Description

This invention relates to new compounds which are useful in photodiagnosis and phototherapy, especially in the detection and treatment of tumors and cancerous tissues in the human or animal body.

It is known to irradiate tumors and cancerous tissues in the human body with intensive light following administration of a hematoporphyrin derivative in the wavelength range of 626 to 636 namometers to reduce and, at times, destroy the cancerous cells (see PCT published specification WO 83/00811). It is also known that porphyrins, especially the sodium salt of protoporphyrins, can maintain or promote the normal functions of cells and are useful for preventing the genesis, growth, metastasis , and relapse of malignant tumors. Japanese Published Patent Application No. 125737/76 describes the use of porphyrins as tumor inhibiting agents, exemplifying etioporphyrin, mesoporphyrin, protoporphyrin, deuteroporphyrin, hematoporphyrin, coprophyrin, and uroporphyrin.

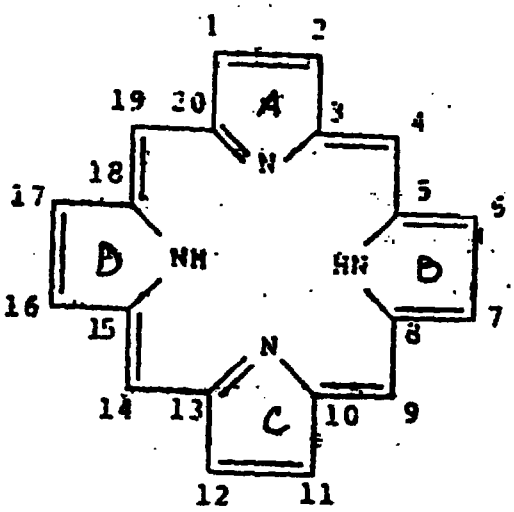
In Tetrahedron Letters No. 23, pp. 2017-2020 (1978), there is described an amino monocarboxylic acid adduct of the pigment bonellin obtained by extraction of principally the body wall of the marine echuroid B. viridis. The structure of these adducts is presumed to be an amide formed through either of the free carboxy groups of bonellin and the amino monocarboxylic acid. Hydrolysis of the adduct yielded a mixture of valine, isoleucine, leucine and alloisoleucine. No use for these amino acid adducts is described in this reference.

Mesoporphyrin and mesohaemin bis amino acid esters and the corresponding acids are described in Chemische Berichte, vol. 90, no. 4, 1957(pp. 470-481). Specific bis amino acid compounds include DL-valine, DL-leucine, DL-phenylalanine, DL-isoleucene and L-glutamic acid esters (and the corresponding free acids) bis-adducts of mesoporphyrin and mesohaemin. No therapeutic use of these compounds is disclosed, however.

PCT Patent Application No. WO 84/01382 describes the use of a new derivative of hematoporphyrin as useful in the localization and treatment of tumors.

That the tetrapyrroles cause intense photosensitivity in animals is well-known and has been documented in numerous articles in literature, e.g., J. Intr. Sci. Vitaminol, 27, 521-527 (1981); Agric. Biol. Chem., 46(9), 2183-2193 (1982); Chem. Abst. 98, 276 (1983) and 88, 69764m (1928).

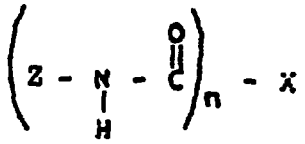
The cyclic tetrapyrroles have as their common parent tetrapyrrole, uroporphyrinogen, and possess the following ring structure:



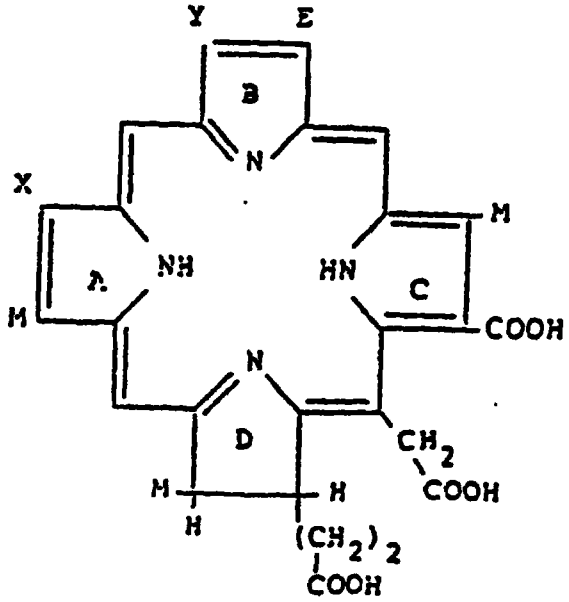
in which the positions in the molecule are numbered 1-20, and the rings identified by letters A, B, C and D, and also include perhydro-, e.g., dihydro- and tetrahydro-, derivatives of the said ring structures, e.g., compounds in which one or more double bonds are absent. There are present in the ring system four pyrrole rings joined through the alpha positions of the respective pyrrole rings by a methine group, i.e., -CH=. The compounds of the present invention are designated as derivatives of the tetrapyrroles for convenience in the disclosure and the appended claims and it will be understood that the term "tetrapyrrole" will designate compounds of the characteristic ring structure designated hereinbefore as well as the corresponding perhydro derivatives.

The tetrapyrroles employed in the present invention are all known or derived by various means and

various alteration procedures from natural tetrapyrroles. The naturally occurring tetrapyrroles have as their common ancestor uroporphyrinogen III, a hexahydroporphyrin reduced at the bridge positions. The present invention is directed to a fluorescent mono, di- or polyamide of an aminodicarboxylic acid and a tetrapyrrole of the structure:



wherein Z is the amino acid residue and X is the residue of a tetrapyrrole of the formula:



wherein;

X = H, vinyl, ethyl, acetyl or formyl;
Y = methyl or formyl;
H = methyl; and
E = ethyl

and "n" is an integer from 1 to 3 inclusive and pharmaceutically-acceptable salts thereof.

A characteristic of the present new compounds is the presence of at least one amide linkage in a substituent at any of the numbered positions of the ring structure. These are present in the instant new compounds together with other substituents as defined hereinafter.

Thus, the present invention contemplates amino acid or peptide derivatives of compounds which contain the chromophore of certain chlorins and bacteriochlorins, as well as related porphyrin compounds. The peptide linkage involves a carboxy group of the chromophore-bearing compound and the amino group of the specified amino acid. The present new compounds embrace, derivatives of the tetrapyrroles which contain three carboxy groups. These derivatives include porphyrins of the major classes of tetrapyrroles: chlorins and bacteriochlorins, which are well-known to those skilled in this art.

The amino acid employed in the present invention to form the aforesaid peptide linkage are amino-dicarboxylic acids in which the amino group, of course, is located on a carbon atom of the dicarboxylic acid. The specific position of the amino group in the carbon atom chain is not critical, the only requirement being that the amino group be available to form the requisite peptide linkage with the carboxyl group of the selected porphyrin. Thus, a variety of amino dicarboxylic acids are useful in the present invention, including α -aminosuccinic (aspartic), α -aminoglutaric (glutamic), beta-aminoglutaric, beta-aminosebacic, 2,6-piperidinedicarboxylic, 2,5-pyrroledicarboxylic, 2-carboxypyrrole-5-acetic, 2-carboxypiperidine-6-propionic, α-aminoadipic, α-aminoazelaic, and similar such acids. These amino acids may be substituted with angular alkyl groups such as methyl and ethyl groups, as well as other groups which do not adversely affect the

capability of the amino group to form the peptide linkage, e.g., alkoxy groups or acyloxy groups, and may also include additional amino groups. The preferred amino acids are the naturally occurring α-amino acids, glutamic and aspartic acids, which are readily available and, up to the present, have provided the best results.

Exemplary compounds of the tetrapyrrole classes are illustrated in Table I in which the numbered positions of the tetrapyrrole ring structure are used to designate the position of the indicated substituent. The absence of double bonds in the ring system is designated under "dihydro" with each set of numbers (ring position) indicating the absence of a double bond between the designated positions.

