DESCRIPTION

RNA PHOTOCLEAVAGE USING TEXAPHYRINS

BACKGROUND OF THE INVENTION

Photodynamic therapy (PDT) is a treatment technique that uses a photosensitizing dye that localizes at, or near, a treatment site, and when irradiated in the presence of oxygen serves to produce cytotoxic materials, such as singlet oxygen ($O_2(^1\Delta_g)$), from benign precursors (e.g. ($O_2(^3\Sigma_g^-)$). Other reactive species such as superoxide, hydroperoxyl, or hydroxyl radicals may be involved. At the doses used, neither the light nor the drug has any independent activity against the disease target.

The effectiveness of PDT is predicated on three main factors: i) Photosensitive dyes used in PDT must have the ability to localize at the treatment site as opposed to surrounding tissue. ii) The high reactivity and short lifetime of activated oxygen means that it has a very short range and is unlikely to escape from the cell in which it is produced; cytotoxicity is therefore restricted to the precise region of tissue absorbing light, perhaps down to the cellular level. iii) Developments in lasers and fiber optics allow a beam of intense light to be delivered precisely to many parts of the body.

For reviews of photodynamic therapy, see U.S. patent 5,252,720 (incorporated by reference herein); Sindelar *et al.*, (1991); Grossweiner, (1991); Henderson and Dougherty, (1992); and Moan and Berg, (1992). In recent years, considerable effort has been devoted to the synthesis and study of new photosensitizers (a review is found in Brown and Truscott, 1993). The development of more effective photochemotherapeutic agents requires the synthesis of compounds which absorb in the spectral region where living tissues are relatively transparent (i.e., 700-1000 nm), have high triplet quantum yields, and are minimally toxic. The present inventors' texaphyrin molecules absorb strongly in the tissue-transparent 730-770 nm range; the diamagnetic complexes sensitize the production of $^1O_2$ in high quantum yield; and the texaphyrins of the present invention, being completely synthetic, can be tuned so as to incorporate desired properties.

Photodynamic cleavage of DNA is known. Praseuth *et al.*, (1986) reported cleavage of plasmid DNA by synthetic water-soluble porphyrins with visible light in the presence of oxygen. Fiel (1989) also reported the photosensitized strand cleavage and

oxidative-reductive strand scission of DNA by iron porphyrins. In another example, Kobayashi *et al.* (1993) reported cleavage of plasmid DNA by sodium pheophorbide (a derivative of chlorophyll) with visible light in the presence of oxygen. Porphyrin-oligonucleotide derivatives were reportedly used to effect sequence specific modifications of DNA substrates followed by cleavage using hot piperidine (Vlassov *et al.*, 1991; Le Doan *et al.*, 1990). The absorption wavelengths for these porphyrin conjugates were below 700 nm, a range that does not penetrate tissue as effectively as longer wavelengths of light. WO 96/09315 relates to DNA photocleavage using texaphyrins.

The use of ultraviolet light with the drug 8-methoxy-psoralen to treat psoriasis is well established. Lee *et al.* (1988) relates to the interaction of psoralen-derivatized oligodeoxyribonucleoside methylphosphonates with single-stranded DNA. Crosslinked photoadducts between pyrimidines and psoralen appear to form. This treatment may result in the development of cancerous cells. Furthermore, irradiation at the short wavelength of about 365 nm does not penetrate the body and is therefore only useful on the body surface. Psoralen-based treatments must allow the drug to leave the body before the patient is exposed to visible light or the reaction will continue on the skin surface.

Sequence-specific cleavage of DNA has also been reported for dark reactions using oligonucleotides derivatized with metal complexes. Some examples include oligonucleotide-EDTA-Fe complexes (Strobel and Dervan, 1989; Lin, *et al.*, 1989; Dreyer and Dervan, 1985), oligonucleotide-tricationic porphyrins with metal binding appendages (Groves and Farrell, 1989), oligonucleotide-phenanthroline-copper complexes (Chen and Sigman, 1988), oligonucleotide-manganese-porphyrins (Meunier *et al.*, 1993), and iron-porphyrins linked to oligonucleotides (Le Doan *et al.*, 1986, 1987).

Current photosensitive molecules lack good tumor selectivity, and require a short wavelength of light to effect the photoexcitation that is prerequisite to photosensitization.

The present invention relates to photosensitive molecules having activity for the photocleavage of RNA, in particular, photo-induced photocleavage of RNA in a biological system. An effective photosensitive molecule for PDT and RNA photocleavage would have the following properties: easy availability, low intrinsic

toxicity, long wavelength absorption, efficient photosensitizer for singlet oxygen production, fair solubility in water, selective uptake in lipophilic tissue such as atheroma or tumor tissue, high affinity for enveloped viruses, quick degradation and/or elimination after use, chemically pure and stable, easily subject to synthetic modification, efficient at physiological temperature and pH, specificity for certain biological substrates, easy administration to a biological system, and amenable to conjugation to site-directing carrier molecules.

The present inventors address these problems and provide herein photosensitizers having capability to cleave RNA, thereby providing a whole new range of targets for photodynamic therapy. These photosensitizers demonstrate tumor localization, absorption in the longer wavelength ranges up to about 800 nm, as well as non-toxicity, lack of skin photosensitivity, and ease of production in a pure form.

LIST OF ABBREVIATIONS

DCC	:	Dicyclohexylcarbodiimide
DMF	:	Dimethylformamide
EDTA	:	Ethylenediamine tetraacetic acid
NHS	:	N-hydroxysuccinimide
NM	:	Nanometers
RNA	:	Ribonucleic acid
TEA	:	Triethylamine
THF	:	Tetrahydrofuran
Txp(txph)(TX)	:	Texaphyrin

SUMMARY OF THE INVENTION

The present invention provides a method of light-induced photocleavage of a polymer of ribonucleic acid. The method comprises the steps of contacting the polymer of ribonucleic acid with a photosensitive texaphyrin and exposing the photosensitive texaphyrin to light for a time sufficient to cleave the polymer. A texaphyrin as used herein is an aromatic pentadentate expanded porphyrin analog with appended functional groups. Such pendant groups may enhance solubility or biolocalization or may provide coupling sites for site-directing molecules such as oligonucleotides.

The polymer of ribonucleic acid may be a solution or a suspension of RNA or may be cellular RNA *in vitro, in vivo*, or *ex vivo*. The ability to specifically photocleave RNA has important implications for the treatment of various diseases; for

destruction of retroviral RNA, messenger RNA, oncogenic mRNA, ribosomal RNA, RNA cofactors, transfer RNA, small nuclear RNA, or small cytoplasmic RNA, thereby providing a multifactorial approach to eliminating diseased, cancerous or other unwanted cells or tissues. A site of desired photocleavage may be an RNA encoding a product deleterious to the host or may be a normal RNA that is deleterious in some way.

The photocleavage of RNA described herein is a photolytic cleavage. It is believed that the cleavage is not hydrolytic where a water molecule is added across a bond to break the bond, nor is the photocleavage believed to be solely oxidative where an oxidation reaction in the absence of light causes breakage of the bond.

The method of site-specific photocleavage of RNA involves at least two sources of specificity. A complementary oligonucleotide is designed to base-pair with the targeted substrate, providing a first source of specificity, and a second source of specificity for *in vitro* or *in vivo* applications is the positioning of the laser light. Such positioning of laser light, either by manual or mechanical means, would be particularly advantageous when the oligonucleotide photocleavage reaction in question is to be effected at a particular biological locus, such as, for instance, a deep-seated tumor site. Here, the fact that the texaphyrins absorb light at wavelengths where bodily tissues are relatively transparent (700-900 nm) is particularly advantageous. This procedure allows for the effective implementation of light-based oligonucleotide strategies at loci deep within the body with relatively little deleterious light-based photosensitization of other tissues where the texaphyrin conjugates are not localized.

A method of treating a host harboring benign or malignant tumor cells is a further embodiment of the present invention. The method comprises administering to the host an effective amount of a photosensitive texaphyrin-oligonucleotide conjugate, the oligonucleotide having sequence complementarity to an RNA molecule of the benign or malignant tumor cells, and photoirradiating the photosensitive texaphyrin in proximity to the tumor cells. This method may further comprise the step of determining localization sites of the photosensitive texaphyrin in the host by reference to the texaphyrin.

A further embodiment of the present invention is a method for targeted intracellular RNA photocleavage. The method comprises the introduction into a cell of a texaphyrin coupled to an oligonucleotide having complementary binding affinity for a

targeted RNA, whereby photocleavage of the targeted RNA is catalyzed by the texaphyrin.

A method for destroying messenger RNA, and thereby inhibiting the expression of a gene in an animal, comprising administration to the animal of a texaphyrin oligonucleotide-conjugate is a further embodiment of the present invention. The oligonucleotide has complementary binding affinity for regions of the messenger RNA molecule, or for small nuclear RNAs involved in the splicing reaction of messenger RNA. A further embodiment of the present invention is a method for inhibiting a tissue specific messenger RNA of an animal comprising administering to the animal a texaphyrin having specificity for the tissue. The texaphyrin may have appended an oligonucleotide complementary to the target messenger RNA.

A further embodiment of the present invention is a texaphyrin conjugate wherein two or more separate texaphyrin complexes are attached to an oligonucleotide, one at the 3', one at the 5' end, and/or one or more at an internal residue. The texaphyrin may be metal-free or may be metallated. A metal ion of each of the texaphyrin complexes may be the same or it may be different. Similarly, each of the texaphyrins may be different. Use of a dual texaphyrin complex-conjugate should effect the photocleavage of RNA with increased efficiency due to concerted activity of the metal complexes. For diagnosis and treatment purposes, the administration of such a conjugate with one texaphyrin complex having a diamagnetic metal species and the other having a paramagnetic metal species would allow binding, imaging, and photocleavage, all effected by one conjugate. In this case, binding is effected by the oligonucleotide, imaging is accomplished by MRI due to the presence of the paramagnetic metal ion, and photocleavage is accomplished by the photosensitive texaphyrin containing a diamagnetic metal cation. Therefore, the biodistribution and cellular penetration of the conjugate may be determined.

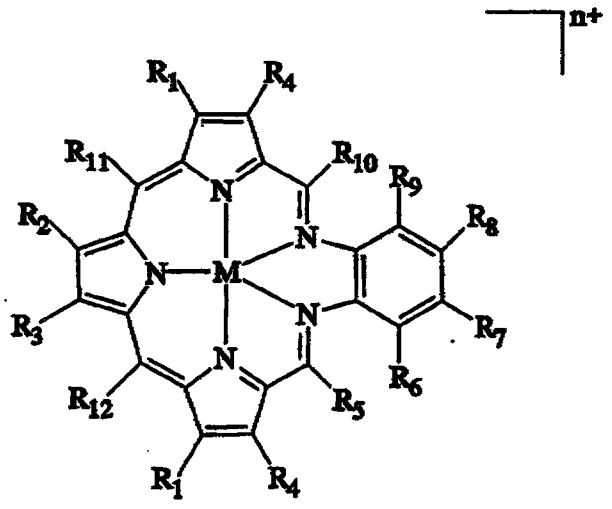
A further aspect of the present invention is use of a photosensitive texaphyrin in the preparation of a pharmaceutical composition for use in photocleaving a polymer of ribonucleic acid. In a preferred embodiment, the polymer of ribonucleic acid is messenger RNA and the photosensitive texaphyrin has structure I or II as provided herein.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

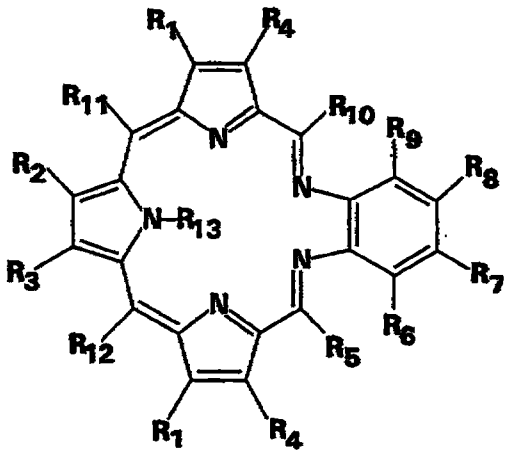
The present invention provides for the use of photosensitive texaphyrins for the photoinduced cleavage of a polymer of ribonucleic acid. The photosensitive texaphyrin may be a free-base texaphyrin or may be metallated with a diamagnetic metal. A preferred diamagnetic metal is Lu(III), La(III), In(III), Y(III), Zn(II), or Cd(II) and a most preferred diamagnetic metal is Lu(III) or Y(III). The term "photosensitive" as used herein means that upon irradiation, texaphyrin effects the generation of oxygen products that are cytotoxic, such as singlet oxygen, hydroxyl radicals, superoxide, or hydroperoxyl radicals, for example, thereby achieving a targeted light-induced therapeutic effect in the vicinity of a photosensitive molecule. It is important to note that while strand breakage, as outlined herein, is useful for quantitating the extent of photochemically derived damage, this damage in itself (e.g., modification of a nucleotide) is known to inhibit biological function of the nucleic acid (for example, translation of RNA, phage infectivity).

