2,3-DIHYDROPORPHINE DERIVATIVES, PROCESSES FOR THEIR PREPARATION, AND PHARMACEUTICAL COMPOSITIONS CONTAINING THEM

Technical field

The present invention relates to compounds of formula 3 or salts thereof, processes for the preparation of compounds of formula 3 or salts thereof, pharmaceutical compositions comprising compounds of formula 3 or salts thereof, the use of compounds of formula 3 or salts thereof as phototherapeutic or photodiagnostic agents, and methods of treatment using compounds of formula 3 or salts thereof.

Background art

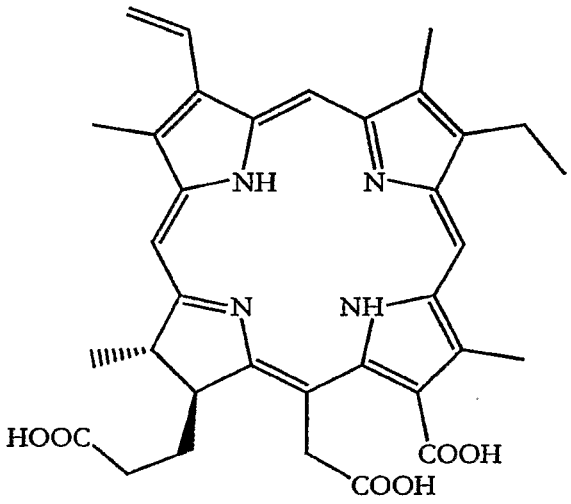
Photodynamic therapy (PDT) is a known treatment that uses light to destroy, for example, cancer tissue. Cytoluminescent therapy (CLT) is a form of photodynamic therapy. In both photodynamic therapy and cytoluminescent therapy, a photosensitizer is administered to a patient, generally orally or intravenously. The photosensitizer collects selectively in cancer tissue and, when exposed to light, becomes activated, releasing a highly energized, free radical form of oxygen known as singlet oxygen. Singlet oxygen destroys cancer cells from the inside out, while leaving normal tissues largely unaffected. The administered photosensitizer can be exposed to light and activated internally using fibre-optic catheters or endoscopes inserted into the body to bring the light directly to the seat of the tumour or externally using light of higher wavelengths, which allows a greater depth of penetration into the body.

Most known photosensitizers have mayor drawbacks, for example, they may be difficult to prepare and purify, or they may only accumulate slowly in tumours. For example, Russian patent RU-2183956 discloses photosensitizers based on a mixture of alkali metal salts, chlorine-e6, purpurine-5 and purpurine-18, which is obtained by extracting Spirulina biomass. However, the photosensitizers disclosed in RU-2183956 have a low selectivity for tumour tissues, a high toxicity to normal organs and tissues, and a low therapeutic photoactivity in tumour cells. Moreover, they are

chemically and photochemically unstable, but are only slowly metabolised and cleared from normal tissues.

It is therefore an object of the present invention to provide photosensitizers with certain desired physical, chemical, photophysical and biological properties, such as high selectivity for tumour tissue, optimum speed of accumulation in tumour tissue, rapid clearance from normal tissue, slow clearance from tumour tissue, high photodynamic activity, low tendency to induce photosensitivity, low cytotoxicity towards normal tissue, homogeneity and chemical stability of medicinal forms during storage, and ease of preparation and purification of industrial quantities.

The inventors of the present invention have investigated the compound of formula **1**, 18-carboxy-20-(carboxymethyl)-8-ethenyl-13-ethyl-2,3-dihydro-3,7,12,17-tetramethyl-21H,23H-porphine-2-propanoic acid, which is also known as phytochlorin or chlorine-e6, and derivatives and metal complexes thereof.

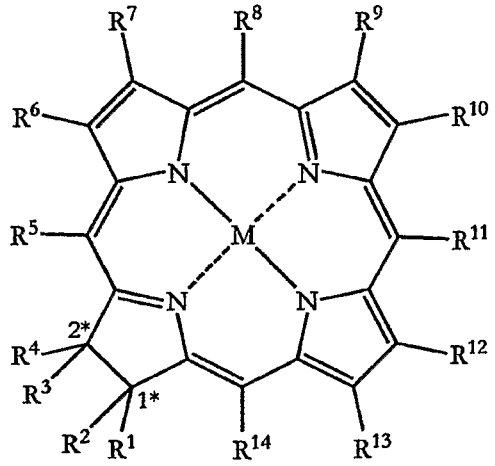


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The inventors of the present invention have further developed a process for the preparation of derivatives and metal complexes of chlorine-e6, which is simple and effective, and provides the derivatives and metal complexes without residual toxic reagents.

Summary of the invention

A first aspect of the present invention is a compound of formula **3**



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or a salt thereof,

wherein

M is a metal atom in the M(II) oxidation state, a metal halide or a metal oxide, where the metal is Ca, Ti, V, Nb, Cr, Mo, Mn, Tc, Ru, Co, Rh, Ni, Pd, Pt, Ag, Au, Zn, Cd, Hg, Al, Ga, In, Ge, Pb or a lanthanide, or M is SiR_2 where R is a C_1-C_8 saturated or unsaturated alkyl group,

each R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} is independently hydrogen, $(CH_2)_n$-CHO, $(CH_2)_n$-CO_2R^{15} or a C_1-C_6 saturated or unsaturated alkyl group optionally substituted with one or more of -OH and $-NH_2$,

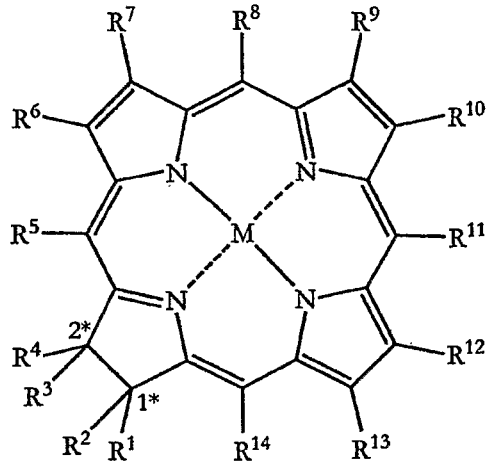
n is 0, 1, 2 or 3, and

each R^{15} is independently hydrogen, lithium, sodium, potassium, magnesium, calcium, a C_1-C_6 saturated or unsaturated alkyl group optionally substituted with one or more of -OH and $-NH_2$, or a naturally occurring amino acid.

The metal halide may be a metal fluoride, chloride, bromide, iodide or a mixture thereof. Preferably M is Zn, Cd, Ca, Mn, Au or Co. More preferably M is Zn.

Preferably the compound is immobilized on a protein, a polypeptide, a polymer or activated charcoal.

A second aspect of the present invention is a compound of formula **3**



3

or a salt thereof,

wherein

M is a metal atom in the M(II) oxidation state, a metal halide, a metal oxide or a silicon with two axial substituents,

each R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} is independently hydrogen, $(CH_2)_n$-CHO, $(CH_2)_n$-CO_2R^{15} or a C_1-C_6 saturated or unsaturated alkyl group optionally substituted with one or more of -OH and -NH_2,

n is 0, 1, 2 or 3,

each R^{15} is independently hydrogen, lithium, sodium, potassium, magnesium, calcium, a C_1-C_6 saturated or unsaturated alkyl group optionally substituted with one or more of -OH and -NH_2, or a naturally occurring amino acid, and

wherein the compound is immobilized on a protein, a polypeptide, a polymer or activated charcoal.

The metal halide may be a metal fluoride, chloride, bromide, iodide or a mixture thereof. Preferably M is Mg, Ca, Ti, V, Nb, Cr, Mo, Mn, Tc, Fe, Ru, Co, Rh, Ni, Pd, Pt, Cu, Ag, Au, Zn, Cd, Hg, Al, Ga, In, Ge, Sn, Pb, a lanthanide or SiR_2 where R is a C_1-C_8 saturated or unsaturated alkyl group. More preferably M is Mg, Ca, Ti, V, Nb, Cr, Mo, Mn, Tc, Fe, Ru, Co, Rh, Ni, Pd, Pt, Cu, Ag, Au, Zn, Cd, Hg, Al, Ga, In, Ge, Sn, Pb or a lanthanide. Even more preferably M is Zn, Cu, Cd, Ca, Mn, Au or Co. Even more preferably M is Zn.

