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(54) Title: AGENTS FOR THERAPY EFFICACY MONITORING AND DEEP TISSUE IMAGING

(57) Abstract: Compounds and methods related to NIR molecular imaging, in-vitro and in-vivo functional imaging, therapy/efficacy monitoring, and cancer and metastatic activity imaging. Compounds and methods demonstrated pertain to the field of peripheral benzodiazepine receptor imaging, metabolic imaging, cellular respiration imaging, cellular proliferation imaging as targeted agents that incorporate signaling agents.

AGENTS FOR THERAPY EFFICACY MONITORING AND DEEP TISSUE IMAGING

Priority Information

[01] This application claims priority to US Patent Application No. 60/641,091, filed January 3, 2005, the contents of which are incorporated herein by reference.

Field of the Invention

[02] This invention relates generally to the field of molecular imaging, and more specifically to the field of functional imaging, including glucose transporters, thymidine kinase activity, and peripheral benzodiazepine receptors as targeted agents that incorporate near-infrared fluorophores as signaling agents.

Background of the Invention

[03] Current state-of-the-art detection and surgical resection tools used in cancer treatment are insufficient. Early stage disease can be missed, resection can be incomplete and these two factors alone are major contributors to morbidity and mortality. Outcomes are intrinsically linked to disease detection and treatment efficacy. Therefore, improvement in the detection of early cellular changes, as well as enhanced visualization of diseased tissue, is of paramount importance.

[04] Optical methods continue to provide a powerful means for studying cell and tissue function. Recent discoveries in Molecular Imaging (MI) are certain to play a vital role in the early detection, diagnosis, and treatment of disease. MI will also aid in the study of biological and biochemical mechanisms, immunology, and neuroscience. MI agents commonly consist of a signaling moiety (fluorophore, radioisotope or Gd^{3+} ion) and a targeting functionality such as an antibody or peptide, sugar or a peripheral benzodiazepine receptor (PBR) ligand. NIR molecular imaging agents are particularly attractive due to the inherently low water and tissue absorption in the NIR spectral region. Additionally, the low scattering cross-section and lack of autofluorescence background in the near infrared (NIR) region facilitate deep penetration and high-resolution images from small interrogated volumes.

[05] While glandular and secretory tissues are normally rich in PBR, other quiescent tissue ordinarily express PBR at relatively low levels. Primarily spanning the bi-layered

mitochondrial membrane, the PBR is expressed almost ubiquitously and thought to be associated with many biological functions including the regulation of cellular proliferation, immunomodulation, porphyrin transport, heme biosynthesis, anion transport, regulation of steroidogenesis and apoptosis. Given the importance of PBR toward regulating mitochondrial function, it is not surprising that PBR density changes have been observed in acute and chronic neurodegenerative states in humans, as well as numerous forms of cancer. For example, temporal cortex obtained from Alzheimer's patients showed an increase in PBR, and correlations with Huntington's disease, multiple sclerosis and gliosis have been demonstrated. Breast cancer generally demonstrates increased PBR expression and represents another potentially attractive target, especially in the NIR. The development of high affinity ligands for PBR (such as, for example, PK-11195, Ro5-4864, DAA1106, and DAA1107) has made non-invasive imaging modalities more suitable.

[06] Other functional imaging targets include the glucose transporter and thymidine kinase 1. By targeting the glucose transporter, [^{18}F]-fluoro-deoxyglucose (FDG) has been successfully employed as a positron emission tomography (PET) agent to determine the metabolic statues (cellular respiration) of suspect tissues. Modest functionalization of glucose at the C-2 position does not hinder sugar uptake but does prevent cellular metabolism, therefore glucose agents can accumulate intracellularly. Since tumor cells metabolize glucose a higher rate than normal cells, the accumulation of glucose mimics (i.e. FDG and similar agents) can facilitate discrimination of tissues based on their metabolic status. While FDG imaging certainly has demonstrated utility to the clinical oncologist, the requirement of a cyclotron and a PET scanner somewhat limit its use.

[07] Recently, in effort to improve the specificity of functional imaging agents like FDG, new probes for cellular proliferation imaging have been developed. Targeting the enzyme thymidine kinase 1 (TK1), an enzyme responsible for DNA replication, [^{18}F]3'-deoxy-3'fluorothymidine (FLT) has been shown to be an attractive complement to FDG imaging. Similar to FDG, FLT is not fully metabolized by cells and accumulates in target tissues, making it a promising imaging agent for rapidly proliferating tissues. When used in combination with FDG, clinical imaging of diseased tissue has the ability to be highly sensitive *and* specific.

[08] It has been shown that NIR emitting Ln-Chelates can be prepared opening the avenue to complexes with spectral properties more compatible with biological imaging such as visible absorption, NIR emission and microsecond-long emission lifetimes. These complexes

have high molar absorptivity and have luminescent lifetimes in the microsecond regime allowing temporal rejection of noise.

[09] The present inventors have demonstrated the synthesis and utility of Eu-PK11195 and Gd-PK11195. Others have prepared PK11195 as a PET agent for use in humans. A NIR Pyropheophorbide agent has been reported for imaging glucose transporters, however this agent was not spectroscopically optimized for deep tissue in-vivo imaging (ex. 679nm, em. 720nm). At present, the authors are unaware of any NIR imaging agents based on thymidine imaging.

Summary of the Invention

[010] The peripheral benzodiazepine receptor (PBR) has been shown an attractive target for contrast-enhanced imaging of disease. See Publication No. 2003/0129579, incorporated herein by reference. Embodiments of the present invention include PBR targeted agents which incorporate near-infrared (NIR) fluorophores as signaling agents. Aspects of the present invention include a previously unknown class of NIR absorbing/emitting PBR targeted contrast agents which utilize a conjugable form of PK11195 as a targeting moiety.

[011] Additionally, aspects of the present invention include the synthesis of NIR-metabolic and proliferation probes. The authors report a sacharide agent suitable for metabolic imaging in similar fashion to ^{18}FDG and a NIR-thymidine probe suitable for imaging cellular proliferation (DNA synthesis). The NIR contrast agents disclosed herein are suitable for optical imaging using spectral and time-gated detection approaches to maximize the signal-to-background ratio. High molar extinction dyes that absorb and emit in the NIR, such as IRdye800CW™ (available from LiCOR) and CY7 (Amersham), as well as NIR Lanthanide chelates are demonstrated. Since thymidine, PK11195 and other PBR ligands have been suggested as therapeutic agents, the molecules demonstrated here could also be useful therapeutics which also offers direct monitoring of dose delivery and therapeutic efficacy.

[012] With absorption and emission closer to the tissue transparency window (780nm, 830nm respectively), the dyes reported here are much more suited for in-vivo imaging. Additionally, no one has demonstrated NIR PBR ligands for imaging PBR expression and/or therapy.

[013] Thus, one aspect of the present invention is a method of imaging a molecular event in a sample, the method steps comprising administering to the sample a probe having an affinity for a target. The probe has at least one of a ligand/signaling agent combination, or

conjugable form of a ligand/signaling agent combination. After the probe is administered, a signal from the probe may be detected. In embodiments of the present invention, the sample can be at least one of cells, tissue, cellular tissue, serum, cell extract, bodily fluids. The bodily fluids may be, for example, breast milk, sputum, vaginal fluids, urine.

[014] Another aspect of the present invention is a method of measuring glucose uptake. This embodiment comprises the steps of administering to a sample a conjugate, the conjugate comprising a conjugable glucosamine compound and a signaling agent; and then detecting a signal from said conjugate. In embodiments of the present invention, the sample is at least one of cells, tissue, cellular tissue, serum, cell extract, bodily fluids.

[015] Another aspect of the present invention is a method of quantifying the progression of a disease state progression that includes the steps of (a) administering to a first sample a conjugate that comprises a conjugable deoxythymidine compound and a signaling agent; (b) detecting a signal from the conjugate; (c) after a period of time from step (b), administering to a second sample a conjugate, (d) detecting a second signal; and (e) comparing the first signal with the second signal to determine the progress of a disease state. Again examples of the sample are at least one of cells, tissue, cellular tissue, serum, cell extract, bodily fluids.

[016] Another aspect of the present invention is the above method, where the conjugate includes a peripheral benzodiazepine affinity ligand or conjugable form thereof and a signaling agent.

[017] Another aspect of the present invention is the above method, where the conjugate includes a glucosamine compound and a signaling agent.

[018] In the above embodiments and other embodiments of the present invention, the administration step is *in vivo* or *in vitro*.

Brief Description of the Drawings

[019] Figure 1 is a photograph that shows white light and fluorescence pictures of dosed cells and un-dosed cells in accordance with the present invention, and is further discussed in Example 6, below. Picture A is a white light picture of dosed cells, Picture B is a fluorescence picture of dosed cells, Picture C is a white light picture of un-dosed cells, and Picture D is a fluorescence picture of un-dosed cells.

[020] Figure 2 is a photograph that shows white light and fluorescence pictures of dosed cells and un-dosed cells in accordance with the present invention, and is further discussed in Example 7, below. Picture A is a white light picture of dosed cells, Picture B is a fluorescence picture of dosed cells, Picture C is a white light picture of un-dosed cells, and Picture D is a fluorescence picture of un-dosed cells.

[021] Figure 3 is a photograph that shows *in vivo* cancer imaging of a small laboratory animal.

[022] Figure 4 is a photograph that shows *in vivo* neurodegenerative imaging of a small laboratory animal.

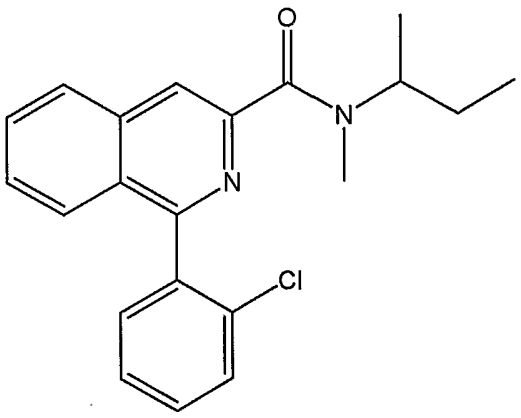
Description of the Invention

[023] Embodiments of the present invention include NIR agents for the PBR based on NIR dyes, including Lanthanide chelates. Additionally, complimentary imaging agents are disclosed using a novel NIR sacharide and NIR thymidine agent. Aspects of the present invention include both being used separately, as well as where the agents are used together as a cocktail whereby both PBR expression and metabolic and or cellular proliferation status could be simultaneously monitored in-vivo.

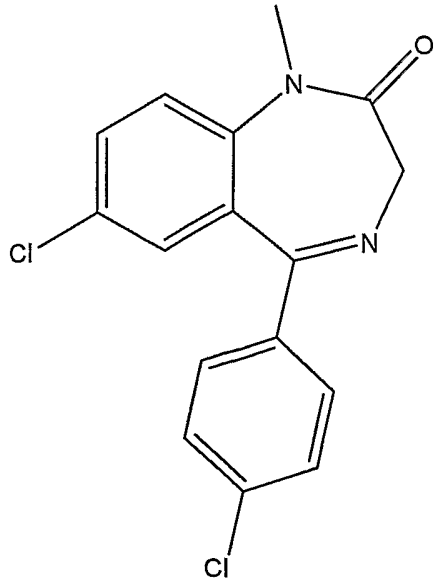
[024] PBR ligands such as PK11195 have been suggested as therapeutic agents. Mitochondria localized anti-death proteins of the Bcl-2 family play a central role in inhibiting apoptosis and therefore present therapeutic targets. PBR shares a close physical association with the permeability transition pore complex (PTPC) and binding of PK11195 has been shown to cause Bcl-2 resistant generation of oxidative stress. The agents reported here are unique in that they facilitate in-vivo monitoring of therapeutic delivery and efficacy.

[025] As stated above, aspects of the present invention include methods of imaging a molecular event. in a sample, the method steps comprising administering to the sample a probe having an affinity for a target. The probe has at least one of a ligand/signaling agent combination, or conjugable form of a ligand/signaling agent combination. One such ligand/signaling agent combination comprises PBR ligands, or conjugable forms thereof.

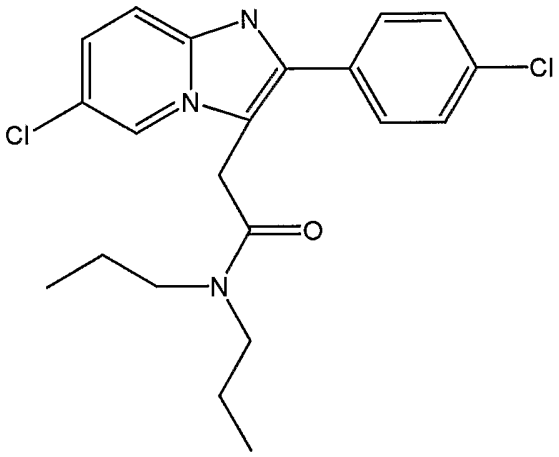
[026] Examples of the PBR ligands of the present invention include conjugable forms, or conjugable analogs of the following compounds:



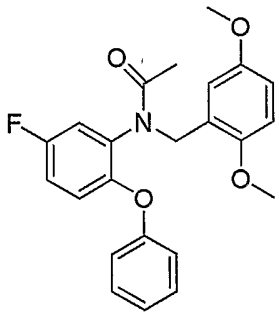
PK11195



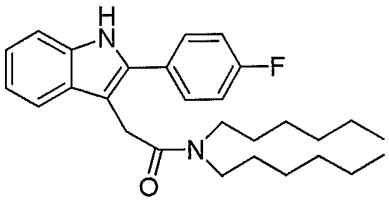
Ro5-4864



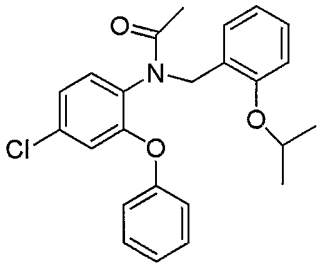
Alpidem



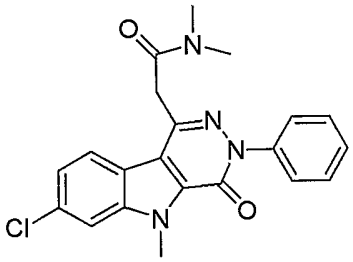
DAA1106



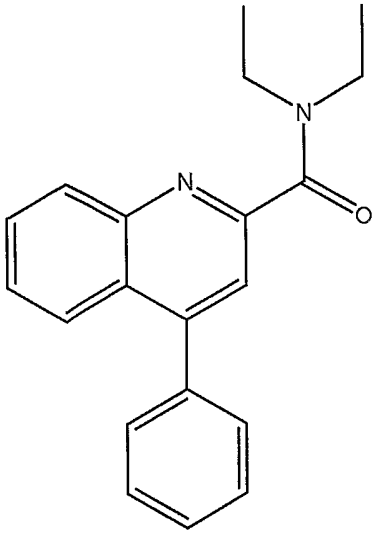
FGIN-1



DAA1097

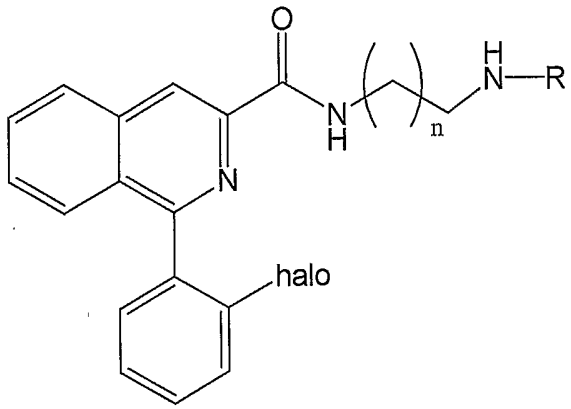


SSR180575 , and



[027] For the purposes of the present invention, the term analog encompases isomers, homologs, or other compounds sufficiently resembling the base compound in terms of structure and do not destroy activity. "Conjugable forms," "conjugable compounds," and similar terms describe a form of the compound that can readily form a covalent form a covalent bond with a signaling agent such as an IR dye.

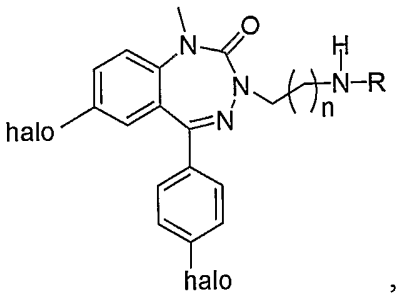
[028] For exemplary purposes, conjugable forms of PK11195, above, include at least the following compounds, and/or analogs or derivatives thereof:



wherein R is H or alkyl, n is 0-10, and "halo" is fluorine, chlorine, bromine, iodine. In other embodiments of the present invention, halo is chlorine.

[029] The term "halo" or "halogen," as used herein, includes radio isotopes of halogen compounds, such as I^{121} and F^{19}.

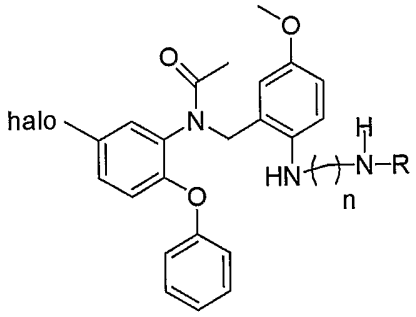
[030] Additionally, for exemplary purposes, conjugable forms of Ro5-4864 include the following and/or analogs or derivatives thereof:



,

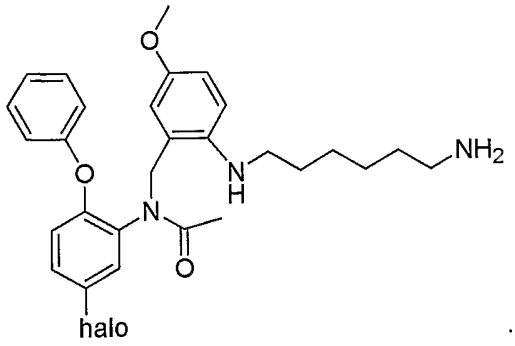
wherein the variables are defined above. In other embodiments of the present invention, halo is chlorine.