In the present light-dependent photocleavage, the light may have a wavelength range of about 650-900 nm, preferably about 700-800 nm, and most preferably about 730-770 nm.

The texaphyrin or texaphyrin metal complex for use in light-induced photocleavage of a polymer of ribonucleic acid may have structure I or II:



I



II

M is H or a diamagnetic metal cation. R_1-R_4, R_7 and R_8 are independently hydrogen, halide, hydroxyl, alkyl, alkenyl, alkynyl, aryl, haloalkyl, nitro, formyl, acyl, hydroxyalkyl, alkoxy, hydroxyalkoxy, hydroxyalkenyl, hydroxyalkynyl, saccharide, carboxy, carboxyalkyl, carboxyamide, carboxyamidealkyl, amino, aminoalkyl, a site-directing molecule, a catalytic group, or a couple that is coupled to a site-directing molecule or to a catalytic group.

R_6 and R_9 are independently selected from the groups of R_1-R_4, R_7 and R_8, with the proviso that the halide is other than iodide and the haloalkyl is other than iodoalkyl.

R_5 and R_{10}-R_{12} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, hydroxyalkyl, alkoxy, hydroxyalkoxy, hydroxyalkenyl, hydroxyalkynyl, carboxyalkyl, carboxyamide, carboxyamidealkyl, amino, aminoalkyl, or a couple that is coupled to a saccharide, to a site-directing molecule, or to a catalytic group; and n is an integer value less than or equal to 5.

R_{13} is alkyl, alkenyl, oxyalkyl, or hydroxyalkyl having up to about 3 carbon atoms and having rotational flexibility around a first-bound carbon atom. Rotational flexibility allows the rest of the group to be positioned outside the plane of the texaphyrin. Thus, for example, a preferred alkenyl is CH_2-CH=CH_2. The pyrrole nitrogen substituent is most preferably a methyl group.

Texaphyrins of the present invention may be metal-free or may be in a complex with a metal. In the above-described structure I, n will typically be an integer value less than or equal to 5. In the context of the basic macrocycle with a divalent or trivalent metal cation, n is 1 or 2; however, one skilled in the art in light of the present

disclosure would realize that the value of n would be altered due to charges present on substituents R_1-R_{12} and charges present on the covalently bound site-directing molecule. It is understood by those skilled in the art that the complexes described in the present invention have one or more additional ligands providing charge neutralization and/or coordinative saturation to the metal ion. Such ligands include chloride, nitrate, acetate, and hydroxide, among others.

Representative examples of alkanes useful as alkyl group substituents of the present invention include methane, ethane, straight-chain, branched or cyclic isomers of propane, butane, pentane, hexane, heptane, octane, nonane and decane, with methane, ethane and propane being preferred. Alkyl groups having up to about thirty, or up to about fifty carbon atoms are contemplated in the present invention. Representative examples of substituted alkyls include alkyls substituted by two or more functional groups as described herein.

Representative examples of alkenes useful as alkenyl group substituents include ethene, straight-chain, branched or cyclic isomers of propene, butene, pentene, hexene, heptene, octene, nonene and decene, with ethene and propene being preferred. Alkenyl groups having up to about thirty or fifty carbon atoms, and up to about five double bonds, or more preferably, up to about three double bonds are contemplated in the present invention.

Representative examples of alkynes useful as alkynyl group substituents include ethyne, straight-chain, branched or cyclic isomers of propyne, butyne, pentyne, hexyne, heptyne, octyne, nonyne and decyne, with ethyne and propyne being preferred. Alkynyl groups having up to about thirty, or up to about fifty carbon atoms, and having up to about five or up to about three triple bonds are contemplated in the present invention.

The aryl may be a compound whose molecules have the ring structure characteristic of benzene, naphthalene, phenanthrene, anthracene, and the like, i.e., either the 6-carbon ring of benzene or the condensed 6-carbon rings of the other aromatic derivatives. For example, an aryl group may be phenyl or naphthyl, unsubstituted or substituted with a nitro, carboxy, sulfonic acid, hydroxy, oxyalkyl or halide substituent. In this case, the substituent on the phenyl or naphthyl may be added in a synthetic step after the condensation step which forms the macrocycle.

Among the halide substituents, chloride, bromide, fluoride and iodide are contemplated in the practice of this invention with the exception of iodide for R_6 and R_9. R_6 and R_9 may have chloride, bromide or fluoride substituents. Representative examples of haloalkyls used in this invention include halides of methane, ethane, propane, butane, pentane, hexane, heptane, octane, nonane and decane, with halides, preferably chlorides or bromides, of methane, ethane and propane being preferred.

"Hydroxyalkyl" means alcohols of alkyl groups. Preferred are hydroxyalkyl groups having one to twenty, more preferably one to ten, hydroxyls. "Hydroxyalkyl" is meant to include glycols and polyglycols; diols of alkyls, with diols of C_{1-10} alkyls being preferred, and diols of C_{1-3} alkyls being more preferred; and polyethylene glycol, polypropylene glycol and polybutylene glycol as well as polyalkylene glycols containing combinations of ethylene, propylene and butylene.

Representative examples of oxyalkyls include the alkyl groups as herein described having ether linkages. "Oxyalkyl" is meant to include polyethers with one or more functional groups. The number of repeating oxyalkyls within a substituent may be up to 200, preferably is from 1-20, and more preferably, is 1-7, and most preferably is 2-3. A preferred oxyalkyl is $O(CH_2CH_2O)_xCH_3$ where x = 1-120, preferably 1-10, and more preferably, 1-5.

Oxyhydroxyalkyl means alkyl groups having ether or ester linkages, hydroxyl groups, substituted hydroxyl groups, carboxyl groups, substituted carboxyl groups or the like.

Representative examples of thioalkyls include thiols of ethane, thiols of straight-chain, branched or cyclic isomers of propane, butane, pentane, hexane, heptane, octane, nonane and decane, with thiols of ethane (ethanethiol, C_2H_5SH) or propane (propanethiol, C_3H_7SH) being preferred. Sulfate-substituted alkyls include alkyls as described above substituted by one or more sulfate groups, a representative example of which is diethyl sulfate ($(C_2H_5)_2SO_4$).

Representative examples of phosphates include phosphate or polyphosphate groups. Representative examples of phosphate-substituted alkyls include alkyls as described above substituted by one or more phosphate or polyphosphate groups. Representative examples of phosphonate-substituted alkyls include alkyls as described above substituted by one or more phosphonate groups.

Representative examples of carboxy groups include carboxylic acids of the alkyls described above as well as aryl carboxylic acids such as benzoic acid. Representative examples of carboxyamides include primary carboxyamides ($CONH_2$), secondary (CONHR') and tertiary (CONR'R") carboxyamides where each of R' and R" is a functional group as described herein.

Representative examples of useful amines include a primary, secondary or tertiary amine of an alkyl as described hereinabove.

"Carboxyamidealkyl" means alkyl groups with secondary or tertiary amide linkages or the like. "Carboxyalkyl" means alkyl groups having hydroxyl groups, carboxyl or amide substituted ethers, ester linkages, tertiary amide linkages removed from the ether or the like.

The term "saccharide" includes oxidized, reduced or substituted saccharide; hexoses such as D-glucose, D-mannose or D-galactose; pentoses such as D-ribose or D-arabinose; ketoses such as D-ribulose or D-fructose; disaccharides such as sucrose, lactose, or maltose; derivatives such as acetals, amines, and phosphorylated sugars; oligosaccharides, as well as open chain forms of various sugars, and the like. Examples of amine-derivatized sugars are galactosamine, glucosamine, sialic acid and D-glucamine derivatives such as 1-amino-1-deoxysorbitol.

In an embodiment of the present invention, texaphyrins are further coupled to site-directing molecules to form conjugates for targeted *in vivo* delivery. "Site-directing" means having specificity for targeted sites. "Specificity for targeted sites" means that upon contacting the texaphyrin-conjugate with the targeted site, for example, under physiological conditions of ionic strength, temperature, pH and the like, specific binding will occur. The interaction may occur due to specific electrostatic, hydrophobic, entropic or other interaction of certain residues of the conjugate with specific residues of the target to form a stable complex under conditions effective to promote the interaction. A site-directing molecule may have binding specificity for localization to a treatment site. Exemplary site-directing molecules contemplated in the present invention include, but are not limited to,: polydeoxyribonucleotides, oligodeoxyribonucleotides, polyribonucleotides, oligoribonucleotides, and analogs thereof; polyamides including peptides having affinity for a biological receptor; proteins such as antibodies; steroids and steroid derivatives;

hormones such as estradiol, or histamine; hormone mimics such as morphine; and further macrocycles such as sapphyrins and rubyrins.

In a presently preferred embodiment, the invention involves the site-specific photocleavage of a polymer of ribonucleic acid using a photosensitive texaphyrin-site-directing molecule conjugate where the site-directing molecule is an oligonucleotide having sequence complementarity to a portion of the RNA to be cleaved.

The data of Example 5 demonstrate that diamagnetic metal-texaphyrin-oligonucleotide conjugates may be developed into RNA antisense reagents. This antisense strategy provides a clear and rational method for new drug design because there is one requirement, namely that the antisense probe hybridize to its target molecule. The hybridization requirement is very well understood via complementary Watson-Crick base-pairing. Unlike the present methods in the art which require screening of thousands of compounds and X-ray crystal structure analysis, the information needed for antisense technology is the sequence of the target. Treating native RNA with a texaphyrin-oligonucleotide conjugate results in the conjugate binding to a complementary RNA sequence via the appended oligonucleotide. The diamagnetic metal-texaphyrin complex then cleaves the RNA proximal to this specific site. Two texaphyrin molecules may be attached to a conjugated oligonucleotide, enhancing the photocleavage activity. Also, a greater number of texaphyrins attached to the oligonucleotide will cause the antisense agent to take on more of the pharmacodynamic and biodistribution properties of the texaphyrin, such as selective localization in tumors.

The texaphyrin oligonucleotide-conjugate would have immediate applications for anti-viral and anti-bacterial therapy as well as cancers (an oligonucleotide complementary to an oncogenic messenger RNA, for example) and inflammatory responses that are caused by the overexpression of certain proteins. Antisense technology is discussed in U.S. Patents 5,194,428, 5,110,802 and 5,216,141, all of which are incorporated by reference herein.

Oligonucleotides have several advantages over traditional drugs, notably their exquisite specificity to target sites and their ease of design. Oligonucleotides may be derivatized at the bases, the sugars, the ends of the chains, or at the phosphate groups of the backbone to promote *in vivo* stability. CpG sequences may be derivatized to minimize degradation; derivatization may be alkylation, and is preferably methylation.

Modifications of the phosphate groups are preferred in one embodiment of the invention since phosphate linkages are sensitive to nuclease activity. Preferred derivatives are the methylphosphonates, phosphotriesters, phosphorothioates, and phosphoramidates. Derivatives may also contain alternating phosphorothioate and unmodified linkages, or alternating methylphosphonate and unmodified linkages, or alternating phosphorothioate and methylphosphonate linkages. Additionally, the phosphate linkages may be completely substituted with non-phosphate linkages such as amide linkages. Appendages to the ends of the oligonucleotide chains also provide exonuclease resistance. The 5' or 3' end may be derivatized or "capped" with a phosphoramidate linkage, an inverted nucleotide conjugated to the oligonucleotide via a 3'-3' linkage, an aminoacridine residue, or poly-L-Lysine.

