The Synthesis of Haematoporphyrin Derivative III and other Novel Porphyrins

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Statement

I declare that this thesis is based on original work done while I was enrolled as a Ph.D. candidate in the Department of Organic Chemistry at the University of Adelaide. To the best of my knowledge and belief this thesis does not contain any material from previous studies conducted at this or any other University, except where due reference is cited.

Sek Sau Yin

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CONTENTS

CHAPTER 3

CHAPTER 4

ABSTRACT

The Synthesis of Haematoporphyrin Derivative III and other Novel Porphyrins

This thesis is divided into four chapters. Chapter 1, which provides an introduction to the work in Chapters 2 and 3, contains a brief account of haematoporphyrin derivative (HPD-IX), an anticancer drug which is currently undergoing final clinical trials in the United States for use in the photodynamic therapy of endobronchial, esophageal and bladder tumours.

Chapter 2 describes a study of the relationship between the regiochemistry of the haematoporphyrins and the biological activity of the material (HPD) derived from them. This involved first, the total synthesis of haematoporphyrin III by two literature routes, both via the copper-mediated cyclization of biladiene-*ac* salts, and then the preparation of haematoporphyrin derivative-III (HPD-III) by standard procedures, using haematoporphyrin III. Investigations of the literature steps in the the total syntheses resulted in significant improvements in the yields in some cases. HPD-III was found (by HPLC, FAB m.s., NMR, visible spectroscopy) to be similar to HPD-IX in that HPD-III contained monomers as well as ether-, ester- and carbon-linked dimers and oligomers. However the proportion of dimeric/oligomeric material was significantly lower in HPD-III than in HPD-IX. In addition HPD-III was less soluble in water and less biologically active than HPD-IX. Reasons for these differences are discussed.

Chapter 3 describes the synthesis of four symmetrical amino-linked diporphyrin dimers with diacetyl-, diethyl-, divinyl and di(1-hydroxyethyl) terminal groups. Some of these dimers are amino analogues of the ether-linked material claimed to be in HPD-IX. Optimization studies for the hydrobromination (using saturated hydrobromic acid/dichloromethane) of ethyl- and acetyl-substituted vinyl-containing porphyrins, and the partial hydrobromination of protoporphyrin dimethyl ester, were undertaken. The amino-linked dimers were characterized by NMR, u.v./vis. absorption spectroscopy and FAB m.s. Tests of *in vivo* anticancer activity indicated that the amino-linked dimers were less active than the corresponding ether-linked dimers. A possible relationship between the nature of the linking group and the biological activity of the dimers was proposed.

The hydrobromination methodology established in Chapter 3 was used for a preliminary study of the synthesis of diporphyrin dimers with long linking groups. Compounds of this type are potential DNA intercalators. Chapter 4 details the synthesis and characterization of diporphyrin dimers linked by propane-1,3-diether, pentane-1,5-diether, decane-1,10-diether and propane-1,3-diamino bridges, as well as monomeric precursors to dimers with amine and amide-containing bridges. The synthesis of a dimer linked by a bridge which contained ether and ester groups was achieved but attempts to prepare and characterize dimers with amine- and amide-containing bridges were hampered by difficulties in obtaining informative FAB mass spectra.

ABBREVIATIONS

General

DMF	N,N-dimethylformamide
FAB	fast atom bombardment
HBr	hydrogen bromide
MIKE	mass analysed ion kinetic energy
NMR	nuclear magnetic resonance
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
R_t	retention time

Porphyrins

HP, HP_{IX}	haematoporphyrin IX
HPdme, HP_{IX}dme	haematoporphyrin IX dimethyl ester
HP_{III}	haematoporphyrin III
HP_{III}dme	haematoporphyrin III dimethyl ester
HV, HV_{IX}	3-(1-hydroxyethyl)-8-vinyldeutero-porphyrin IX
HVdme, HV_{IX}dme	3-(1-hydroxyethyl)-8-vinyldeutero-porphyrin IX dimethyl ester
HV_{III}	3-(1-hydroxyethyl)-7-vinyldeutero-porphyrin III
HV_{III}dme	3-(1-hydroxyethyl)-7-vinyldeutero-porphyrin III dimethyl ester

PP, PP_{IX}	protoporphyrin IX
PPdme, PP_{IX}dme	protoporphyrin IX dimethyl ester
PP_{III}	protoporphyrin III
PP_{III}dme	protoporphyrin III dimethyl ester
HPD, HPD-IX	haematoporphyrin derivative IX
HPD-III	haematoporphyrin derivative III

CHAPTER 1

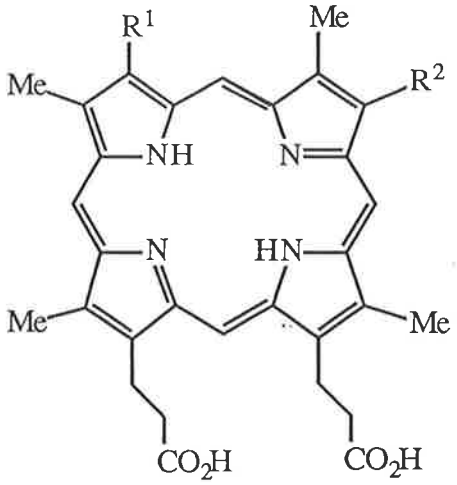
1.1 General Introduction§

The use of porphyrins in cancer therapy dates back to the early 1940's. Initially, impure samples of haematoporphyrin (1) were used for the visualization and delineation[2-5] of tumour tissue because of the preferential accumulation of the porphyrin material in tumour tissue[1,2] and the ability of the porphyrin to fluoresce intensely in the cell. Lipson *et. al.*[6] showed that a material (later termed "haematoporphyrin derivative") derived from haematoporphyrin (1) was a more effective localizer than the impure haematoporphyrin, and he developed an improved method[6-9] for the detection of malignant tissue, which involved the use of this derivative and a light source.

Later,[10,11] it was found that this combination of the haematoporphyrin derivative and light could cause the destruction of human bladder carcinoma. This eventually led to the use of haematoporphyrin derivative in the treatment of cancer by the procedure known as photodynamic therapy (PDT),[14,15] which involves the use of a photosensitizer and light to bring about cell damage. PDT using the haematoporphyrin derivative is currently being subjected to final clinical trials in the United States. The treatment [12,13] involves the injection of a dose of the haematoporphyrin derivative, followed by an

§ References, p. 13.

incubation period of up to three days, then the irradiation of the tumour site with light at the wavelength of 630 nm. Encouraging results have been reported[14,15] on the photodynamic therapy using the haematoporphyrin derivative on a variety of tumours. It is believed[16a] that singlet oxygen, produced by the interaction of ground state oxygen with triplet state photosensitizer (the latter a product of the excitation of the porphyrin by light) is responsible for the damage to the tumour cells.[16b]



(1) $R^1 = R^2 = CH(CH_3)OH$

(2) $R^1 = CH(CH_3)OCOCH_3$, $R^2 = CH(CH_3)OH$

(3) $R^1 = R^2 = CH(CH_3)OCOCH_3$

(4) $R^1 = CH(CH_3)OH$, $R^2 = CH{=}CH_2$

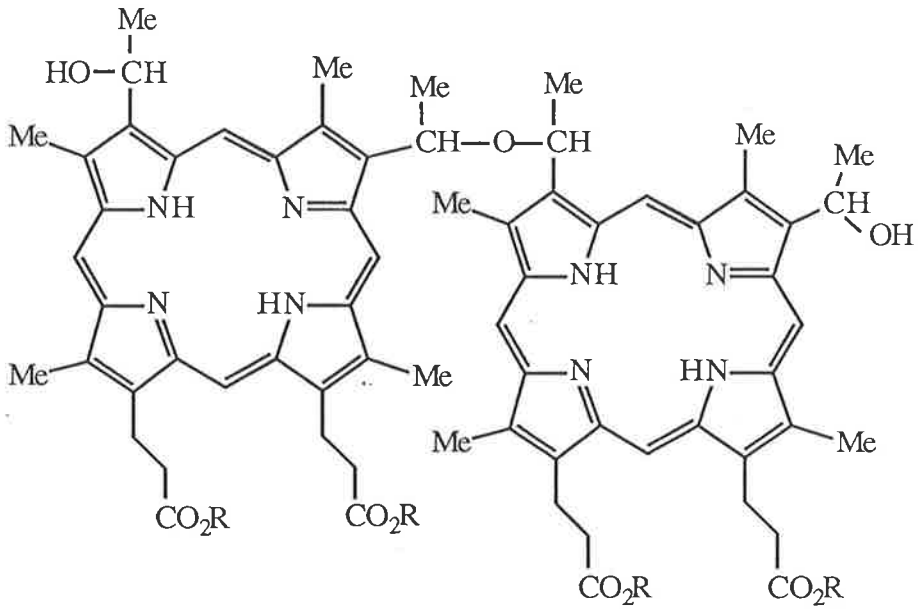
In the Lipson procedure[6,17] for the preparation of haematoporphyrin derivative, haematoporphyrin (1) is first

treated with a mixture of concentrated sulphuric acid and acetic acid (1:19), and neutralised with aqueous sodium acetate. The resulting precipitate, which consists mainly of the mono- (2) and diacetate (3) of haematoporphyrin,[18,19,20] is then dissolved in aqueous sodium hydroxide for one hour, neutralised and diluted with saline. The final isotonic solution is referred to as haematoporphyrin derivative (HPD).

Initial reverse-phase HPLC analysis of HPD[21-24] showed the presence of haematoporphyrin (1), 3-(1-hydroxyethyl)-8-vinyldeuteroporphyrin (4), small amounts of protoporphyrin (7b) and a significant amount of poorly resolved, less polar material which was of an unknown structure. Berenbaum *et. al.*[25,26] fractionated HPD by reverse-phase HPLC and showed that the active material was the poorly resolved fraction that was relatively strongly retained by the column. Separation of the active fraction from the other materials in HPD can also be achieved by size exclusion methods using Bio-Gel P-10[21] or Sephadex LH-20.[27,28] Photofrin II,®[29] the commercially available active fraction, is obtained by an alternative size separation procedure using a porous membrane system[29].

Dougherty *et al.*[30] proposed that the active material was a mixture of regio- and diastereomers of dihaematoporphyrin ether (DHE) (5). This was on the basis of hydrolysis studies on the active material where it was found that the active material was stable towards basic hydrolysis but was cleaved to monomers under acidic conditions. It was reported[30]

that FAB mass spectra of the active material and ^{1}H and ^{13}C NMR data of the corresponding methyl esterified material all supported this structure.[30] Subsequently, the acronym DHE was used to refer to the active material until later studies (see below) showed that this was an inaccurate description of the material. Recently Kessel[31] recommended that DHE no longer be used as the acronym for the active material.



(5) R = H

(9) R = Me

Berenbaum *et al.*[25,26] proposed a dimer or oligomer structure for the active material and suggested either an ether or a carbon bridge on the grounds of the stability of this material towards basic hydrolysis conditions. It was shown by size-exclusion chromatography,[28,32-38] that the active fraction could

be further divided into smaller fractions which have different *in vivo* activity and localizing ability, suggesting that there may be more than one active component in the biologically active fraction. The mass spectrum of the active fraction obtained by Dougherty *et al.*[30] showed the presence of trimeric material as well as dimeric material. Molecular weight determinations on the aqueous solution of HPD and various size separated fractions using ultracentrifugation techniques indicated[39] an oligomeric structure for the active material of HPD. This oligomeric material resembled the stable high molecular weight aggregate that protoporphyrin (7b) also forms[39] in water in that it was stable to ultracentrifugation conditions. However, unlike the protoporphyrin aggregate, this oligomeric material did not revert to a monomeric state upon the addition of organic solvents to the solution,[40] indicating that it was covalently linked. Moan *et. al.*[41] also arrived at the same conclusion from similar studies. The behaviour of the active fraction on Sephadex LH-20 was also consistent with a covalently linked system.[39] FAB mass spectrometry on HPD and the active fraction showed sets of peaks corresponding to various dimers, trimers and tetramers.[19,27] It is difficult to analyze the oligomeric acids by FAB mass spectrometry because of their low volatility under the analysis conditions, and it is assumed[42] that larger oligomers are also present in the active fraction. Esterified material from the active fraction showed peaks up to and including the hexamer region.[19]

The nature of the functional group linking the individual porphyrins units was also the subject of some debate. Initially it was thought that the oligomers were ether-[30] and/or ester-linked.[19,27,32,45-48] Recently, oligomers linked by a three carbon bridge were obtained from HPD and Photofrin II.®[43]

Spectroscopic techniques could in principle be used to distinguish between the ester and ether linkages. However a Fourier Transform infrared study[44] did not support either an ester or an ether linkage. Another infrared study[45] indicated that the active fraction from HPD contained both ester and ether linkages with ester linkages predominating. NMR spectroscopy has not as yet been successfully applied to distinguish between the two functional groups because of the difficulties in obtaining adequately resolved spectra of the active fraction due to its oligomeric nature. For ^{1}H NMR spectra in D_2O, the situation is further complicated by the difficulty in obtaining samples which are completely free of water. Hence most of the signals, particularly those in the 3-4 ppm region, are usually obscured by the signals due to water. However, these problems were alleviated[30] by converting the active fraction into the corresponding methyl ester and recording the spectra in deuterated chloroform. FAB mass spectrometry alone, without prior chemical manipulation of the material, cannot be used to distinguish between isomeric ester and ether linked materials because both species give the same molecular ions, and at present, there is not enough information on relative

fragmentation patterns for this technique to be successfully used for differentiating between the two species.

Chemical degradation has been used with some success to determine the nature of the linking group. Alkyl porphyrin esters are cleaved by acidic and basic hydrolysis conditions[19,27] and are readily reduced by lithium aluminium hydride.[46,47] However alkyl porphyrin ethers are cleaved by acidic hydrolysis conditions only[19,27] and are unaffected by lithium aluminium hydride reduction.[46,47] Carbon-linked oligomers are stable to all three conditions.[43]

Acidic hydrolysis[19,27] of HPD and Photofrin II® provides good yields of monomeric porphyrin products, indicating ether- and ester-linked starting materials. Ward *et. al.*[19] found that their active fraction of HPD showed substantial, but not total, stability to alkaline hydrolysis conditions, suggesting a predominance of ether linkages between the porphyrins. However Kessel's active fraction was extensively hydrolysed with base[27,32] and reduced to monomer products with lithium aluminium hydride[46,47] showing that his material was substantially ester linked. Dougherty[48] and Truscott[45] concluded that their active material was linked by both ether and ester groups on the basis of hydrolysis studies. These conflicting results may be partly due to variations in the preparation of the active fraction. Kessel[27,32] prepared his HPD by Bonnett's[20] procedure which involves the use of acetic anhydride and pyridine in the acetylation step. This modification of the original

Lipson procedure[6,17] forms mainly the haematoporphyrin diacetate in the first step of the reaction. Dougherty[48] and Ward *et. al.*[19] followed the Lipson procedure[6,17] which produces a mixture of monoacetates and diacetate in a 1:2 ratio. (The subsequent step of both methods are essentially the same.) However, freshly prepared active fractions from both methods show[43] similar activity *in vivo*. In addition, ester-linked oligomers gradually convert[19,32] to ether-linked products on standing at pH 7 over 48 hours, without affecting the overall tumour-localizing ability of the active fraction. These results[19,32] suggest that the nature of the linkage between the porphyrins may not be of critical importance in determining biological activity. Presumably any ester linked material would be less stable *in vivo* because of its susceptibility towards hydrolysis by esterases.

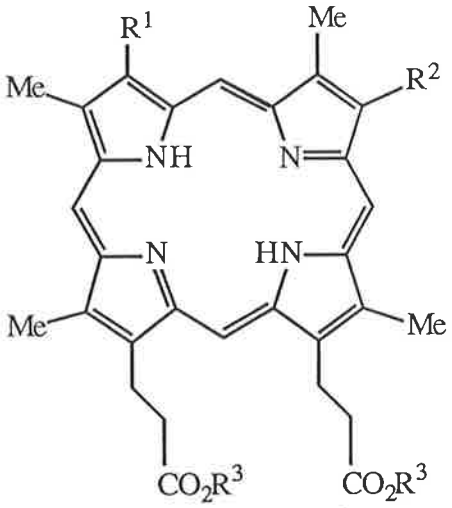
Recently Ward *et. al.*[43] isolated other oligomeric material from HPD and Photofrin II,® which was stable towards both acidic and basic hydrolysis conditions, indicating that the material was not linked by ether or ester bridges. Treatment of the corresponding esterified or fully alkylated[43] product of this material with benzoyl chloride in dimethylformamide produced a derivative from which a new porphyrin dimer and trimer were isolated.[43] NMR and FAB mass spectral studies on the dimer showed that the porphyrin units are linked by a carbon chain.[43] This structure is different from the carbon linked dimer structure that was proposed by Berenbaum *et al.*[25,26]

In order to resolve the apparently conflicting data on the structure of the active fraction of HPD, the synthesis of ester and ether linked porphyrin dimers and oligomers was undertaken by several groups.[27,42,49-54] Two approaches have been used: the incorporation of other porphyrin monomers into the HPD preparation procedure, and more extensively, the unambiguous synthesis of ether and ester linked porphyrin dimers and trimers as model compounds.

Kessel *et al.*[27,49] 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.

Scourides *et al.*[51] prepared ether linked porphyrin materials by firstly reacting haematoporphyrin dimethyl ester (6) or protoporphyrin dimethyl ester (7a) with hydrogen bromide in acetic acid to form the dibromoethyl porphyrin (8). This intermediate product was allowed to react with either haematoporphyrin dimethyl ester (6), or with small amounts of water which produced sufficient haematoporphyrin dimethyl ester *in situ* to react with the remaining bromoethyl compound, to give ether-linked, methyl ester-containing porphyrin material.[51] Each of these products was hydrolysed using basic conditions to form the corresponding acids which were separated into fast running fractions by gel filtration chromatography. All

of these fractions showed similar properties *in vivo* to the active fraction of HPD.[51] Since these products were, by their method of synthesis, ether linked porphyrins, it was concluded that the results provided strong support for ether linkages in HPD itself.



(6) $R^1 = R^2 = CH(CH_3)OH$, $R^3 = CH_3$

(7a) $R^1 = R^2 = CH{=}CH_2$, $R^3 = CH_3$
(7b) $R^1 = R^2 = CH{=}CH_2$, $R^3 = H$

(8) $R^1 = R^2 = CH(CH_3)Br$, $R^3 = CH_3$

Ward *et. al.*[42,50] have also reacted haematoporphyrin dimethyl ester (6) and dihaematoporphyrin ether tetramethyl ester (9) with hydrogen bromide in dichloromethane to form ether-linked oligomers. FAB mass spectral analysis of the products showed peaks up to and including the nonamer region, which was the upper detection limit of their mass spectrometer

for the esterified oligomers and not necessarily the maximum molecular weight of the oligomeric material formed.[42,50]

The synthesis of ether linked porphyrin dimers was recently reported by two groups.[50,55,56] Although the methodology differed slightly, essentially the same chemistry was used in each case. Both groups[50,55] prepared dihaematoporphyrin ether (DHE) (5) and were able to conclude from HPLC comparisons that dihaematoporphyrin ether was unlikely to be present in HPD or Photofrin II® in other than trace amounts. Ward *et. al*[50] have also prepared a range of ether linked dimers and showed that the HPLC peaks of the more hydrophobic ether linked dimers, particularly the divinyl terminated dimer, had similar retention times to those of the active fraction of HPD and Photofrin II.® A vinyl terminated trimer[50] was also found to have an HPLC retention time that is very similar to that of the active fraction of HPD. However, it is not clear whether these more hydrophobic porphyrin systems are present in HPD and Photofrin II® or not. As expected, the ether linked porphyrin dimers are stable to lithium aluminium hydride and basic hydrolysis conditions but are readily cleaved by acidic hydrolysis conditions.[42]

Both groups that have synthesised DHE reported[50,53] that it was inactive *in vivo*. However Dougherty noted[53] that if this material was irradiated soon after injection, rather than waiting twenty four hours it was quite active. Ward *et. al.*[50] observed that purified DHE is inactive but the by-products

obtained from the base hydrolysis of the precursor tetramethyl ester are quite active. DHE is unstable in aqueous solution and on standing it slowly forms a more hydrophobic (by HPLC), higher molecular weight material which is biologically active.[42]

The ester-linked dimer of hematoporphyrin has also been synthesised.[42,52,54] The HPLC retention times of this ester dimer indicated that it was unlikely to be a significant component of HPD.

Other synthetic dimers[42,50,56] vary from inactive to as active as Photofrin II® when tested *in vivo*. The activity appears to correlate with the hydrophobicity of the terminal side chains of the dimers, with the more hydrophobic materials (e.g. the dimer with vinyl side chains) being more active.[50]

It is now generally accepted on the basis of the results of the hydrolysis studies and synthetic work, that Photofrin II® and the active fraction of HPD consist of a mixture of dimers and oligomers which are linked by ether, ester and carbon bridges and/or mixtures of these groups.[57]

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CHAPTER 2

2.1 Introduction[§]

It has been shown (see Chapter 1) that the active fraction of HPD consists of a mixture of ether-, ester- and carbon-linked dimers and oligomers. Each of these dimers and oligomers exist as a mixture of diastereomers and regioisomers. This is because the functional groups that are involved in the linkages between the porphyrins are on chiral carbons and are situated on non-equivalent positions of the porphyrin ring. To date, most studies (see Chapter 1) on the active fraction of HPD have centred on the identification of the functional group(s) in these linkages, but very little work has been done to investigate the relationship between regiochemistry and the biological activity of the active fraction. Recently, Pandey *et. al.*[39] synthesized two regioisomers of a carbon-linked porphyrin dimer and noted that one regioisomer was more cytotoxic than the other, suggesting that biological activity might be influenced by the regiochemistry of the dimers.

In order to address this question in relation to HPD, the synthesis of an analogous derivative using a different regioisomer of haematoporphyrin was undertaken. In principle

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§ References, p. 98.

there are fifteen possible regioisomers of haematoporphyrin (see Table 1, Appendix), although if one were to neglect the restrictions imposed by the biogenesis of the porphyrin, the four methyl, two propionic acid and two hydroxyethyl groups in haematoporphyrin can be arranged in more than fifteen ways.[1] It was decided to limit the investigation initially to the isomers of haematoporphyrin whose propionic acid groups are on adjacent pyrrole subunits, in particular, at the 13 and 17 positions of the porphyrin ring (Fig. 2.0) and it was a choice between haematoporphyrin III (10) and haematoporphyrin XIII (11). Due to the availability of starting pyrroles from previous work[19] and numerous precedents in the literature[12,14,20,22,32] on the total synthesis of related porphyrins, it was decided to start with haematoporphyrin III (10). This isomer (10) differs from the natural haematoporphyrin IX (1) that is used in the standard preparation of HPD, in the position of its hydroxyethyl groups: in haematoporphyrin IX (1), these groups are on the 3 and 8 positions of the porphyrin ring (Fig. 2.0) while in haematoporphyrin III (10), they are on the 3 and 7 positions* . As the 3 and 7 positions are equivalent (because of the plane of symmetry bisecting the molecule), the derivatization of haematoporphyrin III (10) will also produce fewer regioisomers than haematoporphyrin IX (1). This may result in less complicated, better resolved NMR spectra of the active fraction. One of the problems encountered in the analysis of the active

* The semisystematic IUPAC nomenclature[49] is used throughout this thesis but in certain instances (where it is more convenient) the Fischer nomenclature is used as well. An example of the latter is the "type" system for the regioisomers of the porphyrins. See Appendix for details.

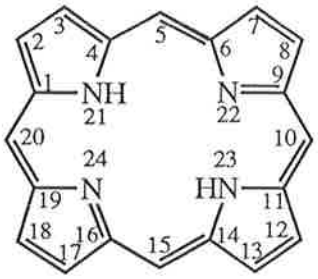
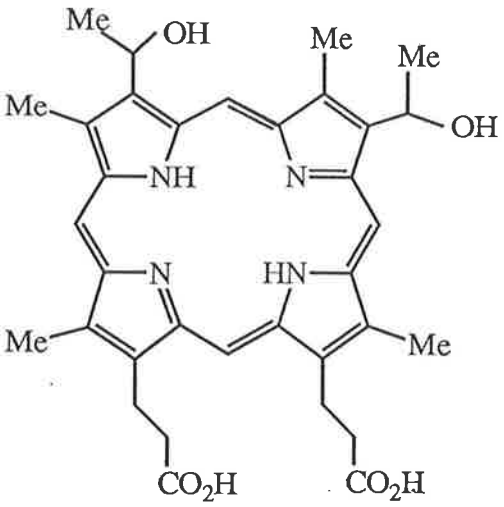
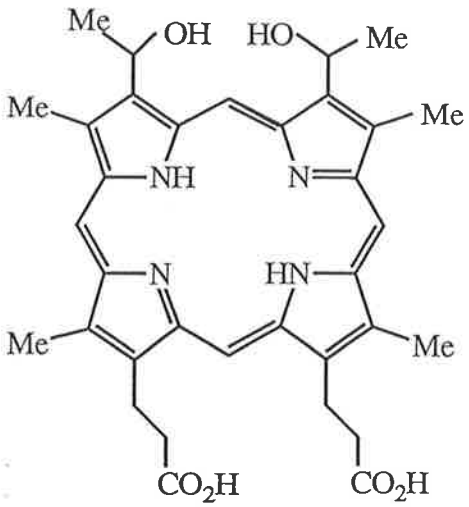


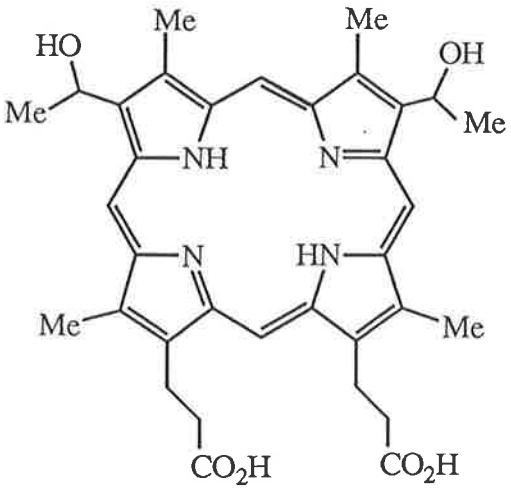
Fig. 2.0 Porporphyrin - IUPAC numbering scheme



(1)



(10)



(11)

fraction of HPD is the poor resolution of the NMR spectra in D_2O due to the fact that the material is a mixture of regio- and diastereoisomers. Haematoporphyrin derivative-III (HPD-III), which is the derivative that is to be prepared from haematoporphyrin III by the Lipson procedure[2] (see Chapter 1), might be a useful model for NMR studies because it will have fewer regioisomers than HPD.

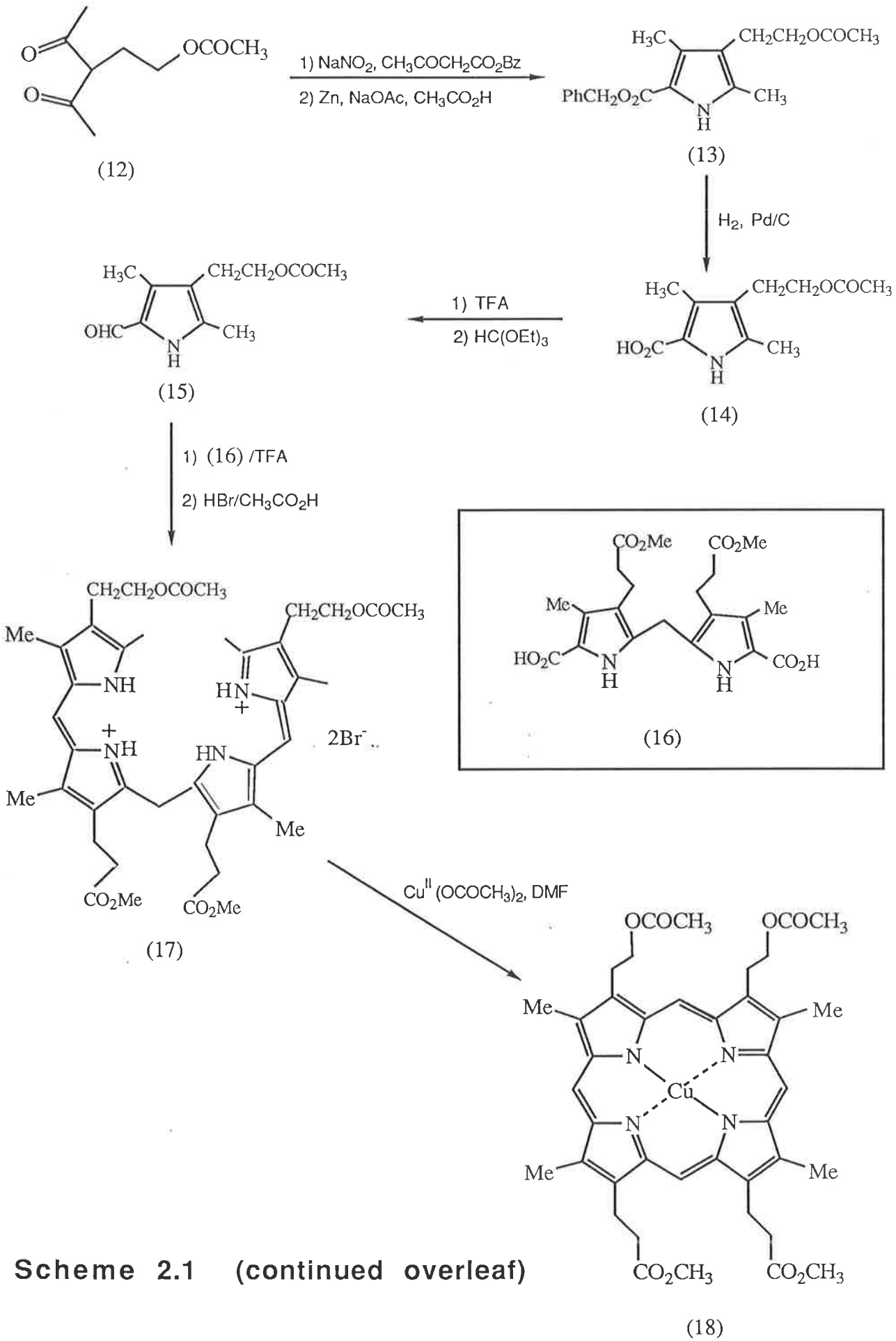
There are a number of general approaches to the total synthesis of porphyrins.[3,4] Polymerization of monopyrroles[3a,4b] is suitable for making porphyrins with identical substituents on all the β positions of the component pyrroles. The condensation of dipyrrolic intermediates[3a,4c] is also somewhat limited in its applicability because of symmetry restrictions, hence it is only useful in the synthesis of porphyrins which possess symmetry in one or both halves of the molecule. The most general approach to the total synthesis of porphyrins involves the use of open chain tetrapyrrolic systems such as bilanes,[3a] oxobilanes,[4a] bilenes,[4a] biladienes[3a,4a] and bilatrienes[3a] (see Appendix for Nomenclature). Unsymmetrically substituted porphyrins can be synthesized by this method because the individual pyrrole units can be linked in a stepwise synthesis, culminating in the cyclization of the tetrapyrrole to give the desired porphyrin.

The method that was selected for the synthesis of haematoporphyrin III involves the cyclization of an biladiene-*ac* salt in the presence of a copper(II) salt and N,N-dimethyl formamide. It has been proposed[5] that the cyclization proceeds

via a bilatriene-*abc* which is formed in the initial oxidation of the biladiene-*ac*. The bilatriene then undergoes ring closure by a radical mechanism. The copper functions as an oxidizing as well as a chelating agent in the reaction.[5] The bridging carbon is derived from one of the terminal methyl groups while the other one is extruded. Similar studies[6] on bilenes have shown that the methyl group is lost during the final aromatization step.

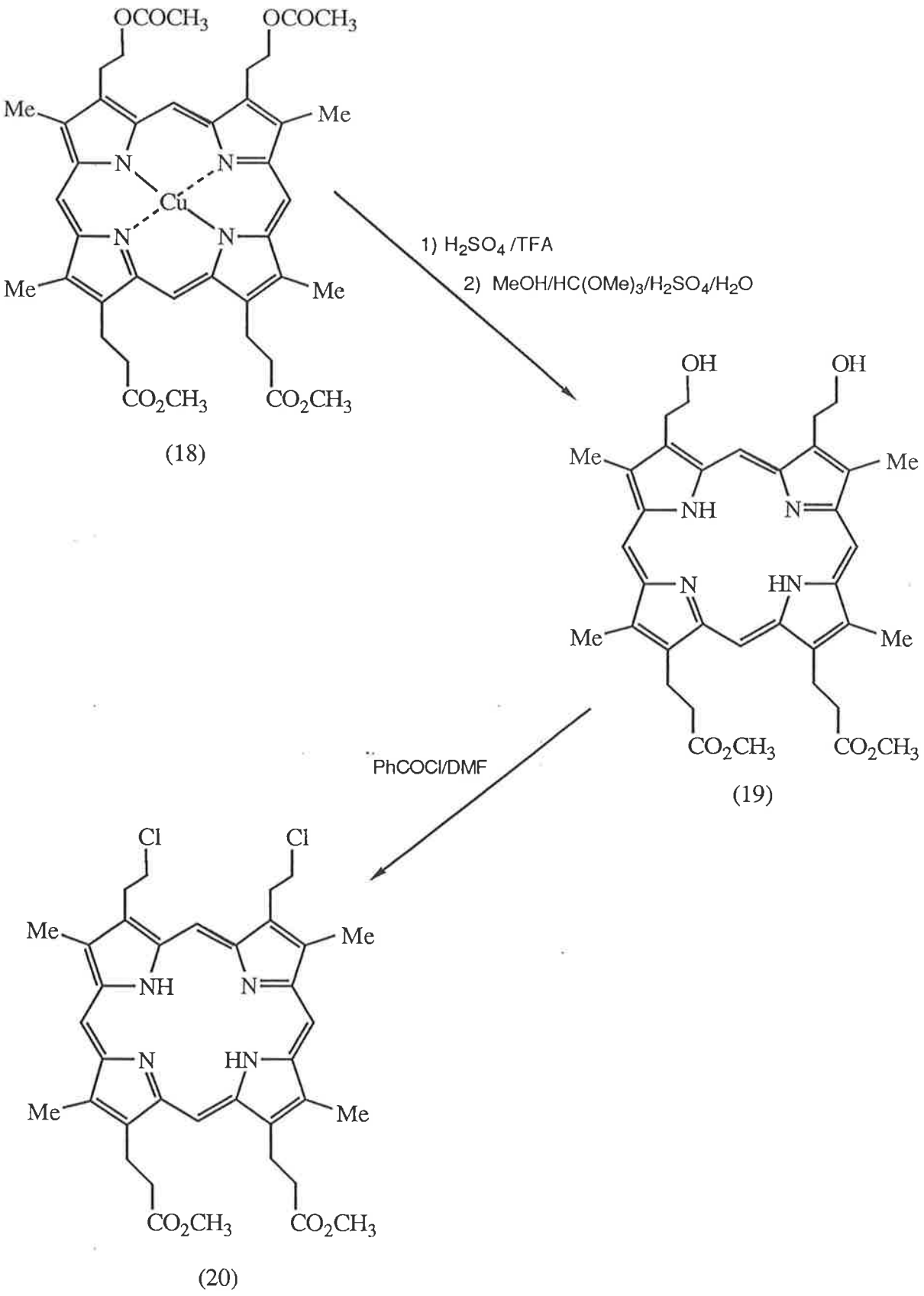
The proposed routes are shown in Schemes 2.1 and 2.2. In Scheme 2.1, protoporphyrin III dimethyl ester (21a) is synthesized by literature methods[7-15] and then converted to haematoporphyrin III dimethyl ester (22) by hydrobromination of the vinyl groups and subsequent hydrolysis of the dibromide. Similarly, Scheme 2.2 outlines the total synthesis of 3,7-diacetyldeuteroporphyrin III dimethyl ester (32),[17-20] which is then treated with sodium borohydride,[22,50] resulting in the reduction of the acetyl groups to the corresponding hydroxyethyl moieties of haematoporphyrin III dimethyl ester (22). It was anticipated[20] that the diacetyl-containing biladiene-*ac* salt (30) may not readily undergo cyclization because of the electron withdrawing effect of the acetyl groups, so yields would be expected to be lower than the 20-40%[3a,4a] usually obtained from copper-mediated cyclizations of non-deactivated biladiene-*ac* salts. Clezy[20] reported a low (6%) yield from the cyclization of the biladiene-*ac* (30) in pyridine-methanol. A Russian group[28] obtained 3,7-diacetyldeuteroporphyrin III dimethyl ester (32) in 18% yield by oxidative cyclization of biladiene-*ac* (30) using methanol/acetic acid as the solvent. However significant

proportions of porphyrin by-products were also produced in the reaction.[28] Due to the symmetrical nature of the target 3,7-diacetyldeuteroporphyrin III dimethyl ester (32), the MacDonald procedure[18] involving the condensation of dipyrrylmethanes would have been a viable alternative. Nevertheless we chose to use the biladiene-*ac* route because of the availability of starting material from previous work.[19]



(12) (13) (14) (15) (16) (17) (18)

Scheme 2.1 (continued overleaf)

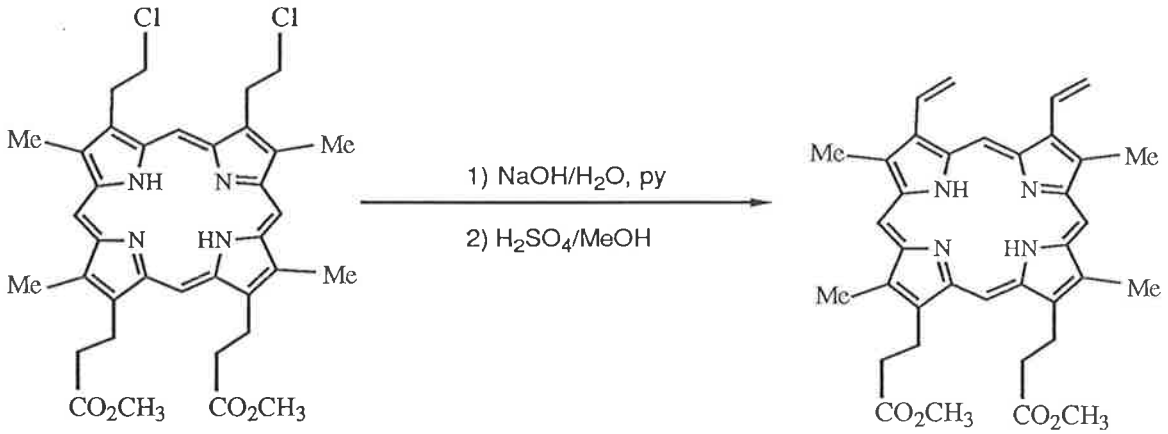


(18)

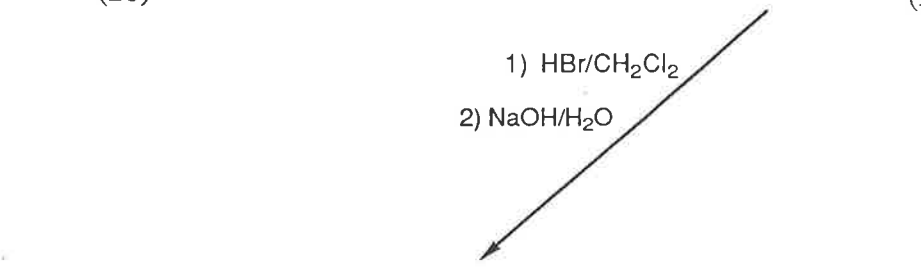
(19)

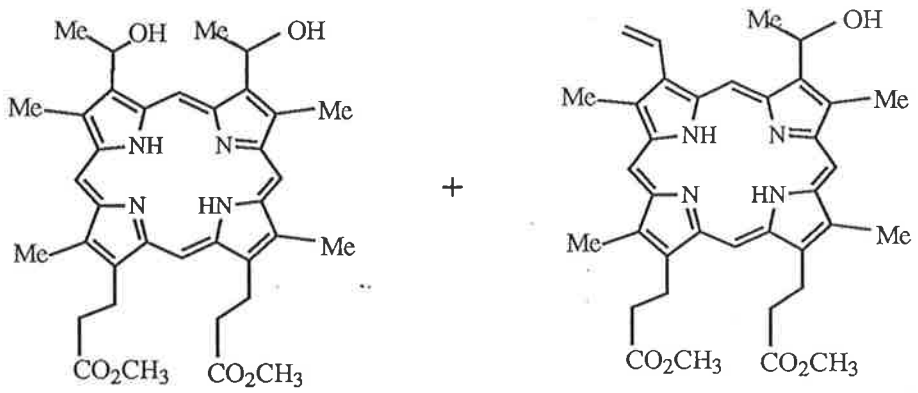
(20)

Scheme 2.1 (continued overleaf)

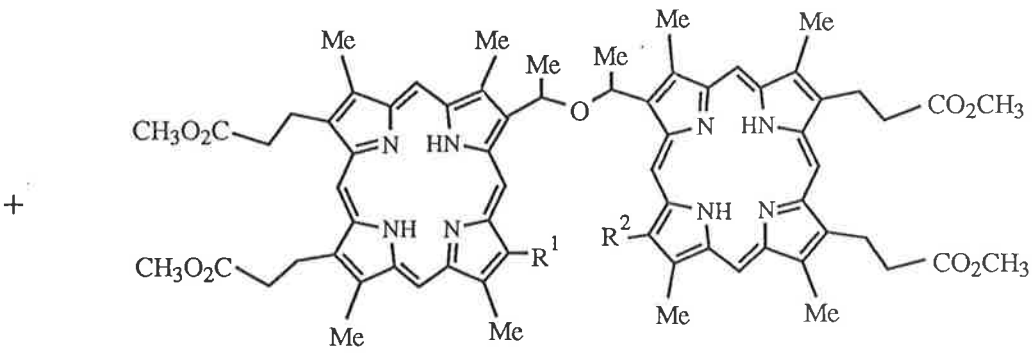


(20) (21a)



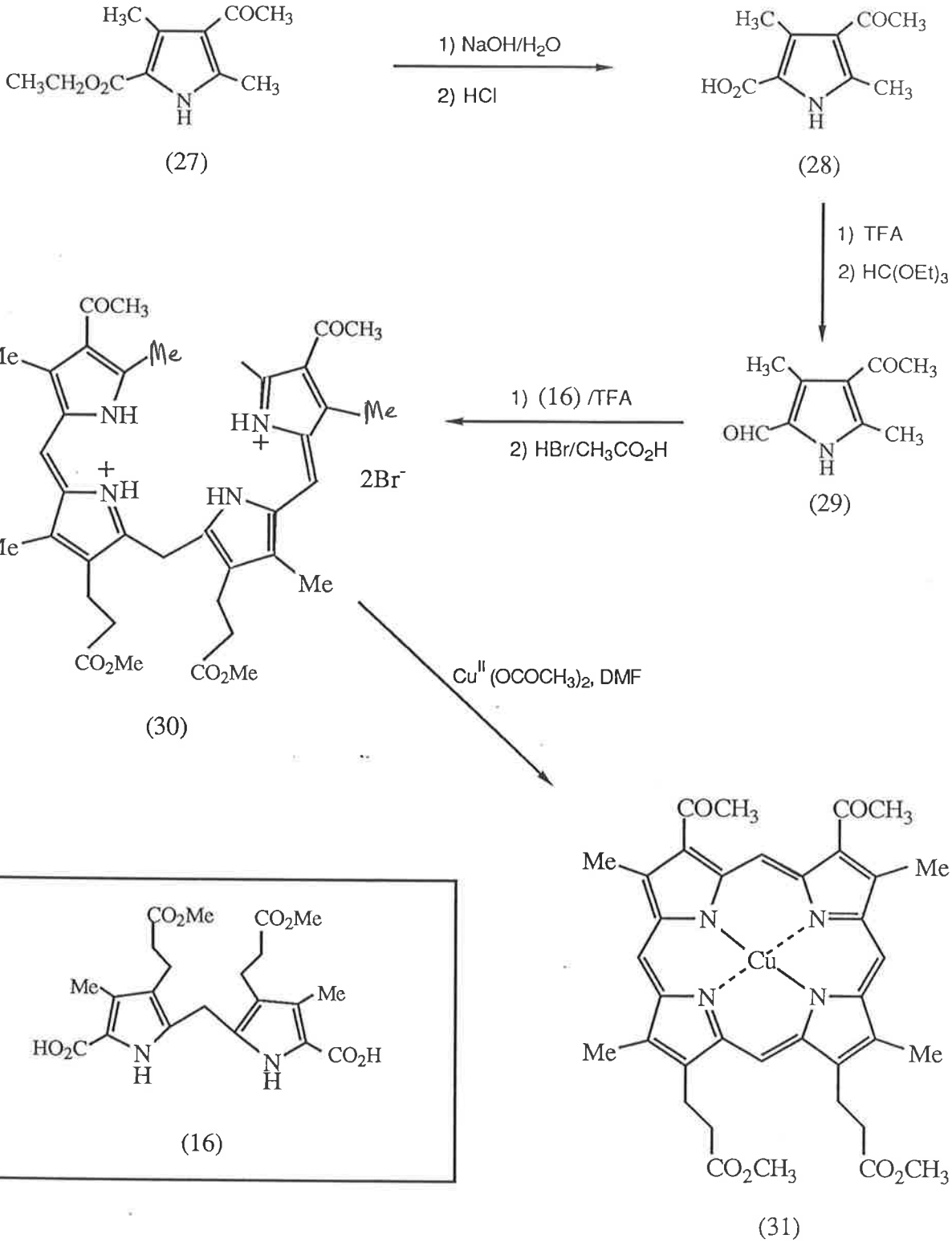


(22) (23a)



(24) $R^1 = R^2 = CH(CH_3)OH$
(25) $R^1 = R^2 = CH{=}CH_2$
(26) $R^1 = CH(CH_3)OH$, $R^2 = CH{=}CH_2$

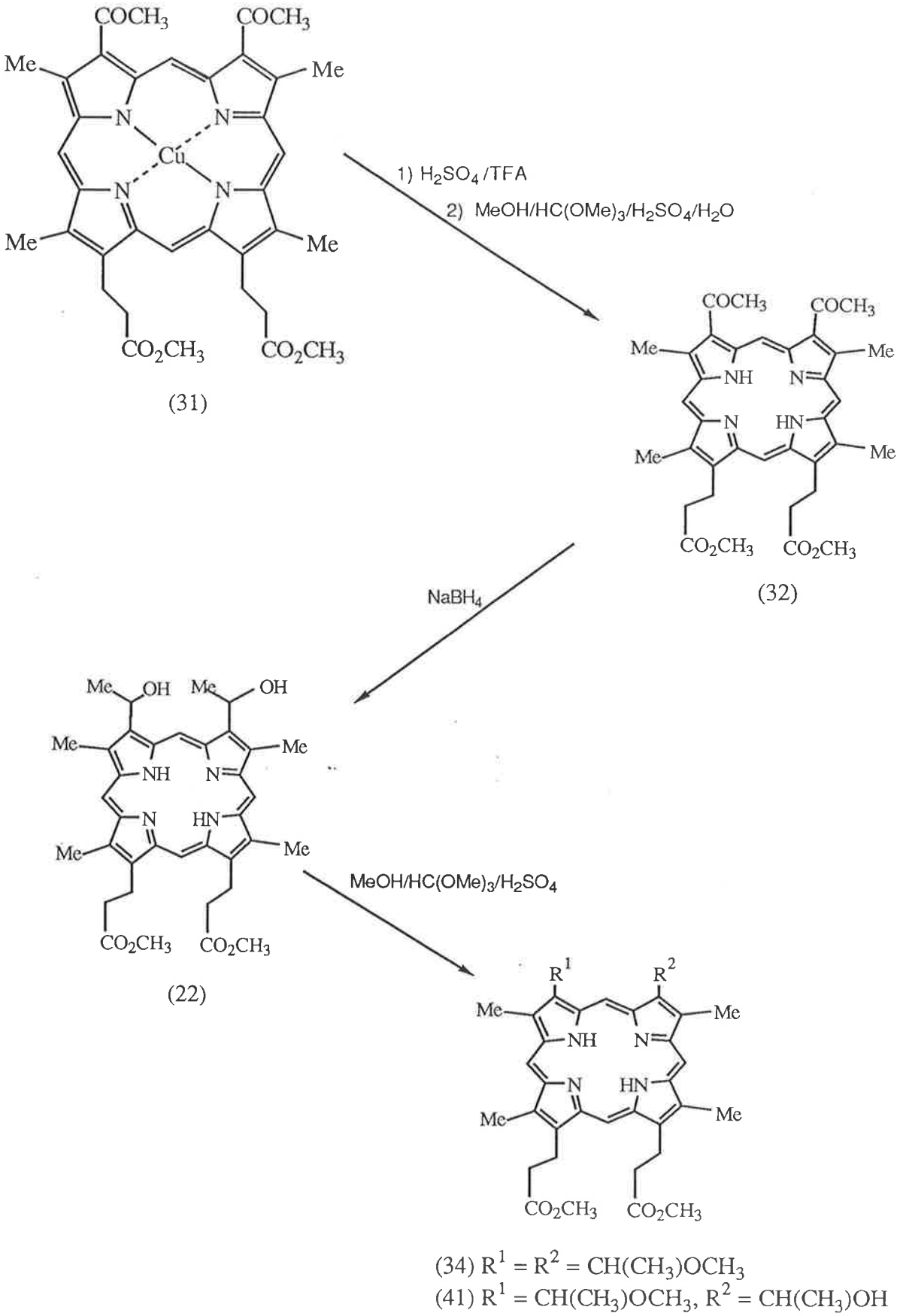
Scheme 2.1 (continued)



(27) (28) (29) (30) (31)

(16)

Scheme 2.2 (continued overleaf)



(31)

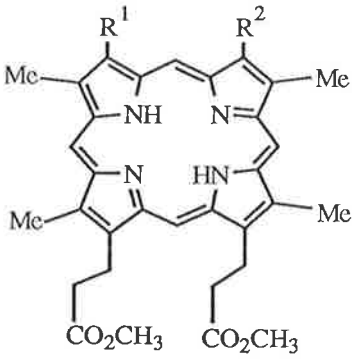
(32)

(22)

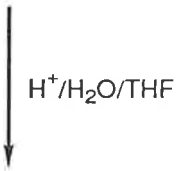
(34) $R^1 = R^2 = CH(CH_3)OCH_3$

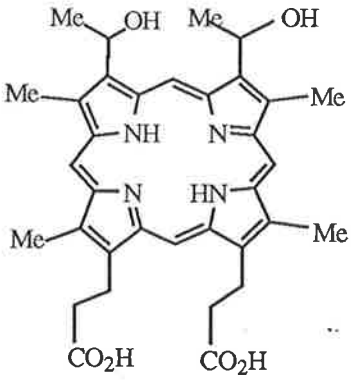
(41) $R^1 = CH(CH_3)OCH_3$, $R^2 = CH(CH_3)OH$

Scheme 2.2 (continued overleaf)



(34) $R^1 = R^2 = CH(CH_3)OCH_3$
(41) $R^1 = CH(CH_3)OCH_3$, $R^2 = CH(CH_3)OH$





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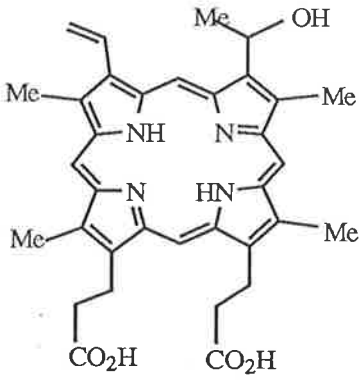
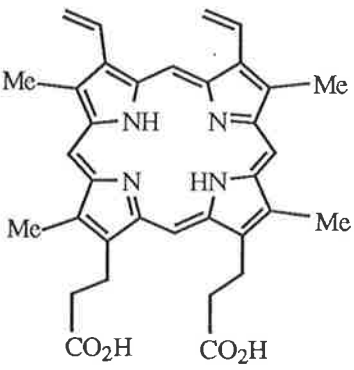


(23b)

+



(21b)

Scheme 2.2 (continued)

2.2 Results and Discussion[§]

2.2.1 Total synthesis of protoporphyrin III dimethyl ester (21a)

Benzyl 4-(2-acetoxyethyl)-3,5-dimethylpyrrole-2-carboxylate (13)[7b] was prepared by the standard Knorr procedure[8] from benzyl acetoacetate[7a] and 3-acetyl-4-oxopentyl acetate (12).[7b] Recrystallization of the crude product gave (13) in a yield of 11%, but another 55% was obtained after chromatography of the mother liquor. This second batch was obtained as a yellow oil, which resisted attempts to solidify it. However TLC and NMR analysis showed that it was identical to the product from the recystallization and it was used in the next step without any problems.

Hydrogenation[9] of pyrrole (13) with palladium on charcoal at atmospheric pressure gave the pyrrole carboxylic acid (14) in 70% yield. The carboxyl function was converted to a formyl group by the procedure developed by Clezy *et. al.*,[10] using TFA to decarboxylate the pyrrole, followed by triethyl orthoformate to formylate the resulting α-unsubstituted pyrrole intermediate *in situ*. The formyl pyrrole (15) was obtained in 43% yield.

The synthesis of the biladiene-*ac* was based on the original procedure by Johnson and Kay.[27] Decarboxylation of the dipyrryl-methane (16)[11] with TFA gave the 5,5'- unsubstituted

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[§] References, p. 98.

intermediate which was reacted *in situ* with 2.1 equivalents of the formyl pyrrole (15). Addition of hydrobromic acid-acetic acid[12] and dry ether resulted in the precipitation of the biladiene-*ac* salt (17) (in 79% yield) which was cyclized[12] in refluxing DMF in the presence of copper(II) acetate. The crude metalloporphyrin (18) was demetallated[12] with concentrated sulphuric acid/TFA (10%). This resulted in the hydrolysis of the acetoxyethyl side chains as well as the propionic esters on the porphyrin. Re-esterification of the crude porphyrin by the trimethyl orthoformate procedure[13] gave 3,7-di(2-hydroxyethyl)deuteroporphyrin III dimethyl ester (19) which was purified by chromatography on silica. The overall yield of porphyrin (19) was 28%, based on the starting biladiene-*ac* salt (17). This yield is consistent with that obtained from the copper-mediated cyclizations in refluxing DMF[12,15] but is lower than the 54% obtained by Clezy *et. al.*[15] using acetic acid /methanol at 100°C. The higher yield from the latter method might have been due to the lower reaction temperature. Smith *et. al.*[25] showed that in some cases cyclizations conducted at room temperature gave better yields than those done in refluxing DMF. However when the cyclization of (17) was done at room temperature, a lower yield (18%) of (19) was obtained.

Chlorination of the di(2-hydroxyethyl) porphyrin (19) with benzoyl chloride/DMF[14] produced the di(2-chloroethyl) derivative (20) in quantitative yield. Dehydrochlorination using aqueous sodium hydroxide/pyridine,[14,15] followed by re-

esterification gave protoporphyrin III dimethyl ester (21a) in 71% yield.

2.2.2 Conversion of Protoporphyrin III dimethyl ester ($PP_{III}dme$) (21a) to Haematoporphyrin III dimethyl ester ($HP_{III}dme$) (22)

Hydrobromination of the vinyl groups in $PP_{III}dme$ (21a) with saturated HBr/dichloromethane (see Chapter 3, Section 3.2.1 for optimization studies) gave the reactive di(1-bromoethyl) intermediate which was then quenched with water. This afforded a mixture which was chromatographed on silica to give the desired $HP_{III}dme$ (22) (12%), the starting material $PP_{III}dme$ (21%), 3-(1-hydroxyethyl)-7-vinyldeuteroporphyrin III dimethyl ester, $HV_{III}dme$ (23a) and the ether-linked dimers (24), (25) and (26). $HV_{III}dme$ (23a) and the ether-linked dimers (24), (25), (26) eluted together in a broad band which accounted for 65% of the total material, with the dimers predominating.

