



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US93/00060 <b>(22) International Filing Date:</b> 7 January 1993 (07.01.93)  <b>(30) Priority data:</b> 822,409                      17 January 1992 (17.01.92)      US  <b>(71) Applicant:</b> HEALTH RESEARCH INC. [US/US]; 666 Elm Street, Buffalo, NY 14263 (US).  <b>(72) Inventors:</b> PANDEY, Ravindra, K. ; 75 Lemay Court, Williamsville, NY 14221 (US). DOUGHERTY, Thomas, J. ; 2306 West Oakfield, Grand Island, NY 14072 (US).  <b>(74) Agents:</b> BOZICEVIC, Karl et al.; Morrison & Foerster, 755 Page Mill Road, Palo Alto, CA 94304-1018 (US).	<b>(81) Designated States:</b> AU, CA, FI, JP, KR, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>With international search report.</i>	
<b>(54) Title:</b> PYROPHEOPHORBIDES AND THEIR USE IN PHOTODYNAMIC THERAPY		
<b>(57) Abstract</b>		
<p>Pyropheophorbide compounds are injected into a host and accumulate in tumor tissue to a higher degree than surrounding normal tissues. When the pyropheophorbide compounds are exposed to a particular wavelength of light the compounds become cytotoxic and destroy the tumor or diseased tissue without causing irreversible normal tissue damage. The pyropheophorbide compounds have shown improved results as compared to drugs currently used in photodynamic therapy. Further, they absorb light further in the red, optimizing tissue penetration and are retained in the skin for short time periods relative to other drugs used in photodynamic therapy.</p>		

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5                                    PYROPHEOPHORBIDES AND THEIR  
   USE IN PHOTODYNAMIC THERAPY

Cross-References

10                                    This application is a continuation-in-part of  
our earlier filed application Serial No. 07/597,786 filed  
October 15, 1990 which is a continuation of application  
Serial No. 07/221,804 filed July 20, 1988 which is now  
U.S. Patent 5,002,962 issued March 26, 1991 both of which  
are incorporated herein by reference and to which we  
15                                    claim priority under 35 USC §120.

FIELD OF THE INVENTION

20                                    This invention relates generally to  
photosensitive therapeutic compounds and photodynamic  
therapy (PDT). More particularly, the invention relates  
to pyropheophorbides, formulations that contain such and  
their use in the treatment of cancer.

25                                    BACKGROUND OF THE INVENTION

As described in U.S. Patent 5,002,962,  
porphyrin related compounds accumulate at higher  
concentrations in tumor tissue as compared to normal  
tissue, and that irradiation of these compounds using  
30                                    light of the proper wavelength results in an energized  
form which, upon decay, results in cytotoxicity. It is  
believed that excitation of the porphyrin or related  
material results in the formation of singlet oxygen which  
is in fact the toxic agent. However, the compounds  
35                                    administered apparently do not degrade in this process.

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Literature relating to the use of "hematoporphyrin derivative" (HPD) describes this process utilizing a preparation obtained when hematoporphyrin dichloride is treated using the procedure of Lipson, R.L., et al, J National Cancer Inst (1961) 26:1-8. More recently, it has been shown that if this hematoporphyrin derivative is treated at a suitable pH, aggregation occurs and the active material in the mixture can be prepared in crude form as a size segregated aggregate (see, for example, U.S. Patent 4,649,151, incorporated herein by reference). This preparation is commercially available under the trademark Photofrin.

The preparation marketed as the Photofrin composition is a mixture. The mixture contains porphyrins joined by ether linkages (Dougherty, T.J., et al, Adv Exp Med Bio (1983) 160:3-13), and more recently, Kessel, D., et al Photochem Photobiol (1987) 46:463-568, has shown that ester linked porphyrins are contained in this mixture as well. Scourides, P.A.; et al, Cancer Res (1987) 47:3439-3445 have synthesized an oligomeric mixture of ether linked porphyrins starting from hematoporphyrin dimethyl esters. The mixture was active in PDT, but was as complex a mixture as the Photofrin preparation. Dimers of hematoporphyrin joined by ester linkages have also been prepared by Pandey, R.K., et al, Cancer Res (in press) and the dimers prepared were shown to be absent from the mixture in the Photofrin composition as well as inactive in an in vitro assay.

Thus, it is known in the art that some elements of a mixture prepared when HPD is aggregated and segregated into higher molecular weight components are active in photodynamic therapy. Earlier, the present inventors prepared single compound compositions useful in PDT as disclosed in U.S. Patent 5,002,962. The purified and defined compositions disclosed in U.S. Patent

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5,002,962 are useful in photodynamic therapy as are compounds and methods disclosed in U.S. Patents 4,920,143 and 4,883,790.

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SUMMARY OF THE INVENTION

Pyropheophorbide compounds and pharmaceutical compositions containing such compounds can be used in methods of photodynamic therapy. The pyropheophorbides are encompassed by the following general structural formula I or II.

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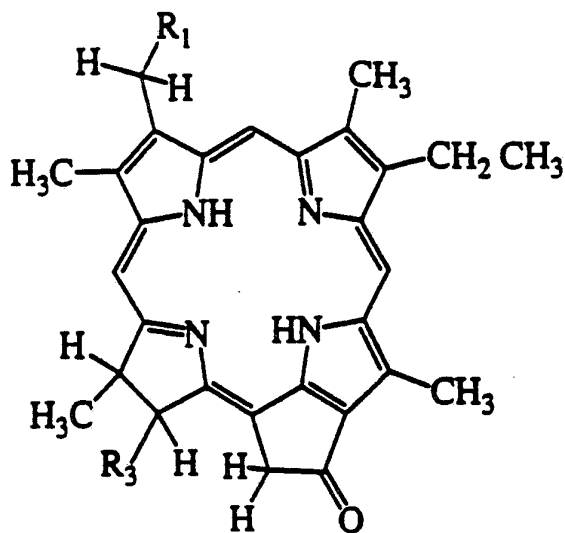
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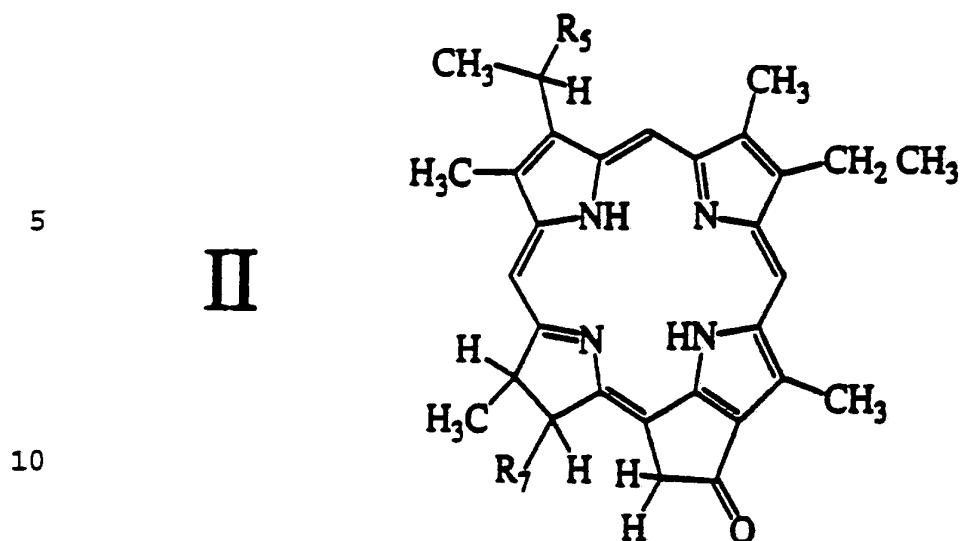
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I



