Synthesis and Chemistry of Hematoporphyrin derived Monomers and Oligomers

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Contents

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Declaration

This thesis contains no material which has been accepted for the award of any other degree of diploma in any university. To the best of my knowledge, this thesis contains no material previously published or written by any other person, except where due reference has been made in the text.

Lorely Vivienne Krippner.

NAME: LORELY VIVIENNE KRIPPNER **COURSE:** PhD

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Abstract

The composition of HPD, an agent used in photodynamic therapy for the treatment of tumours, and made by acetylation of hematoporphyrin with subsequent polymerisation of the acetates using 0.1N sodium hydroxide, was investigated.

Hydrolysis systems for the cleavage of porphyrin ether and esters were established. Hydrolysis studies of HPD made by various methods show it to be a mixture of ether and/or ester linked porphyrins with the amount of ester and ether linkages dependent on the nature of the acetylated HP. The ester-linked material was shown to be unstable in aqueous conditions. A third type of material was detected in the HPD mixture which was not ether or ester-linked by hydrolysis studies. Heating of HP at 90° for 2 days provided a polymeric material which was also neither ester nor ether linked

Dihematoporphyrin ether was synthesised by a Williamson-type reaction. The mesylate provided a suitable leaving group, although elimination was a competing reaction. The *t*-butyldimethylsilyl ethers were shown to be good protecting groups for non-participating alcohol groups.

The ester-linked hematoporphyrin dimer was synthesised by condensation of a porphyrin acid chloride with a 1-hydroxyethyl bearing porphyrin. The 2-(trimethylsilyl)ethyl esters were found to be good protecting groups for the non-reacting porphyrin carboxylic acids and were cleanly removed using fluoride. The 2-trichloroethyl esters were prepared but could not be cleanly removed. Ester-linked porphyrin dimers with acetyl, vinyl or 1-methoxyethyl sidechains were also synthesised. It was shown that the ester-linked hematoporphyrin dimer was not a major component of HPD and that the ester-linked dimers were unstable under aqueous conditions.

Grignard reactions were used to prepare porphyrins with extended sidechains, in the 3 and 8 positions, compared to hematoporphyrin. The monoacetyl mono(1-hydroxypropyl)deuteroporphyrin was synthesised however the diacetyldeuteroporphyrins were resistant to full alkylation. Formyl porphyrins were readily alkylated when the porphyrin was soluble in a suitable solvent. Porphyrins containing 1-hydroxybenzyl, 1-hydroxypropyl or 1-hydroxypentyl sidechains were synthesised and the 1-hydroxybenzyl porphyrin was shown to be without biological activity.

Publications*

Some of the work in this thesis has been reported in the following publications:

C. J. Byrne, L. V. Marshallsay and A. D. Ward, "The Structure of the Active Material in Hematoporphyrin Derivative." *Photochem. Photobiol.*, 1987, **40**, 575.

C. J. Byrne, L. V. Marshallsay and A. D. Ward, "The Composition of Photofrin II." *J. Photochem. Photobiol. B: Biology,* 1990, **6**, 13.

A review of some of the chemistry involved has also been published:

C. J. Byrne, L. V. Marshallsay, S. Y.,Sek and Ward, A. D., in 'Photodynamic therapy of Neoplastic Disease' (Ed. D. Kessel) Vol. 2, p. 131 (CRC Press: Boca Raton 1990)

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* These publications appear under my maiden name; Marshallsay

Abbreviations

Porphyrins

HP	hematoporphyrin
HP.DME	hematoporphyrin dimethyl ester
PP	protoporphyrin
PP.DME	protoporphyrin dimethyl ester
HV	3(8)-(1-hydroxyethyl)-8(3)-vinyldeuteroporphyrin
HV.DME	3(8)-(1-hydroxyethyl)-8(3)-vinyldeuteroporphyrin dimethyl ester
DP	deuteroporphyrin
HPD	hematoporphyrin derivative
DHE	dihematoporphyrin ether

Other compounds and groups

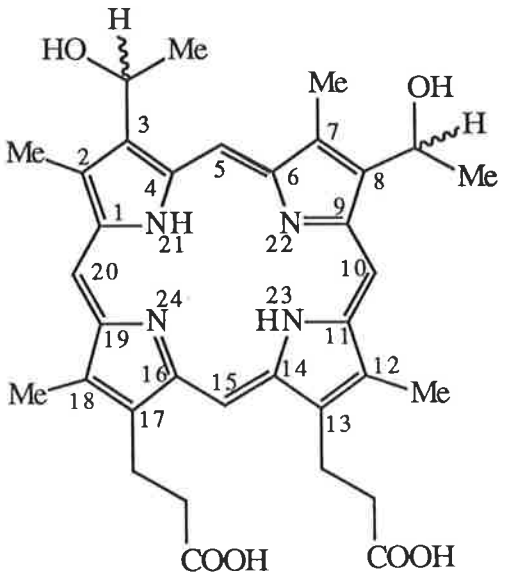
TBDMS	*tert*-butyldimethylsilyl group
Ph	phenyl group
SEM	2-(trimethylsilyl)ethoxymethyl group
DMAP	4-dimethylaminopyridine
DCC	1,3-dicylohexylcarbodiimide
EtMgBr	ethyl magnesium bromide
HCl	hydrogen chloride

Techniques

HPLC	high performance liquid chromatography
tlc	thin layer chromatography
n.m.r.	nuclear magnetic resonance
FAB	fast atom bombardment
MIKES	mass analysed ion kinetic energy spectra
COSY	correlated spectroscopy

Chapter 1. Introduction

Hematoporphyrin [1], abbreviated as HP, is an asymmetric porphyrin substituted with 4 methyl groups at the 2, 7, 12 and 18 positions on the basic porphyrin skeleton, propionic acid sidechains at the 13 and 17 positions and 1-hydroxyethyl sidechains in the 3 and 8 positions* as shown in Figure 1. Due to the chiral centre present in each of the two 1-hydroxyethyl sidechains HP is isolated as a mixture of diastereomers from hemin when hemin is treated with hydrobromic acid in acetic acid followed by an aqueous work up.[2,3] Under certain conditions the diastereomers of HP can be resolved[4] and separated[5] using reverse phase high performance liquid chromatography.



Me = CH_3

hematoporphyrin, HP [1]

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*Nomenclature is semisystematic and based on the IUPAC rules outlined in Merritt and Loenig[1].

Interest in the therapeutic properties of HP began in the early 1900s when experiments showed that animals[6] and humans[7] treated with HP.2HCl became sensitised to light; this is known as a photodynamic effect. In the 1940's and '50's it was discovered that HP accumulated preferentially in tumours rather than normal tissue.[8,9,10] Due to the fluorescing properties of porphyrins this enabled the tumour tissue to be delineated from normal tissue when irradiated with light. However, the HP being used in these experiments was impure and contained, apart from HP, a mixture of other porphyrins[11]. These impurities, and not HP, were found to be the good localisers[11]; HP is a poor localiser in pure form.[11,12,13] Due to the variability in the crude material inconsistent results were obtained in tumour localization experiments using HP. Lipson *et al.*[14,15] found that a derivative, made by treating HP with a mixture of acetic and sulfuric acid, gave a photosensitising agent that was a better tumour localizing agent than crude HP and gave more reproducible results. This product was known as hematoporphyrin derivative, HPD.

The combination of the two properties of some porphyrins, their ability to accumulate in tumour tissue and sensitise tissue to light, was first utilised in 1972[16] when crude HP was administered to subcutaneous gliomas in rats. The gliomas were then subjected to light resulting in massive destruction of the glioma cells; this use of a chemical photosensitiser and exposure to light is known as photodynamic therapy. Since that time interest in photodynamic therapy has bourgeoned. HPD, and its commercial successor, Photofrin II[17], have been used extensively as photosensitising agents for the treatment of a wide variety of cancers by photodynamic therapy.[18,19,20,21] Photofrin II, is currently undergoing final clinical trials in the United States for the treatment of lung, bladder and oesophagus cancer. Photodynamic therapy involves the initial injection of the photosensitising agent and then an incubation time, generally 24 to 48 hours, during which the drug accumulates preferentially in

neoplastic tissue[22]. The tumour site is then irradiated with light of wavelength 630 nm, causing tumour necrosis.[19,22] Fatal cell damage is attributed[23] to the formation of the cytotoxic singlet oxygen, produced when the porphyrin is excited by light to its triplet state and interacts with ground state dioxygen.[19,22]

The components of the mixture generated by the sulfuric and acetic acid treatment of HP used by Lipson *et al.*[14,15] in the preparation of HPD were initially identified by Clezy *et al.*[24] who methylated the mixture and used chromatographic separation to identify the main components of the methylated mixture as the methyl esters of diacetylated HP [2] and the two regioisomers of monoacetylated HP* [3]. Small quantities of the methyl esters of the elimination products; protoporphyrin, PP [4], 3(8)-(1-hydroxyethyl)-8(3)-vinyldeuteroporphyrin, HV [5], and its monoacetylated analogue [6], were identified in the mixture

Bonnett *et al.*[25] independently synthesised the free acid analogues of the compounds identified by Clezy *et al.* [24] and confirmed that they were the main components of the acetylation mixture using reverse phase high performance liquid chromatography. They[25] also noted that base treatment of the acetylation mixture, to form the injectable solution (HPD), gave a product without any acetylated groups but which consisted mainly of HP with some HV [5], a little PP [4] and also some material of porphyrin nature that was strongly retained on the HPLC column.

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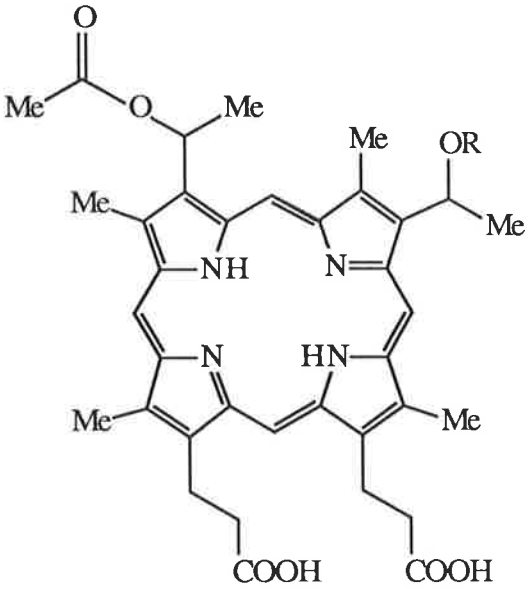
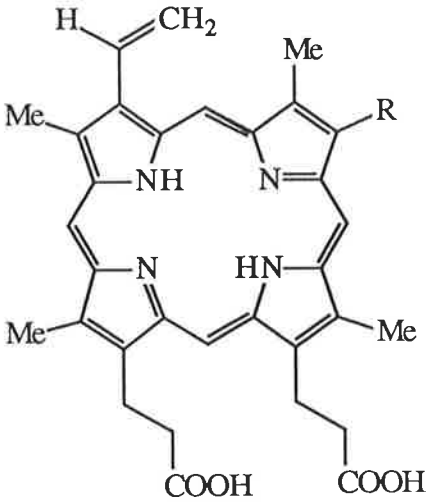
*Use of a name which does not distinguish between structural isomers implies that no attempt has been made to separate these isomers and the compound is a mixture of regioisomers. For simplicity the diagrams in this thesis show only one of the possible regioisomers for a compound.



[2] R = $COCH_3$
[3] R = H



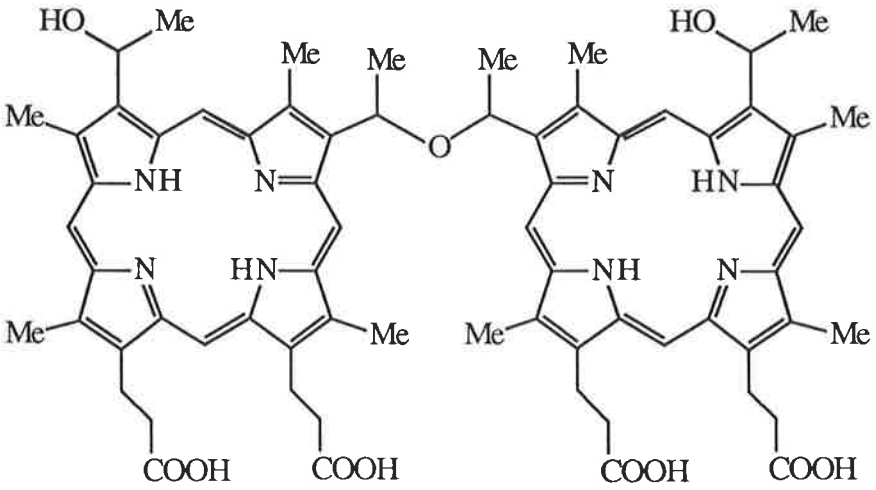
[4] R = $CH{=}CH_2$
[5] R = $CH(OH)CH_3$
[6] R = $CH(OCOCH_3)CH_3$

Further work by Bonnett's group[5,26] showed that the major components of the acetylated mixture, the diacetylated [2] and monoacetylated HP [3], were inactive *in vivo* . The identified components of HPD (HP, HV and PP) were also inactive *in vivo*, but the fraction of HPD which was strongly retained on the reverse phase column was active. They suggested[5,26] that this material was a dimer or oligomer joined by an ether, ester or carbon linkage.

In 1983 Dougherty *et al.*[27] fractionated HPD using size exclusion chromatography to give a rapidly moving fraction which was 40-50% of the total material and contained all the biological activity. This fraction was considered to be aggregate in nature, although more stable than aggregates formed by HP, HV or PP. The slowest fraction to elute from the column consisted of the monomers; HP, HV and PP. A similar separation was

obtained[28,29] using Sephadex LH-20. Photofrin II is obtained from HPD using an alternative size separation process.[17]

Further work by Dougherty *et al.* [30] led him to propose that the active material in HPD was dihematoporphyrin ether [7]. This conclusion was based on FAB mass spectrometry, n.m.r. spectroscopy and hydrolysis studies. The hydrolysis studies showed that the active material was unaffected by base but was hydrolysed by acidic conditions, which is consistent with an ether linkage joining the porphyrins, since an ester linkage would be hydrolysed by both acidic and basic conditions. The mass spectral data was less convincing; it could not distinguish between an ether or an ester linked dimer species, as both have the same molecular weight, and the data presented showed molecular weights corresponding to the presence of trimeric, as well as dimeric, species indicating that a range of oligomers may be present in the active fraction.

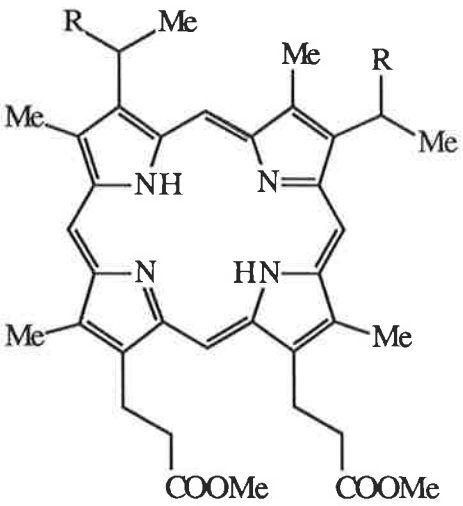


[7] dihematoporphyrin ether, DHE

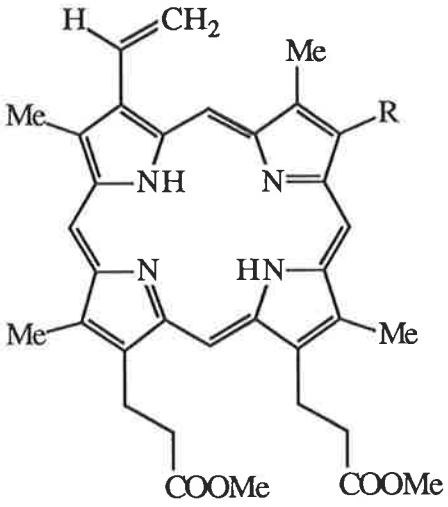
By 1985 a large weight of independent evidence had accumulated to show that the active fraction was oligomeric in nature[29,31,32] and that the oligomers were

covalently linked, rather than aggregated, species[33,34]. Interest in HPD turned to the nature of the linkage between the porphyrin units.

A Fourier transform infrared study[35] of HPD did not favour either an ester or an ether linkage but a later infrared study[36] concluded that the active fraction of HPD contained ether and ester linkages with ester linkages predominating.



[8] R = Br
[10] R = OH



[9] R = $CH{=}CH_2$
[21] R = $CH(OH)CH_3$

Scourides *et al.*[37] synthesised ether linked porphyrin material unambiguously. They formed the dibromoporphyrin [8], by treating protoporphyrin dimethyl ester, PP.DME [9], or hematoporphyrin dimethyl ester, HP.DME [10], with hydrobromic acid in acetic acid. The dibromide [8] was allowed to react with HP.DME to give methyl esterified, ether linked, porphyrin material. This material was hydrolysed, using basic conditions, to give the free acid material which was similar to HPD by HPLC analysis and showed similar *in vivo* properties. The free acid material behaved in a similar manner to HPD when subjected to gel filtration chromatography, with a fast running, aggregate rich, fraction being separated. The ^{13}C n.m.r. spectrum confirmed the presence of ether linkages in this aggregate rich fraction. The similarities between the

ether linked material and HPD provided strong support for the presence of ether linkages in HPD.

Data obtained during an n.m.r. spectroscopy study[33] of HPD favoured an ether linkage between porphyrins.

Kessel *et al.*[28,29] separated HPD on a Sephadex LH-20 column, using organic solvents, to give a fast running fraction in which the active components reside. They found[29,38] that this fraction was hydrolysed to monomers by acidic and basic conditions when tetrahydrofuran was used as a cosolvent. They explained[38] the difference in hydrolysis results, compared to those observed by Dougherty *et al.* [30], by invoking the tetrahydrofuran's ability to break down aggregation between porphyrin units which may protect ester linkages between porphyrin units making them resistant to hydrolysis.

Kessel *et al.*[29,39] also showed that porphyrin monomers that contain only carboxylic acid groups with no alcohol groups can be incorporated into the oligomeric fraction during the preparation of HPD. These extra products were hydrolysed with base which demonstrated that ester linked material could be formed in the preparation of HPD.

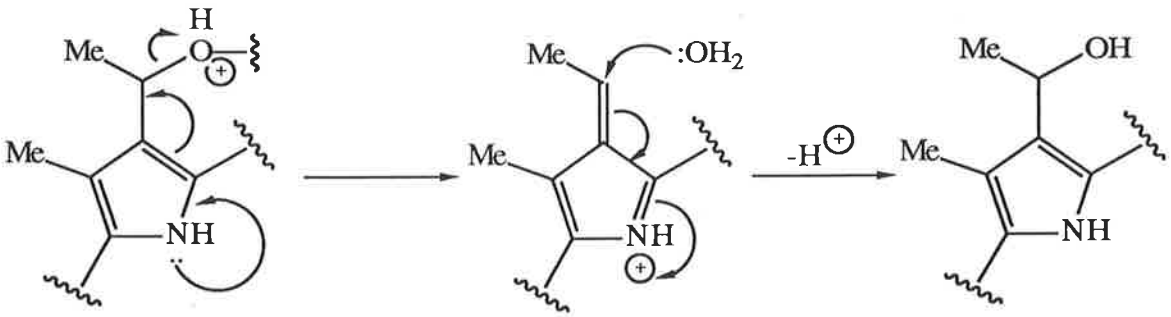
At the commencement of the work presented in this thesis the general consensus was that the aggregate fraction of HPD contained predominantly covalently linked material. However considerable uncertainty existed concerning the nature of the covalent linkage involved, with conflicting evidence being presented by a number of workers. Since the initiation of this project, hydrolysis studies and synthetic work, some of which is presented as part of this thesis, have shown that HPD and Photofrin II consists of a mixture of dimers and oligomers which are linked by ether, ester and/or carbon linkages.[40]

Chapter 2. Hydrolysis studies on HPD and related compounds

2.1 Introduction

At the commencement of this project it was generally accepted that the active fraction of HPD contained covalently linked porphyrin oligomers. The nature of the linking group, or groups, was, however, subject to some controversy. Results obtained by Dougherty[30], Scourides[37,41], and Ward[33,42], and their coworkers, supported an ether linkage whereas Kessel *et al.*[29,43] had presented evidence that strongly indicated that the porphyrin units were linked by ester groups. This chapter discusses work undertaken to establish the nature of the linking group(s) in the active fraction of HPD and discover reasons for the conflicting results that had been obtained by various workers.

Dougherty *et al.*[30] had described the relative stability of HPD to aqueous sodium hydroxide solution and noted that hot, aqueous, acidic solutions rapidly hydrolysed HPD mainly to the expected monomeric porphyrins (HP [1], HV [5] and PP [4]). This behaviour was consistent with that expected for a porphyrin dibenzylic ether linkage; given that such a system would cleave more easily than simple alkyl ethers, which normally require concentrated acid for cleavage[44]. An elimination/addition mechanism (Scheme 1) could also account for the facile hydrolysis of the ether linked porphyrins. Kessel *et al.*[29,38,45] had shown that the aggregate fraction he had obtained from HPD was rapidly hydrolysed to monomer products in aqueous tetrahydrofuran containing sodium hydroxide. Furthermore Kessel *et al.*[45,46] showed that the aggregate fraction could be cleaved by lithium aluminium hydride; a reagent that reductively cleaves esters but not ethers.



Scheme 1: Elimination/addition mechanism

Initially it was decided to establish hydrolysis conditions that would cleanly hydrolyse porphyrin esters but not ethers, and conditions that would hydrolyse both esters and ethers. Using these conditions the amount of ester linkages in HPD could be ascertained by assessing the change in HPD caused by basic hydrolysis. It was expected that hydrolysis using acidic conditions would cleave both ester and ether linkages, whilst basic conditions would only cleave ester linkages. The difference between the amount of hydrolysis occurring under acidic and basic hydrolysis conditions for a sample would provide a measure of the ether linkages present.

The hydrolysis results were analysed using reverse phase high performance liquid chromatography (HPLC). A typical HPLC trace of HPD is shown in Figure 1. It can be seen that there is a clear delineation between the initial half of the trace, which contains the monomers, HP and HV, and the large amount of poorly resolved material observed at longer than 14 to 15 minutes retention time; this material corresponds to the biologically active material obtained by chromatography on Sephadex LH-20[29]. The HPLC traces were analysed by identifying the known monomers. In the case of HPD; HP and HV are identified separately. The HPLC trace is then divided into two parts, based on the delineation already discussed; the material of less than 15 minutes retention time and that of greater than 15 minutes retention time, which as mentioned contains the active material and will be referred to as the long

retention time material, abbreviated as LRTM. Protoporphyrin, PP, is sometimes seen at 22 to 23 minutes in the trace as a peak on the LRTM. As PP is in the same region as the LRTM and can not easily be integrated separately it is included in the LRTM; it is usually only present to the extent of about 5%. The HPLC traces are subject to errors, there is some variation between runs due to changes in the column and slight variations in the solvents. The complexity of this particular material, and the lack of resolution obtainable for it, results in variations in the integration. The porphyrins are detected by their visible absorption at a set wavelength (397 nm). The integration values do not take into account variations in extinction coefficients and their maxima for the various porphyrins. Given these circumstances there may be appreciable errors (5-10%) in the percentage values obtained. Due to the inherent errors in the analysis system and also variations that occur between runs of the same experiment, which seems to be a feature of this porphyrin mixture, this chapter discusses trends rather than absolute values.

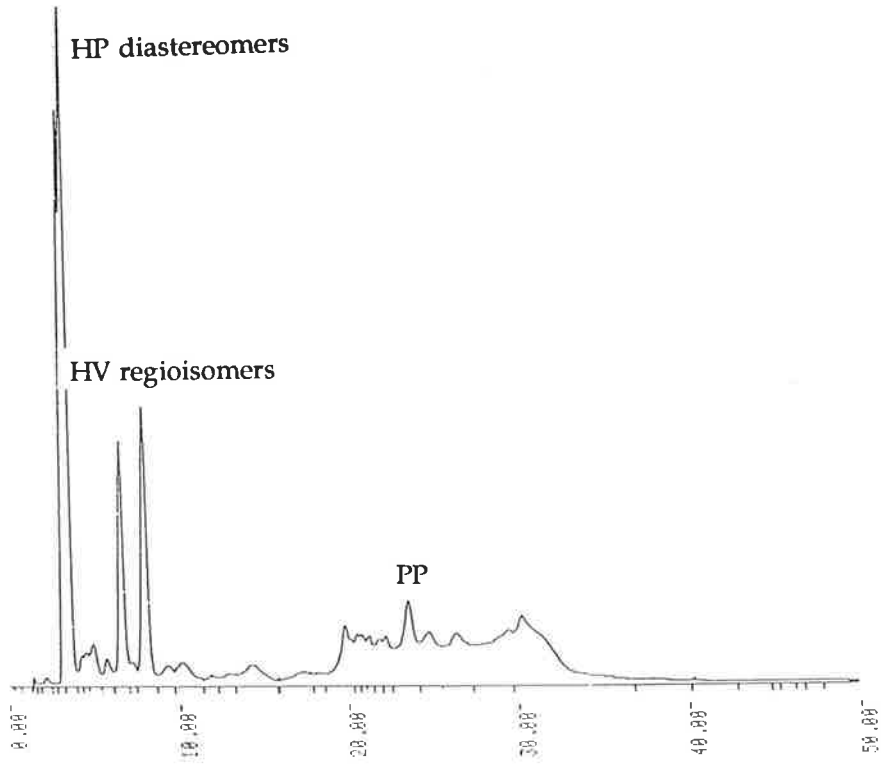
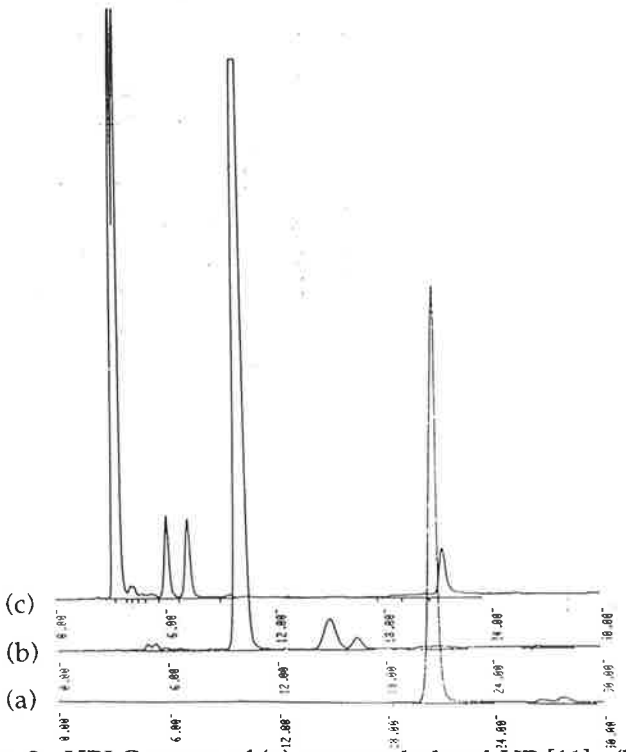
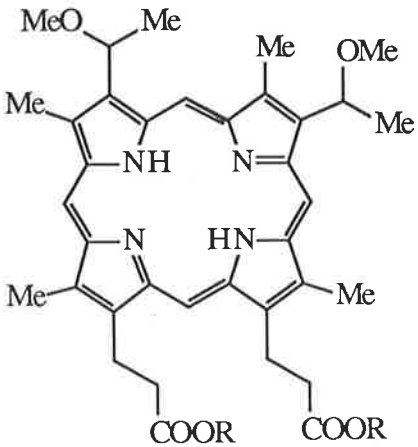


Figure 1. HPLC trace of HPD made by the Lipson procedure.

2.2 Standard hydrolysis conditions

HP dimethyl ether dimethyl ester [11], was used as a model compound for hydrolysis studies on ether and ester groups. This compound was prepared by treating HP.2HCl with trimethyl orthoformate, methanol and sulfuric acid.[47] When the tetramethylated HP [11] (Fig. 2, bottom trace) was refluxed with a 3:2 solution of tetrahydrofuran and 0.1N sodium hydroxide solution for 75 minutes it gave one major product, by HPLC analysis (Fig. 2, middle trace). This product, by its fast atom bombardment (FAB) mass spectrum ($(M+H)^+$, *m/z* 627), proton n.m.r. spectrum, and its nonidentity with HP.DME (HPLC), was the dimethyl ether of HP [12]. Treatment of [11] with 1N hydrochloric acid at reflux for one hour gave the top trace (Fig. 2), the major pair of peaks are due to the two diastereomers of HP with minor peaks due to HV and PP. Elimination of methanol and water from the 1-methoxyethyl and 1-hydroxyethyl sidechains, respectively, is an expected side reaction under acidic conditions[40] to give the conjugated vinyl group





[11] R = Me
[12] R = H

Figure 2. HPLC traces of (a) tetramethylated HP [11], (b) the products from the basic hydrolysis of [11], (c) the products from the acidic hydrolysis of [11].

These results establish that basic conditions hydrolyse porphyrin esters, but not ethers, while acidic conditions hydrolyse both. Although the results were obtained on porphyrin monomers it was expected that these conditions would be appropriate for porphyrin oligomers. Subsequent to this work ether linked dimers, trimers and tetramers have been synthesised and have been shown to be base stable but acid labile[48,49] whilst ester dimers that have been synthesised have been labile under all aqueous conditions[50] (see Chapter 3).

2.3 Hydrolysis studies on HPD and its precursor acetates

Hydrolysis studies using the hydrolysis conditions investigated above, showed that the active fraction of HPD, made by the Lipson procedure*, is substantially, but not totally, stable to basic hydrolysis but labile to the acidic hydrolysis conditions. Figure 3(a), below, shows the active material, obtained by chromatography on Sephadex LH-20, of HPD and which is mainly free of monomers. Figure 3(b) shows the active material after treatment with the basic hydrolysis conditions; some material has been hydrolysed to HP and HV but much remains apparently unchanged. Due to the poor resolution of the LRTM region it is not possible to detect if changes to give products that are still of long retention time are also occurring. The third trace (Fig. 3(c)) is of the active material after acidic hydrolysis and shows almost total hydrolysis to HP, with small amounts of HV and PP being formed. From these hydrolysis results it would appear that the active material consists mainly of ether linked material.

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* The Lipson procedure is widely used to describe the acetylation of HP using acetic and sulfuric acid followed by base treatment of the acetylated mixture to give HPD using the procedure of Gomer and Dougherty[51] which is a variation of the original procedure used by Lipson *et al.*[14,15]

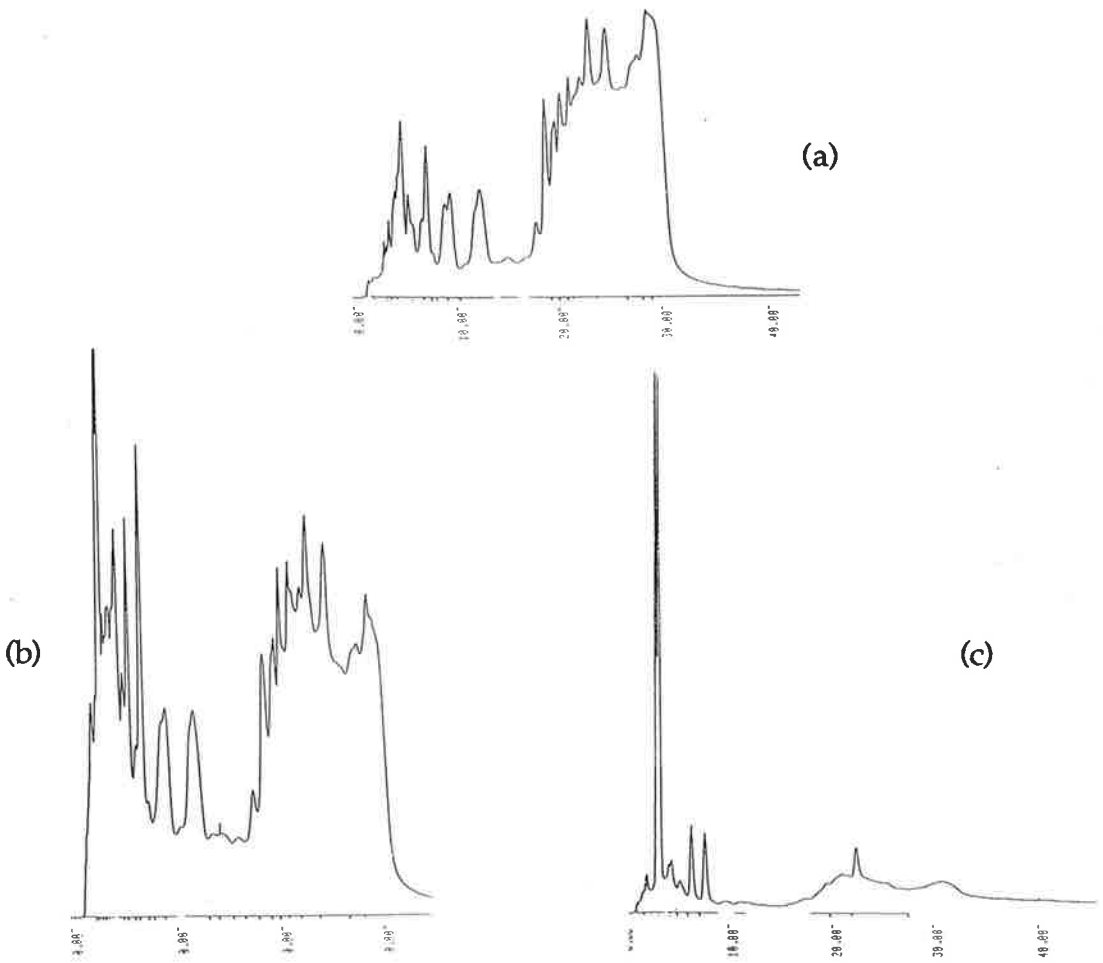

Figure 3. HPLC traces of (a) the fast fraction obtained from HPD after chromatography on a Sephadex LH-20 column. (b) the fast fraction, shown in (a), after basic hydrolysis. (c) the fast fraction, shown in (a), after acidic hydrolysis.

Kessel *et al.*, whose results from hydrolysis studies and reduction studies had shown HPD to contain mainly ester linked material[29,38,45], frequently made HPD [29,45,46,52] from the diacetate [2] rather than from the mixture of mono [3] and diacetates [2] formed by the Lipson procedure. HPLC analysis of HPD formed by these two methods showed them to be similar and the active fraction from HPD made by both methods shows similar cytotoxicity[13]; consequently it has been assumed that they were the same material[45,52].

The diacetate of HP [2] (Fig. 4(a)) was synthesised by the method of Bonnett *et al.*[25]; treatment of HP with a 1:9 mixture of acetic anhydride and pyridine. The diacetate [2], was then treated with 0.1N sodium hydroxide to give HPD (Fig. 4(b)). Use of the diacetate to form HPD, rather than the mixture of HP, mono

[3] and diacetates [2] formed by the acetylation conditions used in the Lipson procedure, results in a higher percentage of active material being formed compared to monomers[4,13,29] (cf Figures 1 and 4(b)). HPD made from the monoacetate [3] alone contains less of the active fraction than HPD made from the diacetate [2].[45]

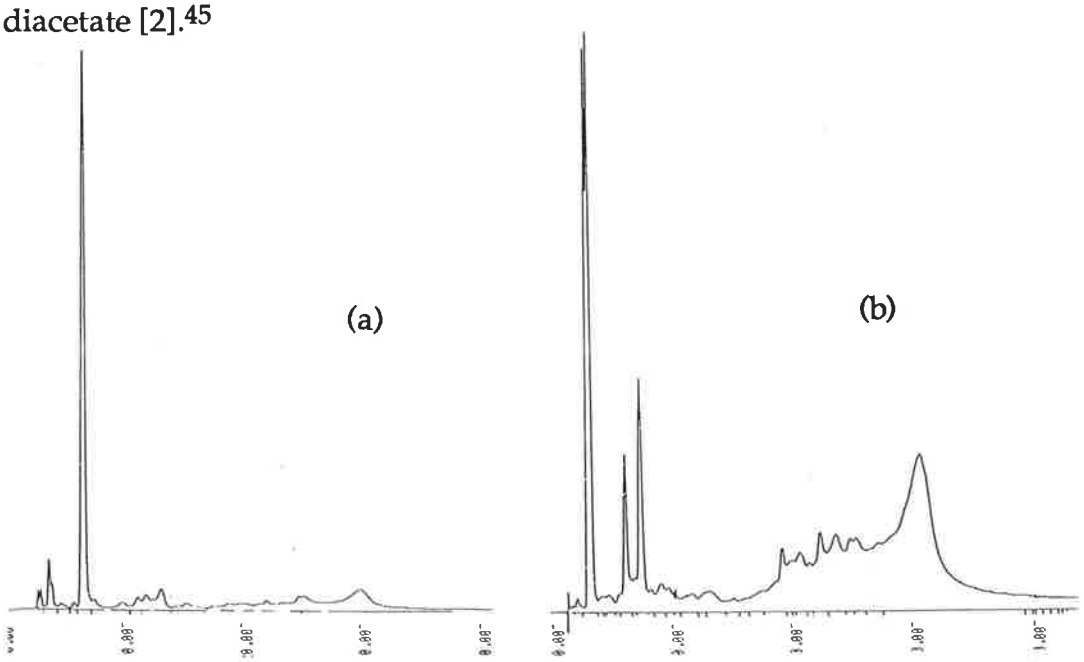

Figure 4. HPLC traces of (a) diacetate [2] formed by the treatment of HP with acetic anhydride/pyridine. (b) HPD formed from the diacetate [2] shown in (a).

The reaction of HP with either acetic acid/sulfuric acid mixture, or the acetic anhydride/pyridine mixture, produces, besides the expected acetates, some material that is poorly resolved in the HPLC trace. This is more evident in the acetic anhydride/pyridine reaction, which also shows resolved impurities. The detection of these impurities by HPLC is dependent on the detection and integration system used. For example, a sample was analysed using an older HPLC system which showed that the sample contained 80% of the diacetate [2], however when the same sample was analysed using the system now in use it was shown to only contain 50% of the diacetate, the rest being unidentified impurities.

For convenience in the following discussion the material prepared from the acetic acid/sulfuric acid method will be referred to as the Lipson acetates,

whilst the product derived from the acetic anhydride/pyridine reaction will be termed the Bonnett acetate; these products are used without purification, Kessel *et al.*[45], also, did not purify their acetates before use. It is important to realise that the Bonnett acetate contains HP diacetate [2] but little or none of the monoacetates [3] whereas the Lipson acetates contains a small amount of HP (<10%) and a mixture of mono [3] and diacetates [2] in a ratio of 1:2 to 1:3, by HPLC analysis. The acetate mixtures are analysed by HPLC in the same manner as HPD. The monomers; HP, monoacetate [3] and diacetate [2] are easily identified[53] and the rest of the material in the trace is categorised as either less than 15 minute or greater than 15 minute retention time material.

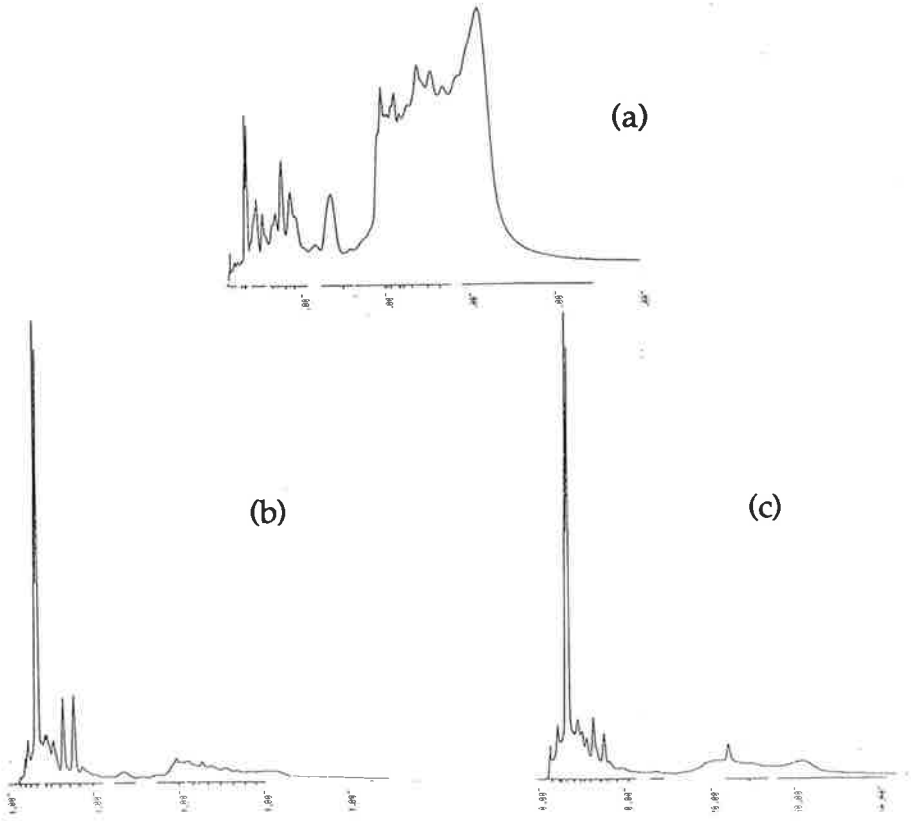

Figure 5. HPLC traces of (a) The fast fraction obtained by chromatography of HPD, formed from the Bonnett acetates, on Sephadex LH-20. (b) the fast fraction, shown in (a), after basic hydrolysis. (c) the fast fraction, shown in (a), after acidic hydrolysis.

The composition of HPD made using the Bonnett acetate was investigated. The active fraction of HPD made from the Bonnett acetate was isolated by chromatography on Sephadex LH-20 (Fig. 5a). Treatment of this material with aqueous acid (Fig. 5c) or base (Fig. 5b) gave substantial hydrolysis to the monomers, HP and HV, using either conditions; indicating that the oligomers in this HPD are mainly ester linked, compared to HPD made by the Lipson procedure which is mainly ether linked.

As the only apparent difference in the formation of the two polymeric products is the nature of the starting material from which they are made, attention was turned to the procedures used to prepare the acetate containing material from which HPD is derived.

The formation of the acetates, [2] and [3], using the using the Lipson acetylation procedure, was investigated with respect to time. Aliquots were removed from the reaction at various times, poured into dilute sodium acetate, extracted with a mixture of dichloromethane/tetrahydrofuran and then dissolved in the HPLC eluting solvent and injected immediately on to the HPLC column. A rapid drop in HP was observed during the first 15 minutes of the reaction, whilst the monoacetate [3] increased to a maxima and then gradually declined as the amount of diacetate [2] increased. The amount of diacetylated HP declined at reaction times longer than 1.5 hours and there was a corresponding increase in unidentified material at long retention times (greater than 15 minutes).

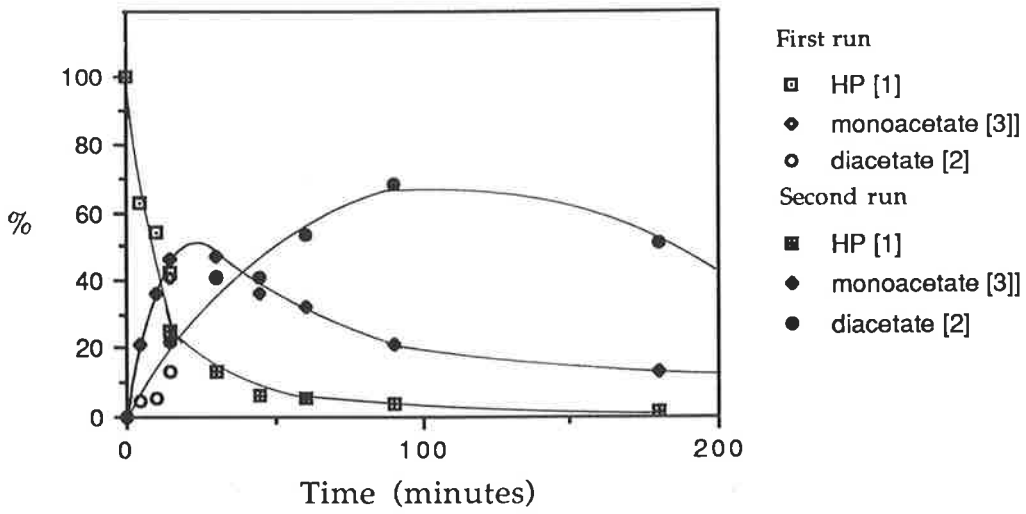

Figure 6. Formation of the mono [2] and diacetates [3] against time when HP is treated with acetic acid/sulfuric acid (19:1).

Figure 6 (above) combines observations made on two separate reactions; the first reaction had aliquots removed at 15, 30, 45, 60, 90, 120 and 180 minutes. A large fraction of the starting material had been consumed within the initial 15 minutes of the reaction so the reaction was repeated and monitored at 5, 10 and 15 minutes. A comparison of the two reactions shows that in the second reaction the starting material was consumed at a slower rate, with less monoacetylated [3] and diacetylated HP [2] having been formed, and less HP consumed, within the first 15 minutes compared with the first reaction.

Both reactions were much slower than those observed by Bonnett *et al.*[25], who found that the maxima for monoacetate presence occurred at about 10 minutes, compared with the above reaction where the maxima occurred at 15 to 30 minutes. It was also noted that by 30 minutes in Bonnett's results the amount of HP present was almost zero whilst in these results it was still greater than 10%. The only apparent difference in the experimental procedures was in the workup; Bonnett used only 3% aqueous sodium acetate,

compared with 5%, and collected his product by centrifugation instead of extraction with organic solvents.

The reaction to give the Lipson acetates used in the production of HPD is generally worked up after one hour[51] which is well before the reaction reaches equilibrium. This variability in the rate of the reaction means that the mixture used in the next step, the base treatment to produce HPD, may vary substantially. A survey of various preparations that have been used in the base treatment to give HPD showed the following ranges of components; HP 3-10%, monoacetylated HP [3] 18-47%, and diacetylated HP [2] 36-57%.

Sulfuric acid (10%) in acetic acid was used for the acetylation of HP to see if the proportion of the diacetylated HP [2] produced could be increased. The reaction gave results similar to those obtained with 5% sulfuric acid in acetic acid (5% HP, 33% monoacetylated HP [3] and 45% diacetylated HP [2]). Treatment of this mixture with Lipson conditions (5% sulfuric acid in acetic acid) resulted in only a small change in the relative amounts of the products (3% HP, 27% monoacetylated [3] and 47% diacetylated HP [2]).

Treatment of the Lipson acetates (HP 10%, monoacetylated HP [3] 26%, diacetylated HP [2] 31%, <15 minutes 10%, >15 minutes 24%) with the Bonnett conditions (10% acetic anhydride in pyridine), for 1 hour, increased the amount of diacetate [2] but there was also an increase in the amount of unidentified material formed (HP 0%, monoacetylated HP [3] 3%, diacetylated HP [2] 40%, <15 minutes 34%, >15 minutes 12%).

As may be expected the formation of diacetate [2] by the Bonnett procedure (Fig. 7) proceeds in a manner similiar to the Lipson acetylation, with a build up in monoacetate [3] followed by its decline as the diacetate [2] is formed. The main difference is that in the Bonnett procedure the monoacetate level decreases to

almost nothing, being converted to the diacetate, but in the Lipson procedure the monoacetate reaches a steady state.

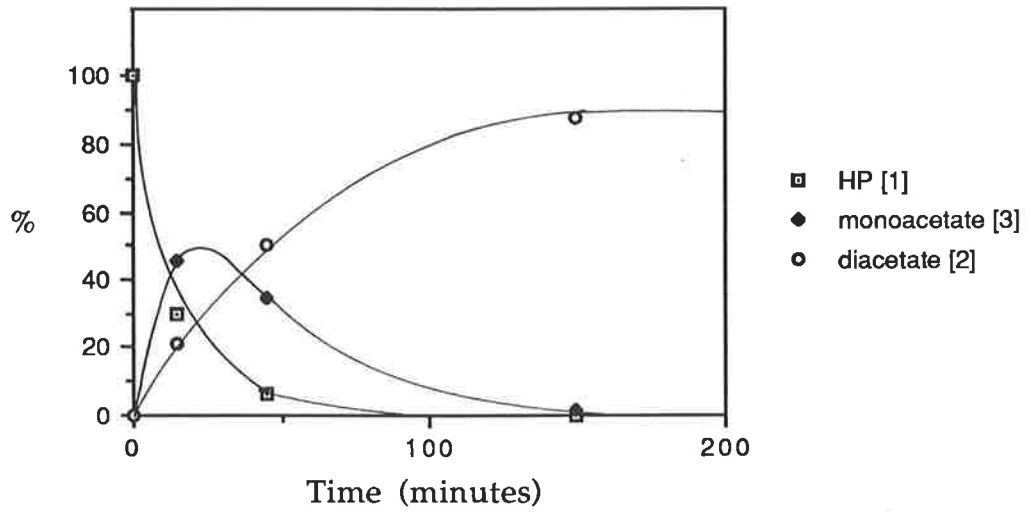

Figure 7. Formation of the diacetate [2]* against time when HP is treated with pyridine/acetic anhydride (9:1).

As discussed above HPD made from Bonnett acetates contains greater quantities of LRTM. Generally the Lipson acetates, both freshly prepared acetates and acetates obtained from the a commercial supplier[54], form HPD containing 40-60% LRTM, whereas HPD made from the Bonnett preparation of diacetylated HP usually contains over 70%.

A monoacetate [3]/diacetate [2] mixture of similar ratio to the Lipson acetates was prepared using the Bonnett reaction conditions for the synthesis of diacetylated HP but with a reduced reaction time. Standard base polymerisation of this mixture yielded a product with about 60% LRTM, this is lower than for the HPD made from the Bonnett diacetate alone, but generally

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* measurements for this experiment were made using a less sensitive detection and integration system, as discussed earlier.

higher than the amount received from the Lipson acetates. This concurs with previous observations[4,29] that the proportion of diacetate [2] used in producing the HPD is important in determining the quantity of LRTM produced.

Although the acetylation results are difficult to reproduce exactly it is clear that acetylation using the acetic acid/sulfuric acid procedure gives rise to a mixture of monoacetates [3] and diacetate [2] whereas the acetic anhydride/pyridine method gives the diacetate [2]. What was not clear was why the aggregate fraction of HPD derived from Bonnett diacetate should be mainly ester linked whereas the Lipson acetate mixture produced mainly ether linked oligomeric material. Accordingly a variety of factors were investigated to establish whether they were of importance in determining the nature of the linking group between the porphyrins in the oligomeric material.

It is well known that porphyrins can aggregate in water and organic solvents[55,56]. If the porphyrins aggregate in a head-to-head (Fig. 8 (B)) manner the 1-hydroxyethyl groups in the 3 and 8 positions, or their derivatives, would be in close proximity on adjacent porphyrins and an ether linkage would be likely. If the porphyrins aggregate in a head-to-tail (Fig. 8 (A)) manner then the 1-hydroxyethyl groups, or their derivatives, would be adjacent to a carboxylic acid, or carboxylate, group and an ester linkage would be more likely. The orientation of the acetates may be dictated by the solvent in which they are formed. The Bonnett diacetate is formed in the basic pyridine/ acetic anhydride solvent and the Lipson acetates are formed in the acidic acetic acid/sulfuric acid solvent. If this interaction persists during the workup and when the acetates are dissolved in base to form the HPD then the orientation may determine the mode of coupling.

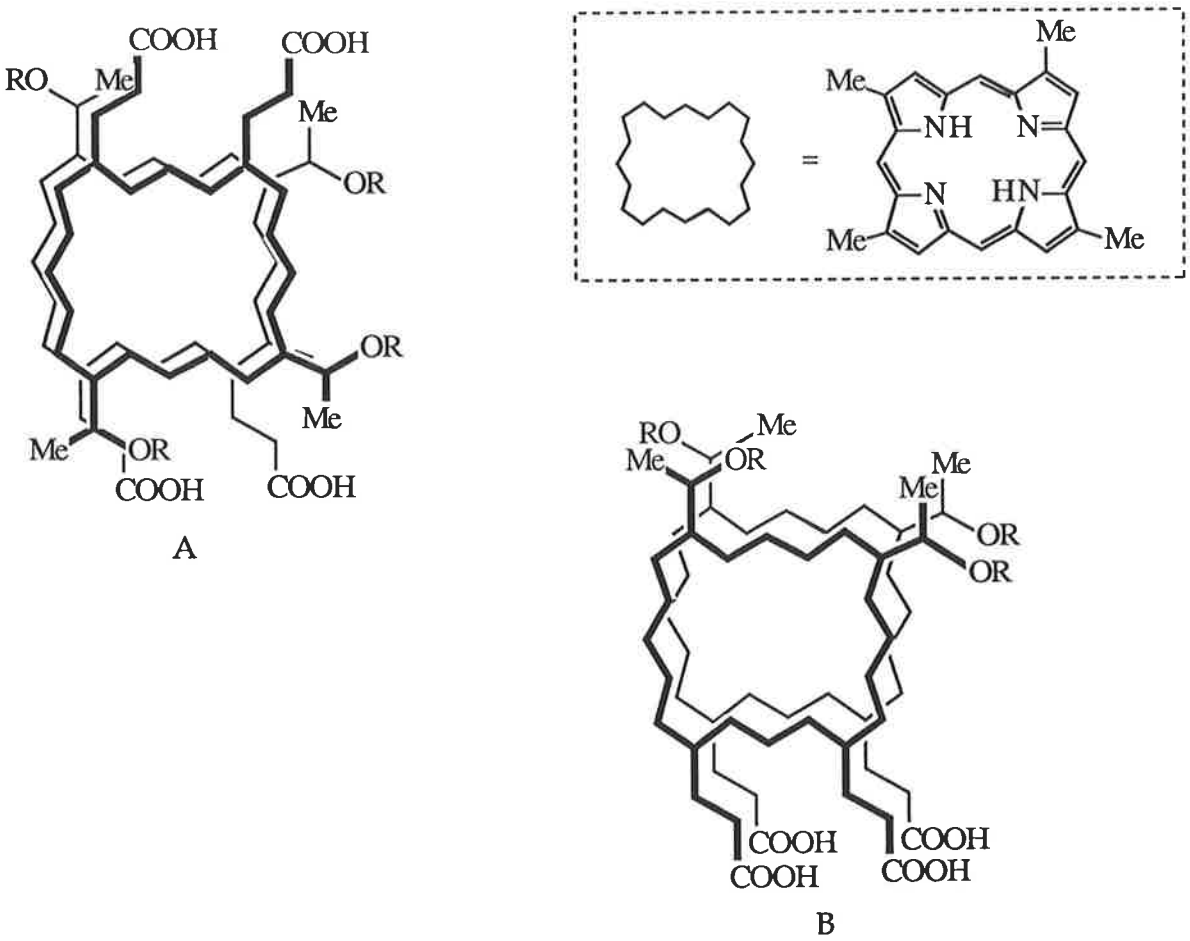

Figure 8. (A) head to tail aggregation. (B) tail to tail aggregation

To investigate the effect, if any, of solvent prior to collection of the acetate mixture a sample of Bonnett acetate was treated briefly with acetic acid/sulfuric acid (19:1), Table 1, and then both materials were converted to HPD (Table 2) and hydrolysed using basic conditions.

Table 1. Treatment of the Bonnett diacetate with acetic acid/sulfuric acid (19:1).

	HPLC (%)		
	diacetate [2]	<15min	>15min
Bonnett acetate (A)	60	22	18
Bonnett acetate after treatment with $AcOH/H_2SO_4$ (B)	60	21	19

Table 2. Formation and basic hydrolysis of HPD from the Bonnett acetates (A) and (B) (Table 1).

	HPLC (%)			
	HP	HV	<15min	>15min
HPD (from Bonnett acetate (A))	7	8	11	74
-basic hydrolysis	43	22	13	22
HPD (from Bonnett acetate (B))	13	13	9	65
-basic hydrolysis	34	21	14	31

The normal Bonnett acetate sample (A) formed 74% of LRTM (Table 2) of which 70%* was ester linked as established by basic hydrolysis. However the Bonnett acetate sample that had been treated with the acetic acid/sulfuric acid mixture; although apparently barely affected by the treatment (Table 1), gave less LRTM and which contained only 53% ester linked material. These results suggest that exposure of the acetylation product to acidic conditions results in the production of less LRTM compared to the untreated acetates but the LRTM produced contains a greater percentage of ether linked material compared to the normal Bonnett procedure.

HPD formed from a mixture of mono [3] and diacetates [2] made using a shortened Bonnett procedure[25] (pyridine/acetic anhydride) consists of about 40 to 60% ester linked material by basic hydrolysis. This figure is less than that formed from the diacetate alone (70% ester linked) and greater than that observed for HPD formed from the Lipson acetates, which generally contains 15%, or less, ester linked material.

Another area where it was perceived differences in the HPD formed may arise was in the workup of the acetate(s) mixture. If the acetates are collected from aqueous media by filtration or centrifugation then they may aggregate differently than if they are extracted from aqueous medium by organic solvents and this may result in a different polymer when the acetates are treated with 0.1N sodium hydroxide to give HPD if orientation is a significant factor in determining the nature of the linkage.

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* The HPD consisted of 74% LRTM, when it was hydrolysed, under basic conditions, the product consisted of only 22% LRTM which is 30% of the initial LRTM. This means that 70% of the original LRTM was hydrolysed by the basic conditions and is therefore ester linked.

A mixture of the mono [3] and diacetates [2] was made from HP using the pyridine and acetic anhydride method of Bonnett[25] and a shortened reaction time. Half of the reaction mixture was worked up following Bonnett's procedure (cooled, acetic acid added, water added and the precipitate collected by centrifugation) to give the acetate mixture (5% HP, 29% monoacetate [3], 35% diacetate [2]), the other half of the mixture was poured into water, the pH adjusted to 5 and the product (7% HP, 23% monoacetate [3], 33% diacetate [2]) extracted into dichloromethane/tetrahydrofuran. The acetate mixtures obtained by both methods were similar, by HPLC, although there was a slightly less monoacetate [3] isolated from the extractive workup.

HPD made from the monoacetate/diacetate mixture that had been collected by a workup involving an extraction into an organic solvent contained 73% LRTM of which 60% was hydrolysed by base. HPD made by the normal Bonnett workup procedure, involving centrifugation from an acidic solution, gave only 59% LRTM, of which only 37% was hydrolysed by base (ie. ester linked). Again the conclusion can be reached that the previous history of the acetylated material may influence the way in which it oligomerises and the extent to which it oligomerises.

In the above experiments it appears that there is some correlation between the amount of LRTM formed and its composition. In both cases where diacetylated HP [2] was exposed to the Lipson conditions, and where the mono-diacetate mixture was isolated by centrifugation rather than organic extraction, the yield of LRTM was reduced but the ratio of ether linkages present was increased. This may indicate that ester formation, where the conditions are favourable, is more rapid than hydrolysis, or elimination, of the acetate groups to form HP or HV, whereas ether formation competes less favourably with these reactions.

Kessel *et al.* lyophilised their acetate material prior to treatment with base to form HPD.[45] It was thought that lyophilisation may assist dehydration leading to the formation of greater amounts of vinyl containing products and hence the effect of drying the Bonnett acetate was investigated. HPLC data showed that the Bonnett acetate was unaffected by being dried under vacuum (0.01mm Hg) at room temperature for seventeen hours or at elevated temperatures (50-70°) for 1.5 hours. The effect of lyophilisation on the Lipson acetates was investigated. Comparison of the Lipson acetates worked up by the normal procedure versus a lyophilisation procedure showed that the lyophilisation had only caused slight changes to the acetate mixture, by HPLC. However the lyophilised acetates formed a greater amount of LRTM (68% vs 57%) which was 21% ester linked compared with the HPD formed from the non-lyophilised material which was virtually all ether linked.

The results obtained indicate that the nature of the linking group in HPD is influenced by the ratio of mono [3] to diacetate [2] used in the formation of HPD used and also by method of preparation of the acetylated material used to form HPD. For example, use of the Bonnett acetate favours formation of ester linkages but exposure of that acetate to the solvent conditions used for the production of the Lipson acetate resulted in a decrease in the amount of active material produced but an increase in the amount of ether linkages. Similarly if the Bonnett acetylation is utilised to give a mixture of mono and diacetates it gives less LRTM in HPD formed from it, which is more ether linked, than HPD formed from the Bonnett acetates.

