Synthesis and characterisation of a selection of phthalocyanines covalently bonded to water-soluble polymers and a ferrocenyl fragment

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ABSTRACT

This thesis is concerned with the synthesis of phthalocyanine derivatives and their anchoring onto water-soluble polyaspartamides to produce compounds that may have biomedical applications, especially in the photodynamic therapy of cancer. The anchoring of a ferrocenyl fragment, which itself is an anti-neoplastic entity, onto both the polymer and a phthalocyanine entity was also accomplished utilizing biodegradable amide bonds. The electrochemical behaviour of selected phthalocyanines and polymer-anchored phthalocyanines is reported.

Phthalocyanines containing both amine and carboxylic acid functional groups were prepared using established methods. The active amine side chains of the polymers derived from aspartic acid and/or lysine were utilized to couple both ferrocenyl and phthalocyanine entities. In addition ferrocenylethylamine was anchored onto a phthalocyanine containing carboxylic acid groups. The phthalocyanine-anchored polymers showed a marked decrease in water-solubility especially when these had additional ferrocenyl fragments anchored onto either the polymeric backbone or onto a polymer-bound phthalocyanine.

The attachment of a ferrocenylethylamine onto a polymer-bound phthalocyanine containing three free carboxylic acid groups appears to be a sterically hindered process as only one of the carboxylic acid groups undergoes amidation even in the presence of excess amine.

OPSOMMING

Hierdie tesis behels die sintese van ftalosianien derivate en hulle koppeling aan wateroplosbare poliaspartamiedes om verbindings te vorm wat moontlike biomediese toepassings mag vind, veral in die fotodinamiese behandeling van kanker. Die koppeling van 'n ferroseniel fragment, wat self 'n neoplastiese fragment is, aan beide die polimeer en 'n ftalosianien entiteit is ook bereik deur van biodegredeerbare amied bindings gebruik te maak. Die elektrochemiese gedrag van geselekteerde ftalosianiene is ook vermeld.

Ftalosianiene wat beide amien en karboksielsuur funksionele groepe bevat is ook berei deur bekende metodes. Die aktiewe amien sykettings van die polimere wat gesintetiseer is vanaf aspartaamsuur en/of lisien is gebruik om beide ferroseniel en ftalosianien entiteite te koppel. Daarby is ferrosenieletielamien gekoppel aan ftalosianienbevattende karboksielsuur groepe. Die ftalosianien gekoppelde polimere het 'n noemenswaardige afname in wateroplosbaarheid getoon, veral wanneer addisionele ferroseniel fragmente gekoppel is aan die polimeriese rugraat of aan 'n polimeergebonde ftalosianien.

Die koppeling van ferrosenieletielamien aan 'n polimeergebonde ftalosianien wat oor drie vrye karboksielsuurgroepe beskik, bleik 'n steries verhinderde proses te wees omdat slegs een van die karboksielsure amiedvorming ondergaan in die teenwoordigheid van oormaat amien.

List of abbreviations

b	-	broad (infrared context)
BTU	-	O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate
cm^{-1}	-	wave number
C_p	-	cyclopentadienyl
CV	-	cyclic voltammogram
DBN	-	1,5-diazabicyclo[4.3.0]non-5-ene
DBU	-	1,8,-diazabicyclo[5.4.0]undec-7-ene
DMF	-	dimethylformamide
DMSO	-	dimethylsulphoxide
$E^{0'}$	-	formal reduction potential
E_{pa}	-	anodic peak potential
E_{pc}	-	cathodic peak potential
^{1}H NMR	-	proton nuclear magnetic resonance
HDL	-	high density lipoprotein
HPD	-	Heamatoporphyrin derivative
HPLC	-	high pressure liquid chromatography
i_{pa}	-	anodic peak current
i_{pc}	-	cathodic peak current
IR	-	infrared
mp	-	melting point
LDL	-	low-density lipoprotein
m	-	medium (infrared context)

MPc	-	metallated phthalocyanine
Nc	-	naphthalocyanine
Pc	-	phthalocyanine
PDT	-	Photodynamic therapy
PPA	-	polyphosphoric acid
ppm	-	parts per million
s	-	sharp (infrared context)
SPc	-	superphthalocyanine
SubPc	-	subphthalocyanine
TBAHFB	-	tetrabutylammonium tetrafluoroborate
TBAP	-	tetrabutylammonium perchlorate
$TBAPF_6$	-	tetrabutylammonium hexafluorophosphate

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1998.

Chapter 1

Introduction

The development of new and more efficient chemotherapeutic agents entered a new era with the commissioning of *cis*-diamminedichloroplatinum(II) (cisplatin) as metal-containing chemotherapeutic agent[1] in 1979. Cisplatin, as a member of the so-called first generation chemotherapeutic drugs, is still the most frequently used metal-containing drug for cancer therapy in the USA, Europe and Japan[2]. The clinical use of all chemotherapeutic agents is, however, restricted due to the severe side effects they induce. Using cisplatin as a representative example, the side effects and undesirable properties of many chemotherapeutic drugs may be summarised as follow:

i) Exceptional toxicity to the kidneys and bone marrow[3,4];

ii) Damage to the linings of the intestines by this and many other drugs is extensive, leading to loss of appetite (anorexia) and eventual starving in the case of rats and mice[5].

iii) Hair loss, nausea, vomiting and audio toxicity is commonly encountered.

iv) The window between the minimum effective (3 mg kg^{-1} test animal – mice – body mass for cisplatin), therapeutic (7 mg kg^{-1} for cisplatin) and 50% lethal dosages (14 mg kg^{-1}) is often small. The therapeutic dosage for cisplatin also represents the 10% lethal dosage.

v) A slow build-up of drug resistance takes place with time[6,7].

vi) Although cisplatin's excretion profile from the body is complex, it has been demonstrated that within 20 hours of administering the drug to the body, 50% of it is excreted. This demonstrates the fact that the quick excretion mechanism of all foreign chemicals in the body causes large drug concentration fluctuations, often beyond the limits of optimum therapeutic and minimum effective levels, over short periods of time[8,9,10,11].

vii) Also, many chemotherapeutic drugs are in themselves moderate carcinogens. Thus it has been demonstrated that cisplatin eventually induces lung cancer, skin papillomas and other sarcomas[12].

In addition to the above chemical and biological side effects, physical properties of many promising pharmaceutical agents are often not conducive to extensive biological applications. Most notorious of these is poor solubility in aqueous media. The solubility of cisplatin in water is only 2.53 g dm^{-3}. Non ionic phthalocyanines or ferrocene derivatives are virtually insoluble in water.

Thus, although second and third generation drug development is continuously taking place, the drugs seldom satisfactorily address all the above mentioned negative aspects.

To address as many of the negative aspects as possible, a multi-disciplinary approach leading to a package of solutions is required. Towards this end, many pharmacological advantages are obtained by tying a pharmacological agent (drug) to a macromolecular drug carrier possessing solubility in water. The clinical administration of a polymer-bound drug may significantly enhance therapeutic effectiveness in terms of:

i) Accelerated and unencumbered drug distribution in the aqueous central circulation system of the body, thereby reducing the risk of premature degradation and excretion.
ii) Cell entry *via* endocytosis – a cell penetration mechanism generally unavailable to non-polymeric compounds, but highly desired for drugs operating intracellularly[13].
iii) More precise controlled drug serum levels (i.e. restriction of drug concentration to the gap between toxic and minimum effective levels).
iv) An enhanced depot effect through delayed drug release from the polymer drug conjugate.

Some of the properties that should be built into a polymeric drug carrier includes bio-compatibility, water-solubility, it must have a large amount of drug attachment sites, it must be biodegradable to allow ultimate elimination of the spent polymeric carrier from the body after its payload of drug has been delivered to the target site and it must be non-toxic, non-antigenic or non-provocative in any other respect.

The central problem in chemotherapy, however, remains selectivity, that is, the capability of a drug to distinguish between healthy and cancerous cells. In this regard, certain phthalocyanines may in future play a significant role in at least two ways. Firstly, aluminium, zinc and gallium phthalocyanine complexes are photodynamically active[14]. In photodynamic cancer therapy a photodynamically active drug is administered to the body. It is totally inactive in the dark. Only when it is irradiated with light of the correct wavelength is it activated and destroys living cells.

This provides a unique way of introducing selective action in cancer therapy. The key to no side effects during photodynamic cancer therapy, therefore, does not hinge on the drug at all but rather on the capability of irradiation of cancerous growths without allowing light to fall on non-cancerous growths. Sadly though, most phthalocyanines are extremely insoluble in any solvent. Even carboxylated and quaternary ammonium salts of phthalocyanines are only sparingly soluble in water. What is needed is a carrier that will allow phthalocyanines to become soluble in an aqueous system. The second way in which phthalocyanines may play a key role in future selective chemotherapeutic drug action is centered on the superphthalocyanines. The superphthalocyanines represent the first example of antineoplastic material that has a pronounced larger affinity for cancer cells than healthy cells. It was recently demonstrated that within 48 hours after administering uranium superphthalocyanine to the body, 98% of it was accumulated in cancer cells while the remainder was evenly spread through non-cancerous cells[15]. In principle, it should therefore be possible to tag an existing drug with a superphthalocyanine and if the properties of the superphthalocyanine dominate, the chemotherapeutic agent should preferentially be carried into the cancer cell.

With this background, the following goals were set for this study:

i) Investigate synthetic routes towards carboxylated and amine functionalised phthalocyanines.

ii) Synthesise phthalocyanine/ferrocene conjugates, preferably linked *via* an amide bond.

iii) Synthesis of potential water-soluble biodegradable polymeric drug carriers.

iv) Develop suitable procedures to allow anchoring of selected phthalocyanines on water-soluble polymeric drug carriers utilising biodegradable amide bonds.

v) Investigate some of the electron transfer properties of the synthesised phthalocyanine derivatives by means of cyclic voltammetry.

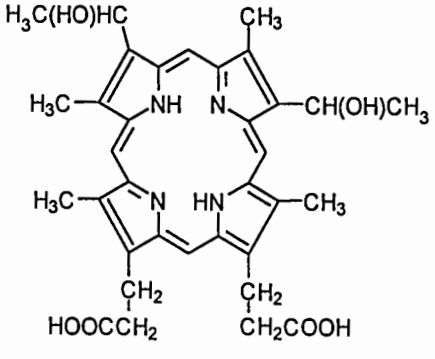
Chapter 2

Literature Survey

2.1 Photodynamic therapy

2.1.1 Introduction

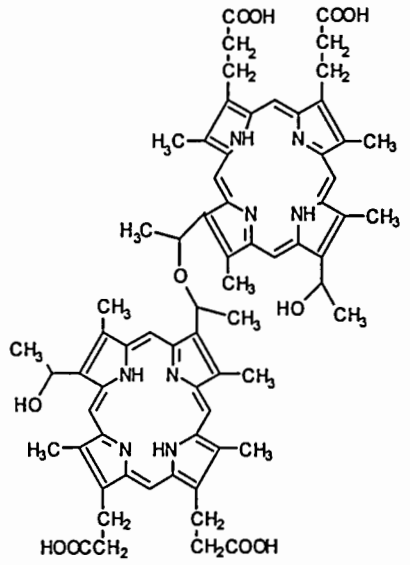
Apart from radical surgery, the two major techniques used for the treatment of cancer are radiotherapy and chemotherapy. Whilst combating tumour growth with some success, both methods can also induce disabling and life threatening side effects mainly because they destroy indiscriminately both normal and tumour tissue[16]. Photodynamic therapy (PDT) has developed as an alternative for the treatment of cancer. In photodynamic cancer therapy, a photodynamically active drug, which is a photosensitizer, is administered to the body. It is totally inactive in the dark. Only when it is irradiated with light of the correct wavelength is it activated and then destroys living cells. This provides a unique way of introducing selective action in cancer therapy. The key to no side-effects during photodynamic cancer therapy therefore does not hinge on the drug at all but rather on the capability of irradiation of cancerous growths without allowing light to fall on non-cancerous growths. It employs the combination of light and a drug called a photosensitizer to selectively destroy tumour tissue. The first scientific observation of this phenomenon is found in the work of Raab[17] who found that paramecia were rapidly killed by visible light in the presence of oxygen and low concentrations of dyes. This mechanism was further exploited after Auler and Banzer[18] established the affinity of various porphyrins, including hematoporphyrin **1**, to malignant as compared to adjacent healthy tissues.



1

Fig. 1 Structure of hematoporphyrin

Treating hematoporphyrin **1** with a 19:1 mixture of acetic acid and sulfuric acid further optimized the tumor localizing ability, the then acetylated product was dissolved in dilute alkali to improve its solubility in water. The new product was then named hematoporphyrin derivative (HPD) which is actually a mixture of products and is currently used widely for the treatment of a variety of malignancies[19]. The purified form of HPD is commercialized under the name of Photofrin II, this then being a mixture of dimers and oligomers in which the active component in the photodynamic action is believed to be the dihematoporphyrin ether[20] **2**



2

Fig. 2 Structure of Dihematoporphyrin ether

2.1.2 Localization of drugs

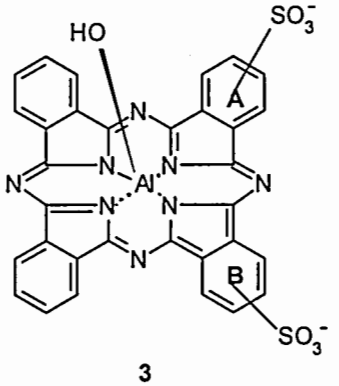
Photodynamic therapy depends on selective cell injury; thus the drugs should be retained selectively in tumor tissue and then, if not totally possible, additional selectivity can be attained by spatial localization of the illumination to the target tissue. Prior to localization at tumour tissue the photosensitizer is transported by the blood and interacts with serum proteins. The serum proteins, albumin and low-density lipoprotein (LDL) have been identified as important natural drug

carriers[21]. Various studies have shown that the more hydrophilic photosensitizers bind to albumin and are localized in the vascular stroma. By contrast, the more hydrophobic photosensitizers are bound progressively more to the lipoprotein[22]. Recent studies also suggest that the pre-binding of high density lipoprotein (HDL) and (LDL) to photosensitizers (porphyrins, benzoporphyrins and phthalocyanines) leads to a significant increase in tumour localization[23]. Recognition of the importance of the delivery mode in the overall therapeutic effectiveness has thus led to the study of liposomes[22], cyclodextrin, pre-binding to proteins[24] and conjugation to monoclonal antibodies[25] as delivery systems in PDT. The advantage of using delivery systems is an increase in solubility and an enhancement of tumour selectivity. Various researchers have suggested that the localization and retention of photosensitizers in malignant tissue is due to:

a) Tumour cells having a higher vascular permeability due to the expression of a protein by the tumor to increase tumour growth[26].
b) Poor lymphatic drainage of tumours due to the underdevelopment of the lymphatic system[27].
c) The interstitial space difference between tumours and normal cells.
d) A deficiency of the ferrochelatase enzyme in cancerous tissue which is responsible for the formation of haem and which is subsequently broken down by haem oxygenase[20].
e) The relatively high collagen concentrations in tumour tissues facilitate the binding of porphyrins to the stroma and vessel walls of tumour tissue[28].
f) Higher acidity of malignant tissue.

2.1.3 Phthalocyanines in cancer therapy

In the search for an ideal photodynamic drug the most important factors to be considered are : that the drug should possess a very low systemic toxicity, show a preferential affinity for malignant tumors, have a high photodynamic efficiency and have a maximum absorption in the red part of the visible spectrum. HPD complies with all the above except that its main absorption is around 400nm, for therapy the dye is activated by red light (λ = 630nm). In contrast the extended conjugated aromatic phthalocyanines possess an intense, more or less Gaussian Q-band at 650-700 nm ($\varepsilon > 10^5$ m^{-1} cm^{-1})[29] and are essentially transparent between λ = 400-630 nm. These compounds, therefore, allow deeper light penetration of tissue and are substantially less efficient in inducing skin photosensitivity, which is a major problem with HPD[30]. The phthalocyanine macrocycle can coordinate with almost every element from the periodic table and can be substituted at the periphery with a variety of substituents. Of all the possible phthalocyanines, the sulphonated zinc and sulphonated aluminium phthalocyanines, especially the di-sulphonated aluminium phthalocyanines **3** wherein the sulphonated benzene rings are located on adjacent pyrrole moieties (indicated in fig. 3 as rings A and B), seem to be the most potent photosensitizers of this class[31].

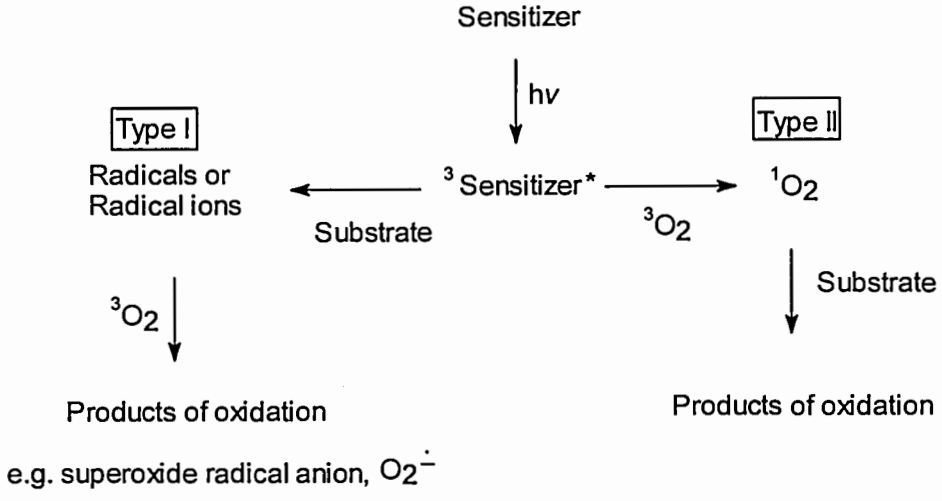


3

Fig. 3 Disulphonated Aluminium Phthalocyanine

2.1.4 Photochemistry of photodynamic therapy

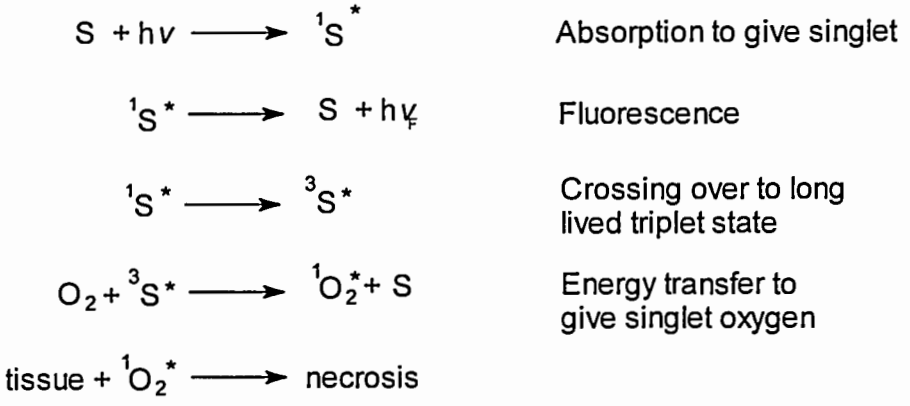
The cytotoxic agent in PDT is produced by one of two different processes, which, in photochemistry is referred to as a type I or a type II process. These processes are mediated by the excited triplet state of the photosensitizer, shown in scheme 1.



Scheme 1 Photochemical processes in PDT

A type I mechanism results in hydrogen atom or electron transfer reactions between the sensitizer and some substrate or the solvent to yield either radicals or radical ions[32]. Radicals and radical ions formed can then react with oxygen to yield superoxide radical anion (scheme 1). The type II process is detailed in scheme 2, page 11, from which it can be seen that energy is transferred from a sensitizer, (such as a metallated phthalocyanine) denoted by the symbol S, to ground-state oxygen which results in singlet state oxygen.

S = metallated phthalocyanine



Scheme 2 Type II mechanism wherein S represents a metallated phthalocyanine.

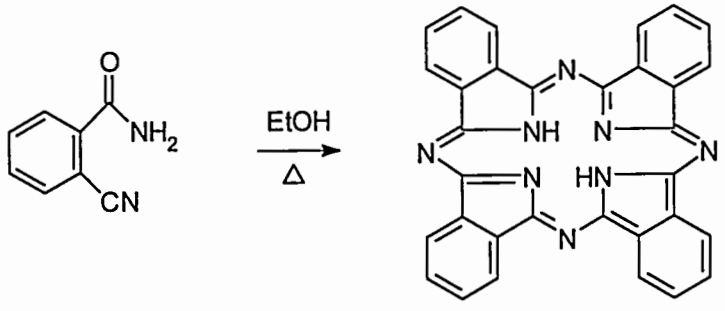
Both singlet oxygen and superoxide are cytotoxic species, causing oxidative destruction of tissue and they constitute the basis for photodynamic cancer therapy[33]. Which mechanism is operative has not yet been firmly established, however, the generation of singlet oxygen via the Type II pathway in solution is picked up by observation of its weak luminescence at λ = 1270 nm using a near infrared photodetector. Such luminescence has been seen widely in *in vitro* studies giving rise to the widespread belief that singlet oxygen is invariably the mediator (active substance) in PDT[27].

2.2 Phthalocyanine synthesis

Since the synthesis and characterisation of various phthalocyanine derivatives constitutes the heart of this research program, it is important to highlight some aspects of phthalocyanine synthesis in this literature survey.

2.2.1 Metal-free unsubstituted phthalocyanine synthesis

The first synthesis of a phthalocyanine **4** was recorded in 1907 when it was found that heating *o*-cyanobenzamide at a high temperature[34] led to the formation of a blue compound (scheme 3) which was only characterised a quarter of a century later[35].



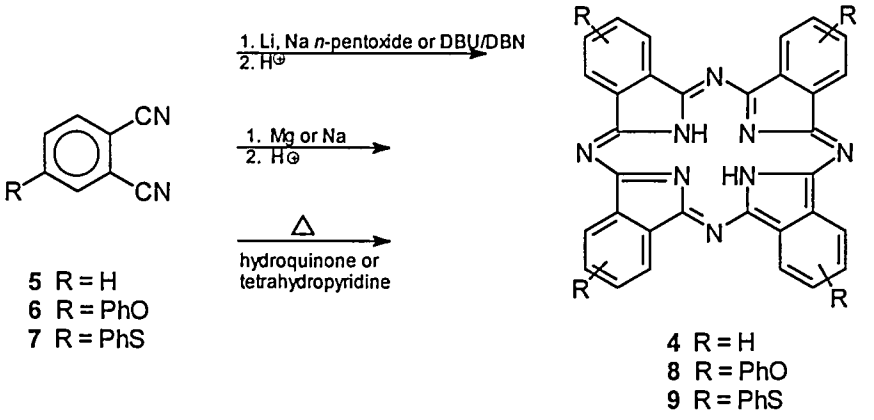
4

Scheme 3 First metal free phthalocyanine synthesis

2.2.2 Metal-free substituted phthalocyanines

2.2.2.1 Phthalonitriles

Despite earlier difficulties in preparing phthalonitriles, Linstead and Lowe showed that phthalonitrile, upon treatment with sodium or lithium *n*-pentoxide in *n*-pentanol at 135-140^{0}C gave disodium phthalocyanine, which could be directly demetallated to phthalocyanine **4** (scheme 4, page 13) with concentrated sulphuric acid[36]. As substituted phthalonitriles are now readily available, the possibility of preparing substituted phthalocyanines by this method is widely used. For example, 4-phenoxyphthalonitrile **6** and 4-thiophenoxyphthalonitrile **7** gives 2,9,16,23-tetraphenoxyphthalocyanine **8** and 2,9,16,23-tetrathiophenoxyphthalocyanine **9** (scheme 4) as mixtures of isomers in 39% and 25% yield respectively[37]. More recently Wöhrle reported that substitution of the alkoxide bases for stronger bases such as 1,8,-diazabicyclo[5.4.0]undec-7-ene (DBU) or 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) gave the previously mentioned phthalocyanines in yields of 77% and 96% respectively[38].

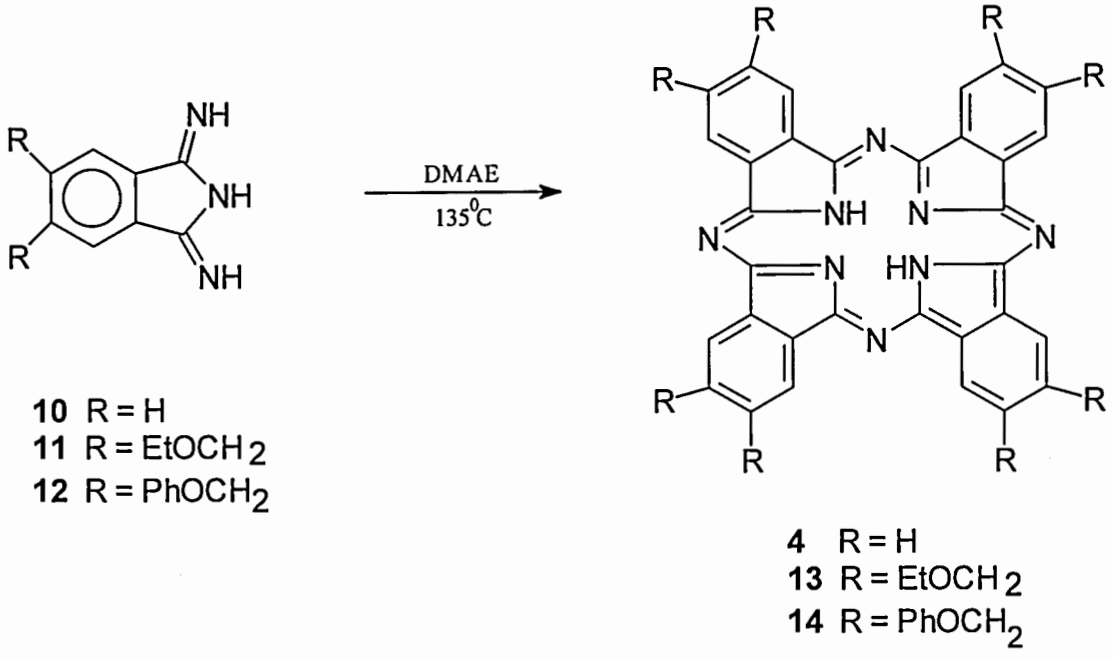


Scheme 4 Using phthalonitriles in phthalocyanine synthesis

The second method in scheme 4 illustrates the reaction of magnesium or sodium metal at 200^{0}C to give magnesium or sodium phthalocyanines[39] from which substituted metal-free phthalocyanines are liberated by treatment with concentrated sulphuric acid. The use of the reducing agents hydroquinone and tetrahydropyridine (scheme 4) as co-reactants in a sealed tube is also illustrated in analogous reactions of substituted phthalonitriles **6** and **7** to afford **8**[40] and **9**[41] in 81% and 43% yield, respectively.

2.2.2.2 Diiminoisoindolines

Diiminoisoindoline may also be employed in the synthesis of phthalocyanine **4**, the reaction takes place in 85% yield by simply refluxing **10** in 2-N,N-dimethylaminoethanol[42] (scheme 5, page 14). Octasubstituted phthalocyanines, for example, have been prepared from 5,6-bis(ethoxymethyl)-1,3-diiminoisoindoline **11** or 5,6-bis(phenoxymethyl)-1,3-diiminoisoindoline **12** to give 2,3,9,10,16,17,23,24-octa(ethoxymethyl)-phthalocyanine **13** and 2,3,9,10,16,17,23,24-octa(phenoxymethyl)phthalocyanine **14** both in 80% yield[43] (scheme 5).



10 R = H
11 R = $EtOCH_2$
12 R = $PhOCH_2$

4 R = H
13 R = $EtOCH_2$
14 R = $PhOCH_2$

Scheme 5 Using diiminoisoindolines to synthesise phthalocyanines

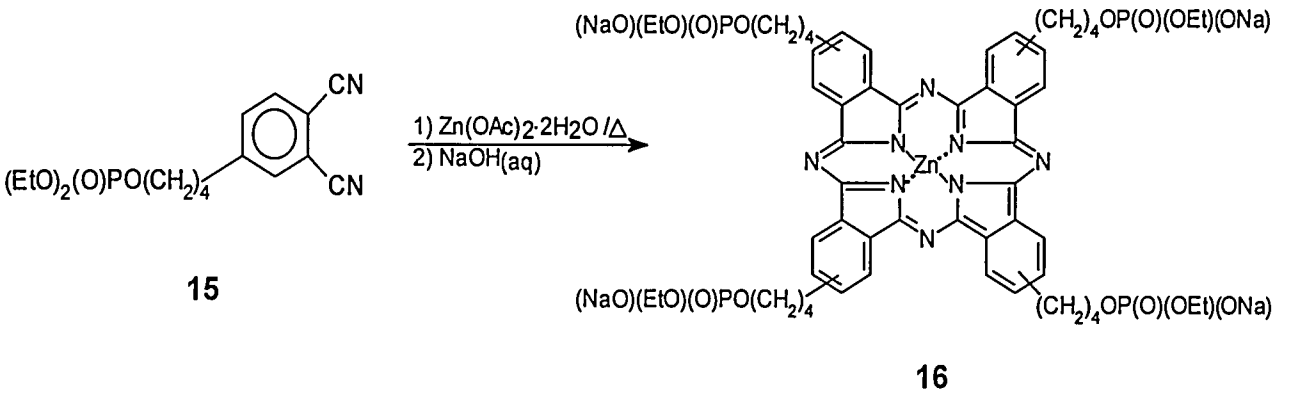
2.2.3 Metallated phthalocyanines

Phthalocyanines have been metallated in conjunction with peripheral functionalization for various applications. Many methods of preparation of metallated phthalocyanines have been developed among which the most popular are : (1) The reaction of phthalonitrile with a metal[44] or a metal salt using a strong base[30], (2) the reaction of phthalic anhydride[45], phthalic acid[46] or phthalimide[47] with urea, metal salts and a catalyst and (3) the reaction of 1,3-diiminoisoindolines[48] with a metal salt in a hydrophilic solvent. In addition, a metal-free phthalocyanine or a metallated phthalocyanine will react with a metal or a metal salt if the product is a more stable entity[49]. As it is not practical to discuss all possible methods the most important are given.

2.2.3.1 Phthalonitrile with metal salt

In the PDT application of phthalocyanines, it has been established that the more water-soluble compounds are more compatible with biological media and as such the disulphonated phthalocyanines were found to be the most effective (see paragraph 2.1.3, page 9). In an attempt to

synthesise different water-soluble phthalocyanines as potential PDT drugs van Lier[30] prepared 2,9,16,23-tetrakis[1-(*O*-ethylphosphonato)butyl]-zinc phthalocyanine sodium salt **16** by using the phthalonitrile diethyl 4-(3,4-dicyanophenyl)butylphosphate **15** and condensing the phthalonitrile in the presence of zinc acetate at 130^{0}C for 3 hours. The phthalocyanine was then purified by medium pressure reverse phase chromatography and then used as the sodium salt in the tumour treatment.



15 **16**

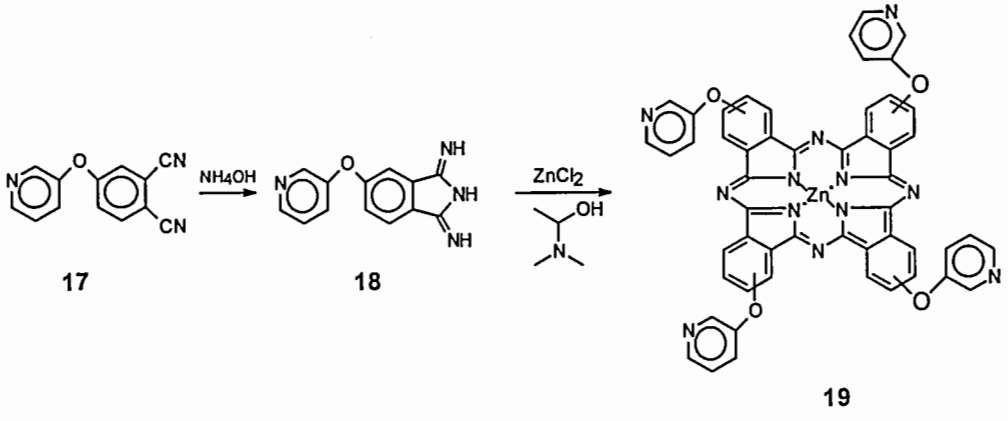
Scheme 6 Using phthalonitriles with a metal salt to synthesise phthalocyanines

Very often however the "nitrile" method requires the use of high boiling solvents such as 1-chloronaphthalene, nitrobenzene, and 1,2-dichlorobenzene. Thus, soluble phthalocyanines, which require the use of distillation for solvent removal, should rather be synthesised by a special variant of the nitrile method with ethylene glycol[50].

2.2.3.2 Diiminoisoindoline with a metal salt

The solubility properties of the phthalocyanines are strongly influenced by the nature of the peripheral groups. The range of such groups capable of effecting water-solubility is relatively small, the usual groups used for this purpose being sulphonic or carboxyl groups. In an interesting variation, Smith[51] synthesised 5-(pyridyloxy)-1,3-diiminoisoindoline **18** from 4-(3-

pyridyloxy)phthalonitrile **17** and used the diiminoisoindoline to attain zinc(II)tetra-(3-pyridyloxy)phthalocyanine **19** in 24% yield.



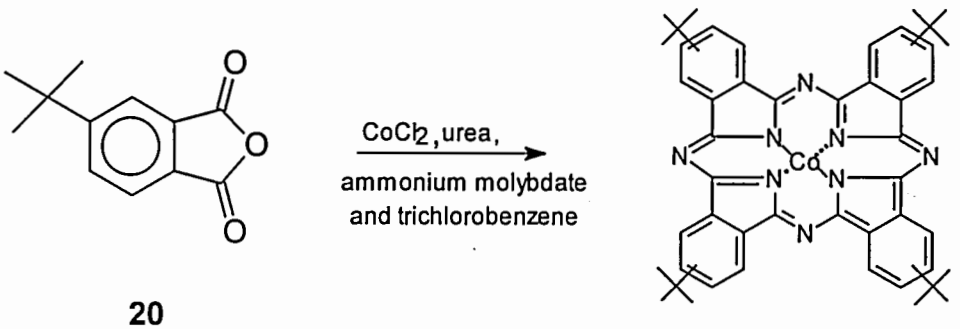
Scheme 7 Using diiminoisoindolines with metal salts

The diiminoisoindoline was used instead of the phthalonitrile due to the milder reaction conditions required for the more reactive diiminoisoindoline to enable the pyridyloxy groups to remain intact. In a surprising observation Smith reports that no isomers could be detected chromatographically which is rather doubtful as in virtually all other condensations reported of 4-substituted phthalonitriles the resultant phthalocyanines were mixtures of isomers and differently substituted compounds. The purification process thus reported was a simple precipitation from solution with ethanol and then washing with water and ethanol followed by air drying at 70^0C. The phthalocyanine may then be rendered water-soluble by converting the peripheral pyridyloxy groups to their pyridinium cationic form by protonation.

2.2.3.3 Anhydride with metal salt

An important field of phthalocyanine chemistry is that of development of one-dimensional organic conductors[52] in which the basic structural feature should be a linear arrangement of transition metal atoms (e.g. Fe, Ru, Co). The phthalocyanine is essentially a planar, tetradentate macrocyclic system

that complexes each metal atom in its equatorial plane[53]. Using the "anhydride" method Hanack[45] synthesised (2,9,16,23-tetra-*tert*-butylphthalocyaninato)cobalt(II) **21** (scheme 8) by reacting 4-*tert*-butylphthalic anhydride **20**, urea and cobalt chloride in trichlorobenzene with a catalytic amount of ammonium molybdate added. The mixture was heated for 4h at 190^{0}C, after addition of petroleum ether and filtration, the filtrate was concentrated, after precipitation the material was purified by successive hydrochloric acid and sodium hydroxide treatment and after a final extraction with methanol the material was dried *in vacuo* for 6 hours to afford **21** in a yield of 31%.

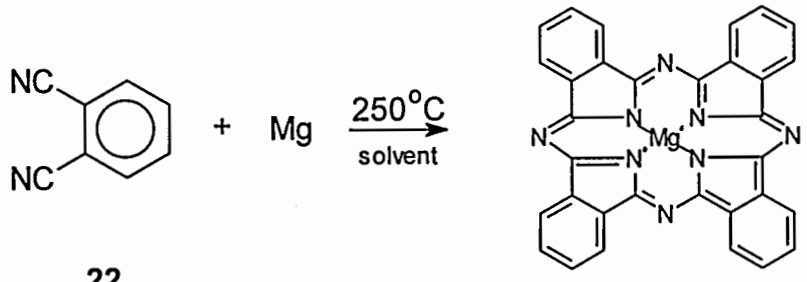


20 **21**

Scheme 8 Using anhydrides to synthesise metallated phthalocyanines

2.2.3.4 Phthalonitrile with metal (sublimation)

The classic use of phthalocyanines as dyestuffs has probably led to the further development of these extremely versatile molecules. The purification procedures are usually very arduous, but in the case of sublimeable molecules, a pure product may be obtained by a very simple procedure. Magnesium phthalocyanine **23** is prepared (scheme 9, page 18) in 79% yield by adding lightly etched magnesium turnings to phthalonitrile **22**[54]. The obtained material is washed with ethanol and dried, upon which sublimation of the product at 5mm Hg results in dark blue needles of pure magnesium phthalocyanine **23**.

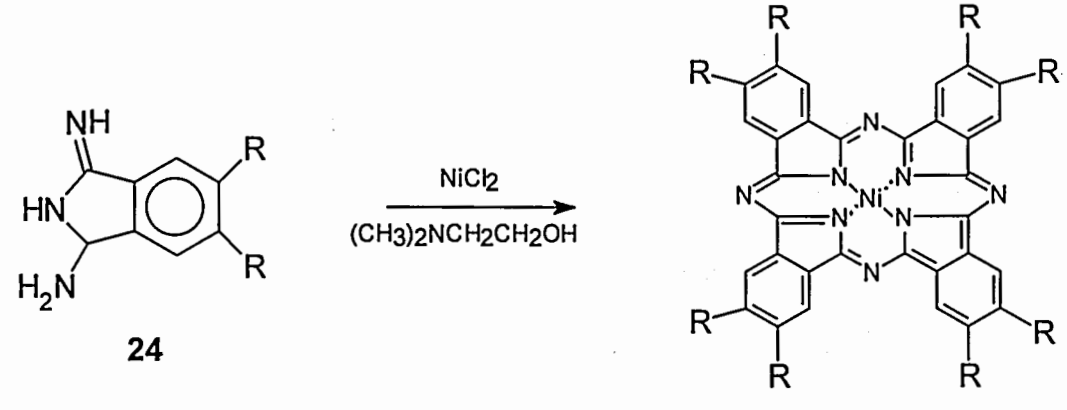


22 23

Scheme 9 Using phthalonitrile with a metal to synthesise phthalocyanines

2.2.3.5 Diiminoisoindoline with base

The method of purification that is used most extensively in the synthesis of phthalocyanines is by means of column chromatography, more specifically flash column chromatography[55]. A typical example is that of Hanack[56] in which 2,3,9,10,16,17,23,24-octa(octyloxymethyl)-phthalocyaninatonickel(II) is synthesised (scheme 10) from 1,3-diimino-4,5-bis(octyloxy methyl)-1,3-dihydroisoindole **24** by heating in boiling dimethylaminoethanol in the presence of nickel chloride for 7 hours. The residue, after solvent evaporation is chromatographed on a silica gel flash column to yield the pure octasubstituted phthalocyanine **25** in 33% yield.



