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(54) Title: METALLATION ENHANCEMENTS IN TUMOR-IMAGING AND PDT THERAPY

(57) Abstract: A compound in the form of a metallized tetrapyrrolic photosensitizer linked to a fluorescent dye where the photosensitizer (PS), is linked by a structure that does not have detrimental radiation emission or absorbing characteristics, to a fluorophore, usually a cyanine dye (CD). The photosensitizer in accordance the invention is a metallized analog of porphyrins, chlorins, purpurinimides, bacterio purpurinimides, phthalocyanines, expanded porphyrins, benzoporphyrin derivatives and purpurins. The fluorophore is usually a cyanine dye with variable substituents. And, A method for determining effectiveness of PDT by comparing proportion of STAT-3 monomer with crosslinked STAT-3 dimer after PDT where the relative proportion of STAT-3 monomer to crosslinked STAT-3 directly correlates to efficacy of the PDT.



METALLATION ENHANCEMENT IN TUMOR-IMAGING AND PDT THERAPY**CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit under 35 U.S.C. §119(e) of U.S. Provisional Application No. 61/361,718, filed July 6, 2010.

Background of the Invention

A major challenge of cancer therapy is preferential destruction of malignant cells with sparing of the normal tissue. Critical for successful eradication of malignant disease are early detection and selective ablation of the malignancy. This proposal addresses both issues.

Multiple, complementary techniques for tumor detection, including magnetic resonance, scintigraphic and optical imaging are under active development, each approach has particular strengths and advantages. Optical imaging includes measurement of absorption of endogenous molecules (e.g hemoglobin) or administered dyes, detection of bioluminescence in preclinical models, and detection of fluorescence from endogenous fluorophores or from targeted exogenous molecules . Fluorescence, which involves absorption of light and re-emission at a longer wavelength, can be highly sensitive: a typical cyanine dye with a lifetime of 0.6 nsec can emit up to 10^{32} photons/second/mole. A sensitive optical detector can image $<10^3$ photons/second. Thus even with low excitation power, low concentrations of fluorescent molecular beacons can be detected.

As with other non-invasive techniques, fluorescence imaging has the potential for performing *in vivo* diagnosis *in situ*, with real time display of the resulting information [46]. Optical tomographic techniques are being devised to visualize the fluorescent probes within tissue volumes. Optical imaging instruments may be simpler and less expensive to operate than those required for other imaging technologies, permitting their eventual application by less specialized medical centers. Therapeutically in applications such as endoscopic examination, fluorescence imaging can allow precise assessment of the location and size of a tumor, and provide information on its invasiveness. During debulking surgery, where malignant loci can be difficult to identify, the presence of a fluorescent signal might assist the surgeon in identifying the diseased site.

The optimal wavelength range for *in vivo* fluorescence excitation and emission is determined by tissue optical properties. Hemoglobin has strong absorption at wavelengths less than about 600 nm and there can be significant background fluorescence from endogenous biomolecules up to about 680 nm. At longer wavelengths into the near infrared (NIR), tissue absorption and scattering decrease with wavelength. As shown in Figure 1 there is a large increase in light penetration as wavelengths increase from ~600 to 800 nm. In addition, the difference between the fluorophore's absorption and emission bands (i.e. its Stokes shift), should be at least 20 nm, to readily discriminate between the excitation and emission light. Many NIR fluorescent dyes are based on carbocyanine molecules such as indocyanine green (ICG), an FDA-approved agent with a 730 nm excitation and 830 nm emission maxima. Various novel ICG analogs have been evaluated because of the high biocompatibility and desirable spectral properties of the carbocyanines.

A challenge is to deliver the dyes selectively and in high enough concentration to detect small tumors. Use of ICG alone to image hypervascular or "leaky" angiogenic vessels around tumors has been disappointing, due to the dye's limited intrinsic tumor selectivity. Multiple approaches have been employed to improve optical probe localization, including administering it in a quenched form that is activated within tumors, or coupling the fluorescent agents to antibodies or small molecules such as receptor ligands. Recent studies have focused on developing dye conjugates of small bioactive molecules, to improve rapid diffusion to target tissue, incorporate combinatorial and high throughput strategies to identify and optimize new probes, and enhance *in vivo* stability of the compounds. Some of the peptide and folic-acid analogs of certain ICG derivatives have shown some tumor specificity and are at initial stages of pre-clinical studies.

Recently, a new class of multicarboxylate-containing carbocyanine probes have been reported for use as optical scaffolds that not only serve as fluorescent antennae but also participate in structural assembly of the multivalent molecular construct. The peripheral carboxylic acids that are distal to the chromophore core allow facile conjugation with biomolecules and retain the desirable NIR spectral properties of the dendritic molecule. However, none of these compounds are designed for both tumor detection and therapy. It is important to develop targeting strategies that cope with the heterogeneity of tumors *in vivo*, where there are inconsistent and varying expression of targeable sites. As discussed below,

Photodynamic therapy (PDT) is a clinically effective and still evolving locally selective therapy for cancers. PDT's utility has been demonstrated for varying photosensitizers and multiple types of disease. It is FDA approved for early and late stage lung cancer, obstructive esophageal cancer, high-grade dysplasia associated with Barrett's esophagus, age-related

macular degeneration and actinic keratoses. PDT employs tumor localizing photosensitizers that produce reactive singlet oxygen upon absorption of light. Subsequent oxidation-reduction reactions also can produce superoxide anions, hydrogen peroxide and hydroxyl radicals. Photosensitizers have been designed which localize relatively specifically in certain subcellular structures such as the mitochondria, which are exquisitely sensitive targets. On the tumor tissue level, direct photodynamic tumor cell kill, destruction of the tumor supporting vasculature and possibly activation of the innate and adaptive anti-tumor immune system interact to destroy the malignant tissue. The preferential killing of the targeted cells (e.g. tumor), rather than adjacent normal tissues, is essential for PDT, and the preferential target damage achieved in clinical applications is a major driving force behind the use of the modality. The success of PDT relies on development of tumor-avid molecules that are preferentially retained in malignant cells but cleared from normal tissues.

Clinical PDT initially was developed at Roswell Park Cancer Institute which has one of the world's largest basic and clinical research programs. Initially the RPCI group developed Photofrin®, the first generation FDA approved hematoporphyrin-based compound. Subsequently, our group has investigated the structure activity relationships for tumor selectivity and photosensitizing efficacy, and used the information to design new photosensitizers with high selectivity and desirable pharmacokinetics. Although the mechanism of porphyrin retention by tumors is not well understood, the balance between lipophilicity and hydrophilicity is recognized as an important factor. In efforts to develop effective photosensitizers with the required photophysical characteristics, chlorophyll-a and bacteriochlorophyll-a were as the substrates. An extensive QSAR study on a series of the alkyl ether derivatives of pyropheophorbide-a (660 nm) led to selection of the best candidate, HPPH (hexyl ether derivative), [98, 99] currently in

promising Phase II clinical trials. Our PS development currently is being extended in purpurinimide (700 nm) and bacteriopurpurinimide (780-800 nm) series with high singlet oxygen ($^1\text{O}_2$) producing capability. The long wavelength absorption is important for treating large deep-seated tumors, because it both increases light penetration and minimizes the number of optical fibers needed for light delivery within the tumor.

Some of these compounds are highly tumor avid. As shown in Fig 18 in the Preliminary Data, with an optimized system, 48 and 72 h after administration, ratios of $\sim 6:1$ and $10:1$ between the tumor and surrounding muscle and other body sites have been achieved, except for the liver, spleen and kidney. This *in vivo* selectivity is 2-3 fold greater than that reported for a carbocyanine dye coupled to a somatostatin analog.

Photosensitizers (PS), especially tetrapyrrolic photosensitizers such as porphyrins, are not optimal for tumor detection. Examples of such tetrapyrrolic photosensitizers are intended to include include modified chlorines, bacteriochlorins, hematoporphyrins, porphyrins, purpurins, purpurin imides, and pyropheophorbides. All of the foregoing are referred to herein as porphyrins. Examples of such photodynamic compounds are described in numerous patents in this area that have been applied for and granted world wide on these photodynamic compounds. Reference may be had, for example to the following U.S. Patents which are incorporated herein by reference: 4,649,151; 4,866,168; 4,889,129; 4,932,934; 4,968,715; 5,002,962; 5,015,463; 5,028,621; 5,145,863; 5,198,460; 5,225,433; 5,314,905; 5,459,159; 5,498,710; and 5,591,847.

Such photosensitizers generally fluoresce and the fluorescence properties of these porphyrins *in vivo* has been exploited by several investigators for the detection of early-stage cancers in the lung, bladder and various other sites. In addition, for treatment of early disease or for deep seated tumors the fluorescence can be used to guide the activating light. However, such

photosensitizers are not optimal fluorophores for tumor detection for several reasons: (i) They have low quantum yields. Because the excited state energy is transferred to the triplet state and then to molecular oxygen, efficient photosensitizers tend to have lower fluorescence efficiency (quantum yield) than compounds designed to be fluorophores, such as cyanine dyes, (ii) They have small Stokes shifts. Porphyrin-based photosensitizers have a relatively small difference between the long wavelength absorption band and the fluorescence wavelength (Stokes shift), which makes it technically difficult to separate the fluorescence from the excitation wavelength, (iii) They have relatively short fluorescent wavelengths, < 800 nm, which are not optimal for deep tissue penetration.

