4-(4-Aminophenyl)-*N*-[2-(dimethylamino)ethyl]-1-piperazinecarboxamide Trihydrochloride (42). A solution of 8.0 g (0.02 mol) of 41 in 150 mL of CH_3OH was hydrogenated in a Parr apparatus over 0.25 tsp of 5% Pd/C for 1 h. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was dissolved in ethyl ether and treated with ethereal HCl. The resulting solid was collected by filtration and recrystallized from CH_3OH/H_2O to yield 2.0 g (20%) of 42 as a white solid, mp 114–116 °C. Anal. ($C_{15}H_{25}N_5O\cdot3HCl$) C, H, N.

***N*-[2-(Dimethylamino)ethyl]-4-[4-(dimethylamino)-phenyl]-1-piperazinecarboxamide Trihydrochloride (43).** A solution of 1.7 g (0.005 mol) of 41 and 0.80 mL (0.01 mol) of 37% formalin solution in 200 mL of CH_3OH was hydrogenated in a Parr apparatus over 0.25 tsp of 5% Pd/C until H_2 uptake ceased. The mixture was filtered through Celite, and the filtrate was concentrated. The residue was dissolved in ethyl ether and treated with ethereal HCl. The resulting solid was collected by filtration and recrystallized from $CH_3OH/(C_2H_5)_2O$ to yield 0.4 g (18%) of 43 as a white solid, mp 221–223 °C. Anal. ($C_{17}H_{23}N_5O\cdot3HCl$) C, H, N.

Acknowledgment. We thank Dr. Larry W. Jaques, Ashby F. Johnson, John B. Forehand, Joanne E. Anderson, and Malcolm D. Stone who determined the spectral and analytical data; Marianne H. Foxwell and Laura L. Whitman provided technical assistance in conducting the pharmacological assays; Dr. Brian F. Kilpatrick and Robin D. Tabor determined the H_1 histamine binding data; Drs. William J. Welstead, Jr. and Mark C. Sleevi kindly proofread the manuscript.

Porphyrin Dimers as Photosensitizers in Photodynamic Therapy

Ravindra K. Pandey,* Kevin M. Smith,† and Thomas J. Dougherty‡

Oncologic Foundation of Buffalo, 225 Oak Street, Suite 200, Buffalo, New York 14203, Department of Chemistry, University of California, Davis, California 95616, and Division of Radiation Biology, Department of Radiation Medicine, Roswell Park Memorial Institute, Buffalo, New York 14263. *Received July 27, 1989*

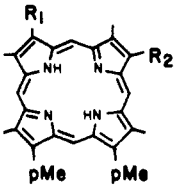
Porphyrin dimers **9** with ether linkages and possible isomers bis[1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-2-vinylporphin-4-yl]ethyl] ether (**10**) bis[1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-4-vinylporphin-2-yl]ethyl] ether (**11**), and 1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-2-vinylporphin-4-yl]ethyl 1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-4-vinylporphin-2-yl]ethyl ether (**12**) were synthesized from the corresponding (1-hydroxyethyl)vinyldeuteroporphyrin IX dimethyl esters (Hvd). The pure Hvd isomers 2-(1-hydroxyethyl)-4-vinyldeuteroporphyrin IX dimethyl ester (**7**) and 4-(1-hydroxyethyl)-2-vinyldeuteroporphyrin IX dimethyl ester (**8**) were obtained from 2-acetyl-4-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**3**) and 4-acetyl-2-(1-hydroxyethyl)deuteroporphyrin IX dimethyl ester (**4**). Porphyrins **3** and **4** were prepared either by partial reduction of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**2**) or by oxidation of hematoporphyrin IX dimethyl ester (**1**) by using tetra-*n*-propylammonium perruthenate (Pr^n_4N)(RuO_4) with *N*-methylmorpholine *N*-oxide as an oxidizing agent. The in vivo photosensitizing ability and therapeutic ratios of dimers **9**–**12** were compared with that of Photofrin II in the SMT-F tumor growing subcutaneously in DBA/2 Ha mice. These dimers were found to have better tumorcidal activity than Photofrin II with reduced skin phototoxicity.

Introduction

Photodynamic therapy (PDT) is a new procedure for the treatment of various types of malignant tumors and involves local photochemical activation following accumulation of the photosensitizers in the tumors.[1,2] Currently, Photofrin II (Quadralogic Technology, Vancouver, Canada, stored <−20 °C) enriched in the active components of hematoporphyrin derivative (Hpd) has been used worldwide for tumor photosensitization and more than 4000 patients have been treated so far. Upon light activation, generally delivered from lasers via fiber optics, the sensitizers generate singlet oxygen, which is apparently the cytotoxic agent, causing both vascular damage and injury to tumor cells.[3] Photofrin II is currently in phase III clinical trials for treatment of obstructive endobronchial tumors, tumors of the esophagus, and superficial bladder tumors.

Hematoporphyrin derivative is prepared in two steps by following Lipson's procedure,[4] as modified by Dougherty.[1] Dougherty et al.[5] isolated the active fraction in Hpd by gel-exclusion chromatography representing approximately 45% of the total mixture. This material was found to be responsible for the tumor-photosensitizing ability, was free from most of the monomers, and also appeared to provide a higher therapeutic ratio (tumor vs skin) than the Hpd mixture. Commercially available Photofrin II is chemically similar to the gel-purified Hpd by HPLC.

Chart I



1. $R_1 = R_2 = -CH(OH)CH_3$
2. $R_1 = R_2 = -COCH_3$
3. $R_1 = COCH_3$, $R_2 = -CH(OH)CH_3$
4. $R_1 = -CH(OH)CH_3$, $R_2 = -COCH_3$
5. $R_1 = -COCH_3$, $R_2 = -CH=CH_2$
6. $R_1 = -CH=CH_2$, $R_2 = -COCH_3$
7. $R_1 = -CH(OH)CH_3$, $R_2 = -CH=CH_2$
8. $R_1 = -CH=CH_2$, $R_2 = -CH(OH)CH_3$

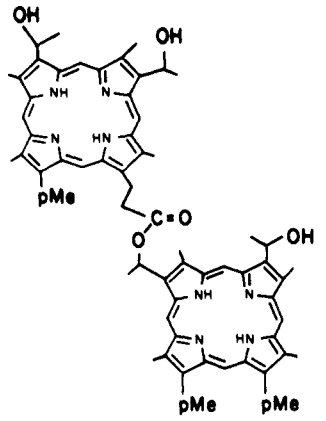
pMe = $-CH_2CH_2CO_2CH_3$

* Address correspondence to Oncologic Foundation of Buffalo.
† University of California.
‡ Roswell Park Memorial Institute.

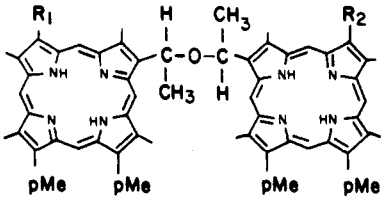
(1) Dougherty, T. J.; Kaufman, J. H.; Goldfarb, A.; Weishaupt, K. R.; Boyle, D.; Mittleman, A. *Cancer Res.* **1976**, *38*, 2628.
(2) Dougherty, T. J. *CRC Crit. Rev. Oncol. Hematol.* **1984**, *2*, 83.
(3) Weishaupt, K. R.; Gomer, C. J.; Dougherty, T. J., *Cancer Res.* **1976**, *36*, 2326.
(4) Lipson, R. L.; Baldes, E. J.; Olsen, A. M. *J. Natl. Cancer Inst.* **1961**, *26*, 1.