Compared to the hydrobromination-hydrolysis reaction with protoporphyrin IX dimethyl ester, $PP_{IX}dme$, (7) (Chapter 3, Section 3.2.1) where $HP_{IX}dme$ (6) was the main product, the corresponding reaction with $PP_{III}dme$ (21) produced an unexpectedly high proportion of ether-linked dimers (24), (25) and (26). This could be due to a combination of steric and aggregation factors, Fig. 2.1. The proximity of the ethyl substituents in the porphyrin would force the two groups to be

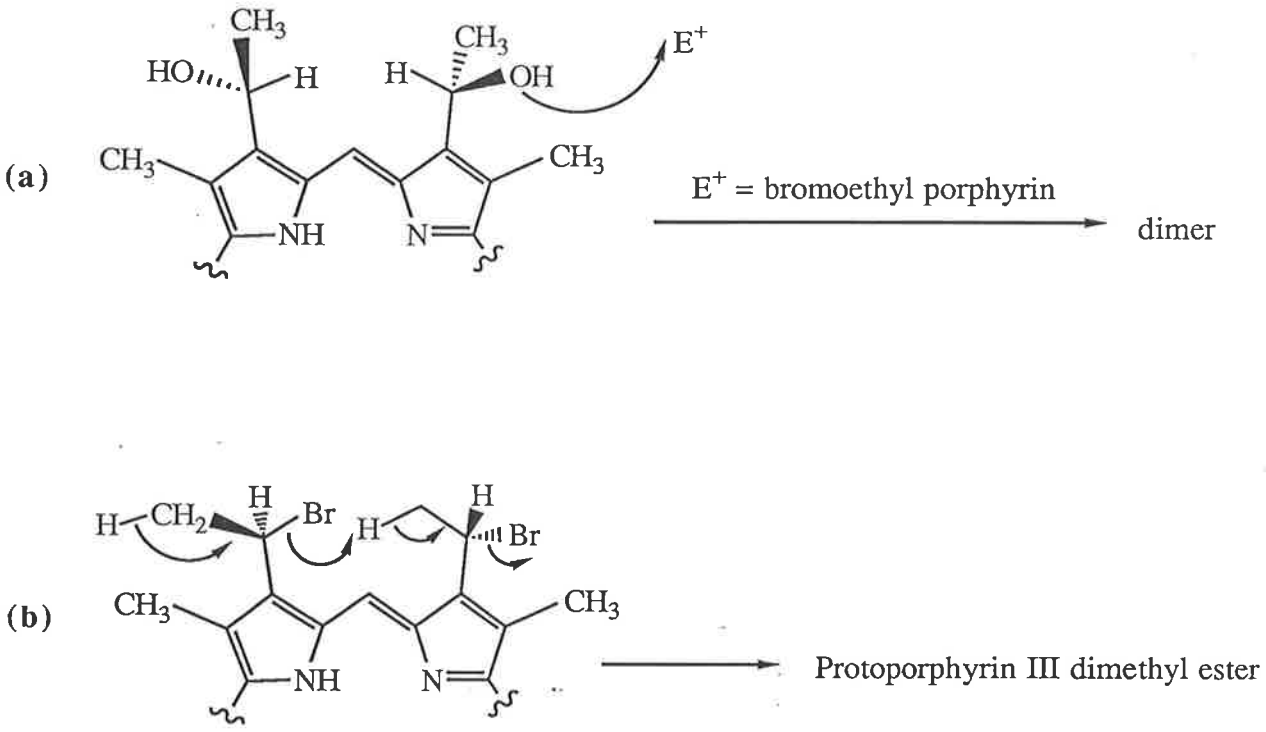


Fig. 2.1 Possible configurations of substituents in the sterically crowded
(a) haematoporphyrin III dimethyl ester, leading to preferential formation of dimers;
(b) 3,7-di(1-bromoethyl) intermediate, favouring dehydrobromination to give protoporphyrin III dimethyl ester.

orientated in such a way as to minimize steric interaction. One possible arrangement, shown in Fig. 2.1, involves the two bulky groups (the methyl and the bromide or the hydroxyl group) directed outwards at opposite sides of the plane of the molecule and therfore are readily accessible to an approaching electrophile such as a molecule of the bromoethyl porphyrin [Fig. 2.1(a)]. The likelihood of another porphyrin molecule in the vicinity is high because of the tendency of porphyrins to aggregate[43] in aqueous solutions, hence the increased yield of dimers. It appears that the steric factor is a major influence in the formation of dimers because the less sterically restricted PP_{IX}dme did not produce a significant proportion of dimers under similar reaction conditions (Chapter 3, Section 3.2.1).

One possible explanation for the 21% recovery of starting PP_{III}dme (21a) is that in the bromoethyl porphyrin the leaving groups are suitably aligned for an intra-molecular reaction [Fig. 2.1(b)]. It is also possible that hydrobromination proceeded more slowly with the sterically restricted PP_{III}dme (21a) compared to PP_{IX}dme (7a), on which the optimization studies were done (Chapter 3, Section 3.2.1). In PP_{III}dme (21a) the vinyl groups might be forced out of the plane of the molecule so that there is a loss of conjugation between the π electrons of the vinyl groups and the porphyrin ring. This loss of conjugation may in turn result in the reduced nucleophilicity of vinyl groups, thereby slowing down hydrobromination.

The dimers with 1-hydroxyethyl side chain(s), (24) and (26), were combined and hydrolysed under reflux using 1M hydrochloric acid/THF,[16] in order to cleave the ether linkages. The product was re-esterified and chromatographed to give HP_{III}dme (22), PP_{III}dme (21) and HV_{III}dme (23a). The overall recovery of HP_{III} dme (22) by this method was 41% based on starting PP_{III}dme (21a).

The HP_{III}dme (22) obtained by this method showed a typical etio-type visible spectrum with absorption maxima which were very similar to those of Clezy *et. al.*[22] which was obtained by borohydride reduction of the diacetyl porphyrin (32). The proton NMR spectrum was also similar to that in the literature;[22] with signals occurring within 0.1 ppm of those in the literature.[22] Variations in chemical shifts are to be expected because proton NMR spectra are concentration dependent.[3b] The signals in the proton NMR spectrum of HP_{III}dme (22) were distinct and the multiplicities were readily identified. The pyrrole hydrogens resonated as a broad singlet at -4.52 ppm; two distinct doublets resonating at 1.79 ppm (J 6.6 Hz) and 1.92 ppm (J 6.6 Hz) were assigned to the methyl protons ($C\underline{H}_3CHOH$). The existence of two signals from equivalent protons could be due to the presence of diastereoisomers.[22] The triplet at 3.19 ppm was assigned to the methylene on the propionate ($C\underline{H}_2CO_2CH_3$), the other triplet at 4.21 ppm to $C\underline{H}_2CH_2CO_2CH_3$ and the singlet at 3.41 ppm to $CO_2C\underline{H}_3$. Overlapping singlets at approximately 3.7 ppm were due to the protons of the ring methyls. The proton $CH_3C\underline{H}OH$ resonated as a

quartet at 5.79 ppm and the meso protons produced four distinct singlets at 9.52, 9.67, 9.70 and 10.44 ppm.

The ^{13}C resonances of HP_{III}dme (22) correlated well with those of the corresponding porphyrin of the IX series.[26] The four ring methyl carbons resonated as two distinct signals (11.1 ppm and 11.5 ppm), one due to the equivalent methyl groups in the 2 and 8 positions and the other to those in the 12 and 18 positions. The methyl carbons $\underline{C}H_3CHOH$ produced two signals at 25.95 ppm and 26.22 ppm, possibly due to the presence of diastereoisomers. The carbon $CH_3\underline{C}HOH$ resonated at 65.54 ppm and the carbons of the propionate groups gave signals at 21.68 ppm ($\underline{C}H_2CH_2CO_2CH_3$), 36.69 ppm ($\underline{C}H_2CO_2CH_3$), 51.77 ppm ($CO_2\underline{C}H_3$) and 173.59 ppm ($\underline{C}O_2CH_3$). The meso carbons resonated at 95.75 ppm, 96.36 ppm and100.4 ppm but the signals due to the pyrrole carbons were broad due to NH tautomerism.[31]

Haematoporphyrin III dimethyl ester (22) was hydrolysed by the action of aqueous sodium hydroxide/THF according to the standard procedure[16] to afford haematoporphyrin III (HP_{III}) (10). The retention time of HP_{III} (approximately 3.2 minutes) under standard reversed phase HPLC conditions[16] was found, as expected, to be similar to that of haematoporphyrin IX (HP_{IX}) (1). [16]

2.2.3 Total synthesis of 3,7-Diacetyldeutero-porphyrin III dimethyl ester (31)

This section is a continuation of an earlier project[19b] involving the total synthesis of 3,7-diacetyldeuteroporphyrin III dimethyl ester (31) by literature procedures. The cyclization of the biladiene-*ac* (30) was attempted[19b] but the resulting porphyrin was not fully characterized.[19b] This present work, outlined in Scheme 2.2 and discussed below, continues from the modification of the existing pyrrole[19b] (27) through to the desired haematoporphyrin III (10).

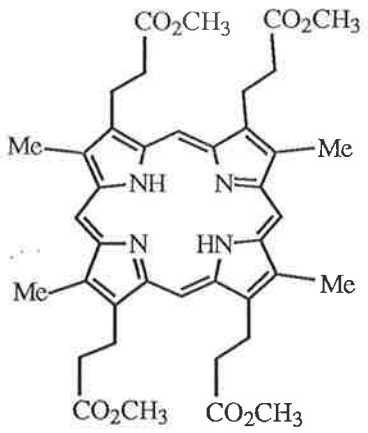
Ethyl 3-acetyl-2,4-dimethylpyrrole-5-carboxylate[17,19b] (27) was saponified to give the corresponding pyrrole carboxylic acid (28) which was decarboxylated and formylated using the procedure of Clezy *et. al.*[10,19b] Previous studies[19] using pyrrole acid (28) showed that the formyl pyrrole (29) was obtained in 42% yield under the following conditions: decarboxylation with TFA (5 ml/g of pyrrole) for 30 min at room temperature, followed by *in situ* formylation with triethyl orthoformate (2 ml/g of pyrrole) for 4 min at 0°C; appreciable amounts (~40%) of the starting pyrrole acid (28) were recovered[19] from the reaction. In the light of these results,[19] the possibility of improving the yield of formyl pyrrole (29) was investigated. The high percentage recovery[19] of starting pyrrole acid (28) suggested that the low yield was partly due to incomplete decarboxylation. This was confirmed when the reaction time for decarboxylation was increased from 30 minutes to 95 minutes and the formyl pyrrole (29) was obtained in a

yield of 52%. A longer decarboxylation time of 120 minutes increased the yield of formyl pyrrole to 90%.

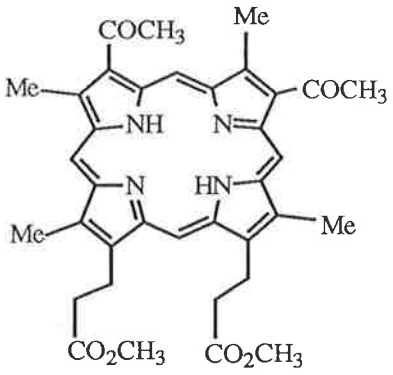
The synthesis of the diacetyl-containing biladiene-*a c* salt (30) was slightly modified from the standard[12] procedure. The formyl pyrrole[19b] (29) was only sparingly soluble in methanol, so the solution containing the decarboxylated dipyrrylmethane[28] (16) in TFA was added to the suspension of the formyl pyrrole (in methanol) instead of the other way round. The mixture was allowed to stir until all the formyl pyrrole had dissolved before HBr/acetic acid was added. This resulted in a biladiene-*ac* (30) yield of 89%. The salt was used immediately in the cyclization step.

Oxidative cyclization with copper(II) acetate in refluxing DMF[5] followed by demetallation, re-esterification and chromatography afforded a porphyrin which gave a molecular ion of m/z 710 by FAB mass spectrometry. The expected molecular ion of m/z 623 corresponding to the desired 3,7-diacetyldeuteroporphyrin III dimethyl ester (32) was not present in the spectrum. The anomalous porphyrin exhibited an etio-type absorption spectrum with maxima at 402 nm, 498 nm, 532 nm, 567 nm and 621 nm. The wavelength of band I at 621 nm indicated that there were no electron-withdrawing groups (such as acetyl groups) directly attached to the porphyrin ring because a shift to a longer wavelength of approximately 640 nm is expected of porphyrins with electron-withdrawing groups on adjacent pyrrole subunits.[3c] ^{1}H and ^{13}C NMR data suggested that

the porphyrin contained four propionic ester groups and four ring methyl groups. A possible structure for this porphyrin would be (35), which has a molecular weight of 710. The formation of this porphyrin is puzzling because redistribution[3a] reactions tend to occur mainly in more saturated tetrapyrroles such as bilanes,[4a] bilenes[4a] and the reduced porphyrins like porphyrinogens[3a] (see Appendix for nomenclature). The high temperature during cyclization is likely to have been the cause of this anomalous result because when the temperature was kept below the boiling point of DMF ie. at 135-138°C, the desired 3,7-diacetyl-deuteroporphyrin III dimethyl ester (32) was obtained. Evidence for this structure was the characteristic red shift of bands I-IV in the absorption spectrum of the crude free base product, in particular, the maximum at 640 nm for band I. A molecular ion at m/z 623 in the FAB mass spectrum indicated that the porphyrin was the desired 3,7-diacetyldeuteroporphyrin III dimethyl ester (32). TLC comparisons with 3,8-diacetyldeuteroporphyrin IX dimethyl ester (36) showed that the porphyrin from the III series (32) had an R_F value and a characteristic green-brown colour which was similar to that of the natural IX isomer (36).



(35)

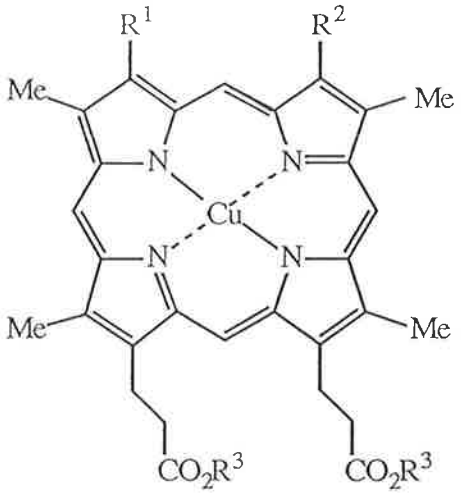


(36)

Problems were encountered during extraction of the diacetyl-containing porphyrin as intractable emulsions formed during extraction in the aqueous work-up procedures that followed the cyclization, demetallation and re-esterification steps. Attempts to disperse the emulsion by filtration through Celite and/or filter paper under vacuum failed because the filters were quickly blocked by a fine precipitate that was present in the emulsion. It was later discovered that the desired 3,7-diacetyldeuteroporphyrin III dimethyl ester (32) and its Cu-metalloporphyrin (31) were only sparingly soluble in dichloromethane, THF, acetone and combination of these solvents. This lack of solubility was a major problem during chromatography. In addition, demetallation under standard[12,15] conditions did not proceed to completion but this was not detected in the absorption spectrum of the crude product because of the very poor resolution of the individual peaks in the overall spectrum due to the impurities in the material. Purified Cu^{II}-3,7-diacetyldeuteroporphyrin III dimethyl ester (31) readily demetallated under standard[12,15] conditions (see later). It is

likely that the low yields[20] from the cyclization of the biladiene-*ac* salt are not only due to the deactivating influence of the acetyl groups but also to the low solubility of the product.

Since the target porphyrin, $HP_{III}dme$, did not give any solubility problems, it was decided to convert crude Cu^{II}-3,7-diacetyldeuteroporphyrin III dimethyl ester (31) to the corresponding Cu^{II}-3,7-di(1-hydroxyethyl) porphyrin (Cu^{II}-$HP_{III}dme$) (37a). Attempts to reduce the crude metalloporphyrin with sodium borohydride failed despite the use of excess amounts of reducing agent. It is possible that the borohydride was quenched by the impurities present in the crude metalloporphyrin. Purified Cu^{II}-3,7-diacetyldeuteroporphyrin III dimethyl ester (31) was readily reduced by sodium borohydride to give Cu^{II}-$HP_{III}dme$ (37a).



(31) $R^1 = R^2 = COCH_3$, $R^3 = CH_3$
(37a) $R^1 = R^2 = CH(CH_3)OH$, $R^3 = CH_3$
(38) $R^1 = R^2 = CH(CH_3)OCH_3$, $R^3 = CH_3$

(21c) $R^1 = R^2 = CH{=}CH_2$, $R^3 = H$
(23c) $R^1 = CH(CH_3)OH$, $R^2 = CH{=}CH_2$, $R^3 = H$
(37b) $R^1 = R^2 = CH(CH_3)OH$, $R^3 = H$

In an attempt to determine if it was feasible to delay demetallation to a later stage, purified 3,7-diacetyldeuteroporphyrin III dimethyl ester (32) which contained some (≤25%) Cu^{II}-metalloporphyrin (31) (the latter a result of the incomplete demetallation mentioned above) was treated with sodium borohydride. Reduction proceeded cleanly to give HP_{III}dme (22) and its Cu^{II}-metalloporphyrin (37a), both of which were soluble in dichloromethane.

In order to facilitate chromatography, the crude HP_{III}dme was etherified using trimethyl orthoformate/ methanol/concentrated sulphuric acid (5:5:1)[13] to give 3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (34) and its

corresponding Cu^{II}-metalloporphyrin (38) after chromatography. This mixture was subjected to standard demetallation conditions in order to remove the chelating metal and to determine if the conditions would simultaneously hydrolyse the methoxy and propionic ester groups to give HP_{III} (10). While the demetallation proceeded as expected (evidence for which was the etio-type absorption of the product), the reversed phase HPLC chromatogram of the crude material showed that it was a complicated mixture of HP_{III} (10) (7%), HV_{III} (23b) (36%), PP_{III} (21b) (~5%) and at least six other (unidentified) by-products. The high proportion of HV_{III} (23b) suggested that elimination of the methoxy group and/or dehydration of 1-hydroxyethyl group had occurred along with demetallation and hydrolysis.

The demetallation of Cu^{II}-HP_{III} (37b) was also investigated. Cu^{II}-HP_{III} was obtained from the acidic hydrolysis (see later) of Cu^{II}-3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (38). Studies using the more readily available 3,8-di(1-methoxyethyl)deuteroporphyrin IX dimethyl ester (39) as a model for the III isomer (34) indicated that heating under reflux for 7 hours in a mixture of 1M hydrochloric acid/THF was required to hydrolyse the porphyrin to give HP_{IX} (1) (88%) and 3-(1-hydroxyethyl)-8-vinyldeuteroporphyrin IX (HV_{IX}) (4) (12%). Working on the expectation that the reactivity of the metalloporphyrin was similar to that of the free base analogue, the same conditions were used on Cu^{II}-3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (38). Cu^{II}-

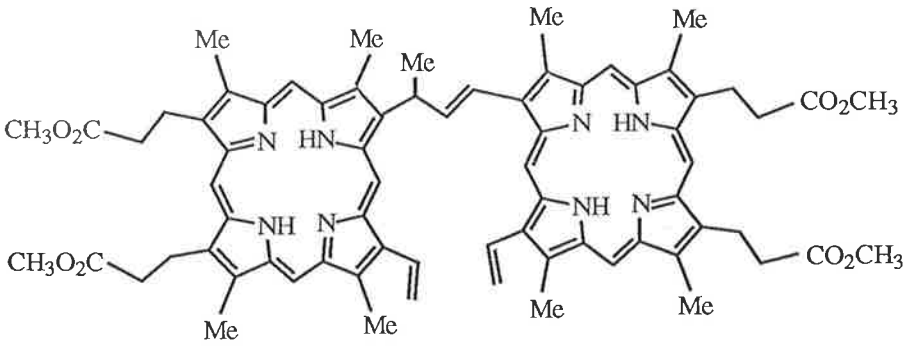
HP_{III} (37b) (72%), Cu^{II}-HV_{III} (23c) (8%) and Cu^{II}-PP_{III} (21c) (10%) were obtained.

The mixture of Cu^{II}-HP_{III} (37b), Cu^{II}-HV_{III} (23c) and Cu^{II}-PP_{III} (21c) was demetallated under standard conditions[12,15] using concentrated sulphuric acid/TFA (5%). The product exhibited an etio-type absorption spectrum (indicating that demetallation had been achieved) but FAB mass spectrometry did not yield any molecular ions However this may be due to the low mass spectrometric sensitivity for propionic acid-containing porphyrins[21,22] although, the molecular ions for monomers are usually observed. Reversed phase HPLC analysis of the crude product indicated the presence of non-polar (R_t > 20 min)† material, which accounted for ~82% of the product. None of the expected porphyrins, namely HP_{III} (10), HV_{III} (23b) and PP_{III} (21b), were present. A polar product (R_t 1.44 min, 18%) formed the remainder of the material. The crude material was reesterified with concentrated sulphuric acid/methanol and the product was analysed by FAB mass spectrometry. Again, no molecular ions were detected.

The long retention time of the major portion of the material suggested that it was polymeric in nature.† In an attempt to determine the type(s) of linkages involved and to salvage the HP_{III}, the crude material was hydrolysed with 1 M

† Other studies[16] have indicated that with this solvent gradient, polar porphyrins like HP_{IX} and HV_{IX} elute before 10 minutes and less polar material like PP_{IX} and dimeric and oligomeric porphyrins have retention times which are longer than 20 minutes.

hydrochloric acid/THF. The HPLC chromatogram of the product was similar to that of the unhydrolysed material, indicating that the original material was stable towards acidic hydrolysis. This suggested that the linkages between the porphyrins are not esters or ethers because these groups are cleaved by acidic hydrolysis.[16] A portion of the hydrolysed material was reesterified (in order to convert any acid groups to the corresponding esters because the sensitivity of the mass spectrometer towards acid-containing porphyrins is low) and analysed by FAB mass spectrometry. Molecular ions of *m/z* 711, 811, 825 and 1181 were detected. The mass of 1181 m.u. corresponds to the carbon-linked dimer (40). No attempts were made to detect higher oligomers. The stability of the material towards acidic hydrolysis is consistent with the nature of the linkage on the proposed structure (40), which is similar to the dimer that was isolated from HPD by Ward *et. al.*.[41] Pandey *et. al.*[39] synthesized a similar carbon-linked porphyrin dimer using a 1-hydroxyethyl containing porphyrin and triflic acid. The reaction is believed[39] to proceed via the initial dehydration of the 1-hydroxyethyl group followed by the reaction of the resulting vinyl porphyrin with another porphyrin carbocation. It is possible that dimer (40) was formed from HP_{III} (10), HV_{III} (23b) and PP_{III} (21b) by the same mechanism but using the demetallating reagents, namely concentrated sulphuric acid /TFA instead of triflic acid.



[**Note** : the stereochemistry of the double bond in the bridge is not known]

(40)

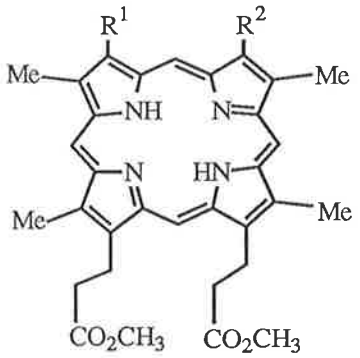
These results suggested that it was necessary for demetallation to be done in the beginning of the sequence, preferably after cyclization of the biladiene-*ac* salt and that due to the inherent difficulties caused by the low solubility of the diacetyl-containing porphyrin, low yields are unavoidable. A few modifications to the literature procedure[12,20] were made: during work-up, the extracts containing diacetyl porphyrins were not washed with water before evaporation; the crude Cu^{II}-3,7-diacetyldeuteroporphyrin III dimethyl ester (31) was chromatographed on Grade III alumina before demetallation. The chromatography removed most, but not all, of the low R_F impurities that are normally formed in the cyclization step and it was found that the small proportion of impurities that remained did not adversely affect the subsequent steps. Reduction of the acetyl groups with sodium borohydride afforded HP_{III}dme (22) which was etherified (using the orthoformate method[13]) to facilitate chromatography. 3,7-Di(1-

methoxyethyl)deuteroporphyrin III dimethyl ester (34), 3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41) and HP_{III}dme (22) were separated from the crude product. The total yield of porphyrin was 12% based on the starting biladiene-*ac* salt. The mixture of methoxyethyl porphyrins (34) and (41) ~~were~~ as combined with HP_{III}dme (22) and hydrolysed using acidic reflux to give mainly HP_{III} (10) whose HPLC chromatogram is shown in Fig. 2.4(a) (see Section 2.2.5). This crude mixture of HP_{III} (10) (>75%) with small amounts (≤10%) of HV_{III} (23b) and PP_{III} (21b) was used to make HPD-III without prior purification. This is because the corresponding HPD is usually prepared from commercial HP_{IX} (1) which contains similar impurities. Commercial HP_{IX} (1) is contaminated with HV_{IX} (4) (5–8%) and smaller amounts of PP_{IX} (7) as well as dimeric and oligomeric material [13,29]

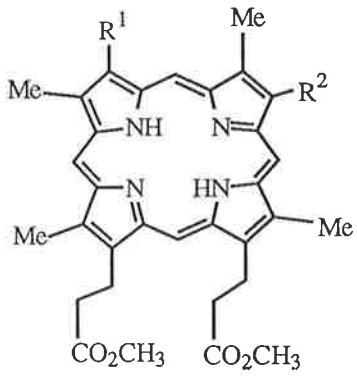
2.2.4.1 Spectral analysis of 3,7-Di(1-methoxyethyl)-deuteroporphyrin III dimethyl ester (34)

The proton NMR spectrum of 3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (34), was well resolved and the multiplicities were clearly discernable. The signals duc to the methyl protons $C\underline{H}_3CHOCH_3$ resonated as a doublets at 2.3 ppm (J 6.6 Hz), while the methoxy protons $CH_3CHOC\underline{H}_3$ produced a singlet at 3.7 ppm. The signal at 6.1 ppm, due to the proton $CH_3C\underline{H}OCH_3$, was a multiplet resulting from two overlapping quartets, possibly due to the presence of

diastereomers. All the chemical shifts correlated well with those of the IX isomer (39).[13,29] Similarly the resonances of the protons on the other sidechains, namely the propionates at 4.2 ppm ($C\underline{H}_2CH_2CO_2CH_3$), 3.2 ppm ($C\underline{H}_2CO_2CH_3$) and 3.7 ppm ($CH_2CO_2C\underline{H}_3$); and the ring methyls at 3.5 – 3.6 ppm also appeared in the expected[29] regions of the spectrum. The resonances of the



(34) $R^1 = R^2 = CH(CH_3)OCH_3$
(41) $R^1 = CH(CH_3)OCH_3$, $R^2 = CH(CH_3)OH$



(39) $R^1 = R^2 = CH(CH_3)OCH_3$
(42) $R^1 = CH(CH_3)OCH_3$, $R^2 = CH(CH_3)OH$

propionate and ring methyl sidechains also correlated well with those of other known porphyrins.[3b,30] The signals due to the meso protons were distinct singlets at 9.8 ppm, 10.0 ppm, 10.93 ppm. The pyrrole protons resonated as broad signals centred at -3.8 ppm. The main difference between the proton NMR spectrum of 3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (34) and its IX isomer (39)[29] is the good resolution of the former, which is encouragingly in line with the anticipation that the more symmetrical III isomer will produce better resolved proton and ^{13}C spectra.

The ^{13}C NMR spectrum of 3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (34) was very similar to that of its IX isomer (39).[29] The carbons of the ring methyls resonated at 11.53 ppm while the corresponding carbons in the IX isomer[29] resonated at 11.62 ppm; the carbons of the propionates in (34) gave signals at 21.69 ppm ($C\underline{H}_2CH_2CO_2CH_3$, the corresponding signal in the IX isomer[29] was at 21.66 ppm), 36.82 ppm ($CH_2\underline{C}O_2CH_3$, 36.95 in the IX isomer[29]), 51.57 ppm ($CO_2C\underline{H}_3$, 51.72 ppm in the IX isomer[29]) and 173.47 ppm ($\underline{C}O_2CH_3$, 173.59 ppm in the IX isomer[29]). The four meso carbon signals appeared at 96.07 ppm, 96.60 ppm, 99.59 ppm and 99.73 ppm, while the signals due to the pyrrole carbons were broad (as a result of NH tautomerism[31]) and appeared in the region of 135 – 145 ppm. The methoxy carbon $CH_3CHO\underline{C}H_3$ resonated at 57.07 ppm, the methyl $\underline{C}H_3CHOCH_3$ at 25.11 ppm and the methine $CH_3\underline{C}HOCH_3$ at 74.93 ppm, all of which were

approximately 0.2 ppm downfield from the corresponding signal in the ^{13}C NMR spectrum of the IX isomer.[29]

The FAB mass spectrum of 3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (34) showed a strong molecular ion at m/z 655 and the absorption spectrum exhibited a typical etio-type pattern with maxima at 401 nm, 499 nm, 532 nm, 568 nm and 622 nm.

2.2.4.2 Spectral analysis of 3-(1-Hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41)

A notable difference between the proton NMR spectrum of 3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41), and that of its IX isomer (42), was the absence, in the former, of a signal in the 5.4 – 5.8 ppm region of the spectrum normally[29] due to the methine proton $CH_3C\underline{H}OH$. A heteronuclear ^{13}C-proton correlation spectrum of 3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41) revealed that the proton signal was considerably further upfield at 3.9 ppm. In contrast the corresponding proton in the IX isomer[29] resonated between 5.5 – 5.7 ppm. In haematoporphyrin IX dimethyl ester[22] the $CH_3C\underline{H}OH$ signal appears at 6.45 ppm. The upfield shift of the proton $CH_3C\underline{H}OH$ signal in 3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41) suggests

that the 1-hydroxyethyl group in the III isomer (41) may be distorted to relieve steric interaction with the nearby 1-methoxyethyl group and in doing so the proton $CH_3C\underline{H}OH$ is forced out of its normal position, apparently in a direction that lessens the deshielding influence of the porphyrin ring current. The methyl protons $C\underline{H}_3CHOH$ and $C\underline{H}_3CHOCH_3$ in (41) resonated at 2.3 ppm and 2.5 ppm, respectively, while those in the IX isomer[29] (42) resonated at 2.4 ppm. Again, this may suggest that the two methyl groups $C\underline{H}_3CHOH$ and $C\underline{H}_3CHOCH_3$ are experiencing some steric restrictions which may be forcing them to occupy two different environments. However this postulate was not tested with Nuclear Overhauser Enhancement[15] studies. The methoxy protons $CH_3CHOC\underline{H}_3$ resonated at 3.7 ppm, which is in agreement with the signal of the corresponding protons in the IX isomer[29] (42). The signals of the other sidechain and ring protons in 3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41) appeared in regions that are similar to those of the corresponding protons in its IX isomer[29] (42).

Most of the signals in the ^{13}C NMR spectrum of 3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41) appeared at similar regions to those of corresponding signals in the spectrum of the IX isomer[29] (42), except for the signals due to the carbons of the 1-hydroxyethyl and 1-methoxyethyl groups. This could be an indication of the steric interaction experienced by these groups. The methyl carbon $\underline{C}H_3CHOH$ in (41) resonated at 25.1 ppm while the signal of the corresponding carbon in the IX isomer appeared at 26.0 ppm.

The methine carbon $CH_3\underline{C}HOH$ (62 ppm) in (41) exhibited an upfield shift of 3.4 ppm relative to the corresponding signal ($CH_3\underline{C}HOH$, 65.4 ppm) in the IX isomer.[29] This upfield shift is comparable to that observed in sterically hindered cyclohexanes.[34] A surprising downfield shift of 9.6 ppm in the resonance of the methyl carbon $\underline{C}H_3CHOCH_3$ (35.5 ppm) in the III isomer (41) compared to that of its IX analogue[29] (42) ($\underline{C}H_3CHOCH_3$, 26.0 ppm) appeared to contradict the argument of steric interaction between the 1-hydroxyethyl and 1-methoxyethyl groups in (41) because steric hindrance generally results in upfield shifts in the resonances of the hindered carbon(s).[34] However this anomalous shift is similar to that observed by Kroschwitz *et.al.*[35] in their study of sterically crowded substituted isopropyl hydrocarbons. They[35] attributed a downfield shift of the methyl carbon to an elongation of the C-CH_3 bond to relieve strain. This explanation may apply to the C-CH_3 bond in the CH_3CHOCH_3 group in 3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41). The major flaw in the steric hindrance argument is that similar shifts in resonances were not observed in the presumably more hindered 3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (34) discussed in Section 2.2.4.1 above. However if one were to consider the possibility of intramolecular hydrogen-bonding between the 1-hydroxyethyl and 1-methoxyethyl groups in 3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41), Fig. 2.2, then the upfield shift of the methine proton $CH_3C\underline{H}OH$ can be

explained by the increased electron density in its adjacent oxygen, and the distortion of the C-CH_3 bond in the 1-methoxyethyl group is therefore due to the necessity to minimize interaction with the neighbouring methyl group on the 8-position of the porphyrin. This would also explain the chemical shifts of the methoxy carbon $CH_3CHO\underline{C}H_3$ and the methine carbon $CH_3\underline{C}HOCH_3$ in (34) (Section 2.2.4.1) which are no different from those of the less hindered IX isomer[29] (39), because there is no possibility of intramolecular hydrogen bonding in the di(1-methoxyethyl) porphyrin (34).

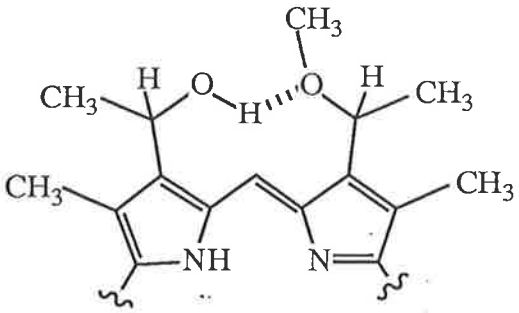


Fig. 2.2 Intramolecular hydrogen bonding in 3(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41)

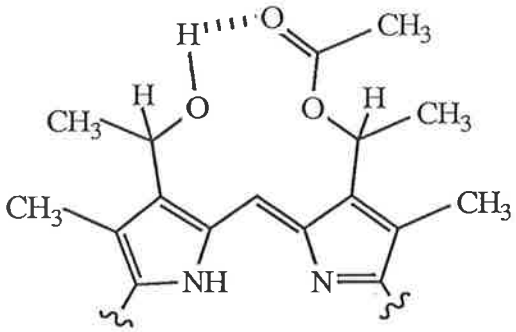
3-(1-Hydroxyethyl)-7-(1-methoxyethyl)deutero-porphyrin III dimethyl ester (41) exhibited a typical etio-type absorption spectrum with maxima at 400 nm, 499 nm, 532 nm, 568 nm and 622 nm; and produced a molecular ion at *m/z* 641 in the FAB mass spectrum.

2.2.5 HPD-III

HPD-III was prepared from crude HP_{III} (10) (containing >75% HP_{III}) according to the Lipson procedure.[2] The porphyrin was stirred in concentrated sulphuric acid/acetic acid

(1:19) for 1 hour and then treated with aqueous sodium acetate solution. The precipitate that formed was shown by HPLC to consist of more than ten components, but the main components were haematoporphyrin III monoacetate (HP_{III} monoacetate, R_t 3.5 minutes) (43) and haematoporphyrin III diacetate (HP_{III} diacetate, R_t 6.2 minutes) (44) which were present in the ratio of approximately 2:3. The total yield of HP_{III} acetates was 49% and most of the by-products eluted before 20 minutes, indicating that they were not polymeric in nature.[16] A similar ratio (2:3) of HP_{III} monoacetate (43) to HP_{III} diacetate (44) was obtained when "clean" HP_{III} (≥86% pure) was used. However fewer by-products were formed in the reaction using "clean" HP_{III} and the total yield (75%) of HP_{III} mono- and diacetates was higher than the 49% obtained using crude HP_{III}. The HP_{III} acetates have retention times which are similar to those of the analogous systems of the IX series.[16] The relative proportion of HP_{III} monoacetate compared to diacetate is higher than that obtained from the IX isomer.[37] This suggests that, due to the steric restrictions in the III isomer and the possibility of forming intramolecular hydrogen bonds in HP_{III} monoacetate, Fig. 2.3(a), the formation of HP_{III} diacetate (44) is a less favourable process. The reversed phase HPLC chromatogram of the HP_{III} monoacetate/diacetate mixture showed that material which eluted after 20 minutes (namely the porphyrins which are dimeric or oligomeric in nature[16]) accounted for less than 10% of the crude material indicating, as expected,[16] that polymerization did not occur in the acetylation step.

(a)



(b)

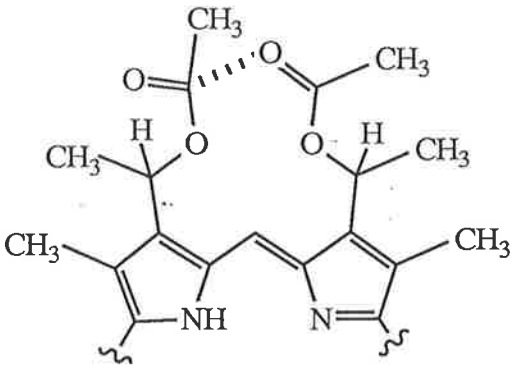
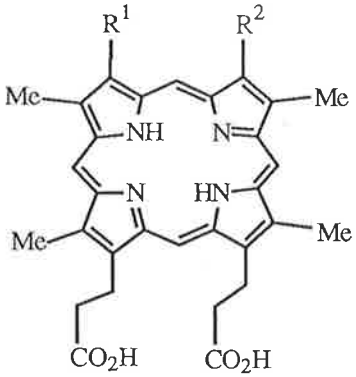


Fig. 2.3 Intramolecular hydrogen bonding in **(a)** haematoporphyrin III monoacetate (43), and **(b)** haematoporphyrin III diacetate (44)



(43) $R^1 = CH(CH_3)OH$, $R^2 = CH(CH_3)OCOCH_3$
(44) $R^1 = R^2 = CH(CH_3)OCOCH_3$

The mixture of HP_{III} acetates was treated with aqueous sodium hydroxide for 1 hour, then neutralized and diluted with saline to make up a solution with a porphyrin concentration of 5 mg/ml which is the usual concentration of commercially available HPD solutions.[24,38] The solution was then filtered through a 0.45 μm filter to give HPD-III. HPD-III was diluted 1000 times with 0.1 N sodium hydroxide/ethanol (1:1) and analysed by ultraviolet spectroscopy. The resulting etio-type spectrum had maxima at 395.7 nm, 499 nm, 533 nm, 569 nm and 621 nm. The absorbance at 397 nm was 0.372 units. This spectrum was compared to that of the crude HPD-III solution prior to filtration ("unfiltered HPD-III") which had been diluted in the same manner. "Unfiltered HPD-III" exhibited an etio-type spectrum with maxima at 395 nm, 501 nm, 534 nm, 570 nm and 620 nm, which is nearly identical to that of the filtered HPD-III. The main difference between the two spectra was the magnitude of the absorbances. The absorbance of "unfiltered HPD-III" at 397 nm was 0.808 units, which is twice as large as that of HPD-

III. The differences in the ultraviolet absorbances of "unfiltered HPD-III" and HPD-III at 397 nm indicated that approximately 54% of the material that was formed during the preparation of HPD-III had been removed by filtration. The HPLC chromatogram (Fig. 2.4(c)) of the material that was retained by the filter was identical to that of HPD-III. This indicated that both the filtrate (ie. HPD-III) and the residue from the filtration were the same material and that HPD-III was only sparingly soluble in saline at the normal[38] HPD concentration of 5 mg/ml. Assuming that an HPD solution with an absorbance of 0.8 units at 397 nm had a porphyrin concentration of 5 mg/ml[38] it follows then that the concentration of HPD-III was approximately 2.3 mg/ml.

The reversed phase HPLC analysis of HPD-III using standard solvents[16] showed a chromatogram (Fig. 2.4(b)) which could be arbitrarily divided into two sections – sharp peaks with retention times below 10 minutes and a poorly resolved section which eluted after approximately 15 minutes. Most (60 – 70%) of the material was in the short retention time (R_t < 10 minutes) section of the chromatogram while only 30 –40% of the material was in the less polar section ($R_t \geq$ 15 minutes). As expected[16] polar material like HP_{III} and HV_{III} were in the short retention time (R_t < 10 minutes) section, although the two monomers only accounted for 25 –30% of the material. A considerable amount (30 – 40%) of other (unidentified) material was also present in this section of the chromatogram. Although the chromatogram

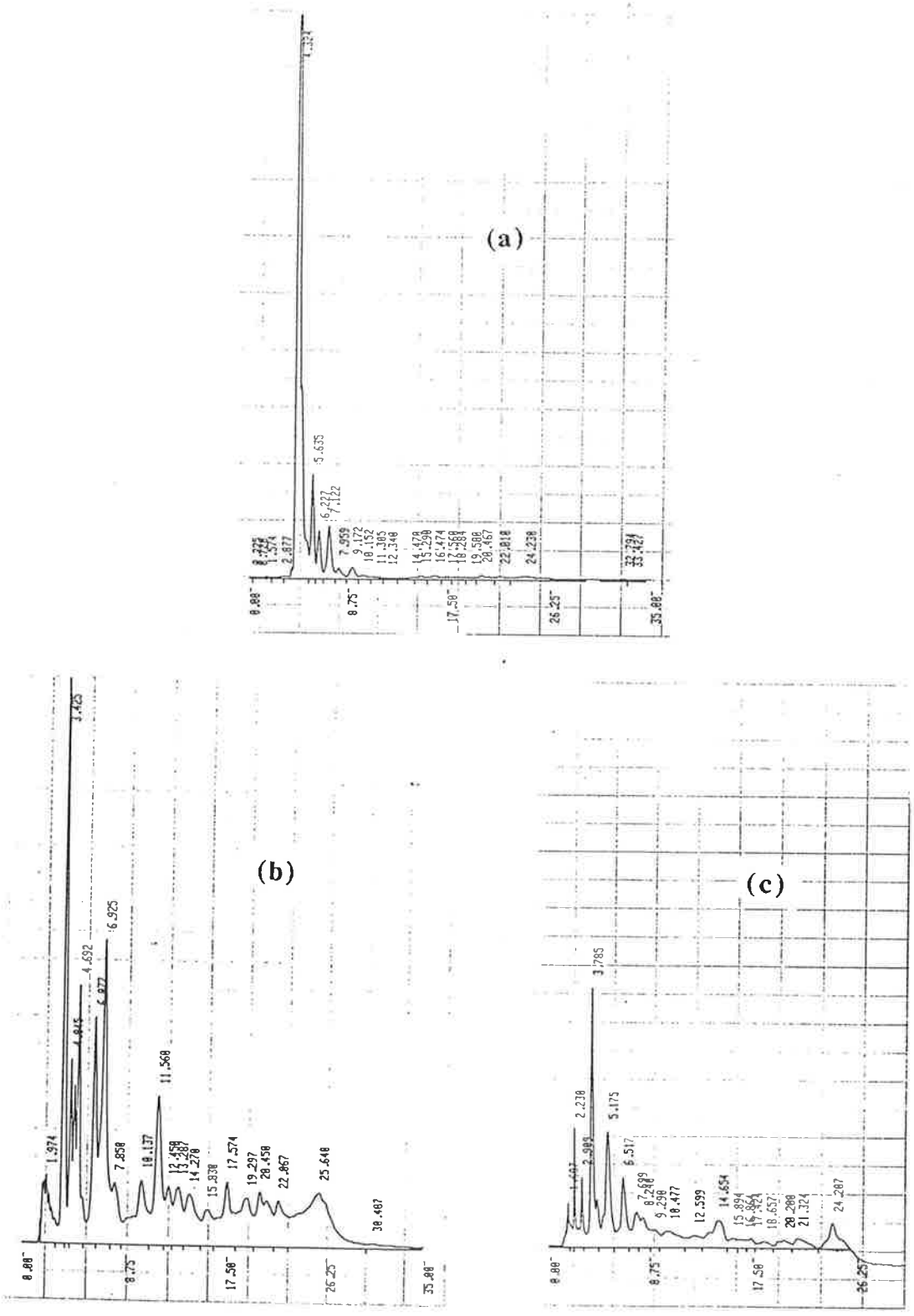


Fig. 2.4 Reversed phase HPLC chromatograms of (a) haematoporphyrin III, HP_{III}, (10) from Section 2.2.3; (b) HPD-III; (c) residue from filtration of crude HPD-III.

of HPD-IX,* obtained using the same solvents,[16] had the same general pattern (polar and less-polar material) the relative proportion of polar and less polar material was significantly different. HPD-IX contains a smaller proportion of polar material (R_t < 10 minutes, 35 – 40%) of which nearly all are HP and HV, while the less polar material accounts for a larger proportion (55 – 60%) of the total material.[21] The HPD-III that was prepared from pure HP_{III} contained approximately 38% of less polar material which is similar to that in HPD-III prepared from crude HP_{III}. This suggests that the low yield of less polar material in HPD-III (prepared from crude HP_{III}) is not due to the impurities present in the starting HP_{III} and probably not due to the the considerable amount of by-products formed during the acetylation step (see above).

The lower yield of less polar material ($R_t \geq 15$ minutes) in HPD-III suggested that polymerization was not occurring readily during the preparation of HPD-III. This might be due to steric restrictions caused by the proximity of the two reactive groups, whether they be 1-acetoxyethyl or 1-hydroxyethyl groups. In addition, the possibility of intramolecular hydrogen-bonding within the HP_{III} monoacetate and HP_{III} diacetate molecules, Fig. 2.3, could reduce the probability of an intermolecular reaction.

Size exclusion chromatography of HPD-III on Sephadex LH-20 gave four fractions, although the resolution was

* In order to differentiate between the haematoporphyrin derivatives in this discussion, the abbreviation "HPD-IX" is used to refer to normal HPD, which is prepared from haematoporphyrin IX.

quite poor after the first fraction. In contrast, HPD-IX separated quite effectively into two main fractions and an intermediate fraction[29] on Sephadex LH-20. The fast-running fraction from HPD-III was shown by HPLC to consist mainly of the less polar material ($R_t \geq 15$ minutes), as expected.[29] However, while the slowest fraction contained relatively more polar material than the first, it was not devoid of the less polar material. In contrast the slowest fraction obtained from HPD-IX was composed entirely of polar monomeric material.[29] It is possible that the column was not sufficiently long for the separation of HPD-III, although it had been used to successfully separate a similar amount of HPD-IX.[21,29] This suggested that HPD-III did not separate as efficiently on Sephadex LH-20 as did HPD-IX.

The proton NMR spectrum of the fast-running fraction of HPD-III in D_2O was extremely poorly resolved, similar to that of the corresponding fraction of HPD-IX. The FAB mass spectrum of the fast-running fraction of HPD-III did not yield any molecular ions but the corresponding esterified (concentrated sulphuric acid/methanol) material showed the presence of monomeric (*m/z* 563, 581, 599), dimeric (*m/z* 1202, 1217), trimeric (*m/z* 1810, 1827) and tetrameric (*m/z* 2420, 2436) ions. No attempt was made to detect higher peaks. The monomeric molecular ions might have been the result of fragmentation of the dimers and oligomers under the mass spectrometric conditions, which is known[21] to occur. In any case, the mass spectrum was similar to that of the corresponding material from HPD-IX.[23] This esterified material was unusually

difficult to redissolve in dichloromethane or deuterochloroform after it had been evaporated to dryness but it was sparingly soluble in acetone.

Despite the presence of fewer regioisomers in HPD-III (see Introduction, Section 2.1) the NMR spectra of the esterified fast-running fraction (the dimeric and/or oligomeric material), which were recorded in deuterochloroform were still disappointingly lacking in resolution. The proton NMR spectrum was very poorly resolved due to the complicated nature of the material, which is a mixture of diastereo- and regioisomers of dimers and oligomers linked by different bridges (see later). Hence it was not possible to obtain any information from the spectrum. The ^{13}C NMR spectrum of this material in deuterated acetone was weak due to the low solubility of the material. Nevertheless it was possible to make out the the stronger signals such those due to the carbons of the ring methyls (11.5 – 12.0 ppm), the carbons in the propionic ester groups ($\underline{C}H_2CH_2CO_2^-$, 21.5 – 21.8 ppm; $\underline{C}H_2CO_2^-$, 36 – 37 ppm; $\underline{C}O_2^-$, ~174 ppm; $CO_2\underline{C}H_3$, ~57 ppm). The meso and pyrrole carbon (C_α, C_β)* signals were broad and barely discernable.

Standard[16] acidic and basic hydrolyses were done on HPD-III in order to determine the type(s) of functional groups linking the porphyrin dimers/oligomers, and, if possible, to determine the relative proportion of dimers/oligomers joined by each type of linkage. The reversed phase HPLC chromatogram of the material obtained after standard[16] basic hydrolysis of HPD-

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* See Appendix for for definition.

III showed a decrease from approximately 30% to approximately 21% (ie. a 30% decrease) in the amount of less polar material ($R_t \geq$ 15 minutes). This was complemented with an increase of approximately similar magnitude (23%) in the proportion of HP_{III} (10) and HV_{III} (23b) (which are two of the expected products from the basic hydrolysis of ester-linked material[16]). Due to the small values involved, minor differences in the integration would translate into large values, making the estimates less reliable. Therefore under these circumstances it is likely that the figures of 30% and 23% do correspond. Another problem with yields based on the peaks of the chromatogram was that any decrease or increase in the proportion of dimeric/oligomeric material would also be expected to result in a change in the proportion of PP_{III} (21b), whose retention time of 22 minutes places it in the retention time range of the dimer/oligomer material. It is not possible to subtract the amount of PP_{III} (21b) from that of the $R_t \geq$ 15 minutes portion because accurate integrations of PP_{III} (21b) cannot be obtained due to the poor resolution of that section of the chromatogram. Admittedly this problem might be overcome by using a solvent gradient that would separate PP_{III} (21b) from the dimeric/oligomeric portion, however the large experimental errors expected from this method of calculation (see above) indicated that that might not be a worthwhile exercise.

Suffice to say that there was a decrease of approximately 30% in the proportion of dimeric/oligomeric material after standard[16] basic hydrolysis of HPD-III indicating

that there was some ester-linked material in the dimeric/oligomeric portion of HPD-III. This conclusion was made on the assumption that the reactivity of ester-linked material in HPD-III was similar to that of HPD-IX.[16] Most of the ester-linked material in freshly prepared HPD-IX converts rapidly (over a period of 48 hours[16]) to ether-linked material on standing,[16,40] until a plateau level of approximately 15% ester-linked material remains.[21] It is interesting to note that although the hydrolysis was done 23 days after the HPD-III had been prepared, there was still a significant amount (30%) of base-labile material present in HPD-III. This suggested that ester-linked material in HPD-III did not convert to ether-linked material as rapidly (if at all) as its IX series analogue.[16,40] Freshly prepared HPD-III was not analysed for its content of ester-linked material.

Acidic hydrolysis[16] of HPD-III resulted in an approximately 80% decrease (from 30% to 6%) in the proportion of dimeric/oligomeric material. This indicated that there was a substantial amount of ether- and ester-linked dimers and/or oligomers in HPD-III because studies with the dimeric/oligomeric material in HPD-IX (Photofrin II)[16] have shown that these groups are readily cleaved under acidic conditions. Deducting the proportion due to ester-linked material (30%, see above) leaves approximately 50% which is due to ether-linked material. The remainder of the $R_t \geq 15$ minutes material amounted to 6% which is 20% of the original less polar material (this includes an indeterminate amount of PP_{III}). On the basis of studies on the IX

analogue[21,41] the dimeric/oligomeric material that was left after acidic hydrolysis is probably carbon-linked.

The FAB mass spectrum of the product from acidic hydrolysis of HPD-III showed a strong peak at *m/z* 599 (corresponding to HP_{III}) and weak peaks at *m/z* 1143 and 1161. Higher mass peaks were not observed but this is not unusual due to the low sensitivity of the mass spectrometer for high mass acid-containing porphyrins.[21] The mass spectrum of the acid-hydrolysed HPD-III resembled that of Ward *et. al.*'s acid-hydrolysed Photofrin II®[21] which was shown to contain carbon-linked dimers and higher oligomers. However, due to the lack of material, the chemical manipulations[21,41] were not done on the material obtained from the acidic hydrolysis of HPD-III so it cannot be conclusively said that the $R_t \geq 15$ minutes material that survived acidic hydrolysis was carbon-linked.

The results from the hydrolysis studies indicated that the composition of HPD-III was similar to that of HPD-IX. Both HPD-IX and HPD-III contain monomers (HP_{IX}, HV_{IX} and PP_{IX} in HPD-IX, and HP_{III}, HV_{III} and PP_{III} in HPD-III) and dimers/oligomers which are linked by ether, ester and carbon bridges. However the relative proportion of the components was significantly different. HPD-III contained less dimeric/oligomeric material and more polar (monomeric) material than HPD-IX. There was also a significant amount of (unidentified) polar by-products in HPD-III which were not present in HPD-IX. Nearly half the dimers/oligomers in HPD-III were ether-linked; of the remainder, there was more ester-linked

than carbon-linked material. In contrast HPD-IX contains nearly twice as much dimeric/oligomeric material and less monomeric (HP_{IX}, HV_{IX} and PP_{IX}) material than HPD-III. Other than the monomers HP_{IX}, PP_{IX} and HV_{IX} there are virtually no other polar impurities in HPD-IX. Ward *et. al.*[21] showed that there was a significant amount (40%) of acid-stable (carbon-linked) material in the dimer/oligomer fraction of HPD-IX (Photofrin II).[21] This is twice the amount of acid-stable material found in the dimer/oligomer portion of HPD-III. Like HPD-III, most of the acid-labile material in the dimer/oligomer fraction of HPD-IX was ether-linked.[21] However there was more ester-linked material (~30%) in HPD-III than in HPD-IX (which contained 10 — 20% ester-linked material) [21] The conversion of ester- to ether-linked material[16,40] appeared to be slower (if it occured at all) in HPD-III.

Freshly prepared HPD-III was tested for *in vivo* phototoxicity using the assay described in the literature.[24] C57BL mice which had been implanted with Lewis Lung Carcinoma cells were given intraperitoneal injections of HPD-III at a dose of 50 mg/kg porphyrin.[24] Twenty four hours later a 1-cm area over the tumour was irradiated with red light (600 — 700 nm).[24] The end point (TC_{50}) was the number of days for 50% of the mice to show a recurrence of tumour.[24] Preliminary tests with HPD-III gave TC_{50} = 0 (HPD-IX, TC_{50} = 5).[24] This was a surprising result considering that HPD-III was similar, in many respects, to HPD-IX. However the absence of any antitumour activity in HPD-III could be due to the low porphyrin concentration in the solution.

As indicated above, the porphyrin concentration in HPD-III was approximately 2.3 mg/ml, which is half that of the usual HPD-IX solutions.[24,38] As a result of that the amount of active material in HPD-III is likely to below the threshold level[42] necessary for biological activity. Hence it is not possible on this basis to form a definite conclusion regarding the relationship between regiochemistry and the biological activity of HPD-IX. It would have been interesting to see if doubling the dose of HPD-III (and hence bringing the amount of injected porphyrin up to the standard[24,38] 5 mg/ml level) would have any effect on its overall *in vivo* activity. However this was not done due to the lack of time and material.

2.2.6 Conclusion

The total synthesis of HP_{III} was undertaken by two literature[7-20,22] routes, both of which involved the cyclization of a biladiene-*ac* salt. The yields of porphyrin were low but comparable to those obtained in the literature.[14,20] NMR data of a porphyrin which had a 1-methoxyethyl group and was a precursor to HP_{III} indicated that there was some steric interaction and intramolecular hydrogen bonding occurring between the two groups on the 3 and 7 positions of the porphyrin.

HPD-III was prepared from crude (75%) HP_{III} as well as from "clean" HP_{III} (approximately 85% pure), and was found to be less soluble in saline than HPD-IX. HPLC and spectral analysis of HPD-III showed that it was similar to HPD-IX in that it contained monomers (HP_{III}, HV_{III} and PP_{III}), dimers and

oligomers. However HPD-III was contaminated with a significant amount of (unidentified) polar impurities which was not present in HPD-IX. HPD-III contained less dimeric/oligomeric material than HPD-IX. Standard[16] acidic and basic hydrolyses of HPD-III showed that the dimers/oligomers were linked by ether, ester and carbon linkages, with the ether-linked material predominating. Tests[24] for *in vivo* antitumour activity indicated that HPD-III was virtually inactive. However this may be due to the low proportion of active material in HPD-III and not necessarily to the regiochemistry of the material.

Recently Pandey *et. al.*[38] showed that two regioisomers of a synthetic porphyrin dimer had different tumoricidal activity. It is possible that regiochemistry might influence the biological activity of HPD-IX in a similar way so that one (or more) of the other fourteen regioisomers (see Appendix) might produce an HPD that is more effective than the present HPD-IX prepared from haematoporphyrin IX. Although this study did not go as far as to address the question of influence (if any) of regiochemistry on the biological activity of HPD, it does indicate that the III (regio)isomer of haematoporphyrin is probably not a good choice for the preparation of HPD because it does not polymerize readily. A better alternative might be the less sterically restricted haematoporphyrin XIII.

ADDENDUM

Subsequent to the writing of this thesis an *in vitro* comparison of the phototoxicity of HPD-IX and HPD-III was made using MBT-2 cells, courtesy of Dr. John Hill, Higginbotham Neuroscience Research Institute, Royal Melbourne Hospital. This study, in which the cells are incubated with excess porphyrin (i.e. concentration differences are unimportant), showed that HPD-III was less phototoxic towards these cells than HPD-IX, both at low and high light exposures.

2.3 Experimental[§]

2.3.0 General

Melting points for non-porphyrin compounds were determined on a Kofler hot-stage apparatus and are uncorrected. Due to the fact that the porphyrins were isomerically impure, melting points for these compounds were not recorded.

Electronic spectra were recorded on a Pye Unicam SP8-100 ultraviolet spectrometer, using 1 cm quartz cells and, unless otherwise stated, dichloromethane as solvent. Absorbances are quoted relative to the intensity of the Soret band.

NMR spectra were obtained on a Bruker CXP300 spectrometer operating at 300 MHz for ^{1}H and 75.47 MHz for ^{13}C spectra. Low resolution proton NMR spectra were recorded on a Varian T-60 spectrometer operating at 60 Mz. Unless otherwise stated, all spectra were obtained in deuterochloroform solutions. Chemical shifts are quoted on the δ scale relative to tetramethylsilane (internal standard). The following abbreviations were used: br broad; d doublet; m multiplet; q quartet; s singlet; t triplet.

Normal (electron impact, E.I.) mass spectra were recorded on an A.E.I. MS-3074 spectrometer at 70 eV. Fast Atom Bombardment (FAB) mass spectra were obtained on a VG ZAB-2HF mass spectrometer using Argon atoms accelerated to 8 kV. Samples were dissolved in a 3-nitrobenzyl alcohol matrix. In the

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[§] References, p. 98.

spectra of mixtures, peak intensities are sometimes described by the abbreviations m moderate, s strong or w weak.

Reversed phase high performance liquid chromatography (HPLC) was carried out according to the literature[16] procedure on a Waters Novapak C18 cartridge using a Waters dual solvent delivery system controlled by a Waters model 680 Automated Gradient Controller and a U6K injector with a Waters Z-module. The absorbance detector (Waters Model 481AZ) was set at 397 nm and peaks were integrated using a Waters 740 Data Module. All HPLC solvents were distilled and filtered (through a 0.45 μm Millipore filter) before use. Aqueous methanol solutions, 15 : 85 ("Solvent B") and 10 : 90 ("Solvent A"), each containing 2.5×10^4 mM tetra-*n*-butylammonium phosphate were adjusted to pH 3.6 and 7.5 respectively by the addition of phosphoric acid and, when necessary, triethylamine. The column was equilibrated with Solvent B (85% methanol/water) for at least 10 min before the sample was injected. The eluting solvent was changed exponentially to Solvent A 10 min after injection. A typical run lasted 35 min.