[031] Additionally, for exemplary purposes, conjugable forms of DAA1106 include the following and/or analogs or derivatives thereof:



,

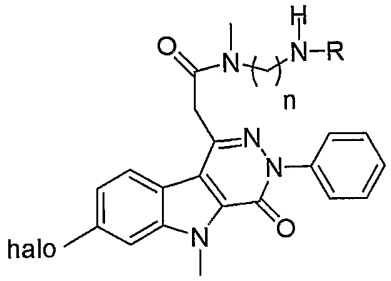
wherein the variables are defined above, and:



.

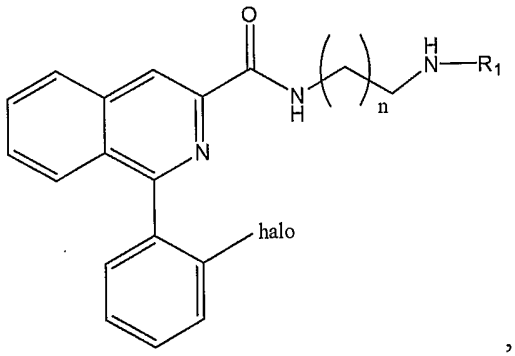
[032] In other embodiments of the present invention, halo is chlorine or fluorine.

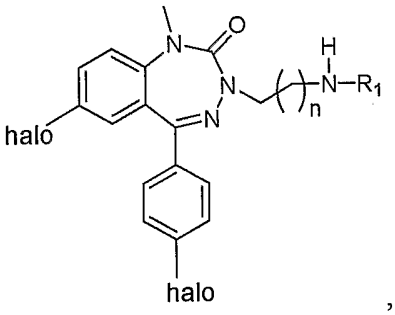
[033] Additionally, for exemplary purposes, conjugable forms of SSR180575 include the following and/or analogs or derivatives thereof:

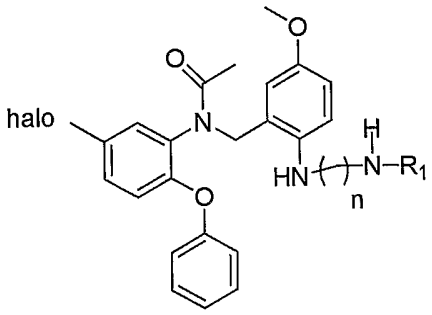


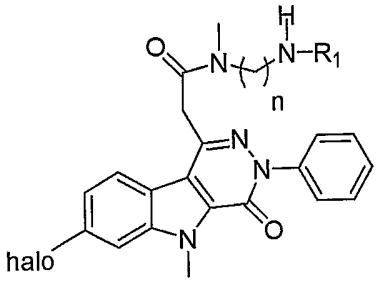
wherein the variables are defined above. In other embodiments of the present invention, halo is chlorine.

[034] Non-limited examples of PBR ligands and signaling moieties include the following compounds:









or an analog thereof, wherein R_1 is a signaling moiety; "halo" is fluorine, chlorine, bromine, iodine; and n is 0-10.

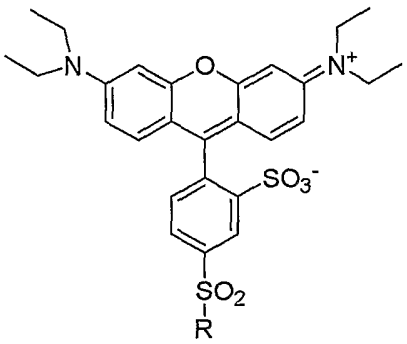
[035] Preferably, in the above examples, the signaling moiety is a dye, such as a cyanine dye.

[036] Additionally, other aspects of the present invention include NIR-sacharide agents suitable for metabolic imaging in similar fashion to ^{18}FDG. Given the ubiquitous clinical use of ^{18}FDG, 2-deoxyglucose derivatives have been extensively biologically characterized. See Czernin, J.; Phelps, M. E. *Annu Rev Med* **2002**, *53*, 89-112. These derivatives are useful metabolic imaging agents given the overexpression of glucose transporters and increased hexokinase activity in tumors. See Medina, R. A.; Owen, G. I. *Biol Res* **2002**, *35*, 9-26. 2-deoxyglucose imaging agents are incorporated into cells via the glucose transporter and are subsequently phosphorylated by hexokinase. In phosphorylating the probe, the neutral molecule becomes anionic and membrane impermeable. Functionalization at the 2-position prevents further metabolism, and thus the probe is trapped in the cells, with further uptake leading to significant accumulation. See Zhang, M.; Zhang, Z. H.; Blessington, D.; Li, H.; Busch, T. M.; Madrak, V.; Miles, J.; Chance, B.; Glickson, J. D.; Zheng, G. *Bioconjugate Chem* **2003**, *14*, 709-714.

[037] Additionally, other aspects of the present invention include a NIR-thymidine probe for monitoring cellular proliferation, similar in fashion to [^{18}F]3'-deoxy-3'fluorothymidine (FLT). FLT has been used clinically and extensively compared to FDG. See Halter et al. *General Thoracic Surgery* **2004**, *127*, 1093-1099 and Francis et al. *Eur J Nucl Med Mol Imaging* **2003**, *30*, 988-994. In proliferating cells, FLT metabolism takes place within the anabolic arm of the DNA salvage pathway. TK1 controls entry into the salvage pathway and converts FLT to the mono-phosphate species. The agent is further phosphorylated, but can not be incorporated into DNA due to its lack of a hydroxyl group at 3'.

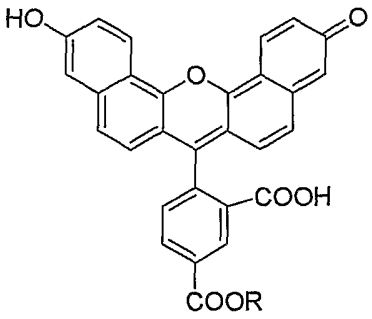
[038] With respect to the signaling agents used in connection with the present invention, embodiments include near infrared signaling agents. Also includes are dyes, such as, for example, near-infrared fluorophores/fluorescent dyes. Examples include cyanine dyes which have been used to label various biomolecules. See US 5,268,486, which discloses fluorescent arylsulfonated cyanine dyes having large extinction coefficients and quantum yields for the purpose of detection and quantification of labeled components.

[039] Additional examples include compounds of the following formulas:

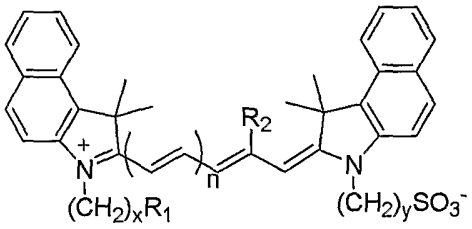


Lissamine-Rhodamine abs/em = 560nm, 590nm

;

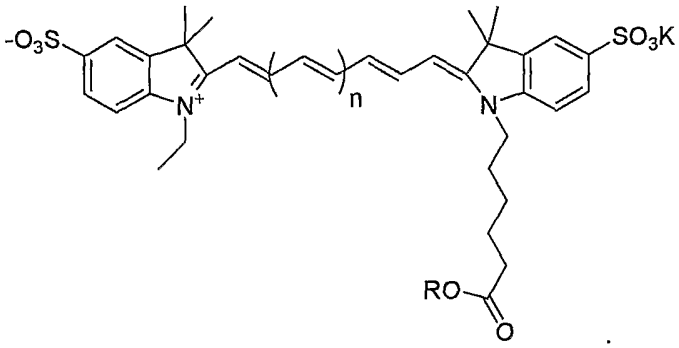


Carboxynaphthofluorescein abs/em = 580nm, 690nm



General Cyanine dye

;



CY-Family of dyes

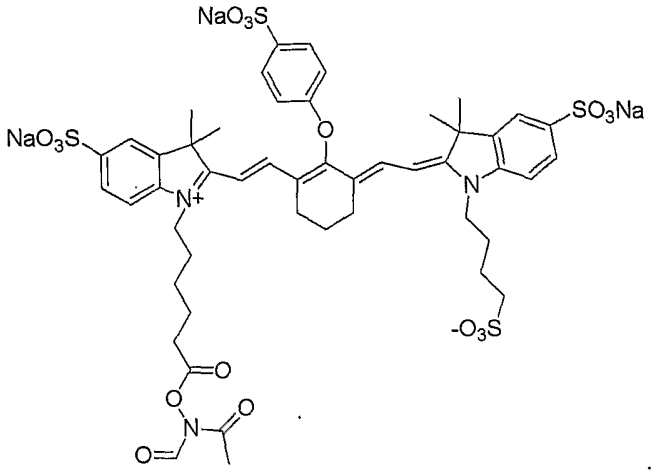
;

and analogs thereof.

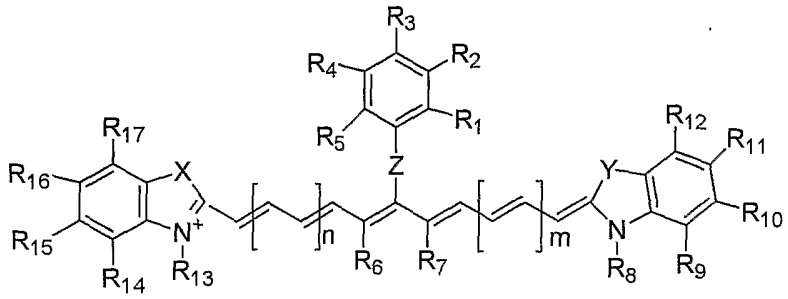
[040] Additional examples include dyes available from Li-Cor, such as IR Dye 800CW™, available from Li-Cor.

[041] Thus, examples of dyes for use in connection with the present invention include those disclosed in US Patent Application Publication Number 2004/0014981, the contents of which are incorporated herein by reference.

[042] The following dye is a specific example:



[043] US Patent Application Publication Number 2004/0014981 additionally discloses the following dyes, all of which, when joined with a probe, are embodiments of the present invention:



[044] wherein, Z is a heteroatom having at least one lone pair of electrons. In one embodiment, Z is O, S, or N_{35}, wherein R_{35} is H or alkyl. In embodiments, Z is of such a structure that only one atom is in the direct linkage between the benzene ring bonded to Z and to the polyene chain of:  , bonded to Z. Side chains on the linkage between the benzene ring and the polyene chain are acceptable. In those embodiments having side chains, lower alkyl side chains may be used.

[045] R_1-R_5 are each independently H, alkyl, halo, carboxyl, amino, or SO_3-Cat^+, wherein Cat^+ is a cation and at least one of R_1-R_5 is SO_3-Cat^+. In embodiments, R_3 is SO_3-Cat^+. In other embodiments, Cat^+ is H^+ or an alkali metal ion such as Na^+.

[046] R_6 and R_7 are each H, alkyl, or optionally, together with the  group to which they are bonded, form a ring. In embodiments, R_6 and R_7 together with the atoms to

which they are bonded form a ring. These rings may have 4 to 10 member atoms, more preferably 5 or 6 member atoms. In one embodiment, the ring including R_6 and R_7 is substituted, with, for example, a sulfonato radical.

[047] The integers m and n are each independently integers from 0 to 5. In embodiments, both the sum of m and n is two. Additionally, the sum of m and n may be one. In other embodiments, both m and n are zero. As the sum of m and n rises, so too does the wavelength of the dye. Generally, the addition of each double bond in the polyene chain can increase the wavelength by about 40 to 120 nm. For the absorption changes accompanied with trimethine to pentamethine or pentamethine to heptamethine, there is a typically a bathochromic shift (red shift) of about 100 nm. For example, when m and n are both 0, the wavelength of the preferred dye is about 770 nm. When m and n are both 1, the wavelength of the preferred dye is about 950 nm. The most preferred dyes operate in the NIR spectrum (600-1000 nm).

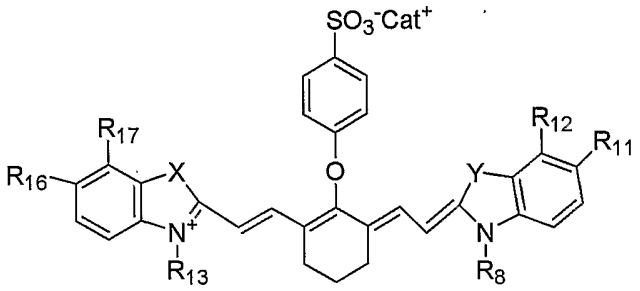
[048] X and Y are each independently O, S, Se, or $CR_{19}R_{20}$, wherein R_{19} and R_{20} are each independently alkyl, or optionally form a ring together with the carbon atom to which they are bonded. In embodiments, X and Y are a heteroatom such as O, S, and Se. When X or Y is $CR_{19}R_{20}$, both R_{19} and R_{20} may be both lower alkyl, including methyl.

[049] R_8 and R_{13} are each independently alkyl, $(CH_2)_rR_{25}$ or $(CH_2)_rR_{19}$; wherein at least one of R_8 and R_{13} is $(CH_2)_rR_{18}$ and wherein r is an integer from 1 to 50, and R_{25} is a functional group that does not directly react with a carboxyl, hydroxyl, amino, or a thiol group, and R_{18} is a functional group that can react with a carboxyl, hydroxyl, amino, or thiol group. In one embodiment, one of R_8 and R_{13} is $(CH_2)_rR_{18}$ and the other is $(CH_2)_rR_{25}$. In other words, one of R_8 and R_{13} reacts with a biomolecule to form a bond to that biomolecule, and that the other does not react. The R_{18} group must be able to covalently bond with the biomolecule being labeled. R_{18} groups include mercapto, carboxyl, amino, haloalkyl, phosphoramidityl, N-hydroxy succinimidyl ester, sulfo N-hydroxy succinimidyl ester, isothiocyanato, iodoacetamidyl, and maleimidyl. R_{25} groups include hydroxyl, thioacetyl, and sulfonato.

[050] R_9-R_{12} and R_{14}-R_{17} are each independently H, alkyl, halo, amino, sulfonato, $R_{21}COOH$, $R_{21}OR_{22}$, $R_{21}SR_{22}$, or $R_{21}COOR_{22}$ wherein R_{21} is a bond or alkylene and R_{22} is alkyl, or optionally R_{11} and R_{12} together with the atoms to which they are bonded form an aromatic ring, or optionally R_{16} and R_{17} together with the atoms to which they are bonded form an aromatic ring. In one embodiment, one or both of R_{11} and R_{16} is sulfonato. In another embodiment, when R_{11} and R_{12} together with the atoms to which they are bonded form an aromatic ring, the ring is substituted in at least one position with a sulfonato group. In another

embodiment, when R_{16} and R_{17} together with the atoms to which they are bonded form an aromatic ring, the ring is substituted in at least one position with a sulfonato group, a halo group, an alkyl substituent, or an amino substituent.

[051] Another cyanine dye that can be used with the present invention is of the following formula:



[052] Cat^+ is a cation. In embodiments, Cat^+ is H^+ or a metal ion. More preferably, Cat^+ is an alkali metal ion, most preferably Na^+ . X and Y are each independently O, S, Se, or $(CH_3)_2C$.

[053] R_8 and R_{13} are each independently alkyl, $(CH_2)_rR_{25}$ or $(CH_2)_rR_{19}$; wherein at least one of R_8 and R_{13} is $(CH_2)_rR_{18}$ and wherein r is an integer from 1 to 50, and R_{25} is a functional group that does not directly react with a carboxyl, hydroxyl, amino, or a thiol group, and R_{18} is a functional group that can react with a carboxyl, hydroxyl, amino, or thiol group. In one embodiment, one of R_8 and R_{13} is $(CH_2)_rR_{18}$ and the other is $(CH_2)_rR_{25}$. In other words, one of R_8 and R_{13} reacts with a biomolecule to form a bond to that biomolecule, and that the other does not react. The R_{18} group must be able to covalently bond with the biomolecule being labeled. R_{18} groups include mercapto, carboxyl, amino, haloalkyl, phosphoramidityl, N-hydroxy succinimidyl ester, sulfo N-hydroxy succinimidyl ester, isothiocyanato, iodoacetamidyl, and maleimidyl. R_{25} groups include hydroxyl, thioacetyl, and sulfonato.

[054] $R_{11}$and R_{12} are either H, sulfonato, or together with the atoms to which they are bonded form an aromatic ring. In a preferred embodiment, $R_{11}$is sulfonato. In another preferred embodiment, when $R_{11}$and R_{12} together with the atoms to which they are bonded form an aromatic ring, the ring is substituted in at least one position with a sulfonato group.

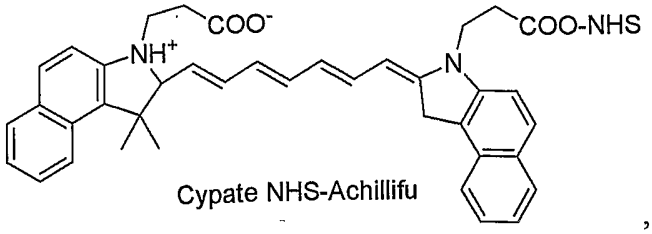
[055] R_{16} and R_{17} are either H, sulfonato, or together with the atoms to which they are bonded form an aromatic ring. In a preferred embodiment, R_{16} is sulfonato. In another

preferred embodiment, when R_{16} and R_{17} together with the atoms to which they are bonded form an aromatic ring, the ring is substituted in at least one position with a sulfonato group.

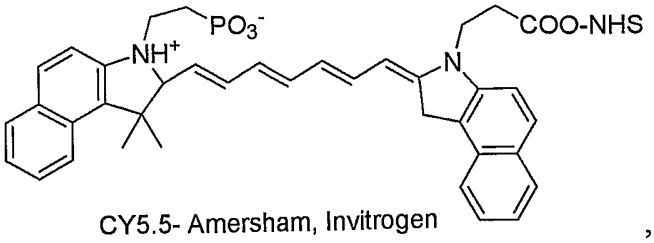
[056] Further examples of cyanine dyes that can be used in connection with the present invention are those cyanine dyes that can be excited efficiently by commercially available equipment purchasable through companies such as Toshiba, Phillips, Blue Sky Research, and NEC.

