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# The Photodynamic Therapy (PDT) Anticancer Activity of a Range of Porphyrin Dimers and Related Compounds Derived from Hematoporphyrin

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The synthesis of diporphyrins and analogous compounds related to those present in the oligomeric fraction (Photofrin II) obtained from hematoporphyrin derivative (HPD) is described. The photodynamic activity of these compounds, in vivo, varies from inactive to as active as Photofrin II. Factors that are important in determining this anticancer activity of the synthetic compounds are the presence of hydrophobic side chains, as well as the propionic acid side chains of the hematoporphyrin derived materials, and the nature of the linking group between the porphyrins.

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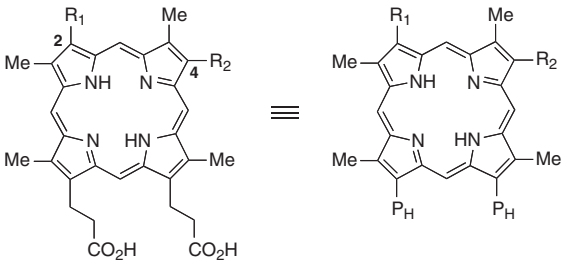
## Introduction

Photodynamic therapy (PDT) is a treatment modality for cancer, which involves the activation of a suitable photosensitizer within a tumour cell with light of an appropriate wavelength, usually delivered by an optical fibre from a laser source.[1] The cytotoxic effects result from activation of cellular apoptosis by reactive oxygen species.[2] In particular, apoptotic death of the cells of the vasculature leads to severe compromise of blood flow and the tumours are destroyed in part by infarct.[3] A successful early photosensitizer was Photofrin II, the oligomeric fraction of hematoporphyrin derivative (HPD) produced in two steps from hematoporphyrin IX, **1** ($R_1 = R_2 = CH_3CH(OH)$, Scheme 1).[4] This active fraction is a complex mixture of porphyrin dimers and oligomers. The dimers and oligomers are linked by the combination of two of the 1-hydroxyethyl side chains of hematoporphyrin to form either an ether-linked dimer (with the linking unit being ─CH(Me)OCH(Me)─) or a carbon chain link formed from the same side chains (with the linking unit being either or both of ─CH(Me)$CH_2$CH(OH)─ and ─CH(Me)CH═CH─).[4,5]




**1** $R_1 = R_2 = CH(OH)Me$
$P_H = CH_2CH_2CO_2H$

**Scheme 1.** Structure of hematoporphyrin **1**.

The separation and determination of the structures of the active components proved to be a difficult and time-consuming task.[4–22] The active component of HPD was first thought[23,24] to be the ether-linked hematoporphyrin dimer **2** or an ester-linked dimer of hematoporphyrin.[10,14,17,25] Further work[11,12,15] showed that several ether-linked dimers were present in HPD and subsequent work[26,27] established the presence of hematoporphyrin-derived dimers linked by a three-carbon chain as well as by these ether-linked dimers. The presence of trimers and higher oligomers of these systems is also likely in the active fraction of HPD.[4,5,16,17,28] The syntheses[15,19–21,26,29–32] of these possible components of HPD were necessary before the overall composition could be clarified. The synthetic work established that the ether-linked dimer **2** was inactive and that the ester-linked hematoporphyrin dimer rapidly hydrolyzed in neutral aqueous solution. It is now generally agreed that the active material is a mixture of ether- and carbon-linked dimers and oligomers, with mixed linkages possible in trimers and higher oligomers.

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## Results and Discussion

### *Synthesis of the Compounds Tested*

When it became apparent that ether-linked hematoporphyrin-derived diporphyrins were likely to be among the major components of the active fraction of HPD, we embarked on a synthetic program to prepare the ether-linked diporphyrins and triporphyrins for comparison with those in Photofrin II, and analogues of these compounds.[29,33] A major aim of this investigation was to examine the effects of changing the side chains around the porphyrin nucleus and the linkage between the porphyrins on PDT activity. It was decided, as a first approach, to prepare the diporphyrins etc. from the much more readily available starting materials such as hematoporphyrin and protoporphyrin. Because of the regioisomerism associated with the functional groups at positions 2 and 4 of these porphyrins (Scheme 1), the resulting diporphyrins can be linked by the 2 and 2′ positions on the two porphyrin rings, or by the 2-position on one ring and the 4-position on the other, or by the 4- and 4′-positions. In addition, the side chains at the 2- and 4-positions of hematoporphyrin both contain a chiral centre, which means that linkages that involve these chiral atoms will lead to the formation of diastereomers as well as regioisomers. Hence ether-linked diporphyrins can be a complex mixture of regio- and diastereo-isomers. In this paper we describe the synthesis of a much wider range of diporphyrins and related compounds and compare their activity with that of our previously prepared compounds. Our results indicate which side chains and linking groups are important for maximizing in vivo PDT activity and form a base from which the individual regioisomers of the most active compounds could be prepared separately for further examination, using the chemistry we have established as part of this initial investigation.

Ether-linked diporphyrins were made[29,33] by reacting an alcohol-containing side chain on one porphyrin with the appropriate alkyl bromide side chain on the second porphyrin. The alkyl bromide side chain was formed from a hydroxymethyl, a 1-hydroxyethyl, or a vinyl group by reaction with a saturated solution of anhydrous HBr in anhydrous dichloromethane. These alkyl bromides were unstable and were used immediately after removal of solvent and excess reagent under high vacuum.

The ester dimers were more difficult to prepare since one porphyrin diacid chloride, with appropriate side chains, had to react with another containing appropriate side chains and protected propionic acid units. The protecting groups had to be capable of being removed without cleaving the ester group that had just been formed. This approach inevitably meant that some trimer was formed as well. Because of the lability of the 1-hydroxyethyl side chains to some of the reagents used they were usually 'protected' as the corresponding acetyl side chain and reduced back at a later stage in the sequence. Our approach to dihematoporphyrin ester was very similar to that used independently by another group.[21] All of the ester-linked dimers proved to be unstable in the aqueous solution necessary for injection into mice, a feature also noted by others.[21] Hence their lack of activity reflects the general lack of activity of porphyrin monomers to which they would be hydrolyzed.

Other diporphyrins were prepared by similar procedures or by chemical modification of side chains. 2(4)-Acetyl-4(2)-vinyldeuteroporphyrin dimethyl ester was reacted with the appropriate dienophile to make the corresponding chlorin adduct which was then reduced to give the hydroxyethyl containing material which was coupled, using the procedure described above, with the HBr adduct of 2(4)-(1-hydroxyethyl)-4(2)-vinyldeuteroporphyrin dimethyl ester to provide the porphyrin–chlorin compounds **50** and **51**. The mono dimethyl acetylenedicarboxylate (DMAD) adduct of protoporphyrin dimethyl ester was converted into a benzoporphyrin adduct using a literature procedure,[34] and then coupled with 2(4)-ethyl-4(2)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester, using the procedures described above, to give **49**. The dichlorin **52** was prepared from the mono DMAD adduct of protoporphyrin dimethyl ester using the above coupling methodology.

These diporphyrins and higher oligomers, in their methyl ester form, were purified by chromatography on silica to remove any residual porphyrin monomers. Purified diporphyrin fractions were checked by thin-layer chromatography (TLC) to confirm the absence of porphyrin monomers. Except in a few cases, where relatively straightforward chromatographic fractionation was possible, the dimers were obtained as a mixture of regio- and diastereomers and were tested as such. With fractionated material, the α fraction refers to material eluting more quickly from the chromatographic adsorbent and the β fraction refers to the more slowly eluting material.

The dimers were characterized by fast atom bombardment mass spectrometric data (usually the molecular ion was also the base peak) and $^1$H NMR spectroscopy, and, in many cases, $^{13}$C NMR spectroscopy. Because many of the products were mixtures of regioisomers their $^1$H NMR spectra often consisted of rather complex sets of peaks and were often more useful for establishing the absence of possible impurities (e.g., vinyl-containing products that could be formed by dehydrobromination of the 1-bromoethyl starting materials). The methyl ester groups present in the coupled porphyrins were hydrolyzed, using basic conditions, to the corresponding carboxylic acids and the products were analyzed by high-performance liquid chromatography (HPLC) (whereby the monomers and dimers etc. are usually well separated) and UV instrumentation before being used for the biological testing.

### *In Vivo Biological Testing Procedure*

Our treatment protocol using an incandescent lamp[35,36] was designed to deliver a sub-lethal treatment to subcutaneous Lewis lung carcinomas in C57B1/6J black mice in order to measure, as an indication of the efficacy of the porphyrin, the rate of recurrence of the tumour as defined by the number of days for 50% of the tumours to recur ($TC_{50}$). This procedure is very similar to that of Dougherty et al.[37] with the only major differences being the use, by Dougherty, of a different tumour cell line (SMT-F) in DBA/2 HeLa mice and a different lamp at a higher dose rate (288 J cm$^{-2}$). The need to use higher

amounts of porphyrin in our case, compared with that used by Dougherty et al.[37] is a reflection of the greater sensitivity of the SMT-F cell line to PDT and to the fact that our light dosage was lower than that used by Dougherty. The concentration of porphyrin was determined by Dougherty by measurement at 500 nm whereas we used a measurement at 397 nm because of the greater sensitivity associated with the Soret band.

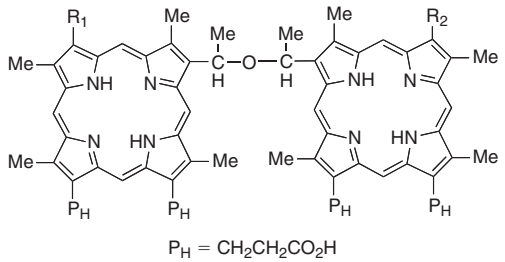
Provided uniformly sized tumours were used, the reproducibility of $TC_{50}$ was excellent. Using a standard dose of HPD (50 mg $kg^{-1}$), $TC_{50}$ was $4.6 \pm 0.5$ days, calculated from nine separate assays. Using our methodology, an HPD sample that had a $TC_{50}$ of 7 days in the Dougherty assay had a $TC_{50}$ of 5 days. Similarly Photofrin II, the partially purified oligomer-containing fraction of HPD, had a $TC_{50}$ of 7 days in our system and a $TC_{50}$ of 9 days in the Dougherty assay. A solution of the divinyl dimer **4**, which had a $TC_{50}$ of 7 days using our procedure, had a $TC_{50}$ of 9 days using the Dougherty procedure.[38] Our assay has previously been used to demonstrate relative differences in efficacy of several porphyrin mixtures derived from HPD.[36]

*In Vivo PDT Activity*

All of the possible hematoporphyrin-derived, ether-linked dimers that could be present in HPD were synthesized and tested for their anticancer activity.[29] The results are displayed in Table 1 (see also Scheme 2). The dihematoporphyrin ether **2** was inactive as a photodynamic agent. The partially dehydrated system **3** showed some activity but it was the completely dehydrated material, the divinyl dimer **4**, that had the greatest activity of this group. Since the only difference between these dimers is the nature of the substituents at the 2- and 4-positions of each porphyrin unit, it is immediately apparent that these substituents have a significant bearing on the anticancer activity of the dimers. Hydroxyl-containing substituents are hydrophilic since they contain a polar substituent that is also capable of forming hydrogen bonds. In contrast, the vinyl side chain present in **3** and **4** is hydrophobic and can be involved in π–π interactions with the other double bond or the aromatic system of the other porphyrin unit. The results outlined in Table 1 suggest hydrophobic groups are necessary for activity and that hydrophilic or polar groups do not facilitate anticancer activity in these dimers.

