(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
05 March 2020 (05.03.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/046543 A2

(51) International Patent Classification:
Not classified

(21) International Application Number:
PCT/US2019/045250

(22) International Filing Date:
06 August 2019 (06.08.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/714,856 06 August 2018 (06.08.2018) US

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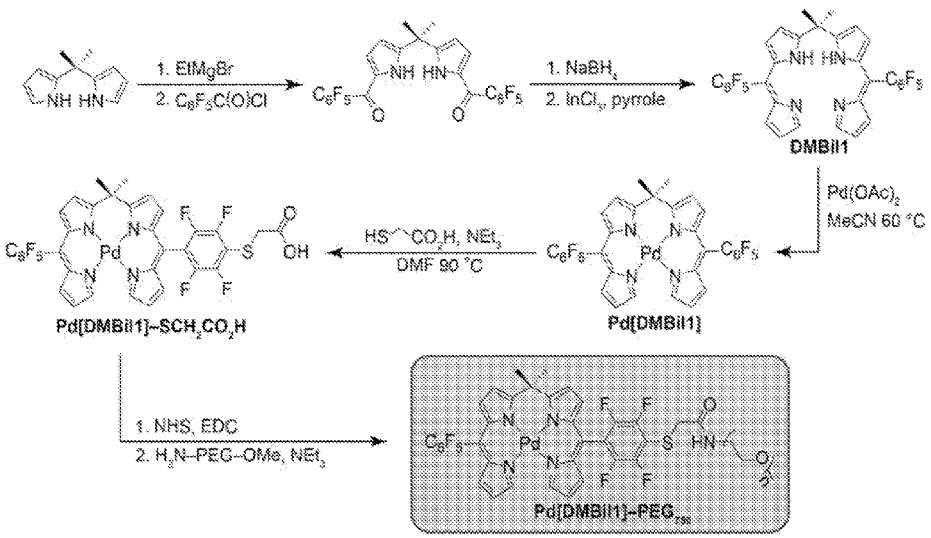
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV,

(54) Title: WATER SOLUBLE TETRAPYRROLE COMPLEXES CONTAINING BILADIENE LIGANDS USEFUL IN PHOTODYNAMIC THERAPY

Fig. 1



(57) Abstract: Water soluble tetrapyrrole complexes of metals are useful in photodynamic therapy, wherein the complexes are capable of functioning as photosensitizers which when activated by light lead to the generation of singlet oxygen. The singlet oxygen thereby produced is toxic to cancer cells. The tetrapyrrole ligands present in such complexes are characterized by the presence of at least one water-solubilizing segment (which may be provided in the form of a poly(oxyalkylene)-containing substituent, for example), which helps to promote the biocompatibility of the complex.

[Continued on next page]

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MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

WATER SOLUBLE TETRAPYRROLE COMPLEXES CONTAINING BILADIENE LIGANDS USEFUL IN PHOTODYNAMIC THERAPY

Cross-Reference to Related Application

This application claims priority to United States Provisional Application No. 62/714,856, filed 6 August 2018, the entire disclosure of which is incorporated herein by reference in its entirety for all purposes.

Government License Rights

This invention was made with government support under Contract No. CHE1352120 awarded by the National Science Foundation (NSF) and Contract No. P20GM104316 awarded by the National Institutes of Health (NIH). The government has certain rights in the invention.

Field of the Invention

The present invention relates to substituted derivatives of linear tetrapyrroles (biladienes) which are capable of forming water soluble metal complexes useful as photochemotherapeutic agents in photodynamic therapy and other applications. Such complexes are referred to herein as "tetrapyrrole complexes" and are described in more detail below.

Background of the Invention

Photodynamic therapy (PDT) represents a minimally invasive and highly localized treatment strategy to ablate tumors in patients with few side effects. In PDT, photosensitizers embedded within tumors are activated by light and undergo intersystem crossing followed by energy transfer to molecular oxygen, resulting in the production of toxic singlet oxygen. The singlet oxygen thereby produced is capable of destroying tumor cells. In PDT, photosensitizing compounds are either intratumorally or intraveneously injected and circulated throughout the body prior to irradiation of a tumor site.

PDT is a promising treatment strategy for at least certain types of cancers and skin conditions because it is less invasive than surgical options, has fewer side effects than radiation or chemotherapy, and has been shown to stimulate antitumor responses. Despite these promising benefits of PDT, its widespread clinical use is met with three key limitations. First, photosensitizers often have high toxicity to healthy tissues even without light application, which limits the allowable administered dosages. Further, successful PDT requires sufficient oxygen presence in the native tissue to produce toxic $^1O_2$. However, the microenvironment deep within solid tumors is often hypoxic, thereby rendering the photosensitizers ineffective in these regions. Lastly,

most photosensitizers for PDT are activated by short wavelengths of light (<600 nm) that cannot deeply penetrate tissue, resulting in uneven therapeutic effects throughout the tumor space. Photosensitizers have been developed that can be activated with longer wavelengths of light for enhanced tissue penetration, but unfortunately this approach is still ineffective in hypoxic tumor regions and these photosensitizers still suffer from relatively high off-target toxicities even without light application.

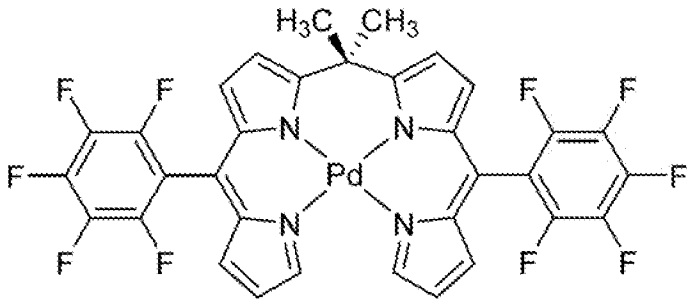
Ideally, a photosensitizer intended for use in PDT has the following characteristics:

- Low dark toxicity
- A high degree of chemical purity
- Easy/inexpensive to synthesize
- High $^1O_2$ quantum yield
- Soluble in aqueous environments
- Selective for tumor cells
- Strong absorption between 600 and 850 nm

Achieving all of these characteristics simultaneously have proven to be very challenging. Thus, there is still a significant need for improved photosensitizers useful in PDT.

Summary of the Invention

A robust, linear tetrapyrrole palladium(II) complex, referred to as **Pd[DMBil1]** and having the following structure, has previously been reported. See Potocny et al., Electrochemical, Spectroscopic, and $^1O_2$ Sensitization Characteristics of Synthetically Accessible Linear Tetrapyrrole Complexes of Palladium and Platinum. *Inorg. Chem.* 2017, *56*, 12703-12711.



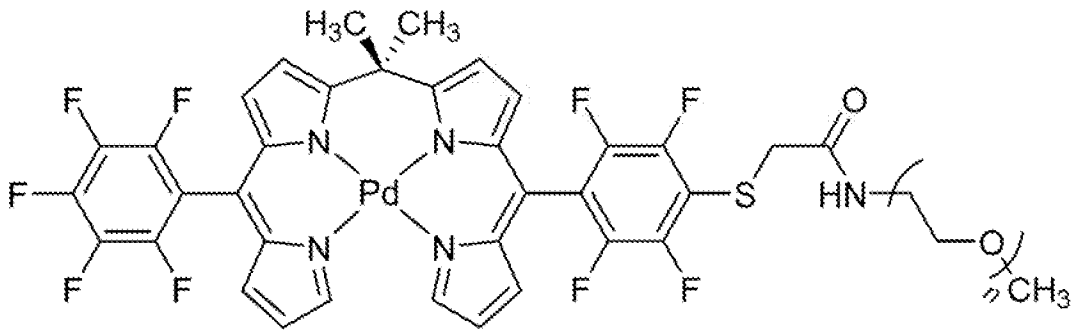
Pd[DMBil1]

This complex is characterized by its facile and modular synthesis, broad absorption profile, and efficient $^1O_2$ quantum yield of $\Phi_\Delta = 0.8$ in organic media. However, the insolubility of this porphrinoid derivative in aqueous solution prevents its use under biologically relevant conditions.

Water soluble diorganobiladiene derivatives have now been developed that are appended with one or more water-solubilizing segments, in particular poly(alkylene) glycol functionalities

such as a poly(ethylene) glycol functionality. Such derivatives provide tetrapyrrole complexes which are comprised of a metal, such as Pd or Pt, complexed by a 10,10-diorgano-5,15-diarylbiladiene ligand which bears at least one substituent comprised of a water-solubilizing segment.

Such functionalized complexes, exemplified by **Pd[DMBil1]-PEG$_{750}$**, are capable of maintaining the attractive photophysical properties of the corresponding parent complexes under biologically relevant conditions. Introduction of the poly(alkylene) glycol functionality has been found to overcome the inherent hydrophobicity of the biladiene architecture. The addition of this functionality endows the linear tetrapyrrole complex with water solubility while having little effect on its photophysical properties, thus generating a biocompatible compound that retains the ability to generate singlet oxygen with a high quantum yield under biologically relevant conditions. While such functionalized biladiene complexes are highly nontoxic in the dark, they can serve as extremely potent chemotherapeutic agents for treatment of cancer cells and drive apoptotic cell death with a high phototoxicity index.



Pd[DMBil1]-PEG$_{750}$ (n = ca. 17)

More specifically , the absorption profile of **Pd[DMBil1]-PEG$_{750}$**, for example, closely matches that of **Pd[DMBil1]** and obeys the Beer-Lambert Law, suggesting that the complex does not aggregate under biologically relevant conditions. Additionally, the emission spectrum of **Pd[DMBil1]-PEG$_{750}$** retains the fluorescence and phosphorescence features characteristic of **Pd[DMBil1]**. Importantly, the PEGylated photosensitizer generates $^1O_2$ with $\Phi_\Delta \approx 0.57$, which is well within the range to warrant interrogation as a potential photodynamic therapy (PDT) agent for treatment of cancer cells. The **Pd[DMBil1]-PEG$_{750}$** is biologically compatible, as it is taken up by MDA-MB-231 triple negative breast cancer (TNBC) cells and has an ED_{50} of only 0.354 μM when exposed to $\lambda_{ex} > 500$ nm light for 30 minutes. Impressively, the LD_{50} of **Pd[DMBil1]-PEG$_{750}$** without light exposure is 1.87 mM, leading to a remarkably high phototoxicity index of ~5300, which is vastly superior to existing photosensitizers that form the basis for clinical PDT treatments. Further, through flow cytometry experiments, it has been shown that PDT with **Pd[DMBil1]-PEG$_{750}$** induces

primarily apoptotic cell death in MDA-MB-231 cells. Overall, these results demonstrate that **Pd[DMBil1]-PEG$_{750}$** is an easily prepared, biologically compatible, and well-tolerated photochemotherapeutic agent that can efficiently drive the photoinduced apoptotic death of TNBC cells.

As described in more detail below, the tetrapyrrole complexes of the present invention are useful as PDT photosensitizers for treatment of cancers. Such complexes have been found to be well tolerated by cancer cells such as TNBC cells and are potent $^1O_2$ sensitizers. Moreover, the inventive tetrapyrrole complexes may be readily synthesized using a comparatively small number of steps from commercially available starting materials and can be purified and isolated using modular methods. Further, the properties and characteristics of the complexes can be easily tuned as may be desired for particular end use applications by varying the different substituents present on the biladiene skeleton of the ligand.

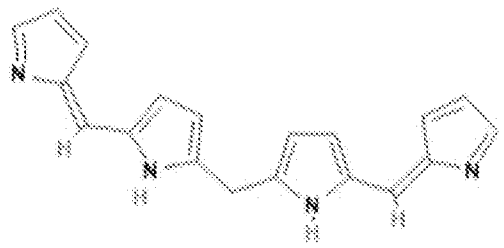
Description of the Drawings

Fig. 1 shows, in summary form, a reaction sequence which can be used to prepare a water soluble tetrapyrrole complex in accordance with the invention.

Detailed Description of Embodiments of the Invention

Tetrapyrrole Complexes

The tetrapyrrole complexes of the present invention may be described as compounds in which a metal such as palladium or platinum is complexed by a 10,10-diorgano-5,15-diarylbiladiene ligand which bears at least one substituent comprised of a water-solubilizing segment such as poly(oxyalkylene) segment. Such ligands have an a,c-biladiene framework structure containing four linked pyrrole rings, which is non-cyclic ("linear"):



The complexed metal may be, for example, Pd or Pt, but any other metal may also be employed such as platinum group metals generally (Pd, Pt, Ru, Rh, Os, Ir), transition metals (such as Ni, Cu, Zn, Fe, Cd), Group 13 metals (e.g., Al, In, Th, Ga), and other metals (e.g., Mg). The metal may be in ionic form, for example as a divalent, trivalent or tetravalent ion. One or more other anions may be present and

associated with the complex if needed to compensate for any charges that may exist as a result of the complexed metal being selected and its valency.

The ligand is disubstituted at the 10 position with organo groups, which may be the same as or different from each other. The organo groups may be hydrocarbon groups, but could also be organo groups containing one or more heteroatoms such as halogen, nitrogen, oxygen, sulfur and so forth. Suitable hydrocarbon groups include, for example, alkyl groups and/or aryl groups. For example, the alkyl groups may be C1-C6 alkyl groups, straight chain or branched, such as methyl, ethyl, propyl, butyl and the like. Suitable aryl groups include phenyl groups and other aromatic (including hetroaromatic) or conjugated groups. The alkyl or aryl groups may be substituted, for example with halo groups or other heteroatom-containing groups. According to various embodiments, the 10 position is substituted with two alkyl groups (e.g., two methyl groups), two aryl groups (e.g., two phenyl groups), or both an alkyl group and an aryl group (e.g., a methyl group and a phenyl group). Having organo groups present as substituents at the 10 position of the ligand helps to improve the oxidative stability of the tetrapyrrole ligand and metal complexes thereof. If the organo groups are replaced by hydrogens, the ligand can rapidly decompose in the presence of air.

The 10,10-diorgano-5,15-diarylbiladiene ligand is substituted with one or more substituents comprised of a water-solubilizing segment. The water-solubilizing segment(s) help to improve the water solubility, and thus the biocompatibility, of the tetrapyrrole complex, owing to the hydrophilicity of the water-solubilizing segment. As used herein, the term "water-solubilizing segment" refers to a segment (moiety) within the ligand that functions to increase the solubility in water of a complex comprising such ligand as compared to the solubility in water of a complex comprising an analogous ligand that is identical in structure except that it does not comprise such a segment.

According to certain embodiments of the invention, the water-solubilizing segment is oligomeric or polymeric in character and is comprised of two or more hydrophilic repeating units. Suitable water-solubilizing segments include, for example, poly(oxyalkylene) segments, polysaccharide segments, polypeptide segments, poly(thioalkylene) segments, poly(aminoalkylene) segments, polyvinylpyrrolidone segments, aliphatic polyester segments, polyamide segments, polyvinyl alcohol segments, polyacrylic acid segments, polyacrylamide segments, polyoxazoline segments, aliphatic polycarbonate segments, polyphosphate segments, and polyphosphazene segments. According to certain embodiments of the invention, the water-solubilizing segment may contain an average of at least three monomer units with a combined mass of at least 200 g/mol (in another embodiment, a combined mass

of at least 300 g/mol). By increasing the size and/or average molecular weight of the water-solubilizing segment, the water solubility of the resulting tetrapyrrole complex may generally be increased. For example, the number average molecular weight of the water-solublizing segment may be as high as 50,000, 40,000, 30,000, 20,000, 10,000 or 5000 g/mol, although the use of even higher number average molecular weight water-solubilizing segments is possible.

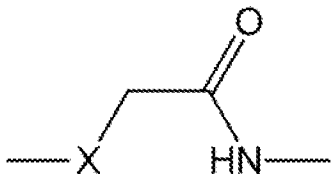
The ligand may bear one, two, three, four or more substituents comprising water-solubilizing segments and such substituent or substituents may be present at any position of the biladiene skeleton, provided such positioning does not interfere with the ability of the ligand to complex with a selected metal. In particular, the substituent or substituents comprising a water-solubilizing segment, such as a poly(oxyalkylene) segment, may for example be substituted on one or both of the aryl groups which are present at the 5 and 15 positions of the a,c-biladiene framework of the ligand.

If a poly(oxyalkylene) segment or poly(oxyalkylene) segments is or are utilized, such segment(s) may be an aliphatic polyether moiety containing repeating oxyalkylene units, such as oxymethylene, oxyethylene, oxypropylene and oxybutylene units or combinations thereof (for example, oxyethylene/oxypropylene). Such poly(oxyalkylene) segments may be formed, for example, by the ring-opening polymerization of cyclic ethers (such as oxetanes, epoxides, and oxolanes) and/or by the condensation of aliphatic glycols such as ethylene glycol, propylene glycol and butylene glycol. According to preferred embodiments of the invention, the poly(oxyalkylene) segment(s) may be poly(oxyethylene) segments. Such poly(oxyethylene segments may correspond to the structure $-(CH_2CH_2O)_n-$, wherein n is an integer from 3 to 250. Depending upon how the ligand is synthetically prepared, there may be a distribution of poly(oxyethylene) segments in which the value of n varies. In such cases, n may refer to the average number of oxyethylene repeating units per segment. According to certain embodiments, n may be from about 3 to about 250 on average, for example. According to other embodiments, the poly(oxyalkylene) segment may have a number average molecular weight of at least 200 g/mol.

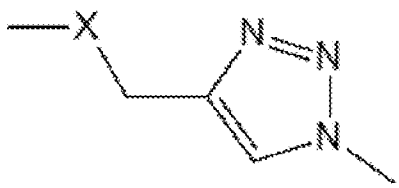
The at least one substituent comprised of a water-solubilizing segment such as a poly(oxyalkylene) segment may be additionally comprised of a terminal group selected from the group consisting of alkyl groups and alkyl groups substituted with at least one functional group. Suitable alkyl groups include, for, example, methyl, ethyl, propyl, butyl and the like, which may be straight chain, branched or cyclic. Suitable functional groups include, without limitation, -SR, NR_2, CO_2R, $-C(=O)NR_2$, $-SO_3R$, $-PO_3R$, $-PR_3^+$, or $-NR_3^+$, wherein each R is independently H or an organo group (such as alkyl, aryl or the like).

Further, the at least one substituent comprised of at least one water-solubilizing segment (e.g., a poly(oxyalkylene) segment) may be additionally comprised of a linking moiety which links (directly or indirectly) the water-solubilizing segment to the biladiene skeleton of the ligand (for example, to an aryl group pendant to the biladiene skeleton). Any of the linking moieties known in the art may be employed.

According to certain embodiments, the linking moiety may for example be selected from the group consisting of:



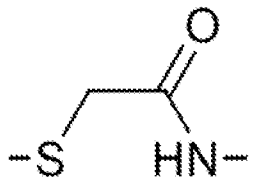
and



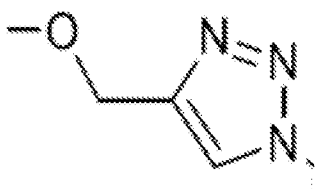
wherein X is O, S, Se, Te, NH, NR, CH_2, CHR, and CR_2, with R being an organo group (e.g., an alkyl group).

For example, the linking moiety may be divalent and may be selected from the group consisting of $-SCH_2C(=O)NH-$ (wherein the nitrogen atom is covalently bonded to a carbon atom of the water-solubilizing segment, e.g., a poly(oxyethylene) segment) and $-O-CH_2$-triazole- (wherein a nitrogen atom of the triazole ring is covalently bonded to a carbon atom of the water-solubilizing segment (e.g., a poly(oxyalkylene) segment).