[057] Examples of how the above cyanine dyes may be prepared are shown in US 2004/0014981. That is, the cyanine dyes disclosed herein are prepared using methods that are well known in the art. Generally, cyanine dyes are prepared according to the procedures taught in Hamer, F. M., Cyanine Dyes and Related Compounds, Weissberger, M. A., ed. Wiley Interscience, N.Y. 1964. Further, U.S. Pat. Nos. 4,337,063; 4,404,289 and 4,405,711, incorporated herein by reference, describe a synthesis for a variety of cyanine dyes having N-hydroxysuccinimide active ester groups. U.S. Pat. No. 4,981,977, incorporated herein by reference, describes a synthesis for cyanine dyes having carboxylic acid groups. U.S. Pat. No. 5,268,486, incorporated herein by reference, discloses a method for making arylsulfonate cyanine dyes. U.S. Pat. No. 6,027,709, discussed below, and incorporated herein by reference, discloses methods for making cyanine dyes having phosphoramidite groups. U.S. Pat. No. 6,048,982, incorporated herein by reference, discloses methods for making cyanine dyes having a reactive group selected from the group consisting of isothiocyanate, isocyanate, phosphoramidite, monochlorotriazine, dichlorotriazine, mono- or di-halogen substituted pyridine, mono- or di-halogen substituted diazine, aziridine, sulfonyl halide, acid halide, hydroxysuccinimide ester, hydroxy sulfosuccinimide ester, imido ester, glyoxal and aldehyde.

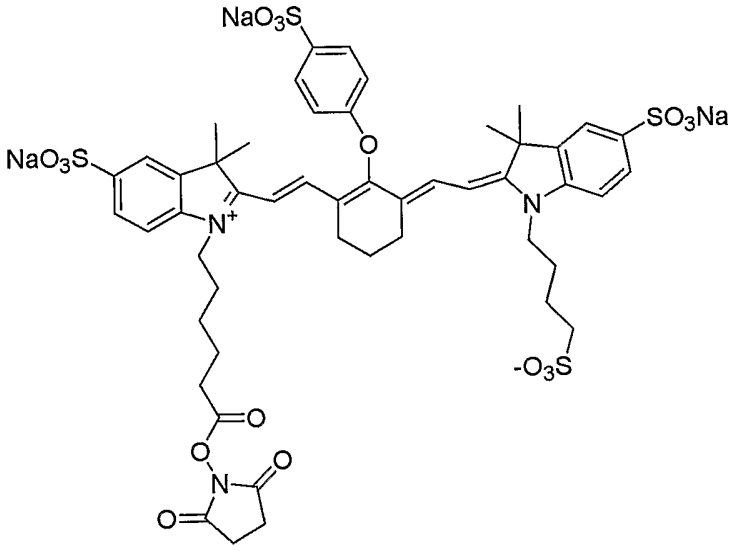
[058] Additional dyes that can be used with the present invention are the following:



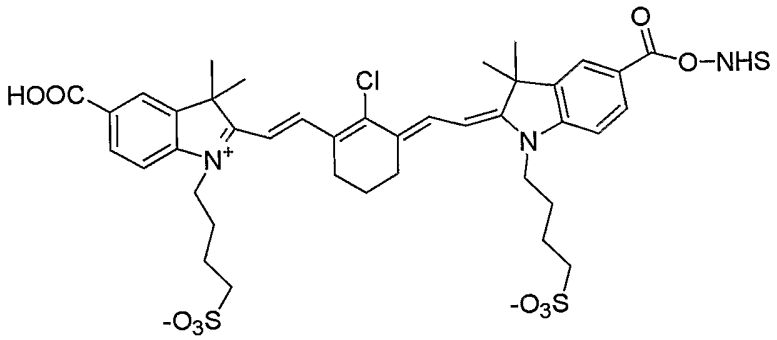
Cypate NHS-Achillifu ,



CY5.5- Amersham, Invitrogen ,



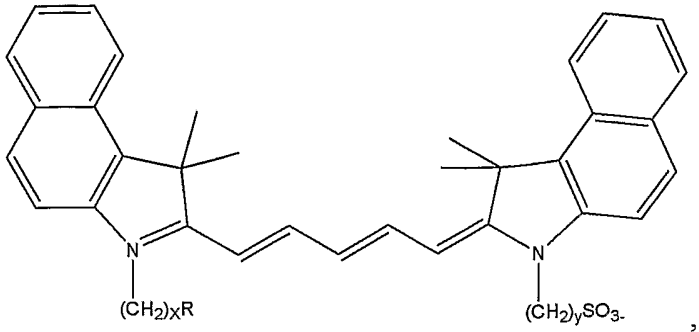
, and



NIR-820 Pham, Tung et al.

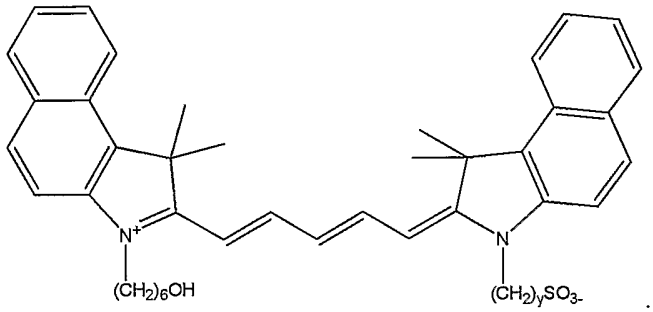
[059] Even further examples include the dyes disclosed in US 6,027,709.

[060] US '709 discloses dyes which have the following general formula:

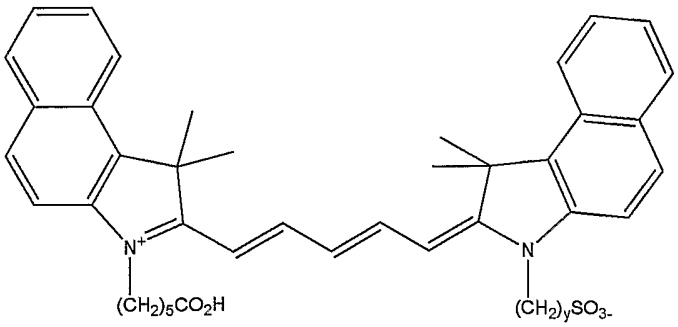


wherein R is -OH, -CO_2H, -NH_2, or -NCS and each of x and y, independently, is an integer selected from 1 to about 10. In preferred embodiments, each of x and y, independently, is an integer between about 2 and 6.

[061] In one embodiment, the dye is N-(6-hydroxyhexyl)N'-(4-sulfonatobutyl)-3,3,3',3'-tetramnethylbenz(e)indod icarbocyanine, which has the formula:



[062] In a second embodiment, the dye is N-(5-carboxypentyl)N'-(4-sulfonatobutyl)3,3,3', 3'-tetramethylbenz(e)indodicarbocyanine, which has the formula:



[063] These two dyes are embodiments because they have commercially available precursors for the linking groups: 6-bromohexanol, 6-bromohexanoic acid and 1,4-butane sultone (all available from Aldrich Chemical Co., Milwaukee, Wis.). The linking groups provide adequate distance between the dye and the biomolecule for efficient attachment without imparting excessive hydrophobicity. The resulting labeled biomolecules retain their solubility in water and are well-accepted by enzymes.

[064] These dyes, wherein R is $-CO_2H$ or -OH can be synthesized, as set forth in detail in the US '709 patent, by reacting the appropriate N-(carboxyalkyl)- or N-(hydroxyalkyl)-1,1,2-trimethyl-1H-benz(e)indolinium halide, preferably bromide, with sulfonatobutyl-1,1,2-trimethyl-1H-benz(e)indole at a relative molar ratio of about 0.9:1 to about 1:0.9, preferably 1:1 in an organic solvent, such as pyridine, and heated to reflux, followed by the addition of 1,3,3-trimethoxypropene in a relative molar ratio of about 1:1 to about 3:1 to the reaction product and continued reflux. The mixture subsequently is cooled and poured into an organic solvent such as ether. The resulting solid or semi-solid can be purified by chromatography on a silica gel column using a series of methanol/chloroform solvents.

[065] As an alternative, two-step, synthesis procedure, also detailed in U.S. '709, N-4-sulfonatobutyl-1,1,2-trimethyl-1H-benz(e)indole and malonaldehyde bis(phenylimine)-monohydrochloride in a 1:1 molar ratio can be dissolved in acetic anhydride and the mixture heated. The acetic anhydride is removed under high vacuum and the residue washed with an organic solvent such as ether. The residual solid obtained is dried and subsequently mixed with the appropriate N-(carboxyalkyl)- or N-(hydroxyalkyl)-1,1,2-trimethyl-1H-benz(e)indolinium halide in the presence of an organic solvent, such as pyridine. The reaction mixture is heated, then the solvent is removed under vacuum, leaving the crude desired dye compound. The procedure was adapted from the two step procedure set forth in Ernst, L. A., et al., Cytometry 10:3-10 (1989).

[066] The dyes also can be prepared with an amine or isothiocyanate terminating group. For example, N-(omega.-amino-alkyl)-1,1,2-trimethyl-1H-benz(e)indolenium bromide hydrobromide (synthesized as in N. Narayanan and G. Patonay, J. Org. Chem. 60:2391-5 (1995)) can be reacted to form dyes of formula 1 wherein R is $-NH_2$. Salts of these amino dyes can be converted to the corresponding isothiocyanates by treatment at room temperature with thiophosgene in an organic solvent such as chloroform and aqueous sodium carbonate.

[067] These dyes have a maximum light absorption which occurs near 680 nm. They thus can be excited efficiently by commercially available laser diodes that are compact, reliable and inexpensive and emit light at this wavelength. Suitable commercially available lasers include, for example, Toshiba TOLD9225, TOLD9140 and TOLD9150, Phillips CQL806D, Blue Sky Research PS 015-00 and NEC NDL 3230SU. This near infrared/far red wavelength also is advantageous in that the background fluorescence in this region normally is low in biological systems and high sensitivity can be achieved.

[068] The hydroxyl, carboxyl and isothiocyanate groups of the dyes provide linking groups for attachment to a wide variety of biologically important molecules, including

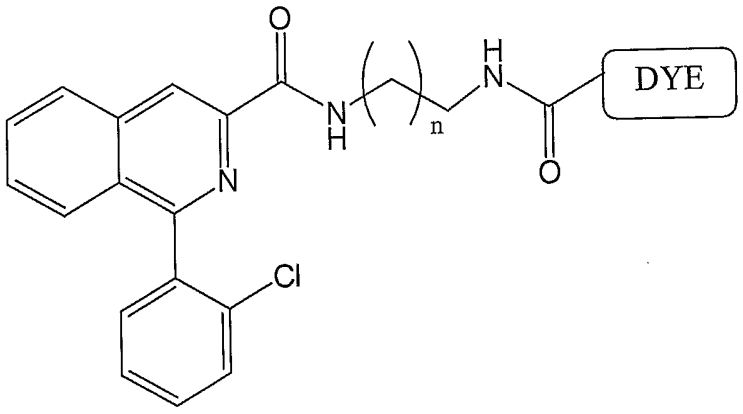
proteins, peptides, enzyme substrates, hormones, antibodies, antigens, haptens, avidin, streptavidin, carbohydrates, oligosaccharides, polysaccharides, nucleic acids, deoxy nucleic acids, fragments of DNA or RNA, cells and synthetic combinations of biological fragments such as peptide nucleic acids (PNAs).

[069] In another embodiment of the present invention, the ligands of the present invention may be conjugated to a lissamine dye, such as lissamine rhodamine B sulfonyl chloride. For example, a conjugable form of DAA1106 may be conjugated with lissamine rhodamine B sulfonyl chloride to form a compound of the present invention.

[070] Lissamine dyes are typically inexpensive dyes with attractive spectral properties. For example, examples have a molar extinction coefficient of 88,000 $cm^{-1}M^{-1}$ and good quantum efficient of about 95%. It absorbs at about 568 nm and emits at about 583 nm (in methanol) with a decent stokes shift and thus bright fluorescence.

[071] Coupling procedures for the PBR ligands and Glucosamine proceed via standard methods and will be recognized by those skilled in the art. In general, the nucleophilic N terminus of the targeting moieties are reactive towards activated carbonyls, for example an NHS (N-hydroxysuccinimide ester), sulfonyl chlorides, or other electrophile bearing species. Solvent of choice for coupling reactions can be dye specific, but include dimethyl sulfoxide (DMSO), chloroform, and/or phosphate buffered saline (PBS buffer). The resulting conjugates, amides, sulfonamides, etc. resist hydrolysis under physiological conditions, and are thus useful for *in-vivo* and *in-vitro* application.

[072] The following are examples of compounds of the present invention:



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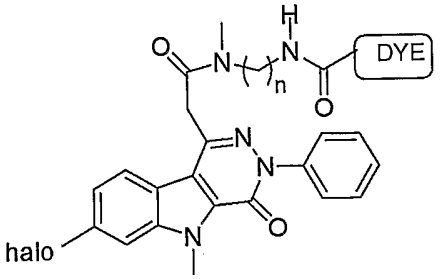

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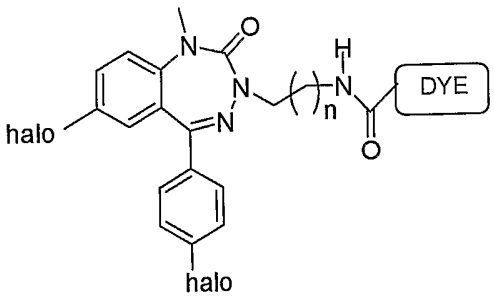

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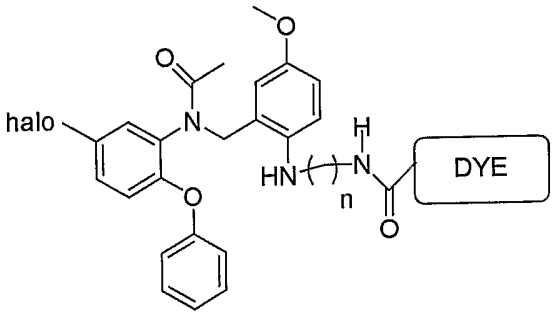

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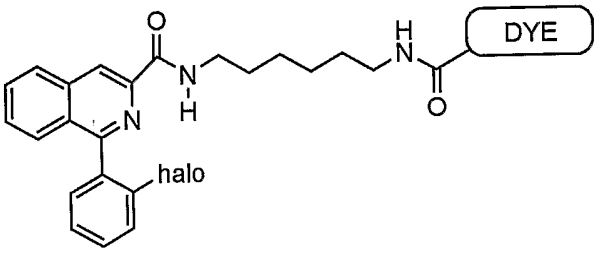

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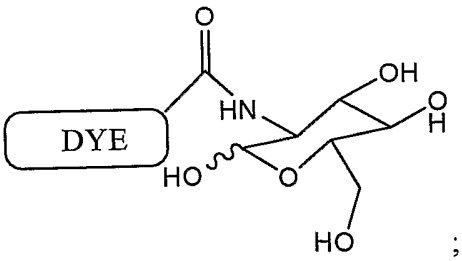
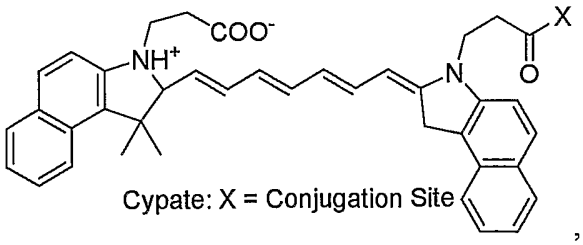
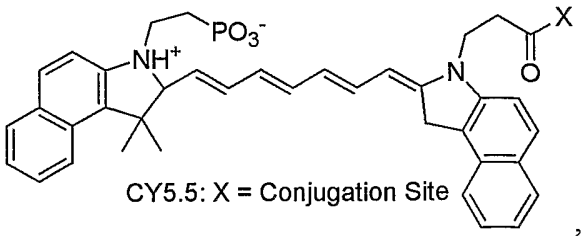


;

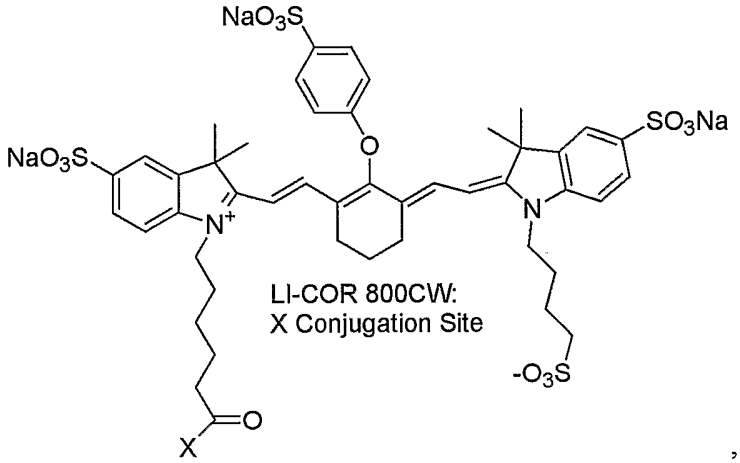
[073] The following are examples of dyes in conjugable form



