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UMI
Novel Water-Soluble Phthalocyanines for Photodynamic Therapy and Nuclear Imaging

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en vue de l'obtention de grade de
maître en sciences (M.Sc.)

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"The most beautiful experience we can have is the mysterious - the fundamental emotion which stands at the cradle of true art and true science."

Albert Einstein

"It happens also in chemistry as in architecture that beautiful edifices, that is, symmetrical and simple, are also the most sturdy: in short, the same thing happens with molecules as with the cupolas of cathedrals or the arches of bridges."

Primo Levi
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Abbreviations and Symbols

Å  angstroms
DBU  1,8-diazabicyclo[5.4.0]undec-7-ene
DCC  dicyclohexylcarbodiimide
Δ  heat, reflux
δ  chemical shift
DMF  dimethylformamide
DMSO  dimethylsulfoxide
DNA  deoxyribonucleic acid
DTPA  diethylenetriaminepentaacetic acid
ε  molar extinction coefficient
EDC  1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EDTA  ethylenediaminetetraacetic acid
FAB  fast atom bombardment
FT-IR  Fourier transform infrared
GC-MS  gas chromatography-mass spectroscopy
HEDP  1-hydroxyethylidene diphosphonate
HMPAO  hexamethylpropylene amine oxime
HOMO  highest occupied molecular orbital
HpD  hematoporphyrin derivative
HPLC  high performance liquid chromatography
IR  infrared
λ_max  maximum absorption wavelength
Li_2Pc  lithium phthalocyanine
LUMO  lowest unoccupied molecular orbital
LuPc_2  lutetium phthalocyanine
MAG3  mercaptoacetylglucylglycylglycine
MIBI  methoxyisobutyl isocyanide
MgPc  magnesium phthalocyanine
MS    mass spectroscopy
NMR   nuclear magnetic resonance
NHSS  N-hydroxysulfosuccinimide
Pc, Pcs phthalocyanine, phthalocyanines
PDT   photodynamic therapy
ppm   parts per million
rt    room temperature
r_t   retention time
SnCl_2Pc stannic phthalocyanine dichloride
SnPc_2 stannous diphthalocyanine
subPc boron subphthalocyanine
\( \tau_T \) triplet state lifetime
\( \phi_T \) quantum yield of the triplet state
\( T_b \) biological half-life
\( T_e \) effective half-life
\( T_p \) physical half-life
\( t_{1/2} \) half-life
THF   tetrahydrofuran
TLC   thin layer chromatography
UV    ultraviolet
UO_2Pc uranium superphthalocyanine
\(^{235}\text{UPc} \) uranium-235 superphthalocyanine
ZnPc  zinc phthalocyanine
Abstract-Macrocyclic dyes such as phthalocyanines have found applications in an ever increasing number of wide-ranging and diverse fields. Among these are such fields as solar cells, chemical catalysis and photodynamic therapy. New applications such as these call for new synthetic methods for the preparation of well-defined phthalocyanines bearing novel substituents.

A series of phthalocyanine derivatives bearing phosphonate substituents directly bound to the aromatic rings of the phthalocyanine macrocycle were synthesized and characterized. The precursor, 4-diethoxyphosphonophthalonitrile was prepared by phosphorylation of 4-iodophthalonitrile with diethylphosphite in the presence of tetrakis(triphenylphosphine)palladium(0) and triethylamine at 100°C followed by extensive purification via column chromatography on silica gel along with recrystallizations from acetonitrile/ether and from methanol. Condensation of this precursor in high boiling, basic solvents such as quinoline or imidazole afforded the corresponding tetra(diethoxyphosphono)-substituted metal phthalocyanine (M = Zn, Cu, Co, Ni) in preparative yields. The hydrolysis of the phosphonate esters was accomplished by refluxing in 6N HCl for 20 hours and readily gave the corresponding tetraphosphonated derivatives in quantitative yields. The zinc tetraphosphonophthalocyanine was examined as a potential photosensitizer for photodynamic therapy and as a possible technetium chelator for nuclear medicine.

Zinc octacarboglycylglycylphthalocyanine and its aza analog, zinc tetra-2,3-(5,6-carboglycylglycylpyrazino)porphyrazine were synthesized from the corresponding octacarboxy-substituted compounds. Glycylglycine was added to the carboxy groups of the phthalocyanine as its ethyl ester using dicyclohexylcarbodiimide as a coupling agent and 1-hydroxybenzotriazole as an auxiliary nucleophile in the presence of triethylamine. Following purification via column chromatography on silica gel, the ethyl esters were hydrolyzed using simple alkaline conditions (for the phthalocyanine derivative) or using
3:1 saturated methanolic NaOH/water (for the aza analog). Attempts were made to use the resulting tetradeutate N₄ core to chelate technetium.
Résumé-Les teintures macrocycliques comme les phthalocyanines ont trouvé les applications dans des domaines divers comme les éléments solaires, les catalyseurs et dans la thérapie photodynamique du cancer. Ces nouvelles applications requièrent des nouvelles méthodes synthétiques pour la préparation des phthalocyanine bien-définies portant les substituants nouveaux.

Une série de phthalocyanines portant les substituants phosphonates liés directement aux cycles aromatiques du macrocycle phthalocyanine ont été synthétisées et caractérisées. Le précurseur 4-diéthoxyphosphonophthalonitrile a été préparé par phosphorylation de 4-iodophthalonitrile avec diéthylphosphite en présence de tétrakis(triphenylphosphine) palladium(0) et triéthylamine dans le toluène à 100°C, suivi par la purification chromatographique sur la silice et la recristallisation dans l'acétonitrile/éther et dans le méthanol. La condensation de ce précurseur avec les sels métalliques (M = Zn, Cu, Co, Ni) dans les solvants basiques comme la quinoline ou l'imidazole donne des phthalocyanines substituées aux groupements diéthoxyphosphonates avec des rendements élevés. L'hydrolyse des esters phosphoniques a été accomplie par un reflux dans 6N HCl pendant 20 heures et a facilement donné les dérivés tetraphosphonates correspondants dans les rendements quantitatifs. Le zinc tetraphosphonophthalocyanine a été étudié comme photosensibilisateurs pour la thérapie photodynamique et comme un chélateur du technetium pour la médecine nucléaire.

La zinc octacarboglycylglyclylphthalocyanine et son analogue aza, zinc tétra-2,3-(5,6-carboglycylglycylpyrazino)porphyrazine ont été synthétisés à partir des composés substitués aux groupements carboxyliques correspondants. Glycylglycine a été ajouté aux groupements carboxylique de la phthalocyanine comme son ester d'éthyl en utilisant le dicyclohexylcarbodiimide comme un agent de couplage et le 1-hydroxybenzotriazole comme nucléophile auxiliaire en présence de triéthylamine. Après la purification chromatographique sur la silice, les esters d'éthyl ont été hydrolysés en utilisant les conditions basiques simples (pour le dérivé phthalocyanine) ou en utilisant un mélange de
méthanol saturé avec NaOH et de l'eau (3:1) (pour l'analogue aza). Des essais ont été faits pour utiliser le noyau N₄ résultant pour la chelation du technitium.
1. Introduction
1. Introduction

1.1 Historical Outlook on Phthalocyanines

The term phthalocyanine finds its origin in the Greek words "naptha" which means rock oil and "cyanine" which means dark blue. It was first used to describe this class of macrocyclic compounds by Sir Reginald Linstead in 1933 during his pioneering work on the subject (Linstead, 1934). He coined the term due to the origin of the compound (naptha) and their intense blue-green colour (cyanine).

In hindsight, the first recorded observation of a phthalocyanine occurred in 1907 (Braun and Tscherniac, 1907). During the synthesis of o-cyanobenzamide from

![Chemical Structure](image)

Figure 1: The first synthesis of a phthalocyanine as reported by Braun and Tscherniac

phthalamide and acetic anhydride, Braun and Tscherniac observed the production of a coloured impurity of unknown structure and origin. However, this coloured by-product was not studied any further.

It was not until 1927 that a second preparation of a phthalocyanine was reported. During their study into the synthesis of nitriles, de Diesbach and von der Weid reacted o-dibromobenzene with copper cyanide in pyridine at elevated temperature in a sealed tube in the hopes of synthesizing the corresponding o-dicyanobenzene (de Diesbach and von der Weid, 1927). To their surprise, they obtained a deeply coloured blue coordination
complex which they perceived to be a pyridine salt of the type \([\text{C}_6\text{H}_4(\text{CN})_2 + \text{C}_5\text{H}_5\text{N}]_2\text{Cu}\)
(i.e.: as a complex copper salt of the o-dicyanobenzene and pyridine ). This novel
complex was found to be extremely stable to both acidic and basic conditions as

\[
\begin{align*}
&\text{Br} & \quad \text{Br} \\
&+ & \quad \text{CuCN} \\
\rightarrow & \quad \text{pyridine} \\
& & \quad [\text{C}_6\text{H}_4(\text{CN})_2 + \text{C}_5\text{H}_5\text{N}]_2\text{Cu} \\
\end{align*}
\]

Figure 2: The synthesis of an unknown complex by de Diesbach and von der Weid

well as to heat and could only be decomposed using hot concentrated nitric acid. The
work was further expanded to show that the same complex could be synthesized by
directly heating o-dicyanobenzene, which was prepared from phthalic acid using a copper
bromide catalyst. Furthermore, similar complexes were obtained in the case of both 1,2-
dimethyl-4,5-dibromobenzene and for 1,2-dibromonaphthaleine. However, the exact nature
of these compounds was not known until 1931 when Heilbron and Irving suggested that
these pyridine salts were in reality copper phthalocyanines . This was later confirmed by
Linstead (Linstead and Lowe, 1934a).

A third observation of phthalocyanines occurred in 1928 at Scottish Dyes, Ltd.
(Linstead, 1934) during an industrial synthesis of phthalimide. The process involved
passing ammonia through molten phthalic anhydride in a iron reaction vessel. During
certain preparations, trace amounts of a dark blue impurity were formed. A preliminary
examination of this iron-containing compound was carried out by Dunsworth and
Drescher at Scottish Dyes, Ltd. (Moser and Thomas, 1983). They found the greenish-
blue product to be highly stable to numerous reagents and conditions. It was also
observed that the iron contained by these compounds could not be removed using sulfuric
acid.
Scottish Dyes, Ltd. immediately recognized the potential usefulness of these intensely coloured compounds as highly stable dyes and colouring agents. As early as 1929, the first patent with respect to the compounds that we now know as phthalocyanines was granted to Dandridge, Drescher and Thomas of Scottish Dyes, Ltd (Dandridge et al., 1929). In this patent, the preparation of ferric phthalocyanine from iron filings, phthalimide and ammonia was described. Furthermore, the first phthalocyanine dye, a polysulphonated Pc was also mentioned.

In addition to the immediate applications, it was realized that such compounds would also be of great academic interest. As such, starting in 1929, Linstead and his group, supported by grants from the Research Committee of the Dyestuffs Group of Imperial Chemical Industries, Ltd., began their work on phthalocyanines which lead to the determination of their structure in the early 1930’s (Linstead and Lowe, 1934b; Dent et al., 1934; Linstead and Robertson; 1936). The synthesis of Pcs (Byrne et al., 1934; Linstead and Lowe, 1934a; Dent and Linstead, 1934) and their relationship to porphyrins (Dent, 1938), along with further examination into the intricate structure of Pcs (Dent and Linstead, 1934), their planar nature, their complexes with metal ions (Dent and Linstead, 1934; Barrett et al., 1936; Linstead and Robertson, 1936; Barrett et al., 1938) and their stability (Linstead, 1934; Dent and Linstead, 1934) were also studied at this time. The structure of Pcs was later confirmed by Robertson via x-ray crystallography in a series of classic papers (Robertson, 1935; Robertson, 1936; Robertson and Woodward, 1937; Robertson and Woodward, 1940).
1.2 Structure of Phthalocyanines

1.21 General Discussion

![Phthalocyanine structure](image)

Figure 4: The classic structure of a tetrasubstituted phthalocyanine

Phthalocyanines are azaporphyrin derivatives and mimic naturally occurring porphyrins, such as heme, in many ways. Their central macrocycle consists of a cyclic tetapyrrole unit where, unlike porphyrins, the individual pyrrole units are linked by nitrogen atoms and not methine bridges. This difference greatly affects the chemistry of phthalocyanines as compared to porphyrins. It results in an entirely different electronic spectra as well as giving the molecule more pronounced aromaticity, higher sensitivity to oxidizers, and stronger, more stable metal complexes. Furthermore, it substantially decreases the proton acceptor (Lewis base) properties of PCs as compared to porphyrins. However, it is well-known that the substitution of a nitrogen atom for a methine bridge in heterocycles does little to change the actual physical structure and the same holds true in this case. The tetapyrrolic macrocycle of PCs is similar to that of porphyrins in terms of
shape and size (Berezin, 1981) with slight differences being found in the size of the central core of the macrocycle where the nitrogen-nitrogen diagonal distance is smaller for PCs (396 pm) than for porphyrins (402 pm) (Stillman and Nyokong, 1989).

Unlike porphyrins, the chromophore of a Pc is extended by the presence of benzene rings on the periphery of the macrocycle. This also affects the chemistry of phthalocyanines, in particular the electronic spectra where PCs have much stronger absorbances at longer wavelengths than do porphyrins. Furthermore, the presence of the benzene rings allows for the addition of functional groups (R-) onto the phthalocyanine framework without greatly affecting their electronic properties.

By examining the physical and chemical properties of the compound, Linstead was able to propose the structure shown in Figure 4 for phthalocyanines (Dent et al., 1934). This general structure was later confirmed by Robertson in a series of classical X-ray crystallographic studies (Robertson, 1935; Robertson, 1936; Robertson and Woodward, 1937; Robertson and Woodward, 1940). The Pc molecule itself is planar and highly symmetric, displaying $D_{4h}$ symmetry. This planarity is readily seen in both the structure proposed by Linstead and the X-ray work accomplished by Robertson. However, Robertson also found that the carbon-nitrogen bond lengths in the central macrocycle are on the order of 1.34 angstroms and are constant throughout the molecule (Robertson, 1936). Such a bond length points to a single bond-double bond resonance between all the carbon and nitrogen atoms in the molecule. Linstead's structure fails to explain this. Furthermore, Robertson found that the carbon-carbon bonds that link the benzene rings to the inner tetrapyrrolic macrocycle are also of a constant length of 1.49 Å. By using an empirical formula proposed by Pauling to determine the double bond character from the bond length, it can be determined that these bonds have from 12% to 15% double bond character (Robertson, 1936). This is significantly less than what would be expected from Linstead's structure. Finally, because Linstead's structure has one benzene in the quinoid form, he was also unable to explain the homogeneity of the oxidation products (Dent et
al., 1934; Robertson, 1935). Therefore, while the structural formula proposed by Linstead and generally used to describe phthalocyanaines is adequate, it fails to completely explain the exact structure of PCs.

One potential explanation for these inconsistencies is in the aromatic nature of the phthalocyanine, in particular the distribution of the \( \pi \) electrons. Berezin proposed the following structure (Figure 5) for a metal free phthalocyanine as a more adequate representation of the compound (Berezin, 1981). In this structure, the (*) represent the 24 \( \pi \) electrons of the four benzene rings, the (+) the 16 \( \pi \) electrons inherent to the tetrapyrole macrocycle and the (-) the two ionization electrons resulting from the internal ionization of two imino-hydrogen bonds. The four sextets of \( \pi \) electrons of the benzene rings obviously form intrinsically stable aromatic shells that participate only in a weak interaction with the \( \pi \) electron of the macroring. This would help explain the constant length of the C-C bonds that link the benzene rings to the macrocycle and the very weak double bond character that they possess. In the meantime, the remaining 18 \( \pi \) electrons occupy molecular orbitals of the tetrapyrrolic macrocycle and independently form an aromatic system that obeys Hückel's 4N+2 rule for aromaticity. It is this aromaticity that
can be used to explain the uniform lengths of all the C-N bonds as well as the homogeneity of the oxidation products.

One consequence of this representation of a phthalocyanine is that the inner protons within the phthalocyanine core are in the electrostatic field of three nitrogens at once. This turns out to be acceptable from a quantum mechanical viewpoint. It is energetically productive to have the proton in the electrostatic field of several nuclei. Furthermore, the formation of such protons liberates the two ionization electrons which augments the conjugation of the macrocycle to aromaticity which is also very energetically favored. There does exist a small amount of experimental data that would seem to verify the existence of such bonding. It has been shown by Robertson that there is a significant distortion from true tetragonal symmetry in metal-free phthalocyanines that is not seen in the case of the metal complexes (Robertson, 1936). Robertson tried to explain this through hydrogen bond formation. However, weak hydrogen bond forces would not be strong enough to cause such a significant distortion of a strong aromatic system such as that found in phthalocyanines. On the other hand, the three-center bonding proposed by Berezin would readily explain the existence of this distortion. Furthermore, it has been shown that, in the NMR spectra of metal-free phthalocyanines, the signal due to the two strongly shielded protons found in the phthalocyanine macrocyclic cavity (δ ≈ -5.00 ppm) disappears as a result of deuterium exchange (Dabak et al., 1994). This would hardly be the case for protons that are covalently bound such as in the structure proposed by Linstead. The protons involved in three-center bonding would, however, be susceptible to deuterium exchange. As such, the structure proposed by Berezin should be considered a more accurate representation of the real structure of phthalocyanines.

Phthalocyanines and their metal complexes are intensely stable species, primarily due to their aromaticity and the inherent difficulties in breaking open such highly stable species. They are known to remain unaffected by extremely high temperatures and are often purified by sublimation. For example, copper phthalocyanines can be sublimed at
580°C without decomposition (Dent and Linstead, 1934). It is not until over 750°C that cobalt and nickel phthalocyanines decompose, liberating the metal and nitrogen gas, and leaves behind a carbonaceous mass as the temperature nears 800°C (Moser and Thomas, 1983). In the meanwhile, the more labile tin(IV) phthalocyanine (SnCl$_2$Pc) has been shown to be more susceptible to heat, with the tin(IV) being reduced to tin(II) to form tin(II)Pc between 500-530°C. The entire phthalocyanine structure then falls apart around 550°C with the evolution of HCN, benzene and phenyl cyanide (Moser and Thomas, 1983). This loss of stability is most likely due to the large increase in the ionic radius of the central metal atom. In crystals, it is known that Sn(IV) has a ionic radius of 0.71 Å while Sn(II) is significantly larger at 0.93 Å (Lide, 1992). This would put significant strain on the phthalocyanine ligand, causing a loss of planarity and therefore, a reduction in aromatic stability due to the ineffective overlap of the atomic orbitals.

The phthalocyanine ligand itself also owes its exceptional stability to its aromaticity. These highly conjugated systems can only be broken open by strong reducing agents such as metallic lithium or hydrosulphite or when oxidized by hydrogen peroxide or permanganate in acidic media (Berezin, 1981). They readily withstand attack by Cl$_2$, Br$_2$, H$_2$SO$_4$ and oleum, preferring to undergo electrophilic aromatic substitution onto the peripheral benzene rings. Apparently, the transformation of a higher energy $\pi$ electron into a $\sigma$ electron that could be involved in bonding with the attacking species would lead to a rearrangement of all the bonding $\pi$ orbitals towards higher energy. This rearrangement to higher energy would be due to a loss of conjugation and therefore, a loss of aromatic stabilization. As such, it would not be energetically favorable for the $\pi$ electrons of the phthalocyanine to take part in addition reactions. Hence, the phthalocyanine core remains highly unreactive, leading to an extremely stable ligand for the chelation of metals.
1.22 UV-Visible Spectra

Of all the properties that phthalocyanines exhibit, the most interesting and perhaps the most important are its electronic spectral properties. After all, it was their intense blue-green colour that first captured the attention of chemists and it is because of their beautiful colour and their unusual spectral properties that they have found so many of their numerous potential applications.

The UV-visible spectra of a typical phthalocyanine is shown in Figure 6. It is highlighted by the sharp, strong Q band absorption found around 670 nm with a corresponding high molar extinction coefficient of greater than $10^5 \text{ M}^{-1}\text{cm}^{-1}$. Unlike most molecules that absorb strongly in the UV-visible portion of the spectrum, the next set of
absorptions are much less intense and lie at a considerably higher energies. The Soret or B band, as it is called, occurs around 340 nm for phthalocyanines and has a much weaker extinction coefficient than the Q band, it being around $10^4 \text{ M}^{-1}\cdot\text{cm}^{-1}$. It is this rather unique spectra with the isolation of such an intense absorption in the visible region of the spectrum that results in the incredible purity of the blues that are characteristic of these compounds.

The Q band absorption found in the spectra of phthalocyanines has been extensively studied because of its singular appearance and isolation away from interfering bands. It has been shown that the Q band is relatively insensitive to a change in the nature of the central metal atom or in the axial ligands that is coordinated to it. Table 1 shows

<table>
<thead>
<tr>
<th>Metal phthalocyanine</th>
<th>$\lambda_{\text{max}}$ of the Q band</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li$_2$Pc</td>
<td>651 nm</td>
</tr>
<tr>
<td>FePc</td>
<td>637 nm</td>
</tr>
<tr>
<td>Co(II)Pc</td>
<td>638 nm</td>
</tr>
<tr>
<td>[(CN)$^-_2$Co(III)Pc$^-]$</td>
<td>656 nm</td>
</tr>
<tr>
<td>NiPc</td>
<td>652 nm</td>
</tr>
<tr>
<td>ZnPc</td>
<td>657 nm</td>
</tr>
</tbody>
</table>

Table 1: The $\lambda_{\text{max}}$ for a series of phthalocyanines (values from Stillman and Nyokong, 1989)

some $\lambda_{\text{max}}$ values for a series of unsubstituted Pcs containing different central metal atoms. Clearly, the position of this strong absorption varies little even with such a drastic change to the complex. The axial ligand, which would affect the electron density around the metal, will obviously have little effect as well. All this readily reveals that the UV-visible spectra in general, and the Q band in particular, are due to electronic transitions in the ligand and that the central metal ion has only a slight effect on these transitions. Furthermore, while having a minor effect on the position and intensity of the Q band,
changing the functional groups substituted onto the periphery of the phthalocyanine does not have nearly the same effect on the electronic spectra as it does in porphyrins. This is due to the fact that the electronic transition resulting in the Q band absorption is essentially symmetry allowed for phthalocyanines, which is not the case for porphyrins. As such, the transition is not so dependent upon the gains in intensity caused by differences in substituents. Furthermore, the presence of the benzene rings on the periphery of the phthalocyanine and their relative electronic independence from the phthalocyanine core makes it so that substituents have little effect on the energy of the transition involved.

In contrast, the Soret band is extremely sensitive to changes in the structure of the phthalocyanine. This is presumably due to the symmetry and orientation of the molecular orbitals from which these transitions arise. They must be more susceptible to changes in the electron density surrounding them, leading to shifts in the wavelength (and therefore energy) of the Soret band along with changes in the overall intensity of the absorption.

In order to understand the electronic origin of the absorbances seen in the UV-visible spectra of phthalocyanines, the nature and the energy of the molecular orbitals involved need to be known. Several detailed theoretical calculations for the molecular orbitals of phthalocyanines have been considered (Lee et al., 1982; Hale et al., 1987; Stillman and Nyokong, 1989), primarily because of the interesting spectral properties of these compounds. They provide an excellent guide into the accuracy of the theoretical treatment used and therefore, help to validate the theory itself. Of these, one of the more easily understood treatments involves the use of a four orbital model to help explain the first two or three allowed transitions in the UV-visible region of the spectrum (Stillman and Nyokong, 1989). Only the top two occupied molecular orbitals (HOMOs) and the degenerate lowest unoccupied molecular orbital (LUMO) need be considered. This theory is displayed diagrammatically in Figure 7. The terms on the left of each orbital are symmetry elements and refer to the symmetry of the orbital involved.
For phthalocyanines, the Q band absorption is the result of a transition from the HOMO orbital with \(a_{1u}\) symmetry to the LUMO orbital which has \(e_g\) symmetry. In the meanwhile, the Soret or B band is due to a transition from the HOMO orbitals having \(a_{2u}\) and \(b_{2u}\) symmetry to the LUMO orbital. It should be noted here that this model predicts that the Soret band is in fact split into two components, \(B_1\) and \(B_2\), which occur at about the same energy and result in the broad band seen in the spectra. This splitting of the B band has been observed in the spectra of ZnPc, MgPc, and Li\(_2\)Pc but continues to be in dispute.

![Diagram of molecular orbitals for Phthalocyanine and Porphyrin](image)

**Figure 7:** A molecular orbital explanation for the origin of the UV-visible spectra of phthalocyanines (adapted from Stillman and Nyokong, 1989)
As can be seen in Figure 7, there is quite a large energy gap between the \(a_{1u}\) and the \(a_{2u}\) HOMO orbitals. This gap leads to the isolation of the Q band in the UV-visible spectra of phthalocyanines. In the meanwhile, for porphyrins, the \(a_{1u}\) and \(a_{2u}\) orbitals are accidentally degenerate, leading to extensive interactions between the Q and B bands and therefore, the lack of a nice, isolated band in their spectra.

This model can also be used to explain the effect of aggregation on the electronic spectra of phthalocyanines. It is well-known that phthalocyanines tend to aggregate via stacking in solution. This is primarily due to the hydrophobic nature of the benzene rings and the aromatic phthalocyanine core. Furthermore, the planar nature of phthalocyanines readily allows them to stack one on top of another, leading to a stabilizing overlap of their \(\pi\) electron clouds. This has the effect of broadening the Q and B bands, with a corresponding hypsochromic or blue shift in their \(\lambda\) values. The Q band, for instance, is regularly shifted to around 620 nm. An explanation for this is shown in Figure 8.

![Diagram](image)

Figure 8: The effect of aggregation on the LUMO orbitals of phthalocyanines

The aggregation of two phthalocyanines and the corresponding overlap of their \(\pi\) electron clouds results in a loss of degeneracy in the LUMO orbital, causing it to split into different
energies. Knowing that transitions must transform with either $E_u$ or $A_{2u}$ symmetry according to quantum mechanical theories, only transitions to the higher energy states are allowed and therefore, the resulting $\lambda_{\text{max}}$ is shifted to a higher energy (or a shorter wavelength). The broadening of the bands is the result of a few factors. First of all, even though the transition to the lower energy LUMO orbital is forbidden, it can still occur to a small extent, resulting in a wide absorption. Also, the magnitude of the aggregation effect is governed by several factors including the closeness of the rings, their overlap position, their tilt angle and the overall strength ($e$) of the band being studied. All these factors basically dictate how well the $\pi$ electron clouds actually overlap and surely, they will vary for each aggregate in solution. As such, the size of the splitting between the non-degenerate LUMO differs for each aggregate, leading to a broad absorption, since each aggregate will absorb at a slightly different wavelength.

The UV-visible spectrum of metal-free phthalocyanines is slightly different than for

![Figure 9: The UV-visible spectra for a typical metal free phthalocyanine](image)
phthalocyanine complexes. Figure 9 shows a typical spectrum where the Q band is split into a doublet at a slightly longer wavelength than is found in the metal complexes.

Symmetry is the primary factor used to explain this. Metal-free phthalocyanines, because of the presence of the two protons in the inner core, are less symmetric than are metal-phthalocyanine complexes, displaying $D_{2h}$ instead of $D_{4h}$ symmetry. This loss of symmetry makes all the molecular orbitals non-degenerate, causing a similar splitting as is seen in the case of aggregation. However, in this case, the transitions are polarized in either the $x$ or $y$ directions, making it so that both transitions are allowed, leading to a split Q band. The doublet is lost when the phthalocyanine is metallated since symmetry is returned to the molecule. The absorption occurs at slightly longer wavelengths (and as such, are of less energy) because the four pairs of electrons from the pyrrolic nitrogens are available to occupy antibonding $\pi$ orbitals. This raises the energy of the HOMO orbital

\[ \text{H}_2\text{Pc} \quad \text{ZnPc} \]

*Figure 10: Filling of the antibonding $\pi$ orbitals of the phthalocyanine macroring*
and as such, the transition is of less energy (see Figure 10). Complexation of a metal makes these four electron pairs unavailable as they are involved in bonding to the metal. As such, the HOMO orbital is of less energy and the Q band occurs at shorter wavelengths for metallated complexes.