TABLE I - Ring Position

PORPHYRIN	A 1	A 2	B 6	B 7	C 11	C 12	C 14	D 16	D 17	Dihydro
Chlorin e_6	Me	V	Me	Et	Me	CO_2H	Ac	H	Me	16,17
								Pr		
Mesochlurin e_6	Me	Et	Me	Et	Me	CO_2H	Ac	H	H	16,17
								Pr	Me	
Bacteriochlorin e_6	Me	ACL	H	H	Me	CO_2H	Ac	H	H	6,7
			Me	Et				Pr	Me	16,17
2-Desvinylchlorin e_6 (or Deuterochlorin e_6)	Me	H	Me	Et	Me	CO_2H	Ac	H	H	16,17
								Pr	Me	
2-Acetylchlorin e_6	Me	ACL	Me	Et	Me	CO_2H	Ac	H	H	16,17
								Pr	Me	
2-Formylchlorin e_6	Me	CHO	Me	Et	Me	CO_2H	Ac	H	H	16,17
								Pr	Me	
Rhodin g_7	Me	V	CHO	Et	Me	CO_2H	Ac	H	H	16,17
								Pr	Me	

Notes:

Me: $-CH_3$ (Methyl group)

Pr: $-CH_2CH_2COOH$ (Propionic acid group)

V: $-CH=CH_2$ (Vinyl group)

Et: $-CH_2CH_3$ (Ethyl group)

Ac: $-CH_2COOH$ (Acetic acid group)

ACL: CH_3-CO- (Acetyl group)

Particularly preferred compounds of this invention include:

Chlorin Derivatives

Mono, di and triaspartyl chlorin e_6

Mono, di and triaspartyl mesochlorin e_6

Mono, di and triglutamyl chlorin e_6

Mono, di and triglutamyl mesochlorin e_6
Moni, di and triaspartyl acetylchlorin e_6
Mono, di and triaspartyl rhodin g_7
Mono, di and triaspartyl formylchlorin e_6
Mono, di and triglutamyl rhodin g_7
Mono, di and triglutamyl acetylchlorin e_6
Mono, di and triglutamyl acetylchlorin e_6
Mono, di and triglutamyl formylchlorin e_6
Mono, di and triaspartyl deuterochlorin e_6
Mono, di and triglutamyl deuterchlorin e_6

Bacteriochlorin Derivatives
Mono, di and triaspartyl bacteriochlorin e_6
Mono, di and triglutamyl bacteriochlorin e_6

The present new compounds form salts with either acids or bases. The acid salts are particularly useful for purification and/or separation of the final amide products as are the salts formed with bases. The base salts, however, are particularly preferred for diagnostic and therapeutic use as hereindescribed.

The acid salts are formed with a variety of acids such as the mineral acids, hydrochloric, hydrobromic, nitric and sulfuric acids, organic acids such as toluenesulfonic and benezenesulfonic acids.

The base salts include, for example, sodium, potassium, calcium, magnesium, ammonium, triethylammonium, trimethylammonium, morpholine and piperidine salts and similar such salts.

The acid and base salts are formed by the simple expediency of dissolving the selected amino acid tetrapyrrole amide in an aqueous solution of the acid or base and evaporation of the solution to dryness. The use of a water-miscible solvent for the amide can assist in dissolving the amide.

The final amide products can also be converted to metal complexes for example by reaction with metal salts. The magnesium complexes may be useful for the same purpose as the adduct product. Other metal complexes, as well as the magnesium complex, including, for example, iron and zinc, are useful to preclude contamination during processing of the adduct product by metals such as nickel, cobalt and copper, which are difficult to remove. Zinc and magnesium are readily removed from the final adduct product after processing is completed.

Since many of the aminodicarboxylic acids exist in both the D- and L-forms, and also are employed in mixtures of these forms as well as the D,L-form, the selection of the starting amino acid will, of course, result in products in which the respective isomer or mixture of isomers exist. The present invention contemplates the use of all such isomers, but the L-form is particularly preferred.

The present new compounds are prepared by the usual peptide synthetic routes which generally include any amide-forming reaction between the selected amino acid and the specific tetrapyrrole. Thus, any amide-forming derivative of the tetrapyrrole carboxylic acid can be employed in producing the present new peptides, e.g., lower alkyl esters, anhydrides and mixed anhydrides.

The preferred preparative methods use mixed anhydrides of the carboxylic acid or carbodiimides. The reactants are merely contacted in a suitable solvent therefor and allowed to react. Temperatures up to the reflux temperature can be used, with the higher temperatures merely reducing the reaction time. Excessively high temperatures are usually not preferred so as to avoid unwanted secondary reactions however.

The procedures for forming the instant peptides are well known in this art and are provided in detail in the accompanying examples.

When the selected tetrapyrrole contains at least three carboxyl groups, then mixtures of products can be formed including isomeric monopeptide products and di- and even tri- or higher peptide products, depending on the number of carboxyl groups and depending on the selected stoichiometry. Thus, when equimolar mixtures of amino acid and tetrapyrrole are reactd, not only monopeptides but also dipeptides are obtained, although the monopeptide would predominate. With higher molar ratios, the nature of the products will similarly vary. It is generally possible to separate the monopeptides and higher peptides using known chromatographic techniques. However, such separations are not necessary since the mixed peptides are usually comparable to the separated products in their ultimate use. Thus, mixtures of the mono-, di- and tripeptides of the same tetrapyrrole can be used.

Usually, unreacted tetrapyrrole is separated from the peptide products of the invention during purification as, for example, by chromatographic techniques.

Photodiagnosis and Phototherapy

The compounds of the present invention are useful for the photodiagnosis and phototherapy of tumor, cancer and malignant tissue (hereinafter referred to as "tumor").

When a man or animal having tumor is treated with doses of a compound of the present invention and when appropriate light rays or electromagnetic waves are applied, the compound emits light, i.e., fluorescence. Thereby the existence, position and size of tumor can be detected, i.e., photodiagnosis.

When the tumor is irradiated with light of proper wavelength and intensity, the compound is activated to exert a cell killing effect against the tumor. This is called "phototherapy".

Compounds intended for photodiagnosis and phototherapy ideally should have the following properties:

(a) non-toxic at normal therapeutic dosage unless and until activated by light;

(b) should be selectively photoactive;

(c) when light rays or electromagnetic waves are applied, they should emit characteristic and detectable fluorescence;

(d) when irradiated with light rays or electromagnetic waves are applied, they are activated to an extent to exert a cell killing effect against tumor; and

(e) easily metabolized or excreted after treatment.

In accordance with testing up to the present, the present new compounds have the foregoing properties and are also characterized by reasonable solubility in saline at physiological pH.

The present new compounds possess greater fluorescence in tumors than do the corresponding basic tetrapyrroles, and even peptides formed with amino monocarboxylic acids, e.g., alanine and epsilon aminocaproic acid. Their use provides the best contrast in tumors compared to normal tissue around the tumor. The instant compounds absorb activating energy for phototherapy in the convenient range of 600 to 800 nanometers, with the preferred compounds absorbing in the 620-760 nanometer range, i.e., light of longer wavelengths which more readily permits penetration of energy into the tumor for phototherapeutic purpose.

In present experience, the present compounds more uniformly distribute throughout the tumor than the basic tetrapyrrole permitting the use of considerably lower dosage (to about 1/10th of the required normal dose of the basic tetrapyrrole) which lessens, if not eliminates, photosensitization in the host. They also possess a more consistent fluorescence whereas some of the corresponding tetrapyrroles show inconsistent fluorescence or the fluorescence varies from day to day in the host.

A particularly advantageous property of the present compounds resides in the ease with which they are excreted by the host. Generally, within 24 to 72 hours of intravenous or intraperitonal administration, there are little or no detectable amounts in normal muscle tissue. Up to about 50% of the present compounds are recovered from the feces of the host within 24-72 hours of injection whereas under equivalent circumstances, substantial amounts of the corresponding tetrapyrroles and peptides formed with amino monocarboxylic acids remain. This property is extremely important in that it contributes to minimization of photosensitivity in the host.

The instant compounds can be used for diagnosis and therapeutic treatment of a broad range of tumors. Examples of tumors are gastric cancer, enteric cancer, lung cancer, breast cancer, uterine cancer, esophageal cancer, ovarian cancer, pancreatic cancer, pharyngeal cancer, sarcomas, hepatic cancer, cancer of the urinary bladder, cancer of the upper jaw, cancer of the bile duct, cancer of the tongue, cerebral tumor, skin cancer, malignant goiter, prostatic cancer, cancer of the parotid gland, Hodgkins's disease, multiple myeloma, renal cancer, leukemia, and malignant lymphocytoma. For diagnosis, the sole requirement is that the tumor be capable of selectively fluorescing when exposed to proper light. For treatment, the tumor must be penetrable by the activation energy. For diagnosis, light of shorter wavelength is used whereas for therapeutic purposes light of longer wavelength is used to permit ready penetration of the tumor tissue. Thus, for diagnosis, light of from 360-760 nanometers can be used, and for treatment, from 620 to 760, depending on the individual characteristics of the tetrapyrrole. The absorption characteristics of the present new compounds are substantially the same as the tetrapyrrole from which derived.

It is necessary that the light rays be so intense as to cause the compounds to emit fluorescence for diagnosis and to exert a cell killing effect for therapy.

The source of irradiation for photodiagnosis and phototherapy is not restricted, however, but the laser beam is preferable because intensive light rays in a desired wavelength range can be selectively applied. For example, in photodiagnosis, the compound of the invention is administered to a human or animal body, and after a certain period of time, light rays are applied to the part to be examined. When an endoscope can be used for the affected part, such as lungs, gullet, stomach, womb, urinary bladder or rectum, it is irradiated using the endoscope, and the tumor portion selectively emits fluorescence. This portion is observed visually, or observed through an adapted fiber scope by eye or on a CRT screen.