wherein  $R_1$  is  $CH_2OR_2$  where  $R_2$  is a primary or secondary alkyl containing 1 to 20 carbons; and  $R_3$  is  $-CO_2R_4$  where  $R_4$  is H or an alkyl containing 1 to 20 carbons. Other compounds of the invention are encompassed by formula II as follows:

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wherein  $R_5$  is  $-OR_6$  where  $R_6$  is a primary or secondary alkyl containing 1 to 20 carbons and  $R_7$  is  $-CO_2R_8$  where  $R_8$  is H or an alkyl containing 1 to 20 carbons. Particularly preferred compounds are where  $R_5$  is -O-hexyl and  $R_7$  is  $-CO_2H$  or  $-CO_2CH_3$ . The pyropheophorbides of the invention are combined with excipients to provide pharmaceutically acceptable formulations suitable for use in photodynamic therapy.

The invention also includes methods of synthesizing compounds of formula I and II.

The invention includes injectable pharmaceutical compositions containing the pyropheophorbide compounds of the invention as active ingredients and to methods of conducting photodynamic therapy using the compounds and compositions of the invention.

The invention also includes the pyropheophorbide compounds of the invention conjugated to a ligand which is capable of binding a specific receptor such as a cellular receptor, or an antibody which is capable of binding to a particular antigen and to compositions containing these conjugates and methods of

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conducting photodynamic therapy using the conjugates and their compositions.

A primary object of the invention is to provide pyropheophorbide compounds, pharmaceutical compositions  
5 containing such compounds and method of treatment carried out using such compounds in a photodynamic therapy.

Other objects are to provide methods of treating humans with tumor cells which cells replicate abnormally fast, treating atherosclerosis or inactivating  
10 bacteria or virus infections.

A feature of the present invention is that the pyropheophorbide compounds of the invention absorb light further into the red portion of the spectrum as compared with conventional compounds used in photodynamic therapy.

15 An advantage of the present invention is that the pyropheophorbide compounds and pharmaceutical compositions of the invention optimize tissue penetration and are retained in the skin for relatively short periods of time as compared with other compounds used in  
20 photodynamic therapy.

Another advantage of the present invention is that the pyropheophorbide compounds of the invention have a greater toxicity with respect to tumor cells and diseased tissue as compared with the toxicity of  
25 conventional compounds used in photodynamic therapy.

Another advantage of the invention is that the pyropheophorbides can be synthesized as free acids (e.g. in formula I or II when  $R_3$  or  $R_7$  are  $-CO_2H$ ) allowing ease in formulation without the need for liposomes or  
30 detergents.

Another advantage of the invention is the pyropheophorbide of the invention are active at very low doses of injected material as compared to conventional photosensitizers used in photodynamic therapy.

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These and other objects, advantages and features of the present invention will become apparent to those persons skilled in the art upon reading the details of the structure, synthesis and usage as more fully set forth below, reference being made to the accompanying structural formals forming a part herein wherein like symbols refer to like molecular moieties throughout.

#### Brief Description of the Drawings

Figure 1 is a FAB mass spectrum of the compound of formula II(a).

#### DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

Before the present pyropheophorbide compounds, pharmaceutical compositions, methods of synthesizing and using such compounds are disclosed, it is to be understood that this invention is not limited to the particular compounds, compositions, methods of use or synthesis as described as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims.

It is to be noted that as used in this specification and the appended claims, the singular forms "a", "and" and "the" include plural reference unless the context clearly dictates otherwise thus, for example, reference to "a pyropheophorbide" includes mixtures of such pyropheophorbides, reference to "an antibody" includes mixtures of such antibodies and reference to "the method of treatment" includes reference to like methods which will become apparent to those skilled in the art upon reading this disclosure.

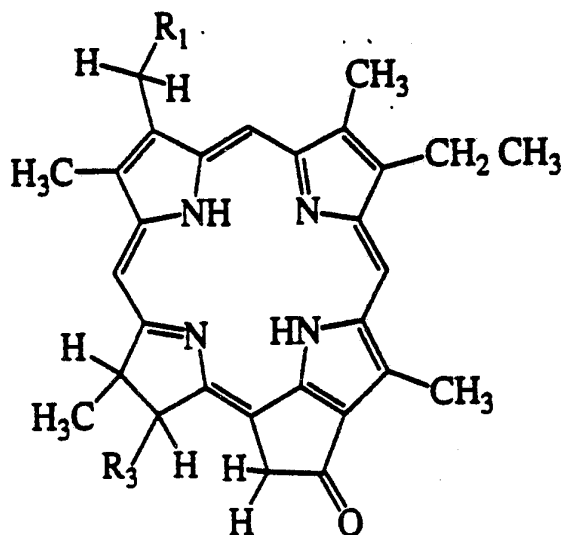


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Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art of photodynamic therapy. Although any methods and materials similar or equivalent to those described herein may be used in the practice or testing of the present invention, attempts have been made to describe preferred methods and materials below.

The essence of the invention is the disclosure of novel compounds and pharmaceutical compositions containing such compounds which have been found to be highly effective in the treatment of cancer when used in connection with a photodynamic therapy. More specifically, the compounds are pyropheophorbide compounds which are encompassed by the following general structural formulae I and II.