The compound of the first aspect of the present invention is preferably immobilized on a protein, a polypeptide, a polymer or activated charcoal. The compound of the second aspect of the present invention is immobilized on a protein, a polypeptide, a polymer or activated charcoal. Either way, preferably the compound is immobilized in monomer form. Preferably the protein is serum humane albumin (SHA) or bovine serum albumin (BSA), more preferably serum humane albumin (SHA). Preferably the polypeptide is a low molecular weight polypeptide, more preferably polylysine or polyasparagine. Preferably the polymer is polyvinylpyrrolidone (PVP).

For the purposes of this invention, a "salt" of a compound of the present invention is formed between a carboxylic acid functionality of a compound of the present invention and a suitable cation. Suitable cations include, but are not limited to lithium, sodium, potassium, magnesium, calcium and ammonium. Preferably the salt is a pharmaceutically acceptable salt. The salt may be a mono-, di- or tri-salt. Preferably the salt is a mono- or di-lithium, sodium, potassium, magnesium, calcium or ammonium salt. More preferably the salt is a mono- or di-sodium salt.

Preferably each R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} is independently hydrogen, methyl, ethyl, propyl, allyl, CO_2H, CH_2CO_2H or $(CH_2)_2CO_2H$. Preferably R^1 and R^3 are hydrogen. Preferably R^5, R^8 and R^{11} are hydrogen.

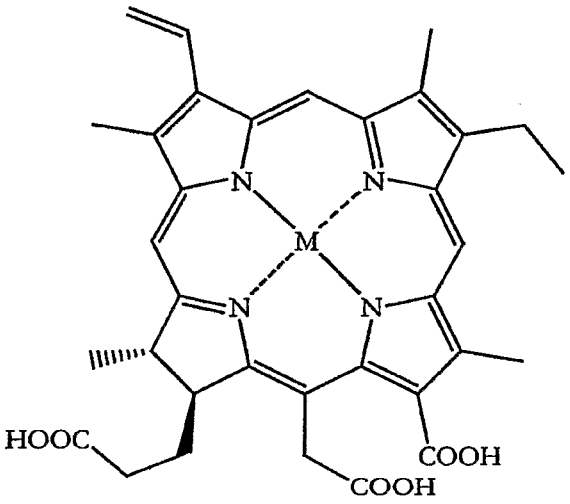
Preferably R^{15} is hydrogen, sodium, a C_1-C_6 saturated or unsaturated alkyl group or a naturally occurring amino acid, such as aspartic acid or lysine.

The compound of formula 3 has two chiral centres, 1* and 2*, and can therefore exist in the form of four stereoisomers. The present invention embraces all of these stereoisomers and mixtures thereof. Mixtures of the stereoisomers can be resolved by conventional methods, for example, chiral chromatography, fractional recrystallisation, derivatisation to form diastereomers and subsequent resolution, and resolution using enzymes. Alternatively, the compound of formula 3 can be prepared directly in substantially enantiomerically pure form by enantioselective or stereoselective synthesis.

The compound of formula **3** preferably comprises at least 95% of one enantiomer, preferably at least 98% of one enantiomer, and more preferably at least 99% of one enantiomer. Preferably the compound of formula **3** is substantially enantiomerically pure, which is defined for the purposes of the present invention as meaning that the compound of formula **3** comprises at least 99% of one enantiomer.

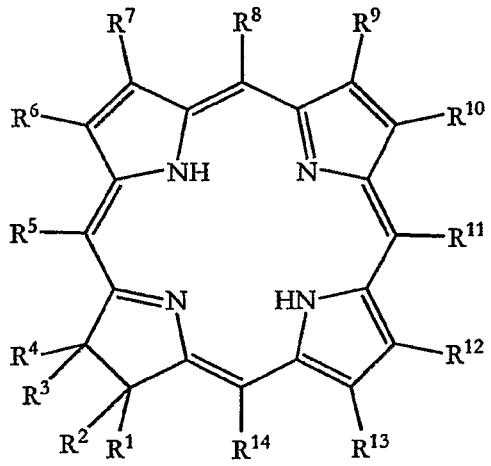
Preferably R^1 and R^3 are hydrogen, and R^1 is in the down-configuration and R^3 is in the up-configuration in formula **3** as shown. More preferably R^1 and R^3 are hydrogen, R^2 is $(CH_2)_2CO_2H$, R^4 is CO_2H, and chiral centres 1* and 2* are in the (S)-configuration.

In the most preferred embodiment, the compound of the present invention is of formula **2**



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A third aspect of the present invention is a process for the preparation of a compound of formula 3 or a salt thereof, comprising the step of mixing a compound of formula 4

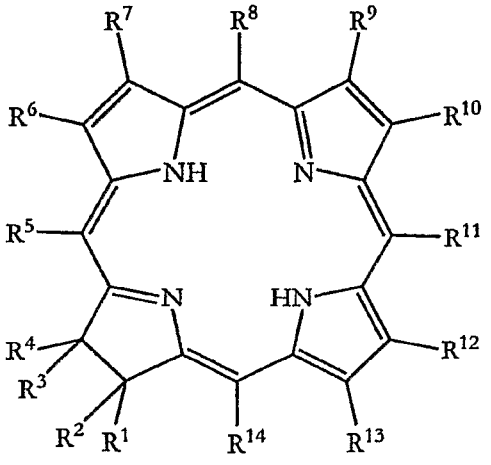


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or a salt thereof with a metal compound in an aqueous solution having a pH $\geq$ 9 to yield the compound of formula 3 or the salt thereof.

An immobilizer can be added to the compound of formula 3 upon formation. Alternatively an immobilizer can be added to the compound of formula 4 prior to the mixing with the metal compound. Preferably the immobilizer is added to an aqueous solution having a pH $\geq$ 9.

The third aspect of the present invention further provides a process for the preparation of a compound of formula 3 or a salt thereof, comprising the steps of (i) mixing a compound of formula 4



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or a salt thereof with an immobilizer in an aqueous solution having a pH ≥ 9 to yield an immobilized compound 4, and (ii) adding a metal compound to the immobilized compound 4 to yield an immobilized compound of formula 3 or the salt thereof.

Preferably the immobilizer is a protein, a polypeptide, a polymer or activated charcoal. Preferably the protein is serum humane albumin (SHA) or bovine serum albumin (BSA), more preferably serum humane albumin (SHA). Preferably the polypeptide is a low molecular weight polypeptide, more preferably polylysine or polyasparagine. Preferably the polymer is polyvinylpyrrolidone (PVP). Preferably the immobilizer immobilizes the compound of formula 3 in monomer form.

Preferably the compound of formula 4 and the metal compound are mixed in a ratio of about 1:1. Preferably the compound of formula 4, the metal compound and the immobilizer are mixed in a ratio of about 1:1:1.

Preferably the metal compound is an organometallic compound. Preferably the metal compound is a carboxylic acid metal salt. Preferably the metal compound is a Zn compound, such as zinc acetate. Alternatively the metal compound may be a Cd or Cu compound, such as cadmium acetate or copper acetate.

Preferably the aqueous solution is provided with a pH ≥ 9 by the addition of ammonia. Preferably the aqueous solution is provided with a pH of from 9 to 10.

Preferably the step of mixing the immobilized or non-immobilized compound of formula 4 with the metal compound is carried out at a temperature of from 10°C to 100°C. Preferably the step of mixing the compound of formula 3 or 4 with the immobilizer is carried out at a temperature of from 10°C to 100°C. More preferably the steps are carried out at a temperature of from 15°C to 40°C, even more preferably at a temperature of from 18°C to 20°C.

