Use of the Chlorophyll Derivative, Purpurin-18, for Syntheses of Sensitizers for Use in Photodynamic Therapy

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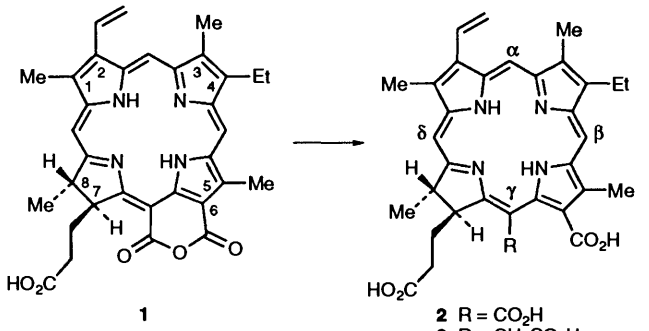
Reactions of purpurin-18 methyl ester **6** with various nucleophiles have been investigated as a means for synthesis of sensitizers for use in photodynamic therapy of tumours. Use of butylamine as the nucleophile resulted in ring-opening of the purpurin-18 anhydride ring to give a mono-amide-γ-carboxylic acid **8**, the purpurin-imide **11**, and the di-amide-γ-carboxylic acid **12**. When lysine or its esters were used as nucleophile, then lysylamide-γ-carboxylic acids **18**, **20** and **23** were obtained, along with the purpurin lysyl-imides **21** and **22**. Esterification and hydrolysis of the various carboxy functions were investigated as a means for obtaining stable water-soluble sensitizers. Similar reactions between ornithine and purpurin-18 methyl ester gave the corresponding ornithyl-chlorin-p_6 derivatives (*e.g.* **31**). The lysyl-chlorin-p_6 **18** was shown (in separate work) to be a highly effective tumour sensitizer.

Photodynamic therapy (PDT) is a new procedure for the treatment of various types of malignant tumours and involves local photochemical activation following accumulation of the photosensitizers in the tumours.[1,2] Currently, Photofrin II® which is enriched in the active components of hematoporphyrin derivative (Hpd), has been used world-wide for tumour photosensitization, and more than 4000 patients have been treated so far. Upon light activation, generally delivered from lasers by way of fibre optics, the sensitizers apparently generate singlet oxygen which causes both vascular damage and injury to tumour cells.[3] Photofrin II®, a gross mixture of components (hematoporphyrin dimer, trimer, tetramer, pentamer, and their dehydration products with ether, ester or carbon–carbon linkages[4–14]), is currently in phase III clinical trials for a number of indications.

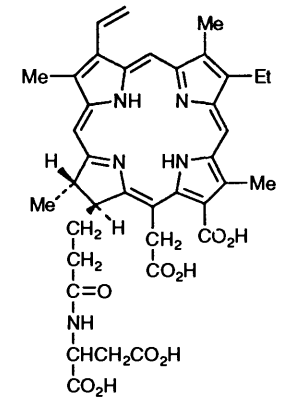
For a photosensitizer to be clinically useful it should be non-toxic, selectively taken up and/or retained in malignant tissues, activated by penetrating light (λ_{max} > 600 nm) and photochemically efficient. Photofrin II® meets the above criteria reasonably well but lacks rapid clearance from normal tissues including skin, thus rendering patients photosensitive for a month or longer after treatment. It has the disadvantage of being chemically complex and its longest wavelength absorbance peak at 630 nm is therapeutically sub-optimal for tissue penetration. During the last few years, a number of dyes which absorb strongly in the range of 650–780 nm,[15] such as phthalocyanines,[16–18] naphthalocyanines,[19] purpurins,[20] verdins,[21] pheophytins,[22] benzoporphyrin derivatives,[23] and bacteriochlorophyll and its derivatives[24,25] have been reported as potential photosensitizers. In the chlorin series, purpurin-18 **1** and chlorin-p_6 **2**[26,27] have been shown to be effective *in vitro* photosensitizers. The aspartyl derivatives of chlorin-e_6,[28] monoaspartyl chlorin-e_6 (**4**, MACE) and diaspartyl chlorin-e_6 (**5**, DACE) have been reported as effective photosensitizers *in vitro* when compared with Photofrin II®.[29] *In vivo* studies show that MACE is an effective tumour photosensitizer with rapid clearance properties if the light treatments were performed 3–4 h after drug administration. Neither tumour cures nor normal skin damage could be induced when the drug was administered (up to 50 mg kg^{-1}) 24 h prior to light treatment.[30]

MACE is prepared by standard peptide coupling reactions on chlorin-e_6 **3**. In our hands, MACE was not particularly simple to synthesize, mostly because of the troublesome contamination with DACE, which required high performance liquid chromatography for separation.[29] Under the name NPe6, MACE is currently in clinical PDT trials and the problems we encountered have, presumably, been solved on the commercial scale. Meanwhile, we determined that the anhydride ring in purpurin-18 methyl ester **6** should be easy to open with amino acid (and other) nucleophiles, and that in such a reaction *only one amino acid residue could be attached.* The present paper reports our results in this area.



1

2 R = CO_2H
3 R = CH_2CO_2H



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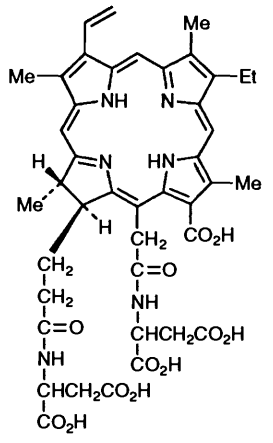

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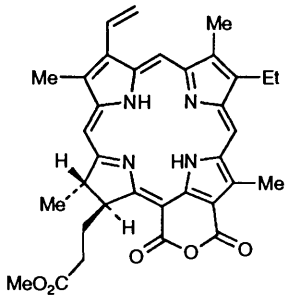
Results and Discussion

Methyl pheophorbide-a **7** was extracted from the alga *Spirulina*



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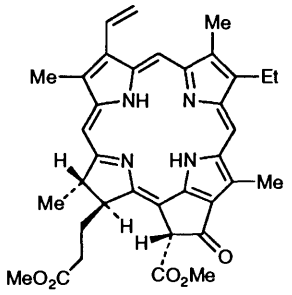
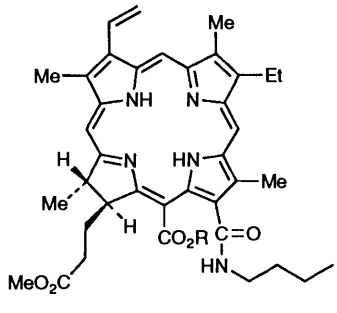


7

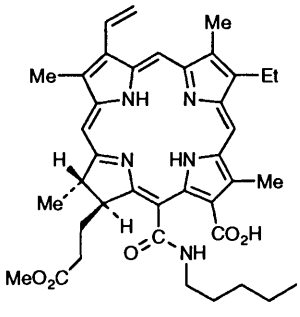
maxima in excellent yield by following the literature procedure.[31] It was transformed into purpurin-18 methyl ester **6** by dissolution in pyridine, dilution with ether and addition of KOH in propanol. After aeration for 30 min Fischer's 'unstable chlorin'[32] was obtained. Repeated evaporation to dryness and addition of dichloromethane–benzene gave purpurin-18 **1**, which was converted into its methyl ester **6** using diazomethane. The overall yield from **7** was 67%.

Our plan was to attach amino acids, such as lysine and ornithine, to purpurin-18 methyl ester, making use of the inbuilt activation provided by the anhydride ring in **6**. As a prelude, however, and because **6** possesses two anhydride carbonyl groups which were potentially reactive (though the carbonyl at the 6-position appeared to be less sterically encumbered) we began our studies using butylamine as a model for the aminobutyl group in lysine.

Reactions of Purpurin-18 Methyl Ester with Butylamine.—Excess of butylamine was added to a solution of the purpurin-18 methyl ester **6** in dichloromethane at room temperature. Spectrophotometry showed the characteristic purpurin-18 absorption at 700 nm to be progressively replaced with a new peak at 664 nm. The product was chromatographed to give a single green product in 90% yield with a λ_{max} at 664 nm. Though we favoured structure **8** for the product, as mentioned above, two isomeric products, **8** and **10**, are possible. The ^{1}H NMR spectrum of the product **8** showed that the resonances remain unchanged in the 11–5 ppm region along with one additional broad triplet amide proton at 6.85 ppm. Low resolution (Liquid SIMS) mass spectrometry gave a MH^+ (652) peak and elemental combustion analysis indicated that the product is a mono butylamine adduct. One important characteristic of **8** is that it was somewhat unstable with time and reverted to a compound with an electronic absorption maximum around 700 nm. The degradation product (*vide infra*) is presumably the imide **11**.[33]



8 R = H
9 R = Me



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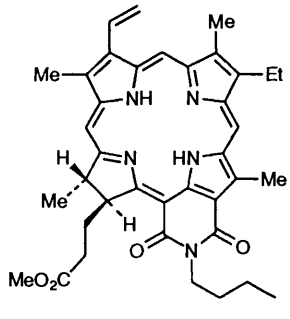

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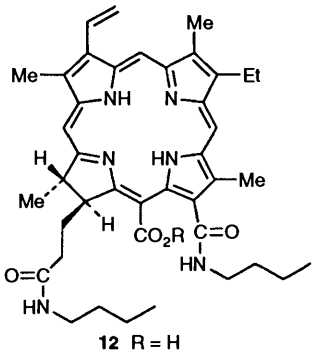