Analytical thin layer chromatography (TLC) was performed on Merck Kieselgel 60 PF_{254}. The plates were developed in mixtures of methanol/dichloromethane. Preparative thin layer chromatography was done on pre-coated (0.2 mm) Merck Kieselgel 60 (indicator free) plates. Squat column chromatography was performed using silica gel (Merck Kieselgel type 60G), unless otherwise stated.

Solvents were dried (where applicable) by the literature[48] procedure. Gaseous hydrogen bromide (Matheson) was used without prior treatment. "Concentrated ammonium hydroxide" refers to strong ammonia solution of S. G. 0.90 (approximately).

2.3.1 Benzyl 4-(2-acetoxyethyl)-3,5-dimethylpyrrole -2-carboxylate.[7b] (13)

Benzyl acetoacetate[7a] (7.74 g, 40.3 mmol) in acetic acid (13 ml) was nitrosylated[7] at *ca.* 30°C by the addition of aqueous sodium nitrite (10 M, 4 ml) and the mixture was stirred at room temperature for 5 h to yield the corresponding hydroxyimino compound.

A solution of 3-acetyl-4-oxopentyl acetate[7b] (12) (7.5 g, 40.3 mmol) in acetic acid (13 ml) was heated to 70°C. Heating was discontinued and a mixture of zinc dust (6.26 g) and sodium acetate (6.26 g) was added portionwise with stirring while the above hydroxyimino compound was added dropwise. The additions were regulated so that the zinc mixture was always in excess and the temperature of the reaction. mixture was maintained at 90-100°C. After the addition, the mixture was heated under reflux for 15 min and then allowed to cool to room temperature. The yellow oil was poured into ice water (*ca.* 300 ml) with vigorous stirring. A yellow precipitate was obtained after overnight refrigeration (5°C) and was collected and crystallized from aqueous ethanol to give the *title pyrrole* (1.34 g, 11%) m.p. 70.5-72°C [lit.[7] 72-74°C]. ^{1}H NMR (60 MHz, $CDCl_3$), δ ppm : 2.05 (s, 3H, OCOC$\underline{H}_3$), 2.25, 2.30 (6H, 2 × s, 2 × ring C$\underline{H}_3$), 2.68

(t, 2H, $C\underline{H}_2CH_2OCOCH_3$), 4.02 (t, 2H, $C\underline{H}_2OCOCH_3$), 5.30 (s, 2H, $PhC\underline{H}_2O_2C$), 7.37 (s, 5H, Ph). The mother liquor was evaporated to dryness, leaving an oil which, by NMR analysis, was mainly the desired pyrrole. Chromatography of this material on a silica squat column and elution with dichloromethane and gave a yellow oil (7.0 g, 55%). TLC and NMR data of this sample matched those of the crystallized material.

2.3.2 4-(2-Acetoxyethyl)-3,5-dimethylpyrrole-2-carboxylic acid.[9] (14)

A mixture consisting of benzyl 4-(2-acetoxyethyl)-3,5-dimethylpyrrole-2-carboxylate (13) (1.34 g, 4.26 mmol), palladium on charcoal (5%, 1.0 g) and triethylamine (12 drops) in tetrahydrofuran (15 ml) was hydrogenated at room temperature at atmospheric pressure until hydrogen uptake had ceased. The catalyst was removed by vacuum filtration on Kenite and washed with hot THF. Evaporation of the solvent gave a gray solid which was recrystallized from THF/methanol to give the *title pyrrole* (0.66 g, 70%) m.p. 163°C(d) [lit. m.p.[9] 168°C]. ^1H NMR (60 MHz, $CDCl_3/d_6$-DMSO), δ ppm : 2.00 (s, 3H, $OCOC\underline{H}_3$), 2.68 (t, 2H, $C\underline{H}_2CH_2OCOCH_3$), 2.18 (s, 6H, 2 × ring $C\underline{H}_3$), 4.05 (t, 2H, $C\underline{H}_2OCOCH_3$). Mass spec. (E.I.), *m/e* 225.

2.3.3 2-Formyl-4-(2-acetoxyethyl)-3,5-dimethyl-pyrrole (15)

4-(2-Acetoxyethyl)-3,5-dimethylpyrrole-2-carboxylic acid (14) (0.65 g, 2.9 mmol) was dissolved in trifluoroacetic acid (6 ml) and stirred for 30 min at room temperature. Triethyl

orthoformate (2 ml) was then added in one lot with stirring. The mixture was stirred at room temperature for 5 min, then water (60 ml) was added and the resulting solution was neutralized with 10% aqueous sodium bicarbonate. The solution was extracted with dichloromethane and the organic extracts were washed with brine, dried ($MgSO_4$) and evaporated to dryness. The solid residue was recrystallized from aqueous ethanol to give the *title formyl pyrrole* (0.26 g, 43%), m.p. 99-100.5°C. ^{1}H NMR, δ ppm: 2.07 (s, 3H, OCOC$\underline{H}_3$), 2.33 (s, 6H, 2 × pyrrole-C$\underline{H}_3$), 2.71 (t, 2H, C$\underline{H}_2$CH$_2$OCOCH$_3$), 4.12 (t, 2H, C$\underline{H}_2$OCOCH$_3$), 9.58 (s, 1H, C$\underline{H}$O). Mass spec. (E.I.) *m/e* 209.

2.3.4 1,19-Dideoxy-2,18-di(2-acetoxyethyl)-8,12-di(2-methoxycarbonylethyl)-1,3,7,13,17,19-hexamethylbiladiene-ac dihydrobromide (17)

To 3,3'-di(2-methoxycarbonylethyl)-4,4-dimethyl-2,2'-dipyrrylmethane-5,5'-dicarboxylic acid[11] (16) (0.40 g, 0.9 mmol) was added trifluoroacetic acid (5 ml). The mixture was stirred under nitrogen at room temperature for 10 min until the evolution of carbon dioxide had ceased.

A solution of 2-formyl-4-(2-acetoxyethyl)-3,5-dimethylpyrrole (15) (0.4 g, 1.9 mmol) in methanol (15 ml) was added dropwise with stirring to the solution of the above dipyrrylmethane in trifluoroacetic acid. Then hydrobromic acid-acetic acid (33%, 5 ml) was added dropwise and stirring was continued for 45 min. Dry ether (80 ml) was added dropwise to precipitate the biladiene-*ac* salt. The suspension was refrigerated (5°C) overnight, and the salt was collected by

vacuum filtration and washed thoroughly with ether. The *title biladiene-ac salt* (0.64 g, 79%) was used without further purification. λ_{max} (rel. abs.) 453 nm (69%), 526 (100%). ^{1}H NMR (60 MHz), δ ppm: 1.8 (s, 6H, 2 × $OCOC\underline{H}_3$), 2.05, 2.28, 2.36 (s, each 6H, 6 × $C\underline{H}_3$), 2.75 (2 × $C\underline{H}_2CO_2CH_3$, 2 × $C\underline{H}_2CH_2OCOCH_3$), 3.47 (2 × $C\underline{H}_2CH_2CO_2$, 2 × $OC\underline{H}_3$), 4.13 (t, 2 × $C\underline{H}_2OCOCH_3$), 5.27 (br, 2H, methine CH_2), 7.16 (br, s, 2 × methine C<u>H</u>), 13.5 13.6 (br, 2 × 2H, 4 × pyrrole H).

2.3.5.1 3,7-Di(2-hydroxyethyl)-13,17-di(2-methoxycarbonylethyl)-2,8,12,18-tetramethylporphyrin. [*3,7-Di(2-hydroxyethyl)deuteroporphyrin III dimethyl ester.*][14] (19)

The foregoing biladiene-*ac* salt (17) (0.60 g, 0.68 mmol) was added to a solution of copper-II acetate (4.9 g, 20.4 mmol, 30 equivalents) in dry DMF (20 ml) stirring at 155-160°C under nitrogen. Stirring was continued for 6 min, then the reaction mixture was cooled briefly and poured into water (230 ml). The resulting emulsion was extracted with dichloromethane (6 × 60 ml). The combined organic phase was washed with water (4 × 40 ml), dried (Na_2SO_4) and evaporated to dryness, leaving a black solid (0.513 g). λ_{max}(rel. abs.) 402 nm (100%), 525 (17.7%), 561 (26.0%)

The crude metalloporphyrin (18) was taken up in concentrated sulphuric acid/trifluoroacetic acid (10%, 30 ml) and the mixture was stirred in the dark at room temperature for 65 min, then diluted with dichloromethane (150 ml) and poured into water. The organic phase was separated and the remaining

aqueous phase was extracted further with dichloromethane. The combined organic phase was washed with water (3 × 50 ml), dried (Na_2SO_4) and evaporated to dryness. The solid residue was dissolved in a mixture of methanol/trimethyl orthoformate (1:1, 10 ml). Water (0.5 ml) was added and the solution was cooled in an ice-bath. Concentrated sulphuric acid (1 ml) was added dropwise with stirring. The mixture was stirred in the dark overnight.

The reaction mixture was diluted with dichloromethane poured into 10% aqueous sodium hydroxide solution, neutralized and extracted with more dichloromethane. The combined organic extracts were washed with water, dried (Na_2SO_4) and evaporated to dryness. The residue, λ_{max} (rel. abs.) 409 nm (100%), 498 (25.9%), 531 (19.4%), 567 (15.5%), 621 (11.3%), was chromatographed on a silica squat column, where the fraction containing the *title porphyrin* was eluted with 2% methanol/dichloromethane. Evaporation of the solvent gave 3,7-di(2-hydroxyethyl)deuteroporphyrin-III dimethyl ester (19) (0.118 g, 28%). ^{1}H NMR, δ ppm (-4.6) — (-5.3) (2 × pyrrole H), 3.3-3.8 (2 × $CH_2C\underline{H}_2OH$, 2 × $C\underline{H}_2CH_2CO_2CH_3$, 4 × ring $C\underline{H}_3$, 2 × $CO_2C\underline{H}_3$), 4.20 (2 × $C\underline{H}_2CH_2OH$), 4.43 (2 × $CH_2C\underline{H}_2CO_2CH_3$), 10.13 (4 × meso H). FAB m.s. *m/z* 627.

2.3.5.2 Oxidative cyclization of (17) at room temperature[25]

The biladiene-*ac* salt (17) (40 mg, 0.045 mmol), prepared by the procedure outlined in Section 2.3.4, was added in one lot to a solution of dry DMF (4 ml) containing copper(II)

acetate (0.27 g, 1.35 mmol, 30 equivalents). The mixture was stirred at room temperature for 2 h and then worked up in the usual manner (see Section 2.3.5.1). After demetallation and reesterification, the crude material was chromatographed on silica to give 3,7-di(2-hydroxyethyl)deuteroporphyrin-III dimethyl ester (19) (4 mg, 18% based on the starting a,c-biladiene salt). The porphyrin was identical (FAB m.s., vis. spec. and TLC) to the sample obtained by oxidative cyclization in refluxing DMF above (Section 2.3.5.1).

2.3.6 3,7-Di(2-chloroethyl)-13,17-di(2-methoxycarbonylethyl)-2,8,12,18-tetramethylporphyrin.

[*3,7-Di(2-chloroethyl)deuteroporphyrin III dimethyl ester.*][14,15] (20)

3,7-Di(2-hydroxyethyl)deuteroporphyrin III dimethyl ester (19) (0.118 g, 0.188 mmol) was dissolved in a mixture of benzoyl chloride (2 ml) and dimethylformamide (20 ml) and stirred at 95°C for 75 min under nitrogen. The reaction mixture was cooled to room temperature and aqueous triethylamine (6%, 120 ml) was added with stirring. The resulting emulsion was extracted with dichloromethane (4 × 50 ml), and the combined organic phases were washed with water (5 × 30 ml). Evaporation of the solvent gave a red-brown solid which was chromatographed on a silica squat column. The desired fraction was eluted with dichloromethane. Evaporation of the solvent gave the *title porphyrin* (0.124 g, 99%). λ_{max} (rel. abs.) 398.5 nm (100%), 498 (9.0%), 530 (6.1%), 566.5 (4.4%), 620 (2.7%). ^{13}C NMR ($CDCl_3$ + CD_3COCD_3), δ ppm 11.59, 11.79 (ring $\underline{C}H_3$), 21.61

($\underline{C}H_2CH_2CO_2CH_3$), ~30.09 ($\underline{C}H_2CH_2Cl$),* 36.69 ($CH_2\underline{C}H_2CO_2CH_3$), 45.43 ($CH_2\underline{C}H_2Cl$), 51.64 ($O\underline{C}H_3$) 96.08, 96.54, 96.97 (meso C), 173.46 ($\underline{C}O_2CH_3$); the signals due to the pyrrole carbons were not distinguishable from the baseline. ^1H NMR ($CDCl_3$ + CD_3COCD_3), δ ppm: -3.89 (br, pyrrole H), 2.90 (2 × $CH_2C\underline{H}_2Cl$), 3.04 ($C\underline{H}_2CO_2CH_3$), 3.21 ($OC\underline{H}_3$), 3.57 (ring $C\underline{H}_3$), 4.24-4.43 (2 × $C\underline{H}_2CH_2CO_2CH_3$, 2 × $C\underline{H}_2CH_2Cl$), 9.86-10.11 (meso H). FAB mass spec., *m/z* 663, 665, 667 (M^+).

2.3.7 13,17-Di(2-methoxycarboxyethyl)-2,8,12,18-tetramethyl-3,7-divinylporphyrin.

[*Protoporphyrin III dimethyl ester.*][14,15] (21a)

3,7-Di(2-chloroethyl)deuteroporphyrin III dimethyl ester (20) (0.165 g, 0.25 mmol) was dehydrochlorinated according to the literature procedure[14,15] using deoxygenated pyridine (64 ml) and aqueous sodium hydroxide solution (3%, 14 ml).

The crude porphyrin was dissolved in concentrated sulfuric acid/methanol (5%, 20 ml) and the mixture was left to stand in the dark for two days. The mixture was diluted with dichloromethane (30 ml) and poured into water, neutralized and extracted with more dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. The solid residue was chromatographed on a silica squat column. *Protoporphyrin III dimethyl ester* (21a) (0.105 g, 71%) was eluted with 0.5% acetone/dichloromethane. λ_{max} (rel. abs.) 407 nm (100%), 506 (8.7%), 541 (7.4%), 576 (5.1%), 630 (3.8%). ^{13}C

* obscured by $(CD_3)_2CO$ signals.

NMR ($CDCl_3$ + CD_3COCD_3), δ ppm 11.71, 12.69 (ring $\underline{C}H_3$), 21.67 ($\underline{C}H_2CH_2CO_2CH_3$), 36.95 ($\underline{C}H_2CO_2CH_3$), 51.77 ($O\underline{C}H_3$), 96.16-98.49 (meso C), 120.87 ($CH=\underline{C}H_2$), 130.31 ($\underline{C}H=CH_2$), 173.60 ($\underline{C}O_2CH_3$). ^1H NMR ($CDCl_3$ + CD_3COCD_3), δ ppm -3.63 (2 × pyrrole H), 3.26 (m, 4 H, 2 × $CH_2C\underline{H}_2CO_2CH_3$), 3.63 (2 × $CO_2C\underline{H}_3$), 3.66 (× ring $C\underline{H}_3$), 4.38 (m, 4 H, 2 × $C\underline{H}_2CH_2CO_2CH_3$), 6.15-6.39 (2 × d, $CH=C\underline{H}_2$), 8.18-8.28 (m, $C\underline{H}=CH_2$), 9.97, 10.00, 1025 (4 H, 4 × meso H). FAB m.s. *m/z* 591.

2.3.8 3,7-Di(2-hydroxyethyl)-13,17-di(2-methoxycarbonylethyl-2,8,12,18-tetramethylporphyrin [*Haematoporphyrin III dimethyl ester*] (22)

To protoporphyrin III dimethyl ester (21a) (80 mg, 0.135 mmol) was added saturated HBr/dichloromethane† (8 ml) and the mixture was stirred for 100 min at room temperature. The reaction mixture was then diluted with dry THF (8 ml) and added dropwise rapidly into ice-water (*ca.* 20 ml) with stirring. The resulting mixture was stirred for 5 minutes, then neutralised and extracted with dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. The solid residue was placed on a silica squat column. The porphyrins were eluted with solvents of increasing polarity: protoporphyrin III dimethyl ester (17 mg), identical (TLC, vis. spec., FAB m.s.) to authentic sample in Section 2.3.7, was eluted with dichloromethane; 3(1-hydroxyethyl)-7-vinyldeuteroporphyrin III dimethyl ester (23a) λ_{max} (rel. abs.) 402 nm (100%), 501

† Prepared by bubbling gaseous HBr into dry dichloromethane at a rate of approximately 1 min/ml dichloromethane.

(8.4%), 536 (6.7%), 572 (5.2%), 626 (3.3%), FAB m.s. *m/z* 609, along with the ether-linked dimers (24), (25) and (26) (total 52 mg) (TLC on silica in 5% methanol/dichloromethane, R_F 0.5 – 0.9, similar to those of the corresponding IX isomers[45]) with methanol/dichloromethane (0.4-0.8%) and *haematoporphyrin III dimethyl ester* (10 mg, 12%) was eluted with methanol/dichloromethane (1-1.5%), λ_{max} (rel. abs.) 401 nm (100%), 499 (9.8%), 533 (5.7%), 569 (4.3%), 622 (2.4%). ^{13}C NMR, δ ppm 11.09, 11.51 (ring $\underline{C}H_3$), 21.68 ($\underline{C}H_2CH_2CO_2CH_3$), 25.95, 26.22 ($\underline{C}H_3CHOH$), 36.69 ($\underline{C}H_2CO_2CH_3$), 51.77 ($CO_2\underline{C}H_3$) 65.54 ($CH_3\underline{C}HOH$), 95.75, 96.36, 100.42 (meso C), 136.3, 138.06 (Cβ), 141.73 (br, Cα), 173.59 ($\underline{C}O_2CH_3$). ^1H NMR, δ ppm: -4.52 (br, 2 × pyrrole H), 1.79(d) (J 6.6 Hz), 1.92(d) (J 6.6 Hz) (2 × d, $C\underline{H}_3CHOH$), 3.19 (t, $C\underline{H}_2CO_2CH_3$), 3.41 ($CO_2C\underline{H}_3$), 3.663 (ring $C\underline{H}_3$), 4.21 (m, $C\underline{H}_2CH_2CO_2CH_3$), 5.74-5.84 (q, $CH_3C\underline{H}OH$), 9.52, 9.67, 9.70, 10.44 (meso H). FAB m.s., *m/z*: 627.

The 1-hydroxyethyl substituted ether-linked dimers (24) and (26) (40 mg) from above were combined and dissolved in THF (6 ml). To this was added aqueous hydrochloric acid (1M, 2 ml) and the mixture was refluxed for 2 h, then allowed to cool to room temperature. Water (8 ml) was added, and the mixture was extracted with THF/dichloromethane. The combined organic phases were washed with water and evaporated to dryness. The solid residue was redissolved in concentrated sulphuric acid/methanol (5%, 10 ml) and left to stand in the dark overnight. The mixture was diluted with water (20 ml), neutralised and extracted with dichloromethane. The combined

organic phase was washed with water and evaporated to dryness. The solid residue was chromatographed on a silica squat column as outlined above to give protoporphyrin III dimethyl ester (21a) (8 mg) identical (TLC, vis. spec., FAB m.s.) to authentic sample in Section 2.3.7; 3(1-hydroxyethyl)-7-vinyldeuteroporphyrin III dimethyl ester (23a) (7 mg), physical data (TLC, FAB m.s., vis. spec.) similar to those of the sample above and haematoporphyrin III dimethyl ester (22) (25 mg, 29%), identical (vis. spec., TLC, FAB m.s., NMR) to the sample obtained above.

Haematoporphyrin III dimethyl ester (35 mg) from above was dissolved in a mixture of THF (5 ml) and 1.0 M sodium hydroxide. The solution was stirred at room temperature in the dark for 20 h, then diluted with water (20 ml), acidified (pH 5) and extracted with THF/dichloromethane. The extracts were combined, washed with water and evaporated to dryness to afford *haematoporphyrin III* (10), FAB m.s. *m/z* 599, HPLC R_t 3.3 min (86%, haematoporphyrin III),.5.02 min (4%), 6.25 min (2%), 7.84 min (2%).

2.3.9 3-Acetyl-5-formyl-2,4-dimethylpyrrole[19] (29)

Ethyl 3-acetyl-2,4-dimethylpyrrole-5-carboxylate[45] (27) (10 g, 47.8 mmol) was suspended in a mixture of ethanol (20 ml) and aqueous sodium hydroxide solution (10%, 30 ml) and the mixture was refluxed for 2 h. The reaction mixture was cooled, diluted with water (20 ml) and acidified with concentrated hydrochloric acid. The white precipitate was collected and recrystallized from methanol to give 3-acetyl-2,4-

dimethylpyrrole-5-carboxylic acid (28) (7.56 g, 87%), m.p. 207-210°C (lit.[46] 208–210°C).

(a) Modification of the decarboxylation/formylation procedure[19b]

3-Acetyl-2,4-dimethylpyrrole-5-carboxylic acid (28) (2.5 g, 13.8 mmol) was dissolved in trifluoroacetic acid (12 ml) and stirred under nitrogen at room temperature for 95 min, then cooled in an ice-bath. Triethyl orthoformate (6 ml) was added to the mixture in one lot and the resulting orange-red solution was stirred at 0°C for 4 min, then poured into aqueous sodium hydroxide (10%, 100 ml) with stirring. The pH was adjusted to 10 by addition of aqueous hydrochloric acid (10%), and the mixture was extracted with dichloromethane (4 × 50 ml). Combined organic extracts were washed with brine (2 × 30 ml), dried over Na_2SO_4 and evaporated to dryness. The solid residue was recrystallized with aqueous ethanol to give *3-acetyl-5-formyl-2,4-dimethylpyrrole* (29) (1.19 g, 52%), m.p. 165-167°C (lit.[47] 166°C) ^{1}H NMR (60 MHz), δ ppm: 2.5 (s, 3H, $COC\underline{H}_3$), 2.67 (s, 6H, 2 × $C\underline{H}_3$), 9.8 (s, 1H, $C\underline{H}O$).

(b) Using a longer decarboxylation time.

The pyrrole carboxylic acid (28) (0.874 g, 4.83 mmol) was treated with TFA (6 ml) and the mixture was stirred at room temperature under nitrogen for 2 h. The reaction mixture was cooled in an ice-bath and triethyl orthoformate (3 ml) was added in one lot with stirring. Stirring was continued for 4 min and then the mixture was poured into aqueous sodium hydroxide.

After work-up and recrystallization in the manner outlined above, the *title formyl pyrrole* (29) (0.714 g, 90%) was obtained. NMR and m.p. of this sample matched those obtained above.

2.3.10 1,19-Deoxy-2,18-diacetyl-8,12-di(2-methoxycarbonylethyl)-1,3,7,13,17,19-hexamethylbiladiene-*ac* dihydrobromide (30).

A solution of 3,3'-di(2-methoxycarbonyl-ethyl)-4,4'-dimethyl-2,2'-dipyrrylmethane-5,5'-dicarboxylic acid[11] (16) (0.685 g, 1.58 mmol) in trifluoroacetic acid (6.5 ml) was added dropwise to a suspension of 3-acetyl-5-formyl-2,4-dimethylpyrrole (29) (0.6 g, 3.64 mmol) in methanol (20 ml) and the mixture was stirred at room temperature for 15 min. Hydrobromic acid in acetic acid (45%, 3 ml) was added dropwise to the mixture which was stirred for a further 35 min, then ether (160 ml) was added dropwise with stirring at 0°C. The crude *biladiene-ac salt* (30) (1.13 g, 89%) was collected 90 min later, washed with ether, dried and used immediately. λ_{max} (rel. abs.) 450 nm (55%), 481 (45%), 521 (100%). ^{1}H NMR (60 MHz), δ ppm 2.03 (s, 6H, 2 × ($COC\underline{H}_3$), 2.36, 2.53, 2.65 (s, each 6H, 6 × $C\underline{H}_3$), 3.0 (2 × $C\underline{H}_2CO_2$), 3.52 (10 H, 2 × $C\underline{H}_2CH_2CO_2$, 2 × $OC\underline{H}_3$), 5.43 (br, 2 × 2H, methine CH_2), 7.43 (s, 2H, methine CH), 13.57, 14.17 (br, 2H, 2 × pyrrole H).

2.3.11 3,7-Diacetyl-13,17-di(2-methoxycarbonylethyl)-2,8,12,18-tetramethylporphyrin.

[*3,7-Diacetyldeuteroporphyrin III dimethyl ester*] (32)

The foregoing biladiene-*ac* salt (30) (2.056 g, 2.57 mmol) was added in one lot to a solution of copper(II) acetate (16.9 g, 84.6 mmol) in dry DMF (160 ml) heated to 138°C under nitrogen. The mixture was stirred at that temperature for 6 min, then cooled to room temperature and poured into water (600 ml) and extracted with dichloromethane. The combined organic extracts were washed with water, whereupon an emulsion formed. Attempts were made to break up the emulsion by filtering it through Kenite and/or filter paper, but in both cases the filters were quickly blocked. In another attempt THF was added to the emulsion and swirled gently but the emulsion persisted. So the emulsion was extracted repeatedly with dichloromethane (6 × 30 ml) until no more colour entered the organic phase. The combined organic phases were evaporated to dryness without chemical drying. (Residual dimethylformamide was removed under high vacuum.)

The aqueous emulsion was diluted with water (*ca.* 300 ml) and extracted with dichloromethane (5 × 30 ml). Most of the brown-black colour was extracted into the organic phase, leaving the aqueous phase pale blue. The combined organic phases were evaporated to dryness and the solid residue was combined with that obtained above.

The crude metalloporphyrin was dissolved in concentrated sulphuric acid/TFA (5%, 20 ml) and stirred at room

temperature for 75 min. The mixture was then diluted with water (*ca.* 100 ml) and extracted with THF/dichloromethane (4 × 25 ml). The combined organic phases were washed with dilute hydrochloric acid and evaporated to dryness without chemical drying. The solid residue had λ_{max} (rel. abs.) 418 nm (100%), 476 (17.6%), 510 (14.7%), 548 (13.7%), 587 (13.0%), 640 (8.2%).

This crude porphyrin was taken up in concentrated sulphuric acid/methanol (5%, 20 ml) and stirred in the dark overnight. The mixture was diluted with dichloromethane (*ca.* 20 ml) and poured into water (100 ml). Dilute aqueous sodium hydroxide solution (10%) was added until the pH of the solution was pH 10. When an attempt was made to extract the mixture, an intractible emulsion formed. This was extracted repeatedly (while gently swirling the separating funnel) with dichloromethane (6 × 20 ml). Then the remaining emulsion was diluted with water (300 ml) and extracted again with dichloromethane (3×) until no more colour entered the organic phase. The combined organic phases were evaporated to dryness, leaving a black solid.

When dichloromethane (3 ml) was added to the residue in order to redissolve it for chromatography, the material did not readily dissolve — a fine, black suspension formed instead. The suspension did not redissolve when acetone and THF were added to the solution. So more dichloromethane (15 ml) was added to the suspension which was then filtered. The filter paper was quickly blocked so filtration was abandoned.

The filtrate and remaining suspension were combined and concentrated to *ca.* 10 ml.

One portion (5 ml) of this suspension was placed on a short squat column packed with silica. Dichloromethane was run through the column until the crude material had settled, then the polarity of the eluting solvent was gradually increased to 4% acetone/dichloromethane. Elution was stopped when the eluate was no longer red. The main fraction (red-purple band) was evaporated to give a solid (50 mg). FAB m.s., *m/z*: 623 (M^+), 684. (3,7-diacetyldeuteroporphyrin III dimethyl ester (32) and Cu^{II}-3,7-diacetyldeuteroporphyrin III dimethyl ester (31), respectively). TLC (silica, 5% methanol/dichloromethane): main spot, R_F 0.70, some material was present on the baseline.

The remaining portion of the crude porphyrin suspension (5 ml) was placed on a squat column packed with alumina (Brockmann Grade I). Similar prolonged elution with 10% acetone/dichloromethane and evaporation of the solvent gave a solid (100 mg) which was found (by TLC) to be similar to that obtained from the silica column above.

2.3.12 Haematoporphyrin III dimethyl ester (22) : from the reduction of diacetyldeuteroporphyrin III dimethyl ester (32).

Purified 3,7-diacetyldeuteroporphyrin III dimethyl ester (32) (0.15 g, 0.2 mmol) from Section 2.3.11 was suspended in methanol/dichloromethane (20% v/v, 5 ml). To this was added sodium borohydride (50 mg) and the mixture was stirred at room

temperature in the dark for 1 h, until all the porphyrin had dissolved, forming a bright red solution.

Water (6 ml) was added to the mixture, which was then acidified with dilute hydrochloric acid and extracted with dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. The solid residue was taken up in methanol/trimethyl orthoformate/concentrated sulphuric acid/water (5 : 5 : 1 : 0.5; 11.5 ml) and the mixture was stirred at room temperature in the dark for 1 h. Then, it was diluted with water, neutralised and extracted with dichloromethane. The organic extracts were washed with water and evaporated to dryness to yield a dark solid, λ_{max} (rel. abs.) 411 nm (100%), 500 (10.2%), 530 (6.4%), 565 (4.9%), 621 (2.7%)

The crude product was chromatographed on a squat column packed with alumina (Grade I). Three fractions were collected: Fraction 1 (65 mg), eluted with dichloromethane, had FAB m.s. *m/z* 655, 716 (3,7-di(1-methoxyethyl)-deuteroporphyrin III dimethyl ester (34) and its Cu-metalloporphyrin (38), respectively); Fraction 2 (50 mg), eluted with 1-5% acetone/dichloromethane, had FAB m.s. *m/z* 627, 684 m.u. (HP_{III}dme and Cu-3,7-diacetyldeuteroporphyrin III dimethyl ester (31), respectively) and Fraction 3 (20 mg), eluted with 10% acetone/dichloromethane had FAB m.s. *m/z* 627, 684 m.u. (as in Fraction 2).

2.3.13 Investigation into the conditions needed for the acidic hydrolysis of 3,7-di(1-methoxyethyl)-deuteroporphyrin III dimethyl ester, using 3,8-di(1-methoxyethyl) deuteroporphyrin IX dimethyl ester (39) as the model system

(a) Room temperature

3,8-Di(1-methoxyethyl)deuteroporphyrin IX dimethyl ester[13,29] (39) (10 mg) was dissolved in a mixture of 1 M hydrochloric acid/THF (1:1, 6 ml) and stirred at room temperature in darkness for 16 h. The reaction mixture was diluted with water (20 ml) and extracted with THF/dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. The residue, analysed by HPLC, gave HP_{IX} (1) (R_t 5.8 min, 0.6%), HV_{IX} (4) (R_t 7.93 min, 4.5%), 3(1-hydroxyethyl)-8-(1-methoxyethyl)-deuteroporphyrin IX (R_t 11.41 min, 12.3%), 3,8-di(1-methoxyethyl)deuteroporphyrin IX (R_t 15.84 min, >36%), 3,8-di(1-methoxyethyl)deuteroporphyrin IX dimethyl ester (39) (R_t 21.12 min, 33.6%). The retention times were similar to those in the literature.[16]

(b) Reflux

3,8-Di(1-methoxyethyl)deuteroporphyrin IX dimethyl ester[13,29] (39) (50 mg) was dissolved in 1M hydrochloric acid/THF (1:1, 16 ml) and heated under reflux. Aliquots (*ca.* 1ml) were removed at intervals and worked-up in the manner

described in Section 2.3.13.(a) above. Results from the HPLC analyses are summarised in Table 2.1.

2.3.14 Attempted reduction of crude Cu^{II}-3,7-diacetyl-deuteroporphyrin III dimethyl ester (31)

The crude Cu^{II}-3,7-diacetyldeuteroporphyrin III dimethyl ester (31) (188 mg) from the Section 2.3.11, prior to chromatography, was suspended in methanol/dichloromethane (1 : 4, 10 ml). To this was added sodium borohydride (*ca.* 100 mg) and the mixture was stirred at room temperature in darkness for 20 h. The the reaction mixture was diluted with water (5 ml) and acidified with dilute hydrochloric acid. The mixture was extracted with dichloromethane (3 × 20 ml), the combined organic extracts were washed with water and evaporated to dryness without chemical drying.

The solid residue was taken up in a mixture of methanol/trimethyl orthoformate/conc. sulphuric acid/water (5 : 5 : 1 : 0.5, 11.5 ml)[13] and stirred for 1 h. Then the reaction mixture was diluted with water (20 ml), neutralised and extracted with dichloromethane. The combined organic extracts were washed with water and evaporated to yield a solid whose TLC (silica, 5% methanol/dichloromethane) showed a major spot R_F 0.47 corresponding to Cu^{II}-3,7-diacetyldeuteroporphyrin III dimethyl ester (31). Below this point, there were mainly poorly resolved stretching from the baseline. The desired Cu^{II}-3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (38) was present in only trace amounts.

Table 2.1 HPLC analyses of the material obtained from the acidic hydrolysis (reflux) of 3,8-di(1-methoxyethyl)deutero-porphyrin IX dimethyl ester (39).

Reaction time (hours) under reflux	Products and yields[a] HP	HV	PP	Others
1	5	-[b]	-[b]	R_t 4.7-6.1 min (53%), R_t 11.07 min(26%), R_t 11.9-25.3 min(14%),
2	16	15	1	R_t 4.7-6.1 min(58%), R_t 11.1-21.9 min(8%)
6	74	11	2	R_t 4.8-6.3 min(13%)
7	88	12	-[c]	None
24	57	15	8	R_t 7-8.5 min(15%), R_t 22- 25.1 min(9%),

a. The approximate retention times are: HP_{IX} (3.8-4.2 min), HV_{IX} (7.6-9.6 min), PP_{IX} (~ 22 min), 3,8-di(1-methoxyethyl)deuteroporphyrin IX dimethyl ester (21 min), all of which are similar to those in the literature.[16] Yields were estimated from the peaks in the HPLC chromatogram

b. Obscured by other peaks.

c. PP_{IX} was not present in the mixture.

2.3.15 Demetallation of crude 3,7-di(1-methoxyethyl) deuteroporphyrin III dimethyl ester (34) containing residual copper^{II} - porphyrin impurities

The material from Section 2.3.12 (Fraction 1) (65 mg) was taken up in conc. sulphuric acid/TFA (5%, 10 ml) and stirred at room temperature for 50 min. Water (50 ml) was added to the mixture which was then extracted with THF/dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. λ_{max} (rel. abs.) (methanol) 397 nm (100%), 500 (11%), 535 (8%), 569 (6.7%), 620 (3.3%). Reversed phase HPLC R_t 1.5 – 2.2 min (3%), 3.1 – 4.0 min (3%), 5.1 min (7%, HP_{III} – confirmed by comparison with an authentic sample), 7.3 min (36%, HV_{III} – by comparison with an authentic sample), 8.8 min (2%), 9.8 min (4%), 11.4 min (4%), 15.4 min (17%), 19.22 min (7%), 24 min (5%, PP_{III} – by comparison with an authentic sample), 25 –34 min (6%).

2.3.16 Borohydride reduction of purified Cu^{II}-3,7-diacetyldeuteroporphyrin III dimethyl ester (31) followed by acidic hydrolysis to give Cu^{II}-HP$_{III}$

The crude metalloporphyrin (31) from Section 2.3.11 was chromatographed on alumina (Brockmann, Grade III), and a sample of the purified metalloporphyrin (31) (58 mg) was taken up in methanol/dichloromethane (1 : 4, 5 ml). To this was added

sodium borohydride (*ca.* 50 mg) and the mixture was stirred at room temperature in darkness for 155 min. The mixture was acidified with dilute hydrochloric acid, diluted with water and extracted with dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. The solid residue was taken up in a mixture of methanol/trimethyl orthoformate/concentrated sulphuric acid/water (5 : 5 : 1 : 0.5; 11.5 ml),[13] stirred for 1 h and worked-up in the standard[13] manner.

The product was chromatographed on a squat column (Grade I alumina). The fraction containing Cu^{II}-3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (38) (FAB m.s. *m/z* 716) was dissolved in 1 M hydrochloric acid/THF (20 ml) and heated under reflux for 7 h, then allowed to cool to room temperature over 8 h. Water (30 ml) was added to the reaction mixture which was then extracted with THF/dichloromethane. The combined organic extracts were washed with water and evaporated to dryness to give Cu^{II}-HP_{III} (37b), λ_{max} (rel. abs.) (methanol + aq. NaOH at pH 12) 397 nm (100%), 524 (10.9%), 561 (17.8%) Reversed phase HPLC R_t 3.5 min (72%, Cu^{II}-HP_{III}, (37b)), 6.2 min (8%, Cu^{II}-HV_{III}, (23c)), 22 min (10%, Cu^{II}-PP_{III}, (21c)). Retention times correlated well with those of the free base analogues (Section 2.3.24).

2.3.17 Demetallation of Cu^{II}-Haematoporphyrin III (37b)

The crude Cu^{II}-haematoporphyrin III (37b) obtained from Section 2.3.16 was dissolved in conc. sulphuric acid/TFA (5%, 10 ml) and stirred in darkness at toom temperature for 35 min. The reaction mixture was diluted with water (30 ml) and extracted with THF/dichloromethane. The combined organic extracts were washed with water and evaporated to dryness, leaving a solid. λ_{max} (rel. abs.) 397 nm (100%), 500 (11.0%), 535 (8.0%), 569 (6.7%), 620 (3.3%). FAB m.s.: no molecular ions. Reversed phase HPLC : R_t 1.44 min (18.3%), very broad peaks centred at 22.27 min (46.4%) and 26.49 min (35.3%), the latter characteristic of polymeric material.[16]

A small portion (ca. 5 mg) of this crude product was dissolved in conc. sulphuric acid/methanol (5%, 10 ml) and left to stand in the dark for 24 h. The mixture was diluted with water (20 ml), neutralized and extracted with THF/dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. The solid residue did not give an informative mass spectrum (FAB m.s.: no molecular ions were detected). TLC (silica, 5% methanol/dichloromethane): the main portion (>75%) of the material remained on the baseline, again suggesting that it might be polymeric in nature.

In an effort to salvage any HP_{III} from the material obtained from demetallation of Cu^{II}-HP_{III} above, an acid hydrolysis was attempted. The material from above was dissolved in THF (8 ml). To this was added 1 M hydrochloric acid

(8 ml) and the mixture was refluxed for 6 h, then allowed to cool to room temperature for 16 h. As before, the mixture was diluted with water, extracted with THF/dichloromethane, the combined organic extracts were washed with water and evaporated to dryness. Reversed phase HPLC analysis of the hydrolysed material showed R_t 1.76 min (7%), 2.66 min (2%), 4.45 min (3.7%), 5.06 min (3%), 5.63 min (3.5%), 7.24 min (2%), 12.0-34.0 min (76.3%), indicating that most of the material obtained from the demetallation of Cu^{II}-HP_{III} (37b) above was acid stable, polymeric material.

In an effort to obtain adequate FAB spectra, the hydrolysed material was esterified using conc. sulphuric acid/ methanol (10 ml). The reaction mixture was stirred in darkness at room temperature for 19 h and then diluted with water, neutralized and extracted with dichloromethane. The combined organic extracts were washed with water and evaporated to dryness, leaving a solid residue. TLC (silica, 5% methanol /dichloromethane): major component (>60%) of the material was at the baseline, suggesting that it might be polymeric. FAB m.s. *m/z* 710, 810, 825, 840, 1167, 1181, ~1290, 1400 (all ions were weak).

2.3.18 Attempted cyclization of the diacetyl-containing biladiene-*ac* salt (30) in refluxing DMF

The diacetyl-containing biladiene-*ac* salt (30) (Section 2.3.10) (1.84 g) was added in one lot to refluxing DMF (oil bath temperature 165°C) containing copper(II) acetate (14.0 g, 30 equivalents) under an atmosphere of nitrogen. The mixture was stirred for 6 min, then cooled briefly and poured into water (250 ml). The solution was extracted repeatedly with dichloromethane until the aqueous phase was clear. The extracts were combined and washed with water, during which an emulsion formed. The emulsion was extracted repeatedly with dichloromethane until all the organic material was recovered. The combined organic extracts were evaporated to dryness (residual DMF was removed under high vacuum).

The resulting solid residue was demetallated by dissolving it in concentrated sulphuric acid/TFA (10%, 20 ml) and stirring the mixture at room temperature in darkness for 30 min. Then water (50 ml) was added and the solution was extracted with dichloromethane. The combined organic extracts were evaporated to dryness.

The solid residue was dissolved in concentrated sulphuric acid/methanol (5%, 20 ml). The mixture was stirred at room temperature for 48 h, then diluted with dichloromethane and poured into water. The mixture was neutralized and

extracted with more dichloromethane. As before, an intractable emulsion formed during extraction but most of the material was recovered with repeated extraction with gentle swirling. The organic extracts were combined and dried over anhydrous sodium sulphate. Evaporation of the solvent afforded a solid. This was chromatographed on a short squat column (silica gel-60G). The porphyrin product (0.117 g) was eluted with dichloromethane. λ_{max} (rel. abs.): 403 nm (100%), 460 (13%), 525 (22.5%), 562 (40%).

The metalloporphyrin was taken up in conc. sulphuric acid/TFA (10%, 15 ml) again and stirred at room temperature for 45 min. Water (30 ml) was added to the mixture and the solution was extracted with dichloromethane. The combined organic phases were washed with water and evaporated to dryness leaving a solid residue which had λ_{max} (rel. abs.): 413 nm (100%), 499 (18%), 534 (12%), 569 (9%), 622 (5%).

The crude porphyrin was re-esterified by stirring in conc. sulphuric acid/methanol (5%, 10 ml) at room temperature over 2 days. The mixture was diluted with dichloromethane (20 ml) and poured into water, neutralized, and extracted with dichloromethane. The organic extracts were combined, washed with water and evaporated to dryness. The residue was chromatographed on a silica squat column. The porphyrin-containing band was eluted with 2% acetone/dichloromethane. Evaporation of the solvent afforded a solid (71 mg). λ_{max} (rel. abs.): 402 nm (100%), 498 (9.0%), 532 (6.7%), 567 (5.1%), 621

(2.8%). ^{13}C NMR, δ ppm 11.65 (ring $\underline{C}H_3$), 21.68 ($\underline{C}H_2CH_2CO_2CH_3$), 36.96 ($CH_2\underline{C}H_2CO_2CH_3$), 51.70 ($CO_2\underline{C}H_3$), 96.60 (meso C), 136.53, 138.46 (Cβ), 142.5-145.7 (Cα), 173.53 ($\underline{C}O_2CH_3$). ^1H NMR, δ ppm -3.87 (2 H, 2 × pyrrole H), 3.27 (t, 8 H, 4 × $CH_2C\underline{H}_2CO_2CH_3$), 3.61-3.75 (~23 H, 4 × ring $C\underline{H}_3$, 4 × $CO_2C\underline{H}_3$), 4.39 (t, 8 H, 4 × $C\underline{H}_2CH_2CO_2CH_3$), 10.035 (4 H, 4 × meso H). FAB m.s., *m/z* 710.

2.3.19 Cyclization of the diacetyl-containing biladiene-*ac* salt (30) at 135°C

The biladiene-*ac* salt (30) (1.52 g, 1.9 mmol), prepared according to the procedure outlined in Section 2.3.10, was added in one lot to a solution of copper(II) acetate (12.0 g, 60 mmol, 32 equivalents) in dry DMF heated to 135°C, under an atmosphere of nitrogen. The mixture was stirred at 135°C for a further 6 min, then quickly cooled to room temperature and poured into water (*ca.* 400 ml) The mixture was extracted repeatedly with dichloromethane until the aqueous phase was colourless. The combined organic extracts were evaporated to dryness, leaving a gum which was then chromatographed on an alumina (Grade III) column. The initial eluate (an orange band) eluted with dichloromethane, was discarded. The desired fraction containing Cu^{II}-3,7-diacetyldeuteroporphyrin III dimethyl ester (31) (230 mg) was eluted with 5-10% acetone/dichloromethane.

The foregoing metalloporphyrin (31) was dissolved in concentrated sulphuric acid/TFA (5%, 10 ml) and stirred at room temperature in the dark for 55 min. Water (80 ml) was added to the reaction mixture which was then extracted repeatedly with dichloromethane until the aqueous phase was clear. The extracts were combined and evaporated to dryness leaving a solid residue. λ_{max} (rel. abs.) 424 nm (100%), 513 (16%), 551 (11%), 586 (8%), 640 (6%).

The crude 3,7-diacetyldeuteroporphyrin III was dissolved in a mixture of methanol/trimethyl orthoformate (1:1, 10 ml) and cooled in an ice-bath. To this solution was added concentrated sulphuric acid (1 ml) dropwise with swirling at 0°C. The mixture was then stirred at room temperature for 1 h. Water (70 ml) was added and the pH of the solution was adjusted to pH 5 with aqueous sodium hydroxide (10%). The mixture was extracted repeatedly with dichloromethane. The extracts were not washed with water. (As a test, an aliquot was placed in at separating funnel and washed with water. The organic phase immediately emulsified and a fine, brown precipitate formed.) The organic extracts were combined and evaporated to dryness to afford a solid residue. λ_{max} (rel. abs.), (CH_2Cl_2 + trace of NEt_3), 423 nm (100%), 514 (16%), 551 (11%), 586 (8%), 640 (5%). Due to the low solubility of the porphyrin in $CDCl_3$, $(CD_3)_2CO$, CD_3OD and combinations of these, NMR spectra were not recorded. FAB m.s., *m/z* 622.

2.3.20 Borohydride reduction of crude 3,7-diacetyl-deuteroporphyrin III dimethyl ester (32)

The foregoing porphyrin (32) (*ca.* 200 mg) was suspended in methanol/dichloromethane (1 : 4, 10 ml). To this was added sodium borohydride (*ca.* 200 mg) and the mixture was stirred at room temperature for 75 min, then acidified with dilute hydrochloric acid. The mixture was poured into water and

extracted with dichloromethane. The organic phase was evaporated to dryness. TLC (silica, 5% methanol/dichloromethane) of the residue: 3,7-diacetyldeutero-porphyrin III dimethyl ester (32) (R_F 0.75) - major component, HP_{III}dme (22) (R_F 0.22) - minor.

The crude product was taken up in methanol/ dichloromethane (1 : 4, 10 ml) again and treated further with sodium borohydride (200 mg). After work-up in the manner described above, TLC analysis of the product showed that unreacted 3,7-diacetyldeutero- porphyrin III dimethyl ester was still present, so the crude material was taken up in methanol/dichloromethane (1 : 4, 10 ml) again and treated with excess sodium borohydride until unreacted sodium borohydride was present in the reaction mixture. Stirring was continued for 60 min, then dilute hydrochloric acid was added dropwise to the reaction mixture to quench the excess sodium borohydride. The mixture was poured into water and extracted with more dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. TLC (silica, 5% methanol/dichloromethane) of the residue indicated that HP_{III}dme was the main product.

The crude HP_{III}dme was dissolved in methanol/trimethyl orthoformate (1:1, 5 ml). To this was added conc. sulphuric acid (1 ml) dropwise with swirling at 0°C. The mixture was stirred at room temperature for 70 min, then diluted with water (40 ml). The mixture was neutralized and

extracted with dichloromethane. The combined organic extracts were washed with water and evaporated to dryness, leaving a solid residue. TLC (silica, 5% methanol/dichloromethane), R_F 0.8 (3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (34)), R_F 0.5 (3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41)), R_F 0.3 (HP_{III}dme) in the approximate ratio of 3:2:1. The crude material was chromatographed on alumina (Grade I) using a squat column. 3,7-di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (34) (92 mg, 0.14 mmol) was eluted with 2% acetone/dichloromethane. λ_{max} (rel. abs.) 401 nm (100%), 499 (9.3%), 532 (6.0%), 568 (4.5%), 622 (2.8%). ^{13}C NMR, δ ppm 11.53 (ring $\underline{C}H_3$), 21.69 ($\underline{C}H_2CH_2CO_2CH_3$), 25.11 ($\underline{C}H_3CHOCH_2$), 36.82 ($\underline{C}H_2CO_2CH_3$), 51.57 ($CO_2\underline{C}H_3$), 57.07 ($CH_3CHO\underline{C}H_3$), 74.93 ($CH_3\underline{C}HOCH_3$), 90.07, 96.60, 99.59, 99.73 (meso C), 136.47, 136.74, 138.26, 139.96 ($C\beta$), 144.23 (br, $C\alpha$), 173.47 ($\underline{C}O_2CH_3$). ^1H NMR, δ ppm -3.83 (2 × pyrrole H), 2.26 - 2.29 (2 × d, 2 × $C\underline{H}_3CHOCH3$), 3.18 (t, 2 × $C\underline{H}_2CO_2CH_3$), 3.49 (s, 2 × $CO_2C\underline{H}_3$), 3.59, 3.61, 3.62 (4 × ring $C\underline{H}_3$), 3.67 (s, 2 × $CH_3CHOC\underline{H}_3$), 4.22 (t, 2 × $C\underline{H}_2CH_2CO_2CH_3$), 6.06 (m, 2 × $CH_3C\underline{H}OCH_3$), 9.83, 10.01, 10.93, 10.93 (4 × meso H). FAB m.s., *m/z* 655. 3(1-hydroxyethyl)-7(1-methoxyethyl)-deuteroporphyrin III dimethyl ester (41) (39 mg, 0.06 mmol) was eluted with 10% acetone/dichloromethane. λ_{max} (rel. abs.) 400 nm (100%), 499 (8.1%), 532 (5.3%), 568 (4.2%), 622 (2.5%). ^{13}C NMR, δ ppm 11.5 (ring $\underline{C}H_3$), 21.94 ($\underline{C}H_2CH_2CO_2CH_3$), 25.10 ($\underline{C}H_3CHOH$), 35.53 ($CH_3CHOCH_3$), 37.01 ($\underline{C}H_2CO_2CH_3$), 51.77 ($CO_2\underline{C}H_3$) 57.09 ($CH_3CHO\underline{C}H_3$), 61.94 ($CH_3\underline{C}HOH$), 75.05

($CH_3\underline{C}HOCH_3$), 96.49 – 96.61, 99.47 – 99.36 (meso $\underline{C}H$), 136.84 – 139.97 (Cβ), 143.24 – 146.67 (br., Cα), 174.06 ($\underline{C}O_2CH_3$). ^1H NMR, δ ppm -3.75 (br, 2 × pyrrole H), 2.25-2.28 ($C\underline{H}_3CHOH$), 2.53 ($C\underline{H}_3CHOCH_3$), 3.17 (2 × $C\underline{H}_2CO_2CH_3$), 3.52 (2 × $CO_2C\underline{H}_3$), 3.60 - 3.62 (4 × ring $C\underline{H}_3$), 3.69 – 3.70 (2 × $CO_2C\underline{H}_3$, $CH_3CHOC\underline{H}_3$), 3.91 – 3.95 ($CH_3C\underline{H}OH$), 4.09, 4.27 (2 × $C\underline{H}_2CH_2CO_2CH_3$), 6.03 – 6.11 (m, $CH_3C\underline{H}OCH_3$), 10.05 – 10.08, 10.90 – 10.91 (4 × meso H). FAB m.s. *m/z*: 641. HP_{III}dme (23 mg, 0.03 mmol) was eluted with 30% acetone/dichloromethane and was identical (TLC, FAB m.s., NMR, u.v.) to the authentic sample in Section 2.3.8. Total yield of porphyrin: 0.23 mmol (12% based on starting biladiene-*ac* salt in Section 2.3.19)

2.3.21 Acidic hydrolysis of the methoxyether porphyrins (34) and (41), and haematoporphyrin III dimethyl ester (22) to give haematoporphyrin III (HP_{III}) (10)

3,7-Di(1-methoxyethyl)deuteroporphyrin III dimethyl ester (34) (92 mg), 3-(1-hydroxyethyl)-7-(1-methoxyethyl)deuteroporphyrin III dimethyl ester (41) (39 mg) and HP_{III}dme (22) (43 mg) from Section 2.3.20 were combined and dissolved in distilled THF (15 ml). To this was added 1 M hydrochloric acid (15 ml) and the solution was heated under reflux for 6.5 h. The reaction mixture was diluted with water (50 ml) and the pH of the solution was adjusted to pH 5 by the addition of aqueous sodium bicarbonate (10%). The mixture was extracted with THF/dichloromethane (4 × 25 ml), the organic

extracts were combined and washed with water and evaporated to dryness leaving a solid (160 mg). Standard[16] reversed phase HPLC R_t 4.32 min (75%, HP_{III} (10)), R_t 7.12 min (6%, HV_{III} (23b)), other components (R_t 5.64 min, 8.5%; R_t 6.23 min, 4.5%). FAB m.s. *m/z* 581, 559 (M^+), 613.

2.3.22 Preparation of haematoporphyrin derivative-III (HPD-III) by the Lipson[2a] procedure as modified by Gomer *et. al.*[2b]

(a) "Clean" HP_{III} (10) (57 mg) (obtained by base hydrolysis of HP_{III}dme as outlined in Section 2.3.8) was dissolved in glacial acetic acid (1.08 ml). To this was added conc. sulphuric acid (0.06 ml) and the mixture was stirred in darkness at room temperature for 1 h. The mixture was then added dropwise to aqueous sodium acetate solution (5%, 7.6 ml) with swirling. The reaction flask was rinsed with water (*ca.* 7 ml) and the rinsing were added to the sodium acetate solution. The resulting suspension was collected by centrifugation. Water (*ca.* 3 ml) was added to the solid and the resulting suspension was centrifuged. The water was drawn off and the remaining solid was washed again with water. The procedure was repeated until the washings were only weakly acidic (pH 5.5-6). The water was drawn off leaving a soggy paste. HPLC analysis R_t 3.52 min (30%, HP_{III} monoacetate (43)), 6.21 min (45%, HP_{III} diacetate (44)) and minor peaks R_t 1.75 min (2%), 2.47 min (2%), 2.84 min

(5%), 4.71 min (1%), 5.44 min (7%), 7.11 min (6.7%), 10.94 min (0.7%), 15.1 min (0.7%).

The paste was treated with 0.1 M sodium hydroxide (2.85 ml), but did not completely dissolve. So 1.0 M sodium hydroxide solution was added dropwise (*ca.* 3 drops) with swirling until all the solid had dissolved. The mixture was stirred in darkness at room temperature for 1 h, then neutralized (pH 7.24) with dilute hydrochloric acid. The resulting solution was diluted with saline. Aliquots (10 µl) were removed and diluted 1000 times with 0.1 M NaOH/ethanol (50%) and the u.v. absorbance at 397 nm was measured. Saline was added to the crude HPD-III until the u.v. absorbance of the 0.1 M NaOH/ethanol (5%) solution at 397 nm was 0.833 units (Abs_{397} 0.833). The crude HPD-III, λ_{max} (rel. abs.) (5% 0.1 M NaOH/ethanol) 397 nm (0.833, 100%), 502 (0.078, 9.4%), 535 (0.055, 6.6%), 568 (0.044, 5.3%), 620 (0.032, 3.8%) was then filtered through a 0.45 µm pore size filter to give HPD-III. HPLC analysis of HPD-III, R_t 2.85 min (20.2%, HP_{III}); unidentified peaks R_t 3.35 min (13%), 5.08 min (8.1%), 7.56 min (3%), 9.32 min (3%),11.4 min (1%), $R_t \geq 15$ min (38%).

(b) Crude HP_{III} (75%, 0.153 g) from Section 2.3.24 was acetylated for 1 h using a mixture of glacial acetic acid (2.85 ml) and concentrated sulphuric acid (0.15 ml) in the manner described above. After treatment with aqueous sodium acetate (5%, 20 ml), centrifugation and washing with water, the crude

HP_{III} acetates mixture was obtained. HPLC analysis R_t 4.04 - 4.35 min (20%, HP_{III} monoacetate), 6.87 min (29%, HP_{III} diacetate), and minor peaks R_t1.73 min (4.6%), 3.39 min (2.8%), 6.13 min (5.6%), 7.9 min (6.4%), 9.0 min (0.7%), 10.09 min (1.6%), 11.48 min (5.8%), 12.35 min (3.2 min%), 13.24 min (2.7%), 14.16 min (2.0%), 15.72 min (1.2%), 17.45 min (1.4%), 18.48 min (0.9%), 19.16 min (1.3%), 20 - 33 min (7.1%).

The crude HP_{III} acetates mixture from above was treated with 0.1 M sodium hydroxide (7.5 ml). 1.0 M sodium hydroxide (*ca.* 5 drops) was added to completely dissolve the paste. The resulting solution was then stirred at room temperature in the dark for 1 h. After neutralization (pH 7.20) and dilution with saline in the manner described above, the crude "unfiltered HPD-III" λ_{max} (rel. abs.) (50% 0.1 M NaOH/ethanol) 395 nm (0.809, 100%), 501 (0.077, 9.5%), 534 (0.056, 6.9%), 570 (0.046, 5.7%), 620 (0.034, 4.2%), Abs_{397} 0.808, was filtered through a 0.45 μm pore size filter to give HPD-III. HPLC analysis, R_t 2.2 min (7%), 2.9 min (2%), 3.8 min (21%,HP_{III}), 5.3 min (11%), 6.6 min (9%, HV_{III}), 7.8 — 11 min (14%), $R_t \geq 15$ min (31%). An aliquot (10 μl) of HPD-III was diluted 1000 times with 0.1 M NaOH/ethanol (5%) to give λ_{max} (rel. abs.) (50% 0.1 M NaOH/ethanol) 395.7 nm (0.373, 100%), 499 (0.024, 6.4%), 533 (0.017, 4.5%), 569 (0.013, 3.5%), 620 (0.006, 1.6%), Abs_{397} 0.372. The residue from the filtration, λ_{max} (rel. abs.) (methanol) 396 nm (100%), 500 (8.7%), 534 (6.2%), 569 (4.7%), 622 (2.6%), HPLC, was identical to HPD-III obtained after filtration.