One problem in studying the electronic spectra of phthalocyanines is their insolubility in most useful solvents. The most effective and practically the only solvent for unsubstituted phthalocyanines is sulphuric acid at concentrations greater than 8M. However, this is of only limited usefulness for acquiring UV-visible spectra. The absorption spectra of Pcs in organic solvent and in H₂SO₄ differs to such an extent that there must be a strong chemical interaction involved. Quite obviously, the sulphuric acid acts to protonate the phthalocyanine at one of the intracyclic nitrogen atoms. This leads to the macroring becoming strongly polarized and the resulting strong dipole decreases the energy of all the electronic transitions by raising the energy of the HOMO orbitals. Subsequently, there is a bathochromic or red shift in the wavelengths of the Q and B bands by 80-120 nm. For example, zinc octabutoxyphthalocyanine absorbs at 678 nm in DMF. However, in concentrated sulphuric acid, the Q band is shifted to 827 nm (Stillman and Nyokong, 1989). Therefore, while using sulphuric acid as a solvent is useful, it is less than ideal for acquiring electronic spectra of phthalocyanines.

1.23 Inorganic Viewpoint

Inorganic chemistry was at one time described as the chemistry of all the elements except for carbon. While the growth in such fields as organometallic and coordination chemistry has somewhat clouded this original definition, it can still serve as a rough guideline. Therefore, in terms of phthalocyanine chemistry, inorganic chemists are more concerned with the central metal atom, its environment and the nature of the bonding
around it. The phthalocyanine molecule itself is viewed as a simple tetradeantate ligand capable of forming 4 dative σ bonds with the central metal atom.

Usually, phthalocyanine complexes display octahedral geometry (Figure 11) with the phthalocyanine ligand occupying the equatorial plane and axial ligands coordinated to the metal both above and below the xy plane. The nature of these axial ligands depends greatly upon the central metal atom, the solvent involved and even the synthetic method used (vide infra).

Stable phthalocyanine complexes are a result of the formation of 4 equivalent dative σ bonds between the pyrrolic nitrogen atoms of the Pc and the central metal atom. This involves the filling of vacant s, p_x, p_y and d_{xy}, d_{y^2} orbitals of the metal (those orientated towards the nitrogens) with the lone pair electrons of the nitrogen atoms to form stable dative bonds where both electrons involved in bonding come from the same atom. These σ bonds are the most stable when the best possible overlap of orbitals occurs. Such is the case for metal cations whose covalent radius is around 1.35 Å, the radius of the inner core of phthalocyanines (Berezin, 1981). As such, the metal fits tightly into the inner space and there is very effective orbital overlap and strong σ bonds are
formed. Metal ions such as \( \text{Cu}^{2+}, \text{Zn}^{2+}, \text{Co}^{2+}, \text{Ni}^{2+}, \text{Pt}^{2+}, \text{Pd}^{2+}, \text{Al}^{3+}, \text{Ga}^{3+} \) and \( \text{VO}^{2+} \) fit this criteria and are known to form extremely stable phthalocyanine complexes (Berezin, 1981).

In addition to \( \sigma \) bonds (N\(\rightarrow\)M), metals with filled d-orbitals of \( \pi \) symmetry (d\(_{xy}\), d\(_{xz}\), d\(_{yz}\)) can also be involved in dative \( \pi \) backbonding. In this case, the metal serves as the electron donor, with the ligand accepting electrons into its antibonding \( \pi^* \) molecular orbitals. Phthalocyanines have an unusually high capacity to form \( \pi \) backbonds with metals, which has the effect of further strengthening the bonding around the metal and thus, increasing the stability of the complex. Such would be the case for \( \text{Zn}^{2+} \) which has a completely filled d-shell and can thus form very strong \( \pi \) backbonds.

Not all phthalocyanine complexes are stable. More labile ones are formed with metal cations that are not capable of forming strong \( \sigma \) bonds. This is due to their weak electron affinity (\( \text{Li}^+, \text{Na}^+, \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Ag}^+, \text{Mn}^{2+}, \text{Sn}^{2+}, \text{Pb}^{2+} \)) or their inability to form a planar arrangement of orbitals suitable for \( \sigma \) bond formation with the ligand. This is the case for most of the metal ions mentioned above plus \( \text{Be}^{2+}, \text{Cd}^{2+} \) and \( \text{Hg}^{2+} \). Complexes with these ions feature mainly ionic bonding and the metal cations can be easily removed by treatment with sulphuric acid. This reaction is quite often used in order to produce metal-free phthalocyanines. Furthermore, complexes with lithium (\( \text{Li}_2\text{Pc} \)) will readily react with alcoholic solutions of anhydrous metal salts that are incapable of intensive solvolysis (such as \( \text{ZnCl}_2 \) and \( \text{CoCl}_2 \)) to yield pure phthalocyanine complexes with the other metal. Salts like \( \text{AlCl}_3, \text{SnCl}_2 \) or \( \text{ThCl}_4 \), which readily lend themselves to solvolysis, lead only to metal-free phthalocyanine as the reaction with the metal would call for desolvolyis, which requires considerable energy. In aprotic solvents such as DMF, these lithium complexes readily react with such salts to form metal complexes since the solvolysis involved is far less intense.

Depending upon the strength of the coordinative metal-phthalocyanine interaction, which itself is determined by the strength of the covalent M-N bonds and the formal
charge of the metal cation, metal ions can be divided as either being coordinatively saturated or unsaturated. Coordinatively saturated metals have a formal charge of +2 and form extremely stable covalent complexes with both strong σ forward- and π backbonding. These include metal ions such as Ni^{2+}, Pt^{2+} and Pd^{2+}. Phthalocyanine complexes with these metals lack low energy level antibonding orbitals at the metal and they show no tendency towards attachment of axial ligands. d^7 metal ions like Ni^{2+}, Pd^{2+}, and Pt^{2+} are well-known to form highly stable square planar complexes in most cases (Butler and Herrod, 1989) and they act no differently here. Their phthalocyanine complexes show the same square planar geometry, with the metal and the phthalocyanine ligand occupying the same equatorial plane.

Coordinatively unsaturated metals include those that have no tendency towards dsp^2 hybridization at a formal charge of +2 (Zn^{2+}, Fe^{2+}, Cr^{2+}, Mn^{2+}), feature unstable oxidation states (Co^{2+}, Re^{2+}, Cu^0), or form primarily ionic complexes (Be^{2+}, Mg^{2+}, Cd^{2+}, Hg^{2+}, Ca^{2+}, Ga^{2+}). These metals tend to complex axial ligands in solution and thus display octahedral symmetry like that shown previously (Figure 11). In most cases, the axial ligand turns out to be solvent molecules, with an atom capable of electron donation within the solvent molecule becoming coordinated to the metal. However, anions such as Cl^- or H_2O^- can also act as the axial ligand if they are present in solution. Finally, in aggregates, another phthalocyanine can actually be seen as an axial ligand. It is interesting to see how phthalocyanines that tend to aggregate when dissolved in water or alcohols become monomers in solvents like DMF or pyridine. This is because these solvents are more strongly coordinating and as such, remain tightly bound to the metal in solution, preventing stacking from occurring and reducing π interactions. It should be noted however, that in crystals, these complexes do not have axial ligands and usually form crystals via some sort of stacking.

Coordinatively unsaturated metals in phthalocyanine complexes also include any metal with a formal charge exceeding +2, irrespective of the strength of the dative σ and π
interactions. These complexes readily attach axial ligands in order to help neutralize the charge of the metal ion. In this case, the axial ligand is more tightly bound to the metal and is present even in their solid crystals. Binding of axial ligands involves the s, p_z and d_{z^2} orbitals of the metal, along with any free dsp^2-hybridized orbitals, as they are the ones orientated towards the position occupied by the axial ligands. In these cases, these phthalocyanines do carry axial ligands as solids and therefore, they form slightly different crystal structures.

One last thing to consider is the magnetism of the system, as it has a serious effect on some of the properties of phthalocyanine complexes. It is well-known that phthalocyanine complexes of Cu^{2+}, Ni^{2+} and Fe^{2+} are paramagnetic as they contain unpaired d-orbital electrons. In contrast, complexes with Zn^{2+}, Ga^{3+} and Al^{3+} have no unpaired d-orbital electrons and are diamagnetic. This can be clearly shown by examining the behaviour of each in a magnetic field (Hickie, 1992). It is obvious that Zn^{2+}, Ga^{3+} and Al^{3+} are diamagnetic since they are d^{10} ions and as such, their d-shell is filled. However, Ru(II) phthalocyanines are also paramagnetic even though Ru(II) is a d^6 ion. Since it requires energy to pair electrons into the same orbital, in most cases, atoms and molecules tend to fill degenerate orbitals (like the d-orbitals) one at a time before pairing electrons up. Consequently, something must be causing the d-orbitals to lose their

![Diagram](attachment:image.png)

Figure 12: The crystal field diagram for an octahedral complex

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degeneracy in this case. This loss of degeneracy can be explained using crystal field theory. The electrostatic interactions between the d-orbitals of the metal and the ligand cause the normally degenerate d-orbitals to split as shown in Figure 12, with the orbitals pointing towards the ligands being destabilized (d_{x^2-y^2}, d_{z^2}) while the others (d_{xy}, d_{xz}, d_{yz}) are stabilized as compared to an uncomplexed metal ion because of the presence of the ligand. In the case of Ru(II) phthalocyanines, it is energetically favorable to pair electrons into the lower energy d-orbitals rather than having them remain unpaired and fill the higher energy d-orbitals. As such, all the electrons are paired, filling the d_{xy}, d_{xz}, d_{yz} orbitals and the complex is diamagnetic. Such diamagnetism does have a significant effect on some of the physical properties of phthalocyanine complexes. For one, PCs complexed with a diamagnetic central metal atom are known to have high triplet yields and long triplet lifetimes. In the meanwhile, the singlet state for PCs with paramagnetic central metal atoms is rapidly deactivated, leading to poor triplet yields. This is important in some phthalocyanine applications, in particular photodynamic therapy, which requires high triplet yields and long triplet lifetimes in order to effectively produce the cytotoxic species needed to induce the desired biological effect.

Phthalocyanines are essentially organic molecules. However, it is their complexes that hold the promise of so many potential applications. As such, the inorganic origins of the structure and properties of phthalocyanines must be understood and examined as well.

1.24 Novel Phthalocyanine Analogs

A number of novel phthalocyanine analogs have been prepared in order to better study phthalocyanines in general. Novel analogs can be used to examine structure-activity relationships and other phthalocyanine properties by observing the effect caused by slight modifications to the Pc structure. Furthermore, the absorption spectra can be "fine-tuned" to the desired wavelength and the potential exhibited by phthalocyanines for various
application can be optimized by studying and using new and improved phthalocyanine derivatives.

**a) Boron Subphthalocyanine**

Because of its smaller atomic radius and its strong Lewis acidity, boron readily forms subphthalocyanine complexes (Figure 13) when reacted with phthalonitriles or diiminoisoindolines. These lower homologs to phthalocyanines are characterized by their inner macrocycle, which consist of a tripyrrolic system rather than the tetrapyrrolic one found in Pcs. This loss of an indoline unit causes a hypsochromic or blue shift in the $\lambda_{\text{max}}$ of the Q band down to around 560 nm, where the boron subphthalocyanine solution takes on a reddish colour. However, the inner macrocycle of boron subphthalocyanines is still aromatic in nature, obeying Huckel's 4N+2 rule for aromaticity, as there are 14 $\pi$ electrons occupying $\pi$ orbitals of the tripyrrolic unit.

![Figure 13: A substituted boron subphthalocyanine](image)

First reported in 1972 by Meller and Ossoko (Meller and Ossoko, 1972), boron subphthalocyanines were originally synthesized from phthalonitrile after having condensed
gaseous boron trichloride into the reaction vessel and heating to 250°C. This rather difficult method was soon replaced by commercially available solutions of boron trihalide, which lead to increased yields and cleaner, safer reactions (Rauschnabel and Hanack, 1995). One limitation of this method, however, is the production of halogenated by-products (Dabak et al., 1994; Hanack, and Geyer, 1994; Weitemeyer et al., 1996). Halogen (Cl₂, Br₂) is generated during the formation of the subphthalocyanine via this procedure. As a consequence, the aromatic rings surrounding the subphthalocyanine are subject to electrophilic attack by the halogen, an electrophilic aromatic substitution reaction that is known to be catalyzed by boron trihalide (March, 1992). Thus, some halogenated subphthalocyanine is produced and this can be very difficult to remove from the final product. However, this can be avoided in certain cases by using triphenyl boron as the boron source in the presence of an organic base like DBU, which would avoid the production of halogen in the reaction mixture (Hanack and Geyer, 1994; Rauschnabel and Hanack, 1995). This newer procedure also has disadvantages as well. Triphenyl boron is significantly less reactive than the other boron reagents and requires the presence of organic bases in order to react. This limits its usefulness to the more reactive phthalocyanine precursors and to those that are unaffected by the organic base. Furthermore, this reagent leads to much lower yields than those observed for boron trihalide, even though a more pure product is obtained.

The boron in these complexes is in the +3 oxidation state and therefore is coordinatively unsaturated. As such, it has a σ-bonded axial ligand. In most cases, this axial ligand is a halide which is freed during the synthesis of the subphthalocyanine. However, when the boron reagent used contains a phenyl group, such as PhBCl₂ or Ph₃B, the phenyl group becomes axially coordinated to the boron. This is presumably due to the ability of the π orbitals of the phenyl group to overlap with π-oriented d-orbitals of the metal to form stabilizing dative backbonding. This leads to a stronger coordination and therefore, a more stable complex.
The one major difference between phthalocyanines and subphthalocyanines is in their stability and reactivity. While phthalocyanines are extremely stable compounds, subphthalocyanines are quite unstable and react readily with compounds like diiminoisoindoline to form metal-free phthalocyanines (vide infra). This reactivity can be explained by examining the x-ray crystal structure of subphthalocyanines (Rauschnabel and Hanack, 1995). Instead of being planar in nature, subphthalocyanines have a bowl-shaped structure with the boron coordinated in a tetrahedral geometry. This loss of planarity is due to the steric hindrance between the indoline units in the more crowded subphthalocyanine. An ineffective overlap of the p-orbitals in the macrocoring results, leading to a strained and unstable structure with loss of some aromatic stabilization. This leads to the high reactivity of these compounds and gives them one of their most important applications, in the synthesis of unsymmetrical substituted phthalocyanines.

b) Out-of-Plane and Sandwich Complexes

![Diagram of out-of-plane and sandwich phthalocyanine]

Figure 14: Diagrams of an out-of-plane and sandwich phthalocyanine

Out-of-plane and sandwich phthalocyanine complexes are formed when the metal ion is too large to fit into the phthalocyanine cavity. Such is the case for several 3d and 4d metal ions along with all of the lanthanides. Out-of-plane complexes, diagrammatically shown in Figure 14, exist with the metal ion sitting just above the plane of the phthalocyanine nitrogen atoms. In dichlorotin (IV) phthalocyanine (SnCl₂Pc), there is a
1.11 Å displacement of the tin atom above the plane of the phthalocyanine (Kroenke and Kenney, 1964). This results in the complex having a distorted geometry and weaker σ bonds because of the poor overlap of the orbitals involved. Hence, these out-of-plane complexes are more labile and will more readily lose the metal ion from the complex. It has been known for a long time that dichlorotin (IV) phthalocyanines are significantly less stable to alkali conditions than are most phthalocyanine complexes. For instance, successive treatment with ammonia under pressure or boiling quinoline will give to rise to demetallation, yielding stannic oxide and free phthalocyanine (Barrett et al., 1936).

When dichlorotin phthalocyanine is refluxed with metal-free phthalocyanine in chloronaphthalene, tin (IV) or stannic phthalocyanine (SnPc2) is produced (Barrett et al., 1936). Similar sandwich compounds (see Figure 14) are produced for large multivalent metal ions such as the lanthanides by direct reaction with phthalonitriles. In fact, by controlling the amount of precursor, it is sometimes possible to produce either the out-of-plane or the sandwich compound, depending on which product is desired.

Sandwich compounds such as these have very interesting properties. They display electrochromism and are especially interesting in electroconductivity. It has been established that sandwich Pcs have a very rich redox chemistry, especially LuPc2, and it has been reported that thin films made by cosubliming LuPc2 and an electron acceptor display semiconductor properties (Stillman and Nyokong, 1989). Sandwich phthalocyanines are also useful in studying the effects of aggregation on the electronic spectra of phthalocyanines. As the size of the central metal atom goes down, the rings come closer together. This, much like in the aggregation of phthalocyanines in solution, causes a blue shift in the λmax (Kasuga et al., 1990). Finally, sandwich compounds such as these offer a great deal of control over the amphilicity of the molecule. Obviously, one of the phthalocyanine rings can be substituted with hydrophobic groups while the other with hydrophilic groups. Such amphilicity could be extremely useful in a number of applications.
c) Superphthalocyanines

When the metal atom gets bigger still, as in the case of the actinides, they will react with phthalonitriles to form the next homolog up in the series, superphthalocyanines. As shown in Figure 15, these phthalocyanine derivatives contain an extra isoindoline unit. This is primarily due to the large size of the metal ion and its propensity to form seven coordinate pentagonal bipyramidal complexes. The addition of the extra isoindoline unit has the expected effect on the UV-visible spectra with a strong bathochromic shift of the Q band to 900 nm (Moser and Thomas, 1983).

The formation of superphthalocyanines causes a lot of steric strain within the ligand. X-ray studies on crystals have clearly shown that these phthalocyanine derivatives

![Diagram](image.png)

Figure 15: A superphthalocyanine

are not planar and have a somewhat wavy structure (Day et al., 1975). Like in the case of subphthalocyanines, this causes an incomplete overlap of the p-orbitals and a loss of aromatic stability. In addition, like subphthalocyanines, superphthalocyanines are labile,
reacting readily with metal ions such as Cu\textsuperscript{2+}, Zn\textsuperscript{2+}, Co\textsuperscript{2+} or Ni\textsuperscript{2+} to expel one phthalonitrile to yield a simple phthalocyanine complex and an actinide salt (Moser and Thomas, 1983). Such a ligand contraction reaction is quite unprecedented in macrocycle coordination chemistry. However, the reason for such a reaction is clear. The driving force is the return of complete aromatic stability with the subsequent loss of the steric strain within the molecule.

It should be noted that actinides such as uranium and thorium can also form labile out-of-plane complexes with phthalocyanines. In this case, the extra coordination sites must be occupied by another ligand. Often, a chelator, such as acetylacetate, is added as it will take advantage of the stability inherent in multidentate ligands. However, it can also be the metal oxide that is complexed, leading to compounds of the type UO\textsubscript{2}Pc.

One interesting property of these out-of-plane complexes is in their biodistribution. It has been found that the uranyl sulphonated phthalocyanine is retained by damaged brain tissue (Frigerio, 1962). In fact, tumor-to-normal tissue ratios of up to 50:1 have been observed. This makes these compound of potential interest in both tumor detection and treatment, where a slow beam of neutrons could provide local deposition of radiation where the \textsuperscript{235}U-Pc is retained.
1.3 Synthesis of Phthalocyanines

1.31 General Synthesis

As can be seen through their accidental discovery, phthalocyanines are quite easily synthesized. During his pioneering work, Linstead showed that PCs and their metal complexes can be readily synthesized from any number of possible methods (Byrne et al., 1934; Linstead and Lowe, 1934a; Barrett et al., 1936; Barrett et al., 1938). In general, they are formed via a template reaction by heating an appropriate phthalocyanine precursor in the presence of a metal ion source. Among possible precursors are phthalonitriles, phthalimides, phthalic anhydrides, diiminoisoindolines, o-cyanobenzamides and other o-phthalic acid derivatives (Figure 16). Of these, the most useful are the

![Chemical structures](image)

Figure 16: Phthalocyanine precursors
phthalonitriles, which readily form complexes with most metals, except for mercury and silver, in good yield. For example, the reaction of phthalonitrile with copper bronze at 190-270°C gives the corresponding copper phthalocyanine in an excellent yield of 75-90% based on the nitrile (Dent and Linseman, 1934). Furthermore, it should be noted that imides and anhydrides require aminating agents such as urea and a catalyst like ammonium molybdate or boric acid in order to get complex formation. Thus, their potential usefulness is greatly decreased in certain cases.

Template reactions like these can be carried out in either a melt of reagents or in an inert, high boiling solvent such as quinoline (163.2°C), imidazole (257°C), tetrahydronaphthalene (207°C) or nitrobenzene (210-211°C). Among metal ion sources that can be used are pure metals (Zn, Co, Cu, Fe), their salts (NiCl₂, AlCl₃, PtCl₂, Zn(OAc)₂), oxides (CaO, PbO, MnO₂) and sulphates (PbSO₄, BaSO₄) with the specific one to be used depending upon the conditions involved in each case. The reaction conditions themselves can vary as well. Reaction temperatures are usually quite elevated, lying between 150-300°C. However, more reactive precursors have been prepared that allow the complexation reaction to occur at temperatures as low as -10 to -20°C (Greenberg et al., 1988). Reaction times, on the other hand, can run a few minutes to several days, again depending upon the reactivity of the starting materials and the reaction conditions in use. However, usually these reactions occur quite rapidly, with typical reaction times being on the order of 5 minutes to 1 hour.

Condensation reactions of this type are notoriously messy and the resulting products contain a great deal of impurities. These comprise unreacted starting materials, linear and cyclic polymers of a non-phthalocyanine nature, metal ion salts and other resinoid materials along with metal-free phthalocyanines. As a result of this, numerous purification methods have been used in order to obtain a pure product. The more classical methods, such as column, thin layer and high performance liquid chromatography, have been applied to this problem. In fact, solid supports have been designed especially to
achieve this task. In the meanwhile, other techniques have taken advantage of the high stability of these compounds. Sublimation at elevated temperature has been used successfully to purify phthalocyanines as has dissolution in concentrated sulphuric acid followed by reprecipitation in ice. However, these methods are limited by the stability of the Pc in question and on the type of substitution it carries. Finally, the solubility of phthalocyanines and their impurities (or lack thereof) has been utilized by either washing insoluble phthalocyanines with various solvents or by extracting soluble Pcs from insoluble by-products. But, as in all cases of purification via extraction, impurities that share solubility with the desired product remain. Clearly, purification of phthalocyanines can be quite a tedious affair.

For their part, free phthalocyanine can be readily prepared via a similar template reaction using either sodium or potassium alkoxides or metallic magnesium or lithium as the metal ion source. In these cases, the metal can be easily removed by dilute aqueous acid to yield the desired free phthalocyanine. Free phthalocyanine can also be acquired by heating phthalonitriles with either an organic base such as piperidine or cyclohexylamine or with hydrogen in dioxane. In fact, these last two procedures are those used commercially to manufacture phthalocyanine free base (Jackson, 1978).

A free phthalocyanine can be transformed into its metal complex, in certain cases, by heating it in the presence of a metal ion source. However, this method is rather limited. The core of the phthalocyanine is rather small, measuring 1.35 Å from the pyrrolic nitrogens to the center of the core (Berezin, 1981). As such, only metal ions with a similar or smaller radius can readily complex with a pre-existing phthalocyanine ligand. These would include metal ions like Cu$^{2+}$, Zn$^{2+}$, Co$^{2+}$, Ni$^{2+}$, Pt$^{2+}$, Pd$^{2+}$, Al$^{3+}$, Ga$^{3+}$ and VO$^{2+}$. In the meanwhile, metal ions with radii larger than this can only form phthalocyanine complexes via a template reaction. The resulting phthalocyanine is strained to a certain extent in order to accommodate the large metal. This strain causes the phthalocyanine to lose some of its planarity, resulting in a loss of some aromatic
stabilization and overall, forming a less stable compound. Out-of-plane phthalocyanines would fit in with this group. Finally, as has been described before, lithium phthalocyanine can be used to form other metal Pc complexes, depending upon the metal salt and the solvent used.

1.32 Substitution on the Phthalocyanine Framework

Unsubstituted phthalocyanines and their metal complexes are extremely insoluble in water and the vast majority of organic solvents. This can be primarily traced to the high degree of hydrophobicity of the aromatic phthalocyanine core and due to the planarity of the molecule, which leads it to form highly stable crystal structures with high molecular lattice energies. The solubility of unsubstituted phthalocyanines in the more universal organic solvents like sulpholane, dimethyl sulfoxide (DMSO) and N,N-dimethyl formamide (DMF) is negligible. Even highly aromatic solvents such as quinoline or α-chloronaphthalene rarely give solutions of concentrations exceeding 10⁻⁵ M (Berezin, 1981). The only effective solvent is sulphuric acid at concentrations greater than 8 M. However, even in this case, the solvent acts to protonate the phthalocyanine, thus changing its properties somewhat (see previously). Therefore, even its usefulness is extremely limited.

The insolubility of unsubstituted phthalocyanines has led to extensive work being carried out in order to add substituents to the phthalocyanine framework, not only to increase their solubility in the solvent of choice but also to enhance their potential usefulness in countless possible applications. The number of possible substituents is as countless as colours in a rainbow and a vast array of chemical functionality has been added to phthalocyanines via substitution onto the Pc backbone. These run from aliphatic chains and higher order aromatics to amines, thiols, halides and acids. Even more exotic
substituents such as crown ethers and porphyrins have been added to the Pc backbone in the hopes of increasing their potential in specific applications.

In general, substitution onto a phthalocyanine can be accomplished by one of two basic methods. The first involves direct substitution onto a pre-existing phthalocyanine. An example of this is the sulphonation of phthalocyanines, which can be accomplished by heating a phthalocyanine in oleum (concentrated sulphuric acid containing 20-30% free SO₃) (Ali et al., 1988). Such harsh reaction conditions can result in substitution at any or all of the available positions (see Figure 17), leading to complex isomeric mixtures and varying degrees of substitution, thus making isolation and purification of the desired

![Diagram of a phthalocyanine molecule](image)

**Figure 17:** Potential sites for phthalocyanine substitution

product extremely difficult.

The second basic method is by condensation of a substituted precursor. This may entail the use of a commercially available precursor, synthesis of an appropriately substituted precursor or complexation of a substituted precursor followed by chemical modification of the Pc periphery. Obviously, this leads to a far cleaner reaction mixture in terms of the degree of substitution and number of isomers produced. However, while the
degree of substitution is more well-defined, this method still leads to constitutional isomers such as those shown in Figure 18. Again, isolation of these constitutional isomers can be very difficult. However, it is theoretically possible to achieve this separation due to their differing geometries and this has been accomplished recently, to a certain extent, for

Figure 18: The four constitutional isomers for a tetrasubstituted phthalocyanine
very specific phthalocyanines using specially designed HPLC columns (Hanack et al., 1993a; Hanack et al., 1993b; Schmid et al., 1996). The first complete separation of a 1,4
tetrasubstituted phthalocyanine, namely the 1,4 tetrakis(2-ethylhexyloxy)phthalocyanine
nickel complex, used a nitrophenyl-modified silica gel HPLC column (Hanack et al.,
1993b). However, in the case of 2,3 substituted PCs, complete separation is still not
possible, even with nitrophenylquinoline-based HPLC columns designed to take advantage
of π-π interactions between the solid phase of the column and the phthalocyanine. Only
enriched isomeric fractions could be obtained for phthalocyanines fitted with sterically
bulky substituents (Schmid et al., 1996).

Further complicating matters are mixed condensations, where phthalocyanines
substituted with different chemical groups are desired. This is the case in several
phthalocyanine applications. For instance, their use as Langmuir-Blodgett films requires
different substituents in order to achieve the molecular orientations necessary so that
transferred films will have similar orientations (Snow and Jarvis, 1989). For the most part,
unsymmetrically substituted phthalocyanines are synthesized by a statistical condensation
of appropriately substituted precursors followed by chromatographical isolation of the
desired product. Although this method, via trial and error, can lead to reaction mixtures
Figure 19: The six possible products synthesized during a mixed condensation (A and B represent two differently substituted isoindoline units)

enriched with the product with the desired degree of substitution, it still leads to the production of six possible products (see Figure 19). Furthermore, while column chromatography and HPLC can partially separate the different products, this is often very difficult and will still lead to products contaminated with differently substituted phthalocyanines. Thus, quite obviously, new synthetic procedures are needed in order to synthesis phthalocyanines of exact composition and of pure isomeric distribution.
1.33 Novel Synthetic Approaches

Because of the need for isomerically pure phthalocyanines and for unsymmetrically substituted phthalocyanines with precise degrees of substitution, a number of novel synthetic approaches for the synthesis of phthalocyanines have been developed. These range from the use of axial ligands to impart solubility to the Pc to the use of boron subphthalocyanines as a template in the synthesis of 3:1 substituted phthalocyanines. A few of these newer techniques will be described below.

a) Axial Ligands

As has been stated previously, the central metal atom in phthalocyanine complexes is octahedrally coordinated, with the phthalocyanine occupying the xy-plane and axial ligands in the z-plane. Therefore, one possible approach to avoid the synthesis of complex isomeric mixtures is to leave the periphery of the phthalocyanine unsubstituted and to use the nature of the axial ligand to impart solubility to the compound. Though this is not truly a novel method for the synthesis of Pcs, it is very interesting nonetheless.