Bifunctional photosensitizer-fluorophore conjugates can optimize tumor detection and treatment. Certain bifunctional conjugates have been recently developed that use tumor-avid photosensitizers to target the NIR fluorophores to the tumor. The function of the fluorophore is to visualize the tumor location and treatment site. The presence of the photosensitizer allows subsequent tumor ablation. A compound that effectively functions both as a fluorescence imaging agent and a photosensitizer would create an entirely new paradigm for tumor detection and therapy. The optical imaging allows the clinician performing photodynamic therapy to continuously acquire and display patient data in real-time. This "see and treat" approach may determine where to treat superficial carcinomas and how to reach deep-seated tumors in sites such as the breast with optical fibers delivering the photoactivating light.

Metallized photodynamic compounds have shown promise in *in vivo* PDT efficacy and fluorescence imaging potential. However, the therapeutic dose was significantly higher than the therapeutic dose and a considerable fluorescence resonance energy transfer (FRET) was observed between the two chromophores.

Brief Description of the Several Views of the Drawings

Figure 1 is a graph showing results of treatment of tumors in mice with HPPH-cyanine dye(CD) conjugate in different concentrations.

Figure 2 is a graph showing results of treatment of tumors in mice with metal chelates of HPPH-cyanine dye (CD) conjugates.

Figure 3 shows electrophoresis gels of relative proportions of STAT-3 dimers and STAT-3 monomers after treatment with compounds of the invention.

Figure 4 shows fluorescence images of tumors with HPPH-CD alone compared with complexes of In, Ga, and Pd.

Brief Summary of the Invention

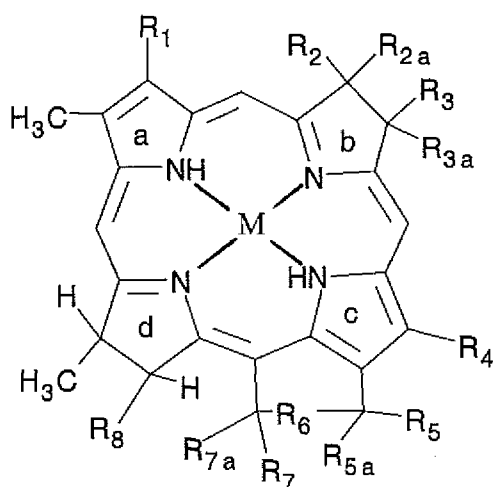
We used two approaches to solve the above problems.

(i) To introduce those metals in the photosensitizers, which are known for enhancing the singlet oxygen yields, and

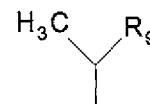
(ii) to link the chromophores with variable linkers (flexible or rigid) and investigate the effect of the length of linkers in FRET its correlation with PDT.

In particular, we prepared a novel compound in the form of a metallized tetrapyrrolic photosensitizer linked to a fluorescent dye.

Compounds of the invention have the general formula:



where



R_1 is, substituted or unsubstituted, $-\text{CH}=\text{CH}_2$, $-\text{CHO}$, COOH , or

where $R_9 = -\text{OR}_{10}$ where R_{10} is lower alkyl of 1 through 8 carbon atoms, or $-(\text{CH}_2-\text{O})_n\text{CH}_3$; R_2 , R_{2a} , R_3 , R_{3a} , R_4 , R_5 , R_{5a} , R_7 , and R_{7a} are independently hydrogen, lower alkyl, substituted lower alkyl, lower alkylene or substituted lower alkylene or two R_2 , R_{2a} , R_3 , and R_{3a} groups on adjacent carbon atoms may be taken together to form a covalent bond or two R_2 , R_{2a} , R_3 , R_{3a} , R_5 , R_{5a} , R_7 , and R_{7a} groups on the same carbon atom may form a double bond to a divalent pendant group; R_2 and R_3 may together form a 5 or 6 membered heterocyclic ring containing oxygen, nitrogen or sulfur; R_6 is $-\text{CH}_2-$, $-\text{NR}_{11}-$, where R_{11} is, substituted or unsubstituted, lower alkyl, or lower alkylene; or a R_6 is a covalent bond; R_8 is $-(\text{CH}_2)_2\text{CO}_2R_{12}$ where R_{12} is a non-toxic fluorescent dye group that causes the conjugate to preferentially emit (fluoresce) at a wave length of 800 to about 900 nm and M is In, Ga or Pd,

Detailed Description of the Invention

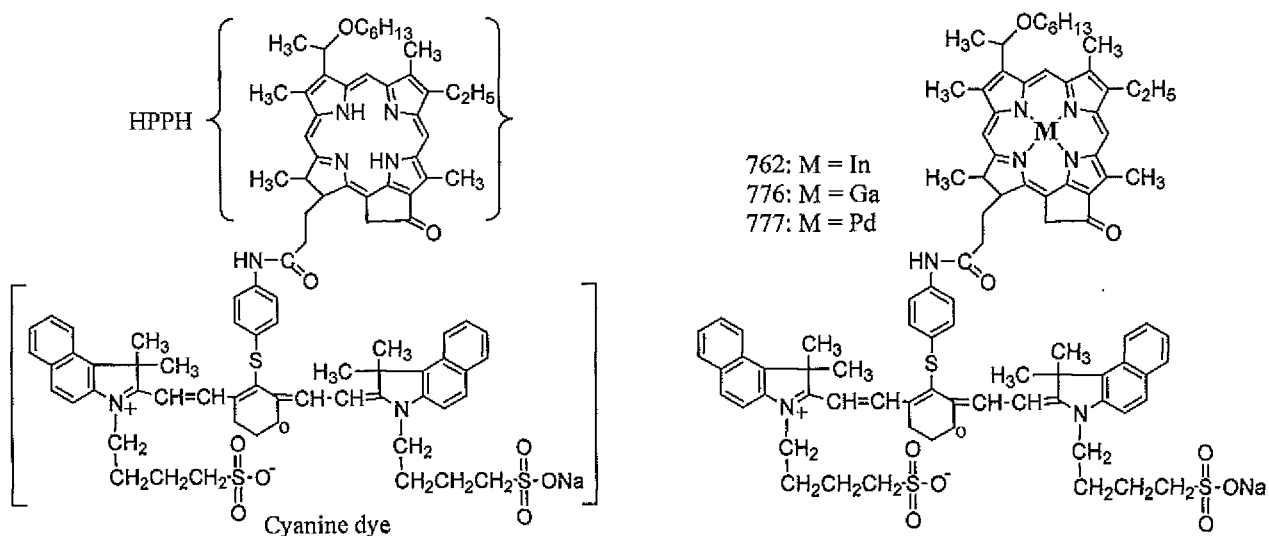
The compound in accordance with the invention is a photosensitizer (PS), linked by essentially any structure that does not have detrimental radiation emission or absorbing characteristics, to a fluorophore, usually a cyanine dye (CD). The photosensitizer in accordance with the invention is a metallized analog of porphyrins, chlorins, purpurinimides, bacterio purpurinimides, phthalocyanines, expanded porphyrins, benzoporphyrin derivatives and purpurins.

The fluorophore is usually a cyanine dye with variable substituents.

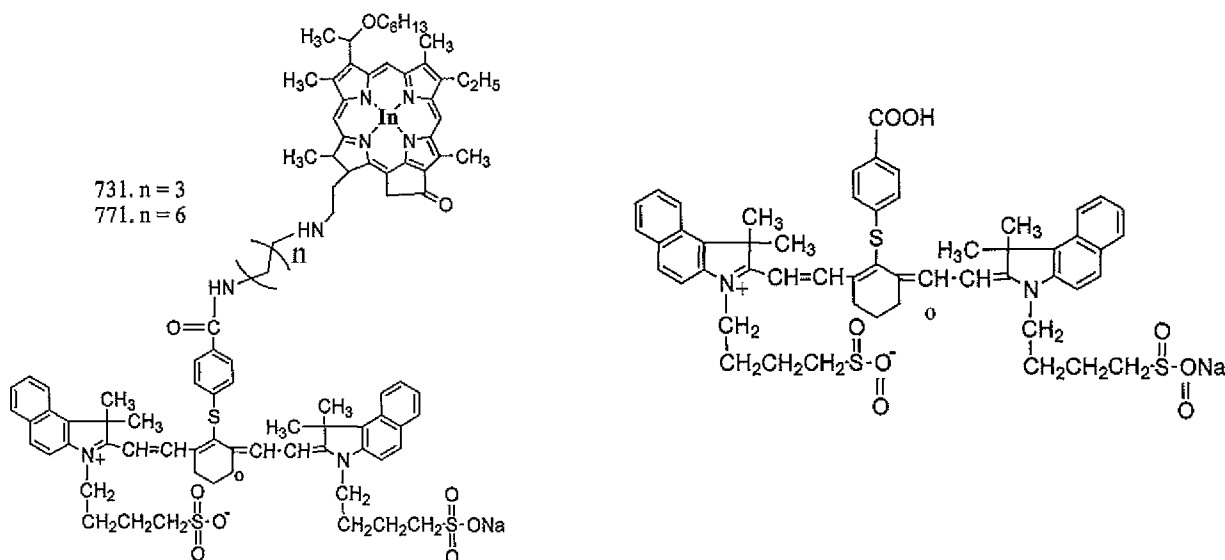
Structures, photophysical, tumor-imaging characteristics and in vivo efficacy of the metallated analogs of the HPPH-Cyanine dye conjugates:

Indium, Gallium and Palladium analogs of HPPH-Cyanine Dye Conjugate:

HPPH-CD conjugate



HPPH-CD linked with variable length of carbon linkages:



Insertion of metal (In, Ga and Pd) in HPPH-CD conjugate enhances PDT effects. Among the metallated analogs, the corresponding In(III) analog produced the best efficacy and was almost 8-fold more effective than the non metallated derivative. See Figures 1 and 2.