2.3.23 Chromatographic separation of HPD-III on Sephadex LH-20

HPD-III (*ca.* 4 ml) was diluted with THF (*ca.* 2 ml) and placed on a Sephadex LH-20 column. The fractions were eluted with THF/methanol/water (2:1:1, ~pH 5).[16] Four fractions were collected.

The fractions were diluted with water, acidified with 1 M hydrochloric acid solution (2 drops) and extracted with THF/ dichloromethane. The extracts were evaporated to dryness. The residue from Fraction 1 showed ^{1}H and ^{13}C NMR (D_2O, t-BuOH internal standard) – all the signals in the ^{1}H NMR spectrum were obscured by H_2O signals while only broad signals which were barely discernable from the baseline were present in the ^{13}C NMR spectrum. FAB m.s. of Fraction 1: no molecular ions were detected.

2.3.24 Reesterification of Fraction 1 of HPD-III

Fraction 1 of HPD-III in D_2O, from Section 2.3.23, was diluted with water (30 ml). 1 M HCl (5 drops) was added and the mixture was extracted with THF/dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. The solid residue was dissolved in concentrated sulphuric acid/methanol (5%, 10 ml). The reaction

mixture was stirred in darkness at room temperature for 25 h, then water was added and the mixture was extracted with THF/dichloromethane. The organic phases were combined and washed with water. Evaporation of the solvent afforded a solid residue. ^{13}C NMR (CD_3Cl + CD_3COCD_3 suspension, ~25% in solution), δ ppm 10.64 – 13.22 (ring $\underline{C}H_3$), 20.95 – 21.87 ($\underline{C}H_2CH_2CO_2CH_3$), 25.74 –26.29 ($\underline{C}H_3CHOR$), 36.07 – 36.64 ($CH_2\underline{C}H_2CO_2CH_3$), 50.69 ($CO_2\underline{C}H_3$), 95.64 (br, meso C), ~137.1 (br, pyrrole C), 172.7 ($\underline{C}O_2CH_3$). FAB m.s. *m/z*: 591, 609, 627, 701 (w), 719 (w), 1202, 1217, 1291 (w), 1310 (m), 1327 (w), 1810, 1827, 1902 (w), 1290 (w), ~2420, 2436.

2.3.28 Standard hydrolyses[16] of HPD-III

(a) Acidic hydrolysis[16]

To HPD-III (1 ml) was added THF (2 ml) and 1 M hydrochloric acid (1 ml). The mixture was refluxed for 60 min, then diluted with water (20 ml) and extracted with THF/dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. HPLC analysis R_t 3.84 min (45%, HP_{III}), 4.89 min (11%), 5.7 min (3%), 6.55 min (14%, HV_{III}), 7.3 - 14.3 min (17%), $R_t \geq 15$ min (6%)

(b) Basic hydrolysis[16]

To HPD-III (1 ml) was added THF (2 ml) and aqueous sodium hydroxide (10%, 4 drops). The mixture was refluxed for

90 min, then diluted with water (20 ml), acidified (pH 4 - 5) and extracted with THF/dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. HPLC analysis R_t 3.9 min (25%, HP_{III}), 4.3 -5.8 min (22%), 6.7 min (7%), 7.5 - 14.8 min (15%), $R_t \geq 15$ min (21%).

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CHAPTER 3

3.1 Introduction[§]

In the studies towards the structure elucidation of HPD,* several porphyrin dimers were synthesized and compared with HPD (see Chapter 1). In particular, some porphyrin dimers with ether[1—4,6] bridges were found to be as active as Photofrin II.[1,2] Since then, emphasis has extended to the design of improved photosensitizers – the so-called "second generation" photosensitizers.[8] At the start of this project it was thought that ether-linked dimers were the most promising class of dimers in terms of having anti-cancer activity which was comparable to that of Photofrin II. Hence it was decided to focus our studies on the synthesis of analogues to the ether-linked dimers.[1—4,6]

Ward *et. al.*[1,2] treated 1-hydroxyethyl-containing porphyrins with hydrobromic acid in dichloromethane for a short period, during which initially formed 1-bromoethyl derivatives were trapped *in situ* by the unreacted 1-hydroxyethyl-containing porphyrin to give ether-linked dimers. This *in situ* reaction is a convenient route to symmetrical dimers,[1,2] which can also be prepared by a stepwise reaction involving the initial synthesis of the 1-bromoethyl porphyrin followed by reaction with the hydroxyethyl-containing porphyrin in a separate step.

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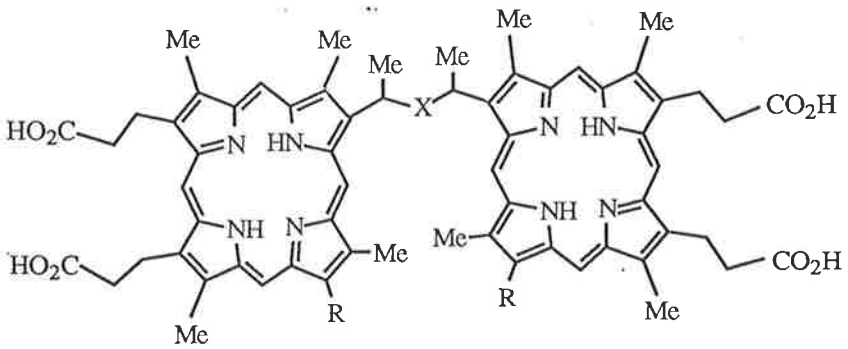
§ References, p. 171.

* In the following chapters, all the porphyrins are of the naturally occurring IX series. Although only one regioisomer of each compound is shown in the diagrams, the presence of other regioisomer(s) is(are) implied.

The two step approach is suitable for the synthesis of unsymmetrical dimers.[1,2] Pandey *et. al.*[6] synthesized ether-linked dimers by first preparing the bromoethyl derivative of hydroxyethyl-containing porphyrins and then reacting these reactive bromoethyl compounds with hydroxyethyl-containing porphyrins in a separate step.[6] The bromoethyl compounds were prepared by treating the starting hydroxyethyl-containing porphyrins with either hydrobromic acid in acetic acid[6b] or bromine in dichloromethane.[6a] Alternatively, the hydroxyl group in the precursor porphyrin was converted to a mesylate which was then treated with lithium bromide to form the bromoethyl compound.[6a] The bromoethyl compound can also be prepared by the hydrobromination of vinyl substituents on the porphyrin[2,3,5,7] as was done in the synthesis of other porphyrin ether[2,3,5] and thioether[7] derivatives.

It has been suggested[1,12] that biological activity of the ether-linked dimers may be related to the hydrophobicity of the terminal sidechains, with the dimers bearing more hydrophobic terminal sidechains (for example the vinyl-terminated dimer (49)[1]) producing greater anticancer activity than those with hydrophilic groups, such as the hydroxyethyl-terminated dimer (2).[1] In an order to determine whether the hydrophobic requirement for biological activity also extended to the type of linking group between the porphyrins, it was of interest to synthesize porphyrin dimers with other heteroatoms in the bridging position. Dimers with hydrophobic linking groups such as the hydrocarbon-[9] and ester-linked porphyrins[13] have

been prepared, and it was found that the hydrocarbon-linked porphyrins[9] showed moderate anti-tumour activity[9,29] compared to Photofrin II (see Chapter 1) while the ester-linked porphyrin dimers[13] were either too unstable in normal aqueous solutions[13a] to have any significant activity *in vivo* or were virtually inactive[13c] It was decided to concentrate our study on the synthesis of dimers with hydrophilic linking groups, in this case amino-linked dimers. The synthesis of four amino-linked bis-porphyrin dimers which complement the known ether-linked dimers (5), (49 – (51),[1,2] namely the dimers with divinyl- (45), diethyl- (46), diacetyl- (47) and di(1-hydroxyethyl)- (48) terminal groups was undertaken with the aim of comparing their *in vivo* activity with those of the analogous ether dimers.[1,2]

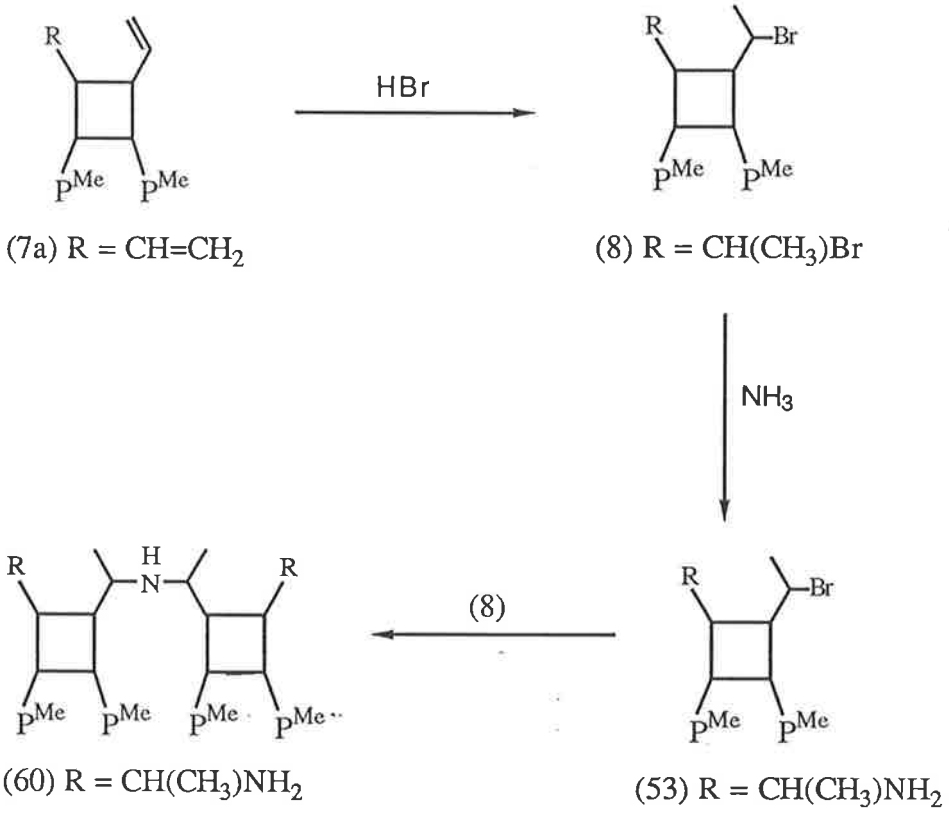


(45) R = $CH{=}CH_2$, X = NH
(46) R = CH_2CH_3, X = NH
(47) R = $COCH_3$, X = NH
(48) R = $CH(CH_3)OH$, X = NH

(5) R = $CH(CH_3)OH$, X = O
(49) R = $CH{=}CH_2$, X = O
(50) R = $COCH_3$, X = O
(51) R = CH_2CH_3, X = O

The proposed route, shown in Scheme 3.1, involved the initial synthesis of the suitably substituted 1-bromoethyl-containing intermediate which would then be reacted with ammonia to give the 1-aminoethyl porphyrin. Reaction between the 1-aminoethyl porphyrin and another molecule of the 1-bromoethyl-containing intermediate in a separate step would then give the desired dimer.

Scheme 3.1



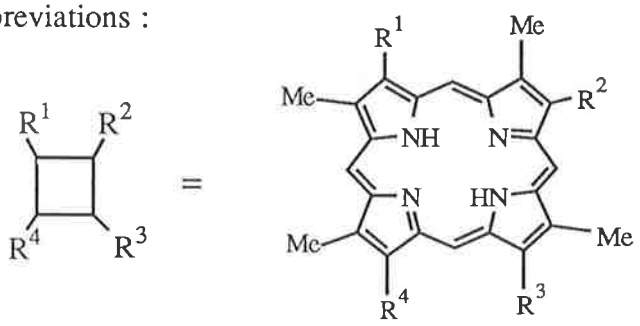
(7a) R = $CH{=}CH_2$

(8) R = $CH(CH_3)Br$

(60) R = $CH(CH_3)NH_2$

(53) R = $CH(CH_3)NH_2$

Abbreviations :



$P^{Me} = CH_2CH_2CO_2Me$

3.2 Results and Discussion[§]

3.2.1 Hydrobromination with hydrogen bromide/ dichloromethane

The general procedure for the synthesis of ether-linked porphyrin dimers[1,2,3] involves the use of hydrobromic acid in acetic acid, either to hydrobrominate vinyl substituents[3] or to effect the protonation and subsequent nucleophilic substitution of hydroxyl groups.[1,2,3] However, Ward *et. al.*[1,2] expressed concern that the acetic acid solvent participates to some extent in the reaction to form acetylated by-products. To avoid this problem dichloromethane saturated with hydrogen bromide was used instead. The added advantage of using dichloromethane as the solvent is that it can be easily removed under reduced pressure without any need for heating, thereby reducing the risk of elimination of the bromide to form vinyl products (see later).

Since the overall aim was to replace the oxygen atom on the 1-position of the ethyl substituent of the porphyrin with nitrogen, a 1-hydroxyethyl containing porphyrin such as those used in the synthesis of ether-linked dimers,[1,2,5] would not be a suitable starting material because it readily self-condenses under acidic conditions.[1,2,5] Although the ether-linkages in the resulting products would eventually be cleaved if high concentrations of hydrogen bromide and long reaction times were used, the water

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[§] References, p. 171.

that is extruded in the process may cause other unwanted side reactions to take place. Hence it was decided to start with a vinyl-containing porphyrin like protoporphyrin dimethyl ester, PPdme, (7a). Reaction with HBr would result in hydrobromination of the vinyl groups[2] to give the di-(1-bromoethyl)deuteroporphyrin dimethyl ester (8) which could then react with ammonia to give the desired di(1-aminoethyl) product (53), Scheme 3.1.

Since there were no precedents for using dichloromethane as the solvent for the hydrobromination of the vinyl-containing porphyrins, it was necessary to establish the conditions for the minimum time required for complete reaction. In a preliminary study, equal volumes of saturated HBr/dichloromethane solution[a] were added to a solution of protoporphyrin dimethyl ester (PPdme)[b] (7a) in dichloromethane (thereby halving the concentration of HBr in the solution) and the mixture was stirred at room temperature under an atmosphere of nitrogen. Aliquots were removed at intervals and hydrolysed with a mixture of aqueous sodium bicarbonate in THF. The product (obtained after extraction with dichloromethane) was analysed by TLC. The hydrolysis step was necessary because the bromoethyl porphyrin was a reactive intermediate and could not

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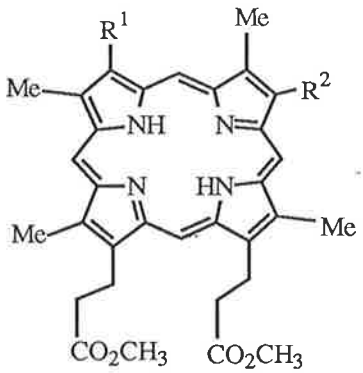
[a] Prepared by bubbling gaseous HBr into dry dichloromethane at the rate of approximately 1min/ml dichloromethane.

[b] Throughout these, and subsequent chapters, the naturally occurring porphyrins, in this case, those of the IX series (see Appendix for nomenclature), were used in the syntheses. The porphyrin regioisomers were not separated. and unless otherwise stated, the numbering of the sidechains is arbitrary and the corresponding regioisomer(s) is(are) assumed to be also present.

be isolated.[23] Trapping it with water forms the stable, known 1-hydroxyethyl products, namely haematoporphyrin dimethyl ester, HPdme (6), and/or 3(8)-(1-hydroxyethyl-8(3)-vinyldeuteroporphyrin dimethyl ester, HVdme (52), and possibly some ether-linked dimers (9), (55) and (56). These dimers were not expected to be major products because ~~because~~ dilute aqueous base was being used. This is in contrast with the procedure of Scourides *et. al.*[3] where saturated sodium chloride was used, and the reaction between the bromoethyl compound and the sodium chloride solution produced significant amounts of dimeric and/or oligomeric material.[3]



(6) $R^1 = R^2 = CH(CH_3)OH$
(7a) $R^1 = R^2 = CH{=}CH_2$
(8) $R^1 = R^2 = CH(CH_3)Br$
(52) $R^1 = CH(CH_3)OH$, $R^2 = CH{=}CH_2$

It was found that the hydrobromination of PPdme (7a) proceeded very slowly compared to the reaction between HBr/dichloromethane and HPdme.[1,2] After 30 minutes, the major product from the hydrolysis of the bromoethyl compound was the starting material, PPdme (7a), while HVdme (52) and HPdme (6) were present in only trace amounts. With increasing time, the

proportion of HVdme (52) began to gradually increase and, after 19 hours HVdme was the major product but HPdme was still a minor component and there were still traces of PPdme present in the product mixture. After 22 hours, the proportion of HPdme (6) and HVdme (52) were almost equal, but PPdme (7a) was still present in the product mixture. The reaction was allowed to proceed for a total of 90 hours, and reversed phase HPLC analysis of the products after work-up showed that the relative proportion of (the main products) HVdme (52) to HPdme (6) to PPdme was 13:11:3.

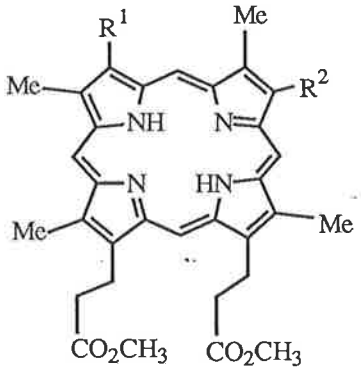
The effect of increasing the concentration of HBr in the reaction mixture was then investigated. HBr gas was bubbled directly into the solution of PPdme (7a) in dichloromethane and the mixture was stirred at 0°C under an atmosphere of nitrogen. As before, aliquots were removed from the reaction mixture, hydrolysed with aqueous base and the products were analysed by TLC. A significant amount of hydrobromination was found to have occurred, after 1 minute as shown by the hydrolysis products where PPdme (7a), HPdme (6) and HVdme (52) were present in approximately equal amounts. After 20 minutes, the major component was HPdme (6), while PPdme (7a) and HVdme (52) were still present but represented only about 30% of the product. HPdme (6) was the main component after 40 minutes, although traces of PPdme (7a) and HVdme (52) were still present. Allowing the hydrobromination to proceed for 50 minutes did not eliminate the PPdme (7a) and/or HVdme (52) from the product mixture. It is likely that the small amounts of

the monovinyl- (HVdme) (52) and divinylporphyrin (PPdme) (7a) are the products of the competing elimination (dehydrobromination) reaction by the nucleophile/base. Raising the reaction temperature of the hydrobromination step from 0°C to room temperature and extending the reaction time to 2 hours at room temperature did not have any effect on the relative yields of HPdme (6), HVdme (52) and PPdme (7a).

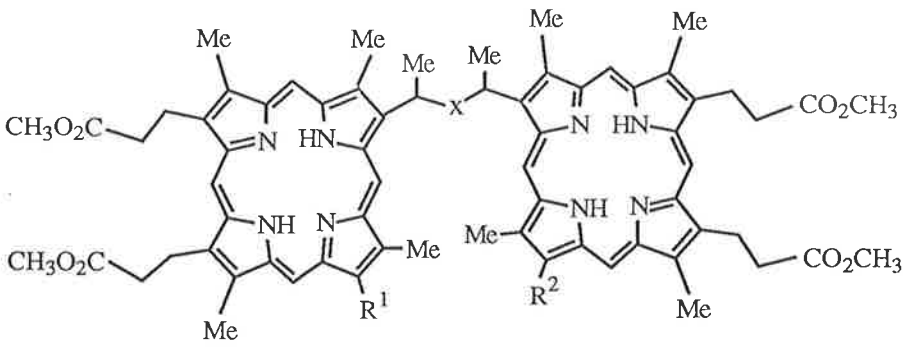
As it was wasteful to pass excess amounts of gaseous HBr into the reaction mixture, the procedure was slightly modified : freshly prepared saturated solutions of HBr in dry dichloromethane were added to the solid porphyrin in the ratio of 1 ml of HBr/dichloromethane to 10 mg of porphyrin and the resulting mixture was stirred for 90 minutes at room temperature. In most cases, except when copious amounts of the nucleophile (eg. water, ammonia - see later) were used, most of the excess hydrogen bromide, along with the solvent, were removed by evaporation under reduced pressure at 0°C over 20-30 minutes until a gum was obtained. This was done to minimize protonation (and thereby inactivation) of the nucleophile in the next step. The crude bromoethyl porphyrin (8) was used without further purification.

3.2.2 Nucleophilic substitution with concentrated ammonium hydroxide

In a preliminary study, the reactive 3,8-di(1-bromoethyl)deuteroporphyrin dimethyl ester (8) was treated with concentrated ammonium hydroxide at 0°C for 7 minutes. FAB mass spectral analysis of the product after aqueous work-up showed strong molecular ions which corresponded to the amino monomers (53), (57) and dimers (58), (59), (60). However, these



(6) $R^1 = R^2 = CH(CH_3)OH$
(53) $R^1 = R^2 = CH(CH_3)NH_2$
(57) $R^1 = CH(CH_3)NH_2$, $R^2 = CH{=}CH_2$



(9) $R^1 = R^2 = CH(CH_3)OH$, X = O
(55) $R^1 = R^2 = CH{=}CH_2$, X = O
(56) $R^1 = CH(CH_3)OH$, $R^2 = CH{=}CH_2$, X = O
(58) $R^1 = R^2 = CH{=}CH_2$, X = NH
(59) $R^1 = CH(CH_3)NH_2$, $R^2 = CH{=}CH_2$, X = NH
(60) $R^1 = R^2 = CH(CH_3)NH_2$, X = NH

amino systems have molecular weights that are very similar to those of the analogous hydroxyl containing monomers and/ or ether-linked dimers[2] (which could have formed during the aqueous work-up procedure). For example, the di(1-hydroxyethyl) containing porphyrin (HPdme) (6) has a molecular weight of 627 while its diamino analogue (53) has a molecular weight of 625. Similarly with the dimers, the divinyl-terminated ether-linked dimer[1,2] (55) has a molecular weight of 1200 while the molecular weight of its amino analogue (58) is 1199. TLC analysis of the crude material on silica (developed in 3% methanol/dichloromethane) showed that the major portion of the material (>90%) did not migrate from the baseline. This suggested that this material was mainly the desired amino derivatives; however it does not preclude the possibility that the propionic esters had been hydrolysed under the strongly basic conditions to give the corresponding propionic acids. Both the propionic acid

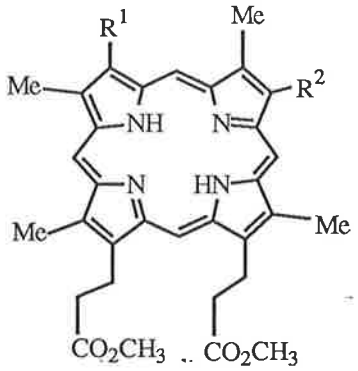
and amino containing porphyrins would coordinate strongly to silica and hence would not migrate very far from the baseline.

The porphyrins with propionic acid side chains would be less readily detected in the FAB mass spectrum of the mixture because porphyrins with propionic acid functionalities generally do not give strong molecular ions.[2,12] So it was possible that the molecular ions which were observed in the FAB mass spectrum of the mixture were due to the trace amounts of porphyrins whose propionic esters were still intact, and did not necessarily represent the total composition of the product mixture.

The material was esterified in a mixture of methanol/trimethyl orthoformate/water/concentrated sulphuric acid.[11] Following aqueous work-up, the product was found to be identical to the unesterified material by TLC and FAB mass spectrometry. TLC comparison of the esterified material with HPdme (6), HVdme (52) and the ether-linked dimers (9), (55) and (56) (which have similar molecular weights to the amino derivative(s) and which could have been produced during work-up procedure in the synthesis of the latter) indicated that these hydroxyl and/or ether systems were present in only trace amounts. These results suggested that the amino derivatives were the major products from the reaction and that they had low R_F values (< 0.2) on silica in 3% methanol/dichloromethane, as anticipated.

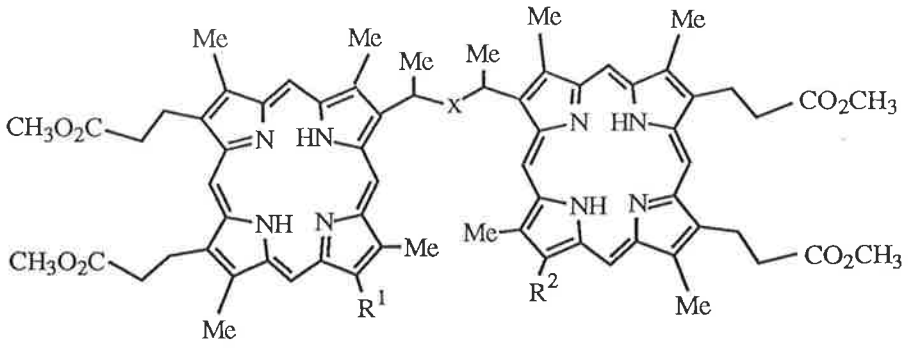
Attempts to separate the mixture on silica or alumina using combinations of methanol/dichloromethane were

unsuccessful because the compounds were being retained by the adsorbent; so the amino groups were acetylated by standard procedures[24] using pyridine/acetyl chloride. Preparative chromatography of the product on deactivated silica produced six components, namely PPdme (7a) (*m/z* 591 by FAB m.s.), the divinyl-terminated amino-linked dimer* (58) (*m/z* 1199) and the N-acetylated products (61) (*m/z* 650), (62) (*m/z* 709), (63) (*m/z* 1258), (64) (*m/z* 1317). The dimers altogether accounted for 59% of the total product, and PPdme, 14%.



(61) $R^1 = CH(CH_3)NHCOCH_3$, $R^2 = CH{=}CH_2$
(62) $R^1 = R^2 = CH(CH_3)NHCOCH_3$

* See Appendix for the naming of new compounds.

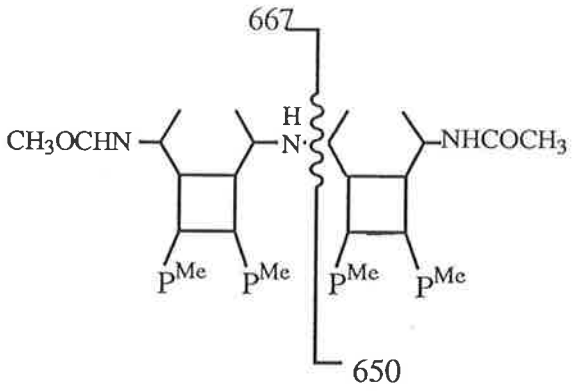


(60) $R^1 = R^2 = CH(CH_3)NH_2$, X = NH
(63) $R^1 = CH(CH_3)NHCOCH_3$, $R^2 = CH{=}CH_2$, X = NH
(64) $R^1 = R^2 = CH(CH_3)NHCOCH_3$, X = NH
(65) $R^1 = CH(CH_3)NHCOCH_3$, $R^2 = CH(CH_3)NH_2$,
X = $NCOCH_3$
(66) $R^1 = R^2 = CH(CH_3)NHCOCH_3$, X = $NCOCH_3$

The molecular ion at *m/z* 1317 suggested that only two of the three amino groups on dimer (60) had been acetylated. A Mass analysed Ion Kinetic Energy (MIKE) spectrum of the dimer ion *m/z* 1317 showed peaks at *m/z* 650 and 664, possibly due to cleavage at the bridging group, Fig.3.1(a). This indicated that the acetyl groups were only attached to the terminal amino groups, and that the bridging nitrogen was not acetylated. If the bridging nitrogen had been acetylated, as in (65), ions *m/z* 609 and *m/z* 708, resulting from the second cleavage on the bridging group, Fig. 3.1(b) would also have been present in the MIKE spectrum of *m/z* 1317. In addition, there was no evidence in the mass spectrum (of the crude acetylated product) of peaks which corresponded to the triacetylated dimer (66), further indicating that under the conditions used, the bridging nitrogen did not acetylate.

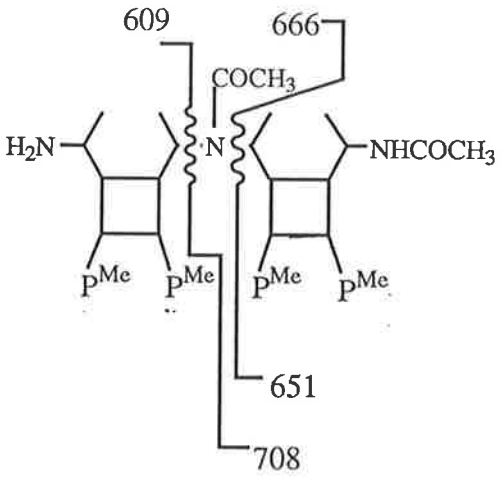
Fig. 3.1 MIKES analysis of *m/z* 1317.

(a)

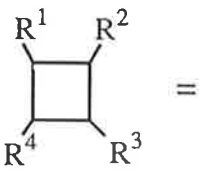


and, *m/z* 667 - 3H = *m/z* 664

(b)



Abbreviations :



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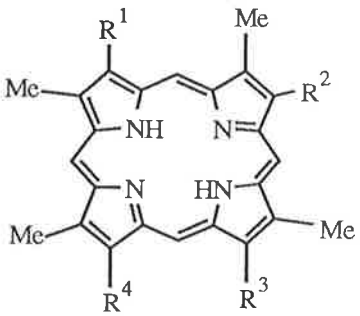
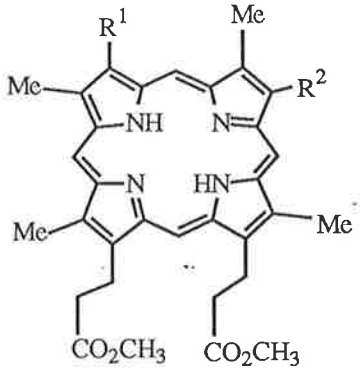


$P^{Me} = CH_2CH_2CO_2Me$

As was mentioned before, the amino-linked dimers accounted for 59% of the total product. This tendency to form dimers is possibly due to the aggregation[25] of the porphyrins in the aqueous reaction mixture, such that an initially formed 1-aminoethyl substituted porphyrin is favourably aligned with another bromoethyl porphyrin for a pseudo-intramolecular reaction to form a dimer. Since the dimer was the desired product in this study, efforts were concentrated on improving the yield of dimers and not so much on the preferential synthesis of the monomer.



(53) $R^1 = R^2 = CH(CH_3)NH_2$
(54) $R^1 = R^2 = C(CH_3)NOH$

Previous attempts by Morris[2a] to synthesize the diamino monomer (53) via the oxime (54) were unsuccessful : catalytic reduction with palladium on charcoal at atmospheric pressure resulted in ring reduction; in another attempt using sodium cyanoborohydride, the oxime failed to react.[2a]

The presence of vinyl-containing products suggested that either the bromide on the bromoethyl porphyrin was being eliminated during the course of the reaction, or that

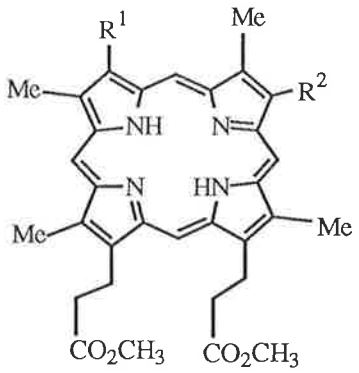
hydrobromination of the vinyl groups (Section 3.2.1) had not gone to completion. However studies with aqueous base (base hydrolysis of the bromoethyl porphyrin - see 3.2.1) had indicated that 90 minutes was sufficient for complete hydrobromination of both vinyl groups on protoporphyrin dimethyl ester. Therefore elimination of the bromide by the nucleophile/base (ammonia) was more likely to have occurred. This dehydrobromination is not unusual because the bromoethyl compound is known[23,30] to be very unstable. For example, attempts by Rapoport *et. al.* [30] to isolate the free base of the di(1-bromoethyl) porphyrin (8) resulted in the elimination of the bromide to give PPdme (7a).

It was difficult to accurately determine the proportion of each component in the product mixture by preparative TLC because some of the compounds have similar R_F values (for instance, the vinyl containing dimers (58) and (63) eluted together in a broad band). It was however necessary to determine quantitatively the composition of the product mixture before optimization studies could be done. So an attempt was made to analyse the product mixture by reversed phase HPLC on the C-18 Novapak column using existing conditions.[21] Reversed phase HPLC analysis of the unacetylated amino derivatives (with propionic esters) and the acetylated (propionic ester) products was unsuccessful because the material eluted with very poor separation and resolution. The corresponding hydrolysed material (ie. porphyrins containing propionic acid side chains) did not elute with the standard[21] solvents. In all cases it was possible to elute all the material off the column with THF. These results

indicated that a new solvent system would have to be developed for the amino systems. Also, the possibility of using a different type of column might have to be considered. However due to the length of time needed to set up a suitable HPLC assay[22] it was decided to explore quicker, albeit less accurate, solutions to the problem of quantifying the products from the nucleophilic substitution of the 1-bromoethyl compound with amines.

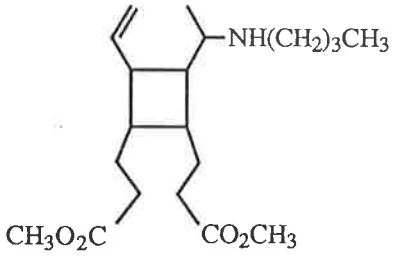
3.2.3 Nucleophilic substitution with *n*-butylamine

The possibility of using a primary amine, namely *n*-butylamine, as the nucleophile for optimization studies was investigated. It was hoped that the less reactive secondary amine that would be formed initially, namely the di-(1-*n*-butylamino)ethyl derivative (67), would not react with another molecule of 1-bromoethyl porphyrin (8) in the reaction mixture and hence a less complicated product mixture would be obtained. Another anticipated advantage of using *n*-butylamine was that the products would be less polar than the ammonia derivatives, and could be chromatographed easily.

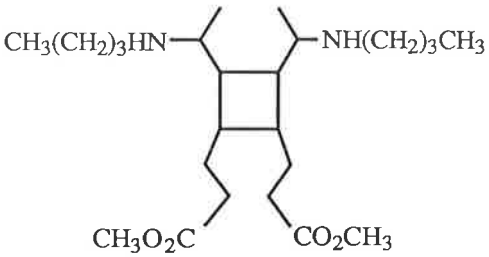


(67) $R^1 = R^2 = CH(CH_3)NH(CH_2)_3CH_3$
(68) $R^1 = CH(CH_3)NH(CH_2)_3CH_3$, $R^2 = CH{=}CH_2$

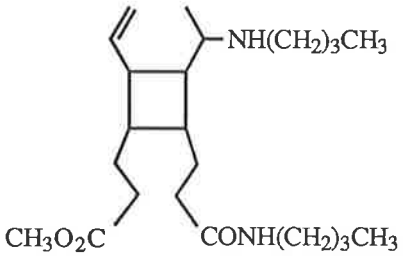
The bromoethyl porphyrin (8), prepared by the procedure established in Section 3.2.1, was dissolved in dry dichloromethane and added to an excess of *n*-butylamine. The reaction mixture was stirred at 0°C for 3 minutes, and then evaporated to dryness. A crude mixture consisting of mono- (68) (FAB *m/z* 663) and diamino-substituted (67) (FAB *m/z* 737) porphyrin monomers was obtained. These monomers were readily separated by TLC on silica (5% methanol/dichloromethane) at R_F 0.3 (di-*n*-butylamino monomer, (67)) and R_F 0.5 (3-*n*-butylamino-8-vinyl monomer, (68)). Separation of the mixture by column chromatography on deactivated silica afforded first, a minor fraction consisting of the 3-(1-(*n*-butylamino)ethyl-8-vinyldeuteroporphyrin dimethyl ester (68) (4% of the total product), followed by a major band which contained the 3,8-di-(1-(*n*-butylamino)ethyl)-deuteroporphyrin dimethyl ester (67) (84%). FAB mass spectra of the di-(1-*n*-butylamino)ethyl- (67) and the 1-(*n*-butylamino)ethyl-vinyl monomer (68) gave molecular ions at *m/z* 737 and *m/z* 663, respectively; the base peak in both



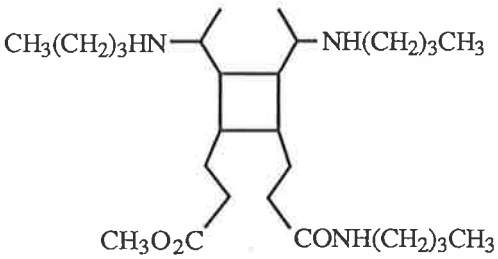
(68)



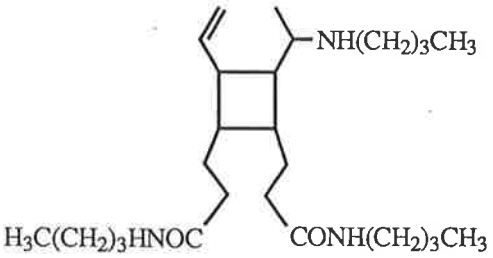
(67)



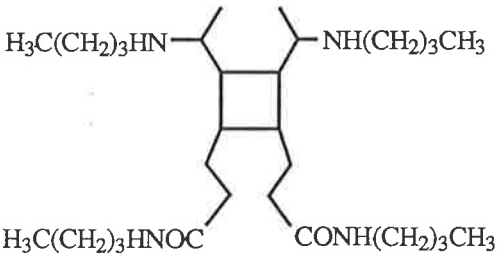
(69a)



(69b)

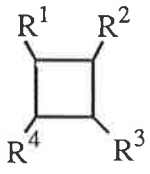


(69c)



(69d)

Abbreviations :



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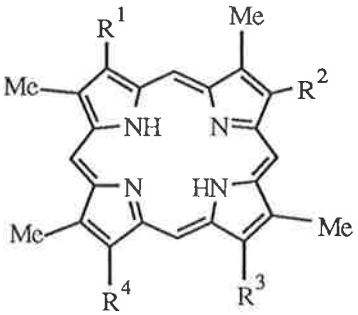


spectra was *m/z* 591 which is the fragment arising from the loss of the *n*-butylamino group to give a stabilized benzylic ion. Similar losses of large groups at the β position to an aromatic system have been observed in branched alkylthiophenes.[14]

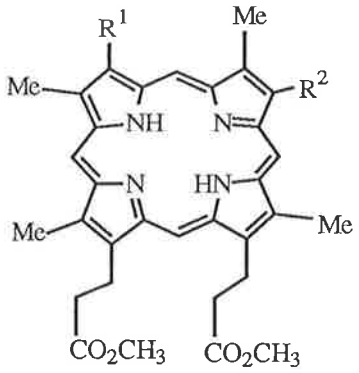
Increasing the reaction time of the nucleophilic substitution from 3 minutes to 20 minutes resulted in the amminolysis of the propionic ester groups, so that in addition to the expected products (67), (68), four other products (69a) (*m/z* 704), (69b) (*m/z* 778), (69c) (*m/z* 745) and (69d) (*m/z* 819), all containing amides, were also observed in the FAB mass spectrum of the crude material.

Having established the optimum conditions for hydrobromination using HBr/dichloromethane (90 minutes at room temperature) and nucleophilic substitution (3 minutes at 0°C), attention was then turned to the synthesis of amino-linked porphyrin dimers with diethyl- (70), divinyl- (58), diacetyl- (71) and dihydroxyethyl- (72) terminal groups (see later).

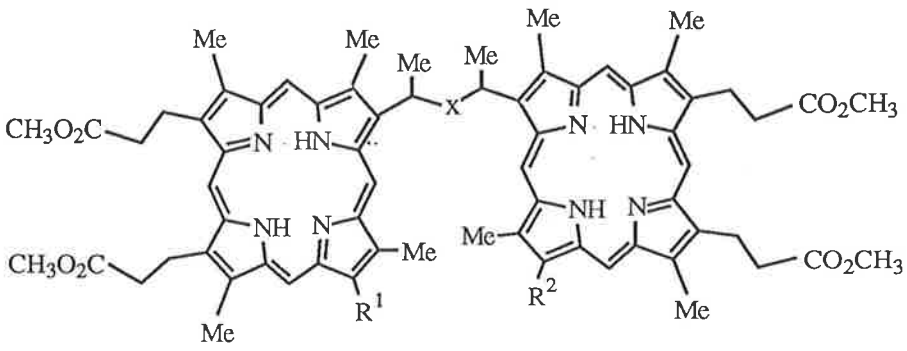
3.2.4 Divinyl-terminated amino-linked dimer (58)

There were two possible routes to this dimer (58) : [1] via the benzoyl chloride/DMF dehydration[2b,17] of the hydroxyl groups in the di(1-hydroxyethyl)-terminated amino-linked dimer (72) (Section 3.2.7), or [2] via the reaction between 3(1-bromoethyl)-8-vinyldeuteroporphyrin dimethyl ester (73) and

concentrated ammonium hydroxide (which was expected to proceed to give mainly dimers - see Section 3.2.2).



(73) R^1 = $CH(CH_3)Br$, R^2 = $CH{=}CH_2$
(74) R^1 = $COCH_3$, R^2 = $CH{=}CH_2$



(58) $R^1 = R^2 = CH{=}CH_2$, X = NH
(59) R^1 = $CH(CH_3)NH_2$, R^2 = $CH{=}CH_2$, X = NH
(60) $R^1 = R^2$ = $CH(CH_3)NH_2$, X = NH
(71) $R^1 = R^2$ = $COCH_3$, X = NH
(72) $R^1 = R^2$ = $CH(CH_3)OH$, X = NH

The disadvantage with the first route was that there would be five steps in going from the starting porphyrin, HPdme, to the dimer, namely the partial oxidation of HPdme,[2] then

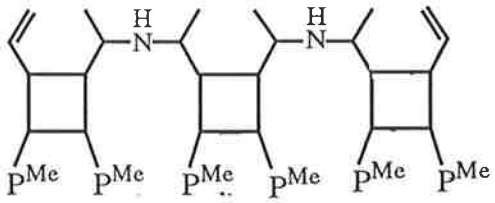
dehydration of the remaining hydroxyl group to give the acetyl-vinyl precursor porphyrin[2] (74); hydrobromination of the vinyl group, followed by reaction with ammonia would give the diacetyl-terminated dimer (71); the acetyl groups on this dimer (71) would then have to be reduced[2] to the corresponding 1-hydroxyethyl functionalities and finally, dehydration[2] would give the desired divinyl-terminated dimer. Some of these steps were expected, by analogy with the ether-linked dimers,[2a] to be low yielding, making this route even less favourable.

The alternative route, starting with PPdme, involved the partial hydrobromination of the vinyl groups followed by reaction with concentrated ammonium hydroxide. Although it was possible to obtain the desired 3-(1-bromoethyl)-8-vinyldeuteroporphyrin dimethyl ester (73) intermediate (see Section 3.2.1), the reaction between PPdme and HBr was difficult to control and there would always be by-products arising from fully hydrobrominated PPdme. So the expected products from the reaction with concentrated ammonium hydroxide would be the desired divinyl-terminated amino-linked dimer (58), unreacted PPdme (7a), the (1-aminoethyl)-vinyl-terminated amino-linked dimer (59), the di(1-aminoethyl)-terminated amino-linked dimer (60) and possibly larger oligomers with similar terminal groups. The main problem with this route was that the yield of divinyl-terminated amino-linked dimer might be low, but if the conditions which produce PPdme (7a) as the major by-product could be established, then the problem would be lessened because PPdme (7a) could be readily recycled.

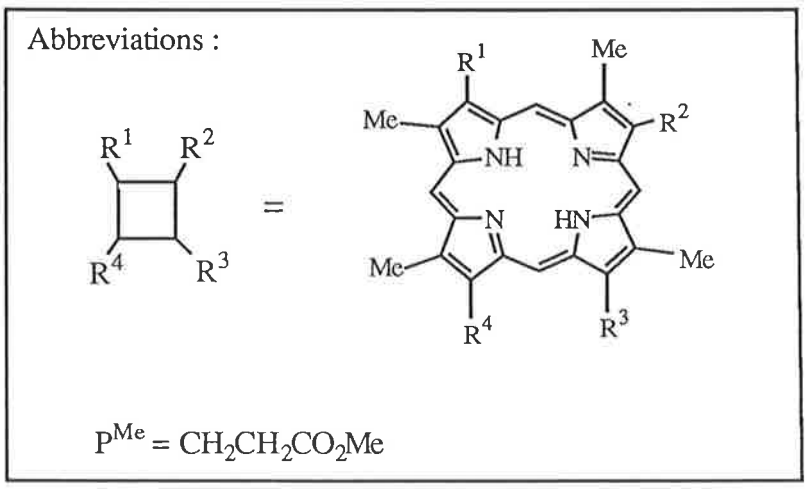
In Section 3.2.1 it was found that when PPdme (7a) was stirred in a half-saturated solution of HBr/dichloromethane at room temperature for 90 hours, the main products after hydrolysis were HVdme, HPdme and PPdme in a ratio of 13 : 11 : 3. It was possible that after one molecule of HBr had reacted with one of the vinyl groups on PPdme, the resulting (electron-withdrawing) benzylic bromide could exert a long-range negative inductive effect that is transmitted mesomerically to the second vinyl group making the latter less nucleophilic, and in this way, slowing down the hydrobromination of the second vinyl group. This effect is not very strong so, this vinyl group would still react with HBr, but not as quickly as the first one. Therefore it might be possible to trap the bromo-intermediate before the second vinyl group hydrobrominates. In an attempt to shorten the reaction time (from 90 hours) and to lower the proportion of di(1-bromoethyl)deuteroporphyrin dimethyl ester (8) intermediate, the effect of using a higher concentration of HBr / dichloromethane was investigated.

Gaseous hydrogen bromide was bubbled into a solution of PPdme in dichloromethane for 90 seconds and the mixture was stirred at 0°C for a further 10 minutes. Excess HBr and dichloromethane were removed at 0°C over 20 minutes under reduced pressure. The residue was dissolved in dry dichloromethane and an aliquot was hydrolysed with aqueous base as in Section 3.2.1. The thin layer chromatogram of the product showed that HVdme and HPdme were the major

components (in an approximately 1:1 ratio) and PPdme was present in trace amounts, indicating that most of the starting PPdme had reacted with HBr and that hydrobromination of both vinyl groups in PPdme had occurred to a significant extent. The main portion of this solution of the bromoethyl intermediate in dichloromethane was reacted with concentrated ammonium hydroxide/THF at 0°C for 30 minutes. As expected FAB mass spectral and TLC analyses of the crude product indicated that it consisted of a mixture of amino-containing monomers (53), (57), dimers (58) (59) and trimer (75). No attempts were made to



(75)



determine whether higher oligomers were present. Unlike the n-butylamino derivative (Section 3.2.3), no ammonolysis products

were detected despite the long reaction time with ammonia. The TLC chromatogram of the product showed that most of the material was at the baseline (suggesting the presence of diamino-substituted material). The desired divinyl-terminated amino-linked dimer (58), which was present in insignificant amounts, readily separated from the more polar (1-aminoethyl)-substituted material.

Next, the reaction time of hydrobromination was lowered from 10 minutes to 4 minutes. As before, the reaction mixture was evaporated to a gum, redissolved in dichloromethane and reacted with concentrated ammonium hydroxide. The product was chromatographed on deactivated silica to give PPdme (12%), the desired divinyl-terminated amino-linked dimer (58) (20%) and the other 1-aminoethyl-containing products (53), (57), (59) and (60) (>60%).

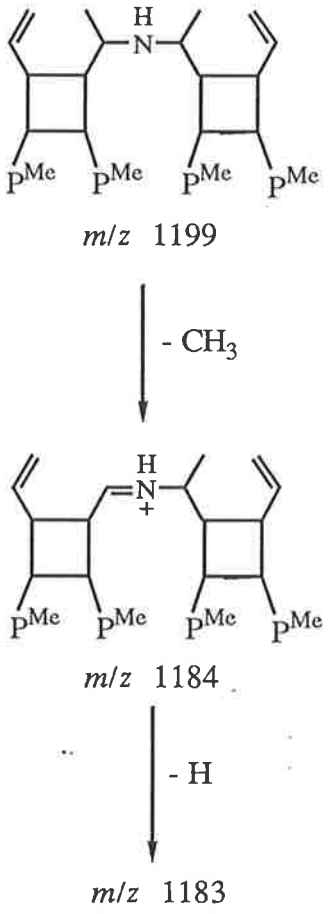
. When the reaction time in HBr was lowered to 2 minutes, the yield of divinyl-terminated amino-linked dimer (58) fell to 8% and 60% of the starting PPdme was recovered. A 3 minute reaction time for hydrobromination resulted in a higher yield of the desired dimer (58) (32%). PPdme (33%) and the other polar 1-aminoethyl-containing compounds were the other products.

It was likely that reaction with HBr was still occurring while the HBr/dichloromethane solution was being removed under reduced pressure over the standard 20 minutes so a modification of the procedure was tried. This involved pouring

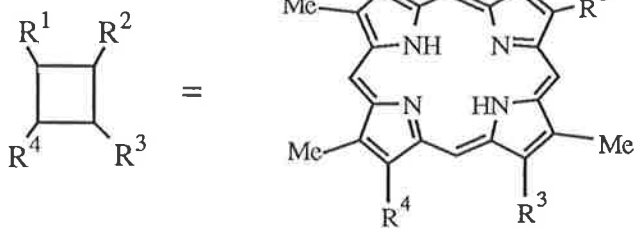
the hydrobromination reaction mixture directly into excess concentrated ammonium hydroxide whereby the HBr should be instantly quenched by the base and hence removed from the reaction mixture, leaving the 1-bromoethyl compound to react with any unreacted ammonia. In addition, instead of bubbling HBr into a solution of PPdme in dichloromethane, a saturated solution of HBr in dichloromethane (see Section 3.2.1) was added to solid PPdme.

In a trial run, solid PPdme (7a) was treated with saturated with HBr/dichloromethane and the mixture was stirred at room temperature for 22 minutes, then poured into excess concentrated ammonium hydroxide at 0°C. The heterogeneous mixture was stirred for 10 minutes, then worked up in the usual way. This afforded PPdme (7a)(18%), the desired dimer (58) (31%), the vinyl-(1-aminoethyl)-terminated dimer (59) (44%) and other more polar products. A shorter hydrobromination time of 16 minutes followed by reaction with concentrated ammonium hydroxide/THF gave a higher yield (40%) of the desired dimer while PPdme (37%) and the other 1-aminoethyl containing by-products were the minor components. This was in contrast with the original results where a 10 minute hydrobromination time produced mainly material resulting from over-hydrobromination of PPdme, and it suggests that hydrobromination was still occuring while the excess HBr was being removed over the standard 20 minutes.

Fig. 3.2 MIKES fragmentation of dimer (58) to give the stabilized iminium ion (m/z 1184).



Abbreviations :



$P^{Me} = CH_2CH_2CO_2Me$

The visible absorption spectrum of dimer (58) had the typical etio-type pattern, with a Soret band at 395 nm and bands I - IV at 628 nm, 575 nm, 540 nm, 507 nm. The FAB mass spectrum of (58) gave a weak molecular ion at *m/z* 1199 and a more intense dimer ion at *m/z* 1197 (M^+ - 2), as well as the fragment peaks *m/z* 607 and *m/z* 591 (the base peak). The Mass analysed Ion Kinetic Energy (MIKE) spectrum of the dimer ion *m/z* 1199 showed peaks at *m/z* 591 and *m/z* 606 as the major fragments, probably resulting from the loss of 2H and the cleavage of the C-N bond in the bridge to give the component porphyrin ions (*m/z* 591 and *m/z* 606). Minor fragmentations to *m/z* 1183 (M^+ - CH_3, H) and *m/z* 1157 (M^+ - CH_2CO)[10b,20] were also observed. α-Cleavage to give iminium ions is a common occurrence in aliphatic amines.[19] With the amino-linked dimer (58), this fragmentation may be further driven by the stabilization of the resulting iminium ion by the porphyrin ring, Fig. 3.2. A subsequent loss of $H^{\bullet}$ would give the observed ion *m/z* 1183. Cleavage of the propionates[10b,20] such as (M^+ - CH_2CO) is a well known process and, in this case, accounts for the ion at *m/z* 1157. The ^1H and ^{13}C NMR analysis of this dimer are discussed in Section 3.2.8.

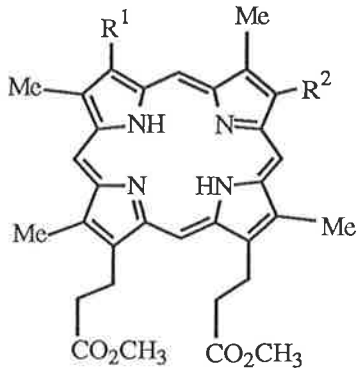
3.2.5 Diethyl-terminated amino-linked dimer (70)

The precursor vinyl porphyrin (76) for this dimer (70) was obtained from the more readily available

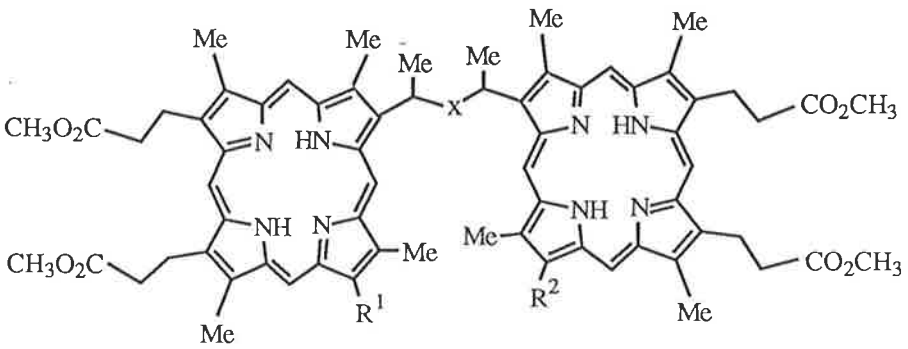
haematoporphyrin by standard reduction[2,16] and dehydration[2,17] procedures. The reduction of one of the hydroxyl groups on haematoporphyrin dimethyl ester to an ethyl group was achieved by treatment with a mixture of zinc iodide and sodium cyanoborohydride in dichloroethane.[16] Dehydration of the remaining 1-hydroxyethyl group with DMF-benzoyl chloride[17] afforded the mixture consisting of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76a) and its regioisomer 8-ethyl-3-vinyldeuteroporphyrin dimethyl ester (76b), which were the required precursors for the ethyl-terminated dimer (70). This mixture of regioisomers, which was not separable by chromatography, was used as such.

The 3-(1-bromoethyl)-8-ethyldeuteroporphyrin dimethyl ester (77) intermediate was prepared by stirring 3-vinyl-8-ethyldeuteroporphyrin dimethyl ester (76) in a solution of saturated of HBr/dichloromethane at room temperature under an atmosphere of nitrogen for 90 minutes. The excess HBr and dichloromethane were then removed under reduced pressure at 0°C over 20 minutes. The gummy residue was redissolved in dry dichloromethane and added to a mixture of concentrated ammonium hydroxide/THF (2 : 1) at 0°C and stirred for 10 minutes. After aqueous work-up, TLC analysis of the crude product on silica (developed in 10% methanol/dichloromethane) showed that the desired diethyl-terminated amino-linked dimer (70), R_F 0.8, was the major component, while the monomer 3-(1-aminoethyl)-8-ethyldeuteroporphyrin dimethyl ester (78), R_F 0.3, and the starting material (76) together accounted for less

than 25% of the product. The proportion of products from the competing reaction between the 1-bromoethyl intermediate (77) and water, namely the diethyl-terminated ether-linked dimer (79) and 3-(1-hydroxyethyl)-8-ethyldeuteroporphyrin dimethyl ester (80) was negligible. The crude mixture was separated by



(76) a. $R^1 = CH_2CH_3$, $R^2 = CH{=}CH_2$
b. $R^1 = CH{=}CH_2$, $R^2 = CH_2CH_3$
(77) $R^1 = CH(CH_3)Br$, $R^2 = CH_2CH_3$
(78) $R^1 = CH(CH_3)NH_2$, $R^2 = CH_2CH_3$
(80) $R^1 = CH(CH_3)OH$, $R^2 = CH_2CH_3$



(70) $R^1 = R^2 = CH_2CH_3$, X = NH
(79) $R^1 = R^2 = CH_2CH_3$, X = O

preparative chromatography on deactivated silica to give the desired dimer (70) in 69% yield and the monomer 3(1-aminoethyl)-8-ethyldeuteroporphyrin dimethyl ester (78) (17%). A small amount (10%) of the the starting porphyrin, 3-ethyl-8-vinyl deuteroporphyrin dimethyl ester (76) was also recovered.

Several variations of the procedure for the second step (reaction with concentrated ammonium hydroxide) were tried in an attempt to maximize the yield of diethyl-terminated amino-linked (70). In one experiment the 1-bromoethyl compound (77) in dichloromethane was added to concentrated ammonium hydroxide (3 equivalent volumes) at 0°C for 12 minutes in order to determine the effect of doing the reaction in a heterogeneous mixture. The product mixture was similar to that obtained from the homogeneous reaction mixture above, in that the dimer (70) was the major product, 3-(1-aminoethyl)-8-ethyldeutero-porphyrin dimethyl ester (78) was a minor product and the starting ethyl-vinyl deuteroporphyrin (76) was present in a smaller proportion than the monomer (78). In another variation, the excess HBr and dichloromethane were not removed, but the hydrobromination reaction mixture was added dropwise to a mixture concentrated ammonium hydroxide/THF (3:1, 4 equivalent volumes of the hydrobromination reaction mixture). The mixture was stirred at 0°C for 5 minutes and worked-up in the standard manner. The TLC chromatogram of the crude product was similar to those obtained in the previous runs. Similar results were obtained when a mixture of concentrated

ammonium hydroxide/THF was added to the solid (crude) 1-bromoethyl intermediate (77). In all cases, the desired dimer (70) was the main product but significant amounts of monomer (78) and starting porphyrin (76) were also recovered.