In phototherapy, after administration of the dosage, the irradiation is carried out by laser beams from

the tip of quartz fibers. Besides the irradiation of the surface of tumor, the internal part of the tumor can be irradiated by inserting the tip of quartz fibers into the tumor. The irradiation can be visually observed or imaged on a CRT screen.

For photodiagnosis, light of wavelengths between 360 and 760 nm. is suitable for activating the present tetrapyrrole compounds. Of course, each compound has a specific optimal wavelength of activation. A long wavelength ultraviolet lamp is particularly suitable for photodiagnosis. Similar methods for viewing of the treated tumor can be used as already described for phototherapy.

The dosages of the present new compounds will vary depending on the desired effect, whether for diagnosis or for treatment. For diagnosis, doses of as little as 1 mg/kg will be effective, and up to about 7.5 mg/kg can be used. For treatment, the dose will usually approximate about 0.5 mg/kg. Of course, the dosage for either diagnosis or treatment can be varied widely in view of aforesaid advantageous properties of the present compounds, e.g., the ease of elimination from the host, for one.

The present compounds are apparently non-toxic at the dosage levels employed for diagnosis or treatment. No mortality of test animals due the present compounds has been noted in studies employing dosage levels up to 20 mg/kg.

For both diagnosis and treatment, the present compounds can be administered by the oral, intravenous, or intramuscular routes. They can be formulated as lyophilized sterile, pyrogen-free compounds, preferably in the form of basic salts, e.g., sodium salt. The preferred dosage forms are provided as injectable solutions (isotonic).

The irradiation source used in treatment of tumors containing compounds of this invention is a filtered, high-intensity, continuous source or pumped dye, or other lasers and light delivery system, which is capable of performing within the following limits: power intensity 20-500 mW/cm^2 at wavelengths between 620 and 680 nm for the porphyrins and chlorins and 700-760 nm for the bacteriochlorins and a total output of at least 500 mW or greater. Several currently commercially available lasers meet these criteria. The tetrapyrroles can be prepared by various synthetic methods which are found in the literature, e.g.,

Chlorin e_6

Willstatter, R., Stoll, A.; Investigations on Chlorophyll, (Trans., Schertz, F.M., Merz, A.R.,) p. 176. Science Printing Press, Lancaster, Pennsylvani, 1928.

Willstatter, R., Isler, M.; Ann. Chem., 390, 269 (1912).

Fisher, H., Baumler, R.; Ann. Chem., 474, 65 (1929).

Fisher, H., Siebel, H.; Ann. Chem., 499, 84 (1932).

Conant, J.B., Mayer, W.W.; J. Amer. Chem. Soc., 52, 3013 (1930).

Chlorin e_6, e_4, *mesochlorin* e_6, *bacteriochlorin* e_6

Fischer and Orth, "Des Chemie des Pyrrole" Akademische Verlazsgesellschaft, Leipzig, 1940, Vol. II, Part 2.

General Reference for Porphyrins

"Porphyrins and Metalloporphyrins" ed. Kevin M. Smith, Elsevier 1975 N.Y.

The compounds of the present invention can be administered to the host in a variety of forms adapted to the chosen route of administration, i.e., orally, intraveneously, intramuscularly or subcutaneous routes.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 50 and 300 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain,

In addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

The active compound may also be administered parenterally or intraperitoneally. Solutions of the active compound as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporanous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

The present new compounds may also be applied directly to tumors, whether internal or external, in the host in topical compositions. Exemplary compositions include solutions of the new compounds in solvents, particularly aqueous solvents, most preferably water. Alternatively, for topical application particularly to skin tumors, the present new compounds may be dispersed in the usual cream or salve formulations commonly used for this purpose or may be provided in the form of spray solutions or suspensions which may include a propellant usually employed in aerosol preparations.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatable with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of tumors in living subjects.

EXAMPLE 1

Mono(L)-aspartylchlorin e_6 (carbodiimide method)

150 mg of chlorin e_6 and 250 mg of L aspartic acid di-t.butyl ester hydrochloride were dissolved in 20 ml of dimethyl formamide. There was made a total of 3-100 mg additions of N,N'-dicyclohexyl-carbodiimide at one hour intervals. After 4 hours, the reaction mixture was diluted with 300 ml ether, washed twice with 200 ml H_2O then extracted with 40 ml 1 M KOH. The KOH solution was allowed to hydrolyze overnight, then heated to 70° C. for 10 minutes.

The pH of the solution was adjusted to 7, then any residual ether was removed by flash evaporation. The solution was then applied to a reverse phase (C-18 silica) column (1.5 cm x 30 cm). The product was purified by a stepwise elution of methanol/.01 M pH 6.85 KPO_4 buffer. Eluted with 5% methanol until unwanted polar pigments were removed. Monoaspartyl chlorin e_6 was eluted off with 6-8% methanol, and unreacted chlorin e_6 was removed with 25% methanol.

The product was precipitated at pH 3 after flash evaporating briefly to remove methanol, then washed at the centrifuge 3 times with dilute acetic acid.

The product was dried under vacuum. Yield of Mono(L)-aspartylchlorin e_6 was 50 mg.

EXAMPLE 2

Mono(L)-glutamyl chlorin e_6 (carbomide method)

130 mg of chlorin e_6 and 260 mg L glutamic acid dimethyl ester hydrochloride was dissolved in 18 ml of dimethylformamide. 100 mg of N,N'-dicyclohexyl-carbodiimide was added and the reaction mixture stirred for 1 hour. 50 mg more carbodiimide was then added. After 1 hour, the reaction mixture appeared to contain 75-80% of the monosubstituted product by reverse phase TLC (C-18 plates with 70% MeOH, 30% .01 M KPO_4 pH 6.85). 200 ml Diethyl ether was added, washed twice with 100 ml H_2O, then extracted with 30 ml 1 M KOH.

The product was allowed to hydrolyze in the dark in the KOH solution for 12 hours, then was heated to 70° C for 10 minutes, to complete the hydrolysis of the ester groups. The product was then separated by reverse phase column chromatography (C-18 reverse phase silica 1.5 cm x 30 cm), using stepwise gradient elution with methanol in buffer .01 M KPO_4 pH 6.85. 5% Methanol removed polar impurities. The monoglutamyl chlorin e_6 was eluted with 6-8% methanol. Chlorin e_6 was eluted off the column with 25% methanol. The methanol was removed by flash evaporation and the Mono(L)-glutamyl chlorin e_6 was precipitated at pH 3, collected and washed 3 times at the centrifuge with dilute acetic acid, and dried under vacuum. Yield 40 mg.

EXAMPLE 3

Mono and Di (L) Aspartyl Chlorin e_6 (Carbodiimide Method)

400 mg of chlorin e_6 and 1 g of L-aspartic acid dibenzyl ester p-tosylate were dissolved in 75 ml of dimethyl formamide. Temperature of the solution was maintained at 65-70° C. with stirring and 100 mg of N,N'-dicyclohexyl carbodiimide was added. (A total of 3 additions were made at 2 hour intervals). The solution was allowed to stir at this temperature for a total of 20 hrs., then checked by TLC (reverse phase) (C-18 silica) plate, 70% methanol, 30% .01 M pH 6.85 KPO_4 buffer. The TLC showed greater than 50% monosubstitution with some di-substitution.

150 ml of ether was added, and agitated with 100 ml of water and several drops of glacial acetic acid. The ether phase was separated and the aqueous phase extracted several more times with 100 ml of ether. The ether extracts were combined and washed with water (100 ml) four times to remove dimethyl formamide.

The aspartyl chlorin e_6 esters were then extracted into 100 ml of 1 M KOH (4 extractions of 25 ml each). The KOH solution was allowed to stand at ambient temperature for 24 hours to hydrolyze. The components were separated by neutralizing the solution to pH 7 and applying to a reverse phase (C-18 silica) column (1.5 cm x 30 cm). The elution was performed using a 1 liter gradient of 30 % methanol to 80% methanol with 0.01 M pH 6.85 KPO_4 buffer. Fractions were collected and characterized by TLC. The order of elution was di (L) diaspartyl chlorin e_6, L-monoaspartyl chlorin e_6 and chlorin e_6. Methanol was removed was flash evaporation and the individual components precipitated at pH 3, using HCl.

The products were collected by centrifugation, washed several times with very dilute acetic acid and dried under vacuum. Yield was 23.8 mg. of the disubstituted product and 112 mg. of the monosubstituted chlorin e_6.

EXAMPLE 4

Di (D,L) aspartyl rhodin g_7 (Carbodiimide Method)

140 mg of rhodin g_7 and 200mg of (DL) aspartic acid dimethyl ester hydrochloride are dissolved in 30 ml of dimethyl formamide. 300 mg of N,N'-dicyclohexyl-carbodiimide is added. The reaction is allowed to stand for one hour, then another 300 mg of carbodiimide is added. This procedure is repeated twice and then the reaction mixture is allowed to stand overnight. The reaction may be monitored by thin layer chromatography on silica, using solvent benzene/methanol/88% formic acid 8.5/1.5/0.13 V/V/V.