Cypate: X = Conjugation Site ,

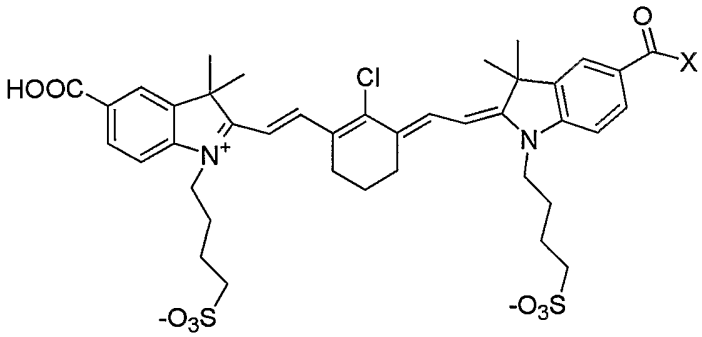


CY5.5: X = Conjugation Site ,



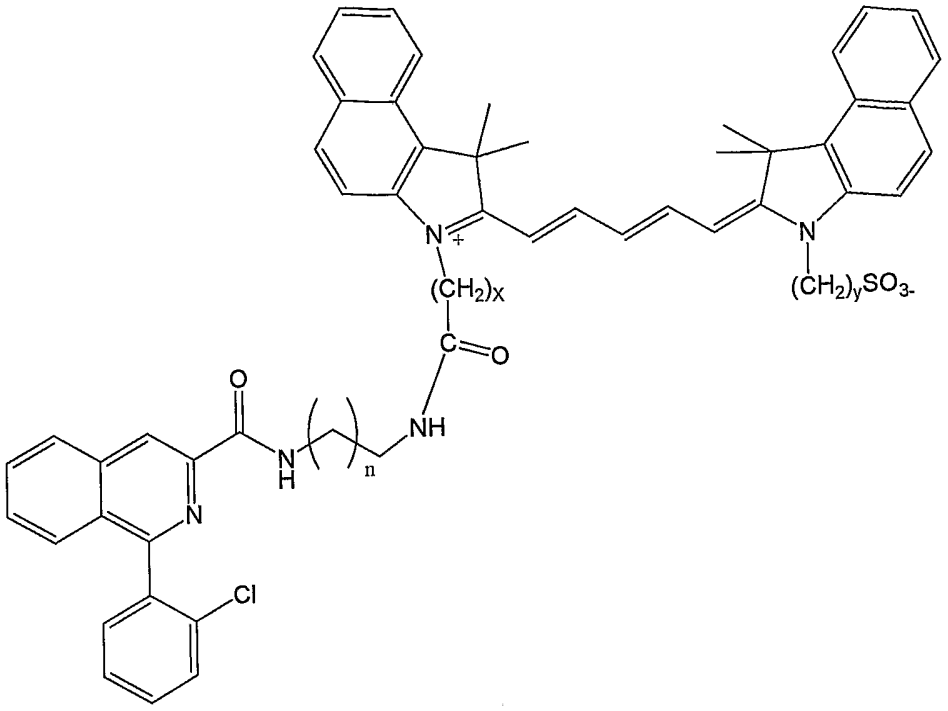
LI-COR 800CW:
X Conjugation Site

, and



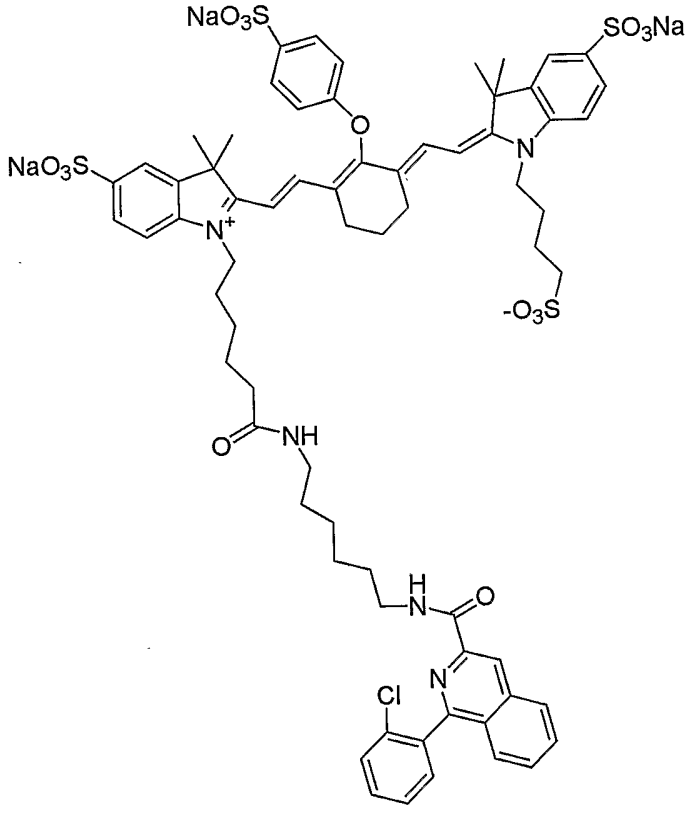
NIR-820: X Conjugation Site .

[074] The following compound is an example of one of the coupled compounds described above:



and analogs thereof, wherein n and x are integers from 1 to 10.

[075] The following is an additional example of a compound of the present invention, comprising a probe and a signaling moiety conjugated thereto:

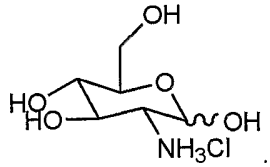


and analogs thereof.

[076] As stated above, the compounds of the present invention can be employed as signaling agents in NIR imaging. The resulting signal may be used to image a molecular event. Non-limiting examples of specific molecular events associated with the present invention include at least one of peripheral benzodiazepine expression, cell proliferation, glucose uptake, epidermal growth factor receptor expression, coronary disease.

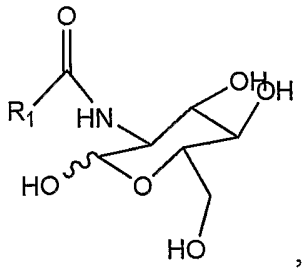
[077] Thus, the resulting signal may be used to diagnose a disease state such as, for example, cancer, neurodegenerative disease, multiple sclerosis, epilepsy, coronary disease, etc. Specifically, brain cancer and breast cancer are two cancers that may be diagnosed with the compounds and methods of the present invention. Two additional examples are non-Hodgkin's lymphoma and colon cancer.

[078] Another embodiment of the present invention is a method of measuring glucose uptake. This method comprises, comprises administering to a sample a conjugate, the conjugate comprising a conjugable glucosamine compound and a signaling agent; and detecting a signal from said conjugate. As in the other methods, the sample is at least one of cells, tissue, cellular tissue, serum or cell extract. An example of a conjugable glucosamine includes the following compound and conjugable analog thereof:

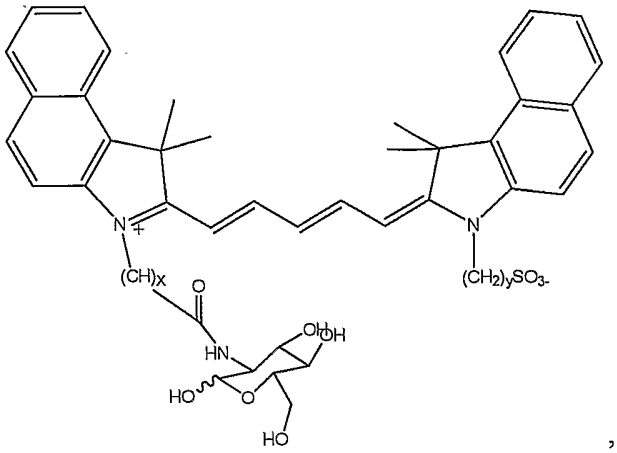


[079] The administration step may be *in vivo* administration or *in vitro* administration. The *in vivo* administration step further comprises at least one time course imaging determination, and in other embodiments, the *in vivo* administration step further comprises at least one bio distribution determination.

[080] Other embodiments of the present invention include conjugable compounds associated with this glucosamine method, specifically including the following:



where R_1 is a signaling moiety, and



and analogs thereof.

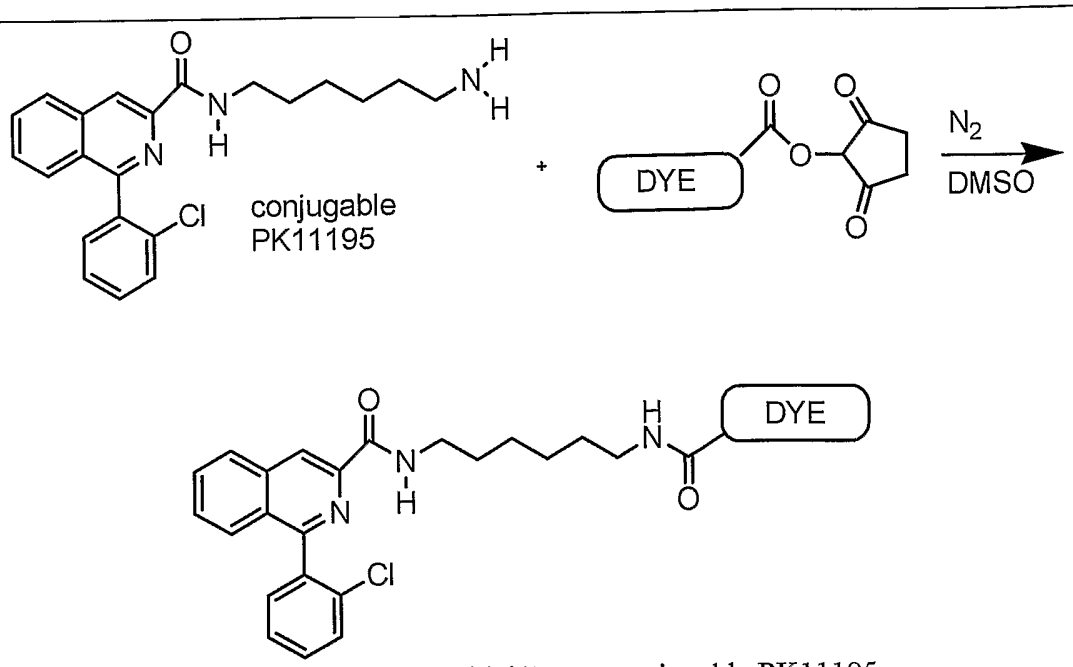
Examples

[081] The following examples are presented purely for exemplary purposes, and as such the material in this section should be considered as embodiments of the present invention and not to be limiting thereof.

Example 1

[082] This example demonstrates the conjugation of a NIR dye of the present invention and a conjugable analog or conjugable form of PK11195 for deep tissue imaging. In this example, IRDye800CW (LiCOR) is coupled to conjugable PK11195.

[083] *Dye800CW-PK11195 (Scheme 1)*- To a 10mL round bottom flask, about 196.5μL of a 1mg/ml conjugable PK11195 solution (DMSO) is mixed with about 300 μL of an about 1mg/mL Dye800CW (DMSO). The reaction proceeds under nitrogen flow for about 1 hour at RT. Reaction progress is monitored via HPLC and ESI MS.



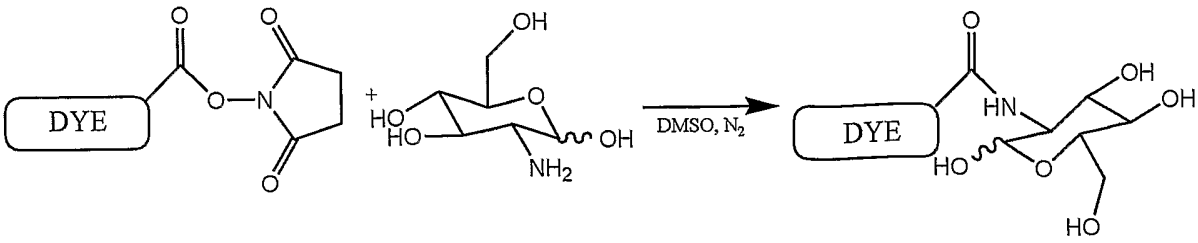
Scheme 1- Conjugation of Succinimidyl Dye to conjugable PK11195

[084] Yield is about 99% and requires no further purification.

Example 2

[085] This example demonstrates an example of the formulation of a NIR-glucosamine conjugate of the present invention.

[086] *Dye800CW-glucosamine (Scheme 2)*- To a 10mL round bottom flask, about 9.3mg sodium methoxide and about 37mg D-glucosamine hydrochloride are reacted in about 2mL DMSO. The solution is stirred under nitrogen for about 3 hours at RT. Next, about 3μL of the resulting solution are mixed with about 150 μL of an about 1mg/mL Dye800CW/DMSO solution in a separate 10mL flask. The mixture is stirred under nitrogen for another 1.5 hours at RT.



Scheme 2- Conjugation of Succinimidyl Dye to glucosamine

[087] Reaction progress was monitored via HPLC and ESI MS and the reaction yielded 98% pure conjugate.

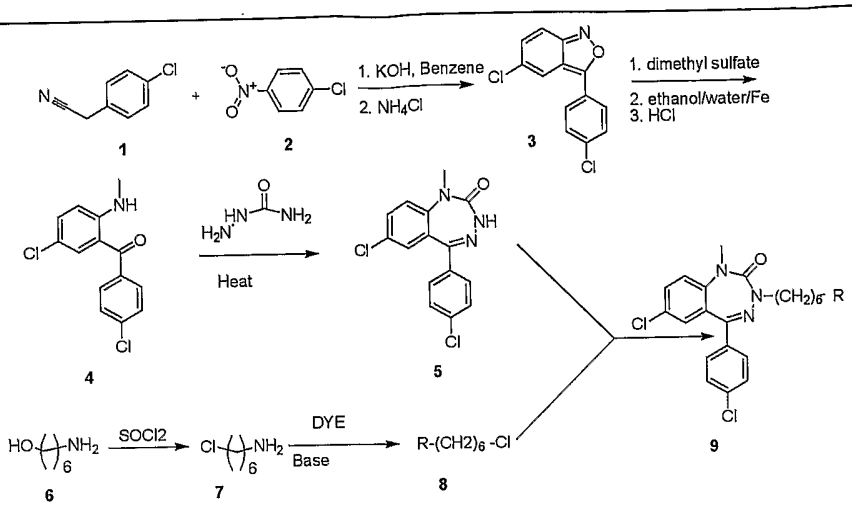
Example 3

[088] This example demonstrates the use of compounds of the present invention in ESI (Electrospray Ionization) mass spectra.

[089] Initially, about 20μL of the reaction solution of Example 1 is diluted to about 180 μL using 5mM ammonium acetate aqueous solution containing about 0.05% acetic acid. The sample is injected the sample immediately into a Mariner ESI mass spectrometer. Some major instrument settings are: spray tip at about 3.4kv, nozzle potential at about 200v, quadrupole temperature at about 150 °C and nozzle temperature at about 150 °C. Spectra is collected every 100 seconds. In spectrum for Dye800W-glucosamine complex, the expected molecular peak is observed at 1164Da. In the spectrum for Dye800CW-PK11195 complex, the expected molecular peak is observed at 1365.9Da.

Example 4

[090] This example shows a synthetic pathway yielding a conjugable Ro5-4864 of the present invention, and conjugation to an imaging agent, such as Lanthanide chelate or NIR-dye.

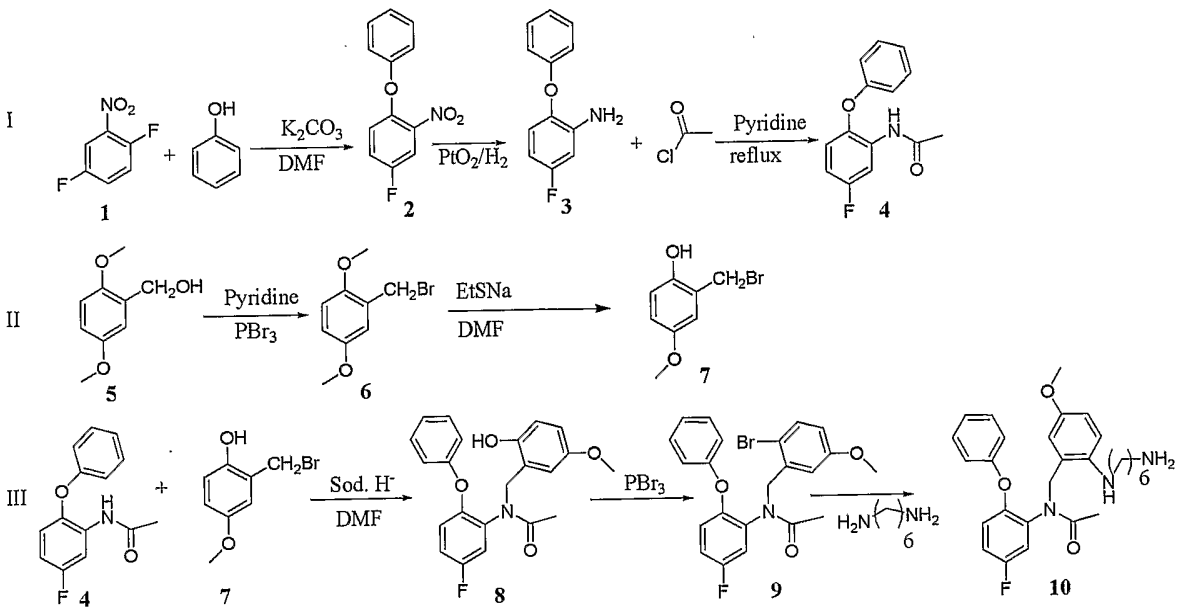


Scheme 3--Conjugation of Ro5-4864 to Lanthanide Chelator or DYE

[091] A conjugable form of compounds similar to Ro5-4864 has been previously reported (see US Patent No. 5,901,381) and a synthetic procedure in Scheme 3 will be used to synthesize a conjugable form of Ro5-4864. A solution of KOH in methanol will be treated with a solution of 4- chlorophenyl-acetonitrile **1** and 4-chloronitro-benzene **2** in benzene. The mixture will be stirred for 3 hours and then poured to ammonium chloride solution. Compound **3** will then precipitate out. Compound **4** will be produced by stirring compound **3** and dimethyl sulfate for 5 hours, followed by being treated with ethanol, water, iron fillings and hydrochloric acid. See Vejdelek Z, Polivka Z, Protiva M. Synthesis of 7-Chloro-5-(4-Chlorophenyl)-1-Methyl-1,3-Dihydro-1,4-Benzodiazepin-2-One. Collection of Czechoslovak Chemical Communications 1985;50:1064-1069. Compound **4** and semicarbazide, after heated to 210°C, will produce compound **5**. Compound **7** will be used as a linker to combine compound **5** and lanthanide chelate/dye800cw. Compound **7** can be synthesized by the reaction between compound **6** and thionyl chloride. Lanthanide chelate (with carboxylic acid group) or dye800cw (a N-hydroxysuccinimide ester) can then react with compound **7** in basic solution to produce a compound in the form of compound **8**. The chlorine on the signaling part will react with N-H group in compound **5** to produce the final imaging agent (compound **9**). The product can be further chelated by adding lanthanide chloride solution ($LnCl_3$, $EuCl_3$ etc) into product solution with pH 6.5. The synthetic pathway for lanthanide chelate has been reported. See Griffin JMM, Skwierawska AM, Manning HC, Marx JN, Bornhop DJ. Simple, high yielding synthesis of trifunctional fluorescent lanthanide chelates. Tetrahedron Letters 2001;42:3823-3825.

Example 5

[092] This Example shows a scheme for the synthesis of a conjugable form of DAA1106 of the present invention, which cnathen be conjugated to an imaging agent.