One example of such a compound is shown in Figure 20. In this case, the phthalocyanine is left unsubstituted and two triphenylphosphine monosulphonate ligands are used as axial ligands coordinated to the ruthenium central metal atom (Charlesworth et al., 1994). Even though the ruthenium central metal atom is only in the +2 state, such complexes are quite stable because of the nature of the axial ligand. It is well-known that triphenylphosphine ligands form very stable complexes with most metal ions (Butler and Herrod, 1989). This is due to the strong electron donation of the aromatic rings to the phosphorus, which increases the availability of its lone pair to form strong dative $\alpha$--bonds to the metal. Furthermore, the P atom has vacant d-orbitals of $\pi$ symmetry that can participate in $\pi$ backbonding with the metal, thus further stabilizing the complex. It turns
Figure 20: Dipotassium[bis(triphenylphosphinemonosulphonate)]Ru(II)phthalocyanine
(Charlesworth et al., 1994)

out that this compound has been studied as a possible photosensitizer for the photodynamic therapy of cancer and initial results are promising. The main advantage of this compound is the bulkiness of the axial ligand, which helps prevent aggregation, thus keeping the compound more photoactive. A second example of using the axial ligand to impart the desired properties to a phthalocyanine is found in Pc4 (Oleinick et al., 1993; Rywkin et al., 1994), a silicon-based phthalocyanine that is now involved in clinical trials as a photosensitizer for the photodynamic therapy of cancer (Brown, 1996b).

Using the nature of the axial ligand to determine the desired physical properties does affect the chemistry of phthalocyanines to a certain extent, especially in terms of their aggregation. Bulky axial ligands, such as the ones mentioned above, greatly prevent aggregation from occurring by inhibiting the aromatic stacking displayed by phthalocyanines. This can greatly enhance their applicability in certain applications, especially those that utilize the photophysical and photochemical properties of phthalocyanines since self-quenching caused by such aggregation is reduced. However, their ability to stack is also very important in other application where the rather unique structure of phthalocyanines is of more importance. Therefore, the loss of this ability
would greatly decrease or even eliminate other application. As such, the use of the axial ligand to increase solubility and improve other physical properties is very useful in some cases but is extremely detrimental in others.

b) Novel Phthalocyanine Precursors

The general synthesis of phthalocyanines is a very symmetrical one, with the condensation occurring from any number of possible orientations. It is this symmetry that leads to the production of the constitutional isomers seen for tetrasubstituted phthalocyanines. As such, novel phthalocyanine precursors have been studied that eliminate this symmetry and force the condensation to occur in one direction, thus producing one isomer exclusively.

Iminothioamides have been envisioned as precursors that could be used in the synthesis of isomerically pure phthalocyanines (Baguley and Elvidge, 1957). It has been shown that dithioamides readily form phthalocyanines at relatively low temperatures (around 80-90°C) (Leznoff et al., 1987). Therefore, the use of a substituted iminothioamides should lead to the production of one single isomer. The reasoning behind this is that the imino group of one molecule should selectively displace the methylthiol group of the second, particularly at the reaction temperatures used.

![Synthesis of substituted iminothioamides](image)

Figure 21: Synthesis of substituted iminothioamides

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The substituted iminothioamide was synthesized as shown in Figure 21 (Greenberg et al., 1988). S-methylation was necessary in order for the compound to condense to form phthalocyanines. The resulting 5-substituted S-methyliminothioamide readily self-condensed to the tetrasubstituted phthalocyanine at room temperature as a mixture of isomers. However, if the condensation is carried out at -20°C in DMF using zinc acetate as a template, a single isomer has been obtained for smaller scale reactions (Figure 22). But larger scale reactions again lead to a mixture of isomers. It should be noted that the statistical distribution of isomers found above is not the same as is found for the condensation of a substituted diiminoisoindoline (Greenberg et al., 1988). Thus, a different reaction pathway must be leading to this isomeric mixture and perhaps future study along these lines will lead to the production of pure isomers.

![Chemical structure diagram](image)

R= (CH₃)₃CCH₂O

**Figure 22: Synthesis of pure 2,9,16,23 tetrasubstituted phthalocyanine**

40
Precursors such as these can also be used to produce pure disubstituted phthalocyanines during a mixed condensation. An example of this is the condensation of 1,3,3-trichloroisindoline with 5-phenyl-1,3-diiminoisoindoline (Idelson, 1977). In the presence of a base such as triethylamine and a reducing agent like hydroquinone, these condense to form exclusively the trans-diphenylphthalocyanine (Figure 31). The principals here are the same as those above. At the lower reaction temperature used for this reaction (room temperature), the diiminoisoindoline does not self-condense and, therefore, the imino group reacts only with the more reactive 1,3,3-trichloroisindoline to give substitution on opposite sides of the molecule.

Attempts have been made to extend this work to dithioamides. However, a mixture of substituted products was obtained, apparently showing a certain degree of self-condensation between the imino groups of the isoindolines and the thio groups of the dithioamides as shown in Figure 32. Such self-condensation to form phthalocyanine ligands could also be used to explain the mixed results obtained in the case of iminothioamides, where reaction between the imino groups or the thio groups could lead to the isomeric mixtures produced.

c) Polymer Support

The polymer support method can be used to synthesize unsymmetrically substituted phthalocyanines which combine two different isoindoline units in a 3:1 ratio. Such unsymmetrically substituted Pcs are of increased interest because of their potential applications in non-linear optics. Furthermore, monofunctionalized phthalocyanines provide the ability to bind Pcs to polymers or to biomolecules, such as monoclonal antibodies, without the possibility of crosslinking reactions occurring. Basically, in this method, one of the substituted precursors is covalently linked to an insoluble polymer support and this precursor is then reacted with a large excess of a second precursor in
solution. The desired unsymmetrically substituted phthalocyanine can then be readily separated from the symmetrically substituted phthalocyanine that is produced by self-condensation of the unbound precursor by a simple filtration and can be isolated by a reaction to liberate the phthalocyanine from the polymer.

An example of the use of this method is shown in Figure 23. Polymer-bound

![Polymer-bound structure](image)

Figure 23: Polymer-based synthesis of pure (4'-hydroxyphenyl)-9,16,23-triphenoxyphtalocyaninato zinc(II)

4-benzyloxyphenylphthalonitrile was reacted with an excess of 4-phenoxyphthalonitrile in the presence of zinc acetate in an appropriate solvent to give both zinc tetraphenoxyphe-
phthalocyanine (from the self-condensation of the phthalonitrile in solution) and the unsymmetrically substituted metallated product (Leznoff, 1989). Soxhlet extraction easily removes the free phthalocyanine and acid cleavage releases the desired product in a 22% yield.

Although this method is very clean and produces the desired product in good yields, it does have several limitations. If the phthalocyanine that needs to be produced is viewed as an ABBB system where A is the isoindole unit found in the phthalocyanine once and the B the unit found 3 times, then it must be possible to physically bind the precursor A to the polymer, while the precursor B must be soluble in an appropriate solvent. Thus, the number of phthalocyanines that can be produced is somewhat limited. Furthermore, a large excess of the unbound precursor is used and is effectively lost since it self-condenses to form the symmetrically tetrasubstituted phthalocyanine. If the unbound precursor is hard to come by, it is hardly desirable to lose such an excess of reagent. Finally, while yields are often very good, only small amounts of product can be obtained due to the low binding capacity of the polymeric carrier. Thus, while small scale reactions work very well, the polymer-support method is not appropriate for large scale synthesis.

d) Boron Subphthalocyanines

The organization of three isoindoline units in boron subphthalocyanines make them extremely attractive reagents for the synthesis of unsymmetrically substituted phthalocyanines. A simple ring enlargement reaction with various diiminoisoindolines should readily yield the desired unsymmetrical Pc with identical substituents on three of the peripheral benzene rings and a different one on the fourth. Such ring enlargement reactions are known to occur because of the steric strain and the resulting ineffective p-orbital overlap found in boron subPcs. However, despite its bright promise, this method
has had only limited success to date, with more recent studies finding that its potential usefulness may be quite limited.

![Image](image-url)

Figure 24: Synthesis of unsymmetrically substituted phthalocyanines using boron subphthalocyanines

Initial research (Kobayashi et al., 1990) seemed to back up the immense potential of this procedure (Figure 24). Reacting tri(t-butyl) boron subphthalocyanine with a series of diiminoisoindolines of increasing aromaticity, the predicted 3:1 substituted phthalocyanine was obtained, following column chromatography, in yields of 8-20%. Analytical and spectrographical data was used to verify the purity and identify the product as a single pure compound. The advantages of this new method were immediately recognized. The yields were good, even at 8-20%, compared to the other methods, since a pure compound could be obtained in relatively large amounts. Furthermore, the purification of the reaction mixture was deemed simple as only two easily separated bands existed, one reddish fraction containing the unreacted subphthalocyanine and one blue fraction containing the phthalocyanine product. Finally, judging from the analytical and spectrographical data, it was felt that no other Pc analogs were produced. Only the 3:1 unsymmetrically substituted phthalocyanine was obtained. As such, this new synthetic
approach seemed ideal. This conclusion was verified by the synthesis of pure monosubstituted phthalocyanines fitted with crown ether substituents using this method (Musluoglu et al., 1992). Characterization of the product in this case also pointed to a pure monosubstituted product being formed in good yield.

Following these very promising initial studies, extensive work was carried out using this method in order to synthesize other unsymmetrically substituted phthalocyanines and it was found that the reaction is not so straightforward. First of all, several studies have reported the presence of chlorinated products in the reaction mixture (Weitemeyer et al., 1995; Dabak et al., 1994; Rauschnabel and Hanack, 1995). This is not all that surprising since, as was stated before, halogenated subphthalocyanines are a by-product formed via a BCl$_3$-catalyzed electrophilic aromatic substitution reaction during the preparation of subPcs. As such, the ring enlargement reaction involving these chlorinated subPcs would lead to chlorinated phthalocyanines. The separation of the chlorinated compounds from either the intermediate subphthalocyanine or from the final product is very difficult and thus, an impurity has been added to the final product that can affect its

![Chemical Reaction Diagram]

Figure 25: Attempted synthesis of pure monoamino- and mononitro-substituted phthalocyanines using a boron subphthalocyanine
usefulness in several applications.

Of far more importance are the cases where a pure 3:1 substitution ratio is not obtained. Instead, a mixture of differently substituted phthalocyanines is produced. This is found in several instances, for example, in the reaction of boron subphthalocyanine with both 5-amino-1,3-diiminoisoindoline and 5-nitro-1,3-diiminoisoindoline (as shown in Figure 25) (Sastre et al., 1995). The reaction with the amino-substituted isoindoline lead to the production of the desired monoaminophthalocyanine along with some unsubstituted Pc. In the meanwhile, the nitro-substituted iminoisoindoline yields to all the possible

![Molecular structure](image)

**Figure 26**: Proposed mechanism for the ring enlargement of boron subphthalocyanines

substituted phthalocyanines, having either none, one, two, three or four nitro groups. To help explain this previously unobserved behavior, the following mechanism was proposed (Figure 26). In it, it was assumed that the diiminoisoindoline unit reacts with the subphthalocyanine to form an open four-membered intermediate (A) which can cyclize to
give the desired monosubstituted product. However, A can also undergo a cleavage, promoted either thermally or by the attack of the diiminoisoindoline or solvent molecules to yield dimers such as B and C. Self-condensation of B in the case of amino-substitution would lead to the unsubstituted Pc while condensation of B and C would produce the desired monosubstituted product as well.

This reaction mechanism should be general for all ring enlargement reactions of subphthalocyanines. The relative importance of each pathway will be dependent upon the substituents involved (and therefore the reactivity of the starting material) and on the reaction conditions used. As such, the products given for the reaction involving 5-nitro-1,3-diiminoisoindoline can also be explained. The increased reactivity of the nitro compound would promote self-condensation and this fact, along with the intermediates proposed in the mechanism above, can be used to readily explain the mixture of phthalocyanines produced.

Similar findings were obtained for the reaction of boron subphthalocyanine with both 5-(4-t-butylyphenoxy)-1,3-diiminoisoindoline and 5,6-dimethyl-1,3-diiminoisoindoline (Weitemeyer et al., 1995). In this case, a series of experiments were carried out under the conditions used for the reaction (2:1 mixture of DMSO and chloronaphthalene, 80-90°C, 24 hours) in order to explain the formation of the product mixture. First of all, the stability of the boron subphthalocyanine was studied and it was found to be stable under these conditions. The boron subPc and phthalonitriles (instead of diiminoisoindolines) did not react to form phthalocyanines. Furthermore, the diiminoisoindolines used only produced traces of the corresponding tetrasubstituted product under these conditions. And finally, under the influence of the weakly basic zinc(II) acetate dihydrate, the subPc was converted into unsubstituted ZnPc whereas no reaction was observed in the case of the neutral zinc(II) chloride dihydrate. All these experiments led to the assumption that the subPc did not undergo a concerted ring enlargement reaction but instead was involved in a multi-step process. The first step was theorized to be a base-catalyzed decomposition
of the subPc. These would most likely be followed by a second step where the reactive fragments produced by this decomposition would react with each other and with the diiminisoindolines present in the reaction mixture, leading to ring closure and formation of the differently substituted phthalocyanines. Furthermore, these reactions might not lead to ring closure, thus producing polynitriles, which were observed in the UV-visible spectra and have been isolated from such reaction mixtures (Wohrle, 1972). Finally, such a multi-step reaction pathway would help explain the poor yields of these ring enlargement reactions, which can not be really explained by a concerted process, that should usually give good yields.

Despite these drawbacks, this method still has some utility. In work done using more soluble substituted boron subphthalocyanines, mixtures of substituted phthalocyanines were not observed in the final product (Dabak et al., 1994; Kudrevich et al., 1996). It was assumed that, in these cases, the lower reaction temperatures (40°C instead of 80°C), the shorter reaction times and the nature of the substituents on the boron subphthalocyanine (either an alkyl thiol or a sulfo group) could have caused the ring enlargement reaction to occur cleanly.

1.34 Mechanism of Phthalocyanine Formation

Though it has been the subject of considerable study and speculation, the mechanism of formation of phthalocyanines is still not fully understood. One of the main problems involved in determining the mechanism for these reactions is that phthalocyanines can be prepared by many different routes and although some of them may proceed via common intermediates, it is not necessarily true that all follow the same mechanism. In addition, due to the high reaction temperatures used, the high relative reaction rates and their very nature, the intermediates are themselves difficult to isolate and identify.
Even with these inherent difficulties, there are several definite clues as to the mechanism involved that give a tantalizing picture of phthalocyanine formation. During his initial work with phthalocyanines, Linstead concluded that, for the condensation of o-cyanobenzamide, iminophthalimidine could be an intermediate (Byrne et al., 1934). He based this on the fact that iminophthalimidine is readily formed via the isomerization of o-cyanobenzamide above its melting point. Further proof that iminophthalimidine is an intermediate in phthalocyanine formation comes from the synthesis of phthalocyanines

![Chemical structures](image)

**Figure 27:** Iminophthalimidine and diiminiosoindoline as intermediates in the synthesis of phthalocyanines

using phthalic anhydride as the precursor and urea as an amminating agent. In this case, it can be readily shown via $^{14}$C-labeling experiments (Broomfield et al., 1964) that the urea
provides the nitrogen for the macrocycle but none of the carbon. The phthalic anhydride must have been converted to iminophthalimidine and then to diiminoisoindoline before Pc formation. Both of these two intermediates have been isolated from reaction mixtures and their conversion to Pcs has been shown (Elvidge and Linstead, 1955).

Diiminoisoindolines are known to readily react to form phthalocyanines and are in fact extensively used in the synthesis of substituted phthalocyanines using subphthalocyanines. As such, the possibility of them being intermediates in the formation of phthalocyanines via other routes cannot be denied. Evidence showing the plausibility of diiminoisoindoline intermediates comes from the reaction of diiminoisoindolines with diamines, which readily give a stable 2:1 adduct (Figure 28) (Elvidge and Golden, 1957).

![Reaction Diagram]

Figure 28: The 2:1 adduct products by the reaction of diiminoisoindoline and diamines

Such a product would seem to show the pathway for imidine-imidine condensation and the relevance of such intermediates in the synthesis of Pcs.

Although it is difficult to isolate reaction intermediates in the case of phthalocyanines, a few have been obtained and identified and these few examples provide further clues into the nature of these reactions (Figure 28). For instance, in the preparation of metal-free phthalocyanines using sodium methoxide, a sodium salt of methoxyiminoisoindoline (I) was isolated (Borodkin, 1958). Such an intermediate would seem to indicate that the iminophthalimidine is a possible step along the route towards
condensation. In the meantime, nickel complexes (II and III) were isolated during the synthesis of nickel phthalocyanine (Hurley et al., 1967). In this case, these intermediates show that the metal ion involved in the condensation can act like a template around which the phthalocyanine can be formed. Finally, lithium salts such as IV have been isolated during the synthesis of a lithium tetranitrophthalocyanine (Oliver and Smith, 1987). This
Figure 30: Dimeric and trimeric intermediates postulated in phthalocyanine formation

reaction must proceed by a stepwise approach with the intermediate diiminoisoindoline derivative condensing to form dimers and trimers before finally giving the desired phthalocyanine (Figure 30).

In addition to more direct evidence for the mechanism of Pc formation, there is a lot of indirect evidence, in particular in the synthesis of substituted phthalocyanines and in their resulting isomeric distribution. Of special interest are syntheses that involve novel phthalocyanine intermediates. An example of this is shown in Figure 31, which is a rare

Figure 31: Synthesis of a pure disubstituted phthalocyanine
bona fide example of the synthesis of a pure disubstituted Pc (Idelson, 1977). When 5-phenyl-1,3-diiminoisoindoline is reacted with 1,3,3-trichloroisoindoline at room temperature in the presence of a base such as triethylamine and a reducing agent such as hydroquinone, pure trans disubstituted phthalocyanine is produced in a 7% yield. It is interesting to note that no cis disubstituted product is produced. Such a unique result gives information on the mechanism involved. In this case, the self-condensation of the substituted diiminoisoindoline, which would lead to a tetrasubstituted product, does not occur because of the low temperature used for this reaction. Therefore, the imidine must react readily with the 1,3,3-trichloroisoindoline to yield dimers that react rapidly to produce the phthalocyanine. Furthermore, since only the trans product is observed, the imidine end of one of the dimers must react with the chloro end of the second. It should be noted that such dimeric intermediates are most likely far more reactive than the 5-phenyl-1,3-diiminoisoindoline starting material since the intramolecular reaction occurs far more readily than the intermolecular reaction. As such, these dimers cannot be intercepted nor can their existence be proven.

A second such cross condensation reaction is shown in Figure 32 where an unsubstituted 1,3-diiminoisoindoline is condensed with 5-neopentoxy-1H-isoindole-1,3(2H)dithione, in a 1:1 or a 15:1 ratio, in N,N-dimethylaminoethanol (Leznoff et al., 1987). It was expected that this reaction would proceed like the one discussed previously, with the only product being the cis disubstituted product. However, in this case, all the possible isomers where obtained, from the unsubstituted Pc through the mono, di, tri and tetra substituted ones. At the reaction conditions used in this case, neither of the starting materials should self-condense, so the formation of substituted phthalocyanines other than the desired disubstituted product was a surprise. However, it did give some insight into the possible mechanism involved. Because of the distribution of the products, dimeric and trimeric intermediates like those shown in Figure 30 were postulated. However, even though such intermediates are possible and even reasonable, they have not been isolated

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Figure 32: Dithiones in the synthesis of pure single phthalocyanine isomers and, as such, cannot be proven.

The synthesis of a zinc tetrabutylphthalocyanine gives extensive clues into the mechanism of Pc formation (Gaspard and Maillard, 1987). As was stated previously, in most cases, tetrasubstituted phthalocyanines are produced in a statistical distribution of four possible isomers (see Figure 18). However, when 4-t-butyl-phthalonitrile was condensed with a reducing metal such as zinc, only one isomer was produced. This was proven by NMR analysis of the final product which readily shows that there were two sets of magnetically equivalent t-buty1 groups. This is only possible for one of the four potential isomers. Similar observations have been made for germanium tetra-t-butyl phthalocyanine. However, in a NMR analysis of a zinc tetrakis(ethyl)phthalocyanine, no such distinction could be made as several signals existed in the range for the protons of the methyl group, seemingly indicating that all the possible isomers were produced.

One possible explanation for this phenomenon is steric hindrance, which would explain why this was observed for bulky t-butyl groups but not for the much smaller methyl substituents. However, a second possible explanation, proposed by Gaspard and Maillard (Gaspard and Maillard, 1987), would seem to help elucidate the mechanism of phthalocyanine formation. In effect, the two nitrile groups on the 4-t-butyl-phthalonitrile can be seen as being non-equivalent, with the nitrile in the para position to the t-butyl substituent being far more polarized due to the presence of an electron-donating group.
such a t-butyl group para to it. In addition, electrochemical studies have shown that two of the four phthalonitriles involved in these condensations are reduced (Yang et al., 1980). Therefore, intermediates such as those shown in Figure 33 have been suggested as possible intermediates in the formation of the Pcs (Baumann et al., 1956; Gaspard and Maillard, 1987). However, these intermediates do not fully explain the production of only one isomer. Since two neutral intermediates combine with two reduced intermediates during this reaction to form the phthalocyanine, it is quite clear that they can combine in one of two ways (Figure 34). From the NMR data, however, it is known that only combination A occurs, as it is the only one that will lead to two sets of magnetically equivalent tertiary butyl groups. Thus, for some reason (either electrostatic or steric), only this combination occurs, leading to the single pure isomer that is obtained. As can be seen, combination A leads to the combination of two reduced species and two neutral species. Perhaps, the reduced and neutral species react more rapidly with their own kind for some unknown reason, leading to dimers of reduced-reduced and neutral-neutral
Figure 34: The two possible combinations for the condensation of Pc intermediates (Gaspard and Maillard, 1987). The R represents the reduced species while the N shows the neutral species.
nature. This would drive the reaction towards combination A, leading to the observed single product. However, it should not be forgotten that this is a template reaction and the zinc may also play a part in this process.

In conclusion, both Elvidge and Linstead have pointed out that during the condensation of diiminoisoindoline, a hydrophthalocyanine should be obtained and, therefore, a reductant must act sometime during the formation of the Pc in order to get the desired product (Elvidge and Linstead, 1955). The above work theorizes that this reduction occurs on the phthalonitrile (or precursors) prior to complexation. However, it is not known when this reductant acts for sure. Hence, as can be clearly seen, although several clues exist for the mechanism of phthalocyanine formation, a lot of work is still left to be done.
1.4 Applications of Phthalocyanines

Traditionally, phthalocyanines have been used as dyes and colouring agents due to their intense blue-green colour. In fact, over 45 000 tons of phthalocyanine were produced by industry in 1987 alone (Leznoff and Lever, 1996). They are used primarily in inks for ballpoint pens, as a colourant of plastics and metal surfaces and as dyestuff for jeans and other clothing. Much of the early work done on these compounds was supported by the dye and textile industry who recognized the potential that phthalocyanines had in their business. More recently, the use of phthalocyanines as photoconducting agents in photocopying devices has also been demonstrated and put into practice (Leznoff and Lever, 1996).

Even with these rather important industrial uses, it is in the coming decades that the field of phthalocyanines is expected to explode. Few other classes of compounds have been heralded as being of potential usefulness in such a vast array of widely diverging fields. Because of their unique electronic spectra, phthalocyanines have been publicized for its utility in 1) photodynamic therapy as a treatment of cancer (Spikes, 1986; Rosenthal and Ben-Hur, 1989; van Lier and Spikes, 1989; Foote, 1990; van Lier, 1990; Paquette and van Lier, 1991; Oleinick et al., 1993; Charlesworth et al., 1994; Rosenthal, 1996) and a method for the sterilization of blood components (Rywkin et al., 1994; Allen et al., 1995; Rosenthal, 1996), 2) optical storage devices and other computer applications (Shirk, 1996), 3) electrochromic displays (Bardin et al., 1989; Shirk, 1996), 4) solar cells (Wöhrle et al., 1996), and 5) sensing elements in chemical sensors (Snow and Barger, 1989). However, it is the peculiar structure with its high degree of aromatic stability and its planar nature that has lead to the study of phthalocyanines as 1) catalysts for numerous chemical reactions, especially for the control of sulfur in effluents and gasoline (Moser and Thomas, 1983, Schneider et al., 1994, Chen and Rathke, 1996; Kasuga, 1996), 2) liquid crystals (Piechocki et al., 1982), 3) molecular metals and conducting polymers (Wöhrle,
1989), 4) Langmuir-Blodgett films (Snow and Jarvis, 1984; Snow and Barger, 1989; Wöhrle et al., 1996), and 5) electrocatalyst in fuel cell generation (Jasinski, 1964; Jasinski, 1965; Jahnke, 1968; Jahnke et al., 1976; Moser and Thomas, 1983). As can be clearly seen, phthalocyanines have enormous possibilities with implications in such wide ranging fields as medicine, the environment, energy and information. Clearly, phthalocyanines can be expected to have significant ramifications in the future even if only some of their potential is fulfilled.

Because of the numerous applications possible for phthalocyanines, both those presently used and those proposed for the future, a general look into the more important and more interesting possibilities is all that can be accomplished here.

1.41 Dyes and Colouring Agents

As was stated previously, phthalocyanines are first and foremost of interest as dyes and colour agents. Their extremely high thermal stability, inertness to both acidic and alkaline conditions, insolubility in most solvents and fastness to light along with their high dyeing power and the incredible purity and intensity of their colour has ensured the reputation of phthalocyanines in the painting, dyeing, textile and paper industry as superior quality blue, blue-green and green pigments. Industry produced over 45 000 tons of phthalocyanine in 1987 alone, primarily as dyestuff for jeans and clothing and as colourant for inks in ballpoint pens (Leznoff and Lever, 1996). Due to their ease of synthesis and the ready availability of the starting materials, phthalocyanine dyes are inexpensive to produce. In 1983, phthalocyanines sold for between $7-10 per pound in the United States (Moser and Thomas, 1983), a remarkably low price considering the intensity of their colour and the minimal amounts needed in colouring processes.

Unsubstituted phthalocyanines cannot be used directly as dyestuffs because of their extreme insolubility in the vast majority of solvents. However, some metallo-
phthalocyanine complexes can be reduced by dithionite to give more soluble vat dyes (Struve, 1955; Jackson, 1978). When steeped onto textile material and exposed to air, these dyes are reoxidized to the insoluble form and precipitate in the fiber, leading to a coloured product. An example of this is cobalt phthalocyanine, which is known as indanthrene brilliant blue 4G (Berezin, 1981).

In the meanwhile, a number of more soluble phthalocyanines have been synthesized and investigated as potential dyestuffs. In fact, the first patent involving phthalocyanines and their use as dyes, granted in 1929, included a water-soluble polysulphonated phthalocyanine (Dandridge et al., 1929). Since then, functional groups such as sulphonic acids, sulphonyl chlorides, amides, thiols, and tertiary and quaternary ammonium compounds, among others, have been added to the phthalocyanine to impart solubility and to increase their ability to fix to fibers (Bigelow, 1955; Struve, 1955; Booth, 1971; Vollman, 1971). Sulpho derivatives of copper phthalocyanine are valuable direct dyes for cotton and are known as Sirius turquoise and direct light-fast turquoise (Berezin, 1981). Thiol-substituted phthalocyanines can also be applied to cotton in a sulfide bath by oxidation to the disulfide (Booth, 1971; Struve, 1955; Jackson, 1978). In the meanwhile, carboxy-substituted phthalocyanines are very useful as pigments in bulk colouring of polyamides and polyesters when added during the course of polymerization (Struve, 1955; Jackson, 1978). Finally, one of the more popular methods used by the textile industry is phthalogenic dyeing of fabrics (especially cotton and other cellulose material) (Vollman, 1971; Jackson, 1978). This involved the synthesis of the phthalocyanines directly on the fibers, using diiminoisoindoline precursors as intermediates.

Most metal ions that form stable complexes with phthalocyanines have been used in dyes (Bigelow, 1955). These include cobalt (II), nickel (II), zinc (II) and aluminum (III). However, the most widely used of the metallophthalocyanines are the copper (II) complexes. They accounted for around 25% of the total pigment production in the United States in 1978 (Jackson, 1978). This popularity is mainly due to the extreme stability of
the copper complexes and their ease of preparation. Furthermore, pigmentary copper phthalocyanine has increased fastness as compared to other phthalocyanine pigments and often has a sharper, darker colour. For instance, nickel phthalocyanine pigments are distinctly duller and more green in colour (Bigelow, 1955) and as such, are less desirable for most applications. In contrast, cobalt dyes are more reddish in colour and are more susceptible to oxidation, making them unsatisfactory as a colourant in paints. Whereas the cobalt-based vat dyes (Indanthrene Brilliant Blue 4G) are as stable as any vat dye, their fastness to peroxide or chlorine bleach is poor (Bigelow, 1955). As such, copper phthalocyanines are the dyes of choice in the vast majority of cases.

