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(54) **ECL LABELS HAVING IMPROVED NON-SPECIFIC BINDING PROPERTIES, METHODS OF USING AND KITS CONTAINING THE SAME**

ECL-MARKER MIT VERBESSERTEN NICHTSPEZIFISCHEN BINDUNGSEIGENSCHAFTEN, VERFAHREN ZU DEREN VERWENDUNG UND DIESE ENTHALTENDE KITS

MARQUEURS ELECTROCHIMILUMINESCENTS POSSEDANT DES PROPRIETES DE LIAISON NON SPECIFIQUE AMELIOREES, PROCEDES D'UTILISATION ET KITS ASSOCIES

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(56) References cited:
WO-A-03/068947 **US-A- 5 310 687**
US-A- 5 597 910 **US-A- 5 695 890**

- **PIPPIN, C. G. ET AL: "Kinetics of electron transfer reactions between dioxoactinide(VI) ions and a viologen radical anion" JOURNAL OF THE LESS-COMMON METALS , VOLUME DATE 1988, 149, 193-6 CODEN: JCOMAH; ISSN: 0022-5088, 1989, XP002333473**
- **WILLNER I. ET AL.: 'Photosensitized reduction of CO2 to CH4 and H2 evolution in the presence of ruthenium and osmium colloids: strategies to design selectively of products distribution' JOURNAL OF THE AMERICAN CHEMICAL SOCIETY vol. 109, no. 20, 1987, pages 6080 - 6086, XP002959297**
- **OKURA I. ET AL.: 'Photoinduced hydrogen evolution from water using bipyridinium salts as electron carriers' INORGANICA CHIMICA ACTA vol. 101, 1985, pages L25 - L26, XP002959298**
- **TAKEYAMA N. ET AL.: 'Photo-induced charge separation in carbazole-containing bilayer membrane in the presence of various electron acceptors' CHEMISTRY LETTERS vol. 11, 1985, pages 1735 - 1738, XP002959299**

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(Cont. next page)

- **PENICAUD V. ET AL.: 'New water-soluble diamine complexes as catalysts for the hydrogenation of ketones under hydrogen pressure' EUROPEAN JOURNAL OF ORGANIC CHEMISTRY vol. 7, 1999, pages 1745 - 1748, XP002927664**
- **ZABAN A. ET AL.: 'Relative energetics at the semiconductor/sensitizing dye/electrolyte interface' JOURNAL OF PHYSICAL CHEMISTRY B vol. 102, 1998, pages 452 - 460, XP002959300**
- **YAN S. ET AL.: 'Evaluating interfacial charge transfer kinetics and energetics via laser based spectroelectrochemical methods' PROCEEDINGS-ELECTROCHEMICAL SOCIETY vol. 96, no. 9, 1996, pages 53 - 64, XP002959601**
- **YAN S. ET AL.: 'Semiconductor-based interfacial electron-transfer reactivity: decoupling kinetics from pH-dependent band energetics in a dye-sensitized titanium dioxide/aqueous solution system' JOURNAL OF PHYSICAL CHEMISTRY vol. 100, no. 17, 25 April 1996, pages 6867 - 6870, XP000925275**
- **YAN S. ET AL.: 'Energetics of electron transfer at the nanocrystalline titanium dioxide semiconductor/aqueous solution interface: pH invariance of the metal-based formal potential of a representative surface-attached dye couple' JOURNAL OF PHYSICAL CHEMISTRY B vol. 101, 1997, pages 1493 - 1495, XP002959602**
- **WILL G. ET AL.: 'Potentiostatic modulation of the lifetime of light-induced charge separation in a heterosupermolecule' JOURNAL OF PHYSICAL CHEMISTRY B vol. 103, 1999, pages 8067 - 8079, XP002959603**

Description

CROSS-REFERENCE TO RELATED APPLICATIONS

5 **[0001]** Reference is made to the following issued patents: U.S. Patent Nos. 5,310,687; 5,591,581; 5,597,910; 5,705,402; 5,846,485; 6,066,448; 6,214,552; and 6,207,369.

FIELD OF INVENTION

10 **[0002]** The present invention relates to labels, preferably metal-containing labels for use in assays. In particular, the invention relates to metal labels having ligands substituted with hydrophilic and/or charged groups that prevent the non-specific binding ("NSB") of labeled substances to other materials. The invention also relates to methods for conducting assays, preferably luminescence assays, that use these labels and to kits and compositions containing these labels.

15 ## BACKGROUND OF THE INVENTION

[0003] Documents cited in this application relate to the state-of-the-art to which this invention pertains.

[0004] Electrochemiluminescence (ECL) detection has become an important analytical technique and has been applied in general analysis and diagnostic procedures. Electrochemiluminescence involves electrogenerated species and the 20 emission of light. For example, electrochemiluminescence may involve luminescence generated by a process in which one or more reactants are generated electrochemically and undergo one or more chemical reactions to produce species that emits light, preferably repeatedly.

[0005] In practice, most ECL-based assays involve the use of electrochemiluminescent compounds as labels. The presence of a labeled substance or the participation of a labeled substance in a binding reaction is determined via 25 detection of electrochemiluminescence from the ECL label. Assays for analytes based on the use of labeled binding reagents specific for an analyte of interest may be homogenous in nature (see US Patent No. 5,591,581 and Published PCT Application WO87/06706) or may involve binding reactions occurring on a solid phase such as a magnetic particle (see US Patent No. 5,705,402) or an electrode surface (see US Patent Nos. 6,066,448 and 6,207,369 and Published PCT Application WO98/12539).

30 **[0006]** An important class of ECL labels is organometallic complexes of ruthenium, osmium or rhenium having one or more polydentate heterocyclic nitrogen containing ligands (e.g., bipyridine, phenanthroline, bipyrazine, bipyrimidine, etc., or substituted derivatives thereof) such as those described in US Patent Nos. 5,310,687; 5,597,910; and 5,591,581 and Published PCT Application WO87/06706. These types of labels (in particular, labels based on tris-bipyridyl ruthenium complexes) have found considerable use because of their stability and the efficiency at which they produce ECL.

35 **[0007]** In commercial ECL instrumentation, ECL from these labels is typically produced by oxidizing the labels in the presence of an ECL coreactant, such as tripropylamine. The ECL coreactant is also oxidized at the electrode to produce a strong reductant (see, e.g., US Patent No. 5,846,485). The highly energetic reaction of the reductant and the oxidized label leads to reduction and excitation of the label to a luminescent excited state. Emission of a photon regenerates the label in its original state and allows for detection of the label.

40 **[0008]** Electrochemiluminescence is an extremely sensitive detection technique. The sensitivity of the detection technique is often, however, not the determining factor for the sensitivity of a particular assay. In assays that involve the specific binding interaction between a labeled binding reagent and a binding partner (e.g., an analyte), the sensitivity is often limited by the background signal resulting from the non-specific binding (NSB) of the labeled binding reagent with substances other than the binding partner, e.g., other components of crude samples, other assay reagents, or in the 45 case of solid phase binding assays, the solid phase itself. In some cases, NSB may also lead to a lowering of signals through loss of reagent on the surfaces of containers, pipettes, etc. While ECL labels generally have better NSB properties than other classes of labels, under certain conditions NSB may be a limiting factor in assay sensitivity. This occurs, for example, in i) ECL assays using labeled binding reagents, where the binding reagent itself exhibits high levels of NSB; ii) ECL assays using binding reagents labeled with large numbers of labels and iii) ECL assays carried out using low 50 concentrations of blockers of NSB (such as blocking proteins or detergents) or carried out in the absence of such blockers.

SUMMARY OF THE INVENTION

[0009] The present invention relates to substituted bipyridines and phenanthrolines having at least one and preferably 55 two substituents, the substituents comprising negatively charged groups, preferably sulfate or sulfonate groups. These substituted bipyridines and phenanthrolines, when present as a ligand in a metal complex, reduce the NSB of the complex relative to analogous unsubstituted bipyridines or phenanthrolines. In addition, the present invention relates to organometallic complexes comprising such ligands and labeled assay reagents comprising such organometallic complexes.

[0010] The present invention also relates to luminescent metal complexes having the structure

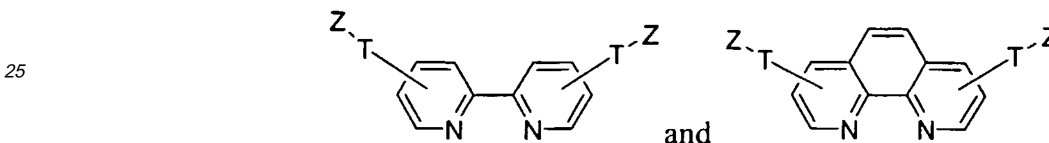
$ML^1L^2_2$

wherein
M is Os or Ru;
L^1 is L^2 as described below or a substituted bipyridine or phenanthroline ligand having at least one substituent that is covalently linked to i) a biological material and/or an assay reagent useful in an assay or ii) a moiety that can participate in a reaction with a biological material and/or an assay reagent useful in an assay so as to form such a covalent linkage; and
L^2 is a substituted bipyridine or phenanthroline ligand that comprises a negatively charged group, preferably a sulfate or sulfonate group, said group acting to reduce the NSB of the complex relative the analogous complex in which L^2 is unsubstituted bipyridine or phenanthroline. Alternatively, L^2 is a substituted bipyridine or phenanthroline ligand that comprises a neutral hydrophilic group, preferably a hydroxyl group or a carboxamide, or a positively charged group, preferably, a guanidinium group.

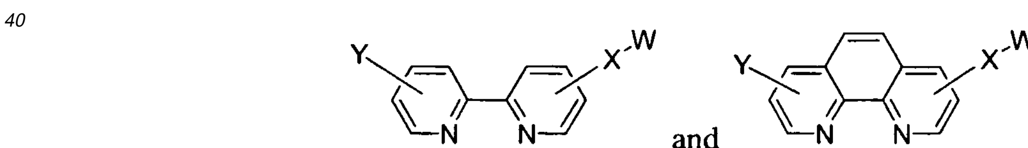
[0011] The present invention also relates to luminescent metal complexes having the structure

$ML^1L^2_2$

wherein
M is Os or Ru;
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is a linker group comprising an alkyl, alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-PO_3^{2-}$, $-PO_3H^-$, $-PO_3H_2$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl; and
L^1 is a substituted bipyridine or phenanthroline ligand having at least one substituent that is covalently linked to i) a biological material and/or a binding reagent useful in an assay or ii) a moiety that can participate in a reaction with a biological material and/or a binding reagent useful in an assay so as to form such a covalent linkage; and L^1 is, preferably, selected from the group consisting of



wherein,
X is a linker group comprising an alkyl, alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Y is H or alkyl and
W is a functional group that is linked to a biological molecule, binding reagent, enzyme substrate or other assay reagent or W is a functional group that when present on the ligand is suitable for conjugating the ligand to a biological material, binding reagent, enzyme substrate or other assay reagent.

[0012] The present invention also relates to labeled materials having one or more metal complexes of the invention, preferably luminescent metal complexes, linked to a material. In one embodiment, the invention relates to labeled materials having the structure $[A]_i[B]_j$, wherein A is a luminescent metal complex of the invention, B is a substance (preferably a biological material and/or an assay reagent useful in an assay) covalently linked to one or more A, i is an integer greater than zero and j is an integer greater than zero (preferably, one). Preferably, A is a metal complex with the structure $ML^1L^2_2$ as described above and A and B are covalently linked via a functional group on L^1.

[0013] The present invention also relates to the use of the luminescent metal complexes of the invention for the generation of luminescence. The complexes may be used in luminescence-based assays such as assays based on the measurement of photoluminescence intensity, time resolved photoluminescence, luminescence energy transfer, luminescence quenching, luminescence lifetime, luminescence polarization, chemiluminescence or, preferably, electrochemiluminescence. The invention also includes the use of complexes of the invention in non-luminescent assays such as electrochemical assays (i.e., assays involving the measurement of current or voltage associated with the oxidation or reduction of the complexes) including electrochemical assays that use the metal complex as a redox label and electrochemical assays that use the metal complex as a redox mediator for measuring the reduction or oxidation of an analyte (e.g., DNA). Preferably, the use of the metal complexes of the invention leads to improved assay performance through low non-specific binding of the complex relative to analogous complexes not presenting low NSB functional groups.

[0014] The present invention also relates to methods of measuring the labeled materials of the invention comprising the steps of i) contacting the labeled materials with a binding reagent and, optionally, a solid phase support; ii) forming a binding complex comprising the binding reagent, the labeled materials, and, optionally, the solid phase support; iii) inducing the labeled materials to produce a signal, preferably, luminescence, more preferably ECL and iv) measuring the signal so as to measure the luminescent metal complex. Preferably, the use of the metal complexes of the invention leads to improved assay performance through low non-specific binding of the metal complex relative to analogous metal complexes not presenting low NSB functional groups.

[0015] The present invention also relates to methods of measuring an analyte in a sample comprising the steps of i) contacting the sample with a labeled binding reagent and optionally a solid phase support; ii) forming a binding complex comprising the binding reagent, the analyte and, optionally, the solid phase support; iii) inducing labels in the labeled binding reagent to produce a signal, preferably, luminescence, more preferably ECL and iv) measuring the signal so as to measure the analyte in the sample; wherein the labeled binding reagent comprises one or more of the low NSB labels described above covalently linked to a binding reagent specific for the analyte. Preferably, the use of the labels of the invention leads to improved assay performance through low non-specific binding of the labels relative to analogous labels not presenting low NSB functional groups.

[0016] The present invention also relates to methods of measuring an analyte in a sample comprising the steps of i) contacting the sample with a labeled analog of the analyte, a binding reagent and, optionally, a solid phase support; ii) forming a binding complex comprising the labeled analog of the analyte, the binding reagent and, optionally, the solid phase support; iii) inducing labels in the labeled analog of the analyte to produce a signal, preferably, luminescence, more preferably ECL and iv) measuring the signal so as to measure the analyte in the sample; wherein the labeled analog of the analyte comprises one or more of the low NSB labels described above covalently linked to an analog of the analyte, and wherein said analog of the analyte competes with the analyte for binding to the binding reagent. Preferably, the use of the labels of the invention leads to improved assay performance through low non-specific binding of the complex relative to analogous labels not presenting low NSB functional groups.

[0017] The present invention also relates to methods of measuring an analyte or a chemical or biological activity in a sample comprising the steps of i) contacting a sample containing the analyte or the chemical or biological activity (or a sample containing substrates or products of the activity) with a low NSB metal complex of the invention; ii) inducing the metal complex to produce a signal, preferably luminescence, more preferably electrochemiluminescence and iii) measuring the signal so as to detect or measure the chemical or biological activity.

[0018] The present invention also relates to methods of improving existing assays employing metal complexes by replacing the metal complexes or ligands on the metal complexes with the low NSB labels or ligands of the invention.

[0019] The invention further relates to kits and compositions containing the low NSB ligands and metal complexes of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020]

Figure 1 shows the results of a comparison of ECL-based sandwich immunoassays on carbon composite electrodes using antibodies labeled with complexes **20, 8,** and **10.** The figure shows the non-specific and specific signals as a function of the number of labels per antibody.

Figure 2 shows the results of a comparison of ECL-based sandwich immunoassays on carbon composite electrodes using antibodies labeled with complexes **20, 2, 15, 12, 17** and **15.** The figure shows the non-specific and specific signals as a function of the number of labels per antibody.

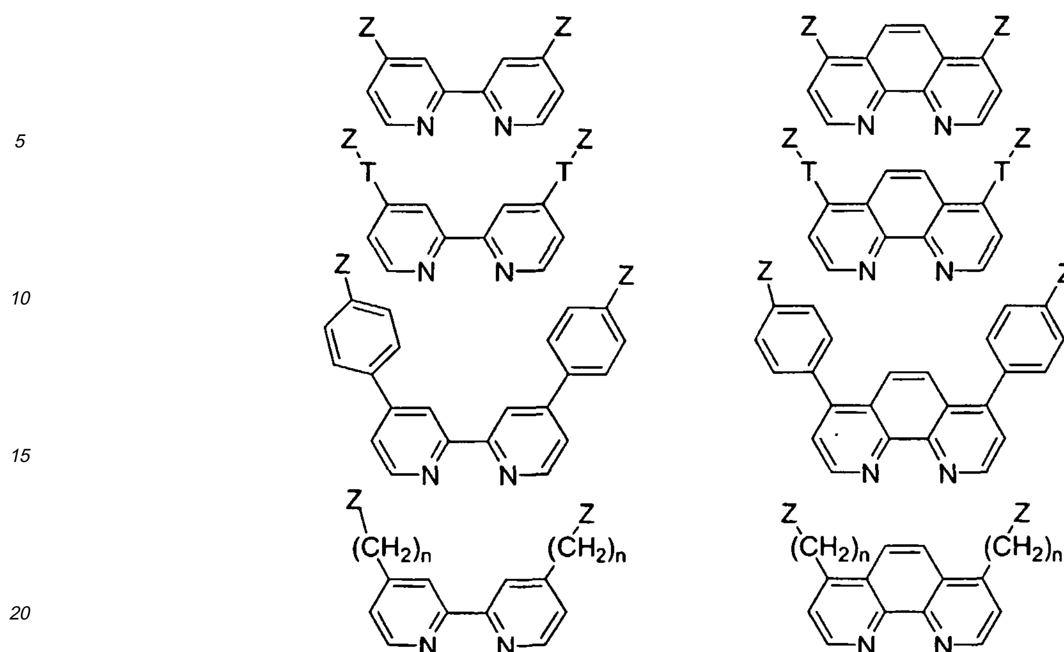
Figure 3 shows the results of a comparison of ECL-based sandwich immunoassays carried out on magnetic particles using antibodies labeled with complexes **20** and **2**. The figure shows the non-specific and specific signals as a function of the number of labels per antibody.

DETAILED DESCRIPTION OF THE INVENTION

[0021] The present invention relates to improved labels for use in assays for measuring an analyte of interest. The word "measurement" and verb forms of "to measure" as used herein refer to both quantitative and qualitative determinations. "Measurement" is understood to include the comparison of a quantity relative to one or more threshhold values or standards, as well as determinations carried out to detect the presence of something and/or the occurrence of an event (e.g., the presence of an analyte or label in a solution or the emission of luminescence). One preferred embodiment of the invention relates to luminescent organometallic complexes useful as labels in ECL assays. The complexes comprise one or more substituted phenanthroline or bipyridine metal ligands, at least one of the metal ligands having substituents that prevent non-specific binding of the ligand (such ligands are referred to hereafter as low NSB ligands). Preferred low NSB substituents comprise negatively charged groups. Negatively charged groups are understood to include neutral or positively charged groups that may be deprotonated in water to a negatively charged state (i.e., having pK_as between 0 and 14). Suitable negatively charged groups include carboxylates, phosphates, phosphonates, sulfates and sulfonates (as well as their protonated forms). Preferably, the negatively charged group will not react under the conditions used to couple amines and carboxylic acids through amide bonds. Preferred negatively charged groups include sulfates and, most preferably, sulfonate groups due to their high stability, low pK_a, and relative insensitivity to the conditions and reagents used to couple amines and carboxylic acids to form amide bonds (e.g., a sulfonate on one ligand of a metal complex will not interfere with the use of a carbodiimide to couple a carboxylic acid on another ligand with an amine-containing reagent. Phosphates and phosphonates are also useful, although they will react with some carboxylic acid activating reagents such as carbodiimides; when these reactions occur under aqueous conditions the products of these reactions are often unstable and do not affect the final result of a conjugation reaction. In an alternate embodiment of the invention, the bipyridine or phenanthroline ligands are substituted with substituents that comprise neutral hydrophilic groups, preferably hydroxyl groups or carboxamides, or positively charged groups, preferably, guanidinium groups (such ligands are also most preferably chosen so as to be insensitive to standard conditions used to make amide bonds).

[0022] The ligands are designed so that organometallic complexes comprising the ligands will give, relative to analogous organometallic complexes comprising unsubstituted bipyridines or phenanthrolines, lower non-specific binding while, preferably, also giving comparable (within a factor of 2) or better ECL signals on a per label basis. The negatively charged groups are, preferably, not directly linked to the phenanthroline or bipyridine rings but are attached through linkers such as alkyl, alkenyl, alkynyl and/or phenyl linkers so as to not have detrimental effect on the ECL properties of organometallic complexes comprising the ligand (e.g., by affecting the redox properties, quantum yields of luminescence, energy of the excited states, or label stability). Such linkers may include heteroatoms in the linking chain or as substituents, although these heteroatoms are, preferably, not directly bonded to the bipyridine or phenanthroline rings (e.g., carbons in an alkyl linking chain may be replaced with oxygens to form one or more alkyl ether linkages or oligoethylene glycol linkers). Preferred linkers are phenyl and alkyl groups. Short (one to five carbon) alkyl groups are especially preferred due their relatively minor influence on ECL and their relative inability to unfold or denature proteins when incorporated in organometallic labels attached to the protein. Single carbon linkers are most preferred since they screen the bipyridine or phenanthroline moieties from the low NSB functional group while not providing additional hydrophobic surface area or substantially increasing the effective volume of the label.

[0023] The ligands are preferably disubstituted at the 5 and 5' positions or, most preferably, the 4 and 4' positions for bipyridyl ligands or the 3 and 8 or, most preferably, the 4 and 7 positions for phenanthrolyl ligands; in metal complexes, these positions have high solvent accessibility and exhibit limited steric crowding. Exemplary low NSB ligands are shown below for bipyridines substituted at the 4 and 4' positions and phenanthrolines substituted at the 4 and 7 positions. Other substitution patterns are not shown but can be defined by analogy.



Where

T = a linker group, preferably, an alkyl, alkenyl, alkynyl or phenyl linker or a combination thereof, optionally having one or more chain carbons replaced by a heteroatom

Z = $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-PO_3^{2-}$, $-PO_3H^-$, $-PO_3H_2$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$

n = an integer, preferably, between 1-5, most preferably 1

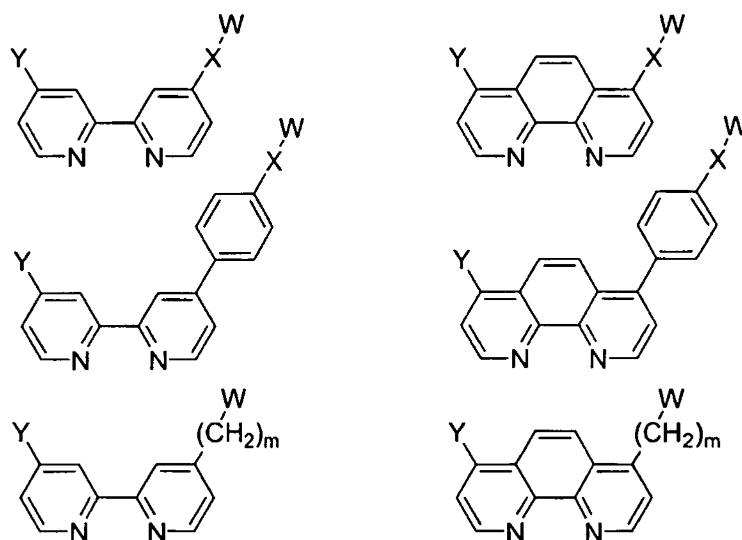
R = alkyl, preferably methyl.

[0024] The present invention relates to organometallic complexes comprising one of the aforementioned low NSB ligands chelated to a metal atom, e.g., Co, Ni, Cu, Ru, Rh, Pd, Ag, Re, Os, Ir, Pt, Fe, Tc, Cr, Mo and W. An important class of such complexes is luminescent ECL-active complexes of Ru and Os, preferably in the Ru(II) or Os(II) oxidation states. The invention includes complexes with the formula ML^2_3, where L^2 is a low NSB ligand of the invention and M is Ru or Os, preferably in the Ru(II) or Os(II) oxidation states. It is understood that notations like ML^2_3 or $ML^1L^2_2$, as used herein, do not exclude the complexes from having an overall net charge (as determined by the charge state of the ligands and the oxidation state of the metal) and that the complexes may be associated with counterions so as to form a composition with an overall neutral charge. The ML^2_3 class of complexes is especially useful in applications that use free unconjugated ECL-active compounds (see, e.g., US Patent No. 5,641,623) and should reduce adsorption of complex to most electrode surfaces.