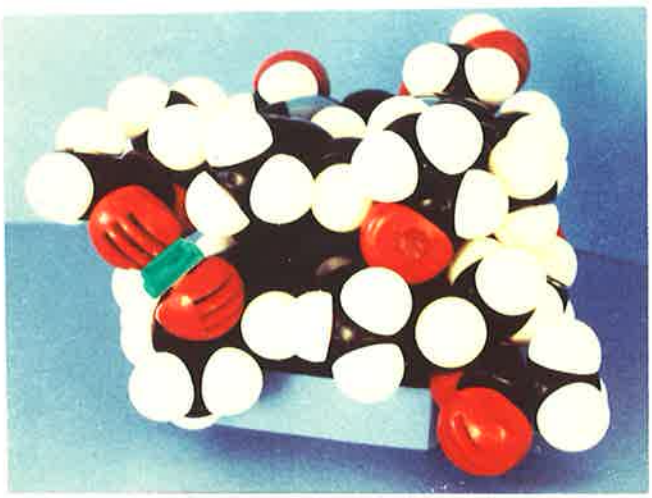
The preference of the diacetate [2] to form esters can be rationalised in the following manner. Porphyrins are known to aggregate via $\pi-\pi$ interactions between the porphyrin rings[55,56]. Dicarboxylic porphyrins with electron withdrawing groups in the 3 and 8 positions tend to aggregate in an offset[57]

head-to-tail manner[58], as illustrated in Figure 8 (A), due to dipole-dipole interactions. Examination of molecular models show that this is also the most favourable configuration sterically for the diacetates. In this configuration it can be seen that the carboxylate is placed in a favourable position to execute an S_N2 nucleophilic attack on the acetate to form the ester linkage (Fig. 9a).

An investigation of aggregation between two monoacetylated HP [3] molecules, using models, shows that, although the dipole-dipole interactions discussed above may also favour a head-to-tail orientation in this case a head-to-head orientation is less sterically hindered and hydrogen bonding can occur between the hydroxyl group on one porphyrin and the acetate on the other, stabilising the arrangement. Aggregation in this manner places the free hydroxyl, in a position to displace the acetate on the other porphyrin to form the ether linkage (Fig. 9(b) and (c)). The hydrogen bonding may occur with either the carbonyl oxygen (Fig. 9(b)) or the alkyl oxygen (Fig. 9(c)) of the acetate group, with the initial case being favoured electronically but the latter case giving a more satisfactory approach for nucleophilic attack as indicated by the models. If prior displacement of the acetate occurs by the elimination/addition sequence, shown in Scheme 1, then the same arguments apply to the addition of the free hydroxyl to the alkene carbon.

Although ether formation is favoured between monoacetates [3] it still does not account for the extent of ether formation in HPD made using the Lipson acetates, where there may be as much as 57% of the diacetate [2] in the acetate mixture used. Hydrogen bonding to stabilise the head-to-head orientation of porphyrins may occur between any porphyrin with a hydroxyl group and one with an acetate group, hence a diacetate and monoacetate may align head-to-head, stabilised by hydrogen bonding, and as one of the acetate groups is hydrolysed then the ether linkage is formed. This mechanism for ether

(a)



(b)

(c)

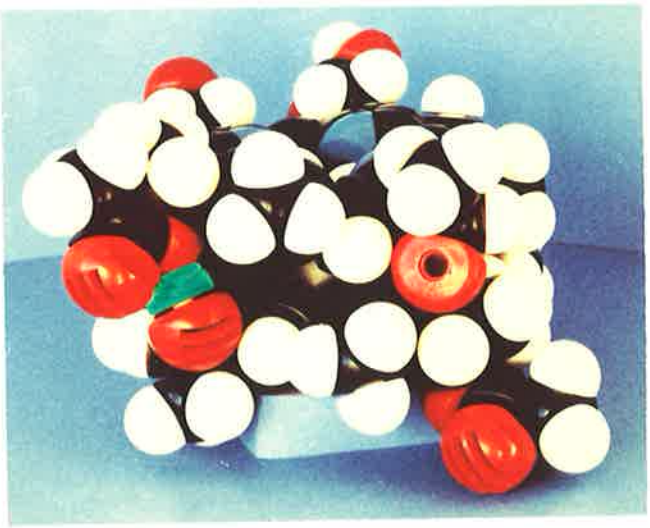


Figure 9. Two diacetate [2] molecules aggregated in a head-to-tail manner (a) showing the favourable configuration for formation of the ester linkage. Two aggregated monoacetate [3] molecules exhibiting hydrogen bonding (shown in green) between (b) the carbonyl oxygen of the acetate, or (c) the alkyl oxygen, on one molecule and the hydroxyl group on the other molecule giving a favourable configuration for formation of the ether linkage. The leaving acetate group in the three diagrams is seen in the lower right hand corner of each.

formation only accounts for dimer formation, oligomer formation requires disruption to the initial hydrogen bonding for further ether formation.

The ester formation by head-to-tail orientation is extendable to oligomer formation as it allows stacking of multiple porphyrin units and formation of ester linked oligomers as observed in HPD made from the Bonnett acetate.

When the Bonnett and Lipson acetates were chromatographed on Sephadex LH-20 the LRTM fractions obtained were substantially hydrolysed by base indicating the impurities are ester linked material.[59] The Lipson acetates form almost entirely ether linked material, indicating that the ester linked material is hydrolysed during the polymerisation step. It is possible that some of the ester linked material in HPD formed from the Bonnett acetate is due to the initial presence of these ester-linked impurities in the Bonnett acetate.

It appears from the results obtained that the formation of ester and ether linkages is influenced by a number of factors. The Bonnett acetate forms more ester linked material in HPD than Lipson acetates. Mono/diacetate mixtures formed using the Bonnett acetylation procedure results in greater quantities of ester linked material than mono/diacetate mixtures made by the Lipson acetylation and the workup used to isolate the acetates affects the formation of HPD. Kessel *et al.* had also found that the amount of active material formed was also influenced by the concentration of the acetates[60] in the base polymerisation step and the pH[45] of the base solution used for polymerisation.

2.4 The stability of HPD in solution

The stability of HPD, derived from the Bonnett acetate, was investigated. A solution of HPD (13% HP, 10% HV, 4% <15 min., 74% >15 min.) was divided into three portions. One portion was kept as a neutral control (pH 7.1), and one portion was made basic (pH 9.0). Attempts to acidify the other portion to

pH 5 were unsuccessful as precipitation of porphyrin material occurred when the pH of the solution was lowered below 6.4. The standard basic hydrolysis of the 'neutral' and 'basic' solutions was investigated over time.

Basic hydrolysis of the 'neutral' and 'basic' solutions 24 hours after formation showed that the 'basic' solution contained only 12% ether linked material (basic hydrolysis: 44% HP, 30% HV, 16% <15 min., 9% >15 min.), the ester linked material having been hydrolysed during the standard basic hydrolysis or on standing in the basic conditions of the solution. The 'neutral' solution contained 34% ether linked material (basic hydrolysis: 43% HP, 21% HV, 11% <15 min., 25% >15 min.). The pH of both solutions had dropped; from 9.00 to 7.14 for the 'basic' solution and 7.10 to 6.59 for the 'neutral' solution. After 48 hours the 'basic' solution showed a further decrease in pH, to 6.96, and a marked increase in the amount of ether linked material (basic hydrolysis: 34% HP, 22% HV, 12% <15 min., 32% >15 min.) to 43%, after a further 48 hours the hydrolysis results showed little change (basic hydrolysis: 38% HP, 20% HV, 9% <15 min., 34% >15 min.) and the pH had stabilised at 6.8.

The 'neutral' solution, after 96 hours also showed an increase in ether linked material (10% <15 min., 39% >15 min.) to 53% and the pH had stabilised at 6.4.

The results show that some of the ester linked material is interconverting to a base stable material, apparently the ether linked material. The decrease in the pH of the solutions with time is consistent with hydrolysis of the ester groups to form carboxylic acids and alcohols. It may also result from absorption of carbon dioxide from the atmosphere to give a buffered solution.

The stability under neutral conditions of HPD (20% HP, 13% HV, 3% <15 min., 64% >15 min.), derived from a monoacetate/diacetate mixture formed by a shortened Bonnett procedure (acetic anhydride/pyridine) was also investigated.

In this case there were smaller changes from the initial situation. About 40% of the oligomer material was stable to basic hydrolysis (basic hydrolysis; 45% HP, 21% HV, 11% <15 min., 24% >15 min.) when the solution was initially made and the figure increased with time (basic hydrolysis after 48 hours; 34% HP, 21% HV, 16% <15 min., 29% >15 min.) but not as markedly as with the diacetate derived material. A slight increase in pH of 0.04 was observed.

Basic hydrolysis of HPD made from the Lipson acetates (32% HP, 21% HV, 7% <15 min., 41% >15 min.), showed it to be substantially ether linked initially (basic hydrolysis: 30% HP, 26% HV, 11% <15 min., 34% >15 min.), with a slight increase in base stable material over one week (basic hydrolysis; 38% HP, 15% HV, 11% <15 min., 37% >15 min.).

Overall it can be seen that the ester linked material, formed mainly when the Bonnett diacetate is used to make HPD, is not stable in aqueous conditions but is converted to a base stable material assumed to be ether linked material, a result also noted by Kessel[61].

2.5 Acidic hydrolyses of HPD

To this stage the investigation had concentrated upon the analysis of ester versus ether linkages which was assessed by basic hydrolysis. However the acidic hydrolyses which would be expected to show complete hydrolysis of both ester linked and ether linked porphyrin oligomers, were providing some puzzling results. An initial perusal of Fig 2(c) would indicate that the acidic hydrolysis conditions cleave the porphyrin linkages to give mainly HP and small amounts of HV and PP. However a more careful analysis, using electronic integration of the trace, showed that substantial amounts (30-50%) of poorly resolved material is contained in the 20-30 minute region of the trace.

This result was reproducible and was not due variations caused by solvent changes.

Kessel *et al.*[38] had suggested that the use of THF may increase the hydrolysability of material by breaking up aggregation that may occur under aqueous conditions and result in incomplete hydrolysis. A comparison of aqueous hydrolysis conditions and those containing THF showed that hydrolysis by both sets of conditions gave the the same result (HPLC).

It was possible that the acidic hydrolysis conditions were giving rise to the hydrolysis resistant material. However, when commercial HP.2HCl was treated with the acidic hydrolysis conditions there was a decrease in the amount of non-HP material. No LRTM was produced in the acidic hydrolysis of tetramethyl HP [11], see Fig.2.

Chromatography of acid hydrolysed HPD (100mg), using a Sephadex LH-20 column, which separates mainly based on size, and eluting with tetrahydrofuran, methanol and water[29] gave four fractions which were analysed by HPLC (Table 3).

Table 3. HPLC analysis of Sephadex fractions.

	HPLC (%)				
fraction	HP	HV	<15min	>15min	quantity
A	0	1	0	99	12mg
B	2	5	7	87	29mg
C	6	9	26	59	19mg
D	57	34	3	6*	36mg

*contains 2% PP

This result confirms the presence of quite large quantities of material that are not hydrolysed by the acidic conditions. To see if LRTM was being formed during chromatography a sample of HPD that had been hydrolysed using acidic conditions (44% HP, 23% HV, 5% <15 min., 28% >15 min.), was chromatagraphed on Sephadex and the fractions recombined (39% HP, 18% HV, 12% <15 min., 31% >15 min.). This showed that while some unidentified material of less than 15 minute retention time was formed other significant changes had not occurred during chromatography.

In an effort to distinguish at which stage of the procedure this acid stable oligomeric material was being formed the acetate mixture from which HPD is derived was investigated. A sample of the Lipson acetates (3% HP, 26% monoacetate [3], 51% diacetate [2], 9% <15 min, 12% >15 min) was chromatographed on a Sephadex LH-20 column (Table 4).

Table 4. Fractions obtained from Sephadex LH-20 chromatography of Lipson acetates.

	HPLC (%)					
Fraction	HP	mono-acetate	diacetate	<15min	>15min	Weight
A	7	0	0	2	91	5mg
B	0	16	58	8	18	53mg
C	5	32	45	11	8	34mg
original	3	26	51	9	12	100mg

Acidic hydrolysis of fraction A showed that all but a small amount (15%) of the LRTM was hydrolysed by acid.

Rechromatography of Fraction B gave a fast-running, LRTM enriched fraction (0% HP, 1% monoacetate [3], 17% diacetate [2], 3% <15 min, 79% >15 min), which was hydrolysed substantially by both base (62% HP, 7% HV, 14% <15 min, 18% >15 min) and acid (68% HP, 10% HV, 10% <15 min, 12% >15 min) giving the same ratio of non-acid hydrolysable LRTM as Fraction A.

Fraction C, which contained only 8% LRTM, was converted to HPD, containing 50% LRTM, when treated with 0.1N sodium hydroxide. Basic hydrolysis of this material caused only 10% hydrolysis of the LRTM and acidic hydrolysis caused only 30% hydrolysis, indicating the presence of a substantial amount of acid stable LRTM (Table 5).

Table 5. Hydrolysis of HPD formed from Fraction C.

	HPLC (%)			
	HP	HV	<15min	>15min
HPD	24	20	6	50
basic hydrolysis	26	21	8	45
acidic hydrolysis	33	22	10	35

From these results it is apparent that the majority of the acid stable material is generated during the second step of the Lipson procedure, the treatment of the acetates with sodium hydroxide.

Some time after this phase of the research was completed it was shown that the acid stable material, in HPD, comprised carbon linked oligomeric material[62]. This carbon linked material was only found in HPD made from the Lipson acetates, but not in HPD made using the Bonnett diacetate.

The above results, while clear in terms of trends and general indications, indicate that a detailed analysis of the situation is complicated by the complex nature of the materials being studied and the variations in the results obtained from one run to another. Accordingly it was decided that synthetic dimers and trimers linked unambiguously by either ether or ester bonds were required as model compounds to study the complicated chemistry further.

2.6 'Healux' material

At the time that these hydrolyses were being investigated another 'porphyrinal' material was developed that was apparently oligomeric by HPLC

analysis. This, commercially available[63], material was known as Healux and was believed[64] to by formed by heating HP in water at 90°.

The formation of this material was investigated. HP.2HCl was dissolved in water and the pH of the solution was adjusted to 7.1 using aqueous sodium hydroxide. The solution was heated at 90° to 93°. After 2 hours the solution appeared slightly browner in colouration and reverse phase HPLC analysis showed an increase in HV, but very little polymerisation. After 22 hours heating not all the HP had been consumed so the solution was heated for a further 26 hours. Figure 10 shows an HPLC trace of the material at the end of the 50 hours heating.

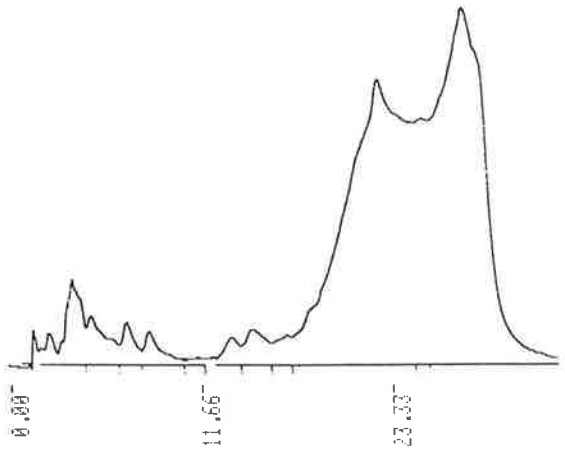


Figure 10. HPLC trace of material obtained when HP is heated at 90° for 50 hours.

The product was chromatographed on Sephadex LH-20 to remove small amounts of the residual monomers, HV and PP. The chromatography had to be done with the exclusion of light as the compound bleaches and adheres to the column when exposed to diffuse light.

The product showed a basically 'etiotype' visible spectrum[65] with a small additional absorbance observed at 640nm. The absorbance at 640 nm is probably due to formation of photoproducts; photoproducts have been observed to form when concentrated solutions of HP (>10^{-5} M) were exposed to

light[66]. Although the reaction was shielded from direct light, total light exclusion during the reaction and product isolation was not attempted.

When the material was treated with the standard basic hydrolysis conditions there was very little change in the HPLC trace (Fig 11 (a)) compared to the original (Fig 11 (b)) and no recognizable monomers were produced. Acidic hydrolysis conditions caused some cleavage to give HP and HV and there was a change in the general shape of the LRTM (Fig 11 (c)), this may indicate some hydrolysis is occurring but the resulting products are still oligomeric. The material was inactive when tested *in vivo* for anticancer activity.

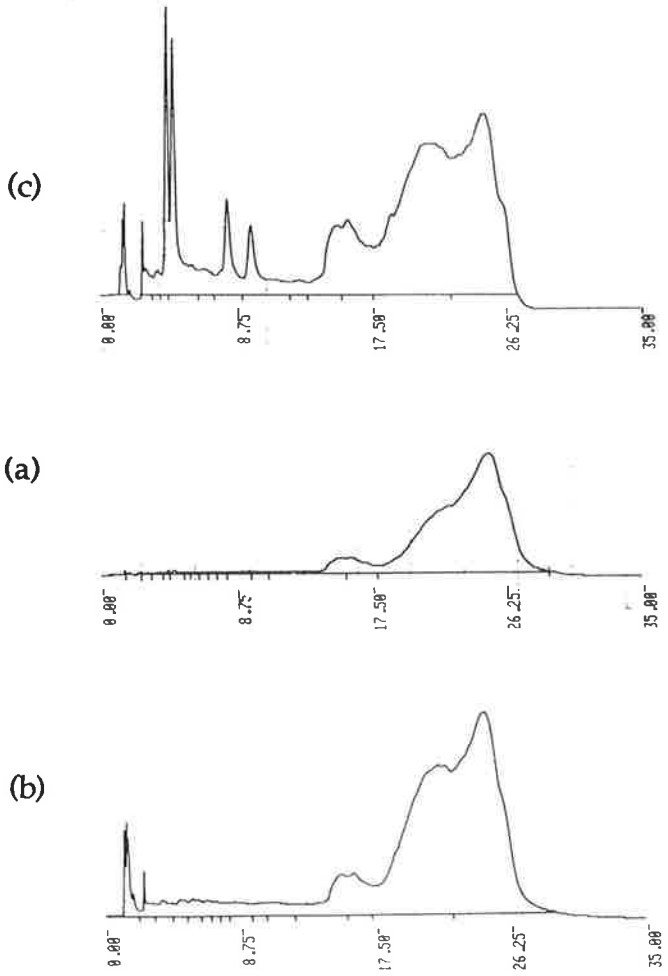


Figure 11. HPLC traces of (a) The fast fraction obtained by chromatography of the 'Healux' material on Sephadex LH-20. (b) the fast fraction, shown in (a), after basic hydrolysis. (c) the fast fraction, shown in (a), after acidic hydrolysis.

The product was methylated using methanol and trimethyl orthoformate[47]. The FAB mass spectrum of the methylated product showed peaks compatible with compounds containing at least five porphyin units. A peak at *m/z* 591 was compatible with PP.DME and one at *m/z* 623 may be due to the methyl ether of HV.DME. Both of these products may arise from fragmentations. Peaks in the dimer region at *m/z* 1231 and 1217 are compatible with ether, ester and carbon linked dimers which are not fully methylated but a major peak in the dimer region (*m/z* 1247) is compatible with a fully methylated monodehydrated carbon linked dimer (Fig. 12) rather than any of the possible ether or ester linked dimers. At higher mass units, the trimer and tetramer region, the spectrum becomes harder to interpret, due to lower resolution, but the peaks are more compatible with the carbon linked material and its partially dehydrated analogues than the ether or esters, showing peaks at higher mass than may be expected for fully methylated ether or ester trimers and tetramers, which contain less hydroxyl groups than the carbon linked analogue. Some of the peaks, such as *m/z* 1201, do not correspond to any of the expected peaks but may be due to fragmentation of higher order oligomers or polymers.

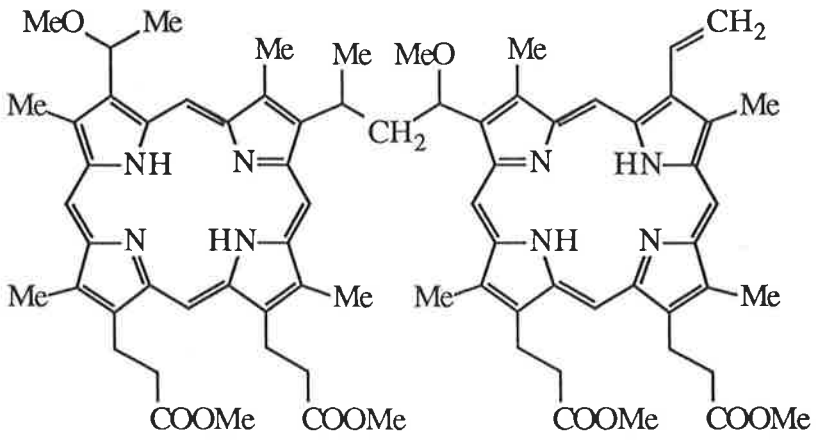


Figure 12. One of the possible isomers for a fully methylated monodehydrated carbon linked dimer.

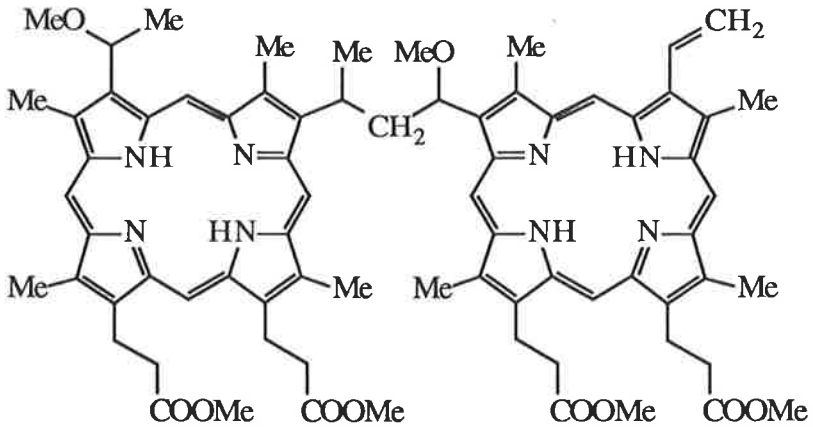


Figure 12. One of the possible isomers for a fully methylated monodehydrated carbon linked dimer.

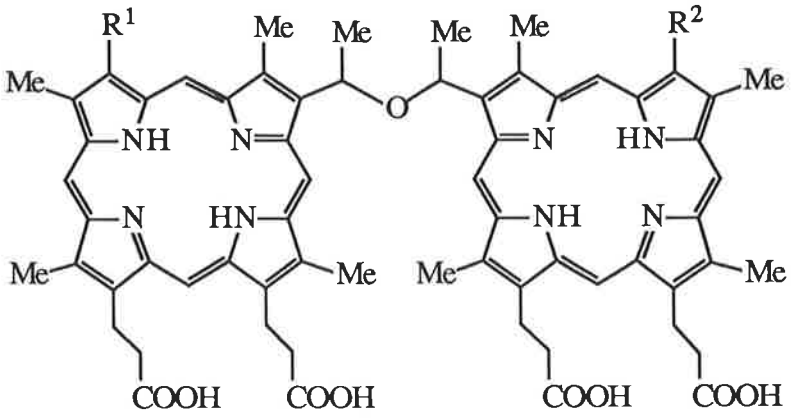
The proton n.m.r. spectrum of the methylated material was very poorly resolved and provided no structural information. The ^{13}C n.m.r. spectrum of the material showed most of the expected porphyrin resonances; the ring methyl carbons, propionic side chains, methine carbons, pyrrole ring carbons and the ester carbonyl resonances. A peak at 51.6 ppm is due to the methyl ester groups. There are no peaks between 25 and 26 ppm which is where the methyl carbons of the 1-hydroxyethyl and 1-alkoxyethyl sidechains normally resonate. Unidentified resonances occur at 30 ppm and a weak multiple set of peaks at 58 ppm, which may be due to methyl ethers. Two peaks are seen at 35.6 and 36.6 ppm, one, or both, of which are due to methylene protons in the propionic sidechain.

The product is clearly not ether or ester linked to any great degree based on its ^{13}C n.m.r. spectrum and hydrolysis studies. It may be the carbon linked material identified in HPD and Photofrin II.[62,67] However this can not be confirmed or discounted as the carbon linked material has only been isolated as the fully dehydrated.analogue and the alcohol containing material has not been prepared.

Chapter 3. Synthesis of dihematoporphyrin ether.

3.1 Introduction

In 1984 dihematoporphyrin ether, DHE* [7], was proposed[30] as the active fraction of HPD. Some of the evidence for this structure was ambiguous and did not eliminate the possibility of higher order polymers[29,31,33]. Synthesis of the dihematoporphyrin ether [7] would enable comparison with HPD to determine if DHE [7] is a component of the active fraction of HPD and whether it is active in its own right. The techniques employed in the synthesis of the dihematoporphyrin ether [7] should be extendable to the synthesis of other dimers that may conceivably be present in HPD such as the mono [13] and didehydrated [14] analogues of DHE and the higher molecular weight analogues, for example the trimers and tetramers, in order to allow them to be compared with HPD.



[13] $R^1 = CH(OH)CH_3$ $R^2 = CH{=}CH_2$
[14] $R^1 = R^2 = CH{=}CH_2$

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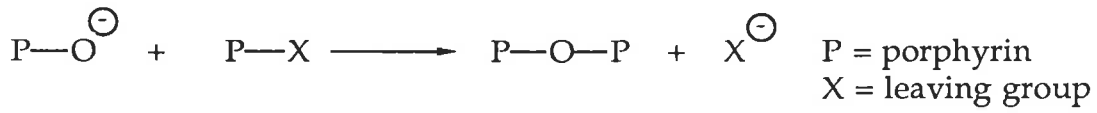
* The abbreviation, DHE, has often been used to describe the total active fraction of HPD. It has been proposed that this should no longer occur and consequently, in this thesis, the term dihematoporphyrin ether, DHE, will only be used to describe the compound [7].

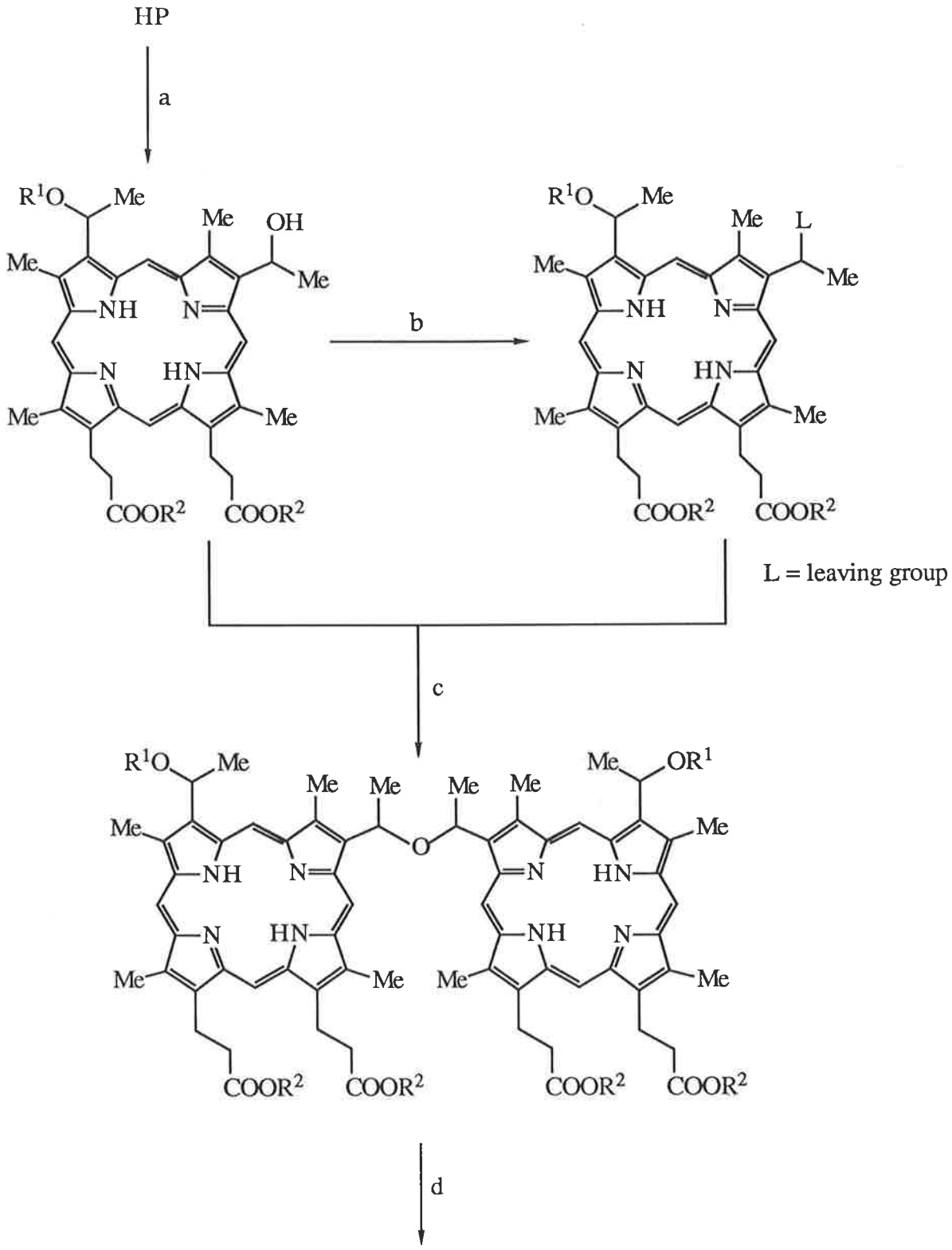
This chapter describes approaches to the synthesis of DHE [7] via a number of methods which are based on the classical Williamson ether synthesis[44], as shown in the generalised scheme below (Scheme 1).



Scheme 1. Generalised ether synthesis.

To enable the synthesis to be unambiguous a number of conditions needed to be met. First, the conditions for the coupling must not cause hydrolysis of the ester groups. If free carboxylic acids were formed in this manner they could participate in the reaction to form ester linked dimers. Second, only one hydroxyl on each porphyrin should be free to react, or oligomers of various length can be formed without control. With these conditions in mind the following synthetic route was proposed (Scheme 2).

The formation of a suitable protecting group for the non-reacting hydroxyl of HP and for the propionic acids was investigated first. It was considered that the propionic acids may be protected as their methyl esters, which could be removed by basic hydrolysis. From hydrolysis studies of tetramethylated HP [11], discussed in Chapter 2, it was known that the methyl esters could be hydrolysed under basic conditions without affecting the methyl ethers and it was considered that this selectivity should extend to the diporphyrin ether. It was possible, however, that the doubly benzylic ether in the dimer may be more labile than the singly benzylic ether in tetramethyl HP [11]. It was also possible that rearrangements, such as those that appear to occur during the formation of HPD to give carbon linked polymers (see Chapter 2) may occur under basic aqueous conditions for hydrolysis. The best scenario would be to



if $R^1 \neq R^2$ then steps a and d may each consist of 2 steps

Scheme 2. Synthesis of dihematoporphyrin ether [7]

have hydroxyl and acid protecting groups formed from the same reagents (Scheme 2, $R^1 = R^2$) in a one pot reaction and for these groups to be removed simultaneously under nonaqueous conditions without affecting the newly formed ether linkage.

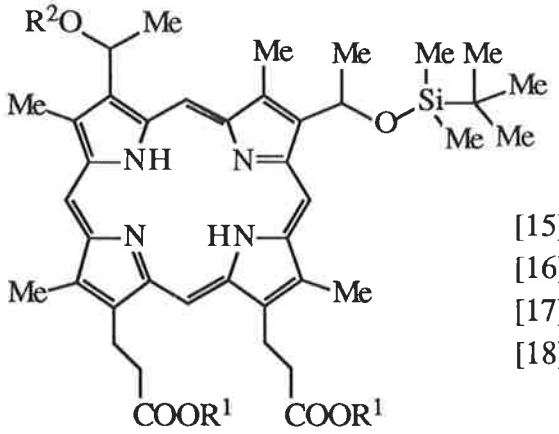
3.2 *tert*-Butyldimethylsilyl ethers and esters

tert-Butyldimethylsilyl [TBDMS][69] and benzyl[70] systems were selected as protecting groups to investigate first. *t*-Butyldimethylsilyl [TBDMS] groups on the hydroxyl[69] and carboxylic acids[69] should be removable using fluoride ions in organic solvents[69,71,72,73]. *t*-Butyldimethylsilyl ethers are stable to a wide range of conditions[69] however TBDMS esters are much less stable[70].

It had already been established[74] that *t*-butyldimethylsilyl trifluoromethanesulfonate (TBDMS triflate)[75] was the reagent of choice for the silylation of HP.DME [10] as both N-(*t*-butyldimethylsilyl) acetamide[76] and *t*-butyldimethylsilyl chloride[77] were extremely slow and inefficient as silylating reagents for this system.

TBDMS triflate can be prepared from *t*-butyldimethylsilyl chloride and trifluoromethanesulfonic acid[75] although it is now commercially available[78].

Both hydroxyl functions on HP.DME [10] were silylated by treating HP.DME with a large excess of TBDMS triflate and 4-dimethylaminopyridine (DMAP) as the base. When less TBDMS triflate and/or shorter reaction times were used a mixture of bissilylated [15], monosilylated [16] and unreacted HP.DME [10] was produced. Hematoporphyrin $3^1(8^1)$-(*t*-butyldimethylsilyl) ether dimethyl ester [16] was isolated by chromatography on silica, using 1% methanol in dichloromethane, in 30-43% yield.



[15] $R^1 = CH_3$ $R^2 = Si(CH_3)_2C(CH_3)_3$
[16] $R^1 = CH_3$ $R^2 = H$
[17] $R^1 = R^2 = Si(CH_3)_2C(CH_3)_3$
[18] $R^1 = H$ $R^2 = Si(CH_3)_2C(CH_3)_3$

The FAB mass spectrum of the bissilylated compound [15] showed a strong (M+H) peak at *m/z* 855 with a minor peak at *m/z* 723, due to elimination of *t*-butyldimethylsilanol. The FAB mass spectrum of the monosilylated compound [16] also showed a strong (M+H) peak at *m/z* 741 with two main fragmentations to *m/z* 723 (loss of water) and 609 (loss of TBDMS-OH). Elimination of water[79,80] and alcohol[81] from the 1-hydroxyethyl and 1-alkoxyethyl sidechains, respectively, in the mass spectrometer is well documented under electron impact (E. I.) conditions. A MIKES spectrum of *m/z* 741 confirmed the peaks at *m/z* 723 and 609 to be at least partially due to fragmentations of the molecular ion.

The ^1H n.m.r. spectrum of bissilylated HP.DME [15] has the resonances[56] expected for a derivative of HP.DME and peaks attributable to the TBDMS groups at 0.09 ppm, 0.32 ppm and 1.04 ppm in a 1:1:3 ratio. Multiple peaks are observed at each of these shifts due to the mixture of isomers and also to minor anistropic affects described below. The most interesting feature of the spectrum are the two different shifts at -0.09 and 0.32 ppm for the diastereotopic methyl groups atta ched to the silicon atom. An examination of a space filling model of [15] showed that the bulky *t*-butyl group blocks free rotation of the TBDMS group which assumes a preferred conformation in which one of the methyl groups is placed closer to the porphyrin nucleus and

is affected by the deshielding anisotropic affect of the ring causing a downfield shift compared to the other methyl group. The proton on the 1-ethyl carbon is seen as a poorly resolved quartet at 6.5 ppm.

The ^{13}C n.m.r. spectrum of [15] shows resonances expected for a derivative of HP.DME[56] and resonances due to the TBDMS group. The methyl carbons adjacent to the silicon atom resonate at -4.8 ppm and the *t*-butyl group shows a strong resonance at 26 ppm, due to the methyl carbons, and a weak resonance at 18 ppm due to the quaternary carbon atta ched to silicon.

The monosilylated porphyrin [16] is a mixture of regioisomers as well as stereoisomers and this is reflected in its more complex proton n.m.r. spectrum where all the resonances show multiple peaks. The anisotropic effect of the porphyrin ring on the methyl groups attatched to the silicon atom, observed in the bissilylated material, is again observed with resonances at -0.9 and 0.3 ppm. A quartet is observed at 6.51 ppm for the proton at the 1-position on the 1-(*t*-butyldimethylsiloxyethyl) sidechain, but the proton at the 1-position on the 1-hydroxyethyl sidechain is observed as two multiplets at 6.0 and 6.2 ppm, this may either be due to the two regioisomers or diastereomers. The ^{13}C n.m.r. spectrum contained similar resonances to those observed in the bissilylated compound [15], although multiple peaks were observed at some shifts due to the presence of the regioisomers. A pair of additional resonances, at 65.9 ppm and 65.8 ppm, were observed for the carbon bearing the hydroxyl group.

Johnstone[74] had found that reacting HP.2HCl with a large excess of DMAP and TBDMS triflate formed a single compound which was fast running on tlc, he was able to isolate this material by flash chromatography and characterise it as hematoporphyrin $3^1,8^1$-bis(*t*-butyldimethylsilyl) ether (*t*-butyldimethylsilyl) ester [17]. He also noted its instability. Repetition of this reaction showed that,

although the reaction, by tlc, appeared to proceed entirely to the tetrasilylated material [17], the product was very difficult to isolate. The most successful method, eluting the reaction mixture from a flash column which had been prewashed with 1% methanol in dichloromethane, resulted in only 27% yield. The mass spectrum of this compound did not show a molecular ion, the major peak was at *m/z* 827 with a minor peak at *m/z* 695, these peaks are probably due to bissilylated HP [18] and its expected fragment ion; the TBDMS esters having cleaved in the mass spectrometer. The silyl esters were particularly sensitive to hydrolysis under aqueous conditions. Washing a solution of the [17] with dilute aqueous acid or base resulted in formation of a polar material (R_f 0.0). This material showed a single peak in the reverse phase HPLC trace, and a mass spectrum compatible with bissilylated HP [18]. Obviously the TBDMS esters are too unstable to be of any synthetic value.

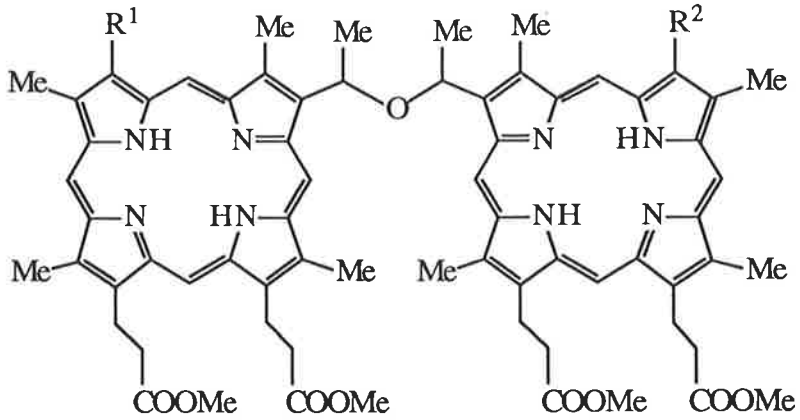
The TBDMS groups were removed from the bissilylated dimethyl ester [15] to give HP.DME [10] using tetrabutylammonium fluoride in tetrahydrofuran at room temperature.

3.3 Benzyl ethers and esters.

The other protecting system investigated was the benzyl group. HP dibenzyl ester [19] has been synthesized by transesterification of HP.DME with benzyl alcohol[24] using a catalytic amount of sodium and can be removed by catalytic hydrogenation.[24] If the benzyl ethers can be synthesised then both esters and ethers may be removable by hydrogenolysis simultaneously.

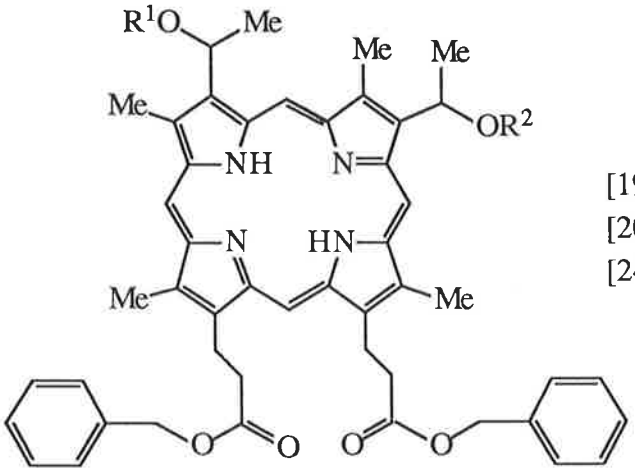
It was anticipated that the benzyl ethers may be formed by the reaction of 1-bromoethyl porphyrins with benzyl alcohol. The 1-bromoethyl side chain may be formed by addition of hydrogen bromide to either a vinyl sidechain[3,37,82] or the 1-hydroxyethyl side chain[37,83,84].

HP.DME dissolved in dichloromethane was treated with a solution of hydrogen bromide in acetic acid, the volatiles were removed, and the crude dibromide was redissolved in dichloromethane and benzyl alcohol added. The reaction gave a mixture of products (tlc) with very little in the high R_f area expected for hematoporphyrin dibenzyl ether dimethyl ester [20] apart from a faint spot due to PP.DME. The FAB mass spectrum of this mixture showed peaks compatible with the elimination products, PP.DME [9] and HV.DME [21], and some unidentified peaks, but no peaks compatible with the desired benzylated compounds were observed. Although PP.DME was the major compound by mass spectral analysis, its presence by tlc was minor, indicating that the PP.DME observed in the mass spectrum, is due mainly to fragmentation of higher molecular weight peaks. An important feature of the mass spectrum was the presence of peaks corresponding to dimeric material. Two small peaks were observed in the dimer region at m/z 1199 and 1217; these masses correspond to the mono [22] and didehydrated [23] methyl esterified dimers. The actual dimer concentration in the sample is probably greater than is indicated by the mass spectrum. The higher mass of the dimers make them less volatile in the source of the mass spectrometer[85] than monomers and their ability to fragment to components such as PP.DME also reduces their intensity in the mass spectrum. From the presence of dimeric and vinyl compounds, rather than the desired benzylated compounds, it appears that elimination of either hydrogen bromide or water from the 1-bromoethyl or 1-hydroxyethyl sidechains, respectively, or condensation between two porphyrin units, is occurring in preference to reaction with benzyl alcohol or before the addition of benzyl alcohol. Although this method did not appear useful for the synthesis of the benzyl ethers, it appeared to have potential for the synthesis of the dimers; this aspect of the reaction is discussed later in this chapter.



[22] $R^1 = CH(OH)CH_3$ $R^2 = CH{=}CH_2$
[23] $R^1 = R^2 = CH{=}CH_2$

Alkyl ethers and esters of HP have been formed using a mixture of the alkyl alcohol and its corresponding trialkyl orthoformate.[47] A trial reaction, treating HP with tribenzyl orthoformate and benzyl alcohol, and a small amount of concentrated hydrochloric acid showed promising results, with HP monobenzyl ether dibenzyl ester [24] being the main product[86] by mass spectral analysis. Altering the reaction temperature and/or the acid concentration did not significantly alter the product ratio. Use of trifluoroacetic acid, in place of concentrated hydrochloric acid, increased the relative amount of the tetrabenzylated material [20].



[19] $R^1 = R^2 = H$
[20] $R^1 = R^2 = CH_2C_6H_5$
[24] $R^1 = H$ $R^2 = CH_2C_6H_5$

The reaction was done on a preparative scale. With simple alkylations the nonporphyrin byproducts and excess reagents may be removed at low temperature under vacuum, however, in this reaction the byproducts tend to cochromatograph with the porphyrins, especially with the tetrabenzylated HP [20], making purification difficult. Careful chromatography enabled the monobenzyl ether dibenzyl ester [24] and the dibenzyl ester [19] to be purified and further chromatography allowed the tetrabenzylated HP [20] to be isolated.

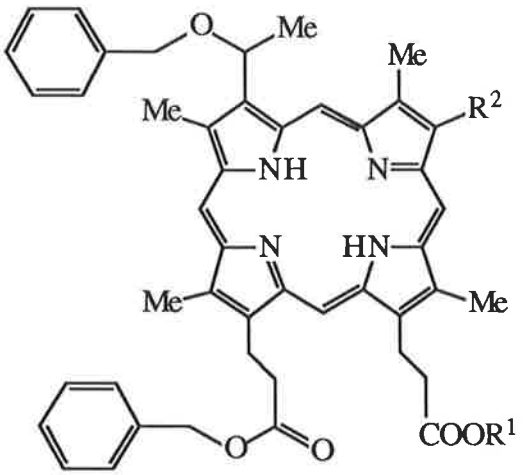
The ^{1}H n.m.r. spectrum of the tetrabenzylated HP [20] displays, apart from the basic porphyrin resonances,[56] resonances attributable to the benzyl groups. Two resonances of multiplets at 7.01 and 7.35 ppm in a 1:1 ratio are due to the aryl protons of the benzyl group. Comparison with the known HP dibenzyl ester [19][24] allows the resonance at 7.01 ppm to be assigned to the aryl protons of the benzyl ester and hence the resonance at 7.35 ppm to be assigned to the benzyl ether. The benzyl ethers are deshielded relative to the esters due to their proximity to the deshielding anisotropic affect of the porphyrin ring. Resonances at 4.70, 4.87 and 5.04 ppm, in a 1:1:2 ratio, are due to the methylene protons in the benzyl moiety. The resonance at 5.04 ppm is due to the benzyl esters[24] and the two resonances at 4.70 and 4.87 ppm are due to the diastereotopic hydrogens of the benzyl ether.

The ^{13}C n.m.r. spectrum is as expected with resonances due to the benzyl groups at 71.1 and 72.2 ppm for the methylene carbons and 127.6, 128.0, 128.2 and 128.4 ppm for the aromatic carbons in addition to the usual porphyrin resonances[56].

The ^{1}H n.m.r. spectrum of the HP monobenzyl ether dibenzyl ester [24] exhibits similar chemical shifts to the spectrum of the tetrabenzylated HP [20] but it is more complex, due to the presence of regioisomers in addition to diastereomers, and exhibits two additional resonances at 5.7 and 5.9 ppm

which are attributable to the methine proton on the 1-hydroxyethyl sidechains in the 3 and 8 positions. The benzyl ether and ester resonances are present in a 1:2 ratio.

In the ^{13}C n.m.r. spectrum increased complexity is seen with multiple peaks observed for the ring methyls and additional resonances at 26.1 ppm, due to the methyl carbon of the 1-hydroxyethyl sidechain, and 65.7 and 65.6 ppm due to the tertiary carbon of the 1-hydroxyethyl sidechain.



[25] $R^1 = CH_2C_6H_5$ $R^2 = CH{=}CH_2$
[26] $R^1 = H$ $R^2 = CH(OCH_2C_6H_5)CH_3$

In an effort to remove the excess reagents and nonporphyrin byproducts prior to chromatography, the reaction mixture was heated to 100° under vacuum (0.01mm Hg). This procedure resulted in a product that was brown, rather than red, in colour. Chromatography of this product gave two major fractions, the first eluted fraction contained a mixture of tetrabenzyl HP [20] and the monovinyl product [25], by mass spectral analysis. None of the monobenzyl ether [24] was recovered from the reaction. Elimination of water from the 1-hydroxyethyl sidechain has been observed when HP and similar compounds are heated in a vacuum[87], which suggests that the monobenzyl ether [24] has been dehydrated to give the vinyl compound [25]. The second fraction contained, by mass spectral analysis, a product containing three benzyl groups, which was not, by tlc, the monobenzyl ether [24]. The high resolution ^1H n.m.r. of this compound and the ^{13}C n.m.r. showed that this compound was

HP dibenzyl ether monobenzyl ester [26]. This product also appears to have been present in other preparations of the tetra and tribenzylated material but it had not been characterised previously.

The proton n.m.r. spectrum of [26] shows a 2:1 ratio for the benzyl ether resonances to the benzyl ester resonances and a multiplet at 6.19 ppm compatible with the methine proton on the 1-benzyloxyethyl sidechain but no resonances for the 1-hydroxyethyl sidechain. The ^{13}C n.m.r. spectrum exhibits two peaks in the carboxy region at 177.1 and 173.6 ppm, due to the carboxylic acid and the benzyl ester respectively, and two peaks are observed for each of the methylene carbons of the propionic sidechain.

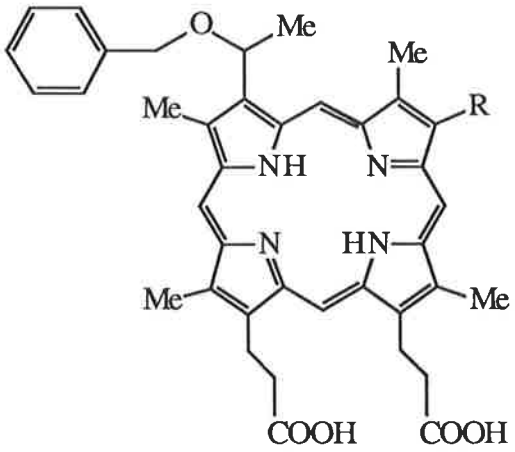
The removal of the benzyl ethers and esters was investigated. Hydrogenation in the presence of a palladium catalyst has previously been used to remove benzyl esters from porphyrins.[24]

When tetrabenzyl HP [20] was hydrogenated in the presence of 5% palladium on carbon in methanol/dichloromethane solvent the proton n.m.r. spectrum of the product showed only one resonance, at 7.4 ppm, in the aryl proton region; this corresponds to the shift for the benzyl ether indicating that the benzyl esters have been removed. HPLC of the product revealed a complex mixture with a major peak at 12.3 minutes. Further hydrogenation of the product resulted in a relative increase in the peak at 12 minutes. No HP or monobenzyl ether HP [27] was observed in the HPLC trace which would have indicated that the reaction was removing the benzyl ether groups as well as the esters.

The use of tetrahydrofuran as the solvent resulted in incomplete hydrogenolysis of the ester functions with some tetrabenzylated HP [20] and monobenzyl ether [24] still present by tlc and mass spectrum. The ^{1}H n.m.r.

spectrum showed a small resonance at 7.0 ppm, compatible with residual esters, and a larger peak at 7.3 ppm due to the benzyl ethers.

The use of 10% palladium on carbon in tetrahydrofuran for hydrogenolysis resulted in a complex mixture of compounds by HPLC and FAB mass spectral analysis. The mass spectral data contained only one mass which corresponded to an expected compound; the dibenzyl ether HP [28] (*m/z* 779), which was observed in the HPLC trace in 30% abundance.



[27] R = $CH(OH)CH_3$
[28] R = $CH(OCH_2C_6H_5)CH_3$
[29] R = $CH{=}CH_2$

Catalytic transfer hydrogenation using various hydrogen donors[88,89,90] has been used for the removal of benzyl ethers[88,89,90] and esters[89,90]. Hydrogenolysis of a mixture of tetra [20] and tribenzylated [24] HP in acetic acid with 10% palladium on carbon and the catalyst, 1,4-cyclohexadiene,[90] resulted in a product which was contained a number of peaks in the HPLC trace, with a major component at 12 minutes retention time. The mass spectrum showed a single major peak at *m/z* 671, which is compatible with the monovinyl compound [29].

Removal of the benzyl groups was attempted using trifluoroacetic acid and sulphuric acid; a method which has been used to cleave benzyl esters.[91] An initial attempt at this reaction gave a complex mixture of products (by reverse phase HPLC) whilst a second attempt gave a mixture of material that was intractable and material which would not extract into organic solvents. HPLC

analysis of water soluble material showed a peak with retention time of 1.5 minutes whilst the rest of the material (76%) was strongly retained on the column, suggesting it may be polymeric in nature. The strongly acidic nature of this reaction means that it is unlikely to be useful in the ether synthesis even if it had been efficient for removing the benzyl protecting groups.

The benzyl ether group's resistance to hydrogenolysis, makes it unsuitable as a protecting group for the synthesis of DHE [7]. It was later shown that benzyl esters were unsuitable as protecting groups in the synthesis of ether and ester linked porphyrins as the hydrogenolysis conditions used to remove the benzyl esters cleaves both ester and ether dimer linkages.[90]

3.4 Formation of the ether linkage

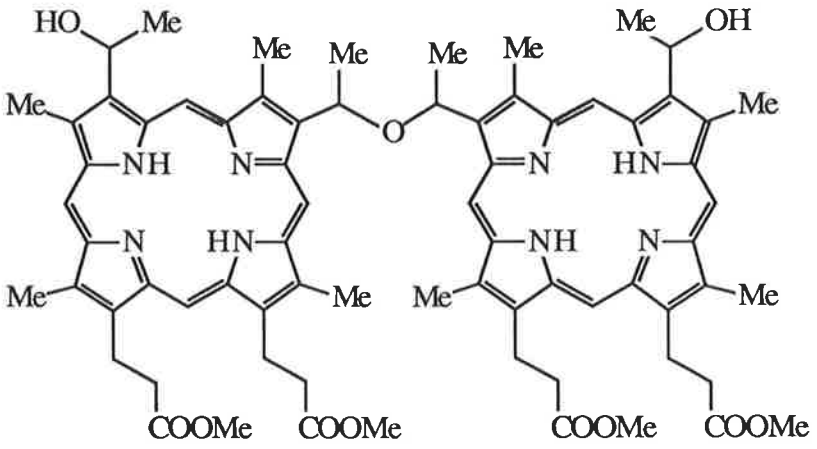
The TBDMS ethers may be a suitable protecting group for the hydroxyl group as the mono-TBDMS ether [16] can be formed and the silyl group can be removed using tetrabutylammonium fluoride which should not affect the ether linkage between porphyrins. As discussed (Part 3.1) the methyl esters should provide a suitable protecting group for the carboxylic acids. The formation of a suitable leaving group (L in Scheme 2) for dimer formation was investigated.

3.4.1 Bromide as the leaving group

As discussed above (Part 3.3) treatment of HP.DME with hydrobromic acid in acetic acid, followed by the addition of benzyl alcohol, resulted in the formation of some dimeric material, believed to be the methyl esterified monovinyl terminated dimer [22] and methyl esterified divinyl terminated ether linked dimer [23].

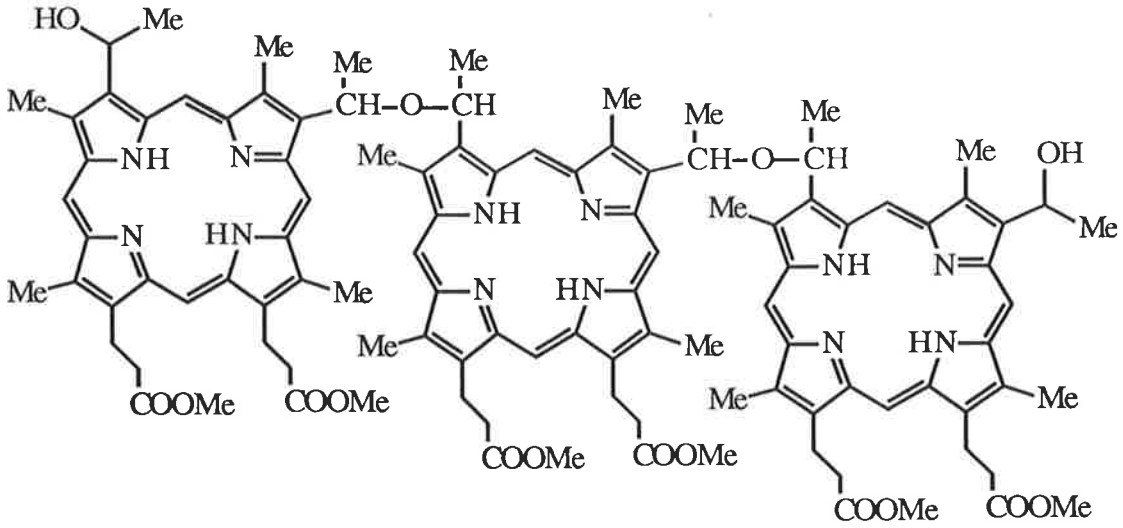
This reaction was repeated but, in this case, the crude dibromide was treated with an equivalent of HP.DME rather than benzyl alcohol. The reaction gave a

similar product (tlc) to the benzyl alcohol quenched reaction except for the presence of unreacted HP.DME. Tlc of the product showed three main products, in addition to HP.DME, between R_f 0.3 and 0.1.



[30]

Repetition of this reaction and isolation of the products by preparative thin layer chromatography, showed that each of the three main spots previously observed by tlc were composed of a number of individual components. PP.DME and HP.DME were isolated and identified by mass spectroscopy and by comparison with authentic samples. Peaks corresponding to HV.DME were identified in the mass spectrum of several of the bands and could be due to either fragmentation in the mass spectrometer or the presence of the material in the sample. One band contained predominantly the methyl esterified monovinyl terminated dimer [22] (*m*/*z* 1217) and another contained a major amount of methyl esterified DHE [30] (*m*/*z* 1235) as well as a small amount of trimer [31] (*m*/*z* 1845).



[31]

From another preparation a small amount of monovinyl dimer [22] (14%) was isolated by radial chromatography. The mass spectrum of this material showed a major peak at *m/z* 1217 and two peaks at *m/z* 609 and 591; which are compatible with the fragmentations expected for the dimer, based on the fragmentations arising from elimination observed for the benzyl and TBDMS ethers and the alcohols.

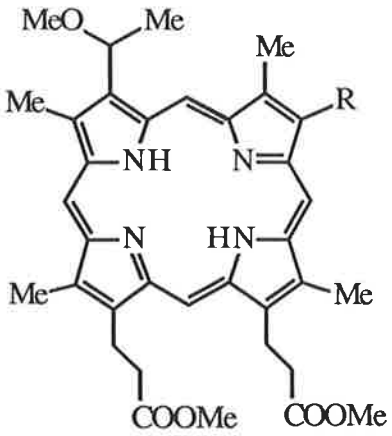
The dimer [22] was hydrolysed using aqueous sodium hydroxide to give the free acid. Mass spectral analysis of the acid showed peaks at *m/z* 1161, the free acid, and 1183, its monosodium salt. HPLC analysis of the free acid showed a complex set of peaks from 6 to 10 minutes which has been shown[59,67,92] to be typical of the ether dimers formed from monomers which are not isomerically pure. No HP was formed during the hydrolysis indicating that the ether linkage had not been compromised.

A trial reaction treating monoTBDMS HP.DME [16] in dichloromethane with HBr in acetic acid and quenching with water gave a similar product mixture (by FAB mass spectrum) to the reaction using HP.DME, above. The mass spectrum of the mixture showed no peaks corresponding to silylated material.

PP.DME, HV.DME and HP.DME were all formed in the reaction (tlc and mass spectral analysis) as well as the three methyl esterified dimers; methyl esterified DHE [30], monovinyl terminated dimer [22] and divinyl terminated dimer [23] (mass spectral analysis). It appears that the TBDMS ethers were unstable to the acidic conditions. The TBDMS groups are stable in acetic acid and acetic acid/water for extended periods of time but they are obviously unstable to the strong hydrobromic acid. The presence of HP.DME indicates that the silyl group has been displaced to form the 1-bromoethyl porphyrin, either by an elimination/addition reaction or nucleophilic substitution, which is then hydrolysed during the aqueous workup to the hydroxyethyl porphyrin.

Formation of the 1-bromoethyl porphyrins using less acidic bromination conditions should result in less by-products due to elimination of water and/or protecting groups. Kenner *et al.*[93] have formed the 2-bromoethyl porphyrin from the corresponding 2-hydroxyethyl porphyrin using tetrabromomethane and triphenylphosphine in dichloromethane. It was envisaged that these reagents could be used to form the 1-bromoethyl from the 1-hydroxyethyl porphyrin.

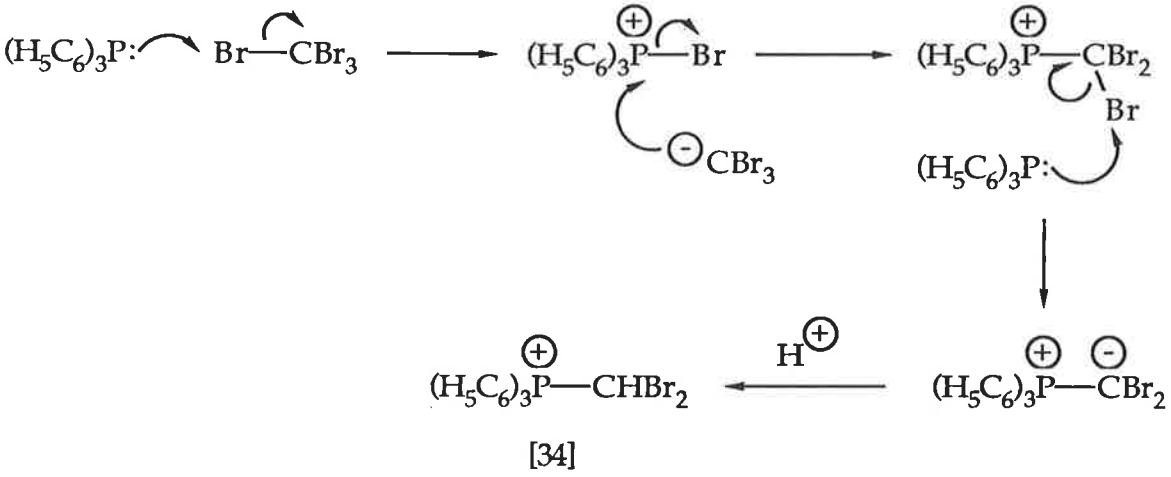
The reaction was attempted using Kenner's conditions[93] on HP.DME. The crude reaction mixture appeared complex by tlc and was unstable to the conditions described by Kenner *et al.* for the isolation of his dibromo product. The reaction was worked up by removing the solvent, dissolving the residue in methanol and washing with light petroleum to remove unreacted triphenylphosphine. FAB mass spectral analysis of the mixture showed peaks compatible with methylation of HP.DME and/or elimination from the 1-hydroxyethyl sidechains (trimethylated HP [32], methoxyvinyl [33], HV.DME, PP.DME) and dimeric material. The methylation and dimerisation indicates that bromination is occurring but that the 2º bromide, being benzylic, is much less stable than the 1º bromide.