Overall, it is very important to remember that while phthalocyanines have so many possible and far reaching applications in the future, all the preliminary work done on this class of compounds was because of its ability as a colouring agent. And it is there that phthalocyanines have found their most important use to date.

1.42 Photodynamic Therapy

Photodynamic therapy (PDT) is a promising new modality for the treatment of cancer and viral infections and the sterilization of blood products. It is based upon the dye-sensitized photooxidation of biological matter in the target tissue and requires three basic components: 1) a photosensitizer, which is a compound capable of absorbing light of a specific wavelength and transforming it into useful energy, 2) light of that specific wavelength and 3) molecular oxygen. One of the main attractions of PDT is in its basic concept of combining two therapeutic agents, the photosensitizer and light. Neither are harmful by themselves but together, along with molecular oxygen, they ultimately will cause the desired biological damage, be that tumor destruction or viral kill.

It is thought that photodynamic therapy invokes its photooxidation via two major pathways, as shown in Figure 35 (MacRoberts et al., 1989; Foote, 1990). In both
pathways, the photosensitizer absorbs light and jumps to an excited singlet state. Then, through intersystem crossing, a percentage of these excited molecules shift to the longer lived triplet state. In the type I mechanism, the excited photosensitizer reacts with the biological substrate, producing free radicals and radical ions. These radical sites are then fixed by the oxygen present in the tissue to produce oxygenated products that will ultimately lead to the cytotoxic effects seen in PDT. The type II mechanism, on the other hand, has the triplet state of the photosensitizer imparting its excitation energy to

Figure 35: The proposed mechanism of photodynamic action (adapted from Foote, 1990)
molecular oxygen, resulting in excited oxygen species such as singlet oxygen. These then react with the substrate to give the oxygenated products that cause the desired biological effect.

The photodynamic effect has probably been known for centuries, with early civilizations using plant extracts and sunlight to cure skin afflictions. As early as 1400 B.C., psoralens, which are well-known photosensitizers, were extracted from the seeds of Psoralea corylifolia and used as a treatment for vitiligo (Daniell and Hill, 1991). However, photodynamic action was not formally introduced until 1900 when Raab noticed the effects of acridine orange on paramecia and its dependence on the intensity of the light in the laboratory (Raab, 1900). The phrase "photodynamic action" was first coined in 1907 by von Tappeiner and Jodlbauer who extensively studied this effect (von Tappeiner and Jodlbauer, 1907). Since then, a tremendous amount of work has been done in studying the plausibility of using photodynamic therapy as a treatment against cancer and viruses. At present, several photosensitizers have either been accepted into clinic or are now involved in clinical trials. The most important of these is Photofrin®, a hematoporphyrin derivative enriched in the more active ether and ester oligomers (Dougherty, 1996). To date, Photofrin® has been approved in Canada for the palliative treatment of oesophageal cancer and the prophylactic treatment of superficial bladder cancer. In the Netherlands, its use in the treatment of superficial lung cancer and in the palliation of advanced lung and oesophageal cancer has been accepted. France has also approved its use in the treatment of recurrent lung and oesophageal cancer while the treatment of inoperable superficial oesophageal and gastric cancer, early lung and cervical cancers and cervical dysplasia with Photofrin® has been authorized in Japan. Finally, the United States has accepted its use in the palliative treatment of oesophageal cancer in patients deemed unsuitable for Nd:YAG laser therapy (Brown, 1996a). Methylene blue, a well-known phenothiazine tricyclic dye, is being used by the Swiss and German Red Cross for the sterilization of freshly frozen plasma units (Mohr et al., 1995). Among other potential photosensitizers in clinical trials
are benzoporphyrin derivative and aminolaevulinic acid (Dierickx and Anderson, 1996), which itself is not a photosensitizer but is metabolized in situ into protoporphyrin IX, a precursor to heme. It is the protoporphyrin IX that acts as the photosensitizer in this case.

Being the first generation photosensitizer, Photofrin® has several serious disadvantages, including the unknown composition of the mixture, its relative weak absorbance at the wavelength of interest (Rosenthal and Ben-Hur, 1989) and its retention by epidermal tissue, which leads to prolonged skin photosensitivity (Dierickx and Anderson, 1996). Furthermore, it shows only marginally higher tumor retention as compared to most surrounding healthy tissue (van Lier and Spikes, 1989). Therefore, second generation photosensitizers are being examined. An ideal photosensitizer should have the following characteristics (MacRoberts et al., 1989):

1) It should be chemically homogeneous and of known and constant composition.

2) It should have minimal dark toxicity and only be harmful in the presence of light.

3) It should be preferentially retained by the target tissue.

4) It should be rapidly excreted from the body, thus inducing a low systemic toxicity.

5) It should have high photochemical reactivity (high $\phi_T$, long $\tau_T$) and be able to effectively produce singlet oxygen and other reactive oxygen species.

6) It should have a strong absorbance, with a high extinction coefficient ($\varepsilon$), at a
longer wavelength, between 600 and 800 nm, where tissue penetration is at a maximum while still being energetic enough to produce singlet oxygen. Furthermore, cheaper diode lasers can be used in this range, increasing the potential usefulness of this technique in a clinical setting.

Phthalocyanines meet most of these criteria. They are easily synthesized, are highly stable and can be obtained in a high degree of chemical purity. They are of known composition and show a minimal dark toxicity (Rosenthal and Ben-Hur, 1989). Preferential tumor uptakes have been demonstrated for some phthalocyanines at 24 to 48 hours post-injection while normal tissues show a maximum uptake between 2 to 3 hours post-injection (van Lier and Spikes, 1989; van Lier, 1990; Paquette and van Lier, 1991). Monomeric phthalocyanines complexed with a diamagnetic central metal atom have high triplet yields ($\phi_T > 0.4$) and long triplet lifetimes ($\tau_T > 0.1$ msec) and can effectively produce singlet oxygen, which is believed to be one of the more important reactive species involved in PDT (Spikes, 1986). The triplet energy for phthalocyanines is between 110-126 kJ/mole while the energy needed to produce singlet oxygen is 94.5 kJ/mole (van Lier and Spikes, 1989). Finally, the Q band absorption for phthalocyanines is red-shifted as compared to Photofrin® ($\lambda_{max}$ for Pc = 680 nm, $\lambda_{max}$ for HpD = 630 nm) towards more favorable wavelengths, where tissue penetration is greater while still being energetic enough to produce singlet oxygen. In addition, this absorption is of two orders of magnitude greater in strength when compared to the Q-band of Photofrin® ($\varepsilon$ for Pc > $10^5$ M$^{-1}$cm$^{-1}$, $\varepsilon$ for HpD = $10^3$M$^{-1}$cm$^{-1}$) (Dolphin, 1994). Due to these characteristics, phthalocyanines have been extensively studied as photosensitizers in photodynamic therapy. In fact, one phthalocyanine, chloro-aluminum sulphonated phthalocyanine is presently involved in clinical trials (Dierickx and Anderson, 1996) while two others, a silicon-based phthalocyanine (Pc4) and a disulphonated compound are about to enter clinic trials as well (Brown, 1996b). In addition, numerous phthalocyanines with different
central metal atoms, peripheral substituents and axial ligands have been examined for their potential as PDT agents (Boyle and Dolphin, 1996). Third generation photosensitizers are also being designed and include antibody conjugates and photosensitizers that absorb between 700-800 nm, where red light is still more tissue penetrating (Dierickx and Anderson, 1996). Phthalocyanines are among the more promising third generation photosensitizers as well, due to the ease by which substituents can be added to their framework and the small effect these functional groups have on their electronic properties. Furthermore, extending the aromaticity of phthalocyanines to form napthalocyanines readily yields photosensitizers that absorb at higher wavelengths (740-780 nm) while still being able to produce singlet oxygen (van Lier and Spikes, 1989). Naphthalocyanines have been studies as PDT agents. However, they appear to be somewhat limited due to their strong tendency to aggregate in solution and their propensity towards degradation via photooxidation.

Overall, phthalocyanines are very promising photosensitizers for photodynamic therapy and numerous comprehensive investigations have been undertaken to determine the prospects of using various phthalocyanine derivatives in the PDT of cancer and viral infections and in the sterilization of blood components. With several phthalocyanines either in or about to enter clinical trials, their future in this field seems secure.

1.43 Phthalocyanine Catalysts

Unlike in their use as both dyes and photosensitizers, phthalocyanine catalysts rely not on the photochemical properties of the compound but on their unique structure. The planar nature of phthalocyanine complexes, with easy access to the central metal atom and the presence of extra coordination sites, make them ideal for chemisorption and the variability of the central metal atom make them extremely useful in electrochemical reactions.
Phthalocyanines as heterogeneous catalysts for reactions involving
dehydrogenation, oxidation and electrochemistry have several advantages (Moser and
Thomas, 1983):

1) They form complexes with a vast array of metal ions, making them extremely
useful as electrocatalyst.

2) They are extremely stable to variations in temperature and in pH.

3) They form square planar complexes with the metal, thus
leaving two octahedral sites available for complexation of additional ligands that
could be involved in the reaction.

4) The benzene rings give the opportunity to introduce chemical substituents on
the periphery of the macroring $\pi$ system. By carefully selecting the functional
group that is added, electrons can either be donated or withdrawn from the $\pi$
system, which would be useful in electrochemical processes as well as in
chemisorption of molecules onto the Pc surface.

5) Substituted complexes can be dissolved in organic solvents. This allows the
study of the physical and chemical properties of the metal ion in solution, where it
is in the same environment as it would be in as a surface catalyst.

Thus, it is clear that phthalocyanines can be extremely useful as catalysts in numerous
chemical reactions.

One of the more interesting reactions catalyzed by phthalocyanines is the
electroreduction of oxygen. The underlying importance of this is the development of
useful fuel cells such as those used in manned spacecrafts. In a typical fuel cell (Jahnke, 1976; Moser and Thomas, 1983), the two gases (hydrogen and oxygen) are taken into the cell where each comes into contact with a porous electrode of either nickel or graphite. These two electrodes are separated by an electrolyte such as potassium hydroxide. The theoretical reaction at the cathode is:

\[ \text{O}_2 + 2 \text{H}_2\text{O} + 4e^- \rightarrow 4\text{OH}^- \]

However, in practice, the actual reaction involves the formation of peroxide ions:

\[ \text{O}_2 + \text{H}_2\text{O} + 2e^- \rightarrow \text{HO}_2^- + \text{OH}^- \]

Embedded on the porous electrode are catalyst that hasten the decomposition of the peroxide ions, leading to further production of hydroxide ions:

\[ 2\text{HO}_2^- \rightarrow \text{O}_2 + 2\text{OH}^- \]

The hydroxide ions then diffuse to the anode, where they are involved in the oxidation of the hydrogen:

\[ \text{H}_2 + 2\text{OH}^- \rightarrow 2\text{H}_2\text{O} + 2e^- \]

Thus, the overall reaction is therefore:

\[ \text{O}_2 + 2\text{H}_2 \rightarrow 2\text{H}_2\text{O} \]

In separate work, Jasinski and Jahnke first recognized that phthalocyanines catalyzed the oxygen reduction reaction at the cathode (Jasinski, 1964; Jasinski, 1965;
Jahnke, 1968; Jahnke et al., 1976). Since then, extensive work has been done to fully utilize and understand these catalysts. It is believed that phthalocyanine catalysts work like is depicted in Figure 36 (Beck, 1973). The oxygen molecule becomes adsorbed onto the phthalocyanine, which is chemically bound to a solid support. The phthalocyanine is then oxidized by the oxygen, leading to the production of the reduced oxygen species. The catalyst is then reduced in an electrochemical follow-up step.

![Figure 36: Mechanism of phthalocyanine catalysis](image)

The nature of this catalytic activity must be linked to the adsorption of oxygen onto the phthalocyanine catalyst. There are several lines of experimental evidence to back up this hypothesis. It is well-known that all phthalocyanines display some catalytic activity and that the amount of activity depends greatly on the nature of the central metal atom. The order of increasing catalytic performance is Fe > Co > Ni > Cu > H₂, with the metal-free phthalocyanine showing very little activity (Randin, 1974). This would seem to verify that the oxygen becomes absorbed onto the phthalocyanine as an axial ligand and the nature of the central metal atom affects the electrochemical steps of the reaction.

Further evidence comes from the fact that addition of CN⁻, PO₄²⁻ or ethylenediaminetetraacetic acid (EDTA) to the reaction poisons the catalyst (Kozawa et
al., 1971; Beyer and Von Sturm, 1972). This can be readily explained since these species are much stronger ligands than oxygen and, as such, will readily displace the oxygen from the axial ligand position of the phthalocyanine, thus making the catalyst ineffective. Other possible ligands, such as halide ions (Cl\(^{-}\), Br\(^{-}\)) or SO\(_4\)\(^{-2}\), which are weaker ligands, only slightly decrease the effectiveness of the catalyst (Kozawa et al., 1971) as they only compete for the last coordination position and will not entirely lock out the oxygen as would a ligand like EDTA.

One final line of evidence rests in the findings that the electrochemical activity of the catalyst depends upon the crystal structure of the phthalocyanine (Moser and Thomas, 1983). Most solid compounds can exist in any number of crystal forms, depending upon the origination of the molecules in the crystal. It has been shown that phthalocyanines may exist in as many as eight different crystal forms (\(\alpha\), \(\beta\), \(\epsilon\), \(\delta\), \(\pi\), \(\gamma\), \(X\) and \(R\)) with each having the individual phthalocyanine molecules orientated differently within the crystal (Moser and Thomas, 1983). The nature of each phthalocyanine crystal depends greatly on the method of preparation. For instance, the \(\alpha\) phase crystal can be obtained by fast vacuum deposition and has the phthalocyanine molecules in a planar orientation. On the other hand, the \(\beta\) phase is acquired by slow vacuum deposition with the phthalocyanines in a more diagonal alignment. It turns out that the \(\alpha\) form has a higher catalytic activity than does the \(\beta\) form (Moser and Thomas, 1983), which would follow from the above assumptions since the phthalocyanine in the \(\alpha\) form is more available for oxygen adsorption.

Phthalocyanines have also found use as a catalyst in the oxidation of sulfur-containing compounds, which has very important implications in terms of the environment. In fact, Pc catalysts are involved in a patented process for the treatment of aqueous effluent (Urban and Rosenwald, 1973a; Urban and Rosenwald, 1973b), where the catalyst removes ammonium hydrosulfide salt contained in waste water. Primarily used are cobalt and vanadium phthalocyanines, though iron, nickel, copper, molybdenum, manganese and
tungsten have also been shown to be useful. Furthermore, any suitably substituted phthalocyanine can also be used. The basic process involves the treatment of the aqueous waste stream that contains NH₄HS to produce treated water that is substantially free of (NH₄)₂S₂O₃ and hopefully with the recovery elemental sulfur and ammonia. This procedure is particularly important in gasoline refinement and the petroleum industry where removal of sulfur from effluents is extremely important. Numerous other patents have been granted for sulfur removal processes that are catalyzed by phthalocyanines (Moser and Thomas, 1983). In addition to this, phthalocyanine complexes have also been examined for their ability to oxidize sulfur-containing compounds in gasoline and other petroleum products (Chen and Rathke, 1996) and in smokestack effluents (Moser and Thomas, 1983).

Phthalocyanines have also been shown to catalyze several other oxidation reactions, including the oxidation of hydrocarbons, phenols, sugars, alcohols, aldehydes and ketones (Moser and Thomas, 1983, Chen and Rathke, 1996). In addition, the reduction of acetylenes, hydrogenation reactions, epoxidations, and the decomposition of hydrogen peroxide, organic peroxides and formic acid have been demonstrated using phthalocyanines as a catalyst (Moser, 1983). Finally, the chemical fixation of carbon dioxide into organic molecules has been shown to be aided by the presence of a phthalocyanine catalyst (Kasuga, 1996). This reaction is of special importance in synthetic organic chemistry where the addition of a carbonyl group to a molecule not only increases its size by one carbon atom but also increases its chemical versatility. From all this, it can be clearly seen that phthalocyanines are extremely important compounds as catalysts in organic, inorganic and environmental chemistry.
1.5 Radiopharmaceuticals

1.51 General Discussion

A radiopharmaceutical can be defined as a radioactive compound used in the diagnosis and therapeutic treatment of human diseases. In nuclear medicine, the vast majority of such radiopharmaceuticals are used for diagnostic purposes, with over 95% of them being used for the detection and examination of disease and other abnormalities (Saha, 1992). A radiopharmaceutical basically has two components, a radionuclide and a pharmaceutical, and the overall value of the compound is dictated by the characteristics of these two components. The pharmaceutical portion of the molecule is chosen so that the compound is preferentially localized in the target tissue or is involved in the physiological process that is to be examined. The radionuclide that can effectively tag the pharmaceutical is then chosen so that the compound can be detected within the body and the morphological structure or the physiological function of the organ of interest can be accessed.

Radiopharmaceuticals should possess certain very important characteristics, especially since they are to be administered to humans. In addition, there are severe limitations in the detection of radiation with currently used instruments and this must be taken into account as well. The characteristics needed include the following (Saha, 1992):

1) These compounds should be easily synthesized and purified and ideally be available in a kit so that the manipulations done by nuclear medicine technicians are minimized. They should be readily available and inexpensive so that any nuclear medicine department can have access to them.
2) They should have short effective half-lives, where an effective half-life takes into account both the physical half-life of the radionuclide and the biological half-life of the radiopharmaceutical. This is given by:

$$T_e = \frac{T_p \times T_b}{T_p + T_b}$$

Overall, the effective half-life of the compound should be no longer than the time necessary to complete the study in question.

3) The radionuclide involved should have minimal particle emission so that the radiation doses given to the patient is as small as possible.

4) To avoid particle emission, it is favourable that the radionuclide decay via electron capture or isomeric transition. Obviously, positron emission is also a possibility but the common isotopes used in PET imaging have very short half-lives and are not as widely available because they can only be produced using a cyclotron. Furthermore, PET cameras are not as widely used as their SPECT counterparts, thus again limiting their usefulness. Furthermore, the radionuclide should emit a $\gamma$ radiation with energy between 30 and 300 keV. Below 30 keV, there is too much attenuation by the body and the signal is lost. Above 300 keV, the sensitivity and selectivity of presently used detectors decreases, thus leading to poorer results.

5) The radiopharmaceutical should have a high target-to-nontarget ratio, with preferentially target tissue uptakes as to minimize the amount of radiation that needs to be administer to the patient in order to get a good picture that is easy to analyze.
Overall, these criteria are so stringent that very few radiopharmaceuticals are ideal. The ones used in any given situation is a compromise between these characteristics.

1.52 Technetium

Technetium is a Group VIIIB transition metal in the same family as manganese and rhenium. No stable isotopes of technetium exist in nature and it was, in fact, the first artificially produced element, being first detected by Perrier and Segre in 1937 in a sample of molybdenum that had been bombarded with deuterons (Lide, 1992). Since this detection, searches for this element in terrestrial matter have been undertaken without success. However, evidence for its presence has been found in the spectrum of S-, M-, and N-type stars and its existence in stellar matter has lead to new theories for the production of heavy elements in stars (Lide, 1992).

Having an atomic number of 43, the electronic structure of neutral technetium is 1s^2 2s^2 2p^6 3s^2 3p^6 3d^10 4s^2 24p^6 4d^6 5s^1. As such, technetium can exist in eight oxidation states, ranging from -1 to +7, resulting from the loss of 4d and 5s electrons or the gain of an electron in a 4d orbital (Saha, 1992). The stability of these oxidation states depends greatly on the ligands surrounding the technetium ion and its chemical environment (Schwochau and Pleger, 1993). The +4 and +7 states are the most stable and are found in the oxides, sulfides, halides and pertechnetates. However, the lower states (-1, +1, +2 and +3) are possible and are normally stabilized by complexation with ligands. For instance, the +1 state is found in the radiopharmaceutical ^{99m}Tc	ext{-}sestamibi, where technetium is complexed to 6 isonitrile groups (Hjelstuen, 1995).

Nineteen isotopes of technetium are known, with mass numbers ranging from 90 to 108 (Lide, 1992). However, the most interesting of all these is metastable technetium 99 (^{99m}Tc). It turns out that this isotope is ideal for use in nuclear medicine (Saha, 1992; Schwochau, 1994; Hjelstuen, 1995). It has a short half-life of only 6 hours and its decay
to ground state $^{99}$Tc results in a $\gamma$ transition of 140 keV, which is ideal for presently used SPECT imaging devices. There is no particle emission, thus keeping the radiation doses given to the patient as low as possible and allowing the injection of activities of more than 30 mCi without tissue damage (Schwochau, 1994). Finally, it is readily available from Moly generators, where it is eluted off a column as a decay product of bound $^{99}$Mo (Saha, 1992). To show the importance of $^{99m}$Tc in nuclear medicine, it should be noted that over 80% (Saha, 1992), and maybe as high as 90% (Hjelstuen, 1995), of all radiopharmaceuticals used today are $^{99m}$Tc-labeled compounds.

It should be noted that $^{99m}$Tc can only be used in tracer amounts and therefore, it is very difficult to characterize its complexes. More often than not, the chemical behavior of technetium complexes is determined with the help of the much longer lived $^{99}$Tc isotope, with its half-life of 2.13x10$^5$ years (Grummon et al., 1995; Tisato et al., 1995), which can be used at a slightly large scale (even in gram quantities). However, care must be taken since $^{99}$Tc is radioactive, decaying via a $\beta^-$ emission of 293 keV. $^{99}$Tc has a specific activity of 6.2x10$^8$ Becquerels/gram (17 $\mu$Ci/mg) and therefore must be considered a contamination hazard (Lide, 1992). However, if it is handled carefully in a small scale, it should not present a health hazard.

1.53 Technetium Complexes

The technetium-$99m$ that is used in nuclear medicine is acquired from commercially available Moly generator as sodium pertechnetate ($\text{Na}^{99m}\text{TcO}_4$). This salt, with the technetium in the +7 oxidation state, is a rather unreactive species and does not label any compounds directly. Because of this, it is necessary to reduce the technetium in the presence of a suitable ligand in order to obtain stable complexes. Among potential reducing agents are stannous chloride, stannous citrate, stannous tartarate, concentrated HCl, sodium borohydride and sodium hydrosulfite. Of these, the most important is
stannous chloride as it is used in the vast majority of cases in which the labeling is done in acidic aqueous media. However, the others are useful in specific cases as well. For instance, with stannous tartarate, the tartarate ion can act as a trans-chelator, complexing to the reduced technetium species and protects it from hydrolysis before it is complexed by the desired compound. On the other hand, sodium hydrosulfite is useful under more basic conditions.

The resulting reduced $^{99m}$Tc species (usually in the +5 oxidation state, thought it really depends upon the nature of the ligand and the reducing agent used) are chemically reactive and can form complexes with any number of possible ligands (Saha, 1992). Complexes exist with the technetium having coordination numbers ranging from 4 to 9 (Schwochau and Pleger, 1993). In analogy to the central metal atom in phthalocyanine complexes, the ligands form dative σ bonds with the metal by donating a pair of electrons to empty orbitals of the metal. As such, the ligands usually involve functional groups such as carboxylic acids (-COOH), alcohols (-OH), amines (-NH$_2$) and thiols (-SH) that can effectively donate electrons to the metal. General technetium complexes are shown in Figure 37, where the technetium is shown in either the square pyramidal or octahedral geometry (Saha, 1992; Grummon et al., 1995; Tisato et al., 1995). Furthermore, it should be noted that the technetium is often bonded with one or two oxygen atoms when complexed, as is shown below.

![Figure 37: Structures of oxotechnetium complexes](image)

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As was stated previously, $^{99m}$Tc complexes may make up as much as 90% of all compounds used in nuclear medicine. These complexes cover a wide range of pharmaceuticals that are intended to carry the $^{99m}$Tc to the desired location within the body. They are used to study a large number of physiological functions and to image the vast majority of organs and tissues in the body. Everything from the regional cerebral blood flow in the brain to the functioning of the hepatobiliary system can be visualized and studied using $^{99m}$Tc complexes, some of which are shown in Figure 38. For instance, hexamethylpropylene amine oxime (HMPAO), a tetracentate ligand, is used to measure the regional cerebral blood flow within the brain (Hjelstuen, 1995). Another tetracentate ligand, mercaptoacetylglycylglycylglycine (MAG3), on the other hand, is used routinely in the assessment of renal function and in determining effective renal plasma flow (Fritzberg et al., 1986; Cleynhens et al., 1994; Hjelstuen, 1995). Bone and skeletal abnormalities are imaged using phosphates and phosphonates such as 1-hydroxyethylidene diphosphonate (HEDP) (Saha, 1992; Hjelstuen, 1995). The heart traditionally calls for the use of isonitriles such as methoxyisobutyl isocyanide (MIBI) which is a octahedral complex with the $^{99m}$Tc coordinated to 6 isocyanate groups (Hjelstuen, 1995). In addition to synthetic complexes such as these, it is also possible to label proteins such as albumin (used in blood pool imaging studies), colloids such as sulfur colloids (used in the imaging of liver morphology and in gastric emptying studies), aggregates and microspheres such as macroaggregated albumin (used in lung perfusion imaging) and even red blood cells (used for blood loss studies) (Saha, 1992). Thus, the usefulness of $^{99m}$Tc complexes in nuclear medicine is clear.

One major weakness in $^{99m}$Tc complexes is that no $^{99m}$Tc radiopharmaceutical exists for the imaging of tumors, unless the tumor causes a physiological change in the tissue that can be seen using one of the complexes mentioned above. At present, the most widely used radiopharmaceutical for the imaging of tumors, $^{67}$gallium citrate, has several
limitations (Ali, 1996). It lacks selectivity, resulting in poor tumor uptakes in many common malignancies. Higher uptakes are seen for normal tissue, such as in the liver or bones, which obscures imaging in those regions. Finally, $^{67}$Ga citrate is slowly cleared from the blood, leading to high background readings. In order to get around these drawbacks, attempts have been made to label tumor-specific antibodies with $^{99m}$Tc, either directly to the sulfhydryl groups of the antibody (Griffiths et al., 1994) or by attaching a chelator to the antibody that is capable of forming stable complexes with technetium (Ram
and Buchsbaum, 1994). However, to date, these methods have been of limited usefulness in nuclear medicine, primarily because of loss of specificity.

1.54 Radiolabeled Phthalocyanines

The idea that radiolabeled phthalocyanines might be useful in the imaging of tumors is based on the finding of Policard, who in 1924 observed an intense red fluorescence in tumors when they were exposed to ultraviolet light (Policard, 1924). He proposed that this was due to the accumulation of porphyrins from microbial sources present in the necrotic and infected portions of the tumor. Shortly after, it was established that this same effect could be induced in tumors by injecting hematoporphyrin, thus suggesting a preferential tumor uptake (Korbler, 1932). However, further work showed that this phenomenon was not only restricted to cancer cells but could be seen for all tissues in the state of rapid cell division, be it neoplastic, embryonic or traumatized tissue (Figge et al., 1948). Even with this, it seemed like porphyrins could provide a useful diagnostic tool for tumor detection in nuclear medicine. And, since phthalocyanines are stable porphyrin derivatives, logic dictates that radiolabeled phthalocyanines might too be of interest. Furthermore, radiolabeling of phthalocyanines would also be beneficial in helping to optimize photodynamic therapy protocols as well as to aid in the determination of the biodistribution of such photosensitizers. Finally, by choosing the appropriately labeled phthalocyanine, one can envision the phthalocyanine being used to locate the tumor and treat it via PDT all in one step.

Phthalocyanines can be radiolabeled by three basic methodologies. First of all, radioactive isotopes can be synthetically added either into ($^{14}$C) or onto ($^{3}$H, radioactive iodine) the phthalocyanine ligand. Tritium, for instance, can be easily added to the phthalocyanine by catalytic exchange with the hydrogen atoms in the parent molecule. Phthalocyanines labeled in this way are only really useful in autoradiographic studies.
however and some ambiguous results can be obtained due to in vivo exchange (Ali, 1996). Carbon-14 can be added synthetically to the molecule by using $^{14}$Cphthalic acid as a precursor (Rousseau et al., 1990). Being a particle emitter, these compounds are useful in the determination of the in vivo and in vitro distribution of phthalocyanines. However, they are of little use in nuclear medicine. The main advantage found in the use of tritium or carbon-14 is that the chemical and pharmacological properties of the substrate are not altered as would be the case upon the addition of a chelator or by using a different central metal atom. However, as radionuclides, they are of little use in nuclear medicine.