As a consequence of the observation that the activity of the ether-linked dimers varied with the nature of the substituents at positions 2(4) and 4(2) of the dimer systems, a range of ether-linked porphyrin dimers were prepared that differed only in the nature of these substituents. The activity of these compounds is summarized in Table 2 (Scheme 2).

When these dimers have functional groups in the side chains capable of being involved in hydrogen bonding, such as the hydroxyl groups of **2** and **7**, the compounds have no anticancer activity. In contrast hydrophobic groups, such as vinyl in **4**, methyl in **5**, and ethyl in **6**, make the dimers very active. However, it is clear that hydrophobicity is not the only important factor as is evidenced by a comparison of the activity of the dimers **9**–**12** with ether-containing side chains. In these compounds the activity decreases as the size of the alkyl part of the ether group increases. The hydrophobicity of this alkyl group will increase as the size of the alkyl group increases, but the steric requirements of the alkyl groups will also increase as the size of the alkyl groups increase. The activity of these ether-substituted systems **9**–**12** thus suggests




**Scheme 2.** Structure of compounds in Tables 1, 2, and 3 (the 4,4′-regioisomer only is shown).

**Table 1. PDT activity of ether-linked diporphyrins (Scheme 2) that could be present in HPD**

| Compound no. and reference | $R_1$ | $R_2$ | $TC_{50}$ [days] |
|---|---|---|---|
| **2**[29] | CH(OH)Me | CH(OH)Me | 0 |
| **3**[29] | CH(OH)Me | CH=$CH_2$ | 3 |
| **4**[29] | CH=$CH_2$ | CH=$CH_2$ | 7 |

**Table 2. PDT activity of ether-linked dimers (Scheme 2; $R_1 = R_2$)**

| Compound no. and reference | $R_1$ and $R_2$ | $TC_{50}$ [days] |
|---|---|---|
| **5** | Me | 6 |
| **7**[33] | $CH_2OH$ | 0 |
| **13**[33] | CH=O | 5 |
| **8** | $CH_2OMe$ | 3 |
| **6**[33] | Et | 7 |
| **6** (α fraction) | Et | 6 |
| **6** (β fraction) | Et | 6 |
| **2**[29] | CH(OH)Me | 0 |
| **9**[33] | CH(OMe)Me | 7 |
| **10**[33] | CH(OEt)Me | 3 |
| **11**[33] | CH(OPr$^i$)Me | 3 |
| **12**[33] | CH(OBu$^n$)Me | 2 |
| **14**[29] | COMe | 4 |
| **15**[29,33] | C(Me)=NOH | 0 |
| **4**[29] | CH=$CH_2$ | 7 |
| **16** | CH=$CHCO_2H$ | 0 |

**Table 3. PDT activity of ether-linked diporphyrins (Scheme 2, $R_1 \neq R_2$)**

| Compound no. and reference | $R_1$ | $R_2$ | $TC_{50}$ [days] |
|---|---|---|---|
| **3**[29] | CH=$CH_2$ | CH(OH)Me | 3 |
| **17**[29] | CH=$CH_2$ | COMe | 4 |
| **18** | CH=$CH_2$ | CH(OMe)Me | 5 |
| **19** | Et | CH(OMe)Me | 6 |
| **20** | Et | CH(OEt)Me | 4 |
| **21**[33] | Et | CH($OC_6H_5$)Me | 5 |
| **22** | Et | $CONHC_6H_5$ | 2 |

that both hydrophobicity of the side chains and the size of the side chain are important factors in determining the extent of PDT activity.

The difference in the activity of the ether **8** compared with that of the ether **9** could then be attributed to the greater hydrophobicity of the longer carbon chain in the substituents present in **9**. The more polar double bond-containing side chains of **13** and **14** make those compounds less active than the less polar, vinyl-containing analogue **4**. On this basis the oxime-containing system **15** and the conjugated carboxylic side chain-containing compound **16** both contain a polar hydroxyl group and a polar double bond, which makes these compounds inactive. Other systems **2** and **7**, which have hydroxyl groups in their side chains, are also inactive.

The activity of **4** and **6** are the same, indicating that any π–π interactions of the vinyl groups of **4** may not be of great significance. This also indicates that a smaller group, such as ethyl or vinyl, may lead to a greater activity than compounds that have larger hydrophobic groups such as **11** and **12**. However, **5** with small methyl groups, is slightly less active than **6** with larger ethyl groups, which may indicate that there is an optimum requirement for hydrophobicity at these positions.

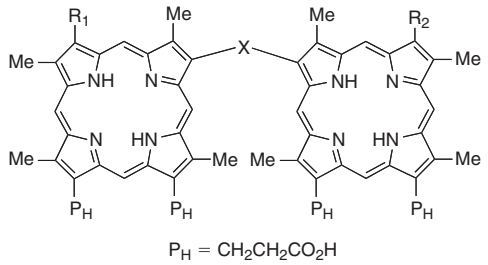
The dimerization procedure provides material that contains four methyl ester groups and these are converted into the required carboxylic acids by brief treatment with sodium hydroxide solution. The initial ester material is insufficiently soluble in water to be able to be injected intraperitoneally. Chromatographic purification of the methyl esters was achieved on silica that did not contain any zinc-containing indicators, such as silica $HF_{254}$. The dimers will sequester zinc from sources such as these. To ascertain whether this was a factor in the PDT activity of the dimers, the mono zinc salt of the dimer **6** was synthesized. Although the product turned out to be an approximately equal mixture of the dimer **6** and its monozinc salt, the fact that the mixture was less than half as active as **6** indicated that the monozinc salt had little activity and hence zinc salt formation is not important in determining activity. It was also of interest to ascertain whether the carboxylic acid groups could be modified to enhance activity. The monobenzamide, formed from one of the carboxylic acid groups of **6**, was prepared but it too was less than half as active as **6** and had a $TC_{50}$ of 3.

An analysis of Table 3 (Scheme 2), which lists the activity of ether-linked diporphyrins where $R_1 \neq R_2$, also leads to the same conclusions reached from a consideration of the data in Tables 1 and 2. In all compounds listed in Table 3 one of the R side chains is hydrophobic and hence all these systems show some activity. The less active ones are those, such as **3** and **22**, that contain more polar $R_2$ side chains with hydrophilic functional groups. Those with two hydrophobic side chains, such as **18**–**21**, are more active. The more polar (more hydrophilic) carbonyl of **17** makes it less active than **18**, which has a less polar ether group.

A comparison of the activity of compounds **19** and **20** indicates that the activity decreases as the size of the alkyl group of the ether-containing side chain ($R_2$) increases, as also noted for compounds **9**–**12**. However, the phenyl ether-containing **21** is as active as **19** suggesting that factors other than size are also important in determining the activity of diporphyrins with ether-containing side chains.

An examination of space-filling molecular models of these ether-linked diporphyrins shows that the ─CH(Me)─O─CH(Me)─ group connecting the two porphyrin units is of the right size and flexibility to fold in such a way that the porphyrin units are partially superimposed and hence could achieve a favourable offset stacked conformation, which maximizes the attraction of the two porphyrins, particularly in aqueous solution.[39] On this basis one possibility that may explain the increased activity of **21** compared with **20** could be the extra π–π interaction provided by one or both of the benzene and porphyrin rings of one section being able to interact more strongly with an aromatic portion of the other section of the dimer. A diporphyrin in a folded conformation may be more likely to be able to bind to a porphyrin binding site of porphyrin carriers, such as albumin, since the spatial characteristics and binding interactions of one face of a folded dimer would be little different to that of the porphyrin molecules normally carried by the protein. This would then allow the diporphyrins, particularly those that prefer to be in a folded conformation in an aqueous environment, to be more readily transported in the blood and hence be able to reach tumour sites by the blood supply to the tumour.

Table 4 (Scheme 3) lists the activity of a group of diporphyrins that are linked by groups other than ─CHMeOCHMe─. Compound **23**, which has a less hydrophobic linker than the similarly substituted **6**, is less active than **6**. Compound **24** shows no activity, possibly because it cannot stabilize a folded dimer by π–π interactions. The order of activity of the amine-linked compounds **25**, **26**, and **27** parallels that of their ether-linked counterparts **4**, **6**, and **14**. Both **28** and the related **2**, with hydrophilic side chains, are inactive. The decreased activity of the amine-linked dimers




**Scheme 3.** Structure of compounds in Table 4 (the 4,4′-regioisomer only is shown).

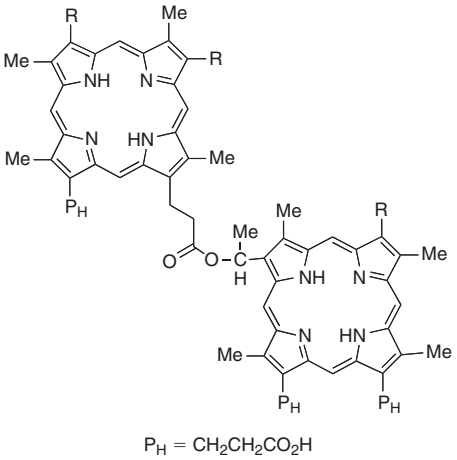
**Table 4. PDT activity of other ether- and amine-linked diporphyrins (Scheme 3)**

| Compound no. | $R_1$ | Linker (R) | $TC_{50}$ [days] |
|---|---|---|---|
| **23** | Et | $CH_2OCH_2$ | 3 |
| **24** | Et | $CHMeO(CH_2)_5OCHMe$ | 0 |
| **25** | $CH{=}CH_2$ | CHMeNHCHMe | 4 |
| **26** | Et | CHMeNHCHMe | 5 |
| **27** | COMe | CHMeNHCHMe | 2 |
| **28** | CH(OH)Me | CHMeNHCHMe | 0 |
| **29** | Et | $CH_2NHCH_2$ | 4 |
| **30** | Et | $CH_2$ | 3 |

may be attributed to the fact that the amine unit will be substantially in the protonated form (and therefore hydrophilic) in either the bloodstream or within cells. Compound **30** has a short linker that should preclude π–π interactions and which may explain its low activity.

Three ester-linked porphyrin dimers (with the ester linkage from one of the carboxylic acid groups of one porphyrin to the hydroxyl group of the CH(OH)Me group of the other) were prepared (with side chains R = CH(OH)Me, CH(OMe)Me, and $CH{=}CH_2$, respectively, Scheme 4). None of them showed any PDT activity. All were unstable in aqueous solution to the extent that the material tested was likely to have been, at least partially, a mixture of the component porphyrin monomers rather than the ester-linked dimers, particularly given that these ester-linked dimers would also have been exposed to esterases once they had been injected. Others[21] have noted that the ester-linked hematoporphyrin dimer is inactive and unstable in aqueous solution.

