PYRIDYL-SUBSTITUTED PORPHYRIN COMPOUNDS AND METHODS OF USE THEREOF

This application claims the benefit of U.S. Provisional Application No. 60/557,551, filed March 29, 2004 and U.S. Provisional Application No. 60/628,465, filed November 16, 2004, which are incorporated by reference herein in their entirety.

1. FIELD OF THE INVENTION

The present invention relates to Pyridyl-Substituted Porphyrin Compounds, compositions comprising an effective amount of a Pyridyl-Substituted Porphyrin Compound and methods for treating or preventing injury due to exposure to a reactive species, erectile dysfunction due to surgery, lung disease, hyperoxia, neurodegenerative disease, liver disease, myocardial damage during cardioplegia, an inflammatory condition, a reperfusion injury, an ischemic condition, a cardiovascular disease, diabetes, a diabetic complication, cancer, a side effect of cancer chemotherapy, or a radiation-induced injury, or to prolong the half-life of an oxidation-prone compound, comprising administering to a subject in need thereof an effective amount of a Pyridyl-Substituted Porphyrin Compound.

2. BACKGROUND OF THE INVENTION

Oxidants are normal by-products of cell metabolism. However, reactive oxygen species such as superoxide ("O_2^-") and reactive intermediates formed from O_2^- are known to damage biological targets. For example, J. Lee et al., *J. Am. Chem. Soc.* 120:7493-7501 (1998) discloses that reactive oxygen and nitrogen species play a role in the regulation and inhibition of mitochondrial respiration and apoptosis.

S. Cuzzocrea et al., Pharm. *Rev.*, 53:135-159 (2001) discloses that biologically relevant free-radicals derived from oxygen include O_2^-, perhydroxyl radical ("HO_2^-"), and nitric oxide ("NO"). One source of O_2^- is a proinflammatory cytokine, which produces O_2^- during reperfusion following ischemia. This reference discloses that reaction of NO with O_2^- forms the reactive peroxynitrite ion ("$ONOO^-$") according to the reaction:

$$NO + O_2^- \longrightarrow ONOO^-$$

The reference further discloses that formation of ONOO- enhances the cytotoxic potential of NO and O_2^-.

In animals, a superoxide dismutase ("SOD") counters the effects of these reactive species. SODs are metalloenzymes that catalyze the conversion of O_2^- to hydrogen peroxide and oxygen according to the reaction:

$$2\,O_2^- + 2\,H^+ \longrightarrow H_2O_2 + O_2$$

It is reported that certain synthetic metallomacrocyles also catalyze the transformation of reactive species into less reactive products. U.S. Patent No. 6,204,259 to Riley et al. discloses that a pentazamacrocycle comprising a Mn(II) or Mn(III) metal can catalyze the conversion of O_2^- into oxygen and hydrogen peroxide.

Spasojevic et al., *Biology and Chemistry* 4(5):526-533 (2000) discloses that tetrakis-5,10,15,20-(2-N-ethylpyridinium)porphyrinato complexes of manganese(II) and manganese(III) are catalytic scavengers of oxygen.

J. Lee et al., *J. Am. Chem. Soc.* 120:7493-7501 (1998) discloses that O_2^- and $ONOO^-$ are decomposed by the metalloporphyrin 5,10,15,20-tetrakis(N-methyl-4-pyridyl)porphinatoiron(III).

Lee et al., *Bioorg. Med. Chem. Letters* 7:2913-2918 (1997) discloses that 5,10,15,20-tetrakis(N-methyl-4-pyridinium)porphinatomanganese(III) catalyzes the reduction of $ONOO^-$ in the presence of biological antioxidants such as vitamin C, gluthionate, and vitamin E.

U.S. Patent No. 5,630,137 to Nguyen et al. discloses a cosmetic composition containing SODs in combination with metalloporphyrins that is allegedly useful to treat skin and hair disorders caused by free radicals. This patent discloses the use of naturally occurring metalloporphyrins such as chlorophyll, chlorophyllin and hemoglobin to allegedly reinforce the anti-free radical action of the SOD.

German Patent Publication No. DE 19647640 A1 discloses a metalloporphyrin dimer in which two metalloporphyrin compounds are covalently joined at the meso position of the porphyrin rings. The patent publication alleges that the dimer is useful for catalyzing oxygen-transfer processes.

International Publication No. WO 99/55388 discloses meso-substituted metalloporphyrin complexes in which the meso substituents are ester, alkyl, alkyl halide, and amide groups. This publication further alleges that such compounds are

useful for modulating the cellular levels of oxidants and the processes in which these oxidants participate.

Metalloporphyrins are also reported to inhibit telomerase activity by binding to quadraplex DNA. For example, Shi et al., *J. Med. Chem.* 44:4509-4523 (2002) discloses that cationic forms of meso-tetrakis(N-methylpyridinium)metalloporphyrins interact with the quadraplex structure of DNA.

U.S. Patent No. 6,087,493 to Wheelhouse et al. discloses meso-tetrakis(pyridyl)metalloporphyrins in which the nitrogen atom of the pyridyl rings are substituted with hydrogen, alkyl, alkylhydroxy, alkylamine, alkylacetate or alkylsulfate groups. This patent alleges that such compounds are useful as telomerase inhibitors.

U.S. Patent No. 6,204,259 to Riley et al. discloses that pentazamacrocycles comprising a Mn(II) or Mn(III) metal are allegedly useful for treating inflammatory disease states and reperfusion injury.

U.S. Patent No. 6,127,356 to Crapo et al. discloses meso-substituted metalloporphyrins in which the meso substituents are aryl, substituted aryl, cycloalkyl, 4-pyridyl or N-substituted 4-pyridyl groups. This patent further discloses meso-tetrakis(pyridinium)metalloporphyrins in which the nitrogen atom of the pyridyl ring is substituted with an alkyl group, alkylhydroxy, alkylamine, alkylcarboxylate, alkysulfate or alkylphospate. The patent alleges that the disclosed metalloporphyrins act as mimetics of SODs.

Misko et al., *J. Biol. Chem.* 273:15646-15653 (1998) discloses that 5,10,15,20-tetrakis(N-methyl-4-pyridinium)porphinatoiron(III) catalyzes the conversion of $ONOO^-$ into nitrate. The authors also disclose that 5,10,15,20-tetrakis(N-methyl-4-pyridinium) porphinatoiron(III) is allegedly useful for reducing cellular damage at sites of inflammation.

International Publication No. WO 00/75144 A2 discloses 5,10,15,20-tetrakis(N-alkylpyridinum)metalloporphyrins in which the pyridyl fragments are joined to the meso carbon atoms of the porphyrin ring at the 2("ortho"), 3("meta") or 4("para") position of the pyridyl ring relative to the nitrogen atom. The publication alleges that the meso-tetrakis(N-alkylpyridinium)metalloporphyrins are useful for treating inflammation diseases including arthritis, inflammatory bowel disease and

acute respiratory disease syndrome, and for the treatment of ischemia-reperfusion injury.

U.S. Patent No. 5,994,339 to Crapo et al. discloses Mn-, Fe- and Cu-based 5,10,15,20-tetrakis(N-alkyl-4-pyridinium)metalloporphyrins in which the nitrogen atom of the pyridyl ring is substituted with an alkyl, alkylhydroxy, alkylamine, alkylcarboxylate, alkylsulfate or alkyphosphate group. This patent also alleges that 5,10,15,20-tetrakis(N-alkyl-4-pyridinium) metalloporphyrins are useful as mimetics of SODs and for the treatment of an inflammatory condition.

U.S. Patent No. 6,245,758 B1 to Stern et al. discloses the use of 5,10,15,20-tetrakis(pyridyl)metalloporphyrins, and their corresponding N-alkylpyridinium salts, to allegedly treat disorders including inflammation disease and ischemic reperfusion. Metals allegedly useful in the metalloporphyrins include Mn, Fe, Ni and V.

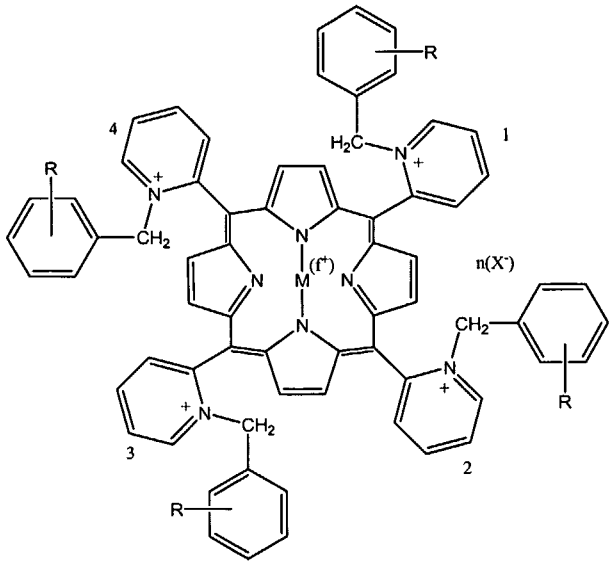
U.S. Patent Application Publication 2002/0042407 to Fridovich et al. discloses that 5,10,15,20-tetrakis(N-alkylpyridinium)metalloporphyrins are allegedly useful for modulating the intra-or extracellular levels of oxidants such as $O_2^{\cdot}$. Metals allegedly useful in the metalloporphyrins include Fe, Mn, Co, Ni and Zn. The publication also discloses methods for using these 5,10,15,20-tetrakis(N-alkylpyridinium)metalloporphyrins to allegedly treat disorders such as inflammatory diseases of the skin and lungs, ischemia reperfusion injury; eye disorders such as glaucoma, macular degeneration and cataracts; and diseases of the central nervous system.

There remains, however, a clear need for compounds, compositions and methods for that are useful for treating or preventing injury due to exposure to a reactive species, erectile dysfunction due to surgery, lung disease, hyperoxia, neurodegenerative disease, liver disease, myocardial damage during cardioplegia, an inflammatory condition, a reperfusion injury, an ischemic condition, a cardiovascular disease, diabetes, a diabetic complication, cancer, a side- effect of cancer chemotherapy, or a radiation-induced injury, or to prolong the half-life of an oxidation-prone compound.

Citation of any reference in Section 2 of this application is not an admission that the reference is prior art to this application.

3. SUMMARY OF THE INVENTION

The present invention encompasses compounds having the Formula (A):



(A)

wherein:

M is Fe or Mn;

f is 0 or 1;

each R is independently –C(O)OH or –C(O)O$^-$;

each X$^-$ is independently a negatively-charged counterion; and

n = (f) + (the total number of R groups where R is –C(O)OH).

A compound of Formula (A) (a "Pyridyl-Substituted Porphyrin Compound") is useful for treating or preventing injury due to exposure to a reactive species, erectile dysfunction due to surgery, lung disease, hyperoxia, neurodegenerative disease, liver disease, myocardial damage during cardioplegia, an inflammatory condition, a reperfusion injury, an ischemic condition, a cardiovascular disease, diabetes, a diabetic complication, cancer, a side effect of cancer chemotherapy, or a radiation-induced injury, or to prolong the half-life of an oxidation-prone compound, (each being a "Condition") in a subject.

The invention also relates to compositions comprising an effective amount of a Pyridyl-Substituted Porphyrin Compound, and a physiologically

acceptable carrier or vehicle. The compositions are useful for treating or preventing a Condition in a subject.

The invention further relates to methods for treating or preventing a Condition, comprising administering to a subject in need thereof an effective amount of a Pyridyl-Substituted Porphyrin Compound.

The present invention may be understood more fully by reference to the following detailed description, figures, and illustrative examples, which are intended to exemplify non-limiting embodiments of the invention.

4. BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 shows a survival curve for Balb/c mice that were pre-treated with Compound **3A**, prior to exposure to 6 Gy of ionizing radiation. The x-axis represents days post-irradition and the y-axis represents the ratio of surviving mice to the total number of irradiated mice. Line -■- represents mice (n=10) treated with Compound **3A**, administered at a dose of 2 mg/kg, two hours prior to irradiation followed by administration of post-irradition doses of 2 mg/kg every 12 hours until death. Line -□- represents a non-treated control group of mice (n=10).

FIG. 2 shows a survival curve for Balb/c mice that were treated with Compound **3A**, after being exposed to 6 Gy of ionizing radiation. The x-axis represents days post-irradition and the y-axis represents the ratio of surviving mice to the total number of irradiated mice. Line -■- represents mice (n=10) treated with Compound **3A**, administered at a dose of 2 mg/kg, ten minutes post-irradiation, followed by repeated administration of 2 mg/kg doses every 12 hours until death. Line -□- represents a non-treated control group of mice (n=10).

FIG. 3 shows a survival curve for Balb/c mice that were treated with Compound **3A**, after being exposed to 6 Gy of ionizing radiation. The x-axis represents days post-irradition and the y-axis represents the ratio of surviving mice to the total number of irradiated mice. Line -■- represents mice (n=10) treated with Compound **3A**, administered at a dose of 10 mg/kg, ten minutes post-irradiation, followed by repeated administration of 10 mg/kg doses every 12 hours for a time period of 30 days. Line -□- represents a non-treated control group of mice (n=10).

FIG. 4 shows the effect of Compound **3** on mitochondrial respiration in human A549 and murine RAW cells exposed to hydrogen peroxide, peroxynitrite, superoxide generated from xanthine oxidase and hypoxanthine, or nitroxyl radical generated by Angeli's salt. The four right-hand most bar graphs represent cytotoxin plus μM of Compound **3**.

FIG. 5 shows the effect of Compound **3** on creatine kinase (CK) release, a marker of myocardial necrosis induced by left anterior descending coronary artery (LAD) occlusion and reperfusion in the rat. The three bars represent, from left to right, before ischemia, vehicle, and 6 mg/kg Compound **3**.

FIG. 6 shows the effect of Compound **3** on myocardial necrosis induced by LAD occlusion and reperfusion in the rat, where AAV represents area at risk and LV represents left ventricular. The four bars in each graph represent, from left to right, vehicle, 1 mg/kg Compound **3**, 3 mg/kg Compound **3**, and 6 mg/kg Compound **3**.

FIG. 7 shows time course of mean blood pressure (BP) and mean survival time in rats subjected to hemorrhagic shock and resuscitation. Values are shown as means ± SEM. *, $P < 0.05$ vs. vehicle treated rats.

FIG. 8 shows cardiac function in vehicle treated and Compound **3** treated rats at 1 hour after resuscitation. The three bars in each graph represent, from left to right, sham, vehicle treated rats, and Compound **3** treated (6 mg/kg, i.v.) rats. Values are means ± SEM. Sham (n = 4), vehicle (n = 8), Compound **3** (n = 7); *, $P<0.05$ vs. vehicle treated rats; #, $P<0.05$ vs. sham rats.

FIG. 9 shows plasma levels of alanine aminotransferase (ALT) and creatine (CRE) in vehicle treated and Compound **3** treated groups. The three bars in each graph represent, from left to right, sham, vehicle treated rats, and Compound **3** treated (6 mg/kg, i.v.) rats. Values are means ± SEM. Sham (n = 4), vehicle (n = 8), Compound **3** (n = 7); *, $P<0.05$ vs. vehicle treated rats; #, $P<0.05$ vs. sham rats.

FIG. 10 shows pulmonary myeloperoxidase level (MPO) in sham animals in vehicle treated and Compound **3** treated hemorrhagic shock groups. The three bars in the graph represent, from left to right, sham, vehicle treated, and Compound **3** treated rats. Values are means ± SEM. Sham (n = 4), vehicle (n = 8), Compound **3** (n = 7); *, $P<0.05$ vs. vehicle treated rats; #, $P<0.05$ vs. sham rats.

FIG. 11 shows cardiac hypertrophy (heart weight (HW)/body weight (BW) ratio, or left ventricular weight (LVW)/body weight (BW) ratio) in sham and banded animals with and without Compound **3** treatment for 2 months. The left-hand most set of bars in each graph represents vehicle treated and the right-hand most set of bars in each graph represents Compound **3** treated. Values are means ± SEM. Sham (n = 4), vehicle (n = 8), Compound **3** (n = 7); *, P<0.05 vs. vehicle treated rats; #, P<0.05 vs. sham rats.

FIG. 12 shows the effect of Compound **3** at 0.3 mg/kg/day, i.p., and low-dose cyclosporine (2.5 mg/kg i.p.) on rat allografts. A: untreated, B: low-dose cyclosporine (2.5 mg/kg), C: Compound **3** at 0.3 mg/kg/day, D: Compound **3** at 1 mg/kg/day, E: Combination of Compound **3** at 0.3 mg/kg/day and cyclosporine at 2.5 mg/kg/day.

FIG. 13 shows the effect of Compound **3** at 1 mg/kg b.i.d., s.c., on a balloon-induced vascular injury in the rat. Line -♦- represents control right (control) side, n = 6. Line -◇- represents control left (injured) side, n = 5. Line -■- represents Compound **3** right (control) side, n = 7.5. Line -□- represents Compound **3** left (injured) side, n = 4.5.

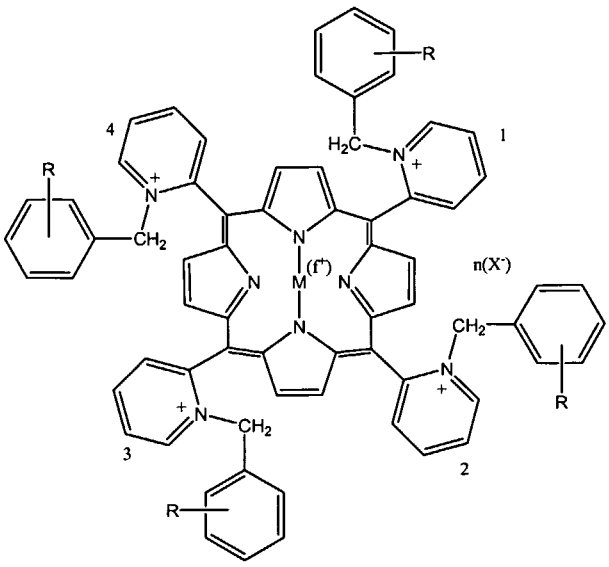
FIG. 14 shows the effect of Compound **3** at 1 mg/kg b.i.d., s.c., on balloon-induced vascular injury in the rat (n = 4-7). The left bar indicates control and the right bar indicates Compound **3**.

FIG. 15 shows the effect of Compound **3** (3 and 10 mg/kg/day i.p.) on streptozotocin (STZ)-induced hyperglycemia (left and middle panels) and loss of pancreatic insulin content (right panel), (n = 20).

5. DETAILED DESCRIPTION OF THE INVENTION

5.1 PYRIDYL-SUBSTITUTED PORPHYRIN COMPOUNDS OF FORMULA (A)

As stated above, the present invention encompasses Pyridyl-Substituted Porphyrin Compounds of Formula (A)



(A)

wherein M, f, R, X^- and n are defined above.

In one embodiment M is Fe.

In another embodiment, M is Mn.

In one embodiment, f is 1.

In another embodiment, f is 0.

In one embodiment, X^- is Cl^- or Br^-.

In one embodiment, X^- is $CH_3C(O)O^-$, 2-methylbenzoate, 3-methylbenzoate, or 4-methylbenzoate.

In one embodiment, an X^- forms a bond with M.

In one embodiment, an X^- that forms a bond with M is the same as an X^- that does not form a bond with M.

In one embodiment, an X^- that forms a bond with M is different from an X^- that does not form a bond with M.

In one embodiment, an X^- that does not form a bond with M is different from another X^- that does not form a bond with M.

In another embodiment, each X^- is independently F^-, Cl^-, Br^-, I^-, HO^-, or $CH_3C(O)O^-$.

In one embodiment, each R is $-C(O)O^-$.

In another embodiment, each R is –C(O)OH.

In one embodiment, n is 0.

In one embodiment, n is 1.

In another embodiment, n is 5.

In one embodiment M is Fe, f is 1, and X^- is Cl^-.

In another embodiment, M is Fe, f is 1, X^- is Cl^-, and each occurrence of R is

$-C(O)O^-$.

In one embodiment, each R is in the *ortho* position.

In one embodiment, each R is in the *meta* position.

In one embodiment, each R is in the *para* position.

In one embodiment, the total number of –C(O)OH R groups is 4.

In another embodiment, the total number of –C(O)OH R groups is 3.

In another embodiment, the total number of –C(O)OH R groups is 2.

In a further embodiment, the total number of –C(O)OH R groups is 1.

In another embodiment, the total number of –C(O)OH R groups is 0.

In one embodiment, the Pyridyl-Substituted Porphyrin Compounds of Formula (A) are in isolated and purified form.

The Pyridyl-Subsituted Porphyrin Compounds of Formula (A) contain four pyridyl groups. Due to steric factors, each pyridyl group's nitrogen atom can exist: (1) above the plane of the porphyrin ring (this conformation is herein referred to as the β-position); or (2) below the plane of the porphyrin ring (this conformation is herein referred to as the α-position).

In certain embodiments, the Pyridyl-Subsituted Porphyrin Compounds of Formula (A) can exist in one of the following isomeric forms, denoted as Isomer Nos. 1-8, as described in the table below, or a mixture thereof, with the pyridyl groups being numbered 1-4 as shown in Formula (A):

	Pyridyl Group #			
Isomer No.	**1**	**2**	**3**	**4**
1	α	α	α	α
2	α	α	α	β
3	α	α	β	α
4	α	β	α	α
5	β	α	α	α
6	α	α	β	β
7	α	β	β	α
8	α	β	α	β

In the above table, "α" signifies that the pyridyl group's nitrogen atom is in the α-position, and "β" signifies that the pyridyl group's nitrogen atom is in the β-position.

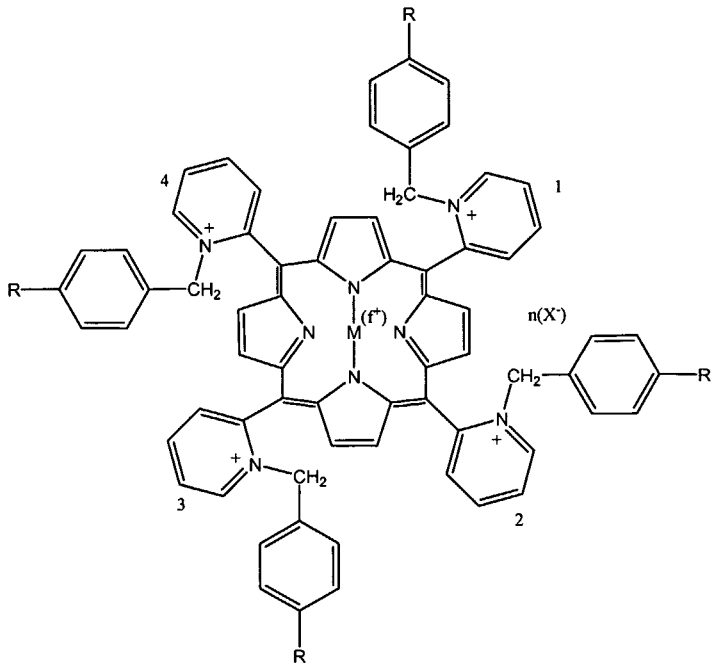
In one embodiment, the X^- that forms a bond with M exists above the plane of the porphyrin ring. In another embodiment, the X^- that forms a bond with M exists below the plane of the porphyrin ring.

In one embodiment, a Pyridyl-Subsituted Porphyrin Compound of Formula (A) is substantially free of its corresponding other isomers.