Scheme 4 - Synthesis of conjugatable DAA1106

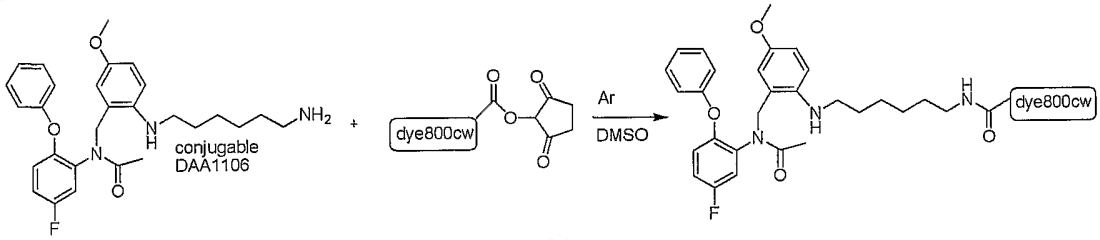
[093] The synthetic pathway for conjugable DAA1106 is shown in Scheme 4. Compound 2 will be obtained by reaction of compound 1 with phenol in DMF. Compound 2 will then be reduced by PtO2 under hydrogen flow in methanol. Compound 3 can react with acetyl chloride in pyridine to produce compound 4 after the reaction refluxes for 2 hours. The hydroxyl group in compound 5 will be substituted by bromide to produce compound 6. One hydroxyl group in compound 6 will be deprotected in DMF by Sodium ethanethiolate to produce compound 7. Compound 4 and 7 will then react in DMF with the presence of sodium hydride. After compound 8 is obtained, the hydroxyl group will be brominated to form compound 9. Conjugable DAA1106 (compound 10) is prepared by treatment of compound 9 with hexane-1, 6-diamine. The conjugation position on DAA1106 is determined according to another conjugation that has been done on DAA1106 which did not affect the biological activity of DAA1106. See Zhang MR, Maeda J, Furutsuka K, Yoshida Y, Ogawa M, Suhara T, et al. [F-18]FMDAA1106 and [F-18]FEDAA1106: Two positron-emitter labeled ligands for peripheral benzodiazepine receptor (PBR). Bioorganic & Medicinal Chemistry Letters 2003;13:201-204. The product should be conjugable to lanthanide chelator in

water/DMF/dioxane/TEA mixture. The conjugate will be further chelated by adding Lanthanide chloride solution ($LnCl_3$, $EuCl_3$ etc) into pH 6.5 product solution.

Example 6

[094] This example shows an example of the synthesis, characterization, and preliminary cell study for an embodiment of the present invention, a dye800cw-DAA1106 conjugation, as well as the conjugation of the PBR ligand DAA1106 to a NIR dye, followed by cell uptake.

[095] In this example, dye800CW (5 mg, 4.3 μmol) and conjugable DAA1106 (5 mg, 10 μmol) is mixed in DMSO (1 mL) in a 10 mL round bottom flask. The solution is stirred under argon flow for 10 hours. The reaction scheme is shown in Scheme 5, below. Product is purified through neutral alumina column using 0.1 M triethyl ammonium acetate in 80/20 acetonitrile/water solution.



Scheme 5. Reaction scheme for dye800CW-DAA1106

[096] Upon preparing dye800CW-DAA1106, absorption and emission spectra (Table 1) are obtained at room temperature with a Shimadzu 1700 UV-vis spectrophotometer and ISS PCI spectrofluorometer respectively. The same sample (2 μM) is used for taking both UV and fluorescence spectrum. UV spectrum was scanned from 190 nm to 900nm with sampling rate of 1 nm. Cuvette path length was 1 cm. Fluorescence sample was excited at 797 nm. Spectrum was collected from 700 nm to 900 nm with scan rate 1 nm/second. Slit width was set to 1.5. Photo multiplier tube (PMT) voltage was at 75 watts. Dye800CW-DAA1106 has maximum absorption at 779nm and fluorescence at 801nm in methanol.

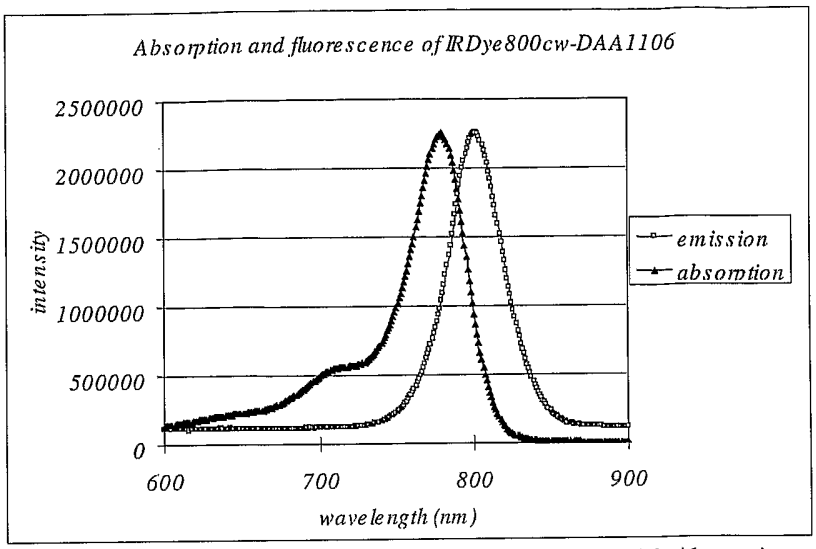


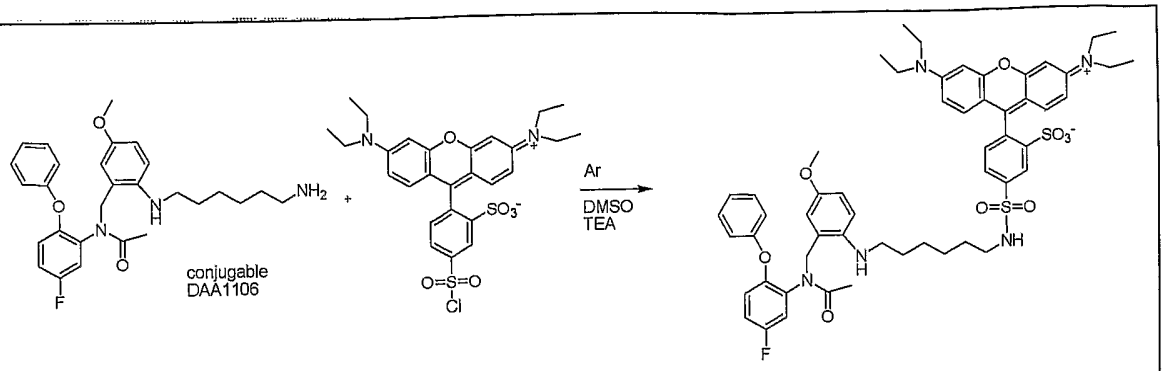
Table 1. Absorption and fluorescence of dye800CW-DAA1106. Absorption λ_{max}=779 nm and fluorescence λ_{max}=801 nm

[097] Regarding cell uptake, C6 glioma cell lines are a widely used cell line in neurobiological research that has high PBR expression. C6 cells were incubated with 10 μM dye800CW-DAA1106 in culture media for half hour and then rinsed and re-incubated with saline before imaging. Figure 1 shows white light and fluorescence pictures of dosed and un-dosed cells. Instrument used is Nikon epifluorescence microscope equipped with Ludl Qimaging camera, Nikon S fluor 20x/0.75 objective, mercury lamp and ICG filter set. Picture B shows cell take-up of dye800CW-DAA1106, while un-dosed cell (picture D) does not show any significant fluorescence.

Example 7

[098] This example shows the synthesis, characterization and preliminary cell study of a lissamine-DAA1106 conjugation. An example of a lissamine dye has a molar extinction coefficient of 88,000 $cm^{-1}M^{-1}$ and good quantum efficient of about 95%. It absorbs at 568 nm and emits at 583 nm (in methanol) with a decent stokes shift and thus bright fluorescence.

[099] Lissamine rhodamine B sulfonyl chloride (4 mg, 6.9 μmol), conjugable DAA1106 (5 mg, 10 μmol) and tri-ethylamine (10 μL) was mixed in dichloromethane (0.8 mL) in a 10mL round bottom flask. The solution was stirred under argon flow for 3 hours. The reaction scheme is shown in Scheme 6. Product was purified through column chromatography (silica gel) using 19/1 dichloromethane/methanol solution.



Scheme 6. Lissamine-DAA1106 reaction scheme

[0100] Upon preparing lissamine-DAA1106, absorption and emission spectra (Table 2) was obtained with a Shimadzu 1700 UV-vis spectrophotometer and ISS PTI spectrofluorometer at room temperature. The same sample (2 μM) was used for taking both UV and fluorescence spectrum. UV spectrum was scanned from 190 nm to 900nm with sampling rate of 1 nm. Cuvette path length was 1 cm. Fluorescence sample was excited at 561 nm. Spectrum was collected from 700 nm to 900 nm with scan rate 1 nm/second. Slit width was set to 1.5. Photo multiplier tube (PMT) voltage was at 75 watts. Lissamine-DAA1106 has maximum absorption at 561 nm and fluorescence at 579 nm in methanol.

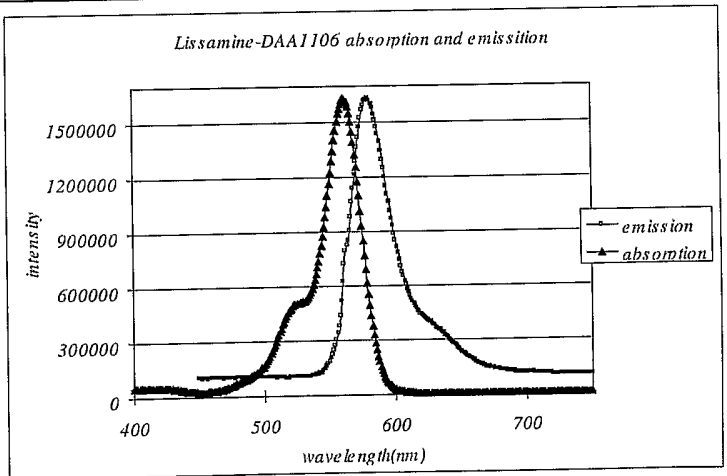


Table 2. Lissamine-DAA1106 absorption and fluorescenceAbsorption λ_{max}=561 nm and fluorescence λ_{max}=579 nm

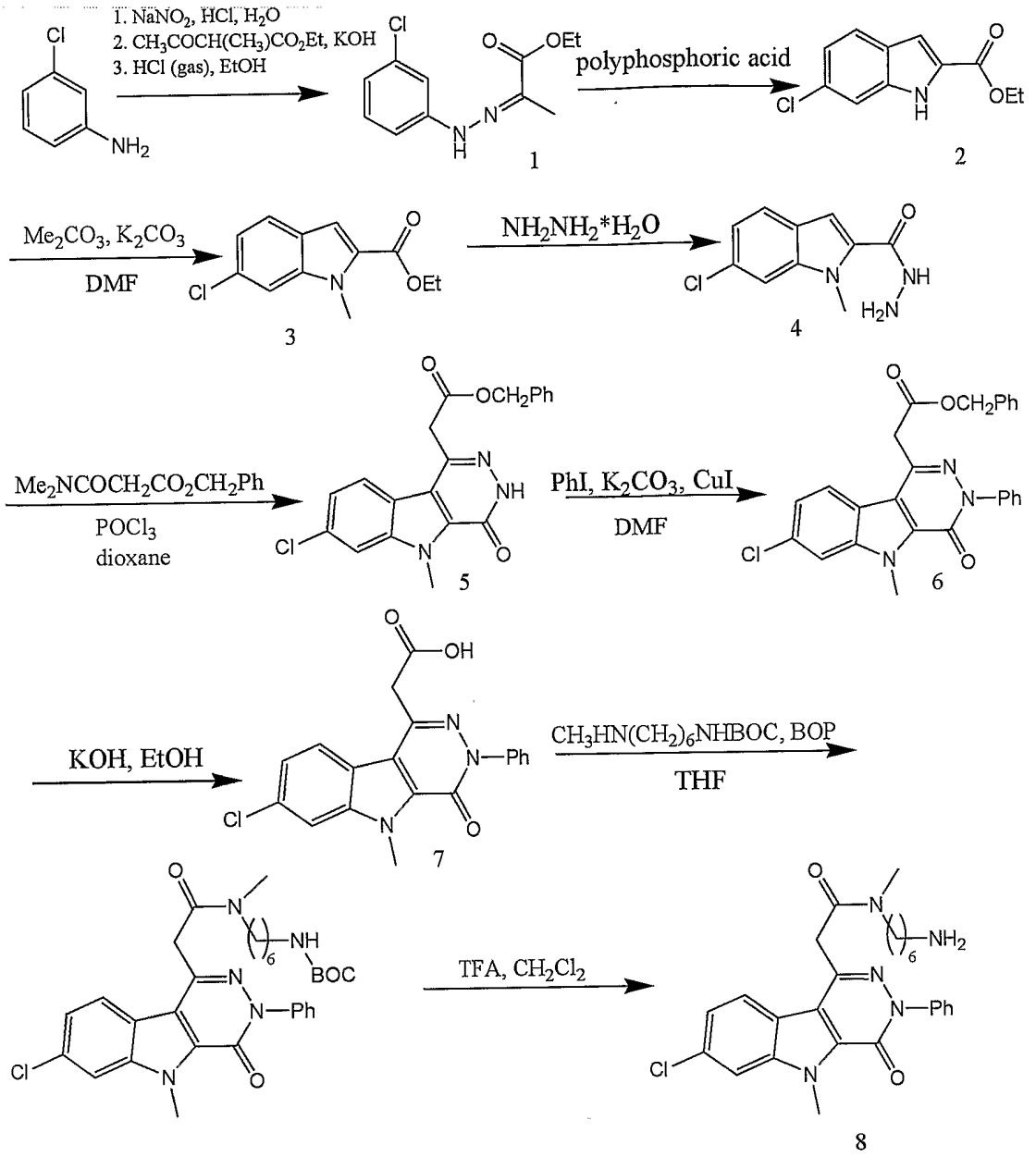
[0101] C6 cells were incubated with 10 μM lissamine-DAA1106 in culture media for half hour and then rinsed and re-incubated with saline before imaging. Figure 2 shows white light and fluorescence pictures of dosed and un-dosed cells. Instrument used was Nikon

epifluorescence microscope equipped with Ludl Qimaging camera, Nikon S fluor 20x/0.75 objective, mercury lamp and Texas red filter set. Picture B shows cell take-up of lissamine-DAA1106 at perinuclear location. This observation was expected since PBR is a mitochondrial protein. Un-dosed cells (picture D) exhibited no fluorescence.

Example 8

[0102] This example shows an example of a synthetic pathway yielding a conjugable form of a SSR180575 compound of the present invention.

[0103] Starting from m – chloroaniline, which was diazotised and coupled with ethyl α - methylacetoacetate, the azo – ester was converted into ethyl pyruvate m – chlorophenylhydrazone **1** (the Japp – Klingeman reaction). Polyphosphoric acid facilitated the conversion to molecule **2**. Next, N – methylation with dimethylcarbonate in presence K_2CO_3 yielded the ester **3** and was treated with hydrazine and converted into hydrazide **4**. The ring was closed in the presence of $POCl_3$ and compound **5** was obtained. N – phenylation with using PhI and CuI (as catalyst) provide compound **6**. Mild hydrolysis with dilute KOH in EtOH yield acid **7**. To conjugated mono – N – BOC protected N – methyl – 1,6 – hexanediamine to **7** used BOP. Removal of protecting group with TFA in CH_2Cl_2 is yields **8**.



Example 9

[0104] Example 9 demonstrates specific, *in vivo* tumor labeling using a method of the present invention. A NIR-PK 11195 deep tissue imaging agent was made as shown in Example 1. Tumor bearing Smad3 gene knockout mice and control animals were injected with 10 nmoles of the imaging agent and imaged about 14 hours following injection. Specific labeling was observed in the abdominal region of tumor animals and clearance in the control animals. This selective uptake is shown in Figure 3. A post-imaging autopsy confirmed localization of the imaging agent in the SMAD3 animal.

Example 10

[0105] This Example shows NIR-PK 11195 imaging in connection with neurodegenerative processes in experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis. Additionally, this Example shows the use of the present invention to monitor the progression of a disease state. A conjugated imaging agent NIR-PK 11195 was made in accordance with Example 1. An EAE induced and control animals were injected with NIR-PK 11195 and imaged. EAE animals demonstrate strong fluorescence along the spinal column indicating activated T cell and macrophage response which signal the onset of the demylenation processes characteristic to EAE. The EAE/treated mouse was treated with a curcumin composition.

[0106] Figure 3 shows images associated with this example that confirm insignificant uptake of the imaging agent in the control, but indicate full onset of a disease state in the EAE mice. Subsequent imaging shows the progression of the disease after a disease state treatment is administered.

References

[0107] Throughout this application, various publications are referenced. All such publications, specifically including the publications listed below, are incorporated herein by reference in their entirety.

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[0108] It will be apparent to those skilled in the art that various modifications and variations can be made in the present invention without departing from the scope or spirit of the invention. Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the Specification and Example be considered as exemplary only, and not intended to limit the scope and spirit of the invention.

[0109] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as reaction conditions, and so forth used in the Specification and Claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the Specification and Claims are approximations that may vary depending upon the desired properties sought to be determined by the present invention.

[0110] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the experimental or example sections are reported as precisely as possible. Any numerical value,

however, inherently contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

We claim:

1. A method of imaging a molecular event in a sample, comprising:

(a) administering to said sample a probe having an affinity for a target, the probe comprising at least one of a ligand/signaling agent combination, or conjugable form of a ligand/signaling agent combination; and

(b) detecting a signal from said probe.

2. The method of claim 1, wherein said sample is at least one of cells, tissue, cellular tissue, serum, cell extract, bodily fluids.

3. The method of claim 2, wherein the bodily fluids are breast secretion, sputum, vaginal fluids, urine.

4. The method of claim 1, wherein the administering step is *in vivo* or *in vitro*.

5. The method of claim 1, wherein said molecular event is at least one of peripheral benzodiazepine receptor expression, cell proliferation, glucose uptake, epidermal growth factor receptor expression, microglial activation, apoptosis, matrix metalloproteinase activation.

