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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
08 March 2018 (08.03.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/045385 A1

(51) International Patent Classification:
G01N 21/76 (2006.01) *C07D 471/22* (2006.01)
C07D 401/14 (2006.01)

(21) International Application Number:
PCT/US2017/050118

(22) International Filing Date:
05 September 2017 (05.09.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/383,205 02 September 2016 (02.09.2016) US

(71) Applicant: **LUMIPHORE, INC.** [US/US]; 600 Bancroft Way, Suite B, Berkeley, CA 94710-2224 (US).

(72) Inventors: **TATUM, David**; 1642 Allston Way, Berkeley, CA 94703 (US). **XU, Jide**; 2716 Carlson Blvd., Richmond, CA 94804 (US). **MAGDA, Darren**; 1368 Graff Avenue, San Leandro, CA 94577 (US). **BUTLIN, Nathaniel**; 46 San Jose Avenue, Pacifica, CA 94044 (US).

(74) Agent: **MANN, Jeffry, S.** et al.; Morgan, Lewis & Bockius LLP, One Market, Spear Street Tower, San Francisco, CA 94105 (US).

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

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
— *with international search report (Art. 21(3))*

WO 2018/045385 A1

(54) Title: MACROCYCLIC LIGANDS WITH PENDANT CHELATING MOIETIES AND COMPLEXES THEREOF

(57) Abstract: The invention relates to chemical compounds and complexes that can be used in therapeutic and diagnostic applications.

MACROCYCLIC LIGANDS WITH PENDANT CHELATING MOIETIES AND COMPLEXES THEREOF

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 62/383,205, filed on September 2, 2016, which is incorporated herein by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The invention relates to chemical compounds and complexes that can be used in therapeutic and diagnostic applications.

BACKGROUND

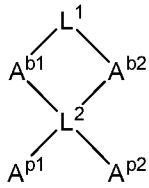
[0003] Current radioimmunotherapy practice makes use of two classes of chelating agents: acyclic species based on diethylenetriamine pentaacetic acid (DTPA) or macrocyclic derivatives similar to 1,4,7,20-tetraazacyclododeccane N,N′,N″,N‴-tetraacetic acid (DOTA). The former display more rapid association kinetics, while the DOTA-like compounds tend to produce a more stable complex, with the caveat that complexation typically requires harsher conditions such as high temperatures. A list of radiometals currently under clinical investigation (according to clinicaltrials.gov) includes actinium-225, bismuth-213, copper-64, gallium-67, gallium-68, holmium-166, indium-111, lutetium-177, rubidium-82, samarium-153, zirconium-89, strontium-89, technetium-99m, lead-212, and yttrium-90.

[0004] Lanthanide and actinide radiometal cations, in the absence of chelation, are largely deposited in bone, a significant concern given the potential for bone marrow suppression. Toxicity concerns that have arisen recently following the use of MRI contrast agents such as Gd^{+3} DTPA, clearly underscore the insufficient control of the metal cation biodistribution by this chelating group. Similarly, radiometal loss can lead to a loss of signal specificity by targeted radiodiagnostics. Therefore, there is a recognized, compelling need for improved chelating agents for use in radioimmunotherapy. Such chelating agents and complexes and methods of their use are provided by the present invention.

SUMMARY OF INVENTION

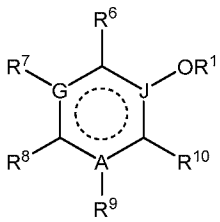
[0005] The present invention provides a new class of ligands and metal complexes of these ligands which are particularly useful in therapeutic or diagnostic applications. The

compounds (ligands) of this invention comprise a mixture of bridging chelating moieties and pendant chelating moieties that are linked together to have a structure of:

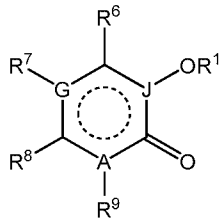


wherein L^1 and L^2 are independently selected scaffold moieties; A^{b1} and A^{b2} are independently selected bridging chelating moieties; and A^{p1} and A^{p2} are independently selected pendant chelating moieties.