24

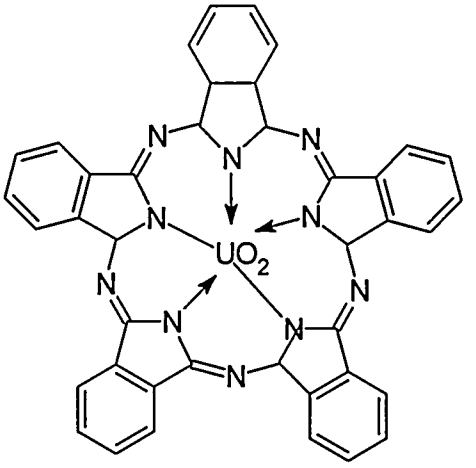
R = $CH_2OC_8H_{17}$

25

Scheme 10 Using diiminoisoindoline with a base to synthesise phthalocyanines

2.2.4 Superphthalocyanines (SPc's)

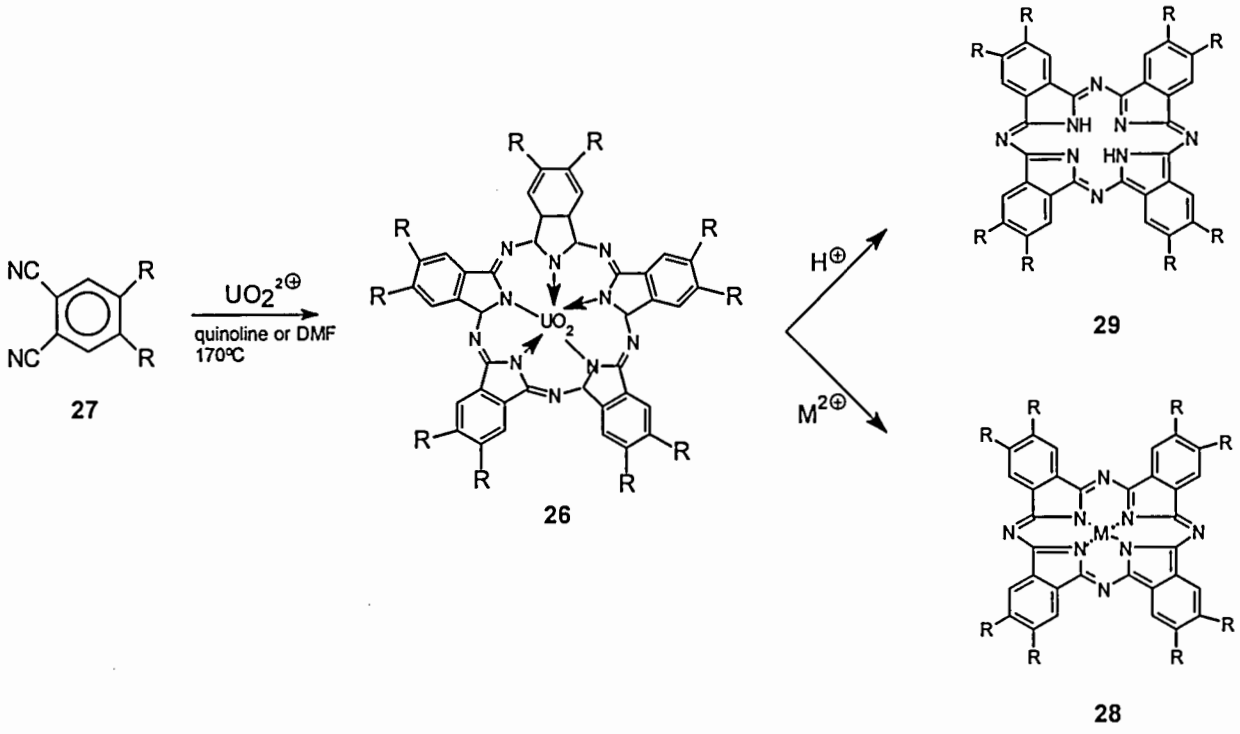
A superphthalocyanine, uranyl superphthalocyanine **26** was synthesised as early as 1964[57], and its structure was confirmed by x-ray crystallography in 1975[58].



26

Fig. 4 Structure of uranyl superphthalocyanine

In the superphthalocyanine (SPc) molecule, the uranyl ion serves as a nucleus around which five (not the usual four) phthalonitrile moieties may assemble to produce the five-subunit uranyl superphthalocyanine. The molecule may be prepared by heating phthalonitrile in the presence of anhydrous uranyl chloride in DMF at 170^{0}C for one hour[59] (scheme 11, page 20). The use of other uranyl salts and the presence of moisture decreased the yield significantly. In a similar fashion, alkylated SPc's were prepared using substituted phthalonitriles, in very low yields[60]. Furthermore, due to the inherent instability of SPc's, octaalkylated phthalocyanines may be obtained by reacting the corresponding superphthalocyanine complexes with either acid (e.g., acetic acid in chlorobenzene), generating the metal free complex **29** or a metal salt (e.g. copper acetate in DMF) to generate the metallated complex[59] **28**. These transformations are summarized in scheme 11.



Scheme 11 Preparation of phthalocyanines from uranyl superphthalocyanine

A quantitative determination of the solubilities of some phthalocyanines and analogous superphthalocyanines are presented in table 1, page 21. It can be seen that the superphthalocyanines are substantially more soluble than the phthalocyanines. In each case, as might be expected, alkyl substitution increases the solubility. Also very noticeable is the relatively high solubility of less symmetrical $(4\text{-Me})_4PcH_2$, compared to $(4,5\text{-Me}_2)_4PcH_2$ and $(4,5\text{-Bu}_2)_4PcH_2$ in spite of the larger number of "solubilising" groups present in the latter complexes. In accordance with this trend, tetra-*tert*-butylphthalocyanine is reported to be even more soluble, dissolving appreciably even in nonaromatic solvents[61].

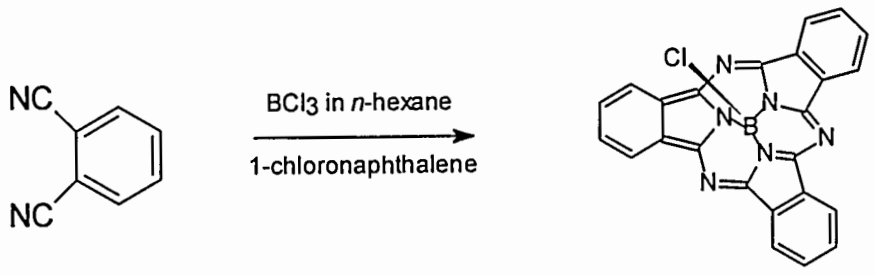
Table 1 Solubilities of phthalocyanines and superphthalocyanines

Compound (Solvent)	Solubility mol·dm^{-3}
PcH_2 (trichlorobenzene)	9.5×10^{-6}
$(4\text{-}Me)_4PcH_2$ (trichlorobenzene)	8.5×10^{-4}
$(4\text{-}Me)_4PcH_2$ (toluene)	2.7×10^{-5}
$(4,5\text{-}Me_2)_4PcH_2$ (trichlorobenzene)	$<1 \times 10^{-5}$
$(4,5\text{-}Bu_2)_4PcH_2$ (trichlorobenzene)	6.8×10^{-5}
CuPc (trichlorobenzene)	9.8×10^{-6}
$Ni(4,5\text{-}Me_2)_4Pc$ (trichlorobenzene)	1.5×10^{-5}
$Ni(4,5\text{-}Bu_2)_4Pc$ (trichlorobenzene)	4.2×10^{-5}
$Ni(4,5\text{-}Bu_2)_4Pc$ (toluene)	8.8×10^{-6}
$SPcUO_2$ (trichlorobenzene)	1.4×10^{-3}
$SPcUO_2$ (toluene)	6.8×10^{-5}
$(4\text{-}Me)_5SPcUO_2$ (toluene)	9.4×10^{-4}
$(4,5\text{-}Bu_2)_5SPcUO_2$ (trichlorobenzene)	9.0×10^{-2}
$(4,5\text{-}Bu_2)_5SPcUO_2$ (toluene)	5.8×10^{-2}

X-ray structural analysis of **26** (scheme 11, page 20) revealed a structure severely and irregularly distorted from planarity, and this was attributed to steric strain within the macrocycle[58].

2.2.5 Subphthalocyanines

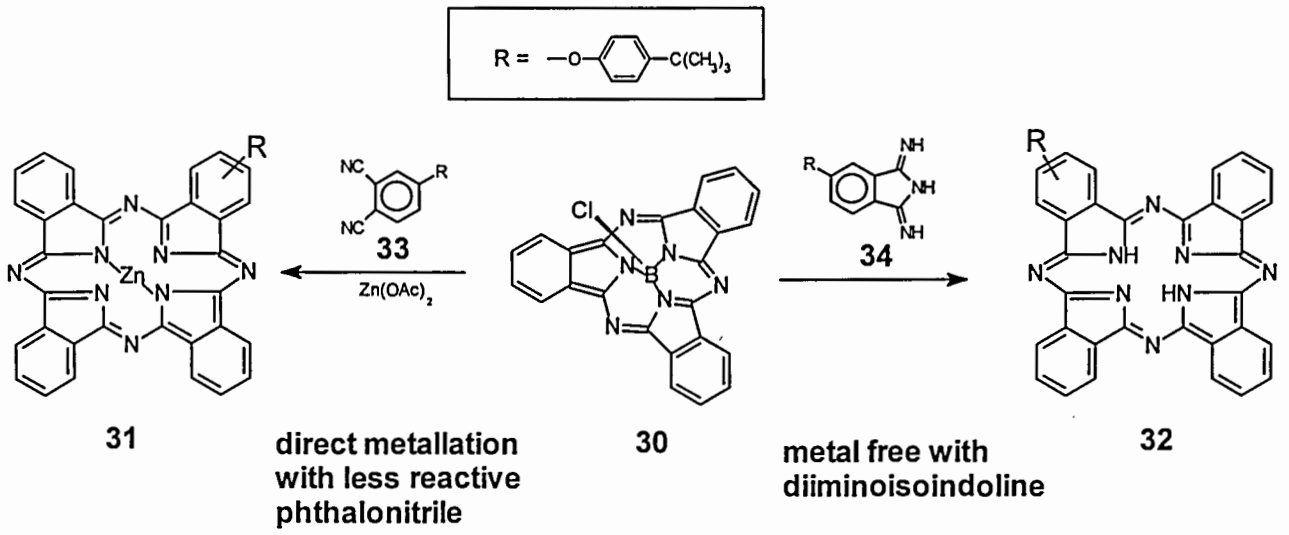
An important, recent consideration, in the chemistry of phthalocyanines is the preparation of phthalocyanines substituted unsymmetrically with regards to the peripheral substituents and especially monosubstituted phthalocyanines that have apparent application in the field of nonlinear optics[62] and thin film formation[63]. Monofunctional phthalocyanines with one reactive functional group have the advantage of polymer binding without the disadvantage of crosslinking reactions[64]. The subphthalocyanine method was firstly employed by Meller et al[65] by condensing boron trichloride (BCl_3) on a reaction vessel containing phthalonitrile **22** and heating to 250^{0}C yielding 40% of product **30**. Recently however the procedure was improved[66] with milder conditions (scheme 12, page 22) and approximately 20% higher yield by replacing gaseous boron trichloride with the commercially available 1M solution of boron trichloride in *n*-hexane.



22 **30**

Scheme 12 Synthesis of subphthalocyanine

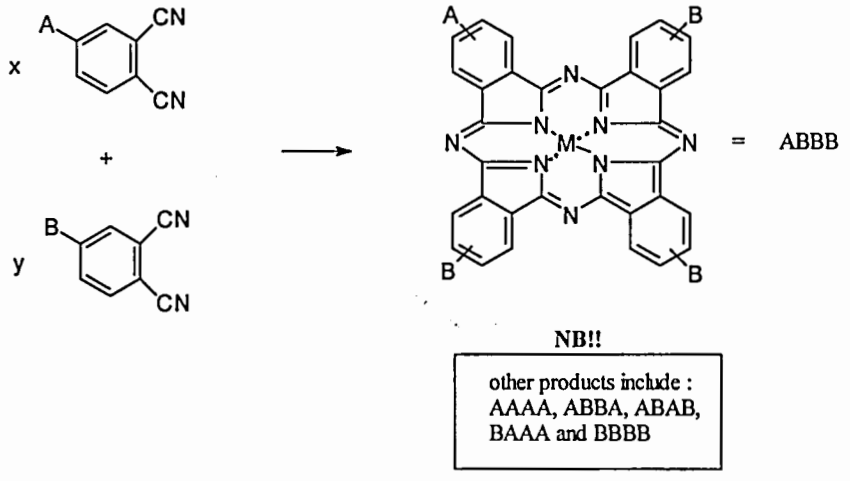
The ring enlargement reaction of unsubstituted chloro-subphthalocyanine **30** to produce monosubstituted phthalocyanines (scheme 13, page 23) is illustrated by Wöhrle[66] who synthesised, firstly the metal free 2-(4-*tert*-butylphenoxy)phthalocyanine **32**, which after reaction he found to be not only the monosubstituted phthalocyanine but also a mixture of unsubstituted, differently substituted and various chlorinated, including ring chlorinated, phthalocyanines. It is not possible to separate these products by recrystallization or column chromatography[67]. The products of the ring enlargement reaction are found to be insoluble in common organic solvents and to affect separation had to be metallated and then could be separated by preparative HPLC[38]. Since the metallation of the phthalocyanines is required in most cases, a single step procedure for their preparation is advantageous. Zinc metallation cannot be done with the previously mentioned diiminoisoindolines due to the tendency of diiminoisoindolines to cyclotetramerise with zinc salts. The less reactive phthalonitrile **33** was used instead to affect direct metallation (scheme 13, page 23) and after preparative HPLC the monosubstituted phthalocyanine **31** was attained in 22% yield[66].



Scheme 13 Monofunctional phthalocyanines from subphthalocyanine

2.2.5.1 Synthesizing unsymmetrical phthalocyanines

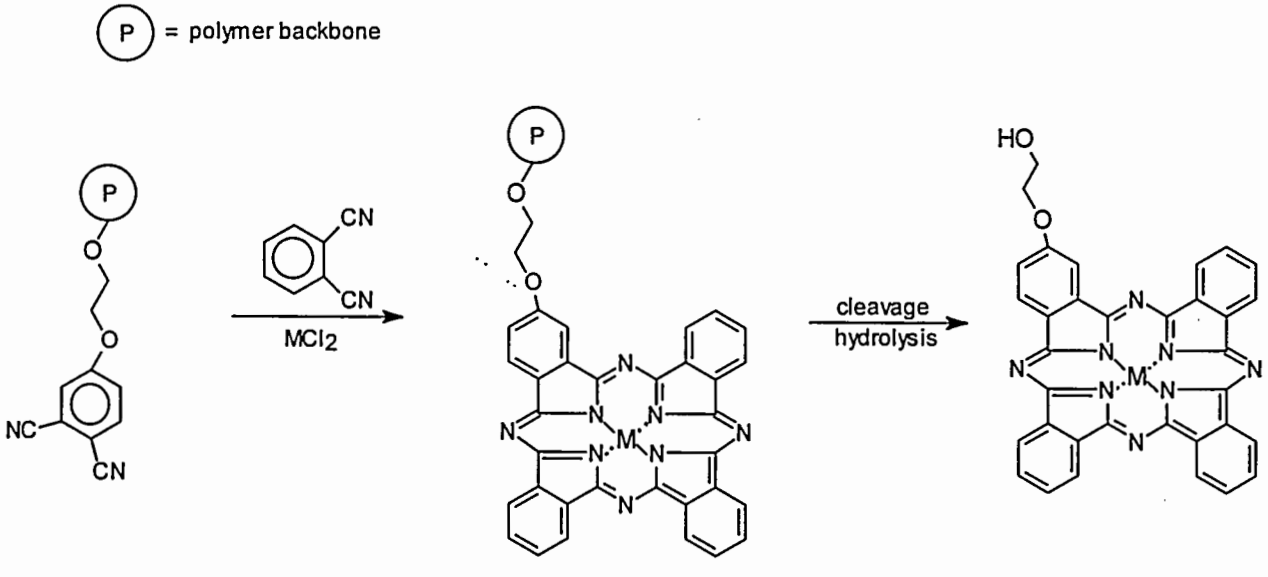
Apart from the subphthalocyanine route, it is possible to obtain unsymmetrical phthalocyanines by the statistical condensation route[68] and the polymer support route[69]. The statistical condensation route suffers from the very difficult separation of the different products (scheme 14) that are invariably formed in the synthesis of these phthalocyanines.



Scheme 14 Differently substituted phthalocyanines by using a statistical mixture of phthalonitriles

In a statistical condensation of 2 different phthalonitriles, as depicted in scheme 14 for an ideal situation, if we regard A and B as two different phthalonitriles then six different compounds are expected namely those phthalocyanines with substituents AAAA, BBBB, AAAB, ABBB, ABAB and AABB. It has been reported that such mixtures are difficult to separate by common chromatographic methods due to their tendency toward aggregation[70]. It is also possible to reduce the number of products by using phthalonitriles containing bulky groups on the 3,6-positions[30]. By substituting the anellated benzene rings with certain functionalities, it is possible to increase solubility, thus, creating a possibility of chromatographic separation as Wöhrle has done[44] by introducing long chain ether substituents onto the phthalocyanine molecule. Cook[71] also showed that carefully chosen phthalonitrile ratios would lead to predominantly two isomers.

An elegant way of creating unsymmetrical phthalocyanines is by the polymer support method. In principle, a phthalonitrile or a more reactive diiminoisoindoline unit, is attached to a polymeric backbone by means of spacer groups attached to the polymer. The polymer is then reacted with an excess of phthalonitrile (scheme 15).

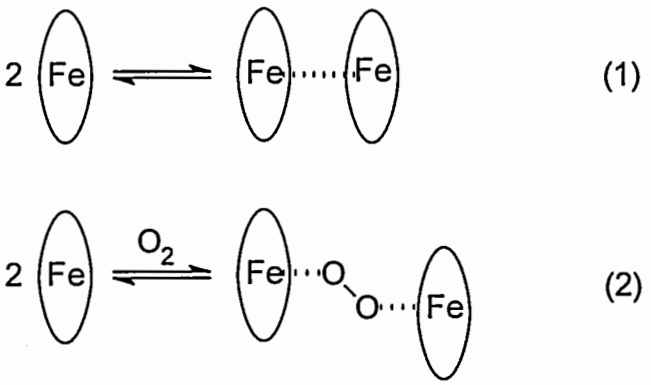


Scheme 15 Polymer bound phthalonitrile leading to a monosubstituted phthalocyanine

All non-polymer bound reaction products are removed upon which the polymer bound phthalocyanine unit may be cleaved chemically to liberate the pure monofunctional phthalocyanine.

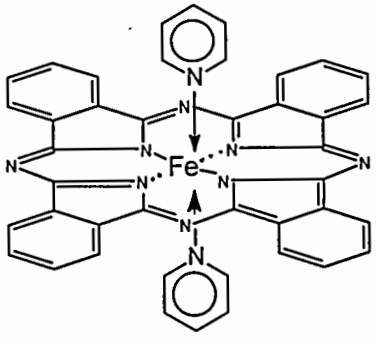
2.2.6 Aggregation

Probably the most notorious property of many phthalocyanines is their tendency to aggregate. Aggregation usually prevents easy purification of isomers and lowers solubility. Like most other large planar molecules most phthalocyanines substituted with *hydrophilic* groups form stacked aggregates[72]. The apparent *driving forces* for this aggregation is *hydrophobic* in character and is the result of the propensity of the phthalocyanine skeleton to avoid contact with the water molecules[73]. The aggregates exist mainly in the form of dimers[74] but the existence of higher aggregates are observed as a *progressive* blue shift from approximately 670 nm to about 630 nm in the visible spectrum with increasing phthalocyanine concentrations[75]. In general, metallophthalocyanine rings tend to form dimers as shown in scheme 16, in the absence of oxygen (equation 1) and in the presence of oxygen (equation 2).



Scheme 16 Dimer formation in iron phthalocyanine

It is seemingly possible to prevent aggregation by introducing bulky substituents into the benzene rings. For example, 2,9,16,23-tetra-*t*-butylphthalocyanine exhibits an enhanced tendency to sublime[76] when compared to the unsubstituted compound because the bulky *t*-butyl groups limit aggregation. Furthermore, the addition of organic solvents, which then act as axial ligands that coordinate with the central metal ion like methanol, DMSO and pyridine may prevent aggregation of phthalocyanines[77] (fig. 5).



35

Fig. 5 Axial coordination by pyridine in iron phthalocyanine enhances solubility

It has also been reported that phthalocyanines with lipophilic substituents dissolve and associate in organic solvents such as benzene[78].

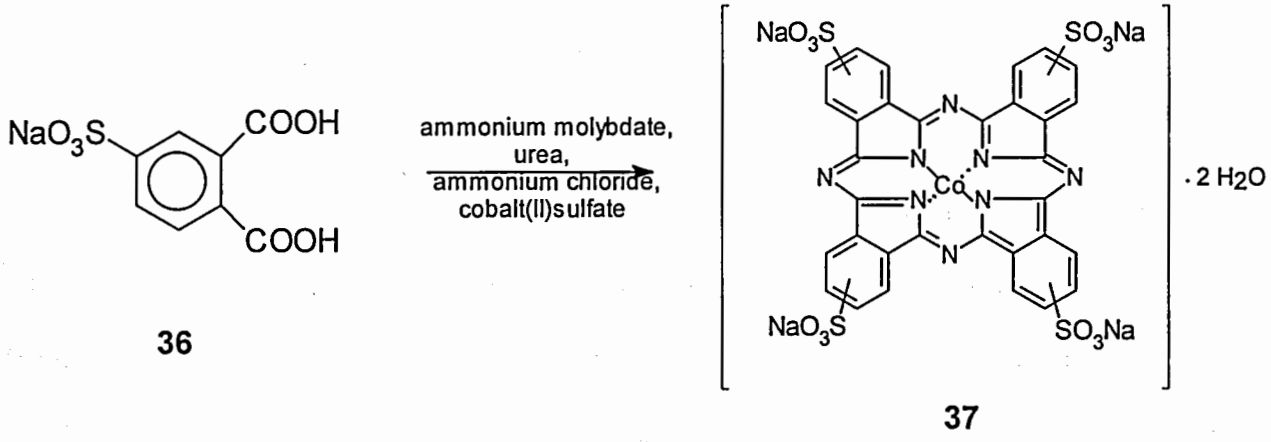
2.2.7 Solubility manipulations

There is now a very substantial amount of literature devoted to the preparation of substituted phthalocyanines. The synthesis of water-soluble phthalocyanines can be achieved in several ways, the most important consideration being to create a polar substance by means of functional chains on the anellated benzene rings. Organic solvent solubility may also be achieved by anellated benzene ring functionalization with groups that would then overwhelm the phthalocyanine properties. The

synthesis of peripherally substituted, metallated phthalocyanines with various metal ions may also lead to a disaggregated, soluble and bridged molecules.

2.2.7.1 Water solubility

Phthalocyanines may be rendered water-soluble in several ways such as incorporating sulfonic acid groups on the anellated benzene rings of the phthalocyanine as was done by Weber et al[79] wherein the monosodium salt of 4-sulphophthalic acid, ammonium chloride, urea, ammonium molybdate and cobalt(II)sulfate-7-hydrate were heated in nitrobenzene for 6 hours at 180^{0}C. After purification by basic and acid washing the water-soluble 4,4',4",4'"-tetrasulfophthalocyanine cobalt(II) dihydrate **37** was obtained in 80% yield (scheme 17).



36 **37**

Scheme 17 Synthesis of water-soluble tetra(sodium sulphonate)cobalt(II)- phthalocyanine

Other functionalities, which may be employed in the water-solubilisation of phthalocyanines, are *inter alia* carboxylic acid[80] **39**, phosphonic acid **40** and concurrently butyl chains terminating in diesterified phosphate groups[81] **16** (fig. 6, page 28)

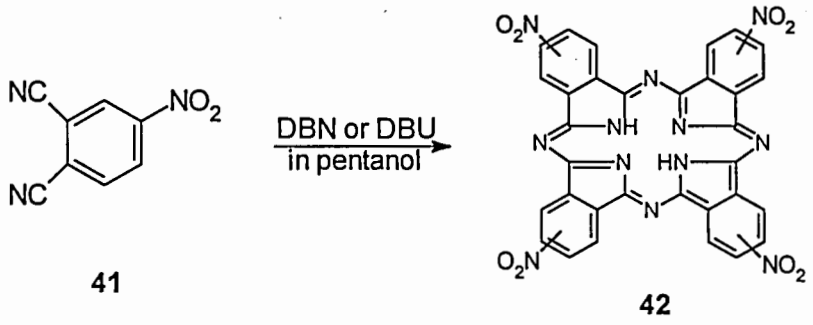
R = SO_3H **38**; CH_2COOH **39**; P(O)(OEt)(ONa) **40**; $(CH_2)_4OP(O)(OEt)(ONa)$ **16**

Fig. 6 Water solubilising functionalities

It is the declared aim of this study to introduce water-solubility into non-ionic phthalocyanine derivatives by anchoring them covalently to a water-soluble polymeric drug carrier.

2.2.7.2 Organic solvent solubility

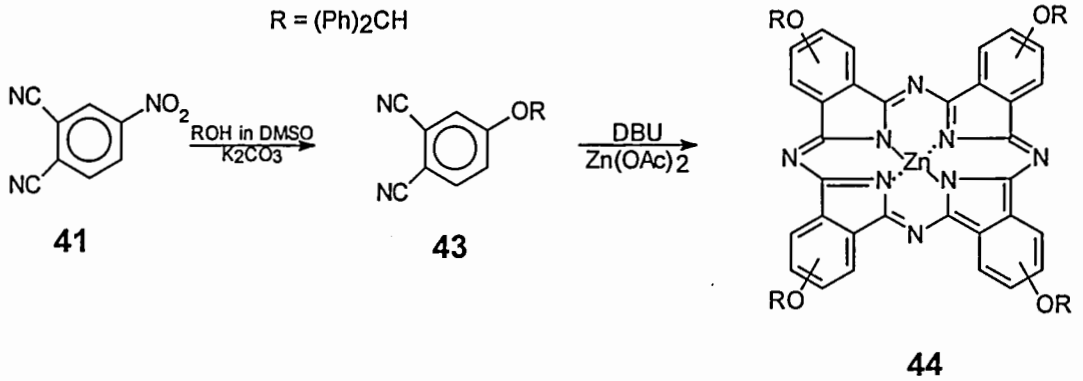
Recently, it has been shown that the use of a strong base greatly enhances the yields in the formation of metallated as well as metal-free phthalocyanines[38]. On heating 4-nitrophthalonitrile **41** with the strong bases 1,5-diazabicyclo[4.3.0]non-5-en (DBN) or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in pentanol at 200^{0}C for 4 hours the pyridine soluble tetranitrophthalocyanine **42** (Scheme 18) is obtained in 95 % yield.



41 **42**

Scheme 18 Pyridine soluble metal free tetranitrophthalocyanine

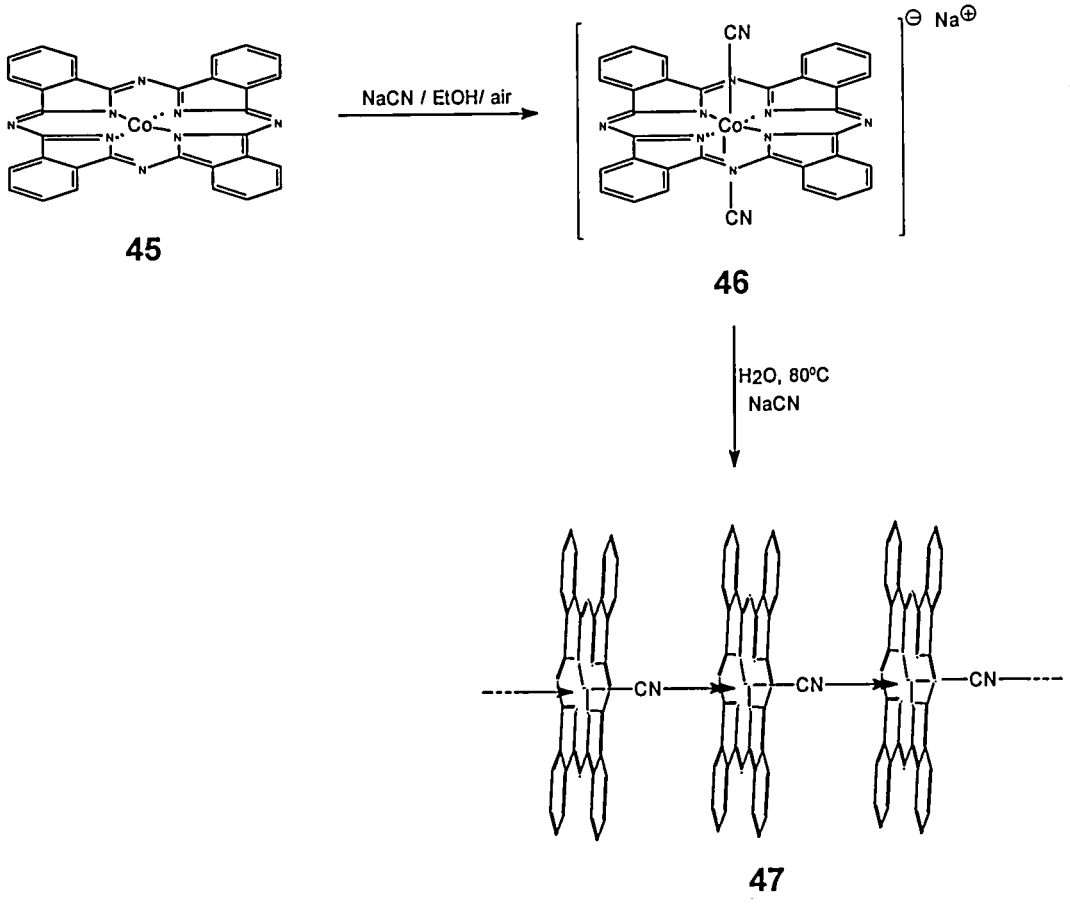
Metallated phthalocyanines may also be obtained by this method as Leznoff et al[82] did by using the phthalonitrile, 4-diphenylmethoxyphthalonitrile **43** which was heated at 100^{0}C using DBU as a base and zinc acetate as a template during which ammonia gas was introduced into the reaction to synthesise the toluene soluble 2,9,16,23-tetra(diphenylmethoxy)phthalocyaninato zinc(II) **44** in 65% yield. The organic solvent solubility of the obtained phthalocyanine **44** in toluene is increased by the presence of the diphenylmethoxy groups, which dominate the solubility properties of the formed molecule.



Scheme 19 Toluene soluble metallated tetra(diphenylmethoxy)phthalocyanine

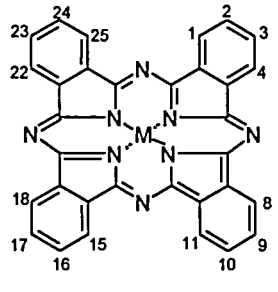
2.2.7.3 Axial substitution

Another method of improving the solubility of phthalocyanines is by substituting the axial ligand at the central metal ion of the phthalocyanine. When the ligand used for substitution is bifunctional, a cofacially stacked coordinative polymeric phthalocyanine such as **47** arises (scheme 20, page 30). If the ligand is monofunctional, a monomeric compound such as **35** (fig. 5, page 26) is obtained. Beck et al[83] illustrated the synthesis of a cofacially stacked polymeric phthalocyanine by coordinating two cyanide ions onto phthalocyanine **45** by reaction with sodium cyanide in refluxing ethanol for 2 days to yield sodium dicyano(phthalocyaninato)cobalt(III) **46** in 83% yield. A solution of **46** in dichloromethane is then added to hot water and left for 1 hour upon which the chloroform soluble polymer, poly[μ-cyano(phthalocyaninato)cobalt(III)], **47**, is obtained.



Scheme 20 Axially substituted cobalt(II)phthalocyanine

2.2.8 Spectroscopic effects of 2,9,16,23- and 1,8,15,22- substituted phthalocyanines as compared to naphthalocyanines



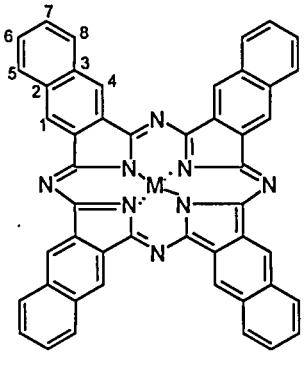
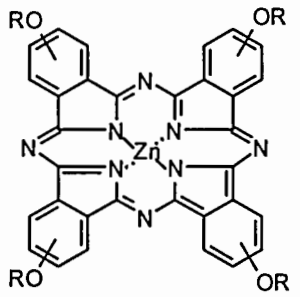


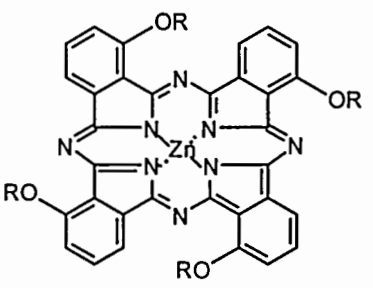
Fig. 7 Numbering of phthalocyanines and naphthalocyanines

The designation of tetrasubstituted phthalocyanines is generally used to express the positioning of substituents on the tetrasubstituted phthalocyanine annelated rings. The numbering in current use is shown in fig. 7, page 30. Preparation of phthalocyanines with 4-substituted phthalonitriles results in a mixture of isomers which then is referred to as 2,9,16,23-phthalocyanines (fig. 8) but actually consists of a statistical mixture of their 2,9,16,23-, 2,10,16,24-, 2,9,17,24-, and 2,9,16,24- isomers[84]. Similarly, by using 3-substituted phthalonitriles a mixture may result (depending on the functional group on the 3-position) in which case the isomers of the phthalocyanine would be generally referred to as the 1,8,15,22- phthalocyanine but actually consist of the 1,8,15,22-, 1,8,15,25-, 1,11,15,25-, and 1,11,18,22- isomers[85].



2,9,16,23 - phthalocyanine

50 R = *p*-*n*-BuPhCH$_2$
51 R = H



1,8,15,22 - phthalocyanine (single isomer)

52 R = *p*-*n*-BuPhCH$_2$
53 R = H

Fig. 8 Two types of tetrasubstituted phthalocyanines

2.2.8.1 ^{1}H NMR spectroscopy of phthalocyanines and naphthalocyanines

The 2,9,16,23-tetrasubstituted phthalocyanines generally exhibit broad absorptions in the aromatic and other spectral regions due to the presence of four isomers[86] as can be seen in the ^{1}H NMR spectrum of **50** in fig. 9, spectrum (a). Similarly 1,8,15,22-tetrasubstituted phthalocyanines or analogs can exist as a distribution of four isomers[87]. Leznoff et al[88], however, found that by condensing 3-*p*-*n*-butylbenzyloxyphthalonitrile in lithium octoxide the resultant 1,8,15,22-tetra(*p*-*n*-butylbenzyl-oxy)phthalocyanine **52** existed as a single isomer[88] as could be seen in the very resolved ^{1}H NMR spectrum of **52** in fig. 9, spectrum (b). The presence of the bulky butylbenzyloxy groups thus sterically inhibits the formation of isomers.

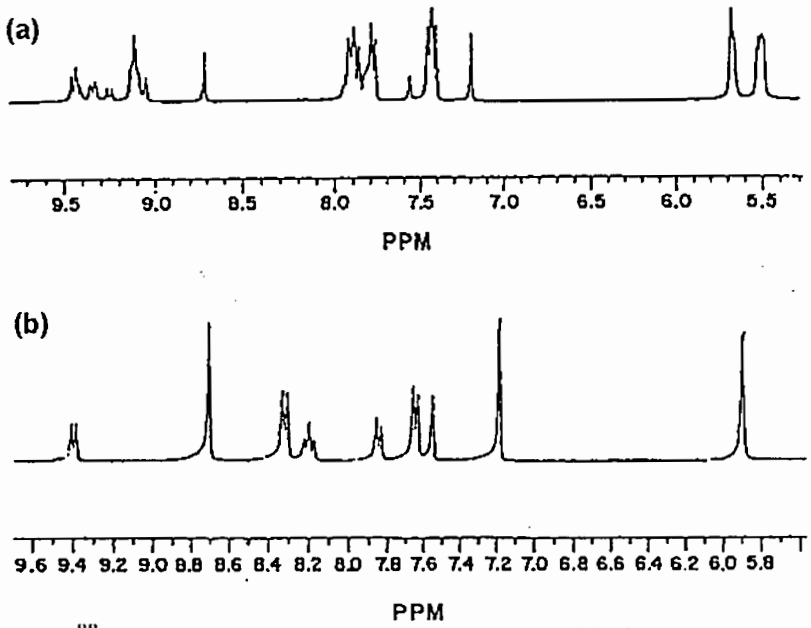


Fig. 9 ^{1}H-NMR spectra[88] of a) a phthalocyanine with inseparable isomers **50** and b) a single isomeric phthalocyanine **52**

The ^{1}H-NMR spectra of metallated naphthalocyanines is strongly dependent on the central metal ion as is illustrated by comparing the ^{1}H-NMR spectra of zinc(II)naphthalocyanine (ZnNc) **48** with the axially substituted alkyl silicon naphthalocyanine **49**. The axially alkylated silicon naphthalocyanine **49** in deuterated chloroform shows three resonances at 9.96, 8.54 and 7.77 ppm (fig. 10, page 33), corresponding to the 1,4, 5,8, and 6,7 protons illustrated in fig. 10. The alkyl

protons appeared as low as at ~ -2.5, -1.2 and 0.5 ppm (fig. 10) due to the ring current effect of the Nc ring[89]. In contrast, the spectrum of ZnNc **48** in deuterated DMSO shows three resonances with the same multiplicity as the alkylated silicon naphthalocyanine at 7.17 (singlet), 6.14 (quartet) and 5.38 (quartet) ppm[90].

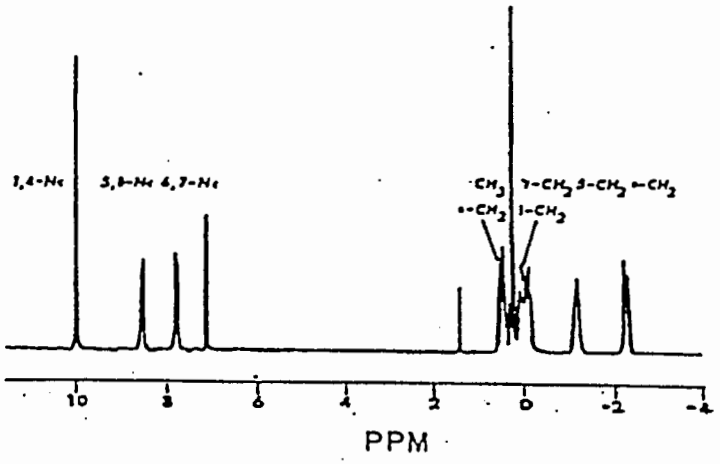


Fig. 10 ^{1}H-NMR spectra of axially substituted SiNc **49**

2.2.8.2 UV-spectroscopy of phthalocyanines and naphthalocyanines

The UV visible spectra of **52** and **53** (for structures see fig. 8 page 31) contrast strongly with those of **50** and **51**, because **53** exhibits a strong Q-band at 719 nm compared to **51** at 684 nm[91]. **52** has a Q-band at 696 nm compared to **50** at 682 nm. These results demonstrate the tendency of 1,8,15,22-substituted phthalocyanines to induce an upward Q-band shift, that is, a shift towards longer wavelengths for Q-band maximas.

Typically, the Q-band maxima of most metallated Ncs without peripheral substituent groups lie at wavelengths shorter than 800 nm, while those of poly-substituted Ncs shift to wavelengths longer than 800 nm. Of particular interest is the effect of alkoxy[92] and the amino[93] groups. For example, a tetraamino-substituted VONc shows the Q-band maximum at 870 nm in quinoline. Kobayashi et

al[94] also reported accumulated data in which the absorption coefficients of the Q band of metallated naphthalocyanines are generally found larger than those of phthalocyanines with similar substituents. Together with the longer wavelength shift of the Q-band, the Soret band region also spreads towards longer wavelengths on going from phthalocyanines to naphthalocyanines. The absorption spectrum of ZnNc[95] **48** in DMSO is shown in fig. 11.