[0025] In applications that use ECL-active complexes as labels, a particularly useful class of complexes have the formula $ML^1L^2_2$, where M is Ru or Os (preferably in the Ru(II) or Os(II) oxidation states), L^2 is a low NSB ligand as described above, and L^1 is a substituted bipyridine or phenanthroline that is covalently linked to a biological molecule, binding reagent, enzyme substrate or other assay reagent (i.e., to form a labeled reagent, where "label" refers to the metal complex component of the labeled reagent) or, alternatively, has a substituent that is capable of being covalently conjugated to a biological molecule, binding reagent, enzyme substrate or other assay reagent so as to form a labeled reagent. Such ligands may be referred to herein as linking ligands. Biological materials refers herein to materials of biological origin or synthetic analogs thereof, e.g., amino acids, nucleosides, nucleotides, proteins, peptides (and peptidomimetics), nucleic acids (as well as analogs having modified bases or unnatural linkages, e.g., PNAs), hormones, vitamins, sugars, second messengers, polysaccharides, steroids, lipids, phospholipids, cells, organelles, subcellular fragments, viruses, prions, etc. Binding reagents are reagents capable of participating in a specific binding interaction with another material. Examples of binding reagents includes enzymes, antibodies (and fragments thereof), receptors, ligands of biological receptors, metal ligands, nucleic acids, nucleic acid intercalators, nucleic acid major and minor groove binders, haptens, avidin, streptavidin, biotin, purification tags (such as FLAG, myc, glutathione S-transferase, His-tag, etc.), binding partners of purification tags (such as specific antibodies, glutathione, nitrilotriacetic acid, iminodiacetic acid, etc.), etc. Enzyme substrates include molecules that are transformed in enzyme-catalyzed reactions and include cofactors, nucleic acids that are joined or cleaved by enzyme, and peptides that are joined or cleaved by enzymes.

[0026] Examples of functional groups that when present on the ligand are suitable for conjugating the ligand to a biological material, binding reagent, enzyme substrate or other assay reagent include functional groups known in the

art of conjugation chemistry such as: amines, thiols, hydrazides, carboxylic acids, activated carboxylic acids (e.g., acyl chlorides and active esters such as N-hydroxysuccinimide esters), hydroxyls, alkyl halides, isocyanates, isothiocyanates, sulfonyl chlorides, activated phosphates, phosphonates or phosphoramidites, alkenes, alkynes, active carbamates and carbonates, aldehydes, ketones, maleimides, disulfides, α,β unsaturated carbonyls, carbon linked to leaving groups such as halides, mesyl, tosyl, tresyl, etc. For further information on useful functional groups for conjugating labels to reagents and useful conjugation techniques, the reader is directed to G. Hermanson, A. Mallia and P. Smith, *Immobilized Affinity Ligand Techniques* (Academic Press, San Diego, 1992) and G. Hermanson, *Bioconjugate Techniques* (Academic Press, San Diego, 1996). Preferred functional groups include amines, carboxylic acids, active esters and, most preferably, N-hydroxy succinimide esters. Preferably, such functional groups are linked to a bipyridine or phenanthroline ring via a linker chain comprising alkyl, alkenyl, alkynyl and/or phenyl groups so as to limit the effect of the functional group on ECL. Such linkers may include heteroatoms in the linking chain or as substituents, although these heteroatoms are, preferably, not directly bonded to the bipyridine or phenanthroline rings (e.g., carbons in an alkyl linking chain may be replaced with oxygens to form alkyl ethers or oligo(ethylene glycol linkers). The ligands are preferably substituted at the 5 and/or 5' positions or most preferably at the 4 and/or 4' positions for bipyridyl ligands or preferably substituted at the 3 and/or 8 or most preferably at the 4 and/or 7 positions for phenanthrolyl ligands; in metal complexes, these positions exhibit limited steric crowding. Examples of suitable linking ligands are shown below. The ligands are shown as being substituted at the 4 and 4' positions for bipyridine or the 4 and 7 positions for phenanthroline, however, the structures of ligands with other substitution patterns are clear by analogy.



Where

X = a linker group, preferably, an alkyl, alkenyl, alkynyl or phenyl linker or a combination thereof, optionally having a chain carbon replaced by a heteroatom

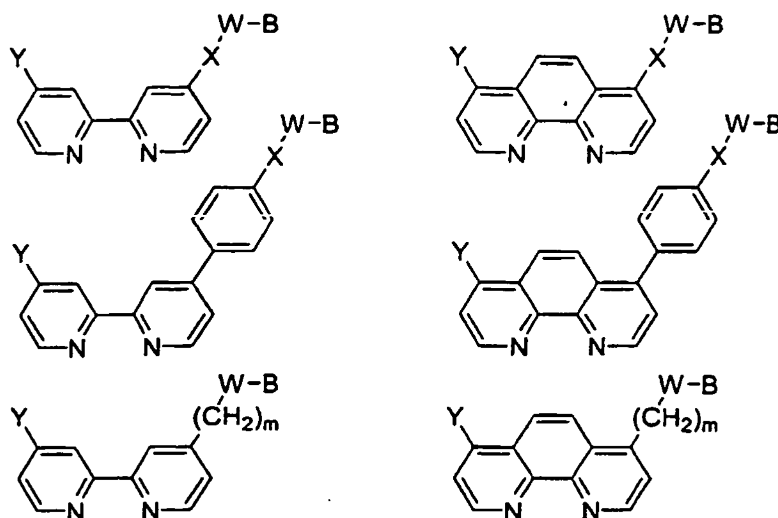
Y = H, or alkyl, preferably, $-CH_3$

W = a functional group that is linked to a biological molecule, binding reagent, enzyme substrate or other assay reagent or a functional group that when present on the ligand is suitable for conjugating the ligand to a biological material, binding reagent, enzyme substrate or other assay reagent

m = an integer, preferably, between 1-5

[0027] In some embodiments of the invention, the labels of the invention are covalently linked to another substance (e.g., a biological material, binding reagent, enzyme substrate or other assay reagent) and are used as labels to allow the measurement of the substance. A preferred embodiment relates to labeled materials having the structure $[A]_i[B]_j$, wherein A is a luminescent metal complex of the invention, B is a substance (preferably, a biological material, binding reagent, enzyme substrate or other assay reagent) covalently linked to one or more A, i is an integer greater than zero and j is an integer greater than zero (preferably, one). The covalent linkage may be provided by a variety of covalent linkages known in the art of conjugation chemistry, e.g., amide bonds, amine linkages, ethers, thioethers, carbamates, ureas, thioureas, Schiff's Bases, carbon-carbon bonds, esters, phosphate esters, sulfonamides, etc. Most preferably, A is a metal complex with the structure $ML^1L^2_2$, wherein M is Ru or Os (preferably in the Ru(II) or Os(II) oxidation states), L^2 is a low NSB ligand of the invention as described above and A and B are covalently linked via a functional group on a linking ligand L^1 as described above. Another embodiment of the invention relates to a labeled substance with the structure $ML^1L^2_2$, wherein M is Ru or Os (preferably in the Ru(II) or Os(II) oxidation states), L^2 is a low NSB ligand of

the invention and L^1 is a linking ligand that is linked to another substance (preferably, a biological material, binding reagent, enzyme substrate or other assay reagent). Examples of suitable L^1 according to this aspect of the invention are shown below. The ligands are shown as being substituted at the 4 and 4' positions for bipyridine or the 4 and 7 positions for phenanthroline, however, the structures of ligands with other substitution patterns are clear by analogy.



Where
X = a linker group, preferably, an alkyl, alkenyl, alkynyl or phenyl linker or a combination thereof, optionally having a chain carbon replaced by a heteroatom
Y = H, or alkyl, preferably, $-CH_3$
B = a substance, preferably, a biological molecule, binding reagent, enzyme substrate or other assay reagent
W = a functional group that is linked to B
m = an integer, preferably, between 1-5

[0028] The labels and labeled materials of the invention are useful in a wide variety of known assay formats employing metal complexes, preferably, luminescent metal complexes, e.g., assays based on the measurement of photoluminescence intensity, time resolved photoluminescence, luminescence energy transfer, luminescence quenching, luminescence lifetime, luminescence polarization, chemiluminescence or, preferably, electrochemiluminescence. For examples of methods for conducting ECL assays, the reader is directed towards U.S. Patents Nos. 5,591,581; 5,641,623; 5,643,713; 5,705,402; 6,066,448; 6,165,708; 6,207,369; and 6,214,552 and Published PCT Applications WO87/06706 and WO98/12539. Preferably, the use of the luminescent metal complexes of the invention leads to improved assay performance through low non-specific binding of the complex relative to analogous complexes not presenting low NSB functional groups. The use of the luminescent metal complexes, preferably, leads to an improvement in a given assay in the ratio of specific to non-specific signal of greater than or equal to a factor of two, or more preferably, greater than or equal to a factor of 5.

[0029] In some applications, the luminescent metal complexes of the invention are used as labels to allow the monitoring of assay reagents such as enzyme substrates or binding reagents. We have found that reagents labeled with the luminescent metal complexes of the invention (especially complexes presenting negatively charged groups) show significant decreases in non-specific binding relative to reagents labeled with analogous complexes that do not present low NSB functional groups. The use of the luminescent metal complexes of the invention significantly reduces the requirement for blocking reagents to reduce non-specific binding, reduces the loss of reagents on the surfaces of containers and fluidic lines, and reduces non-specific signals due to non-specific interactions of labeled binding reagents. These effects are most pronounced when reagents, e.g., antibodies and other proteins, are linked to multiple labels. We have prepared reagents linked to 4-7, 7-10, 10-15, 15-20 and greater than 20 labels and observed only minor amounts of non-specific binding. By going to higher numbers of labels per reagent, assay signals may be increased (relative to assays using luminescent metal complexes not presenting low NSB functional groups) 2-5 fold, 5-10 fold, or greater, while maintaining equivalent or significantly improved ratios of specific to non-specific signals. Alternatively, under conditions that give equivalent specific signals, the ratio of specific to non-specific signal is greatly improved.

[0030] Many assay formats employ solid-phase supports so as to couple the measurement of an analyte or activity to the separation of labeled reagents into solution-phase and solid phase supported portions. Examples include solid-phase binding assays that measure the formation of a complex of a material and its specific binding partner (one of the pair being immobilized, or capable of being immobilized, on the solid phase support), the formation of sandwich complexes

(including a capture reagent that is immobilized, or capable of being immobilized, on the solid phase support), the competition of two competitors for a binding partner (the binding partner or one of the competitors being immobilized, or capable of being immobilized, on the solid phase support), the enzymatic or chemical cleavage of a label (or labeled material) from a reagent that is immobilized, or capable of being immobilized on a solid phase support and the enzymatic or chemical attachment of a label (or labeled material) to a reagent that is immobilized or capable of being immobilized on a solid-phase support. The amount of analyte or activity is determined by measuring the amount of label on the solid phase support and/or in solution, measurements typically being conducted via a surface selective technique, a solution selective technique, or after separation of the two phases. The term "capable of being immobilized" is used herein to refer to reagents that may participate in reactions in solution and subsequently be captured on a solid phase during or prior to the detection step. For example, the reagent may be captured using a specific binding partner of the reagent that is immobilized on the solid phase. Alternatively, the reagent is linked to a capture moiety and a specific binding partner of the capture moiety is immobilized on the solid phase. Examples of useful capture moiety-binding partner pairs include biotinstreptavidin (or avidin), antibody-hapten, receptor-ligand, nucleic acid - complementary nucleic acid, etc.

[0031] It is particularly important in solid phase binding assays that labeled reagents do not bind non-specifically to the solid phase because that binding can result in non-specific signal and significantly reduce the sensitivity of assays. We have tested reagents labeled with labels of the invention in ECL assays using magnetic particles as solid phase supports and ECL assays using electrodes as solid phase supports. In both cases, comparisons to assays using labels that do not present low NSB functional groups showed that assays using the labels of the invention could be optimized to work with more heavily labeled reagents (e.g., 4-7, 7-10, 10-15, 15-20 and greater than 20 labels per reagent), to give higher signals (e.g., 2-5 fold, 5-10 fold, or greater improvements) and to produce higher ratios of specific to non-specific signals (e.g.,1-2 fold, 2-5 fold or greater than 5-fold improvements). The labels of the invention have proved to be particularly beneficial in preventing the non-specific binding of labels and labeled reagents to carbon-containing electrodes used as supports for solid phase assays and/or as electrodes for inducing ECL. Such electrodes include electrodes comprising carbon fibrils or other carbon particles such as plastic composite electrodes comprising carbon fibrils or carbon particles dispersed in a polymeric matrix. Beneficial effects have also been observed in assays carried out on electrodes comprising a thin layer of carbon ink supported on a support (as described in copending application meanwhile published as U.S. Patent No. 6,977,722 entitled "Assay Plates, Reader Systems and Methods for Luminescence Test Measurements", filed on even date.)

[0032] Another aspect of the invention relates to kits for use in conducting assays, preferably luminescence assays, more preferably electrochemiluminescence assays, comprising the ligands and/or metal complexes of the invention and at least one assay component selected from the group consisting of: (a) at least one electro-chemiluminescence coreactant; (b) one or more binding reagents; (c) one or more pH buffers; (d) one or more blocking reagents; (e) one or more preservatives; (f) one or more stabilizing agents; (g) one or more enzymes; (h) one or more enzyme substrates; (i) one or more magnetic particles; (j) one or more electrodes suitable for inducing ECL and (k) one or more detergents. Preferably, at least one of said assay components is covalently linked to a ligand or metal complex of the invention.

[0033] Preferably, the kit comprises the ligands and/or metal complexes of the invention and at least one assay component(s) in one or more, preferably two or more, more preferably three or more containers.

[0034] According to one embodiment, the kit comprises the ligands and/or metal complexes of the invention and one or more of the assay components in one or more containers in dry form.

[0035] According to one embodiment, the ligands and/or metal complexes of the invention and assay components are in separate containers.

[0036] One preferred embodiment relates to a kit for use in conducting electrochemiluminescence assays comprising at least one label according to the invention and at least one electrochemiluminescence coreactant.

[0037] According to one preferred embodiment, the kit comprises the label of the invention and further comprises at least one bioreagent selected from: antibodies, fragments of antibodies, proteins, enzymes, enzyme substrates, inhibitors, cofactors, antigens, haptens, lipoproteins, liposaccharides, cells, sub-cellular components, cell receptors, viruses, nucleic acids, antigens, lipids, glycoproteins, carbohydrates, peptides, amino acids, hormones, protein-binding ligands, pharmacological agents or combinations thereof.

[0038] Another aspect of the invention relates to compositions comprising the metal ligand or metal complex of the invention attached to an assay-performance substance.

[0039] One embodiment of the invention relates to a composition for the detection of an analyte of interest present in a sample, which composition comprises:

(i) the metal complex of the invention containing a functional group and
(ii) an assay-performance-substance linked to said functional group, said assay-performance-substance being capable of binding to the analyte-of-interest or being bound to the analyte-of-interest.

[0040] Preferably, the composition further comprises at least one substance selected from the group consisting of

(i) added analyte of interest or added analogue of said analyte;
(ii) a binding partner of said analyte or a binding partner of said analogue; and
(iii) a reactive component capable of binding with (i) or (ii).

5 **[0041]** Yet another embodiment of the invention relates to a composition for the detection of an analyte of interest present in a sample, which composition comprises:

(a) the metal complex of the invention and
(b) an assay-performance-substance linked to said complex, wherein said assay-performance-substance contains
10 at least one substance selected from the group consisting of:

(i) added analyte of interest or added analogue of said analyte;
(ii) a binding partner of said analyte or a binding partner of an analogue of said analyte; and
(iii) a reactive component capable of binding with (i) or (ii).

15

[0042] A still further embodiment of the invention relates to a composition of matter for use as a reagent in an assay comprising the metal ligand or metal complex of the invention bound to an assay-performance-substance and at least one other component selected from the group consisting of:

20 (a) electrolyte;
(b) analyte of interest or an analog of the analyte of interest;
(c) a binding partner of the analyte of interest or of its analog;
(d) a reactive component capable of reacting with (b) or (c); and
(e) an ECL coreactant and

25

provided, however, that no two components contained within any reagent composition are reactive with one another during storage so as to impair their function in the intended assay.

EXAMPLES

30

ECL Instrumentation.

[0043] ECL examples presented here were conducted on a variety of ECL instrumentation. Some methods used magnetic particles as solid phase supports for binding assays as described in US Patents Nos. 5,935,779; 6,133,043;
35 and 6,200,531. Such ECL measurements involve the collection of the magnetic particles on a platinum electrode, applying electrical energy to the electrode and measuring the emitted luminescence. Other examples employed composite electrodes comprising carbon fibrils dispersed in a polymeric matrix as both the solid phase support for binding assays and the electrode for the induction of ECL (as described in US Patent No. 6,207,369 and Published PCT Application WO98/12539). Because of differences in electrodes, instrumentation, light detectors, detector gains, etc., the ECL signals
40 reported in the different examples should not be directly compared.

Example 1. Synthesis of Ligand **1**

[0044]

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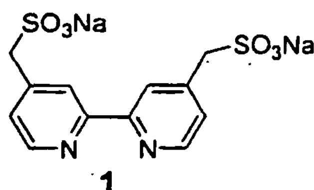

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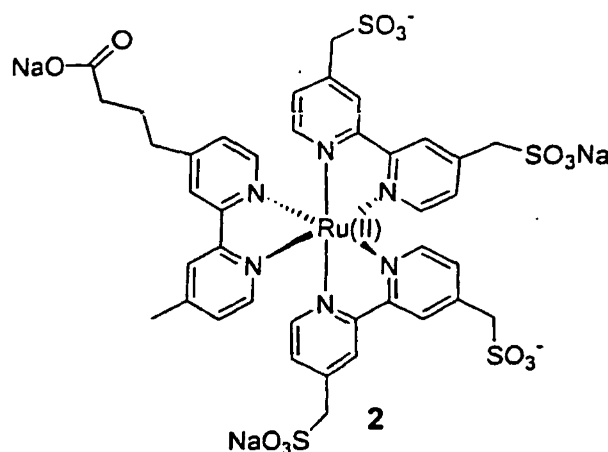
[0045] Sodium sulfite (1.3g) was added to a suspension containing 1.7 g of 4,4'-*bis*-bromomethylbipyridine (Fraser
55 et al., J. Org. Chem. 1997, 62, 9314-9317) in 250 mL of a 7:3 water/methanol mixture. The mixture was refluxed for 60 min, cooled, and then concentrated to ~ 50% of its original volume by rotary evaporation. The remaining aqueous solution was washed with 2 x 20 mL of ethyl acetate. The aqueous phase was concentrated to dryness by rotary evaporation. The remaining solid material was further dried under high vacuum. The product was extracted from the solid material

into 3 x 100 mL portions of boiling 1% water/methanol (by stirring the solid in each portion for 5 min before collecting the supernatant by filtration). The filtrates were combined and concentrated by rotary evaporation to give a crude solid product. The product was purified by recrystallization from water/isopropanol and washed with 1:4 water/isopropanol, isopropanol and ether to give a pure white solid. Typical yields are - 50% but may be improved by recovering product from the recrystallization supernatant.

Example 2. Synthesis of Complex **2**

[0046]

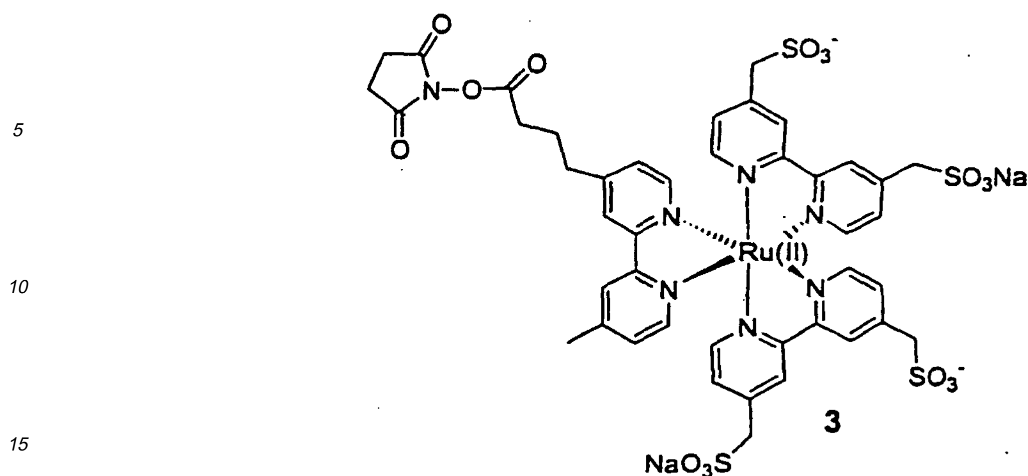


[0047] The first step of the synthesis involved preparing the complex of the ligand 4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid with $RuCl_3$ by a modification of the procedure of Anderson et al. (J. Chem. Research (S) 1979, 74-75). $RuCl_3$ hydrate (0.94 g) was combined with 4 mL of IN HCl and 8 mL of water and dissolved by vigorous mixing. While mixing the resulting solution, 1.02 g of the ligand 4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid (Published PCT Application No. WO87/06706) was added. The mixture was mixed vigorously for 36 hours during which time the ligand-$RuCl_3$ complex precipitated from solution. The product suspension was cooled and the product collected by filtration, washed with 1 N HCl, water and ether, and dried under vacuum. Typical yields were about 60 %.

[0048] The resulting complex (0.47 g) was combined with 0.78 g of the sulfonate-containing bipyridine ligand **1** in 75 mL of ethylene glycol. The solution was refluxed and monitored by visible adsorption at 455 nm to determine when product formation was complete (less than 2.5 hours). The solvent was removed by rotary evaporation (using a bath temperature < 100°C) and the residue was dissolved in 15 mL of I N NaOH and incubated in the dark for 2 hours to hydrolyze any esters that may have formed during the formation of the complex. The solution was diluted to 750 mL with 10 mM formic acid and loaded on a 100 mL column of QAE-Sephadex A-25 (previously equilibrated with 500 mM NaCl, 10 mM formic acid and then washed with copious amounts of 10 mM formic acid). The product was eluted using a gradient of 0-250 mM NaCl in 10 mM formic acid. The product fraction was concentrated by rotary evaporation. Most of the salt was removed by extracting the solid product into 5 x 10 mL of cold methanol (with the addition of a small amount of water if necessary), concentrating the extract and repeating the extraction procedure one more time. The product was then purified on reverse phase silica using 5:95:0.1 acetonitrile/water/trifluoroacetic acid as the eluent. The reverse phase purified product was dissolved in ~ 2 mL of water and the pH adjusted to 6-8 with 1 N NaOH. The resulting solution was applied to a 100 mL column of Sephadex G-15 and eluted with water. The product fraction was lyophilized to give the product as a pure orange solid. Typical yields were - 50%. The three chromatography steps could be replaced with a single purification on QAE-Sephadex A-25 by using a gradient of a volatile salt such as ammonium acetate.

Example 3. Synthesis of Complex **3**

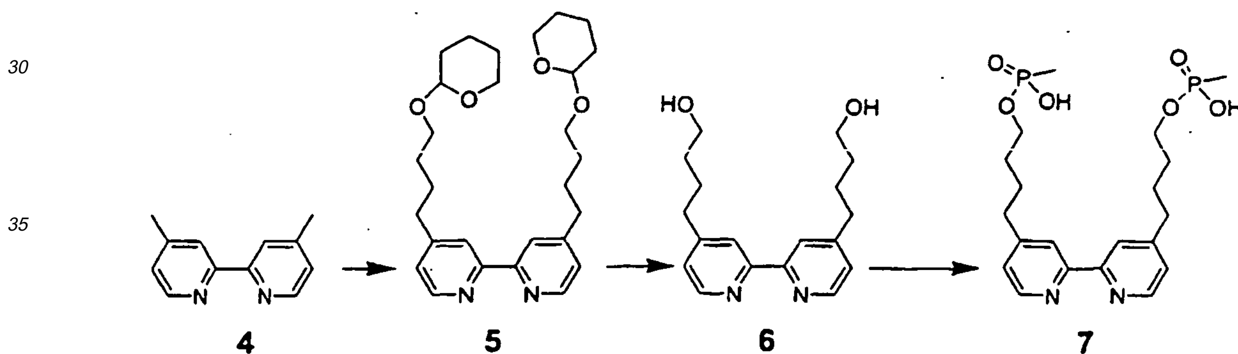
[0049]



[0050] Complex **2** was activated as the NHS ester by the following procedure. Complex 2 (22 mg) was dissolved in 1 mL of 40 mM HCl. While mixing, 5.8 mg of N-hydroxy succinimide (NHS) was added followed by 39 mg of N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide (EDC). The reaction was allowed to incubate for 30 min at room temperature after which it was immediately passed through a 30 mL column of Sephadex G-15 (preequilibrated at 4°C in water) and eluted with water. The product fraction was immediately frozen and lyophilized to give the product as a pure orange solid.