0022-2623/90/1833-2032$02.50/0

Chart II



13



14. $R_1 = R_2 = -CH(OH)CH_3$

15. $R_1 = -CH(OH)CH_3$, $R_2 = -CH=CH_2$

pMe = $-CH_2CH_2CO_2CH_3$

Only one of the possible isomers is shown

Recently, a number of studies have been reported on the structure(s) of the active components of Hpd. In summary, it is believed that Photofrin II is a mixture of porphyrins in which from two to six hematoporphyrins, and their dehydration products, are linked either by ether and/or ester linkages.[6-11] To gain further chemical insight into the nature of Photofrin II, we have synthesized related porphyrin dimers with ester linkages[12] and also a series of dimers and higher oligomers with ether linkages,[13,14] as shown in Chart II. Porphyrin dimers with ester linkages were found to have little tumorcidal activity compared with Photofrin II. However, for the Hp dimer with ether linkage, we observed that replacement of one or both 1-hydroxyethyl groups with vinyl substituents made a remarkable difference in photosensitizing ability.[14-16] The best results were obtained when both the 1-hydroxyethyl groups in Hp dimer were replaced with vinyl substituents (i.e. 9).

We present herein the syntheses and tumorcidal activity of divinyl dimer 9 and its possible isomers 10–12. We also report a new and efficient route for the syntheses of (hydroxyethyl)vinyldeuteroporphyrin dimethyl ester and its isomers (7 and 8) from hematoporphyrin IX dimethyl ester (1) (Chart I). Biological studies (tumorcidal activity) were performed by following the procedure as described previously by Dougherty et al.[5] and are discussed briefly in the Experimental Section.

Results and Discussion

Treatment of hematoporphyrin IX dimethyl ester, obtained by esterification of commercially available hematoporphyrin (Hp), with a catalytic amount of tetra-*n*-propylammonium perruthenate (TPAP; Aldrich) with *N*-methylmorpholine *N*-oxide (NMO)[16] gave a mixture of porphyrins. Porphyrins 3 and 4 were the major products along with some minor amounts of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (2) and hematoporphyrin IX dimethyl ester (1). If excess TRAP was used, both of the secondary alcohol groups of Hp were oxidized and 2,4-diacetyldeuteroporphyrin IX dimethyl ester (4) was obtained in quantitative yield. Porphyrins 3 and 4, as a mixture, can also be obtained in >75% yield by partial reduction of porphyrin 2 with sodium borohydride. In an effort to further explore the utility of the TPAP reagent, we also found it to be an excellent oxidizing agent for converting primary alcohols to aldehydes and secondary alcohols to ketones in chlorin and pyrrole chemistry.[31] As reported by Griffith et al.,[16] we also found addition of 4-Å molecular sieves to be beneficial since they remove both the water formed during reaction and the water of crystallization of NMO. 2,4-Diacetyldeuteroporphyrin IX dimethyl ester (2) has been used as a starting material for the syntheses of several porphyrins with biological importance as well as for the preparation of deuterium- and carbon-13-labeled hemins, which have been used frequently in heme protein NMR studies as a probe of the heme environment and heme orientational disorder, as well as in resonance Raman studies.[17-24] Smith et al. have shown that protons of the ring methyl groups adjacent to (conjugated with) acetyl substituents in porphyrin 2 can be easily exchanged with deuterium under mildly basic conditions[25] and this chemistry has been used in the syntheses of a series of deuterium-labeled hemins. Syntheses of these porphyrins would otherwise involve a large number of steps starting with specifically labeled

(5) Dougherty, T. J.; Boyle, D. G.; Weishaupt, K. R.; Henderson, B. A.; Potter, W. R.; Bellnier, D. A.; and Wityk, K. E. *Adv. Exp. Med. Biol.* **1983**, *160*, 3.

(6) Bonnett, R.; Berenbaum, M. C. *Adv. Exp. Med. Biol.* **1983**, *160*, 241.

(7) Dougherty, T. J.; Potter, W. R.; Weishaupt, K. R. *Adv. Med. Biol.* **1984**, 301.

(8) Kessel, D. *Photochem. Photobiol.* **1986**, *44*, 193.

(9) Kessel, D.; Thompson, P.; Musselman, B.; Chang, C. K. *Cancer Res.* **1987**, *47*, 4642.

(10) Scourides, P. A.; Bohmer, R. M.; Kaye, A. H.; Morstyn, G. *Cancer Res.* **1987**, *47*, 3439.

(11) Morris, I. K.; Ward, A. D. *Tetrahedron Lett.* **1988**, *29*, 2501.

(12) Pandey, R. K.; Dougherty, T. J. *Cancer Res.* **1989**, *49*, 2042.

(13) Pandey, R. K.; Dougherty, T. J.; Smith, K. M. *Tetrahedron Lett.* **1988**, *29*, 4657.

(14) Pandey, R. K.; Majchrzycki, D.; Smith, K. M.; Dougherty, T. J. *SPIE Proc.* **1989**, *1065*, 164.

(15) Pandey, R. K.; Dougherty, T. J. *Photochem. Photobiol.* **1988**, *47*, 769.

(16) Griffith, W. P.; Ley, S. V.; Whitcombe, P.; and White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, 1625.

(17) La Mar, G. N.; Budd, D. L.; Viscio, D. B.; Smith, K. M.; Langry, K. C. *Proc. Natl. Acad. Sci., U.S.A.* **1978**, *75*, 5755.

(18) Levy, M. J.; La Mar, G. N.; Jue, T.; Smith, K. M.; Pandey, R. K.; Smith, W. S.; Livingston, D. J.; Brown, W. D. *J. Biol. Chem.* **1985**, *260*, 13694.

(19) La Mar, G. N.; Yamamoto, Y.; Jue, T.; Smith, K. M.; Pandey, R. K. *Biochemistry* **1985**, *24*, 3626.

(20) Pande, U.; La Mar, G. N.; Lecomte, J. T.; Ascoli, F.; Brunori, M.; Smith, K. M.; Pandey, R. K.; Parish, D. W.; Thanbal, V. *Biochemistry* **1986**, *25*, 6538.

(21) Chatfield, M. J.; La Mar, G. N.; Smith, K. M.; Leung, H.-K.; Pandey, R. K. *Biochemistry* **1988**, *27*, 1500.

(22) Constantinidis, I.; Satterlee, J. D.; Pandey, R. K.; leung, H.-K.; Smith, K. M. *Biochemistry* **1988**, *27*, 3069.

(23) La Mar, G. N.; Pande, U.; Hauksson, J. B.; Pandey, R. K.; Smith, K. M. *J. Am. Chem. Soc.* **1989**, *111*, 485.

(24) Lee, H.; Kitagawa, T.; Abe, M.; Pandey, R. K.; Leung, H.-K.; Smith, K. M. *J. Mol. Struct.* **1986**, *146*, 329.

