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NOTICE OF ENTITLEMENT

We, **Board of Regents, The University of Texas System** AND **Pharmacyclics, Inc.** the applicant/nominated person in respect of Application No. 70526/94, state the following:-

The Nominated persons are entitled to the grant of the patent because the Nominated person Board of Regents, The University of Texas System derives title to the invention from the inventors Jonathan L. Sessler, Daniel A. Smith, Vladimir A. Kral & Brent Iverson by assignment and the Nominated Person, Pharmacyclics, Inc. derives title to the invention from the inventors Richard A. Miller, Meredith Wright, Gregory W. Hemmi, Kevin L. Ross, William C. Dow, Darren Magda & Tarak Mody by assignment.

The Nominated persons are entitled to claim priority from the applications listed in the declaration under Article 8 of the PCT because the Nominated Persons are the assignees of the applicants in respect of the applications listed in the declaration under Article 8 of the PCT.

DATED this FOURTH day of JANUARY, 1996.

.....

a member of the firm of
DAVIES COLLISON CAVE
for and on behalf of the
applicant(s)

(DCC ref:1789419)

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TEXAPHYRIN METAL COMPLEX MEDIATED ESTER HYDROLYSIS

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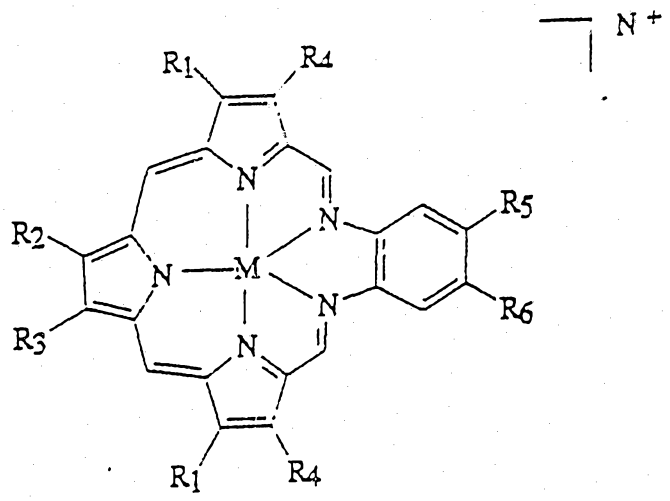
(57) Claim

1. A texaphyrin having the structure:



where:

M is a divalent metal cation selected from the group consisting of Ca^{+2}, Mn^{+2}, Co^{+2}, Ni^{+2}, Zn^{+2}, Cd^{+2}, Hg^{+2},

.../2

Fe^{+2}, Sm^{+2} and UO^{+2} or a trivalent metal cation selected from the group consisting of Mn^{+3}, Co^{+3}, Ni^{+3}, Fe^{+3}, Ho^{+3}, Ce^{-3}, Y^{+3}, In^{+3}, Pr^{+3}, Nd^{+3}, Sm^{+3}, Eu^{+3}, Gd^{+3}, Tb^{-3}, Dy^{+3}, Er^{+3}, Tm^{+3}, Yb^{+3}, Lu^{+3}, La^{+3}, and U^{+3}; R_1, R_2, R_3, R_4, R_5 and R_6 are independently hydrogen, hydroxyl, alkyl, hydroxyalkyl, alkoxy, hydroxyalkoxy, saccharide, carboxyalkyl, carboxyamidealkyl, a site-directed molecule, a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group, and at least one R_1, R_2, R_3, R_4, R_5 and R_6 is a site-directed molecule, a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group; and

N is an integer less than or equal to 2.

23. A method of phosphate ester hydrolysis, the method comprising:

Obtaining an aqueous phosphate ester solution, and

incubating the solution with a metal complex having catalytic activity for ester bond hydrolysis, said incubation being under conditions and for a time sufficient to hydrolyze the phosphate ester, characterized in that the metal complex is a texaphyrin metal complex as hereinbefore described.

PCT

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(57) Abstract

A method of phosphate ester hydrolysis including incubating a solution of an aqueous phosphate ester with a texaphyrin metal complex. The metal is a metal cation having catalytic activity for ester bond hydrolysis in aqueous solution, in particular, a lanthanide metal cation, preferably Eu(III) or Dy(III). A preferred substrate is RNA and a preferred texaphyrin is a derivatized texaphyrin having binding specificity, in particular, a texaphyrin covalently coupled to a site-directed molecule, preferably an oligonucleotide.

DESCRIPTION

TEXAPHYRIN METAL COMPLEX MEDIATED ESTER HYDROLYSIS

BACKGROUND OF THE INVENTION

The present invention relates to catalysts for the cleavage of ester bonds, in particular, phosphate ester bonds related to a biological system. An effective catalyst would have the following properties:

1) Efficiency at physiological temperature and pH;
2) Specificity for certain biological substrates;
3) Low toxicity for physiological systems; and
4) Easy administration to a biological system and ready subsequent elimination.

Various biologically important phosphate esters may be hydrolyzed according to the methods of the present invention, including RNA, phospholipids, phosphate anhydrides, UDP-glucose or a widely used substrate for assays, p-nitrophenylphosphate ester.

Many divalent and trivalent metal salts have been shown to promote the hydrolysis of phosphate ester bonds. Komiyama et al. (1992) reported the hydrolysis of adenylyl(3′-5′)adenosine and uridyl(3′-5′)uridine at pH 8.0, 30°C by rare earth metal(III) ions. A Cerium(III) hydroxide cluster has been reported to hydrolyze 3′,5′-cyclic adenosine monophosphate (Sumaoka, et al. 1992). Browne and Bruice (1992) reported the hydrolysis of bis(8-hydroxyquinoline)phosphate in the presence of divalent cations. However, in order to convey a degree of specificity to catalysis by the metal ion, complexes of metals with various ligands have been studied. The ligand may serve a number of roles in catalysis

including, modulation of catalytic efficiency, and maintenance of the metal ion in solution, while also allowing for coupling of reagents having a binding specificity for a desired substrate.

Ligands complexing metal ions for use in the hydrolysis of phosphate ester bonds include: tris(aminopropyl)amine (trpn), 1,4,7,10-tetraazacyclododecane (cyclen), tris(2-aminoethyl)amine (tren), triethylenetetramine (trien), tetraethylenepentamine (tetren), bipyridine conjugates, imidazole, cyclodextrin derivatives, lysine, terpyridine (trpy), 1,2-diaminoethane, a bis(diaquo) complex, "metallomicelles" and a phenanthroline-polyamine tridentate complex (Basile *et al.* 1987, Menger *et al.* 1987, Chung *et al.* 1990, Hendry and Sargeson, 1989, Shelton and Morrow, 1991, Ranganathan *et al.* 1993, Breslow and Huang, 1991, Modak *et al.* 1991, Kim and Chin, 1992, Chin *et al.* 1989, Chin and Banaszczyk, 1989a,b, Chin and Zou, 1987).

In order for a metal complex to function catalytically *in vivo*, the complex should not release bound metal ion. Morrow *et al.* (1992) have studied the cleavage of RNA by a lanthanide(III) hexamine Schiff-base (HAM) macrocyclic complex. Cleavage of the dinucleotide adenylyl-3',5'uridine 3'-monophosphate (ApUp) or of oligomers of adenylic acid (A12-A18) was reported at 37°C after 4 hours by several lanthanide complexes. Other hexadentate ligands such as EDTA formed lanthanide(III) complexes that are completely inactive in RNA cleavage under similar conditions. Inertness of the macrocyclic complex to metal release was reported to change dramatically throughout the lanthanide series. These complexes have some serious disadvantages, including high toxicity of the HAM ligand, weak coordination and dissociation of the lanthanide metals. Further, the

ligand cannot be easily modified which precludes the generation of derivatives with substrate specificity.

Given the limitations of the HAM complex, it is clear that the development of new macrocycles, capable of chelating lanthanide metals and forming stable complexes which are able to cleave RNA, would be of utility.

LIST OF ABBREVIATIONS

DEPC	:	Diethylpyrocarbonate
dm	:	decimeter
EDTA	:	Ethylenediamine tetraacetic acid
EuOAc	:	Eu(III)(acetate)$_3$
Txp(txph) (TX)	:	Texaphyrin

SUMMARY OF THE INVENTION

The present invention seeks to solve these problems by providing texaphyrin metal complexes and texaphyrin metal complex-conjugates for ester hydrolysis that provide stable chelation for an otherwise toxic metallic cation, specificity for targeted sites in a therapeutic application, and sufficient nontoxicity for *in vivo* use.

The present invention involves the discovery that texaphyrin metal complexes catalyze the hydrolysis of ester linkages. Texaphyrins are unique molecules in that they chelate the metal in a very stable complex but allow access to the metal coordination sites, thus preserving the metal's reactivity and its ability to hydrolyze phosphoester bonds. Furthermore, the texaphyrin molecule allows for derivatization for various biological applications. The texaphyrin complex is far less toxic than the HAM complex as well.

U.S. Patent 5,252,720, incorporated herein by reference, demonstrated the stability and utility of texaphyrin metal complexes for *in vivo* use in magnetic resonance imaging protocols. The Y^{3+}, Gd^{+3}, and In^{3+} complexes of texaphyrin were found to be hydrolytically stable in 1:1 methanol-water mixtures with half-lives for decomplexation and/or ligand decomposition exceeding 3 weeks in both cases. A T2B2 texaphyrin-gadolinium complex showed low toxicity and good tissue selectivity in magnetic resonance imaging enhancement. The texaphyrin metal complex-conjugates of the present invention are expected to have similar stability for chelating lanthanide metal cations and similar low toxicity for *in vivo* applications.

Texaphyrin metal complexes possess inherent biolocalization specificity as described in the '720 patent. In one embodiment of the present invention, the texaphyrin metal complexes are further coupled to site-directed molecules to form conjugates for targeted *in vivo* delivery. "Specificity for targeted sites" means that upon contacting the texaphyrin metal complex-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 nucleotide, amino acid or glycolytic residues of the target to form a stable complex under the conditions effective to promote the interaction. In the present invention, this interaction will allow cleavage of an ester linkage that is in the vicinity of the specific binding.

Exemplary site-directed molecules contemplated in the present invention include but are not limited to: polynucleotides and oligonucleotides such as antisense

oligonucleotides; polyamides including peptides having affinity for a biological receptor and proteins such as antibodies; steroids and steroid derivatives; hormones such as estradiol, morphine or histamine; and further macrocycles such as sapphyrins and rubyrins.

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.

Hydrolytic cleavage of RNA by lanthanide(III) texaphyrins may be enhanced by additional catalytic groups appended to the texaphyrin metal complex-conjugate. The term "additional catalytic groups" means chemical functional groups that assist catalysis by acting as a general acid, Brønsted acid, general base, Brønsted base, nucleophile, or any other means by which the activation barrier to reaction is lowered or the ground state energy of the substrate is increased. Exemplary catalytic groups contemplated in the present invention 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 most preferably 2-7, and the like, derivatives thereof and texaphyrin metal complexes. The term "appended to the texaphyrin metal complex-conjugate" means that the catalytic groups are attached either directly to the texaphyrin metal complex via a linker or couple of variable length, or attached to the ligand portion of a texaphyrin complex-ligand conjugate. For example, a catalytic group attached to the oligonucleotide portion

of a texaphyrin metal complex-oligonucleotide conjugate is placed in the vicinity of the substrate upon binding the targeted RNA substrate.

A conjugate group having site specificity or catalytic activity may be covalently coupled to a texaphyrin directly on the macrocycle ring or through various couples. A couple may be described as a linker, i.e. a reactive group for covalently attaching another molecule at a distance from the texaphyrin macrocycle. Exemplary linkers or couples are amides, thiols, thioether or ether covalent bonds as described in the examples for attachment of oligonucleotides and antibodies. In most preferred embodiments, conjugates and appended groups are covalently bonded to the texaphyrin via a carbon-carbon, a carbon-nitrogen or a carbon-oxygen bond.

It will be apparent to one of skill in the art in light of the present disclosure that a variety of ester linkages may be cleaved by the molecules of the present invention. Exemplary ester linkages cleaved by the molecules of the present invention include phosphate monoester and diester linkages, especially physiologically important phosphate linkages present in nucleic acids such as RNA; important mediators of metabolism such as nucleotides ATP, ADP, AMP, cAMP, UDP, NADH, NADPH, FAD or $FADH_2$, for example; and phospholipids such as phosphatidyl choline and sphingomyelin that are important in nerve and brain functions.

An embodiment of the present invention provides a method of phosphate ester hydrolysis. The method comprises the steps of obtaining an aqueous phosphate ester solution, and incubating the solution with a texaphyrin metal complex having catalytic activity for ester bond hydrolysis, the incubation being under

conditions and for a time sufficient to hydrolyze the phosphate ester. A texaphyrin metal complex as used herein throughout the description and claims is an aromatic pentadentate expanded prophyrin analog metal complex with appended functional groups. Such pendant groups may enhance solubility or biolocalization or may provide coupling sites for site-directed molecules or catalytic groups.

The metal is a divalent or a trivalent metal cation having catalytic activity for ester bond hydrolysis in aqueous solution, in particular, the metal is a lanthanide cation or a Lewis acidic cation such as Y(III), In(III), or Sc(III). Exemplary metals include lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, lutetium, scandium or yttrium. In particular, the metal may be La(III), Nd(III), Sm(III), Gd(III), Tm(III), or Lu(III), or preferably, Eu(III) or Dy(III).

In a preferred embodiment, the phosphate ester is a nucleic acid ester. The aqueous phosphate ester solution may be a solution or a suspension of nucleic acid, for example, a solution or suspension of RNA or DNA. RNA is more preferably hydrolyzed than DNA by a factor of about 10^{12}. When the phosphate ester is RNA, the metal is preferably Dy(III) or Eu(III).

The texaphyrin metal complex may be a water soluble compound having the structure:

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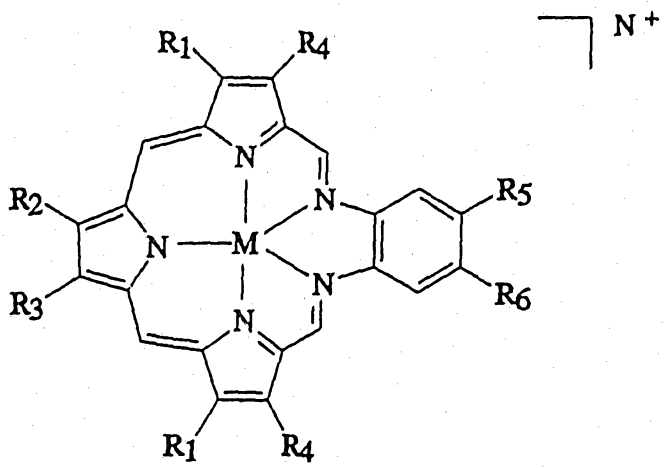

In this texaphyrin metal complex, M is a divalent or a trivalent metal cation catalyzing ester bond hydrolysis in aqueous solution. R_1, R_2, R_3, R_4, R_5 and R_6 are independently hydrogen, hydroxyl, alkyl, hydroxyalkyl, alkoxy, hydroxyalkoxy, saccharide, carboxyalkyl, carboxyamidealkyl, a site-directed molecule, a catalytic group, or a couple to a site-directed molecule or to a catalytic group. A site-directed molecule is the conjugate of a texaphyrin metal complex-conjugate. A catalytic group is also the conjugate of a texaphyrin metal complex-conjugate and may include, but is not limited to, imidazole, guanidine or texaphyrin.

N will typically be an integer less than or equal to 2. In the context of the basic macrocycle with a divalent or trivalent metal cation, N is 1 or 2; however, with a site-directed molecule covalently attached, 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 the site-directed molecule, for example, charges of the phosphate groups on an oligonucleotide.

R_1, R_2, R_3, R_4, R_5 and R_6 may also independently be amino, carboxy, carboxamide, ester, amide sulfonato, aminoalkyl, sulfonatoalkyl, amidealkyl, aryl, etheramide or equivalent structures conferring the desired properties. In a preferred embodiment, at least one of R_1, R_2, R_3, R_4, R_5 and R_6 is a site-directed molecule or is a couple to a site-directed molecule. For bulky R groups on the benzene ring portion of the molecule such as antibodies, peptides or oligonucleotides, one skilled in the art would realize that derivatization at one position on the benzene portion is more preferred.

Hydroxyalkyl means alkyl groups having hydroxyl groups attached. Alkoxy means alkyl groups attached to an oxygen. Hydroxyalkoxy means alkyl groups having ether or ester linkages, hydroxyl groups, substituted hydroxyl groups, carboxyl groups, substituted carboxyl groups or the like. Saccharide includes oxidized, reduced or substituted saccharide. Carboxyamidealkyl means alkyl groups with hydroxyl groups, 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.

For the above-described texaphyrins, hydroxyalkoxy may be alkyl having independently hydroxy substituents and ether branches or may be $C_{(n-x)}H_{((2n+1)-2x)}O_xO_y$ (Polyhydroxy Polyether (c-bound)) or $OC_{(n-x)}H_{((2n+1)-2x)}O_xO_y$ (Polyhydroxy Polyether (o-bound)) where n is a positive integer from 1 to 10, x is zero or a positive integer less than or equal to n, and y is zero or a positive integer less than or equal to ((2n+1)-2x). The hydroxyalkoxy or saccharide may be $C_nH_{((2n+1)-q)}O_yR^a_q$, $OC_nH_{((2n+1)-q)}O_yR^a_q$ or $(CH_2)_nCO_2R^a$ where n is a positive integer from 1 to 10, y is zero or a positive integer less than ((2n+1)-q), q is zero or a positive integer less than or equal to 2n+1, and R^a is independently H, alkyl, hydroxyalkyl, saccharide,

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$C_{(m-w)}H_{((2m+1)-2w)}O_wO_z$ (Polyhydroxy Polether (c-bound)) $O_2CC_{(m-2)}H_{((2m+1)-2w)}O_wO_z$ (Polyhydroxy Polyether (carboxylate bound)) or $N(R)OCC_{(m-w)}H_{((2m+1)-2w)}O_wO_z$ (Polyhydroxy Polyether (amide-bound). In this case, m is a positive integer from 1 to 10, w is zero or a positive integer less than or equal to m, z is zero or a positive integer less than or equal to ((2m+1)-2w), and R is H, alkyl, hydroxyalkyl, or $C_mH_{((2m+1)-r)}O_zR^b_r$ where m is a positive integer from 1 to 10, z is zero or a positive integer less than ((2m+1)-r), r is zero or a positive integer less than or equal to 2m+1, and R^b is independently H, alkyl, hydroxyalkyl, or saccharide.

Carboxyamidealkyl may be alkyl having secondary or tertiary amide linkages or $(CH_2)_nCONHR^a$, $O(CH_2)_nCONHR^a$, $(CH_2)_nCON(R^a)_2$, or $O(CH_2)_nCON(R^a)_2$ where n is a positive integer from 1 to 10, and R^a is independently H, alkyl, hydroxyalkyl, saccharide. $C_{(m-w)}H_{((2m+1)-2w)}O_wO_z$ (Polyhydroxy Polyether (c-bound)), $O_2CC_{(m-w)}H_{((2m+1)-2w)}O_wO_z$ (Polyhydroxy Polyether (carboxylate-bound)), $N(R)OCC_{(m-w)}H_{((2m+1)-2w)}O_wO_z$ (Polyhydroxy Polyether (amide-bound)), or a site-directed molecule or catalytic group. In this case, m is a positive integer from 1 to 10, w is zero or a positive integer less than or equal to m, z is zero or a positive integer less than or equal to ((2m+1)-2w), and R is H, alkyl, hydroxyalkyl, or $C_mH_{((2m+1)-r)}O_zR^b_r$. In this case, m is a positive integer from 1 to 10, z is zero or a positive integer less than ((2m+1)-r), r is zero or a positive integer less than or equal to 2m+1, and R^b is independently H, alkyl, hydroxyalkyl, or saccharide. In a preferred embodiment, R^a is an oligonucleotide.

Carboxyalkyl may be alkyl having a carboxyl substituted ether, an amide substituted ether or a tertiary amide removed from an ether or $C_nH_{((2n+1)-q)}O_yR^c_q$ or $OC_nH_{((2n+1)-q)}O_yR^c_q$ where n is a positive integer from 1 to 10; y is zero or a positive integer less than ((2n+1)-q), q is zero or a positive integer less than or equal to 2n+1, and R^c is $(CH_2)_nCO_2R^d$, $(CH_2)_nCONHR^d$, $(CH_2)_nCON(R^d)_2$ or a site-directed molecule or catalytic group. In this

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case, n is a positive integer from 1 to 10, R^d is independently H, alkyl, hydroxyalkyl, saccharide, $C_{(m-w)}H_{((2m+1)-2w)}O_wO_z$ (Polyhydroxy Polyether (c-bound)), $O_2CC_{(m-w)}H_{((2m+1)-2w)}O_wO_z$ (Polyhydroxy Polyether (carboxylate-bound)) or $N(R)OCC_{(m-w)}H_{((2m+1)-2w)}O_wO_z$ (Polyhydroxy Polyether (amide-bound)). In this case, m is a positive integer from 1 to 10, w is zero or a positive integer less than or equal to m, z is zero or a positive integer less than or equal to ((2m+1)-2w), and R is H, alkyl, hydroxyalkyl, or $C_mH_{((2m+1)-r)}O_zR^b_r$. In this case, m is a positive integer from 1 to 10, z is zero or a positive integer less than ((2m+1)-r), r is zero or a positive integer less than or equal to 2m+1, and R^b is independently H, alkyl, hydroxyalkyl, or saccharide. In a preffered embodiment, R^c is an oligonucleotide.

In an embodiment of the present invention, M may be a divalent metal cation selected from the group consisting of CA^{+2}, Mn^{+2}, Co^{+2}, Ni^{+2}, Zn^{+2}, Cd^{+2}, Hg^{+2}, Fe^{+2}, Sm^{+2} and UO^{+2} or a trivalent metal cation selected from the group consisting of Mn^{+3}, Co^{+3}, Ni^{+3}, Fe^{+3}, Ho^{+3}, Ce^{+3}, Y^{+3}, In^{+3}, Pr^{+3}, Nd^{+3}, Sm^{+3}, Eu^{+3}, Gd^{+3}, Tb^{+3}, Dy^{+3}, Er^{+3}, Tm^{+3}, Yb^{+3}, Lu^{+3}, La^{+3}, and U^{+3}.

In presently preferred texaphyrins, R_1 is hydroxyalkyl and R_2, R_3 and R_4 are alkyl. Alternatively, R_3 may be a site-directed molecule or a couple to a site-directed molecule, preferably an oligonucleotide or a couple to an oligonucleotide. In a further preferred texaphyrin, R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3 and R_5 and R_6 are $OCH_2CH_2CH_2OH$. Alternatively, R_5 is a site-directed molecule or a couple thereto, preferably an oligonucleotide or a couple thereto, more preferably $O(CH_2)_nCO$-oligonucleotide where n is 1-7 and preferably 1-3; and R_6 is H. 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, R_5 is $O(CH_2CH_2O)_2CH_2CH_2OCH_3$ and R_6 is a site-directed

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molecule or a couple thereto, preferably an oligonucleotide or a couple thereto, more preferably $O(CH_2)_nCO$-oligonucleotide where n is 1-7 and preferably 1-3. In other presently preferred embodiments, R_1-R_6 are as in Table 1 for texaphyrins A1-A29.

In a further embodiment of the present invention, at least one of R_1, R_2, R_3, R_4, R_5 and R_6 is a site-directed molecule or is a couple to a site-directed molecule. In a more preferred embodiment, the site-directed molecule is an oligonucleotide or is a couple to an oligonucleotide and most preferably, the oligonucleotide is a deoxyribonucleotide and the phosphate ester to be cleaved is RNA. The oligonucleotide has complementary binding affinity for the RNA in a region proximal to the phosphate ester bond being hydrolyzed. The oligonucleotide may have complementary binding affinity for viral RNA, in particular, retroviral RNA, or for bacterial ribosomal RNA, thereby cleaving the viral RNA or bacterial RNA and killing the organism. When the phosphate ester is RNA, the metal cation is preferably a lanthanide metal cation, more preferably, Eu(III) or Dy(III).

The oligonucleotide may be a deoxyribonucleotide and have complementary binding affinity for oncogenes. The oligonucleotide, antibody, hormone, sapphyrin or rubyrin may have binding specificity for localization to a treatment site and the biological receptor may be localized to a treatment site.

Another embodiment of the present invention is a method of phosphate ester hydrolysis comprising the steps of i) obtaining an aqueous phosphate ester solution and ii) incubating the solution with a texaphyrin metal complex linked to a sapphyrin. Sapphyrins have binding specificity for phosphate esters as described in Example

12. The incubation is under conditions and for a time sufficient to hydrolyze the phosphate ester. Sapphyrin compounds are disclosed in U.S. Patents 5,159,065 and 5,120,411, incorporated by reference herein.

Another embodiment of the present invention is a method for targeted intracellular mRNA hydrolysis. The method comprises the introduction into a cell of a texaphyrin metal complex coupled to an oligonucleotide having complementary binding affinity for a targeted RNA, whereby hydrolysis of the targeted mRNA is catalyzed by the texaphyrin metal complex. The mRNA may be transcribed from an oncogene or it may encode a growth factor, for example. The mRNA may be a normal mRNA which needs to be destroyed, for example, due to improper timing of expression.

A method for inhibiting the expression of a gene in an animal comprising the administration to the animal of a texaphyrin metal complex-conjugate is a further embodiment of the present invention. The oligonucleotide may have complementary binding affinity for messenger RNA transcribed from said gene or may be complementary to either strand of the DNA surrounding the gene or encoding the gene. The gene may be an oncogene or it may encode a growth factor, for example. A further embodiment of the present invention is a method for inhibiting the expression of a gene in a particular tissue of an animal comprising administering to the animal a texaphyrin metal complex-conjugate having specificity for the tissue. The texaphyrin metal complex-conjugate may have appended an oligonucleotide complementary to the target gene and a further appended tissue-specific molecule like estradiol, for example, or an antibody directed for said tissue or a peptide having affinity for a biological receptor on said tissue.

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

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1C schematically summarize the synthesis of B2T2TXP($\mathbf{1}_H$, $\mathbf{1}_I$) and lanthanide metal complexes of B2T2, $\mathbf{1}_J$-$\mathbf{1}_W$.

Figures 2A-2B schematically summarize the synthesis of an oligonucleotide conjugate of a texaphyrin metal complex, $\mathbf{2}_H$.

Figures 3A-3C demonstrate the covalent coupling of texaphyrin metal complexes with amine, thiol or hydroxy linked oligonucleotides to form preferred compounds of the present invention.

Figures 4A-4D demonstrate the synthesis of diformyl monoacid tripyrrane 4_H and oligonucleotide conjugate 4_J.

Figure 5 shows the recombinant plasmid from which ^{32}P-labelled 2000 base pair RNA transcripts were made for the RNA hydrolysis experiments of Example 6. R1 = *Eco*RI, MCS = multi-cloning sequence.

Figure 6 shows the autoradiogram of control experiments where RNA transcripts were incubated with europium acetate and with EuOAc and EDTA. Lane 1, control , no EuOAc; lane 2, control with EDTA, no EuOAc; lanes 3-7, EuOAc, 100 μM, 50 μM, 25 μM, 10 μM, and 5 μM; lane 8, EuOAc, 100 μM and EDTA 500 μM; lane 9, EuOAc, 100 μM and EDTA, 300 μM; lane 10, EuOAc, 100 μM and EDTA, 100 μM; lane 11, EuOAc, 100 μM and EDTA, 10 μM; lane 12, MW std; lane 13, control, no EuOAc; lane 14, MW std.

Figure 7 shows the autoradiogram of experiments where RNA transcripts were incubated with europium B2T2 txph. Lane 1, control, no EuB2T2 txph; lane 2, control with EDTA; lanes 3-7, EuB2T2 txph, 100 μM, 50 μM, 25 μM, 10 μM and 5 μM; lanes 8-10, EuB2T2 txph, 100 μM and EDTA at 500 μM, 300 μM and 100 μM; lane 11, M.W. std; lane 12, control, no EuB2T2 txph; lane 13, EuB2T2 txph, 100 μM and EDTA, 10 μM.

Figure 8 shows presently preferred texaphyrin-europium complexes of the present invention.

Figure 9 shows europium (III) texaphyrin (EuTx) DNA conjugates of Example 7, dysprosium (III) texaphyrin (DyTx) conjugates of Example 8, oligonucleotides conjugated at the 3′ end to texaphyrin and dual conjugates, i.e., oligonucleotides having a texaphyrin conjugated to the 5′ and to the 3′ end. Oligonucleotides are written in a 5′ -- 3′ direction.

Figure 10 shows a schematic representation of cleavage of an RNA 31-mer by EuTx-DNA conjugate and of an RNA 36-mer by Dy-Tx-DNA conjugate; the arrows show sites of metal-catalyzed hydrolysis.