The structure of the linking moiety $-SCH_2C(=O)NH-$ may be depicted as:



The structure of the linking moiety $-O-CH_2$-triazole- may be depicted as:



The at least one substituent comprised of at least one water-solubilizing segment may comprise at least one biologically active group (which may be a part of the water-solubilizing segment, linking moiety and/or terminal group, but may also be present in the water-solubilizing segment in addition to the water-solubilizing segment, linking moiety or terminal group). The term "biologically active group" can be any group that selectively promotes the accumulation, elimination, binding rate, or tightness of binding in a particular biological environment. For example, one category of biologically active groups is the substituents derived from sugars, specifically, (1) aldoses such as glyceraldehyde, erythrose, threose, ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose, and talose; (2) ketoses such as hydroxyacetone, erythrulose, rebulose, xylulose, psicose, fructose, verbose, and tagatose; (3) pyranoses such as glucopyranose; (4) furanoses such as fructo-furanose; (5) O-acyl derivatives such as penta-O-acetyl-a-glucose; (6) O-methyl derivatives such as methyl a-glucoside, methyl p-glucoside, methyl a-glucopyranoside and methyl-2,3,4,6-tetra-O-methyl glucopyranoside; (7) phenylosazones such as glucose phenylosazone; (8) sugar alcohols such as sorbitol, mannitol, glycerol, and myo-inositol; (9) sugar acids such as gluconic acid, glucaric acid and glucuronic acid, o-gluconolactone, 5-glucuronolactone, ascorbic acid, and dehydroascorbic acid; (10) phosphoric acid esters such as a-glucose 1-phosphoric acid, a-glucose 6-phosphoric acid, a-fructose 1,6-diphosphoric acid, and a-fructose 6-phosphoric acid; (11) deoxy sugars such as 2-deoxy-ribose, rhammose (deoxy-mannose), and fructose (6-deoxy-galactose); (12) amino sugars such as glucosamine and galactosamine; muramic acid and neurarninic acid; (13) disaccharides such as maltose, sucrose and trehalose; (14) trisaccharides such as raffinose (fructose, glucose, galactose) and melezitose (glucose, fructose, glucose); (15) polysaccharides (glycans) such as glucans and mannans; and (16) storage polysaccharides such as a-amylose, amylopectin, dextrins, and dextrans.

Amino acid derivatives are also useful biologically active groups, such as those derived from valine, leucine, isoleucine, threonine, methionine, phenylalanine, tryptophan, alanine, arginine, aspartic acid, cystine, cysteine, glutamic acid, glycine, histidine, proline, serine, tyrosine, asparagine and glutamine. Also useful are peptides, particularly pHlip peptides, cell-penetrating peptides and those peptides known to have affinity for specific receptors, for example, oxytocin, vasopressin, bradykinin, LHRH, thrombin and the like.

Another useful group of biologically active groups are those derived from nucleosides, for example, ribonucleosides such as adenosine, guanosine, cytidine, and uridine; and 2'-deoxyribonucleosides, such as 2'-deoxyadenosine, 2'-deoxyquanosine, 2'-deoxycytidine, and 2'-deoxythymidine.

Another category of biologically active groups that is particularly useful is any ligand that is specific for a particular biological receptor. The term "ligand specific for a receptor" refers to a moiety that binds a receptor at cell surfaces, and thus contains contours and charge patterns that are complementary to those of the biological receptor. The ligand is not the receptor itself, but a substance complementary to it. It is well understood that a wide variety of cell types have specific receptors designed to bind hormones, growth factors, or neurotransmitters. However, while these embodiments of ligands specific for receptors are known and understood, the phrase "ligand specific for a receptor", as used herein, refers to any substance, natural or synthetic, that binds specifically to a receptor.

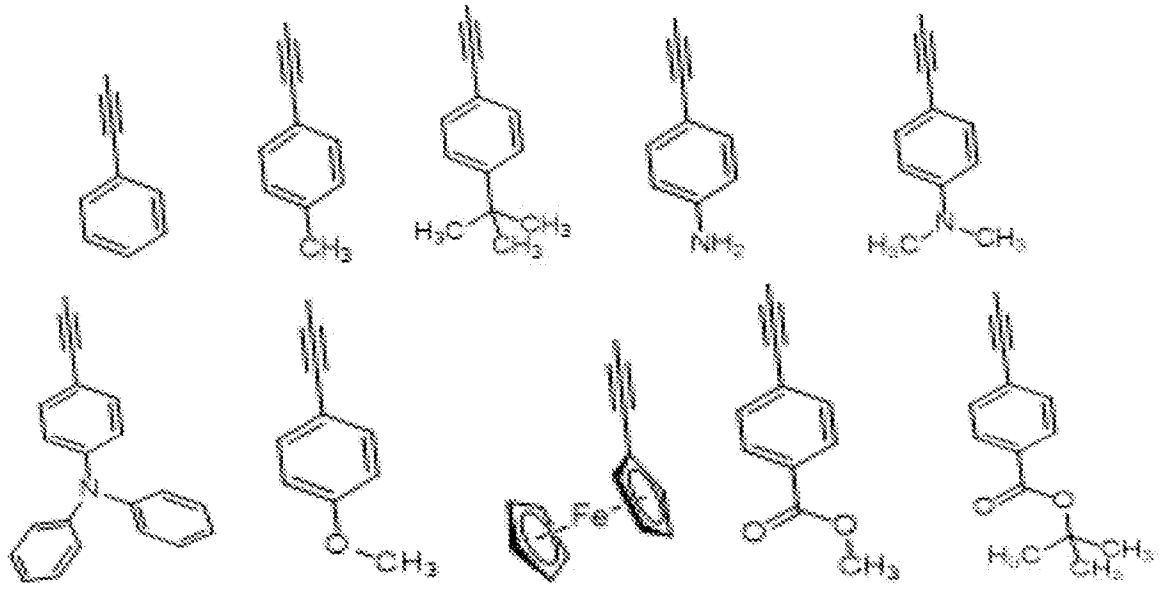
Examples of such ligands include: (1) the steroid hormones, such as progesterone, estrogens, androgens, and the adrenal cortical hormones; (2) growth factors, such as epidermal growth factor, nerve growth factor, fibroblast growth factor, and the like; (3) other protein hormones, such as human growth hormone, parathyroid hormone, and the like; (4) neurotransmitters, such as acetylcholine, serotonin, dopamine, and the like; and (5) antibodies. Any analog of these substances that also succeeds in binding to a biological receptor is also included.

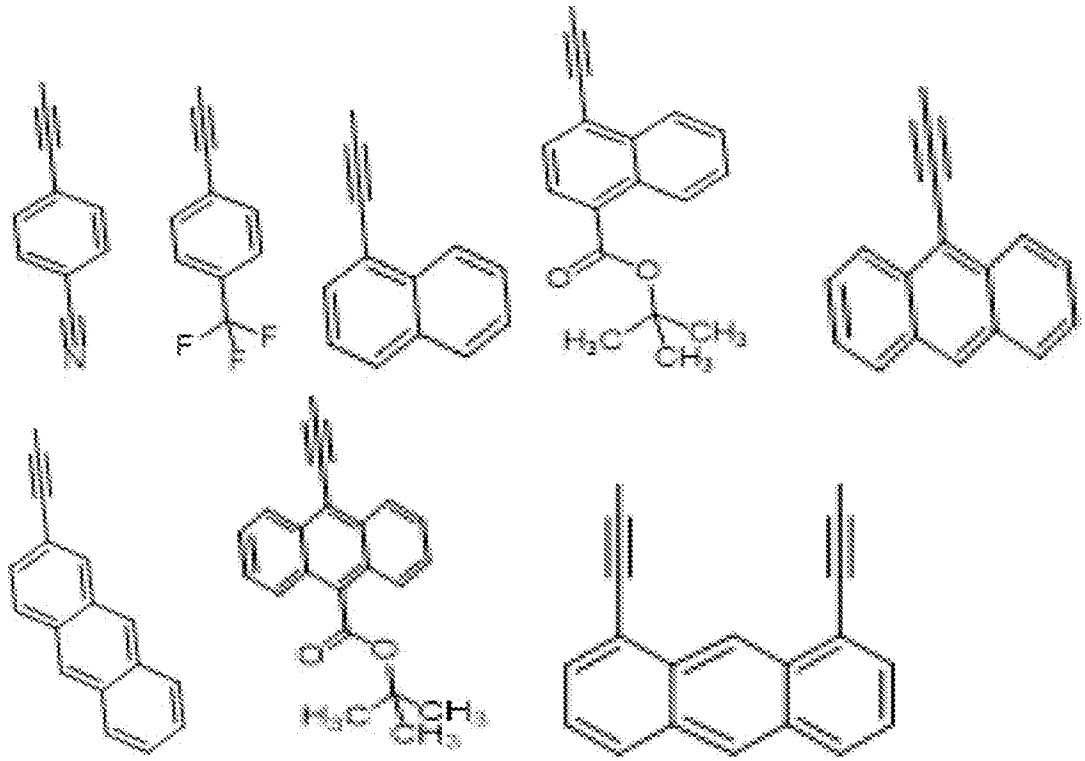
The aryl groups substituted at the 5 and 15 positions of the 10,10-diorgano-5,15-diarylbiladiene ligand may be substituted phenyl groups having one or more substituents selected from the group consisting of halogen, alkyl, nitrogen-containing substituents (e.g., amine groups), sulfur-containing substituents (e.g., thiol, thio ether), oxygen-containing substituents (e.g., hydroxyl, carboxylate, hydroxyalkyl, alkoxy) and combinations thereof, subject to the proviso that at least one of the substituted phenyl groups is substituted by at least one water-solubilizing segment-containing substituent (such as a poly(oxyalkylene) segment-containing substituent). As used herein, the term "substituted phenyl group" means a phenyl group which is substituted at at least one carbon of the aromatic ring with a substituent other than hydrogen. According to one embodiment, all substituents on the substituted phenyl groups other than water-solubilizing segment-containing substituents are halogen (e.g., fluorine). In other embodiments, a water-solubilizing segment-containing substituent, such as a poly(oxyalkylene) segment-containing substituent, is attached to the position on the phenyl group which is para to the carbon atom which attaches the phenyl group to the 5 or 15 position of the 10,10-diorgano-5,15-diarylbiladiene ligand.

The 10,10-diorgano-5,15-diarylbiladiene ligand, according to certain embodiments of the invention, may be substituted at one or both of the 2 and 18 positions with a π-conjugation-extending substituent. A π-conjugation-extending substituent is a

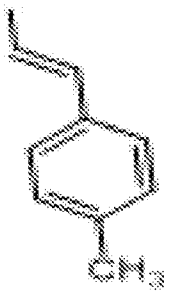
substituent which extends the π-conjugation present within the 10,10-diorgano-5,15-diarylbiladiene ligand. For example, the π-conjugation-extending substituent may be selected from the group consisting of carbonyl-containing substituents, imine-containing substituents, aromatic substituents, vinylaromatic substituents and ethynylaromatic substituents. Suitable aromatic substituents include phenyl groups, naphthyl groups, anthrenyl groups and the like (including both unsubstituted and substituted versions thereof). A vinylaromatic substituent may have structure –CH=CH-Ar, wherein Ar is an aryl group such as phenyl, which may be substituted or unsubstituted. An ethynylaromatic substituent may have structure –C≡C-Ar, wherein Ar is an aryl group such as phenyl, naphthyl or anthrenyl, which may be substituted or unsubstituted. According to certain embodiments, a π-conjugation-extending substituent is present at the 2 and 18 positions of the 10,10-diorgano-5,15-diarylbiladiene ligand. It is also possible for a single π-conjugation-extending substituent to bridge between the 2 and 18 positions of the 10,10-diorgano-5,15-diarylbiladiene ligand (thereby making the ligand macrocyclic rather than linear).

Exemplary ethynylaromatic substituents include:





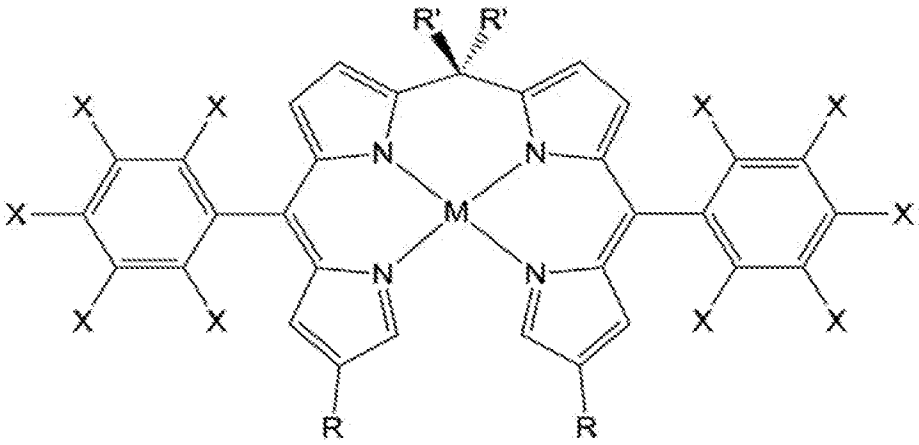
Exemplary vinylaromatic substituents include:



and analogues of any of the ethynylaromatic substituents depicted above, where the carbon-carbon triple bond is replaced by a carbon-carbon double bond.

It is also possible for one or more of the pyrrole rings to be substituted, particularly at the 2 and/or 18 position of the biladiene, for example with substituents such as bromine or other halogen. In this context, "substituted" means that a hydrogen atom otherwise present on the biladiene skeleton is replaced by a non-hydrogen substituent.

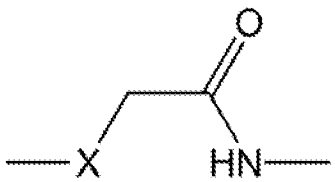
According to certain embodiments of the invention, the tetrapyrrole complex has a structure corresponding to Formula (I):



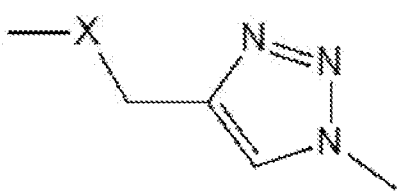
(I)

wherein M is a metal; each X is independently selected from the group consisting of hydrogen, halogen, alkyl, and water-solubilizing segment-containing substituents, subject to the proviso that at least one X is a water-solubilizing segment-containing substituent, each R is independently subjected from the group consisting of hydrogen, halogen, and π-conjugation-extending substituents, and each R' is independently selected from the group consisting of alkyl groups and aryl groups.

M may be Pd or Pt or any of the other metals previously described. R' and R' may independently be methyl or phenyl. The poly(oxyalkylene) segment may have a number average molecular weight of from 300 to 2000 g/mol. The poly(oxyalkylene) segment may be a poly(oxyethylene) segment. The at least one substituent comprised of a poly(oxyalkylene) segment-containing substituent may have a terminal group selected from the group consisting of alkyl groups and alkyl groups substituted with at least one functional group. The at least one substituent comprised of a poly(oxyalkylene) segment-containing substituent may be linked to a phenyl group through a linking moiety. The linking moiety may, for example, be selected from the group consisting of

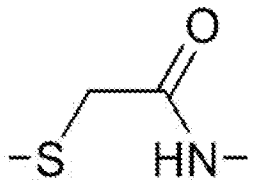


and

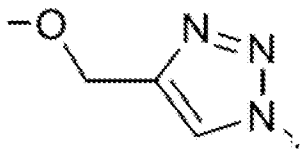


wherein X is O, S, Se, Te, NH, NR, CH_2, CHR, and CR_2, with R being an organo group such as an alkyl group.

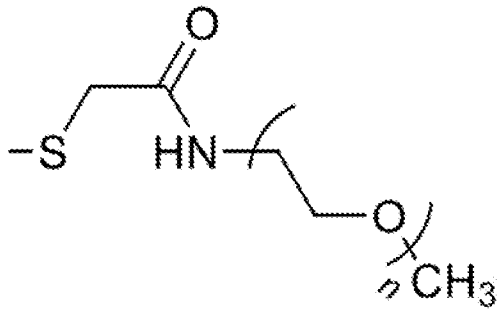
Specific illustrative examples of suitable linking moieties include:



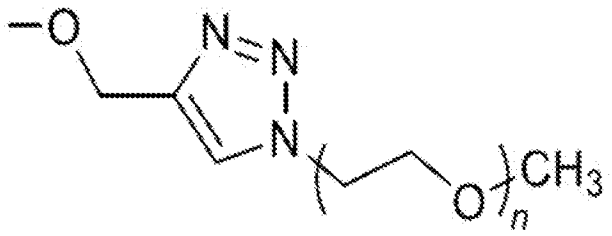
and



According to certain embodiments, one or two X groups (in Formula (I)) may be poly(oxyalkylene) segment-containing substituents and the remaining X groups may be fluorine. The water-solubilizing segment-containing substitutent(s) (e.g., poly(oxyalkylene) segment-containing substituents) may be attached to the phenyl group in the para position. Each R may be a π-conjugation-extending substituent selected from the group consisting of carbonyl-containing substituents, imine-containing substituents, aromatic substituents, vinylaromatic substituents and ethynylaromatic substituents. The water-solubilizing segment may have structure $-(CH_2CH_2O)_n-$ wherein n is from about 3 to about 250 on average. According to particular embodiments of the invention, the poly(oxyalkylene) segment-containing substituent(s) may be selected from:

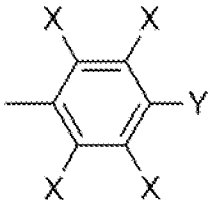


or



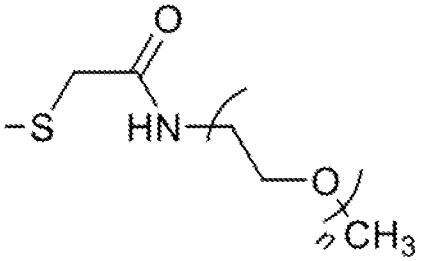
wherein n is from about 3 to about 250 on average.

In certain embodiments, the tetrapyrrole complex is characterized by having a phenyl group of Formula (II) attached at one or both of the 2 position and the 15 position of the biladiene skeleton:

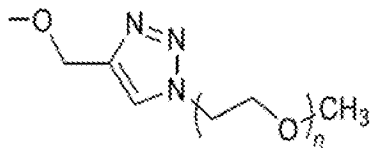


(II)

wherein each X is the same or different and is selected from the group consisting of hydrogen, halogen, alkyl, nitrogen-containing substituents (e.g., amine groups), sulfur-containing substituents (e.g., thiol, thio ether), oxygen-containing substituents (e.g., hydroxyl, carboxylate, hydroxyalkyl, alkoxy) and combinations thereof, and Y is a poly(oxyalkylene) segment-containing substituent (in particular, a poly(oxyethylene) segment-containing substituent). According to certain embodiments, Y is:

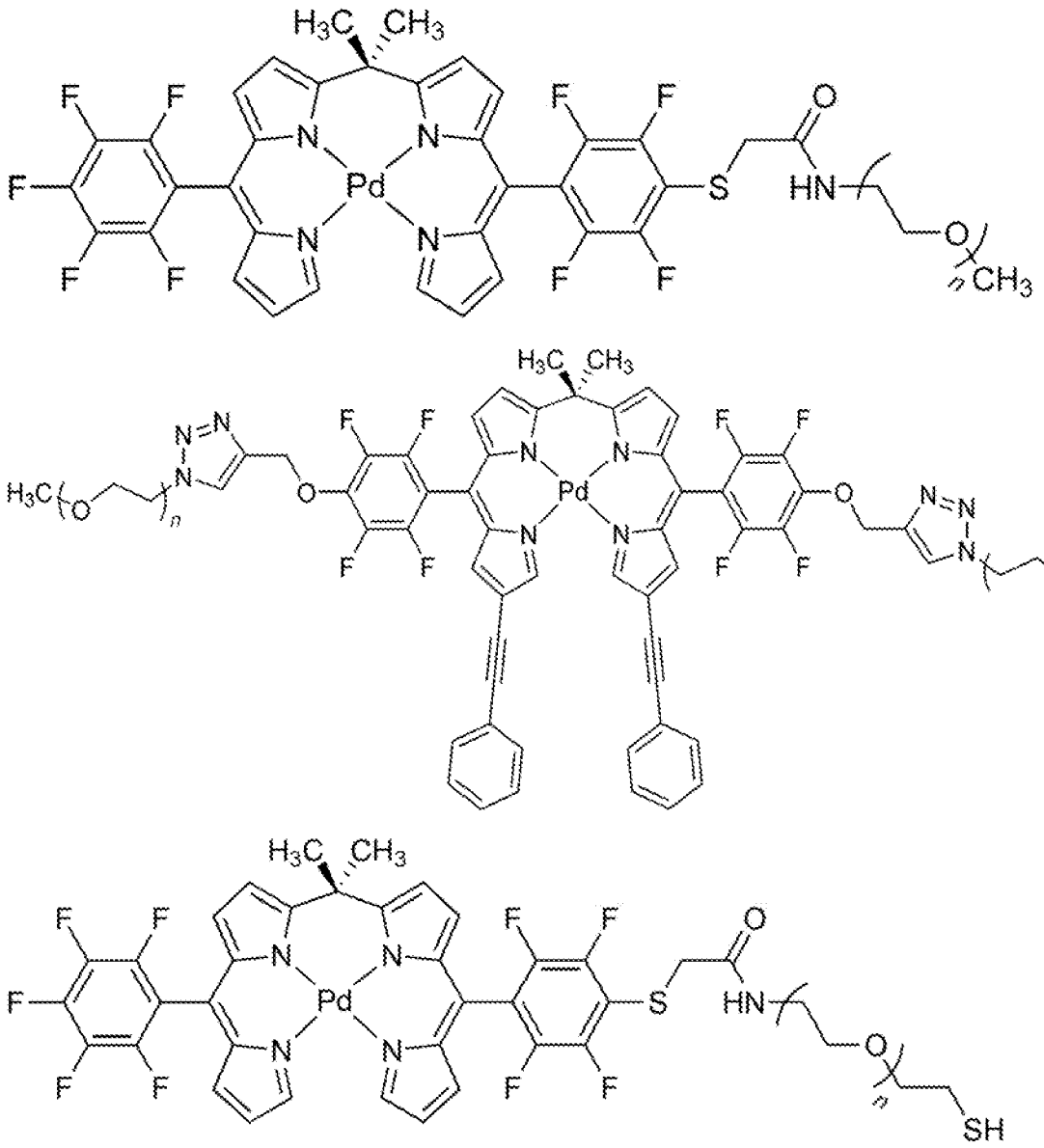


or



wherein n is from about 3 to about 250 on average.