Example 4. Synthesis of Ligand 7

[0051]



[0052] Intermediate **5** was prepared from compound **4** in two separate alkylation steps. A solution was prepared containing 1.38 g of 4,4-dimethyl-2,2-bipyridine in 60 mL of dry THF under inert atmosphere and cooled to 0°C. Added 5.25 mL of lithium diisopropylamine (LDA, 1.5 M solution in cyclohexane) dropwise with stirring and incubated for 30 min at 0 C. Added 1.4 mL of 2-(3-bromopropyloxy)tetrahydro-2H pyran and incubated 3 hrs more at 0°C. The reaction was quenched with sat'd NH_4Cl and concentrated under reduced pressure to remove THF. The remaining aqueous solution was extracted with methylene chloride. The organic fractions were dried over $MgSO_4$, concentrated, and purified by silica chromatography using 4% methanol/methylene chloride as the eluent to the mono-alkylated product in 95% yield. The mono-alkylated product (1.925 g) was dissolved in dry THF and reacted with 4 mL of LDA and 1.05 mL of 2-(3-bromopropyloxy)tetrahydro-2*H*-pyran under the conditions used for the first alkylation. The reaction was worked up by quenching with sat'd NH_4Cl, adjusting the pH to - 8 with potassium carbonate, extracting the product into methylene chloride, drying over $MgSO_4$ and purifying by chromatography on basic alumina (activity I) using 10% ethyl acetate/hexane as eluent. The overall yield of the dialylated intermediate 5 from starting material **4** was 73%.

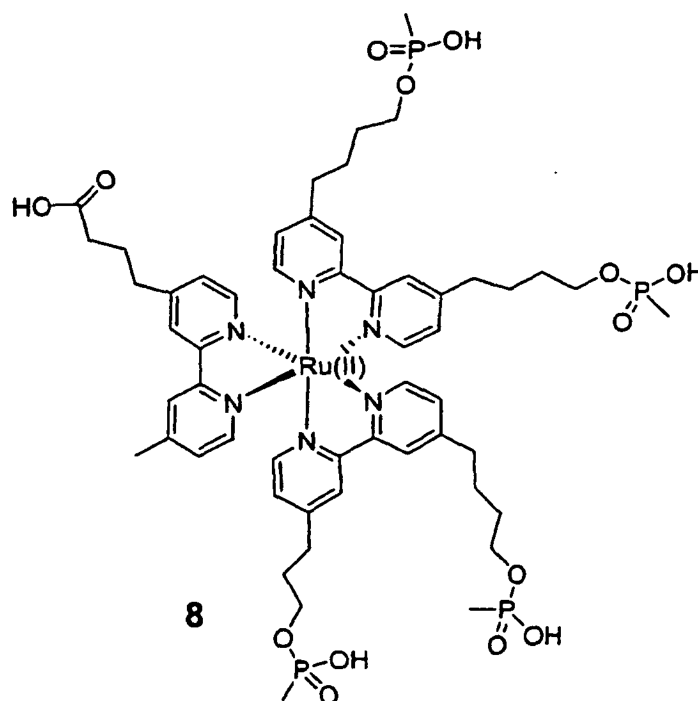
[0053] The intermediate **5** was deprotected to give diol **6** under acidic conditions. Intermediate 5 (1.277 g) was dissolved in 17 mL of 1:1 1 N HCl/methanol. The solution was incubated overnight at room temperature. The pH of the solution was adjusted to - 9 with concentrated ammonium hydroxide and the product was extracted into methylene chloride, dried over $MgSO_4$, and purified by chromatography over basic alumina using 3 % methanol/methylene chloride as the eluent. The diol **6** was collected in 84% yield.

[0054] Preparation of the diphosphonate ester **7** was achieved by reacting the diol 6 with methyldichlorophospite. Diol

6 (60 mg) was dissolved in ~ 8 mL of pyridine. Most of the pyridine was distilled off leaving - 1 mL of solution. The remaining solution was added dropwise with stirring to a chilled (0 C) solution containing 106 mg of methydichlorophosphite in 0.5 mL of methylene chloride. The reaction was allowed to proceed for 45 min at 0°C. The reaction was quenched by the addition of 2 mL of 2 M potassium carbonate. The solution was concentrated to dryness by rotary evaporation. Water was added and evaporated off several times to remove all the pyridine. The product was purified by HPLC chromatography on a C18 reverse phase column using a gradient of acetonitrile in water containing 0.1 % trifluoracetic acid (TFA).

Example 5. Synthesis of Complex **8**

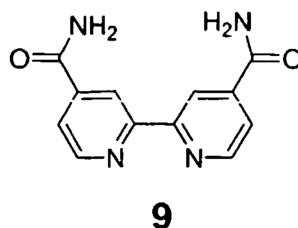
[0055]



[0056] Complex **8** was prepared from ligand 7, the ligand 4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid and $RuCl_3$ by a procedure analogous to that used to prepare complex 2 from ligand 1. Purification was achieved by chromatography on reverse phase silica using 30 % acetonitrile/water + 0.1 % TFA as eluent, ion exchange chromatography on QAE-Sephadex A25 using a gradient of triethylammonium acetate, pH 5.5 as eluent, and desalting on a column of reverse phase silica using a gradient of acetonitrile in water + 0.1% TFA as eluent to give the TFA salt.

Example 6. Synthesis of Ligand **9**

[0057]

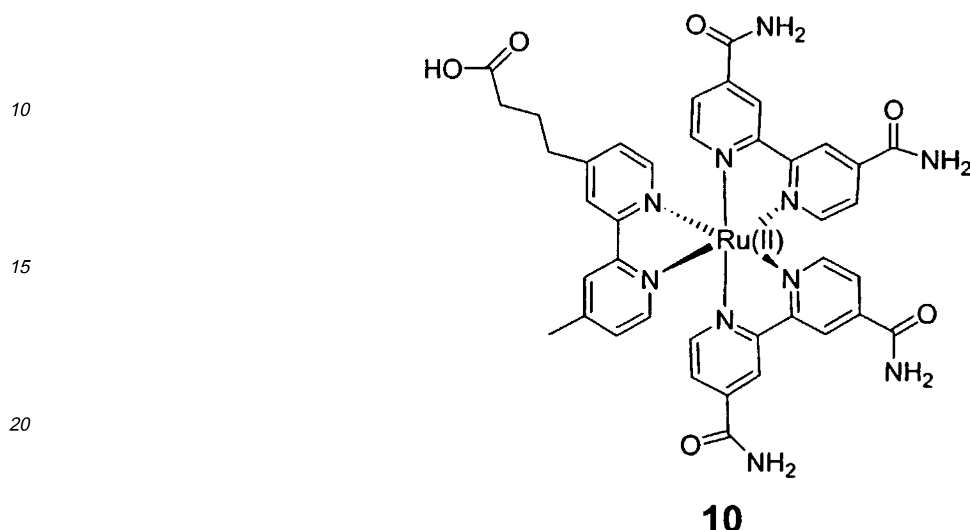


[0058] A suspension containing 1.02 g of 4,4'-dicarboxy-2,2'-bipyridine dimethyl ester in 200 mL of methanol was heated to boiling to dissolve as much of the dimethyl ester as possible. After allowing the solution to cool to room temperature, ammonia gas was bubbled into the solution. The reaction was allowed to proceed until TLC showed the

disappearance of the starting material. The insoluble product **9** was collected by filtration.

Example 7. Synthesis of Complex **1**0

[0059]



[0060] Ligand **9** (300 mg) was combined with 162 mg of $RuCl_3$ hydrate in DMF and refluxed overnight. The insoluble RuL_2Cl_2 intermediate was collected by filtration and washed with acetone and water. A portion of the intermediate (49.8 mg) was combined with 21.8 mg of the ligand 4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid in ~100-150 mL of 1:1 methanol/water and the mixture was refluxed overnight. The resulting solution was decanted to remove insoluble impurities and concentrated by rotary evaporation. Purification was achieved by silica chromatography using methanol saturated with NaCl as the eluent. After removal of the solvent by rotary evaporation, the product was dissolved in concentrated ammonium hexafluorophosphate. The solution was applied to a C18 silica column. Water was used to wash away excess salt. The pure product **10** (as the PF_6 salt) was then eluted using acetonitrile.

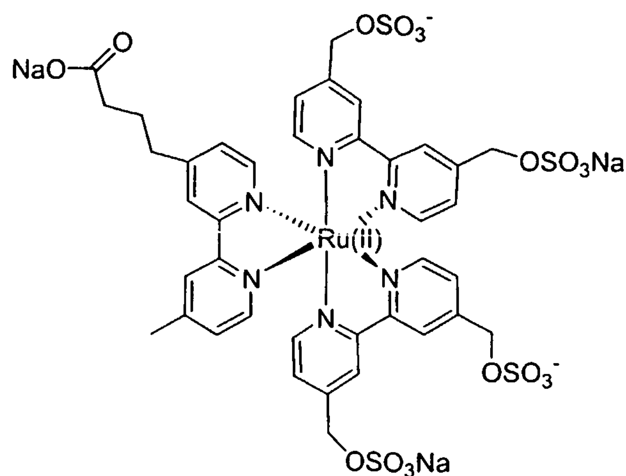
Example 8. Synthesis of Ligand **11**

[0061]

[0062] Ligand **11** was prepared by the reaction of 4,4'-*bis*-hydroxymethyl-2,2'-bipyridine (prepared by the sodium borohydride reduction of 4,4'-*bis*-carboxy-2,2-bipyridine dimethyl ester in refluxing ethanol) with sulfur trioxide pyridine complex (SO_3·pyr). SO_3·pyr (107 mg) was added to 35.3 mg of 4,4'-*bis*-hydroxymethyl-2,2'-bipyridine in 0.75 mL of DMF. After 1.5 hours, the product was precipitated with chloroform and redissolved in water. The aqueous solution was washed with chloroform, neutralized with NaOH solution, and lyophilized to a powder. The product was purified by reverse phase HPLC using a C18 column and a gradient of acetonitrile in water containing 0.1% TFA.

Example 9. Synthesis of Complex **12**

[0063]



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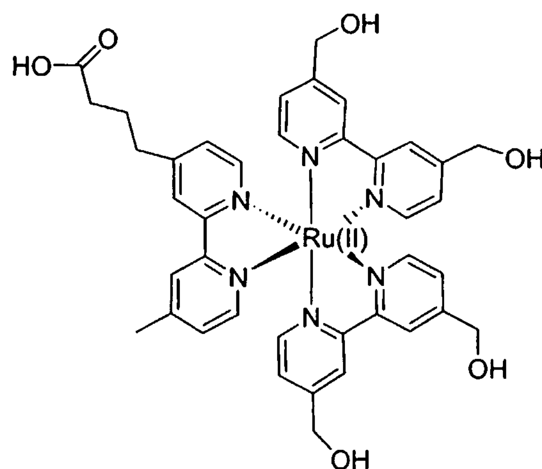
[0064] $RuCl_3$ hydrate (11.7 mg) was combined with 37.6 mg of ligand **11** in 75 mL of 4:1 methanol/water. The mixture was refluxed for 4 hours. The ligand 4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid (10.3 mg) was added and the mixture refluxed overnight. The solution was filtered and the filtrate concentrated by rotary evaporation. The product was purified by reverse phase chromatography using a gradient of acetonitrile in water and silica gel chromatography using methanol in acetonitrile as eluent.

Example 10. Synthesis of Complex **13**

[0065]

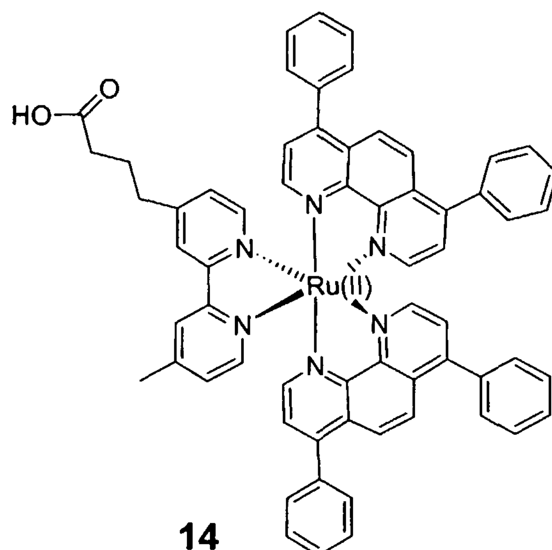


13

[0066] The ligand 4,4'-*bis*-hydroxymethyl-2,2'-bipyridine (58.7 mg) was combined with 58.1 mg of the complex of the ligand 4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid with $RuCl_3$ (see Example 2) in 50 mL of 1:1 methanol/water. The solution was refluxed overnight. The solution was then filtered and concentrated under vacuum. The product was dissolved in 5% acetonitrile/water, loaded on a column of C 18 silica and eluted with 66% acetonitrile/water. The product was redissolved in 5% acetonitrile/water containing ammonium hexafluorphosphate, loaded onto C18 silica and eluted with 40% acetonitrile/water to give the pure complex in 45% yield.

Example 11. Synthesis of Complex **14**

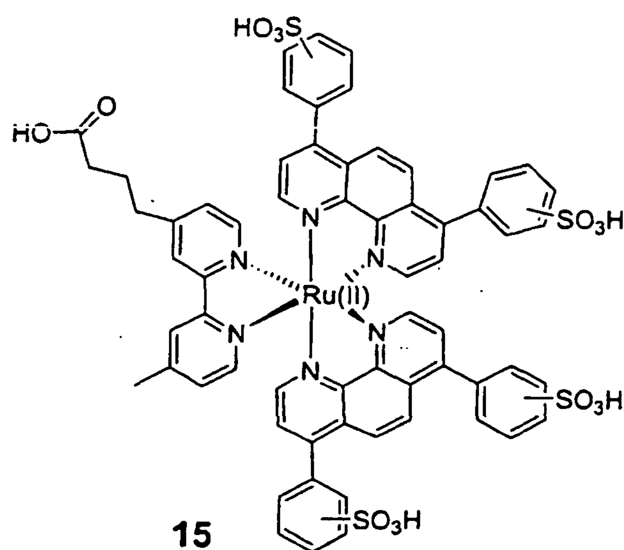
[0067]



[0068] $RuCl_3$ hydrate was combined with two equivalents of 4,7-diphenylphenanthroline in DMF and refluxed overnight to give the bis-diphenylphenanthroline ruthenium dichloride intermediate as a black solid precipitate. The precipitate was collected by filtration, dried and combined with one equivalent of 4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid in 1: 1 dioxane/water and refluxed overnight. The product was purified by reverse phase HPLC on C18 silica using a gradient of acetonitrile in water containing 0.1% TFA.

Example 12. Synthesis of Complex **15**

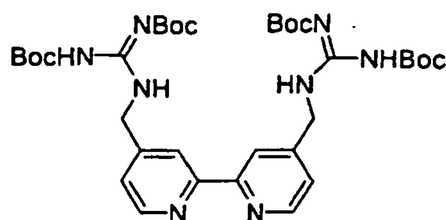
[0069]



[0070] Complex **15** was prepared from 4,7-diphenyl-1,10-phenanthrolinedisulfonic acid (sodium salt) and [4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid]$RuCl_3$ using a procedure analogous to that which was reported above for the preparation of complex 2, except that purification was achieved by a single reverse phase HPLC purification using a C18 silica column and a gradient of acetonitrile in water containing 0.1% TFA.

Example 13. Synthesis of Ligand **16**

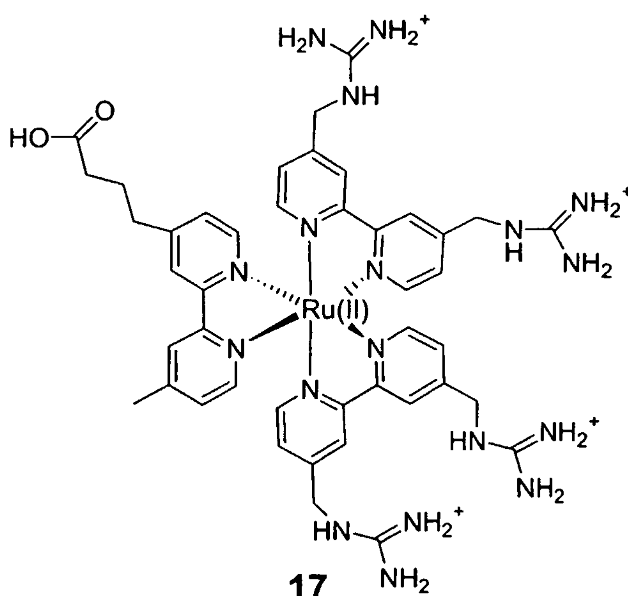
[0071]



[0072] The ligand 4,4'-*bis*-hydroxymethyl-2,2'-bipyridine (108 mg) was combined with 520 mg of *N,N'*-bis-butyloxy-carbonyl-guanidine and 393 mg of triphenylphosphine in 20 mL of 1:1 THF/toluene and cooled down to 0°C. Diisopropyl azodicarboylate (300 uL) was added over the course of 30 min. The mixture was stirred for 3 hours at 0°C and 3 hours at room temperature. The reaction was quenched with 5 mL of water and the solvent removed under vacuum. Trituration with a 1:1 mixture of methanol and acetonitrile gave a white solid. The product was used without further purification. The structure of the product is shown as having the guanidine linked to the bipyridine via the unprotected nitrogen; linkage may have occurred via one of the protected nitrogens.

Example 14. Synthesis of Complex **17**

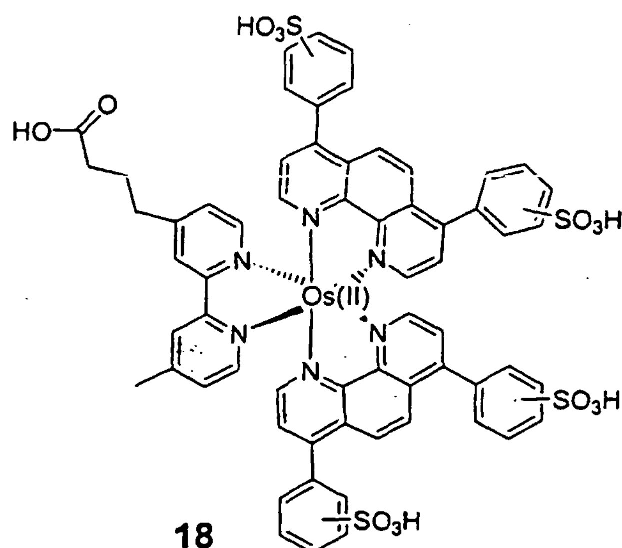
[0073]



[0074] Ligand **16** (28.8 mg) was deprotected in 0.8 mL of 1:1 TFA/CH_2Cl_2. The solvent was removed under reduced vacuum and the deprotected ligand was used without further purification. The deprotected ligand was combined with the complex [4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid]Ru(II)$(DMSO)_2Cl_2$ (prepared by the overnight reaction of 4.8 mg of 4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid with 10.2 mg of Ru$(DMSO)_4Cl_2$ in 0.6 mL of methanol at room temperature) in 1.0 mL of ethanol and the reaction mixture was refluxed for 4 hours. The product was purified by ion exchange on SP-Sephadex C25 using a gradient of TFA in water and by reverse phase chromatography on reverse phase silica using an acetonitrile/water mixture containing 0.1% TFA as the eluent.

Example 15. Synthesis of Complex **18**

[0075]



[0076] A mixture was prepared combining I g of $(NH_4)_2OsCl_2$ and 4,7-diphenyl-1,10-phenanthrolinedisulfonic acid (sodium salt) in 40 mL of 3 N HCl. The mixture was heated for 2 hours at 80°C. The solution was cooled and aqueous potassium carbonate was added until the pH reached ~ 9. The solution was concentrated under vacuum to dryness. Another equivalent of 4,7-diphenyl-1,10-phenanthrolinedisulfonic acid (sodium salt) was added in 20 mL of DMF. The solution was refluxed for 8 hr. After cooling, 200 mg of sodium dithionite was added in a small amount of water. The solution was concentrated under vacuum to - 10 mL after which the *bis*-diphenylphenanthroline osmium dichloride intermediate was precipitated by the addition of acetone. The material was purified once more by precipitating the product from methanol by the addition of acetone.

[0077] A portion (266 mg) of the *bis*-diphenylphenanthroline osmium dichloride intermediate was combined with 60 mg of 4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid in 1:1 dioxane/water and refluxed for 18 hours. The solution was concentrated to dryness under vacuum and the residue redissolved in 10 mL of water. After addition of 200 mg of ammonium hexafluorophosphate, the solution was again concentrated to dryness and the residue was washed with 10 mL portions of acetonitrile. The product was purified on reverse phase silica using methanol as the eluent.

Example 16. Synthesis of NHS Esters.

[0078] The NHS ester of [4-(4-methyl-2,2-bipyridine-4'-yl)-butyric acid]Ru(II)$[bpy]_2$ (Complex **19**) was obtained in purified form (NHS TAG, IGEN International) or was prepared in crude form from the corresponding carboxylic acid (Complex **20**) by one of the methods described below. The NHS ester-containing complex **3** was made in purified form according to Example 3 or was prepared in crude form from complex **2** by one of the methods described below. NHS esters could be prepared in crude form under aprotic conditions or in water. Typically, aprotic conditions were used if possible. Water soluble complexes that were insoluble in typical aprotic solvents were prepared under aqueous conditions.

[0079] Aprotic conditions: The carboxylic acid containing complex was dissolved in methylene chloride to which was added a small excess of NHS and EDC. Some complexes that were not soluble in methylene chloride were reacted in acetonitrile or DMF or mixtures of these solvents with methylene chloride. After allowing the reaction to go to completion (as determined by HPLC or TLC), the solvent was removed under vacuum and the crude product was redissolved in DMF or DMSO to provide a stock solution for use in labeling reactions.

[0080] Aqueous conditions: To a 20 mM aqueous solution of the carboxylic acid-containing complex was added, in order of addition: i) 100 mM morpholinoethanesulfonic acid (MES) buffer, pH 6.0, 50 mM NHS and 200 mM EDC·HCl. The reaction was incubated from 15-30 minutes. The NHS ester product could be used as is. In some experiments, excess EDC was removed immediately after the preparation of the NHS ester by one of the following methods: i) purification of the product by size exclusion chromatography on Sephadex G-15 using water as the eluent; ii) passage of the product through a short column of SP-Sephadex C25 in the sodium form and elution with water or iii) loading of the product on a reverse phase chromatography medium and elution with water/acetonitrile. NHS ester made under one of these aqueous conditions was used immediately after preparation/purification (as described in Example 17).

Example 17. Labeling of Antibodies with NHS Esters

[0081] Antibodies were dissolved in PBS-1 buffer (150 mM potassium phosphate, 150 mM NaCl, pH 7.8) at concen-

trations greater than or equal to 2 mg/mL. ECL labels functionalized with NHS esters (prepared as described in Example 16) were added in water, DMF or DMSO (the final concentration of organic solvent should remain < 20%) and the reaction was allowed to proceed for at least 2 hours at room temperature. The labeled proteins were purified by size exclusion chromatography on Sephadex G-25 (positively charged or neutral complexes) or Sephadex G-50 (negatively charged complexes). A variety of molar ratios of label to protein were generally tried since the efficiency of the coupling reaction could vary from label to label. The average number of labels per protein in the purified product was determined by using a colorimetric protein assay (BCA Assay or Coomassie Blue Assay, Pierce Chemicals) to determine the concentration of protein and the visible absorbance (typically at 455 nm for ruthenium-based labels) to determine the concentration of labels.

Example 18. A comparison of sandwich immunoassays on carbon electrodes using antibodies labeled with complexes **20, 8,** and **10.**

[0082] Plastic composite electrodes comprising carbon nanotubes (fibrils) dispersed in ethylene-*co*-vinyl acetate (EVA) were treated with an ammonia/nitrogen plasma so as to expose fibrils on the surface and introduce amine groups. An immobilized layer of streptavidin was introduced by treating the surface with SMCC (Pierce Chemical) and then reacting the surface with streptavidin (labeled with Traut's reagent to introduce thiol groups).