The porphyrin monomers, hematoporphyrin **1**, the monodehydrated compound from hematoporphyrin **31**, and the didehydrated hematoporphyrin (i.e., protoporphyrin **32**), all of which are components of HPD, were all inactive (Table 5, Scheme 1). Protoporphyrin has shown activity in other test systems[40,41] in vitro, and when synthesized within cancer cells in vivo,[42] but its insolubility in water makes it very difficult to prepare an adequate solution for testing using our procedure. Other porphyrin monomers **33**–**38**, related to the dimers discussed above, were either inactive or only weakly active with $TC_{50} \leq 3$ (Table 5). However, a few monomeric porphyrin, chlorin, and phthalocyanine compounds have shown significant PDT activity and have been assessed for clinical use.[1,4,43]




**Scheme 4.** Structure of one regioisomer of an ester-linked porphyrin dimer.

For some time it was believed, on the basis of the evidence available at the time, that the active components of HPD were ether-linked diporphyrins, such as **2**, **3**, and **4**. It was then shown[27] that porphyrin dimers linked by a three-carbon unit were also present in HPD. The linking units either contained a methyl substituent and a hydroxyl group, [—CH(Me)—$CH_2$—CH(OH)—], or a methyl group and a double bond [—CH(Me)$CH{=}CH_2$—], the latter resulting from the dehydration of the former. Porphyrin dimers, trimers, and a mixture **41** containing dimers, trimers, and oligomers all linked by the —CH(Me)$CH{=}CH_2$— group were obtained as a result of this study. The PDT activity of these carbon-linked, vinyl-containing systems is shown in Table 6. Also shown is the activity of a related compound **42** (not present in HPD) prepared by the sodium cyanoborohydride reduction, catalyzed by zinc iodide, of **39**.

The procedure used to prepare the ether-linked diporphyrins can easily be extended to provide ether-linked triporphyrins. The triporphyrins listed in Table 7 (Scheme 5) were prepared and tested for PDT activity. The results are similar to those of the corresponding diporphyrins with the triporphyrin generally being slightly less active than the corresponding diporphyrin. In the case of the ethyl-terminated trimer **47**, it was possible to separate the trimer in its methyl ester form into two fractions by chromatography, as could be achieved for the corresponding dimer. In the trimer case, insufficient β fraction was obtained for it to be tested. An ether-linked tetramer, with terminal ethyl groups, was also prepared using the same general procedure. This material was tested as the

**Table 6. PDT activity of carbon chain-linked oligomers**
The oligomer in compound **41** refers to the mixture of the total vinyl side chain-containing, carbon-linked material from Photofrin II, with the protoporphyrin removed

| Compound no. and reference | R | $TC_{50}$ [days] |
|---|---|---|
| **39**[5] | $CH{=}CH_2$ (dimer) | 5 |
| **40**[5] | $CH{=}CH_2$ (trimer) | 5 |
| **41**[5] | $CH{=}CH_2$ (oligomer) | 5 |
| **42** | Et (dimer) | 3 |

**Table 5. PDT activity of porphyrin monomers (Scheme 1)**

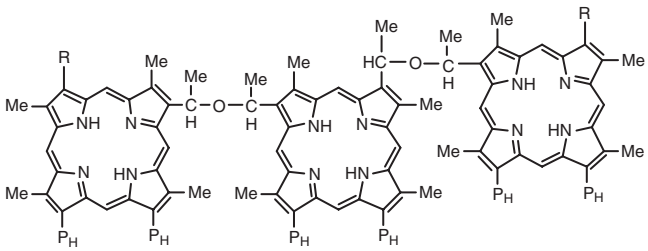
| Compound no. and reference | $R_1$ | $R_2$ | $TC_{50}$ [days] |
|---|---|---|---|
| **1** (hematoporphyrin) | CH(OH)Me | CH(OH)Me | 0 |
| **31**[29] | CH(OH)Me | $CH{=}CH_2$ | 0 |
| **32** (protoporphyrin) | $CH{=}CH_2$ | $CH{=}CH_2$ | 0 |
| **33**[33] | CH(OMe)Me | CH(OMe)Me | 3 |
| **34** | $CH(OC_5H_{11})Me$ | $CH(OC_5H_{11})Me$ | 2 |
| **35**[44] | $CH{=}CH_2$ | CH(OEt)Me | 2 |
| **36** | $CH{=}CH_2$ | $CH[O–(4–MeC_6H_4)]Me$ | 0 |
| **37** | Et | $CH(OH)C_6H_5$ | 0 |
| **38** | $CH(NHC_4H_9)Me$ | $CH(NHC_4H_9)Me$ | 3 |

total mixture and separately as the α fraction obtained as the major fraction after chromatography. The total mixture was inactive, while the α fraction had slight activity with a $TC_{50}$ of 3. The vinyl-terminated dimer **4** and the vinyl-terminated trimer **44** were both sensitive to light and air and quite rapidly turned green (presumably because of addition of oxygen to form chlorin adducts) on standing under these conditions.

Because of the stability and activity of the ether-linked porphyrins **3** and **4**, the analogous trimers **44** and **48** and of the carbon-linked compounds, **39**–**41**, in aqueous solution, it is likely that the PDT activity of Photofrin II is attributable largely to a combination of these compounds.

The vinyl group of protoporphyrin and related vinyl-substituted porphyrins is part of a diene system that is reactive towards various dienophiles. Addition of a dienophile converts the porphyrin system into a chlorin. Because chlorins have a strong absorption near 680 nm in their visible spectrum, compared with a weak absorption near 630 nm for the hematoporphyrin-derived porphyrins, they have been of interest as PDT agents since red light penetrates tissue better than light of other wavelengths, and stronger absorption towards the red end of the spectrum would, in principle, result in better PDT agents. Hence it was of interest to prepare porphyrin–chlorin systems and related compounds to evaluate their PDT activity. Table 8 lists the activity of a range of these types (see also Scheme 6). Since the compounds listed in Table 8 vary considerably in the nature of the fused carbocyclic ring and in the type of substituent provided by the dienophile, it is difficult to ascribe the variation in $TC_{50}$ for these compounds to any particular factor or factors.

The hematoporphyrin referred to above is the naturally occurring material usually obtained from blood. As mentioned, it is an asymmetrically substituted porphyrin and is strictly named hematoporphyrin IX to indicate which one of the sixteen possible isomers it is, all of which contain four methyl groups, two propionic acid, and two hydroxyethyl substituents attached to a porphyrin nucleus. It was



$P_H = CH_2CH_2CO_2H$

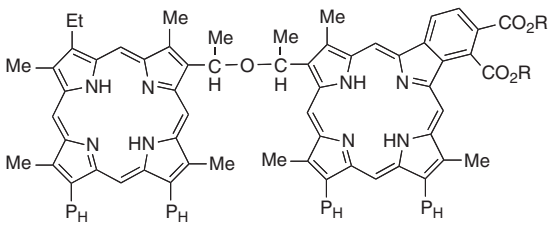
**Scheme 5.** Structure of the ether-linked porphyrin trimers (the 4,4′ : 2′,2″-regioisomer only is shown).

**Table 7. PDT activity of ether-linked triporphyrins (Scheme 5)**
Only the α fraction was tested for compound **47**

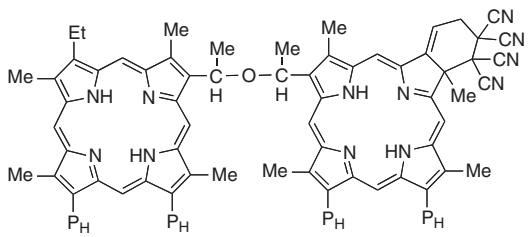
| Compound no. and reference | $R_1$ | $R_2$ | $TC_{50}$ [days] |
|---|---|---|---|
| **43**[29] | CH(OH)Me | CH(OH)Me | 2 |
| **44**[29] | $CH=CH_2$ | $CH=CH_2$ | 5 |
| **45** | CH(OMe)Me | CH(OMe)Me | 6 |
| **46** | $CH(OPr^i)Me$ | $CH(OPr^i)Me$ | 3 |
| **47**[33] | Et | Et | 6 |
| **48**[29] | CH(OH)Me | $CH=CH_2$ | 3 |

**Table 8. PDT activity of 49, 50, 51, and 52 (Scheme 6)**
In compound **49**, the FAB mass spectrum of the total sample indicated that there had been incomplete hydrolysis of the methyl esters prior to testing. Since the ester groups on the starting porphyrin materials hydrolyze readily under our standard hydrolysis conditions this presumably indicates that at least one of the methyl esters from the DMAD derived portion was slow to hydrolyze. In compound **52**, the sample was at half the usual concentration but absorbed strongly at 680 nm.

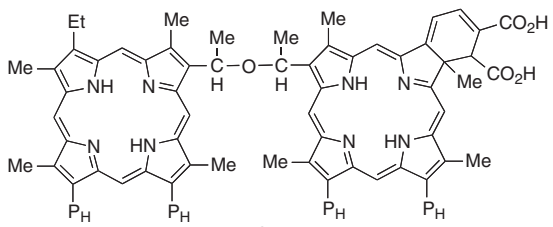
| Compound no. | $TC_{50}$ [days] |
|---|---|
| **49** (total) | 0 |
| **49** (β-fraction) | 3 |
| **50** | 6 |
| **51** | 5 |
| **52** | 0 |



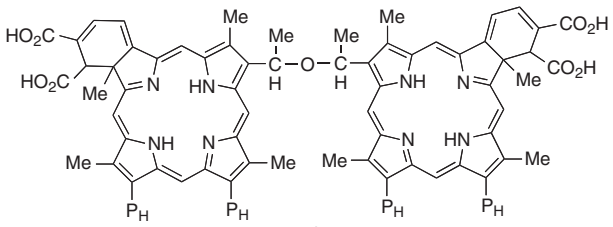
**49** R = H or Me



**51**



**50**



**52**

**Scheme 6.** Structures of the 'benzoporphyrin' compounds. In each case only one regioisomer is shown. In all cases $P_H = CH_2CH_2CO_2H$.

thus of interest to evaluate HPD made from one of the other isomers that still contains the two methyl and two propionic acid side chains on the 'bottom' of the porphyrin nucleus but retains the hydroxyethyl groups on the 'top'. We chose to prepare HPD made from the symmetrical hematoporphyrin III, which has the two hydroxyethyl side chains in 'adjacent' positions on the nucleus.[45] This HPD III was inactive whereas HPD IX has a $TC_{50}$ of 5. A possible explanation for this rather surprising difference is that HPD III was appreciably less soluble in water than HPD IX and only had a maximum solubility of 2.3 mg $mL^{-1}$ compared with significantly more than 5 mg $mL^{-1}$ (the concentration used for testing) for HPD IX. The amount of oligomeric material (the active fraction) in HPD III was approx. 30% less than that in HPD IX. An examination of molecular models suggests that the hydroxyethyl and related vinyl side chains that would be expected to be present in the diporphyrins present in HPD III are sufficiently close to each other for them to intramolecularly hydrogen bond or π–π interact with each other. This could affect the chance for these groups to interact with other porphyrins to form the oligomers, and may affect their interaction with water molecules and hence decrease the ability of these monomers and diporphyrins to dissolve in water.