The following are illustrative examples of specific tetrapyrrole complexes in accordance with the present invention:



In each case, n may be from 3 to 250 on average.

Methods of Making Tetrapyrrole Complexes

Tetrapyrrole complexes in accordance with the present invention are synthetically accessible by introducing water-solubilizing (e.g., poly(oxyalkylene)) functionality into a base tetrapyrrole complex that does not contain such functionality. Suitable unfunctionalized base tetrapyrrole complexes are known in the art; their synthesis (and that of related tetrapyrroles containing sp^3 hybridized *meso*-carbons) is described, for example, in the following publications, the entire disclosure of each of which is incorporated herein by reference in its entirety for all purposes: Potocny et al., Electrochemical, Spectroscopic, and $^1O_2$ Sensitization Characteristics of Synthetically Accessible Linear Tetrapyrrole Complexes of Palladium and Platinum. *Inorg. Chem.*

2017, *56*, 12703-12711; Pistner et al., Electrochemical, Spectroscopic and Singlet Oxygen Sensitization Characteristics of 10,10-Dimethylbiladiene Complexes of Zinc and Copper. *J Phys. Chem A.*, 2014, *118*, 10639-10648; Pistner et al., A Tetrapyrrole Macrocycle Displaying a Multielectron Redox Chemistry and Tunable Absorbance Profile. *J. Phys. Chem C*, 2012, *116*, 16918-16924; Pistner et al., Synthesis, Electrochemistry and Photophysics of a Family of Phlorin Macrocycles that Display Cooperative Fluoride Binding. *J. Am. Chem. Soc.*, 2013, *135*, 6601-6607; and Pistner et al., Factors Controlling the Spectroscopic Properties and Spramolecular Chemistry of an Electron Deficient 5,5-Dimethylphlorin Architecture. *J. Phys. Chem C.*, 2014, *118*, 14124-14132.

According to one embodiment, a leaving group on an aryl group attached to the 5 or 15 position of the tetrapyrrole (biladiene) ligand backbone can be displaced via nucleophilic aromatic substitution. The leaving group can be a halogen, for example (e.g., fluorine), and can be positioned as a *para* substituent on a phenyl group. The nucleophile may be a thiol-containing compound, such as mercaptoacetic acid, that either already contains a poly(oxyalkylene) segment or contains a functional group, such as a carboxylic acid group, that is capable of being derivatized to introduce a poly(oxyalkylene) segment. For example, where the functional group that can be derivatized to provide a poly(oxyalkylene) segment is a carboxylic acid group, the poly(oxyalkylene) segment can be provided in the form of an amino-functionalized poly(oxyalkylene) glycol, wherein the amino functionality reacts with the carboxylic acid group to form an amide linkage. Carbodiimide coupling chemistry may be used, for example, to convert a mercaptoacetic acid substituent into an N-(methoxyPEG)mercaptoacetamide. An $-SCH_2CO_2H$ functionalized complex may be initially reacted with *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC) to form an NHS mercaptoacetate intermediate. Further treatment with a tertiary amine such as trimethylamine and a methoxy-PEG-amine results in the formation of a (methoxy-PEG)-containing substituent on the complex.

Suitable amino-functionalized poly(oxyalkylene) glycols include, for example, amino-functionalized poly(oxyethylene) glycols corresponding to Formula (III):

$$HN(R^1)CH_2CH_2O(CH_2CH_2O)_nR^2 \quad (III)$$

wherein R^1 is hydrogen or alkyl (e.g., C1-C6 alkyl), R^2 is alkyl (e.g., C1-C6 alkyl) or $-CH_2CH_2R^3$, wherein R^3 is –OH, -OAlk, $-NH_2$, -NHAlk, -NHAcyl, $-N(Alk)_2$, $-C(=O)R^4$, or $-OCH_2C(=O)R^4$, Alk is alkyl, R^4 is –OH, -OAlk, $-NH_2$, -NHAlk or $-N(Alk)_2$, and n is 2 to 249.

Besides thiol groups, other functional groups that are capable of acting as nucleophiles in a nucleophilic aromatic substitution reaction include any of the

nucleophiles known in the art of nucleophilic aromatic substitution, such as hydroxyl groups and primary or secondary amino groups.

A specific, illustrative example of this synthetic approach to tetrapyrrole complexes in accordance with the present invention is provided in Fig. 1 and further described in detail in the Examples.

Another way to link a water-solubilizing segment-containing substituent to an aryl group is to first react a leaving group (e.g., a halogen such as fluorine) on an aryl group attached to one or both of the 5 or 15 positions of the tetrapyrrole (biladiene) ligand backbone with propargyl alcohol to introduce a propargyl substituent on the aryl group, then react the ethynyl functionality of the propargyl substituent with an azide-functionalized reactant also containing a water-solubilizing segment such as a poly(oxyalkylene segment). The ethynyl functionality reacts with the azide group to form a triazole linkage.

Suitable azide-functionalized poly(oxyalkylene) glycols include, for example, azide-functionalized poly(oxyethylene) glycols corresponding to Formula (IV):

N_3-$CH_2CH_2O(CH_2CH_2O)_nR^1$ (IV)

wherein R^1 is alkyl (e.g., C1-C6 alkyl) or $-CH_2CH_2R^3$, wherein R^3 is –OH, -OAlk, $-NH_2$, -NHAlk, -NHAcyl, $-N(Alk)_2$, $-C(=O)R^4$, or $-OCH_2C(=O)R^4$, Alk is alkyl, R^4 is –OH, -OAlk, $-NH_2$, -NHAlk or $-N(Alk)_2$, and n is 2 to 249.

It is also possible to attach a water-solubilizing segment-containing substituent directly to an aryl group using a reactant that contains a water-solubilizing segment as well as a nucleophilic group capable of displacing a leaving group on the aryl group in a nucleophilic aromatic substitution reaction.

The one or more water-solubilizing segment-containing substituents need not be substituted on an aryl group attached to one or both of the 5 or 15 positions of the tetrapyrrole (biladiene) ligand backbone. Reactive functionality anywhere in a starting tetrapyrrole ligand may be utilized for the purpose of introducing a water-solubilizing segment-containing substituent. Such reactive functionality may include, for example, an ethynyl group, a primary or secondary amino group, a halogen group, or a carboxylic acid group. A reactant containing both a functional group reactive with the reactive functionality present in the starting tetrapyrrole ligand and a water-solubilizing segment may be reacted with the starting tetrapyrrole ligand.

The tetrapyrrole complexes of the present invention may also be prepared by first preparing a suitable 10,10-diorgano-5,15-diarylbiladiene ligand which bears at least one substituent comprised of a water-solubilizing segment and then reacting such ligand with a source of the metal to be complexed (for example, a metal salt). Such

ligands may, for example, have a structure corresponding to that of Formula (I) wherein M is replaced by two hydrogens.

Formulations containing Tetrapyrrole Complexes

The tetrapyrrole complexes disclosed herein can be prepared as or formulated into a formulation (pharmaceutical composition) suitable for use in the treatment, therapeutic, or diagnostic methods also described herein. For example, the formulations can further comprise one or more pharmaceutically acceptable excipient(s) and/or carrier(s), in addition to one or more tetrapyrrole complexes. The pharmaceutically-acceptable excipient(s) and/or carrier(s) can be administered with the tetrapyrrole complexes disclosed above as well as possibly other components such as nanoparticles which emit heat in response to laser light. The formulation can be administered *in vivo* in a pharmaceutically acceptable carrier. In view of the water solubility of the tetrapyrrole complexes, the use of water or other aqueous-based carrier is preferred in at least certain embodiments. The tetrapyrrole complex may be fully or partially dissolved in the carrier. By "pharmaceutically acceptable" is meant a material selected to minimize any degradation of the active ingredient(s) and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

Suitable carriers and excipients are described in Remington: The Science and Practice of Pharmacy (19th ed.) ed. A. R. Gennaro, Mack Publishing Company, Easton, Pa. 1995. An appropriate amount of a pharmaceutically-acceptable salt may be used in the formulation to render the formulation isotonic. Examples of pharmaceutically-acceptable carriers include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of tetrapyrrole complex and possibly other components being administered.

The formulations can be administered orally, parenterally (e.g., via intravenous injection, intraperitoneal injection, intramuscular injection, intratumoral injection, intraarterial injection), transdermally, extracorporeally, topically or the like, including by topical intranasal administration or administration by inhalant, or a combination thereof. As used herein, "topical intranasal administration" means delivery of the formulation into the nose and nasal passages through one or both of the nostrils and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the formulation. Administration of the formulation by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery

can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the formulation required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the type of disorder or disease being treated, the location of the diseased tissue being treated, the particular tetrapyrrole complex, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every formulation. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

Formulations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for topical administration of the tetrapyrrole complexes can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be desirable.

The formulations can further include, in addition to one or more tetrapyrrole complexes in accordance with the present invention, one or more active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics, and the like. Nanoparticles which emit heat in response to laser light, as described elsewhere herein, could also be included in the formulations, so that tetrapyrrole complex(es) and such nanoparticles may be co-administered.

Methods of Using Tetrapyrrole Complexes/Methods of Treatment

The disclosed tetrapyrrole complexes, and formulations comprising them, can be administered to an individual to kill endogenous tissue or cells. The tissue can be undesirable tissue that has arisen due to transformation, such as a tumor, cancer, or endometriosis; adipose tissue; plaques present in vascular tissue and over-proliferation such as those formed in restenosis; birthmarks and other vascular lesions of the skin; scars and adhesions; and irregularities in connective tissue or bone, such as bone spurs. As used herein, the term "cancer" includes a wide variety of malignant solid neoplasms. These can be caused by viral infection, naturally occurring transformation,

or exposure to environmental agents. Parasitic infections and infections with organisms, especially fungal, that lead to disease may also be targeted.

In some examples, the tetrapyrrole complexes of the present invention and formulations containing such complexes can be useful for causing photodynamic damage to cancer cells. Photodynamic damages to cancer cells include, but are not limited to, preventing or reducing the development of a cancer, reducing the symptoms of cancer, suppressing or inhibiting the growth of an established cancer, preventing metastasis and/or invasion of an existing cancer, promoting or inducing regression of the cancer, inhibiting or suppressing the proliferation of cancerous cells, reducing angiogenesis or increasing the amount of apoptotic cancer cells, thereby treating cancer.

Generally, the methods can include contacting a cell with an effective amount of the tetrapyrrole complex or a formulation comprising the tetrapyrrole complex as described herein. One of skill in the art recognizes that an amount can be considered therapeutically effective even if the condition is not totally eradicated but improved partially. The formulations can be injected directly into the target tissue, or can be administered systemically. More specifically, the formulations can be administered using any suitable method including intravenous (i.v.), intraperitoneal (i.p.), intramuscular (i.m.), intratumoral (i.t), intraarterial (i.a.), topically, and/or by inhalation. Intravenous administration is particularly preferred for solid tumors, while i.p. administration is preferred for pancreatic, liver, and gastric tumors. Advantageously, even when administered systemically, the tetrapyrrole complexes preferentially accumulate in the cancerous tissue, and preferably actively integrate in the cancerous tissue, as opposed to surrounding healthy tissue.

The disclosed methods can also include the application of external ionizing radiation for the purpose of exciting the tetrapyrrole complex. The rate and time at which the cancerous cells are irradiated may depend on the results required. The cancerous cells can be irradiated at an effective fluence rate and time to cause therapeutic injury resulting in the reduction of at least one of the surface area, the depth, and the amount of the tissue affected by the cancerous condition. The irradiation regime may also be dependent on the structure of the tetrapyrrole complex, the maximum safe dose of radiation that can be tolerated by the patient, or the targeted cell or material.

Embodiments of the present invention are directed to methods of inhibiting the growth of cancer cells, *in vitro* or *in vivo*, comprising the steps of contacting the cancer cells with a tetrapyrrole complex of the present invention, and exposing the cancer cells to an effective amount of artificial irradiation. In one aspect, the invention

provides methods of inhibiting the growth of cancer cells, such as breast, lung, pancreas, bladder, ovarian, testicular, prostate, retinoblastoma, Wilm's tumor, adrenocarcinoma or melanoma.

In accordance with the practice of this invention, the subject may be a human, equine, porcine, bovine, murine, canine, feline, and avian subjects. Other warm blooded animals are also included with the scope of this invention.

The present invention also provides a method for treating a subject suffering from cancer. The subject may be a human, dog, cat, mouse, rat, rabbit, horse, goat, sheep, cow, chicken. The cancer may be identified as a breast, lung, pancreas, bladder, ovarian, testicular, prostate, retinoblastoma, Wilm's tumor, adrenocarcinoma or melonoma and is generally characterized as a group of cells which over-express and/or have an over-abundance of the target. This method comprises the steps of administering to the subject a cancer killing amount of one or more tetrapyrrole complex of the present invention.

Also provided is a method of inhibiting the proliferation of mammalian tumor cells which comprises the steps of contacting the mammalian tumor cells with a sufficient concentration of the tetrapyrrole complex of the invention, and exposing the mammalian tumor cells to artificial irradiation.

The subject invention further provides methods for inhibiting the growth of human tumor cells, treating a tumor in a subject, and treating a proliferative-type disease in a subject. These methods comprise the steps of administering to the subject an effective amount of the tetrapyrrole complex of the invention.

The present invention also provides for a method of treating a disease state comprising administering to a target tissue of a patient a tetrapyrrole complex of the present invention and irradiating the tetrapyrrole complex, which functions as a photosensitizer, thereby killing the target tissue. Irradiation of the tetrapyrrole complex can lead to generation of singlet oxygen in proximity to the target tissue.

The present invention encompasses formulations (pharmaceutical compositions), combinations and methods for treating human carcinomas. For example, the invention includes formulations (pharmaceutical compositions) for use in the treatment of human carcinomas comprising a pharmaceutically effective amount of one or more tetrapyrrole complexes in accordance with the present invention and a pharmaceutically acceptable carrier. Such formulations may additionally include other drugs or antibodies effective for treating carcinomas.

The tetrapyrrole complexes of the invention can be administered using conventional modes of administration including, but not limited to, intravenous, intraperitoneal, oral, intralymphatic, or administration directly into a tumor.

The tetrapyrrole complexes of the invention may be provided in a variety of dosage forms which include, but are not limited to, liquid solutions or suspension, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The form depends upon, among other things, the mode of administration and the therapeutic application.

The most effective mode of administration and dosage regimen for the tetrapyrrole complexes of this invention and formulations comprising such complexes depends upon the severity and course of the disease, the patient's health and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the tetrapyrrole complexes should be titrated to the individual patient. Nevertheless, an effective dose of the tetrapyrrole complexes of this invention may be in the range of from about 1 to about 2000 mg/kg. The dosage can also be from about 2 to about 1000 mg/kg, about 4 to about 400 mg/kg, or about 5 to about 100 mg/kg.

Adjustments in the dosage regimen may be made to optimize the tumor cell growth inhibiting and killing response, e.g., doses may be divided and administered on a daily basis or the dose reduced proportionally depending upon the situation (e.g., several divided doses may be administered daily or proportionally reduced depending on the specific therapeutic situation). In certain embodiments, the tetrapyrrole complex may be administered on a one time basis or on an as-needed basis, depending upon the patent's response to previously-administered doses.

The dose of the tetrapyrrole complex of the invention required to achieve cures or remission may be further reduced with schedule optimization.

The human or other subject is preferentially exposed to artificial irradiation which is selected from the group consisting of artificial ultraviolet, infrared (IR), gamma-irradiation, x-ray and visible light. In certain embodiments the irradiation is IR or near-infrared (NIR). According to certain embodiments, the artificial irradiation is light having a wavelength of from 350 to 1000 nm. The artificial irradiation can be applied about 5 minutes to about 3 hours after administering the tetrapyrrole complex of the present invention or the artificial irradiation is applied about 10 to about 60 minutes after administering the tetrapyrrole complex of the present invention.

In another embodiment, in the methods of treating cancer of the present invention, the artificial irradiation can be applied for about 10 seconds to about 60 minutes, or the artificial irradiation is applied for about 15 seconds to about 30 minutes.

In yet another embodiment, the present invention further provides pharmaceutical compositions which comprise the tetrapyrrole complexes of the present invention and a pharmaceutically acceptable carrier.

The present invention further provides a method for treating cancer in a subject having cancer comprising the steps of administering to the subject a therapeutically effective amount of a tetrapyrrole complex in accordance with the present invention.

The tetrapyrrole complexes of the present invention and formulations containing such compounds are also useful in applications other than dynamic phototherapy, such as diagnostic imaging.

Use of Tetrapyrrole Complexes in Combination with Light-Activated Nanoparticles

As previously mentioned, the tetrapyrrole complexes of the present invention may be employed in combination with nanoparticles that are capable of emitting heat when irradiated (that is, materials in nanoparticulate form that are capable of converting light into heat, sometimes referred to as light-activated heating nanoparticles). Such combinations make possible the implementation of dual photothermal therapy/photodynamic therapy (PTT/PDT) for treatment of cancer and other disorders. The use of both nanoparticles and tetrapyrrole complexes in accordance with the present invention may help overcome certain limitations of each type of therapy itself to provide a more comprehensive and effective solution for treatment of cancers such as solid tumor cancers. Under at least some conditions, PTT (using light-activated heating nanoparticles) and PDT (using tetrapyrrole complexes in accordance with the present invention) have been found to work synergistically to induce more cell death than either therapy alone. Dual PTT/PDT in accordance with the invention may primarily induce apoptotic cell death over necrosis at low light dosages

As an example, nanoparticles and tetrapyrrole complexes may be introduced into a subject, either consecutively or simultaneously, by a suitable means such as intravenous injection and allowed to accumulate within targeted tissue (such as a solid tumor) based on the enhanced permeability and retention (EPR) effect. Then, sources of artificial light effective to activate each of the administered components may be applied. For example, a 700 nm to 1000 nm continuous wave laser and 350 to 600 nm wavelength light may be applied to activate the nanoparticles (e.g., nanoparticles having a silica core and a gold shell coated by polyethylene glycol) and the tetrapyrrole complex, respectively, to produce heat and singlet oxygen that is toxic to the surrounding cancer cells. In some embodiments, the irradiation source effective to activate the nanoparticles can comprise a single emission wavelength or a range of emission wavelengths. In some embodiments, the emission wavelength range can be a wavelength range that causes minimal or no cellular damage. In some embodiments, the emission wavelength range can be in the near-infrared wavelength range, e.g., from about 750 nm to about 1250 nm. In some embodiments, the irradiation source can comprise a single emission wavelength from about 750 nm to about 1250 nm. In

some embodiments, the irradiation source can be a laser with a single emission wavelength of from about 750 nm to about 1250 nm. In some embodiments, the irradiation source can be a laser with an emission wavelength range of from about 750 nm to about 1250 nm. In some embodiments, the irradiation source can be an 808 nm diode laser.

The types of nanoparticles useful in such PTT/PDT treatment strategies is not particularly limited and any of the nanoparticles known in the PTT field may be utilized, such as gold nanospheres, hollow gold nanocages, gold nanostars and gold nanorods. Suitable nanoparticles include nanoparticles characterized by containing non-metallic cores (e.g., silica cores) and metallic shells (e.g., gold shells), which are sometimes referred to as nanoshells. Other types of metal-containing nanoparticles may also be employed. The use of nanoparticles which do not contain metal, such as graphite nanoparticles, graphene nanoparticles, carbon nanotubes or other carbon-based nanoparticles is also possible.