Figures 11A and 11B show cleavage of synthetic RNA 30-mer $\mathbf{10}_B$ by EuTx-DNA conjugates or free europium complex $\mathbf{8}_B$. Autoradiograph of a 20% high resolution denaturing polyacrylamide gel labelled with ^{32}P at the 3′ end. Ca. 1.5 X 10^5 cpm of substrate was incubated for ca. 24h at 37°C in a total volume of 20 μL of buffer containing 50 mM HEPES, pH 7.5, 100 mM NaCl, 25 μM EDTA and 5 μg/mL calf thymus DNA. Key: lane 1, no DNA control; lane 2, control with unmodified oligodeoxynucleotide $\mathbf{9}_E$; lane 3, 2.5μM $\mathbf{8}_B$; lane 4, 25 μM $\mathbf{8}_B$; lanes 5-7, $\mathbf{9}_E$ and 250 nM, 2.5 μM, and 25 μM $\mathbf{8}_B$, respectively; lane 8, $\mathbf{9}_A$; lane 9, $\mathbf{9}_C$; lane 10, $\mathbf{9}_B$; lanes 11-14, $\mathbf{9}_D$ at 2.5 nM, 25 nM, 250 nM, and 2.5 μM, respectively. All other oligonucleotides were at 2.5 μM final concentration. Nucleotide sequence analysis was determined by partial digestion with base-specific ribonucleases: T1(G); U2 (A>G); Phy M (U+A); B, cereus (U+C); HCO_3 (alkaline hydrolysis); PNK (polynucleotide kinase-treated HCO_3 lane).

Figure 12 shows cleavage of 5′ end labelled RNA 30-mer $\mathbf{10}_B$ by EuTx-DNA conjugate $\mathbf{10}_A$ ($\mathbf{9}_D$). Labelled substrate was incubated with 2.5 μM EuTx-DNA conjugate for 24h at 37°C (lanes 3-6) or 25°C (lanes 7-14) in buffers containing 100 mM NaCl. Key: lane 1, non-incubated RNA control; lane 2, ribonuclease T1 (G) reaction; lanes 3, 8, 50 mM HEPES, pH 7.5, 25 μM EDTA; lanes 4, 9, 50 mM HEPES, pH 7.0, 25 μM EDTA; lane 5, 10:50 mM TrisAcetate pH 7.5, 25 μM EDTA; lanes 6,11,14, 50 mM TrisAcetate pH 7.0, 25 μM EDTA; lane 7, 50 mM HEPES, pH 7.5; lanes 12, 13, 50 mM TrisAcetate, pH 7.0;

lane 13, 5 μM Fe(II), 4mM DTT, 5 μM non-modified oligo 9_E; lane 14, no conjugate control. EuTx: conjugate-derived cleavage; Bkgd: background autocleavage.

Figure 13 shows cleavage of 5′- end labelled RNA 30-mer 10_B by EuTx-DNA conjugate 10_A (9_D). Labelled substrate was incubated for 25h at 37°C in 50 μm HEPES (or TrisAcetate, lane 6) buffer containing 100 mM NaCl, 25 μM EDTA, and 5 μg/mL calf thymus DNA. Key: lane 1, no DNA control; lane 2, non-modified oligodeoxynucleotide 9_E (2.5 μM); lane 3, 9_B (2.5 μM); lane 4, 9_C (2.5 μM); lanes 5-9, 9_D (10_A) (250 nM). EuTx: conjugate-derived cleavage; Bkgd: background autocleavage.

Figure 14 shows the hydrolysis of cUMP to uridine in the presence of EuTXP.

Figure 15 shows the formation of amide linked derivatives of texaphyrin: R = sapphyrin, texaphyrin, porphyrin or a catalytic group; $(NH_2)_m$ represents monoamines $NH_2(CH_2)_x$, x=0-12; diamines NH_2-$(CH_2)_x$-NH_2, x=2-12; triamines $(NH_2(CH_2)_x)_3N$, x=2-6; or tetramines $(NH_2(CH_2)_x)_2N(CH_2)_yN((CH_2)_xNH_2)_2$, x=2-4, y=2-6.

Figure 16 shows the synthesis of a texaphyrin-sapphyrin conjugate, R may be H, OH, alkyl, hydroxyalkyl or alkoxy, M = Ln and n = 1-5.

Figure 17 shows a dimer or conjugate of texaphyrins. The metals may be the same or different.

Figures 18A and 18B show examples of the oligonucleotide conjugate having a one base deletion and, therefore, causing the substrate to have a one-base loop with enhanced susceptibility to hydrolysis.

Figures 19A-19B schematically summarize the synthesis of a dysprosium texaphyrin having an imidazole catalytic group.

Figures 20A-20B show europium texaphyrins having a histanine group attached (20A) and an arginine methyl ester group attached (20B).

Figure 21 shows the use of a set of texaphyrin metal complex-conjugates targeted for the same hydrolysis site on the RNA template.

Figure 22 shows cleavage of 5′-end labelled RNA 30-mer $\mathbf{10}_B$ (lanes 1-9) or 36-mer $\mathbf{10}_D$ (lanes 12-18) by EuTx-conjugates $\mathbf{9}_B$ and $\mathbf{9}_D$ or DyTx-conjugates $\mathbf{9}_I$ and $\mathbf{9}_H$. Autoradiograph of a 20% denaturing polyacrylamide gel. Labelled substrate was incubated with or without 250nM conjugate for 24h at 37°C in buffer containing 100mM NaCl, 50mM HEPES pH 7.5, 25μM EDTA. Key: lanes 1, 12, no conjugate control; lanes 2, 6, 13, $\mathbf{9}_B$; lanes 3, 7, 14, $\mathbf{9}_D$; lanes 4, 8, 15, $\mathbf{9}_I$; lanes 5, 9, 16, $\mathbf{9}_H$; lanes 10, 17, HCO_3 sequencing; lanes 11, 18, base-specific ribonuclease U2 (A) sequencing. Reactions corresponding to lanes 1-5 were conducted under inert (argon) atmosphere, while reactions corresponding to lanes 12-16 were conducted under oxygen. Lnlx, conjugate-derived cleavage; Bkgd, background autocleavage.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention involves the use of texaphyrin metal complexes, in particular, water soluble texaphyrin metal complexes for the hydrolytic cleavage of ester bonds. More particularly, the invention involves the cleavage of phosphate ester bonds of a diester, a monoester and an RNA substrate using a lanthanide metal complex of a hydroxylated texaphyrin. Although a survey

of lanthanide (III) texaphyrin complexes indicates that all of the metal complexes examined are capable of hydrolytically cleaving RNA, complexes of europium (III) and dysprosium (III) cleave RNA faster than other lanthanides of those tested thus far.

An individual skilled in the art of organic synthesis in light of the present disclosure is able to prepare a large variety of lanthanide texaphyrins, all of which are expected to hydrolytically cleave phosphate esters, an important biological species. Potential particular applications for this process include the specific cleavage and possible subsequent recombination of RNA, destruction of viral RNA, digestion of cell membrane components such as phosphatidyl cholines and sphingomyelin, disruption of the transfer of free energy in cells by hydrolyzing ATP, ADP, NADH, NADPH, FAD or $FADH_2$, treatment of liver diseases by preventing the formation of glycogen, regulation of hormones by hydrolysis of cAMP, hydrolysis of mutagenic and carcinogenic di- and trialkyl phosphates commonly used as solvents, and the detoxification of phosphate ester nerve gases and insecticides by hydrolysis of phosphate ester bonds.

Texaphyrin compounds and methods for making are described in U.S. Patents 4,935,498, 5,162,509, 5,252,720, 5,272,142 and 5,256,399, each of which is incorporated by reference herein.

Generally, the introduction of hydroxy substituents on the B (benzene ring) portion of the texaphyrin molecule is accomplished by their attachment to phenylenediamine in the 4 and 5 positions of the phenyl subunit of the molecule or they may be added in a synthetic step following the condensation step that forms the macrocycle metal complex as described in the above-

named patents. The introduction of hydroxy substituents on the T (tripyrrole or tripyrrane) portion of the molecule is accomplished by appropriate functionalization of the alkyl substituents in the 3 and/or 4 positions of the pyrrole rings at a synthetic step prior to condensation with the substituted phenylenediamine. Standard deprotection methodology such as ester hydrolysis may be used to unmask free hydroxyl substituents. Alternatively, they may be prepared as the result of ester reduction. These derivatives exhibit significant solubility in aqueous media, up to 1 mM or better, yet they retain affinity for lipid rich regions which allows them to be useful in biological environments.

Divalent and trivalent metal complexes of texaphyrins are by convention shown with a formal charge of N^+, where N=1 or 2, respectively. 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.

Exemplary texaphyrins of the present invention are listed in Table 1.

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

TXP	R_1	R_2	R_3	R_4	R_5	R_6
A1	$CH_2(CH_2)_3OH$	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-directed molecule texaphyrin-to-ether-hydrocarbon-amide-linker n=1-7	"
A4	"	"	"	"	$O(CH_2)_nCON$-linker-site-directed molecule texaphyrin-to-ether-hydrocarbon-amide-linker	H
A5	"	"	"	"	OCH_2CO-poly-L-lysine	"
A6	"	"	"	"	OCH_2CO-hormome	"
A7	"	"	"	"	$O(CH_2CH_2O)_3CH_3$	"
A8	"	"	"	"	OCH_2CON-linker-site-directed molecule texaphyrin-to-ether-hydrocarbon-amide-linker	$O(CH_2CH_2O)_3CH_3$
A9	"	"	"	"	OCH_2CO-hormone	"
A10	"	"	"	"	OCH_2CON-oligo texaphyrin-ether-methylene-amide-oligonucleotide	$O(CH_2CH_2O)_3CH_2$-CH_2-N-imidazole texaphyrin-polyetheramide-imidazole

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TABLE 1. - CONTINUED

TXP	R_1	R_2	R_3	R_4	R_5	R_6
A11	"	"	"	"	$O(CH_2CH_2O)_{120}CH_3$	"
A12	"	"	"	"	saccharide	H
A13	"	"	"	"	$OCH_2CON(CH_2CH_2OH)_2$	"
A14	"	"	"	"	$CH_2CON(CH_3)CH_2$-$(CHOH)_4CH_2OH$	"
A15	"	COOH	COOH	"	"	"
A16	"	$COOCH_2CH_3$	$COOCH_2CH_3$	"	"	"
A17	$CH_2CH_2CON(CH_2CH_2OH)_2$	CH_2CH_3	CH_2CH_3	"	"	"
A18	$CH_2CH_2ON(CH_3)CH_2$-$(CHOH)_4CH_2OH$	"	"	"	OCH_3	OCH_3
A19	CH_2CH_3	"	"	"	OCH_2CO_2-glucosamine	H
A20	$CH_2(CH_2)_2OH$	"	"	"	$O(CH_2)_nCOOH$, n=1-7	"
A21	"	"	"	"	$(CH_2)_n$-CON-linker-site-directed molecule, n=1-7	"

TABLE 1. - CONTINUED

TXP	R_1	R_2	R_3	R_4	R_5	R_6
A22	"	"	"	"	$YCOCH_2$-linker-site-directed molecule Y=NH,O	"
A23	CH_2CH	CH	CH_2CH_2COOH	"	$O(CH_2)_2CH_2OH$	$O(CH_2)_2CH_2OH$
A24	"	"	CH_2CH_2CON-oligo texaphyrin-ethylene-amide-oligonucleotide	"	"	"
A25	$CH_2(CH_2)_3OH$	CH_2CH	CH_2CH_2	"	OCH_2COOH	$O(CH_2CH_2O)CH_2$-CH_2-N-imidazole
A26	"	"	"	"	$O(CH_2)CO$-histamine	H
A27	"	"	"	"	$O(CH_2)CO$-arginine methyl ester	"
A28	"	"	"	"	$O(CH_2)_nCO$-amino acid, n=1-7	"
A29	"	"	"	"	$O(CH_2)_nCON$-catalytic group texaphyrin-ether-hydrocarbon-amide-catalytic group, n=1-7	"

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Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Unless mentioned otherwise, the techniques employed herein are standard methodologies well known to one of ordinary skill in the art.

EXAMPLE 1

Synthesis of B2T2 TXP, see Fig. 1A-1C.

2,5-Bis[(5-benzyloxycarbonyl-4-methyl-3-methoxycarbonylethylpyrrol-2-yl)methyl]-3,4-diethylpyrrole. 1_C, *Fig. 1A.* In a 500 mL round bottom flask was placed 250 mL of ethanol from an unopened bottle and this was then purged with dry nitrogen for ten minutes. 3,4-Diethylpyrrole 1_B (1.29 g, 0.01 mol) and 2-acetoxymethyl-5-benzyloxycarbonyl-4-methyl-3-methoxycarbonylethylpyrrole 1_A (7.83 g, 0.02 mol) were added and the mixture heated until all of the pyrroles dissolved. p-Toluenesulfonic acid (65 mg) was added and the reaction temperature maintained at 60° C. The reaction slowly changed color from a clear yellow to a dark red with the product precipitating out of the solution as the reaction progressed. After ten hours the reaction was cooled to room temperature, the volume reduced to one half on a rotary evaporator, and then placed in the freezer for several hours. The product was collected by filtration, washed with a small amount of cold ethanol to afford 4.61 g of an off white fine powder (61%): ^{1}H NMR ($CDCl_3$, 250 MHz): δ 1.14 (6H, t, CH_2CH_3), 2.23 (6H, s, pyrrole-CH_3), 2.31 (4H, t, $CH_2CH_2CO_2CH_3$), 2.50 (4H, q, CH_2CH_3), 2.64 (4H, t, $CH_2CH_2CO_2CH_3$), 3.60 (10H, br s, CH_3CO_2- and (pyrrole)$_2$-CH_2), 4.44 (4H, br s,

$C_6H_5CH_2$), 6.99-7.02 (4H, m, aromatic), 7.22-7.26 (6H, m, aromatic), 8.72 (1H, s, NH), 10.88 (2H, br s, NH); ^{13}C NMR ($CDCl_3$, 250 MHz): δ 10.97, 16.78, 17.71, 19.40, 22.07, 35.09, 51.46, 65.32, 117.37, 119.34, 122.14, 126.58, 126.79, 127.36, 128.19, 133.55, 136.62, 162.35, 173.49; CI MS $(M+H)^+$ 750; HRMS 749.3676 (calc. for $C_{44}H_{51}N_3O_8$: 749.3676).

2,5-Bis[(5-benzyloxycarbonyl-3-(3-hydroxypropyl)-4-methylpyrrol-2yl)methyl]-3,4-diethylpyrrole. 1_D, Fig.1A. 2,5-Bis[(5-benzyloxycarbonyl-4-methyl-3-methoxycarbonylethylpyrrol-2-yl)methyl]-3,4-diethylpyrrole 1_C (5.00 g, 0.007 mol) was placed in a three necked 100 mL round bottom flask and vacuum dried for at least 30 minutes. The flask was equipped with a thermometer, an addition funnel, a nitrogen inlet tube, and a magnetic stir bar. After the tripyrrane was partially dissolved into 10 mL of dry THF, 29 mL of borane (1M BH_3 in THF) was added dropwise with stirring. The reaction became mildly exothermic and was cooled with a cool water bath. The tripyrrane slowly dissolved to form a homogeneous orange solution which turned to a bright fluorescent orange color as the reaction went to completion. After stirring the reaction for one hour at room temperature, the reaction was quenched by adding methanol dropwise until the vigorous effervescence ceased. The solvents were removed under reduced pressure and the resulting white solid redissolved into CH_2Cl_2. The tripyrrane was washed three times with 0.5M HCl (200 mL total), dried over anhydrous K_2CO_3, filtered, and the CH_2Cl_2 removed under reduced pressure until crystals of the tripyrrane just started to form. Hexanes (50 mL) was added and the tripyrrane allowed to crystallize in the freezer for several hours. The product was filtered and again recrystallized from CH_2Cl_2/ethanol. The product was collected by filtration and vacuum dried to yield 3.69 g

of an orangish white solid (76%): mp 172-173° C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.11 (6H, t, CH_2CH_3), 1.57 (4H, p, $CH_2CH_2CH_2OH$), 2.23 (6H, s, pyrrole-CH_3), 2.39-2.49 (8H, m, CH_2CH_3 and $CH_2CH_2CH_2OH$), 3.50 (4H, t, $CH_2CH_2CH_2OH$), 3.66 (4H, s, $(pyrrole)_2$-CH_2), 4.83 (4H, s, C_6H_5-CH_2), 7.17-7.20 (4H, m, aromatic), 7.25-7.30 (6H, m, aromatic), 8.64 (1H, s, NH), 9.92 (2H, s, NH); ^{13}C NMR ($CDCl_3$, 300 MHz): δ 10.97, 16.72, 17.68, 20.00, 22.38, 33.22, 62.01, 65.43, 117.20, 119.75, 120.72, 122.24, 127.23, 127.62, 128.30, 132.95, 136.60, 162.13; FAB MS (M^+) 693.

2,5-Bis[(3-(3-hydroxypropyl)-5-carboxyl-4-methylpyrrol-2-yl) methyl]-3,4-diethylpyrrole 1_E, *Fig. 1B.* 2,5-Bis[(3-(3-hydroxypropyl)-5-benzyloxycarbonyl-4-methylpyrrol-2-yl)methyl]-3,4-diethylpyrrole 1_D (15.0 g, 0.02 mol) was placed in a 1 L round bottom flask and dried *in vacuo* for ca. 30 min. The tripyrrane was dissolved in dry THF (600 mL) with triethylamine (10 drops) and 10% Pd on carbon (600 mg) and the reaction was stirred at room temperature under one atmosphere of H_2. After 15 h, the suspension was filtered through celite to remove the catalyst and the resulting clear solution was concentrated under reduced pressure to yield a light pink solid. This material, obtained in near quantitative yield, was taken on to the next step without further purification.

2,5-Bis[(5-formyl-3-(3-hydroxypropyl)-4-methylpyrrol-2-yl) methyl]-3,4-diethylpyrrole 1_F, *Fig. 1B.* 2,5-Bis[(3-(3-(hydroxypropyl)-5-carboxyl-4-methylpyrrol-2-yl)methyl]-3,4-diethylpyrrole 1_E (10 g, 0.02 mol) was placed in a 250 mL round bottom flask and dried *in vacuo* for ca. 1 h. At room temperature under nitrogen, trifluoroacetic acid (31 mL, 0.40 mol) was added dropwise via syringe. The tripyrrane dissolved with visible evolution of CO_2 to form a homogeneous

yellow solution. The reaction was stirred at room temperature for ca. 15 min, then cooled to -20°C using a dry ice/CCl_4 bath. Freshly distilled triethylorthoformate (31 mL, 0.20 mol, dried over CaH_2) was added via a syringe to produce a dark orange/yellow solution. This mixture was stirred an additional 10 min at -20°C, then the cold bath was removed and 100 mL of distilled water was added dropwise to the solution. The resulting brown suspension was stirred at room temperature for 15 min. The product was collected by filtration, washed several times with water and re-suspended into a 50 mL/100 mL/50 mL (H_2O:EtOH:NH_4OH, v/v) mixture. The yellow/brown suspension was stirred for 1 h, filtered, washed several times with water, and then rinsed with a small amount of cold 95% ethanol. At this point, TLC analysis showed a mixture of tripyrranes. Therefore, the crude dialdehyde tripyrrane and $LiOH \cdot H_2O$ (2.10 g, 0.05 mol) were added to 400 mL of degassed 95% MeOH and the suspension heated to reflux under a N_2 atmosphere. The reaction became homogeneous when heated and after ca. 1 h, it was slowly cooled to room temperature. The reaction mixture was concentrated under reduced pressure to 75 mL and the resulting slurry placed in the freezer for several hours. The product was filtered and then purified by forming a slurry with 400 mL of methanol and 50 mL of water and heating close to boiling. The suspension was slowly cooled to room temperature, reduced to 150 mL under reduced pressure, and placed in the freezer for several hours. The purified dialdehyde tripyrrane was filtered, rinsed with water and dried *in vacuo* for 24 h to yield 7.65 g (80%) of a light tan powder. For $\mathbf{1}_F$: mp 164-166°C; ^1H NMR (CD_3OD): δ 0.96 (t, 6H, CH_2CH_3), 1.49 (p, 4H, $CH_2CH_2CH_2OH$), 2.25 (s, 6H, pyrr-CH_3), 2.32-2.43 (m, 8H, CH_2CH_3 and $CH_2CH_2CH_2OH$), 3.46 (t, 4H, $CH_2CH_2CH_2OH$), 3.85 (s, 4H,

(pyrr)$_2$-CH_2), 9.34 (s, 2H, CHO); CI MS, M^+: m/e 480; HR MS, M^+: m/e 481.2942 (calcd. for $C_{28}H_{39}N_3O_4$, 481.2941).

4,5-Diethyl-10,23-dimethyl-9,24-bis(3-hydroxypropyl)-16,17-bis(3-hydroxypropyloxy)-13,20,25,26,27-pentaazapentacyclo [20.2.1.1^{3,6}.1^{8,11}.0^{14,19}] heptacosa-3,5,8,10,12,14,16,18,20,22,24-undecaene (1_H, Fig. 1B). 2,5-Bis[(5-formyl-3-(3-hydroxypropyl)-4-methylpyrrol-2-yl)methyl]-3,4-diethylpyrrole 1_F (1.00 g, 0.002 mol) and 1,2-diamino-4,5-bis(3-hydroxypropyloxy)benzene 1_G (0.52 g, 0.002 mol) were placed in a 2 L round bottom flask with 1000 mL of toluene and 200 mL of methanol. The solvents were purged with nitrogen prior to use. Concentrated HCl (0.5 mL) was added and the reaction heated to reflux under nitrogen. The reaction went from a clear suspension of starting materials to a dark red homogeneous solution as the reaction proceeded. After 5 h the reaction was cooled to room temperature and the solvents removed under reduced pressure until the product precipitated out of solution. The remainder of the solvent was decanted off and the macrocycle dried *in vacuo*. The dark red product was recrystallized from methanol/diethylether and yielded 1.4-1.5g (90-100%). For 1_H: mp 190°C dec; ^{1}H NMR (CD_3OD): δ 1.11 (t, 6H, CH_2CH_3), 1.76 (p, 4H, pyrr-$CH_2CH_2CH_2OH$), 2.03 (p, 4H, $OCH_2CH_2CH_2OH$), 2.36 (s, 6H, pyrr-CH_3), 2.46 (q, 4H, CH_2CH_3), 2.64 (t, 4H, pyrr-$CH_2CH_2CH_2OH$), 3.61 (t, 4H, pyrr-$CH_2CH_2CH_2OH$), 3.77 (t, 4H, $OCH_2CH_2CH_2OH$), 4.10 (s, 4H, (pyrr)$_2$-CH_2), 4.22 (t, 4H, $OCH_2CH_2CH_2OH$), 7.41 (s, 2H, PhH), 8.30 (s, 2H, HC=N); ^{13}C NMR (CD_3OD): δ 10.0, 17.2, 18.6, 20.9, 24.5, 33.2, 33.5, 59.6, 61.9, 67.8, 107.1, 120.7 123.8, 125.0, 125.8, 128.7, 144.8, 145.0, 150.7, 154.6; UV/vis (CH_3OH) [λ_{max}, nm] 365; FAB MS, $(M+H)^+$: m/e 703; HRMS, M^+: m/e 701.4120 (calcd. for $C_{40}H_{55}N_5O_6$: 701.4152). Anal. calcd. [$C_{40}H_{55}N_5O_6$](HCl)(CH_3OH): C,

63.92; H, 7.85; N, 9.09; Cl, 4.60. Found: C, 64.17; H, 7.68; N, 9.39; Cl, 4.70.

General procedure for the synthesis of water soluble lanthanide (III) 4,5-diethyl-10,23-dimethyl-9,24-bis(3-hydroxypropyl)-16,17-(3-hydroxypropyloxy)-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),12,14,16,18,20,22(25),23-tridecaene, B2T2TXP (1_I, Fig. 1C). One equivalent of the hydrochloride salt of the macrocycle, 4,5-diethyl-10,23-dimethyl-9,24-bis(3-hydroxypropyl)-16,17-bis(3-hydroxypropyloxy)-13,20,25,26,27-pentaazapentacyclo [20.2.1.1^{3,6}.1^{8,11}.0^{14,19}]heptacosa-3,5,8,10,12,14,16,18,20,22,24-undecaene 1_H, 1.5 equivalents of the $Ln(NO_3)_3 \cdot XH_2O$ metal salt, 2-3 equivalents of tetrabutylammonium nitrate ($TBANO_3$) and triethylamine (ca. 1 mL) were mixed together in methanol and heated to reflux under air. After completion of the reaction (as judged by the UV/vis spectrum of the reaction mixture), the deep green solution was cooled to room temperature, the solvent removed under reduced pressure and the crude complex dried *in vacuo* for several hours. A solution of dichloromethane/methanol (99:1 v/v) was added to the crude complex and the suspension was sonicated a few min. The green suspension was filtered in order to remove red/brown colored impurities in the filtrate (incomplete oxidation products and excess triethylamine). The resulting deep green solid was first dissolved in methanol and then chloroform was added to reduce the polarity of the mixture (1:2 v/v). This solution was filtered through celite and loaded on a (pre-treated/pre-washed 1M $NaNO_3$) neutral alumina column (10 cm). The column was first eluted with a 1:10 (v/v) methanol/chloroform solution by gravity to remove a reddish brown impurity. The metal complex was then obtained by eluting the column with chloroform containing increasing amounts of methanol (20-50%). The purified

lanthanide (III) texaphyrin complex was recrystallized by dissolving the complex in methanol/chloroform and carefully layering the dark green solution with a small amount of methanol, then with diethylether. The layered solution was kept at room temperature in the dark for a few days. Some of the lanthanide (III) texaphyrin complexes formed single crystals by this method. Other complexes were recrystallized twice for analytically pure measurements and characterizations.

Lanthanum(III) complex, 1_J. The hydrochloride salt of macrocycle $\mathbf{1}_H$ (300 mg, 0.407 mmol), $La(NO_3)_3 \cdot 6H_2O$ (350 mg, 0.814 mmol), $TBANO_3$ (305 mg, 1.0 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 10 h. After workup using the general procedure outlined above, 132 mg of the complex was obtained (34%). For $\mathbf{1}_J$: ^1H NMR (CD_3OD): δ 1.68 (t, 6H, CH_2CH_3), 2.22-2.30 (m, 4H, pyrr-$CH_2CH_2CH_2OH$ and 4H, $OCH_2CH_2CH_2OH$), 3.20 (s, 6H, pyrr-CH_3), 3.72-3.78 (m, 4H, CH_2CH_3 and 4H, pyrr-$CH_2CH_2CH_2OH$ and 4H, pyrr-$CH_2CH_2CH_2OH$), 3.94 (t, 4H, $OCH_2CH_2CH_2OH$), 4.78 (m, 4H, $OCH_2CH_2CH_2OH$), 9.37 (s, 2H, ArH), 9.87 (s, 2H, $(pyrr)_2C=CH$), 11.71 (s, 2H, HC=N); ^{13}C NMR (CD_3OD): δ 11.0, 18.9, 20.3, 23.0, 33.3, 36.3, 59.7, 62.2, 68.1, 101.5, 118.5, 137.1, 140.3, 144.6, 147.5, 148.2, 152.9, 154.9, 159.4; UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 355 (4.34), 417 (4.73), 476 (5.06), 685.5 (4.08), 746 (4.59); FAB MS, M^+: m/e 835; HRMS, $(M+H)^+$: m/e 836.2919 (calcd. for $C_{40}H_{51}N_5O_6{}^{139}La$, 836.2903). Anal. calcd. for $[C_{40}H_{50}N_5O_6La](NO_3)_2(H_2O)_2$: C, 48.23; H, 5.47; N, 9.85. Found: C, 47.93; H, 5.41; N, 9.77.

Cerium(III) complex 1_K. The hydrochloride salt of macrocycle $\mathbf{1}_H$ (300 mg, 0.407 mmol), $Ce(NO_3)_3 \cdot 6H_2O$ (265 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.0 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux

under air for 7 h. It is important to note that initially the reaction mixture formed a suspension, however, as the product formed the solution became homogeneous. After workup using the general procedure outlined above, 143 mg of dark green crystals were obtained (37%). This material was suitable for X-ray diffraction analysis. For $\mathbf{1}_K$: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 349.5 (4.34), 416.5 (4.70) 476.5 (5.05), 684 (4.07), 741 (4.56); FAB MS, M^+: m/e 836; HRMS, $(M+H)^+$: m/e 836.2807 (calcd. for $C_{40}H_{51}N_5O_6{}^{140}Ce$, 836.2816). Anal. calcd. for $[C_{40}H_{50}N_5O_6Ce](NO_3)_2(H_2O)_3$: C, 47.32; H, 5.56; N, 9.66. Found: C, 46.98; H, 5.22; N, 9.63.

Praseodymium(III) complex 1_L. The hydrochloride salt of macrocycle $\mathbf{1}_H$ (300 mg, 0.407 mmol), $Pr(NO_3)_3 \cdot 5H_2O$ (255 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.0 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 10 h. After workup using the general procedure outlined above, 200 mg of the complex was obtained (51%). For $\mathbf{1}_L$: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 352 (4.32), 416.5 (4.69), 476.5 (5.04), 689 (4.07), 744.5 (4.57); FAB MS, M^+: m/e 838; HRMS, M^+: m/e 837.2823 (calcd. for $C_{40}H_{50}N_5O_6{}^{141}Pr$, 837.2838). Anal. calcd. for $[C_{40}H_{50}N_5O_6Pr](NO_3)_2(CH_3OH)(H_2O)$: C, 48.65; H, 5.58; N, 9.69. Found: C, 48.75; H, 5.52; N, 9.71.