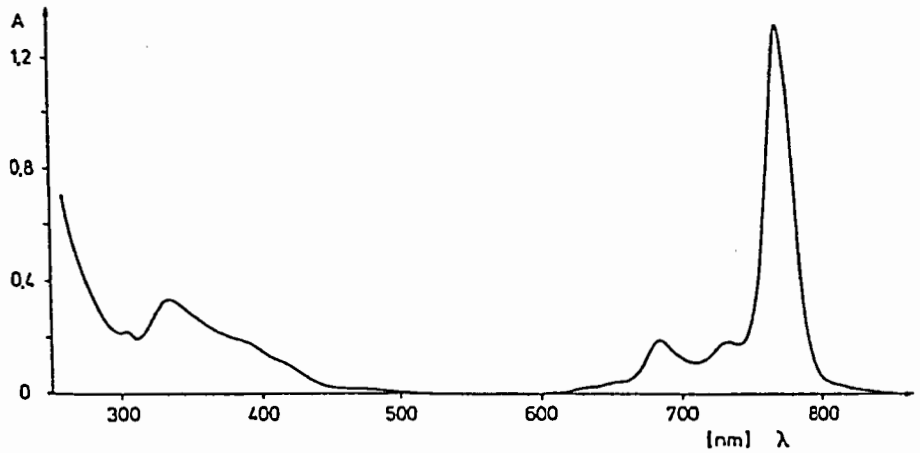


Fig. 11 Absorption spectrum of ZnNc **48.** A = Absorption, λ = wavelength in nm.

2.2.8.3 IR spectroscopy

The infrared spectra of various tetra-*tert*-butylated phthalocyanines **54** to **57** are shown in fig. 12, page 35. The spectra of the phthalocyanines are somewhat complex. Except for the bands assignable to the *tert*-butylated phthalocyanines, the characteristic bands common to metallated phthalocyanines are observed at 670-700, 750-790, 840-850, 940-945, 1090-1120, 1140-1147, 1200-1210, 1240-1290, 1305-1320, 1400-1430, 1490-1540 and 1600-1625[96]. The bands at 670-700, 750-790, 1240-1300 and 1400-1430 are well-defined doublets and those at 1090-1120 quite intense.

Naphthalocyanines have characteristic infrared bands observable at 470-472, 724-725, 742-749, 808-812, 888-901, 946-947, 1082-1088, 1100-1104, 1142-1144 and 1343-1359 cm^{-1}. Of these, the

bands at 470-472 are typical of skeletal vibrations of naphthalene[97] those at 888-901 cm^{-1} are a doublet, and those at 1343-1359 cm^{-1} are a multiplet. The bands at 1082-1088, 1100-1104 and 1343-1359 cm^{-1} are particularly intense[96]. The infrared spectra are shown for various tetra-*tert*-butylated naphthalocyanines **58** to **61** in fig. 12.

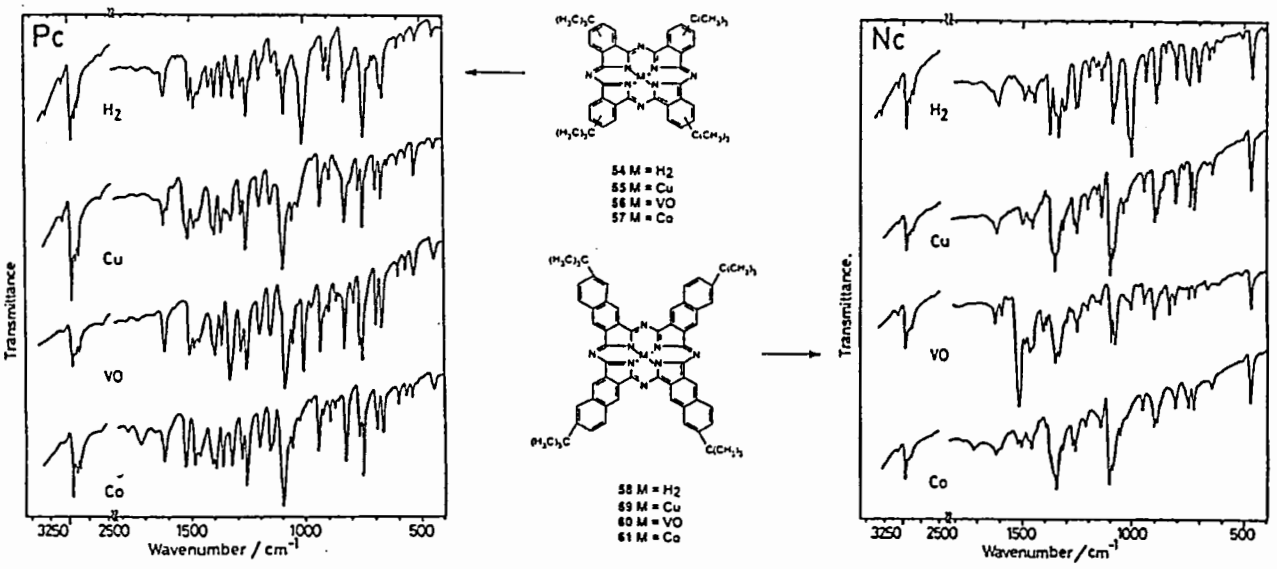


Fig. 12 Infrared spectra of naphthalocyanines and phthalocyanines

2.2.9 Electrochemistry of phthalocyanines and naphthalocyanines

Since the fifth objective of this study is to determine some electrochemical properties of selected phthalocyanines, it is necessary to include in this literature survey examples of cyclic voltammograms of selected compounds to illustrate the basic cyclic voltammetric behaviour of the phthalocyanines.

The electrochemistry of metallophthalocyanine species is very rich with many redox processes. Incorporation of different metals into the core of the phthalocyanine ring and variations in the substituents on the periphery of the ring result in complexes that have varied properties[98]. Redox processes occurring in MPc complexes may be centred at the phthalocyanine ring or at the central metal and are affected by several factors[99], including i) the nature of the substituents on the phthalocyanine ring ii) the nature and oxidation state of the central metal and iii) the nature of the axial ligands and solvents.

Changes in the oxidation states often result in reversible and dramatic colour changes because of ring based redox processes in metallophthalocyanine MPc complexes[98]. Depending on the relative energies of the metal d orbitals and the ring π orbitals (fig. 13), it is possible to observe two successive one-electron oxidations of the phthalocyanine ring by removal of electrons from the a_{1u} orbital and four successive one-electron reductions into the e_g orbital. If metal orbitals lie at energies within the HOMO-LUMO gap of the ring, oxidation or reduction, or both, may occur at the central metal[98].

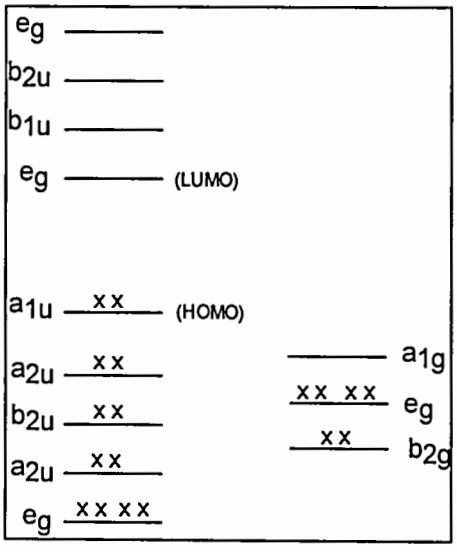


Fig. 13 Energy levels of phthalocyanines

To illustrate a typical ring based redox process[100] the cyclic voltammogram of zinc(II)tetraneopentoxyphthalocyanine **62** in 1,2 dichlorobenzene containing 0.2M TBAP vs Ag/AgCl is shown in fig. 14.

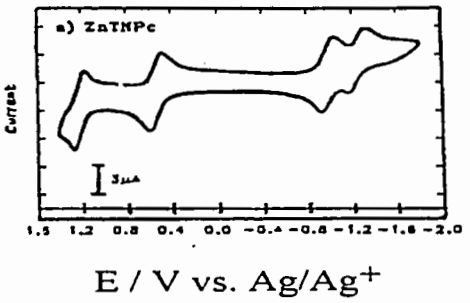


Fig. 14 Cyclic voltammogram at 100 mV s^{-1} of zinc(II)tetraneopentoxyphthalocyanine

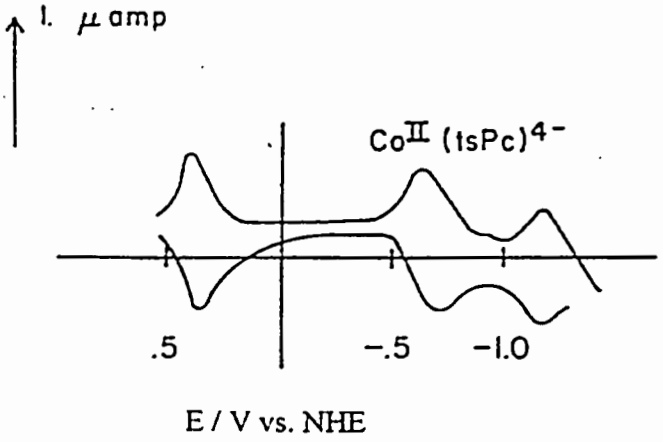
Two oxidation couples at 1.23 V and 0.55 V and two reduction couples at -0.98 V and -1.24 V were observed. The oxidation couples correspond to two one electron removals from the phthalocyanine ligand and thus the formation of the π-cation species $[ZnTnPc(1-)]^{+}$ / $[ZnTnPc(0)]^{2+}$ and $[ZnTnPc(2-)]$ / $[ZnTnPc(1-)]^{+}$. The two reduction couples are summarised in table 2.

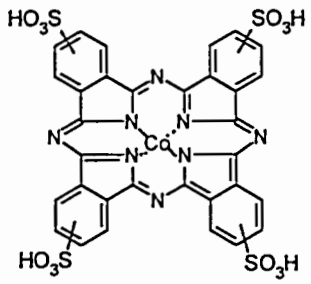
Table 2 Reduction couples of zinc(II)tetraneopentoxyphthalocyanine

$E_{½}$ / V vs SCE	Assignment
-0.98	$Pc^{2-} \rightarrow Pc^{3-}$
-1.24	$Pc^{3-} \rightarrow Pc^{4-}$

In cyclic voltammetry the reduction or oxidation of the metal center of metallated phthalocyanines can also be observed, for example, the oxidation of cobalt in cobalt(II)tetrasulfonated phthalocyanine **63** occurs at lower potentials than the oxidation of the ligand[101], the cyclic

voltammogram shown in fig. 15 together with the previously mentioned cyclic voltammogram in which the ligand is oxidized is typical of metallated phthalocyanines[102].





63

Fig. 15 Cyclic voltammogram of cobalt tetrasulfonated phthalocyanine with the structure inserted

It is typically found that the cyclic voltammograms of metallated silicon naphthalocyanines exhibit two reductions and two oxidations as is seen in the cyclic voltammogram of bis(tri-*n*-hexylsiloxy) (2,3,naphthalocyaninato) silicon [SiNc(OR)$_2$][103] **49** (for structure see fig. 7, page 30) which shows two reversible one electron transfers (fig. 16).

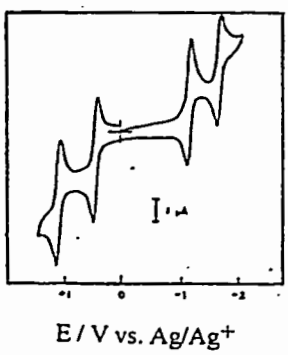


Fig. 16 Cyclic voltammogram of naphthalocyanine **49**

The average values of the reduction potentials of naphthalocyanine **49** and its phthalocyanine analog obtained at several scan rates are given in table 3. Noticeable is that while it is easier to oxidize **49** than its phthalocyanine analog, potentials for the reduction processes are both nearly the same.

Table 3 E^0 values for $SiPc(OR)_2$ and $SiNc(OR)_2$ **49** in CH_2Cl_2

	$SiPc(OR)_2$, V vs. SCE	$SiNc(OR)_2$, V vs. SCE
2^{nd} ox.		+ 1.24
1^{st} ox.	+1.00	+0.58
1^{st} red.	-0.90	-1.01
2^{nd} red.	-1.48	-1.55

2.3 Polymeric drug carriers

2.3.1 Introduction

Many good pharmaceutical agents are dose limited due to poor solubility in an aqueous media or to the severe side effects they exhibit at high concentrations. For others, the inability of many of these drugs to gain access to the diseased site at suitable dosages diminishes their *in vivo* therapeutic effectiveness. In addition, the metastatic nature of tumour cells additionally require that total tumour cell destruction be achieved early in treatment, before resistance to the drug is developed, or a metastatic cancer cell line render the drug totally ineffective. The multitude of problems associated with chemotherapy imply that vehicles are required that are capable of rapidly and selectively carrying cytotoxic agents in a concentrated form to the tumour tissue, while largely avoiding healthy surrounding cells. This goal may, in principle, be achieved if the drug is bound to a suitable polymeric drug carrier. The detrimental properties and side effects of many drugs may

also be overcome to a large extent if the pharmaceutical agent is covalently anchored to a polymeric drug carrier. Therefore, part of the aim of this study is to synthesise water-soluble polymeric drug carriers and to demonstrate the feasibility of anchoring selected phthalocyanine and other antineoplastic drugs on them.

2.3.2 The selective action of drugs

Central to the goal of selective action of any cytotoxic agent is the inability of present drugs to distinguish between healthy and cancerous cells. Ideally one would like a cytotoxic agent that can discriminate between neoplastic and non-neoplastic cells and would be able to "zoom" into the neoplastic cells. This approach requires the existence of some molecular characteristic that differs between the target and non-target cells. This might be a surface receptor, a structural membrane protein, an intercellular enzyme or an altered sequence in the genome. Such a specific trait has not been identified yet but early results strongly indicate that it might be possible in the future[104,105,106,107]. The illusive goal of developing so-called "homing devices" is further aggravated by the knowledge that neoplastic cell populations are not static but ever changing. Tumours have an amazing ability to neutralize the mechanism of action of therapeutic agents that do not rapidly enough enforce necrosis[108]. Some common examples of this evasive ability are[109]:

(a) The loss of specific receptors;

(b) Down regulation of tumour associated antigens;

(c) Shedding of antigens into the body fluids.

Development of these "homing devices" is still in early stages with relatively few results available, but indications are that polymeric drug carriers may play a significant role in achieving this goal.

2.3.3 Natural macromolecular drug carriers

Two classes of polymeric drug carriers may be defined, namely, natural and synthetic drug carriers. Examples of natural macromolecular drug carriers are liposomes[110] and tumour specific antibodies[111,112,113]. Both the natural polymers have a great advantage of complete specificity for infectious or generally unhealthy cells. Natural polymers can, however, only be loaded to a limited extent by a drug due to a limited amount of available binding sites.

2.3.4 Synthetic macromolecules as drug carriers

2.3.4.1 Introduction

An important property of any successful drug carrier should be an ability to avoid rapid clearance from bodily circulation *via* the reticuloendothelial system and certain synthetic polymers appear to have a capacity to do that. Thus, by avoiding reticuloendothelial cells, the probability of the carrier reaching targets such as tumour tissue, muscle and parenchymal tissue is greatly enhanced. A theoretical model for the design of synthetic polymers that could serve as drug carriers has been described[114] and the important characteristics outlined there provide a firm basis for drug design. Some of the properties that should be built into a polymeric drug carrier includes biocompatibility, water-solubility, a large amount of drug attachment sites, binding of the drug to these drug anchoring sites must proceed easily without side reactions to generate a biodegradable bond between drug and polymer, it must have a sufficiently large molecular mass to prevent quick excretion from the body yet it must be biodegradable to allow ultimate elimination of the spent polymeric carrier from the body after its payload of drug has been delivered to the target site and it must be non-toxic, non-antigenic or non-provocative in any other respect[115]. Ideally drugs that act intracellularly should gain access to the cell by the process of endocytosis and upon penetration, activation of the release mechanism should deposit the drug inside the target cell.

2.3.4.2 Mechanism of cell entry - Endocytosis

A major disadvantage of most low molecular mass pharmacological agents in use is their rapid excretion from the body. This means that these drugs have to be administered repeatedly in large dosages in order to maintain a therapeutic effect. Also, their low molecular masses allow them to readily penetrate all cell types. Thus, healthy and unhealthy cells are destroyed alike, causing deleterious side effects. Macromolecular drug carriers of sufficient high molecular mass can overcome the quick excretion mechanism of the body. This means the polymer may act as a store of drugs in the body and offers the possibility of sustained release over a prolonged period. Furthermore, it limits the uptake of drug by cells to the process of endocytosis - a cell membrane penetration mechanism generally unavailable to non-polymeric compounds but highly desired for drugs operating intracellularly.

Endocytototic uptake[116,117,118] of material by cells occurs by means of either phagocytosis or pinocytosis. The term phagocytosis[119] describes the process by which particulate matter (usually > 1μm in diameter) is internalized in a cell but will not be considered further as soluble macromolecules do not follow this route. Pinocytosis[120] describes the capture of extracellular fluid by invaginations of the cell membrane that form smaller membrane-bound vacuoles or vesicles (see fig. 17, page 43). The formation of the vacuoles or vesicles is a continuous process with no obvious rate control mechanism and is common to most, if not all cell types.

The macromolecules may enter a cell via two distinct pathways during pinocytosis, either by internalizing in a random way known as "fluid phase" pinocytosis or by adsorbing on the plasma membrane which, upon internal migration carries the macromolecule with it. This process, called adsorptive pinocytosis, is thus responsible for the specificity of uptake in the forming vesicles and is

a consequence of the fact that certain cell types do possess unique recognition receptors, particularly for certain sugar residues[121].

The newly formed macromolecule-containing vesicles separate from the cell wall (membrane) and migrate into the cytoplasm where it may fuse, amongst others, with newly formed lysosomes, also called primary lysosomes. The lysosomes contain in excess of seventy hydrolytic enzymes[122] capable of degrading amongst others, peptides (i.e. amide bonds constructed from L-amino acids) to monomeric constituents. After fusion, the lysosomes are referred to as secondary lysosomes[123]. Hydrolytic degradation then takes place and the low-molecular mass products are released and pass through the secondary lysosomal membrane into the cytoplasm, either for re-utilization or for removal from the cell. Non-biodegradable macromolecules accumulate within secondary lysosomes and are slowly released by exocytosis or because of cell death.

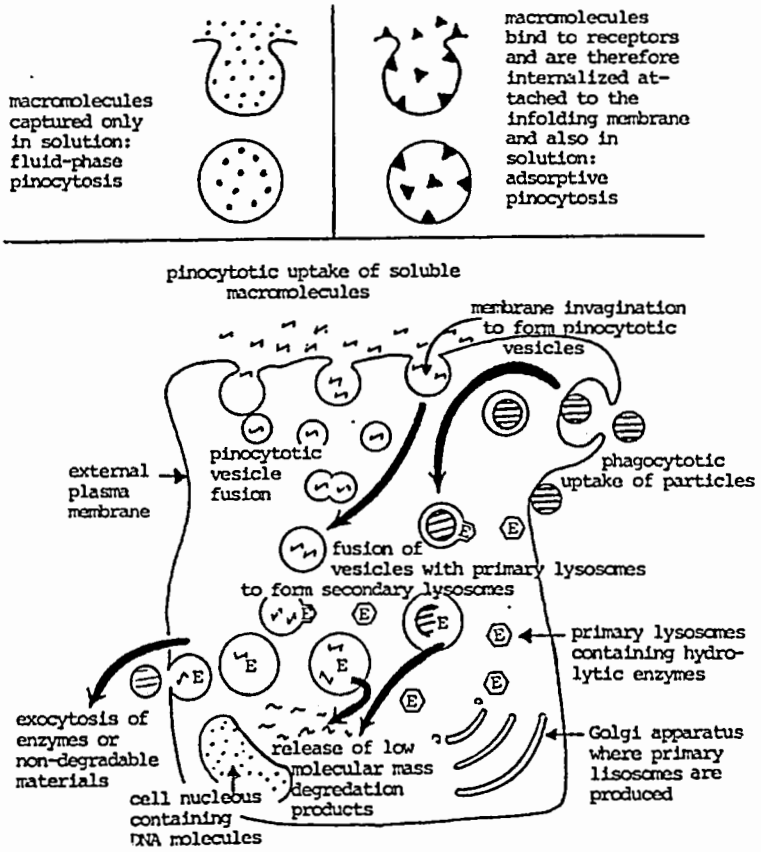


Fig. 17 Cellular pinocytotic uptake of polymers (figure adapted from reference 13)

Pinocytotic uptake has various properties favourable for utilization as a drug delivery route:

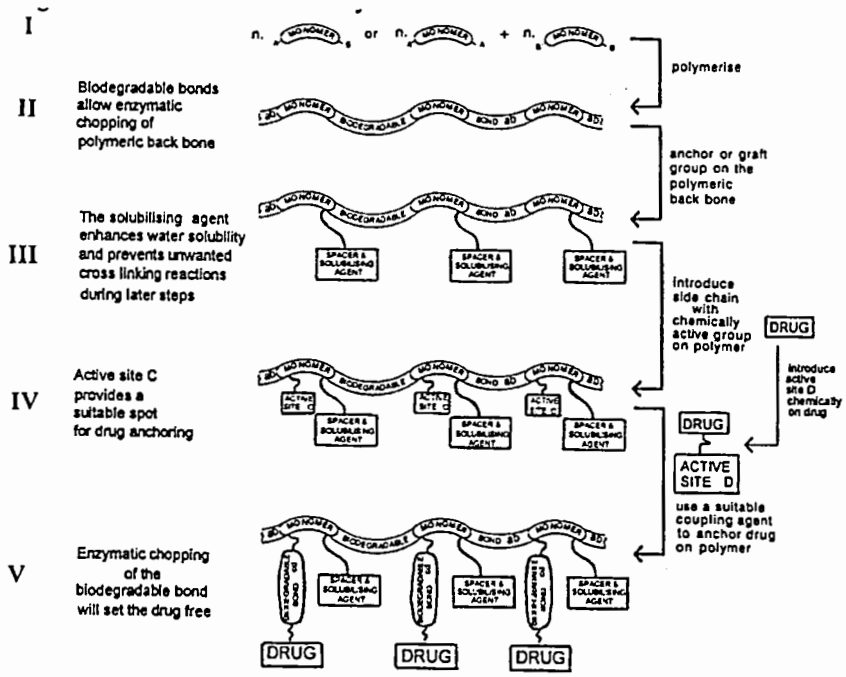
(a) All cell types capture macromolecules pinocytotically, thus, the range of therapy is great.

(b) Cell specific targeting of pharmaceutical agents may be possible by utilizing the receptor systems.

(c) Drug carriers can be designed that are only cleaved intralysosomally and are stable in an extracellular environment.

2.4 Practical considerations in the design of a polymeric drug carrier

In the design of a polymeric drug carrier, the first consideration should be to the structure of the backbone, the main chain of the polymer. The polymerization process should result in a linear polymer to ensure solubility. Even a tiny amount of crosslinking will render the polymer insoluble. Thus, a careful choice of difunctional monomers is required. The polymerization process should create biodegradable links as is seen in entry II of scheme 21.



Scheme 21 General guidelines for the synthesis of a polymeric drug carrier / drug conjugate

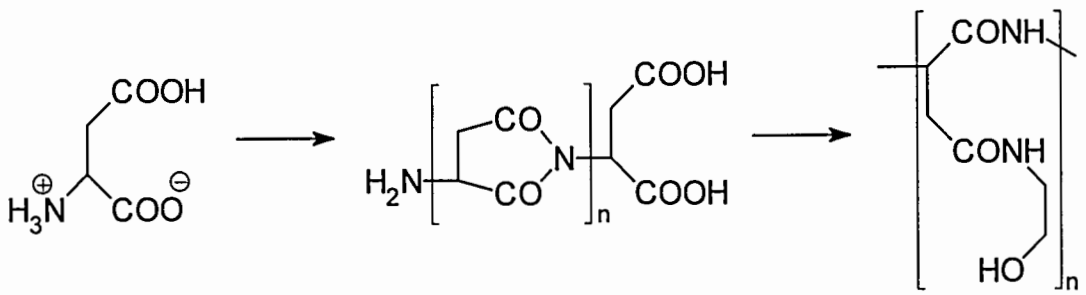
To enable the polymer to act as a drug carrier, a primary objective is to effect water-solubility. Water-solubility is enhanced by anchoring or grafting a solubilising agent onto the polymer backbone as is seen in entry III of scheme 21. The spacer and solubilising agent combination not only enhances the polymer solubility in water but also prevents crosslinking reactions during drug anchoring reactions. The next step in the design of a polymeric drug carrier is to introduce active sites as side chains on the main chain polymeric backbone as seen in entry IV (denoted as active site C). When referring to an active site, it is understood to imply a chemically active group that may undergo further reactions, specifically to allow drug anchoring.

Before the anchoring of the required therapeutic drug to the polymeric drug carrier can be achieved, an active site D must be introduced into the drug that is capable of reacting with active site C on the polymer. The final step in producing a polymeric drug carrier / drug conjugate requires that the polymeric drug carrier be coupled with the drug itself by reacting active sites C and D to generate a biodegradable bond cd as is seen in the final step of scheme 21.

2.5 Selected examples of polymeric drug carriers

One of the important factors to take into consideration toward the success of a polymeric drug carrier is whether the carrier is immunogenic or not. It has been shown that water-soluble polymers significantly decrease the antigenic properties of known immunogenic compounds[124,125]. Mere water-solubility, however, does not guarantee loss of immunogenecity. Even if neither the drug nor the carrier is immunogenic, coupling of the two may result in the so-called Hapten effect[126] whereby immunogenecity is introduced into the single coupled moiety. Water-soluble poly(L-α-amino acids) do often show immunogenic tendencies, nevertheless they are attractive choices of carriers since they are easily biodegradable. Co-polymerization with hydrophilic moieties, such as ethylene glycols, has been shown to completely eliminate immunogenic properties of certain poly(α

-amino acids)[127]. Recently, several attempts have been made to synthesise non-immunogenic poly (L-α-amino acids) for biological use[128]. Outstanding among these are derivatives of aspartic acid **64**, which can be polymerized thermally to polysuccinimide **65** (scheme 22). After ring opening of **65** by ethanolamine, an extremely biocompatible compound poly(2-hydroxyethyl-α,β-L-aspartamide) **66** is obtained, which has been suggested as a blood plasma expander[129].



64 **65** **66**

Scheme 22 Poly (2-hydroxyethyl-α,β-L-aspartamide), a proposed blood plasma expander

The carrier polymer (or monomer) may be linked to the drug by a variety of bonds[130]. For biodegradability and biocompatibility the peptide (i.e. amide) saccharide and nucleotide bonds may prove to be most advantageous although ester (very hydrolytically unstable), urethane, thio-ethers and disulphides may also be convenient. The cytostatic group bis(2-chloroethyl)amine has been linked to methacrylic acid derivatives *via* a urethane bond and O-acylated hydroxamic acid bonds[131]. Co-polymerization with the hydrophilic monomer 2-(methyl-sulfinyl)ethyl methacrylate yielded the biologically active compounds of structure **67**, **68** and **69** in fig. 18, page 47.

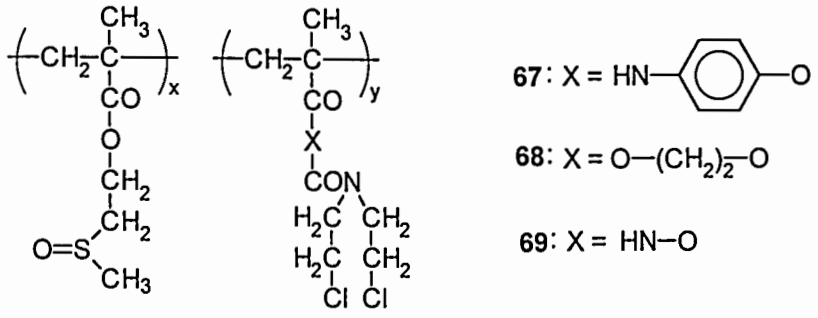
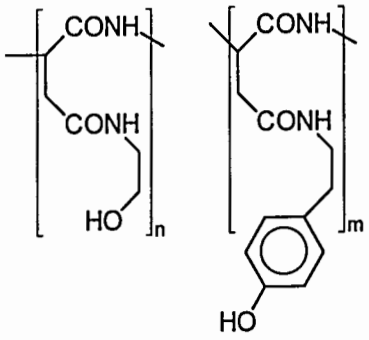


Fig. 18 Ester, amide, urethane and O-acylated hydroxamic acid bonds were utilised to anchor the cytotoxic agent bis(2-chloroethyl)amine onto a methacrylate based polymeric drug carrier. The main chain of this polymeric system is not biodegradable.

The incorporation of phenolic residues into polymers such as in poly(2-hydroxyethyl-α,β-D,L-aspartamide) **70** greatly increases the rate of pinocytotic capture[132] of the polymer. Here the amide bond was used for anchoring purposes.

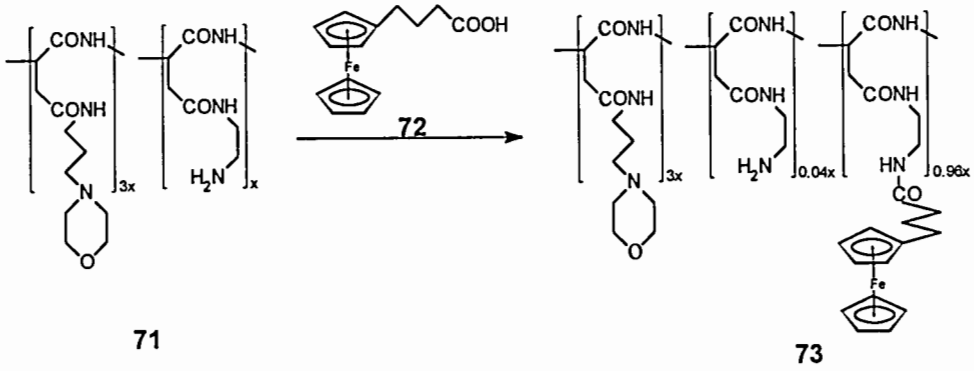


70

Fig. 19 The phenolic residue on polymer **70** enhances pinocytotic cell penetration

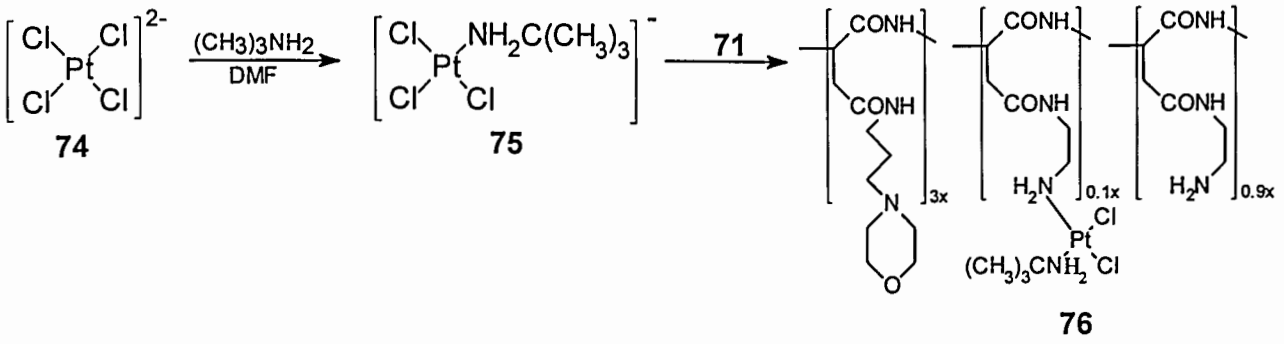
Polymer **73** (scheme 23, page 48) demonstrates that more than one pharmacological agent can be carried simultaneously on the same polymeric drug carrier. Polymer **73**[133] has anticancer as well as anti bloating properties. This dual action in one drug is seldom achieved. Normally, to obtain more

than one pharmacological effect, use is made of combination therapy i.e. treatment of a patient with more than one drug simultaneously. The anchoring of the antineoplastic ferrocenyl moiety onto the biodegradable and a water-soluble polymeric drug carrier **71** has been achieved with 96% success *via* an amide bond[133] according to scheme 23. The polymeric drug **73** was isolated in 71% yield. The N-(3-aminopropyl)morpholine portion of polymer **73** has anti bloating properties and it enhances water-solubility as well.



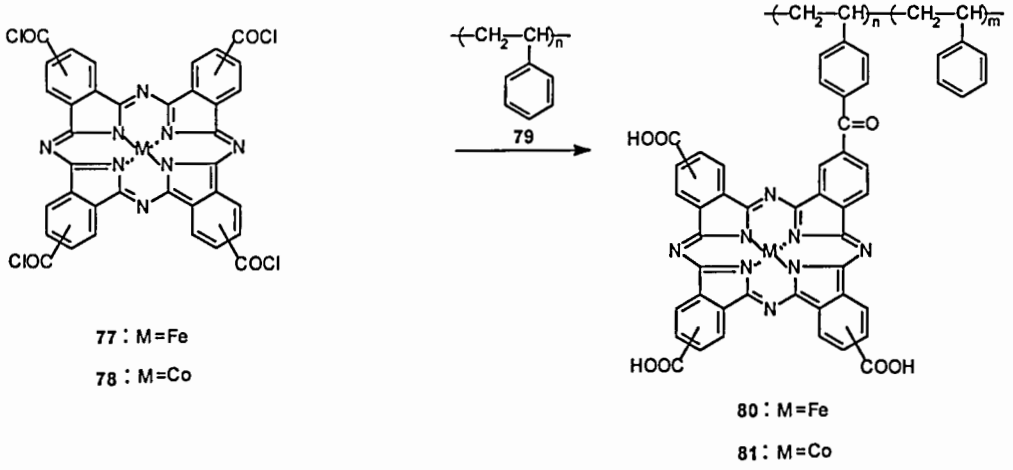
Scheme 23 The anchoring of a ferrocenyl moiety onto a water-soluble polymeric carrier

In another experiment, using the same carrier system as described for polymer **73**, the chemotherapeutic drug [potassium ammonium-*tert*-butylaminedichoro-platinate(II)] was simulated by anchoring [potassium *tert*-butylaminetrichoro-platinate(II)] (prepared by the method of Pasini[134]) **75** onto polymer **71** to produce polymer **76**[135] in 14% yield (scheme 24, page 49). However, only 10% of the available amine side chains were complexed with the platinum (II) nucleus.



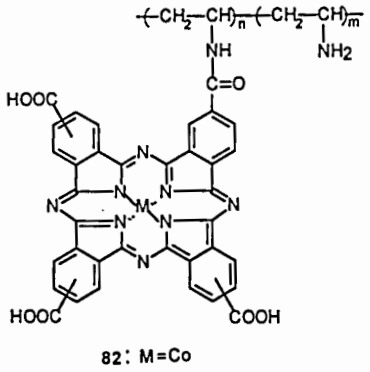
Scheme 24 The anchoring of [potassium *tert*-butylaminetrichoroplatinate(II)] onto a water-soluble polymeric carrier

The anchoring of the phthalocyanine moiety onto several polymers has also been demonstrated. Kobayashi[136] et al. anchored iron(III)tetracarboxylchloride phthalocyanine **77** and cobalt(II)tetra-carboxylchloride phthalocyanine **78** onto polystyrene **79** (scheme 25) by Friedel-Crafts reaction with a 50 times molar excess of polystyrene. The resultant polymers **80** and **81** both contained approximately 4 mole % bonded metal phthalocyanines.



Scheme 25 Anchoring of phthalocyanines onto polystyrene

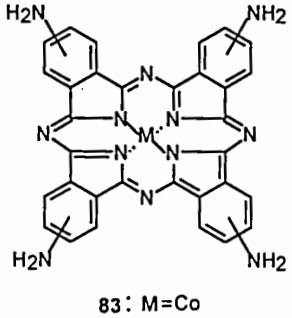
In a similar experiment, the amide bond was utilized to attach **78** to polyvinylamine in the presence of dicyclohexylcarbodiimide[137] fig. 20. The resultant polymer **82** contained 0.013 mole % of the phthalocyanine.



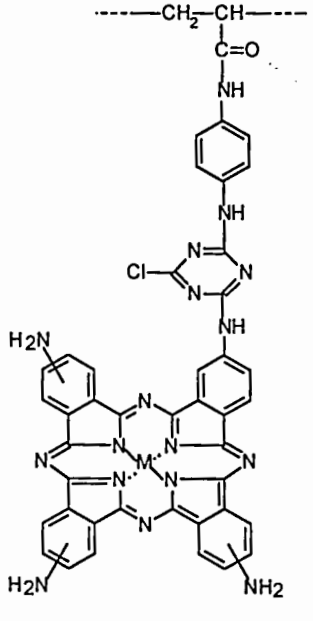
82: M=Co

Fig. 20 Amide bond utilized in attachment of a phthalocyanine to polyvinylamine

The above phthalocyanine reactions all employed carboxylic acid groups for coupling but analogously cobalt(II)tetraminophthalocyanine **20** was coupled to cyanuric chloride and this conjugate then attached to polyacrylamide[138] to yield the polymer **21** (fig. 21) with a 15 mole % phthalocyanine content.



83: M=Co



84: M=Co

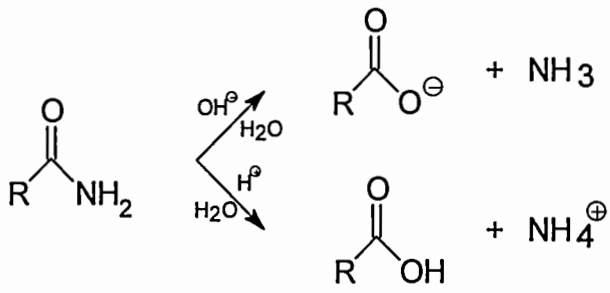
Fig. 21 Coupling of cobalt(II)tetraaminophthalocyanine to polyacrylamide

2.6 Synthetic methods

2.6.1 Carboxylic acid synthesis

Since it is the aim of this study to anchor drugs onto a polymeric drug carrier or a ferrocene-containing compound *via* biodegradable amide bonds (goals 2 and 4 in chapter 1), synthesis of carboxylic acid-containing phthalocyanines is a necessity.

The most common methods for the synthesis of carboxylic acids are oxidation, oxidation - reduction reactions, carbonation of organometallics, condensation reactions and hydrolysis reactions. In the course of this study the only method employed in carboxylation of a phthalocyanine moiety was that of primary amide hydrolysis. The hydrolysis of primary amides may be achieved with either acidic or basic conditions (scheme 26)



Scheme 26 Carboxylic acid formation by amide hydrolysis

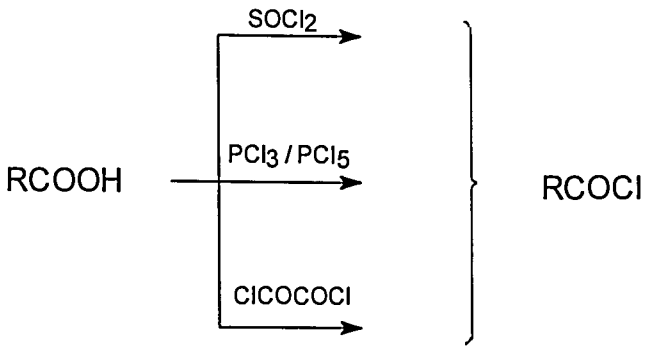
Since the amino group is a very poor leaving group (less even than the alkoxide groups)[139], water alone cannot hydrolyze the amide compound, very often prolonged heating is required even with acidic or basic catalysts[140]. For cases in which the hydrolysis is difficult, compounds such as nitrous acid, nitrogen hypochlorite or nitrous oxide may be used[141]. Apart from the described hydrolysis of amides, many other methods exist for the synthesis of carboxylic acid products. Some of these are shown in table 4, page 52.