Sugar modifications may include groups, such as halo, alkyl, alkenyl or alkoxy groups, attached to an oxygen of a ribose moiety in a ribonucleotide. In a preferred embodiment, the group will be attached to the 2' oxygen of the ribose. In particular, halogen moieties such as fluoro may be used. The alkoxy group may be methoxy, ethoxy or propoxy. The alkenyl group is preferably allyl. The alkyl group is preferably a methyl group and the methyl group is attached to the 2' oxygen of the ribose. Other alkyl groups may be ethyl or propyl. The O-methylation derivatization serves to protect the ribonucleotide from degradation.

It is understood that the terms "nucleotide", "polynucleotide" and "oligonucleotide", as used herein and in the appended claims, refer to both naturally-occurring and synthetic nucleotides, poly- and oligonucleotides and to analogs and derivatives thereof such as methylphosphonates, phosphotriesters, phosphorothioates and phosphoramidates and the like.

The term "texaphyrin-oligonucleotide conjugate" means that an oligonucleotide is attached to the texaphyrin in a 5' linkage, a 3' linkage, or both types of linkages that would allow the texaphyrin to be an internal residue in the conjugate. The term can also refer to a texaphyrin that is linked to an internal base of the oligonucleotide. The oligonucleotide or other site-directing molecule may be attached either directly to the texaphyrin or to a texaphyrin via a linker or a couple of variable length. During catalysis, for example, the texaphyrin portion of a texaphyrin metal complex-oligonucleotide conjugate is placed in the vicinity of the RNA substrate upon binding of the oligonucleotide to the targeted nucleic acid substrate. A "sapphyrin-oligonucleotide

conjugate" is referred to in the same way as described above for a texaphyrin-oligonucleotide conjugate except that the texaphyrin molecule is replaced with a sapphyrin molecule.

An exemplary method for delivering texaphyrin-oligonucleotide conjugates into a cell is the use of glycoconjugates for carrying oligonucleotides specific for targeted sequences. Oligonucleotides protected at both ends and linked through a disulfide bridge to a glycoconjugate are significantly more effective in reaching a target site than the corresponding free oligonucleotides. Poly-L-lysine can be substituted by three components: a sugar as a recognition signal, a therapeutic oligonucleotide element, and gluconoic acid as a neutralizing and solubilizing agent. This type of neutral, highly water-soluble glycosylated polymer is an efficient carrier to deliver drugs into cells according to the nature of the sugar attached to the polymer.

Representative examples of useful steroids include any of the steroid hormones of the following five categories: progestagens such as progesterone, glucocorticoids such as cortisol, mineralocorticoids such as aldosterone, androgens such as testosterone or androstenedione, and estrogens such as estrone or estradiol.

The term "a peptide having affinity for a biological receptor" means that upon contacting the peptide with the biological receptor, for example, under appropriate conditions of ionic strength, temperature, pH and the like, specific binding will occur. The interaction may occur due to specific electrostatic, hydrophobic, entropic or other interaction of certain amino acid or glycolytic residues of the peptide with specific amino acid or glycolytic residues of the receptor to form a stable complex under the conditions effective to promote the interaction. The interaction may alter the three dimensional conformation and the function or activity of either or both the peptide and the receptor involved in the interaction. A peptide having affinity for a biological receptor may include naturally occurring or synthetic peptides such as an endorphin, an enkephalin, a growth factor, e.g. epidermal growth factor, poly-L-lysine, a hormone, a peptide region of a protein and the like. Specific examples include e.g., insulin, ribonuclease, or β-endorphin.

A "catalytic group" appended to a texaphyrin complex or to a texaphyrin complex-site directing conjugate means a chemical functional group that may act as a general acid, Brønsted acid, general base, Brønsted base, nucleophile, or any other means by which an activation barrier to reaction is lowered or the ground state energy

of a substrate is increased. Exemplary catalytic groups contemplated include, but are not limited to, imidazole; guanidine; substituted saccharides such as D-glucosamine, D-mannosamine,, D-galactosamine, D-glucamine, and the like; amino acids such as L-histidine and L-arginine; derivatives of amino acids such as histamine; polymers of amino acids such as poly-L-lysine, $(LysAla)_n$, $(LysLeuAla)_n$ where n is from 1-30 or preferably 1-10 or more preferably 2-7 and the like; derivatives thereof; and texaphyrin metal complexes.

The term "appended" to a texaphyrin or texaphyrin-conjugate means that the catalytic group is attached either directly to the texaphyrin or to the conjugate via a linker or couple of variable length.

A couple may be described as a linker, i.e., the covalent product formed by reaction of a reactive group designed to attach covalently another molecule at a distance from the texaphyrin macrocycle. Exemplary linkers or couples are amides, amine, disulfide, thioether, ether, ester, or phosphate covalent bonds. In most preferred embodiments, conjugates and appended groups are covalently bonded to the texaphyrin via a carbon-carbon, carbon-nitrogen, carbon-sulfur, or a carbon-oxygen bond, more preferably a carbon-oxygen or a carbon-nitrogen bond.

Preferred functionalizations are: when R_6 and R_9 are other than hydrogen, then R_5 and R_{10} are hydrogen or methyl; and when R_5 and R_{10} are other than hydrogen, then R_6 and R_9 are hydrogen, hydroxyl, or halide other than iodide. Other preferred functionalizations are where R_6 and R_9 are hydrogen, then R_5, R_{10}, R_{11} and R_{12} are lower alkyl or lower hydroxyalkyl. The lower alkyl is preferably methyl or ethyl, more preferably methyl. The lower hydroxyalkyl is preferably of 1 to 6 carbons and 1 to 4 hydroxy groups, more preferably 3-hydroxypropyl.

In a preferred embodiment of the present invention, at least one of R_1, R_2, R_3, R_7 and R_8 is a site-directing molecule or a couple that is coupled to a site-directing molecule. In a more preferred embodiment, at least one of R_1, R_2, R_3, R_7 and R_8 is an oligonucleotide, or a couple that is coupled to an oligonucleotide.

In a presently preferred texaphyrin, R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, R_8 is a site-directing molecule or a couple that is coupled to a site-directing molecule, and R_7 is H.

In another preferred texaphyrin, the substituent R_1 is $(CH_2)_2CH_2OH$; R_2 and R_3 are CH_2CH_3; R_4 is CH_3; R_7 is $O(CH_2CH_2O)_2CH_2CH_2OCH_3$, H, or OCH_3; and R_8 is a site-directing molecule or a couple that is coupled to a site-directing molecule.

A couple that is coupled to an oligonucleotide may be further described as $O(CH_2)_nCO$-oligonucleotide where n is 1-7 and preferably 1-3.

In a further presently preferred embodiment, R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, and R_7 and R_8 are $O(CH_2CH_2O)_2CH_2CH_2OCH_3$.

In presently preferred texaphyrin compounds, R_1-R_4, R_7, and R_8 are as in Table 1 for texaphyrins A1-A22, R_5, R_6, and R_9-R_{12} are H, and M is as defined hereinabove. However, while the above-described texaphyrins are presently preferred compounds for use in the present invention, the invention is not limited thereto and any photosensitive texaphyrin may be useful in the practice of the invention.

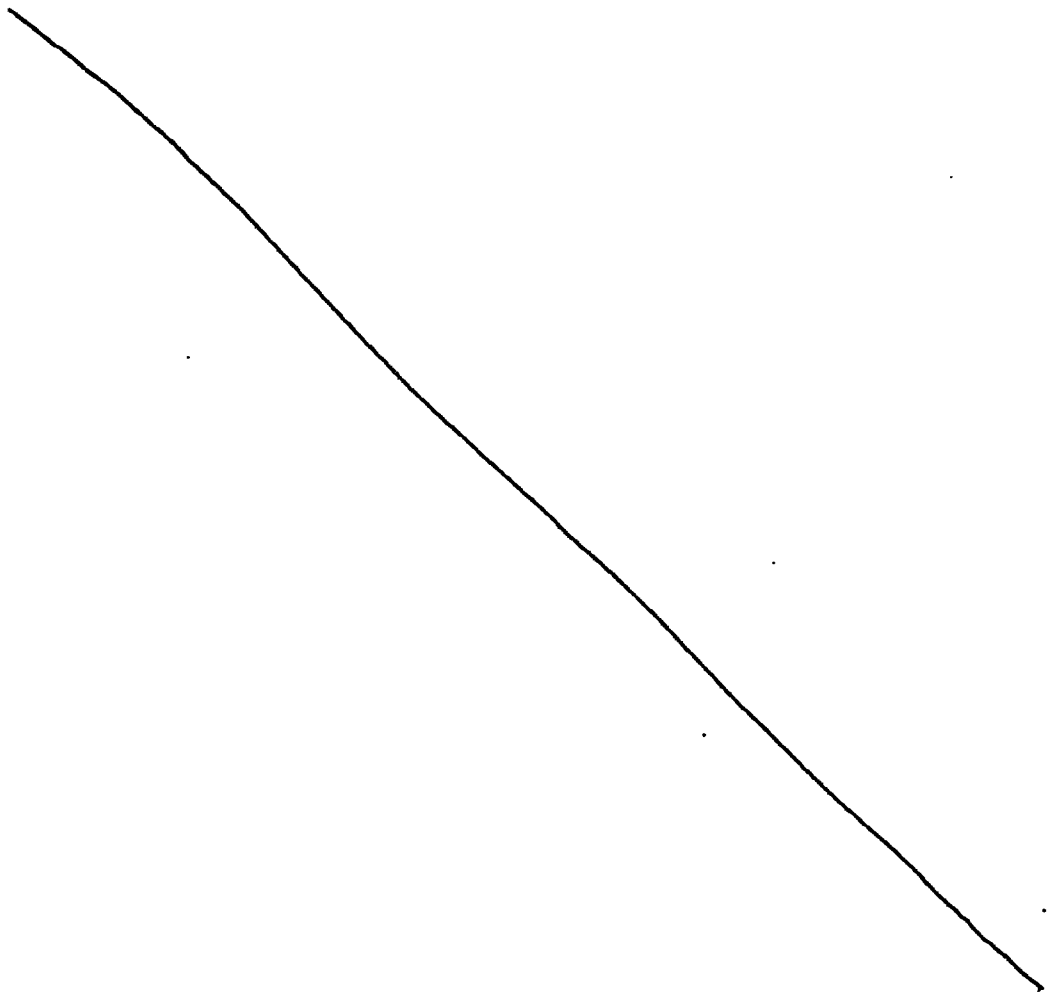


TABLE 1.
Representative Substituents for
Texaphyrin Macrocycles of the Present Invention

TXP	R_1	R_2	R_3	R_4	R_6	R_7
A1	$CH_2(CH_2)_2OH$	CH_2CH_3	CH_2CH_3	CH_3	$O(CH_2)_3OH$	$O(CH_2)_3OH$
A2	"	"	"	"	$O(CH_2CH_2O)_3CH_3$	$O(CH_2CH_2O)_3CH_3$
A3	"	"	"	"	$O(CH_2)_nCON$-linker-site-directing molecule, n=1-10	"
A4	"	"	"	"	$O(CH_2)_nCON$-linker-site-directing molecule	H
A5	"	"	"	"	OCH_2CO-hormone	"
A6	"	"	"	"	$O(CH_2CH_2O)_3CH_3$	"
A7	"	"	"	"	OCH_2CON-linker-site-directing molecule	$O(CH_2CH_2O)_3CH_3$
A8	"	"	"	"	OCH_2CO-hormone	"
A9	"	"	"	"	$O(CH_2CH_2O)_{120}CH_3$	$O(CH_2CH_2O)_3CH_2$-CH_2-N-imidazole
A10	"	"	"	"	saccharide	H
A11	"	"	"	"	$OCH_2CON(CH_2CH_2OH)_2$	"
A12	"	"	"	"	$CH_2CON(CH_3)CH_2$-$(CHOH)_4CH_2OH$	"
A13	"	COOH	COOH	"	"	"

