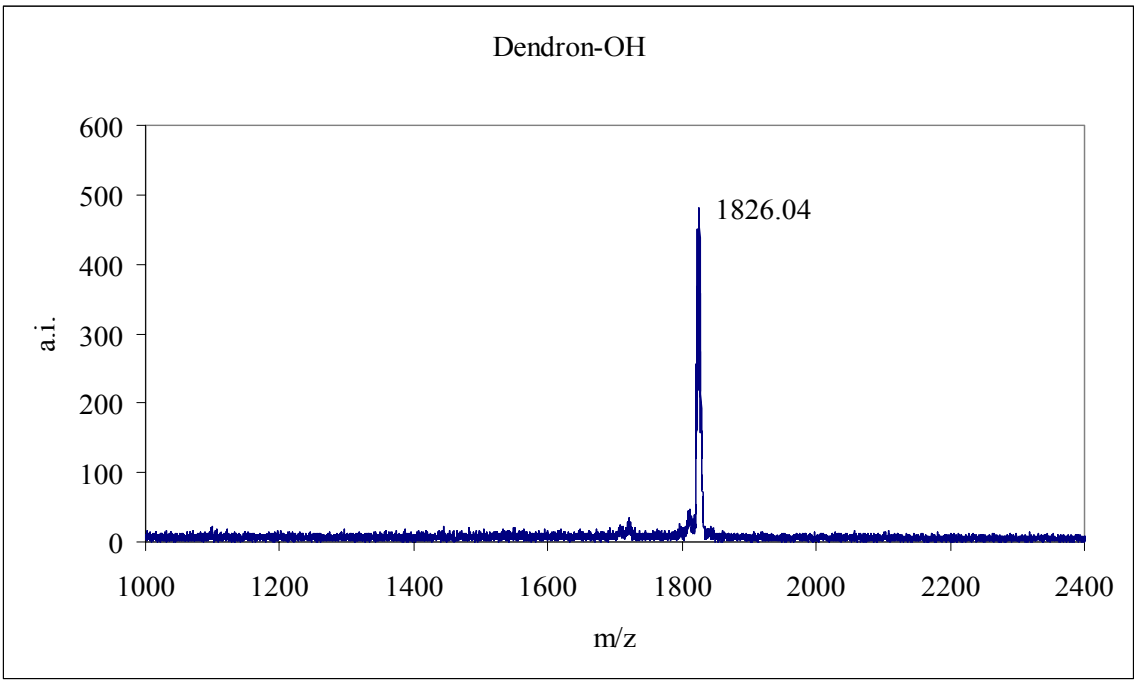


Figure 3.20 Laser desorption mass spectrum of dendron **9** (theoretical mass is 1824.85).

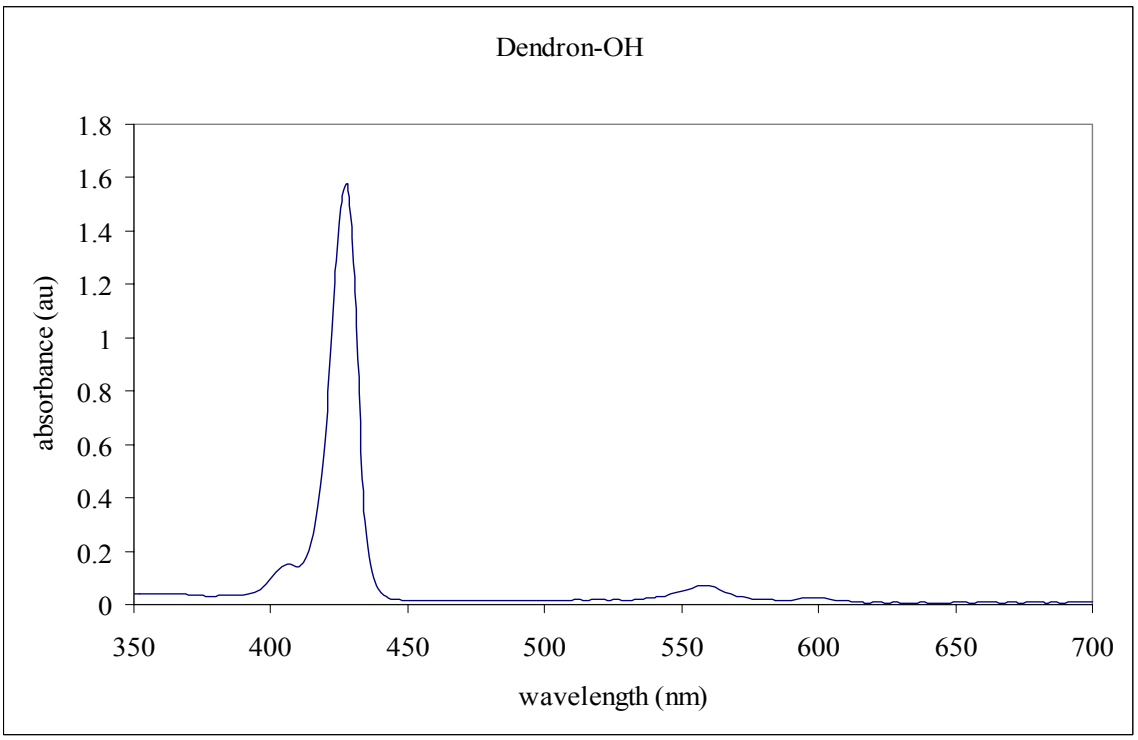


Figure 3.21 UV/VIS spectrum of dendron **9**.

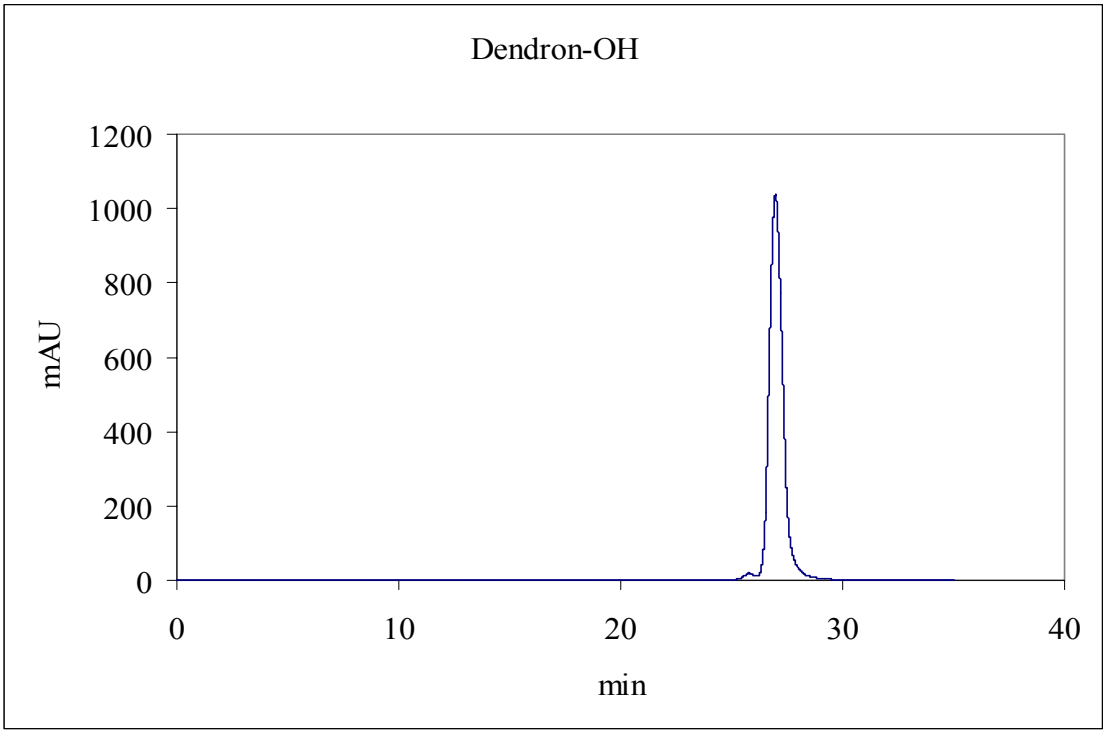


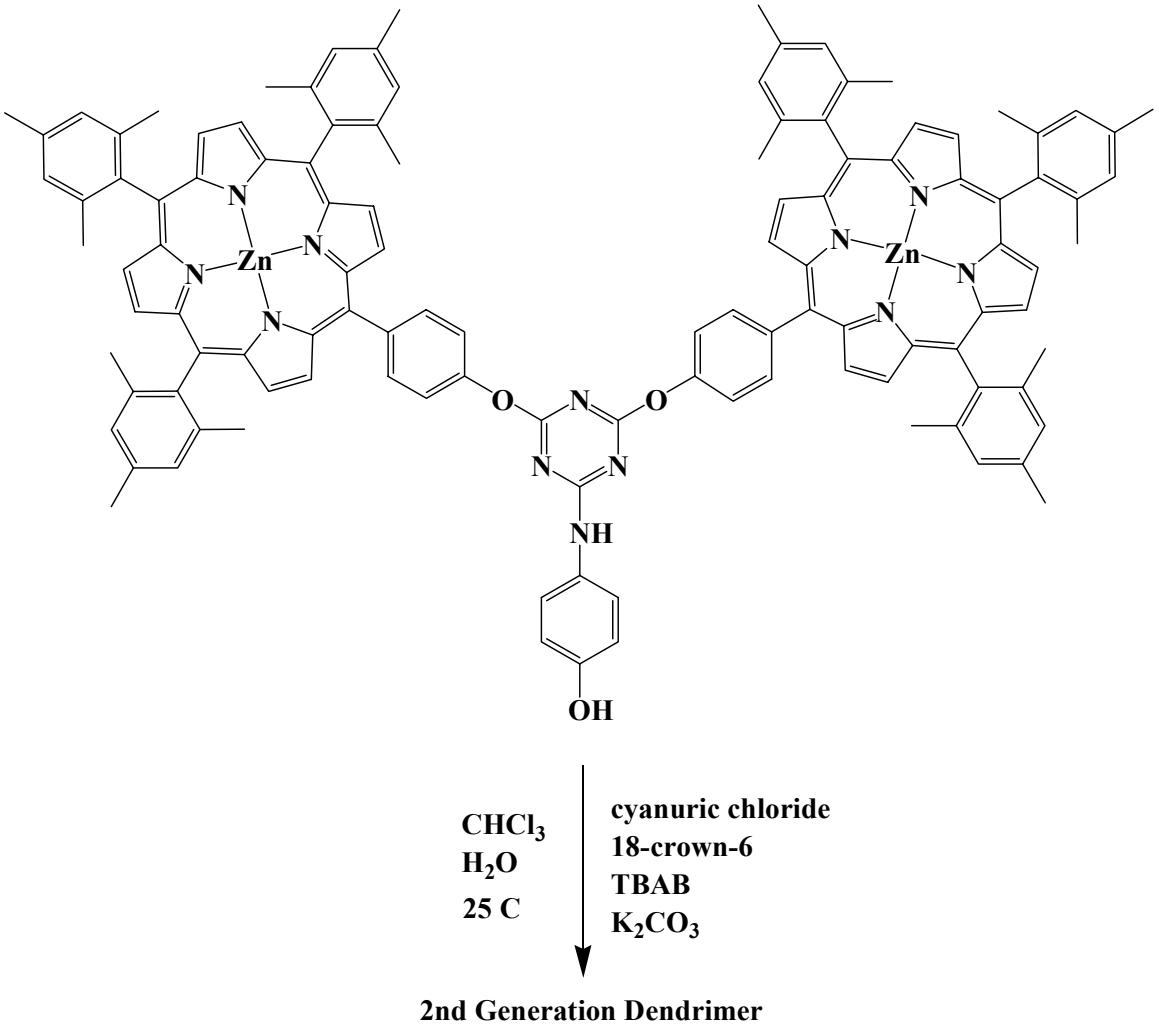
Figure 3.22 SEC of dendron **9**, retention time is 26.97 min.

It was discovered that this reaction could be safely heated to 45ºC with no undesirable by-products, and the reaction time was lowered from overnight, to only a few hours. The phenol peak, and the secondary amine peak appeared in the ^{1}H NMR, with correct integration. This further proved a selective coupling of the amine versus phenol to triazine.