(25) Smith, K. M.; Leung, H.-K.; Parish, D. W. *J. Chem. Res. (S)* **1986**, *324*; *J. Chem. Res. (M)* **1986**, 2743.

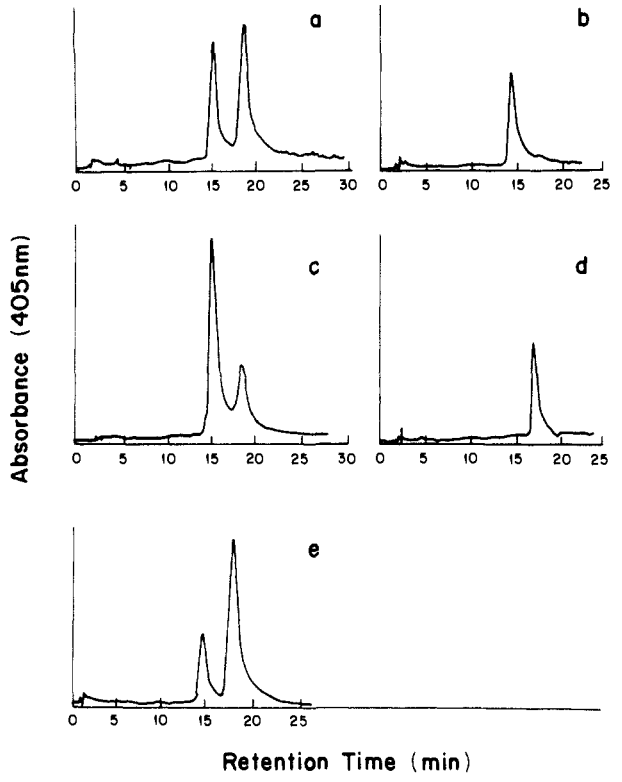


Figure 1. HPLC chromatograms, as carboxylic acids of (a) (hydroxyethyl)vinyldeuteroporphyrin (Hvd), a commercial product, (b) 2-(1-hydroxyethyl)-4-vinyldeuteroporphyrin (**7**), (c) Hvd (commercial product), coinjected with porphyrin **7**, (d) 4-(1-hydroxyethyl)-2-vinyldeuteroporphyrin (**8**), (e) Hvd (commercial product), coinjected with porphyrin **8**. Solvent A (see the text) was used in isocratic mode at a flow rate of 1.5 mL/min with fixed wavelength at 405 nm (column, Partisil 5 C-8).

pyrroles.[26,27] Thus, the preparation of 2,4-diacetyldeuteroporphyrin IX which involves five steps by following the literature procedure can be achieved quantitatively and in only one step from hematoporphyrin (**1**) by using TPAP/NMO (Scheme II). Porphyrin **2** was also prepared by following Jones' oxidation, as reported by Clezy et al.,[28] and under these conditions porphyrins **3** and **4** (as a mixture) were isolated in very low yield. The workup of these reaction was also problematic.

Our next step was to separate the mixture of **3** and **4** into the individual isomers, and this was accomplished by simple preparative TLC, eluting with 2% methanol in dichloromethane. Before proceeding further, the isomeric structures of porphyrins **3** and **4** were confirmed by proton NMR spectroscopy and by melting point comparisons. These porphyrins were then individually dehydrated to the vinyl analogues **5** and **6** by refluxing in 1,2-dichlorobenzene containing a small amount of *p*-toluenesulfonic acid. Reduction with sodium borohydride afforded the corresponding porphyrins **7** and **8** in >75% yield. As the methyl esters, both the isomers have the same retention time in HPLC. However, as the corresponding carboxylic acids, the commercially available Hvd mixture has two peaks with retention times of 14.8 and 17.8 min. As shown in Figure 1, the peak with retention time 14.8 min corresponds to 2-(1-hydroxyethyl)-4-vinyldeuteroporphyrin IX

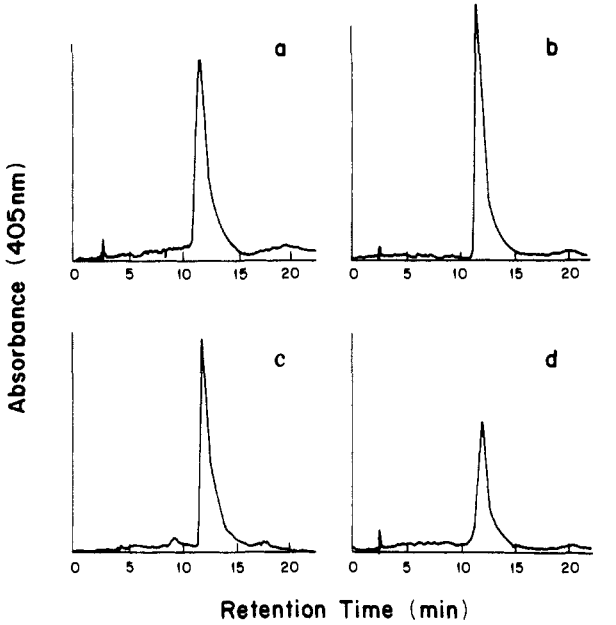


Figure 2. HPLC chromatograms, as methyl esters of (a) porphyrin dimer **9** (as a mixture of isomers), (b) porphyrin dimer **10**, (c) porphyrin dimer **11**, (d) porphyrin dimer **12**. Solvent B (see the text) was used in isocratic mode at a flow rate of 1.5 mL/min with fixed wavelength at 405 nm (column, Partisil 5 C-8).

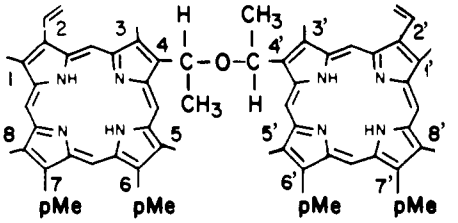
and that with retention time 17.8 min is 4-(1-hydroxyethyl)-2-vinyldeuteroporphyrin IX. These findings were further confirmed by coinjecting the individual isomers along with the mixture which showed enhancement of the particular peak.

For the syntheses of dimers **9–12** we followed the approach reported earlier for the preparation of hematoporphyrin dimers with ether linkages (Scheme I).[13] In brief, for the synthesis of dimer **10**, porphyrin **8** was first reacted with methanesulfonyl chloride (<–70 °C) and the mesylate so obtained was converted into the bromo derivative upon reaction with lithium bromide in THF. The bromo derivative was not isolated but was immediately condensed with porphyrin **8** and the desired dimer **10** was obtained in 30% yield. Other dimers were synthesized along similar lines, as shown in Scheme I. To further confirm that in dimer **12** porphyrins **7** and **8** are linked together, dimer **12** (as the carboxylic acid) was reacted with hydrochloric acid (10%), which provided both of the Hvd isomers with HPLC retention times of 14.8 and 17.8 min. These dimers, as methyl esters, were purified by preparative TLC and purity was ascertained by HPLC. Under our HPLC conditions (Figure 2) we observed the same retention time (12.0 min) for all the methyl ester isomers. However, as carboxylic acids the HPLC chromatogram (Figure 3) of **9** has peaks with retention times of 23.4 and 25.8 min along with minor peaks at 24.7 and 26.9 min. Peaks at 25.8 and 26.9 min were due to dimer **10** as a mixture of diastereomers (*S,S*; *R,R*; *S,R*; *R,S*). Dimers **11** and **12**, as a diastereomeric mixture, were eluted at the same retention times of 23.4 and 24.7 min. However in the case of dimers **10** and **11**, the *R,S* and *S,R* compounds are the same (due to a 180° rotation of the entire molecule in the plane of the page). All the porphyrins, either as monomers or dimers, have either "etio" or "rhodo" type electronic absorption spectra. The different type of absorption spectra are due to the presence of either electron-donating or electron-withdrawing substituents at the peripheral positions of the porphyrin core. The structures of the compounds were confirmed by NMR spectroscopy, mass spectroscopy, and by elemental analyses or high-