TABLE 1. - CONTINUED

TXP	R_1	R_2	R_3	R_4	R_6	R_7
A14	"	$COOCH_2CH_3$	$COOCH_2CH_3$	"	"	"
A15	$CH_2CH_2CON(CH_2CH_2OH)_2$	CH_2CH_3	CH_2CH_3	"	"	"
A16	$CH_2CH_2ON(CH_3)CH_2-(CHOH)_4CH_2OH$	"	"	"	OCH_3	OCH_3
A17	$CH_2(CH_2)_2OH$	"	"	"	$O(CH_2)_nCOOH$, n=1-10	H
A18	"	"	"	"	$(CH_2)_n$-CON-linker-site-directing molecule, n=1-10	"
A19	"	"	"	"	$YCOCH_2$-linker-site-directing molecule Y=NH,O	"
A20	CH_2CH_3	CH_3	CH_2CH_2COOH	"	$O(CH_2)_2CH_2OH$	$O(CH_2)_2CH_2OH$
A21	"	"	CH_2CH_2CON-oligo	"	"	"
A22	$CH_2(CH_2)_2OH$	CH_2CH_3	CH_2CH_3	"	$O(CH_2)_3CO$-histamine	H

Texaphyrin metal complexes possess inherent biolocalization specificity as described in U.S. Patent 5,252,720, localizing in lipid-rich regions such as, for example, liver, kidney, tumor and atheroma. Importantly, hydroxylated texaphyrins have a lipid-water distribution coefficient that is optimal for localization to lipophilic regions, yet are sufficiently water soluble to allow ease of handling. "Water soluble" means soluble in aqueous fluids to about 1 mM or better. "Localization to lipophilic regions" means having greater affinity for lipid rich tissues or materials than surrounding nonlipid rich tissues or materials and, in the case of viruses in suspension, the term means having affinity for the membranous coat of the virus. "Lipid rich" means having a greater amount of triglyceride, cholesterol, fatty acids or the like.

Determining localization sites of a texaphyrin "by reference to the texaphyrin" as used herein means that the location may be found by localization such as magnetic resonance imaging if the texaphyrin contains a metal that is paramagnetic, gamma ray detection if the metal is gamma-emitting, or by using monochromatic X-ray photon sources or fluorescent spectroscopy. Gamma-emitting metals for radioimmunodiagnostics are described in U.S. Patent 5,252,720, incorporated by reference herein. A preferred gamma-emitting metal is ^{111}In(III). The nonmetallated form of texaphyrin may be used, in particular, where fluorescence is the preferred means of detection of the texaphyrin.

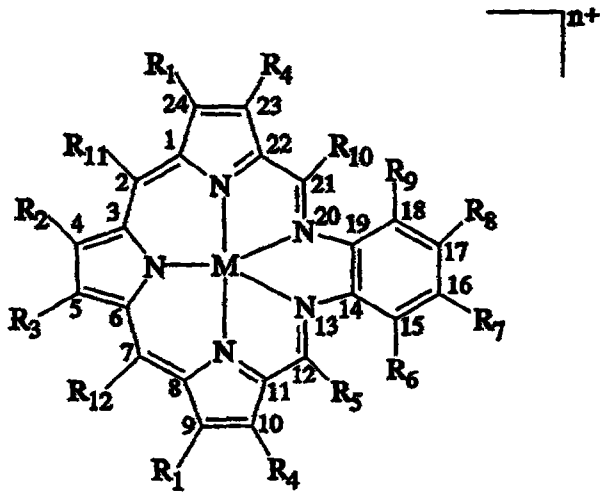
Metal-free and diamagnetic metallated texaphyrin compounds, methods for making and methods for using them are described in U.S. Patents 4,935,498; 5,162,509; 5,252,720; 5,256,399; 5,272,142; 5,292,414; 5,369,101; 5,432,171; 5,439,570; 5,451,576; 5,457,183; and 5,475,104; and in pending applications USSN 08/098,514, 08/196,964, 08/227,370, and 08/207,845; each patent and application is incorporated by reference herein. Sapphyrin compounds are disclosed in U.S. Patents 5,041,078; 5,159,065; 5,120,411; 5,302,714; and 5,457,195; each patent is incorporated by reference herein.

One skilled in the art of organic synthesis in light of the present disclosure and the disclosures in the patents, applications and publications incorporated by reference herein could extend and refine the above basic synthetic chemistry to produce photosensitive texaphyrins having various substituents. For example, polyether-linked polyhydroxylated groups, saccharide substitutions in which the saccharide is appended via an acetal-like glycosidic linkage, an oligosaccharide or a polysaccharide may be

similarly linked to a texaphyrin. A doubly carboxylated texaphyrin in which the carboxyl groups are linked to the texaphyrin core via aryl ethers or functionalized alkyl substituents could be converted to various esterified products wherein the ester linkages serve to append further hydroxyl-containing substituents. Polyhydroxylated texaphyrin derivatives may be synthesized via the use of secondary amide linkages. Saccharide moieties may be appended via amide bonds. Polyhydroxylated texaphyrin derivatives containing branched polyhydroxyl (polyol) subunits may be appended to the texaphyrin core via aryl ethers or ester linkages.

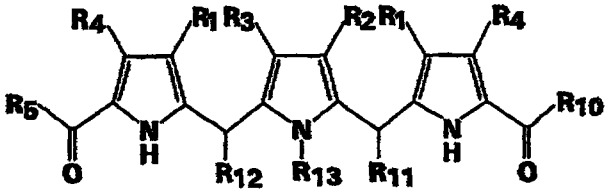
Treatment of carboxylated texaphyrins with thionyl chloride or *p*-nitrophenol acetate would generate activated acyl species suitable for attachment to monoclonal antibodies or other biomolecules of interest. Standard in situ coupling methods (e.g., 1,1'-carbonyldiimidazole) could be used to effect the conjugation.

The following structure shows a correlation of the IUPAC nomenclature for the positions of the atoms around the periphery of the macrocycle with the positions of the R groups of the present invention.

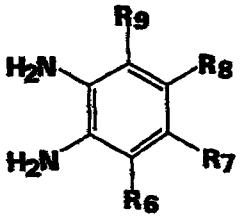


Substituents at the R_6 and R_9 positions on the B (benzene ring) portion of the macrocycle are incorporated into the macrocycle by their attachment to *ortho*-phenylenediamine in the 3 and 6 positions of the molecule. Substituents at the R_5 and R_{10} positions on the T (tripyrrane) portion of the macrocycle are incorporated by appropriate functionalization of carboxyl groups in the 5 positions of the tripyrrane at a synthetic step prior to condensation with a substituted *ortho*-phenylenediamine.

The nonaromatic texaphyrin is conveniently produced by condensation of a tripyrrane aldehyde or ketone having structure A; and a substituted *ortho*-phenylenediamine having structure B:



A



B

Substituents R_1-R_{12} are as described herein. In a preferred method of synthesis, the Brønsted base is triethylamine or N,N,N',N'-tetramethyl-1,8-diaminonaphthalene ("proton sponge") and the oxidant is air saturating the organic solvent, oxygen, platinum oxide, *o*-chloranil or 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. The stirring or heating at reflux step may comprise stirring or heating at reflux the mixture for at least 24 hours and the organic solvent may comprise methanol, or methanol and chloroform, or methanol and benzene, or methanol and dimethylformamide.

PCT publication, WO 94/29316 is incorporated by reference herein for the synthesis of texaphyrin oligonucleotide conjugates, particularly texaphyrin molecules where substituent R_2, R_3, R_7, or R_8 is an oligonucleotide or is a couple that is coupled to an oligonucleotide. Amides, ethers and thioethers are representative of couples which may be used for this purpose. Oligonucleotides functionalized with amines at the 5'-end, the 3'-end, or internally at sugar or base residues may be modified post-synthetically with an activated carboxylic ester derivative of the texaphyrin complex. Alternatively, oligonucleotide analogs containing one or more thiophosphate or thiol groups may be selectively alkylated at the sulfur atom(s) with an alkyl halide derivative of the texaphyrin complex. The resultant oligonucleotide-complex conjugates may be designed so as to provide optimal catalytic interaction between a target nucleic acid and the bound texaphyrin. The oligonucleotide may be large enough to bind probably at least about 8 nucleotides of complementary nucleic acid.

For general reviews of synthesis of DNA, RNA, and their analogues, see *Oligonucleotides and Analogues*, F. Eckstein, Ed., 1991, IRL Press, New York; *Oligonucleotide Synthesis*, M.J. Gait, Ed., 1984, IRL Press Oxford, England;

Caracciolo *et al.* (1989); *Bioconjugate Chemistry*, Goodchild, J. (1990); or for phosphonate synthesis, Matteucci, MD. *et al.*, *Nucleic Acids Res.* 14:5399 (1986) (the references are incorporated by reference herein).

In general, there are three commonly used solid phase-based approaches to the synthesis of oligonucleotides containing conventional 5'-3' linkages. These are the phosphoramidite method, the phosphonate method, and the triester method.

A brief description of a current method used commercially to synthesize oligomeric DNA is as follows: Oligomers up to ca. 100 residues in length are prepared on a commercial synthesizer, eg., Applied Biosystems Inc. (ABI) model 392, that uses phosphoramidite chemistry. DNA is synthesized from the 3' to the 5' direction through the sequential addition of highly reactive phosphorous(III) reagents called phosphoramidites. The initial 3' residue is covalently attached to a controlled porosity silica solid support, which greatly facilitates manipulation of the polymer. After each residue is coupled to the growing polymer chain, the phosphorus(III) is oxidized to the more stable phosphorus(V) state by a short treatment with iodine solution. Unreacted residues are capped with acetic anhydride, the 5'- protective group is removed with weak acid, and the cycle may be repeated to add a further residue until the desired DNA polymer is synthesized. The full length polymer is released from the solid support, with concomitant removal of remaining protective groups, by exposure to base. A common protocol uses saturated ethanolic ammonia.

The phosphonate based synthesis is conducted by the reaction of a suitably protected nucleotide containing a phosphonate moiety at a position to be coupled with a solid phase-derivatized nucleotide chain having a free hydroxyl group, in the presence of a suitable activator to obtain a phosphonate ester linkage, which is stable to acid. Thus, the oxidation to the phosphate or thiophosphate can be conducted at any point during synthesis of the oligonucleotide or after synthesis of the oligonucleotide is complete. The phosphonates can also be converted to phosphoramidate derivatives by reaction with a primary or secondary amine in the presence of carbon tetrachloride.

In the triester synthesis, a protected phosphodiester nucleotide is condensed with the free hydroxyl of a growing nucleotide chain derivatized to a solid support in the presence of coupling agent. The reaction yields a protected phosphate linkage which may be treated with an oximate solution to form unprotected oligonucleotide.

To indicate the three approaches generically, the incoming nucleotide is regarded as having an "activated" phosphite/phosphate group. In addition to employing commonly used solid phase synthesis techniques, oligonucleotides may also be synthesized using solution phase methods such as diester synthesis. The methods are workable, but in general, less efficient for oligonucleotides of any substantial length.

Preferred oligonucleotides resistant to *in vivo* hydrolysis may contain a phosphorothioate substitution at each base (*J. Org. Chem.*, 55:4693-4699, (1990) and Agrawal, (1990)). Oligodeoxynucleotides or their phosphorothioate analogues may be synthesized using an Applied Biosystem 380B DNA synthesizer (Applied Biosystems, Inc., Foster City, CA).

For the above-described uses, texaphyrins are provided as pharmaceutical preparations. A pharmaceutical preparation of a texaphyrin may be administered alone or in combination with pharmaceutically acceptable carriers, in either single or multiple doses. Suitable pharmaceutical carriers include inert solid diluents or fillers, sterile aqueous solution and various organic solvents. The pharmaceutical compositions formed by combining a texaphyrin of the present invention and the pharmaceutically acceptable carriers are then easily administered in a variety of dosage forms. Administration may be intravenous, intraperitoneal, parenteral, intramuscular, subcutaneous, oral, or topical, with topical and intravenous administration being preferred, and intravenous being more preferred.

Solutions of the texaphyrin in sesame or peanut oil, aqueous propylene glycol, or in sterile aqueous solution may be employed. Such aqueous solutions should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. Topical creams, emulsions, solutions, and the like are contemplated for applications to surface areas of the body. Topical application may also be by iontophoresis.