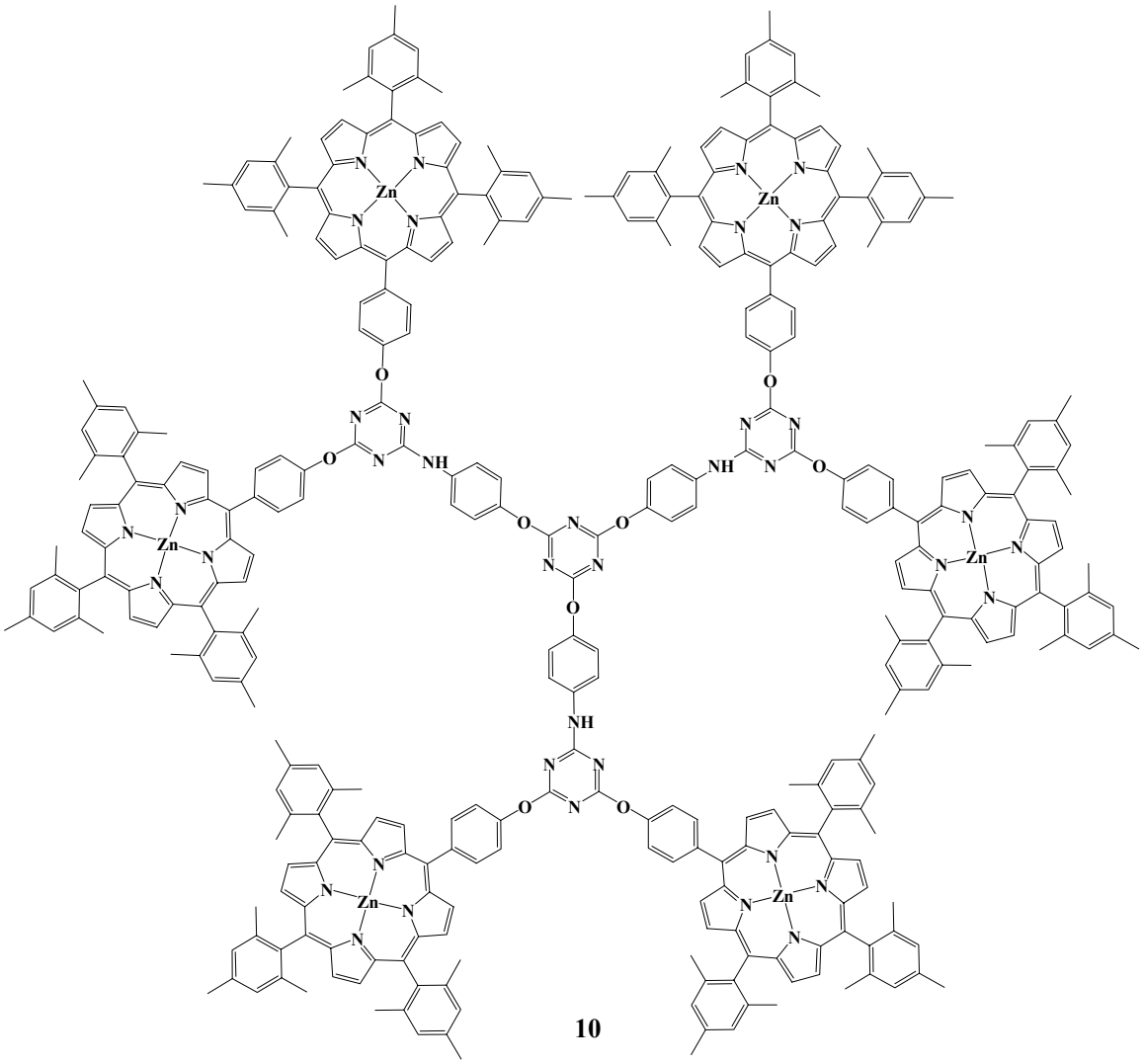
3.4.5 2nd generation dendrimer synthesis

With all of the prerequisite building blocks in hand, the next step was to assemble a 2nd generation dendrimer **10** using the same biphasic coupling conditions as in the 1st generation dendrimer formation (**scheme 3.11**). This reaction was very efficient for the 1st generation dendrimer synthesis, and the results were the same for the 2nd generation

synthesis. Three equivalents of dendron-OH **9** were reacted with one equivalent of triazine using biphasic conditions (K_2CO_3, TBAB, and 18-crown-6 at 25º C in $CHCl_3/H_2O$). The 2nd generation dendrimer **10** (**figure 3.23**) was characterized by mass spectrometry (**figure 3.24**), UV/VIS spectroscopy (**figure 3.25**), SEC (**figure 3.26**), and ^1H NMR (**appendix A**), and ^{13}C NMR (**appendix A**) spectroscopies.



Scheme 3.11 Synthesis of 2nd generation dendrimer **10**.



10

Figure 3.23 2nd generation dendrimer **10**.

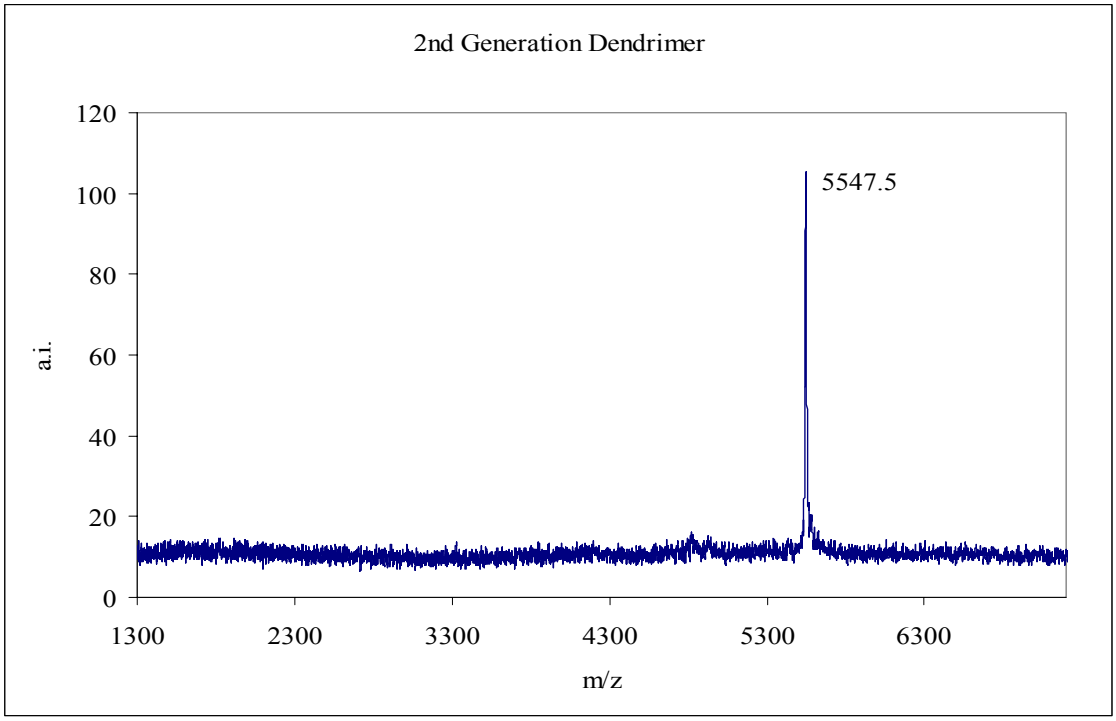


Figure 3.24 Laser desorption mass spectrum of 2nd generation dendrimer **10** (theoretical mass is 5549.58).

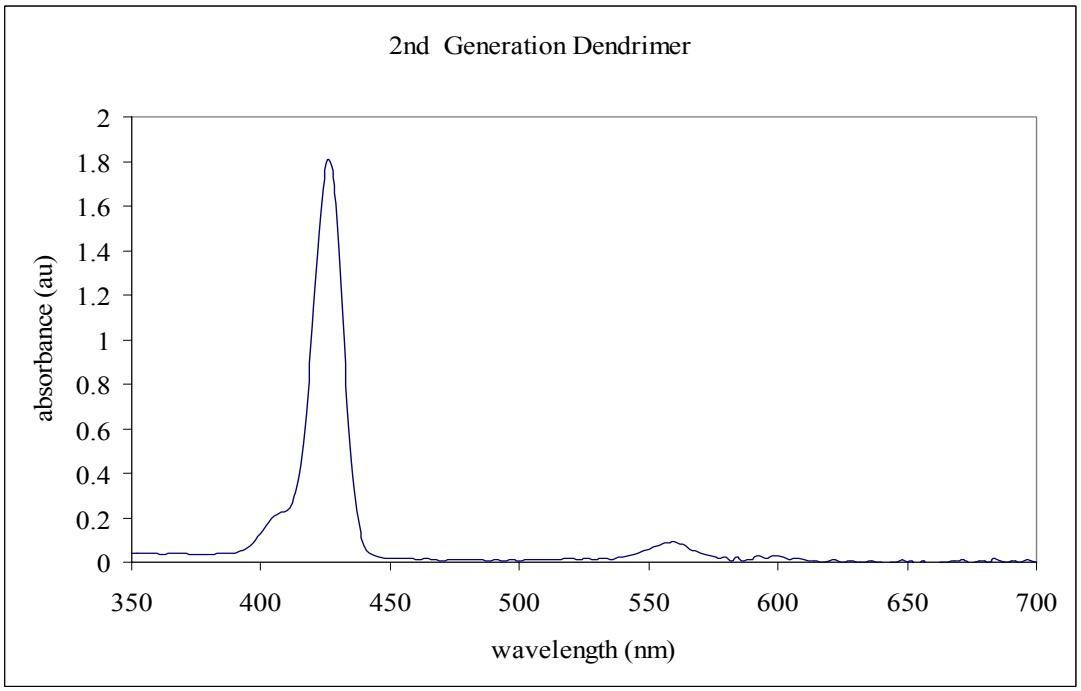


Figure 3.25 UV/VIS spectrum of 2nd generation dendrimer **10**.

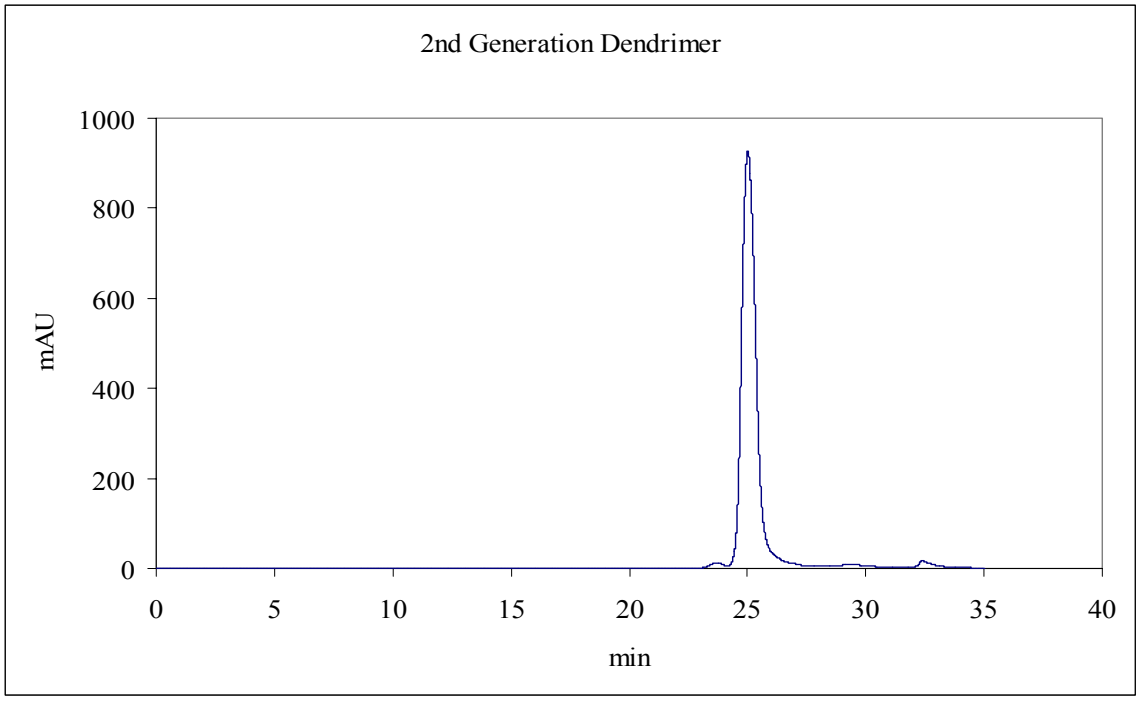


Figure 3.26 SEC of 2nd generation dendrimer **10**, retention time is 25.020 min.

It should be noted that the dendron:triazine ratio used in this reaction was 3:1. This afforded the 2nd generation dendrimer **10**, but some di-substituted triazine **11**(**figure 3.28**) remained in the mixture because there was not any dendron **9** left to further react with it (**figure 3.27, figure 3.29**).

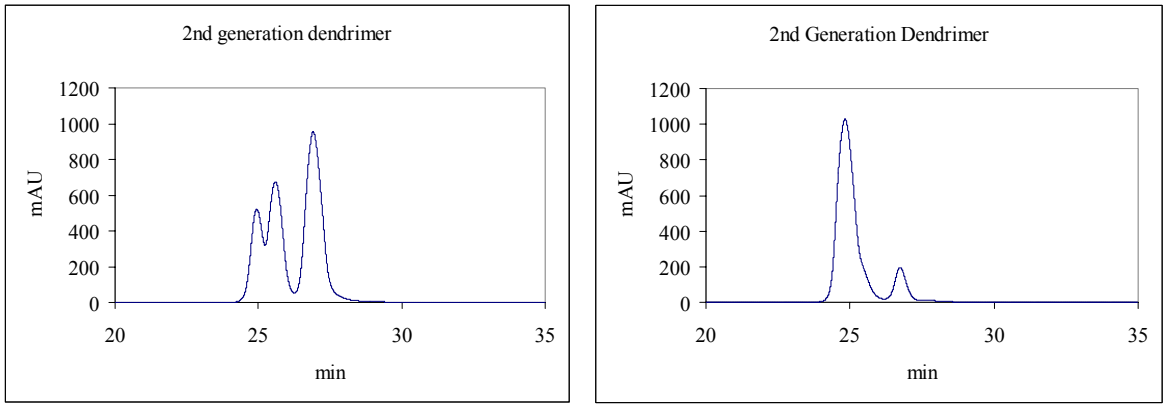


Figure 3.27 SEC of 2nd generation dendrimer **10** formation reaction at 1 h 50 min (left), and when the reaction was stopped (right).

This "deletion error", seen as a small shoulder on the dendrimer SEC peak, created some difficulty in purification, and the dendrimer **10** was obtained only after multiple purifications by chromatotron using silica as the stationary phase.