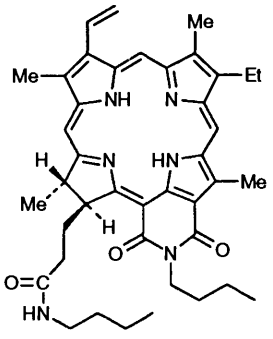
12 R = H
13 R = Me

In order to eliminate the troublesome decomposition and fully characterize the mono-amide product, compound **8** was esterified with diazomethane to give the corresponding stable methyl ester **9**. The ^{1}H NMR assignments for **9** were based on decoupling and a COSY spectrum (not shown).[34] The meso proton chemical shifts (9.72, 9.62 and 8.77 ppm) confirmed the chlorin structure. Decoupling allowed assignment of the butylamide group. One triplet proton observed at 6.21 ppm was assigned as the amide proton; irradiation of this triplet simplified both multiplets at 3.87 and 3.70 ppm. When either of these were irradiated, the multiplet at 1.77 ppm collapsed, which was further found to be coupled with the multiplet at 1.55 ppm. A triplet at 1.04 ppm was assigned as the terminal butyl-CH_3, and was coupled (J 7.8 Hz) to the multiplet at 1.55 ppm. One singlet observed at 4.22 ppm was assigned as the γ-meso methyl ester, which was further downfield compared with four other methyl groups due to its conjugation with the macro ring. The pairs of methylene protons in the 7-side-chain are slightly upfield from those in purpurin-18 methyl ester.

When the reaction was conducted in pure butylamine it gave two major green products in a ratio of about 10:1. These were separated by preparative silica gel TLC and the major green product was obtained in 82% yield. This was shown to be identical with compound **8** described above. The minor green product was obtained in 7% yield. The visible absorption spectrum of this was similar to that of **8** but with a slightly lower extinction coefficient. As expected, no molecular ion peak in high resolution electron impact (HREI) was observed; the broad ^{1}H NMR spectrum and high melting point (> 300 °C) indicated the continued presence of a carboxylic acid group in the molecule. Structural isomer **10** was initially suspected to be the minor product, but the Liquid SIMS mass spectrum gave a peak at m/z 693, corresponding to a molecular weight of chlorin-p_6 with *two* butylamine groups attached (*i.e.* **12**). Compound **12** is also unstable, as is **8**, and readily undergoes decomposition to a compound with an optical spectrum similar to that of purpurin-18 methyl ester **6**, and also **11**, but more polar than purpurin-18 methyl ester. This brown compound is presumably **14**.

Compound **12** was also treated with diazomethane to give the corresponding stable methyl ester **13**. The ^{1}H NMR spectrum showed two triplet amide protons at 6.39 and 4.65 ppm, indicating that two butylamine functions were attached. Moreover, the missing singlet methyl group in the 3–4 ppm region and the presence of a singlet methyl group at 4.22 ppm suggested that the second butylamine is attached at the 7-propionic group. The chemical shift assignments were again based on decoupling and a COSY spectrum (not shown).[34]

Reactions of Purpurin-18 Methyl Ester with Methanol.—The successful ring-opening of the purpurin-18 anhydride with butylamine indicated that similar reactions with lysine should be productive, and also should yield a water-soluble product. Purpurin-18 methyl ester **6** was dissolved in dichloromethane followed by addition of a lysine–methanol solution. After 1 h spectrophotometry and TLC showed complete consumption of purpurin-18 methyl ester. After work up and purification a new



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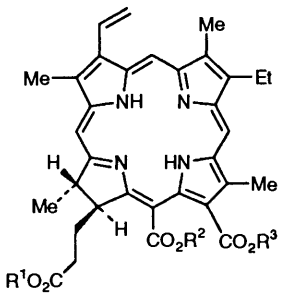
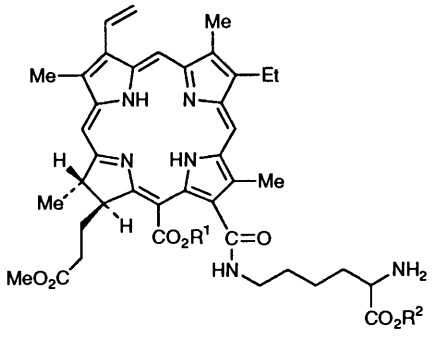
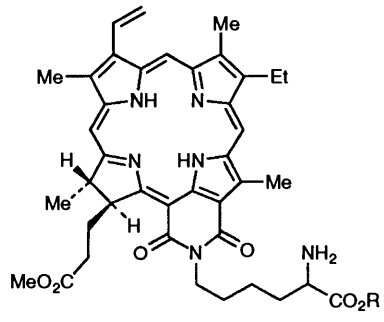


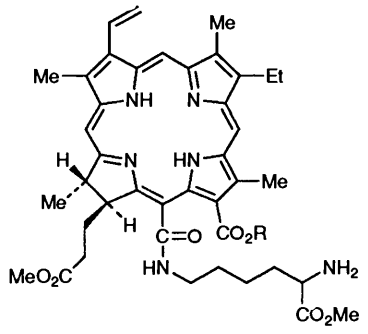
15 $R^1 = R^2 = R^3 = Me$
16 $R^1 = R^3 = Me$; $R^2 = H$
17 $R^1 = R^2 = Me$; $R^3 = H$



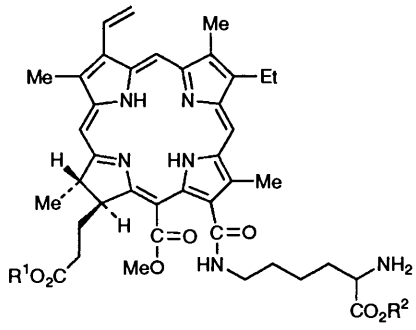
18 $R^1 = R^2 = H$
19 $R^1 = R^2 = Me$
20 $R^1 = H$, $R^2 = Me$



21 R = Me
22 R = Et



23 R = H
24 R = Me



25 $R^1 = H$, $R^2 = Me$
26 $R^1 = Me$, $R^2 = H$
27 $R^1 = Me$, $R^2 = Et$
28 $R^1 = H$, $R^2 = Et$

green compound was isolated in 80% yield. The ^1H NMR spectrum did not show a triplet amide proton in the 6–7 ppm region and an unexpected three-hydrogen singlet appeared at 4.26 ppm accompanied by an additional small peak beside it, presumably from a structural isomer. The corresponding fully esterified compound with visible absorption at 668 nm was obtained using diazomethane in 90% yield. ^1H NMR spectroscopy identified this compound as the chlorin-p_6 trimethyl ester **15**. The methanol solvent (rather than lysine) apparently attacked the anhydride ring to produce **16** and **17** in a ratio of about 7:1 (from ^1H NMR integration). This is surprising because purpurin-18 methyl ester **6** was usually recrystallized from dichloromethane and methanol. A solution of purpurin-18 methyl ester in dichloromethane with a maximal amount of methanol (avoiding precipitation) was stirred for 3 days but no reaction occurred, indicating that the presence of lysine (a weak base) is obligatory in the above case for catalysis of the anhydride methanolysis. Reaction between purpurin-18 methyl ester in dichloromethane and methanol in the presence of a catalytic amount of triethylamine was complete in 1 h, and after treatment with diazomethane, gave chlorin-p_6 trimethyl ester **15** in 80% yield. The above results show that the anhydride ring in purpurin-18 methyl ester is labile in weakly basic solution.

Reactions of Purpurin-18 Methyl Ester with Lysine.—Purpurin-18 methyl ester was treated with aqueous lysine and reaction was complete after 12 h (spectrophotometry). HPLC of the crude mixture showed only one peak. The crude product was purified using a reversed-phase C_{18} SepPak cartridge (Waters Associates). Attempts to remove all the eluted solvents using a Rotovapor caused imide-ring cyclization as with butylamine, but freeze-drying gave pure chlorin-p_6 6-N^{ε}-lysylamide-7-methyl ester **18**.

Compound **18** is very soluble in water and failed to give a reasonable ^1H NMR spectrum in D_2O or [$^2H_4$]methanol. The spectrum was too broad to assign peaks. Low resolution (Liquid SIMS) mass spectrometry gave the desired MH^+ (*m/z* 725) peak. The optical spectrum showed characteristic chlorin-p_6 absorption and only one peak was detected by reversed phase HPLC. We therefore decided to fully characterize its corresponding methyl ester since this compound should be more easy to purify and handle. The di-acid **18** was treated with ethereal diazomethane at 0 °C. Many solvent permutations were explored but the yield of **19** from the esterification was very low (~ 10%); however high resolution electron impact (HREI) mass spectrometry did show a molecular ion at *m/z* 752.3861 (calculated 752.3897).