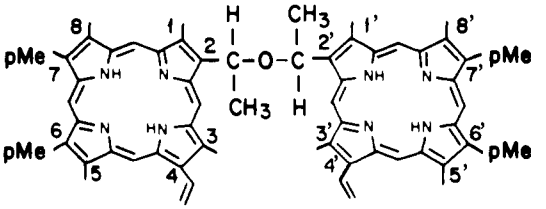
(26) Smith, K. M.; Eivazi, F.; Langry, K. C.; Almeida, J. A. P. B.; Kenner, G. W. *Bio-org. Chem.* **1979**, *8*, 485.
(27) Smith, K. M.; Pandey, R. K. *J. Heterocycl. Chem.* **1983**, *20*, 1383.
(28) Clezy, P. S.; Hai, T. T.; Henderson, R. W.; Thuc, L. *Aust. J. Chem.* **1980**, *33*, 585.

Scheme I

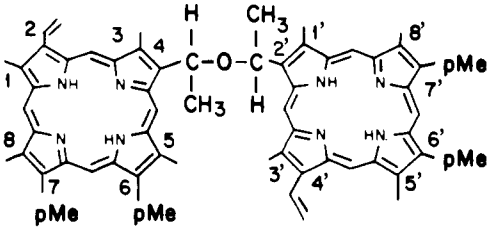
Mix of 7 and 8 → (I) CH_3SO_2Cl, (II) LiBr, (III) Mix of 7 and 8 → Divinyl dimer **9** (Mix of 10, 11 and 12)

Porphyrin 8 → (I) CH_3SO_2Cl, (II) LiBr, (III) Porphyrin 8 → 

10

Porphyrin 7 → (I) CH_3SO_2Cl, (II) LiBr, (III) Porphyrin 7 → 

11

Porphyrin 8 → (I) CH_3SO_2Cl, (II) LiBr, (III) Porphyrin 7 → 

12

pMe = $-CH_2CH_2CO_2CH_3$

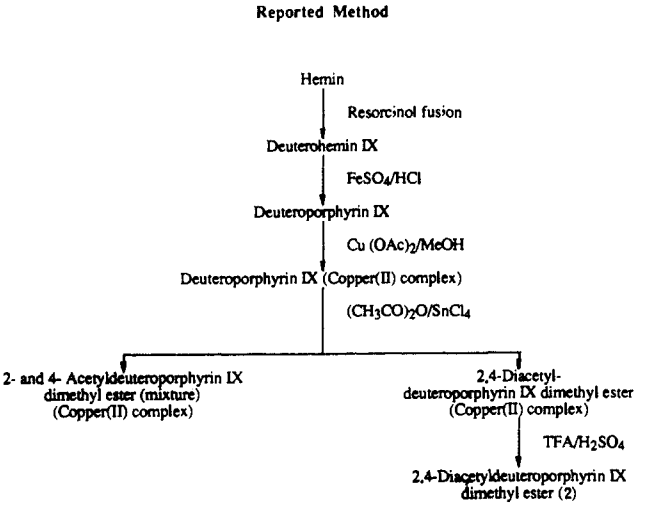
resolution mass spectroscopy. The proton NMR spectra of all the monomers were easy to characterize; the meso protons were observed in the range of 9.5–10.5 ppm and the NH protons were present at -4 to -5 ppm due to the shielding of these protons by the porphyrin ring current. In the case of dimers, we did not observe the NH protons and the meso proton region was complex due to the presence of a mixture of diastereomers. However, we could identify the resonances for almost all the protons; in case of dimer **10**, a quartet at 6.60 ppm was observed for the C*H*(CH_3)O protons, which integrated for two protons. A six-proton doublet at 1.50 ppm corresponds to the 2-CH(CH_3)O resonance. The vinyl resonances were present at 8.00 (—CH=) and 6.20 (=CH_2) ppm. All of the methyl groups and methoxyls were present in the range of 3.0–4.0 ppm. Propionate α-methylenes (nearest to the porphyrin ring) were observed as a multiplet at 4.42 ppm and the resonances for propionate β-methylenes (away from porphyrin ring) were identified at 3.20 ppm. In the case of dimer **11**, the resonances for most of the protons were almost at the same positions as described for dimer **10**, except there was an upfield shift for the vinyl (=CH_2) protons which were observed at 5.52 and 5.30 ppm. In the case of dimer **12**, vinyl resonances were observed at 8.00 ppm as well as 5.52 and 5.30 ppm. This upfield shift might be due to the shielding and possibly depends on porphyrin–porphyrin association, which may vary with the polarity of the NMR solvent. However, we have used only deuterochloroform as the NMR solvent and have not investigated this further. In the mass spectra all the dimers show mass peaks at *m/e* 1199 (M + 1, 100) and simple ether cleavage mass peaks at *m/e* 609 and 591 corresponding to Hvd and its dehydration product (i.e. protoporphyrin IX dimethyl ester).

Biological Results

These dimers were tested for in vivo tumorcidal photosensitizing activity and compared with Photofrin II. The tumor system (subcutaneously implanted SMT-F tumor in DBA/2 mice) and the biological test procedure was followed as described previously.[5] Normal tissue response was tested by exposing the mouse foot in a manner identical with that used in the tumor response test. Water-insoluble porphyrins were first dissolved in Tween 80 overnight and then diluted 10-fold in normal saline for ip injection. The results are summarized in Table I.