Neodymium(III) complex 1_M. The hydrochloride salt macrocycle $\mathbf{1}_H$ (300 mg, 0.407 mmol), $Nd(NO_3)_3 \cdot 6H_2O$(267 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.0 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 12 h. After workup using the general procedure outlined above, 125 mg of the complex was obtained (32%). For $\mathbf{1}_M$: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 353.5 (4.32), 416 (4.68), 476 (5.05), 688 (4.06), 742.5 (4.56); FAB MS, M^+: m/e 839; HRMS, M^+: m/e 838.2828 (calcd. for $C_{40}H_{50}N_5O_6{}^{142}Nd$, 838.2838). Anal. calcd. for

$[C_{40}H_{50}N_5O_6Nd](NO_3)_2(CH_3OH)$: C, 49.48; H, 5.47; N, 9.86. Found: C, 49.23; H, 5.49; N, 9.83.

Samarium(III) complex 1_N. The hydrochloride salt of macrocycle 1_H (300 mg, 0.407 mmol), $Sm(NO_3)_3 \cdot 5H_2O$ (270 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.0 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 7 h. After workup using the general procedure outlined above, 183 mg of a dark green crystalline solid was obtained (46%). This material has the potential for X-ray diffraction. For 1_N: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 354.5 (4.36), 415.5 (4.71), 475.5 (5.09), 682 (4.09), 741 (4.61); FAB MS, M^+: m/e 849; HRMS, M^+: m/e 848.2957 (calcd. for $C_{40}H_{50}N_5O_6{}^{152}Sm$, 848.2959). Anal. calcd. for $[C_{40}H_{50}N_5O_6Sm](NO_3)_2(CH_3OH)$: C, 48.99; H, 5.42; N, 9.76. Found: C, 48.79; H, 5.64; N, 9.43.

Europium(III) complex 1_O. The hydrochloride salt of macrocycle 1_H (400 mg, 0.543 mmol), $Eu(NO_3)_3 \cdot 5H_2O$ (290 mg, 0.65 mmol), $TBANO_3$ (500 mg, 1.64 mmol) and triethylamine (ca 1 mL) in 350 mL methanol were heated to reflux under air for 16 h. After workup using the general procedure outlined above, 255 mg of a dark green crystalline solid was obtained (48%). This material was suitable for X-ray diffraction analysis. For 1_O: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 414 (4.72), 475.5 (5.10), 678 (4.08), 739.5 (4.63); FAB MS, $(M+H)^+$: m/e 850; HRMS, M^+: m/e 849.2961 (calcd. for $C_{40}H_{50}N_5O_6{}^{153}Eu$, 849.2974). Anal. calcd. for $[C_{40}H_{50}N_5O_6Eu](NO_3)_2(H_2O)$: C, 47.56; H, 5.39; N, 9.71. Found: C, 47.47; H, 5.45; N, 9.64.

Gadolinium (III) complex 1_P. The hydrochloride salt of macrocycle 1_H (750 mg, 1 mmol), $Gd(NO_3)_3 \cdot 5H_2O$ (660 mg, 1.5 mmol), $TBANO_3$ (930 mg 3.0 mmol) and triethylamine

(ca. 1 mL) in 600 mL methanol were heated to reflux under air for 12 h. After workup using the procedure outlined above, the dark green complex was recrystallized from chloroform/methanol/diethylether to yield 700 mg (72%) of a deep green crystalline solid. X-ray quality single crystals were obtained by dissolving the complex in methanol/chloroform and carefully layering the dark green solution with a small amount of methanol, then with diethylether. The layered solution was kept at room temperature in the dark for a few days. For $\mathbf{1}_P$: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 358 (4.33), 416 (4.72), 478 (5.12), 678 (4.03), 737.5 (4.64); [(H_2O) λ_{max}, nm (log ϵ)]: 347 (4.43), 419 (4.75), 469 (5.08), 740 (4.60). IR (KBr, cm^{-1}, major peaks): υ 3299 (OH), 1647 (C=N), 1601 (C=N), 1507, 1456, 1437, 1385 (NO_3-), 1290, 1221, 1098, 1082. FAB MS, M^+: m/e 854; HRMS, M^+: m/e 854.2989 (calcd. for $C_{40}H_{50}N_5O_6{}^{158}Gd$, 854.300. Anal. calcd. for $[C_{40}H_{50}N_5O_6Gd](NO_3)_2(CH_3OH)(H_2O)$: C, 47.85; H, 5.49; N, 9.53. Found: C, 47.62; H, 5.37; N, 9.54.

NOTE: If the alumina is not pre-treated with a $NaNO_3$ wash, the Gd(III) will not have two nitrate counter anions, instead it will have one nitrate and one chloride counter anion: Anal. calcd. for $[C_{40}H_{50}N_5O_6Gd](NO_3)Cl(H_2O)_2$: C, 48.65; H, 5.51; N, 8.51; Cl, 3.59. Found: C, 48.21; H, 5.58; N, 8.34; Cl, 3.62.

Terbium(III) complex $\mathbf{1}_Q$. The hydrochloride salt of macrocycle $\mathbf{1}_H$ (300 mg, 0.407 mmol), $Tb(NO_3)_3 \cdot 6H_2O$ (276 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.64 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 12 h. After workup using the general procedure outlined above, 152 mg of the complex was obtained (38%). For $\mathbf{1}_Q$: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 353 (4.35), 414 (4.71), 474.5 (5.09), 680 (4.08), 737 (4.62); FAB MS, M^+: m/e 856; HRMS, M^+: m/e 855.3017 (calcd. for $C_{40}H_{50}N_5O_6{}^{159}Tb$, 855.3015). Anal. calcd. for

$[C_{40}H_{50}N_5O_6Tb](NO_3)_2(CH_3OH)(H_2O)$: C, 47.80; H, 5.48; N, 9.52. Found: C, 48.11; H, 5.28; N, 9.75.

Dysprosium(III) complex 1_R. The hydrochloride salt of macrocycle 1_H (300 mg, 0.407 mmol), $Dy(NO_3)_3 \cdot 5H_2O$ (266 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.64 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 5 h. After workup using the general procedure outlined above, 250 mg of the complex was obtained (62%). For 1_R: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 354 (4.32), 414 (4.68), 475 (5.07), 677.5 (4.03), 735.5 (4.60); FAB MS, $(M+H)^+$: m/e 861; HRMS, M^+: m/e 860.3048 (calcd. for $C_{40}H_{50}N_5O_6{}^{164}Dy$, 860.3053). Anal. calcd. for $[C_{40}H_{50}N_5O_6Dy](NO_3)_2(H_2O)$: C, 47.89; H, 5.23; N, 9.78. Found: C, 47.97; H, 5.22; N, 9.72.

Holmium(III) complex 1_S. The hydrochloride salt of macrocycle 1_H (300 mg, 0.407 mmol), $Ho(NO_3)_3 \cdot 5H_2O$ (269 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.64 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 12 h. After workup using the general procedure outlined above, 220 mg of the complex was obtained (55%). For 1_S: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 354 (4.35), 414 (4.72), 475.5 (5.12), 677 (4.08), 734 (4.65); FAB MS, M^+: m/e 862; HRMS, M^+: m/e 861.3044 (calcd. for $C_{40}H_{50}N_5O_6{}^{165}Ho$, 861.3064). Anal. calcd. for $[C_{40}H_{50}N_5O_6Ho](NO_3)_2(CH_3OH)(H_2O)$: C, 47.52; H, 5.45; N. 9.47. Found: C, 47.55; H, 5.26; N, 9.30.

Erbium(III) complex 1_T. The hydrochloride salt of macrocycle 1_H (300 mg, 0.407 mmol), $Er(NO_3)_3 \cdot 5H_2O$ (270 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.64 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 12 h. After workup using the general procedure outlined above, 143 mg of the complex was

obtained (36%). For 1_T: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 355.5 (4.36), 414.5 (4.72), 477 (5.13), 672 (4.08), 732 (4.66); FAB MS, M^+: m/e 863; HRMS, M^+: m/e 865.3110 (calcd. for $C_{40}H_{50}N_5O_6{}^{166}Er$, 862.3064). Anal. calcd. for $[C_{40}H_{50}N_5O_6Er](NO_3)_2(CH_3OH)$: C, 48.32; H, 5.34; N, 9.63. Found: C, 48.14; H, 5.14; N, 9.55.

Thulium(III) complex 1_U. The hydrochloride salt of macrocycle 1_H (300 mg, 0.407 mmol), $Tm(NO_3)_3 \cdot 5H_2O$ (274 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.64 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 22 h. After workup using the general procedure outlined above, 150 mg of the complex was obtained (37%). This complex is more difficult to purify due to its lower solubility in methanol/chloroform solutions, which leads to its lower yield. For 1_U: UV/vis: [(MeOH) λ_{max}, nm (log ϵ)]: 355.5 (4.36), 414.5 (4.72), 477 (5.13), 672 (4.08), 732 (4.66); FAB MS, M^+: m/e 866; HRMS, M^+: m/e 865.3110 (calcd. for $C_{40}H_{50}N_5O_6{}^{169}Tm$, 865.3103). Anal. calcd. for $[C_{40}H_{50}N_5O_6Tm](NO_3)_2(H_2O)_2$: C, 46.82; H, 5.31; N, 9.56. Found: C, 46.85; H, 5.23; N, 9.38.

Ytterbium(III) complex 1_V. The hydrochloride salt of macrocycle 1_H (300 mg, 0.407 mmol), $Yb(NO_3)_3 \cdot 5H_2O$ (274 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.64 mmol) and triethylamine (ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 24 h. After workup using the general procedure outlined above, 220 mg of the complex was obtained (54%). For 1_V: FAB MS, M^+: m/e 870; HRMS, M^+: m/e 870.3132 (calcd. for $C_{40}H_{50}N_5O_6{}^{174}Yb$, 870.3149).

Lutetium(III) complex 1_W. The hydrochloride salt of macrocycle 1_H (300 mg, 0.407 mmol), $Lu(NO_3)_3 \cdot H_2O$ (220 mg, 0.611 mmol), $TBANO_3$ (305 mg, 1.64 mmol) and triethylamine

(ca. 0.5 mL) in 350 mL methanol were heated to reflux under air for 24 h. After workup using the general procedure outlined above, 150 mg of the complex was obtained (37%). This complex has very low solubility in methanol/chloroform solution. Almost half of the product remained on the column during purification. For 1_W: FAB MS, M^+: m/e 872; HRMS, M^+: m/e 871.3154 (calcd. for $C_{40}H_{50}N_5O_6{}^{175}Lu$, 871.3169).

Acid-catalyzed Schiff-base condensation between 1_G and 1_F produced the so-called "sp^3" nonaromatic macrocycle 1_H in quantitative yield. Macrocycle 1_H is quite stable, decomposing only slightly over a period of months when stored in freezer. Oxidation and metallation of 1_H in the presence of 1.5 equivalents of lanthanide(III) metal salt, triethylamine, and air in boiling methanol produces a deep green metal complex within 3-24 hours (as judged by UV-Vis) of reaction time. All the lanthanide(III) [La-Lu, except Pm] texaphyrin complexes 1_J-1_W were isolated with unoptimized yields ranging from 34%-75%. Satisfactory spectroscopic and mass spectrometric data were obtained for all new compounds. Single crystals suitable for X-ray diffraction analysis of the Eu(III) and Gd(III) complexes 1_O and 1_P, respectively, were obtained by dissolving each complex in $MeOH/CHCl_3$ and layering with diethyl ether.

EXAMPLE 2

Synthesis of Europium(III) T2B1 TXP-oligo conjugate.

The present example provides for the synthesis of a texaphyrin metal complex-oligonucleotide conjugate useful for site-directed hydrolysis of ester bonds of a complementary nucleic acid (see Figures 2A and 2B).

4-Amino-1-[1-(ethyloxy)acetyl-2-oxy]-3-nitrobenzene 2_B, *n=1*. Potassium carbonate (14.0 g, 101 mmol) and 4-amino-3-nitrophenol 2_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 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 2_B, n=1 (94.1%). TLC: Rf = 0.43, $CHCl_3$.

4-Amino-1-[1-(hydroxy)acetyl-2-oxy]-3-nitrobenzene 2_C, *n=1*. 4-Amino-1-[1-(ethyloxy)acetyl-2-oxy]-3-nitrobenzene 2_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 2_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 2_E, *n=1*. 4-Amino-1-[1-(hydroxy)acetyl-2-oxy]-3-nitrobenzene 2_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'. 2,5-Bis[(3-hydroxypropyl-5-formyl-4-methylpyrrol-2-yl)methyl]-3,4-diethylpyrrole 2_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 2_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).

Europium(III) 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 2_F, *n=1*. 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 2_E, n=1 (500 mg, 753 μmol), europium(III) acetate pentahydrate (334 mg, 797 μmol) and triethylamine (1.33 mL, 8.0 mmol) in 150 mL of methanol were 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 (200 mL) was added, and the suspension was stirred on a rotary evaporator for 2 h. The suspension was filtered and the precipitate was 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 2_F, n=1 was obtained as a olive precipitate (303 mg, 48.4%) after filtering and drying in vacuo. An analytical sample was prepared by treating 50 mg of 2_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. Analysis. Calculated for $(C_{36}H_{39}N_5O_5Eu)(CH_3CO_2)(H_2O)$: C, 53.66; H, 5.21, N, 8.23. Found: C, 53.39, H, 5.50, N, 8.25. HR FAB mass spectrum, M^+: Calculated for $C_{36}H_{40}N_5O_5Eu$, 773.2228. Found: 773.2239. UV/vis (MeOH) [λ_{max}, nm (log ε)]: 330.0 (4.47), 464.0 (4.72), 708.0 (3.90), 762.0 (4.35).

Postsynthetic modification of oligodeoxynucleotide-amine 2_G (9_E) with europium(III) complex 2_F, n=1. The europium(III) 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 2_F, n=1, (25 mg, 30.1 μmol) and N-hydroxysuccinimide (5 mg, 43 μmol) were dried together overnight in vacuo. The compounds were dissolved in dimethylformamide (anhydrous, 500 μL) and dicyclohexyl-carbodiimide (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 2_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 which formed was filtered through 0.45 μm nylon microfilterfuge tubes, and the eppendorf tube was washed with 250 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 ^{0}C for ca. 2′, and loaded on a 20% denaturing polyacrylamide gel. The yellow band corresponding to conjugate 2_H, n=1 (9_D, 10_A) 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 determined using UV/vis spectroscopy (calculated ϵ^{260}= 187,110) was 21.6 μM in conjugate 2_H, n=1 (9_D, 10_A) (12%).

EXAMPLE 3

Synthesis of texaphyrin metal complexes with amine, thiol or hydroxy linked oligonucleotides, see Fig. 3A-3C.

Amides, ethers and thioethers are representative of linkages which may be used for coupling site-directed molecules such as oligonucleotides to texaphyrin metal complexes (see Figures 3A-3C). Oligonucleotides or other site-directed molecules functionalized with amines 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 the texaphyrin complex. 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 phosphoester backbone and the texaphyrin-bound lanthanide cation(s).

Oligonucleotides are used to bind selectively compounds which include the complementary nucleotide or oligo- or poly-nucleotides containing substantially complementary sequences. 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.

Oligonucleotides are used, for example, as hybridization probes in blot analyses, primers for polymerase chain reaction (PCR) amplification, and for

site-specific mutagenesis. Oligonucleotide-derived products are being used for the detection of genetic diseases and for proviral HIV, the causative agent of Acquired Immunodeficiency Syndrome (AIDS). They are also being considered as potential chemotherapeutic agents, for example, in gene therapy, and in an antisense capacity.

The oligonucleotide may be large enough to bind probably at least 9 nucleotides of complementary nucleic acid.

A general method for preparing oligonucleotides of various lengths and sequences is described by Caracciolo *et al.* (1989) *Science*, 245:1107.

In general, there are two commonly used solid phase-based approaches to the synthesis of oligonucleotides containing conventional 5′-3′ linkages, one involving intermediate phosphoramidites and the other involving intermediate phosphonate linkages. In the phosphoramidite synthesis a suitably protected nucleotide having a cyanoethylphosphoramidate at the position to coupled is reacted with the free hydroxyl of a growing nucleotide chain derivatized to a solid support. The reaction yields a cyanoethylphosphite, which linkage must be oxidized to the cyanoethylphosphate at each intermediate step, since the reduced form is unstable to acid.

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. To indicate the two 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 triester 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)). Oligodeoxy- nucleotides or their phosphorothioate analogues may be synthesized using an Applied Biosystem 380B DNA synthesizer (Applied Biosystems, Inc., Foster City, CA).

EXAMPLE 4

Synthesis of diformyl monoacid tripyrrane 4_H and oligonucleotide conjugate 4_J, see Fig. 4A-4D.

Dimethylester Dibenzylester Dipyrromethane 4_B: A three-neck 1000 ml round-bottom flask set with a magnetic stirring bar, a thermometer, a heating mantle, and a reflux condenser attached to an argon line was charged with methylester acetoxypyrrole 4_A (100.00 g; 267.8 mmol), 200 proof ethyl alcohol (580 ml), and deionized water (30 ml.) The reaction mixture was heated up and when the resulting solution began to reflux, 12 N aq. hydrochloric acid (22 ml) was added all at once. The

flask contents were stirred under reflux for two hours. The heating element was replaced by a 0 °C bath and the resulting thick mixture was stirred for two hours prior to placing it in the freezer overnight.

The mixture was filtered over medium fritted glass funnel, pressed with a rubber dam, and washed with hexanes until the filtrate came out colorless. The collected solids were set for overnight high vacuum drying at 30 °C to afford slightly yellowish solids (65.85 g, 214.3 mmol, 80.0 % yield.)

Dimethylester Diacid Dipyrromethane, 4_C: All the glassware was oven dried. A three-neck 2000 ml round-bottom flask set with a magnetic stirring bar, a hydrogen line, and a vacuum line was charged with dimethylester dibenzylester dipyrromethane 4_B (33.07 g, 53.80 mmol), anhydrous tetrahydrofuran (1500 ml), and 10 % palladium on charcoal (3.15 g.) The flask was filled with dry hydrogen gas after each of several purges of the flask atmosphere prior to stirring the reaction suspension under a hydrogen atmosphere for 24 hours.

The solvent of the reaction suspension was removed under reduced pressure. The resulting solids were dried under high vacuum overnight.

The dry solids were suspended in a mixture of saturated aqueous sodium bicarbonate (1500 ml) and ethyl alcohol (200 ml), and stirred at its boiling point for five minutes. The hot suspension was filtered over celite. The filtrate was cooled down to room temperature and acidified to pH 6 with 12 N aqueous hydrochloric acid. The resulting mixture was filtered over medium fritted glass. The collected solids were dried under high vacuum to constant weight (21.63 g, 49.78 mmol, 92.5 % yield.)

Methylester Dibenzylester Tripyrrane, 4_E A three-neck 2000 ml round-bottom flask set with a heating mantle, a magnetic stirring bar, a thermometer, and a reflux condenser attached to an argon line was charged with dimethylester diacid dipyrromethane 4_C (21.00 g, 48.33 mmol), ethyl acetoxy pyrrole 4_D (30.50 g), p-toluenesulfonic acid monohydrate (1.94 g), trifluoroacetic acid (39 ml), and methyl alcohol (1350 ml.) The flask contents were heated and stirred under reflux for two hours. The heating element was replaced with a 0 °C and the stirring was continued for half an hour prior to placing the resulting mixture in a freezer overnight.

The cold mixture was filtered over medium fritted glass. The collected solids were washed with hexanes and dried under high vacuum overnight (13.05 g, 19.25 mmol, 39.8 % yield.)

Methylester Diacid Tripyrrane 4_F: All the glassware was oven dried. A three-neck 500 ml round-bottom flask set with a magnetic stirring bar, a hydrogen line, and a vacuum line was charged with methylester dibenzylester tripyrrane 4_E (12.97 g, 19.13 mmol), anhydrous tetrahydrofuran (365 ml), and 10 % palladium on charcoal (1.13 g.) The flask was filled with dry hydrogen gas after each of several purges of the flask atmosphere prior to stirring the reaction suspension for 24 hours under a hydrogen atmosphere at room temperature.

The reaction suspension was filtered over celite. The solvent of the filtrate was removed under reduced pressure to obtain a foam which was dried under high vacuum overnight (10.94 g, 21.99 mmol, 87.0 % pure.)

Monoacid Tripyrrane 4_H: All the glassware was oven dried. A three-neck 500 ml round-bottom flask set with a

mechanical stirrer, a thermometer, a 0 °C bath, and an addition funnel set with an argon line was charged with methylester diacid tripyrrane 4_F (10.20 g, 17.83 mmol). Trifluoroacetic acid (32.5 ml) was dripped into the reaction flask from the addition funnel over a 45 minute period keeping the flask contents below 5 °C. The resulting reaction solution was stirred at 0 °C for 15 minutes, and then at 20 °C for three hours. Triethylorthoformate (32.5 ml) was dripped into the flask from the addition funnel over a 20 minute period keeping the flask contents below -25 °C by means of a dry ice/ethylene glycol bath. The reaction solution was stirred for one hour at -25 °C and then a 0 °C bath was set up. Deionized water (32.5 ml) was dripped into the reaction flask from the addition funnel keeping the flask contents below 10 °C. The resulting two phase mixture was stirred at room temperature for 75 minutes and then added 1-butanol (200 ml.) The solvents were removed under reduced pressure. The resulting dark oil was dried under high vacuum overnight to obtain black solids (11.64 g.)

A three-neck 2000 ml round-bottom flask set with a thermometer, a heating mantle, a magnetic stirring bar, and a reflux condenser attached to an argon line, was charged with the crude methylester diformyl tripyrrane 4_G (11.64 g), methyl alcohol (900 ml), deionized water (60 ml), and lithium hydroxide monohydrate (4.7 g.) The flask contents were heated, stirred under reflux for two hours, cooled down to room temperature, added deionized water (250 ml), acidified with 12 N aq. HCl to pH 5, and then stirred at 0 °C for one hour. The resulting mixture was filtered over medium fritted glass funnel. The collected solids were dried under high vacuum to constant weight prior to their purification by column chromatography (silica gel, MeOH in CH_2Cl_2, 0-10 %; 3.64 g, 8.06 mmol, 45.2 % yield.)

The monoacid tripyrrane 4_H is condensed with a derivatized *ortho*-phenylene diamine, for example, 1_G to form a nonaromatic precursor which is then oxidized to an aromatic metal complex, for example, 4_I. An oligonucleotide amine may be reacted with the carboxylic acid derivatized texaphyrin 4_I to form the conjugate 4_J having the site-directed molecule on the T portion of the molecule rather than the B portion.

EXAMPLE 5

Hydrolysis of Monoesters by Lanthanide (III) T2B2 Texaphyrin

The present example provides the utility of the present invention in the use of texaphyrin metal complexes for the hydrolysis of monoesters, in particular, the hydrolysis of UpU, cUMP, 3′-UMP and 2′-UMP.

Cytosine, uridine, uridine-2′ and 3′-monophosphate disodium salt (2′-UMP and 3′-UMP), uridine-2′,3′-cyclicmonophosphate sodium salt (cUMP), and uridylyl(3′→5′) uridine ammonium salt (UpU) were purchased from Sigma (St. Louis, MO) and used without further purification. The lanthanide texaphyrins were prepared as in the previous examples. All solutions, unless otherwise stated, were prepared from a stock solution of 5.0 mM N-(2-hydroxyethyl) piperazine-N′-ethanesulfonic acid (HEPES), in Milli-Q purified water, adjusted to pH 7.0. Solutions were stored and reactions conducted in RNAse free plastic vials further sterilized by heating at 120°C for 20 minutes in an autoclave. Gloves were worn at all times during solution preparation and reaction sampling. All kinetic runs were thermostated at 37°C in a water bath.

High-performance liquid chromatography (HPLC) was performed on a Waters 501 equipped with a Waters model 440 absorbance detector, monitoring at 254 nm. A YMC, Inc., USA ODS-AQ column (150 mm x 4.6 mm I.D.) was used. Satisfactory separation was achieved with an isocratic gradient (10 mM NaH_2PO_4 adjusted to pH 5.6 with 1% methanol) with a flow rate of 1.0 ml/min. A Beckman DU-7 spectrometer was used to confirm the concentrations of EuB2T2 txp.

$Eu(NO_3)_3$. In the control experiment, the reaction solutions were prepared by diluting 100 µl of UpU (2.94 mM), 25 µl of $Eu(NO_3)_3$ (3.5 µm), and 100 µl of cytosine (0.423 mM), as internal standard, in 375 µl of 5.0 mM HEPES solution. The reactions were carried out as for EuB2T2 txp. The pseudo-zero order rate constant for the control reaction was determined to be $k = (2.2 \pm 0.8) \times 10^{-4}$ mM/h.

EuB2T2 txp. In a typical kinetics experiment, the reaction solutions were prepared by diluting 100 µl of UpU (2.94 mM), 50 µl of EuB2T2 txp (7.8 mM), and 100 µl of cytosine (0.423 mM), as internal standard, in 350 µl of 5.0 mM HEPES solution. The rate of UpU hydrolysis was monitored by removing 15 µl aliquots which were frozen until HPLC analysis was possible. All samples were microfiltered (0.2 µm) prior to injection on the HPLC. All runs were performed in triplicate. The background as determined from the simultaneous control containing no metal complex was negligible. The pseudo-zero order rate constant for the reaction was determined to be $k = (9.1 \pm 1.6) \times 10^{-4}$ mM/h at 37°C, pH 7.0.

The pseudo-zero order rate constant for the hydrolytic cleavage of a ribodinucleotide by the nitrate salt of the water soluble EuB2T2 texaphyrin has been examined. Investigations indicate that a 0.15 mM aqueous

solution of Eu(B2T2 txph)$^{2+}$ hydrolytically cleaved uridylyl (3′→5′) uridine, UpU, (0.49 mM) with a pseudo-zero order rate of (9.1 ± 1.6) x 10^{-4} mM/h at 37°C, pH 7.0. In the absence of the metal complex no evidence of RNA cleavage was observed by HPLC. The reaction was followed by HPLC, monitoring the formation of uridine. Uridine-2′-monophosphate, uridine-3′-monophosphate, and uridine-2′:3′-cyclicmonophosphate (cUMP) were also observed by HPLC; this indicates a hydrolytic rather than an oxidative mechanism for the cleavage reaction. Uridine-2′:3′-cyclicmonophosphate reached a steady state concentration, implying that the texaphyrin complex hydrolyzed cUMP as well. Under identical conditions, a 0.15 mM aqueous solution of $Eu(NO_3)_3$ has a pseudo-zero order rate constant of 2.2 ± 0.35) x 10^{-4} mM/h. Therefore, small traces of free metal ions cannot account for the hydrolysis observed in the presence of the texaphyrin metal complex. Under these conditions, the Eu(III) complex of HAM displayed a pseudo-zero order rate constant of 4.1 x 10^{-4} mM/h. Thus, the texaphyrin complex is found to be more effective than the HAM system.

A survey of other lanthanide (III) complexes of the B2T2 texaphyrin indicates that these complexes are also capable of RNA hydrolysis. Results are summarized in Table 2.

TABLE 2

Rate Constants (Pseudo-Zero Order) for the Hydrolysis of UpU by Lanthanide (III) B2T2 Texaphyrin Complexes[a]

LANTHANIDE CATION	k mM/h
La(III)	1.16×10^{-4}
Nd(III)	4.69×10^{-4}
Sm(III)	6.3×10^{-4}
Eu(III)	4.99×10^{-3}
Gd(III)	1.44×10^{-4}
Dy(III)	6.0×10^{-3}
Tm(III)	4.16×10^{-4}
Lu(III)	1.91×10^{-4}

[a]The concentrations of the Lanthanide(III)B2T2 txph$(NO_3)_2$ are all approximately 0.25 mM.

Further evidence supporting the catalytic effect of the texaphyrin metal complex was obtained by monitoring the formation of uridine produced from the Eu(T2B2Txp)$^{+2}$ catalyzed decomposition of uridine-2',3'-cyclicmonophosphate (cUMP). The decomposition of cUMP (0.10 mM) catalyzed by Eu(T2B2Txp)$^{+2}$ (0.15 mM), when incubated at 37°C and pH=7.0 (5 mM HEPES buffer), had a pseudo-zero order rate of 6.94×10^{-5} mMh^{-1} for the production of uridine. Examination of the reaction products by HPLC indicated that initially, cUMP is isomerized to uridine-3'-monophosphate (3'-UMP) and uridine-2'-monophosphate (2'-UMP) which are subsequently hydrolytically cleaved to produce uridine (Figure 14).