Reactions of Purpurin-18 Methyl Ester with Lysine Esters.—Lysine methyl ester·2HCl was neutralized with 2 equiv. of NaOH followed by extraction into $CHCl_3$. The organic layer

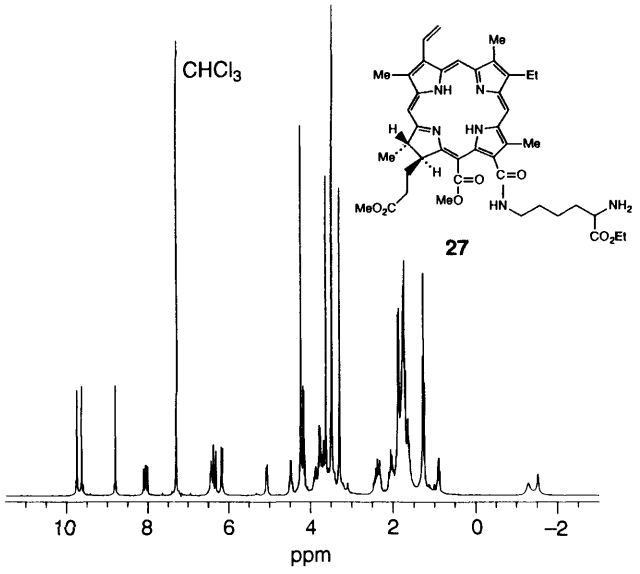
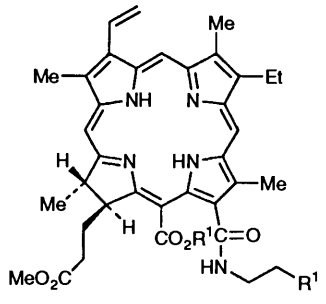


Fig. 1 The ^1H NMR spectrum, in $CDCl_3$, of lysylamide **27**



29 $R^1 = H$, $R^2 = CH_2CH(NH_2)CO_2Et$
30 $R^1 = H$, $R^2 = CH(NH_2)CO_2Me$
31 $R^1 = Me$, $R = CH(NH_2)CO_2Me$

was evaporated under reduced pressure at temperatures below 40 °C to avoid diketopiperazine formation. The resulting lysine methyl ester syrup was then added slowly into a $CHCl_3$ solution of purpurin-18 methyl ester **6**. The reaction was completed within 1 h (by spectrophotometry and TLC) to give two green bands in a ratio of 10:1. The ^1H NMR spectrum of the major product **20** showed a broad amide signal at 6.87 ppm. Gradual degradation to imide **21** was also apparent using spectrophotometry. The minor component in the mixture was shown to be **23**. Low resolution (Liquid SIMS) mass spectra of both products **20** and **23** gave similar fragmentation patterns and a protonated molecular ion at 739 (MH^+), and the optical spectra of **20** and **23** were similar.

Esterification of **20** and **23** with diazomethane gave the corresponding methyl esters **19** and **24**. The chemical shift assignments were based on decoupling and COSY spectra (not shown). Overall the chemical shifts of compound **19** are similar to those of chlorin-p_6 6-*N*-butylamide-γ,7-dimethyl ester **9**, except in two places. Instead of a triplet methyl at 1.04 ppm assigned to the terminal methyl of butylamine, there is one additional singlet methyl at 3.70 ppm which is due to the methyl ester of lysine, and the triplet amine peak is slightly downfield for **19** (6.36 ppm) than for **9** (6.21 ppm). As expected, the optical spectra of **19** and **24** are similar. HREI mass spectra indicated that both products are chlorin-p_6 with one lysine adduct, unlike in the butylamine case where the minor product was a dibutylamine chlorin-p_6 derivative.

In order to be tested as photosensitizers the chlorin derivatives should ideally be water soluble. Chlorin-p_6 6-N^ε-lysylmethoxyamide-γ,7-dimethyl ester **19** is soluble in organic solvents but insoluble in water. The corresponding carboxylic acid derivative **20** is partially soluble in water but unstable; its decomposition leads to imide purpurin **21**. To avoid the degradation to imide, the carboxylic function in the γ-position should be esterified and the 7-methyl ester should be hydrolysed to a carboxylic acid which should give a stable water-soluble chlorin **25**. Hydrolysis of chlorin-p_6 6-N^ε-lysylmethoxyamide-γ,7-dimethyl ester **19** gave a mixture of carboxylic acid chlorins **25** and **26**. In order to eliminate the hydrolysis of the methoxyamide group ester, chlorin-p_6 6-N^ε-lysylethoxyamide-γ,7-dimethyl ester **27**, in which the amide group is esterified with an ethyl group, was synthesized and then hydrolysed. The procedure for preparation of chlorin-p_6 6-N^ε-lysylethoxyamide-γ,7-dimethyl ester **27** was analogous to that of the corresponding methoxyamide chlorin **19**, but using lysine ethyl ester instead of lysine methyl ester, to give initially the γ-carboxylic acid **29**; diazomethane esterification of **29** then gave **27**. Fig. 1 shows the ^1H NMR spectrum of **27**. Using lithium hydroxide for hydrolysis of **27** led to decomposition of the starting material. No hydrolysis of the 7-methyl ester occurred when chlorin **27**, dissolved in tetrahydrofuran, was treated with less than 25% hydrochloric acid. When chlorin-p_6 6-N^ε-lysylethoxyamide-γ,7-dimethyl ester **27** in tetrahydrofuran was stirred with 25% hydrochloric acid in water overnight at room temperature, thin layer chromatography showed complete consumption of starting material to give a more polar product. After work-up, the residue was purified by chromatography to give a green product, in 51% yield, with a λ_{max} at 668 nm. HRMS studies and elemental analysis indicated the product to be **28**. The ^1H NMR spectrum of the acid chlorin **28** showed the absence of the signal at 3.61 ppm corresponding to the resonance of 7d-OMe of the starting material **27** (Fig. 1). As expected, this compound was partially soluble in water and more stable than the γ-acid derivative **20**.

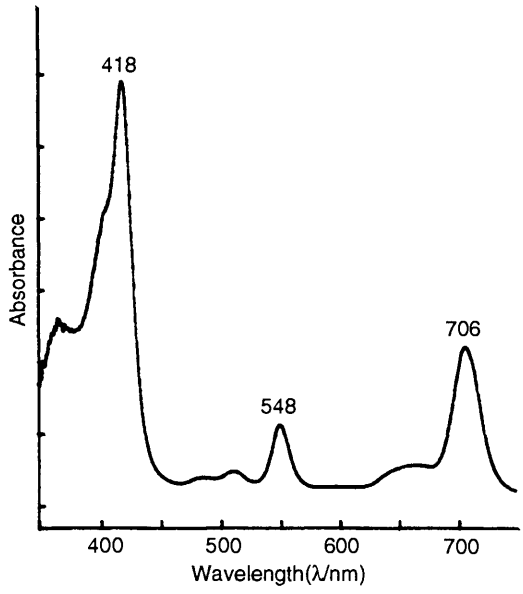


Fig. 2 The optical spectrum, in CH_2Cl_2, of lysylimide **22**

If, prior to treatment with diazomethane, a solution of chlorin-p_6 6-N^ε-lysylethoxyamide-7-methyl ester **29** in THF and/or methanol was stored, the colour changed from green to brown (λ_{max} 706 nm). Work-up and isolation of the brown decomposition product showed it to be the imide **22**. Fig. 2 shows the optical spectrum of this imide.

Reactions of Purpurin-18 Methyl Ester with Ornithine.— Reactions between purpurin-18 methyl ester and ornithine gave

chlorin-p_6 6-N^{δ}-ornithylamide-7-methyl ester **30**. The optical spectrum of **30** is typical of chlorin-p_6. As in the case of the lysine derivative **18**, the poor solubility of **30** made the subsequent esterification step very difficult and resulted in low yields. The yield of chlorin-p_6 6-N^{δ}-ornithylmethoxyamide-γ,7-dimethyl ester **31** upon esterification was very low. The optical spectrum of **31** showed the typical chlorin-p_6 pattern, whereas the ^{1}H NMR spectrum was similar to that of chlorin-p_6 6-N^{ε}-lysylmethoxyamide-γ,7-dimethyl ester **19** and the HREI mass spectrum gave the expected molecular ion.

Biological Studies.—*In vivo*, an energy density-dependent coagulation necrosis of 9L rat glioma cells by chlorin-p_6 6-N^{ε}-lysylamide-7-methyl ester **18** ('lysylchlorin-p_6'; LCP) occurred[35] in treated tumours subcutaneously implanted in the flanks of rats. Significant inhibition of tumour growth was achieved at a dose of 2.5 mg kg^{-1} and an energy density of 100 J cm^{-2}. The *in vitro* phototoxicity, localization, and intracellular changes following irradiation were examined[36] in 9L glioma cells sensitized with LCP. LCP was shown to be 10 to 100 times more toxic than Photofrin-II®. It was taken up by endocytosis and localized in an area of the endocytic compartment and diffusely throughout the perinuclear cytoplasm. Ultrastructural changes after treatment occurred in the mitochondria, Golgi apparatus, perinuclear envelope, and rough endoplasmic reticulum. Biological studies of purpurin imides **21** and **22** are in progress.