A fourth aspect of the present invention is a pharmaceutical composition comprising a compound of formula 3 or a salt thereof and a pharmaceutically acceptable carrier or diluent.

Preferably the pharmaceutical composition is in a form suitable for oral, parental (including intravenous, subcutaneous, intramuscular, intradermal, intratracheal, intraperitoneal, intraarticular, intraabdominal, intracranial and epidural), transdermal, airway (aerosol), rectal, vaginal or topical (including buccal, mucosal and sublingual) administration, most preferably in a form suitable for oral or parental administration.

For oral administration, the pharmaceutical composition is preferably provided in the form of a tablet, capsule, hard or soft gelatine capsule, caplet, troche or lozenge, as a powder or granules, or as an aqueous solution, suspension or dispersion. Moreover, the pharmaceutical composition is preferably in a form suitable for providing 0.01 to 10 mg/kg/day of a compound of formula 3 or a salt thereof, more preferably 0.1 to 5 mg/kg/day, even more preferably about 2 mg/kg/day.

Alternatively, the pharmaceutical composition is in a form suitable for parental, in particular intravenous, administration, in which case the pharmaceutical composition is preferably an aqueous solution or suspension having a pH of from 6 to 8.5.

Preferably the pharmaceutical composition is suitable for use in the photodynamic therapy or cytoluminescent therapy of a human or animal disease. Preferably the human or animal disease is characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation. More preferably the human or animal disease is a benign or malignant tumour.

Preferably the pharmaceutical composition is suitable for the treatment of atherosclerosis, multiple sclerosis, diabetes, a benign or malignant tumour, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic

infectious disease, HIV, hepatitis, herpes simplex, herpes zoster, psoriasis, a cardiovascular disease, or a dermatological condition.

A fifth aspect of the present invention is the use of a compound of formula **3** or a salt thereof for the manufacture of a phototherapeutic agent for the use in photodynamic therapy or cytoluminescent therapy. Preferably the phototherapeutic agent is used for the treatment of a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation. More preferably the phototherapeutic agent is used for the treatment of a benign or malignant tumour.

A sixth aspect of the present invention is the use of a compound of formula **3** or a salt thereof for the manufacture of a medicament for the treatment of atherosclerosis, multiple sclerosis, diabetes, a benign or malignant tumour, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, hepatitis, herpes simplex, herpes zoster, psoriasis, a cardiovascular disease, or a dermatological condition.

A seventh aspect of the present invention is the use of a compound of formula **3** or a salt thereof for the manufacture of a photodiagnostic agent for the identification of an area that is affected by begin or malignant cellular hyperproliferation or by neovascularisation. Preferably the area is a begin or malignant tumour.

An eighth aspect of the present invention is a method of photodynamic therapy or cytoluminescent therapy of a human or animal disease, comprising administering a therapeutically effective amount of a compound of formula **3** or a salt thereof to a human or animal in need thereof and subjecting the human or animal to irradiation or sound. Preferably the human or animal disease is characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation. More preferably the human or animal disease is a benign or malignant tumour. The precise wavelength of the irradiation or sound used depends on the compound administered to the human or animal. However, generally the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm,

preferably from 600nm to 900nm, more preferably from 620nm to 820nm, even more preferably from 630nm to 710nm.

A ninth aspect of the present invention is a method of treating atherosclerosis, multiple sclerosis, diabetes, a benign or malignant tumour, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, hepatitis, herpes simplex, herpes zoster, psoriasis, a cardiovascular disease, or a dermatological condition, comprising administering a therapeutically effective amount of a compound of formula **3** or a salt thereof to a human or animal in need thereof. Optionally the human or animal is further subjected to irradiation or sound. The precise wavelength of the irradiation or sound used depends on the compound administered to the human or animal. However, generally the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm, preferably from 600nm to 900nm, more preferably from 620nm to 820nm, even more preferably from 630nm to 710nm.

Brief description of the drawings

Figure 1 shows the absorption spectra of (1) chlorine-e6 (λ_{max} = 656nm), (2) Zn-chlorine-e6 complex (λ_{max} = 632nm), and (3) Zn-chlorine-e6 complex immobilized on SHA (λ_{max} = 636nm), all in water.

Figure 2 shows the absorption spectrum of chlorine-e6 (λ_{max} = 402, 502 and 656nm) in water.

Figure 3 shows the absorption spectra of (1) chlorine-e6 (λ_{max} = 656nm), (2) chlorine-e6 immobilized on SHA (λ_{max} = 662nm), and (3) Zn-chlorine-e6 complex immobilized on SHA (λ_{max} = 636nm), all in water.

Figure 4 shows the absorption spectra of (1) chlorine-e6 (λ_{max} = 656nm), (2) Zn-chlorine-e6 complex (λ_{max} = 632nm), and (3) Zn-chlorine-e6 complex immobilized on PVP (λ_{max} = 638nm), all in water.

Figure 5 shows the absorption spectra of (1) chlorine-e6 (λ_{max} = 656nm), (2) chlorine-e6 immobilized on PVP (λ_{max} = 662nm), and (3) Zn-chlorine-e6 complex immobilized on PVP (λ_{max} = 638nm), all in water.

Figures 6 to 8 show the absorption spectra of Zn-chlorine-e6 complex (λ_{max} = 414 and 634nm), Zn-chlorine-e6 complex immobilized on SHA (λ_{max} = 418 and 636nm), and Zn-chlorine-e6 complex immobilized on PVP (λ_{max} = 416 and 638nm), all in water, respectively.

Figures 9 and 10 show the fluorescence spectrum (λ_{max} = 643nm) and the fluorescence stimulation spectrum (λ_{max} = 412 and 607nm) of Zn-chlorine-e6 complex in water respectively.

Figures 11 and 12 show the fluorescence spectrum (λ_{max} = 645nm) and the fluorescence stimulation spectrum (λ_{max} = 446 and 673nm) of Zn-chlorine-e6 complex immobilized on SHA in water respectively.

Figures 13 and 14 show the fluorescence spectrum (λ_{max} = 645nm) and the fluorescence stimulation spectrum (λ_{max} = 429 and 727nm) of Zn-chlorine-e6 complex immobilized on PVP in water respectively.

Figures 15 and 16 show the fluorescence spectrum (λ_{max} = 645nm) and the fluorescence stimulation spectrum (λ_{max} = 418 and 641nm) of a biological sample taken from the liquid above the sediment of an ascite tumour taken from an experimental animal (mouse), which had previously been injected intraabdominally with a preparation comprising Zn-chlorine-e6 complex immobilized on SHA.

Figure 17 shows the absorption spectra of (1) chlorine-e6 (λ_{max} = 656nm), (2) chlorine-e6 immobilized on PVP (λ_{max} = 662nm), and (3) Cd-chlorine-e6 complex immobilized on PVP (λ_{max} = 646nm), all in water.

Figure 18 shows the absorption spectrum of Cd-chlorine-e6 complex immobilized on PVP (λ_{max} = 424 and 646nm) in water.

Figure 19 shows the absorption spectra of (1) chlorine-e6 (λ_{max} = 656nm), (2) chlorine-e6 immobilized on PVP (λ_{max} = 662nm), and (3) Cu-chlorine-e6 complex immobilized on PVP (λ_{max} = 636nm), all in water.

Figure 20 shows the absorption spectrum of Cu-chlorine-e6 complex immobilized on PVP (λ_{max} = 410, 505 and 636nm) in water.

Figure 21 shows the results of pharmacokinetic distribution studies. The pharmacokinetic distribution of Zn-chlorine-e6 complex immobilized on SHA over 30 hours in organs, tissues, biological liquids and tumours (embryocarcinoma) was studied.

Detailed description of the invention

The present invention provides two routes to compounds of formula **3**.

The first route (see Examples 1 and 2 below) comprises the step of mixing a compound of formula 4, also called chlorine-e6, which is commercially available, with a metal compound in an aqueous solution having a pH $\geq$ 9 to yield the compound of formula **3**. The compound of formula **3** may be immobilized in monomer form on an immobilizer, such as a protein, a polypeptide, a polymer or activated charcoal, by adding the immobilizer to the compound of formula **3** upon formation.