[0083] Sandwich immunoassays for α-fetoprotein (AFP) were carried out using 3/16" disks of the streptavidin-coated electrodes as solid phase supports and using antibodies, antibody diluents and calibrator diluents from the Elecsys AFP Assay (Roche Diagnostics). The Roche kit uses a biotin-labeled capture antibody and a detection antibody labeled with complex **20.** To compare the labels of the invention, the labeled detection antibody of the kit was replaced with the same antibody but labeled with varying amounts of labels **20, 8,** and **10** as described in Example 17. The streptavidin-coated electrode was contacted with the capture antibody, one of the labeled detection antibodies and a sample containing calibrator diluent (an artificial serum substitute containing bovine serum albumin and bovine IgG) spiked with 1864 ng/mL AFP. A negative control was also run using unspiked calibrator diluent. The assay mixtures were incubated over the electrodes to allow the sandwich complex to form after which the disks were washed with phosphate buffer and transferred to an ECL cell. ECL was measured by contacting the composite electrode with a solution containing tripropylamine (ORIGEN Assay Buffer, IGEN) and scanning the potential at the composite electrode to -2.3 V.

[0084] Figure 1 shows the non-specific signal (i.e., the signal in the absence of analyte) and the specific signal (the difference between the signal in the presence of analyte and the signal in the absence of analyte). The figure shows that labels **8** (presenting negatively charged phosphonate ester groups) and **10** (presenting neutral hydrophilic carboxamide groups) gave roughly the same specific signal as the conventional ECL label **20** and that the specific signal increased roughly linearly with the number of labels per protein. The labels showed significant and unexpected differences, however, in the amount of non-specific binding. The non-specific signal of label **20** increased exponentially with the number of labels per protein. By contrast, label **10** presenting neutral hydrophilic carboxamide groups showed less NSB and label **8** presenting negatively charged showed negligible NSB even at high numbers of labels per protein. Assay signal (S) as well as assay signal to background (S/B) can be improved by using label **8** because the number of labels per antibody can be increased (relative to conventional labels) without increasing NSB signals. The peak S/B measured with label **8** was greater than twice that measure with label **20.** Using the optimal ratios of label to protein (as determined by S/B), the signal measured with label **8** was eight times greater than that measured with label **20.**

Example 19. A comparison of sandwich immunoassays on carbon electrodes using antibodies labeled with complexes **20**, **2**, **15**, **12**, **17** and **15**.

[0085] AFP assays were carried out as described in Example 18 except that the capture antibody was directly adsorbed on oxygen plasma treated fibril-EVA composite electrodes prior to conducting the assay and the calibrators (0 and 2200 ng/mL) were prepared in human serum. Figure 2 shows that the specific signals from antibodies labeled with complexes **20**, **2**, and **15** were roughly similar except for antibodies highly labeled with the bathophenanthroline containing complex **15**; we hypothesize that at high labeling ratios the large planar aromatic structure was able to either sterically block binding or denature the antibody and affect its activity. As also observed in Example 18, Figure 2 shows that the non-specific signals from antibodies labeled with complexes having negatively charged ligands were low even at high numbers of labels per antibody, while the non-specific signal from the conventional label **20** went up exponentially with number of labels per antibody. The optimized S/B obtained using label **2** was greater than 5 times the optimized S/B obtained with label **20.** Using the optimal ratios of label to protein (as determined by S/B), the signal measured with label **2** was more than three times greater than that measured with label **20**. In similar assays (data not shown), antibodies labeled with a complex presenting sulfate groups (complex **12**) behaved similarly to those labeled with complex **2**. Antibodies labeled with the Os analog of complex **15**, in general, showed similar low non-specific binding although the specific signals tended to be roughly 30-50% of the Ru complex. Antibodies labeled with the non-sulfonated version of complex

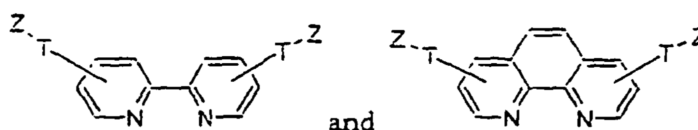
15 (i.e., complex **14**), in general, gave higher backgrounds and lower signals even at low ratios of label to protein. We hypothesize that the highly hydrophobic complex interfered with antibody function. In similar assays, the highly positively charged complex **17** gave extremely high non-specific signals (at - 5-6 labels per antibody, the non-specific signal was more than 100 times the non-specific signal from antibodies labeled with complex **2**). This result shows that the excellent behavior of the negatively charged complexes is not simply due to having hydrophilic charged groups but that the sign of the charge is important. We hypothesize that complex **17** may find use in some selected applications where assays are carried out near highly positively charged surfaces (the positive charge would be beneficial in those conditions for preventing NSB). Complex **17** and other complexes having ligand **16** may also find use in applications requiring the adsorption of a luminescent label to an electrode (e.g., in luminescence-based sensors, ECL-based HPLC detectors or ECL displays using ECL labels adsorbed on electrodes or in films, such as Nafion films, deposited on an electrode).

Example 20. A comparison of sandwich immunoassays on magnetic particles using antibodies labeled with complexes **20** and **2**.

[0086] AFP assays were carried out using the Elecsys AFP Assay (Roche Diagnostics) reagents except that the standard detection antibody was replaced with the same antibody but labeled with varying amounts of complexes **20** or **2.** A sample (0.007 mL) containing a known concentration of AFP (0 or 1000 ng/mL) in a buffered solution containing blocking proteins was combined with 0.035 mL of the biotin-labeled capture antibody (0.0045 mg/mL) and 0.035 mL of the detection antibody (0.012 mg/mL). The reaction mixture was incubated at room temperature for 15 minutes after which 0.035 mL of streptavidin-coated magnetic particles (0.72 mg/mL) and 0.139 mL of a buffered solution of tripropylamine (ORIGEN Assay Buffer, IGEN International) was added. The reaction mixture was incubated for an additional 15 minutes, after which the suspension was analyzed using an ORIGEN M-8 Instrument (IGEN International) and ECL detection. Figure 3 shows that label **2** gave slightly less specific signal than label **20**, but drastically less non-specific signal, especially for heavily labeled antibodies. The optimized S/B obtained using label **2** was more than two times the optimized S/B obtained with label **20**.

Claims

1. A bipyridine or phenanthroline ligand selected from the group consisting of:



wherein,
T is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OS_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl,
provided that the ligand is not a 4,4'-dipropylsulfonate-2,2'-bipyridinium radical anion.

2. The ligand of claim 1, wherein T is $-(CH_2)_n-$ and n is an integer between 1 and 5.

3. The ligand of claim or 2, wherein Z is $-SO_3$- or $-SO_3H$.

4. The ligand of claim or 2, wherein Z is $-OSO_3$- or $-OSO_3H$.

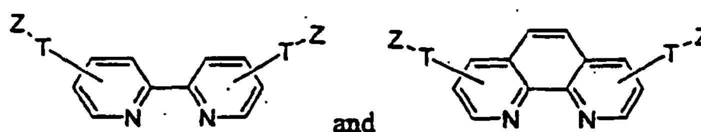
5. The ligand of claim or 2, wherein Z is $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(Me)O_2^-$ or $-OP(Me)O_2H$.

6. The ligand of claim or 2, wherein Z is $-[NHC(NH_2)_2]^+$ or $-NHC(NH)NH_2$.

7. A luminescent metal complex comprising the ligand of claim 2, and a metal atom, the metal atom being bound to the ring nitrogens of the ligand.

8. A method for conducting a luminescence-based assay comprising the steps of:

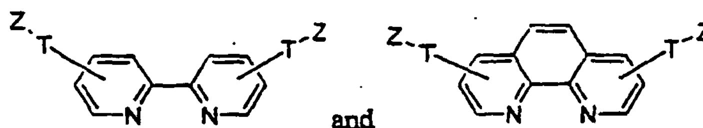
(a) using a luminescent metal complex comprising a ligand selected from the group consisting of



wherein,
T is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $NHC(NH)NH_2$;
R is alkyl; and
said metal complex comprises a metal atom that is bound to the ring nitrogens of said ligand;
(b) inducing said metal complex to emit luminescence; and
(c) measuring the emitted luminescence.

9. A method as recited in claim 8, wherein said metal complex exhibits reduced non-specific binding in said assay relative to the analogous complex having Z = H.

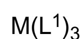
10. A luminescent metal complex having the structure
wherein
M is Os or Ru; and
L^1 is a ligand selected from the group consisting of:



wherein,
T is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, OPO_3^{2-}, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl.

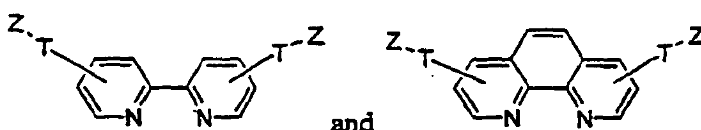
11. A method for conducting a luminescence-based assay comprising the steps of:

(a) using a luminescent metal complex having the structure



$$M(L^1)_3$$

wherein
M is Os or Ru; and
L^1 is a ligand selected from the group consisting of:



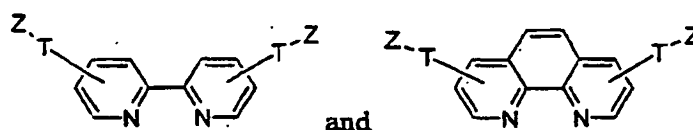
wherein,
T is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom,
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)CO_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl;
(b) inducing said metal complex to emit luminescence; and
(c) measuring the emitted luminescence.

12. A method as recited in claim 11, wherein said metal complex exhibits reduced non-specific binding in said assay relative to the analogous complex having Z = H.

13. A luminescent metal complex having the structure

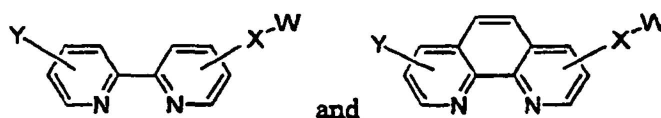
$$ML^1L^2_2$$

wherein
M is Os or Ru;
L^1 is a substituted bipyridine or phenanthroline ligand having at least one substituent that can react with a biological material, binding reagent, enzyme substrate or other assay reagent so as to form a covalent linkage; and
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is a linker group comprising an alkyl, alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $- OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl; and wherein
the biological material, binding reagent, enzyme substrate or other assay reagent is selected from the group consisting of amino acids, nucleosides, nucleotides, proteins, peptides, peptidomimetics, nucleic acids, and peptide nucleic acids.

14. The luminescent metal complex of claim 13, wherein L^1 is selected from the group consisting of



wherein,
X is a linker group comprising an alkyl, alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Y is H or alkyl and
W is a substituent that can react with the biological material, binding reagent, enzyme substrate or other assay reagent so as to form a covalent linkage.

15. The luminescent metal complex of claim 13, wherein Z is $-SO_3$- or $-SO_3H$.

16. The luminescent metal complex of claim 13, wherein Z is $-OSO_3$- or $-OSO_3H$.

17. The luminescent metal complex of claim 13, wherein Z is $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(Me)O_2^-$ or $-OP(Me)O_2H$.

18. The luminescent metal complex of claim 13, wherein Z is $-[NHC(NH_2)_2]^+$ or $-NHC(NH)NH_2$.

19. The luminescent metal complex of claim 13, wherein M is Ru.

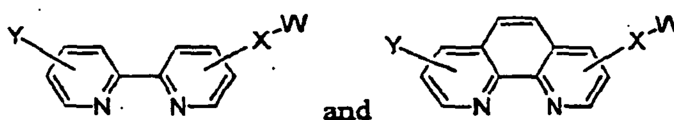
20. The luminescent metal complex of claim 13, wherein W is an acyl chloride or an N-hydroxysuccinimide ester.

21. The luminescent metal complex of claim 13, wherein W is a carboxylic acid group, an amine group, or a hydroxyl group.

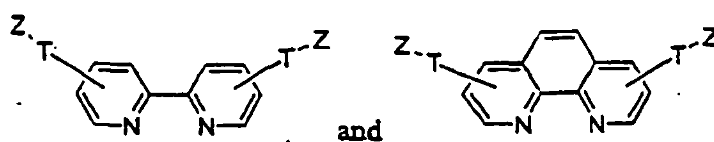
22. A luminescent metal complex having the structure

$$ML^1L^2_2$$

wherein
M is Os or Ru;
L^1 is selected from the group consisting of



wherein,
X is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom;
Y is H or alkyl and
W is a functional group that can react with a biological material, binding reagent, enzyme substrate or other assay reagent so as to form a covalent linkage; and
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom; and
Z is $-SO_3^-$ or $-SO_3H$; and wherein
the biological material, binding reagent, enzyme substrate or other assay reagent is selected from the group consisting of amino acids, nucleosides, nucleotides, proteins, peptides, peptidomimetics, nucleic acids, and peptide nucleic acids.

23. The luminescent metal complex according to claim 22, wherein M is Ru.

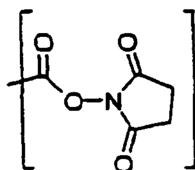
24. The luminescent metal complex according to claim 22, wherein T is $-(CH_2)_n-$, X is $-(CH_2)_m-$ and n and m are each integers between 1 and 5.

25. The luminescent metal complex of claim 22, wherein L^2 is a phenanthroline, ligand substituted at the 4 and 7 positions or L^2 is a bypyridine ligand substituted at the 4 and 4' positions.

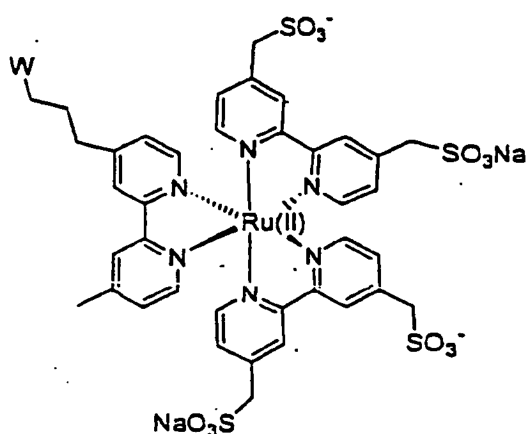
26. The luminescent metal complex of claim 22, wherein W is an acyl chloride or an N-hydroxysuccinimide ester.

27. The luminescent metal complex of claim 22, wherein W is a carboxylic acid group, an amino group, or a thiol group.

28. The luminescent metal complex of claim 22, wherein W is



29. A luminescent metal complex with the structure:

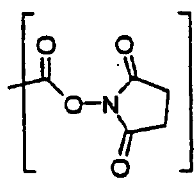


wherein W is a functional group that can react with a biological material, binding reagent, enzyme substrate or other assay reagent so as to form a covalent linkage; and wherein
the biological material, binding reagent, enzyme substrate or other assay reagent is selected from the group consisting of amino acids, nucleosides, nucleotides, proteins, peptides, peptidomimetics, nucleic acids, and peptide nucleic acids.

30. The luminescent metal complex according to claim 29, wherein W is an acyl chloride or an N-hydroxysuccinimide ester.

31. The luminescent metal complex according to claim 29, wherein W is a carboxylic acid group, an amino group, or a hydroxyl group.

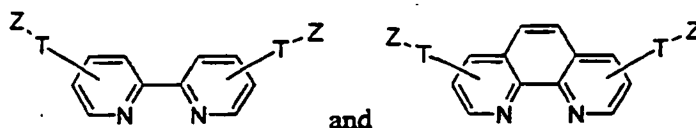
32. The luminescent metal complex of claim 29, wherein W is



33. A labeled material comprising a luminescent metal complex having the structure

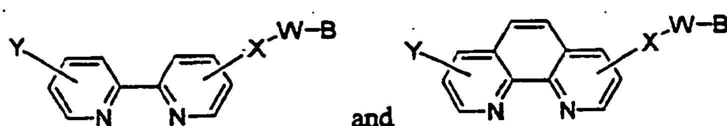
$ML^1L^2_2$

wherein
M is Os or Ru;
L^1 is a substituted bipyridine or phenanthrolines ligand having at least one substituent that is covalently linked to a biological material, binding reagent, enzyme substrate or other assay reagent; and
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is a linker group comprising an alkyl, alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3$-, $-SO_3H$, $-OSO_3$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl; and wherein
the biological material, binding reagent, enzyme substrate or other assay reagent is selected from the group consisting of amino acids, nucleosides, nucleotides, proteins, peptides, peptidomimetics, nucleic acids, and peptide nucleic acids.

34. The labeled material of claim 33, wherein L^1 is selected from the group consisting of



wherein,
X is a linker group comprising an alkyl, alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Y is H or alkyl;
B is the biological material, binding reagent, enzyme substrate or other assay reagent; and
W is a functional group that is linked to B.

35. The labeled material of claim 33, wherein Z is $-SO_3$- or $-SO_3H$.

36. The labeled material of claim 33, wherein Z is $-OSO_3$- or $-OSO_3H$.

37. The labeled material of claim 33, wherein Z is $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(Me)O_2^-$ or $-OP(Me)O_2H$.

38. The labeled material of claim 33, wherein Z is $-[NHC(NH_2)_2]^+$ or $-NHC(NH)NH_2$.

39. The labeled material of claim 33, wherein M is Ru.

40. The labeled material of claim 33, wherein the complex exhibits less non-specific binding than the analogous complex in which Z is H.

41. The labeled material of claim 33, wherein said biological material, binding reagent, enzyme substrate or other assay reagent is linked to five or more of said luminescent metal complexes.

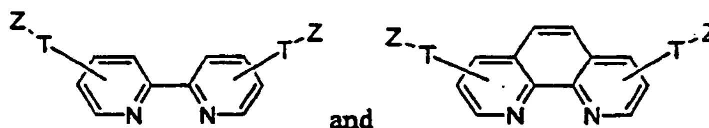
42. The labeled material of claim 33, wherein said biological material, binding reagent, enzyme substrate or other assay reagent is linked to ten or more of said luminescent metal complexes.

43. A method for conducting a luminescence-based assay comprising the steps of:

(a) using a labeled material comprising a luminescent metal complex having the structure

$ML^1L^2_2$

wherein
M is Os or Ru;
L^1 is a substituted bipyridine or phenanthroline ligand having at least one substituent that is covalently linked to a biological material, binding reagent, enzyme substrate or other assay reagent; and
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is a linker group comprising an alkyl, alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl;
(b) inducing said metal complex to emit luminescence; and
(c) measuring the emitted luminescence; and wherein

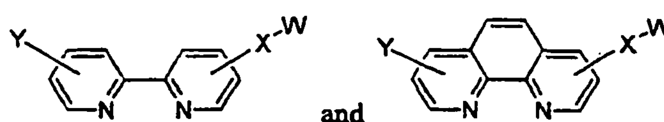
the biological material, binding reagent, enzyme substrate or other assay reagent is selected from the group consisting of amino acids, nucleosides, nucleotides, proteins, peptides, peptidomimetics, nucleic acids, and peptide nucleic acids.

44. The method according to claim 43, wherein said labeled material exhibits reduced non-specific binding in said assay relative to the analogous labeled material having Z = H.

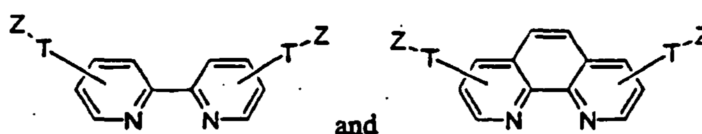
45. A labeled material comprising a luminescent metal complex having the structure

$ML^1L^2_2$

wherein
M is Os or Ru;
L^1 is selected from the group consisting of



wherein,
X is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom;
Y is H or alkyl and
W is a functional group that is linked to a biological material, binding reagent, enzyme substrate or other assay reagent; and
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom; and
Z is $-SO_3^-$ or $-SO_3H$; and wherein
the biological material, binding reagent, enzyme substrate or other assay reagent is selected from the group consisting of amino acids, nucleosides, nucleotides, proteins, peptides, peptidomimetics, nucleic acids, and peptide nucleic acids.

46. The labeled material according to claim 45, wherein M is Ru.

47. The labeled materal according to claim 45, wherein T is $-(CH_2)_n-$, X is $-(CH_2)_m-$and n and m are each integers between 1 and 5.

48. The labeled material according to claim 45 wherein L^2 is a phenanthroline ligand substituted at the 4 and 7 positions or L^2 is a bypyridine ligand substituted at the 4 and 4'. positions.

49. The labeled material according to claim 45, wherein the complex exhibits less non-specific.binding than the analogous complex in which Z is H.

50. The labeled material according to claim 45, wherein said biological material, binding reagent, enzyme substrate or other assay reagent is linked to 5 or more of said luminescent metal complexes.

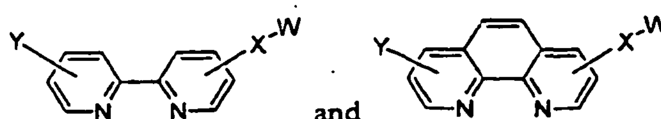
51. The labeled material according to claim 45, wherein said biological material, binding reagent, enzyme substrate or other assay reagent is linked to 10 or more of said luminescent metal complexes.

52. A method for conducting a luminescence-based assay comprising the steps of:

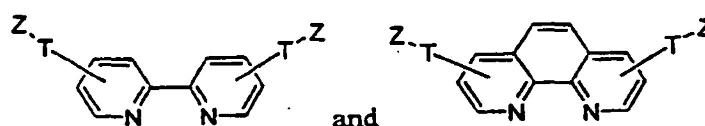
(a) using a labeled material comprising a luminescent metal complex having the structure

$$ML^1L^2_2$$

wherein
M is Os or Ru;
L^1 is selected from the group consisting of



wherein,
X is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom;
Y is H or alkyl; and
W is a functional group that is linked to a biological material, binding reagent, enzyme substrate or other assay reagent;
selected from the group consisting of amino acids, nucleosides, nucleotides, proteins, peptides, peptidomimetics, nucleic acids, and peptide nucleic acids and
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom; and

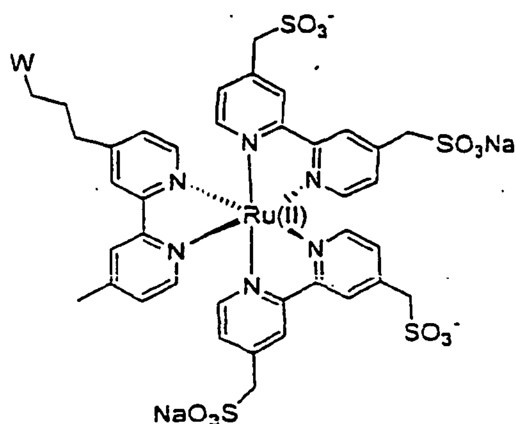
Z is $-SO_3^-$ or $-SO_3H$;

(b) inducing said metal complex to emit luminescence; and

(c) measuring the emitted luminescence.

53. The method according to claim 52, wherein said labeled material exhibits reduced non-specific binding in said assay relative to the analogous labeled material having Z = H.

54. A labeled material comprising a luminescent metal complex with the structure:



wherein W is a functional group that is linked to a biological material, binding reagent, enzyme substrate or other assay reagent; and wherein

the biological material, binding reagent, enzyme substrate or other assay reagent is selected from the group consisting of amino acids, nucleosides, nucleotides, proteins, peptides, peptidomimetics, nucleic acids, and peptide nucleic acids.

55. The labeled material according to claim 54 wherein (i) W is -C(O)-, and (ii) said biological material, binding reagent, enzyme substrate or other assay reagent is linked to W via an amide bond.

56. The labeled material according to claim 54, where said biological material, binding reagent, enzyme substrate or other assay reagent is linked to 5 or more of said luminescent metal complexes.

57. The labeled material according to claim 54, wherein said biological material, binding reagent, enzyme substrate or other assay reagent is linked to 10 or more of said luminescent metal complexes.

58. A method for conducting a luminescence-based assay comprising the steps of:

(a) using the labeled material of claim 54

(b) inducing said labeled material to emit luminescence; and

(c) measuring the emitted luminescence.

59. The method according to claim 58, wherein said labeled material exhibits reduced non-specific binding in said assay relative to the analogous labeled material having Z = H.

60. A method of measuring an analyte, chemical activity or biological activity in a sample comprising the steps of i) contacting a sample containing the analyte, chemical activity, biological activity, a product of the biological activity or a product of the chemical activity with the luminescent metal complex of claim 13; ii) inducing the metal complex to emit luminescence and iii)

measuring the luminescence so as to detect or measure the chemical or biological activity.

61. The method of claim 60, wherein said labeled material exhibits less non-specific binding in said assay when compared to a labeled material having an analogous ligand that does not present non-specific binding reducing functional groups.