Table 4 Methods of carboxylic acid formation

Reagent / Reagents	**Reference**	**Starting material**
$KMnO_4$ or K_2CrO_7	142	Alkylbenzenes
Ag^+ in ammonia *(Tollens reagent)*	143	Aldehydes
CrO_3, $KMnO_4$ or Na_2CrO_7	144	Primary alcohols
$KMnO_4$ in hot alkaline medium	145	Ketone
dicyclohexano-18-crown-6[146] or HIO_4 with trace K^+ ion *(Lemieux von Rudloff reagent)* $NaIO_4^-$ and ruthenium tetroxide	147 148 149	Vinyl molecules
NaCN *(Von Richter rearrangement)*	150	Aromatic nitro compounds
Halogen gas with base *(Haloform reaction)*	151	Aliphatic methyl ketones
Mg then CO_2 gas *(Grignard reaction)*	152	Aliphatic or aromatic halides

2.6.2 Formation of acid chlorides

To obtain amide bonds it is necessary to condense an amine with a carboxylic acid. However, this condensation reaction is sluggish and normally requires high temperatures and long reaction times. These severe conditions may lead to undesired side reactions and degradation of labile moieties in the reacting molecules. To allow amide formation under less drastic conditions the carboxylic acid should be converted to a more reactive compound such as an activated ester, an anhydride or an acid chloride. A carboxylic acid is more often converted into the acid chloride than into any other of its functional derivatives. From the highly reactive acid chloride there can be obtained many other types of compounds, including esters and amides. The acid chloride is prepared by substitution of the carboxylic acids hydroxy (OH) for chlorine (Cl). Scheme 27, page 53, illustrates three of the many reactions and reagents that may be used to effect the transformation.

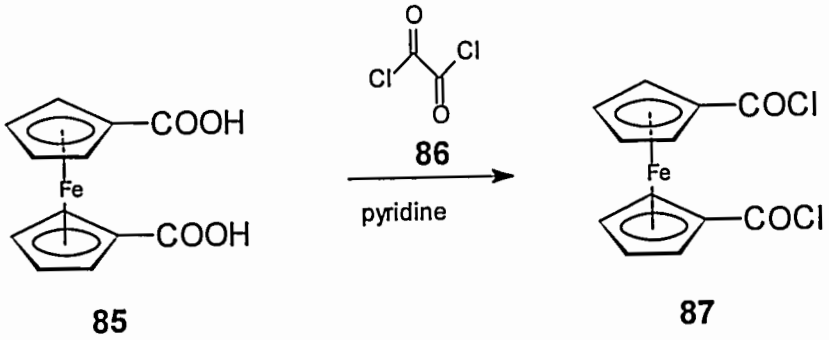


Scheme 27 Reagents for acid chloride synthesis

With respect to the methods used in this thesis use was firstly made of thionyl chloride ($SOCl_2$). Thionyl chloride is usually used in excess and is particularly convenient since the products formed, besides the acid chloride are gases (CO_2 and HCl)[153], are easily separated from the formed acid chloride. Any excess of the low boiling thionyl chloride (bp.79°C) is easily removed by distillation. Many reactions require the use of a catalyst such as pyridine[154,155], triethylamine[156,157] and DMF[158] in addition to thionyl chloride.

2.6.2.1 Oxalyl chloride

Oxalyl chloride is quite useful for converting carboxylic acids to their acid chlorides because the residual oxalic acid decomposes to carbon monoxide (CO) and carbon dioxide gas, thus, driving the equilibrium to the side of the acyl halide. Oxalyl chloride, with pyridine as catalyst, is used for heat -or strong acid/base sensitive compounds, such as 1,1'-ferrocenedicarboxylic acid **85** (scheme 28, page 54). The acid **85** upon reaction with oxalyl chloride and pyridine produces the acid chloride **87** in 90% yield[159]. Other catalysts that may be used include DMF[160] and for base insensitive compounds, the strong bases KOH and NaOH[161].



85 87

Scheme 28 Synthesis of 1,1'-ferrocenecarboxylic acid chloride

Table 5 summarizes various other reagents that may be used to effect conversion

Table 5 Reagents that may be used in acid chloride synthesis

Reagent / Reagents	**Reference**
PCl_3	162
PCl_5	163
Cyanuric chloride	164
Triphenylphosphine	165
Benzoyl chloride (*chloride exchange*)	166

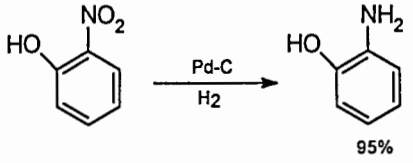
2.6.3 Primary amine Synthesis

2.6.3.1 Nitro reductions

An amine is the reacting partner of a carboxylic acid to form amides. The amino group (-NH_2) can only be directly incorporated into those aromatic compounds that are strongly activated to nucleophiles[167]. The reduction of aromatic nitro compounds that lead to amines is thus important because the nitro group can be introduced into a variety of aromatic systems by simple nitration.

2.6.3.1.1 Catalytic hydrogenation

It is usually the amino group that is required from reduction of a nitro-containing compound. When other reducible functional groups are also present in the molecule, it is often problematic to find a selective reductant that will leave other reducible groups intact. Catalytic hydrogenation of nitro compounds over platinum is clean and gives high yields, but is often incompatible with the presence elsewhere in the molecule of other reducible groups, such as unsaturated functionalities or carbonyl groups. Hydrogenation may be carried out with hydrogen gas and one of many catalysts such as raney nickel[168], palladium on carbon[169], platinum oxide[170] and ruthenium metal[171]. Typical yields in these hydrogenations are very high as is shown in scheme 29 in the reduction of *o*-nitrophenol[172] **88**.

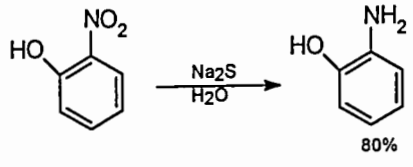


88 **89**

Scheme 29 Hydrogenation of nitro compounds

2.6.3.1.2 Sodium sulfide

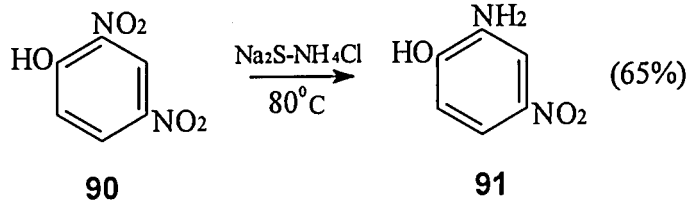
Sulfides such as NaHS, $(NH_4)_2S$ and various polysulfides have been used to reduce nitro compounds[173]. Sodium sulfide (Na_2S) is a very mild reducing agent for nitro reductions and can be employed to reduce only one of two or three nitro groups in a molecule. For example, the reduction of of *o*-nitrophenol **88** with sodium sulfide was done by Hartmann[174] in water medium (scheme 30) in 80% yield.



88 **89**

Scheme 30 Nitro compound reduction by sodium sulphide

It is also possible to reduce one nitro group in the presence of another with the use of sodium sulfide and ammonium chloride as is seen in scheme 27 with *o-p*-dinitrophenol[175] **81**.

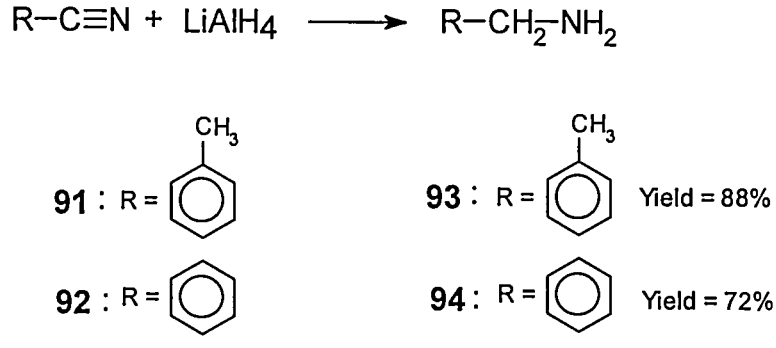


90 **91**

Scheme 31 Selective reduction of one nitro group in a multi nitrated compound

2.6.3.1.3 Nitrile reduction (One carbon gain)

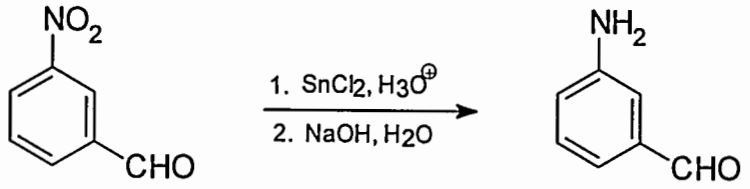
A variety of reducing agents may be used to reduce nitriles to primary amines including $LiAlH_4$[176], BH_3-Me_2S[177] and hydrogen with various catalysts[178]. $NaBH_4$ does not generally reduce nitriles but does so in alcoholic solvents when a $CoCl_2$ catalyst is added[179] or in the presence of raney nickel. Synthesis via reduction of nitriles has the special feature of increasing the length of the carbon chain, producing a primary amine that has one more carbon than the alkyl halide from which the nitrile was synthesised. Typical reactions illustrating the use of $LiAlH_4$ was conducted by Nystrom[180] (scheme 32).



Scheme 32 Amine synthesis by nitrile reduction

2.6.3.1.4 Metals and tin chloride

Iron, zinc, tin and stannous chloride ($SnCl_2$) are also effective for nitro reduction when used in acidic aqueous solution[181]. The acid usually used is hydrochloric acid but occasionally glacial acetic acid is used to advantage, especially when the latter may have a hydrolytic effect as in the case of acetylated nitroamines[182]. Stannous chloride is particularly mild and is often used when other reducible functional groups are present e.g. *m*-nitrobenzaldehyde **95** is reduced to *m*-aminobenzaldehyde **96** in 90% yield[183].



95 **96**

Scheme 33 Amine formation by stannous chloride reduction of a nitro group

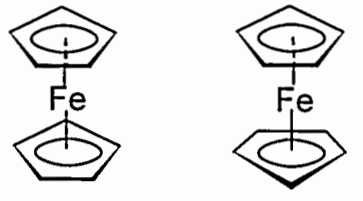
Many other methods are used to reduce nitro compounds to amines; some of the more important methods are summarized in table 6.

Table 6 Reagents for amine synthesis

Reagent / Reagents used	Reference	Starting material
$LiAlH_4$, $NaBH_4$, PPh_3 (Staudinger reaction)	184	Alkyl halide
Strong base (e.g. KOH) and phthalimide	185	Alkyl halide
$NaBH_4$ or $LiAlH_4$	186	Amides
NaOH, Br_2 (Hoffman)	187	Amides
ammonia	188	Ketones or Aldehydes
Water (heat) (Curtius)	189	Acyl azide

2.7 Synthesis of ferrocenes

During the course of this study some use is made of ferrocene derivatives due to their established anti-tumour activity[190] and thus deserves some attention. In particular, it is a goal of this study to synthesise ferrocene / phthalocyanine conjugates to obtain a molecule with potential synergistic antineoplastic effects. Although several reviews on the general organic chemistry are available elsewhere[191,192,193,194], it was considered important to introduce the reader to some characteristic properties and reactions of ferrocene that were regarded as important within the context of this thesis. Ferrocene has remarkable geometry in that it possesses a sandwich structure, in which the two cyclopentadienyl rings lie parallel to one another and the iron cation is buried in the π-electron cloud between them. Two conformations (fig. 22) are possible for ferrocene, one in which the cyclopentadienyl rings (Cp rings) are in the eclipsed (D_{5h} symmetry) formation, the other in which it is in the staggered conformation possessing D_{5d} symmetry. Although the energy to Cp ring rotation is less than 4 KJ $mole^{-1}$, many substituted derivatives approach the eclipsed conformation very closely.

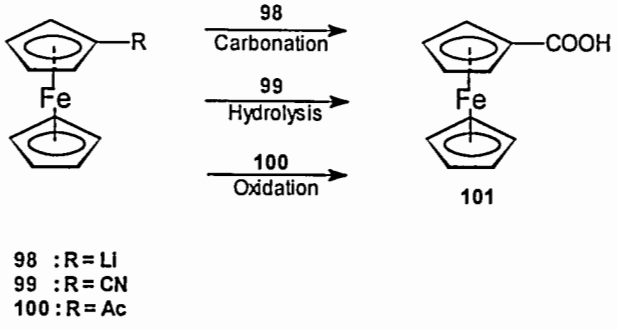


97

Fig. 22 The eclipsed and staggered conformations of ferrocene

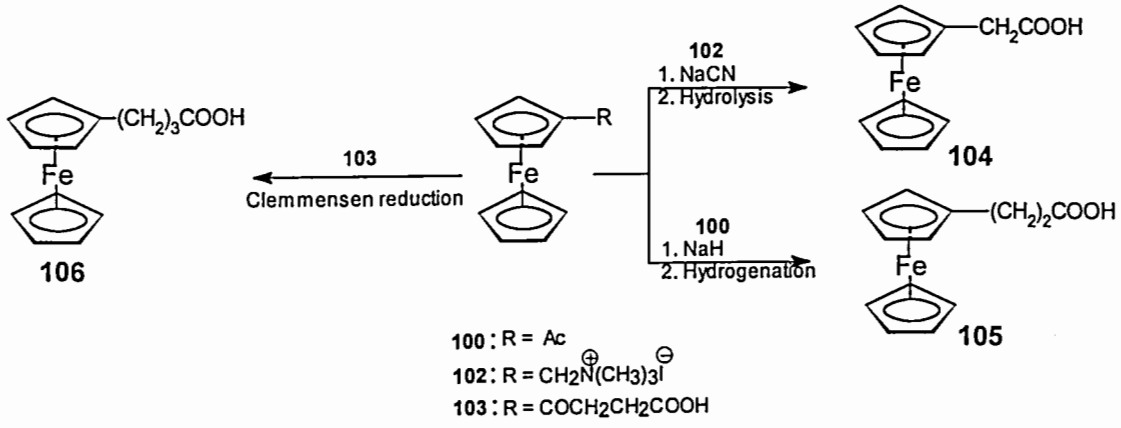
2.7.1 Ferrocene carboxylic acids

Ferrocenoic acid (ferrocenecarboxylic acid) **101** has been prepared in many ways[195], the most important being carbonation of lithioferrocene[196] **98**, hydrolysis of cyanoferrocene[197] **99** and commercially by oxidation of acetylferrocene[195,198] **100**.



Scheme 34 Preparation of ferrocenoic acid

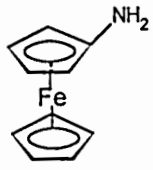
Ferrocenylacetic acid **104** may be prepared from N,N-dimethylaminomethylferrocene methiodide[199,200,201] **102** after treatment with sodium cyanide and further hydrolysis of the formed ferrocenylacetonitrile[202]. Ferrocenylpropanoic acid **105** results from the hydrogenation of ethyl ferrocenoylacetate[203], which in turn is prepared from acetylferrocene **100** and finally ferrocenylbutanoic acid **106** can be prepared by the Clemmensen reduction of β-ferrocenoylpropanoic acid[204] **103**.



Scheme 35 Preparation of various ferrocene carboxylic acids

2.7.2 Aminoferrocenes

Aminoferrocene **107** is most commonly synthesised by the hydrogenolysis of an N-ferrocenylurethane[205,206].



107

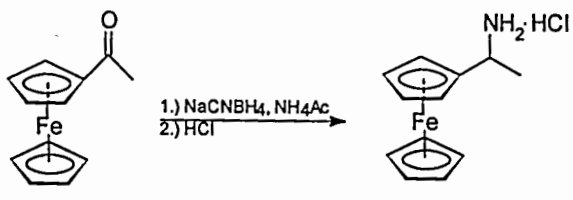
Fig. 23 Structure of aminoferrocene

Some alternative ways of synthesizing **107** are summarized in table 7.

Table 7 Reagents for aminoferrocene preparation

Reagent / Reagents used	Reference	Starting material
O-benzylhydroxylamine or methoxyamine	207 208	ferrocenyllithium
Iron and HCl	209	Nitroferrocene
$LiAlH_4$	210	Ferrocenylazide
Phthalimide copper salt, hydrazine (*Gabriel synthesis*)	211	Bromoferrocene

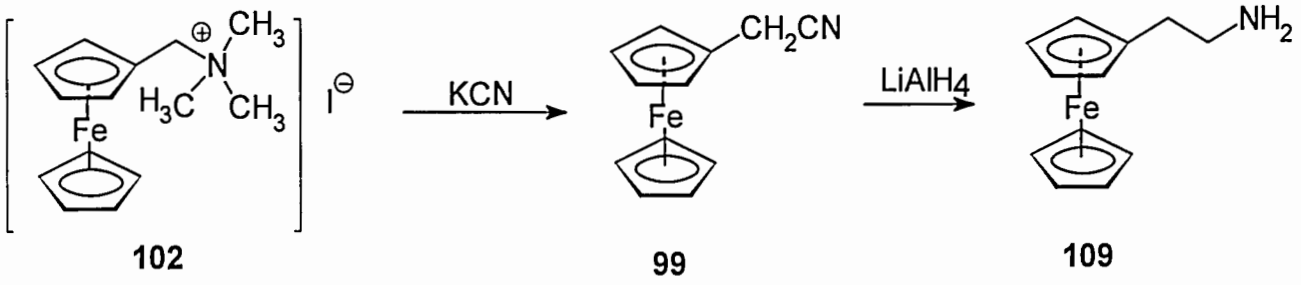
1-Ferrocenylethylamine hydrochloride **108** is prepared from acetylferrocene **100** upon reaction with ammonium acetate and sodium cyanoborohydride and then treatment with hydrochloric acid to prepare the hydrochloride[212] **108**. The preparation of the hydrochloride is necessitated by the instability of the free amine.



100 **108**

Scheme 36 Synthesis of 1-ferrocenylethylamine hydrochloride

Finally, the preparation of an amine functionality slightly removed from the ferrocenyl moiety by two methylene spacers is done by the synthesis of ferrocenylethylamine **109** which is accomplished by the reduction of ferrocenylacetonitrile with $LiAlH_4$[202]. Ferrocenylacetonitrile **99** is obtained from ferrocenylmethyl(trimethylammonium)iodide[199,200,201] **102**.



102 **99** **109**

Scheme 37 Synthesis of ferrocenylethylamine

Chapter 3

Results and discussion

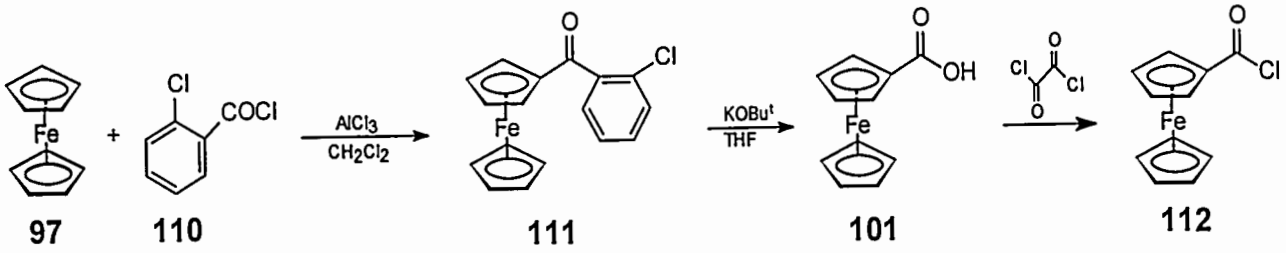
3.1 Introduction

In this research program results are ordered firstly to discuss the synthesis of amine- and carboxylic acid-functionalized ferrocene and phthalocyanine derivatives. Some of these compounds are new, or utilize different synthetic procedures than published. In the third section, results are presented that describe the coupling of some of the ferrocene and phthalocyanine derivatives *via* an amide bond. The resulting conjugates were very insoluble and hence, apart from IR spectroscopy, could not be well characterised. This is followed by sections four and five which describe the syntheses of water-soluble drug carriers and the techniques used to anchor the aforementioned antineoplastic ferrocene and phthalocyanine derivatives on the polymeric drug carriers respectively. Criteria are established that will allow the polymeric drug conjugates to possess good water-solubility. The developed coupling techniques were sufficient to allow virtually quantitative coupling of all ferrocene derivatives, but the success rate of phthalocyanine anchoring was not more than 30%. All the compounds described in sections 3.3-3.5 are new. The final part of this discussion is devoted to the cyclo voltammetric study of a selection of the compounds synthesised.

3.2 Synthesis of ferrocenyl compounds

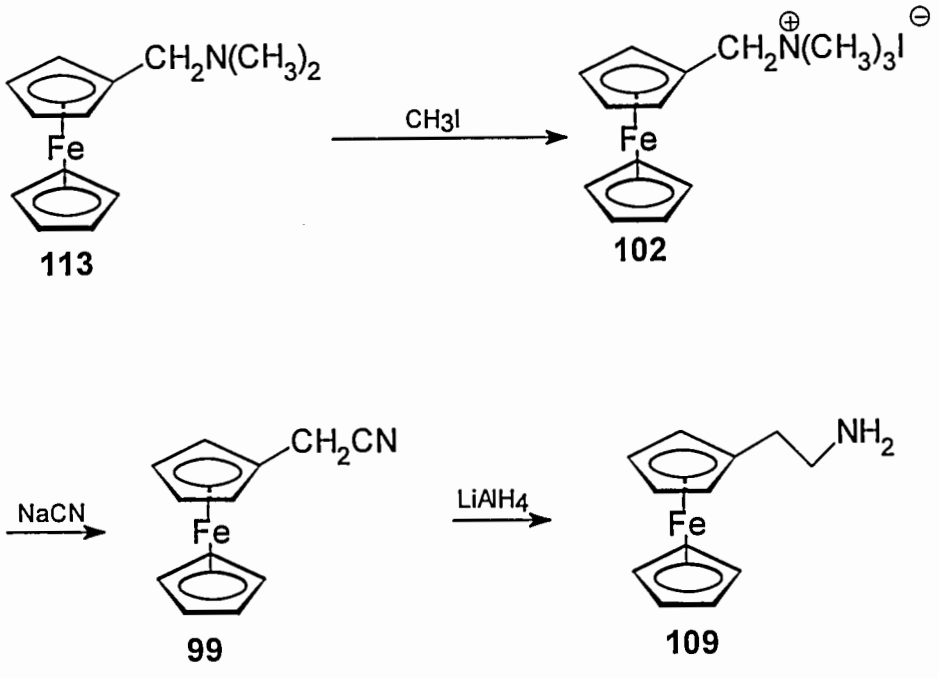
In order to attach the antineoplastic drug ferrocene to the water-soluble polymers and the phthalocyanines it is necessary to functionalize ferrocene. One of the goals of this study are to achieve coupling by means of amide bonds, thus carboxylic and amine-containing side chains were introduced onto the ferrocenyl molecule.

The first ferrocene compound that was prepared was ferrocenoic acid[213] **101** (scheme 38, page 64) in a two step procedure from ferrocene **97**. The first step was a Friedel crafts reaction to produce (2-chlorobenzoyl)ferrocene **111**. Treatment of **111** with potassium *tert*-butoxide gave ferrocenoic acid **101** in 70% yield. Conversion of the ferrocenoic acid **101** to the more reactive ferrocenoyl chloride **112**[214] was achieved virtually quantitatively by means of reaction with oxalyl chloride.



Scheme 38 The preparation of ferrocenoyl chloride **112**

The relative instability of aminoferrocene **107** and difficulty of preparation of that compound[215] led to the use instead of 2-ferrocenylethylamine **109**.

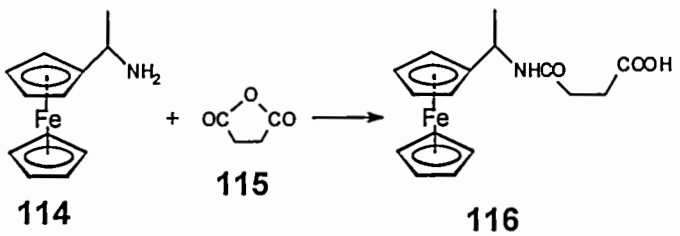


Scheme 39 The preparation of 2-ferrocenylethylamine

In a three step procedure to synthesise **109**, the first step is the synthesis of ferrocenylmethyl-(trimethylammonium)iodide[201] **102** (scheme 39). This is done by reaction of dimethylaminomethylferrocene **113** with methyl iodide to give **102** in 80% yield. Ferrocenylacetonitrile[202] **99** was prepared by refluxing the quaternary salt with sodium cyanide for

2 hours under nitrogen to give the acetonitrile **99** in 42% yield. Isolation of **99** from the reaction mixture proved to be difficult and tended to lower yields dramatically. The last reaction in the sequence is the reduction of the ferrocenylacetonitrile[202] **99** by $LiAlH_4$ in dry ether. The reduction proceeds overnight and after workup the ferrocenylethylamine **109** is obtained as a red oil in 49% yield. Compound **109**, as all other aminated ferrocenes, are not stable but decompose with time. Therefore, it was used immediately after synthesis. Normally feroceneamines would be stored as the more stable hydrochloride salt.

Previous studies in this laboratory showed that the ferrocenyl compound, 1-ferrocenylethylamine **114**[216] is very poorly nucleophilic. It was found that the anchoring of the amine **114** onto a polymeric drug carrier consistently occurred in yields of less than 6%[217]. It was therefore decided to react 1-ferrocenylethylamine **114** with succinic anhydride **115** to give the new carboxylic acid derivative **116** in 60 % yield. It was hoped that **116** would allow more facile ferrocenyl anchoring later with a suitable polymeric drug carrier (results of this reaction is presented in paragraph 3.5, page 87)



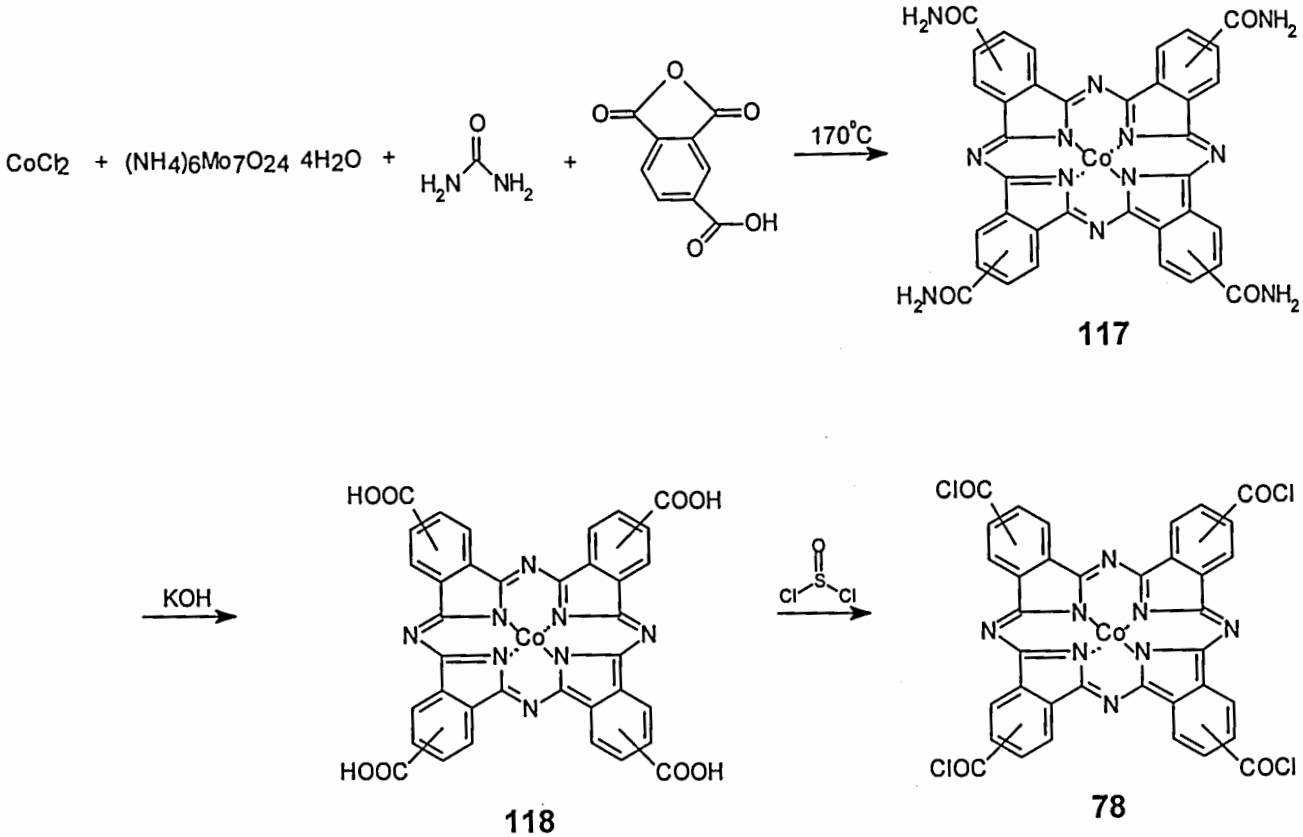
Scheme 40 Synthesis of the ferrocenyl carboxylic acid derivative **116**

3.3 Synthesis of phthalocyanines

Having synthesised the amine and carboxylic acid ferrocene derivatives, attention was focussed on the phthalocyanines. Reaction conditions were mostly determined by the preparation of cobalt phthalocyanines. Zinc phthalocyanines were also prepared. In an electrochemical study (cyclic voltammetry), cobalt is also redox reactive while zinc is not.

3.3.1 2,9,16,23-Substituted phthalocyanines

The first phthalocyanine that was synthesised was one that contained reactive carboxylic acid groups by the method of Kobayashi[218]. Thus navy blue cobalt(II)-2,9,16,23-tetracarboxy-phthalocyanine **118*** was prepared according to scheme 41. Trimeliticanhydride, urea, cobaltous chloride and ammonium molybdate were reacted under reflux (170^{0}C) for 3 hours to give the intermediate amide, cobalt(II)-2,9,16,23-tetracarboxamidophthalocyanine **117** in 52 % yield.



Scheme 41 Synthesis of cobalt(II)-2,9,16,23-tetracarboxylchloride phthalocyanine

This reaction demonstrated the use of an anhydride to obtain a phthalocyanine. The insoluble navy blue amide **117** was characterised by infrared spectroscopy and cobalt determination only. The infrared spectrum showed a broad N-H stretch at 3150 cm^{-1} (fig. 24, page 67) as well as a carbonyl

* Although the numbers 2,9,16 and 23 are used in naming the product 2,9,16,23-tetracarboxyphthalocyanine it should be noted that 118 and most other tetra substituted phthalocyanines really consist of a mixture of different isomers as discussed in paragraph 2.2.8, page 30.

stretching band at 1659 cm^{-1} and the characteristic N-H bend band at 1620 cm^{-1}. In fig. 24 the IR spectrum of **117** is shown together with inserts and assignments of the IR spectra of **118** and **78**.

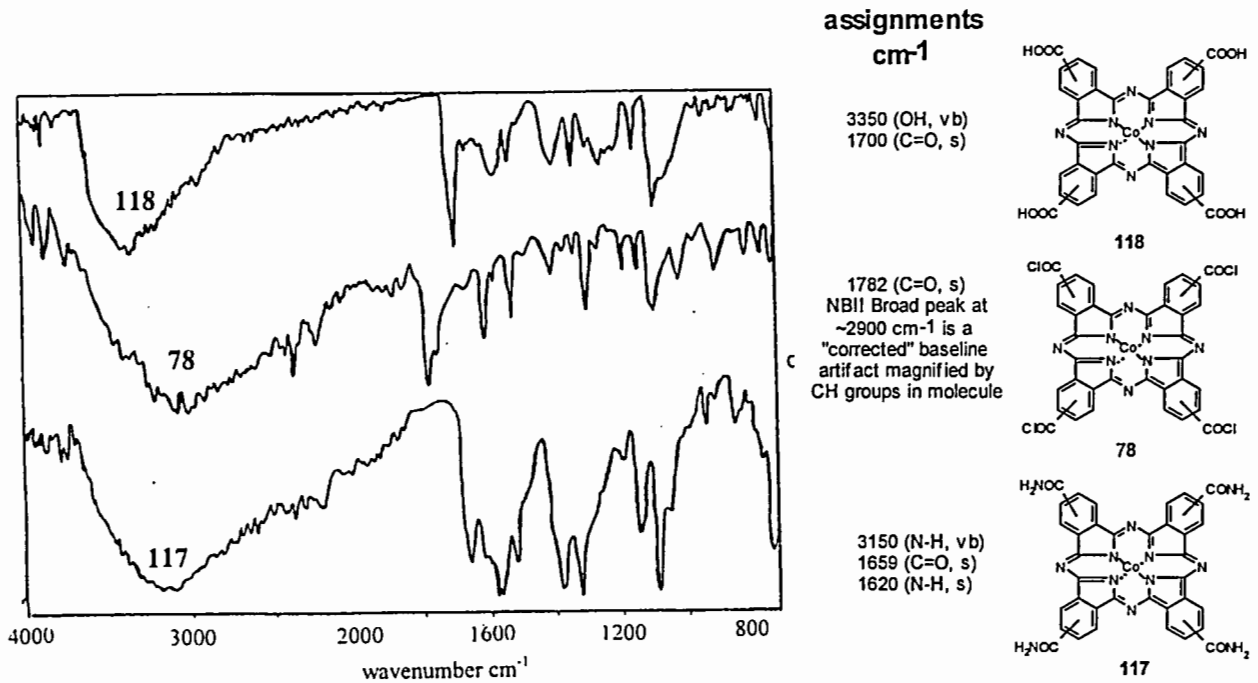
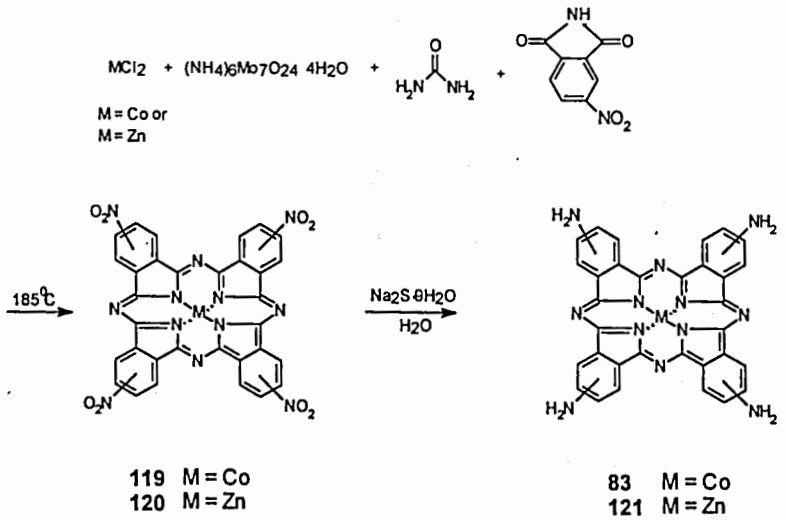


Fig. 24 Infrared spectrum of cobalt(II)-2,9,16,23-tetracarboxamidophthalocyanine **117** together with inserts and assignments of the IR spectra of **118** and **78**. (vb = very broad,, s = sharp)

Hydrolysis of **117** was achieved in refluxing aqueous potassium hydroxide to yield the navy blue cobalt(II)-2,9,16,23-tetracarboxyphthalocyanine **118**. The presence of the carboxylic acid groups, as expected, rendered the phthalocyanine water-soluble in strongly basic (10% sodium hydroxide) medium. The infrared spectra of the carboxylic acid showed a carbonyl peak shift to 1700 cm^{-1} and a broad hydroxyl band at ~ 3350 cm^{-1} (see insert in fig. 24). The ^{1}H-NMR spectrum of the sodium salt in deuterium oxide clearly shows two aromatic signals at δ 7.78 and δ 8.30 integrating for 8 and 4 protons respectively (spectrum 7). Formation of amides by reaction of an amine with a carboxylic acid normally requires acidic conditions and high temperatures. This may prove detrimental for acid labile moieties in other parts of the reacting molecules. To circumvent this, the acid is often

activated either by the addition of a coupling agent, of which there are many, or by conversion of the carboxylic acid functionality into carboxylic acid chlorides. Therefore the tetracarboxyphthalocyanine **118** was treated with thionyl chloride and a catalytic amount of pyridine in benzene to quantitatively yield the neon green tetracarboxylchloridephthalocyanine **78**. The infrared spectrum of the acid chloride showed a carbonyl stretch shift to 1782 cm^{-1} indicating the presence of a strong electron withdrawing group attached to the carbonyl carbon (see fig. 24 page 67). The second target compound was a phthalocyanine containing amine groups for coupling to carboxylic acid moieties. Zinc and cobalt phthalocyanines were synthesised. This time, rather than an anhydride, an imide precursor was used to obtain the phthalocyanine according to the method of Achar[219]. 4-Nitrophthalimide, zinc chloride or cobaltous chloride, ammonium chloride, ammonium molybdate and urea were refluxed for 5 hours at 185^{0}C and after an extensive workup yielded dark green 2,9,16,23-cobalt(II)tetranitrophthalocyanine **119** (see footnote page 66) and analogously green-blue 2,9,16,23-zinc(II)tetranitrophthalocyanine **120** in 80% and 45% yields respectively according to scheme 42. Since the desired functionality on the phthalocyanines was an amine, the nitro groups of the cobalt and zinc tetranitrophthalocyanines were reduced with sodium sulfide nonahydrate in water to yield the dark green 2,9,16,23-cobalt(II)tetraaminophthalocyanine **83** and the dark green-blue 2,9,16,23-zinc(II)tetraaminophthalocyanine **121**.



119 M = Co
120 M = Zn

83 M = Co
121 M = Zn

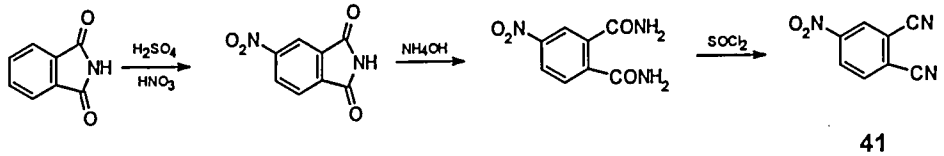
Scheme 42 The synthesis and amination of Co(II) and Zn(II)-tetranitrophthalocyanine

The IR spectrum of the zinc(II)tetranitrophthalocyanine **120** showed typical nitro group bands at 1338 cm^{-1} and 1521 cm^{-1}. Upon reduction the IR spectrum of **121** had a broad N-H band at 3350 $cm^{-1}$and an N-H bending band at 1618 cm^{-1}. The same IR trends were observed for the cobalt analogs.