In another embodiment, a Pyridyl-Subsituted Porphyrin Compound of Formula (A) exists as a mixture of two or more isomers.

5.1.1 PYRIDYL-SUBSTITUTED PORPHYRIN COMPOUNDS OF FORMULA (I)

In one embodiment, the Pyridyl-Substituted Porphyrin Compounds have the Formula (I)



(I)

wherein M, f, R, X^- and n are defined above for the Pyridyl-Substituted Porphyrin Compounds of Formula (A).

In one embodiment M is Fe.

In another embodiment, M is Mn.

In one embodiment, f is 1.

In another embodiment, f is 0.

In one embodiment, X^- is Cl^- or Br^-.

In one embodiment, X^- is $CH_3C(O)O^-$ or 4-methylbenzoate.

In one embodiment, an X^- forms a bond with M.

In one embodiment, an X^- that forms a bond with M is the same as an X^- that does not form a bond with M.

In one embodiment, an X^- that forms a bond with M is different from an X^- that does not form a bond with M.

In one embodiment, an X^- that does not form a bond with M is different from another X^- that does not form a bond with M.

In another embodiment, each X^- is independently F^-, Cl^-, Br^-, I^-, HO^-, or $CH_3C(O)O^-$.

In one embodiment, each R is $-C(O)O^-$.

In another embodiment, each R is –C(O)OH.

In one embodiment, n is 0.

In one embodiment, n is 1.

In another embodiment, n is 5.

In one embodiment M is Fe, f is 1, and X^- is Cl^-.

In another embodiment, M is Fe, f is 1, X^- is Cl^-, and each occurrence of R is –C(O)O$^-$.

In one embodiment, the total number of –C(O)OH R groups is 4.

In another embodiment, the total number of –C(O)OH R groups is 3.

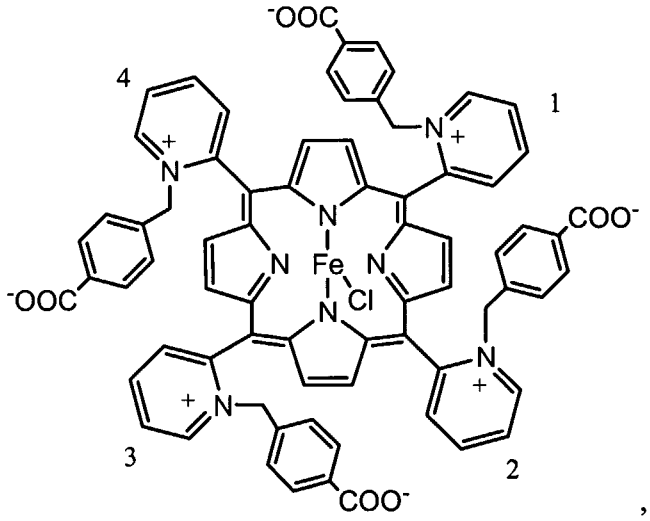
In another embodiment, the total number of –C(O)OH R groups is 2.

In a further embodiment, the total number of –C(O)OH R groups is 1.

In another embodiment, the total number of –C(O)OH R groups is 0.

In one embodiment, the Pyridyl-Substituted Porphyrin Compounds of Formula (I) are in isolated and purified form.

Illustrative examples of the compounds of Formula (I) are as set forth below:



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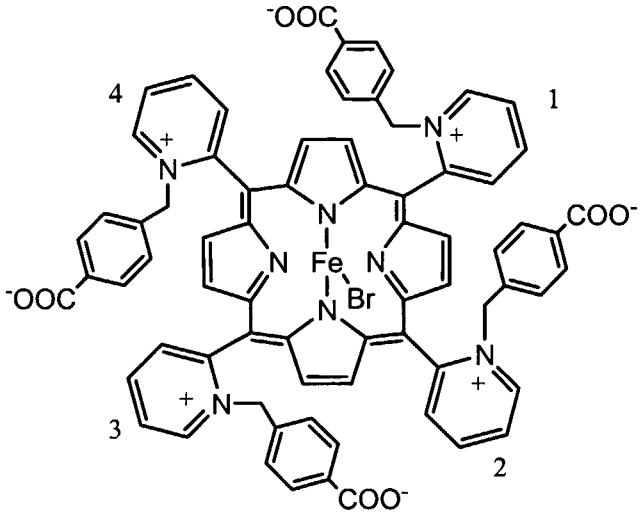

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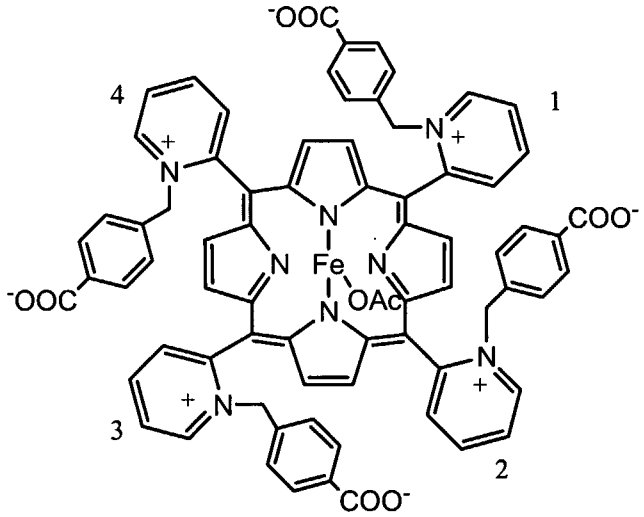
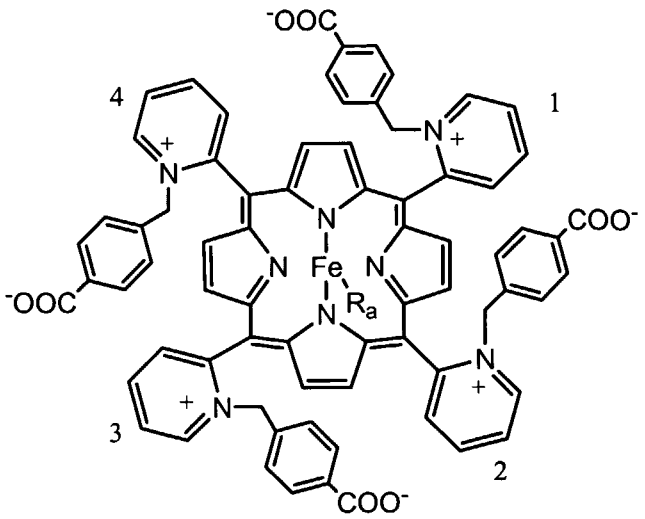


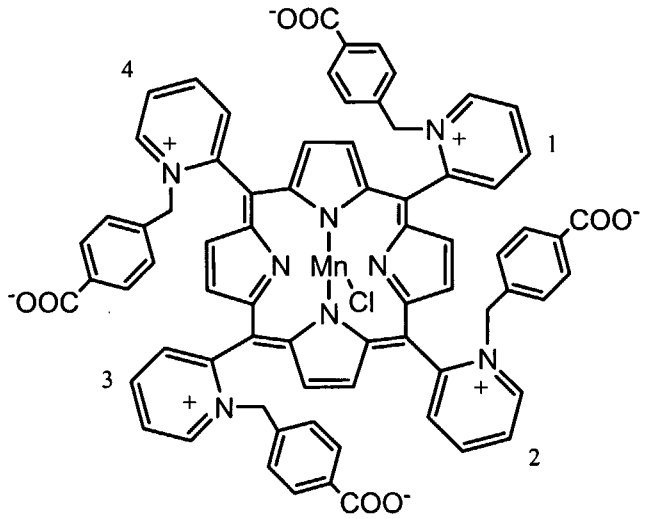
5, and



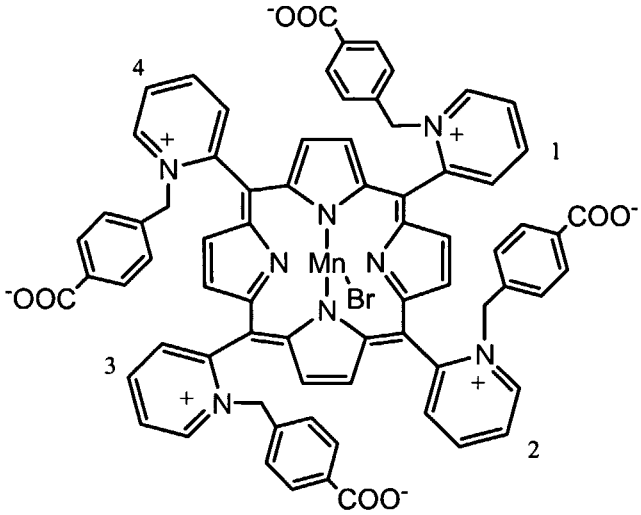
R_a = 4-methylbenzoate

23.

Additional illustrative examples of the compounds of Formula (I) are as set forth below:



6,



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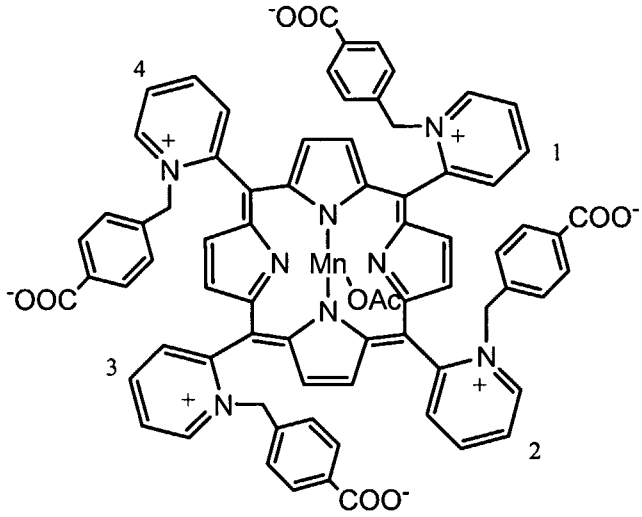
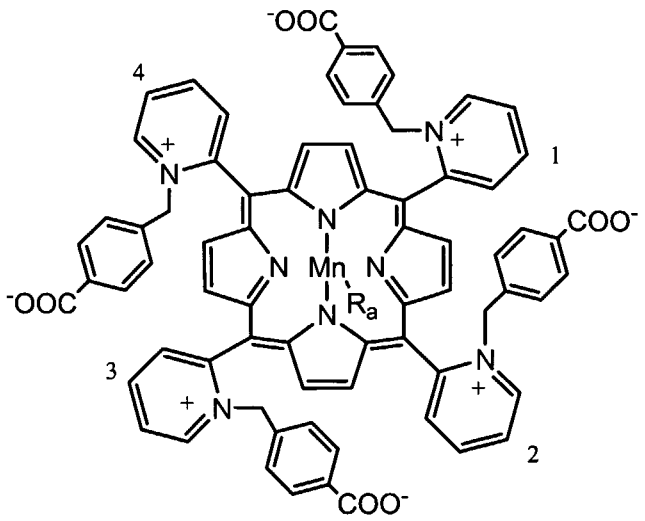


8,

and



R_a = 4-methylbenzoate

24.

The Pyridyl-Subsituted Porphyrin Compounds of Formula (I) contain four pyridyl groups. Due to steric factors, each pyridyl group's nitrogen atom can exist: (1) above the plane of the porphyrin ring (this conformation is herein referred to as the β-position); or (2) below the plane of the porphyrin ring (this conformation is herein referred to as the α-position).

In certain embodiments, the Pyridyl-Subsituted Porphyrin Compounds of Formula (I) can exist in one of the following isomeric forms, denoted as Isomer Nos. 1-8, as described in the table below, or a mixture thereof, with the pyridyl groups being numbered 1-4 as shown in Formula (I):

	Pyridyl Group #			
Isomer No.	**1**	**2**	**3**	**4**
1	α	α	α	α
2	α	α	α	β
3	α	α	β	α
4	α	β	α	α
5	β	α	α	α
6	α	α	β	β
7	α	β	β	α
8	α	β	α	β

In the above table, "α" signifies that the pyridyl group's nitrogen atom is in the α-position, and "β" signifies that the pyridyl group's nitrogen atom is in the β-position.

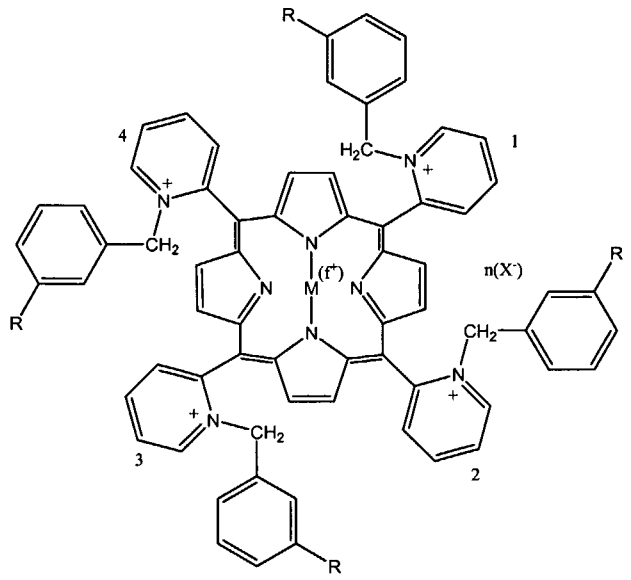
In one embodiment, the X^- that forms a bond with M exists above the plane of the porphyrin ring. In another embodiment, the X^- that forms a bond with M exists below the plane of the porphyrin ring.

In one embodiment, a Pyridyl-Subsituted Porphyrin Compound of Formula (I) is substantially free of its corresponding other isomers.

In another embodiment, a Pyridyl-Subsituted Porphyrin Compound of Formula (I) exists as a mixture of two or more isomers.

5.1.2 PYRIDYL-SUBSTITUTED PORPHYRIN COMPOUNDS OF FORMULA (II)

In one embodiment, the Pyridyl-Substituted Porphyrin Compounds have the Formula (II)



(II)

wherein M, f, R, X^- and n are defined above for the Pyridyl-Substituted Porphyrin Compounds of Formula (A).

In one embodiment M is Fe.

In another embodiment, M is Mn.

In one embodiment, f is 1.

In another embodiment, f is 0.

In one embodiment, X^- is Cl^- or Br^-.

In one embodiment, X^- is $CH_3C(O)O^-$ or 3-methylbenzoate.

In one embodiment, an X^- forms a bond with M.

In one embodiment, an X^- that forms a bond with M is the same as an X^- that does not form a bond with M.

In one embodiment, an X^- that forms a bond with M is different from an X^- that does not form a bond with M.

In one embodiment, an X^- that does not form a bond with M is different from another X^- that does not form a bond with M.

In another embodiment, each X^- is independently F^-, Cl^-, Br^-, I^-, HO^-, or $CH_3C(O)O^-$.

In one embodiment, each R is $-C(O)O^-$.

In another embodiment, each R is $-C(O)OH$.

In one embodiment, n is 0.

In one embodiment, n is 1.

In another embodiment, n is 5.

In one embodiment M is Fe, f is 1, and X^- is Cl^-.

In another embodiment, M is Fe, f is 1, X^- is Cl^-, and each occurrence of R is –C(O)O⁻.

In one embodiment, the total number of –C(O)OH R groups is 4.

In another embodiment, the total number of –C(O)OH R groups is 3.

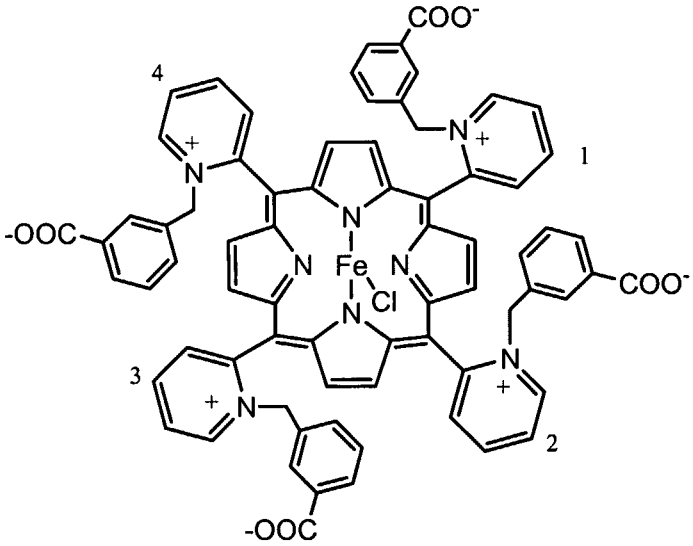
In another embodiment, the total number of –C(O)OH R groups is 2.

In a further embodiment, the total number of –C(O)OH R groups is 1.

In another embodiment, the total number of –C(O)OH R groups is 0.

In one embodiment, the Pyridyl-Substituted Porphyrin Compounds of Formula (II) are in isolated and purified form.

Illustrative examples of the compounds of Formula (II) are as set forth below:



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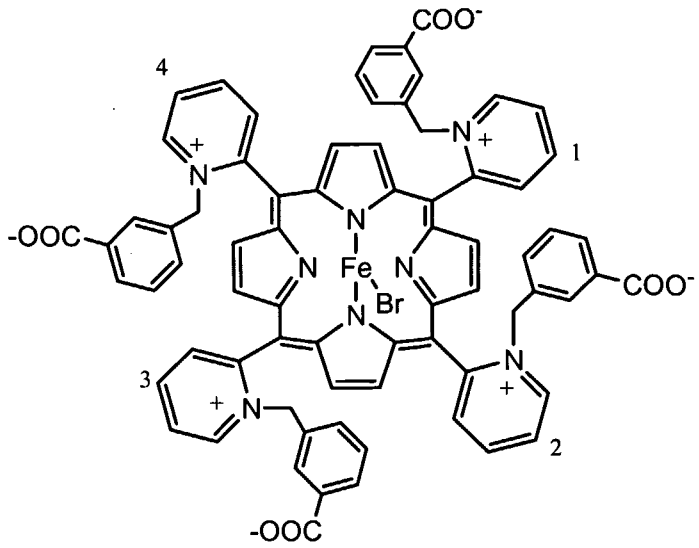

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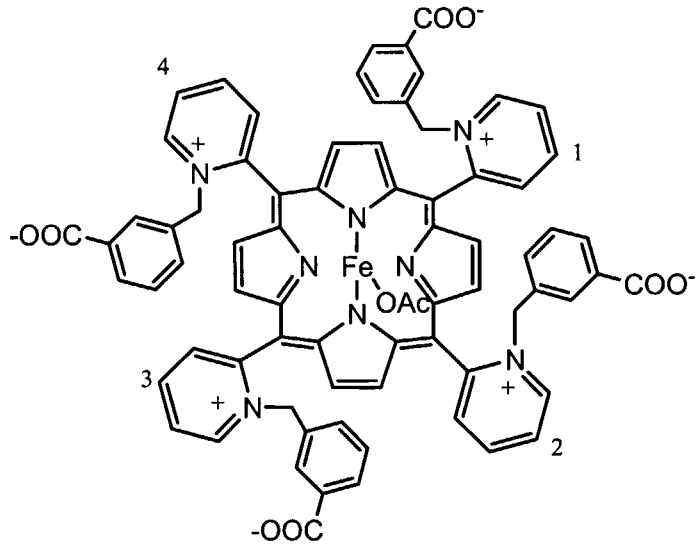

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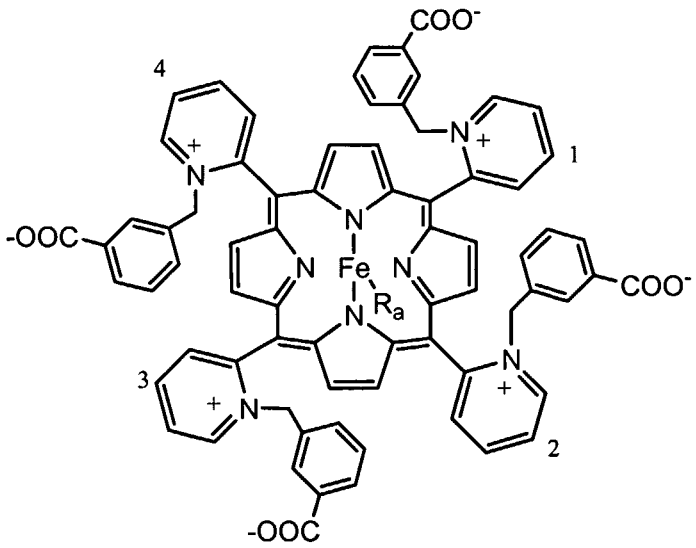
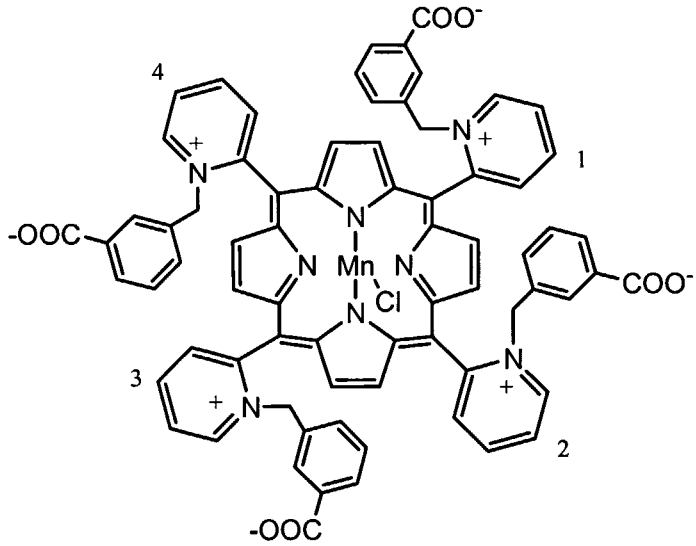


R_a = 3-methylbenzoate

25.