As shown in Table I, Photofrin II on day 7 shows 50–60% tumor response (i.e. absence of palpable tumors in 50–60% of mice) at a dose of 4.2 mg/kg with acute skin phototoxicity. If the Photofrin II dose is reduced to 2.0 mg/kg, it was found to be inactive but to have almost the same phototoxicity. We have recently reported that the Hp dimer with ester linkages **13** had little photosensitizing capability.[12] Among porphyrin dimers with ether linkages, Hp dimer **14** did not show any activity at doses similar to those used for Photofrin II. Dimer **15** with one 1-

Scheme II. Synthesis of 2,4-Diacetyldeuteroporphyrin IX Dimethyl Ester (4)



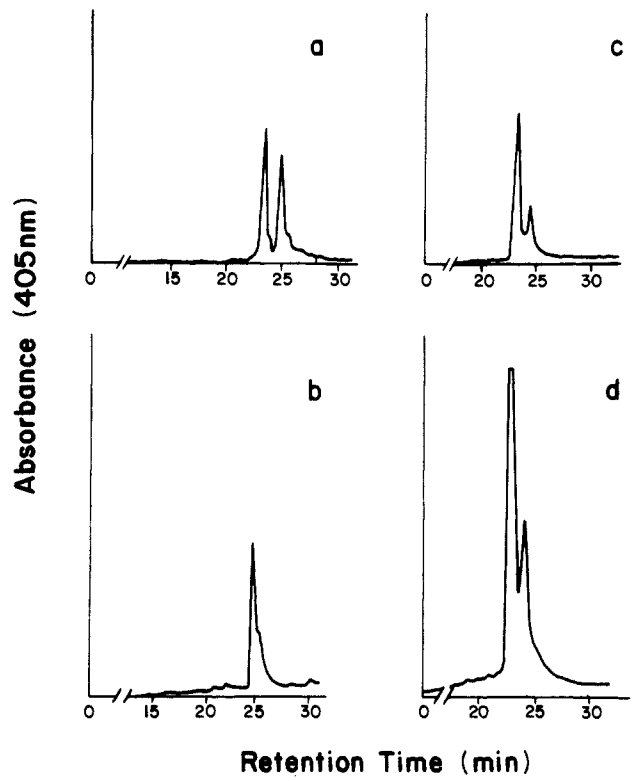


Figure 3. HPLC chromatograms, as carboxylic acids of (a) dimer **9**, (b) dimer **10**, (c) dimer **11**, (d) dimer **12**. Gradient program with solvents A and B was used (see the text) at a constant flow rate of 1.5 mL/min with fixed wavelength at 405 nm (column, Partisil 5 C-8).

hydroxyethyl and one vinyl group showed promising photosensitizing activity with almost no normal tissue (foot) toxicity after day 2, even at a dose of 1 mg/kg if both light and heat (40–41 °C) were used. Surprisingly, if only light was used dimer **15** was found to be inactive, even at a dose of 4.2 mg/kg. Further work in this direction is in progress. However, dimer **9** at a dose of 2.1 mg/kg demonstrated excellent tumorcidal activity (without using heat) with no skin phototoxicity elicited on day 5. These results suggest that dimer **9** clears from the system much faster than does Photofrin II. On the other hand Photofrin II remains in the system for a much longer time and patients are advised to avoid direct sunlight for at least 30 days after the treatment. Dimer **9**, as the methyl ester or as the carboxylic acid, gave almost the same tumor response. Preliminary results show that dimers **10–12** also showed the same efficacy as did dimer **9** (a mixture of **10–12**). Thus, the excellent tumorcidal activity and reduced skin phototoxicity give divinyl dimer **9** a potential advantage over Photofrin II. Further biological studies like distribution and elimination of these dimers in mice as we have previously described with Photofrin II[29] are in progress with ^{14}C-labeled porphyrin dimers.

We also report a one-step synthesis of 2,4-diacetyldeuteroporphyrin IX dimethyl ester (**2**) from hematoporphyrin IX (**1**), along with an improved method for the preparation of Hvd.

Table I. In Vivo Photosensitizing Activity[a]

material	dose, mg/kg	% tumor response[b] day 1–2	day 7
Photofrin II	4.2	100	50
	5.0	100	70
	2.0	0	
Hp dimer ester linkage (**13**)	4.2	0	
Hp dimer ether linkage (**14**)	4.2	0	
Hp/Hvd dimer ether linkage (**15**)	2.1[c]	100	100
	1.0[c]	100	100
	4.2	0	
divinyl dimer (**9**) as methyl ester	2.0	100	90
divinyl dimer (**9**) as carboxylic acid	2.0	100	90
hematoporphyrin[d]	5.0	0	
protoporphyrin[e]	5.0	10	0
(hydroxyethyl)vinyldeuteroporphyrin	5.0	0	

[a] 4.5–5.5 mm diameter tumors were exposed to light from a filtered Xe-arc lamp (283.5 J/cm^2) 20–24 h post ip injection. [b] Percent of nonpalpable tumors; 30 mice, 10 mice/group. [c] When both light and heat (40–41 °C) were used (unpublished results). [d] Dougherty, T. J. (ref 30). [e] Dougherty, T. J., unpublished results.

Experimental Section

Melting points were measured on a hot-stage apparatus and were uncorrected. Silica gel 60 (70–230 mesh, Merck) or neutral alumina (Merck) was 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 was performed with Merck 60 F254 silica gel (precoated sheets, 0.2 mm thick). Reactions were monitored by thin-layer chromatography and spectrophotometry and were carried out under nitrogen and in the dark. Proton NMR spectra were obtained in deuterochloroform solution at 270 MHz with a JEOL FX270 spectrometer. The NMR values are expressed in ppm. Electronic absorption spectra were measured in dichloromethane solution with a Bausch and Lomb

(29) Bellnier, D. A.; Ho, Y. K.; Pandey, R. K.; Missert, J. R.; Dougherty, T. J. *Photochem. Photobiol.* **1989**, *50*, 221.
(30) Dougherty, T. J. *Photochem. Photobiol.* **1983**, *38*, 377.
(31) Pandey, R. K.; Shiau, F. Y.; Smith, K. M., unpublished results.
(32) Abbreviations: PDT, photodynamic therapy; Hpd, hematoporphyrin derivative; Photofrin II, a purified commercial preparation of Hpd; Hp, hematoporphyrin; Hvd, (1-hydroxyethyl)vinyldeuteroporphyrin IX; TPAP, tetra-*n*-propylammonium perruthenate; NMO, *N*-methylmorpholine *N*-oxide; TLC, thick-layer chromatography; NMR, nuclear magnetic resonance; ppm, parts per million; s, singlet; d, doublet; t, triplet; m, multiplet; br, broad; PTS, *p*-toluenesulfonic acid; THF, tetrahydrofuran; HPLC, high-performance liquid chromatography.

Spectronic 2000 spectrophotometer. Mass spectra were obtained on a Kratos MS-80 RFA mass spectrometer with a DS 90 data system. Samples were run in the positive ion mode at 1000 resolving power and a scan rate of 10–30 s per decade. The xenon atom gun was operated at 8–10 kV with approximately 1.0 mA total discharge count. The instrument was calibrated over the appropiate mass region with sodium and cesium iodide salts. HPLC was carried out on a Spectra Physics SP8700 instrument using a Partisil 5 C-8 column connected to a SP4270 integrator and LDCUV III monitor with a fixed wavelength at 405 nm. Two solvent compositions were used in the HPLC analyses: (1) Solvent A was prepared by dissolving anhydrous dibasic sodium phosphate (1.0 g) in 400 mL of water. To this was added HPLC-grade methanol (600 mL). The pH of the solution was adjusted to 7.5 with phosphoric acid. (2) Solvent B was prepared by dissolving anhydrous dibasic sodium phosphate (0.3 g) in 100 mL of water, and to this was added methanol (900 mL) and the pH was adjusted to 7.5 with phosphoric acid. For dimers 9–12 as methyl esters, solvent B was used in the isocratic mode at a flow rate of 1.5 mL/min. For dimers with carboxylic acids, the following gradient program was used at a constant flow rate of 1.5 mL/min: solvent A only from 0 to 10 min (linear gradient), solvent A to solvent B (100%) from 10 to 40 min, finally solvent B from 40 to 55 min. HPLC analysis for Hvd and its isomers was performed by using solvent A in the isocratic mode at a flow rate of 1.5 mL/min. Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN.