3.3.2 Unsymmetrically substituted phthalocyanines

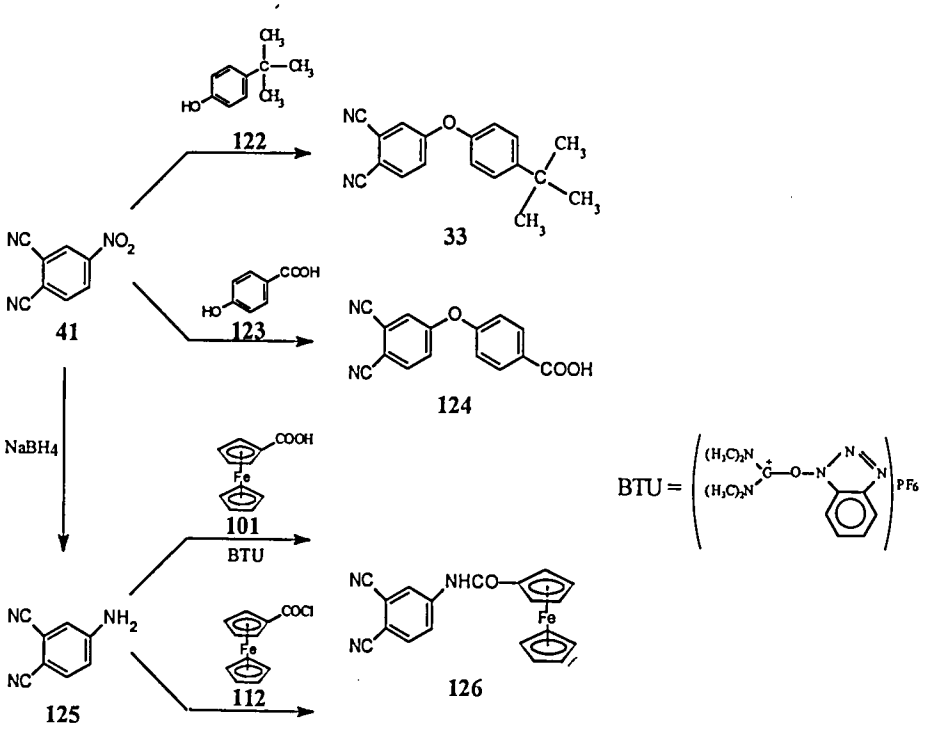
The synthesis of phthalocyanines containing only one reactive substituent, i.e. monofunctionalized with carboxylic or amine groups, is an important prerequisite for the coupling of phthalocyanines to polymers. Since the phthalocyanines so far described have four reactive substituents, crosslinking is expected to occur during polymer anchoring. This will render the polymer insoluble. Obviously a monofunctionalized phthalocyanine will not induce polymer crosslinking during coupling reactions. Two methods are generally used to synthesise monofunctional phthalocyanines: Ring enlargement of a subphthalocyanine and statistical condensation of two different phthalonitriles. Both methods were used in this study. However, literature reports indicate that ring chlorinated side products accompany the products obtained in the ring enlargement of subphthalocyanines. These side products are not separable from unchlorinated phthalocyanines. Hence the current method of choice for the preparation of monosubstituted phthalocyanines is the statistical condensation of two different phthalonitriles. In this method, the separation of a mixture of phthalocyanines with no or unpolar substituents is difficult due to the formation of dimers and oligomers, caused by the aggregation of the molecules. Wöhrle[44] has demonstrated that phthalocyanines with long-chain ether substituents are easily separated by chromatography. They are also soluble in solvents as DMF or THF.

3.3.2.1 Phthalonitrile derivatization



Scheme 43 Synthesis of 4-nitrophthalonitrile

All the phthalonitriles needed in this study could be derived from 4-nitrophthalonitrile **41**, which is commercially available. The purchase price of **41** however, led to the decision to synthesise it locally by the pathway depicted in scheme 43. The relative expense of all steps in the reaction are shown in scheme 43, thus in total $13.73 for a yield of 8.5g compared to a purchase price of $165.75 for 8.5g of **41**. Two known phenoxy-substituted phthalonitriles[44], 4-(4-*tert*-butylphenoxy)-1,2-benzenedicarbonitrile **33** and 4-(3,4-dicyanophenoxy)benzoic acid **124**, as well as 4-amino phthalonitrile[220] **125** and a ferrocene containing derivative **126** were synthesised according to scheme 44.



Scheme 44 Synthesis of phenoxy substituted phthalonitriles, aminophthalonitrile **125** and a ferrocene-containing phthalonitrile **126**

Nitro displacement by phenols or alcohols is generally slow. The reaction between 4-nitrophthalonitrile **41** and p-*tert*-butylphenol **122** needed 2 days for completion while the carboxylic acid **123** needed 5 days. 4-(4-*Tert*-butylphenoxy)-1,2-benzenedicarbonitrile **33** and 4-(3,4-dicyanophenoxy)benzoic acid **124** were obtained in yields above 50 %. 4-Aminophthalonitrile **125** was obtained by reduction of 4-nitrophthalonitrile **41** with sodium borohydride in the presence of 10% Pd on charcoal. The amine **125** is not a stable compound and decomposes rapidly, thus it is used immediately upon formation. The synthesis of 4-(ferrocenylamido)phthalonitrile **126** was done as shown in scheme 44, page 70, firstly utilizing a coupling agent, O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate, referred to hereafter as BTU, and ferrocenoic acid **101** to give the red compound in 45 % yield. In a second approach ferrocenoyl chloride **112** was used instead of the carboxylic acid **101**, using pyridine as a catalyst to give the amide **126** in 70 % yield. The ^{1}H-NMR spectrum of the amide **126** does not convincingly indicate the presence of an amide proton (usually occurring as a broad signal between δ5-8). Thus a ^{1}H-NMR 2-D cosy spectrum (fig. 25) was used to confirm the presence of the amide proton which is indicated as A after the correlation showed that the ABX system of the phenyl protons consists of the doublet of doublets at C, the doublet at B and the doublet at D.

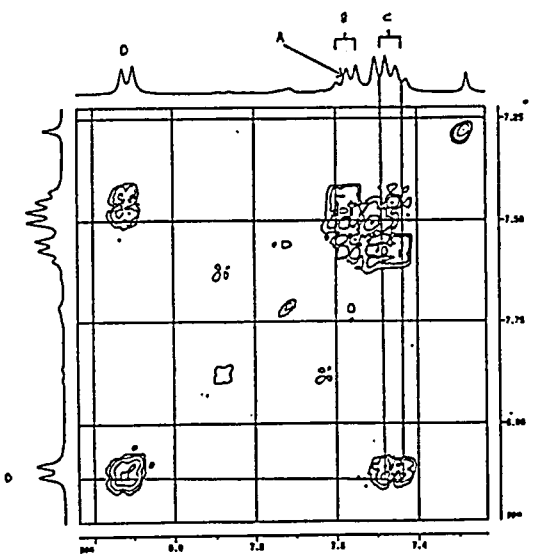


Fig. 25 2D cosy spectrum of 4-(ferrocenylamido)phthalonitrile

The phthalonitriles prepared, as well as their precursors, display a variety of functional groups. The infrared spectra with certain functional group assignments are shown in fig. 26.

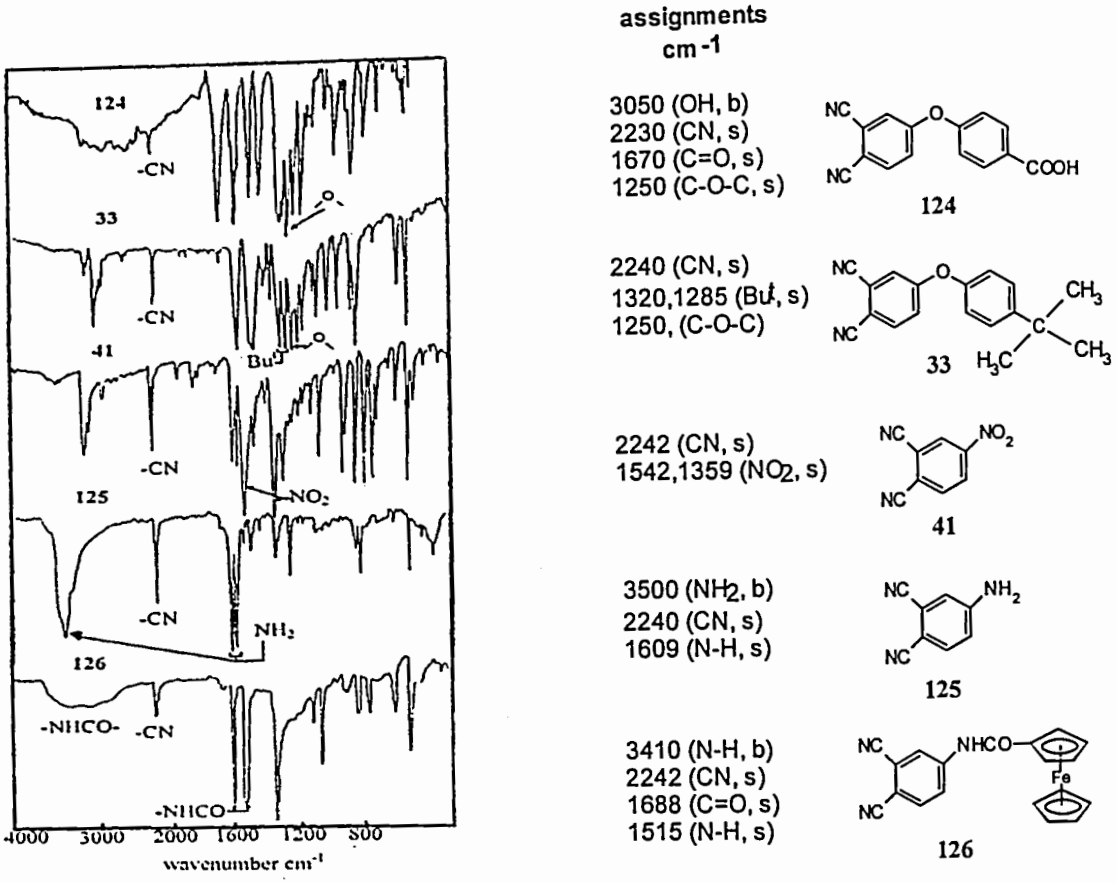


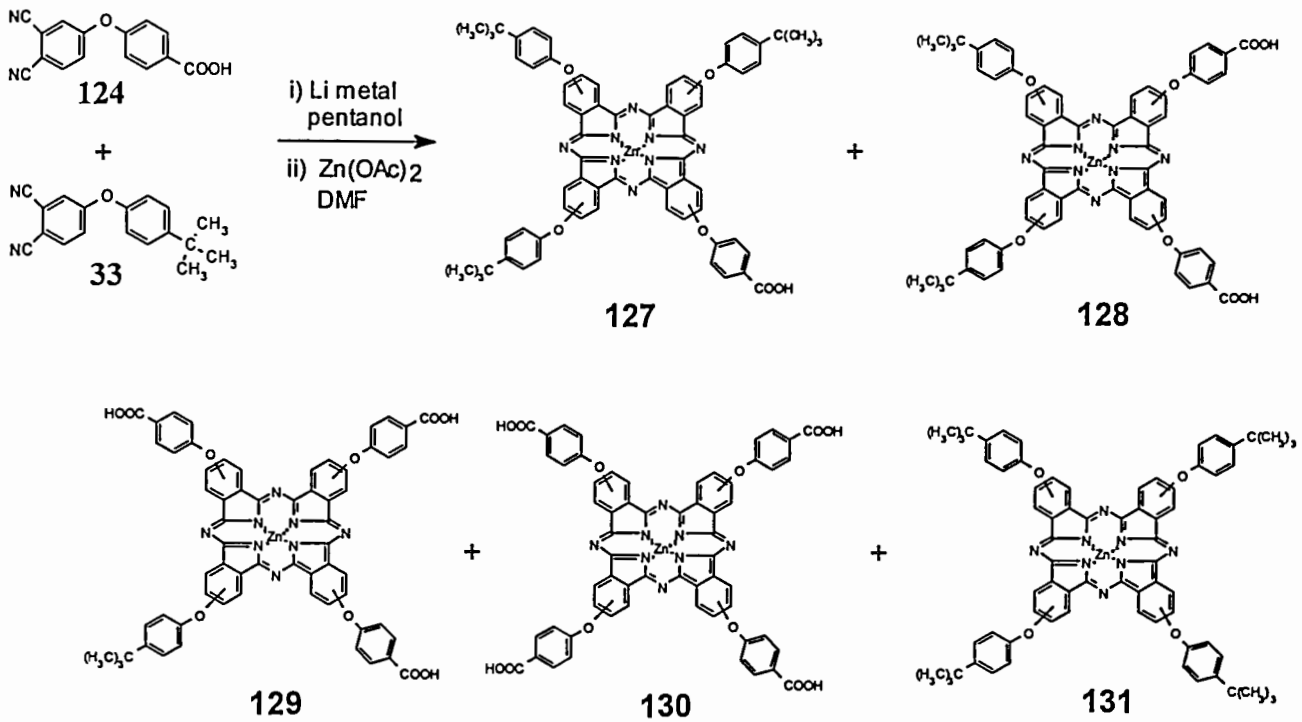
Fig. 26 The infrared spectra of phthalonitriles **33**, **41**, **124**, **125** and **126** (b = broad, s = sharp)

3.3.2.2 Statistical condensation of phthalonitriles

For the synthesis of the phthalocyanines, the lithium alkoxide-catalysed reaction was selected using the method of Cook[92]. This two step procedure is preferred to existing direct metallation procedures[38] due to higher expected yields.

Cook's protocol requires that the dinitriles be dissolved under inert atmosphere in pentanol at 140^{0}C followed by lithium metal addition. Lithium pentoxide is formed *in situ* and the appearance of a green solution is almost instantaneous. Delithiation of the formed dilithium phthalocyanine is achieved by treatment with glacial acetic acid. Metallation of the metal free phthalocyanine with zinc or cobalt is achieved directly by reaction with zinc(II)acetate or the required metal salt in DMF

at 80^{0}C for approximately 12 hours. With the introduction of polar substituents, the separation of the phthalocyanine compounds is possible by flash chromatography using silica gel (Si 60) with a 6:4 ethyl acetate-petroleum ether (60^{0}C -80^{0}C) mixture. Accordingly, phthalocyanines **127-131** were synthesised as seen in scheme 45 by reacting dinitriles **33** and **124** in a molar ratio of 1 : 1.5.



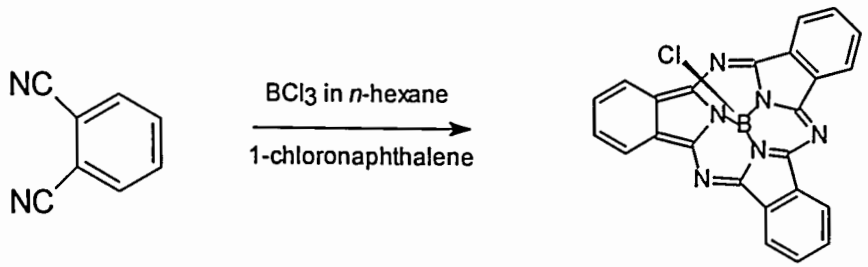
Scheme 45 Condensation of phthalonitriles to yield phthalocyanines

TLC of the phthalocyanine mixture in a 6:4 solution of ethyl acetate and petroleum ether showed three eluted spots (R_f = 0.97, R_f = 0.60 and R_f = 0.35) and a spot at the origin. Chromatography was thus carried out using the same solvent system wherein the first fraction (R_f = 0.97) was the [2,9,16,23-tetra(4-*tert*-butylphenoxy)phthalocyanato]zinc(II) **131** species determined by ^{1}H-NMR (spectrum 10) in 20% yield. The second fraction (R_f = 0.60) was the required [9,16,23-tris(4-*tert*-butylphenoxy)-2-(4-carboxyphenoxy)phthalocyanato]zinc(II) **127** wherein the ^{1}H-NMR spectrum (spectrum 9) showed peaks at δ9.1-9.3 corresponding to 4 protons, δ8.6-8.8 also 4 protons, δ8.0-

8.2 integrating 4 protons, δ7.4-7.9 corresponding to 16 protons and finally δ1.2-1.4 corresponding to 27 protons. The yield of the monofunctional phthalocyanine **127** obtained was approximately 15%. The third fraction (R_f = 0.35) was [9,23-bis(4-*tert*-butylphenoxy)-2,16-bis(4carboxyphenoxy)phthalo-cyanato]zinc(II) **128** or the bifunctional phthalocyanine also determined by ^{1}H-NMR spectroscopy (spectrum 8), obtained in 2% yield. The fractions that did not elute were presumably the phthalocyanines **129** and **130**, but could also be some very polar or polymeric impurities. Since phthalocyanine **130** would be required for independent reactions, but was not separable from the obtained reaction mixture a further experiment was done using only dinitrile **124**. The lithium pentoxide method (as described above) was thus used to obtain phthalocyanine **130** in 67 % yield (spectrum11).

3.3.2.3 Subphthalocyanine condensation with a phthalonitrile

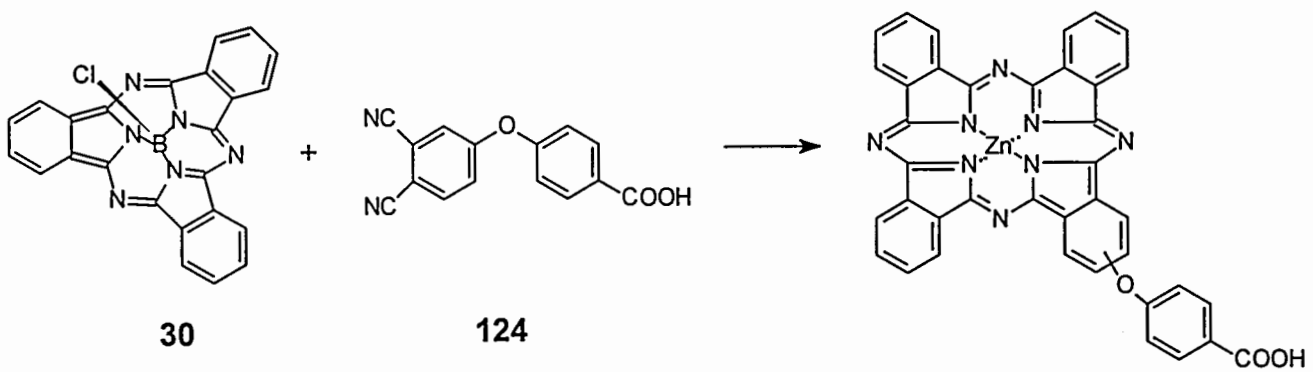
The IUPAC designation of chloro subphthalocyanine **30** is chloro[7,12;14,19-diimino-21,5-nitrilo-5*H*-tribenzo[*c,h,m*][1,16,1]triazacyclopentadecinato (2-) N^{22}, N^{23}, N^{24}] boron but for convenience sake will be referred to subsequently as SubPc. In the ring enlargement reaction of SubPc **30** - diiminoisoindolines or 1,2-benzenedicarbonitriles (phthalonitriles) are employed in enlarging the ring to a phthalocyanine structure. Since the very reactive diiminoisoindolines seem to favour cyclotetramerization with zinc salts to yield mostly tetrasubstituted phthalocyanines rather than monosubstituted phthalocyanines, use was solely made of phthalonitriles, as only the zinc phthalocyanines were targeted in this part of the research program. SubPc **30** was synthesised using the improved method developed by Wöhrle[66] (scheme 46, page 75). Hence phthalonitrile **22** was condensed with boron trichloride to yield a violet mixture which, after purification, gave the brown chlorosubphthalocyanine **30** in 58 % yield (spectrum 5).



22 30

Scheme 46 Synthesis of chlorosubphthalocyanine **30**

The ring enlargement reaction was conducted by the method of Wöhrle[66] using the strong base DBU and a solvent mixture of DMSO and 1-chloronaphthalene wherein SubPc **30** and zinc(II)acetate were added to 4-(3,4-dicyanophenoxy)benzoic acid **124** at 130^{0}C (scheme 47). The product was purified by flash chromatography and further by preparative TLC using toluene:DMF (98:2) to give the blue monofunctional phthalocyanine **132** in 15% yield.



30 **124** **132**

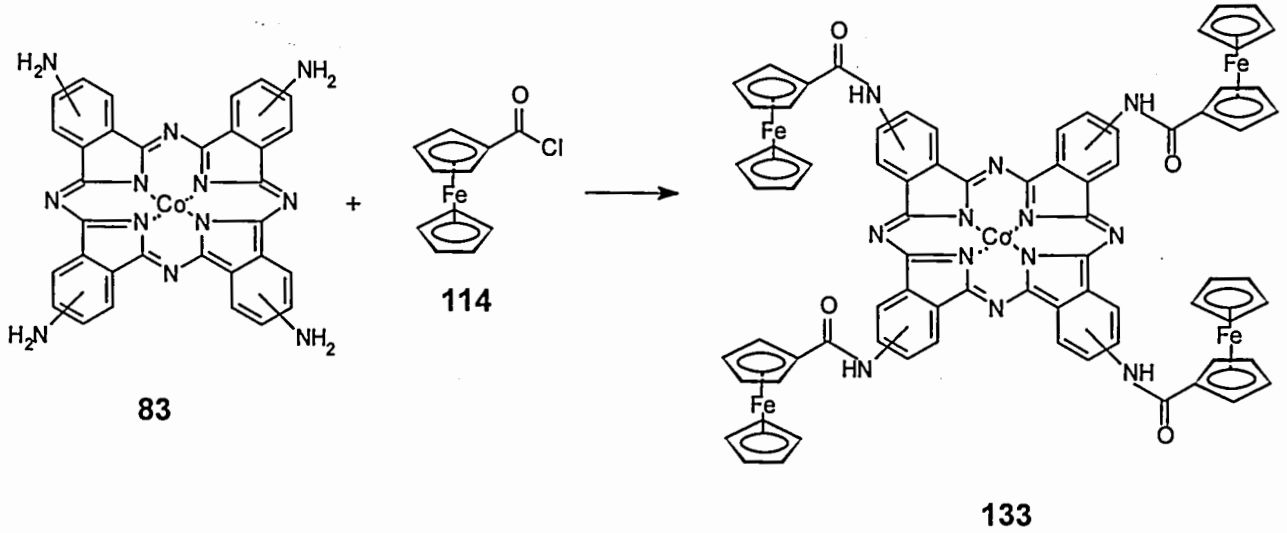
Scheme 47 Monofunctional phthalocyanine **132** synthesis by the SubPc **30** route

The SubPc reaction produces the monosubstituted phthalocyanine **132** in low yields (~15%-20%), thus it is possible that the SubPc **30** does not undergo a concerted reaction with the phthalonitriles but indeed a multistep reaction, whereby the first step would be a base catalyzed decomposition of the SubPc **30**. The second step may then be the reaction of the activated fragments with each other

or the phthalonitriles followed by ring closure to differently substituted phthalocyanines and also probably also polynitriles. The infrared spectrum of the phthalocyanine **125** has certain additional bands in the 650 cm^{-1} to 720cm^{-1} range as compared to the tetrasubstituted counterpart, thus confirming that there may be a certain degree of ring-chlorination that has occurred

3.3.3 The synthesis of ferrocene-phthalocyanine conjugates

One of the goals of this thesis is to prepare a conjugate between a ferrocene and a phthalocyanine species by means of an amide linkage. The previously prepared 2,9,16,23-cobalt(II)tetraaminophthalocyanine **83** was selected for coupling to ferrocenoyl chloride **114**. The best results were obtained by reaction in DMF upon which the 2,9,16,23-cobalt(II)tetraamidoferrocenylphthalocyanine **133** (see footnote page 66) is presumably formed (scheme 48). Mixtures of mono, di and tri ferrocenylated derivatives (not shown) are also probably present in the crude product mixture.



Scheme 48 Synthesis of 2,9,16,23-cobalt(II)tetraamidoferrocenylphthalocyanine **126** – a phthalocyanine-ferrocene conjugate (see footnote page 66)

The product obtained is very insoluble in all common organic solvents and thus defied all attempts to determine the ferrocene content quantitatively by spectroscopic methods. It was hoped that the introduction of the ferrocenyl group into the phthalocyanine would prevent phthalocyanine stacking. Although this may well have happened, the tetraamide system of **133** is probably inherently sparingly soluble anyway. Confirmation of this was observed in the synthesis of **116** (scheme 40, page 65). The mono-amide **116** was also observed to be much less soluble in common organic solvents like DMF, ether and chloroform than expected. The infrared spectrum of the crude product that should contain phthalocyanine **133** showed the formation of the amide bond did take place because the IR amine N-H bend peak at 1601 cm^{-1} reduced very much in intensity and a new peak associated with the formed amide appeared at 1659 cm^{-1} (Amide I band) for the amide. The amide II band was also seen at 1479 cm^{-1} (see fig. 27). The spectrum did not show any obvious intense signals associated with remnants of unreacted amine or carboxylic acid.

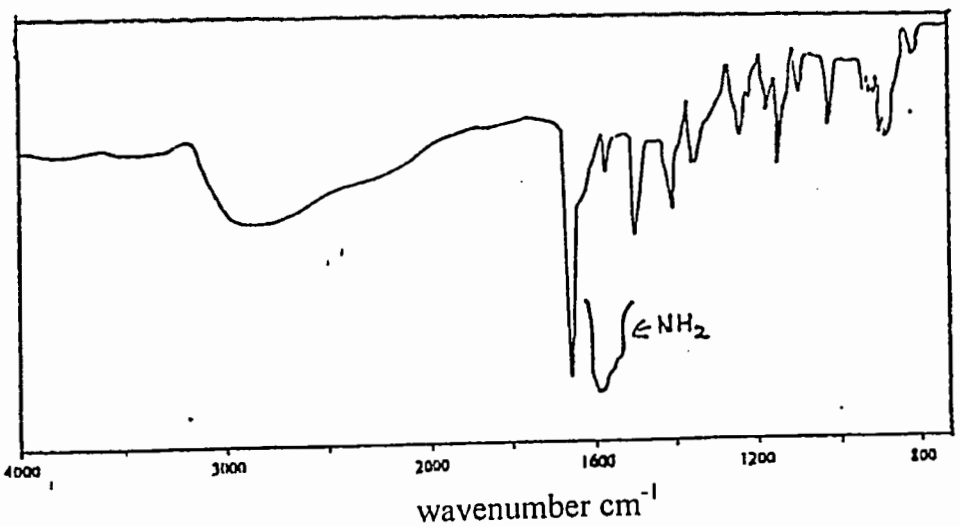
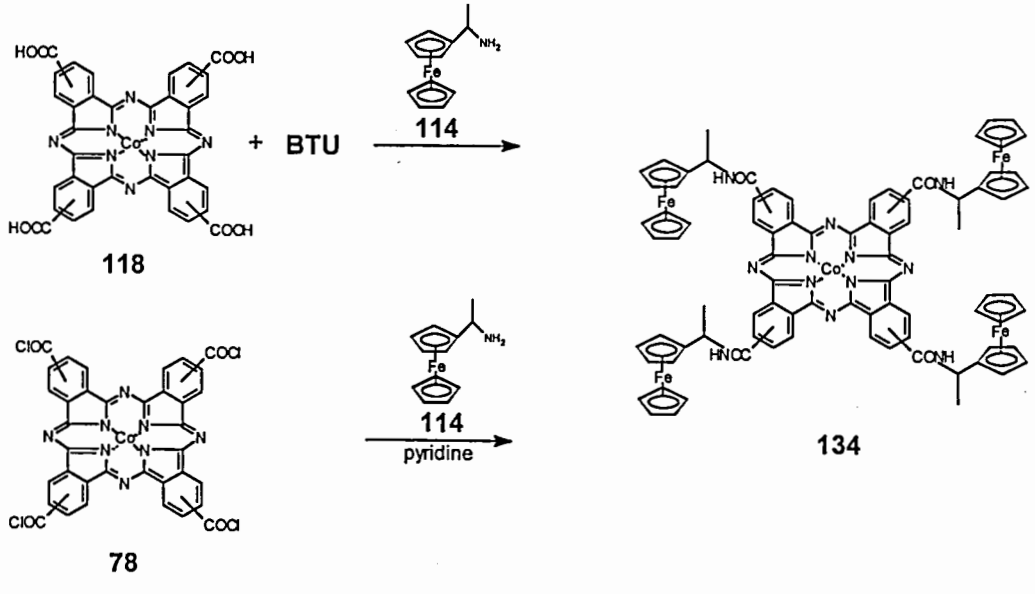


Fig. 27 The infrared spectrum of 2,9,16,23-cobalt(II)tetraamidoferrocenylphthalocyanine **133**

The reaction between **83** and **114** was also conducted by stirring the reactants in chloroform at room temperature and under reflux. Although **114** is soluble in chloroform, **83** is not, and even after

prolonged periods of time, no significant reaction was observed. By substituting solid ferrocenoyl chloride with liquid benzoyl chloride, phthalocyanine **83** was refluxed in an excess of this reagent. With this procedure, the infrared spectrum of the crude solid reaction product did indicate partial amide formation, but the product remained insoluble in organic solvents. In a last reaction, **83** was reacted with ferrocenoic acid **114** in the presence of the coupling agent BTU (for structure see scheme 44, page 70) in DMF. Again, after workup, the infrared spectra of the crude product showed partial amide formation did take place, which indicated **133** and / or mixtures of the mono, di and tri substituted compound did form.

The second approach consisted of reversing the order of functionalities, using the tetracarboxylic acid phthalocyanine **118** and its acid chloride derivative **78**. The amine used was the previously described 1-ferrocenylethylamine **114**. The coupling reactions performed were, as indicated in scheme 49, using BTU with **118** and pyridine as catalyst with **78**.



Scheme 49 A second approach to the synthesis of a phthalocyanine-ferrocene conjugate

The obtained products were again insoluble but the formation of amide bonds was seen in their infrared spectra as for **133**.

The insolubility of products **133** and **134** led us, however, to abandon any further investigation of these compounds within the framework of this study. In order to characterise **133** and **134** properly, or to utilize them, for example, in PDT, it must be made soluble in a suitable solvent. Anchoring of derivatives of it on a polymeric drug carrier will render it soluble, and is the subject of another research program.

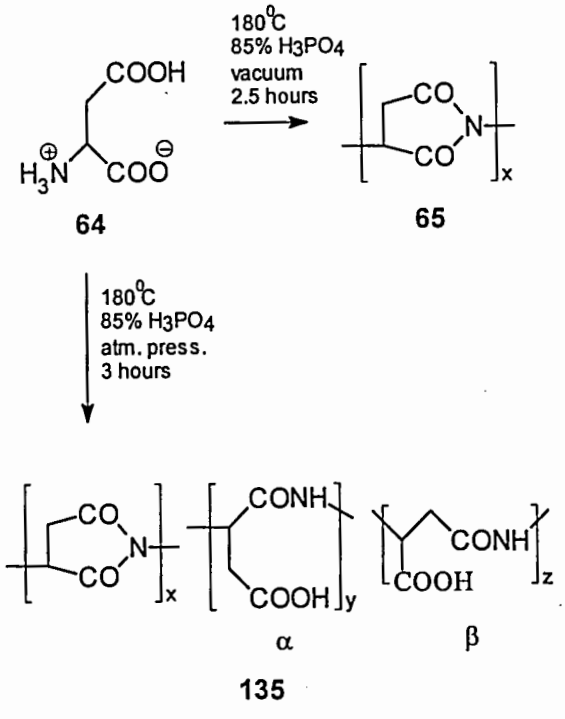
3.4 Preparation of polymeric drug carriers

The selection of the drug carriers to be used in this thesis took into consideration the properties mentioned earlier (paragraph 2.3.4, page 41). In this regard, the drug carriers selected to demonstrate the anchoring reactions of the phthalocyanine and ferrocene drugs were mostly modifications of polysuccinimide **65** and to a lesser extent, co-polymers of the amino acid lysine in the protected form **138** and aspartic acid **64**. Polysuccinimide **65** is not a water-soluble polymer but by conversion to polyaspartamides it is rendered so. The degradation of the polymers in a biological environment is possible by means of amide bond hydrolysis. This was an important consideration in the selection of these polymers. Another factor contributing to the selection of the two mentioned types of polymers is the potential selective action of the polymers. Although no specific recognition systems for cancer cells exist in the structure of the polyaspartamides, aspartic acid **64** and lysine are the monomers that were used to create the backbone of the polymeric drug carriers. Taking into consideration the greater need for nutrients and metabolic precursors, such as amino acids and peptides, by tumour cells as compared to healthy cells, it may be expected that those polymers containing these units would be preferentially taken up by cancer cells. This will

introduce a crude form of selective drug delivery. The lack of well characterised tumour homing devices necessitates the investigation of such systems.

3.4.1 Homopolymers from aspartic acid

The known[221] thermal polymerization of aspartic acid **64** at 180°C and under reduced pressure for 2.5 hours leads to polysuccinimide **65** in 90% yield (scheme 50). The molecular mass of **65** is known to be in the order of 70000 g mol^{-1}



Scheme 50 The effect of different experimental conditions on the polymerization of aspartic acid

It was demonstrated elsewhere[222] that less harsh polymerization conditions lead to a polymer in which not all the aspartic acid that polymerized underwent imide cyclization. Part of the monomer remained in the uncyclized state. This observation is important because it explains low yields when aspartic acid **64** is copolymerized with lysine during the latter stages of this study (paragraph 3.4.2, page 84). Polysuccinimide **65** is recovered by dissolving the crude reaction mixture in DMF and

precipitation with water. It was found that polysuccinimide **65** becomes increasingly water-soluble with a decreasing percentage of imide rings in the main polymeric chain. The succinimide fractions of the polymer is characterised by NMR signals at 5.25 ppm (1H; CH), 3.18 ppm (1H,CH_2) and 2.67 ppm(1H,CH_2) in DMSO-d_6 (fig. 28)

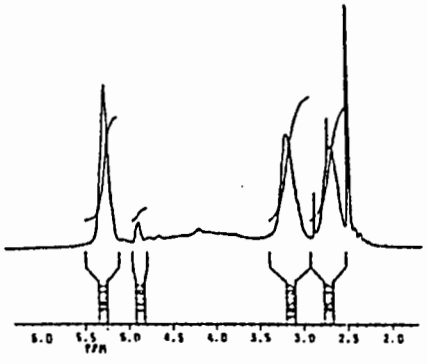


Fig. 28 ^{1}H-NMR signals of polysuccinimide

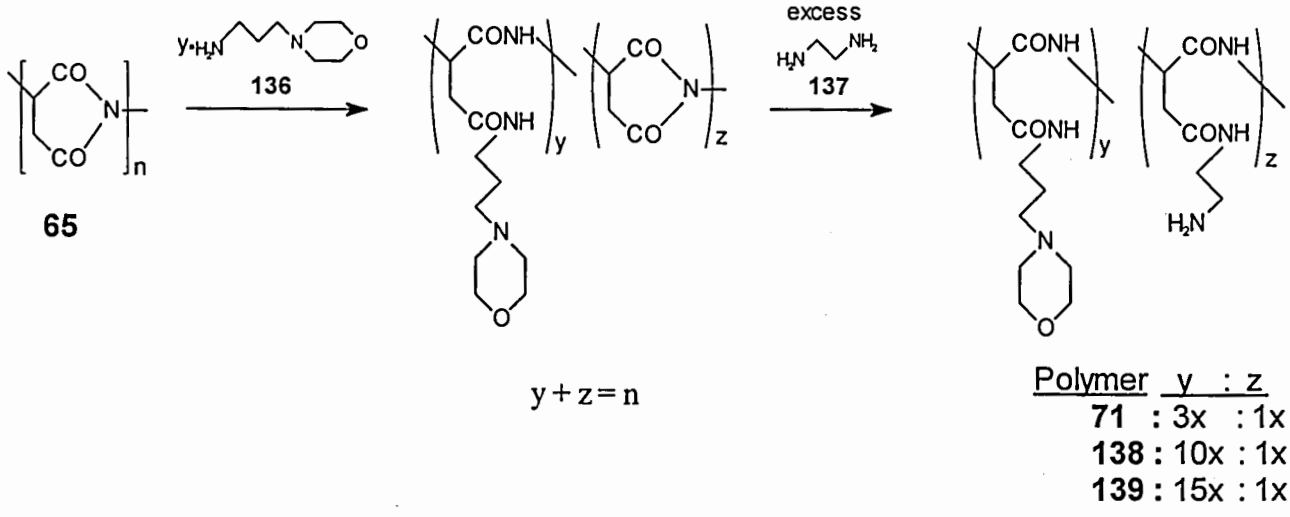
Uncyclized aspartic acid units in the polymeric backbone* are seen by the peak at 4.90 ppm (1H,CH) in d_6-DMSO (fig. 28). In D_2O this peak is found at 4.2-4.5 ppm.

The first step in the synthesis of the polymeric drug carriers is the opening of the imide rings of polysuccinimide **65**. Polymers **71, 138** and **139**† (scheme 51, page 82) were obtained by reacting **65** with the indicated amounts of N-(3-aminopropyl)-morpholine **136** followed by treating the intermediate polymers with a slight excess of ethylenediamine **137**. The N-(3-aminopropyl)-morpholine unit in the polymers act as a solubilising agent and has antibloating properties[223]. The ethylenediamine fragment provides an anchoring site for acid-functionalized ferrocenes and phthalocyanines. The different monomer ratio's of polymers **71, 138** and **139** may also be helpful in determining carrier capacity of the polymeric carriers without losing water-solubility. The N-(3-aminopropyl)-morpholine unit in polymers **71, 138** and **139** is inert toward carboxylic or amine

* All polymers containing uncyclised fragments of aspartic acid will have random mixtures of the α - and β - isomers as shown in polymer 135 on page 80. Henceforth, only the α - isomer will consistently be shown. This will also apply to all polyaspartamides reported later in this study.

† All polymers in this study actually have a random distribution of monomers and by no means should the reader deduce from the structure shown that ordered or block polymers have been synthesised

functional groups, thus it should not interfere with the anchoring of the drugs onto the polymer. An additional function of the bulky morpholine group on the polymer would be to sufficiently separate the reactive amino side groups. This will minimize possible unwanted side reactions such as polymer crossbinding during subsequent anchoring reactions of tetra carboxylic acid phthalocyanine derivatives. The reactions were all done by adapting the procedure of Swarts[224] to synthesise the new polymers **138** and **139**. The y : z monomer ratios of three and ten to one were easily obtained. The ratio of 15 : 1 became difficult to control due to the small margin available for weighing errors on small scale synthesis. However, it is entirely conceivable that steric effects at very large y : x ratios may cause slower morpholine anchoring during the latter stages of reaction. This would create the impression that large y : z monomer ratios (see scheme 51) are not easily attainable.



Scheme 51 Preparation of co-polyaspartamides

Polymers **71**, **138** and **139** were dialyzed in water in a 12000 molecular mass cut off membrane tubing to eliminate all small molecule reactants and oligomers and then freeze dried. Solution viscometry in water gave inherent viscosities between 0.1 and 0.2 dl g^{-1}. Polymers **71**, **138** and **139** were obtained in yields of 46, 51 and 39% respectively.

The structure of **71** was confirmed earlier by elemental analysis, IR, ^{1}H and ^{13}C NMR spectroscopy. For the purposes of this thesis, monomer ratios y : x in all polymers was determined by a simplified treatment of ^{1}H NMR data only. To illustrate this procedure, polymer **138** will be discussed in detail. The ^{1}H NMR spectrum of **138** as well as the important peak assignments to specific protons can be found in fig. 29

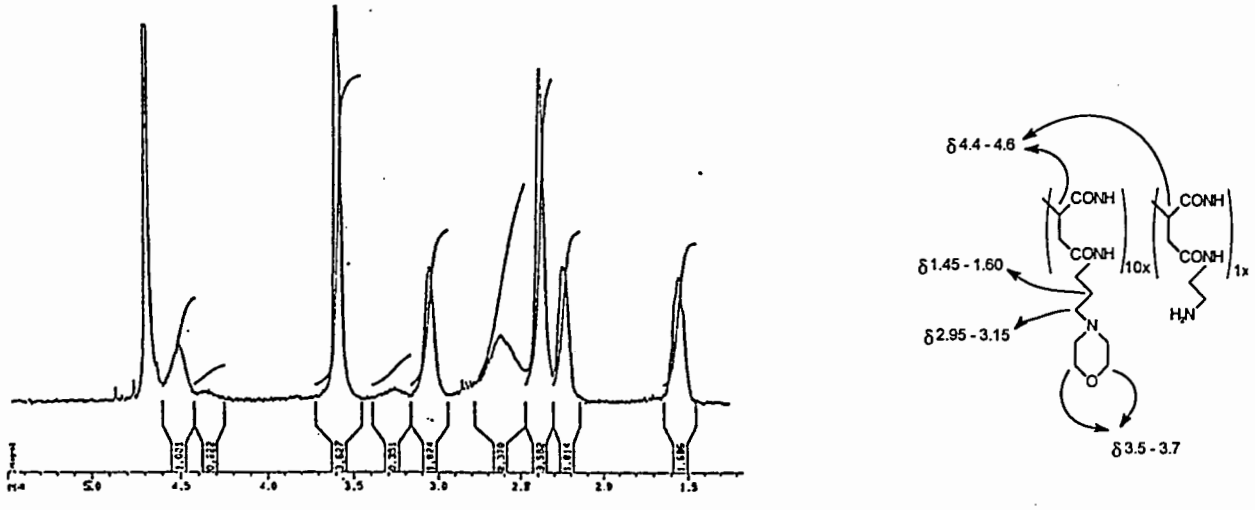
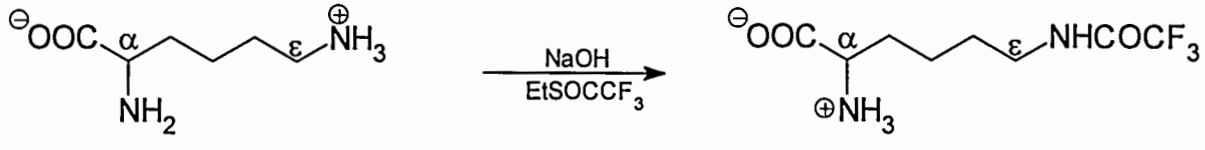


Fig. 29 The proton assignment in the ^{1}H-NMR spectrum of polymer **138**

As can be seen above in the spectrum of polymer **138** possessing a 10 : 1 ratio, the peak at δ 4.3-4.5 is the combination of the aspartyl methine protons of the two different repeating units. The peak at δ 3.5-3.7 is assigned to the 10 x 4 = 40 morpholine protons adjacent to the oxygen atom of the cyclic ether. The combined integral of these 40 protons is 3.627 or 0.091 integral units per proton. If the expected ratio of y : z = 10:1 was achieved during synthesis, the methine protons of the aspartyl groups should integrate for 11 protons, that is 11 x 0.091 = 1.001. In practice it was found to integrate to 1.00 which confirms the expected monomer ratio.