The disubstituted rhodin g_7 has the the highest R_f value, the unsubstituted rhodin g_7 has the lowest, with the monosubstituted isomers in between and unresolved.

After standing overnight, the reaction mixture appears to contain at least 50% of the disubstituted rhodin g_7. The solvent is removed under vacuum and the remaining solid dissolved in 50 ml of 3N HCl.

The solution is allowed to stand at room temperature for 48 hours to hydrolyze the ester groups, then the chlorin mixture is precipitated at pH 2.5-3 and collected and washed with water at the centrifuge.

The rhodin g_7 mixture is purified by dissolving in 0.05 M NH_4OH and applying to a reverse phase (C-18 silica) column 2.5 cm x 30 cm. The elution procedure is a linear gradient from 40 to 70% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The leading rhodin g_7 band is collected and flash evaporated to remove the methyl alcohol; the solution is then precipitated at pH 2.5-3 and collected and washed 3 times at the centrifuge with dilute acetic acid. The product is dried under vacuum.

EXAMPLE 5

Di and Mono (L) glutamyl rhodin g_7 (mixed anhydride method)

50 mg (0.000087 moles) of rhodin g_7 is dissolved in 100ml of tetrahydrofuran (THF) .210 1 (0.002 moles) of triethylamine is added with stirring. After 10 minutes, 195 µl (0.00179 moles) of ethyl-chloroformate is added. After stirring 10 minutes, 50 ml (0.01 moles) of 0.2 M KOH containing 250 mg (0.00169 moles) of (L) glutamic acid is added dropwise with stirring to the THF solution. This mixture is stirred 60 minutes at room temperature.

The organic solvent is flashed off and the reaction mixture is checked by silica TLC for product. Benzene/methanol/88% formic acid (8.5/1.5/0.13) is used to develop the chromatogram.

After checking for product, the solution is adjusted to pH 7.5-8.0 and placed on a reverse phase (C-18 silica) column 2.5 x 30 cm. The reaction mixture is resolved using a linear gradient of 40-80% methanol in 0.01 M KPO_4 buffer pH 6.85 (1 liter total volume).

The column effluent is collected via fraction collector and the tube contents were pooled according to individual components. The order of elution is di (L) glutamyl rhodin g_7, mono (L) glutamyl rhodin g_7 and unsubstituted rhodin g_7.

The methanol is flashed off and the material is precipitated at pH 2.5-3.0. The precipitate is washed 3 times with dilute acetic acid in water and the product dried under vacuum.

Using the procedures of the foregoing examples, the following amides are prepared:

mono and di-L-aspartyl 2-acetylchlorin e_6
mono and di-L-aspartyl 2-formylchlorin e_6
mono and di-L-aspartyl mesochlorin e_6
mono and di-L-aspartyl 2-deuterochlorin e_6
mono and di-L-glutamyl mesochlorin e_6
mono and di-L-glutamyl 2-deuterochlorin e_6
mono and di-L-glutamyl bacteriochlorin e_6
mono and di-L-aspartyl bacteriochlorin e_6

Physical characteristics of representative compounds (relative polarity) is measured by a standard chromatographic system.

TLC Plate Baker Si-C18 20 um particle size 200 mm coating thickness
Solven System 75% methanol 25% 0.01 M KPO_4 buffer pH 6.85

Compound	Derivative	R_f
Chlorin e_6	-	.66
"	mono(L) aspartyl	.77
"	di(L) aspartyl	.84
"	mono(L)glutamyl	.79

The visible absorption spectrum in pyridine for all of the aminodicarboxylic acid derivatives are identical to the parent porphyrin chlorin or bacteriochlorin.

Comparative Spectroscopic Absorption Data

Solvent in All Cases is P-dioxane.

Compounds	Absorption Maxima (nm) in Visible Region	mM Extinction Coefficient (EmM) ± 10%	Soret Band nm	mM Extinction Coefficient (EmM) ± 10%
Chlorin e_6	665.6	42	402	124
Mono (L) aspartyl chlorin e_6	663.5	38	401.7	111

The preparation of pharmacological dosages for the administration of the active ingredient, that is, the amino acid porphyrin adducts, which were prepared in Examples 1-5 hereinabove, is as follows:

EXAMPLE 6

A tablet base was prepared by blending the following ingredient in the proportion by weight indicated:

	Grams
Sucrose, USP	80.3
Tapioca Starch	13.2
Magnesium Stearate	4.4

Into this base, there was blended sufficient amino acid porphyrin adducts to provide tablets each containing 100 mg. of active ingredient.

EXAMPLE 7

A blend was prepared containing the following ingredients:

	Grams
Calcium Phosphate	17.6
Dicalcium phosphate	18.8
Magnesium trisilicate, USP	5.2
Lactose, U.S.P.	5.2
Potato Starch	5.2
Magnesium Stearate A	0.8
Magnesium Stearate B	0.32
Porphyrin Amino Acid Adducts	20

This blend was divided and formed into capsules each containing 25 mg of active ingredient.

EXAMPLE 8

To a commercially available raspberry flavored sugar syrup is added the equivalent of 40 mg of the amino acid porphyrin adduct per milliliter and the mixture is homogenized in a mechanical device for this purpose. This mixture is especially suitable for oral administration containing 200 mg of the active ingredient.

EXAMPLE 9

A sterile solution of the following composition is prepared: 200 mg of the sodium salt of the amino acid porphyrin adduct is dissolved in a 0.9% NaCl solution so that the final concentration is 20 mg/ml.

This solution is suitable for I.V. and I.M. administration.

EXAMPLE 10

The sodium salt of the amino acid porphyrin adduct is dissolved in 0.9% NaCl solution so that the final concentration is 5 mg/ml. This is placed in an aerosal dispenser with a hydrocarbon propellant. This preparation is suitable for topical application.

EXAMPLE 11

PREPAPATION OF A METAL SALT

The sodium salt of the porphyrin amino acid adduct is prepared by dissolving said adduct in water containing an equimolar amount of sodium hydroxide and freeze drying the resulting mixture.

In this fashion, other metal salts are prepared including potassium, calcium, and lithium salts.

PREPAPATION OF AN ACID SALT

The amino acid porphyrin adducts described in the preceding examples are converted to acid salts, e.g., hydrochloride, by dissolving in an aqueous solution containing an equivalent amount of acid, e.g., hydrochloric acid, and the solution is evaporated to dryness to obtain the solid salt. Alternately, alcoholic solutions of hydrogen chloride gas, dissolved in ethanol can be used in lieu of the aqueous acid solution and the acid salt is obtained by evaporation of the solvent or crystallization from the alcohol, e.g., by addition of a non-solvent.

The following protocols describe the procedure for the utilization of these new compounds of the present invention in the treatment of rat tumors.

EXAMPLE 12

The photodynamic therapy experiments have been carried out using the compound mono- (L)-aspartyl chlorin e_6. Two transplantable tumor lines in Buffalo rats have been used, Morris Hepatoma 7777 and Morris Hepatoma 5123 tc. The tumors were transplanted subcutaneously on the outside of the thigh. During treatment, the tumors ranged in size between 1 and 2.5 cm in diameter.

The general treatment regime is as follows. The rats are injected with a solution of the chlorin prepared as follows: 20 mg of the sodium salt of the chlorin was dissolved in 1 ml of 0.9% NaCl. The chlorin solution was then injected intravenously through the external jugular while the rat was anesthetized with ether. The volume of solution injected was calculated based upon the weight of the animal and the dosage, on a weight to weight basis, for the particular experiment. A specified time interval was then allowed to elapse before light treatment was instigated.

Light treatment of the rats was without anesthesia. The rats were restrained, the hair removed in the treatment area and treated with laser light from a Cooper Aurora argon pumped, tunable dye laser.

The laser was equipped with a fiber optic light delivery system coupled to a microlens system developed by Dr. Daniel Doiron, D.R.D. Consulting, Santa Barbara, California.

The lens disperses the laser beam, providing a circular distribution of light with homogenous light intensity throughout the area of the incident light beam. The wavelength of light was adjusted using a Hartridge reversion spectroscope. The light intensity was determined using a Yellow Springs Instrument, Model 65A, radiometer.

The micro lens was positioned at such a distance from the skin of the animal so as to provide an illumination diameter of 1.5cm, and the light flux was varied by control of the laser output.

Subsequent to illumination, the animal was returned to its cage and, 24 hours later, it was treated intravenously in the external jugular vein with 14 mg of Evans Blue dye, dissolved in 250 µl of 0.9% NaCl. Two hours after injection, the rat was sacrificed and the tumor cross-sectioned. The extent of tumor necrosis was assessed by the lack of dye uptake (1), and the depth of the necrotic cross section of the tumor was recorded in millimeters.

Table II summarizes the effects of these drugs on tumors and includes a range of wavelengths, dosages, intensities, and time intervals for treatment. This has been necessary, in order to attempt to establish the optimal conditions for phototherapy utilizing this new drug. The conditions described result in measurable and significant damage to the tumors.