[32] R = $CH(OH)CH_3$
[33] R = $CH{=}CH_2$

Basic hydrolysis of the product resulted in an extremely complex mixture (HPLC) and a mass spectrum compatible with loss of methyl esters from the above material.

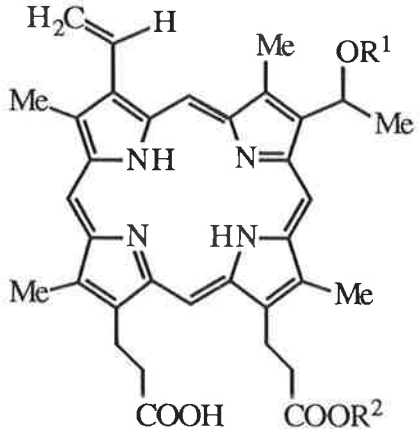
The reaction was investigated using the monomethyl ether [32]. The monomethyl ether [32] was treated with triphenylphosphine and tetrabromomethane. After refluxing the solution for 3 hours there was still monomethyl ether [32] present, addition of further tetrabromomethane and triphenylphosphine resulted in all the monomethyl ether [32] being consumed after a further 15 minutes at reflux. The reaction was quenched by the addition of excess ethanol. The FAB mass spectrum of the product was dominated by a set of peaks at *m/z* 433/435/437 in a 1:2:1 ratio. The two mass unit separation and the 1:2:1 peak ratio indicates the presences of two bromine atoms; this, and the mass, is compatible with the species $Ph_3\overset{+}{P}CBr_2H$ [34] which may be envisaged as arising from the reaction outlined in Scheme 3[94]. The formation of this byproduct would account for the reaction stopping as the triphenylphosphine was consumed; this was counteracted in later reactions by increasing the ratio of triphenylphosphine to tetrabromomethane.



[34]

Scheme 3 Mechanism for the formation of [34]

The mass spectrum also showed peaks which were compatible with the elimination products, the vinylmethoxy compound [33] and HV.DME [21], and two other main peaks at *m/z* 651 and 595. The peak at *m/z* 595 is compatible with hydroxyvinyl monomethyl ester [35] and/or methyoxyvinyl HP [36], indicating that some hydrolysis of the ester groups may be occurring. The peak at *m/z* 609, attributed to HV.DME may also be may be due to the methoxyvinyl monomethyl ester [37] if ester hydrolysis is occurring. The peak at *m/z* 651 maybe due to ethylation of the vinylmethoxy compound [33] at the nitrogen. N-alkylated porphyrins have been formed from alkyl halides[95,96], and ethyl bromide may have been formed in this reaction during the workup, by reaction with excess triphenylphosphine and tetrabromomethane.

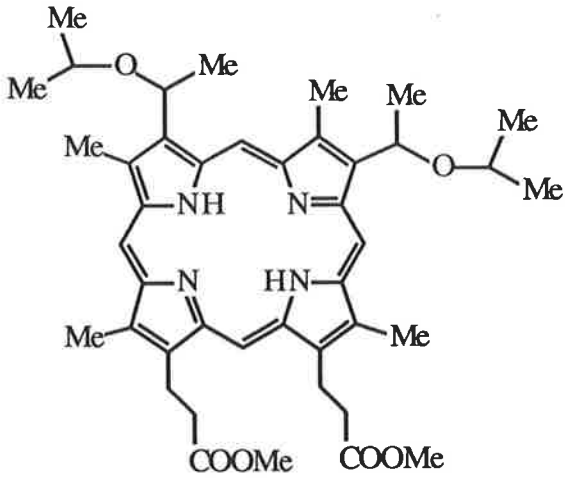


[35] $R^1 = H$ $R^2 = CH_3$
[36] $R^1 = CH_3$ $R^2 = H$
[37] $R^1 = CH_3$ $R^2 = CH_3$

HP.DME in dichloromethane was added to a refluxing solution of triphenylphosphine and carbon tetrabromide in dichloromethane and the solution was refluxed for a further hour and then quenched with methanol. Mass spectral analysis of the product indicated varying degrees of methylation. The product contained, according to the mass spectrum; tetramethylated HP [11], trimethylated HP [32], HP.DME, the methoxyvinyl [33] and HV.DME [21]. A small amount of material at *m/z* 669 may be due to alkylation of the porphyrin at the nitrogen, which was noted in the previous reaction.

Reducing the length of reflux after the addition of the HP.DME from one hour to five minutes and allowing the solution to stand at room temperature after the addition of the methanol resulted in a similar mixture to that obtained above.

The extensive methylation and elimination suggest that the bromide is forming. In an attempt to form the dimer a second equivalent of HP.DME was added, instead of methanol, to the reaction mixture. The reaction was cooled to 0° and HP.DME was added. After 2 hours at 0° no appreciable change had occurred to the reaction mixture (tlc) so it was allowed to warm to room temperature and stirred for 17 hours; there was still an appreciable amount of HP.DME remaining so the solution was refluxed and 2-propanol, a model for the 1-hydroxyethyl sidechain, was added to quench any unreacted bromo porphyrin. Analysis of the product by mass spectroscopy showed no peaks in the dimer range. The major peaks observed were at *m/z* 711, which corresponds to diisopropyl ether of HP.DME [38], and *m/z* 696, which does not correspond to an expected product but may be a fragmentation peak produced by loss of methyl from the isopropyl ether.[97]



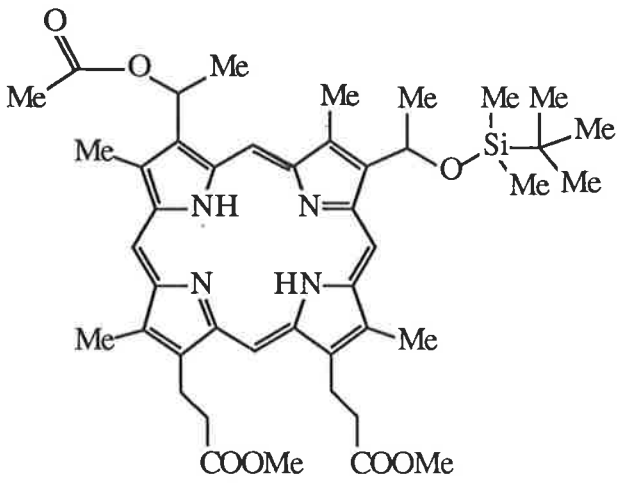
[38]

Elimination from the ethyl side chain, due to the lability of the 2º bromide when formed, was proving to be a major problem and it was considered that the chloro derivative may be more stable. In an attempt to make the 1-chloroethyl derivative HP.DME was reacted with tetrachloromethane and triphenylphosphine in dichloromethane at reflux for 2 hours until all the HP.DME was consumed (tlc contained mainly baseline material). The volatiles were removed, the residue was dissolved in dichloromethane and further HP.DME was added to see if dimer would form. The reaction mixture was allowed to stand for 2 days and then refluxed for 3 hours but HP.DME was still present (tlc). Mass spectral analysis of the products showed no dimeric material or chlorinated products, only HP.DME and its elimination products; HV.DME and PP.DME. Obviously the chloride, although slower to form than the bromide, is still too unstable under these conditions to be useful in this synthesis, consequently other leaving groups were investigated.

3.4.2 Acetate as the leaving group

Treatment of acetylated monomers with base causes ether linked oligomers to be formed in the production of HPD (Chapters 1 and 2), consequently it was expected that the acetate may be a good leaving group for the synthesis of dimers under more controlled conditions.

The acetate [39] was synthesised by treating monoTBDMS HP.DME [16] with a 9:1 mixture of pyridine and acetic anhydride. The reaction proceeded cleanly to give one product (tlc), which, by mass spectral analysis, was the acetate [39] (*m/z* 783), and which was used without further purification.

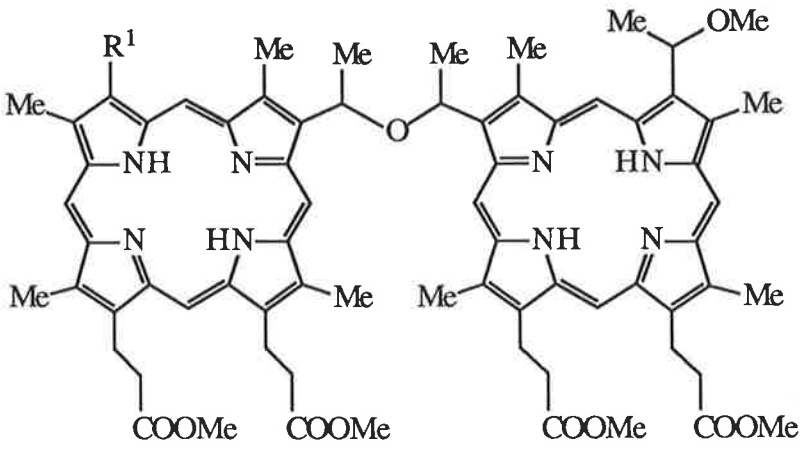


[39]

Equal amounts of the acetate [39] and the monoTBDMS porphyrin [16] were dissolved in dry dichloromethane. After two hours stirring no reaction had occurred so a small amount of triethylamine was added to see if base would catalyse the coupling. No reaction occurred after extended standing at room temperature or when the solution was refluxed.

Equal amounts of the acetate [39] and the monoTBDMS HP.DME [16] were dissolved in dry tetrahydrofuran and a small amount of sodium hydride was added. At room temperature a small amount of the starting materials were converted to polar material, as observed by tlc, which indicates that some

hydrolysis of the methyl esters may be occurring. When the reaction mixture was refluxed for one hour all the starting material was converted to polar material.* After workup the residue was reesterified using trimethyl orthoformate, water and concentrated sulfuric acid.[47] FAB mass spectral analysis of the esterified product showed some peaks compatible with the dimers, [30], [22], [40], and [41], but the spectrum mainly showed peaks for monomers with varying degrees of methylation and dehydration. No TBDMS containing polymers were present indicating that they were unstable to either the basic conditions of the reaction or the acidic conditions of the reesterification. Some methyl etherification was observed both in the dimers and the monomers, where normally the conditions used should only lead to esterification[47]. These particular acetates were not reacting readily under mild conditions so other leaving groups were considered.

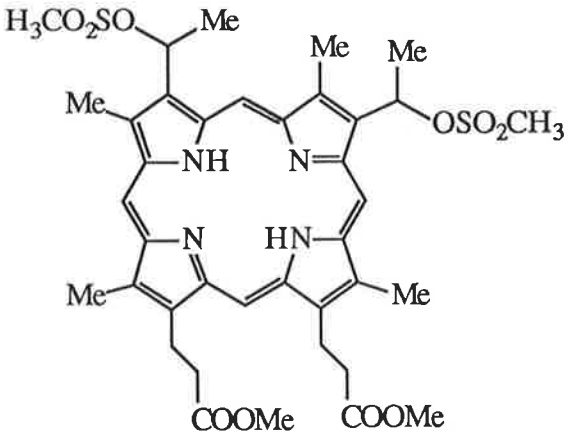


[40] R = $CH(OH)CH_3$
[41] R = $CH{=}CH_2$

*The source of water is possibly due to hydroxide contamination of the sodium hydride as hydrolysis was not observed in the first reaction. The reaction was done on a tlc scale and only a miniscule amount of hydroxide would be required for hydrolysis.

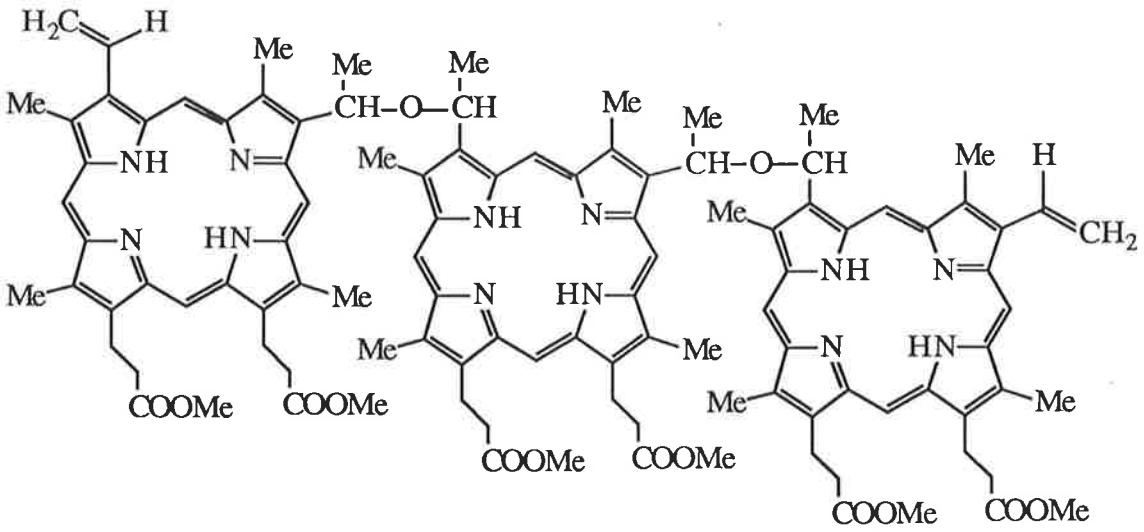
3.4.3 Mesylate as the leaving group

The mesylate group was investigated as a leaving group for the ether dimer synthesis.



[42]

Synthesis of the dimesylate of HP.DME [42] was attempted using the method of Crossland and Servis[98] for the formation of the mesylate. A solution of HP.DME and triethylamine in dichloromethane was treated with methanesulfonyl chloride at 0°; after 15 minutes HP.DME was still present so further methanesulfonyl chloride was added and the reaction was warmed to room temperature prior to work up. The product was a mixture (tlc) which by mass spectral analysis contained no mesylated HP.DME but predominantly contained PP.DME and some methyl esterified divinyl terminated dimer [23] and a small amount of the methyl esterified divinyl terminated trimer [43]. When the reaction was worked up without warming to ambient temperature the products included some methyl esterified DHE [30] and monovinyl terminated dimer [22] (by mass spectral analysis) as well as starting material and HV.DME.



[43]

The use of large excesses of methanesulfonyl chloride and triethylamine gave a complex mixture with some hydrolysis to give polar compounds.

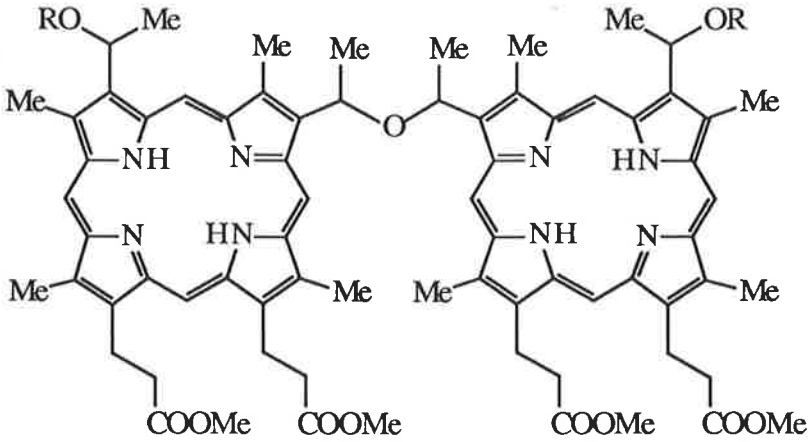
Treatment of tetramethyl HP [11] with triethylamine and methanesulfonyl chloride resulted in no significant change to the tetramethyl HP [11]; no hydrolysis of the esters and no elimination of methanol from the sidechains. This indicates that the methyl esters and ethers are stable to the reaction conditions and the extensive elimination observed is occurring via the mesylate derivative.

Treatment of HP.DME in dichloromethane with methanesulfonyl chloride and no base, in an effort to reduce elimination, resulted in very little reaction. Pyridine has been used as the base and solvent in the formation of mesylates[99] however starting material and a small amount of elimination products only were observed when HP.DME dissolved in pyridine was treated with methanesulfonyl chloride. Increasing the amount of methanesulfonyl chloride used and the reaction time resulted in no significant increase in reaction.

The formation of the tosylate as the leaving group, instead of the mesylate, was attempted. The tosylate should be somewhat more stable than the mesylate, resulting in less elimination. It may also be possible to isolate the tosylate prior to reacting it to form the dimer, this would allow purification of the tosylate and a more controlled dimerisation, enabling non-symmetrical dimers to be formed. When the monomethyl ether [32] was treated with *p*-toluenesulfonyl chloride in pyridine at 0° for up to three weeks, and the reaction quenched with methanol, mainly starting material was returned (tlc, mass spectrum). If the tosylate had been formed the expected product would be the tetramethylated HP [11], or the tosylate itself, if it were particularly stable.

The mesylation may not have occurred in the absence of base, or in the weaker base, pyridine, due to lack of formation of the alkoxide of HP.DME, although pyridine is a sufficient proton acceptor to drive the acetylation reaction of HP.DME. It may also be because methanesulfonyl chloride forms the sulfene in the presence of base.[100] The sulfene has a low steric requirement, which makes nucleophilic attack by a bulky alcohol more favourable for the sulfene than for the sulfonyl group. HP.DME may be too hindered to attack the sulfonyl chloride and may only form the mesylate from the sulfene, consequently, in the absence of base, no mesylation occurs. Pyridine forms sulfenes at a much slower rate than triethylamine[101] which may account for the lack of reaction when pyridine was used as the base. The lack of tosylation may be rationalised in the same way; tosyl chloride lacks α-hydrogens and may not form the more accessible sulfene and, as discussed, pyridine may be too weak a base to drive the reaction.

When the mesylation is done at 0° in the presence of triethylamine the mesylate is obviousy forming and then reacting with second porphyrin molecule to form the dimer.



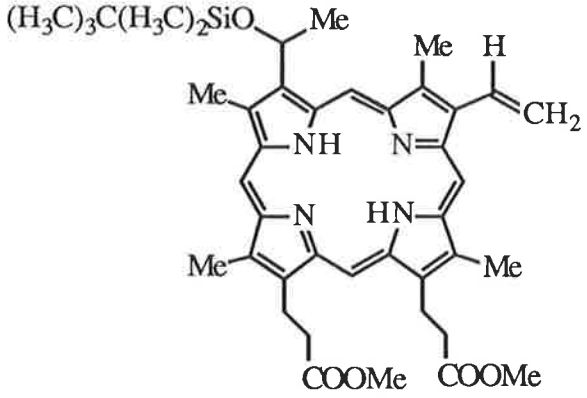
[44] R = CH_3
[47] R = $Si(CH_3)_2C(CH_3)_3$

Synthesis of the methoxy terminated dimer [44] was investigated first as a trial reaction as it has already been established that the methyl ethers are stable to the mesylation conditions. Trimethyl HP [32] when treated with triethylamine (1.8 equivalents) and methanesulfonyl chloride (1.3 equivalents) gave a mixture of four compounds by tlc analysis. Preparative tlc enabled the four components to be separated. One of the compounds was identified as starting material by FAB mass spectral analysis and comparison with authentic material (tlc). The mass spectra of the two compounds of highest R_f were compatible with the two regioisomers of the methoxyvinyl compound [33]. The other compound, recovered in 45% yield, was the methoxy terminated dimer [44] based on its mass spectrum (m/z 1263 (52%, $(M+H)^+$), 1231 (14), 623 (100)), indicating that this is a viable pathway for the formation of ether-linked porphyrin dimers.

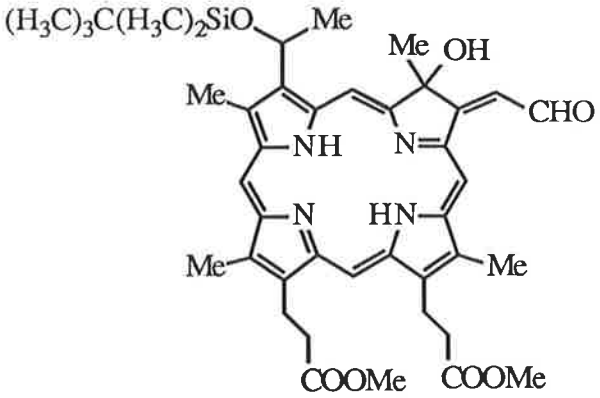
Synthesis of the required dimer, dihematoporphyrin ether [7], was attempted using the mesylate as the leaving group, TBDMS as the protecting group for the nonreacting alcohol and methyl esters as the protecting group for the carboxylic acids (Scheme 2; L = H_3CSO_3, $R^1 = (CH_3)_3C(CH_3)_2Si$).

The monoTBDMS porphyrin [16] was treated with methanesulfonyl chloride (1.1 equivalents) and triethylamine (1.5 equivalents) in a trial reaction. The products were treated with tetrabutylammonium fluoride in tetrahydrofuran, to remove the TBDMS groups, and then separated by preparative thin layer chromatography. The highest R_f band consisted of HV.DME (*m/z* 609) and the three lower R_f bands contained DHE tetramethyl ester (*m/z* 1235) by FAB mass spectrum. The mass spectra also showed a large peak for *m/z* 609, however a MIKES spectrum of *m/z* 1235 and a B^2/E linked spectrum of *m/z* 609 confirmed that *m/z* 609 was a fragmentation peak of the dimer.

The reaction was repeated on a larger scale. The products were separated on a squat column. The first product to elute was the monoTBDMS vinyl compound [45]. Tlc analysis of this compound showed that it rapidly developed two green impurities, of lower R_f, when exposed to light. A sample exposed to natural light for 10 hours was converted entirely to the green compounds. The visible spectra of the compound was typical of a chlorin[65] and the mass spectra was 32 units greater than the original material, indicating addition of oxygen. If kept out of light the compound remained unchanged. This light induced addition of oxygen has been reported for a number of porphyrins containing vinyl sidechains[102,103,104,105] and is believed to occur by addition of oxygen in a Diels-Alder fashion to the vinyl substituted pyrrole ring of the porphyrin[106,107]. The products formed in this case would be the chlorin [46] and its regioisomer.



[45]



[46]

The second product to elute was the TBDMS terminated dimer [47], which was identified by FAB mass spectral analysis (*m/z* 1463 ((M+H), 100%), 723 (72%)) and proton n.m.r. spectroscopy; the spectrum is extremely complex due to the number of isomers that may exist. Resonances for the TBDMS groups occur from -0.4 to 0.3 ppm for the methyl protons atta ched to the silicon and 0.8 to 1.1 ppm for the *t*-butyl group protons. The protons α to the ethers, both the silyl ethers and the linking ether, occur between 6.1 and 6.8 ppm.

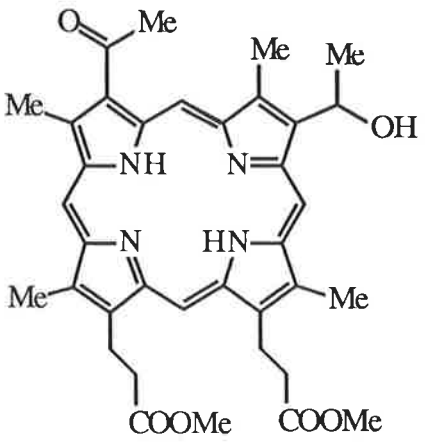
The TBDMS groups were removed using tetrabutylammonium fluoride in tetrahydrofuran to give the methyl esterified DHE [30] (*m/z* 1235 ((M+H), , 100%), 609 (73)). Again the proton n.m.r. spectrum is complex but both it and the ^{13}C n.m.r. are in agreement with data for known DHE tetramethyl ester.[92,108]

The methyl esters were hydrolysed using 0.1N sodium hydroxide in tetrahydrofuran to give DHE [7] (*m/z* 1179). The mass spectrum also showed a peak at *m/z* 1161 which corresponds to the monovinyl dimer [13], this compound may be present due to fragmentation in the mass spectrometer or some elimination during the hydrolysis reaction. The HPLC trace of the product was complex, due to the number of isomers possible for the dimer, but

showed only a small amount of HP indicating that the ether linkage was relatively stable to basic hydrolysis. Some poorly resolved, long retention time material was observed in the trace, it has been shown that this can be removed by reverse phase chromatography.[48] The product was cochromatographed on HPLC with a sample of known DHE[109] synthesised by a different route[108] and showed good agreement. The major peaks occur at 9-10 minutes and 16 minutes. The region from 8 to 15 minutes in the HPLC trace of HPD is sparsely populated (Fig. 1, Chapter 2) indicating that DHE is not a major component of HPD.

Diisopropylamine could be used in place of triethylamine in the preparation of bisTBDMS DHE tetramethyl ester [47] with similar results.

Whilst mesylation was being studied Pandey *et al*[49]. described the preparation of the mesylate of monoacetyl HP.DME [48] using methanesulfonyl chloride at -70°. The mesylate was then treated with lithium bromide to convert it to the corresponding bromo compound and then condensed with an equivalent of [48] to give the diacetyl terminated ether linked dimer [89]. Treatment of the dimer with sodium borohydride and then hydrolysis with sodium hydroxide to remove the methyl esters yielded DHE [7].



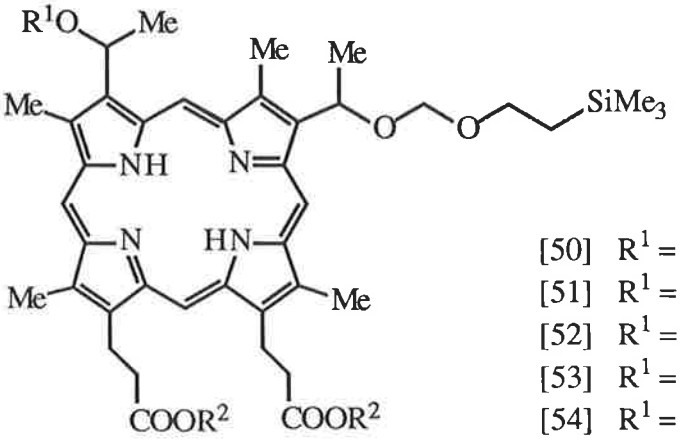
[48]

Within our group it was discovered that DHE could be formed most conveniently by treating HP.DME with hydrogen bromide dissolved in dichloromethane to give DHE tetramethyl ester [30] and hence DHE.[108] It could also be formed using the monoacetyl [48], treating that with hydrogen bromide dissolved in dichloromethane to give the diacetyl DHE tetramethyl ester [89] which could be reduced and the methyl esters hydrolysed to give DHE [7].

3.5. 2-(Trimethylsilyl)ethoxymethyl (SEM) ethers and esters

The 2-(trimethylsilyl)ethoxymethyl group, commonly known as SEM,[110] was investigated for its suitability as a protecting group for hydroxyl and carboxylic acid groups during ether and ester linked dimer synthesis. 2-(Trimethylsilyl)ethoxymethyl has been used to protect hydroxyl groups as the SEM ether.[110]

The silylating reagent, 2-(trimethylsilyl)ethoxymethyl chloride (SEM-chloride) was prepared[110] by reacting 2-(trimethylsilyl)ethanol with paraformaldehyde in the presence of gaseous hydrogen chloride.



[50] $R^1 = H$ $R^2 = CH_3$
[51] $R^1 = CH_2OCH_2CH_2Si(CH_3)_3$ $R^2 = CH_3$
[52] $R^1 = CH_2OCH_2CH_2Si(CH_3)_3$ $R^2 = H$
[53] $R^1 = R^2 = CH_2OCH_2CH_2Si(CH_3)_3$
[54] $R^1 = OH$ $R^2 = CH_2OCH_2CH_2Si(CH_3)_3$
[56] $R^1 = H$ $R^2 = H$

The formation of the SEM ethers was investigated by reacting HP.DME with SEM-chloride and diisopropylethylamine in dichloromethane at 30°. An

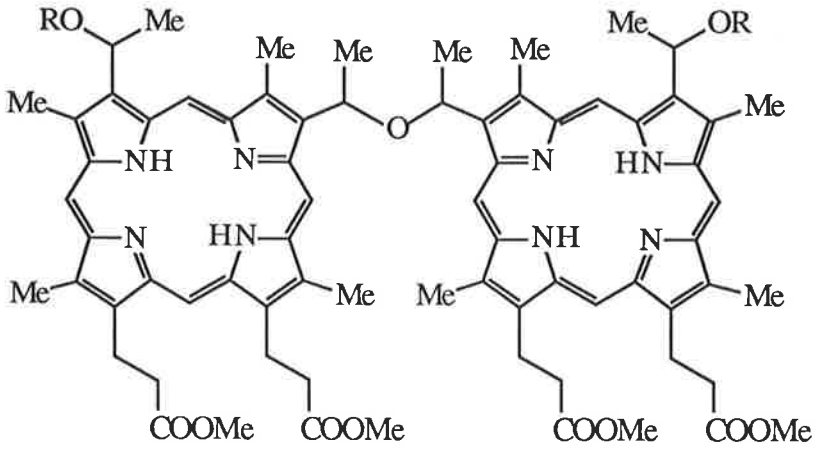
initial small scale reaction showed that 6 equivalents of SEM-Cl gave a mixture of 3 compounds, one of which was starting material. The reaction was repeated on a larger scale using 5 equivalents of SEM-Cl and 4 equivalents of diisopropylethylamine to try and maximise the formation of the monoSEM ether [50]. However, this reaction yielded, after chromatographic separation, the bisSEM ether [51], identified by mass spectral analysis and its proton n.m.r. spectrum, and only 20% of the monoSEM ether [50], identified by mass spectral analysis.

The SEM ethers were found to be stable to basic hydrolysis conditions but not acidic hydrolysis conditions. When the bisSEM ether [50] was treated with the standard basic hydrolysis conditions used in Chapter 2, the starting material was consumed to give polar material (tlc) whose mass spectral analysis was compatible with the bisSEM ether diacid [52]. Methylation of this material returned the bisSEM ether dimethyl ester [51]. Under acidic hydrolysis conditions (10% HCl, reflux for 1.5 hours) the starting material was consumed and the product, when treated with diazomethane in methanol, gave HP.DME (tlc, mass spectrum), indicating that the SEM ethers were unstable to the acidic hydrolysis conditions.

Simultaneous synthesis of SEM ethers and esters was also investigated. HP.2HCl, suspended in dichloromethane, was treated with SEM-chloride and diisopropylethylamine at 30° for 45 minutes. Tlc analysis of the solution showed the presence of 3 components which were not stable to the aqueous workup used. The reaction was repeated and worked up by removing the solvent and chromatographing the residue on a silica column which had been partially deactivated to reduce ester cleavage. The chromatography yielded two impure fractions; the first fraction to elute contained a mixture of tetrasilylated HP [53] and trisilylated HP [54], based on the mass spectrum and tlc behaviour.

The second fraction contained four products (tlc) with the major component being trisilylated HP [54], by mass spectral analysis.

To determine if the SEM esters and ethers were suitable protecting groups for the synthesis of DHE the dimerisation via the mesylate was attempted using the impure product from above. If stable to the conditions the tetrasilylated HP [53] in the fraction should not react and may be separated from the desired products. Tetrasilylated HP [53], was still present after the dimerisation reaction, indicating that the SEM groups were stable to the conditions used for forming the mesylate. Some unreacted trisilylated HP [54] was also identified in the mixture and some unidentified products were also observed. Mass spectral analysis showed, apart from the starting materials, the presence of some dimeric material.



[55] R = $CH_2OCH_2CH_2Si(CH_3)_3$

The crude product was treated with tetrabutylammonium fluoride in tetrahydrofuran for 2 hours, with no reaction, and then refluxed for 1 hour to give polar products (R_f 0.0). The residue , after workup, was chromatographed on a Sephadex LH-20 to separate dimers from the monomers. Three fractions were separated; the first fraction to elute contained the SEM terminated dimer [55], by mass spectral analysis, as well as tetraSEM HP [53] and bisSEM HP [52].

The tetraSEM HP [53], although abundant in the mass spectrum, was not visible by tlc. This suggests that only a small amount of this compound is present but that it is much more volatile in the mass spectrum than the free acids. The other fractions corresponded to bisSEM HP [52] and monoSEM HP [56] indicating that the SEM ethers had not been cleaved by the reaction with tetrabutylammonium fluoride.

The SEM protecting groups were not investigated further, the SEM ethers are not readily cleaved by fluoride, as the *t*-butyldimethylsilyl groups are, and the SEM esters, although stable to these dimerisation conditions are readily cleaved under aqueous conditions. Pandey *et al.*[49] reported the use of SEM ethers in synthesising ether linked dimers and trimers and found that the conditions necessary to cleave the SEM ethers also resulted in extensive decomposition of the dimer and trimer.

In summary this chapter describes the following results. The dihematoporphyrin ether [7] has been synthesised. A protecting group which would protect both hydroxyl and carboxylic acid functions was not found. TBDMS ethers were used to protect the nonreacting hydroxyl group and could be removed using tetrabutylammonium fluoride without cleaving the newly formed ether linkage. The methyl esters proved to be suitable for the protection of the carboxylic acids and could be removed under basic hydrolysis conditions. The mesylate proved to be the best leaving group for the ether formation, although elimination of methanesulphonic acid was a competing reaction. It has been shown that dihematoporphyrin ether is not a major component of HPD and that dibenzylic ether linkage between porphyrins is stable to basic hydrolysis.

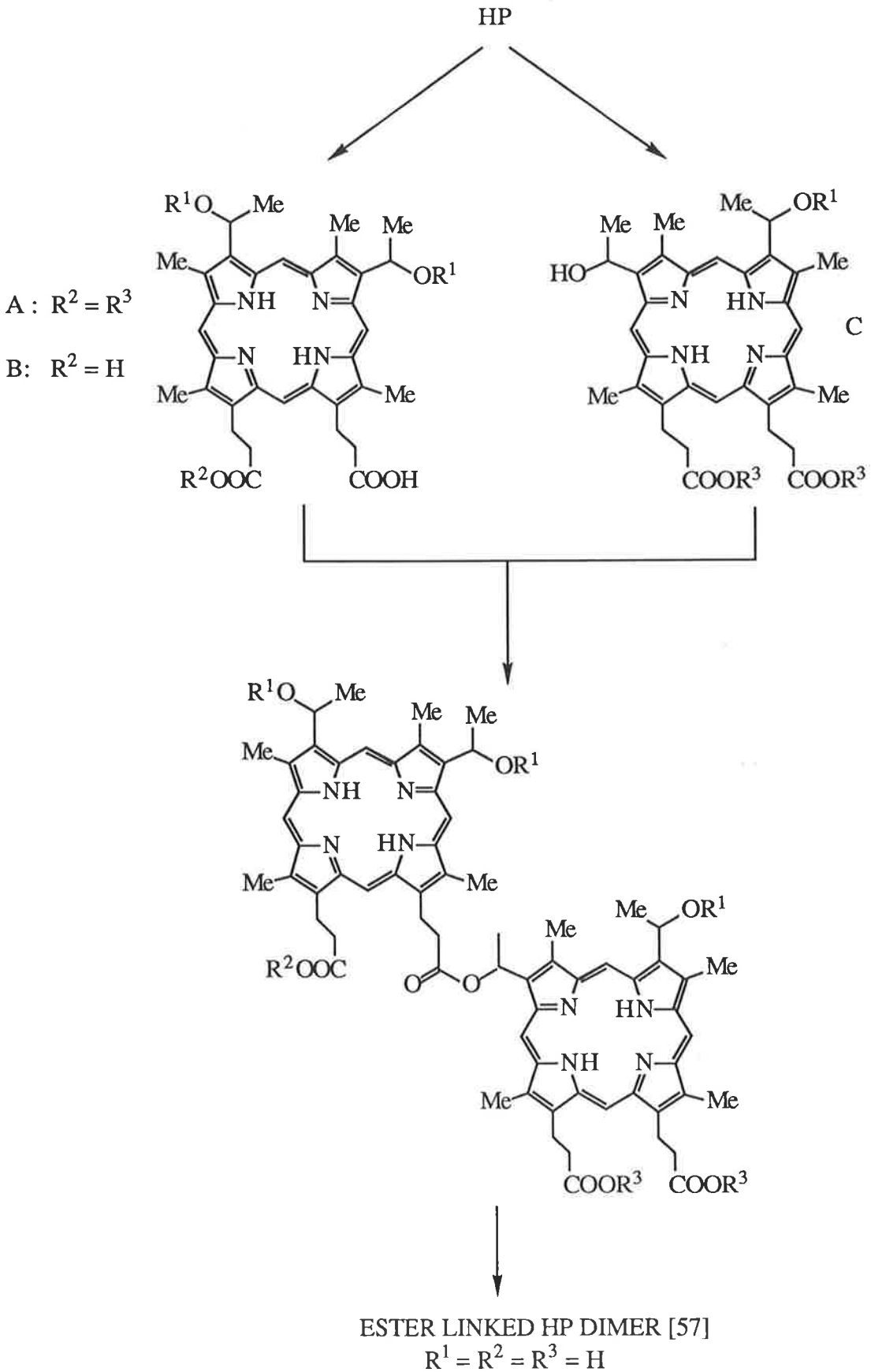
Chapter 4. Synthesis of the hematoporphyrin ester-linked dimer

4.1 Introduction

The active fraction of HPD was believed to consist of mainly ether and/or ester linked oligomeric material (see Chapter 2 and references therein). Chapter 3 discussed the synthesis of dihematoporphyrin ether; other syntheses of dihematoporphyrin ether[48,49] and other ether linked dimers[48,49,108,111,112,113] and oligomers[37,48,49,113] have been reported. In this chapter the synthesis of the ester linked dimer of HP is investigated.

A general scheme for the synthesis of the ester linked dimer is outlined below (Scheme 1).

The synthesis of the ester linked material presented a number of challenges as the ester linkage was expected to be less stable than the ether linkage. The first challenge was to provide suitable protection for the non-reacting alcohol and carboxylic acid groups of the porphyrins. The methyl ester, which was used as a protecting group for the carboxylic acid in the synthesis of DHE [7] (Chapter 3), is not suitable for the synthesis of ester linked dimers. The aqueous hydrolysis conditions used to remove the methyl groups would be expected to cleave the ester linkage between the porphyrins. The TBDMS ethers developed in Chapter3 were expected to be suitable as protecting groups for the alcohols as they are readily prepared and are removable under mild, nonaqueous conditions. However the TBDMS, and SEM, esters were too unstable to be used as protecting groups for the carboxylic acids (Chapter 3).



Scheme 1. Synthesis of the ester-linked dimer

It may not be necessary to form the monoesterified porphyrin (A, Scheme 1). The coupling reaction may be done using the dicarboxylic acid (B, Scheme 1) and one or less equivalents of the reacting species (C, Scheme 1) and then separating the dimer from any trimer that may be formed. The presence of a free carboxylic acid on the dimer should make it more polar than the trimer and allow separation by chromatography.

The ester linkage was expected to be formed using current methodology for ester formation.[114]

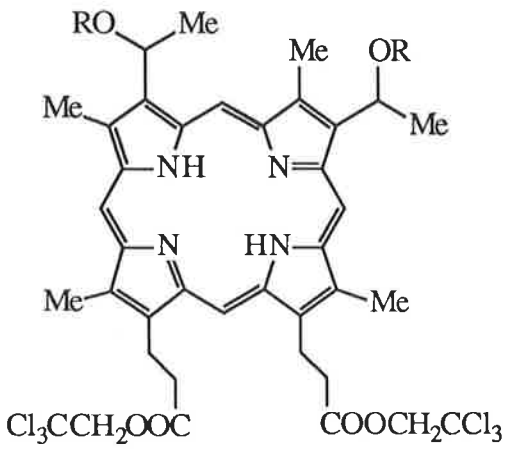
4.2 Formation of protecting groups for the carboxylic esters.

2,2,2-Trichloroethyl esters[115,116] and 2-(trimethylsilyl)ethyl esters[117,118] were investigated as protecting groups for the carboxylic acids. The 2-(trimethylsilyl)ethyl esters can be removed using fluoride in nonaqueous conditions[117,118] whilst the trichloroethyl esters can be removed under mild conditions using zinc dust[116,119]. The use of zinc, however, may cause metallation of the porphyrin. Initial attempts at preparing the trimethylsilylethyl esters proved unsuccessful, and are discussed later; consequently the formation of trichloroethyl esters was investigated first. It was also considered that preparation of the trichloroethyl esters would act as a good model for the preparation of the more expensive trimethylsilyethyl esters.

4.2.1 2,2,2-Trichloroethyl esters

Trichloroethyl esters have been formed using 2,2,2-trichloroethanol and *p*-toluenesulfonic acid as a catalyst.[115,116] The synthesis of HP bis(2,2,2-trichloroethyl) ester [58] from HP using this method was attempted. HP.2HCl was dissolved in 2,2,2-trichloroethanol, dichloromethane was used as a cosolvent to reduce the viscosity of the mixture, and *p* -toluenesulfonic acid

was added. No reaction was observed after 24 hours. Sulfuric acid was added and the reaction mixture heated under reflux for 5 hours to give one major product. After purification the product was identified as the bis(2,2,2-trichloroethyl) ether bis(2,2,2-trichloroethyl) ester [59]. FAB mass spectral analysis showed a more complex set of peaks for the molecular ion (*m/z* 1125), than is usual for a porphyrin due to the presence of multiple chlorine atoms. The proton n.m.r. spectrum contained, apart from the expected porphyrin resonances, resonances at 4.31 ppm due to the methylene protons in the trichloroethyl ether group and at 4.77 ppm due to the methylene protons of the trichloroethyl ester group. The ^{13}C n.m.r. spectrum was assigned with the aid of comparisons with the n.m.r. spectra of the bis(trichloroethyl) ester [58] and its dimethyl ether [60], whose syntheses are discussed later. Peaks at 74.1 ppm and 94.9 ppm were common to all three compounds and were assigned as the methylene carbon and the trichlorinated carbon, respectively, of the trichloroethyl ester. A resonance at 81.9 ppm was assigned to the methylene carbon of the trichloroethyl ether whilst the trichlorinated carbon of that group was coincident with the quaternary carbon of the trichloroethyl ester at 94.9 ppm.

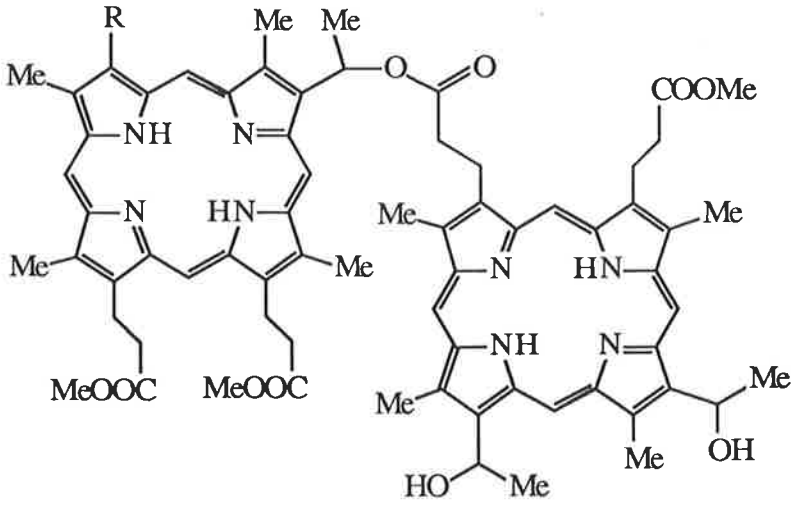


[58] R = H
[59] R = CH_2CCl_3
[60] R = Me
[65] R = $COCH_3$

Preparation of [58], the esterified only material, obviously required more specific conditions. Trichloroethyl esters[115] and other esters[114,120,121], have been prepared from the relevant alcohol using 1,3-dicyclohexylcarbodiimide (DCC) and base so some trial reactions were done on formation of esters using DCC. It was also considered that this method may be used for forming the ester linkage between porphyrins.

Formation of the simple methyl esters was investigated. A suspension of HP.2HCl in dichloromethane containing 4-dimethylaminopyridine and methanol at 0° was treated with DCC. The reaction mixture was warmed to room temperature and stirred for 3.5 hours during which time all the porphyrin appeared to have dissolved. Tlc analysis of the reaction mixture showed HP.DME to be the major product, with some material at the baseline which may be unreacted HP. However, after the workup, which included filtration to remove dicyclohexylurea and washing with aqueous ammonium chloride to remove the base, tlc analysis showed a large number of compounds in addition to HP.DME. Mass spectral analysis of this product showed peaks at *m/z* 1221 and 1203 which correspond to the ester-linked dimer trimethyl ester [61] and its monodehydrated analogues [62], which may be due to fragmentation in the mass spetrometer. The peak at *m/z* 1235 corresponds to the monomethyl ether of the dimer [61] and/or the ether linked dimer, DHE, and the peak at *m/z* 1217 corresponds to their monodehydrated analogues; the formation of any of these compounds indicates that some etherification is occurring. The major peak in the mass spectrum was due to HP.DME.

When the reaction mixture was worked up by elution from a small silica column, to remove the base and the urea, a slightly cleaner product, by tlc, was obtained.



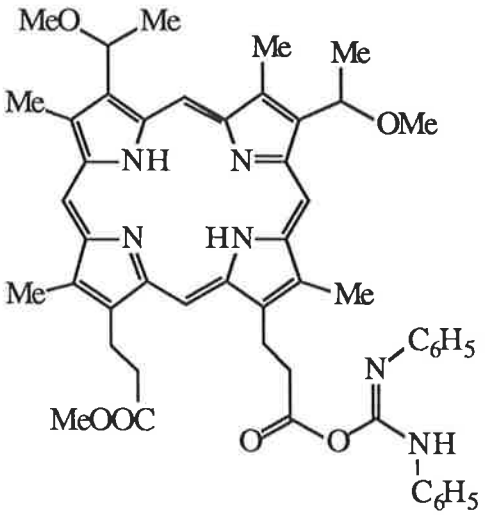
[61] R = $CH(OH)CH_3$
[62] R = $CH{=}CH_2$

The reaction was repeated with the addition of tetrahydrofuran to aid solubility of the starting material. The reaction product was chromatographed to give HP.DME, in 36% yield. The dimers observed above were also seen in this reaction.

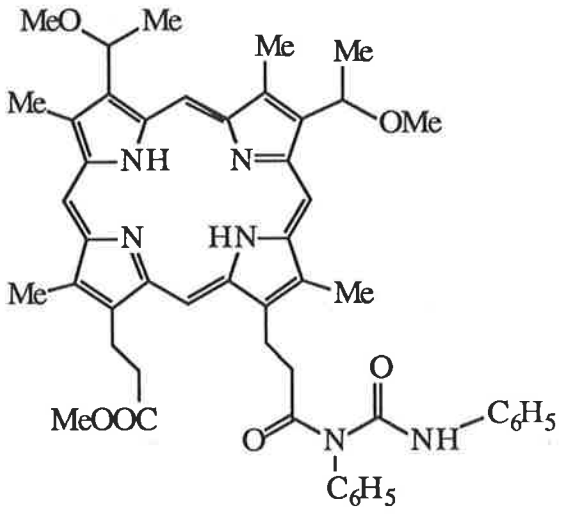
The reaction was investigated using the dimethyl ether of HP [12] to stop dimerisation. The reaction product was much cleaner giving one major product, tetramethylated HP [11], by tlc and mass spectroscopy. No porphyrin dimers were observed in the mass spectrum of the reaction product; a peak observed at *m/z* 847 in the mass spectrum may be attributed to the compounds, [63] and/or its rearrangement product, the N-acylurea [64]. N-acylurea formation is a common problem in DCC reactions[122,123], however the intermediate O-acylurea has been isolated from an attempted esterification of HP[24].

When the hydroxyethyl sidechains are unprotected they are competing with the alkyl alcohol to form ester dimers. The porphyrin alcohol would not normally be favoured as the esterifying group in the presence of excess methanol, however, if the intermediate O-acylurea is present when the

reaction is worked up the removal of solvent, including excess methanol, will increase the concentration of porphyrin alcohols and favour the formation of porphyrin dimer esters. This would account for the first esterification reaction appearing cleaner prior to the workup and also for the appearance of either of the adducts, [63] and [64], in the reaction where the porphyrin hydroxyl groups are protected and can not react with the O-acylurea [63] to form dimers. Using the alcohol required for esterification as the solvent, rather than in a five-fold excess used in the above reactions may increase the alkyl ester formation and decrease dimer formation. Conversely, doing the reaction at high porphyrin concentrations without other alcohols present should enable the ester-linked porphyrin dimers to be formed.



[63]



[64]

The bis(2,2,2-trichloroethyl) esterified material [58] was formed in 66% yield using 2,2,2-trichloroethanol as the solvent with dichloromethane to reduce the viscosity and DMAP as the base. The compound showed the expected molecular ion at m/z 861 and its proton n.m.r. spectrum exhibited a single resonance at 4.75 ppm due to the methylene protons of the trichloroethyl ester as well as the resonances expected for the hematoporphyrin system. The ^{13}C

n.m.r. spectrum contained resonances at 74.1 and 94.9 ppm due to the trichloroethyl ester group as discussed above.

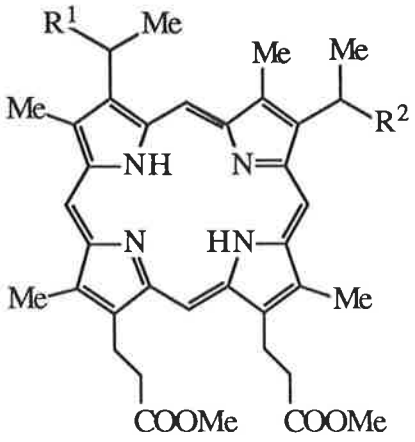
The dimethyl ether bis(trichloroethyl) ester [60] was prepared by the above method in 71% yield. Its mass spectrum showed the expected peak at *m/z* 889. The protons of the methoxy groups resonated in the same region as the ring methyl protons in the proton n.m.r. spectrum and could not be distinguished from them. The methine protons α to the methoxy group are seen at 6.6 ppm. The resonances in the ^{13}C spectrum are similar to those seen for [58] except for the tertiary carbon of the 1-methoxyethyl sidechain which resonates at 75.1 ppm compared with the tertiary carbon of the 1-hydroxyethyl sidechain in [58] which resonates at 65.4 ppm. A peak at 57.3 ppm is due to the carbon of the methyl ether.

Trial reactions were undertaken to determine a method for cleaving the trichloroethyl esters. The diester [58] was dissolved in tetrahydrofuran, buffered with potassium dihydrogen phosphate solution (1M), and powdered zinc was added; the reaction was stirred for 15 minutes and then worked up. HPLC analysis of the reaction product showed that there was still a large amount of the starting material [58] present (46%) and only a small amount of the desired HP (6%). The reaction was repeated on this product but there was only a minor change in the product composition by HPLC. A further repetition of the procedure, using an extended reaction time of 20 hours, caused a slight decrease in the amount of starting material, to 41%, and a slight increase in the HP formed to 7%. Mass spectra confirmed that [58] was the major component.

The method for cleavage of 2,2,2-trichloroethyl esters used by Woodward *et al.*[115] was investigated. The diester [58] was dissolved in 90% acetic acid in water and then treated with zinc at 0°. The reaction was incomplete (tlc) after 2

hours so further zinc was added and stirring was continued for a further hour. The HPLC of the product was complex, no HP was evident but a peak of high retention time was noted. The FAB mass spectrum of the product was of weak intensity but it did show a peak at *m/z* 945 which could correspond to the diacetate bis(trichloroethyl) ester [65].

A 50% acetic acid in water solvent was used in the above reaction to try and stop acetylation of the hydroxyl group, however, after four hours stirring, the reaction mixture still contained a large amount of starting material, by HPLC, as well as an unidentified material of slightly longer retention time. The reaction was repeated with the reaction time extended to 24 hours. HPLC analysis of the product revealed a highly complex mixture. FAB mass spectral analysis of the product showed three main sets of peaks at *m/z* 903 (84%), 861 (100%) and 843 (70%). *M/z* 861 corresponds to starting material, *m/z* 903 corresponds to the addition of one acetate group, and *m/z* 843 corresponds to the monodehydrated starting material.



[66] $R^1 = OH$ $R^2 = OCOCH_3$
[67] $R^1 = R^2 = OCOCH_3$
[68] $R^1 = R^2 = H$
[69] $R^1 = H$ $R^2 = OCOCH_3$

The complex mixtures being obtained may be due to reduction of the hydroxyethyl sidechains by zinc as well as solvolysis of the hydroxyl group. The effect of the reaction conditions on HP.DME was investigated. HP.DME was dissolved in 90% acetic acid in water at 0° and zinc was added, the reaction was worked up after stirring for 45 minutes. HPLC analysis of the reaction

product showed three main peaks. The mass spectrum of the product contained complex patterns which appeared to contain zinc so the product was demetallated using dilute hydrochloric acid. Mass spectrum of the product after zinc removal contained peaks compatible with mono and diacetylation of HP.DME, [66] and [67], and the reduction products, mesoporphyrin dimethyl ester [68] and the monoacetate [69]. As the trichloroethyl esters could not be removed cleanly without causing solvolysis and reduction of the sidechains they were abandoned as protecting groups for the ester synthesis.

4.2.2 2-(Trimethylsilyl)ethyl esters

The preparation of the 2-(trimethylsilyl)ethyl esters was investigated. It was envisaged that the 2-(trimethylsilyl)ethyl ester could be prepared by forming the acid chloride from the free carboxylic acid and then treating it with 2-(trimethylsilyl)ethanol. A suspension of HP.2HCl in dichloromethane was treated with oxalyl chloride for 10 minutes, the volatiles were removed and the residue treated with methanol. Tlc analysis of the product showed only baseline material and no HP.DME, the expected product. When HP, rather than HP.2HCl, was used and five equivalents of pyridine were added, to quench the hydrogen chloride produced, the reaction gave a complex mixture by tlc and HPLC.

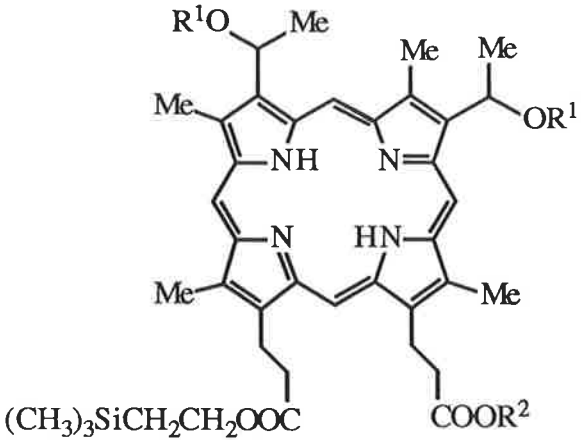
The reaction was attempted on HP dimethyl ether [12], in case the oxalyl chloride was reacting with the hydroxyethyl sidechains. The dimethyl ether [12] was suspended in dichloromethane and dissolved gradually upon the addition of oxayl chloride. The volatiles were removed and the residue was treated with trimethylsilylethanol in dichloromethane. Tlc analysis of the product was complex with a large amount of baseline material present as well as a variety of higher running compounds. These initial reactions to make the

2-(trimethylsilyl)ethyl ester via the acid chloride were not encouraging so other methods were explored.

Transesterification was investigated. HP.DME was stirred with 2-(trimethylsilyl)ethanol in dichloromethane over 4Å molecular sieves, to trap liberated methanol, but no reaction was observed when the reaction was run at room temperature for extended periods or at reflux. Sodium metal was added to the reactants to form some of the alkoxide but again no reaction was observed.

In an effort to drive the equilibrium to favour the trimethylsilylethoxy ester, HP.DME was dissolved in benzene and trimethylsilylethanol and sodium metal were added, the reaction was refluxed for 5 hours and then benzene was distilled from the mixture, in an effort to azeotrope methanol, whilst fresh benzene was added. The product, by tlc analysis, was mainly polar material; mass spectral analysis indicated that HP was the main product, although a peak at *m/z* 699 may be due to monotrimethylsilylethoxy HP [70].

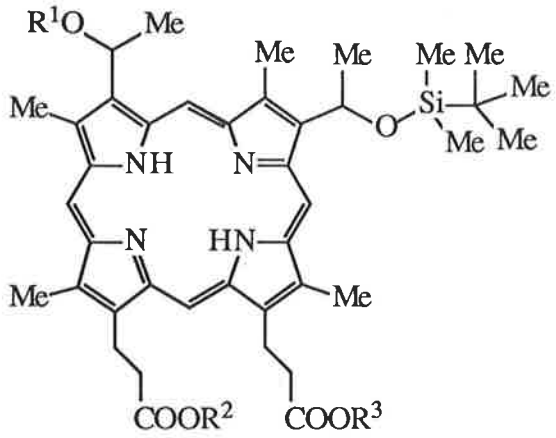
2-(Trimethylsilyl)ethoxide was formed, and the excess sodium removed prior to the addition of the porphyrin. HP.DME was added to the alkoxide and benzene removed by distillation whilst fresh benzene was added. Tlc analysis of the product showed one major nonpolar product which was isolated by preparative tlc. Mass spectral analysis of this product showed one peak (*m/z* 799); which was compatible with bis(2-(trimethylsilyl)ethyl) ester [71]. This reaction was attempted on an increased scale but the reaction would only proceed to partial completion, even after the product was subjected to the reaction conditions again. Replacing benzene with toluene as the solvent in the reaction resulted in an intractable material.



[70] $R^1 = H$ $R^2 = H$
[71] $R^1 = H$ $R^2 = CH_2CH_2Si(CH_3)_3$
[72] $R^1 = CH_3$ $R^2 = CH_2CH_2Si(CH_3)_3$

Tetramethylated HP [11] was treated with 2-(trimethylsilyl)ethoxide in benzene as above. Tlc analysis of the product showed one running spot and mainly baseline material. Mass spectral analysis of the product showed only one peak (*m/z* 827); which was compatible with the dimethyl ether [72].

Another approach was to transesterify the highly reactive tetraTBDMS HP [17] (see Chapter3) with 2-(trimethylsilyl)ethanol to give the trimethylsilyl ester [73]. Careful manipulation of the reaction to form tetraTBDMS HP [17] to give quantities of the monoTBDMS ether bisTBDMS ester [74] and treatment with 2-(trimethylsilyl)ethanol would enable the corresponding 2-(trimethylsilyl)ethyl ester [75] to be synthesised; a useful precursor to the ester linked dimer [57] (Scheme1). TetraTBDMS HP [17] was prepared from HP and TBDMS triflate as before (Chapter3), but 2-(trimethylsilyl)ethanol was added prior to the reaction being worked up. Tlc analysis of the product showed two running spots plus some baseline material; mass spectral analysis showed peaks compatible with the diester [73] (*m/z* 1027) and the mono(2-(trimethylsilyl)ether) ester [76] (*m/z* 928), indicating that this is a potentially useful reaction for the formation of 2-(trimethylsilyl)ethyl ester [75].

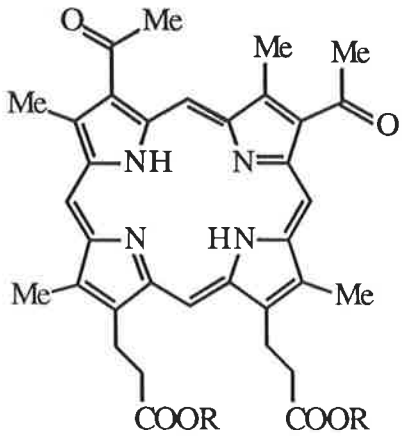


[73] $R^1 = Si(CH_3)_2C(CH_3)_3$ $R^2 = R^3 = CH_2CH_2Si(CH_3)_3$
[74] $R^1 = H$ $R^2 = R^3 = Si(CH_3)_2C(CH_3)_3$
[75] $R^1 = H$ $R^2 = R^3 = CH_2CH_2Si(CH_3)_3$
[76] $R^1 = Si(CH_3)_2C(CH_3)_3$ $R^2 = CH_2CH_2Si(CH_3)_3$ $R^3 = H$

Protecting the 1-hydroxyethyl sidechain as the acetyl has been used successfully in the synthesis of dihematoporphyrin ether[49,108] so its applicability to ester synthesis was investigated. Diacetyl DP [77] was treated with 2-(trimethylsilyl)ethanol and dicyclohexylcarbodiimide using DMAP as the base and dimethylformamide as the solvent; the diacetyl [77] was insoluble in dichloromethane. TLC analysis showed three spots but mass spectral analysis showed only one major peak (*m/z* 795) which is consistent with the expected diacetyl bis(2-(trimethylsilyl)ethyl ester) [78].