Suitable nanoparticles may be from 10 to 300 nm in diameter or 100 to 200 nm in diameter, for example. The nanoparticles may be coated with or conjugated to a hydrophilic or passivating material or ligand such as poly(ethylene glycol) (PEG). Such treatments can increase the biocompatibility of the nanoparticles. The hydrophilic or passivating material may be attached to the outer surface of the gold shell using gold-thiol conjugation chemistry. In some embodiments, the nanoparticles can absorb wavelengths of light in the near-infrared (NIR) spectrum. In some embodiments, the nanoparticles can absorb wavelengths of light between about 750 nm and about 1250 nm. In some embodiments, the nanoparticles can have a maximum absorption peak of about 800-810 nm (in other words, the nanoparticles can have a UV-vis maximum absorption peak of about 800-810 nm).

Various non-limiting aspects of the present invention may be summarized as follows:

Aspect 1: A tetrapyrrole complex comprising a metal complexed by a 10,10-diorgano-5,15-diarylbiladiene ligand which bears at least one substituent comprised of a water-solubilizing segment.

Aspect 2: The tetrapyrrole complex of Aspect 1, wherein the water-solubilizing segment is selected from the group consisting of poly(oxyalkylene) segments, polysaccharide segments, polypeptide segments, poly(thioalkylene) segments, poly(aminoalkylene) segments, polyvinylpyrrolidone segments, aliphatic polyester segments, polyamide segments, polyvinyl alcohol segments, polyacrylic acid segments, polyacrylamide segments, polyoxazoline segments, aliphatic polycarbonate segments, polyphosphate segments, and polyphosphazene segments.

Aspect 3: The tetrapyrrole complex of Aspect 1, wherein the water-solubilizing segment is a poly(oxyalkylene) segment.

Aspect 4: The tetrapyrrole complex of any of Aspects 1 to 3, wherein at least one aryl group substituted at the 5 or 15 position of the 10,10-diorgano-5,15-diarylbiladiene ligand bears at least one substituent comprised of a water-solubilizing segment.

Aspect 5: The tetrapyrrole complex of any of Aspects 1 to 4, wherein at least one aryl group substituted at the 5 or 15 position of the 10,10-diorgano-5,15-diarylbiladiene ligand bears at least one substituent comprised of a water-solubilizing segment selected from the group consisting of poly(oxyalkylene) segments, polysaccharide segments, polypeptide segments, poly(thioalkylene) segments, poly(aminoalkylene) segments, polyvinylpyrrolidone segments, aliphatic polyester segments, polyamide segments, polyvinyl alcohol segments, polyacrylic acid segments, polyacrylamide segments, polyoxazoline segments, aliphatic polycarbonate segments, polyphosphate segments, and polyphosphazene segments.

Aspect 6: The tetrapyrrole complex of any of Aspects 1 to 5, wherein at least one aryl group substituted at the 5 or 15 position of the 10,10-diorgano-5,15-diarylbiladiene ligand bears at least one substituent comprised of a poly(oxyalkylene) segment.

Aspect 7: The tetrapyrrole complex of any of Aspects 1 to 6, wherein the metal is Pd or Pt.

Aspect 8: The tetrapyrrole complex of any of Aspects 1 to 7, wherein the 10,10-diorgano-5,15-diarylbiladiene ligand is a 10,10-dialkyl-5,15-diarylbiladiene ligand, a 10-alkyl,10-aryl-5,15-diarylbiladiene ligand, or a 10,10-diaryl-5,15-diarylbiladiene ligand.

Aspect 9: The tetrapyrrole complex of any of Aspects 1 to 8, wherein the 10,10-diorgano-5,15-diarylbiladiene ligand is a 10,10-dimethyl-5,15-diarylbiladiene ligand, a 10-methyl,10-phenyl-5,15-diarylbiladiene ligand, or a 10,10-diphenyl-5,15-diarylbiladiene ligand.

Aspect 10: The tetrapyrrole complex of any of Aspects 1 to 9, wherein the water-solubilizing segment is a poly(oxyalkylene) segment having a number average molecular weight of at least 200 g/mol.

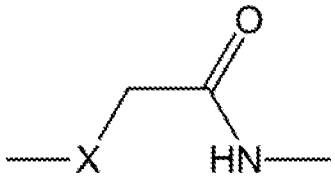
Aspect 11: The tetrapyrrole complex of any of Aspects 1 to 10, wherein the water-solubilizing segment is a poly(oxyethylene) segment.

Aspect 12: The tetrapyrrole complex of any of Aspects 1 to 11, wherein the at least one substituent comprised of a water-solubilizing segment is additionally comprised of a terminal group selected from the group consisting of alkyl groups and

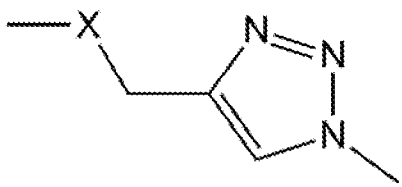
alkyl groups substituted with at least one functional group selected from –SR, NR_2, CO_2R, -C(=O)NR_2, -SO_3R, -PO_3R, -PR_3^+, or -NR_3^+, wherein each R is independently H or an organo group.

Aspect 13: The tetrapyrrole complex of any of Aspects 1 to 12, wherein the at least one substituent comprised of a water-solubilizing segment is additionally comprised of a linking moiety which links the water-solubilizing segment to the 10,10-diorgano-5,15-diarylbiladiene ligand.

Aspect 14: The tetrapyrrole complex of Aspect 13, wherein the linking moiety is selected from the group consisting of:



and



wherein X is O, S, Se, Te, NH, NR, CH_2, CHR, and CR_2, with R being an organo group such as an alkyl group.

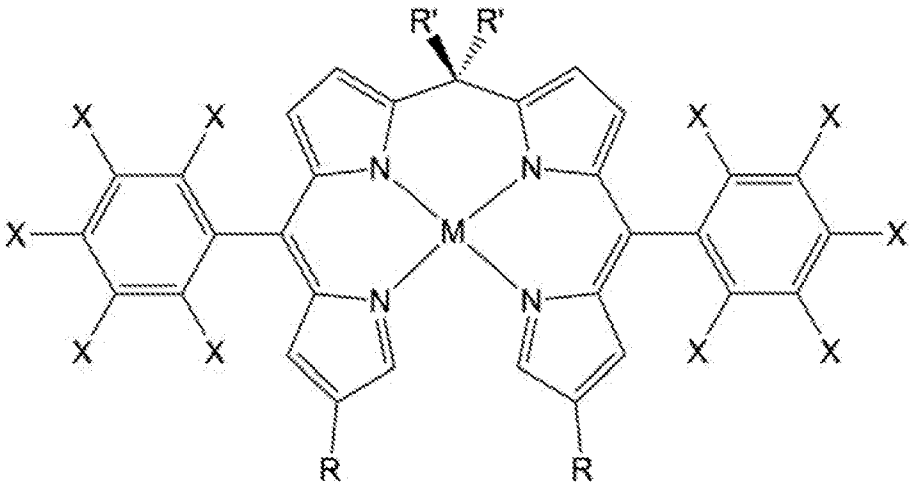
Aspect 15: The tetrapyrrole complex of any of Aspects 1 to 14, wherein the aryl groups substituted at the 5 and 15 positions of the 10,10-diorgano-5,15-diarylbiladiene ligand are substituted phenyl groups having one or more substituents selected from the group consisting of halo, alkyl, oxygen-containing substituents, sulfur-containing substituents and nitrogen-containing substituents, subject to the proviso that at least one of the substituted phenyl groups is substituted by at least one water-solubilizing segment-containing substituent.

Aspect 16: The tetrapyrrole complex of any of Aspects 1 to 15, wherein the aryl groups substituted at the 5 and 15 positions of the 10,10-diorgano-5,15-diarylbiladiene ligand are substituted phenyl groups, at least one of the substituted phenyl groups is substituted by a poly(oxyalkylene) segment-containing substituent, and all substituents on the substituted phenyl groups other than poly(oxyalkylene) segment-containing substituents are fluorine.

Aspect 17: The tetrapyrrole complex of any of Aspects 1 to 16, wherein the 10,10-diorgano-5,15-diarylbiladiene ligand is substituted at one or both of the 2 and 18 positions with a π-conjugation-extending substituent.

Aspect 18: The tetrapyrrole complex of Aspect 17, wherein the π-conjugation-extending substituent is selected from the group consisting of carbonyl-containing substituents, imine-containing substituents, aromatic substituents, vinylaromatic substituents and ethynylaromatic substituents.

Aspect 19: The tetrapyrrole complex of Aspect 1, wherein the tetrapyrrole complex has a structure corresponding to Formula (I):



(I)

wherein M is a metal; each X is independently selected from the group consisting of hydrogen, halogen, alkyl, and water-solubilizing segment-containing substituents, subject to the proviso that at least one X is a water-solubilizing segment-containing substituent, each R is independently subjected from the group consisting of hydrogen, halogen, and π-conjugation-extending substituents, and each R' is independently selected from the group consisting of alkyl groups and aryl groups.

Aspect 20: The tetrapyrrole complex of Aspect 19, wherein M is Pd or Pt.

Aspect 21: The tetrapyrrole complex of Aspect 19 or 20, wherein R' and R' are independently methyl or phenyl.

Aspect 22: The tetrapyrrole complex of any of Aspects 19 to 21, wherein the water-solubilizing segment-containing substituent(s) comprise(s) at least one water-solubilizing segment selected from the group consisting of poly(oxyalkylene) segments, polysaccharide segments, polypeptide segments, poly(thioalkylene) segments, poly(aminoalkylene) segments, polyvinylpyrrolidone segments, aliphatic polyester segments, polyamide segments, polyvinyl alcohol segments, polyacrylic acid segments, polyacrylamide segments, polyoxazoline segments, aliphatic polycarbonate segments, polyphosphate segments, and polyphosphazene segments.

Aspect 23: The tetrapyrrole complex of any of Aspects 19 to 22, wherein the water-solubilizing segment-containing substituent(s) comprise(s) at least one poly(oxyalkylene) segment.

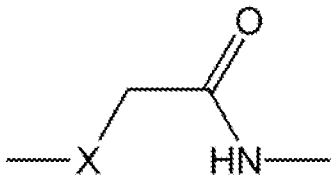
Aspect 24: The tetrapyrrole complex of Aspect 23, wherein the at least one poly(oxyalkylene) segment has a number average molecular weight of at least 200 g/mol (e.g., 300 to 5000 g/mol).

Aspect 25: The tetrapyrrole complex of Aspect 23 or 24, wherein the at least one poly(oxyalkylene) segment is a poly(oxyethylene) segment.

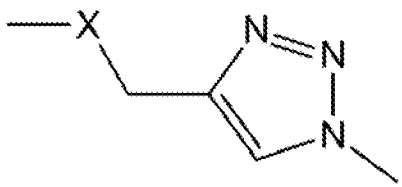
Aspect 26: The tetrapyrrole complex of any of Aspects 23 to 25, wherein the water-solubilizing segment-containing substituent has a terminal group selected from the group consisting of alkyl groups and alkyl groups substituted with at least one functional group.

Aspect 27: The tetrapyrrole complex of any of Aspects 19 to 26, wherein the water-solubilizing segment-containing substituent is linked to a phenyl group through a linking moiety.

Aspect 28: The tetrapyrrole complex of Aspect 27, wherein the linking moiety is selected from the group consisting of:



and



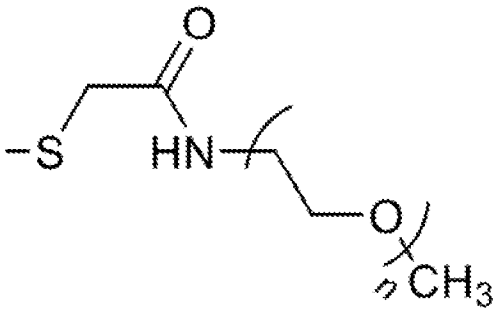
wherein X is O, S, Se, Te, NH, NR, CH_2, CHR, and CR_2, with R being an organo group such as an alkyl group.

Aspect 29: The tetrapyrrole complex of any of Aspects 19 to 28, wherein at least one X group is a poly(oxyalkylene) segment-containing substituent and the remaining X groups are fluorine.

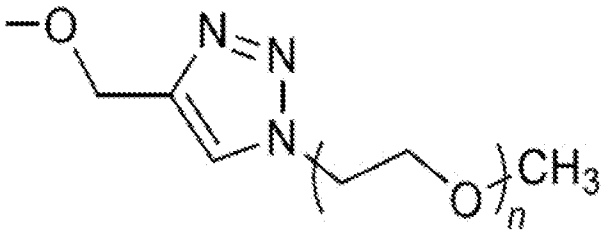
Aspect 30: The tetrapyrrole complex of any of Aspects 19 to 29, wherein each R is a π-conjugation-extending substituent selected from the group consisting of carbonyl-containing substituents, imine-containing substituents, aromatic substituents, vinylaromatic substituents and ethynylaromatic substituents.

Aspect 31: The tetrapyrrole complex of Aspect 25, wherein the poly(oxyethylene) segment has structure $-(CH_2CH_2O)_n-$ and n is from about 3 to about 250 on average.

Aspect 32: The tetrapyrrole complex of any of Aspects 19 to 31, wherein the water-solubilizing segment-containing substituent(s) is or are selected from:



or



wherein n is from about 3 to about 250 on average.

Aspect 33: A method of using a tetrapyrrole complex in accordance with any of Aspects 1 to 32, comprising administering the tetrapyrrole complex to a patient and, after a period of time, irradiating targeted tissue of the patient with an energy source that excites the tetrapyrrole complex thereby producing a desired therapeutic response in the targeted tissue.

Aspect 34: The method of Aspect 33, wherein the targeted tissue comprises tumor cells.

Aspect 35: The method of Aspect 33 or 34, wherein the tetrapyrrole complex is administered intravenously or intratumorally.

Aspect 36: The method of any of Aspects 33 to 35, wherein the tetrapyrrole complex is administered intravenously as an aqueous solution.

Aspect 37: A process of photodynamic therapy for treatment of diseased tissues comprising a) delivering a formulation comprised of a tetrapyrrole complex in accordance with any of Aspects 1 to 32 and a pharmaceutically acceptable vehicle to diseased tissue at a specific treatment site; b) allowing the formulation to preferentially accumulate in the diseased tissue; and c) irradiating the specific treatment site with light of a sufficient power and wavelength to activate the tetrapyrrole complex.

Aspect 38: The process of Aspect 37, wherein the light has a wavelength of from 350 to 1000 nm.

Aspect 39: The process of Aspect 37 or 38, wherein the process is carried out in coordination with photothermal therapy.

Aspect 40: The process of Aspect 39, wherein the photothermal therapy comprises embedding within the diseased tissue nanoparticles which emit heat in response to laser light.

Aspect 41: The process of Aspect 40, wherein the nanoparticles are comprised of a silica-containing core and a gold-containing shell.

Aspect 42: A formulation useful for photodynamic therapy, comprising a tetrapyrrole complex in accordance with any of Aspects 1 to 32 and a pharmaceutically acceptable vehicle.

Aspect 43: The formulation of Aspect 42, wherein the pharmaceutically acceptable vehicle is comprised of water.

Aspect 44: A tetrapyrrole ligand, wherein the tetrapyrrole ligand is a 10,10-diorgano-5,15-diarylbiladiene ligand which bears at least one substituent comprised of a water-solubilizing segment.

The examples below explain the invention in more detail. The following preparations and examples are given to enable those skilled in the art to more clearly understand and to practice the present invention. The present invention, however, is not limited in scope by the exemplified embodiments, which are intended as illustrations of aspects of the invention only, and methods and components which are functionally equivalent are within the scope of the invention. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

Examples

General Experimental Methods

General Materials and Methods. Air sensitive reactions were carried out under a nitrogen atmosphere using standard schlenk techniques and flasks fitted with Suba-seal® rubber septa. Solvents used in synthesis were of reagent grade or better, and anhydrous solvents were dried before use via passage through activated alumina. Phosphate buffered saline (PBS) solutions were obtained by dissolving one PBS tablet, purchased from Sigma-Aldrich, per 200 ml of Millipore® water. All PBS solutions were 0.0027 M in potassium chloride, 0.137 M in sodium chloride, and had a pH of 7.4.

Mercaptoacetic acid and the methoxy-PEG-amine were purchased from Sigma-Aldrich. Triethylamine, dimethylformamide, methanol, chloroform, hexane and ethyl acetate were purchased from Fisher. *N*-hydroxysuccinimide was manufactured by Alfa Aesar. 1-Ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and ethyltrichlorosilane were manufactured by Acros. Dichloromethane was purchased from Fisher or VWR. All deuterated solvents were purchased from Cambridge Isotopes Laboratories. Column chromatography was carried out using 40-63 μm silica gel from Silicycle. Thin layer chromatography (TLC) was done on precoated glass plates from Silicycle, and visualized with UV light. **Pd[DMBil1]** was prepared according to previously published procedures. Cell culture reagents, including cell culture media components and the Alamar blue viability reagent, were purchased from VWR and Thermo Fisher, respectively.

Preparation of C_2-silica. 500 g of 40-63 μm silica gel from Silicycle was added to 1.0 L of chloroform in a round bottomed flask, which was cooled in an ice bath. The reaction flask was sparged with N_2 for 15 mins. Ethyltrichlorosilane (24.8 g, 151.4 mmol) was added slowly under an N_2 atmosphere and allowed to stir while allowing the reaction mixture to slowly warm to room temperature overnight. The functionalized silica was then collected via vacuum filtration, washed 4 times with 500 mL portions of chloroform followed by 6 washes with 500 mL portions of methanol, and dried in an oven at 160 °C overnight.

Tetrapyrrole complex characterization. The ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra of **Pd[DMBil1]-SCH_2CO_2H** were measured at 25 °C on a Bruker 400 MHz spectrometer with a cryogenic QNP probe. The ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra of **Pd[DMBil1]-PEG_{750}** were measured at 25 °C on a Bruker 600 MHz spectrometer with a 5-mm Bruker SMART probe. Proton spectra are referenced to the residual proton resonance of the deuterated solvent ($CDCl_3$ = δ 7.26) and carbon spectra are referenced to the carbon resonances of the solvent ($CDCl_3$ = δ 77.16). Fluorine spectra are referenced to an external trifluoroacetic acid standard (TFA = δ -76.55 in CD_3CN). Chemical shifts are reported using the standard δ notation in parts-per-million. High resolution mass spectrometry analysis was performed at the Mass Spectrometry Laboratory in the Department of Chemistry and Biochemistry at the University of Delaware. Liquid injection field desorption ionization mass spectrometry was carried out using a Waters GCT Premier high-resolution time of flight mass spectrometer. Electrospray ionization mass spectrometry was conducted with a Thermo Q-Extractive Orbitrap high resolution mass spectrometer UV-Vis Absorption Experiments. All UV-visible absorbance spectra were measured using a

StellarNet CCD array UV-vis spectrometer. Samples were prepared in quartz cuvettes (6Q) with 1.0 cm pathlength manufactured by Firefly Scientific. Absorption spectra were collected for solutions of **Pd[DMBil1]-PEG$_{750}$** in room temperature methanol or PBS at concentrations of 4.0, 8.0, 12.0, 16.0, 20.0, and 24.0 μM.

Photodegradation Experiments. A 1.0 cm pathlength quartz cuvette (6Q) fused to a graded quartz to pyrex tube was filled nearly to the top with 10 mL of a 24.0 μM solution of **Pd[DMBil1]-PEG$_{750}$** in PBS. The cuvette setup was sealed with a kontes valve, and the solution was stirred for 2 hr at room temperature while the quartz to pyrex tube was illuminated with light from a 150 Watt halogen lamp (Nikon, Inc., Model MKII) fitted with a 10 nm FWHM bandpass filter centered at 550 nm (Thor Labs, FB550-10). Over the course of the 2 hr illumination period, the absorption spectrum of the solution was recorded every minute by a StellarNet CCD array UV-vis spectrometer.