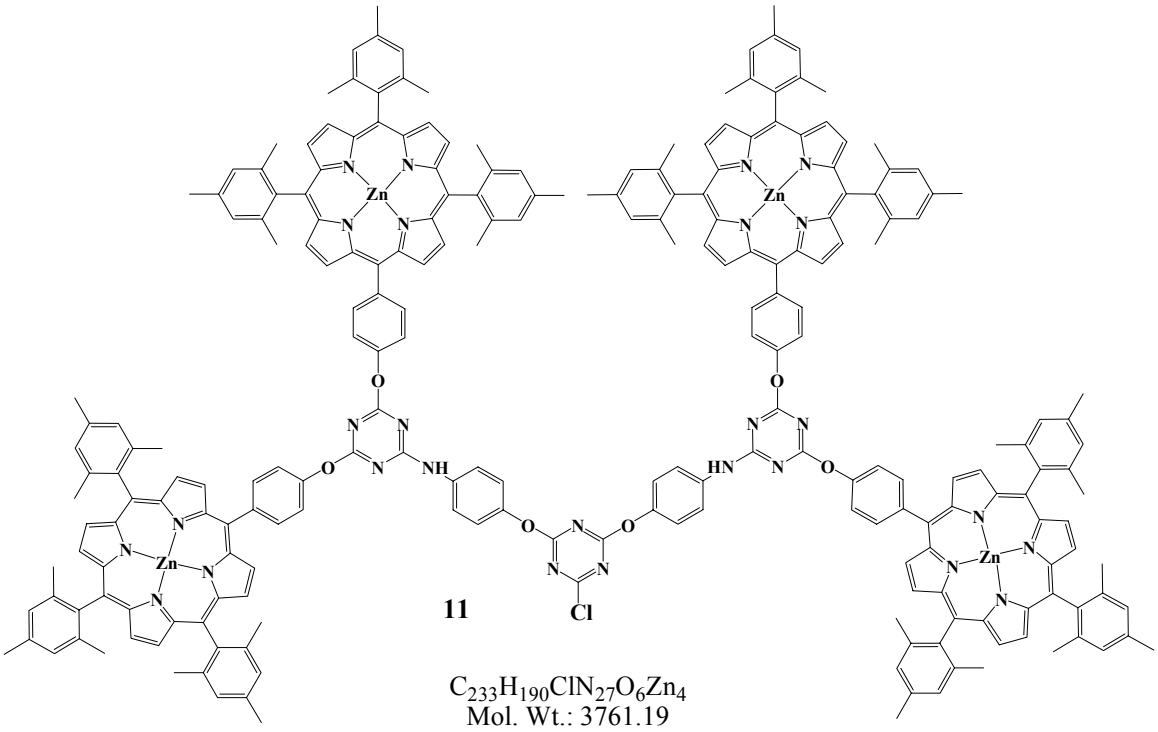


Figure 3.28 Impurity "deletion error" formed during 2nd generation dendrimer reaction.

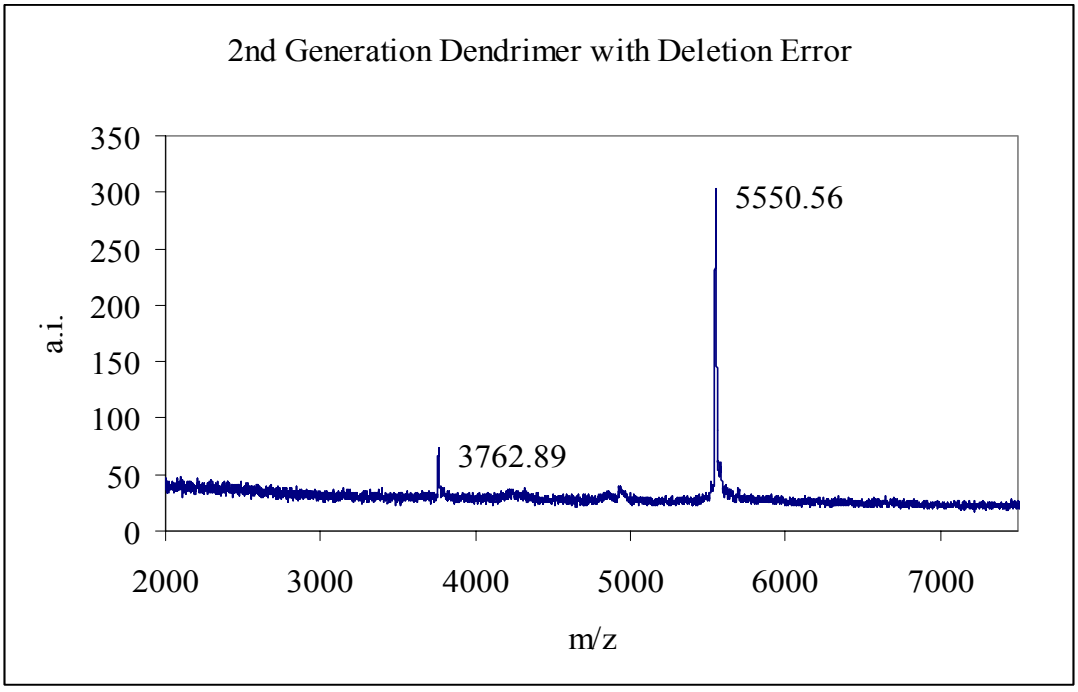


Figure 3.29 Laser desorption mass spectrum of 2nd generation dendrimer **10** containing a deletion error impurity **11**.

The difficulty of purification can be prevented in future repeats of this synthesis by using a slight excess of the dendron-OH **9**, which will "push" the formation of 2nd generation dendrimer **10**, and use up all of the di-substituted triazine **11**. The size difference between the dendron-OH **9** and the 2nd generation dendrimer **10** is great enough so that they can be separated on a size exclusion column.

The 1st and 2nd generation dendrimers, as well as the dendrons, were synthesized quickly, in high yields, and with a minimal number of synthetic steps. The synthesis of higher generation dendrimers using this same strategy is currently underway, and predicted to be successful.

3.5 Conclusions

For a dendrimer to be a viable therapeutic delivery system, it must have a mild, efficient synthesis which can afford sufficient quantities for practical use. This chapter presented the design and synthesis of a triazine based porphyrinic dendrimer which was synthesized in high yields, with short reaction times, and under mild conditions. The dendrimer was synthesized with the intent of being tested as a candidate for a photosensitizer in PDT, and as a contrast agent in MRI. Based on the EPR effect, the size of a dendrimer imparts an active targeting for tumor tissue versus healthy tissue, thereby minimizing any harmful side effects caused by the delivery of monomeric porphyrins to healthy tissues. The modular, convergent dendrimer synthesis facilitates future optimizations of size, biocompatibility, solubility, and efficiency of singlet oxygen production, by simply changing out a building block. Most triazine-based reactions

require higher temperatures, long reaction times, and result in lower yields. This triazine-based dendrimer assembly is significant in that the mild conditions would allow more sensitive functionality to be present.

3.6 Experimental

General Procedures

1H NMR spectra (400 MHz, Bruker AMX400 and 400MHz, Varian Mercury Plus 400), 13C NMR (400 MHz, Bruker AMX400 and 400MHz, Varian Mercury Plus 400) and absorption spectra (HP 8452A) were routinely collected. Column chromatography was performed using silica gel (Merck 70 – 230 mesh). CH_2Cl_2 and $CHCl_3$ were subjected to simple distillation from K_2CO_3. All other solvents were used as received. Pyrrole was distilled at atmospheric pressure from CaH_2. All reagents, unless otherwise noted, were received from Aldrich and used as provided. TLC was performed using silica gel plates.

Metallation of porphyrins

Porphyrins were metallated with Zn^{2+} by the following procedure. The free base porphyrin was dissolved into a minimal amount of dichloromethane:methanol (9:1, v/v) solution. The solution was degassed, by bubbling with argon, for 10 minutes, then 1.2 equivalents of $Zn(AcO)_2$ were added, and stirred overnight at RT. NMR spectroscopy and UV/Vis spectroscopy were used to monitor the completion of the metallation reactions. The reaction mixture was washed with 50 mL of $NaHCO_3$ and 50 mL of H_2O three times,

and then dried over $MgSO_4$. The solvent was removed to obtain Zn-metalloporphyrins in 95~98% yield (**figure 3.30**).

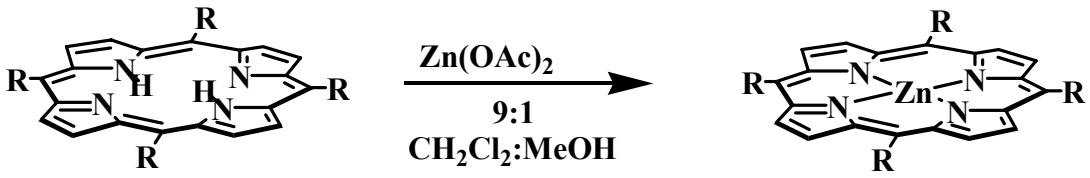


Figure 3.30 Insertion of Zn(II) into porphyrins.

General procedure for the synthesis of porphyrins

The porphyrins were prepared following standard procedures.[20,21] Porphyrin formation was measured by oxidizing aliquots from the reaction mixture with excess DDQ, followed by absorption spectroscopy. Porphyrin yield was estimated from the intensity of the Soret band (420 nm, ε = 500,000 $M^{-1}cm^{-1}$). After the spectroscopic yield of porphyrin reached a plateau, the reaction was quenched with DDQ. Purification usually involved adsorption and/or size exclusion chromatography.

Analytical size exclusion chromatography:

Analytical size exclusion columns (styrene-divinylbenzene copolymer) were purchased from Phenomonex. Analytical SEC was performed with a Hewlett-Packard 1100 HPLC using 500Å (300 x 7.8 mm), 5 10^4 Å (300 x 7.8 mm), and 5 10^5 Å (300 x 7.8 mm) columns in series eluting with THF (flow rate = 1.0 mL/min; void volume ~ 16 min). Molecular weight results were based on seven polystyrene standards (MW = 114 00, 18 700, 13 700, 3 700, 2 430 and 760). Reaction monitoring was performed by removing 25 μL aliquots and diluting to 1.0 mL with THF (Fisher, HPLC). Sample

detection was achieved by absorption spectroscopy using a diode array detector with quantitation at 450 nm (+/- 80 nm bandwidth).

Preparative size exclusion chromatography:

Samples were purified with S-X3 Bio-Beads from BIO-RAD. The beads were 200-400 mesh, and were expanded in toluene, and packed in toluene.

General procedure for mass spectrometry:

The samples were analyzed on a Bruker Reflex MALDI TOF mass spectrometer updated with delayed extraction, and using reflectron mode. The samples were used without any matrix (laser desorption).

4-(*tert*-butyldiphenylsiloxy)benzaldehyde (2). 4-aminophenol **1** (10 g, 81.9 mmol) was dissolved in dry DMF (80 mL), and *tert*-butyldiphenylsilyl chloride (22.51 g, 81.9 mmol) was added. Upon the addition of imidazole (12 g, 180 mmol), the reaction was stirred at RT, and reaction progress was followed by TLC. After 5 h, H_2O was added to the reaction mixture, and the product precipitated from solution. The solid precipitate was extracted into ether, and the ether layer was washed with 0.1N NaOH, water, brine, and then concentrated. The white solid was recrystallized in ethanol to afford a 95% yield of 4-(*tert*-butyldiphenylsiloxy)benzaldehyde **2**. ^{1}H NMR ($CDCl_3$) 9.817 s (1 H); 7.716 d (4 H) J=6.891; 7.666 d (2 H) J=8.860; 7.462 m (6H); 6.877 d (2 H) J=8.860; 1.118 s (9 H).

5,10,15-trimesityl-20-[(4-*tert*-butyldiphenylsiloxy)phenylporphyrin (4).

Mesitaldehyde **3** (5.18 g, 35 mmol) and TBDPS protected 4-hydroxybenzaldehyde **2** (3.97 g, 11 mmol) were dissolved in 4 L of dry chloroform. The solution was stirred at RT and degassed with argon for 15 min. Pyrrole (3.08 g, 46 mmol) was added to the mixture, and the reaction flask was shielded from ambient light. Upon the addition of 1.65 mL $BF_3(OEt)_2$ the reaction was stirred at RT. The reaction was monitored by UV/VIS spectroscopy (36 % spectroscopic yield), and the porphyrinogen was oxidized after 1 h by the addition of DDQ (7.8 g, 35 mmol). The reaction mixture was partially purified by silica chromatography (with 70:30 (chloroform : heptane) as the eluent), and a mixture of porphyrins was obtained.

Zn(II)-5,10,15-trimesityl-20-[(4-hydroxy)phenyl]porphyrin (5). Without any further purification, the porphyrin mixture obtained from the first column was concentrated, and then redissolved in dry chloroform. The porphyrins were metallated following standard procedures, the solution was evaporated, and then the solid porphyrin material was redissolved in dry chloroform. Excess tetrabutylammonium fluoride (TBAF) was added, and the mixture was stirred overnight at RT. The TBDPS deprotected porphyrin mixture was purified by adsorption chromatography (silica gel) with chloroform as the eluent. To remove additional impurities, the porphyrin was further purified by size exclusion chromatography with S-X3 biobeads (in toluene), resulting in an overall isolated 8% yield. ^{1}H NMR ($CDCl_3$) 18.942 d (2 H) J=4.695; 8.825 d (2 H) J=4.695; 8.774 s (4 H); 8.103 d (2 H) J=8.608; 7.323 s (6 H); 7.118 d (2 H) J=7.825; 2.666 s (9 H); 1.916 d (18 H). ^{13}C NMR ($CDCl_3$) 155.204; 150.495; 150.144; 150.083; 150.007; 139.583; 139.385;

139.278; 137.647; 135.727; 135.681; 132.417; 131.367; 130.799; 127.908; 120.044; 119.040; 118.705; 113.691; 22.085; 21.994; 21.778.

