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(54) **WATER SOLUBLE TETRAPYROLLIC PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY**

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(58) **Field of Classification Search** 540/140, 540/145; 534/14; 514/185, 410; 424/9.362, 424/9.6

See application file for complete search history.

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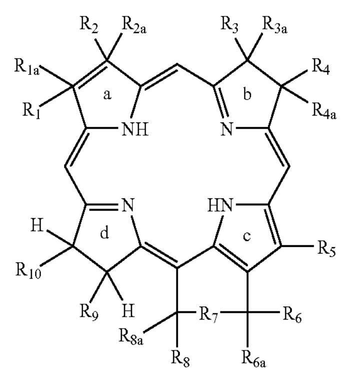
(57) **ABSTRACT**

A tetrapyrollic photosensitizer compound having at least one pendant —$CH_2CH_2CON(CH_2CON(CH_2COOH)_2)_2$ or —$N(CH_2COOH)_2$ group or esters thereof said tetrapyrollic compound being a chlorin, bacteriochlorin, porphyrin, pyropheophorbide, purpurinimide, or bacteriopurpurinimide. Desirably the compound has the formula:



or a phamaceutically acceptable derivative thereof, wherein R_1-R_8 and R_{10} are various substituents and R_9 is substituted or unsubstituted —$CH_2CH_2CON(CH_2CON(CH_2COOH)_2)_2$; or —$N(CH_2COOH)_2$. The invention also includes a method of treatment by photodynamic therapy by treatment with light after injecting the compound and a method of imaging by fluorescence after injection of the compound.

12 Claims, 5 Drawing Sheets

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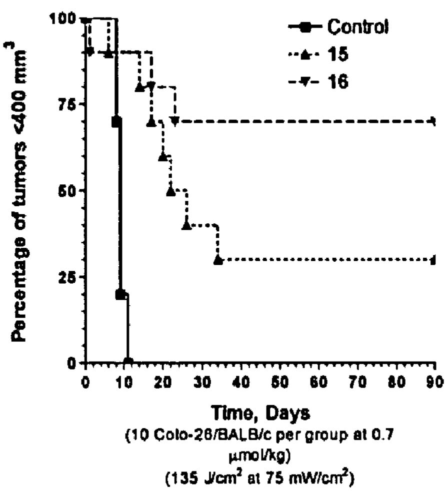


Figure 1A

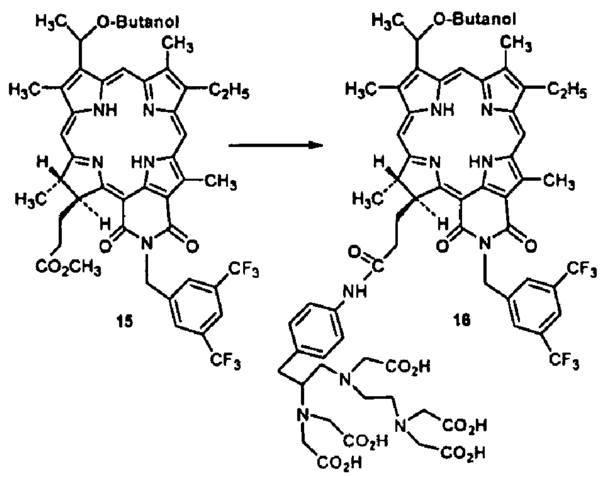
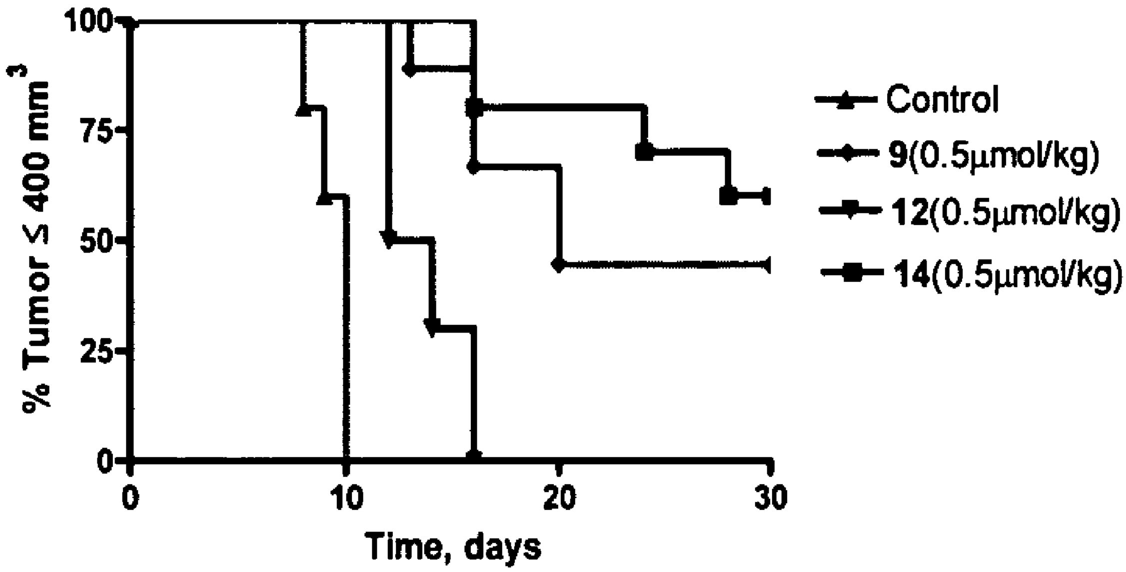


Figure 1B



100
75
50
25
0
% Tumor ≤ 400 mm3
0
10
20
30
Time, days
Control
9(0.5μmol/kg)
12(0.5μmol/kg)
14(0.5μmol/kg)