In all cases except where noted, tissue damage occurred selectively to the tumor tissue as assayed by the Evans Blue method, even though, in nearly all cases, normal skin overlayed the tumor and the treatment area overlapped significant areas of normal muscle tissue.

(1) M.C. Berenbaum, Br. J. Cancer 45: 571(1982)

The photodynamic therapy date is presented in tabular form. Column No. 2 is the total light dose administered in terms of Joules per square centimeter. Column No. 3 is the dose of mono(L)aspartyl chlorin e_6 administered in terms of mg of drug per kilogram of rat body weight. Column No. 4 is the time lapse between administration of drug and treatment with laser light. Column No. 5 is the wavelength of treatment light in nanometers. Column No.6 is the intensity of the treatment light in milliwatts per square centimeter. In Column No. 7, $\bar{x}$ is the mean depth of necrosis in millimeters of the tumor tissue, i.e., the distance from the necrotic top of the tumor next to the skin to the necrotic edge of the tumor most distant from the skin.

S.D. is the standard deviation of $\bar{x}$.

(N) is the number of tumors or legs involved in the experiment.

Column No. 8 is the range of depth of necrosis in millimeters within the group.

TABLE II

tumor	joules/ cm2	drug dose mg/kg	time in hrs. btwn drug & light	wave-lnth nm	intensity mW/cm2	$\bar{x}$ ± s.d. (n)	range mm
7777	10	20	24	655	100	2.8 ± 1.6 (10)	1-6
	10	20	24	665	200	2.8 ± 1.0 (3)	2-4
	10	20	24	650	200	2.9 ± 1.1 (5)	1.5-4.5
	10	20	24	660	100	4.6 ± 1.9 (7)	2.5-8
	10	20	24	660	200	3.6 ± 1.4 (6)	1-6
	10	20	24	665	100	5.9 ± 2.4 (7)	2.5-9
	10	20	48	655	200	7.5 ± 4.2 (2)	4.5-10.5
	20	20	24	655	100	4.1 ± 1.3 (17)	2-6
	20	20	24	660	100	5.4 ± 1.9 (8)	2-7.5
	28.4	20	24	655	200	5.4 ± 1.6 (29)	2.5-11
	28.4	15	24	655	200	4.0 (2)	
	56.8	15	24	655	200	4.5 ± 0.7 (2)	4-5
	56.8	20	24	655	200	4.5 ± 0.7 (2)	4-5
	113	15	24	655	200	Damage Non Specific (3)	
	113	20	24	655	200	4.0 ± 1.2 (4)	3-5
	113	5	48	655	200	3.8 ± 1.2 (6)	2-5
	169	15	24	655	200	Damage Non Specific (5)	
	169	20	24	655	200	5.0 (2)	
	169	5	48	655	200	4.8 ± 0.8 (6)	3.5-6
5123tc	10	20	24	655	100	4.0 (1)	
	20	20	24	655	100	2.7 ± 1.0 (7)	1-4
	20	20	48	655	200	2.0 (2)	
	20	20	24	665	100	4.9 ± 1.0 (8)	3.5-4
	20	20	24	655	200	4.3 ± 1.1 (8)	2.5-6 2/10*
	28.4	20	24	655	100	4.1 ± 1.3 (4)	3-6 1/5*
	28.4	20	24	655	200	3.2 ± 0.3 (3)	3-3.5 4/7*
	30	20	24	655	100	4.4 ± 1.0 (4)	3.5-5.5
	56.8	20	24	655	200	3.0 (1)	
	56.8	5	48	655	200	No effect (9)	
	113	5	48	655	200	No effect (8)	

*No effect

Control Rats: No Tumor

10 20 24 665 100 (10)
24 hr Evaluation: 5 showed some increased dye uptake in the skin at point of treatment.

20 20 24 665 100 (10)
24 hr Evaluation: 6 showed some increased dye uptake in the skin at point of treatment.

10 20 24 665 100 (10)
14 day Evaluation: none showed signs of skin or tumor necrosis and hair had regrown normally.

20 20 24 665 100 (10)
14 day Evaluation: one leg of one animal showed some sign of muscle necrosis. Skin appeared normal and hair regrew normally on all animals.

EXAMPLE 13

PDT Experiments with Mice and mono-L-Aspartyl Chlorin e_6

PDT with the mono-L-aspartyl chlorin e_6 tetrasodium salt was evaluated in another animal/tumor model system.

The tumor, SMT-F, was transplanted subcutaneously into the shoulder/rib area (one side only) of DBA/2 Ha ROS D+ Ha mice. The treatment regime was started when the tumors had reached a dimension of approximately 1.5 - 2 cm long by 1 cm wide and 0.7 to 1 cm deep, (approximately 7 to 8 days after transplant). The drug was administered through intraperitoneal injection at a concentration of 4 mg/ml. Specific parameters and results are listed in the following table. The evaluation was done 24 hours after light treatment using the vital stain Evans Blue in a procedure similar to that which was used for evaluating tumor necrosis in the Buffalo rats, the only difference being the intraperitoneal injection of the dye at a dose of 5 mg per mouse. The headings of each column are the same as the rat system.

Joules/cm^2	Drug Dose mg/kg	Time in Hrs btwn drug & light	Wave-length nm	Intensity mW/cm^2	$\bar{X}$ (mm)	s.d.	(n)	$\bar{D}$ (mm)	s.d.
40	40	24	665	100	$6.6^{\pm}$	2.0	7	$10.3^{\pm}$	3.2

No indication of necrosis of normal tissue (muscle or skin) was observed.

Similar results are obtained when the compounds in Examples 1-5 are administered to a similarly pretreated mice.

EXAMPLE 14

PDT Experiments with Rats and mono-L-Glutamyl Chlorin e_6

Buffalo rats with Morris Hepatoma 7777 transplanted subcutaneously on the outside of each hind leg were subjected to photodynamic therapy, using mono-L-glutamyl chlorin e_6 tetrasodium salt as the drug.

The experimental procedure was the same as is employed for testing of the mono-L-aspartyl chlorin e_6. Specific parameters and results are listed in the table below.

No visible damage - as assessed by the Evans Blue method - to the overlying skin or normal muscle tissue surrounding the tumor was observed, although the 1.5 cm diameter area of light treatment overlapped normal tissue in several cases.

Column No. 1 is the total light dose administered in terms of Joules per square centimeter. Column No. 2 is the dose of chlorin administered in terms of mg of drug per kilogram of rat body weight. Column No. 3 is the time lapse between administration of drug and treatment with laser light. Column No. 4 is the wavelength of treatment light in nanometers. Column No. 5 is the intensity of the treatment light in milliwatts, per square centimeter. In Column No. 6, X is the mean depth of necrosis in millimeters of the tumor tissue, i.e., the distance from the necrotic top of the tumor next to the skin to the necrotic edge of the tumor most distant from the skin. s.d. is the standard deviation of X, (n) is the number of tumors or legs involved in the experiment. D is the mean diameter of tumor necrosis with the following s.d. the standard deviation for D.

Joules/cm^2	Drug Dose mg/kg	Time in Hrs btwn drug & light	Wave-length nm	Intensity mW/cm^2	$\bar{X}$ (mm)	s.d.	(n)	$\bar{D}$ (mm)	s.d.
20	20	24	665	100	$3.4^{\pm}$	1.3	17	$9.6^{\pm}$	3.6

Similar results are obtained when Compounds 1-5 of the preceding examples are administered to similarly pretreated rats.

EXAMPLE 15

PDT EXPERIMENTS WITH MICE AND MONO-L-ASPARTYL CHLORIN e_6

The SMT-F tumor in DBA/2 Ha ROS D+ Ha mouse system was used to evaluate the photodynamic effect of mono-L-glutamyl chlorin e_6 tetrasodium salt.

The protocol is the same as the experiment involving mono - L - aspartyl chlorin e_6 , and the column headings are the same as those used in this system and the rat system.

Joules/ cm^2	Drug Dose mg/kg	Time in Hrs btwn drug & light	Wave-length nm	Intensity mW/cm^2	$\bar{X}$ (mm)	s.d.	* (n)	$\bar{D}$ (mm)	s.d.
40	40	24	665	100	7.9	± 2.9	8	13.9	±3.5

*** A ninth mouse showed no response and was not included in the above statistical analysis. This is because of the possibility that drug was injected into the gut instead of the peritoneum.**

EXAMPLE 16

Human cells (HeLa, strain D98/AH2) were incubated in 25 cm^2 Plastic culture flasks for 24 h to permit attachment. They were then rinsed, incubated for 10 minute periods in Ham's F-12 medium containing porphyrins, rinsed again in Ham's F-12 medium without porphyrins for 5 minutes, then illuminated for various periods, and cultured at 37° C in complete medium for 24 h. Cell counts were then made using a phase contrast microscope of the fraction of the surviving cells. The broad band incandescent light source used was adjusted to give an incident light intensity of 5×10^5 erg cm^{-2} sec^{-1}. A positioning device permitted illuminating each of five areas of a flask for different times; one area was not illuminated and served as a dark control. This gave a four light dose survival curve from a single flask; the technique is thus suitable for the rapid and economical screening of large numbers of potential photosensitizing agents. The results of this experiment are shown in Table III.