20



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I

30

wherein  $R_1$  is  $CH_2OR_2$  where  $R_2$  is a primary or secondary alkyl containing 1 to 20 (preferably 5-20) carbons; and  $R_3$  is  $-CO_2R_4$  where  $R_4$  is H or an alkyl containing 1 to 20

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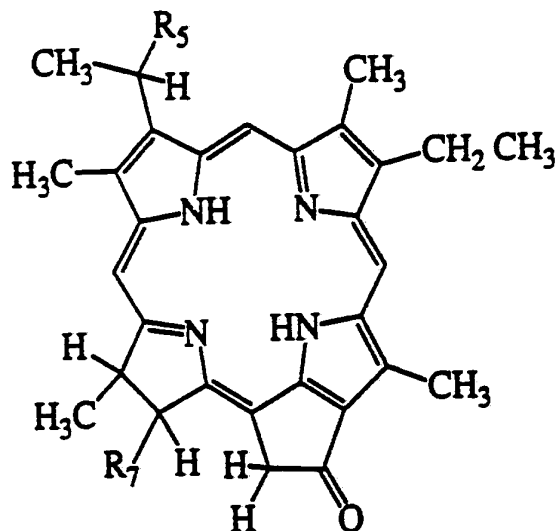
carbons. Preferred compounds are when  $R_1$  is  $-\text{CH}_2-\text{O}-\text{hexyl}$  and  $R_3$  is  $-\text{CO}_2\text{CH}_3$  or  $-\text{CO}_2\text{H}$ . Other compounds of the invention are encompassed by formula II as follows:

5

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II

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wherein  $R_5$  is  $-\text{OR}_6$  where  $R_6$  is a primary or secondary  
20 alkyl containing 1 to 20 (preferably 5-20) carbons and  $R_7$   
is  $-\text{CO}_2\text{R}_8$  where  $R_8$  is H or an alkyl containing 1 to 20  
carbons. Particularly preferred compounds are where  $R_5$   
is  $-\text{O}-\text{hexyl}$  and  $R_7$  is  $-\text{CO}_2\text{H}$  or  $-\text{CO}_2\text{CH}_3$ .

The pyropheophorbide compounds of structural  
25 formulae I and II can be formulated into pharmaceutical  
compositions and administered to patients in  
therapeutically effective amounts in order to treat  
cancer.

Although the invention encompasses all of the  
30 compounds of structural formulae I and II it has been  
found that the compound of structural formula IIa is

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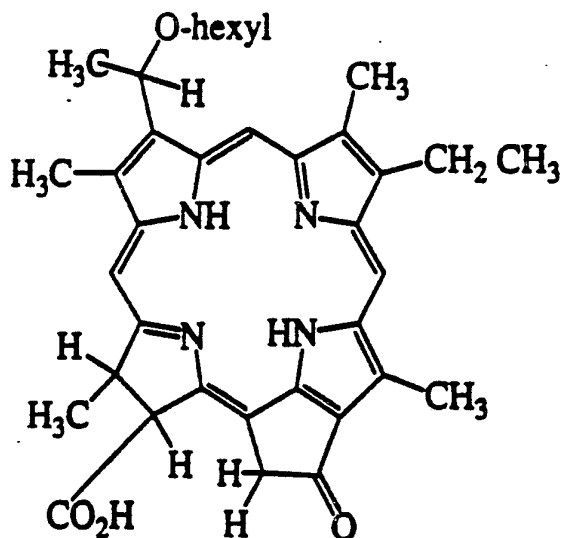
particularly effective in the treatment of cancer when used in connection with photodynamic therapy. Structural formula IIa is put forth below:

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IIa



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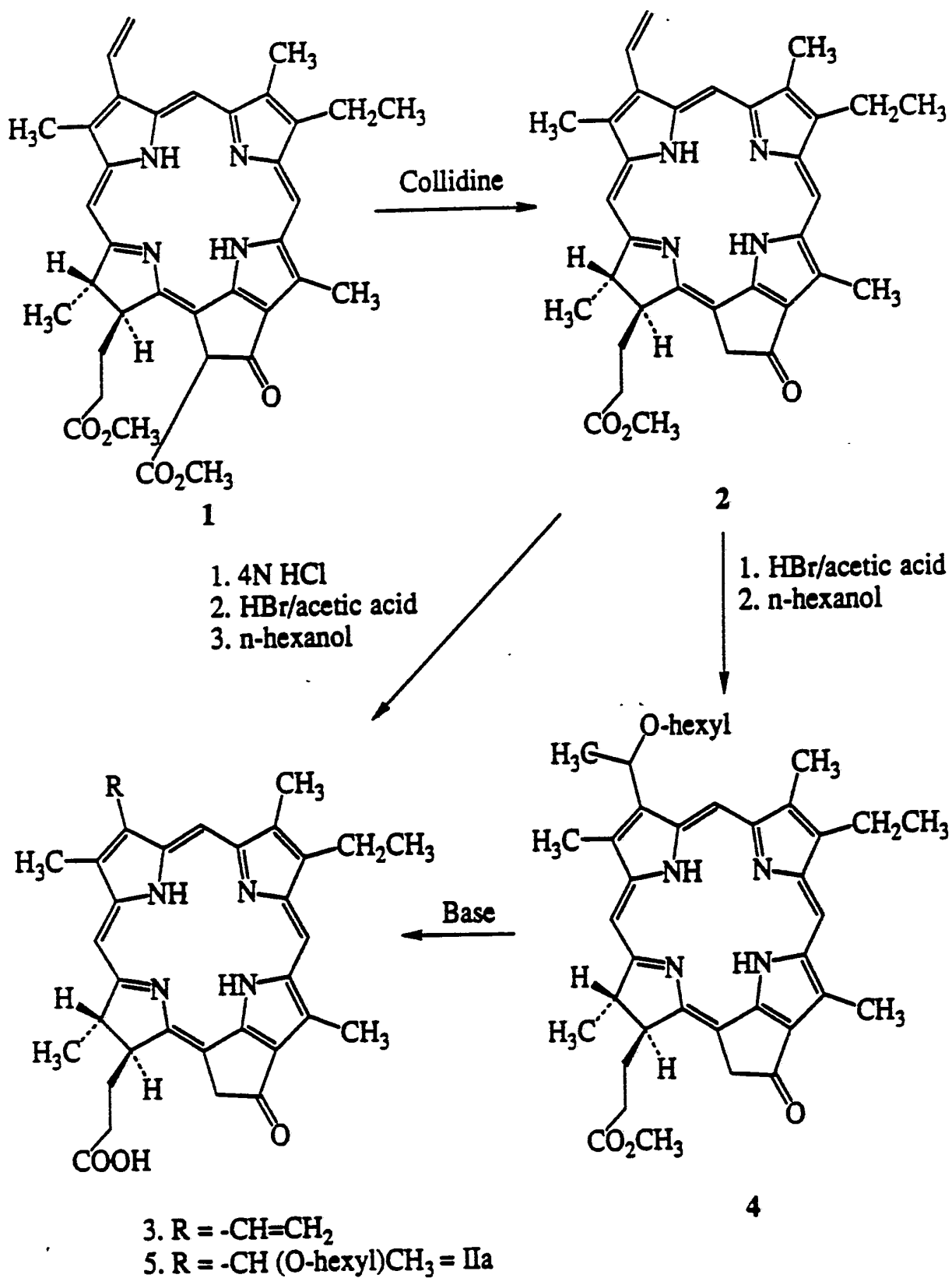
A generalized reaction scheme for the synthesis of the compound of structural formula IIa is put forth below:

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Scheme 1

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### Starting Materials

The starting material for preparation of the red light-absorbing compounds is methyl pheophorbide-a, which is isolated from Spirulina destridratada by the method of Smith and Goff (D. Goff, Ph.D. Thesis, Univ. of Calif., Davis, CA 95616, 1984 incorporated herein by reference). Briefly, 500 gm dried Spirulina was slurried in a large volume of acetone and then liquid nitrogen was added to form a frozen slush. The slush was transferred to a 3-necked, 5-liter round bottom flask and heated to reflux under nitrogen with stirring for 2 hours. The mixture was filtered through Whatman paper on a Buchner funnel with extensive acetone washing. The extraction and filtration process was repeated 2 more times; all green color could not be removed from the solid.