Figure 2

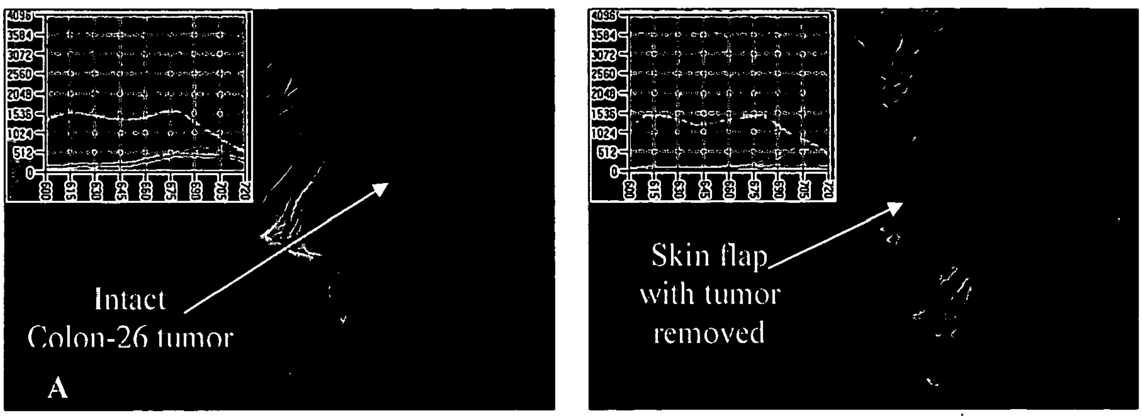


Figure 3

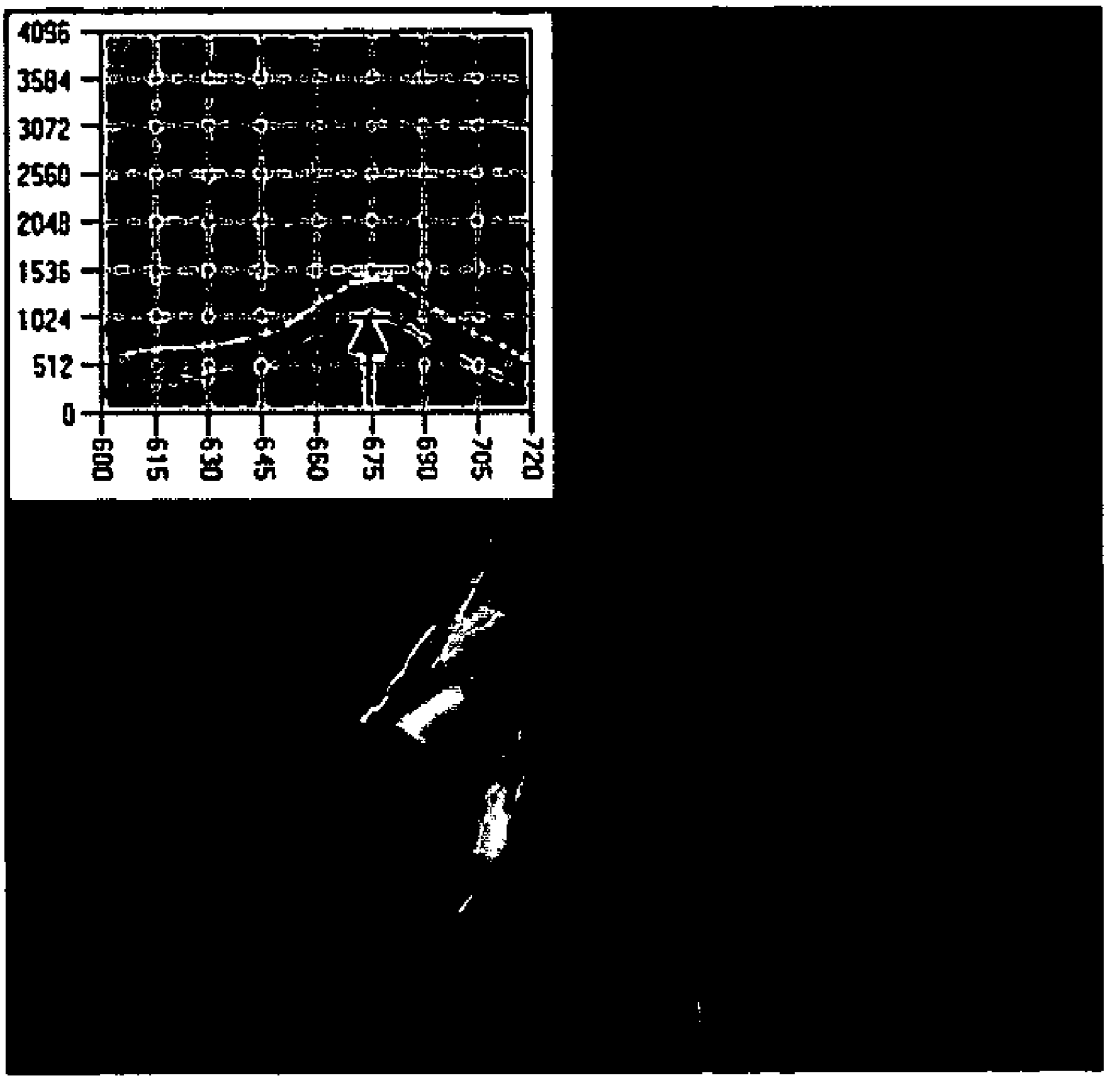
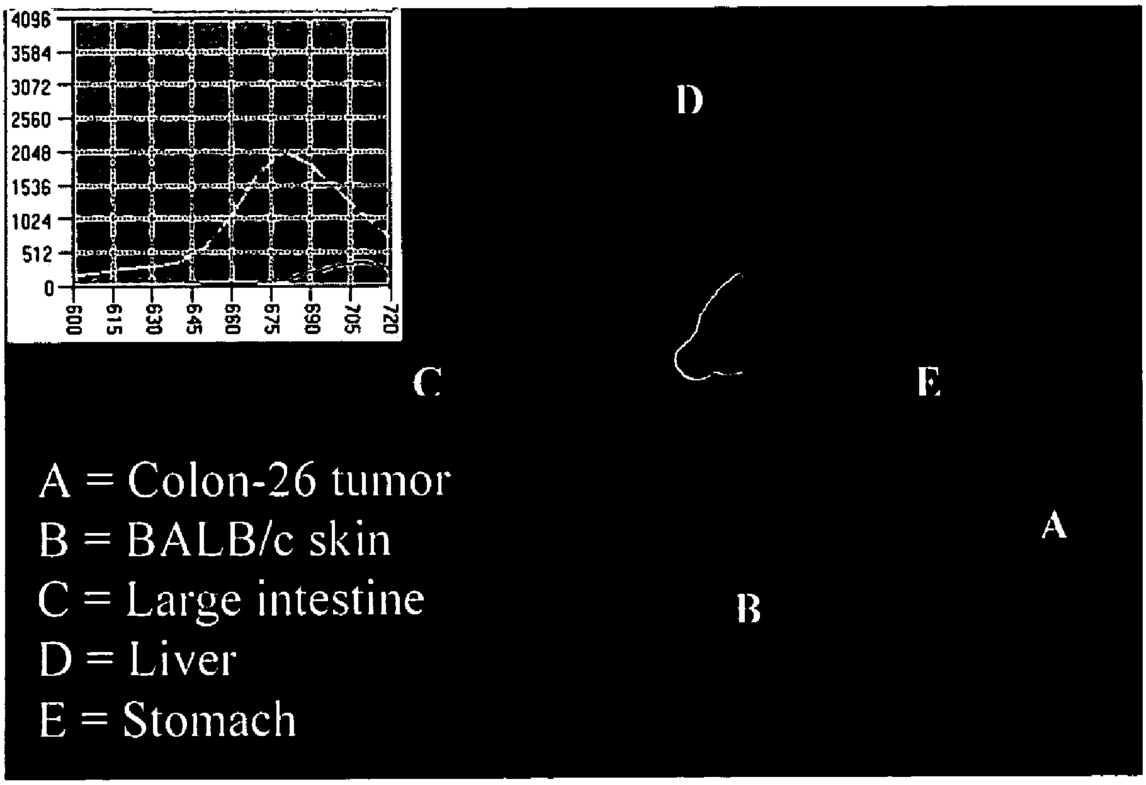

4096
3584
3072
2560
2048
1536
1024
512
0
600
615
630
645
660
675
690
705
720

Figure 3A


4096
3584
3072
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2048
1536
1024
512
0
600
615
630
645
660
675
690
705
720
D
C
E
A
B
A = Colon-26 tumor
B = BALB/c skin
C = Large intestine
D = Liver
E = Stomach

Figure 4

WATER SOLUBLE TETRAPYRROLIC PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation-in-part of U.S. patent application Ser. No. 10/607,922 to Pandey et al. filed Jun. 27, 2003 now U.S. Pat. No. 7,166,719 entitled FLUORINATED PHOTOSENSITIZERS RELATED TO CHLORINS AND BACTERIOCHLORINS FOR PHOTODYNAMIC THERAPY which in turn claims priority from Provisional Application Ser. No. 60/392,473 to Pandey et al. filed Jun. 27, 2002 entitled FLUORINATED PHOTOSENSITIZERS RELATED TO CHLORINS AND BACTERIOCHLORINS FOR PHOTODYNAMIC THERAPY.

The above applications are incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with funding from the National Institute of Health Grant Number NIH CA55791. The United States Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

For a number of years, attempts have been underway in various laboratories to replace Photofrin® with new porphyrin-based photosensitizers (PS). To date, most PS are amphiphilic in nature in that they contain both hydrophilic and hydrophobic substituents. Due to their #-conjugated systems, a phenomenon known as aggregation has become a concern such that it can: "decrease fluorescence quantum yields, shorten a photosensitizer's triplet excited state lifetime or reduce its photosensitizing efficiency". Most of these compounds, therefore, are visibly aggregated in solution, so the challenge remains to be the synthesis of effective water-soluble photosensitizers that accumulate in the tumor, yet clear at a suitable time as to limit toxicity. Several researchers have either incorporated sugar residues on the periphery or ionic groups such as pyridinium, sulfonato or carboxylate groups as a means to enhance photosensitizers' aqueous solubility. The 5, 10, 15, 20-tetrakis(4-sulfonatophenyl)-porphyrin ($TPPS_4$) is a known tetrasodium salt that although soluble in water still absorbs weakly at ~630 nm. Core modifications have been made to $TPPS_4$ in which chalcogen atoms such as sulfur, selenium and tellurium have aided in the water solubility of the PS, as well as, increasing the wavelength maximum to ~695 nm. Unfortunately, these compounds were found to be toxic Therefore, the aim of the present invention was to synthesize effective and non-toxic water-soluble long wavelength absorbing photosensitizers with high singlet oxygen ability, singlet oxygen being a key cytotoxic agent for PDT. Tetrapyrollic compounds, especially porphyrin related compounds, have played a key role in developing a variety of photosensitizers. Inventors herein have recently shown that porphyrin-based compounds can also be used (i) as PET and SPECT imaging agents and (ii) as vehicles to deliver the required contrast agents (MRI, Fluorescence etc.) to image tumors. These approaches have been extremely useful in developing multimodality agents. However, one major drawback with most of these compounds is their limited solubility in water. Therefore, most of the formulations require a biocompatible surfactant, e.g. such as those commonly sold under the trademarks TWEEN-80 or CREMOPHORE. At low concentrations, such formulations are approved by FDA for clinical use, but to avoid a number of disadvantages with such formulations, it would be 'ideal' to design water soluble compounds for tumor imaging and therapy.