2,4-Diacetyl-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,6-tetramethylporphyrin (2). Hematoporphyrin IX dimethyl ester (1, 50 mg) was dissolved in dichloromethane (20 mL) containing a few 4-Å molecular sieves and *N*-methylmorpholine *N*-oxide. The mixture was stirred for 5 min, tetra-*n*-propylammonium perruthenate (7 mg) was added, and the reaction mixture was stirred at room temperature. The reaction was monitored by TLC and was worked up by diluting it with 2% methanol/dichloromethane (50 mL) and then washing with saturated sodium sulfite solution (20 mL), brine (50 mL), and then with saturated copper(II) sulfate solution. It was finally washed with water (3 × 100 mL); the dichloromethane layer was separated and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue which was almost pure with a slight base-line TLC impurity. The residue was passed through a small alumina (Brockmann Grade III) column, eluted with 25% methanol in dichloromethane. Evaporation of the solvent and crystallization with dichloromethane/hexane afforded the title porphyrin in 98% yield (48.5 mg). Vis λ_{max}: 421 nm (ϵ 142400), 514 (13300), 549 (7600), 586 (6200), 638 (3200). NMR (δ, ppm): 10.25, 10.10, 9.35, 9.00 (each s, 1 H, 4 meso H); 4.12 (m, 4 H, 2 $CH_2CH_2CO_2CH_3$); 3.62 and 3.60 (each s, 3 H, 2 CO_2CH_3); 3.50, 3.45, 3.25, 3.22 (each s, 3 H, 4 CH_3); 3.12 and 3.60 (each s, 3 H, 2 $COCH_3$); 3.18 (m, 4 H, 2 $CH_2CH_2CO_2CH_3$); −4.98 (s, 2 H, 2 NH).

2-Acetyl-4-(1-hydroxyethyl)-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethylporphyrin (3) and 4-Acetyl-2-(1-hydroxyethyl)-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethylporphyrin (4). These porphyrins, as a mixture, were prepared by two methods.

(1) **With TPAP as an Oxidizing Agent.** Hematoporphyrin IX dimethyl ester (50 mg) was dissolved in dry dichloromethane (20 mL) and a few 4-Å molecular sieves, along with TPAP (5 mg) and NMO (30 mg), were added. The reaction mixture was stirred for only 15 min and worked up as discussed for the foregoing porphyrin. The residue obtained after evaporation of the solvent was found to be a mixture of three compounds. It was separated on preparative TLC plates, eluting with 5% methanol/dichloromethane. The most mobile band was characterized as porphyrin 2 and the least mobile band was identified as the starting porphyrin. The band in between was found to be the required mixture of porphyrins and was crystallized from dichloromethane/hexane in 60% yield (29.6 mg).

(2) **Partial Reduction of 2,4-Diacetyl-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethylporphyrin.** Porphyrin 2 (250 mg) was dissolved in dichloromethane (50 mL) and cooled to 5 °C by using an ice bath. Sodium borohydride (150 mg) in chilled methanol (10 mL) was added and the reaction mixture was stirred for 20 min (monitored by analytical TLC); acetic acid (3 mL) was added to quench the unreacted sodium borohydride and the mixture was poured into water. It was extracted with dichloromethane (3 × 100 mL) and washed with aqueous sodium bicarbonate (10%) and again with water (2 × 100 mL), and the organic phase was dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue which was found to be a mixture of starting porphyrin and hematoporphyrin, as minor products, and the desired title porphyrins (18.8 mg, 75%).

Separation of Isomers. The mixture of porphyrins 3 and 4 was separated into individual isomers by preparative thin-layer chromatography, eluting with 2% methanol in dichloromethane. Thus, from 300 mg of the mixed porphyrins, 120 mg of 2-acetylporphyrin (3) and 135 of 4-acetylporphyrin (4) were isolated. An intermediate band obtained as a mixture could be reseparated by following the same procedure.

Most Mobile Band: 4-Acetyl-2-(1-hydroxyethyl)deuteroporphyrin Dimethyl Ester (4). Mp: 230–232 °C (lit.[28] mp 229–230 °C). Vis λ_{max}: 409 nm (ϵ 159700), 510 (9600), 547 (10300), 577 (6700), 637 (1700). NMR (δ, ppm): 10.50, 9.56, 9.50, 9.40 (each s, 1 H, 4 meso H); 5.60 (q, 1 H, $CH(OH)CH_3$); 4.20 and 4.10 (each t, 2 H, 2 $CH_2CH_2CO_2CH_3$); 3.68, 3.66 (each s, 3 H, 2 CO_2CH_3); 3.64, 3.60, 3.40, and 3.25 (each s, 3 H, 4 CH_3); 3.15 (m, 4 H, 2 $CH_2CH_2CO_2CH_3$); 3.00 (s, 3 H, $COCH_3$); 1.70 (d, 3 H, $CH(OH)CH_3$); −4.56 (s, 2 H, 2 NH). Anal. Calcd for $C_{36}H_{40}N_4O_6$: C, 69.21; H, 6.45; N, 8.96. Found: C, 69.01; H, 6.35; N, 8.71.

Least Mobile Band: 2-Acetyl-4-(1-hydroxyethyl)deuteroporphyrin IX Dimethyl Ester (3). Mp: 247–249 °C (lit.[28] mp 260–266 °C). Vis λ_{max}: 410 nm (ϵ 156500), 510 (9600), 550 (10300), 580 (6700), 635 (1700). NMR (δ, ppm): 10.48, 10.00, 9.78, 9.58 (each s, 1 H, 4 meso H); 6.20 (q, 1 H, $CH(OH)CH_3$); 4.25 (m, 4 H, 2 $CH_2CH_2CO_2CH_3$); 3.70 and 3.65 (each s, 3 H, 2 CO_2CH_3); 3.52, 3.48, 3.46, 3.40 (each s, 3 H, 4 CH_3); 3.12 (s, 3 H, $COCH_3$); 2.00 (d, $CH(OH)CH_3$); −4.50 (s, 2 H, 2 NH). Anal. Calcd for $C_{36}H_{40}N_4O_6$: C, 69.21; H, 6.45; N, 8.96. Found: C, 68.91; H, 6.39; N, 8.80.