STAT-3 dimerization can be used as biomarker to monitor the PDT response. As seen in Figure 3, photoreactions mediated by HPPH-CD and metallated derivatives. BCC1 cells were used for determining the level of photoreaction resulting in the oxidative crosslinking of STAT3. The uptake of the compounds was carried out under two conditions. The HPPH derivatives were diluted to 400 nM in culture medium containing 10% fetal calf serum and added to subconfluent monolayer cultures of human basal cell carcinoma (BCC-1) cells. The cells were incubated for 2.5 hours at 37°C. Alternatively, the HPPH-derivatives were diluted to 20 nM in serum-free medium and added to BCC-1 cells that had been preincubated for 1.5 hours in serum-free medium. The cells were incubated for 1 hour at 37°C. Following uptake, the cells were washed 3

times with serum-free medium and exposed at 37°C for 9 min to light at the indicated wavelength. In each case, the total fluence was 3 J/cm². Cells were immediately extracted with RIPA buffer, Aliquots of the lysates containing 20 µg protein were analyzed by western blotting for the level of STAT3 proteins (one representative exposure of the immunoblots is reproduced). The enhanced chemiluminescence signals for STAT3 monomeric and crosslinked STAT3 were quantified and the relative amount of crosslinked dimeric STAT3 was expressed as percentage of the total STAT3 signal (indicated above each lane in the figure).

As seen in Figure 4, in contrast to HPPH-CD where the therapeutic dose was almost 10-fold higher than the tumor-imaging dose. The metallated analogs showed a great potential for PDT and tumor-imaging at the same dose, which was almost 8-fold lower than the therapeutic dose of HPPH-cyanine dye.

The synthesis of compound code no. 762:

HPPH-Cyanine dye conjugate (100 nig), indium chloride (300 mg) and sodium bicarbonate (600mg) were put in the solvent mixture of toluene (60 ml) and ethanol (20 ml). The reaction mixture was refluxed for 1 hour. After evaporation, the residue was purified by chromatography using MeOH/CH₂Cl₂ (1:4) as the elute solvent and the title compound was obtained in ~ 80% yield. UV-vis in MeOH: 835 nm (ϵ = 159430), 646 nm (ϵ = 56500), 602 nm (ϵ = 12425), 563 nm (ϵ = 8762), 416 nm (ϵ = 75074)..NMR (CHCl₃), δ (ppm) for compound 762: 9.87 (ss looks like a doublet, 1H, *meso*-H in HPPH part), 9.70 (s, 1H, *meso*-A in HPPH part), 8.39 (s, 1H, *meso*-H in HPPH part), 7.98 (m, 4H, aromatic-H of cyanine dye), 7.84 (br s, 4H, aromatic-H of cyanine dye), 7.50 (br s, 4H, aromatic-H of cyanine dye), 7.37 (br s, 4H, =CH- of cyanine dye), 7.07 (m, 4H, 4H of the linker phenyl group), 5.78 (m, 1H, H-3¹), 5.17 (m, 1H, H-17), 5.03 (m, 1H, H-18), 4.60 (m, 2H, H-13²), 4.40 (m, 2H, N⁺-CH₂), 4.01 (br, 10H, 2H for N-CH₂, 2H for H-17¹, 4H for -CH₂SO₃, 2H for -OC*H₂(CH₂)₄CH₃), 3.78 (s, 3H, 7-CH₃), 3.59 (s, 3H, 12-CH₃), 3.68-3.45 (m, 6H, 4H for SO₃-CH₂C*H₂-(CH₂)₂, 2H for 8-C*H₂CH₃), 3.34 (m, 2H, H-17²), 3.31 (s, 3H, 2-CH₃), 3.12 (m, 4H, SO₃-(CH₂)₂C*H₂-CH₂), 2.04 (m, 11H, 3H for 3-CH₃, 2H for -OCH₂C*H₂(CH₂)₃CH₃), 1.73 (s, 12H, 4X-CH₃ of cyanine dye), 1.33 (m, 3H, 18-CH₃), 1.26 (m, 3H, 8-CH₂C*H₃), 1.15 (m, 6H, -O(CH₂)₂(C*H₂)₃CH₃), 0.71 (m, 3H, -O(CH₂)₅C*H₃). MS for 762: Calculated for C₉₁H₁₀₂N₇O₉S₃InCl: 1682.6, Found: 1682.5.

The synthesis of compound Ga(Cl)-776:

HPPH-CD (100 mg), gallium chloride (400 mg) and sodium bicarbonate (400mg) were put in the solvent mixture of toluene (65 ml) and ethanol (25 ml). The reaction mixture was refluxed for 30 minutes. After evaporation, the residue was purified by chromatography using

MeOH/CH₂Cl₂ (1:4) as the elute solvent and the title compound was obtained in ~ 45% yield. UV-vis in MeOH: 839 nm (ϵ = 159183), 649 nm (ϵ = 55102), 606 nm (ϵ = 12505), 563 nm (ϵ = 8767), 419 nm (ϵ = 144002). NMR (CHCl₃), δ (ppm) for compound 776: 9.70 (s, 1H, *meso*-H in HPPH part), 9.22 (s, 1H, *meso*-H in HPPH part), 8.64 (s, 1H, *meso*-H in HPPH part), 8.12 (m, 4H, aromatic-H of cyanine dye), 8.02 (br s, 4H, aromatic-H of cyanine dye), 7.73 (br s, 4H, aromatic-H of cyanine dye), 7.46 (br s, 4H, =CH- of cyanine dye), 7.18 (m, 4H, 4H of the linker phenyl group), 5.79 (m, 1H, H-3¹), 5.19 (m, 1H, H-17), 5.01 (m, 1H, H-18), 4.63 (m, 2H, H-13²), 4.45 (m, 2H, N⁺-CH₂), 4.07 (br, 10H, 2H for N-CH₂, 2H for H-17¹, 4H for -CH₂SO₃, 2H for -OC*H₂(CH₂)₄CH₃), 3.79 (s, 3H, 7-CH₃), 3.55 (s, 3H, 12-CH₃), 3.69-3.44 (m, 6H, 4H for SO₃-CH₂C*H₂-(CH₂)₂, 2H for 8-C*H₂CH₃), 3.32 (m, 2H, H-17²), 3.36 (s, 3H, 2-CH₃), 3.12 (m, 4H, SO₃-(CH₂)₂C*H₂-CH₂), 2.02 (m, 11H, 3H for 3-CH₃, 2H for ~OCH₂C*H₂(CH₂)₃CH₃), 1.75 (s, 12H, 4X-CH₃ of cyanine dye), 1.31 (m, 3H, 18-CH₃), 1.24 (m, 3H, **8-CH₂C*H₃**), 1.17 (m, 6H, -O(CH₂)₂(C*H₂)₃CH₃), 0.73 (m, 3H, -O(CH₂)₅C*H₃). MS for 776: Calculated for C₉₁H₁₀₂N₇O₉S₃GaCl: 1636.6, Found: 1636.1.