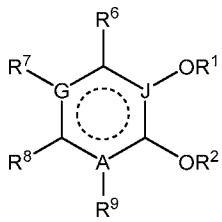
[0006] The bridging chelating moieties and pendant chelating moieties of the present invention are independently selected from:



(I),



(II), and



(III)

wherein A and G are independently selected from carbon, nitrogen and oxygen. J is selected from carbon and nitrogen. Each R^1 and R^2 is independently selected from H, an enzymatically labile group, a hydrolytically labile group, a metabolically labile group, a photolytically labile group and a single negative charge. Each R^6, R^7, R^8, R^9, and R^{10} is independently selected from a bond to L^1 or L^2, alkanediyl attached to L^1 or L^2, H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, halogen, CN, $-CF_3$, $-C(O)R^{17}$, $-SO_2NR^{17}R^{18}$, $-NR^{17}R^{18}$, $-OR^{17}$, $-S(O)_2R^{17}$, $-COOR^{17}$, $-S(O)_2OR^{17}$, $-OC(O)R^{17}$, $-C(O)NR^{17}R^{18}$, $-NR^{17}C(O)R^{18}$, $-NR^{17}SO_2R^{18}$, and $-NO_2$, wherein at least two of R^6, R^7, R^8, R^9, and R^{10} are optionally joined to form a ring system selected from substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. R^{17} and R^{18} are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl; and R^{17} and R^{18}, together with the atoms to which they are attached, are optionally joined to form a 5-, 6- or 7-membered ring. When A is oxygen, R^9 is

not present; and when G is oxygen, R^7 is not present. A^{b1} and A^{b2} are attached to L^1 and L^2 through two members selected from R^6, R^7, R^8, R^9 and R^{10}; and A^{p1} and A^{p2} are attached to L^2 through a member selected from R^6, R^7, R^8, R^9 and R^{10}.

[0007] Advantages of the compounds of the present invention are that such chelating ligands bind the isotope rapidly, so that they are compatible with the practicalities of clinical laboratory preparation. Such compounds also bind the cation stably so that none is released in vivo, at least prior to its decay. These apparently contradictory properties of the compounds are in fact embodied by the pre-organized chelating groups that retain a sufficient degree of flexibility.

[0008] Exemplary compounds of the present invention also comprise a linker to a reactive functional group or a linker to a targeting moiety, therefore, the chelating ligands and their complexes provided herein can be directed to a site of interest for therapeutic or diagnostic purposes.

[0009] Compounds of the present invention and metal ion complexes thereof are particularly useful for targeted radioisotope applications and sensitized luminescence applications (such as Eu sensitized luminescence immunoassays). As shown in the Examples, compounds (ligands) of the present invention stably coordinate metal cations, display facile complexation kinetics, and possess an exceptionally high aqueous quantum yield with Eu(III).

BRIEF DESCRIPTION OF DRAWINGS

[0010] **Figure 1** shows that chromatograms of ligand **13**, **13**·Eu, and **13**·Th, were recorded by absorption at 315 nm. Although multiple chromatogram peaks were observed for the free ligand and **13**·Th complex, there was no significant difference in the absorption spectrum when comparing each peak of the same sample.

[0011] **Figure 2** shows UV-vis spectra of ligand **13**, **13**·Eu, and **13**·Th, recorded in TFA/water/acetonitrile.

[0012] **Figure 3** shows UV-vis spectrum (Figure 3a) and photoluminescence spectrum (Figure 3b) of **9**·Eu measured in TBS buffer, pH 7.6.

[0013] **Figure 4** shows quantum yield determination of **9**·Eu.

[0014] **Figure 5** shows UV-vis spectrum (Figure 5a) and photoluminescence spectrum (Figure 5b) of **13**·Eu measured in TBS buffer, pH 7.6.

[0015] **Figure 6** shows quantum yield determination of **13**·Eu.