2-Acetyl-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-4-vinylporphyrin (5). 2-Acetyl-4-(1-hydroxyethyl)-deuteroporphyrin IX dimethyl ester (150 mg) in 1,2-dichlorobenzene (80 mL) containing *p*-toluenesulfonic acid (380 mg) was heated at 145 °C for 40 min as nitrogen was rapidly passed into the flask, agitating the surface of the solution. The reaction mixture was diluted with dichloromethane (100 mL) and washed with water (3 × 100 mL). The dichloromethane layer was separated and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue which was redissolved in a minimum quantity of dichloromethane and treated with ethereal diazomethane. The solvent was evaporated and the residue was passed through a short alumina (Brockmann Grade III) column, eluted with chloroform. The residue obtained after evaporating the dichloromethane was crystallized from dichloromethane/hexane to give the title porphyrin in 75% yield (110 mg). Mp: 256–258 °C. Vis λ_{max}: 413 nm (ϵ 173400), 512 (12800), 550 (11600), 580 (7800), 637 (2700). NMR (δ, ppm): 10.70, 9.98, 9.85, 9.80 (each s, 1 H, 4 meso H); 8.20 (m, 1 H, $CH=CH_2$); 6.40 and 6.20 (each d, 1 H, $CH=CH_2$); 4.25 (m, 4 H, 2 $CH_2CH_2CO_2CH_3$); 3.72, 3.70 (each s, 3 H, 2 CO_2CH_3); 3.62, 3.56, 3.50, 3.25 (each s, 3 H, 4 CH_3); 3.25 (m, 4 H, 2 $CH_2CH_2CO_2CH_3$); 3.12 (s, 3 H, $COCH_3$); −4.1 (s, 2 H, 2 NH).

4-Acetyl-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-2-vinylporphyrin (6). 4-Acetyl-2-(1-hydroxyethyl)-deuteroporphyrin IX dimethyl ester (120 mg) was dehydrated with *p*-toluenesulfonic acid (310 mg) in *o*-dichlorobenzene (60 mL) along similar lines as discussed for porphyrin 5. The product was obtained in 76% yield (88 mg). Mp: 160–163 °C. Vis λ_{max}: 413 nm (ϵ 165600), 512 (11900), 550 (10600), 580 (7200), 637 (2500). NMR (δ ppm): 10.52, 9.66, 9.54, 9.51 (each s, 1 H, 4 meso H); 7.90 (m, 1 H, $CH=CH_2$); 6.20 and 6.10 (each d, 1 H, $CH=CH_2$); 4.30, 4.20 (each t, 2 H, 2 $CH_2CH_2CO_2CH_3$); 3.70, 6.68 (each s, 3 H, 2 CO_2CH_3); 3.60, 3.50, 3.42, 3.40 (each s, 3 H, 4 CH_3); 3.20 (m, 4 H, 2 $CH_2CH_2CO_2CH_3$); 3.15 (s, 3 H, $COCH_3$); −4.48 (br s, 2 H, 2 NH).

2-(1-Hydroxyethyl)-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-4-vinylporphyrin (7). Porphyrin 5 (80 mg) was reacted with sodium borohydride (100 mg) in chilled methanol (10 mL) by following the method as described for porphyrins 2 and 3. The residue was almost pure with only a slight base-line TLC impurity and thus it was passed through a short alumina

(Brockmann Grade III) column, eluting with 5% methanol/dichloromethane. The product obtained after evaporating the solvent afforded the title porphyrin in 90% yield (54 mg). Mp: 155–158 °C. Vis λ_{max}: 403 nm (ϵ 142900), 502 (11700), 537 (8400), 572 (5500), 625 (3400). NMR (δ, ppm): 10.20, 10.10 (each s, 1 H, 2 meso H); 9.88 (s, 2 H, 2 meso H); 8.28 (m, 1 H, C*H*=CH_2); 6.35, 6.20 (each d, 1 H, CH=C*H*$_2$); 6.18 (q, 1 H, C*H*(OH)CH_3); 4.30 (m, 4 H, 2 C*H*$_2$$CH_2CO_2CH_3$); 3.70 (s, 6 H, 2 CO_2CH_3); 3.60 and 3.48 (each s, 3 H, 2 CH_3); 3.50 (s, 6 H, 2 CH_3); 3.20 (t, 4 H, 2 CH_2C*H*$_2$$CO_2CH_3$); 2.08 (d, 3 H, CH(OH)C*H*$_3$); −4.00 (s, 2 H, 2 NH). Anal. Calcd for $C_{36}H_{40}N_4O_5$: C, 71.03; H, 6.62; N, 9.20. Found: C, 70.57; H, 6.80; N, 9.21.

4-(1-Hydroxyethyl)-6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-2-vinylporphyrin (8). Porphyrin **6** (80 mg) was reduced with sodium borohydride (100 mg) along similar lines to those discussed for the foregoing porphyrin, and the title compound was isolated in 91% yield (73 mg). Mp: 164–166 °C. Vis λ_{max}: 403 nm (ϵ 142000), 502 (11800), 537 (8500), 570 (5500), 625 (3500). NMR (δ, ppm): 10.20, 10.15, 9.98, 9.96 (each s, 1 H, meso H); 8.20 (m, 1 H, C*H*=CH_2); 6.40 and 6.18 (each d, 1 H, CH=C*H*$_2$); 6.20 (q, 1 H, C*H*(OH)CH_3); 4.40 (m, 2 H, 2 C*H*$_2$$CH_2CO_2CH_3$); 3.70 (s, 9 H, 2 CO_2CH_3 and 1 CH_3); 3.58, 3.56, 3.42 (each s, 3 H, 3 CH_3); 3.25 (t, 4 H, 2 CH_2C*H*$_2$$CO_2CH_3$); 2.10 (d, 3 H, CH(OH)C*H*$_3$); −4.00 (s, 2 H, 2 NH). Anal. Calcd for $C_{36}H_{40}N_4O_5$: C, 71.03; H, 6.62; N, 9.20. Found: C, 70.82; H, 6.55; N, 8.84.

Synthesis of Dimer 9. This dimer was obtained as a mixture of isomers **10–12** and was synthesized by reacting a mixture of porphyrins **7** and **8** (50 mg) in dry dichloromethane (15 mL) with methanesulfonyl chloride (200 μL) and triethylamine (350 μL) at −70 °C under a nitrogen atmosphere for 2 h. Lithium bromide (30 mg) in dry THF (5 mL) was then added and the mixture was stirred for an additional 1 h. The bromo analogue was not isolated but was immediately reacted with starting porphyrin mixture (**7** and **8**, 50 mg) in dry THF (10 mL). The stirring was continued for another 1 h. It was then poured into water, extracted with dichloromethane, and washed with aqueous sodium bicarbonate solution (10%) and then again with water. The dichloromethane layer was dried over anhydrous sodium sulfate. Evaporation of the solvent afforded a residue which was found to be a mixture of three components by analytical TLC. The most mobile fraction was identified as protoporphyrin IX dimethyl ester (5%). NMR (δ, ppm): 10.20, 10.18, 10.12, 10.00 (each s, 1 H, 4 meso H); 8.25 (m, 2 H, 2 C*H*=CH_2); 6.15 and 6.40 (each d, 2 H, 2 CH=C*H*$_2$); 4.40 (m, 4 H, 2 C*H*$_2$$CH_2CO_2CH_3$); 3.50–3.70 (singlets merged together, 18 H, 2 CO_2CH_3 and 4 CH_3); 3.26 (t, 4 H, 2 CH_2C*H*$_2$$CO_2CH_3$); −4.0 (s, 2 H, 2 NH). MS: mass peak *m/e* 591 (M + 1, 100). The third fraction was identified as starting material and the fraction in between was characterized as the desired dimer. The NMR spectrum of the mixture was complex. The resonances best assigned are as follows: 9.30–10.00 (meso H); 8.0 (m, C*H*=CH_2); 6.60 (q, C*H*(CH_3)O); 6.05–6.20 (m, CH=C*H*$_2$); 5.30–5.50 (m, CH=C*H*$_2$); 4.40 (m, C*H*$_2$$CH_2CO_2CH_3$); 3.30–4.00 (peripheral CH_3 and CO_2CH_3); 3.36 (m, CH_2C*H*$_2$$CO_2CH_3$) 2.50 (d, CH(C*H*$_3$)O). MS: *m/e* 1199 (M + 1, 100), 609 (10), 591 (30). Yield: 40 mg (39.5%).