## Conclusions

Our results show that diporphyrins can have a range of PDT activity from none to very active. In diporphyrins with the same linking groups these differences can only be attributed to the nature of the variable side chains (i.e., other than the methyl groups and the propionic acid groups which are present in most). Hydrophobic side chains seem to be the most desirable and the best linking groups are the ─CHMeOCHMe─ and the three-carbon linkers. The differences in activity that we, and others,[31] have found for regioisomers and diastereomers indicate that subtle differences in the linking of the two porphyrins in diporphyrin systems can have a major impact on the anticancer activity. The overall activity of HPD may also be, in part, attributable to synergistic effects between the various active compounds that may be able to attack the cancer cell at multiple sites depending on their individual structures. The ability of the two porphyrins in the diporphyrin molecule to fold in such a way that relatively strong π–π interactions can occur between them may also be important. This possible folding, which is likely to be strongest in an aqueous environment, may be important in facilitating transport to the cancer cells by the bloodstream. Alternatively, in hydrophobic areas, such as the cell membrane where folding is less likely, a planar conformation may facilitate the passage of the diporphyrins into the cell. Whether conformational factors are important in the destruction of the cancer cells on irradiation is not clear with the available data.

## Experimental

### *In Vivo Biological Testing*

Lewis lung carcinoma cells were propagated in vitro and transplanted into the back of C57Bl/6J black mice by subcutaneous injection of approx. $1 \times 10^6$ cells. After 7–10 days, when the tumours were 5–7 mm in diameter, mice in groups of 10 were given an intraperitoneal injection of a solution of the porphyrin material in saline at pH 7.24 (250 μL per 25 g bodyweight, corresponding to 25 mg $kg^{-1}$). Twenty-four hours later, mice were anaesthetized with sodium pentobarbitone (60 mg $kg^{-1}$ bodyweight), the fur over the tumour was shaved, and a 1 cm diameter area over the tumour was irradiated for 200 s with 225 J $cm^{-2}$ red light of wavelength 620–720 nm, from an incandescent lamp.[35,36] To reduce any hyperthermic effects during treatment, the skin over the tumour was sprayed with cold water at approx. 30 s intervals. Mice were palpated daily for recurrence of tumour. The end point was the number of days for five out of ten mice to regrow palpable tumour ($TC_{50}$). All animal procedures were approved by the Ethics Committees of the University of Adelaide and the Queen Elizabeth Hospital and were carried out under the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

### *Preparation of Samples for Biological Testing*

The porphyrin methyl esters were hydrolyzed by the literature procedure[29] using a mixture of tetrahydrofuran (60%) and aqueous sodium hydroxide (0.1 M) at room temperature. The resulting porphyrin acids were dissolved in the minimum amount of aqueous sodium hydroxide solution (0.1 M), the stirred solution was then neutralized to pH 7.0–7.3 by the careful addition of dilute hydrochloric acid. Saline was added until the concentration of the solution was approx. 2.5 mg of porphyrin per mL. This concentration was judged to have been obtained when 10 μL of the solution, diluted with 10 mL of sodium hydroxide solution (0.1 M) and ethanol (1 : 1), gave an absorbance reading of $0.40 \pm 0.05$ at 397 nm. The solution was then filtered through a 0.45 μm filter and the concentration checked by absorbance, before use. HPLC analysis[12] of the filtered solution was used to confirm the absence of monomer material.

This hydrolysis procedure was very satisfactory for the hydrolysis of the propionic ester side chains of the materials described below (and for the acrylic ester side chain of **16**) but did not always hydrolyze all of the ester side chains of the DMAD adducts. Amide groups were stable to these conditions.

For the ester-linked dimers, the material was dissolved in the minimum amount of tetrahydrofuran, saline was added, and the pH adjusted to 7.1 by the cautious addition of sodium hydroxide solution (0.1 M). Further saline was added until a concentration of 2.5 mg $mL^{-1}$ was reached as described above. The solution of the ester-linked dimer of hematoporphyrin showed the presence of monomers [hematoporphyrin (43%) and 2(4)-(1-hydroxyethyl)-4(2)vinyldeuteroporphyrin (18%)] after 24 h at room temperature. The solution of the trimethyl ether of the ester-linked dimer of hematoporphyrin showed the presence of monomers as soon as it could be analyzed, and after 2 days at 4°C it showed the presence of 2(4)-(1-hydroxyethyl)-4(2)-(1-methoxyethyl) deuteroporphyrin (13%) and the dimethyl ether of hematoporphyrin (42%).

### *Porphyrin Synthesis*

General details have been described previously.[29,33]

The porphyrins described below show, where appropriate, $^1$H NMR signals in the region $\delta$ −3.6 to −4.1 (NH), 2.5 to 3.7 (side chain Me, ring Me, acetyl Me), ~3.35 ($CH_2CH_2CO_2R$), 3.55 to 3.75 (ester Me), 4.1 to 4.3 ($CH_2CH_2CO_2R$), 5.9 to 6.5 (vinyl $CH_2$), ~6.2 [CH(OR)], 8.1 to 8.4 (vinyl CH), and 8.5 to 11 (meso hydrogens). The chlorins are similar except the NH of the chlorin is usually in the region of −2.5 to −3.0 ppm. The compounds described below show, where appropriate, $^{13}$C NMR signals in the region of 11 to 14 (ring Me), 17 to 21 (side chain Me, side chain methylene), 21 to 24 ($CH_2CH_2CO_2R$), 32 to 33 ($COCH_3$), 35 to 38 ($CH_2CH_2CO_2R$), 50 to 53 (ester Me), 64 to 72 [CH(OR)], 90 to 105 (meso carbons), 118 to 125 (vinyl CH), 128 to 133 (vinyl $CH_2$), 132 to 153 (pyrrole carbons), 172 to 175 ($CO_2R$),186 to 189 (CH=O), and 197 to 200 ppm (COMe). Accordingly, the only NMR data ($\delta_H$, $\delta_C$) given for the compounds below are those signals that are not from the preceding groups and which are significant in establishing their structure.

The position of visible spectroscopic peaks are given in nm, with the relative intensity of each peak in parenthesis and were determined from solutions in dichloromethane.

Fast atom bombardment mass spectrometric data are given as *m*/*z* values with the intensity relative to the base peak (100, usually the molecular ion) in parenthesis.

As mentioned above, the compounds described below were obtained as mixtures of regioisomers and, in many cases, diastereomers.

### *Porphyrin Monomers*

#### *General Procedure for the Formation and Reaction of Bromomethyl- and Bromoethyl-Containing Porphyrins*

A solution of the hydroxymethyl-, 1-hydroxyethyl-, or vinyl-containing porphyrin (100 mg) in anhydrous dichloromethane (5 mL) was saturated with gaseous HBr. The reaction flask was then tightly stoppered and left to stand at room temperature. After 2 h, the solvent was removed under oil pump vacuum (ice bath) to give the crude bromide. The product was used immediately with precautions taken to exclude moisture.

Treatment of the appropriate bromo product with the appropriate nucleophile and purification of the product by chromatography on silica gave the following compounds:

The *dimethyl ester of 2,4-di[1-(pentyloxy)ethyl]deuteroporphyrin* **34** from protoporphyrin dimethyl ester and pentan-1-ol. *m*/*z* 766 (M, 100).

The *dimethyl ester of 2(4)-[1-(ethoxy)ethyl]-4(2)-vinyldeuteroporphyrin* **35** from protoporphyrin dimethyl ester (reaction time with HBr 10 min) and ethanol. *m*/*z* 636 (M, 100).

The *dimethyl ester of 2(4)-[1-(4-methylphenoxy)ethyl]-4(2)-vinyldeuteroporphyrin* **36** from protoporphyrin dimethyl ester (reaction time with HBr 10 min) and *p*-cresol. *m*/*z* 700 (M, 100).

*2(4)-Ethyl-4(2)-[1-(5-hydroxypentyloxy)]ethyldeuteroporphyrin dimethyl ester* from 2(4)-ethyl-4(2)vinyldeuteroporphyrin dimethyl ester[33] and excess pentane-1,5-diol. $\lambda_{max}$ 399 (100), 499 (7.1), 533 (6.5), 565 (6.1), 621 (3.6). *m*/*z* 697 (M, 100).

The *dimethyl ester of 2,4-di[1-(butylamino)ethyl]deuteroporphyrin* **38** from protoporphyrin dimethyl ester and butylamine. Elution with methanol (5%) in dichloromethane gave the title diester. $\lambda_{max}$ 412 (100), 501 (21.8), 538 (14.5), 569 (11.2), 623 (5.1). *m*/*z* 737 (M), 663, 591 (100). $\delta_H$ 1.55 (butyl Me), 1.92 ($NHCH_2CH_2$), 2.21 (*Me*CHNH), 2.61 ($NHCH_2$), 6.01 (MeC*H*NH).

#### *2(4)-[(2-Ethoxycarbonyl)ethene]-4(2)-(1-hydroxyethyl) deuteroporphyrin Dimethyl Ester*

The zinc salt of 2(4)-formyl-4(2)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester[33] was prepared by a literature procedure.[46] To a solution of this salt (160 mg) in ethanol (15 mL) was added ethoxycarbonylmethylidenetriphenylphosphorane (300 mg) and the mixture was stirred at room temperature overnight. Water was then added, the solution was extracted with dichloromethane, and the extracts were dried and evaporated under reduced pressure. The residue was dissolved in a mixture of dichloromethane (10 mL), tetrahydrofuran (10 mL), and hydrochloric acid (1.0 M, 10 mL) and stirred at room temperature for 2.5 h. The mixture was then neutralized with sodium hydroxide solution (1.0 M) and immediately extracted with dichloromethane. The residue from the extraction was chromatographed on silica. Elution with methanol (1.25%) in dichloromethane gave the title porphyrin.

#### *2(4)-Ethyl-4(2)-formyldeuteroporphyrin Dimethyl Ester*

2(4)-Ethyl-4(2)-vinyldeuteroporphyrin dimethyl ester[33] (192 mg) was treated with potassium permanganate in refluxing acetone in an identical procedure to that described[33] for the oxidation of 2(4)-(1-hydroxyethyl)-4(2)-vinyldeuteroporphyrin dimethyl ester. The crude product was chromatographed on silica. Elution with 0.75% methanol in dichloromethane gave the aldehyde (62 mg). $\lambda_{max}$ 413 (100), 516 (5.3), 556 (10.8), 580 (6.6), 640 (1.1). *m*/*z* 594 (100), 593 (72).

#### *Dimethyl Ester of 2(4)-Ethyl-4(2)-(1-hydroxybenzyl) deuteroporphyrin **37***

A solution of phenylmagnesium bromide (2.4 equivalents) in ether was added to a solution of 2(4)-ethyl-4(2)-formyldeuteroporphyrin dimethyl ester (62 mg) in tetrahydrofuran (30 mL). The mixture was stirred at 0°C for 5 min and then poured into ice water. The pH was adjusted to 5 and the mixture extracted with dichloromethane. Chromatography of the residue from the extracts on silica and elution with acetone (2%) in dichloromethane gave the alcohol (37) (60 mg, 90%). $\lambda_{max}$ 408 (100), 499 (18), 537 (13), 568 (9), 622 (5). *m*/*z* 673 (M, 100). $\delta_H$ 6.62, 6.66 ($CHC_6H_5$), 7.16 (m, 3H, ArH), 7.48 (m, 2H, ArH). $\delta_C$ 70.0 (CHOH), 126.4, 126.8, 128.2 ($C_6H_5$).