Formation of the esters via the acid chloride was attempted using the diacetyl [77]. Diacetyl DP [77] was treated with oxalyl chloride in dichloromethane and refluxed for 15 minutes to give the corresponding acid chloride, the volatiles were removed and 2-(trimethylsilyl)ethanol was added. The reaction gave mainly one product by tlc and mass spectral analysis showed a strong peak at *m/z* 795, which is compatible with the diacetyl bis(2-(trimethylsilyl)ethyl) ester

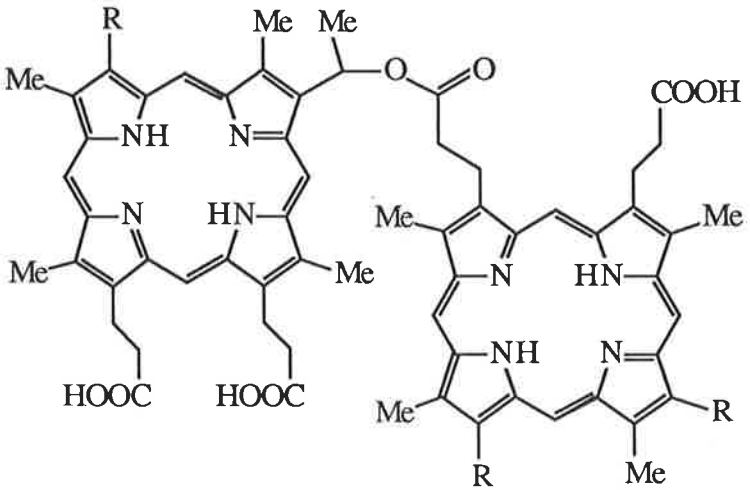
[78]. The proton n.m.r. spectrum of the chromatographically purified product contained peaks at -0.08 and -0.06 ppm ($SiC\underline{H}_3$) and 0.85 ppm ($SiC\underline{H}_2$) for the protons α to the silicon in the 2-(trimethylsilyl)ethyl group. The protons β to the silicon ($SiCH_2C\underline{H}_2$) resonate between 4.06 and 4.22 ppm but could not be distinguished from the peaks due to the methylene protons of the propionic sidechains α to the porphyrin ring. The resonances due to the methyl protons of the acetyl sidechains occurred in the same region (2.9 to 3.7 ppm) as the ring methyl protons and the other protons of the propionic sidechains. The ^{13}C n.m.r. spectrum showed resonances due to the trimethylsilylethyl group at -1.6 ppm ($Si\underline{C}H_3$), 17.3 ppm ($Si\underline{C}H_2$) and 62.8 ppm ($SiCH_2\underline{C}H_2$). Resonances attributable to the acetyl sidechains occur at 32.8 and 33.1 ppm tor the methyl carbons and 197.9 and 198.6 ppm for the carbonyl carbons. The ring methyl carbons show clearly the downfield shift due to the presence of the acetyl groups[56] with two of the resonances at 13.7 and 13.9 ppm for the methyls at positions 2 and 7 on the porphyrin ring compared to those for the 12 and 18 positions at 11.4 and 11.5 ppm.



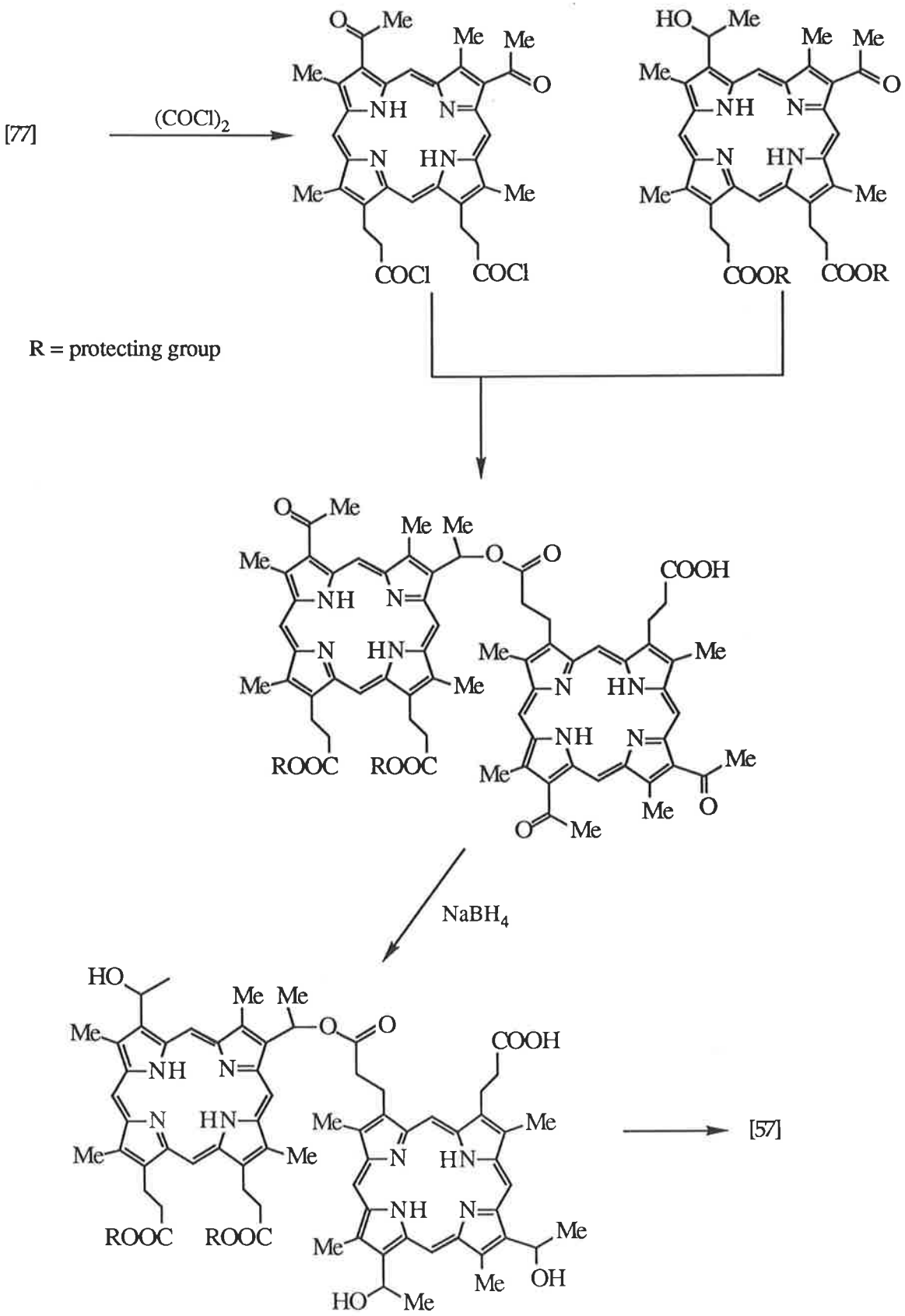
[77] R = H
[78] R = $CH_2CH_2Si(CH_3)_3$

4.3 Ester-linked porphyrin dimers

During the course of this investigation work other members of the research group had synthesised the ester-linked hematoporphyrin dimer with methyl and benzyl ester groups[92] (Scheme 2, R = CH_3, $CH_2C_6H_5$). However the ester protecting groups could not be successfully removed to give the ester-linked hematoporphyrin dimer [57]. Consequently their synthetic route to the ester dimer was investigated using 2-(trimethylsilyl)ethyl ester as the protecting group (Scheme 2, R = 2-(trimethylsilyl)ethyl). The synthesis of the vinyl terminated dimer [79], another possible component of HPD, and the methoxy terminated dimer [80] by a similar route was also investigated



[79] R = $CH{=}CH_2$
[80] R = $CH(OCH_3)CH_3$

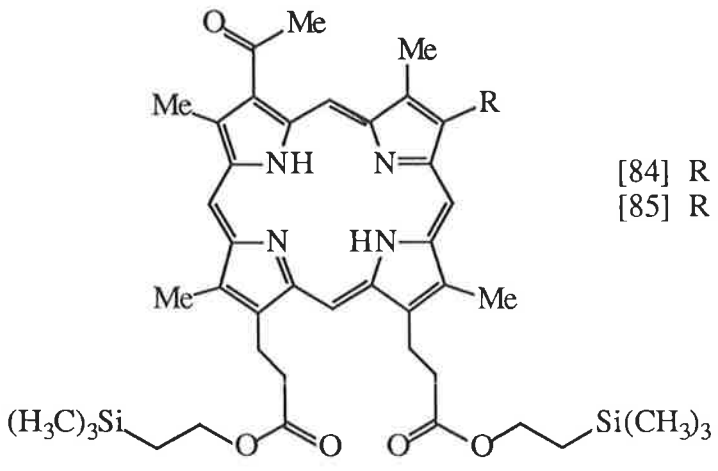


Scheme 2. Proposed synthetic route for the synthesis of the ester-linked hematoporphyrin dimer

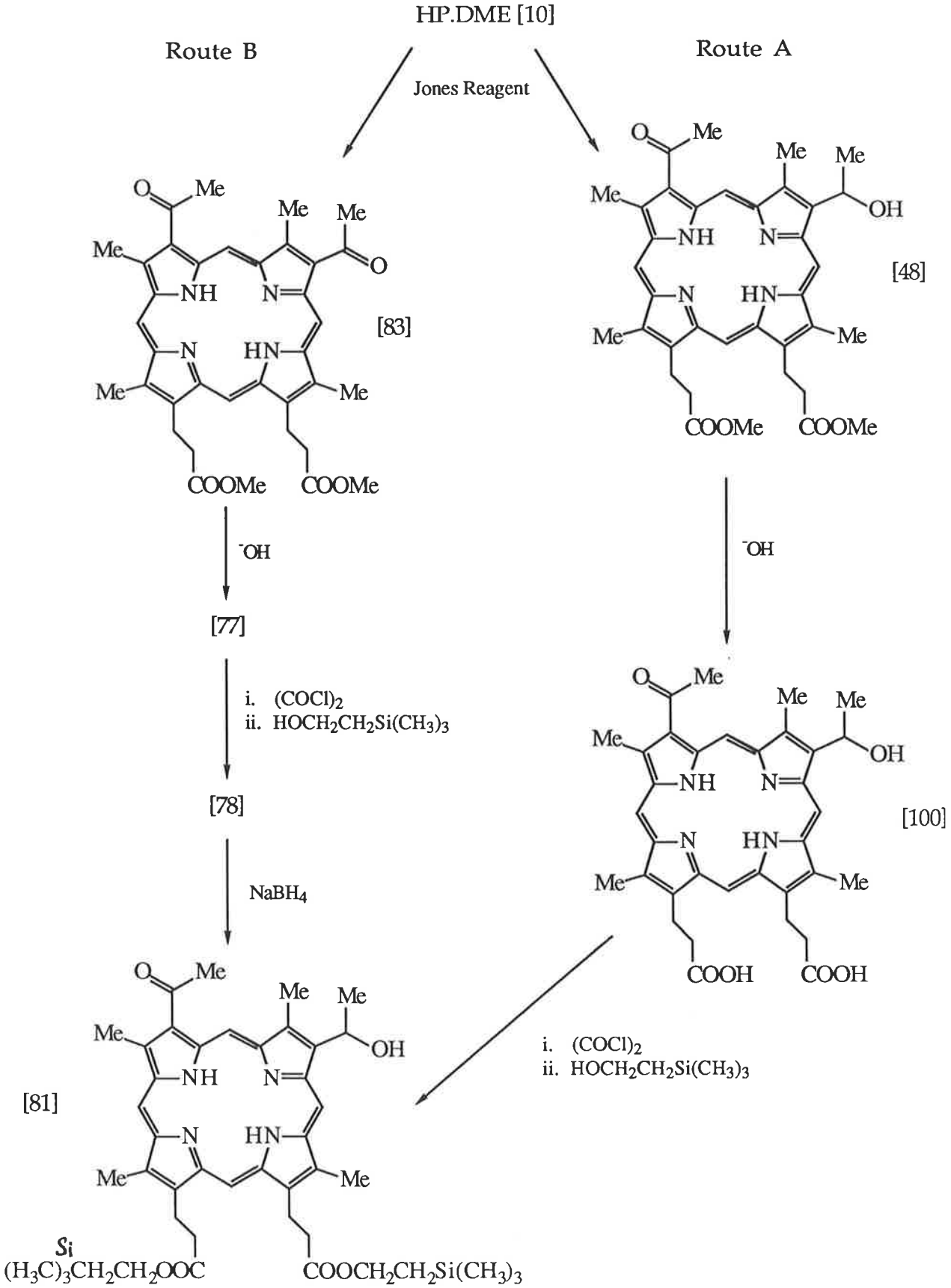
4.3.1 Acetyl terminated ester-linked dimer.

Two routes were investigated for the synthesis of the monohydroxyl precursor [81] (scheme 3). The first, and slightly shorter, route would only work if the oxalyl chloride conditions were mild enough to form the acid chloride only, and not react with the 1-hydroxyethyl sidechain.

The monoacetyl dimethyl ester [48] was prepared by treating HP.DME with Jones reagent[124] to gain a mixture of the mono and diacetyl porphyrins, [48] and [83], and HP.DME which were separated by chromatography. The methyl esters of the monoacetyl porphyrin [48] were hydrolysed under basic conditions and the carboxylic acid groups converted to acid chlorides using oxalyl chloride. The acid chloride was dissolved in dichloromethane and treated with 2-(trimethylsilyl)ethanol to give a major product in 68% yield, after purification. Mass spectral analysis of the product showed a peak at *m/z* 897 and one at *m/z* 779 which was one-tenth as intense. *M/z* 897 coincides with the mass expected for of monoacetyl trisilylated material [84] and *m/z* 779 corresponds to an expected fragmentation of this compound; elimination of 2-(trimethylsilyl)ethanol to the monoacetyl vinyl compound [85]. From this result it is apparent that the 1-hydroxyethyl group was being converted to the chloride during this reaction which then reacted with 2-(trimethylsilyl)ethanol to give the corresponding ether [84].

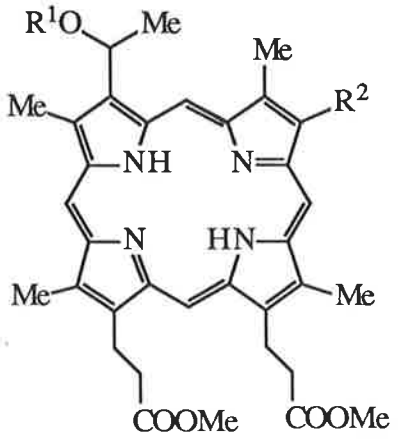


[84] R = $CH(CH_3)OCH_2CH_2Si(CH_3)_3$
[85] R = $CH=CH_2$



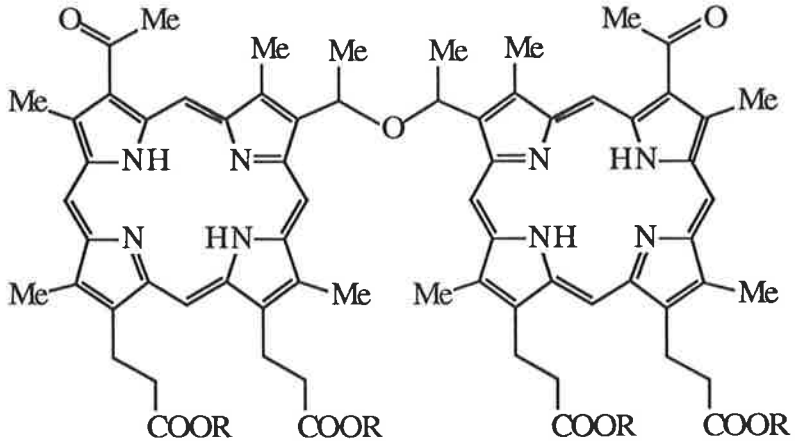
Scheme 3. The two routes for the synthesis of the disilyl ester [81]

This chlorination of the 1-hydroxyethyl sidechain was investigated briefly on a tlc scale. HP.DME was treated with oxalyl chloride. The crude product was treated with triethylamine at room temperature which gave mainly polar material (R_f 0.0), this could be indicative of unreacted chloride, which may bind to the tlc plate. The triethylamine and solvent were removed and methanol was added, the product contained two major components by tlc analysis. Mass spectral analysis of this product showed three peaks (*m/z* 655, 623 and 591). Tetramethylated HP [11] (*m/z* 655) was identified as one of the two major products and PP.DME [9] (*m/z* 591) was identified as a minor product; by comparison (tlc) with authentic samples. The other product was probably the monovinyl trimethylated HP [33] (*m/z* 623). When the chloride was refluxed with triethylamine it gave PP.DME (tlc, *m/z* 591) as the major product showing that elimination of the chloride occurs readily under basic conditions.



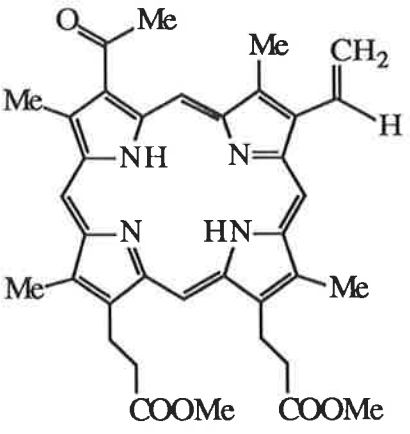
[86] $R^1 = CH_2CH_3$
$R^2 = CH(OCH_2CH_3)CH_3$
[87] $R^1 = CH_2CH_3$ $R^2 = CH{=}CH_2$
[88] $R^1 = (CH_2)_4CH_3$
$R^2 = CH(O(CH_2)_4CH_3)CH_3$

When the chloride of HP.DME was treated with ethanol it gave two compounds by tlc (R_f 0.7 and 0.58) and mass spectrum (*m/z* 683 and 637) which were compatible with the diethyl ether dimethyl ester [86] and its monodehydrated analogue [87]. Addition of pentanol to the chloride was investigated and the dipentyl ether [88] (*m/z* 767) appeared to be formed.

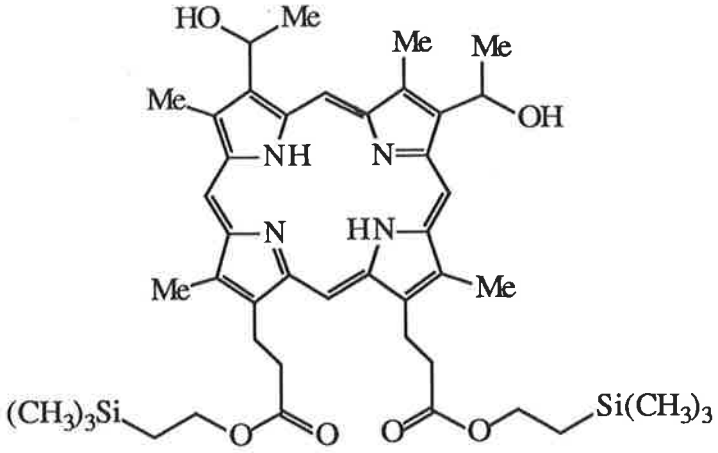


[89] R = CH_3
[94] R = $CH_2CH_2Si(CH_3)_3$

A trial reaction to form the ether-linked dimer using the chloride was performed. The monoacetyl dimethyl ester [48] was treated with oxalyl chloride and then a solution of the monoacetyl [48] in dichloromethane was added and the reaction allowed to stir overnight. Tlc and mass spectral analysis of the product indicated three products; the acetyl terminated dimer [89] (*m/z* 1231), monoacetyl dimethyl ester [48] (*m/z* 625) and its dehydrated analogue [90] (*m/z* 607). From these trial reactions oxalyl chloride appears to be a useful reagent for the conversion of the 1-hydroxyethyl group to the 1-chloroethyl group, which may be used for forming ethers, or eliminated using basic conditions to give the vinyl group. As was observed with the mesylate (chapter 3), the elimination reaction, to form the vinyl, is competing with nucleophilic substitution of the chloride.

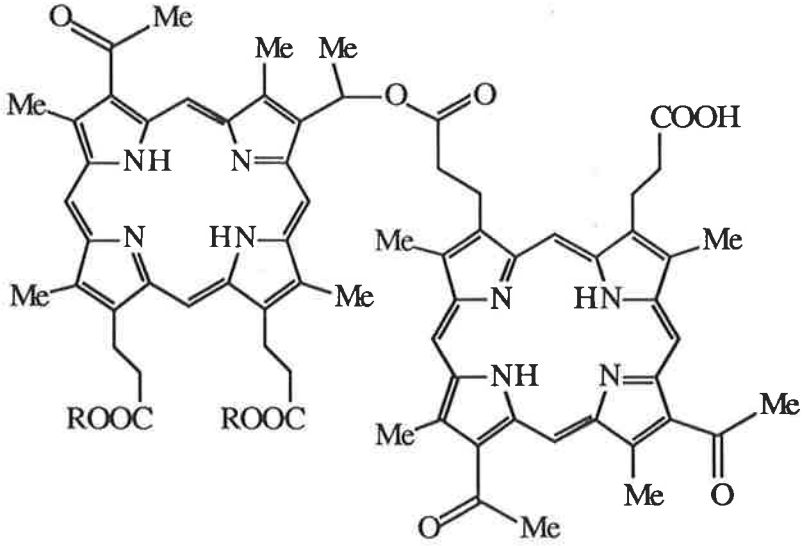


[90]



[91]

The monoacetyl bis(2-(trimethylsilyl)ethyl) ester [81] was formed by reducing the diacetyl silyl ester [78], using sodium borohydride in tetrahydrofuran. The reaction was monitored by tlc until maximum conversion to the monoacetyl product [81] was observed. The reaction yielded up to 51% of the monoacetyl product [81], with starting material [78] and HP.bis(2-(trimethylsilyl)ethyl) ester [91] also being recovered. The proton n.m.r. spectrum of the compound [81] was more complex than for the diacetyl silyl ester [78], exhibiting more resonances for the methine protons and the ring methyl protons. Multiplets were observed at 1.54 and 1.75 ppm attributable to the methyl protons of the 1-hydroxyethyl sidechain and at 5.3 and 5.6 ppm due to the proton under the alcohol. The ratio of the resonances at 1.54 and 1.74 ppm and at 5.3 and 5.6 ppm are both 3:2 (ie 1.54 : 1.74ppm =3:2), this may indicate that some selection between regioisomers has occurred during the reduction. Resonances at 32.8 ppm and 65.1 ppm are observed in the ^{13}C n.m.r. spectrum due to the methyl carbon and the 3^{o} carbon, respectively, of the 1-hydroxyethyl sidechain.

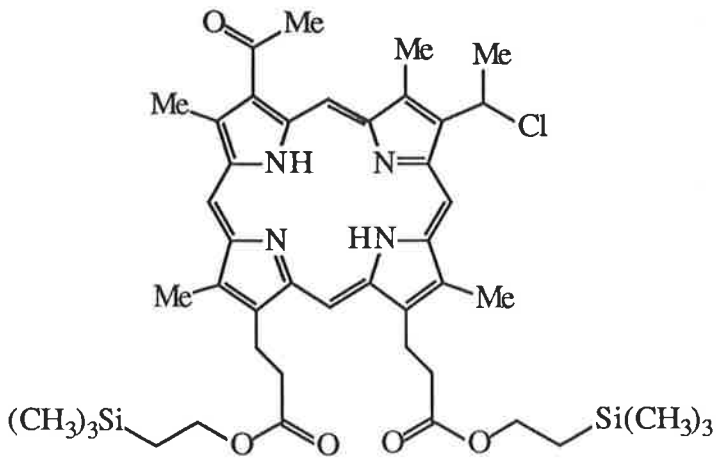


[92] R = $CH_2CH_2Si(CH_3)_3$
[99] R = H

The formation of the acetyl terminated ester-linked dimer [92] was attempted on a small scale initially. The acid chloride of diacetyl DP [77] was formed by treating diacetyl DP [77] with oxalyl chloride. The crude acid chloride was dried under vacuum (45 minutes at 0.01mm Hg), redissolved in dichloromethane, and the monoacetyl silyl ester (1 equivalent) [81] added. The reaction mixture was allowed to stir for 17 hours but still contained mainly starting material. A portion of the reaction mixture was refluxed for one hour with no change. Pyridine was added to the remaining mixture and it was stirred for two hours after which time tlc analysis showed 3 compounds of which one was the monoacetyl [81] (tlc, mass spectrum).

The three components of the product mixture were separated by preparative tlc; the first band (R_f 0.78), on closer inspection, contained two compounds which were separated by further chromatography. The highest R_f band showed peaks in the mass spectrum at *m/z* 1575 and 779 only, which are compatible with the ether linked dimer [94]. The presence of this compound may indicate some oxalyl chloride was still present after drying the acid chloride, allowing a small amount of the chloride [95] to be formed from the

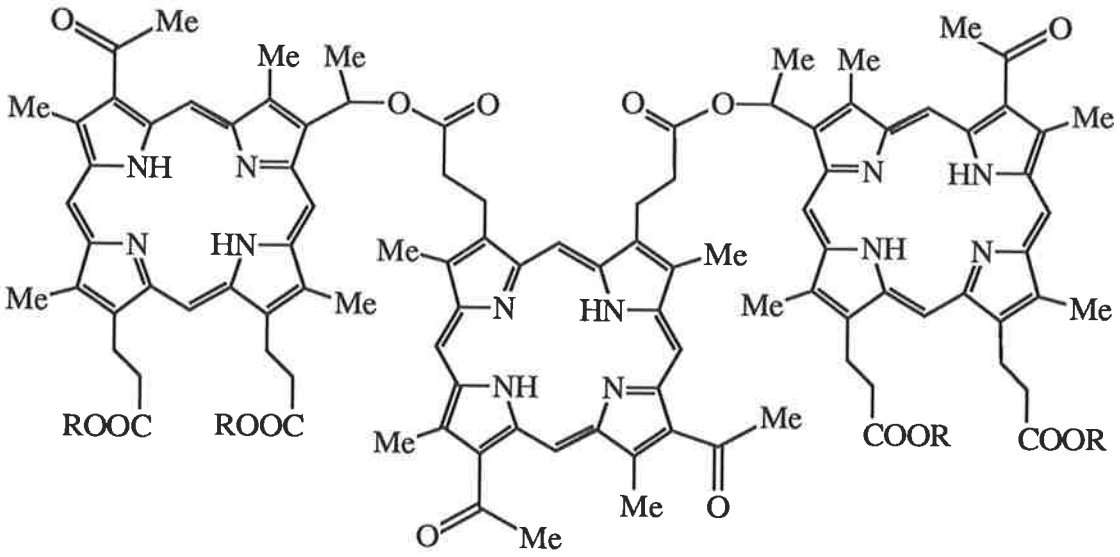
monoacetyl [81] which then condensed with the monoacetyl silyl ester [81] to give the ether-linked dimer. In other preparations the residue was dried for longer and this compound was not observed again. The mass spectrum of the second band contained peaks at *m/z* 2152, 1373, 779 and 595. *M/z* 2152 is compatible with the trimer [96] and shows a strong fragmentation to *m/z* 1373 in the MIKES spectrum. The lowest R_f band of the original preparative tlc corresponded to the acetyl terminated dimer [92] with peaks at *m/z* 1373, 779 and 595 in the mass spectrum. A MIKES spectrum of the peak *m/z* 1373 showed that it fragmented mainly to *m/z* 1356, presumably through loss of OH from the carboxylic acid, and *m/z* 779, which corresponds to the vinyl acetyl compound [85]. The peak *m/z* 595 arises from a number of other peaks as shown by its B^2/E linked spectrum. The fragmentation of the trimer and dimer follows the pattern observed for 1-alkoxyethyl and 1-hydroxyethyl compounds which eliminate to give the vinyl sidechain.



[95]

The reaction was repeated on a larger scale using a small amount of pyridine as the base. The reaction worked well, as indicated by tlc, but some problems were encountered in the separation of the products. When radial chromatography was used material tended to stick to the plate and separation was poor, the crude dimer obtained by radial chromatography had to be

purified by preparative tlc, as did the trimer. On an even larger scale the product was purified most successfully on a short silica column. The trimer eluted well with 4-8% acetone in dichloromethane but the dimer exhibited erratic behaviour; it would begin to elute at 40% acetone in dichloromethane and then its elution would begin to slow, even if the acetone component was gradually raised to 100%. The rest of the dimer was then eluted using 5-10% methanol. This behaviour has been observed for other porphyrin systems.[125]



[96] R = $CH_2CH_2Si(CH_3)_3$

The proton n.m.r. spectrum of the dimer was complex due to the number of possible isomers for the ester linked dimer. The monoacetyl silyl ester [81] used is a racemic mixture of regioisomers and these may form esters with either the 7 or 8 propionic sidechains in the diacetyl DP [77] to give a mixture of 4 regioisomers. It is also possible that the dimer may self aggregate in a cofacial manner in which case conformational isomers may occur. Conformational isomers may exhibit different chemical shifts as has been observed in aggregated chlorophylls[56] and also in porphyrin cofacial dimers[126].

In the proton n.m.r. spectrum the trimethylsilyl methyl protons are readily recognisable at -0.19 to -0.04 ppm and the methylene protons adjacent to the

silicon are seen as a broad multiplet at 0.82 ppm. The other methylene protons of the trimethylsilylethyl group are part of a multiplet at 4.1 to 4.2 ppm which also contains the methylene protons of the propionic sidechains which are adjacent to the porphyrin ring. The acetyl methyl protons, the methylene protons α to the ester and carboxylic acid groups, and the ring methyl protons are all part of a complex region from 2.4 to 3.9 ppm. The methine protons α to the ester linkage appear at 6.8 ppm and the methine protons of the porphyrin ring occur over a large shift range from 7.7 to 9.7 ppm. The ^{13}C n.m.r. spectrum is also reasonably complex with multiple peaks for most of the resonances. The resonances due to the 2-(trimethylsilyl)ethyl groups appear at shifts identified in the monomers. The carbons of the propionic sidechains are seen as a number of peaks, their shifts are affected by the nature of the propionic sidechain terminating group, which is either a carboxylic acid, a porphyrin linking ester or a 2-(trimethylsilyl)ethyl ester, as well as the complication of regioisomers. Three types of carbonyl carbons are identified, ketone carbonyls at 197 to 198.5 ppm, the carboxylic acid at 176.9 and the ester carbonyls at 173 to 174 ppm.

The acetyl terminated trimer gave similar, but more complex, n.m.r. spectra than the dimer. There are no resonances due to the carboxylic acid carbon in the ^{13}C n.m.r. spectrum of the trimer.

The 2-(trimethylsilyl)ethyl esters of the acetylterminated dimer [92] proved to be more difficult to remove than either those of the 1-hydroxyethyl terminated ester-linked dimer [97] or the 1-methoxyethyl terminated ester-linked dimer [98], which are discussed later. Treatment of the acetyl dimer [92] with tetrabutylammonium fluoride[117,118] at room temperature for extended periods resulted in very little desilylation (tlc, HPLC). Treatment with lithium fluoride in tetrahydrofuran did not facilitate removal of the silyl esters even after the addition of boron trifluoride etherate[127]. The use of trifluoroacetic

acid[128] in dichloromethane for 50 minutes resulted in consumption of the acetyl dimer [92] to give a polar product. The mass spectrum of this product showed it to be the acetyl dimer triacid [99] (*m/z* 1173) with peaks at *m/z* 597 and *m/z* 595 due to fragmentation and/or hydrolysis (the HPLC trace showed a minor peak (3%) due to the monoacetyl diacid [100]) HPLC of the product showed two major peaks at 9 and 10 minutes (Fig. 1).

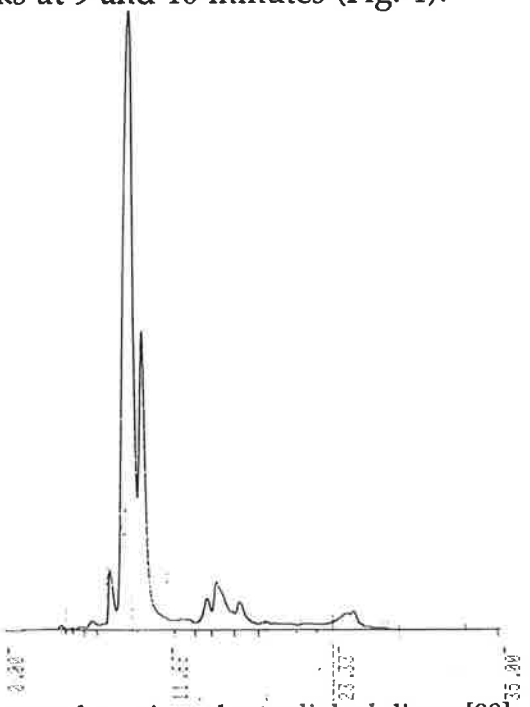
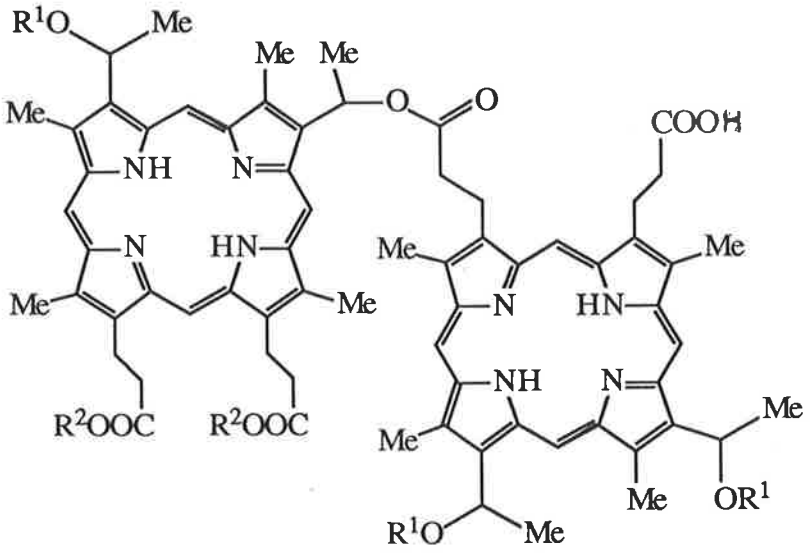


Figure 1. HPLC trace of the acetyl terminated ester-linked dimer [99]

4.3.2 Ester-linked hematoporphyrin dimer

The acetyl dimer bis(2-(trimethylsilyl)ethyl) ester [92] was reduced using sodium borohydride to give the ester-linked HP dimer bis(2-(trimethylsilyl)ethyl) ester [97]. The product consisted of a number of components from R_f 0.17-0.28 but the mass spectrum showed peaks at *m/z* 1379 and *m/z* 781 only, compatible with the 1-hydroxyethyl terminated ester-linked dimer [97]. The multiple spots in the tlc appear to be due to the different isomers of the DHE ester. The reduction was done on a larger scale and dimer [97] (*m/z* 1379) was isolated in 65% yield. The ^{13}C spectrum showed no peaks associated with the acetyl sidechain carbons. Peaks due to the

hydroxyethyl group are present at 25 to 26 ppm for the methyl carbon and 64 to 65 ppm for the methine carbon. During accumulation of the ^{13}C spectrum the compound appeared to decompose forming a precipitate. The precipitate was removed by filtration but attempts to obtain a ^{1}H n.m.r. spectrum on the remaining material gave very poorly resolved signals indicating that the product is unstable in solvent.



[97] $R^1 = H$ $R^2 = CH_2CH_2Si(CH_3)_3$
[98] $R^1 = CH_3$ $R^2 = CH_2CH_2Si(CH_3)_3$

The ester-linked dimer of HP [57] was obtained by treating the corresponding bis(2-(trimethylsilyl)ethyl) ester [97] with tetrabutylammonium fluoride. The mass spectrum contained the (M+H) peak at *m/z* 1179. Peaks in the mass spectrum at *m/z* 599 and 581 are compatible with fragmentions of the dimer to give HP and HV, a peak at *m/z* 621 may be due to the monosodium salt of HP. HPLC of the dimer (Fig 2(a)) showed some HP due to hydrolysis and a mixture of peaks of retention time 8 to 15 minutes. As with the DHE ether the ester [57] has retention times in a region which is generally unoccupied in the HPLC trace of HPD. Therefore the ester-linked hematoporphyrin dimer [57] is not a major component of HPD.

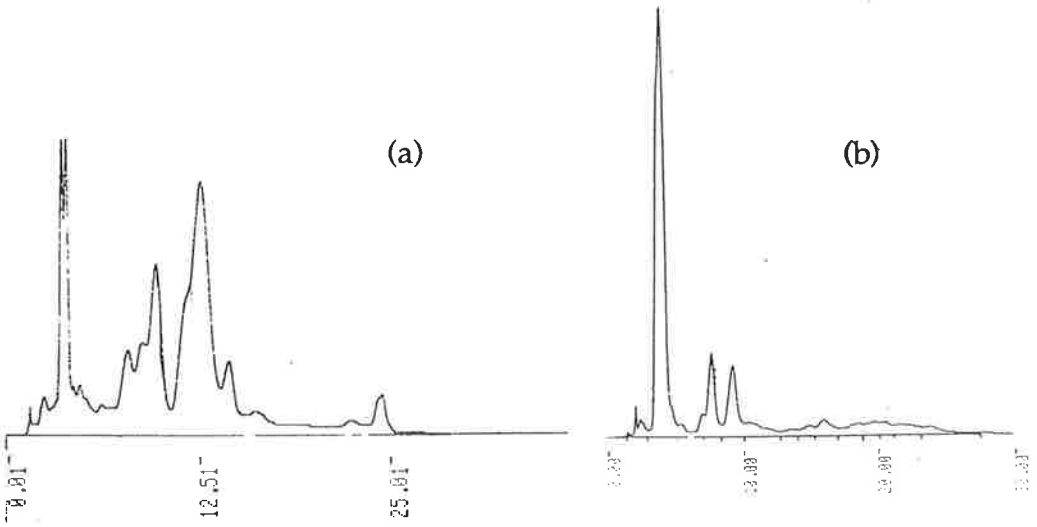


Figure 2. HPLC traces of (a) ester-linked hematoporphyrin dimer [97] and (b) the biological testing sample.

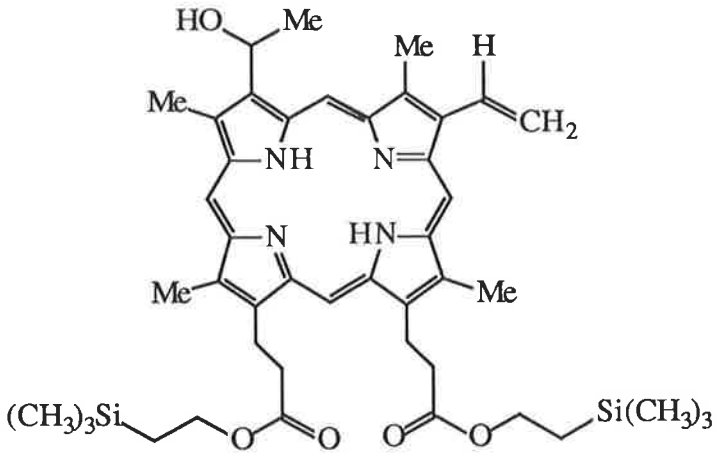
A sample of the ester dimer was prepared with difficulty for biological testing. A solution of the dimer in saline at pH 7.1 and at a specific concentration needed to be prepared for the testing for anticancer activity. The dimer, however, would not dissolve in saline directly so ethanol was used as a cosolvent and then as much ethanol as possible was removed whilst still maintaining a homogeneous solution. An HPLC trace of the final testing solution sample showed that the dimer had been hydrolysed, mainly to HP and HV (Fig. 2(b)), during the sample preparation .

4.3.3 Vinyl terminated ester-linked dimer

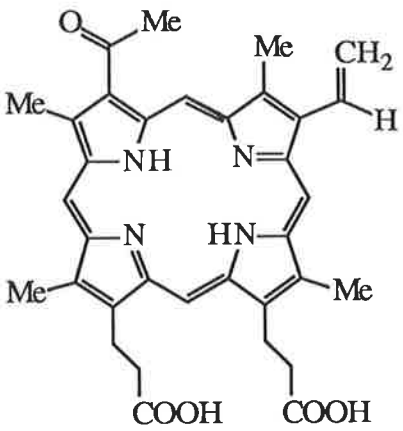
The vinyl terminated ester-linked dimer [79] was made by a similar route (Scheme2) to the acetyl terminated ester-linked dimer [99] except the porphyrins contained vinyl sidechains in place of the acetyl sidechains.

The monovinyl bis(2-(trimethylsilyl)ethyl) ester [101] was synthesised in a similar manner to [81] (Scheme 3, route B). HV.DME [21] was treated with Jones reagent to give the acetylvinyl dimethyl ester [90]. The reaction did not

proceed cleanly and the product mixture had to be chromatographed twice to give pure acetylvinyl [90] (tlc, mass spectrum) in 40% yield. It may be preferable to form this compound by dehydration of the monoacetyl dimethyl ester [48]. The methyl esters were hydrolysed using basic conditions to give 3(8)-acetyl-8(3)-vinyldeuteroporphyrin [102] which was reacted with oxalyl chloride and then 2-(trimethylsilyl)ethanol, as described, to form the 2-(trimethylsilyl)ethyl esterified material [85] in 67% yield (*m/z* 779). Proton n.m.r. of the product showed, apart from the expected porphyrin and trimethylsilylethyl ester resonances, multiple peaks between 6.1 and 6.4 ppm, and 7.8 and 8.2 ppm, due to the terminal and nonterminal vinyl protons respectively. The ^{13}C n.m.r. spectrum showed peaks at 121.2 ppm due to the terminal carbon and 129.5 and 129.7 ppm for the other carbon of the vinyl sidechain. The acetyl sidechain showed peaks at 33.1 and 199.3 ppm for the methyl and carbonyl carbons respectively. Reduction of [85] using sodium borohydride in tetrahydrofuran gave 3(8)-(1-hydroxyethyl)-8(3)-vinyldeuteroporphyrin bis(2-(trimethylsilyl)ethyl) ester [101] in 90% yield. The spectral data on [101] showed no resonances due to the acetyl sidechain but instead showed resonances due to the hydroxyethyl sidechains. The n.m.r. spectra contained doublets at 1.70 and 1.76 ppm due to the methyl protons and 25.8 ppm for the methyl carbon of the 1-hydroxyethyl sidechain. The tertiary carbon and proton of the sidechain were observed at 65.3 ppm and 5.43 and 5.53 ppm respectively.



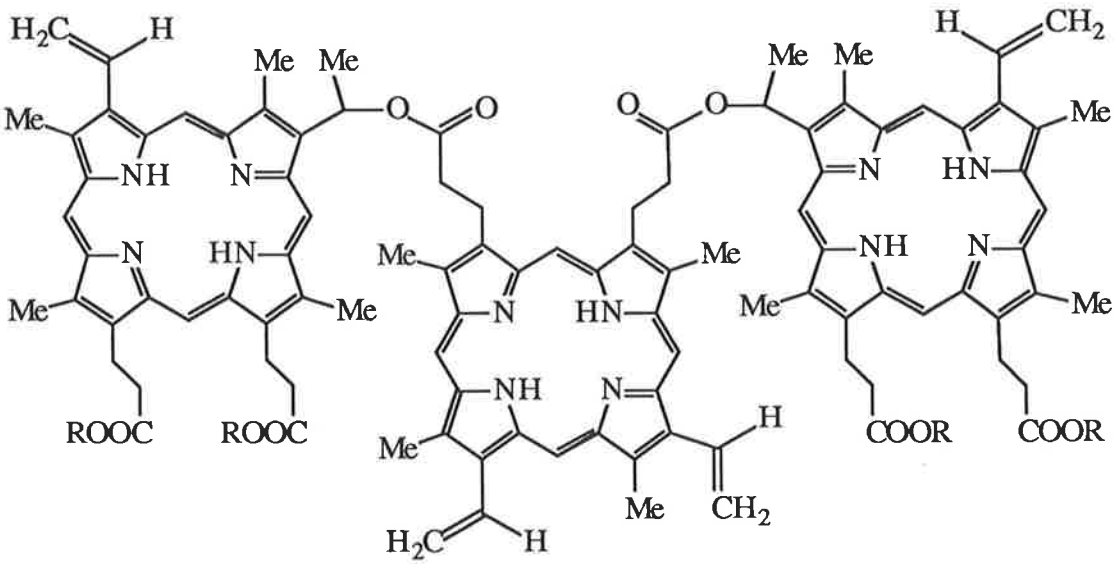
[101]



[102]

The acid chloride of PP was formed using oxalyl chloride by the method which has been described for other compounds. The crude acid chloride, after being dried for 1 hour under vacuum, was dissolved in dichloromethane to which a solution of the monovinyl bis(2-(trimethylsilyl)ethyl) ester [101] and pyridine in dichloromethane was added and the solution allowed to stir for 17 hours. The reaction was worked up and tlc analysis of the residue showed 5 main components, including a small amount of starting material [101]. The mass spectrum showed two main peaks at *m/z* 2089 and *m/z* 1325 and also peaks at *m/z* 779 [101], *m/z* 763 and *m/z* 563. *M/z* 2089 corresponds to the vinyl terminated ester-linked trimer tetra(2-(trimethylsilyl)ethyl) ester [103] and *m/z* 1325 to the vinyl terminated ester-linked dimer [104]. The product was chromatographed (preparative tlc). The highest running band contained starting material [101] and some other compounds by tlc. Two intermediate bands contained one main component by tlc (R_f 0.22) so they were combined and rechromatagraphed to purify the compound, however the chromatography yielded four bands which contained more than one component, indicating that the compound may be unstable. None of the bands separated in either chromatography showed the dimer or trimer as a major component by mass spectral analysis as the analysis of the product

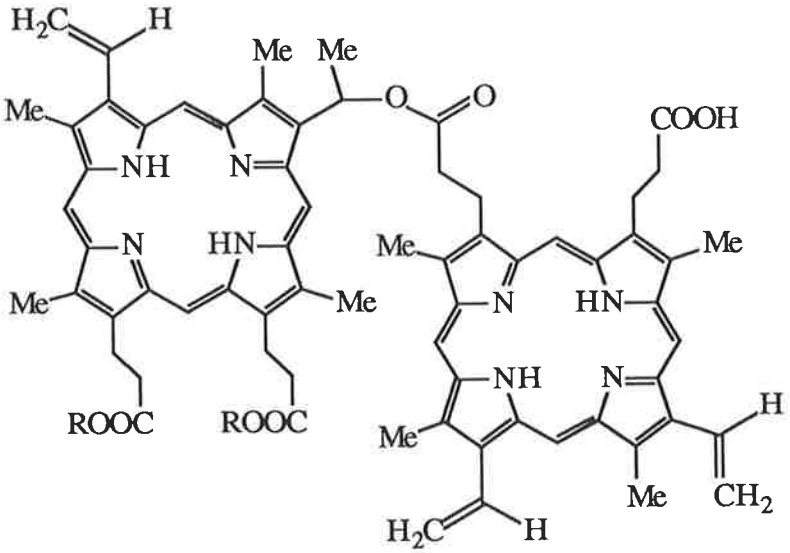
mixture had indicated. Peaks attributable to the dimer and trimer appeared in a number of the fractions but only at low relative intensities. A peak at *m/z* 2121, which corresponds to the addition of oxygen to the trimer [103] was seen in three of the samples and may be due to the formation of the photoproducts of the trimer, which, as discussed in Chapter3, are commonly formed by porphyrins containing vinyl sidechains.[102-107]



[103] R = $CH_2CH_2Si(CH_3)_3$

The reaction was repeated. Tlc of the product showed 3 main running components and baseline material. The component at R_f 0.14 on closer examination consisted of 2 poorly separated components. Mass spectral analysis showed 4 main peaks at *m/z* 2089, 1325, 763 and 563. A MIKES spectrum of *m/z* 2089 showed a major fragmentation to *m/z* 1325. The ion at *m/z* of 1325 showed fragmentations to *m/z* 1282, 763 and a smaller fragmentation to *m/z* 563. The components were separated on a squat column. The first component to elute corresponded to the starting material [101] (tlc, mass spectrum). Elution with 40% acetone in dichloromethane gave impure trimer [103]; *m/z* 2089. Elution with 10 to 20% methanol in dichloromethane gave two fractions which appeared to be the dimer [104]; *m/z*

1325. These fractions were combined and purified by chromatography on a short silica column to give the vinyl dimer in 19% yield. The ^{13}C n.m.r. spectrum showed typical resonances of the porphyrin and the trimethylsilylethyl esters. The methine carbon α to the ester linkage resonates at 67.8 ppm which is within 0.5 ppm of the values seen for the acetyl and hydroxy dimers. The carbons of the vinyl groups appear at 117 ppm (=CH_2) and 130 ppm (=CH) as multiple signals.



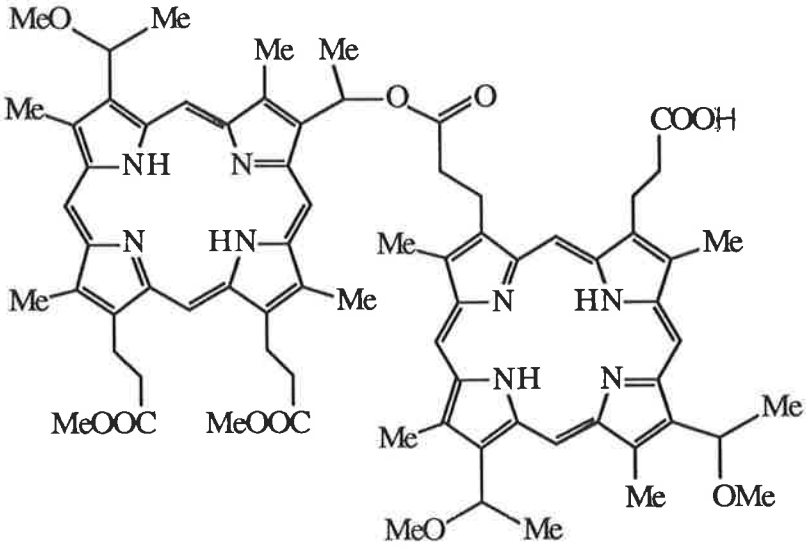
[104] R = $CH_2CH_2Si(CH_3)_3$

The ^1H n.m.r. spectrum was complex, especially in the region 5.5 to 9 ppm which includes the peaks due to the vinyl protons, the methine proton adjacent to the ester linkage and some of the ring methine protons. A homoCOSY n.m.r. spectrum of the product was used to help assign these resonances by indicating coupling between protons. The methyl protons of the oxyethyl sidechain resonated at 1.5 ppm and correlated in the homoCOSY with resonances at 6.98 ppm; due to the methine proton. The set of resonances from 5.7 to 6.1 ppm correlate with resonances from 7.0 to 7.5 ppm, and those from 6.1 to 6.3 ppm correlate with resonances at 7.9 ppm. The resonances at 5.7 to 6.3 ppm may be ascribed to the terminal vinyl protons and those from 7 to 8 ppm to the nonterminal protons. The appearance of two separate sets of peaks

(5.7 to 6.1 ppm which correlate with 7.0 to 7.5 ppm and 6.1 to 6.3 ppm which correlate to 7.9 ppm) may be due to the different vinyls in the dimer or to different regioisomers. The vinyl dimer was not tested for activity as the sample of the trimethylsilylethyl ester dimer [103] had degraded to unidentified materials before testing facilities became available.

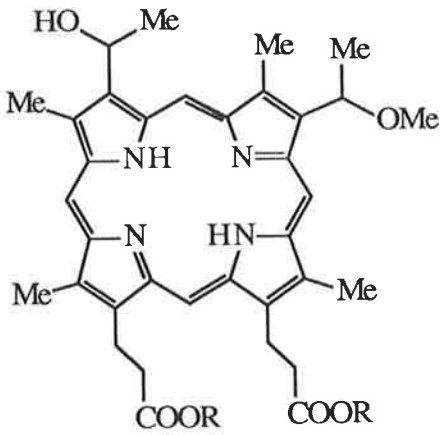
4.3.4 1-Methoxyethyl terminated ester-linked dimer

The 1-methoxyethyl terminated ester-linked dimer [80], was also synthesised to test the activity and stability of low polarity, bulky sidechains.

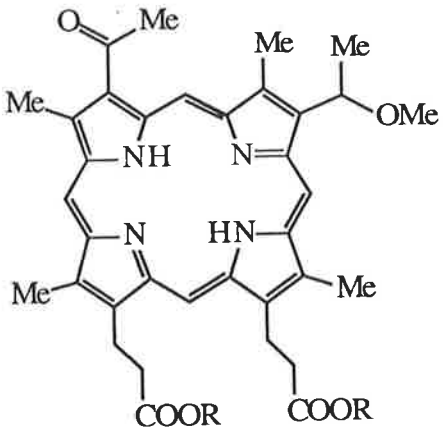


[105]

An initial trial reaction was done to form the 1-methoxyethyl terminated ester-linked dimer dimethyl ester [105] to test the stability of the 1-methoxyethyl sidechains to the reaction conditions. The dimethyl ether of HP [12], obtained by basic hydrolysis of tetramethyl HP [11], was treated with oxalyl chloride to give the acid chloride, which was dried and treated with trimethyl HP [32] in dichloromethane, using pyridine as a base, for 18 hours. After work up the product was separated by preparative tlc and the dimer purified further by chromatography on a short silica column to give the methyl etherified and esterified ester-linked dimer [105] (*m/z* 1249) in 32% yield.



[106] R = $CH_2CH_2Si(CH_3)_3$



[107] R = CH_3
[108] R = H
[109] R = $CH_2CH_2Si(CH_3)_3$

The 1-methoxyethyl terminated ester-linked dimer [80] was synthesised by the route that has been established for the vinyl and acetyl terminated ester-linked dimers. The monomethyl ether [106] was synthesised by the route outlined in Scheme 3, route B. Trimethylated HP [32] was oxidised using Jones reagent to monoacetyl compound [107] in 73% yield and the methyl esters removed by basic hydrolysis to give the monoacetyl monomethyl ether [108]. Treatment of the diacid [108] with oxalyl chloride gave the acid chloride which upon treatment with trimethylsilylethanol and purification gave the bis(2-(trimethylsilyl)ethyl) ester [109]. The mass spectrum of this material showed a molecular ion at m/z 811 with a small peak at m/z 779 (30%) due to elimination of methanol from the 1-methoxyethyl sidechain. The proton and ^{13}C n.m.r. spectra were as expected. In particular a doublet at 2.28 ppm and a quartet at 6.09 ppm, with coupling constants of 6 Hz, in the proton n.m.r. spectrum, were due to the methyl and methine protons of the 1-methoxyethyl sidechain respectively. Resonances due to the methyl of the acetyl sidechain and the methyl ether of the methoxyethyl sidechain were part of the complex region from 3 to 4 ppm. Signals at 25.1, 57.3 and 74.9 ppm, in the ^{13}C n.m.r. spectrum, were due to the methyl, methyl ether and methine protons,

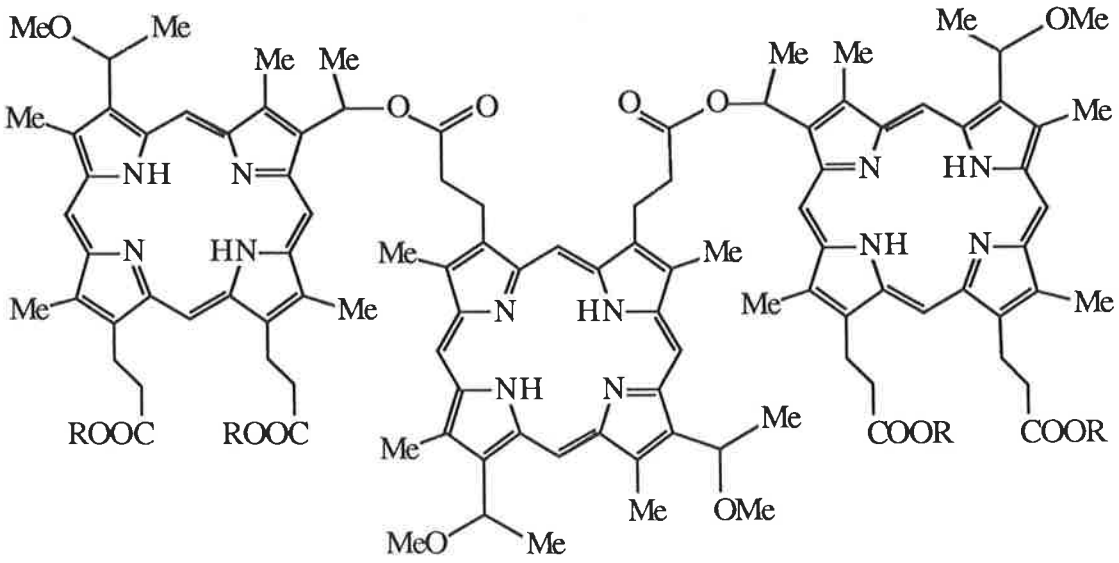
respectively, of the methoxyethyl sidechain and signals at 33.2 ppm and 199.7 ppm were attributable to the acetyl sidechain.

The monoacetyl compound [109] was reduced to the 1-hydroxyethyl compound [106], using sodium borohydride in tetrahydrofuran. The mass spectrum shows an increase in 2 mass units to *m/z* 813 for the molecular ion and two small peaks at *m/z* 795 and *m/z* 781; compatible with the elimination of water and methanol from the sidechains. The proton n.m.r. spectrum contains signals at 2.1 and 6.2 ppm due to the 1-hydroxyethyl sidechain and at 26.2 and 66.0 ppm in the ^{13}C n.m.r. spectrum. Acetyl sidechain peaks at 33 ppm and 199 ppm were not observed.

The dimethyl ether of HP [12] was treated with oxalyl chloride to form the acid chloride which was in turn treated with the monomethyl ether [106] (0.66 equivalents) in the presence of pyridine. Tlc analysis of the reaction product showed two major components (R_f 0.5 and 0.11) which were assumed to be the 1-methoxyethyl terminated trimer [110] (R_f 0.5) and 1-methoxyethyl terminated dimer [98] (R_f 0.11), based on expected polarities. A short alumina column was used to try to separate the components but this was unsuccessful with a large amount of porphyrin material being retained on the column. Some of this material was removed by adding acetic acid to the alumina and then washing with acetone to recover what appeared to be the dimer by tlc. The products were separated and purified using a short silica column prewashed with 1% methanol in dichloromethane to give trimer [110] (12%) and dimer [98] (23%).

The mass spectral analysis of the 1-methoxyethyl terminated ester-linked dimer [98] showed a main peak at *m/z* 1421 with other peaks at *m/z* 1390, 795 and 627. A MIKES spectrum of the *m/z* 1421 peak showed fragmentations to *m/z* 1390, elimination of methanol from the sidechain, *m/z* 794 and 627.

The proton n.m.r. spectrum of the dimer is very complex and less well resolved than the monomers due to the increase in the number of possible isomers. Resonances for the 2-(trimethylsilyl)ethanol groups are at similar chemical shifts to those seen in the monomethoxy HP.bis(trimethylsilyl)ethyl ester [106]; -0.13 to -0.05 ppm, for the methyl protons, 0.86 ppm for the methylene protons atta ched to the silicon and 4.35 ppm for the methylene protons β to the silicon. The methine proton under the methoxy group resonates at 6.03 ppm and a resonance is observed at 7.65 ppm for the methine proton under the porphyrin ester linkage. The methyl protons α to this proton are coincident with the methyl protons of the methoxy ethyl sidechain at 2.23ppm. The ^{13}C n.m.r. is also more complex than the comparable monomers. Resonances of particular interest occur at 23.7 and 24.0 ppm for the methyl carbon α to the ester linkage and at 68.7 and 69.2 for the methine carbon of the ester linkage. Two resonances are seen in the carbonyl region, four peaks at 173 ppm are attributable to the ester carbonyls, including the linking ester, and at 174.8 for the acid carbonyl.



[110] R = $CH_2CH_2Si(CH_3)_3$

The trimer shows peaks at *m/z* 2217, 2187, 1437, 1420, 795 and 627 in the mass spectrum. MIKES of *m/z* 2217 shows fragmentation to 2187, 1420 and 796. The origin of the peak at *m/z* 1437 is unclear. The proton n.m.r. spectrum of the trimer is not as well resolved as n.m.r. spectrum of the dimer but it shows similar chemical shifts to the dimer. The ^{13}C n.m.r. spectrum is similar to the dimer but is slightly less complex with less multiple peaks, especially for the propionic sidechain carbons, this is probably due to the more symmetrical nature of the trimer which does not have any free acids, only propionic sidechains ending as esters. No resonances are seen for acid carbonyls. It is interesting to note that the acetyl compounds did not show a similar decrease in complexity in the ^{13}C n.m.r. spectra in going from the dimer to the trimer; apart from the loss of the carboxylic acid resonances. This may arise because in the acetyl case the introduction of the third porphyrin unit, and a second chiral centre, results in the introduction of diastereomers as well as further regioisomers.