62. The method of claim 60 or 61 wherein said luminescence is electrochemiluminescence..

63. A method of measuring a labeled material comprising the steps of i) contacting the labeled material with a binding reagent and, optionally, a solid phase support; ii) forming a binding complex comprising the binding reagent, the labeled material, and, optionally, the solid phase support; iii) inducing the labeled material to emit luminescence and iv) measuring the emitted luminescence so as to measure the labeled material; wherein the labeled material is prepared from the luminescent metal according to claim 13.

64. The method of claim 63, wherein said labeled material is contacted with said solid phase and said binding complex is formed on said solid phase.

65. The method of claim 63, wherein said labeled material exhibits less non-specific binding in said assay when compared to an analogous labeled material having an analogous ligand that does not present non-specific binding reducing functional groups; wherein the non-specific binding reducing functional groups are selected from the group consisting of $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$.

66. The method of claim 63, wherein said luminescence is electrochemiluminescence.

67. A method of measuring an analyte in a sample comprising the steps of i) contacting the sample with a labeled binding reagent and optionally a solid phase support; ii) forming a binding complex comprising the binding reagent, the analyte and, optionally, the solid phase support; iii) inducing labels in the labeled binding reagent to emit luminescence; preferably ECL and iv) measuring the emitted luminescence so as to measure the analyte in the sample; wherein said labeled binding reagent is prepared from the luminescent metal complex of claim 13.

68. The method of claim 67, wherein said binding complex is formed on said solid phase.

69. The method of claim 67, wherein said labeled binding reagent is contacted with said solid phase and said binding complex is formed on said solid phase.

70. The method of claim 67, wherein said labeled binding reagent exhibits less non-specific binding in said assay when compared to analogous labeled binding reagents that do not have labels that present non-specific binding reducing functional groups.

71. The method of claim 67, wherein said luminescence is electrochemiluminescence.

72. The method of claim 67, wherein said assay is a sandwich assay.

73. The method of claim 67, wherein said assay is a competitive assay.

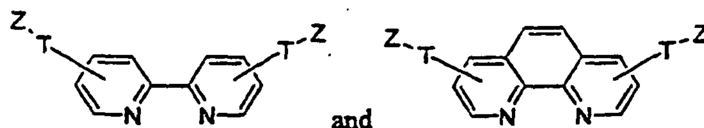
74. A method of measuring an analyte in a sample comprising the steps of i) contacting the sample with a labeled analog of the analyte, a binding reagent and, optionally, a solid phase support; ii) forming a binding complex comprising the labeled analog of the analyte, the binding reagent and, optionally, the solid phase support; iii) inducing labels in the labeled analog of the analyte to emit luminescence, preferably ECL and iv) measuring the emitted luminescence so as to measure the analyte in the sample; wherein said labeled analog of the analyte is prepared from the luminescent metal complex of claim 13.

75. The method of claim 74, wherein said labeled analog of the analyte is contacted with said solid phase and said binding complex is formed on said solid phase.

76. The method of claim 74, wherein said labeled analog of the analyte exhibits less non-specific binding in said assay when compared to analogous reagents that do not present non-specific binding reducing functional groups.

77. The method of claim 74, wherein said luminescence is electrochemiluminscence.

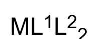
78. A method of improving a luminescence assay that employs a luminescent metal complex that contains a bipyridine, phenanthroline, substituted bipyridine or substituted phenanthroline ligand, said method comprising the step of substituting said ligand with a ligand selected from the group consisting of



wherein,
T is an alkyl linker having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, OPO_3^{2-}, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl.

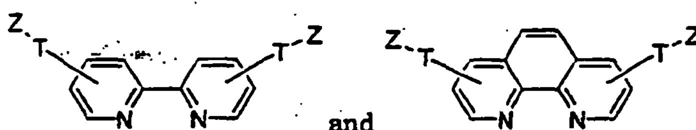
79. The method of claim 78, wherein said replacement improves assay signal to background by a factor of two or greater.

80. A kit comprising, in one or more containers, a labeled material comprising a luminescent metal complex having the structure



$$ML^1L^2_2$$

wherein
M is Os or Ru;
L^1 is a substituted bipyridine or phenanthroline ligand having at least one substituent that is covalently linked to an assay-performance-substance; and
L^2 is a metal ligand selected from the group consisting of:



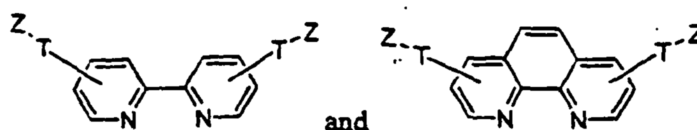
wherein,
T is a linker group comprising an alkyl alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl; and
at least one assay component selected from the group consisting of:

(a) an electrochemiluminescence coreactant;
(b) one or more binding reagents;
(c) one or more pH buffers.

81. A labeled material comprising a luminescent metal complex having the structure

$$ML^1L^2_2$$

wherein
M is Os or Ru;
L^1 is a substituted bipyridine or phenanthroline ligand having at least one substituent that is covalently linked to an assay-performance-substance; and
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is a linker group comprising an alkyl, alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl.

82. A labeled material according to claim 81, wherein said assay-performance-substance is selected from the group consisting of:

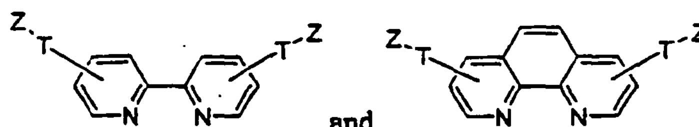
(a) added analyte of interest ;
(b) a binding partner of said analyte ; and
(c) a reactive component capable of binding with (a) or (b).

83. A composition of matter for use as a reagent in an assay comprising:

(a) a labeled material comprising a luminescent metal complex having the structure

$$ML^1L^2_2$$

wherein
M is Os or Ru.;
L^1 is a substituted bipyridine or phenanthroline ligand having at least one substituent that is covalently linked to an assay-performance-substance; and
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is a linker group comprising an alkyl alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom,
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, OPO_3^{2-}, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl; and
(b) at least one additional assay components selected from the group consisting of:

(i) electrolyte;
(ii) analyte of interest;
(iii) a binding partner of the analyte of interest;
(iv) a reactive component capable of reacting with (ii) or (iii); and
(v) an ECL coreactant,

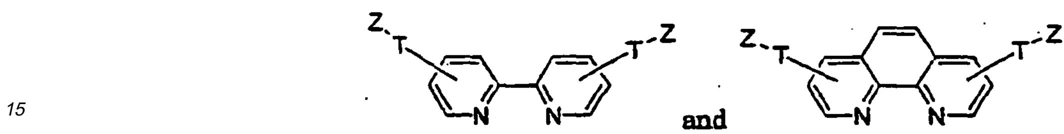
provided, however, that no two components contained within any reagent composition are reactive with one another during storage so as to impair their function in the intended assay.

84. A composition of matter for the detection of an analyte of interest present in a sample, which composition comprises

a labeled material comprising a luminescent metal complex having the structure

$ML^1L^2_2$

wherein
M is Os or Ru;
L^1 is a substituted bipyridine or phenanthroline ligand having at least one substituent that is covalently linked to an assay-performance-substance; and
L^2 is a metal ligand selected from the group consisting of:



wherein,
T is a linker group comprising an alkyl, alkenyl, alkynyl or phenyl linker, or a combination thereof, having, optionally, one or more chain carbons substituted by a heteroatom;
Z is $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$ $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OPO(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, or $-NHC(NH)NH_2$; and
R is alkyl,
said assay-performance-substance being capable of binding to the analyte-of-inerest or being bound to the analyte-of-interest.

85. A composition of matter according to claim 84, wherein said composition contains at least one additional substance selected from the group consisting of

(i) added analyte of interest;
(ii) a binding partner of said analyte; and
(iii) a reactive component capably of binding with (i) or (ii).

86. The ligand of claim 3, wherein n = 1.

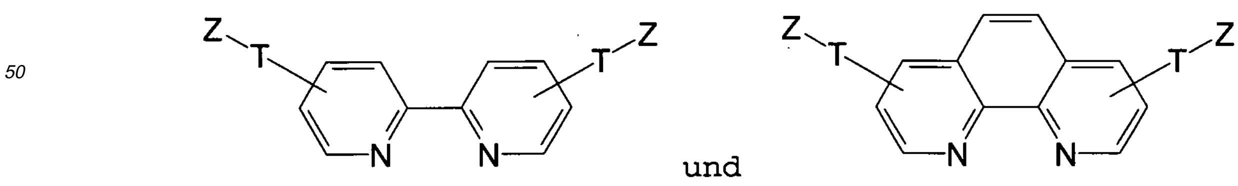
87. The luminescent complex according to claim 22, wherein M is Os.

88. The labeled material of claim 35, wherein M is Ru.

89. The labeled material of claim 45, wherein M is Os.

Patentansprüche

1. Bipyridin- oder Phenanthrolinligand, der ausgewählt ist aus der Gruppe bestehend aus:



worin
T ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;

Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H$-, $-OPO_3H_2$, -OP (R) O_2^-, -OP (R) O_2H, - [NHC $(NH_2)_2]^+$ oder -NHC(NH)NH_2 ist; und
R Alkyl ist,
vorausgesetzt, dass der Ligand kein 4,4'-Dipropylsulfonat-2,2'-bipyridinium Radikalanion ist.

5

2. Ligand gemäß Anspruch 1, worin T $-(CH_2)_n-$ ist und n eine ganze Zahl zwischen 1 und 5 ist.

3. Ligand gemäß Anspruch 1 oder 2, worin Z $-SO_3$- oder $-SO_3H$ ist.

10

4. Ligand gemäß Anspruch 1 oder 2, worin Z $-OSO_3$- oder $-OSO_3H$ ist.

5. Ligand gemäß Anspruch 1 oder 2, worin Z $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, -OP(Me)O_2^- oder -OP(Me)O_2H ist.

6. Ligand gemäß Anspruch 1 oder 2, worin Z -[NHC$(NH_2)_2]^+$ oder -NHC(NH_2)NH_2 ist.

15

7. Lumineszierender Metallkomplex, der den Ligand gemäß Anspruch 2 und ein Metallatom umfasst, wobei das Metallatom an die Ringstickstoffatome des Liganden gebunden ist.

8. Verfahren zur Durchführung eines Lumineszenz-basierten Assays, welches die folgenden Schritte umfasst:

20

(a) Verwendung eines lumineszierenden Metallkomplexes, der einen Liganden umfasst, der ausgewählt ist aus der Gruppe bestehend aus

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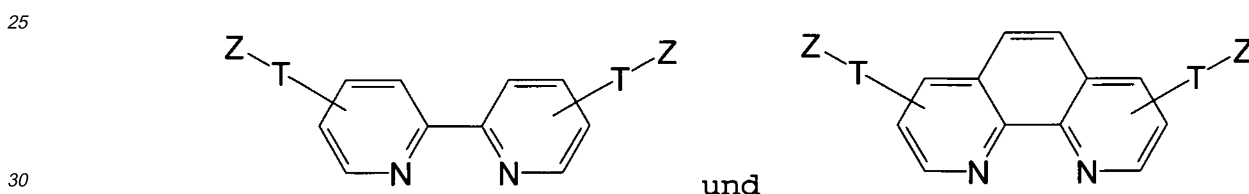

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worin
T ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;

35

Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, -OP(R)O_2^-, -OP(R)O_2H, -[NHC$(NH_2)_2]^+$ oder -NHC(NH)NH_2 ist;
R Alkyl ist; und
der Metallkomplex ein Metallatom umfasst, das an die Ringstickstoffatome des Liganden gebunden ist;

40

(b) Induzieren einer emittierten Lumineszenz des Metallkomplexes; und
(c) Messen der emittierten Lumineszenz.

9. Verfahren gemäß Anspruch 8, worin der Metallkomplex eine reduzierte nicht-spezifische Bindung in dem Assay relativ zu dem Analogkomplex mit Z = H zeigt.

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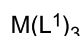
10. Lumineszierender Metallkomplex, der die Struktur



$M(L^1)_3$

50

aufweist, worin M Os oder Ru ist; und
L^1 ein Ligand ist, der ausgewählt ist aus der Gruppe bestehend aus:

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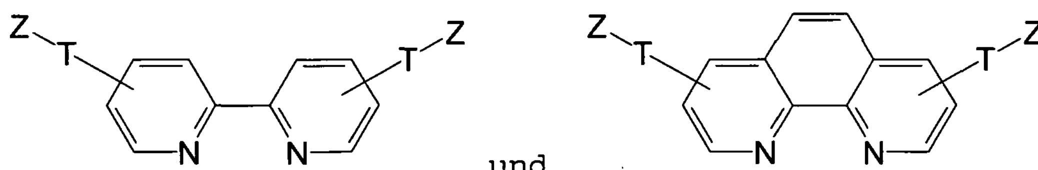
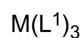


worin
T ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, -OP (R) O_2^-, -OP (R) O_2H, - [NHC $(NH_2)_2$] + oder -NHC(NH)NH_2 ist; und
R Alkyl ist.

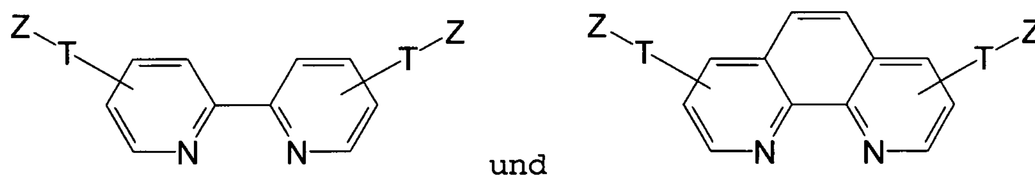
11. Verfahren zur Durchführung eines Lumineszenz-basierten Assays, welches die folgenden Schritte umfasst:

(a) Verwenden eines lumineszierenden Metallkomplexes, der die Struktur



$$M(L^1)_3$$

aufweist, worin M Os oder Ru ist; und
L^1 ein Ligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



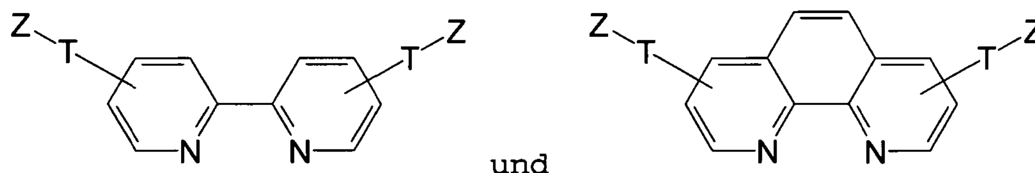
worin
T ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$ oder -NHC(NH)NH_2 ist; und
R Alkyl ist;
(b) Induzieren einer emittierten Lumineszenz des Metallkomplexes; und
(c) Messen der emittierten Lumineszenz.

12. Verfahren gemäß Anspruch 11, worin der Metallkomplex eine reduzierte nicht-spezifische Bindung in dem Assay relativ zu dem Analogkomplex mit Z = H zeigt.

13. Lumineszierender Metallkomplex, der die Struktur

$$ML^1L^2_2$$

aufweist, worin M Os oder Ru ist;
L^1 ein substituierter Bipyridin- oder Phenanthrolinligand ist, der mindestens einen Substituenten aufweist, der mit einem biologischen Material, einem Bindungsreagenz, einem Enzymsubstrat oder anderen Assayreagenz zur Bildung einer kovalenten Verknüpfung reagieren kann; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



worin
T eine Linkergruppe ist, die ein Alkyl-, Alkenyl-, Alkynyl- oder Phenyllinker oder eine Kombination daraus umfasst und wahlweise ein oder mehrere
Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, -OP (R) O_2^-, -OP (R) O_2H, -[NHC $(NH_2)_2]^+$ oder -NHC (NH) NH_2 ist; und
R Alkyl ist; und
worin das biologische Material, das Bindungsreagenz, das Enzymsubstrat oder das andere Assayreagenz ausgewählt ist aus der Gruppe bestehend aus Aminosäure, Nucleosiden, Nucleotiden, Proteinen, Peptiden, Peptidomimetics, Nucleinsäuren und Peptidnucleinsäuren.

14. Lumineszierender Metallkomplex gemäß Anspruch 13, worin L^1 ausgewählt ist aus der Gruppe bestehend aus

worin
X eine Linkergruppe ist, die ein Alkyl-, Alkenyl-, Alkynyl- oder Phenyllinker oder eine Kombination daraus umfasst, und wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Y H oder Alkyl ist und
W ein Substituent ist, der mit dem biologischen Material, dem Bindungsreagenz, dem Enzymsubstrat oder dem anderen Assayreagenz zur Bildung einer kovalenten Verknüpfung reagieren kann.

15. Lumineszierender Metallkomplex gemäß Anspruch 13, worin Z $-SO_3$- oder $-SO_3H$ ist.

16. Lumineszierender Metallkomplex gemäß Anspruch 13, worin Z $-OSO_3$- oder $-OSO_3H$ ist.

17. Lumineszierender Metallkomplex gemäß Anspruch 13, worin Z $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, -OP(Me)O_2^- oder -OP (Me)O_2H ist.

18. Lumineszierender Metallkomplex gemäß Anspruch 13, worin Z - [NHC$(NH_2)_2]^+$ oder -NHC (NH) NH_2 ist.

19. Lumineszierender Metallkomplex gemäß Anspruch 13, worin M Ru ist.

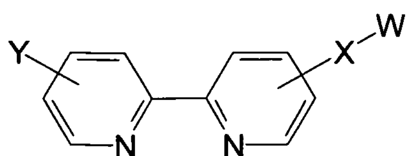
20. Lumineszierender Metallkomplex gemäß Anspruch 13, worin W ein Acylchlorid oder ein N-Hydroxysuccinimidester ist.

21. Lumineszierender Metallkomplex gemäß Anspruch 13, worin W eine Carbonsäuregruppe, eine Amingruppe oder eine Hydroxylgruppe ist.

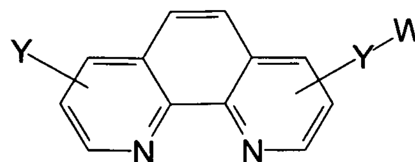
22. Lumineszierender Metallkomplex, der die Struktur

$ML^1L^2_2$

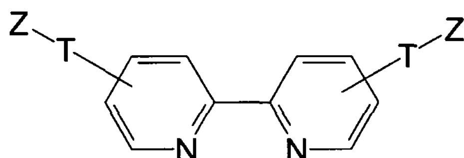
aufweist, worin M Os oder Ru ist;
L^1 ausgewählt ist aus der Gruppe bestehend aus:



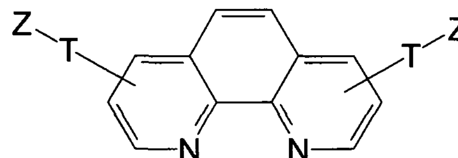
und



worin
X ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heterotatom substituiert sind;
Y H oder Alkyl ist; und
W eine funktionelle Gruppe ist, die mit einem biologischen Material, einem Bindungsreagenz, einem Enzymsubstrat oder einem anderen Assayreagenz zur Bildung einer kovalenten Verknüpfung reagieren kann; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



und



worin
T ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind; und
Z $-SO_3$- oder $-SO_3H$ ist; und
worin das biologische Material, das Bindungsreagenz, das Enzymsubstrat oder das andere Assayreagenz ausgewählt ist aus der Gruppe bestehend aus Aminosäure, Nucleosiden, Nucleotiden, Proteinen, Peptiden, Peptidomimetics, Nucleinsäuren und Peptidnucleinsäuren.

23. Lumineszierender Metallkomplex gemäß Anspruch 22, worin M Ru ist.

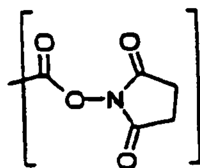
24. Lumineszierender Metallkomplex gemäß Anspruch 22, worin T $-(CH_2)_n-$ ist, X $-(CH_2)_m-$ ist und n und m jeweils ganze Zahlen zwischen 1 und 5 sind.

25. Lumineszierender Metallkomplex gemäß Anspruch 22, worin L^2 ein Phenanthrolinligand ist, der an den Positionen 4 und 7 substituiert ist, oder L^2 ein Bipyridinligand ist, der an den Positionen 4 und 4' substituiert ist.

26. Lumineszierender Metallkomplex gemäß Anspruch 22, worin W ein Acylchlorid oder ein N-Hydroxysuccinimidester ist.

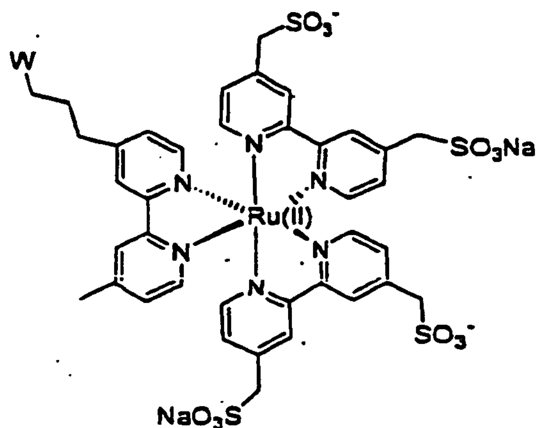
27. Lumineszierender Metallkomplex gemäß Anspruch 22, worin W eine Carbonsäuregruppe, eine Aminogruppe oder eine Thiolgruppe ist.

28. Lumineszierender Metallkomplex gemäß Anspruch 22, worin W



ist.

29. Lumineszierender Metallkomplex mit der Struktur:

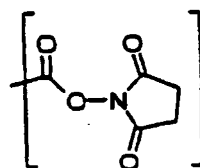


worin W eine funktionelle Gruppe ist, die mit einem biologischen Material, einem Bindungsreagenz, einem Enzymsubstrat oder einem anderen Assayreagenz zur Bildung einer kovalenten Verknüpfung reagieren kann; und worin das biologische Material, das Bindungsreagenz, das Enzymsubstrat oder das andere Assayreagenz ausgewählt ist aus der Gruppe bestehend aus Aminosäure, Nucleosiden, Nucleotiden, Proteinen, Peptiden, Peptidomimetics, Nucleinsäure und Peptidnucleinsäuren.

30. Lumineszierender Metallkomplex gemäß Anspruch 29, worin W ein Acylchlorid oder ein N-Hydroxysuccinimidester ist.

31. Lumineszierender Metallkomplex gemäß Anspruch 29, worin W eine Carbonsäuregruppe, eine Aminogruppe oder eine Hydroxylgruppe ist.

32. Lumineszierender Metallkomplex gemäß Anspruch 29, worin W



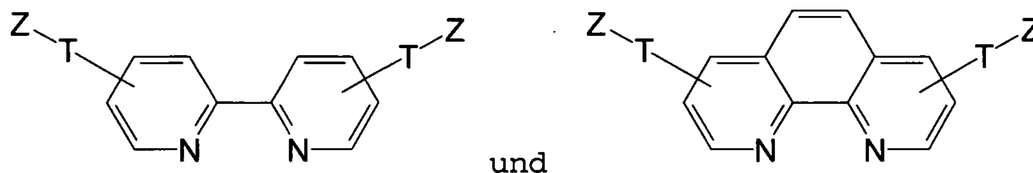
ist.

33. Markiertes Material, das einen lumineszierenden Metallkomplex mit der Struktur

$ML^1L^2_2$

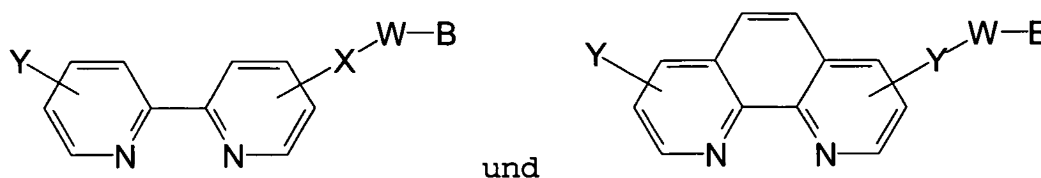
umfasst, worin

M Os oder Ru ist;
L^1 ein substituierter Bipyridin- oder Phenanthrolinligand ist, der mindestens einen Substituenten aufweist, der kovalent an ein biologisches Material, Bindungsreagenz, Enzymsubstrat oder ein anderes Assayreagenz gebunden ist; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



worin
T eine Linkergruppe ist, die ein Alkyl-, Alkenyl-, Alkynyl- oder Phenyllinker oder eine Kombination daraus umfasst, und die wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$ oder $-NHC(NH)NH_2$ ist; und
R Alkyl ist; und
worin das biologische Material, das Bindungsreagenz, das Enzymsubstrat oder das andere Assayreagenz ausgewählt ist aus der Gruppe bestehen aus Aminosäure, Nucleosiden, Nucleotiden, Proteinen, Peptiden, Peptidomimetics, Nucleinsäuren und Peptidnucleinsäuren.