[0016] **Figure 7** shows UV-vis titration of ligand **13** with europium chloride.

[0017] **Figure 8** shows photoluminescent titration of ligand **13** with europium chloride.

[0018] **Figure 9** shows changes in integrated luminescence intensity over time in the presence of ca. 25 mM competitors.

[0019] **Figure 10** shows absorption spectra of samples with various competitors after 5 days at room temperature.

DESCRIPTION OF EMBODIMENTS

Definitions

[0020] Where substituent groups are specified by their conventional chemical formulae, written from left to right, they optionally equally encompass the chemically identical substituents, which would result from writing the structure from right to left, *e.g.*, $-CH_2O-$ is intended to also recite $-OCH_2-$.

[0021] The term "alkyl", by itself or as part of another substituent, means a straight or branched chain hydrocarbon, which may be fully saturated, mono- or polyunsaturated and includes mono-, di- and multivalent radicals. Examples of saturated hydrocarbon radicals include, but are not limited to, groups such as methyl, ethyl, n-propyl, isopropyl, n-butyl, t-butyl, isobutyl, sec-butyl, cyclohexyl, (cyclohexyl)methyl, cyclopropylmethyl, homologs and isomers of, for example, n-pentyl, n-hexyl, n-heptyl, n-octyl, and the like. An unsaturated alkyl group is one having one or more double bonds or triple bonds (i.e., alkenyl and alkynyl moieties). Examples of unsaturated alkyl groups include, but are not limited to, vinyl, 2-propenyl, crotyl, 2-isopentenyl, 2-(butadienyl), 2,4-pentadienyl, 3-(1,4-pentadienyl), ethynyl, 1- and 3-propynyl, 3-butynyl, and the higher homologs and isomers. The term "alkyl" can refer to "alkylene", which by itself or as part of another substituent means a divalent radical derived from an alkane, as exemplified, but not limited, by $-CH_2CH_2CH_2CH_2-$. Typically, an alkyl (or alkylene) group will have from 1 to 30 carbon atoms. A "lower alkyl" or "lower alkylene" is a shorter chain alkyl or alkylene group, generally having eight or fewer carbon atoms. In some embodiments, alkyl refers to an alkyl or combination of alkyls selected from

C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27}, C_{28}, C_{29} and C_{30} alkyl. In some embodiments, alkyl refers to C_1-C_{25} alkyl. In some embodiments, alkyl refers to C_1-C_{20} alkyl. In some embodiments, alkyl refers to C_1-C_{15} alkyl. In some embodiments, alkyl refers to C_1-C_{10} alkyl. In some embodiments, alkyl refers to C_1-C_6 alkyl.