Additional illustrative examples of the compounds of Formula (II) are as set forth below:



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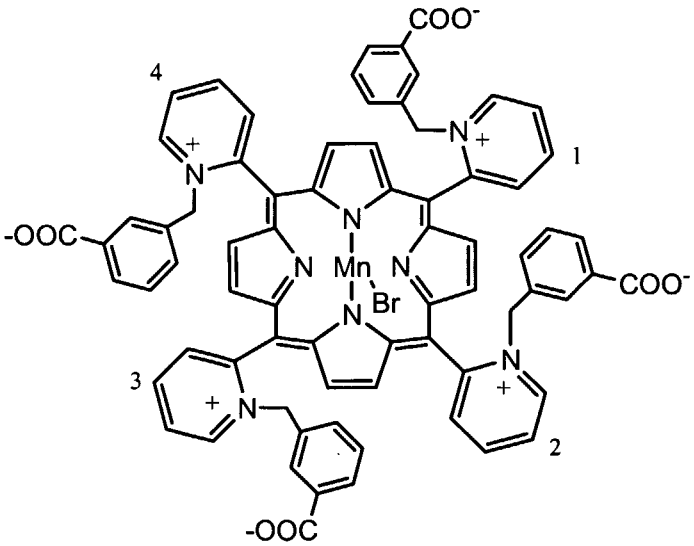
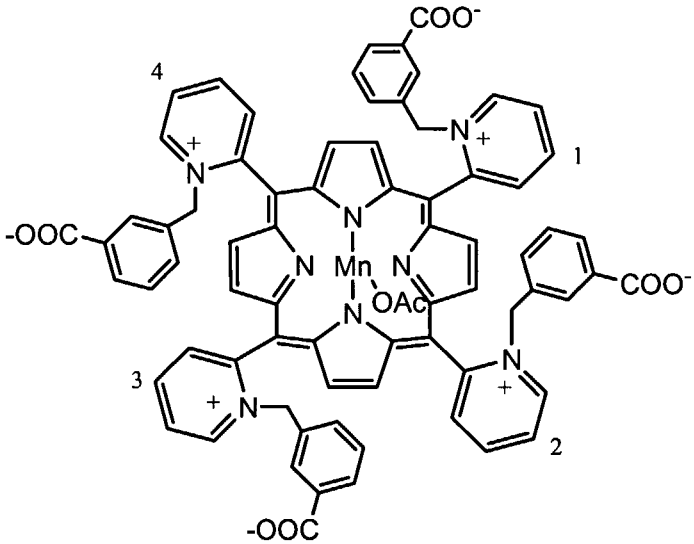
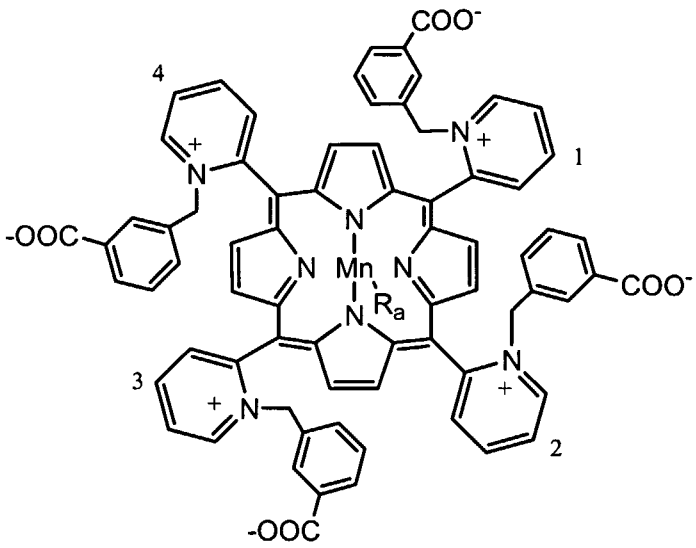

COO-
4
N+
1
N+
-OOC
N
Mn
N
N
Br
N
COO-
N
3
N+
N+
2
-OOC

13,


COO-
4
N+
1
N+
-OOC
N
Mn
N
N
OAc
N
COO-
N
3
N+
N+
2
-OOC

14,

and



R_a = 3-methylbenzoate

26.

The Pyridyl-Subsituted Porphyrin Compounds of Formula (II) contain four pyridyl groups. Due to steric factors, each pyridyl group's nitrogen atom can exist: (1) above the plane of the porphyrin ring (this conformation is herein referred to as the β-position); or (2) below the plane of the porphyrin ring (this conformation is herein referred to as the α-position).

In certain embodiments, the Pyridyl-Subsituted Porphyrin Compounds of Formula (II) can exist in one of the following isomeric forms, denoted as Isomer Nos. 1-8, as described in the table below, or a mixture thereof, with the pyridyl groups being numbered 1-4 as shown in Formula (I):

	Pyridyl Group #			
Isomer No.	**1**	**2**	**3**	**4**
1	α	α	α	α
2	α	α	α	β
3	α	α	β	α
4	α	β	α	α
5	β	α	α	α
6	α	α	β	β
7	α	β	β	α
8	α	β	α	β

In the above table, "α" signifies that the pyridyl group's nitrogen atom is in the α-position, and "β" signifies that the pyridyl group's nitrogen atom is in the β-position.

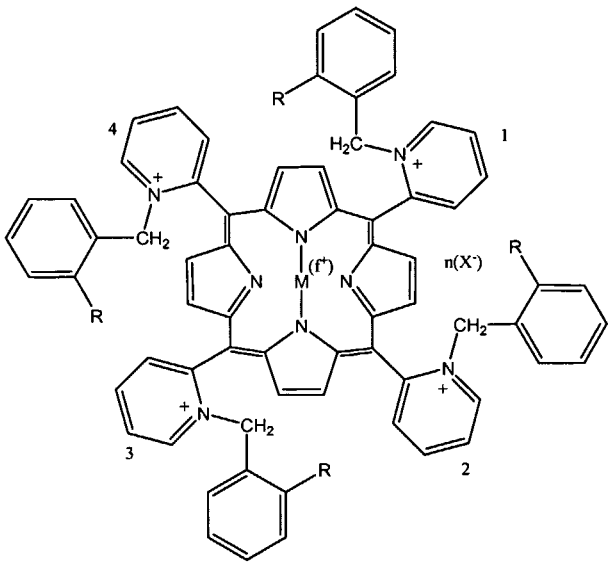
In one embodiment, the X^- that forms a bond with M exists above the plane of the porphyrin ring. In another embodiment, X^- that forms a bond with M exists below the plane of the porphyrin ring.

In one embodiment, a Pyridyl-Subsituted Porphyrin Compound of Formula (II) is substantially free of its corresponding other isomers.

In another embodiment, a Pyridyl-Subsituted Porphyrin Compound of Formula (II) exists as a mixture of two or more isomers.

5.1.3 PYRIDYL-SUBSTITUTED PORPHYRIN COMPOUNDS OF FORMULA (III)

In one embodiment, the Pyridyl-Substituted Porphyrin Compounds have the Formula (III)



(III)

wherein M, f, R, X^- and n are defined above for the Pyridyl-Substituted Porphyrin Compounds of Formula (A).

In one embodiment M is Fe.

In another embodiment, M is Mn.

In one embodiment, f is 1.

In another embodiment, f is 0.

In one embodiment, X^- is Cl^- or Br^-.

In one embodiment, X^- is $CH_3C(O)O^-$ or 2-methylbenzoate.

In one embodiment, an X^- forms a bond with M.

In one embodiment, an X^- that forms a bond with M is the same as an X^- that does not form a bond with M.

In one embodiment, an X^- that forms a bond with M is a different from an X^- that does not form a bond with M.

In one embodiment, an X^- that does not form a bond with M is different from another X^- that does not form a bond with M.

In another embodiment, each X^- is independently F^-, Cl^-, Br^-, I^-, HO^-, or $CH_3C(O)O^-$.

In one embodiment, each R is $-C(O)O^-$.

In another embodiment, each R is $-C(O)OH$.

In one embodiment, n is 0.

In one embodiment, n is 1.

In another embodiment, n is 5.

In one embodiment M is Fe, f is 1, and X^- is Cl^-.

In another embodiment, M is Fe, f is 1, X^- is Cl^-, and each occurrence of R is –C(O)O$^-$.

In one embodiment, the total number of –C(O)OH R groups is 4.

In another embodiment, the total number of –C(O)OH R groups is 3.

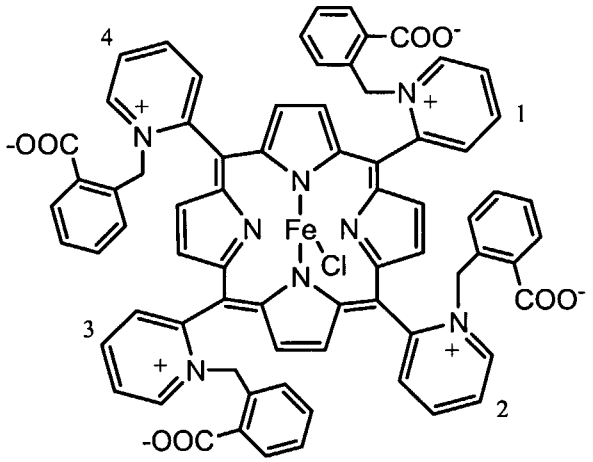
In another embodiment, the total number of –C(O)OH R groups is 2.

In a further embodiment, the total number of –C(O)OH R groups is 1.

In another embodiment, the total number of –C(O)OH R groups is 0.

In one embodiment, the Pyridyl-Substituted Porphyrin Compounds of Formula (III) are in isolated and purified form.

Illustrative examples of the compounds of Formula (III) are as set forth below:



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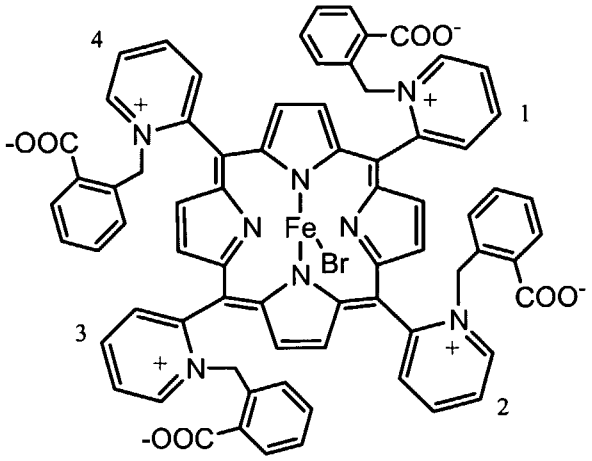

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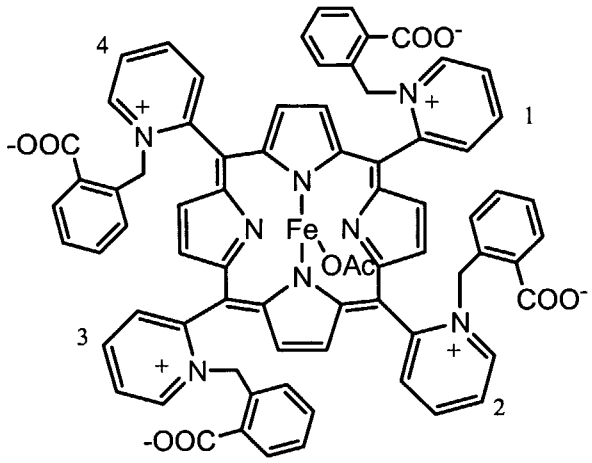

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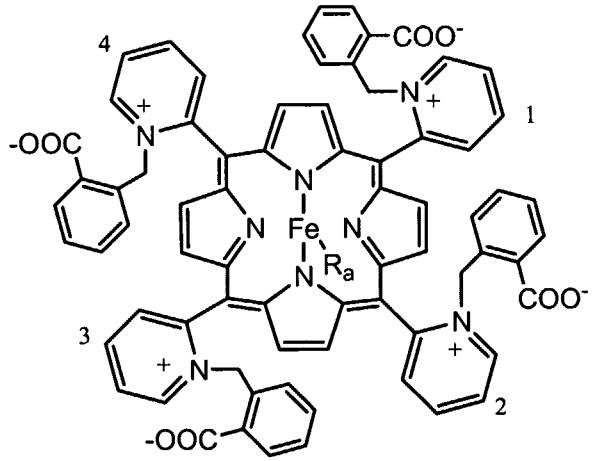
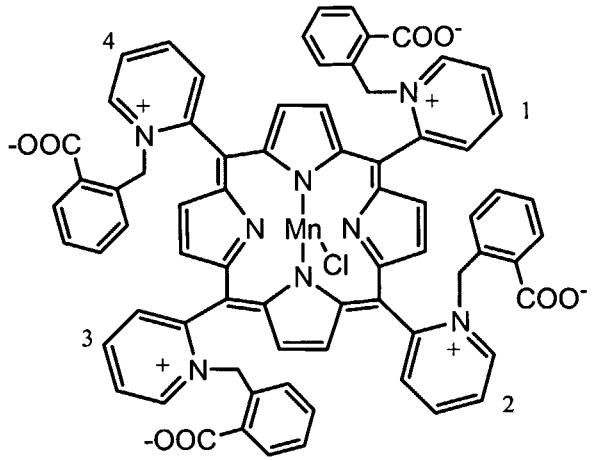


R_a = 2-methylbenzoate

Additional illustrative examples of the compounds of Formula (III) are as set forth below:



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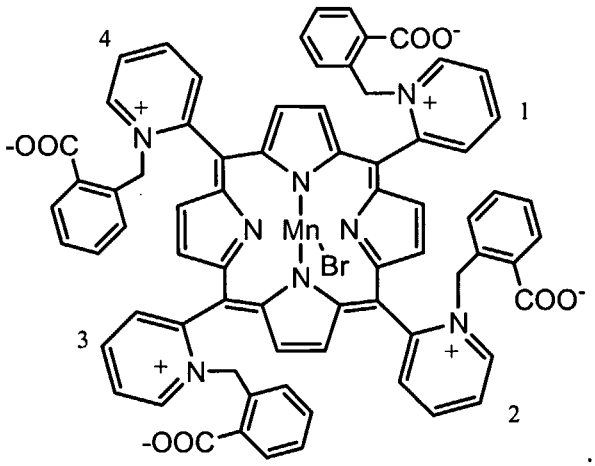

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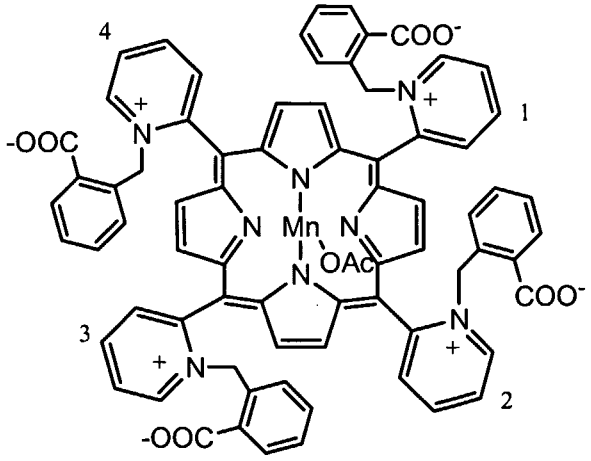

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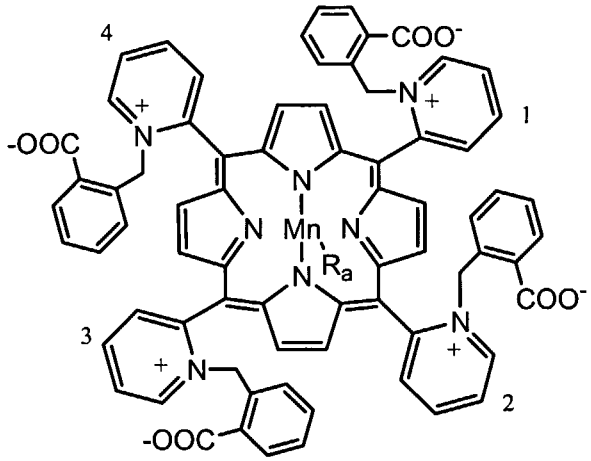
and



R_a = 2-methylbenzoate

28.

The Pyridyl-Subsituted Porphyrin Compounds of Formula (III) contain four pyridyl groups. Due to steric factors, each pyridyl group's nitrogen atom can exist: (1) above the plane of the porphyrin ring (this conformation is herein referred to as the β-position); or (2) below the plane of the porphyrin ring (this conformation is herein referred to as the α-position).

In certain embodiments, the Pyridyl-Subsituted Porphyrin Compounds of Formula (I) can exist in one of the following isomeric forms, denoted as Isomer Nos. 1-8, as described in the table below, or a mixture thereof, with the pyridyl groups being numbered 1-4 as shown in Formula (III):

	Pyridyl Group #			
Isomer No.	**1**	**2**	**3**	**4**
1	α	α	α	α
2	α	α	α	β
3	α	α	β	α
4	α	β	α	α
5	β	α	α	α
6	α	α	β	β
7	α	β	β	α
8	α	β	α	β

In the above table, "α" signifies that the pyridyl group's nitrogen atom is in the α-position, and "β" signifies that the pyridyl group's nitrogen atom is in the β-position.

In one embodiment, the X^- that forms a bond with M exists above the plane of the porphyrin ring. In another embodiment, the X^- that forms a bond with M exists below the plane of the porphyrin ring.

In one embodiment, a Pyridyl-Subsituted Porphyrin Compound of Formula (III) is substantially free of its corresponding other isomers.

In another embodiment, a Pyridyl-Subsituted Porphyrin Compound of Formula (III) exists as a mixture of two or more isomers.

5.2 DEFINITIONS

As used herein, the terms used above and below have the following meaning:

The term "subject," as used herein, includes, but is not limited to, a cow, monkey, horse, sheep, pig, chicken, turkey, quail, cat, dog, mouse, rat, rabbit, guinea pig and human. In one embodiment, a subject is a human.

Illustrative counterions include but are not limited to, sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate,

gluconate, glucaronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, *p*-toluenesulfonate, pamoate (*i.e.*, 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)), camphorsulfonate, 2-methylbenzoate, 3-methylbenzoate, and 4-methylbenzoate counterions.

The term "effective amount" when used in connection with a Pyridyl-Substituted Porphyrin Compound is an amount that is effective to treat or prevent a Condition in a subject.

The term "isolated and purified" as used herein means separated from other components of a reaction mixture or natural source. In certain embodiments, the isolate contains at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 98% of a Pyridyl-Substituted Porphyrin Compound by weight of the isolate. In one embodiment, the isolate contains at least 95% of a Pyridyl-Substituted Porphyrin Compound by weight of the isolate.

The term "is substantially free of its corresponding other isomers" as used herein means no more than about 10% by weight of its corresponding other isomers; in one embodiment, no more than about 5% by weight, in another embodiment, no more than about 2% by weight, in another embodiment, no more than about 1% by weight, and in another embodiment, no more than about 0.1% by weight of its corresponding other isomers.

The term "reactive species" as used herein means a species that can injure a cell or tissue. Exemplary reactive species include oxidants and free radicals. Further exemplary reactive species include a reactive oxygen species, such as superoxide or peroxide, and a reactive nitrogen species, such as $^{-}$ONOO, nitric oxide, $NO^{\cdot}$, NOH, or ONO.

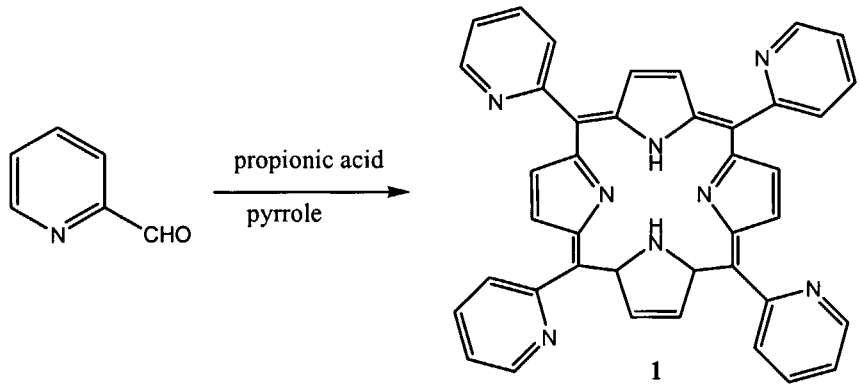
In the Pyridyl-Substituted Porphyrin Compounds it is to be understood that the number of R groups where each R is –C(O)OH is an integer ranging from 0 to 4. Accordingly, n is the sum of f and an integer ranging from 0 to 4. It is to be further understood that n=f when all four R groups are $-C(O)O^{-}$. Whether each R group is $-C(O)O^{-}$ or -C(O)OH can vary due to factors including pH.

5.3 METHODS FOR MAKING THE PYRIDYL-SUBSTITUTED PORPHYRIN COMPOUNDS

The Pyridyl-Substituted Porphyrin Compounds can be made using conventional organic synthesis or by the following illustrative methods shown in Schemes 1-4 below.

Scheme 1 below illustrates a procedure useful for synthesizing porphyrin intermediate **1**, which is useful for making the Pyridyl-Substituted Porphyrin Compounds of Formula (I).

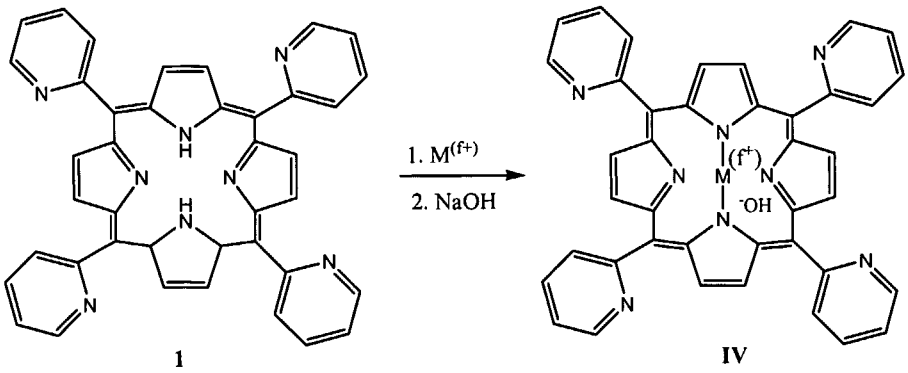
Scheme 1



Pyridine-2-carboxaldehyde can be reacted with propionic acid and pyrrole in the presence of about 10% xylene or toluene at a temperature of from about 120 °C to reflux, for example at a temperature in the range of from about 130 °C to about 140 °C, to provide the pyridyl porphyrin **1**, which is useful for making the Pyridyl-Substituted Porphyrin Compounds of Formula (A).

Scheme 2 below illustrates a method useful for making the hydroxymetallo- porphyrin intermediates of Formula (IV), which are useful for making the Pyridyl-Substituted Porphyrin Compounds of Formula (I) wherein f is 1 and M is defined above for the Pyridyl-Substituted Porphyrin Compounds of Formula (I).

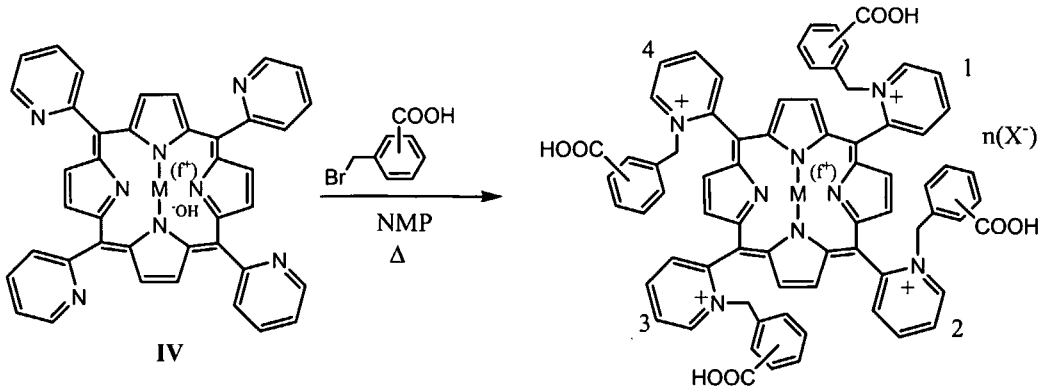
Scheme 2



The porphyrin intermediate **1** can be reacted with a metallating agent in refluxing hydrochloric acid to form a metallated porphyrin complex that can be treated at room temperature with a hydroxide base, such as sodium hydroxide or potassium hydroxide, to provide the hydroxy-metallated porphyrin intermediates of Formula (IV). Metallating agents useful in the method of Scheme 2 include, but are not limited to, ferrous chloride, ferric chloride, ferric sulfate, ferrous acetate, ferrous ammonium sulfate, manganese(III) acetate, manganese(II) acetate, and manganese(II) chloride.

Scheme 3 below shows a method for making the Pyridyl-Substituted Porphyrin Compounds of Formula (A) wherein R is –COOH; n is 4 or 5; and M, f and X^- are as defined above for the Pyridyl-Substituted Porphyrin Compounds of Formula (A).