34. Markiertes Material gemäß Anspruch 33, worin L^1 ausgewählt ist aus der Gruppe bestehend aus:



worin
X eine Linkergruppe ist, die ein Alkyl-, Alkenyl-, Alkynyl- oder Phenyllinker oder eine Kombination daraus umfasst, und die wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Y H oder Alkyl ist;
B das biologische Material, Bindungsreagenz, Enzymsubstrat oder das andere Assayreagenz ist; und
W eine funktionelle Gruppe ist, die an B gebunden ist.

35. Markiertes Material gemäß Anspruch 33, worin Z $-SO_3^-$ oder $-SO_3H$ ist.

36. Markiertes Material gemäß Anspruch 33, worin Z $-OSO_3^-$ oder $-OSO_3H$ ist.

37. Markiertes Material gemäß Anspruch 33, worin Z $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(Me)O_2^-$ oder $-OP(Me)O_2H$ ist.

38. Markiertes Material gemäß Anspruch 33, worin Z $-[NHC(NH_2)_2]^+$ oder $-NHC (NH) NH_2$ ist.

39. Markiertes Material gemäß Anspruch 33, worin M Ru ist.

40. Markiertes Material gemäß Anspruch 33, worin der Komplex eine geringere nicht-spezifische Bindung als der Analogkomplex mit Z = H zeigt.

41. Markiertes Material gemäß Anspruch 33, worin das biologische Material, das Bindungsreagenz, das Enzymsubstrat oder das andere Assayreagenz an 5 oder mehr der lumineszierenden Metallkomplexe gebunden ist.

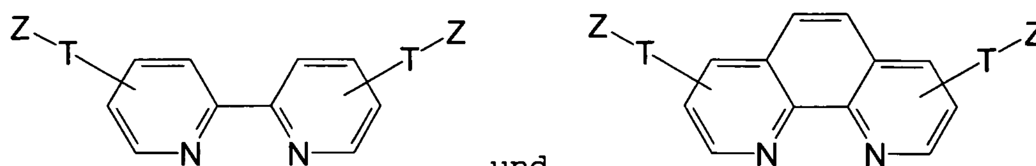
42. Markiertes Material gemäß Anspruch 33, worin das biologische Material, das Bindungsreagenz, das Enzymsubstrat oder das andere Assayreagenz an 10 oder mehr der lumineszierenden Metallkomplexe gebunden ist.

43. Verfahren zur Durchführung eines Lumineszenz-basierten Assays, welches die folgenden Schritte umfasst:

(a) Verwendung eines markierten Materials, das einen lumineszierenden Metallkomplex mit der Struktur

$ML^1L^2_2$

umfasst, worin
M Os oder Ru ist;
L^1 ein substituierter Bipyridin- oder Phenanthrolinligand ist, der mindestens einen Substituenten aufweist, der kovalent an ein biologisches Material, Bindungsreagenz, Enyzmsubstrat oder ein anderes Assayreagenz gebunden ist; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



worin
T eine Linkergruppe ist, die ein Alkyl-, Alkenyl-, Alkynyl- oder Phenyllinker oder eine Kombination daraus umfasst, und die wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$ oder $-NHC(NH)NH_2$ ist; und
R Alkyl ist;
(b) Induzieren einer emittierten Luminszenz des Metallkomplexes; und
(c) Messen der emittierten Lumineszenz; und

worin das biologische Material, Bindungsreagenz, Enzymsubstrat, oder das andere Assayreagenz ausgewählt ist aus der Gruppe bestehend aus Aminosäuren, Nucleosiden, Nucleotiden, Proteinen, Peptiden, Peptidomimetics, Nucleinsäuren und Peptidnucleinsäuren.

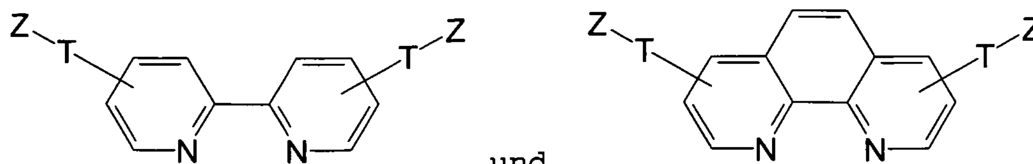
44. Verfahren gemäß Anspruch 43, worin das markierte Material eine geringere nicht-spezifische Bindung in dem Assay, bezogen auf das analog markierte Material mit Z = H, zeigt.

45. Markiertes Material, das ein lumineszierenden Metallkomplex mit der Strukur

$ML^1L^2_2$

umfasst, worin
M Os oder Ru ist;
L^1 ausgewählt ist aus der Gruppe bestehend aus:

worin
X ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Y H oder Alkyl ist und
W eine funktionelle Gruppe ist, die an ein biologisches Material, Bindungsreagenz, Enzymsubstrat oder ein anderes Assayreagenz gebunden ist; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



worin
T ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind; und
Z $-SO_3$- oder $-SO_3H$ ist; und
worin das biologische Material, Bindungsreagenz, Enzymsubstrat oder das andere Assayreagenz ausgewählt ist aus der Gruppe bestehend aus Aminosäuren, Nucleosiden, Nucleotiden, Proteinen, Peptiden, Peptidomimetics, Nucleinsäuren und Peptidnucleinsäuren.

46. Markiertes Material gemäß Anspruch 45, worin M Ru ist.

47. Markiertes Material gemäß Anspruch 45, worin T $-(CH_2)_n$-ist, X $-(CH_2)_m$- ist und n und m jeweils ganze Zahlen zwischen 1 und 5 sind.

48. Markiertes Material gemäß Anspruch 45, worin L^2 ein Phenanthrolinligand ist, der an den Positionen 4 und 7 substituiert ist oder L^2 ein Bipyridinligand ist, der an den Positionen 4 und 4' substituiert ist.

49. Markiertes Material gemäß Anspruch 45, worin der Komplex eine geringere nicht-spezifische Bindung als der Analogkomplex mit Z = H zeigt.

50. Markiertes Material gemäß Anspruch 45, worin das biologische Material, Bindungsreagenz, Enzymsubstrat oder das andere Assayreagenz an 5 oder mehr der lumineszierenden Metallkomplexe gebunden ist.

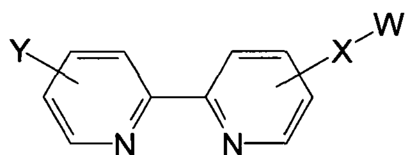
51. Markiertes Material gemäß Anspruch 45, worin das biologische Material, Bindungsreagenz, Enzymsubstrat oder das andere Assayreagenz an 10 oder mehr der lumineszierenden Metallkomplexe gebunden ist.

52. Verfahren zur Durchführung eines Lumineszenz-basierten Assays, welches folgende Schritte umfasst:

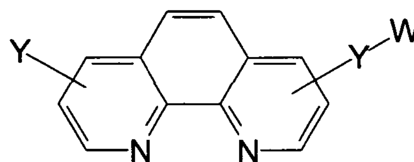
(a) Verwendung eines markierten Materials, das einen lumineszierenden Metallkomplex mit der Struktur

$$ML^1L^2_2$$

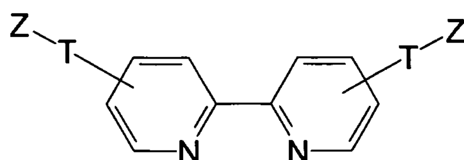
umfasst, worin
M Os oder Ru ist;
L^1 ausgewählt ist aus der Gruppe bestehend aus:



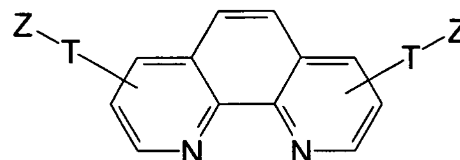
und



worin
X ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Y H oder Alkyl ist und
W eine funktionelle Gruppe ist, die mit einem biologischen Material, Bindungsreagenz, Enzymsubstrat oder einem anderen Assayreagenz verknüpft ist, und ausgewählt ist aus der Gruppe bestehend aus Aminosäuren, Nucleosiden, Nucleotiden, Proteinen, Peptiden, Peptidomimetics, Nucleinsäuren und Peptidnucleinsäuren; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



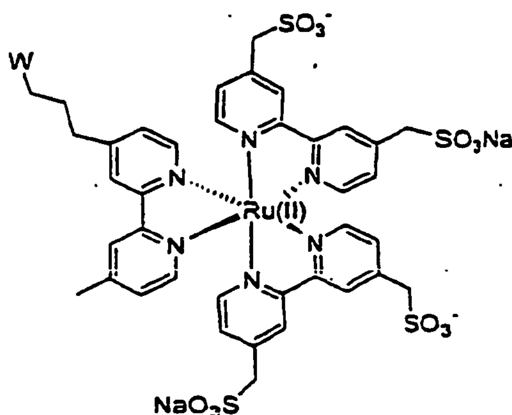
und



worin
T ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind; und
Z $-SO_3$- oder $-SO_3H$ ist
(b) Induzieren einer emittierten Lumineszenz des Metallkomplexes; und
(c) Messen der emittierten Lumineszenz.

53. Verfahren gemäß Anspruch 52, worin das markierte Material eine geringere nicht-spezifische Bindung in dem Assay, bezogen auf das analog markierte Material mit Z = H, zeigt.

54. Markiertes Material, das einen lumineszierenden Metallkomplex mit der Struktur:



umfasst, worin W eine funktionelle Gruppe ist, die an ein biologisches Material, Bindungsreagenz, Enzymsubstrat oder ein anderes Assayreagenz verknüpft ist; und
worin das biologische Material, Bindungsreagenz, Enzymsubstrat oder das andere Assayreagenz ausgewählt ist aus der Gruppe bestehend aus Aminosäuren, Nucleosiden, Nucleotiden, Proteinen, Peptiden, Peptidomimetics,

Nucleinsäuren und Peptidnucleinsäuren.

55. Markiertes Material gemäß Anspruch 54, wobei (i) W -C(O)- ist, und (ii) das biologische Material, Bindungsreagenz, Enzymsubstrat oder andere Assayreagenz an W über eine Amidbindung verknüpft ist.

56. Markiertes Material gemäß Anspruch 54, wobei das biologische Material, Bindungsreagenz, Enzymsubstrat oder andere Assayreagenz an 5 oder mehr der lumineszierenden Metallkomplexe gebunden ist.

57. Markiertes Material gemäß Anspruch 54, wobei das biologische Material, Bindungsreagenz, Enzymsubstrat oder andere Assayreagenz an 10 oder mehr der lumineszierenden Metallkomplexe gebunden ist.

58. Verfahren zur Durchführung eines Lumineszenz-basierten Assays, welches die folgenden Schritte umfasst:

(a) Verwendung des markierten Materials gemäß Anspruch 54;
(b) Induzieren der emittierten Lumineszenz des markierten Materials; und
(c) Messen der emittierten Lumineszenz.

59. Verfahren gemäß Anspruch 58, worin das markierte Material eine geringere nicht-spezifische Bindung in dem Assay, bezogen auf das analog markierte Material mit Z = H, zeigt.

60. Verfahren zur Messung eines Analyten, einer chemischen oder biologischen Aktivität in einer Probe, welches die folgenden Schritte umfasst:

(i) Kontaktieren einer Probe, die den Analyten, die chemische Aktivität, biologische Aktivität, ein Produkt der biologischen Aktivität oder ein Produkt der chemischen Aktivität mit dem lumineszierenden Metallkomplex gemäß Anspruch 13 enthält,
(ii) Induzieren der emittierten Lumineszenz des Metallkomplexes, und
(iii) Messen der Lumineszenz zur Detektion oder Messung der chemischen oder biologischen Aktivität.

61. Verfahren gemäß Anspruch 60, worin das markierte Material eine geringere nicht-spezifische Bindung in dem Assay zeigt, verglichen mit einem markierten Material, das einen analogen Liganden aufweist, der keine nicht-spezifischen bindungsreduzierenden, funktionellen Gruppen zeigt.

62. Verfahren gemäß Anspruch 60 oder 61, worin die Lumineszenz Elektrochemolumineszenz ist.

63. Verfahren zur Messung eines markierten Materials, welches die folgenden Schritte umfasst:

(i) Kontaktieren des markierten Materials mit einem Bindungsreagenz und wahlweise einem Festphasenträger;
(ii) Bilden eines Bindungskomplexes, der das Bindungsreagenz, das markierte Material und wahlweise den Festphasenträger umfasst;
(iii) Induzieren der emittierten Lumineszenz des markierten Materials; und
(iv) Messen der emittierten Lumineszenz zur Messung des markierten Materials;

worin das markierte Material hergestellt ist aus dem lumineszierenden Metall gemäß Anspruch 13.

64. Verfahren gemäß Anspruch 63, worin das markierte Material mit der Festphase kontaktiert wird und der Bindungskomplex auf der Festphase gebildet wird.

65. Verfahren gemäß Anspruch 63, worin das markierte Material eine geringere nicht-spezifische Bindung in dem Assay zeigt, verglichen mit einem analog markierten Material, das einen analogen Liganden aufweist, der keine nicht-spezifischen, bindungsreduzierenden funktionellen Gruppen zeigt;
worin die nicht-spezifischen, bindungsreduzierenden funktionellen Gruppen ausgewählt sind aus der Gruppe bestehend aus $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$ oder $-NHC(NH)NH_2$.

66. Verfahren gemäß Anspruch 63, worin die Lumineszenz Elektrochemolumineszenz ist.

67. Verfahren zur Messung eines Analyten in einer Probe, welches die folgenden Schritte umfasst:

(i) Kontaktieren der Probe mit einem markierten Bindungsreagenz und wahlweise einem Festphasenträger,
(ii) Bilden eines Bindungskomplexes, der das Bindungsreagenz, den Analyten und wahlweise den Festphasenträger umfasst,
(iii) Induzieren der emittierten Lumineszenz der Marker in dem markierten Bindungsreagenz, vorzugsweise ECL, und
(iv) Messen der emittierten Lumineszenz zur Messung des Analyten in der Probe;

worin das markierte Bindungsreagenz hergestellt ist aus dem Lumineszenzmetallkomplex gemäß Anspruch 13.

68. Verfahren gemäß Anspruch 67, worin der Bindungskomplex auf der Festphase gebildet ist.

69. Verfahren gemäß Anspruch 67, worin das markierte Bindungsreagenz mit der Festphase kontaktiert wird und der Bindungskomplex auf der Festphase gebildet wird.

70. Verfahren gemäß Anspruch 67, worin das markierte Bindungsreagenz eine geringere nicht-spezifische Bindung in dem Assay zeigt, verglichen mit einem analog markierten Bindungsreagenz, das keine Marker aufweist, die keine nicht-spezifischen, bindungsreduzierenden funktionellen Gruppen zeigen.

71. Verfahren gemäß Anspruch 67, worin die Lumineszenz Elektrochemolumineszenz ist.

72. Verfahren gemäß Anspruch 67, worin der Assay ein Sandwichassay ist.

73. Verfahren gemäß Anspruch 67, worin der Assay ein Kompetitivassay ist.

74. Verfahren zur Messung eines Analyten in einer Probe, welches die folgenden Schritte umfasst:

(i) Kontaktieren der Probe mit einem markierten Analoga des Analyten, eines Bindungsreagenz und wahlweise eines Festphasenträgers,
(ii) Bilden eines Bindungskomplexes, der das markierte Analoga des Analyten, des Bindungsreagenz und wahlweise den Festphasenträger umfasst,
(iii) Induzieren der emittierten Lumineszenz der Marker in dem markierten Analoga des Analyten, vorzugsweise ECL, und
(iv) Messen der emittierten Lumineszenz zur Messung des Analyten in der Probe;

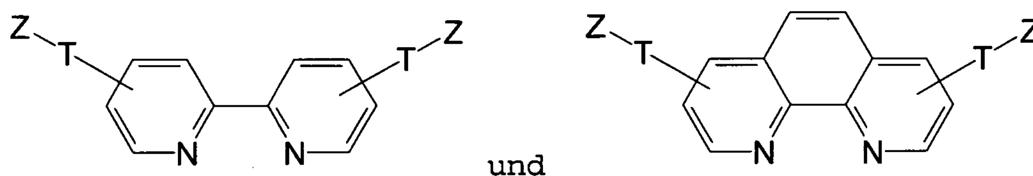
worin das markierte Analoga des Analyten hergestellt ist aus dem Lumineszenzmetallkomplex gemäß Anspruch 13.

75. Verfahren gemäß Anspruch 74, worin das markierte Analoga des Analyten mit der Festphase kontaktiert wird und der Bindungskomplex auf der Festphase gebildet wird.

76. Verfahren gemäß Anspruch 74, worin das markierte Analoga des Analyten eine geringere nicht-spezifische Bindung in dem Assay zeigt, verglichen mit einem analogen Reagenz, das keine nicht-spezifischen, bindungsreduzierenden funktionellen Gruppen zeigt.

77. Verfahren gemäß Anspruch 74, worin die Lumineszenz Elektrochemolumineszenz ist.

78. Verfahren zur Verbesserung eines Lumineszenzassays, der einen Lumineszenzmetallkomplex anwendet, der einen Bipyridin- Phenanthrolin-, substituierten Bipyridin-oder substituierten Phenanthrolinliganden enthält und das Verfahren den Substitutionsschritt des Liganden mit einem Liganden umfasst, der ausgewählt ist aus der Gruppe bestehend aus:

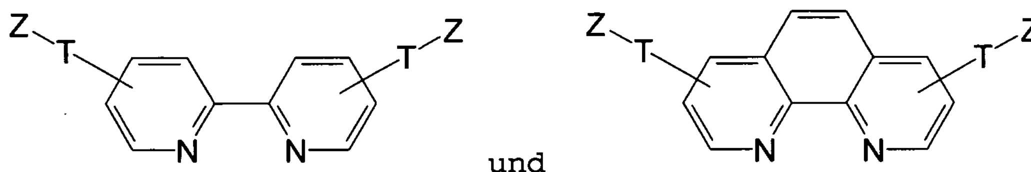


worin
T ein Alkyllinker ist, der wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$ oder $-NHC(NH)NH_2$ ist; und
R Alkyl ist.

79. Verfahren gemäß Anspruch 78, worin der Ersatz das Signal-zu-Rausch-Verhältnis um einen Faktor von 2 oder mehr verbessert.

80. Kit umfassend einen oder mehrere Container, ein markiertes Material, das einen lumineszierenden Metallkomplex mit der Struktur

umfasst, worin
M Os oder Ru ist;
L^1 ein substituierter Bipyridin- oder Phenanthrolinligand mit mindestens einem Substituenten ist, der kovalent an eine Assayfunktionssubstanz gebunden ist; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:

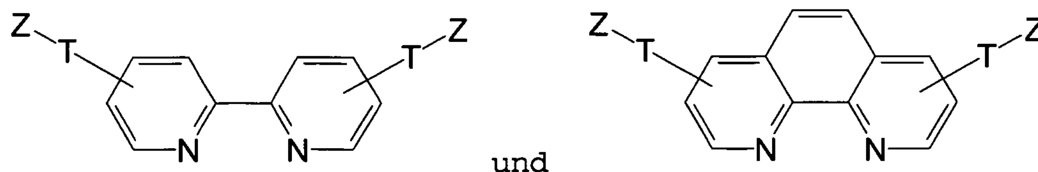


worin
T eine Linkergruppe ist, die einen Alkyl-, Alkenyl-, Alkynyl- oder Phenyllinker oder eine Kombination daraus umfasst und wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$ oder $-NHC(NH)NH_2$ ist; und
R Alkyl ist; und
mindestens eine Assaylkomponente ausgewählt ist aus der Gruppe bestehend aus:

(a) einem Elektrochemolumineszenz-Koreaktant;
(b) einem oder mehreren Bindungsreagenzien;
(c) einem oder mehreren pH-Puffern.

81. Markiertes Material, das einen lumineszierenden Metallkomplex mit der Struktur

umfasst, worin
M Os oder Ru ist;
L^1 ein substituierter Bipyridin- oder Phenanthrolinligand mit mindestens einem Substituenten ist, der kovalent an eine Assayfunktionssubstanz gebunden ist; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



worin
T eine Linkergruppe ist, die einen Alkyl-, Alkenyl-, Alkynyl- oder Phenyllinker oder eine Kombination daraus umfasst und wahlweise ein oder mehrere
Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$ oder -NHC(NH)NH_2 ist; und
R Alkyl ist.

82. Markiertes Material gemäß Anspruch 81, worin die Assayfunktionssubstanz ausgewählt ist aus der Gruppe bestehen aus:

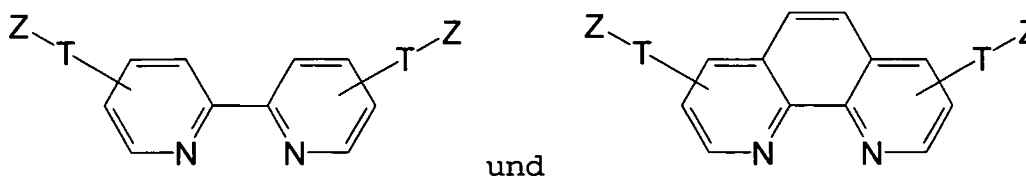
(a) hinzugefügter Analyt von Interesse;
(b) Bindungspartner des Analyten; und
(c) reaktive Komponente, die zur Bindung von (a) oder (b) in der Lage ist.

83. Materiezusammensetzung zur Verwendung als Reagenz in einem Assay, welche folgendes umfasst:

(a) ein markiertes Material, das einen lumineszierenden Metallkomplex mit der Struktur

$$ML^1L^2_2$$

umfasst, worin
M Os oder Ru ist;
L^1 ein substituierter Bipyridin- oder Phenanthrolinligand mit mindestens einem Substituenten ist, der kovalent an eine Assayfunktionssubstanz gebunden ist; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



worin
T eine Linkergruppe ist, die einen Alkyl-, Alkenyl-, Alkynyl- oder Phenyllinker oder eine Kombination daraus umfasst und wahlweise ein oder mehrere Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$ oder -NHC(NH)NH_2 ist; und
R Alkyl ist; und
(b) mindestens eine zusätzliche Assaykompente, die ausgewählt ist aus der Gruppe bestehend aus:

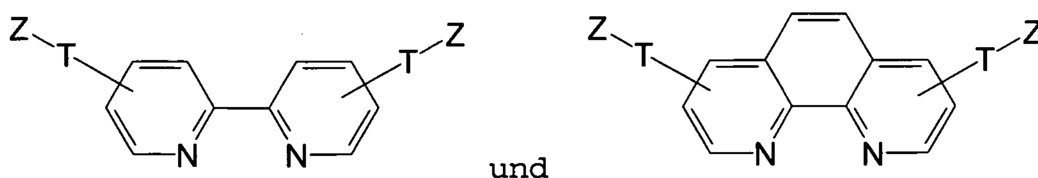
(i) Elektrolyt;
(ii) Analyt von Interesse;
(iii) Bindungspartner des Analyten von Interesse;
(iv) reaktive Komponente, die zur Reaktion mit (ii) oder (iii) in der Lage ist; und
(v) einen ECL-Koreaktanten;

vorausgesetzt, dass keine zwei Komponenten, die innerhalb einer Reagenzzusammensetzung enthalten sind, miteinander während der Lagerung reaktiv sind, wodurch deren Funktion im beabsichtigten Assay verschlechtert wird.

84. Materiezusammensetzung zur Detektion eines Analyten von Interesse in einer Probe, wobei die Zusammensetzung ein markiertes Material umfasst, welches einen lumineszierenden Metallkomplex mit der Struktur

$ML^1L^2_2$

umfasst, worin
M Os oder Ru ist;
L^1 ein substituierter Bipyridin- oder Phenanthrolinligand mit mindestens einem Substituenten ist, der kovalent an eine Assayfunktionssubstanz gebunden ist; und
L^2 ein Metallligand ist, der ausgewählt ist aus der Gruppe bestehend aus:



worin
T eine Linkergruppe ist, die einen Alkyl-, Alkenyl-, Alkynyl- oder Phenyllinker oder eine Kombination daraus umfasst und wahlweise ein oder mehrere
Kettenkohlenstoffatome aufweist, die durch ein Heteroatom substituiert sind;
Z $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, $-OPO_3H^-$, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$ oder $-NHC(NH)NH_2$ ist; und
R Alkyl ist, und
die Assayfunktionssubstanz zur Bindung des Analyten von Interesse in der Lage ist, oder von dem Analyten von Interesse gebunden wird.