Emission Experiments. Emission spectra were collected with an automated Photon Technology International (PTI) QuantaMaster 40 fluorometer equipped with an LPS-220B lamp power supply, a 75-W Xenon arc lamp and a Hamamatsu R2658 photomultiplier tube. Oxygen-free solutions of **Pd[DMBil1]-PEG$_{750}$** in methanol or PBS were prepared in a nitrogen-filled glovebox and transferred to 1.0 cm pathlength quartz cuvettes with screw cap closures from Firefly Scientific. The solution of **Pd[DMBil1]-PEG$_{750}$** in methanol was prepared at a concentration of 21.4 μM such that its absorbance at 500 nm closely matched the A = 500 nm absorbance of a 128 μM solution of $[Ru(bpy)_3](PF_6)_2$ in acetonitrile (used as an emission standard where Φ_{ref} = 0.094 under a nitrogen atmosphere). The sample and standard were then excited at λ_{ex} = 500 nm and emission was monitored from λ_{em} = 515 - 1000 nm. The solution of **Pd[DMBil1]-PEG$_{750}$** in PBS was prepared at a concentration of 80 μM such that its absorbance at 460 nm closely matched that of a 32 μM standard solution of $[Ru(bpy)_3](PF_6)_2$ in acetonitrile at that wavelength. The sample and standard were then excited at λ_{ex} = 460 nm and emission was monitored from λ_{em}= 515 - 1000 nm. Emission spectra of the methanol and PBS solutions of **Pd[DMBil1]-PEG$_{750}$** were re-measured after exposure of the samples to air. All reported spectra are the average of 5 individual acquisitions collected using a step size of 1 nm and an integration time of 0.25 sec. Emission quantum yields were calculated using the following expression:

$$\Phi_s = \Phi_{ref}\left(\frac{I_s}{I_{ref}}\right)\left(\frac{A_{ref}}{A_s}\right)\left(\frac{\eta_s}{\eta_{ref}}\right)^2$$

where Φ_s and Φ_{ref} are the emission quantum yields of the sample and reference, respectively, I_s and I_{ref} are the integrated emission intensities of the sample and reference, respectively, A_s and A_{ref} are the measured absorbances of the sample and reference at the excitation wavelength, and η_s and η_{ref} are the refractive indices of the solvents used to dissolve the sample and reference, respectively.

Singlet Oxygen Experiments. Data was collected using the automated Photon Technology International (PTI) QuantaMaster 40 fluorometer setup mentioned above for the emission experiments. All $^1O_2$ experiments were conducted in 1.0 cm path length quartz cuvettes (6Q) from Firefly Scientific. $^1O_2$ quantum yields were calculated using the following equation:

$$\Phi_s = \Phi_{ref}\left(\frac{m_s}{m_{ref}}\right)\left(\frac{\varepsilon_{ref}}{\varepsilon_s}\right)$$

where Φ_s and Φ_{ref} represent $^1O_2$ quantum yields for the sample and a reference standard, respectively, m_s and m_{ref} are the rates of consumption of a singlet oxygen trapping tetrapyrrole complex in the presence of the sample and standard respectively, and ε_s and ε_{ref} are the extinction coefficients of the sample and standard at the wavelength of irradiation. Reported $^1O_2$ quantum yields are averages obtained from at least three trials.

$^1O_2$ production in methanol was measured by monitoring the attenuation of fluorescence from the trapping agent 1,3-diphenylisobenzofuran (DPBF) as it reacted with $^1O_2$ to form a non-emissive product. $(Ru(bpy)_3](PF_6)_2$ was used as the standard (Φ_{ref} = 0.81). Cuvettes were prepared to contain 2.0 mL of methanol that was 1.0 μM in DPBF and 10.0 μM in either **Pd[DMBil1]-PEG$_{750}$** or $[Ru(bpy)_3](PF_6)_2$. A third cuvette served as a control and contained only 2.0 mL of a 1.0 μM solution of DPBF in methanol. Consumption of DPBF was monitored by observing the decrease in the integrated intensity of its emission profile following 0, 10, 20, 30, and 40 sec of irradiation with light from a 150 Watt halogen lamp (Nikon, Inc., Model MKII) fitted with a 10 nm FWHM bandpass filter centered at 500 nm (Thor Labs, FB500-10). Emission spectra of DPBF were obtained by exciting at λ_{ex} = 405

nm and scanning from λ_{em} = 400 - 600 nm using a step size of 1 nm and an integration time of 0.25 sec. To correct for any absorption of the DPBF emission by the sample or standard, calibration curves were generated to plot the integrated emission intensity as a function of the concentration of unreacted DPBF remaining in the presence of **Pd[DMBil1]-PEG$_{750}$** or $[Ru(bpy)_3](PF_6)_2$. Emission spectra were collected from 10.0 μM solutions of the PEGylated photosensitizer or the reference standard which had not been irradiated and contained 0, 0.25, 0.50, 0.75, 1.0, 1.25, or 1.50 μM concentrations of DPBF. Linear regression lines were then fit to the calibration data from each solution. Linear regression analyses enabled the integrated emission intensity values obtained from the $^1O_2$ experiments to be converted into the corresponding concentrations of unreacted DPBF. Plots of the concentration of unreacted DPBF as a function of the irradiation time of the **Pd[DMBil1]-PEG$_{750}$** and $[Ru(bpy)_3](PF_6)_2$ solutions formed straight lines with slopes m_s and m_{ref} respectively, which were used in the equation above to calculate the $^1O_2$ quantum yield(s).

Singlet oxygen production in PBS was measured by monitoring the enhancement in fluorescence resulting from the reaction of the fluorescent probe Singlet Oxygen Sensor Green (SOSG) with $^1O_2$. Methylene blue was used as the standard (Φ_{ref} = 0.52). Aliquots (2.0 mL) of pure PBS were added to two cuvettes intended to act as controls; two additional cuvettes were prepared with 2.0 ml aliquots of a 10.0 μM solution of **Pd[DMBil1]-PEG$_{750}$** in PBS; and two other cuvettes were filled with 2.0 mL aliquots of a 10.0 μM solution of the methylene blue standard in PBS. In accordance with instructions from the manufacturer, 100 μg of SOSG was dissolved in 33.0 μL of methanol, and the resulting ~ 5 mM solution was then added to 627 μL of deionized water to make a 0.25 mM solution which was 95% water and 5% methanol by volume. A 20.0 μL aliquot of this SOSG solution was then added to each of the six prepared cuvettes such that the final PBS solutions in the cuvettes contained a 2.5 μM concentration of SOSG and 0.05% methanol by volume. Production of $^1O_2$ was monitored in one control cuvette, one cuvette containing **Pd[DMBil1]-PEG$_{750}$**, and one cuvette containing methylene blue by observing the increase in the integrated intensity of the SOSG emission profile following 0, 30, 60, 90, and 120 sec of irradiation with light from a 150 Watt halogen lamp (Nikon, Inc., Model MKII) fitted with a 10 nm FWHM bandpass filter centered at 550 nm (Thor Labs, FB550-10). Emission spectra of SOSG were obtained by exciting at λ_{ex} = 480 nm and scanning from λ_{em} = 500 - 650 nm using a step size of 1 nm and an integration time of 0.25 sec. To correct for the possibility that the 480 nm excitation

light used to collect the SOSG emission spectra might result in small amounts of $^1O_2$ sensitization by the photosensitizers or by SOSG itself, the three remaining control, **Pd[DMBil1]-PEG$_{750}$**, and methylene blue cuvettes served as dark controls which were not exposed to the 30 second intervals of 550 nm light but were still monitored for changes in SOSG emission. The change in integrated emission intensity of each cuvette was plotted as a function of irradiation time, and linear regression lines were fit to the data. The slopes of the regression lines from the dark controls were subtracted from the slopes of the regression lines from the cuvettes exposed to 550 nm light to give values of m_s and m_{ref} used with the equation above to calculate Φ_s.

MDA-MB-231 Cell Culture. MDA-MB-231 cells were purchased from American Type Culture Collection (ATCC) and cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cells were cultured in T75 cell culture flasks and incubated at 37°C in a 5% CO_2 humidified environment. Cells were passaged between flasks or into sample plates by detaching the cells from the flasks with Trypsin-EDTA, diluting the cells with complete medium, and counting the cells with a hemocytometer before transferring to a new flask or well plate.

Cell Uptake Analysis. For flow cytometry experiments, MDA-MB-231 cells were plated at 25,000 cells/well in 24-well plates and were incubated overnight. **Pd[DMBil1]-PEG$_{750}$** was diluted to 0, 0.5, 1.0, or 1.5 mM in complete cell culture media, which was then added to cells. Well plates were incubated with **Pd[DMBil1]-PEG$_{750}$** for 48 hr in the dark and then cells were detached with trypsin-EDTA and transferred to 1.5 ml Eppendorf tubes. The samples were centrifuged to form a cell pellet, the supernatant was removed, and the cells were re-suspended in sterile 1X PBS. Samples were read on a Novocyte flow cytometer (ACEA Biosciences) with the phycoerythrin (PE) filter set (excitation, 488 nm; emission, 572/28 nm) and data analysis was performed using the NovoExpress software package. The median fluorescence intensity (MFI) was calculated by averaging the fluorescence median from three experiments of each photosensitizer concentration. For fluorescence imaging, cells were plated at 15,000 cells/well in a glass bottom 8-well plate with a removable well chamber and were incubated overnight. A solution of **Pd[DMBil1]-PEG$_{750}$** (1 mM) diluted in cell culture media was added to cells, which were then incubated for 24 hr. Cells were then fixed with 4% formaldehyde for 15 min and rinsed 3X with 1X PBS. Cells were stained with DAPI and phalloidan to visualize cell nuclei and F-actin on the cytoskeleton, respectively. Well chambers were removed and slides were mounted

with Vectashield mounting media. Cells were imaged with a Zeiss Axioobserver Z1 Inverted Fluorescent Microscope equipped with an apotome using the FITC (F-actin), DsRed (photosensitizer), and DAPI (nuclei) fluorescence channels.

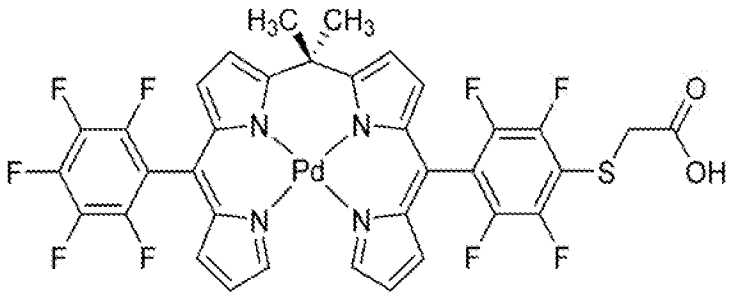
Annexin/PI Staining. MDA-MB-231 cells were plated at 10,000 cells/well in black-walled 96-well plates and incubated overnight. Cells were treated with 0, 4.0, 6.0, or 8.0 μM **Pd[DMBil1]-PEG$_{750}$** diluted in complete cell culture media for 24 hr in the dark. The cells were then irradiated with a LightPad 930 (Artograph) with a long pass λ = 525 nm filter for 0 or 30 min. After incubating the cells for 1 hr, an AnnexinV-FITC stain (Cayman Chemicals) was conducted *via* manufacturer instructions. Briefly, cells were lifted with trypsin, washed 1X with 1X binding buffer (300xg, 5 min), and resuspended in 50 μL binding buffer containing 1:500 AnnexinV-FITC and 1:2000 propidium iodide (PI) stains for 10 min, while protected from light. The samples were then diluted with 150.0 μL of 1X binding buffer and run on the flow cytometer with the FITC (excitation, 488 nm; emission, 530/30 nm) and PerCP (excitation, 488 nm; emission, 675/30 nm) channels. Data analysis was performed using the NovoExpress software package, and positive stained gates were based off of unstained cells. Single stained controls were used for compensation. Data shown are averaged amongst three independent experiments and statistical significance was calculated using t-tests to compare cells treated with and without light for each photosensitizer concentration.

Cell Viability Assays. MDA-MB-231 cells were plated at 10,000 cells/well in black-walled 96-well plates and incubated overnight. The cells were then treated with **Pd[DMBil1]-PEG$_{750}$** at 0-5.0 mM concentrations for the dark toxicity assays or 0-10.0 μM concentrations for the light exposure experiments. For the commercial photosensitizer experiments, isohematoporphyrin (IHP) or hematoporphyrin dihydrochloride (HPDC) were diluted to 0-3.0 mM or 0-700.0 μM for dark toxicity or for light exposure experiments, respectively. All solutions of photosensitizers were prepared by diluting the dried tetrapyrrole complexes in complete cell culture media. To evaluate the inherent photosensitizer toxicity in the dark, well plates were covered with aluminum foil to avoid light contamination and were incubated for 48 hr prior to Alamar blue viability assays. In the PDT experiments with light irradiation, plates were covered with aluminum foil and were incubated for 0 or 24 hr prior to light exposure. The 96-well plates were placed onto a LightPad 930 (Artograph) with a long pass (λ = 525 nm) filter for 0, 10, 20, or 30 min. After light treatment, the **Pd[DMBil1]-PEG$_{750}$**-containing cell culture media was replaced with fresh media. Plates were covered with aluminum foil and were incubated overnight. The media was then removed and the Alamar blue viability reagent (diluted 1:10 in cell culture media) was added per

manufacturer recommendations. Samples were read on a Hybrid Synergy H1M plate reader with excitation and emission wavelengths of 560 nm and 590 nm, respectively. To analyze the data, background (Alamar blue reagent without cells) was subtracted from each well. Then, wells were averaged and normalized to cells treated with 0 μM photosensitizer. Data reported are averaged values from at least three experiments that were each run with triplicate wells per photosensitizer concentration and irradiation time. The **Pd[DMBil1]-PEG$_{750}$** experimental data was analyzed by 2-way ANOVA with post hoc Tukey-Kramer, and the IHP and HPDC data was analyzed by 1-way ANOVA with post hoc Tukey. Phototoxicity indices were calculated by dividing the lethal dose for 50% cell viability (LD50) by the effective dose for 50% cell viability (ED50).

Synthetic Protocols

Palladium 10,10-Dimethyl-5-[para(mercaptoacetic acid) tetrafluorophenyl]-15-(pentafluorophenyl)biladiene (Pd[DMBil]-SCH$_2$CO$_2$H)

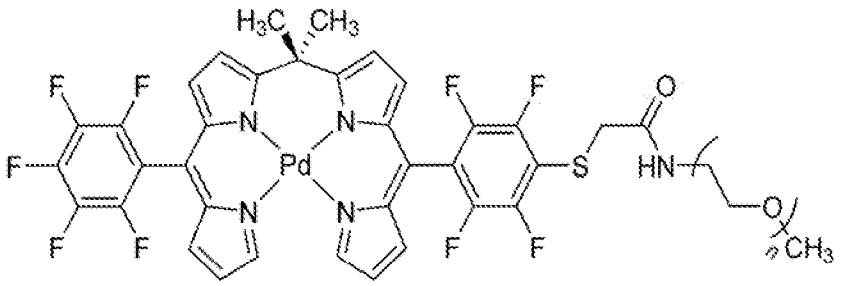


Pd[DMBil]-SCH$_2$CO$_2$H

Pd[DMBil1] (0.204 g, 267 μmol) was dissolved in 75 mL of dimethylformamide in a round bottomed flask. Triethylamine (44.5 μL, 319 μmol) was added and the reaction mixture was sparged with N_2 gas for 15 minutes. Mercaptoacetic acid (22.0 μL, 317 μmol) was added to the vessel and the reaction was heated at 90°C under N_2 for 1 hr. The solvent was removed via rotary evaporation and the resulting residue was redissolved in ethyl acetate and washed 8 times with brine to remove the remaining traces of DMF. The organic layer was collected, dried over Na_2SO_4, and concentrated via rotary evaporation. The crude product was purified by column chromatography using Ci-silica along with hexanes and ethyl acetate (3:1, then 2:1, then 1:1, then pure ethyl acetate) as the eluent. Chromatography yielded 3 red bands: the first band was unreacted **Pd[DMBil1],** the third band was a di-substituted side product (Palladium 10,10-

dimethyl-5,15-bis[para(mercaptoacetic acid)tetrafluorophenyl]biladiene), and the second band gave 156 mg of the desired product as a red solid in 76% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.39 (t, J = 1.3 Hz, 2H), 6.66 (d, J = 4.4 Hz, 2H), 6.62 to 6.52 (m, 4H), 6.48 (dd, J = 4.3, 1.4 Hz, 2H), 3.79 (s, 2H), 1.79 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C) δ/ppm: 173.36, 166.78, 147.98, 147.84, 146.19, 146.01, 145.86, 145.52, 145.37, 143.51, 143.36, 138.70, 135.02, 134.67, 134.39, 134.07, 128.36, 118.08, 113.61, 113.41, 41.76, 35.43. ^{19}F NMR (376 MHz, $CDCl_3$, 25 °C) δ/ppm: -132.33 to -132.70 (m, 2F), -137.51 to -137.80 (m, 2F), -137.88 to -138.12 (m, 2F), -151.65 to -152.15 (m, 1F), -160.48 (td, J = 21.7, 6.6 Hz, 2F). HR-LIFDI-MS: $[M]^+$ m/z: calcd for $C_{39}H_{19}F_9N_4O_2PdS$, 836.0120; found, 836.0132.

Palladium 10,10-Dimethyl-5-[para(N-(methoxypolyethylene glycol)mercaptoacetamide)tetrafluorophenyl]-15-(pentafluorophenyl) biladiene (Pd[DMBil1]-PEG$_{750}$)

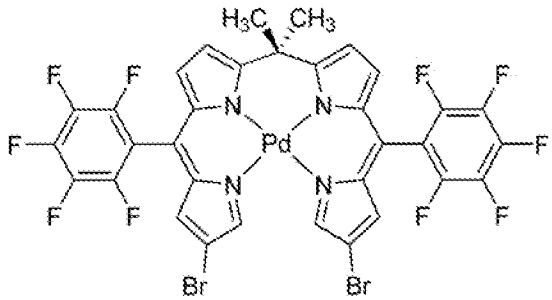


Pd[DMBil1]-PEG$_{750}$

Pd[DMBil]-SCH$_2$CO$_2$H (61 mg, 73 μmol) was combined with *N*-hydroxysuccinimide (17 mg, 148 μmol) and 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (28 mg, 146 μmol) in a round bottomed Schleck flask. The flask was placed under vacuum for 1 hr and then 5.5 mL of dry CH_2Cl_2 was added, and the reaction was stirred under N_2 at room temperature for 20 hrs. The crude reaction mixture was diluted with extra CH_2Cl_2, washed 4 times with deionized water and once with brine, and dried over Na_2SO_4. The solvent was removed via rotary evaporation, and the remaining residue was redissolved in 6 ml of DCM. Methoxy-PEG750-amine (82 mg, 109 μmol) and triethylamine (81 μL, 581 μmol) were added to the resulting solution, which was stirred under air at room temperature for 18 hrs. Following removal of the solvent via rotary evaporation, the crude product was redissolved in CH_2Cl_2, washed 4 times with deionized water and once with brine and dried over Na_2SO_4. The product was further purified by column chromatography on silica by eluting with 3% methanol in CH_2Cl_2, which removed

several impurities. The eluent was then changed to 6% methanol in CH_2Cl_2 and finally to 8% methanol in CH_2Cl_2 to yield 98 mg of the title product as red material in 86% yield. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.39 (d, *J*= 6.4 Hz, 2H), 7.35 (s, 1H), 6.69 (d, *J* = 4.5 Hz, 1H), 6.65 (d, *J* = 4.5 Hz, 1H), 6.61 (d, *J* = 4.2 Hz, 1H), 6.57 (d, *J* = 4.2 Hz, 1H), 6.55 (t, *J* = 5.0 Hz, 2H), 6.50 to 6.45 (m, 2H), 3.71 (s, 2H), 3.64 (d, *J* = 7.0 Hz, 59H), 3.55 (dt, *J* = 13.8, 4.6 Hz, 5H), 3.48 (q, *J* = 5.2 Hz, 2H), 3.37 (s, 3H), 1.80 (s, 6H). ^{13}C NMR (151 MHz,$CDCl_3$,25°C) δ/ppm: 167.27, 166.90, 166.72, 147.51, 145.84, 143.88, 138.24, 136.61, 135.02, 134.72, 134.40, 134.12, 128.35, 117.88, 114.24, 112.35, 72.06, 70.72, 70.42, 69.70, 41.77, 39.91, 37.73. ^{19}F NMR (565 MHz, $CDCl_3$, 25 °C) δ/ppm: -132.62 (dd, *J*= 24.7, 11.7 Hz, 2F), -138.13 (ddd, *J* = 57.5, 23.3, 8.8 Hz, 4F), -151.81 to-152.36 (m, 1F), -160.68 (dt, *J* = 21.8, 10.9 Hz, 2F). HR-ESI-MS: $[M + Na]^+$ *m/z*: calcd for $C_{88}H_{86}F_9N_5O_{17}PdSNa$, 1576.45227; found, 1576.45259. Due to the range of PEG chain lengths present in the original methoxy-PEG-amine, the mass spectrogram of **Pd[DMBil1]-PEG$_{750}$** shows a distribution of peaks between m/z ratios of 700-1050 as well as a distribution between m/z ratios of 1300 - 1800. The peaks at m/z ratios between 700 and 1050 are separated by 22 mass units (half the size of an ethylene glycol monomer), and correspond to $[M + 2Na]^{2+}$ while the peaks at m/z ratios between 1300 and 1800 are separated by 44 mass units (the size of a single ethylene glycol monomer), and correspond to $[M + Na]^+$.