The synthesis of compound 777:

HPPH-Cyanine dye (100 mg), L-ascorbic acid 6-palmitate (220 mg), and palladium acetate (160 mg) were put into the solvent mixture of methanol (80 ml) and chloroform (80 ml). Under argon the reaction mixture was stirred overnight at room temperature. After work-up and evaporation, the residue was purified by chromatography using MeOH/CH₂Cl₂ (1:5) as the elute solvent and the title compound was obtained in ~ 85% yield. UV-vis in MeOH: 839 nm (ϵ = 132340), 635 nm (ϵ = 65069), 589 nm (ϵ = 11949), 536 nm (ϵ = 9091), 415 nm (ϵ = 59484), 389.9 nm (ϵ = 62212). NMR (CHCl₃), δ (ppm) for compound 777: 9.75 (s, 1H, *meso*-H in HPPH

part), 9.61 (s, 1H, *meso-U* in HPPH part), 8.45 (s, 1H, *meso-H* in HPPH part), 7.72 (m, 4H, aromatic-H of cyanine dye), 7.53 (br s, 4H, aromatic-H of cyanine dye), 7.12 (br s, 4H, aromatic-H of cyanine dye), 6.93 (br s, 4H, =CH- of cyanine dye), 6.80 (m, 4H, 4H of the linker phenyl group), 5.64 (m, 1H, H-3¹), 5.32 (m, 1H, H-17), 5.02 (m, 1H, H-18), 4.64 (m, 2H, H-13²), 4.47 (m, 2H, N⁺-CH₂), 4.09 (br, 10H, 2H for N-CH₃, 2H for H-17¹, 4H for -CH₂SO₃, 2H for ~OC*H₂(CH₂)₄CH₃), 3.76 (s, 3H, 7-CH₃), 3.57 (s, 3H, 12-CH₃), 3.67-3.46 (m, 6H, 4H for SO₃-CH₂C*H₂-(CH₂)₂, 2H for 8-C*H₂CH₃), 3.35 (m, 2H, H-17²), 3.31 (s, 3H, 2-CH₃), 3.16 (m, 4H, SO₃-(CH₂)₂C*H₂-CH₂), 2.05 (m, 11H, 3H for 3-CH₃, 2H for -OCH₂C*H₂(CH₂)₃CH₃), 1.73 (s, 12H, 4X-CH₃ of cyanine dye), 1.34 (m, 3H, 18-CH₃), 1.21 (m, 3H, 8-CH₂C*H₃), 1.15 (m, 6H, -O(CH₂)₂(C*H₂)₃CH₃), 0.71 (m, 3H, -O(CH₂)₂C*H₃). MS for 777: Calculated for C₉₁H₁₀₂N₇O₉S₃Pd: 1638.6, Found: 1638.5.

Amino diethyl analog of HPPH:

HPPH (100.0 mg, 0.157 mmol) was taken in a dry RBF (50.0 ml) and dissolved in dry dichloromethane (30.0 ml). To this, N-BOC-ethylenediamine (50.3 mg, 0.314 mmol), *N*-Ethyl-*i*V-(3-dimethylaminopropyl) carbodiimide hydrochloride (60.2 mg, 0.314 mmol) and 4-dimethylamino pyridine (38.36 mg, 0.314 mmol) were added and the resultant mixture was stirred for 12 hr at room temperature under N₂ atmosphere. Reaction mixture was then diluted with dichloromethane (50.0 ml) and washed with brine (50 ml). Organic layer separated, dried over sodium sulfate and concentrated. Product was purified over silica gel column using 1-3 % methanol-dichloromethane as mobile phase. Yield: 105.0 mg (85.9 %). UV-vis λ_{max} (in CH₂Cl₂): 661 nm (ϵ 5.0 x 10⁴), 604 nm (ϵ 0.8 x 10⁴), 537 nm (ϵ 0.9 x 10⁴), 505 nm (ϵ 0.9 x 10⁴), and 410 nm (ϵ 10.5 x 10⁴). ¹HNMR (400MHz, CDCl₃): δ 9.76 (singlet, 1H, *meso-H*), 9.21 (singlet, 1H,

meso-H), 8.52 (singlet, 1H, meso-H), 6.12 (brs, 1H, NH), 5.92 (m, 1H, CH₃CHOhexyl), 5.29 (d, 1H, 15¹-C*HH, J = 19.6 Hz), 5.09 (d, 1H, 15¹-CH*H, J = 20.0 Hz), 4.85 (brs, 1H, NH), 4.52 (q, 1H, 17-H, J = 7.6 Hz), 4.30 (d, 1H, H-18, J = 5.2 Hz), 3.62-3.61 (m, 4H, 8-C*H₂CH₃ & -OC*H₂-Hexyl), 3.38 (singlet, 3H, ring-CH₃), 3.28 (singlet, 3H, ring-CHa), 3.28 (singlet, 3H, ring-CH₃), 3.18 (m, 2H, -(NHCH₂)₂-), 3.08 (m, 2H, -(NHCH₂)₂-), 2.65 (m, 1H, 17²-C*HH), 2.45 (m, 1H, 17²-CH*H), 2.30 (m, 1H, 17¹-CHH), 2.13 (d, 3H, C*H₃CH-Ohexyl, J = 7.2 Hz), 2.05 (m, 1H, 17¹-C*HH), 1.80 (d, 3H, 18-CHs, J = 7.2 Hz), 1.75 (m, 2H, -CH₂-Hexyl), 1.63 (t, 3H, 8-CH₂C*H₃, J = 7.2 Hz), 1.43 (m, 2H, -CH₂-Hexyl), 1.24 (m, 4H, -2CH₂-Hexyl), 1.21 (s, 9H, NH-Boc), 0.80 (t, 3H, CH₃-Hexyl, J = 6.8 Hz), 0.45 (brs, 1H, NH), -1.65 (brs, 1H, NH). MS calculated for C₄₆H₇₂N₆O₅ 779.02. EIMS: 779.3 (M⁺).

Aminohexane analog of HPPH:

HPPH (100.0 mg, 0.157 mmol) was taken in a dry RBF (50.0 ml) and dissolved in dry dichloromethane (30.0 ml). To this, N-BOC-1,6 diaminothexane (60.0 mg, 0.31 mmol), *N*-Ethyl-NV-(3-dimethylaminopropyl) carbodiimide hydrochloride (70.0 mg, 0.31 mmol) and 4-dimethylamino pyridine (38.36 mg, 0.314 mmol) were added and the resultant mixture was stirred for 12 hr at room temperature under N₂ atmosphere. Reaction mixture was then diluted with dichloromethane (50.0 ml) and washed with brine (50 ml). Organic layer separated, dried over sodium sulfate and concentrated. Product was purified over silica gel column using 1-3 % methanol-dichloromethane as mobile phase. Yield: 105.0 mg (85.9 %). UV-vis λ_{max} (in CH₂Cl₂): 661 nm (ϵ 5.0 x 10⁴), 604 nm (ϵ 0.8 x 10⁴), 537 nm (ϵ 0.9 x 10⁴), 505 nm (ϵ 0.9 x 10⁴), and 410 nm (ϵ 10.5 x 10⁴). ¹HNMR (400MHz, CDCl₃): δ 9.76 (singlet, 1H, meso-H), 9.15 (singlet, 1H, meso-H), 8.50 (singlet, 1H, meso-H), 5.92 (m, 1H, CH₃C*HOhexyl), 5.20 (d, 1H, 15¹-C*HH, J =

19.6 Hz), 5.09 (d, 1H, $15^1\text{-CH}^*\text{H}$, $J = 20.0$ Hz), 4.52 (q, 1H, 17-H, $J = 7.6$ Hz), 4.30 (d, 1H, H-18, $J = 5.2$ Hz), 3.62-3.61 (m, 4H, $8\text{-C}^*\text{H}_2\text{CH}_3$ & $\text{-OC}^*\text{H}_2\text{-Hexyl}$), 3.38 (singlet, 3H, ring- CH_3), 3.28 (singlet, 3H, ring- CH_3), 3.28 (singlet, 3H, ring- CH_3), 3.18 (m, 2H, $\text{-(NHCH}_2\text{)}_2\text{-}$), 3.08 (m, 2H, $\text{-(NHCH}_2\text{)}_2\text{-}$), 2.65 (m, 1H, 17^2-CHH), 2.45 (m, 1H, $17^2\text{-CH}^*\text{H}$), 2.30 (m, 1H, $17^1\text{-C}^*\text{HH}$), 2.13 (d, 3H, $\text{C}^*\text{H}_3\text{CH-Ohexyl}$, $J = 7.2$ Hz), 1.95 (m, 1H, $17^1\text{-C}^*\text{HH}$), 1.80 (d, 3H, 18-CH_3 , $J = 7.2$ Hz), 1.75 (m, 2H, $\text{-CH}_2\text{-Hexyl}$), 1.63 (t, 3H, $8\text{-CH}_2\text{CH}_3$, $J = 7.2$ Hz), 1.43 (m, 2H, $\text{-CH}_2\text{-Hexyl}$), 1.40-1.31 (m, 8H, $\text{-(NH CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH)}$), 1.24 (m, 4H, $\text{-2CH}_2\text{-Hexyl}$), 1.21 (s, 9H, NH-Boc), 0.80 (t, 3H, $\text{CH}_3\text{-Hexyl}$, $J = 6.8$ Hz), 0.45 (brs, 1H, NH), -1.65 (brs, 1H, NH). MS calculated for $\text{C}_{50}\text{H}_{70}\text{N}_6\text{O}_5$ 835.13 EIMS: 835.8 (M^+).