For antisense applications, excipients and preservatives that preserve oligonucleotide stability are chosen. The highly negatively charged phosphate or sulfur groups of the backbone of the oligonucleotide may be irritating to epithelial or other surface cells. Counterions may be used for formulation purposes to prevent irritation.

Pharmaceutical forms include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy use with a syringe exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars such as mannitol or dextrose or sodium chloride. A more preferable isotonic agent is a mannitol solution of about 2-8% concentration, and, most preferably, of about 5% concentration. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, permeation enhancers, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar

as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

A reliable assay for RNA photocleavage potency of a texaphyrin-oligonucleotide conjugate is an assay for photocleavage of a complementary ribonucleic acid as described in Example 5. Photocleavage by a conjugate would demonstrate that the conjugate has the intended potency and activity.

Treatment of RNA with 1μM LuB2T2 results in hydrolysis products in the absence, as well as the presence of light, (see PCT publication WO/94/29316, incorporated by reference herein). This reaction with RNA, therefore, is not photoinduced and produces different products than the photocleavage reaction of the present invention. Photooxidatively damaged products include those involving reaction at position 9 of guanine, which generally leads to depurination, strand breakage, and the generation of two smaller pieces that both contain phosphorylated ends. Hydrolysis, on the other hand, leaves a free ribose on one of the two resulting fragments and a phosphate terminus on the other.

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1
Synthesis of a Lutetium Texaphyrin-Oligonucleotide Conjugate

The present example provides for the synthesis of a lutetium texaphyrin-oligonucleotide conjugate useful for site-directed photocleavage of a complementary RNA (see Scheme A).

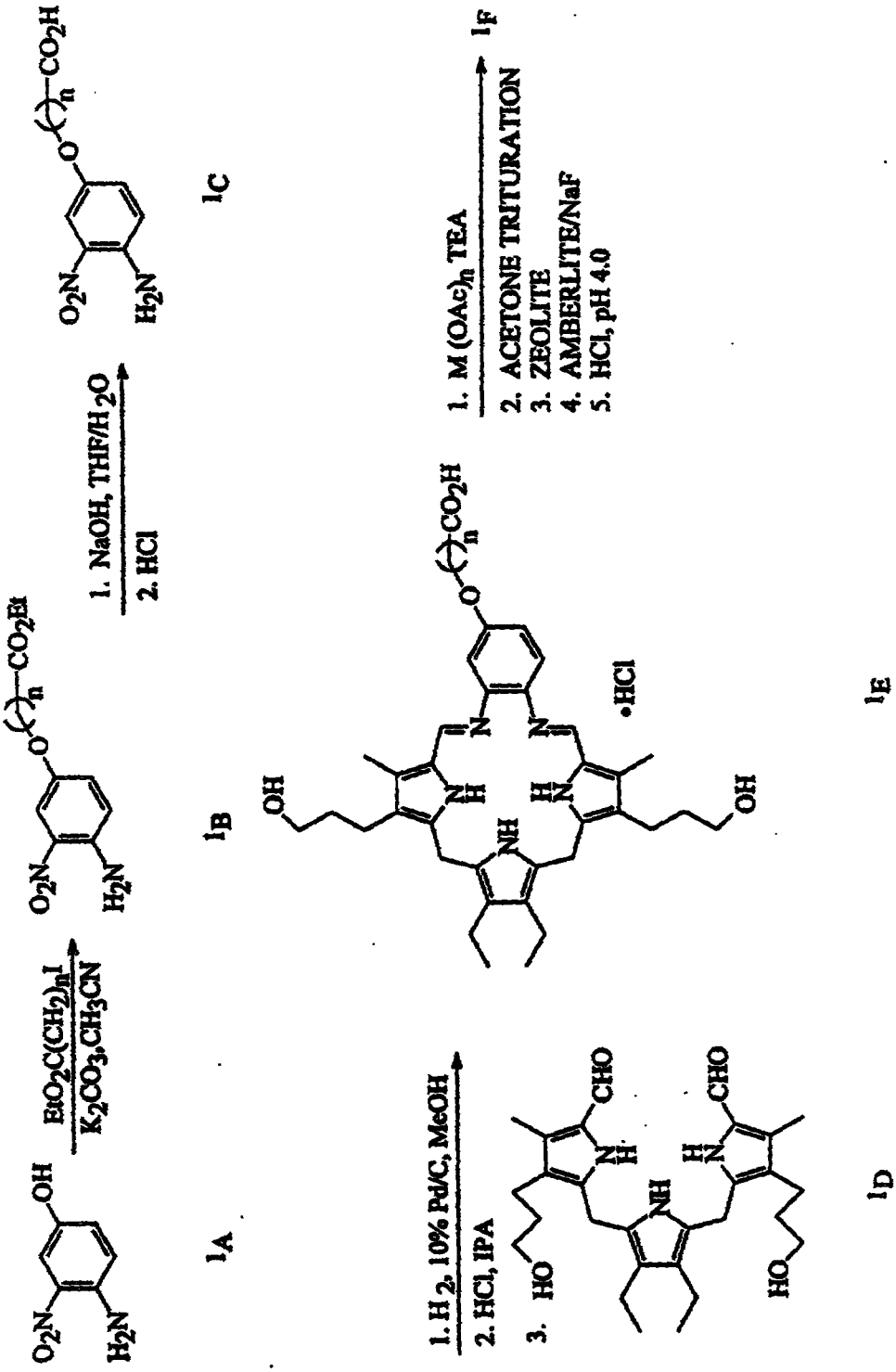
4-Amino-1-[1-(ethyloxy)acetyl-2-oxy]-3-nitrobenzene 1_B, *n=1.* Potassium carbonate (14.0 g, 101 mmol) and 4-amino-3-nitrophenol 1_A (10.0 g, 64.9 mmol) were

suspended in 150 mL dry acetonitrile. Ethyl-2-iodoacetate (10 mL, 84.5 mmol) (or ethyl iodobutyrate may be used, in that case n=3) was added via syringe, and the suspension was stirred at ambient temperature for ca. 21 h. Chloroform (ca. 375 mL) was added and was used to transfer the suspension to a separatory funnel, whereupon it was washed with water (2 x ca. 100 mL). The water washes were in turn washed with $CHCl_3$ (ca. 100 mL) and the combined $CHCl_3$ extracts were washed with water (ca. 100 mL). Solvents were removed on a rotary evaporator, and the residue was redissolved in $CHCl_3$ (ca. 500 mL) and precipitated into hexanes (1.5 L). After standing two days, the precipitate was filtered using a coarse fritted funnel and dried in vacuo to provide 14.67 g compound 1_B, n=1 (94.1%). TLC: Rf = 0.43, $CHCl_3$.

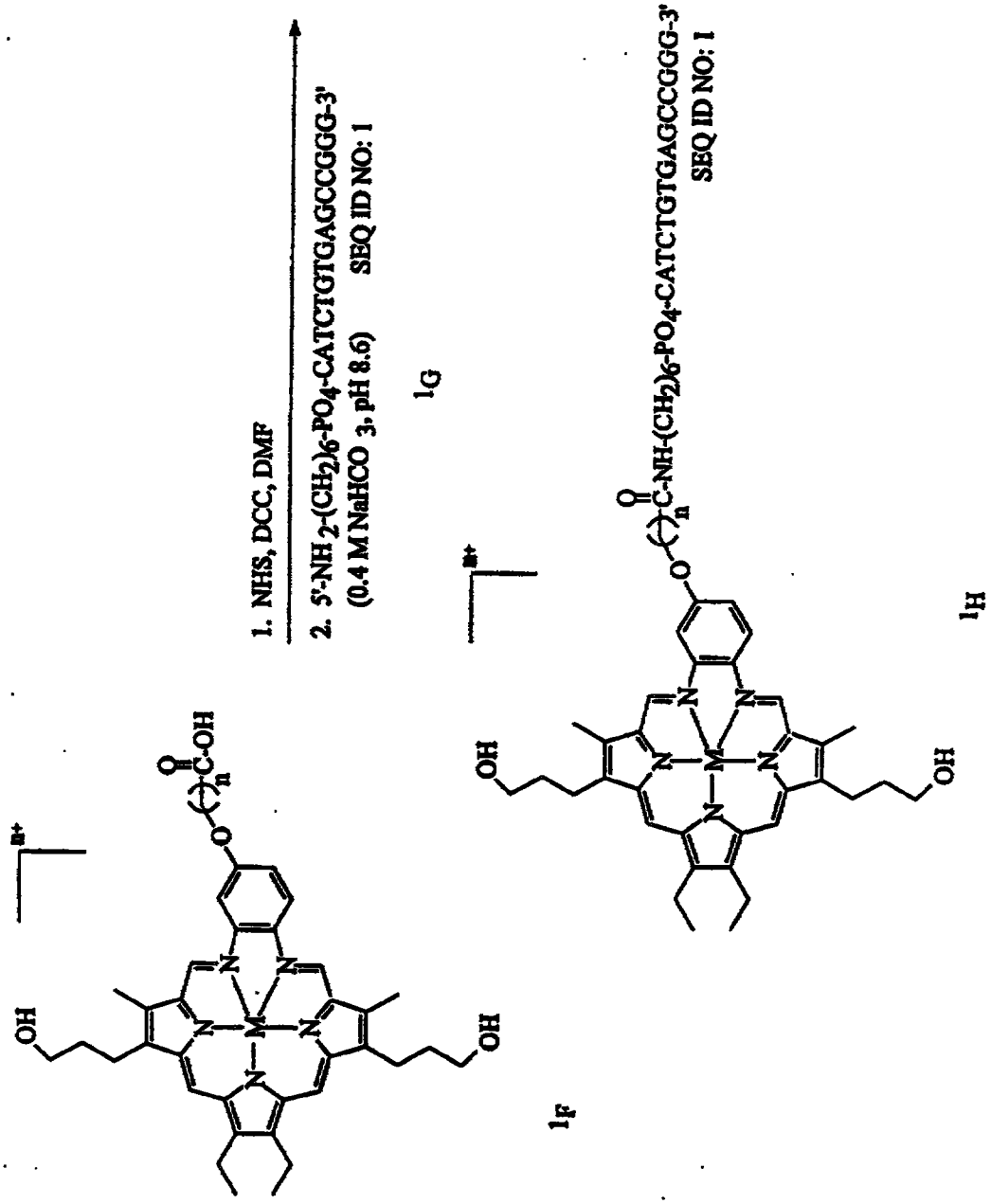
4-Amino-1-[1-(hydroxy)acetyl-2-oxy]-3-nitrobenzene 1_C, *n=1.* 4-Amino-1-[1-(ethyloxy)acetyl-2-oxy]-3-nitrobenzene 1_B, n=1, (10.00 g, 37.3 mmol) was dissolved in tetrahydrofuran (100 mL), aqueous sodium hydroxide (1M solution, 50 mL) was added and the solution was stirred at ambient temperature for ca. 21 h. Tetrahydrofuran was removed on a rotary evaporator, and water (100 mL) was added. The solution was washed with $CHCl_3$ (ca. 200 mL), then neutralized by addition of hydrochloric acid (1M solution, 50 mL). The precipitate which formed was filtered after standing a few minutes, washed with water, and dried in vacuo to provide 8.913 g compound 1_C, n=1 (99.5%). TLC: Rf = 0.65, 10% methanol/$CHCl_3$.

16-[1-(Hydroxy)acetyl-2-oxy]-9, 24-bis(3-hydroxypropyl)-4,5-diethyl-10,23-dimethyl-13,20,25,26,27-pentaazapenta-cyclo[20.2.1.1^{3,6}.1^{8,11}.0^{14,19}] heptacosa-3,5,8,10,12,14(19), 15,17,20,22,24-undecaene 1_E, *n=1.* 4-Amino-1-[1-(hydroxy)acetyl-2-oxy]-3-nitrobenzene 1_C, n=1 (1.800 g, 8.49 mmol) was dissolved in methanol (100 mL) in a 1 L flask. Palladium on carbon (10%, 180 mg) was added, and the atmosphere inside the flask was replaced with hydrogen at ambient pressure. A grey precipitate was formed after ca. 3 h, and the supernatant was clear. Methanol was removed in vacuo, taking precautions to prevent exposure to oxygen, and the compound was dried overnight in vacuo. Isopropyl alcohol (500 mL) and HCl (12 M, 400 μL) were added, and the suspension was allowed to stir for ca. 15'.