1st generation dendrimer (6). The A_3B porphyrin **5** (50 mg, 0.061 mmol) was dissolved in $CHCl_3$ (1 mL), followed by addition of 18-crown-6 (6.1 μL, 0.0061 mmol of a 0.01M solution in chloroform), and TBAB (61 μL, 0.0061 mmol of a 0.1M solution in chloroform). An aqueous solution of K_2CO_3 (12.6 mg, 0.09 mmol in 1 mL H_2O) was added, resulting in a biphasic reaction medium. While stirring at 25°C, a solution of triazine (203 μL, 0.02 mmol of a 0.1M solution in chloroform) was added dropwise. The reaction progress was monitored by SEC and stopped upon the disappearance of the porphyrin peak. Purification was done via size exclusion (S-X3 biobeads in toluene) and adsorption chromatographies (silica gel with chloroform as the eluent), resulting in a 91% yield. ^{1}H NMR ($CDCl_3$) 9.058 d (6 H) J=4.695; 8.801 d (6 H) J=3.913; 8.748 q (12 H) J=4.695, 6.260; 8.446 d (6 H) J=8.608; 7.857 d (6 H) J=7.825; 7.305 s (6 H); 7.242 s (12 H); 2.655 s (9 H); 2.638 s (18 H); 1.885 s (18 H); 1.775 s (36 H). ^{13}C NMR ($CDCl_3$) 174.309; 151.496; 149.845; 149.728; 141.038; 139.243; 139.184; 138.922; 137.336; 135.403; 132.110; 131.134; 131.060; 130.708; 127.616; 127.554; 119.690; 118.853; 118.744; 118.671; 77.186; 29.701; 21.745; 21.610; 21.456; -0.000.

Dimer-Cl (7). The A_3B porphyrin **5** (500 mg, 0.61 mmol) was dissolved in $CHCl_3$ (6 mL) followed by addition of TBAB (609 μL, 0.061 mmol of a 0.1M solution in chloroform). An aqueous solution of K_2CO_3 (126 mg, 0.91 mmol in 6 mL H_2O) was added, resulting in a biphasic reaction medium. This mixture was stirred at 0ºC, then

added dropwise to a solution of triazine (56.23 mg, 0.3 mmol) in $CHCl_3$ (1 mL). The reaction was stirred at 0ºC; reaction progress was monitored by TLC and SEC. The reaction was stopped after 5 h, and the mixture was diluted with more chloroform, then extracted with water. After removal of the solvent, purification was accomplished via size exclusion (S-X3 bio beads in toluene) and adsorption chromatographies (silica gel with chloroform as the eluent), resulting in a 92% yield. ^{1}H NMR ($CDCl_3$) 9.054 d (4 H) J=4.695; 8.882 d (4 H) J=4.695; 8.794 s (8 H); 8.440 d (4 H) J=8.608; 7.761 d (4 H) J=8.608; 7.337 s (4 H); 7.319 s (8 H); 2.679 s (18 H); 1.930 s (12 H); 1.884 s (24 H). ^{13}C NMR ($CDCl_3$) 174.325; 173.201; 151.422; 150.251; 150.218; 150.082; 141.701; 139.551; 139.522; 139.248; 139.201; 137.702; 135.691; 132.283; 131.521; 131.444; 131.110; 127.924; 119.724; 119.268; 119.095; 118.812; 77.479; 30.008; 22.053; 21.941; 21.765.

Dendron-OH (9). The dimer-Cl **7** (300 mg, 0.17 mmol) was dissolved in $CHCl_3$ (3 mL). While stirring at 25ºC, p-aminophenol **8** (94 mg, 0.86 mmol) was added. The reaction ran overnight and was followed by TLC. After the compound was diluted in chloroform and extracted with water, purification was achieved via size exclusion (S-X3 biobeads in toluene) and adsorption chromatographies (silica gel with chloroform as the eluent), resulting in a 94% yield. ^{1}NMR ($CDCl_3$) 9.028 d (2 H) J=3.919; 8.971 d (2 H) J=3.919; 8.829 d (4 H) J=4.695; 8.732 s (8 H); 8.340 d (4 H) J=7.825; 7.698 d (4 H) J=7.825; 7.482 d (2 H) J=5.478; 7.446 s (1 H); 7.288 s (12 H); 6.787 d (2 H) J=8.608; 4.747 s (1 H); 2.639 s (18 H); 1.871 s (12 H); 1.859 s (24 H). ^{13}C NMR ($CDCl_3$) 166.385; 152.396; 151.843; 151.615; 149.911; 149.754; 140.552; 139.244; 138.969;

138.888; 137.400; 135.295; 135.129; 132.125; 131.149; 130.705; 130.465; 127.613; 122.251; 120.134; 119.856; 118.911; 118.701; 118.666; 115.755; 77.309; 77.200; 76.990; 76.669; 76.334; 21.762; 21.685; 21.471; -0.002.

2nd generation dendrimer (10). The dendron **9** (105 mg, 0.058 mmol) was dissolved in $CHCl_3$ (2 mL) followed by addition of 18-crown-6 (5.8 μL, 0.00058 mmol of a 0.1M solution in chloroform), and TBAB (58 μL, 0.0058 mmol of a 0.1M solution in chloroform). An aqueous solution of K_2CO_3 (12 mg, 0.087 mmol in 2 mL H_2O) was added, resulting in a biphasic reaction medium. While stirring at 25ºC, a solution of triazine (192 μL, 0.02 mmol of a 0.1M solution in chloroform) was added dropwise. The reaction was followed by SEC and TLC; upon completion the mixture was diluted in chloroform, extracted with H_2O, and concentrated. Purification was accomplished via size exclusion (S-X3 biobeads in toluene) and adsorption chromatographies (silica gel with chloroform as the eluent, and chromatotron), resulting in a 35% yield. ^{1}NMR (CD_2Cl_2) 8.988 bd (12H); 8.700 m (36 H); 8.331 d (12 H) J=8.608; 7.689 d (12 H) J=8.608; 7.167 m (48 H); 6.555 d (6 H) J=8.606; 2.611 s (18 H); 2.509 s (36 H); 1.827 s (36 H); 1.736 s (72 H) ^{13}C NMR (CD_2Cl_2) 166.829; 150.112; 150.067; 149.987; 149.924; 140.939; 139.536; 139.337; 139.260; 139.125; 137.707; 137.627; 135.571; 135.371; 132.221; 131.273; 131.155; 130.786; 127.784; 127.726; 121.748; 121.355; 120.255; 119.113; 119.039; 54.208; 53.936; 53.659; 53.396; 53.127; 21.644; 21.550; 21.302.

3.7 References

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CHAPTER 4

FUTURE WORK AND CONCLUSIONS

4.1 Abstract

Polyethylene glycols (PEG)s are known to inhibit the immunogenic effect of the body on many drugs.[1-6] If the outer periphery of a drug is composed of PEG, the rest of the drug remains "invisible" to the body, and the immune system will not attack the drug. Future work on the triazine dendrimer project involves incorporating PEG onto the surface of the dendrimer, which would also provide a higher molecular weight and size, thereby increasing selectivity for tumor tissue based on the EPR effect. Additionaly, PEGs provide water solubility, which is a requirement for the administration of a systemic drug. For the above reasons, future dendrimers will contain a PEG periphery.

Other work will include synthesizing higher generation dendrimers, and studying the effects of inserting different metals into the porphyrins. The higher generation dendrimers should have an increased targeting for tumor tissue due to the larger size. By incorporating different metals into the dendrimers, singlet oxygen sensitization and MRI properties can be optimized.

4.2 "PEGylated" Dendrimers

4.2.1 Water Solubility

It is important that our drug candidates be easy to administer systemically; water solubility will be achieved by covalently attaching a layer of PEGs to the periphery of the dendrimer. Despite the two-dimensional representation of the dendrimer in **figure 4.1**, modeling suggests that a dendritic structure is globular, with nonionic PEGs forming the outer sphere of the dendrimer. It is important to note that ionic chelates are hyperosmolar and some of their side effects may be attributed to this property.

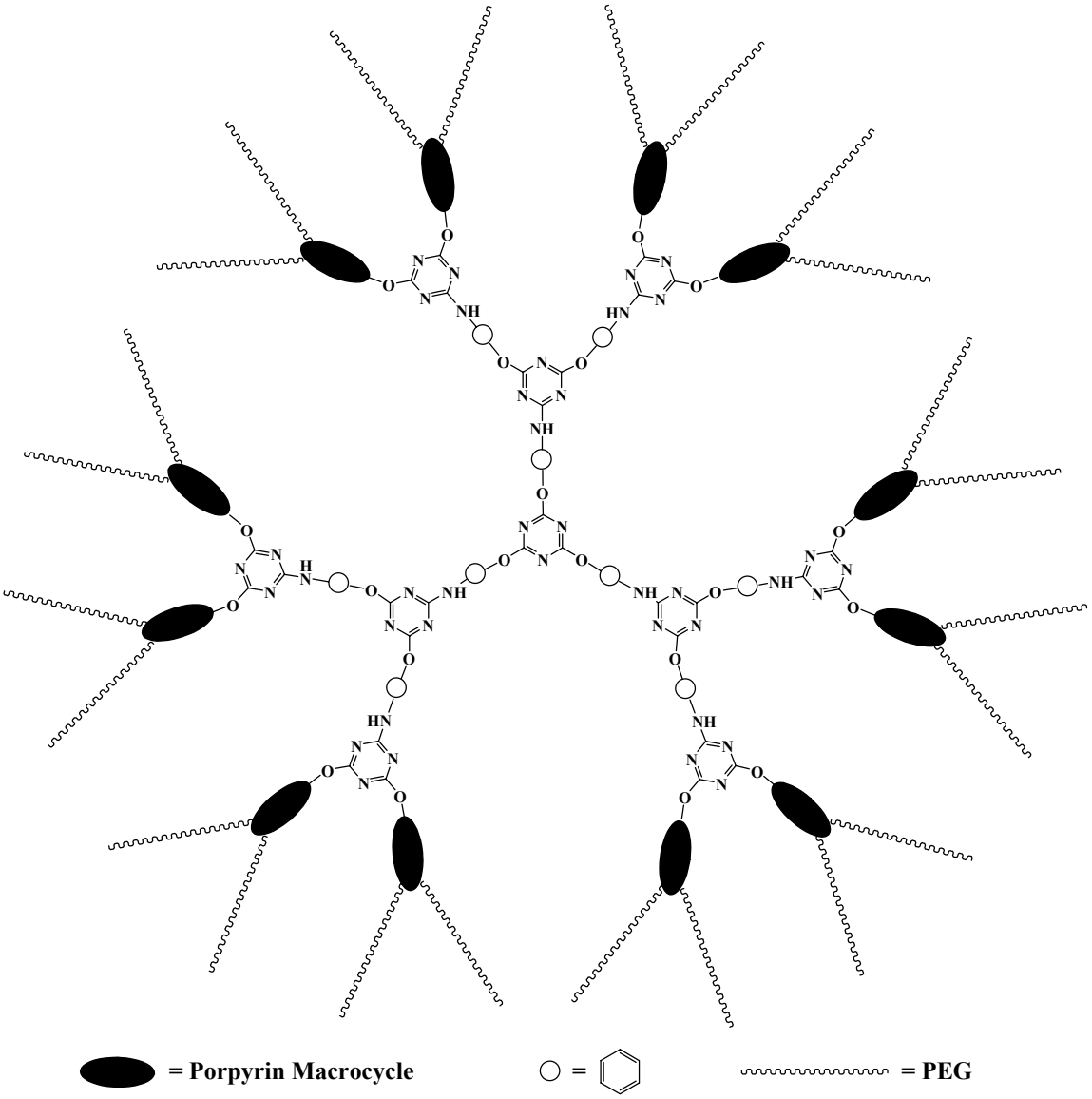

Figure 4.1 Triazine-based porphyrinic dendrimer with a PEG periphery for biocompatibility, larger size, water solubility, and anti-immunogenic properties.

4.2.2 Biocompatibility

In addition to providing increased solubility, the poly(ethylene glycol)s will serve as a biologically-passive coating which, theoretically, enables the dendrimer to achieve prolonged circulation times[6]. This biocompatibility should allow for repetitive monitoring of suspicious lesions or repetitive treatments for large tumors. Also, because

the metalloporphyrins will remain attached to the dendritic structure, the porphyrin should never invade normal tissue and toxic side effects should be minimal.

The hypothesis that PEG provides enhanced biocompatibility is based upon the development of new generation of "stealth liposomes" as a promising antitumor drug delivery system[7,8]. The concept of liposomes as a drug delivery system has existed for decades, but it is only in the last ten years that such a system has achieved the potential for wide ranging applications. The new stealth liposomes have the ability to remain in the vascular circulation for extended periods of time. The prolonged plasma half-life derives from highly water soluble PEGs grafted onto lipids that are assembled in the lipid bilayer of the liposomes. It has been shown that the polymers exert long-range mutual repulsion between adjacent bilayers and presumably prevent the interaction of macromolecules and cellular surfaces in the blood with the liposomes. The hydrophilic nature of PEG causes it to bind water tightly, creating a high surface density. This dense surface inhibits protein absorption and consequent uptake by macrophages resulting in increased circulation time in the body[9]. These polymer-related properties allow stealth liposomes to evade the reticuloendothelial system and thus have longer circulation times. Molecules that have been successfully incorporated into PEGylated liposomes, such as doxorubicin, have shown marked increase in circulation times and improvement in antitumor activity[10-13].

To append PEG to the porphyrinic dendrimer, only minor modifications of the synthetic design are needed. Aldehydes with protected functionality will be used to synthesize the A_3B porphyrin exterior (**figure 4.2**).