HPPH-Cyanine dye joined with a short linker (without any metal):

HPPH-N-Boc ethylenediamine (35.0 mg, 0.052 mmol) was taken in a dry RBF (50.0 ml) and stirred with 50% TFA/DCM (5.0 ml) at RT for 3 hr. Resultant mixture was concentrated and dried under high vacuum to remove trace of TFA. The crude thus obtained was dissolved in anhy. DMF (25 ml) and cyanine dye (50.0 mg, 0.052 mmol), BOP (30.0 mg, 0.067 mmol) and triethyl amine (3-4 drops) were added and the resultant mixture was stirred for 12 hr at room temperature under N_2 atmosphere. Solvent was removed under vacuum and the product was purified over preparative plates using 15 % methanol-dichloromethane to yield the compound in 35% yield. UV-vis λ_{max} (in MeOH): 838.9 nm ($\epsilon 2.04 \times 10^5$), 659.9 nm ($\epsilon 5.13 \times 10^4$), 607.0 nm ($\epsilon 1.16 \times 10^4$), 538 nm ($\epsilon 1.0 \times 10^4$), 408 nm ($\epsilon 8.1 \times 10^4$) $^1\text{HNMR}$ (400Mhz, CD_3OD): 9.73 (s, 1H, H-5 of HPPH part), 9.35 (s, 1H, H-10 of HPPH part), 8.50-8.45 (m, 2H, aromatic protons of cyanine dye part), 8.40 (s, 1H, H-20 of HPPH part), 7.73-7.67 (m, 4H, aromatic protons of cyanine dye part), 7.65-7.62 (m, 2H), 7.44-7.42 (m, 2H), 7.37-7.21 (m, 6H), 7.14-7.11 (m, 2H),

6.76-6.74 (d, 2H), 5.91-5.90 (m, 1H, 3'-H of HPPH part), 5.07-5.07 (m, 18-H of HPPH part), 5.02-4.99 (m, 17-H of HPPH part), 4.26 (d, 1H, 13 C*HH of HPPH part), 3.92 (d, 2H, 13 CH*H of HPPH part), 3.62-3.54 (m, 6H, 2H for $-\text{NC}^*\text{H}_2(\text{CH}_2)_3\text{SO}_3^-$ and 2H for $-\text{NC}^*\text{H}_2(\text{CH}_2)_3\text{SO}_3^-$ and 2H for 8-C* CH_3 , 3.45-3.44 (overlapped, 6H, 2H for $3^1\text{-OC}^*\text{H}_2(\text{CH}_2)_4\text{CH}_3$ of HPPH part, 4H for $2\text{X-N}(\text{CH}_2)_3\text{C}^*\text{H}_2\text{SO}_3^-$), 3.33 (s, 3H, 7- CH_3 of HPPH), 3.31 (s, 3H, 2- CH_3), 3.19 (s, 3H, 12- CH_3), 2.83-2.81 (m, 2H for 17- $\text{CH}_2\text{C}^*\text{H}_2\text{CO}-$), 2.68-2.63 (m, 12H, 8H for $2\text{X-NCH}_2(\text{C}^*\text{H}_2)_2\text{CH}_2\text{SO}_3^-$ of cyanine dye part, 4H for $-\text{CONHC}^*\text{H}_2\text{C}^*\text{H}_2\text{NHCO}-$), 2.43-2.63 (m, 2H for 17-C* $\text{CH}_2\text{CO}-$), 2.10-2.10 (overlapped with other peaks, 3H, 3^2-CH_3 of HPPH part), 1.65-1.57 (m, 6H, cyclohexene CH - of cyanine dye part), 1.53-1.50 (m, 3H for 18- CH_3), 1.29-1.12 (overlapped, 17H, 12H for 4X- CH_3 of cyanine dye part, 2H for $3^1\text{-OCH}_2\text{C}^*\text{H}_2(\text{CH}_2)_3\text{CH}_3$ of HPPH part, 3H for 8- CH_2C^* of HPPH part), 1.17-1.14 (m, 6H for $3^1\text{-O}(\text{CH}_2)_2(\text{C}^*\text{H}_2)_3\text{CH}_3$), 0.66 (m, 3H for $3^1\text{-OCH}_2(\text{CH}_2)_4\text{C}^*\text{H}_3$). MS calculated for $\text{C}_{94}\text{H}_{108}\text{N}_8\text{O}_{10}\text{S}_3$ 1628 EIMS (m/z): 1650 ($\text{M}^+ + \text{Na}$).

Synthesis of HPPH-CD linked with a long, without any metal):

HPPH-N-Boc hexyldiamine (38.0 mg, 0.052 mmol) was taken in a dry RBF (50.0 ml) and stirred with 50% TFA/DCM (5.0 ml) at RT for 3 hr. Resultant mixture was concentrated and dried under high vacuum to remove trace of TFA. The crude thus obtained was dissolved in anhy. DMF (25 ml) and cyanine dye (50.0 mg, 0.052 mmol), BOP (30.0 mg, 0.067 mmol) and triethyl amine (3-4 drops) were added and the resultant mixture was stirred for 12 hr at room temperature under N_2 atmosphere. Solvent was removed under vacuum and the product was purified over preparative plates using 15 % methanol-dichloromethane to yield the compound in 35% yield. UV-vis λ_{max} (in MeOH): 838.1 nm ($\epsilon 2.23 \times 10^5$), 661.0 nm ($\epsilon 5.34 \times 10^4$), 609.0 nm

(ϵ 1.02×10^4), 538 nm (ϵ 8.79×10^4), 408 nm (ϵ 9.07×10^5) ^1H NMR (400MHz, CD_3OD): 9.65 (s, 1H, H-5 of HPPH part), 8.93 (s, 1H, H-10 of HPPH part), 8.75-8.72 (m, 3H, 1H, H-20 of HPPH part, 2H, aromatic protons of cyanine dye part), 7.76 (m, 2H, aromatic protons of cyanine dye part), 7.70-7.67 (m, 4H, aromatic protons of cyanine dye part), 7.46-7.43 (m, 2H), 7.30-7.29 (m, 2H), 7.20-7.19 (m, 4H), 6.87-6.83 (m, 2H), 5.98 (d, 2H), 5.84-5.86 (m, 1H, 3*-H of HPPH part), 5.01-4.99 (m, 18-H of HPPH part), 4.80-4.77 (m, 17-H of HPPH part), 4.38 (d, 1H, 13 C^*HH of HPPH part), 4.05 (d, 1H, 13 CH^*H of HPPH part), 3.70-3.60 (m, 2H, $-\text{NC}^*\text{H}_2(\text{CH}_2)_3\text{SO}_3^-$), 3.50-3.45 (m, 2H, $-\text{NC}^*\text{H}_2(\text{CH}_2)_3\text{SO}_3^-$), 3.38-3.35 (overlapped, 5H, 3H of 7- CH_3 of HPPH and 2H of 8- $\text{C}^*\text{H}_2\text{CH}_3$), 3.33-3.45 (overlapped, 8H, 2H for 3 1 - $\text{OC}^*\text{H}_2(\text{CH}_2)_4\text{CH}_3$ of HPPH part, 3H of 2- CH_3 and 3H of 12- CH_3), 3.25-3.15 (m, 6H, 2H for 17- $\text{CH}_2\text{C}^*\text{H}_2\text{CO}-$ and 4H for 2X- $\text{N}(\text{CH}_2)_3\text{C}^*\text{H}_2\text{SO}_3^-$), 2.80-2.65 (m, 12H, 8H for 2X- $\text{NCH}_2(\text{C}^*\text{H}_2)_2\text{CH}_2\text{SO}_3^-$ of cyanine dye part, 4H for $-\text{CONHC}^*\text{H}_2(\text{CH}_2)_4\text{CH}_2\text{NHCO}-$), 2.30-2.22 (2H for 17- $\text{C}^*\text{H}_2\text{CH}_2\text{CO}$), 2.13-2.08 (overlapped with other peaks, 3H, 3 2 - CH_3 of HPPH part), 1.75-1.60 (m, 9H, 6H for cyclohexene- $(\text{C}^*\text{H}_2)_3-$ of cyanine dye part, and 3H for 18- CH_3), 1.49-1.36 (overlapped, 17H, 12H for 4X- CH_3 of cyanine dye part, 3H for 8- $\text{CH}_2\text{C}^*\text{H}_3$ of HPPH part), 2H for 3 1 - $\text{OCH}_2\text{C}^*\text{H}_2(\text{CH}_2)_3\text{CH}_3$ of HPPH part), 1.35-1.15 (m, 14H, 6H for 3 1 - $\text{O}(\text{CH}_2)_2(\text{C}^*\text{H}_2)_3\text{CH}_3$, 8H for $-\text{CONHCH}_2(\text{C}^*\text{H}_2)_4\text{CH}_2\text{NHCO}-$), 0.65 (m, 3H for 3 1 - $\text{OCH}_2(\text{CH}_2)_4\text{C}^*\text{H}_3$). MS calculated for $\text{C}_{98}\text{H}_{116}\text{N}_8\text{O}_{10}\text{S}_3$ 1684 EIMS (m/z): 1706 ($\text{M}^+ + \text{Na}$).