Experimental

M.p.s were measured on a Thomas/Bristoline microscopic hot-stage apparatus and were uncorrected. Silica gel 60 (70–230 and 230–400 mesh, Merck) or neutral alumina (Merck; usually Brockmann Grade III, *i.e.* deactivated with 6% water) were used for column chromatography. Preparative thin layer chromatography was carried out on 20 × 20 cm glass plates coated with Merck G 254 silica gel (1 mm thick). Analytical thin layer chromatography (TLC) was performed using Merck 60 F254 silica gel (precoated sheets, 0.2 mm thick). Reactions were monitored by TLC and spectrophotometry and were carried out under nitrogen and in the dark. ^{1}H NMR spectra were obtained in deuteriochloroform solution at 300 MHz using a General Electric QE300 spectrometer; chemical shifts are expressed in ppm relative to chloroform (7.258 ppm). Elemental analyses were performed at the Midwest Microlab, Ltd., Indiana, USA. Unless stated otherwise, electronic absorption spectra were measured in dichloromethane solution using a Hewlett–Packard 8450A spectrophotometer. Mass spectra were obtained at the Mass Spectrometry Facility, University of California, San Francisco. Methyl pheophorbide-a **7** was obtained from *Spirulina maxima* or *Spirulina pacifica* alga using the literature method.[31]

Purpurin-18 *Methyl Ester* **6**.—Methyl pheophorbide-a[31] **7** (300 mg, 0.49 mmol) was dissolved in warm pyridine (5 cm^3) and the solution was diluted with ether (500 cm^3). The solution was stirred with a stream of air passing through it, and a solution of KOH (4 g) in propanol (10 cm^3) was added. The bright green mixture (containing precipitated KOH) was stirred and aerated for 30 min and then extracted with water until the ethereal layer was no longer green. The ethereal solution was discarded; the aqueous extracts were combined, adjusted to pH ~ 4 with conc. sulfuric acid (~ 4 cm^3) in water (20 cm^3) (over-acidity should be avoided), and then extracted with dichloromethane until the aqueous layer was no longer green–brown. The combined dichloromethane extract containing 'unstable chlorin' was subjected to repeated evaporation and re-dissolution in THF (distilled) until no further increase in visible absorption at 700 nm was observed. The product was esterified with ethereal diazomethane and then washed with water, dried (Na_2SO_4) and evaporated to dryness. The product was purified on preparative TLC plates, eluting with 2% methanol in dichloromethane to give a purple–brown band. After evaporation of the solvents the residue was crystallized from dichloromethane–methanol to give purpurin-18 methyl ester **6** (143 mg, 50%), m.p. > 260 °C (decomp.) (lit.,[37] 260–280 °C); λ_{max} 698 nm (ε 4.98 × 10^4), 642 (9.85 × 10^3), 592 (3.16 × 10^3), 546 (2.46 × 10^4), 508 (7.52 × 10^3), 478 (5.13 × 10^3) and 410 (1.23 × 10^5); δ_H 9.55 (s, β-meso H), 9.35 (s, α-meso H), 8.57 (s, δ-meso H), 7.88 (dd, *J* 18, 11.7, 2a-H), 6.29 (d, *J* 18, 2b-H), 6.18 (d, *J* 11.7, 2b′-H), 5.17 (d, *J* 9.3, 7-H), 4.38 (q, *J* 7.2, 8-H), 3.76 (s, 3 H), 3.62 (q, *J* 7.5, 4a-CH_2), 3.60 (s, 3 H), 3.34 (s, 3 H), 3.12 (s, 3 H), 2.73 (m, 7b-H), 2.47 (m, 7b′-H), 2.43 (m, 7a-H), 1.99 (m, 7a′-H), 1.74 (d, *J* 7.2, 8-Me), 1.65 (t, *J* 7.5, 4b-Me) and 0.21 and −0.09 (each br s, NH) (HRMS) [Found: *m/z* (HRMS) 578.2509. $C_{34}H_{34}N_4O_5$ requires 578.2529].

Chlorin-p_6 Trimethyl Ester **15**.—(a) Purpurin-18 methyl ester **6** (30 mg, 0.052 mmol) was dissolved in dichloromethane (20 cm^3) to which was added lysine (600 mg, 4 mmol) in methanol (20 cm^3). This mixture was stirred at room temperature in the dark under nitrogen for 2 h whereupon spectrophotometry and TLC showed the absence of starting material. The solvents were evaporated at room temperature, the residue redissolved in dichloromethane, and the solution washed with water (4 × 50 cm^3), dried (Na_2SO_4) and evaporated to dryness. The material was purified by preparative TLC, eluting with dichloromethane–methanol (92:8) to give chlorin-p_6 6,7-dimethyl ester **16** (28.5 mg, 90%). The chlorin-p_6 6,7-dimethyl ester **16** was dissolved in dichloromethane and the solution treated with an excess of ethereal diazomethane and then washed with water, dried (Na_2SO_4) and evaporated to dryness. The product was purified on preparative TLC, eluting with dichloromethane–methanol (98:2). After evaporation of the solvents the residue was crystallized from dichloromethane–hexane to give chlorin-p_6 trimethyl ester **15** (26 mg, 80%), m.p. 235–236 °C (lit.,[38] 236 °C).

(b) Purpurin-18 methyl ester **6** (30 mg, 0.052 mmol) was dissolved in a solution of dichloromethane and methanol (40 cm^3; 1:1), to which was added 3 drops of triethylamine. This mixture was stirred at room temperature in the dark under nitrogen for 2 h, whereupon spectrophotometry and TLC showed the absence of starting material. The solvents were evaporated at room temperature and eventually with an oil pump to remove residual traces of triethylamine. The material was purified as described in method A to give chlorin-p_6 6,7-dimethyl ester **16** (28 mg, 93%) and chlorin-p_6 trimethyl ester **15** (26 mg, 86%).

(c) Purpurin-18 methyl ester **6** (30 mg, 0.052 mmol) was dissolved in 0.1 mol dm^{-3} sodium hydroxide in 50% methanol (20 cm^3). This mixture was stirred at room temperature in the dark under nitrogen for 1 h, whereupon spectrophotometry showed the absence of starting material. The aqueous solution was adjusted to pH ~ 7 with dilute HCl and extracted with dichloromethane until no longer green. The combined organic layers were evaporated at room temperature and the material was purified by preparative TLC, eluting with dichloromethane–methanol (85:13) to give chlorin-p_6 6-methyl ester (18.6 mg, 60%). The chlorin-p_6 6-methyl ester was dissolved in a small amount of methanol and the solution diluted with dichloromethane. It was then immediately treated with an excess of ethereal diazomethane, washed with water, dried (Na_2SO_4) and evaporated to dryness. The product was purified as described on method A to give chlorin-p_6 trimethyl ester **15** (16.2 mg, 50%).

Chlorin-p_6 6,7-dimethyl ester **16**. M.p. 240 °C (lit.,[38] 241–242 °C); δ_H 9.75 (s, β-meso H), 9.51 (s, α-meso H), 8.69 (s, δ-meso H), 8.03 (dd, *J* 17.8, 11.5, 2a-H), 6.38 (dd, *J* 17.8, 1.0, 2b-H), 6.18

(dd, *J* 11.5, 1.0, 2b′-H), 5.33 (d, *J* 11.8, 7-H), 4.46 (q, *J* 7.2, 8-H), 4.26 (s, 3 H), 3.79 (q, *J* 7.5, 4a-CH_2), 3.71 (s, 3 H), 3.53 (s, 3 H), 3.46 (s, 3 H), 3.30 (s, 3 H), 2.43–2.20, 2.04–1.96 (m, 7a and 7b-CH_2CH_2), 1.87 (d, *J* 7.2, 8-Me), 1.73 (t, *J* 7.5, 4b-Me) and −0.63 and −0.77 (each br s, NH).

Chlorin-p$_6$ *6-methyl ester*. δ_H 9.71 (s, β-meso H), 9.50 (s, α-meso H), 8.72 (s, δ-meso H), 7.95 (dd, *J* 17.7, 11.5, 2a-H), 6.33 (d, *J* 17.7, 2b-H), 6.14 (d, *J* 11.5, 2b′-H), 5.40 (d, *J* 12, 7-H), 4.48 (q, *J* 7.2, 8-H), 4.24 (s, 3 H), 3.72 (q, *J* 7.5, 4a-CH_2), 3.63 (s, 3 H), 3.42 (s, 3 H), 3.25 (s, 3 H), 2.67–2.70, 2.34–2.00 (m, 7a and 7b-CH_2CH_2), 1.92 (d, *J* 7.2, 8-Me), 1.71 (t, *J* 7.5, 4b-Me) and −0.71 and −0.89 (each br s, NH).

Chlorin-p$_6$ *trimethyl ester* **15**. M.p. 235–236 °C (lit.,[38] 236 °C); λ_{max} 668 nm (ε 4.09 × 10^4), 614 (4.85 × 10^3), 532 (5.49 × 10^3), 498 (9.92 × 10^3), 402 (1.37 × 10^5); δ_H 9.70 (s, β-meso H), 9.49 (s, α-meso H), 8.66 (s, δ-meso H), 7.98 (dd, *J* 18, 11.7, 2a-H), 6.31 (dd, *J* 18, 0.9, 2b-H), 6.14 (dd, *J* 11.7, 0.9, 2b′-H), 5.17 (d, *J* 12, 7-H), 4.41 (q, *J* 7.2, 8-H), 4.25 (s, 3 H), 4.19 (s, 3 H), 3.74 (q, *J* 7.5, 4a-CH_2), 3.67 (s, 3 H), 3.54 (s, 3 H), 3.41 (s, 3 H), 3.24 (s, 3 H), 2.39 (m, 7b-H), 2.22 (m, 7a-H), 2.07 (m, 7b′-H), 1.91 (m, 7a′-H), 1.87 (d, *J* 7.2, 8-Me), 1.71 (t, *J* 7.5, 4b-Me) and −0.84 and −1.02 (each br s, NH) [Found: *m/z* (HRMS) 624.2926. $C_{36}H_{40}N_4O_6$ requires 624.2947].