EXAMPLE 6

Generalized Hydrolysis of RNA Using a Texaphyrin Metal Complex

This example describes the degradation of a homogenous population of RNA molecules with europium B2T2

texaphyrin. P^{32}-labelled RNA transcripts from an isolated clone was the homogenous RNA substrate. The transcripts and their degradation products were visualized by polyacrylamide gel electrophoresis and autoradiography.

pGEM®-3Z vector and Riboprobe® RNA transcript systems were obtained from Promega Corporation, Madison, Wisconsin. A 4.3 kb fragment of the mouse 1b Multi Drug Resistant gene (MDR) was cloned into the *Eco*RI site of the pGem 3Z vector and its orientation determined (see Fig. 5). The plasmid was used in transcription reactions and when digested with *Bam*HI, T7 RNA polymerase makes a transcript from this template that is approximately 2000 bases long. The transcription reaction consisted of 100 ng of *Bam*HI digested pGem 3Z/4.3 MDR#3, 20 μl of 5X transcription buffer, triphosphate nucleotides (A,C,G) at 500 μM, UTP at 100 μM, 50 μC of ^{32}-P α-UTP (3000 Ci/mmol), 10 mmol DTT, 120 units of RNasin and 70-100 units T7 RNA polymerase. This reaction was brought up to a total volume of 100 μl with DEPC treated double distilled water. The reaction was allowed to incubate at 37° C for 1.5 hours. The entire reaction volume was then run over a G-50 Sephadex column (Nick column, Pharmacia) pre-equilibrated with 20 mM Tris pH 7.0, 2 mM EDTA, 0.1% SDS. The transcript was eluted from the column in the second 400 μl volume applied to the column. Any unincorporated nucleotide was left on the column.

Ten μl aliquots of the transcript were put into separate tubes and stock solutions of Eu(III)B2T2txp 1_0, EDTA or Eu(III) acetate were added so that the final volume was 20 μl. The tubes were allowed to incubate for 2 hr at 37°C. Thirty μl of dye mix (formamide, 0.2% bromphenol blue) was added to each tube. The tubes were mixed and heated at 60°C, 5 min, then the entire content of the reaction was loaded onto a 5% 8M urea polyacrylamide gel and electrophoresis was performed.

The results of the digests of the 2000 base long transcripts with EuB2T2 txp are shown in Figure 7. There is one band in the control and control with EDTA lanes 1, 2 and 12. This band is absent in the lane with 100 μM EuB2T2 txp, lane 3. An increase in lower molecular weight material, i.e. degradation products, is seen as smearing throughout lane 3. The transcript remains intact at the lower EuB2T2 txp concentrations, lanes 4-7. The transcript is degraded with 100 μM EuB2T2 txp in the presence of 500, 300, 100 and 10 μM amounts of EDTA, lanes 8-10 and 13. This experiment eliminates the possibility that free metal in the B2T2 txp solution is causing the degradation. Lane 11 contains molecular weight standards of 1418 and 679 bases. Figure 6 shows that the 100, 50, 25, 10 and 5 μM concentrations of free Europium metal salt (EuOAc) do not digest the transcript, lanes 3-7. These results are not affected by the presence of EDTA, lanes 8-11.

A digestion of total RNA (primarily 28s and 18s ribosomal RNA from K562 cells) with EuOAc, EuT2B2 txp and GdT2B2 txp indicated that all are able to hydrolyze total RNA. The digestions were performed in 50% DMSO and H_2O, the gel was electrophoresed using a 10 μM phosphate buffer, pH 6.8. It is likely that EuOAc digests the homogeneous transcript also but at higher EuOAc concentrations than those used in the present example.

Clearly, EuB2T2 txph is able to hydrolyze RNA substrates. Since the texaphyrins have such versatility for functionalization, this result has significant implications for the construction of site-specific cleaving reagents for nucleic acids and other molecules as discussed further in the following examples.

EXAMPLE 7

Site-Specific Hydrolysis of RNA by Europium(III)-Texaphyrin Conjugated to a Synthetic Oligodeoxyribonucleotide

The present example provides antisense agents using a texaphyrin metal complex-oligonucleotide conjugate that effects the hydrolysis of its RNA complement. Such antisense agents should therefore be able to act *in vivo* without the participation of endogenous nucleases. A DNA-EuTx-oligonucleotide conjugate was synthesized based on the functionalized texaphyrin 8_B, Figure 8, (2_F, Figure 2). This "ribozyme analogue" (cf., 8_C) provides an example of oligodeoxynucleotide-directed, metal catalyzed hydrolysis of a complementary RNA oligomer.

Two 20-mer oligonucleotides were machine-synthesized to contain alkylamine groups at either the 5-position of an internal thymine residue or the 5′-end terminal phosphate. Oligodeoxynucleotide-amines modified on the 5-position of thymine were purchased from Oligo's Etc. (Wilsonville, Oregon); oligodeoxynucleotide-amines modified on the 5′ end were purchased from Keystone Laboratories, Inc. (Menlo Park, California). Oligonucleotides were HPLC purified and precipitated using LiCl prior to use. Reaction of the carboxylic acid functionalized europium(III) texaphyrin complex 8_B with carbodiimide and N-hydroxysuccinimide produced the corresponding activated ester, which was added directly to a solution of the chosen oligodeoxynucleotide amine. The resulting DNA-EuTx conjugates (Figure 9, 9_{A-D}) were purified by electrophoresis.

A synthetic RNA 30-mer (10_B, Figure 10) was obtained as substrate (Keystone Labs, Inc., Menlo Park, California), with a sequence selected from a unique site within the gene transcript for multiple drug resistance.

Sequence is complementary at 1562 bases post-transcriptional start site in mouse multidrug resistance protein mRNA. The 3′-^{32}P-labelled substrate was incubated with an excess of oligodeoxynucleotide conjugate at 37°C for 18-24 h in a buffered salt solution, ethanol precipitated, and assayed on a 20% denaturing polyacrylamide gel. As illustrated schematically in Figure 10 and shown in Figures 11A and 11B, ca. 30% cleavage occurred near the expected location of the europium (III) texaphyrin complex upon hybridization with conjugate $\mathbf{10}_A$. Cleavage yield was measured by densitometry and calculated as ratio of cleavage band to intact material. The corresponding cleavage bands were not observed when this same substrate was incubated with oligonucleotides that were non-complementary in sequence, unmodified, or were modified internally with the complex (Figures 11A-11B, lanes 8-10). Control reactions indicate that ambient light, calf thymus DNA or type of buffer (Tris acetate or HEPES, EDTA, pH 6.0-8.0) had no apparent effect on cleavage efficiency. EDTA inhibits cleavage by free lanthanide (III) cations as observed in Morrow *et al.* (1992).

Figures 12 and 13 demonstrate hydrolysis of 5′end labelled RNA 30-mer $\mathbf{10}_B$ by EuTx-conjugate $\mathbf{10}_A$. Comparison of lanes 3-6 with lanes 7-14 of Figure 12 indicates that hydrolysis occurs more readily at 37°C than at 25°C. Lane 3 of Figure 13 demonstrates that a noncomplementary oligo $\mathbf{9}_B$ does not effect hydrolysis of $\mathbf{10}_B$ and lane 4 demonstrates that a conjugate having a complementary oligo and EuTx bound to an internal T residue does not effect hydrolysis of the RNA substrate. Lanes 5-9 demonstrate hydrolysis of the RNA substrate by $\mathbf{10}_A$, a 5′ EuTx conjugate having an oligonucleotide complementary to substrate $\mathbf{10}_B$.

The cleavage fragments co-migrate with bands in sequencing lanes produced by incubation of substrate under alkaline conditions or subjected to partial digestion with a series of base-specific ribonucleases (For an example of a similar end-product analysis, see Dange *et al.*, 1990). This observation is consistent with a hydrolytic mechanism, presumably involving the EuTx acting as a Lewis acid that facilitates an intramolecular attack of the 2'-hydroxyl group to effect cleavage. There are bands indicating site-specific cleavage of the ribonucleotide target sequence in the absence of any added cleavage reagents (lane 1, Figure 11A). Although the source of this background cleavage is unknown, it is believed to be the direct result of a higher order structure (ie., a hairpin) of the oligoribonucleotide, since hybridization with any complementary oligonucleotide dramatically inhibits the cleavage (eg., lanes 2, 5-7, 9, Figure 11A). This type of structure-dependent cleavage behavior has been seen previously with oligoribonucleotides (Dange *et al.*, 1990, Kazabov *et al.*, 1992).

Maximal cleavage activity of the Eu(III)Txp-oligonucleotide was observed down to 25 nM conjugate. Decreased cleavage below this level may be due to a decrease in hybridized material (as judged by increased background cleavage of the target RNA present at a concentration of about 1nM). By means of comparison, the free europium complex non-specifically hydrolyzed the RNA substrate at 25 μM (lane 4, Figure 11A). In the control reaction containing both complex and the non-derivatized complementary DNA oligomer (lane 7, Figure 11A), cleavage occurred predominantly in the single stranded region, although still at lower efficiency than the Eu-Tx-DNA conjugate at 2.5 nM. Thus, attachment of the EuTx to the DNA probe increases its effective concentration ca. 10,000-fold. A target RNA without the secondary

structure observed here would likely allow for cleavage at lower DNA-EuTx concentrations. These data indicate the utility of such conjugates in antisense applications.

As demonstrated in the present example, the selectivity of the texaphyrin complexes is enhanced by covalently linking oligonucleotides onto the periphery of the macrocycle. Since the metal complexes do cleave RNA over DNA preferentially, the DNA appendages would remain intact during the hydrolysis experiments. The DNA arm will recognize and bind to an appropriate RNA segment, effectively increasing the metal concentration at these loci relative to the overall metal concentration in solution. Phosphate ester hydrolysis will therefore be significantly increased at specific locations along the RNA backbone. In one embodiment, primers (known or deduced) for PCR could be coupled to a hydrolytic divalent or trivalent texaphyrin complex to induce hydrolysis of proximal RNA.

Enhancement of RNA hydrolysis by the texaphyrin metal complex-conjugate may be accomplished by designing the deoxyribonucleotide to bind to the RNA target so as to produce a single-stranded loop which may have increased susceptibility to hydrolysis. The loop may contain one or more nucleotides. In this case, the oligodeoxyribonucleotide acts as a catalytic group by using the energy of its binding to the RNA target to increase the ground state energy of the targeted phosphate diester. Examples are illustrated in Figs. 18A and 18B. Fig. 18A shows a DNA twelve-mer lacking a nucleotide to base pair with the looped out "U" and conjugated at its 5′ end to a Dy(III) texaphyrin complex. Binding to the RNA substrate causes a one-base loop structure that is a site of enhanced susceptibility to hydrolysis by the Dy(III)Txp complex. Fig. 18B shows a DNA ten-mer lacking the same nucleotide, conjugated to a

Dy(III)Txp at its 3′ end and to a second Dy(III)Txp at its 5′ end, binding to the RNA substrate so as to cause the same one-base loop structure. The loop is a site of enhanced susceptibility to hydrolysis, this time by the two Dy(III)Txp complexes which converge on the loop structure upon binding. In this example, one Dy(III)Txp may act as a general acid and the second may act as a general base via a Dy(III)Txp-bound hydroxyl group for activating the RNA towards hydrolysis.

EXAMPLE 8

Site-Specific Hydrolysis of RNA by Dysprosium(III)-Texaphyrin and Europium (III) Texaphyrin Conjugated to Synthetic Oligodeoxyribonucleotides of Varying Lengths

Following the procedures of Example 7, oligodeoxyribonucleotide-amines of varying nucleotide lengths and modified at the 5′ end (obtained from Keystone Labs, Menlo Park, California) were reacted with carboxylic acid functionalized dysprosium(III)-texaphyrin complex 8_B to give DyTx oligo-conjugates 9_F - 9_I (Figure 9). Corresponding EuTx oligo-conjugates were also prepared.

Each of the above-described conjugates was then incubated with the 3′-labeled synthetic RNA 30-mer 10_B following the procedures of Example 7. All of the conjugates having complementary DNA and a texaphyrin complex at either end of the oligo hydrolytically cleaved the RNA, although the RNA was hydrolyzed by the Dy conjugates at about twice the rate (producing ca. 60% cleavage of the RNA) as by the Eu conjugates (Figure 22). At the 250 nM concentration of DNA used, the 12-mer conjugate was found to be as effective as the 20-mer conjugate, the 9-mer conjugate was less effective and only a small amount of cleavage was observed with the EuTx-9 mer conjugate. Increased background cleavage of

the RNA substrate in the presence of the 9-mer conjugate indicates that less specificity for annealing to the substrate is provided by the shorter oligonucleotide.

These data indicate that oligonucleotides having as few as 12 bases provide the specificity needed for accurate and full hydrolytic activity. This aspect is important for drug design, since the ability of an oligonucleotide analog to traverse a cellular membrane is generally found to be inversely proportional to its length.

EXAMPLE 9

Site-Specific Hydrolysis of a 36-Mer RNA by Dysprosium (III)-Texaphyrin Conjugated to a Synthetic Oligodeoxyribonucleotide

A 3'-labelled synthetic RNA 36-mer 10_D (Promega Corp., Madison, WI) (Fig. 10) was incubated with an excess of the DyTx-oligo conjugate $10_C(9_I)$, following the procedures of Example 7, and the hydrolysis observed. As illustrated schematically in Figure 10, the DyTx-oligo conjugate hydrolyzed the RNA at positions indicated by the arrows. This additional result supports the breadth of utility for the texaphyrin-oligonucleotide conjugates in the hydrolysis of ester bonds.

EXAMPLE 10

Synthesis of Texaphyrin-Oligonucleotide Conjugates 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). These oligomers were reacted with carboxylic acid functionalized Eu(III)-texaphyrin complex

8_B or Lu(III)texaphyrin complex following the procedures of Example 7, to give conjugates 9_J, 9_K and 9_N (Fig. 9).

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 oligonucleotides renders the oligonucleotide resistant to cellular nucleases.

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 hydrolysis of RNA using texaphyrin metal complex-oligonucleotide conjugates is the use of a set of two conjugates, one having the texaphyrin metal complex conjugated to the 5′ end of an oligomer and the other having a texaphyrin metal complex 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 texaphyrin metal complexes in proximity to the targeted hydrolysis site as depicted in Figure 21. 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 hydrolysis efficiencies that result upon the simultaneous binding of the two conjugates to the RNA template.

EXAMPLE 11
Synthesis of an Eu(III) 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 the carboxylic acid functionalized Eu(III)-texaphyrin complex 8_B, following the procedures of Example 7, to give the dual conjugate 9_L (Fig. 9) which has an Eu(III)Tx metal complex at both the 3′- and the 5′-ends of the 12-mer.

The use of two texaphyrin-metal complexes conjugated to the same oligonucleotide, one at each end, should effect the hydrolysis of RNA with increased efficiency due to the concerted activity of the metal complexes. In this embodiment, it is preferred that both of the texaphyrin complexes contain the same metal, preferably a lanthanide metal cation and more preferably europium(III) or dysprosium(III). One of the texaphyrin metal complexes may act as an acid and the second may act as a base in order to further activate the RNA towards hydrolysis.

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 ester hydrolysis. Such a dual conjugate allows for 2 functions, imaging and hydrolysis to be effected by one molecule.

The use of texaphyrin metal complexes to cleave RNA *in vivo* as a treatment procedure relies on the effective localization of the complex to the site of desired

cleavage. A site of desired cleavage may be a position novel to undesired organisms in terms of health care. A site of desired cleavage may be a messenger RNA encoding a product deleterious to the host or may be a normal RNA that is deleterious in some way.

The data of Examples 6 through 11 demonstrate that lanthanide texaphyrin complexes may be developed into RNA antisense reagents. The anti-sense approach is efficient in regulating the expression of proteins. Many copies of a particular polypeptide are made from one messenger RNA molecule; therefore, by moving up in the levels of cellular processes and knocking out the message, fewer attacking agents would be required because there would be fewer target sites. The anti-sense strategy provides a clear and rational method for new drug design because there is one requirement, that the anti-sense 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 anti-sense technology is the sequence of the target. Treating native RNA with this new texaphyrin complex results in the texaphyrin complex binding to a complementary RNA sequence via an appended oligonucleotide. The lanthanide texaphyrin complex then cleaves the RNA proximal to this specific site. Either one or two texaphyrin molecules may be attached to the DNA, creating the potential for gene splicing reagents.

The texaphyrin oligo complex would have immediate applications for anti-viral and anti-bacterial therapy as well as cancers (an oligonucleotide complementary to an oncogene, 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.

EXAMPLE 12

Texaphyrin-Sapphyrin Conjugates for Ester Cleavage and Transport Across Membranes

A further means of gaining selectivity is to link covalently the texaphyrin complex to a sapphyrin (sap) molecule, (Sessler *et al.*, 1992; Furuta *et al.*, 1991; Sessler *et al.*, 1991; U.S. Patent 5,159,065; U.S. Patent 5,120,411; U.S. Patent 5,041,078, all incorporated by reference herein). Since sapphyrins bind phosphate, K = 20 M^{-1} in water at pH 7, the linked texaphyrin-sapphyrin complex (txph-sap) could effectively increase the metal concentration at locations adjacent to the sapphyrin binding sites. Since the txph-sap molecule would be quite large it is expected that the ternary structure of RNA will provide a limited number of favorable binding sites. Thus, a degree of structural selectivity for RNA hydrolysis would be expected, with this selectivity being based on the conformations of the substrate molecules.

The synthesis of (Eu)texaphyrin-sapphyrin conjugate (Figures 15 and 16): Synthesis was accomplished by amide bond formation between activated (Eu)texaphyrin carboxylic acid and amino-substituted sapphyrin. By the same strategy, a variety of texaphyrin-sapphyrin conjugates may be prepared including conjugates where other metallic cations may be incorporated into the texaphyrin moiety. Figure 15 shows the formation of exemplary amide linked derivatives of texaphyrin.

The synthesis of (Eu)texaphyrin acid EuT2B1(O(CH$_2$)$_3$CO$_2$H) **16$_B$**, R = CH_2OH, Figure 16. Texaphyrin derivative (T2B1(O(CH$_2$)$_3$CO$_2$H) ·HCl, 0.694g, 1mmol) was dissolved in 80 ml of dry methanol. Eu(OAc)$_3$·H_2O (0.329

g, 1mmol) was added, followed by triethylamine (0.5 ml). The reaction mixture was refluxed (reflux condenser was open to the air) for 6 hours, with the progress of metallation followed by visible spectra. Methanol was evaporated under reduced pressure to give a dry, dark solid which was washed with dichloromethane under vigorous stirring for 2 hours. The product was filtered off, redissolved in MeOH (25 ml), and the solution was treated with zeolite (by standard procedure for removing free europium salt). The product was twice precipitated from methanol by adding diethylether. The collected dark green solid was dried under high vacuum overnight. Yield 91.0%. Characterization data: Elemental analysis for $C_{38}H_{44}N_5O_5Eu \cdot 2(OAc)$ (F.W. 920.855) calc. 54.78% C, 5.47% H, 7.61% N; found 54.46% C, 5.50% H, 7.55% N. FAB HR MS: For $C_{38}H_{43}N_5O_5Eu$ calc. 802.24626; found 802.247752. UV-Vis (EtOH, λ_{max}): 420, 469, 760 nm.

The Synthesis of EuTexaphyrin-Sapphyrin derivative (**16**$_C$, Figure 16): Texaphyrin dihydroxyl, carboxylate derivative as described above (0.092 g, 0.1 mmol) was dissolved in 10 ml dry dimethylformamide (solvent without dimethylamine). The solution was cooled on ice to 0°C. The activating agent (carbodiimide, EDC, 95.5 mg, 0.5 mmol) and hydroxybenzotriazole (5 mg) were added and the mixture was held at 0°C for 45 minutes. For the preparation of the aminosapphyrin derivative **16**$_A$, the sapphyrin monocarboxylic acid was, after activation with DCC, reacted with t-BOC monoprotected ethylenediamine and then subsequently deprotected by treating with TFA at room temperature for 1 hour. The solution of aminosapphyrin derivative 3,8,17,22-tetraethyl-12-[N-(2-aminoethyl)aminocarbonylethyl-2,7,13,18,23-pentamethylsapphyrin (**16**$_A$ 0.067 g, 0.1 mmol) in 5 ml of dimethylformamide and 0.1 ml of dry pyridine was added at the same temperature. The reaction mixture was kept at 0°C for 30 minutes, allowed to warm to room temperature,

and stirred for 3 days. Solvent was evaporated under reduced pressure. The crude product was washed, dissolved in EtOH (10 ml) and diethylether was slowly added. Precipitated product was dried *in vacuo*. Yield 68%. Characterization data: UV-Vis λ_{max} (EtOH): 358, 431, 450, 618, 681, 761 nm. (H_2O, pH7): 358, 408, 455, 623, 675, 765 nm. FAB MS: For $C_{80}H_{95}N_{12}O_5Eu$ calc. 1456.67539; found 1457.

Alternative synthetic approaches to analogous heterodimers may include the coupling of amino-substituted texaphyrins (prepared by the reaction of texaphyrin carboxylate anion with monoprotected ethylenediamine $H_2N(CH_2)_2NHR$, where R is $(CH_3)_3COCO$-(t-BOC), followed by heating to 180°C to effect deprotection) with activated sapphyrin monocarboxylic acid derivatives (e.g., sapphyrin acid chloride, or the products obtained from treatment with dicylohexylcarbodiimide, DCC).

This compound **16**$_C$ was tested (a) for transport of ADP and ATP across bulk liquid membrane. Efficient transport at neutral pH was observed, and (b) phosphodiester hydrolysis - during ATP transport, AMP was formed as a result of hydrolysis. Transport studies were performed using an H_2O-CH_2Cl_2-H_2O three phase Pressman-type U-tube type model membrane system (Araki *et al.*, 1990). Aqueous phase I (source phase) was 5 mM solution of ADP, ATP at pH 7.0, the organic phase was 0.1 mmol solution of (Eu)texaphyrin-sapphyrin conjugate. Aqueous phase II (receiving phase) was water, pH 7.0. The increase in concentration of ADP and ATP in the receiving phase as a result of membrane transport was followed as a function of time. Quantities transported were determined by HPLC analysis of receiving phase using cytosine and/or adenosine as the internal standard(s) (reverse phase analytical column, 10 mM phosphate buffer, pH 6.5). In

this way the initial transport rates for the through-membrane transport of ADP and ATP were derived. Results show that the initial rate of transport for ADP is in range of 5 x 10^{-9} mol/cm^2·h and about five times lower than this for ATP. During the course of the above-described through-membrane transport of ADP and ATP the formation of a new compound in the receiving phase, determined to be AMP by comparison with an authentic sample, was also observed. Since this AMP material was not present in the source phase nor observed when carrier-free control experiments were carried out, its production as the result of the (Eu)texaphyrin-sapphyrin conjugate mediated transport process is taken as indicating that the conjugate is capable of effecting the hydrolysis of a phosphodiester bond.

Texaphyrin-sapphyrin conjugates or analogs thereof should be very useful in antisense applications: Sapphyrin binds to phosphate diesters, such as those of DNA, with high specificity and affinity. Ln(III) texaphyrins bind to anionic phosphates and effect the hydrolytic cleavage of RNA and related species. Thus, a texaphyrin-sapphyrin conjugate should provide an enhanced recognition of RNA/DNA and an improved rate of hydrolysis by virtue of the induced "neighboring group effect".

Along with the potential to cleave RNA specifically the texaphyrin molecule may be designed to pass through cell membranes and selectively attack viral RNA. Such a molecule would have the potential to treat human patients infected with a variety of viruses, including HIV, and to treat blood *in vitro* or *ex vivo* for viral infection to purge or purify blood.

EXAMPLE 13

Synthesis of a Dy(III)-Texaphyrin Having an Imidazole Catalytic Group, Figures 19A-19B

Dysprosium(III) complex of 16-[1-(hydroxy)acetyl-2-oxyl-17-[2-[2-[2-[2-(N-imidazolyl)ethoxy]ethoxy]ethoxy]ethoxy]-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 (**19$_H$**, *Fig. 19B*). 4,5-Dinitrocatechol (**19$_A$**) is reacted with triethylene glycol ditosylate (1 equiv.) in the presence of potassium chloride and methanol. The monotosylate (**19$_B$**) is isolated from the resulting mixture, and is reacted with imidazole (1 equiv.) in the presence of potassium carbonate and methanol to give 4,5-dinitro-2-[2-[2-[2-[2-(N-imidazolyl)ethoxy]ethoxy]ethoxy]ethoxy]phenol (**19$_C$**). Following the procedures described for the synthetic scheme of Figure 2A, and as outlined in Fig. 19A-19B, compound **19$_C$** is reacted with ethyl-2-iodoacetate, and the resulting compound **19$_D$** is saponified and reduced to give the corresponding diamino compound **19$_E$** which is then reacted with tripyrrole **19$_F$**. The resulting nonaromatic macrocycle 16-[1-(hydroxy)acetyl-2-oxy]-17-[2-[2-[2-[2-(N-imidazolyl)ethoxy]ethoxy]ethoxy]ethoxy]-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 (**19$_G$**) is metallated to give the Dy(III)Tx complex **19$_H$** having an imidazole as a catalytic group.

The above-described Dy(III)-Tx complex is an example of a texaphyrin complex having an appended catalytic group, imidazole, attached via a tri(ethylene glycol) linker to a texaphyrin metal complex. Such a catalytic

group may assist as a general base in site-specific hydrolysis of an RNA target. For this purpose, the texaphyrin metal complex is conjugated to an oligodeoxyribonucleotide complementary in sequence to the RNA being cleaved. The oligo is conjugated to the texaphyrin via a linker group, such as the carboxy group at the 16-position of the Dy(III)Tx compound 19_H, following the procedures described herein.

EXAMPLE 14

Synthesis of Eu(III)-Texaphyrin Having an Amino Acid or Amino Acid Derivative Catalytic Group

Eu(III)-Tx carboxylic acid complex 2_F (n=3, 92 mg, 0.1 mmol) was dissolved in 5 mL of dry purified DMF and cooled to 0°C, after which 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC; 57 mg, 0.3 mmol) and 0.5 mL of pyridine were added. The resulting activation reaction was allowed to proceed for 1 hr at 0°C. Histamine dichloride (55 mg, 0.3 mmol) was then added, together with 1-hydroxybenztriazole (5 mg) and dimethylaminopyridine (5 mg) in 3 mL of water. The reaction mixture was then stirred for 1 hr at 0°C, followed by stirring at RT for 5 days. The solvents were evaporated off *in vacuo* at RT and the product was recrystallized from a methanol-benzene mixture to give the final Eu(III)Tx-histamine conjugate shown in Fig. 20A. An analytical sample was prepared by reverse phase column chromatography. UV-Vis (H_2O, pH 7): λ_{max} (nm): 457.5, 761.5; FAB HR MS: for $C_{43}H_{51}N_8O_4Eu$, calcd.: 896.324571, found: 896.323476.

Following the above-described procedures, L-arginine methyl ester dihydrochloride (78 mg, 0.3 mmol) was reacted with the activated Eu(III)Tx carboxylic acid 2_F. The reaction mixture was stirred for 1 hr at 0°C and then for 5 days at RT. The solvents were evaporated off and

the product recrystallized to give the final Eu(III)Tx shown in Fig 20B. UV-Vis (H_2O, pH 7): λ_{max} (nm): 457.0, 761.0; FAB HR MS: for $C_{45}H_{52}ClN_9O_6Eu$, calcd.: 1008.341102, found: 1008.34004.

Following similar procedures, metal complexes of texaphyrin having substituents containing an amine group may be prepared via reaction of the activated acid of the texaphyrin complex with an amine component of the amine-containing substituent. Fig. 15 presents a general synthetic scheme. These amine substituents may function as catalytic groups to assist the texaphyrin metal complex in catalyzing ester hydrolysis. Amine-containing substituents include amine-containing saccharides, polysaccharides, amino acids, amino acid derivatives, polyamino acids and the like.

EXAMPLE 15

Texaphyrin-Texaphyrin Conjugates (Fig. 17)

Texaphyrins may be coupled to form a texaphyrin-texaphyrin conjugate similar to the texaphyrin-sapphyrin conjugate described in Example 12. A carboxylate texaphyrin derivative may be converted to an amino derivative by coupling with diamino compounds, such as $NH_2(CH_2)_nNH_2$, or by coupling with a monoprotected compound of the type $NH_2(CH_2)_nNHR$, where R may be a variety of protecting groups and n is an integer from 1 to 7, preferably from 1 to 3. The individual TX metal complex in a texaphyrin-texaphyrin conjugate may have the same or a different chelated metallic cation from the other TX metal complex. Fig. 17 shows a texaphyrin-texaphyrin conjugate which is prepared by the reaction of 2 equivalents of a metallotexaphyrin (such as Eu-TX) carboxylic acid with an activating reagent, e.g. EDC, followed by coupling with an amino component, for example, ethylenediamine.