More specifically, chlorine-e6 is dissolved in an aqueous solution with a pH $\geq$ 9. A pH $\geq$ 9 can be achieved, for example, by adding ammonia to an aqueous solution. Then an about equimolar quantity of a metal compound, for example zinc acetate, is added to the reaction mixture. When mixing the solution at about room temperature, chlorine-e6 and the metal ion form a complex. The progress and completion of the complex-formation reaction can be monitored with a spectrophotometer.

On completion of the complex-formation reaction, an about equimolar quantity of an immobilizer such as a protein, a polypeptide, a polymer or activated charcoal, for example serum humane albumin (SHA) or polyvinylpyrrolidone (PVP), is added to the reaction mixture. The solution is mixed at about room temperature until the compound of formula **3** is immobilized on the immobilizer. The progress and completion of the immobilization reaction can be monitored with the help of a spectrophotometer.

The second route (see Examples 3 to 6 below) comprises the steps of (i) mixing a compound of formula **4** with an immobilizer in an aqueous solution having a pH $\geq$ 9 to yield an immobilized compound **4**, and (ii) adding a metal compound to the immobilized compound **4** to yield an immobilized compound of formula **3**. Preferably the compound of formula **4** is immobilized in monomer form on a protein, a polypeptide, a polymer or activated charcoal. The progress and completion of the immobilization and the complex-formation reaction can be monitored with a spectrophotometer.

Thus a water-soluble immobilizer, for example serum humane albumin (SHA) or polyvinylpyrrolidone (PVP), is added to the reaction mixture in an about equimolar quantity relative to chlorine-e6, either before (route 2) or after (route 1) carrying out the complex-formation reaction.

The fact that compounds of formula **3** can be immobilized in monomolecular form on the immobilizer is surprising, since monomeric compounds of formula **3** are not particularly stable in aqueous solution. The quantity of the immobilizer required is defined by the number of sites on the molecule to be immobilized, which is one for compounds of formula **3**.

Without wishing to be bound by theory, it is believed that it is the monomer form of the compounds of formula **3**, which is the photoactive form, which may be useful as a phototherapeutic or photodiagnostic agent. However, compounds of formula **3**, which have not been immobilized, have a tendency to form aggregates (dimers, trimers and oligomers of unknown structure) with unpredictable physical, chemical,

photophysical and biological properties, in particular when the compounds of formula 3 are subjected to pHs lower than 9. For example, aggregates of Zn-chlorine-e6 are chemically very stable and attempts to disaggregate the Zn-chlorine-e6 aggregates, for example, by increasing pH, heating, using polar solvents, etc. have failed. Thus the aggregation process is difficult, if not impossible, to reverse. The present invention solves this problem by immobilizing the compounds of formula 3 in monomeric form prior to any aggregation occurring.

The compounds of formula 3 are photosensitizers and therefore useful in pharmaceutical compositions and medicaments for the use in photodynamic therapy. Moreover the photosensitizers of formula 3 can be used as photodiagnostic agents for the identification of areas that are affected by begin or malignant cellular hyperproliferation or by neovascularisation.

The pharmaceutical composition or medicament employed in the present invention can be administered by oral, parental (including intravenous, subcutaneous, intramuscular, intradermal, intratracheal, intraperitoneal, intraarticular, intraabdominal, intracranial and epidural), transdermal, airway (aerosol), rectal, vaginal or topical (including buccal, mucosal and sublingual) administration.

For oral administration, the compounds of the invention will generally be provided in the form of tablets, capsules, hard or soft gelatine capsules, caplets, troches or lozenges, as a powder or granules, or as an aqueous solution, suspension or dispersion.

Tablets for oral use may include the active ingredient mixed with pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavouring agents, colouring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose. Corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatine. The lubricating agent, if present, may be magnesium stearate, stearic acid or talc. If

desired, the tablets may be coated with a material, such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

Capsules for oral use include hard gelatine capsules in which the active ingredient is mixed with a solid diluent, and soft gelatine capsules wherein the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin or olive oil.

Formulations for rectal administration may be presented as a suppository with a suitable base comprising, for example, cocoa butter or a salicylate.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For parenteral use, the compounds of the present invention will generally be provided in a sterile aqueous solution or suspension, buffered to an appropriate pH and isotonicity. Suitable aqueous vehicles include Ringer's solution and isotonic sodium chloride or glucose. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium alginate, polyvinylpyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate. The compounds of the invention may also be presented as liposome formulations.

For topical and transdermal administration, the compounds of the invention will generally be provided in the form of ointments, cataplasms (poultices), pastes, powders, dressings, creams, plasters or patches.

Suitable suspensions and solutions can be used in inhalers for airway (aerosol) administration.

In general, a suitable dose will be in the range of 0.01 to 10 mg per kilogram body weight of the recipient per day, preferably in the range of 0.1 to 5 mg per kilogram

body weight per day, more preferably about 2 mg per kilogram body weight per day. The desired dose is preferably presented once a day, but may be dosed as two, three, four, five, six or more sub-doses administered at appropriate intervals throughout the day. These sub-doses may be administered in unit dosage forms, for example, containing 1 to 1500 mg, preferably 10 to 1000 mg, and most preferably 20 to 500 mg of active ingredient per unit dosage form.

The invention will now be described with reference to the following examples. It will be appreciated that what follows is by way of example only and that modifications to detail may be made whilst still falling within the scope of the invention.

Synthetic experimental details

Example 1

Ammonia was added to water until the pH of the solution was not less than 9. Then chlorine-e6 (1.0g) was dissolved in the aqueous solution. An equimolar quantity of zinc acetate (0.22g) was added and the reaction mixture was stirred for 15 minutes at about 20°C to achieve the complex-formation reaction. The progress and completion of the reaction was monitored with the help of a spectrophotometer. On completion of the complex-formation reaction, serum humane albumin (SHA) (71g) was added to the reaction mixture as an immobilizer. On completion of the immobilization reaction, which was monitored with a spectrophotometer, the product of the reaction, Zn-chlorine-e6 complex immobilized on SHA, was purified by dialysis.

Figure 1 shows the long-wave region of the visible absorption spectra of (1) the starting material chlorine-e6 (λ_{max} = 656nm), (2) Zn-chlorine-e6 complex (λ_{max} = 632nm), and (3) Zn-chlorine-e6 complex immobilized on SHA (λ_{max} = 636nm), all in water.

As can be seen in Figure 1, the formation of the Zn-chlorine-e6 complex is accompanied by a 24nm short-wave shift of the long-wave absorption peak, and the immobilization of Zn-chlorine-e6 on protein causes a 4nm long-wave shift. Such shifts of the long-wave peak are typical for both complex-formation with metal and immobilization on protein and prove the completeness and purity of the reactions. Moreover, the characteristic absorption peak of chlorine-e6 of medium intensity at λ_{max} = 502nm practically disappears for Zn-chlorine-e6, and instead a weak peak at λ_{max} = 514nm appears, which also demonstrates the completeness and purity of the reaction.

For comparison, Figure 2 shows the visible absorption spectrum of the starting material chlorine-e6 in water down to 350nm. The maxima of the main absorption peaks are at λ_{max} = 402, 502 and 656nm.

Example 2

The synthesis of immobilized Zn-chlorine-e6 was carried out as described in Example 1, except that as immobilizer polyvinylpyrrolidone (PVP) (62g) was used instead of SHA.

As can be seen in Figure 4, the spectral picture of the visible absorption spectra of (1) the starting material chlorine-e6 (λ_{max} = 656nm), (2) Zn-chlorine-e6 complex (λ_{max} = 632nm), and (3) Zn-chlorine-e6 complex immobilized on PVP (λ_{max} = 638nm) are practically identical to the ones depicted in Figure 1. One observes a significant 24nm short-wave shift of the long-wave peak upon metal complex formation and a small 6nm long-wave shift upon immobilization on polymer PVP. The medium intensity peak of chlorine-e6 at λ_{max} = 502nm practically disappears, when forming the Zn-chlorine-e6 complex. All of these changes prove the completeness of the reactions and the purity and homogeneity of the products obtained.