3.4.2 Synthesis of lysine-aspartic acid co-polymers

To enable the preparation of co-polymers of the amino acids aspartic acid **64** and lysine **140** it is necessary first to protect one of the two amino groups of lysine to prevent crosslinking during polymerization reactions. Crosslinking will take place because lysine is not di- but trifunctional. Protection of both the α - and ε - amino groups (scheme 52) can be achieved, but in this research program it was decided to protect the ε - amino group to allow easier drug anchoring on the ultimate polymeric carrier. It was established elsewhere[224,225] that drug anchoring becomes progressively easier with more methylene spacers separating polymer (or drug) from the functional group that will be utilized for anchoring purposes. Thus, by the method of Schellenberg[226], lysine **140** is reacted with ethyl thioltrifluoroacetate in basic medium to give N^{ε}-trifluoroacetyl-L-lysine **141** in 34% yield according to scheme 52.

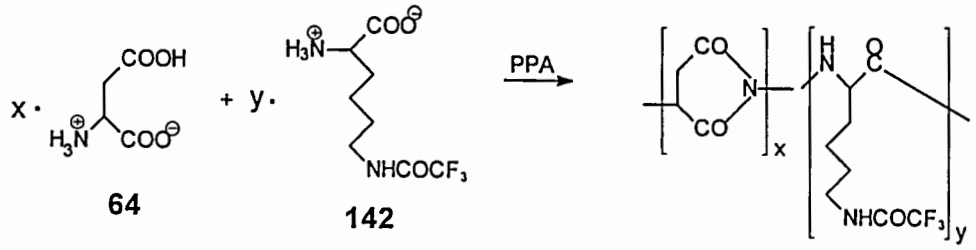


lysine **140**

N^{ε}- trifluoroacetyl-L-lysine **141**

Scheme 52 ε-amino protection of lysine

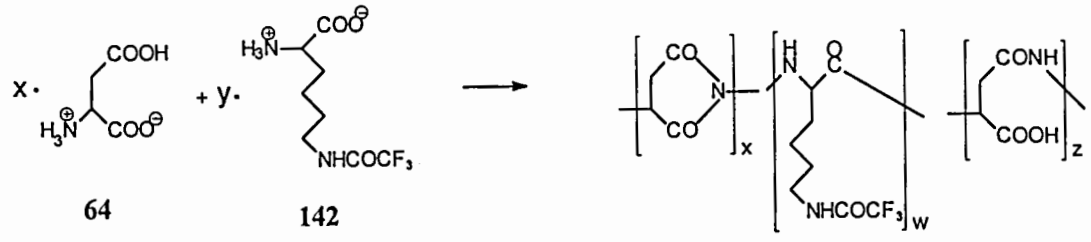
The target co-polymeric drug carrier for this study was co-polymer **143** (scheme 53) with x : y ratio of 2 : 1



64 **142** **143**

Scheme 53 The synthesis of a co-polymer of lysine and aspartic acid

Thermal polymerisation of aspartic acid **64** and N^{ε}-trifluoroacetyl-L-lysine **141** mixed in a molar ratio of 2 : 1 in polyphosphoric acid (PPA) at 180 °C for 2.5 hours at or below 2 torr gave a solid yellow glassy material as crude product. A small portion of this product could be extracted into water, the remainder was soluble in DMF. The water extract was dialyzed in 12000 molecular mass membrane tubing and freeze dried to yield a white polymer in trace amounts. The DMF extract was found to be a co-polymer in which not all the aspartyl moieties were cyclized into succinimide rings. The structure of this polymer, polymer **144** in scheme 54, page 86, is concluded on the basis of ^{1}H-NMR spectroscopy. The monomer ratio's x : w : z is found to be 1 : 0.12 : 0.15. This conclusion was drawn by comparing the ^{1}H NMR peak at the signal at δ5.1-5.4 (methine protons of cyclized aspartic acid – the integral is assigned to represent 1 proton) the signal at δ4.5-4.7 (uncyclized aspartic acid methine protons – the peak accounts for 0.15 protons) and at δ1.0-1.75 (the 6 methylene protons of the lysine residues at the β, γ, δ positions – it accounts only for 0.12 lysine units in the polymer repeating fragments). (see spectrum 13). Thus, only 87% of the aspartic acid monomers in polymer **144** were cyclized. The effective ratio of polymerization between the two monomers was the sum of the aspartic acid groups (1 + 0.15 = 1.15) divided by the average of the six lysine protons (0.69 / 6 = 0.12). Therefore the ratio of aspartic acid to lysine, that is x : y in polymer **143**, scheme 53, is 1.15 : 0.12 = 9.3 : 1 compared to the theoretical expected ratio of 2 : 1. The presence of the protective trifluoroacetyl group was confirmed by infrared spectroscopy. The strong CF_3 band is at 1180 cm^{-1}. ^{13}C-NMR also confirmed it (spectrum 20).



64 142 144

Scheme 54 Co-polymer of lysine and aspartic acid

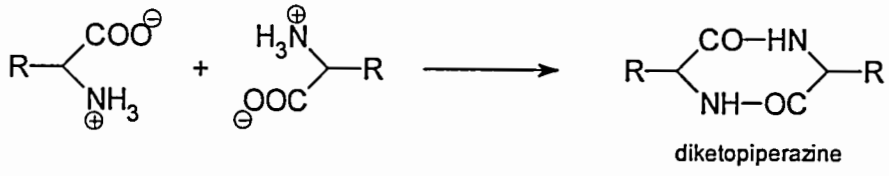
In an attempt to obtain polymer **143** with monomer ratios closer to the theoretically expected, several further experiments were performed. Firstly, the reaction time was increased to 3 hours and in follow-up experiments the temperature was kept at 180^0C for 1½ hours and then increased to 200^0C for a further ½ hour. The result was that these conditions did nothing to affect the ratio's attained, they were both in the order of ~ 10 : 1. Longer reaction times (up to eight hours) resulted in polymers in which the trifluoroacetyl-protecting group was lost. At lower temperatures (140 °C), virtually the entire crude product was soluble in water. The absence of a ^{1}H NMR signal at 5.25 ppm indicated that ring closure of the aspartic acid monomers was not accomplished to a significant extent. Yields were also very poor (6%). Further experiments in which the amount of PPA (or H_3PO_4) also did not significantly change the observed (x + w) : y ratio of polymer **144**. It is concluded that:

(a) On using the thermal polymerization technique, N^{ε}-trifluoroacetyl-L-lysine and aspartic acid have a tendency towards homo-polymerization. This tendency becomes stronger as the reaction conditions become harsher (e.g. higher temperatures, reducing of the pressure above the reacting monomers and longer reaction times). Less harsh reaction conditions decreased the tendency towards homo-polymerization but a lower yield of polymer is obtained.

(b) Ring closure of aspartic acid fragments of the polymer becomes progressively less effective when milder reaction condition are employed.

(c) The trifluoroacetyl protective group is not stable in an acidic environment at elevated temperatures for prolonged periods of time.

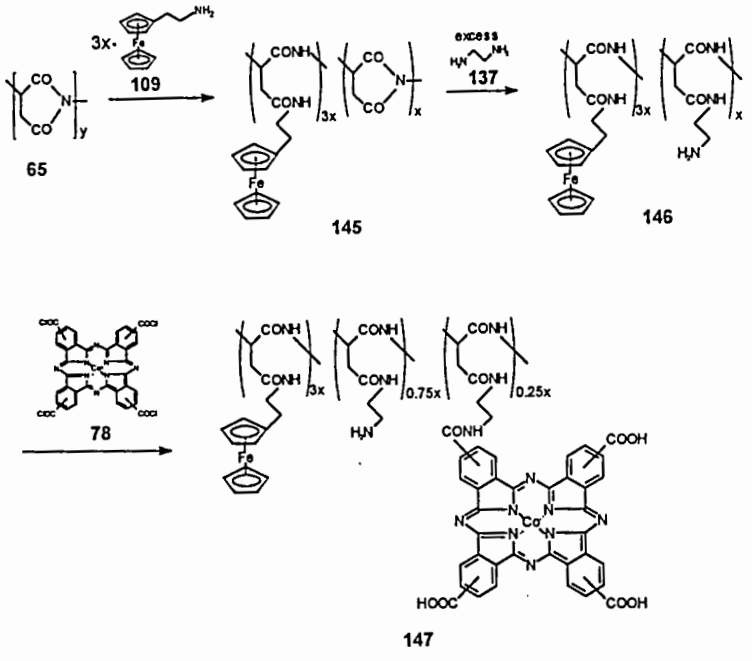
The tendency of α-amino acids to form 6 membered diketopiperazine rings upon heating[227] may also contribute to the obtained results.



Scheme 55 Diketopiperazine formation of α-amino acids

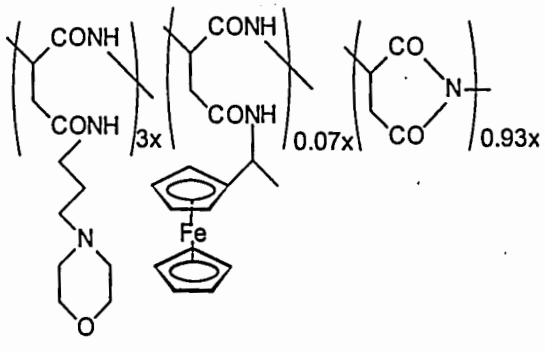
3.5 Drug anchoring onto the polymeric drug carriers

The first attempt, within the goals of this thesis, to synthesise a potential polymeric drug is illustrated by compounds **146** and **147** in scheme 56, page 88. The first part of the synthesis consisted of the anchoring of 2-ferrocenylethylamine **109** directly onto polysuccinimide **65** in a similar way the morpholine group was anchored on **65** in scheme 51, page 82. This meant that polymer **146** would have no hydrophilic moiety attached as a side group to the polymeric backbone. Ethylenediamine **137** was again used to introduce a reactive side chain on polymer **145**. Despite the absence of a water-solubilising group such as 3-aminopropylmorpholine, polymer **146** was still water-soluble. After dialysis and freeze-drying, polymer **146** was obtained in 16% yield, with the side group ratio's of 3 : 1 (ferrocene : amine) as determined by ^{1}H-NMR spectroscopy.



Scheme 56 The anchoring of a ferrocenyl moiety onto the polymer backbone

Polymer **145** is of special interest. It was attempted elsewhere on numerous occasions to synthesise an analogue of **145** (polymer **148** shown in fig. 30)



148

Fig. 30 Previously attempted anchoring of amine **114** onto a polymeric backbone

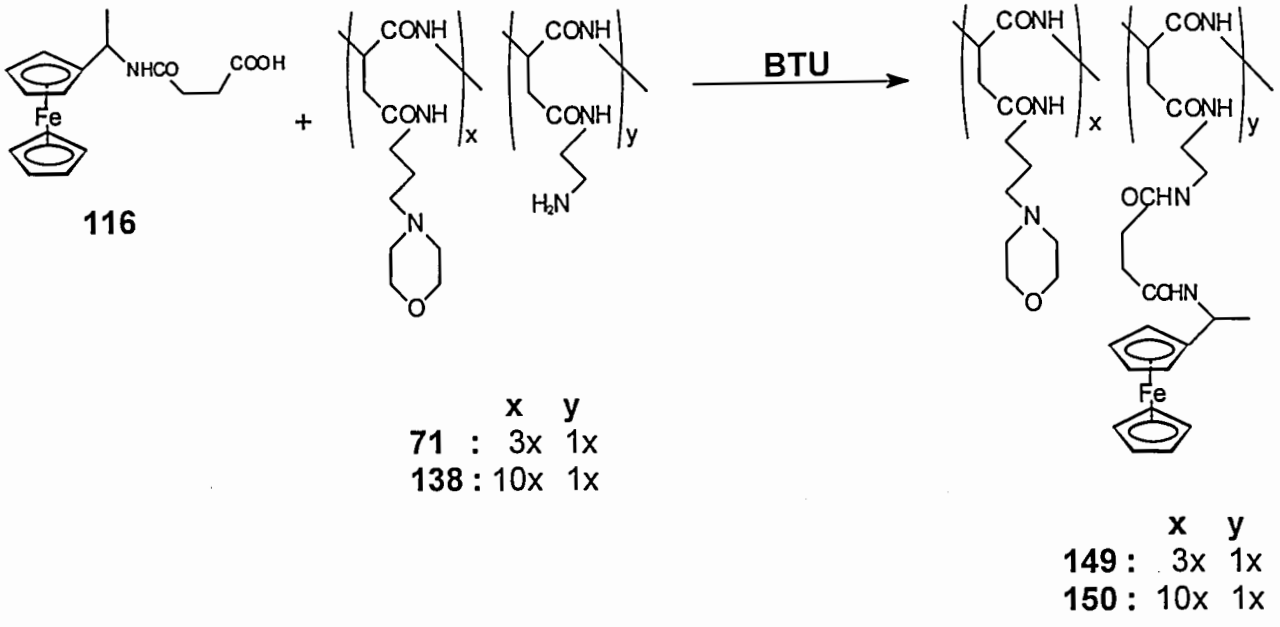
In this case the nucleophile used was not 2-ferrocenylethyl amine **109** but rather 1-ferrocenylethyl amine **114**. The reaction repeatedly failed. At best the success rate of **114** anchoring on polysuccinimide was less than 10%. For **145** (scheme 56, page 88), it was virtually 100%. This indicated that 2-ferrocenylethyl amine **109** is much more nucleophilic than its counterpart 1-ferrocenylethyl amine **114**.

Since polymer **146** was still soluble in water, though to a much lesser extent than the morpholine polymers **71**, **138** and **139**, an attempt was made to anchor cobalt(II)-2,9,16,23-tetracarboxylchloridephthalocyanine **78** onto polymer **146**. The reaction was performed in DMF and was reacted for 20 hours to yield, after dialysis and freeze drying a poorly soluble light green polymer **147**. Approximately 300 ml of water is needed to dissolve 10 mg, therefore, the solubility of **147** is ~0.03g per dm^3. ^{1}H-NMR could not resolve the spectrum at such low concentrations. Cobalt analysis, however, showed that 0.25 equivalents of cobalt was present. Thus, most probably, the structure is as shown in scheme 56, page 88.

Polymer **147** showed that the aqueous solubility of poly(aspartic acid) polymers without the aid of solubilising agents such as 3-aminopropylmorpholine units is not enough to solubilise simultaneously 3 ferrocenyl moieties and a phthalocyanine moiety. However, it cannot entirely be excluded that polymer **147** lost its solubility due to crosslinking of polymer molecules by the tetra functionalized phthalocyanine moiety. This illustrates very elegantly the need for monofunctionalized phthalocyanine carboxylic acids. This then, was addressed in a later stage in the synthesis of various other polymers.

It was previously found, and briefly mentioned in this study, that 1-ferrocenylethyl amine **114**, did not satisfactorily react with polysuccinimide **65**. Polymer **148** (fig. 30, page 88) was found to have

an exceedingly low ferrocenyl content. In contrast, in this research program it was found that the succinoylated derivative **116** can be anchored almost quantitatively to polymers **71** and **138** under the influence of the coupling agent BTU (for structure see scheme 44 page 70) to give polymers **149** and **150** according to scheme 57.



Scheme 57 Synthesis of ferrocene containing water-soluble polymers

The degree of ferrocene-anchoring was again determined by ^{1}H NMR. For instance in the case of polymer **149,** by comparing the integrals of the ^{1}H NMR signals (see fig. 31, page 91) of the methyl group at δ1.2-1.25 (3 protons is therefore associated with the integral), the integral at δ3.5-3.7 (corresponding to 3 x 4 = 12 morpholine protons adjacent to the oxygen atom of the cyclic ether), the integral at δ3.9-4.1 (which corresponds to 9 ferrocenyl protons) and the integral at δ4.3-4.5 (4 aspartyl methine protons) the ratio of x : y = 3 : 1 was confirmed.

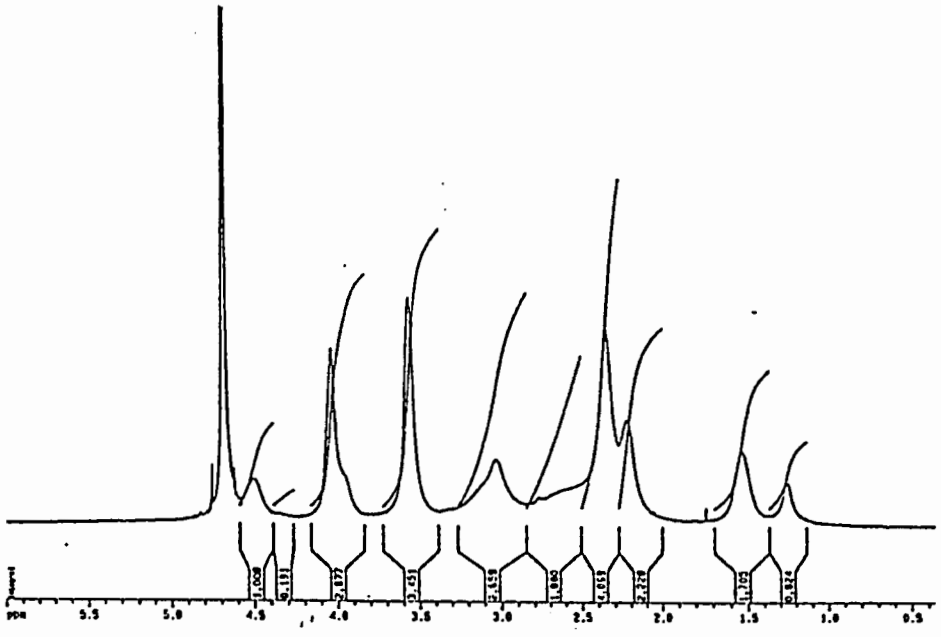
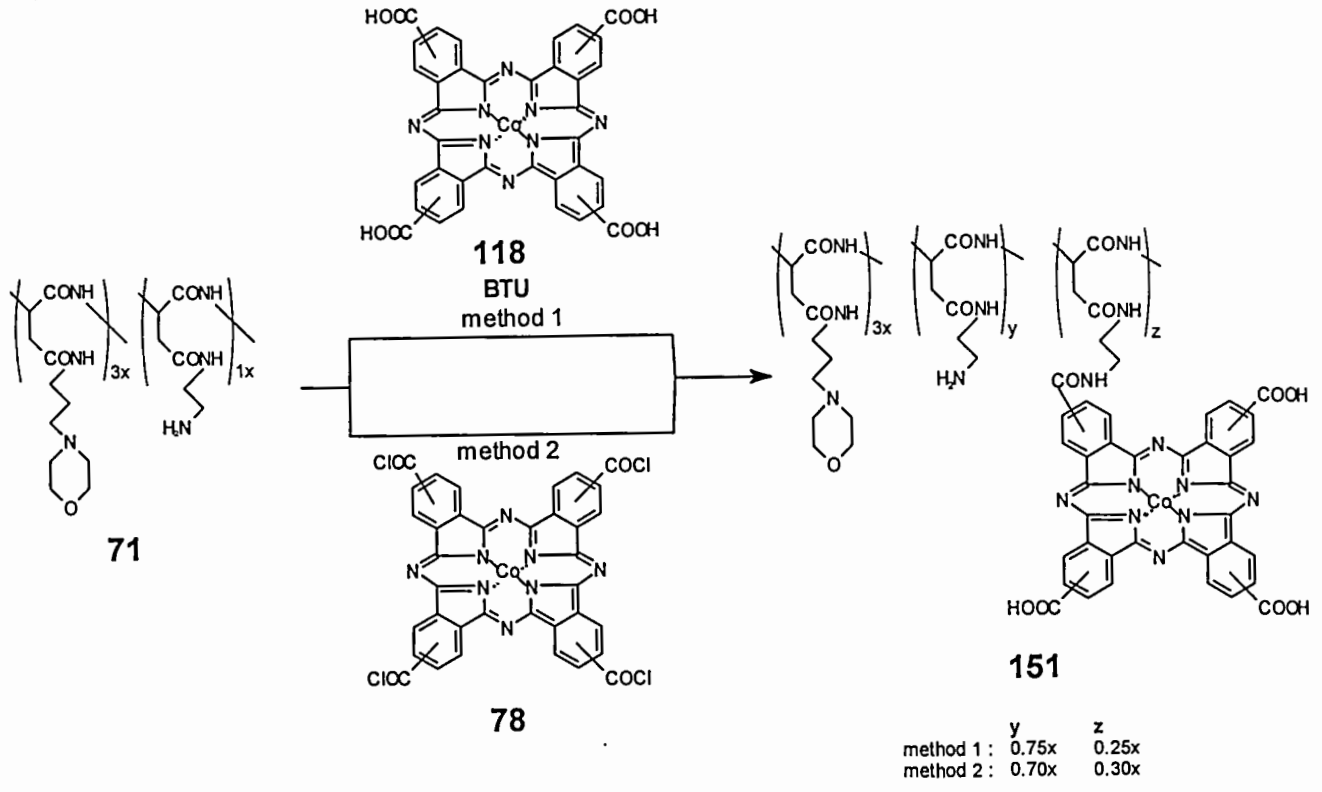


Fig. 31 The ^{1}H-NMR spectrum of polymer **149**

Polymers **149** and **150** were both yellow polymers and obtained in yields of 72% and 70% respectively after dialysis in 12000 molecular mass cut off membrane tubing and freeze drying.

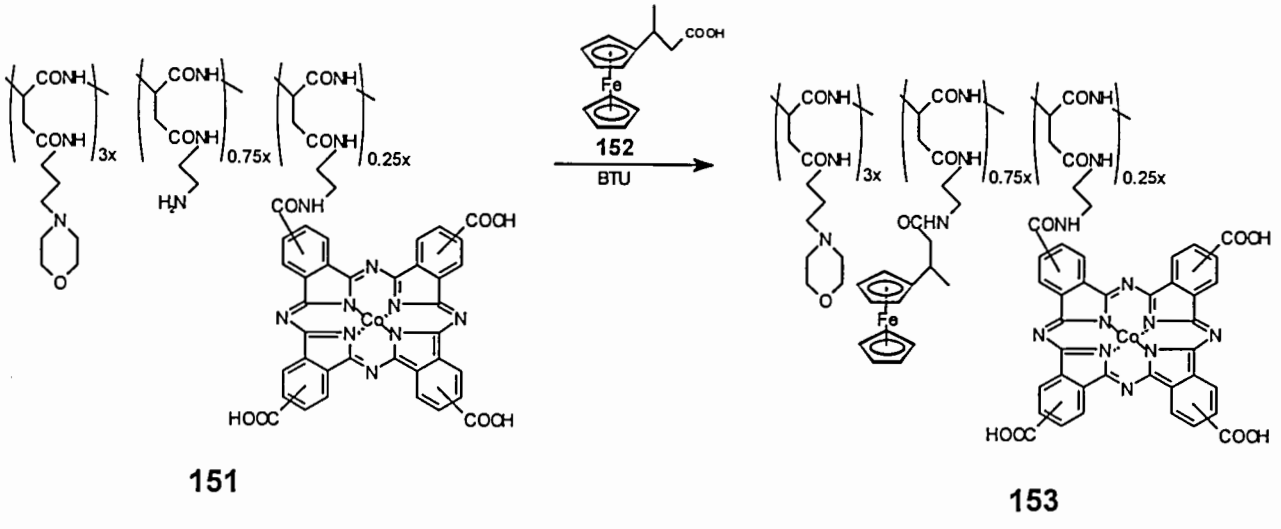
In the first attachment of a phthalocyanine moiety to a polymeric carrier cobalt(II)-2,9,16,23-tetracarboxyphthalocyanine **118** (see footnote page 66) was attached to polymer **71** using the coupling agent BTU according to scheme 58, page 92.



Scheme 58 Coupling of phthalocyanines **78** and **118** to polymer **71**

The polymer obtained was dialyzed in 12000 molecular mass cut off membrane tubing and freeze dried to give a 10% yield of polymer **151**. Using a different method in a second experiment cobalt(II)-2,9,16,23-tetracarboxylchloridephthalocyanine **78** was used to obtain **151** after dialysis and freeze drying in 6% yield. It was apparent in the ^{1}H-NMR spectra that the coupling of the phthalocyanine occurred to a slightly lesser extent (10% less) with the coupling agent BTU than was the case for the acid chloride reaction. The phthalocyanine content of **151** was judged by comparing the aromatic peaks in the ^{1}H-NMR spectrum δ7.3-7.4 (a broadened doublet), δ7.65- 7.70 (broadened doublet) and δ7.78-7.81 (broad singlet), each integrated for 4 protons with the morpholine and methine aspartyl protons as previously described. (see spectrum 15). The lower yield of polymer obtained by the acid chloride route could be ascribed to the possible breakdown of polymer backbone by the acid chloride.

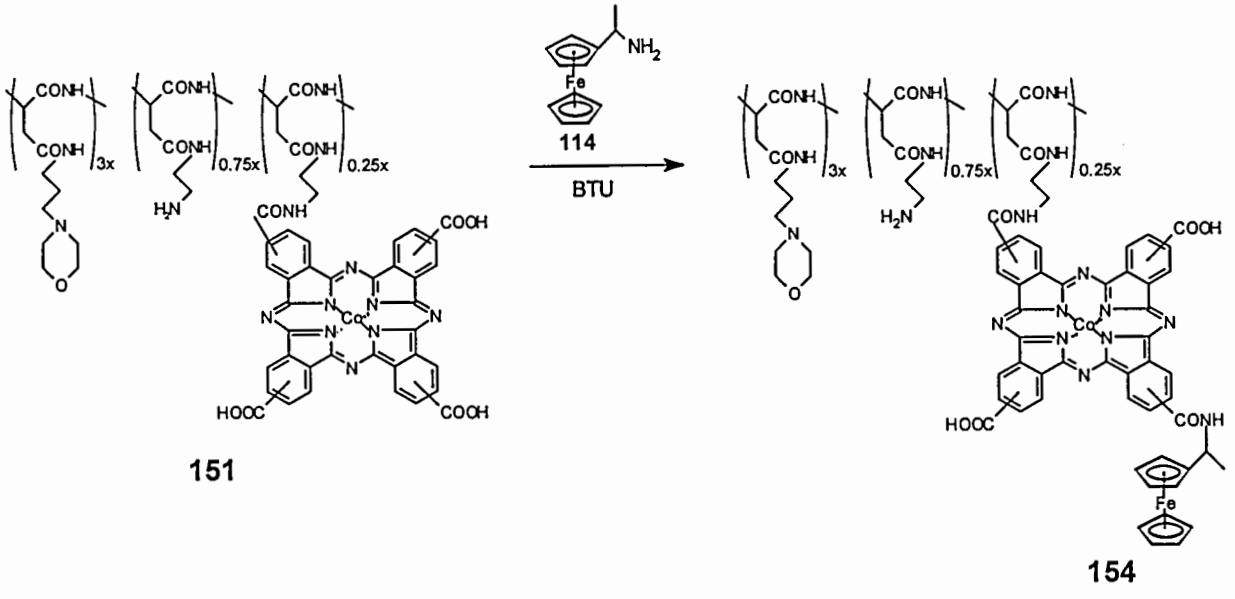
The next reaction that was undertaken was the synthesis of a polymer containing both a phthalocyanine and a ferrocene entity to demonstrate the coupling of two drugs onto the same polymeric carrier with different mechanisms of anti-tumour action. Polymer **151** still had approximately 0.75 equivalents free amine-containing side chains available. Hence **151** was reacted with β-ferrocenylbutanoic acid **152**[228] in the presence of BTU according to scheme 59.



Scheme 59 Synthesis of a polymer with both a ferrocenyl and a phthalocyanyl group anchored onto it.

The methyl protons on **152** is useful as a marker in ^{1}H-NMR analysis. After dialysis and freeze drying the light green polymer **153** was attained in 70% yield and the ^{1}H-NMR spectrum showed that the ferrocene entity was attached four times less than the morpholine group on the polymer. This corresponds to approximately 0.75 equivalents of ferrocenyl units which proved that almost all the amine side groups on **153** had been coupled to a ferrocenyl moiety. It was concluded that steric interference by the polymeric backbone, the phthalocyanine moiety and the morpholine units did not noticeably inhibit ferrocene coupling. The water-solubility of polymer **153** was still satisfactory, especially when compared to the previously prepared polymer **147**

Earlier in this study 2,9,16,23-cobalt(II)tetraamidoferrocenylphthalocyanine **133** a ferrocene-phthalocyanine conjugate described in scheme 48, page 76, was prepared but the characterisation could not include ^{1}H-NMR spectroscopy due to solubility problems. This problem can now be addressed. Consequently an excess of 1-ferrocenylethylamine **114** was reacted with polymer **151** in the presence of BTU according to scheme 60.

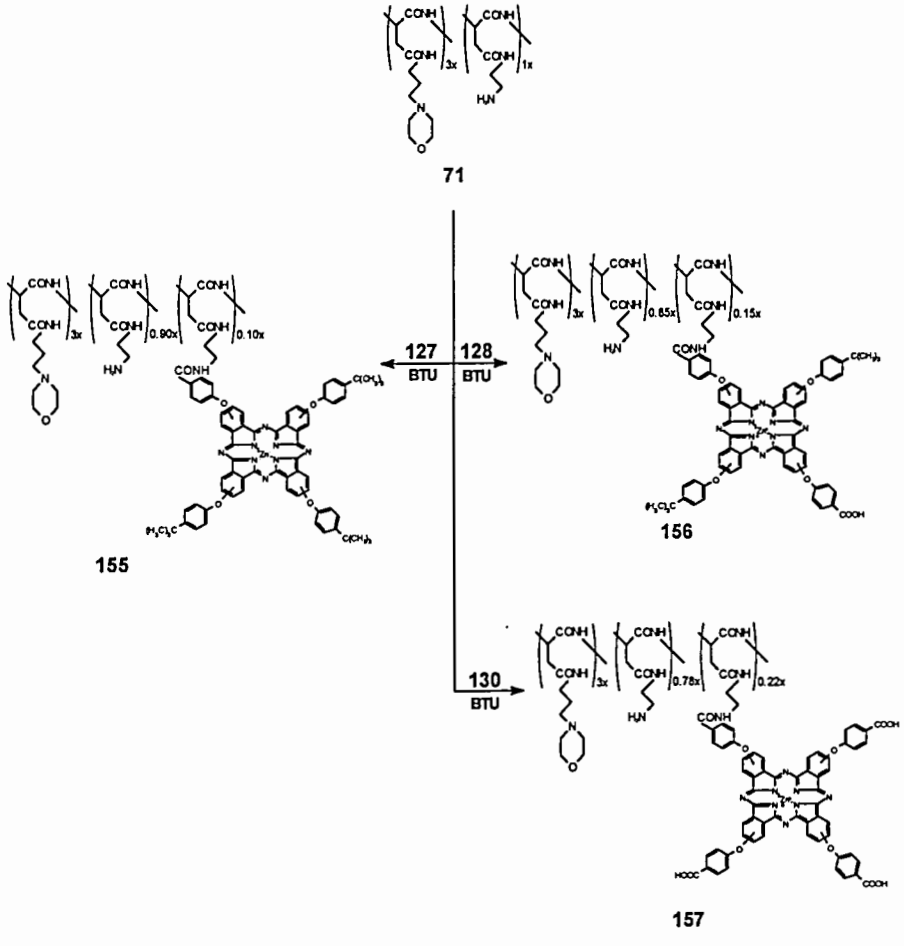


Scheme 60 Synthesis of a ferrocene-phthalocyanine conjugate on a polymeric carrier

After dialysis in 12000 molecular mass cut off molecular tubing and freeze-drying light green polymer **154** was obtained in 68% yield. It was hoped that **114** would bind to all three available carboxylic acid groups on the phthalocyanine portion of polymer **154**. However, the ^{1}H-NMR spectrum indicated that, by average, only one ferrocene unit had been attached on each phthalocyanine unit. Any of the three available carboxylic acid groups on the phthalocyanyl group could react with **114**, but for steric reasons the indicated position is favoured. It is improbable that the reason for the two unreacted carboxylic acid groups in **154** is deactivation of the phthalocyanines carboxylic acid groups. A more feasible explanation would be that the bulky ferrocenyl groups just could not make a steric fit to facilitate binding to the remaining two

carboxylic acid functions. Perhaps this problem will be overcome if the phthalocyanine is anchored onto a longer polymer side chain than is now the case. What was, however, achieved in this experiment was the demonstration of the possible attachment of the ferrocenyl group *via* an amide bond to the phthalocyanine. Further studies on this type of multiple coupled polymers may prove to be most interesting and awarding, but is the topic of a further research program.

The phthalocyanines prepared in the statistical condensation route (see scheme 45, page 73) were the next to be used in coupling reactions. Phthalocyanines **127**, **128** and **130** were coupled with polymer **71** under the influence of BTU according to scheme 61. Yields of **155-157** after dialysis and freeze drying were consistently low ~ 10% (see experimental).



Scheme 61 Polymers **155 - 157** with phthalocyanines prepared in the statistical condensation method

The ^{1}H-NMR spectra of the phthalocyanines led to interesting results in that the amount of COOH groups on the phthalocyanines **127**, **128** and **130** actually had a direct effect on the substitution ratio's that were obtained. The results are summarised in table 8.

Table 8 Correlation between COOH groups and substitution ratio's

Polymer	Number of COOH substituents in monomer	Equivalents of amine substituted
155	1	0.10
156	2	0.15
157	4	0.22

The results in table 8 show that the success of coupling is controlled statistically as the number of COOH groups on the monomeric phthalocyanine directly determine the success of the coupling. Utilizing the same reaction conditions thus leads to coupling patterns as in table 8.

3.6 Electrochemistry of selected compounds

The final aim of this study was to investigate some of the electron transfer properties of selected phthalocyanine derivatives by means of cyclic voltammetry. In addition to this, ferrocenoic acid **101** which had previously shown[229] abnormal behaviour in aqueous solutions, was investigated together with its acid chloride **112** and amide **126** in acetonitrile. Finally, the aqueous electrochemistry of selected water-soluble polymers prepared was also studied.

3.6.1 Cyclic voltammetry of phthalocyanine derivatives

3.6.1.1 Ligand redox processes of phthalocyanines

The zinc phthalocyanines **127** and **130** (for structures see scheme 45, page 73) were selected for the cyclic voltammetric experiments in which the ligand redox processes could be studied in an organic

solvent medium (DMF). The phthalocyanines were prepared in *ca.* 1mM argon purged solutions containing 0.1M $TBAPF_6$ as supporting electrolyte utilising a platinum wire auxiliary electrode with a platinum working electrode and a Ag/Ag^+ non-aqueous reference electrode. The CV curve of phthalocyanine **127** is shown in fig. 32.

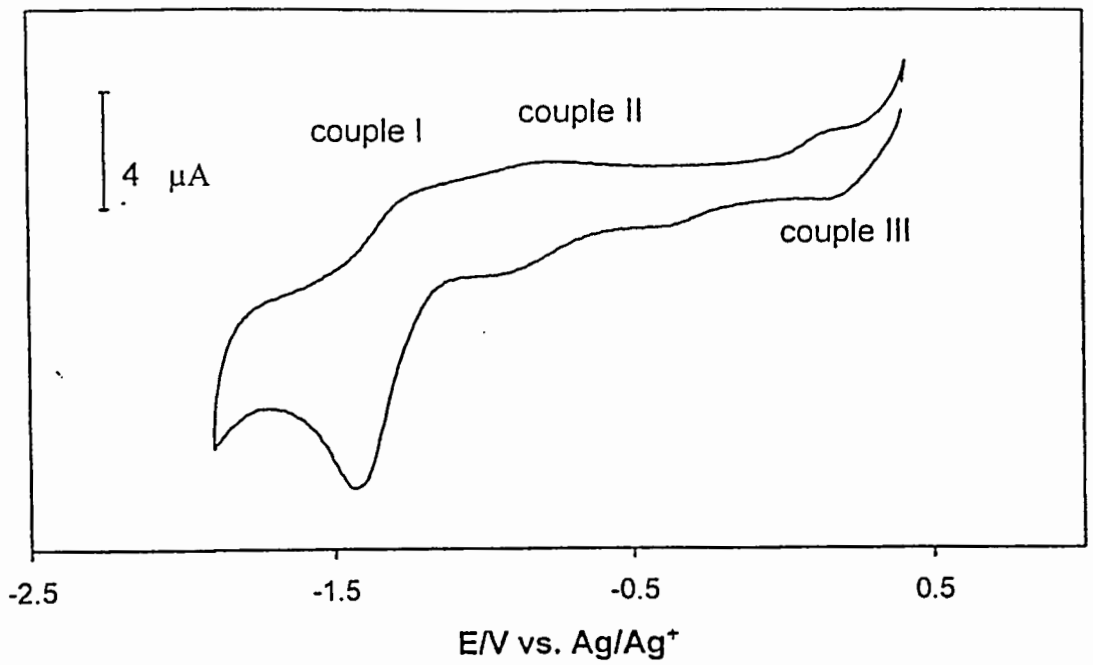


Fig. 32 CV curve of **127** in DMF. Scan rate 150 mV s^{-1}

Fig. 32 represents the typical response observed for phthalocyanines. In the case of phthalocyanine **127** (for structure see scheme 45 page 73) at 150 mV s^{-1} two quasi-reversible reduction couples and one quasi-reversible oxidation couple (couples I, II and III) are observed at $E^{0'}$ = -1.34V and -0.87V as well as the third couple at 0.17V vs Ag/Ag^+. These values compare well to previously found[230,231] ring redox couples of zinc phthalocyanines thus the assignments are as follows $[ZnOPc(3-)]^-$ / $[ZnOPc(4-)]^{2-}$ for couple I, [ZnOPc(2-)] / $[ZnOPc(3-)]^-$ for couple II and $[ZnOPc(1-)]^+$ / [ZnOPc(2-)] for couple III as indicated in fig. 32. The ΔE_p values for the three couples of phthalocyanine **127** are summarised in table 9, page 98.