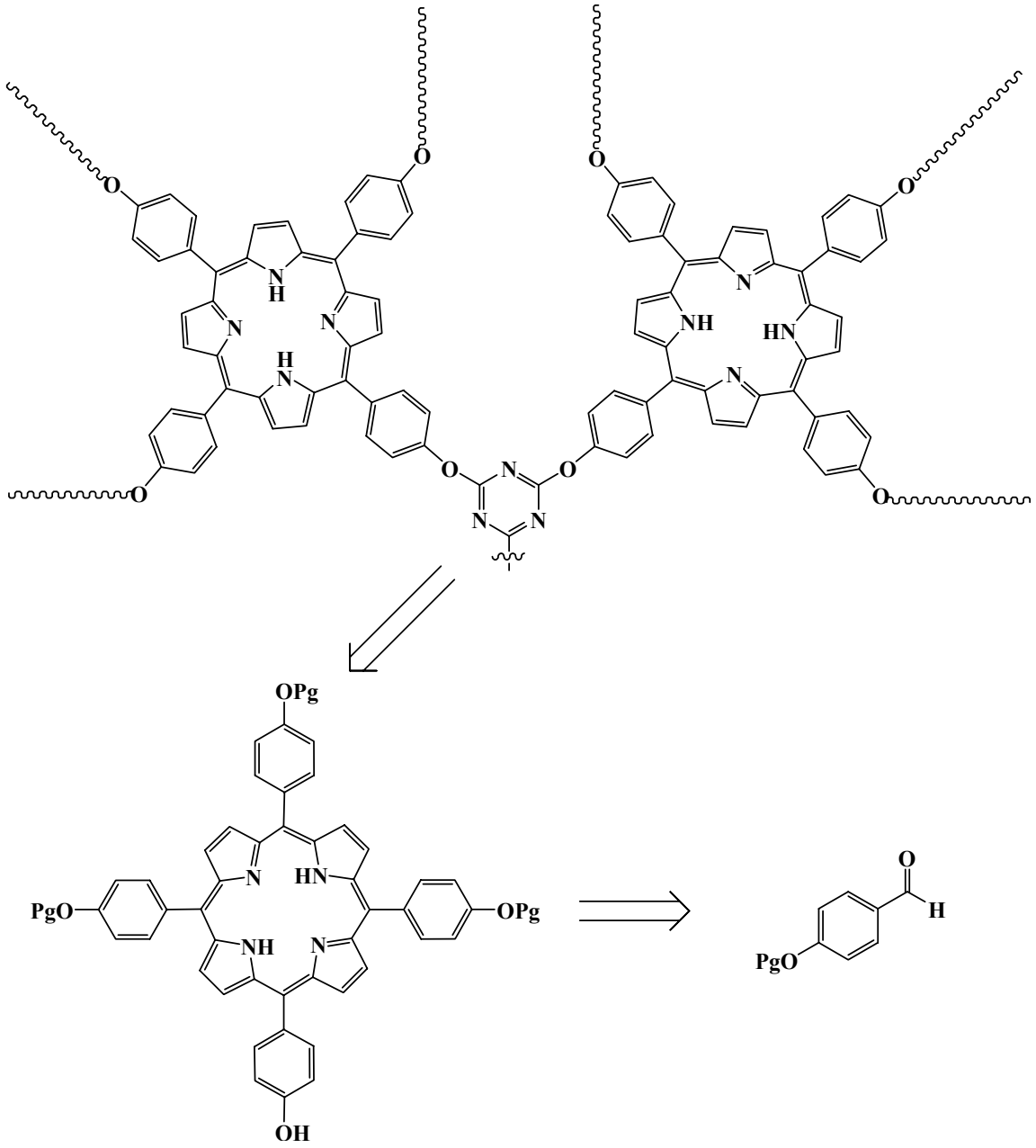

Figure 4.2 Aldehydes containing protected hydroxyl groups which can be used to attach PEG.

The dendrimer synthesis would be accomplished using standard biphasic conditions (**chapter III**). Upon formation of the dendrimer, the hydroxyl protecting groups would be cleaved, and PEG would be attached. This method of deprotecting the periphery of a

dendrimer and attaching PEG to the exterior has been proven effective by Frechet and coworkers, who have synthesized several PEG-containing dendrimers[14].

4.2.3 Molecular Size

4.2.3.1 PEG hydration sphere

Based on the EPR effect, a macromolecule must have sufficient size to enhance the selectivity for tumor tissue. We will explore variations in the density of PEGs making up the periphery of our dendrimers. Given the modular nature of our synthesis these modifications are easily accomplished using commercially available aldehydes **(figure 4.3**).

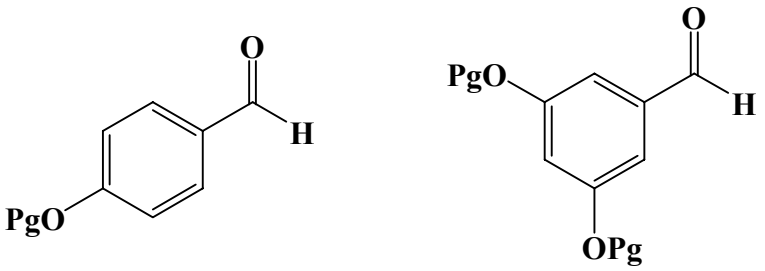

Figure 4.3 An example of two aldehydes which could be used to synthesize the periphery porphyrin. When "PEGylated", the aldehydes would each give a different surface density of PEG.

Also, PEGs carry around a significant hydration sphere. By modifying the PEG sidechains our dendrimers may appear significantly larger than their molecular weight would predict. This would be an advantage in that smaller, less synthetically demanding dendrimers may exhibit the biolocalization characteristics of much larger molecules.

This hydration sphere may also actually increase the relaxivity of its environment, making them more efficient as MRI contrast agents.

4.2.3.2 Higher Generation Dendrimers

In addition to incorporating a PEG surface on the dendrimers, it might be necessary to synthesize higher generation dendrimers to achieve better tumor tissue targeting. This can be accomplished using the conditions developed in Chapter III. Two equivalents of dendron-OH can be coupled to triazine, at 0° C, using TBAB and K_2CO_3 in biphasic conditions, with no 18-crown-6. The resulting dimer-Cl would then be reacted with excess 4-aminophenol to afford a larger "dendron-OH", which can then be coupled to a triazine core to generate the 3rd generation dendrimer (**figure 4.4**).

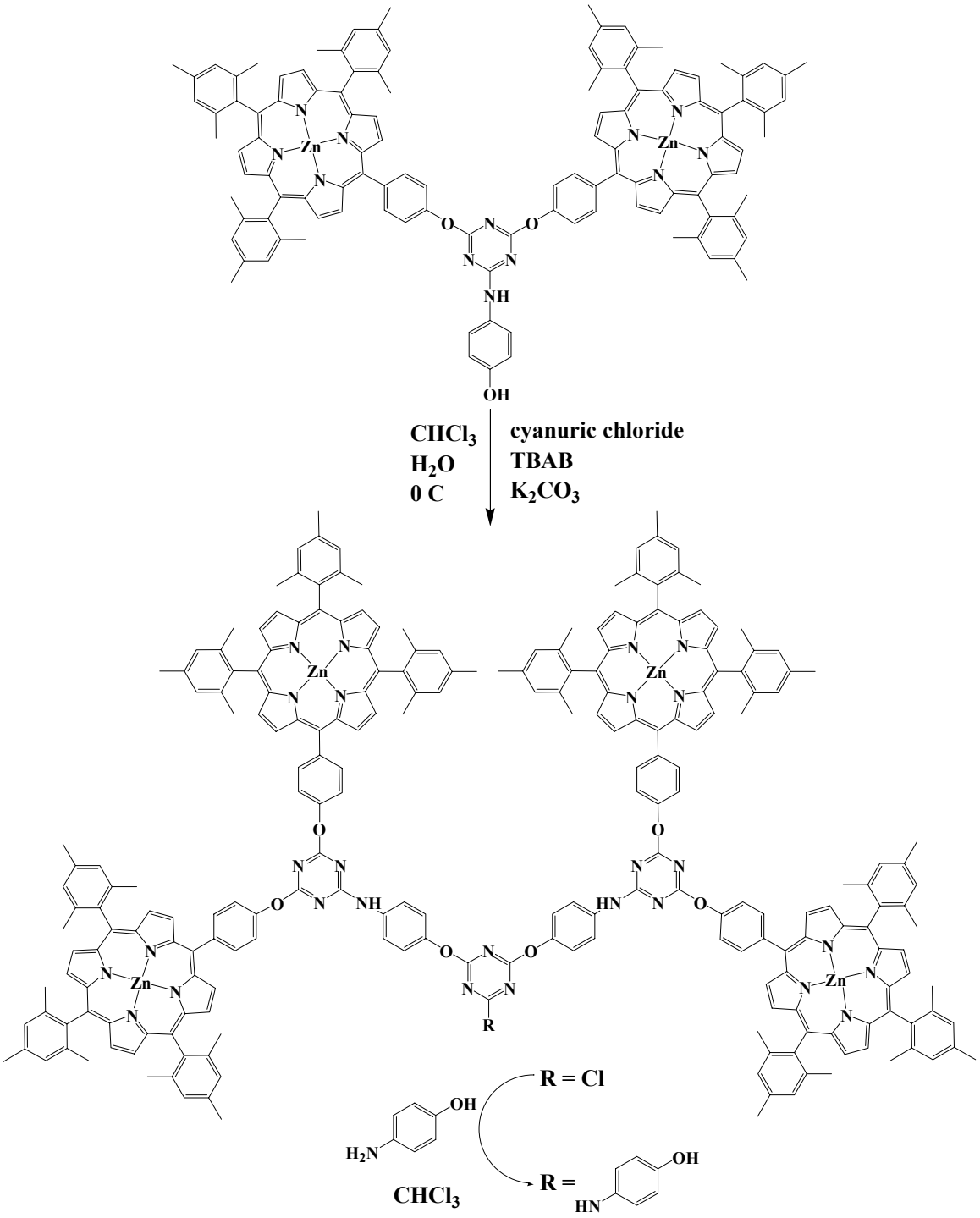

Figure 4.4 Synthesis of dendron needed to form 3rd generation dendrimer

4.3 Conclusions

A high yielding, efficient synthesis of triazine-based porphyrin-containing dendrimers was developed, for the generation of possible drug candidates for PDT and MRI. Reaction control was obtained by careful control of temperature, and use of catalysts. The dendrimer was assembled by controlling the nucleophilicity of a bi-functionalized "linker", thereby avoiding the usual deprotection/transformation step encountered in most dendrimer synthesis. This modular, convergent synthesis allows for easy future modifications of structure to optimize therapeutic properties.

PEG has proven functionality when appended to drugs; it has been shown to increase solubility, circulation time in the plasma, and biocompatibility. Appended to the surface of the triazine-based dendrimers, PEG would provide an increased molecular size, resulting in an enhanced targeting for tumor tissue based on the EPR effect. Future work on this project would consist of synthesizing larger, PEG-containing dendrimers to take advantage of these properties, and beginning *in vivo* and *in vitro* studies with tumor tissue to discover their efficacy as PDT and MRI drug candidates.

4.4 References

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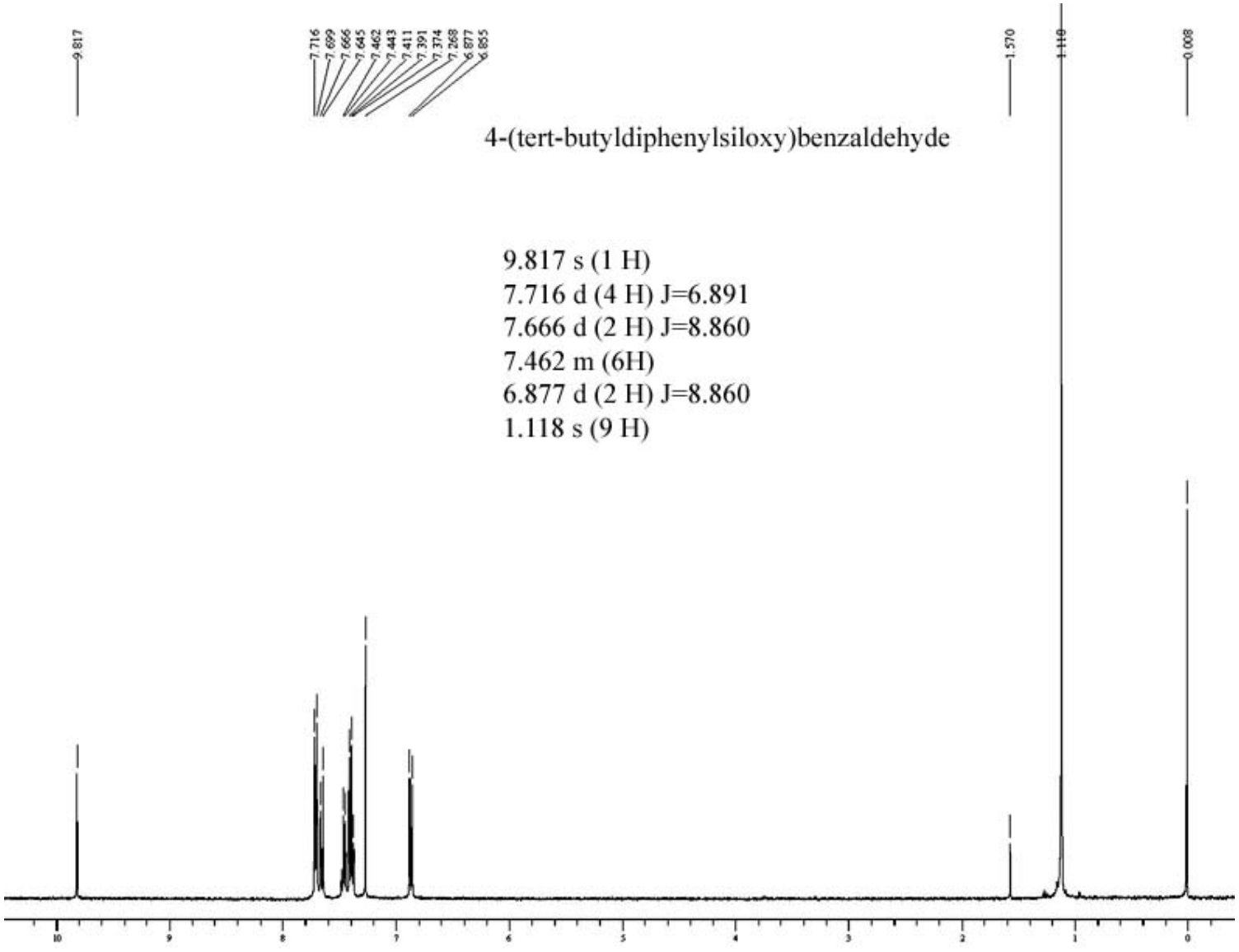
APPENDIX A