Scheme A


EtO2C(CH2)nI
K2CO3,CH3CN
1. NaOH, THF/H2O
2. HCl
1. H2, 10% Pd/C, MeOH
2. HCl, IPA
3. HO
1. M (OAc)n TEA
2. ACETONE TRITURATION
3. ZEOLITE
4. AMBERLITE/NaF
5. HCl, pH 4.0
•HCl

Scheme A (con't)



2,5-Bis[(3-hydroxypropyl-5-formyl-4-methylpyrrol-2-yl)methyl]-3,4-diethylpyrrole 1_D (n=1) (4.084 g, 8.49 mmol) was added, and the reaction stirred at room temperature under argon for 3 hours. Hydrochloric acid was again added (12 M, 400 μL) and the reaction again was allowed to stir for an additional 3.5 h. The resulting red solution was filtered through celite, and the filtercake was washed with isopropyl alcohol until the filtrate was colorless. Solvent was reduced to a volume of ca. 50 mL using a rotary evaporator, whereupon the solution was precipitated into rapidly stirring Et_2O (ca. 700 mL). Compound 1_E (n=1) was obtained as a red solid (5.550 g, 98.4%) upon filtering and drying in vacuo. TLC: R_f = 0.69, 20% methanol/$CHCl_3$ (streaks, turns green on plate with I_2).

Lutetium complex of 16-[1-(hydroxy)acetyl-2-oxy]-9,24-bis(3-hydroxypropyl)-4,5-diethyl-10,23-dimethyl-13,20,25,26,27-pentaazapentacyclo [20.2.1.1^{3,6}.1^{8,11}.0^{14,19}]heptacosa-1,3,5,7,9,11(27),12,14(1 9),15,17,20,22(25),23-tridecaene 1_F, *M=Lu, n=1.* Approximately equal molar amounts of the protonated form of the macrocycle, 16-[1-(hydroxy)acetyl-2-oxy]-9,24-bis(3-hydroxypropyl)-4,5-diethyl-10,23-dimethyl-13,20,25,26,27-pentaazapentacyclo[20.2 .1.1^{3,6}.1^{8,11}.0^{14,19}] heptacosa-3,5,8,10,12,14(19),15,17,20,22,24-undecaene hydrochloride 1_E, n=1, and a lutetium acetate pentahydrate were combined with triethylamine in methanol, and heated to reflux under air for 5.5 h. The reaction was cooled to room temperature, and stored at -20 °C overnight. Solvent was removed on a rotary evaporator, acetone was added, and the suspension was stirred on a rotary evaporator for 2 h. The suspension was filtered and the precipitate dried briefly *in vacuo*, whereupon a solution was formed in methanol (ca. 250 mL) and water (25 mL). The pH was adjusted to 4.0 using HCl (1 M), HCl-washed zeolite LZY54 was added (ca. 5 g) and the suspension was stirred on the rotary evaporator for ca. 6 h. Amberlite™ IRA-900 ion exchange resin (NaF treated, ca. 5 g) was added, and the suspension was stirred for an additional hour. The suspension was filtered, the resin was washed with methanol (ca. 100 mL), and the filtrate was adjusted to pH 4.0 using HCl (1 M). Solvents were removed on a rotary evaporator, using ethanol (abs.) to remove traces of water. After drying *in vacuo*, the compound was dissolved in methanol (25 mL) and precipitated into rapidly stirring Et_2O (300 mL). Compound 1_F, M=Lu and n=1, was obtained as a precipitate after filtering and drying *in vacuo*. An analytical sample was prepared by treating 50

mg of 1_F, n=1, dissolved in methanol (25 mL) with acetic acid-washed zeolite, then acetic acid-washed Amberlite™ for ca. 1 h. After reducing methanol to a minimum volume, the solution was precipitated into rapidly stirring Et_2O (70 mL), filtered, and dried *in vacuo*.

Postsynthetic modification of oligodeoxynucleotide-amine 1_G with lutetium texaphyrin complex 1_F, n=1. The lutetium complex of 16-[1-(hydroxy)acetyl-2-oxy]-9,24-bis(3-hydroxypropyl)-4,5-diethyl-10,23-dimethyl-13,20,25,26,27-pentaazapentacyclo [20.2.1.$1^{3,6}$.$1^{8,11}$.$0^{14,19}$]heptacosa-1,3,5,7,9,11(27),12, 14(19),15,17,20,22(25),23-tridecaene 1_F, M=Lu, n=1, (about 30 μmol) and N-hydroxysuccinimide (43 μmol) were dried together overnight *in vacuo*. The compounds were dissolved in dimethylformamide (anhydrous, 500 μL) and dicyclohexylcarbodiimide (10 mg, 48 μmol) was added. The resulting solution was stirred under argon with protection from light for 8h, whereupon a 110 μL aliquot was added to a solution of oligodeoxynucleotide 1_G (87 nmol) in a volume of 350 μL of 0.4 M sodium bicarbonate buffer in a 1.6 mL Eppendorf tube. After vortexing briefly, the solution was allowed to stand for 23 h with light protection. The suspension was filtered through 0.45 μm nylon microfilterfuge tubes, and the Eppendorf tube was washed with 150 μL sterile water. The combined filtrates were divided into two Eppendorf tubes, and glycogen (20 mg/mL, 2 μL) and sodium acetate (3M, pH 5.4, 30 μL) were added to each tube. After vortexing, ethanol (absolute, 1 mL) was added to each tube to precipitate the DNA. Ethanol was decanted following centrifugation, and the DNA was washed with an additional 1 mL aliquot of ethanol and allowed to air dry. The pellet was dissolved in 50% formamide gel loading buffer (20 μL), denatured at 90 °C for ca. 2', and loaded on a 20% denaturing polyacrylamide gel. The band corresponding to conjugate 1_H, M=Lu, n=1, was cut from the gel, crushed, and soaked in 1X TBE buffer (ca. 7 mL) for 1-2 days. The suspension was filtered through nylon filters (0.45 μm) and desalted using a Sep-pak™ reverse phase cartridge. The conjugate was eluted from the cartridge using 40% acetonitrile, lyophilized overnight, and dissolved in 1mM HEPES buffer, pH 7.0 (500 μL). The solution concentration was determined using UV/vis spectroscopy.

Example 2
Synthesis of texaphyrins or texaphyrin metal complexes with amine-, thiol- or hydroxy-linked oligonucleotides

Amides, ethers, and thioethers are representative of linkages which may be used for coupling site-directing molecules such as oligonucleotides to texaphyrins or texaphyrin metal complexes. PCT publication WO 94/29316 is incorporated by reference herein for providing syntheses of texaphyrin-oligonucleotide conjugates having these types of linkages or couples.

Site-directing molecules having an amine functionality or oligonucleotides functionalized with an amine at the 5'-end, the 3'-end, or internally at sugar or base residues are modified post-synthetically with an activated carboxylic ester derivative of a texaphyrin or texaphyrin metal complex. In the presence of a Lewis acid such as $FeBr_3$, a bromide derivatized texaphyrin will react with an hydroxyl group of an oligonucleotide to form an ether linkage between the texaphyrin linker and the oligonucleotide.

Alternatively, oligonucleotide analogues containing one or more thiophosphate or thiol groups are selectively alkylated at the sulfur atom(s) with an alkyl halide derivative of the texaphyrin complex. Oligodeoxynucleotide-complex conjugates are designed so as to provide optimal catalytic interaction between the targeted RNA phosphodiester backbone and the texaphyrin.

In the present invention, oligonucleotides are used to bind selectively compounds that include the complementary ribonucleotide, or oligoribonucleotide, or polyribonucleotide containing a substantially complementary sequence. As used herein, a substantially complementary sequence is one in which the nucleotides generally base pair with the complementary nucleotide and in which there are very few base pair mismatches. The oligonucleotide may be large enough to bind probably at least 9 nucleotides of complementary nucleic acid. The present inventors envision the texaphyrin-oligonucleotide conjugates of the present invention as being chemotherapeutic agents, for example, in an antisense capacity.

Example 3
Synthesis of a Texaphyrin-Oligonucleotide Conjugate Having a Texaphyrin Attached to the 3' End of the Oligonucleotide

Two oligodeoxyribonucleotides of 12 bases each were synthesized to contain alkylamine groups at the 3' terminal phosphate (Keystone Labs, Menlo Park, California). Oligonucleotides were HPLC purified and precipitated using LiCl prior to use. Reaction of a carboxylic acid functionalized metal texaphyrin complex, such as the Lu(III)texaphyrin complex (1_F where M=Lu(III) and n=1), with carbodiimide and N-hydroxysuccinimide produced the corresponding activated ester, which was added directly to a solution of the chosen oligodeoxynucleotide amine. The resulting texaphyrin-metal complex-oligonucleotide conjugates were purified by electrophoresis.

These 3'-conjugates may be of particular importance in certain embodiments of the present invention, since attachment of large groups (such as the present texaphyrin complexes) to the 3' end of an oligonucleotide renders the oligonucleotide resistant to cellular exonucleases.

In a similar manner, an embodiment of the present invention is the addition of particular ligands to the 3' end of an oligonucleotide having its 5' end conjugated to a texaphyrin. The function of the 3' ligand is to aid in the uptake of the conjugate into the cell. Such ligands are known in the art and include, but are not limited to, cholesterol and polylysine.

A further embodiment of the present invention in the photocleavage of RNA using a texaphyrin or texaphyrin-metal complex-oligonucleotide conjugate is the use of a set of two conjugates, one having the texaphyrin conjugated to the 5' end of an oligomer and the other having a texaphyrin conjugated to the 3' end of an oligomer and the oligomers are complementary to the same RNA substrate, one just upstream from the other, so as to position both texaphyrins in proximity to the targeted photocleavage site. The distance separating the two catalytic groups may be varied by preparing a nested set of oligomer-5'-conjugates of varying lengths and comparing the photocleavage efficiencies that result upon the simultaneous binding of the two conjugates to the RNA template.

Example 4
Synthesis of a Texaphyrin-Oligonucleotide Dual Conjugate

An oligodeoxyribonucleotide having 12 bases was synthesized to contain alkylamine groups at both the 3' and the 5' ends (Keystone Labs, Menlo Park, California). This oligomer was reacted with an excess of a carboxylic acid functionalized metal-texaphyrin complex, following the procedures of Example 3, to give a dual conjugate having a texaphyrin-metal complex at both the 3'- and the 5'-ends of the 12-mer.

The use of two texaphyrins conjugated to the same oligonucleotide, one at each end, should effect the photocleavage of RNA with increased efficiency due to the concerted activity of the metal complexes. In this embodiment, if the texaphyrin is metallated, it is preferred that both of the texaphyrin complexes contain the same metal, preferably a diamagnetic metal cation and more preferably lutetium(III).