6. The method of claim 1, wherein said signaling agent is a near infrared signaling agent.

7. The method of claim 1, wherein said detecting step comprises near infrared detection.

8. The method of claim 8, wherein the near infrared detection included near infrared imaging.

9. The method of claim 1, further comprising the step of:

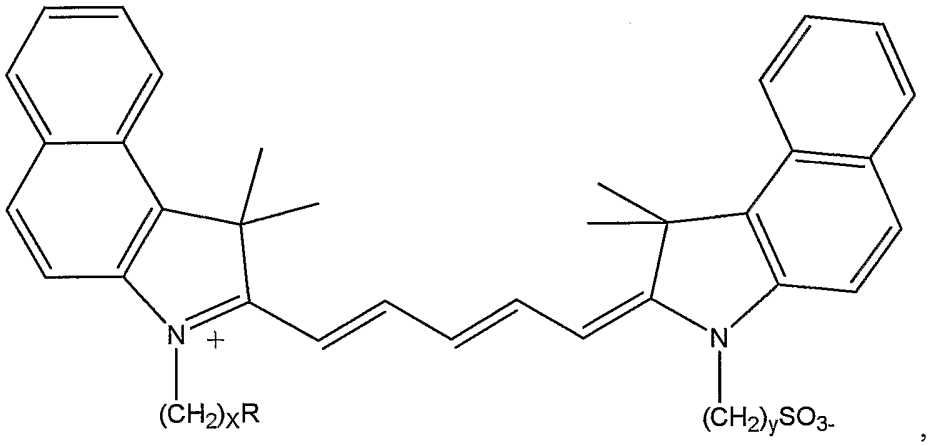
analyzing said signal to diagnose a disease state.

10. The method of claim 7, wherein the disease state is cancer, neurodegenerative disease, cancer, multiple sclerosis, epilepsy.

11. The method of claim 9, wherein the disease state is brain cancer or breast cancer.

12. The method of claim 1, wherein the signaling agent is a cyanine dye.

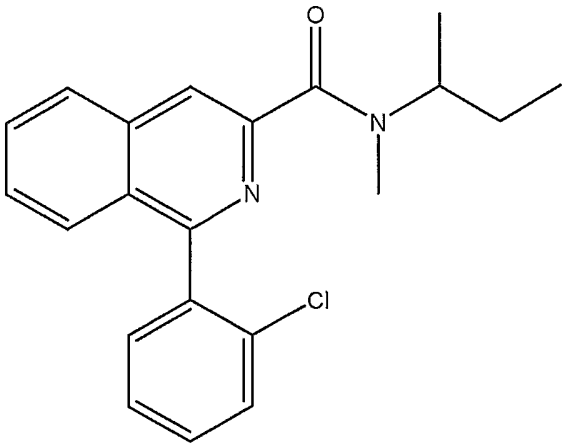
13. The method of claim 1, wherein the signaling agent is a compound of the following formula:



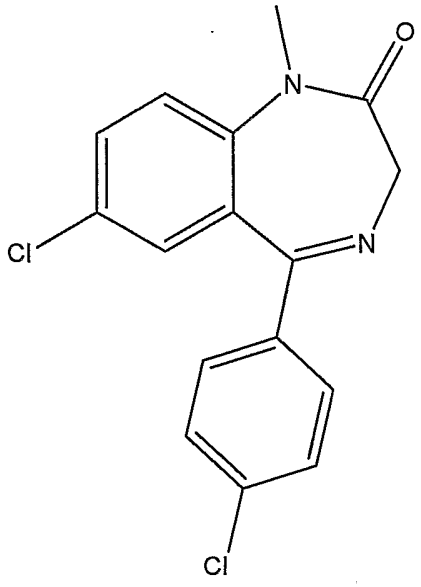
wherein R is -OH, $-CO_2H$, $-NH_2$, -NCS and each of x and y, independently, is an integer selected from 1 to about 10.

14. The method of claim 1, wherein said probe comprises a peripheral benzodiazepine affinity ligand or a conjugable form thereof.

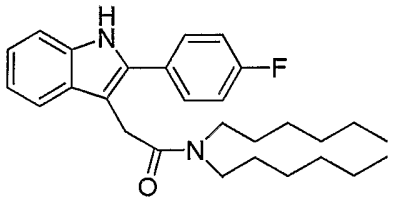
15. The method of claim 14, wherein the peripheral benzodiazepine affinity ligand or conjugable form thereof is at least one of the following compounds:



PK11195



Ro5-4864



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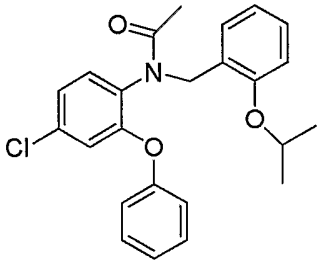

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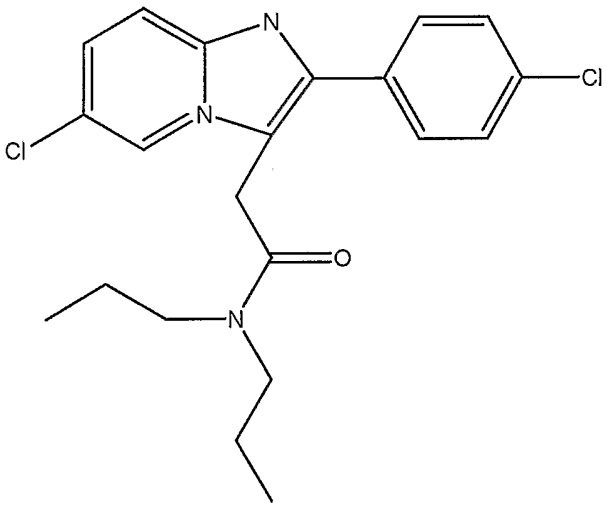
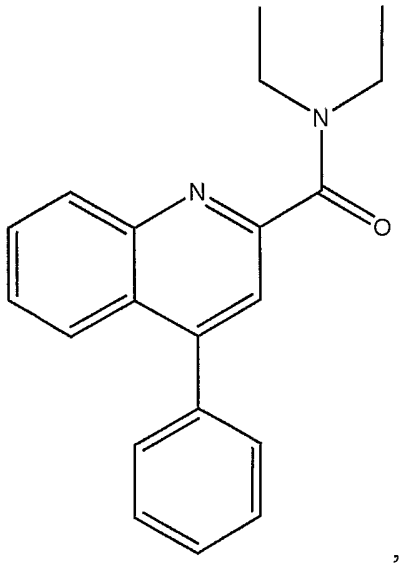
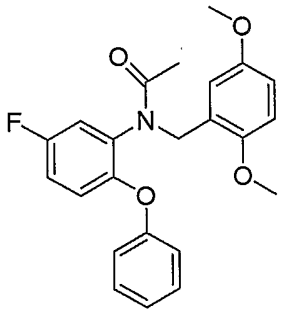
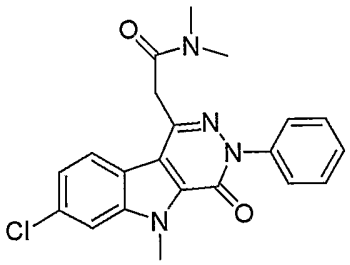


Alpidem





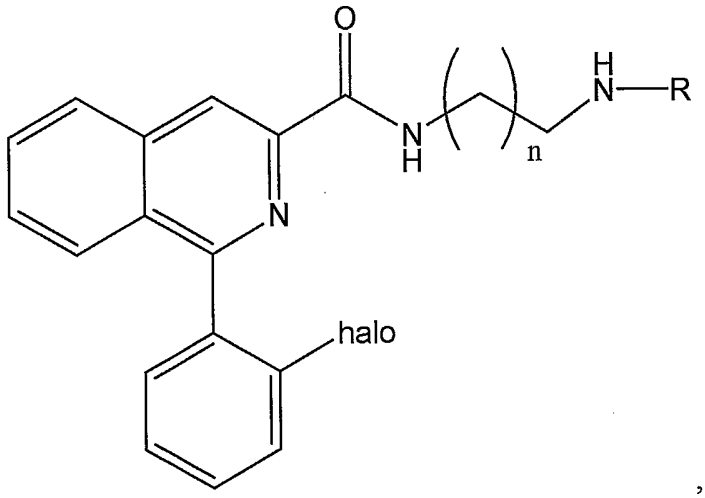
DAA1106 ,



SSR180575 ,

or a conjugable analog thereof.

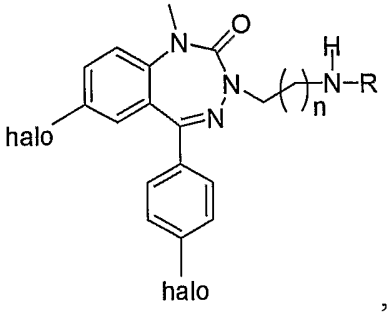
16. The method of claim 14, wherein the peripheral benzodiazepine affinity ligand is a compound of the following formula:



,

or an analog thereof, wherein R is H or alkyl; "halo" is fluorine, chlorine, bromine, iodine; and n is 0-10.

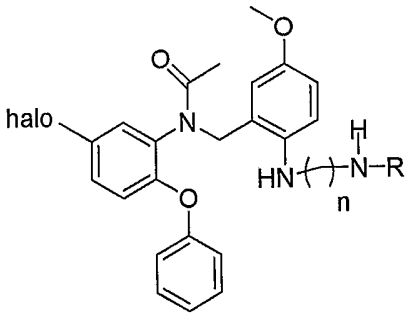
17. The method of claim 14, wherein the peripheral benzodiazepine affinity ligand is a compound of the following formula:



,

or an analog thereof, wherein R is H or alkyl; "halo" is fluorine, chlorine, bromine, iodine; and n is 0-10.

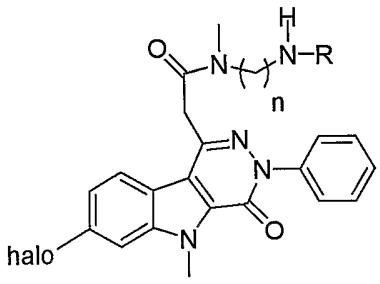
18. The method of claim 14, wherein the peripheral benzodiazepine affinity ligand is a compound of the following formula:



,

or an analog thereof, wherein R is H or alkyl; "halo" is fluorine, chlorine, bromine, iodine; and n is 0-10.

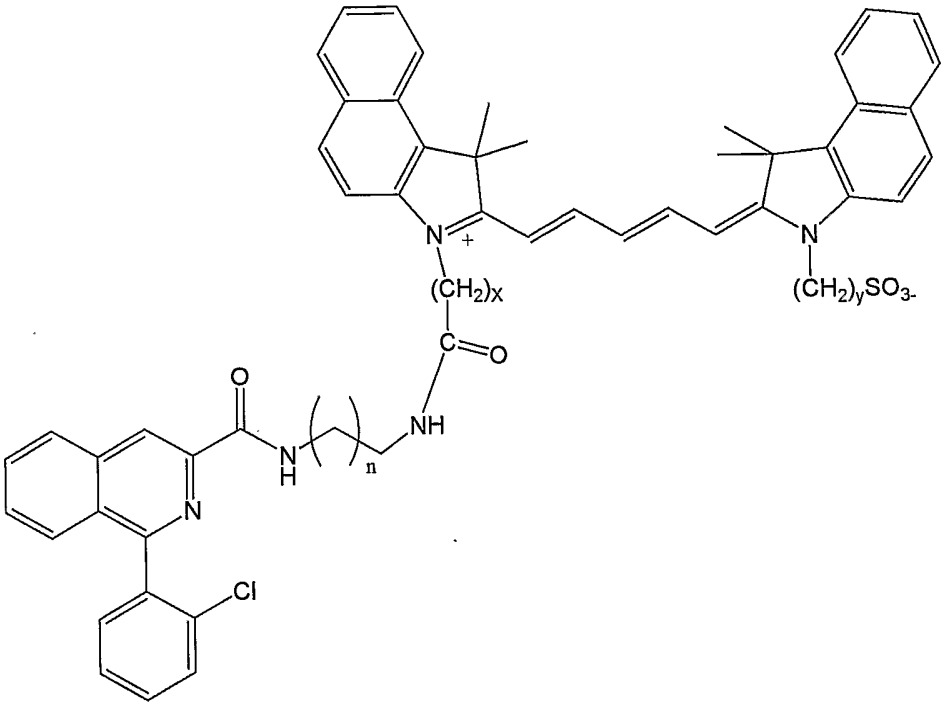
19. The method of claim 14, wherein the peripheral benzodiazepine affinity ligand is a compound of the following formula:



,

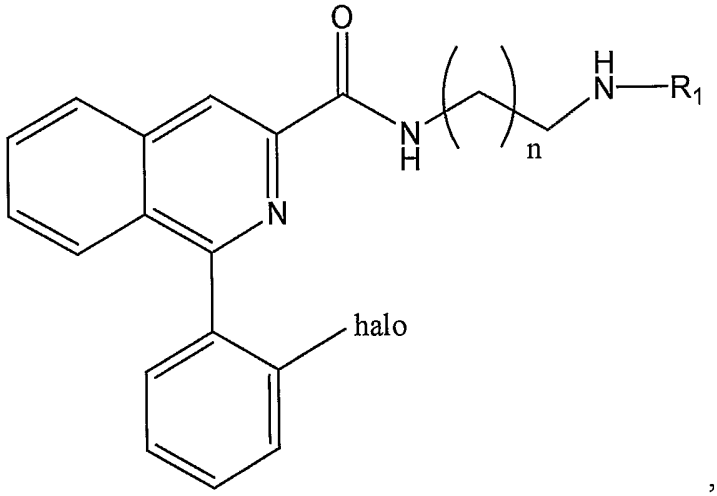
or an analog thereof, wherein R is H or alkyl; "halo" is fluorine, chlorine, bromine, iodine; and n is 0-10.

20. The method of claim 1, wherein the signaling agent is a compound of the formula:



and analogs thereof, wherein n and x are integers from 1 to 10.

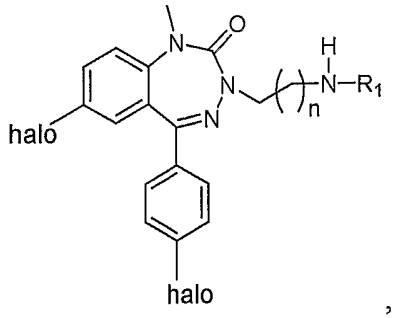
21. A compound of the formula:



or an analog thereof, wherein R_1 is a signaling moiety; "halo" is fluorine, chlorine, bromine, iodine; and n is 0-10.

22. The compound of claim 21, wherein the signaling moiety is a dye.

23. A compound of the following formula:

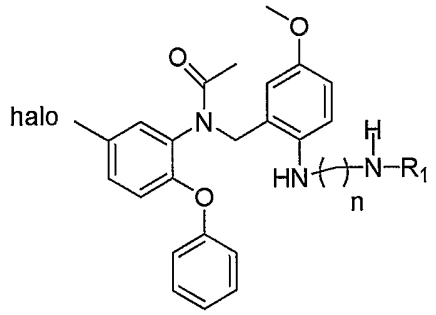


,

or an analog thereof, wherein R_1 is a signaling moiety; "halo" is fluorine, chlorine, bromine, iodine; and n is 0-10.

24. The compound of claim 23, wherein the signaling moiety is a dye.

25. A compound of the following formula:

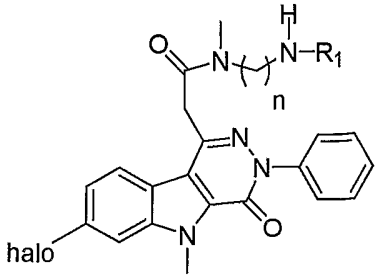


,

or an analog thereof, wherein R_1 is a signaling moiety, "halo" is fluorine, chlorine, bromine, iodine; and n is 0-10.

26. The compound of claim 25, wherein the signaling moiety is a dye.

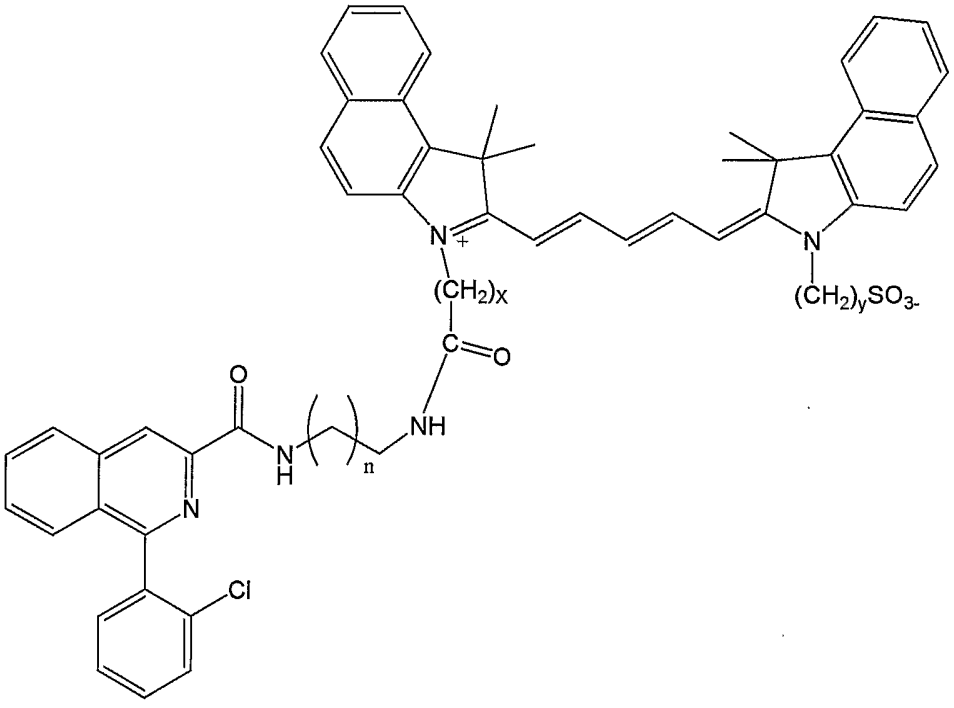
27. A compound of the following formula:



or an analog thereof, wherein R_1 is a signaling moiety; "halo" is fluorine, chlorine, bromine, iodine; and n is 0-10.

28. The compound of claim 27, wherein the signaling moiety is a dye.

29. A compound of the formula:



and analogs thereof, wherein n and x are integers from 1 to 10.

30. A method of measuring glucose uptake, comprising:

(a) administering to a sample a conjugate, the conjugate comprising a conjugable glucosamine compound and a signaling agent; and

(b) detecting a signal from said conjugate.