The FAB mass spectrum of dimer (70) showed a molecular ion at *m/z* 1203 and fragment ions *m/z* 608 and *m/z* 591. As with the divinyl-terminated dimer (58) (Section 3.2.4), *m/z* 591 was the base peak in the spectrum. A MIKE spectrum of the dimer ion (*m/z* 1203) showed major fragmentations to *m/z* 591 and *m/z* 608, which correspond to the component monomers. In addition there were losses of 17 m.u. and 44 m.u., to give peaks at *m/z* 1186 and *m/z* 1159, respectively. As was noted in the preceeding Section, the daughter ion *m/z* 1186 could be due to an initial loss of a methyl fragment from the bridge followed loss of 2H. However this does not preclude the possibility that *m/z* 1186 may be due to the loss of an ammonia fragment from the bridging group. A similar loss of a neutral NH_3 fragment was observed in the fragmentation of 2-aminomethyl-8-quinolinol.[19] The ion at *m/z* 1159 could be due to the loss of CH_2CO and 2H. The loss of CH_2CO is a known process and is due to a rearrangement in the propionate sidechain.[10b,20]

Dimer (70) gave an etio-type absorption spectrum, with maxima at 392 nm, 502 nm, 536 nm, 570 nm and 622 nm. The proton and ^{13}C NMR spectra of (70) are discussed in Section 3.2.8

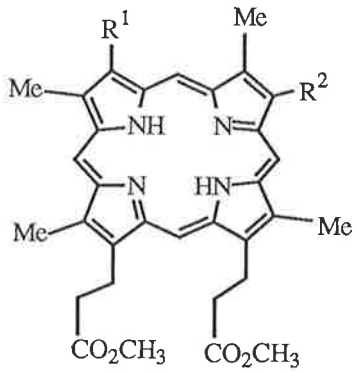
3.2.6 Diacetyl-terminated amino-linked dimer (71).

The starting porphyrin, 3-acetyl-8-vinyldeuteroporphyrin dimethyl ester (74), was synthesized from HPdme in the following way : partial Jones oxidation[2,18] gave a mixture of mainly 3,8-diacetyldeuteroporphyrin dimethyl ester (36) and 3-acetyl-8-(1-hydroxyethyl)deuteroporphyrin dimethyl ester (81).[2] The latter was separated from the crude product and dehydrated using benzoyl chloride/DMF[2,17] to give the desired 3-acetyl-8-vinyldeuteroporphyrin dimethyl ester (74).

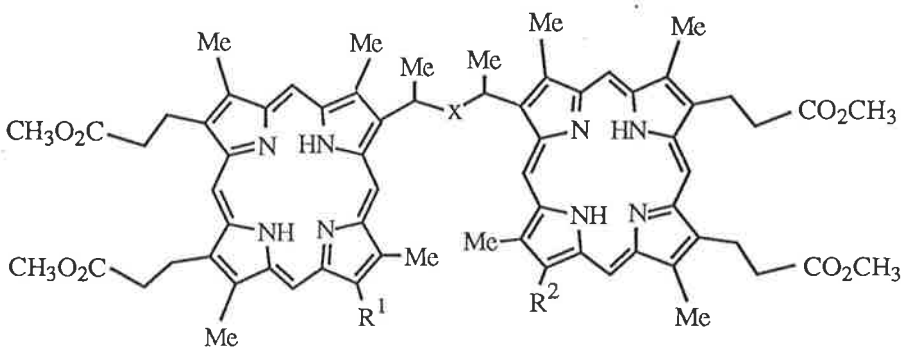
Due to the presence of the electron-withdrawing acetyl group it was anticipated that the reaction between the vinyl-group on 3-acetyl-8-vinyldeuteroporphyrin dimethyl ester (74) and HBr would be slower than the reaction between 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) and HBr (Section 3.2.5). In order to determine the minimum time required to hydrobrominate the vinyl group in (74), preliminary studies involving the use of aqueous base to quench the 1-bromoethyl compound in the manner described in Section 3.2.1 were done. The expected products from the hydrolysis reaction were 3-acetyl-8-(1-hydroxyethyl)deuteroporphyrin dimethyl ester (81), the starting porphyrin (74) and the diacetyl-terminated ether-linked dimer (82), all of which are known compounds.[1,2]

After 90 minutes' hydrobromination followed by hydrolysis, the major component of the crude product was the

starting material (74), as expected. After 160 minutes, the major product was 3-acetyl-8(1-hydroxyethyl)deuteroporphyrin dimethyl ester (81) and the starting porphyrin (74) was a minor component of the crude product. There were only trace amounts of the dimer (82). When this hydrobromination reaction mixture was added to concentrated ammonium hydroxide/THF and stirred



(36) $R^1 = R^2 = COCH_3$
(74) $R^1 = COCH_3$, $R^2 = CH{=}CH_2$
(81) $R^1 = COCH_3$, $R^2 = CH(CH_3)OH$
(83) $R^1 = COCH_3$, $R^2 = CH(CH_3)NH_2$
(84) $R^1 = COCH_3$, $R^2 = CH(CH_3)Br$



(71) $R^1 = R^2 = COCH_3$, X = NH
(82) $R^1 = R^2 = COCH_3$, X = O

for 20 minutes at 0°C, TLC analysis of the crude product indicated that the major product (≥ 50%) was the desired diacetyl-terminated amino-linked dimer (71). The starting porphyrin (74) and the monomer, 3-acetyl-8-(1-aminoethyl)deuteroporphyrin dimethyl ester (83) were minor products and were present in approximately equal amounts. The proportion of by-products (81) and (82) formed from the hydrolysis of the 1-bromoethyl intermediate (84) was negligible.

In a typical reaction the hydrobromination step was allowed to proceed for 3 hours. Then the reaction mixture was cooled to 0°C and added dropwise with stirring to a mixture of concentrated ammonium hydroxide/THF (1 : 1, 1 equivalent volume to the reaction mixture) maintained at 0°C. The mixture was stirred for a further 10 minutes. The product obtained after aqueous work-up was chromatographed on silica to afford the desired diacetyl-terminated amino-linked dimer (71) in 56% yield. The monomer 3-acetyl-8(1-aminoethyl)deuteroporphyrin dimethyl ester (83) (15%) was the minor product and some starting porphyrin (74) (18%) was recovered.

The absorption spectrum of the diacetyl-terminated amino-linked dimer (71) was a cross between an etio- and a rhodo spectrum — the relative intensity of band III (551 nm) was enhanced, but unlike a typical rhodo spectrum, it did not exceed that of band IV (513 nm), (Fig.3.3). There was an overall shift to longer wavelengths : the Soret band appeared at 410 nm while bands I-IV were at 639 nm, 581 nm, 551 nm, and 513 nm

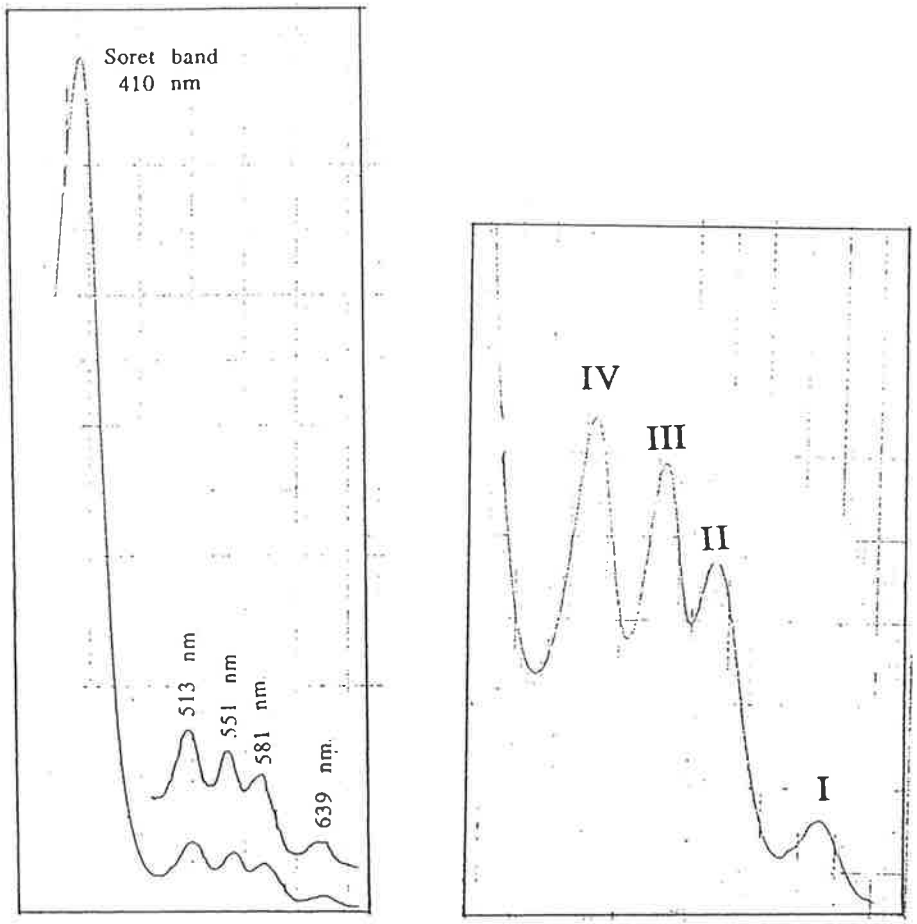


Fig. 3.3 **(a)** u.v./visible absorption spectrum of the diacetyl-terminated amino-linked dimer **(b)** visible portion of spectrum **(a)** showing bands I - IV.

respectively, compared to 392 nm (Soret), 622 nm, 570 nm, 536 nm, and 502 nm (bands I — IV, respectively) of the diethyl-terminated amino-linked dimer (see Section 3.2.5). The red-shift, characteristic of porphyrins with one electron withdrawing (rhodofying)[10a] group, was caused in this case, by the acetyl groups. The presence of the electron donating amino group on the other benzylic position in the molecule might have lessened the effect of the rhodofying group, so that the overall absorption spectrum was a cross between a rhodo- and an etio-type pattern. The monomer, 3-acetyl-8-(1-aminoethyl)deuteroporphyrin dimethyl ester (83), with a primary amino group in the benzylic position, gave a similar rhodo - etio type spectrum with maxima at 411 nm, 509 nm, 548 nm, 577 nm and 634 nm.

The FAB mass spectrum of the diacetyl-terminated amino-linked dimer (71) was similar to those of the other amino-linked dimers (Sections 3.2.4, 3.2.5) : a molecular ion at *m/z* 1230, with fragment ions at *m/z* 624 and *m/z* 607, the latter being the base peak in the spectrum. The MIKE spectrum of the dimer ion *m/z* 1231 showed major fragments at *m/z* 607 and *m/z* 624, which corresponded to its monomeric components. In addition there were the characteristic losses of 17 m.u. and 43 m.u. possibly due to NH_3 or (CH_3 H) and (CH_2CO + H), respectively (see Section 3.2.5).

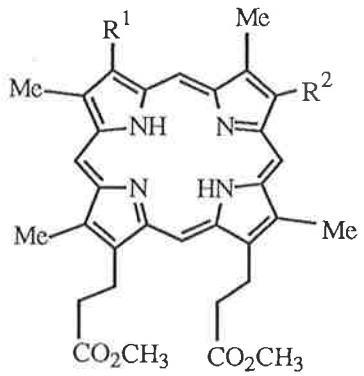
The monomer, 3-acetyl-8-(1-aminoethyl)deutero-porphyrin dimethyl ester (83), gave a FAB mass spectrum with a molecular ion of moderate intensity at *m/z* 624 and a base peak

at *m/z* 607. The approximate ratio of intensity was 1:3. In the MIKE spectrum, the major fragment from *m/z* 624 was *m/z* 607, arising from the loss of NH_3.

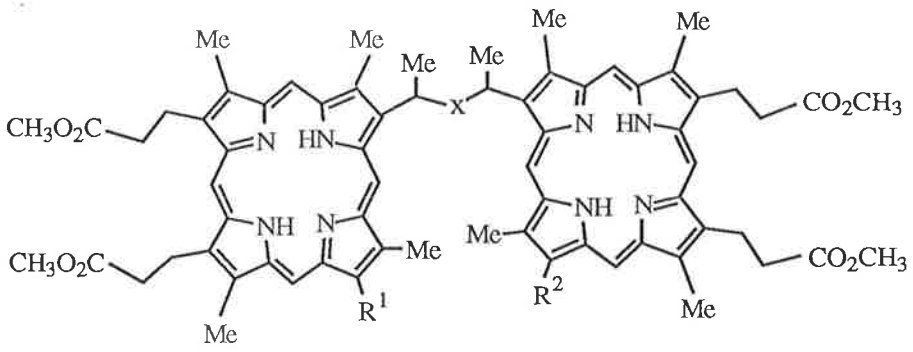
NMR analyses of dimer (71) are discussed in Section 3.2.8.

3.2.7 Di(1-hydroxyethyl)-terminated amino-linked dimer (72).

It was not feasible to synthesize this dimer (72) using the standard hydrobromination — concentrated ammonium hydroxide procedure on vinyl-containing porphyrin monomers because the available and obvious precursors, 3-(1-hydroxyethyl)-8-vinyldeuteroporphyrin dimethyl ester, HVdme (52) or 3-(1-methoxyethyl)-8-vinyldeuteroporphyrin dimethyl ester (85)[2] were known[1,2] to undergo side reactions in addition to hydrobromination. 3-(1-Hydroxyethyl)-8-vinyldeuteroporphyrin dimethyl ester, HVdme (52) self-condenses in the presence of HBr/dichloromethane to give the divinyl-terminated ether-linked dimer (55),[1,2] while the 1-methoxyethyl group on (85) was readily cleaved by HBr/dichloromethane to give a complicated mixture of products.[2a]



(52) $R^1 = CH(CH_3)OH$, $R^2 = CH{=}CH_2$
(85) $R^1 = CH(CH_3)OCH_3$, $R^2 = CH{=}CH_2$



(55) $R^1 = R^2 = CH{=}CH_2$, X = O
(71) $R^1 = R^2 = COCH_3$, X = NH
(72) $R^1 = R^2 = CH(CH_3)OH$, X = NH

This left the reduction of the acetyl groups in the diacetyl-terminated amino-linked dimer (71) as the most feasible approach to the di(1-hydroxyethyl)-terminated amino-linked dimer (72). The dimer (71) (see Section 3.2.6) was treated with an excess of sodium borohydride in the presence of methanol/dichloromethane (1:4) for 1 hour at room temperature. This reduced the acetyl groups cleanly to the corresponding 1-hydroxyethyl groups. Four bands were separated from the crude product by preparative TLC. FAB mass spectral analysis of each of these fractions gave nearly identical spectra (dimer molecular ion at *m/z* 1234, and fragment ions at *m/z* 626 and *m/z* 609; the ion at *m/z* 609 was the base peak in the spectrum). This indicated that the four bands were regio- and/or diastereoisomers of dimer (72). Attempts to achieve a similar separation of the product mixture on a larger scale using a squat column were, however, unsuccessful due to the small separation between each band (the R_F value of the bands were 0.24, 0.31, 0.33 and 0.39, on silica developed in 5% methanol/ dichloromethane).

The MIKE spectrum of the dimer ion, *m/z* 1234, showed a major fragment at *m/z* 1217, resulting from the loss of either an ammonia fragment (see Section 3.2.5) or OH from the end group. There were minor fragment ions at *m/z* 1199 (M^+ - NH_3, H_2O; or M^+ - CH_3, 2H, H_2O), *m/z* 1192 (M^+ - CH_2CO),[20] and *m/z* 1174 (M^+ - $CH_2CO_2CH_3$, H).[10b,20] Fragmentation of the propionate groups (resulting in the ions at *m/z* 1192 and *m/z* 1174) are common processes in porphyrin esters.[20] A notable departure from the general trend in the fragmentation of the amino-linked dimers (Sections 3.2.4 – 6) was the major fragmentation of the molecular ion to another dimer ion (*m/z* 1217) and only minor fragmentation to the monomer components (*m/z* 626 and *m/z* 609). The overall MIKE fragmentation pattern of the di(1-hydroxyethyl)-terminated amino-linked dimer (72) molecular ion was similar to that of the ether-linked analogue.[1,2]

The absorption spectrum of the di(1-hydroxyethyl)-terminated amino-linked dimer (72) was significantly different from its precursor, the diacetyl-terminated amino-linked dimer (71) (Section 3.2.6). Due to the absence of rhodofying (acetyl) groups in dimer (72), its visible absorption spectrum had an etio-type pattern with maxima at 397 nm, 504 nm, 536 nm, 571 nm and 624 nm.

3.2.8 NMR analysis of the amino-linked dimers

The signals in the proton NMR spectra of the amino-linked dimers (58), (70), (71) and (72) were generally broad and poorly resolved due to the presence of regio- and diastereoisomers. Nevertheless most of the signals were readily assigned on the basis of comparisons with the analogous ether-linked dimers.[1,2]

The pyrrole hydrogens resonated between -4.0 and -5.5 ppm, the meso hydrogens around 9 – 10 ppm and those of the ring methyls around 3.3 – 3.8 ppm. The propionate signals occurred around 3.2 – 3.4 ppm ($C\underline{H}_2CO_2CH_3$), 4.3 – 4.5 ppm ($C\underline{H}_2CH_2CO_2$) and 3.5 – 4.0 ppm ($CO_2C\underline{H}_3$). All these signals correlated well with those of corresponding groups in the ether-linked dimers[2] as well as porphyrin monomers.[2,26] Signals at ~2.2 ppm and ~5.6 ppm were assigned to $C\underline{H}_3CHNH$ and $CH_3C\underline{H}NH$ of the bridging group, respectively. As expected, the signal due to the methine proton ($CH_3C\underline{H}NH$) at ~5.6 ppm is upfield relative to the signal of the analogous group ($CH_3C\underline{H}OR$, ~6.5 ppm)[2] in the ether-linked dimers[1,2] as a result of the lower electronegativity of nitrogen compared to oxygen. The chemical shift of the $CH_3C\underline{H}NH$ proton was related to the type of terminal group in the dimer: the electron rich vinyl group produced a small (0.1 ppm) upfield shift in the resonance of the $CH_3C\underline{H}NH$ proton in dimer (58) (relative to the signal in the diethyl-terminated dimer (70)) while the electron-withdrawing acetyl groups in dimer (71) caused a downfield shift of 0.3 ppm in the resonance of $CH_3C\underline{H}NH$.

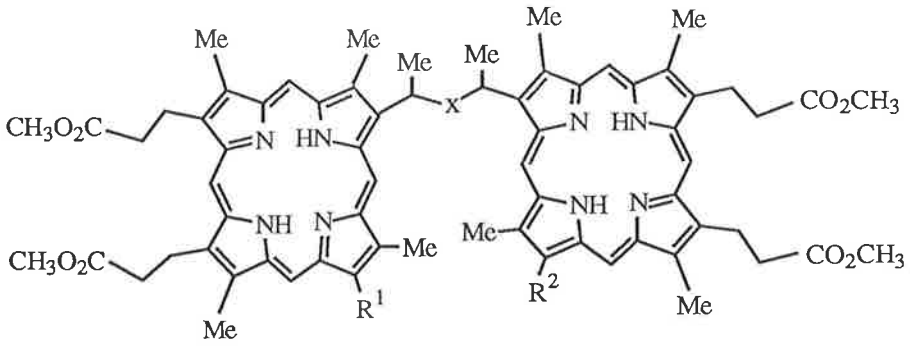
The CH_3CHNH signal (5.6 ppm) in the di(1-hydroxyethyl)-terminated dimer appeared to be unaffected by the 1-hydroxyethyl terminal groups. This is not unexpected because the effect of the 1-hydroxyethyl group would be inductive and therefore not significant over a long distance in contrast with the resonance effects of the acetyl and vinyl groups. The resonances of the protons in the terminal groups were similar to those of analogous groups in known monomers.[26] The vinyl protons resonated at 7.5 – 7.6 ppm ($CH=CH_2$) and ~6.0 ppm ($CH=CH_2$), the ethyls at 3.9 – 4.0 ppm (CH_2CH_3) and 1.8 ppm (CH_2CH_3), the acetyl protons resonated at 2.8, 3.0 ppm ($2 \times COCH_3$), and the 1-hydroxyethyl groups showed signals at 5.9 ppm (CH_3CHOH) and ~2.1, 2.2 ppm ($2 \times CH_3CHOH$).

The ^{13}C NMR spectra of the amino-linked dimers (58), (70), (71) and (72) were well resolved and the signals were also readily assigned on the basis of comparisons with spectra of the analogous ether-linked dimers[1,2] as well as certain monomers.[26] As expected the pyrrole carbons gave broad signals (133 – 150 ppm) due to NH tautomerism.[28] The resonances of the meso carbons (95 – 98 ppm) were complicated multiplets due to the overlap of signals from the two component porphyrins in the molecule. The resonances of the sidechain carbons are summarized in Table 3.1. The carbons of the ring methyls resonated at approximately 11.6 ppm, with the exception of the diacetyl-terminated dimer (71) whose ring methyl carbons resonated over a range of 10.3 – 14.2 ppm. A similar spread in the ring methyl signals was observed in the analogous ether-

linked dimer.[2] The propionate groups produced signals at 21.6 – 22 ppm ($\underline{C}H_2CH_2CO_2$), 36.7 – 37 ppm ($\underline{C}H_2CO_2CH_3$), ~173 ppm ($\underline{C}O_2CH_3$), 51.7 ppm ($CO_2\underline{C}H_3$), all of which correlated well with the corresponding signals in the analogous ether-linked dimers.[1,2]

The carbons in the bridging group were assigned the signals ~24 ppm ($\underline{C}H_3CHNH$-) and ~49 ppm ($CH_3\underline{C}HNH$-). As expected the signal of the benzylic carbons $CH_3\underline{C}HX$- in the amino-linked dimers was upfield relative to those in the ether-linked dimers because of the lower electronegativity of nitrogen compared to oxygen. The signals of the terminal groups in the amino-linked dimers also correlated well with those in the analogous ether-linked dimers,[1,2] as well as monomers.[27] The signals due to the methyl carbon ($\underline{C}H_3CHOH$) of the 1-hydroxyethyl groups in dimer (72) occurred in the expected[1,2] range of 23.7 – 29.2 ppm, which overlapped with the signals of the methyl carbon in the bridging group ($\underline{C}H_3CHNH$-). Without the aid of COSY experiments, a definite assignment could not be made.

A proton-^{13}C COSY spectrum of the diacetyl-terminated dimer (71) showed that the presence of the electron withdrawing acetyl group did not appear to have any effect on the chemical shift of the benzylic carbon on the bridging group ($CH_3\underline{C}HNH$-), which resonated at nearly the same frequency as the corresponding carbon in the ethyl-terminated dimer (70), (Table 3.1).



(58) $R^1 = R^2 = CH{=}CH_2$, X = NH
(70) $R^1 = R^2 = CH_2CH_3$, X = NH
(71) $R^1 = R^2 = COCH_3$, X = NH
(72) $R^1 = R^2 = CH(CH_3)OH$, X = NH

Table 3.1 **^{13}C chemical shifts (δ, ppm) for amino-linked porphyrin dimers.**

Dimer	ring CH_3	Propionic ester CH_2 - CH_2 - CO - O - CH_3				Terminal groups	Linking group CH_3	Linking group CHNH-
(58)	11.6	21.8	37.0	173.6	51.7	$CH{=}CH_2$ 128.8 118 – 120†	24.5	49.6
(70)	11.8	22.0	37.1	173.7	51.7	CH_2-CH_3 17.6 19.8	24.5 24.5	49.9 49.9
(71)	10.3–14.2	21.6	36.7	173.4	51.7	CO-CH_3 198.1, 198.6 33.0	23.6, 24.7	49.2
(72)	11.6	21.9	37.0	173.6	51.7	CH(OH)-CH_3 64.4, 65.5 23.7–29.2*	23.7–29.2*	49.5

† broad signals

* overlapping signals

3.2.9 Biological activity of the amino-linked dimers.

Tests for *in vivo* anticancer activity were done using the literature[15] assay outlined in Chapter 2 (Section 2.2.5). The amino-linked dimers (58), (70), (71) and (72) were each hydrolysed in aqueous base according to the literature procedure.[1,2,9] The product of the hydrolysis was then dissolved in saline to make up a solution with a porphyrin concentration of 2.5 mg/ml.

The testing procedure[15] involved the injection of the aqueous porphyrin solution (at a dose of 50 mg/kg) into mice which had been implanted with Lewis Lung carcinoma. Twenty four hours after administration of the porphyrin solution, the tumour site was irradiated with 600 — 700 nm light from an external light source for 200 seconds, and the size of the tumour was measured daily until the end point (T_C 50), which is the number of days taken for half the mice in the sample to show a recurrence of tumour growth. The results of the tests with the amino-linked dimers are summarized in Table 3.2.

Generally, the amino-linked dimers were less active than their ether-linked analogues.[1,2] This implied that the more basic and hydrophilic amino group had caused a decrease in the biological activity of the dimers, regardless of the type of terminal groups. This complements the results of Ward *et. al.*[1,2] which indicated that the biological activity of ether-linked dimers[1,2] was partly related to the type of terminal groups in

Table 3.2 **Comparisons between the anticancer activity (T_C 50) of amino-linked porphyrin dimers and their ether-linked analogues.**

Terminal group	T_C 50 (days)	
	amino-linked dimer	ether-linked dimer
Diacetyl	2	4
Divinyl	3 – 4	6—7
Diethyl	5	6—7
Di(1-hydroxyethyl)	0	0

the dimers, with the more hydrophobic groups producing greater anticancer activity than hydrophilic groups.

3.2.10 Summary and Conclusion

Conditions for the hydrobromination of 3-acetyl-8-vinyldeuteroporphyrin dimethyl ester (74), 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76), as well as the partial hydrobromination of PPdme (7a) using saturated HBr/dichloromethane were established. Reaction of the resulting bromoethyl compounds with concentrated ammonium hydroxide resulted in moderate yields of amino-linked dimers (71), (70) and (58), respectively. Borohydride reduction of the acetyl groups in dimer (71) furnished the di(1-hydroxyethyl)-terminated dimer (72). The dimers were characterized u.v./visible absorption, NMR spectroscopy and FAB mass spectrometry. The FAB, NMR and visible absorption spectra of the amino-linked dimers were similar to those of the analogous ether-linked systems.[1,2] The NMR resonances of the benzylic CH groups in the bridging position were characteristically upfield relative to the analogous resonances in the ether-linked[1,2] systems due to the lower electronegativity of the adjacent nitrogen atom.

The overall decrease in the biological activity of the dimers when the oxygen atom in the bridging group was replaced with nitrogen suggested that hydrophilic and basic groups such as the amino groups tend to lower the biological activity of the dimers.

3.3 Experimental[§]

3.3.0 General Experimental

The general experimental details are essentially similar to those outlined in Chapter 2, Section 2.3.0.

3.3.1 Hydrobromination of PPdme (7a) using HBr/dichloromethane.

(a) Reaction with half-saturated HBr/dichloromethane.

Gaseous HBr was bubbled into dry dichloromethane (2 ml) for 2 min. This solution was added dropwise to a solution of PPdme (7a) (50 mg) in dichloromethane (2 ml). The mixture was stirred under nitrogen (in darkness) at room temperature. Aliquots (*ca.* 0.5 ml) were removed at intervals and added to aqueous sodium bicarbonate (10%)/THF (1:1, 5 ml), and the mixture was shaken at 0°C for 30 min , then diluted with water and extracted with dichloromethane. The combined organic extracts were washed with water and evaporated to dryness. The residue was analysed by TLC on silica developed in 3% methanol/dichloromethane. Results are summarised in Table 3.3.

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§ References, p. 171.

Table 3.3 Hydrobromination of PPdme using half-saturated HBr/dichloromethane.

Hydrobromination[a] Reaction time	Product Mixture[b]
15 min	Mainly (>85%) PPdme
30 min	PPdme (~80%), HVdme (~15%), HPdme (~15%)
19 h 30 min	PPdme (~20%), HVdme (~60%), HPdme (<20%)
20 h	as for 19 h 30 min
22 h	PPdme (<20%), HVdme (~50%), HPdme (~25%)
24 h	PPdme (~10%), HVdme (~40%), HPdme (~40%)
90 h	PPdme : HVdme : HPdme = 3 : 13 : 11[c]

a . Length of time for which PPdme was stirred in half-saturated HBr/dichloromethane.

b. Products from the hydrolysis of the crude 1-bromoethyl intermediate from the hydrobromination of PPdme. Yields were estimated on the basis of the intensity of the spot on the TLC chromatogram. PPdme (7a), HVdme (52), HPdme (6).

c. Obtained by reversed-phase HPLC using standard conditions[9] (see Section 3.3.0). PPdme eluted at R_t 58.9 min, HVdme at 10.4 – 11.7 min and HPdme at 5.6 min.

(b) Reaction with saturated HBr/dichloromethane at 0°C

PPdme (7a) (30 mg) was dissolved in dry dichloromethane (3 ml) and cooled to 0°C. Gaseous HBr was bubbled into the solution for 45 seconds and the mixture was stirred at 0°C under nitrogen. Aliquots were removed at intervals and hydrolysed with aqueous sodium bicarbonate (10%)/THF (1:1) as outlined in 3.3.1(a), and the products were analysed by TLC, results of which are summarized in Table 3.4.

(c) Reaction with saturated HBr/dichloromethane at room temperature

Into PPdme (7a) (60 mg) dissolved in dry dichloromethane (5 ml) was bubbled HBr for 1 min. The solution was stirred at room temperature for 2 h. An aliquot was removed and hydrolysed in the manner outlined in Section 3.3.1(a). Analysis of the products by TLC (silica, 3% methanol/dichloromethane) showed the following products (estimated yields): PPdme (7a) (<5%) HVdme (52) (<10%), HPdme (6) (>80%), which is similar to the results obtained in the 40 min hydrobromination at 0°C in Section 3.3.1(b).

Table 3.4 Hydrobromination with saturated HBr/dichloromethane.

Reaction time[a]	Product mixture[b]
1 min	PPdme (~20%), HVdme (~40%), HPdme (~40%)
20 min	PPdme (~10%), HVdme (20%), HPdme (> 25%)
40 min	PPdme (< 5%), HVdme (<10%), HPdme (> 80%)
50 min	Similar to results obtained after 40 mins' hydrobromination above.

a. Time during which the reaction mixture was stirred at 0°C.

b. Products of the hydrolysis of the crude 1-bromoethyl intermediate (from the hydrobromination of PPdme). Yields were estimated on the basis of the intensity of the spot on the thin layer chromatogram. PPdme (7a), HVdme (52), HPdme (6).

3.3.2 Nucleophilic substitution with ammonia

(a) Preliminary study

The bromoethyl intermediate from PPdme (7a) was prepared in the following manner: gaseous HBr was bubbled for ~5 min into a solution of PPdme (7a) (60 mg) in dichloromethane (5 ml). The mixture was stirred at room temperature for 2 h, then the excess HBr and dichloromethane were removed under reduced pressure (10 mm Hg) at 0°C until a gum remained. This gum was redissolved in dry dichloromethane (4 ml) and cooled to 0°C. Cold concentrated ammonium hydroxide (90%, 1.5 ml) was added dropwise with stirring. The heterogeneous mixture was stirred vigorously at 0°C for 7 min, then diluted with water (5 ml) and the pH was adjusted to 9 by the addition of 1 M HCl. The mixture was extracted with dichloromethane/THF, the combined organic extracts were washed with water (2 × 20 ml) and evaporated to dryness, leaving a solid which gave FAB m.s. *m/z* 590 (base peak), 607 (m), 624 (m), 1197 (w), 1214 (w), 1230 (w); λ_{max} (rel. abs.) 400 nm (100%), 502(10.6%), 535 (6.5%), 570 (4.8%), 622(2.8%); TLC (silica, 3% methanol/dichloromethane) showed no PPdme, trace amounts of HVdme were present (detected under 254 nm light) and the main portion (>90%) of the material remained on the baseline.

(b) Esterification using the orthoformate method[8]

The crude product (ca. 10 mg) from Section 3.3.2(a) was dissolved in methanol/trimethyl orthoformate (1:1, 4 ml). Water (0.1 ml) was added and the mixture was cooled in an ice-bath. Concentrated sulphuric acid (0.2 ml) was added dropwise with swirling. After the addition the mixture was stirred in the dark for 1 h, then diluted with water (10 ml) and made slightly alkaline (pH 8) by the addition of aqueous sodium bicarbonate. The mixture was extracted with dichloromethane (3 × 20 ml). The combined organic extracts were washed with water and evaporated to dryness, leaving a solid which was similar (TLC, FAB m.s.) to the untreated product from Section 3.3.2(a).

(c) Standard acetylation[24] **with acetyl chloride/pyridine**

The crude product (*ca.* 80 mg) from Section 3.3.2(a) was dissolved in dry dichloromethane (*ca.* 4 ml) and added dropwise with stirring to a mixture containing acetyl chloride (80 mg) and pyridine (2 drops) at 0°C. The mixture was stirred in darkness at room temperature under nitrogen for 2 h. Dichloromethane (10 ml) was added, followed aqueous sodium bicarbonate (10 %) until the solution was slightly alkaline (pH 8). The organic phase was collected and the remaining aqueous phase was extracted with dichloromethane/THF until all the colour had left the aqueous phase. The combined organic extracts were washed with water and evaporated to dryness. The solid

that was obtained gave FAB m.s. *m/z* 590 (base peak), 608 (m), 649 (m), 666 (m), 708 (m), 1197 (w), 1258 (w), 1315 (w).

The acetylated material (*ca.* 60 mg) was chromatographed by preparative TLC on silica developed in 3% methanol/dichloromethane containing concentrated (90%) ammonium hydroxide (1% vol./vol.). Each of the (five) significant bands were collected and stirred in 20% methanol/dichloromethane. Each suspension was filtered through and the filtrate was evaporated to dryness. The residue was redissolved in dichloromethane (or up to 5% methanol/dichloromethane), filtered through Celite and evaporated to dryness. The residues were analysed by FAB m.s. where Band 1 (14%) (the highest R_F) gave *m/z* 590; Band 2 (21%), *m/z* 1199, (58); Band 3 (37%) *m/z* 1199 (58), 1258 (63); Band 4 (1%) *m/z* 1317 (64); Band 5 (12%) *m/z* 709 (62). MIKE spectrum of *m/z* 1315 gave *m/z* 650, 664.

3.3.3 Nucleophilic substitution with *n*-butylamine

(a) 3,8-Di(1-(*n*-butylamino)ethyl)deuteroporphyrin dimethyl ester (67)

PPdme (7a) (20 mg) was dissolved in dry dichloromethane (3 ml) and hydrobromic acid was bubbled into the solution for 2 min. The mixture was stirred in darkness at room temperature under an atmosphere of nitrogen for a further 60 min. The excess HBr and dichloromethane was removed under reduced pressure (10 mm Hg) at 0°C to leave a gum, which

was redissolved in fresh dry dichloromethane (2 ml). The solution was added dropwise at 0°C to neat *n*-butylamine (*ca.* 1.5 ml) in an inert atmosphere. The mixture was stirred at 0°C for 3 min, then the excess *n*-butylamine and dichloromethane were removed rapidly under high vacuum. The gummy residue showed λ_{max} (rel. abs.) 410 nm (100%), 501 (22.3%), 536 (15.0%), 569 (11.7%), 622 (6.3%); FAB m.s. *m/z* 739, 666, 594. The crude product was chromatographed on a deactivated silica (soaked in 5% methanol/dichloromethane before use) squat column. The first significant band, eluted with 5% methanol/dichloromethane, gave *3-(1-n-butylaminoethyl)-8-vinyldeuteroporphyrin dimethyl ester* (68) (1 mg, 4%), FAB m.s. *m/z* 663 (M^+), 607, 591. This was followed by the major band (also eluted with 5% methanol/dichloromethane) which contained *3,8-di(1-(n-butyl-amino)ethyl)deuteroporphyrin dimethyl ester* (67) (21 mg, 84%) which had λ_{max} (rel. abs.) 412 nm (100%), 501 (21.8%), 538 (14.5%), 569 (11.2%), 623 (5.1%); ^{13}C NMR, δ ppm: 11.66 – 14.04 (ring $\underline{C}H_3$), 19.74 ($NHCH_2CH_2CH_2\underline{C}H_3$), 21.80 ($\underline{C}H_2CH_2CO_2$), 22.66 ($NHCH_2CH_2\underline{C}H_2CH_3$), 25.70 ($\underline{C}H_3CHNH$), 27.15 ($NHCH_2\underline{C}H_2$), 31.65 ($NH\underline{C}H_2CH_2$), 36.76 ($\underline{C}H_2CO_2CH_3$), 47.41 ($CH_3\underline{C}HNH$), 51.76 - 53.38 ($CO_2\underline{C}H_3$), 96.60 – 98.77 (meso C), 129.70 (br, Cα), 139.23 (br, Cβ), 172.9 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm: 1.55 ($2 \times NHCH_2CH_2CH_2C\underline{H}_3$), 1.92 ($2 \times NHCH_2C\underline{H}_2$-), 2.21 ($2 \times C\underline{H}_3CHNH$), 2.61 ($2 \times NHC\underline{H}_2CH_2$), 3.22 ($2 \times C\underline{H}_2CO_2CH_3$), 3.57 (4 × ring $C\underline{H}_3$), 3.67, 3.80 ($2 \times CO_2C\underline{H}_3$), 4.34 ($2 \times C\underline{H}_2CH_2CO_2$), 6.01 ($2 \times CH_3C\underline{H}NH$), 10.05, 10.12, 10.36, 10.38, 10.63 (4 × meso H); FAB m.s., *m/z* 737 (M^+), 663, 591 (base peak).

(b) Aminolysis products

The bromoethyl derivative (8) of PPdme (7a) (20 mg) was prepared by the procedure outlined in Section 3.3.3(a), and added dropwise with stirring, to neat *n*-butylamine (*ca.* 1 ml) at 0°C. Throughout the addition, a steady stream of dry nitrogen was bubbled into the reaction mixture. The mixture was then stirred at 0°C for 20 min, after which it was evaporated to dryness, leaving a red solid (45 mg) which gave λ_{max} (rel. abs.) 405 nm (100%), 502.(12.6%), 537.(10.0%), 568.(9.1%), 622.(3.5%); FAB m.s. *m/z* 591, 663 (68), 704 (69a), 745 (69c), 778 (69b), 819 (69d).

3.3.4 Divinyl-terminated amino-linked dimer (58)

(a) Optimization studies for partial hydrobromination of PPdme

PPdme (7a) (10 mg) was dissolved in dichloromethane (4 ml). HBr gas was bubbled into the solution for 90 sec. The reaction mixture then stirred at room temperature under nitrogen for a length of time (see Table 3.3). The reaction mixture was cooled in an ice-bath and the excess HBr and solvent were removed under reduced pressure (10 mm Hg) until a gummy residue was obtained. This usually proceeded for 20 min. The residue was redissolved in dry dichloromethane and

reacted with either (i) aqueous sodium bicarbonate, or (ii) saturated ammonium hydroxide/THF, in the following manner:

(i) Reaction with aqueous sodium bicarbonate

The solution containing the bromoethyl compound (8) in dichloromethane was added to aqueous sodium bicarbonate (10%, 10 equivalent volumes)/ice and shaken for 5 min. The reaction mixture was diluted with dichloromethane, the organic phase was separated and washed with water. Finally, evaporation under reduced pressure afforded the product, which was analysed by TLC (silica, 2% methanol/dichloromethane), HVdme (R_F 0.2 – 0.3) and HPdme (R_F 0.1) were the major products (~1:1), PPdme (R_F 0.8), (<10%).

(ii) Reaction with concentrated ammonium hydroxide/THF

Concentrated ammonium hydroxide (90%)/THF (1:1, 2 ml) was added dropwise to the bromoethyl porphyrin (8) solution at 0°C with stirring. The mixture was stirred at 0°C for 30 min, then diluted with dichloromethane and washed with water (2 × 20 ml). The organic phase was evaporated to dryness leaving a residue which was chromatographed on a squat column packed with deactivated silica (soaked in methanol before use). The column was flushed with dichloromethane before the crude product was placed on it. Typically, elution with 0.5% acetone/dichloromethane afforded PPdme; the divinyl-terminated amino-linked dimer (58) was eluted with 2-3%

acetone/dichloromethane, while the "polar material" containing (1-aminoethyl)-vinyl-terminated amino-linked dimer (59), 3-(1-aminoethyl)-8-vinyldeuteroporphyrin dimethyl ester (57), di(1-aminoethyl)-terminated amino-linked dimer (60) and higher oligomers were eluted with 2 – 60% methanol/dichloromethane. The reaction times for the hydrobromination step were varied and the results of these modifications are summarised in Table 3.5.

Table 3.5 : Results of optimization studies for the partial hydrobromination of PPdme.

Reaction time for hydrobromination[a]	Products of reaction with conc. ammonium hydroxide/THF[b]	
	PPdme	divinyl dimer[c]
2 min	60%	8%
3 min	33%	32%
4 min	12%	20%
10 min	polar material > divinyl dimer ≤ PPdme (TLC)	

a. Time taken from the point after the addition of HBr gas into the PPdme/dichloromethane solution to the point when the excess HBr/dichloromethane was about to be removed (see beginning of Section 3.3.4 for details). PPdme, (7a).

b. Yields (obtained after chromatography) are based on starting PPdme; the remainder of the product was mainly the "polar material" (see Section 3.3.4(ii)).

c. Divinyl-terminated amino-linked dimer (58).

(c) Hydrobromination using saturated HBr/dichloromethane.

Gaseous HBr was bubbled into dry dichloromethane (15 ml) at 0°C for approximately 10 min. 3-(1-bromoethyl)-8-vinyldeuteroporphyrin dimethyl ester (57) was prepared by the addition of the saturated HBr/dichloromethane solution to PPdme (7a) (150 mg, 0.25 mmol). The mixture was stirred in the dark at room temperature under an atmosphere of nitrogen for 16 min, then added dropwise with stirring to concentrated ammonium hydroxide/THF (2:1, 40 ml) at 0°C and stirred for a further 20 min. The mixture was diluted with dichloromethane (*ca.* 20 ml), and poured into water. Dilute hydrochloric acid was added to adjust the pH of the solution to pH 10. The mixture was extracted with dichloromethane, the extracts washed with water and evaporated to dryness without chemical drying. Chromatography of the residue using a silica squat column afforded PPdme (7a) (55 mg, 37%) which was eluted with 0.25% methanol/dichloromethane. Elution with 1% methanol/dichloromethane afforded the *divinyl-terminated amino-linked dimer* (58) (60 mg, 40%), λ_{max} (rel. abs.) 395 nm (100%), 507 (10.4%), 540 (7.7%), 575 (5.7%), 628 (3.3%); ^{13}C NMR, δ ppm: 11.59 (ring $\underline{C}H_3$), 21.78 ($\underline{C}H_2CH_2CO_2CH_3$), 24.48 ($\underline{C}H_3CHNH$-), 36.95 ($CH_2\underline{C}H_2CO_2CH_3$), 49.60 ($CH_3\underline{C}HNH$-), 51.72 ($CO_2\underline{C}H_3$), 95.19-96.11 (meso C), 118.68 – 120.36 ($CH=\underline{C}H_2$), 128.77 – 130.36 ($\underline{C}H=CH_2$), 136.03 – 137.43 (C_β), 140 – 145 (br., C_α), 173.63 ($\underline{C}O_2CH_3$). ^1H NMR, δ ppm : -5.33, -5.12, -4.95, -4.06

(4 × pyrrole H), 2.15, 2.67 (2 × d, 2 × $C\underline{H}_3CHNH$), 3.0 – 3.8 (4 × $CO_2C\underline{H}_3$, 8 × ring $C\underline{H}_3$, 4 × $C\underline{H}_2CO_2CH_3$), 4.31 – 4.39 (4 × $C\underline{H}_2CH_2CO_2CH_3$), 5.47 - 5.53 (br, 2 × $CH_3C\underline{H}NH$-), 5.83 – 5.96 (2 × $CH=C\underline{H}_2$), 7.49 – 7.67 (2 × $C\underline{H}=CH_2$), 9.12 – 9.97 (m, 8 × meso H). FAB m.s. *m/z* 1199 (w, M^+) 1197 (M^+ - 2H), 608, 591 (M^+); MIKE spec. of *m/z* 1199 gave *m/z* 591, 606 (major fragments); 1183, 1157 (minor fragments).

(d) Nucleophilic substitution in a heterogeneous reaction mixture

Gaseous HBr was bubbled into dry dichloromethane (5 ml) at 0°C for approximately 3 min. The saturated HBr/dichloromethane solution was added to PPdme (50 mg) and the mixture was stirred at room temperature under nitrogen for 22 min, then added dropwise with stirring to concentrated ammonium hydroxide (90%, 5 ml) at 0°C. The heterogenous mixture was stirred vigorously at 0°C for 10 min, then diluted with dichloromethane and poured into water. Dilute HCl was added to adjust the basicity of the solution to pH 9, the organic layer was separated and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with water (2 × 20 ml) and then evaporated to dryness without chemical drying, leaving a solid residue which was chromatographed on a normal silica gel squat column. PPdme (9 mg, 18%) was eluted with 0.5% methanol/dichloromethane; the desired *divinyl-terminated amino-linked dimer* (58) (16 mg,

31%), which was eluted with 0.5-1.0% methanol/dichloromethane, was identical (TLC, FAB m.s., NMR) to the sample obtained in Section 3.2.4(c); (1-aminoethyl)-vinyl-terminated amino-linked dimer (59) (23 mg, 44%) which was eluted with 2-10% methanol /dichloromethane had FAB m.s. *m/z* 1216; some porphyrin material remained adhered to the adsorbent and could not be shifted despite attempts to elute it with 50% methanol/dichloromethane.

3.3.5 Diethyl-terminated amino-linked dimer (70)

(a) "Standard method"

To 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester[2,16,17] (76) (30 mg) was added saturated HBr/dichloromethane (3 ml) and the mixture was stirred at room temperature under nitrogen for 90 min. Excess HBr and dichloromethane were removed under reduced pressure (10 mm Hg) at 0°C to leave a gum, which was redissolved in dry dichloromethane (0.5 ml).

The solution of crude bromoethyl porphyrin (77) in dichloromethane was added dropwise with stirring to a mixture of concentrated ammonium hydroxide (90%, 1 ml) and THF (0.5 ml) at 0°C. The mixture was stirred at 0°C for 10 min, then diluted with dichloromethane and poured into water. The mixture was neutralized and extracted with dichloromethane. The organic extracts were washed with water and evaporated to

dryness without chemical drying, leaving a red-brown solid which gave FAB m.s. *m/z* 1203 (M^+), 610, 593 (base peak). The crude product was chromatographed on a squat column packed with deactivated silica (soaked in 20% methanol/dichloromethane for 2 h and flushed with dichloromethane before use). Elution with 0.5-1.0% methanol/dichloromethane afforded the *title dimer* (70) (16 mg) (TLC silica, 5% methanol/dichloromethane, R_F 0.36 – 0.38) which gave λ_{max} (rel. abs.) 392 nm (100%), 502 (10.4%), 536 (7.5%), 569 (6.4%), 622 (3.3%); ^{13}C NMR, δ ppm: 11.78 (ring-$\underline{C}H_3$), 17.61 ($CH_2\underline{C}H_3$), 19.80 ($\underline{C}H_2CH_3$), 22.0 ($\underline{C}H_2CH_2CO_2CH_3$), 36.06 ($\underline{C}H_3CHNH$-), 37.13 ($CH_2\underline{C}H_2CO_2CH_3$), 49.86 ($CH_3\underline{C}HNH$), 51.74 ($O\underline{C}H_3$), 96.0 – 96.2 (meso C), 134-139 (C_β), 141.5 – 146.3 (C_α), 173.70 (-$\underline{C}O_2CH_3$); ^1H NMR, δ ppm* -4.0 (br, 4 × pyrrole H), 1.8 (2 × $C\underline{H}_2CH_3$), 2.2 (2 × $C\underline{H}_3CHNH$), 3.36 – 3.41 (4 × $C\underline{H}_2CO_2CH_3$), 3.5 – 3.8 (4 × $CO_2C\underline{H}_3$, 8 × ring $C\underline{H}_3$,), 3.9 – 4.0 (2 × $CH_2C\underline{H}_3$), 4.4 – 4.5 (4 × $C\underline{H}_2CH_2CO_2CH_3$), 5.57 - 5.63 (br, 2 × $CH_3C\underline{H}NH$-), 9.6 (br), 9.9 (br), 10.1 (br) (8 × meso H); FAB m.s. *m/z* 1203 (M^+), 610, 593 (base peak); MIKE spec. of *m/z* 1203 gave *m/z* 1159, 593 (major fragments); 1186, 608 (minor). Elution with 5% methanol/dichloromethane afforded *3-ethyl-8-(1-aminoethyl)deuteroporphyrin dimethyl ester* (78) (7 mg), which gave TLC silica, 5% methanol/dichloromethane R_F 0.03, λ_{max} (rel. abs.) 400 nm (100%), 500 (8.8%), 536 (6.3%), 568 (4.9%), 622 (2.6%); ^{13}C NMR, δ ppm 11.27–12.17 (ring $\underline{C}H_3$), 17.61 ($CH_2\underline{C}H_3$), 19.74 ($\underline{C}H_2CH_3$), 21.75 ($\underline{C}H_2CH_2C0_2CH_3$), 24.90

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* All signals were broad.

($\underline{C}H_3CHNH_2$), 36.93 ($CH_2\underline{C}H_2C0_2CH_3$), 45.60 ($CH_3\underline{C}HNH_2$), 51.72 ($CO_2\underline{C}H_3$), 96.08-97.77 (meso C), 137.23-139.03 ($C\beta$), 141.63 (br. $C\alpha$), 173.59 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm -4.10 (br., pyrrole H), 1.78-1.87 (t, $CH_2C\underline{H}_3$), 2.04 – 2.10 (d, $C\underline{H}_3CHNH_2$), 3.10 – 3.71 ($CH_2C\underline{H}_2C0_2CH_3$, $C0_2C\underline{H}_3$, ring $C\underline{H}_3$), 4.0 – 4.04 (q, $C\underline{H}_2CH_3$), 4.20 (br., $C\underline{H}_2CH_2C0_2CH_3$), 5.75 (br. $CH_3C\underline{H}NH_2$), 9.61-10.20 (m, meso H); FAB m.s., *m/z* 610 (M^+), 593 (base peak); MIKE spectrum of *m/z* 610 gave *m/z* 595, 582, 568, 537 (major fragments), 551, 523 (minor fragments). The starting 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (6 mg) (TLC silica, 5% methanol/dichloromethane R_F 0.9) was eluted with 0.5% methanol/dichloromethane.

(b) Optimization attempt no.1 : heterogeneous reaction mixture

The bromoethyl compound (77) was prepared according to the procedure outlined in Section 3.3.5(a) above from 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (20 mg) and was dissolved in dry dichloromethane (1 ml). This was added dropwise with vigorous stirring to concentrated ammonium hydroxide (90%, 3 ml) at 0°C. The mixture was stirred at 0°C for 12 min, diluted with dichloromethane and poured into water. After neutralization and extraction as outlined above, FAB m.s. analysis of the crude product showed the presence of the *title dimer* (70), the 1-aminoethyl monomer (78) and the starting porphyrin (76), which were present in an approximate ratio of

2 : 1 : 1 (TLC on silica, 5% methanol/dichloromethane). This was similar to the relative yields in Section 3.3.5(a) above.

(c) Optimization attempt no.2 : reversed order of addition – ammonium hydroxide to the 1-bromoethyl compound

Saturated HBr/dichloromethane (5 ml) was added to 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (30 mg) and the solution was stirred in darkness at room temperature under nitrogen for 100 min and evaporated to a gum under reduced pressure (10 mm Hg) at 0°C. To the gummy residue was added a cold (0°C) solution of concentrated ammonium hydroxide/THF (3:1, 4 ml) dropwise with stirring at 0°C. The mixture was stirred at 0°C for 9 min, then diluted with dichloromethane, poured into water and worked-up as above. TLC and FAB m.s. were nearly identical to those of the preceeding runs (Section 3.3.5(a) – (b))

(d) Optimization attempt no.3 : direct treatment with ammonium hydroxide - without prior removal of excess HBr.

A mixture containing saturated HBr/dichloromethane (5 ml) and 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (30 mg) was stirred in darkness at room temperature under nitrogen for 100 min. The solution was then added dropwise

with stirring to a cold (0°C) mixture of concentrated ammonium hydroxide (90%, 3 ml) and THF (1 ml). Stirring was continued at 0°C for 5 min, then the mixture was diluted with dichloromethane and poured into water. Neutralization, extraction and evaporation of the solvent afforded a solid. FAB m.s. and TLC analysis indicated that the composition of the crude material was nearly identical those of the preceeding runs (Sections 3.3.5(a) – (c))

3.3.7 Diacetyl-terminated amino-linked dimer (71)

(a) Determination of minimum time for hydrobromination

To 3-acetyl-8-vinyldeuteroporphyrin dimethyl ester[2,17,18] (74) (50 mg) was added saturated HBr/dichloromethane (5 ml) and the mixture was stirred at room temperature under nitrogen.

After 90 min an aliquot (0.5 ml) was removed and added to aqueous sodium bicarbonate (10%)/THF (1:1, 2 ml). The mixture was shaken vigorously, then diluted with water and extracted with dichloromethane. The organic phase was washed with water and evaporated to dryness. The residue, analysed by TLC (silica, 2% methanol/dichloromethane), contained mainly (>90%) the starting porphyrin (74) (R_F 0.6—0.8).

Another aliquot (0.5 ml) was removed after 160 min and treated with aqueous base as outlined above. The major

product was 3-acetyl-8-(1-hydroxyethyl)deuteroporphyrin dimethyl ester (81) (R_F 0.1) while the starting porphyrin (74) was present in only negligible amounts.

(b) Reaction between crude 3-acetyl-8-(1-bromoethyl)-deuteroporphyrin dimethyl ester (84) and concentrated ammonium hydroxide

(i) The hydrobromination reaction mixture from Section 3.2.7(a) was allowed to stir for a total of 4 h, then diluted with THF (1 ml) and dichloromethane (1 ml). The mixture was added dropwise with stirring to concentrated ammonium hydroxide (90%, 6 ml) at 0°C. The mixture was stirred at 0°C for 20 min, then diluted with dichloromethane and poured into water. The solution was adjusted to pH 9-10, and the mixture was extracted with dichloromethane. The organic extracts were combined and washed (3 × 20 ml) with water. Evaporation of the solvent gave a solid residue which gave FAB m.s. *m/z* 1231, 625, 607 (base peak). Preparative TLC on a silica gel plate developed in 5% methanol/dichloromethane gave 3 distinct bands : the major band (R_F 0.46-0.56) contained the *diacetyl-terminated amino-linked dimer* (71) (20 mg) which gave λ_{max} (rel. abs.): 410 nm (100%), 513 (13.0%), 551 (11.8%), 581 (9.2%), 639 (2.4%). ^{13}C NMR, δ ppm: 10.30 – 14.16 (ring-$\underline{C}H_3$), 21.61 ($\underline{C}H_2CH_2CO_2CH_3$), 32.97 ($CO\underline{C}H_3$), 23.64 – 24.72 ($\underline{C}H_3CHNH$-), 36.70 ($CH_2\underline{C}H_2CO_2CH_3$), 49.20 ($CH_3\underline{C}HNH$-), 51.71 ($O\underline{C}H_3$), 94.54 - 97.64, 98.78 – 101.58 (meso-C), 133.93 – 140.42 (br, C_β), 144.08 – 150.07 (br, pyrrole-C, C_α), 173.41 ($\underline{C}O_2CH_3$), 198.08, 198.64 ($\underline{C}OCH_3$). ^1H NMR, δ ppm:

-5.72, -5.50, -5.44, -4.99, -4.78, -4.27, -4.13, -4.09 (4 × pyrrole H), 2.27 (d, 2 × $C\underline{H}_3CHNH$-), 2.82, 3.02 (2 × $COC\underline{H}_3$), 3.1 – 3.8 (4 × $CH_2C\underline{H}_2CO_2CH_3$, 4 × $OC\underline{H}_3$, 8 × ring $C\underline{H}_3$), 4.218 – 4.327 (4 × $C\underline{H}_2CH_2CO_2CH_3$), 5.882 – 5.944 (2 × $CH_3C\underline{H}NH$-), 8.942 – 10.257 (m, 8 × meso H). FAB m.s., *m/z* 1231 (w, M^+)1230 (M^+ - H), 624, 607 (base peak). MIKE spec. of *m/z* 1231 gave *m/z* 607, 1188 (major ions), 624, 1214 (minor ions). The two minor bands contained the starting porphyrin (74) (12 mg) (R_F 0.84) and the monomer, 3-acetyl-8-(1-aminoethyl)deuteroporphyrin dimethyl ester (83) (10 mg) (R_F 0.04-0.14), gave λ_{max} (rel. abs.) 411 nm (100%), 509 (11.9%), 548 (11.4%), 577 (8.2%), 634 (2.0%); ^{13}C NMR, δ ppm 11.14 – 11.98 (4 × ring $\underline{C}H_3$), 21.69 (2 × $\underline{C}H_2CHCO_2$-), 26.19 ($\underline{C}H_3CHNH_2$), 33.05 ($CO\underline{C}H_3$), 45.81 ($CH_3\underline{C}HNH_2$), 51.70 (2 × $CH_2CO_2\underline{C}H_3$), 95.52 – 101.33 (m, 4 × meso $\underline{C}H_3$), 132.83 – 140.82 (br, 8 × C_β), 147.46 – 151.47 (br, 8 × C_α), 173.28, 173.55 (2 × $\underline{C}O_2CH_3$), 199.67 ($\underline{C}OCH_3$); ^1H NMR, δ ppm -4.12, -4.04 (2 × pyrrole H), 2.06 – 2.16 (d, $C\underline{H}_3CHNH_2$), 2.43 (br, $COC\underline{H}_3$), 3.12 – 3.80 (2 × $CH_2C\underline{H}_2CO_2CH_3$, 2 × $CH_2CH_2CO_2C\underline{H}_3$, 4 × ring $C\underline{H}_3$), 4.14 – 4.24 (2 × $C\underline{H}_2CH_2CO_2CH_3$), 5.75 – 5.95 ($CH_3C\underline{H}NH_2$), 9.48 – 10.69 (m, 4 × meso H); FAB m.s. 624 (M^+), 607 (base peak); MIKE spec. of *m/z* 624 gave 607 (major ion), 595, 581, 565, 550, 535 (minor ions).