85. Materiezusammensetzung gemäß Anspruch 84, worin die Zusammensetzung mindestens eine zusätzliche Substanz enthält, die ausgewählt ist aus der Gruppe bestehen aus:

(i) hinzugefügtem Analyten von Interesse;
(ii) einem Bindungspartner des Analyten; und
(iii) einer reaktiven Komponente, die zur Bindung von (i) oder (ii) in der Lage ist.

86. Ligand gemäß Anspruch 3, worin n = 1 ist.

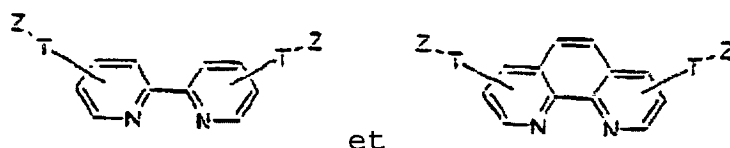
87. Lumineszenzkomplex gemäß Anspruch 22, worin M Os ist.

88. Markiertes Material gemäß Anspruch 35, worin M Ru ist.

89. Markiertes Material gemäß Anspruch 45, worin M Os ist.

Revendications

1. Ligand bipyridine ou phénanthroline choisi parmi le groupe consistant en :



dans lesquelles
T est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle,
à la condition que le ligand ne soit pas un anion de radical 4,4'-dipropylsulfonate-2,2'-bipyridinium.

2. Ligand selon la revendication 1, dans lequel T est $-(CH_2)_n-$ et n est un entier entre 1 et 5.

3. Ligand selon la revendication 1 ou 2, dans lequel Z est $-SO_3$- ou $-SO_3H$.

4. Ligand selon la revendication 1 ou 2, dans lequel Z est $-OSO_3$- ou $-OSO_3H$.

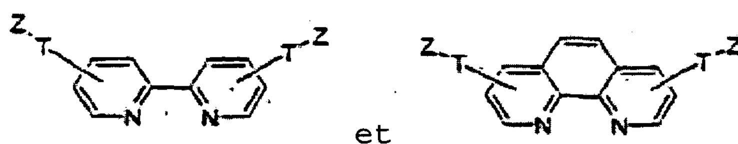
5. Ligand selon la revendication 1 ou 2, dans lequel Z est $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(Me)O_2^-$ ou $-OP(Me)O_2H$.

6. Ligand selon la revendication 1 ou 2, dans lequel Z est $-[NHC(NH_2)_2]^+$ ou $-NHC(NH)NH_2$.

7. Complexe métallique luminescent comprenant le ligand selon la revendication 2, et un atome de métal, l'atome de métal étant lié aux azotes de cycle du ligand.

8. Procédé de conduite d'un dosage basé sur la luminescence comprenant les étapes de :

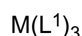
(a) utilisation d'un complexe métallique luminescent comprenant un ligand choisi parmi le groupe consistant en



dans lesquelles
T est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3$, $-SO_3H$, $-OSO_3$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle ; et
ledit complexe métallique comprend un atome de métal qui est lié aux azotes de cycle dudit ligand ;
(b) induction dudit complexe métallique à émettre une luminescence ; et
(c) mesure de la luminescence émise.

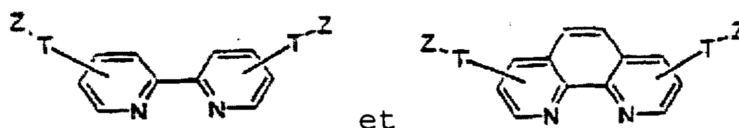
9. Procédé selon la revendication 8, dans lequel ledit complexe métallique affiche une liaison non spécifique réduite dans ledit dosage par rapport au complexe analogue ayant Z = H.

10. Complexe métallique luminescent ayant la structure


$M(L^1)_3$

dans laquelle
M est Os ou Ru ; et

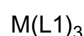
L^1 est un ligand choisi parmi le groupe consistant en :



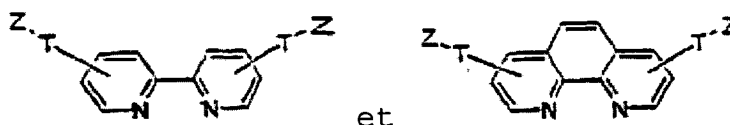
dans lesquelles
T est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3$-, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^2$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle.

11. Procédé de conduite d'un dosage basé sur la luminescence comprenant les étapes de :

(a) utilisation d'un complexe métallique luminescent ayant la structure



dans laquelle
M est Os ou Ru ; et
L^1 est un ligand choisi parmi le groupe consistant en :

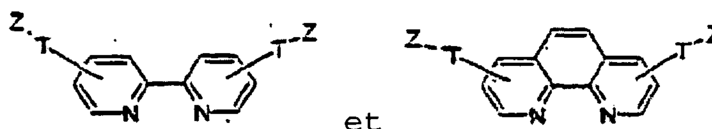


dans lesquelles
T est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3$, $-SO_3H$, $-OSO_3$-, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, - $[NHC(NH_2)_2]^+$, ou -NHC (NH) NH_2 ; et
R est un alkyle ;
(b) induction dudit complexe métallique à émettre une luminescence ; et
(c) mesure de la luminescence émise.

12. Procédé selon la revendication 11, dans lequel ledit complexe métallique affiche une liaison non spécifique réduite dans ledit dosage par rapport au complexe analogue ayant Z = H.

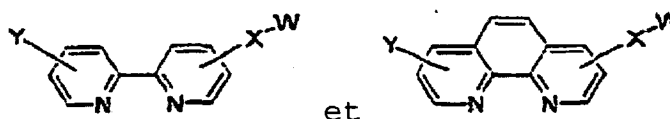
13. Complexe métallique luminescent ayant la structure

dans laquelle
M est Os ou Ru ;
L^1 est un ligand bipyridine ou phénanthroline substitué ayant au moins un substituant qui peut réagir avec un matériau biologique, un réactif de liaison, un substrat enzymatique ou autre réactif de dosage de manière à former une liaison covalente ; et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



dans lesquelles
T est un groupe lieur comprenant un lieur alkyle, alcényle, alcynyle ou phényle, ou une combinaison de ceux-ci, ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3$-, $-SO_3H$, $-OSO_3$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)\ _2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle ; et dans lequel
le matériau biologique, le réactif de liaison, le substrat enzymatique ou autre réactif de dosage est choisi parmi le groupe consistant en des acides aminés, des nucléosides, des nucléotides, des protéines, des peptides, des peptidomimétiques, des acides nucléiques, et des acides nucléiques peptidiques.

14. Complexe métallique luminescent selon la revendication 13, dans lequel L^1 est choisi parmi le groupe consistant en



dans lesquelles
X est un groupe lieur comprenant un lieur alkyle, alcényle, alcynyle ou phényle, ou une combinaison de ceux-ci, ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Y est H ou un alkyle et
W est un substituant qui peut réagir avec le matériau biologique, le réactif de liaison, le substrat enzymatique ou autre réactif de dosage de manière à former une liaison covalente.

15. Complexe métallique luminescent selon la revendication 13, dans lequel Z est $-SO_3$- ou $-SO_3H$.

16. Complexe métallique luminescent selon la revendication 13, dans lequel Z est $-OSO_3$- ou $-OSO_3H$.

17. Complexe métallique luminescent selon la revendication 13, dans lequel Z est $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, -OP (Me)O_2^- ou -OP(Me)O_2H.

18. Complexe métallique luminescent selon la revendication 13, dans lequel Z est - $[NHC(NH_2)_2]^+$ ou $-NHC(NH)NH_2$.

19. Complexe métallique luminescent selon la revendication 13, dans lequel M est Ru.

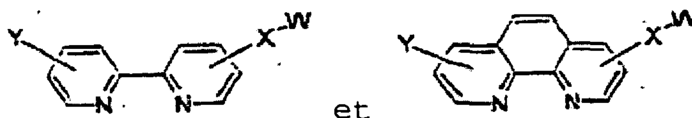
20. Complexe métallique luminescent selon la revendication 13, dans lequel W est un chlorure d'acyle ou un ester de N-hydroxysuccinimide.

21. Complexe métallique luminescent selon la revendication 13, dans lequel W est un groupe acide carboxylique, un groupe amine, ou un groupe hydroxyle.

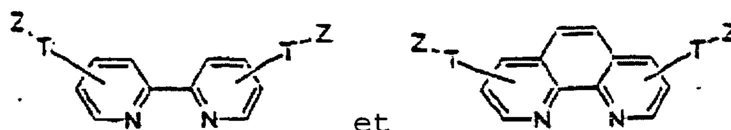
22. Complexe métallique luminescent ayant la structure

$$ML^1L^2_2$$

dans laquelle
M est Os ou Ru ;
L^1 est choisi parmi le groupe consistant en



dans lesquelles
X est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Y est H ou un alkyle et
W est un groupe fonctionnel qui peut réagir avec un matériau biologique, un réactif de liaison, un substrat enzymatique ou autre réactif de dosage de manière à former une liaison covalente ; et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



dans lesquelles
T est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ; et
Z est $-SO_3^-$ ou $-SO_3H$; et dans lequel
le matériau biologique, le réactif de liaison, le substrat enzymatique ou autre réactif de dosage est choisi parmi le groupe consistant en des acides aminés, des nucléosides, des nucléotides, des protéines, des peptides, des peptidomimétiques, des acides nucléiques, et des acides nucléiques peptidiques.

23. Complexe métallique luminescent selon la revendication 22, dans lequel M est Ru.

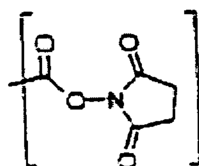
24. Complexe métallique luminescent selon la revendication 22, dans lequel T est $-(CH_2)_n-$, X est $-(CH_2)_m-$et n et m sont chacun des entiers entre 1 à 5.

25. Complexe métallique luminescent selon la revendication 22, dans lequel L^2 est un ligand phénanthroline substitué aux positions 4 et 7 ou L^2 est un ligand bipyridine substitué aux positions 4 et 4'.

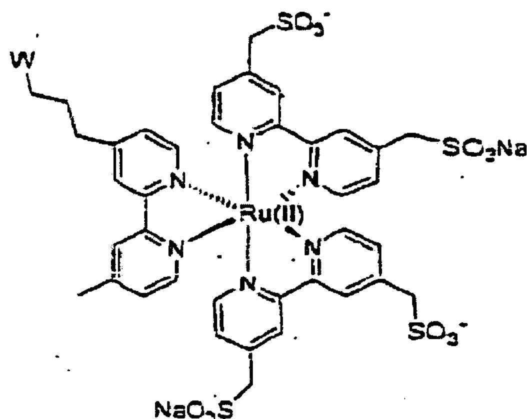
26. Complexe métallique luminescent selon la revendication 22, dans lequel W est un chlorure d'acyle ou un ester de N-hydroxysuccinimide.

27. Complexe métallique luminescent selon la revendication 22, dans lequel W est un groupe acide carboxylique, un groupe amino, ou un groupe thiol.

28. Complexe métallique luminescent selon la revendication 22, dans lequel W est



29. Complexe métallique luminescent avec la structure :

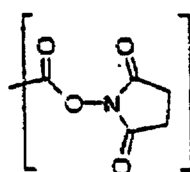


dans laquelle W est un groupe fonctionnel qui peut réagir avec un matériau biologique, un réactif de liaison, un substrat enzymatique ou autre réactif de dosage de manière à former une liaison covalente ; et dans laquelle le matériau biologique, le réactif de liaison, le substrat enzymatique ou autre réactif de dosage est choisi parmi le groupe consistant en des acides aminés, des nucléosides, des nucléotides, des protéines, des peptides, des peptidomimétiques, des acides nucléiques, et des acides nucléiques peptidiques.

30. Complexe métallique luminescent selon la revendication 29, dans lequel W est un chlorure d'acyle ou un ester de N-hydroxysuccinimide.

31. Complexe métallique luminescent selon la revendication 29, dans lequel W est un groupe acide carboxylique, un groupe amino ou un groupe hydroxyle.

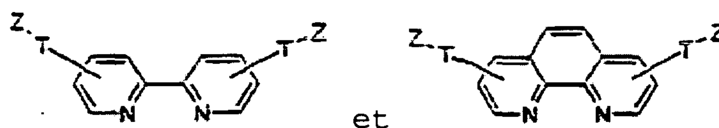
32. Complexe métallique luminescent selon la revendication 29, dans lequel W est



33. Matériau marqué comprenant un complexe métallique luminescent ayant la structure

$ML^1L^2_2$

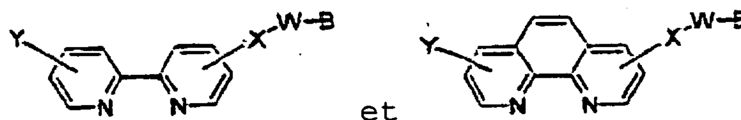
dans laquelle
M est Os ou Ru ;
L^1 est un ligand bipyridine ou phénanthroline substitué ayant au moins un substituant qui est lié de manière covalente avec un matériau biologique, un réactif de liaison, un substrat enzymatique ou autre réactif de dosage ; et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



dans lesquelles

T est un groupe lieur comprenant un lieur alkyle, alcényle, alcynyle ou phényle, ou une combinaison de ceux-ci, ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle ; et dans lequel
le matériau biologique, le réactif de liaison, le substrat enzymatique ou autre réactif de dosage est choisi parmi le groupe consistant en des acides aminés, des nucléosides, des nucléotides, des protéines, des peptides, des peptidomimétiques, des acides nucléiques, et des acides nucléiques peptidiques.

34. Matériau marqué selon la revendication 33, dans lequel L^1 est choisi parmi le groupe consistant en



dans lesquelles
X est un groupe lieur comprenant un lieur alkyle, alcényle, alcynyle ou phényle, ou une combinaison de ceux-ci, ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Y est H ou un alkyle ;
B est le matériau biologique, le réactif de liaison, le substrat enzymatique ou autre réactif de dosage ; et
W est un groupe fonctionnel qui est lié à B.

35. Matériau marqué selon la revendication 33, dans lequel Z est $-SO_3$- ou $-SO_3H$.

36. Matériau marqué selon la revendication 33, dans lequel Z est $-OSO_3$- ou $-OSO_3H$.

37. Matériau marqué selon la revendication 33, dans lequel Z est $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(Me)O_2^-$ ou $-OP(Me)O_2H$.

38. Matériau marqué selon la revendication 33, dans lequel Z est - $[NHC(NH_2)_2]^+$ ou $-NHC(NH)NH_2$.

39. Matériau marqué selon la revendication 33, dans lequel M est Ru.

40. Matériau marqué selon la revendication 33, dans lequel le complexe affiche une liaison non spécifique moindre par rapport au complexe analogue dans lequel Z est H.

41. Matériau marqué selon la revendication 33, dans lequel ledit matériau biologique, ledit réactif de liaison, ledit substrat enzymatique ou autre réactif de dosage est lié à cinq ou plus desdits complexes métalliques luminescents.

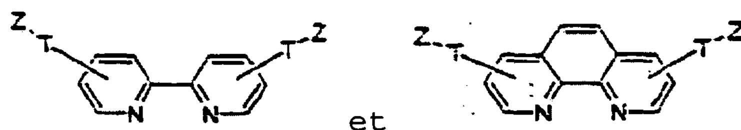
42. Matériau marqué selon la revendication 33, dans lequel ledit matériau biologique, ledit réactif de liaison, ledit substrat enzymatique ou autre réactif de dosage est lié à dix ou plus desdits complexes métalliques luminescents.

43. Procédé de conduite d'un dosage basé sur une luminescence comprenant les étapes de :

(a) utilisation d'un matériau marqué comprenant un complexe métallique luminescent ayant la structure

$$ML^1L^2_2$$

dans laquelle
M est Os ou Ru ;
L^1 est un ligand bipyridine ou phénanthroline substitué ayant au moins un substituant qui est lié de manière covalente avec un matériau biologique, un réactif de liaison, un substrat enzymatique ou autre réactif de dosage ; et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



dans lesquelles
T est un groupe lieur comprenant un lieur alkyle, alcényle, alcynyle ou phényle, ou une combinaison de ceux-ci, ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, ou -NHC (NH) NH_2 ; et
R est un alkyle ;
(b) induction dudit complexe métallique à émettre une luminescence ; et
(c) mesure de la luminescence émise ; et dans lequel

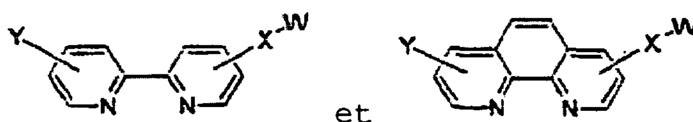
le matériau biologique, le réactif de liaison, le substrat enzymatique ou autre réactif de dosage est choisi parmi le groupe consistant en des acides aminés, des nucléosides, des nucléotides, des protéines, des peptides, des peptidomimétiques, des acides nucléiques, et des acides nucléiques peptidiques.

44. Procédé selon la revendication 43, dans lequel ledit matériau marqué affiche une liaison non spécifique réduite dans ledit dosage par rapport au matériau marqué analogue ayant Z = H.

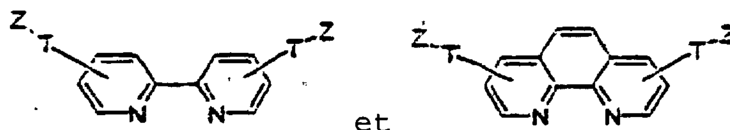
45. Matériau marqué comprenant un complexe métallique luminescent ayant la structure

$ML^1L^2_2$

dans laquelle
M est Os ou Ru ;
L^1 est choisi parmi le groupe consistant en :



dans lesquelles
X est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Y est H ou un alkyle et
W est un groupe fonctionnel qui est lié à un matériau biologique, un réactif de liaison, un substrat enzymatique ou autre réactif de dosage ; et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



dans lesquelles
T est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ; et
Z est $-SO_3^-$ ou $-SO_3H$; et dans lequel
le matériau biologique, le réactif de liaison, le substrat enzymatique ou autre réactif de dosage est choisi parmi le

groupe consistant en des acides aminés, des nucléosides, des nucléotides, des protéines, des peptides, des peptidomimétiques, des acides nucléiques, et des acides nucléiques peptidiques.

46. Matériau marqué selon la revendication 45, dans lequel M est Ru.

47. Matériau marqué selon la revendication 45, dans lequel T est $-(CH_2)_n-$, X est $-(CH_2)_m-$ et n et m sont chacun des entiers entre 1 à 5.

48. Matériau marqué selon la revendication 45, dans lequel L^2 est un ligand phénanthroline substitué aux positions 4 et 7 ou L^2 est un ligand bipyridine substitué aux positions 4 et 4'.

49. Matériau marqué selon la revendication 45, dans lequel le complexe affiche une liaison non spécifique moindre par rapport au complexe analogue dans lequel Z est H.

50. Matériau marqué selon la revendication 45, dans lequel ledit matériau biologique, ledit réactif de liaison, ledit substrat enzymatique ou autre réactif de dosage est lié à 5 ou plus desdits complexes métalliques luminescents.

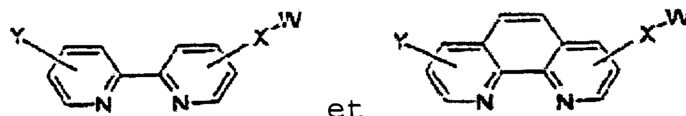
51. Matériau marqué selon la revendication 45, dans lequel ledit matériau biologique, ledit réactif de liaison, ledit substrat enzymatique ou autre réactif de dosage est lié à 10 ou plus desdits complexes métalliques luminescents.

52. Procédé de conduite d'un dosage basé sur une luminescence comprenant les étapes de :

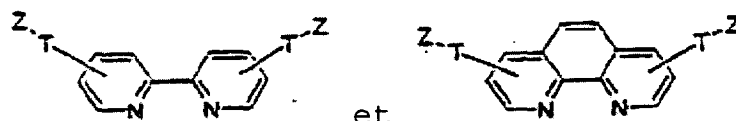
(a) utilisation d'un matériau marqué comprenant un complexe métallique luminescent ayant la structure

$ML^1L^2_2$

dans laquelle
M est Os ou Ru ;
L^1 est choisi parmi le groupe consistant en :



dans lesquelles
X est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Y est H ou un alkyle ; et
W est un groupe fonctionnel qui est lié à un matériau biologique, un réactif de liaison, un substrat enzymatique ou autre réactif de dosage ; choisi parmi le groupe consistant en des acides aminés, des nucléosides, des nucléotides, des protéines, des peptides, des peptidomimétiques, des acides nucléiques, et des acides nucléiques peptidiques et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



dans lesquelles
T est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ; et

Z est $-SO_3$ ou $-SO_3H$;
(b) induction dudit complexe métallique à émettre une luminescence ; et
(c) mesure de la luminescence émise.

5

53. Procédé selon la revendication 52, dans lequel ledit matériau marqué affiche une liaison non spécifique réduite dans ledit dosage par rapport au matériau marqué analogue ayant Z = H.

54. Matériau marqué comprenant un complexe métallique luminescent avec la structure :

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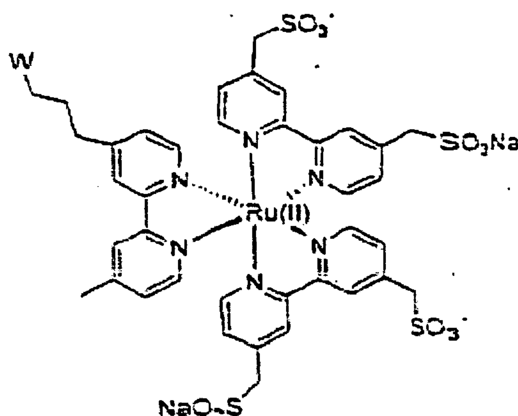

dans laquelle W est un groupe fonctionnel qui est lié à un matériau biologique, un réactif de liaison, un substrat enzymatique ou autre réactif de dosage ; et dans laquelle
le matériau biologique, le réactif de liaison, le substrat enzymatique ou autre réactif de dosage est choisi parmi le groupe consistant en des acides aminés, des nucléosides, des nucléotides, des protéines, des peptides, des peptidomimétiques, des acides nucléiques, et des acides nucléiques peptidiques.

30

55. Matériau marqué selon la revendication 54, dans lequel (i) W est -C(O)-, et (ii) ledit matériau biologique, ledit réactif de liaison, ledit substrat enzymatique ou autre réactif de dosage est lié à W par une liaison amide.

35

56. Matériau marqué selon la revendication 54, dans lequel ledit matériau biologique, ledit réactif de liaison, ledit substrat enzymatique ou autre réactif de dosage est lié à 5 ou plus desdits complexes métalliques luminescents.

57. Matériau marqué selon la revendication 54, dans lequel ledit matériau biologique, ledit réactif de liaison, ledit substrat enzymatique ou autre réactif de dosage est lié à 10 ou plus desdits complexes métalliques luminescents.

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58. Procédé de conduite d'un dosage basé sur une luminescence comprenant les étapes de :

(a) utilisation du matériau marqué selon la revendication 54 ;
(b) induction dudit matériau marqué à émettre une luminescence ; et
(c) mesure de la luminescence émise.

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59. Procédé selon la revendication 58, dans lequel ledit matériau marqué affiche une liaison non spécifique réduite dans ledit dosage par rapport au matériau marqué analogue ayant Z = H.

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60. Procédé de mesure d'un analyte, d'une activité chimique ou d'une activité biologique dans un échantillon comprenant les étapes de i) mise d'un échantillon contenant l'analyte, l'activité chimique, l'activité biologique, un produit de l'activité biologique ou un produit de l'activité chimique en contact avec le complexe métallique luminescent selon la revendication 13 ; ii) induction du complexe métallique à émettre une luminescence ; et iii) mesure de la luminescence de façon à détecter ou mesurer l'activité chimique ou biologique.

55

61. Procédé selon la revendication 60, dans lequel ledit matériau marqué affiche une liaison spécifique moindre dans ledit dosage par rapport à un matériau marqué ayant un ligand analogue qui ne présente pas de groupes fonctionnels

réduisant une liaison non spécifique.