Example 3

Ammonia was added to water until the pH of the solution was not less than 9. Then chlorine-e6 (1.0g) was dissolved in the aqueous solution. An equimolar quantity of SHA (71g) was added and the reaction mixture was stirred for 17 minutes at about 20°C to immobilize chlorine-e6 on SHA. Then an equimolar quantity of zinc acetate (0.22g) was added and the reaction mixture was stirred at room temperature to complex Zn into the chlorine-e6, which was monitored with a spectrophotometer. The product of the reaction, Zn-chlorine-e6 complex immobilized on SHA, was purified by dialysis.

Figure 3 shows the long-wave region of the visible absorption spectra of (1) the starting material chlorine-e6 (λ_{max} = 656nm), (2) chlorine-e6 immobilized on SHA (λ_{max} = 662nm), and (3) Zn-chlorine-e6 complex immobilized on SHA (λ_{max} = 636nm). Unlike the first method of synthesis (see Example 1), when forming chlorine-e6 immobilized on protein, first a 6nm long-wave shift of the absorption peak occurs, and then a 26nm short-wave shift, when forming Zn-chlorine-e6 immobilized on SHA. Such shifts of the absorption peak agree with the properties of the synthesized products and prove the completeness of the reactions and the purity of the products obtained. Moreover, the medium intensity peak of chlorine-e6 (λ_{max} = 502nm) is observed in the spectra of chlorine-e6 as well as of chlorine-e6 immobilized on protein, but then it disappears in the spectrum of Zn-chlorine-e6 complex immobilized on protein and gets transformed into a peak at λ_{max} = 514nm.

Example 4

The synthesis of immobilized Zn-chlorine-e6 was carried out as described in Example 3, except that as immobilizer polyvinylpyrrolidone (PVP) (62g) was used instead of SHA.

Figure 5 shows the long-wave region of the visible absorption spectra of (1) the starting material chlorine-e6 (λ_{max} = 656nm), (2) chlorine-e6 immobilized on PVP (λ_{max} = 662nm), and (3) Zn-chlorine-e6 complex immobilized on PVP (λ_{max} = 638nm). As in Example 3, when immobilising chlorine-e6 on PVP, a 6nm long-wave shift of the absorption peak takes place, and then after introduction of Zn

ions into chlorine-e6 and formation of the Zn-chlorine-e6 complex immobilized on PVP, a 24nm short-wave shift of the absorption peak occurs. These results demonstrate the completeness of the reactions and the purity of the products obtained. They are also evidenced by the behaviour of the medium intensity peak of chlorine-e6 at λ_{max} = 502nm, which is present in the spectra of chlorine-e6 as well as of chlorine-e6 immobilized on PVP, but disappears in the spectrum of Zn-chlorine-e6 complex immobilized on PVP.

The fact that the spectra of the products, synthesised by the two different routes discussed above (route 1: Examples 1 and 2, route 2: Examples 3 and 4), are identical proves that the conclusions drawn in the final paragraphs of Examples 1 to 4 are correct.

Discussion of further spectra

Figures 6 to 8, with a spectral range of 350-700nm, show visible absorption spectra of Zn-chlorine-e6 complex, Zn-chlorine-e6 complex immobilized on SHA and Zn-chlorine-e6 complex immobilized on PVP, all in water, respectively. The absorption spectra have main absorption peaks at λ_{max} = 414 and 634nm for Zn-chlorine-e6 complex, λ_{max} = 418 and 636nm for Zn-chlorine-e6 complex immobilized on SHA, and λ_{max} = 416 and 638nm for Zn-chlorine-e6 complex immobilized on PVP. The conclusions, drawn from these absorption spectra regarding the purity and stability of the monomeric products, were confirmed at every stage of the synthesis with the help of the highly sensitive analytical method of fluorescence spectroscopy (see Figures 9 to 14, discussed below).

Figures 9 and 10 show the fluorescence spectrum and the fluorescence stimulation spectrum of Zn-chlorine-e6 complex in water respectively. The monomeric Zn-chlorine-e6 complex has a characteristic fluorescence spectrum with λ_{max} = 643nm, and a fluorescence stimulation spectrum with main peaks at λ_{max} = 412 and 607nm, i.e. analogous to the peaks observed in the absorption spectrum. This shows that the fluorescence belongs to the monomeric Zn-chlorine-e6 complex and the fluorescence data prove the high purity and homogeneity of the studied product.

Figures 11 and 12 show the fluorescence spectrum and the fluorescence stimulation spectrum of Zn-chlorine-e6 complex immobilized on SHA in water respectively. The fluorescence spectrum is similar to the fluorescence spectrum of Zn-chlorine-e6 complex in water, though slightly shifted into the red region (λ_{max} = 645nm) and with peaks of a smaller half-width, which demonstrates the great structural similarity between the centres of Zn-chlorine-e6 complex and Zn-chlorine-e6 complex immobilized on SHA observed in these spectra. The fluorescence stimulation spectrum of Zn-chlorine-e6 complex immobilized on SHA, shown in Figure 12, is very similar to its absorption spectrum shown in Figure 7 and shows two main peaks at λ_{max} = 446 and 673nm with a smaller half-width and a more regular shape compared to the peaks in the absorption spectrum. This proves that the fluorescence belongs to monomeric Zn-chlorine-e6 complex immobilized on SHA and that the studied product has a high homogeneity and purity.

Figures 13 and 14 show the fluorescence spectrum and the fluorescence stimulation spectrum of Zn-chlorine-e6 complex immobilized on PVP in water respectively. The shape of the fluorescence spectrum is very similar to the fluorescence spectra discussed above and has a peak at λ_{max} = 645nm as in the spectrum of Zn-chlorine-e6 complex immobilized on SHA. The fluorescence stimulation spectrum has main peaks at λ_{max} = 429 and 727nm, which agrees with its absorption spectrum and shows that the fluorescence belongs to Zn-chlorine-e6 complex immobilized on PVP and that the product is highly pure.

Figures 15 and 16 show the fluorescence spectrum and the fluorescence stimulation spectrum of a biological sample taken from the liquid above the sediment of an ascite tumour taken from an experimental animal (mouse), which had previously been injected intraabdominally with a preparation comprising Zn-chlorine-e6 complex immobilized on SHA. As can be seen by comparing the spectra of the biological sample shown in Figures 15 and 16 with the corresponding spectra of the models shown in Figures 9 to 14, the peaks in the spectra of the biological sample occur at similar λ_{max} (fluorescence spectrum in Figure 15: λ_{max} = 645nm; fluorescence stimulation spectrum in Figure 16: λ_{max} = 418 and 641nm) and have a

similar peak shape and peak intensity ratio as the peaks in the spectra of the models. This means that the preparation injected into the experimental animal did not undergo substantial structural changes and comprises Zn-chlorine-e6 with a high structural homogeneity of the absorbing and fluorescent centre as was observed for Zn-chlorine-e6 complex immobilized on SHA.

Example 5

Cd-chlorine-e6 complex immobilized on PVP was synthesized in a similar way to Zn-chlorine-e6 complex immobilized on PVP (see Example 4). Figure 17 shows the long-wave part of the visible absorption spectra of (1) the starting material chlorine-e6 (λ_{max} = 656nm), (2) chlorine-e6 immobilized on PVP (λ_{max} = 662nm), and (3) Cd-chlorine-e6 complex immobilized on PVP (λ_{max} = 646nm). Figure 18 shows the absorption spectrum in the range of 350-750nm of the monomer form of Cd-chlorine-e6 complex immobilized on PVP in water. As can be seen in Figure 18, the spectrum of Cd-chlorine-e6 complex immobilized on PVP in monomer form has two main peaks at λ_{max} = 424 and 646nm respectively.