(ii) A solution of saturated HBr/dichloromethane (15 ml) was added to 3-acetyl-8-vinyldeuteroporphyrin dimethyl ester (74) (0.14 g, 0.23 mmol) and the solution was stirred in the dark at room temperature under an atmosphere of nitrogen for 3 h 15 min. The mixture was then added dropwise with stirring to a

mixture of concentrated ammonium hydroxide (90%, 10 ml) and tetrahydrofuran (10 ml) at 0°C. Stirring was continued at that temperature for 20 min. Then the mixture was diluted with dichloromethane (*ca.* 10 ml), and poured into water. Dilute hydrochloric acid was added to adjust the pH of the solution to 10. The mixture was extracted with dichloromethane, the extracts washed with water and evaporated to dryness without chemical drying. The crude product was separated on a silica squat column where the starting porphyrin 2-acetyl-4-vinyldeuteroporphyrin dimethyl ester (25 mg, 18%) was recovered by elution with 0.5% methanol/dichloromethane The *title dimer* (71) (79 mg, 56%), identical (TLC, FAB m.s., NMR, vis. spec.) to the sample in Section 3.3.7(b), was eluted with 1—1.5% methanol/dichloromethane. Elution with 4% methanol/dichloromethane afforded the monomer, 3-acetyl-8-(1-aminoethyl)deuteroporphyrin dimethyl ester (83) (21 mg, 15%) which was identical to the sample in Section 3.3.7(b).

3.3.8 Di(1-hydroxyethyl)-terminated amino-linked dimer (72)

(a) Trial reaction - borohydride reduction of the diacetyl-terminated amino-linked dimer (71)

The diacetyl-terminated dimer (71) (*ca.* 7 mg) (Section 3.3.7(c)) was dissolved in methanol/dichloromethane (1:1, 3 ml). To this was added sodium borohydride (10 mg)

portionwise and the resulting mixture was stirred at room temperature in darkness for 3 h 45 min. After that the mixture was diluted with dichloromethane and poured into 1 M hydrochloric acid (10 ml). The mixture was extracted with THF/dichloromethane. The combined organic extracts were washed with water (2 × 20 ml) and evaporated to dryness, leaving a solid residue which, by TLC (silica, 5% methanol/dichloromethane), contained two spots, R_F 0.19 and R_F 0.25, with no trace of the starting dimer (71).

The crude product was chromatographed by preparative TLC on silica developed in 5% methanol/dichloromethane. Two major bands at R_F 0.25 (*ca.* 2 mg) and R_F 0.32 (*ca.* 2 mg), and two minor bands at R_F 0.31 (*ca.* 1 mg) and R_F 0.39 (*ca.* 1 mg) were collected. All four bands gave identical FAB m.s. (*m/z* 1234, 626, 609) corresponding to the *di(1-hydroxyethyl)-terminated amino-linked dimer* (72) (see below).

(b) Large scale reduction of diacetyl-terminated amino-linked dimer (71)

To the diacetyl-terminated dimer (71) (0.12g, 0.097 mmol), from Section 3.3.7(c), dissolved in methanol/dichloromethane (1:4, 5 ml) was added sodium borohydride (20 mg). The mixture was stirred in the dark at room temperature for 1 h. Then 1 M hydrochloric acid was

added dropwise into the reaction mixture until the excess borohydride had been quenched. The mixture was poured into water (20 ml) and extracted with more dichloromethane. The combined organic extracts were washed with water and evaporated to dryness, leaving a solid residue which was chromatographed on a silica squat column. Unlike preparative TLC, there was very poor separation between the regio- and/or diastereosomers. The major (broad) band, (TLC, silica, 5% methanol/dichloromethane showed 4 spots at R_F 0.08, 0.19, 0.20, 0.28) eluted with 20% acetone/dichloromethane, contained the *di(1-hydroxyethyl)-terminated amino-linked dimer* (72) (0.120 g, 99%) which gave λ_{max} (rel. abs.) 397 nm (100%), 504 (12%), 563 (7%), 571 (6%), 624 (4%); ^{13}C NMR, δ ppm 11.56 (ring $\underline{C}H_3$), 21.85 ($\underline{C}H_2CH_2CO_2$-), 23.76 – 26.27 ($\underline{C}H_3CHNH$, $\underline{C}H_3CHOH$),* 36.95 ($CH_2\underline{C}H_2CO_2$), 49.49 ($CH_3\underline{C}HNH$), 51.70 ($CO_2\underline{C}H_3$), 64.41 – 65.52 ($CH_3\underline{C}HOH$), 95.14 – 98.36 (meso-C). 133.86-139.38 (Cβ), 141.63 – 144.71 (Cα), 173.57 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm (-5.20) – (-4.24) (m, br, 4 × pyrrole H), 2.06 – 2.17 (2 × $C\underline{H}_3CHOH$), 2.30 – 2.39 (2 × $C\underline{H}_3CHNH$), 3.17 –.3.28 (2 × $C\underline{H}_2CO_2CH_3$), 3.33 – 3.77 (4 × $CO_2C\underline{H}_3$, 8 × ring $C\underline{H}_3$), 4.22 – 4.35 (4 × $C\underline{H}_2CH_2CO_2$), 5.63 (br, 2 × $CH_3C\underline{H}NH$), 5.91 (br, 2 × $CH_3C\underline{H}OH$), 9.42 – 10.00 (m, 8 × meso H); FAB m.s. *m/z* 1235 (w, M^+), 1234 (M^+ - H), 626, 609 (base peak); MIKE spec of *m/z* 1235 gave 1217 , 1192 (major fragment ions); 1199, 1174, 652, 626, 609, 592 (minor ions).

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* Overlapping signals.

3.3.9 Preparation of amino-linked dimer samples for *in vivo* tests for anti-tumour activity – general procedure

(a) Base hydrolysis

The porphyrin dimer tetramethyl ester was dissolved in a mixture of distilled THF/1.0 M sodium hydroxide (1:1, 0.2 ml/mg) and the resulting solution was stirred in darkness at room temperature for 20 h. The reaction mixture was diluted with water and dilute hydrochloric acid was added to adjust the pH to 5 – 6. The mixture was extracted with distilled THF/dichloromethane. The combined organic phases were washed with water and evaporated to dryness leaving a solid residue.

(b) Sample preparation

The solid residue from the base hydrolysis was dissolved in minimal 1 M sodium hydroxide. Dilute hydrochloric acid was added dropwise with stirring until the solution was pH 7.2 (± 0.1). Saline (0.9%) was added and aliquots (10 µl) were removed and diluted 1000 times with ethanol/0.1 N sodium hydroxide (1:1). The u.v. absorbance (at 397 nm) of the resulting solution was measured. Addition of saline was stopped when the absorbance at 397 nm was 0.400 units (± 0.020), ie. Abs_{397} 0.400. (Abs_{397} 0.400 is equivalent to a porphyrin concentration of 2.5 mg/ml.[15]) The saline solution containing the porphyrin was then filtered through a 0.45 µm filter.

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CHAPTER 4

4.1 Introduction§

There has been a growing interest in the use of porphyrin derivatives as DNA intercalators.[1] It is known that certain porphyrins and metalloporphyrins, such as the derivatives of cationic meso-tetrakis(4-N-methylpyridyl) porphyrin[2a] and their metal analogues,[2b] interact readily with nucleic acids. In general, bisintercalators[3] (intercalators which consist of two aromatic systems joined by an extended linking group) are more effective than homonuclear species. The type of linkages between the aromatic systems vary from simple hydrocarbon chains[3] to functionalised bridges containing peptide or amide groups, sulphonamides, amines, ethers, thioethers and generally, groups which can form hydrogen bonds with the bases of the DNA.[3] With porphyrin based intercalators, cationic substituents such as quaternized pyridyl[1,2] groups on the porphyrin greatly enhance the intercalation of the molecule with DNA by forming electrostatic interactions with the phosphate backbone of DNA.[2c] Studies with porphyrin-containing dimers such as the porphyrin-ellipticine hybrid dimer[4] and actinocinyl-bis(hemin)[5] indicate that dimers of porphyrins linked by long chains can be good intercalating-chelating agents[4] and DNA-intercalating cleavage agents.[5]

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Examples of other diporphyrin dimers with extended linkages include the methylene-bridged dimers,[6, 7, 8] aryl-,[9] ethylene-,[10] triene-[11] and amide-linked[12] dimers, dimers with spirobi-indan bridges,[13] rigid cyclic dimers linked by ester groups[14] and the ester-linked systems that are analogues of the dimer components of HPD.[15] It appears that none of these dimers have been investigated for DNA intercalation. HPD was found to not interact effectively with DNA.[40]

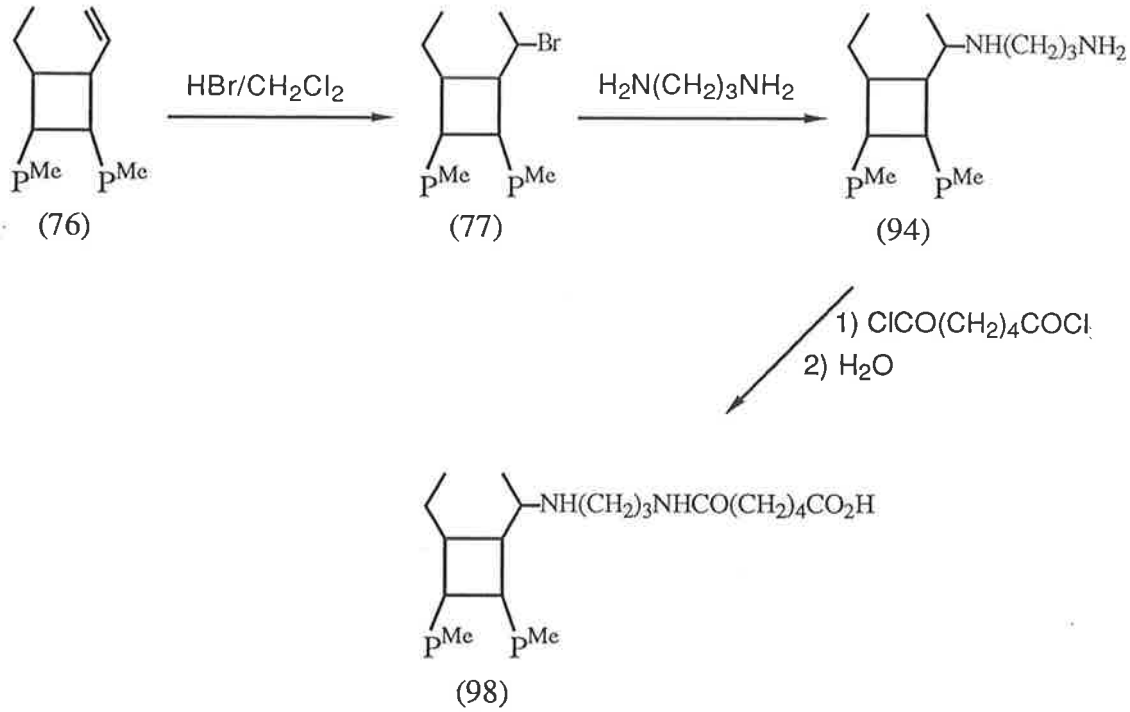
Methods for the synthesis of the dimers vary from the total synthesis of the terminal porphyrin around the linking group,[13] to the manipulation of the functional groups already present on the porphyrin.[6–14] A typical example is the conversion of the carboxylic acid groups to acid chlorides[5,12,14,15] followed by the displacement of the chloride by the bridging system. Alternatively, a suitable functional group (e.g formyl group) is introduced onto the porphyrin which then dimerizes in the presence of a reducing agent. [10]

The aim of this chapter was to use the methodology that had been established in Chapter 3 (for the hydrobromination of vinyl porphyrins) to synthesize other porphyrins of biological interest. In the light of the promising results in the studies[1,2,4,5] of porphyrins as DNA intercalators it was decided to commence studies towards the synthesis of diporphyrin dimers with extended linking groups for the purpose of evaluating their ability to interact with DNA. It was intended that this investigation would be limited to symmetric dimers with an

emphasis on bridging groups that contain amine and/or amide groups (because of the ability[3] of these groups to improve intercalation). 3-Ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) was used as the starting porphyrin in all the syntheses because it was readily available[20] and, being monofunctional (with respect to reaction with HBr), there was less likelihood of complications from side reactions. Although it would be preferable to have cationic groups on the porphyrins for electrostatic interaction with the phosphate backbone of DNA[2c] it was decided to concentrate initial investigations on the synthesis of the long, bridged dimer before focussing attention on the quaternization of the component porphyrins. Ward *et al.*[8] have shown that it is possible to synthesize porphyrins with sidechains containing quaternary ammonium salts by treating a 1-bromoethyl porphyrin intermediate with a tertiary amine such as triethylamine.

Two routes (Schemes 4.1 and 4.2) to the synthesis of porphyrin dimers with amine- and amide-containing linkages were investigated. In Scheme 4.1, the bromethyl intermediate (77) which is prepared by the hydrobromination procedure that was established in Chapter 3 is treated with an α,ω-diamine to give the derivative (94). The diamino derivative (94) is then reacted with a diacid chloride to give the desired dimer (97). In the alternative route, Scheme 4.2, a porphyrin-amino acid derivative (106) is converted to the corresponding acid chloride which is then reacted with an α,ω-diamine to afford the dimer with the amine- and amide-containing bridge (110). The

Scheme 4.1



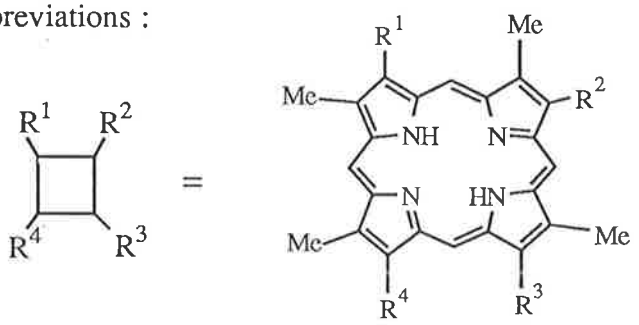
(76) (77) (94)

(98)

+

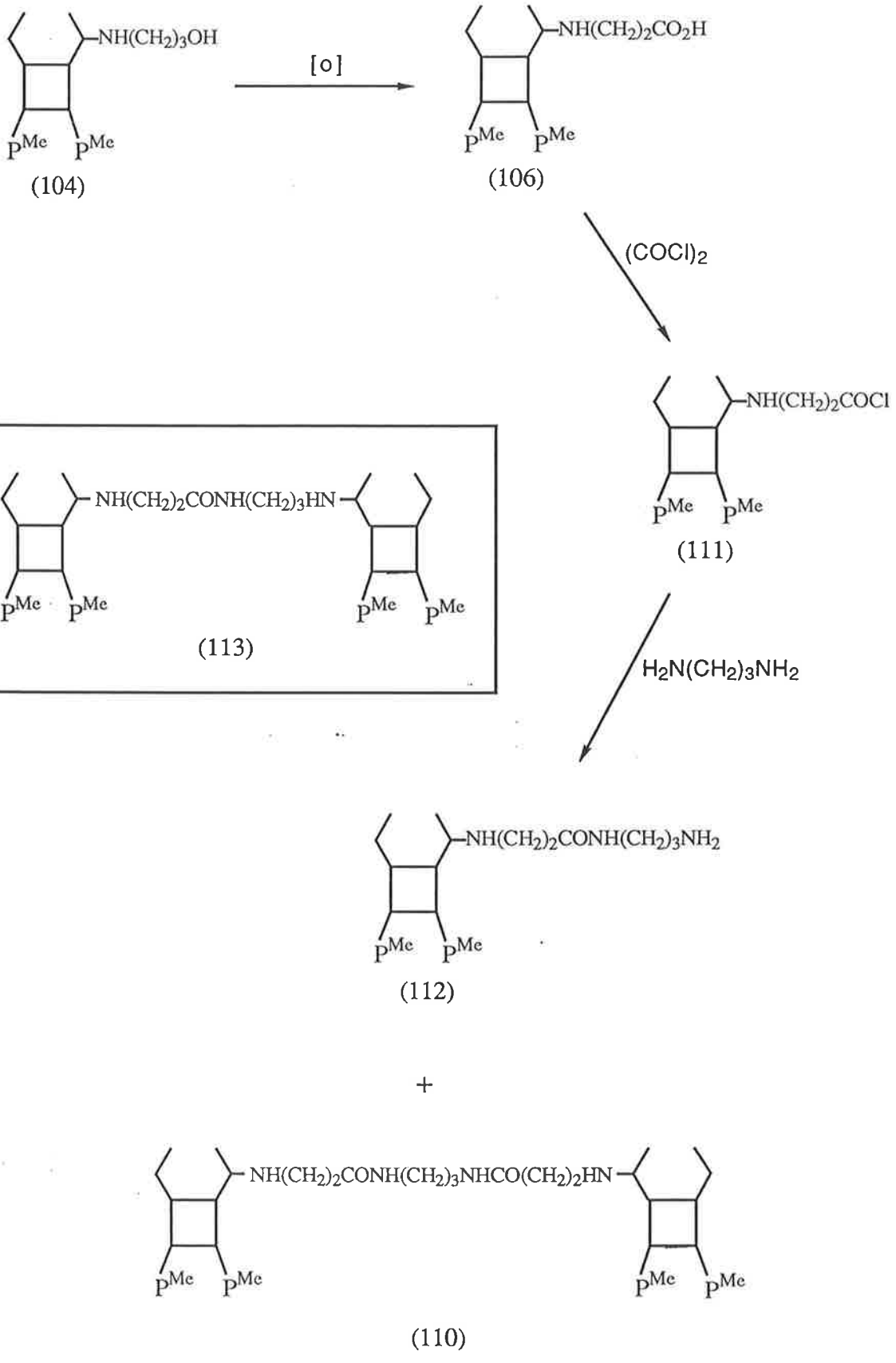
(97)

Abbreviations :



$P^{Me} = CH_2CH_2CO_2Me$

Scheme 4.2


NH(CH2)3OH
[o]
NH(CH2)2CO2H
PMe PMe
(104)
(106)
(COCl)2
NH(CH2)2COCl
(111)
NH(CH2)2CONH(CH2)3HN
(113)
H2N(CH2)3NH2
NH(CH2)2CONH(CH2)3NH2
(112)
+
NH(CH2)2CONH(CH2)3NHCO(CH2)2HN
(110)

monomer by-products from these two Schemes are potentially useful intermediates for other dimers. For example, (112) can be reacted with another molecule of the bromoethyl intermediate to afford an unsymmetrical dimer (113).

Due to the anticipated difficulty in the chromatographic separation of the amine-containing products (see Chapter 3, Section 3.2.2) diols were used in the initial studies to establish reaction conditions. It was of interest to determine the most appropriate methodology for dimer formation, in particular, whether it was more feasible to use a one-step synthesis such as those shown in Schemes 4.1 and 4.2, or a two-step process via the monomers (98) and (112).

4.2 Results and Discussion[§]

4.2.1 Diol derivatives of 3-ethyl-8-vinyldeutero-porphyrin dimethyl ester

4.2.1.1 Monomer derivatives

Hydrobromination of the vinyl group of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76), using the procedure established in Chapter 3 afforded the reactive 1-bromoethyl intermediate (77) which was treated with excess amounts of α,ω-diol to give the desired derivatives. Reaction with 1,3-propanediol gave the monomer (86) in 77% yield. 1,5-Pentanediol afforded (87) in 93% yield and 1,10-decanediol produced (88) in 66% yield. In all cases the yield of dimers (89), (90) and (91) was negligible. This contrasts with the reaction between the bromoethyl compound (77) and concentrated ammonium hydroxide (Chapter 3) where the main product was the amino-linked dimer (70). A possible reason for this is the aggregation of the porphyrins in the aqueous reaction mixture of the latter. Ammonia may have been small enough to fit into the space[14c] between two aggregated porphyrin molecules and so the reacting species are set up favourably for a pseudo-intramolecular reaction (see Chapter 3, Section 3.2.2) but this type of alignment was not possible in the reaction with the diols because aggregation does not occur as much in dichloromethane as it does

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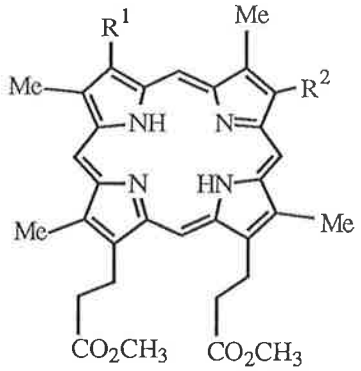
in aqueous solutions.[41] Apart from the large excess of diols in the reaction mixture, another factor that may also contribute to the low yield of dimers is that the diols may be too large to



(39) $R^1 = R^2 = CH(CH_3)OCH_3$
(76) $R^1 = CH_2CH_3$, $R^2 = CH{=}CH_2$
(77) $R^1 = CH(CH_3)Br$, $R^2 = CH_2CH_3$

(86) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_3OH$
(86) b. $R^1 = CH(CH_3)O(CH_2)_3OH$, $R^2 = CH_2CH_3$

(87) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_5OH$
(87) b. $R^1 = CH(CH_3)O(CH_2)_5OH$, $R^2 = CH_2CH_3$

(88) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_{10}OH$
(88) b. $R^1 = CH(CH_3)O(CH_2)_{10}OH$, $R^2 = CH_2CH_3$

insert easily between the porphyrins, and hence there may be little likelihood of any pseudo-intramolecular reaction between the porphyrins (see Chapter 3, Section 3.2.2).

On the TLC chromatogram, each of the diol derivatives* (86), (87) and (88) showed two distinct spots which

* See Appendix for the naming of new compounds.

could be separated preparatively by squat column chromatography into two fractions, designated α and β according to their order of elution. FAB m.s. of each fraction indicated that the two components were isomeric. Since the derivatives contain only one chiral centre and the ethyl and 1-substituted-ethyl groups are on non-equivalent positions (3 and 8) of the porphyrin ring, the derivatives will be mixtures of regioisomers and enantiomers. In principle, enantiomers can only be separated on a chiral column,[42,43] therefore the two fractions are likely to be racemic mixtures of each regioisomer of the diol derivatives. Similar separations of regioisomers of porphyrin monomers[31–33] and dimers[8,20] have been reported.

In general the relative yields of α- and β-isomers were different – the α-isomer (41%) of the 1,3-propanediol derivative (86) predominated over its β-isomer (36%), while the reverse was true in the case of the 1,5-pentanediol derivative (87) (38% α-isomer and 55% β-isomer) and the 1,10-decanediol derivative (88) (22% α-isomer and 40% β-isomer). This difference in the reactivity of the 3 and 8 substituents has also been observed by Pandey *et. al.*[31] and Smith *et. al.*[32]

Clezy *et. al.*[33,34] were able to predict the structure of porphyrin regioisomers on the basis of Fookes rule[34] which relates the proximity of polar groups to chromatographic mobility, whereby the porphyrins with polar groups which are close to each other will be less mobile than those whose polar groups are far apart. Applying this rule[34] to the diol derivatives

(86) – (88), it is predicted that the (more mobile) α-isomers are those with the 1-substituted-ethyl groups in the 3 position, namely isomers (86b), (87b) and (88b), because in these isomers, the polar alcohol group of the 1-substituted-ethyl group is furthest away from the (weakly) polar propionates. The β-isomers are therefore (86a), (87a) and (88a), whose 1-substituted-ethyl groups are in the 8 position. Recently Pandey *et. al.*[31] unambiguously assigned the structures of the regioisomers of monoacetylmono(1-hydroxyethyl)deutero-porphyrin dimethyl ester by means of Nuclear Overhauser Enhancement (NOE) Studies, and their[31] results confirmed the assignments made using the method of Clezy *et. al.*[33,34] An attempt to assign unambiguously the regioisomers of the 1,5-pentanediol derivative (87) using the NOE experiment of Pandey *et. al.*[31] was unsuccessful due to the limitations of the available spectrometer. Crystallization by conventional[35] methods produced crystals which were unfortunately of unsuitable quality (probably because of the racemic nature of the material) for X-Ray Crystallography.

Both the α- and β-regioisomers of the 1,3-propanediol derivative (86) gave similar etio-type absorption spectra with maxima at 400 nm, 498 nm, 533 nm, 567 nm and 621 nm (for the α-isomer) and 401 nm, 499 nm, 533 nm 566 nm and 621 nm (for the β-isomer). The FAB mass spectrum of each of the regioisomers showed a base peak molecular ion at *m/z* 669 and a fragment peak at *m/z* 593 resulting from the loss of the diol

substituent (M^+- $OCH_2CH_2CH_2OH$, H). Proton and ^{13}C NMR analyses are discussed in Section 4.2.1.2.

As expected, the regioisomers of the 1,5-pentanediol derivative, (87a) and (87b), gave nearly identical etio-type absorption spectra with maxima at 399 nm, 499 nm, 533 nm, 565 and 621 nm (for the α-isomer) and 399 nm, 498 nm, 532 nm, 567 nm and 622 nm (for the β-isomer). FAB mass spectral analysis showed a base peak molecular ion at *m/z* 697 and a fragment peak at *m/z* 593, which is consistent with the results obtained from its homologue (86) above. NMR analysis is discussed in Section 4.2.1.2.

Similar results were obtained from the 1,10-decanediol derivative (88a) and (88b) : etio-type absorption spectra, base peak molecular ion at *m/z* 767 with a fragment peak at *m/z* 593 in the FAB spectrum. NMR analysis is discussed in Section 4.2.1.2.

4.2.1.2 NMR analysis of the regioisomers of the diol derivatives (86), (87) and (88)

All the proton spectra of the regioisomers of diol derivatives (86), (87) and (88) were recorded on solutions with concentrations of ≤ 0.05 M in deuterochloroform. In general the spectra were well-resolved and the signals were readily assigned by comparisons with literature chemical shifts.[8,16,17] The NH protons gave broad signals ranging from -3.8 to -3.9 ppm; the

protons of the ring methyls resonated in the range 3.3 – 3.8 ppm; the meso protons at 9.2 – 10.6 ppm; the multiplicities of the protons in the ethyl sidechains were discernable as triplets at 1.8 – 1.9 ppm ($CH_2C\underline{H}_3$) and quartets at 3.8 – 4.1 ppm ($C\underline{H}_2CH_3$) and the propionates gave signals at 4.2 – 4.4 ppm ($C\underline{H}_2CH_2CO_2CH_3$), 3.1 – 3.3 ppm ($C\underline{H}_2CO_2CH_3$) and 3.5 – 3.8 ppm ($CO_2C\underline{H}_3$). The multiplicities of the signals due to the protons bearing the diol substituent were clearly observable as doublets (J 6.6 Hz) around 2.2 – 2.3 ppm ($C\underline{H}_3CHOR$) and quartets at 6.0 – 6.1 ppm ($CH_3C\underline{H}OCH_2$-). The chemical shifts correlate well with the resonances of corresponding protons in 3,8-di(1-methoxyethyl)deuteroporphyrin dimethyl ester (39).[8] Signals produced by the $C\underline{H}_2$ protons of the diol sidechain (which were expected to occur around 1.5 – 2.2 ppm) were obscured by signals from solvent impurities.

Spectra from α– and β–isomers were similar; the only difference between the spectra apppeared to be the chemical shift of the $C\underline{H}_2CH_2CO_2CH_3$ protons. For example, in the spectrum of the α isomer of the 1,5-pentanediol derivative (87), the $C\underline{H}_2CH_2CO_2CH_3$ signals appeared as a multiplet due to two overlapping triplets at 4.04 – 4.12 ppm while the corresponding signals in the β isomer were distinct triplets at 4.35 ppm and 4.44 ppm. However these differences could be due to solvent effects.[16a] Although the samples concentrations were approximately 0.05 M which is the recommended[16a] concentration for proton NMR samples (because it represents the ideal compromise between minimum aggregation in

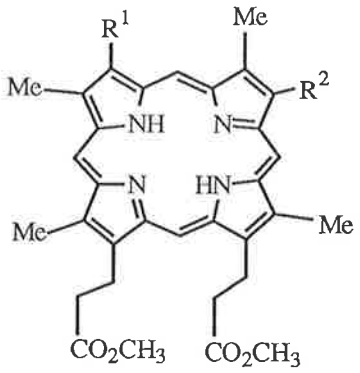
deuterochloroform and maximum signal-to-noise ratio) it is not known whether the diol derivatives exhibit the same general[16a] behaviour in deuterochloroform. Abraham *et. al.*[45] and Honeybourne *et. al.*[46] have reported that even at concentrations which are lower than 0.05 M some porphyrins still show appreciable aggregation effects in deuterochloroform and that this results in upfield shifts in the chemical shifts of the affected protons.

The ^{13}C NMR spectra of the α- and β- isomers of the diol derivatives were well resolved and readily interpreted. In most cases the signals correlated well with those reported.[8,16,20] As usual the signals produced by the ring carbons were broad[8,16,17,20] as a result of NH tautomerism.[17] The meso carbons resonated in the range of 96 – 99 ppm. Chemical shifts of the sidechains are summarized in Table 4.1. With the exception of some of the carbons on the sidechain bearing the diol substituent (see later), the other sidechains gave signals which were comparable to those of other monomers in the literature.[8,16,17] The methine carbon $CH_3\underline{C}HOCH_2$ resonated at 73 – 74 ppm, which is approximately 2 ppm upfield relative to the corresponding signal of 3,8-di(1-methoxyethyl)deuteroporphyrin dimethyl ester (39).[8] This upfield shift is likely to be due to the γ effect[36a] that was being transmitted through the oxygen atom from the diol substituent. Similar results have been observed in aliphatic ethers.[36b] The methylene carbon next to the ether oxygen $CH_3CHO\underline{C}H_2$ resonated at 68.5 – 69.4 ppm while the the corresponding signal in the analogous 3,8-di(1-

methoxyethyl)deuteroporphyrin dimethyl ester (39)[8] appeared at ~58 ppm. This ~10 ppm downfield shift is due to the α-effect of the neighbouring carbon in the chain.[36a] The methylene carbon next to the hydroxyl group, $\underline{C}H_2OH$ was assigned the signal around 62.1 – 62.6 ppm; the assignment was later confirmed when the hydroxyl group was etherified (Sections 4.2.1.3 – 5, synthesis of diether-linked dimers) as the 62.1 – 62.6 ppm signal was not present in the spectrum of the etherified product.

In general the ^{13}C NMR spectra of the α-isomers were very similar to those of the corresponding β-isomers and all the chemical shifts were within 0.2 ppm of each other. It was not possible to differentiate between the regioisomers on the basis of the ^{13}C NMR spectra alone.

Pandey *et. al.*[31] were able to assign the regioisomers of monoacetylmono(1-hydroxyethyl)deuteroporphyrin dimethyl ester by an elegant proton NOE experiment which involved the irradiation of each meso proton (9 – 11 ppm) to identify the signals of the methyl group in the 2 position of the porphyrin ring.[31] Subsequently, irradiation of these methyl signals would reveal the identity of the sidechain on the adjacent 3 position of the ring.[31] This method was tried on the regioisomers of the 1,5-pentanediol derivative (87). Unfortunately, due to the limitations of the available instruments, these experiments were unsuccessful.



(86) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_3OH$
(86) b. $R^1 = CH(CH_3)O(CH_2)_3OH$, $R^2 = CH_2CH_3$

(87) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_5OH$
(87) b. $R^1 = CH(CH_3)O(CH_2)_5OH$, $R^2 = CH_2CH_3$

(88) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_{10}OH$
(88) b. $R^1 = CH(CH_3)O(CH_2)_{10}OH$, $R^2 = CH_2CH_3$

Table 4.1 ^{13}C chemical shifts (δ ppm) of the sidechains in the α and β isomers of the diol derivatives of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester.

Sidechain	1,3-Propanediol derivative (86) isomers		1,5-Pentanediol derivative (87) isomers		1,10-Decanediol derivative (88) isomers	
	α	β	α	β	α	β
ring $\underline{C}H_3$	11.59	11.27, 11.64	11.58, 11.71	11.40, 11.71	11.58, 11.28	11.14,11.46, 11.59,11.71
$CH_2\underline{C}H_3$	17.54	17.54	17.55	17.61	17.60	17.54
$\underline{C}H_2CH_3$	19.74	19.68	19.80	19.74	19.74	19.59
$\underline{C}H_3CHOCH_2$	25.44	25.43	25.44	25.51	25.55	25.56
$CH_3\underline{C}HOCH_2$	73.88	73.83	73.38	73.38	73.31	73.37
$CH_3CHO\underline{C}H_2$	68.53	68.46	69.23	69.24	69.44	69.44
$\underline{C}H_2CH_2CO_2$	21.83	21.81	21.84	21.91	21.82	21.77
$CH_2\underline{C}H_2CO_2$	36.89	36.93	36.99	37.02	36.91	36.95
$CO_2\underline{C}H_3$	51.64	51.65	51.69	51.70	51.64	51.66
$\underline{C}O_2CH_3$	173.5, 173.6	173.6, 173.5	173.5	173.7	173.7, 173.5	173.6
$\underline{C}H_2OH$	62.18	62.12	62.64	62.64	62.64	62.58

4.2.1.3 Pentane-1,5-diether-linked diporphyrin dimer (90)

The title dimer (90) was prepared (76% yield) by reacting the α-isomer* of the 1,5-pentanediol derivative (87) with the bromoethyl compound (77), by the same procedure as that used in the synthesis of the monomer. No attempt was made to synthesize the dimer (90) in a one step reaction by treating the bromoethyl compound (77) with half equivalents of αω-diol in order to effect *in situ* dimerization of initially formed diol derivative (87) and unreacted bromoethyl compound (77) because similar studies[8] have indicated that the two step reactions are generally more controllable and provide better yields of dimers than the *in situ* reactions.[8] Since the bromoethyl intermediate (77) was a mixture of regio- and stereoisomers, the resulting dimer would also be a mixture of regio- and stereoisomers. There was no distinct separation between the isomers of the dimer (90) during chromatography on silica.

The absorption spectrum of dimer (90), which was similar to that of its precursor, was an etio-type spectrum with maxima at 400 nm, 499 nm, 533 nm, 568 nm and 621 nm. The FAB mass spectrum showed a strong molecular ion at *m/z* 1290

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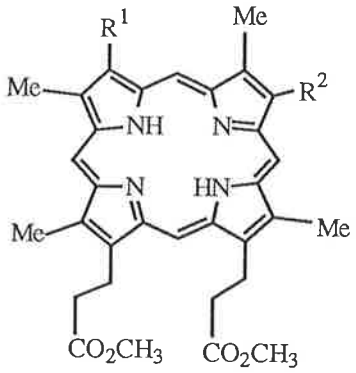
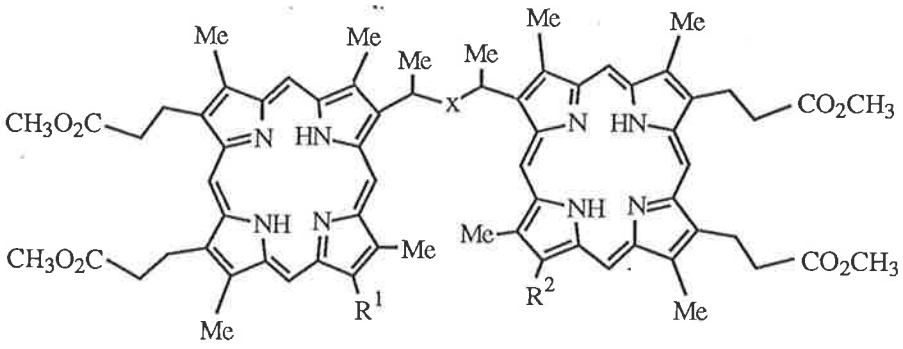
* As the structural assignments of the regioisomers (Section 4.2.1.1) are only tentative, the compound(s) will continue to be represented by all possible structures.

and a peak of equal height at *m/z* 593, the latter a fragment from the molecular ion.



(39) $R^1 = R^2 = CH(CH_3)OCH_3$
(77) $R^1 = CH(CH_3)Br$, $R^2 = CH_2CH_3$
(87) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_5OH$
(87) b. $R^1 = CH(CH_3)O(CH_2)_5OH$, $R^2 = CH_2CH_3$



(90) $X = O(CH_2)_5O$

Proton and ^{13}C NMR spectra were recorded in deuterochloroform on a solution of approximately 0.04 M. The chemical shift assignments are based on those of known porphyrins,[8,16] but have not been confirmed by off resonance decoupling or COSY experiments. Most of the carbon signals

appeared in regions that were similar to those of the corresponding carbons on known porphyrins.[16c] For example, the carbons on the ethyl sidechain resonated at 17.4 ppm ($CH_2\underline{C}H_3$) and 19.7 ppm ($\underline{C}H_2CH_3$) which was in good agreement with those of the corresponding carbons on the ethyl groups in mesoporphyrin IX dimethyl ester,[16c] which were at 17.7 ppm and 19.8 ppm, respectively. The α-carbons on the ring pyrroles gave broad (144 - 149 ppm) signals due to NH tautomerism,[16c,17] while the β-carbon resonances were a complicated set of six peaks ranging from 135 - 142 ppm. The meso carbons resonated at 96.10 - 98.59 ppm, the carbons of the ring methyls at 11.5 ppm and 11.7 ppm (in mesoporphyrin IX dimethyl ester[16c] they also appeared at 11.5 and 11.7 ppm, respectively). Similarly the carbons of the propionates resonated at 21.83 ppm, 21.93 ppm ($\underline{C}H_2CH_2CO_2$), 37.00 ppm ($\underline{C}H_2CO_2$), 173.54 ppm ($\underline{C}O_2CH_3$) and 51.64 ppm ($CO_2\underline{C}H_3$), all of which correlated well with those of known porphyrins.[8,16] The sidechains bearing the diether bridge were assigned the following chemical shifts : the methyl group ($\underline{C}H_3CHOCH_2$), at 25.4 ppm on the basis of the chemical shifts on the corresponding carbons on haematoporphyrin IX dimethyl ester[16c] ($\underline{C}H_3CHOH$, at 26.0 ppm) and 3,8-di(1-methoxy)ethyldeuteroporphyrin dimethyl ester[8,18] (39) ($\underline{C}H_3CHOH$, at 25.26 ppm). The peak at 73.32 ppm was assigned to the benzylic carbon ($CH_3\underline{C}HOCH_2$) on the sidechain. The analogous carbon on its precursor (87) resonated at 73.38 ppm while that of 3,8-di(1-methoxy)ethyldeuteroporphyrin dimethyl ester[8,18] (39) resonated at 74.93 ppm. The upfield peaks at

23.49 ppm and 23.30 ppm were tentatively assigned to the methylene carbons ($OCH_2\underline{C}H_2\underline{C}H_2\underline{C}H_2CH_2O$) of the bridge, because of their distance from any electron-withdrawing groups. The methylene group (C_a) adjacent to the benzylic oxygen was assigned the 69.32 ppm peak. This is considerably further downfield than the methoxy carbon of 3,8-di(1-methoxy)ethyldeuteroporphyrin dimethyl ester[8,18] (39) which resonates at 57.27 ppm but agrees quite well with the analogous carbon in its precursor, the 1,5-pentanediol monomer derivative (87), which was tentatively assigned the signal at 69.24 ppm. Again, this downfield shift might be due to the "α-effect" of neighbouring carbons.[16c,36a] A similar downfield shift of comparable magnitude was observed in an ethoxy substituent (compared to the methoxy) on a benzathiazole.[19]

As expected there were no peaks around the 62 ppm region in the ^{13}C NMR spectrum of the dimer (90). This supports the assignment of the 62.64 ppm peak to the methylene carbon under the hydroxyl group ($\underline{C}H_2OH$) in the precursor porphyrin (see Section 4.2.1.2).

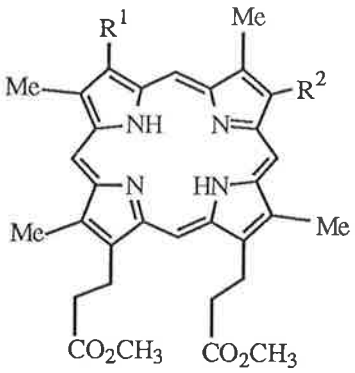
Despite the low concentration of the solution which, at 0.04 M, was below the minimum 0.05 M (in deuterochloroform) that is necessary to minimize aggregation, the proton spectrum of dimer (90) was not as well-resolved as that of its monomer precursor (90) (Section 4.2.1.2), possibly due to the presence of regioisomers as well as the overlap of signals of protons with similar environments from the two component porphyrins in the

molecule. The signals appeared at chemical shifts which were similar to those of the precursor monomer (87) (Section 4.2.1.2) and other standard porphyrins.[8,16] The four pyrrole hydrogens resonated in a broad signal at -3.87 ppm. A multiplet at 1.64 – 1.79 ppm, which was due to 10 protons, was assigned to the two terminal methyl groups on the ethyl sidechains ($CH_2C\underline{H}_3$) and possibly to two methylene groups on the linking group. The complicated nature of the signals prevented a more definite assignment of the resonances. The ubiquitous broad signals between 0.5 – 1.5 ppm (due to solvent impurities) presumably obscured the signals of some of the methylene protons on the bridge. Two distinct sets of doublets at 2.08 ppm (J 6.6 Hz) and 2.16 ppm (J 6.6 Hz) were assigned to the two methyl groups ($C\underline{H}_3CHOCH_2$) on the sidechain bearing the diether bridge. The corresponding methyl protons on the precursor porphyrin (87) (Section 4.2.1.1) resonated at 2.26 ppm (J 6.6 Hz). A broad peak at 3.23 ppm was assigned to the four methylene groups ($C\underline{H}_2CH_2CO_2$) on the propionic sidechain. The intense set of overlapping signals at 3.3 – 3.7 ppm were assigned to the ring methyls, the methyls of the four propionic esters ($CO_2C\underline{H}_3$) and possibly the two methylene groups next to the oxygen atoms on the bridge ($CH_3CHOC\underline{H}_2$). The latter is based on the proton chemical shift of the methoxy group on 3,8-di(1-methoxy)-ethyldeuteroporphyrin dimethyl ester[8,18] (39) which was at 3.67 ppm. The multiplet at 5.90 – 5.99 ppm due to two protons was assigned to the two benzylic hydrogens ($CH_3C\underline{H}OCH_2$) on the bridge. The corresponding proton in the precursor porphyrin

(87) resonated at 6.05 ppm. The methylene group on the ethyl sidechains ($C\underline{H}_2CH_3$) gave a multiplet (due to the overlap of two quartets) at 3.83 – 3.90 ppm. A broad singlet at 4.31 ppm due to eight protons was assigned to the four methylene ($C\underline{H}_2CH_2CO_2$) groups on the propionic sidechains. The eight meso proton signals were distinct singlets at 9.92, 9.95, 9.96, 9.99, 10.00, 10.47, 10.50 and 10.51 ppm.

4.2.1.4 Propane-1,3-diether-linked dimer (89)

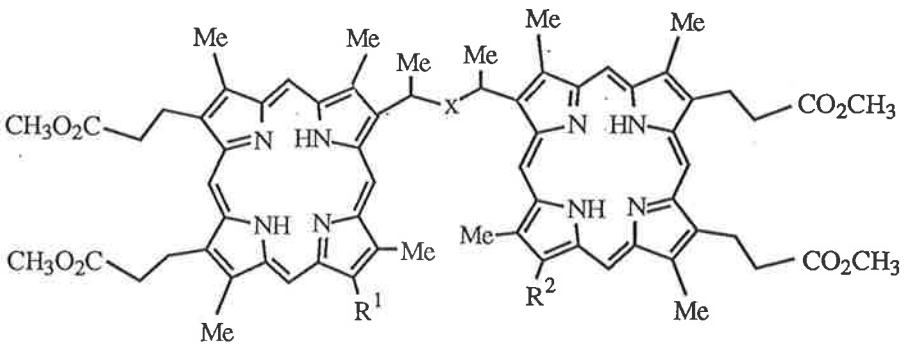
The title dimer (89) was prepared in 67% yield using the α-isomer of the 1,3-propanediol derivative (86) (Section 4.2.1.1) by the procedure used for the synthesis of the pentane-1,5-diether-linked dimer (Section 4.2.1.3). The title dimer (89) exhibited an etio-type absorption spectrum with maxima at 399 nm, 499 nm, 535 nm, 566 nm and 622 nm. A strong molecular ion at *m/z* 1262 and a fragment peak at *m/z* 593 featured in its FAB mass spectrum. The ^{13}C and proton NMR spectra of dimer (89) were, in all respects, similar to those of the pentane-1,5-diether-linked dimer (90) (Section 4.2.1.3).



(77) $R^1 = CH(CH_3)Br$, $R^2 = CH_2CH_3$

(86) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_3OH$
(86) b. $R^1 = CH(CH_3)O(CH_2)_3OH$, $R^2 = CH_2CH_3$

(88) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_{10}OH$
(88) b. $R^1 = CH(CH_3)O(CH_2)_{10}OH$, $R^2 = CH_2CH_3$



(89) $X = O(CH_2)_3O$
(90) $X = O(CH_2)_5O$
(91) $X = O(CH_2)_{10}O$

4.2.1.5 Decane-1,10-diether-linked diporphyrin dimer (91)

In order to determine if there was any difference in the reactivity of the two regioisomers of the monomer (88), the title dimer (91) was synthesized separately from the α- and β-isomers of the 1,10-decanediol monomer (88). The yields of dimers from both dimerizations were similar: 53% from the α-isomer and 57% from the β-isomer of the 1,10-decanediol derivative (88) indicating that there was no regioselectivity in the reaction between the regioisomers of the 1,10-decanediol derivative (88) and the bromoethyl compound (77). As with the other homologues, the TLC distance between the regioisomers of the product was too small for adequate separation to be made. Both isomeric dimer mixtures gave nearly identical etio-type absorption spectra with maxima at approximately 399 – 400 nm, 499 – 500 nm, 532 – 533 nm, 567 nm and 621 – 622 nm.

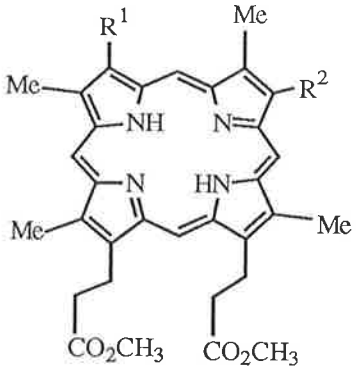
The FAB mass spectra of both dimer (91) mixtures gave a molecular ion at *m/z* 1361 m.u., a weak peak at *m/z* 767 (corresponding to the monomer (88) arising from the cleavage of the benzylic C-O bond on the bridge) and a base peak at *m/z* 593.

The similarity between the two dimer mixtures also extended to the NMR spectra. The ^{13}C NMR spectra of both dimer mixtures were nearly identical, the the sidechain chemical shifts were within a ± 0.1 ppm range and were readily assigned on the

basis of the chemical shifts of its pentane-1,5-diether-linked homologue (Section 4.2.1.3). Most of the proton signals of the decane-1,10-diether-linked dimer (91) were broad while those which were distinct were complicated multiplets because of the overlap of signals. Nevertheless most of the signals appeared at approximately similar chemical shifts as those of the pentane-1,5-diether-linked homologue (90) (Section 4.2.1.3).

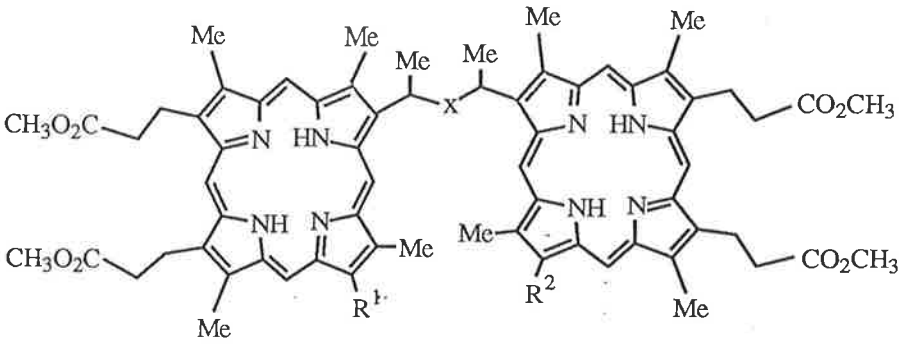
4.2.2 The synthesis of a symmetric diporphyrin dimer linked by an ether- and ester-containing bridge (92)

A preliminary investigation was done on a TLC scale. The α-isomer of the 1,5-pentanediol derivative (87) (Section 4.2.1.1) was dissolved in dichloromethane and added to a mixture containing an excess of malonyl dichloride (approximately 30 equivalents) and a catalytic amount of pyridine. It was not possible, on the scale of the reaction, to accurately weigh out the half molar equivalents of malonyl dichloride that would (presumably) be needed for maximum yields of the dimer. As a result of the excess amounts of diacid chloride, it was anticipated that there would be at least two products, namely the desired dimer (92) and the acid-containing monomer (93).



(87) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_5OH$
(87) b. $R^1 = CH(CH_3)O(CH_2)_5OH$, $R^2 = CH_2CH_3$

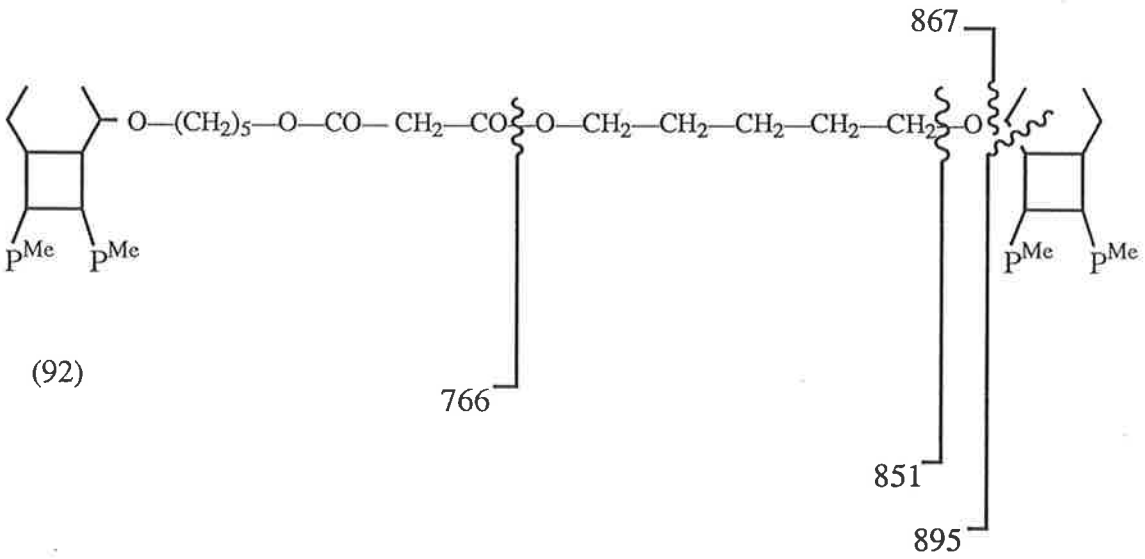
(93) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_5OCOCH_2CO_2H$
OR its regioisomer



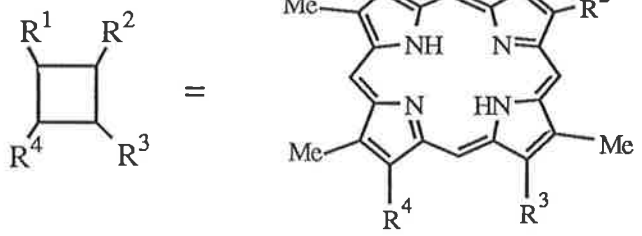
(92) $X = O(CH_2)_5OCOCH_2CO_2(CH_2)_5O$

The crude product obtained after aqueous work-up was shown by TLC to consist of two main components : a major spot at R_F 0.4 and a minor spot at R_F 0.7. Separation by preparative TLC followed by analysis of the fractions by FAB mass spectrometry indicated that the high R_F component was the desired dimer (92) (molecular ion at *m/z* 1462). The MIKE spectrum of the ion *m/z* 1462 showed major peaks at *m/z* 895 and 867, and minor peaks at *m/z* 851 and 765 , all of which result from cleavages along the linking group, Fig 4.1. This

Fig. 4.1 Fragmentations from dimer (92) (*m/z* 1462) under MIKE conditions.



Abbreviations :



$P^{Me} = CH_2CH_2CO_2Me$

fragmentation pattern is similar to that of the diether-linked dimers (Sections 4.2.1.3 – 5).

The low R_F fraction gave an intense molecular ion at *m/z* 783 in its FAB mass spectrum. This corresponds to the expected acid derivative (93). Under MIKE conditions, this *m/z* 783 ion underwent decarboxylation to give the ion at *m/z* 739.

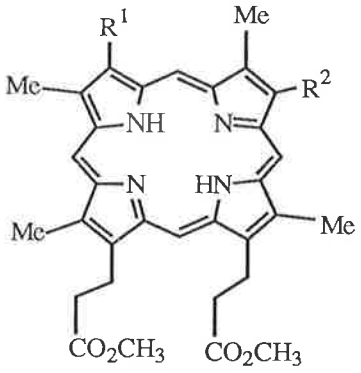
In this reaction the monomer was the major product because of the excess amounts of malonyl dichloride that was used. It should be possible to increase the yield of dimer (92) by decreasing the proportion of acid chloride accordingly. However this was not pursued further because the main objective was to determine if this approach would produce the desired dimer and having done so, use the methodology to synthesize dimers with amine- and amide-containing linkages. This was because the dimer with the ester-containing linking group (92) was not expected to be very stable biologically, due to the likelihood of its being cleaved by estereases *in vivo*. Therefore attention was focused on the synthesis of the dimers with amine- and amide-containing linkages by replacing the diol derivatives in the reaction scheme with a diamino derivative.

4.2.3 1,3-Diaminopropane derivative (94) of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester

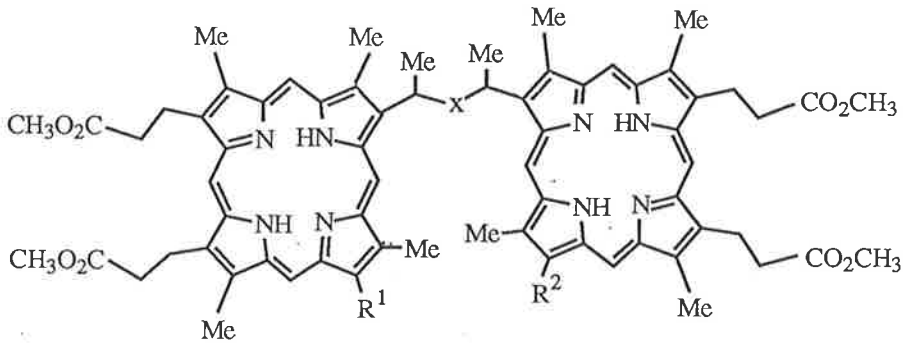
The title porphyrin (94) was prepared from 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) by essentially the

same procedure as that used for the synthesis of the diol derivatives (Section 4.2.1.1), with the following modifications. A smaller excess (10 mol equivalents) of 1,3-diaminopropane was used and the reaction time was kept below 10 minutes. This was done to minimize the aminolysis of the propionic esters, which was found (Chapter 3, Section 3.2.3) to occur to a significant extent when the reaction time was more than 10 minutes.

The crude product was found by FAB mass spectrometry to contain the desired porphyrin (94) (molecular ion at *m/z* 667), some 1,3-diaminopropane-linked dimer (95) (molecular ion *m/z* 1260), starting porphyrin (76) (*m/z* 593) and some oxygen-containing by-products, (80) (*m/z* 610) and (79) (*m/z* 1204), which were presumably formed during aqueous work-up. Attempts to separate this mixture on silica were frustrated by the polarity of the major portion of the material.



(7a) $R^1 = R^2 = CH{=}CH_2$
(76) $R^1 = CH_2CH_3$, $R^2 = CH{=}CH_2$
(80) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)OH$
(94) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3NH_2$



(79) X = O
(95) $X = HN(CH_2)_3NH$

Very little migration occurred on deactivated silica despite the use of polar mixtures of eluting solvents (50% methanol/dichloromethane or even methanol on its own). Grade III alumina did not retain the material as strongly – most of the material eluted with 10% methanol/dichloromethane – but there was no resolution in the separation. Kieselguhr was the most suitable adsorbent : the components were readily separated with relatively weakly polar solvent combinations – the starting

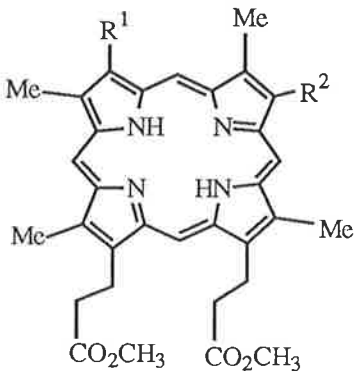
porphyrin (76) and by-products, (79) and (80), were eluted with dichloromethane, the 1,3-diaminopropane-linked dimer (95) with 2% methanol/dichloromethane and the most polar component, namely the title monomer (94), was eluted with 10 – 50% methanol/dichloromethane. Unlike the diol analogue (86) (Section 4.2.1.1) there was no separation between the regioisomers of (94).