#### *2(4)-Acetyl-4(2)-(1-methoxyethyl)deuteroporphyrin Dimethyl Ester*

The monomethyl ether of hematoporphyrin dimethyl ester[33] (450 mg) was dissolved in tetrahydrofuran (150 mL); Jones reagent (0.5 mL) was added and the solution stirred for 5 min. The reaction was then poured into 5% aqueous sodium acetate (150 mL) which was then extracted with dichloromethane/tetrahydrofuran (2 : 1). The organic extracts were washed with water (2 × 50 mL), dried, and the solvent removed under reduced pressure. The residue was purified by chromatography, with dichloromethane as eluent, to give the acetylporphyrin dimethyl ester. *m*/*z* 639 (M, 100), 607 (12).

#### *General Procedure for the Conversion of Porphyrin Diacids into the Corresponding Di(trimethylsilyl)ethyl Esters*

The porphyrin diacid [prepared from the corresponding dimethyl ester by alkaline hydrolysis (see below) followed by extraction with tetrahydrofuran/dichloromethane] was dried for several hours at 0.01 mmHg, and then dissolved in dry dichloromethane; excess oxalyl chloride was added and the solution was heated to reflux for 15 min under nitrogen. The reaction was cooled and the volatiles were removed under reduced pressure. The residue was dried for several hours at 0.01 mmHg and then dissolved in dry dichloromethane; crushed molecular sieves and excess 2-(trimethylsilyl)ethanol were added to the solution, and the mixture was stirred for 2 h. The reaction mixture was diluted with dichloromethane (30 mL) and washed with aqueous sodium acetate (5%, 2 × 30 mL) and water (1 × 50 mL). The solvent was removed under reduced pressure and the residue chromatographed. In this manner the following compounds were prepared.

The *di(2-(trimethylsilyl)ethyl) ester of 2,4-diacetyldeuteroporphyrin*, $\lambda_{max}$ 423 (100), 515 (14), 550 (16), 587 (7), 640 (3), 659 (1). *m*/*z* 795 (M, 100). $\delta_H$ −0.08, −0.06 ($Si(CH_3)_3$), 0.85 (m, $SiCH_2$). $\delta_C$ −1.6 ($Si(CH_3)_3$), 17.2 ($SiCH_2$).

The *di(2-(trimethylsilyl)ethyl) ester of 2(4)-acetyl-4(2)-{1-(2-[(trimethylsilyl)-ethoxy]ethyl}deuteroporphyrin*, *m*/*z* 897 (M, 100), 779 (30).

The *di(2-(trimethylsilyl)ethyl) ester of 2(4)-acetyl-4(2)-vinyldeuteroporphyrin*, $\lambda_{max}$ 413 (100), 510 (9), 549 (8), 580 (5), 635 (2). *m*/*z* 779 (M, 100). $\delta_H$ −4.47, −0.11, −0.09 ($Si(CH_3)_3$), 0.83 ($SiCH_2$), 4.1–4.4 ($CO_2CH_2$). $\delta_C$ −1.6 ($Si(CH_3)_3$), 17.2 ($SiCH_2$), 62.7 ($CO_2CH_2$).

The *di(2-(trimethylsilyl)ethyl) ester of 2(4)-acetyl-4(2)-(1-methoxyethyl)deuteroporphyrin bis(2-(trimethylsilyl)ethyl) ester*, $\lambda_{max}$ 409 (100), 509 (9), 548 (10), 577 (6), 634 (2). *m*/*z* 811 (M, 100), 779 (30). $\delta_H$ −0.10 to −0.07 ($Si(CH_3)_3$), 0.83 ($SiCH_2$), 4.1–4.4 ($CO_2CH_2$). $\delta_C$ −1.67 ($Si(CH_3)_3$), 17.2 ($SiCH_2$), 62.8 ($CO_2CH_2$).

#### *General Procedure for the Reduction of Acetyl- or Formyl-Substituted Porphyrins*

The porphyrin was dissolved in tetrahydrofuran containing a small amount of water. Excess sodium borohydride in water was added and the reaction stirred at room temperature until TLC indicated the absence of starting material. Acetone (0.5 mL) was added followed by water (100 mL). The porphyrins were extracted using dichloromethane (2 × 100 mL) and the organic phase was washed with water (50 mL) and dried ($Na_2SO_4$). The solvent was removed under reduced pressure

and the crude material was purified by chromatography on a silica squat column. In this manner the following compounds were prepared:

Reduction of the di(2-(trimethylsilyl)ethyl) ester of 2,4-diacetyldeuteroporphyrin gave the *di(2-(trimethylsilyl)ethyl) ester of 2(4)-acetyl-4(2)-(1-hydroxyethyl)deuteroporphyrin*. $\lambda_{max}$ 407 (100), 499 (18), 532 (11), 569 (8), 622 (5), 645 (2). *m/z* 797 (100). $\delta_H$ −0.03, −0.02 ($Si(CH_3)_3$), 0.91 ($SiCH_2$), 4.11–4.25 ($CO_2CH_2$). $\delta_C$ −1.6 ($Si(CH_3)_3$), 17.3 ($SiCH_2$), 62.8 ($CO_2CH_2$).

Reduction of the di(2-(trimethylsilyl)ethyl) ester of 2(4)-acetyl-4(2)vinyldeuteroporphyrin dimethyl ester gave the *di(2-(trimethylsilyl) ethyl) ester of 2(4)-(1-hydroxyethyl)-4(2)-vinyldeuteroporphyrin*. $\lambda_{max}$ 407 (100), 499 (18), 532 (11), 569 (8), 622 (5). *m/z* 781 (M, 100). $\delta_H$ −0.04, −0.03 ($Si(CH_3)_3$), 0.88 ($SiCH_2$), 4.18–4.34 ($CO_2CH_2$). $\delta_C$ −1.61 ($Si(CH_3)_3$), 17.3 ($SiCH_2$), 62.8 ($CO_2CH_2$).

Reduction of the di(2-(trimethylsilyl)ethyl) ester of 2(4)-acetyl-4(2)-(1-methoxyethyl)-deuteroporphyrin gave the *di(2-(trimethylsilyl)ethyl) ester of hematoporphyrin mono-2(4)-(1-methoxyethyl) ether*. *m/z* 813 (M, 100), 795 (10), 781 (10). $\delta_H$ −0.09, −0.07 ($Si(CH_3)_3$), 0.88 ($SiCH_2$), 4.39 ($CO_2CH_2$). $\delta_C$ −1.6 ($Si(CH_3)_3$), 17.3 ($SiCH_2$), 62.8 ($CO_2CH_2$).

Reduction of 2(4)-ethyl-4(2)-formyldeuteroporphyrin dimethyl ester gave *2(4)-ethyl-4(2)-hydroxymethyldeuteroporphyrin dimethyl ester*. $\lambda_{max}$ 400 (100), 502 (10.2), 535 (7.6), 571 (5.5), 623 (3.8). *m/z* 596 (M, 100), 579 (31). $\delta_C$ 56.5 ($CH_2OH$).

*Hydrolysis of Deuteroporphyrin Esters*

The deuteroporphyrin ester was dissolved in tetrahydrofuran (20 mL) and aqueous sodium hydroxide (0.1 M, 20 mL) and the mixture was heated to reflux for 2 h. The solution was acidified (dilute HCl), and extracted with a dichloromethane/tetrahydrofuran mixture. The extracts were washed with water, dried, and evaporated to give the diacid. By this means the following compounds were prepared:

*2,4-Di[1-(pentyloxy)ethyl]deuteroporphyrin* **34**, $\lambda_{max}$ 397, 498, 534, 568, 620. *m/z* 740 (M, 100), 695 (33), 652 (29); also 763 (M + Na, 78) and 786 (M + 2Na, 43).

*2(4)-[1-Ethoxyethyl]-4(2)-vinyldeuteroporphyrin* **35**, $\lambda_{max}$ 400, 503, 537, 572, 625.

*2(4)-[1-(4-Methylphenoxy)ethyl]-4(2)-vinyldeuteroporphyrin* **36**, $\lambda_{max}$ 400, 505, 537, 574, 626.

*2(4)-Ethyl-4(2)-(1-hydroxybenzyl)deuteroporphyrin* **37**, $\lambda_{max}$ 405, 498, 533, 568, 621.

*2,4-Di[1-(butylamino)ethyl]deuteroporphyrin* **38**, $\lambda_{max}$ 399, 502, 535, 570, 623.

*Preparation of Ether-Linked Porphyrin Dimers*

*Tetramethyl Ester of the Dimer **5***

2(4)-Formyl-4(2)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester[33] (300 mg) was reduced using a Wolff–Kishner procedure[46] to 2(4)-(1-hydroxyethyl)-4(2)-methyldeuteroporphyrin dimethyl ester (77 mg, 26%). The product was treated with HBr in dichloromethane by the general procedure to form the 1-bromoethyl derivative, which was treated with further 2(4)-(1-hydroxyethyl)-4(2)-methyldeuteroporphyrin dimethyl ester to yield, after chromatography, the dimer **5** (30%). $\lambda_{max}$ 402 (100), 501 (11.5), 535 (8.6), 570 (6.3), 622 (3.2). *m/z* 1175 (M, 100).

*Tetramethyl Ester of the Dimer **8***

A solution of the tetramethyl ester of the dimer **7**[33] (25 mg) in methanol (1 mL), trimethyl orthoformate (1 mL) and concentrated sulfuric acid (0.2 mL) was stood at room temperature for 18 h and then worked up in the usual manner.[44] Chromatography of the crude product on silica gave the tetramethyl ester of the dimer **8** as the major product. *m/z* 1235 (M, 100), 623 (9), 618 (13), 608 (21).

*Diethyltetramethyl Ester of the Dimer **16***

2(4)-[(2-Ethoxycarbonyl)ethene]-4(2)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester in dichloromethane (4 mL) was treated with a solution of dichloromethane saturated with hydrogen bromide (2 mL). After the usual workup[29,33] the residue was purified by chromatography on silica. Elution with methanol (1.25%) in dichloromethane gave the diethyltetramethyl ester of the dimer **16** contaminated with triphenylphosphine oxide. *m/z* 1343 (M, 100), 682 (37), 662 (79). (After basic hydrolysis the phosphine oxide was removed by filtration to yield a solution of the porphyrin dimer in its tetra-acid form, $\lambda_{max}$ 397, 505, 545, 582, 626.)