Palladium 2,18-Dibromo-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBilBr$_2$]):



Pd[DMBilBr$_2$]

To a solution of **Pd[DMBil2]** (490 mg, 0.643 mmol) dissolved in 100 mL of acetonitrile was added dropwise a solution of N-bromosuccinimide (265 mg, 1.488 mmol, 2.3 equivalents) dissolved in 20 mL of dichloromethane. The reaction was stirred at room temperature covered for two hours, afterwhich the solution was poured over 100 mL of deionized H_2O in a 250 mL separatory funnel. The product was extracted with ethyl acetate, washed with brine, then dried over sodium sulfate. Following solvent

removal via rotary evaporation the product was purified by flash chromatography using 10% diethyl ether in hexanes as the eluent to give 565 mg of the target material as a dark red solid in 95% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.23 (s, 2H), 6.73 (d, J = 4.6 Hz, 2H), 6.62 (d, J = 4.6 Hz, 2H), 6.54 (s, 2H), 1.8 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 168.61, 149.38, 146.13, 143.66, 135.41, 133.94, 132.63, 128.48, 128.13, 127.88, 118.20, 104.63, 100.10, 42.23, 31.02. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -138.36 (dd, J = 10.05, 6.275, 4F), -151.39 (t, J = 12.55, 2F), -160.33 (dt, J = 10.05, 3.75, 4F). HR-LIFDI-MS: $[M+H]^+$ *m/z*: calcd for $C_{33}H_{18}N_4F_{10}Br_2Pd$, 921.8617; found 921.8586.

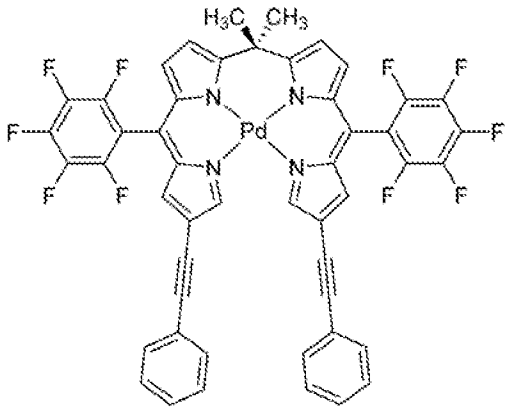
Pd[DMBilBr$_2$] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(Aryl-ethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-X]):

The following general procedure was used to prepare a series of palladium 2,18-bis(aryl-ethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene complexes, which are individually characterized in the following examples.

To a baked 100 mL Schlenk flask copper(I) iodide (9 mg, 0.048 mmol) was added with a stir bar. The flask was placed under vacuum followed by addition of 3 mL of anhydrous triethylamine and corresponding aryl-alkyne (1.5 mmol, 10 equivalents) and stirring for 15 minutes at room temperature. Positive pressure of nitrogen gas was then applied and the septa was removed in order to add Pd[DMBilBr$_2$] (138 mg, 0.15 mmol) and tetrakis-palladium(0) triphenylphosphine (50 mg, 0.044 mmol). The septa was then replaced and anhydrous tetrahydrofuran (40 mL) was cannulaed into solution, where upon the solution was stirred at 75°C for 18 hours. After the time elapsed, the solution was cooled to room temperature then exposed to air and diluted with 50 mL of ethyl acetate. The resulting solution was washed once with saturated ammonium chloride solution and once with brine and dried over sodium sulfate followed by solvent removal via rotary evaporation. The product was purified by column chromatography using corresponding conditions as the eluent to give product material.

Palladium 2,18-Bis(phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2]):

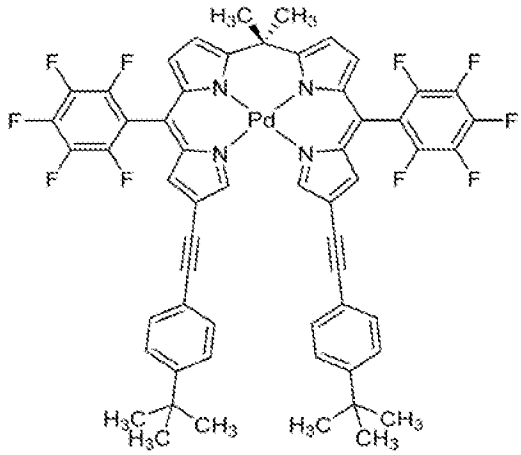


Pd[DMBil2]

The crude material was purified by column chromatography on silica using 10% CH_2Cl_2 in hexanes to yield 121 mg of target material as a maroon solid in 84% yield. ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.57 (s, 2H), 7.45 (d, 4H), 7.28 (m, 6H) 6.74 (d, *J* = 4.8 Hz, 2H), 6.69 (s, 2H), 6.61 (d, *J* = 4.8 Hz, 2H), 1.82 (s, 6H), 1.29 (s, 16H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 168.37, 152.99, 136.02, 133.97, 132.25, 131.46, 129.81, 128.43, 128.11, 123.51, 118.05, 113.72, 91.88, 83.64, 61.92, 42.13, 31.01. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -137.81 (dt, *J* = 8.785, 5.02, 4F), -151.19 (dt, *J* = 8.785, 5.02 Hz, 2F), -160.02 (dt, *J* = 10.05, 3.75 Hz, 4F). HR-ESI-MS: $[M+H]^+$ *m/z*: calcd for $C_{49}H_{25}N_4F_{10}Pd$, 965.09758; found 965.09758.

Pd[DMBil2] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(para-tert-butyl-phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-^{t}Bu]):

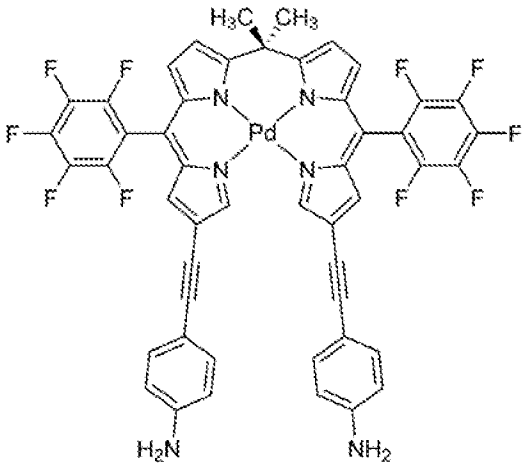


Pd[DMBil2-^{t}Bu]

The crude material was purified by column chromatography on silica using 10% CH_2Cl_2 in hexanes to yield 130 mg of target material as a maroon solid in 81% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.57 (s, 2H), 7.26 (d, *J* = 9.6 Hz, 4H), 7.31 (*J* = 9.6 Hz, 6H) 6.75 (d, *J* = 4.6 Hz, 2H), 6.71 (s, 2H), 6.62 (d, *J* = 4.6Hz, 2H), 1.83 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 168.16, 153.21, 151.32, 135.92, 133.99, 132.07, 131.19, 129.72, 128.35, 125.43, 120.47, 117.87, 114.06, 92.08, 82.89, 77.36, 42.07, 36.66, 34.90, 31.58, 31.30, 31.02. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -138.31 (dd, *J* = 11.295, 3.765, 4F), -151.80 (t, *J* = 13.805, 2F), -160.58 (dt, *J* = 13.805, 3.765, 4F). HR-LIFDI-MS: $[M+H]^+$ *m/z*: calcd for $C_{57}H_{40}N_4F_{10}Pd$, 1076.2128; found 1076.1362.

Pd[DMBil2-^{t}Bu] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(para-amino-phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-NH$_2$]):

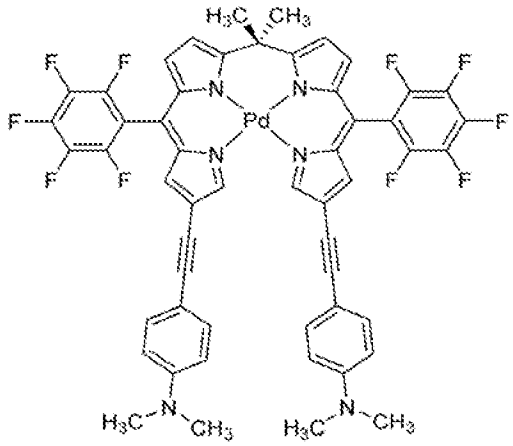


Pd[DMBil2-NH$_2$]

The crude material was purified by column chromatography on silica using 50% ethyl acetate in hexanes to yield 102 mg of target material as a purple solid in 68% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.54 (s, 2H), 7.39 (d, J = 9 Hz, 4H), 6.71 (d, J = 4.6 Hz, 2H), 6.65 (s, 2H), 6.59 (d, J = 4.6 Hz, 2H), 6.58 (d, J = 9 Hz, 4H), 3.78 (s, br, 4H), 1.81 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 167.84, 153.35, 146.54, 146.14, 143.70, 135.76, 134.05, 132.87, 131.76, 129.47, 128.13, 117.56, 114.87, 114.57, 112.88, 92.62, 81.41, 77.36, 41.97, 31.02. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -137.81 (dd, J = 10.04, 3.765, 4F), -151.45 (t, J = 15.06, 2F), -160.18 (dt, J = 15.06, 3.765, 4F). HR-ESI-MS: $[M+H]^+$ m/z: calcd for $C_{49}H_{27}N_6F_{10}Pd$, 995.11723; found 995.11869.

Pd[DMBil2-NH$_2$] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(para{N,N-dimethyl-amino}-phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-NMe$_2$]):

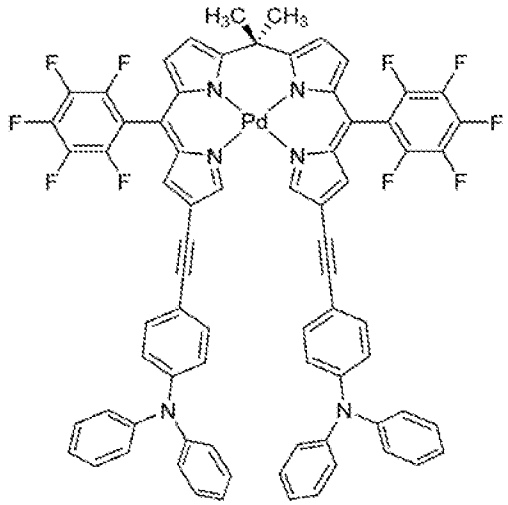


Pd[DMBil2-NMe$_2$]

The crude material was purified by column chromatography on silica using 10% ethyl acetate in hexanes to yield 102 mg of target material as a purple solid in 67% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.56 (s, 2H), 7.34 (d, *J* = 9 Hz, 4H), 6.70 (d, *J* = 4.6 Hz, 2H), 6.65 (s, 2H), 6.61 (d, *J* = 9 Hz, 4H), 6.59 (d, *J* = 4.6 Hz, 2H), 2.96 (s, 12H), 1.82 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 167.64, 153.57, 150.01, 146.20, 143.71, 135.68, 134.13, 132.79, 132.64, 131.57, 129.33, 128.00, 117.38, 114.92, 111.93, 110.27, 93.20, 81.40, 41.92, 40.35, 31.04. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -137.80 (dd, *J* = 11.295, 3.765, 4F), -151.56 (t, *J* = 13.805, 2F), -160.24 (dt, *J* = 13.805, 3.765, 4F). HR-ESI-MS: $[M+H]^+$ *m/z*: calcd for $C_{53}H_{35}N_6F_{10}Pd$, 1051.17984; found 1051.8292.

Pd[DMBil2-NMe$_2$] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(para{N,N-diphenyl-amino}-phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-NPh$_2$]):

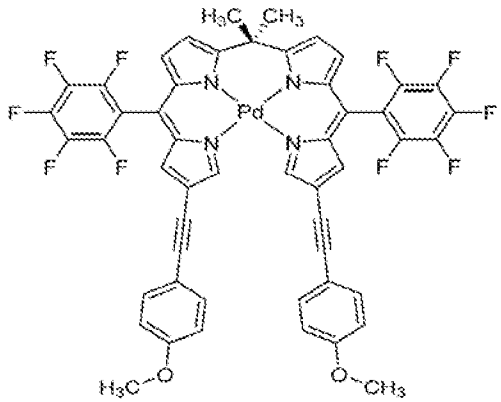


Pd[DMBil2-NPh$_2$]

The crude material was purified by column chromatography on silica using 20% CH_2Cl_2 in hexanes to yield 118 mg of target material as a purple solid in 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.55 (s, 2H), 7.29 (d, *J* = 8 Hz, 4H), 7.25 (t, *J* = 8.8 Hz, 6H), 7.08 (d, *J* = 8 Hz, 6H), 7.04 (t, *J* = 8.8 Hz, 4H), 6.95 (d, *J* = 8Hz, 4H), 6.73 (d, *J* = 4.6 Hz, 2H), 6.66 (s, 2H), 6.61 (d, *J* = 4.6 Hz, 2H), 1.82 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 168.08, 153.19, 147.75, 147.30, 135.89, 134.03, 132.38, 131.98, 129.49, 128.25, 125.06, 123.58, 122.49, 117.80, 116.35, 114.20, 100.11, 92.21, 82.82, 42.06, 31.03. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -137.81 (dd, *J* = 8.785, 3.765, 4F), -151.30 (t, *J* = 13.805, 2F), -160.08 (dt, *J* = 13.805, 3.765, 4F).

Pd[DMBil2-NPh$_2$] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(para-methoxy-phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-OCH$_3$]):

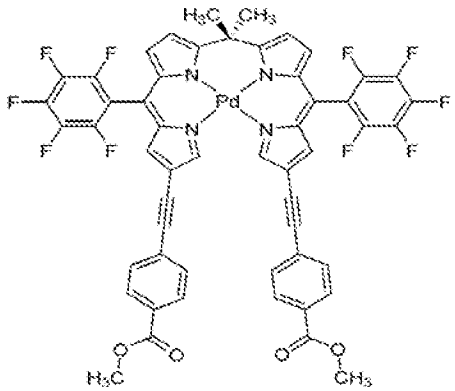


Pd[DMBil2-OCH$_3$]

The crude material was purified by column chromatography on silica using 15% ethyl acetate in hexanes to yield 132 mg of target material as a purple solid in 86% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.57 (s, 2H), 7.45 (m, 4H), 7.28 (m, 4H), 6.78 (d, *J* = 4.6 Hz, 2H), 6.74 (s, 2H), 6.61 (d, *J* = 4.6 Hz, 2H), 3.79 (s, 6H), 1.82 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 168.21, 152.78, 136.20, 134.14, 132.46, 131.79, 131.46, 129.91, 129.44, 128.41, 128.04, 123.58, 117.82, 113.39, 111.08, 91.69, 83.86, 61.92, 42.06, 31.00. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -138.33 (dd, *J* = 10.04, 3.765, 4F), -151.82 (t, *J* = 13.805, 2F), -160.61 (dt, *J* = 13.805, 3.765, 4F). HR-ESI-MS: $[M+H]^+$ *m/z*: calcd for $C_{51}H_{29}N_4F_{10}O_2Pd$, 1081.10640 found 1081.11003.

Pd[DMBil2-OCH$_3$] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(para-methyl-ester-phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-Me Ester]):

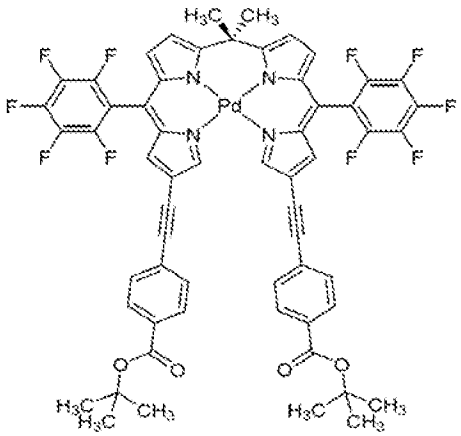


Pd[DMBil2-Me Ester]

The crude material was purified by column chromatography on silica using 10% ethyl acetate in hexanes to yield 118 mg of target material as a maroon solid in 73% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.96 (d, *J* = 8 Hz, 4H), 7.57 (s, 2H), 7.49 (d, *J* = 8 Hz, 4H), 6.78 (d, *J* = 4.6 Hz, 2H), 6.72 (s, 2H), 6.64 (d, *J* = 4.6 Hz, 2H), 3.90 (s, 6H), 1.83 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 168.87, 168.71, 152.59, 136.28, 133.93, 132.69, 131.22, 129.88, 129.64, 129.18, 128.65, 128.29, 118.53, 112.93, 91.26, 86.99, 52.35, 42.26, 30.96. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -137.81 (dd, *J* = 9.415, 3.765, 4F), -150.91 (t, *J* = 13.805, 2F), -159.85 (dt, *J* = 13.805, 3.765, 4F). HR-ESI-MS: $[M+H]^+$ *m/z*: calcd for $C_{53}H_{41}N_4F_{10}O_4Pd$, 1025.11657 found 1025.11705.

Pd[DMBil2-Me Ester] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(para-tert-butyl-ester-phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-^{t}Bu Ester]):

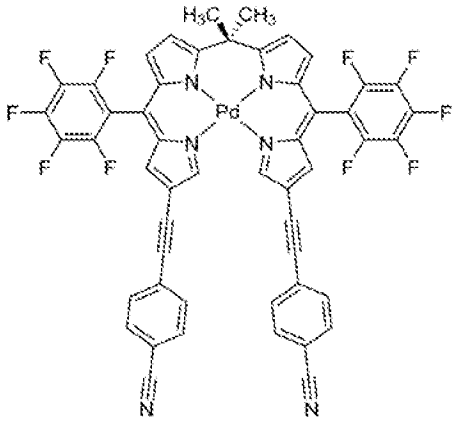


Pd[DMBil2-^{t}Bu Ester]

The crude material was purified by column chromatography on silica using 10% ethyl acetate in hexanes to yield 113 mg of target material as a maroon solid in 65% yield. ^{1}H NMR (400 MHz, CD_3CN, 25 °C) δ/ppm: 7.96 (d, *J* = 8.8 Hz, 4H), 7.57 (s, 2H), 7.47 (d, *J* = 8.8 Hz, 4H), 6.77 (d, *J* = 4.6 Hz, 2H), 6.72 (s, 2H), 6.64 (d, *J* = 4.6 Hz, 2H), 1.83 (s, 6H), 1.57 (s, 18). ^{13}C NMR (100 MHz, CD_3CN, 25 °C) δ/ppm: 170.57, 165.52, 153.46, 137.25, 134.35, 134.23, 132.62, 132.52, 131.59, 131.49, 130.75, 129.68, 129.51, 128.81, 128.33, 120.16, 88.16, 81.65, 42.82, 30.83, 28.17. ^{19}F NMR (251 MHz, CD_3CN, 25 °C) δ/ppm: -140.51 (s, 4F), -155.37 (t, *J* = 13.805, 2F), -163.26 (dt, *J* = 13.805, 3.765, 4F). HR-ESI-MS: $[M+H]^+$ *m/z*: calcd for $C_{59}H_{40}N_4F_{10}O_4Pd$, 1165.20030 found 1165.20529.

Pd[DMBil2-^{t}Bu Ester] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(para-cyano-phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-CN]):

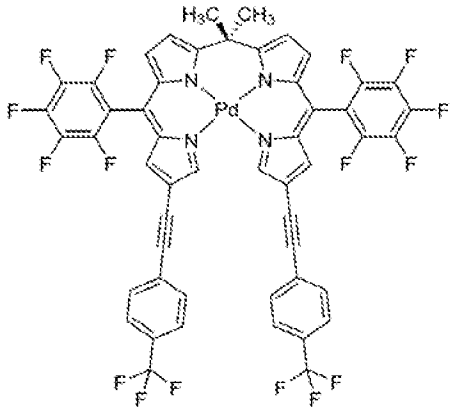


Pd[DMBil2-CN]

The crude material was purified by column chromatography on silica using 10% ethyl acetate in hexanes to yield 86 mg of target material as a maroon solid in 57% yield. ^{1}H NMR (600 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.57 (s, 2H), 7.56 (d, *J* = 8.4 Hz, 4H) 7.49 (d, *J* = 8.4 Hz, 4H), 6.79 (d, *J* = 4.6 Hz, 2H), 6.72 (s, 2H), 6.67 (d, *J* = 4.6 Hz, 2H), 1.83 (s, 6H). ^{13}C NMR (151 MHz, $CDCl_3$, 25 °C) δ/ppm: 169.33, 168.84, 152.22, 136.53, 133.94, 133.06, 132.29, 132.23, 132.16, 132.12, 131.73, 129.82, 128.70, 128.62, 128.59, 118.94, 118.69, 112.34, 111.18, 30.40, 88.62, 42.44, 30.97, 29.86. ^{19}F NMR (377 MHz, $CDCl_3$, 25 °C) δ/ppm: -138.36 (dd, *J* = 10.818, 3.765, 4F), -151.23 (t, *J* = 13.805, 2F), -160.29 (dt, *J* = 13.805, 3.765, 4F). HR-ESI-MS: $[M+H]^+$ *m/z*: calcd for $C_{51}H_{22}N_6F_{10}Pd$, 1015.08593 found 1015.08828.