The desired monomer (94) was the main product (76%) from the reaction. The dimer (95) was formed in only 5% yield. This was consistent with the results from the analogous diol series (Section 4.2.1.1), and again indicates that the aggregation of the porphyrins in aqueous reaction mixtures (see Chapter 3) contributes significantly towards the formation of dimers in the nucleophilic substitution of the bromoethyl compound by a bifunctional nucleophile.

This hydrobromination-nucleophilic substitution procedure is a convenient method for attaching the diamine to the 1-position of the ethyl sidechain of the porphyrin. Under more vigorous conditions, such as those employed by White *et. al.*,[47] which involved refluxing protoporphyrin dimethyl ester (7a) with either neat diamine or diamine in the presence of catalytic amounts of hydrochloric acid, anti-Markownikov addition of the diamine to the double bond occurs, along with aminolysis of the propionates.[47]

The 1,3-diaminopropane derivative (94) exhibited an etio-type absorption spectrum with maxima at 401 nm, 501 nm,

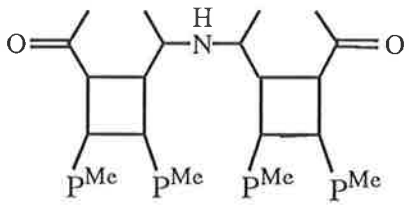
535 nm, 569 nm and 622 nm. The FAB mass spectrum of the derivative (94) showed a weak molecular ion at *m/z* 667 and a base peak at *m/z* 593, the latter resulting from the loss of the 1,3-diaminopropane groups from the benzylic position. Similar fragmentation patterns were observed among the diol derivatives (Section 4.2.1). The driving force for the cleavage is probably the stabilization of the resulting daughter ion by delocalization of the the charge onto the porphyrin ring. The ratio of peak intensities (*m/z* 667 : 593) was approximately 8 : 21. The low intensity of the molecular ion of the 1,3-diaminopropane derivative (94) may have been due to its incompatibility with the FAB matrix (3-nitrobenzyl alcohol[39]) that was used. The matrix is very effective with the oxygen-containing systems[8] but it appears to not work as well with the amino compound.



(86) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_3OH$
(86) b. $R^1 = CH(CH_3)O(CH_2)_3OH$, $R^2 = CH_2CH_3$

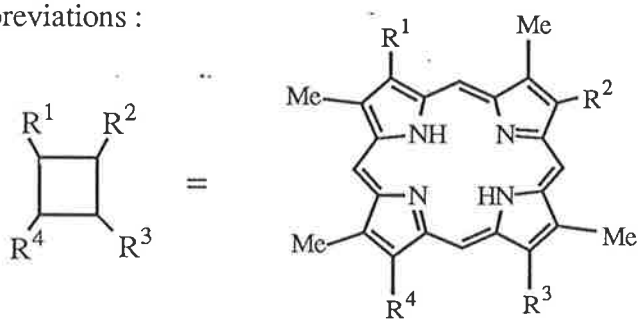
(94) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3NH_2$

NMR spectra were recorded on an approximately 0.02 M solution. Despite this low concentration, the signals in the proton spectrum were very broad and indistinct. The ^{13}C NMR spectrum was however more informative. The assignments of the chemical shifts (see below) were based on those of known porphyrins in the literature[8,16] and by comparison with the 1,3-propanediol derivative (Section 4.2.1.2).



(71)

Abbreviations :

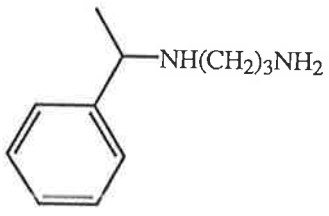


$P^{Me} = CH_2CH_2CO_2Me$

The assignments are tentative and have not been checked with off-resonance decoupling or COSY studies. The carbons of the ring methyls resonated at 11.36 ppm. The methylene group next to the nitrogen ($-NH\underline{C}H_2CH_2CH_2NH_2$) was tentatively assigned the peak at 32.61 ppm; the analogous carbon on the 1,3-propanediol derivative (Section 4.2.1.2) resonated at 32.55 ppm. The carbons

of the ethyl sidechain resonated at 17.52 ppm ($CH_2\underline{C}H_3$) and 19.73 ppm ($\underline{C}H_2CH_3$). By comparison the signals on the analogous carbons of the α-isomer of the 1,3-propanediol derivative (86) (Section 4.2.1.2) appeared at 17.54 ppm and 19.74 ppm, respectively. Good correlations were also obtained in the chemical shifts of the propionic ester sidechains. However the resonances of the meso carbons of the 1,3-diaminopropane derivative (94) were broad (96 – 98 ppm), as were those of the pyrrole carbons (C_α : 144.0 – 152.0 ppm; C_β : 134.0 – 141.7 ppm). The signal at 23.23 ppm was assigned to the carbon of the methyl group ($\underline{C}H_3CHNH$-) that was attached to the benzylic methine carbon. This was based on the chemical shift of the analogous methyl group on the diacetyl-terminated amino-linked dimer (Chapter 3, Section 3.2.9) which had been determined by a ^{13}C-^{1}H chemical shift correlation study to be in the range of 23.6 – 24.8 ppm. The chemical shift of the corresponding methyl carbon on the 1,3-diaminopropane derivative (94) would be expected to be slightly upfield relative to that of the diacetyl dimer (71) (Chapter 3, Section 3.2.9) because it does not have the electron-withdrawing effect of the acetyl group. Similarly the benzylic methine ($CH_3\underline{C}HNH$-) in the 1,3-diaminopropane derivative (94) resonated at 41.81 ppm compared with 45.60 ppm in the diacetyl dimer (71) (Chapter 3, Section 3.2.9). The methylene carbon next to the primary amino group (-$\underline{C}H_2NH_2$) was assigned the signal at 60.18 ppm. It was expected that this carbon would resonate at a chemical shift that was upfield relative to the signal (~62.1 ppm) of the corresponding carbon on the analogous 1,3-propanediol

derivative (Section 4.2.1) because of the lower electronegativity of nitrogen. This assignment was supported by the spectrum of the 1,3-diaminopropane-linked dimer (95) (see later) in which, as expected,the signal around 60 ppm was not present. A signal at 41.09 ppm was tentatively assigned to the methylene carbon next to the secondary amino group (-NH$\underline{C}H_2CH_2$-). This was based on a calculated chemical shift of 43.7 ppm for a similar structure (96) using benzene as an approximation to the porphyrin ring and standard chemical shift correlation tables[21] for alkanes. The corresponding carbon on the analogous 1,3-propanediol derivative (Section 4.2.1) had been assigned a chemical shift of ~68.5 ppm, but there were no peaks in the 60 – 70 ppm region (apart from 60.18 ppm which was assigned to the methylene carbon next to the primary amino group, -$\underline{C}H_2NH_2$) in the spectrum of the 1,3-diaminopropane derivative (94). This big difference between the chemical shift of -NH$\underline{C}H_2CH_2$- and -O$\underline{C}H_2CH_2$- is not without precedent : the methine carbon ($CH_3\underline{C}HOH$) in haematoporphyrin dimethyl ester resonates at 65.03 ppm[16c] while the analogous carbon ($CH_3\underline{C}HNH_2$) in 3-(1-aminoethyl)-8-ethyldeuteroporphyrin dimethyl ester (78) (Chapter 3, Section 3.2.9) resonates around 45 ppm.

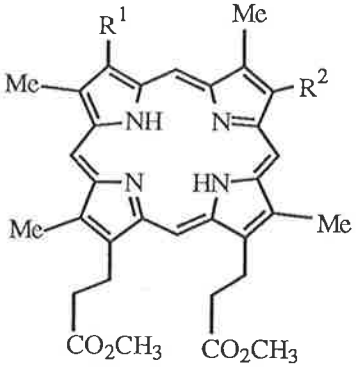


(96)

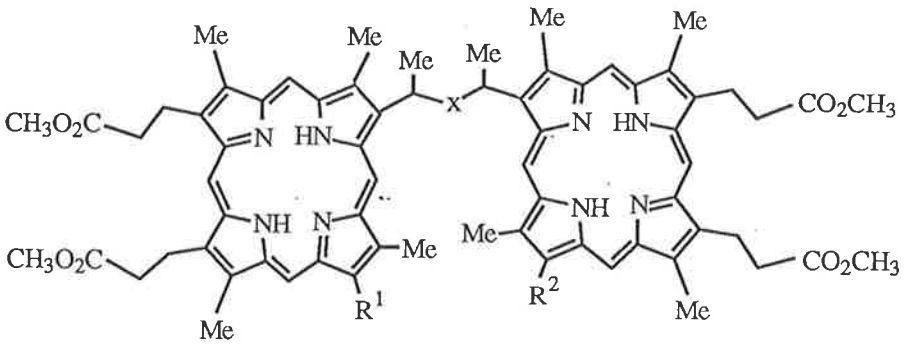
4.2.4 1,3-Diaminopropane-linked diporphyrin dimer (95)

Reaction between the bromoethyl derivative (77) and crude 1,3-diaminopropane derivative (94) (Section 4.2.3) afforded a mixture consisting of the desired dimer (95), unreacted 1,3-diaminopropane derivative (94) (Section 4.2.3), 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76), all of which were present in approximately equal amounts, and small amounts of by-products (79) and (80), presumably formed during the aqueous work-up procedure. The dimer (95) was readily separated on Kieselguhr by elution with 2% methanol/dichloromethane.

The dimerization did not proceed as efficiently as that of the diol derivatives (Sections 4.2.4 – 6), which averaged a yield of 62%. The high percentage recovery of starting 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) suggested that the 1,3-diaminopropane derivative (94) had functioned more as a base than a nucleophile. This is not unusual considering the size of the molecule (94). In contrast the reaction between the bromoethyl compound (77) and the much smaller and less sterically demanding 1,3-diaminopropane (Section 4.2.3) had produced mainly derivative (94) and ≤20% dehydrobromination products, suggesting that in this case, the diamine was functioning mainly as a nucleophile.



(76) $R^1 = CH_2CH_3$, $R^2 = CH{=}CH_2$
(77) $R^1 = CH(CH_3)Br$, $R^2 = CH_2CH_3$
(78) $R^1 = CH(CH_3)NH_2$, $R^2 = CH_2CH_3$
(80) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)OH$
(94) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3NH_2$



(70) X = NH
(79) X = O
(89) X = $O(CH_2)_3O$
(95) X = $HN(CH_2)_3NH$

The title dimer (95) exhibited a typical etio-type absorption spectrum with maxima at 402 nm, 501 nm, 539 nm, 568 nm and 622 nm. The FAB mass spectrum of dimer (95) showed a molecular ion of medium intensity at *m/z* 1260, a weak peak at *m/z* 667 and a base peak at *m/z* 593. This

contrasts with the FAB spectrum of the propane-1,3-diether-linked dimer (89) (Section 4.2.6) where the dimer molecular ion was the base peak in the spectrum. Again, this might be due to the unsuitable FAB matrix (see Section 4.2.3).

The proton and ^{13}C NMR spectra of dimer (95) were recorded in a deuterochloroform solution with a concentration of approximately 0.016 M. The proton NMR spectrum of dimer (95) was very poorly resolved, similar to that of its precursor (94) (4.2.3). Generally, signals were observed in the expected[16] areas, however, due to the broadness of the signals the actual chemical shifts were difficult to determine.

The ^{13}C NMR spectrum was better resolved and the signals could be readily assigned. The carbons of the methyl, ethyl, and propionic ester sidechains resonated at the expected chemical shifts of 11.66 ppm (ring $\underline{C}H_3$) 17.54 ppm ($CH_2\underline{C}H_3$), 19.72 ppm ($\underline{C}H_2CH_3$), 36.95 ppm ($\underline{C}H_2CO_2$), 51.70 ppm ($CO_2\underline{C}H_3$) and 173.59 ($\underline{C}O_2CH_3$). These values correlated well with the literature[16,20] and those of the diol derivatives (Sections 4.2.1 and 4.2.2). The signal due to the methyl on the bridge ($\underline{C}H_3CHNH$-) appeared at 23.88 ppm while the corresponding carbon in its precursor (94) (Section 4.2.3) resonated at 23.23 ppm; the methine carbon ($CH_3\underline{C}HNH$-) was tentatively assigned the signal 52.86 ppm, which is ~10 ppm downfield from the corresponding signal in the precursor monomer (94). There were no peaks in the 40 – 50 ppm region where the ($CH_3\underline{C}HNH$-) signal was expected to appear. This change in chemical shift of the

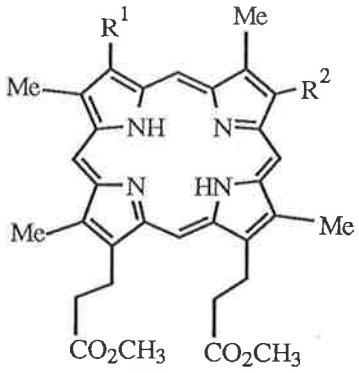
$CH_3\underline{C}HNH$- signal is in contrast with the diether-linked dimers (Sections 4.2.1.3 – 5) where the resonance of the $CH_3\underline{C}HOR$ carbon remained the same after dimerization. The downfield shift of the $CH_3\underline{C}HNH$- signal appears to be unique to the amino-linked dimers : the chemical shift of the $CH_3\underline{C}HNH$- carbon (49.9 ppm) in the diethyl-terminated amino-linked dimer (70) (Chapter 3, Section 3.3.5a.) was ~4 ppm downfield relative to the signal (45.6 ppm) of the corresponding carbon in its monomer (78). In the 1,3-diaminopropane-linked dimer (95), the presence of the hydrocarbon chain appears to have enhanced the downfield shift of the $CH_3\underline{C}HNH$- signal, possibly by α- and β-effects.[3a]

4.2.5 Attempted synthesis of a diporphyrin dimer (97) linked by an amine- and amide-containing bridge

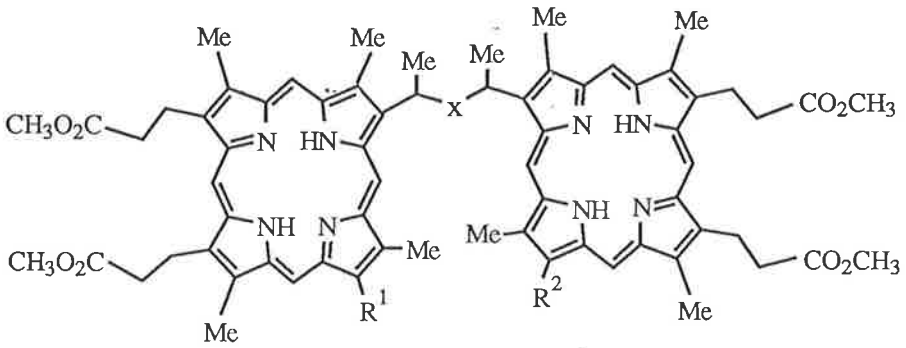
An attempt was made to synthesize dimer (97) using the procedure that had been established in Section 4.2.2. The 1,3-diaminopropane derivative (94) (Section 4.2.3.1) was reacted with adipoyl dichloride in the presence of a catalytic amount of pyridine. After aqueous work-up, a solid was obtained which, by TLC, appeared to be similar to the starting porphyrin (94) in that the main portion (~60%) of it remained at the baseline. FAB mass spectral analysis of the crude material was not very informative because, apart from the base peak at *m/z* 593 (which corresponds to 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76)), there were no other distinct peaks. However there were no peaks corresponding to the starting porphyrin (94), suggesting that reaction had taken place. A very weak peak at *m/z* 796,

which corresponds to the acid-containing porphyrin (98), along with the non-migration of the material on silica indicated that the main product(s) of the reaction might contain acid functionalities.

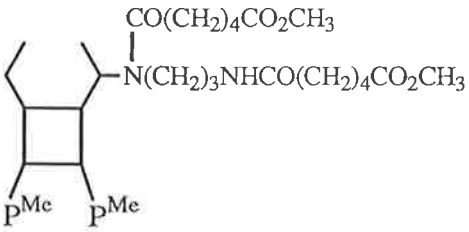
Esterification of the crude material with acidified methanol resulted in a product which gave a better FAB mass spectrum. In addition to the base peak at *m/z* 593, other peaks of medium intensity were detected at *m/z* 952, *m/z* 810, *m/z* 752 and *m/z* 609. A weak peak in the dimer region of ~1530 m.u. was also present. However the desired peak at *m/z* 1444 corresponding to dimer (97) was not present in the spectrum. The peak at *m/z* 810 corresponds to (99), while that at *m/z* 952 corresponds to the diacylated monomer (100), both of which were expected products due to the excess amounts of diacid chloride that was used. The peak at *m/z* 752 matches the molecular weight of the amide-containing monomer (101). If it is the correct structure at all, its formation is puzzling. However peak at *m/z* 752 could also be the result of the fragmentation of monomer (99), Fig 4.2. Fragmentation at the position alpha to the carbonyl group has been shown to occur in dialkyl adipates.[26] The peak at approximately *m/z* 1530 could be due to dimer (102) or (103) which both have the molecular weight of 1528. However an attempt to determine its monomer components by Mass analysed Ion Kinetic Energy spectroscopy was not successful because the *m/z* 1530 peak could not be detected in a second measurement of the spectrum. This suggested that the dimer peak might have been a spurious peak, but bearing in mind the difficulty in obtaining a strong molecular ion for the



(76) $R^1 = CH_2CH_3$, $R^2 = CH{=}CH_2$
(94) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3NH_2$
(98) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3NHCO(CH_2)_4CO_2H$
(99) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3NHCO(CH_2)_4CO_2CH_3$



(97) $X = HN(CH_2)_3NHCO(CH_2)_4COHN(CH_2)_3NH$



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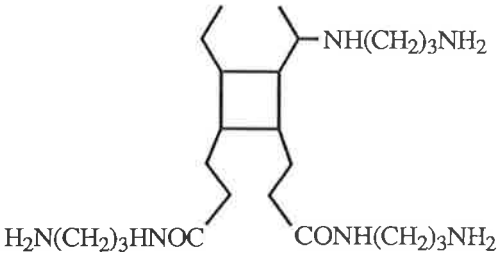

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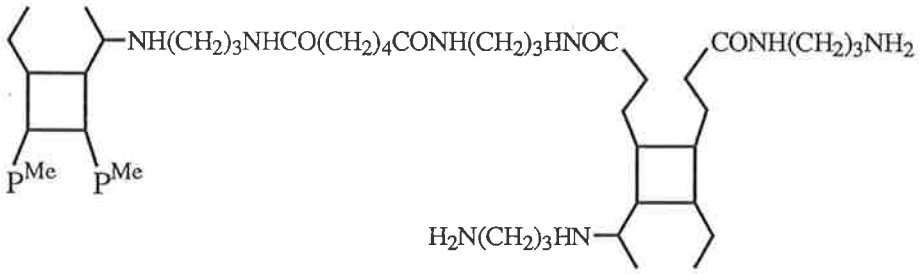
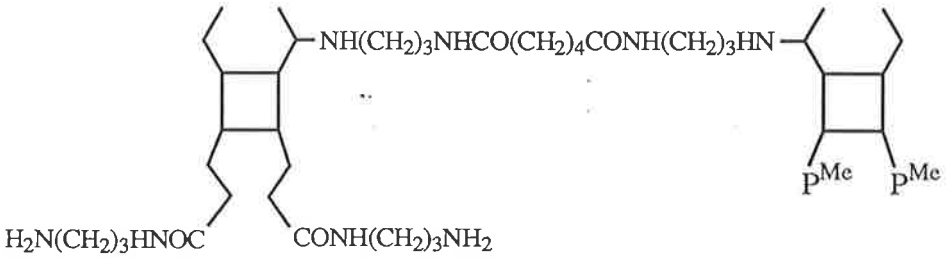
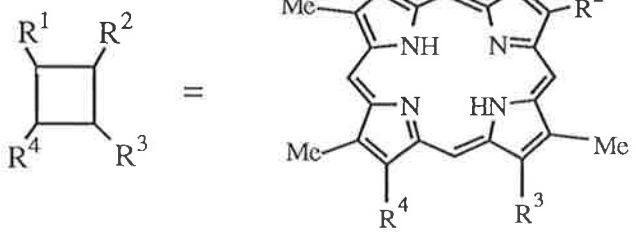


(102)



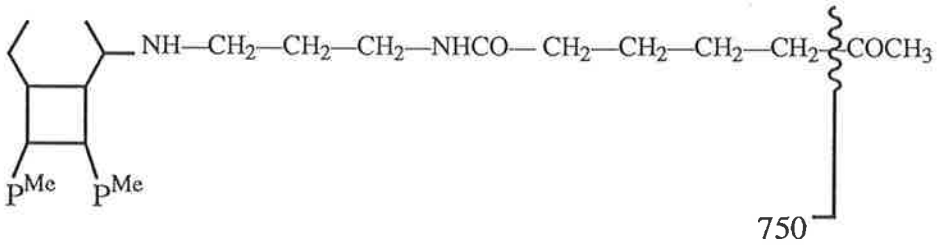
(103)

Abbreviations :



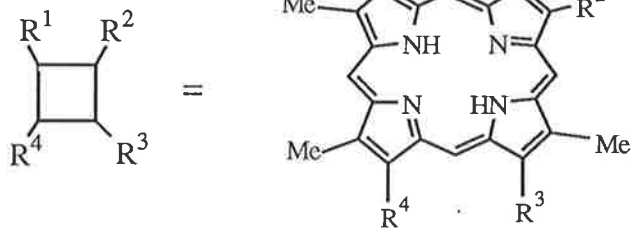
$P^{Me} = CH_2CH_2CO_2Me$

Fig. 4.2 Possible fragmentation of monomer (99) leading to the ion at ~ *m/z* 750.



(99)

Abbreviations :

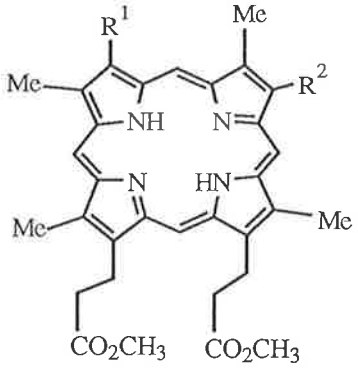


$P^{Me} = CH_2CH_2CO_2Me$

amino derivatives (see, for example, Chapter 3 and Sections 4.2.3 and 4.2.4) it may, on the other hand, indicate the need for a different FAB matrix. Another FAB matrix, namely glycerol, was tried but no improvements were observed in the spectrum. Gower[38] has indicated that mixtures of glycerol and other solvents are effective alternatives to glycerol alone as a FAB matrix.

4.2.6 Propanolamine derivative (104) of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester

The reason for this synthesis was two-fold : first, to determine the relative reactivity of an amino group and a hydroxyl group under the reaction conditions and secondly, because the product, whether it was (104a) or (104b), would be a useful starting material for other dimers. Scourides[23] showed that in the presence of excess amounts of hydrobromic acid in acetic acid, the amino group of L-cysteine was effectively prevented from functioning as a nucleophile by protonation. This is not expected to occur under the conditions that have so far been used because most of the excess hydrobromic acid is removed from the reaction mixture before the addition of the amino alcohol nucleophile and, in order to ensure that there would be free amines in the reaction mixture, twenty equivalents of propanolamine were used.

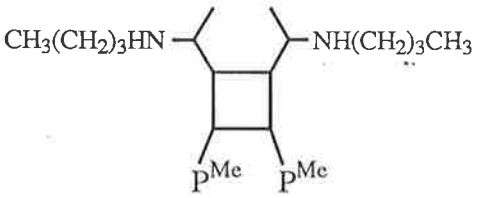


(77) $R^1 = CH(CH_3)Br$, $R^2 = CH_2CH_3$

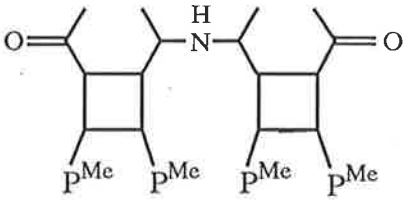
(86) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_3OH$
(86) b. $R^1 = CH(CH_3)O(CH_2)_3OH$, $R^2 = CH_2CH_3$

(94) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3NH_2$

(104a) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3OH$
(104b) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_3NH_2$



(68)



(71)

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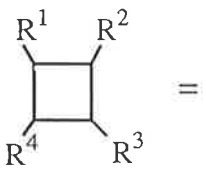

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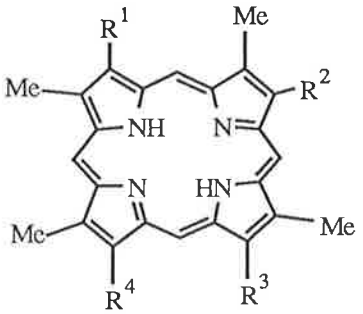


$P^{Me} = CH_2CH_2CO_2Me$

The product that was obtained after aqueous work-up was found by TLC (on silica) to consist mainly of a polar (R_F 0.11) component. This component was readily separated on a preparative scale using deactivated silica and elution with 2% methanol/dichloromethane. The FAB mass spectrum of this material showed a peak at *m/z* 668 of moderate intensity and a base peak at *m/z* 593. The mass of 668 corresponds to the propanolamine isomers (104a) and/or (104b), and on the basis of previous results (Sections 4.2.3 – 5), the peak at *m/z* 593 was probably the result fragmentation of the propanolamine derivative(s) (104). The relative intensity of the two peaks (an intense *m/z* 593 compared to the moderately intense *m/z* 668) suggested that the derivative might have the structure (104a), with the benzylic secondary amine. It was expected, on the basis of spectra of the diol derivatives (Section 4.2.1) and other derivatives[8,20] with benzylic oxygens that the structure (104b) would give a base peak molecular ion. A MIKE spectrum of *m/z* 668 gave a weak peak at *m/z* 653 and a strong peak at *m/z* 593. The *m/z* 653 peak could be due to the loss of the benzylic methyl group to give an iminium ion-type fragment, which is a known pattern of fragmentation among secondary amines,[22] and has also been found to occur to the 1,3-diaminopropane-linked bisporphyrin dimer (95) (Section 4.2.4) and the amino-linked dimers in Chapter 3. On the other hand, the loss of 15 m.u. could also be due to the cleavage of a methyl group from one of the propionic ester groups, which is also known[24] to occur. Hence it

was not possible to unambiguously assign the structure of the product by mass spectrometry alone.

The hydrolysis of the propanolamine derivative (104) under standard[25] acidic hydrolysis conditions which have been shown[25] to efficiently cleave benzyl ethers of porphyrins,[25] gave a product which was then esterified using a mixture of methanol/trimethyl orthoformate/concentrated sulphuric acid.[18] The FAB mass spectrum and the TLC chromatogram of the final product were very similar to those of the starting propanolamine derivative. The stability of the material towards acidic hydrolysis conditions indicated that it was much more likely to have been porphyrin (104a) with the benzylic amino group than the ether (104b), since amines are substantially less acid labile than ethers.[27]

The structure was confirmed by the presence of a signal at 47.75 ppm in the ^{13}C NMR spectrum of the propanolamine derivative. It was shown (Chapter 3, Section 3.2.9) by ^{1}H-^{13}C COSY experiments on the diacetyl-terminated amino-linked dimer (71) (Chapter 3, Section 3.2.9) that the benzylic carbon alpha to the amino group ($CH_3\underline{C}HNH$-) resonated at 49.20 ppm. The chemical shift of this carbon ranged from 41.8 – 49.20 ppm depending on the type of substituents on the porphyrin and the class of the amine. The corresponding carbon on the *n*-butylamine derivative (68) (Chapter 3, Section 3.2.3) resonated at 47.41 ppm. In contrast, the benzylic carbons attached to an alkoxy group ($CH_3\underline{C}HOCH_2$-) such as those in the

diol derivatives (Section 4.2.1), resonated around 73 ppm. Therefore the signal at 47.75 ppm in the ^{13}C NMR spectrum of the propanolamine derivative (104) is due to the carbon attached to an amino group and the absence of any signals in the 68 – 75 ppm region of the spectrum further proves that the group that is attached to the benzylic carbon is not an alkoxy group. This was confirmed by the signal at 62.64 ppm due to the methylene ($\underline{C}H_2OH$) attached to the hydroxyl group. In contrast, a $\underline{C}H_2NH_2$ signal is expected, on the basis of the analogous chemical shift in the 1,3-diaminopropane derivative (94) (Section 4.2.3), to occur near 60 ppm.

These results showed that the main products from the reaction between the bromoethyl porphyrin (77) and propanolamine was the derivative (104a) with the benzylic amino group, and that in a competing reaction between the amino and hydroxyl group, the amino group was the more nucleophilic of the two, as expected. The yield of the propanolamine derivative (104a) was 89%.

The propanolamine derivative (104a) exhibited a typical etio-type absorption spectrum with maxima at 401 nm, 501 nm, 537 nm, 568 nm and 621 nm.

Proton and ^{13}C NMR spectra were recorded on a 0.03 M solution. The proton spectrum was better resolved than most of the other proton spectra (for example in Sections 4.2.1, 4.2.3 and 4.2.4) and the multiplicity of some of the signals were clearly discernable. The pyrrole hydrogens resonated as a broad singlet

at -3.84 ppm. The triplet due to the methyl group on the ethyl sidechain ($CH_2C\underline{H}_3$) occured at 1.85 ppm, which correlates well with the chemical shift (~1.8 ppm) of the corresponding group in mesoporphyrin dimethyl ester.[16c] The methylene protons ($C\underline{H}_2CH_3$) of the ethyl group gave a distinct quartet at 4.07 ppm, again in good agreement with the literature[16c] chemical shift. The protons of the methyl group attached to the benzylic carbon ($C\underline{H}_3CHNH$-) gave a doublet centred at 2.43 ppm. In contrast the protons of methyl group $C\underline{H}_3CHOR$ in the diol derivatives (Section 4.2.1) resonated at ~2.2 ppm. This downfield shift appears to go against the general trend that has so far been observed (Chapter 3 and Sections 4.2.4 and 4.2.5), namely the upfield shift in the signals of protons that are near the nitrogen atom; a possible reason for this is the through space effect of the electron withdrawing hydroxyl group in the other substituent on the benzylic carbon. The proton on the benzylic carbon ($CH_3C\underline{H}NH$-) gave a broad signal at 5.8 ppm. This is slightly upfield relative to the chemical shift of the benzylic proton ($CH_3C\underline{H}OR$) on the diol derivative (Section 4.2.1) which resonated around 6.0 ppm. This upfield shift is due to the lower electronegativity of nitrogen compared to oxygen. Most of the other signals on the proton NMR spectrum of the propanolamine derivative (104a) were not as well resolved but occurred in the general chemical shifts that are expected[16] for those groups.

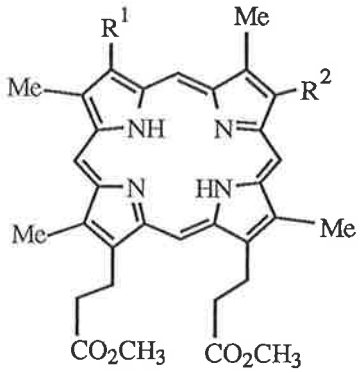
The signals in the ^{13}C NMR spectrum of the propanolamine derivative (104a) were readily assigned on the basis of chemical shifts of analogous porphyrins (Sections 4.2.1,

4.2.3, 4.2.4). The carbons of the ethyl, methyl and propionic ester sidechains resonated at the expected chemical shifts of 17.53 ppm ($CH_2\underline{C}H_3$), 19.73 ppm ($\underline{C}H_2CH_3$), 11.70 ppm (ring $C\underline{H}_3$), 21.87 ppm ($\underline{C}H_2CH_2CO_2$), 36.95 ppm ($\underline{C}H_2CO_2$), 51.65 ppm ($CO_2\underline{C}H_3$) and 173.41 ppm ($\underline{C}O_2CH_3$). The benzylic methine carbon ($CH_3\underline{C}HNH$-) resonated at 47.75 ppm, the methyl carbon ($\underline{C}H_3CHNH$-) at 23.04 ppm and the methylene carbon under the hydroxyl group ($\underline{C}H_2OH$) was assigned the signal at 62.64 ppm, which correlates with the chemical shift (62.18 ppm) of the corresponding carbon in the 1,3-propanediol derivative (86).

Having established the structure of the propanolamine derivative as (104a), attention was then focussed on using this porphyrin to synthesize other bisporphyrin dimers. Due to the earlier difficulties in the analysis of the dimer(s) linked by amino- and amide-containing bridges (Section 4.2.5), it was decided to synthesize another dimer with an amino- and amide-containing bridge and to compare its physical properties, particularly its behaviour under FAB mass spectrometric conditions, with that of the earlier dimer(s) (Section 4.2.5).

4.2.7 Attempted oxidation of the primary alcohol on the porphyrin sidechain

The proposed route, shown in Scheme 4.2, involved the oxidation of the primary alcohol on the sidechain of the porphyrin to a carboxylic acid group. The acid group was subsequently converted to the reactive acid chloride which was



(87) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_5OH$
(87) b. $R^1 = CH(CH_3)O(CH_2)_5OH$, $R^2 = CH_2CH_3$

(105) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_4CO_2H$

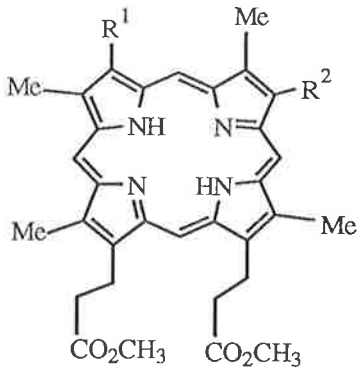
then reacted with a terminal diamine to give the desired bisporphyrin dimer linked by an amine- and amide-containing bridge.

Initial studies on the oxidation were done on the more readily available diol derivatives (Section 4.2.1). An attempt was made to oxidize the hydroxyl group on the 1,5-pentanediol derivative (87) (Section 4.2.1) using neutral permanganate solution.[28] It was hoped that the mild conditions would not cleave the propionic ester groups. Unfortunately oxidation did not proceed at all after a 4.5 hour reflux, and the starting porphyrin was recovered. It was then decided to investigate the use of Jones reagent.[29] The 1,5-pentanediol derivative (87) (from Section 4.2.1) was stirred in an excess of Jones reagent for 5 minutes at room temperature. After aqueous work-up, the product that was obtained was shown by TLC to consist of the starting diol derivative (87) (Section 4.2.1) and another spot below it, at R_F 0.3, in a ratio of 3 : 2. FAB mass spectral analysis of the crude material showed a base peak molecular ion at *m/z* 711, which corresponds to the desired acid (105), and a peak of moderate intensity at *m/z* 697 corresponding to the starting porphyrin (87). When the reaction time was extended from 5 minutes to 7 minutes, the desired acid was obtained in quantitative yield.

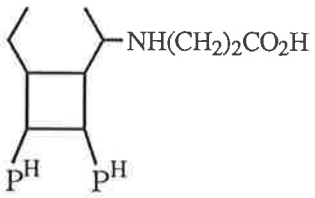
4.2.8 Jones oxidation of the propanolamine derivative (104a)

The propanolamine derivative (104a) (Section 4.2.6) was treated with Jones reagent for 7 minutes according to the procedure that had been established in Section 4.2.7 using the diol derivatives. After work-up the crude product was shown by TLC to consist of two components, namely the starting material (104a) and a lower R_F spot at (R_F 0.3), which were present in the ratio of approximately 2:1. The FAB mass spectrum of the crude material showed a base peak at *m/z* 593 and two other peaks at *m/z* 668 and 682. The *m/z* 668 peak corresponds to the starting porphyrin (104a), while the *m/z* 682 peak is the molecular ion of the desired β-amino acid derivative (106). The presence of the *m/z* 593 base peak was characteristic of benzylic amino-containing porphyrins (see Sections 4.2.3, 4.2.4, 4.2.6 and also Chapter 3). It is likely that the low R_F component was the desired β-amino acid derivative (106) because it is expected to be more polar than its precursor (104a).

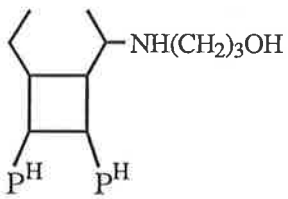
The crude product was treated with Jones reagent again for a further 5 minutes. The product that was obtained after aqueous work-up was similar by TLC to the partially oxidised material in that both the starting propanolamine derivative (104a) (Section 4.2.6) and the low R_F component were present in the chromatogram, but the proportion of the low R_F component had increased so that the relative ratio of the two components was approximately 1:1. In addition to that there was



(104a) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3OH$
(106) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_2CO_2H$
(107) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_2CO_2CH_3$

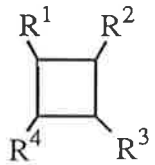


(108)



(109)

Abbreviations :



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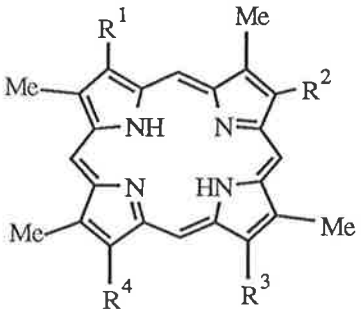


$P^{Me} = CH_2CH_2CO_2Me$

$P^{H} = CH_2CH_2CO_2H$

some polar material (~30% by TLC) on the baseline of the chromatogram, possibly hydrolysed porphyrins. The crude material was not completely soluble in dichloromethane and/or methanol. The insoluble material was separated by filtration and the filtrate was found by TLC to contain the starting propanolamine derivative (104a) (Section 4.2.6) and the low R_F component which was found (see later) to be the desired β-amino acid derivative (106). The residue from the filtration was esterified using the orthoformate esterification procedure[18] and the product was found (by FAB m.s.) to give the ions *m/z* 696 (the triester (107)) and 668 (the propanolamine derivative (104a) (Section 4.2.6)). These results indicated that the triacid (108) and the hydrolysed propanolamine derivative (109) are insoluble in dichloromethane and/or methanol, and that the oxidation had not gone to completion. However the reaction time needs to be kept around 7 minutes in order to minimize the products of competing hydrolysis of the propionic esters. The hydrolysed by-products can be readily separated from the desired product and unreacted propanolamine derivative (104a) by filtration.

The β-amino acid derivative (106), which was the low R_F component, was readily separated from starting material by preparative TLC on silica. The FAB mass spectrum of the porphyrin (106) showed a molecular ion at *m/z* 682 of moderate intensity and a base peak at *m/z* 593. Again, this is consistent with the FAB mass specra of other amino derivatives (see Sections 4.2.3, 4.2.4, 4.2.6 and also Chapter 3). The

porphyrin (106) exhibited a typical etio-type absorption spectrum with maxima at 401 nm, 499 nm, 536 nm, 568 nm and 622 nm.

The proton and ^{13}C NMR spectra of the β-amino acid derivative (106) were recorded in deuterochloroform on a solution of approximately 0.015 M. Despite this low concentration the proton NMR spectrum was uninformative due to the broadness of the signals. On the other hand the ^{13}C NMR spectrum was well resolved and most of the signals could be assigned on the basis of chemical shifts in the literature[16] and other similar porphyrins (Sections 4.2.3, 4.2.4 and 4.2.6). As with the other amino derivatives, the ethyl, methyl, propionic ester sidechains and the ring carbons resonated at chemical shifts that were comparable to those of known[16] porphyrins. The resonances of the carbons of the sidechain bearing the β-amino acid group were also readily assigned. The methyl carbon ($\underline{C}H_3CHNH$-) resonated at 25.64 ppm; by comparison the corresponding carbon on the precursor propanolamine derivative (104a) (Section 4.2.6) was assigned the signal at 23.04 ppm. The signal at 36.56 ppm was assigned to the methylene carbon next to the carboxylic group ($\underline{C}H_2CO_2H$) because of the similarity of the environment of this methylene group with that of $\underline{C}H_2CO_2CH_3$ in the propionic ester group. The latter resonated around 36.8 ppm. The signal at 43.73 ppm was assigned to the benzylic methine carbon ($CH_3\underline{C}HNH$-), which correlated well with with the chemical shift of the corresponding carbon on the other amino-containing porphyrins (see Chapter 3 and Sections 4.2.3, 4.2.4 and 4.2.6).

The quaternary carbon in the carboxylic group ($\underline{C}O_2H$) resonated at 176.76 ppm, which is similar to the resonances of the quaternary carbon in the propionic ester groups ($\underline{C}O_2CH_3$) at approximately 173.5 ppm. These assignments are only tentative as they have not been confirmed with off-resonance decoupling experiments or correlation studies.

4.2.9 Attempted synthesis of a bisporphyrin dimer with an amine- and amide-containing bridge (110)

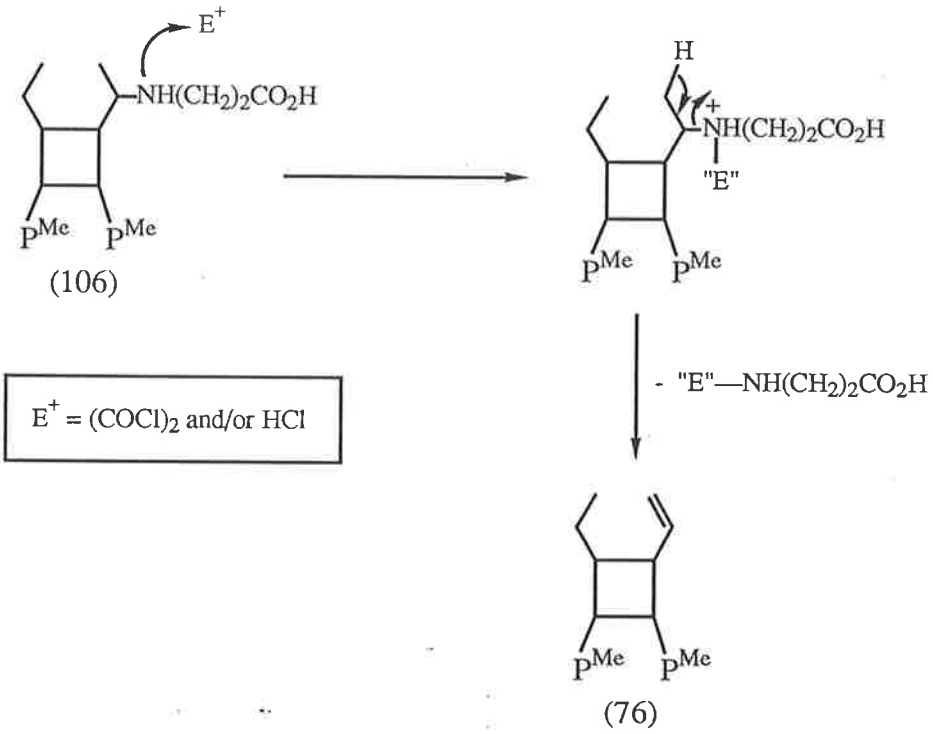
Having obtained the β-amino acid derivative (106) (Section 4.2.8), the next step was to convert it to an acid chloride and then react the acid chloride with a terminal diamine (Scheme 4.2), to give the desired dimer (110).

The acid chloride (111) was prepared by the standard procedure[15a,c] using oxalyl chloride and was treated with 1,3-diaminopropane (~9 equivalents) without prior purification. A smaller proportion (~0.5 equivalents) of diamine would have been preferable but was not possible on the scale of the reaction. However this was not expected to be a problem because it was anticipated on the basis of an earlier study (Section 4.2.2) using excess amounts of reagents that while the large excess of diamine would lower the yield of dimer (110) it would not prevent its formation altogether.

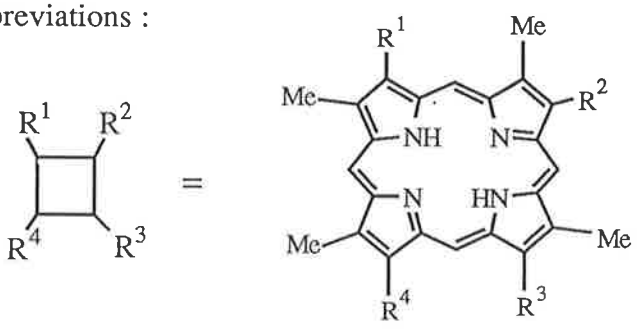
TLC analysis of the crude product showed trace amounts of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) but most (>80%) of the material did not migrate from the baseline. It was expected that the amino monomer (112) which would be the main product (because of the excess amounts of diamine that was used) would not migrate very far from the baseline under the standard TLC conditions that have so far been used; the desired dimer (110) should have a higher R_F than the amino monomer (112) because it does not have any primary amino groups. Therefore on this basis it appeared that that the desired dimer (110) was not a significant product in this reaction. The formation of the 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) could be due to the initial reaction between the secondary amino group in the benzylic position and oxalyl chloride, Scheme 4.3, to form the ammonium salt which could then be eliminated to give 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester.

The FAB mass spectrum of the crude product gave, apart from the peak at *m/z* 593 corresponding to 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76), only very weak peaks. It was only just possible to make out peaks around *m/z* 1330, 1300, 810, ~735 and 680. There were no peaks around *m/z* 1402, which was the molecular weight of the desired dimer (110). The peak at *m/z* 680 corresponds to the starting β-amino acid derivative (106) (Section 4.2.8). The *m/z* ~735 m.u. peak could be due to the derivative (112) (molecular weight 738), or

Scheme 4.3

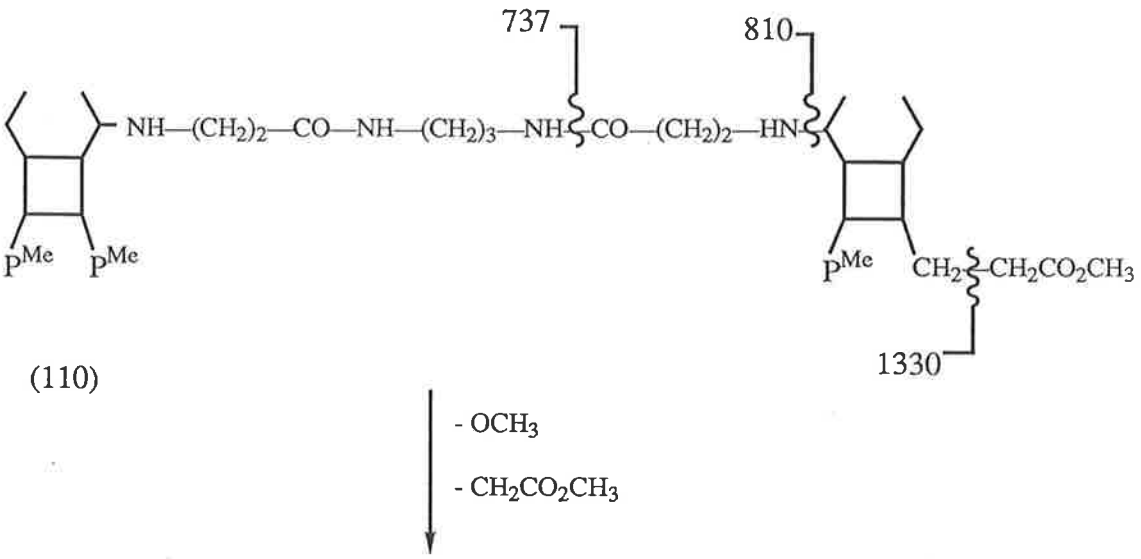


Abbreviations :



$P^{Me} = CH_2CH_2CO_2Me$

Fig. 4.3 Possible fragmentations from dimer (110) (*m/z* 1402).



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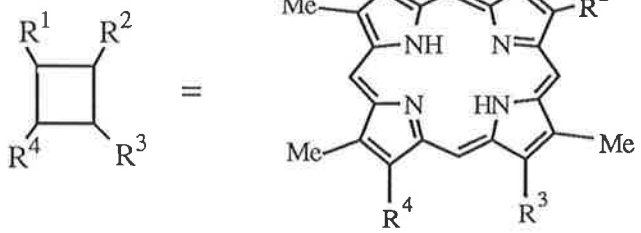
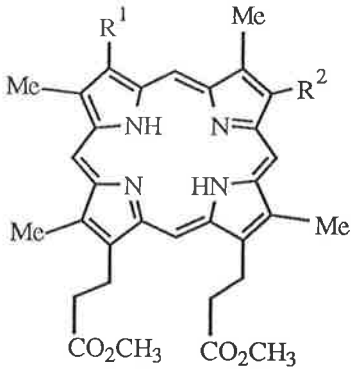


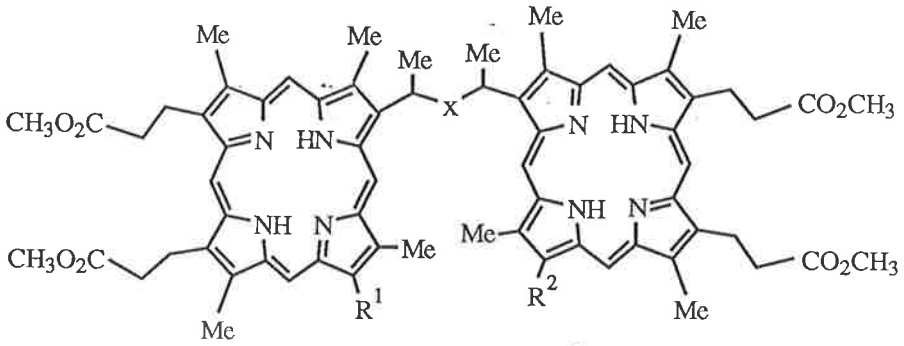
$P^{Me} = CH_2CH_2CO_2Me$

it could be a fragment from the dimer (110), Fig. 4.3. Fragmentation at the C-N bond of an amide is a known[30] process and would be expected to occur in a molecule that is as large as dimer (110). Similarly, the peak at *m/z* ~810 could be due to fragmentation at the benzylic C-N bond, which has been found to occur in all the derivatives with benzylic amino groups (Sections 4.2.4, 4.2.6 and 4.2.8). The weak peak around *m/z* 1330 in the dimer region could also be due to a fragment of the desired dimer (110), Fig. 4.3. On the other hand it could be the molecular ion of the unsymmetrical dimer (110), which could have formed from the substitution of the ammonium salt by the monomer (112), Scheme 4.3. Similarly, the peak at *m/z* 1300 could be due to the partially hydrolysed dimer (114) or the fragmentation of dimer (110), Fig. 4.3. Fragmentation at the propionate group is a common process.[24] Attempts to test these postulates by a Mass analysed Ion Kinetic Energy spectrum of the dimer ions was unsuccessful because the dimer ions appeared to be very short-lived and could not be consistently detected. Therefore it was not known whether the dimer ions were genuine, transient species or artefacts from the mass spectra. Under the synthesis conditions, the dimers are not expected to be formed in significant yields but it was anticipated, by analogy with the work on diol derivatives (Section 4.2.2), that there would be some dimer in the product mixture.

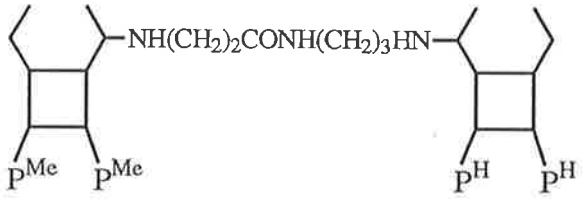
In order to esterify any free acid groups the crude material was stirred in 5:5:1 trimethyl orthoformate/methanol/concentrated sulphuric acid[18]. After



(76) $R^1 = CH_2CH_3$, $R^2 = CH{=}CH_2$
(106) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_2CO_2H$
(107) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_2CO_2CH_3$
(111) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_2COCl$
(112) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_2CONH(CH_2)_3NH_2$

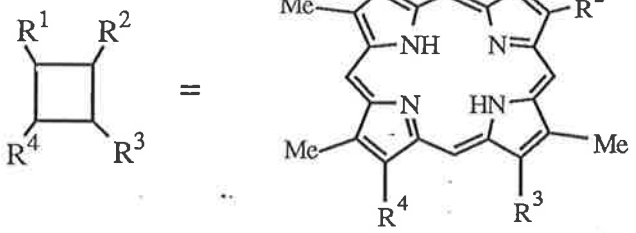


(110) $X = HN(CH_2)_2CONH(CH_2)_3HNCO(CH_2)_2NH$
(113) $X = HN(CH_2)_2CONH(CH_2)_3HN$



(114)

Abbreviations :



$P^{Me} = CH_2CH_2CO_2Me$

$P^{H} = CH_2CH_2CO_2H$

work-up TLC analysis of the crude product showed that it consisted of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76), the triester (107), as expected, and at least two other components. FAB mass spectral analysis of the crude product confirmed the presence of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (*m/z* 593) and the triester (107) (*m/z* 694). In addition, there were weak peaks at *m/z* 1374, 1336, 1258 and 750. However the peaks at *m/z* 1330, 1300 and ~735, which were present in the FAB mass spectrum of the unesterified material, were not present in the spectrum of the esterified material. This was unusual because they were not expected to be altered by esterification. The desired peak at *m/z* 1402 corresponding to the dimer (110) was not present in the spectrum of the esterified material. Attempts to obtain a Mass analysed Ion Kinetic Energy spectrum of the peaks at *m/z* 1374, 1316 and 1258 met with the same difficulty as that which was encountered before – the peaks could not be detected in subsequent runs. Similarly, an attempt to obtain a LINK spectrum of *m/z* 750 that would enable to determine the peak(s) that it originated from was also unsuccessful because the peak could not be detected in a later measurement. These results highlight the need for investigations into the use of different matrices[37,38] and/or mass spectrometric techniques because existing techniques do not appear to give adequate molecular ions of the products.

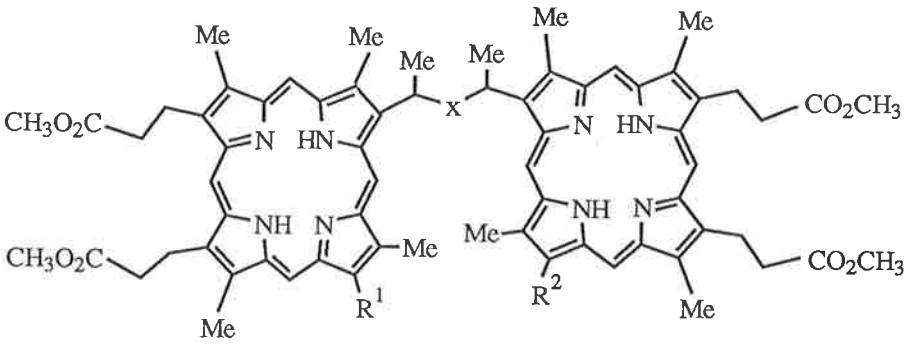
The presence, in the crude product, of the monomer containing the amine and amide groups (112) indicates that acid

chloride formation and subsequent substitution with the diamine had proceeded as expected. The implication from the TLC analysis of the crude product was that the desired dimer (110) (which should have a moderate to high R_F value) was not a significant product in the reaction, suggesting that it would be more convenient to follow a two step synthesis, by preparing the monomer (112) first and then treating it with the acid chloride (111) in a separate step. The competing elimination reaction (to give 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76)) presumably during the formation of the acid chloride will lower the yield of the desired products but it might be possible to minimize the reaction by doing this reaction at a lower temperature. Unfortunately, due to limited time optimization studies could not be undertaken.

4.2.10 Preliminary tests for intercalating ability

The diether-linked dimers (89), (90) and (91) as well as the diaminopropane-linked dimer (95) were tested for possible DNA intercalation using an assay involving spectral titration at 400 nm with calf thymus DNA.[44] All the dimers showed small (≤7%) absorption changes, indicating that the compounds were not significantly interacting with DNA by intercalation.

This absence of any intercalating ability is not unexpected because of the non-cationic nature of the component



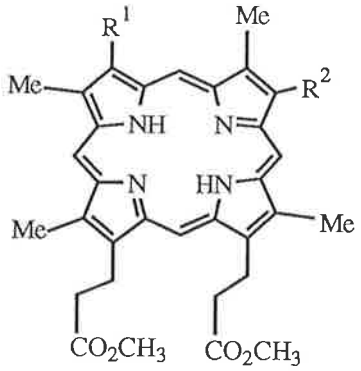
(89) X = -O(CH_2)$_3$O-
(90) X = -O(CH_2)$_5$O-
(91) X = -O(CH_2)$_{10}$O-
(95) X = -HN(CH_2)$_3$NH-

porphyrins in the dimers. Studies with HPD and the anionic meso-tetrakis(phenylsulfonato)porphine (H_2TPPS)[40] have indicated that non-cationic porphyrins do not interact as efficiently with DNA as their cationic analogues.[40]

4.2.11 Summary

The hydrobromination-nucleophilic substitution reaction conditions that had been established in Chapter 3 were used efficiently to synthesize the α,ω-diol derivatives (86), (87), (88), 1,3-diaminopropane derivative (94) and the propanolamine derivative (104a) in good yields. The regioisomers of the α,ω-diol derivatives (86), (87) and (88) were readily separated on silica by squat column chromatography. This derivatization of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) with an α,ω-diol, particularly the 1,5-pentanediol, represents a convenient and efficient way of separating the regioisomers of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76). All the compounds were characterized by u.v./visible and NMR spectroscopy and FAB mass spectrometry. The structures of the α-isomers of the diol derivatives (86), (87) and (88) were tentatively assigned (86b), (87b), (88b) and the β-isomers to (86a), (87a) and (88a), according to Fookes rule.