Table 9 ΔE_p and $E^{0'}$ values obtained for phthalocyanine **127**

Scan rate *V*/m v s^{-1}	Couple I				Couple II				Couple III			
	E_{pa}/V	E_{pc}/V	ΔE_p/ mV	$E^{0'}$/ V	E_{pa}/V	E_{pc}/V	ΔE_p/ mV	$E^{0'}$/ V	E_{pa}/V	E_{pc}/V	ΔE_p/ mV	$E^{0'}$/ mV
50	-1.228	-1.421	193	-1.33	-0.803	-0.897	94	-0.85	0.195	0.135	60	165
100	-1.236	-1.428	192	-1.33	-0.761	-0.965	204	-0.86	0.204	0.148	56	176
150	-1.253	-1.45	197	-1.35	-0.764	-0.97	206	-0.87	0.213	0.148	65	181
200	-1.183	-1.369	186	-1.28	-0.791	-1.016	225	-0.90	0.198	0.144	54	171
250	-1.176	-1.401	225	-1.29	-0.816	-1.022	206	-0.92	0.19	0.141	49	166
300	-	-	-	-	-0.767	-0.965	198	-0.87	0.199	0.129	71	164

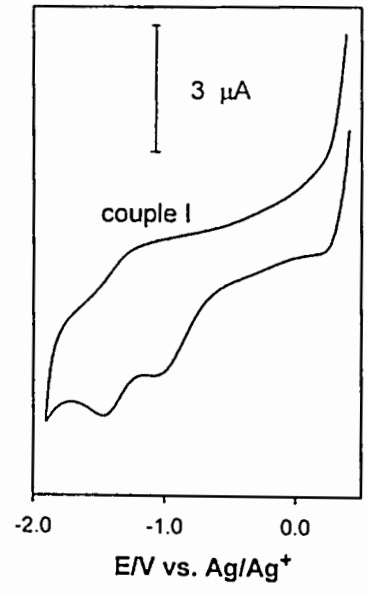


Fig. 33 CV curve of **130** in DMF. Scan rate 150 mV s^{-1}

Phthalocyanine **130** (for structure see scheme 45 page 73) displayed a different CV curve to that observed for **127.** Only the reduction of couple I (fig. 33) at a scan rate of 150 mV s^{-1} ($E^{0'}$ = -1.28V vs. Ag/Ag^+) was observed corresponding to the quasi-reversible $[ZnOPc(3-)]^-$ / $[ZnOPc(4-)]^{2-}$ couple. The anodic sweep did not show an oxidation corresponding to the reduction at E = -1.04V vs. Ag/Ag^+. In addition, no ligand oxidation or reduction was observed in the same region of couple III in the CV curve of phthalocyanine **127** (fig. 32). The ΔE_p and $E^{0'}$ values of couple I in fig. 33 are summarised in table 10.

Table 10 ΔE_p and $E^{0'}$ values obtained for phthalocyanine **130**

scan rate v/mV s^{-1}	E_{pa}/V	E_{pc}/V	ΔE_p/mV	$E^{0'}$/V
150	-1.201	-1.411	210	-1.31
200	-1.194	-1.441	247	-1.32
250	-1.187	-1.477	290	-1.33
300	-1.212	-1.507	295	-1.36

For a compound to undergo a one-electron diffusion-controlled, electrochemically reversible process it should have a ΔE_p value of approximately 59 mV[232]. Deviations larger than 59 mV point toward electrochemical irreversibility, which may be caused by slow electron exchange of the redox species with the working electrode. Thus it was interesting to note that phthalocyanine **127** shows both reversible (couple III, fig. 32) and essentially non-reversible (couples I and II, fig. 32) behaviour of the ligand in its different oxidation states, indicating that electron exchange is favoured by, in this case, a positively charged species.

3.6.1.2 Aqueous electrochemistry of phthalocyanines 118 and 130

The electrode system used for aqueous cyclic voltammetric measurements consisted of a platinum wire auxiliary electrode, a platinum wire working electrode and a Ag/AgCl reference electrode. The supporting electrolyte was always 1M KCl for aqueous measurements and the two phthalocyanines **118** (for structure see scheme 41 page 66) and **130** (structure in scheme 45, page 73) were studied in argon purged aqueous medium as their sodium salts at concentrations of *ca* 1mM. The pH of both solutions was *ca.* 9.5. The cyclic voltammograms are shown in fig. 34. (a) for **118** and (b) for **130**.

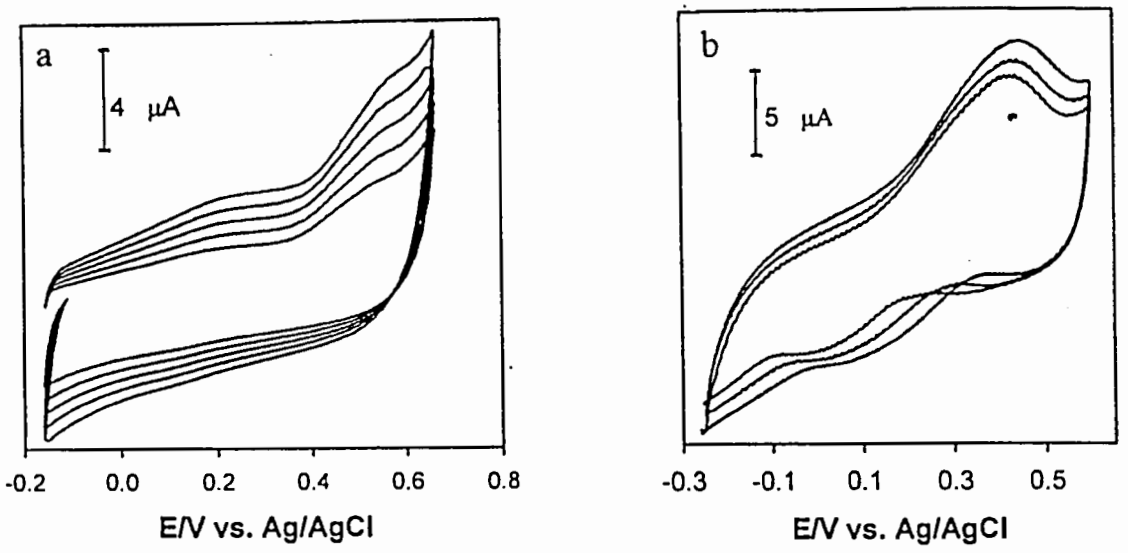


Fig. 34 CV curves for phthalocyanines **118** [voltammogram (a)] and **130** [voltammogram (b)]

Both the phthalocyanines underwent what appeared to be an irreversible oxidation peak at 0.54 V for **118** and 0.44 V for **130** vs Ag/AgCl and thus based on findings by Lever[230] they were assigned to the ligand redox process $[MPc(1-)]^{+}$ / [MPc(2-)] for each respective phthalocyanine. Furthermore, the CV curve for **118** showed an oxidation peak at *ca.* 0.25 V vs Ag/AgCl that compares well with the values previously found by Kobayashi et. al.[231] for a $Co^{III}Pc(-2)$ / $Co^{II}Pc(-2)$ process. The irreversibility of the ligand redox couple in the studied voltage range probably has a direct influence on the inability of the cobalt nucleus to be reduced in the range studied. The reason for both the water-soluble phthalocyanines not showing reductions in the cathodic sweep is unclear at this stage.

3.6.2 Cyclic voltammetry of ferrocenyl derivatives 101, 112 and 126

The cyclic voltammetric study carried out on the ferrocenyl derivatives (for structures see scheme 44 page 70) was done at 25^0C in argon purged acetonitrile. All analytes were prepared in concentrations of *ca.* 1 mM and contained 0.1M $TBAPF_6$ as supporting electrolyte. The electrode system used was a platinum wire auxiliary electrode with a platinum working electrode and a Ag/Ag^+ non-aqueous reference electrode. Scan rates varied over the range 50-250 mV s^{-1}. The anodic (E_{pa}) and cathodic (E_{pc}) peak potentials (referenced against Ag / Ag^+) as well as their differences (ΔE_p), ratio of anodic and cathodic peak currents (i_{pa} / i_{pc}) determined by the method of Nicholson[233] and formal potentials ($E^{0'}$) of the ferrocenyl derivatives are given in table 11 page 102.

The average ΔE_p values of **112** and **126** were found to be within reasonable range of the theoretical value (74 mV and 88 mV vs. Ag / Ag^+ respectively). Ferrocenoic acid **101** was found to be 159 mV, which is a large deviation from the theoretical value. The reason for this deviation is not clear. A previous study[229], however, found that ferrocenoic acid **101** in an ethanol/water mixture had an average ΔE_p value of 118 mV and the sodium salt of the carboxylic acid **101** in pure water had an average ΔE_p value of 65 mV. Thus, besides the solvent medium playing a pivotal role in the ΔE_p value it appears as if ferrocenyl carboxylate salts have a faster electron exchange reaction with the working electrode in aqueous medium. The low i_{pa} / i_{pc} values for all three ferrrocenyl products are not clear at this stage and further studies are needed to clarify this behaviour.

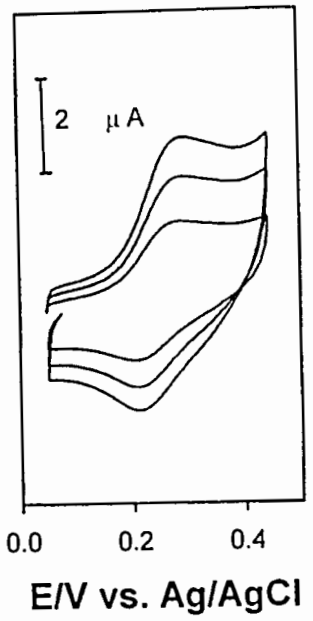


Fig. 36 CV curve of polymer **149** shown for scan rates 50, 150 and 250 mV s^{-1}

The curve displays typical ferrocene conjugate behaviour, the broadened peaks being due to the polymeric nature of the compound. Some electrochemical data obtained from the spectrum are shown in table 12.

Table 12 Electrochemical data obtained for polymer 149

v / mV s^{-1}	E_{pa} / mV	E_{pc} / mV	ΔE_p / mV	i_{pa} / i_{pc}	$E^{0'}$ / mV
50	286	206	80	0.85	246
100	295	208	87	0.86	251
150	294	211	83	0.87	252
200	293	210	83	0.88	252
250	296	214	81	0.89	255
average			***83***	***0.87***	***251***

Firstly, from table 12 it can be assumed that the redox couple was essentially a one electron process as the ΔE_p value is comparable to the theoretical value of 59 mV. The i_{pa} / i_{pc} values of this compound were slightly lower than unity which could be explained in terms of possible amine side chain remnants in the polymer interacting chemically with the formed ferrocinium ion as has been found by Swarts[229].

Two water-soluble polymers **151** (structure in scheme 58, page 92) and **155** (structure in scheme 61 page 95) containing phthalocyanine entities were also studied by cyclic voltammetry and displayed very much the same type of behaviour as their related monomeric entities in aqueous medium. The pH of the polymeric solutions were *ca.* 7.1 and the solutions in which the monomeric sodium salts were studied *ca.* 9.5. Polymer **151** showed in its CV curve a redox couple with an average ΔE_p value of 213 mV at an average formal potential of 217 mV (table 13) corresponding to the $Co^{III}Pc(-2)$ / $Co^{II}Pc(-2)$ couple, as was seen in the monomeric phthalocyanine **118** (see paragraph 3.6.1.2, page 99). Some ΔE_p and $E^{0'}$ values are shown for polymer **151** (table 13). Polymer **155** displayed an irreversible oxidation at 40 mV vs. Ag/AgCl, which correlates well with the previously found $[MPc(1-)]^+$ / [MPc(2-)] process described for the monomeric zinc phthalocyanine **130** (see paragraph 3.6.1.2 page 99).

Table 13 ΔE_p and $E^{0'}$ values for polymer **151**

scan rate v / mv s^{-1}	ΔE_p / mV	$E^{0'}$ / mV
150	220	240
200	220	200
250	200	210
average	*213*	*217*

Chapter 4

Conclusions and future perspectives

Cobalt and zinc phthalocyanines tetrafunctionalized with carboxy and amine peripheral groups were easily prepared using standard procedures. These products had low solubility, but could still be utilized for further amide bonding. Soluble zinc phthalocyanines were prepared from carboxylic acid and *tert*-butyl peripherally substituted phenoxy phthalonitriles. These phthalonitriles were also used to prepare a carboxylic acid "monofunctionalized" phthalocyanine that was eventually useful in the prevention of polymer crosslinking. An important aspect for the preparation of the "monofunctionalized" phthalocyanine was the route by which it was obtained, two different methods were used to do this namely the statistical condensation route and the so called subphthalocyanine route. The subphthalocyanine route, which utilised boron trichloride, led to ring chlorinated phthalocyanines and these were not considered appropriate as the separation of the ring chlorinated products was not possible. The ring chlorination could possibly be prevented in future attempted reactions by replacing boron trichloride with boron tribromide. In terms of the photodynamic therapy application many possiblilities still exist for varying the structure of synthesised phthalocyanines such as replacing zinc with aluminium and gallium.

The cobalt phthalocyanines that were tetrafunctionalized with carboxylic acid and amine groups were reacted with a ferrocenyl amine and a ferrocenyl carboxylic acid respectively to yield insoluble compounds that could only be characterised by means of infrared spectroscopy and cobalt analysis. It could not be determined if, in fact, these compounds were tetrafunctionalized or if they consisted of mixtures of differently substituted compounds. The solubility of these compounds could be improved in further investigations by incorporating instead of amide groups, other functionalities such as ether, ester or even longer aliphatic linkages.

Two polymers were synthesised in the coarse of this study, firstly polysuccinimide in which the aspartic acid is cyclized only by using appropriately harsh reaction conditions. A co-polymer of aspartic acid and lysine was also prepared, but due to apparent homopolymerization a ratio of aspartic acid : lysine of *ca* 10 : 1 was consistently obtained even when monomer ratio's of aspartic acid : lysine of 2 : 1 were employed under different reaction conditions. The polysuccinimide was

selected for further investigation and as such was co-polymerized by ring opening reactions with both N-(3-aminopropyl)-morpholine and ethylenediamine. In these polyaspartamides the morpholine enforces water-solubility and the resultant free amine of the ethylenediamine is available for further drug anchoring. Three polyaspartamides were prepared with ratio's of morpholine to the amine of 15 : 1, 10 : 1 and 3 : 1 to determine the effect on polymer solubility. The polymer with a ratio of 3 : 1 was found to possess a high level of water-solubility and was thus used in subsequent drug anchoring reactions.

The work done in this study also conclusively demonstrated the ability of water-soluble polyaspartamides to retain their water-solubility upon attachment of phthalocyanine moieties. However, further anchoring of ferrocenyl fragments onto the polymeric backbone already containing phthalocyanines or directly onto a polymer-bound phthalocyanine drastically reduced their water-solubility. This behaviour was found for all polymers with attached phthalocyanines. On average ony 15% of the available amine side chains were found to have reacted with the phthalocyanines. This problem may be overcome in future studies by increasing the length of the spacer (aliphatic chain) between the polymeric backbone and the phthalocyanine drug. The polymers containing the phthalocyanine drugs are not stable entities but are found to degrade with subsequent reactions and upon standing in light, thus besides storing the polymers in the dark and in an oxygen-free environment reactions should be conducted in oxygen-free media in the dark. Additionally the polymers with a higher morpholine content may be employed to ascertain whether the water-solubility could be improved upon drug anchoring. The further attachment of a superphthalocyanine molecule in addition to a phthalocyanine onto the polymeric drug carrier may even improve selectivity for cancerous cells.

The electrochemistry of selected phthalocyanines was done in acetonitrile and water. Reversible and non-reversible ligand redox processes, as well as a cobalt redox process were identified in the cyclic voltammograms obtained. Selected polymer bound phthalocyanines were also studied by cyclic voltammetry in water and were found to display similar curves to their monomeric equivalents in water. In addition to the phthalocyanines, three ferrocenyl compounds, substituted

with groups that have differing electron withdrawing capacities were studied by cyclic voltammetry. The results obtained showed that a linear correlation exists between the formal potentials obtained and the electron withdrawing ability (determined by ^{1}H-NMR) of the groups attached to the ferrocene unit. An interesting study could be an extensive electrochemical study of different electron withdrawing groups on ferrocene units. A study on the effect of direct conjugation of different electron withdrawing groups on metallated phthalocyanines may also lead to similar results.

Chapter 5

Experimental

Equipment and Chemicals

Organic solvents used were distilled prior to use. Water was double distilled. Chemicals were from Merck, Aldrich, Sigma, Fluka and Strem Chemicals and were used without further purification, unless otherwise stated. Melting points were uncorrected. NMR spectra were obtained on Bruker Advance DPX 300 instrument. Chemical shifts are presented as δ-values (ppm) relative to the solvent peak. IR spectra (cm^{-1}) were recorded on a Hitachi spectrophotometer model 270-50 with data processor; for solid state spectra a KBr matrix was used, thin films were prepared between NaCl plates. Viscosity measurements were made in a Cannon-Fenske tube at 35,2°C in water, unless otherwise stated. Dialyses were performed in 12 000 molecular mass cut-off cellulose membrane tubing. Electrochemical measurements were made with a computer controlled BAS voltammograph, model CV-27 using a Ag/Ag^+ (acetonitrile, 0.01M $AgNO_3$, 0.1M $TBAPF_6$) reference electrode, a platinum auxiliary electrode and a platinum working electrode at 25 °C for DMF and acetonitrile solutions, the supporting electrolyte was always 0.1M TBAHFB for the organic solvents. Both organic solvents were argon purged prior to use. Aqueous measurements were done with a platinum wire auxiliary electrode, a platinum wire working electrode and a Ag/AgCl (3M KCl) reference electrode. The supporting electrolyte was always 1M KCl and the water was argon purged prior to use. The analytes in aqueous and non-aqueous solutions were prepared in concentrations of 1mM except for polymer **155,** which was studied at a concentration of 0.1 mM.

5.1 Synthesis of ferrocenyl compounds

5.1.1 (2-Chlorobenzoyl)ferrocene[213] (111) [scheme 38 page 64]

To a solution of ferrocene **97** (18.6 g, 0.1 mol) in dichloromethane (200 cm^3) is added 2-chlorobenzoylchloride **110** (17.5g, 0.1 mol) and this mixture is cooled in an ice-bath while under nitrogen atmosphere. Anhydrous $AlCl_3$ (14.0 g, 0.1 mol) is added over 20 min. A dark blue

solution results almost instantaneously and the stirring is continued for 30 min. in the ice-bath and a further 3 hrs. at room temperature. The blue solution is cooled in an ice-bath and water (200 cm^3) is added slowly, the resultant two-phase mixture is stirred vigorously for a further 30 minutes. The layers were separated and the aqueous layer extracted with dichloromethane (3 x 30 cm^3). The combined dichloromethane solutions are washed with water (50 cm^3), 10% NaOH (2 x 50 cm^3) and then dried over magnesium sulphate. Filtration and evaporation to dryness at reduced pressure yields a viscous red liquid which gradually solidifies to give the dark red (2-chlorobenzoyl)ferrocene **111** (27.1g, 85%); mp. 94-97^{0}C. δ_H($CDCl_3$, spectrum 1) 7.30-7.55 (4H, m, phenyl-H), 4.76 (2H, t, C_5H_4), 4.62 (2H, t, C_5H_4), 4.29 (5H, s, C_5H_5). ν_{max}/cm^{-1} (KBr) 1644 (s) (C=O)

5.1.2 Ferrocenoic acid[213] (101) [scheme 38 page 64]

To a mixture of potassium *tert*-butoxide* (3.6 g, 0.03 mol) in THF (20 cm^3) and water (0.3 cm^3) in a nitrogen atmosphere is added (2-chlorobenzoyl)ferrocene **111** and the resultant slurry is refluxed for one hour upon which a tan coloured mixture develops. After cooling and adding to 1 litre of water the ferrocenoic acid **101** is extracted with ether (3 x 15 cm^3) washed with a 10% NaOH solution thus forcing the sodium salt into the aqueous phase and washed with ether. The basic solution is then acidified to pH2 with hydrochloric acid to reprecipitate the desired product. After filtration and redissolving in ether, the ether solution is dried with magnesium sulphate. Filtration and solvent removal under reduced pressure gives acid **101** (4.1 g, 55%); mp. 210-212^{0}C (decomp.). δ_H($CDCl_3$) 4.88 (2H, t, C_5H_4), 4.48 (2H, t, C_5H_4), 4.29 (5H, s, C_5H_5). ν_{max}/cm^{-1} (KBr) 2950 (b) (O-H str.), 1659 (s) (C=O).

* The potassium *tert*-butoxide is prepared by adding *tert*-butanol (8.2g, 0.11 mol) over one hour under nitrogen atmosphere to potassium metal (4.6 g, 0.12 mol) in dry THF. The mixture is heated to 40^{0}C and reacted overnight. THF (20 cm^3) is added and the residual potassium removed to yield the white butoxide.

5.1.3 Ferrocenoyl chloride[159] (112) [scheme 38 page 64]

To ferrocenoic acid **101** (1g, 4.39 mmol) dissolved in dry dichloromethane (20 cm^3) was added oxalyl chloride (2 cm^3, 23 mmol) and a drop of pyridine, the mixture was stirred for 10 hrs at 25^0C and then refluxed at 45^0C for 8 hrs. The red solution was then recrystallized after concentration from the 65^0C-72^0C fraction of petroleum ether to yield wine red needles of the ferrocenoyl chloride **112** (0.65 g, 59%); $\delta_H(CDCl_3$, spectrum 2) 4.91 (2-H, t, C_5H_4), 4.65 (2-H, t, C_5H_4), 4.32 (5-H, s, C_5H_5), ν_{max}/cm^{-1} (KBr) 1758 (s) (C=O str.).

5.1.4 N, N-dimethylaminomethylferrocene methiodide[201] (102) [scheme 39 page 64]

Methyl iodide (10 g, 0.07 mol) was added to N, N-dimethylaminomethylferrocene **113** (12.15 g, 0.05 mol) while stirring, the solution was heated to 100^0C for 5 minutes and then cooled to room temperature after which ether (50 cm^3) is added. The methiodide **102** crystallizes after a few minutes and is filtered on a büchner funnel, washed with ether and dried under reduced pressure at room temperature to yield **102** (16.09 g, 80%); mp. 200-203^0C (degrad.). $\delta_H(CDCl_3)$ 4.78 (2H, t, C_5H_4), 4.40 (2H, t, C_5H_4), 4.22 (5H, s, C_5H_5), 1.21 (9-H, m, $-(CH_3)_3$)

5.1.5 Ferrocenylacetonitrile[202] (99) [scheme 39 page 64]

N,N dimethylferrocenemethiodide **102** (20.11 g, 0.05 mol) and NaCN (20.24 g, 0.41 mol) were suspended in water (200 cm^3) and refluxed for 2h at 105^0C. The ferrocenylacetonitrile **99** was extracted with ether (3 x 100 cm^3), the ethereal layer washed with water (3 x 100 cm^3) and dried with $MgSO_4$ to afford, after solvent removal under reduced pressure and recrystallization from hexane, the acetonitrile **99** as yellow crystals (4.74 g, 42 0%); mp. 77-80^0C. $\delta_H(CDCl_3)$ 4.1-4.3 (9H, m, C_5H_5 and C_5H_4), 3.35 (2H, d, CH_2). ν_{max}/cm^{-1} (KBr) 2228 (s) (C≡N)

5.1.6 2-(Ferrocenyl)ethylamine[202] (101) [scheme 39 page 64]

A solution of ferrocenylacetonitrile **99** (1.1 g, 4.9 mmol) was added dropwise over 2 minutes to $LiAlH_4$ (0.42 g, 11.1 mmol) in dry ether (50 cm^3). The mixture was stirred at room temperature overnight before moist ether (100 cm^3) was added to destroy excess hydride. The ethereal solution was washed twice with water (150 cm^3), dried with $MgSO_4$ and evaporated off under reduced pressure. The residual oil was distilled at 110^0C to 115^0C at 0.5 torr yielding 2-(ferrocenyl)ethylamine **101** (0.55 g, 50%); bp. $116\text{-}118^0C$. $\delta_H(CDCl_3)$ 4.3 –4.6 (4-H, broad s, NH_2 and C_5H_4), 4.25 (2-H, t, C_5H_4), 4.09 (5-H, s, C_5H_5), 2.95 (2-H, t, CH_2), 2.65 (2-H, t, CH_2). ν_{max}/cm^{-1} (NaCl disks) 3372 (b) (N-H str.), 1646 (m) (N-H def.)

5.2 Preparation of reagents for phthalocyanine synthesis

5.2.1 4-Nitrophthalimide[234] [scheme 43 page 70]

Fuming nitric acid* (30 cm^3) is slowly added to concentrated sulphuric acid (180 cm^3) and the mixture is cooled in an ice bath. When the temperature of the mixed acids reaches 12^0C - phthalimide (50.0 g, 0.3 mol) is stirred in as quickly as possible while the temperature is maintained between 10^0C and 15^0C using an ice-bath. The solution is then allowed to reach room temperature and left to stand overnight. The yellow solution obtained is poured on ice (1.1 Kg) while rapidly stirring the solution to yield a beige suspension, which is removed by filtration under reduced pressure. The beige solid is washed with ice-water (6 x 150 cm^3) to yield the 4-nitrophthalimide (25.9 g, 40.0%); mp. $197\text{-}200^0C$. $\delta_H(CDCl_3)$ 8.72 (1H, d, Ar-H), 8.68 (1H, dd, Ar-H), 8.09 (1H, d, Ar-H), 7.96 (1H, s, N-H). ν_{max}/cm^{-1} (KBr) 1780 (s) and 1720 (s) (Co-NH-CO), 1536 (vs) (NO_2 assym.), 1349 (vs) (NO_2 sym.)

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* Distilling a mixture with equal volumes of 55% nitric acid and concentrated sulphuric acid gave purified nitric acid (sp. gr. 1.45 compare to fuming nitric acid sp. gr. 1.50). The experiment was also repeated using 55% nitric acid and the yield was found to be 38 % compared to the obtained yield of 40%, thus there is no actual need for purification as described.

5.2.2 4-Nitrophthalamide[235] [scheme 43 page 70]

4-Nitrophthalimide (20 g, 0.1 mol) was stirred in 25 % aqueous ammonia solution (300 cm^3) for 24h before a further 100 cm^3 25 % aqueous ammonia solution was added. After 24 hours the yellowish product was filtered off and washed with water (3 x 200 ml) to yield the 4-nitrophthalamide (18.7 g, 89 %); mp. 180-183^{0}C. $\delta_H(CDCl_3)$ 8.42 (1H, d, Ar-H) 8.24 (1H, dd, Ar-H), 7.89 (1H, d, Ar-H). ν_{max}/cm^{-1} 3340 (NH_2 str.), 1679 (C=O str.), 1615 (NH_2 def.).

5.2.3 4-Nitrophthalonitrile[235](41) [scheme 43 page 70]

Freshly distilled thionyl chloride (70 cm^3, 0.07.mole) was added while stirring at 0^{0}C to dry dimethylformamide (100 cm^3) in a nitrogen atmosphere. After 2 hours, dry 4-nitrophthalamide (170 g , 0.081 mol) was added. The mixture was stirred for 5h at 0^{0}C and then at room temperature overnight before it was added to ice water (approx. 500 cm^3), filtered and washed with water (6 x 100 cm^3). After recrystallizing twice from methanol light yellow 4-nitrophthalonitrile (**41**) was obtained (8.5 g, 60.4 %); mp. 144-147^{0}C. $\delta_H(CDCl_3)$ 8.69 (1H, d, Ar-H), 8.62 (1H, dd, Ar-H), 8.11 (1H, d, Ar-H),. ν_{max}/cm^{-1} (KBr) 2242 (s) (CN), 1542 (vs) (NO_2 assym), 1359 (vs) (NO_2 sym)

5.2.4 4-(4-*Tert*-butylphenoxy)-1,2-benzenedicarbonitrile (33) [scheme 44 page 70]

4-*Tert*-butylphenol **122** (11.27 g, 75 mmol) and 4-nitrophthalonitrile **41** (8.65 g, 50 mmol) were dissolved in dry DMSO (100 cm^3) under nitrogen. To this suspension was added dry potassium carbonate (13.82 g, 100 mmol) and the mixture stirred at room temperature. Further aliquots of potassium carbonate (0.64 g, 4.63 mmol) were added after 4h and 24h of stirring. After 48h total reaction time the mixture was poured into 1M HCl (500 cm^3) thereby precipitating a brownish-yellow product which was then filtered. The product was recrystallized from acetone to yield light brown crystals of the *tert*-butyl compound **33** (7.42 g, 51%); mp. 105-108^{0}C. δ_H (D_6-DMSO, spectrum 6) 7.73 (1-H, dd), 7.49 (2-H, m, 3, 5-H), 7.26 (2-H, m, 3', 5'-H), 7.01 (2-H, m, 2, 6-H), 1.38 (9-H, s, Bu^t). ν_{max}/cm^{-1} (KBr) 2240 (s) (C≡N), 1320 (m) (C-H def.), 1250 (C-O -C str.).

5.2.5 4-(3,4-Dicyanophenoxy)bezoic acid[44] (124) [Scheme 43 page 70].

Dry potassium carbonate (0.64 g, 4.63 mmol) was suspended in dried DMSO (5.5 cm^3) under a nitrogen atmosphere. To this suspension was added 4-hydroxybenzoic acid **123** (0.43 g, 3.12 mmol) and 4-nitrophthalonitrile **41** (0.36 g, 2.1 mmol). Further aliquots of potassium carbonate (0.64 g, 4.63 mmol) were added after 4h and 24h of stirring at room temperature The suspension turned a milky yellow colour and was then further stirred at room temperature for 5d under nitrogen throughout. The precipitate that formed was dissolved in water (50 cm^3) and the pH adjusted to 1 by adding concentrated HCl. The filtered product was recrystallised from methanol to yield the white acid **124** (0.29 g, 52 %); mp. 144-147^{0}C. δ_H (D_6-DMSO, spectrum 3) 13.05 (1-H, s, COOH), 7.86 (2-H, m, 3, 5-H), 7.85 (1-H, d, 6'-H), 7.46 (1H, d, 3'-H), 7.36 (1H, dd, 5'-H), 7.10 (2H, m, 2,6-H). ν_{max}/cm^{-1} (KBr) 3050 (b) (OH), 2230 (s) (C≡N str.), 1670 (s) (C=O), 1250 (vs) (C-O str.).

5.2.6 4-Aminophthalonitrile[220] (125) [scheme 44, page 70]

4-Nitrophthalonitrile **41** (1.0 g, 5.77 mmol) in THF (25 cm^3) is cooled in an ice bath. To this solution is added 10% Pd on carbon (0.24 g) in three portions over 10 minutes $NaBH_4$ (0.57 g, 15 mmol). The mixture is stirred for 30 minutes at room temperature upon which 2M HCl is added to destroy excess $NaBH_4$ (pH = 6). Ether (60 cm^3) is added and the solid filtered off. The filtrate is washed with water (2 x 10 cm^3) and dried over $MgSO_4$, the solvent is removed under reduced pressure with no heat applied to yield† the off-white amine **125** (0.62 g, 75%); mp. 176-179^{0}C. ν_{max}/cm^{-1} (KBr) 3500 (b) (N-H str.), 2240 (s) (C≡N str.), 1609 (N-H def.), 1280 (s) (C-N str.). The product does deteriorate on standing and is thus used directly.

† The product may be further purified by flash chromatography by using ethyl acetate : petroleum ether 1:1 as eluent

5.2.7 4-(Ferrocenylamido)phthalonitrile (126) [scheme 44, page 70]

The synthesis was done employing two methods firstly by using the previously prepared ferrocenoyl chloride **112** and secondly using the ferrocenoic acid **101** with the coupling agent BTU.

i) Ferrocenoyl chloride **112** (0.52 g, 2.1 mmol) was quickly added to a solution of 4-aminophthalonitrile **125** (0.30 g , 2.1 mmol) and triethylamine (0.61 cm^3, 4.2 mmol) in dry ether (30 cm^3). The solution was stirred for 4 hours at room temperature and the red mixture was extracted with ether (3 x 50 cm^3). The combined ethereal portions were washed with 10% NaOH (3 x 20 cm^3), water (2 x 20 cm^3), concentrated HCl (2 x 20 cm^3) and again with water (20 cm^3). The ethereal layer was concentrated and the amide **126** was obtained after preparative TLC (R_f = 0.65 1:1 ethyl acetate : petroleum ether). (0.52 g, 70%); mp. 150-154^{0}C. δ_H($CDCl_3$, spectrum 4) 8.12 (1-H, d, Ar-H), 7.57 (1-H, d, Ar-H) 7.57 (1-H, s, N-H), 7.46 (1-H, dd, Ar-H), 5.07 (2-H, t, C_5H_4), 4.64 (2-H, t, C_5H_4), 4.41 (5-H, s, C_5H_5). ν_{max}/cm^{-1} (KBr) 3410 (N-H, b), 1688 (s) (C=O str.), 1515 (s) (N-H def.)

ii) Ferrocenoic acid **101** (0.065 g, 0.3 mmol) and 4 drops of triethylamine were added to 4-aminophthalonitrile **125** (0.0429 g, 0.3 mmol) in DMF (10 cm^3). BTU (0.136 g, 0.3 mmol) was then added to the solution which was stirred for 2 hours at room temperature. Water (75 cm^3) was added to the reaction mixture to precipitate a red product, which was extracted with ether (3 x 25 cm^3). The ethereal layer was washed with water (2 x 20 cm^3), concentrated HCl (2 x 25 cm^3), 10% NaOH (2 x 25 cm^3) and then again with water (2 x 25 cm^3). The ethereal layer was concentrated and the amide **126** was obtained after preparative TLC (R_f = 0.65 1:1 ethyl acetate : petroleum ether) (0.047 g, 45%). mp. 120-124^{0}C. δ_H($CDCl_3$, spectrum 4) 8.12 (1-H, d, Ar-H), 7.57 (1-H, d, Ar-H) 7.57 (1-H, s, N-H), 7.46 (1-H, dd, Ar-H), 5.07 (2-H, t, C_5H_4), 4.64 (2-H, t, C_5H_4), 4.41 (5-H, s, C_5H_5). ν_{max}/cm^{-1} (KBr) 3410 (b) (N-H), 1688 (s) (C=O str.), 1515 (s) (N-H def.)

5.2.8 Chloro[7, 12; 14, 19-diimino-21, 5-nitrilo-5*H*-tribenzo[*c, h, m*]- [1, 16, 1] triazacyclopentadecinato-(2-) N^{22}, N^{23}, N^{24}] boron[56] (SubPc) (30) [scheme 46 page 75]

Phthalonitrile **22** (5.0 g, 40 mmol) was suspended in freshly distilled 1–chloronaphthalene (12 cm^3) under nitrogen. The suspension was cooled to -3^0C on an electrical cold-plate and boron trichloride (20.5 cm^3, 20 mmol; 1M solution in n-hexane) was added slowly through and injection syringe. The yellowish-green solid was slowly heated to 120^0C under vigorous stirring, the colour changed to black, n-hexane was distilled off at this temperature and the heating continued at 250^0C for 10 minutes, by now the solution was violet. The solvent was then removed under reduced pressure at this temperature. The violet product was then extracted by stirring in petroleum ether (80^0C – 100^0C fraction) for 24h at room temperature, the petroleum ether was filtered off and then the remaining solid was extracted a further 2 hours by stirring in toluene. After filtration and recrystallization from ethanol the crude solid residue was then filtered and recrystalized from ethanol to give brown SubPc **30** (3.30 g, 58%); mp. > 230^0C. δ_H (D_6-DMSO, spectrum 5) 8.86 (6-H, dd), 8.21 (6-H, dd). λ_{max} (DMF) 566, 300 nm

5.3. Preparation of phthalocyanines

5.3.1 Zinc(II)-2,9,16,23-tetranitrophthalocyanine[219](120) [scheme 42 page 68]

4-Nitrophthalimide (1.81 g, 9.45 mmol), zinc chloride (0.3529 g, 2.59 mmol), ammonium chloride (0.2474 g, 4.63 mmol), ammonium molybdate (0.0282 g, 0.02 mmol) and excess urea (3 g) were finely ground and added to nitrobenzene (10 cm^3) in a 500 cm^3 flask. The mixture was refluxed for 5h at 185°C ± 5^0C after a gradual temperature increase from 25 to 185^0C over 30 minutes. After filtration and washing with methanol to remove the nitrobenzene, the deep purple solid was added to 1M hydrochloric acid (30 cm^3) which had previously been saturated with sodium chloride. This suspension was boiled for about 5 min and filtered after cooling. The solid was added to 1M sodium hydroxide (30 cm^3) containing sodium chloride (11 g) and heated at 90°C for 30 min. After

filtration the solid product was treated with 1M hydrochloric acid (30 cm^3) saturated with sodium chloride and 1M sodium hydroxide, also saturated with sodium chloride as before twice alternately and each time separated by centrifugation. The green compound obtained was washed with water (100 cm^3) and dried overnight at 135°C to yield the nitro compound **120** (0.79 g, 45%); mp > 230^0C*. ν_{max}/cm^{-1} (KBr) 1521 (s) (NO_2 assym), 1338 (vs) (NO_2 sym)

5.3.2 Cobalt(II)-2,9,16,23-tetranitrophthalocyanine[219] (119) [scheme 42 page 68]

The cobalt phthalocyanine **119** was prepared analogously to the zinc phthalocyanine **120** in 5.3.1, using cobaltous chloride (0.594 g, 2.5 mmol), 4-nitrophthalimide (1.921 g, 10 mmol), ammonium chloride (0.267 g, 4.9 mmol), ammonium molybdate (0.04 g) and an excess of urea (3 g). After the same extensive workup as in 4.3.1, the dark green cobalt phthalocyanine **119** was attained (1.50 g, 80%); ν_{max}/cm^{-1} (KBr) 1538 (s) (NO_2 assym), 1350 (vs) (NO_2 sym)

5.3.3 Zinc(II)-2,9,16,23-tetraaminophthalocyanine 2-hydrate[219] (121) [scheme 42 page 68]

Zinc(II)-4,9,16,23-tetranitrophthalocyanine **120** (0.74 g, 0.98 mmol) was finely ground and suspended in water (20 cm^3), sodium sulfide nonahydrate (3.7 g, 15.41 mmol) was added and the mixture stirred at 50°C for 5 hours. The dark green product was separated by centrifugation and treated with 1M hydrochloric acid (60 cm^3). After removal of residual solids by centrifuging, the acidic solution was stirred in 1M sodium hydroxide (40 cm^3) for 1h and centrifuged again. The obtained green product was washed with water (40 cm^3) and dried overnight at 140°C to yield the green amine **121** (0.59 g, 95%). ν_{max}/cm^{-1} (KBr) 3350(w) (N-H str), 1618(s) (NH_2 def)

* The melting points of all the phthalocyanines prepared had melting points greater than 230^0C, thus no further mention will be made of the melting points of these compounds.

5.3.4 Cobalt(II)-2,9,16,23-tetraaminophthalocyanine 2-hydrate[219] (83) [scheme 42 page 68]

Cobalt(II)-4,9,16,23-tetranitrophthalocyanine **119** (1.40 g, 1.86 mmol) was finely ground and suspended in water (60 cm^3), sodium sulfide nonahydrate (11.1 g, 45 mmol) was added and the mixture stirred at 50°C for 5h. The green product was separated by centrifugation and treated with 1M hydrochloric acid (180 cm^3). After removal of residual solids by centrifuging, the acidic solution was stirred in 1M sodium hydroxide (120 cm^3) for 1h and centrifuged again. The obtained green product was washed with water (120 cm^3) and dried overnight at 140°C to yield the green amine **83** (1.11 g, 95%). ν_{max}/cm^{-1} (KBr) 3330 (w) (N-H str.), 1610 (s) (NH_2 def.) .

5.3.5 Cobalt(II)-2,9,16,23-tetracarboxamidophthalocyanine[231] (117) [scheme 41 page 66]

Trimeliticanhydride (10.0 g, 0.05 mol), urea (30 g, 0.50 mol), cobaltous chloride (7.14 g, 0.03 mol) and ammonium molybdate (1.0 g, 0.001 mol) were finely ground and to this was added nitrobenzene (150 cm^3). The mixture was refluxed at an internal temperature of 165^{0}C for 3 h after which the dark blue-green solid was filtered off and washed with methanol until no nitrobenzene was left. The blue slurry was air dried for 30 min after which it was dried overnight in an abderhalden drying tube to yield the dark blue insoluble phthalocyanine **117** (11.5 g, 51.6%). ν_{max}/cm^{-1} (KBr) 3150 (b) (N-H str.), 1659 (vs) (C=O), 1620 (s) (NH_2 def), 1578 (m) (N-H).