31. The method of claim 30, wherein the sample is at least one of cells, tissue, cellular tissue, serum or cell extract.

32. The method of claim 30, wherein the bodily fluids are breast milk, sputum, vaginal fluids, urine.

33. The method of claim 30, wherein the signaling agent is a near infrared signaling agent.

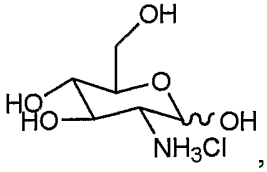
34. The method of claim 30, wherein the administration step is *in vivo* or *in vitro*.

35. The method of claim 34, wherein the *in vivo* administration step further comprises at least one time course imaging determination.

36. The method of claim 34, wherein the *in vivo* administration step further comprises at least one bio distribution determination.

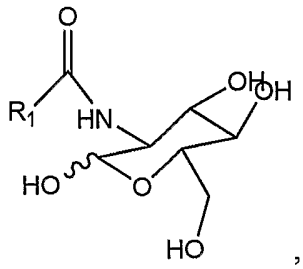
37. The method of claim 30, wherein the detecting step comprising near infrared imaging.

38. The method of claim 30, wherein the conjugable compound is of the following formula:



or a signaling agent-conjugable analog thereof.

39. The method of claim 30, wherein the conjugate compound is a compound of the following formula:



,

where R_1 is a signaling moiety.

40. The method of claim 39, wherein the signaling moiety is a dye.

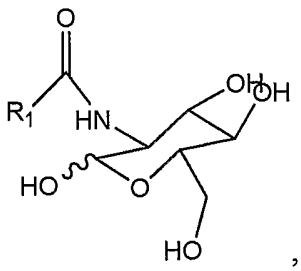
41. The method of claim 30, further comprising the step of:

analyzing said signal to diagnose a disease state.

42. The method of claim 41, wherein the disease state is cancer, neurodegenerative disease, coronary disease.

43. The method of claim 41, wherein the disease state is brain cancer or breast cancer.

44. A compound of the following formula:

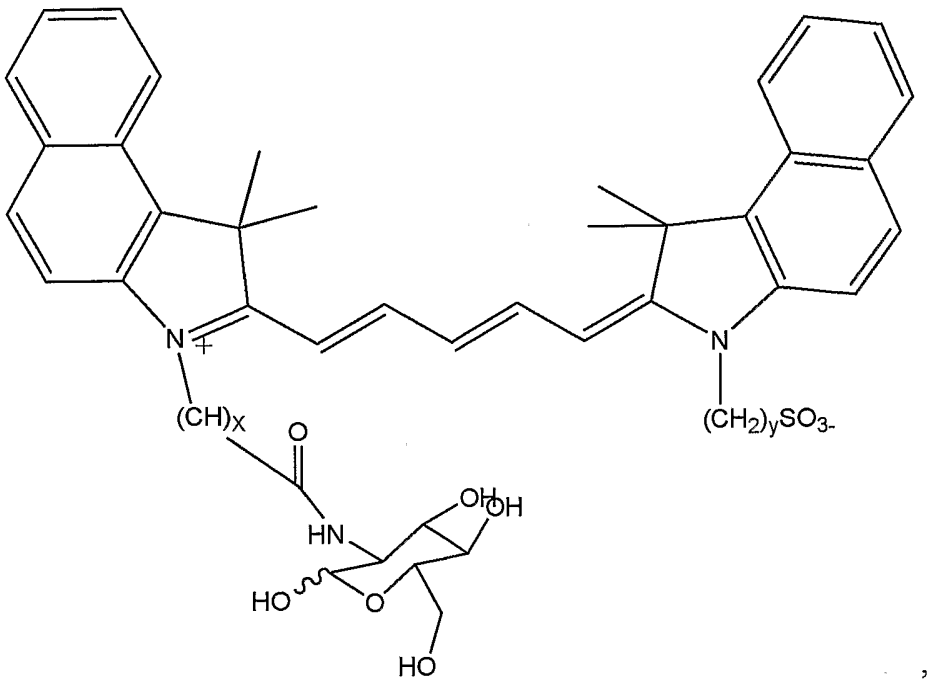


,

where R_1 is a signaling moiety.

45. The compound of claim 44, wherein the signaling moiety is a dye.

46. The compound of claim 44, of the following formula:



and analogs thereof.

47. A method of quantifying the progression of a disease state in a subject, comprising:

(a) administering to a first sample of the subject a conjugate, the conjugate comprising a conjugable deoxythymidine compound and a signaling agent;

(b) detecting a signal from said conjugate;

(c) after a period of time from step (b), administering to a second sample of the subject a conjugate, the conjugate comprising a conjugable deoxythymidine compound and a signaling agent;

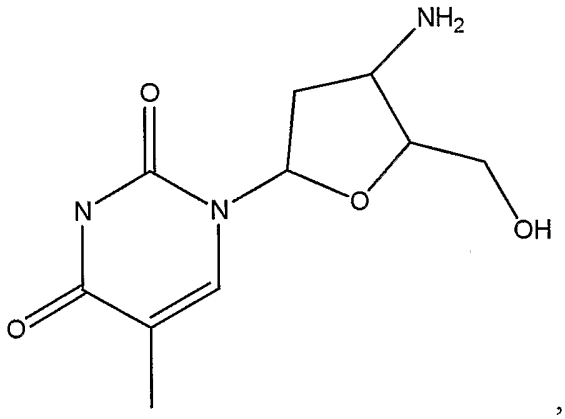
(d) detecting a second signal; and

(e) comparing the first signal with the second signal to determine the progress of a disease state.

48. The method of claim 47, wherein said sample is at least one of cells, tissue, cellular tissue, serum, cell extract, bodily fluid.

49. The method of claim 48, wherein the bodily fluid is selected from breast milk, urine, vaginal fluids, sputum.

50. The method of claim 47, wherein the conjugable compound is of the following formula:

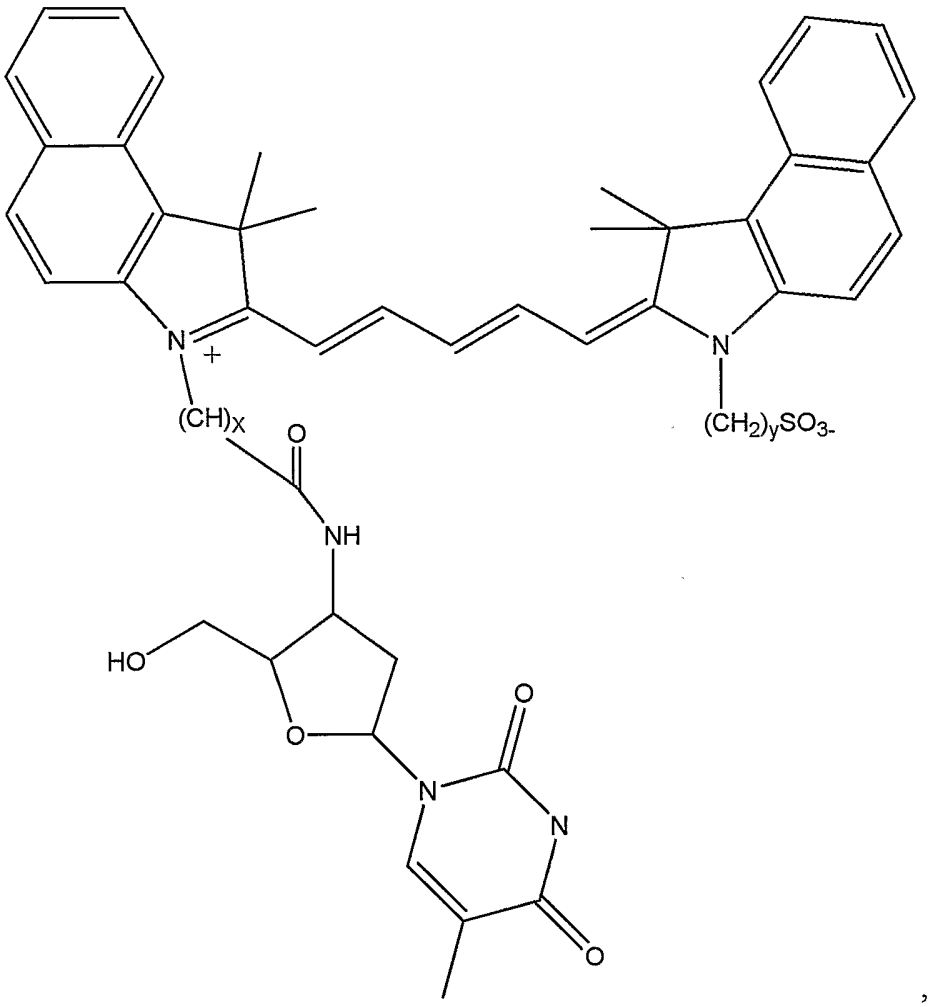


and analogs thereof.

51. The method of claim 47, wherein the disease state is cancer, neurodegenerative disease, coronary disease.

52. The method of claim 47, wherein the disease state is breast cancer, non-Hodgkin's lymphoma, or colon cancer.

53. The method of claim 47, wherein the conjugable compound of the following formula:



and analogues thereof, wherein x and y are independently an integer from 1 to 10.

54. The method of claim 47, wherein the period of time between step (b) and the administering to a second sample step, includes a treatment step for said disease state.

55. A method of quantifying the progression of a disease state in a subject, comprising:

(a) administering to a first sample of the subject a conjugate, the conjugate comprising a peripheral benzodiazepine affinity ligand or conjugable form thereof, and a signaling agent;

(b) detecting a signal from said conjugate;

(c) after a period of time from step (b), administering to a second sample of the subject a conjugate, the conjugate comprising a peripheral benzodiazepine affinity ligand or conjugable form thereof and a signaling agent;

(d) detecting a second signal; and

(e) comparing the first signal with the second signal to determine the progress of a disease state.

56. The method of claim 55, wherein the comparing step includes a determination of the efficacy of a treatment for said disease state.

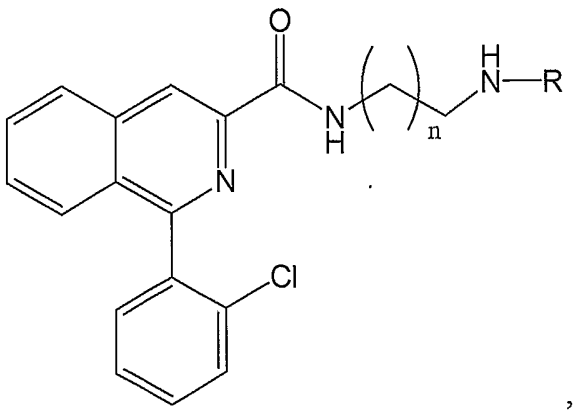
57. The method of claim 55, wherein said sample is at least one of cells, tissue, cellular tissue, serum, cell extract, bodily fluids.

58. The method of claim 57, wherein the bodily fluids are selected from vaginal fluid, breast milk, urine, sputum.

59. The method of claim 55, wherein the disease state is at least one of cancer, neurodegenerative disease, cancer, multiple sclerosis, epilepsy, coronary disease.

60. The method of claim 55, where in the disease state is breast cancer, non-Hodgkin's lymphoma, colon cancer.

61. The method of claim 55, wherein the peripheral benzodiazepine affinity ligand is a compound of the following formula:



or an analog thereof, wherein R is H or alkyl, and n is 0-10.

62. The method of claim 55, wherein the period of time between step (b) and the administering to a second sample step, includes a treatment step for said disease state.

63. A method of quantifying the progression of a disease state, comprising:

(a) administering to a first sample a conjugate, the conjugate comprising a glucosamine compound, and a signaling agent;

(b) detecting a signal from said conjugate;

(c) after a period of time from step (b), administering to a second sample a conjugate, the conjugate comprising a glucosamine compound and a signaling agent;

(d) detecting a second signal; and

(e) comparing the first signal with the second signal to determine the progress of a disease state.

64. The method of claim 63, wherein the comparing step includes a determination of the efficacy of a treatment for said disease state.

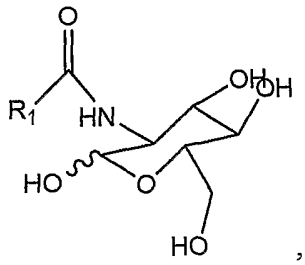
65. The method of claim 63, wherein said sample is at least one of cells, tissue, cellular tissue, serum, cell extract, bodily fluids.

66. The method of claim 65, where the bodily fluids include breast milk, urine, vaginal fluids, sputum.

67. The method of claim 63, wherein the disease state is at least one of cancer, neurodegenerative disease, cancer, multiple sclerosis, epilepsy, coronary disease.

68. The method of claim 63, where in the disease state is breast cancer, non-Hodgkin's lymphoma, or colon cancer.

69. The method of claim 63, wherein the conjugate is a compound of the following formula:

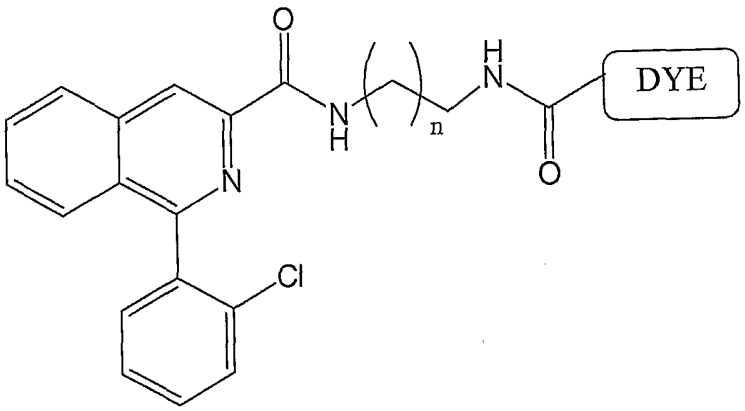


,

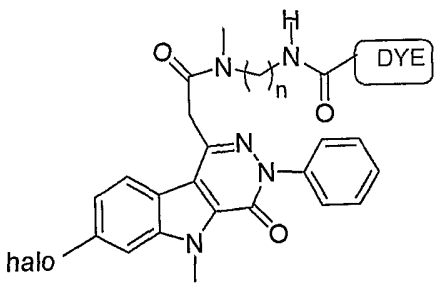
wherein R_1 is a signaling moiety, or an analog thereof.

70. A method of imaging a molecular event, comprising:

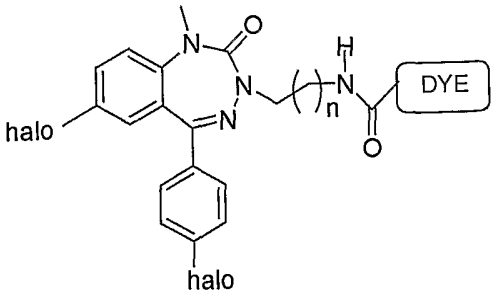
(a) administering a probe having an affinity for a target, the probe comprising a compound of the following formulae:



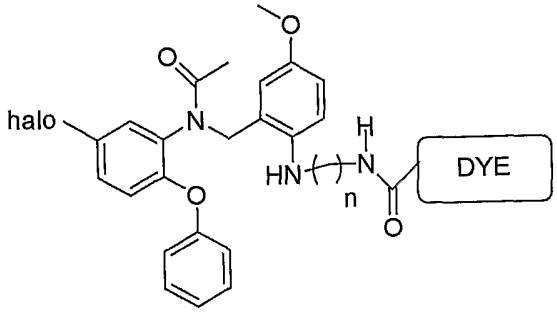
;



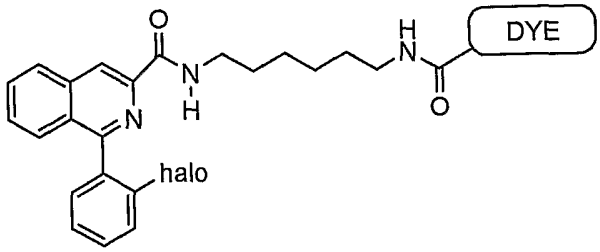
;



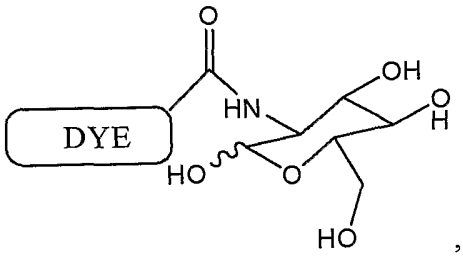
;



;



; or

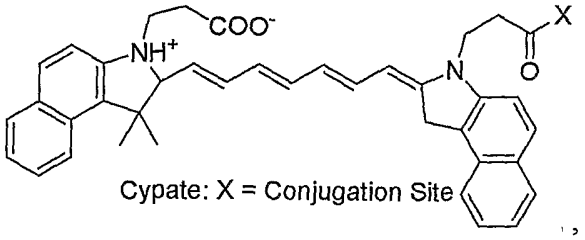


,

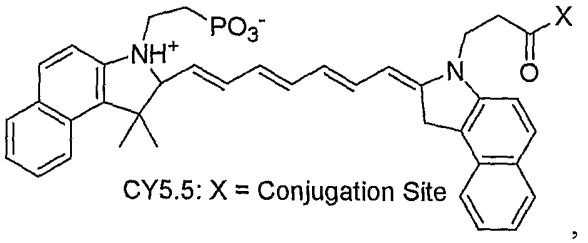
wherein n is an integer from 1 to 10, and analogs thereof.