Synthesis of (HPPH-CD linked with a long linker, without.) 731:

To a round bottom flask the foregoing conjugate (25 mg) in toluene (50 ml) and DMF (1-2 ml) was added Indium chloride (75 mg) and sodium bicarbonate (150 mg). Mixture was refluxed for 3 hours under argon. The solvent was removed under vacuum and the product was

purified over preparative plates using 15 % methanol-dichloromethane to yield the compound in 65% yield. UV-vis λ_{max} (in MeOH): 836.0 nm (ϵ 1.91×10^5), 646.0 nm (ϵ 7.55×10^4), 602.0 nm (ϵ 1.20×10^4), 564 nm (ϵ 6.79×10^4), 417 nm (ϵ 9.30×10^5) ^1H NMR (400Mhz, CH_3OD): 10.07 (s, 1H, H-5 of HPPH part), 9.90 (s, 1H, H-10 of HPPH part), 8.75-8.72 (m, 3H, 1H, H-20 of HPPH part, 2H, aromatic protons of cyanine dye part), 8.07 (m, 2H, aromatic protons of cyanine dye part), 7.93-7.89 (m, 4H, aromatic protons of cyanine dye part), 7.55-7.40 (m, 8H), 7.18-7.17 (m, 2H), 6.29 (d, 2H), 5.83-5.80 (m, 1H, 3'-H of HPPH part), 5.33-5.28 (m, 18-H of HPPH part), 5.09-5.05 (m, 17-H of HPPH part), 4.25-4.15 (m, 2H, 13²-H of HPPH part), 3.90-3.88 (m, 2H, $-\text{NC}^*\text{H}_2(\text{CH}_2)_3\text{SO}_3^-$), 3.73-3.70 (bs, 5H, 2H for $-\text{NC}^*\text{H}_2(\text{CH}_2)_3\text{SO}_3^-$, 3H and 7- CH_3 of HPPH), 3.61-3.57 (m, 2H, $8\text{-C}^*\text{H}_2\text{CH}_3$), 3.38-3.35 (overlapped, 6H, 2H for 3¹- $\text{OC}^*\text{H}_2(\text{CH}_2)_4\text{CH}_3$ of HPPH part, 4H for $2\text{X-N}(\text{CH}_2)_3\text{C}^*\text{H}_2\text{SO}_3^-$), 3.40 (s, 6H, 2- CH_3 and 12- CH_3), 3.34-3.11 (m, 4H for 17- $\text{C}^*\text{H}_2\text{C}^*\text{H}_2\text{CO}-$), 2.85-2.44 (m, 12H, 8H for $2\text{X-NCH}_2(\text{C}^*\text{H}_2)_2\text{CH}_2\text{SO}_3^-$ of cyanine dye part, 4H for $-\text{CONHC}^*\text{H}_2\text{C}^*\text{H}_2\text{NHCO}-$), 2.12-2.10 (overlapped with other peaks, 3H, 3²- CH_3 of HPPH part), 1.90 (m, 6H, cyclohexene- $(\text{C}^*\text{H}_2)_3-$ of cyanine dye part), 1.82 (d, 3H for 18- CH_3), 1.79-1.72 (overlapped, 17H, 12H for 4X- CH_3 of cyanine dye part, 3H for 8- $\text{CH}_2\text{C}^*\text{H}_3$ of HPPH part, 2H for 3¹- $\text{OCH}_2\text{C}^*\text{H}_2(\text{CH}_2)_3\text{CH}_3$ of HPPH part), 1.33-1.11 (m, 6H for 3¹- $\text{O}(\text{CH}_2)_2(\text{C}^*\text{H}_2)_3\text{CH}_3$, 0.71 (t, 3H for $\text{S}^1\text{-OCH}_2(\text{CH}_2)_4\text{C}^*\text{H}_3$) MS calculated for $\text{C}_{94}\text{H}_{106}\text{ClInN}_8\text{O}_{10}\text{S}_3$ 1754.36 EIMS (m/z): 1754.7 (M^+).

Synthesis of In(III) complex of HPPH-CD linked with a long linker 771 :

To a round bottom flask containing HPPH-CD linked with a long carbon chain (25 mg) in toluene (50 ml) and DMF (1-2 ml) was added Indium chloride (75 mg) and sodium bicarbonate (150 mg), mixture was refluxed for 3 hours under argon. The solvent was removed under vacuum and

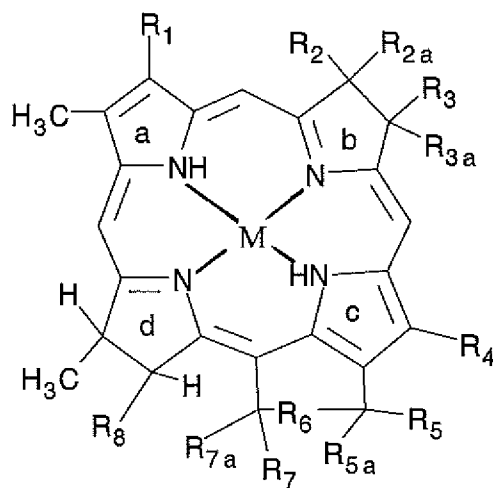
the product was purified over preparative plates using 15 % methanol-dichloromethane to yield the compound in 70 % yield. **UV-vis** λ_{max} (in MeOH): 836.0 nm (ϵ 1.98×10^5), 646.0 nm (ϵ 7.33×10^4), 602.0 nm (ϵ 1.24×10^4), 565 nm (ϵ 6.79×10^4), 418.1 nm (ϵ 9.57×10^5) **¹H NMR** (400 MHz, CH₃OD): 10.08 (s, 1H, H-5 of HPPH part), 9.91 (s, 1H, H-10 of HPPH part), 8.83-8.75 (m, 3H, 1H, H-20 of HPPH part, 2H, aromatic protons of cyanine dye part), 8.09 (m, 2H, aromatic protons of cyanine dye part), 7.94-7.91 (m, 4H, aromatic protons of cyanine dye part), 7.71-7.66 (2H, protons of cyanine dye part), 7.58-7.50 (m, 4H), 7.43 (t, 2H), 7.35-7.32 (m, 2H), 6.32 (d, 2H), 5.85 (m, 1H, 3¹-H of HPPH part), 5.29-5.34 (m, 18-H of HPPH part), 5.03 (m, 17-H of HPPH part), 4.26-4.22 (m, 2H, 13²-H of HPPH part), 3.92-3.90 (m, 2H, -NC*H₂(CH₂)₃SO₃⁻), 3.75-3.73 (m, 2H, -NC*H₂(CH₂)₃SO₃⁻), 3.69 (s, 3H, 7-CH₃ of HPPH), 3.63-3.58 (m, 2H, 8-C*H₂CH₃), 3.33-3.40 (overlapped, 6H, 2H for 3¹-OC*H₂(CH₂)₄CH₃ of HPPH part, 4H for 2X-N(CH₂)₃C*H₂SO₃⁻), 3.40 (s, 3H, 2-CH₃), 3.34 (s, 3H, 12-CH₃), 3.20-3.18 (m, 2H for 17-C*H₂CH₂CO-), 2.94-2.66 (m, 14H, 8H for 2X-NCH₂(C*H₂)₂CH₂SO₃⁻ of cyanine dye part, 2H for 17-C*H₂CH₂CO-, 4H for -CONHC*H₂(CH₂)₄C*H₂NHCO-), 2.13-2.08 (overlapped with other peaks, 3H, 3²-CH₃ of HPPH part), 1.97-1.96 (m, 6H, cyclohexene-(C*H₂)₃- of cyanine dye part), 1.88 (d, 3H for 18-CH₃), 1.80-1.76 (overlapped, 17H, 12H for 4X-CH₃ of cyanine dye part, 3H for 8-CH₂C*H₂ of HPPH part), 2H for 3¹-OCH₂C*H₂(CH₂)₃CH₃ of HPPH part), 1.33-1.11 (m, 14H, 6H for 3¹-O(CH₂)₂(C*H₂)₃CH₃, 8H for -CONHCH₂(C*H₂)₄CH₂NHCO-), 0.75-0.70 (m, 3H for 3¹-OCH₂(CH₂)₄C*H₃). MS calculated for C₉₅H₁₁₄ClInN₈O₃ 1810.4 EIMS (m/z): 1775.5 (M⁺ - Cl).

We Claim:

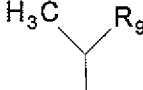
1. A photosensitizing imaging compound characterized in that it is in the form of a metallized tetrapyrrolic photosensitizer linked to a fluorescent dye.
2. The compound of claim 1 characterized in that the photosensitizer (PS), is linked by a structure that does not have detrimental radiation emission or absorbing characteristics, to a fluorophore, usually a cyanine dye (CD). The photosensitizer in accordance the invention is a metallized analog of porphyrins, chlorins, purpurinimides, bacterio purpurinimides, phthalocyanines, expanded porphyrins, benzoporphyrin derivatives and purpurins.

The fluorophore is usually a cyanine dye with variable substituents.