An approach for increasing the water solubility is to introduce hydrophilic substituents (e.g., —COOH, PEG, amino acids, charged species etc.) in the desired molecules. Unfortunately such incorporation can limit biological efficacy.

The following references are incorporated by reference as background art.

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BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A shows a graph of In vivo photosensitizing efficacy of PS 15 and the corresponding water-soluble analog PS 16 (24 h p.i.) BALB-C mice were implanted with Colo-26 tumors. The tumors were exposed with laser light (135 J/cm^2, 75 mW/cm^2 for 30 min) 24 h post injection.

FIG. **1**B shows a schematic preparation of compound 16 from compound 15.

FIG. **2** shows a graph of In vivo photosensitizing efficacy of compounds 9, 12 and 14. BALB-C mice were implanted with Colo-26 tumors. The tumors were exposed with laser light (135 J/cm^2, 75 mW/cm^2 for 30 min) 24 h post injection.

FIG. **3** shows an In vivo fluorescence image of PS 16 (24 h p.i.). A: intact tumor; B: skin flap with tumor removed so that PS fluorescence could be imaged on underside.

FIG. **3**A shows a BALB/c Colon-26 background fluorescence image prior to PS injection.

FIG. **4** shows an In vivo fluorescence image of PS 16 in various organs (24 h p.i.). A: Colon-26 tumor; B: skin over tumor; C: large intestine; D: liver; E: stomach.

FIG. **5** shows In vivo quantitation of PS 16 fluorescence normalized to controls (ex: 417 nm; em: ~710 nm).

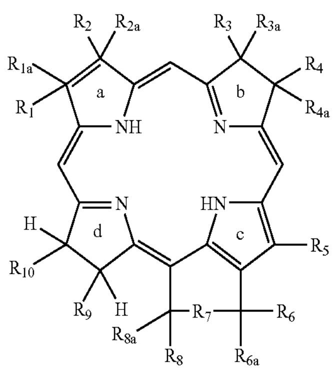
BRIEF DESCRIPTION OF THE INVENTION

In accordance with the present invention, a series of water soluble purpurinimides were prepared and some of these compounds were found to be quite effective both for PDT efficacy and tumor imaging (fluorescence).

The photosensitizers are tetrapyrollic photosensitizers having at least one pendant —$CH_2CH_2CON(CH_2CON(CH_2COOH)_2)_2$ or —$N(CH_2COOH)_2$ group or esters thereof. The substituted tetrapyrollic compound is usually a chlorin, bacteriochlorin, porphyrin, pyropheophorbide, purpurinimide, or bacteriopurpurinimide.

DETAILED DESCRIPTION OF THE INVENTION

In a preferred embodiment the compound of the invention has the formula:



or a phamaceutically acceptable derivative thereof.

R_1 and R_2 are each independently substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, —$C(O)R_a$ or —$COOR_a$ or —$CH(CH_3)(OR)$ or —$CH(CH_3)(O(CH_2)_nXR)$ where R_a is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, or substituted or unsubstituted cycloalkyl where R_2 may be $CH{=}CH_2$, $CH(OR_{20})CH_3$, $C(O)Me$, $C({=}NR_{21})CH_3$ or $CH(NHR_{21})CH_3$.

X is an aryl or heteroaryl group.

n is an integer of 0 to 6.

R and R' are independently H or lower alkyl of 1 through 8 carbon atoms.

R_{20} is methyl, butyl, heptyl, docecyl or 3,5-bis(trifluoromethyl)-benzyl.

R_{21} is 3,5,-bis(trifluoromethyl)benzyl.

R_{1a} and R_{2a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond.

R_3 and R_4 are each independently hydrogen or substituted or unsubstituted alkyl.

R_{3a} and R_{4a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond.

R_5 is hydrogen or substituted or unsubstituted alkyl.

R_6 and R_{6a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form =O.

R_7 is a covalent bond, alkylene, azaalkyl, or azaaraalkyl or $=NR_{20}$ where R_{20} is hydrogen or lower alkyl of 1 through 8 carbon atoms or —CH_2-3,5-bis(tri-fluoromethyl)benzyl or —CH_2X—R_1 or —YR^1 where Y is an aryl or heteroaryl group.

R_8 and R_{8a} are each independently hydrogen or substituted or unsubstituted alkyl or together form =O.

R_9 is —$CH_2CH_2CON(CH_2CON(CH_2COOA)_2)_2$ or —$N(CH_2COOH)_2$; where A is —OH or -lower alkyl.

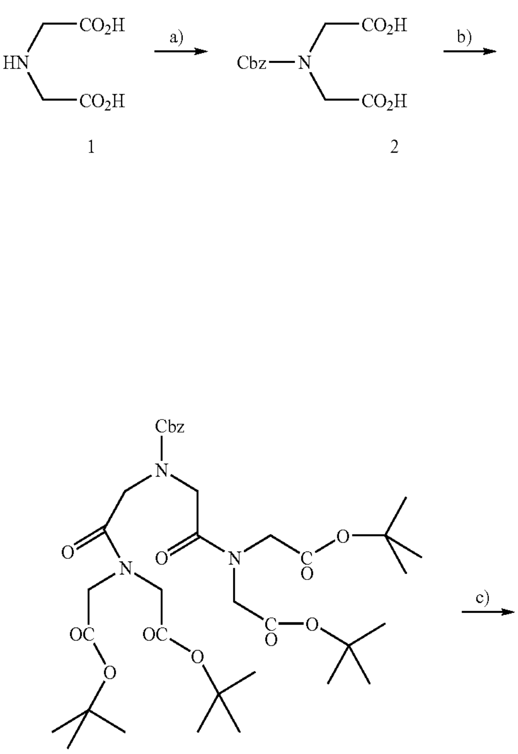
R_{10} is hydrogen, or substituted or unsubstituted alkyl.

Each of R_1-R_{10}, when substituted, is substituted with one or more substituents each independently selected from Q, where Q is alkyl, haloalkyl, halo, pseudohalo, or —$COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or —$CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or —NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or $=NR_h$, where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue;

each Q is independently unsubstituted or is substituted with one or more substituents each independently selected from Q_1, where Q_1 is alkyl, haloalkyl, halo, pseudohalo, or —$COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or $=NR_h$, where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue.