Other dimers **10–12** were prepared along similar lines. The quantity of the starting porphyrin(s) used, yield, melting point and other analytical data are as follows.

Bis[1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-2-vinylporphin-4-yl]ethyl] Ether (10). Porphyrin **8** (25 mg) in dry dichloromethane (10 mL) and triethylamine (200 μL) was reacted with methanesulfonyl chloride (100 μL) and lithium bromide (15 mg) in dry THF (5 mL). It was reacted with porphyrin **8** (25 mg) and the product was isolated in 38.5% (19 mg) yield. Mp: 106–108 °C. Vis λ_{max} (CH_2Cl_2): 399 nm (ϵ 177000), 505 (17800), 537 (12000), 574 (8300), 626 (4550). NMR (δ, ppm): 9.2–10.0 (multiple peaks, 8 H, 8 meso H); 8.00 (m, 2 H, 2 C*H*=CH_2); 6.60 (q, 2 H, 2 C*H*(O)CH_3); 6.10, 6.20 (each d, 2 H, 2 CH=C*H*$_2$); 4.40 (m, 8 H, 4 C*H*$_2$$CH_2CO_2CH_3$); 3.75, 3.73 (each s, 6 H, 4 $CH_2CH_2CO_2$C*H*$_3$); 3.68, 3.70 (each s, 6 H, peripheral CH_3); 3.40 and 3.20 (each s, 6 H, 4 CH_3); 3.39 (m, 8 H, 4 CH_2C*H*$_2$$CO_2CH_3$); 2.50 (d, 6 H, 2 CH(O)C*H*$_3$). MS: *m/e* 1200 (M + 2, 100), 608 (15), 590 (30). HRMS: calcd for $C_{72}H_{78}N_8O_9$ 1199.5970 (M + 1), found 1199.5983 (M + 1).

Bis[1-[6,7-bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-4-vinylporphin-2-yl]ethyl] Ether (11). Porphyrin **7** (25 mg) in dry dichloromethane (10 mL) and triethylamine (200 μL) was reacted with methanesulfonyl chloride (100 μL) and lithium bromide (15 mg) in dry THF (5 mL). The product was reacted with porphyrin **7** (25 mg) and the title compound was isolated in 36% yield (17 mg). Mp: 108–110 °C. Vis λ_{max}: 400 nm (ϵ 178000), 505 (18000), 537 (8400), 626 (4600). NMR (δ, ppm): 9.40–10.0 (8 H, 8 meso H); 6.62 (q, 2 H, 2 C*H*(CH_3)O); 5.55 and 5.30 (m, 4 H, 2 CH=C*H*$_2$); 4.40–4.60 (m, 8 H, 4 C*H*$_2$$CH_2CO_2CH_3$); 3.70 (s, 18 H, 4 CO_2CH_3, 2 CH_3); 3.60, 3.52, 3.22 (each s, 6 H, 6 CH_3); 3.35 (m, 8 H, 4 CH_2C*H*$_2$$CO_2CH_3$); 2.50 (d, 6 H, 2 CH(C*H*$_3$)O). MS: *m/e* 1199 (M + 1, 100), 609 (25), 591 (15). HRMS: calcd for $C_{72}H_{78}N_8O_9$ 1199.5970 (M + 1), found 1199.5980 (M + 1).

1-[6,7-Bis[2-(methoxycarbonyl)ethyl]-1,3,5,8-tetramethyl-2-vinylporphin-4-yl]ethyl 1-[6′,7′-Bis[2′-(methoxycarbonyl)ethyl]-1′,3′,5′,8′-tetramethyl-4′-vinylporphin-2′-yl]ethyl Ether (12). Porphyrin **8** (14 mg) in dry dichloromethane (5 mL) was reacted with methanesulfonyl chloride (70 μL), triethylamine (150 μL), and lithium bromide (10 mg) in dry THF (3 mL). It was then reacted with porphyrin **7** (14 mg) and the title dimer was isolated in 36% yield (10 mg). Mp: 97–98 °C. Vis λ_{max}: 400 nm (ϵ 178000), 505 (17900), 538 (12000), 574 (8100), 626 (4500). NMR (δ, ppm): 9.50–10.0 (8 H, 8 meso H), 8.00 (H, CH=CH_2), 6.65 (q, 2 H, 2 0C*H*CH_3), 6.10, 5.50, 5.30 (each m, 2-CH=CH_2 and 4′-CH=CH_2), 4,48 (m, 8 H, C*H*$_2$$CH_2CO_2CH_3$), 3.80, 3.78, 3.70, 3.56, 3.30 (each s, CH_3 and CO_2CH_3), 3.35 (m, 8 H, CH_2C*H*$_2$$CO_2CH_3$), 2.50 (d, 6 H, OCHC*H*$_3$), −4.5 (s, 4 NH). MS: *m/e* 1199 (M + 1, 100), 609 (10), 591 (30). HRMS: calcd for $C_{72}H_{78}N_8O_9$ 1199.5970 (M + 1), found 1199.5978 (M + 1).

Biological Studies. For studies of tumorcidal activity, female DBA/2 Ha-DD mice were used. When the mice were 5–6 weeks old and weighed between 16 and 22 g, each was implanted subcutaneously with a single 1-mm section of SMT-F tumor obtained from a donor mouse of the same strain and sex. These tumors grew to approximately 5 × 5 mm in orthogonal directions in about 5 days, at which time the mice were injected intraperitoneally with the drug doses as shown in Table I. After approximately 24 h the mice were restrained in aluminum holders so that the tumor plus a 2 cm diameter margin were exposed to the activating light. Illuminations were performed with a 1000-W Xe-arc lamp filtered to pass only light at wavelengths between 610 and 690 nm. The fluence rate was 157.5 mW/cm^2 as measured with a Coherent 210 power meter. Each tumor was illuminated for 30 min, yielding an incident fluence of 283.5 J/cm^2. Tumor size as well as appearance of the overlying skin were recorded daily; lack of the palpable tumor mass on day 7 after illumination was considered a short-term cure. There were ten mice/group per photosensitizer tested. Photosensitizers that yielded at least 50% short-term cure were considered photoactively equivalent to Photofrin II. Porphyrins, as methyl esters, were dissolved in a minimum quantity of Tween 80 and then diluted with saline solution. Porphyrins as carboxylic acids and Photofrin II were injected either in 0.9% saline or in Tween 80/saline solution. We have found that Tween 80 does not affect the uptake/retention of the photosensitizers.

Acknowledgment. We thank Adam Sumlin (Roswell Park) for his help in the biological studies and Joseph Missert (Oncologic Foundation) for his assistance. We appreciate the help rendered by Dr. James H. McReynolds and thank Thomas Smith (Department of Biophysics, SUNY, Buffalo) for mass spectral and NMR studies.