Scheme 3

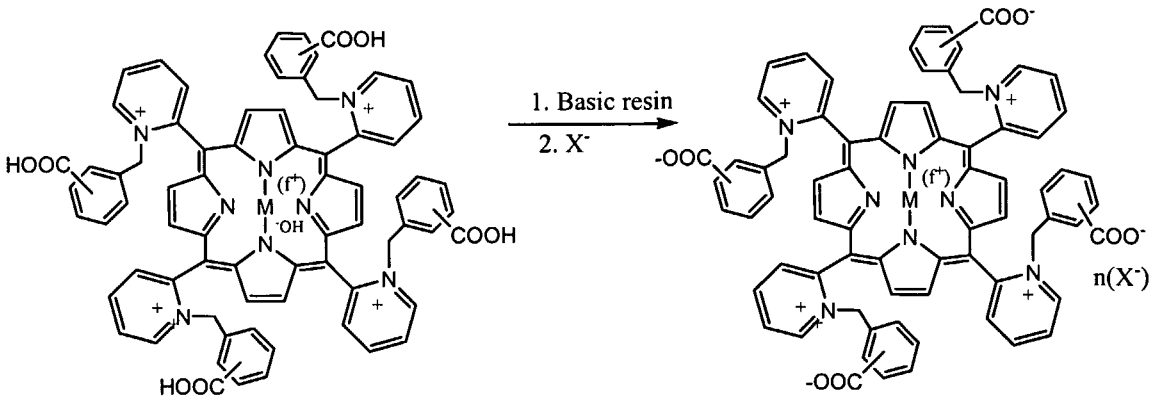


Pyridyl-Substituted Porphyrin Compounds of Formula (A) wherein R is -COOH

The pyridyl groups of the hydroxy-metallated porphyrin intermediates of Formula (IV) can be N-benzylated using excess α-bromo toluic acid in N-methylpyrrolidinone (NMP) at elevated temperature (about 50 °C – 130 °C). This method provides Pyridyl-Substituted Porphyrin Compounds of Formula (A) wherein R = -COOH; n = f; X^- is Br^-; and M, f, and n are as defined above for the Pyridyl-Substituted Porphyrin Compounds of Formula (A).

Scheme 4 below shows a method for ion exchange of a Pyridyl-Substituted Porphyrin Compound of Formula (A) having a Br^- metal counterion. This method is useful for making Pyridyl-Substituted Porphyrin Compounds of Formula (A) wherein R is $-COO^-$; n = f; X^- is other than Br^-; and M, f, and n are as defined above for the Pyridyl-Substituted Porphyrin Compounds of Formula (A).

Scheme 4



Pyridyl-Substituted Porphyrin Compounds of Formula (A) wherein R is -COOH, f is 1 and X- is OH-

Pyridyl-Substituted Porphyrin Compounds of Formula (A) wherein R is -COO-

The Pyridyl-Substituted Porphyrin Compounds of Formula (A) wherein R is –COOH can be further derivatized by deprotonation of the carboxylic acid units using a basic resin (e.g., Dowex Marathon WBA-2 resin), followed by counterion exchange using a negative counterion source, including, but not limited to, an alkali metal halide; or a resin that can act as a source of a negative counterion, such as Amberlite IRA-402 chloride resin, to provide the Pyridyl-Substituted Porphyrin Compounds of Formula (A) wherein R is $–COO^-$ and X^- is other than Br^-.

If desired, the Pyridyl-Substituted Porphyrin Compounds of Formula (A) can be purified using methods well-known to one of ordinary skill in the relevant art including, but not limited to, flash column chromatography, high-performance liquid chromatograpy (HPLC), medium-pressure liquid chromatography (MPLC), preparative thin-layer chromatograpy, anion-exchange chromatography, and recrystallization.

5.4 THERAPEUTIC USES OF THE PYRIDYL-SUBSTITUTED PORPHYRIN COMPOUNDS

In accordance with the invention, the Pyridyl-Substituted Porphyrin Compounds can be administered to a subject in need of treatment or prevention of a Condition.

In one embodiment, the Pyridyl-Substituted Porphyrin Compounds treat or prevent a Condition by scavenging or neutralizing one or more reactive species that are generated *in vivo* due to the interaction of ionizing radiation with a subject's tissue. Such reactive species include, but are not limited to, reactive oxygen species, including superoxides and peroxides; and reactive nitrogen species, including $^{-}$ONOO, nitric oxide, and nitroxyl species, such as NO^{-}, NOH, or ONO.

5.4.1 TREATMENT OR PREVENTION OF INJURY DUE TO EXPOSURE TO A REACTIVE SPECIES

The Pyridyl-Substituted Porphyrin Compounds can be used to treat or prevent cell or tissue injury due to exposure to a reactive species. In one embodiment, the reactive species is an oxidant or a free radical, including, but not limited to reactive oxygen species, such as superoxides and peroxides, and reactive nitrogen species, such as $^{-}$ONOO, nitric oxide, and nitroxyl species, such as NO^{-}, NOH, and ONO.

Examples of injury due to exposure to a reactive species are skin wrinkling, skin aging, sunburn erythema, UV-induced skin injury, and UV-induced skin disease.

5.4.2 PROLONGING THE HALF-LIFE OF AN OXIDATION-PRONE COMPOUND

The Pyridyl-Substituted Porphyrin Compound can be used to prolong the half-life of an oxidation-prone compound *in vivo*. In another embodiment, a Pyridyl-Substituted Porphyrin Compound can be administered to a subject in combination with an oxidation-prone drug or biomaterial to treat or prevent oxidative injury due to, or biodegradation of, the oxidation-prone drug or biomaterial *in vivo* or *in vitro*. In one embodiment, the oxidation-prone drug or biomaterial is hyaluronic acid.

5.4.3 TREATMENT OR PREVENTION OF ERECTILE DYSFUNCTION DUE TO SURGERY

The Pyridyl-Substituted Porphyrin Compounds can be used to treat or prevent erectile dysfunction caused by surgery. In one embodiment, the surgery is surgery of the prostate or the colon.

5.4.4 TREATMENT OR PREVENTION OF LUNG DISEASE

The Pyridyl-Substituted Porphyrin Compounds can be used to treat or prevent a lung disease. In one embodiment, the lung disease is cystic fibrosis, hyperoxic lung injury, emphysema, or adult respiratory distress syndrome.

5.4.5 TREATMENT OR PREVENTION OF HYPEROXIA

The Pyridyl-Substituted Porphyrin Compounds can be used to treat or prevent injury due to hyperoxia. In one embodiment, the injury due to hyperoxia is hyperoxia-induced eye injury or hyperoxia-induced lung injury.

5.4.6 TREATMENT OR PREVENTION OF NEURODEGENERATIVE DISEASE

The Pyridyl-Substituted Porphyrin Compounds can be used to treat or prevent a neurodegenerative disease. In one embodiment, the neurodegenerative disease is Parkinson's disease, Alzheimer's disease, Huntington's disease, or amyotrophic lateral sclerosis.

5.4.7 TREATMENT OR PREVENTION OF LIVER DISEASE

The Pyridyl-Substituted Porphyrin Compound can be used to treat or prevent a liver disease. In one embodiment, the liver disease is hepatitis, liver failure, or drug-induced liver injury.

5.4.8 PROTECTING A SUBJECT'S HEART AGAINST MYOCARDIAL DAMAGE DURING CARDIOPLEGIA

In one embodiment, the invention provides methods for inducing cardioplegia comprising administering to a subject in need thereof an effective amount of a cardioplegia-inducing agent and a Pyridyl-Substituted Porphyrin Compound. Cardioplegia-inducing agents useful in the present invention include, but are not limited to, potassium chloride, procaine, lidocaine, novocaine, bupivocaine, nicorandil, pinacidil, halothane, St. Thomas solution, Fremes solution, 2,3-butanedione monoxime, or esmolol.

In one embodiment, the cardioplegia-inducing agent is lidocaine.

In one embodiment, a cardioplegia-inducing agent and a Pyridyl-Substituted Porphyrin Compound are present within the same composition. The present methods for inducing cardioplegia are useful for preventing or minimizing myocardial damage from occurring during cardioplegia.

In still another embodiment, the invention provides methods for protecting a subject's heart against myocardial damage during cardioplegia, the method comprising administering to an animal in need thereof an effective amount of:

(a) a cardioplegia-inducing agent; and

(b) a Pyridyl-Substituted Porphyrin Compound.

In one embodiment, the cardioplegia-inducing agent is administered prior to the administration of the Pyridyl-Substituted Porphyrin Compound.

In another embodiment, Pyridyl-Substituted Porphyrin Compound is administered prior to the administration of the cardioplegia-inducing agent.

In a further embodiment, the cardioplegia-inducing agent and the Pyridyl-Substituted Porphyrin Compound are administered concurrently.

In another embodiment, the cardioplegia-inducing agent and the Pyridyl-Substituted Porphyrin Compound are administered such that the Pyridyl-Substituted Porphyrin Compound exerts its prophylactic effect of protection against myocardial damage while the cardioplegia-inducing agent exerts its cardioplegic effect.

5.4.9 TREATMENT OR PREVENTION OF AN INFLAMMATORY CONDITION

The Pyridyl-Substituted Porphyrin Compounds can be used to treat or prevent an inflammatory condition. Inflammatory conditions can arise where there is an inflammation of the body tissue. Examples of inflammatory conditions treatable or preventable using the Pyridyl-Substituted Porphyrin Compounds include, but are not limited to, transplant rejection; chronic inflammatory disorders of the joints, such as arthritis, rheumatoid arthritis, osteoarthritis and bone diseases associated with increased bone resorption; inflammatory bowel diseases such as ileitis, ulcerative colitis, Barrett's syndrome, and Crohn's disease; inflammatory lung disorders such as asthma, adult respiratory distress syndrome (ARDS), and chronic obstructive airway disease; inflammatory disorders of the eye such as corneal dystrophy, trachoma, onchocerciasis, uveitis, sympathetic ophthalmitis and endophthalmitis; chronic

inflammatory disorders of the gum, such as gingivitis and periodontitis; tuberculosis; leprosy; inflammatory diseases of the kidney such as uremic complications, glomerulonephritis and nephrosis; inflammatory disorders of the skin such as sclerodermatitis, psoriasis and eczema; inflammatory diseases of the central nervous system, such as chronic demyelinating diseases of the nervous system, multiple sclerosis, AIDS-related neurodegeneration and Alzheimer's disease, infectious meningitis, encephalomyelitis, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis and viral or autoimmune encephalitis; autoimmune diseases such as diabetes mellitus, immune-complex vasculitis, systemic lupus erythematosus (SLE); inflammatory diseases of the heart such as cardiomyopathy, ischemic heart disease hypercholesterolemia, and atherosclerosis; as well as inflammation resulting from various diseases such as preeclampsia, chronic liver failure, brain and spinal cord trauma, and cancer. The Pyridyl-Substituted Porphyrin Compounds can also be used to treat or prevent reduce the progression of an inflammatory condition and/or to reduce the symptoms of the inflammatory condition. In one embodiment, the Pyridyl-Substituted Porphyrin Compounds are useful for treating or preventing pain associated with an inflammatory condition.

The inflammatory condition treatable or preventable by administration of an effective amount of a Pyridyl-Substituted Porphyrin Compound can also be a systemic inflammation of the body. Examples of systemic inflammation include but are not limited to, gram-positive or gram negative shock, sepsis, septic shock, hemorrhagic or anaphylactic shock, (SIRS), or shock induced by cancer chemotherapy in response to a pro-inflammatory cytokine such as IL-2, interferon-γ, or GM-CSF.

In one embodiment, the inflammatory condition is circulatory shock, sepsis, systemic inflammatory response syndrome, hemorrhagic shock, cardiogenic shock, or systemic inflammation induced by an anticancer immunotherapy such as IL-2.

In one embodiment, a Pyridyl-Substituted Porphyrin Compound can be used to treat or prevent an inflammatory skin disease. In one embodiment, the inflammatory skin disease is contact dermatitis, erythema, or psoriasis.

In one embodiment, the inflammatory condition is in a cell or tissue that is exposed to a reactive species.

5.4.10 TREATMENT OR PREVENTION OF A REPERFUSION INJURY

A reperfusion injury can be treated or prevented by administration of an effective amount of a Pyridyl-Substituted Porphyrin Compound. Reperfusion injury can result following a naturally occurring episode, such as a myocardial infarction, stroke, or during a surgical procedure where blood flow in vessels is intentionally or unintentionally blocked.

Reperfusion injuries that can be treated or prevented by administering an effective amount of a Pyridyl-Substituted Porphyrin Compound include, but are not limited to, intestinal reperfusion injury, stroke, neurotrauma, neuroinjury, myocardial infarction, and reperfusion injury resulting from cardiopulmonary bypass surgery, organ transplantation surgery, thoracoabrominal aneurysm repair surgery, carotid endarerectomy surgery, or hemorrhagic shock.

In one embodiment, the reperfusion injury results from cardiopulmonary bypass surgery, thoracoabrominal aneurysm repair surgery, carotid endarerectomy surgery or hemorrhagic shock.

In one embodiment, a Pyridyl-Substituted Porphyrin Compound is administered during myocardial reperfusion. In one embodiment, the reperfusion results from cardiopulmonary bypass. In another embodiment, the reperfusion results in a myocardial infarction injury.

In one embodiment, the reperfusion injury is a reoxygenation injury resulting from surgery, particularly organ transplantation surgery.

In one embodiment, the organ transplantation is cardiac transplantation or kidney transplantation.

In another embodiment, the organ transplantation is heart transplantation, kidney transplantation, liver transplantation, or lung transplantation.

In one embodiment, the reperfusion injury is in a cell or tissue that is exposed to a reactive species.

5.4.11 TREATMENT OR PREVENTION OF AN ISCHEMIC CONDITION

An ischemic condition can be treated or prevented by administration of an effective amount of a Pyridyl-Substituted Porphyrin Compound.

Ischemic conditions that can be treated or prevented by administering an effective amount of a Pyridyl-Substituted Porphyrin Compound include, but are

not limited to, stable angina, unstable angina, myocardial ischemia, hepatic ischemia, mesenteric artery ischemia, intestinal ischemia, critical limb ischemia, chronic critical limb ischemia, erebral ischemia, acute cardiac ischemia, and an ischemic disease of the central nervous system, such as stroke or cerebral ischemia.

In one embodiment, the ischemic condition is myocardial ischemia, stable angina, unstable angina, stroke, ischemic heart disease or cerebral ischemia.

In one embodiment, the ischemic condition is in a cell or tissue that is exposed to a reactive species.

5.4.12 TREATMENT OR PREVENTION OF A CARDIOVASCULAR DISEASE

A cardiovascular disease can be treated or prevented by administration of an effective amount of a Pyridyl-Substituted Porphyrin Compound.

Cardiovascular diseases that can be treated or prevented by administering an effective amount of a Pyridyl-Substituted Porphyrin Compound include, but are not limited to, chronic heart failure, atherosclerosis, congestive heart failure, circulatory shock, cardiomyopathy, cardiac transplant, myocardial infarction, and a cardiac arrhythmia, such as atrial fibrillation, supraventricular tachycardia, atrial flutter, and paroxysmal atrial tachycardia.

In one embodiment, the cardiovascular disease is a cardiac arrhythmia, congestive heart failure, circulatory shock or cardiomyopathy.

In another embodiment, the cardiac arrhythmia is atrial fibrillation, supraventricular tachycardia, atrial flutter or paroxysmal atrial tachycardia.

In one embodiment, the cardiovascular disease is heart failure.

In another embodiment, the cardiovascular disease is balloon-induced vascular injury, coronary stenting, atherosclerosis, or restenosis.

In another embodiment, the cardiovascular disease is acute heart failure, chronic heart failure, ischemic heart failure, drug-induced heart failure, idiopathic heart failure, alcoholic heart failure, or cardiac arrhythmia.

In one embodiment, the cardiovascular disease is in a cell or tissue that is exposed to a reactive species.

5.4.13 TREATMENT OR PREVENTION OF DIABETES OR A DIABETIC COMPLICATION

Diabetes or a diabetic complication can be treated or prevented by administration of an effective amount of a Pyridyl-Substituted Porphyrin Compound.

Types of diabetes that can be treated or prevented by administering an effective amount of a Pyridyl-Substituted Porphyrin Compounds include, but are not limited to, Type I diabetes (Insulin Dependent Diabetes Mellitus), Type II diabetes (Non-Insulin Dependent Diabetes Mellitus), gestational diabetes, an insulinopathy, diabetes resulting from pancreatic disease, diabetes resulting from another endocrine disease (such as Cushing's Syndrome, acromegaly, pheochromocytoma, glucagonoma, primary aldosteronism or somatostatinoma), Type A insulin resistance syndrome, Type B insulin resistance syndrome, lipatrophic diabetes, and diabetes induced by β-cell toxins.

The Pyridyl-Substituted Porphyrin Compounds can also be used to treat or prevent a diabetic complication. Examples of diabetic complications treatable or preventable by administering an effective amount of a Pyridyl-Substituted Porphyrin Compound include, but are not limited to, diabetic cataract, glaucoma, retinopathy, nephropathy (such as microaluminuria and progressive diabetic nephropathy), polyneuropathy, gangrene of the feet, atherosclerotic coronary arterial disease, peripheral arterial disease, nonketotic hyperglycemic-hyperosmolar coma, mononeuropathy, autonomic neuropathy, a skin or mucous membrane complication (such as an infection, a shin spot, a candidal infection or necrobiosis lipoidica diabeticorumobesity), a peripheral vascular disesase, hyperlipidemia, hypertension, syndrome of insulin resistance, coronary artery disease, diabetic neuropathy, mononeuropathy, a foot ulcer, a joint disease, a fungal infection, a bacterial infection, neuropathy, angiopathy, cardiomyopathy, and erectile dysfunction.

5.4.14 TREATMENT OR PREVENTION OF A SIDE EFFECT OF CANCER CHEMOTHERAPY

A side effect of cancer chemotherapy can be treated or prevented by administration of an effective amount of a Pyridyl-Substituted Porphyrin Compound.

Examples of a side effect of cancer chemotherapy include, but are not limited to, nausea, vomiting, alopecia, myelosuppression, anorexia, neuropathy, headache, pain, dry mouth, mouth sores, bone marrow suppression,

hyperpigmentation, skin rash, fluid retention, diarrhea, cardiotoxicity, anaphylaxis, fever and chills, leucopenia, thrombocytopenia, lethargy, nephrotoxicity, ototoxicity, hot flashes, hyperglycemia, and pancreatitis.

In one embodiment, the cancer chemotherapy comprises administering a platinum-based antitumor agent. Accordingly, the present invention encompasses methods for treating or preventing a side effect resulting from administration of a platinum-based antitumor agent, comprising administering to a subject in need thereof an effective amount of a Pyridyl-Substituted Porphyrin Compound. Side effects resulting from administration of a platinum-based antitumor agent are those side effects of cancer chemotherapy listed above. In certain embodiments, platinum-based antitumor agents include, but are not limted to, cisplatin, carboplatin, aroplatin, and oxaliplatin.

In one embodiment, the cancer chemotherapy comprises admininstering doxorubicin.

In a specific embodiment, a Pyridyl-Substituted Porphyrin Compound is administered to a subject in need of treatment or prevention of a side effect of doxorubicin.

In another specific embodiment, a Pyridyl-Substituted Porphyrin Compound is administered to a subject in need of treatment or prevention of a side effect of cisplatin.

5.4.15 TREATMENT OR PREVENTION OF A RADIATION-INDUCED INJURY

A radiation-induced injury can be treated or prevented by administration of an effective amount of a Pyridyl-Substituted Porphyrin Compound to a subject.

Examples of a radiation-induced injury treatable or preventable using the present methods include, but are not limited to, an acute radiation syndrome, such as a cerebral syndrome; a gastrointestinal syndrome; a hematopoietic syndrome; acute radiation sickness; pulmonary fibrosis; radiation proctitis; neuropathy; nausea; vomiting; alopecia; pain; headache; esophageal stricture; gastric ulcer; radiation pneumonitis; and cardiomyopathy.

In one embodiment, treating a radiation-induced injury includes increasing a subject's survival time following exposure to radiation.

In another embodiment, death is an example of a radiation-induced injury that is preventable according to the present invention.

The Pyridyl-Substituted Porphyrin Compounds are also useful for protecting bystander healthy tissue from a radiation-induced injury during administration of therapeutic radiation.

A radiation-induced injury may result from exposure of a subject to ionizing radiation from numerous sources including, but not limited to, a nuclear weapon, such as an atomic bomb, a neutron bomb, or a "dirty bomb;" an industrial source, such as a nuclear power plant, a nuclear submarine, or a nuclear waste disposal site; a diagnostic or therapeutic medical or dental application, such as x-rays, CT scans, external radiation therapy, internal radiation therapy (e.g., radioactive "seed" implants used in cancer therapy). The injury might result from an accident, an act of war or terrorism, cumulative exposure at the home or workplace, or purposeful exposure during medical diagnosis or treatment.

In one embodiment, the injury is induced by radiation from a nuclear weapon.

In another embodiment, the injury is induced by radiation from a nuclear power plant.

In still another embodiment, the injury is induced by radiation from radiation therapy that the subject is receiving for the treatment of a non-radiation related disorder.

In still another embodiment, the injury is induced by radiation from radiation therapy that the subject is receiving for the treatment of cancer.

In one embodiment, the injury is induced by radiation from a radioactive material that is ingested by a subject.

In one embodiment, the radiation-induced injury is in a cell or tissue that is exposed to a reactive species.

5.4.16 TREATMENT OR PREVENTION OF CANCER

The invention encompasses methods for treating or preventing cancer, comprising administering to a subject in need thereof an effective amount of a Pyridyl-Substituted Porphyrin Compound.

Examples of cancers treatable or preventable using the Pyridyl-Substituted Porphyrin Compounds include, but are not limited to, the cancers disclosed below in Table 1 and metastases thereof.

TABLE 1

Solid tumors, including but not limited to:

fibrosarcoma
myxosarcoma
liposarcoma
chondrosarcoma
osteogenic sarcoma
chordoma
angiosarcoma
endotheliosarcoma
lymphangiosarcoma
lymphangioendotheliosarcoma
synovioma
mesothelioma
Ewing's tumor
leiomyosarcoma
rhabdomyosarcoma
colon cancer
colorectal cancer
kidney cancer
pancreatic cancer
bone cancer
breast cancer
ovarian cancer
prostate cancer
esophageal cancer
stomach cancer
oral cancer
nasal cancer
throat cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatoma

bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
uterine cancer
testicular cancer
small cell lung carcinoma
bladder carcinoma
lung cancer
epithelial carcinoma
glioma
glioblastoma multiforme
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
meningioma
skin cancer
melanoma
neuroblastoma
retinoblastoma

blood-borne cancers, including but not limited to:

acute lymphoblastic leukemia ("ALL")
acute lymphoblastic B-cell leukemia
acute lymphoblastic T-cell leukemia
acute myeloblastic leukemia ("AML")
acute promyelocytic leukemia ("APL")
acute monoblastic leukemia
acute erythroleukemic leukemia
acute megakaryoblastic leukemia
acute myelomonocytic leukemia
acute nonlymphocyctic leukemia
acute undifferentiated leukemia
chronic myelocytic leukemia ("CML")
chronic lymphocytic leukemia ("CLL")
hairy cell leukemia
multiple myeloma

acute and chronic leukemias:

lymphoblastic
myelogenous

lymphocytic
myelocytic leukemias

Lymphomas:

Hodgkin's disease
non-Hodgkin's Lymphoma
Multiple myeloma
Waldenström's macroglobulinemia
Heavy chain disease
Polycythemia vera

In one embodiment, the cancer is pancreatic cancer, colorectal cancer, mesothelioma, a malignant pleural effusion, peritoneal carcinomatosis, peritoneal sarcomatosis, renal cell carcinoma, small cell lung cancer, non-small cell lung cancer, testicular cancer, bladder cancer, breast cancer, head and neck cancer, or ovarian cancer.