The green filtrate was evaporated and purified by flash chromatography on Grade V neutral Alumina, eluting first with n-hexane to remove a fast running yellow band and then with dichloromethane to obtain the major blue/gray peak containing pheophytin-a. Treatment of pheophytin-a with 500 ml sulfuric acid in methanol for 12 hours at room temperature in the dark under nitrogen was followed by dilution with dichloromethane. The reaction mixture was rinsed with water and then 10% aqueous sodium bicarbonate and the organic layer was dried, evaporated, and the residue recrystallized from dichloromethane/methanol to obtain 1.8 gm methyl pheophorbide-a. Methyl pheophorbide-a appears to be inactive in the in vivo tumoricidal activity assay when injected at a dose of 5 mg/kg.

### Conjugates and Labeled Pyropheophorbides

In addition to using compositions which consist essentially of the above-defined compounds or preparations as active ingredient, it is possible to use

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derivatized forms in order to provide a specific targeting mechanism. Commonly used target-specific components include monoclonal antibodies and ligands which bind to a cellular receptor. The compositions can also be conveniently labeled.

The target-specific component can then be, for example, an immunoglobulin or portion thereof or a ligand specific for a particular receptor. The immunoglobulin component can be any of a variety of materials. It may be derived from polyclonal or monoclonal antibody preparations and may contain whole antibodies or immunologically reactive fragments of these antibodies such as  $F(ab')_2$ , FAB, or FAB' fragments. Use of such immunologically reactive fragments as substitutes for whole antibodies is well known in the art. See, for example, Spiegelberg, H.L., in "Immunoassays in the Clinical Laboratory" (1978) 3:1-23 incorporated herein by reference.

Polyclonal anti-sera are prepared in conventional ways by injecting a suitable mammal with antigen to which antibody is desired, assaying the antibody level in serum against the antigen, and preparing anti-sera when the titers are high. Monoclonal antibody preparations may also be prepared conventionally such as by the method of Koehler and Milstein using peripheral blood lymphocytes or spleen cells from immunized animals and immortalizing these cells either by viral infection, by fusion with myelomas, or by other conventional procedures, and screening for production of the desired antibodies by isolated colonies. Formation of the fragments from either monoclonal or polyclonal preparations is effected by conventional means as described by Spiegelberg, H.L., supra.

Particularly useful antibodies include the monoclonal antibody preparation CAMAL1 which can be

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prepared as described by Malcolm, A., et al, Ex Hematol  
(1984) 12:539-547; polyclonal or monoclonal preparations  
of anti-M1 antibody as described by New, D., et al, J  
Immunol (1983) 130:1473-1477 (supra) and B16G antibody  
5 which is prepared as described by Maier, T., et al, J  
Immunol (1983) 131:1843; Steele, J.K., et al, Cell  
Immunol (1984) 90:303 all of which publications are  
incorporated herein by reference.

The foregoing list is exemplary and certainly  
10 not limiting; once the target tissue is known, antibody  
specific for this tissue may be prepared by conventional  
means. Therefore the invention is applicable to  
effecting toxicity against any desired target.

The ligand specific for receptor refers to a  
15 moiety which binds a receptor at cell surfaces, and thus  
contains contours and charge patterns which are  
complementary to those of the receptor. It is well  
understood that a wide variety of cell types have  
specific receptors designed to bind hormones, growth  
20 factors, or neurotransmitters. However, while these  
embodiments of ligands specific for receptor are known  
and understood, the phrase "ligand specific for  
receptor," as used herein, refers to any substance,  
natural or synthetic, which binds specifically to a  
25 receptor.

Examples of such ligands include the steroid  
hormones, such as progesterone, estrogens, androgens, and  
the adrenal cortical hormones; growth factors, such as  
epidermal growth factor, nerve growth factor, fibroblast  
30 growth factor, and so forth; other protein hormones, such  
as human growth hormone, parathyroid hormone, and so  
forth; and neurotransmitters, such as acetylcholine,  
serotonin, and dopamine. Any analog of these substances  
which succeeds in binding to the receptor is also  
35 included.

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The conjugation of the target-cell-specific component to the compounds of the invention can be effected by any convenient means. For proteins, such as Ig and certain receptor ligands, a direct covalent bond  
5 between these moieties may be effected, for example, using a dehydrating agent such as a carbodiimide. A particularly preferred method of covalently binding the compounds of the invention to the immunoglobulin moiety is treatment with 1-ethyl-3-(3-dimethylamino propyl)  
10 carbodiimide (EDCI) in the presence of a reaction medium consisting essentially of dimethyl sulfoxide (DMSO).

Of course, other dehydrating agents such as dicyclohexylcarbodiimide or diethylcarbodiimide could also be used as well as conventional aqueous and  
15 partially aqueous media.

Nonprotein receptor ligands can be conjugated to be dimers and trimers according to their relevant functional groups by means known in the art.

The active moieties of the conjugate may also  
20 be conjugated through linker compounds which are bifunctional, and are capable of covalently binding each of the two active components. A large variety of these linkers is commercially available, and a typical list would include those found, for example, in the catalog of  
25 the Pierce Chemical Co. These linkers are either homo- or heterobifunctional moieties and include functionalities capable of forming disulfides, amides, hydrazones, and a wide variety of other linkages.

Other linkers include polymers such as  
30 polyamines, polyethers, polyamine alcohols, derivatized to the components by means of ketones, acids, aldehydes, isocyanates, or a variety of other groups.

The techniques employed in conjugating the active moieties of the conjugate to the target-specific  
35 component include any standard means and the method for



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conjugation does not form part of the invention.

Therefore, any effective technique known in the art to produce such conjugates falls within the scope of the invention, and the linker moiety is accordingly broadly  
5 defined only as being either a covalent bond or any linker moiety available in the art or derivable therefrom using standard techniques.

The compounds of the invention per se or the conjugates may be further derivatized to a compound or  
10 ion which labels the drug. A wide variety of labeling moieties can be used, including radioisotopes and fluorescent labels. Radioisotope labeling is preferred, as it can be readily detected in vivo.

The compounds which are alone or are conjugates  
15 with a specific binding substance can be labeled with radioisotopes by coordination of a suitable radioactive cation in the porphyrin system. Useful cations include technetium and indium. In the conjugates, the specific binding substances can also be linked to label.