[0022] The term "heteroalkyl," by itself or in combination with another term, means an alkyl in which one or more carbons are replaced with one or more heteroatoms selected from the group consisting of O, N, Si and S, (preferably O, N and S), wherein the nitrogen and sulfur atoms may optionally be oxidized and the nitrogen heteroatom may optionally be quaternized. The heteroatoms O, N, Si and S may be placed at any interior position of the heteroalkyl group or at the position at which the alkyl group is attached to the remainder of the molecule. In some embodiments, depending on whether a heteroatom terminates a chain or is in an interior position, the heteroatom may be bonded to one or more H or substituents such as (C_1, C_2, C_3, C_4, C_5 or C_6) alkyl according to the valence of the heteroatom. Examples of heteroalkyl groups include, but are not limited to, -CH_2-CH_2-O-CH_3, -CH_2-CH_2-NH-CH_3, -CH_2-CH_2-N(CH_3)-CH_3, -CH_2-S-CH_2-CH_3, -CH_2-CH_2,-S(O)-CH_3, -CH_2-CH_2-S($O)_2$-CH_3, -CH=CH-O-CH_3, -Si(CH_3)$_3$, -CH_2-CH=N-OCH_3, and –CH=CH-N(CH_3)-CH_3. No more than two heteroatoms may be consecutive, as in, for example, -CH_2-NH-OCH_3 and –CH_2-O-Si(CH_3)$_3$, and in some instances, this may place a limit on the number of heteroatom substitutions. Similarly, the term "heteroalkylene" by itself or as part of another substituent means a divalent radical derived from heteroalkyl, as exemplified, but not limited by, -CH_2-CH_2-S-CH_2-CH_2- and –CH_2-S-CH_2-CH_2-NH-CH_2-. The designated number of carbons in heteroforms of alkyl, alkenyl and alkynyl includes the heteroatom count. For example, a (C_1, C_2, C_3, C_4, C_5 or C_6) heteroalkyl will contain, respectively, 1, 2, 3, 4, 5 or 6 atoms selected from C, N, O, Si and S such that the heteroalkyl contains at least one C atom and at least one heteroatom, for example 1-5 C and 1 N or 1-4 C and 2 N. Further, a heteroalkyl may also contain one or more carbonyl groups. In some embodiments, a heteroalkyl is any C_2-C_{30} alkyl, C_2-C_{25} alkyl, C_2-C_{20} alkyl, C_2-C_{15} alkyl, C_2-C_{10} alkyl or C_2-C_6 alkyl in any of which one or more carbons are replaced by one or more heteroatoms selected from O, N, Si and S (or from O, N and S). In some embodiments, each of 1, 2, 3, 4 or 5 carbons is replaced with a heteroatom.The terms "alkoxy," "alkylamino" and "alkylthio" (or thioalkoxy) are used in their conventional sense, and refer to those alkyl and heteroalkyl groups attached to the

remainder of the molecule via an oxygen atom, a nitrogen atom (e.g., an amine group), or a sulfur atom, respectively.

[0023] The terms "cycloalkyl" and "heterocycloalkyl", by themselves or in combination with other terms, refer to cyclic versions of "alkyl" and "heteroalkyl", respectively. Additionally, for heterocycloalkyl, a heteroatom can occupy the position at which the heterocycle is attached to the remainder of the molecule. Examples of cycloalkyl include, but are not limited to, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, cycloheptyl, and the like. Examples of heterocycloalkyl include, but are not limited to, 1 –(1,2,5,6-tetrahydropyridyl), 1-piperidinyl, 2-piperidinyl, 3-piperidinyl, 4-morpholinyl, 3-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1–piperazinyl, 2-piperazinyl, and the like.

[0024] The term "aryl" means a polyunsaturated, aromatic substituent that can be a single ring or optionally multiple rings (preferably 1, 2 or 3 rings) that are fused together or linked covalently. In some embodiments, aryl is a 3, 4, 5, 6, 7 or 8 membered ring, which is optionally fused to one or two other 3, 4, 5, 6, 7 or 8 membered rings. The term "heteroaryl" refers to aryl groups (or rings) that contain 1, 2, 3 or 4 heteroatoms selected from N, O, and S, wherein the nitrogen and sulfur atoms are optionally oxidized, and the nitrogen atom(s) are optionally quaternized. A heteroaryl group can be attached to the remainder of the molecule through a heteroatom. Non-limiting examples of aryl and heteroaryl groups include phenyl, 1-naphthyl, 2-naphthyl, 4-biphenyl, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, 3-pyrazolyl, 2-imidazolyl, 4-imidazolyl, pyrazinyl, 2-oxazolyl, 4-oxazolyl, 2-phenyl-4-oxazolyl, 5-oxazolyl, 3-isoxazolyl, 4-isoxazolyl, 5-isoxazolyl, 2-thiazolyl, 4-thiazolyl, 5-thiazolyl, 2-furyl, 3-furyl, 2-thienyl, 3-thienyl, 2-pyridyl, 3-pyridyl, 4-pyridyl, 2-pyrimidyl, 4-pyrimidyl, 5-benzothiazolyl, purinyl, 2-benzimidazolyl, 5-indolyl, 1-isoquinolyl, 5-isoquinolyl, 2-quinoxalinyl, 5-quinoxalinyl, 3-quinolyl, and 6-quinolyl.