Synthetic details for the preparation of examples of water soluble photosensitizers of the invention are depicted in Schemes 1-4 as follow:

Scheme 1



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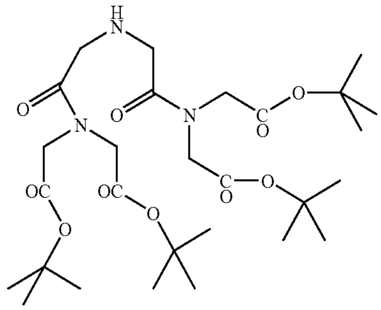

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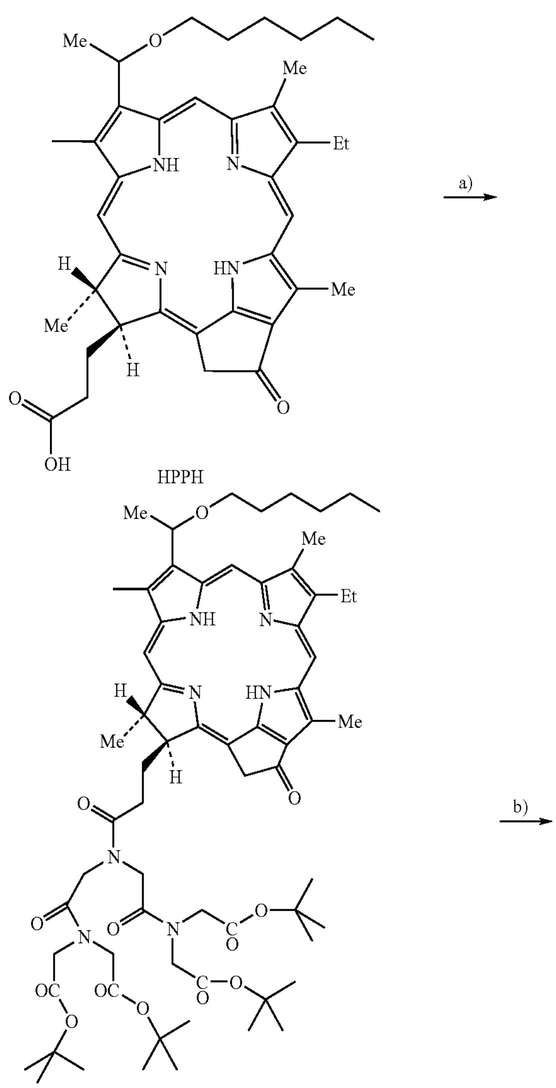
Reagents: a) Cbz-Cl, $KHCO_3$, H_2O, 0° C., RT, 6 hr
b) Di-tert-butyl iminodiacetate, EDCl, DMAP, Dry DCM, RT, 16 hr
c) Pd/C 10%, MeOH, H_2, 2 hr, RT

Scheme 2



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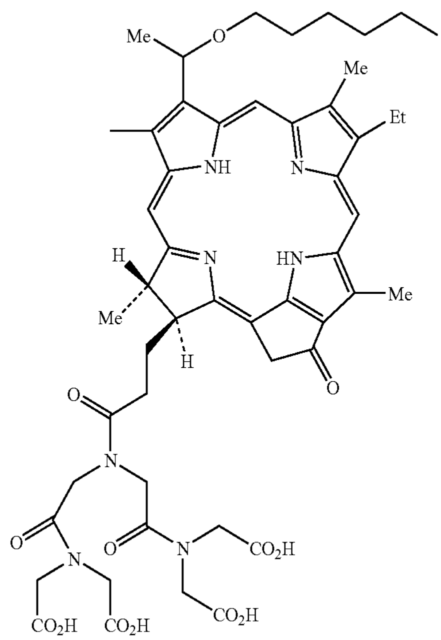
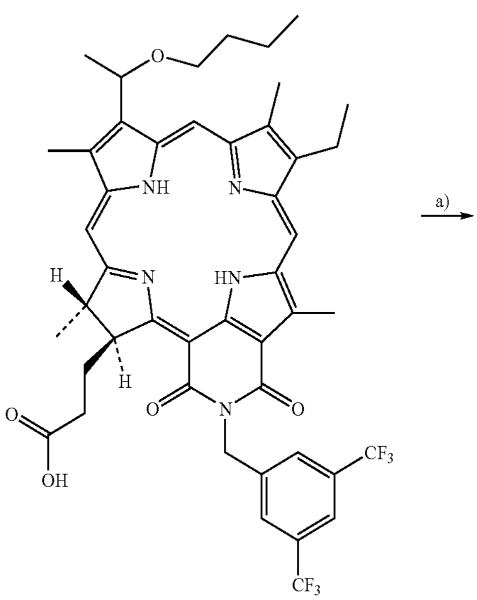


6

Reagents: a) 3, EDCl, DMAP, Dry DCM, RT, 16 hr
b) 70% TFA/DCM, 3 hr, RT

Scheme 3



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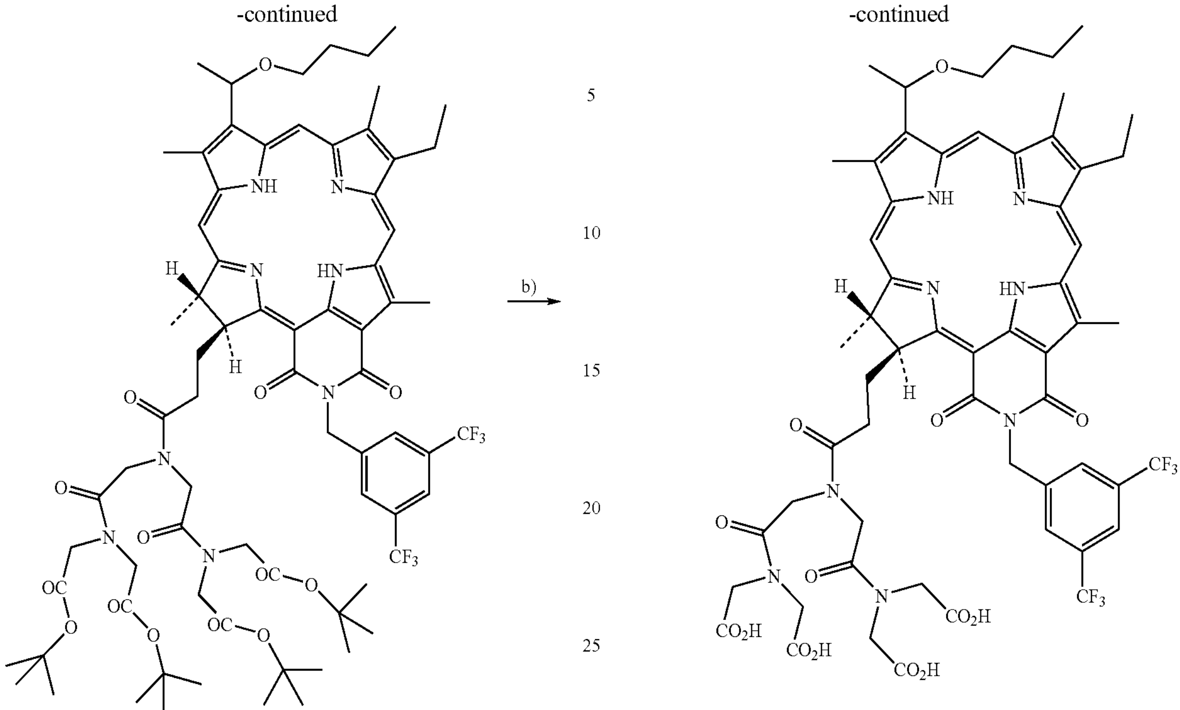

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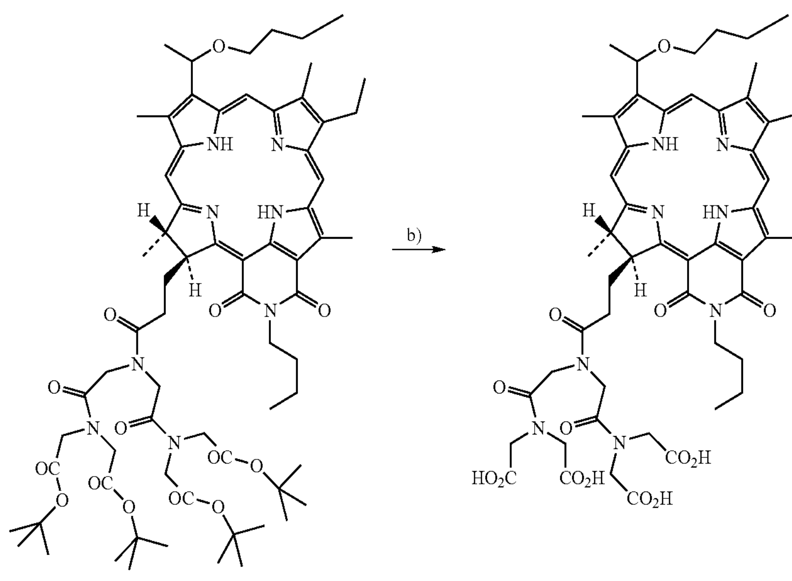
9