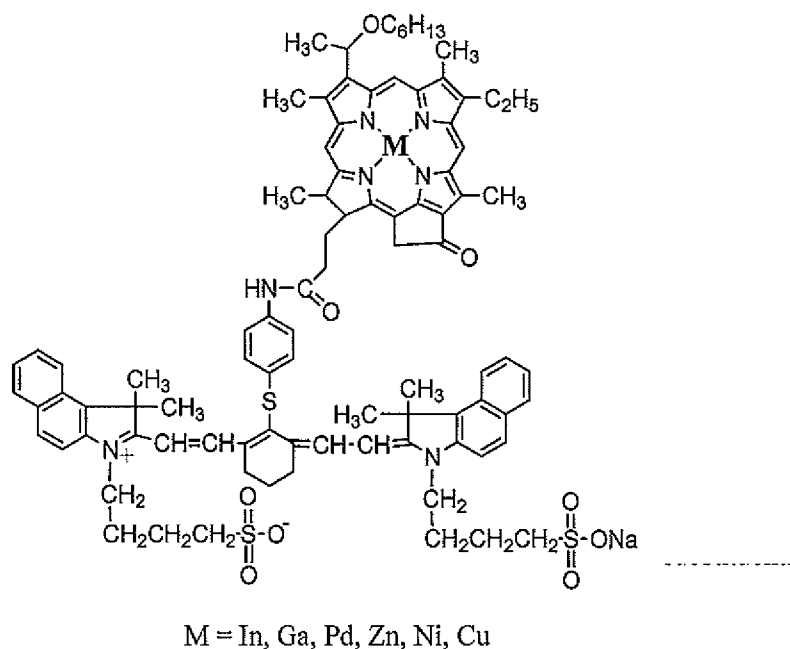
3. The compound of claim 1 characterized in that it has the formula:



where

R_{ns} , substituted or unsubstituted, $-\text{CH}=\text{CH}_2$, $-\text{CHO}$, COOH , or ,
 where $R_9 = -\text{OR}_{10}$ where R_{10} is lower alkyl of 1 through 8 carbon atoms, or $-(\text{CH}_2\text{-O})_n\text{CH}_3$; R_2 ,
 R_{2a} , R_3 , R_{3a} , R_4 , R_5 , R_{5a} , R_7 , and R_{7a} are independently hydrogen, lower alkyl, substituted lower
 alkyl, lower alkylene or substituted lower alkylene or two R_2 , R_{2a} , R_3 , and R_{3a} groups on adjacent
 carbon atoms may be taken together to form a covalent bond or two R_2 , R_{2a} , R_3 , R_{3a} , R_5 , R_{5a} , R_7 ,
 and R_{7a} groups on the same carbon atom may form a double bond to a divalent pendant group;
 R_2 and R_3 may together form a 5 or 6 membered heterocyclic ring containing oxygen, nitrogen or
 sulfur; R_e is $-\text{C}^{\text{N}}-$, $-\text{NR}_{11}-$, where R_{11} is, substituted or unsubstituted, lower alkyl, or lower
 alkylene; or a R_6 is a covalent bond; R_8 is $-(\text{CH}_2)_2\text{CO}_2\text{R}_{12}$ where R_{12} is a non-toxic fluorescent
 dye group that causes the conjugate to preferentially emit (fluoresce) at a wave length of 800 to
 about 900 nm and M is In, Ga or Pd.

4. The compound of claim 1 characterized in that it has the formula:



5. A method for determining effectiveness of PDT using the compound of claim 1 characterized in that it has the step of comparing proportion of STAT-3 monomer with crosslinked STAT-3 dimer after PDT where the relative proportion of STAT-3 monomer to crosslinked STAT-3 directly correlates to efficacy of the PDT.
6. The method of claim 5 characterized in that the relative proportion of STAT-3 monomer to crosslinked STAT-3 is determined by electrophoresis.