TABLE III

Sensitizer	Percent of Cells Surviving 24 Hours After Illumination					
	Time of Illumination in minutes					
	0	.35	.75	.15	3	5	8
Chlorin e_6 mono-L-aspartic acid	100	99	98	82	1	0	0

EXAMPLE 17

SCREENING OF PORPHYRIN FLUORESCENCE AS A FUNCTION OF MOLECULAR STRUCTURE

Two transplantable tumor lines in Buffalo rats were used, Morris Hepatoma 7777 and Morris Hepatoma 5123tc. The tumors were transplanted intramuscularly on the rear of the thigh of the rats. After 10-14 days, when the tumors reached the appropriate size, 2 mg (0.5 ml) of an amino acid porphyrin adduct solution were introduced intraperitoneally into the rats. The amino acid porphyrin adduct solution was prepared as

follows: 4 mg of the amino acid porphyrin was dissolved in 0.1 M NaOH and adjusted to physiological pH with 1 M HCl.

The rats were killed 24 hours after the injection. The tumor was bisected in situ. The porphyrin fluorescence was determined under a constant intensity UV light source.

Tables IV and V list the porphyrin derivatives tested. The compounds are grouped alphabetically.

Following the name of the porphyrin is a number that indicates the total number of tumors examined. The next column of figures (A) is a number calculated as follows: the porphyrin fluorescence within the tumor was ranked visually by one person under a constant intensity U.V. light source according to the scale 0, +½, 1, 2, 3, 4. This number was then multiplied by the percent of the tumor demonstrating this fluorescence, i.e. (+½) (80%) + (+1) (10%) = 50. More often than not, the A value in the table represent averages obtained in several series of separate experiments conducted at different times.

The "C value" for each tumor is the "A value" for that tumor divided by the average diameter of the tumor, in cm.

A time study of 12-72 hours was also conducted on some of the tumors. The procedure is the same as above, except 1 mg of the amino acid adduct was utilized. The results are also indicated in Table IV.

TABLE IV

SCREENING EXPERIMENTS*

Porphyrin Derivative	Dsg	Time	# of Tumors	Avg Diam	% Fluor	A	C
Chlorin e_6 mono-L-glutamyl	2mg	24hr	17	2.41	90	67	28
Chlorin e_6 mono-L-aspartyl	2mg	24hr	18	2.51	88	74	30
Chlorin e_6 mono-L-aspartyl	2mg	24hr	10	1.5	90.6	46.2	38.5
Control			4	3.05	29	15	5
Control			2	2.6	57.5	32.5	20.3
Chlorin e_6 mono-L-glutamyl	2mg	24hr	16	2.7	59.9	34.7	21.1
TIME STUDY							
Chlorin e_6 mono-L-aspartyl	1mg	12hr	6	2.2	81.7	42.1	20.1
Chlorin e_6 mono-L-aspartyl	1mg	18hr	6	1.3	95	47.7	43.4
Chlorin e_6 mono-L-aspartyl	1mg	36hr	6	1.8	93.5	61.8	47.5
Chlorin e_6 mono-L-aspartyl	1mg	48hr	6	1.5	80.8	42.1	35.1
Chlorin e_6 mono-L-aspartyl	1mg	72hr	6	1.7	89.2	48.3	37.2

*Tumor - Horris Hepatoma 7777

TABLE V

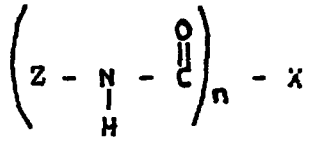
TUMOR LINE: MORRIS HEPATOMA 5123TC

PORPHYRIN	DERIVATIVE	% OF TUMORS	AVG DIAM OF TUMORS (cm)	% FLUORS	A	C
Chlorin e_6	mono (L) aspartyl	11	1.10	26	26	24
"	mono (L) glutamyl	13	1.34	24	12	10

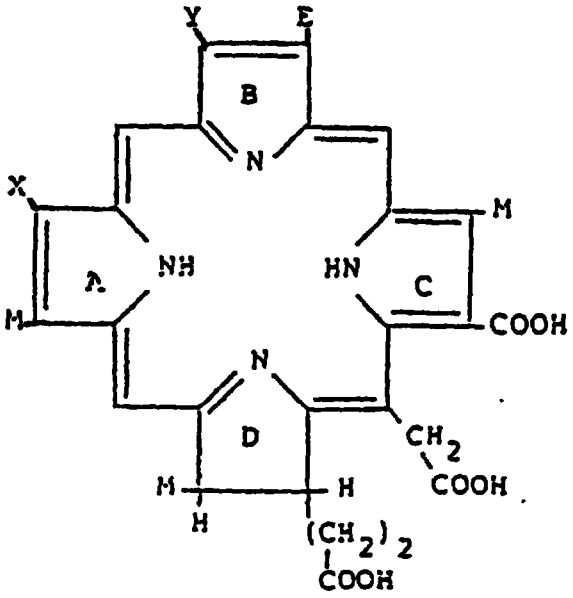
Claims

Claims for the following Contracting States: BE, CH, DE, FR, GB, IT, LI, LU,NL, SE

1. A fluorescent mono, di- or polyamide of an amino dicarboxylic acid and a tetrapyrrole of the structure:



wherein Z is the amino acid residue and X is the residue of a tetrapyrrole of the formula:



wherein;

X = H, vinyl, ethyl, acetyl or formyl;
Y = methyl or formyl;
M = methyl; and
E = ethyl

and "n" is an integer from 1 to 3 inclusive and pharmaceutically-acceptable salts thereof.

2. The amide according to Claim 1 wherein the amino acid is an alpha amino acid.

3. A mono- or diamide according to Claim 1.

4. Mono- or di-L-aspartyl chlorin e_6 according to Claim 1.

TABLE V

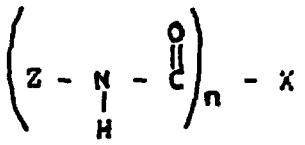
TUMOR LINE: MORRIS HEPATOMA 5123TC

PORPHYRIN	DERIVATIVE	# OF TUMORS	AVG DIAM OF TUMORS (cm)	% FLUORS	A	C
Chlorin e_6	mono (L) aspartyl	11	1.10	26	26	24
"	mono (L) glutamyl	13	1.34	24	12	10

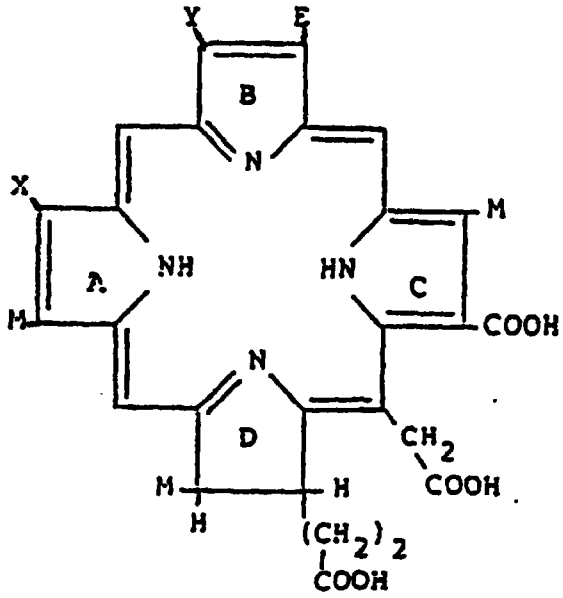
Claims

Claims for the following Contracting States: BE, CH, DE, FR, GB, IT, LI, LU,NL, SE

1. A fluorescent mono, di- or polyamide of an amino dicarboxylic acid and a tetrapyrrole of the structure:



wherein Z is the amino acid residue and X is the residue of a tetrapyrrole of the formula:



wherein;

X = H, vinyl, ethyl, acetyl or formyl;
Y = methyl or formyl;
M = methyl; and
E = ethyl

and "n" is an integer from 1 to 3 inclusive and pharmaceutically-acceptable salts thereof.

2. The amide according to Claim 1 wherein the amino acid is an alpha amino acid.

3. A mono- or diamide according to Claim 1.

4. Mono- or di-L-aspartyl chlorin e_6 according to Claim 1.

5. Mono- or di-L-glutamyl chlorin e_6 according to Claim 1.

6. Mono- or di-L-aspartyl bacteriochlorin e_6 according to Claim 1.

7. Mono- or di-L-glutamyl bacteriochlorin e_6 according to Claim 1.

8. Mono- or di-L-aspartyl rhodin g_7 according to Claim 1.

9. Mono- or di-L-glutamyl rhodin g_7 according to Claim 1.

10. Mono- or di-L-glutamyl mesochlorin e_6 according to Claim 1.

11. Mono- or di-L-aspartyl mesochlorin e_6 according to Claim 1.

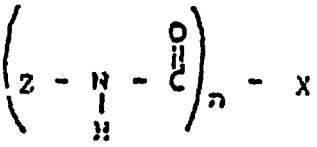
12. A process for preparing the compounds of Claims 1 to 11 which comprises contacting a tetrapyrrole as defined in Claim 1 with an aminodicarboxylic acid in a suitable solvent and optionally converting the product to a salt thereof.