[0025] In some embodiments, any of alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl and heteroaryl is optionally substituted. That is, in some embodiments, any of these groups is substituted or unsubstituted. In some embodiments, substituents for each type of radical are selected from those provided below.

[0026] Substituents for the alkyl, heteroalkyl, cycloalkyl and heterocycloalkyl radicals (including those groups often referred to as alkylene, alkenyl, heteroalkylene, heteroalkenyl, alkynyl, cycloalkyl, heterocycloalkyl, cycloalkenyl, and heterocycloalkenyl) are generically

referred to as "alkyl group substituents". In some embodiments, an alkyl group substituent is selected from -halogen, -OR', =O, =NR', =N-OR', -NR'R", -SR', -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NR-C(NR'R"R"')=NR"", -NR-C(NR'R")=NR"', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', -CN and -NO_2 in a number ranging from zero to (2m'+1), where m' is the total number of carbon atoms in such radical. In one embodiment, R', R", R"' and R"" are each independently selected from hydrogen, alkyl (e.g., C_1, C_2, C_3, C_4, C_5 and C_6 alkyl). In one embodiment, R', R", R"' and R"" each independently refer to hydrogen, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, *e.g.*, aryl substituted with 1-3 halogens, substituted or unsubstituted alkyl, alkoxy or thioalkoxy groups, or arylalkyl groups. In one embodiment, R', R", R"' and R"" are each independently selected from hydrogen, alkyl, heteroalkyl, cycloalkyl, heterocycloalkyl, aryl, heteroaryl, alkoxy, thioalkoxy groups, and arylalkyl. When R' and R" are attached to the same nitrogen atom, they can be combined with the nitrogen atom to form a 5-, 6-, or 7-membered ring. For example, -NR'R" can include 1-pyrrolidinyl and 4-morpholinyl. In some embodiments, an alkyl group substituent is selected from substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl.

[0027] Similar to the substituents described for the alkyl radical, substituents for the aryl and heteroaryl groups are generically referred to as "aryl group substituents". In some embodiments, an aryl group substituent is selected from -halogen, -OR', =O, =NR', =N-OR', -NR'R", -SR', -SiR'R"R"', -OC(O)R', -C(O)R', -CO_2R', -CONR'R", -OC(O)NR'R", -NR"C(O)R', -NR'-C(O)NR"R"', -NR"$C(O)_2$R', -NR-C(NR'R"R"')=NR"", -NR-C(NR'R")=NR"', -S(O)R', -$S(O)_2$R', -$S(O)_2$NR'R", -$NRSO_2$R', -CN and -NO_2, -R', -N_3, -$CH(Ph)_2$, fluoro(C_1-C_4)alkoxy, and fluoro(C_1-C_4)alkyl, in a number ranging from zero to the total number of open valences on the aromatic ring system. In some embodiments, R', R", R"' and R"'' are independently selected from hydrogen and alkyl (e.g., C_1, C_2, C_3, C_4, C_5 and C_6 alkyl). In some embodiments, R', R", R"' and R"" are independently selected from hydrogen, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. In some embodiments, R', R", R"' and R"" are independently selected from hydrogen, alkyl, heteroalkyl, aryl and heteroaryl. In some embodiments, an aryl group substituent is selected from substituted or

unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl.

[0028] Two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -T-C(O)-$(CRR')_q$-U-, wherein T and U are independently -NR-, -O-, -CRR'- or a single bond, and q is an integer of from 0 to 3. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -A-$(CH_2)_r$-B-, wherein A and B are independently –CRR'-, -O-, -NR-, -S-, -S(O)-, -$S(O)_2$-, -$S(O)_2$NR'- or a single bond, and r is an integer of from 1 to 4. One of the single bonds of the new ring so formed may optionally be replaced with a double bond. Alternatively, two of the substituents on adjacent atoms of the aryl or heteroaryl ring may optionally be replaced with a substituent of the formula -$(CRR')_s$-X-$(CR''R''')_d$-, where s and d are independently integers of from 0 to 3, and X is -O-, -NR'-, -S-, -S(O)-, -$S(O)_2$-, or –$S(O)_2$NR'-. The substituents R, R', R'' and R''' are preferably independently selected from hydrogen or substituted or unsubstituted (C_1-C_6)alkyl.