Chlorin-p$_6$ 6-N-*Butylamide-7-methyl Ester* **8**.—A solution of purpurin-18 methyl ester **6** (30 mg, 0.052 mmol) in dichloromethane (20 cm^3) was cooled to 0 °C and butylamine (0.5 cm^3, 5 mmol) was added to it. This mixture was stirred at room temperature in the dark under nitrogen for 2 h, whereupon spectrophotometry and TLC showed the absence of starting material. The reaction mixture was then diluted with dichloromethane (100 cm^3), washed with water (2 × 50 cm^3), dried (Na_2SO_4), and evaporated to dryness, eventually with an oil pump to remove traces of butylamine. The product was purified by preparative TLC, eluting with dichloromethane–methanol (88:12). After evaporation of the solvents the residue was crystallized from dichloromethane–hexane to give the title chlorin-p_6 derivative **8** (30 mg, 90%), m.p. > 300 °C (Found: C, 68.0; H, 6.7; N, 9.9. $C_{38}H_{45}N_5O_5$ requires C, 67.74; H, 6.58; N, 10.39%); λ_{max}/nm 664 (ε 4.56 × 10^4), 608 (1.08 × 10^4), 532 (1.13 × 10^4), 500 (1.90 × 10^4) and 404 (1.37 × 10^5); δ_H 9.65 (s, β-meso H), 9.60 (s, α-meso H), 8.60 (s, δ-meso H), 8.09 (br dd, *J* 18, 11, 2a-H), 6.33 (d, *J* 18, 2b-H), 6.12 (d, *J* 11, 2b′-H), 6.85 (br t, 6-butylamine-CONH), 4.88 (br d, 7-H), 3.98 (br q, 8-H), 3.77 (m, 6-butylamine-a-CH_2), 3.63 (br q, 4a-CH_2), 3.40 (br s, 2 × 3 H), 3.33 (br s, 2 × 3 H), 2.50–2.00 (m, 7a- and 7b-CH_2CH_2), 1.75 (br d, 8-Me), 1.43 (br t, 4b-Me), 1.26–1.00 (br m, 6-butylamine-b and c-CH_2CH_2), 0.54 (br t, 6-butylamine-d-CH_3) and −1.72 and −2.06 (each br s, NH); *m/z* (LRMS) 652 (MH^+). The product was observed to be erratically unstable in solution, reverting to a new compound with a λ_{max} around 700 nm (reminiscent of the optical spectrum of purpurin-18 methyl ester); this degradation product (see text) is probably purpurin-N-butylimide **11**, but was not further characterized.

Chlorin-p$_6$ 6,7-*Di*-N-*butylamide* **12**.—Purpurin-18 methyl ester **6** (30 mg, 0.052 mmol) was dissolved in butylamine (20 cm^3). This mixture was stirred at room temperature in the dark under nitrogen for 30 min, whereupon spectrophotometry showed the absence of starting material. The reaction mixture was then diluted with dichloromethane (200 cm^3), washed with water (2 × 50 cm^3), dried (Na_2SO_4) and evaporated to dryness, eventually with an oil pump to remove residual traces of butylamine. The crude product was purified by preparative TLC, eluting with dichloromethane–methanol (88:12) to give two major products in the ratio of 7:3. The faster-running green band was the chlorin-p_6 6-*N*-butylamide-7-methyl ester **8** the slower-running green band was the title chlorin-p_6 6,7-di-*N*-butylamide **12**. Both green compounds were crystallized from dichloromethane–hexane to give **8** (21 mg, 63%) and **12** (8 mg, 22%) respectively. The title compound had m.p. > 300 °C; λ_{max}/nm 664 (ε 3.55 × 10^4), 610 (4.03 × 10^3), 528 (3.48 × 10^3), 500 (1.08 × 10^4) and 404 (1.27 × 10^5); *m/z* (LRMS) 693 (MH^+). Both **8** and **12** were unstable in solution, presumably degrading to **11** and **14**, respectively (*vide infra*), which were not further characterized. These compounds had λ_{max} around 700 nm.

Chlorin-p$_6$ 6-N-*Butylamide-γ,7-dimethyl Ester* **9**.—The crude product of chlorin-p_6 6-*N*-butylamide-7-methyl ester **8** (30 mg, 0.046 mmol) was dissolved in dichloromethane and treated with an excess of ethereal diazomethane. The reaction mixture was then washed with water, dried (Na_2SO_4) and evaporated to dryness. The product was purified by preparative TLC, eluting with dichloromethane–methanol (96:4). After evaporation of the solvents the residue was crystallized from dichloromethane–hexane to give the title compound (27.5 mg, 90%), m.p. 229–231 °C (Found: C, 70.2; H, 7.2; N, 10.4. $C_{39}H_{47}N_5O_5$ requires: C, 70.35; H, 7.12; N, 10.52%); λ_{max}/nm 666 (ε 4.34 × 10^4), 612 (3.47 × 10^3), 562 (3.90 × 10^2), 528 (3.30 × 10^3), 498 (1.12 × 10^4) and 400 (1.50 × 10^5); δ_H 9.72 (s, β-meso H), 9.62 (s, α-meso H), 8.77 (s, δ-meso H), 8.04 (dd, *J* 17.7, 11.4, 2a-H), 6.34 (dd, *J* 17.7, 1.5, 2b-H), 6.21 (t, *J* 5.7, 6-butylamine-CONH), 6.15 (dd, *J* 11.4, 1.5, 2b′-H), 5.04 (d, *J* 8.1, 7-H), 4.44 (q, *J* 7.2, 8-H), 4.22 (s, 3 H), 3.87 (m, 6-butylamine-a-H), 3.75 (q, *J* 7.5, 4a-CH_2), 3.70 (m, 6-butylamine-a′-H), 3.62 (s, 3 H), 3.46 (s, 3 H), 3.45 (s, 3 H), 3.28 (s, 3 H), 2.40 (m, 7b-H), 2.29 (m, 7a-H), 2.03 (m, 7b′-H), 1.98 (m, 7a′-H), 1.84 (d, *J* 7.2, 8-Me), 1.77 (m, 6-butylamine-b-CH_2), 1.70 (t, *J* 7.5, 4b-Me), 1.55 (m, 6-butylamine-c-CH_2), 1.04 (t, *J* 7.8, 6-butylamine-d-Me) and −1.32 and −1.55 (each br s, NH) [Found: *m/z* (HRMS) 665.3564. $C_{39}H_{47}N_5O_5$ requires 665.3577].

Chlorin-p$_6$ 6,7-*Di*-N-*butylamide-γ-methyl Ester* **13**.—The chlorin-p_6 6,7-di-*N*-butylamide **12** (6.0 mg, 0.0087 mmol) was esterified with an excess of ethereal diazomethane and then washed with water, dried (Na_2SO_4) and evaporated to dryness. The product was purified by preparative TLC, eluting with dichloromethane–methanol (95:5). After evaporation of the solvents the residue was crystallized from dichloromethane–hexane to give the title compound (3.0 mg, 50%), m.p. 114–115 °C; λ_{max}/nm 666 (ε 4.22 × 10^4), 614 (4.57 × 10^3), 564 (1.53 × 10^3), 530 (4.45 × 10^3), 498 (1.21 × 10^4), 400 (1.44 × 10^5). δ_H 9.73 (s, β-meso H), 9.61 (s, α-meso H), 8.76 (s, δ-meso H), 8.04 (dd, *J* 17.9, 11.6, 2a-H), 6.39 (t, *J* 5.7 Hz, 6-butylamine-CONH), 6.34 (dd, *J* 17.9, 1.0, 2b-H), 6.15 (dd, *J* 11.6, 1.0, 2b′-H), 5.03 (dd, *J* 7.2, 2.4, 7-H), 4.65 (t, *J* 5.1, 7c-butylamine-CONH), 4.46 (q, *J* 7.2 8-H), 4.20 (s, 3 H), 3.86 (m, 6-butylamine-a-H), 3.76 (q, *J* 7.5, 4a-CH_2), 3.69 (m, 6-butylamine-a′-H), 3.62 (s, 3 H), 3.46 (s, 3 H), 3.29 (s, 3 H), 2.75 (m, 7c-butylamine-a-H), 2.49 (m, 7c-butylamine-a′-H), 2.36 (m, 7a-H), 2.24 (m, 7a′-H), 1.97 (m, 6-butylamine-b-H), 1.83 (d, *J* 7.2, 8-Me), 1.77 (m, 6-butylamine-b′-H), 1.71 (t, *J* 7.5, 4b-Me), 1.56 (m, 7b-CH_2), 1.45 (m, 6-butylamine-c-CH_2), 1.04 (t, *J* 7.5, 6-butylamine-d-Me), 0.93 (m, 7c-butylamine-b-CH_2), 0.82 (m, 7c-butylamine-c-CH_2), 0.63 (t, *J* 6.9, 7c-butylamine-d-Me) and −1.26 and −1.48 (each br s, NH) [Found: *m/z* (HRMS), 706.4194. $C_{42}H_{54}N_6O_4$ requires 706.4206].