Diporphyrin dimers linked by diether bridges (89), (90) and (91) were synthesized in moderate yields by a two step reaction involving the treatment of the bromoethyl compound



(76) $R^1 = CH_2CH_3$, $R^2 = CH{=}CH_2$
(77) $R^1 = CH(CH_3)Br$, $R^2 = CH_2CH_3$

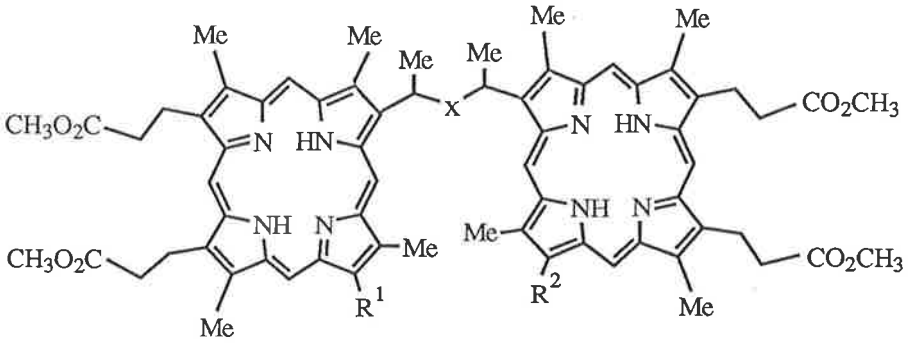
(86) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_3OH$
(86) b. $R^1 = CH(CH_3)O(CH_2)_3OH$, $R^2 = CH_2CH_3$

(87) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_5OH$
(87) b. $R^1 = CH(CH_3)O(CH_2)_5OH$, $R^2 = CH_2CH_3$

(88) a. $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)O(CH_2)_{10}OH$
(88) b. $R^1 = CH(CH_3)O(CH_2)_{10}OH$, $R^2 = CH_2CH_3$

(94) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3NH_2$

(104a) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_3OH$
(106) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_2CO_2H$
(107) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_2CO_2CH_3$
(112) $R^1 = CH_2CH_3$, $R^2 = CH(CH_3)NH(CH_2)_2CONH(CH_2)_3NH_2$



(89) X = $-O(CH_2)_3O-$
(90) X = $-O(CH_2)_5O-$
(91) X = $-O(CH_2)_{10}O-$
(92) X = $-O(CH_2)_5OCOCH_2CO_2(CH_2)_5O-$
(95) X = $-HN(CH_2)_3NH-$
(97) X = $-HN(CH_2)_3NHCO(CH_2)_4COHN(CH_2)_3NH-$

(77) with the appropriate α,ω-diol derivatives. The dimers were characterized by u.v./visible and NMR spectroscopy and FAB mass spectrometry. A dimer (92) linked by a bridge containing ester and ether groups was synthesized; and analysed by FAB and MIKE mass spectrometry.

The 1,3-diaminopropane-linked dimer (95) was prepared in a low yield from the 1,3-diaminopropane derivative (94) and the bromoethyl compound (77) by the same procedure as that used for the synthesis of the diether-linked dimers (see above). An attempt to synthesize the dimer (97) with an amine- and amide-containing bridge using the procedure that had been established for the synthesis of the analogous dimer (92) linked by a bridge containing ester and ether groups (see above) gave inconclusive results because of difficulties in analysing the product by FAB and MIKE mass spectrometry An alternative approach (Scheme 4.2) to another dimer (110) linked by an amine- and amide-containing bridge was investigated. Jones oxidation of the propanolamine derivative (104a) furnished the monomer precursor (106) in low yield due to competing hydrolysis of the reactant and product during the reaction. Conversion of the acid group in (106) to an acid chloride (107) followed by reaction of the latter with 1,3-diaminopropane gave a complicated product mixture of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76), the amine- and amide-containing monomer (112), and some unidentified monomeric and dimeric material. Analysis of the crude material

by mass spectrometry was hampered by difficulties in obtaining reproducible spectra.

Preliminary analyses of the diether-linked dimers (89), (90) and (91), and the 1,3-diaminopropane-linked dimer (95) for DNA intercalating ability indicated that the dimers were poor intercalators, possibly due to the non-cationic nature of the component porphyrins. Future efforts in the synthesis of dimers which are potential DNA intercalators would have to be focussed on obtaining long-bridged dimers of cationic porphyrins.

4.3 Experimental§

4.3.0 General

The general experimental details are similar to those outlined in Chapter 2, Section 2.3.0.

4.3.1 1,3-Propanediol derivative of 3-ethyl-8-vinyl-deuteroporphyrin dimethyl ester (86)

To 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (0.17 g, 0.29 mmol) was added cold saturated HBr/dichloromethane (10 ml) (see Chapter 3, Section 3.2.1) and the resulting red-green solution was stirred at room temperature under an atmosphere of nitrogen for 100 min. Then the excess acid and solvent were removed under reduced pressure at 0°C. The residual gum was redissolved in dry dichloromethane (2 ml) and added dropwise with stirring to 1,3-propanediol (2.2 g, 29 mmol, 100 equivalents) at 0°C. The mixture was stirred for a further 35 min at 0°C, then diluted with dichloromethane (ca. 20 ml), poured into water and washed with water (3 × 20 ml). The organic phase was evaporated to dryness without chemical drying, leaving a solid residue. The crude porphyrin was chromatographed on a silica squat column. The first major band, eluted with 2-4% acetone/dichloromethane, contained the *α-isomer of the 1,3-propanediol derivative* (86) (79 mg, 41%) which

§ References, p. 251.

gave λ_{max} (rel. abs.) 400 nm (100%), 498 (8.9%), 533 (6.5%), 567 (5.1%), 621 (2.8%); ^{13}C NMR, δ ppm 11.59 (ring $\underline{C}H_3$), 17.54 ($CH_2\underline{C}H_3$), 19.74 ($\underline{C}H_2CH_3$), 21.83 ($\underline{C}H_2CH_2CO_2CH_3$), 25.44 ($\underline{C}H_3CHOCH_2$-), 32.55 ($OCH_2\underline{C}H_2CH_2OH$), 36.89 ($\underline{C}H_2CO_2CH_3$), 51.64 ($CO_2\underline{C}H_3$), 62.18 ($\underline{C}H_2OH$), 68.53 ($O\underline{C}H_2$-), 73.88 ($CH_3\underline{C}HO$-), 96,31, 96.87, 98.17 (meso C), 134.9 - 141.4 (m, C_β), 145-150 (br, C_α), 173.47, 173.59 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm -3.82 (br, 2H, 2 × pyrrole H), 1.86 (t, $CH_2C\underline{H}_3$), 1.93 (m, -$OCH_2C\underline{H}_2CH_2O$-), 2.26 (d, 3H, $C\underline{H}_3CHOR$), 3.25 (t, 2 × $C\underline{H}_2CO_2CH_3$), 3.5 - 3.7 (4 × ring $C\underline{H}_3$, 2 × CO_2CH_3), 3.87 (br, $OC\underline{H}_2CH_2$-), 4.08 (q, $C\underline{H}_2CH_3$), 4.36 (2 × t, 2 x $C\underline{H}_2CH_2CO_2CH_3$), 6.07 (q, 1H, $C\underline{H}_3CHOCH_2$-), 9.95, 9.98, 10.00, 10.03, 10,08, 10.52 (meso H); FAB m.s. *m/z* 669 (base peak), 593; while elution with 4% acetone/dichloromethane afforded the *β-isomer* (70 mg, 36%) which had λ_{max} (rel. abs.) 410 (100%), 499 (26%), 533 (19%), 566.5 (13%), 621 (8%); ^{13}C NMR, δ ppm 11.27, 11.64 (ring $\underline{C}H_3$), 17.54 ($CH_2\underline{C}H_3$), 19.68 ($\underline{C}H_2CH_3$), 21.81 ($\underline{C}H_2CH_2CO_2$), 25.43 ($\underline{C}H_3CHOCH_2$-), 32.49 (-$OCH_2\underline{C}H_2$-), 36.93 ($\underline{C}H_2CO_2CH_3$), 51.65 ($CO_2\underline{C}H_3$), 62.12 ($\underline{C}H_2OH$), 68.46 ($O\underline{C}H_2CH_2$-), 73.83 ($CH_3\underline{C}HOCH_2$), 96.14, 96.50, 98.16 (meso C), 134.20 – 140.5 (m, C_β), 145 – 150 (br, C_α), 173.60, 173.46 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm -3.92 (br, 2H 2 × pyrrole H), 1.80 (t, $CH_2C\underline{H}_3$), 1.92 (m, -$OCH_2C\underline{H}_2CH_2O$-),* 2.27 (d, 3H, $C\underline{H}_3CHOCH_2$-), 3.15 – 3.28 (2 × t, $CH_2C\underline{H}_2CO_2CH_3$), 3.41(s), 3.60 – 3.67 (m) (2 × $CO_2C\underline{H}_3$, 4 × ring $C\underline{H}_3$, -OCH-), 3.79 – 3.96 (m, $C\underline{H}_2CH_3$, -$OC\underline{H}_2CH_2$-),* 4.20, 4.37 (2 × t, $C\underline{H}_2CH_2CO_2CH_3$), 6.06 (q, 1H, $CH_3C\underline{H}OCH_2$-), 9.80, 9.92, 10.01, 10.52 (4H, all s, 4 × meso H); FAB m.s. was identical to that of the α-isomer.

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* Arbitrary assignments.

*Arbitrary assignments.

4.3.2 1,5-Pentanediol derivative of 3-ethyl-8-vinyl-deuteroporphyrin dimethyl ester (87)

The title compound (87) was prepared by the method previously described for the 1,3-propanediol derivative. 3-Ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (80 mg, 0.135 mmol) and 1,5-pentanediol (1.5 g, 14 mmol) produced the *α-isomer of the 1,5-pentanediol derivative* (87) (36 mg, 38%) which had λ_{max}(rel. abs.) 399 nm (100%), 499 (7.1%), 533 (6.5%), 565 (6.1%), 621 (3.6%); ^{13}C NMR, δ ppm 11.58, 11.71 (ring $\underline{C}H_3$), 17.55 ($CH_2\underline{C}H_3$), 19.80 ($\underline{C}H_2CH_3$), 21.84 ($\underline{C}H_2CH_2CO_2CH_3$), 22.53 (bridge $\underline{C}H_2$), 25.44 ($\underline{C}H_3CHOCH_2$-), 36.99 ($\underline{C}H_2CO_2CH_3$), 32.42 (bridge $\underline{C}H_2$), 51.69 ($CO_2\underline{C}H_3$), 62.64 ($\underline{C}H_2OH$), 69.23 ($CH_3CHO\underline{C}H_2$), 73.38 ($CH_3\underline{C}HOCH_2$), 96.16, 96.34, 96.80, 98.61 (meso C), 135 – 138 (br, C_β), 140 – 144 (br, C_α), 173.54 ($\underline{C}O_2CH_3$); ^{1}H NMR, δ ppm -3.80 (br, 2H, 2 × pyrrole H), 1.85 (t, $CH_2C\underline{H}_3$), 2.26 (d, $C\underline{H}_3CHOCH_2$), 3.26 (t, $C\underline{H}_2CO_2CH_3$), 3.35 – 3.76 (4 × ring $C\underline{H}_3$, 2 × $CO_2C\underline{H}_3$, $CHOC\underline{H}_2$), 4.08 (q, $C\underline{H}_2CH_3$), 4.04 - 4.12 (4H, 2 × t, 2 × $C\underline{H}_2CH_2CO_2$), 6.05 (q, 1H, $CH_3C\underline{H}OCH_2$), 10.02, 10.04, 10.09, 10.58 (4 × meso H); FAB m.s. *m/z* 697 (M^+, base peak), 593; and the *β-isomer* (52 mg, 55%), λ_{max}(rel. abs.) 399 nm (100%), 498 (7.6%), 532 (6.1%), 567 (5.33%), 622 (3.2%); ^{13}C NMR, δ ppm 11.40, 11.71 (ring $\underline{C}H_3$), 17.61 ($CH_2\underline{C}H_3$), 19.74 ($\underline{C}H_2CH_3$), 21.90 ($\underline{C}H_2CH_2CO_2$), 22.59, 32.43 (bridge $\underline{C}H_2$), 25.51 ($\underline{C}H_3CHOCH_2$), 37.02 ($\underline{C}H_2CO_2CH_3$), 51.70 ($CO_2\underline{C}H_3$), 62.64 ($\underline{C}H_2OH$), 69.24 ($O\underline{C}H_2CH_2$), 73.38 ($\underline{C}H_3CHOCH_2$), 96.15, 96.56, 98.68 (meso C), 134 – 138 (br,

C_β), 141 – 146 (br, C_α), 173.65 ($\underline{C}O_2CH_3$); ^{1}H NMR, δ ppm -3.80 (s, 2H, 2 × pyrrole H), 1.84 (t, $CH_2C\underline{H}_3$), 2.26 (d, 3H, $C\underline{H}_3CHOCH_2$), 3.24 – 3.32 (m, 2 × $C\underline{H}_2CO_2CH_3$), 3.39 – 3.41, 3.52, 3.56 –3.77 (4 × ring $C\underline{H}_3$, 2 × $CO_2C\underline{H}_3$, $OC\underline{H}_2CH_2$), 4.06 (q, $C\underline{H}_2CH_3$), 4.35, 4.44 (2 × t, 4H, 2 × $C\underline{H}_2CH_2CO_2$), 6.06 (q, 1H, $CH_3C\underline{H}OCH_2$), 10.00, 10.04, 10.08, 10.60 (4 × meso H); FAB m.s. *m/z* 697 (M^+, base peak), 593 (as above).

4.3.3 1,10-Decanediol derivative of 3-ethyl-8-vinyl-deuteroporphyrin dimethyl ester (88)

The title compound was prepared from 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (170 mg, 0.29 mmol) and 1,10-decanediol (5 g, 29 mmol) by the method described in Section 4.3.2 above, with the following modifications : the 1,10-decanediol was finely powdered and suspended in dry THF (3 ml) before the addition of the bromoethyl porphyrin. The resulting reaction mixture was stirred at room temperature under an atmosphere of nitrogen for 19 h, then diluted with dichloromethane and washed with water in the manner described in Section 4.3.2. An initial attempt to chromatograph the crude product on a silica squat column resulted in both the α- and β - isomers eluting together with 2% acetone/dichloromethane. This fraction was re-chromatographed by preparative TLC on silica developed in 2% methanol/dichloromethane. This gave the *α-isomer of the title porphyrin* (88) (50 mg, 22%) which had λ_{max}(rel. abs.) 399 nm (100%), 498 (7.6%), 532 (6.4%), 567 (5.4%), 621 (3.2%); ^{13}C NMR,

δ ppm 11.58, 11.28 (ring $\underline{C}H_3$), 17.60 ($CH_2\underline{C}H_3$), 19.74 ($\underline{C}H_2CH_3$), 21.82 ($\underline{C}H_2CH_2CO_2$), 26.35, ~32.5 (bridge $\underline{C}H_2$), 25.55 ($\underline{C}H_3CHOCH_2$), 36.91 ($\underline{C}H_2CO_2CH_3$), 51.64 ($CO_2\underline{C}H_3$), 62.64 ($\underline{C}H_2OH$), 69.44 ($O\underline{C}H_2CH_2$), 73.31 ($CH_3\underline{C}HOCH_2$), 96.04, 96.22, 96.54, 96.73, 98.72 (meso C), 135.03, 135.50, 137.27, 139.09, 141.47 (C_β), 145-151 (br, C_α), 173.66, 173.53 ($\underline{C}O_2CH_3$); ^{1}H NMR, δ ppm -3.86 (br, 2H, 2 × pyrrole H), 1.83 (t, $CH_2C\underline{H}_3$), 2.25 (d, $CH_3C\underline{H}OCH_2$), 3.20 (t, $C\underline{H}_2CO_2CH_3$), 3.41 – 3.45 ($OC\underline{H}_2CH_2$),* 3.50 – 3.73 (4 × ring $C\underline{H}_3$, 2 × $CO_2C\underline{H}_3$), 4.03 (q, $C\underline{H}_2CH_3$), 4.28 (2 × $C\underline{H}_2CH_2CO_2$), 6.05 (q, 1H, $CH_3C\underline{H}OCH_2$), 9.91, 9.95, 10.02, 10.60 (4H, 4 × meso H); FAB m.s. *m/z* 767 (M^+, base peak), 593 (as above); and the *β-isomer of the title porphyrin* (88) (90 mg, 40%), λ_{max}(rel. abs.) 399 nm (100%), 497 (7.4%), 533 (6.3%), 567 (5.4%), 621 (3.1%); ^{13}C NMR, δ ppm 11.14, 11.46, 11.59, 11.71 (ring $\underline{C}H_3$), 17.54 ($CH_2\underline{C}H_3$), 19.59 ($\underline{C}H_2CH_3$), 21.77 ($\underline{C}H_2CH_2CO_2$), 25.56 ($\underline{C}H_3CHOCH_2$), 26.38, 30.29, 32.42, 32.55 (bridge $\underline{C}H_2$), 36.95 ($\underline{C}H_2CO_2CH_3$), 51.66 ($CO_2\underline{C}H_3$), 62.58 ($\underline{C}H_2OH$), 69.44 ($CH_3CHO\underline{C}H_2$), 73.37 ($CH_3\underline{C}HOCH_2$), 95.96, 96.28, 96.47, 98.73 (meso C), 134.20, 135.94, 137.32, 138.96, 141.17, 141.43 (C_β), 142 – 147 (br, C_α), 173.59 ($\underline{C}O_2CH_3$); ^{1}H NMR, δ ppm -3.94 (2H, 2 × pyrrole H), 1.77 ($CH_2C\underline{H}_3$), 2.27 (d, $C\underline{H}_3CHOCH_2$), 3.14 – 3.74 (2 × $C\underline{H}_2CH_2CO_2$, 4 × ring CH_3, 2 × $CO_2C\underline{H}_3$), 4.18 (q, $C\underline{H}_2CH_3$), 4.36 ($C\underline{H}_2CH_2CO_2$), 6.05 (q, 1H, $CH_3C\underline{H}OCH_2$), 9.27, 9,89, 9.98, 10.62 (4 × meso H); FAB m.s. *m/z* 767 (M^+, base peak), 593.

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* Tentative assignment.

4.3.4 Pentane-1,5-diether-linked diporphyrin dimer (90)

The bromoethyl compound (77) was prepared as outlined in Section 4.3.1 from 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (30 mg, 0.05 mmol) and saturated HBr/dichloromethane (3 ml). After evaporation of most of the HBr and solvent, the gummy residue was dissolved in dry dichloromethane and added dropwise with stirring to a solution of the α-isomer of the 1,5-pentanediol derivative of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (87) (27 mg, 0.04 mmol), from Section 4.3.2, in dry dichloromethane (1 ml) at O°C. The mixture was stirred at O°C for 100 min, then diluted with dichloromethane (20 ml) and poured into water. The mixture was washed with water (2 × 10 ml) and then evaporated to dryness leaving a solid residue which was chromatographed on a silica squat column. The *title dimer* (90) (39 mg, 76%) λ_{max} 400 nm (100%), 499 (8.3%), 533 (6.5%), 568 (5%), 621 (0.133, 3%); ^{13}C NMR, δ ppm 11.53, 11.71 (ring CH_3), 17.47 ($CH_2\underline{C}H_3$), 19.65 ($\underline{C}H_2CH_3$), 21.83, 21.93 ($\underline{C}H_2CH_2CO_2$), 23.49, 23.30 (bridge $\underline{C}H_2$), 25.38 ($CH_3\underline{C}HOCH_2$), 37.00 ($\underline{C}H_2CO_2$), 51.64 ($CO_2\underline{C}H_3$), 69.32 ($O\underline{C}H_2CH_2$), 73.32 ($CH_3\underline{C}HOCH_2$), 96.10, 96.21, 96.41, 96.74, 98.59 (meso C), 134.97, 135.56, 137.34, 139.12, 141.06, 141.46 (C_β), 143 – 148 (br, C_α), 173.54 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm -3.87 (br, 4H, 4 × pyrrole H), 1.64 – 1.79 (m, 10H, 2 × $CH_2C\underline{H}_3$, 2 × bridge $C\underline{H}_2$), 2.08, 2.16 (2 × d, 2 × $C\underline{H}_3CHOCH_2$), 3.23 (br, 4 × $C\underline{H}_2CH_2CO_2$), 3.34, 3.39 – 3.72 (8 × ring $C\underline{H}_3$, 4 × $CO_2C\underline{H}_3$, 2 x $OC\underline{H}_2CH_2$), 5.90 – 5.99 (m, 2H, 2 × $CH_3C\underline{H}OCH_2$), 3.83 – 3.90 (m, 2 × $C\underline{H}_2CH_3$), 4.31 (br,

8H, 4 × $C\underline{H}_2CH_2CO_2$), 9.92, 9.95, 9.96, 9.99, 10.00, 10.47, 10.50, 10.51 (8H, all s, 8 × meso H); FAB m.s. *m/z* 1290, 593 (equal intensity); was eluted with 2 – 4% acetone/dichloromethane and unreacted 1,5-pentanediol monomer derivative (87) (*ca.* 10 mg), with 4% acetone/dichloromethane.

4.3.5 Decane-1,10-diether-linked diporphyrin dimer

(a) From the α-isomer of the 1,10-decanediol derivative (88)

The bromoethyl porphyrin was prepared by the standard procedure (Section 4.3.1) from 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (50 mg, 0.084 mmol) and saturated HBr/dichloromethane, and was redissolved (after evaporation of the excess HBr/dichloromethane) in dry dichloromethane (2 ml). The resulting solution was added dropwise with stirring to a solution of the α-isomer of the 1,10-decanediol derivative of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (88) (60 mg, 0.078 mmol) from Section 4.3.3 in dichloromethane (2 ml) at 0°C. The mixture was stirred in the dark at room temperature for 48 h, then diluted with dichloromethane (20 ml) and washed with water. The organic phase was separated and evaporated to dryness. The residue was chromatographed on a silica squat column. Initial elution with 0.5% acetone/dichloromethane afforded starting 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester. The *decane-1,10-diether-linked dimer* (91) (56 mg, 53%), which had λ_{max} (rel. abs.) 400 nm (100%), 500 (9.2%), 532 (6.9%), 567 (5.5%), 621 (3.7%); ^{13}C NMR, δ ppm 11.57, 11.71 (ring $\underline{C}H_3$), 17.54 ($CH_2\underline{C}H_3$), 19.75

($\underline{C}H_2CH_3$), 21.93 ($\underline{C}H_2CH_2CO_2$), 25.50 ($\underline{C}H_3CHOCH_2$-), 26.36, 30.28 (bridge $\underline{C}H_2$), 37.00 ($\underline{C}H_2CO_2$), 51.64 ($CO_2\underline{C}H_3$), 69.50 ($O\underline{C}H_2CH_2$), 73.31 ($CH_3\underline{C}HOCH_2$), 96.11, 96.27, 96.53, 96.74, 98.68 (meso C), 135.04, 135.60, 137.37, 139.11, 141.14, 141.51 (C_β), 145-150 (br, C_α), 173.57 ($\underline{C}O_2CH_3$); ^{1}H NMR, δ ppm -3.80 (4 × pyrrole H), 1.79 (br, 2 × $CH_2C\underline{H}_3$), 2.20 (br, 2 × $C\underline{H}_3CHOCH_2$), 3.24 (br, 4 × $C\underline{H}_2CO_2CH_3$), 3.5 – 3.7 (8 × ring $C\underline{H}_3$, 4 × $CO_2C\underline{H}_3$, 2 × $OC\underline{H}_2CH_2$), 4.01 (m, 2 × $C\underline{H}_2CH_3$), 4.35 (br, 4 × $C\underline{H}_2CH_2CO_2$), 6.02 (m, 2 × $CH_3C\underline{H}OCH_2$), 9.94, 9.97, 9.98, 10.00, 10.02, 10.04, 10.55 (meso H); FAB m.s., *m/z* 1361 (M^+, base peak), 767, 593; was eluted as a broad band with 1% acetone/dichloromethane.

(b) From the β-isomer of the 1,10-decanediol derivative (88)

The title dimer (91) was prepared as above from 3-ethyl-8-vinyl-deuteroporphyrin dimethyl ester (90 mg, 0.15 mmol) and the β-isomer of the 1,10-decanediol derivative of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (88) (82 mg, 0.107 mmol) according to the procedure described in Section 4.3.3. Chromatography (silica squat column) afforded the *title dimer* (91) (83 mg, 57%), λ_{max}(rel. abs.) 401 nm (100%), 499 (9.3%), 533 (7.1%), 567.4 (5.6%), 622 (3.6%); ^{13}C NMR, δ ppm 11.27, 11.59 (ring $\underline{C}H_3$), 17.54 ($CH_2\underline{C}H_3$), 19.68 ($\underline{C}H_2CH_3$), 21.87 ($\underline{C}H_2CH_2CO_2$), 25.50 ($\underline{C}H_3CHOCH_2$), 26.34, 30.24 (bridge $\underline{C}H_2$), 36.91 ($\underline{C}H_2CO_2CH_3$), 51.64 ($CO_2\underline{C}H_3$), 69.49 ($O\underline{C}H_2CH_2$), 73.31 ($CH_3\underline{C}HOCH_2$), 96.04, 96.21, 96.35, 96.47, 96.68, 98.65 (meso C), 134.31, 134.98, 135.48, 135.99, 137.33, 139.04, 141.16, 141.55 (C_β), 144 – 149 (br, C_α), 173.59, 173.47 ($\underline{C}O_2CH_3$); ^{1}H NMR, δ ppm -3.86 (br, 4 ×

pyrrole H), 1.77 (m, 2 × $CH_2C\underline{H}_3$), 2.21 (d, 2 × $C\underline{H}_3CHOCH_2$), 3.21 (br, 2 × $C\underline{H}_2CO_2CH_3$), 3.44 – 3.70 (8 × ring $C\underline{H}_3$, 4 × $CO_2C\underline{H}_3$, 2 × $OC\underline{H}_2CH_2$), 3.95 (m, 2 × $C\underline{H}_2CH_3$), 4.29 (br, 2 × $C\underline{H}_2CH_2CO_2$), 6.02 (br, 2 × $CH_3C\underline{H}OCH_2$), 9.87, 9.93, 9.95, 9.96, 9.97, 10.01, 10.55, 10.56, 10.56 (meso H); FAB m.s. *m/z* 1361 (s), 767 (s), 593 (as in Section 4.3.5(a)), upon elution with 1.25% acetone/ dichloromethane.

4.3.6 Propane-1,3-diether-linked dimer (89)

The product of the hydrobromination of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (40 mg, 0.067 mmol) was reacted with the β-isomer of the 1,3-propanediol derivative (86) (35 mg, 0.052 mmol) in the manner described for the preparation of the other diether-linked dimers (Sections 4.3.5 and 4.3.5). Chromatography of the crude product on silica, upon elution with 2% acetone/dichloromethane, afforded the *title dimer* (89), which gave λ_{max} (rel. abs.) 399 nm (100%), 499 (8.1%), 535 (6.4%), 566 (5.47%), 622 (3.2%); ^{13}C NMR, δ ppm 11.40 (ring $\underline{C}H_3$), 17.42, 17.54 ($CH_2\underline{C}H_3$), 19.74 ($\underline{C}H_2CH_3$), 21.93 ($\underline{C}H_2CH_2CO_2$), 25.11 ($\underline{C}H_3CHOCH_2$-), 31.13 (bridge $\underline{C}H_2$), 36.38, 37.01 ($\underline{C}H_2CO_2$), 51.64 ($CO_2\underline{C}H_3$), 66.64 ($O\underline{C}H_2CH_2$), 73.31 ($CH_3\underline{C}HOCH_2$), 96 – 96.5, 98.56 (meso C), 134 – 152 (br, $C_\alpha + C_\beta$), 173.60 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm -3.85 (br, 4 × pyrrole H), 1.65 – 1.90 (br, 2 × $CH_2C\underline{H}_3$, bridge $OCH_2C\underline{H}_2CH_2O$), 2.15 – 2.30 (br, 2 × $C\underline{H}_3CHOCH_2$), 3.1 – 3.7 (4 × $C\underline{H}_2CO_2CH_3$, 8 × ring $C\underline{H}_3$, 4 × $CO_2C\underline{H}_3$), 3.8 – 4.1 (br, 2 × $C\underline{H}_2CH_3$, 2 × $OC\underline{H}_2CH_2$), 4.2 – 4.5 (br, 4 × $C\underline{H}_2CH_2CO_2$), 5.76 (m, 2 × $CH_3C\underline{H}OCH_2$-), 9.9 – 10.1, 10.45 (8H, 8 × meso H); FAB m.s. *m/z* 1262 (M^+, base peak), 668, 593.

4.3.7 A diporphyrin dimer (92) linked by an ether- and ester- containing bridge - a preliminary study

To malonyl dichloride (*ca.* 60 mg) was added dry pyridine (3 drops) with stirring at 0°C. To the resulting paste was added the α-isomer of the 1,5-pentanediol derivative of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (87) (*ca.* 10 mg) (from Section 4.3.4) in dry dichloromethane (2 ml), with stirring at 0°C. The mixture was stirred at room temperature for 4 h. Dichloromethane (20 ml) was added to the reaction mixture which was then poured into water (50 ml). The mixture was neutralized with dilute sodium hydroxide and extracted with dichloromethane. The combined organic extracts were washed with water and evaporated to dryness leaving a solid residue which was chromatographed by preparative TLC on silica developed in 5% methanol/dichloromethane. This afforded two major bands: Band 1 (R_F 0.36) which contained the *monomer* (93) gave FAB m.s. *m/z* 783 (M^+); MIKE spectrum of *m/z* 783 gave 739; Band 2 (R_F 0.72), containing the *desired dimer* (92), gave FAB m.s. *m/z* 1462 (M^+); MIKE spectrum of *m/z* 1462 gave *m/z* 895 (s), 867 (s), 1289 (w), 1155 (w), 851 (w), 765 (w), 592 (w).

4.3.8 1,3-Diaminopropane derivative of 3-ethyl-8-vinyldeuteroporplyrin dimethyl ester (94)

The bromoethyl porphyrin which was prepared in the standard manner (see Section 4.3.1) from 3-ethyl-8-vinyl-deuteroporphyrin dimethyl ester (76) (90 mg, 0.15 mmol) was

dissolved in dichloromethane and added dropwise with stirring to 1,3-diaminopropane (0.12 g, 1.62 mmol, ~10 equivalents) in dichloromethane (1 ml) at 0°C. Stirring was continued at 0°C for 9 min, then the mixture was diluted with dichloromethane (10 ml) and poured into water. The organic phase was collected and the remaining aqueous phase was extracted with dichloromethane; the extracts were combined, washed with water, then evaporated to dryness. The crude product was separated by flash chromatography on Kieselguhr. The first fraction, containing 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (15 mg), was eluted with dichloromethane. The 1,3-diaminopropane-linked dimer (95) (10 mg) (see Section 4.3.8) was eluted with 2% methanol/dichloromethane and finally, elution with 10 – 50% methanol/dichloromethane afforded the *1,3-diaminopropane derivative* (94) (76 mg, 76%), λ_{max}(rel. abs.) 401 nm (100%), 501 (9.1%), 535 (6.5%), 569 (4.7%), 622 (2.7%);. ^{13}C NMR, δ ppm 11.36 (ring $\underline{C}H_3$), 14.29 (possibly $NHCH_2\underline{C}H_2CH_2NH$-), 17.52 ($CH_2\underline{C}H_3$), 19.73 ($\underline{C}H_2CH_3$), 21.90 ($\underline{C}H_2CH_2CO_2CH_3$), 23.23 ($\underline{C}H_3CHNH$-), 32.61 ($NH\underline{C}H_2CH_2$-), 36.96 ($CH_2\underline{C}H_2CO_2CH_3$), 41.09, 41.81 ($CH_3\underline{C}HNH$-), 51.69 ($CO_2\underline{C}H_3$), 60.18 ($CH_2\underline{C}H_2NH_2$), 96.15 (br, meso C), 134.0 – 141.7 (br, C_β), 144.0 - 152.0 (br, C_α), 173.46 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm (all peaks were either broad or in clusters; the following chemical shifts correspond to the tallest peak in a cluster) -4.22, -4.04, -3.74 (br, pyrrole H), 1.87 ($CH_2C\underline{H}_3$), 2.30 ($C\underline{H}_3CHNH$-), 3.26 (br, $C\underline{H}_2CO_2CH_3$), 3.4 – 3.8 (ring $C\underline{H}_3$, $CO_2C\underline{H}_3$), 4.11 (q, $C\underline{H}_2CH_3$), 4.30 (br, $C\underline{H}_2CH_2CO_2CH_3$), 5.8 (br, $CH_3C\underline{H}NH$-), 9.97, 10.04, 10.09 (br, meso

H); FAB m.s. *m/z* 667 (M^+), 593 (base peak) (intensity ratio was 8 : 21).

4.3.9 1,3-Diaminopropane-linked diporphyrin dimer (95)

The bromoethyl porphyrin, which was prepared by the standard procedure from 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (87 mg), was redissolved in dichloromethane and added dropwise with stirring to the crude 1,3-diaminopropane derivative (94) (91 mg) (from Section 4.3.8) in dichloromethane (1 ml) at 0°C. The reaction mixture was stirred in the dark at room temperature for 22 h, then diluted with dichloromethane (20 ml), washed with water (2 × 20 ml) and evaporated to dryness. The crude product was chromotographed on Kieselguhr in the manner described in Section 4.3.8. 3-Ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (60 mg), 3-ethyl-8(1-hydroxyethyl)deuteroporphyrin dimethyl ester (80) and the ether-linked diethyl-terminated dimer (79) (combined, 66 mg) were eluted with dichloromethane. The *dimer* (95) (55 mg), which gave λ_{max}(rel. abs.) 402 nm (100%), 501 (7.2%), 539 (6.4%), 568 (4.0%), 622 (1.5%); ^{13}C NMR, δ ppm 11.66 (ring $\underline{C}H_3$), 14.29 (possibly $NHCH_2\underline{C}H_2CH_2NH$-), 17.54 ($CH_2\underline{C}H_3$), 19.72 ($\underline{C}H_2CH_3$), 21.86 ($\underline{C}H_2CH_2CO_2CH_3$), 23.88 ($\underline{C}H_3CHNH$-), 31.93 ($NH\underline{C}H_2CH_2$-), 36.95 ($CH_2\underline{C}H_2CO_2CH_3$), 51.70 ($CO_2\underline{C}H_3$), 52.86 ($CH_3\underline{C}HNH$-), 96.44 (br, meso C), 132 – 142 (br, C_β), 145 - 154 (br, C_α), 173.59 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm (all peaks were broad) the following chemical shifts correspond to the tallest peak in the

broad signal(s): -4.42, -4.18, -3.89 (br, pyrrole H), 1.8 – 2.4 ($CH_2C\underline{H}_3$, $C\underline{H}_3CHNH$-), 3.24 (br, $C\underline{H}_2CO_2CH_3$), 3.4 – 3.8 (ring $C\underline{H}_3$, $CO_2C\underline{H}_3$), 4.05 (br, $C\underline{H}_2CH_3$), 4.2 – 4,5 (br, $C\underline{H}_2CH_2CO_2CH_3$), 5.9 (br, $CH_3C\underline{H}NH$-), 9.7 – 10.1 (br, meso H); FAB m.s., *m/z* 1260 (M^+, m), 667 (w), 593 (base peak); was eluted with 2% methanol/ dichloromethane and unreacted 1,3-diaminopropane monomer derivative ( ) (40 mg) (identical with the authentic sample from Section 4.3.8) was eluted with 10-50% methanol/dichloromethane.

4.3.10 Attempted synthesis of a diporphyrin dimer (97) linked by an amine-and amide-containing bridge

Dry pyridine (1 drop) was added with stirring to a solution of adipoyl chloride (10 mg, 0.055 mmol) in dry dichloromethane (1 ml) at 0°C. To the resulting suspension was added a solution of the 1,3-diaminopropane monomer derivative (94) (from Section 4.3.7) (15 mg, 0.022 mmol) in dry dichloromethane dropwise with stirring at 0°C. The mixture was stirred in darkness at room temperature for 28 h. Dichloromethane (20 ml) was added to the mixture which was then poured into water, neutralized and extracted with more dichloromethane. The combined organic extracts were washed with water and evaporated to dryness, leaving a solid residue which gave FAB m.s. *m/z* 855 (w), 796 (w), 593 (base peak) but no starting porphyrin (*m/z* 667).

The crude product was dissolved in concentrated sulphuric acid/methanol (5%, 10 ml) and stirred in the dark at room temperature for 28 h. The reaction mixture was diluted with water, neutralized and extracted with dichloromethane. The extracts were combined, washed with water and evaporated to dryness leaving a solid residue which gave TLC (silica, 10% methanol/dichloromethane): 5 components, at R_F 0.38, 0.45-0.66, 0.73, 0.81, 0.91; FAB m.s. *m/z* ~1530 (w), 952 (m), 810 (m), 753 (m), 609 (m), 593 (base peak).

4.3.11 Propanolamine derivative of 3-ethyl-8-vinyl-deuteroporphyrin dimethyl ester (104a)

(a) Synthesis

As before (see Section 4.3.1), the 1-bromoethyl porphyrin was prepared from 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (76) (94 mg, 0.16 mmol) using saturated HBr/dichloromethane (10 ml). After evaporation of excess HBr/dichloromethane the crude 1-bromoethyl porphyrin (77) was redissolved in dichloromethane (1.5 ml) and added dropwise with stirring to a solution of propanolamine (250 mg, 3.3 mmol) in dichloromethane at 0°C. The reaction mixture was stirred at 0°C for 10 min after the addition, then dichloromethane (20 ml) was added and the mixture was poured into water, neutralized and extracted with more dichloromethane. The combined organic extracts were washed with water and evaporated to dryness leaving a solid residue which was chromatographed on deactivated silica (silica gel which had been soaked in 5%

methanol/dichloromethane for 2 h, then packed into a squat column and the column was flushed with dichloromethane before use). The *title porphyrin* (104a) (95 mg, 89%), which was eluted with 4-8% methanol/dichloromethane, gave λ_{max}(rel. abs.) 401 nm (100%), 501 (7.5%), 537 (6.3%), 568 (4.1%), 621 (1.9%); ^{13}C NMR, δ ppm 11.70 (ring $\underline{C}H_3$), 17.53 ($CH_2\underline{C}H_3$), 19.73 ($\underline{C}H_2CH_3$), 21.87 ($\underline{C}H_2CH_2CO_2CH_3$), 23.04 ($\underline{C}H_3CHNH$-), 30.00 ($NH\underline{C}H_2CH_2$-), 36.95 ($CH_2\underline{C}H_2CO_2CH_3$), 47.75 ($CH_3\underline{C}HNH$-), 51.65, 52.87 ($CO_2\underline{C}H_3$), 62.64 ($\underline{C}H_2OH$), ($CH_2\underline{C}H_2NH_2$), 96.41 – 97.41 (br, meso C), 138 – 141 (br, C_β), 145 – 150 (br, C_α), 173.41 ($\underline{C}O_2CH_3$); ^1H NMR, δ ppm -3.84 (br, pyrrole H), 1.69 ($C\underline{H}_2CH_2OH$), 1.85 (t, 3H, $CH_2C\underline{H}_3$), 2.05 ($NHC\underline{H}_2CH_2$), 2.43 (d, 3H, $C\underline{H}_3CHNH$), 3.05 (br, 2H, $C\underline{H}_2OH$), 3.26 (2 × t, 4H, $C\underline{H}_2CO_2CH_3$), 3.5 – 3.9 (18H, 4 × ring $C\underline{H}_3$, 2 × $CO_2C\underline{H}_3$), 4.07 (q, 2H, $C\underline{H}_2CH_3$), 4.33 (m, 4H, 2 × $C\underline{H}_2CH_2CO_2CH_3$), 5.8 (br, 1H, $CH_3C\underline{H}NH$-), 10.00, 10.02, 10.04, 10.05, 10.12, 10.34 (4H, meso H); FAB m.s. *m/z* 668 (M^+), 593 (base peak); MIKE spectrum of *m/z* 668 gave 653 (w), 593 (s).

(b) Acidic hydrolysis of the propanolamine derivative of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (104) — determination of the functional groups on the benzylic position

The propanolamine derivative (104) (5 mg) from Section 4.3.11(a) was dissolved in a mixture of 1.0 M hydrochloric acid/THF (2 : 1, 3 ml).; The resulting solution was refluxed for 90 min. After cooling to room temperature the mixture was diluted with water and extracted with THF/dichloromethane. The organic phase was washed with water and evaporated to dryness. The residue was dissolved in a mixture of methanol/trimethyl orthoformate/concentrated sulphuric acid (5:5:1, 11 ml) and stirred at room temperature for 2 h. Then the mixture was diluted with water, neutralized and extracted with dichloromethane, washed with water and evaporated to dryness leaving a solid residue which was similar (TLC, FAB m.s. *m/z* 593 (base peak), 669) to the non-hydrolysed material from Section 4.3.11(a).

4.3.12 Oxidation of the primary alcohol on the porphyrin sidechain

(a) Using neutral permanganate[28]

The β-isomer of the 1,5-pentanediol derivative (87) of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (see Section 4.3.2) (*ca.* 5 mg) was dissolved in distilled THF (4 ml). To this was added aqueous potassium permanganate solution[28] (10%, 2 ml) and the mixture was heated under reflux for 90 min. After

the reaction mixture had cooled to room temperature, methanol was added to quench the excess permanganate. Dichloromethane was then added and the organic phase was separated and washed with water (3 × 30 ml). Evaporation of the solvent afforded a solid residue which (by TLC) was mainly starting porphyrin. This crude material was redissolved in THF (4 ml), aqueous potassium permanganate (10%, 2 ml) was added and the mixture was heated under reflux for 4.5 h. After work-up (as above) a solid residue was obtained which was similar (by TLC, FAB m.s.) to the starting porphyrin (as above).

(b) Jones oxidation[29]

To the β-isomer of the 1,5-pentanediol derivative (87) (from Section 4.3.4) (5 mg) dissolved in THF (1 ml) was added Jones reagent[29] (10 drops), with stirring. The mixture was stirred at room temperature for 5 min, then poured into aqueous sodium hydroxide (10%, 10 ml). The pH of the solution was adjusted to 5 and the mixture was extracted with dichloromethane. The organic phases were combined, washed with water and evaporated to dryness. The residue (TLC, silica, 5% methanol/dichloromethane : 2 spots at R_F 0.54, 0.27) contained the *acid derivative* (105), which had FAB m.s. *m/z* 711 (M^+, base peak), 697 and TLC R_F 0.3; and the starting 1,5-pentanediol derivative (87), R_F 0.5.

(c) Jones oxidation the 1,3-propanediol derivative (86)

The α-isomer of the 1,3-propanediol derivative of 3-ethyl-8-vinyldeuteroporphyrin dimethyl ester (86) (5 mg) was dissolved in THF (1 ml). To this was added Jones reagent[29] (10 drops) and the resulting mixture was stirred at room temperature for 7 min. The reaction mixture was poured into aqueous sodium hydroxide solution (10 ml) and dilute hydrochloric acid was added to adjust the pH of the solution to pH 4. This was then extracted with THF/dichloromethane; the combined organic extracts were washed with water and evaporated to dryness. The residue, on silica TLC (5% methanol/dichloromethane) : one spot (R_F 0.26); gave FAB m.s. *m/z* 684.

4.3.13 Jones oxidation of the propanolamine derivative (104a) of 3-ethyl-8-vinyldeutero-porphyrin dimethyl ester

The propanolamine derivative (104a) from Section 4.3.11 (78 mg) was dissolved in distilled THF (4 ml). To this was added Jones reagent (30 drops) with stirring. The mixture was stirred at room temperature for 7 min, then poured into aqueous sodium hydroxide solution, neutralized and extracted with THF/dichloromethane. The combined organic phases were washed with water and evaporated to dryness. The residue, silica TLC (10% methanol/dichloromethane) gave R_F 0.4 (starting porphyrin) and R_F 0.3 (*β-amino acid derivative* (106), see later); FAB m.s. *m/z* 682 (M^+, *β-amino acid derivative* (106), see later),

668 (M^+, starting porphyrin), 593 (base peak), was redissolved in THF/dichloromethane/ acetone (4:2:1, 7 ml). Jones reagent (18 drops) was added with stirring. The mixture was stirred for 5 min at room temperature, then poured into aqueous sodium hydroxide, neutralized and extracted as outlined above. The crude product was only sparingly soluble in dichloromethane, so it was filtered and the filtrate was chromatographed by preparative TLC on silica developed in 10% methanol/dichloromethane. Two major bands were separated : band 1 (R_F 0.3) contained the desired *β-amino acid derivative* (106) (10 mg), λ_{max}(rel. abs.) 401 nm (100%), 499 (8.7%), 536 (6.6%), 568 (4.3%), 622 (2.3%); ^{13}C NMR, δ ppm 11.33 – 11.78 (ring $\underline{C}H_3$), 17.22, 17.48 ($CH_2\underline{C}H_3$), 19.40, 19.68 ($\underline{C}H_2CH_3$), 21.49, 21.93 ($\underline{C}H_2CH_2CO_2CH_3$), 25.64 ($\underline{C}H_3CHNH$-), 32.37 ($NH\underline{C}H_2CH_2CO_2H$), 36.56 ($NHCH_2\underline{C}H_2CO_2H$), 36.83, 36.96 ($\underline{C}H_2CO_2CH_3$), 43.78 ($CH_3\underline{C}HNH$-), 51.69 ($CO_2\underline{C}H_3$), 96.48, 96.74, 97.13 (meso C), 135.75, 136.77, 138.60, 140.45, 141.36 (C_β), 145.33 – 152.33 (br, C_α), 173.40, 173.60 ($\underline{C}O_2CH_3$), 176.76 ($\underline{C}O_2H$); ^1H NMR, δ ppm -3.89 (2H, pyrrole H), 1.76 ($NHC\underline{H}_2CH_2CO_2H$), 1.85 ($CH_2C\underline{H}_3$), 2.44 ($C\underline{H}_3CHNH$-), 3.22 – 3.27 (2 × $C\underline{H}_2CO_2CH_3$, $C\underline{H}_2CO_2H$), 3.4 – 3.8 (4 × ring $C\underline{H}_3$, 2 × $CO_2C\underline{H}_3$), 3.98 (br, $C\underline{H}_2CH_3$), 4.34 (br, $C\underline{H}_2CH_2CO_2CH_3$), 5.98 (br, $CH_3C\underline{H}NH$-), 9.85, 9.89, 10.02, 10.18 (4H, meso H); FAB m.s., *m/z* 682, 593 (base peak); and Band 2 contained the starting porphyrin (104a) (15 mg). The residue from the filtration was dissolved in a mixture of trimethyl orthoformate/methanol/concentrated sulphuric acid (5:5:1, 11 ml) and stirred in darkness at room temperature for 1 h. Water

(30 ml) was added, the mixture was neutralized and extracted with THF/dichloromethane. The combined organic extracts were washed with water and evaporated to dryness, leaving a solid residue which was a mixture of the triester (107), FAB m.s. *m/z* 696 (M^+), 593 (base peak), and the propanolamine derivative (104a) which was identical (TLC, FAB) to the material from Section 4.3.11.

4.3.14 Attempted synthesis of a diporphyrin dimer (110) linked by an amine- and amide-containing bridge, using the β-amino acid substituted porphyrin (106)

The β-amino acid derivative (106) from Section 4.3.13 (10 mg) was dissolved in dry dichloromethane (3 ml). To this was added oxalyl chloride (10 drops) and the mixture was refluxed for 15 min, then evaporated to dryness under reduced pressure. The residue was redissolved in dry dichloromethane (2 ml) and pyridine (2 drops) was added with stirring at 0°C. To this was added a solution of 1,3-diaminopropane (10 mg) in dry dichloromethane (1 ml) dropwise with stirring at 0°C. The mixture was stirred in the dark at room temperature, under an atmosphere of nitrogen for 24 h. The reaction mixture was diluted with dichloro-methane (*ca.* 20 ml), poured into water (25 ml), neutralized and extracted with THF/dichloromethane. The organic extracts were combined, washed with water and evaporated to dryness, leaving a solid residue whose TLC (silica, 10% methanol/dichloromethane) showed the main portion (~80%)

of the material on the baseline; FAB m.s. of the crude product showed *m/z* ~1327 (w), 1300 (w), 1158 (w), 898 (w), 866 (w), 810 (m), 792 (w), ~736 (w), 680 (w), 593 (base peak); MIKE spectrum of 738 gave *m/z* 721 (m), 710 (m), 593 (s), 567 (w).

The crude material was dissolved in a mixture of methanol/trimethyl orthoformate/concentrated sulphuric acid (5:5:1; 5.5 ml) and stirred at room temperature for 1 h. Water (*ca.* 30 ml) was added to the mixture which was then neutralized and extracted with dichloromethane. The organic extracts were combined, washed with water and evaporated to dryness leaving a solid residue which gave by silica TLC (10% methanol/dichloromethane) : 4 major but poorly resolved components, R_F 0.8, 0.5, 0.5 – 0.3, 0.1 – 0; the residue gave FAB m.s. *m/z* ~1374 (w), 1316 (w), 1258 (w), 750 (m), 694 (w), 593 (base peak).

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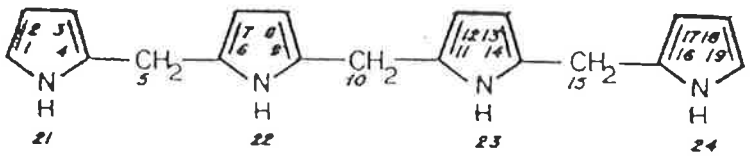
APPENDIX

NOMENCLATURE§

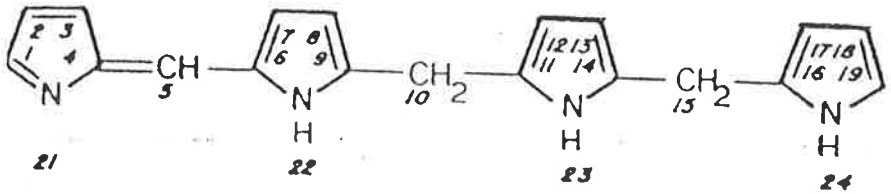
A Linear Tetrapyrroles

The fundamental linear (or open-chain) tetrapyrrole system is the "bilane" (Fig. 1[i]). Other oxidized forms are shown in Fig. 1[ii] – [v]. The location of the double bond(s) and/or oxygen in the oxidized bilanes is indicated by the letters a, b or c corresponding to the bridge positions 5, 10 and 15, respectively.

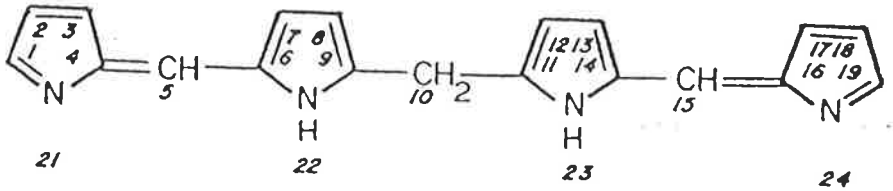
§ Based on the 1986 IUPAC recommendations (ref. 49, Chapter 2), unless otherwise indicated.



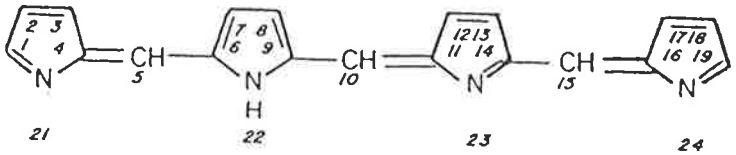
[i] Bilane



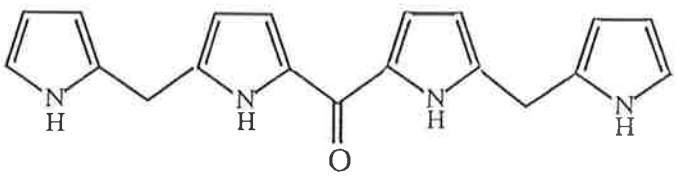
[ii] Bilene-*a*



[iii] Biladiene-*ac*



[iv] Bilin (Bilatriene-*abc*)



[v] oxobilane-*b* †

Fig. 1

† reference 4d, Chapter 2.

B Porphyrins

(i) Numbering

Semisystemic nomenclature was used throughout this thesis although, in a few instances, systematic nomenclature was also adopted, particularly in the Experimental Section of Chapter 2 (Section 2.3). In the Semisystematic Nomenclature, the porphyrin ring is numbered according to the IUPAC 1— 24 numbering scheme (Fig. 2) but the numbering of the point of attachment of substituents (locants) is based on that of the trivial names in the Fischer nomenclature, which is different from that of the IUPAC system on which systematic nomenclature is based. In the semisystematic nomenclature, the propionic acid groups ($CH_2CH_2CO_2H$) are usually placed on the pyrroles in lower the half of the molecule (positions 12, 13, 17 and 18); while in the systematic nomenclature these groups, being the principal group named as suffix are placed in the lowest locants (starting from position 2). So for example, with the naturally occurring protoporphyrin IX (see later for Type nomenclature), the propionic acid groups are in the 13 and 17 positions according to the semisystematic nomenclature, but they are in the 2 and 18 positions when numbered according to the semisystematic nomenclature.

In the discussions of NMR spectra, the term "meso-position" is used to refer collectively to positions 5, 10, 15 and 20. Positions 1, 4, 6, 9, 11, 14, 16 and 19 are referred to as the "α-

positions" while positions 2, 3, 7, 8, 12, 13, 17, and 18 are the "β-positions".

(ii) Type isomers

Although the use of Roman numeral type notation to differentiate between the regioisomers of the porphyrins is discouraged for systems with more than four possible regioisomers, it is more convenient in this thesis to retain their use, particularly for Chapter 2. The fifteen regioisomers of haematoporphyrin are listed in Table 1. The notation is also applicable to derivatives of haematoporphyrin such as protoporphyrin (for $-CH(CH_3)OH$ read $-CH=CH_2$), deuteroporphyrin (for $-CH(CH_3)OH$ read H) and derivatives thereof. The naturally occurring isomer of haematoporphyrin is haematoporphyrin IX.

Table 1 The fifteen regioisomers of haematoporphyrin (locants are numbered according to the semisystematic nomenclature)

HP isomers	Substituents at positions							
	2	3	7	8	12	13	17	18
I	Me	CHMeOH	Me	CHMeOH	Me	Cet	Me	Cet
II	Me	CHMeOH	Me	Cet	Me	CHMeOH	Me	Cet
III	Me	CHMeOH	CHMeOH	Me	Me	Cet	Cet	Me
IV	CHMeOH	Me	Me	CHMeOH	Cet	Me	Me	Cet
V	CHMeOH	Me	Me	Cet	CHMeOH	Me	Me	Cet
VI	Me	CHMeOH	CHMeOH	Me	Me	Cet	Me	Cet
VII	CHMeOH	Me	Me	Cet	Me	CHMeOH	Me	Cet
VIII	CHMeOH	Me	Me	CHMeOH	Cet	Me	Cet	Me
IX	Me	CHMeOH	Me	CHMeOH	Me	Cet	Cet	Me
X	CHMeOH	Me	Cet	Me	CHMeOH	Me	Me	Cet
XI	Me	CHMeOH	Me	CHMeOH	Cet	Me	Me	Cet
XII	Me	CHMeOH	CHMeOH	Me	Cet	Me	Me	Cet
XIII	CHMeOH	Me	Me	CHMeOH	Me	Cet	Cet	Me
XIV	Me	CHMeOH	Me	CHMeOH	Cet	Me	Cet	Me
XV	CHMeOH	Me	Cet	Me	Me	CHMeOH	Me	Cet

Abbreviations : Me, CH_3 ; Cet, $CH_2CH_2CO_2H$.

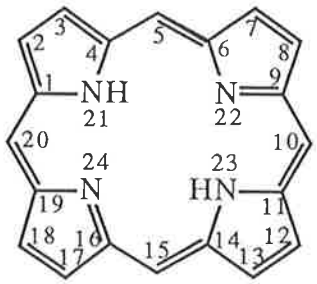


Fig. 2 Porphyrin - IUPAC numbering scheme

(iii) New compounds

In Chapters 1 and 2 the word "derivative" is used in a different context from the usual generic sense. When used in the term "haematoporphyrin derivative" it is meant to refer specifically to the material obtained from the Lipson procedure* for the preparation of the anticancer drug HPD.

There are at present very few (if any) rules concerning the naming of porphyrin dimers and porphyrins with complicated sidechains. Attempts to use existing IUPAC rules to name these compounds generally produce clumsy results. Also, in most cases the compounds are mixtures of regio- and diastereoisomers, therefore it is not accurate to ascribe one name to the mixture. The common practice has been to name only one regioisomer while implying that the other regio- and diastereoisomer(s) are also present in the mixture. Due to the ambiguity of this method and the aforementioned limitations of the IUPAC rules, the new porphyrins reported in this thesis, particularly those in Chapters 3 and 4, have not been named, but have instead been referred to by number and, where possible, by general descriptions like "the diethyl-terminated amino-linked dimer" for (70) (Chapter 3, Section 3.2.5) or "the propanolamine derivative" for (104a) (Chapter 4, Section 4.2.6). In these cases the word "derivative" is used in the usual generic sense. This situation is currently accepted by the Australian Journal of Chemistry.**

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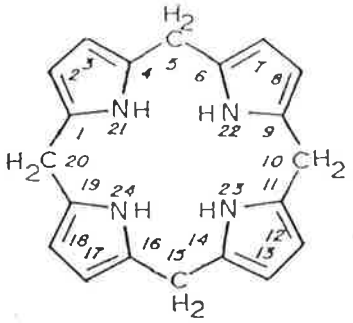
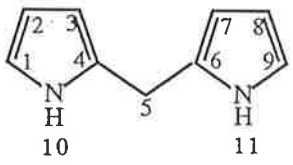
* references 6 and 17, Chapter 1.

** For example, in reference 2b, Chapter 3.

C Other Fundamental Parents

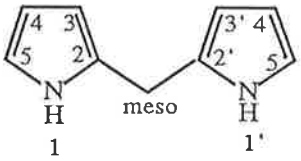


Porphyrinogen



Dipyrromethane

formerly



2,2'-dipyrrylmethane,
dipyrrolylmethane,
pyrromethane

For convenience the use of the Fischer nomenclature for naming the pyrroles and dipyrrolic systems has been retained in this thesis.