In addition to this, radioactive iodine can be added to the benzene rings of the phthalocyanine via nucleophilic aromatic substitution reaction (Lenaerts et al., 1995). This tends to be done by substitution of a diazo salt and therefore, it must be done prior to complexation. As such, extensive purification is needed in order to obtain a single product, and this greatly reduces the usefulness of this method for nuclear medicine purposes. Furthermore, poor yield are generally obtained (25-50%) and the resulting phthalocyanine tends to be less soluble in water and would require a vehicle to transport it to the target.

Quite obviously, radioactivity can be introduced into the molecule by using a radioactive metal ion as the central metal atom and complexes with radioactive zinc, copper and gallium have been produced. This has been accomplished by either direct insertion of the radioactive metal ion into a pre-existing phthalocyanine ligand or by forming the ligand around the metal ion via a template reaction. Such radiolabeled phthalocyanines have shown some interesting biological properties. For instance, a tetrasulphonated phthalocyanine using $^{64}$Cu as the central metal atom has been shown to accumulate in considerably greater amounts in sites of cerebral injury and in brain tumors than it does in the surrounding normal tissue (Figge et al., 1948). $^{67}$Ga tetrasulphonated phthalocyanine, while having a decreased tumor uptake as compared to the standard $^{67}$Ga citrate, showed higher tumor-to-blood and tumor-to-muscle ratios (Rousseau et al., 1985;
Rousseau et al., 1990; Šcasnár and van Lier, 1993). This would indicate that it is more rapidly cleared from the blood than is the gallium citrate. Finally, $^{65}$Zn was used to radiolabel a series of sulphonated phthalocyanines in order to determine their in vivo biodistribution and it was found that the tri- and tetrsubsstituted phthalocyanines accumulated the most with optimal tumor-to-blood ratios occurring 24-48 hours post-injection (Ali, 1996). However, despite these promising results, such compounds are of only limited usefulness in nuclear medicine because of poor isotopic properties and inefficiency in labeling. $^{65}$Zn, for one, is unsuitable for medical purposes because of its long half-life of 243.8 days and its energetic $\gamma$ of 1.12 MeV (Ali, 1996). The synthesis of $^{67}$Ga tetrarsulphonated phthalocyanine, on the other hand, results in poor yields, especially by nuclear medicine standards, with the direct chelation with a phthalocyanine having maximum yields of only 63% while the less beneficial template reaction gives labeling yields of around 33% (Rousseau et al., 1985). Finally, while these reactions are quite simple, they still require extensive purification in order to obtain a pure labeled compound.

As such, they are still far from the simple add-and-shake kit preparations preferred in nuclear medicine. In addition, their isotopes, while available, are not as widely used in nuclear medicine, where over 80% of all labeled compounds involve $^{99m}$Tc and the majority of the rest use a radioactive iodine (Saha, 1992).

Because of this last point, work has been done to incorporate technetium into phthalocyanines as their central metal atom. It turns out that direct labeling of a pre-existing phthalocyanine ligand with technetium fails, leading to no detectable complex formation (Rousseau et al., 1983; Rousseau et al., 1985). This is despite attempts using different reducing agents such as sodium dithionite, sodium bisulfite, formamidine sulfinate, hydroxylamine, ascorbate-sulphuric acid, borohydride and concentrated hydrochloric acid. Various concentrations of reagents and reaction times were also tried without success. This lack of complex formation is presumably due to the bulkiness of the reduced oxotechnetium species and the confined size of the macrocyclic cavity. It is
known that $^{99m}$Tc is large, with a radius of 1.358 Å (Schwochau and Pleger, 1993) and that the technetium often chelates as a oxospecies which further increases its size. As such, the reduced technetium species simply cannot fit into the phthalocyanine core directly to form a radiolabeled product. To circumvent this problem, a water-soluble tetrasulphonated phthalocyanine was synthesized using technetium as the central metal atom via a template reaction. This involved heating a mixture of sodium sulphopthalic acid, urea, ammonium chloride and ammonium molybdate in the presence of sodium pertechnetate ($[^{99m}$Tc]$\text{NaTcO}_4$) and a reducing agent, hydroxylamine, at 230°C (Rousseau et al., 1985). In this case, hydroxylamine was chosen as the reducing agent mainly because the standard stannous chloride reducing agent could lead to the production of tin complexes. Three major Pc-based products were isolated from this reaction after extensive purification. It has been clearly established that one of these compounds has a specific activity corresponding to one mole of $^{99m}$Tc per phthalocyanine ligand while the other two fit closer to 2 Pc ligands per $^{99m}$Tc. It has been proposed that the first compound corresponded to an out-of-plane phthalocyanine complex, with the Tc sitting just above the plane of the flat Pc and being chelated to three of the four pyrrolic nitrogens (Rousseau et al., 1983; Rousseau et al., 1985). The remaining coordination sites would be occupied by either oxygen, hydroxyl groups or water molecules. This fits with known data for N-substituted iminodiacetates, which form stable octahedral Tc(III) dimer complexes (Loberg and Field, 1978). Furthermore, a similar tridentate complex has been suggested for porphyrin complexes of lower oxidation state technetium (Tsutsui et al., 1975). The other two complexes are most likely sandwich compounds, with the technetium perhaps chelated to three of the four pyrrolic nitrogens in each ring, therefore fitting with the preference that technetium shows to form 6 coordinate complexes. Such bonding would help explain the existence of two chromatographically different sandwich complexes as there would be geometrical differences depending upon which nitrogens were involved in bonding. The two complexes could be seen as being either cis or trans,
depending upon whether the triplet of bonding nitrogens were on the same side or on different sides of the technetium ion. However, the existence of two different sandwich compounds could also be the result of different oxidation states of the technetium being involved in each of the two complexes.

The in vivo stability of the $^{99m}$Tc phthalocyanine tetrasulphonated complex was indicated by the absence of specificity to the thyroid and stomach where free pertechnetate would accumulate (Rousseau et al., 1985). The rest of the in vivo distribution of these compounds is not as promising however. Tumor/muscle and tumor/blood ratios were similar to free pertechnetate and did not reach important values within 24 hours post-injection. The technetium chelates accumulated rapidly in the kidneys while also showing relatively high accumulations in both the liver and the spleen. Interestingly, the uterus and ovarian follicles retain the radiolabeled compound as well. In contrast, gallium phthalocyanine tetrasulphonate has been shown to have a preferential tumor/blood and tumor/muscle ratios and was taken up more by the liver than by the kidneys (Rousseau et al., 1985). This would seem to confirm the differences in structure as discussed above as a simple change in the nature of the central metal atom would not be expected to cause such a drastic change in the pharmacological properties of the compound.

In addition to the unfavourable tumor/blood and tumor/muscle ratios, which could possibly be corrected by changing the substitution pattern of the phthalocyanine, the major disadvantage of labeling in this way is the method of production. Since the technetium cannot be added to a pre-existing phthalocyanine, there is a need for extensive purification in order to obtain a pure compound that could be of use in a nuclear medicine setting. This makes the template reaction useless in terms of nuclear medicine because the time required to get the pure compound results in a great loss of radioactivity. Furthermore, there is no possibility of a kit preparation using this method, especially with the extensive column and HPLC chromatography that would be required. Therefore, phthalocyanine ligands containing $^{99m}$Tc as the central metal atom have not been applicable to nuclear
imaging and have only found applications in determining pharmacokinetics and biodistribution of photosensitizers, along with helping to optimize PDT protocols.

Phthalocyanines can also be labeled by addition of a chelating agent (a molecule that can bind to a metal ion by more than one coordinate covalent bond and that can be covalently attached to the molecule of interest) to the periphery of the molecule. Diethylenetriaminepentaacetic acid (DTPA) has been conjugated to phthalocyanines via amine linkages in order to bind $^{111}$Indium to the macrocycle (Ali, 1996). $^{111}$In has chemical and radiochemical properties similar to $^{99m}$Tc ($T_1/2 = 2.8$ days, $\gamma = 171.2$ keV and 245.3 keV) and has found applications in the labeling of leukocytes, platelets and antibodies (Saha, 1992). The metal does not form stable complexes with phthalocyanines (Ali, 1996) but will readily complexed with tetradeutate chelators such as DTPA when they are attached to the phthalocyanine framework. A series of DTPA-substituted phthalocyanines have been prepared and each has been successfully labeled with indium-111. These stable complexes revealed a significant liver and spleen retention in animal models, with the mono-DTPA-substituted phthalocyanine leading to the best tumor/blood and tumor/muscle ratios (Ali, 1996). This procedure for radiolabeling phthalocyanines, unlike the previous one, can be readily incorporated into a kit with the simple addition of the radioactive element to the chelator leading to a radiolabeled product. Furthermore, this chemistry can be transferred over to technetium due to the similarity between its coordination properties and those of indium. Finally, by carefully choosing the correct chelator to add to the Pc framework, a useful technetium complex may be acquired. The only disadvantage of this method of labeling phthalocyanines is that the addition of a chelating handle to the periphery of the phthalocyanine will change its localizing properties, thus making this method less appropriate for determining the biodistribution of these compounds. However, by carefully selecting the chelator added, the tumor uptake and the tumor/normal tissue ratios can perhaps be optimized.
Radiolabeled phthalocyanines have to date found only limited usefulness, mainly in the determination of the pharmacokinetics and biodistribution of complexes that have potential as photodynamic therapy agents. However, since they have been shown to have preferential tumor uptakes, they still have potential in nuclear medicine.
1.6 Research Objectives

The objective of this research was the synthesis of water-soluble phthalocyanines bearing novel substituents. These phthalocyanines would be selected so as to be of potential usefulness in both photodynamic therapy and nuclear imaging of cancer. Substituents were chosen in order to impart water-solubility to the macrocycle while making the compound more biocompatible. Furthermore, the functional groups added to the phthalocyanine periphery were carefully selected so that they would have the potential to chelate metastable technetium-99m, which is extensively used in nuclear medicine.
2. Material and Methods
2. Material and Methods

2.1 Synthesis:

Unless otherwise stated, all reagents were purchased from Aldrich Canada (Mississauga, Ontario) and were used without further purification. All common solvents were purchased from BDH Inc. (Toronto, Ontario) with the exception of absolute ethanol, which came from Commercial Alcohols Inc. (Brampton, Ontario). Nitrogen was acquired from Praxair (Mississauga, Ontario).

4-Aminophthalonitrile (1).

This compound was prepared following a modification of the reported literature procedure (Marcuccio et al., 1985). To 300 ml of absolute ethanol and 15 ml of distilled water in a 500 ml round bottom flask were added 5.06 g (2.92x10^{-2} moles) of 4-nitrophthalonitrile. 564 mg of 10% palladium on carbon were added as a catalyst and the flask was stoppered with a septum. Hydrogen (Praxair, Mississauga, Ontario) was bubbled through the suspension with stirring for 6 hours. The progress of the reaction was followed by thin layer chromatography (TLC) using 5% acetonitrile in dichloromethane as eluant (PE Sil G/UV silica gel TLC plates, Whatman Paper Ltd., Kent, UK). Once all the starting material had been reacted and there remained only one spot on the TLC, the reaction mixture was filtered and the solvent removed under vacuum to give the product as a pale yellow solid in a nearly quantitative yield (96%). (Reaction Scheme 1)
4-Iodophthalonitrile (2).

Using a slightly modified method (Marcuccio et al., 1985), 3.58 g of 4-aminophthalonitrile (2.50x10^{-2} moles) were suspended in 100 ml of 2.5 M sulphuric acid (36N stock solution from J. T. Baker Inc., Phillipsburg, New Jersey). This was cooled to -5°C in an ice/salt bath and a solution of 2.20 g of sodium nitrite (3.19x10^{-2} moles) in 10 ml of distilled water was added dropwise. Once all of this solution had been added, the reaction mixture was stirred at -5°C for 30 minutes.

After 30 minutes, the reaction mixture was filtered cold (-5°C) and the resulting orange filtrate was added portionwise, with stirring, at -5°C to a solution of 5.22 g of potassium iodide (3.14x10^{-2} moles) in 30 ml of distilled water. A brown, foamy solid immediately was produced. Once all the KI solution had been added, the reaction mixture was warmed to room temperature and the brown solid was collected by filtration.

This brown solid was dissolved in benzene and this solution was washed successively with 25 ml of cold distilled water, 5% sodium bicarbonate in water, cold distilled water, saturated sodium thiosulphate in water and brine. The organic layer was then dried over anhydrous sodium sulphate, filtered and evaporated under vacuum to dryness. This gave the product as a slightly yellow compound in a 73% yield. (Reaction Scheme 1)

4-Diethoxyphosphonophthalonitrile (3).

The basic phosphorylation procedure was adapted from Bigge (Bigge et al., 1992). To a 100 ml round bottom flask were added 3 ml of diethyl phosphite (2.33x10^{-2} moles) and 15 ml of triethylamine (Fisher Scientific, Nepean, Ontario). This was flushed with nitrogen for 15 minutes and then 989 mg of tetrakis(triphenylphosphine)palladium(0) were added. All this was kept under an inert nitrogen atmosphere.

In the meanwhile, 4.88 g of 4-iodophthalonitrile (1.92x10^{-2} moles) were dissolved in 30 ml of toluene with the aid of sonication. The solution was degassed and quickly
added to the diethylphosphite reaction mixture. The reaction was then heated to 100°C under nitrogen for 18 hours.

After 18 hours, the reaction mixture was cooled to room temperature and the solvent was removed under vacuum. The resulting dark oil was then purified by column chromatography on silica gel using 40% methanol in toluene as eluant (Silica gel 60, particle size 0.063-0.2 mm, 70-230 mesh, E. M. Science, Gibbstown, New Jersey). The fractions containing the desired product were pooled and evaporated to dryness. The resulting yellowish solid was recrystallized from methanol and from acetonitrile/ether to yield the product as a pure, white solid in a 60% yield. (Reaction Scheme 1)

**Zinc tetra(diethoxyphosphono)phthalocyanine (4).**

123.2 mg of diethoxyphosphonophthalonitrile (4.66x10^{-4} moles) were added to 109.4 mg of zinc acetate dihydrate (4.98x10^{-4} moles) and 470.2 mg of imidazole. After grounding the solids up and thoroughly mixing them with a mortar and pestle, the mixture was added to a 10 ml test tube and the solid mixture was heated at 180°C in a sand bath. The imidazole quickly melted and the reaction mixture soon became dark green. After 20 minutes, the reaction mixture was cooled to room temperature and was washed with cold methanol until the methanol washings were colourless. It was then washed successively with distilled water, DMF and methanol. Following this, the product was dried at 100°C overnight. Note that similar phthalocyanine complexes were made using cuprous chloride, nickel chloride hexahydrate, cobalt chloride dihydrate, aluminum chloride hexahydrate and ferric chloride, with the metal ion source in similar molar excesses as above. (Reaction Scheme 2)

**Zinc tetraphosphonophthalocyanine (5).**

Crude zinc tetra(diethoxyphosphono)phthalocyanine (roughly 100 mg) was suspended in 10 ml of 6N hydrochloric acid (12 N stocks from Anachemia, Toronto,
Ontario) and was refluxed under an inert atmosphere (nitrogen). After 20 hours, the reaction was cooled to room temperature and the resulting blue solid was collected by centrifugation. It was then washed three times with 45 ml of 1N HCl and then with 45 ml of methanol. The resulting blue solid was dried in an oven at 100°C for 16 hours to yield the desired product as a blue solid in good yield. Note that complexes containing copper, nickel, cobalt, aluminum and iron were also prepared using the same methodology. (Reaction Scheme 3)

*Metal-free octacyanophthalocyanine (6).*

Using a slightly modified procedure (Wöhrle et al., 1980), 4.65 g of 1,2,4,5-tetracyanobenzene (2.61x10⁻² moles) were suspended in 250 ml of 1-propanol and added to a 500 ml three-necked round bottom flask fitted with a condenser and a dropping funnel. This suspension was set refluxing.

In the meanwhile, 70.4 mg of lithium metal (1.01x10⁻² moles) were dissolved in 50 ml of 1-propanol via sonication. Once all was dissolved, this lithium propylate solution was added dropwise to the refluxing reaction mixture. Quite rapidly, the 1,2,4,5-tetracyanobenzene dissolved and the reaction mixture turned dark green. After all of the lithium propylate had been added, refluxing was continued for another hour.

The reaction was then cooled to room temperature and filtered. The filtrate was evaporated to dryness under vacuum to give a dark green solid. This solid was washed thoroughly with diethyl ether in a Buchner funnel until the ether washings were colourless. The yield of the desired product was approximately 40%. (Reaction Scheme 4)

*Metal-free octacarboxyphthalocyanine (7).*

Based on the procedure suggested in the literature (Wöhrle et al., 1980), 60 ml of tri(ethyleneglycol), 5 ml of distilled water and 6 g of sodium hydroxide (0.15 moles) (Fisher Scientific, Nepean, Ontario) were placed into a 100 ml round bottom flask. In
order to completely dissolve the sodium hydroxide, this mixture was sonicated for 2 hours. 1.01 g of the metal-free octacyanophthalocyanine (1.41x10⁻³ moles) were added to this solution and the reaction mixture was heated to 160°C for 4 days with the addition of 1 ml of distilled water every day through the condenser.

After 4 days, the hot reaction mixture was added to 500 ml of cold water. The hard pellet that remained fixed in the reaction vessel was dissolved in water (with sonication), filtered and added to the above aqueous solution. Acidification with concentrated HCl caused the product to precipitate from solution and this solid was collected by centrifugation. This green solid was redissolved in 1N NaOH and reprecipitated by the addition of 1N HCl. The solid was again collected by centrifugation and was washed twice with 45 ml of methanol. Following drying at 100°C in an oven, the desired product was obtained as a blue solid in a 55% yield. (Reaction Scheme 4)

**Zinc octacarboxyphthalocyanine (8).**

509.5 mg of metal-free octacarboxyphthalocyanine (5.87x10⁻⁴ moles) were dissolved in 25 ml of DMF along with 174.2 mg of zinc acetate dihydrate (7.94x10⁻⁴ moles) (Fisher Scientific, Nepean, Ontario). This was heated to 100°C for 3 hours.

The reaction was cooled to room temperature and the solvent was removed under vacuum. The resulting blue solid was washed extensively with distilled water and with acetone. Following this, it was dissolved in 1N NaOH and precipitated by the addition of 1N HCl. This was repeated three times. The product was then washed with methanol until the methanol retained a slight green colour. Drying in the oven at 100°C gave the desired product in nearly quantitative yields. (Reaction Scheme 4)

**Diethylidioxsuccinate (9).**

This compound was prepared according to a described procedure (Fox, 1947). 10.21 g of disodium dihydroxytartarate (3.89x10⁻² moles) (Janssen Chimica, Beerse,
Belgium) were suspended in 180 ml of absolute ethanol. This was cooled to 0°C in an ice/salt bath and hydrogen chloride gas (Canadian Liquid Air Ltd., Montreal, Quebec) was bubbled through the suspension at a moderate rate for 3 hours. The reaction was then stoppered tightly and was stored at 4°C for 3 days.

After 3 days, the reaction mixture was filtered to remove the precipitated sodium chloride and the filtrate was evaporated to dryness under vacuum to give a yellowish oil. This crude oil was distilled under water aspiration (10 mmHg) in order to obtain the desired product, which was obtained between 120°C and 124°C as a yellowish oil in a 65% yield. (Reaction Scheme 5)

2,3-Dicyano-5,6-diethoxycarbonylpyrazine (10).

As has been reported in the literature (Kudrevich et al., 1994), 2.76 g of diethyldioxosuccinate (2.55×10^{-2} moles) and 5.11 g of diaminomaleonitrile (2.53×10^{-2} moles) were added to a mixture of 500 ml of absolute ethanol and 20 ml of glacial acetic acid (Fisher Scientific, Nepean, Ontario). Following refluxing for 3 hours, the reaction was cooled to room temperature and the resulting solid was removed by filtration. The solvent was removed from the filtrate under vacuum and a brown solid was obtained. This solid was purified by column chromatography on silica gel 60 using chloroform as eluant. Yellowish crystals were obtained. Further purification was accomplished by recrystallization from ethanol. This gave the desired product as white crystals in a 72% yield. (Reaction Scheme 5)

Zinc tetra-2,3-(5,6-diethoxycarbonylpyrazino)porphyrazine (11).

The literature procedure for the synthesis of this compound was followed (Kudrevich et al., 1994). 520 mg of 2,3-dicyano-5,6-diethoxycarbonylpyrazine (2.11×10^{-3} moles) were mixed thoroughly with 478 mg of zinc acetate dihydrate (2.17×10^{-3} moles). The resulting solid mixture was added to a 50 ml round bottom flask and was wet with
quinoline (Fisher Scientific, Nepean, Ontario). This was heated at 170°C until the reaction became blue-green.

The reaction was cooled to room temperature. Following the addition of dichloromethane, the reaction was filtered through a cotton plug and the solvent evaporated. The resulting green solid was then purified over silica gel 60 using dichloromethane and 1:1 acetone/dichloromethane as eluant. This led to the desired product being obtained as a green solid in a 26% yield. (Reaction Scheme 5)

**Zinc tetra-2,3-(5,6-dicarboxyphyrazino)porphyrazine sodium salt (12).**

The deesterification of zinc tetra-2,3-(5,6-dioxycarboxylpyrazino)porphyrazine was done as reported in the literature (Kudrevich et al., 1994). 42.7 mg of the ester (3.67x10^-5 moles) were suspended in a solution of 45 ml of saturated methanolic NaOH and 15 ml of distilled water. This suspension was sonicated for 30 minutes and solid obtained was collected by centrifugation. It was washed twice with 45 ml of a 3:1 methanol/water solution.

The remaining solid was dissolved in a minimum amount of water and was acidified with 1.2N HCl (about 2 drops). Ethanol was then added to the solution until the product precipitated from solution. The product was collected and this procedure was repeated a second time. The resulting product was then dried at 100°C. Yields were quantitative. (Reaction Scheme 5)

**Zinc tetra-2,3-(5,6-dichloroformylpyrazino)porphyrazine (13).**

Prior to reaction, a sample of zinc tetra-2,3-(5,6-dicarboxyphyrazino)porphyrazine sodium salt was acidified by dissolving a sample of the salt in distilled water and carefully reprecipitating by adding concentrated HCl dropwise until precipitate appeared. The resulting solid was then washed with distilled water until the washings were neutral.
128.5 mg of this sample \((1.38 \times 10^{-4} \text{ moles})\) were dissolved in 6 ml of DMF. Once dissolved, 1 ml of thionyl chloride \((\text{SOCl}_2)\) \((\text{J. T. Baker, Phillipsburg, New Jersey})\) was added and the reaction was heated at 80°C for 4 hours. After 4 hours, the reaction was cooled to room temperature and the excess \(\text{SOCl}_2\) was removed under vacuum. This crude solution of acid chloride was used directly in the next step. (Reaction Scheme 6)

**Zinc tetra-2,3-(5,6-dicarboglycylglyclypyrazino)porphyrazine (14) Method A.**

To the crude DMF solution of acid chloride prepared above was added a 13.3 fold excess of glycylglycine \((243 \text{ mg})\) \((1.84 \times 10^{-3} \text{ moles})\). This was allowed to stir for 2 hours at 100°C. Following cooling to room temperature, the solvent was removed via rotary evaporation to yield an impure green solid in a low yield \((38\%)\). HPLC chromatography of the reaction mixture indicated the presence of multiple reaction products (see Appendix B-2). (Reaction Scheme 6)

**Zinc tetra-2,3-(5,6-dicarboxsuccinimidylpyrazino)porphyrazine (15).**

173 mg of zinc tetra-2,3-(5,6-dicarboxypyrazino)porphyrazine \((1.85 \times 10^{-4} \text{ moles})\) were added to 15 ml of tetrahydrofuran \((\text{THF})\) \((\text{BDH Inc., Toronto, Ontario})\). After cooling to 0°C in an ice bath, 208.8 mg of N-hydroxysuccinimide \((1.81 \times 10^{-3} \text{ moles})\) were added and the reaction was stirred for 5 minutes. At this time, a solution of 363 mg of dicyclohexylcarbodiimide \((\text{DCC})\) \((1.75 \times 10^{-3} \text{ moles})\) in 10 ml of \(\text{THF}\) was added dropwise and the reaction was maintained at 0°C for 2 hours. It was then warmed to room temperature and stirred overnight.

Following 18 hours, the reaction was quenched by the addition of 1 ml of glacial acetic acid. The solution was stirred for 1 hour at room temperature \((\text{RT})\) and was then filtered. The resulting filtrate was evaporated to dryness to give the active ester as a green solid. This crude product was used as is in the next step. (Reaction Scheme 7)
Zinc tetra-2,3-(5,6-dicarboglycylglycylpyrazino)porphyrazine (14) Method B.

The crude green product obtained above was dissolved in 10 ml of methanol. To this was added a solution of 325.2 mg of glycylglycine (2.46x10^{-3} moles) in 5 ml of distilled water. The reaction was then refluxed for 4 hours. The reaction was cooled to 60°C and stirred for 1.5 hours and was then cooled further, to room temperature, where it remained overnight.

After 24 hours of total reaction time, the solvent was removed by bubbling air through the solution. The resulting solid was taken up in water and the pH was adjusted to 12. Acetone was added to the solution until a blue precipitate formed. The precipitate was collected by centrifugation and was washed twice with 10 ml of acetone and once with 10 ml of acetone containing 2 drops of 1N HCl. The product was dried in the oven at 100°C overnight. A crude green solid was obtained in a 27.6% yield from the starting zinc tetra-2,3-(5,6-dicarboxypyrazino)porphyrazine. HPLC analysis of this green material indicated a number of different products being present (see Appendix B-3). (Reaction Scheme 7)

Zinc tetra-2,3-(5,6-dicarboxylsulfosuccinimidylpyrazino)porphyrazine (16).

17 mg of zinc tetra-2,3-(5,6-dicarboxypyrazino)porphyrazine (1.82x10^{-5} moles) and 51.5 mg of N-hydroxysulfosuccinimide (2.37x10^{-4} moles) (Pierce, Rockford, Illinois) were dissolved in a minimum amount of distilled water and were cooled to -5°C in a ice/salt bath. Once cooled, a solution of 96.6 mg of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) (5.04x10^{-4} moles) (Pierce, Rockford, Illinois) in 5 ml of distilled water was added dropwise to the reaction mixture over a period of 10 minutes. The reaction was then stirred at -5°C and the progress of the reaction was followed by HPLC.

After 5 hours, the solvent was removed under vacuum and the resulting solid was purified via column chromatography on a C-18 column using an increasing gradient of
methanol in water as eluant. This led to two distinct fractions, one being unreacted starting material and the other possibly being the desired product. Yields for the desired product were very low (<5%) (Reaction Scheme 8)

**Zinc tetra-2,3-(5,6-dicarboglycylglycylpyrazino)porphyrazine (14) Method C.**

A small amount of the product synthesized above was dissolved in 10 ml of distilled water. To this was added 98 mg of glycylglycine (7.14x10^{-4} moles). The reaction was refluxed for 2 hours and then stirred overnight at room temperature.

After 18 hours, the reaction was checked by HPLC and the chromatogram was compared to the starting material. No noticeable changes had occurred. (Reaction Scheme 8)

**Zinc tetra-2,3-(5,6-dicarboglycylglycylpyrazino)porphyrazine (14) Method D.**

A 494 μM solution of zinc tetra-2,3-(5,6-dicarboxypyrazino)porphyrazine in distilled water was prepared. 1 ml of this solution (4.94x10^{-7} moles) was added to three separate 10 ml glass test tubes. To each was added a large excess of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and N-hydroxysulfoseuccinimide (NHSS). Each reaction was shaken at room temperature for 1 hour, at which time the EDC was removed from the reaction mixture using C_{18} Sep-Pak® cartridges (Millipore, Mississauga, Ontario). Elution with 10 mM sodium phosphate buffer pH 5 only removed the EDC from the cartridge. The green fraction was then removed from the cartridge using 60% methanol in sodium phosphate buffer.

The resulting solutions containing the green fraction were concentrated to 1/2 ml and an excess of glycylglycine was added. The pH was adjusted to 8 using 1N NaOH and the reaction mixture was shaken for 3 hour at room temperature and then was stored at 4°C overnight.