Reagents: a) 3, EDCl, DMAP, Dry DCM, RT, 16 hr
b) 70% TFA/DCM, 3 hr, RT

Scheme 4



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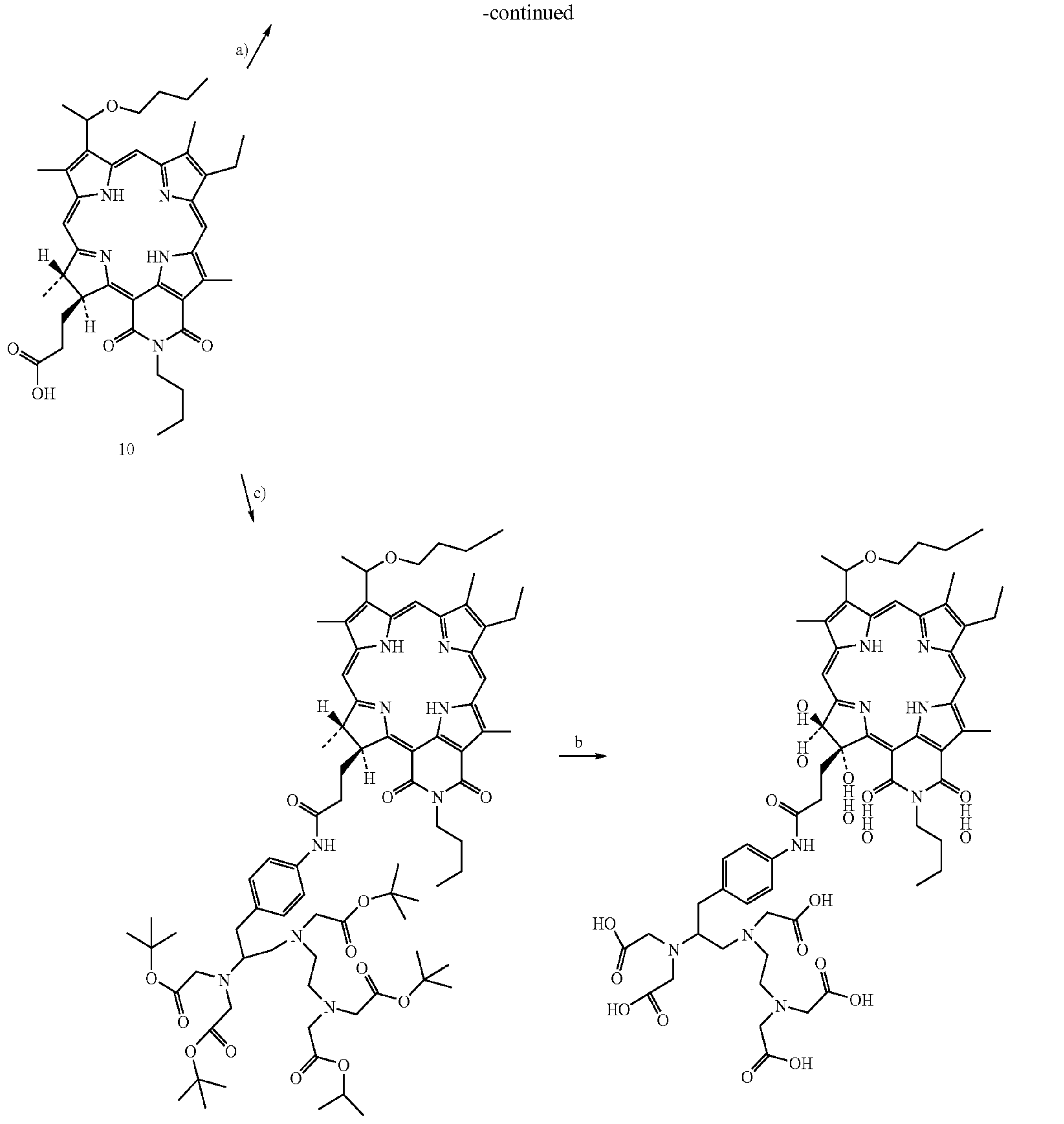


10 13 14

Reagents: a) 3, EDCI/DMAP, Dry DCM, RT, 16 hr
b) 70% TFA/DCM, 3 hr, RT
c) aminobenzyl DTPA(tBu), EDCI, DMAP, Dry DCM, RT, 16 hr

All the intermediates and the final products were characterized by NMR and mass spectrometry analyses. The purity was ascertained by analytical TLC. The starting photosensitizers (e.g. HPPH, fluorinated purpurinimide 7 and the N-butyl-purpurinimide 10 were synthesized by following published methodologies that were developed in our laboratory) The Synthetic details are as follows:

Compound No. 2

Iminodiacetic acid (5.0 gm, 0.03756 mole) was taken in a 500 ml RBF, water (150 ml) and THF (50 ml) were added to it. Resultant mixture was cooled to 0° C. using an ice bath. K_2CO_3 (25.9 gm, 0.187 mole) was added to it in portions keeping temperature of reaction mixture below 10° C. After 10 min of stirring at the same temperature Cbz-Cl (7.9 ml, 0.056 mole) was added to it drop wise. Resultant mixture was stirred for 6 hr at room temperature, concentrated partially to remove THF. Reaction mixture was washed with ether to remove excess of Cbz-Cl, aq layer was separated, acidified with dil HCl and extracted with EtOAc (100 ml×3). Organic layers were separated, combined and washed with H_2O (100 ml), dried over sodium sulfate and concentrated to give 2 as viscous oil in quantitative yield.

Yield: 9.6 gm (95.7%).

^{1}HNMR (400 MHz, $CDCl_3$): δ 7.36-7.30 (m, 5H, Ph), 5.16 (s, 2H, Ph$\underline{CH_2}$O), 4.15 (s, 2H, CH2), 4.12 (s, 2H, CH_2).

EIMS: 267(m^+).

Compound No. 3

Di-acid 2 (0.5 gm, 1.88 mmol), Di-tert-butyl iminodiacetate (0.92 gm, 3.77 mmol), EDCI (1.0 gm, 5.6 mmol) and DMAP (0.36 gm, 5.6 mmol) were dissolved in dry DCM (30 ml). Resultant mixture was stirred at room temperature for 16 hr under N_2 atm, diluted with DCM (100 ml) and washed with brine (50 ml). Organic layer was separated, dried over sodium sulfate and concentrated. Crude was purified on silica gel column using EtOAc/hexane (20-40%) as eluent to give product 3. Yield: 1.0 gm (75%).

^{1}HNMR (400 MHz, $CDCl_3$): δ 7.34-7.28 (m, 5H, Ph), 5.12 (s, 2H, Ph<u>CH$_2$</u>O), 4.28 (d, 1H, J=6.4 Hz), 4.24 (d, 1H, J=6.8 Hz), 4.18-4.14 (m, 1H), 4.05 (m, 4H), 3.91 (m, 1H), 3.74 (d, 1H, J=8.0 Hz), 3.67 (d, 1H, J=10.8 Hz), 1.47 (s, 9H, CO_2Bu^t), 1.45 (s, 9H, CO_2Bu^t), 1.44 (s, 9H, CO_2Bu^t), 1.40 (s, 9H, CO_2Bu^t). EIMS: 744(m+Na^+).

Compound No. 4

Compound 3 (0.9 gm, 1.24 mmol), Pd/C (10%, 1.0 gm), MeOH (60 ml) were stirred together under H_2 atm for 2 hr. Reaction mixture was filtered over celite, filtrate was concentrated and chromatographed over silica get using MeOH/DCM (1-3%) as eluent. Yield: 0.6 gm (82.5%).

^{1}HNMR (400 MHz, $CDCl_3$): δ 4.06 (s, 4H, CH_2), 4.01 (s, 4H, CH_2), 3.46 (s, 4H, CH_2), 1.46 (s, 36H, CO_2Bu^t). EIMS: 587(m^+).