The 1-methoxyethyl terminated dimer [80] was formed by treating the corresponding silyl ester [98] with tetrabutylammonium fluoride in tetrahydrofuran. The mass spectrum of the product showed a peak at *m/z* 1221 ($M+H^+$) and peaks at *m/z* 627 and 595, compatible with expected fragmentations. The HPLC trace of the dimer [80] (Fig. 3(a)) was similar to the trace observed for the 1-hydroxyethyl terminated dimer [57] but at longer retention times. The 1-methoxyethyl terminated dimer [80] was tested for anticancer activity but proved to be inactive. The dimer was unstable in the testing solution and marked hydrolysis of the sample was noted after two days at 4^o (Fig. 3(b)).

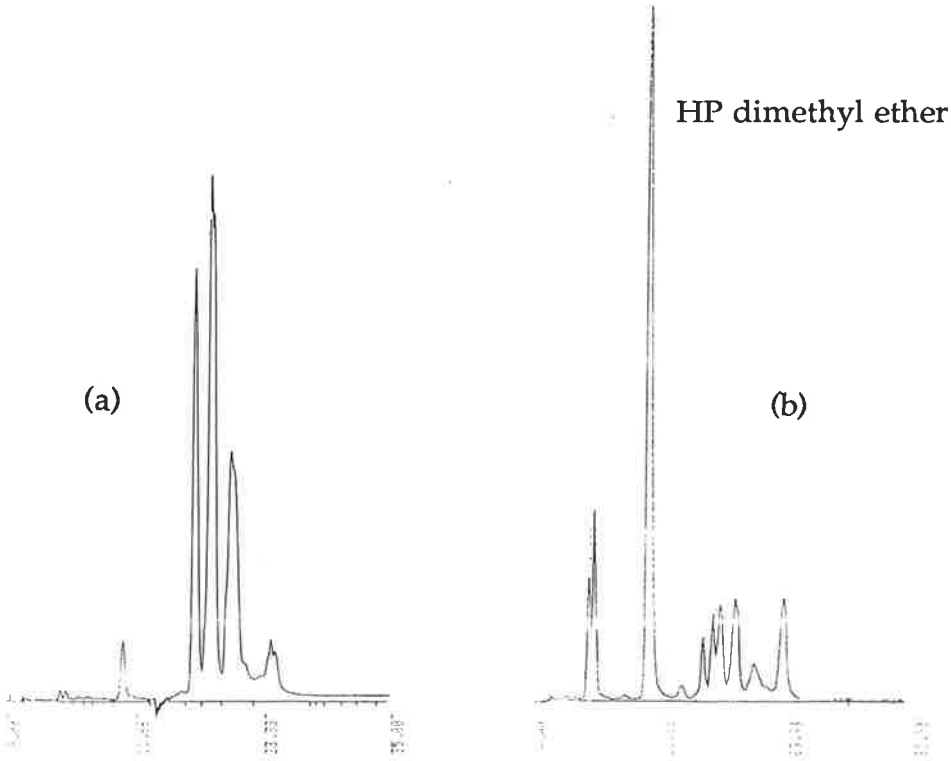


Figure 3. HPLC traces of (a) the 1-methoxyethyl terminated ester-linked dimer [80] and (b) the testing solution of [80] after standing at 4^{o} for 2 days.

A report of the synthesis of the ester-linked hematoporphyrin dimer [57], by an almost identical route to the one described in this chapter, was published[50] near the completion of this section of work. The authors had synthesised ester-linked dimer [57] and a didehydrated analogue using 2-(trimethylsilyl)ethyl esters and acetyl sidechains as protecting groups for the carboxylic acids and 1-hydroxyethyl sidechains respectively. A direct comparison of the proton n.m.r. spectra for the di [70] and monoacetyl [81] monomers and the acetyl dimer [92] was hampered by differences in chemical shifts which probably arise from different sample concentrations[56] used in accumulating the spectra. However the spectral data is in general agreement with the data presented in this chapter. Full spectral data for the ester-linked hematoporphyrin dimer [57] is not quoted by the authors. They did synthesise ^{13}C enriched ester-linked hematoporphyrin dimer and showed that resonances at 65.8 and 68.2 ppm were due to the carbons attached to the alcohols and the alkyl carbon of the ester linkage respectively. This concurs with the data presented above which show resonances at 64-66 ppm and 68.3 ppm

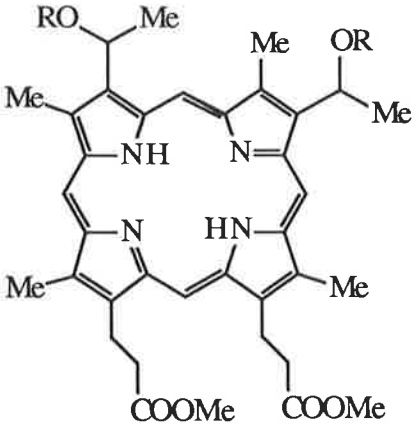
respectively. The authors also observed that the ester-linked dimer [57] was unstable in the saline injection solution for biological testing and was hydrolysed by acidic and basic conditions.

In conclusion, it has been shown that the ester linked porphyrin dimers and trimers can be synthesised and that ester linked hematoporphyrin dimer [57] is not present in any significant degree in HPD or Photofrin II by HPLC comparison. The ester linked dimers synthesised were not very stable in aqueous conditions so it is unlikely that ester dimers exist long enough, in the conditions for formation and injection of HPD or Photofrin II, for them to be the active components of these materials. This does not, however, preclude ester linked oligomers from being active components as the increased bulk and possible aggregation factors within the oligomers may protect the ester linkage from hydrolysis.

Chapter 5. Synthesis of analogues of HP

5.1 Introduction

In addition to the investigation and synthesis of possible components of HPD that may be active in photodynamic therapy, the synthesis of a group of analogues of HP was investigated. Evenson *et al.*[129] synthesised a number of HP dialkyl ethers of varying alkyl chain length and found that the anticancer activity of these diethers was dependent on their polarity. The short chain, more polar diethers, HP dimethyl ether [12] and HP diethyl ether [111] were inactive but the activity increased going from HP dipropyl ether [112] to HP dipentyl ether [113], which showed similar activity to Photofrin II. It had also been observed that the activity of the diporphyrin ethers was dependent on the sidechains in the terminating 3 and/or 8 positions.[48,113] In general, and in contrast with the observations for the monomers above, the activity of ether linked dimers with alkoxyethyl terminating sidechains decreases as the alkoxy group increases in size.[113] Hydrophobic terminating sidechains (vinyl, ethyl) have been found to be more active than hydrophilic terminating sidechains (1-hydroxyethyl); the vinyl terminated ether-linked dimer [14] showed similar activity to Photofrin II and dihematoporphyrin ether [7] showed no activity while the ether dimer [13] containing one vinyl and one hydroxyl group showed an intermediate activity.[48] The ether linked tetramers and trimers show less activity than the corresponding dimers.[113]



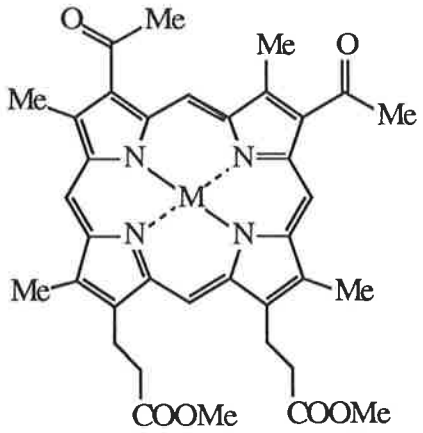
[10] R = CH_3
[111] R = CH_2CH_3
[112] R = $CH_2CH_2CH_3$
[113] R = $(CH_2)_4CH_3$

increasing activity

The synthesis of porphyrin sidechains of varying polarity in the 3 and 8 positions, compared to the 1-hydroxyethyl groups of HP, and sidechains containing tertiary hydroxyl groups, was of interest. It was envisaged that these sidechains may be synthesised by reacting porphyrins containing acetyl sidechains with Grignard reagents. Grignard reagents react faster with ketones than esters[130] so it was considered that the acetyl groups may be alkylated selectively in the presence of the methyl esters. Grignard reagents have been used in the past to include magnesium into porphyrins[131,132]; although this is no longer a favoured procedure, they have also been used to alkylate nickel *meso*-formyl octaethylporphyrin at the formyl group[133] and to form the 1-hydroxyethyl group from the formyl group[107,133].

5.2 Grignard reactions on the diacetyl porphyrins

Grignard reactions are traditionally carried out using ether, benzene or tetrahydrofuran as the solvent.[134] Diacetyl deuteroporphyrin dimethyl ester [83] was not soluble in ether or benzene and was only slightly soluble in tetrahydrofuran so the more soluble zinc metalloporphyrin analogue [114] was prepared by treating the diacetyl porphyrin [83] with zinc acetate in methanol according to a literature procedure.[124]



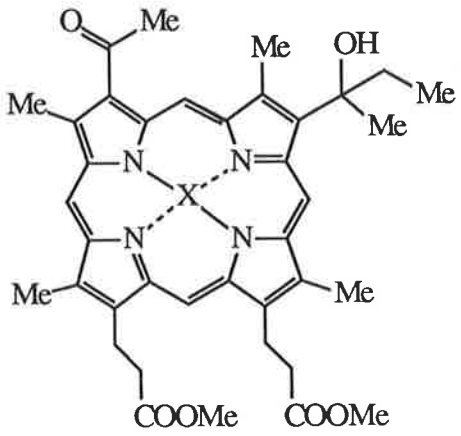
[114] M = Zn
[115] M = Mg

Zinc diacetyldeuteroporphyrin dimethyl ester [114] was dissolved in dry tetrahydrofuran and 1.25 equivalents* of ethyl magnesium bromide were added at zero degrees. The solution immediately went from a light pink colour to a red-green colour; this observation was typical of the Grignard reactions done using the zinc porphyrin [114]. After 40 seconds the reaction was worked up and the products analysed by tlc. This analysis showed two major components, neither of which were starting material, as well as some minor components. The visible spectrum of the product showed three distinct peaks, apart from the Soret band (427 nm), at 560, 602 and 642 nm in a ratio of 11 : 8 : 2. Mass spectral analysis of the product showed peaks at *m/z* 674 and 644 which correspond to the masses required for magnesium diacetyldeuteroporphyrin dimethyl ester [115] and the corresponding monoethylated product [116]. When shaken with acid the product showed a visible spectrum typical for a demetallated monocation[65], with the addition of triethylamine to the sample the spectrum became a typical 'etiotype' spectrum[65] with a small extra absorption at 661 nm. The mass spectrum of the product showed two major peaks at *m/z* 653 and 623 which corresponds to

*the number of equivalents is given with respect to the moles of carbonyl groups; not including the ester groups.

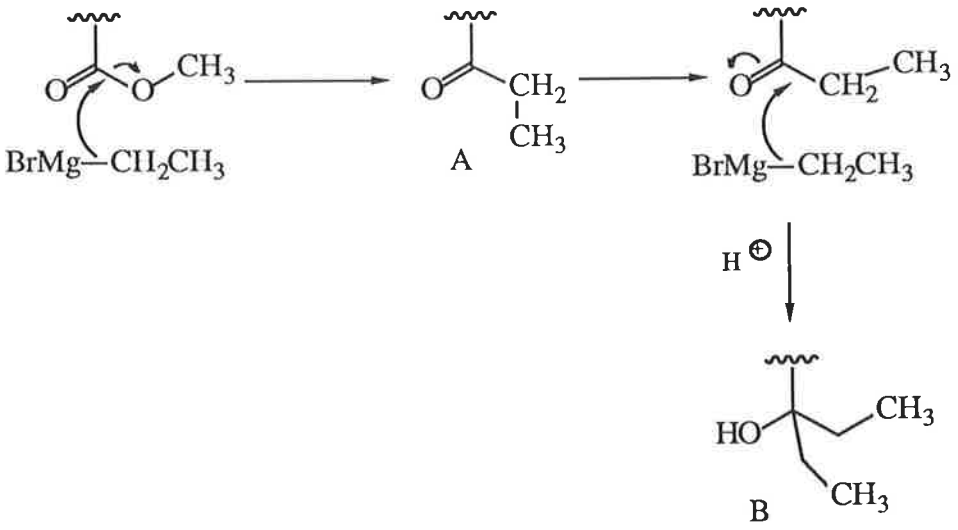
removal of magnesium from the compounds, [115] and [116], above. The two major components were separated by preparative tlc; the higher R_f material was identified as the diacetyl dimethyl ester [83] (*m/z* 623). The other compound showed one major peak in the mass spectrum (*m/z* 653); this value corresponds to the monoethylated product [117].

It is suprising that the zinc porphyrin is transmetallated by magnesium in this reaction as zinc has a higher stability index than magnesium (4.46 compared with 3.64 in the octaalkylporphyrins)[135] indicating that the transmetallation should be unfavourable.



[116] R = Mg
[117] R = H, H

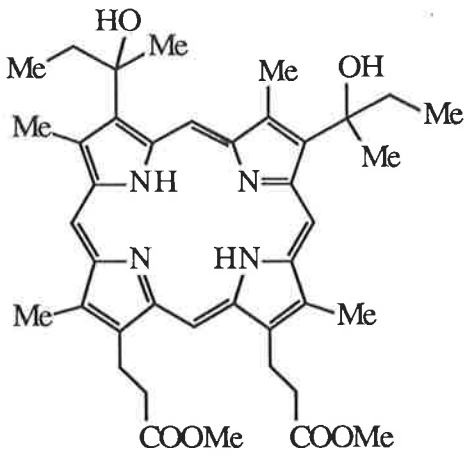
Using 1.9 equivalents of EtMgBr increased the amount of alkylation. Tlc analysis showed two major spots and the mass spectral analysis of the product, after treatment with dilute acid, showed peaks at *m/z* 683, 653 and 623, indicating that some dialkylation had occurred. The reaction was investigated over time at 1, 2, 4 and 8 minutes by removing aliquots and analysing them by tlc. The analysis showed that the bulk of the reaction occurred in the first minute to give three main products. Mass spectral analysis of the final product showed peaks at *m/z* 683, 653 and 623. The mass spectrum also contained a small peak at *m/z* 681; this may indicate attack by the Grignard reagent at the methyl ester to give the ketone (A, Scheme 1).



Scheme 1. Alkylation of the methyl ester groups.

The use of 5 equivalents of EtMgBr and a two minute reaction time gave a slightly more complex mixture by tlc, with four distinguishable components. Mass spectral analysis showed three peaks at m/z 683, 653 and 623, with relative intensities in the mass spectrum of 1 : 2 : 1; suggesting that the fourth component, seen by tlc, was an isomer, possibly a regioisomer, of one of these compounds.

A reaction was attempted using the free base; the diacetyl dimethyl ester [83]. The diacetyl dimethyl ester [83] was partially dissolved in tetrahydrofuran and treated with 5 equivalents of EtMgBr for 2 minutes. Not suprisingly tlc and mass spectral analysis showed that the product was mainly starting material. Small amounts of the mono and diethylated species, [117] and [118], and their magnesium containing analogues were present by mass spectral analysis.



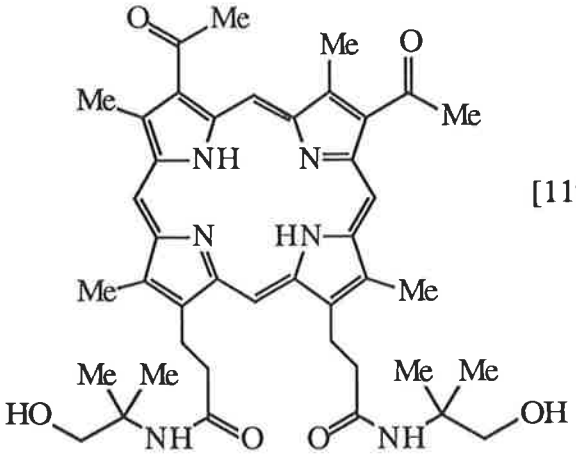
[118]

The alkylation reactions appeared to be halting partway so large equivalents of EtMgBr were used. Using 21 equivalents of EtMgBr gave a complex mixture containing no starting material by tlc. Mass spectral analysis showed peaks at *m/z* 739, 711, 709, 683, 681 and 653. Increasing the equivalence to 35 gave, in the mass spectrum, peaks at *m/z* 811, 739, 711 and 709; the latter three peaks correspond to products formed by alkylation of the esters; the peak, *m/z* 811, does not correspond to an expected product. The infrared spectrum of the product showed the virtual disappearance of the acetyl stretch (1660 cm^{-1}) and a much weaker ester carbonyl peak (1734 cm^{-1}) than is observed for the diacetyl dimethyl ester [83].

5.3 Oxazolines and amides as ester protecting groups

The acetyl sidechains were not alkylating fully and the use of longer reaction times or more equivalents of EtMgBr caused alkylation at the methyl esters. To stop this undesirable alkylation protecting groups for the esters were investigated. Oxazolines are stable to the Grignard conditions[136,137], so the synthesis of the oxazoline of diacetyl deuteroporphyrin [77] was attempted. Diacetyl deuteroporphyrin [77] was refluxed with oxalyl chloride in dichloromethane for 15 minutes, the solvent was removed and the crude dichloride dried *under vacuo*. The dichloride was dissolved in dichloromethane and 2-amino-2-methylpropanol and pyridine were added;

the reaction was stirred for 17 hours and then worked up to give, by mass spectral analysis, the diacetyldeuteroporphyrin bis(amidomethylpropanol) [119] (*m*/*z* 737) in 80% crude yield.

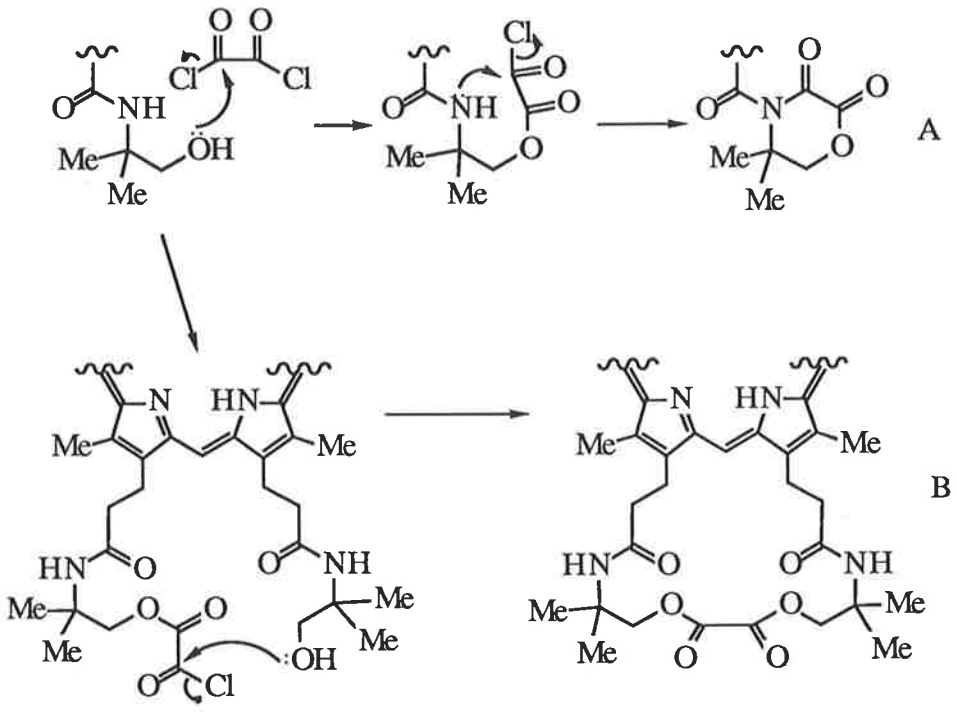


[119]

Thionyl chloride has been used to induce the cyclisation of the amide to the oxazoline[138,139], however the milder oxalyl chloride was investigated first. The diamide [119] was dissolved in dichloromethane, oxalyl chloride was added and the reaction refluxed for 15 minutes. Tlc analysis showed that the product was still mainly starting material. The reaction was repeated with twice the oxalyl chloride concentration and refluxed for 18 minutes. The tlc of the reaction product was complex with a strong baseline component. Mass spectral analysis showed no peaks compatible with cyclisation; a peak at *m*/*z* 667 may correspond to the product of cleavage of one of the amide groups. Other major peaks were observed at *m*/*z* 791 and 809.

When the reaction was not refluxed but stirred at room temperature for 3 hours the tlc of the product was again complex with a strong baseline component. Mass spectral analysis of the product showed peaks at *m*/*z* 737 and 719, which correspond to starting material [119] and the monocyclised product, but the base peak of the spectrum was *m*/*z* 791. This peak corresponds

to the addition of 54 mass units to the diamide [119]; two possible rationales for this addition are outlined in the scheme below (Scheme 2). The peak at *m/z* 809 observed in the previous reaction could then be accounted for by partial hydrolysis of either of the species (A or B in Scheme 2) which may have been formed.



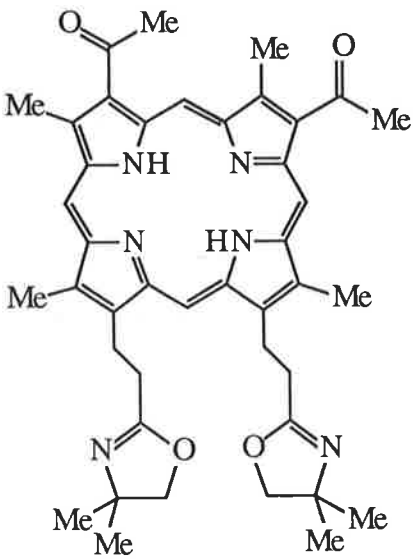
Scheme 2. Mechanisms for the addition of 54 m.u. to the amide [119]

Oxazolines have been formed by reacting benzoic acids with amino alcohols in refluxing toluene with azeotropic removal of water.[140] This cyclisation was attempted by refluxing a solution of the diamide [119] in toluene under azeotrope conditions, but after 3 days the product was still mainly starting material.

The diamide [119] was treated with thionyl chloride in dichloromethane at room temperature for 5 minutes. Tlc analysis showed that the mixture contained 3 components, the most polar of these being the starting material [119]. Mass spectral analysis of the product showed three peaks at *m/z* 737, 719 and 701 which indicates that some cyclisation is occurring causing loss of water

from the starting material [119] (*m*/z 737). When the residue from this reaction was treated again with thionyl chloride in an attempt to complete cyclisation tlc analysis of the resulting product showed negligible starting material remaining and two spots, one of higher R_f than the starting material and one of lower R_f. The mass spectrum of the product showed peaks at *m*/z 811, 737, 719, and the two major peaks at m/z 550 and 522. No peak was observed for the dicyclised material [120] (expected *m*/z 701), indicating that other reactions may be occurring during further treatment with thionyl chloride.

When the diamide [119] was treated with twice the concentration of thionyl chloride it gave a mixture (tlc) with one component predominating. Mass spectral analysis of the product showed one major peak (*m*/z 701) which corresponds to the required dioxazoline [120].

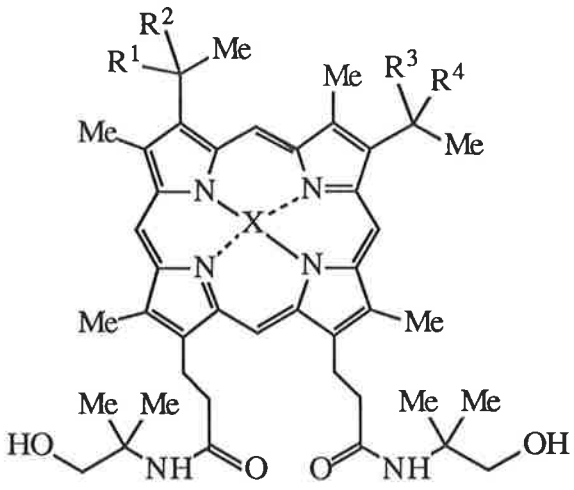


[120]

The dioxazoline [120] was isolated in 81% yield upon treatment of the diamide [119] for five minutes with thionyl chloride. The proton n.m.r. spectrum of the dioxazoline [120] showed two peaks at 1.22 and 1.26 ppm for the methyl

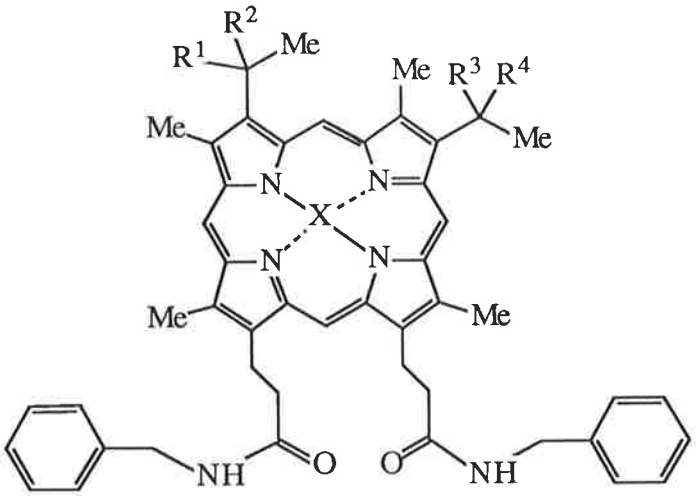
protons on the oxazoline ring and at 3.95 and 3.98 ppm for the methylene protons in the oxazoline ring. It was again hard to distinguish the ring methyls from the methyls adjacent to the ketone. In the ^{13}C n.m.r. spectrum the ring methyls showed their characteristic shift diversity due to the influence of the acetyl groups. An intense resonance at 22.8 ppm was due to the methyl carbons on the oxazoline ring. The methylene carbon in the propionic side chain which is α to the oxazoline ring has shifted from about 36 ppm in the ester compounds to 31 ppm in the dioxazoline. The resonances at 67.1 and 79.2 ppm are due to the tertiary carbon and the methylene carbon of the oxazoline ring respectively. The sp^2 carbon of the oxazoline ring resonates at 165 ppm in the oxazoline compared with 173 ppm in the comparable ester compounds.

Whilst the conditions for cyclisation to the oxazoline were being explored the suitability of the amide as a protecting group for the Grignard reaction was investigated. It was considered that the amide may be more stable to the Grignard conditions than the ester. As the diamide [119] was already synthesised it was tested in the Grignard reaction. It is not the most suitable amide due to the presence of the alcohol groups which will react in an acid-base reaction with some of the Grignard reagent and may adversely affect the solubility. The amidopropanol [119] was not initially very soluble in tetrahydofuran but appeared to dissolve on stirring. Treatment of [119] with 15 equivalents of EtMgBr gave some monoethylated material [121] (*m/z* 767) by mass spectral analysis. The solubility of the amidopropanol [119] was improved by forming the zinc metalloporphyrin analogue [122]. When this compound was treated with 14 equivalents of EtMgBr it gave, by mass spectral analysis, a 3 : 6 : 4 ratio of [119] (*m/z* 737) to monoethylated [121] (m/z 767) to diethylated [123] (*m/z* 797) material.



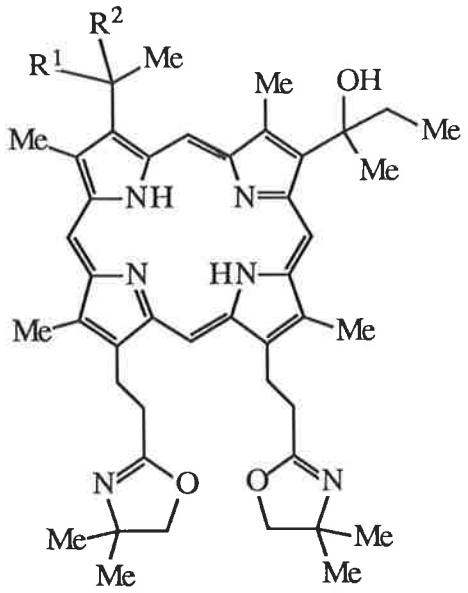
[121] $R^1, R^2 = O$ $R^3 = OH$ $R^4 = CH_2CH_3$ $X = 2H$
[122] $R^1, R^2 = O$ $R^3, R^4 = O$ $X = Zn$
[123] $R^1 = R^3 = OH$ $R^2 = R^4 = CH_2CH_3$ $X = 2H$

The dibenzyl amide of diacetyl deuteroporphyrin [77] was made from from the diacid chloride of diacetyl deuteroporphyrin [77] and benzylamine, however it was only partially soluble in tetrahydrofuran and even less soluble in ether and benzene. Treatment of the diacetyl dibenzylamide [124], which had been partially dissolved in tetrahydrofuran, with 5 equivalents of EtMgBr gave a mixture of mono [125] and diethylated [126] material as well as starting material [124]. The use of 12 equivalents of Grignard reagent still gave a mixture of mono [125] and diethylated [126] material as well as starting material. The zinc metalloporphyrin analogue [127] of the diacetyl dibenzylamide [124] was made. It dissolved in tetrahydrofuran when warmed but treatment with 3 equivalents of EtMgBr gave, by mass spectral analysis, monoethylated material [125] and the dibenzylamide [124] only.



[124] $R^1, R^2 = O$ $R^3, R^4 = O$ $X = 2H$
[125] $R^1, R^2 = O$ $R^3 = OH$ $R^4 = CH_2CH_3$ $X = 2H$
[126] $R^1 = R^3 = OH$ $R^2 = R^4 = CH_2CH_3$ $X = 2H$
[127] $R^1, R^2 = O$ $R^3, R^4 = O$ $X = Zn$

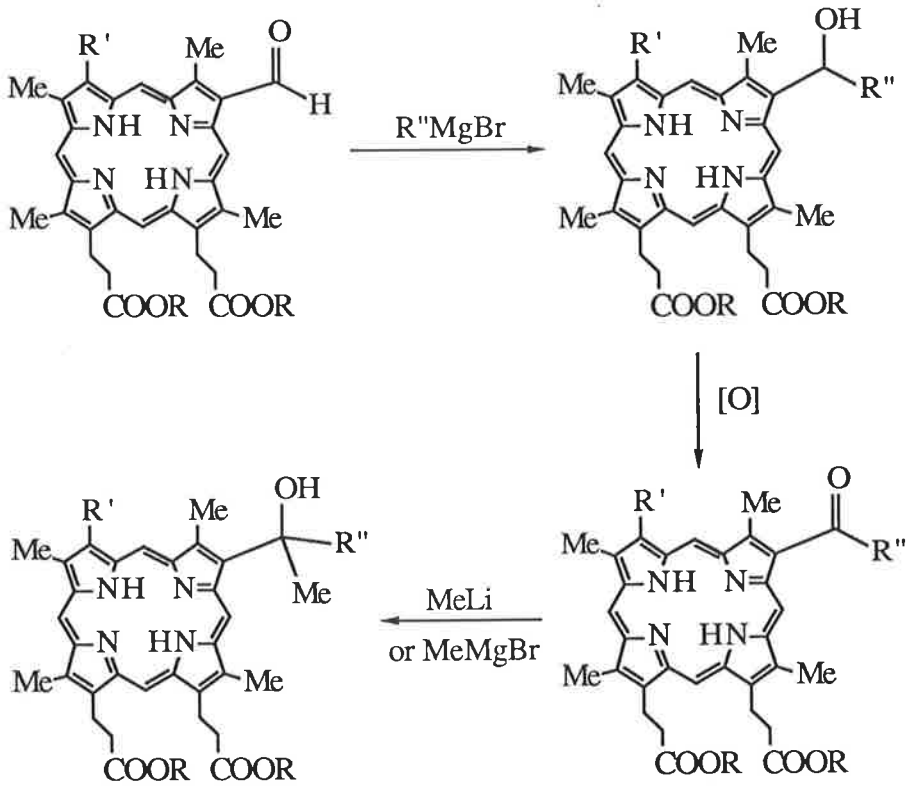
The dioxazoline [120] was more soluble than either of the other amides in tetrahydrofuran. When the dioxazoline [120] was treated with 7 equivalents of EtMgBr it gave a complex mixture of products by tlc analysis. Mass spectral analysis of the reaction product showed peaks compatible with mono [128] and diethylation [129] (*m/z* 731 and 761). It also showed peaks of 18 m.u. higher than these; which suggests that some hydrolysis of the oxazalines may be occurring, possibly during the work up when the oxazoline is shaken with dilute hydrochloric acid to remove any magnesium incorporated in the porphyrin. Oxazolines are hydrolysed to the corresponding acid by acid hydrolysis, although the conditions usually require elevated temperatures and 3N, or greater, hydrochloric acid concentrations.[136,137,138]



[128] $R^1, R^2 = O$
[129] $R^1 = OH, \quad R^2 = CH_2CH_3$

5.4 Grignard reactions on formyl porphyrins

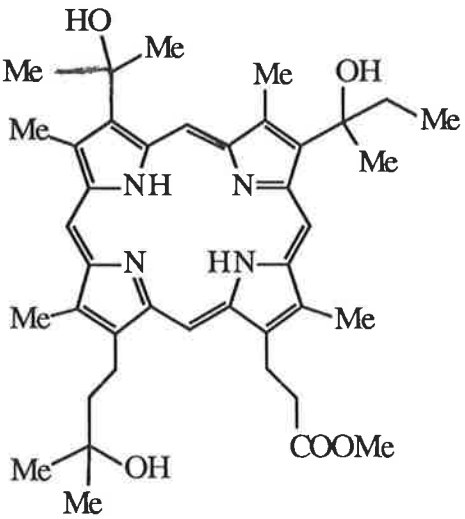
The diacetyl porphyrins were proving particularly recalcitrant to full alkylation. The second carbonyl group is hard to alkylate even when attack at the esters is no longer a competing process, so it was decided to investigate the reaction of Grignard reagents on the formyl porphyrins, as the formyl groups should be more labile to alkylation than the acetyl groups. It may be possible to synthesise 3° alcohols by alkylating the formyl group and then oxidising the 2° alcohol formed to the ketone and alkylating with methyl lithium or methyl magnesium bromide (Scheme 3); as attack by a methyl group at the acetyl carbonyl may occur more readily than attack by a larger group. Methyl lithium has been used to alkylate the ketone in copper pyrrohodin[141] and methyl magnesium bromide has been used to alkylate formyl groups[107,133].



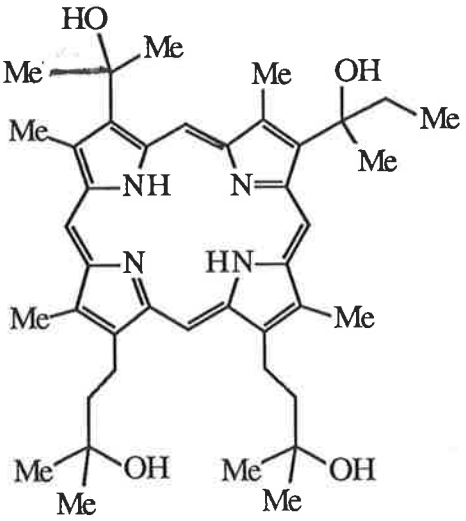
Scheme 3. Dialkylation of formyl groups.

A trial reaction was done by treating the zinc diacetyl porphyrin [114] with methyl lithium, 6 equivalents. The reaction was monitored by tlc analysis which showed components of R_f 0.13, 0.10, 0.06, and 0.0 for all samples from 45 seconds until the workup of the reaction at 16 minutes. The relative presence of the compounds of R_f 0.06 and 0.0 increased with time and the final product showed a component of R_f 0.03 as well. The reaction was repeated using a 30 second reaction time and the three major products separated by preparative tlc. Analysis of the products was difficult because most of the products due to alkylation of the esters had masses equivalent to the desired acetyl alkylation products. The first fraction showed, by mass spectral analysis, a major peak at *m/z* 623. Although the mass corresponded to the diacetyl dimethyl ester [83] the product did not cochromatograph with it, and could be any of four products which result from alkylation of the ester groups instead of, or in addition to, alkylation at one of the acetyl sidechains. The second fraction

showed a mass of *m/z* 642 which does not correspond to any of the expected methylation products. The third fraction showed a major peak at *m/z* 655 and a minor peak at *m/z* 676 which does not correspond to any of the expected products. The mass at *m/z* 655 correlates with methylation of both acetyl groups, it does not however preclude ester alkylation as well, as the peak at *m/z* 655 is also compatible with the alcohols [130] and [131] and the high polarity of the compounds was in keeping with replacement of esters groups with alcohols. Obviously the reaction with methyl lithium would require the ester groups to be protected but the appearance of unexpected products that could not be rationalised (*m/z* 676 and *m/z* 642) suggests that this reaction may not be useful for this system.



[130]



[131]

The alkylation of the formyl group, using Grignard reagents, was investigated. The diformyl dimethyl ester [132] was not soluble in ether and only partially soluble in tetrahydrofuran. Partially dissolved diformyl porphyrin [132] was treated with 1 equivalent of EtMgBr for 5 minutes at 0°. Tlc analysis of the product showed 2 main components. Chromatography by preparative tlc (3%

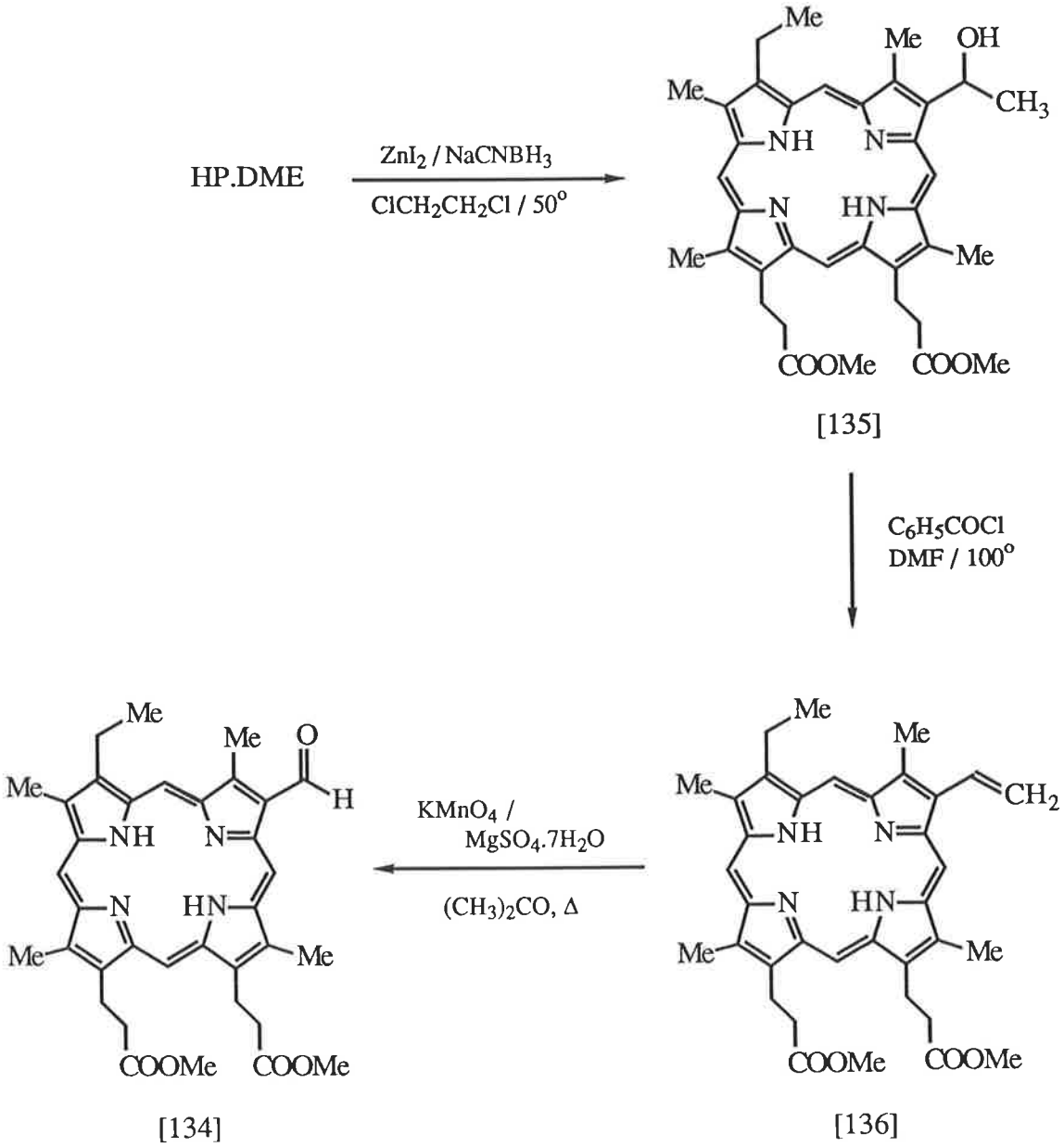
methanol in dichloromethane) enabled the two components to be separated, both in 49% yield. One of the products was the starting material, [132], the other main component showed a main peak in its mass spectrum at *m/z* 655 which correlates with the diethylated porphyrin [133]. A mass spectrum obtained for a lower R_f band from the plate showed peaks at *m/z* 711, 681 and 653; masses that indicate alkylation may be occurring, to a minor degree, at the ester groups.

The reaction was repeated using a 10 minute reaction time and also with a 5 minute reaction time using 2.2 equivalents of EtMgBr instead of one. Both of these reactions gave a similar mixture of products to the initial reaction, above, by tlc analysis. Using 4 equivalents resulted in a relatively stronger spot for the ester alkylated species and the baseline material. Analysis, by tlc, of the reaction products for both reactions showed similar product ratios to the above reaction. A reaction using 1 equivalent was monitored at 10, 20, 30 and 50 minutes. After 30 minutes more polar material began to appear in the reaction mixture. Tlc analysis showed an increase in baseline material over the 50 minutes but otherwise a similar product ratio was observed compared to the other reactions.

Obviously the reaction with the diformyl porphyrin [132] is being hindered by the lack of solubility of the starting material. From the lack of monoalkylated material it is assumed that the dissolved material is reacting readily to give the di(1-hydroxypropyl) porphyrin [133] and the undissolved material is not reacting.

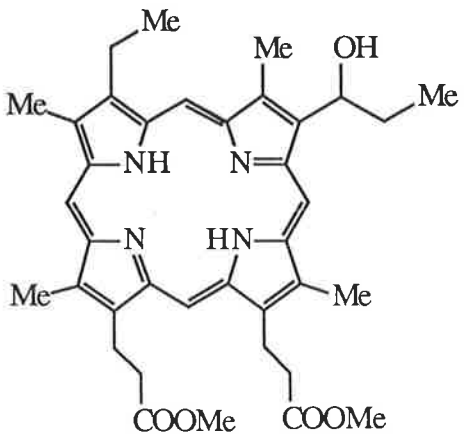
The ethylformyl dimethyl ester[142] [134] was synthesised from HP.DME as shown (Scheme 4) to give a compound which was more soluble than the diformyl [132]. The ethylformyl dimethyl ester [134] was dissolved in tetrahydrofuran and treated with 1.8 equivalents of EtMgBr. The reaction was

analysed by tlc at 5 minutes which showed that the starting material had been consumed to give one major product, which was of lower R_f than the starting material [134], two minor products of low R_f, and some baseline material. The reaction was worked up after 10 minutes; by tlc it appeared that there had been a slight increase in the amount of low R_f and baseline material. The mass spectrum of the product showed a single major component, m/z 625, which corresponds to the ethylated material [137].



Scheme 4. Formation of the 3(8)-ethyl-8 (3)-deuteroporphyrin dimethyl ester [134]

The reaction was repeated on a larger scale using 1.3 equivalents of EtMgBr and a 5 minute reaction time. In this reaction not all of the ethylformyl porphyrin [134] was consumed; 17% was recovered after chromatography. When the product was chromatographed on silica the main component resolved into two bands which could be separated successfully; these were termed for convenience, fraction α, the first band to elute, and fraction β. Mass spectral analysis of both fractions showed identical spectra with a molecular ions at *m/z* 625, which corresponds to the monoethylated product [137]. HPLC analysis of the free acid of fraction α, obtained by basic hydrolysis of the ester, showed a main peak at 9.3 minutes (85%) with a small peak at 10.4 minutes of only 6%. The free acid of fraction β showed a main peak at 10.4 minutes (96%) with a small peak at 9.3 minutes (4%). An HPLC trace of a combined sample of the free acids of α and β contained two peaks only, indicating that each of the compounds contain a small amount of the other isomer.



[137]

Proton n.m.r. spectra of the two fractions showed only minor differences. The two fractions must arise from the two regioisomers; 3-ethyl-8-(1-hydroxypropyl)deuteroporphyrin dimethyl ester and 8-ethyl-3-(1-hydroxypropyl)deuteroporphyrin dimethyl ester. Recent work[113] has indicated that in some cases, notably the ethyl-terminated ether-linked dimer, chromatographic separation appears to occur between diastereotomers rather

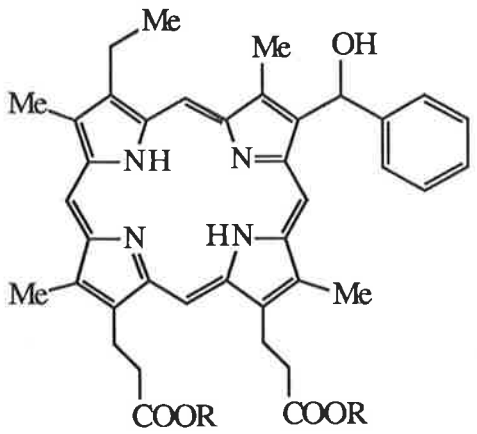
than regioisomers. However in this case the lack of a second chiral centre in the molecules means that the separation is due to the regioisomers. It was not possible to assign the structures of the regioisomers from their n.m.r. spectra; NOE experiments have been used to assign regioisomers[143] of porphyrins but unfortunately these facilities were not available. The regiochemistry of the isomers may be inferred from their chromatographic behaviour. Fooke's rule[144] predicts that the isomer with the polar groups closest together will be less mobile chromatographically. This has been applied successfully to systems containing a 1-hydroxyethyl sidechain and a less polar sidechain in the 3 and 8 positions.[24] Based on this rule fraction α should correspond to the 8-ethyl-3-(1-hydroxypropyl)deuteroporphyrin dimethyl ester and fraction β to the other regioisomer.

Both fractions showed resonances for the propyl sidechain at 1.0 ppm for the methyl protons, 2.4-2.5 ppm for the methylene protons and 5.86 (fraction α) and 5.7 ppm (fraction β) for the methine proton. The ethyl sidechain showed resonances at 1.8 ppm and 4.2 ppm for the methyl and methylene protons respectively. In fraction α the porphyrin methine protons are resolved into 3 peaks but in fraction β they appear as only 2 peaks. The concentration of both samples was similar.

The ^{13}C n.m.r. spectrum of the combined fractions showed peaks at 17.6 and 19.7 ppm due to the methyl and methylene carbons of the 1-hydroxypropyl sidechain respectively. Resonances for the methylene and methine carbons of the 1-hydroxypropyl sidechain were observed at 33.2 and 71.5 ppm respectively. The signal for the methyl carbon appears to occur in the same region as the ring methyls.

A trial reaction was done on the ethylformyl porphyrin [134] using 2.5 equivalents of phenyl magnesium bromide as the alkylating reagent and a 5

minute reaction time. Tlc analysis showed one main product and a small amount of starting material. The reaction was repeated and 3(8)-ethyl-8(3)-(1-hydroxybenzyl)deuteroporphyrin dimethyl ester [138] (*m/z* 673) was isolated in 91% yield and 7% starting material was recovered. A trial reaction using phenyl magnesium bromide (3.7 equivalents) and a 2 minute reaction time gave only the 1-hydroxybenzyl ethyl dimethyl ester [138] by tlc. The product did not separate into two distinct fractions when chromatographed.

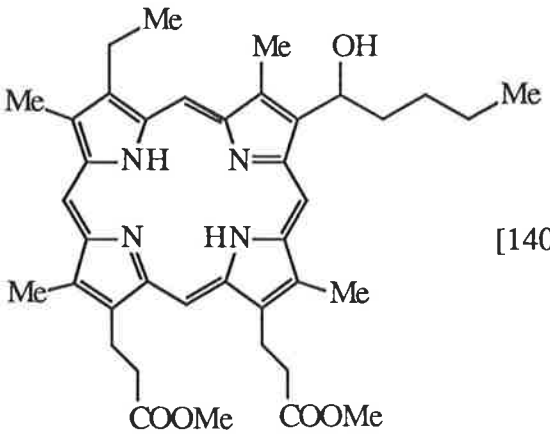


[138] R = CH_3
[139] R = H

The proton n.m.r. spectrum of the 1-hydroxybenzyl porphyrin [138] shows similar shifts for the ethyl sidechain (1.7 and 3.7 ppm) to those seen for 1-hydroxypropyl porphyrin [137] . The aryl protons resonate at 7.2 and 7.5 ppm. The methine proton attached to the hydroxyl bearing carbon resonates at 6.6 ppm which is is downfield from that seen in the 1-hydroxypropyl porphyrin [137] (5.8 ppm) due to its position α to a phenyl group. The ^{13}C n.m.r. spectrum shows resonances at 17.5 and 19.6 ppm due the ethyl sidechain. The aryl carbons appear at 126 to 128 ppm and the hydroxyl bearing carbon is observed at 70 ppm.

The hydroxybenzyl porphyrin [138] was hydrolysed using basic conditions to give the free acid [139], confirmed by mass spectral analysis (*m/z* 646); HPLC

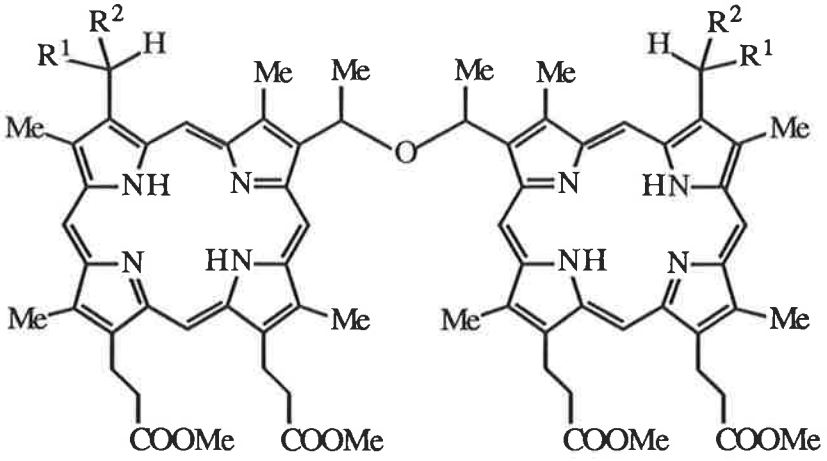
analysis of the free acid showed resolution into two peaks due to the presence of regioisomers.



[140]

3(8)-Ethyl-8(3)-(1-hydroxypentyl)deuteroporphyrin dimethyl ester [140] was synthesised from the ethylformyl porphyrin [134] using *n*-butyl magnesium bromide (2.2 equivalents) and a five minute reaction time. The desired product (*m/z* 653) was isolated in 72% yield and 21% of starting material was recovered. The proton n.m.r. spectrum showed resonances attributable to the butyl group at 0.76 ppm due to the methyl protons, 1.27 ppm due to the protons of the middle methylene groups, and 2.4 ppm due to the methylene protons α to the hydroxyl group. The proton attached to the hydroxyl bearing carbon has a 5.7 ppm chemical shift. The other resonances were typical of ethyl porphyrins, [137] and [138], already discussed. The ^{13}C n.m.r. spectrum showed carbons at 13.9, 22.6, 28.5,and 39.9 ppm due to the butyl group, the other resonances were similar to those seen for the other alkylated materials.

Due to limited testing facilities only one of the above compounds could be tested for anticancer activity. The hydroxybenzyl porphyrin [139] was tested as it was the most novel of the three compounds, and the effect of the benzene ring could not be predicted. The hydroxybenzyl porphyrin [139] was inactive.



[141] $R^1, R^2 = O$
[142] $R^1 = OH$ $R^2 = CH_2CH_3$

Due to the ease of alkylation of the formyl ethyl monomers a trial reaction was done on the diformyl terminated ether-linked dimer [141][113] to see if longer sidechains could be incorporated into the dimer system. The dimer [141], dissolved in tetrahydrofuran, was treated with 4 equivalents of EtMgBr. Samples of the reaction mixture were worked up at 2, 5, 10 and 20 minutes, they all contained similar products by tlc analysis, with the main running spot at R_f 0.32. All the starting material was consumed within the first 2 minutes and there was an increase in the baseline material during the 20 minutes. The major peak in the mass spectrum of the 2 minute sample was at *m/z* 1263 which corresponds to the product [142], formed by diethylation of the dimer [141]. A smaller peak at *m/z* 623 is probably due to the fragmentation that is typical for an ether linked dimer (see Chapter 3). The mass spectrum of the 20 minute sample showed *m/z* 1263 and 623 as the main peaks but peaks at *m/z* 1291 and 1319 were observed at less than 20% relative intensity. The peak at *m/z* 1291 may correspond to dialkylation of an ester group and *m/z* 1319 may correspond to the dialkylation of a second ester group. These results show that

alkylation of formyl terminated ether-linked dimers using Grignard reagents is an efficient way of forming dimers with long terminating sidechains. If short reaction times are used then the methyl esters are not affected.

5.5 Grignard reactions using dichloromethane as the solvent

Dichloromethane can be used[145] as the reaction solvent in Grignard reactions when using preprepared Grignard reagents and is stable to Grignard reagents for short periods of time. It was considered that this may be a more suitable solvent for reactions using the diformyl dimethyl ester [132] and the diacetyl dimethyl ester [83]. The diformyl porphyrin [132] was less soluble in dichloromethane than tetrahydrofuran but the diacetyl porphyrin [83] was more soluble. A solution of the diacetyl porphyrin [83] in dichloromethane was treated with 1 equivalent of EtMgBr and the reaction mixture analysed at 2, 5 and 10 minutes. The tlc analysis showed essentially the same product mixture, including some starting material [83], for all times. Mass spectral analysis showed the main products to be the monoethylated material [117] and starting material [83], with some overalkylated and a little dialkylated material. The reaction was repeated using 2 equivalents and a 2 minute reaction time but essentially the same products were formed (tlc).

The reaction was done on a larger scale using 1 equivalent of Grignard reagent and a 3 minute reaction time. The starting material and the monoethylated product were difficult to separate on silica but separated more readily when eluted from alumina, to give diacetyl dimethyl ester [83] (19%) and monoethylated material (34%). Although in the trial reactions there appeared to be only one component of lower R_f than the monoalkylated material [117] on closer inspection, with a larger sample, this proved to be made up of a number of components (tlc), including the dialkylated [118] and overalkylated material, by mass spectral analysis, which could not be separated satisfactorily.

The monoethylated material showed two separate signals for each set of protons in the new tertiary alcohol containing sidechain due to the regioisomers. The methyl protons of the ethyl chain show poorly resolved triplets at 0.65 and 0.77 ppm and the methylene protons have chemical shifts of 2.35 and 2.18 ppm. The protons of the methyl group α to the hydroxyl group appear at 1.8 and 1.9 ppm. The ^{13}C n.m.r. spectrum shows resonances at 8.9 ppm due to the methyl carbon and 38.5 ppm for the methylene carbon of the ethyl chain and the quaternary carbon is just distinguishable within the deuterochloroform peaks at 77.2 ppm. The methyl carbon α to the hydroxyl group resonates at 30.8 and 31.1 ppm and the acetyl sidechain showed resonances at 33.0 and 199.7 ppm.

From the results obtained it can be seen that the formyl groups are readily alkylated under Grignard conditions when the porphyrin is soluble. The acetyl groups react less readily than the formyl groups, which is expected for a ketone group compared with an aldehyde. The second acetyl group of the diacetyl porphyrin is particularly difficult to alkylate, this decreased reactivity may be due to electronic factors. The acetyl groups are electron withdrawing and when attached to the electron rich porphyrin ring they are deactivated to attack by the nucleophilic Grignard reagent. The presence of two acetyl groups decreases the electron density on each group; however when one of the groups is removed through alkylation the other group becomes more electron rich and nucleophilic attack becomes less favourable.

This chapter has shown that Grignard reagents can be used to extend formyl sidechains in porphyrins when the porphyrin is soluble in a suitable solvent for the Grignard reaction. Alkylation of porphyrins with one formyl and one ethyl sidechain proceeds readily and in good yield for a variety of Grignard

reagents. Extension of acetyl sidechains using Grignard reagents is less successful due to the lower reactivity of these groups.

Chapter 6. Experimental

6.1 General experimental

The compounds described are almost entirely mixtures of diastereomers and many are mixtures of regioisomers, for this reason their melting points and ε values were not determined. The purity of the compounds was assessed by mass spectroscopy, n.m.r. spectroscopy and their behaviour on tlc.

High performance liquid chromatography (HPLC) analyses were performed using a Waters dual solvent delivery system controlled by a Waters Model 680 Automated Gradient Controller, a U6K injector with a Waters Z-module and a Waters Novapak C18 cartridge, with a Waters Model 481AZ absorbance detector operating at 397 nm and a Waters 740 Data module. Aqueous methanol solutions, 15 : 85 and 10 : 90, each containing 2.5×10^{-4} ml of tetra-*n*-butylammonium phosphate, were adjusted to pH 3.6 and 7.5 respectively by the addition of phosphoric acid and triethylamine (if necessary). Analyses were run, unless otherwise indicated, with a solvent system which used the 85% methanol solution for 10 minutes and then changed exponentially, over 5 minutes, to the 90% methanol solution. The column was equilibrated for at least 10 minutes prior to injection with the first solvent to be used and each analysis was of 35 to 40 minutes duration.

Absorption spectra were measured on a Pye Unicam SP8-100 ultraviolet spectrometer, using 1 cm quartz cells and dichloromethane as the solvent, unless otherwise stated. The peak intensities are quoted relative to Soret band intensity, the abbreviation, sh, is used to denote a shoulder on a peak. Infrared spectra were measured on a Hitachi 270-30 spectrometer.

N.m.r spectra were recorded, unless otherwise indicated, on a Bruker CXP300 spectrometer operating at 300 MHz for ^{1}H and 75.47 MHz for ^{13}C spectra, using

deuterated chloroform as the solvent. Proton n.m.r. spectra recorded at 60MHz (as indicated) were recorded on a Varian T-60 spectrometer. Trimethylsilane was used as an internal standard except where the porphyrin contained (2-trimethylsilyl)ethyl or *t*-butyldimethylsilyl groups, in which case chloroform was used as the reference. All ^{13}C n.m.r. spectra are fully decoupled. All chemical shifts are reported as ppm and coupling constants as hertz. Abbreviations used are; d (doublet), t (triplet), q (quartet) and br. s. (broad singlet). The terms *meso* carbons and 'methine protons' refer to the carbons of the porphyrin ring in the 5, 10, 15 and 20 positions and their protons respectively.

Fast atom bombardment (FAB) mass spectrometry was carried out on a VG ZAB-2HF mass spectrometer. Argon atoms accelerated to 8kV were used as the bombarding particles and 3-nitrobenzyl alcohol was used as the matrix. Spectra were generally recorded from *m/z* 500, or 550. Porphyrins give clusters of peaks in the mass spectrum; the tallest peak in the cluster is recorded here. FAB mass spectrometry does not give a true molecular ion but gives an (M+H) peak.[85]

Chromatography using Sephadex LH-20 followed the method of Kessel *et al.*[29] using tetrahydrofuran : methanol : water (2 : 1 : 1), adjusted to pH 4.5 by the addition of dilute hydrochloric acid, as the eluant. Crude products were purified by chromatography on silica (Merck Keiselgel type 60G), unless otherwise indicated. Alumina columns were prepared from U.G. basic alumina, 200-300 mesh, Brockman activity II-III. Squat column chromatograhy refers to the method of Harwood[146], although for these compounds it was found that better results were obtained if the column was not run dry between solvent additions. Radial column chromatography was performed using a Harrison Research Inc. 7924 Chromatotron, with rotors coated with a 2 mm layer of silica gel (Merck PF_{254} type 60).

Analytical thin layer chromatography (tlc) was carried out on Merck Kieselgel 60 F_{254} plates using 3% methanol in dichloromethane, unless otherwise stated. Preparative tlc of small samples (less than about 30 mg) was carried out on precoated (0.2 mm) Merck Keiselgel 60 (indicator free) plates. Preparative tlc of larger samples was carried out on plates (25 x 25cm) prepared from 35 grams of Merck Keiselgel G.

Reagents and solvents were purified, and dried where necessary by standard laboratory procedures.[147] Light petroleum refers to the fraction of boiling point 40-60°. Reactions were generally carried out under nitrogen and were protected from direct light.

Samples were tested for their anticancer activity* in an *in vivo* mouse model.[148] Samples for testing were prepared by dissolving the sample in saline and adjusting the concentration of the sample by addition of saline until the absorbance (at 397 nm) of the solution (diluted by 1 in 1000 with 1:1 ethanol/0.1N aqueous sodium hydroxide) was approximately 0.4. This gives a concentration of approximately 2.5 mg/ml of sample in saline.

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* Samples were tested by the courtesy of Dr. P. Cowled.

6.2 **Chapter 2**: Experimental

The experimental section for this chapter covers the basic procedures referred to in Chapter 2 and also covers more fully some of the results obtained in the chapter.

2.1 General procedures

Standard basic hydrolysis.

i. of solid samples.