^{1}H NMR and ^{13}C NMR Spectra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7.666
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7.462
7.443
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7.374
7.268
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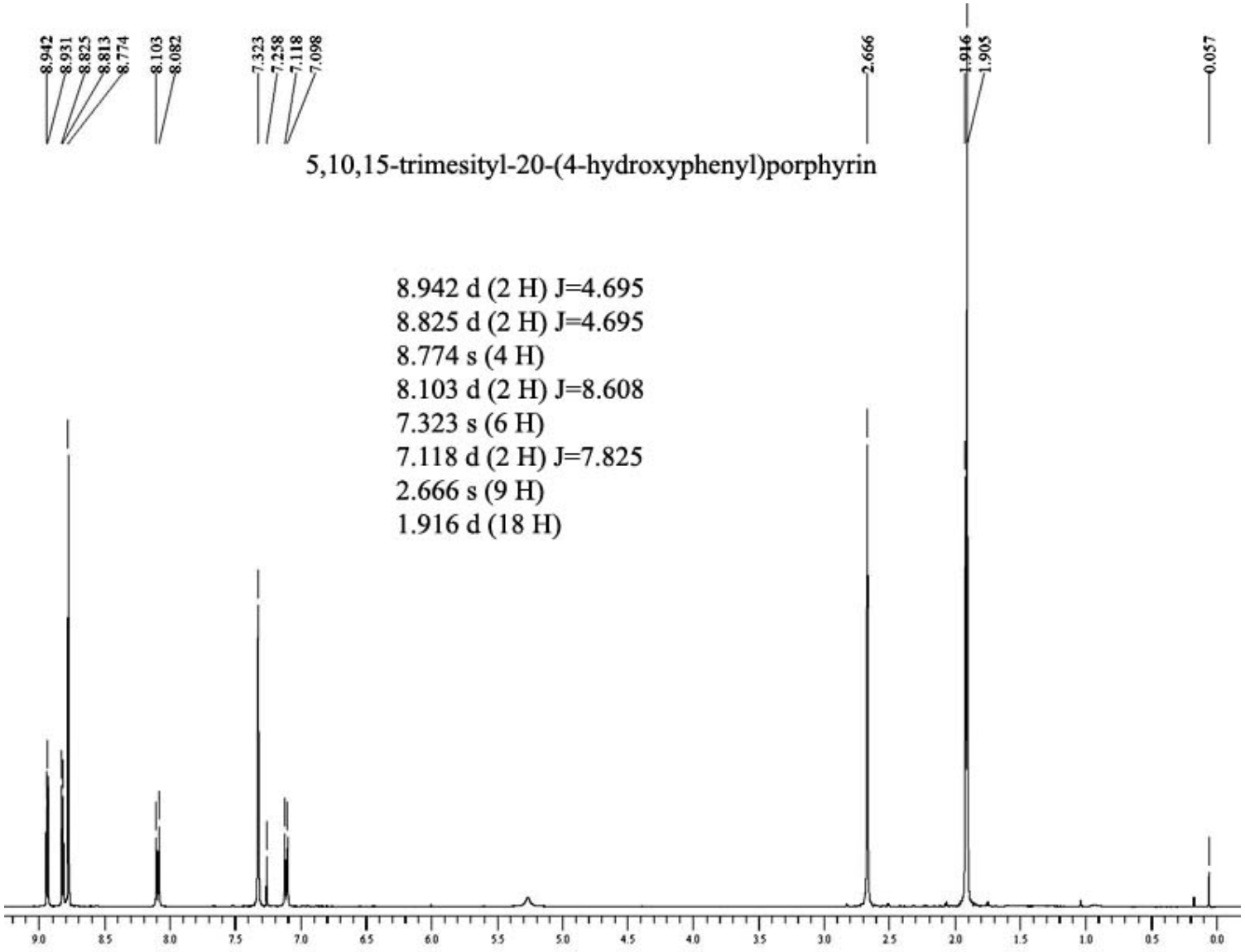
6.855
1.570
1.118
0.008
4-(tert-butyldiphenylsiloxy)benzaldehyde
9.817 s (1 H)
7.716 d (4 H) J=6.891
7.666 d (2 H) J=8.860
7.462 m (6H)
6.877 d (2 H) J=8.860
1.118 s (9 H)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8.825
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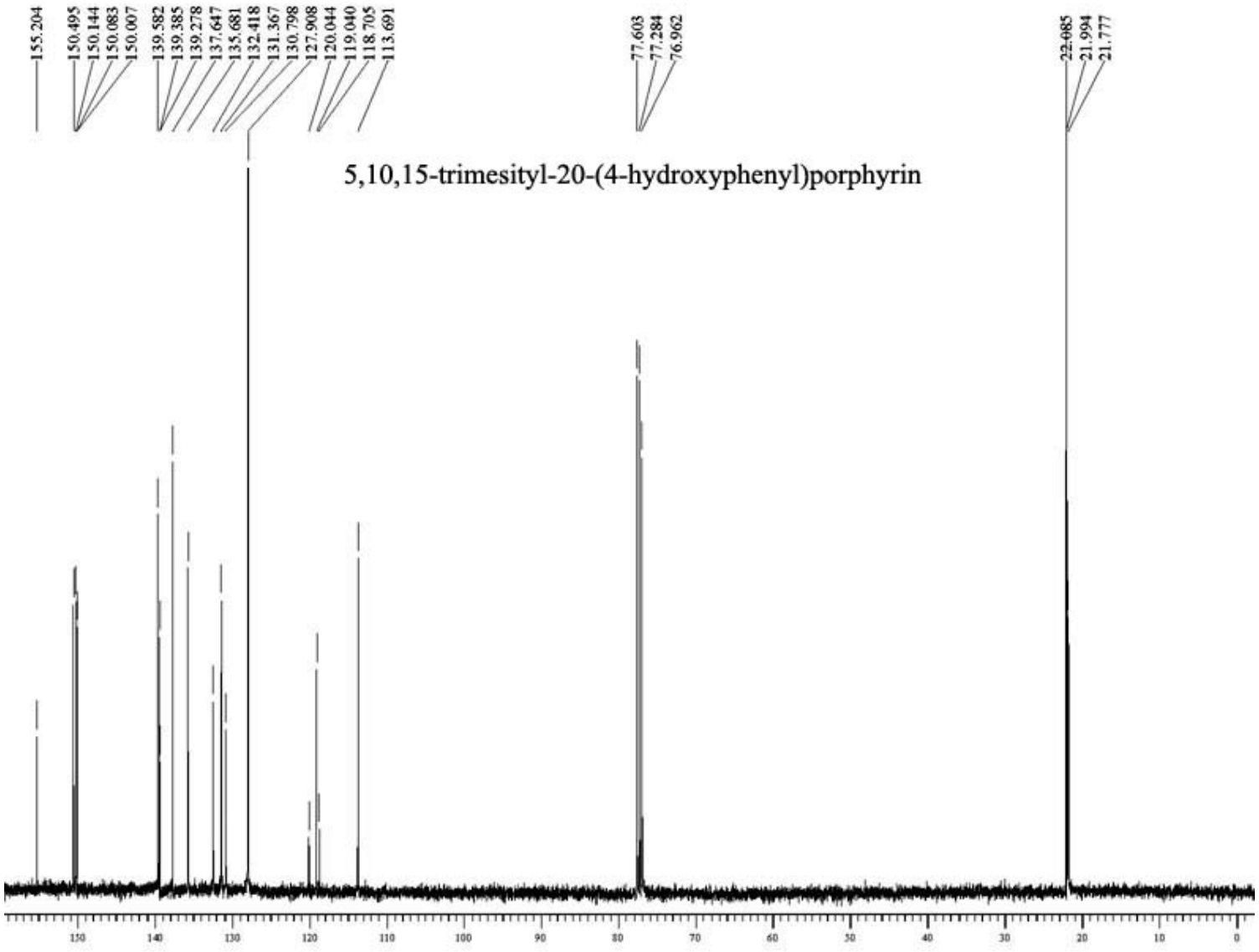
7.098
2.666
1.916
1.905
0.057
5,10,15-trimesityl-20-(4-hydroxyphenyl)porphyrin
8.942 d (2 H) J=4.695
8.825 d (2 H) J=4.695
8.774 s (4 H)
8.103 d (2 H) J=8.608
7.323 s (6 H)
7.118 d (2 H) J=7.825
2.666 s (9 H)
1.916 d (18 H)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5,10,15-trimesityl-20-(4-hydroxyphenyl)porphyrin
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10
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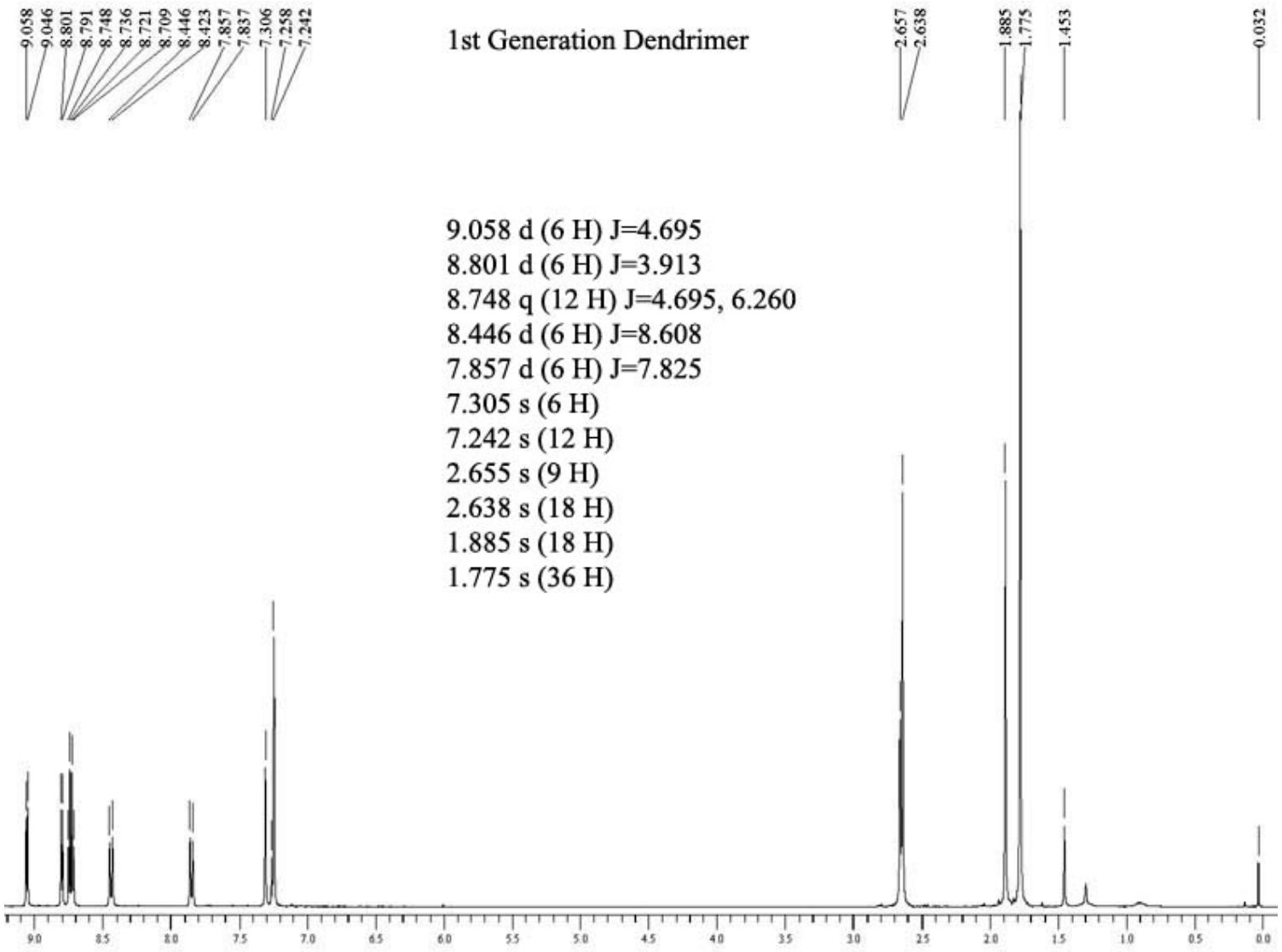