Compound No. 5

HPPH (100.0 mg, 0.157 mmol), amine 4 (184.5 mg, 0.314 mmol), EDCI (90.4 mg, 0.471 mmol) and DMAP (57.5 mg, 0.471 mmol) were dissolved in dry DCM (30 ml). Resultant mixture was stirred at room temperature for 16 hr under N_2 atm, diluted with DCM (100 ml) and washed with brine (50 ml). Organic layer was separated, dried over sodium sulfate and concentrated. Crude was purified on silica gel column using MeOH/DCM (1-3%) as eluent to give product 5. Yield: 120.0 mg (63.35%). UV-vis (λmax cm^{-1}, dichloromethane): 409, 505, 535, 606 & 661.

^{1}HNMR (400 MHz, $CDCl_3$): δ 9.74 (s, 1H, meso-H), 9.51 (s, 1H, meso-H), 8.52 (s, 1H, meso-H), 5.91 (m, 1H, CH_3 <u>CH</u>Ohexyl), 5.35 (d, 1H, 15^1-<u>CH</u>, J=20.0 Hz), 5.13 (d, 1H, 15^1-<u>CH</u>, J=20.0 Hz), 4.52-4.49 (m, 2H, H-17 & H-18), 4.29-4.27 (m, 4H), 4.11 (m, 2H), 4.09-4.04 (m, 4H), 3.88-3.85 (m, 2H, CH_2), 3.74-3.72 (m, 2H, O<u>CH$_2$</u>hexyl), 3.67 (s, 3H, ring-CH_3), 3.66-3.59 (m, 2H, 8^1-<u>CH$_2$</u>), 3.36 (s, 3H, ring-CH_3), 3.26 (s, 3H, ring-CH_3), 2.78-2.66 (m, 2H, 17^2-CH_2), 2.53-2.49 (m, 1H, 17^1-CH), 2.15 (m, 1H, 17'-CH), 2.11 (d, 3H, <u>CH$_3$</u>CHOhexyl, J=6.8 Hz), 1.79 (d, 3H, 18-CH_3, J=7.6 Hz), 1.74 (t, 3H, 8-CH_2<u>CH$_3$</u>, J=7.6 Hz) 1.63 (m, 4H, CH_2-hexyl), 1.47-1.43 (four singlets each for CO_2Bu^t, 36H), 1.20 (m, 4H, CH_2-hexyl), 0.77 (t, 3H, CH_3-hexyl, J=6.4 Hz), 0.37 (brs, 1H, NH), −1.82 (brs, 1H, NH).EIMS: 1206 (m^+).

Compound No. 6

Compound 5 (70.0 mg) was stirred in 5 ml of 70% TFA/DCM for 3 hr at room temperature. The reaction mixture was concentrated and dried under high vacuum to give 6 in quantitative yield.

Yield: 50.0 mg (87.7%).UV-vis (λmax cm^{-1}, THF): 408, 505, 538, 605 & 660. EIMS: 983 (m^{30} +1).

Compound No. 8

Acid 7 (100.0 mg, 0.115 mmol), amine 4 (136.0 mg, 0.231 mmol), EDCI (44.4 mg, 0.231 mmol) and DMAP (28.27 mg, 0.231 mmol) were dissolved in dry DCM (30 ml). Resultant mixture was stirred at room temperature for 16 hr under N_2 atm, diluted with DCM (100 ml) and washed with brine (50 ml). Organic layer was separated, dried over sodium sulfate and concentrated. Crude was purified on silica gel column using MeOH/DCM (1-3%) as eluent to give product 8. Yield: 80.0 mg (48%). UV-vis (λmax cm^{-1}, dichloromethane): 365, 414, 548 & 701. ^{1}HNMR (400 MHz, $CDCl_3$): δ 9.74 (s, 1H, meso-H), 9.60 (s, 1H, meso-H), 8.51 (s, 1H, meso-H), 8.20 (s, 2H, bis-$CF_3C_6H_3$), 7.79 (s, 1H, bis-$CF_3C_6H_3$), 5.79 (s, 2H, benzylic CH_2), 5.75 (m, 1H, CH_3<u>CH</u>Obutyl), 5.19-5.16 (m, 1H, H-17), 4.60-4.49 (m, 2H, CH_2), 4.40-4.31 (m, 2H, CH_2), 4.18-3.96 (m, 8H, 4CH_2), 3.62 (s, 3H, ring-CH_3), 3.61-3.60 (m, 4H, 2CH_2), 3.26 (s, 3H, ring-CH_3), 3.16 (s, 3H, ring-CH_3), 2.94-2.87 (m, 1H, 17^2-CH), 2.76-2.69 (m, 1H, 17^2-CH), 2.40-2.34 (m, 1H, 17^1-CH), 2.05 (d, 3H, <u>CH$_3$</u>CHObutyl, J=10.2 Hz), 1.77-1.64 (m, 11H, 17^1-CH, 18-CH_3, 2CH_2butyl, 8-CH_2<u>CH$_3$</u>), 1.48 (s, 9H, CO_2Bu^t), 1.46 (s, 9H, CO_2Bu^t), 1.39 (s, 9H, CO_2Bu^t), 1.38 (s, 9H, CO_2Bu^t), 0.89-0.85 (spitted t, 3H, CH_3-butyl), 0.21 (brs, 1H, NH), 0.07 (brs, 1H, NH). EIMS: 1403 (m^+).

Compound No. 9

Compound 8 (60.0 mg) was stirred in 5 ml of 70% TFA/DCM for 3 hr at room temperature. Reaction mixture was concentrated and dried under high vacuum to give 9 in quantitative yield.

Yield: 40.0 mg (77.36%). UV-vis (λmax cm^{-1}, THF): 363, 414, 546 & 699. EIMS: 211 (m^++1).

Compound No. 11

Acid 10 (50.0 mg, 0.072 mmol), amine 4 (84.7 mg, 0.144 mmol), EDCI (34.5 mg, 0.18 mmol) and DMAP (22.0 mg, 0.18 mmol) were dissolved in dry DCM (30 ml). Resultant mixture was stirred at room temperature for 16 hr under N_2 atm, diluted with DCM (100 ml) and washed with brine (50 ml). Organic layer was separated, dried over sodium sulfate and concentrated. Crude was purified on silica gel column using MeOH/DCM (1-2%) as eluent to give product 11.

Yield: 65.0 mg (71.42%). UV-vis (λmax cm^{-1}, dichloromethane): 363, 415, 508, 547 & 701. ^{1}HNMR (400 MHz, $CDCl_3$): δ 9.72 (s, 1H, meso-H), 9.63 (s, 1H, meso-H), 8.52 (s, 1H, meso-H), 5.79 (m, 1H, CH_3<u>CH</u>Obutyl), 5.22 (m, 1H, H-17), 4.66 (m, 2H, CH_2), 4.45 (t, 2H, OCH_2butyl, J=7.6 Hz), 4.33 (m, 1H, H-18), 4.18-4.00 (m, 4H, 2CH_2), 3.97-3.95 (m, 4H, 2CH_2), 3.84 (s, 3H, ring-CH_3), 3.68-3.61 (m, 4H, 8-<u>CH$_2$</u>CH_3, CH_2), 3.30 (s, 3H, ring-CH_3), 3.18 (s, 3H, ring-CH_3), 3.00-2.90 (m, 1H, 17^2-CH), 2.74-2.69 (m, 1H, 17^2-CH), 2.45-2.39 (m, 1H, 17^1-CH), 2.06 (d, 3H, <u>CH$_3$</u>CHObutyl, J=6.8 Hz), 2.01-1.96 (m, 2H, NCH_2-butyl), 1.70 (m, 1H, 17^1-CH), 1.68-1.61 (m, 10H, 18-CH_3, 2CH_2butyl, 8-CH_2<u>CH$_3$</u>), 1.51, 1.49, 1.37 & 1.36 (each singlet for 36H, CO_2Bu^t), 1.10 (t, 3H, CH_3-Obutyl, J=7.6 Hz), 0.87 (t, 3H, CH_3-Nbutyl, J=7.4 Hz), −0.02 (brs, 1H, NH), −0.12 (brs, 1H, NH). EIMS: 1263 (m^+).