Texaphyrin metal complexes, especially paramagnetic metal complexes, bind phosphate anions, nucleotides and oligonucleotides as indicated by up to 200 ppm shifts in ^{1}HNMR spectra induced by the binding of these compounds. The apparent association constant for formation of a monoadduct with diphenylphosphate monoanion in methanol/chloroform is 290 dm^3 mol^{-1} and for formation of a diadduct is 74 dm^3 mol^{-1}. Similar changes in spectral patterns were observed from binding of phenyl phosphate dianion, phenylphosphonate monoanion, uridine 5′-monophosphate, uridine 2′,3′- monophosphate, guanosine 5′- monophosphate and oligonucleotide mixtures. The lanthanide adducts of texaphyrin-texaphyrin conjugates may be useful for effecting hydrolysis of di- or triphosphate species such as ATP, hydrolysis at multiple sites in a phosphate polyester, such as RNA or for effecting simultaneous binding and hydrolysis of such RNA -type substrates. For MRI and PDT, a combined diamagnetic/paramagnetic species would allow for binding, imaging and dual cleavage possibilities via hydrolysis and photo-activation.

The use of two proximal texaphyrins should effect the hydrolysis of RNA with increased efficiency due to the concerted activity of the metal complexes. For example, one complex may act as a Lewis acid while the second serves to generate a metal-bound hydroxide general base at neutral pH. Similar mechanisms have been proposed based on crystallographic data obtained from single crystals of DNA hydrolyzing enzymes such as the 3′-5′ exonuclease activity of DNA polymerase.

EXAMPLE 16

Further Uses for Lanthanide Texaphyrins Coupled to Site-Directed Molecules

U.S. Patent 5,252,720 describes magnetic resonance imaging experiments with B2T2 gadolinium complex *in vivo*. The results show that the B2T2 gadolinium complex demonstrates *in vivo* affinity for tissue high in lipid content, atheroma, the liver, kidneys and tumors, and has low toxicity in rodents. As exemplified herein, the chemical properties of this texaphyrin class of macrocyclic ligands can be varied by peripheral substitution, which allows biological properties to be optimized in terms of biodistribution, pharmacokinetics and toxicity.

Texaphyrin metal complexes are especially suited for acting as bifunctional chelating agents in a variety of targeted treatment regimens. Texaphyrin-metal complexes are effective in antibody conjugate-based treatment since they have functional groups suitable for conjugation to the antibody. They form covalent linkages that are stable *in vivo* which do not destroy the immunological competence of the antibody, they are relatively nontoxic, and they are readily soluble in a physiological environment. A further advantage of these texaphyrins is that many would be suitable for further functionalization. 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 (CDI)) could be used to effect the conjugation.

Many cell membranes are partially constructed from phospholipids. Thus, lanthanide texaphyrins may be

developed into synthetic specific phospholipases. One skilled in the art in light of the present disclosure could then determine precisely the lipid side chain that is connected via the phosphate ester bond in a given phospholipid. An extension of this process would be to digest cell membrane components such as phosphatidyl choline and sphingomyelin. This is important since the latter participates in nerve and brain functions.

The development of potent cytotoxins from lanthanide texaphyrins may be accomplished by developing reagents that specifically hydrolyze ATP, ADP, NADH, or $FADH_2$. Said cytotoxins may disrupt, in a biologically specific way, the flow of free energy in the cell and essentially starve the organism. This could allow for the death of undesired plants and animals or the treatment of mammalian cancers.

Liver diseases causing the accumulation of glycogen may be treated by hydrolyzing uridine diphosphate glucose (UDP), the phosphodiester precursor to glycogen. The present invention demonstrates that uridine phosphates are hydrolytically cleaved by lanthanide texaphyrins and previous work with texaphyrin (U.S. Patent 5,252,720) has shown that they localize in the liver. Thus, one skilled in the art would realize that the basic features of this approach have already been demonstrated by experiment.

Cyclic adenosine monophosphate (cAMP) is believed to play an important part in regulating various hormones. Hydrolyzing cAMP to the linear adenosine monophosphate (AMP) impedes certain hormone regulation. Texaphyrin complexes may therefore be used as hormone regulation drugs.

A further use for texaphyrin metal complexes may be as hydrolysis reagents for the detoxification of di- and

trialkyl phosphate esters. Alkyl phosphate esters have a wide range of uses including solvents in chemical reactions, insecticides (e.g., parathion) and chemical nerve gases (e.g., diisopropyl phosphofluoridate, DIPF). Hydrolysis and detoxification of these agents in the environment is often slow by natural processes. Developing catalysts for the hydrolysis of alkyl phosphate esters could greatly improve the lives of many people. Texaphyrin complexes could further be developed as treatment for patients that have been exposed to such nerve agents. Here, the key feature is that texaphyrins are known to hydrolyze phosphate esters quickly.

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

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It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

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

CTCGGCCATA GCGAATGTTC 20

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CATCTGTGAG CCGGGTGTTG 20

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAACAUUCGC UAUGGCCGAG AAGAUGUCAC C 31

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GAACAUUCGC UAUGGCCGAG AAGAUG 26

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCGGCCATA GC 12

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

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

CTCGGCCAT 9

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

ATAGCGATGT TC 12

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAACAUUCGC UAUGGCCGAG AAGAUGUCAC 30

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATAGCGATGT 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TAGCGAATCT TTC 13

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACACACCCGG CUCACAGAUG AAGUCUCCAA AAUAAA 36

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CTGTGAGCCG GGTGTTG 17

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13

TTTATTTTGG AGACTT 16

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14

TGTGACGGGG GTGTTG 16

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCTGTGAGCC GGGTGTTG 18

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

ATCTGTGAGC CGGGTGTTG 19

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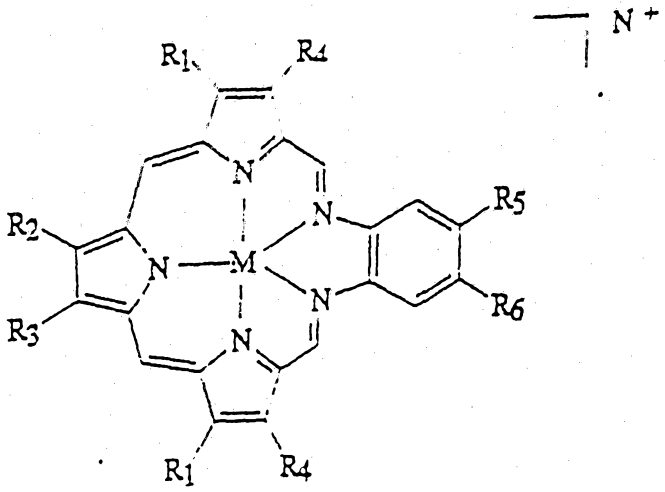
THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A texaphyrin having the structure:



where:

M is a divalent metal cation selected from the group consisting of Ca^{+2}, Mn^{+2}, Co^{+2}, Ni^{+2}, Zn^{+2}, Cd^{+2}, Hg^{+2}, Fe^{+2}, Sm^{+2} and UO^{+2} or a trivalent metal cation selected from the group consisting of Mn^{+3}, Co^{+3}, Ni^{+3}, Fe^{+3}, Ho^{+3}, Ce^{+3}, Y^{+3}, In^{+3}, Pr^{+3}, Nd^{+3}, Sm^{+3}, Eu^{+3}, Gd^{+3}, Tb^{+3}, Dy^{+3}, Er^{+3}, Tm^{+3}, Yb^{+3}, Lu^{+3}, La^{+3}, and U^{+3}; R_1, R_2, R_3, R_4, R_5 and R_6 are independently hydrogen, hydroxyl, alkyl, hydroxyalkyl, alkoxy, hydroxyalkoxy, saccharide, carboxyalkyl, carboxyamidealkyl, a site-directed molecule, a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group, and at least one R_1, R_2, R_3, R_4, R_5 and R_6 is a site-directed molecule,

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a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group; and

N is an integer less than or equal to 2.

2. The texaphyrin of claim 1 wherein the catalytic group is imidazole, guanidine, an amino acid, an amino acid derivative, a polyamino acid, an amino-substituted saccharide or a texaphyrin metal complex.

3. The texaphyrin of claim 1 wherein the site-directed molecule is an oligonucleotide, a hormone, an antibody, a peptide having affinity for a biological receptor, or a sapphyrin molecule.

4. The texaphyrin of claim 1 wherein the site-directed molecule is an oligonucleotide.

5. The texaphyrin of claim 1 wherein the site-directed molecule is an oligodeoxyribonucleotide.

6 The texaphyrin of claim 1 wherein the site-directed molecule is an oligonucleotide conjugated to a catalytic group.

7 The texaphyrin of claim 6 wherein the catalytic group is a texaphyrin metal complex.

8 The texaphyrin of claim 4 wherein the oligonucleotide has complementary binding affinity for oncogenes.

9. The texaphyrin of claim 4 wherein the oligonucleotide has complementary binding affinity for RNA in a region proximal to a phosphate ester bond to be hydrolyzed.

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10. The texaphyrin of claim 4 wherein the oligonucleotide has complementary binding affinity for a targeted intracellular mRNA.

11. The texaphyrin of claim 1 wherein one of R_3, R_5, and R_6 is an oligonucleotide or a couple that is coupled to an oligonucleotide.

12. The texaphyrin of claim 1 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, R_5 is a site-directed molecule, a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group and R_6 is H.

13. The texaphyrin of claim 12 wherein R_5 is $O(CH_2)_nCO$-oligonucleotide where n is 1-7.

14. The texaphyrin of claim 1 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, R_5 is a $(couple)_t$-site directed molecule-catalytic group conjugate where t is zero or 1 and R_6 is H.

15. The texaphyrin of claim 14 wherein R_5 is $O(CH_2)_nCO$-oligonucleotide-texaphyrin metal complex conjugate where n is 1-7.

16. The texaphyrin of claim 1 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, R_5 is $O(CH_2CH_2O)_2CH_2CH_2OCH_3$, and R_6 is a site-directed molecule, a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group.

17. The texaphyrin of claim 16 wherein R_6 is $O(CH_2)_nCO$-oligonucleotide where n is 1-7.

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18. The texaphyrin of claim 13, 15 or 17 wherein n is 1 or 3.

19. The texaphyrin of claim 1 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, R_5 is a catalytic group or a couple that is coupled to a catalytic group, and R_6 is a site-directed molecule or a couple that is coupled to a site-directed molecule.

20. The texaphyrin of claim 1 wherein R_1-R_6 are as follows for texaphyrins A3-A6, A8-A12, A19, A21, A22 and A24-29:

TXP	R_1	R_2	R_3	R_4	R_5	R_6
A3	$CH_2(CH_2)_2OH$	CH_2CH_3	CH_2CH_3	CH_3	$O(CH_2)_nCON$-linker-site-directed molecule, n=1-7	$O(CH_2CH_2O)_3CH_3$
A4	"	"	"	"	$O(CH_2)_nCON$-linker-site-directed molecule	H
A5	"	"	"	"	OCH_2CO-poly-L-lysine	"
A6	"	"	"	"	OCH_2CO-hormone	"
A8	"	"	"	"	OCH_2CON-linker-site-directed molecule	$O(CH_2CH_2O)_2CH_3$
A9	"	"	"	"	OCH_2CO-hormone	"
A10	"	"	"	"	OCH_2CON-oligo	$O(CH_2CH_2O)_3CH_2$-CH_2-N-imidazole
A11	"	"	"	"	$O(CH_2CH_2O)_{120}CH_3$	"

TXP	R_1	R_2	R_3	R_4	R_5	R_6
A12	"	"	"	"	saccharide	H
A19	CH_2CH_2	"	"	"	OCH_2CO_2-glucosamine	H
A21	$CH_2(CH_2)_2OH$	"	"	"	$(CH_2)_n$-CON-linker-site-directed molecule, n=1-7	"
A22	"	"	"	"	$YCOCH_2$-linker-site-directed molecule Y=NH,O	"
A24	CH_2CH_3	CH_3	CH_2CH_2 CON-oligo	"	$O(CH_2)_2CH_2OH$	$O(CH_2)_2CH_2OH$
A25	$CH_2(CH_2)_2OH$	CH_2CH_3	CH_2CH_3	"	OCH_2COOH	$O(CH_2CH_2O)_2CH_2$-CH_2-N-imidazole
A26	"	"	"	"	$O(CH_2)_2CO$-histamine	H
A27	"	"	"	"	$O(CH_2)_2CO$-arginine methyl ester	"
A28	"	"	"	"	$O(CH_2)_nCO$-amino acid, n=1-7	"
A29	"	"	"	"	$O(CH_2)_nCON$-catalytic group, n=1-7	"

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21. The texaphyrin of claim 1 or claims 11 to 20 wherein the metal is a lanthanide cation.

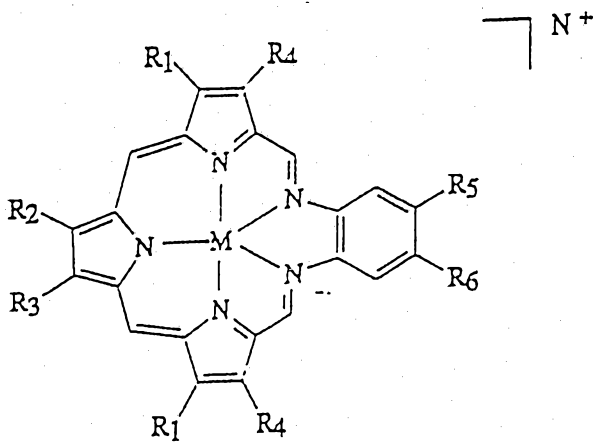
22. The texaphyrin of claim 1 or claims 11 to 20 wherein the metal is Eu(III) or Dy(III).

23. A method of phosphate ester hydrolysis, the method comprising:

Obtaining an aqueous phosphate ester solution, and

incubating the solution with a metal complex having catalytic activity for ester bond hydrolysis, said incubation being under conditions and for a time sufficient to hydrolyze the phosphate ester, characterized in that the metal complex is a texaphyrin metal complex as hereinbefore described.

24. The method of claim 23 where the texaphyrin metal complex has the structure:



where:

M is a divalent or a trivalent metal cation catalyzing ester bond hydrolysis in aqueous solution;

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R_1, R_2, R_3, R_4, R_5 and R_6 are independently hydrogen, hydroxyl, alkyl, hydroxyalkyl, alkoxy, hydroxyalkoxy, saccharide, carboxyalkyl, carboxyamidealkyl, a site-directed molecule, a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group; and

N is an integer less than or equal to 2.

25. The method of claim 23 or 24 wherein the phosphate ester is RNA.

26. The method of claim 24 wherein at least one of R_1, R_2, R_3, R_4, R_5 and R_6 is a site-directed molecule, a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group.

27. The method of claim 26 wherein the catalytic group is imidazole, guanidine, an amino acid, an amino acid derivative, a polyamino acid, an amine-substituted saccharide or a texaphyrin metal complex.

28. The method of claim 26 wherein the site-directed molecule is an oligonucleotide, a hormone, an antibody, a peptide having affinity for a biological receptor, or a sapphyrin molecule.

29. The method of claim 26 wherein the site-directed molecule is an oligonucleotide.

30. The method of claim 26 wherein the site-directed molecule is an oligodeoxyribonucleotide.

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31. The method of claim 26 wherein the site-directed molecule is an oligonucleotide conjugated to a catalytic group.

32. The method of claim 31 wherein the catalytic group is a texaphyrin metal complex.

33. The method of claim 29 wherein the oligonucleotide has complementary binding affinity for oncogenes.

34. The method of claim 29 wherein the phosphate ester is RNA and the oligonucleotide has complementary binding affinity for the RNA in a region proximal to the phosphate ester bond being hydrolyzed.

35. The method of claim 29 wherein the oligonucleotide has complementary binding affinity for a targeted intracellular mRNA.

36. The method of claim 26 wherein one of R_3, R_5 and R_6 is an oligonucleotide or a couple that is coupled to an oligonucleotide.

37. The method of claim 24 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3 and R_5 and R_6 are $OCH_2CH_2CH_2OH$.

38. The method of claim 24 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, R_5 is a site-directed molecule, a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group and R_6 is H.

39. The method of claim 38 wherein R_5 is $O(CH_2)_nCO$-oligonucleotide where n is 1-7.

40. The method of claim 24 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, R_5 is a $(couple)_t$-site directed

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molecule-catalytic group conjugate where t is zero or 1 and R_6 is H.

41. The method of claim 40 wherein R_5 is $O(CH_2)_nCO$-oligonucleotide-texaphyrin metal complex conjugate where n is 1-7.

42. The method of claim 24 wherein R_1 is $(CH_2)_2CH$[illegible] and R_3 are CH_2CH_3, R_4 is CH_3, R_5 is $O(CH_2CH_2O)_2CH_2CH_2$[illegible] and R_6 is a site-directed molecule, a catalytic group, or a couple that is coupled to a site-directed molecule or to a catalytic group.

43. The method of claim 42 wherein R_6 is $O(CH_2)_nCO$-oligonucleotide where n is 1-7.

44. The method of claim 39, 41 or 43 wherein n is 1 or 3.

45. The method of claim 24 wherein R_1 is $(CH_2)_2CH_2OH$, R_2 and R_3 are CH_2CH_3, R_4 is CH_3, R_5 is a catalytic group or a couple that is coupled to a catalytic group, and R_6 is a site-directed molecule or a couple that is coupled to a site-directed molecule.

46. The method of claim 24 wherein R_1-R_6 are as follows for texaphyrins A1-A29:

TXP	R_1	R_2	R_3	R_4	R_5	R_6
A1	$CH_2(CH_2)_2OH$	CH_2CH_3	CH_2CH_3	CH_3	$O(CH_2)_2OH$	$O(CH_2)_2OH$
A2	"	"	"	"	$O(CH_2CH_2O)_2CH_3$	$O(CH_2CH_2O)_2CH_3$
A3	"	"	"	"	$O(CH_2)_nCON$-linker-site-directed molecule, n=1-7	"
A4	"	"	"	"	$O(CH_2)_nCON$-linker-site-directed molecule	H
A5	"	"	"	"	OCH_2CO-poly-L-lysine	"
A6	"	"	"	"	OCH_2CO-hormone	"
A7	"	"	"	"	$O(CH_2CH_2O)_2CH_3$	"
A8	"	"	"	"	OCH_2CON-linker-site-directed molecule	$O(CH_2CH_2O)_3CH_3$
A9	"	"	"	"	OCH_2CO-hormone	"
A10	"	"	"	"	OCH_2CON-oligo	$O(CH_2CH_2O)_2CH_2CH_2$-N-imidazole

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TXP	R_1	R_2	R_3	R_4	R_5	R_6
A11	"	"	"	"	$O(CH_2CH_2O)_{120}CH_3$	"
A12	"	"	"	"	saccharide	H
A13	"	"	"	"	$OCH_2CON(CH_2CH_2OH)_2$	"
A14	"	"	"	"	$CH_2CON(CH_3)CH_2$-$(CHOH)_4CH_2OH$	"
A15	"	COOH	COOH	"	"	"
A16	"	$COOCH_2$-CH_2	$COOCH_2$-CH_2	"	"	"
A17	$CH_2CH_2CON(CH_2$-$CH_2OH)_2$	CH_2CH_3	CH_2CH_3	"	"	"
A18	$CH_2CH_2ON(CH_3)$-CH_2-$(CHOH)_4CH_2OH$	"	"	"	OCH_3	OCH_3
A19	CH_2CH_3	"	"	"	OCH_2CO_2-glucosamine	H
A20	$CH_3(CH_2)_nOH$	"	"	"	$O(CH_2)_nCOOH$, n=1-7	"
A21	"	"	"	"	$(CH_2)_n$-CON-linker-site-directed molecule, n=1-7	"

TXP	R_1	R_2	R_3	R_4	R_5	R_6
A22	"	"	"	"	$YCOCH_2$-linker-site-directed molecule Y=NH,O	"
A23	CH_2CH_3	CH_3	CH_2CH_2-COOH	"	$O(CH_2)_2CH_2OH$	$O(CH_2)_2CH_2OH$
A24	"	"	CH_2CH_2-CON-oligo	"	"	"
A25	$CH_2(CH_2)_2OH$	CH_2CH_3	CH_2CH_3	"	OCH_2COOH	$O(CH_2CH_2O)_3CH_2$-CH_2-N-imidazole
A26	"	"	"	"	$O(CH_2)_3CO$-histamine	H
A27	"	"	"	"	$O(CH_2)_3CO$-arginine methyl ester	"
A28	"	"	"	"	$O(CH_2)_nCO$-amino acid, n=1-7	"
A29	"	"	"	"	$O(CH_2)_nCON$-catalytic group, n=1-7	"

47. The method of claims 23, 24 or claims 37 to 46 wherein the metal is a lanthanide cation.

The method of claims 23, 24 or 37 to 46 wherein the metal is Eu(III) or Dy(III).

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PCT/US94/06284 10526/94

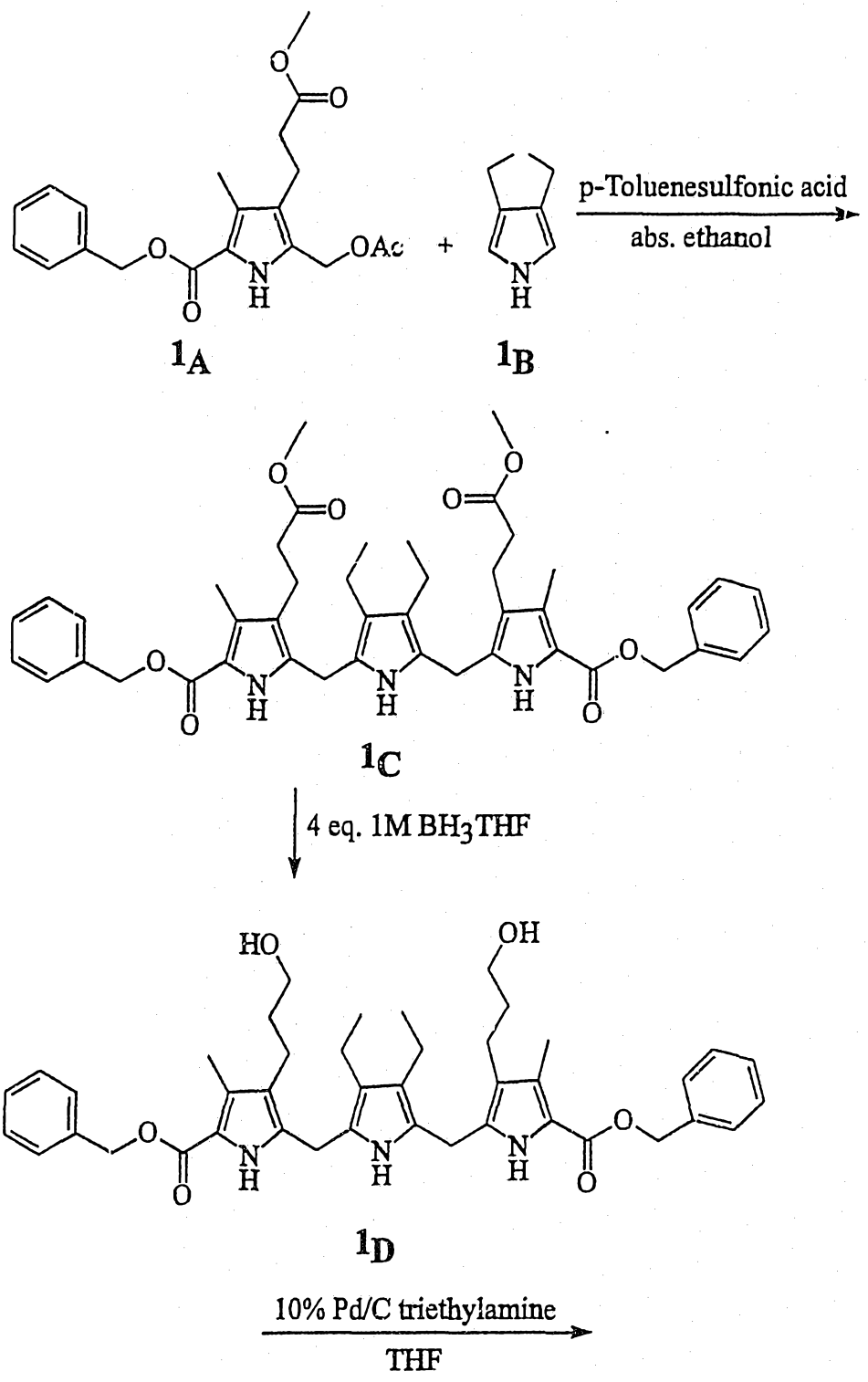


FIGURE 1A

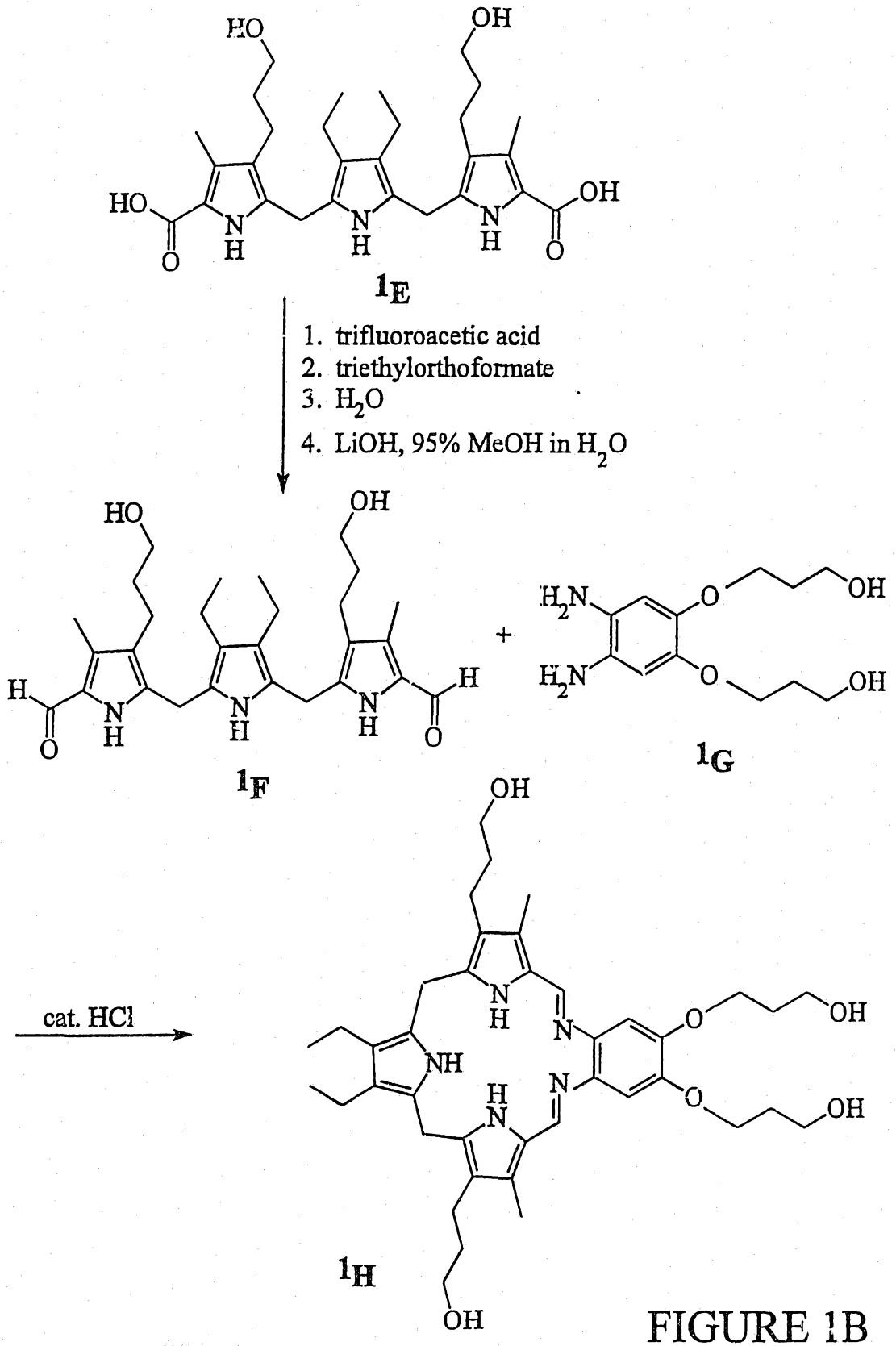
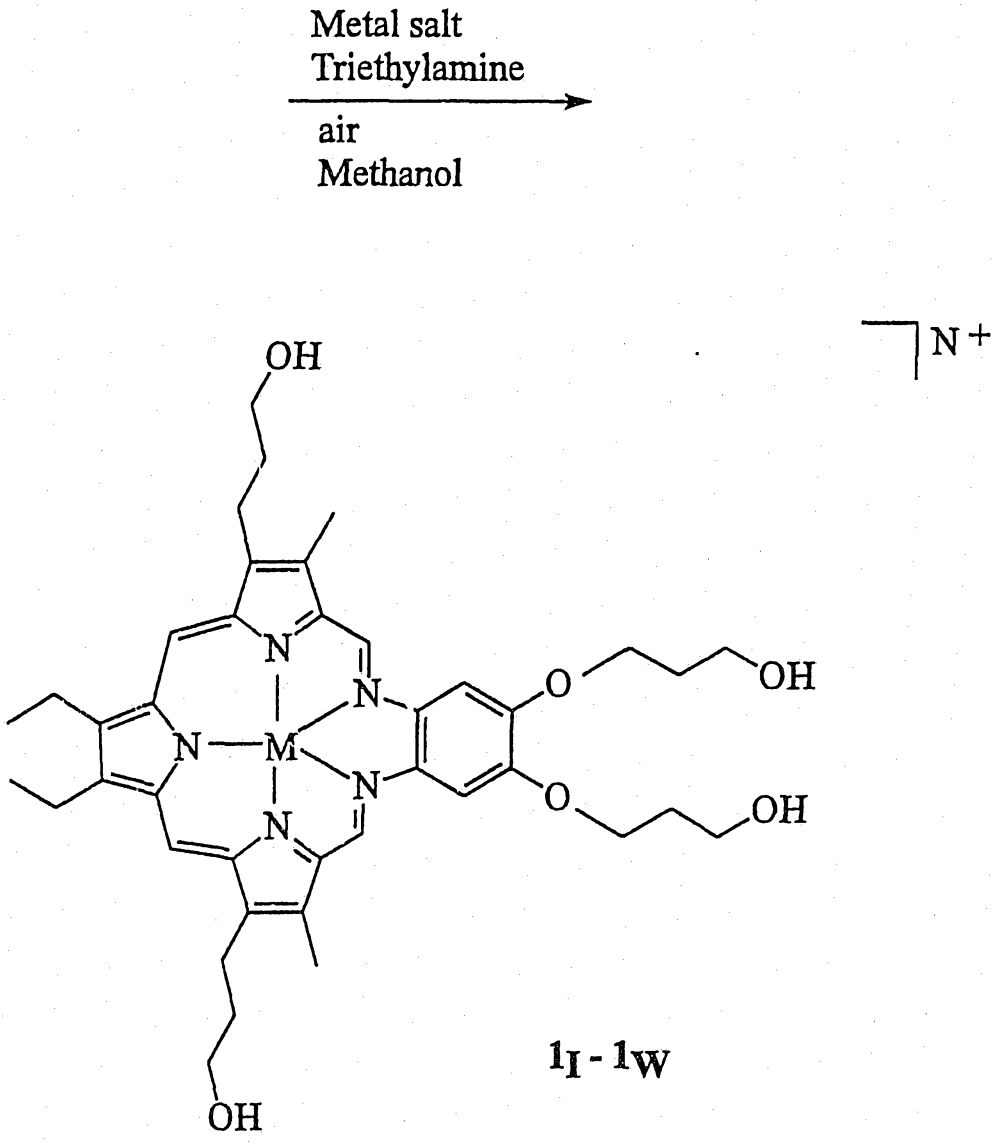