20

#### Administration and Use

In general, the pyropheophorbide compounds of the invention are administered to a host such as a human suffering from cancer in therapeutically effective  
25 amounts by any suitable means such as injection which may be IV or IM or may be administered transdermally. The pyropheophorbide compounds of the invention accumulate in tumor cells to a much higher degree than they accumulate in surrounding normal tissues. After being provided with  
30 sufficient time so as to accumulate in the tumor tissue, the pyropheophorbide compounds are exposed to a particular wavelength of light which causes the compounds to become cytotoxic, thus destroying the tumor or diseased tissue which the pyropheophorbide compounds have  
35 accumulated in. This is accomplished without causing

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irreversible damage to surrounding normal tissues wherein there has not been an accumulation of the pyropheophorbide compounds.

The compounds and their conjugates with target-specific substances of the invention are useful, in general, in the manner known in the art for hematoporphyrin derivative and for Photofrin II compositions. These compositions are useful in sensitizing neoplastic cells or other abnormal tissue to destruction by irradiation using visible light -- upon photoactivation, the compositions have no direct effect, nor are they entered into any biological event; however the energy of photoactivation is believed to be transferred to endogenous oxygen to convert it to singlet oxygen. This singlet oxygen is thought to be responsible for the cytotoxic effect. In addition, the photoactivated forms of porphyrin fluorescence which fluoresce can aid in localizing the tumor. Thus, the dimer and trimer compounds of the invention are not consumed or altered in exerting their biological effects.

Typical indications, known in the art, include destruction of tumor tissue in solid tumors, dissolution of plaques in blood vessels (see, e.g., U.S. patent 4,512,762); treatment of topical conditions such as acne, athlete's foot, warts, papilloma, and psoriasis and treatment of biological products (such as blood for transfusion) for infectious agents, since the presence of a membrane in such agents promotes the accumulation of the drug. Other uses include treating humans suffering from atherosclerosis and inactivating bacterial or viral infections.

The compositions are formulated into pharmaceutical compositions for administration to the subject or applied to an in vitro target using techniques known in the art generally. A summary of such

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pharmaceutical compositions may be found, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania, latest edition. The compositions, labeled or unlabeled, can be administered systemically, in particular by injection, or can be used topically.

Injection may be intravenous, subcutaneous, intramuscular, or even intraperitoneal. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid form suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol and the like. Of course, these compositions may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and so forth.

Systemic administration can also be implemented through implantation of a slow release or sustained release system, by suppository, or, if properly formulated, orally. Formulations for these modes of administration are well known in the art, and a summary of such methods may be found, for example, in Remington's Pharmaceutical Sciences (*supra*).

If the treatment is to be localized, such as for the treatment of superficial tumors or skin disorders, the compositions may be topically administered using standard topical compositions involving lotions, suspensions, or pastes.

The quantity of compound to be administered depends on the choice of active ingredient, the condition to be treated, the mode of administration, the individual subject, and the judgment of the practitioner. Depending on the specificity of the preparation, smaller or larger doses may be needed. For compositions which are highly specific to target tissue, such as those which comprise conjugates with a highly specific monoclonal

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immunoglobulin preparation or specific receptor ligand, dosages in the range of 0.05-1 mg/kg are suggested. For compositions which are less specific to the target tissue, larger doses, up to 1-10 mg/kg may be needed.

5 The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large and considerable excursions from these recommended values are expected. Further, because of slight solubility in water, certain compounds of the invention may be administered directly in saline or 5%  
10 glucose solution, thus avoiding the complications of detergents or other solubilizing agents.

Those skilled in the art of photodynamic therapy and compounds related to the present invention will be better able to determine an appropriate dosage and overall dosage regime when taking a number of factors into consideration. For example, the size, weight and condition of the patient must be considered as must be the responsiveness of the patient and their disease to the particular therapy. It is believed that even  
15 relatively small doses administered a single time can have a beneficial effect. Further, extremely large doses could, of course, be toxic. Accordingly, rather than providing specific information on dosage amount and intervals between dosing, attention should be paid to  
25 conventional factors used in determining such dosing while considering that the pyropheophorbide compounds of the invention have a greater degree of toxicity with respect to tumor cells and therefore can generally be  
30 administered in smaller amounts than the conventional compounds used in connection with photodynamic therapy.

EXAMPLES

5           The following examples are put forth so as to  
provide those of ordinary skill in the art with a  
complete disclosure and description of how to make the  
pyropheophorbide compounds and pharmaceutical  
10           compositions of the invention and are not intended to  
limit the scope of what the inventors regard as their  
invention. Efforts have been made to ensure accuracy  
with respect to numbers used (e.g., amounts, temperature,  
etc.), but some experimental errors and deviations should  
15           be accounted for. Unless indicated otherwise, parts are  
parts by weight, temperature is in degrees Centigrade,  
and pressure is at or near atmospheric.

EXAMPLE 1

**Methyl pyropheophorbide-a (2):** Methyl  
20           pheophorbide-a (1, 1.0 g) was obtained from alga  
Spirulina destridratada by following the procedure  
described in K.M. Smith, D.A. Goff and D.J. Simpson,  
J. Am. Chem. Soc., 1985, 107, 4941-4954; and R.K. Pandey,  
D.A. Bellnier, K.M. Smith and T.J. Dougherty, Photochem.  
25           Photobiol., 1991, 53, 65-72, both of which are  
incorporated herein by reference. The methyl  
pheophorbide-a was heated under reflux in collidine (100  
ml) for 90 min during slow passage of a stream of  
nitrogen. See G.W. Kenner, S.W. McCombie and K.M. Smith,  
30           J. Chem. Soc. Perkin Trans. 1973, 1, 2517-2523,  
incorporated herein by reference. The solution is  
evaporated (0.1 mm Hg) and the residue was recrystallized  
from dichloromethane/methanol. Yield 820 mg; 91%, m.p.  
217-219°C, lit. 220-225°C; H. Fisher and A. Stern, Die  
35           Chemie des Pyrrole, vol II, Part 2, pp. 64 and 74,

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Akademische Verlag, Leipzig incorporated herein by reference. Vis: (max) 410 (112 000); 508 (11 000); 536 (9 600); 610 (8 200); 666 (45 000); NMR, ppm; 9.50, 9.38, 8.52 (each s, 3H, 3 meso H); 7.95-8.05 (m, 1H, CH=CH<sub>2</sub>); 5 6.30 and 6.15 (each s, 1H, CH-CH<sub>2</sub>); 5.27 to 5.12 (q, 2H, 10-CH<sub>2</sub>); 4.50 (m, 8-HO); 4.28 (m, 7-H); 3.70 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>); 3.68 (s, 3H, CHCHCO<sub>2</sub>CH<sub>3</sub>); 3.62, 3.40, 3.22 (each s, 3H, 3CH<sub>3</sub>); 2.70 (7a-H); 2.31(7a'-H); 2.56 (7b-H); 2.29 (7b'-H); 1.82 (d, 3H, 8-CH<sub>3</sub>); 1.70 (t, 3H, CH<sub>2</sub>CH<sub>3</sub>); - 10 1.70 (s, 2H, 2 NH)