Pd[DMBil2-CN] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

Palladium 2,18-Bis(para-trifluoromethyl-phenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-CF_3]):

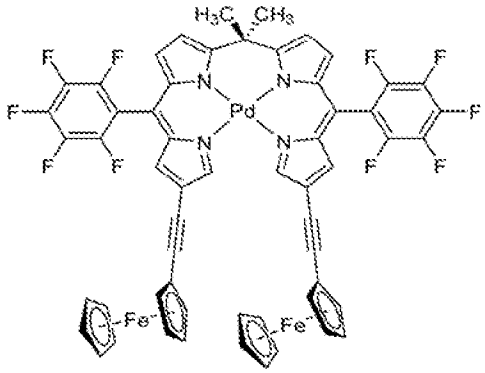


Pd[DMBil2-CF_3]

The crude material was purified by column chromatography on silica using 10% ethyl acetate in hexanes to yield 127 mg of target material as a purple solid in 77% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.57 (s, 2H), 7.54 (s, 8H), 6.78 (d, *J* = 4.6 Hz, 2H), 6.72 (s, 2H), 6.65 (d, *J* = 4.6 Hz, 2H), 1.83 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 168.85, 152.35, 136.19, 133.77, 132.65, 131.38, 129.72, 129.66, 128.56, 127.25, 125.28, 125.24, 125.20, 122.28, 118.50, 112.58, 90.40, 86.19, 42.17, 30.84, 29.73. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -62.24 (s, 6F), -137.85 (dd, *J* = 9.415, 3.765, 4F), -150.86 (t, *J* = 12.55, 2F), -159.82 (dt, *J* = 12.55, 3.765, 4F). HR-ESI-MS: $[M+H]^+$ *m/z*: calcd for $C_{51}H_{23}N_4F_{16}Pd$, 1101.07021; found 1101.07022.

Pd[DMBil2-CF_3] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG_{750}** and **Pd[DMBil2]-diPEG_{550}**.

Palladium 2,18-Bis(ferrocenylethynyl)-10,10-dimethyl-5,15-dipentafluorophenylbiladiene (Pd[DMBil2-Fc]):

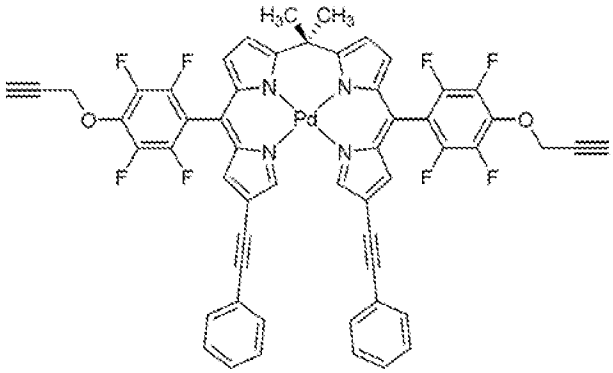


Pd[DMBil2-Fc]

The crude material was purified by column chromatography on silica using 10% ethyl acetate in hexanes to yield 156 mg of target material as a purple solid in 88% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.53 (s, 2H), 6.72 (d, *J* = 4.6 Hz, 2H), 6.64 (s, 2H), 6.60 (d, *J* = 4.6 Hz, 2H), 4.43 (t, *J* = 1.8 Hz, 4H), 4.20 (s, 10H), 4.18 (t, *J* = 1.8 Hz, 4H), 1.82 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 167.91, 153.31, 135.83, 134.01, 131.85, 129.28, 125.67, 117.68, 114.55, 60.91, 79.73, 71.35, 70.08, 68.88, 65.55, 42.04, 31.04, 30.45, 29.5. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -138.34 (dd, *J* = 10.04, 3.765, 4F), -151.84 (t, *J* = 13.805, 2F), -160.59 (dt, *J* = 13.805, 3.765, 4F). HR-ESI-MS: $[M+H]^+$ *m/z*: calcd for $C_{57}H_{32}N_4F_{10}Fe_2Pd$, 1180.02010; found 1180.02420.

Pd[DMBil2-Fc] may be converted into a water soluble tetrapyrrole complex containing one or more poly(oxyethylene) segment-containing substituents in accordance with the present invention by utilizing the synthetic chemistry described herein for the preparation of **Pd[DMBil1]-PEG$_{750}$** and **Pd[DMBil2]-diPEG$_{550}$**.

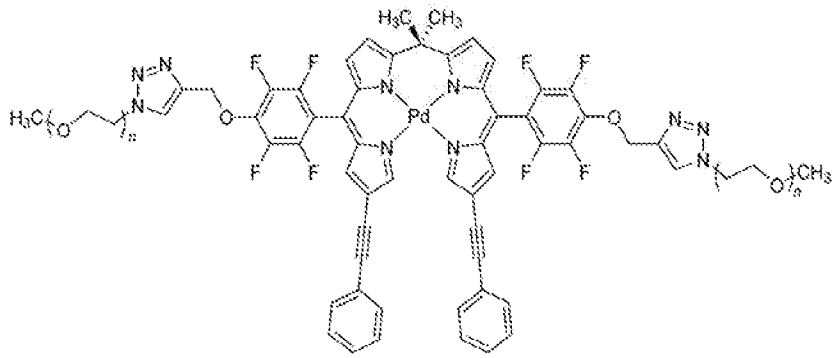
Palladium 2,18-Bis(phenylacetylene)-10,10-dimethyl-5,15-di[para-(propargyl-ether)-tetrafluorophenylbiladiene (Pd[DMBil2]-bis(propargyl)):



Pd[DMBil2]-bis(propargyl)

Pd[DMBil2] (127 mg, 0.13 mmol) was dissolved in 25 mL of tetrahydrofuran in a 100 mL round bottomed flask. Propargyl alcohol (0.083 ml, 1.3 mmol) was added to the solution followed by KOH (42 mg, 0.75 mmol). The reaction headspace was evacuated, and the solution was heated to 40 °C for 16 hours. The reaction was cooled to room temperature and diluted with 50 mL of ethyl acetate. The organic solution was then washed once with deionized water, once with saturated $NaHCO_3$ solution, and once with brine. The solution was then dried over Na_2SO_4, followed by removal of solvent via rotary evaporation. The crude material was then purified by column chromatography on silica using 40% CH_2Cl_2 in hexanes to yield 112 mg of target material as a purple solid in 83% yield. ^1H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 7.58 (s, 2H), 7.46 (d, 4H), 7.28 (m, 6H) 6.77 (d, J = 4.6 Hz, 2H), 6.73 (s, 2H), 6.61 (d, J = 4.6Hz, 2H), 5.00 (s, 4H), 2.68 (s, 2H), 1.83 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 168.20, 152.79, 136.20, 134.15, 132.46, 131.46, 129.92, 129.45, 128.42, 128.05, 123.58, 117.82, 113.40, 111.25, 91.69, 83.86, 61.92, 42.07, 31.00. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -62.24 (s, 6F), -139.45 (dt, J = 11.295, 5.02, 4F), -154.27 (dt, J = 8.785, 5.02, 4F). HR-ESI-MS: $[M+H]^+$ m/z: calcd for $C_{55}H_{30}N_4F_8O_2Pd$, 1037.13541; found 1037.13878.

Palladium 2,18-Bis(phenylacetylene)-10,10-dimethyl-5,15-di[para-triazole(methoxypolyethyleneglycol)-ether-tetrafluorophenylbiladiene (Pd[DMBil2]-diPEG$_{550}$):



Pd[DMBil2]-diPEG$_{550}$

Pd[DMBil2]-bis(propargyl) (103 mg, 0.1 mmol), copper(II) sulfate pentahydrate (48 mg, 0.2 mmol) and sodium ascorbate (60 mg, 0.3 mmol) were dissolved in 10 mL of tetrahydrofuran solution containing PEG$_{550}$-azide (326 mg, 0.54 mmol) in a 20 mL Scintillation vial. Vial was capped with a septum and the reaction headspace was evacuated and the solution was stirred at 55 °C, for 16 hours. The solution was then cooled to room temperature and poured over 100 mL of deionized water in a 250 mL separatory funnel. The aqueous layer was then extracted with 30 mL of CH_2Cl_2 five times, then the organic layers were combined and washed with brine then dried over Na_2SO_4. Following solvent removal via rotary evaporation, the crude material was then purified by column chromatography on silica using 2% methanol in CH_2Cl_2 to remove impurities, then 10% methanol in CH_2Cl_2 to collect 165 mg of target product as a purple material in 78% yield. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C) δ/ppm: 8.03 (s, 2H), 7.55 (s, 2H), 7.46 (d, 4H), 7.27 (m, 6H) 6.77 (d, *J* = 4.6 Hz, 2H), 6.71 (s, 2H), 6.61 (d, *J* = 4.6Hz, 2H), 5.51 (s, 4H), 4.61 (t, 4H), 3.89 (t, 4H), 3.62 (m, 76H), 3.53 (t, 4H), 3.37 (s, 6H), 1.81 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) δ/ppm: 168.19, 152.63, 146.17, 143.69, 142.62, 139.85, 136.20, 134.13, 132.60, 131.42, 129.91, 129.48, 128.38, 127.98, 125.18, 123.61, 117.83, 113.30, 110.69, 91.61, 83.93, 72.59, 72.02, 70.70, 70.62, 70.58, 69.51, 67.92, 59.18, 50.59, 42.04, 30.98. ^{19}F NMR (251 MHz, $CDCl_3$, 25 °C) δ/ppm: -139.63 (dt, *J* = 8.785, 5.02, 4F), -154.55 (dt, *J* = 8.785, 5.02, 4F). HR-ESI-MS: $[M+2H]^{2+}$ *m/z*: calcd for $C_{105}H_{134}N_{10}F_8O_{26}Pd$, 1104.41107; found 1104.42081. Due to the range of PEG chain lengths present in prepared methoxy-PEG-azide, the mass spectrum of shows a distrubtion of peaks between 970 and 1220 that are separated by 22 mass units (half the size for an ethylene glycol monomer), corresponding to the $[M+2H]^{2+}$.

Discussion of Experimental Results

The visible absorption profile of **Pd[DMBil1]-PEG$_{750}$** has three features centered at 402, 483 and 540 nm in methanol. Aside from showing a slight enhancement of the feature at 483 nm, this absorption profile is nearly identical to that of **Pd[DMBil1]** in methanol, suggesting that introduction of the thioether and PEG chain at the *para*-position of one biladiene C_6F_5 substituent has little effect on the electronic structure of the palladium tetrapyrrole core. In a pH 7.4 PBS solution the absorption spectrum of **Pd[DMBil1]-PEG$_{750}$** shows a bathochromic shift relative to its appearance in methanol. Although the full width at half maximum (FWHM) of the most prominent absorption feature of the PEGylated derivative increases slightly from 63 nm in methanol to 67 nm in PBS, both of these values are smaller than the FWHM of the corresponding absorption feature of **Pd[DMBil1]** in methanol (73.5 nm). Furthermore, solutions of **Pd[DMBil1]-PEG$_{750}$** behave in accordance with the Beer-Lambert Law when dissolved in either methanol or PBS at concentrations ranging from 4 to 24 μM. Self-aggregation of other tetrapyrroles under aqueous conditions has been associated with broadened absorption features and deviations from the Beer-Lambert Law, so the observation that **Pd[DMBil1]-PEG$_{750}$** does not exhibit such behavior indicates that aggregation for this system should be insignificant. The **Pd[DMBil1]-PEG$_{750}$** complex is also resistant to photodegradation, as the UV-vis absorption spectrum for this construct in PBS (24.0 μM) showed no substantial changes over the course of two hours of irradiation with 500 or 550 nm light.

The emission properties of the **Pd[DMBil1]** complex in nitrogen-saturated methanol are also largely unaffected by PEGylation. As has been previously observed for **Pd[DMBil1]**, excitation of **Pd[DMBil1]-PEG$_{750}$** with 500 nm light elicits weak fluorescence from 500-700 nm as well as phosphorescence from 700-850 nm. The experimental results show that while the fluorescence emission maximum of **Pd[DMBil1]-PEG$_{750}$** in methanol is red-shifted by 11 nm compared with that of **Pd[DMBil1]**, the fluorescence quantum yield of the PEGylated derivative (Φ_fl = 1.4 x 10^{-4}) is essentially identical to that of the parent compound (Φ_fl = 1.3 x 10^{-4}). The phosphorescence emission maximum shows a much smaller red shift from 753 nm for **Pd[DMBil1]** to 756 nm for **Pd[DMBil1]-PEG$_{750}$** and the phosphorescence quantum yield decreases slightly from Φ_{ph} = 1.3 x 10^{-4} for **Pd[DMBil1]** to Φ_{ph} = 7.8 x 10^{-5} for **Pd[DMBil1]-PEG$_{750}$.**

The emission characteristics of **Pd[DMBil1]-PEG$_{750}$** in nitrogen-saturated PBS (pH 7.4) were also investigated, however, irradiation with 460 nm light under these

conditions only resulted in one emission feature stretching from 500 - 700 nm corresponding to fluorescence. As compared with its fluorescence in methanol, the fluorescence of **Pd[DMBil1]-PEG$_{750}$** in PBS is further red-shifted with an emission maximum at 580 nm, and a quantum yield of $\Phi_fl = 2.0 \times 10^{-4}$. Given that fluorescence quenching is a well-documented consequence of porphyrinoid self-aggregation, the lack of attenuation of Φ_fl in PBS provides further evidence that **Pd[DMBil1]-PEG$_{750}$** does not tend to aggregate in aqueous environments. Failure to detect phosphorescence from the PEGylated derivative in PBS may be attributed to shortening of the triplet excited state lifetime via energy transfer to an H_2O overtone.

In prior work, it had been shown that air efficiently quenches the excited triplet state of **Pd[DMBil1]** in methanol, resulting in quenching of the biladiene's phosphorescence due to efficient energy transfer to molecular oxygen. This phenomenon was also manifest in the ability of **Pd[DMBil1]** to photosensitize $^1O_2$ generation with an impressive quantum yield of $\Phi_\Delta = 0.8$, as quantified using diphenylisobenzofuran (DPBF) as a $^1O_2$ probe and $[Ru(bpy)_3][PF_6]_2$ as an actinometer ($\Phi_\Delta = 0.81$). Similarly, the phosphorescence observed for **Pd[DMBil1]-PEG$_{750}$** in methanol under an inert atmosphere was quenched upon introduction of air to the sample. Quantification of the ability of the **Pd[DMBil1]-PEG$_{750}$** complex to sensitize the formation of $^1O_2$ produced a quantum yield of $\Phi_\Delta = 0.57$ following irradiation with 550 nm light. The slight decrease in Φ_Δ value for the PEGylated biladiene complex may be attributed to a decrease in the O_2 diffusion rate within the immediate vicinity of the photosensitizer due to partial shielding by the PEG moiety. Nonetheless, the ability of **Pd[DMBil1]-PEG$_{750}$** to sensitize the formation of $^1O_2$ ($\Phi_\Delta = 0.57$) is competitive with commercial photosensitizers employed for PDT.

Although **Pd[DMBil1]-PEG$_{750}$** does not exhibit phosphorescence in PBS solutions (vide supra) this complex is capable of sensitizing the formation of $^1O_2$ under aqueous conditions. Through use of the probe Singlet Oxygen Sensor Green (SOSG) and methylene blue as a reference photosensitizer ($\Phi_\Delta = 0.52$), the ability of **Pd[DMBil1]-PEG750** to sensitize singlet oxygen was determined to be $\Phi_\Delta \sim 0.23$ upon irradiation with 550 nm light. The apparent attenuation of Φ_Δ in PBS relative to that in methanol is not surprising given that detecting $^1O_2$ in aqueous environments is more challenging due to its shorter lifetime as well as the lower solubility of oxygen in water as compared to organic solvents. Importantly, however, the aqueous Φ_Δ measured for **Pd[DMBil1]-PEG$_{750}$** is high enough to enable PDT in biological samples (vide infra).

Triple negative breast cancer (TNBC) accounts for ~15-20% of diagnosed breast cancer cases and is associated with earlier relapse, higher mortality rates, and

significantly decreased progression-free survival compared to non-TN breast cancers. TNBC patients are unsusceptible to available targeted or hormonal therapies because the cells in these tumors do not express the necessary surface receptors. Therefore, these patients are treated with aggressive chemotherapies and surgeries that have harmful side effects and are often unsuccessful, necessitating the development of new treatment strategies for this disease. Due to its high potency and specificity, PDT has been recognized as a promising therapeutic approach for TNBC.

To evaluate the inherent toxicity of **Pd[DMBil1]-PEG$_{750}$**, TNBC MDA-MB-231 cells were treated with up to 5.0 mM **Pd[DMBil1]-PEG$_{750}$** for 48 hrs in the dark and then subjected to an Alamar blue cell viability assay. MDA-MB-231 cells were completely viable at **Pd[DMBil1]-PEG$_{750}$** concentrations up to 0.5 mM. Further, the lethal dose required for 50% cell death was found to be LD_{50} =1.87 mM, an exceptionally high value, which demonstrates that in the dark **Pd[DMBil1]-PEG$_{750}$** is biocompatible and well-tolerated by the TNBC cells.

To further demonstrate the safety profile of **Pd[DMBil1]-PEG$_{750}$**, and facilitate a comparison with a set of commercially available $^1O_2$ photosensitizers that form the basis for common PDT treatments, cell viability assays were also conducted for Hematoporphyrin dihydrochloride (HPDC), and Isohematoporphyrin (IHP). Cell viability following treatment with up to 3.0 mM of either HPDC or IHP, revealed lethal dose values of LD_{50} = 1.22 mM and LD_{50} = 0.64 mM, respectively, which are both lower than the LD_{50} obtained for **Pd[DMBil1]-PEG$_{750}$**. These results demonstrate that **Pd[DMBil1]-PEG$_{750}$** is less toxic than two commercially available photosensitizers suggesting that it may be used as a photochemotherapeutic without causing off-target side effects that often limit the efficacy of PDT agents.

To assess whether **Pd[DMBil1]-PEG$_{750}$** is taken up by TNBC cells, MDA-MB-231 cells were treated with up to 1.5 mM **Pd[DMBil1]-PEG$_{750}$** for 48 hr and the cellular fluorescence was analyzed by fluorescence imaging and flow cytometry. As discussed above, **Pd[DMBil1]-PEG$_{750}$** retains the emission properties of **Pd[DMBil1]**, enabling its detection by fluorescence microscopy. Fluorescence imaging revealed that **Pd[DMBil1]-PEG$_{750}$** is taken up by cells during the incubation period, as indicated by the red fluorescent signal. Further, flow cytometry analysis confirmed this result, as cells treated with **Pd[DMBil1]-PEG$_{750}$** experienced a 2-fold increase in median fluorescence intensity (MFI) compared to untreated cells. These results were encouraging given that cellular uptake is important for any compound to be used for PDT,

and led to the assessment of the efficacy of **Pd[DMBil1]-PEG$_{750}$** as a photochemotherapeutic agent.

To investigate the ability of **Pd[DMBil1]-PEG$_{750}$** to mediate PDT, MDA-MB-231 TNBC cells were treated with the biladiene complex at concentrations ranging from 0-10.0 μM for 4 hours. At each concentration surveyed, cells were irradiated (λ_{ex} > 500 nm) for either 0, 10, 20 or 30 minutes. Viability assays were conducted to assess cell death 16 hours after irradiation. Cells incubated with **Pd[DMBil1]-PEG$_{750}$** for 24 hours prior to irradiation were highly susceptible to PDT-mediated cell death compared to cells irradiated immediately after adding **Pd[DMBil1]-PEG$_{750}$.** More specifically, cells irradiated immediately following the addition of **Pd[DMBil1]-PEG$_{750}$** required concentrations of at least 1 μM and 30 min of light exposure before any loss of cell viability was observed. By contrast, cells incubated with **Pd[DMBil1]-PEG$_{750}$** for 24 hr prior to light application were susceptible to PDT with concentrations of photoseositizer as low as 0.25 μM, and 10 μM treatment resulted in complete loss of viability. This enhancement in photoinduced toxicity in cells incubated with the photosensitizer before light treatment provides further evidence of cellular uptake of **Pd[DMBil1]-PEG$_{750}$**. From these results, it was also determined that the effective dose of **Pd[DMBil1]-PEG$_{750}$** required to reduce cell viability by 50%, which was found to be ED_{50} = 0.354 μM for cells incubated with the photosensitizer for 24 hours and then subjected to 30 minutes of light exposure. The ratio of LD_{50}/ED_{50} delivers the phototoxicity index (PI) of a given photochemotherapeutic agent, which provides an indication of the potency of a photodrug in relation to its inherent dark toxicity. For **Pd[DMBil1]-PEG$_{750}$** the phototoxicity index is determined to be PI ~ 5300, which is exceptionally high, especially when compared to porphyrinoids typically employed for PDT (vide infra).