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8.446
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7.857
7.837
7.306
7.258
7.242
1st Generation Dendrimer
2.657
2.638
1.885
1.775
1.453
0.032
9.058 d (6 H) J=4.695
8.801 d (6 H) J=3.913
8.748 q (12 H) J=4.695, 6.260
8.446 d (6 H) J=8.608
7.857 d (6 H) J=7.825
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7.242 s (12 H)
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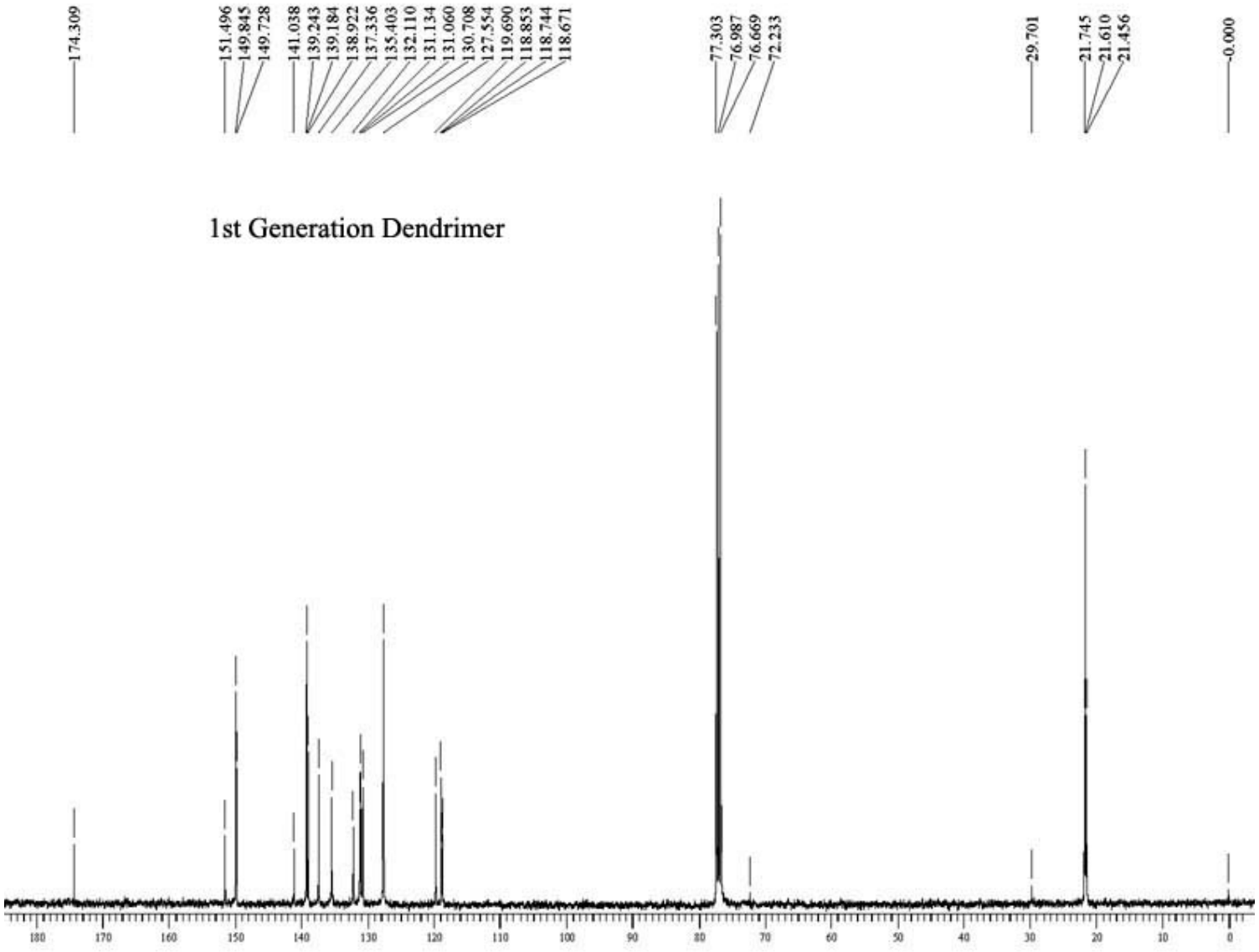
1.775 s (36 H)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0.000
1st Generation Dendrimer
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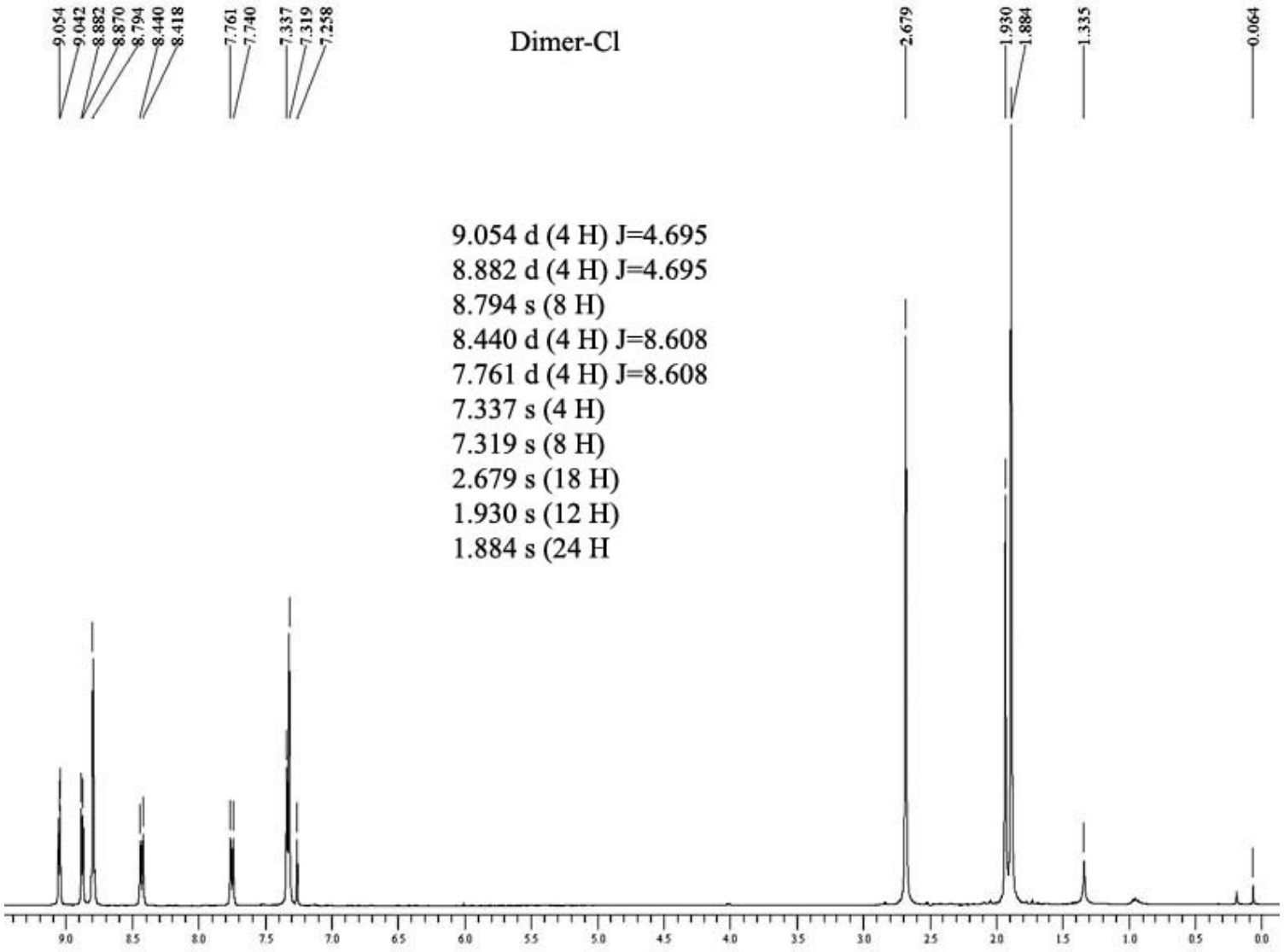
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Dimer-Cl
2.679
1.930
1.884
1.335
0.064
9.054 d (4 H) J=4.695
8.882 d (4 H) J=4.695
8.794 s (8 H)
8.440 d (4 H) J=8.608
7.761 d (4 H) J=8.608
7.337 s (4 H)
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2.679 s (18 H)
1.930 s (12 H)
1.884 s (24 H
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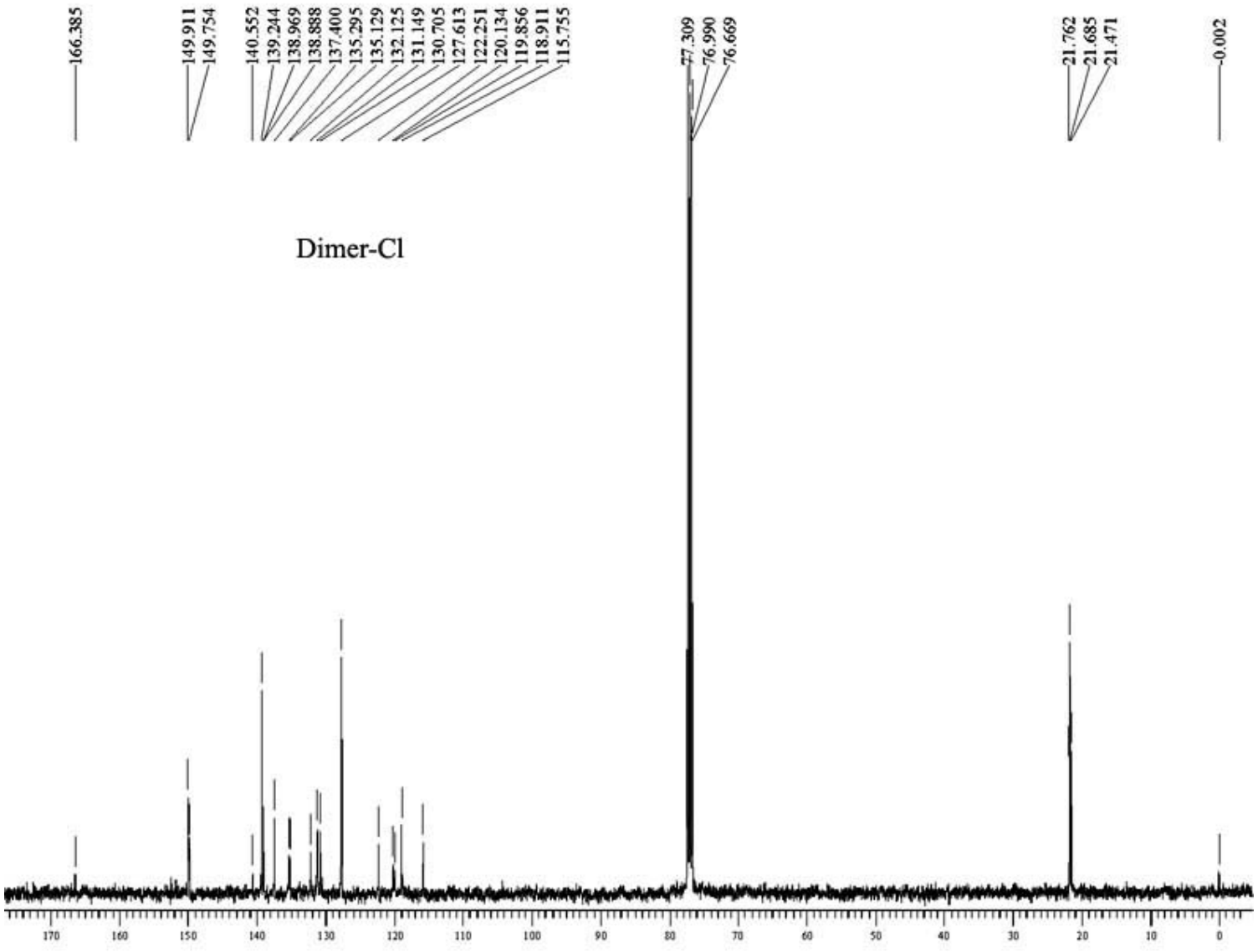