Example 6

Cd-chlorine-e6 complex immobilized on PVP was synthesized in a similar way to Zn-chlorine-e6 complex immobilized on PVP (see Example 4). Figure 19 shows the long-wave part of the visible absorption spectra of (1) the starting material chlorine-e6 (λ_{max} = 656nm), (2) chlorine-e6 immobilized on PVP (λ_{max} = 662nm), and (3) Cu-chlorine-e6 complex immobilized on PVP (λ_{max} = 636nm). Figure 20 shows the absorption spectrum in the range of 350-750nm of the monomer form of Cu-chlorine-e6 complex immobilized on PVP in water. As can be seen in Figure 20, the absorption spectrum of Cu-chlorine-e6 complex immobilized on PVP in monomer form differs from the monomer spectra of Zn-chlorine-e6 immobilized complex and Cd-chlorine-e6 immobilized complex. The absorption spectrum of Cu-chlorine-e6 complex immobilized on PVP in monomer form has three main peaks at λ_{max} = 410, 505 and 636nm respectively.

Preclinical pharmacokinetic studies

A remarkable and important feature of the immobilized monomer Zn-chlorine-e6 complex is the possibility of preparing a stable form of the monomeric Zn-chlorine-e6 complex at a pH of from 6 to 8.5, which is required for injection usage. Zn-chlorine-e6 complex preparations suitable for injection may be prepared by acidifying the reaction medium after completion of the synthesis. Pharmaceutically acceptable additives, which do not interfere with the structural stability of the Zn-chlorine-e6 complex and the homogeneity of the preparation, may be added to such preparations suitable for injection.

The pharmacokinetic distribution of Zn-chlorine-e6 complex immobilized on SHA over 30 hours in organs, tissues, biological liquids and tumours (embryocarcinoma) was studied. Female mice of the line Balb/c weighing 20-21g were used as experimental animals. The pharmacokinetic studies were carried out using a Perkin-Elmer spectrofluorimeter on homogetates of organs and tumours, taken after the intraabdominal injection of Zn-chlorine-e6 complex immobilized on SHA at a dose of 25 mg/kg weight. The results of these pharmacokinetic distribution studies are depicted in Figure 21 and summarised in Table 1 below.

Organ	Amount of Zn-chlorine-e6 complex immobilized on SHA accumulated in organ X hours after injection in relative units				
	X = 1	X = 5	X = 15	X = 24	X = 30
1. Blood	4	9	6	1	1
2. Urine	7	0	0	0	0
3. Small intestine	98	175	136	90	46
4. Liver	86	147	66	49	33
5. Spleen	11	17	17	12	13
6. Kidney	38	64	25	11	8
7. Lungs	18	15	26	11	8
8. Tumour	8	40	99	67	79
9. Skin	9	8	19	7	15
10. Muscle	25	7	33	6	18

Table 1

Results:

A. Intraabdominal injection of Zn-chlorine-e6 complex immobilized on SHA at a dose of 25 mg/kg weight was well endured by the animals without any signs of toxicity and did not affect their behavioural reaction, both immediately and 30 hours after the injection.

B. The immobilized complex was rapidly absorbed from the abdominal cavity into the blood and was deposited in the liver during the first hours after injection. Its content in the liver tissues was 10-14 times higher than its level in the blood.

C. A significant quantity of the immobilized complex was also accumulated in the kidneys in the first 12 hours after injection (only 2-2.5 times less than in the liver), however, the immobilized complex was practically absent from the urine. During the next 18 hours, the immobilized complex was washed out intensely from the kidney tissue into the blood. The kidneys' secretion function was not affected during the whole observation period.

D. The maximum concentrations of the immobilized complex in the liver were found during the first 8 hours after injection. During the next 24 hours, the surplus of the immobilized complex was discharged intensely into the small intestines. The dynamics of the distribution curves of the liver and small intestines correlate precisely with one another. It may be sufficient to inject 5-10 times smaller doses of the immobilized complex in order to achieve maximum concentrations in the tumour.

E. 5-8 times less of the immobilized complex accumulated in the spleen and the lungs compared to the liver or tumour, and 24 hours after injection the spleen and lungs had phone readings.

F. Skin and muscle tissue both had practically the same accumulation dynamics, the only difference being that the immobilized complex content in the muscle was

1.5 times higher in the first 15 hours than the immobilized complex content in the skin.

G. The accumulation of the immobilized complex in tumour increased progressively from the moment of injection and reached its maximum 15 hours after injection. The maximum concentration plateau (12-20 hours) was found to be much longer than after chlorine-e6 injection, and after an insignificant fall by the end of the first 24 hours, a second increase of immobilized complex concentration up to the maximum readings of the concentration plateau was observed between 24 and 30 hours. A "scissors" effect (the immobilized complex concentration in the tumour is increasing, while the immobilized complex concentration in the liver is decreasing) was observed twice for the liver and tumour, once 12 hours after injection and once, even more pronounced, 24 hours after injection.

To summarise, after the absorption of the immobilized complex in the abdominal cavity, redistribution from the blood into the organs and washing out of the immobilized complex surplus by the liver during the first 24 hours, the immobilized complex accumulated in the tumour tissue in a concentration of 2.5 times greater than in the liver and 6 times greater than in the skin, muscle and other parenchymal organs. In comparison with the pharmacokinetics of the dimer form, the monomer form demonstrated much greater tumour selectivity and stability of the chemical structure in tissues.

The spectroscopic data (see Figures 1 to 20) and pharmacokinetic data (see Figure 21) discussed above show that the immobilized preparations preserve the monomeric structure, purity and chemical stability of the porphyrin nucleus of the Zn-chlorine-e6 complex.

Definition of acute toxicity parameters:

To define parameters LD_{10} and LD_{50} three preparation doses were chosen (100, 125 and 150 mg/kg weight) for single intraabdominal injection. Chlorine-e6 readings

were taken as a prototype, where LD_{10} is 119 mg/kg weight and LD_{50} is 160 mg/kg weight.

After injection of Zn-chlorine-e6 complex immobilized on SHA in the above stated doses, a first reaction to the injection was observed only with the third animal group (150 mg/kg weight), because the preparation was injected in 3ml physiological solution, which caused temporal animal stillness due to abdominal swelling. After the absorption of the surplus liquid, however, these animals did not differ from the animals in the other two groups in their behavioural reactions (moving activity, defence reflex, food reflex, coat condition).

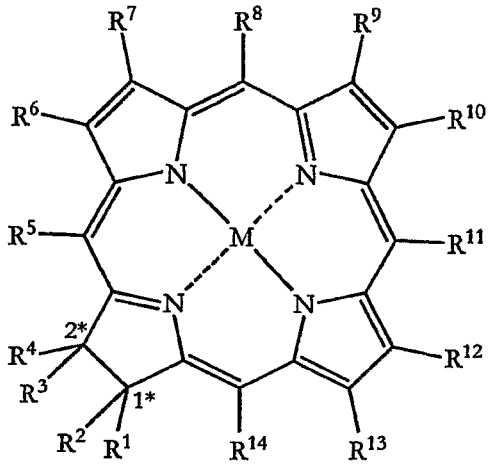
During the following 72 hours, signs of acute toxicity (slow reaction, hollow sides, diarrhoea, defence and food reflex absence) did not appear. The animals were kept under observation for a further fortnight.

Further tests were carried out similarly with intraabdominal injections of 175, 200 and 225 mg/kg weight, as well as 300, 350 and 450 mg/kg weight. None of these concentrations proved toxic.

It will be understood that the present invention has been described above by way of example only. The examples are not intended to limit the scope of the invention. Various modifications and embodiments can be made without departing from the scope of the invention, which is defined by the following claims.