Porphyrin (5 mg) was dissolved in tetrahydrofuran/0.1N aqueous sodium hydroxide (1:1, 2 ml total) and the solution refluxed for 90 minutes. The solution was cooled, diluted with water (20 ml), the pH adjusted to 4.5 using dilute hydrochloric acid and the porphyrin extracted using dichloromethane/tetrahydrofuran (2:1). The extracts were combined, washed with water and the solvent removed under reduced pressure.

ii. of aqueous solutions (e.g. HPD solution)

The sample was diluted with an equal volume of tetrahydrofuran and the pH adjusted to 12 using 2N aqueous sodium hydroxide. The solution was refluxed for 90 minutes and then worked up as above.

Standard acidic hydrolysis.

i. of solid samples.

Porphyrin (5 mg) was dissolved in 1N aqueous hydrochloric acid (1 ml) and the solution refluxed for 60 minutes. The solution was cooled , diluted with water (20

ml), the pH adjusted to 4.5 using aqueous sodium hydroxide and the porphyrin extracted using dichloromethane /tetrahydrofuran (2:1). The extracts were combined, washed with water and the solvent removed under reduced pressure.

ii. of aqueous solutions (e.g. HPD solution)

The sample was diluted with an equal volume of 2N aqueous hydrochloric acid. The solution was then refluxed for 90 minutes and then worked up as above.

Chromatography

Chromatography was done on Sephadex LH-20 columns (see 6.1 General experimental). Samples in aqueous solution were diluted with an equivalent volume of tetrahydrofuran prior to loading on the column.

Formation of acetates.

1. Lipson acetates

The Lipson acetates were made by treating HP.2HCl with acetic acid/sulfuric acid (19:1) following the procedure of Gomer and Dougherty.[51]

2. Bonnett diacetate

Bonnett diacetate was prepared from HP.2HCl by a literature procedure[25] using acetic anhydride and pyridine.

3. Mono and diacetate mixture formed using a shortened Bonnett procedure[25]

Reduction of the reaction time in the procedure for the Bonnett diacetate (above), to approximately 0.5 hours, gives a mixture of the mono [3] and diacetate [2] and a small amount of HP.

Hematoporphyrin derivative (HPD)

HPD was prepared by the literature procedure.[51] The acetates, Lipson acetates or Bonnett diacetate, (100 mg) were dissolved in 0.1N aqueous sodium hydroxide (5 ml). The solution was stirred for 1 hour and neutralized to pH 7.1 using 0.1N hydrochloric acid to give HPD in solution.

2.2 Determination of standard hydrolysis conditions

Hematoporphyrin 3,8-dimethyl ether dimethyl ester [11].

The tetramethylated porphyrin [11] was prepared using a literature procedure.[47] HPLC; 20.1 min (90%), 26.7 (2), 27.8 (4).

1. Standard basic hydrolysis of [11]

HPLC of hydrolysis product; 10.1 min (82%), 15.0 (6), 16.5 (2). M.s. *m/z* (relative intensity); 627 (100%),

The basic hydrolysis was done on a larger scale (100 mg of [1.1]) to obtain an n.m.r. spectrum. ^{1}H nmr (60 MIIz): 2.27, d, J=7Hz, $CHCH_3$, 6H; 3.35, $CH_2CH_2CO_2$, 4H; 3.5-3.8, OCH_3 and ring methyls, 18H; 4.49, $CH_2CH_2CO_2$, 4H; 6.18, d, J=7Hz, H_3CCHO, 2H; 10.23, 10.71, methines.

2. Standard acidic hydrolysis of [11]

HPLC of hydrolysis product; 3.2 and 3.5 min (HP, 68%), 6.1 and 7.3 (HV, 15), 21.3 (PP, 9).

2.3 Hydrolyses of HPD and its precursor acetates

1. HPD formed using mono [3] and diacetylated HP [2] material made using the shortened Bonnett procedure (see 2.1).

Treatment of a mixture of mono and diacetates (monoacetate [3] 21%, diacetate [2] 38%) with the 0.1N aqueous sodium hydroxide for 1 hour, as described above, gave HPD (HP 20%, HV 13%, <15 min 3%, >15min 64%).

2. The affect of the workup conditions on the acetates and HPD.

A mixture of mono and diacetates, [3] and [2], was prepared, up to the point of work up, using the shortened diacetate synthesis (above).

i. extractive work up

Half of the mixture was poured into water, the pH adusted to 5, using dilute hydrochloric acid, and the porphyrin was extracted using dichoromethane/tetrahydrofuran (2:1). The volatiles were removed under reduced pressure to give; 7% HP, 23% monoacetate [3], 33% diacetate [2] and 5% IIV.

ii. Bonnett work up

The other half of the mixture was worked up by the literature procedure[25] (cooled, acetic acid added and the porphyrin precipitated from water) to give; 5% HP, 29% monoacetate [3], 35% diacetate [3] and 8% HV.

HPD was made from both of these acetate mixtures by treatment with 0.1N sodium hydroxide (above) and then hydrolysed using the standard basic hydrolysis conditions to give:-

	HPLC (%)			
	HP	HV	<15min	>15min
HPD (part i)	23	8	2	67
-basic hydrolysis	43	14	12	29
HPD (part ii)	22	13	5	59
-basic hydrolysis	44	18	12	27

3. the affect of lyophilising the acetates prior to HPD formation

A sample of Lipson acetates was prepared. Half of the sample, collected by centrifugation, was immediately converted to HPD by treatment with 0.1N sodium hydroxide and then hydrolysed using the standard basic hydrolysis conditions. The remaining material was suspended in water and lyophilised overnight. This material was converted to HPD and then hydrolysed using the standard basic hydrolysis conditions.

	HPLC (%)				
Lipson acetates	HP	[1.4]	[1.3]	<15 min	>15 min
non-lyophilised	5	23	36	3	33
lyophilised	3	19	38	5	36

	HPLC (%)			
	HP	HV	<15min	>15min
HPD (from non-lyophilised acetates)	20	17	6	57
-basic hydrolysis	21	16	9	57
HPD (from lyophilised acetates)	13	15	4	68
-basic hydrolysis	21	19	6	54

2.4 The stability of HPD in solution

i. at various pH; HPD made from the Bonnett diacetate.

HPD made from the Bonnett diacetate was divided into 3 portions, one portion was left at pH 7.1, the 'neutral' solution, one portion was basified using 0.1N sodium hydroxide, the 'basic' solution. Attempts to lower the pH of the third portion to 5 resulted in precipitation of porphyrin material at pH lower than 6.4. The stability of the 'neutral' and 'basic' solutions to the standard base hydrolysis conditions was tested over four days, the results are shown below. After 5 days the pH of the solutions had stabilised at 6.79 for the neutral solution and 6.38 for the basic solution.

	HPLC (%)				
	HP	HV	<15min	>15min	pH of solution
original HPD	13	10	4	74	7.10

basic hydrolysis 24 hours after making HPD.

					pH prior to hydrolysis
neutral solution	43	21	11	25	6.59
basic solution	44	30	16	9	7.14

basic hydrolysis after 48 hours

basic solution	34	22	12	32	6.96

basic hydrolysis after 96 hours

neutral solution	25	25	10	39	6.40
basic solution	38	20	9	34	6.82

ii. of HPD made from mono and diacetates produced by the shortened Bonnett procedure.

HPD was made using a mixture of acetates formed from the shortened Bonnett procedure (2.1) (HP 3%, monoacetate [3] 21%, diacetate [2] 38%, <15 min 29%, >15 min 9%).

	HPLC (%)				
	HP	HV	<15min	>15min	pH of solution
HPD solution	20	13	3	64	7.15
basic hydrolysis: - initial	45	21	11	24	
- after 48 hours	34	21	16	29	7.19

2.5 Acidic hydrolyses of HPD

Acidic hydrolysis using tetrahydrofuran as a cosolvent.

	HPLC (%)			
conditions (38° for 18 hours)	HP	HV	<15min	>15min
1ml 2N HCl/0.5ml THF/ 0.5ml HPD	35	21	8	37
1ml 2N HCl/1ml HPD	34	19	8	38

2.6 'Healux' material

1. Preparation of 'Healux' material

HP.2HCl (104 mg) was dissolved in water (15 ml) and the pH adjusted to 7.1 using 0.1N aqueous sodium hydroxide. The solution was heated at 90° for 72 hours, most of the water was removed under reduced pressure and the residue was diluted with an equal quantity of tetrahydrofuran. The mixture was then

chromatagraphed on Sephadex LH-20 to remove a small amount of remaining monomeric material. The eluant was diluted to three times its volume with water and the porphyrin material extracted with dichloromethane/ tetrahydrofuran (2:1). The organic extract was washed with water and the solvent removed under reduced pressure to give the product. HPLC; <15 min. (4%), >15 min (96%). λ_{max} (relative intensity); 385 nm (100), 502 (17), 532 (10), 572 (8), 624 (5), 640, sh, (3).

Testing solution:

'Healux' material (15mg) was dissolved in saline solution using 10% aqueous sodium hydroxide to affect dissolution and 10% aqueous hydrochloric acid to readjust the pH to 7.1 and the concentration adjusted as described in 6.1 General experimental (The absorbance was measured at 385 nm in this case).

2. Methylation of the 'Healux' material

The product from above was dissolved in methanol /trimethylorthoformate (1:1, 10 ml) and concentrated sulfuric acid (1 ml) was added carefully. The solution was stirred at room temperature for 0.5 hours and then diluted with water (50 ml). The pH of the solution was adjusted to 5 using 1N sodium hydroxide and the porphyrins extracted with dichloromethane/ tetrahydrofuran (2:1). The solvent was removed under reduced pressure to give a solid. M.s. *m/z* (relative intensity); (the values above 1300 are approximate) 3098, 3085, 3070, 3039, 3020 (0.5%), 2493, 2476, 2461, 2445, 2429, 2415 (1.5%), 1870, 1852, 1839, 1824, 1806 (6), 1261 (3), 1247 (15), 1233 (12), 1231 (10), 1217 (16), 1215 (17), 1201 (8), 1193 (8), 623 (43), 607 (37), 591 (100). ^{13}C n.m.r.: δ 12.1, br, ring methyls; 21.8, $CH_2CH_2CO_2$; 30.3, unassigned; 35.6, 36.6, $CH_2CH_2CO_2$ (and another carbon?); 51.8, OCH_3; 57.8, m, unassigned; 93-101, *meso* carbons; 135-146, pyrrole carbons; 173.1, CO_2Me

6.3 **Chapter 3** Experimental

3.2 *t*-Butyldimethylsilyl ethers and esters

t-Butyldimethylsilyl trifluoromethanesulfonate

This compound was prepared according to a literature procedure.[75] B.p. 53°/5.3 mm Hg (lit.[75] b.p. 60°/7 mm Hg).

Hematoporphyrin $3^1,8^1$-bis(*t*-butyltyldimethylsilyl) ether dimethyl ester [15] and **hematoporphyrin $3^1(8^1)$-(*t*-butyldimethylsilyl) ether dimethyl ester** [16]

TBDMS triflate (50 µl, 0.2 mmol) was added to a stirred solution of HP.DME (104 mg, 0.17 mmol) and 4-dimethylaminopyridine (104 mg, 0.85 mmol) in dry dichloromethane (7 ml). The reaction mixture was allowed to stir for 2.5 hours. The crude reaction mixture was chromatographed on a dry silica column. Elution with dichloromethane gave **hematoporphyrin $3^1(8^1)$, $8^1(3^1)$-bis(*t*-butyldimethylsilyl) ether dimethyl ester** [15] (28mg, 19%). ^{1}H n.m.r.: δ -3.68, NH, 2H; -0.11, -0.84, $SiCH_3$, 6H; 0.31, 0.32, 0.33, $SiCH_3$, 6H; 1.025, 1.03, 1.04, 1.045, $SiC(CH_3)_3$, 18H; 2.3, d, J=6.6 Hz, H_3CHCO; 3.3, m, $CH_2CO_2CH_3$, 4H; 3.6-3.7, ring CH_3 and CO_2CH_3, 18H; 4.5, m, $CH_2CH_2CO_2CH_3$, 4H; 6.5, q, J=6.6 Hz, HCOSi, 2H; 10.10, 10.11, 10.75, 10.79, methine protons. ^{13}C n.m.r.: δ -4.8, $SiCH_3$; 11.6, ring methyls; 18.3, $C(CH_3)_3$; 21.9, $CH_2CH_2CO_2CH_3$; 25.9, $C(CH_3)_3$; 28.5, H_3CCH(OTBDMS); 37.0, $CH_2CO_2CH_3$; 51.7, CO_2CH_3; 66.9, H_3CCH(OTBDMS); 95.9-100.0, *meso* carbons; 134-145, pyrrole carbons; 173.6, CO_2CH_3. M.s. *m/z* (relative intensity): 855 ((M+H), 100%), 723 ((M-OTBDMS), 21). λ_{max} (relative intensity); 409 (100%), 499 (20), 532 (13), 568 (9), 622 (6). Elution with 1% methanol/dichloromethane gave **hematoporphyrin $3^1(8^1)$-(*tert*-butyldimethylsilyl) ether dimethyl ester** [16] (53mg, 43%). ^{1}H n.m.r.: δ -3.86, -3.90, NH, 2H; -0.12,-0.11,-0.08,-0.07, $SiCH_3$, 3H; 0.30, 0.31, 0.33, $SiCH_3$, 3H; 1.01, 1.03, 1.04, 1.05, $SiC(CH_3)_3$, 9H; 2.02-2.30, H_3CHCO; 3.2, m, $CH_2CO_2CH_3$, 4H;

3.4-3.7, ring CH_3 and CO_2CH_3, 18H; 4.3, m, $CH_2CH_2CO_2CH_3$, 4H; 6.0, m, 6.2, m, HCOH, 1H; 6.51, q, J=6.6 Hz, HCOSi, 1H; 9.77, 9.92, 9.94, 10.06, 10.75, 10.78, methine protons. ^{13}C n.m.r.: δ -4.8, $SiCH_3$; 9.3, 11.3, 11.6, ring methyls; 18.3, $C(CH_3)_3$; 21.7, 21.9, $CH_2CH_2CO_2CH_3$; 25.9, $C(CH_3)_3$; 26.1, $H_3CCH(OH)$; 28.5, $H_3CCH(OTBDMS)$; 36.9, $CH_2CO_2CH_3$; 51.7, CO_2CH_3; 65.8, 65.9, $H_3CCH(OH)$; 66.9, $H_3CCH(OTBDMS)$; 95.9-100.0, *meso* carbons; 134-136, pyrrole carbons; 173.5, CO_2CH_3. M.s. *m/z* (relative intensity): 741 ((M+H),100%), 723 ((M-OH), 7), 609 ((M-OTBDMS), 10). MIKES of *m/z* 741: 723 (92%), 681 (68), 666 (53), 609 (100). λ_{max} (relative intensity); 412 (100), 499 (30), 532 (19), 568 (13), 622 (9).

Hematoporphyrin $3^1,8^1$-bis(*t*-butyldimethylsilyl) ether dimethyl ester [15] was also prepared by dissolving HP.DME (39 mg, $6x10^{-5}$ mol) in dichloromethane (5 ml) and adding 4-dimethylaminopyridine (154 mg, $1.3x10^{-3}$ mol). The solution was stirred for 5 minutes, TBDMS triflate (30 µl, $1.3x10^{-4}$mol) was added, and stirring continued for 18 hours. The reaction mixture was diluted with dichloromethane (20 ml) and washed with saturated aqueous ammonium chloride (3 x 15 ml) and water (15 ml). The volatiles were removed under reduced pressure. Radial chromatography of the residue using 0-3% methanol in dichloromethane gave the bissilylated HP.DME [15] (18 mg, 34%).

Hematoporphyrin $3^1,8^1$-bis(*t*-butyldimethylsilyl) ether bis(*t*-butyldimethylsilyl) ester [17]

HP.2HCl (104 mg, $1.6x10^{-4}$mol) was dissolved in a solution of 4-dimethyl-aminopyridine (548 mg, $4.4x10^{-3}$ mol) in dichloromethane (8 ml). TBDMS triflate (400 µl, $1.7x10^{-3}$ mol) was added to the stirred solution and stirring continued for 2.5 hours. The reaction was incomplete (tlc). Further TBDMS triflate (100 µl, $4x10^{-3}$ mol) was added and the solution stirred for 17 hours to give one product; tlc R_f 0.75. The reaction was concentrated under reduced pressure and purified by flash chromatography, on a column which had been

prewashed with 1% methanol in dichloromethane. Elution with 1% methanol in dichloromethane gave the title compound [17] (46 mg, 27%). M.s. *m/z* (relative intensity); 827 (100%), 695 (14). A large amount of porphyrin material was retained on the column.

Washing a solution of [17] in dichloromethane with 10% aqueous sodium hydroxide or 10% aqueous hydrochloric acid followed by water gave a product upon removal of the solvent (under reduced pressure) with R_f 0.0 (tlc) and HPLC; 21.2 min.(86%). M.s. *m/z* (relative intensity); 827 (100%), 781 (21), 695 (21).

3.2.1 Stability of the TBDMS ethers

i. in acetic acid.

The bissilyl dimethyl ester [15] (5mg) was dissolved in acetic acid and the solution was allowed to stand for 24 hours. There was no change from the starting material (tlc).

ii. in acetic acid/water

The bissilyl dimethyl ester [15] (5mg) was dissolved in acetic acid/water (1:1) and the solution was allowed to stand for 24 hours without reaction (tlc).

3.2.2 Deprotection of the TBDMS ethers

The bissilyl dimethyl ester [15] (5 mg, 5.8×10^{-6} mol) was dissolved in tetrahydrofuran (1 ml) and tetrabutylammonium fluoride (1N in tetrahydrofuran, 1µl, 1×10^{-6} mol) was added and the solution was stirred for 1 hour; tlc analysis showed the reaction was incomplete (tlc R_f; 0.75 ([15]), 0.34, 0.08 (HP.DME)). Tetrabutylammonium fluoride (1N in tetrahydrofuran, 20µl, 20×10^{-6} mol) was added and the solution was stirred for another hour; by tlc analysis only HP.DME was present.

3.3 Benzyl ethers and esters

3.3.1 the attempted formation of the benzyl ether via the dibromo adduct of HP.DME

HP.DME (50 mg, $8x10^{-5}$ mol) was dissolved in dry dichloromethane (5 ml) and hydrogen bromide (45% w/v in acetic acid, 50 µl, $3x10^{-4}$ mol) was added. The solution was stirred for 1.25 hours and the volatiles removed under reduced pressure to give a purple solid. The solid was dissolved in dry dichloromethane (5 ml) and benzyl alcohol (32 µl, $3x10^{-4}$ mol) was added, the solution was stirred for 18 hours and the solvent was removed under reduced pressure. The residue was dissolved in dichloromethane (30 ml) and the solution washed with saturated aqueous sodium bicarbonate (20 ml) and then water. The solvent was removed under reduced pressure to leave a solid. Tlc R_f 0.52 (faint), 0.22, 0.17, 0.11, 0.07, 0.0. M.s. *m/z* (relative intensity); 1217 (1%), 1199 (2), 897 (4), 609 (47), 591 (100).

3.3.2 formation of benzyl ethers and esters using tribenzyl orthoformate

Tribenzyl orthoformate

This compound was prepared by a literature procedure[149]. ^1H n.m.r. (60MHz): δ 4.72, s, $C_6H_5CH_2O$, 6H; 5.49, s, CH, 1H; 7.33, s, aryl protons, 15H.

Hematoporphyrin $3^1,8^1$-dibenzyl ether dimethyl ester [20].

HP.2HCl (0.5 g, $7.4x10^{-3}$ mol) was dissolved in a 1:1 mixture of benzyl alcohol and tribenzyl orthoformate (20 ml) and concentrated HCl (1.6 ml) and the mixture was heated at 50° for 3.5 hours. The reaction mixture was added to water (100 ml), the pH adjusted to 5, using saturated aqueous sodium bicarbonate, and extracted with dichloromethane until the aqueous layer was colourless. The volatiles were removed from the combined organic layers

under reduced pressure. The residue was dissolved in light petroleum (50 ml) and the solution allowed to stand until a precipitate had formed, and the liquid was then decanted. This procedure was repeated on the residue twice, although on the second repetition the residue did not dissolve in the light petroleum so the mixture was allowed to stand overnight prior to decanting. The products were separated using radial chromatography. The tetrabenzylated HP [20] was eluted using 50% light petroleum in dichloromethane, proton n.m.r. spectroscopy showed the fraction was impure with large resonances at δ 4.25 ppm and 7.0 ppm indicating benzyl containing impurities. The compound was purified by chromatography on a squat column. Elution using light petroleum and increasing amounts of dichloromethane gave **hematoporphyrin $3^1,8^1$-dibenzyl ether dibenzyl ester** [20] (65 mg, 9%) ^{1}H n.m.r. δ -3.63, br, NH, 2H; 2.29, 2.31, 2.32, H_3CCHO; 3.34, t, J 7.5 Hz, CH_2CO_2, 4H; 3.54, 3.60, 3.62, 3.68, s, ring methyls, 12H; 4.42, t, J=7.5 Hz, $CH_2CH_2CO_2$, 4H; 4.70, m, $H_5C_6CH_2OCH$, 2H; 4.87, m, $H_5C_6CH_2OCH$, 2H; 5.03, 5.O5, $H_5C_6CH_2O_2C$, 4H; 6.20, m, CH_3CHO, 2H; 7.01, m, $H_5C_6CH_2O_2C$, 10H; 7.35, m, $H_5C_6CH_2OCH$, 10H; 10.11, 10.12,.10.58 ,10.64, methine protons. ^{13}C n.m.r.: δ 11.7, ring methyls; 21.9, $CH_2CH_2CO_2CH_2C_6H_5$; 25.3, $H_3CCH(OCH_2C_6H_5)$; 37.1, $CH_2CO_2CH_2C_6H_5$; 66.3, $H_3CCH(OCH_2C_6H_5)$; 71.1, 72.2, $CH_2C_6H_5$; 96.4, 96.9, 98.6, 98.8, *meso* carbons; 127.6, 128.0, 128.2, 128.4, $CH_2C_6H_5$; 135-143, pyrrole carbons; 173.0, $CO_2CH_2C_6H_5$. M.s. *m/z* (relative intensity): 959 ((M+H), 100%), 851 ((M-$OCH_2C_6H_5$), 45). λ_{max} (relative intensity); 404 (100), 499 (14), 533 (8), 569 (6), 623 (4).

Elution with dichloromethane gave **hematoporphyrin $3^1(8^1)$-benzyl ether dibenzyl ester** [24] (193 mg, 29%). ^{1}H n.m.r.: δ -3.95, -3.92, NH, 2H; 1.92, 1.86, $H_3CCH(OH)$, 3H; 2.31, 2.32, 2.33, 2.35, $H_3CCH(OCH_2C_6H_5)$, 3H; 3.06-3.65, m, CH_2CO_2 and ring methyls, 16H; 4.27, m, $CH_2CH_2CO_2$, 4H; 4.69, m, $H_5C_6CH_2OCH$, 2H; 4.87, m, $H_5C_6CH_2OCH$, 2H; 5.00, 5.O4, $H_5C_6CH_2O_2C$, 4H;

5.69, 5.87, CH_3CHOH, 1H; 6.19, m, $CH_3CHOCH_2C_6H_5$, 1H; 7.0, m, $H_5C_6CH_2O_2C$, 10H; 7.36, m, $H_5C_6CH_2OCH$, 5H; 9.88, 9.92, 10.01, 10.12, 10.55, 10.58, methine protons. ^{13}C n.m.r.: δ 11.1, 11.5, 11.7, ring methyls; 21.6, $CH_2CH_2CO_2CH_2C_6H_5$; 25.3, $H_3CCH(OCH_2C_6H_5)$; 26.1, $H_3CCH(OH)$; 37.0, $CH_2CO_2CH_2C_6H_5$; 65.6, 65.7, $H_3CCH(OH)$; 66.3, $H_3CCH(OCH_2C_6H_5)$; 71.1, 72.2, $CH_2C_6H_5$; 96.2 - 98.5, *meso* carbons; 127.6, 127.9, 128.2, 128.4, $CH_2C_6H_5$; 135-145, pyrrole carbons; 172.9, $CO_2CH_2C_6H_5$. M.s. *m/z* (relative intensity): 869 ((M+H), 100%), 761 ((M-$OCH_2H_5C_6$), 14%). λ_{max} (relative intensity); 404 (100%), 499 (15), 533 (9), 569 (7), 623 (4).

Elution with 3% methanol/dichloromethane gave **hematoporphyrin dibenzyl ester** [19][24] (153 mg, 26%). ^1H n.m.r. (60 MHz): δ 1.7, m, H_3CCHO, 6H; 2.9-3.3, ring methyls and CH_2CO_2, 16H; 4.0, m, $CH_2CH_2CO_2$, 4H; 4.9, $H_5C_6CH_2O_2C$,4H; 5.5, CH_3CHOH, 2H; 6.9, s, $H_5C_6CH_2O_2C$, 10H; 9.2-9.5, methine protons, 4H. M.s. *m/z* (relative intensity); 779 ((M+H), 100%), 717 ((M-$OCH_2H_5C_6$), 61).

Hematoporphyrin $3^1,8^1$-dibenzyl ether $13^3(17^3)$-monobenzyl ester [26].

The reaction, above, was worked up by removing the volatiles, and then heating the liquid residue under reduced pressure (0.01 mm Hg) at increasing temperature until almost all of the liquid had been removed; at 100° the residue was brown in colour rather than red, as it had been previously. This residue was chromatagraphed on a squat column and gave two main fractions. Elution with dichloromethane gave a brown product. Tlc R_f; 0.8, 0.77 [20]. M.s. *m/z* (relative intensity); 959 (90%), 851 (100). Elution with 4-10% methanol/dichloromethane gave, after further purification on a squat column, **hematoporphyrin $3^1,8^1$-dibenzyl ether $13^3(17^3)$-monobenzyl ester** [26] (38 mg, 10%). ^1H n.m.r.: δ -4.8, br, NH, 2H; 2.27, 2.29, 2.31, H_3CCHO; 3.29, m, CH_2CO_2, 4H; 3.51, 3.55, 3.56, 3.59, 3.61, 3.66, ring methyls; 4.38, m, $CH_2CH_2CO_2$, 4H; 4.68, m, $H_5C_6CH_2OCH$, 2H; 4.86, m, $H_5C_6CH_2OCH$, 2H; 5.02, 5.O4,

$H_5C_6CH_2O_2C$, 2H; 6.19, m, CH_3CHO, 2H; 6.9, m, $H_5C_6CH_2O_2C$, 5H; 7.34, m, $H_5C_6CH_2OCH$, 10H; 10.08, 10.11, 10.56, 10.59, 10.63, methine protons. ^{13}C n.m.r.: δ 11.6, ring methyls; 21.8, 22.0, $CH_2CH_2CO_2CH_2C_6H_5$; 25.3, $H_3CCH(OCH_2C_6H_5)$; 36.7, 37.2, $CH_2CO_2CH_2C_6H_5$; 66.7, $H_3CCH(OCH_2C_6H_5)$; 71.1, 72.1, $CH_2C_6H_5$; 96.4 - 98.5, *meso* carbons; 127.6, 128.0, 128.2, 128.4, $CH_2C_6H_5$; 135-143, pyrrole carbons; 173.6, $CO_2CH_2C_6H_5$; 177.1, CO_2H. M.s. *m/z* (relative intensity); 869 ((M+H), 100%), 761 ((M-$OCH_2C_6H_5$), 23). λ_{max} (relative intensity); 404 (100%), 499 (16), 533 (10), 569 (7), 623 (4).

3.3.3 Deprotection of the benzyl ethers and esters

HPLC standards.

Tetrabenzyl HP [20] and the monobenzyl ether [24] were hydrolysed to the corresponding free acids using the standard base hydrolysis conditions described in Chapter 2.

Hematoporphyrin $3^1,8^1$-dibenzyl ether. HPLC; 14.9 min. (89%).

Hematoporphyrin $3^1(8^1)$-monobenzyl ether. HPLC; 6.9 and 7.4 min. (87%).

1. Hydrogenation

i. using palladium on carbon (5%) and dichloromethane/methanol solvent.

Tetrabenzylated HP [20] (63 mg, $2x10^{-4}$ mol) was dissolved in methanol (10 ml) and dichloromethane (5 ml). Palladium on carbon (5%, 20mg) was added and the mixture stirred under an atmosphere of hydrogen for 18 hours. The mixture was filtered through celite and the volatiles evaporated under reduced pressure to give a residue. ^1H n.m.r. (60 MHz) δ 6.0-9.0ppm: δ 7.4, br. s, $H_5C_6CH_2O_2C$. HPLC: 5.4 min (10%), 6.5 (6), 7.7 (14), 9.7 (12), 12.2 (30), 16.0 (20). The residue was dissolved in methanol and the hydrogenation repeated as above. HPLC: 5.3 min (10%), 6.2 (7), 6.8 (5), 7.5 (10), 9.5 (7),.10.8 (6), 12.4 (41), 14.1 (6). M.s. *m/z*..(relative intensity); 633 (85%), 611 (100), weak spectrum.

ii. using palladium on carbon (5%) and tetrahydrofuran solvent.

Tetrabenzylated HP [20] (63 mg, $2x10^{-4}$ mol) was dissolved in tetrahydrofuran (10 ml). Palladium on carbon (5%, 18mg) was added and the mixture stirred under an atmosphere of hydrogen for 18 hours. The mixture was worked up as above. Tlc (3% $MeOH/CH_2Cl_2$): 0.83 [20], 0.28 [24], 0.2, 0. ^1H n.m.r. (60 MHz) δ 6.0-9.0ppm: δ 7.0, m, $H_5C_6CH_2O_2C$; 7.3, m, $H_5C_6CH_2OCH$; ratio 1:6. The hydrogenation was repeated on the residue to give a product. M.s. *m/z* (relative intensity); 959 (45%), 897 (83), 869 (72), 807 (100).

iii. using palladium on carbon (10%) and tetrahydrofuran solvent.

Tetrabenzylated HP [20] (10 mg, $2x10^{-4}$ mol) was dissolved in tetrahydrofuran (7 ml). Palladium on carbon (10%, 7mg) was added and the mixture stirred under an atmosphere of hydrogen until hydrogen uptake had ceased. The mixture was worked up as above. Tlc (3% $MeOH/CH_2Cl_2$): 0.78 [20], 0.17, 0. HPLC: 16.2 min (48%), 19.4 (39). The residue was redissolved in tetrahydrofuran (7 ml); palladium on carbon (10%, 4.5 mg) was added and triethylamine (1 drop), to neutralise any acidity due to the catalyst. The mixture was stirred under an atmosphere of hydrogen for 1.5 hours and worked up. HPLC: 7.4 min (9%), 11.4 (13), 13 (12), 15.3 (30, HP dibenzyl ether [28]), 18.2 (7), 19.2 (29). M.s. *m/z* (relative intensity); 807 (41%), 791 (56), 779 ([28] 59), 669 (62), 625 (82), 581 (100).

iv. Catalyst transfer hydrogenation

1:1 tetrabenzyl [20] and tribenzyl HP [24] (25 mg) was dissolved in glacial acetic acid (0.5 ml). Palladium on carbon (10%, 0.1 g) and 1,4-cyclohexadiene (20 mg, $2.5x10^{-4}$mol) were added to the solution and the mixture stirred under a hydrogen atmosphere until it had finished taking up hydrogen, 2 hours. The reaction was worked up as above. HPLC: 1.9 min (8%), 7.6 (8), 12.0 (41), 17.5 (21), 18.8 (22). M.s. *m/z* (relative intensity): 671 (100%), 627 (10%).

2. using trifluoroacetic acid and sulfuric acid

A 1:1 mixture of tetrabenzyl [20] and tribenzyl HP [24] (13 mg) was dissolved in trifluoroacetic acid (18 ml) and concentrated sulfuric acid (2 ml). The solution was stirred for 45 minutes under nitrogen and then poured into ice cold, aqueous sodium acetate solution. The aqueous layer was extracted with dichloromethane and the organic layer was washed with 10% aqueous sodium bicarbonate and then water. The volatiles were removed under reduced pressure. HPLC: 1.4 min (4%), 3.5 (6.7), 4.6 (17), 5.7 (19), 6.1 (14), 8.1 (11), 8.6 (12), 13.4 (10). The reaction was repeated. Some precipitate formed during the reaction and was intractable. The rest of the material could not be extracted from the aqueous layer using dichloromethane and/or tetrahydrofuran and adjusting the pH of the aqueous fraction. HPLC of the aqueous layer showed a single peak at 1.5 min using the standard HPLC conditions, and stripping the column after 28 min with tetrahydrofuran eluted 76% of the material. HPLC: 1.5 min (24%), 28.5 (76%).

3.4 Formation of the ether linkage

3.4.1 Bromide as the leaving group

1. formation of the bromide using hydrogen bromide in acetic acid.

i. trial reaction

HP.DME (11 mg, $2x10^{-5}$ mol) was dissolved in dry dichloromethane (1 ml) and hydrogen bromide (45% w/v in acetic acid, 10 µl, $6x10^{-5}$ mol) was added. The solution was stirred for 1.25 hours and the volatiles removed under reduced pressure to give a purple solid. The solid was dissolved in dry dichloromethane (1 ml) and HP.DME (10 mg, $1.6x10^{-5}$ mol) was added, the solution was stirred for 18 hours and the solvent removed under reduced

pressure. The residue was dissolved in dichloromethane (20 ml) and the solution washed with saturated aqueous sodium bicarbonate, until the aqueous fraction was clear, and then water. The solvent was removed under reduced pressure to leave a solid. Tlc R_f; 0.41 (faint), 0.25, 0.21, 0.13, 0.10 (HP.DME).

ii. separation of products by preparative tlc.

The above reaction was repeated using HP.DME (9 mg, 1.4×10^{-5} mol) and then adding further HP.DME (9 mg, 1.4×10^{-5} mol) after formation of the bromide. The final solid was chromatographed by preparative tlc using 6% methanol in dichloromethane. A number of bands separated were analysed by mass spectroscopy.

Band A, R_f. 0.71; PP.DME by comparison with authentic sample. M.s. m/z (relative intensity): 591 ((M+H), 100%).

Band B, R_f 0.59; M.s. m/z (relative intensity): 1235 (34%), 1217 (53), 655 (58), 591 (100).

Band C, R_f 0.47 - 0.34, consisted of 6 bands; M.s. m/z (relative intensity): 1279 (21%), 1217 (100), 640 (75), 609 (84), 591 (84).

Band D, R_f 0.25 - 0.21, consisted of 3 bands; M.s. m/z (relative intensity): 1845 (17%), 1235 (50), 609 (100).

Band E, R_f 0.12; HP.DME by comparison with authentic material.

iii. Isolation of the monovinyl terminated dimer [22].

HP.DME (51 mg, 8×10^{-5} mol) was dissolved in dry dichloromethane (5 ml) and hydrogen bromide (45% w/v in acetic acid, 0.1 ml, 6×10^{-4} mol) was added. The solution was stirred for 1 hour and the volatiles removed under reduced pressure to give a purple solid. The solid was dissolved in dry dichloromethane (30 ml) and HP.DME (50 mg, 8×10^{-5} mol) was added. The solution was stirred for 18 hours and the solvent removed under reduced

pressure. The reaction was worked up as for part 3.4.1.(i). The solid was dissolved in dichloromethane and run through silica to remove polar impurities. The product was then chromatagraphed on silica using radial chromatography to give three major fractions. The first fraction eluted with dichloromethane and was shown to be PP.DME by comparison with an authentic sample. The two other fractions eluted with 1% methanol in dichloromethane.

Fraction A (14 mg), R_f 0.22; M.s. *m/z* (relative intensity): 1217 (100%), 609 (27).

Fraction B (5 mg), R_f 0.18; M.s. *m/z* (relative intensity): 1217 (90%), 641 (100), 609 (75).

Hydrolysis of Fraction A.

Fraction A (4 mg) was dissolved methanol (1 ml) and sodium hydroxide (10% in water, 0.5 ml) and the solution stirred for 2 hours. Tlc of the reaction showed starting material had been consumed to give polar material (R_f 0.0) only. The solution was diluted with water (10 ml) and the pH adjusted using saturated aqueous ammonium chloride solution until the product could be extracted into dichloromethane. The solvent was removed under reduced pressure to give a solid. M.s. *m/z* (relative intensity); 1217 (100). The residue was dissolved in water (7 ml), methanol (1 ml) and sodium hydroxide (10% in water, 0.5 ml) and refluxed for 1 hour. The solution was worked up as before. M.s. *m/z* (relative intensity); 1185 (90%), 1163 (100). HPLC 1.4 minutes (3%), 4.3 (2), 5.4 (10), 6.1 (5), 6.8(14), 7.3 (13), 9.2 (26), 9.9 (27) (these times are not directly comparable with HPD as a slightly different solvent gradient was used).

iv. using monoTBDMS ether [16]

Reaction 3.4.1.(i) was repeated using the monoTBDMS ether [16] (4 mg, $5x10^{-6}$ mol), in place of HP.DME, and hydrogen bromide (45% w/v in acetic acid, 5 µl, $3x10^{-5}$ mol). The product showed; tlc (6% methanol in dichloromethane) R_f

0.61, 0.43, 0.35, 0.26, 0.14 (HP.DME). M.s. *m/z* (relative intensity): 1235 (26%), 1217 (52), 1199 (35), 627 (52), 609 (100), 591 (48). *M/z* 1235 is compatible with DHE tetramethyl ester [30], and*m/z* 1217 and 1199 are compatible with the mono and didehydrated dimers, [22] and [23].

2 Brominations using triphenylphosphine and tetrabromomethane

i. using HP.DME

Tetrabromomethane (98mg, $3x10^{-4}$ mol) and triphenylphosphine (60 mg, $2.3x10^{-4}$ mol) in dichloromethane (1 ml) was added to a solution of HP.DME (11 mg, $2x10^{-5}$ mol) in dichloromethane (5 ml) and the solution was refluxed for 1 hour. A small portion of the solution was worked up by the literature procedure[93] but it would not elute from an alumina column using dichloromethane. The volatiles were removed from the remaining solution under reduced pressure and the residue was dissolved in methanol (20 ml) and washed with light petroleum (2x5 ml). The solvent was removed under reduced pressure leaving a red-purple solid which gave a complex tlc. M.s. *m/z* (relative intensity); 1263 (10%), 1249 (12), 1235 (13), 1217 (11), 1203 (10), 641 (64), 627 (91), 623 (54), 609 (75), 593 (48), 511 (100). M/z 1235 is compatible with esterified DHE [30], *m/z* 1263 and 1249 are compatible with its di and monoetherified analogues, *m/z* 1217 its dehydrated analogue.

ii. using hematoporphyrin $3^1(8^1)$-monomethyl ether dimethyl ester [32]

A solution of trimethylated HP [32] (5mg, $2.3x10^{-6}$ mol) in dichloromethane (2.5 ml) was added to a solution of tetrabromomethane (42mg, $1.3x10^{-4}$ mol) and triphenylphosphine (30 mg, $1.1x10^{-4}$ mol) in dichloromethane (0.5 ml) and refluxed for 3hours. Tlc showed that the reaction mixture had not changed after the initial 30 minutes and still contained trimethylated HP [32]. Further tetrabromomethane (36mg, $1.1x10^{-4}$ mol) and triphenylphosphine (30 mg, $1.1x10^{-4}$ mol) in dichloromethane (0.5 ml) was added and after 15 minutes

reflux all the starting material was consumed. Ethanol (0.5 ml) was added and the reaction refluxed for 2 hours then cooled and worked up as above. M.s. *m/z* (relative intensity); 651 (1.7%), 623 (1.5), 609 (3), 595 (2.4), 433 (50), 435 (100), 437 (50)

iii. using HP.DME and working up the reaction with methanol

A solution of HP.DME (5 mg, $8x10^{-6}$ mol) in dichloromethane (5 ml) was added slowly to a refluxed solution of tetrabromomethane (46mg, $1.4x10^{-4}$ mol) and triphenylphosphine (57 mg, $2x10^{-4}$ mol) in dichloromethane (5 ml). After completion of the addition the reflux was continued for a further 1 hour. Methanol (5 ml, 0.12 mol) was added and the solution was refluxed for 1 hour. The solution was cooled and the volatiles evaporated under reduced pressure. The residue was dissolved in methanol and dilute HCl and the solution washed with light petroleum (4x), neutralised to pH 5, and extracted with dichloromethane and tetrahydrofuran. The solvents were removed under reduced pressure to give a red-purple solid which gave a complex tlc with discernible spots at R_f; 0.65, 0.58, 0.53, 0.37, 0.05. M.s. *m/z* (relative intensity): 669 (15%), 655 (100), 641 (94), 627 (32), 623 (56), 609 (35).

iv. slow addition of HP.DME

A solution of HP.DME (6 mg, $9.6x10^{-6}$ mol) in dichloromethane (10 ml) was added slowly to a refluxed solution of tetrabromomethane (46mg, $1.4x10^{-4}$ mol) and triphenylphosphine (57 mg, $2x10^{-4}$ mol) in dichloromethane (5 ml). After completion of the addition the reflux was continued for a further 5 minutes and methanol (5 ml) was added. The solution was allowed to stand at room temperature for 45 minutes prior to removal of the volatiles under reduced pressure. The residue was then worked up as above. Tlc R_f; 0.66, 0.60, 0.52 (most intense spot). 0.36, 0.11 (faint, HP.DME), 0.02, 0.00. M.s. *m/z* (relative

intensity): 669 (17%), 655 (41), 641 (37), 627 (HP.DME, 41), 623 (37), 609 (32), 595 (18), 579 (100).

v. bromination followed by addition of HP.DME and then 2-propanol

A solution of HP.DME (6 mg, $9.6x10^{-6}$ mol) in dichloromethane (5 ml) was added slowly to a refluxed solution of carbon tetrachloride (13 µl, $5.3x10^{-5}$ mol) and triphenylphosphine (60 mg, $2.3x10^{-4}$ mol) in dichloromethane (5 ml). After completion of the addition the reflux was continued for a further 2 hours. The solution was cooled and the volatiles evaporated under reduced pressure. The residue was dissolved in dichloromethane and HP.DME (6 mg) was added, the solution was stirred for 70hours and then refluxed for 3 hours. 2-Propanol (1 ml) was added and the solution refluxed for 1 hour. The volatiles were removed under reduced pressure, and the residue was worked up as above. Tlc R_f. 0.46 (faint), 0.08, 0.0. M.s. *m/z* (relative intensity): 711 (76%), 696 (100), 683 (32), 669 (23), 654(26), 652 (20), 638 (25).

3.4.2. Acetate as the leaving group

1. Acetylation of hematoporphyrin t-butyldimethylsilyl ether dimethyl ester [16].

The monoTBDMS ether [16] (25 mg, $3.4x10^{-5}$) was dissolved in a pyridine and acetic anhydride solution (9:1, 2 ml) and stirred for 1 hour. The solution was condensed to half its volume under reduced pressure, the residue diluted with dichloromethane (20 ml) and washed with saturated ammonium chloride (3x10 ml) and water (20 ml). The volatiles were removed under reduced pressure to give the acetylated product [39], one compound by tlc R_f 0.75, used without further purification. M.s. *m/z* (relative intensity): 783 ((M+H), 100%), 723 ((M-$OCOCH_3$), 31).

2. Attempted dimer formation.

i. in dichloromethane using triethylamine as the base.

The acetate [39] (2.5 mg) and the monoTBDMS ether [16] (2.5 mg) were dissolved in dry dichloromethane (1 ml) and the solution stirred for 2.5 hours. By tlc no reaction had occurred so triethylamine (1 drop) was added and the solution was allowed to stand for 18 hours, still no reaction had occurred . The volatiles were removed under reduced pressure The residue was dissolved in triethylamine (1 ml) refluxed for 2 hours and then allowed to stand at room temperature for 20 hours; by tlc no reaction has occurred.

ii. using sodium hydride as the base

The acetate [39] (2.5 mg) and the monoTBDMS ether [16] (2.5 mg) were dissolved in dry tetrahydrofuran (1 ml) and sodium hydride (80% dispersion in oil, 1 mg) was added. The mixture was stirred for 20 hours at room temperature but still contained mainly starting materials by tlc with some polar material (R_f 0) being formed. The mixture was refluxed for 1 hour; all the material had been converted to polar material, suggesting that the esters had been hydrolysed. The mixture was poured into water (5 ml) and the products extracted with dichloromethane and tetrahydrofuran. The solvents were removed under reduced pressure. The residue was reesterified[47] by treating it with trimethylorthoformate (2 ml), water (0.4 ml) and concentrated sulphuric acid (0.2 ml) for 1 hour at room temperature. The solution was diluted with water (10 ml), the pH adjusted to 5, using 10% aqueous sodium hydroxide, and the products extracted with dichloromethane. The volatiles were removed under reduced pressure. M.s. *m/z* (relative intensity): 1249 (22%), 1235 (30), 1231 (15), 1217 (24), 655 (65), 641 (85), 627 (78), 609 (100).

3.4.3. Mesylate as the leaving group

i. initial trial reaction

A solution of HP.DME (20 mg, $3.2x10^{-5}$mol) and triethylamine (15 µl, $1.1x10^{-4}$ mol) in dichloromethane (0.4 ml) was cooled to below 0° using an ice-salt bath and methanesulfonyl chloride (7 µl, $9x10^{-5}$ mol) was added. The solution was stirred for 15 min, tlc showed that starting material was still present so further methanesulfonyl chloride (20 µl) was added and the reaction warmed slowly to room temperature. After two days the reaction was diluted with dichloromethane, washed with 10% aqueous. HCl, saturated aqueous. sodium bicarbonate and water and the volatiles were removed under reduced pressure to give a residue. M.s. *m/z* (relative intensity): 1809 (3%), 1217 (6), 1199 (16), 609 (19), 591 (100).

ii. reaction kept at 0°

A solution of HP.DME (5 mg, $7.8x10^{-6}$mol) and triethylamine (5 µl, $3.6x10^{-5}$ mol) in dichloromethane (0.4 ml) was cooled to 0° and methanesulfonyl chloride (2 µl, $2.6x10^{-5}$ mol) was added. The solution was stirred at 0° for 4 hours and then worked up as for part (i). Tlc; R_f 0.73, 0.42, 0.23, 0.11 (HP.DME). M.s. *m/z* (relative intensity): 1235 (11%), 1221 (9), 1217 (8), 1203 (16), 627 (100), 609 (50).

iii. without base

HP.DME (5.5 mg, $8.8x10^{-6}$mol) in dichloromethane (2 ml) was cooled to 0° and methanesulfonyl chloride (1.5 µl, $1.9x10^{-5}$ mol) was added. The solution was allowed to warm gradually to room temperature (Tlc R_f 0.35 (faint), 0.12 (HP.DME)). The solution was cooled to 0°, further methanesulfonyl chloride (1.5 µl, $1.9x10^{-5}$ mol) was added and the solution was allowed to warm to room

temperature overnight and worked up as for part 3.4.3.(i). Tlc of the product showed that it was starting material.

iv. using pyridine as the base

HP.DME (5 mg, 7.8×10^{-6}mol) dissolved in dry pyridine (1 ml) was cooled to 0° and methanesulfonyl chloride (3 µl, 3.9×10^{-5} mol) was added. After 5 min at 0° the reaction was allowed to stand at room temperature for 5 min and was poured into iced water (20 ml). The product was extracted using diethyl ether (2x15ml) and the combined organic layers were washed with cold 10% aqueous HCl (1x10ml), dilute aqueous sodium bicarbonate (1x10ml) and water (1x20 ml). The solvent was removed under reduced pressure and HP.DME was shown by tlc analysis to be the only product.

This reaction was repeated as above except methanesulfonyl chloride (5 µl, 6.5×10^{-5} mol) was used and the reaction was allowed to stand for 0.5 hours at 0° prior to work up. Tlc analysis showed the main product to be HP.DME (R_f 0.1) with minor spots at R_f 0.33 and 0.0.

v. using excess reagents

Triethylamine (26 µl, 1.9×10^{-4} mol) and methanesulfonyl chloride (12 µl, 1.6×10^{-4} mol) were added to a solution of HP.DME (4.7 mg, 7.5×10^{-6}mol) in dichloromethane (1 ml) at 0°. The solution was stirred for 1 hour and then worked up as for part (i). Tlc analysis was complex with major components at R_f 0.74, 0.62, 0.39, 0.30, 0.11, 0.0.

vi. attempted formation of the tosylate

Trimethyl HP (6.6 mg, 1×10^{-5} mol) was dissolved in dry pyridine (2 ml) and cooled to 0°. *p*-Toluenesulfonyl chloride (15 mg, 8×10^{-5}mol) was added and the reaction swirled and allowed to stand at 4°. After 48 hours an aliquot (3 drops)

was removed and added to methanol (0.5 ml) after standing for an hour tlc analysis of the product showed it to still be mainly starting material. The rest of the reaction mixture was allowed to stand for a further 3 weeks at 4° and then poured into methanol (5 ml) and allowed to stand for 17 hours. Most of the methanol was evaporated under reduced pressure and the residue dissolved in dichloromethane (20 ml), washed with saturated aqueous ammonium chloride (3x) and water and the volatiles evaporated under reduced pressure to give a residue which was starting material by tlc. M.s. *m/z* (relative intensity): 641 (100%), 623 (67).

1-Methoxyethyl terminated dihematoporphyrin ether tetramethyl ester [44]

Trimethylated HP [32][47] (20 mg, 3.1×10^{-5}mol) and crushed molecular sieves were dried on a vacuum pump (0.01mm Hg) for 30 min. Dry dichloromethane (3 ml) was added and then triethylamine (8 µl, 5.7×10^{-5} mol) and methanesulfonyl chloride (3 µl, 1.6×10^{-5} mol). After stirring for 10 minutes tlc analysis showed starting material was still present so further triethylamine (1µl) and methanesulfonyl chloride (0.5 µl) were added, the reaction was stirred for 5 min and worked up as above. The reaction mixture was separated by preparative tlc (3% methanol/dichloromethane) into four bands which were analysed by FAB mass spectroscopy.

Band A. R_f 0.5; M.s. *m/z* (relative intensity): 623 (100%).

Band B. R_f 0.45; M.s. *m/z* (relative intensity): 623 (100%).

Band C. R_f.0.23; M.s. *m/z* (relative intensity): 1263 (52),1231 (14), 623 (100%); 9` mg.

Band D. R_f 0.13; M.s. *m/z* (relative intensity): 641 (100%).

Dihematoporphyrin ether [7]

i. trial reaction

MonoTBDMS ether [16] (11 mg, $1.5x10^{-5}$mol) and crushed molecular sieves were dried on a vacuum pump (0.01mm Hg) for 0.5 hours. Dry dichloromethane (2 ml) was added, the solution cooled to 0°. Triethylamine (3.5 µl, $2.5x10^{-5}$ mol) and methanesulfonyl chloride (1.5 µl, $1.7x10^{-5}$ mol) were added and the solution stirred for 15 min at 0°. The reaction was poured into ice water (20 ml) and extracted with dichloromethane (3x10 ml). The combined organic layers were washed with saturated aqueous ammonium chloride (4x15ml) and water (2x15 ml) and the solvent evaporated under reduced pressure to give a solid.

The residue from above was dissolved in tetrahydrofuran (1 ml) and tetrabutylammonium fluoride (1N in tetrahydrofuran, 0.05 ml) was added. The solution was stirred for 1 hour then diluted with dichloromethane (10 ml) and washed with saturated aqueous ammonium chloride (3x5 ml) and water (10 ml). The volatiles were removed under reduced pressure and products separated by preparative tlc (3% methanol in dichloromethane).
Band A. R_f 0.66; M.s. *m/z* (relative intensity): 741 (10%), 623 (9), 609 (100).
Band B. R_f 0.61; M.s. *m/z* (relative intensity): 1349 (5%), 1217 (6), 609 (100).
Band C. R_f 0.37; M.s. *m/z* (relative intensity): 1235 (73%), 1217 (19), 609 (100).
M/z 1235 is compatible with DHE tetramethyl ester [30]
Band D. R_f 0.33; M.s. *m/z* (relative intensity): 1235 (100%), 609 (100).
Band E. R_f 0.33; M.s. *m/z* (relative intensity): 1235 (100%), 609 (73).

ii. formation of the protected DHE [7]

MonoTBDMS ether [16] (34 mg, $4.6x10^{-5}$mol) and crushed molecular sieves were dried on a vacuum pump (0.01mm Hg) for 1 hour. Dry dichloromethane

(2 ml) was added and the solution cooled to 0°. Triethylamine (10 µl, $2.4x10^{-5}$ mol) and methanesulfonyl chloride (8 µl, $1.0x10^{-4}$ mol) were added.and the solution stirred for 10 min at 0°. The reaction was worked up as for the trial reaction. The products were separated on a squat column. MonoTBDMS monovinyl HP.DME [45] (14 mg, 42%) eluted using 0.5% acetone in dichloromethane. R_f 0.73. M.s. *m/z* (relative intensity): 723 (100%). λ_{max} (relative intensity); 406 (100), 503 (14), 537(10), 572 (7), 626 (4). When exposed to diffuse light for 10 hours this compound converted to two green compounds. R_f 0.46, 0.36. M.s. *m/z* (relative intensity): 755 (100%). λ_{max} (relative intensity); 398 (100), 440 sh (70), 569 (12), 606 (0.09), 664 (34).

The TBDMS terminated dimer [47] (11mg, 32%) was eluted using 1% acetone in dichloromethane. M.s. *m/z* (relative intensity): 1463 ((M+H), 100%), 723 (73). ^1H n.m.r. (ppm) δ-3.93 to -3.49, NH, 4H; -0.49 to 0.28, $SiCH_3$, 12H; 0.81 - 1.06, $SiC(CH_3)_3$, 18H; 2.16 - 2.54, H_3CHCO, 12H; 3.1 - 3.7, m, $CH_2CO_2CH_3$, ring CH_3 and CO_2CH_3, 44H; 4.47, m, $CH_2CH_2CO_2CH_3$, 8H; 6.1 - 6.8, m, HCOSi and HCOCH, 4H; 9.6 - 10.9, methine protons.

A small amount, 4 mg, of the monoTBDMS ether [16] was also recovered.

Removal of the TBDMS groups

BisTBDMS DHE tetramethyl ester [47] (11 mg, $6.8x10^{-6}$ mol) was dissolved in tetrahydrofuran (1 ml) and tetrabutylammonium fluoride (1N in tetrahydrofuran, 0.05 ml) was added. The solution was stirred for 1 hour then diluted with dichloromethane (10 ml) and washed with saturated aqueous ammonium chloride (3x5 ml) and water (10 ml). The volatiles were removed under reduced pressure and the residue purified by chromatography on a small squat column. **DHE tetramethyl ester** [30] (7.3 mg, 79%) was eluted using 1% methanol in dichloromethane. M.s. *m/z* (relative intensity): 1235 ((M+H), 100%), 609 (86). ^1H n.m.r.: δ -3.71 to -4.89, NH, 4H; 2.10 - 2.57, H_3CHCO,

12H; 3.3-3.8, m, $CH_2CO_2CH_3$, ring CH_3 and CO_2CH_3, 44H; 4.49, m ,$CH_2CH_2CO_2CH_3$, 8H; 6.0 - 6.7, m, HCOCH, 4H; 9.3-10.5, methine protons, 8H. ^{13}C n.m.r.: δ 11.8, ring methyls; 22.0, $CH_2CH_2CO_2CH_3$; 23 - 27, $H_3CCH(O-)$; 37.0, $CH_2CO_2CH_3$; 51.8, CO_2CH_3; 64-72, $HC(CH_3)O$; 95-97.5, *meso* carbons; 135-142, pyrrole carbons; 173.6, CO_2CH_3.

Dihematoporphyrin ether [7]

DHE tetramethyl ester [30] (5 mg) was dissolved in tetrahydrofuran (1 ml) and 0.1 N sodium hydroxide (1 ml) and the mixture refluxed for 45 min. Water (10 ml) was added, the pH adjusted to 5 and the coloured material extracted with dichloromethane and tetrahydrofuran. The volatiles were removed under reduced pressure. M.s. *m/z* (relative intensity): 1179 ((M+H), 24%), 1161 (24), 597 (100), 579 (40). HPLC 3.75 and 4.1 min (3.8%, HP), 7.46 (3.4), 8.18 (2.6), 9.35 (13.5),10.21 (9.8), 16.8 (15.6), 19.2 (5.0), 20.83 (10.3), 21.99 (4.3).

3.5 2-(Trimethylsilyl)ethoxymethyl (SEM) ethers and esters

2-(Trimethylsilyl)ethoxymethyl chloride

2-(Trimethylsilyl)ethoxymethyl chloride (SEM chloride) was prepared by a literature procedure.[110] It was distilled at 66-68^0/20 mm Hg (lit.[110] 57-59^0/8 mm Hg.). ^1H n.m.r.: δ 0.0, $(CH_3)_3Si$, 9H; 0.9, t, J = 8Hz, CH_2Si, 2H; 3.7, t, J = 8Hz, CH_2O, 2H; 5.4, CH_2Cl.

3.5.1 SEM ethers.

1. formation of the SEM ethers

HP.DME (dried at 0.1 mm Hg for 0.5 hours, 5.6 mg, $9x10^{-6}$ mol) was dissolved in dry dichloromethane (1 ml) and the solution warmed to 33^o. Diisopropylethylamine (13 µl, $3.7x10^{-5}$mol) and SEM chloride (9µl, $2.7x10^{-5}$mol) were added and the reaction was stirred for 2 hours at 33^o and at room

temperature for 17 hours but still contained starting material (tlc). Further diisopropylethylamine (12 µl, $3.4x10^{-5}$mol) and SEM chloride (8µl, $2.4x10^{-5}$mol) were added and the reaction was stirred for 3 hours at 33°, and then for 17 hours at room temperature. The reaction was poured into water (10 ml), extracted with dichloromethane and washed with saturated ammonium chloride (3x) and water. Tlc R_f 0.66, 0.34, 0.09 (HP.DME), 0.0.

Hematoporphyrin $3^1,8^1$-bis(2-(trimethylsilyl)ethoxymethyl) ether dimethyl ester [51]

HP.DME (dried at 0.1 mmHg for 0.5 hours, 206 mg, $3.3x10^{-4}$ mol) was dissolved in dry dichloromethane (10 ml) and the solution warmed to 33°. Diisopropylethylamine (500 µl, $2.9x10^{-3}$mol) and SEM chloride (300µl, $1.7x10^{-3}$mol) were added and the reaction was stirred for 1.5 hours at 30° and then worked up as for the trial reaction. Tlc R_f 0.4 (main component), 0.07, 0.0. The residue was chromatographed on a squat column. Elution with 1% methanol in dichloromethane gave the bissilyl ether [51]. M.s. m/z (relative intensity); 887 ((M+H), 100%), 827 (10), 739 (40). ^{1}H n.m.r. (60 MHz): δ 0.0, $(CH_3)_3Si$, 18H; 1.03, t, J = 8Hz, CH_2Si, 4H; 2.36, d, J = 7 Hz, CH_3CH, 6H; 3.13, m, CH_2CO_2Me, 4H; 3.47-3.86, m, OCH_3, CH_2CH_2O, ring methyls, 22H; 4.23, m, $CH_2CH_2CO_2Me$, 4H; 5.10, OCH_2O, 4H; 6.56, q, J = 7 Hz, $HCOCH_2$, 2H; 10.0, 10.10, 10.66, 10.73, methine protons, 4H.

Elution with 3% methanol in dichloromethane gave a product compatible with monoSEM porphyrin [50] (47 mg, 20%). M.s. *m/z* (relative intensity); 757 ((M+H), 100%), 739 (34).

2. stability of the SEM ethers

i. to basic hydrolysis conditions

The bissilyl ether [51] (9 mg) was treated with the standard basic hydrolysis conditions described in Chapter 2 experimental to give a product with tlc (6% methanol in dichloromethane) R_f 0.0 with very faint spots at 0.73 [51] and 0.13. M.s. m/z (relative intensity); 859 (100%), 814 (19), 703 (29). The product was methylated by dissolving in methanol and adding diazomethane in ether (0.5 ml) and stirring for 20 minutes. The volatiles were removed under reduced pressure. Tlc analysis showed the bissilyl dimethyl ester [51] as the only product. M.s. m/z (relative intensity); 887 ([51], 100%), 739 (41).

ii. to acidic hydrolysis conditions

The bissilyl ether [51] (9 mg) was suspended in 1N hydrochloric acid (2 ml); tetrahydrofuran (2 drops) was added and the porphyrin dissolved slightly. The mixture was refluxed for 1.5 hours during which time the porphyrin fully dissolved. The solution was diluted with water, the pH adjusted to 5 and the product extracted with tetrahydrofuran and dichloromethane. The combined organic extracts were washed with water and the solvent removed under reduced pressure. Tlc (6% methanol in dichloromethane) R_f 0.0. The product was methylated by dissolving in methanol and adding diazomethane in ether (0.5 ml) and stirring for 20 minutes. The volatiles were removed under reduced pressure. Tlc analysis showed HP.DME as the only product. M.s. m/z (relative intensity); 627 (HP.DME, 100%), 609 (34).