13. A pharmaceutical composition containing a compound according to Claims 1 to 11 together with a pharmaceutical acceptable carrier.

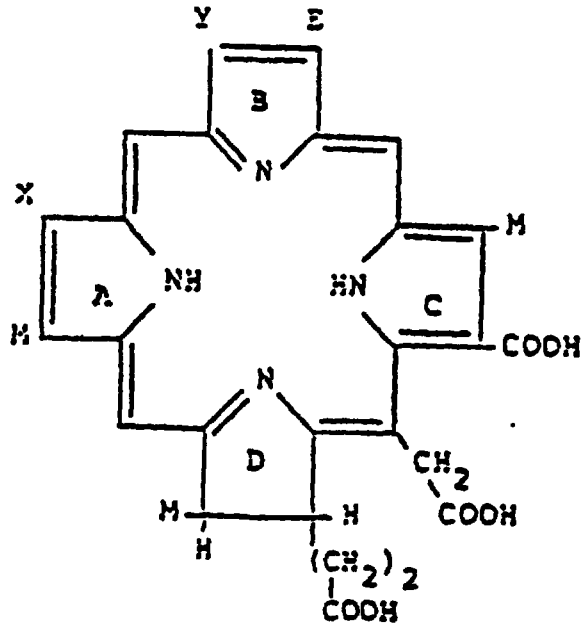
14. Use of a compound according to Claims 1 to 11 in the preparation of a pharmaceutical composition for the detection and treatment of tumors and cancerous tissues in the human or animal body.

Claims for the Contracting State: AT

1. A process for preparing fluorescent mono, di- or polyamide of an aminodicarboxylic acid and a tetrapyrrole of the structure:



wherein Z is the amino acid residue and X is the residue of a tetrapyrrole of the formula:



wherein;

X = H, vinyl, ethyl, acetyl or formyl.
Y = methyl or formyl;
M = methyl; and
E = ethyl

and "n" is an integer from 1 to 3 inclusive which comprises contacting the tetrapyrrole with an aminodicarboxylic acid in a suitable solvent and optionally converting the product to a salt thereof.

2. The process according to Claim 1 wherein the amino acid is an alpha amino acid.

3. The process according to Claim 1 wherein the product is a mono- or diamide.

4. The process according to Claim 3 wherein the product is mono- or di-L-aspartyl chlorin e_6.

5. The process according to Claim 3 wherein the product is mono- or di-L-glutamyl chlorin e_6.

6. The process according to Claim 3 wherein the product is mono- or di-L-aspartyl bacteriochlorin e_6.

7. The process according to Claim 3 wherein the product is mono- or di-L-glutamyl bacteriochlorin e_6.

8. The process according to Claim 3 wherein the product is mono- or di-L-aspartyl rhodin g_7.

9. The process according to Claim 3 wherein the product is mono- or di-L-glutamyl rhodin g_7.

10. The process according to Claim 3 wherein the product is mono- or di-L- glutamyl mesochlorin e_6.

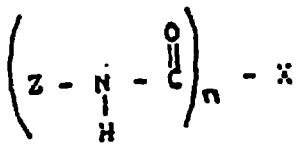
11. The process according to Claim 3 wherein the product is mono- or di-L-aspartyl mesochlorin e_6.

12. Use of a compound prepared according to the process of Claims 1 to 11 for the preparation of a pharmaceutical composition for the detection and treatment of tumors andcancerous tissues in the human or animal body.

Revendications

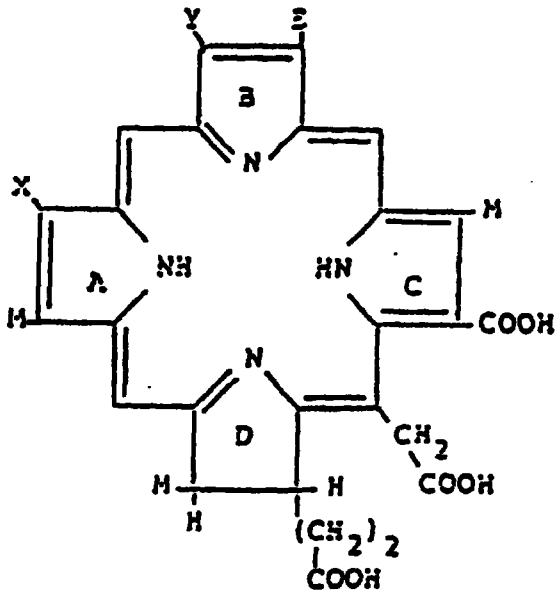
Revendications pour les Etats contractants suivants: BE, CH, DE, GB, IT, LI, LU, NL, SE

1. Mono-, di- ou polyamide fluorescent d'un acide amino dicarboxylique et d'un tétrapyrrole de structure :



dans laquelle :

- Z est le reste d'acide aminé ; et
- X est le reste d'un tétrapyrrole de formule :



dans laquelle :

- X = H, vinyle, éthyle, acétyle ou formyle ;
- Y = méthyle ou formyle ;
- M = méthyle ; et
- E = éthyle ; et

- "n" est un entier de 1 à 3 inclus,

et leurs sels pharmaceutiquement acceptables.

2. Amide selon la revendication 1, dans lequel l'acide aminé est un alpha amino acide.

3. Mono- ou diamide selon la revendication 1.

4. Mono- ou di-L-aspartyl chlorine e_6 selon la revendication 1.

5. Mono- ou di-L-glutamyl chlorine e_6 selon la revendication 1.

6. Mono- ou di-L-aspartyl bactériochlorine e_6 selon la revendication 1.

7. Mono- ou di-L-glutamyl bactériochlorine e_6 selon la revendication 1.

8. Mono- ou di-L-aspartyl rhodine g_6 selon la revendication 1.

9. Mono- ou di-L-glutamyl chlorine g_6 selon la revendication 1.

10. Mono- ou di-L-glutamyl mésochlorine e_6 selon la revendication 1.

11. Mono- ou di-L-aspartyl mésochlorine e_6 selon la revendication 1.

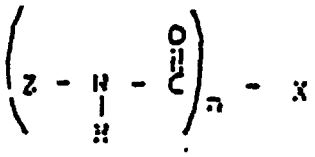
12. Procédé de préparation des composés des revendications 1 à 11, qui comprend la mise en contact d'un tétrapyrrole tel que défini à la revendication 1 avec un acide amino dicarboxylique, dans un solvant approprié, et la conversion facultative du produit en un sel de celui-ci.

13. Composition pharmaceutique contenant un composé selon les revendications 1 à 11 conjointement avec un support pharmaceutique acceptable.

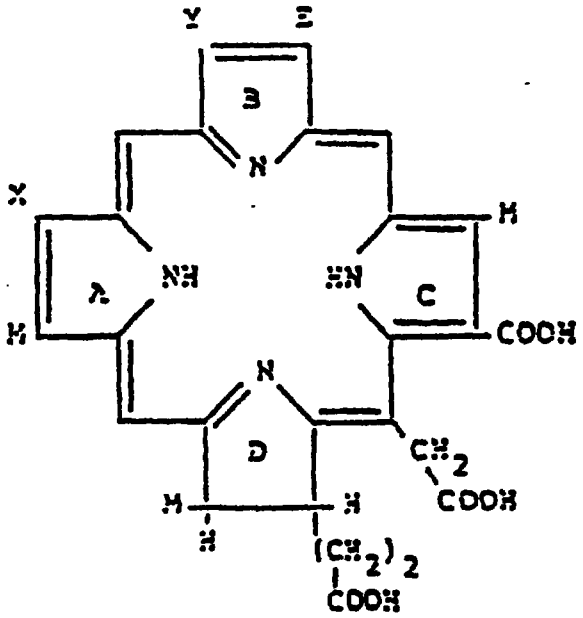
14. Utilisation d'un composé selon les revendications 1 à 11 dans la préparation d'une composition pharmaceutique pour la détection et le traitement de tumeurs et de tissus cancéreux dans le corps humain ou animal.

Revendications pour l'Etat contractant suivant: AT

1. Procédé de préparation d'un mono-, di- ou polyamide fluorescent d'un acide amino dicarboxylique et d'un tétrapyrrole, ayant la structure :



dans laquelle :
- Z est le reste d'acide aminé ; et
- X est le reste d'un tétrapyrrole de formule :



dans laquelle :
- X = H, vinyle, éthyle, acétyle ou formyle ;
- Y = méthyle ou formyle ;
- M = méthyle ; et
- E = éthyle ; et
- "n" est un entier de 1 à 3 inclus,

qui comprend la mise en contact du tétrapyrrole avec un acide amino dicarboxylique, dans un solvant approprié, et la conversion facultative du produit en un sel de celui-ci.