To compare the phototoxicity index of **Pd[DMBil1]-PEG$_{750}$** with those for the commercially available photosensitizers, the same photodynamic activity experiments were conducted with hematoporphyrin dihydrochloride (HPDC) and isohematoporphyrin (IHP). Treatment of MDA-MB-231 cells with each photosensitizer for 24 hours followed by a 30 minute period of irradiation (λ_{ex} > 500 nm) revealed effective doses of ED_{50} = 48.65 μM for HPDC, and ED_{50} = 327.56 μM for IHP. The LD50 and ED50 values for HPDC and IHP correlate to phototoxicity indices of PI ~ 25

for HPDC and PTI ~ 2 for IHP. By comparison, the phototoxicity index of **Pd[DMBil1]-PEG$_{750}$** is quite impressive as it is approximately 200x and 3000x higher than those of HPDC and IHP, respectively.

With the realization that **Pd[DMBil1]-PEG$_{750}$** is highly potent and effective for PDT via $^1O2$ sensitization, the possible mechanism by which the biladiene phototriggers cell death was investigated. Ideally PDT will induce cellular apoptosis as opposed to necrosis, as the latter can cause the release of intra-cellular compartments, and local inflammation that can stimulate tumor growth. By contrast, apoptosis is anti-inflammatory and therefore discourages disease progression. To assess the mechanism by which **Pd[DMBil1]-PEG$_{750}$** photoinduces cell death, MDA-MB-231 cells were treated with 4.0, 6.0, or 8.0 μM **Pd[DMBil1]-PEG$_{750}$** for 24 hours, irradiated for 30 min (λ_{ex} > 500 nm) and then incubated for 1 hour prior to AnnexinV (FITC channel) and PI (PerCP channel) staining. Control experiments in which none of the biladiene photosensitizer were added to the cells were also carried out as controls. For these experiments, the MDA-MB-231 cells were treated with higher **Pd[DMBil1]-PEG$_{750}$** concentrations than in the viability experiments because the cells were analyzed only 1 hour post light treatment (as opposed to overnight). Although these photosensitizer concentrations are higher than required for effective PDT, they are still more than two orders of magnitude lower than the LD_{50} determined for **Pd[DMBil1]-PEG$_{750}$.**

The results of the apoptosis and necrosis assay showed that PDT with **Pd[DMBil1]-PEG$_{750}$** induces primarily apoptotic cell death, and that the percentage of apoptotic cells increases with higher photosensitizer concentrations. The maximum amount of positively stained cells resulted from treatment with 8.0 μM **Pd[DMBil1]-PEG$_{750}$.** The average of three separate assays showed that 20.21% of cells were positive for early apoptosis, 17.08% of cells were positive for late apoptosis, and 8.17% of cells stained positive for necrosis only. Alternatively, cells that did not undergo light treatment experienced only minimal cell death by any mechanism. These results demonstrate that almost half of the total cell populations treated with **Pd[DMBil1]-PEG$_{750}$** and that are exposed to light for 30 minutes experience primarily apoptotic cell death, and approximately 82% of the cells killed by the phototreatment expire via apoptosis rather than necrosis. Given that apoptosis is much preferred

over necrosis for cancer treatment, these results make **Pd[DMBil1]-PEG_{750}** even more attractive as a potential photosensitizer for use in PDT.

What is claimed is:

1. A tetrapyrrole complex comprising a metal complexed by a 10,10-diorgano-5,15-diarylbiladiene ligand which bears at least one substituent comprised of a water-solubilizing segment.
2. The tetrapyrrole complex of claim 1, wherein the water-solubilizing segment is selected from the group consisting of poly(oxyalkylene) segments, polysaccharide segments, polypeptide segments, poly(thioalkylene) segments, poly(aminoalkylene) segments, polyvinylpyrrolidone segments, aliphatic polyester segments, polyamide segments, polyvinyl alcohol segments, polyacrylic acid segments, polyacrylamide segments, polyoxazoline segments, aliphatic polycarbonate segments, polyphosphate segments, and polyphosphazene segments.
3. The tetrapyrrole complex of claim 1, wherein the water-solubilizing segment is a poly(oxyalkylene) segment.
4. The tetrapyrrole complex of claim 1, wherein at least one aryl group substituted at the 5 or 15 position of the 10,10-diorgano-5,15-diarylbiladiene ligand bears at least one substituent comprised of a water-solubilizing segment.
5. The tetrapyrrole complex of claim 1, wherein at least one aryl group substituted at the 5 or 15 position of the 10,10-diorgano-5,15-diarylbiladiene ligand bears at least one substituent comprised of a water-solubilizing segment selected from the group consisting of poly(oxyalkylene) segments, polysaccharide segments, polypeptide segments, poly(thioalkylene) segments, poly(aminoalkylene) segments, polyvinylpyrrolidone segments, aliphatic polyester segments, polyamide segments, polyvinyl alcohol segments, polyacrylic acid segments, polyacrylamide segments, polyoxazoline segments, aliphatic polycarbonate segments, polyphosphate segments, and polyphosphazene segments.
6. The tetrapyrrole complex of claim 1, wherein at least one aryl group substituted at the 5 or 15 position of the 10,10-diorgano-5,15-diarylbiladiene ligand bears at least one substituent comprised of a poly(oxyalkylene) segment.
7. The tetrapyrrole complex of claim 1, wherein the metal is Pd or Pt.
8. The tetrapyrrole complex of claim 1, wherein the 10,10-diorgano-5,15-diarylbiladiene ligand is a 10,10-dialkyl-5,15-diarylbiladiene ligand, a 10-alkyl,10-aryl-5,15-diarylbiladiene ligand, or a 10,10-diaryl-5,15-diarylbiladiene ligand.
9. The tetrapyrrole complex of claim 1, wherein the 10,10-diorgano-5,15-diarylbiladiene ligand is a 10,10-dimethyl-5,15-diarylbiladiene ligand, a 10-

methyl,10-phenyl-5,15-diarylbiladiene ligand, or a 10,10-diphenyl-5,15-diarylbiladiene ligand.

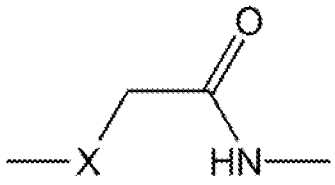
10. The tetrapyrrole complex of claim 1, wherein the water-solubilizing segment is a poly(oxyalkylene) segment having a number average molecular weight of at least 200 g/mol.

11. The tetrapyrrole complex of claim 1, wherein the water-solubilizing segment is a poly(oxyethylene) segment.

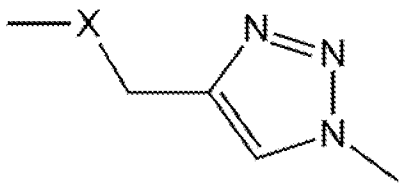
12. The tetrapyrrole complex of claim 1, wherein the at least one substituent comprised of a water-solubilizing segment is additionally comprised of a terminal group selected from the group consisting of alkyl groups and alkyl groups substituted with at least one functional group selected from –SR, NR_2, CO_2R, -C(=O)NR_2, -SO_3R, -PO_3R, -PR_3^+, or -NR_3^+, wherein each R is independently H or an organo group.

13. The tetrapyrrole complex of claim 1, wherein the at least one substituent comprised of a water-solubilizing segment is additionally comprised of a linking moiety which links the water-solubilizing segment to the 10,10-diorgano-5,15-diarylbiladiene ligand.

14. The tetrapyrrole complex of claim 13, wherein the linking moiety is selected from the group consisting of:



and



wherein X is O, S, Se, Te, NH, NR, CH_2, CHR, and CR_2, with R being an organo group.

15. The tetrapyrrole complex of claim 1, wherein the aryl groups substituted at the 5 and 15 positions of the 10,10-diorgano-5,15-diarylbiladiene ligand are substituted phenyl groups having one or more substituents selected from the group consisting of halo, alkyl, oxygen-containing substituents, sulfur-containing substituents and nitrogen-containing substituents, subject to the proviso that at

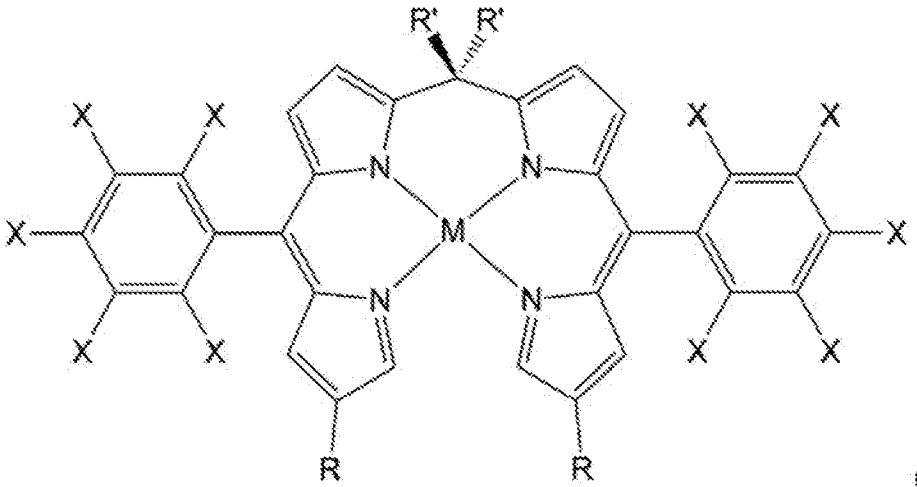
least one of the substituted phenyl groups is substituted by at least one water-solubilizing segment-containing substituent.

16. The tetrapyrrole complex of claim 1, wherein the aryl groups substituted at the 5 and 15 positions of the 10,10-diorgano-5,15-diarylbiladiene ligand are substituted phenyl groups, at least one of the substituted phenyl groups is substituted by a poly(oxyalkylene) segment-containing substituent, and all substituents on the substituted phenyl groups other than poly(oxyalkylene) segment-containing substituents are fluorine.

17. The tetrapyrrole complex of claim 1, wherein the 10,10-diorgano-5,15-diarylbiladiene ligand is substituted at one or both of the 2 and 18 positions with a π-conjugation-extending substituent.

18. The tetrapyrrole complex of claim 17, wherein the π-conjugation-extending substituent is selected from the group consisting of carbonyl-containing substituents, imine-containing substituents, aromatic substituents, vinylaromatic substituents and ethynylaromatic substituents.

19. The tetrapyrrole complex of claim 1, wherein the tetrapyrrole complex has a structure corresponding to Formula (I):



(I)

wherein M is a metal; each X is independently selected from the group consisting of hydrogen, halogen, alkyl, and water-solubilizing segment-containing substituents, subject to the proviso that at least one X is a water-solubilizing segment-containing substituent, each R is independently subjected from the group consisting of hydrogen, halogen, and π-conjugation-extending substituents, and each R' is independently selected from the group consisting of alkyl groups and aryl groups.

20. The tetrapyrrole complex of claim 19, wherein M is Pd or Pt.

21. The tetrapyrrole complex of claim 19, wherein R' and R' are independently methyl or phenyl.

22. The tetrapyrrole complex of claim 19, wherein the water-solubilizing segment-containing substituent(s) comprise(s) at least one water-solubilizing segment selected from the group consisting of poly(oxyalkylene) segments, polysaccharide segments, polypeptide segments, poly(thioalkylene) segments, poly(aminoalkylene) segments, polyvinylpyrrolidone segments, aliphatic polyester segments, polyamide segments, polyvinyl alcohol segments, polyacrylic acid segments, polyacrylamide segments, polyoxazoline segments, aliphatic polycarbonate segments, polyphosphate segments, and polyphosphazene segments.

23. The tetrapyrrole complex of claim 19, wherein the water-solubilizing segment-containing substituent(s) comprise(s) at least one poly(oxyalkylene) segment.

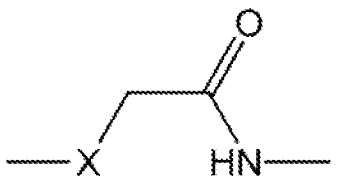
24. The tetrapyrrole complex of claim 23, wherein the at least one poly(oxyalkylene) segment has a number average molecular weight of at least 200 g/mol.

25. The tetrapyrrole complex of claim 23, wherein the at least one poly(oxyalkylene) segment is a poly(oxyethylene) segment.

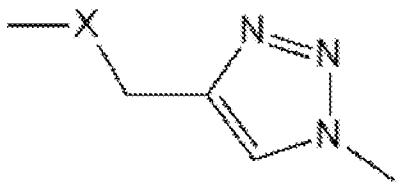
26. The tetrapyrrole complex of claim 23, wherein the water-solubilizing segment-containing substituent has a terminal group selected from the group consisting of alkyl groups and alkyl groups substituted with at least one functional group.

27. The tetrapyrrole complex of claim 23, wherein the water-solubilizing segment-containing substituent is linked to a phenyl group through a linking moiety.

28. The tetrapyrrole complex of claim 27, wherein the linking moiety is selected from the group consisting of:



and



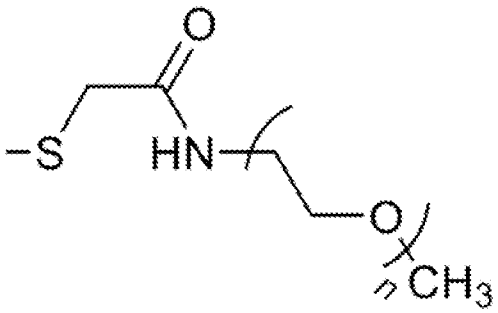
wherein X is O, S, Se, Te, NH, NR, CH_2, CHR, and CR_2, with R being an organo group.

29. The tetrapyrrole complex of claim 19, wherein at least one X group is a poly(oxyalkylene) segment-containing substituents and the remaining X groups are fluorine.

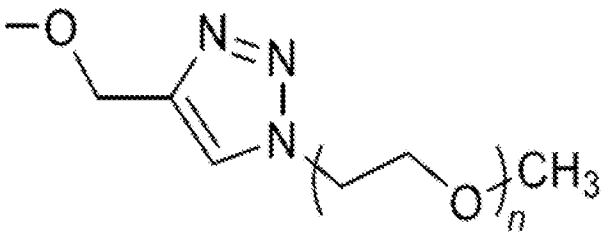
30. The tetrapyrrole complex of claim 19, wherein each R is a π-conjugation-extending substituent selected from the group consisting of carbonyl-containing substituents, imine-containing substituents, aromatic substituents, vinylaromatic substituents and ethynylaromatic substituents.

31. The tetrapyrrole complex of claim 25, wherein the poly(oxyethylene) segment has structure $-(CH_2CH_2O)_n-$ and n is from about 3 to about 250 on average.

32. The tetrapyrrole complex of claim 19, wherein the water-solubilizing segment-containing substituent(s) is or are selected from:



or



wherein n is from about 3 to about 250 on average.

33. A method of using a tetrapyrrole complex in accordance with any of claims 1 to 32, comprising administering the tetrapyrrole complex to a patient and, after a period of time, irradiating targeted tissue of the patient with an energy source that excites the tetrapyrrole complex thereby producing a desired therapeutic response in the targeted tissue.

34. The method of claim 33, wherein the targeted tissue comprises tumor cells.

35. The method of claim 33, wherein the tetrapyrrole complex is administered intravenously or intratumorally.

36. The method of claim 33, wherein the tetrapyrrole complex is administered intravenously as an aqueous solution.

37. A process of photodynamic therapy for treatment of diseased tissues comprising a) delivering a formulation comprised of a tetrapyrrole complex in accordance with any of claims 1 to 32 and a pharmaceutically acceptable vehicle to diseased tissue at a specific treatment site; b) allowing the formulation to preferentially accumulate in the diseased tissue; and c) irradiating the specific treatment site with light of a sufficient power and wavelength to activate the tetrapyrrole complex.

38. The process of claim 37, wherein the light has a wavelength of from 350 to 1000 nm.

39. The process of claim 37, wherein the process is carried out in coordination with photothermal therapy.

40. The process of claim 39, wherein the photothermal therapy comprises embedding within the diseased tissue nanoparticles which emit heat in response to laser light.

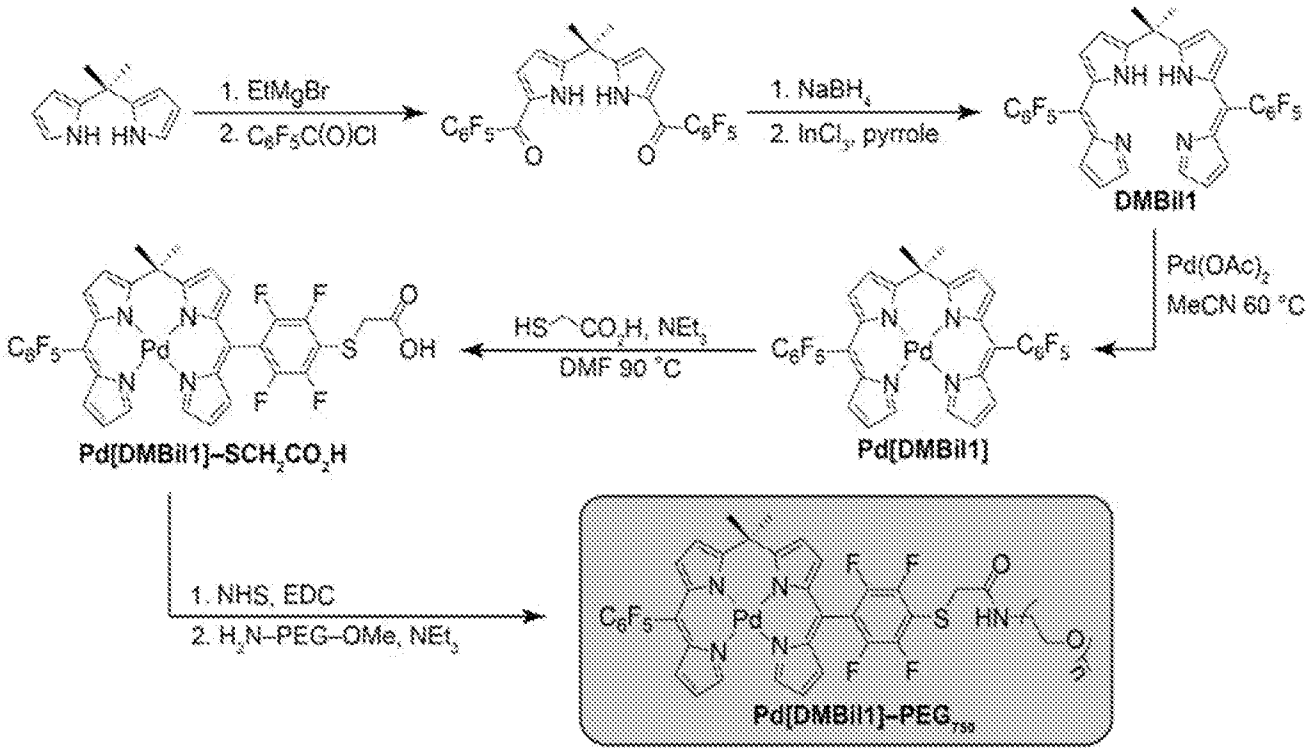
41. The process of claim 40, wherein the nanoparticles are comprised of a silica-containing core and a gold-containing shell.

42. A formulation useful for photodynamic therapy, comprising a tetrapyrrole complex in accordance with any of claims 1 to 32 and a pharmaceutically acceptable vehicle.

43. The formulation of claim 42, wherein the pharmaceutically acceptable vehicle is comprised of water.

44. A tetrapyrrole ligand, wherein the tetrapyrrole ligand is a 10,10-diorgano-5,15-diarylbiladiene ligand which bears at least one substituent comprised of a water-solubilizing segment.

Fig. 1


1. EtMgBr
2. C6F5C(O)Cl
1. NaBH4
2. InCl3, pyrrole
DMBII1
Pd(OAc)2
MeCN 60 °C
HS CO2H, NEt3
DMF 90 °C
Pd[DMBII1]
Pd[DMBII1]–SCH2CO2H
1. NHS, EDC
2. H2N–PEG–OMe, NEt3
Pd[DMBII1]–PEGn