Chlorin-p$_6$ 6-N$^\varepsilon$-*Lysylamide-7-methyl Ester* **18**.—Lysine (600 mg, 4 mmol) in water (15 cm^3) was added dropwise to a solution of purpurin-18 methyl ester **6** (30 mg, 0.052 mmol) dissolved in pyridine (50 cm^3), to make a homogeneous solution. This mixture was stirred at room temperature in the dark under nitrogen for 3 h, whereupon spectrophotometry showed the absence of starting material. The reaction mixture was then evaporated to dryness with an oil pump at temperatures below

40 °C. The crude product was dissolved in a minimum amount of water and purified by a C-18 Sep-pak cartridge, eluting first with water (1 dm^3) to remove lysine, with water–methanol (80:20; 10 cm^3) to remove some yellow and green impurities, and finally with water–methanol (20:80) to remove the desired green compound. Methanol was partially removed under reduced pressure before complete removal of water by a freeze-dryer to give the title chlorin-p_6 lysylamide (30 mg, 80%), m.p. > 300 °C (Found: C, 65.9; H, 6.85; N, 10.9. $C_{40}H_{48}N_6O_7$ requires C, 66.28; H, 6.67; N, 11.59%); λ_{max}(MeOH)/nm 660 (ε 3.96 × 10^4), 606 (3.92 × 10^3), 528 (3.38 × 10^3), 500 (1.09 × 10^4) and 400 (1.45 × 10^5); *m/z* (LRMS) 725 (MH^+).

Chlorin-p_6 6-N^ε-*Lysylmethoxyamide*-7-*methyl Ester* **20**.—Lysine methyl ester dihydrochloride (5.827 g, 25 mmol) was dissolved in water (20 cm^3) to which was added aqueous sodium hydroxide (2.5 mol dm^{-3}; 20 cm^3). The resulting solution was extracted with chloroform (4 × 500 cm^3) and the combined extracts were evaporated (45 °C) to give a syrup. To this syrup was added purpurin-18 methyl ester **6** (30 mg, 0.052 mmol) in chloroform (20 cm^3). This mixture was stirred at room temperature in the dark under nitrogen for 3 h, whereupon spectrophotometry showed the absence of starting material. The reaction mixture was then poured into a mixture of dichloromethane (200 cm^3) and water (100 cm^3) followed by addition of dilute hydrochloric acid to adjust the aqueous layer to pH ~ 6. The organic layer was separated, washed with water, dried (Na_2SO_4) and evaporated to dryness. The product was purified by preparative TLC, eluting with dichloromethane–methanol (80:20) to give chlorin-p_6 6-N^ε-lysylmethoxyamide-7-methyl ester **20** as the major product, along with a more polar, minor green compound which was assigned the structure chlorin-p_6 γ-N^ε-lysylmethoxyamide-7-methyl ester **23**. Crystallization from dichloromethane–hexane gave compound **20** (26 mg, 70%) and (**23**) (2.6 mg, 7%), respectively. The title compound had m.p. 185–186 °C (Found: C, 65.5; H, 6.7; N, 10.8. $C_{41}H_{50}N_6O_7 \cdot H_2O$ requires: C, 65.05; H, 6.93; N, 11.11%); λ_{max}/nm 666 (ε 4.22 × 10^4), 612 (4.61 × 10^3), 560 (1.76 × 10^3), 530 (4.51 × 10^3), 500 (1.18 × 10^4) and 402 (133 × 10^5); δ_H 9.69 (s, β-meso H), 9.66 (s, α-meso H), 8.76 (s, δ-meso H), 8.12 (dd, *J* 17.7, 11.5, 2a-H), 6.87 (br t, 6-lysine-CONH), 6.38 (d, *J* 17.7, 2b-H), 6.16 (d, *J* 11.5, 2b′-H), 5.03 (d, *J* 8.7, 7-H), 4.25 (m, 6-lysine-ε-CH_2), 3.97 (br q, 8-H), 3.76 (q, *J* 7.5, 4a-CH_2), 3.57 (s, 3 H), 3.50 (s, 3 H), 3.42 (m, 6-lysine-α-H), 3.35 (s, 3 H), 2.84 (s, 3 H), 2.78 (s, 3 H), 2.36, 2.17 (m, 7a- and 7b-CH_2CH_2), 2.05–1.54 (m, 6-lysine-β,γ,δ-$CH_2CH_2CH_2$), 1.74 (br d, 8-Me), 1.72 (br t, *J* 7.5, 4b-Me) and −1.61 and −1.97 (each br s, NH); *m/z* (LRMS) 739 (MH^+). *Chlorin-*p_6 γ-N^ε-*lysylmethoxyamide*-7-*methyl ester* **23** had m.p. 133–134 °C; λ_{max}/nm 666 (ε 4.19 × 10^4), 612 (4.42 × 10^3), 530 (4.66 × 10^3), 500 (1.12 × 10^4) and 402 (1.35 × 10^5); δ_H 9.67 (s, β-meso H), 9.64 (s, α-meso H), 8.77 (s, δ-meso H), 8.09 (dd, *J* 18, 11.5, 2a-H), 6.69 (br t, γ-lysine-CONH), 6.36 (d, *J* 18, 2b-H), 6.15 (d, *J* 11.5, 2b′-H), 5.15 (br d, 7-H), 4.37 (br q, 8-H), 4.00 (m, γ-lysine-ε-CH_2), 3.80 (br q, 4a-CH_2), 3.74 (s, 3 H), 3.58 (s, 3 H), 3.55 (s, 3 H), 3.50 (m, γ-lysine-α-H), 3.30 (s, 3 H), 3.12 (s, 3 H), 2.70–2.50, 2.35–2.10 (m, 7a- and 7b-CH_2CH_2), 2.02–1.50 (br m, γ-lysine-β,γ,δ-$CH_2CH_2CH_2$), 1.80 (br d, 8-Me), 1.69 (br t, 4b-Me) and −1.51 and −1.87 (each br s, NH); *m/z* (LRMS) 739 (MH^+). Compound **20** was erratically unstable in solution, reverting to purpurin-*N*-lysylimide **21**; this material was fully characterized in the case of the lysyl ethyl ester derivative **22** (*vide infra*).

Chlorin-p_6 6-N^ε-*Lysylmethoxyamide*-γ,7-*dimethyl Ester* **19**.—Chlorin-p_6 6-N^ε-lysylmethoxyamide-7-methyl ester **20** (26 mg, 0.036 mmol) was first dissolved in a minimum amount of methanol and the solution diluted with dichloromethane before treatment with an excess of ethereal diazomethane. After this it was washed with water, dried (Na_2SO_4) and evaporated to dryness. The product was purified by preparative TLC, eluting with dichloromethane–methanol (96:4), and crystallization from dichloromethane–hexane to give the title compound (17 mg, 63%), m.p. 108–109 °C (Found: C, 66.7; H, 7.0; N, 10.8. $C_{42}H_{52}N_6O_7$ requires C, 67.00; H, 6.96; N, 11.16%); λ_{max}/nm 666 (ε 4.71 × 10^4), 610 (4.90 × 10^3), 530 (4.75 × 10^3), 500 (1.29 × 10^4) and 402 (1.57 × 10^5); δ_H 9.71 (s, β-meso H), 9.61 (s, α-meso H), 8.77 (s, δ-meso H), 8.03 (dd, *J* 17.5, 11.5, 2a-H), 6.36 (t, *J* 5.7, 6-lysine-CONH), 6.33 (dd, *J* 17.5, 1.5, 2b-H), 6.15 (dd, *J* 11.5, 1.5, 2b′-H), 5.04 (d, *J* 8.7, 7-H), 4.45 (q, *J* 7.2, 8-H), 4.21 (s, 3 H), 3.87 (m, 6-lysine-ε-H), 3.74 (q, *J* 7.5, 4a-CH_2), 3.70 (s, 3 H), 3.66 (m, 6-lysine-ε′-H), 3.61 (s, 3 H), 3.48 (m, 6-lysine-α-H), 3.45 (s, 3 H), 3.44 (s, 3 H), 3.27 (s, 3 H), 2.38 (m, 7b-H), 2.28 (m, 7a-H), 2.04 (m, 7b′-H), 1.96 (m, 7a′-H), 1.87 (m, 6-lysine-γ-CH_2), 1.84 (d, *J* 7.2, 8-Me), 1.78 (m, 6-lysine-δ-CH_2), 1.70 (t, *J* 7.5, 4b-Me), 1.59 (m, 6-lysine-β-CH_2) and −1.32 and −1.55 (each br s, NH) [Found: *m/z* (HRMS) 752.3861. $C_{42}H_{52}N_6O_7$ requires 752.3897].

Chlorin-p_6 γ-N^ε-*Lysylmethoxyamide*-6,7-*dimethyl Ester* **24**.—Chlorin-p_6 γ-N^ε-lysylmethoxylamide-7-methyl ester **23** (2.6 mg, 0.0035 mmol) was dissolved in a minimum amount of methanol and the solution diluted with dichloromethane before treatment with an excess of ethereal diazomethane. The mixture was then washed with water, dried (Na_2SO_4) and evaporated to dryness. The product was purified by preparative TLC, eluting with dichloromethane–methanol (96:4) and crystallization from dichloromethane–hexane to give the title chlorin (1.06 mg, 40%), m.p. 129–130 °C; λ_{max}($CHCl_3$)/nm (relative absorbance) 666 (30.9), 612 (3.9), 530 (4.3), 500 (8.9) and 402 (100); δ_H 9.74 (s, β-meso H), 9.58 (s, α-meso H), 8.76 (s, δ-meso H), 8.94 (dd, *J* 17.7, 11.4, 2a-H), 6.34 (dd, *J* 17.7, 1.2, 2b-H), 6.26 (br t, γ-lysine-CONH), 6.17 (dd, *J* 11.4, 1.2, 2b′-H), 5.27 (br d, 7-H), 4.40 (q, *J* 7.2, 8-H), 4.22 (s, 3 H), 3.96 (m, γ-lysine-ε-H), 3.76 (q, *J* 7.5, 4a-CH_2), 3.69 (m, γ-lysine-ε′-H), 3.66 (s, 3 H), 3.65 (s, 3 H), 3.42 (m, γ-lysine-α-H), 3.45 (s, 3 H), 3.29 (s, 3 H), 3.13 (s, 3 H), 2.38–2.28, 2.04–1.96 (m, 7a- and 7b-CH_2CH_2), 1.84 (d, *J* 7.2, 8-Me), 1.70 (t, *J* 7.5, 4b-Me), 1.87–1.59 (m, γ-lysine-β,γ,δ-$CH_2CH_2CH_2$), −1.28 and −1.42 (each br s, NH) [Found: *m/z* (HRMS) 752.3857. $C_{42}H_{52}N_6O_7$ requires 752.3897].