*Tetramethyl Ester of the Diporphyrin **18***

Protoporphyrin dimethyl ester was treated with a saturated solution of HBr in dichloromethane for 20 min before the solvent and excess reagent were removed under reduced pressure. The residue was dissolved in dichloromethane and a solution of 2(4)-(1-hydroxyethyl)-4(2)-(1-methoxyethyl)deuteroporphyrin dimethyl ester in dichloromethane was added. After the usual workup,[29,33] the product was chromatographed on silica. Elution with methanol (0.75%) in dichloromethane gave the tetramethyl ester of the diporphyrin **18**. *m/z* 1231 (M, 100), 623 (34), 591 (49).

In a similar manner the following compounds were prepared:

The *tetramethyl ester of the diporphyrin **19*** from 2(4)-ethyl-4(2)-vinyldeuteroporphyrin dimethyl ester[33] and 2(4)-(1-hydroxyethyl)-4(2)-(1-methoxyethyl)-deuteroporphyrin dimethyl ester,[33] *m/z* 1233 (M, 100), 623 (17), 593 (41).

The *dimethyldiethyl ester of the diporphyrin **20*** from 2(4)-ethyl-4(2)-vinyldeuteroporphyrin dimethyl ester[33] and 2(4)-(1-hydroxyethyl)-4(2)-(1-ethoxyethyl)deuteroporphyrin diethyl ester,[33] *m/z* 1274 (M, 100), 666 (23).

2(4)-Ethyl-4(2)-vinyldeuteroporphyrin dimethyl ester[33] and 2(4)-(1-hydroxyethyl)-4(2)-carboxydeuteroporphyrin dimethyl ester[33] were caused to react by the above procedure to yield the corresponding diporphyrin [$\lambda_{max}$ 396 (100), 508 (10.1), 543 (7.2), 581 (5.8), 626 (2.4). *m/z* 1219 (M, 100), 610 (25), 592 (28)], which was treated with oxalyl chloride in dichloromethane for 20 min under reflux. The solution was evaporated, the residue was dissolved in dichloromethane, aniline (excess) was added, and the solution was allowed to stand at room temperature for 1 h. Water was then added and the organic extract was washed with dilute hydrochloric acid, dried, and evaporated The residue was chromatographed on silica; elution with methanol (1%) in dichloromethane gave the *tetramethyl ester of the diporphyrin* **22**, $\lambda_{max}$ 404 (100), 505 (11.4), 538 (7.7), 572 (6.2), 623 (2.3). *m/z* 1295 (M, 100), 592 (19).

The *tetramethyl ester of the dimer* **24** from 2(4)-ethyl-4(2) vinyldeuteroporphyrin dimethyl ester[33] and 2(4)-ethyl-4(2)-[1-(5-hydroxypentyloxy)]ethyldeuteroporphyrin dimethyl ester. $\lambda_{max}$ 400 (100), 499 (8.3), 533 (6.5), 568 (5.0), 621 (3.0). *m/z* 1290 (M, 100), 593 (100).

The tetramethyl ester of the dimer **23** was made in the following way. A solution of 2(4)-ethyl-4(2)-hydroxymethyldeuteroporphyrin dimethyl ester (30 mg) in anhydrous dichloromethane was saturated with gaseous HBr. After 2 h at room temperature, the solvent was removed under reduced pressure. The residue was dissolved in anhydrous dichloromethane and a solution of 2(4)-ethyl-4(2)-hydroxymethyldeuteroporphyrin dimethyl ester (30 mg) in anhydrous dichloromethane was added. The mixture was heated to reflux for 30 min, and then cooled and water added. The organic layer was separated, washed with water, and the solvent evaporated. The residue was chromatographed on alumina; elution with acetone (6%) in dichloromethane gave the crude dimer, which was purified by chromatography on silica; elution with methanol (0.75%) in dichloromethane gave the tetramethyl ester of the dimer **23** (32 mg). $\lambda_{max}$ 398 (100), 503 (8.9), 537 (7.1), 569 (5.7), 623 (3.2). *m/z* 1175 (100), 595 (18), 579 (50). $\delta_C$ 63.4 ($CH_2OCH_2$).

*Tetramethyl Ester of the Monozinc Salt of the Dimer **5***

The zinc salt of 2(4)-ethyl-4(2)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester was prepared using a published procedure.[46] [$\lambda_{max}$ 407 (100), 532 (19.9), 569 (26.0)]. This salt in dichloromethane was treated with the bromoethyl monomer prepared[33] from 2(4)-ethyl-4(2)-vinyldeuteroporphyrin dimethyl ester. Chromatography of the product

on silica, with methanol (0.25–0.40%) in dichloromethane as eluent, gave an approximately equal mixture of the tetramethyl ester of the monozinc salt of the dimer **5** and the tetramethyl ester of the dimer **5**. (The HPLC trace of the product, obtained after alkaline hydrolysis, had peaks consistent with the presence of both the tetraacid and the mono zinc salt of the tetraacid.) $\lambda_{max}$ 399 (100), 500 (7.9), 534 (7.5), 571 (6.7), 624 (1.7). *m/z* 1268–1263 (M, 88), 1201 (100), 659–654 (11), 595–592 (33).

*Trimethyl Ester, Mono-*N*-phenylamide of the Dimer **5***

The dimethyl ester of 2(4)-acetyl-4(2)-(ethyl)deuteroporphyrin was dissolved in tetrahydrofuran (60%) and aqueous sodium hydroxide (0.1 M) and the resulting mixture stood at room temperature until TLC indicated that the amount of monomethylester monoacid was at a maximum. The solution was then neutralized, the porphyrin material was extracted into dichloromethane, and the monoacid was purified by chromatography on silica. Elution with methanol (5%) in dichloromethane gave the monomethylester monoacid. *m/z* 580 (M, 100). This material was dissolved in dichloromethane, excess oxalyl chloride was added, and the mixture was heated to reflux for 15 min. The solvent was evaporated under reduced pressure and dichloromethane containing excess aniline was added. After 1 h at room temperature the solution was washed with dilute hydrochloric acid. The resulting organic extract was evaporated and the residue chromatographed on silica. Elution with methanol (1%) in dichloromethane gave the monomethyl ester monoamide [*m/z* 655 (M, 100)], which was reduced with sodium borohydride to give the monomethyl ester mono-*N*-phenylamide of 2(4)-ethyl-4(2)-(1-hydroxyethyl)-deuteroporphyrin [*m/z* 657 (M, 100)], which was dissolved in dichloromethane and reacted with the alkyl bromide prepared from 2(4)ethyl-4(2)-(1-hydroxyethyl)deuteroporphyrin dimethyl ester. After the usual workup, the product was chromatographed on silica. Elution with methanol (0.75%) in dichloromethane gave the trimethyl ester mono-*N*-phenylamide of the dimer **5**. *m/z* 1164 (M, 100).

*Preparation of the Tetramethyl Esters of the –CH(Me)NHCH(Me)-Linked Dimers **25**–**28***

The tetramethyl ester of the dimer **25** was prepared by treating protoporphyrin dimethyl ester with a saturated solution of HBr in dichloromethane for 16 min and then adding the resulting solution dropwise to a solution of conc. ammonia (1 mL) and tetrahydrofuran (1 mL) at 0°C. After 20 min further stirring, the mixture was diluted with dichloromethane and water was added. The pH of the aqueous layer was adjusted to pH 10 by the addition of dilute hydrochloric acid and the mixture was extracted with dichloromethane. Chromatography of the organic extracts on silica and elution with methanol (0.25%) in dichloromethane gave the *tetramethyl ester of the dimer* **25** (40%). $\lambda_{max}$ 395 (100), 507 (10.4), 540 (7.7), 575 (5.7), 628 (3.3). *m/z* 1199 (M, 100), 608 (82), 591 (79).

The *tetramethyl ester of the dimer* **26** was prepared as for the tetramethyl ester of the dimer **25** but by using 2(4)-ethyl-4(2)-vinyldeuteroporphyrin dimethyl ester instead of protoporphyrin dimethyl ester. $\lambda_{max}$ 392 (100), 502 (10.4), 536 (7.5), 569 (6.4), 622 (3.3). *m/z* 1203 (M, 52), 609 (28), 593 (100).

The *tetramethyl ester of the dimer* **27** was prepared by treating 2(4)-acetyl-4(2)-vinyldeuteroporphyrin dimethyl ester[29] with HBr in dichloromethane for 3 h and then adding a solution of concentrated ammonium hydroxide in tetrahydrofuran (1 : 1) at 0°C and stirring the mixture for 10 min. After workup as described for dimer **25**, and chromatography on silica, elution with methanol (5%) in dichloromethane gave the tetramethyl ester. $\lambda_{max}$ 410 (100), 513 (13), 551 (11.8), 581 (9.2), 639 (2.4). *m/z* 1231 (M, 38), 1230 (M – H, 54), 624 (23), 607 (100). $\delta_H$ 2.27 ($CH_3CHNH$), 5.90 ($CH_3CHNH$). $\delta_C$ 24.0 ($CH_3CHNH$), 49.2 ($CH_3CHNH$).

Reduction of the tetramethyl ester of the dimer **27** with sodium borohydride gave the *tetramethyl ester of the dimer* **28** after chromatography on silica and elution with acetone (20%) in dichloromethane. $\lambda_{max}$ 397 (100), 504 (12), 563 (7), 571 (6), 624 (4). $\delta_H$ 2.35 ($CH_3CHNH$), 5.63 ($CH_3CHNH$). $\delta_C$ 23.7–26.3 ($CH_3CHNH$, $CH_3CHO$), 49.5 ($CH_3CHNH$).

*Preparation of Ester-Linked Dimers*

*Hematoporphyrin Ester-Linked Dimer, R = CH(OH)Me*

This dimer was prepared by the literature procedure[21] as an unstable solid. *m/z* 1179 (M, 59), 1163 (16), 621 (598 + Na, 100), 598 (92), 581 (97).