Claims

1. A compound of formula **3**



3

or a salt thereof,

wherein

M is a metal atom in the M(II) oxidation state, a metal halide or a metal oxide, where the metal is Ca, Ti, V, Nb, Cr, Mo, Mn, Tc, Ru, Co, Rh, Ni, Pd, Pt, Ag, Au, Zn, Cd, Hg, Al, Ga, In, Ge, Pb or a lanthanide, or M is SiR_2 where R is a C_1-C_8 saturated or unsaturated alkyl group,

each R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} is independently hydrogen, $(CH_2)_n$-CHO, $(CH_2)_n$-CO_2R^{15} or a C_1-C_6 saturated or unsaturated alkyl group optionally substituted with one or more of -OH and $-NH_2$,

n is 0, 1, 2 or 3, and

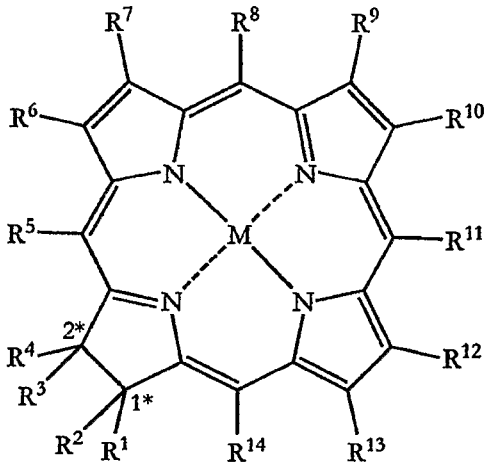
each R^{15} is independently hydrogen, lithium, sodium, potassium, magnesium, calcium, a C_1-C_6 saturated or unsaturated alkyl group optionally substituted with one or more of -OH and $-NH_2$, or a naturally occurring amino acid.

2. A compound as claimed in claim 1, wherein M is Zn, Cd, Ca, Mn, Au or Co.

3. A compound as claimed in claim 2, wherein M is Zn.

4. A compound as claimed in any preceding claim, wherein the compound is immobilized on a protein, a polypeptide, a polymer or activated charcoal.

5. A compound of formula 3



3

or a salt thereof,

wherein

M is a metal atom in the M(II) oxidation state, a metal halide, a metal oxide or a silicon with two axial substituents,

each R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} is independently hydrogen, $(CH_2)_n$-CHO, $(CH_2)_n$-CO_2R^{15} or a C_1-C_6 saturated or unsaturated alkyl group optionally substituted with one or more of -OH and -NH_2,

n is 0, 1, 2 or 3,

each R^{15} is independently hydrogen, lithium, sodium, potassium, magnesium, calcium, a C_1-C_6 saturated or unsaturated alkyl group optionally substituted with one or more of -OH and -NH_2, or a naturally occurring amino acid, and

wherein the compound is immobilized on a protein, a polypeptide, a polymer or activated charcoal.

6. A compound as claimed in claim 5, wherein M is Mg, Ca, Ti, V, Nb, Cr, Mo, Mn, Tc, Fe, Ru, Co, Rh, Ni, Pd, Pt, Cu, Ag, Au, Zn, Cd, Hg, Al, Ga, In, Ge, Sn, Pb, a lanthanide or SiR_2 where R is a C_1-C_8 saturated or unsaturated alkyl group.

7. A compound as claimed in claim 6, wherein M is Mg, Ca, Ti, V, Nb, Cr, Mo, Mn, Tc, Fe, Ru, Co, Rh, Ni, Pd, Pt, Cu, Ag, Au, Zn, Cd, Hg, Al, Ga, In, Ge, Sn, Pb or a lanthanide.

8. A compound as claimed in claim 7, wherein M is Zn, Cu, Cd, Ca, Mn, Au or Co.

9. A compound as claimed in claim 8, wherein M is Zn.

10. A compound as claimed in any one of claims 4 to 9, wherein the compound is immobilized in monomer form.

11. A compound as claimed in any one of claims 4 to 10, wherein the compound is immobilized on serum humane albumin (SHA), bovine serum albumin (BSA) or polyvinylpyrrolidone (PVP).

12. A compound as claimed in any one of claims 4 to 10, wherein the compound is immobilized on a low molecular weight polypeptide.

13. A compound as claimed in claim 12, wherein the compound is immobilized on polylysine or polyasparagine.

14. A compound as claimed in any preceding claim, wherein each R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} is independently hydrogen, methyl, ethyl, propyl, allyl, CO_2H, CH_2CO_2H or $(CH_2)_2CO_2H$.

15. A compound as claimed in any preceding claim, wherein R^1 and R^3 are hydrogen.

16. A compound as claimed in any preceding claim, wherein R^5, R^8 and R^{11} are hydrogen.

17. A compound as claimed in any preceding claim, wherein R^{15} is hydrogen, sodium, a C_1-C_6 saturated or unsaturated alkyl group or a naturally occurring amino acid.

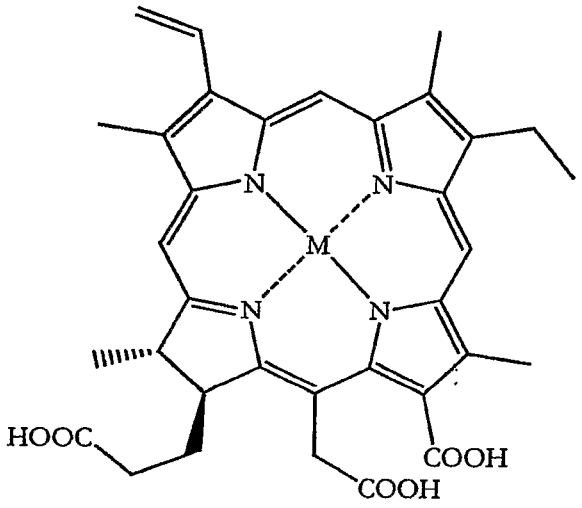
18. A compound as claimed in any preceding claim, wherein R^{15} is aspartic acid or lysine.

19. A compound as claimed in any preceding claim, wherein the compound is substantially enantiomerically pure.

20. A compound as claimed in any preceding claim, wherein R^1 and R^3 are hydrogen, and R^1 is in the down-configuration and R^3 is in the up-configuration in formula 3 as shown.

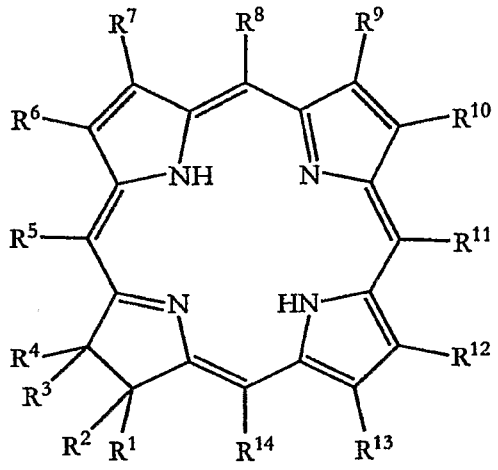
21. A compound as claimed in any preceding claim, wherein R^1 and R^3 are hydrogen, R^2 is $(CH_2)_2CO_2H$, R^4 is CO_2H, and chiral centres 1* and 2* are in the (S)-configuration.

22. A compound as claimed in any preceding claim, wherein the compound is of formula 2



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23. A process for the preparation of a compound of formula 3 or a salt thereof as claimed in any one of claim 1 to 22, comprising the step of mixing a compound of formula 4



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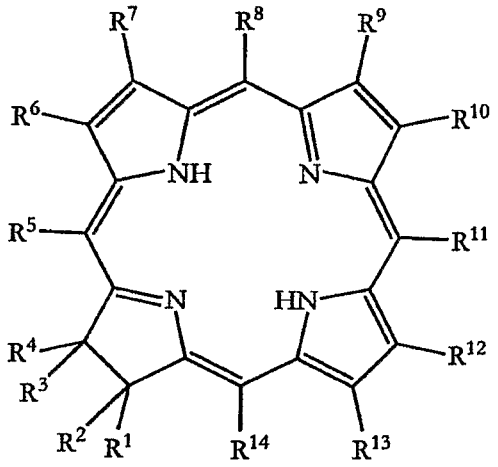
or a salt thereof with a metal compound in an aqueous solution having a pH ≥ 9 to yield the compound of formula 3 or the salt thereof.