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The three reactions were combined and purified on a C18 column using pH 5 sodium phosphate buffer 5 mM and methanol as solvent. Five distinct fractions were obtained, each in very small amounts (<10%) (see Appendix B-4). (Reaction Scheme 8)

**Zinc tetra-2,3-(5,6-dicarboethoxyglycylglycylpyrazino)porphyrinate (17).**

500 mg of zinc tetra-2,3-(5,6-dicarboxypyrazino)porphyrinate (5.33x10^{-4} moles) were dissolved in 20 ml of DMF using sonication. To this were added 20 ml of dichloromethane and the reaction was cooled to 0°C. Then, added successively were 643 mg of 1-hydroxybenzotriazole (4.76x10^{-3} moles) (TCI America, Portland, Oregon), 900 mg of dicyclohexylcarbodiimide (DCC) (4.36x10^{-3} moles), 1.02g of diglycine ethyl ester hydrochloride (5.18x10^{-3} moles) (Sigma, St. Louis, Missouri) and 0.8 ml of triethylamine (5.73x10^{-3} moles). It should be noted that the reaction mixture was stirred for 10 minutes between the addition of the DCC and the diglycine ethyl ester hydrochloride. The reaction was stirred at 0°C for 1/2 hour and was then slowly warmed to room temperature, where it remained overnight.

After 16 hours, the solvent was removed from the reaction mixture under vacuum and the resulting solid was purified by column chromatography on silica gel 60 using methanol as solvent. HPLC analysis of the resulting green eluate showed one peak with shoulders at a retention time of 28 minutes (see Appendix B-5). (Reaction Scheme 9)

**Zinc tetra-2,3-(5,6-dicarboglycylglycylpyrazino)porphyrinate (14) Method E.**

Hydrolysis of the ethyl esters was accomplished using the same method used for zinc tetra-2,3-(5,6-diethoxycarbonylpyrazino)porphyrinate. The product was taken up in 10 ml of a 3:1 mixture of saturated methanolic NaOH and water and was sonicated for 15 minutes. The resulting solid was collected by centrifugation. It was washed twice with 10 ml of a 3:1 methanol/water solution and was dried in an oven at 100°C.
This solid was then dissolved in a minimum amount of distilled water and this was carefully acidified with 1.0 N HCl. Ethanol was then added to the solution until precipitation occurs. This product was collected by centrifugation, and washed with ethanol. It was then dried at 100°C. HPLC of this solid gave one peak with shoulders at a retention time of 13.2 minutes (see Appendix B-6). (Reaction Scheme 10)

**Zinc octacarboethoxyglyclyglyclyphanthalocyanine (18).**

A procedure similar to Method E above was followed. 556 mg of zinc octacarboxyphanthalocyanine (5.98x10^{-4} moles) were dissolved in 20 ml of DMF with sonication. 20 ml of dichloromethane were then added and the reaction was cooled to -5°C in a ice/salt bath. To this were added in succession 1.70 g of N-hydroxysuccinimide (1.48x10^{-2} moles), 3.07 g of dicyclohexylcarbodiimide (1.49x10^{-2} moles), 2.70 g of glyclyglyclycine ethyl ester hydrochloride (1.37x10^{-2} moles) and 2.5 ml of triethylamine (1.79x10^{-2} moles). Note that again the reaction was left to stir for 15 minutes between the addition of the DCC and the glyclyglyclycine ethyl ester hydrochloride. The reaction was maintained at -5°C for 2 hours and was then warmed slowly to room temperature and maintained there overnight.

After 18 hours, the reaction mixture was filtered and the solvent removed by rotary evaporation. The resulting solid was purified by column chromatography on silica gel 60 using 20% methanol in toluene as eluant. From this, the desired compound as its ethyl ester was obtained as a green solid. A single peak with a retention time of 33.5 minutes was obtained upon HPLC analysis of this product (see Appendix B-7). (Reaction Scheme 11)

**Zinc octaglyclyglyclyphanthalocyanine (19).**

Hydrolysis of the ethyl ester groups in this case was accomplished under simple alkaline conditions. A sample of the product above was dissolved in 1N NaOH and was
stirred for 4 hours. The desired product was then precipitated from solution via the addition of concentrated HCl. The product was then washed with water and dried in the oven.

The corresponding salt was obtained by dissolving the acid produced above in base and lowering the pH to around 7. The salt was then precipitated from solution by adding ethanol. Following collection by centrifugation, the salt was washed with ethanol and dried at 100°C. HPLC chromatography of this product lead to a single peak with a retention time of 10.75 minutes (see Appendix B-8). (Reaction Scheme 12)

2.2 Labeling with Technetium-99m:

Labeling with $^{99m}$Tc requires the use of a reducing agent and the following solutions were prepared fresh in all cases:

1) Stannous chloride: 12.5 mg of stannous chloride dihydrate were dissolved in 250 μL of 6N HCl and this was diluted to 25 ml with distilled water.

2) Stannous tartarate: A saturated solution of stannous tartarate in distilled water was used.

3) Formamidinesulfinic acid: 12 mg of formamidinesulfinic acid were dissolved in 100 ml of distilled water.

The radioactivity in each fraction was determined using a Commmugamma Model 1282 gamma counter (LKB, Wallac, Finland).
Labeling of Zinc tetraphosphonophthalocyanine (5).

Method A:

1 ml of a 458 µM solution of zinc tetraphosphonophthalocyanine in water was placed in a 10 ml glass test tube. To this were added 100 µL of the stannous chloride solution, which caused the blue solution to turn green. This was purged with nitrogen at 60°C for 30 minutes. Then, 368.5 MBq of fresh $^{99m}$TcO$_4^-$ (in saline) ($^{99m}$Mo/$^{99m}$Tc generator, DuPont Pharma) were added to the reaction and this reaction was kept at 60°C for 1/2 hour.

After 30 minutes, a sample was removed from the reaction (~ 400 µL). This sample was loaded onto a C$_{18}$ Sep-Pak® cartridge and the cartridge was rinsed with distilled water in order to removed the free pertechnetate and then the majority of the blue phthalocyanine fraction. This blue fraction was collected, the cartridge was washed thoroughly with water and then was purged with methanol, which removed the remaining colour. Each fraction was counted as the following: initial = 169.2 MBq, Sep-Pak® = 16.46 MBq, initial aqueous layer = 10.74 MBq, aqueous blue layer = 89.3 MBq, aqueous wash = 4.77 MBq, methanol layer = 47 MBq.

High performance liquid chromatography (HPLC) was run on both the blue aqueous and methanol layers using a HPLC fitted to both a radioactivity and UV-visible detector to verify the progress of the reaction (see Appendix B-9). Both gave very similar HPLC chromatograms, with an intense radioactive peak corresponding to an absorption peak at 680 nm. Each is clearly seen at around 7 minutes.

Method B:

1 ml of a 512 µM solution of zinc tetraphosphonophthalocyanine in 0.5 N NaOH was added to a 1 ml glass test tube. After flushing with nitrogen, 100 µL of the
formamidinesulfinic acid solution were added along with 181.95 MBq of fresh $^{99m}$TcO$_4^-$ (in saline). This was heated at 60°C for 1 hour. The pH of the reaction was checked using universal pH paper and found to be around 7.

After 1 hour, the reaction was passed through a C$_{18}$ Sep-Pak® cartridge. Rinsing with water removed all the blue phthalocyanine fraction from the cartridge. However, as before, the Sep-Pak® was washed with water thoroughly and then purged with methanol. The radioactivity of each fraction was again determined: Sep-Pak® = 0 MBq, blue aqueous layer = 64.3 MBq, aqueous wash = 1.99 MBq, methanol = 0.02 MBq.

The reaction was checked by HPLC of the blue aqueous layer. A similar chromatogram was obtained as in Method A.

*Labeling of Zinc octacarboethoxyglycylglycylphthalocyanine (18).*

Method A:

To 1 ml of a 225 μM solution of zinc octacarboethoxyglycylglycylphthalocyanine in methanol were added 100 μL of stannous chloride solution. The reaction was purged with nitrogen and 36 MBq of fresh $^{99m}$TcO$_4^-$ (approximately 100 μL) were added. The reaction was heated at 60°C for 15 minutes.

After 15 minutes, a sample was removed and passed through a C$_{18}$ Sep-Pak® cartridge. After washing extensively with pH 5.5 sodium phosphate buffer (10 mM), the blue fraction was removed using methanol. The radioactivity of each fraction was as follows: initial = 4.44 MBq, Sep-Pak® = 2.98 MBq, aqueous wash = 0.21 MBq, blue methanol solution = 0.30 MBq.

HPLC analysis of the methanol fraction was performed as in the case of the labeling of zinc tetraphosphonophthalocyanine (see Appendix B-10). However, in this case, there was no radioactive peak corresponding to the UV-visible peak (640 nm) which was clearly present around 32 minutes.
The reaction was continued and samples were removed at 1 hour and 7 hours. Both were purified using a C18 Sep-Pak® and the HPLCs were checked. As before, no radioactive peaks were seen that corresponded to the peak seen by the UV-visible detector.

Method B:

100 µl of a 225 µM solution of zinc octacarboethoxyglycylglycylphthalocyanine in methanol were added to a 10 ml glass test tube. To this were added 900 µl of methanol and the solution was purged with nitrogen for 15 minutes. Then, 76.5 MBq of fresh 99mTcO₄⁻ were added, along with 200 µl of the formamidinesulfinic acid solution. The reaction temperature was raised to 60°C and maintained there for 45 minutes.

A sample was removed and, as before, the compound was run through a C18 Sep-Pak cartridge. Radioactivity of each fraction was: initial = 38 MBq, Sep-Pak® = 0 MBq, buffer washing = 35.3 MBq, blue methanol wash = 0.05 MBq. Quite obviously, no labeling had occur as all the radioactivity was removed by the aqueous washings.

Method C:

500 µl of the stock 225 µM solution of zinc octacarboethoxyglycylglycylphthalocyanine in methanol were mixed with 500 µl of methanol, 60.5 MBq of fresh 99mTcO₄⁻ and 200 µl of a saturated solution of stannous tartarate in water. The reaction was heated at 60°C.

A fraction was removed after 45 minutes and was passed through a C18 Sep-Pak® as before. The radioactivity in the different fractions was: initial = 25.2 MBq, Sep-Pak® = 0.61 MBq, buffer wash = 21.7 MBq, blue methanol wash = 1.20 MBq.

The HPLC of the blue methanol was checked as before and no radioactive peak was seen corresponding to the peak given by the UV-visible detector (640 nm).
The reaction was checked again after 3 hours and 16 hours of reaction time and no labeling was observed, as indicated by the HPLC chromatograms of the blue methanol fractions (see Appendix B-11).

*Labeling of Zinc octacarboglycylglycylphthalocyanine (19).*

Labeling of zinc octacarboglycylglycylphthalocyanine was attempted using both stannous chloride and stannous tartarate as reducing agents in a similar method to that used for the labeling of zinc tetraphosphonophthalocyanine (Method A) and zinc octaethoxyglycylglycylphthalocyanine (Method A and C). Note that in this case, the phthalocyanine was soluble in water and as such, the reaction was carried out in water and not methanol. The reaction were checked by HPLC and no labeling was observed after 15 minutes, 1 hour and 5 hours in the case of stannous chloride and 45 minutes, 1 1/2 hours and 4 hours in the case of stannous tartarate.

*Labeling of Zinc tetra-2,3-(5,6-dicarboglycylglyclylpyrazino)porphyrazine (14).*

Labeling of zinc tetra-2,3-(5,6-dicarboglycylglyclylpyrazino)porphyrazine (synthesized from Method E) was also attempted using both stannous chloride and stannous tartarate as reducing agents. No labeling was observed (as judged by HPLC) following 15 minutes and 1 hour of reaction time.

**2.3 High Performance Liquid Chromatography:**

High performance liquid chromatography of cold products was done using a Varian 500 Liquid Chromatograph fitted with a Water RCM 8x10 module containing a Nova-Pak Radial-Pak C18 cartridge (8 mm x 100 mm) (particle size = 4 μm, pore size = 60 A) (Waters Chromatography Division, Mississauga, Ontario). It was operated with a linear gradient running from 100% aqueous sodium phosphate buffer pH 5.5 (10 mM) to
100% methanol (HPLC grade, Fisher Scientific, Nepean, Ontario) over a period of 30 minutes, with a flow rate of 1.5 ml/minutes. Compounds were detected from their UV-visible absorption, at the appropriate wavelength, using the UV-visible detector found within the Varian 500 Liquid Chromatograph instrument.

HPLC of radioactive samples was done using a similar Varian 500 Liquid Chromatograph fitted with a Water RCM 8x10 module containing a Nova-Pak Radial-Pak C_{18} cartridge (8 mm x 100 mm) (particle size = 4μm, pore size = 60 A). It was operated with a linear gradient running from 100% aqueous sodium phosphate buffer pH 5.5 (10 MM) to 100% methanol over a period of 30 minutes, with a flow rate of 1.5 ml/minutes. Eluates were detected using an external Shimadzu SPD-6AV UV-visible spectrophotometric detector set at the appropriate wavelength. Radioactivity was also detected using an external radioactivity detector.

2.4 Spectroscopy:

The UV-visible spectra of these compounds were recorded using a Hitachi U-2000 spectrophotometer. The NMR spectra of selected compounds was recorded on a Bruker Ac-300 300 MHz nuclear magnetic resonance spectrometer. The IR spectra of selected compounds was recorded using a Varian 3000 FT-IR spectrometer. The low resolution mass spectra of non-phthalocyanine precursors was obtained using a Hewlett Packard 5988 mass spectrometer. High resolution mass spectra of these compounds were obtained using a V9 Micro-mass model ZAB-1F apparatus.

4-Diethoxyphosphonophthalonitride (3).

Selected spectroscopic data: High resolution mass spectra (EI) Theoretical 264.0664 Experimental 264.0660 (see Appendix A-1). IR ν/cm\(^{-1}\) (KBr) 3096, 3066, 3035 (aromatic C-H), 2974, 2934, 2895 (aliphatic C-H), 2235 (C=O), 1232 (phosphate ester P=O), 1093-
1049 (P-O-C) (see Appendix A-2). 300 MHz $^1$H-NMR δ (DMSO-d$_6$) 8.0-8.2 (3 protons, multiplet, aromatic), 3.3 (4 protons, broad, CH$_2$ ester protons), 0.9-1.0 (6 protons, triplet, methyl CH$_3$ protons) (see Appendix A-3). 300 MHz $^{13}$C-NMR δ 136.2 (aromatic carbon), 133.6 (aromatic carbon), 132.9 (aromatic carbon), 130.25 (aromatic carbon), 115.9 (C=\ N carbon), 114.4 (aromatic carbon), 59.5 (CH$_2$ ester carbon), 16.5 (methyl CH$_3$ carbon) (see Appendix A-4). UV-visible, λ/\nm (ε/M$^{-1}$cm$^{-1}$) 299 (1970), 289 (1940), 260 (4790) (see Appendix A-5).

**Zinc tetra(diethoxyphosphono)phthalocyanine (4).**

Due to the extremely insolubility of these phthalocyanines, it was impossible to obtain FAB mass spectra results as well as a NMR spectra. Furthermore, extinction coefficients could not be determined as the compounds were only sparsely soluble in the solvents used. Selected spectroscopic data: IR ν/cm$^{-1}$ (KBr pellet) 3050 (aromatic C-H), 2965-2840 (aliphatic C-H), 1163-1112 (phosphate ester P=O), 990 (P-O-C) (see Appendix A-6). UV visible λ$_{\text{max}}$/\nm 689 [in DMSO], 685 [in triphenylphosphite] (see Appendix A-7).

**Zinc tetrAPHosphonophthalocyanine (5).**

Selected spectroscopic data: IR, ν/cm$^{-1}$ (KBR pellet) 3210 (aromatic C-H), 2900 (P-O-H stretching), 1152-1120 (P=O), 951-943 (P-O-H bending) (see Appendix A-8). UV-visible, λ/\nm (log ε) [in 0.01 N NaOH] 679 (5.35), 616 (4.51), 339 (4.82) (see Appendix A-9). [in pH 5 sodium phosphate buffer 10mM] 616 (4.76) (see Appendix A-10). HPLC retention time: 8.3 minutes (λ = 688 nm) (see Appendix B-1).

**Zinc octaCABOETHOXYGlycylglycylphthalocyanine (18).**

Selected spectroscopic data: UV-visible, λ/\nm [in methanol] 640.5 (see Appendix A-14). HPLC retention time: 33.5 minutes (λ = 680 nm) (see Appendix B-7).
Zinc octacarboglycylglyclylphthalocyanine (19).
Selected spectroscopic data: UV-visible, $\lambda$/nm [in distilled water] 685.0, 618.0 (see Appendix A-15). HPLC retention time: 10.75 minutes ($\lambda = 640$ nm) (see Appendix B-8).

Zinc tetra-2,3-(5,6-dicarboethoxyglycylglycylpyrazino)porphyrazine (17).
Selected spectroscopic data: UV-visible, $\lambda$/nm [in methanol] 656.0 (see Appendix A-12). HPLC retention time: 28 minutes (with shoulders) ($\lambda = 640$ nm) (see Appendix B-5)

Zinc tetra-2,3-(5,6-dicarboglycylglycylpyrazino)porphyrazine (14).
Selected spectroscopic data: UV-visible, $\lambda$/nm [in distilled water] 650.0 (see Appendix A-13). HPLC retention time: 13.2 minutes (with shoulder) ($\lambda = 640$ nm) (see Appendix B-6)
Reaction Scheme 1: The synthesis of diethoxyphosphonophthalonitrile
Reaction Scheme 2: Condensation of diethoxyphosphonophthalonitrile

**Solvents:**
1) imidazole
2) quinoline
3) urea

**Metal Ion Sources:**
1) Zn(OAc)$_2$
2) CuCl$_2$
3) NiCl$_2$
4) CoCl$_2$
5) AlCl$_3$
6) FeCl$_2$
Reaction Scheme 3: Hydrolysis of the phosphodiester bonds

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Reaction Scheme 4: Synthesis of zinc octacarboxyphthalocyanine
Reaction Scheme 5: Synthesis of zinc tetra-2,3-(5,6-dicarboxypyrrozino)porphyazine
Reaction Scheme 6: The acid chloride method
Reaction Scheme 7: Succinimidy active ester method
Reaction Scheme 8: Water-soluble succinimidyl active ester method
Reaction Scheme 10: Hydrolysis to obtain zinc tetra-2,3-(5,6-dicarboxyglycylglycylpyrazino)porphyrazine
Reaction Scheme 11: Synthesis of zinc octacarboxyglycyglycylphthalocyanine

1-Hydroxybenzotriazole

DCC

Diglycine ethyl ester

$\text{Et}_3\text{N}$

$\text{COOEt}$
Reaction Scheme 12: Hydrolysis of the ester bonds to form zinc octacarboxyglycylphthalocyanine
3. Results
3. Results

3.1 Zinc Tetraphosphonophthalocyanine:

The synthesis of a novel water-soluble complex, zinc tetraphosphonophthalocyanine has been successfully completed (Reaction Schemes 1, 2 and 3) (Sharman et al., 1996). The basic synthesis is as follows. Commercially available 4-nitrophthalonitrile was hydrogenated in 95% ethanol using 10% palladium on carbon as a catalyst to give 4-aminophthalonitrile (Marcuccio et al., 1985). Following diazotization with sodium nitrite in acid, the compound was treated with potassium iodide to readily yield the corresponding 4-iodophthalonitrile (Marcuccio et al., 1985). Phosphorylation of this compound was accomplished using a procedure first described by Hirao (Hirao et al., 1981) and Xu (Xu et al., 1983) and put into practical use by Bigge et al (Bigge et al., 1992) in the synthesis of N-methyl-D-aspartic acid receptor antagonist. Basically, 4-iodophthalonitrile was reacted with diethylphosphite in the presence of tetrakis(triphenylphosphine)palladium(0) as a catalyst and triethylamine at 100°C under an inert atmosphere to give a brown oil, which according to gas chromatography-mass spectroscopy (GC-MS) contained the desired product, 4-diethoxyphosphonophthalonitrile. Extensive purification by column chromatography on silica gel using 40% methanol in toluene as eluant, followed by recrystallizations from both methanol and from acetonitrile/ether gave the desired product, 4-diethoxyphosphonophthalonitrile, as a pure white solid in good yields (= 60%).

The structure of this compound was confirmed using various spectroscopic methods. High resolution electron ionization mass spectroscopy gave an experimental mass for the compound that was correct within experimental limits (Theor. 264.0664 amu, Exp. 264.0660 amu) (see Appendix A-1). The infrared spectrum of the product readily showed the existence of both aliphatic (2974 cm⁻¹, 2934 cm⁻¹, 2895 cm⁻¹) and aromatic
(3096 cm\(^{-1}\), 3066 cm\(^{-1}\), 3035 cm\(^{-1}\)) C-H bonds as well as nitrile groups (C\(=\)N stretching at 2235 cm\(^{-1}\)). Both the P=O and P-O-C bond stretching of phosphate esters (P=O stretching at 1232 cm\(^{-1}\) and P-O-C stretching at 1093 cm\(^{-1}\) and 1049 cm\(^{-1}\)) were also present in the spectra (see Appendix A-2). Finally, all the expected protons can be found in the \(^1\)H-NMR spectra as can all the required carbon atoms in the \(^{13}\)C-NMR (see Appendix A-3 and A-4).

As a result of the extensive purification necessary to obtain pure samples of 4-diethoxyphosphonophthalonitrile, a second literature procedure for the phosphorylation of aromatic halides was also attempted (Czech et al., 1992). This involved reacting 4-iodophthalonitrile with triethylphosphite at 160-180°C with the use of active copper as the catalyst. However, as was somewhat expected, the elevated temperatures of this reaction, along with the presence of a metal ion source (active copper), lead to the condensation of the phthalonitrile to yield copper phthalocyanines instead of the desired phosphorylated product. Furthermore, the phthalocyanine obtained in this reaction remained insoluble in 1N NaOH even after stirring for several hours. This made it highly unlikely that phosphorylation had occurred to any great extent. Hence, this method was not examined any further.

Attempts to extend this work to 4,5-dichlorophthalonitrile failed to give the corresponding diphosphorylated compound. However, this too was not totally unexpected. The original work carried out by Hirao (Hirao et al., 1981) showed that the reaction was limited to aryl bromides and iodides and that aryl chlorides failed to react under these conditions. This was presumable due to the increased strength of C-Cl bonds as compared to C-Br and C-I bonds (Sykes, 1986). Furthermore, it was shown that ortho-dibromo compounds gave only mono-substitution while para-dibromides lead to di-substitution. This is most likely due to both steric and electronic effects. Therefore, the desired product, 4,5-di(diethoxyphosphonophthalonitrile could not be produced using this methodology.
This phosphorylation reaction most likely proceeds via an insertion of the palladium into the aryl halide bond. This would make the compound more susceptible to nucleophilic attack by the diethylphosphite and thus allowing the reaction to occur. The lack of reactivity displayed by aryl chlorides and aryl fluorides could be explained using this mechanism as aryl chloride and aryl fluoride bonds are significantly stronger than their aryl bromide and aryl iodide counterparts. As such, this insertion step cannot occur in their case and the reaction does not proceed.

Complexation of 4-diethoxyphosphonophthalonitrile (Reaction Scheme 2) was unsuccessful in the absence of solvent. Furthermore, the use of high-boiling, neutral solvents such as 2,4,6 trichlorobenzene, 1-chloronaphthalene, tetrahydronaphthalene or sulpholane also failed to produce condensation of the phthalonitrile. However, this template reaction could be carried out in high-boiling, basic solvents such as quinoline and imidazole, with the use of imidazole being preferential as it led to clearer reactions and slightly higher yields. Therefore, the complexation of this dinitrile was accomplished by heating the compound in the presence of a metal ion source at 160-180°C using imidazole as the solvent. While the zinc complex was the most intensely studied, complexes of nickel, iron, cobalt, aluminum and copper were also produced with yields varying from 30% to 60%.

Due to the highly insoluble nature of these complexes, purification was limited to washing with methanol, hot water, DMF and acetone. Furthermore, spectroscopic examination was limited by this insolubility to only infrared and UV-visible analysis. The IR spectrum, recorded as a KBr pellet, contained absorptions corresponding to P=O ester stretching (1163 and 1112 cm⁻¹) and P-O-C ester stretching (950 cm⁻¹). Also present were aromatic and aliphatic C-H stretching frequencies which were due to the presence of both the aromatic benzene rings and the aliphatic ethoxy groups. Furthermore, the absence of a C≡N nitrile stretch around 2200 cm⁻¹ would appear to indicate complex formation (see Appendix A-6).
Also strongly indicating complex formation was the classic blue-green phthalocyanine colour of the resulting solid product. While only soluble in spectroscopic quantities (concentrations of around $10^{-6}$ M), the UV-visible spectra of these compounds could still be obtained and they readily displayed the strong, sharp Q band of a phthalocyanine with a $\lambda_{\text{max}}$ for the zinc complex lying at 689 nm in DMSO and 685 nm in triphenylphosphite (see Appendix A-7). Finally, evidence for the incorporation of a metal was based on the presence of a slight shift in the $\lambda_{\text{max}}$ of the Q band between the zinc (689 nm) and the copper (687 nm) complexes in DMSO.

Complete hydrolysis of the phosphodiester bonds (Reaction Scheme 3) could not be done using traditional alkaline hydrolysis conditions. Only a partially hydrolyzed product could be obtained in this manner. The same result was obtained by dissolving the zinc diethoxyphosphonophthalocyanine in concentrated sulphuric acid followed by recrystallization in cold water. Finally, the use of bromotrimethylsilane, which is often used in the selective removal of alkyl phosphonate esters in the presence of alkyl carboxylate esters, carbamates, acetylenes, ketones and halides (McKenna and Schmidhauser, 1979; Fieser et al., 1981), failed to give the desired product, leading to only partial hydrolysis. Complete hydrolysis could only be accomplished by refluxing the compound in 6N HCl over a period of 20 hours. Yields for this reaction were nearly quantitative with little to no phthalocyanine degradation.

Infrared analysis of the zinc tetraphosphonophthalocyanine would seem to show the hydrolyzed nature of the product. There was a slight shift in the P=O stretching frequencies to 1152 and 1120 cm$^{-1}$ (from 1163 and 1112 cm$^{-1}$) as would be expected when moving from a phosphonate ester to a simple phosphonate (Silverstein, 1991). Furthermore, the appearance of P-O-H bending (2900 cm$^{-1}$) and P-O-H stretching (951 and 943 cm$^{-1}$) frequencies also indicated that hydrolysis had occurred. Finally, the absence of an absorption around 990 cm$^{-1}$ would apparently imply the lack of any P-O-C ester bonds and thus, there is most likely complete hydrolysis (see Appendix A-8).
HPLC of a sample of this compound dissolved in water showed only one peak, according to a UV-visible detector set at 680 nm, with a retention time of 11 minutes (see Appendix B-1). This would suggest complete hydrolysis of the phosphonate ester bonds as multiple peaks of increasing retention times corresponding to an increasing number of hydrophobic ethoxy groups on the molecule would be expected for incompletely hydrolyzed products.

The UV-visible spectra of the completely hydrolyzed product showed a definite pH dependence. In 0.1 N NaOH, the classic sharp Q band of a phthalocyanine was clearly seen at a wavelength of 679 nm (see Appendix A-9) with a corresponding large extinction coefficient of 224 000 M\(^{-1}\) cm\(^{-1}\). However, in sodium phosphate buffer pH 5.5 (10 mM), there was a very strong hypsochromic shift in the \(\lambda_{\text{max}}\) to 616 nm (see Appendix A-10) with a sharp decrease in the \(\varepsilon\) to 57 500 M\(^{-1}\) cm\(^{-1}\). In contrast, no such dependence on the pH was seen in the case of the alkaline hydrolyzed product. Therefore, this results provided indirect evidence for the complete hydrolysis of the phosphodiester bonds by using refluxing in 6N HCl.

Labeling of phosphates and phosphonates with technetium is well-known. Phosphonates such as the one in Figure 38 are extensively used in nuclear medicine, primarily in the imaging of bones and skeletal abnormalities, as phosphonates readily accumulate in bones. They are also used for myocardial infarct imaging and in the labeling of red blood cells for gated blood pool and gastrointestinal blood loss studies (Saha, 1992). Therefore, preliminary attempts were made to label the zinc tetraphosphonophthalocyanine with \(^{99m}\text{Tc}\). Using the traditional reducing agent, stannous chloride, in acidic media appeared to give a \(^{99m}\text{Tc}\)-labeled product. According to the HPLC chromatogram (see Appendix B-9), there is a strong radioactive peak corresponding to the only UV-visible peak, which was detected at 640 nm. However, this reaction was not as straightforward as it appears. Special care must be taken under these reaction conditions because of the nature of the phosphonate moiety. The starting phthalocyanine
complex can easily precipitate under these acidic conditions and this has occurred during initial trials. Furthermore, under these conditions, more than one third of the labeled product (34%) adhered to the Sep-Pak® cartridge during the water washings and could only be removed using methanol. HPLC of this green methanol wash gave the same chromatogram as the green aqueous layer, with corresponding UV-visible and radioactive peaks. This would suggest that it is the same product in both cases and some phenomena caused some of the labeled product to remain on the C$_{18}$ packing of the Sep-Pak® during the aqueous washing.