Chlorin-p_6 6-N^ε-*Lysylethoxyamide*-γ,7-*dimethyl Ester* **27**.—To lysine ethyl ester, prepared by neutralization of lysine ethyl ester dihydrochloride (5 mg, 20 mmol) with aqueous KOH, purpurin-18 methyl ester **6** (350 mg, 0.606 mmol) in chloroform (100 cm^3) was added. This mixture was stirred at room temperature under nitrogen overnight. After work-up similar to that used in the preparation of chlorin-p_6 6-N^ε-lysylmethoxyamide-7-methyl ester **20**, the resulting chlorin-p_6 6-N^ε-lysylethoxyamide-7-methyl ester **29** was not isolated (see below) but instead was dissolved in tetrahydrofuran (50 cm^3) and immediately treated with an excess of ethereal diazomethane. After evaporation to dryness the residue was subjected to preparative silica gel TLC, eluting with dichloromethane–methanol (95:5), and crystallized from dichloromethane–hexane to give the title chlorin-p_6 derivative (258 mg, 56%), m.p. 78 °C (Found: C, 67.2; H, 7.3; N, 10.4. $C_{43}H_{54}N_6O_7$ requires C, 67.33; H, 7.10; N, 10.96%); λ_{max}/nm 666 (ε 4.81 × 10^4), 612 (8.16 × 10^3), 560 (5.16 × 10^3), 528 (8.18 × 10^3), 498 (1.60 × 10^4) and 400 (1.52 × 10^5); δ_H(Fig. 1) 9.72 (s, β-meso H), 9.62 (s, α-meso H), 8.79 (s, δ-meso H), 8.04 (dd, *J* 19.0, 12.6, 2a-H), 6.44 (t, *J* 6.0, 6-lysine-CONH), 6.35 (d, *J* 19.0, 2b-H), 6.15 (d, *J* 12.6, 2b′-H), 5.05 (d, *J* 8.5, 7-H), 4.47 (q, *J* 8, 8-H), 4.23 (s, 3 H), 4.15 (q, *J* 8.3, 6-lysine-$COCH_2$), 3.86 (m, 6-lysine-ε-H), 3.76 (q, *J* 8.6, 4a-CH_2), 3.67 (m, 6-lysine-ε′-H), 3.61 (s, 3 H), 3.55 (m, 6-lysine-α-H), 3.46 (s, 6 H), 3.28 (s, 3 H), 2.45–2.20 (m, 7-CH_2CH_2), 1.85 (d, *J* 8, 8-Me), 1.71 (t, *J* 8.6, 4b-Me), 1.64 (q, *J* 8, 6-lysine-δ-

CH_2), 1.25 (t, *J* 8.3, 6-lysine-CO_2Me), 0.89 (q, *J* 8, 6-lysine-β-CH_2), −1.30 (br s, NH) and −1.53 (br s, NH) [Found: *m/z* (HRMS) 766.4065. $C_{43}H_{54}N_6O_7$ requires 766.4046].

Chlorin-p$_6$ 6-N$^\varepsilon$-*Lysylethoxyamide-γ-methyl Ester* **28**.—A solution of chlorin-p$_6$ 6-*N*$^\varepsilon$-lysylmethoxyamide-7,γ-dimethyl ester **27** (150 mg, 0.19 mmol) dissolved in tetrahydrofuran (20 cm^3) was stirred with aqueous hydrochloric acid (25%; 10 cm^3) at room temperature under nitrogen overnight. The organic layer was then separated, diluted with dichloromethane (100 cm^3), washed with water until neutral, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by preparative silica gel TLC, eluting with dichloromethane–methanol (90 : 10), and crystallized from dichloromethane–hexane to give the title chlorin-p$_6$ derivative (75 mg, 51%), m.p. 140 °C (Found: C, 66.0; H, 7.05; N, 10.6. $C_{42}H_{52}N_6O_7 \cdot CH_3OH$ requires C, 65.78; H, 7.19; N, 10.71%); λ_{max}/nm 668 (ε 4.19 × 10^4), 612 (8.01 × 10^3), 532 (8.72 × 10^3), 498 (1.54 × 10^4) and 402 (1.44 × 10^5); δ_H 9.52 (s, β-meso H), 9.12 (s, α-meso H), 8.71 (s, δ-meso H), 8.03 (dd, *J* 19.3, 13.0, 2a-H), 6.84 (br t, 6-lysine-CONH), 6.33 (d, *J* 19.3, 2b-H), 6.15 (d, *J* 13.0, 2b′-H), 4.85 (d, *J* 8.6, 7-H), 4.38 (q, *J* 8, 8-H), 4.12 (s, 3 H), 3.99 (m, 6-lysine-$COCH_2$), 3.65 (q, *J* 8.6, 4a-CH_2), 3.66 (m, 6-lysine-ε-CH_2), 3.42 (s, 3 H), 3.29 (s, 3 H), 3.17 (s, 3 H), 2.45–2.20 (m, 7-CH_2CH_2), 1.75 (d, *J* 8, 8-Me), 1.44 (t, *J* 8.6, 4b-Me), 1.26 (m, 6-lysine-δ-CH_2), 1.08 (m, 6-lysine-CO_2Me), 0.88 (q, *J* 9.0, 6-lysine-β-CH_2) and −1.72 (br s, 2 NH); *m/z* (LRMS) 753.3 ($C_{42}H_{52}N_6O_7$ requires 752.2).

Chlorin-p$_6$ 6,γN$^\varepsilon$-*Lysylethoxyimide-7-methyl Ester* **22**.—During the preparation of **27**, solutions of chlorin-p$_6$ 6-*N*$^\varepsilon$-lysylethoxyamide-7-methyl ester **29** in organic solvents at room temperature decomposed with time to give a brown product. For example, compound **29** in THF and/or methanol, prior to treatment with diazomethane, slowly but erratically decomposed to give **22**, which was significantly less polar on TLC and absorbed at 706 nm instead of *ca.* 668 nm; the decomposition was, therefore, monitored using both TLC and spectrophotometry. After evaporation to dryness the residue was chromatographed on silica gel, eluting with dichloromethane to give the title imide which was crystallized from dichloromethane–hexane; m.p. 132 °C (Found: C, 68.7; H, 6.7; N, 11.3. $C_{42}H_{50}N_6O_6$ requires: C, 68.63; H, 6.86; N, 11.44%); λ_{max}/nm 706 (ε 4.42 × 10^4), 664 (1.15 × 10^4), 548 (2.28 × 10^4), 510 (9.69 × 10^3), 484 (8.08 × 10^3), 418 (1.18 × 10^5) (Fig. 2); δ_H 9.56 (s, β-meso H), 9.33 (s, α-meso H), 8.59 (s, δ-meso H), 7.89 (dd, *J* 19.6, 12.6, 2a-H), 6.29 (d, *J* 19.6, 2b-H), 6.16 (d, *J* 12.6, 2b′-H), 5.40 (d, *J* 8.5, 7-H), 4.49 (m, 6-lysine-ε CH_2), 4.36 (q, *J* 8, 8-H), 4.22 (q, *J* 8.6, 6-lysine-CO_2CH_2), 3.81 (s, 3 H), 3.60 (q, 4a-CH_2), 3.58 (s, 3 H), 3.38 (s, 3 H), 3.14 (s, 3 H), 2.80–2.30 (m, 7-CH_2CH_2), 2.46 (m, 6-lysine-H), 1.79 (d, *J* 8, 8-Me), 1.66 (t, *J* 8.6, 4b-Me), 1.31 (t, *J* 8.3, 6-lysine-$CO_2CH_2CH_3$), −0.08 (br s, NH) and −0.17 (br s, NH) [Found: *m/z* (HRMS) 734.3784. $C_{42}H_{50}N_6O_6$ requires 734.3792].