*Protoporphyrin Ester-Linked Dimer, R = CH = $CH_2$*

Protoporphyrin (176 mg) was suspended in dichloromethane (10 mL), oxalyl chloride (0.2 mL) was added, and the mixture was heated to reflux for 15 min. After cooling, the volatiles were removed under reduced pressure and the residue was dried (1.5 h, 0.01 mmHg). The residue was dissolved in dichloromethane (10 mL) and a solution of the bis(2-(trimethylsilyl)ethyl) ester of 2(4)-(1-hydroxyethyl)-4(2)-vinyldeuteroporphyrin (180 mg) and pyridine (0.02 mL) were added. The reaction was stirred for 17 h, poured into 5% aqueous sodium acetate (200 mL) and the product extracted with dichloromethane. The organic extracts were washed with saturated ammonium chloride (50 mL) and water (2 × 50 mL) and the solvent removed under reduced pressure. The crude product, *m/z* 2089 (100), 1326 (80), 789 (17), 782 (40), was chromatographed on a squat column that had been washed with methanol (25 mL) and then dichloromethane (3 × 50 mL). Elution with 0–1% acetone gave impure protoporphyrin bis(2-(trimethylsilyl)ethyl ester) [*m/z* 763 (100)]. Elution with 40–80% acetone gave impure vinyl-terminated ester-linked trimer tetrasilyl ester [*m/z* 2089 (100)]. Elution with 10–20% methanol in dichloromethane gave material [*m/z* 1325 (61)] that was rechromatographed on a short squat column (1 cm). Elution with 80% acetone gave the vinyl-terminated ester-linked dimer disilyl ester. *m/z* 1325 (60), 763 (100), 563 (59). $\delta_H$ (assigned with the aid of a proton homoCOSY spectrum) −8.7 (4H, br s, NH), −0.13, −0.08, −0.02, 0.02, 0.05 ($Si(CH_3)_3$), 0.89 (m, $SiCH_2$), 1.53 (m, $CH_3CHO$), 2.3–3.8 (ring methyls, $CH_2CO_2$), 3.9–4.6 (m, $CH_2CH_2CO_2$, $CO_2CH_2$), 5.7–6.1 ($CH_3CHO$) and 6.0–6.32 ($=CH_2$), 6.9–7.5 and 8.01–8.19 (=CH), 8.2–9.90 (methine protons). $\delta_C$ −1.6 ($Si(CH_3)_3$), 10.4–12.8 (ring methyls), 17.3 ($SiCH_2$), 21.3, 21.9, 22.8 ($CH_2CH_2CO_2$), 23.8 ($CH_3CHOCO$), 25.1 ($CH_3CHOMe$), 36.7, 37.4, 38.0 ($CH_2CH_2CO_2$), 62.8 ($CO_2CH_2$), 67.8 (CHOCO), 93–98 (*meso* carbons), 116.8 (m, $=CH_2$), 129.7 (m, =CH), 133–146 (pyrrole carbons), 173.3 ($CO_2CH_2$, COOCH), 177 ($CO_2H$). This material was unstable, both in solution and as a solid, and was not sufficiently pure to warrant desilylation to prepare a sample for testing.

*Trimethyl Ether of the Hematoporphyrin Ester-Linked Dimer, R = CH(OMe)Me*

Hematoporphyrin dimethyl ether (167 mg) was suspended in dichloromethane (10 mL) and oxalyl chloride (0.2 mL) was added and the mixture was heated to reflux for 15 min. After cooling, the volatiles were removed under reduced pressure and the residue was dried (4 h, 0.005 mmHg). The residue was dissolved in dichloromethane (10 mL) and a solution of 2(4)-(1-hydroxyethyl)-4(2)-(1-methoxyethyl) deuteroporphyrin di(trimethylsilylethyl) ester and pyridine (0.05 mL) in dichloromethane (5 mL) was added. The reaction was stirred for 17 h, poured into 5% aqueous sodium acetate (200 mL), and the product extracted with dichloromethane. The organic extracts were washed with saturated ammonium chloride (50 mL) and water (2 × 50 mL), and the solvent was removed under reduced pressure to give a residue, *m/z* 2217 (7), 2185 (5), 1421 (41), 1389 (15), 795 (100), 627 (29). The residue was chromatographed on a silica squat column that had been washed with methanol (25 mL) and then dichloromethane (3 × 50 mL). Elution with acetone (5%) in dichloromethane gave the diester-linked trimer (30 mg), *m/z* 2217 (M, 16).

The ditrimethylsilylethyl ester of the trimethyl ether of the hematoporphyrin ester-linked dimer (71 mg) was eluted using acetone (30–80%) in dichloromethane. *m/z* 1421 (M, 100%), 795 (38), 627 (61). $\delta_H$ −0.13 to −0.05 ($Si(CH_3)_3$), 0.86 (m, $SiCH_2$), 2.43 ($OCOCHCH_3$), 4.35 (m, $CO_2CH_2$), 7.65 (m, $CH_3CHOCO$). $\delta_C$ −1.7 ($Si(CH_3)_3$), 17.2 ($SiCH_2$), 23.7, 24.0 ($CH_3CHOCO$), 62.7 ($CO_2CH_2$), 66.7, 69.2 (CHOCO), 173.2 ($CO_2CH_2$), 174.8 ($CO_2H$).

This ester-linked dimer was dissolved in tetrahydrofuran (10 mL), *n*-tetrabutylammonium fluoride (10 drops) was added, and the solution was stirred for 40 min. The mixture was then poured into water (20 mL) and extracted with dichloromethane to give the trimethyl ether of the hematoporphyrin ester-linked dimer. $\lambda_{max}$ 391, 502, 536, 576, 620. *m/z* 1221 (M + H, 53%), 627 (100), 595 (79).

*Tetramethyl Ester of the Dimer **29***

A solution of anhydrous dichloromethane saturated with gaseous ammonia was added to a solution of the bromide prepared from 2(4)-ethyl-4(2)-hydroxymethyldeuteroporphyrin dimethyl ester and the mixture was heated to reflux for 15 min. Upon cooling, water was added and the organic layer separated. The extract was washed with water and the crude product was chromatographed on a silica column; elution with 2% methanol in dichloromethane gave the amine-linked dimer **29** (19 mg). $\lambda_{max}$ 407 (100), 502 (11.6), 535 (7.8), 569 (5.7), 622 (3.2). *m/z* 1175 (M, 100), 1145 (30), 580 (60).

Further elution with this solvent gave the starting alcohol and elution with 10% methanol in dichloromethane gave 2(4)-aminomethyl-4(2)-ethyldeuteroporphyrin dimethyl ester. $\lambda_{max}$ 399 (100), 499 (8.1), 532 (5.3), 568 (3.6), 620 (2.3). *m/z* 595 (M, 100), 579 (78).

*Tetramethyl Ester of the Dimer **30***

A hot solution of nickel(II) acetate in methanol was added to a refluxing solution of 2(4)-ethyl-4(2)-hydroxymethyldeuteroporphyrin dimethyl ester in chloroform and the solution was heated to reflux until no free base porphyrin was detected by visible spectroscopy (44 h). After cooling, water was added, the organic layer was extracted and washed with water before the solvent was removed under reduced pressure. The product was purified on an alumina column; elution with a gradient of 20–40% acetone in dichloromethane gave the nickel(II) salt of 2(4)-ethyl-4(2)-hydroxymethyldeuteroporphyrin dimethyl ester [$\lambda_{max}$ 392 (100), 518 (6.5), 553 (16.1). *m/z* 653 (M, 72%), 652 (M, 98), 651 (M, 100), 596 (33)], which was dissolved in dimethylformamide (10 mL) containing concentrated sulfuric acid (80 mg) and the resulting solution was heated to reflux for 90 min. After cooling, the mixture was diluted with water (5 mL) and dilute aqueous sodium hydroxide was added until precipitation was complete. The product was filtered using Celite, which was washed with water, and the organic material was dissolved using tetrahydrofuran/dichloromethane (1 : 2). The solution was washed with water before the organic phase was evaporated under reduced pressure. The residue was dissolved in concentrated sulfuric acid (3 mL) and stirred for 2.75 h, after which it was carefully added dropwise to ice-water (100 mL). Aqueous sodium hydroxide was added to adjust the pH of the solution to 4.5 before the porphyrin was extracted with tetrahydrofuran/dichloromethane (1 : 2). The extract was washed with water before the solvent was removed under reduced pressure. The crude product was redissolved in methanol (2 mL), trimethyl orthoformate (2 mL), and water (0.4 mL), and then stirred in a cold water bath while concentrated sulfuric acid (0.4 mL) was added dropwise. After 45 min, water was added, the pH was adjusted to 4.5, and the product was extracted with dichloromethane. After washing with water, the solvent was removed under reduced pressure to yield the crude metal-free product. Polar impurities were removed by passage through an alumina column, eluting with 1% methanol in dichloromethane. The eluate was then chromatographed on silica. Elution with methanol (1%) in dichloromethane gave the dimer **30**. $\lambda_{max}$ 408 (100), 501 (13.5), 535 (10.2), 568 (7.4), 622 (4.9). *m/z* 1145 (M, 100), 579 (15). $\delta_C$ 24.3 ($CH_2$ bridge).

*Tetramethyl Ester of the Dimer **42***

The porphyrin dimer **39**[5] was dissolved in dry chloroform (15 mL) and excess zinc iodide and sodium cyanoborohydride were added. The mixture was heated to reflux for 30 min and the solvent was evaporated to give the monozinc salt of the dimer ester [*m/z* 1241, (M, 100)]. Tetrahydrofuran (3 mL), dichloromethane (3 mL), and hydrochloric acid (1 M, 3 mL) were added to the residue. After 60 min, the mixture was diluted with water and extracted with dichloromethane. Chromatography of the residue from the organic extracts on silica and elution with methanol (0.5%) in dichloromethane gave the tetramethyl ester of the dimer **42**.

This compound was also prepared[28] by the action of trifluoromethanesulfonic acid on 2(4)-ethyl-4(2)-vinyldeuteroporphyrin dimethyl ester. *m/z* 1185 (M, 100). $\delta_H$ 7.75 (m), 8.35 (m), equal integration, (these were the only signals in the region $\delta$ 6.0–9.7). $\delta_C$ 122.6, 143.1 (these were the only strong signals between 100 and 170 ppm).

*Hexamethyl Ester of the Trimer **45***

Protoporphyrin dimethyl ester (100 mg) was dissolved in anhydrous dichloromethane saturated with HBr (10 mL) and the mixture was stirred for 90 min. The solution was evaporated under reduced pressure and a solution of 2(4)-(1-hydroxyethyl)-4(2)-(1-methoxyethyl)-deuteroporphyrin dimethyl ester[33] (250 mg) in dichloromethane (10 mL) was added. After 90 min at room temperature, water was added and the organic layer separated. The solvent was removed and the residue was chromatographed on silica. Elution with methanol (0.75%) in dichloromethane gave the title trimer. *m/z* 1872 (M, 100), 1842 (18), 1248 (14), 1233 (19), 642 (49), 623 (88).

*Hexamethyl Ester of the Trimer **46***

In a similar manner to that described above for the trimer ester **45**, the trimer ester **46** was prepared from protoporphyrin dimethyl ester and 2(4)-(1-hydroxyethyl)-4(2)-(1-isopropoxyethyl)deuteroporphyrin dimethyl ester,[33] and purified by chromatography on silica. *m/z* 1927 (M, 100), 1868 (21), 1318 (35), 1259 (18), 650 (39).

*Hexamethyl Ester of **49***

A solution of the monovinyl monobenzoporphyrin monomer, $\lambda_{max}$ 412, 510, 549, 574, 629, (36 mg), prepared[34] from the mono addition product of dimethyl acetylene dicarboxylate and protoporphyrin dimethyl ester, was converted into the HBr adduct by the procedure described above and then treated with a solution of the 2(4)-ethyl-4(2)-1-hydroxyethyldeuteroporphyrin dimethyl ester in the usual manner.[29,33] Chromatography of the crude product on silica and elution with methanol (0.5%) in dichloromethane gave the hexamethyl ester of **52** (45 mg). $\lambda_{max}$ 391 (100), 412 (90.7), 507 (7.8), 548 (9.2), 577 (5.8), 632 (3.5). *m/z* 1327 (M, 100), 718 (11), 594 (26).