5.3.6 Cobalt(II)-2,9,16,23-tetracarboxyphthalocyanine[231] (118) [scheme 41 page 66]

To the amide **117** (1.0 g, 1.34 mmol) was added 10% potassium hydroxide (50 cm^3) and the heterogenous mixture was refluxed for 5h allowed to cool to room temperature and centrifuged. The cooled liquid was acidified with cold concentrated hydrochloric acid to precipitate the carboxylic acid **118**. The blue solid was then filtered and washed with water (3 x 50 cm^3) and then dried in an abderhalden drying tube overnight to yield the blue carboxylic acid **118** (0.2 g, 20 %);

δ_H (D_2O, spectrum 7*) 8.31 (4-H, Ar-H), 7.77 (8-H, Ar-H). ν_{max}/cm^{-1} (KBr) 3350 (vb) (OH str.), 1700 (s) (C=O str)

5.3.7 Cobalt(II)-2,9,16,23-tetracarboxylchloridephthalocyanine[231] (78) [scheme 41 page 66]

The carboxylic acid **118** (0.15 g, 0.20 mmol), thionyl chloride (2 cm^3, 0.002 mol) and 1 drop of pyridine are mixed in dry benzene (2 cm^3) and refluxed for 10 h. The liquid is removed from the solid neon-green product by centrifugation upon which it is washed with dry benzene (3 x 30 cm^3). The solid is then dried in an abden-halden drying tube to yield the neon green acid chloride **78** (0.16 g, 95%). ν_{max}/cm^{-1} (KBr) 1782 (vs) (C=O str.)

5.3.8 Cobalt(II)-2,9,16,23-tetraamidoferrocenylphthalocyanine (133) [scheme 48 page 76] and (134) [scheme 49 page 78]

The preparation of amides **133** and **134** are given in points a and bi, bii, respectively, the last two being the two methods used for preparation of **134**.

a)To a suspension of the amine **83** (0.0536 g, 0.084 mmol) in DMF (25 cm^3) was added firstly triethylamine (0.385 g, 3.8 mmol) and then ferrocenoyl chloride **114** (0.115 g, 0.47mmol). The reaction was stirred for 3 hrs. at room temperature and after centrifugation the solid was treated with 1M HCl (50 cm^3), 10 % NaOH (50 cm^3) and water (50 cm^3) each time separating the solid by centrifugation. The residual green product was dried at 70^0C for 24hrs to yield an insoluble amide **133** with unknown amount of ferrocenes anchored. ν_{max}/cm^{-1} (KBr) 1659 (C=O str.), 1479 (N-H def.)

* Spectrum was obtained as the sodium salt

bi) To a slightly dissolved mixture of the carboxylic acid **118** (0.110 g, 0.13 mmol) in DMF (10 cm^3) is added triethylamine (0.056 g, 0.56 mmol) and then BTU (0.20 g, 0.52 mmol). The amine **114** (0.031 g, 0.14 mmol) in DMF (3 cm^3) is added quickly and the suspended mixture is stirred for 10 hours at room temperature. The obtained crude product was then washed with 10% sodium hydroxide (50 cm^3). The solid product was separated by centrifugation and washed with concentrated HCl (50 cm^3), centrifuged again and finally washed with water, after overnight drying at 70^{0}C **134** was obtained again with an unknown amount of ferrocenes. ν_{max}/cm^{-1} (KBr) 1665 (C=O str.), 1505 (N-H def.)

bii) To a mixture of the acid chloride **78** (0.10 g, 0.12 mmol) and 2 drops pyridine in DMF (15 cm^3) was added the amine **114** (0.03 g, 0.13 mmol) and this mixture was stirred for 10 hours at room temperature. After extraction with 10% NaOH, concentrated HCl and water, each time separating the water extract by centrifugation the crude product was dried at 70^{0}C overnight to yield **134** with an unknown amount of ferrocene units. ν_{max}/cm^{-1} (KBr) 1665 (C=O str.), 1505 (N-H def.)

5.3.9 Phthalocyanines (127), (128) and (131) obtained from the statistical condensation method[92] [scheme 45, page 73]

4-(3,4-Dicyanophenoxy)benzoic acid **124** (0.19 g, 0.72 mmol) and 4-(4-*tert*-butylphenoxy)-1,2-benzenedicarbonitrile **33** (0.29 g, 1.08 mmol) were suspended in pentanol (10 cm^3) at 140^{0}C under nitrogen. Lithium metal (0.1 g, 14 mmol) was added in one portion and the suspension turned green immediately. The reaction was complete after 10 minutes (TLC) and allowed to cool to room temperature. Cold glacial acetic acid was added to the mixture and the resulting precipitates were centrifuged and washed with water (3 x 50 cm^3). The metallation proceeded directly with the addition of excess zinc acetate dihydrate (1.5 g, 7 mmol) and DMF (30 cm^3) this suspension was heated for 12h at 80^{0}C to yield a mixture of phthalocyanines **127**, **128** and **131**. The DMF was

removed under reduced pressure until the minimal amount was left for dissolution of the blue products. Preparative TLC with elution by hexane:ethylacetate:DMF (10:85:5) led to the isolation of three products.

i) [2,9,16,23-tetra (4-*tert*-butylphenoxy)phthalocyanato]zinc(II) **131** (0.11 g, 19.7%), (R_f = 0.97); δ_H (D_6-DMSO, spectrum 10) 8.7-8.9 (8-H, phenyl-H), 8.3-8.4 (4-H, phenyl-H), 7.05-7.90 (16-H, phenyl-H), 1.15-1.47 (36-H, ^{t}Bu). ν_{max}/cm^{-1} (KBr) 1399 (^{t}Bu), 1365 (^{t}Bu), 1236 (C-O-C).

ii) [9,16,23-tris(4-*tert*-butylphenoxy)-2-(4-carboxyphenoxy)phthalocyanato]zinc(II) **127** (0.075 g, 14.5%) (R_f = 0.60); δ_H (D_6-DMSO, spectrum 9) 9.1-9.3 (4-H, phenyl-H), 8.6-8.8 (4-H, phenyl-H), 7.9-8.2 (4-H, phenyl-H) 7.00-7.92 (16-H, phenyl-H), 1.08-1.43 (27-H, ^{t}Bu). ν_{max}/cm^{-1} (KBr) 3290 (O-H), 1701 (C=O str.), 1395 (^{t}Bu),1362 (^{t}Bu), 1236 (C-O-C)

iii) [9,23-bis(4-*tert*-butylphenoxy)-2,16-bis(4carboxyphenoxy)phthalocyanato]zinc(II) (**128**) (0.01 g, 1.9%) (0.04 g, (R_f = 0.35);); δ_H (D_6-DMSO, spectrum 8) 8.9-9.1 (4-H, phenyl-H), 8.5-8.6 (4-H, phenyl-H), 6.9-8.2 (20-H, phenyl-H), 1.10-1.45 (18-H, ^{t}Bu). ν_{max}/cm^{-1} (KBr) 3310 (O-H), 1710 (C=O str.), 1398 (^{t}Bu), 1362 (^{t}Bu), 1239, (C-O-C).

5.3.10 [2,9,16,23-tetra (4-carboxyphenoxy) phthalocyanato] zinc(II)[92](130) [scheme 45 page 73]

4-(3,4-Dicyanophenoxy)benzoic acid **124** (0.21 g, 0.80 mmol) was suspended in pentanol (10 cm^3) at 140^{0}C under nitrogen. Lithium metal (0.1 g, 14 mmol) was added in one portion and the suspension turned green immediately. After 10 minutes cold glacial acetic acid was added to the mixture and the resulting precipitate was centrifuged and washed with water (3 x 50 cm^3). The metallation proceeded directly with the addition of excess zinc acetate dihydrate (1.5 g, 7 mmol)

and DMF (30 cm^3) this suspension was heated for 12h at 80^0C to yield the green phthalocyanine **123**. The product was purified by precipitation from the DMF solution with water, filtration, washing with 10% NaOH solution to allow the phthalocyanine **129** to dissolve in water, the water layer was washed with ethyl acetate (2 x 50 cm^3) and then acidification of the water layer precipitated the phthalocyanine **129** which was dried at 70^0C for 24h (0.156 g, 67 %). δ_H (D_6-DMSO, spectrum 11) 8.3-9.2 (8-H, phenyl-H) 7.00 - 8.15 (20-H, phenyl-H). ν_{max}/cm^{-1} (KBr) 3310 (O-H), 1710 (C=O str.), 1239 (C-O-C)

5.3.11 Zinc(II)-2-(4-carboxyphenoxy)pthalocyanine (132)[56] [scheme 47 page 75]

4-(3,4-Dicyanophenoxy)benzoic acid **124** (0.24 g, 0.92 mmol) was added to a mixture of DMSO (4 cm^3), 1-chloronaphthalene (2 cm^3) and DBU (0.1 g, 0.65 mmol). This mixture was heated to 130^0C and then a suspension of SubPc **30** and zinc acetate dihydrate (0.11 g, 0.5 mmol) in a solvent mixture of DMSO (13 cm^3), 1-chloronaphthalene (6.5 cm^3) and pentanol (0.5 cm^3) was added dropwise over 1h. The mixture was cooled, glacial acetic acid (0.04 g, 0.65 mmol) was added and the solvents removed until the blue compound was nearly dry. Methanol (50 cm^3) was added and after centrifugation the blue product was dissolved in the minimum amount of DMF and this solution was flash-chromatographed with THF/DMF (95:5), further preparative TLC using toluene/DMF (98:2) to yield phthalocyanine **132** (0.025 g, 15%). δ_H (D_6-DMSO) 8.45-9.10 (8-H, m), 7.90-8.15 (6-H, m), 7.59-7.70 (3-H, m), 7.46 (2-H, s). ν_{max}/cm^{-1} (KBr) 3250 (O-H), 1695 (C=O str.)

5.4 Synthesis co-polyaspartamides

5.4.1 Polysuccinimide (65) [scheme 50 page 80]

A method developed in our laboratory was used.

Finely ground DL-aspartic acid (5.00 g, 37.8 mmol) and 85% orthophosphoric acid (5.00 g) were mixed by using a strong stainless steel spatula. The exothermic mixing process is cooled by running tap water. After thorough mixing the flask is connected to a rotary evaporator fitted with an oil pump and monometer. The flask is submerged into an oil bath preheated to 200^0C with slow rotation, taking care that the developing froth does not rise above the neck of the flask. After 5 minutes the temperature gradually lowered and kept between 170^0C and 190^0C for 2.5 hours under reduced pressure (< 2 torr). The reaction vessel was then cooled to *c/a.* 50^0C and then DMF (30 cm^3) was added to the warm reaction mixture. The mixture was then extracted overnight by rotating the DMF mixture overnight on the rotary evaporator at room temperature to afford a light brown solution. The polymer was precipitated by addition to water (500 cm^3), filtered, washed thoroughly with water and ground under liquid nitrogen to small flakes, again washed with water, and dried at 105^0C under reduced pressure over phosphorous pentoxide in an abderhalden drying tube, using boiling toluene as heat source to yield polymer **65** (3.30 g, 87%). η_{inh} 0.31 dl g^{-1}. δ_H (D_6-DMSO, spectrum 19) 5.25 (1-H, s, C-H), 3.18 (1-H, s, CH_2), 2.67 (1-H, s, CH_2). ν_{max}/cm^{-1} (KBr) 1780 (C=O str.), 1710 (C=O str.), 1400 (C-N), 1370 (C-N)

5.4.2 Polymer (71) [scheme 51 page 82]

To a solution of polysuccinimide **65** (0.97 g, 10.0 mmol) in dry DMF (8 cm^3) was added N–(3-aminopropyl) morpholine **136** (1.08 g, 7.5 mmol) in DMF (4 cm^3) over 10 min. in an ice-bath. The solution was stirred for 25 min. in the ice-bath and then for 5 h at room temperature. After cooling the solution again in an ice-bath ethylenediamine **137** (0.6 g, 10 mmol) in DMF (4 cm^3) was added over 20 min. to the stirred cold solution. The mixture was stirred for 20 minutes in the ice-bath and

then at room temperature for 5 h. The solution was then dialyzed for 16 hours in a 8000 molecular mass cut-off membrane tubing and freeze dried to yield a fluffy white powder (1.02 g, 46 %); mp. 104^0C-107^0C. η_{inh} 0.12 dl g^{-1} δ_H (D_2O) 4.52 (4-H, asp-CH), 3.55 (12-H, ε-CH_2), 2.99 (6-H, α-CH_2), 2.52 (12-H, 4 asp-CH_2 + α'-CH_2 + β'-CH_2), 2.30 (12-H, δ-CH_2), 2.17 (6-H, γ-CH_2), 1.48 (6-H, β-CH_2). ν_{max}/cm^{-1} (KBr) 1663 (s) (C=O str.), 1545 (m) (N-H def.).

5.4.3 Polymers (138) and (139) [scheme 51 page 82]

To a solution of polysuccinimide **65** (0.97 g, 10.0 mmol) in dry DMF (8 cm^3) was added N – (3-aminopropyl) morpholine **136** (1.31 g, 9.1 mmol) for target polymer **138** and (1.35 g, 9.37 mmol) for target polymer **139** in DMF (4 cm^3) over 10 min. in an ice-bath. The solutions were stirred for 25 min. in the ice-bath and then for 5 h at room temperature. After cooling the solutions again in an ice-bath ethylenediamine **137** (0.6 g, 10 mmol) in DMF (4 cm^3) was added over 20 min. to both the stirred cold solutions. The mixtures were stirred for 20 minutes in an ice-bath and then at room temperature for 5h. The solutions were then dialyzed for 16 h in a 8000 molecular mass cut-off membrane tubing and freeze dried to yield fluffy white powders of **138** (1.19 g, 51 %) and **139** (0.88 g, 39%); mp. **138** 97-100^0C η_{inh} 0.17 dl g^{-1} and **139** 99-101^0C η_{inh} 0.18 dl g^{-1}. δ_H (D_2O, spectrum 12) 4.52 (4-H, asp-CH), 3.55 (12-H, ε-CH_2), 2.99 (6-H, α-CH_2), 2.52 (12-H, 4 asp-CH_2 + α'-CH_2 + β'-CH_2), 2.30 (12-H, δ-CH_2), 2.17 (6-H, γ-CH_2), 1.48 (6-H, β-CH_2). ν_{max}/cm^{-1} (KBr) 1663 (s) (C=O str.), 1545 (m) (N-H def.)

5.5 Co-polymers of lysine and aspartic acid

5.5.1 N^{ε} - trifluoroacetyl – L – lysine[226] (141) [scheme 52 page 84]

To a solution of L – lysine monohydrochloride **140** (18.3 g, 100 mmol) in 1M sodium hydroxide (100 cm^3) was added ethyl thioltrifluoroacetate (20 cm^3, 160 mmol). The heterogenous mixture was stirred vigorously for 6h, the solution turned turbid after which a precipitation was observed.

The reaction mixture was cooled to 0^0 C and filtered, the crude white compound was dissolved in boiling water (100 cm^3) and diluted with hot ethanol (150 cm^3). N^{ε} - trifluoroacetyl – L – lysine **141** (8.21 g, 34 %) crystallized overnight from an ice-cooled solution; mp. > 230^0C. δ_H (D_2O) 3.51 - 4.05 (1-H, t, CH), 3.10 (2-H, t, CH_2), 1.63 (2-H, m, CH_2), 1.39 (2-H, m, CH_2), 1.10 - 1.29 (2-H, m, CH_2). ν_{max}/cm^{-1} (KBr) 1690 (C=O str.), 1180 (CF_3)

5.5.2 Polymer (144) [scheme 54 page 86]

Aspartic acid **64** (1.33 g, 10 mmol) and N^{ε} - trifluoroacetyl-L-lycine **142** (1.21 g, 5 mmol) were manually homogenized in a round bottomed flask with a spatula upon which was added polyphosphoric acid (1.27 g), the flask was then mounted on a rotary evaporator and lowered into a oil bath pre-heated to 180^0C, the pressure was reduced to below 5 mm Hg, the flask was slowly rotated for 2.5 hours. The yellow glass-like product was allowed to cool to room temperature and thereafter extracted with water overnight by means of stirring after ensuring that all the product was loosened by the magnet. The residue was dissolved in DMF, filtered and reprecipitated with ethanol to yield **144** (0.47 g, 8%); mp. > 230^0C. δ_H (D_6-DMSO, spectrum 13) 5.1-5.4 (1-H, C-H of succinimide), 4.5-4.65 (1-H, C-H of aspartic acid), 1.1-1.6 (6-H, β, γ and δ CH_2 of lysine). ν_{max}/cm^{-1} (KBr) 1795 and 1719 (C=O imide), 1170 (CF_3)

5.6 Drug anchoring onto the water-soluble polymers

5.6.1 Polymer (146) [scheme 56 page 88]

To a solution of polysuccinimide **65** (0.97 g, 10.0 mmol) in dry DMF (8 cm^3) was added ferrocenylethylamine **109** (0.17 g, 0.75 mmol) in DMF (4 cm^3) over 10 min. in an ice-bath. The solution was stirred for 25 min. in the ice-bath and then for 5 h at room temperature. After cooling the solution again in an ice-bath ethylenediamine **137** (0.6 g, 10 mmol) in DMF (4 cm^3) was added over 20 min. to the stirred cold solution. The mixture was stirred for 20 minutes in the ice-bath and

then at room temperature for 5 h. The solution was then dialyzed for 16 h in a 8000 molecular mass cut-off membrane tubing and freeze dried to yield a fluffy yellow powder (0.45 g, 16 %); mp. 114^0C-117^0C. δ_H (D_2O) 4.05-4.15 (9-H, $C_{10}H_9$), 2.6-2.7 (4-H, α'-CH_2 + β'-CH_2), 2.5-2.6 (4-H, α-CH_2 + β-CH_2). ν_{max}/cm^{-1} (KBr) 1660 (s) (C=O str.), 1552 (m) (N-H def.)

5.6.2 Polymer (147) [scheme 56 page 88]

To polymer **146** (0.05 g, 0.044 mmol) in DMF (15 cm^3) was added phthalocyanine **78** (0.11 g, 0.132 mmol) and 2 drops of triethylamine. The solution was stirred for 20 hours at room temperature. Water was added to the reaction mixture and after centrifugation the liquid was dialyzed for 16 hrs in 8000 molecular mass cut-off membrane tubing and freeze dried to yield the light green polymer **147** (0.076 g, 11 %); mp. 145^0C (degrad.) (Found: Co, 1.2 %; requires Co, 1.1 %). ν_{max}/cm^{-1} (KBr) 3300 (b) (N-H + O-H), 1663 (s) (C=O str.), 1648 (C=O str.), 1545 (m) (N-H def.)

5.6.3 Polymers (149) and (150) [scheme 57 page 90]

To polymers **71** (0.132 g, 0.15 mmol) and **138** (0.385 g, 0.15 mmol) in DMF (5 cm^3) were added 2 drops of triethylamine and to these stirred solutions were added the succinoylated derivative **116** (0.05 g, 0.15 mmol) in DMF (0.5 cm^3) and BTU (0.0681 g, 0.18 mmol). The solutions were stirred for 4 hours at room temperature and thereafter dialyzed for 16 h in a 8000 molecular mass cut-off dialysis tubing. After freeze-drying light yellow fluffy powders of polymer **149** (0.13 g , 72 %) and polymer **150** (0.30 g ,70 %) were obtained.

149; δ_H (D_2O, spectrum 14) 4.52 (4-H, asp-CH), 3.81-4.10 (9-H, C_5H_9), 3.55 (12-H, ε-CH_2), 3.05 (6-H, α-CH_2), 2.52 (12-H, 4 asp-CH_2 + α'-CH_2 + β'-CH_2), 2.30 (12-H, δ-CH_2), 2.17 (6-H, γ-CH_2), 1.48 (6-H, β-CH_2), 1.25 (3-H, CH_3). ν_{max}/cm^{-1} (KBr) 1660 (s) (C=O str.), 1543 (m) (N-H def.)

Polymer **155** was obtained in 10% yield by allowing **71** to react with **127**, BTU and triethylamine resulting in a blue polymer; mp. > 230^{0}C. δ_H (D_2O, spectrum 18) 6.70-7.35 (16-H, phenyl-H), 4.52 (4-H, asp-CH), 3.60 (12-H, ε-CH_2), 3.05 (6-H, α-CH_2), 2.52 (12-H, 4 asp-CH_2 + α'-CH_2 + β'-CH_2), 2.30 (12-H, δ-CH_2), 2.17 (6-H, γ-CH_2), 1.55 (6-H, β-CH_2), 1.1-1.3 (27-H, ^{t}Bu). ν_{max}/cm^{-1} (KBr) 3400 (b) , (N-H) 1667 (s) (C=O str.), 1545 (m) (N-H def.)

Polymer **156** was obtained in 7% yield by allowing **71** to react with **128**, BTU and triethylamine resulting in a blue polymer; mp. > 230^{0}C. δ_H (D_2O) 6.65-7.60 (16-H, phenyl-H), 4.55 (4-H, asp-CH), 3.60 (12-H, ε-CH_2), 3.05 (6-H, α-CH_2), 2.55 (12-H, 4 asp-CH_2 + α'-CH_2 + β'-CH_2), 2.38 (12-H, δ-CH_2), 2.25 (6-H, γ-CH_2), 1.55 (6-H, β-CH_2) 1.1-1.3 (18-H, ^{t}Bu). ν_{max}/cm^{-1} (KBr) 3400 (b) (N-H), 1695 (m) (C=O str.), 1677 (s) (C=O str.), 1555 (m) (N-H def.).

Polymer **157** was obtained in 9% yield by allowing **71** to react with **130**, BTU and triethylamine resulting in a blue polymer; mp. > 230^{0}C. δ_H (D_2O) 6.40-7.45 (16-H, phenyl-H), 4.55 (4-H, asp-CH), 3.60 (12-H, ε-CH_2), 3.15 (6-H, α-CH_2), 2.65 (12-H, 4 asp-CH_2 + α'-CH_2 + β'-CH_2), 2.41 (12-H, δ-CH_2), 2.25 (6-H, γ-CH_2), 1.60 (6-H, β-CH_2). ν_{max}/cm^{-1} (KBr) 3320 (b) (N-H + O-H), 1703 (s) (C=O str.), 1677 (s) (C=O str.), 1555 (m) (N-H def.)

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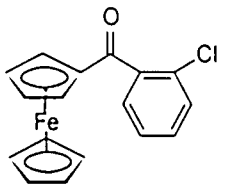
Nuclear magnetic resonance spectra

Spectrum 1



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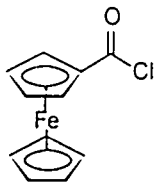
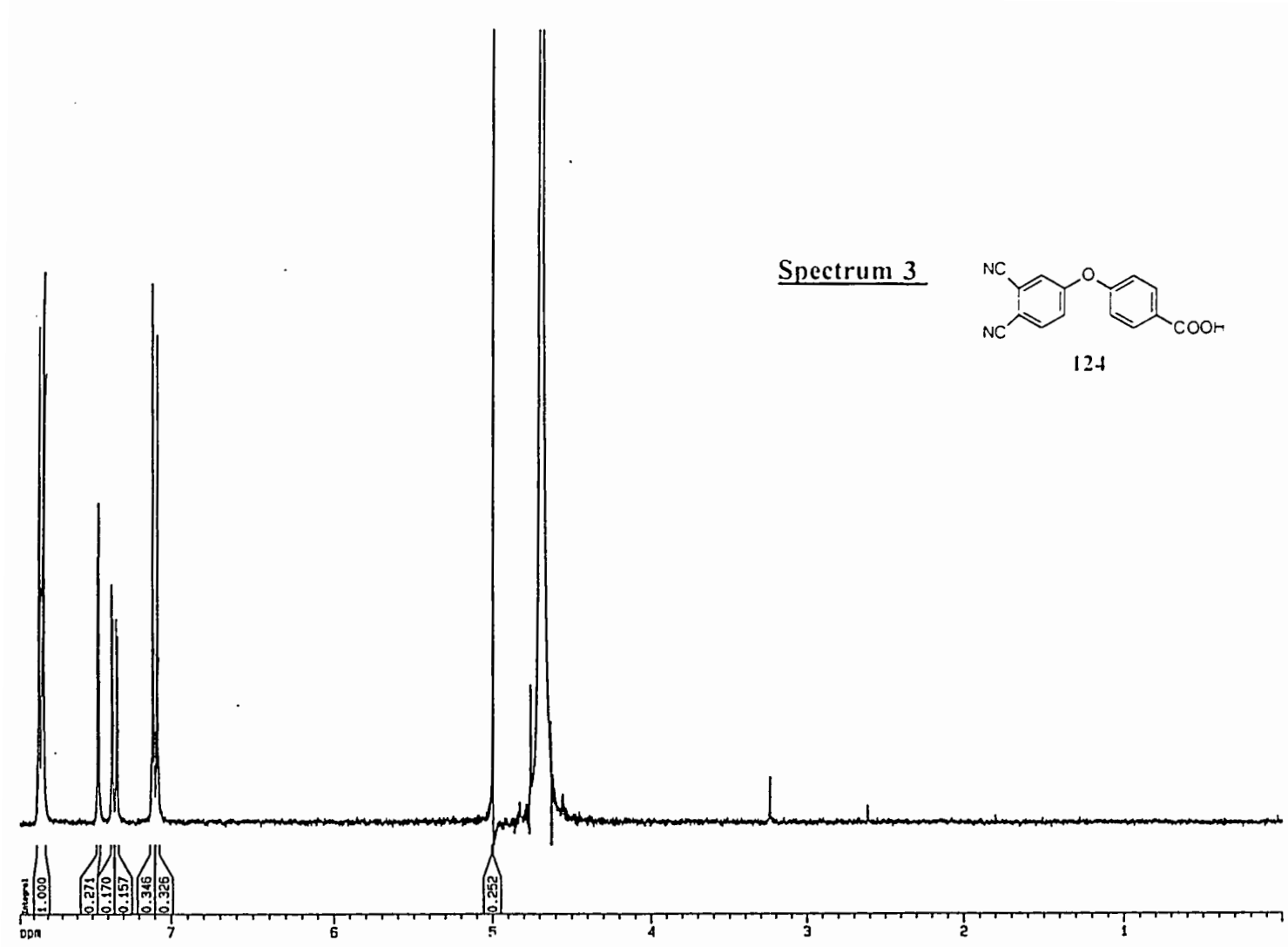
Spectrum 2

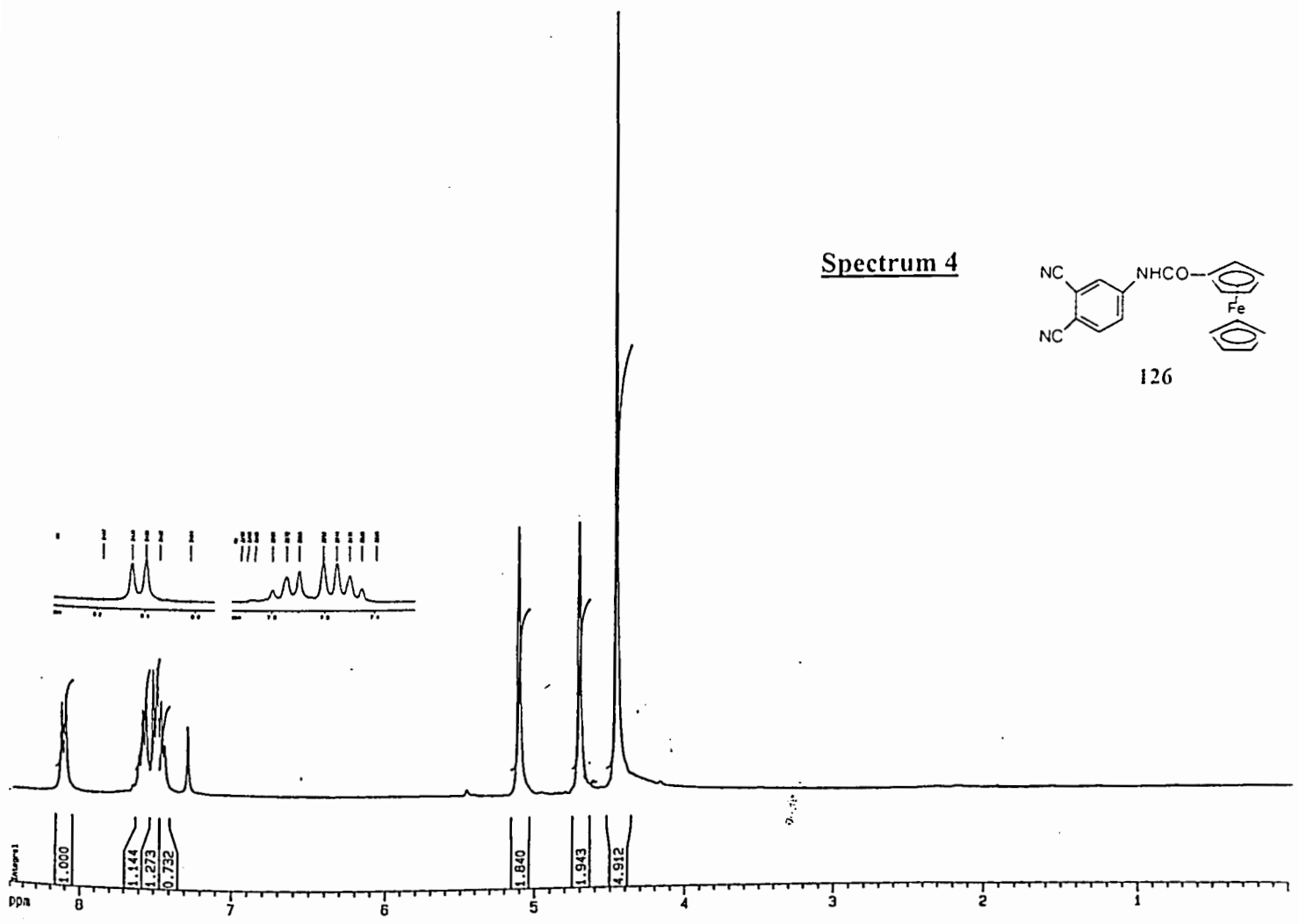


112



Spectrum 3

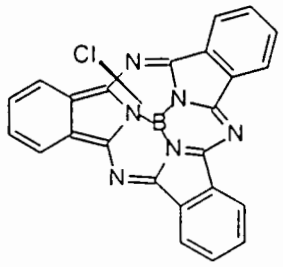
124



Spectrum 4

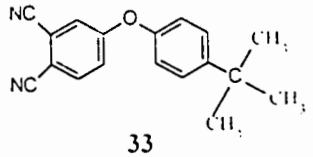
126

Spectrum 5



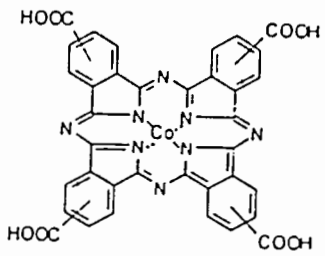
30

Spectrum 6



33

Spectrum 7



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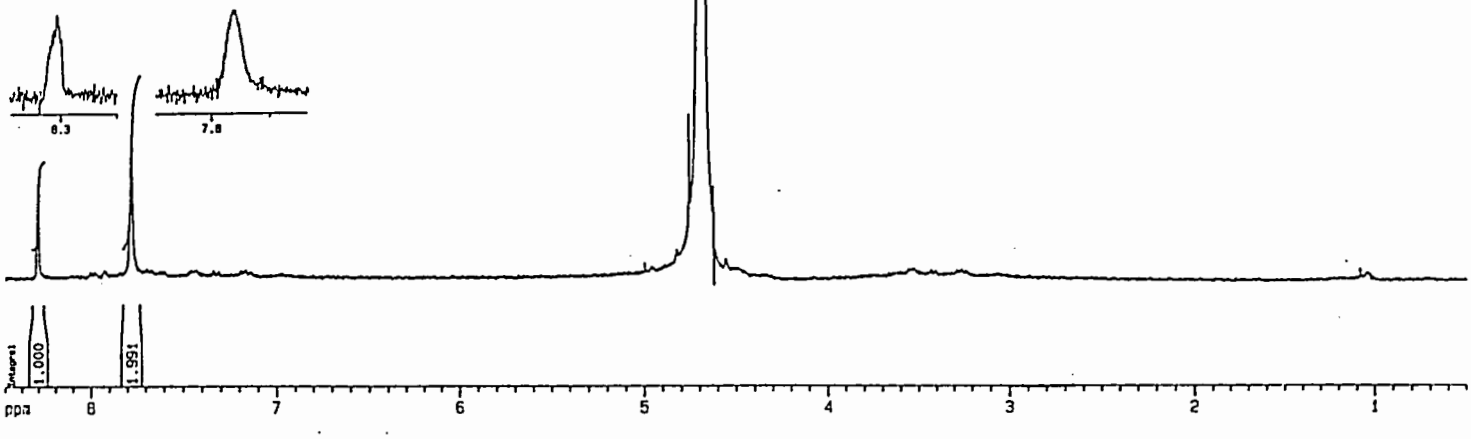
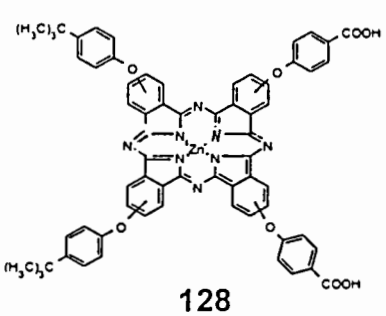


Spectrum 8



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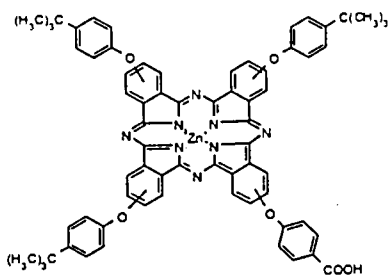

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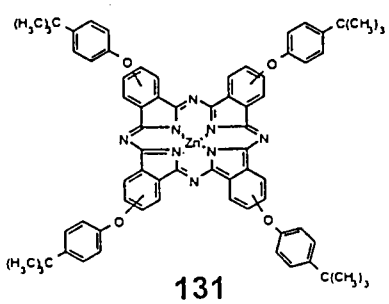

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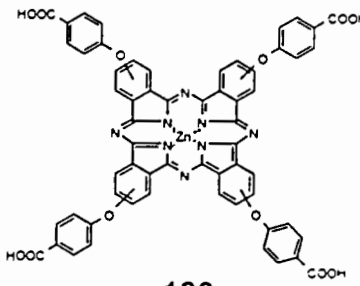

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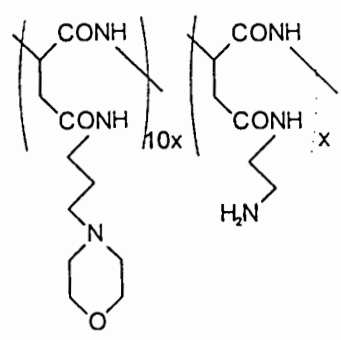

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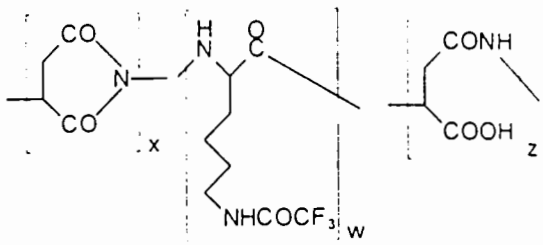

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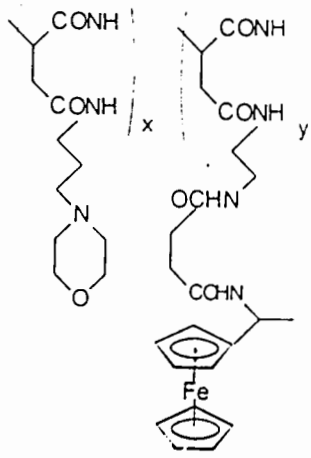
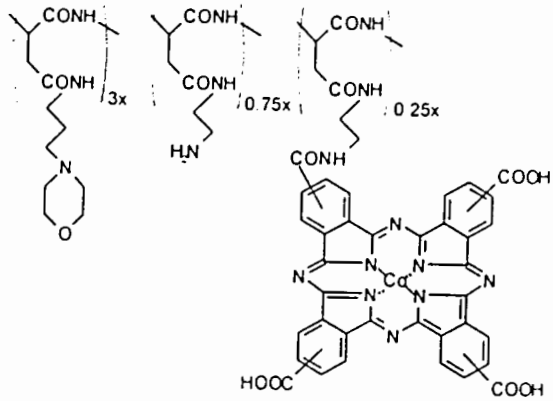


Spectrum 15



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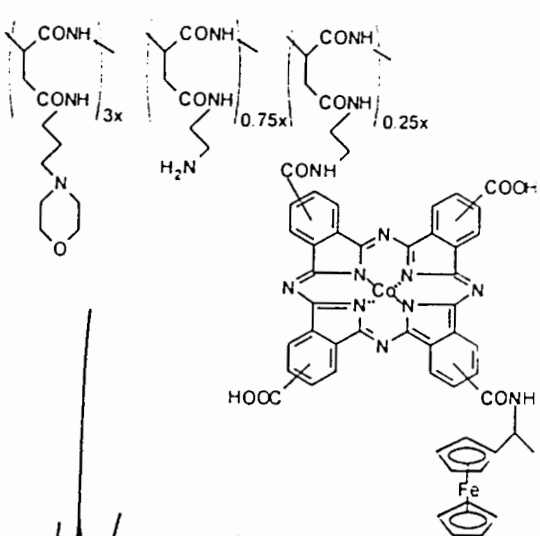

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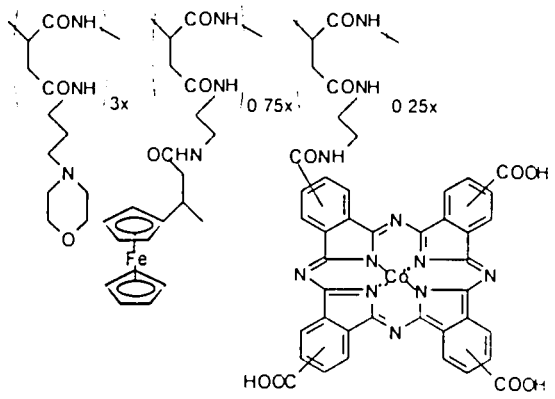
Spectrum 17



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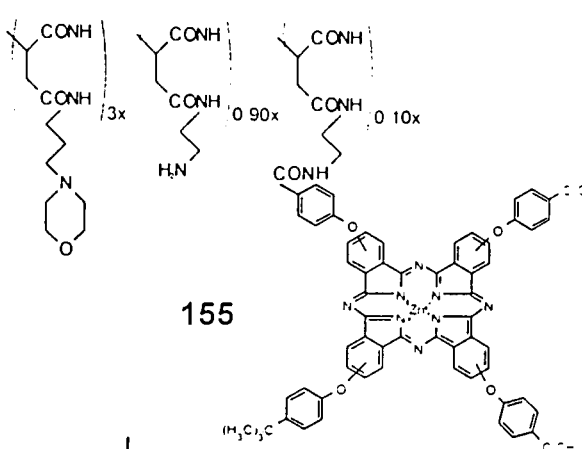
Spectrum 18



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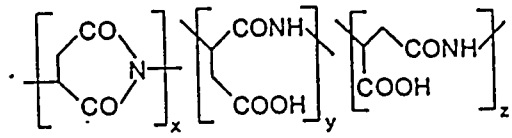

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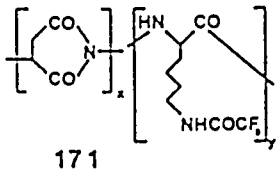

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