2. Procédé selon la revendication 1, dans lequel l'acide aminé est un alpha amino acide.

3. Procédé selon la revendication 1, dans lequel le produit est un mono- ou diamide.

4. Procédé selon la revendication 3, dans lequel le produit est la mono- ou di-L-aspartyl chlorine e_6.

5. Procédé selon la revendication 3, dans lequel le produit est la mono- ou di-L-glutamyl chlorine e_6.

6. Procédé selon la revendication 3, dans lequel le produit est la mono- ou di-L-aspartyl bactériochlorine e_6.

7. Procédé selon la revendication 3, dans lequel le produit est la mono- ou di-L-glutamyl bactériochlorine e_6.

8. Procédé selon la revendication 3, dans lequel le produit est la mono- ou di-L-aspartyl rhodine g_7.

9. Procédé selon la revendication 3, dans lequel le produit est la mono- ou di-L-glutamyl rhodine g_7.

10. Procédé selon la revendication 3, dans lequel le produit est la mono- ou di-L-glutamyl mésochlorine e_6.

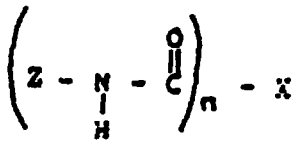
11. Procédé selon la revendication 3, dans lequel le produit est la mono- ou di-L-aspartyl mésochlorine e_6.

12. Utilisation d'un composé préparé selon le procédé des revendications 1 à 11, pour la préparation d'une composition pharmaceutique pour la détection et le traitement de tumeurs et de tissus cancéreux dans le corps humain ou animal.

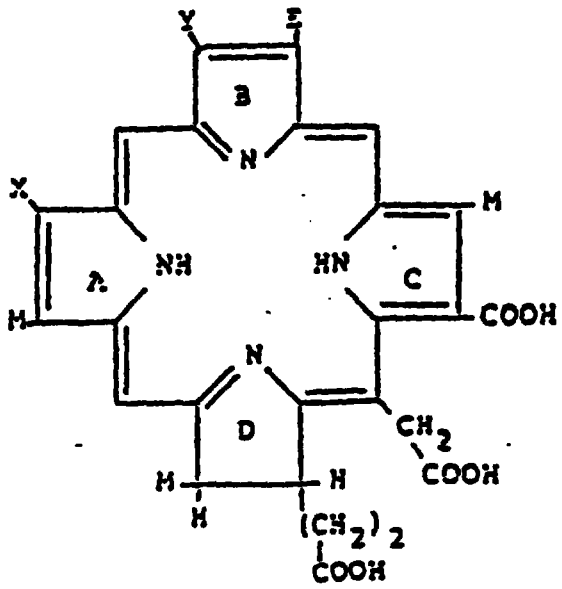
Patentansprüche

Patentansprüche für folgende Vertragsstaaten: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Fluoreszierendes Mono-, Di- oder Polyamid einer Aminodicarbonsäure und eines Tetrapyrrols der Struktur:



wobei Z der Aminosäurerest ist, und X den Rest eines Tetrapyrrols der Formel:



darstellt, wobei X = H, Vinyl, Ethyl, Acetyl oder Formyl;
Y = Methyl oder Formyl;
M = Methyl; und
E = Ethyl
ist, und "n" eine Zahl von 1 bis 3 einschließlich ist, und pharmazeutisch verträgliche Salze davon.

2. Amid nach Anspruch 1, wobei die Aminosäure eine Alphaaminosäure ist.

3. Ein Mono- oder Diamid gemäß Anspruch 1.

4. Mono- oder Di-L-Aspartylchlorin e_6 gemäß Anspruch 1.

5. Mono- oder Di-L-Glutamylchlorin e_6 gemäß Anspruch 1.

6. Mono- oder Di-L-Aspartylbacteriochlorin e_6 gemäß Anspruch 1.

7. Mono- oder Di-L-Glutamylbacteriochlorin e_6 gemäß Anspruch 1.

8. Mono- oder Di-L-Aspartylrhodin g_7 gemäß Anspruch 1.

9. Mono- oder Di-L-Glutamylrhodin g_7 gemäß Anspruch 1.

10. Mono- oder Di-L-Glutamylmesochlorin e_6 gemäß Anspruch 1.

11. Mono- oder Di-L-Aspartylmesochlorin e_6 gemäß Anspruch 1.

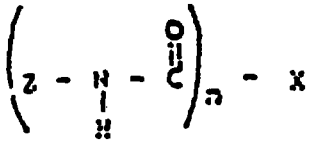
12. Verfahren zur Herstellung der Verbindungen der Ansprüch 1 bis 11, das das Kontaktieren eines Tetrapyrrols gemäß Anspruch 1 mit einer Aminodicarbonsäure in einem geeigneten Lösungsmittel und gegebenenfalls das Umwandeln des Produktes in ein Salz davon umfaßt.

13. Pharmazeutische Zubereitung, die eine Verbindung gemäß der Ansprüche 1 bis 11 zusammen mit einem pharmazeutisch verträglichen Träger enthält.

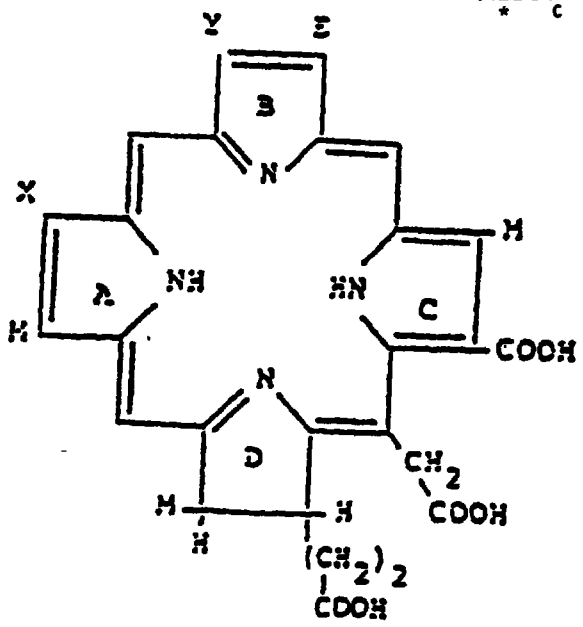
14. Verwendung einer Verbindung gemäß Anspruch 1 bis 11 bei der Herstellung einer pharmazeutischen Zusammensetzung zum Nachweis und zur Behandlung von Tumoren und Krebsgeweben im menschlichen oder tierischen Körper.

Patentansprüche für folgenden Vertragsstaat: AT

1. Verfahren zur Herstellung eines fluoreszierenden Mono-, Di- oder Polyamids einer Aminodicarbonsäure und eines Tetrapyrrols der Struktur:



wobei Z der Aminosäurerest ist, und X den Rest eines Tetrapyrrols der Formel:



darstellt, wobei X = H, Vinyl, Ethyl, Acetyl oder Formyl;
Y = Methyl oder Formyl;
M = Methyl; und
E = Ethyl
ist, und "n" eine Zahl von 1 bis 3 einschließlich ist, das das Kontaktieren des Tetrapyrrols mit einer Aminodicarbonsäure in einem geeigneten Lösungsmittel und gegebenenfalls das Umwandeln des Produkts in ein Salz davon umfaßt.

2. Verfahren nach Anspruch 1, wobei die Aminosäure eine Alphaaminosäure ist.

3. Verfahren nach Anspruch 1, wobei das Produkt ein Mono- oder Diamid ist.

4. Verfahren nach Anspruch 3, wobei das Produkt Mono- oder Di-L-Aspartylchlorin e_6 ist.

5. Verfahren nach Anspruch 3, wobei das Produkt Mono- oder oder Di-L-Glutamylchlorin e_6 ist.

6. Verfahren nach Anspruch 3, wobei das Produkt Mono- oder Di-L-Aspartylbacteriochlorin e_6 ist.

7. Verfahren nach Anspruch 3, wobei das Produkt Mono- oder Di-L-Glutamylbacteriochlorin e_6 ist.

8. Verfahren nach Anspruch 3, wobei das Produkt Mono- oder Di-L-Aspartylrhodin g_7 ist.

9. Verfahren nach Anspruch 3, wobei das Produkt Mono- oder Di-L-Glutamylrhodin g_7 ist.

10. Verfahren nach Anspruch 3, wobei das Produkt Mono- oder Di-L-Glutamylmesochlorin e_6 ist.

11. Verfahren nach Anspruch 3, wobei das Produkt Mono- oder Di-L-Aspartylmesochlorin e_6 ist.

12. Verwendung einer Verbindung, hergestellt gemäß dem Verfahren der Ansprüche 1 bis 11, zur Herstellung einer pharamzeutischen Zusammensetzung zum Nachweis und Behandlung von Tumoren und Krebsgeweben im menschlichen oder tierischen Körper.