HO
OH
HO
OH
N
H
O
1E
1. trifluoroacetic acid
2. triethylorthoformate
3. H2O
4. LiOH, 95% MeOH in H2O
H2N
1F
1G
cat. HCl
1H

FIGURE 1B



1_I - 1_W

FIGURE 1C

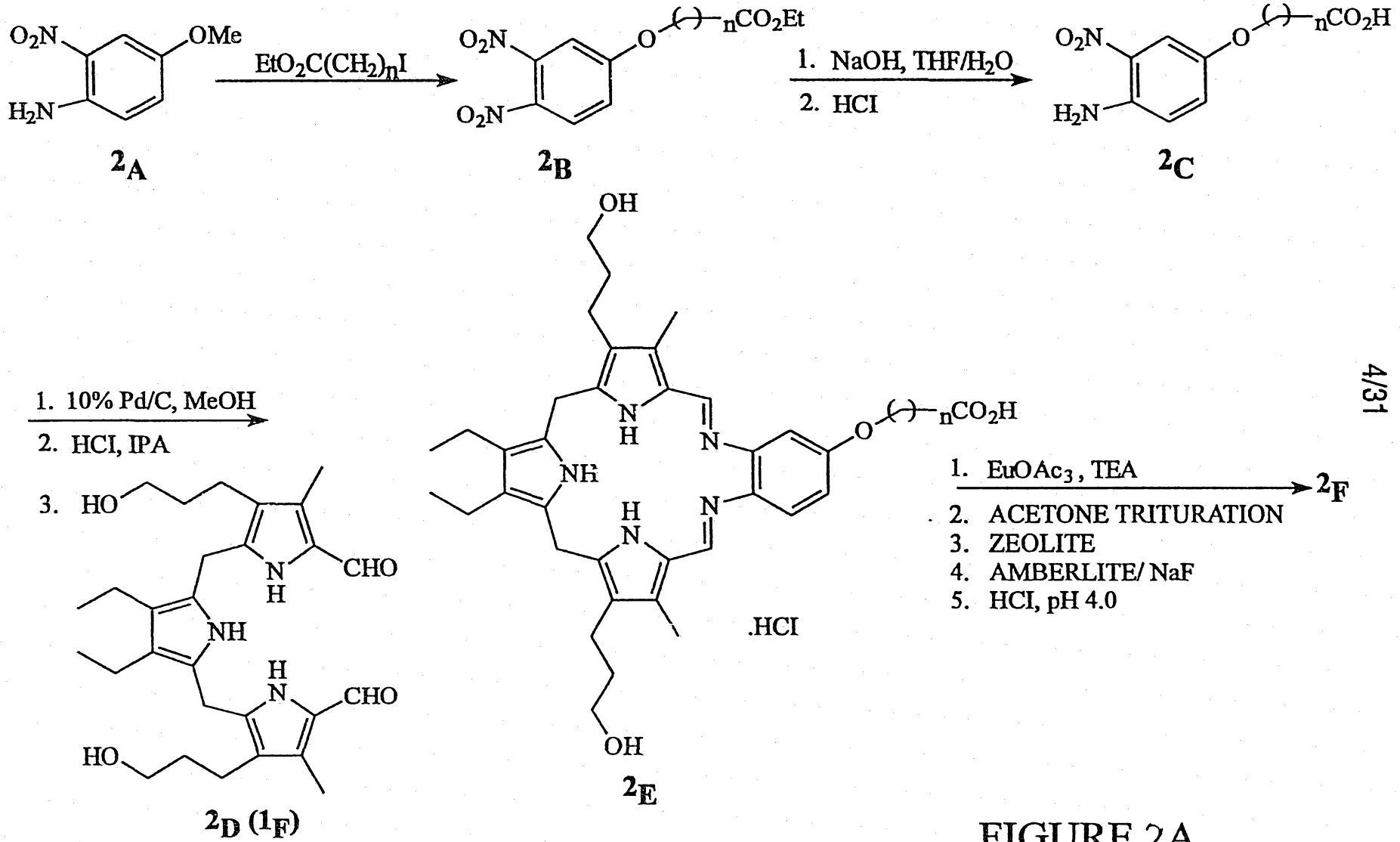

O_2N
OMe
H_2N
$EtO_2C(CH_2)_nI$
O_2N
O
nCO2Et
O_2N
1. NaOH, THF/H2O
2. HCl
O_2N
O
nCO2H
H_2N
2A
2B
2C
1. 10% Pd/C, MeOH
2. HCl, IPA
3. HO
CHO
NH
CHO
HO
2D (1F)
OH
N
H
N
NH
N
H
N
O
nCO2H
.HCl
OH
2E
1. EuOAc3, TEA
2. ACETONE TRITURATION
3. ZEOLITE
4. AMBERLITE/ NaF
5. HCl, pH 4.0
2F

FIGURE 2A

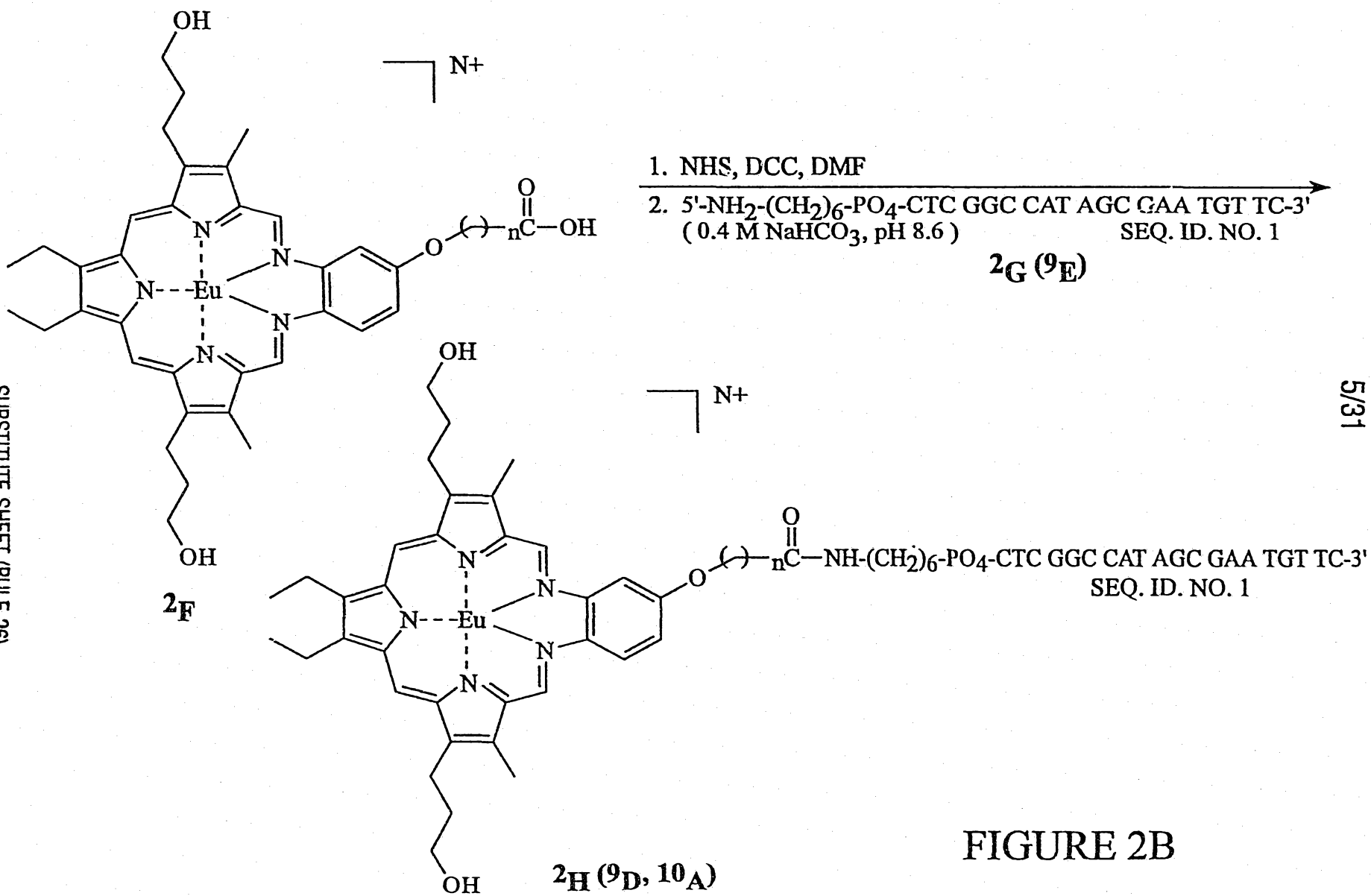

OH
N+
N
N
N
N
N
Eu
O
O
nC—OH
1. NHS, DCC, DMF
2. 5'-NH2-(CH2)6-PO4-CTC GGC CAT AGC GAA TGT TC-3'
(0.4 M NaHCO3, pH 8.6)
SEQ. ID. NO. 1
2G (9E)
OH
N+
N
N
N
N
N
Eu
O
O
nC—NH-(CH2)6-PO4-CTC GGC CAT AGC GAA TGT TC-3'
SEQ. ID. NO. 1
OH
2F
2H (9D, 10A)

FIGURE 2B

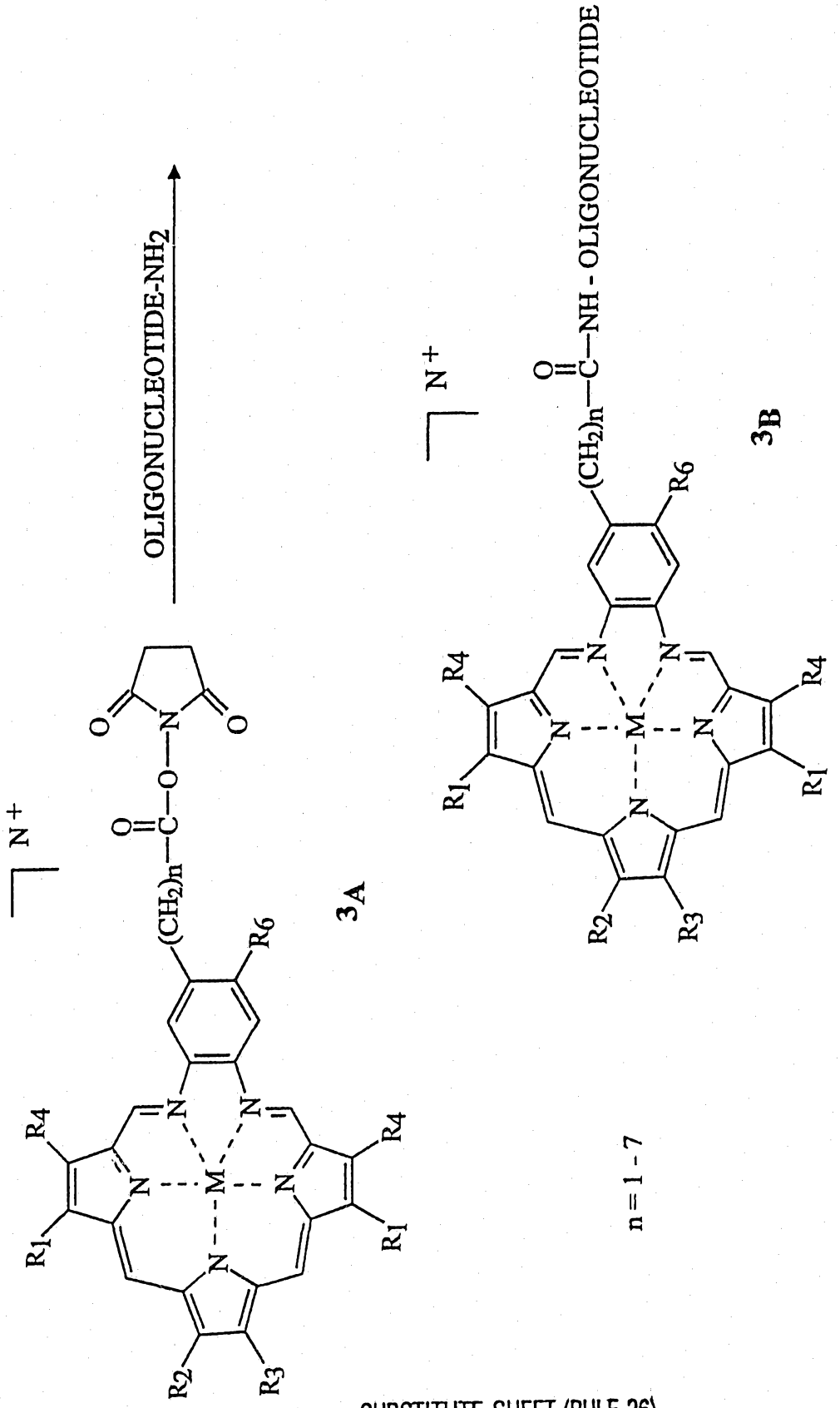


FIGURE 3A

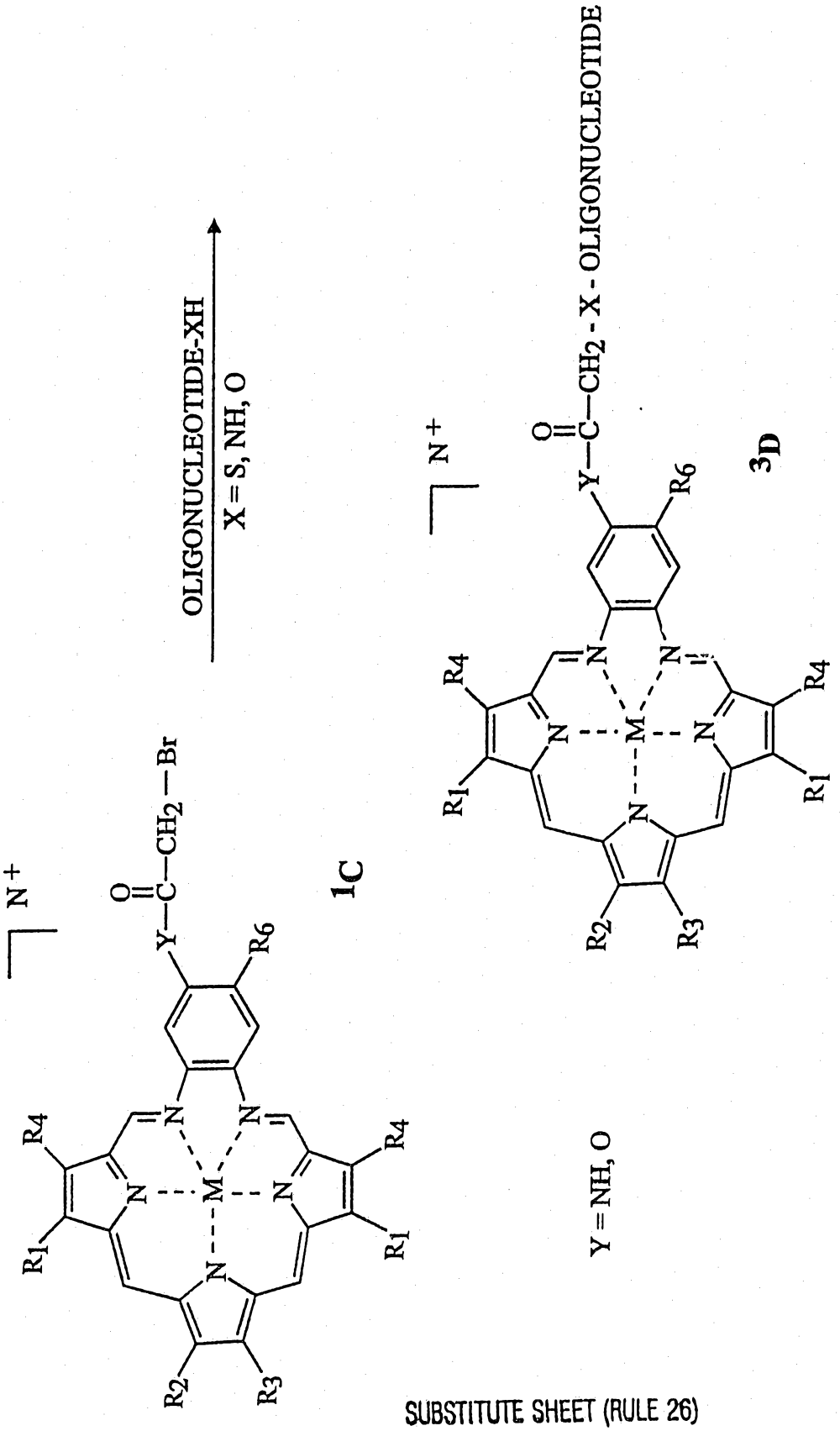


FIGURE 3B

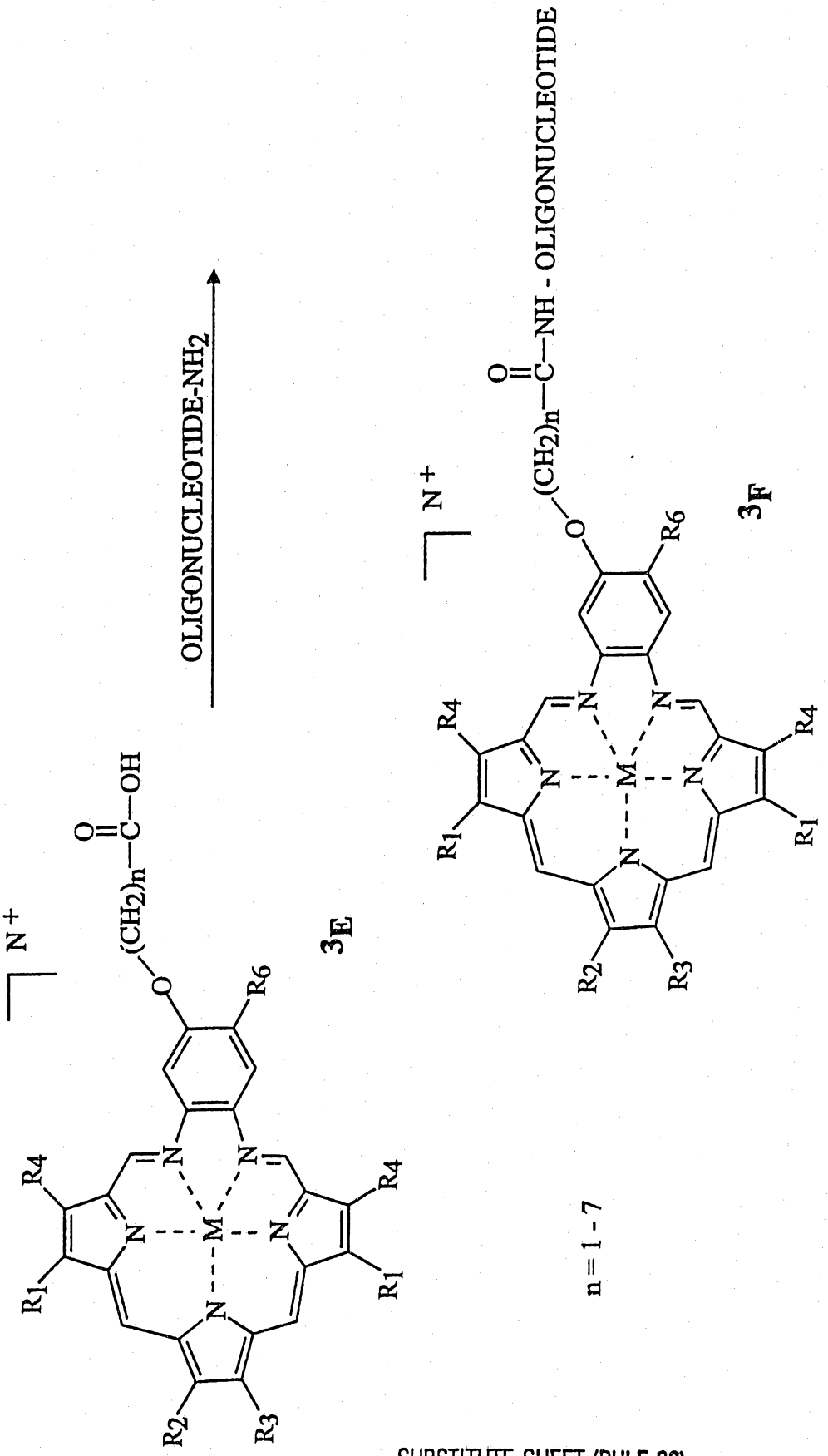


FIGURE 3C

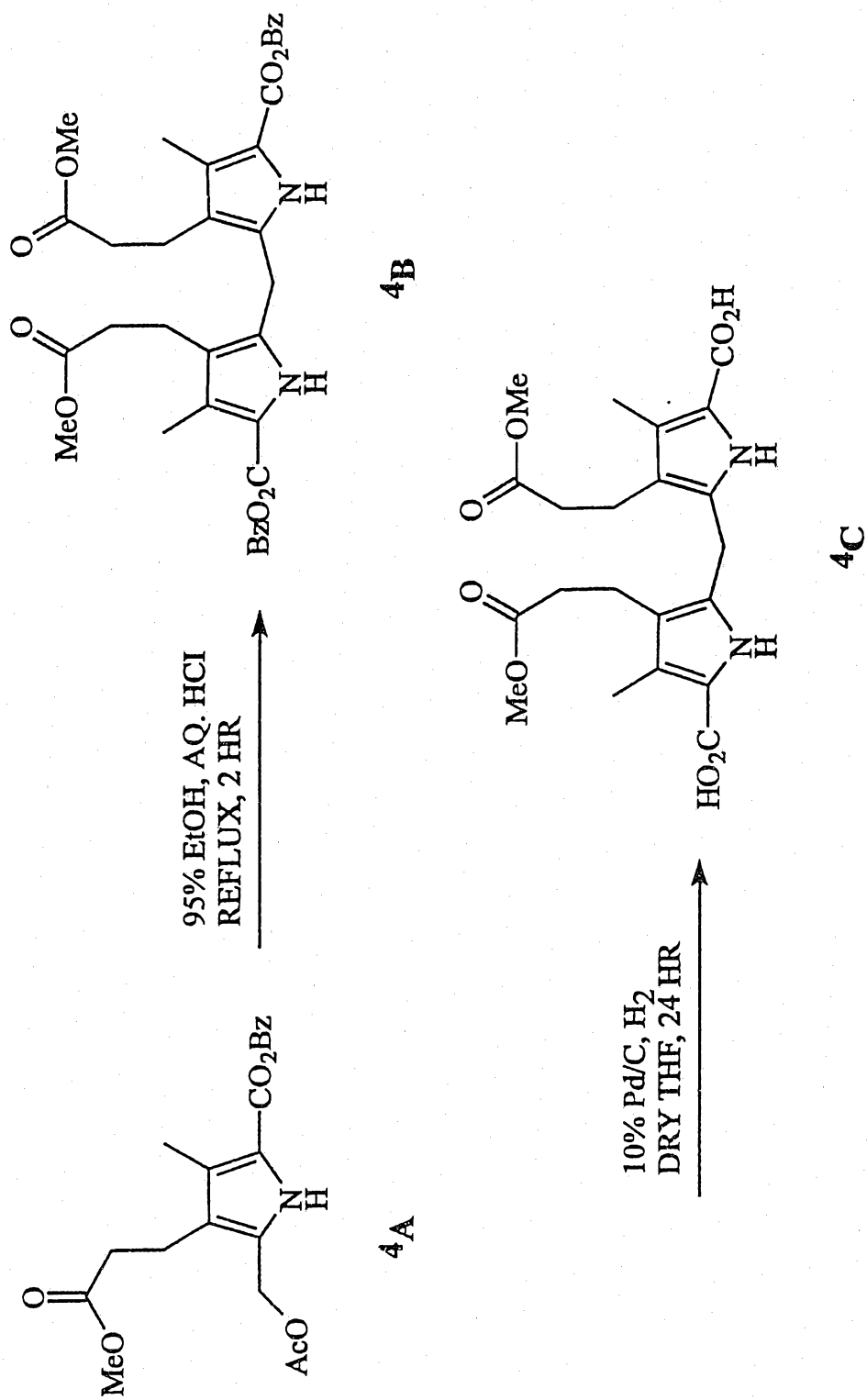


FIGURE 4A

MeO
O
O
OMe
HO2C
CO2H
N
H
N
H
+
AcO
CO2Bz
N
H
pTSA, MeOH, TFA
REFLUX, 2 HR
4C
4D
MeO
O
BzO2C
CO2Bz
N
H
N
H
N
H
10% Pd/C, H2
DRY THF, 24 HR
4E
4F