In still another embodiment, the subject in need of treatment has previously undergone treatment for cancer. Such previous treatments include, but are not limited to, prior chemotherapy, radiation therapy, surgery or immunotherapy, such as cancer vaccines.

The Pyridyl-Substituted Porphyrin Compounds are also useful for the treatment or prevention of a cancer caused by a virus. For example, human papilloma virus can lead to cervical cancer (see, *e.g.*, Hernandez-Avila *et al.*, Archives of Medical Research (1997) 28:265-271), Epstein-Barr virus (EBV) can lead to lymphoma (see, *e.g.*, Herrmann *et al.*, J Pathol (2003) 199(2):140-5), hepatitis B or C virus can lead to liver carcinoma (see, *e.g.*, El-Serag, J Clin Gastroenterol (2002) 35(5 Suppl 2):S72-8), human T cell leukemia virus (HTLV)-I can lead to T-cell leukemia (see *e.g.*, Mortreux *et al.*, Leukemia (2003) 17(1):26-38), human herpesvirus-8 infection can lead to Kaposi's sarcoma (see, *e.g.*, Kadow *et al.*, Curr Opin Investig Drugs (2002) 3(11):1574-9), and Human Immune deficiency Virus (HIV) infection contribute to cancer development as a consequence of immunodeficiency (see, *e.g.*, Dal Maso *et al.*, Lancet Oncol (2003) 4(2):110-9).

The Pyridyl-Substituted Porphyrin Compounds can also be administered to prevent the progression of a cancer, including but not limited to the cancers listed in Table 1. Such prophylactic use is indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-

neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, Basic Pathology, 68-79 (2d ed. 1976). Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. For example, endometrial hyperplasia often precedes endometrial cancer and precancerous colon polyps often transform into cancerous lesions. Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. A typical metaplasia involves a somewhat disorderly metaplastic epithelium. Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a subject, can indicate the desirability of prophylactic/therapeutic administration of a Pyridyl-Substituted Porphyrin Compound. Such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, disappearance of the 250,000 dalton cell surface protein. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions that can be treated or prevented according to the present invention.

In another embodiment, fibrocystic disease (e.g., cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) that can be treated or prevented according to the present invention.

In other embodiments, cancer in a subject who exhibits one or more of the following predisposing factors for malignancy can be treated by administration of an effective amount of a Pyridyl-Substituted Porphyrin Compound: a chromosomal translocation associated with a malignancy, *e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia or t(14;18) for follicular lymphoma; familial polyposis or Gardner's syndrome; benign monoclonal gammopathy; a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern, *e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome (see Robbins and Angell, Basic Pathology, 112-112 (2d ed. 1976); and exposure to carcinogens, *e.g.*, smoking, and inhalation of or contacting with certain chemicals.

In another specific embodiment, the Pyridyl-Substituted Porphyrin Compounds are administered to a human subject to prevent progression to breast, colon, ovarian, or cervical cancer.

5.5 THERAPEUTIC/PROPHYLACTIC ADMINISTRATION AND COMPOSITIONS OF THE INVENTION

Due to their activity, the Pyridyl-Substituted Porphyrin Compounds are advantageously useful in veterinary and human medicine. As described above, the Pyridyl-Substituted Porphyrin Compounds are useful for treating or preventing a Condition in a subject in need thereof.

When administered to a subject, the Pyridyl-Substituted Porphyrin Compounds can be administered as a component of a composition that comprises a physiologically acceptable carrier or vehicle. The present compositions, which comprise a Pyridyl-Substituted Porphyrin Compound, can be administered orally.

The Pyridyl-Substituted Porphyrin Compounds of the invention can also be administered by any other convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral, rectal, and intestinal mucosa) and can be administered together with another biologically active agent. Administration can be systemic or local. Various delivery systems are known, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, capsules, and can be administered.

Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, ocular, subcutaneous, intranasal, epidural, oral, sublingual, intracerebral, intravaginal, transdermal, rectal, by inhalation, or topical, particularly to the ears, nose, eyes, or skin. In some instances, administration will result in the release of the Pyridyl-Substituted Porphyrin Compounds into the bloodstream. The mode of administration can be left to the discretion of the practitioner.

In one embodiment, the Pyridyl-Substituted Porphyrin Compounds are administered orally.

In other embodiments, it can be desirable to administer the Pyridyl-Substituted Porphyrin Compounds locally. This can be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, *e.g.*, in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository or enema, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

In certain embodiments, it can be desirable to introduce the Pyridyl-Substituted Porphyrin Compounds into the central nervous system or gastrointestinal tract by any suitable route, including intraventricular, intrathecal, and epidural injection, and enema. Intraventricular injection can be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

Pulmonary administration can also be employed, *e.g.*, by use of an inhaler of nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon oar, synthetic pulmonary surfactant. In certain embodiments, the

Pyridyl-Substituted Porphyrin Compounds can be formulated as a suppository, with traditional binders and excipients such as triglycerides.

In another embodiment the Pyridyl-Substituted Porphyrin Compounds can be delivered in a vesicle, in particular a liposome (*see* Langer, *Science* 249:1527-1533 (1990) and Treat or prevent *et al.*, *Liposomes in the Therapy of Infectious Disease and Cancer* 317-327 and 353-365 (1989)).

In yet another embodiment the Pyridyl-Substituted Porphyrin Compounds can be delivered in a controlled-release system or sustained-release system (*see, e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)). Other controlled or sustained-release systems discussed in the review by Langer, *Science* 249:1527-1533 (1990) can be used. In one embodiment a pump can be used (Langer, *Science* 249:1527-1533 (1990); Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald *et al.*, *Surgery* 88:507 (1980); and Saudek *et al.*, *N. Engl. J Med.* 321:574 (1989)). In another embodiment polymeric materials can be used (see *Medical Applications of Controlled Release* (Langer and Wise eds., 1974); *Controlled Drug Bioavailability, Drug Product Design and Performance* (Smolen and Ball eds., 1984); Ranger and Peppas, *J. Macromol. Sci. Rev. Macromol. Chem.* 2:61 (1983); Levy *et al.*, *Science* 228:190 (1935); During *et al.*, *Ann. Neural.* 25:351 (1989); and Howard *et al.*, *J. Neurosurg.* 71:105 (1989)).

In yet another embodiment a controlled- or sustained-release system can be placed in proximity of a target of the Pyridyl-Substituted Porphyrin Compounds, *e.g.*, the spinal column, brain, skin, lung, thyroid gland, colon or gastrointestinal tract, thus requiring only a fraction of the systemic dose.

The present compositions can optionally comprise a suitable amount of a pharmaceutically acceptable excipient so as to provide the form for proper administration to the subject.

Such pharmaceutical excipients can be liquids, such as water and oils, including those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The pharmaceutical excipients can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea and the like. In addition, auxiliary, stabilizing, thickening, lubricating, and coloring agents can be used. In one embodiment the pharmaceutically acceptable excipients are sterile when administered to a subject. Water is a particularly useful excipient

when the Pyridyl-Substituted Porphyrin Compound is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, particularly for injectable solutions. Suitable pharmaceutical excipients also include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. In one embodiment the composition is in the form of a capsule (see *e.g.* U.S. Patent No. 5,698,155). Other examples of suitable pharmaceutical excipients are described in *Remington's Pharmaceutical Sciences* 1447-1676 (Alfonso R. Gennaro eds., 19th ed. 1995), incorporated herein by reference.

In one embodiment the Pyridyl-Substituted Porphyrin Compounds are formulated in accordance with routine procedures as a composition adapted for oral administration to human beings. Compositions for oral delivery can be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs for example. Orally administered compositions can contain one or more agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving a Pyridyl-Substituted Porphyrin Compound are also suitable for orally administered compositions. In these latter platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero-order delivery profile as opposed to the spiked profiles of immediate release formulations. A

time-delay material such as glycerol monostearate or glycerol stearate can also be used. Oral compositions can include standard excipients such as mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, and magnesium carbonate. In one embodiment the excipients are of pharmaceutical grade.

In another embodiment the Pyridyl-Substituted Porphyrin Compounds can be formulated for intravenous administration. Typically, compositions for intravenous administration comprise sterile isotonic aqueous buffer. Where necessary, the compositions can also include a solubilizing agent. Compositions for intravenous administration can optionally include a local anesthetic such as lignocaine to lessen pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized-powder or water free concentrate in a hermetically sealed container such as an ampule or sachette indicating the quantity of active agent. Where the Pyridyl-Substituted Porphyrin Compounds are to be administered by infusion, they can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the Pyridyl-Substituted Porphyrin Compounds are administered by injection, an ampule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

The Pyridyl-Substituted Porphyrin Compounds can be administered by controlled-release or sustained-release means or by delivery devices that are well known to those of ordinary skill in the art. Examples include, but are not limited to, those described in U.S. Patent Nos. 3,845,770; 3,916,899; 3,536,809; 3,598,123; 4,008,719; 5,674,533; 5,059,595; 5,591,767; 5,120,548; 5,073,543; 5,639,476; 5,354,556; and 5,733,556, each of which is incorporated herein by reference. Such dosage forms can be used to provide controlled- or sustained-release of one or more active ingredients using, for example, hydropropylmethyl cellulose, other polymer matrices, gels, permeable membranes, osmotic systems, multilayer coatings, microparticles, liposomes, microspheres, or a combination thereof to provide the desired release profile in varying proportions. Suitable controlled- or sustained-release formulations known to those skilled in the art, including those described herein, can be readily selected for use with the active ingredients of the invention. The invention thus encompasses single unit dosage forms suitable for oral

administration such as, but not limited to, tablets, capsules, gelcaps, and caplets that are adapted for controlled- or sustained-release.

In one embodiment a controlled- or sustained-release composition comprises a minimal amount of a Pyridyl-Substituted Porphyrin Compound to treat or prevent the Condition in a minimal amount of time. Advantages of controlled- or sustained-release compositions include extended activity of the drug, reduced dosage frequency, and increased subject compliance. In addition, controlled- or sustained-release compositions can favorably affect the time of onset of action or other characteristics, such as blood levels of the Pyridyl-Substituted Porphyrin Compound, and can thus reduce the occurrence of adverse side effects.

Controlled- or sustained-release compositions can initially release an amount of a Pyridyl-Substituted Porphyrin Compound that promptly produces the desired therapeutic or prophylactic effect, and gradually and continually release other amounts of the Pyridyl-Substituted Porphyrin Compound to maintain this level of therapeutic or prophylactic effect over an extended period of time. To maintain a constant level of the Pyridyl-Substituted Porphyrin Compoundin the body, the Pyridyl-Substituted Porphyrin Compound can be released from the dosage form at a rate that will replace the amount of Pyridyl-Substituted Porphyrin Compound being metabolized and excreted from the body. Controlled- or sustained-release of an active ingredient can be stimulated by various conditions, including but not limited to, changes in pH, changes in temperature, concentration or availability of enzymes, concentration or availability of water, or other physiological conditions or compounds.

The amount of the Pyridyl-Substituted Porphyrin Compound that is effective in the treatment or prevention of a Condition can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed can also depend on the route of administration, the time of the subject's exposure to radiation, the amount of radiation that a subject is exposed to, or the seriousness of the Condition being prevented or treated. Suitable effective dosage amounts, however, range from about 10 micrograms to about 5 grams about every 4 h, although they are typically about 500 mg or less per every 4 hours. In one embodiment the effective dosage is about 0.01 mg, 0.5 mg, about 1 mg, about 50 mg, about 100 mg, about 200

mg, about 300 mg, about 400 mg, about 500 mg, about 600 mg, about 700 mg, about 800 mg, about 900 mg, about 1 g, about 1.2 g, about 1.4 g, about 1.6 g, about 1.8 g, about 2.0 g, about 2.2 g, about 2.4 g, about 2.6 g, about 2.8 g, about 3.0 g, about 3.2 g, about 3.4 g, about 3.6 g, about 3.8 g, about 4.0g, about 4.2 g, about 4.4 g, about 4.6 g, about 4.8 g, and about 5.0 g, every 4 hours. Equivalent dosages may be administered over various time periods including, but not limited to, about every 2 hours, about every 6 hours, about every 8 hours, about every 12 hours, about every 24 hours, about every 36 hours, about every 48 hours, about every 72 hours, about every week, about every two weeks, about every three weeks, about every month, and about every two months. The effective dosage amounts described herein refer to total amounts administered; that is, if more than one Pyridyl-Substituted Porphyrin Compound is administered, the effective dosage amounts correspond to the total amount administered.

When the Pyridyl-Substituted Porphyrin Compounds are administered for prevention of a radiation-inducted therapy injury, the Pyridyl-Substituted Porphyrin Compounds can be administered 48 hours or less time prior to exposure to radiation. Administration may be repeated at regular intervals as set forth above.

In one embodiment, an intial dose of a Pyridyl-Substituted Porphyrin Compound is administered from about 5 minutes to about one hour prior to exposure to radiation with repeated doses optionally administered at regular intervals thereafter.

The Pyridyl-Substituted Porphyrin Compounds can be assayed *in vitro* or *in vivo* for the desired therapeutic or prophylactic activity prior to use in humans. Animal model systems can be used to demonstrate safety and efficacy.

The present methods for treating or preventing a Condition in a subject in need thereof can further comprise administering another therapeutic agent to the subject being administered a Pyridyl-Substituted Porphyrin Compound. In one embodiment the other therapeutic agent is administered in an effective amount.

Effective amounts of the other therapeutic agents are well known to those skilled in the art. However, it is well within the skilled artisan's purview to determine the other therapeutic agent's optimal effective amount range. In one embodiment of the invention, where another therapeutic agent is administered to a subject, the effective amount of the Pyridyl-Substituted Porphyrin Compound is less than its effective amount would be where the other therapeutic agent is not

administered. In this case, without being bound by theory, it is believed that the Pyridyl-Substituted Porphyrin Compounds and the other therapeutic agent act synergistically to treat or prevent a Condition.

The other therapeutic agent can be an anti-inflammatory agent. Examples of useful anti-inflammatory agents include, but are not limited to, adrenocorticosteroids, such as cortisol, cortisone, fludrocortisone, prednisone, prednisolone, 6a-methylprednisolone, triamcinolone, betamethasone, and dexamethasone; and non-steroidal anti-inflammatory agents (NSAIDs), such as aspirin, acetaminophen, indomethacin, sulindac, tolmetin, diclofenac, ketorolac, ibuprofen, naproxen, flurbiprofen, ketoprofen, fenoprofen, oxaprozin, mefenamic acid, meclofenamic acid, piroxicam, meloxicam, nabumetone, rofecoxib, celecoxib, etodolac, and nimesulide.

The other therapeutic agent can be an anti-diabetic agent. Examples of useful anti-diabetic agents include, but are not limited to, glucagons; somatostatin; diazoxide; sulfonylureas, such as tolbutamide, acetohexamide, tolazamide, chloropropamide, glybenclamide, glipizide, gliclazide, and glimepiride; insulin secretagogues, such as repaglinide, and nateglinide; biguanides, such as metformin and phenformin; thiazolidinediones, such as pioglitazone, rosiglitazone, and troglitazone; and α-glucosidase inhibitors, such as acarbose and miglitol.

The other therapeutic agent can be an anti-cardiovascular disease agent. Examples of useful anti-cardiovascular disease agents include, but are not limited to, carnitine; thiamine; and muscarinic receptor antagonists, such as atropine, scopolamine, homatropine, tropicamide, pirenzipine, ipratropium, tiotropium, and tolterodine.

The other therapeutic agent can be an immunosuppressive agent. Examples of useful immunosuppressive agents include a corticosteroid, a calcineurin inhibitor, an antiproliferative agent, a monoclonal antilymphocyte antibody, a polyclonal antilymphocyte antibody, prednisone, methylprednisolone, cyclosporine, tacrolimus, mycophenolate mofetil, azathioprine, sirolimus, muromonab-CD3, interleukin-2 receptor antagonist, daclizumab, antithymocyte globulin-equine, and antithymocyte globulin-rabbit. In one embodiment, the methods for treating or preventing a reoxygenation injury resulting from organ transplantation further comprises administering an immunosuppressive agent.

The other therapeutic agent can be an antiemetic agent. Examples of useful antiemetic agents include, but are not limited to, metoclopromide, domperidone, prochlorperazine, promethazine, chlorpromazine, trimethobenzamide, ondansetron, granisetron, hydroxyzine, acetylleucine monoethanolamine, alizapride, azasetron, benzquinamide, bietanautine, bromopride, buclizine, clebopride, cyclizine, dimenhydrinate, diphenidol, dolasetron, meclizine, methallatal, metopimazine, nabilone, oxyperndyl, pipamazine, scopolamine, sulpiride, tetrahydrocannabinol, thiethylperazine, thioproperazine, tropisetron, and mixtures thereof.

The other therapeutic agent can be an anticancer agent. The Pyridyl-Substituted Porphyrin Compound and the other anticancer agent can act additively or synergistically. A synergistic use of a Pyridyl-Substituted Porphyrin Compound and another anticancer agent permits the use of lower dosages of one or more of these agents and/or less frequent administration of the agents to a subject with cancer. The ability to utilize lower dosages of a Pyridyl-Substituted Porphyrin Compound and/or additional anticancer agents and/or to administer the agents less frequently can reduce the toxicity associated with the administration of the agents to a subject without reducing the efficacy of the agents in the treatment of cancer. In addition, a synergistic effect can result in the improved efficacy of these agents in the treatment of cancer and/or the reduction of adverse or unwanted side effects associated with the use of either agent alone.

In one embodiment, the Pyridyl-Substituted Porphyrin Compound and the anticancer agent can act synergistically when administered in doses typically employed when such agents are used as monotherapy for the treatment of cancer. In another embodiment, the Pyridyl-Substituted Porphyrin Compound and the anticancer agent can act synergistically when administered in doses that are less than doses typically employed when such agents are used as monotherapy for the treatment of cancer.

In one embodiment, the additional anticancer agent can be, but is not limited to, a drug listed in Table 2.

TABLE 2

Alkylating agents	
Nitrogen mustards:	Cyclophosphamide

	Ifosfamide
	Trofosfamide
	Chlorambucil
Nitrosoureas:	Carmustine (BCNU)
	Lomustine (CCNU)
Alkylsulphonates:	Busulfan
	Treosulfan
Triazenes:	Dacarbazine
Platinum containing complexes:	Cisplatin
	Carboplatin
	Aroplatin
	Oxaliplatin
Plant Alkaloids	
Vinca alkaloids:	Vincristine
	Vinblastine
	Vindesine
	Vinorelbine
Taxoids:	Paclitaxel
	Docetaxel
DNA Topoisomerase Inhibitors	
Epipodophyllins:	Etoposide
	Teniposide
	Topotecan
	9-aminocamptothecin
	Camptothecin
	Crisnatol
Mitomycins:	Mitomycin C
	Anti-metabolites
Anti-folates:	
DHFR inhibitors:	Methotrexate
	Trimetrexate
IMP dehydrogenase Inhibitors:	Mycophenolic acid

	Tiazofurin
	Ribavirin
	EICAR
Ribonuclotide reductase Inhibitors:	Hydroxyurea
	Deferoxamine
Pyrimidine analogs:	
Uracil analogs:	5-Fluorouracil
	Fluoxuridine
	Doxifluridine
	Ralitrexed
Cytosine analogs:	Cytarabine (ara C)
	Cytosine arabinoside
	Fludarabine
	Gemcitabine
	Capecitabine
Purine analogs:	Mercaptopurine
	Thioguanine
DNA Antimetabolites:	3-HP
	2′-deoxy-5-fluorouridine
	5-HP
	alpha-TGDR
	aphidicolin glycinate
	ara-C
	5-aza-2′-deoxycytidine
	beta-TGDR
	cyclocytidine
	guanazole
	inosine glycodialdehyde
	macebecin II
	Pyrazoloimidazole
Hormonal therapies:	
Receptor antagonists:	

Anti-estrogen:	Tamoxifen
	Raloxifene
	Megestrol
LHRH agonists:	Goscrclin
	Leuprolide acetate
Anti-androgens:	Flutamide
	Bicalutamide
Retinoids/Deltoids	
	Cis-retinoic acid
Vitamin A derivative:	All-trans retinoic acid (ATRA-IV)
Vitamin D3 analogs:	EB 1089
	CB 1093
	KH 1060
Photodynamic therapies:	Vertoporfin (BPD-MA)
	Phthalocyanine
	Photosensitizer Pc4
	Demethoxy-hypocrellin A (2BA-2-DMHA)
Cytokines:	Interferon-α
	Interferon-β
	Interferon-γ
	Tumor necrosis factor
Angiogenesis Inhibitors:	Angiostatin (plasminogen fragment)
	antiangiogenic antithrombin III
	Angiozyme
	ABT-627
	Bay 12-9566
	Benefin
	Bevacizumab
	BMS-275291
	cartilage-derived inhibitor (CDI)

CAI
CD59 complement fragment
CEP-7055
Col 3
Combretastatin A-4
Endostatin (collagen XVIII fragment)
Fibronectin fragment
Gro-beta
Halofuginone
Heparinases
Heparin hexasaccharide fragment
HMV833
Human chorionic gonadotropin (hCG)
IM-862
Interferon alpha/beta/gamma
Interferon inducible protein (IP-10)
Interleukin-12
Kringle 5 (plasminogen fragment)
Marimastat
Metalloproteinase inhibitors (TIMPs)
2-Methoxyestradiol
MMI 270 (CGS 27023A)
MoAb IMC-1C11
Neovastat
NM-3
Panzem
PI-88
Placental ribonuclease inhibitor
Plasminogen activator inhibitor

Platelet factor-4 (PF4)
Prinomastat
Prolactin 16kD fragment
Proliferin-related protein (PRP)
PTK 787/ZK 222594
Retinoids
Solimastat
Squalamine
SS 3304
SU 5416
SU6668
SU11248
Tetrahydrocortisol-S
Tetrathiomolybdate
Thalidomide
Thrombospondin-1 (TSP-1)
TNP-470
Transforming growth factor-beta (TGF-β)
Vasculostatin
Vasostatin (calreticulin fragment)
ZD6126
ZD 6474
farnesyl transferase inhibitors (FTI)
Bisphosphonates

Antimitotic agents: Allocolchicine
Halichondrin B
Colchicine
colchicine derivative
dolstatin 10
Maytansine

	Rhizoxin
	Thiocolchicine
	trityl cysteine
Others:	
Isoprenylation inhibitors:	
Dopaminergic neurotoxins:	1-methyl-4-phenylpyridinium ion
Cell cycle inhibitors:	Staurosporine
Actinomycins:	Actinomycin D
	Dactinomycin
Bleomycins:	Bleomycin A2
	Bleomycin B2
	Peplomycin
Anthracyclines:	Daunorubicin
	Doxorubicin (adriamycin)
	Idarubicin
	Epirubicin
	Pirarubicin
	Zorubicin
	Mitoxantrone
MDR inhibitors:	Verapamil
Ca^{2+}ATPase inhibitors:	Thapsigargin

5.5.1 MULTI- THERAPY FOR CANCER

The Pyridyl-Substituted Porphyrin Compounds can be administered to a subject that has undergone, is currently undergoing, or is about to undergo one or more additional anticancer treatments including, but not limited to, surgery, radiation therapy, or immunotherapy, such as administration of a cancer vaccine.