**Pyropheophorbide-a (3): Methyl**

pyropheophorbide-a (2, 250 mg) was dissolved in distilled tetrahydrofuran (50 ml) and 4N HCl (125 ml) was added in one lot. The reaction mixture was stirred under nitrogen 15 atmosphere at room temperature for 4 hours. The reaction was monitored by analytical tlc (silica plates), using 10% methanol/dichloromethane as a mobile phase. The reaction mixture was then poured in ice water, extracted with dichloromethane. The dichloromethane layer was 20 washed several times with water (3X200 ml). The organic layer was separated and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was crystallized from dichloromethane/hexane. Yield, 225 mg. The purity of the compound was 25 ascertained by tlc and the structure was confirmed by NMR spectroscopy. The NMR data were similar as described for 2 except the resonances for the -OCH<sub>3</sub> protons of the propionic ester (-CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>) were missing.

**Methyl -2 - {1(O-hexyl)ethyl} - devinyl**

30 pyropheophorbide (4): Pyropheophorbide-a (2, 200 mg) was dissolved in 30% HBr/acetic acid (5.0 ml) and the reaction mixture was stirred in a glass stoppered flask (rubber septum can also be used) at room temperature for 2.5 hours. The solvent was removed under high vacuum (1

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mm Hg) and the resulting 1-bromo ethyl derivative was immediately treated with n-hexanol (3.0 ml) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 45 min, diluted with dichloromethane (100 ml). The dichloromethane layer was washed with water (3X200 ml) till the aqueous phase is neutral and then dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed over Alumina Grade III (6% water/neutral Alumina) and eluted with dichloromethane. The first fraction was a mixture of the starting material and the desired product (minor quantity). Further elution with same solvent gave the desired product. The appropriate eluates were combined. Evaporation of the solvent afforded a sticky solid, which can be crystallized from dichloromethane/hexane. Yield 70%. (see Scheme-1), Vis, (max); 408 (90 000); 471 (3 200), 506 (8600); 536 (8,500); 604 (7,250); 660 (41 500). NMR, ppm; 9.79, 9.51, 8.53 (each s, 1H, meso H); 5.90 (q, 2H, -CH (O-hexyl)CH<sub>3</sub>); 5.08-5.30 (q, 2H, 10 - CH<sub>2</sub>); 4.47 (m, 8H); 4.29 (m, 7-HO); 3.75 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>); 3.67 (s, 3H, CH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub>CH<sub>3</sub>), 3.67 (s, 6H, 2 X CH<sub>3</sub>); 3.38 and 3.27 (each s, CH<sub>3</sub>); 2.68 (7a-H) 2.28 (7a'-H), 2.55 (7b-H); 2.20 (7b'-H); 1.80 (d, 3H, CH<sub>2</sub>CH<sub>3</sub>); -1.70 (s, 2H, 2 NH); for the hexyl group, 3.72 (t, 2H, O-CH<sub>2</sub>CH<sub>2</sub>); 1.73 (2H, CH<sub>2</sub>); 1.25 [bs, merged, 6H, (CH<sub>2</sub>)<sub>3</sub>]; 0.78 (t, 3H, CH<sub>3</sub>). (see fig. 1).

**2-{1(O-hexyl)ethyl}devinyl pyropheophorbide-a (5):** Pyropheophorbide-a (3, 200 mg) was reacted with 30% HBr/acetic acid and then with n-hexanol by following the method as discussed for 4 and the desired product was isolated in 60 to 65% yield. The structure was confirmed by NMR spectroscopy.

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EXAMPLE 2Tumor Treatment

When 2-[1-(O-hexyl)ethyl] devinyl  
5 pyropheophorbide-a - structure (5) in Scheme 1: S-RO-  
where R = (CH<sub>2</sub>)<sub>5</sub>CH<sub>3</sub> and m = H, (formula IIa) synthesized  
as indicated (5.0 mg) is dissolved in Tween 80 (0.1 ml)  
and mixed with 10 ml Hanks Balanced Salt Solution (HBSS),  
a solution of approximately 0.5 mg/ml in 0.1% Tween 80 is  
10 produced after filtration through a 0.22 μM Millipore  
filter. Ten DBA/2 mice with 0.4-0.5 mm diameter  
subcutaneous SMT-F tumors in the axilla are injected  
intravenously with 0.3 mg/kg body weight of the above  
solution (after diluting in HBSS so that the injected  
15 volume per mouse is approximately 0.2 ml). Approximately  
24 h later the tumor area (having been shaved and  
depilated prior to tumor implant) is exposed to laser  
light at 660-670 nm for 30 min at a power of 75 mW/cm<sup>2</sup> to  
deliver 135 Joules/cm<sup>2</sup>. Alternately, a Xenon arc lamp  
20 filtered to emit a broader band width near 670 nm and  
approximately 283 Joules/cm<sup>2</sup> can be used.

The day after light treatment all the tumors  
are seen to be flat (non-palpable) and slight skin  
blanching over the area is noted. This progresses to  
25 frank tumor necrosis over the next few days. At 7 days  
post treatment all tumors remain non-palpable and  
necrotic. At 30 days post treatment, six of the 10  
tumors remain non-palpable, and one remains tumor-free to  
90 days post treatment.

30

EXAMPLE 3Skin Clearance

Six albino Swiss mice (HaICR) are injected  
intravenously with a dose of 0.1 mg/kg body weight of the  
35 compound of formula IIa prepared as in Example 1. After



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approximately 24 h, the hind foot of the animal is exposed to the same dose of either laser light at 660-670 nm (135 Joules/cm<sup>2</sup>) or the Xenon arc lamp (283 Joules/cm<sup>2</sup>) as above. The reaction of the foot is scored  
5 for damage over the next few days to determine the maximum effect, which in this case is a value 0.3 equivalent to slight edema. If the interval between the injection and light treatment is extended to  
10 approximately 48 h, the foot reaction is zero (no damage incurred), indicating either clearance or metabolism of the sensitizer.

Data obtained as a result of experiment carried out is put forth below in Table 1.