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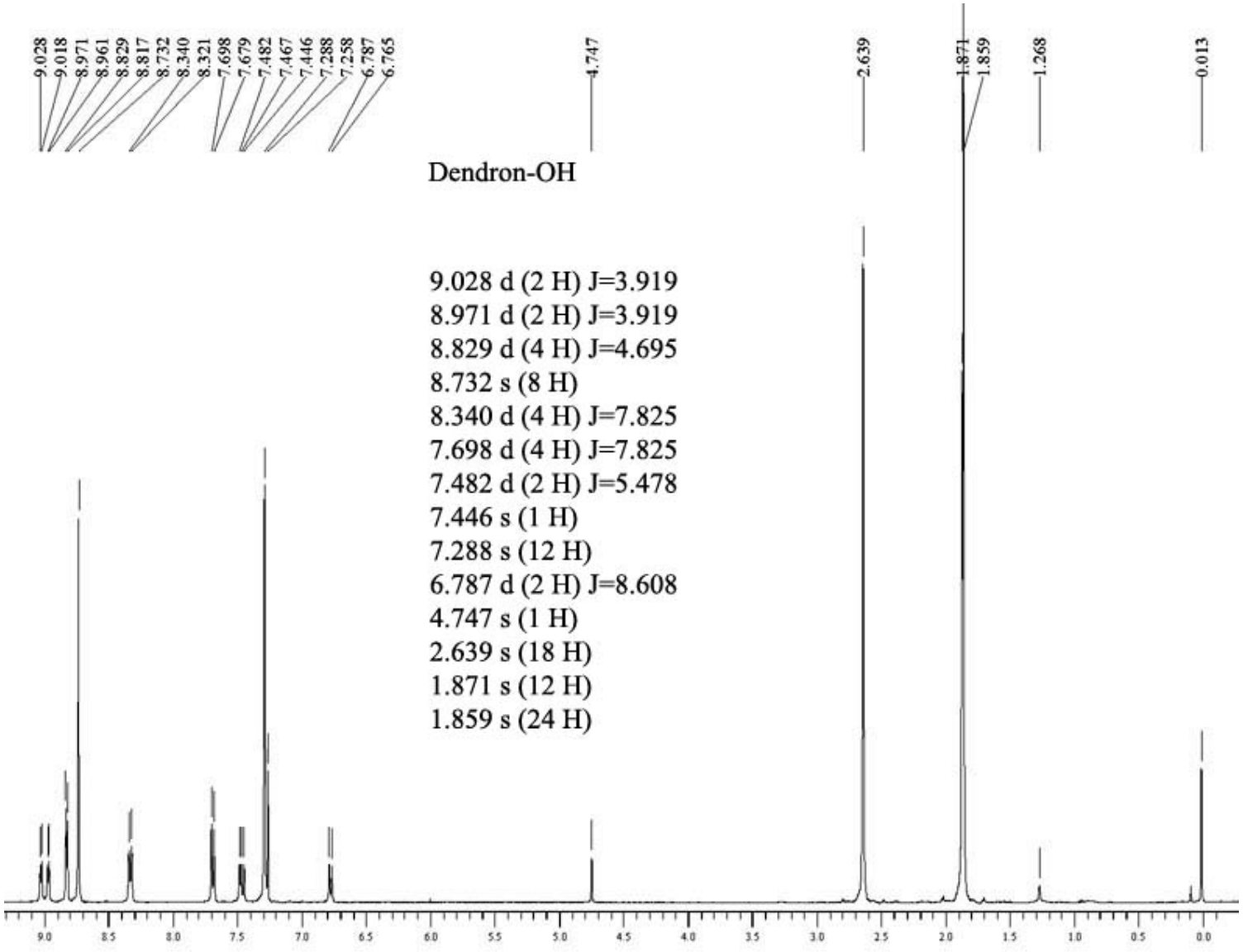
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7.258
6.787
6.765
4.747
2.639
1.871
1.859
1.268
0.013
Dendron-OH
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8.971 d (2 H) J=3.919
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8.732 s (8 H)
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7.698 d (4 H) J=7.825
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7.446 s (1 H)
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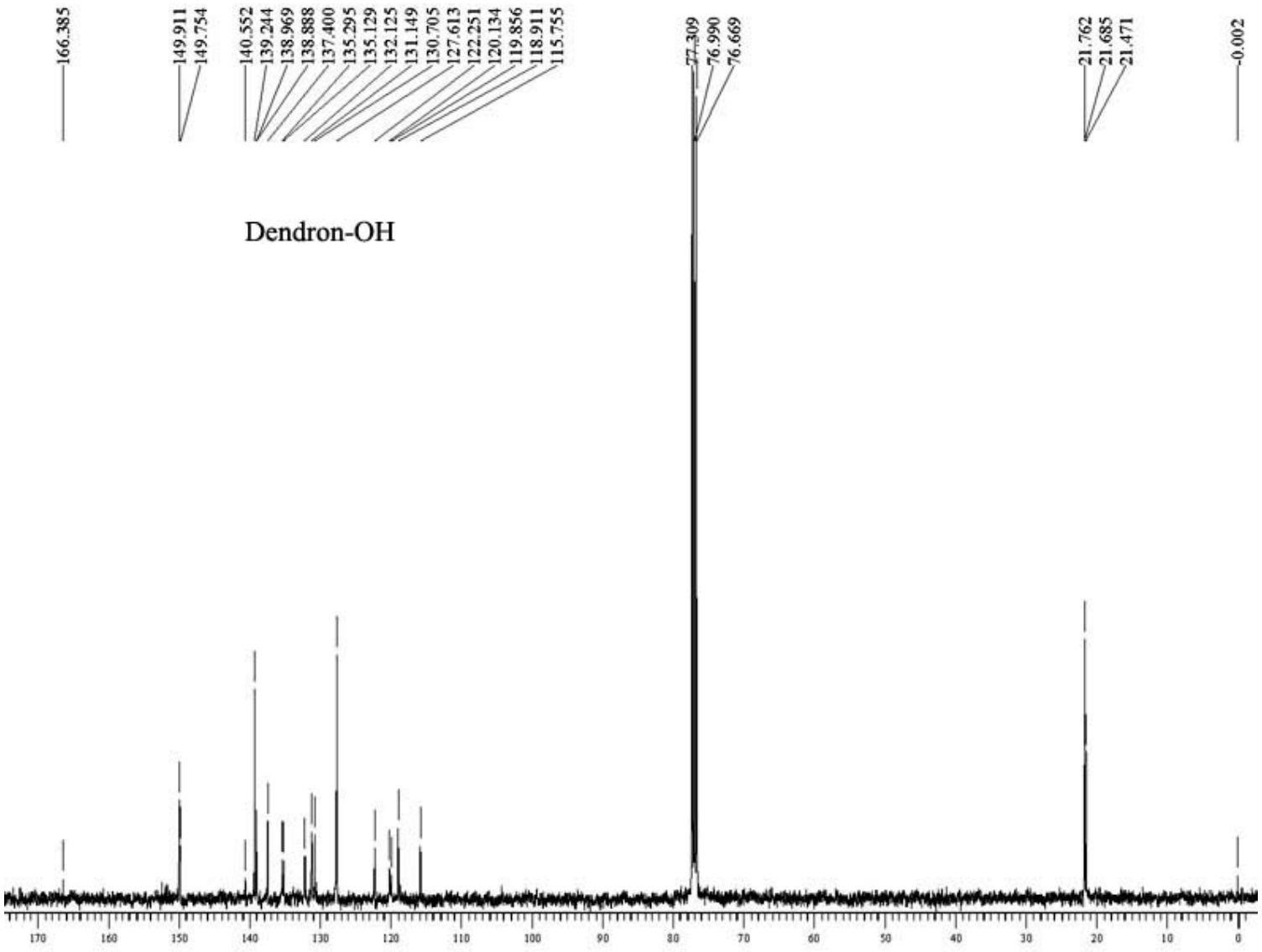
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76.990
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Dendron-OH
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30
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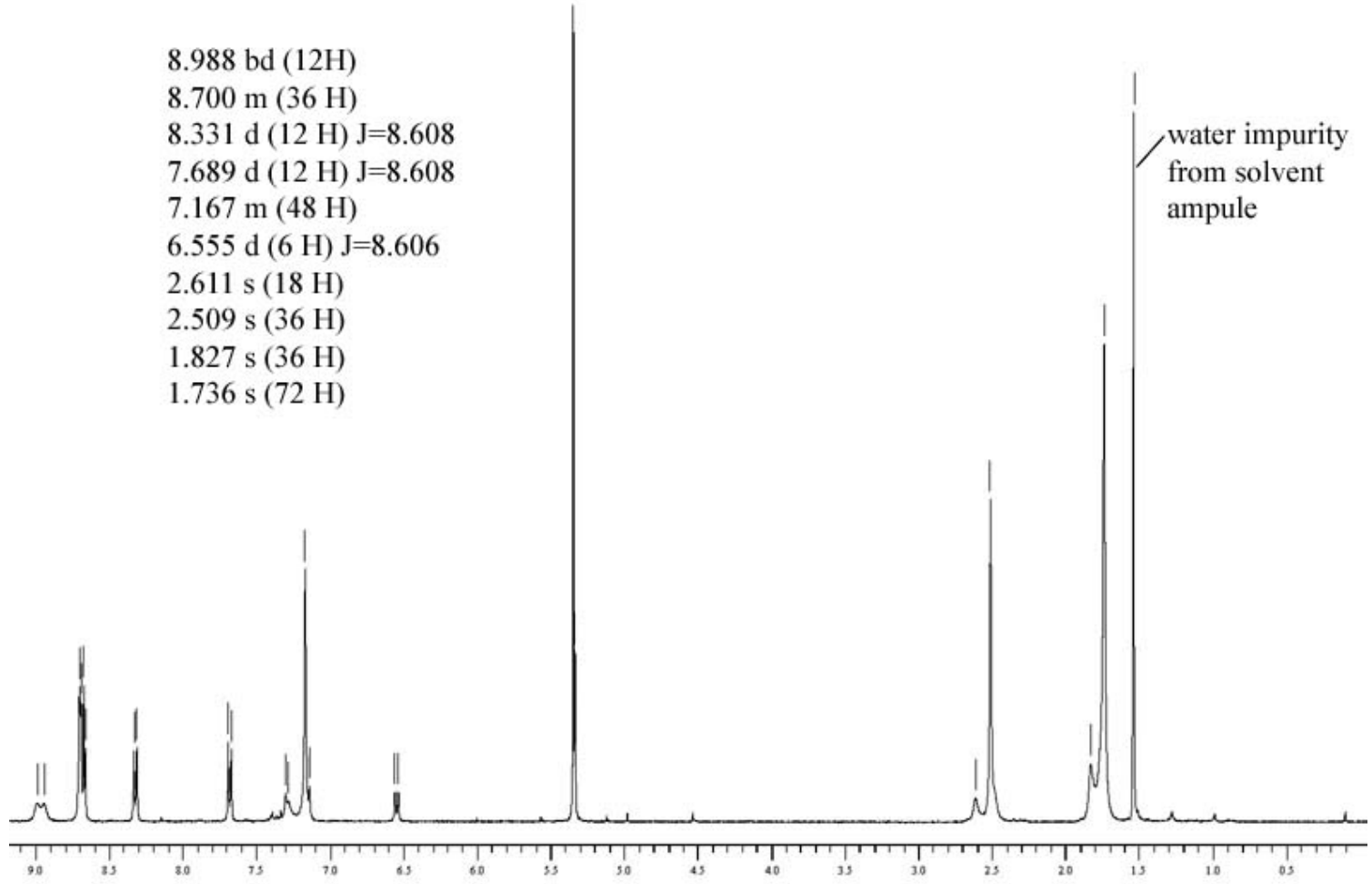
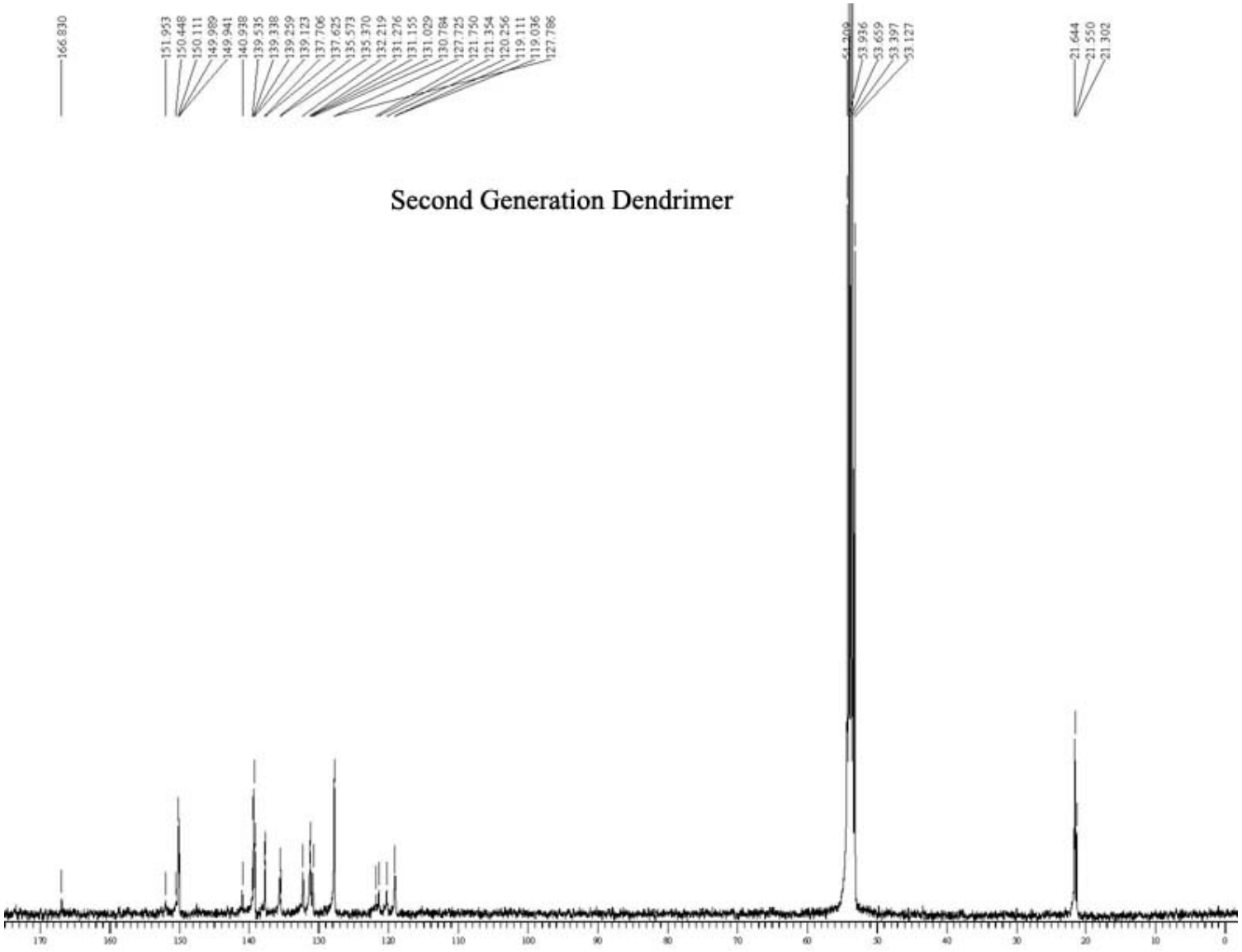
2nd Generation Dendrimer

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53.659
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53.127
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21.550
21.302
Second Generation Dendrimer