The present methods for treating cancer can further comprise administering surgery, radiation therapy, or immunotherapy.

In one embodiment, the anticancer treatment is immunotherapy.

In one embodiment, the immunotherapy is a cancer vaccine.

In one embodiment, the anticancer treatment is radiation therapy.

In another embodiment, the anticancer treatment is surgery.

In a specific embodiment, a Pyridyl-Substituted Porphyrin Compound is administered concurrently with radiation therapy. In another specific embodiment, the additional anticancer treatment is administered prior or subsequent to the administration of the Pyridyl-Substituted Porphyrin Compound, in one embodiment at least an hour, five hours, 12 hours, a day, a week, a month, or several months (e.g., up to three months), prior or subsequent to administration of the Pyridyl-Substituted Porphyrin Compounds.

When the additional anticancer treatment is radiation therapy, any radiation therapy protocol can be used depending upon the type of cancer to be treated or prevented. For example, but not by way of limitation, X-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater that 1 MeV energy) can be used for deep tumors, and electron beam and orthovoltage X-ray radiation can be used for skin cancers. Gamma-ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt and other elements, can also be administered.

Additionally, the invention provides methods of treatment of cancer using the Pyridyl-Substituted Porphyrin Compounds as an alternative to chemotherapy or radiation therapy where the chemotherapy or the radiation therapy results in negative side effects in the subject being treated. The subject being treated can, optionally, be treated with another anticancer treatment modality such as surgery, radiation therapy, or immunotherapy.

The Pyridyl-Substituted Porphyrin Compounds can also be used *in vitro* or *ex vivo*, such as for the treatment of certain cancers, including, but not limited to leukemias and lymphomas, such treatment involving autologous stem cell transplants. This can involve a process in which the subject's autologous hematopoietic stem cells are harvested and purged of all cancer cells, the subject's remaining bone-marrow cell population is then eradicated via the administration of a Pyridyl-Substituted Porphyrin Compound and/or radiation therapy, and the stem cell graft is infused back into the subject.

A Pyridyl-Substituted Porphyrin Compound and the other therapeutic agent can act additively or, in one embodiment synergistically. In one embodiment a Pyridyl-Substituted Porphyrin Compound is administered concurrently with another therapeutic agent. In one embodiment a composition comprising an effective amount

of a Pyridyl-Substituted Porphyrin Compound and an effective amount of another therapeutic agent can be administered. Alternatively, a composition comprising an effective amount of a Pyridyl-Substituted Porphyrin Compound and a different composition comprising an effective amount of another therapeutic agent can be concurrently administered. In another embodiment, an effective amount of a Pyridyl-Substituted Porphyrin Compound is administered prior or subsequent to administration of an effective amount of another therapeutic agent. In this embodiment the Pyridyl-Substituted Porphyrin Compound is administered while the other therapeutic agent exerts its therapeutic effect, or the other therapeutic agent is administered while the Pyridyl-Substituted Porphyrin Compound exerts its preventative or therapeutic effect for treating or preventing a Condition.

A composition of the invention can be prepared by a method comprising admixing a Pyridyl-Substituted Porphyrin Compound and a physiologically acceptable carrier or vehicle. Admixing can be accomplished using methods well known for admixing a compound and a physiologically acceptable carrier or vehicle. In one embodiment the Pyridyl-Substituted Porphyrin Compound is present in the composition in an effective amount.

5.6 KITS

The invention encompasses kits that can simplify the administration of a Pyridyl-Substituted Porphyrin Compound to a subject.

A typical kit of the invention comprises a unit dosage form of a Pyridyl-Substituted Porphyrin Compound. In one embodiment the unit dosage form is within a container, which can be sterile, containing an effective amount of a Pyridyl-Substituted Porphyrin Compound and a physiologically acceptable carrier or vehicle. The kit can further comprise a label or printed instructions instructing the use of the Pyridyl-Substituted Porphyrin Compound to treat or prevent a Condition. The kit can also further comprise a unit dosage form of another therapeutic agent, for example, a container containing an effective amount of the other therapeutic agent. In one embodiment the kit comprises a container containing an effective amount of a Pyridyl-Substituted Porphyrin Compound and an effective amount of another therapeutic agent. Examples of other therapeutic agents include, but are not limited to, those listed above.

Kits of the invention can further comprise a device that is useful for administering the unit dosage forms. Examples of such a device include, but are not limited to, a syringe, a drip bag, a patch, an inhaler, and an enema bag.

The following examples are set forth to assist in understanding the invention and should not, of course, be construed as specifically limiting the invention described and claimed herein. Such variations of the invention, including the substitution of all equivalents now known or later developed, which would be within the purview of those skilled in the art, and changes in formulation or minor changes in experimental design, are to be considered to fall within the scope of the invention incorporated herein.

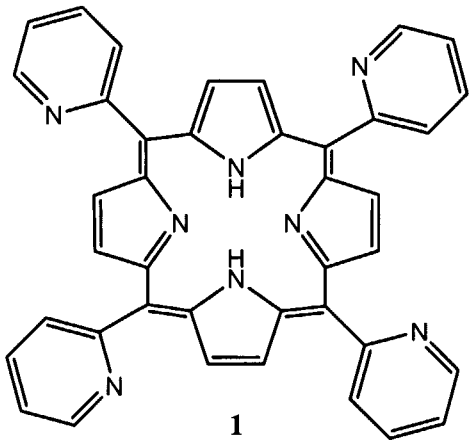
6. EXAMPLES

General Methods

Proton NMR spectra were obtained using a Varian 300 MHz spectrophotometer and chemical shift values (δ) are reported in parts per million (ppm). TLC was performed using TLC plates precoated with silica gel 60 F-254. Intermediates and final compounds were characterized on the basis of ^{1}H NMR and MS data, HPLC, elemental analysis.

6.1 EXAMPLE 1

Synthesis of Compound 1



1

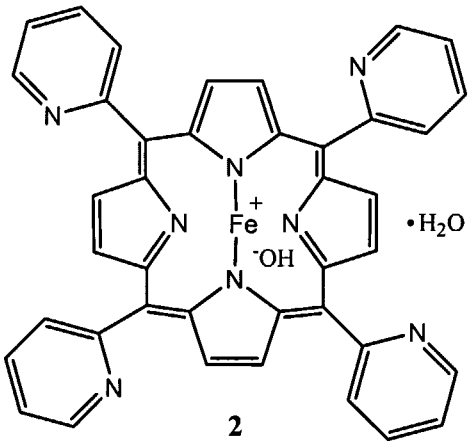
A 50 L three-neck reaction flask containing propionic acid (30 L) was equipped with two addition funnels and a reflux condenser. One addition funnel was charged with a solution of pyrrole (417 mL, 6.0 mol) in toluene (583 mL), and the second addition funnel was charged with a solution of 2-pyridinecarboxaldehyde (568 mL, 6.0 mol) in toluene (432 mL).

The propionic acid was heated to reflux and then the contents of the addition funnels were added simultaneously at approximately equal rates over 2 hours, with vigorous stirring to the refluxing propionic acid. The resultant dark red-brown reaction mixture was heated at reflux for 1 hour, then the heat source was removed and the reaction mixture was allowed to stir for about 18 hours at room temperature. The resultant black solution was filtered through #1 filter paper and concentrated *in vacuo* to provide a black oily residue. The black oily residue was diluted with toluene (5 L) and the resultant solution was stirred for 1 minute, then concentrated *in vacuo*. This dilution/concentration was repeated three times and the resultant black solid residue was diluted with ethyl acetate (5 L) and the resultant solution was stirred at room temperature for about 18 hours. The resultant solution was filtered through #1 filter paper, the collected solids were diluted with dichloromethane (2 L) and the resultant solution was purified using flash column chromatography on silica gel (10 kg) using dichloromethane: triethylamine (98:2 vol:vol) as eluent. The relevant fractions were combined and concentrated *in vacuo*, and the resultant black granular solid was

diluted with 10% aqueous ammonium hydroxide (2 L), and the resultant suspension was stirred vigorously for 2 hours. The resultant suspension was filtered through #1 filter paper, and the collected black solids were washed with deionized water (4 x 1L). The washed solids were then suspended in ethyl acetate (2 L), and the resultant solution was stirred for 1 hour then filtered through #1 filter paper. The collected eggplant-colored granular solid was diluted with 1,2-dichloroethane (1 L) and the resultant solution was stirred for 2 hours, then filtered through #1 filter paper. The collected solids were washed with 1,2-dichloroethane (4 x 200 mL), then dried *in vacuo* overnight to provide Compound **1** as a brilliant deep metallic purple solid. Yield = 64.26 g (7%). R_f=0.56 (silica, 9:1 dichloromethane: 7 N ammonia in methanol); ^{1}H NMR ($CDCl_3$) δ 9.14 (d, J=3.9 Hz, 4H), 8.87 (S, 8H), 8.21 (d, J=7.5 Hz, 4H), 8.10 (dt, J_1=1.8 Hz, J_2=7.8 Hz, 4H), 7.71 (dd, J_1=5.1 Hz, J_2=7.5 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 160.7, 148.8, 134.9, 132.2, 130.6, 122.6, 122.6, 119.0; mass spectrum ("MS") m/z=619 (M+H).

6.2 EXAMPLE 2

Synthesis of Compound 2



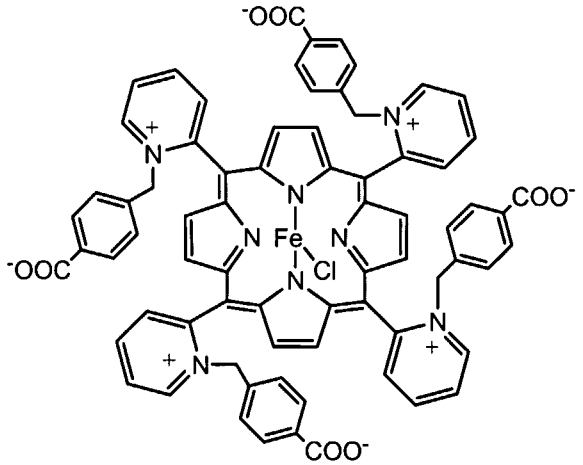
2

Ferric chloride (14.3 g; 88.89 mmol) was added to a suspension of Compound **1** (50.0 g, 80.39 mmol) in 1 N hydrochloric acid (245 mL, 3 eq.) and the resultant reaction mixture was heated to reflux and stirred for about 18 hours. The resultant dark brown reaction mixture was cooled to room temperature and basified using 5N sodium hydroxide (160 mL). The resultant precipitate was vacuum filtered through Whatman #50 filter paper and washed sequentially with deionized water (4 x

1.5 L) and diethyl ether (1.5 L). The resultant purple-black solid was subsequently dried *in vacuo* for 3 days at 100 °C, then dissolved in dichloromethane (200 mL) and vacuum filtered through a one-inch pad of Celite. The Celite cake was washed with a solution of 9:1(vol:vol) dichloromethane: methanol until the filtrate was nearly colorless. The filtrate was then concentrated *in vacuo* to provide Compound **2** as its monohydrate as a purple-black iridescent powdered solid. Yield = 25.74g (47%). MS m/z=672(M+). Anal. Calc. for $C_{40}H_{27}FeN_8O_2$:67.91% C, 3.82% H, 7.90% Fe, 15.85% N, 4.53% O. Found: 67.84%C, 3.63% H, 7.70% Fe, 15.92% N.

6.3 EXAMPLE 3

Synthesis of Compound 3



3

Method 1:

Compound **2** (25 g) was diluted in N-methyl pyrrolidinone (250 mL) and stirred to form a slurry. α-Bromo-*p*-toluic acid (157 g, 20 eq.) was then added to the slurry and the resultant reaction mixture was stirred under nitrogen atmosphere at 130 °C for about 70 hours. The reaction mixture was cooled to room temperature and poured slowly into a vigorously stirring volume of chloroform (2.75 L). The resultant suspension was filtered through a three-inch pad of Celite and the dark-brown precipitate was removed from the filter funnel along with the top one-inch of the Celite pad. The combined precipitate and Celite were extracted with chloroform (1.5 L) in a Soxhlet extractor for about 55 hours. The extracted solid was removed from the Soxhlet thimble and diluted in 2.5 L of a mixture of MeOH:H_2O (1:1) and the resultant solution was filtered through a medium porosity glass fritted funnel. The

filtrate was mixed with Dowex Marathon WBA-2 weakly basic anion exchange resin (340 mL, 16 eq.) and the resultant solution was stirred for about 20 hours, then filtered. The resin was washed with 500 mL of 1:1 MeOH:H_2O and the combined filtrate was eluted down a one-inch O.D. glass column of 340 mL of Dowex Marathon WBA-2 resin at a flow rate of 15-20 mL/min. using 500 mL of 1:1 MeOH:H_2O as eluent. The combined filtrates were mixed with 240 mL (8 eq.) of Amberlite IRA-402 chloride form strongly basic anion-exchange resin and stirred for about 4 hours. The resultant solution was filtered using a coarse porosity glass fritted funnel and the resin resin was washed with 500 mL of 1:1 MeOH:H_2O and the combined filtrate was eluted down a one-inch O.D. glass column of 340 mL of Dowex Marathon WBA-2 resin at a flow rate of 15-20 mL/min. using 500 mL of 1:1 MeOH:H_2O as eluent. The filtrate was passed through a one-inch O.D. glass column of 240 mL of Amberlite IRA-402 chloride resin at a flow rate of 15-20 mL/min. The resin was then washed with 500 mL of 1:1 MeOH:H_2O and the combined filtrates were vacuum filtered though a 0.22 μm membrane and concentrated *in vacuo* to a volume of about 2 L. This solution was then shell-frozen and lyophilized to provide Compound **3** as a black solid.

Method 2:

A 12 L reactor was charged with with 7.9 L of *N*-methyl pyrrolidinone (NMP) and heated to 120°C. 787.5 g of Compound **21** (Example 11) was added, followed by 4.521 kg of α-bromo-*p*-toluic acid. The reaction mixture was stirred under nitrogen atmosphere at 120°C for 6- 7 hours, then poured slowly into a 30 L flask containing 10 L of vigorously stirred chloroform. The remaining residue in the 12 L reactor was rinsed into the stirred chloroform mixture with 6 L of chloroform. The resultant suspension was filtered through a 3" thick celite bed in an 18 inch filter funnel, and the black, product-containing layer of celite was removed from the filter bed and transferred to a 12 L reactor equipped with a mechanical stirrer. 5 L of chloroform was added to the reactor, and the resultant mixture was stirred and heated at reflux for 15 minutes. The chloroform suspension was hot-filtered, the filtered solids were returned to the 12 L reactor, and the aforementioned extraction procedure was repeated twice. The solids were again returned to the 12 L reactor, 5 L of methanol was added, and the resultant mixture stirred for 15 minutes at ambient

temperature. The solids were removed by vacuum filtration and returned to the 12 L reactor, and the methanol extraction was repeated until the filtrate was substantially clear. The combined methanol extracts were concentrated *in vacuo* to provide 1.544 kg of crude Compound **3**.

HPLC Analysis of Compound 3

The black solid Compound **3** (1 mg, prepared using Method 1, described above) was dissolved in 1 mL of 0.1M HCl. 10 μL of the resultant solution was injected onto a Phenomenex Synergi POLAR-RP HPLC column (4 μM, 80 Å, 105 mm x 4.6 mm). The column was eluted at 1 mL/minute using a two-component mixture of (1) water with 0.1% trifluoroacetic acid ("solvent 1"); and (2) methanol with 0.1% trifluoroacetic acid ("solvent 2") in the following gradient:

Time (min)	Solvent 1	Solvent 2	Flow Rate (mL/min)
0	65%	35%	1
13	65%	35%	1
20	40%	60%	1
21	10%	90%	1
25	10%	90%	1

Results show that the Compound **3** comprises three isomers: an isomer (Compound **3A**) having a retention time of about 4 minutes; an isomer (Compound **3B**) having a retention time of about 10 minutes; and an isomer (Compound **3C**) having a retention time of about 17.2 minutes. Each of Compounds **3A**, **3B**, and **3C** is one of Isomer Nos. 1-8 of Compound **3**.

6.4 EXAMPLE 4

Isolation of Compound 3A

Method 1:

Step 1 – pH titration

A Compound **3** mixture of isomers (5 g, prepared using the method described in Example 3, Method 1) was diluted using 0.1M HCl (100 mL), and to the

resultant solution was added 1M NaOH (about 18 mL) dropwise until the pH was about 6.0. This solution was then filtered through a 0.2 μM nylon filter and the collected solid was washed with water (about 50 mL). The filtrate and wash were combined and concentrated *in vacuo*, then further dried in a vacuum oven to provide 4.2 g of a solid crude residue.

Step 2 – Removal of hydrophobes via water elution

The solid crude residue (1 g, prepared using the method of Step 1) was dissolved in water (10 mL), and the resultant solution was loaded onto a polymeric resin column (8-inch effective length, 0.5-inch internal diameter, 12 cm packed bed length, packed with 10 g of MCI gel CHP20P stryene divinylbenzene polymeric resin) and equilibrated using 300 mL of water). The column was eluted at a flow rate of about 5 mL/minute using water as the mobile phase and 20 mL fractions were collected. After collecting 15 fractions, the column was sequentially eluted with methanol (15 mL), and 0.1M HCl (25 mL), and then flushed with methanol and stored for subsequent use. Fractions 2-12 were combined and concentrated *in vacuo* to provide a residue. The residue was analyzed using HPLC and shown to comprise Compound **3A**, Compound **3C**, and a few minor unidentified impurities.

Step 3 – Isolation and Purification of Compound 3A and Compound 3C

The residue obtained from reduced fractions 2-12 (150 mg), as described in Step 2, was dissolved in 5 mM HCl (3 mL), and the resultant solution was loaded onto an equilibrated flash chromatograpy column (12-inch effective length, 0.5-inch internal diameter) using Phenomenex Sepra Phenyl resin (50 mM, 65 Å) as stationary phase (20 g of resin was packed as a slurry in methanol and equilibrated using 500 mL of 5 mM HCl prior to loading). The column was eluted at a flow rate of about 3 mL/minute using 5 mM HCl (pH of about 2.5, degassed for about 30 minutes prior to elution) as the mobile phase and 30 mL fractions were collected from the point of loading. Fractions shown by HPLC analysis to contain Compound **3A** (at > 95 are % using the HPLC analysis described in Example 3) were combined to provide the "Compound **3A** pool" (total volume of combined fractions = about 600 mL). The stationary phase was then sequentially washed using 0.1M HCl

(30 mL), methanol (300 mL) and stored for subsequent use. The washes were then combined to provide the "Compound **3C** pool" (about 100 mL total volume).

Step 4 – Solvent exchange of the Compound *3A pool*

A Compound **3A** (600 mL) pool obtained using the method described in Step 3, was adjusted to pH 1.0 using concentrated HCl (about 30 mL), and the resultant solution was loaded onto a polymeric resin column (12 inch effective length, 1.0 inch internal diameter, packed with 35 g of MCI gel CHP20P stryene divinylbenzene polymeric resin (Supelco, St. Louis, Mo.)) and equilibrated using 500 mL of 0.1M HCl. After loading, additional 0.1M HCl (100 mL) was loaded onto the column to complete adsorption. The column was then eluted at a flow rate of about 5 mL/minute using methanol as the mobile phase and all fractions containing Compound **3A** were combined to provide a subsequent Compound **3A** pool of 300 mL (in methanol).

Step 5 – Counterion Removal Using Ion-Exchange

A Compound **3A** pool (300 mL), obtained using the method described in Step 4, was concentrated *in vacuo* to a final volume of about 300 mL and then stirred with DOWEX Marathon WBA-2 weakly basic ion-exchange resin (50 mL of aqueous solution of settled resin) for about 18 hours at room temperature and vacuum filtered through a 0.2 μM nylon filter. The filtrate was then passed through a column containing fresh DOWEX Marathon WBA-2 weakly basic ion-exchange resin (50 mL of aqueous solution of settled resin) and the filtrate was pooled. The resin was then washed with methanol and the methanol wash was added to the pooled filtrate.

Step 6 – Chlorine Counterion Bonding to Iron

The filtrate pool obtained using the method described in Step 5 was stirred with Amberlite IRA-402 strongly basic ion-exchange resin (10 mL of aqueous solution of settled resin) for about 3 hours at room temperature and vacuum filtered through a 0.2 μM nylon filter. The filtrate was then passed through a column containing fresh Amberlite IRA-402 strongly basic ion-exchange resin (1o mL of aqueous solution of settled resin) and the filtrate was collected and pooled. The resin was then washed with methanol and the methanol wash was added to the pooled

filtrate. The pooled filtrate was concentrated *in vacuo* and dried on high vacuum for about 18 hours to provide a solid residue which was pulverized into a fine powder, transferred to a drying dish and dried in a vacuum oven at 45 °C for about 72 hours to provide Compound **3A** as a powdered solid (50 mg, > 96% purity by HPLC).

Method 2:

1.544 kg of crude Compound **3** (Example 3, Method 2) was dissolved in 50 L of 0.1 N aqueous hydrochloric acid, and the resultant solution was stirred and titrated to pH 6 with 5 N aqueous sodium hydroxide and stirred for 1 h. The resultant suspension was vacuum filtered through a 3" thick bed of celite in an 18" filter funnel, and the filtered solids were washed with 20 L of water. The solids were highly enriched in Compound **3B** (approximately 80% by HPLC), which was extracted from the celite with 1:1 1 N HCl:MeOH. The solvent was removed *in vacuo* to provide Compound **3B** in sufficient purity for further purification by preparatory HPLC.

The aqueous filtrate was subsequently titrated to pH 0.5 with concentrated (12.1 N) aqueous HCl. 23.5 L of the pH 0.5 aqueous solution was loaded onto a column of 5.0 kg of MCI- gel divinylbenzene polymeric resin in 0.1 N HCl. Compounds **3A** and **3C** adsorbed to the column in a narrow band, and were eluted with 0.1 N HCl. Fractions of 20L, then 4 x 5L, then 20 L were collected. Fractions containing greater than 50% of Compound **3A** (retention time = 4 min) were combined, as were fractions containing greater than 50% of Compound **3C** (retention time = 17 min). The column was then washed with 20 L of 1:1 MeOH: 1N HCl, followed by 20 L of methanol, and 10 L of 0.1 N HCl.

This procedure was repeated twice with 23.3 L volumes of the pH 0.5 aqueous solution. Fractions containing Compound **3A** and those containing Compound **3C** were combined and concentrated *in vacuo* to provide 217 g of Compound **3A** in sufficient purity for further purification by preparatory HPLC.

8.0 grams of Compound **3A** fraction pool were then dissolved in 425 mL of water having 0.1% trifluoroacetic acid (vol/vol) and mixed for no less than 15 minutes. The solution was filtered through a 0.22μm nylon membrane providing 450 mL of a column-injectable solution.

The column used for purification was packed with 345 grams of Phenomenex, Synergi, POLAR-RP, 10μm particle size, 80 A pore size resin. The

column dimensions were 310 mm x 50 mm (diam.) and the resin was packed into the column via a Dynamic Axial Compression method.

The column was equilibrated before injection by using water with 0.1% trifluoroacetic acid (vol/vol) and at a flow rate of 80 mL/min for a minimum period of 45 minutes.