Because of these discrepancies, the labeling of zinc tetraphosphonophthalocyanine with $^{99m}$Tc was also attempted using formamidinesulfonic acid as the reducing agent (Fritzberg et al., 1977). The main advantage of using this reducing agent is that it allows for the labeling reaction to be carried out under neutral conditions, where stannous chloride remains insoluble. In addition, it has also been successfully used in the labeling of weaker chelators and in cases where the use of stannous chloride in an acidic milieu failed. In this particular reaction, labeling was carried out at pH 7, where there is little chance of the starting phthalocyanine precipitating. After heating the reaction mixture at 60°C for 1 hour, HPLC analysis of the reaction mixture, following purification over a Sep-Pak® cartridge, indicated the presence of a labeled product with a retention time almost identical to the one obtained using stannous chloride. Furthermore, the green layer was completely eluted off the C$_{18}$ Sep-Pak® using distilled water. Thus, using either stannous chloride under acidic conditions or formamidinesulfonic acid under neutral conditions appeared to lead to the same labeled product. Hence, there appears to be some sort of pH dependent process at work that causes the labeled product to behave differently under either acidic or neutral conditions.
3.2 Glycylglycyl-Substituted Phthalocyanine Derivatives:

Both of the main phthalocyanine building blocks used, the zinc octacarboxy-
phthalocyanine (Wöhrle et al., 1980) and its aza analog, zinc tetra-2,3-(5,6-dicarboxy-
pyrazino)porphyrazine (Kudrevich et al., 1994b), were synthesized using procedures
described in the literature. Slight modifications were made in each case in order to help
increase the overall yield and the purity of the final products.

Basically, zinc tetra-2,3-(5,6-dicarboxypyrazino)porphyrazine was synthesized as
follows (Reaction Scheme 5). Commercially available disodium dihydroxytartrate was
treated with HCl in ethanol at 0°C for 3 days to yield, after distillation,
diethylidioxosuccinate (Fox, 1947). Reacting this with diaminomaleonitrile in ethanol
under reflux in the presence of glacial acetic acid produced the necessary precursor, 2,3-
dicyano-5,6-diethoxycarbonylpyrazine in good yields (>70%). Condensation of this
dinitrile was accomplished by heating to 170°C in quinoline using zinc acetate dihydrate as
the metal ion source. Following column chromatography on silica gel using
dichloromethane and 1:1 acetone/dichloromethane as eluant, the desired product, zinc
tetra-2,3-(5,6-diethoxycarbonylpyrazino)porphyrazine was obtained in a 26% yield.

Deesterification of this compound proved difficult. It is well-known that the aza
analogs of phthalocyanines are much less stable and quite sensitive to both acidic and basic
conditions. As such, special attention must be taken when hydrolyzing the ester bonds in
these compounds as the more traditional acidic or alkaline conditions can lead to oxidation
and degradation of the complex. Thus, deesterification was completed by suspending the
octaester in a 3:1 mixture of saturated methanolic NaOH/distilled water. Following
sonication for 30 minutes, the hydrolysis product was collected by centrifugation and
washed with a similar 3:1 mixture of methanol and water. The compound was then
dissolved in a minimum amount of water and carefully acidified with 1.2 N HCl. Addition
of excess ethanol to this solution caused the desired compound to precipitate from solution as its sodium salt.

The octacarboxy phthalocyanine itself was synthesized (Reaction Scheme 4) from commercially available 1,2,4,5-tetracyanobenzene. Complexation of this compound occurred by refluxing with lithium propylate in 1-propanol. The free nitrile groups of the resulting dilithium octacyanophthalocyanine were hydrolyzed under extreme conditions. The procedure involved heating the phthalocyanine at 160°C in a solution of sodium hydroxide in tri(ethyleneglycol) with traces of water over a period of 4 days. The resulting metal-free octacarboxyphthalocyanine was then collected by adding the reaction mixture to cold water, filtering and acidifying with concentrated HCl. The product readily precipitated from solution and was collected by centrifugation.

While the literature described the synthesis of the copper complex (Wöhrle et al., 1980) the zinc octacarboxyphthalocyanine was prepared in this case by refluxing the metal-free compound with zinc acetate in DMF. Metallation reactions of this type generally occur rapidly and with good yields and this case is no exception. The reaction was complete after one hour (judging from the UV-visible spectra where there is a loss of the Q-band doublet as symmetry is restored to the molecule upon metallation) and yields were nearly quantitative.

It is important to note that the zinc octacarboxyphthalocyanine was prepared primarily due to its diamagnetic nature. This makes this highly water-soluble phthalocyanine a potential photosensitizer for photodynamic therapy. It also allowed for comparison with its aza analog in order to help determine structure-activity relationships involved in PDT. This could help in the design of new and more potent photosensitizers with better tissue localizing and photochemical properties.

Due to its facile synthesis and availability, the preliminary attempts to substitute glycylglycine onto a phthalocyanine were made using zinc tetra-2,3-(5,6-dicarboxy-pyrazino)porphyrizine. Carboxylic acids and especially benzoic acid derivatives are
relatively unreactive towards nucleophilic attack. Therefore, the carboxylic acid must be activated in some way in order to get it to react with the free amine of the glycylglycine to form an amide linkage so as to synthesize the desired product.

One of the more traditional ways of synthesizing amides from carboxylic acids is to first prepare the corresponding acyl chloride. Acyl chlorides are extremely reactive species and readily react with most nucleophiles (including weaker ones such as water), mainly due to the high partial positive charge on the carbonyl carbon and the presence of an extremely good leaving group in the chloride ion. Great care must be taken when using acid chlorides as even moisture in the air can cause them to hydrolyze back to their carboxylic acid.

It turns out that most general methods used for the synthesis of acid chlorides use either benzene, chloroform or dichloromethane as the solvent (Vogel, 1989). However, the starting phthalocyanine analog is insoluble in these solvents. Other procedures call for the reagent, thionyl chloride, to act as the solvent (Vogel, 1989). However, the solubility of the starting material is limited in SOCl₂ as well. It is known that DMF can act like a catalyst in the formation of acyl chlorides (Fieser and Fieser, 1967) and phthalocyanines and their analogs usually have increased solubility in this solvent. Therefore, the formation of the octa acyl chloride was attempted using a large excess of thionyl chloride in DMF (Reaction Scheme 6). After refluxing for 4 hours, the excess thionyl chloride was removed under vacuum and a 14 times excess of glycylglycine was added to the resulting DMF solution of the acyl chloride. The acyl chloride was not isolated because of its ease of hydrolysis and excessive reactivity. Overall, this reaction pathway lead to extensive loss of porphyrazine, with only a 38% recovery of blue compound. Furthermore, HPLC of the final product revealed the presence of multiple reaction products (see Appendix B-2). Due to these factors and the existence of other promising methods, this procedure was abandoned.

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When carboxylic acids themselves are treated with amines, salts are generally obtained, with the amine acting as a base and accepting the acidic proton of the carboxylic acid. Hence, the coveted amide is not formed. However, amides can be formed at room temperature with the use of coupling agents such as dicyclohexylcarbodiimide (March, 1992). Moreover, the use of active esters are well-known in peptide synthesis (Bodanszky, 1984; Bodanszky, 1989) as a way to activate the carboxylic acid group and to prevent racemization. In fact, active esters have been used quite extensively in the synthesis of technetium ligands, in particular MAG₃ (Brandau et al., 1988), which is used in nuclear medicine for the imaging of renal function.

The first attempt to use active esters in the synthesis of the desired octa-substituted porphyrazine was done using a methodology similar to that used in the synthesis of MAG₃ (Brandau et al., 1988) (Reaction Scheme 7). It involved the coupling of the carboxy groups of the porphyrzinie with N-hydroxysuccinimide using dicyclohexylcarbodiimide (DCC) as a coupling agent. The reaction was performed in tetrahydrofuran (THF). To avoid cross coupling of the carboxylic acids, the DCC was added dropwise at 0°C as a solution in THF and the reaction was allowed to proceed for 18 hours at room temperature. One particular problem with this reaction was the limited solubility of the starting porphyrzinie in THF, which definitely limited the scale of the reaction as well as causing lower yields and more impurities in the final product.

Following quenching with glacial acetic acid and evaporation of the solvent, the crude zinc tetra-2,3-(5,6-dicarboxydicarboximido)pyrazino-porphyrzinie was reacted with glycyglycine at reflux for 4 hours and then for 20 hours at room temperature in a 2:1 mixture of methanol and water. The yield of this reaction was only 27%. In addition, the reaction was very impure, leading to several different porphyrzinie-based products as judged by the HPLC chromatograms (see Appendix B-3).

Because of the solubility problems of the above reaction, its poor yield and extensive impurities, addition of glycyglycine was attempted using a water-soluble
coupling agent, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and a water-soluble succinimide, N-hydroxysulfosuccinimide (Staros, 1986) (Reaction Scheme 8). These reagents allow for the reaction to be carried out under aqueous condition, where the solubility of the starting acid is greatly increased. Trials were done using both a one step and two step procedure. The two step procedure involved the preparation of the active ester through the reaction of the porphyrazine with N-hydroxysulfosuccinimide and EDC in water. The active ester was then isolated and purified prior to the reaction with glycyglycine. This purification was done using a C18 reverse-phase column with 10 mM sodium phosphate buffer pH 5.5 and methanol as eluant. The yield of active ester formation was extremely small and no observable reaction was obtained when it was treated with diglycine in water, as judged by HPLC.

The one step procedure entailed generation of the active esters in situ, using EDC and sulfo-NHS in water. The coupling agent was then removed from the reaction mixture using a series of C18 Sep-Pak® cartridges. EDC eluted off the "mini column" with pH 5 10 mM sodium phosphate buffer while the blue product was not removed until 60% methanol in buffer was used. The diglycine was then added directly to the blue eluate and the pH was carefully adjusted to 8. After 3 hours of shaking at room temperature and 18 hours at 4°C, the reaction mixture was purified over a C18 reverse phase column using a gradually increasing percentage of methanol in 10 mM sodium phosphate buffer pH 5 as eluant. This led to the isolation of 5 distinct coloured fractions, all in very small amounts (less than 10% yield for each fraction) (see Appendix B-4 for HPLC of the crude product).

Despite the difficulties encountered above, a third potential protocol was found in the literature (Cleyhens et al., 1994) and was used in an attempt to produce the desired octaglyglycylglycyl-substituted product (Reaction Scheme 9). In this case, the reaction was done completely in situ, using DCC as the coupling agent and 1-hydroxybenzotriazole as an auxiliary nucleophile in order to produce the necessary active acylamino ester. The
reaction was carried out in a 1:1 mixture of DMF and dichloromethane, which turned out to be highly advantageous in this case because of the solubility of zinc tetra-2,3-(5,6-dicarboxypyrazino)porphyrazine in DMF. Because of the presence of the coupling agent in the reaction mixture, the protected glycyglycine ethyl ester was used so as to avoid cross-coupling of this reagent. One further benefit was that this reagent proved to be more soluble than the unprotected diglycine in the reaction solvent used. Finally, triethylamine was added to the reaction mixture to act as a base for the acid that evolves from the reaction.

The porphyrazine was dissolved in DMF and to this was added an equal amount of dichloromethane. After cooling to 0°C, roughly nine equivalents of each reagent were added successively, starting with 1-hydroxybenzotriazole, and followed by DCC, diglycine ethyl ester hydrochloride and triethylamine. It should be noted that 30 minutes were allowed to pass between the addition of the DCC and diglycine. The reaction was continued at 0°C for 1/2 hour and then was slowly warmed to room temperature, where it remained for 16 hours.

Column chromatography of the resulting product on silica gel using methanol as solvent yielded two fractions. According to HPLC, the minor fraction appeared to be unreacted starting material as it displayed a retention time of approximately 10 minutes. The second major fraction was significantly different, with a retention time of 28 minutes. However, this peak in the HPLC did have shoulders, indicating possible impurities (see Appendix B-5). Yields for this reaction were decent.

Due to the sensitivity of the aza compound to alkaline conditions, hydrolysis of the ester bonds was accomplished using the same method as the one used for zinc tetra-2,3-(5,6-diethoxycarbonylpyrazino)porphyrazine (Reaction Scheme 10). This called for the addition of the octaester to a 3:1 mixture of saturated methanolic NaOH and water and sonication for 15 minutes. The resulting product was then collected by centrifugation and washed with 3:1 methanol/water. It was then dissolved in a minimum amount of water,
acidified with 1.0 N HCl and precipitated upon the addition of ethanol. HPLC analysis of
the product led to a single peak, with shoulders, at a retention time of 13.2 minutes (see
Appendix B-6).

With the apparent success of this reaction, attempts to add diglycine onto the
corresponding zinc octacarboxyphthalocyanine were made using the same methodology
(Reaction Scheme 11). Zinc octacarboxyphthalocyanine was dissolved in DMF and an
equal amount of dichloromethane was added. This solution was cooled to -5°C and, as
before, the reagents were added in succession. However, in this reaction, approximately
twenty-five equivalents of each reagent were used instead of the nine used for the
porphyrazine. The reaction was carried out as before, with purification of the reaction
mixture being done on silica gel using 20% methanol in toluene as eluant. In this case,
one major fraction was obtained, which had a HPLC retention time of 33.5 minutes (see
Appendix B-7). No shoulders were apparent on this peak.

Hydrolysis of the ethyl ester bonds was done using simple alkaline conditions
(Reaction Scheme 12), by stirring the zinc octacarboxethoxyglycylglycylphthalocyanine in
1N NaOH for 4 hours. The corresponding octa acid was obtained by precipitation using
concentrated HCl. After washing with water, the compound was dissolved in base and the
pH was lowered to approximately 7. The salt was then precipitated from solution upon
addition of ethanol. A single peak with a retention time of 10.75 minutes was obtained by
HPLC (see Appendix B-8).

Despite a lack of spectral data, labeling of both the zinc tetra-2,3-(5,6-
dicarboglycylglycylpyrazino)porphyrazine and the zinc octacarboxglycylglycyl-
phthalocyanine in aqueous solution, as well as the labeling of zinc octacarboxethoxy-
glycylglycylphthalocyanine in methanol was attempted. Trials were carried out using
either stannous chloride, stannous tartarate and formamidinesulfonic acid as the reducing
agents. However, results were negative, with no radioactivity associated with the main
UV-visible peak (see Appendix B-10 and B-11).
4. Discussion
4. Discussion

Phosphates and phosphonates are ubiquitously found throughout nature. Phosphate groups form a major component of the backbone of DNA while phospholipids are key constituents of biological membranes such as those which surround every living cell. Moreover, phosphorylation is a key step in numerous metabolic and biochemical pathways. Therefore, phthalocyanines bearing phosphonate moieties directly bound to the aromatic rings of the macrocycle may provide a more biocompatible water-soluble photosensitizer as compared to the more commonly used sulphonated PCs. As such, the synthesis of zinc tetraphosphonophthalocyanine was completed (Sharman et al., 1996).

The synthesis of the main precursor, 4-diethoxyphosphonophthalonitrile, was straightforward, with the key step being the phosphorylation of 4-iodophthalonitrile with diethylphosphite in the presence of a tetrakis(triphenylphosphine)palladium(0) catalyst. This nucleophilic aromatic substitution reaction most likely proceeded via an insertion reaction, with the Pd⁰ inserting into the aryl iodide bond. This makes the site more susceptible to nucleophilic attack (Hirao et al., 1981; Sykes, 1986: Butler and Herrod, 1989). It should be noted that this aromatic system is already more susceptible to nucleophilic attack due to the electron-withdrawing nitrile groups, which lead to the benzene ring having less electron density localized on it.

Complexation of this dinitrile failed in the absence of solvent, evidently due to the high melting point of this reagent (it decomposes at temperatures greater than 220°C without melting). The use of non-basic, high boiling solvents also proved unsuccessful. The use of urea, which usually facilitates complexation of dinitriles, proved inappropriate due to its possible interaction with the phosphonate moiety. Therefore, the reaction was carried out in high-boiling, basic solvents (such as quinoline or imidazole) as they provided an acceptor for the evolving acid while sparing the phosphonate ester bonds.
The difficulties encountered in the hydrolysis of the phosphodiester bonds found in zinc tetradiethoxyphosphono-phthalocyanine are not uncommon. Previous work in our laboratory led to the synthesis of phosphonoalkyl-substituted phthalocyanines via the Arbuzov reaction (Boyle and van Lier, 1992), where the phosphonate moiety was separated from the benzene rings by alkyl chains. In this case, hydrolysis using aqueous sodium hydroxide led only to the partial hydrolyzed product. Furthermore, attempts to completely hydrolyze the compound using either acidic conditions or bromotrimethylsilane failed. It was presumed that this failure was due to the extensive aggregation or the insolubility of the phthalocyanine in each reaction solvent. This could also be the case in the attempts made here to hydrolyze the ester bonds with bromotrimethylsilane, where solubility of the starting material was minimal. In contrast, diethylphosphonates produced as proton-ionizable pendant groups for crown ethers were successfully hydrolyzed using bromotrimethylsilane (Czech et al., 1992). However, the solubility of such crown ethers far exceeds that of phthalocyanines in this reagent. Once more, only partial hydrolysis was obtained for these diethylphosphonates using alkaline conditions, where refluxing in a mixture of ethanol and 10% aqueous NaOH was used as the reaction conditions.

Complete hydrolysis of the phosphodiester bonds of zinc tetradiethoxyphosphono-phthalocyanine could only be accomplished by refluxing in 6N HCl over a prolonged period of time (16-20 hours). This procedure was used in the hydrolysis of phosphonate ester bonds in the synthesis of potential competitive N-methyl-D-aspartic acid antagonists (Bigge et al., 1992). It proved to be a useful methodology despite the apparent lack of solubility of the macrocycle under these acidic conditions. It is important to note that yields of this reaction were essentially quantitative, thus indicating the stability of these phthalocyanines towards acidic conditions.

Of particular interest are the aggregation properties of these novel phosphorylated phthalocyanines. In 0.1 N NaOH (pH > 12), their solutions are green, leading to the classic UV-visible spectrum of a monomeric phthalocyanines, with a sharp, strong Q band
at around 680 nm with a strong extinction coefficient of almost 224 000 M⁻¹ cm⁻¹. In the meanwhile, in pH 5 buffer, these phthalocyanines give a blue solution with a drastic hypsochromic shift in the UV-visible spectrum with a much weaker and broader $\lambda_{\text{max}}$ at 615 nm with an extinction coefficient of only 40 000 M⁻¹ cm⁻¹ (see Appendix A-11). This phenomenon is most likely due to the diacidity of the phosphonate moiety (Figure 39). At lower pH, the phosphonate moiety is completely protonated, causing it to precipitate. This is the case for all weaker acids in stronger acids and has been used extensively in the present work in order to purify and collect acidic products. At higher pH, the phosphonates are completely deprotonated, leading to a large amount of negative charge on the periphery of the phthalocyanine. This would most likely prevent aggregation via electrostatic repulsion and would lead to the UV-visible spectrum of a monomeric phthalocyanine as is seen at pH 12. However, at more intermediate pH, the phosphonate groups are presumably only partially ionized. In this case, there would be less negative charge on the phthalocyanine periphery that would have helped prevent aggregation. Furthermore, intermolecular hydrogen bonds can potentially form between the ionized portion of one phosphonate group and the protonated portion of another (Figure 40). Such intermolecular hydrogen bonding between phosphonate groups on adjacent phthalocyanines would in fact enhance aggregation as can be seen in Figure 40.
Figure 40: Intermolecular hydrogen bonding between adjacent phosphonates

Therefore, the classical UV-visible spectrum of an aggregated phthalocyanine is obtained at pH 5.

The novel water-soluble zinc tetraphosphonophthalocyanine is of potential interest as a photosensitizer in photodynamic therapy and initial trial, both in vitro and in vivo, have been carried out. Preliminary results are not promising. (Brasseur, unpublished results). However, this is not completely unexpected. It is well-known that the more water-soluble hydrophilic phthalocyanines do not work as well as their hydrophobic and amphiphilic counterparts (Paquette et al., 1988; Brasseur et al., 1988; van Lier and Spikes, 1988; van Lier, 1990). For instance, it has been shown that the lower sulphonated zinc phthalocyanine fractions are more efficient photosensitizers for V-79 cell killing while the tri- and tetra-sulphonated dyes were totally inactive under the same conditions (Brasseur et al., 1988). In vivo tumor response and cure also followed a similar trend with the tetrasulphonated zinc Pc showing no tumor response (Brasseur et al., 1988). This is mainly due to the poor cell penetrating properties of the more hydrophilic compounds as they cannot pass the lipid membrane and enter the cytoplasm (Paquette et al., 1988).

It has been shown that the photochemical properties of sulphonated gallium...
phthalocyanines is unaffected by the number of sulphonate substituents as long as the dye is in its monomeric form (Wagner et al., 1987). The same should be the case for phosphono-substituted phthalocyanines as well. However, at physiological pH, the zinc tetraphosphonophthalocyanine is highly aggregated in solution, with its phosphonate groups likely only partially ionized. As such, the intermolecular hydrogen bonding that was described earlier is probably enhancing the aggregation of these phthalocyanines at this pH. Aggregation of this type is known to make phthalocyanines photodynamically inactive. This inactivation arises from enhanced decay of the singlet state of the photosensitizer, thus leading to a low triplet yield (Wagner et al., 1987), and therefore, ineffective production of the reactive species responsible for the biological effect.

The synthesis of zinc tetraphosphonophthalocyanine reported here involves the condensation of a substituted dinitrile, namely 4-diethoxyphosphonophthalonitrile. This provides the opportunity to synthesize unsymmetrically substituted phthalocyanines containing this functional group. In analogy with the series of sulphonated phthalocyanine, a mixed condensation product containing phosphonate groups may prove to be more useful in PDT. In all likelihood, it would be a more amphiphilic compound as these have been shown to be the more biological active dyes (Paquette et al., 1988, Brasseur et al., 1988, Allen et al., 1995). Furthermore, phosphonic acid monoethyl ester groups can be produced by the partial hydrolysis of the phosphodiesters in the starting material under alkaline conditions. These compounds could not take part in intermolecular hydrogen bonding and hence, aggregation would be less a problem. Also, the presence of an acid and an ester in the same functional group provides amphilicity in the actual substituents on the phthalocyanine framework. Compounds containing this type of group perhaps would be more biologically active as well. However, in biological screening of phosphonoalkyl-substituted phthalocyanines, it was found that the partially deprotected tetrsubstituted zinc compound was devoid of photodynamic activity (Boyle
and van Lier, 1992). Hence, it remains to be seen if phosphonated phthalocyanines have any future as photosensitizers in photodynamic therapy.

As has been stated previously, phosphates and phosphonates readily chelate technetium and their technetium complexes are extensively used in nuclear medicine. In fact, one of the most common examinations in nuclear medicine and oncology is bone scans for metastases from cancer prostat a and cancer mamma (Hjelstuen, 1995). Such phosphonates are distributed throughout the whole skeleton and absorbed by all bone cells due to their phosphorus content, which is used as a building block for the bone substance hydroxyapatite. Metastases and other skeletal abnormalities show increased uptake of the labeled compound due to increased metabolic activity. In addition to this important application, $^{99m}$Tc-labeled phosphates have also been used for myocardial infarct imaging and in the labeling of red blood cells for gated blood pool and gastrointestinal blood loss studies (Saha, 1992). Uptake in the case of damaged myocardium has been attributed to the deposition of granules in the enlarged mitochondria of damaged myocardium. These granules are composed of calcium and phosphate similar to hydroxyapatite crystals in bones and as such, a similar uptake is observed.

\[ \text{Figure 41: } ^{99m}\text{Tc-MDP complexes seen as a dimer} \]
Phosphonates such as those presently used in nuclear medicine are thought to act as dimers, chelating technetium in an octahedral complex with each MDP molecule acting as a bidentate ligand (Hjoelstuen, 1995) (Figure 41). However, other phosphonate chelators have been investigated whereby the phosphonate end of the molecule would act as a handle for the chelation and transportation of technetium and the remaining portion of the molecule would dictate its biodistribution (Castronovo et al., 1974). Primarily investigated was a combination of an α-aminocarboxyl grouping of an amino acid and a phosphonate moiety (Figure 42). The α-aminocarboxyl grouping was chosen because of its association with protein synthesis in the hopes that the compound would accumulate in areas of increased amino acid turnover instead of in the bones, as would be expected if the phosphonate group dictated the biodistribution. It turns out that the labeled compound remained intravascular after 4 hours with some accumulation in the liver, kidneys and intestines. Hence, it would appear that the biodistribution of such compounds is determined by the amino acid portion of the molecule and not the phosphonate end.

Since phthalocyanines are known to show preferential tumor uptake (Paquette and van Lier, 1991), the labeling of the tetraphosphonophthalocyanine was attempted using both stannous chloride and formamidinesulfinic acid as the reducing agents. Judging from HPLC analysis on a chromatograph fitted with both UV-visible and radioactivity detectors, both methods appeared to be successful, producing a labeled product. Clearly,
the HPLC chromatogram showed a radioactive peak corresponding to the UV-visible peak (at 640 nm) (see Appendix B-9). Though these results are very promising, they are also very preliminary. $^{99m}$Tc-labeled complexes need to be characterized using the long-lived $^{99}$Tc isotope, whose lower specific activity allows it to be used in gram quantities. In addition, phosphonates are notoriously weak chelators and tend to degrade with time (Saha, 1992). Hence, competition studies need to be completed in order to determine the stability of these complexes. Finally, animal studies using tumor-bearing mice and rabbits need to be performed so as to determine the potential usefulness of these chelators in nuclear medicine. This would also aid in the determination of the stability of the complexes, especially in vivo, where free $^{99m}$TcO$_4^-$ is known to accumulate in the thyroid due to its similarity to iodide.

One final point that needs to be explained is the differences seen in the two labeling procedures. When stannous chloride in weak acid was used as the reducing agent, a portion of the labeled product remained bound to the C$_{18}$ Sep-Pak® cartridge following elution with distilled water and could only be removed using methanol as eluant. However, HPLC of each fraction indicated that both were essentially the same, with basically identical retention times. No such phenomenon was seen in the case where formamidinesulfinic acid was used under neutral conditions. One possible explanation is the degree of aggregation of the phthalocyanine. Under more acidic conditions, these phthalocyanines would be highly aggregated, with intermolecular hydrogen bonding increasing this effect. Thus, following labeling with $^{99m}$Tc, part of the sample could remain aggregated and might not be eluted from the C$_{18}$ Sep-Pak® cartridge with distilled water. However, when analyzed by HPLC, the sample is greatly diluted and disaggregation will occur, leading to similarity product peaks in the HPLC. At more neutral pH, aggregation, while still present, is not as effective. Thus, the product can be completely eluted off the small C$_{18}$ Sep-Pak® cartridge.
While the preparation of phosphonated phthalocyanines proved successful, the synthesis of octaglycylglycyl-substituted Pcs was not nearly as straightforward. Numerous reaction pathways needed to be attempted before the desired product could be obtained.