3. Synthesis of SEM ethers and esters.

i. using an aqueous workup

HP.2HCl (dried at 0.05 mmHg for 0.5 hours, 18 mg, $2.7x10^{-5}$ mol) was suspended in dry dichloromethane (1.5 ml) and the solution warmed to 33°. Diisopropylethylamine (80 µl, $4.5x10^{-4}$ mol) and SEM chloride (30µl, $1.7x10^{-3}$ mol) were added and the reaction was stirred for 45 minutes at 33°.# Tlc analysis showed 3 spots of similar intensity at R_f 0.75, 0.43, and 0.17. The reaction was poured into water (10 ml), extracted with dichloromethane and washed with saturated ammonium chloride (1x).and water and the solvent removed under reduced pressure to give a residue, tlc R_f 0.0, which indicates the ester groups have been hydrolysed.

ii. using a nonaqueous workup

The above reaction was repeated up to # and the volatiles removed under reduced pressure. The residue was chromatographed on a squat column which had been prewashed with 50% methanol in dichloromethane followed by dichloromethane (3x). Elution with 6% methanol gave two fractions. The first fraction contained two components (tlc R_f 0.65, 0.33), these were compatible with the tetra and trisilylated compounds, [53] and [54]. M.s. *m/z* (relative intensity); 1119 (100%), 989 (54), 971 (54). The second fraction contained a number of components. Tlc 0.25, 0.18, 0.10, 0.05. M.s. m/z (relative intensity); 1119 (19%), 1018 (19), 989 (100), 858 (50), 841 (42).

3.5.3 Trial dimerisation

The mixture of tetra [53] and trisilylated material [54] obtained above (11 mg, dried on oil pump 15 minutes) was dissolved in dichloromethane (2 ml) and crushed molecular sieves added. Triethylamine (5 µl) and methanesulfonyl

chloride (3 µl,) were added and the solution stirred for 10 minutes and then poured into water. The product was extracted with dichloromethane (2x) and the organic extracts washed with water. Tlc analysis (6% methanol in dichloromethane), of the product showed, R_f 0.84, tetrasilylated HP [53] (major product), 0.56 trisilylatedHP [54], some poorly defined material between 0.84 and 0.56 and a small amount of material from 0.32 to 0.0. M.s. m/z (relative intensity); 1961 (5%), 1831 (3), 1813 (5), 1119 ([54], 100%), 989 ([53], 30), 971 (100), 842 (50), 823 (42).

attempted desilylation

The product was dissolved in tetrahydrofuran and tetrabutylammonium fluoride in tetrahydrofuran (0.5 ml, 0.1M) was added and the reaction stirred for 2 hours with no reaction (tlc). The solution was refluxed for 1 hour which gave polar material (tlc R_f, 0.0). The reaction was poured into water and the product extracted with dichloromethane. The combined organic extracts were washed with saturated ammonium chloride (4x) and water. The residue was chromatagraphed on a small sephadex column, using THF, water, methanol, to give three fractions.

Fraction 1. (brown colouration). M.s. m/z (relative intensity); 1439 (18), 1290 (7) 1119 (11), 859 (100), 711 (71).

Fraction 2. M.s. m/z (relative intensity); 859 (100%), 711 (33).

Fraction 3. M.s. m/z (relative intensity); 729 (100%), 711 (96).

6.4 Chapter 4 Experimental

4.2.1 2,2,2-Trichloroethyl esters

Hematoporphyrin $3^1,8^1$-bis(2,2,2-trichloroethyl) ether $13^3,17^3$-bis(2,2,2-trichloroethyl) ester [59]

HP.2HCl (120 mg, $1.7x10^{-4}$ mol) was dissolved in 2,2,2-trichloroethanol (3 ml) and dichloromethane (3 ml) and *p*-toluenesulfonic acid (2 crystals) were added. The reaction mixture was allowed to stir for 2 hours but by tlc no reaction had occurred. The reaction mixture was divided into two; one half was allowed to stand overnight but there was still no reaction (tlc). Sulfuric acid (5 drops) was added to the other half and the reaction allowed to stand overnight and then refluxed for 5 hours. Analysis by tlc showed one major product (R_f 0.67). The reaction mixture was diluted with dichloromethane (20 ml) and washed with saturated aqueous sodium bicarbonate (3x10 ml) and water (10 ml). The volatiles were removed under reduced pressure and the product purified on a squat column. Elution with dichloromethane gave **hematoporphyrin $3^1,8^1$-bis(2,2,2-trichloroethyl) ether $13^3,17^3$-bis(2,2,2-trichloroethyl) ester** [59] (32 mg, 63%). M.s. *m/z* (relative intensity): 1125 ((M+H), 100%), 1089 (23), 993 (20), 975 (57). λ_{max} (relative intensity); 403 (100%), 500 (13), 533 (8), 569 (6), 623 (4). ^1H n.m.r.: δ -3.67, NH, 2H; 2.39, 2.41, $CHCH_3$, 6H; 3.45, m, $CH_2CH_2CO_2$, 4H; 3.66, 3.70, 3.71, 3.74, ring methyls, 12H; 4.29, 4.30, 4.32, 4.33, $COCH_2CCl_3$, 4H; 4.49, m, $CH_2CH_2CO_2$, 4H; 4.76, 4.78, 4.79, $COOCH_2CCl_3$, 4H; 6.43, m, H_3CCHO, 2H; 10.13, 10.61, 10.62, 10.64, methine protons, 4H. ^{13}C n.m.r.: δ 11.8, ring methyls; 21.6, $CH_2CH_2CO_2$; 25.3, H_3CCHO; 36.6, $CH_2CH_2CO_2$; 74.1, $Cl_3CCH_2O_2C$; 75.3, $HCOCH_2$; 81.9, Cl_3CCH_2OC; 94.9, CCl_3; 96.4-98.6, *meso* carbons; 137-145, pyrrole carbons; 171.5, CO_2CH_2.

4.2.1a Esterifications using dicyclohexylcarbodiimide.

1. Esterification of HP.

HP.2HCl (109 mg, $1.6x10^{-4}$ mol) was suspended in dichloromethane (20 ml); 4- Dimethylaminopyridine, DMAP, (46 mg, $3.7x10^{-4}$ mol) and methanol (60 µl, $1.5x10^{-3}$ mol) were added and the mixture cooled to 0°. Dicyclohexylcarbodiimide, DCC, (72 mg, $3.5x10^{-4}$ mol) was added and the mixture stirred at 0° for 10 minutes and then at room temperature for 3.5 hours during which time the HP appeared to dissolve.# Tlc analysis of the solution showed one main product (HP.DME) and a small spot at R_f 0.0. The solution was filtered through celite, to remove dicyclohexylurea, and the solvent removed under reduced pressure. The residue was dissolved in dichloromethane (10 ml), filtered, and then washed with saturated aqueous ammonium chloride (2x20 ml), 10% aqueous sodium bicarbonate (20 ml) and water (10 ml), the solvent was removed under reduced pressure to give a solid. Tlc R_f 0.61 (faint), 0.45, 0.38, 0.30-0.15 (complex mixture), 0.15 (main product, HP.DME), 0.10, 0.0. M.s. *m/z* (relative intensity): 1235 (5%), 1221 (5), 1217 (3), 1203 (3), 627 (100, HP.DME), 609 (71).

2. Nonaqueous workup.

The reaction above was repeated up to #. The reaction mixture was eluted from a short (1cm) silica column using 6% methanol/dichloromethane. The solvent was removed under reduced pressure. The residue was dissolved in dichloromethane and filtered through celite to remove further urea and the solvent removed under reduced pressure. Tlc R_f 0.56 (faint), 0.46, 0.28-0.12 (complex mixture), 0.10 (main product, HP.DME), 0.0.

3. Tetrahydrofuran as a cosolvent.

The reaction was repeated using tetrahydrofuran (2 ml) in addition to the dichloromethane as the solvent. The product was chromatographed on a preparative tlc plate (6% methanol in dichloromethane) to give three main fractions.

Band A: R_f 0.5; M.s. *m/z* (relative intensity): 1249 (6%), 1235 (5), 1217 (11), 1203 (4), 669 (60), 641 (60), 609 (100).

Band B: R_f 0.4; M.s. *m/z* (relative intensity): 1235 (28%), 1221 (26), 1203 (13), 627 (41), 613 (41), 609 (100), 595 (45).

Band C: R_f 0.3, HP.DME (36 mg, 36%); M.s. *m/z* (relative intensity): 627 (100, HP.DME), 609 (24).

4. Esterification of HP dimethyl ether [12].

HP dimethyl ether [12] (12 mg, 1.8×10^{-5} mol), was partially dissolved in dichloromethane (1.5 ml). DMAP (1.9 mg, 1.5×10^{-5} mol) and dry methanol (8µl, 2×10^{-4} mol) were added and the reaction mixture was cooled to 0°. DCC (9.7 mg, $x10^{-5}$ mol) was added with stirring. The mixture was stirred at 0° for 10 minutes and then at room temperature for 2 hours; it was worked up as above to give a solid. M.s. *m/z* (relative intensity): 847 (23%), 723 (9), 655 (100, tetramethylated HP [11]), 623 (26). *M/z* 847 is compatible with adducts, [63] and [64] of HP $3^1,8^1$-dimethyl ether methyl ester and DCC.

Hematoporphyrin $13^3,17^3$-bis(2,2,2-trichloroethyl) ester [58]

HP.2HCl (78 mg, 1.2×10^{-4} mol), dried at 0.002 mm Hg for 30 minutes, was dissolved in 2,2,2-trichloroethanol (2 ml) and dichloromethane (1.5 ml). DMAP (28 mg, 2.3×10^{-4} mol) was added, the reaction mixture was cooled to 0° C, and DCC (14.3 mg, 6.8×10^{-5} mol) was added with stirring. The mixture was stirred at 0° for 10 minutes and then at room temperature for 2 hours; it was

then filtered through a short column of silica. The product was purified by elution from a squat column using 1% methanol in dichloromethane to give **hematoporphyrin $13^3,17^3$-bis(2,2,2-trichloroethyl) ester** [58] (66 mg, 65%). M.s. *m/z* (relative intensity): 861 (M+H, 100%), 843 (M-OH, 30). λ_{max} (relative intensity); 402 (100%), 499 (12), 533 (7), 568 (5), 622 (3). ^{1}H n.m.r.: δ -4.54, NH, 2H; 1.69, 1.76, $CHCH_3$, 12H; 2.94, m, $CH_2CH_2CO_2$, 4H; 3.28, 3.29, 3.30, ring methyls, 12H; 4.14, m, $CH_2CH_2CO_2$, 4H; 4.71, 4.75, CH_2CCl_3, 4H; 5.5, m, H_3CCHO, 2H; 9.50, 9.55, 9.59, 9.69, 9.73, 9.76, methine protons. ^{13}C n.m.r.: δ 11.0, 11.1, 11.5, ring methyls; 21.3, $CH_2CH_2CO_2$; 26.0, H_3CCHO; 36.4, $CH_2CH_2CO_2$; 65.4, CHOH, 74.1, $Cl_3CCH_2O_2C$; 94.9, CCl_3; 95.6-98.4, *meso* carbons; 136.0-145, pyrrole carbons; 171.5, CO_2CH_2.

Hematoporphyrin $3^1,8^1$-dimethyl ether $13^3,17^3$-bis(2,2,2-trichloroethyl) ester [60]

HP dimethyl ether [12] (17.5 mg, $3x10^{-5}$ mol), dried at 0.002 mm Hg for 30 minutes, and DMAP (2.1 mg, $1.7x10^{-5}$mol) were dissolved in 2,2,2-trichloroethanol (1 ml) and dichloromethane (1.5 ml). The reaction mixture was cooled to 0° and DCC (14.3 mg, $6.8x10^{-5}$ mol) was added with stirring. The mixture was stirred at 0° for 10 minutes and then at room temperature for 2 hours; it was then filtered through a short column of silica. The product was purified on a squat column, eluting with 10% acetone in dichloromethane to give **hematoporphyrin $3^1,8^1$-dimethyl ether $13^3,17^3$-bis(2,2,2-trichloroethyl) ester** [60] (19 mg, 71%). M.s. *m/z* (relative intensity): 889 (M+H, 100%), 857 (29), 713 (13). λ_{max} (relative intensity); 402 (100%), 499 (12), 532 (8), 568 (6), 622 (4). ^{1}H n.m.r.: δ -3.69, NH, 2H; 2.24, 2.25, 2.27, 2.28, $CHCH_3$, 6H; 3.46, t, J = 7.5 Hz, $CH_2CH_2CO_2$, 4H; 3.62, 3.68, 3.71, OCH_3 and ring methyls, 18H; 4.49, t, J = 7.5 Hz, $CH_2CH_2CO_2$, 4H; 4.76, 4.77, CH_2CCl_3, 4H; 6.06, m, H_3CCHO, 2H; 10.12, 10.52, 10.56, methine protons. ^{13}C n.m.r.: δ 11.6, 11.8, ring methyls; 21.7, $CH_2CH_2CO_2$; 25.3, H_3CCHO; 36.7, $CH_2CH_2CO_2$; 57.3, OCH_3; 74.1, $Cl_3CCH_2O_2$;

75.1, $HCOCH_3$; 94.9, CCl_3; 96.1-98.6, *meso* carbons; 137-145, pyrrole carbons; 171.5, CO_2CH_2.

4.2.1b Exploratory reactions for cleavage of the 2,2,2-trichloroethyl ester.

i. using zinc/tetrahydrofuran/buffer.

The diester [58] (3.4 mg) was dissolved in tetrahydrofuran. To the rapidly stirred solution zinc (1 mg) and potassium dihydrogen phosphate (1M, 0.2 ml) were added and the stirring continued for 15 minutes. The mixture was filtered through cotton wool and the solvents removed under reduced pressure. The residue showed; λ_{max} (relative intensity); 400 (100), 498 (9), 530 (6), 566 (5), 621 (3). HPLC ; 3.6 and 4.0 (6%, HP), 4.9 (5), 5.3 (6), 10.2 and 10.8 (45, diester [58]), 13.1 (11), 20.3 (5), 25.6 (5), 27.8 (5). The residue was treated again as above. HPLC; 3.5 and 3.8 (6%, HP), 4.8 (4), 5.2 (7), 9.6 and 10.2 (45, diester [58]), 12.7 (10), 20.8 (3). The residue was again treated as above but the stirring was continued for 17 hours. HPLC; 3.7 and 4.0 (7%, HP), 4.9 (4), 5.3 (6), 10.3 and 10.9 (41, diester [58]), 16.7 (3), 26.0 (3). M.s. *m/z* (relative intensity): 861 ([58]+H, 100%), 843 ([58]-OH, 57).

ii. using zinc/90% acetic acid.

The diester [58] (5 mg) was dissolved in 90% acetic acid in water (2 ml), the solution was cooled to 0° and zinc (1 mg) was added. The mixture was allowed to stir at 0° for 2 hours. Tlc analysis showed that [58] was still present so further zinc (1 mg) was added and stirring was continued for 1 hour, when tlc showed only baseline material. The reaction mixture was filtered through celite, diluted with dichloromethane (25 ml) and washed with 0.1N aqueous sodium hydroxide (2x10 ml) and water (10 ml). The solvent was removed under reduced pressure. HPLC; 4.3 (2%), 5.4 (2), 6.6 (2), 7.4 (4), 9.1 (9), 10.3 (3), 11.4 (8), 12.2 (7), 15.6 (7), 20.4 (5), 28.8 (14). The mass spectrum was of weak intensity. M.s. *m/z* (relative intensity): 945 (100%), 909 (20), 903 (54), 887 (64), 814 (40), 843

(30), 754 (50). *M/z* 945 and 903 are compatible with the addition of two acetates to the diester [58].

iii. using zinc and 50% acetic acid/water.

The diester [58] (3 mg) was dissolved in 50% acetic acid in water (1.4 ml), the solution was cooled to 0° and zinc (1 mg) was added. The mixture was allowed to stir at 0° for 4.5 hours and was then worked up as above (ii). HPLC; 6.5 (2), 8.5 (2), 9.9 (4), 10.6 (22), 13.9 (4), 14.9 (15), 16.0 (8), 20.9 (3.7), 23.5 (5). The reaction was repeated using a reaction time of 24 hours. HPLC showed main peaks at 5.5 (7%), 7.3 (5), 9.9 (6), 11.2 (9). 11.9 (25.2), 19.0 (10.7), 21.8 (4), 23.2 (6). M.s. *m/z* (relative intensity): 903 ([58] + $OCOCH_3$, 84%), 861 ([58], 100), 843 (70).

iv. The effect of the cleavage conditions on HP.DME [10]

HP.DME [10] (7 mg) was dissolved in 90% acetic acid in water (1 ml), the solution was cooled to 0° and zinc (1 mg) was added. The mixture was allowed to stir at 0° for 45 minutes. Tlc analysis showed no HP.DME still present so the reaction mixture was worked up as above (ii). HPLC; 4.7 (3%), 5.4 (4), 6.6 (11), 10.3 (14). M.s. *m/z* (relative intensity): 774 (69%), 713 (50), 653 (59), 595 (100). λ_{max} (relative intensity); 402 (100%), 496 (5), 533 (12), 569 (13). The product was dissolved in dichloromethane (20 ml) and shaken with 10% hydrochloric acid (20 ml) for 10 minutes, the organic phase was then washed with 10% sodium hydroxide (2x 10 ml) and water (10 ml). M.s. *m/z* (relative intensity): 711 (HP.DME + 2($COCH_3$), 100%), 669 (HP.DME + $COCH_3$, 50), 653 ([68] + $OCOCH_3$, 85), 595 ([68], 100).

4.2.2 2-(Trimethylsilyl)ethyl esters

1. Using oxalyl chloride to form the acid chloride.

i. at room temperature.

HP.2HCl (10 mg) was suspended in 1:1 dichloromethane/tetrahydrofuran (2 ml) and oxalyl chloride (3 drops) was added, the suspension was stirred for 10 minutes, but very little solid dissolved. The volatiles were removed under reduced pressure and the residue was treated with methanol (1 ml). The methanol was removed under reduced pressure. Tlc analysis of the product showed baseline material only.

ii. using HP and pyridine.

HP.2HCl was dissolved in 0.1N sodium hydroxide and the pH adjusted to 5 using 10% HCl. The porphyrin was extracted from the aqueous fraction using dichloromethane and tetrahydrofuran. The solvent was removed under reduced pressure to give HP which was then dried under vacuum (0.05mm Hg) for 1 hour.

HP (8 mg,$1.3x10^{-5}$ mol) was suspended in dichloromethane (1 ml) and pyridine (5 µl, $6.2x10^{-5}$ mol) was added and the mixture stirred for a few minutes until the HP had dissolved. Oxalyl chloride (10µl, $1.2x10^{-4}$ mol) was added and the solution stirred for 5 minutes. The volatiles were removed under reduced pressure and methanol (1 ml) was added to the residue. After stirring the solution for 5 minutes the methanol was removed under reduced pressure. Tlc analysis of the product showed multiple products from R_f 0 to 0.64 with distinguishable components at R_f 0.64, 0.18 and 0. HPLC: 3.7 minutes (3%), 4.2 (3), 4.9 (3), 5.8 (7), 6.4 (3), 7.3 (5), 8.1 (14), 10.1 (10), 11.2 (3), 11.8 (8), 14.3 (7), 18.7 (23), 20.7 (3).

iii. using HP dimethyl ether [12]

HP dimethyl ether [12] (5 mg) was suspended in dichloromethane (1 ml) and oxalyl chloride (3 drops) was added, the suspension was stirred for 5 minutes during which time the solid dissolved. The volatiles were removed under reduced pressure and the residue was treated with 2-(trimethylsilyl)ethanol (2 drops) in dichloromethane and the solution stirred for 5 minutes. The volatiles were removed under reduced pressure. Tlc analysis of the product showed a complex mixture from R_f 0 to 0.53.

2. Transesterification of HP.DME using 2-(trimethylsilyl)ethanol

i. using 4 Å molecular sieves to remove methanol

HP.DME (7.2 mg, $1.1x10^{-5}$ mol) was dissolved in dichloromethane (1.5 ml) and activated 4 Å molecular sieves were added. 2-(Trimethylsilyl)ethanol (10 µl, $7x10^{-5}$ mol) was added and the reaction stirred for 18 hours, tlc analysis showed only HP.DME. Further 2-(trimethylsilyl)ethanol (10 µl) was added and the solution refluxed for 5 hours and then stirred at room temperature for 36 hours. Tlc analysis of the mixture showed HP.DME was the main component with a minor amount of material at R_f 0.42.

ii. addition of sodium metal to form the alkoxide of 2-(trimethylsilyl)ethanol

To a stirred solution of 2-(trimethylsilyl)ethanol (25 µl, $7x10^{-4}$ mol) and sodium metal (2 pieces, 2x2x4 mm) with activated 4 Å molecular sieves in dichloromethane was added HP.DME (20 mg, $3.2x10^{-5}$ mol) in tetrahydrofuran (3 ml) and the mixture stirred for 5 hours. Tlc analysis of the mixture showed no reaction had taken place.

iii. removal of methanol as an azeotrope

HP.DME (38 mg, $6.1x10^{-5}$ mol) was dissolved in benzene (10 ml) and 2-(trimethylsilyl)ethanol (100 µl, $7x10^{-4}$ mol) and sodium metal were added and the reaction refluxed for 3 hours. Benzene was distilled from the reaction as fresh benzene was added over 2 hours and then most of the remaining benzene was distilled from the reaction. The residue was diluted with dichloromethane, tetrahydrofuran and methanol but was only partially soluble. The solvents were removed under reduced pressure and the residue dissolved in dilute hydrochloric acid, the pH adjusted to 5 using dilute sodium hydroxide and the product extracted with dichloromethane/tetrahydrofuran (2:1). The solvents were removed under reduced pressure. Tlc analysis of the product showed mainly R_f 0 material with a faint spot at R_f 0.15. M.s. *m/z* (relative intensity): 699 (14%, HP $13^3(17^3)$-(2-(trimethylsilyl)ethyl) ester [70]), 599 (100, HP).

iv. preformation of 2-(trimethylsilyl)ethoxide combined with removal of methanol as an azeotrope.

Sodium metal was added to a solution of 2-(trimethylsilyl)ethanol (50 µl, $3.5x10^{-4}$ mol) in dry benzene (5 ml) and the mixture heated to just below reflux for 1.5 hours. The excess sodium metal was removed manually and HP.DME (10 mg, $1.6x10^{-5}$ mol) was added to the solution. Benzene was distilled from the reaction as fresh benzene was added over 1 hour (tlc analysis (6% methanol/ dichloromethane) showed one spot at R_f 0.31 and some baseline material), the reaction was cooled and acetic acid (1 drop) was added. The volatiles were removed under reduced pressure. The product was purified by preparative tlc (3% methanol in dichloromethane), (7 mg, 55%). M.s. *m/z* (relative intensity): 799 (100%, HP bis(2-(trimethylsilyl)ethyl) ester [71]).

3. Transesterification of the tetramethylated HP [11]

The above reaction (2.iv) was repeated using tetramethylated HP [11] (10 mg, $1.5x10^{-5}$ mol) in place of HP.DME.and the reaction time was extended to 2.5 hours at reflux, the reaction was incomplete so the reaction was stirred at room temperature for 17 hours and then worked up as above. Tlc analysis showed one running spot (R_f 0.63) and a large amount of polar material (R_f 0). M.s. *m/z* (relative intensity): 827 (100%), which is compatible with HP dimethyl ether bis(2-(trimethylsilyl)ethyl) ester [72].

4. Transesterification of HP tetraTBDMS [17].

TBDMS triflate (20 µl, $8x10^{-5}$ mol) was added to a stirred solution of HP.2HCl (8 mg, $1x10^{-5}$ mol) and DMAP (18 mg, $1.5x10^{-4}$ mol) in dichloromethane (3 ml) and the stirring continued for 1 hour, tlc analysis of the reaction indicated HP tetraTBDMS [17] (R_f 0.78) was the single product. 2-(Trimethylsilyl)ethanol (200 µl, $1.4x10^{-3}$ mol) was added and the reaction mixture stirred for 17 hours. The mixture was diluted with dichloromethane (20 ml) and washed with saturated aqueous ammonium chloride (3 x 10 ml) and water (2 x 10 ml) and the solvent removed under reduced pressure to give a solid. Tlc R_f 0.79, 0.52, 0. M.s. *m/z* (relative intensity): 1027 (83%, [73]), 927 (100, [76]), 895 (58).

3,8-Diacetyldeuteroporphyrin [77]

3,8-Diacetyldeuteroporphyrin dimethyl ester [83][48] (200 mg, $3.2x10^{-4}$ mol) was dissolved in tetrahydrofuran (20 ml) and 0.1N aqueous sodium hydroxide (20 ml) and the mixture refluxed for 2 hours. The mixture was poured into dilute HCl and the precipitate, 3,8-diacetyldeuteroporphyrin [77] (140 mg, 75%) was collected by filtration. M.s. *m/z* (relative intensity): 595 (100%). The product was 85% pure (HPLC) and was used without further purification.

3,8-Diacetyldeuteroporphyrin 13^3,17^3-bis(2-(trimethylsilyl)ethyl) ester [78]

i. esterification of 3,8-diacetyldeuteroporphyrin using DCC.

3,8-Diacetyldeuteroporphyrin [77] (5 mg, 8.4×10^{-6} mol) was dissolved in dry dimethylformamide (1ml); 2-(trimethylsilyl)ethanol (250 µl, 1.8×10^{-3} mol) and DMAP (2 mg, 1.6×10^{-5} mol) were added and the solution was cooled to 0°. DCC, (5 mg, 2.4×10^{-5} mol) was added and the solution stirred for 10 minutes at 0° and at room temperature for 48 hours. The volatiles were removed under reduced pressure, the residue was dissolved in dichloromethane, filtered through celite to remove urea and the dichloromethane removed under reduced pressure. Tlc R_f 0.8, 0.65, 0.4, 0 (minor). M.s. *m/z* (relative intensity): 795 (100%, 3,8-diacetyldeuteroporphyrin 13^3,17^3-bis(2-(trimethylsilyl)ethyl) ester [78]), 649 (9).

When dichloromethane was used as the solvent diacetyl DP [77] was only slightly soluble and no reaction occurred (tlc).

ii. via the acid chloride

3,8-Diacetyldeuteroporphyrin (243 mg, 4.1×10^{-4} mol), dried for 3 hours at 0.01mm Hg, was dissolved in dry dichloromethane (30 ml), oxalyl chloride (250 µl) was added and the solution was refluxed for 15 minutes under nitrogen. The reaction was cooled and the volatiles removed under reduced pressure. The residue was dried for 2.5 hours at 0.01mm Hg and then dissolved in dry dichloromethane (30 ml); crushed molecular sieves and 2-(trimethylsilyl)-ethanol (200 µl, 1.4×10^{-3} mol) were added to the solution and the mixture was stirred for 2 hours. The reaction was diluted with dichloromethane (30 ml) and washed with 5% aqueous sodium acetate (2x30 ml) and water (1x50 ml). The solvent was removed under reduced pressure and the residue chromatographed. **3,8-Diacetyldeuteroporphyrin 13^3,17^3-bis(2-(trimethyl-**

silyl)ethyl) ester [78] was eluted with 1-2% acetone in dichloromethane (230 mg, 71%). λ_{max} (relative intensity); 423 (100%), 515 (14), 550 (16), 587 (7), 640 (3), 659 (1). M.s. *m/z* (relative intensity): 795 (100%). ^{1}H n.m.r.: δ -0.08, -0.06, $Si(CH_3)_3$, 18H; 0.85, m, $SiCH_2$, 4H; 2.85-3.68, ring methyls, CH_2CO_2, CH_3CO, 22H; 4.06-4.22, $CH_2CH_2CO_2$, CO_2CH_2, 8H; 8.76, 8.77, 9.29, 9.82, 10.13, methine protons, 4H. ^{13}C n.m.r.: δ -1.6, $Si(CH_3)_3$; 11.1, 11.5, 13.7, 13.9, ring methyls; 17.2, $SiCH_2$; 21.4, 21.5, $CH_2CH_2CO_2$; 32.8, 33.1, H_3CCO; 36.7, $CH_2CH_2CO_2$; 62.8, CO_2CH_2; 95.2, 96.7, 99.6, 101.6, *meso* carbons; 135-147, pyrrole carbons; 173.0, CO_2CH_2; 197.9, 198.6, CH_3CO.

3(8)-acetyl-8(3)-(1-hydroxyethyl)deuteroporphyrin [100]

3(8)-acetyl-8(3)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester [82][48] (86 mg, $1.4x10^{-4}$ mol) was refluxed in 1:1 tetrahydrofuran and 0.1N aqueous sodium hydroxide (10 ml) for 1 hour. The pH of the solution was adjusted to 5 and the product extracted using dichloromethane/ tetrahydrofuran (2:1). The combined organic extracts were washed with water and the solvent removed under reduced pressure to give **3(8)-acetyl-8(3)-(1-hydroxyethyl)-deuteroporphyrin** (82 mg, 98%). Tlc was baseline, HPLC showed a single peak at 4.6 min (71%).

4.3.1 Acetyl terminated ester-linked dimers

3(8)-acetyl-8(3)-[1-(2-(trimethylsilyl)ethoxy)ethyl]deuteroporphyrin 13^3,17^3-bis(2-(trimethylsilyl)ethyl) ester [84]

The diacid [100] (dried for 1.5 hour at 0.01mm Hg, 42 mg, $7.0x10^{-5}$ mol) was dissolved in dry dichloromethane (5 ml), oxalyl chloride (10 drops) was added and the solution was refluxed for 15 minutes. The reaction was cooled and the volatiles removed under reduced pressure. The residue was dried for 1.5 hour at 0.01mm Hg and then dissolved in dichloromethane (5 ml) and

2-(trimethylsilyl)ethanol (50 µl) was added and the reaction stirred for 2 hours. Tlc analysis showed a one major spot at R_f 0.54. The product was purified on an alumina column (12x2cm). Elution with dichloromethane gave a small amount of material (4 mg); M.s. *m/z* (relative intensity): 1000 (100%), 882 (30). Further elution with dichloromethane gave a material compatible with 3(8)-acetyl-8(3)-(1-2-(trimethylsilyl)ethoxyethyl)deuteroporphyrin bis(2-(trimethylsilyl)ethyl) ester [84].(43 mg, 68%); M.s. *m/z* (relative intensity): 897 (100%), 779 (30). Elution with 2% acetone gave a material (7 mg); M.s. *m/z* (relative intensity): 1573 (10%), 899 (30), 779 (100).

4.2.2a Reactions utilising the 1-chloroethyl sidechain formed using oxalyl chloride

i. treatment of the chloro derivative with triethylamine and methanol

HP.DME (dried for 1.5 hour at 0.01mm Hg, 17 mg, $2.7x10^{-5}$ mol) was dissolved in dry dichloromethane (5 ml); oxalyl chloride (3 drops) was added and the solution was refluxed for 15 minutes. The reaction was cooled and the volatiles removed under reduced pressure. The residue was dried for 1.5 hour at 0.01mm Hg# and then dissolved in dichloromethane (5 ml); triethylamine (100 µl) was added and the reaction stirred for 1 hour. Tlc analysis showed mainly baseline material. The volatiles were removed and methanol (5 ml) was added and the solution stirred for 17 hours and the methanol removed under reduced pressure. Tlc analysis showed two major spots at R_f 0.52, 0.44 (tetramethylated HP [11]) and some components at 0.07 and 0.0. M.s. *m/z* (relative intensity): 655 (52%, [11]), 623 (100), 591 (40). *M/z* 623 is compatible with 3(8)-(1-methoxy)ethyl-8(3)-vinyldeuteroporphyrin dimethyl ester [33].

ii. treatment with triethylamine at reflux

The above reaction was repeated using HP.DME (10 mg) up to #. The dried residue was dissolved in dichloromethane (5 ml) and triethylamine (200 µl) was added and the reaction mixture was refluxed for 1 hour and then stirred at room temperature for 17 hours. Dichloromethane (20 ml) was added and the solution was washed with saturated ammonium chloride (2x) and water (2x). The volatiles were removed under reduced pressure. Tlc analysis of the residue showed a major component at R_f 0.76 (PP.DME) and two minor components at 0.54 and 0.0. M.s. *m/z* (relative intensity): 591 (100%, PP.DME).

iii. treatment with ethanol

The above reaction was repeated but ethanol (0.2 ml) was added instead of triethylamine, and the reaction was allowed to stand overnight. The reaction was worked up as above. Tlc analysis of the residue showed a major component at R_f 0.84, and a.slightly less intense one at 0.79. M.s. *m/z* (relative intensity): 683 (100%), 637 (63), which correspond to the masses required for the diethyl ether of HP.DME [86] and the mono(1-ethoxyethyl) monovinyl dimethyl ester [87].

iv. treatment with 1-pentanol

The use of 1-pentanol instead of ethanol in the above reaction gave a residue, M.s. *m/z* (relative intensity): 767 (100%), 697 (16), which corresponds to the masses for the mono and dipentyl [88] ethers of HP.DME.

v. Condensation of 3(8)-acetyl-8(3)-(1-chloroethyl)deuteroporphyrin dimethyl ester with 3(8)-acetyl-8(3)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester [82].

The monoacetyl dimethyl ester [82] (dried for 1 hour at 0.01mm Hg, 10 mg, $1.6x10^{-5}$ mol) was dissolved in dry dichloromethane (5 ml); oxalyl chloride (3 drops) was added and the solution was refluxed for 15 minutes. The reaction was cooled and the volatiles removed under reduced pressure. The residue was dried for 1.5 hour at 0.01mm Hg and then dissolved in dichloromethane (5 ml) and monoacetyl dimethyl ester [82] (dried for 1 hour at 0.01mm Hg, 10 mg, $x10^{-5}$ mol) was added and the reaction stirred for 17 hours and then worked up as above. The volatiles were removed under reduced pressure. Tlc analysis of the residue showed two major components at R_f 0.48 and 0.28 [82] and a minor component at 0.65. M.s. *m/z* (relative intensity): 1231 (71%), 625 ([82],100), 607 (97). *M/z* 1231 is compatible with the acetyl terminated ether-linked dimer [89].

3(8)-acetyl-8(3)-(1-hydroxyethyl)deuteroporphyrin $13^3,17^3$-bis(2-(trimethylsilyl)-ethyl) ester [81]

Diacetyldeuteroporphyrin bis(2-(trimethylsilyl)ethyl) ester [78] (550 mg, $6.9x10^{-4}$ mol) was dissolved in tetrahydrofuran (140 ml) and water (20 ml) and sodium borohydride (550 mg, $6.9x10^{-4}$ mol) was added. The reaction was stirred at room temperature for 80 minutes at which time tlc analysis indicated that a maximum yield of the partial reduction product was present. The reaction mixture was diluted with water (200 ml) and the product extracted with dichloromethane (2x100 ml) and washed with water (2x50 ml). The reaction was chromatographed on a squat column. The diacetyl [78] (186 mg, 34%) was eluted with 0-3% acetone in dichloromethane. **3(8)-acetyl-8(3)-(1-hydroxyethyl)deuteroporphyrin bis2-(trimethylsilyl)ethyl ester** [81] (280 mg,

51%) was eluted with 3-4% acetone in dichloromethane. M.s. m/z (relative intensity): 797 (100%). λ_{max} (relative intensity); 407 (100%), 499 (18), 532 (11), 569 (8), 622 (5), 645 (2). ^{1}H n.m.r.: δ -5.1, -4.9, NH, 2H; -0.03, -0.02, $Si(CH_3)_3$, 18H; 0.91, m, $SiCH_2$, 4H; 1.53, 1.55, 1.74, 1.76, $CHCH_3$, 3H; 2.82-3.54, ring methyls, CH_2CO_2, CH_3CO, 19H; 4.11-4.25, $CH_2CH_2CO_2$, CO_2CH_2, 8H; 5.26, 5.28, 5.30, 5.57, H_3CCHO, 1H; 9.10, 9.34, 9.49, 9.53, 9.56, 10.13, 10.42, methine protons, 4H. ^{13}C n.m.r.: δ -1.6, $Si(CH_3)_3$; 10.6, 11.1, 11.5, 11.6, 13.7, ring methyls; 17.3, $SiCH_2$; 21.4, 21.6, $CH_2CH_2CO_2$; 25.8, $HOCHCH_3$ 32.8, H_3CCO; 36.7, 37.2, $CH_2CH_2CO_2$; 62.8, CO_2CH_2; 65.1, CHOH; 95.3-100.7, *meso* carbons; 136-155, pyrrole carbons; 173.0, 173.2, CO_2CH_2; 199.5, CH_3CO. HP.bis(2-(trimethylsilyl)ethyl ester) [91] (57 mg, 10%) was eluted with 10% acetone in dichloromethane. M.s. m/z (relative intensity): 799 (100%).

3,8-diacetyldeuteroporphyrin 13^3-(8-acetyl-3-ethyldeuteroporphyrin-3^1-yl) ester and its regioisomers [99].

i. 3,8-Diacetyldeuteroporphyrin [77] (7 mg, $1.2x10^{-5}$ mol) was dissolved in dry dichloromethane (1 ml); oxalyl chloride (10 drops) was added and the solution refluxed for 15 minutes under nitrogen. The reaction was cooled and the volatiles removed under reduced pressure. The residue was dried for 45 minutes at 0.01mm Hg and then dissolved in dry dichloromethane (5 ml) and crushed molecular sieves added. 3(8)-acetyl-8(3)-(1-hydroxyethyl) bis(2-(trimethylsilyl)ethyl ester) [81] (10 mg, $1.3x10^{-5}$ mol) in dichloromethane (3 ml) was added to the solution and the mixture was stirred for 17 hours, tlc analysis of the reaction mixture indicated that it contained mainly starting materials.

A portion of the mixture (1 ml) was diluted with dichloromethane (1 ml) and refluxed for one hour with no change (tlc).

To the rest of the reaction mixture was added pyridine (1 drop) and the solution stirred for 2 hours, the reaction was poured into 5% sodium acetate

(20 ml), extracted with dichloromethane (20 ml) and washed with water (1x10 ml). The solvent was removed under reduced pressure and the residue chromatographed by preparative tlc (10X20cm, 6% MeOH/CH_2Cl_2) to give three bands; A (R_f 0.78), B (0.57) and C (0.16). The first band, A, was rechromatographed by preparative tlc (10x20cm, 3% methanol/ dichloromethane) to give two bands; A1, highest R_f band, and A2.

Band A1: M.s. *m/z* (relative intensity); 1575 (61%), 779 (100).

Band A2: M.s. *m/z* (relative intensity); 2152 (15%), 1373 (16), 779 (100), 595 (68). MIKES of 2154; 2110 (28%), 2011 (7), 1554 (2), 1373 (100), 1329 (12), 780 (16).

Band B: M.s. *m/z* (relative intensity); 779 (100%, [81]).

Band C: M.s. *m/z* (relative intensity); 1373 (58%), 779 (83), 595 (100). MIKES of 1373; 1356 (100%), 1332 (27), 1285 (9), 779 (67), 595 (6). B^2/E linked scan of 595; 610 (57), 624 (29), 645 (50), 695 (100), 795 (6), 1375 (8).

ii. The above procedure (i) was repeated using diacetyldeuteroporphyrin [77] (33 mg, $5.5x10^{-5}$ mol) and oxalyl chloride (20 drops) in dichloromethane (5 ml) and adding the monoacetyl mono(1-hydroxyethyl) diester [81] (10 mg, $1.3x10^{-5}$ mol) in dichloromethane (5 ml) with dry pyridine (0.02 ml) to the crude acid chloride (dried for 2 hours at 0.01mm Hg) in dichloromethane (5 ml). Chromatography was attempted using radial chromatography (2 mm thick plate), a first fraction eluted using 1-3% methanol in dichloromethane and a second fraction eluted slowly with 6-10% methanol, although porphyrin material remained behind on the plate and could not be removed with 100% methanol. The second fraction was rechromatographed by preparative tlc to give the acetyl terminated dimers [92] (33 mg, 55%).

iii. Repeated, using diacetyldeuteroporphyrin [77] (300 mg, $5x10^{-4}$ mol) and oxalyl chloride (0.4 ml,$4.6x10^{-4}$ mol) in dichloromethane (20 ml) and adding monoacetyl mono(1-hydroxyethyl) diester [81] (300 mg, $3.5x10^{-4}$ mol) in dichloromethane (20 ml) with dry pyridine (0.05 ml) to the crude acid chloride

(dried for 2 hours at 0.01mm Hg) in dichloromethane (20 ml). The products were purified by chromatography on a silica squat column (7 cm wide x 2 cm high). **3,8-Diacetyldeuteroporphyrin $13^3,17^3$-bis[(8(3)-acetyl-3(8)-ethyldeuteroporphyrin-3^1-yl $13^3,17^3$-bis(2-(trimethylsilyl)ethyl) ester] ester** and its regioisomers [96] were eluted with 4-8% acetone in dichloromethane (139 mg, 19%) and the **3,8-diacetyldeuteroporphyrin 13^3-[8-acetyl-3-ethyldeuteroporphyrin-3^1-yl $13^3,17^3$-bis(2-(trimethylsilyl)ethyl) ester] ester** and its regioisomers [92] was eluted using 40-100% acetone and then 6-10% methanol in dichloromethane (291 mg, 61%).

Acetyl terminated ester-linked dimer [92]: M.s. *m/z* (relative intensity); 1373 (M+H, 73%), 779 (95), 595 (100). λ_{max} (relative intensity); 404 (100%), 519 (8), 554 (6), 588 (5), 645 (2). ^{1}H n.m.r.: δ -5.6, to -4.8, NH, 4H; -0.19 to -0.04, $Si(CH_3)_3$, 18H; 0.82, m, $SiCH_2$, 4H; 1.26-1.41, $OCOCH(CH_3)$, m, 3H; 2.38-3.88, ring methyls, CH_2CO_2, CH_3CO, 41H; 4.08-4.22, $CH_2CH_2CO_2$, CO_2CH_2, 12H; 6.8, m, H_3CCHO, 1H; 7.74-9.72, methine protons, 8H. ^{13}C n.m.r.: δ -1.7, $Si(CH_3)_3$; 10.5 to 12.2, 13.7, ring methyls; 17.2, $SiCH_2$; 21.4 to 22.7, $CH_2CH_2CO_2$; 23.6, 23.7, H_3CCHO; 32.4, 32.7, H_3CCO; 36.6 to 37.9, $CH_2CH_2CO_2$; 62.6, 62.7 CO_2CH_2; 67.2, H_3CCHO; 93.8-100.4, *meso* carbons; 134-148, pyrrole carbons; 173.1, 173.2, 173.7. 173.9, CO_2CH_2, CO_2CH; 176.8, 176.9, CO_2H; 198.2, CH_3CO.

Acetyl terminated ester-linked trimer [96]: M.s. *m/z* (relative intensity); 2154 (M^+, 37%), 1373 (18), 780 (100), 597 (68). λ_{max} (relative intensity); 403 (100%), 516 (7), 554 (6), 585 (5), 644 (1). ^{1}H nmr -9.7, -9.5, -7.8, NH, 6H; -0.24 to -0.09, $Si(CH_3)_3$, 36H; 0.77, m, $SiCH_2$, 8H; 1.22, m, $OCOCH(CH_3)$, 6H; 2.4 to 3.8, ring methyls, CH_2CO_2, CH_3CO, 60H; 4.0-4.5, $CH_2CH_2CO_2$, CO_2CH_2, 12H; 6.83, m, H_3CCHO, 2H; 7.8 to 9.6, methine protons, 12H. ^{13}C nmr -1.63, -1.60, $Si(CH_3)_3$; 10.2 to 11.6, 13.5 to 14.5, ring methyls; 17.15, 17.20, 17.26, $SiCH_2$; 21.1-22.4, $CH_2CH_2CO_2$; 23.5 to 23.9, H_3CCHO; 32.7 to 33.4, H_3CCO; 36.6 to 37.7, $CH_2CH_2CO_2$; 62.3, 62.5, 62.7, 62.8, CO_2CH_2; 66.8, 67.0, 67.2, H_3CCHO; 93.0-99.5,

meso carbons; 130-150, pyrrole carbons; 172.8 to 174.0, CO_2CH_2, CO_2CH; 195.8 to 198.4, CH_3CO.

Removal of the 2-(trimethylsilyl)ethyl ester groups

i. using *n*-tetrabutylammonium fluoride

The acetyl terminated ester-linked dimer [92] was stable to N-tetrabutylammonium fluoride tetrahydrofuran for 10 days at room temperature.

ii. using lithium fluoride and then boron trifluoride

The dimer [92] (2 mg) was added to lithium fluoride (2 mg) in tetrahydrofuran (2 ml) and the solution stirred for 21 hours with no change (tlc). Boron trifluoride (2 drops) was added to the solution but after one week the product was still mainly starting material (tlc).

iii. using trifluoroacetic acid

The dimer [92] (8 mg) was dissolved in dichloromethane (1 ml) and trifluoroacetic acid (2 ml) was added. Aliquots of the solution were worked up at 15 and 30 minutes by adding to 0.1N aqueous sodium hydroxide and then adjusting the pH to 5 and extracting with dichloromethane. Tlc at 15 minutes; 0.26 (dimer), 0.16, 0; 30 minutes; 0.13, 0. The rest of the solution was worked up as above at 50 minutes to give **3,8-diacetyldeuteroporphyrin 13^3-(8-acetyl-3-ethyldeuteroporphyrin-3^1-yl) ester** and its regioisomers [99]. M.s. *m/z* (relative intensity); 1173 (25%), 779 (67), 595 (100). HPLC (changed from 85 to 90% methanol on injection); 7.6 min. (3%, [100]), 8.7 (56), 9.7 (20), 16.7 (3%), 24.7 (8).

4.3.2 Ester-linked hematoporphyrin dimer

Hematoporphyrin 13^3-(3^1-hydroxymesoporphyrin-8^1-yl) ester and its regioisomers [57]

The acetyl terminated ester-linked dimer disilyl ester [92] (33 mg, $2.4x10^{-5}$ mol) was dissolved in tetrahydrofuran (10 ml) and water (0.2 ml). Sodium borohydride (8 mg, $2.1x10^{-4}$ mol) was added and the solution was stirred at room temperature for 15 minutes. Acetone (0.1 ml) was added and the solution diluted with water (10 ml), acidified with 10% aqueous HCl to pH 5 and the product extracted using dichloromethane. The extracts were washed with water and the solvent removed under reduced pressure. The product still contained starting material (tlc) so the procedure was repeated. The product was purified by preparative tlc (1:1 acetone/dichloromethane) to give the 1-hydroxyethyl terminated ester-linked dimer disilyl ester [97] (22 mg, 68% yield). M.s. *m/z* (relative intensity); 1402 (M + Na, 21%), 1379 (50), 798 (54), 599 (28). The dimer appeared to decompose during the accumulation of the spectra. After the ^{13}C n.m.r. spectrum was run some precipitate had formed this was removed by filtration prior to the running of the ^1H n.m.r. spectrum was run but more precipitate formed as the spectra was run resulting in a very poorly resolved spectrum. ^{13}C n.m.r.: δ -1.7 $Si(CH_3)_3$; 9.6 to 11.8, ring methyls; 17.1 $SiCH_2$; 21.4, m, $CH_2CH_2CO_2$; 23.9, H_3CCHO; 37.2, $CH_2CH_2CO_2$; 62.7, CO_2CH_2; 64.1 to 65.5, CHOH; 68.2, 68.3, H_3CCHO; 93-98, *meso* carbons; 130-150, pyrrole carbons; 173.1 CO_2CH_2, 175.1 CO_2CH.

Removal of the 2-(trimethylsilyl)ethyl ester groups

The 1-hydroxyethyl terminated disilyl ester dimer [97] (22 mg, $2.4x10^{-5}$ mol) was dissolved in tetrahydrofuran (5 ml); *n*-tetrabutylammonium fluoride (3 drops) was added and the solution was stirred for 5 minutes and then poured

into water (20 ml) and extracted with dichloromethane until the extracts were colourless. Tlc analysis indicated esterified dimer still present. The residue was redissolved in tetrahydrofuran (5 ml), *n*-tetrabutylammonium fluoride (5 drops) was added and the solution was stirred for 5 minutes and worked up as above to give **hematoporphyrin 13^3-(3^1-hydroxymesoporphyrin-8^1-yl) ester** and its regioisomers [57]. Tlc R_f 0.0. M.s. *m/z* (relative intensity); 1179 (M+H, 59%), 1163 (16), 621 (100), 599 (92). 581 (97). HPLC; 3.68 and 4.00 minutes (15%, HP), 8.0 (6), 9.0 (5), 9.9 (13), 12.8 (33), 14.6 (6).

Testing sample

The dimer was insoluble in saline so it was dissolved in ethanol and most of the solvent removed under reduced pressure. Saline (2 ml) was added but precipitation of the porphyrin occurred. Ethanol was added until the precipitate dissolved and then the solvent was reduced under reduced pressure The concentration of the solution was adjusted by the addition of saline (6.1 General experimental). HPLC of the testing solution showed that most of the dimer had been hydrolysed. HPLC; retention time (%); 4.1 (53%, HP), 7.1 and 9.3 (18, HV), 10.6 (4), 16.1 (3), 18.6 (3), 21.7 (3).

4.3.3 Vinyl terminated ester-linked dimer

3(8)-acetyl-8(3)-vinyldeuteroporphyrin dimethyl ester [90]

HV.DME [21] (430 mg, 7.1×10^{-4} mol) was dissolved in tetrahydrofuran (150 ml); Jones reagent (0.5 ml) was added and the solution stirred for 5 minutes. The reaction was then poured into 5% aqueous sodium acetate (150 ml) and the porphyrins extracted with dichloromethane/tetrahydrofuran (2:1). The organic extracts were washed with water (2x50ml) and the solvents removed under reduced pressure. The product was chromatagraphed twice on a squat column using dichloromethane to give **3(8)-acetyl-8(3)-vinyldeuteroporphyrin**

dimethyl ester [90] (170 mg, 40%). M.s. *m/z* (relative intensity); 607 (M+H, 100%).

3(8)-acetyl-8(3)-vinyldeuteroporphyrin [102]

The dimethyl ester [90] (500 mg, 8.2×10^{-4} mol) was dissolved in tetrahydrofuran (40 ml) and 0.1N aqueous sodium hydroxide (40 ml) and the solution refluxed for 2 hours. The reaction was poured into water (50 ml), the pH adjusted to 5 and the porphyrins extracted using dichloromethane and tetrahydrofuran. The organic extracts were washed with water and the solvents removed under reduced pressure to give **3(8)-acetyl-8(3)-vinyldeuteroporphyrin** [102] (388 mg, 81%), which was used without further purification.

3(8)-acetyl-8(3)-vinyldeuteroporphyrin bis(2-(trimethylsilyl)ethyl) ester [85]

The acid [102] (388 mg, 6.7×10^{-4} mol) was suspended in dichloromethane; oxalyl chloride (0.1ml) was added and the mixture refluxed for 15 minutes and then cooled. The volatiles were removed under reduced pressure and the residue dried for 1.5 hours at 0.01 mm Hg. The residue was dissolved in dichloromethane (20 ml) and 2-(trimethylsilyl)ethanol (500µl, 3.5×10^{-3} mol) was added. The reaction was stirred for 20 hours, poured into 5% aqueous sodium acetate (50 ml) and the product extracted with dichloromethane (2x50ml). The organic extracts were washed with water and the solvent removed under reduced pressure. Chromatography on a squat column (1.6 cm deep x4 cm wide) of the residue gave **3(8)-acetyl-8(3)-vinyldeuteroporphyrin bis(2-(trimethylsilyl)ethyl ester** [85] (351 mg, 67%). M.s. *m/z* (relative intensity); 779 (M+H, 100%). λ_{max} (relative intensity); 413 (100), 510 (9), 549 (8), 580 (5), 635 (2). ^{1}H n.m.r.: δ -4.47, -4.45, NH, 2H; -0.11, -0.09, $Si(CH_3)_3$, 18H; 0.83, m, $SiCH_2$, 4H; 3.12-3.72, ring methyls, CH_2CO_2, CH_3CO, 19H; 4.11-4.38, m, $CH_2CH_2CO_2$, CO_2CH_2, 8H; 6.06, 6.10, 6.15, 6.21, 6.31, 6.37, $=CH_2$, 2H; 7.82, 7.86, 7.88, 7.92, 8.06, 8.10, 8.12, 8.16, CH=, 1H; 9.92, 9.55, 9.66, 9.72, 9.76, 10.50, 10.54,

methine protons, 4H. ^{13}C n.m.r.: δ -1.67, $Si(CH_3)_3$; 11.3, 11.6, 12.3, 12.6, 14.2, ring methyls; 17.2, $SiCH_2$; 21.5, 21.6, 21.8, $CH_2CH_2CO_2$; 33.1, CH_3CO; 37.0, 37.2, $CH_2CH_2CO_2$; 62.7, CO_2CH_2; 95.6-101.5, *meso* carbons; 121.2, $=CH_2$; 129.5, 129.7, HC=; 135-153, pyrrole carbons; 173.0, 173.2, CO_2CH_2; 199.3, CH_3CO.

3(8)-(1-hydroxy)ethyl-8(3)-vinyldeuteroporphyrin bis(2-(trimethylsilyl)ethyl ester) [101]

The monoacetyl monovinyl disilyl ester [85] (240 mg,$3.1x10^{-4}$ mol) was dissolved in tetrahydrofuran (75 ml) and water (10 ml). Sodium borohydride (37 mg, $9.8x10^{-4}$ mol) was added and the reaction stirred for 2 hours; by tlc the reaction was incomplete so further sodium borohydride (5 mg, $1.3x10^{-4}$ mol) was added and the reaction stirred for 45 minutes. Acetone (0.5 ml) was added followed by water (100ml). The porphyrins were extracted using dichloromethane (2x100 ml) and the organic phase washed with water (50 ml). The solvent was removed under reduced pressure and the crude material purified by chromatography on a squat column. Elution with 2% acetone in dichloromethane gave **3(8)-(1-hydroxy)ethyl-8(3)-vinyldeuteroporphyrin bis(2-(trimethylsilyl)ethyl ester)** [101] (219mg, 90%). M.s. m/z (relative intensity); 781 (M+H, 100%). λ_{max} (relative intensity); 407 (100), 499 (18), 532 (11), 569 (8), 622 (5)..^1H n.m.r.: δ -4.39, NH, 2H; -0.04, -0.03, $Si(CH_3)_3$, 18H; 0.88, m, $SiCH_2$, 4H; 1.76, d, 1.70, d, J=6.4Hz, CH_3CHO, 3H; 3.03-3.45, ring methyls, CH_2CO_2, 16H; 4.18-4.34, m, $CH_2CH_2CO_2$, CO_2CH_2, 8H; 5.43, 5.53, CH_3CHO, 1H; 6.08 - 6.32, $=CH_2$, 2H; 8.01 - 8.19, CH=, 1H; 9.60-9.90, methine protons, 4H. ^{13}C n.m.r.: δ -1.61, $Si(CH_3)_3$; 11.0, 11.6, 12.5, ring methyls; 17.3, $SiCH_2$; 21.6, $CH_2CH_2CO_2$; 25.8, CH_3CHOH; 37.3, $CH_2CH_2CO_2$; 62.8, CO_2CH_2; 65.3, CH_3CHOH 95.8-98.5, *meso* carbons; 120.3, $=CH_2$; 130.3, HC=; 135-153, pyrrole carbons; 173.2, CO_2CH_2.

Protoporphyrin 13^3-[3-ethyl-8-vinyldeuteroporphyrin-3^1-yl 13^3,17^3-bis(2-(trimethylsilyl)ethyl) ester] ester and its regioisomers [104]

Protoporphyrin (84 mg, $1.5x10^{-4}$ mol) was suspended in dichloromethane (10 ml); oxalyl chloride (10 drops) was added and the mixture refluxed for 15 minutes. After cooling the volatiles were removed under reduced pressure and the residue dried (1.5 hours, 0.01 mm Hg). The residue was dissolved in dichloromethane (10 ml) and a solution of hydroxyethyl vinyl disilyl ester [101] (62 mg, $8x10^{-5}$ mol) and pyridine (0.05 ml) in dichloromethane (5 ml) was added. The reaction was stirred for 17 hours, poured into 5% aqueous sodium acetate (200 ml) and the product extracted with dichloromethane. The organic extracts were washed with saturated ammonium chloride (50 ml) and water (2x50 ml) and the solvent removed under reduced pressure. Tlc R_f 0.65, 0.55 [101], 0.43, 0.38, 0.28. M.s. *m/z* (relative intensity) 2089 (100), 1325 (80), 789 (17), 782 (40). The product was chromatographed by preparative tlc (13% acetone in dichloromethane) to give 4 fractions;

Band D; R_f 0.83, 0.75, 0.60; M.s. *m/z* (relative intensity); 2089 (100), 1353 (27), 1339 (13), 1326 (7), 780 (18), 762 (100).

Band C; R_f 0.75, 0.6, 0.38, 0.22 (main component).

Band B; R_f 0.38, 0.22 (main component).

Band A; R_f 0.14, 0; M.s. *m/z* (relative intensity); 2121 (7), 1545 (1), 1325 (3), 1338 (5), 1325 (7), 779 (18), 763 (100). Bands C and B were combined and chromatographed (5% acetone) again.

Band D; R_f 0.9; M.s. *m/z* (relative intensity) 2121 (4), 1358 (5), 1353 (5) (27), 1340 (4), 1326 (2), 780 (17), 763 (100). λ_{max} (relative intensity); 408 (100), 506 (13), 540 (10), 576 (7), 626 (4), 675 (4).

Band C; R_f 0.69, 0.34; M.s. *m/z* (relative intensity) 2121 (3), 2089 (5), 1324 (9), 780 (51), 764 (57), 562 (100).

Band B; R_f 0.34, 0.30. M.s. *m/z* (relative intensity) 2089 (7), 1324 (9), 764 (57), 562 (100).

Band A; R_f 0.14, 0; M.s. *m/z* (relative intensity) 2089 (10), 1326 (9), 764 (47), 562 (100).

The reaction above was repeated using protoporphyrin (176 mg, $3.1x10^{-4}$ mol) and oxalyl chloride (0.2 ml). The solution for addition contained hydroxyethyl vinyl disilyl ester [101] (180 mg, $2.3x10^{-4}$ mol) and pyridine (0.02 ml). The product showed; tlc (3% methanol) R_f 0.65, 0.55 [101], 0.43, 0.38, 0.28. M.s. *m/z* (relative intensity) 2089 (100), 1326 (80), 789 (17), 782 (40). The product was chromatographed on a squat column which had been washed with methanol (25 ml) and then dichloromethane (3x50 ml). Elution with 0 to 1% acetone gave a material (M.s. *m/z* (relative intensity) 1547 (11), 1528 (4), 781 (10), 763 (100)) which corresponds to impure protoporphyrin bis(2-(trimethylsilyl)ethyl ester). Elution with 40% acetone gave impure vinyl terminated ester-linked trimer tetrasilyl ester [103]; M.s. *m/z* (relative intensity) 2089 (100), 1544 (46), 1326 (26), 763 (90), 563 (23). Elution with 60% acetone gave a material; M.s. *m/z* (relative intensity) 2089 (22), 1325 (10), 763 (54), 562 (100). Elution with 80% acetone gave a material; M.s. *m/z* (relative intensity) 2089 (2), 1443 (5), 1325 (20), 881 (7), 763 (33), 562 (100). The amount of material being eluted gradually dropped off as the percentage of acetone was increased from 60% to 100%. The material remaining was eluted with 10-20% methanol in dichloromethane in two fractions; a) M.s. *m/z* (relative intensity) 1325 (61), 794 (94), 763 (78), 562 (100). b). M.s. *m/z* (relative intensity) 1325 (31), 794 (53), 763 (67), 562 (100). These two fractions and the fraction eluted with 80% acetone were combined and purified by chromatographing on a short squat column (1 cm). Elution with 80% acetone gave the vinyl terminated ester-linked dimer disilyl ester [104], Tlc analysis showed one spot at R_f 0.2. M.s. *m/z* (relative intensity) 1325 (60), 763 (100), 563 (59).