The column injectable solution was injected as outlined below, and the chromatographic separation was carried out using a two-component system of water with 0.1% trifluoroacetic acid ("solvent 1") and methanol with 0.1% trifluoroacetic acid ("Solvent 2") under the following gradient conditions:

Time (min:sec)	Solvent 1	Solvent 2	Flow Rate (mL/min)
0:00	100%	0%	80
0:30	100%	0%	80
0:31 – injection until fully loaded (about 8 minutes)	100%	0%	80
10:00	100%	0%	80
50:00	70%	30%	80
58:00	70%	30%	80
60:00	10%	90%	80
80:00	10%	90%	80
82:00	100%	0%	80

Fractions were collected beginning at about 24 minutes (run time), when Compound **3A** began to elute. The first fraction volume taken was 100 mL; all subsequent fraction volumes were 320 mL. Fraction collection ended at about 58 minutes.

Fractions that contained Compound **3A** at >98 area % (using the HPLC analysis method described in Example 3) were combined to provide the "Compound **3A** Prep LC Pool." The total volume of combined fractions was about 2.5 L.

The Compound **3A** Prep LC Pool (2.5L) was concentrated *in vacuo* to a final volume of 300 mL and then stirred with AMBERLITE IRA-402 (Chloride form) strongly-basic anion-exchange resin (120 mL of aqueous solution of settled

resin) for a period of 3 hours at room temperature, and subsequently vacuum filtered through a 0.22μm nylon membrane. The filtrate was passed through a column containing fresh AMBERLITE IRA-402 (Chloride form) strongly-basic anion-exchange resin (120 mL of aqueous solution of settled resin) and the product effluent was collected. The resin was washed with methanol and the methanol wash was added to the collected product effluent.

The product effluent was vacuum filtered through a 0.22μm nylon membrane, concentrated *in vacuo,* and dried under high vacuum for about 18 hours. The resultant solid residue was pulverized into a fine powder, transferred to a drying dish and dried *in vacuo* at 45°C for about 72 hours to provide Compound **3A** as a powdered solid, pentachloride salt (n = 5) (5.5 grams, >98% purity by HPLC).

6.5 EXAMPLE 5

Isolation and Purification of Compound 3C

Step 1 – Solvent exchange on Compound 3C pool

30 mg of a residue obtained from *in vacuo* concentration of the Compound **3C** pool (obtained using the method described in Example 4, Step 3) was dissolved in about 6 mL of water. The resultant solution was filtered through a 0.2 μM nylon syringe filter. The filtered solution was then injected onto a Phenomenex Synergi POLAR-RP HPLC column (10 μM, 80 Å, 250 mm x 50 mm). The column was eluted at 120 mL/minute using a two-component mixture of (1) water with 0.1% trifluoroacetic acid ("solvent 1"); and (2) methanol with 0.1% trifluoroacetic acid ("solvent 2") in the following gradient:

Time (min)	Solvent 1	Solvent 2	Flow Rate (mL/min)
0	65%	35%	120
13	65%	35%	120
20	40%	60%	120
21	10%	90%	120
25	10%	90%	120

Fractions (30 mL each) eluting between 13 and 17 minutes at the sustained flow rate of 120 mL/min were collected and analyzed. Fractions shown to contain Compound **3C** at > 95 area % (using the HPLC analysis method described in Example 3) were combined to provide the "Compound **3C** prep LC pool" (total volume of combined fractions = about 240 mL).

Step 2 – Counterion Removal Using Ion-Exchange

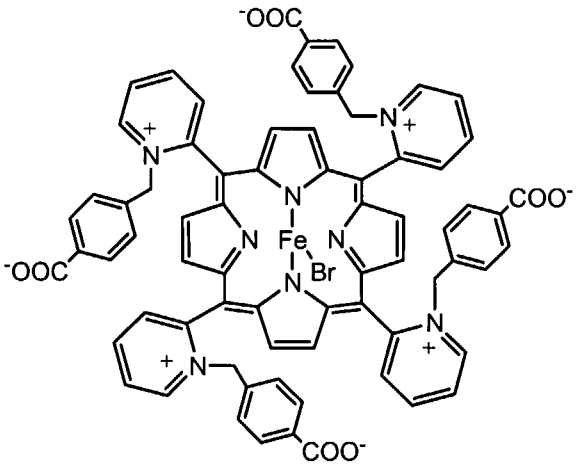
The Compound **3C** prep LC pool (240 mL, obtained in Step 1) was concentrated *in vacuo* to a final volume of about 50 mL and then sirred with DOWEX Marathon WBA-2 weakly basic ion-exchange resin (10 mL of aqueous solution of settled resin) for about 18 hours at room temperature and vacuum filtered through a 0.2 μM nylon filter. The filtrate was then passed through a column containing fresh DOWEX Marathon WBA-2 weakly basic ion-exchange resin (10 mL of aqueous solution of settled resin) and the filtrate was collected and pooled. The resin was then washed with methanol and the methanol wash was added to the pooled filtrate.

Step 3 – Chlorine Ligand Attachment to Iron Center

The pooled filtrate from Step 2 was concentrated *in vacuo* to a final volume of about 10 mL and then stirred with Amberlite IRA-402 strongly basic ion-exchange resin (1 mL of aqueous solution of settled resin) for about 3 hours at room temperature and vacuum filtered through a 0.2 μM nylon filter. The filtrate was then passed through a column containing fresh Amberlite IRA-402 strongly basic ion-exchange resin (1 mL of aqueous solution of settled resin) and the filtrate was collected and pooled. The resin was then washed with methanol and the methanol wash was added to the pooled filtrate. The pooled filtrate was concentrated *in vacuo* and dried on high vacuum for about 18 hours to provide a solid residue which was pulverized into a fine powder, transferred to a drying dish and dried in a vacuum oven at 45 °C for about 72 hours to provide Compound **3C** as a powdered solid (6.1 mg, > 96% purity by HPLC).

6.6 EXAMPLE 6

Synthesis of Compound 4

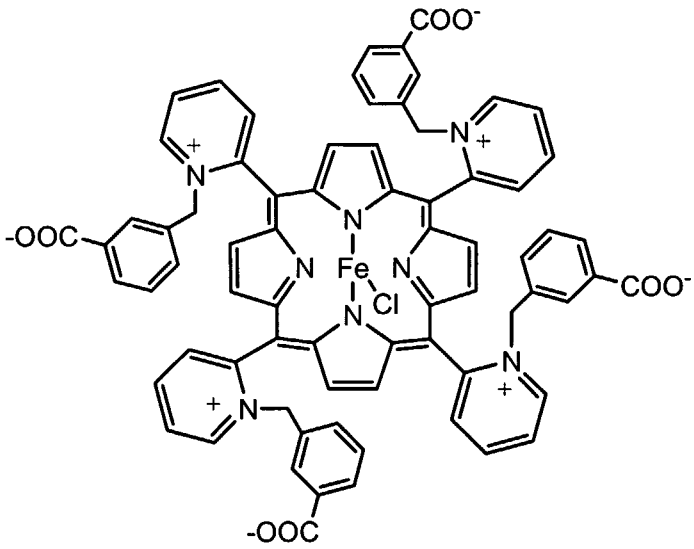


4

Following the procedure described in Example 3, but omitting the step of adding the Amberlite IRA-402 chloride form strongly basic anion-exchange resin, Compound **4** was obtained.

6.7 EXAMPLE 7

Synthesis of Compound 9



9

Synthesis of 3-bromomethylbenzoic acid:

In a 1 L round bottom flask fitted with a reflux condenser, a stirred suspension of 10.00 g of *m*-toluic acid and 14.37 g (1.1 eq.) *N*-bromosuccinimide in 735 mL chloroform was sparged for 0.5 h with nitrogen. The sparging was discontinued, and the suspension was stirred and irradiated under nitrogen atmosphere using a 500 W quartz halogen lamp at 75% power, causing the solids to dissolve and the chloroform to reflux. The red color of the reaction mixture disappeared after 1.25 h, and 14.37 g of *N*-bromosuccinimide was added. The reaction mixture was stirred and irradiated under nitrogen atmosphere with a 500 W quartz halogen lamp at 75% power for another 1.5 h, at which time the solution became colorless. The solvent volume was reduced *in vacuo* to about 100 mL, and then cooled to –20 °C. The resultant suspension was vacuum filtered through a bed of dry silica. The silica was washed with 800 mL of chloroform. The chloroform filtrate was reduced *in vacuo* to about 100 mL, and then cooled to –20 °C. The resultant crystals were vacuum filtered, washed with 30 mL of chloroform followed by 50 mL of hexanes, then dissolved in 250 mL chloroform and washed in a separatory funnel with 3 x 300 mL volumes of water followed by one 300 mL volume of brine to remove traces of succinimide. The organic phase was dried with magnesium sulfate, vacuum filtered, and the solvent was removed *in vacuo* to provide 9.56 g (61%) of 3-bromomethylbenzoic acid as a white crystalline power.

*Formation of Compound **9**:*

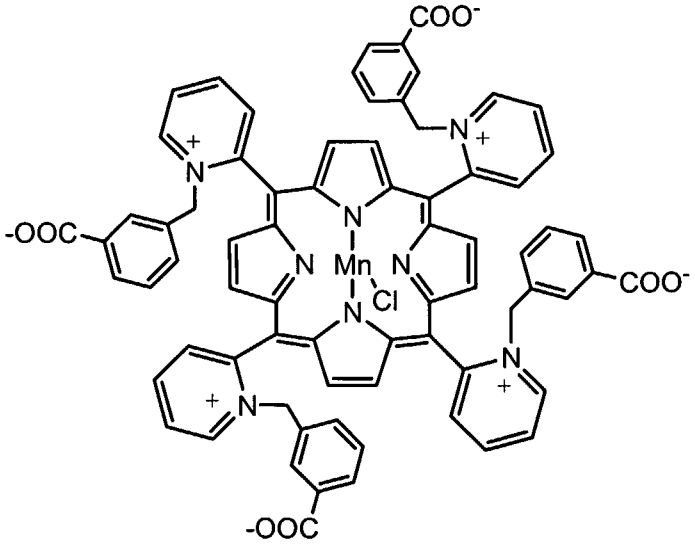
Compound **9** is obtained according to Method 2 in Example 3, but by substituting 3-bromomethylbenzoic acid for α-Bromo-*p*-toluic acid.

Isolation of Compound 9:

Compound **9** is isolated as set forth in Example 4, Method 2, but the initial titration is pH 6 and the precipitation of Compound **9** is omitted. Crude Compound **9** is dissolved in 0.1 N HCl and loaded onto the MCI-gel column directly.

6.8 EXAMPLE 8

Synthesis of Compound 12



12

Formation of Compound 12:

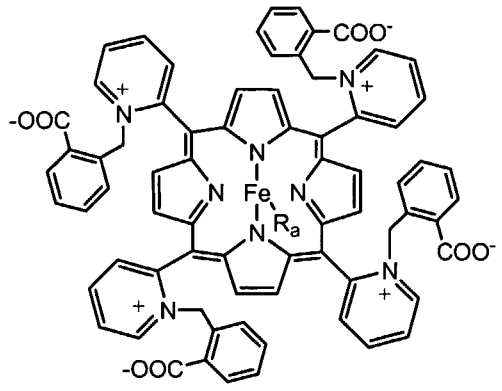
Compound **12** is obtained according to Examples 12, 3 (Method 2), and 7.

Isolation of Compound 12:

Compound **12** is isolated as set forth in Example 4, Method 2, but the initial titration to pH 6 and the precipitation of Compound **12** was omitted. Crude Compound **12** was dissolved in 0.1 N HCl and loaded onto the MCI-gel column directly.

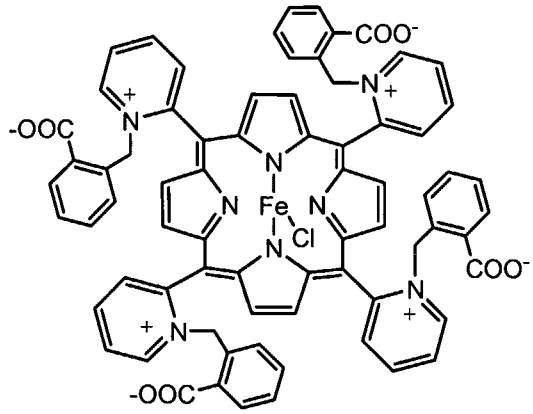
6.9 EXAMPLE 9

Synthesis of Compound 27 and Compound 15



Ra = 2-methylbenzoate

27



15

Synthesis of 2-bromomethylbenzoic acid:

In a 1 L round bottom flask fitted with a reflux condenser, a stirred suspension of 10.00 g of *o*-toluic acid and 19.56 g (1.5 eq.) *N*-bromosuccinimide in 735 mL chloroform was sparged for 0.5 h with nitrogen. The sparging was discontinued, and the suspension was stirred and irradiated under nitrogen atmosphere using a 500 W quartz halogen lamp at 75% power, causing the solids to dissolve and the chloroform to reflux. The red color of the reaction mixture disappeared after 1.5 h, and 6.52 g (0.5 eq.) of *N*-bromosuccinimide was added. The reaction mixture was stirred and irradiated under nitrogen atmosphere with a 500 W quartz halogen lamp at 75% power for another 1.5 h, at which time the solution became colorless. The solvent volume was reduced *in vacuo* to about 100 mL, and then cooled to –20° C. The resultant suspension was vacuum filtered through a 1 cm bed of dry silica in a 150 mL fritted funnel. The silica was washed with 2.5 L of chloroform. The chloroform filtrate was reduced *in vacuo* to about 1 L, washed in a separatory funnel with 3 x 1 L volumes of water followed by one 3 x 1 L volume of brine to remove traces of succinimide, then dried with magnesium sulfate and vacuum filtered. The chloroform was reduced by rotary evaporation at reflux at 1 atmosphere to 250 mL and cooled at –20° C for 3 days. The resultant crystals were vacuum filtered, washed with 30 mL of chloroform followed by 50 mL of hexanes, then dried in a vacuum

oven at room temperature overnight, providing 8.48 g (54%) 2-bromomethylbenzoic acid as a white crystalline power.

Formation of Compound 27:

Compound **27** was obtained according to Method 2 in Example 3, but by substituting 2-bromomethylbenzoic acid for α-Bromo-*p*-toluic acid.

Isolation of Compound 27:

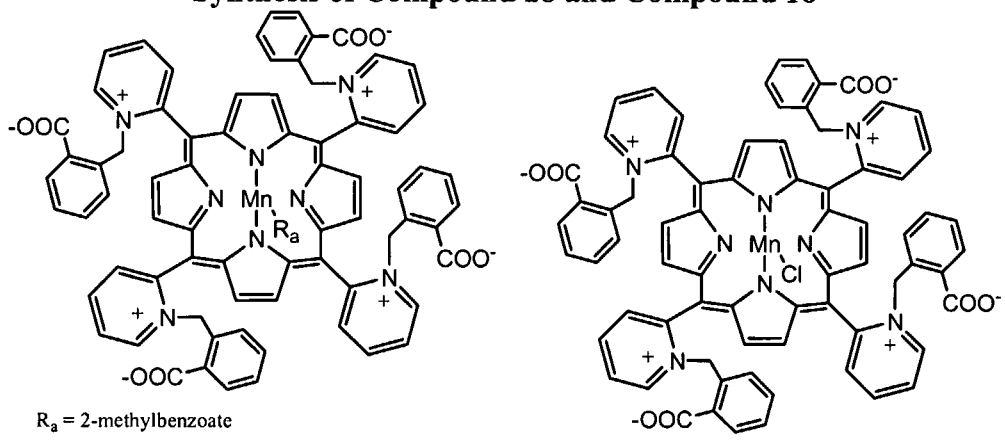
816 mg of Compound **27** obtained above was dissolved in 25 mL of 0.1 N HCl and loaded onto a column of 9.5 g MCI-gel prepared in 0.1 N HCl. The column was eluted with 250 mL of 0.1 N HCl, followed by 250 mL of 0.5 N HCl, and 250 mL of 1 N HCl. Fractions having purity greater than 95% were combined and concentrated *in vacuo* to provide 436 mg (44% yield) of Compound **27** (95% purity).

Formation of Compound 15:

Compound **15** is obtained by passing a solution of Compound **27** through a column containing chloride-form anion-exchange resin, e.g., AMBERLITE IRA-402 (chloride form) strongly-basis anion-exchange resin. The effluent is concentrated *in vacuo* to provide Compound **15**.

6.10 EXAMPLE 10

Synthesis of Compound 28 and Compound 18



28 **18**

Formation of Compound 28:

Compound **28** was obtained according to Examples 12, 3 (Method 2), and 9.

Isolation of Compound 28:

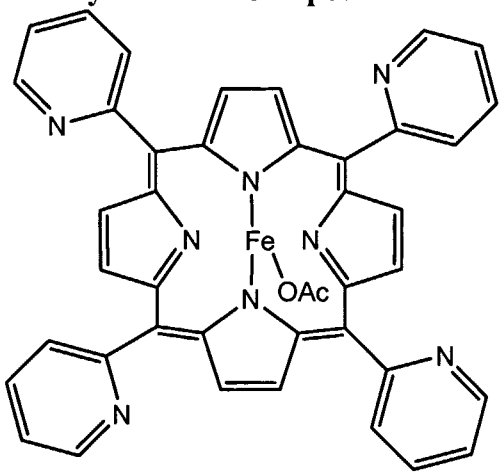
Compound **28** was isolated as set forth in Example 9, but Compound **28** was isolated as its pentachloride salt (n = 5) (95% purity).

Formation of Compound 18:

Compound **18** is obtained by passing a solution of Compound **28** through a column containing chloride-form anion-exchange resin, e.g., AMBERLITE IRA-402 (chloride form) strongly-basis anion-exchange resin. The effluent is concentrated *in vacuo* to provide Compound **18**.

6.11 EXAMPLE 11

Synthesis of Compound 21

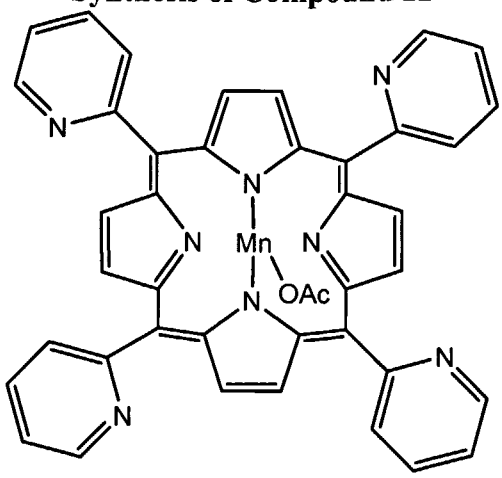


21

Compound **1** (200.0 g) was suspended in 3.2 L of acetic acid and 800 mL of deionized water, and 253.3 g (2.0 eq.) of ferrous ammonium sulfate hexahydrate were added. Air was bubbled slowly through the reaction mixture, which was then refluxed overnight. The hot reaction mixture was transferred to a rotary evaporator, and the solvent was removed *in vacuo*. The resultant solids were suspended with vigorous stirring for 3 hours in 4 L of 10% ammonium hydroxide, vacuum filtered through #50 paper, and washed four times with 1 L portions of deionized water. The slightly damp solids were stirred for 1 hour in 24 L ethanol and vacuum filtered through 500 g celite in a medium fritted funnel. The filtrate was transferred to the rotary evaporator and concentrated *in vacuo*. The resultant solids were dried under vacuum at 40 °C for 1 day to provide 164.0 g (68%) of Compound **21** as a deep-purple solid. MS m/z= 672 (M^+).

6.12 EXAMPLE 12

Synthesis of Compound 22



22

Compound **1** (1.00 g) was suspended in 10 mL of acetic acid, and 440 mg (1.01 eq.) of manganese (III) acetate dihydrate was added. The reaction mixture was refluxed overnight, cooled to room temperature, and the solvent was transferred to an evaporator flask and removed *in vacuo*. Ammonium hydroxide (30% aqueous, 20 mL) was added to the evaporator flask and subsequently removed *in vacuo*. The resultant solids were twice dissolved in methanol (20 mL), which was subsequently evaporated. The resultant black solid was dissolved in 50 mL of dichloromethane and vacuum filtered through a 3 cm thick bed of celite. The filtrate was concentrated *in vacuo* and the resultant solid was dried overnight to provide 1.28 g (95%) of Compound **22** as a metallic black solid. MS m/z= 671 (M^+).

6.13 EXAMPLE 13

In Vivo Efficacy of an Illustrative Porphyrin Compound Against Radiation-Induced Death

Materials and Methods

Balb/c mice used in the following experiments were 8 weeks old, either male or female, and had an average body weight of 24 g. Compound **3A** (obtained using the methods outlined in Examples 3 and 4) was administered to the treated animals subcutaneously as a solution in 0.9% normal saline with each individual dose administered in a total solution volume of 0.1 mL. Both treated and control mice were exposed to a 6 Gy dose of ionizing radiation, delivered via a

Gammacell 3000 Elan Irradiator (MDS Nordion, Ontario, Canada). To administer the radiation dose, a mouse was placed in a beaker in the irradiation chamber with the sealed radiation source for approximately one minute to deliver a dose of 6 Gy. The animals' "survival ratio" was calculated by dividing the number of surviving mice by the total number of irradiated mice.

Pre-irradiation treatment of Animals with Compound 3A

Balb/c mice were divided into two groups of about ten mice each: a control group and a treatment group. Each mouse in the control group was subcutaneously administered 0.1 mL saline two hours prior to irradiation, followed by repeated subcutaneous administrations of 0.1 mL saline every 12 hours afterward. Each mouse in the treatment group was subcutaneously administered a 2 mg/kg dose of Compound **3A** (in 0.1 mL saline) two hours prior to irradiation, followed by repeated subcutaneous administrations of a 2 mg/kg dose of Compound **3A** (in 0.1 mL saline) every 12 hours afterward. Dosing was continued in each animal in both the control and treatment groups until the death of all of the mice in the control group. Mice in the treatment group survived longer than the control mice with mortality being prevented in 20% of treated animals (FIG. 1). Accordingly, Compound **3A**, an illustrative Pyridyl-Substituted Porphyrin Compound, is useful for preventing radiation-induced death in a subject.

Post-irradiation treatment of Mice with Compound 3A at Dosage of 2 mg/kg

Balb/c mice were divided into two groups of about ten mice each: a control group and a treatment group. Each mouse in the control group was subcutaneously administered 0.1 mL saline ten minutes after irradition, followed by repeated subcutaneous administrations of 0.1 mL saline every 12 hours afterward. Each mouse in the treatment group was subcutaneously administered a 2 mg/kg dose of Compound **3A** (in 0.1 mL saline) ten minutes after irradiation, followed by repeated subcutaneous administrations of a 2 mg/kg dose of Compound **3A** (in 0.1 mL saline) every 12 hours afterward. Dosing was continued in each animal in both the control and treatment groups until the death of all of the mice in the control group. Mice in the treatment group survived longer than the control mice by approximately 2-4 days (FIG. 2). Accordingly, Compound **3A**, an illustrative Pyridyl-Substituted

Porphyrin Compound, is useful for increasing a subject's survival time following exposure to radiation.