The reasoning behind the addition of glycylglycine to the carboxy groups of octacarboxyphthalocyanine derivatives is based on the knowledge that tetradentate ligands provide the basic framework of the majority of technetium imaging agents (Tisato et al., 1995). This is due to their intrinsic chelate effect, which leads to clean reactions that yield stable products. Among these are MAG3 and HMPAO, which are depicted in Figure 38. Others include EDC, which is used in brain perfusion imaging (Saha, 1992), and MRP20 (Tisato et al., 1995). The unusual strength of multidentate ligands with certain degrees of separation between ligating atoms is known as the chelate effect and is a result of both enthalpic and entropic contributions (Butler and Herrod, 1989). The enthalpic effect is mainly due to the lack of an increase in electrostatic repulsion that would result from bringing two or more negatively charged ligands together. This is avoided with multidentate ligands since the ligating atoms are already close enough together within a molecular framework. The entropic effect arises because, after the initial attachment of one ligating atom to the metal center, there is no further loss of translation entropy accompanying the bonding of the subsequent ligating atoms as the number of particles in the system remains the same. The addition of glycylglycine to the periphery of phthalocyanines should provide a tetradentate N4 core for the chelation of 99mTc (Reaction Scheme 9 through 12). The preferential tumor uptake shown by phthalocyanines 24 to 48 hours post-injection (while normal tissue have maximum uptakes 2-3 hours post-injection) (Paquette and van Lier, 1991) make radiolabeled phthalocyanines of potential usefulness in nuclear medicine, where the most extensively used imaging agent in oncology, 67Ga citrate, lacks selectivity, has high uptake in normal tissues and is slowly excreted from the body (Ali, 1996).
One of the main obstacles that needed to be overcome was the decreased reactivity of carboxylic acids, especially aromatic carboxylic acid derivatives like those involved here. In most cases, formation of carboxylic acid derivatives, such as amides, esters, anhydrides etc., requires activation of the carboxylic acid functional group in some way, either by using a dehydrating agent or harsh reaction conditions (March, 1992). In addition, more reactive acyl species can be prepared that will more readily react with the substrate to give the desired acyl derivative. All these methods can prove to be particularly difficult when aromatic carboxylic acid derivatives are involved. Their unreactivity is well-documented, with the aromatic system donating charge to the carboxylic acid functional group. This has the effect of decreasing the partial positive (δ+) charge on the carbonyl carbon, thus decreasing its reactivity towards nucleophilic attack. In addition, benzoic acid is a significantly stronger acid than the corresponding cyclohexane carboxylic acid (pKₐ = 4.20 and 4.87 respectively) (Sykes, 1986). The strength of the acid increases further when electron-withdrawing groups are added to the benzene ring, as is the case in phthalocyanines, whose inner aromatic macrocycle acts to withdraw electrons from the outer benzene rings. Increases in the acidity further decreases the reactivity of these functional groups, making the synthesis of the desired amides more troublesome.

An aza phthalocyanine analog, zinc tetra-2,3-(5,6-dicarboxypyrrazino)-porphyrazine, was used to carry out initial trials in the addition of diglycine to the periphery of the macrocycle. This was mainly done because of the easier synthesis and ready availability of this compound as compared to zinc octacarboxyphthalocyanine. However, this compound is not without its problems. These aza analogs are known to be more sensitive to acidic and especially alkaline conditions (Kudrevich et al., 1994) and this surely affected some of the results. For instance, the preparation of acyl chlorides using thionyl chloride leads to the production of acidic byproducts, including gaseous HCl
(Fieser and Fieser, 1967). This could lead to some decomposition of the porphyrizine, helping to explain the small recovery of coloured products.

While the synthesis of acyl chlorides using thionyl chloride, followed by reaction with compounds with active hydrogen atoms such as amines and alcohols, is one of the most commonly used methods to synthesize carboxylic acid derivatives, several problems appear to preclude its use here. In addition to the problems with acidic byproducts, some aromatic acids, especially those with electron-withdrawing substituents, do not react with thionyl chloride (Vogel, 1989). More often than not, phosphorus pentachloride is the preferred reagent for these less reactive compounds (March, 1992). However, while the byproducts using thionyl chloride are gaseous (SO₂ and HCl), POCl₃ is formed when PCl₅ is used (Fieser and Fieser, 1967) and must be removed from the reaction mixture. DMF has also been shown to catalyze the formation of acyl chlorides using thionyl chloride (Fieser and Fieser, 1967) and this has also been used to help synthesize acyl chlorides from carboxylic acids that fail to react with thionyl chloride alone. With the starting octacarboxy porphyrizine being readily soluble in DMF, it was felt that carrying out the reaction using DMF as the solvent instead of SOCl₂ would not only increase the concentration of the octacarboxy compound in the reaction mixture but would also promote the formation of the acyl chlorides.

This choice of reaction conditions, which had to be used due to the low solubility of the starting material and the unreactivity of its carboxylic acid groups, most likely

![Chemical reaction diagram](image-url)

Figure 43: Formation of dimethylformiminium chloride
played a role in the poor results obtained. Thionyl chloride reacts with DMF to form a reactive, hydroscopic intermediate, dimethylformiminium chloride (Figure 43), which is the species responsible for the increased reactivity (Fieser and Fieser, 1967). Often, the DMF is only present in catalytic proportions in these reactions, therefore decreasing the amount of this reactive species in solution. In this case, however, DMF was used as the solvent for the reaction and the thionyl chloride was added in large excess. While reaction protocols of this type are common in the literature (Fieser and Fieser, 1967), they do lead to a high concentration of dimethylformiminium chloride in the reaction mixture. This might have contributed to degradation of the macrocycle. This reactive intermediate has been shown to be useful in formylation, chlorination and dehydration reactions (Fieser and Fieser, 1967; March, 1992), any one of which could result in both the presence of macrocyclic impurities and degradation.

Finally, in most cases, the excess thionyl chloride is removed from the reaction mixture via distillation (Vogel, 1989). Yet, the reactive species in this reaction is a salt, dimethylformiminium chloride, which would not be removed from the reaction in this manner. As such, it would still be present upon the addition of diglycine, whose carboxy group is not protected from attack. Thus, cross-reactions between diglycines are likely, leading to diglycine dimers, trimers and oligimers in the reaction mixture. These could have in turn reacted with the acid chloride of the porphyrazine, resulting in a number of different glycylglycyl-substituted products. This could explain the presence of multiple porphyrazine-based products in the reaction mixture.

In spite of all this, the acyl chloride method may still prove to be useful, especially for the more acid-stable phthalocyanine. It is possible that the solubility and reactivity of the octa acids is sufficient in thionyl chloride for acyl chloride formation to occur. If not, the addition of pyridine, which has been shown to prevent decomposition in the synthesis of certain acid chlorides (Fieser and Fieser, 1967), should increase the solubility and decrease the aggregation of the macrocycle through its complexation as the axial ligand to
the metal (Berezin, 1981). Catalytic amounts of DMF can act to catalyze the reaction and could possibly behave like pyridine and increase the solubility of the starting material in SOCl₂. It is well-known that even small amounts of DMF in solution can greatly increase the solubility of phthalocyanines. Moreover, if thionyl chloride turns out to be completely unreactive and these modifications cause too many side reactions, perhaps the more reactive phosphorus pentachloride would be of use. Finally, while most reaction protocols call for the use of thionyl chloride in large excess (often 100% excess), (Fieser and Fieser, 1967; Vogel, 1989), it has been shown that this is often not necessary. Clearly, any reduction in the amount of reactive starting material could prove to be beneficial. Ultimately, isolation and purification of the acid chloride, though difficult because of its hydroscopic nature and high reactivity, may be necessary as it would eliminate any reactive species that could interfere with the ensuing steps.

Acyl chloride formation has been attempted on the zinc tetracarboxyphthalocyanine by refluxing with a large excess of thionyl chloride in the presence of a small amount of pyridine (Schneider et al., 1994). While this reaction proceeded smoothly, only the diacid chloride could be obtained. Therefore, questions were raised as to the utility of this pathway in the synthesis of an octa amine, where the synthesis of the octa acyl chloride is essential. Therefore, due to this and the problems encountered above, this reaction pathway was abandoned.

Glycylglycine is the simplest of all peptides. Therefore, it was thought that methods used in the synthesis of peptides could be useful. After all, the overall goal here is the production of amide bonds between the carboxylic acid groups of the macrocycle and the amine group of the diglycine. Since a peptide bond is by definition an amide bond, methods involved in peptide synthesis are of potential usefulness here as well. Among the possible methods that could be employed to create the amide bonds in the synthesis of peptides, the use of acylamino acid active esters have been used comprehensively in the production of ligands for the chelation of ⁹⁹ᵐTc (Schneider et al., 1984; Brandau et al.,
1988; Cleyhens et al., 1994; Grummon et al., 1995). Variations of these protocols were used in attempts to synthesis the desired octa-substituted porphyrazine.

Active esters of both N-hydroxsuccinimide (Anderson et al., 1964; Anderson et al., 1967; Bodanszky, 1989) and 1-hydroxybenzotriazole (Windridge and Jorgensen, 1971; Bodanszky, 1989) have been used extensively in peptide chemistry (Figure 44). Initial

![Chemical structures](image)

**N-hydroxsuccinimide**  
**1-hydroxybenzotriazole**

**Dicyclohexylcarbodiimide**

Figure 44: Commonly used reagents in the synthesis of active esters

coupling of these compounds with carboxylic acids is accomplished using a carbodiimide such as dicyclohexylcarbodiimide (DCC). This coupling proceeds through an O-acyl-isourea intermediate (Figure 45) that is produced via the interaction of the DCC with the carboxylic acid (Bodanszky, 1989). In this intermediate, the N=C group provides a powerful activation which can readily lead to coupling. The corresponding acylamino acid active ester is easily produced by adding either N-hydroxsuccinimide or 1-hydroxybenzotriazole in the presence of this O-acyl-isourea intermediate via an
esterification reaction. While DCC itself can act as a coupling agent in the production of peptides (see Figure 45), the use of auxiliary nucleophiles have been shown to suppress

![Figure 45: O-acylisourea intermediate](image)

both racemization and N-acylurea formation (an unreactive byproduct produced by an O→N acyl migration) while improving yields significantly (Bodanszky, 1989). This follows from the rapid rate at which these N-hydroxy compounds react with the O-acylisourea

![Figure 46: Anchimeric assistance in the synthesis of peptides using esters of 1-hydroxybenzotriazole](image)

intermediate. This in turn lowers the concentration and the lifetime of these overactive intermediates and therefore helps to diminish the extent of O→N acyl migration as well as eliminate racemization. Furthermore, the resulting active esters are more potent acylating agents than the corresponding O-acylisourea. Thus, reaction rates and yields are
improved. This is probably due to anchimeric assistance (Figure 46), where the other functional groups in the active ester act to pull the reagents in close proximity via the formation of hydrogen bonds, thus increasing the chances of a reaction occurring.

While the development of reactive esters has continued since the early 1950's, only N-protected amino acid p-nitrophenyl esters and esters of N-hydroxysuccinimide are presently commercially available (Bodanszky, 1989). In addition, succinimidyl active esters are used in the synthesis of a number of tetradeutate $^{99m}$Tc cores. The most important of these is MAG$_3$ (Brandau et al., 1988), where an active ester of N-hydroxysuccinimide has been used to add triglycine to S-benzoylthioglycolic acid. Thus, with the popularity of succinimidyl active esters and the apparent similarities between the MAG$_3$ protocol and what was desired in this case, trials were conducted using N-hydroxysuccinimide-based active esters.

It turned out that the solvent severely limited the scope of this reaction. In most cases, tetrahydrofuran is the solvent of choice in the preparation of succinimidyl esters. However, the starting zinc tetra-2,3-(4,5-dicarboxypyrazino)porphyrinate has only a limited solubility in this solvent. This greatly restricts the scale of the reaction and leads to incomplete reactions and the recovery of a large amount of unreacted starting material. This insolubility also hampers the purification of the active ester. While the active ester is purified by recrystallization with ethyl acetate in the synthesis of MAG$_3$ (Brandau et al., 1988), the porphyrinate remains insoluble in this and most other common organic solvents. Column chromatography can also be counted out, both because of the lack of a suitable solvent and the acidity of silica gel, which will lead to some decomposition of the product. As such, the crude active ester was used directly. A mixture of products was obtained for the addition of the glycylglycine and the yield was poor.

With the insolubility of the starting material in the reaction solvent, it was thought that a reaction in aqueous media might be more suitable. Using N-hydroxysulfo-succinimide with a water-soluble carbodiimide, 1-ethyl-3-(3-dimethylaminopropyl)-
carbodiimide might allow the reaction to be conducted using water as a solvent (Staros, 1986). Trials where attempted involving both the isolation and purification of the active ester and the in situ generation of the active ester with the removal of the coupling agent prior to glycylglycine addition. Yields using these methodologies were poor and multiple reaction products were obtained. This is probably due to several factors. First of all, as was stated previously, aromatic acids are considerably less reactive than their aliphatic counterparts due to the donation of charge by the aromatic system to the carboxylic acid. Therefore, under these reaction conditions, the aromatic acids will be relatively more inert, leading to incomplete reactions and a poor yield of active ester, as was seen in the first case. In addition, while N-hydroxysuccinimide is extensively used in the synthesis of peptides, its application is sometimes less than ideal. It is known that the carbonyl groups of the succinimide are not entirely inert towards amine addition (Bodanszky, 1989). Therefore, there is a competition between the reaction of the amine with the carboxylic acid carbonyl carbon and that of the succinimide (Figure 47). Quite obviously, this competition will become more important as the reactivity of the carboxylic acid carbonyl carbon decreases, as it would be when an aromatic carboxylic acid is involved.

![Diagram](image)

Figure 47: Competition between the reaction of the amine with the carbonyl carbon of the succinimide and that of the active ester
Furthermore, the presence of an electron-withdrawing sulfo group on N-hydroxysulfo-
succinimide will probably make this effect even more important with the carbonyl of the
succinimide having an even greater partial positive charge (δ⁺) due to electron withdrawal
towards the sulfo group. In addition to this, other side reactions have been reported in the
synthesis of some peptides when using DCC and N-hydroxysuccinimide (Windridge and
Jorgensen, 1971). These would also result in decreased yields for the desired product.
Finally, these reactions have a definite pH dependence and with the increased acidity of
aromatic acids as compared to amino acids, this could easily be a factor here as well.

While succinimidyI active esters are the most popular in the synthesis of peptides,
1-hydroxybenzotriazole can also be used as the auxiliary nucleophile. It behaves in much
the same way, by attacking the O-acylisourea intermediate to form the corresponding
acylamino acid active ester. It turns out that 1-hydroxybenzotriazole has a number of
advantages over N-hydroxysuccinimide. Yields in the synthesis of certain peptides were
found to be unacceptably low using N-hydroxysuccinimide but were greatly improved by
using 1-hydroxybenzotriazole. First of all, the lack of carbonyl carbons in 1-
hydroxybenzotriazole prevents the competition seen in the case of succinimidyI active
esters. In addition to this, active esters of 1-hydroxybenzotriazole exhibit stronger
achimeric assistance in amide bond formation, helping them to show increased reaction
rates and higher yields in a number of cases. Finally, the solubility of 1-hydroxy-
benzotriazole allows for the reaction to be carried out in DMF, which is of great
advantage here, especially with the limited solubility of the starting macrocycles in most
other solvents.

With its solubility in DMF, 1-hydroxybenzotriazole would appear to be a very
attractive alternative in the synthesis of octaglycylglycyl-substituted phthalocyanines and a
procedure was drawn up based on the method used in the synthesis of cysteiny1triglycine
(Cleyhens et al., 1994). In order to increase the solubility of the porphyrazine and later, of
the phthalocyanine, a 1:1 mixture of dichloromethane and DMF was used as the reaction
solvent instead of the 4:1 mixture that was used in the literature. The general procedure involved the in situ generation of the active ester and coupling of the diglycine in a one pot procedure. As such, due to the presence of the DCC coupling agent, the carboxylic acid functional group of the diglycine needed to be protected as its ethyl ester. This had the added effect of increasing the solubility of the compound. This could have proven useful in some of the previous attempts to prepare the octa-substituted compound, in particular the acid chloride synthetic method. Not only would the diglycine ethyl ester have been more soluble in organic solvents, but it would have also protected the peptide from cross-coupling reactions. In hindsight, it is clear that while unprotected triglycine was used in the synthesis of MAG₃, the reaction was done in 0.1N NaOH (Brandau et al., 1988). Under these conditions, the carboxylic acid would have been essentially protected as its sodium salt. In the meanwhile, due to the lack of stability of the porphyrazine under alkaline conditions, such reaction conditions could not be used in this case and the carboxylic acid end of the diglycine was free to become involved in cross-coupling reactions.

This reaction proceeded straightforwardly, with good yields (60-80%) and clean products. HPLC of the reaction mixture gave only one major peak with shoulders for the zinc tetra-2,3-(4,5-diethoxyglycylglycylpyrazino)porphyrazine. However, only a nine times excess of reagents was used in this reaction while eight amide bonds need to be formed in order to reach completion. As such, the complete addition of eight glycylglycines to the octacarboxy-substituted macrocycle most likely did not occur and the shoulders seen in the HPLC chromatogram are most likely due to compounds substituted to a lesser degree with diglycine ethyl ester.

One of the reasons behind this increased yield and cleaner reaction is most likely the addition of the organic base triethylamine to the reaction mixture. Not only would it act to protect the porphyrazine from the evolving acid produced during the reaction but it would help drive the reaction towards completion by removing the acid from solution.
This would have the effect of pushing the reaction equilibrium towards the products by removing one of the products, as stated by Le Chatelier's principle.

The only significant problem with this reaction in the case of the porphyrinate is that it leads to an ester which needs to be hydrolyzed in order to obtain the desired water-soluble compound. As was seen in the synthesis of zinc tetra-2,3-(4,5-dicarboxy-pyrazino)porphyrinate, these compounds are sensitive to alkaline conditions and the hydrolysis of the precursor ester needed to be done with great care. However, hydrolysis of the diglycine ester groups was successfully done using the same method as in the original synthesis of the octacarboxyporphyrinate. This involved sonication in a 3:1 mixture of methanolic NaOH and water. Under these conditions, the complex, once hydrolyzed, will precipitate from solution, thus protecting it from the alkaline conditions of the reaction.

With the apparent success of this reaction in the case of the porphyrinate, a similar procedure was also used to added glycylglycine to the carboxy groups of zinc octacarboxyphthalocyanine. The reaction was done using a large twenty-five times excess of reagents in order to ensure that the reaction proceeded to completion. The resulting octa ethyl ester showed a single peak on the HPLC at a retention time of 33.5 minutes. This was as expected, with the addition of eight ethyl esters to the Pc periphery greatly increasing the hydrophobicity of the compound. No shoulders were present in the HPLC chromatogram, presumably showing that the reaction went to completion.

Hydrolysis of the ethyl esters was significantly easier with these compounds. Phthalocyanines are extremely stable and the hydrolysis reaction could be carried out using simple alkaline conditions. The use of 1N NaOH at room temperature allowed the reaction to occur, with hydrolysis being completed after 4 hours, as seen by HPLC. The resulting zinc octacarboglycylglycylphthalocyanine had a retention time similar to the starting material ($R_t = 10.75$ minutes as compared to 10 minutes). Simple agarose electrophoresis was also used to show that the resulting compound was completely
hydrolyzed as it migrated the same distance as the zinc tetra-2,3-(5,6-dicarboxy-
pyrazino)porphyrine octa sodium salt (i.e. both having a -8 charge).

Labeling of these compounds with $^{99m}$Tc was attempted. However, even the use
of numerous different reducing agents (stannous chloride, formaminidinesulfinic acid and
stannous tartarate), different solvents (methanol, distilled water) and different reaction
conditions (acidic, neutral), all attempts surprisingly failed to lead to a labeled product.
Explanation for this failure is not obvious. It is known that the addition of an electron-
withdrawing group to the C-terminal end of MAG$_3$, in this case a p-nitrophenol group
(Figure 48), prevents chelation of technetium (Schaffland et al., 1995). It is possible that
an electronic effect similar to this is at work here too, especially with the large ring current
and the high degree of aromaticity found in phthalocyanines.

![MAG$_3$ structure](image)

Figure 48: MAG$_3$ substituted with a p-nitrophenol group on the C-terminal end

Chelation of technetium often requires the ionization of hydrogens from one or
more of the ligating atoms (Slater et al., 1982). It is possible that the ability to ionize the
N-H bonds in these tetraamide cores does not allow complexation to occur under the
conditions investigated. The pKa of amides is usually around 16 (Francesconi et al.,

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1994), which is not particularly high as compared a C-H bonds (pKa = 43) (Sykes, 1986). However, among tetridentate ligands examined as potential $^{99m}$Tc chelators, the most extensively studied are $N_2S_2$ cores (Grunmon et al., 1995), while $N_4$ cores, like those produced in this work, usually contain some combination of nitrogen-containing functional groups. The pKa of S-H bonds is significantly lower than a N-H bond, being somewhere between 6 and 8 (Francesconi et al., 1994). Furthermore, in $N_2S_2$ cores containing amines (pKa = 33-35), the N-H bonds have been shown not to be ionized at all (Francesconi et al., 1994). Therefore, it is possible that the $N_4$ cores prepared in this work just are not effective in chelating technetium.

A few $N_4$ cores have been studied as technetium chelators. However, few if any of these are composed of a tetraamide core. Most often, they involve some combination of nitrogen-containing functional groups, such as oximes, amines, amides, etc. This combination plays an important role in the stability of the complex as well as the condition under which complexation can occur. For instance, a series of tetridentate cores were examined in order to determine the factors that influence the labeling efficiency for

\[
\text{CO}_2\text{H} \\
\text{O} \text{NH} \text{HN} \text{CO}_2\text{H} \\
\text{SH} \text{HS} \\
\text{H}_3\text{C} \text{SH} \text{HN} \text{CO}_2\text{H} \\
\text{(CH}_2\text{)}_3
\]

$N_2S_2$ diamidedithiol

$N_3S$ triamidethiol
Figure 49: A series of tetradeinate ligands used to study the factors that influence the labeling efficiency with technetium (Liu and Edwards, 1995)

tetradeinate chelators (Liu and Edwards, 1995) (Figure 49). It was found that the substitution of an amine group for an amide group greatly enhanced the kinetics of $^{99m}\text{Tc}$-labeling and changed the conditions necessary for labeling. While $N_2S_2$ monoamide monoaminedithiol and $N_2S_2$ diaminedithiol chelators were readily labeled at a pH between 5 and 11, the $N_2S_2$ diamidedithiol and $N_3S$ triamidethiol cores required strong alkaline conditions ($pH > 10$) in order to obtain a labeled product. In fact, radiochemical yields for the triamide, which is the most similar to the one prepared in this work, were less than 2% when the pH of the reaction was at 7.5 and did not improve until the pH was greater than 9. Hence, obviously, pH can play a very important role in determining the labeling efficiency for chelators with technetium. As such, while the porphyrizaine cannot be labeled under these alkaline conditions due to its instability under these conditions, perhaps labeling of the zinc octacarboglycylglycylphthalocyanine can be accomplished under more basic conditions.
5. Acknowledgements
5. Acknowledgements

I would like to offer my deepest thanks to Dr. J. E. van Lier for allowing me the opportunity to undertake this work and further my education. A sincere thank you also goes out to Dr. S. V. Kudrevich for all her helpful ideas and fruitful discussions. I would also like to thank all the members of Dr. van Lier's laboratory for all their help, especially in doing those little things without which research would grind to a halt. In particular, I should thank Dr. N. Brasseur for doing the biological testing of the zinc tetraphosphonophthalocyanine.

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Thanks everyone for all your help, support and encouragement.
6. Bibliography and References
6.0 Bibliography and References


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7. Appendices
Appendix A: Spectral Data

The UV-visible spectra of these compounds was recorded using a Hitachi U-2000 spectrophotometer. The NMR spectra of selected compounds was recorded on a Bruker Ac-300 300 MHz nuclear magnetic resonance spectrometer. The IR spectra of selected compounds was recorded using a Varian 3000 FT-IR spectrometer. The low resolution mass spectra of non-phthalocyanine precursors was obtained using a Hewlett Packard 5988 mass spectrometer. High resolution mass spectra of these compounds were obtained using a V9 Micro-mass model ZAB-1F apparatus.
Appendix A-1: Mass spectra of 4-diethoxyphosphonophthalonitrile

\[
m_{r/e} \text{ (theoretical)} = 264.0664 \text{ amu} \\
m_{r/e} \text{ (experimental)} = 264.0660 \pm 0.0007 \text{ amu}
\]
Appendix A-2: Infrared spectrum of 4-diethoxyphosphonophthalonitrile (as a KBr pellet)
Appendix A-4: 13C NMR of 4-dilethoxyphosphonophthalonitrile (in d$_6$-DMSO)
Appendix A-5: UV-visible spectra of 4-diethoxyphosphonophthalonitrile (in DMSO)

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<td>298.5 nm</td>
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<td>289.0 nm</td>
<td>0.235</td>
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<tr>
<td>259.5 nm</td>
<td>0.572</td>
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\[
\text{[']} = 1.21 \times 10^{-4} \text{ M}
\]
Appendix A-6: Infrared spectrum of zinc tetra(diethoxyphosphono)Pc (as a KBr pellet)
Appendix A-7 UV-visible spectra of zinc tetra(dilethoxyphosphono)Pc

(in triphenylphosphite)

Wavelength          | Absorbance
685.0 nm            | 0.065
616.0 nm            | 0.018
382.0 nm            | 0.078
344.0 nm            | 0.112

[ ] < 10^{-6} M
Appendix A-8: Infrared spectrum of zinc tetraphosphonophenoPc (as a KBr pellet)
Appendix A-9: UV-visible spectra of zinc tetraphosphonoPc
(in pH 12 buffer)

Wavelength | Absorbance
--- | ---
682.0 nm | 0.505
617.5 nm | 0.112

$\lbrack \rbrack = 3.90 \times 10^{-7}$ M
Appendix A-10: UV-visible spectra of zinc tetraphosphonophene (in pH 5 buffer)

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</table>

\[ [ ] = 7.80 \times 10^{-7} \text{ M} \]
Appendix A-12: UV-visible spectra of zinc tetra-2,3-(5,6-dicarboethoxyglycylglycylpyrazino)porphyrizine

(in methanol)

Wavelength  
656.0 nm

Absorbance  
0.177

[ ] = unknown
Appendix A-13: UV-visible spectra of zinc tetra-2,3-(5,6-dicarboxylicylglycylpyrazino)porphyrazine

(in distilled water)

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[] = unknown
Appendix A-14: UV-visible spectra of zinc octacarboxyethylglycylglycylphthalocyanine (in methanol)

Wavelength
640.5 nm

Absorbance
0.294

[ ] = unknown
Appendix A-15: UV-visible spectra of zinc octacarboglycylglyclylphthalocyanine

(in distilled water)

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<td>618.0 nm</td>
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[ ] = unknown
Appendix B: HPLC Data

High performance liquid chromatography of cold products was done using a Varian 500 Liquid Chromatograph fitted with a Water RCM 8x10 module containing a Nova-Pak Radial-Pak C$_{18}$ cartridge (8 mm x 100 mm) (particle size = 4 µm, pore size = 60 Å) (Waters Chromatography Division, Mississauga, Ontario). It was operated with a linear gradient running from 100% aqueous sodium phosphate buffer pH 5.5 (10 mM) to 100% methanol (HPLC grade, Fisher Scientific, Nepean, Ontario) over a period of 30 minutes, with a flow rate of 1.5 ml/minutes. Compounds were detected from their UV-visible absorption, at the appropriate wavelength, using the UV-visible detector found within the Varian 500 Liquid Chromatograph instrument.

HPLC of radioactive samples was done using a similar Varian 500 Liquid Chromatograph fitted with a Water RCM 8x10 module containing a Nova-Pak Radial-Pak C$_{18}$ cartridge (8 mm x 100 mm) (particle size = 4 µm, pore size = 60 Å). It was operated with a linear gradient running from 100% aqueous sodium phosphate buffer pH 5.5 (10 mM) to 100% methanol over a period of 30 minutes, with a flow rate of 1.5 ml/minutes. Elutes were detected using an external Shimadzu SPD-6AV UV-visible spectrophotometric detector set at the appropriate wavelength. Radioactivity was also detected using an external detector.
Appendix B-1: HPLC chromatogram of zinc tetraphosphonoPc

Retention time = 8.3 minutes
Appendix B-2: HPLC chromatogram of the acyl chloride reaction mixture
Appendix B-3: HPLC chromatogram of the succinimidyl active ester

reaction mixture
Appendix B-4: HPLC chromatogram of the water-soluble succinimidyl active ester reaction mixture
Appendix B-5: HPLC chromatogram of zinc tetra-2,3-(5,6-dicarboethoxyglycylglycylpyrazino)porphyrazine
Appendix B-6: HPLC chromatogram of zinc tetra-2,3-(5,6-dicarboxyglycylglycylpyrazino)porphyrazine

Retention time = 13.2 minutes
Appendix B-7: HPLC chromatogram of zinc octacarboethoxyglycylglycylphthalocyanine

Retention time = 33.5 minutes
Appendix B-8: HPLC chromatogram of zinc octacarboxyglycylglycylphthalocyanine

Retention time = 10.75 minutes
Appendix B-9: HPLC chromatogram of $^{99m}$Tc-labeled zinc tetraphosphonophthalocyanine
Appendix B-10: HPLC chromatogram of the attempted labeling of zinc octacarboxyglycyglyclylphthalocyanine with $^{99m}$Tc using stannous chloride in weak acid as the reducing agent
Appendix B-11: HPLC chromatogram of the attempted labeling of zinc octacarboethoxyglycylglycylphthalocyanine with 99mTc using stannous tartarate in water as the reducing agent