62. Procédé selon la revendication 60 ou 61, dans lequel ladite luminescence est une électrochimioluminescence.

63. Procédé de mesure d'un matériau marqué comprenant les étapes de i) mise du matériau marqué en contact avec un réactif de liaison et, facultativement, un support en phase solide ; ii) formation d'un complexe de liaison comprenant le réactif de liaison, le matériau marqué, et, facultativement, le support en phase solide ; iii) induction du matériau marqué à émettre une luminescence ; et iv) mesure de la luminescence émise de façon à mesurer le matériau marqué ; dans lequel le matériau marqué est préparé à partir du complexe métallique luminescent selon la revendication 13.

64. Procédé selon la revendication 63, dans lequel ledit matériau marqué est mis en contact avec ladite phase solide et ledit complexe de liaison est formé sur ladite phase solide.

65. Procédé selon la revendication 63, dans lequel ledit matériau marqué affiche une liaison spécifique moindre dans ledit dosage par rapport à un matériau marqué analogue ayant un ligand analogue qui ne présente pas de groupes fonctionnels réduisant une liaison non spécifique ;
dans lequel les groupes fonctionnels réduisant une liaison non spécifique sont choisis parmi le groupe consistant en $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$.

66. Procédé selon la revendication 63, dans lequel ladite luminescence est une électrochimioluminescence.

67. Procédé de mesure d'un analyte dans un échantillon comprenant les étapes de i) mise de l'échantillon en contact avec un réactif de liaison marqué et, facultativement, un support en phase solide ; ii) formation d'un complexe de liaison comprenant le réactif de liaison, l'analyte, et, facultativement, le support en phase solide ; iii) induction des marqueurs dans le réactif de liaison marqué à émettre une luminescence, préférablement une ECL ; et iv) mesure de la luminescence émise de façon à mesurer l'analyte dans l'échantillon ; dans lequel ledit réactif de liaison marqué est préparé à partir du complexe métallique luminescent selon la revendication 13.

68. Procédé selon la revendication 67, dans lequel ledit complexe de liaison est formé sur ladite phase solide.

69. Procédé selon la revendication 67, dans lequel ledit réactif de liaison marqué est mis en contact avec ladite phase solide et ledit complexe de liaison est formé sur ladite phase solide.

70. Procédé selon la revendication 67, dans lequel ledit réactif de liaison marqué affiche une liaison spécifique moindre dans ledit dosage par rapport à des réactifs de liaison marqués analogues qui n'ont pas de marqueurs qui présentent des groupes fonctionnels réduisant une liaison non spécifique.

71. Procédé selon la revendication 67, dans lequel ladite luminescence est une électrochimioluminescence.

72. Procédé selon la revendication 67, dans lequel ledit dosage est un dosage en sandwich.

73. Procédé selon la revendication 67, dans lequel ledit dosage est un dosage compétitif.

74. Procédé de mesure d'un analyte dans un échantillon comprenant les étapes de i) mise de l'échantillon en contact avec analogue marqué de l'analyte, un réactif de liaison et, facultativement, un support en phase solide ; ii) formation d'un complexe de liaison comprenant l'analogue marqué de l'analyte, le réactif de liaison et, facultativement, le support en phase solide ; iii) induction des marqueurs dans l'analogue marqué de l'analyte à émettre une luminescence, préférablement une ECL ; et iv) mesure de la luminescence émise de façon à mesurer l'analyte dans l'échantillon ; dans lequel ledit analogue marqué de l'analyte est préparé à partir du complexe métallique luminescent selon la revendication 13.

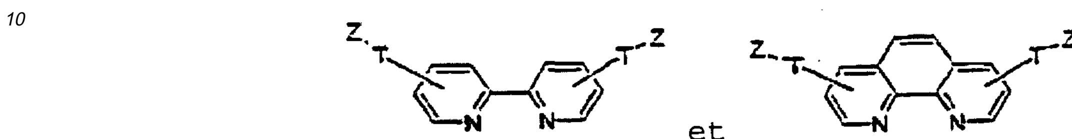
75. Procédé selon la revendication 74, dans lequel ledit analogue marqué de l'analyte est mis en contact avec ladite phase solide et ledit complexe de liaison est formé sur ladite phase solide.

76. Procédé selon la revendication 74, dans lequel ledit analogue marqué de l'analyte affiche une liaison spécifique moindre dans ledit dosage par rapport à des réactifs analogues qui ne présentent pas des groupes fonctionnels

réduisant une liaison non spécifique.

77. Procédé selon la revendication 74, dans lequel ladite luminescence est une électrochimioluminescence.

78. Procédé d'amélioration d'un dosage par luminescence qui emploie un complexe métallique luminescent qui contient un ligand bipyridine, phénanthroline, bipyridine substitué ou phénanthroline substitué, ledit procédé comprenant l'étape de substitution dudit ligand avec un ligand choisi parmi le groupe consistant en



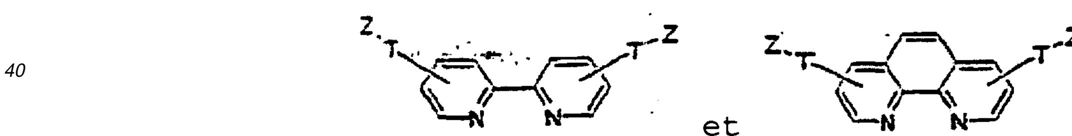
dans lesquelles
T est un lieur alkyle ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle.

79. Procédé selon la revendication 78, dans lequel ledit remplacement améliore un signal de dosage par rapport au fond d'un facteur de deux ou plus.

80. Trousse comprenant, dans un ou plusieurs récipient(s), un matériau marqué comprenant un complexe métallique luminescent ayant la structure

$ML^1L^2_2$

dans laquelle
M est Os ou Ru ;
L^1 est un ligand bipyridine ou phénanthroline substitué ayant au moins un substituant qui est lié de manière covalente avec une substance de performance de dosage ; et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



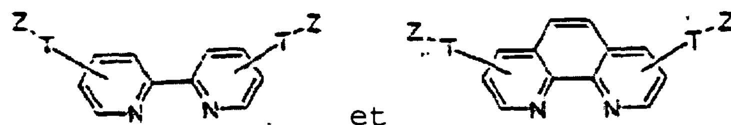
dans lesquelles
T est un groupe lieur comprenant un lieur alkyle, alcényle, alcynyle ou phényle, ou une combinaison de ceux-ci, ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, - $[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle ; et
au moins un composant de dosage choisi parmi le groupe consistant en :

(a) un coréactif d'électrochimioluminescence ;
(b) un ou plusieurs réactif(s) de liaison ;
(c) un ou plusieurs tampon(s) de pH.

81. Matériau marqué comprenant un complexe métallique luminescent ayant la structure

$ML^1L^2_2$

dans laquelle
M est Os ou Ru ;
L^1 est un ligand bipyridine ou phénanthroline substitué ayant au moins un substituant qui est lié de manière covalente avec une substance de performance de dosage ; et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



dans lesquelles
T est un groupe lieur comprenant un lieur alkyle, alcényle, alcynyle ou phényle, ou une combinaison de ceux-ci, ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle.

82. Matériau marqué selon la revendication 81, dans lequel ladite substance de performance de dosage est choisie parmi le groupe consistant en :

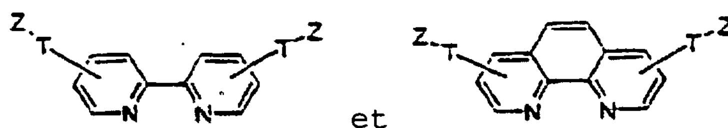
(a) un analyte d'intérêt ajouté ;
(b) un partenaire de liaison dudit analyte ; et
(c) un composant réactif apte à se lier avec (a) ou (b).

83. Composition de matières destinée à une utilisation comme un réactif dans un dosage comprenant :

(a) un matériau marqué comprenant un complexe métallique luminescent ayant la structure

$ML^1L^2_2$

dans laquelle
M est Os ou Ru ;
L^1 est un ligand bipyridine ou phénanthroline substitué ayant au moins un substituant qui est lié de manière covalente avec une substance de performance de dosage ; et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



dans lesquelles
T est un groupe lieur comprenant un lieur alkyle, alcényle, alcynyle ou phényle, ou une combinaison de ceux-ci, ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O2^-$, $-OP(R)O_2H$, $-[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle ; et
(b) au moins un composant de dosage additionnel choisi parmi le groupe consistant en :

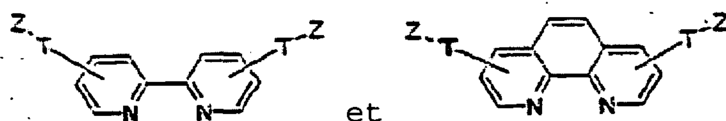
(i) un électrolyte ;
(ii) un analyte d'intérêt ;
(iii) un partenaire de liaison de l'analyte d'intérêt ;
(iv) un composant réactif apte à réagir avec (ii) ou (iii) ; et
(v) un coréactif d'ECL,

à la condition, toutefois, que deux composants contenus au sein d'une quelconque composition de réactifs ne soient pas réactifs l'un avec l'autre pendant le stockage de façon à altérer leur fonction dans le dosage prévu.

84. Composition de matières pour la détection d'un analyte d'intérêt présent dans un échantillon, laquelle composition comprend un matériau marqué comprenant un complexe métallique luminescent ayant la structure

$ML^1L^2_2$

dans laquelle
M est Os ou Ru ;
L^1 est un ligand bipyridine ou phénanthroline substitué ayant au moins un substituant qui est lié de manière covalente avec une substance de performance de dosage ; et
L^2 est un ligand métallique choisi parmi le groupe consistant en :



dans lesquelles
T est un groupe lieur comprenant un lieur alkyle, alcényle, alcynyle ou phényle, ou une combinaison de ceux-ci, ayant, facultativement, un ou plusieurs carbone(s) de chaîne substitué(s) par un hétéroatome ;
Z est $-SO_3^-$, $-SO_3H$, $-OSO_3^-$, $-OSO_3H$, $-OPO_3^{2-}$, OPO_3H^-, $-OPO_3H_2$, $-OP(R)O_2^-$, $-OP(R)O_2H$, $[NHC(NH_2)_2]^+$, ou $-NHC(NH)NH_2$; et
R est un alkyle,
ladite substance de performance de dosage étant apte à se lier à l'analyte d'intérêt ou étant liée à l'analyte d'intérêt.

85. Composition de matières selon la revendication 84, dans laquelle ladite composition contient au moins une substance additionnelle choisie parmi le groupe consistant en

(i) un analyte d'intérêt ajouté ;
(ii) un partenaire de liaison dudit analyte ; et
(iii) un composant réactif apte à se lier avec (i) ou (ii).

86. Ligand selon la revendication 3, dans lequel n = 1.

87. Complexe luminescent selon la revendication 22, dans lequel M est Os.

88. Matériau marqué selon la revendication 35, dans lequel M est Ru.

89. Matériau marqué selon la revendication 45, dans lequel M est Os.

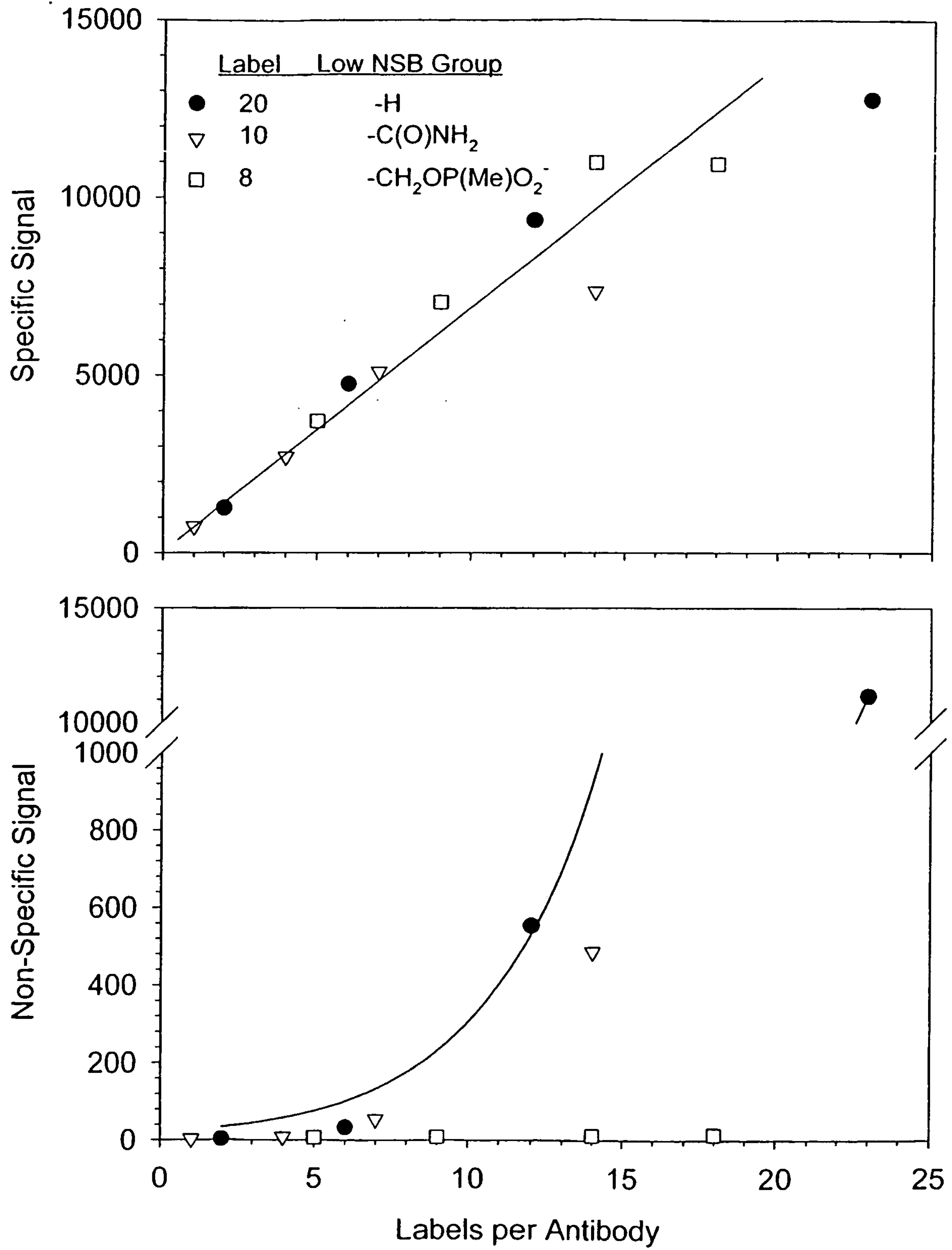


Figure 1.

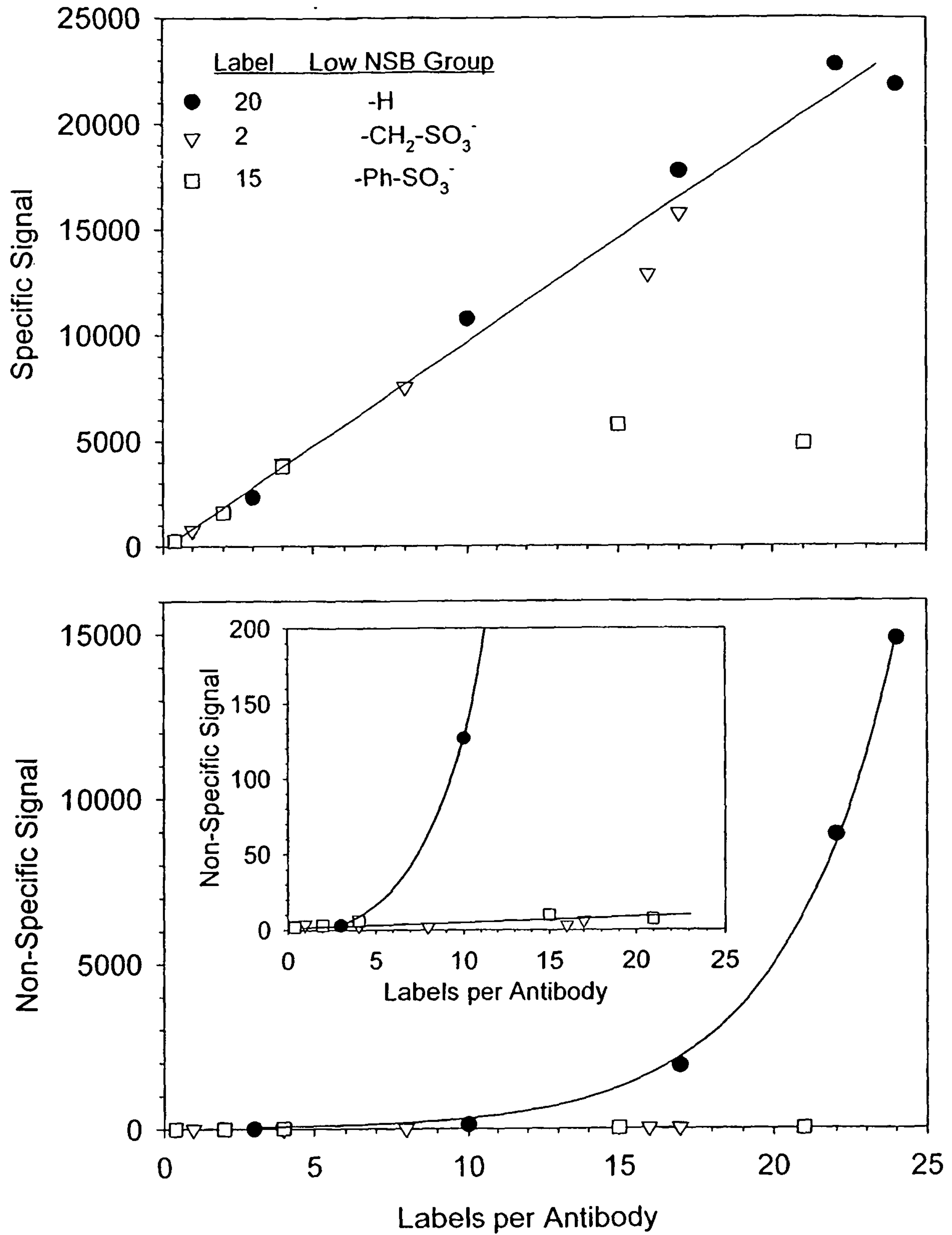


Figure 2.

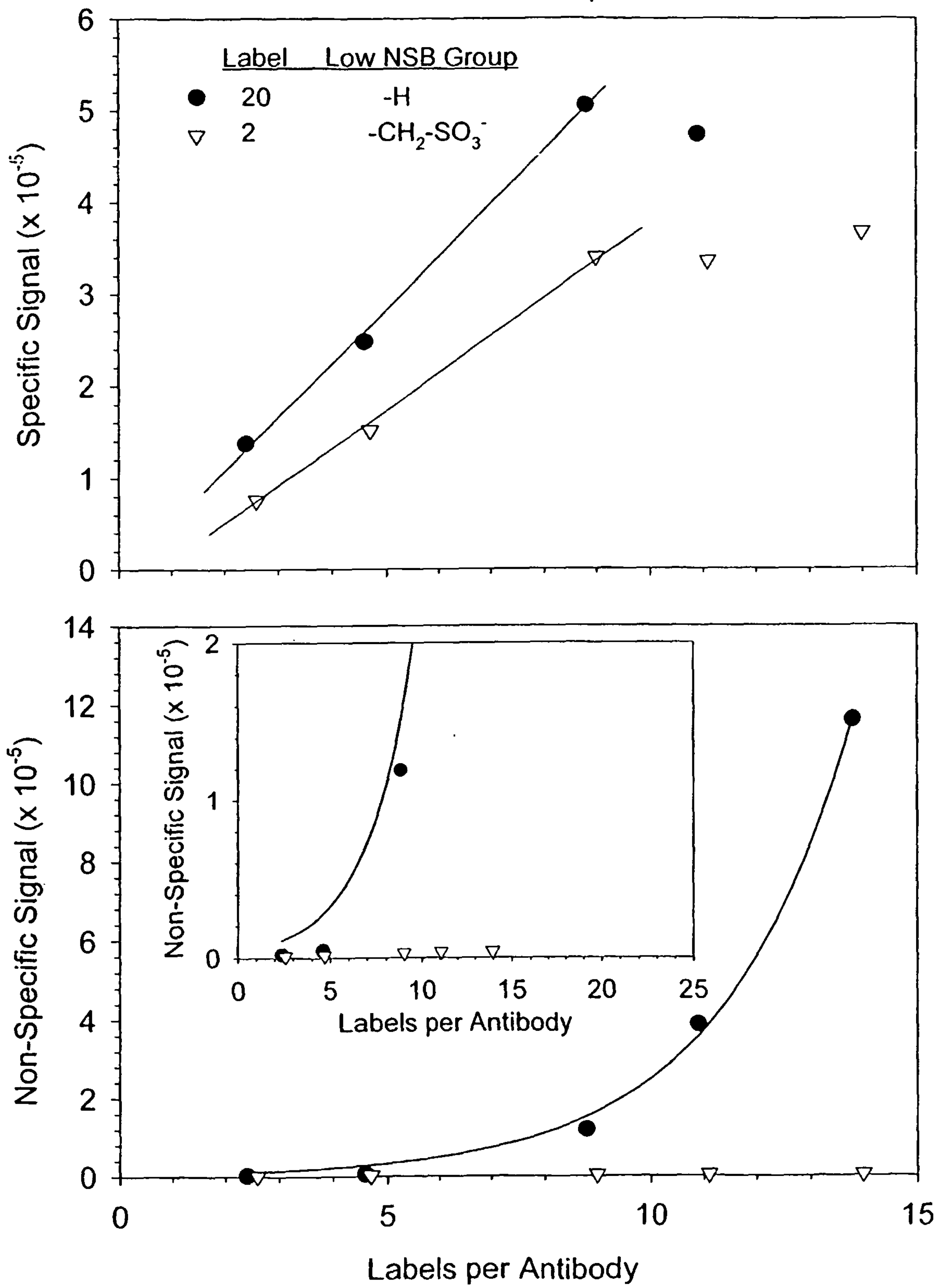


Figure 3.

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 5310687 A **[0001] [0006]**
- US 5591581 A **[0001] [0005] [0006] [0028]**
- US 5597910 A **[0001] [0006]**
- US 5705402 A **[0001] [0005] [0028]**
- US 5846485 A **[0001] [0007]**
- US 6066448 A **[0001] [0005] [0028]**
- US 6214552 A **[0001] [0028]**
- US 6207369 A **[0001] [0028]**
- WO 8706706 A **[0005] [0006] [0028] [0047]**
- US 6207369 B **[0005] [0043]**
- WO 9812539 A **[0005] [0028] [0043]**
- US 5641623 A **[0024] [0028]**
- US 5643713 A **[0028]**
- US 6165708 A **[0028]**
- US 6977722 B **[0031]**
- US 5935779 A **[0043]**
- US 6133043 A **[0043]**
- US 6200531 A **[0043]**

Non-patent literature cited in the description

- **FRASER et al.** *J. Org. Chem.,* 1997, vol. 62, 9314-9317 **[0045]**
- **ANDERSON et al.** *J. Chem. Research (S,* 1979, 74-75 **[0047]**

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专利名称(译)	ECL标签具有改善的非特异性结合特性，使用方法和含有它们的试剂盒		
公开(公告)号	EP1409459B1	公开(公告)日	2012-07-04
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当前申请(专利权)人(译)	BIOVERIS CORPORATION		
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发明人	SIGAL, GEORGE, B. TJIONG, HOWIE DONG, LIWEN MASOOD, ATHAR TITMAS, RICHARD, C.		
IPC分类号	C07D213/22 G01N33/532 G01N33/533 C12Q1/68 C07K1/13 C07D471/04 C07F9/58 C07F9/6561 C07D213/30 C07D213/40 C07D213/81 C07D213/34 C07F15/00 G01N33/58 C07D213/53 C07D213/55 C07D401/14 G01N27/416		
CPC分类号	C07D213/30 C07D213/34 C07D213/40 C07D213/81 C07D471/04 C07F9/58 C07F9/6561 C07F15/0053 C09B57/00 C09B57/10 G01N33/582 G01N2458/40		
优先权	09/896974 2001-06-29 US		
其他公开文献	EP1409459A2 EP1409459A4		
外部链接	Espacenet		

摘要(译)

具有防止非特异性结合的官能团的联吡啶或菲咯啉配体（特别是不受标准条件影响的带负电的官能团，用于通过酰胺键缀合生物试剂）被描述为包含这些配体的发光金属络合物。在电化学发光测定中使用包含这些配体的发光钌和锇络合物表明，相对于使用不具有官能团的类似标记物标记的试剂进行的测定，使用这些标记物可以显着减少观察到的非特异性结合的量。减少非特异性结合。