FIGURE 4B

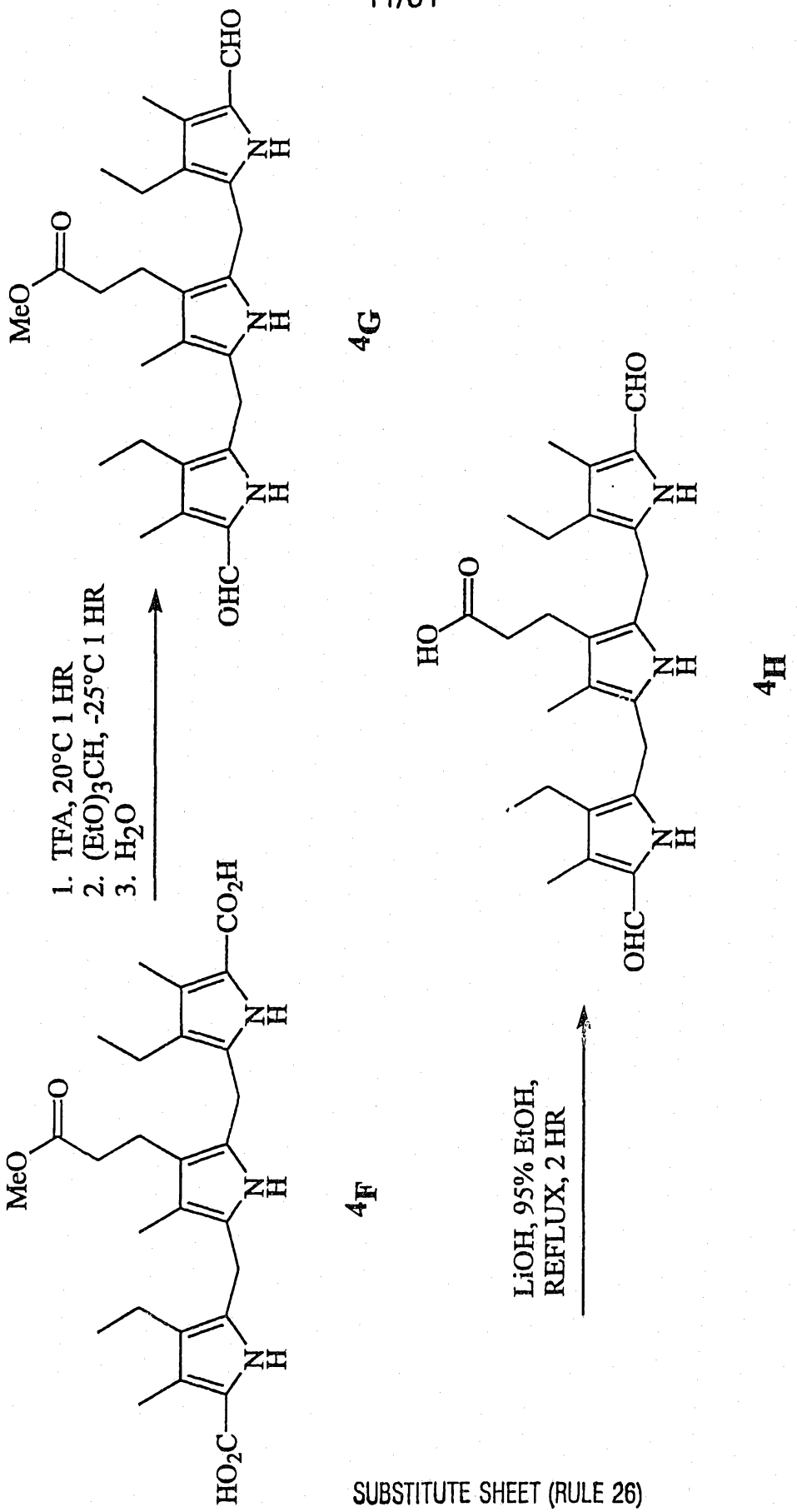

1. TFA, 20°C 1 HR
2. $(EtO)_3CH$, -25°C 1 HR
3. H_2O
LiOH, 95% EtOH,
REFLUX, 2 HR
MeO
HO
OHC
CHO
CO_2H
HO_2C
4_F
4_G
4_H

FIGURE 4C

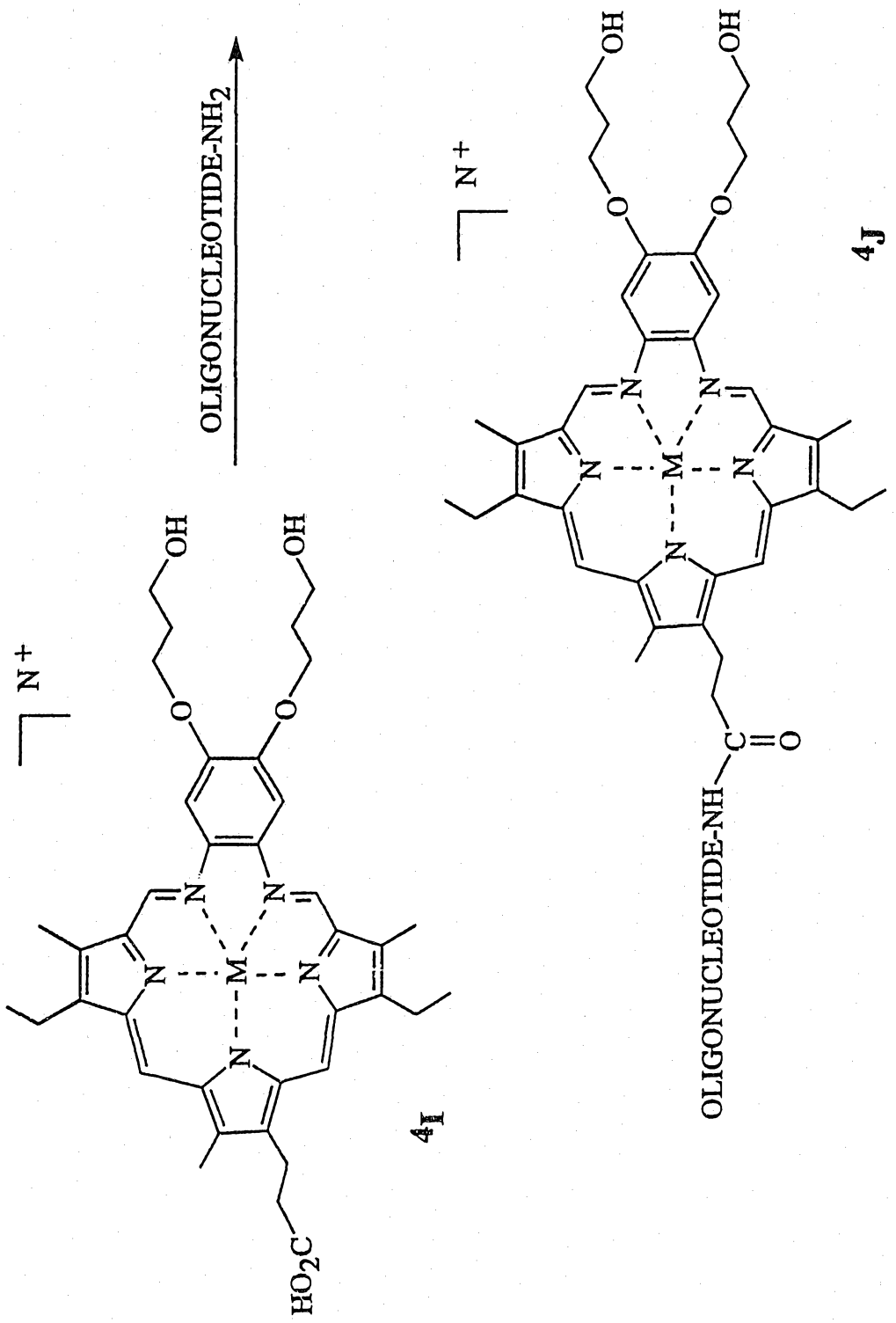

OLIGONUCLEOTIDE-NH2
OH
OH
O
O
N+
N
N
M
N
N
N
HO2C
4I
OH
OH
O
O
N+
N
N
M
N
N
N
C=O
OLIGONUCLEOTIDE-NH
4J

FIGURE 4D

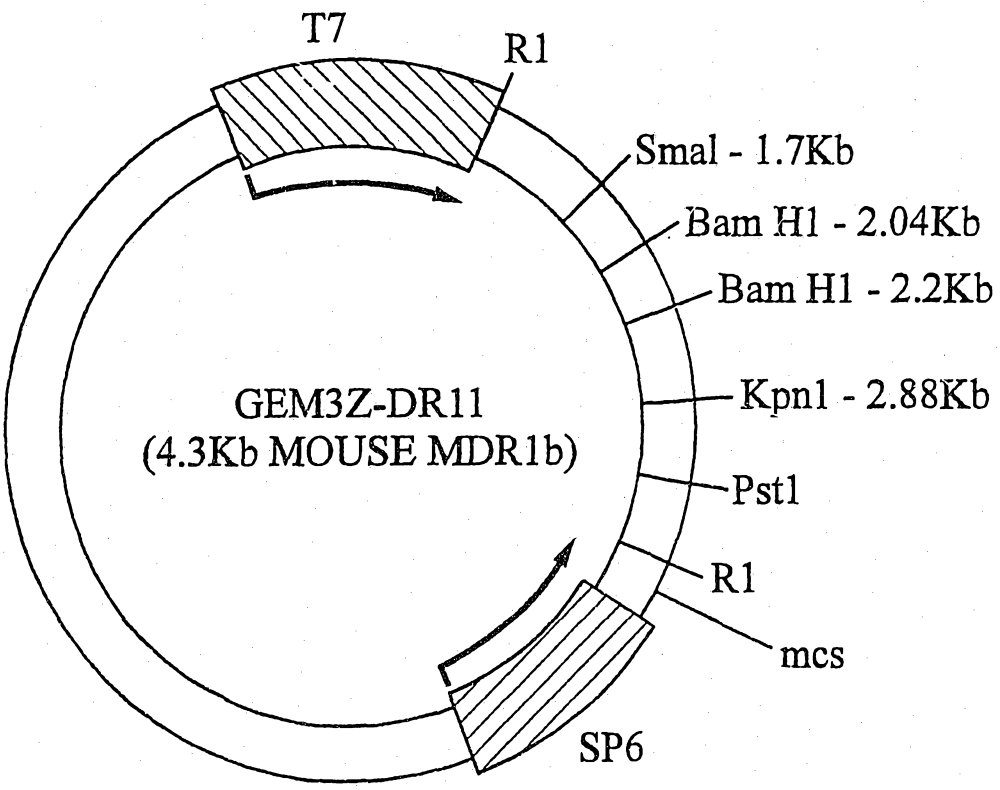

T7
R1
SmaI - 1.7Kb
Bam H1 - 2.04Kb
Bam H1 - 2.2Kb
Kpn1 - 2.88Kb
Pst1
R1
mcs
SP6
GEM3Z-DR11
(4.3Kb MOUSE MDR1b)

FIGURE 5

FIGURE 6

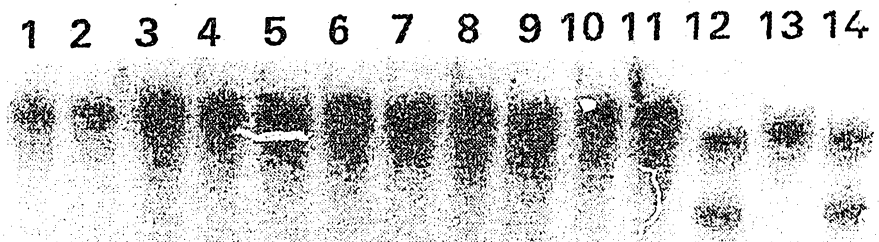
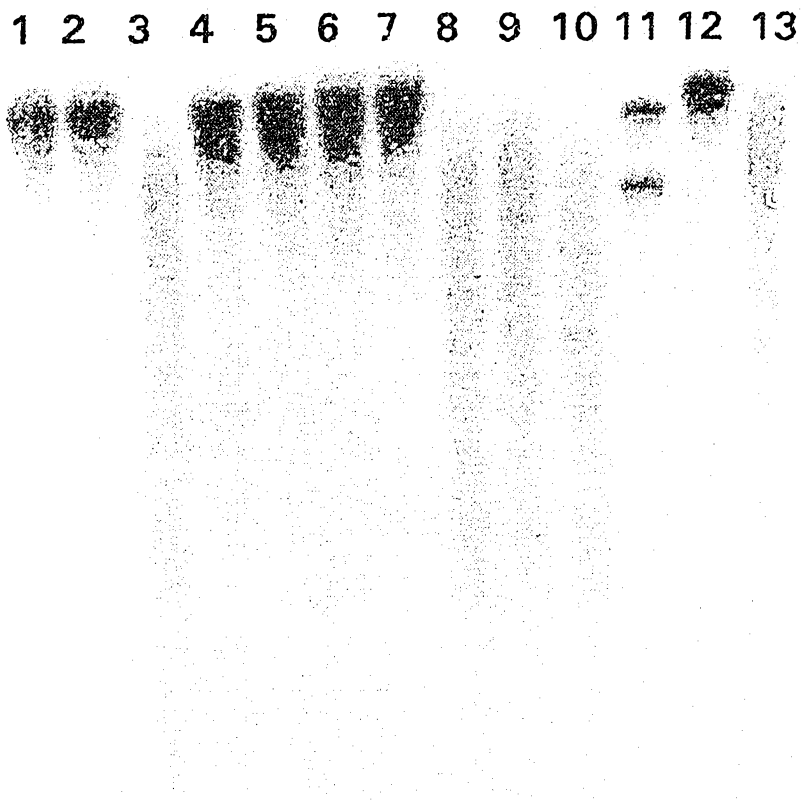
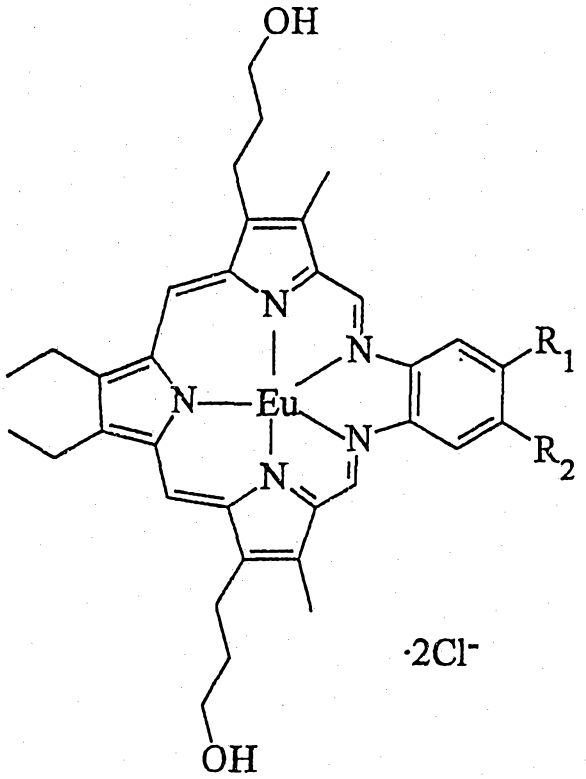


FIGURE 7



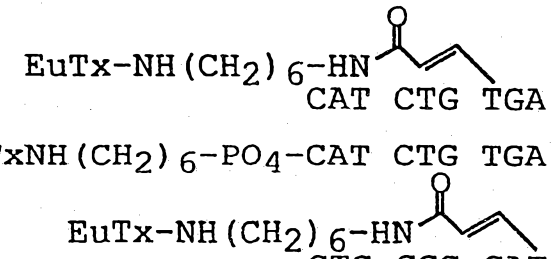


^{8}A $R_1 = R_2 = OCH_2CH_2CH_2OH$

^{8}B $R_1 = OCH_2CO_2H$, $R_2 = H$

^{8}C $R_1 = OCH_2CO\text{-}DNA$, $R_2 = H$

FIGURE 8



9_A	SEQ. ID. NO. 2
9_B	SEQ. ID. NO. 2
9_C	SEQ. ID. NO. 1
$9_D(10_A, 2_H)$	SEQ. ID. NO. 1
9_E	SEQ. ID. NO. 1
9_F	SEQ. ID. NO. 6
9_G	SEQ. ID. NO. 5
9_H	SEQ. ID. NO. 1
$9_I(10_C)$	SEQ. ID. NO. 2
9_J	SEQ. ID. NO. 5
9_K	SEQ. ID. NO. 5
9_L	SEQ. ID. NO. 5
9_M	SEQ. ID. NO. 10
9_N	SEQ. ID. NO. 5
9_O	SEQ. ID. NO. 5

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FIGURE 9

SEQ. ID. NO. 1

5'-EuTx-NH-$(CH_2)_6$-PO_4-CTC GGC CAT AGC GAA TGT TC-3' 10_A (9_D, 2_H)

3'-pC*C ACU GUA GAA GAG CCG GUA UCG CUU ACA AG-5' 10_B

SEQ. ID. NO. 3

SEQ. ID. NO. 2

5'-DyTxNH-$(CH_2)_6$-PO_4-CAT CTG TGA GCC GGG TGT TG-3' 10_C (9_I)

3'-A AAU AAA ACC UCU GAAGUA GAC ACU CGG CCC ACA AC-5' 10_D

SEQ. ID. NO. 11

FIGURE 10

SUBSTITUTE SHEET (RULE 26)

FIGURE 11A

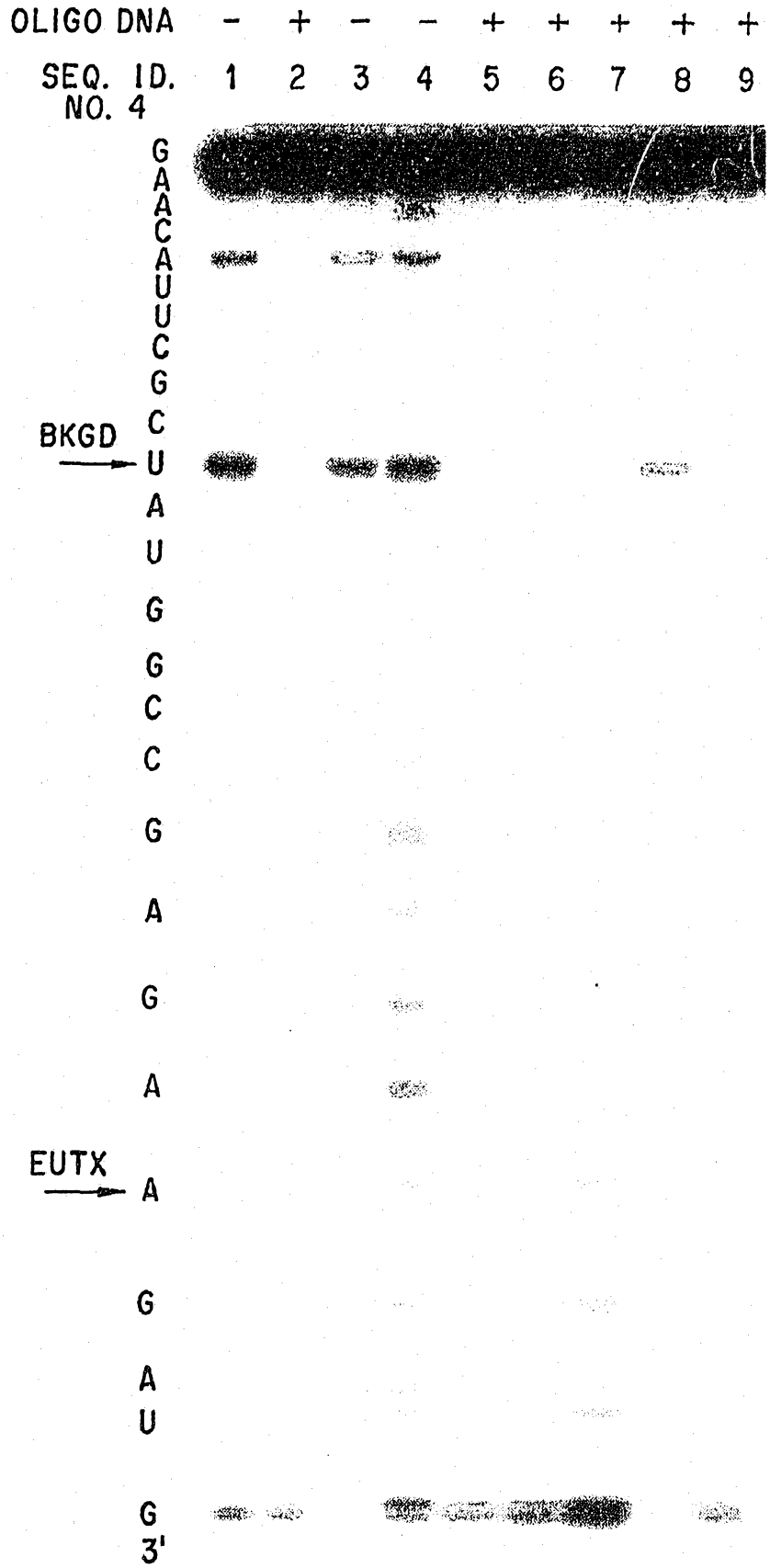

OLIGO DNA - + - - + + + + +
SEQ. ID. NO. 4 1 2 3 4 5 6 7 8 9
G
A
A
C
A
U
U
C
G
C
BKGD
U
A
U
G
G
C
C
G
A
G
A
EUTX
A
G
A
U
G
3'

FIGURE 11B

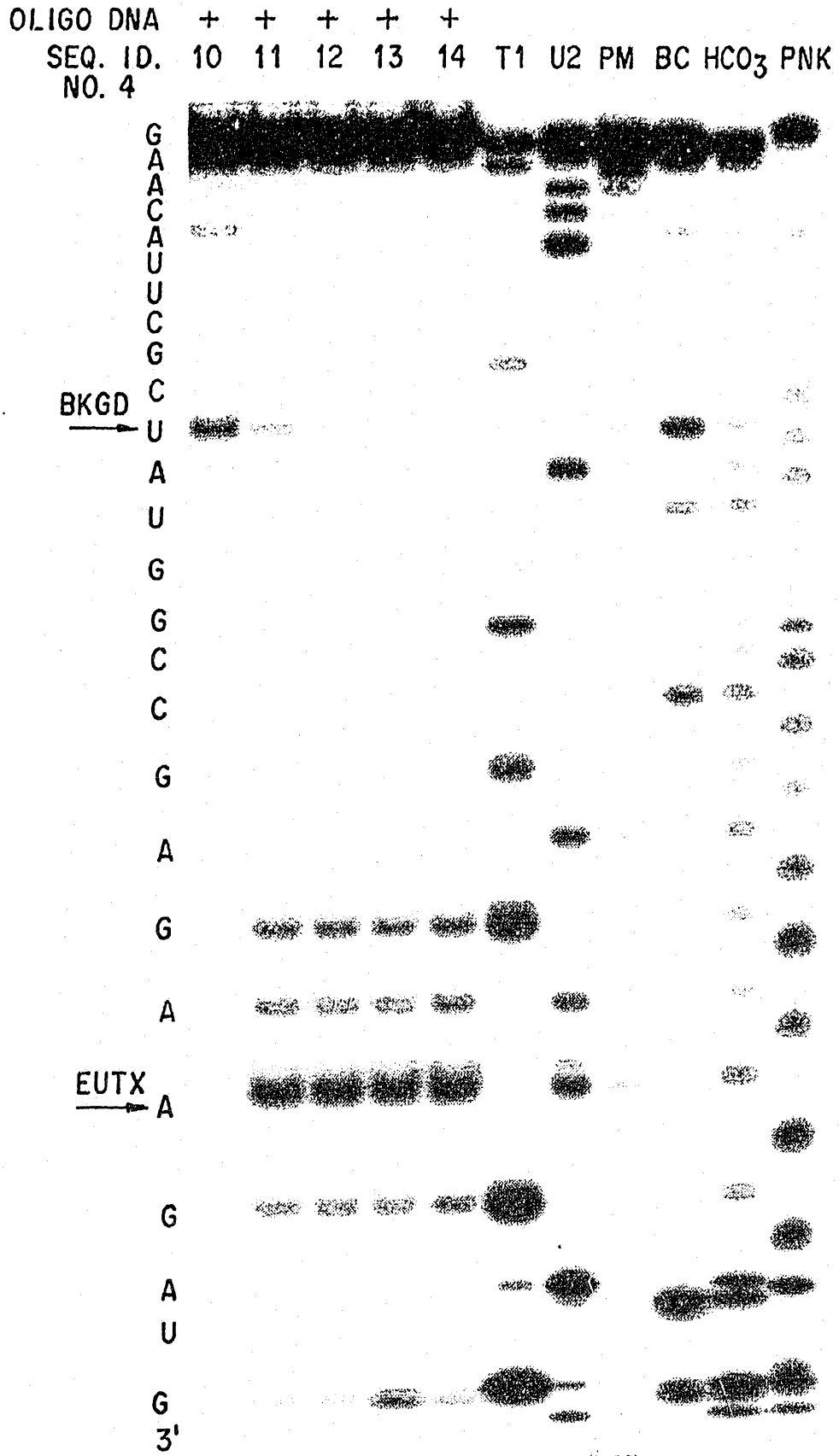

OLIGO DNA + + + + +
SEQ. ID. NO. 4 10 11 12 13 14 T1 U2 PM BC HCO3 PNK
G
A
A
C
A
U
U
C
G
C
BKGD
U
A
U
G
G
C
C
G
A
G
A
EUTX
A
G
A
U
G
3'

FIGURE 12

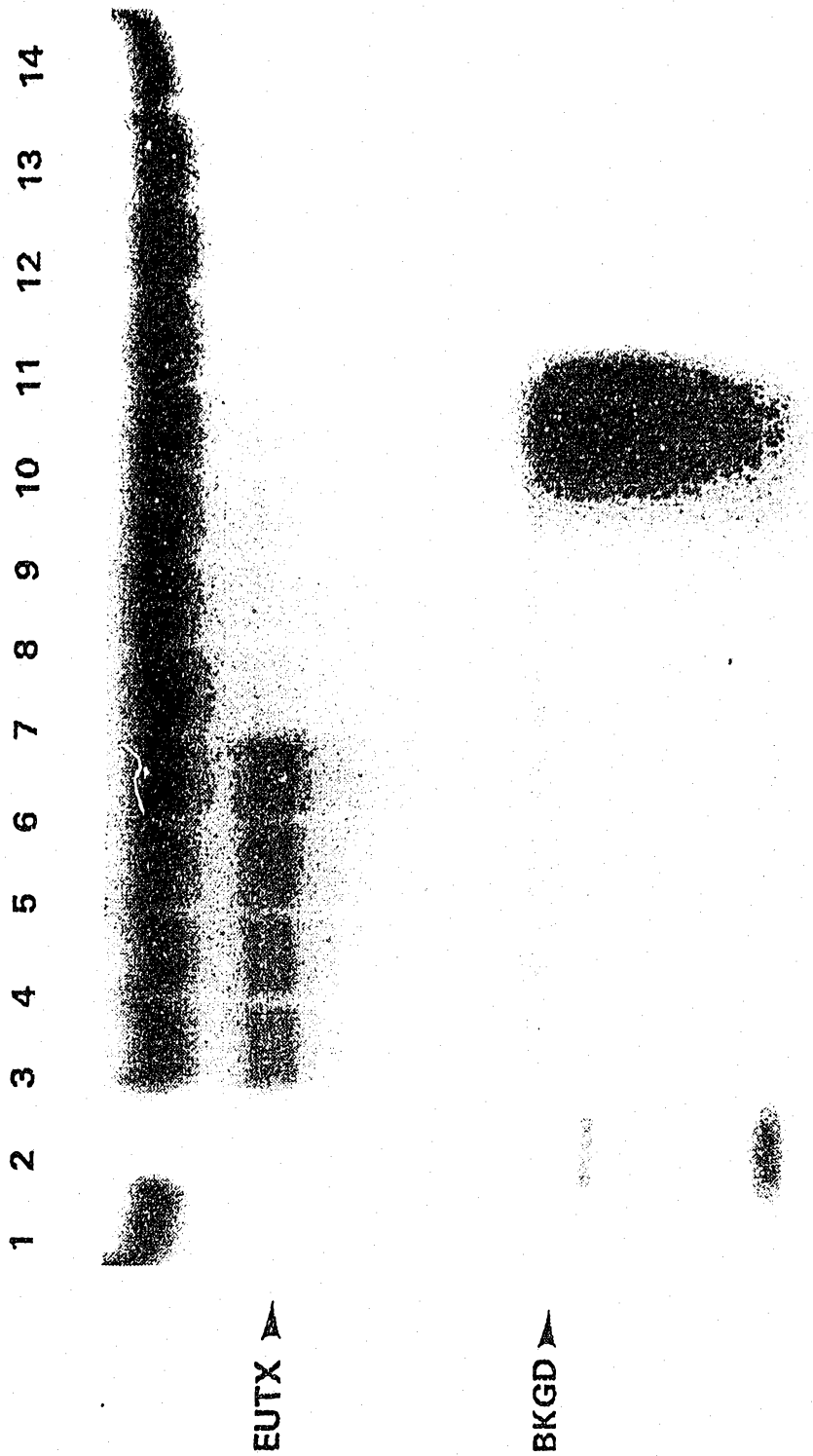
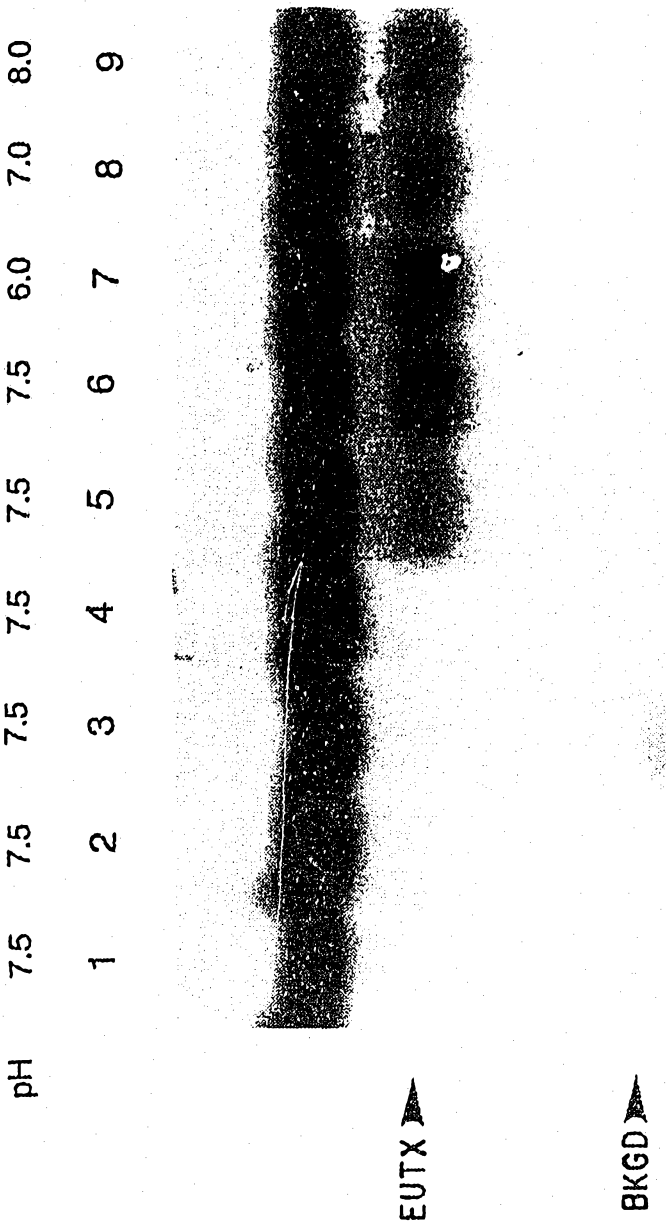