24. A process as claimed in claim 23, wherein an immobilizer is added to the compound of formula 3 upon formation.

25. A process as claimed in claim 23, wherein an immobilizer is added to the compound of formula 4 prior to the mixing with the metal compound.

26. A process as claimed in claim 24 or claim 25, wherein the immobilizer is added to an aqueous solution having a pH ≥ 9.

27. A process for the preparation of a compound of formula 3 or a salt thereof as claimed in any one of claim 1 to 22, comprising the steps of (i) mixing a compound of formula 4



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or a salt thereof with an immobilizer in an aqueous solution having a pH ≥ 9 to yield an immobilized compound 4, and (ii) adding a metal compound to the immobilized compound 4 to yield an immobilized compound of formula 3 or the salt thereof.

28. A process as claimed in any one of claims 24 to 27, wherein the immobilizer is a protein, a polypeptide, a polymer or activated charcoal.

29. A process as claimed in claim 28, wherein the immobilizer is serum humane albumin (SHA), bovine serum albumin (BSA) or polyvinylpyrrolidone (PVP).

30. A process as claimed in claim 28, wherein the immobilizer is a low molecular weight polypeptide.

31. A process as claimed in claim 30, wherein the immobilizer is polylysine or polyasparagine.

32. A process as claimed in any one of claims 24 to 31, wherein the immobilizer immobilizes the compound of formula 3 in monomer form.

33. A process as claimed in any one of claims 24 to 32, wherein the compound of formula 4, the metal compound and the immobilizer are mixed in a ratio of about 1:1:1.

34. A process as claimed in any one of claims 23 to 33, wherein the compound of formula 4 and the metal compound are mixed in a ratio of about 1:1.

35. A process as claimed in any one of claims 23 to 34, wherein the metal compound is an organometallic compound.

36. A process as claimed in any one of claims 23 to 35, wherein the metal compound is a carboxylic acid metal salt.

37. A process as claimed in any one of claims 23 to 36, wherein the metal compound is a Zn, Cd or Cu compound.

38. A process as claimed in claim 36 or claim 37, wherein the metal compound is zinc acetate, cadmium acetate or copper acetate.

39. A process as claimed in any one of claims 23 to 38, wherein the aqueous solution is provided with a pH $\geq$ 9 by the addition of ammonia.

40. A process as claimed in any one of claims 23 to 39, wherein the aqueous solution is provided with a pH of from 9 to 10.

41. A process as claimed in any one of claims 23 to 40, wherein the step of mixing the immobilized or non-immobilized compound of formula 4 with the metal compound is carried out at a temperature of from 10°C to 100°C.

42. A process as claimed in any one of claims 23 to 41, wherein the step of mixing the compound of formula 3 or 4 with the immobilizer is carried out at a temperature of from 10°C to 100°C.

43. A process as claimed in claim 41 or claim 42, wherein the step is carried out at a temperature of from 15°C to 40°C.

44. A process as claimed in claim 43, wherein the step is carried out at a temperature of from 18°C to 20°C.

45. A pharmaceutical composition comprising a compound as claimed in any one of claims 1 to 22, and a pharmaceutically acceptable carrier or diluent.

46. A pharmaceutical composition as claimed in claim 45, wherein the pharmaceutical composition is in a form suitable for oral, parental (including intravenous, subcutaneous, intramuscular, intradermal, intratracheal, intraperitoneal, intraarticular, intraabdominal, intracranial and epidural), transdermal, airway (aerosol), rectal, vaginal or topical (including buccal, mucosal and sublingual) administration.

47. A pharmaceutical composition as claimed in claim 46, wherein the pharmaceutical composition is in a form suitable for oral administration.

48. A pharmaceutical composition as claimed in claim 47, wherein the pharmaceutical composition is provided in the form of a tablet, capsule, hard or soft gelatine capsule, caplet, troche or lozenge, as a powder or granules, or as an aqueous solution, suspension or dispersion.

49. A pharmaceutical composition as claimed in claim 47 or claim 48, wherein the pharmaceutical composition is in a form suitable for providing 0.01 to 10 mg/kg/day of a compound as claimed in any one of claims 1 to 22.

50. A pharmaceutical composition as claimed in claim 49, wherein the pharmaceutical composition is in a form suitable for providing 0.1 to 5 mg/kg/day of the compound.

51. A pharmaceutical composition as claimed in claim 50, wherein the pharmaceutical composition is in a form suitable for providing about 2 mg/kg/day of the compound.

52. A pharmaceutical composition as claimed in claim 46, wherein the pharmaceutical composition is in a form suitable for parental administration.

53. A pharmaceutical composition as claimed in claim 52, wherein the pharmaceutical composition is in a form suitable for intravenous administration.

54. A pharmaceutical composition as claimed in claim 52 or claim 53, wherein the pharmaceutical composition is an aqueous solution or suspension having a pH of from 6 to 8.5.

55. A pharmaceutical composition as claimed in any one of claims 45 to 54, for use in the photodynamic therapy or cytoluminescent therapy of a human or animal disease.

56. A pharmaceutical composition as claimed in claim 55, wherein the human or animal disease is characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation.

57. A pharmaceutical composition as claimed in claim 55 or claim 56, wherein the human or animal disease is a benign or malignant tumour.

58. A pharmaceutical composition as claimed in any one of claims 45 to 57, for the treatment of atherosclerosis, multiple sclerosis, diabetes, a benign or malignant tumour, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, hepatitis, herpes simplex, herpes zoster, psoriasis, a cardiovascular disease, or a dermatological condition.

59. Use of a compound as claimed in any one of claims 1 to 22, for the manufacture of a phototherapeutic agent for the use in photodynamic therapy or cytoluminescent therapy.

60. A use as claimed in claim 59, wherein the phototherapeutic agent is used for the treatment of a disease characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation.

61. A use as claimed in claim 59 or claim 60, wherein the phototherapeutic agent is used for the treatment of a begin or malignant tumour.

62. Use of a compound as claimed in any one of claims 1 to 22, for the manufacture of a medicament for the treatment of atherosclerosis, multiple sclerosis, diabetes, a benign or malignant tumour, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, hepatitis, herpes simplex, herpes zoster, psoriasis, a cardiovascular disease, or a dermatological condition.

63. Use of a compound as claimed in any one of claims 1 to 22, for the manufacture of a photodiagnostic agent for the identification of an area that is affected by begin or malignant cellular hyperproliferation or by neovascularisation.

64. A use as claimed in claim 63, wherein the area is a begin or malignant tumour.

65. A method of photodynamic therapy or cytoluminescent therapy of a human or animal disease, comprising administering a therapeutically effective amount of a compound as claimed in any one of claims 1 to 22 to a human or animal in need thereof and subjecting the human or animal to irradiation or sound.

66. A method as claimed in claim 65, wherein the human or animal disease is characterised by begin or malignant cellular hyperproliferation or by areas of neovascularisation.

67. A method as claimed in claim 65 or claim 66, wherein the human or animal disease is a benign or malignant tumour.

68. A method of treating atherosclerosis, multiple sclerosis, diabetes, a benign or malignant tumour, arthritis, rheumatoid arthritis, a fungal, viral, chlamydial, bacterial, nanobacterial or parasitic infectious disease, HIV, hepatitis, herpes simplex, herpes zoster, psoriasis, a cardiovascular disease, or a dermatological condition, comprising administering a therapeutically effective amount of a compound as claimed in any one of claims 1 to 22 to a human or animal in need thereof.

69. A method as claimed in claim 68, wherein the human or animal is further subjected to irradiation or sound.

70. A method as claimed in any one of claims 65 to 67 or claim 69, wherein the irradiation is electromagnetic radiation with a wavelength in the range of from 500nm to 1000nm.

71. A method as claimed in claim 70, wherein the irradiation is electromagnetic radiation with a wavelength in the range of from 600nm to 900nm.

72. A method as claimed in claim 71, wherein the irradiation is electromagnetic radiation with a wavelength in the range of from 620nm to 820nm.

73. A method as claimed in claim 72, wherein the irradiation is electromagnetic radiation with a wavelength in the range of from 630nm to 710nm.