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*In Vivo* Tumor Photosensitizing Activity of Pyrophosphoribide Ethers<sup>1</sup>

Injected Dose (mg/kg) Formula II R <sub>2</sub> = -O-(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> R <sub>1</sub> = -CO <sub>2</sub> H	Time Interval (hours)	Wavelength	Tumor Response <sup>2</sup>				Normal Foot Response <sup>3</sup>		Maximum Reaction
			Day 1	Day 7	Day 30	Day 90	Time Interval (hours)		
0.05	24	659	0/40	-	-	-	-	-	-
0.1	24	659	6/6	6/6	1/6	1/6	24	0.3	0.3
0.3	24	659	5/5	4/5	0/5	-	48-72	0	0
0.3	24	655	0/10	-	-	-	-	-	-
0.3	24	665	10/10	10/10	2/10	0/10	-	-	-
0.3	24	670	10/10	10/10	6/10	1/10	-	-	-
0.3	24	680	8/10	0/10	-	-	-	-	-
Formula II R <sub>2</sub> = -O-(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> R <sub>1</sub> = -CO <sub>2</sub> CH <sub>3</sub>									
0.3	24	660	6/6	6/6	3/6	3/6	-	-	-
0.1	24	660	5/5	3.5	0/5	-	-	-	-
Formula II R <sub>2</sub> = -O-CH <sub>3</sub> R <sub>1</sub> = -CO <sub>2</sub> CH <sub>3</sub>									
0.1	24	660	0/6	-	-	-	-	-	-
0.5	3	660	0/6	-	-	-	-	-	-
Formula I R <sub>2</sub> = -CH <sub>2</sub> O(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub> R <sub>1</sub> = -CO <sub>2</sub> CH <sub>3</sub>									
0.3	24	660	6/6	2/6	0/6	-	-	-	-

<sup>1</sup>SMT-F tumor in DBA/2 mice; 135 J/cm<sup>2</sup> light from laser at 75 mW/cm<sup>2</sup>

<sup>2</sup>Number of non-palpable tumors/Number treated tumors post light treatment on Day indicated

<sup>3</sup>White Albino Swiss mice; foot exposed using same conditions as for tumor treatment. Score of 0.3 = light edema; 0 = no reaction.

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The data put forth in Table 1 clearly demonstrates that the pyropheophorbide compounds of the invention are activated by light having a wavelength of about 660 nm. Further, when the compound were  
5 administered by injection and subjected to light having a wavelength of about 660 nm, the treatment was found to be highly effective with respect to reducing tumor size in as little as seven days.

Further, the data of Table 1 show compounds of  
10 the invention clear skin over a period of 24-48 hours after administration. This is a desirable feature in that the patient is not subjected to prolonged cutaneous photosensitivity. The data of Table 1 also show that the hexyl ethers of formula II are preferred over methyl  
15 ethers in terms of effecting tumor growth when used in photodynamic therapy.

While the present invention has been described with reference to specific compounds, formulations and methods, it is to be understood by those skilled in the  
20 art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt to a particular individual, method of administration, process of synthesizing, etc., which  
25 are within the scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

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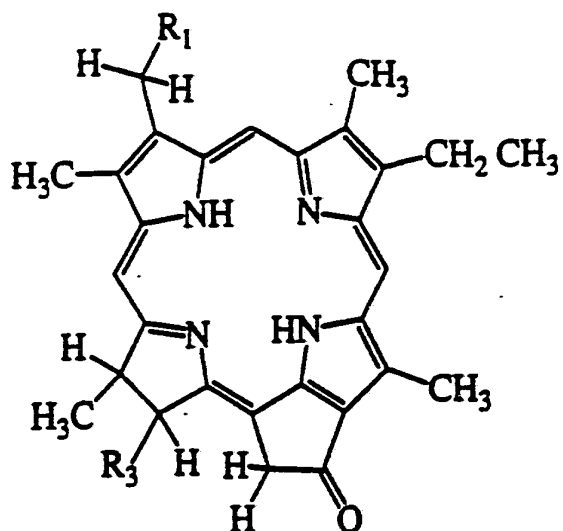
CLAIMS

1. A compound of formula I:

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**I**

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wherein  $R_1$  is  $\text{CH}_2\text{OR}_2$  where  $R_2$  is a primary or secondary alkyl containing 1 to 20 carbons; and  $R_3$  is  $-\text{CO}_2\text{R}_4$  where  $R_4$  is H or an alkyl containing 1 to 20 carbons.

25

2. The compound as claimed in claim 1, wherein  $R_1$  is  $\text{CH}_2\text{-O-hexyl}$ ;  $R_2$  is  $-\text{CH}_3$  and  $R_3$  is  $-\text{CO}_2\text{CH}_3$ .

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3. A method to effect the destruction of target virus, cells or tissue, comprising:

contacting said target with an effective amount of compound of claim 1; and

irradiating with light absorbed by said compound.

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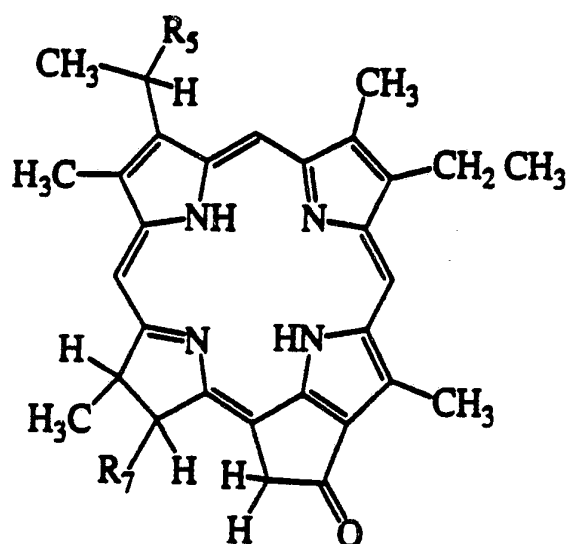
4. A pharmaceutical composition useful in treatment of a target virus, cells or tissue, comprising:  
 an effective amount of the compound of claim 1 in admixture with a pharmaceutically acceptable  
 5 excipient.

5. A conjugate which consists essentially of the compound of claim 1 covalently bound to a target-specific component selected from the group consisting of  
 10 an immunoglobulin and a receptor ligand.

6. A pharmaceutical composition useful for labeling malignant tissue which comprises the compound of claim 1 associated with a label.  
 15

7. A compound of formula II:

II



20  
 25  
 30 wherein R<sub>5</sub> is -OR<sub>6</sub> where R<sub>6</sub> is a primary or secondary alkyl containing 1 to 20 carbons and R<sub>7</sub> is -CO<sub>2</sub>R<sub>8</sub> where R<sub>8</sub> is H or an alkyl containing 1 to 20 carbons.

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8. The compound as claimed in claim 7, wherein R<sub>5</sub> is selected from the group consisting of -O-hexyl and -O-(CH<sub>2</sub>)<sub>5</sub>CH<sub>3</sub> and R<sub>7</sub> is selected from the group consisting of -CO<sub>2</sub>CH<sub>3</sub> and -CO<sub>2</sub>H.

5

9. A method to effect the destruction of target virus, cells or tissue, comprising:

contacting said target with an effective amount of compound of claim 7; and

10 irradiating with light absorbed by said compound.