1/3

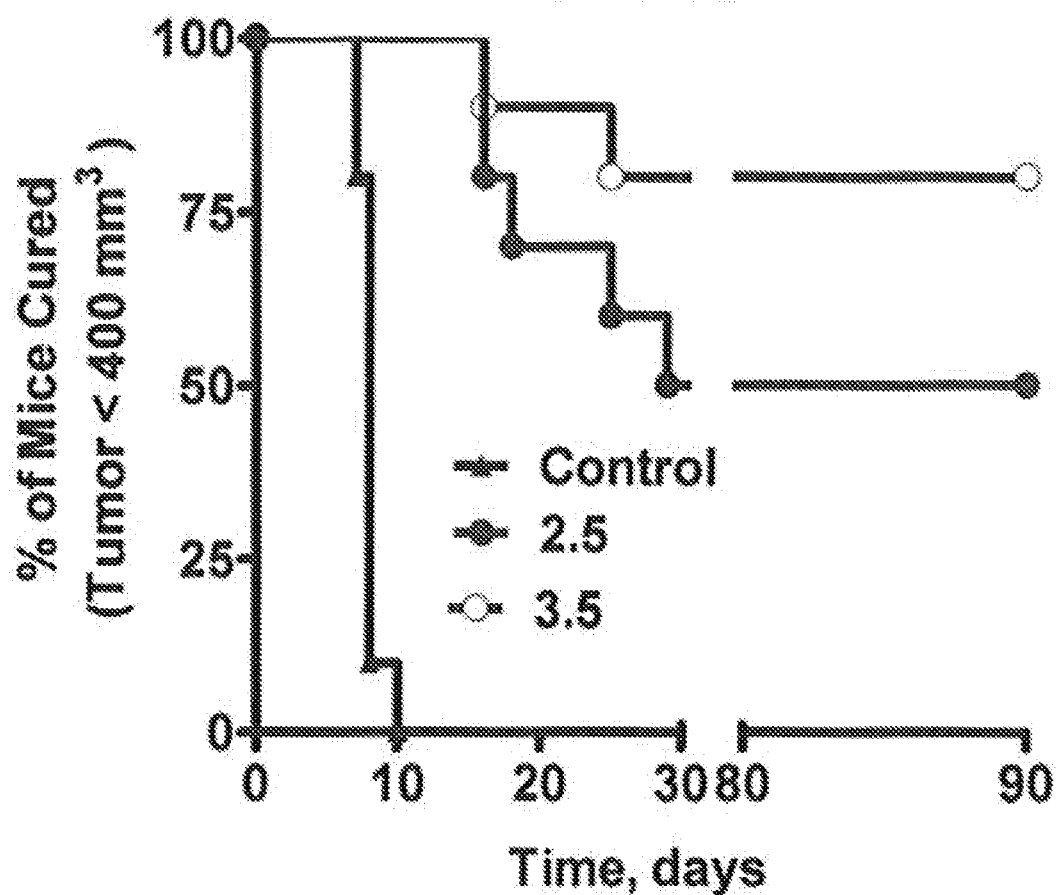
RIF tumor treated with
HPPH-CD

FIGURE 1

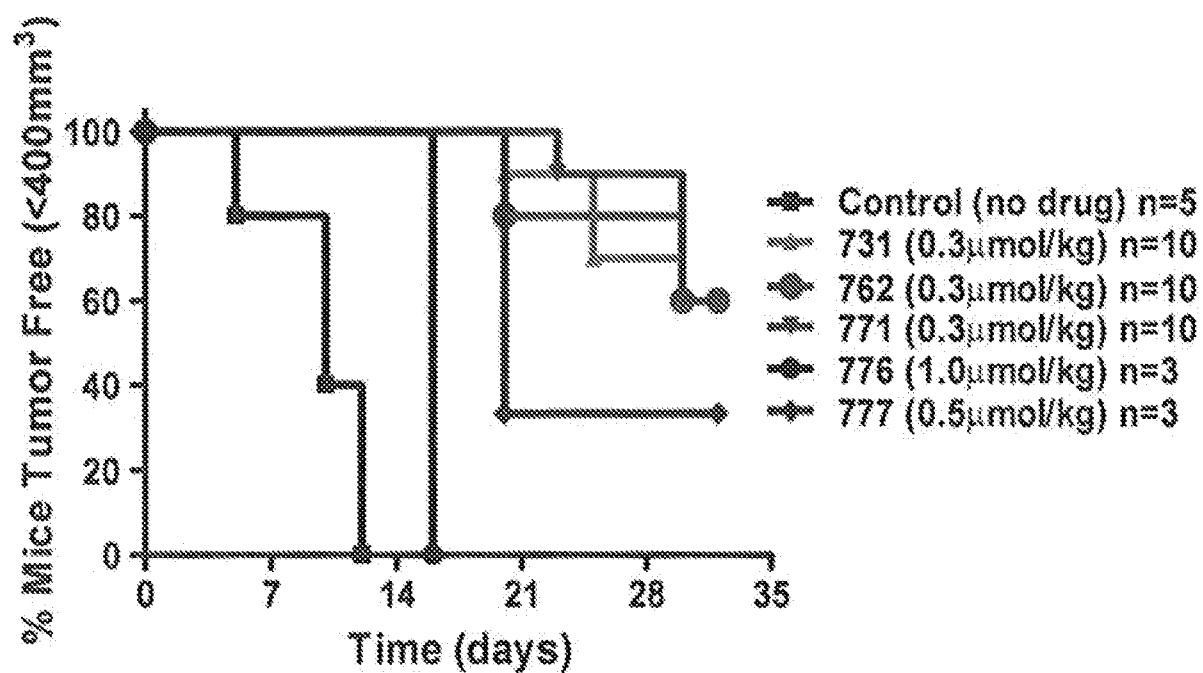


FIGURE 2

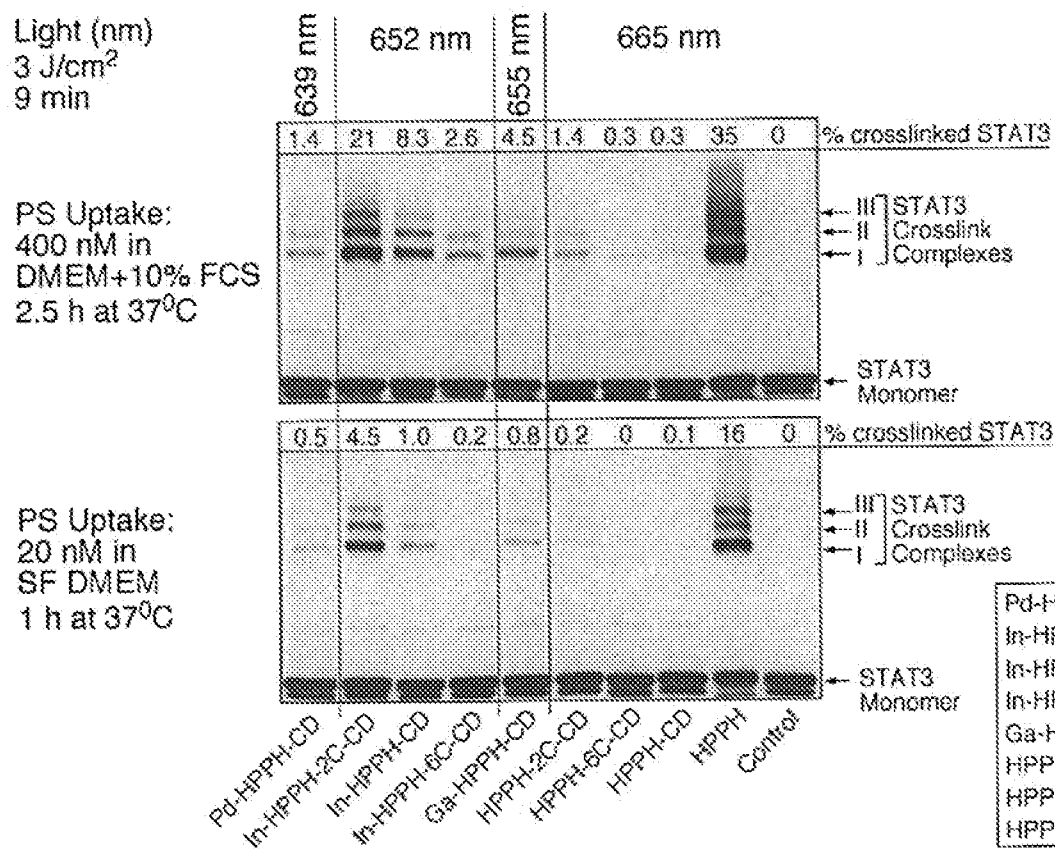


FIGURE 3

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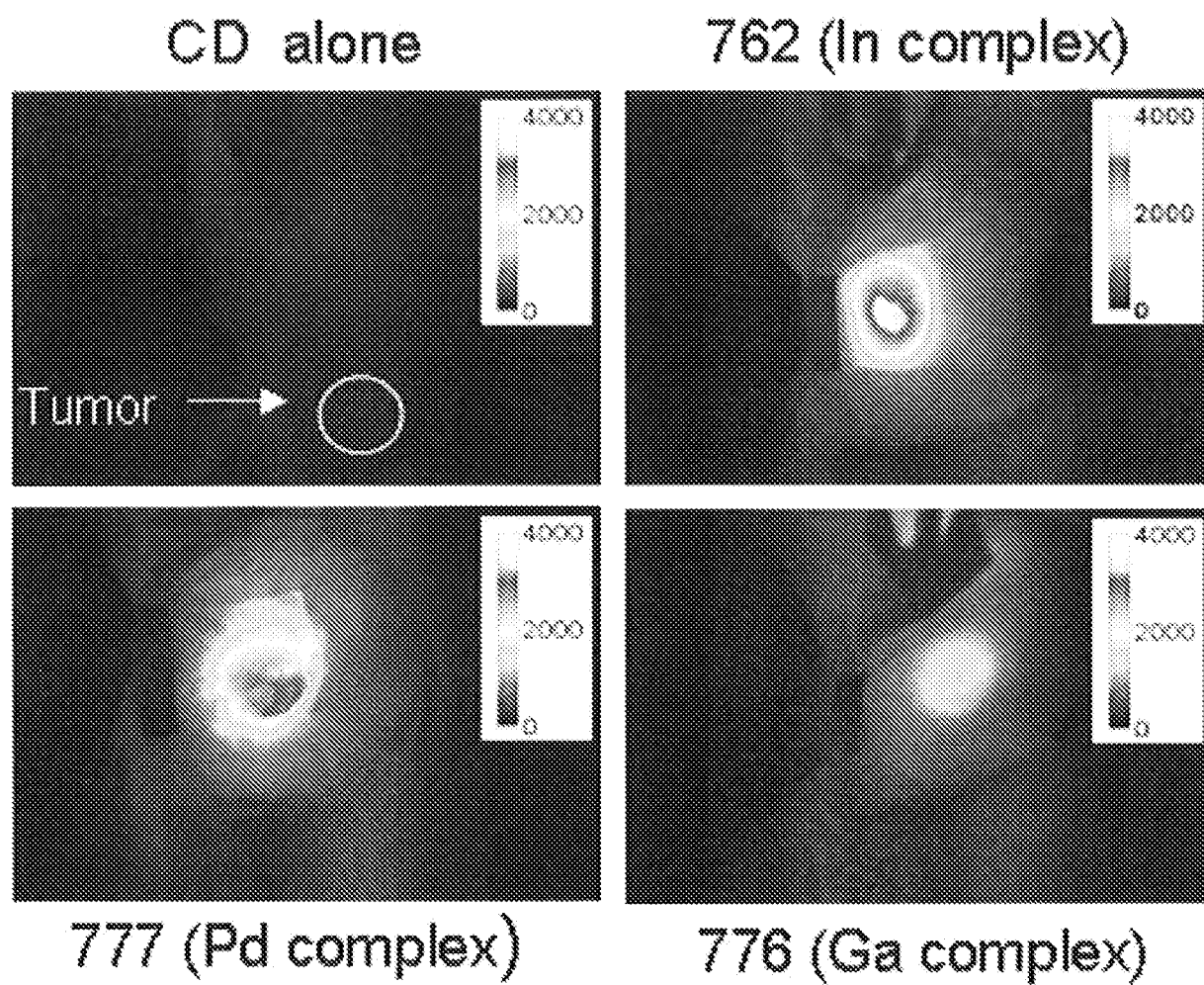


FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/41998

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C09B 67/00; C07B 47/00; C07D 487/22 (201 1.01)
USPC - 540/135

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 540/135

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 540/145 (text search) Find search terms below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB,USPT,EPAB,JPAB), Google Scholar
photodynamic therapy, PDT, porphyrin, cholin, purpurin, phthalocyanine, benzoporphyrin, metallation, metalation, metallized, metalized, palladium, indium, gallium, fluorophore, dye, conjugat\$. -

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2009/0043090 A1 (PANDEY et al.) 12 February 2009 (12.02.2009) para [0022]-[0024], [0046], [0058]-[0061], Fig. 1-Fig.7; para [0064]	1-6
Y	KEE et al. 'Photophysical Characterization of Imidazolium-Substituted Pd(II), In(III), and Zn(II) Porphyrins as Photosensitizers for Photodynamic Therapy', J Photochem Photobiol A Chem. 2008 December 15, Vol 200(2-3), pages 346-355. Downloaded from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2615400/ pg 19, Chart 1; pg 2, para 4; pg 10, para 2 to pg 11, para 4	1-6
Y	LIU et al. 'Photodynamic Therapy Causes Cross-linking of Signal Transducer and Activator of Transcription Proteins and Attenuation of Interleukin-6 Cytokine Responsiveness in Epithelial Cells', Cancer Res 2004, Vol.64, pages 6579-6587. Published online September 16, 2004. Downloaded from cancerres.aacrjournals.org pg 6581, col 2, para 2 to pg 6584, col 1, para 4; Fig 2; Fig 4	5-6
A	US 6,054,449 A (ROBINSON et al.) 25 April 2000 (25.04.2000) Fig 1-Fig 11; col 5, ln 54	1-6

☐ Further documents are listed in the continuation of Box C.

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

Date of the actual completion of the international search
13 November 2011 (13.1 1.201 1)

Date of mailing of the international search report

29 NOV 2011

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