*Hexamethyl Ester of **50***

The addition product of dimethyl acetylenedicarboxylate and 2(4)-acetyl-4(2-vinyl-deuteroporphyrin dimethyl ester [*m/z* 749 (M, 100)] was reduced with sodium borohydride to the alcohol [*m/z* 751 (M, 100)] which was then converted into the HBr adduct by the general procedure and reacted with 2(4)-ethyl-4(2)-1-hydroxyethyldeuteroporphyrin dimethyl ester in the usual manner. Purification on silica, with methanol (0.5%) in dichloromethane as eluent, gave the hexamethyl ester of **49**. $\lambda_{max}$ 400 (100), 503 (20.6), 533 (15.5), 568 (10.4), 627 (6.7), 659 (21.0). *m/z* 1343 (M, 100).

*Tetramethyl Ester of **51***

A solution of 2(4)-vinyl-4(2)-acetyldeuteroporphyrin dimethyl ester (53 mg) and tetracyanoethylene (53 mg) in anhydrous chloroform (50 mL) was heated to reflux for 21 h.[47] The solution was evaporated under reduced pressure, the residue was washed with petroleum ether and then chromatographed on silica. Elution with methanol (1%) in dichloromethane gave the chlorin adduct (32 mg), $\lambda_{max}$ 412, 504, 537, 613, 667. *m/z* 735 (M, 100), 605 (61). Reduction of the adduct with sodium borohydride gave the alcohol, $\lambda_{max}$ 402 (100), 498 (6.3), 534 (4.8), 600 (1.9), 627 (1.5), 657 (26). *m/z* 737 (M, 100), 607 (64). The alcohol and 2(4)-(1-bromoethyl)-4(2)-ethyldeuteroporphyrin dimethyl ester were dissolved in dichloromethane and the solution was stood at room temperature for 45 min. The usual workup and chromatography on silica, with methanol (0.5%) in dichloromethane as eluent, gave the tetramethyl ester of **50**. $\lambda_{max}$ 401 (100), 503 (10.2), 533 (6.0), 569 (2.9), 598 (1.9), 626 (2.4), 654 (17.8). *m/z* 1329 (M, 10), 1302 (M − CN), 1202 (M − TCNE).

*Octamethyl Ester of **52***

The mono DMAD adduct[34] of protoporphyrin dimethyl ester (54 mg) was converted into the HBr adduct by the procedure described above. Water and dichloromethane were added to this adduct. The organic solvent soluble material from the reaction was chromatographed on alumina; elution with acetone (6%) in dichloromethane returned the mono DMAD adduct (28 mg) and elution with acetone (40%) in dichloromethane gave the alcohol (14 mg, *m*/*z* 751), which was reacted with further HBr adduct (from the mono DMAD adduct, 28 mg). After the usual workup, chromatography on alumina, eluting with acetone (20%) in dichloromethane, gave the octamethyl ester of **51**. $\lambda_{max}$ 404 (100), 564 (13.6), 621 (7.2), 680 (32). *m*/*z* 1484 (M, 100).

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## References

[1] (*a*) *Photosensitizing Compounds. Their Chemistry, Biology and Clinical Use (Ciba Foundation Symposium 146)* (Eds G. Bock, S. Harnett) **1989** (John Wiley & Sons: Chichester).
(*b*) *Photodynamic Therapy. Basic Principles and Clinical Applications* (Eds T. J. Dougherty, B. W. Henderson) **1992** (Marcel Dekker: New York, NY).
(*c*) *Photodynamic Therapy of Cancer (SPIE Proceedings Vol. 2078)* (Eds G. Jori, J. Moan, W. M. Star) **1994** (SPIE: Washington, DC).
(*d*) N. Lane, *Sci. Am.* **2003**, *288*, 38.
(*e*) L. Milgrom, S. MacRobert, *Chem. Br.* **1998**, *34*, 45.

[2] N. L. Oleinick, R. L. Morris, I. Belichenko, *Photochem. Photobiol. Sci.* **2002**, *1*, 1. doi:10.1039/B108586G

[3] B. Krammer, *Anticancer Res.* **2001**, *21*, 4271.

[4] R. Bonnett, *Chemical Aspects of Photodynamic Therapy* **2000** (Taylor and Francis: London).

[5] C. J. Byrne, L. M. Marshallsay, A. D. Ward, *J. Photochem. Photobiol. B* **1990**, *6*, 13. doi:10.1016/1011-1344(90)85070-D

[6] *Porphyrin Photosensitization* (Eds D. Kessel, T. J. Dougherty) **1983** (Plenum: New York, NY).

[7] *Porphyrin Localization and Treatment of Tumors* (Eds D. R. Doiron, C. J. Gomer) **1984** (Alan R. Liss: New York, NY).

[8] *Methods in Porphyrin Photosensitization* (Ed. D. Kessel) **1985** (Plenum: New York, NY).

[9] J. Moan, C. Rimington, A. Western, *Clin. Chim. Acta* **1985**, *145*, 227. doi:10.1016/0009-8981(85)90028-2

[10] D. Kessel, P. Thompson, B. Musselman, C. K. Chang, *Photochem. Photobiol.* **1987**, *46*, 563.

[11] T. J. Dougherty, *Photochem. Photobiol.* **1987**, *46*, 569.

[12] C. J. Byrne, L. V. Marshallsay, A. D. Ward, *Photochem. Photobiol.* **1987**, *46*, 575.

[13] W. F. Keir, E. J. Land, A. H. MacLennan, D. J. McGarvey, T. G. Truscott, *Photochem. Photobiol.* **1987**, *46*, 587.

[14] D. Kessel, P. Thompson, B. Musselman, C. K. Chang, *Cancer Res.* **1987**, *47*, 4642.

[15] P. A. Scourides, R. M. Bohmer, A. H. Kaye, G. Morstyn, *Cancer Res.* **1987**, *47*, 3439.

[16] B. Musselman, C. K. Chang, *Proc. Soc. Photo-Opt. Instrum. Eng*. **1987**, *847*, 96.

[17] B. Musselman, D. Kessel, C. K. Chang, *Biomed. Environ. Mass Spectrom.* **1988**, *15*, 257.

[18] R. K. Pandey, T. J. Dougherty, *Photochem. Photobiol.* **1988**, *47*, 769.

[19] I. K. Morris, A. D. Ward, *Tetrahedron Lett.* **1988**, *29*, 2501. doi:10.1016/S0040-4039(00)87918-6

[20] R. K. Pandey, T. J. Dougherty, K. M. Smith, *Tetrahedron Lett.* **1988**, *29*, 4657. doi:10.1016/S0040-4039(00)80573-0

[21] R. K. Pandey, T. J. Dougherty, *Cancer Res.* **1989**, *49*, 2042.

[22] C. J. Byrne, L. V. Marshallsay, S. Y. Sek, A. D. Ward, in *Photodynamic Therapy of Neoplastic Disease* (Ed. D. Kessel) **1990**, Vol II, p. 131 (CRC: Boca Raton, FL).

[23] T. J. Dougherty, W. R. Potter, K. R. Weishaupt, in *Porphyrin Localization and Treatment of Tumors* (Eds D. R. Doiron, C. J. Gomer) **1983**, p. 301 (Alan R. Liss: New York, NY).

[24] T. J. Dougherty, W. R. Potter, K. R. Weishaupt, in *Porphyrins in Tumor Phototherapy* (Eds A. Andreoni, R. Cubeddu) **1984**, p. 23 (Plenum Press: New York, NY).

[25] R. W. Boyle, W. F. Keir, A. H. MacLennan, G. Maguire, T. G. Truscott, *Cancer Lett.* **1987**, *38*, 9. doi:10.1016/0304-3835(87)90194-7

[26] R. K. Pandey, F.-Y. Shiau, C. J. Medforth, T. J. Dougherty, K. M. Smith, *Tetrahedron Lett.* **1990**, *31*, 789. doi:10.1016/S0040-4039(00)94628-8

[27] C. J. Byrne, A. D. Ward, *Tetrahedron Lett.* **1989**, *30*, 6211. doi:10.1016/S0040-4039(01)93345-3

[28] R. K. Pandey, M. M. Siegel, R. Tsao, J. H. McReynolds, T. J. Dougherty, *Biomed. Environ. Mass Spectrom.* **1990**, *19*, 405.

[29] C. J. Byrne, I. K. Morris, A. D. Ward, *Aust. J. Chem.* **1990**, *43*, 1889.

[30] R. K. Pandey, K. M. Smith, T. J. Dougherty, *J. Med. Chem.* **1990**, *33*, 2032.

[31] R. K. Pandey, F.-Y. Shiau, T. J. Dougherty, K. M. Smith, *Tetrahedron* **1991**, *47*, 9571. doi:10.1016/S0040-4020(01)91025-7

[32] A. F. Mironov, A. N. Nihnik, I. V. Deruzhenko, R. Bonnett, *Tetrahedron Lett.* **1990**, *31*, 6409. doi:10.1016/S0040-4039(00)97078-3

[33] C. J. Byrne, A. D. Ward, *Aust. J. Chem.* **1991**, *44*, 411.

[34] A. R. Morgan, V. Scherrer Pangka, D. Dolphin, *J. Chem. Soc., Chem. Commun.* **1984**, 1047. doi:10.1039/C39840001047

[35] I. J. Forbes, P. A. Cowled, A. S. Y. Leong, A. D. Ward, R. B. Black, A. J. Blake, F. J. Jacka, *Med. J. Aust.* **1980**, *2*, 489.

[36] P. A. Cowled, I. J. Forbes, *Cancer Lett.* **1985**, *28*, 111. doi:10.1016/0304-3835(85)90099-0

[37] T. J. Dougherty, D. G. Boyle, K. R. Weishaupt, B. A. Henderson, W. R. Potter, D. A. Bellnier, K. E. Wityk, in *Porphyrin Photosensitization* (Eds D. Kessel, T. J. Dougherty) **1983**, p. 3 (Plenum: New York, NY).

[38] D. Kessel, private communication.

[39] C. A. Hunter, K. R. Lawson, J. Perkins, C. J. Urch, *J. Chem. Soc., Perkin Trans. 2* **2001**, 651.

[40] A. Girotti, *Biochemistry* **1979**, *18*, 4403.

[41] T. Dubbelman, A. De Goeij, J. Van Steveninck, *Biochem. Biophys. Acta* **1980**, *595*, 133. doi:10.1016/0005-2736(80)90255-2

[42] J. C. Kennedy, W. H. Pottier, D. C. Pross, *J. Photochem. Photobiol. B: Biol.* **1990**, *6*, 143. doi:10.1016/1011-1344(90)85083-9

[43] S. B. Brown, T. G. Truscott, *Chem. Br.* **1993**, *29*, 955.

[44] C. J. Byrne, A. D. Ward, *Tetrahedron Lett.* **1988**, *29*, 1421. doi:10.1016/S0040-4039(00)80313-5

[45] S. Y. Sek, G. D. Reynolds, A. D. Ward, in *Photodynamic Therapy and Biomedical Lasers* (Eds P. Spinelli, M. Dal Fante, R. Marchesini) **1992**, p. 127 (Excerpta Medica: Amsterdam).

[46] J.-H. Fuhrhop, K. M. Smith, in *Porphyrins and Metalloporphyrins* (Ed. K. M. Smith) **1975**, p. 757 (Elsevier: Amsterdam).

[47] R. K. DiNello, D. Dolphin, *J. Org. Chem.* **1980**, *45*, 5196.