10. A pharmaceutical composition useful in treatment of a target virus, cells or tissue, comprising:

15 an effective amount of the compound of claim 7 in admixture with a pharmaceutically acceptable excipient.

11. A conjugate which consists essentially of 20 the compound of claim 7 covalently bound to a target-specific component selected from the group consisting of an immunoglobulin and a receptor ligand.

12. A method of treating a human with abnormal 25 cells which replicate at an abnormally high rate, comprising the steps of:

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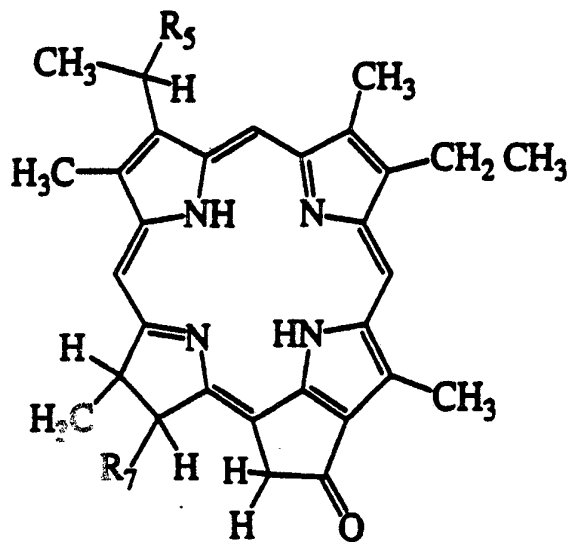
administering to the human a therapeutically effective amount of a compound of formula II

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II



wherein  $R_5$  is  $OR_6$  where  $R_6$  is a primary or secondary alkyl containing 5 to 20 carbons and  $R_7$  is  $-CO_2R_8$  where  $R_8$  is H or  $-CH_3$ ;

allowing the compound of formula I to accumulate on the abnormal cells; and

irradiating the compound of formula I with a wavelength of light which is absorbed by the compound of formula I and thereby generating a cytotoxic effect with respect to the abnormal cells.

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13. The method as claimed in claim 12 wherein the compound is administered in an amount in the range of 0.01 mg/kg to 1.0 mg/kg of body weight and is administered at timed intervals in the range of from every 3 hours to every 72 hours for over a period of from 1 day to 30 days and the wavelength of the light is in the range of 600 to 700 nm.

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14. The method as claimed in claim 12, wherein  $R_5$  is -O-hexyl and  $R_7$  is  $-CO_2H$  and wherein the wavelength of the light is about 660 nm.

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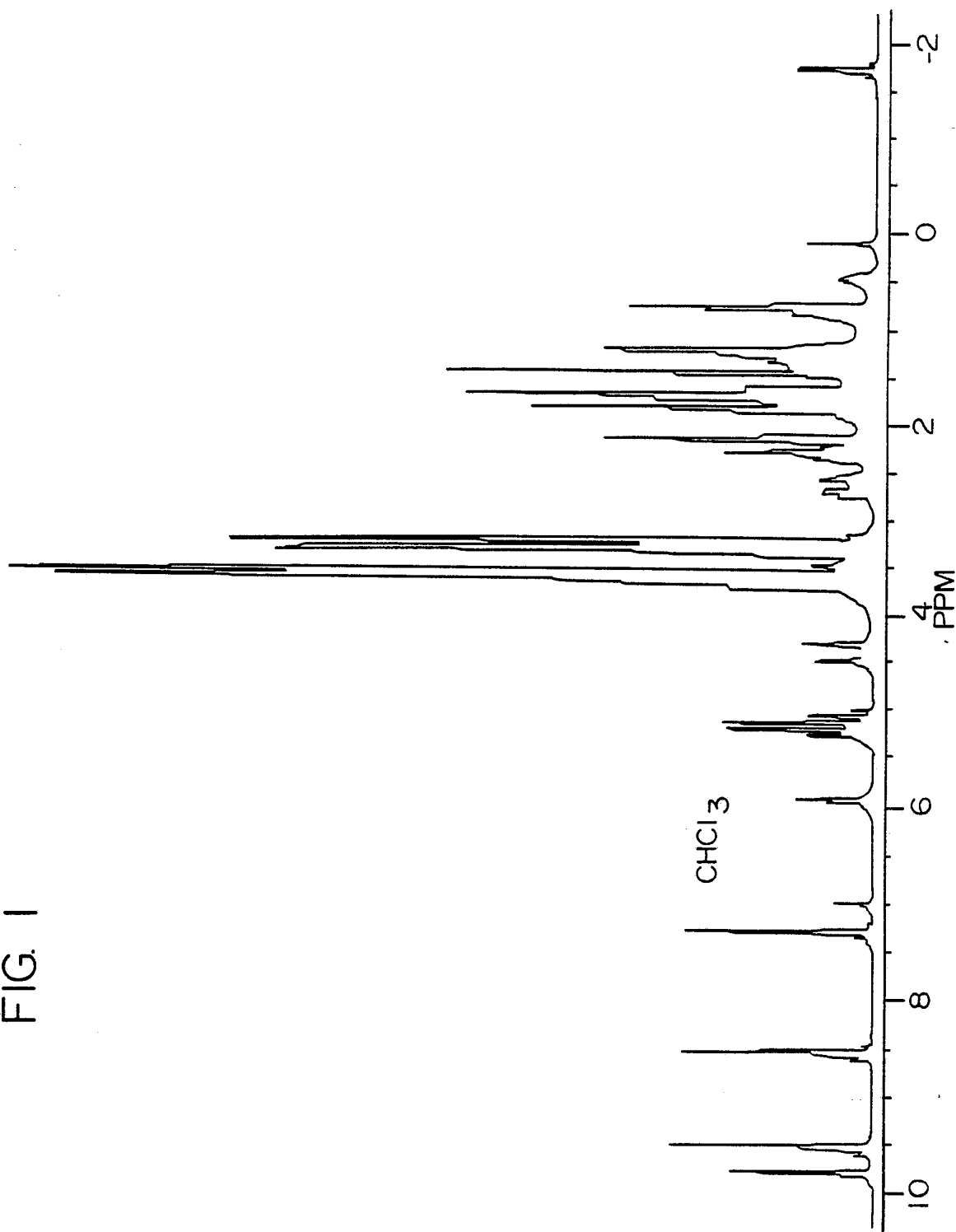
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FIG. 1



INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/00060

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC(5) :A61K 31/40,37/04,37/00; C07D 487/22, 519/00  
 US CL :514/2,410; 530/391; 540/145  
 According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**  
 Minimum documentation searched (classification system followed by classification symbols)  
 U.S. : N/A

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
 CAS ONLINE: Structural search using "PORPHN".

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US,A, 4,949,151 (DOUGHERTY ET AL) 10 MARCH 1987 See abstract	1-14

Further documents are listed in the continuation of Box C.  See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 15 MARCH 1993	Date of mailing of the international search report 31 MAR 1993
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