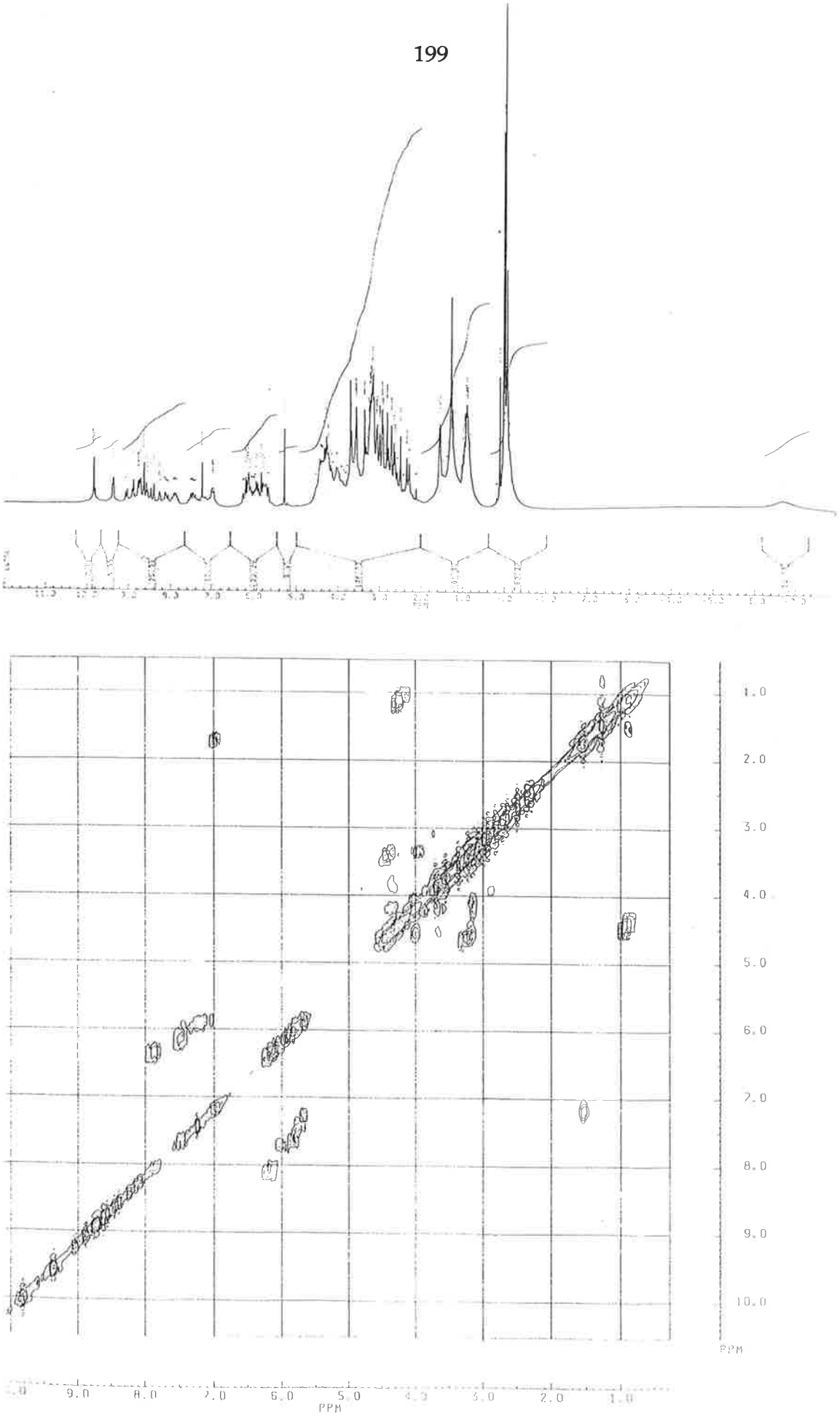

Figure 1. HomoCOSY spectra of the vinyl terminated disilyl ester [104]

The proton n.m.r. was assigned with the aid of a proton homoCOSY spectrum (Fig. 1). ^{1}H n.m.r.: δ -8.7, br, NH, 4H; -0.13, -0.08, -0.02, 0.02, 0.05, $Si(CH_3)_3$, 18H; 0.89, m, $SiCH_2$, 4H; 1.24, grease or water impurity; 1.53, m, CH_3CHO, 3H; 2.3 - 3.8, ring methyls, CH_2CO_2, 32H; 3.9 - 4.6, m, $CH_2CH_2CO_2$, CO_2CH_2, 12H; 5.7 - 6.1, CH_3CHO, and 6.0 - 6.32, $=CH_2$, 7H; 6.9 - 7.5 and 8.01 - 8.19, CH=, includes $CHCl_3$ peak; 8.2 - 9.90, methine protons, 8H. ^{13}C n.m.r.: δ -1.6, $Si(CH_3)_3$; 10.4 - 12.8, ring methyls; 17.3, $SiCH_2$; 21.3, 21.9, 22.8, $CH_2CH_2CO_2$; 23.8, $H_3CCHOCO$; 25.1, $H_3CCHOMe$; 36.7, 37.4, 38.0, $CH_2CH_2CO_2$; 62.8, CO_2CH_2; 67.8, CHOCO; 93-98, *meso* carbons; 116.8, m, $=CH_2$; 129.7, m, =CH; 133-146, pyrrole carbons; 173.3, CO_2CH_2, COOCH; 177, CO_2H.

4.3.4 1-Methoxyethyl terminated ester-linked dimers

Trial reaction of coupling using the dimethyl esterified monomers

HP dimethyl ether [12] (25 mg, $4x10^{-5}$ mol) was suspended in dichloromethane (10 ml); oxalyl chloride (0.2 ml, $2.3x10^{-3}$ mol) was added and the mixture refluxed for 15 minutes. After cooling the volatiles were removed under reduced pressure and the residue dried (2 hours, 0.005 mm Hg). The residue was dissolved in dichloromethane (10 ml) and a solution of trimethylated HP [32] and pyridine (0.02 ml) in dichloromethane (5 ml) was added. The reaction was stirred for 17 hours, poured into 5% aqueous sodium acetate (200 ml) and the product extracted with dichloromethane. The organic extracts were washed with saturated ammonium chloride (50 ml) and water (2x50 ml) and the solvent removed under reduced pressure. The residue was chromatographed on a squat column. Trimethylated HP [32] was eluted with 0-3% acetone/ dichloromethane. Material compatible with the dimer [105] (16 mg, 32%), M.s. *m/z* (relative intensity); 1249 (M+H, 80%), 623 (100), was eluted using 10-40% acetone in dichloromethane.

3(8)-acetyl-8(3)-(1-methoxyethyl)deuteroporphyrin dimethyl ester [107]

Trimethylated HP [32] (450 mg, $7.1x10^{-4}$ mol) was dissolved in tetrahydrofuran (150 ml) and Jones reagent (0.5 ml) was added and the solution stirred for 5 minutes. The reaction was then poured into 5% sodium acetate (150 ml) and the porphyrins extracted with dichloromethane/tetrahydrofuran (2:1). The organic extracts were washed with water (2x50ml) and the solvents removed under reduced pressure. The product was chromatagraphed on a squat column using dichloromethane to give **3(8)-acetyl-8(3)-(1-methoxyethyl)deuteroporphyrin dimethyl ester** [107] (326 mg, 73%). M.s. *m/z* (relative intensity); 639 (M+H, 100%), 607 (12).

3(8)-acetyl-8(3)-(1-methoxy)ethyldeuteroporphyrin [108]

The acetyl dimethyl ester [107] (250 mg, $3.9x10^{-4}$ mol) was dissolved in tetrahydrofuran (10 ml) and 0.1N aqueous sodium hydroxide (6 ml) and the solution refluxed for 2 hours. The solution was poured into dilute hydrochloric acid (30 ml) and pH adjusted to 5, the product was extracted with dichloromethane and tetrahydrofuran and the organic extracts washed with water (50 ml). The solvent was removed under reduced pressure to give a residue. Tlc 0.0.

3(8)-acetyl-8(3)-(1-methoxy)ethyldeuteroporphyrin bis(2-(trimethylsilyl)ethyl) ester [109]

The porphyrin diacid [108] (270 mg, $4.4x10^{-4}$ mol) was suspended in dichloromethane and oxalyl chloride (25 drops) was added and the mixture refluxed for 15 minutes and then cooled. The volatiles were removed under reduced pressure and the residue dried for 2 hours at 0.01 mm Hg. The residue was dissolved in dichloromethane (20 ml) and 2-(trimethylsilyl)ethanol (350µl, $2x10^{-3}$ mol) was added. The reaction was stirred for 17 hours, poured into 5% aqueous sodium acetate (50 ml) and the product extracted with

dichloromethane. The organic extracts were washed with water and the solvent removed. Chromatography on a squat column (4x1.6 cm) of the residue gave **3(8)-acetyl-8(3)-(1-methoxy)ethyldeuteroporphyrin bis(2-(trimethylsilyl)ethyl) ester** [109] (215 mg, 61%). M.s. *m/z* (relative intensity); 811 (M^+, 100%), 779 (30). λ_{max} (relative intensity); 409 (100), 509 (9), 548 (10), 577 (6), 634 (2). ^1H n.m.r.: δ -0.10, -0.09, -0.07, $Si(CH_3)_3$, 18H; 0.83, m, $SiCH_2$, 4H; 2.28, d, J=6.6Hz, $CHCH_3$, 3H; 3.23-3.92, ring methyls, CH_2CO_2, CH_3CO, CH_3O, 22H; 4.13 - 4.38, $CH_2CH_2CO_2$, CO_2CH_2, 8H; 6.09, $H_3CCHOMe$, q, J=6.6Hz, 1H; 9.93, 9.94, 9.96, 10.0, 10.44, 10.65, 10.78, 10.84, methine protons, 4H. ^{13}C n.m.r.: δ -1.67, $Si(CH_3)_3$; 11.5, 11.6, 11.7, 11.9, 14.3, 14.5, ring methyls; 17.2, $SiCH_2$; 21.6, 21.7, $CH_2CH_2CO_2$; 33.2, H_3CCO; 37.0, 37.3, $CH_2CH_2CO_2$; 57.3, OCH_3, 62.8, CO_2CH_2; 74.9, 75.0, CHOMe; 96.0-101.5, *meso* carbons; 135-155, pyrrole carbons; 173.0, 173.2, CO_2CH_2; 199.7, 199.8, CH_3CO.

Hematoporphyrin 3(8)-methyl ether bis(2-(trimethylsilyl)ethyl) ester [106]

The monoacetyl disilyl ester [109] (215 mg, $2.7x10^{-4}$ mol) was dissolved in tetrahydrofuran (50 ml) and water (10 ml) was added slowly. The solution was cooled to 0°, sodium borohydride (20 mg, $5.2x10^{-4}$ mol) was added and the reaction was allowed to warm to room temperature. The reaction was stirred for 4 hours, acetone (2 ml) was added and stirring continued for a minute. The reaction was diluted with water (100 ml) and extracted with dichloromethane until colourless. The organic extracts were washed with water (2x50 ml) and the solvent removed under reduced pressure. Purification on a squat column (3-4% acetone/dichloromethane gave **hematoporphyrin 3(8)-methyl ether bis(2-(trimethylsilyl)ethyl) ester** [106] (191 mg, 89%). M.s. *m/z* (relative intensity); 813 (M^+, 100%), 795 (10), 781 (10). ^1H n.m.r.: δ -3.8, NH, 2H; -0.09, -0.07, $Si(CH_3)_3$, 18H; 0.88, m, $SiCH_2$, 4H; 2.10, $HOCHCH_3$, 3H; 2.27, 2.29, 2.30, m, $MeOCHCH_3$, 3H; 3.24, m, CH_2CO_2, 4H; 3.42-3.71, ring methyls, CH_3O, 15H; 4.16, m, $CH_2CH_2CO_2$, 4H; 4.39, m, CO_2CH_2, 8H; 6.06, $H_3CCHOMe$, q, J=6.6Hz,

1H; 6.19, m, H_3CCHOH, 1H; 9.92, 10.03, 10.09, 10.34, 10.50, 10.51, methine protons, 4H. ^{13}C n.m.r.: δ -1.6, $Si(CH_3)_3$; 11.7, ring methyls; 17.3, $SiCH_2$; 21.9, $CH_2CH_2CO_2$; 25.2, $H_3CCHOMe$; 26.2, H_3CCHOH; 37.3, $CH_2CH_2CO_2$; 57.3, OCH_3; 62.8, CO_2CH_2; 66.0, CHOH; 75.1, CHOMe; 96.3-98.6, *meso* carbons; 136-145, pyrrole carbons; 173.2, CO_2CH_2.

$3^1,8^1$-dimethoxymesoporphyrin 13^3-[3^1-methoxymesoporphyrin-8^1-yl $13^3,17^3$-bis(2-(trimethylsilyl)ethyl) ester] ester and its regioisomers [98]

HP dimethyl ether [12] (167 mg, $2.7x10^{-4}$ mol) was suspended in dichloromethane (10 ml) and oxalyl chloride (0.2 ml, $2.3x10^{-3}$ mol) was added and the mixture refluxed for 15 minutes. After cooling the volatiles were removed under reduced pressure and the residue dried (4 hours, 0.005 mm Hg). The residue was dissolved in dichloromethane (10 ml) and a solution of monomethyl ether disilyl ester [106] and pyridine (0.05 ml) in dichloromethane (5 ml) was added. The reaction was stirred for 17 hours, poured into 5% aqueous sodium acetate (200 ml) and the product extracted with dichloromethane. The organic extracts were washed with saturated ammonium chloride (50 ml) and water (2x50 ml) and the solvent removed under reduced pressure. Tlc R_f 0.5, 0.11, 0.0. M.s. *m/z* (relative intensity); 2217 (7%), 2185 (5), 1421 (41), 1389 (15), 795 (100), 627 (29). The residue chromatograph on an alumina squat column, some material eluted but a large amount of coloured material remained which could not be removed with methanol, tetrahydrofuran of acetone. The alumina from the column was suspended in acetic acid (25 ml) and the alumina filtered off and washed with acetone, the solvents were removed under reduced pressure to leave a residue. M.s. *m/z* (relative intensity); 1443 (17%), 1422 (7), 1412 (7), 854 (74), 795 (100). The material which had initially been eluted from the alumina column was chromatographed on a silica squat column which had been washed with methanol (25 ml) and then dichloromethane (3x50 ml). **$3^1,8^1$-dimethoxy-**

mesoporphyrin $13^3,17^3$-bis[3^1-methoxymesoporphyrin-8^1-yl $13^3,17^3$-bis(2-(trimethylsilyl)ethyl) ester] ester and its regioisomers [110] (30 mg, 12%) was eluted using 0-5% acetone in dichloromethane. M.s. *m/z* (relative intensity); 2217 (16%), 2185 (5), 1437 (11), 1421 (24), 795 (100). ^{1}H n.m.r.: δ -0.1, $Si(CH_3)_3$, 36H; 0.8, m, $SiCH_2$, 8H; 2.2, m, $MeOCHCH_3$, $OCOCHCH_3$, 18H; 2.8 - 4.0, CH_3O, CH_2CO_2, ring methyls, 60H; 4.2, m, $CH_2CH_2CO_2$, 12H; 4.4, m, CO_2CH_2, 8H; 5.9, $H_3CCHOMe$, m, 4H; 7.5, m, $H_3CCHOCO$, 2H; 9.2-10.6, methine protons, 12H. ^{13}C n.m.r.: δ -1.61, $Si(CH_3)_3$; 11.6, 11.8, ring methyls; 17.3, $SiCH_2$; 21.9, $CH_2CH_2CO_2$; 23.9, $H_3CCHOCO$; 252, $H_3CCHOMe$; 37.0, 37.3, 37.7, $CH_2CH_2CO_2$; 57.2, OCH_3, 62.8, CO_2CH_2; 66.4, CHOCO; 75.0, CHOMe; 96.2, 98.0, *meso* carbons; 136-148, pyrrole carbons; 173.2, CO_2CH_2, CO_2CH.

The dimer [98] (71 mg, 23%) was eluted using 30-80% acetone in dichloromethane. M.s. *m/z* (relative intensity); 1421 (M+H, 100%), 1390 (10), 795 (38), 627 (61). MIKES (from 1421); 1390 (1), 1379 (3), 794 (2), 627 (5). ^{1}H n.m.r.: δ -3.8, NH, 4H; -0.13, -0.10, -0.07, -0.05, $Si(CH_3)_3$, 18H; 0.86, m, $SiCH_2$, 4H; 2.43, m, $MeOCHCH_3$, $OCOCHCH_3$, 12H; 2.8 - 4.0, CH_3O, CH_2CO_2, ring methyls, 41H; 4.18, m, $CH_2CH_2CO_2$, 8H; 4.35, m, CO_2CH_2, 4H; 6.03, $H_3CCHOMe$, m, 3H; 7.65, m, $H_3CCHOCO$, 1H; 9.4-10.59, methine protons, 8H. ^{13}C n.m.r.: δ -1.7, $Si(CH_3)_3$; 11.1, 11.5, 11.8, 12.1, ring methyls; 17.2, $SiCH_2$; 20.8, 21.2, 21.5, 21.8, 22.2, $CH_2CH_2CO_2$; 23.7, 24.0, $H_3CCHOCO$; 25.1, $H_3CCHOMe$; 36.0, 36.2, 36.3, 37.1, 37.3, 37.8, $CH_2CH_2CO_2$; 57.2, OCH_3, 62.7, CO_2CH_2; 66.7, 69.2, CHOCO; 75.0, 75.3, CHOMe; 96.2-98.9, *meso* carbons; 137-148, pyrrole carbons; 173.2, CO_2CH_2; 174.8, CO_2H.

Removal of the 2-(trimethylsilyl)ethyl ester groups

The 1-methoxyethyl terminated dimer [98] was dissolved in tetrahydrofuran (10 ml), *n*-tetrabutylammonium fluoride (5 drops) was added and the solution was stirred for 5 minutes and then poured into water (20 ml) and extracted

with dichloromethane until colourless. Tlc analysis indicated esterified dimer still present. The residue was redissolved in tetrahydrofuran (10 ml), N-tetrabutylammonium fluoride (10 drops) was added and the solution was stirred for 40 minutes and worked up as above to give **$3^1,8^1$-dimethoxymesoporphyrin 13^3-(3^1-methoxymesoporphyrin-8^1-yl) ester** and its regioisomers [80]. M.s. *m/z* (relative intensity); 1221 (M+H, 53%), 627 (100), 595 (79). B^2E linked scan of 627; 643 (2), 661 (1), 698 (4),1220 (3). HPLC; 10.6 min (3%), 17.1 (21), 18.5 (36), 20.4 (25), 24.0 (9).

Testing sample

The dimer [80] (29 mg) was dissolved in acetone (2 ml) and saline (3 ml) added, a precipitate formed which was not dissolved by the addition of further acetone (2 ml). Tetrahydofuran (2 ml) was added and dissolved the precipitate, removal of the organic solvent under reduced pressure resulted in the precipitate forming again. The precipitate was redissolved by further additions of tetrahydrofuran and acetone and the pH of the solution was adjusted to 7.1 using 0.1N aqueous sodium hydroxide. Removing the organic solvents under reduced pressure resulted in only a small amount of precipitation. The sample was filtered (0.45 μm filter) and the concentration adjusted (6.1 General experimental). pH 6.95. λ_{max} (relative intensity) (ethanol); 391 (100%), 502 (8), 536 (5), 576 (4), 620 (3). HPLC; 5.4 minutes (2%), 5.9 (2), 11.1 (13) HP dimethyl ether [12], 17.5 (20), 18.9 (35), 20.8 (13), 23.3 (8). The compound was inactive *in vivo*.

After 2 days at 4^o the testing sample showed: HPLC; 5.1 minutes (5%), 5.6 (8), 10.5 (42, HP dimethyl ether [12]), 15.6 (4), 16.4 (4), 17.1 (6), 18.5 (10), 20.2 (6), 22.9 (10).

6.5 **Chapter 5** Experimental

All Grignard reactions were done under nitrogen, protected from light and using dry reagents.

Ethyl magnesium bromide (EtMgBr), butyl magnesium bromide and phenyl magnesium bromide were prepared using the method described by Vogel.[151] The molarities of the Grignard reagents were assessed by titration.

5.2. Grignard reactions on the diacetyl porphyrins

Zinc 3,8-diacetyldeuteroporphyrin dimethyl ester [114]

Zinc diacetyldeuteroporphyrin dimethyl ester [114] was prepared by the general method of Fuhrhop and Smith.[124]

A saturated solution of zinc diacetate in methanol (0.5 ml) was added to a refluxing solution of 3,8-diacetyldeuteroporphyrin dimethyl ester [83] (82 mg, 1.3×10^{-3} mol) in dichloromethane (5 ml). After 20 minutes. most of the dichloromethane was removed under reduced pressure; methanol (10 ml) was added and the solution cooled on ice. The resulting precipitate was collected by vacuum filtration and dried over phosphorous pentoxide *in vacuo* (0.01mm Hg) for 2.5 hours to give **zinc diacetyldeuteroporphyrin dimethyl ester** [114] (59 mg, 62%). λ_{max} (relative intensity); 428 (100), 549 (17), 585 (16).

i. using 1.25 equivalents of Grignard reagent.

A solution of the zinc diacetyl porphyrin [114] (10 mg, 1.4×10^{-5} mol) in tetrahydrofuran (5 ml) was cooled to 0° and EtMgBr (20 µl, 1.85 M in ether, 3.8×10^{-5} mol) was added. The solution immediately changed from a light pink-red colour to a darker red-green colour. The reaction was stirred for 30 seconds, poured into iced water (20 ml) and the pH adjusted to about 5. The

aqueous layer was extracted until colourless using dichloromethane/tetrahydrofuran (2:1) and the solvents were removed from the combined organic extracts under reduced pressure. Tlc analysis of the residue showed minor components at R_f 0.76, 0.44 and 0.13 to 0.0; two major components were seen at R_f 0.36 and 0.2; the starting material had R_f 0.44. The residue had λ_{max} (relative intensity); 429 (100), 520 (3) sh, 560, (11), 602 (8), 642 (2). M.s. *m/z* (relative intensity); 674, (71%), 644 (100). The residue was dissolved in dichloromethane (50 ml) and shaken with dilute hydrochloric acid (20 ml) for 5 minutes to give a product. λ_{max} (relative intensity); 430 (100), 526 sh, (5), 570 (17), 614 (8), 634 sh, (3). λ_{max} (relative intensity); (+triethylamine) 425 (100), 513 (12), 549 (8), 583 (6), 639 (3), 661 (2). The two main components were separated by preparative tlc (3% methanol in dichloromethane) to give the diacetyl dimethyl ester [83] (identified by tlc), M.s. *m/z* (relative intensity); 623, (100%) and the monoethylated product, M.s. *m/z* (relative intensity); 653 (100%).

ii. using 1.9 equivalents of Grignard reagent.

The zinc diacetyl [114] (6.8 mg, 9.9×10^{-6} mol) in tetrahydrofuran (5 ml) was cooled to 0° and EtMgBr (20 µl, 1.85 M in ether, 3.7×10^{-5} mol) was added, the reaction stirred for 35 seconds and then poured into iced water (10 ml). The pH was adjusted to about 5 using dilute hydrochloric acid and the product extracted using dichloromethane/tetrahydrofuran and the solvent removed under reduced pressure. Tlc R_f 0.62, 0.48. The residue was dissolved in dichloromethane (30 ml) and shaken with dilute hydrochloric acid (10 ml) for 5 minutes. The two layers were separated and the organic layer was shaken with 5% aqueous sodium acetate (10 ml) for 3 minutes and water (2x10 ml). The solvent was removed under reduced pressure. λ_{max} (relative intensity); 433 (100), 513 (11), 549 (7), 583 (5), 640 (3), 655 (2). M.s. *m/z* (relative intensity); 683 (17%), 653 (82), 623 (100).

iii. investigation of reaction at various times

The above reaction was repeated using the zinc diacetyl porphyrin [114] (5 mg, $7.3x10^{-6}$ mol) and EtMgBr (10 µl, 1.85 M in ether, $1.9x10^{-5}$ mol). The reaction time was extended to 17 minutes with aliquots analysed by tlc at 1, 2, 4 and 8 minutes. Tlc analysis showed at 1 minute minor components at R_f 0.79 and 0.2 to 0.0 and two major components at 0.56 and 0.39. Over the 8 minutes there was a slight increase in material with R_f 0.2 to 0.0 and a decrease in the relative amounts of the two components at 0.56 and 0.39. The reaction was worked up as above to give the product. λ_{max} (relative intensity); 425 (100), 507 (10), 549 (7), 576 (5), 624 (2), 652 (3). M.s. *m/z* (relative intensity); 683 (100%), 681 (37), 653 (89), 623 (43).

iv. using 5 equivalents of Grignard reagent

The procedure used in 5.2(ii), using the zinc diacetyl porphyrin [114] (5.5 mg, $8.0x10^{-6}$ mol) and EtMgBr (45 µl, 1.85 M in ether, $8.3x10^{-5}$ mol) for 2 minutes gave a complex mixture by tlc, the major components were at R_f 0.64, 0.51, 0.39 and 0.21. The product was washed with acid as above. M.s. *m/z* (relative intensity); 683 (47%), 653 (100), 623 (50).

v. using the free base, diacetyl deuteroporphyrin dimethyl ester [83]

The diacetyl dimethyl ester [83] (5.5 mg, $8.0x10^{-6}$ mol) was partially dissolved in tetrahydrofuran with warming and the mixture cooled to 0°. EtMgBr (45 µl,1.85 M in ether, $8.3x10^{-5}$ mol) was added to the solution and the reaction stirred for 2 minutes. The reaction was worked up as in 5.2(ii), including the acid wash procedure. Tlc analysis showed the major product to be starting material [83]. M.s. *m/z* (relative intensity); 683 (23%), 675 (27), 653 (25), 646 (20), 623 (100). The product was dried (1.5 hours, 0.01mm Hg) and the reaction repeated, the product was more soluble than the original starting material. Tlc

analysis showed a number of components in the region R_f 0.17 to 0.0. λ_{max} (relative intensity); 411 (100), 501 (7), 539 (5), 585 (4), 601 (3), 649 (4). M.s. *m/z* (relative intensity); 739 (100%), 709 (98).

vi. using 21 equivalents of Grignard reagent

As for part 5.2(ii). using zinc diacetyl porphyrin [114] (10 mg, $1.4x10^{-5}$ mol) in tetrahydrofuran (10 ml) and adding EtMgBr (0.5 ml, 2 M in ether, $1.0x10^{-3}$ mol) for 5 minutes. Tlc R_f; 0.26, 0.14 - 0.0. M.s. *m/z* (relative intensity); 739 (68%), 711 (100), 709 (62), 683 (92), 681 (72), 653 (48).

vii. using 35 equivalents of Grignard reagent

As for part 5.2(ii) using zinc diacetyl porphyrin [114] (10 mg, $1.4x10^{-5}$ mol) in tetrahydrofuran (10 ml) and adding EtMgBr (0.5 ml, 2 M in ether, $1.0x10^{-3}$ mol) for 5 minutes. Tlc R_f; 0.29, 0.21. M.s. *m/z* (relative intensity); 811 (73%), 739 (100), 711 (73), 709 (71). I.R. cm^{-1}, (% transmittance); 3311 (48.7) 2955 (11.6), 1734 (10.7), 1660 (32.7). Diacetyl deuteroporphyrin dimethyl ester [83]; I.R. cm^{-1}, (% transmittance); 3310 (81.2), 2956 (74.2), 1734 (25.6), 1660 (41.8).

5.3 Oxazolines and amides as protecting groups

5.3.1 Synthesis of the dioxazoline [120]

3,8-Diacetyl-13,17-bis[N-(1,1-dimethylethan-2-ol)propanamide]-2,7,12,18-tetramethylporphyrin [119]

3,8-Diacetyldeuteroporphyrin [77] (300 mg, $5x10^{-4}$ mol) was suspended in dichloromethane (20 ml); oxalyl chloride (0.1 ml) was added and the mixture was refluxed for 15 minutes. The mixture was cooled and the volatiles removed under reduced pressure. The residue was dried (1.5 hours, 0.01mm Hg) and dissolved in dichloromethane (30 ml). A solution of 2-amino-2-methylpropan-1-ol (225 mg, $2.5x10^{-3}$ mol) and pyridine (0.1 ml) in dichloro-

methane (20 ml) was added and the reaction mixture was stirred for 17 hours. The reaction mixture was diluted with dichloromethane (20 ml) and washed with saturated aqueous ammonium chloride (2x20 ml) and water (2x20 ml). The volatiles were removed under reduced pressure to give the title compound [119] (297 mg, 80%) which was pure by tlc. M.s. *m/z* (relative intensity); 737 (100%).

Exploratory reactions for the cyclisation of the hydroxy amide [119]

1. using oxalyl chloride

i. at room temperature for 15 minutes

The diamide [119] (10 mg) was dissolved in dichloromethane (5 ml), oxalyl chloride (3 drops) was added and the reaction was stirred at room temperature for 15 minutes. The solution was poured into water (10 ml) and the pH adjusted to 5 using dilute hydrochloric acid. The two phases were separated and the aqueous layer was washed with dichloromethane. The combined organic layers were washed with water and the solvent was removed under reduced pressure to give a green residue. Tlc R_f 0.5, 0.3, 0. λ_{max} (relative intensity); 431nm (100), 533 sh (4), 569 (16), 614 (6). The residue was dissolved in dichloromethane (20 ml) and shaken with 1N aqueous sodium hydroxide (20 ml), the pH was adjusted to 6 and the two layers were separated. The organic layer was washed with water and the solvent removed under reduced pressure. Tlc analysis showed that the product was predominantly starting material [119].

ii. double the oxalyl chloride concentration at reflux

The above reaction was repeated using only 5 mg of diacetyl diamide [119] and refluxing the reaction mixture for 18 minutes. After shaking with base the porphyrins were difficult to extract from the aqueous phase. Tlc showed a

number of components from R_f 0.4 to 0.0; R_f 0.0 was a major component. M.s. *m/z* (relative intensity); 809 (32%), 791 (64), 737 (100), 666 (36).

iii. at room temperature for 3 hours

Part (i) was repeated using 8 mg of the diamide [119] and stirring the reaction mixture for 3 hours. Tlc analysis showed a strong baseline component, a reasonably well defined spot at R_f 0.37, and some weak spots in between. M.s. *m/z* (relative intensity); 791 (100%), 737 (40), 719 (33). λ_{max} (relative intensity); 422 (100), 518 (10), 548 (7), 584 (6), 639 (2).

2. by azeotropic removal of water.

The diamide [119] (10 mg) was suspended in toluene in a flask equipped with a Dean Stark apparatus, and the mixture refluxed; the solid gradually dissolved. The reaction mixture was refluxed for 3 days, tlc analysis showed that the mixture contained mainly starting material.

3. using thionyl chloride

i. 5 minutes at ambient temperature

The diamide [119] (5 mg) was slowly dissolved in dichloromethane (5 ml), thionyl chloride (3 drops) was added and the mixture was stirred for 5 minutes. The reaction mixture was poured into 0.05N aqueous sodium hydroxide (10 ml) and the two phases separated. The organic phase was shaken with 0.1N aqueous sodium hydroxide (5 ml) and washed with brine (5 ml) and water (2x10 ml). Tlc 0.50, 0.38 and 0.24 (SM). M.s. *m/z* (relative intensity); 737 (76%), 719 (100), 701 (59). The reaction was repeated on the residue. Tlc R_f 0.57, 0.18 and 0.0. M.s. *m/z* (relative intensity); 811 (23%), 737 (66), 719 (23), 550 (85), 522 (100).

ii. double the concentration of chloride.

The above reaction was repeated but using thionyl chloride (6 drops) and a 10 minute reaction time. Tlc R_f 0.68 (main component), 0.36. M.s. *m/z* (relative intensity); 719 (100%), 701 (28).

Formation of the dioxazoline [120] from 3,8-diacetyl-13,17-bis[N-(1,1-dimethylethan-2-ol)propanamide]-2,7,12,18-tetramethylporphyrin [119]

The diamide [119] (250 mg, $3.4x10^{-4}$ mol) was dissolved in dichloromethane (75 ml) and tetrahydrofuran (5 ml); thionyl chloride (0.5 ml) was added and the mixture was stirred for 5 minutes. The reaction mixture was poured into water and the pH adjusted to 6, the two phases were separated and the organic phase shaken with 0.1 N aqueous sodium hydroxide, the pH was adjusted to 5 and the two layers separated. The aqueous layer was back extracted with dichloromethane and the organic phases combined and washed with water. The solvent was removed under reduced pressure. Elution of the residue from a squat column (25mm high x 45mm wide) with 0-5% methanol in dichloromethane gave the dioxazaline [120] (193 mg, 81%). M.s. *m/z* (relative intensity); 701 (100%). ^{1}H n.m.r.: δ 1.22, 1.26, $NC(CH_3)_2$, 12H; 2.85, 3.11, 3.15, 3.23, 3.41, 3.50, ring methyls, CH_3CO, 18H; 3.05, m, CH_2CO_2, 4H; 3.95, 3.98, $NCOCH_2$, 4H; 4.18, m, $CH_2CH_2CO_2$, 4H; 9.03, 9.53, 9.99, 10.31, methine protons, 4H. ^{13}C n.m.r.: δ 11.3, 11.7, 13.7, 13.9, ring methyls; 22.8, $CH_2CH_2C{=}N(O\text{-})$; 28.4, $C(CH_3)_2$; 30.9, $CH_2CH_2C{=}N(O\text{-})$; 32.8, 33.0, H_3CCO; 67.1, $NC(CH_3)_2C$; 79.2, $C(CH_3)_2CH_2O$; 95.4, 97.2, 99.7, 102.1, *meso* carbons; 135-147, pyrrole carbons; 165.0, $NCOCH_2$; 198.2, 198.8, CH_3CO

5.3.2 Grignard reactions using amides as protecting groups

1. using 3,8-Diacetyl-13,17-bis[N-(1,1-dimethylethan-2-ol)propanamide]-2,7,12,18-tetramethylporphyrin [119]

The diamide [119] (8.4 mg, $1.1x10^{-5}$mol), was partially dissolved in tetrahydrofuran (5 ml) and the solution was cooled to 0°. EtMgBr (80 µl, 2 M in ether, $1.6x10^{-4}$ mol) was added and the reaction stirred for 5 minutes and then poured into iced water (30 ml). The pH was adjusted to about 5 using dilute HCl and the product extracted using dichloromethane/tetrahydrofuran and the solvent removed under reduced pressure. Tlc R_f 0.09-0.0. M.s. *m/z* (relative intensity); 737 (97%), 767 (100).

1b. using the zinc analogue [122] of the diamide [119]

Zinc was incorporated into the diamide [119] (8 mg, $1.1x10^{-5}$mol) using the method outlined for the synthesis of the zinc diacetyl dimethyl ester [114] (part 5.2).to give the zinc diamide [122] (10 mg, 69%). λ_{max} (relative intensity); 440 (100), 556 (18), 594 (12). M.s. *m/z* (relative intensity); 800 (100%).

The zinc diamide [122] (9 mg, $1.1x10^{-5}$ mol) was dissolved in tetrahydrofuran (10 ml) and the mixture cooled to 0°. EtMgBr (80 µl, 2 M in ether, $1.6x10^{-5}$ mol) was added and the reaction stirred for 5 minutes and then poured into iced water (50 ml). The porphyrin was extracted with dichloromethane (2x20 ml) and the extracts shaken with dilute aqueous hydrochloric acid (50 ml) for 5 minutes. The organic phase was washed with dilute aqueous sodium hydroxide and water and the solvent removed under reduced pressure. M.s. *m/z* (relative intensity); 797 (67%), 767 (100), 737 (50).

3. using 3,8-Diacetyl-13,17-bis[N-benzylpropanamide]-2,7,12,18-tetramethyl-porphyrin [124]

i. Synthesis of the dibenzylamide [124]

Diacetyl deuteroporphyrin [77] (70 mg, $1.2x10^{-4}$ mol) was suspended in dichloromethane (10 ml), oxalyl chloride (10 drops) was added and the mixture refluxed for 15 minutes. The mixture was cooled and the volatiles removed under reduced pressure. The residue was dried (2 hours, 0.01mm Hg) and dissolved in dichloromethane (10 ml). Benzylamine (75 µl, $6.8x10^{-4}$ mol) was added and the reaction mixture was stirred for 2 hours. The reaction mixture was diluted with dichloromethane (20 ml) and washed with saturated aqueous ammonium chloride (2x20 ml) and water (2x20 ml). The volatiles were removed under reduced pressure. The residue was dissolved in dichloromethane and filtered. The filtrate was chromatographed on a squat column. Elution with 2-4% methanol in dichloromethane gave the diacetyl dibenzylamide [124] (51 mg, 54%) which was identified by mass spectral analysis; M.s. *m/z* (relative intensity); 773 (100%). The product was only slightly soluble in ether and benzene and only partially soluble in tetrahydrofuran.

ii. Grignard reaction

The dibenzylamide [124] (12 mg, $1.6x10^{-5}$mol) was partially dissolved in tetrahydrofuran (10 ml) and the mixture cooled to 0°. EtMgBr (200 µl, 2 M in ether, $4x10^{-4}$ mol) was added and the reaction stirred for 5 minutes and then worked up as for part 5.2(ii). M.s. *m/z* (relative intensity); 833 (100%), 803 (80), 773 (60).

2b. using the zinc analogue [124]

Zinc was incorporated into the.dibenzylamide [4.14] (8.6 mg, $1.1x10^{-5}$mol) using the standard procedure[124] (part 5.2) to give the zinc dibenzylamide [124] (10 mg, 69%). λ_{max} (relative intensity); 434 (100), 556 (24), 594 (16). M.s. *m/z* (relative intensity); 836 (100%).

The procedure in part 2(ii). was repeated using the zinc dibenzylamide [124] (5 mg, $6x10^{-6}$mol), which dissolved in tetrahydrofuran (10 ml), with warming, and EtMgBr (25 µl,2 M in ether, $5x10^{-4}$ mol) to give a product; M.s. *m/z* (relative intensity); 803 (90%), 773 (100).

3. using the dioxazoline porphyrin [120]

The procedure in part 5.3.2.1. was repeated using dioxazoline porphyrin [120] (8.5 mg, $1.2x10^{-5}$ mol) and EtMgBr (100 µl, 2 M in ether, $1.7x10^{-4}$ mol). The product showed tlc (10% methanol/dichloromethane) R_f 0.61, 0.51, 0.42, 0.22, 0.19, 0.0. M.s. *m/z* (relative intensity); 779 (48%), 761 (100), 749 (62), 731 (93).

5.4 Grignard reactions on formyl porphyrins

5.4.1. Methyl lithium trial reactions

The zinc diacetyl dimethyl ester [114] (5 mg, $7.3x10^{-6}$mol) was dissolved in tetrahydrofuran (5 ml) and methyl lithium (50µl, 2M in ether, $1x10^{-4}$ mol) was added. Aliquots of the reaction mixture were taken at 45 sec, 1 min, 2 min, 4 min and 8 min, added to aqueous acid and the products extracted with dichloromethane. All the samples showed similar tlc analysis with components at R_f 0.13, 0.10, 0.06 and 0. The remaining reaction mixture was worked up after 16 minutes by pouring into acidic iced water and extracting with dichloromethane. The solvent was removed under reduced pressure. Tlc of the product showed the same components were present as for the

aliquots but a further component (R_f 0.03) was observed and the relative intensities of the components at R_f 0.06 and 0.0 had increased with respect to the other components. None of the products were consistent with the zinc diacetyl dimethyl ester [114] (R_f 0.25) or its free base diacetyl dimethyl ester [83] (R_f 0.28). λ_{max} (relative intensity); 425 (100), 513 (15), 550 (12), 583 (9) and 642 (5).

The reaction was repeated and worked up as above at 30 seconds. Tlc of the product was similar to above with 3 main components at R_f 0.13, 0.10 and 0.06. λ_{max} (relative intensity); 425 (100), 509 (20), 535 (19), 548 (19), 572 (18), 623 (4.0), 631 (3.1). The reaction mixture was redissolved in dichloromethane and washed with dilute hydrochloric acid and water. The main components were separated by preparative tlc (12.5 x 25 cm plate, 3% methanol in dichloromethane) to give:-

Band A (R_f 0.13): M.s. *m/z* (relative intensity); 637 (30%), 623 (100).

Band B (R_f 0.10): M.s. *m/z* (relative intensity); 642 (100%), 625 (14).

Band C (R_f 0.06): M.s. *m/z* (relative intensity); 676 (17%), 655 (100), 637 (11).

The peak *m/z* 655 is compatible with the product formed by methylation of both acetyl groups, it is also compatible with alkylation of the ester groups to give the mono or dialcohols, [130] and [131]. The peak at *m/z* 637 may be due to monodehydration of these compounds.

5.4.2 Grignard reactions on diformyldeuteroporphyrin [132].

3,8-Diformyldeuteroporphyrin dimethyl ester [132]

3,8-Diformyldeuteroporphyrin dimethyl ester [132] was prepared from protoporphyrin dimethyl ester in 25% yield using a literature procedure.[152] M.s. *m/z* (relative intensity); 595 (100%).

Grignard reactions

i. using 1 equivalent of Grignard reagent

The diformyl [132] (4.3 mg, 7.2×10^{-6} mol) was partially dissolved in tetrahydrofuran (10 ml), the mixture cooled to 0° and EtMgBr (10 µl, 1.4 M in ether, 1.4×10^{-5} mol) was added. The reaction mixture was stirred for 5 minutes, poured into iced water (25 ml) and the products extracted with dichloromethane. Tlc R_f 0.23, 0.07, 0.03 and 0.0. The two main components (R_f 0.23 and 0.07) were separated by preparative tlc (3% methanol) to give diformyl dimethyl ester [132] (2.1 mg, 49%, identified by tlc) and a product compatible with the diethylated material [133] (2.3 mg, 49%). M.s. *m/z* (relative intensity); 655 (100%). A weak band with R_f 0.03 was also isolated. M.s. *m/z* (relative intensity); 711 (80%), 681 (100), 653 (63).

ii. using a 10 minute reaction time

The reaction in above (i) was repeated using the diformyl dimethyl ester [132] (4.9 mg, 8.3×10^{-6}mol), EtMgBr (12 µl, 1.4 M in ether, 1.7×10^{-5} mol) and reaction time 10 minutes. The reaction was poured into iced water (25 ml) and extracted with dichloromethane. The organic phase was washed with water and the solvent removed under reduced pressure. Tlc analysis; R_f 0.0.36 (diformyl dimethyl ester [132]), 0.16 (diethylated material [133], as above), 0.10, 0.06, 0.

iii. using 2.2 equivalents of Grignard reagent and a 5 minute reaction time.

The reaction (i), above, was repeated using the diformyl dimethyl ester [132] (4.4 mg, 7.5×10^{-6}mol), EtMgBr (24 µl, 1.4 M in ether, 3.3×10^{-5} mol) and a reaction time of 5 minutes. The product showed similar tlc behaviour to the product from part (ii).

iv. using 4 equivalents of Grignard reagent

The diformyl dimethyl ester (3.8 mg, $6.4x10^{-6}$mol) was suspended in ether (10 ml) but it would not dissolve so the solvent was removed under reduced pressure and the residue partially dissolved in tetrahydrofuran. The mixture was cooled to 0° and EtMgBr (38 µl, 1.4 M in ether, $5.2x10^{-5}$ mol) was added; the reaction was stirred for 10 minutes and worked up as above. Tlc analysis contained the same components as part (ii) but comparatively greater amounts of 0.15 (diethylated material), 0.12 and 0.0 components.

v. using 1 equivalent of Grignard reagent and extended reaction time

The diformyl dimethyl ester [132] (4.1 mg, $6.8x10^{-6}$mol) was reacted with EtMgBr (10 µl, 1.4 M in ether, $1.4x10^{-5}$ mol) as in (i). Aliquots were taken at 10, 20 and 30 minutes and analysed by tlc, at 30 minutes a large amount of precipitate appeared to be forming in the reaction mixture, so the reaction mixture was worked up as above. Tlc analysis were similar to (ii) with a slight increase in baseline material seen over time.

5.4.3 Grignard reactions on the ethyl formyl porphyrin [134]

3(8)-Ethyl-8(3)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester [135]

3(8)-Ethyl-8(3)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester [135] was prepared in 29% yield from hematoporphyrin dimethyl ester using a literature procedure.[113] M.s. *m/z* (relative intensity); 611 (100%), 594 (12)

3(8)-Ethyl-8(3)-vinyldeuteroporphyrin dimethyl ester [136]

3(8)-Ethyl-8(3)-vinyldeuteroporphyrin dimethyl ester [136] was prepared in 80% yield from [135] using a literature procedure.[113] M.s. *m/z* (relative intensity); 593 (100%).

3(8)-Ethyl-8(3)-formyldeuteroporphyrin dimethyl ester [134].

A solution of potassium permanganate (760 mg) and magnesium sulfate (1.6g as the heptahydrate) in water (38 ml) was added over 35 minutes to a refluxing solution of the ethylvinyl dimethyl ester [136] (760 mg, 1.3×10^{-3} mol) in acetone. The mixture was refluxed for a further 5 minutes, cooled, and filtered through Celite which was then washed with acetone and dichloromethane. The filtrate and washings were combined and concentrated by removal of solvent under reduced pressure. The porphyrin was extracted using chloroform. The organic fraction was washed with water and the solvent removed under reduced pressure. The residue was chromatographed on alumina. Elution with 0-2% methanol in dichloromethane gave the ethylvinyl dimethyl ester [136]. Elution with 4% methanol in dichloromethane gave by comparison with an authentic sample[59] 3(8)-ethyl-8(3)-formyldeuteroporphyrin dimethyl ester [134].(260 mg, 31%). M.s. *m/z* (relative intensity); 595 (100%).

Grignard reactions

3(8)-ethyl-8(3)-(1-hydroxypropyl)deuteroporphyrin dimethyl ester [137].

a). Ethylformyl dimethyl ester [134] (4.5 mg, 7.6×10^{-6} mol) was dissolved in tetrahydrofuran (5 ml), the mixture cooled to 0°, and EtMgBr (10 µl, 1.4 M in ether, 1.4×10^{-5} mol) was added. The reaction mixture was stirred for 10 minutes and then poured into iced water (25 ml) and extracted with dichloromethane. The solvent was removed to give crude **3(8)-(1-hydroxypropyl)deuteroporphyrin dimethyl ester** [137]. Tlc R_f 0.49 (major component), 0.06, 0.03 and 0.0. λ_{max} (relative intensity); 404 (100%), 499 (12), 532 (8), 567 (7), 622 (3), 643 (1) M.s. *m/z* (relative intensity); 625 (100%).

b). Ethylformyl dimethyl ester [134] (59mg, $9.9x10^{-5}$ mol) was dissolved in tetrahydrofuran (30 ml), the mixture cooled to 0°, and EtMgBr (90 µl, 1.4 M in ether, $1.3x10^{-4}$ mol) was added. The reaction mixture was stirred for 5 minutes and then poured into iced water (75 ml). The pH was adjusted to 5 and the porphyrins extracted with dichloromethane. The solvent was removed and the products separated by chromatography using a squat column with dichloromethane containing 0.4% methanol. Three fractions were isolated; ethylformyl dimethyl ester [134] (9.8 mg, 17%) and two fractions for the ethylated product [137]; termed α and β for the first and second eluting fractions respectively. **Fraction** α (14.7 mg, 24%); M.s. *m/z* (relative intensity); 625 (M+H, 100%). ^{1}H n.m.r.: δ - 4.09, NH, 2H; 1.04, t, J=7.5 Hz, $H_3CCH_2CH(OH)$, 3H; 1.83, t, J=7.5 Hz, H_3CCH_2, 3H; 2.52, m, $H_3CCH_2CH(OH)$, 2H; 3.18, t, 3.22, t, J=7.8 Hz, CH_2CO_2, 2H; 3.41, 3.46, 3.52, 3.53, ring methyls, 12H; 3.63, 3.66, CH_3OC, 6H; 4.02, q, J=7.5 Hz, H_3CCH_2, 4.22, t, 4.26, t, J=7.8 Hz, $CH_2CH_2CO_2$, 4H; 5.86, m, $H_3CCH_2CH(OH)$, 1H; 9.82, 9.93, 10.21, methine protons, 4H. **Fraction** β (13 mg, 21%); M.s. *m/z* (relative intensity); 625 (M+H, 100%). ^{1}H n.m.r.: δ - 4.17, NH, 2H; 0.96, t, J=7.5 Hz, $H_3CCH_2CH(OH)$, 3H; 1.80, t, J=7.5 Hz, H_3CCH_2, 3H; 2.43, m, $H_3CCH_2CH(OH)$, 2H; 3.17, t, 3.19, t, J=7.5 Hz, CH_2CO_2, 2H; 3.35, 3.42, 3.46, ring methyls, 12H; 3.64, CH_3OC, 6H; 3.91, q, J=7.5 Hz, H_3CCH_2, 2H; 4.18, t, 4.27, t, J=7.8 Hz, $CH_2CH_2CO_2$, 4H; 5.70, m, $H_3CCH_2CH(OH)$, 1H; 9.80, 9.82, 10.06, methine protons, 4H. Both samples were made up in 1ml deuterochloroform. ^{13}C n.m.r. (combined sample of fractions α and β): δ 10.88, 10.92, 11.38,11.46, 11.58, 11.63, 11.71, 11.80, ring methyls, CH_3CH_2CHOH; 17.6, H_3CCH_2; 19.7, H_3CCH_2; 21.7, 21.8, $CH_2CH_2CO_2$; 33.2, CH_3CH_2CHOH; 36.9, 37.0, $CH_2CH_2CO_2$; 51.8, OCH_3; 71.5, HCOH; 96.0, 96.2, 96.4, 96.6, 96.7, 98.7, *meso* carbons; 134-148, pyrrole carbons; 173.6, 173.7, CO_2Me.

Hydrolysis of the methyl esters

Fraction α (< 1mg) was dissolved in tetrahydrofuran (1 ml) and 0.1N aqueous sodium hydroxide (0.5 ml) and the solution stirred overnight at 38°. The solution was diluted with water, the pH adjusted to 5,and the product extracted with dichloromethane and tetrahydrofuran. The combined extracts were washed with water and the solvent removed under reduced pressure to give a solid. Tlc R_f 0.0. HPLC; 9.29 minutes (85%), 10.41 (6%).

Fraction β was treated as above. Tlc R_f 0.0. HPLC; 9.36 minutes (4%), 10.42 (96%). A combined HPLC (isocratic) of α and β gave two peaks only; 9.11 and 10.82 minutes.

3(8)-ethyl-8(3)-(1-hydroxybenzyl)deuteroporphyrin dimethyl ester [138]

1. using 2.5 equivalents of Grignard reagent

a) Ethylformyl dimethyl ester [134] (4.0 mg, 6.7×10^{-6} mol) was dissolved in tetrahydrofuran (5 ml), the mixture cooled to 0°, and phenyl magnesium bromide (10 µl, 1.7 M in ether, 1.7×10^{-5} mol) was added. Aliquots were taken at 2, 5 and 10 minutes and the reaction worked up at 20 minutes as before. Tlc analysis showed one major product (R_f 0.83) with some starting material still present at 5 minutes. M.s. (20 minute sample) *m/z* (relative intensity); 673 (100%).

b). Ethylformyl dimethyl ester [134] (62 mg, 1.0×10^{-4} mol) was dissolved in tetrahydrofuran (30 ml), the mixture cooled to 0°, and phenyl magnesium bromide (140 µl, 1.7 M in ether, 2.4×10^{-4} mol) was added. The reaction mixture was stirred for 5 minutes and then poured into iced water (75 ml). The pH was adjusted to 5 and the porphyrins extracted with dichloromethane. The solvent was removed and the products separated by chromatography using a squat

column with 2% acetone in dichloromethane. Two fractions were isolated; starting material [134] (7 mg, 7%) and 3(8)-ethyl-8(3)-(1-hydroxybenzyl)deuteroporphyrin dimethyl ester [138] (60 mg, 90%) M.s. *m/z* (relative intensity); 673 (M+H, 100%). λ_{max} (relative intensity); 408 (100), 499 (18), 537 (13), 568 (9), 622 (5). ^{1}H n.m.r.: δ - 4.34, NH, 2H; 1.73, m, H_3CCH_2, 3H; 3.01 - 3.35, CH_2CO_2, ring methyls, 16H; 3.58, 3.59, 3.60, 3.62, CH_3OC, 6H; 3.72, m, H_3CCH_2, 2H; 4.02, m, $CH_2CH_2CO_2$, 4H; 6.62, 6.66, CHC_6H_5, 1H; 7.16, m, (3H), 7.48, m, (2H), C_6H_5, 5H; 9.51, 9.55, 9.58, 9.62, 9.69, 9.71, 9.73, methine protons, 4H. ^{13}C n.m.r.: δ 11.2, 11.9, ring methyls; 17.5, H_3CCH_2; 19.6, H_3CCH_2; 21.6, $CH_2CH_2CO_2$; 36.8, 36.9, $CH_2CH_2CO_2$; 51.7, OCH_3; 70.0, HCOH; 95.8, 96.0, 96.2, 96.6, 96.7, 99.0 *meso* carbons; 126.4, 126.8, 128.2, C_6H_5; 133-149, pyrrole carbons; 173.5, 173.7, CO_2Me

2. using 3.7 equivalents and a two minute reaction time

Repeated as for part 1(a) using phenyl magnesium bromide (15 µl, 1.7 M in ether, $2.6x10^{-5}$ mol) and stirring for only two minutes prior to work up. Tlc R_f 0.8. M.s. *m/z* (relative intensity); 673 (100%).

Hydrolysis of the methyl esters of [138]

The methyl esters of the hydroxybenzyl dimethyl ester [138] (18mg) were hydrolysed using the procedure outlined for hydrolysis of the methyl esters of the hydroxypropyl porphyrin [137] (part 5.4.3.1) to give 3(8)-ethyl-8(3)-(1-hydroxybenzyl)deuteroporphyrin [139] Tlc R_f 0.0. M.s. *m/z* (relative intensity); 646 (M+H, 100%). λ_{max} (relative intensity); 405 (100), 498 (18.6), 533 (13.2), 568 (9.0), 621 (5.6).

Testing sample;

A sample for testing of hydroxybenzyl porphyrin [139], was prepared as described in 6.1 General experimental. HPLC 10.7 min (34%), 12.0 (48), 24.6 (10%). The sample was inactive.

3(8)-ethyl-8(3)-(1-hydroxypentyl)deuteroporphyrin dimethyl ester [140]

Ethylformyl dimethyl ester [134] (23 mg, 3.9×10^{-4} mol) was dissolved in tetrahydrofuran (10 ml), the mixture cooled to 0°, and *n*-butyl magnesium bromide (85 µl, 1.0 M in ether, 8.5×10^{-4} mol) was added. The reaction mixture was stirred for 5 minutes and then poured into iced water (35 ml). The pH was adjusted to 5 and the porphyrins extracted with dichloromethane. The solvent was removed and the products separated by chromatography using a squat column. Elution with 2% acetone in dichloromethane gave ethylformyl dimethyl ester [134] (5 mg, 21%). Elution with 3% acetone in dichloromethane gave **3(8)-ethyl-8(3)-(1-hydroxypentyl)deuteroporphyrin dimethyl ester** [140] (18 mg, 72%) M.s. *m/z* (relative intensity); 653 (M+H,100%). λ_{max} (relative intensity); 410 (100), 499 (26.8), 533 (18.6), 569 (12.6), 622 (8.1). ^{1}H n.m.r.: δ -4.15, NH, 2H; 0.76, m, $CH_2CH_2CH_3$, 3H; 1.27, m, $CH_2CH_2CH_3$, 4H; 1.81, m, H_3CCH_2, 3H; 2.4, m, $CH_2CH(OH)$; 3.21, m, CH_2CO_2, 4H; 3.35, 3.36, 3.43, 3.45, 3.48, 3.51, ring methyls, 12H; 3.63, 3.65, 3.66, CH_3OC, 6H; 3.96, m, H_3CCH_2, 2H; 4.24, m, $CH_2CH_2CO_2$, 4H; 5.72, m, CHOH, 1H; 9.73, 9.75, 9.79, 9.83, 10.0, 10.1, methine protons, 4H. ^{13}C n.m.r.: δ 11.4, 11.5, 11.7, ring methyls; 13.9, $CH_2CH_2CH_3$; 17.5, H_3CCH_2; 19.7, H_3CCH_2; 21.7, $CH_2CH_2CO_2$; 22.6, $CH_2CH_2CH_3$; 28.5, $CH_2CH_2CH_2CH_3$; 36.8, 36.9, $CH_2CH_2CO_2$; 39.9, $CH(OH)CH_2CH_2$; 51.6, OCH_3; 69.9, CHOH; 95.9, 96.2, 96.3, 96.5, 96.7, 98.5 *meso* carbons; 133-147, pyrrole carbons; 173.5, 173.6, CO_2Me

5.4.4 Grignard reaction on the diformyl ether-linked dimer [141]

The diformyl ether-linked dimer[113] [141] (4.0 mg, $3.3x10^{-6}$ mol) was dissolved in tetrahydrofuran (5 ml), the mixture cooled to 0°, and EtMgBr (10 µl, 1.4 M in ether, $1.4x10^{-5}$ mol) was added. The reaction mixture was stirred for 2 minutes and an aliquot removed, added to iced water and the porphyrins extracted with dichloromethane. Tlc analysis; R_f 0.34 (major component), 0.23, 0.02, 0. M.s. *m/z* (relative intensity); 1263 (100%), 623 (70). Further aliquots were taken at 5 and 10 minutes and the reaction was worked up at 20 minutes; all gave similar tlc analyses to the two minute aliquot but there was a gradual increase in baseline material over the 20 minutes. M.s. (20 minute sample) *m/z* (relative intensity); 1319 (13%), 1291 (19) 1263 (39), 623 (100). *M/z* 1263 is compatible with the diethylated product.

5.5 Grignard reactions using dichloromethane as the solvent

i. using 1 equivalent of EtMgBr

The diacetyl dimethyl ester [83] (4.2 mg, $6.7x10^{-6}$ mol) was dissolved in dichloromethane (5 ml) and the solution cooled to 0°, EtMgBr (10 µl,1.38 M in ether, $1.4x10^{-5}$ mol) was added and the reaction stirred at 0°. Aliquots were taken at 2, 5 and 10 minutes, added to acidified (1 drop ammonium chloride solution) iced water, and the porphyrins extracted with dichloromethane. Tlc (2 minute sample), R_f 0.66 (diacetyl DP.DME), 0.53, 0.32, 0. Tlc analysis at the other times were the same. The 3 samples were combined and shaken with dilute hydrochloric acid for 5 minutes and then washed with dilute aqueous sodium hydroxide and water. Tlc R_f 0.54 (starting material), 0.38, 0.23. M.s. *m/z* (relative intensity); 683 (28%), 681 (40), 653 (100), 623 (84).

ii. using 2 equivalents of EtMgBr and a 2 minute reaction time

The above procedure was repeated using EtMgBr (20 µl,1.38 M in ether, $2.8x10^{-5}$ mol) and a 2 minute reaction time. Tlc analysis was the same as for part (i).

3(8)-Acetyl-8(3)-(1-hydroxy-1-methylpropyl)deuteroporphyrin dimethyl ester [142]

The diacetyl dimethyl ester [83] (103 mg, $1.7x10^{-4}$ mol) was dissolved in dichloromethane (40 ml) and the solution cooled to 0° and EtMgBr (240 µl,1.4 M in ether, $3.4x10^{-4}$ mol) was added and the reaction stirred for 3 minutes and then poured into iced water (75 ml). The pH was adjusted to 5 and the porphyrins extracted with dichloromethane, the combined organic phases were shaken with dilute hydrochloric acid for 5 minutes and then washed with dilute aqueous sodium hydroxide and water. The solvent was removed and the products partially separated by chromatography using a squat column. Elution with solvents ranging from 0-2% methanol gave poor separation into 8 fractions. The first three fractions which contained mainly the diacetyl dimethyl ester [83] and the other main product, by mass spectral analysis, were chromatographed on silica using 2% acetone in dichloromethane to give 8 fractions. The final two fractions contained mixtures of the major product and starting material, these were combined and chromatographed on an alumina column. Elution with10% acetone in dichloromethane gave diacetyl dimethyl ester [83] (20 mg, 19%). Elution with 10-12% acetone in dichloromethane gave **3(8)-acetyl-8(3)-(1-hydroxy-1-methylpropyl)deuteroporphyrin dimethyl ester** [142] (38 mg, 34%). M.s. *m/z* (relative intensity); 653 (M+H, 100%). λ_{max} (relative intensity); 412 (100), 510 (10), 533 (8), 569 (6), 622 (2). ^{1}H n.m.r.: δ - 4.46, -4.37, NH, 2H; 0.65, t, J=7.2Hz, 0.77, t, J=7.2 Hz, CH_2CH_3, 3H; 1.80, 1.93, $HOCCH_3$, 3H; 2.18, 2.35, m, H_3CCH_2COH, 2H; 3.06-3.57, m, CH_2CO_2, ring methyls, $COCH_3$ 19H; 3.61, 3.62, 3.66, 3.68, CH_3OCO, 6H; 4.10, m, $CH_2CH_2CO_2$,

4H; 9.50, 9.51, 9.61, 10.42, 10.48, 10.50, 10.52, methine protons, 4H. ^{13}C n.m.r.: δ 8.7, CH_2CH_3, 11.0, 11.3, 11.7, 13.5, 13.7, 14.0, 14.1, ring methyls; 21.4, 21.7, $CH_2CH_2CO_2$; 30.8, 31.1, CH_3COH; 33.0, $H_3CC{=}O$; 36.5, 36.8, $CH_2CH_2CO_2$; 38.5, $HOCCH_2CH_3$; 51.7, OCH_3; 95.1, 95.4, 96.0, 97.1, 100.6, 102.6, *meso* carbons; 132-152, pyrrole carbons; 173.3, 173.5, CO_2Me

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