Post-irradiation treatment of Mice with Compound 3A at Dosage of 10 mg/kg

Balb/c mice were divided into two groups of about ten mice each; a control group and a treatment group. Each mouse in the control group was subcutaneously administered 0.1 mL saline ten minutes after irradition, followed by repeated subcutaneous administrations of 0.1 mL saline every 12 hours afterward. Each mouse in the treatment group was subcutaneously administered a 10 mg/kg dose of Compound **3A** (in 0.1 mL saline) ten minutes after irradiation, followed by repeated subcutaneous administrations of a 10 mg/kg dose of Compound **3A** (in 0.1 mL saline) every 12 hours afterward. Dosing was continued in each animal in both the control and treatment groups until the death of all of the mice in the control group. This dosing regimen prevented radiation-induced death in all of the treated mice, while all mice in the control group died (FIG. 3). Accordingly, Compound **3A**, an illustrative Pyridyl-Substituted Porphyrin Compound, is useful for preventing radiation-induced death in a subject.

6.14 EFFECT OF AN ILLUSTRATIVE PYRIDYL-SUBSTITUTED PORPHYRIN COMPOUND IN VARIOUS DISEASE MODELS

Effect of Compound 3 on oxidant or free-radical damage

A549 human epithelial cells and RAW murine macrophages were grown and cultured, then treated with oxidants and free radicals in the presence or absence of varying concentrations of Compound **3** according to the method of C. Szabo et al., *Mol Med.*, 2002 Oct;8(10):571-80. Compound **3** dose-dependently protected against the suppression of cell viability (FIG. 4). Protection is by 3-100 μM of Compound **3**.

These data indicate that Compound **3** is useful for protecting cells or tissue from damage from reactive species including oxidants and free radicals, and for treating or preventing various forms of shock, inflammation, reperfusion injury, heart failure, vascular disease, or radiation-induced injury.

Effect of Compound 3 on myocardial infarction in rats

Rats were subjected to myocardial infarction by occlusion and reperfusion of the left anterior descending coronary artery as previously described in C.Y. Xiao et al., *J Pharmacol Exp Ther.*, 2004 Aug;310(2):498-504. Compound **3** was administered at doses of 1, 3, or 6 mg/kg i.v., 5 minutes prior to reperfusion. At 6 mg/kg, Compound **3** reduced plasma levels of creatine kinase (indicative of reduced myocardial necrosis, FIG. 5). 6 mg/kg of Compound **3** produced a significant protective effect. Compound **3** was also effective for reducing infarct size in the rats (FIG. 6).

These data indicate that Compound **3** is useful for protecting myocardial tissues from damage when administered during myocardial reperfusion, and for treating or preventing myocardial infarction and reperfusion injury resulting from cardiopulmonary bypass.

Effect of Compound 3 on hemorrhagic shock in rats

Rats were subjected to 2 hours of hemorrhage, followed by resuscitation as previously described in O.V. Evgenov et al., *Crit Care Med.*, 2003 Oct;31(10):2429-36. Compound **3** was administered at a dose of 6 mg/kg i.v., 5 minutes prior to resuscitation. Compound **3** reduced plasma levels of creatine kinase and ALT (indicative of reduced cell necrosis). Compound **3** was also effective for stabilizing blood pressure and increasing survival rate in the rats (FIGS. 7-10). To obtain the results shown in FIG. 7, rats were bled to reach mean BP of 40 mm Hg. This mean BP was maintained for 2 hours, followed by resuscitation with saline at a volume of 2x the shed blood volume. Rats were then observed for 3 hours, and the survival time was recorded. Compound **3** (6 mg/kg) was administered intravenously before the start of resuscitation. To obtain the results shown in FIG. 8, left intraventricular systolic pressure (LVSP), dP/dt, -dP/dt were monitored continuously for 20 minutes from 40 minutes after resuscitation. Compound **3** (6 mg/kg) was administered intravenously before the start of resuscitation. To obtain the results shown in FIG. 9, blood was taken 1 hour after resuscitation. Compound **3** (6 mg/kg) was administered intravenously before the start of resuscitation. To obtain the results shown in FIG. 10, blood was taken at 1 hour after resuscitation. Compound **3** (6 mg/kg) was administered intravenously before the start of resuscitation.

These data indicate that Compound **3** is useful for protecting a subject from various forms of circulatory shock and for treating or preventing sepsis, systemic inflammatory response syndrome, hemorrhagic shock, cardiogenic shock, and systemic inflammation induced by anticancer therapies, such as IL-2.

Effect of Compound 3 on heart failure in mice

Mice were subjected to heart failure induced by aortic banding as previously described in C.Y. Xiao et al., *J Pharmacol Exp Ther.*, 2005 Mar;312(3):891-8. Compound **3** was administered at a dose of 3 mg/kg/day orally. Compound **3** reduced the degree of myocardial hypertrophy (FIG. 11).

These data indicate that Compound **3** is useful for treating heart failure.

Effect of Compound 3 on rejection of hearts during heterotopic heart transplantation

Rats were subjected to heterotopic heart transplantation as described previously in H. Jiang et al., *Transplantation*, 2002 Jun 15;73(11):1808-17. Compound **3** was administered at a dose of 10 mg/kg/day orally. Compound **3** reduced the degree of myocardial hypertrophy (FIG. 12).

These data indicate that Compound **3** is useful for treating or preventing a reperfusion injury resulting from organ transplantation.

Effect of Compound 3 on vascular injury

Rats were subjected to balloon-induced vascular injury of the carotid artery as previously described in C. Zhang et al., *Am J Physiol Heart Circ Physiol.*, 2004 Aug;287(2):H659-66. Compound **3** (1 mg/kg bid) prevented the development of endothelial dysfunction after balloon-induced vascular injury and reduced the degree of intimal hypertrophy (FIG. 13 and FIG. 14). As shown in FIG. 13, the injured rat showed an impairment of the endothelium-dependent relaxations, compared to the non-injured (control) side, and Compound **3** treatment completely prevented this loss of the endothelial function (n = 4-7).

These data indicate that Compound **3** is useful for reducing the degree of vascular injury associated with cardiovascular diseases including balloon-induced vascular injury, coronary stenting, and atherosclerosis.

*Effect of Compound **3** on diabetes mellitus*

Mice were subjected to multiple low dose streptozocin diabetes as previously described in J.G. Mableyet al., *Br J Pharmacol.*, 2001 Jul;133(6):909-19. Compound **3** (3 or 10 mg/kg/day ip) prevented the development of hyperglycemia and normalized pancreatic insulin content (FIG. 15).

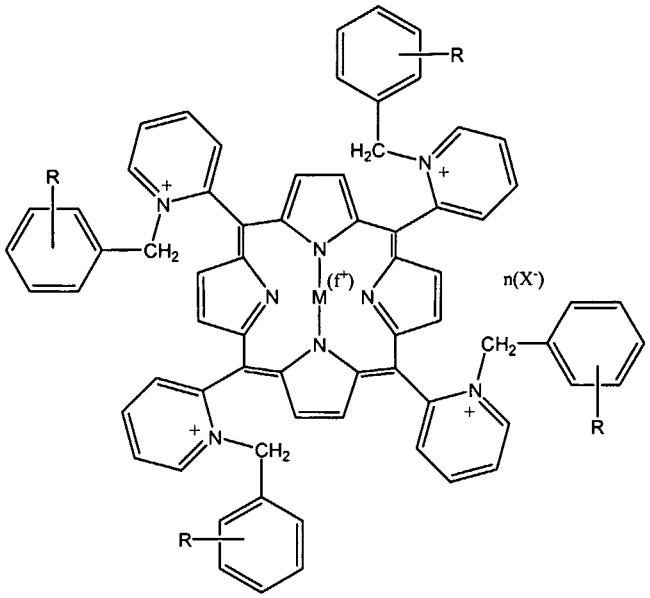
These data indicate that Compound **3** is useful for treating or preventing diabetes or one or more of its complications.

The present invention is not to be limited in scope by the specific embodiments disclosed in the examples, which are intended as illustrations of a few aspects of the invention and any embodiments that are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the scope of the appended claims.

All references cited herein are incorporated by reference in their entirety.

What is claimed is:

1. A compound having the formula



(A)

wherein:

M is Fe or Mn;

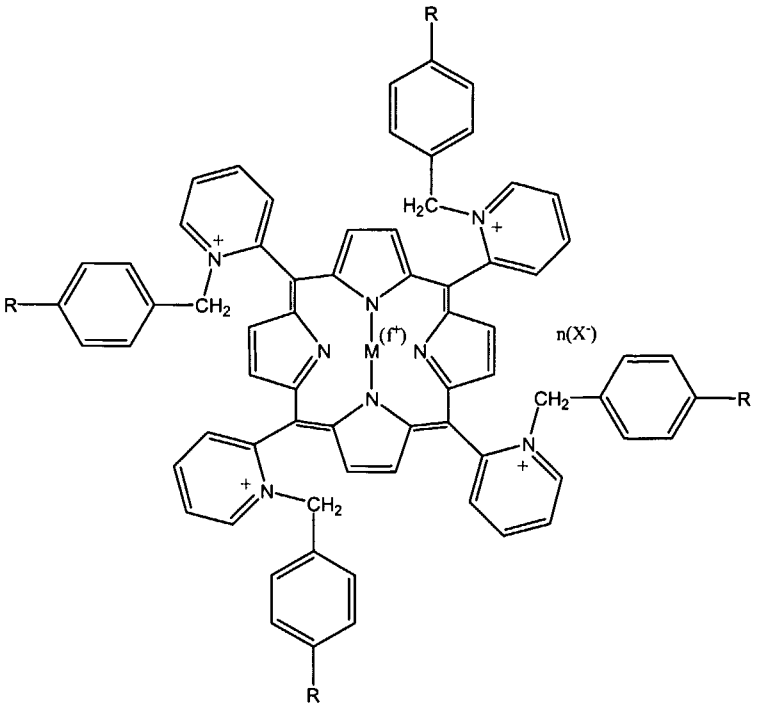
f is 0 or 1;

each R is independently –C(O)OH or –C(O)O$^-$;

each X$^-$ is independently a negatively-charged counterion; and

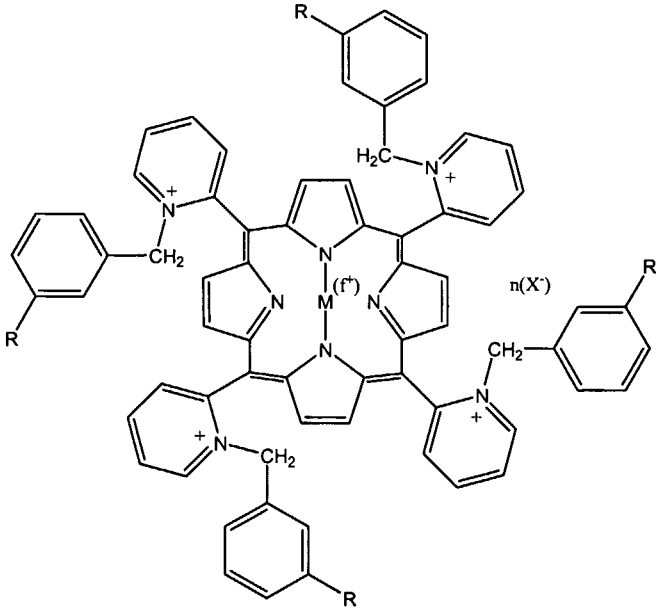
n = (f) + (the total number of R groups where R is –C(O)OH).

2. The compound of claim 1, having the formula:



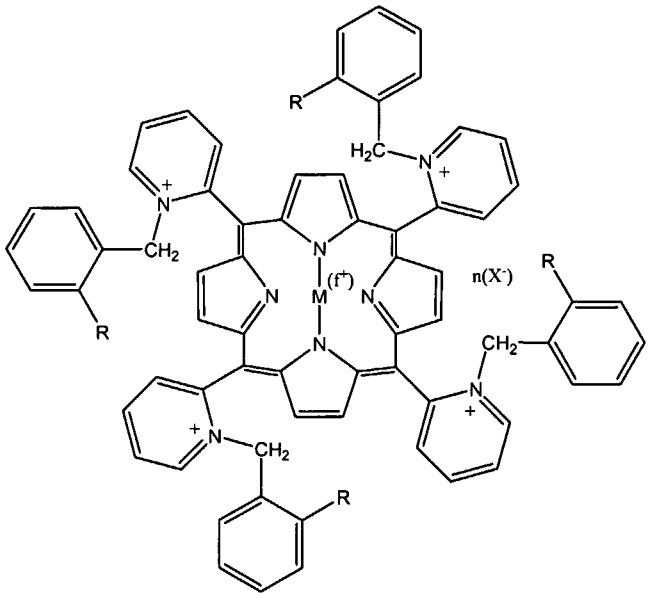
(I).

3. The compound of claim 1, having the formula



(II).

4. The compound of claim 1, having the formula



(III).

5. The compound of claim 1, wherein M is Fe.

6. The compound of claim 1, wherein M is Mn.

7. The compound of claim 1, wherein f is 0.

8. The compound of claim 1, wherein f is 1.

9. The compound of claim 1, wherein X^- is Cl^- or Br^-.

10. The compound of claim 1, wherein an X^- forms a bond with M.

11. The compound of claim 1, wherein X^- is F^-, Cl^-, Br^-, I^-, HO^-, or $CH_3C(O)O^-$.

12. The compound of claim 1, wherein each R is $-C(O)O^-$.

13. The compound of claim 1, wherein each R is $-C(O)OH$.

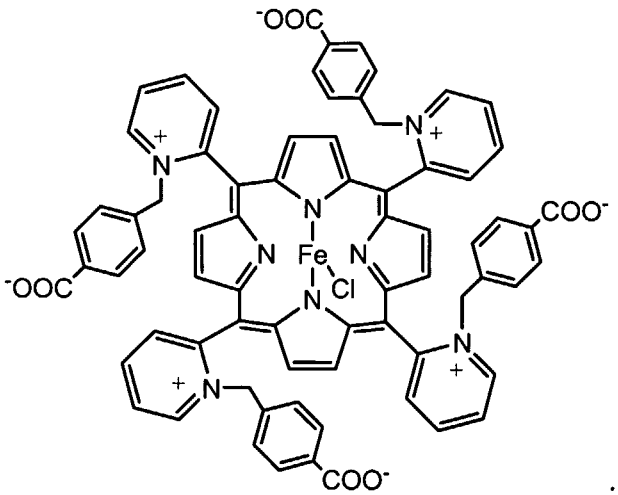
14. The compound of claim 1, wherein n is 0.

15. The compound of claim 1, wherein n is 5.

16. The compound of claim 5, wherein f is 1, and X is Cl^-.

17. The compound of claim 16, wherein each R is –C(O)O⁻.

18. The compound of claim 1, having the structure



19. A composition comprising an effective amount of the compound of claim 1 and a physiologically acceptable carrier or vehicle.

20. A method for treating an inflammatory condition, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

21. A method for treating an inflammatory condition, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

22. The method of claim 20, wherein the inflammatory condition is an inflammatory condition of a joint, a chronic inflammatory condition of the gum, an inflammatory bowel disease, an inflammatory lung disease, an inflammatory condition of the central nervous system, an inflammatory condition of the eye, gram-positive shock, gram negative shock, hemorrhagic shock, anaphylactic shock, traumatic shock, chemotherapeutic shock, or shock induced in response to administration of a pro-inflammatory cytokine.

23. The method of claim 21, wherein the inflammatory condition is an inflammatory condition of a joint, a chronic inflammatory condition of the gum, an

inflammatory bowel disease, an inflammatory lung disease, an inflammatory condition of the central nervous system, an inflammatory condition of the eye, gram-positive shock, gram negative shock, hemorrhagic shock, anaphylactic shock, traumatic shock chemotherapeutic shock, or shock induced in response to administration of a pro-inflammatory cytokine.

24. A method for treating a reperfusion injury, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

25. A method for treating a reperfusion injury, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

26. The method of claim 24, wherein the reperfusion injury is stroke, myocardial infarction, or a reoxygenation injury resulting from organ transplantation.

27. The method of claim 25, wherein the reperfusion injury is stroke, myocardial infarction, or a reoxygenation injury resulting from organ transplantation.

28. The method of claim 26, wherein the organ transplantation is cardiac transplantation or kidney transplantation.

29. The method of claim 27, wherein the organ transplantation is cardiac transplantation or kidney transplantation.

30. A method for treating an ischemic condition, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

31. A method for treating an ischemic condition, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

32. The method of claim 20, wherein the ischemic condition is myocardial ischemia, stable angina, unstable angina, stroke, ischemic heart disease or cerebral ischemia.

33. The method of claim 31, wherein the ischemic condition is myocardial ischemia, stable angina, unstable angina, stroke, ischemic heart disease or cerebral ischemia.

34. A method for treating a radiation-induced injury, the method comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

35. A method for treating a radiation-induced injury, the method comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

36. The method of claim 34, wherein the radiation-induced injury is acute radiation sickness.

37. The method of claim 35, wherein the radiation-induced injury is acute radiation sickness.

38. A method for treating diabetes, the method comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

39. A method for treating diabetes, the method comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

40. The method of claim 38, wherein the diabetes is diabetes mellitus.

41. The method of claim 39, wherein the diabetes is diabetes mellitus.

42. The method of claim 38, wherein the diabetes is Type I diabetes or Type II diabetes.

43. The method of claim 39, wherein the diabetes is Type I diabetes or Type II diabetes.

44. A method for treating a diabetic complication, the method comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

45. A method for treating a diabetic complication, the method comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

46. The method of claim 44, wherein the diabetic complication is diabetic neuropathy, retinopathy, neuropathy, angiopathy, cardiomyopathy, or erectile dysfunction.

47. The method of claim 45, wherein the diabetic complication is diabetic neuropathy, retinopathy, neuropathy, angiopathy, cardiomyopathy, or erectile dysfunction.

48. A method for treating a cardiovascular disease, the method comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

49. A method for treating a cardiovascular disease, the method comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

50. The method of claim 48, wherein the cardiovascular disease is acute heart failure, chronic heart failure, ischemic heart failure, drug-induced heart failure, idiopathic heart failure, alcoholic heart failure, or a cardiac arrhythmia.

51. The method of claim 49, wherein the cardiovascular disease is acute heart failure, chronic heart failure, ischemic heart failure, drug-induced heart failure, idiopathic heart failure, alcoholic heart failure, or a cardiac arrhythmia.

52. The method of claim 48, wherein the cardiovascular disease is balloon-induced vascular injury, coronary stenting, atherosclerosis, or restenosis.

53. The method of claim 49, wherein the cardiovascular disease is balloon-induced vascular injury, coronary stenting, atherosclerosis, or restenosis.

54. A method for treating cancer, comprising administering to a subject in need thereof an effective amount of a compound of claim 1.

55. A method for treating cancer, comprising administering to a subject in need thereof an effective amount of a compound of claim 18.

56. The method of claim 54, wherein the cancer is colorectal cancer, lung cancer, pancreatic cancer, esophageal cancer, stomach cancer, skin cancer, leukemia, lymphoma, testicular cancer, bladder cancer, breast cancer, prostate cancer, head and neck cancer or ovarian cancer.

57. The method of claim 55, wherein the cancer is colorectal cancer, lung cancer, pancreatic cancer, esophageal cancer, stomach cancer, skin cancer, leukemia, lymphoma, testicular cancer, bladder cancer, breast cancer, prostate cancer, head and neck cancer or ovarian cancer.

58. A method for treating a side effect of cancer chemotherapy, comprising administering to a subject in need thereof an effective amount of a compound of claim 1.

59. A method for treating a side effect of cancer chemotherapy, comprising administering to a subject in need thereof an effective amount of a compound of claim 18.

60. The method of claim 58, wherein the cancer chemotherapy comprises administering a platinum-based chemotherapy agent to the subject.

61. The method of claim 59, wherein the cancer chemotherapy comprises administering a platinum-based chemotherapy agent to the subject.

62. The method of claim 60, wherein the platinum-based chemotherapy agent is cisplatin.

63. The method of claim 61, wherein the platinum-based chemotherapy agent is cisplatin.

64. The method of claim 36, wherein the radiation-induced injury is caused by radiation therapy administered to a subject for the treatment of cancer.

65. The method of claim 37, wherein the radiation-induced injury is caused by radiation therapy administered to a subject for the treatment of cancer.

66. A method for preventing radiation-induced death, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

67. A method for preventing radiation-induced death, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

68. A method for increasing a subject's survival time following exposure to radiation, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

69. A method for increasing a subject's survival time following exposure to radiation, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

70. A method for treating or preventing injury due to exposure to a reactive species, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

71. A method for treating or preventing injury due to exposure to a reactive species, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

72. A method for treating or preventing an inflammatory skin disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

73. A method for treating or preventing an inflammatory skin disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

74. The method of claim 72, wherein the inflammatory skin disease is contact dermatitis, erythema, or psoriasis.

75. The method of claim 73, wherein the inflammatory skin disease is contact dermatitis, erythema, or psoriasis.

76. A method for treating or preventing skin wrinkling, skin aging, sunburn erythema, UV-induced skin injury, or UV-induced skin disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

77. A method for treating or preventing skin wrinkling, skin aging, sunburn erythema, UV-induced skin injury, or UV-induced skin disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

78. A method of treating or preventing erectile dysfunction caused by surgery, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

79. A method of treating or preventing erectile dysfunction caused by surgery, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

80. The method of claim 78, wherein the surgery is surgery of the prostate or the colon.

81. The method of claim 79, wherein the surgery is surgery of the prostate or the colon.

82. A method for treating or preventing a lung disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

83. A method for treating or preventing a lung disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

84. The method of claim 82, wherein the lung disease is cystic fibrosis, hyperoxic lung injury, emphysema, or adult respiratory distress syndrome.

85. The method of claim 83, wherein the lung disease is cystic fibrosis, hyperoxic lung injury, emphysema, or adult respiratory distress syndrome.

86. A method for treating or preventing injury due to hyperoxia, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

87. A method for treating or preventing injury due to hyperoxia, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

88. The method of claim 86, wherein the injury due to hyperoxia is hyperoxia-induced eye injury or hyperoxia-induced lung injury.

89. The method of claim 87, wherein the injury due to hyperoxia is hyperoxia-induced eye injury or hyperoxia-induced lung injury.

90. A method for treating or preventing a neurodegenerative disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

91. A method for treating or preventing a neurodegenerative disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

92. The method of claim 90, wherein the neurodegenerative disease is Parkinson's disease, Alzheimer's disease, Huntington's disease, or amyotrophic lateral sclerosis.

93. The method of claim 91, wherein the neurodegenerative disease is Parkinson's disease, Alzheimer's disease, Huntington's disease, or amyotrophic lateral sclerosis.

94. A method for treating or preventing a liver disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

95. A method for treating or preventing a liver disease, comprising administering to a subject in need thereof an effective amount of the compound of claim 18.

96. The method of claim 94, wherein the liver disease is hepatitis, liver failure, or drug-induced liver injury.

97. The method of claim 95, wherein the liver disease is hepatitis, liver failure, or drug-induced liver injury.

98. A method for method for inducing cardioplegia comprising administering to a subject in need thereof an effective amount of a cardioplegia-inducing agent and the compound of claim 1.

99. A method for method for inducing cardioplegia comprising administering to a subject in need thereof an effective amount of a cardioplegia-inducing agent and the compound of claim 18.

100. A method for prolonging the half-life of an oxidation-prone compound *in vivo* comprising administering to a subject in need thereof an effective amount of the compound of claim 1.

101. A method for prolonging the half-life of an oxidation-prone compound *in vivo* comprising administering to a subject in need thereof an effective amount of the compound of claim 18.