Compound No. 12

Compound 11 (60.0 mg) was stirred in 5 ml of 70% TFA/DCM for 3 hr at room temperature. Reaction mixture was concentrated and dried under high vacuum to give 12 in quantitative yield.

Yield: 42.0 mg (85.19%).UV-vis (λmax cm^{-1}, dichloromethane): 363, 415, 508, 547 & 701. EIMS: 1039 (m^+).

In Vivo Photosensitizing Efficacy

The experiments were performed in female BALB/c mice (6-8 weeks of age) purchased from Clarence Reeder (National Cancer Institute Fredrick Cancer Research Facility, Fredrick, Md.). The mice were injected s.c. in the axilla with 10^6 Colo-26 cells in 50 μL complete RPMI-1640 and were used for experimentation when the tumors reached 5-6 mm. All experiments were performed under the approved protocols of the RPCI Animal Care and Use Committee and followed DLAR regulations.

(a) Comparative Photosensitizing Efficacy of 15 vs its water soluble analog 16:

BALB/c mice inoculated with Colon-26 tumors were injected with 0.7λmoles/kg of either PS 15 or 16 and at ~24 h p.i., the mice were treated with PDT for a total fluence of 135 J/cm^2 at 75 mW/cm^2 (30 minute treatment). Preliminary studies had shown that PS 15 was only 30% effective using the 135 J/cm^2 at 75 mW/cm^2 (30 minute) PDT regimen. However, when its water-soluble analog was tested, the PDT response enhanced to 70% mice tumor-free by day 90.

Three explanations for this may be that (1) the slight charge from the carboxylate groups may be contributing to differing localization sites of PS 16 in comparison to 15 (as mentioned above), (2) the PDT-induced mechanism of action may differ in comparison to 16 or (3) the increased PS uptake in the tumor compared to the skin of 16 could be contributing to the enhanced PDT response. The main purpose of these experiments was to determine if the water-soluble PS could be utilized as both a PDT agent and diagnostic imaging tool. The initial in vivo experiments displayed the advantage of the water-soluble PS over its parent compound, 15.

Comparative Photosensitizing Efficacy Water-soluble Photosensitizers 9 and 12

The in vivo photosensitizing efficacy of water-soluble photosensitizers 9 and 12 was determined in BALB-C mice bearing Colo-26 tumors at similar treatment conditions. At 24 h postinjction of the photosensitizer (i. v., 0.5 μmol/Kg), the tumors were exposed to laser light (at the photosensitizer's longest wavelength absorption (135J/cm^2, 75 mW/cm^2 for 30 min) and the tumor regrowth was measured daily. The results are summarised in Figure X. As can be seen among the three candidates, compared to 12, compounds 9 and 12 were found to be more effective.

In Vivo Fluorescence Imaging With the Water-Soluble Analog 16

Measurement of PS accumulation in the tumor and skin via fluorescence measurements using a non-invasive optical imaging camera system was performed. When tumors reached 4-5 mm in diameter, the BALB/c mice were imaged prior to PS injection (using body weight of Ketamine Xylazine or 80 mg/kg of Pentobarbital Sodium anesthesia) to make certain that no endogenous chromophores were excited at the particular wavelengths utilized (425/50 nm or 540/40 nm excitation filters). Background fluorescence measurements had been a concern for previous researchers because it was found that the current diet of the mice contained chlorophyll (λ_{max} fluorescence=676 nm). When evaluating a photosensitizer such as HPPH, the PS emission peak at ~668 nm overlapped with that of chlorophyll. Therefore, the fluorescence images obtained were not particularly specific for only PS fluorescence. For instance, when the background mice were imaged (No PS) using an excitation wavelength of 425/50 nm the chlorophyll from the diet was present in both the hair (yellow) and BALB/c skin (red) exhibiting an emission peak at ~676 nm. For the experiments with PS 15 and 16, there was no concern that the emission peak of chlorophyll would overlap with that of the PS (emission at ~710 nm).

For non-invasive in vivo imaging of PS fluorescence, the Nuance™ Imaging Camera was beneficial in that once anesthetized the whole body of the mouse could be placed into the imaging LT-9CABINET, which provided the proper light insulation required for measurement and the ILLUMATOOL low power light source necessary for keeping the amount of light delivered to each mouse constant (3 mice per time point). This imaging technology was quite beneficial due to the fact that it was minimally invasive, so that there was no need to sacrifice the animal in order to obtain information about where the PS was localized. Previous studies have involved invasive procedures in which a mouse was sacrificed, the tumor or skin was excised and histological staining was performed on the paraffin blocks. Below are fluorescence images of PS 16 excited using the 425/50 nm filter and collected via the non-invasive CCD Nuance Imaging Camera (Princeton Instruments Inc.). This system was capable of taking qualitative hyperspectral images in the specific range of 650-720 nm focused on 710 nm. Attached to the small animal images are the spectral properties of the hair (yellow), skin (blue) and tumor (red).

From FIG. **3**, it can be seen that PS 16 showed a significant selectivity for tumors (peak fluorescence at ~710 nm), but when the skin flap was performed there appeared to be a noticeable amount of PS remaining in the underside of the skin after tumor removal. It is important to remember that these are qualitative images of PS accumulation in the tumor and skin. As a means to determine the exact uptake of the PS in the tumor versus the skin and other organs, a skin-flap excision, as well as, an ex vivo biodistribution study were performed. Once removed, the organs (tumor, skin, heart, spleen, muscle, kidney, stomach, intestine, lung and liver) were placed on a plexiglass plate and their fluorescence was collected (425/50 nm excitation). The fluorescence image displayed fluorescence peaks at ~675 (yellow spectrum characteristic of chlorophyll-a from diet) and ~710 nm (red spectrum characteristic of PS 16) with visible fluorescence in the tumor, skin, large intestine, liver and stomach. The organs were homogenized, dissolved in Solvable and read on the Fluoromax II Fluorimeter at 417 nm. After reading the fluorescence of all the organ samples, it was determined that the tumor and liver retained PS 16 (peak emission ~710 nm), while the skin, stomach and intestine retained material characteristic of chlorophyll-a (peak emission 676 nm). The average fluorescence per mg/mL of protein was normalized to background mice (no PS) and plotted for each organ (avg. of 3 samples per organ).

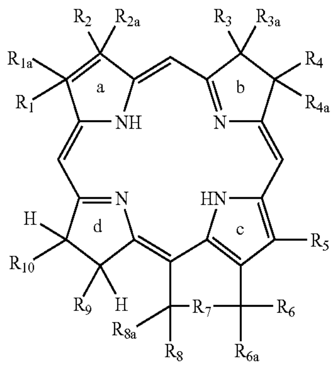
This invention describes the successful synthesis of a new long wavelength water-soluble PS. The in vitro and in vivo PDT photosensitizing experiments indicated that PS 16 was superior to its parent compound, 15

At its therapeutic PDT dose of 0.7 μmoles/kg (70% mice were tumor-free by day 60, 7/10 mice), PS 16 displayed selective tumor uptake at 24 h p.i. as visualized by Nuance™ imaging and confirmed by the fluorescence extraction experiments. This is the first report of a water-soluble fluorinated purpurinimide being utilized as a dual PDT-imaging agent.

What is claimed is:

1. A tetrapyrollic photosensitizer compound having at least one pendant —$CH_2CH_2CON(CH_2CON(CH_2COOH)_2)_2$ or —$N(CH_2COOH)_2$ group or esters thereof said tetrapyrollic compound being a chlorin, bacteriochlorin, porphyrin, pyropheophorbide, purpurinimide, or bacteriopurpurinimide.