71. The method of claim 70, wherein the dye is a cyanine dye.

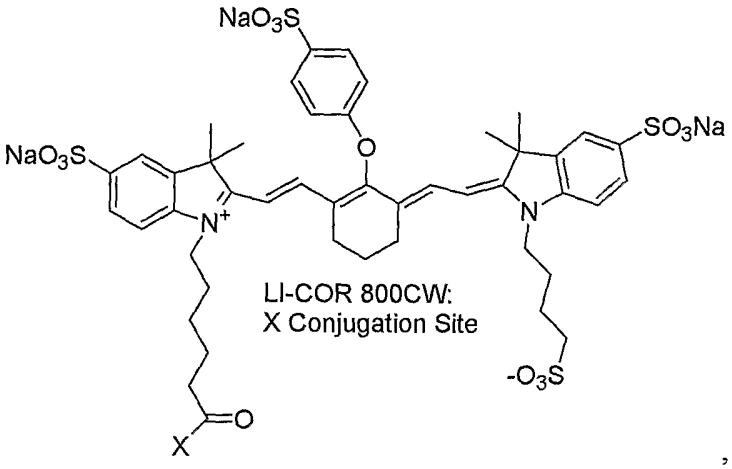
72. The method of claim 70, wherein the dye is selected from the following formula:



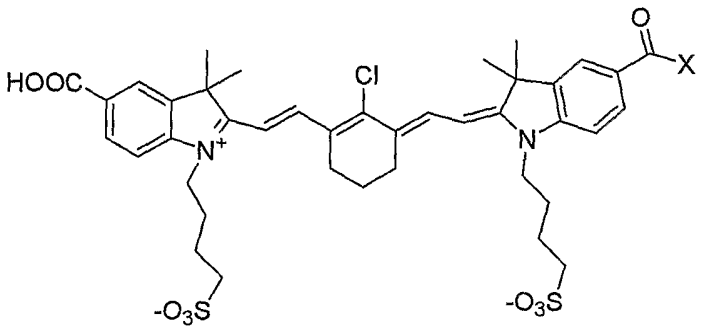
Cypate: X = Conjugation Site ,



CY5.5: X = Conjugation Site ,

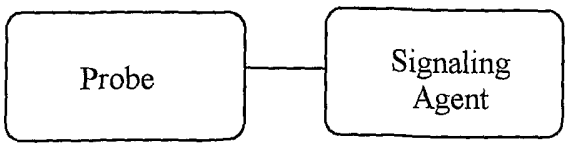


, and



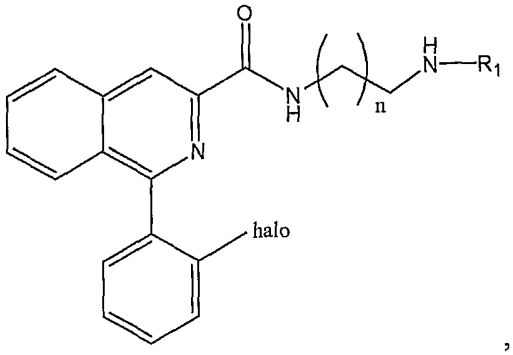
NIR-820: X Conjugation Site .

73. A compound of the following formula:

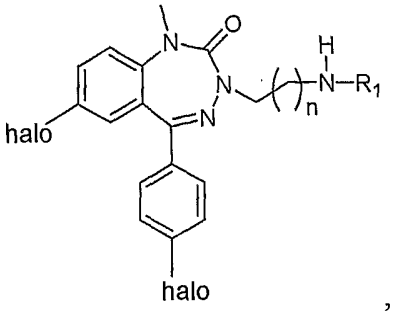


, wherein

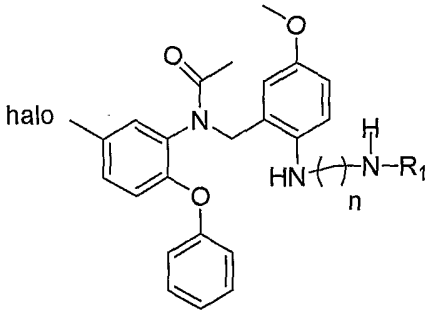
the probe is selected from a compound of the following formula:



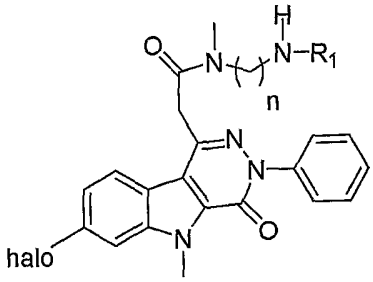
,



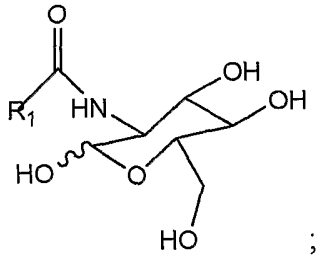
,



,

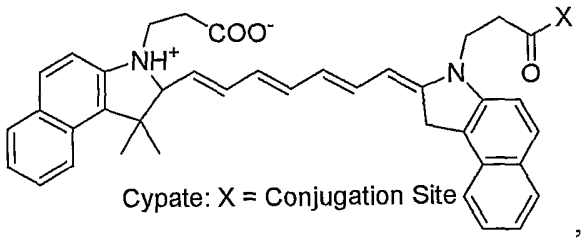


, or

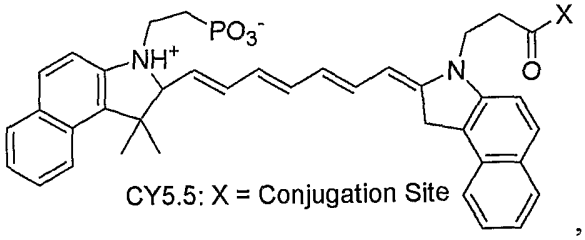


; and

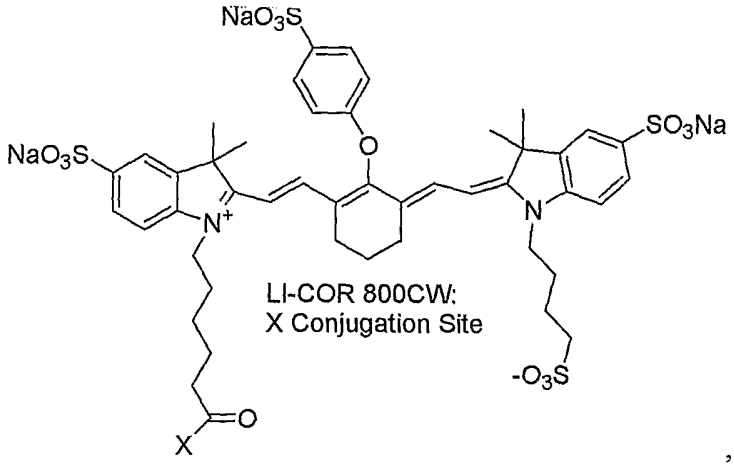
wherein the signaling moiety is selected from the following formula:



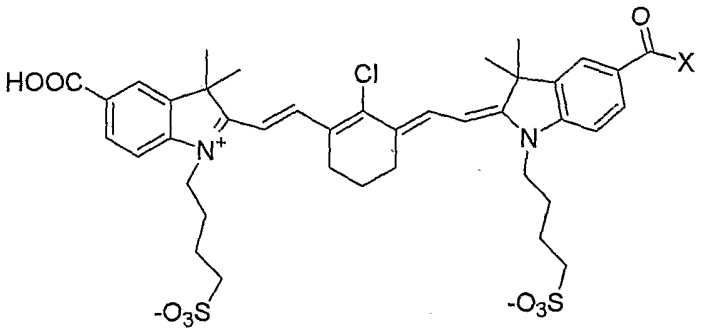
,



,

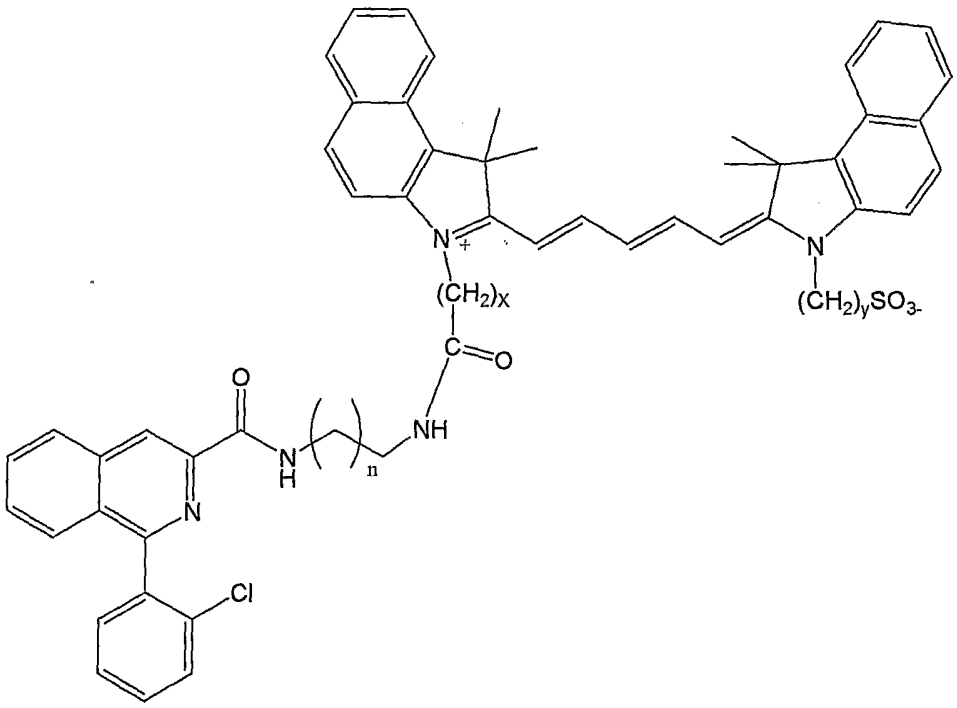


, and



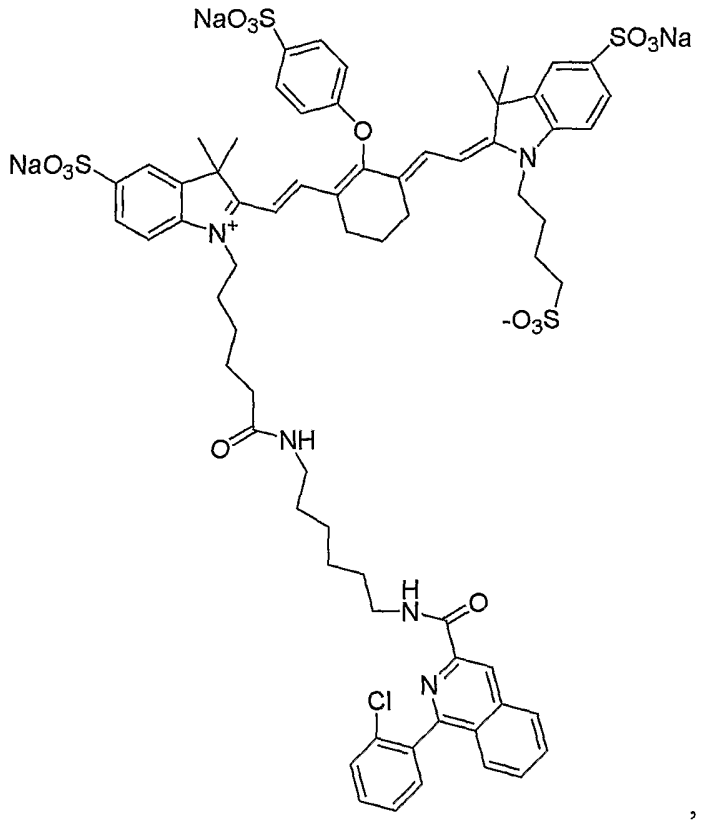
NIR-820: X Conjugation Site , wherein n is an integer from 1 to 10, and R_1-X is the conjugation site.

74. A compound of claim 73, of the following formula:

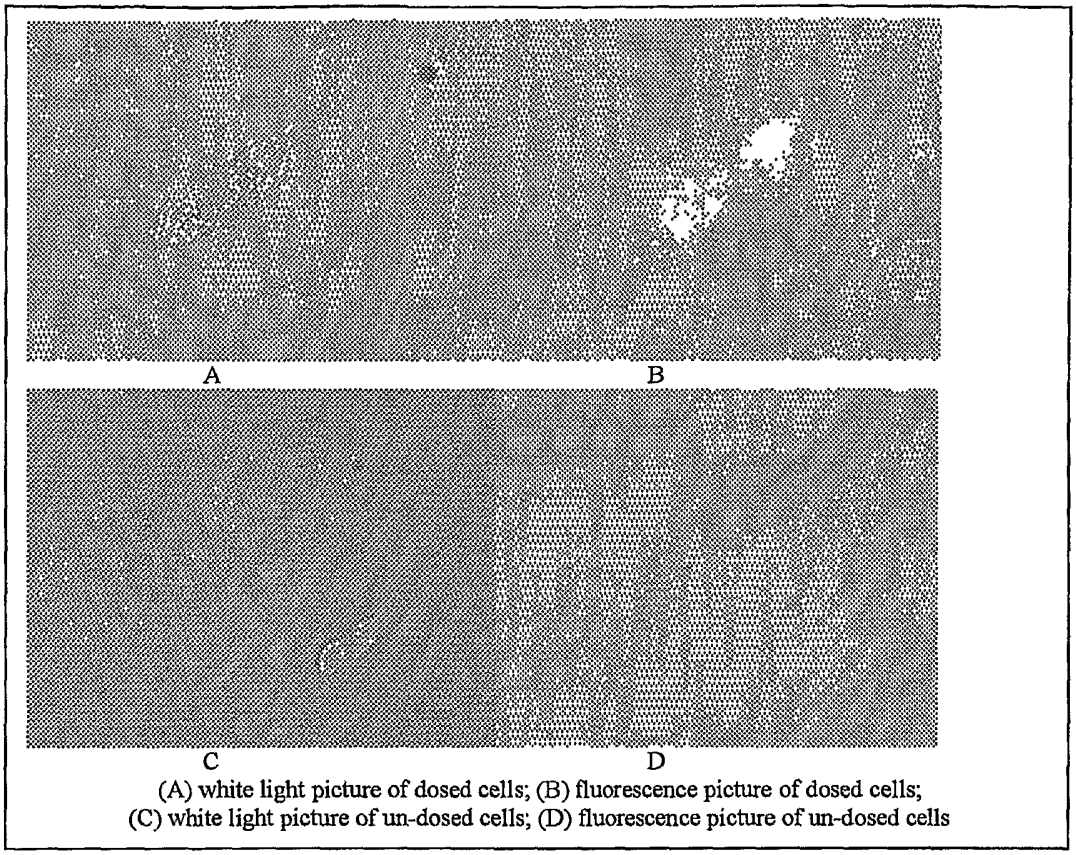


and analogs thereof, wherein n and x are integers from 1 to 10.

75. A compound of claim 73, of the following formula:



and analogs thereof.



(A) white light picture of dosed cells; (B) fluorescence picture of dosed cells;
(C) white light picture of un-dosed cells; (D) fluorescence picture of un-dosed cells

FIG. 1

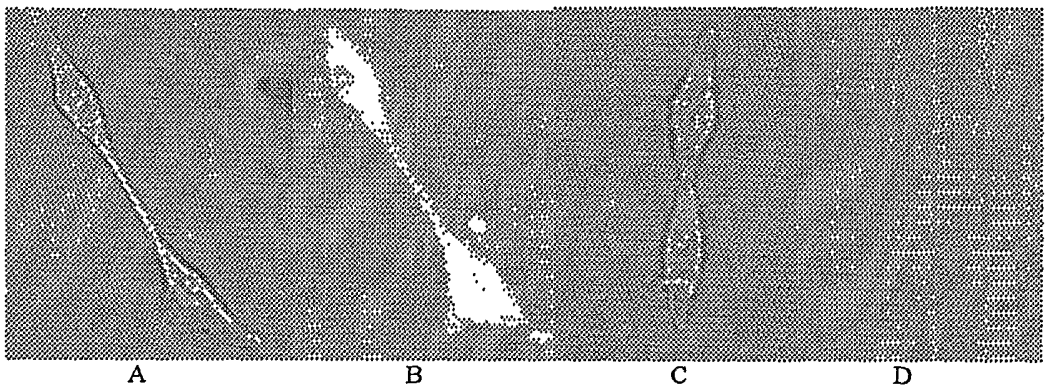
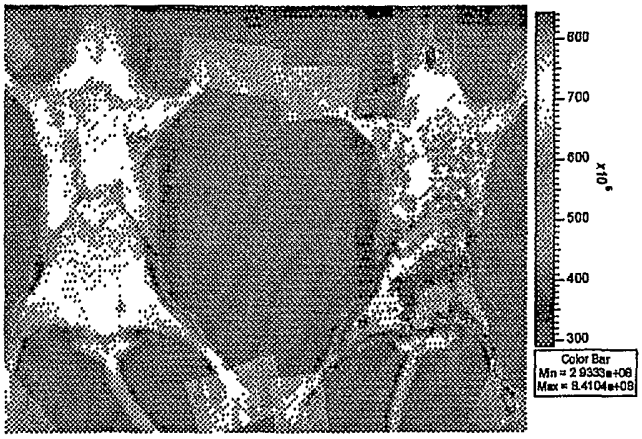


Figure 3.8 (A) white light picture of dosed cells; (B) fluorescence picture of dosed cells; (C) white light picture of un-dosed cells; (D) fluorescence picture of un-dosed cells

FIG. 2



control Smad3KO

FIG. 3

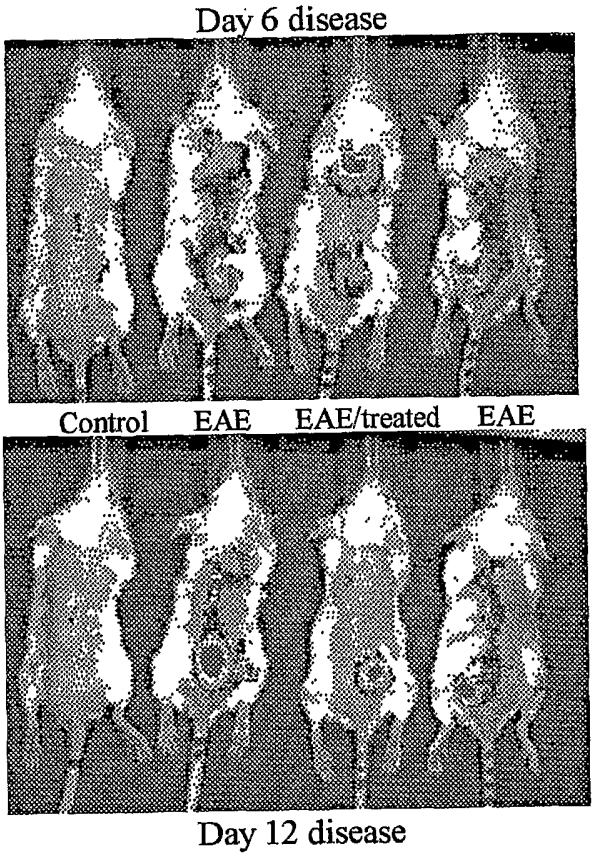


FIG. 4