Chlorin-p$_6$ 6-N$^\varepsilon$-*Ornithylmethoxyamide-γ,7-dimethyl Ester* **31**.—To ornithine hydrochloride (16.8 g, 0.1 mol) dissolved in water (20 cm^3) was added sodium hydroxide (4 g, 0.1 mol). After complete evaporation of the water using a freeze-drier, methanol (100 cm^3) was added to the residue, and the suspension was stirred at room temperature overnight and then filtered. The clear methanol filtrate was concentrated slowly under reduced pressure to *ca.* 10 cm^3 to give a white slurry; the white solid was collected and air dried to give ornithine (9.2 g 70%). Ornithine (5 g, 370 mmol) was dissolved in water (2 cm^3) to which was slowly added pyridine until ornithine was about to precipitate. Purpurin-18 methyl ester **6** (30 mg, 0.052 mmol) was dissolved in pyridine (5 cm^3) to which was added water slowly until purpurin-18 methyl ester was about to precipitate. The water–pyridine solution of ornithine was added dropwise over 2 days to the stirred water–pyridine solution of purpurin-18 methyl ester at room temperature in the dark, precipitation of either ornithine or purpurin-18 methyl ester being avoided by addition of minimal amounts of water or pyridine. After the addition the reaction mixture was stirred at 55 °C until spectrophotometry showed no remaining starting material. The reaction mixture was then evaporated to dryness under an oil pump vacuum at temperatures < 40 °C. The crude product was dissolved in water and purified using a C-18 SepPak cartridge (Waters Associates), eluting first with water to remove ornithine, then with water–methanol (80 : 20) to remove some yellow and green impurities, and finally with water–methanol (20 : 80) to elute the desired green compound. Methanol was partially removed under reduced pressure at temperatures < 40 °C before complete removal of water by using a freeze dryer to give chlorin-p$_6$ 6-*N*$^\delta$-ornithylmethoxyamide-7-methyl ester **30** (7.5 mg, 20%). This was immediately dissolved in a minimum amount of methanol and diluted with dichloromethane before being treated with an excess of ethereal diazomethane. The final product was purified by preparative TLC, eluting with dichloromethane–methanol (96 : 4). Evaporation of the solvents gave the title compound **31** (3 mg, 8%), m.p. 129–130 °C; λ_{max}/nm 662 (ε 4.27 × 10^4), 608 (5.02 × 10^3), 528 (4.52 × 10^3), 498 (1.25 × 10^4) and 400 (1.50 × 10^5); δ_H 9.73 (s, β-meso H), 9.62 (s, α-meso H), 8.76 (s, δ-meso H), 8.05 (dd, *J* 17.5, 11.7, 2a-H), 7.10 (t, *J* 6.6, 6-ornithine-CONH), 6.34 (dd, *J* 17.5, 1.2, 2b-H), 6.16 (dd, *J* 11.7, 1.2, 2b′-H), 5.04 (d, *J* 8.7, 7-H), 4.44 (q, *J* 7.2, 8-H), 4.21 (s, 3 H), 3.93 (m, 6-ornithine-δ-H), 3.77 (q, *J* 7.5, 4a-CH_2), 3.71 (s, 3 H), 3.65 (m, 6-ornithine-δ′-H), 3.62 (s, 3 H), 3.50 (m, 6-ornithine-α-H), 3.46 (s, 3 H), 3.44 (s, 3 H), 3.29 (s, 3 H), 2.38–2.27, 2.02–1.94 (m, 7a- and 7b-CH_2CH_2), 1.87–1.69 (m, 6-ornithine-β,γ-CH_2CH_2), 1.83 (d, *J* 6.9, 8-Me), 1.71 (t, *J* 7.5, 4b-Me) and −1.30 and −1.55 (each br s, NH) [Found: *m/z* (HRMS) 738.3683. $C_{41}H_{50}N_6O_7$ requires 738.3741].

Acknowledgements

This research was supported by a grant from the National Science Foundation (CHE-90-01381). We thank our colleagues Michael W. Leach and Robert J. Higgins for carrying out the *in vivo* and *in vitro* studies using compound **18**. Mass spectrometric analyses were performed by the UCSF Mass Spectrometry Facility (A. L. Burlingame, Director) supported by the Biomedical Research Technology Program of the National Center for Research Resources, NIH NCRR BRTP 01614.

References

1 T. J. Dougherty, J. H. Kaufman, A. Goldfarb, K. R. Weishaupt, D. Boyle, and A. Mittleman, *Cancer Res.*, 1976, **38**, 2628.
2 T. J. Dougherty, in *CRC Critical Rev. Oncol. Hematol.*, 1984, **2**, 83.
3 K. R. Weishaput, C. J. Gomer and T. J. Dougherty, *Cancer Res.*, 1976, **36**, 2326.
4 R. Bonnett and M. C. Berenbaum, *Adv. Exp. Biol. Med.*, 1983, **160**, 241.
5 T. J. Dougherty, W. R. Potter and K. R. Weishaupt, *Adv. Exp. Biol. Med.*, 1984, **170**, 301.
6 D. Kessel, *Photochem. Photobiol.*, 1986, **44**, 193.
7 D. Kessel, P. Thompson, B. Musselman and C. K. Chang, *Cancer Res.*, 1987, **47**, 4642.
8 D. Kessel, C. K. Chang and B. Musselman, in *Methods of Porphyrin Photosensitization*, ed. D. Kessel, Plenum Press, New York, 1985, pp. 237–243.
9 P. A. Scourides, R. M. Bohmer, A. H. Kaye and G. Morstyn, *Cancer Res.*, 1987, **47**, 3439.
10 I. K. Morris and A. D. Ward, *Tetrahedron Lett.*, 1988, **29**, 2501.
11 R. K. Pandey, T. J. Dougherty and K. M. Smith, *Tetrahedron Lett.*, 1988, **29**, 4567.

12 R. K. Pandey and T. J. Dougherty, *Cancer Res.*, 1989, **49**, 2042.
13 R. K. Pandey, D. Majchrzycki, K. M. Smith and T. J. Dougherty, *Proc. SPIE*, 1989, **1065**, 164.
14 R. K. Pandey, K. M. Smith and T. J. Dougherty, *J. Med. Chem.*, 1990, **33**, 2032.
15 *Photodynamic Therapy and Biomedical Lasers*, ed. P. Spinelli, M. D. Fante and R. Marchesini, Excerpta Medica (International Congress Series, vol. 1011), Amsterdam, 1992.
16 J. D. Spikes, *Photochem. Photobiol.*, 1986, **43**, 691.
17 N. Brasseur, H. Ali, D. Autenrieth, R. Langlois and J. E. Van Lier, *Photochem. Photobiol.*, 1985, **42**, 515.
18 N. Brassuer, H. Ali, R. Langlois, R. Wagner, J. Rorreau and J. E. Van Lier, *Photochem. Photobiol.*, 1987, **45**, 582.
19 D. A. Firey and M. A. J. Rodgers, *Photochem. Photobiol.*, 1987, **45**, 535.
20 A. R. Morgan, G. M. Garbo, R. W. Keck and S. H. Selman, *Cancer Res.*, 1988, **48**, 194.
21 A. R. Morgan, A. Rampersaud, R. W. Keck and S. H. Selman, *Photochem. Photobiol.*, 1987, **46**, 441.
22 D. Kessel and K. M. Smith, *Photochem. Photobiol.*, 1989, **49**, 157, and references therein.
23 A. M. Richter, B. Kelly, J. Chaw, J. D. Liu, G. H. N. Towers, D. Dolphin and J. G. Levy, *J. Natl. Cancer Inst.*, 1987, **79**, 1322.
24 E. M. Beems, T. M. A. R. Dubbalman, J. Lugtenburg, J. A. V. Best, M. F. M. A. Smeets and J. P. J. Boegheim, *Photochem. Photobiol.*, 1987, **46**, 639.
25 B. W. Henderson, W. R. Potter, A. B. Sumlin, F. S. Owczarczak and T. J. Dougherty, *Proc. SPIE*, 1990, **1203**, 211.
26 G. W. Kenner, S. W. McCombie and K. M. Smith, *J. Chem. Soc., Perkin Trans. 1*, 1973, 2517.
27 J. K. Hoober, T. W. Sery and N. Yamamoto, *Photochem. Photobiol.*, 1988, **48**, 579.
28 J. C. Bommer and B. F. Burnham, *Tetrapyrrole Compounds*, Eur. Pat. Appl. EP 169,831 (*Chem. Abstr.*, 1986, **105**, 133666e).
29 W. G. Roberts, F.-Y. Shiau, J. S. Nelson, K. M. Smith and M. W. Berns, *J. Natl. Cancer Inst.*, 1988, **80**, 330.
30 C. J. Gomer and A. Ferrario, *Cancer Res.*, 1990, **50**, 3985.
31 K. M. Smith, D. A. Goff and D. J. Simpson, *J. Am. Chem. Soc.*, 1985, **107**, 4946.
32 H. Fisher and A. Stern, *Die Chemie des Pyrrols*, Akademische Verlag, Leipzig, vol. II, part 2, 1940, p. 109.
33 For the reaction of naphthalene-1,4,5,8-tetracarboxylic anhydride with amines to give bis-imides, see: P. Guptasarma, D. Balasubramanian, S. Matsugo and I. Saito, *Biochemistry*, 1992, **31**, 4296.
34 S.-J. Lee, Ph.D. Dissertation, University of California, Davis, 1991.
35 M. W. Leach, R. J. Higgins, J. E. Boggan, S.-J. Lee, S. A. Autry and K. M. Smith, *Cancer Res.*, 1992, **52**, 1235.
36 M. W. Leach, R. J. Higgins, S. A. Autry, J. E. Boggan, S.-J. Lee and K. M. Smith, unpublished results.
37 H. Fisher and A. Stern, *Die Chemie des Pyrrols*, Akademische Verlag, Leipzig, vol. II, part 2, 1940, p. 118.
38 H. Fisher and A. Stern, *Die Chemie des Pyrrols*, Akademische Verlag, Leipzig, vol. II, part 2, 1940, p. 122.

Paper 3/00943B
Received 16*th February* 1993
Accepted 2*nd June* 1993