FIGURE 13



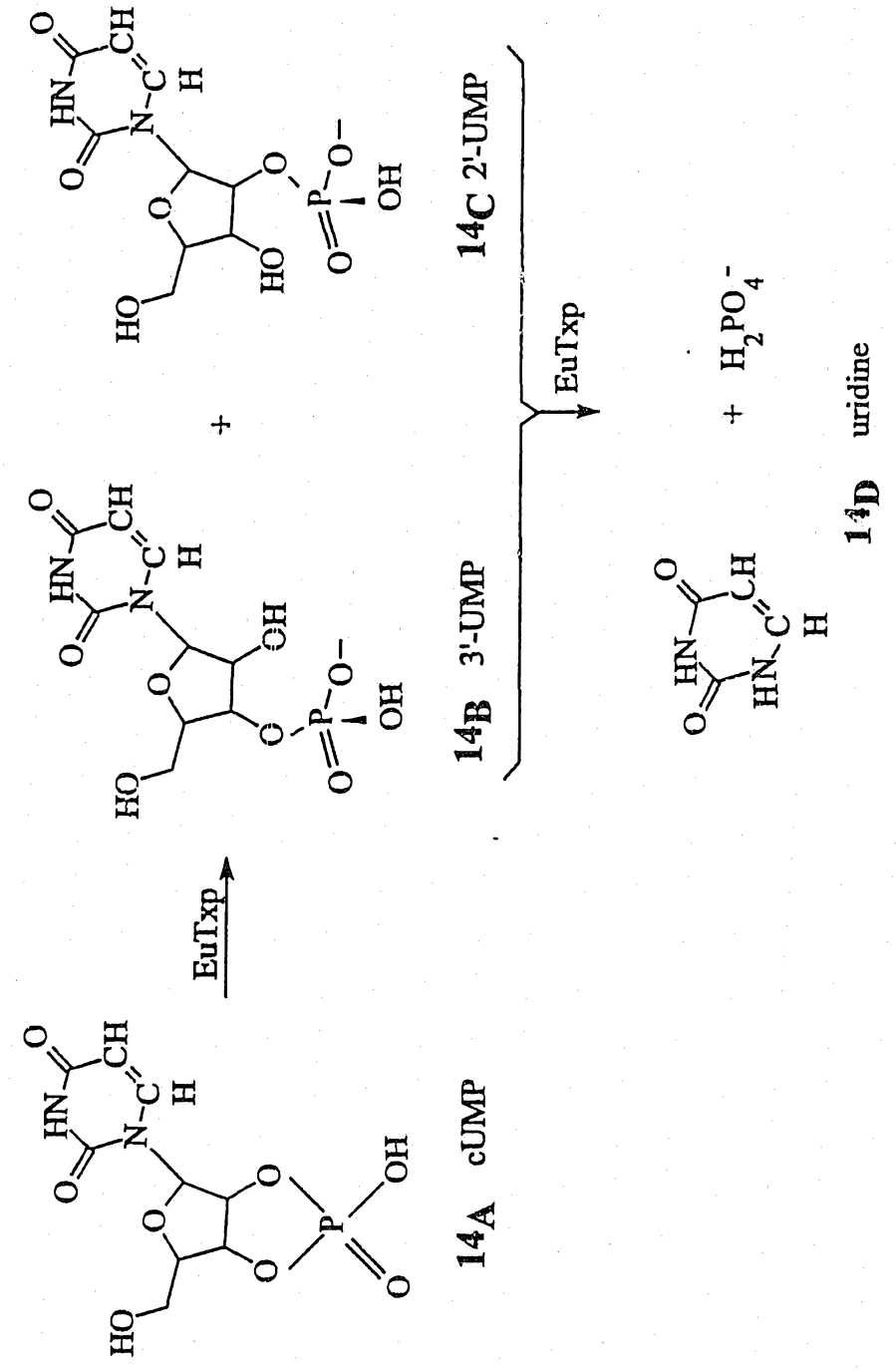
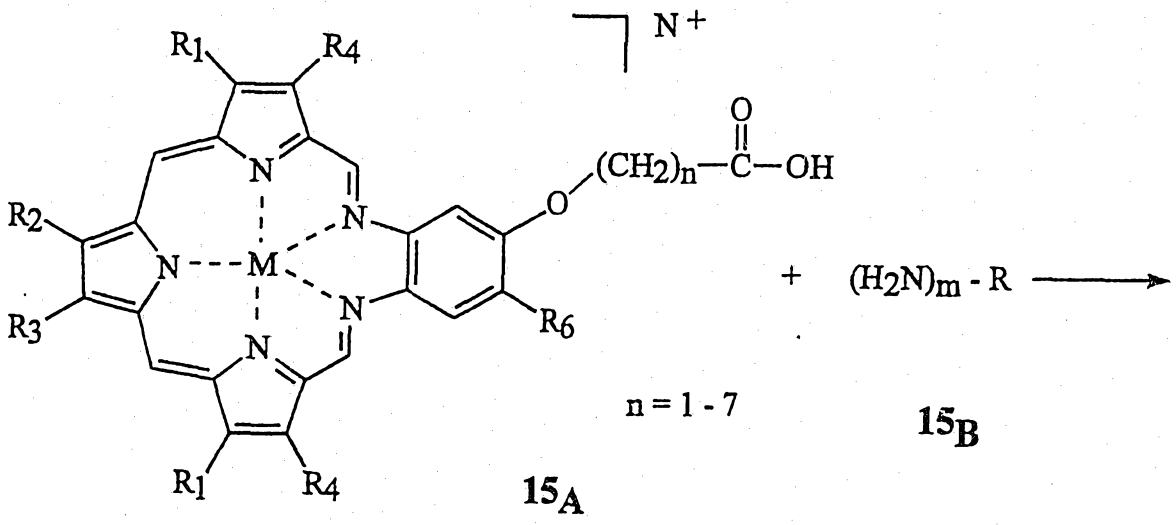


FIGURE 14


N+
R1
R4
R2
R3
R6
N
M
O
(CH2)n—C—OH
+
(H2N)m - R
n = 1 - 7
15A
15B

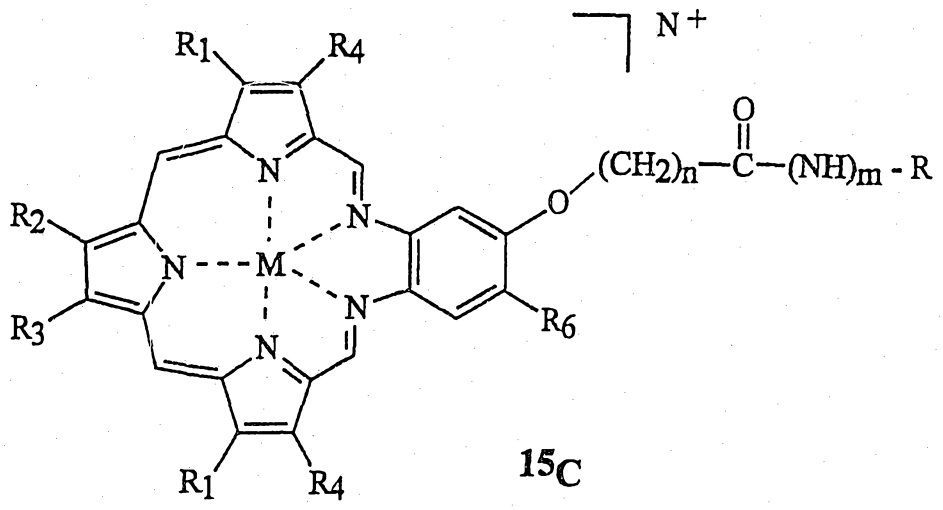

N+
R1
R4
R2
R3
R6
N
M
O
(CH2)n—C—(NH)m - R
15C

FIGURE 15

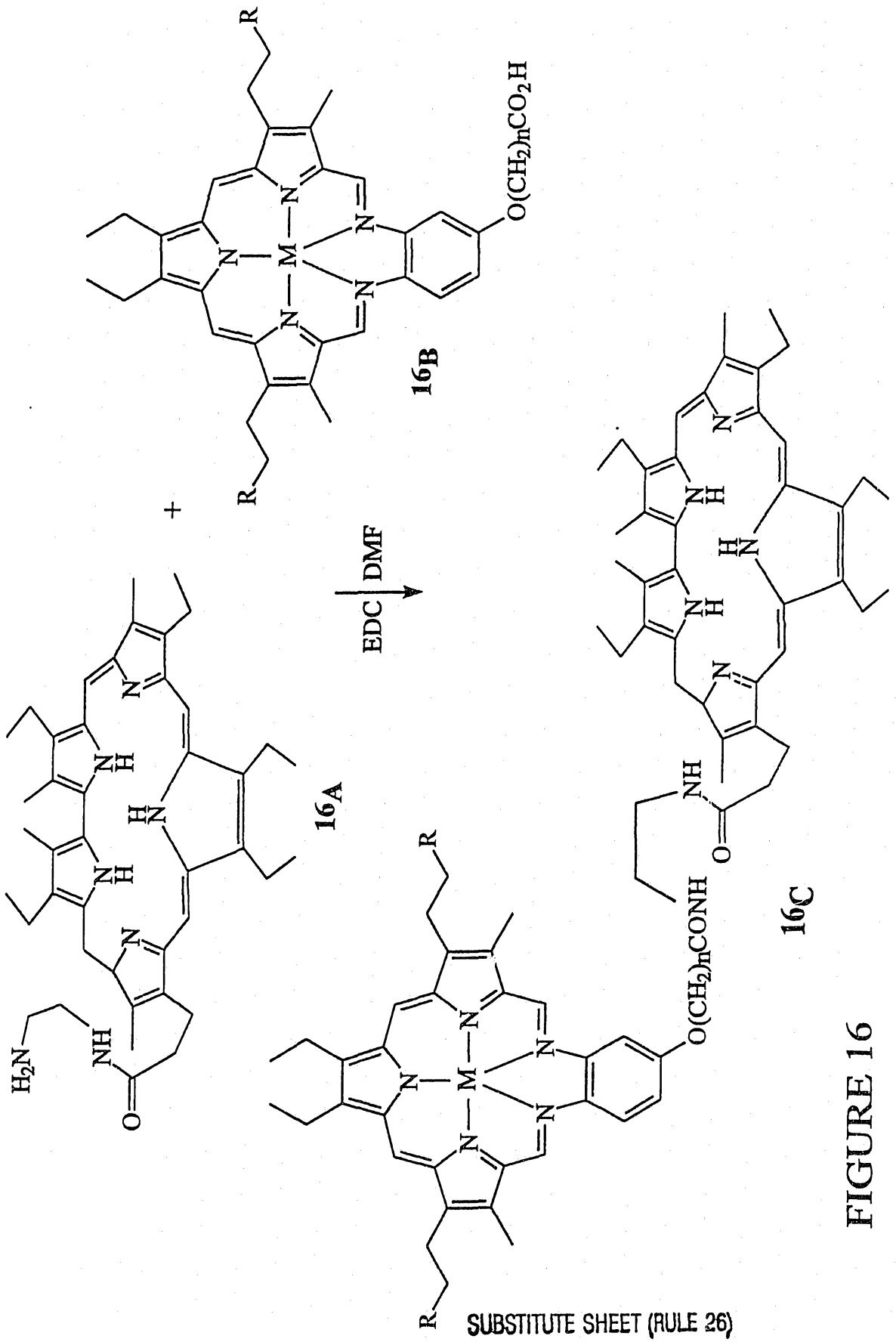


FIGURE 16

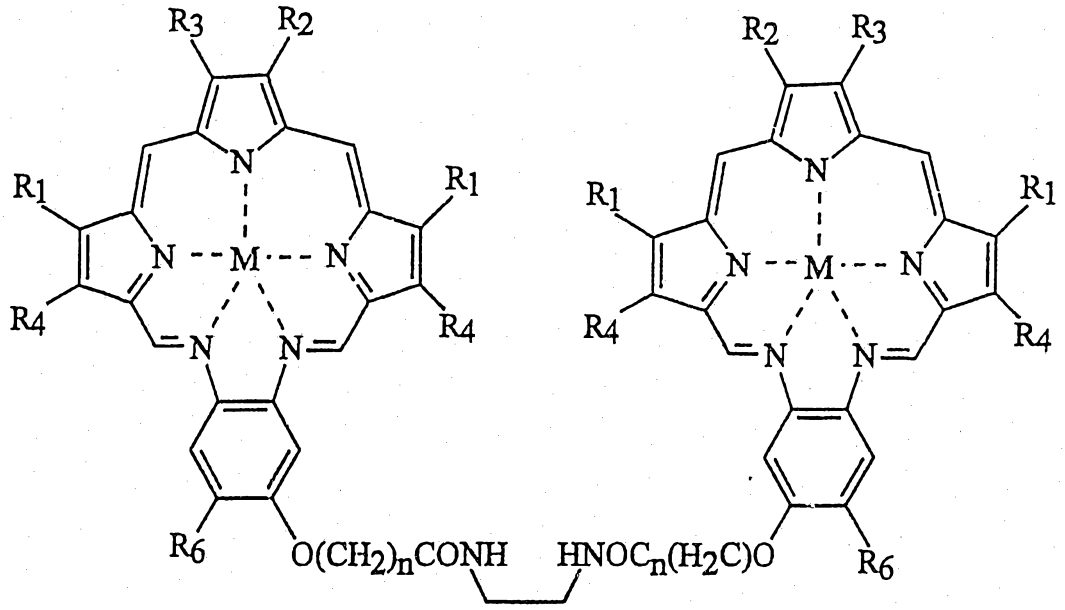


FIGURE 17

SEQ. ID. NO. 7

5'-AT AGC G A TGT TC-3'

3'-C ACU GUA GAA GAG CCG GUA UCG C U ACA AG-5'

Dy

U

SEQ. ID. NO. 8

FIGURE 18A

SEQ. ID. NO. 9

5'-AT AGC G A TGT-3'

3'-C ACU GUA GAA GAG CCG GUA UCG C U ACA AG-5'

Dy

U

Dy

SEQ. ID. NO. 8

FIGURE 18B

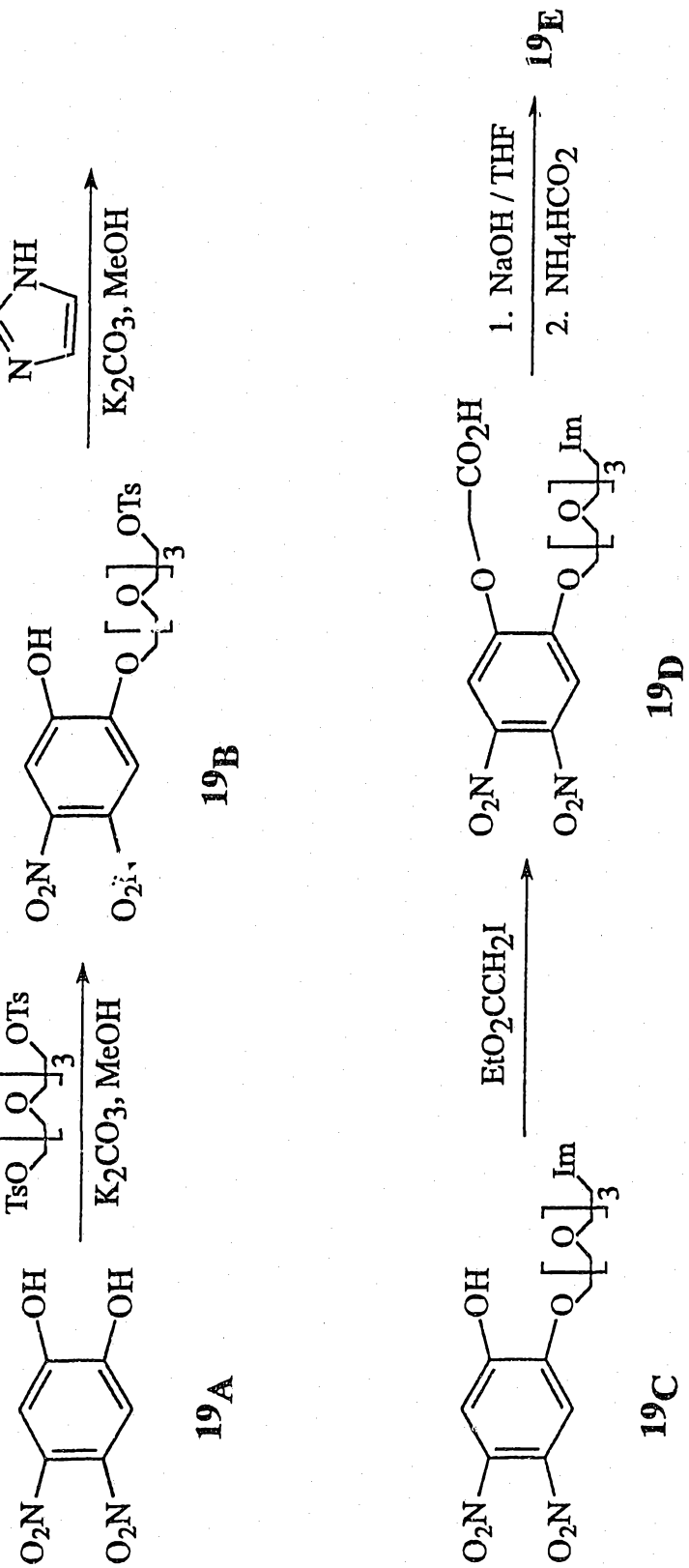

TsO
OTs
3
K2CO3, MeOH
OH
OH
O2N
O2N
19A
N
NH
K2CO3, MeOH
OTs
3
OH
O2N
19B
EtO2CCH2I
Im
3
OH
O2N
O2N
19C
1. NaOH / THF
2. NH4HCO2
CO2H
Im
3
O2N
O2N
19D
19E

FIGURE 19A

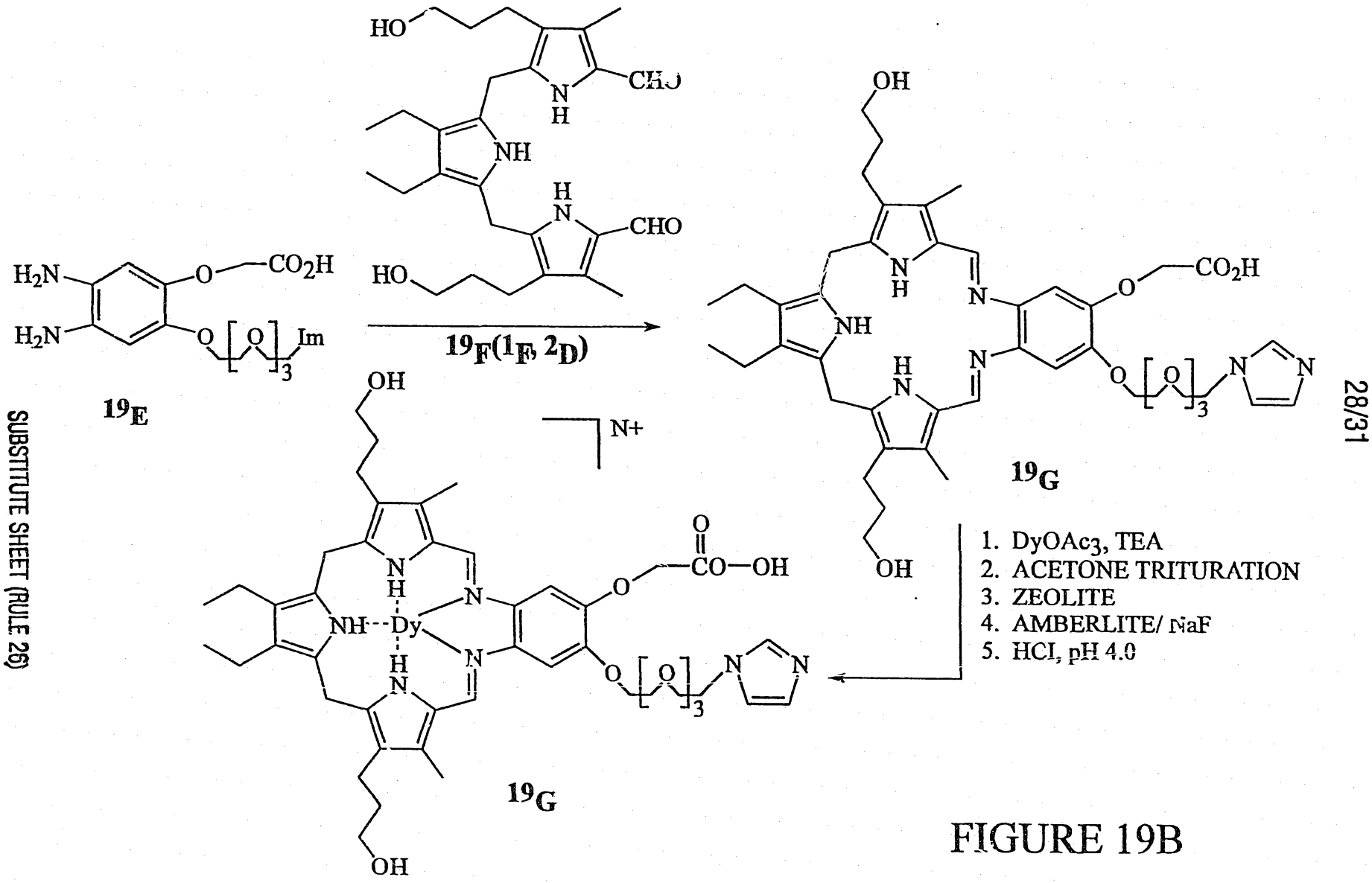


FIGURE 19B

SUBSTITUTE SHEET (RULE 26)

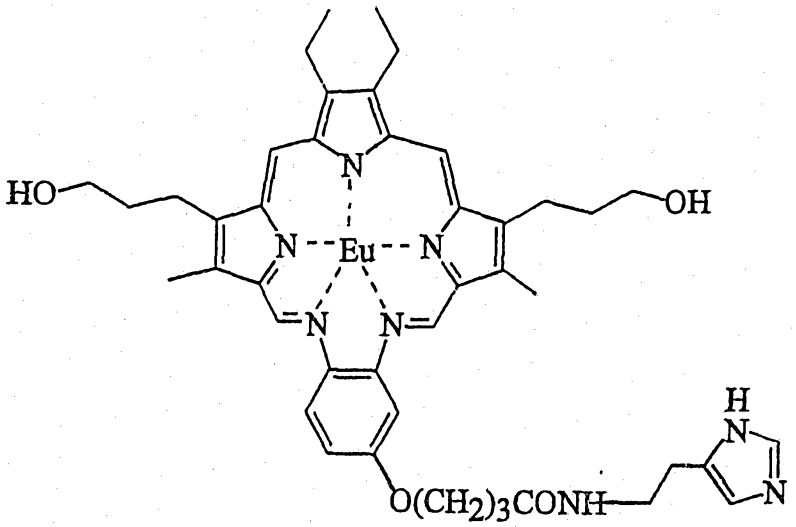


FIGURE 20A

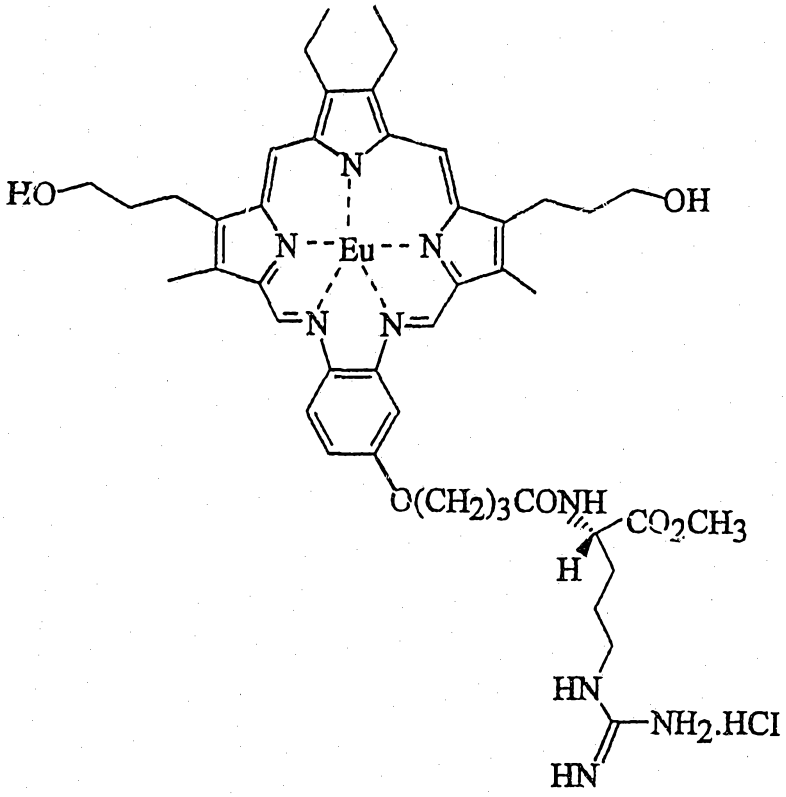
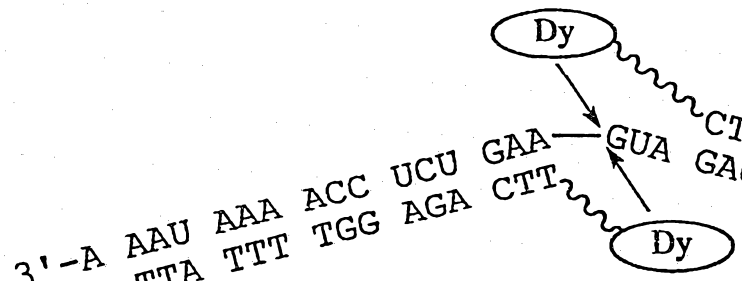


FIGURE 20B



SEQ. ID. NO. 12
SEQ. ID. NO. 11

SEQ. ID. NO. 13

5'-DyTxNH-$(CH_2)_6$-PO_4-TG TGA GCC GGG TGT TG-3' SEQ. ID. NO. 14
5'-DyTxNH-$(CH_2)_6$-PO_4-CTG TGA GCC GGG TGT TG-3' SEQ. ID. NO. 12
5'-DyTxNH-$(CH_2)_6$-PO_4-T CTG TGA GCC GGG TGT TG-3' SEQ. ID. NO. 15
5'-DyTxNH-$(CH_2)_6$-PO_4-AT CTG TGA GCC GGG TGT TG-3' SEQ. ID. NO. 16
5'-DyTxNH-$(CH_2)_6$-PO_4-CAT CTG TGA GCC GGG TGT TG-3' SEQ. ID. NO. 2
3'-A AAU AAA ACC UCU GAA GUA GAC ACU CGG CCC ACA AC-5' SEQ. ID. NO. 11
5'-T TTA TTT TGG AGA CTT-PO_4-$CH_2CHOHCH_2$-NHDyTx-3' SEQ. ID. NO. 13
5'-T TTA TTT TGG AGA CTT-PO_4-$CH_2CH(CH_2OH)(CH_2)_4$-NHDyTx-3' SEQ. ID. NO. 13

FIGURE 21

SUBSTITUTE SHEET (RULE 26)

FIGURE 22



INTERNATIONAL SEARCH REPORT

Intern. ..nal Application No
PCT/US 94/06284

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C07D487/22 C12N15/10 C12Q1/68 B01J31/18

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07D C12N C12Q B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	WO,A,93 14093 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 22 July 1993 cited in the application	
A	WO,A,90 10633 (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM) 20 September 1990	
A	WO,A,92 01781 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 6 February 1992 SEE ESPECIALLY CLAIM 6, PAGE 9, LINE 9	
A	WO,A,91 19730 (MONTESANTO CO.) 26 December 1991	

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

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 1994	06. 12. 94
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Devisme, F

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. al Application No
PCT/US 94/06284

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9314093	22-07-93	US-A-	5252720	12-10-93
		AU-B-	3436793	03-08-93
		CA-A-	2127530	22-07-93
WO-A-9010633	20-09-90	US-A-	4935498	19-06-90
		AU-A-	5278190	09-10-90
		EP-A-	0462206	27-12-91
		JP-T-	4505612	01-10-92
		US-A-	5041078	20-08-91
		US-A-	5162509	10-11-92
		US-A-	5252720	12-10-93
		US-A-	5292414	08-03-94
		US-A-	5256399	26-10-93
		US-A-	5272142	21-12-93
WO-A-9201781	06-02-92	US-A-	5219732	15-06-93
		AU-B-	646290	17-02-94
		AU-A-	8404691	18-02-92
		EP-A-	0540687	12-05-93
		JP-T-	6500098	06-01-94
WO-A-9119730	26-12-91	AU-B-	643542	18-11-93
		AU-A-	8067991	07-01-92
		EP-A-	0533753	31-03-93