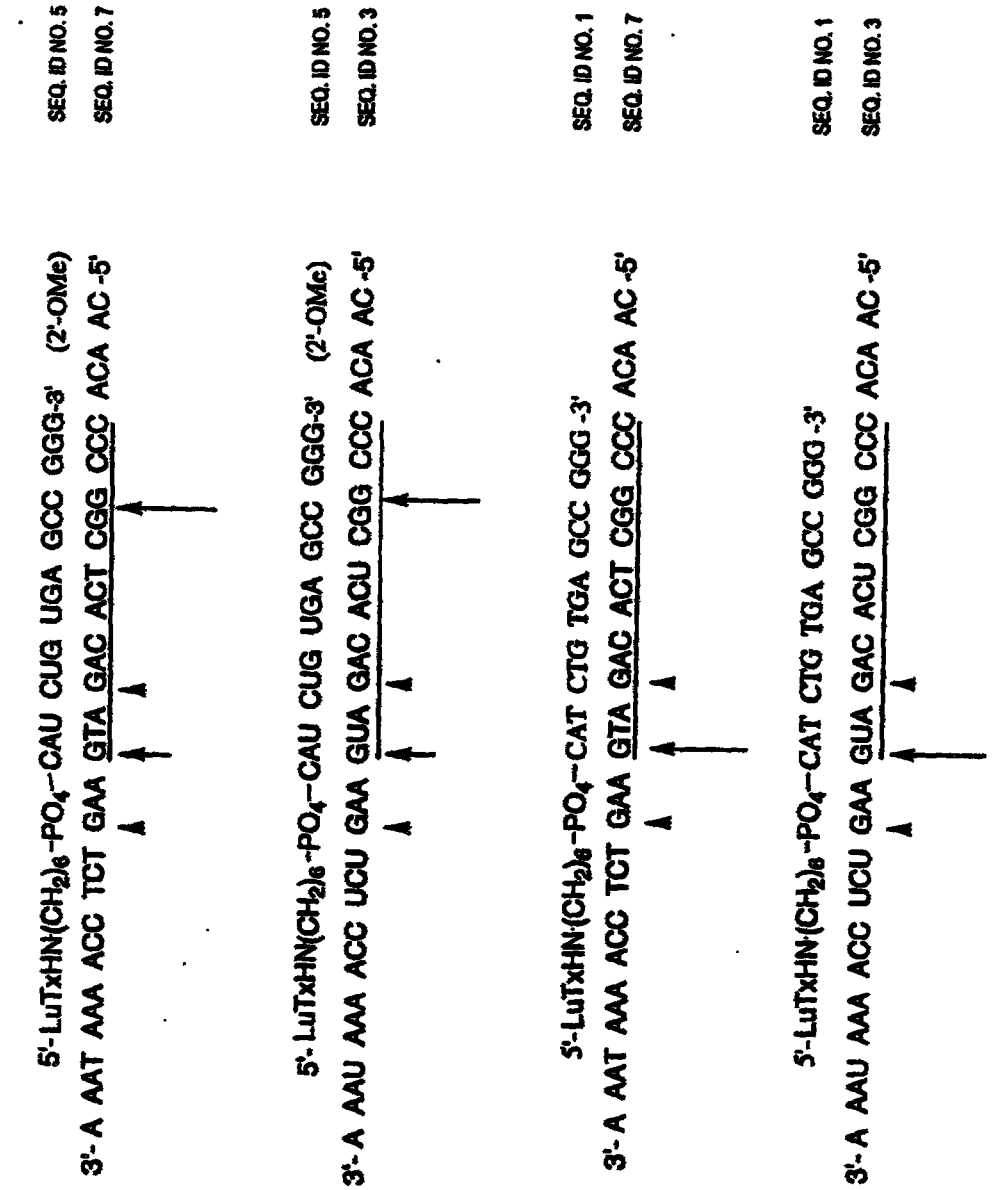
Further, a dual conjugate provides versatility in the functions that may be accomplished by this one molecule. For example, the oligonucleotide provides binding specificity, one texaphyrin metal complex may provide for imaging (having Gd(III) as the metal ion, for example) while the other provides for RNA photocleavage. Such a dual conjugate allows for 2 functions, imaging and photocleavage, to be effected by one molecule.

Example 5
Site-Specific Light-Dependent Cleavage of RNA by LuTxp-Oligonucleotide Conjugate

The present example provides for the site-specific light-dependent photocleavage of RNA by four different lutetium texaphyrin-oligonucleotide conjugates. Photocleavage of the corresponding DNA substrates by the texaphyrin oligonucleotide conjugates serves as a control study and demonstrates that photocleavage occurs at guanine residues with the RNA and DNA substrates.

Reaction mixtures were prepared by adding ca. 100,000 cpm of 5'-^{32}P-labeled RNA 36-mer or DNA 36-mer substrate to solutions made from lutetium texaphyrin-oligonucleotide conjugate as shown in Schemes B and C, 4X buffer (5μL), carrier DNA (1 μL) and water to produce a final volume of 20 μL. Final conjugate concentration was 50 nM. The 4X buffer is 400 mM NaCl, 200 mM HEPES, pH 7.5, 100 μM EDTA.

Scheme B


SEQ. ID NO. 5
SEQ. ID NO. 7
5'-LuTxHN(CH2)6-PO4-CAU CUG UGA GCC GGG-3' (2'-OMe)
3'- A AAT AAA ACC TCT GAA GTA GAC ACT CGG CCC ACA AC -5'
SEQ. ID NO. 5
SEQ. ID NO. 3
5'-LuTxHN(CH2)6-PO4-CAU CUG UGA GCC GGG-3' (2'-OMe)
3'- A AAU AAA ACC UCU GAA GUA GAC ACU CGG CCC ACA AC -5'
SEQ. ID NO. 1
SEQ. ID NO. 7
5'-LuTxHN(CH2)6-PO4-CAT CTG TGA GCC GGG -3'
3'- A AAT AAA ACC TCT GAA GTA GAC ACT CGG CCC ACA AC -5'
SEQ. ID NO. 1
SEQ. ID NO. 3
5'-LuTxHN(CH2)6-PO4-CAT CTG TGA GCC GGG -3'
3'- A AAU AAA ACC UCU GAA GUA GAC ACU CGG CCC ACA AC -5'

Scheme C

5'- LuTxHN-(CH2)6-PO4-CUC GGC CAU AGC GAA-3' (2'-OMe)
SEQ. ID NO. 6
3'- G CGC CAG AGA GGT GAG CCG GTA TCG CTT ACA AGA CA -5'
SEQ. ID NO. 8
5'- LuTxHN-(CH2)6-PO4-CUC GGC CAU AGC GAA-3' (2'-OMe)
SEQ. ID NO. 6
3'- G CGC CAG AGA GGU GAG CCG GUA UCG CUU ACA AGA CA -5'
SEQ. ID NO. 4
5'- LuTxHN-(CH2)6-PO4-CTC GGC CAT AGC GAA-3'
SEQ. ID NO. 2
3'- G CGC CAG AGA GGT GAG CCG GTA TCG CTT ACA AGA CA -5'
SEQ. ID NO. 8
5'- LuTxHN-(CH2)6-PO4-CTC GGC CAT AGC GAA-3'
SEQ. ID NO. 2
3'- G CGC CAG AGA GGU GAG CCG GUA UCG CUU ACA AGA CA -5'
SEQ. ID NO. 4

All samples were irradiated for 15 minutes at ambient temperature using a dye laser (Coherent, Palo Alto, CA) tuned to 732 nm using a power density of 150 mW/cm^2. Following irradiation, the RNA or DNA was precipitated with ethanol using standard methods.

Samples containing radiolabeled DNA were dissolved in 10% aqueous piperidine solution (50 μL) and heated at 90°C for 30 minutes. Samples containing radiolabeled RNA were dissolved in 1:1:8 aniline/acetic acid/water (50 μL) and heated at 58°C for 30 minutes. Water (500 μL) was added to all samples, which were then dried on a Speedvac. All samples were resuspended in 50% formamide loading buffer, denatured at 60°C for 5 minutes, and analyzed by electrophoresis on a 20% denaturing polyacrylamide gel. The autoradiograph indicated substantial photocleavage only in those lanes that contained the appropriate complementary 15-mer LuTx conjugate. A texaphyrin conjugated to a noncomplementary oligonucleotide did not effect photocleavage of the substrate. In Schemes B and C, arrows indicate observed sites of photocleavage. The Lu-Tx-mediated photocleavage bands comigrated with bands generated by dimethylsulfate in guanine-specific sequencing lanes run as a control. The intensity of photocleavage was greater at sites proximal to the expected location of the LuTx complex. These observations are consistent with a model whereby hybridization of the LuTx conjugates to their complementary sequences of RNA or DNA effects site-specific photomodification at guanine residues, and results in site-specific photocleavage upon workup under basic conditions.

In comparing 2'-O-methyl RNA and DNA oligonucleotide conjugates, a greater degree of photocleavage was found to occur at lower positions on the gel, corresponding to photomodification at sites along and across the major groove of the duplex formed between antisense conjugate and target. These differences in photocleavage pattern apparently relate to conformational differences between 2'-O-methyl RNA- and DNA- derived duplexes, with 2'-O-methyl RNA conjugates leading to a greater overall photocleavage efficiency.

Photocleavage efficiency ranged from 70-90% for the DNA substrates. Photocleavage patterns of RNA substrates paralleled that of their DNA analogues, albeit occurring in lower yield. This likely reflects less efficient exposure of the photoinduced lesions by the milder aniline treatment. (The RNA was not subjected to the piperidine treatment due to its greater lability under alkaline conditions). The

combination of substrate SEQ ID NO: 4 and texaphyrin-oligonucleotide conjugate labeled SEQ ID NO: 2 showed relatively little photocleavage in comparison to other lanes containing complementary conjugate. This may indicate poorer binding of the DNA-LuTx conjugate to this RNA sequence, and further evidences the superiority of the 2'-O-methyl RNA conjugate as used in this application.

Although the present inventors were aware of texaphyrins having photocleavage activity for polymers of DNA (WO 96/09315), it was not clear that RNA would also be photocleaved. The 2' site is protected by a hydroxyl group in RNA, the conformation of the polymer is different than DNA, and the electronic effects from the C9 position of guanine are different than in DNA. Even though the photocleavage demonstrated in this example is with a texaphyrin conjugated to an oligonucleotide, the inventors expect that unconjugated texaphyrin would be effective at photocleavage also, although at higher concentrations than used herein.

Example 6
Fluorescent Localization of Texaphyrin-Oligonucleotide Conjugates Within Eukaryotic Cells

The present inventors have demonstrated that texaphyrin-oligonucleotide conjugates are taken up by eukaryotic cells, as observed by fluorescent localization.

HL-60 cells (human promyelocytic leukemia cell line) were incubated with a solution (5μmol final conc.) of a texaphyrin-oligonucleotide conjugate complexed with either a Y(III) metal ion or a Lu(III) metal ion (where the oligonucleotide is a phosphorothioate with 15 bases). The cells were incubated for a minimum of 10 min. and up to about 60 min., after which the cells were washed. Fluorescence was measured with a confocal argon laser, which excites at 488 nm. To view the fluorescence created by the texaphyrin, a cut-off filter was used to eliminate wavelengths below 700 nm. The resulting fluorescence images showed diffuse cytoplasmic fluorescence with some evidence of local "hot spots" of concentrated fluorescence.

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations

may be applied to the composition, methods, and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit, and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope, and concept of the invention as defined by the appended claims.

The following references are incorporated in pertinent part by reference herein for the reasons cited herein.

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USSN 08/112,872.

USSN 08/227,370.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Pharmacyclics, Inc.
Board of Regents, The University of Texas System

(ii) TITLE OF INVENTION: RNA PHOTOCLEAVAGE USING TEXAPHYRINS

(iii) NUMBER OF SEQUENCES: 8

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(F) ZIP: 78701

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT unknown
(B) FILING DATE: Concurrently herewith
(C) CLASSIFICATION: Unknown

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(C) TELEX: 79-0924

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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(D) TOPOLOGY: linear

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

(A) DESCRIPTION: /desc = "RNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CUCGGCCAUA GCGAA 15

(2) INFORMATION FOR SEQ ID NO:7:

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "DNA"

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
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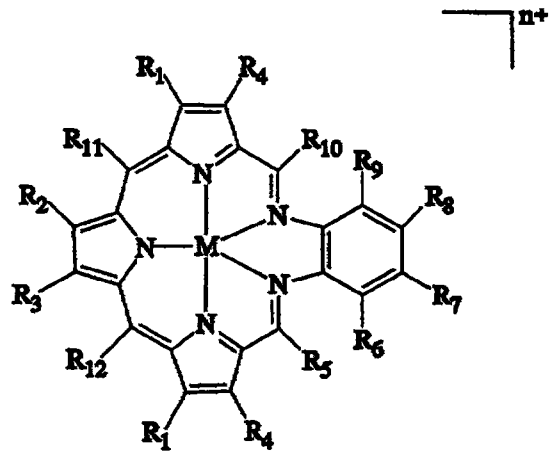
CLAIMS:

1. A method of light-induced photocleavage of a polymer of ribonucleic acid, the method comprising:

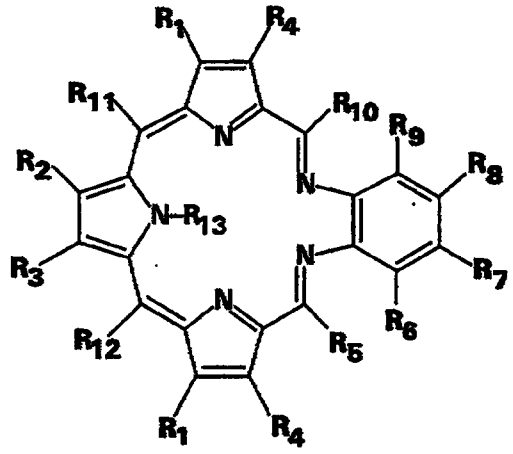
contacting the polymer of ribonucleic acid with a photosensitive texaphyrin; and

exposing the photosensitive texaphyrin to light for a time sufficient to photocleave the polymer.