2. A compound of the formula:



or a phamaceutically acceptable derivative thereof, wherein:

R_1 and R_2 are each independently substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl,—C(O)R_a or —$COOR_a$ or —$CH(CH_3)(OR)$ or —$CH(CH_3)(O(CH_2)_nXR)$ where R_a is hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, or substituted or unsubstituted cycloalkyl where R_2 may be $CH{=}CH_2$, $CH(OR_{20})CH_3$, C(O)Me, $C({=}NR_{21})CH_3$ or $CH(NHR_{21})CH_3$;

where X is an aryl or heteroaryl group;

n is an integer of 0 to 6;

R and R' are independently H or lower alkyl of 1 through 8 carbon atoms;

where R_{20} is methyl, butyl, heptyl, docecyl or 3,5-bis(trifluoromethyl)-benzyl; and

R_{21} is 3,5, -bis(trifluoromethyl)benzyl;

R_{1a} and R_{2a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond;

R_3 and R_4 are each independently hydrogen or substituted or unsubstituted alkyl;

R_{3a} and R_{4a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form a covalent bond;

R_5 is hydrogen or substituted or unsubstituted alkyl;

R_6 and R_{6a} are each independently hydrogen or substituted or unsubstituted alkyl, or together form ═O ;

R_7 is a covalent bond, alkylene, azaalkyl, or azaaraalkyl or $=NR_{20}$ where R_{20} is hydrogen or lower alkyl of 1 through 8 carbon atoms or —CH_2-3,5-bis(tri-fluoromethyl) benzyl or —CH_2X-R^1 or —YR^1 where Y is an aryl or heteroaryl group;

R_8 and R_{8a} are each independently hydrogen or substituted or unsubstituted alkyl or together form ═O;

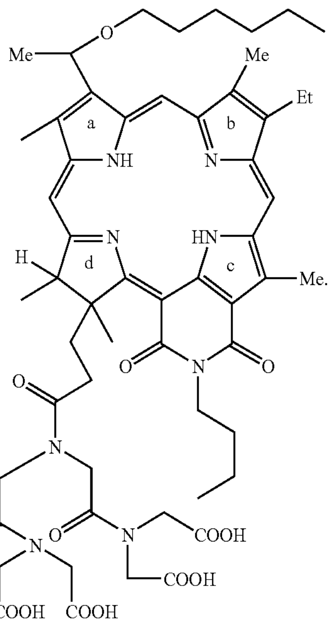
R_9 is —$CH_2CH_2CON(CH_2CON(CH_2COOH)_2)_2$; or —$N(CH_2COOH)_2$

R_{10} is hydrogen, or substituted or unsubstituted alkyl and;

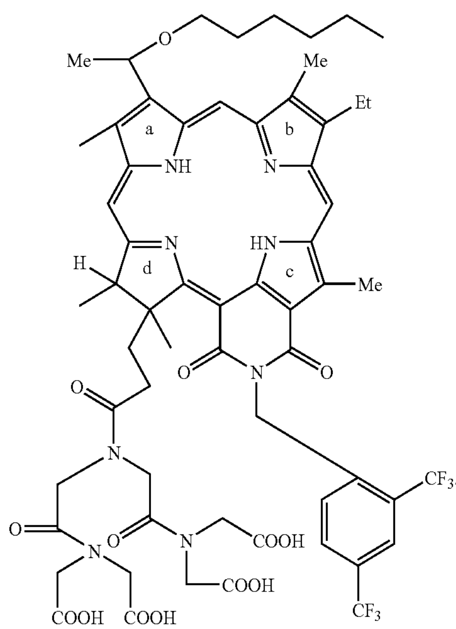
each of R_1-R_{10}, when substituted, is substituted with one or more substituents each independently selected from Q, where Q is alkyl, haloalkyl, halo, pseudohalo, or —$COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or $=NR_h$ where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue;

each Q is independently unsubstituted or is substituted with one or more substituents each independently selected from Q_1, where Q_1 is alkyl, haloalkyl, halo, pseudohalo, or —$COOR_b$ where R_b is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, araalkyl, or OR_c where R_c is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl or $CONR_dR_e$ where R_d and R_e are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or NR_fR_g where R_f and R_g are each independently hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or $=NR_h$ where R_h is hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, or aryl, or is an amino acid residue.

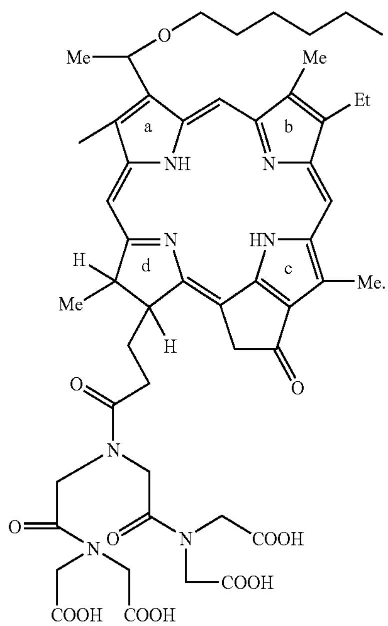
3. A compound according to claim **2** having the formula:



4. A compound according to claim **2** having the formula:



5. A compound according to claim **2** having the formula:



6. The compound of claim **2**, wherein:

R_1 is substituted or unsubstituted alkyl;

R_2 is substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, or —C(O)R_a,

where R_a is substituted or unsubstituted alkyl;

R_{1a} and R_{2a} together form a covalent bond;

R_3 and R_4 are each independently substituted or unsubstituted alkyl;

R_{3a} and R_{4a} are each independently hydrogen, or together form a covalent bond;

R_5 is substituted or unsubstituted alkyl;

R_6 and R_{6a} together form ═O;

R_7 is azaalkyl, or azaaralkyl;

R_8 and R_{8a} together form ═O;

R_9 and R_{10} are each independently substituted or unsubstituted alkyl;

each of R_1-R_{10}, when substituted, is substituted with one or more substituents each independently selected from Q, where Q is halo, pseudohalo, haloalkyl, $COOR_b$ where R_b is hydrogen or alkyl, OR_c where R_c is alkyl or aralkyl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl or aralkyl, or ═NR_h where R_h is aralkyl;

each Q is independently unsubstituted or is substituted with one or more substituents each independently selected from Q_1, where Q_1 is halo, pseudohalo, or haloalkyl.

7. The compound of claim **2**, wherein:

R_1 is unsubstituted alkyl;

R_2 is substituted or unsubstituted alkyl, unsubstituted alkenyl, or —C(O)R_a, where R_a is unsubstituted alkyl;

R_{1a} and R_{2a} together form a covalent bond;

R_3 and R_4 are each independently unsubstituted alkyl;

R_{3a} and R_{4a} are each independently hydrogen, or together form a covalent bond;

R_5 is unsubstituted alkyl;

R_6 and R_{6a} together form ═O;

R_7 is azaalkyl, or azaaralkyl;

R_8 and R_{8a} together form ═O;

R_{10} is unsubstituted alkyl;

each of R_1-R_{10}, when substituted, is substituted with one or more substituents each independently selected from Q, where Q is halo, pseudohalo, haloalkyl, $COOR_b$ where R_b is hydrogen or alkyl, OR_c where R_c is alkyl or aralkyl, NR_fR_g where R_f and R_g are each independently hydrogen, alkyl or aralkyl, or ═NR_h where R_h is aralkyl;

each Q is independently unsubstituted or is substituted with one or more substituents each independently selected from Q_1, where Q_1 is halo, pseudohalo, or haloalkyl.

8. The compound of claim **2**, wherein:

R_1 is methyl;

R_{1a} and R_{2a} together form a covalent bond;

R_3 is methyl;

R_4 is ethyl;

R_{3a} and R_{4a} are each independently hydrogen, or together form a covalent bond;

R_5 is methyl; and

R_{10} is methyl.

9. The compound claim **2**, wherein:

R_2 is $CH{=}CH_2$, $CH(OR_{20})CH_3$, C(O)Me, $C({=}NR_{21})CH_3$ or $CH(NHR_{21})CH_3$;

where R_{20} is methyl, butyl, heptyl, dodecyl or 3,5-bis(trifluoromethyl)-benzyl; and

R_{21} is 3,5-bis(trifluoromethyl)benzyl.

10. The compound of claim **2**, wherein:

R_7 is ═NR_{20}, where R_{20} is methyl, butyl, heptyl, dodecyl or 3,5-bis(trifluoromethyl)-benzyl.

11. A pharmaceutical composition, comprising a compound of claim **1** or a pharmaceutically acceptable derivative thereof in a pharmaceutically acceptable carrier.

12. A pharmaceutical composition, comprising a compound of claim **2** or a pharmaceutically acceptable derivative thereof in a pharmaceutically acceptable carrier.

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