2. The method of claim 1 where the photosensitive texaphyrin has structure I or II:



I



II

wherein

M is H or a diamagnetic metal cation;

R_1-R_4, R_7 and R_8 are independently hydrogen, halide, hydroxyl, alkyl, alkenyl, alkynyl, aryl, haloalkyl, nitro, formyl, acyl, hydroxyalkyl, alkoxy, hydroxyalkoxy, hydroxyalkenyl, hydroxyalkynyl, saccharide, carboxy, carboxyalkyl, carboxyamide, carboxyamidealkyl, amino, aminoalkyl, a site-directing molecule, a catalytic group, or a couple that is coupled to a site-directing molecule or to a catalytic group;

R_6 and R_9 are independently selected from the groups of R_1-R_4, R_7 and R_8, with the proviso that the halide is other than iodide and the haloalkyl is other than iodoalkyl;

R_5 and R_{10}-R_{12} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, hydroxyalkyl, alkoxy, hydroxyalkoxy, hydroxyalkenyl, hydroxyalkynyl, carboxyalkyl, carboxyamide, carboxyamidealkyl, amino, aminoalkyl, or a couple that is coupled to a saccharide, to a site-directing molecule, or to a catalytic group;

R_{13} is alkyl, alkenyl, oxyalkyl, or hydroxyalkyl having up to about 3 carbon atoms and having rotational flexibility around a first-bound carbon atom; and

n is an integer value less than or equal to 5.

3. The method of claim 2 wherein the site-directing molecule is an oligonucleotide, an antibody, a hormone, a peptide having affinity for a biological receptor, or a sapphyrin molecule.

4. The method of claim 2 wherein at least one of R_1, R_2, R_3, R_7 and R_8 is a site-directing molecule, or a couple that is coupled to a site-directing molecule.

5. The method of claim 2 wherein at least one of R_1, R_2, R_3, R_7 and R_8 is an oligonucleotide, or a couple that is coupled to an oligonucleotide.

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6. The method of claim 2 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, R_8 is a site-directing molecule, or a couple that is coupled to a site-directing molecule, and R_7 is H.

7. The method of claim 2 where R_1 is $(CH_2)_2CH_2OH$; R_2 and R_3 are CH_2CH_3; R_4 is CH_3; R_7 is $O(CH_2CH_2O)_2CH_2CH_2OCH_3$, H, or OCH_3; and R_8 is a site-directing molecule, or a couple that is coupled to a site-directing molecule.

8. The method of claim 7 where the site-directing molecule is an oligonucleotide.

9. The method of claim 2 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, and R_7 and R_8 are $O(CH_2CH_2O)_2CH_2CH_2OCH_3$.

10. The method of claim 2 wherein R_1-R_4, R_7, and R_8 are as in Table 1 for texaphyrins A1-A22.

11. The method of claim 2 wherein M is a diamagnetic metal cation and the diamagnetic metal cation is Lu(III), La(III), In(III), Y(III), Zn(II) or Cd(II).

12. The method of claim 2 wherein M is a diamagnetic metal cation and the diamagnetic metal cation is Lu(III).

13. The method of claim 1 wherein the light has a wavelength range of about 700 to 800 nanometers.

14. The method of claim 1 where the polymer of ribonucleic acid is a messenger ribonucleic acid of a cell targeted for treatment and the photosensitive texaphyrin is conjugated to an oligonucleotide having sequence complementarity with the messenger ribonucleic acid.

15. A method of treating a host harboring benign or malignant tumor cells comprising:
administering to the host an effective amount of a photosensitive texaphyrin-oligonucleotide conjugate, the oligonucleotide having sequence

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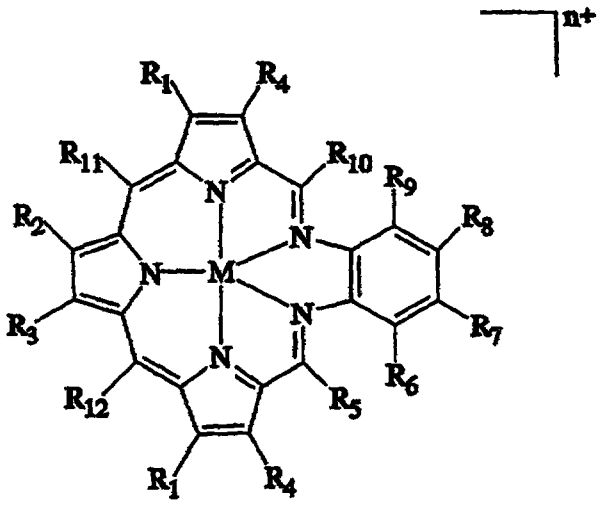
complementarity to an RNA molecule of the benign or malignant tumor cells; and

photoirradiating the photosensitive texaphyrin in proximity to the tumor cells.

16. The method of claim 15 further comprising the step of determining localization sites of the photosensitive texaphyrin in the host by reference to the texaphyrin.

17. The method of claim 15 further comprising a step of administering to the host a photosensitive texaphyrin diamagnetic metal complex.

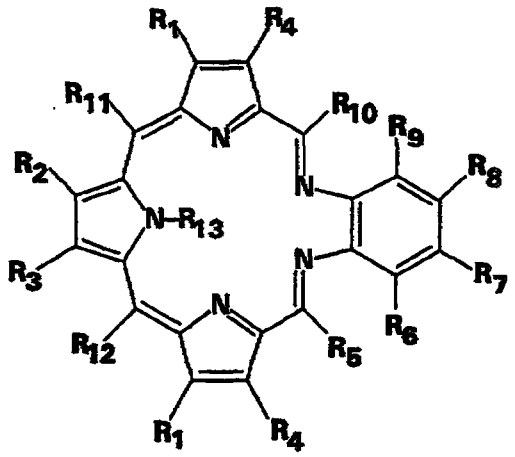
18. The method of claim 15 where the photosensitive texaphyrin-oligonucleotide conjugate has structure I or II:



I

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II

wherein

M is H or a diamagnetic metal cation;

R_1-R_4, R_7 and R_8 are independently hydrogen, halide, hydroxyl, alkyl, alkenyl, alkynyl, aryl, haloalkyl, nitro, formyl, acyl, hydroxyalkyl, alkoxy, hydroxyalkoxy, hydroxyalkenyl, hydroxyalkynyl, saccharide, carboxy, carboxyalkyl, carboxyamide, carboxyamidealkyl, amino, aminoalkyl, an oligonucleotide, or a couple that is coupled to an oligonucleotide;

R_6 and R_9 are independently selected from the groups of R_1-R_4, R_7 and R_8, with the proviso that the halide is other than iodide and the haloalkyl is other than iodoalkyl;

R_5 and R_{10}-R_{12} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, hydroxyalkyl, alkoxy, hydroxyalkoxy, hydroxyalkenyl, hydroxyalkynyl, carboxyalkyl, carboxyamide, carboxyamidealkyl, amino, aminoalkyl, or a couple that is coupled to an oligonucleotide;

R_{13} is alkyl, alkenyl, oxyalkyl, or hydroxyalkyl having up to about 3 carbon atoms and having rotational flexibility around a first-bound carbon atom;

at least one of R_1, R_2, R_3, R_7 and R_8 is an oligonucleotide, or a couple that is coupled to an oligonucleotide; and

n is an integer value less than or equal to 5.

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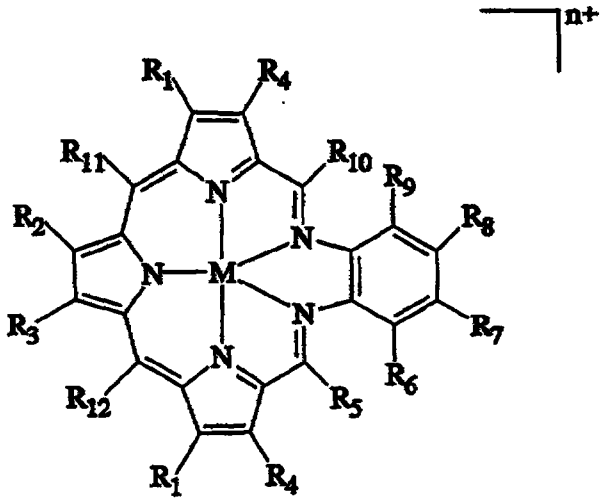
19. The method of claim 18 where R_1 is $(CH_2)_2CH_2OH$; R_2 and R_3 are CH_2CH_3; R_4 is CH_3; R_7 is $O(CH_2CH_2O)_2CH_2CH_2OCH_3$, H, or OCH_3; and R_8 is an oligonucleotide, or a couple that is coupled to an oligonucleotide.

20. The method of claim 19 wherein M is a diamagnetic metal cation and the diamagnetic metal cation is Lu(III), La(III), In(III), Y(III), Zn(II) or Cd(II).

21. Use of a photosensitive texaphyrin in the preparation of a pharmaceutical composition for use in photocleaving a polymer of ribonucleic acid.

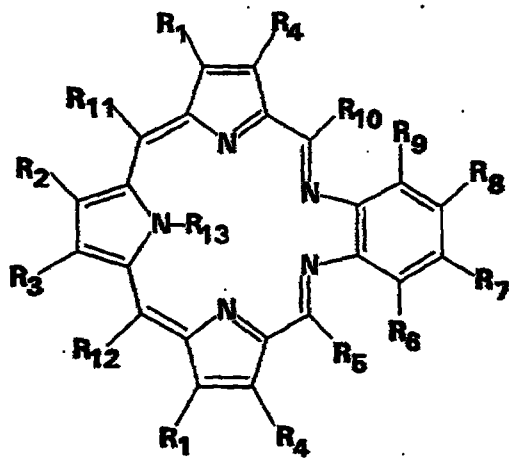
22. The use of claim 21 where the polymer of ribonucleic acid is messenger RNA.

23. The use of claim 22 where the photosensitive texaphyrin has structure I or II:



I

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II

wherein

M is H or a diamagnetic metal cation;

R_1-R_4, R_7 and R_8 are independently hydrogen, halide, hydroxyl, alkyl, alkenyl, alkynyl, aryl, haloalkyl, nitro, formyl, acyl, hydroxyalkyl, alkoxy, hydroxyalkoxy, hydroxyalkenyl, hydroxyalkynyl, saccharide, carboxy, carboxyalkyl, carboxyamide, carboxyamidealkyl, amino, aminoalkyl, an oligonucleotide, or a couple that is coupled to an oligonucleotide;

R_6 and R_9 are independently selected from the groups of R_1-R_4, R_7 and R_8, with the proviso that the halide is other than iodide and the haloalkyl is other than iodoalkyl;

R_5 and R_{10}-R_{12} are independently hydrogen, alkyl, alkenyl, alkynyl, aryl, hydroxyalkyl, alkoxy, hydroxyalkoxy, hydroxyalkenyl, hydroxyalkynyl, carboxyalkyl, carboxyamide, carboxyamidealkyl, amino, aminoalkyl, or a couple that is coupled to an oligonucleotide;

R_{13} is alkyl, alkenyl, oxyalkyl, or hydroxyalkyl having up to about 3 carbon atoms and having rotational flexibility around a first-bound carbon atom;

at least one of R_1, R_2, R_3, R_7 and R_8 is an oligonucleotide, or a couple that is coupled to an oligonucleotide; and

n is an integer value less than or equal to 5.