[0029] The term "acyl" refers to a species that includes the moiety –C(O)R, where R has the meaning defined herein. Exemplary species for R include H, halogen, substituted or unsubstituted alkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, and substituted or unsubstituted heterocycloalkyl. In some embodiments, R is selected from H and (C_1-C_6)alkyl.

[0030] The terms "halo" or "halogen," by themselves or as part of another substituent, mean, unless otherwise stated, a fluorine, chlorine, bromine, or iodine atom. Additionally, terms such as "haloalkyl," are meant to include monohaloalkyl and polyhaloalkyl. For example, the term "halo(C_1-C_4)alkyl" is mean to include, but not be limited to, trifluoromethyl, 2,2,2-trifluoroethyl, 4-chlorobutyl, 3-bromopropyl, and the like. In some embodiments, halogen refers to an atom selected from F, Cl and Br.

[0031] The term "heteroatom" includes oxygen (O), nitrogen (N), sulfur (S) and silicon (Si). In some embodiments, a heteroatom is selected from N and S. In some embodiments, the heteroatom is O.

[0032] Unless otherwise specified, the symbol "R" is a general abbreviation that represents a substituent group that is selected from acyl, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted

heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. When a compound includes more than one R, R', R", R'" and R"" group, they are each independently selected.

[0033] For groups with solvent exchangeable protons, the ionized form is equally contemplated. For example, -COOH also refers to $-COO^-$ and -OH also refers to $-O^-$.

[0034] Any of the compounds disclosed herein can be made into a pharmaceutically acceptable salt. The term "pharmaceutically acceptable salts" includes salts of compounds that are prepared with relatively nontoxic acids or bases, depending on the particular substituents found on the compounds described herein. When compounds of the present invention contain relatively acidic functionalities, base addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired base, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable base addition salts include sodium, potassium, calcium, ammonium, organic amino, or magnesium salt, or a similar salt. When compounds of the present invention contain relatively basic functionalities, acid addition salts can be obtained by contacting the neutral form of such compounds with a sufficient amount of the desired acid, either neat or in a suitable inert solvent. Examples of pharmaceutically acceptable acid addition salts include those derived from inorganic acids like hydrochloric, hydrobromic, nitric, carbonic, monohydrogencarbonic, phosphoric, monohydrogenphosphoric, dihydrogenphosphoric, sulfuric, monohydrogensulfuric, hydriodic, or phosphorous acids and the like, as well as the salts derived from relatively nontoxic organic acids like acetic, propionic, isobutyric, maleic, malonic, benzoic, succinic, suberic, fumaric, lactic, mandelic, phthalic, benzenesulfonic, p-tolylsulfonic, citric, tartaric, methanesulfonic, and the like. Also included are salts of amino acids such as arginate and the like, and salts of organic acids like glucuronic or galactunoric acids and the like (see, for example, Berge *et al.*, *Journal of Pharmaceutical Science*, **66**: 1-19 (1977)). Certain specific compounds of the present invention contain both basic and acidic functionalities that allow the compounds to be converted into either base or acid addition salts. The neutral forms of the compounds are preferably regenerated by contacting the salt with a base or acid and isolating the parent compound in the conventional manner. The parent form of the compound differs from the various salt forms in certain physical properties, such as solubility in polar solvents, but otherwise the salts are equivalent to the parent form of the compound for the purposes of the present invention.

[0035] In addition to salt forms, the present invention provides any of the compounds disclosed herein in a prodrug form. Prodrugs of the compounds described herein are those compounds that readily undergo chemical changes under physiological conditions to provide the compounds of the present invention.

[0036] Certain compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms are equivalent to unsolvated forms and are encompassed within the scope of the present invention. Certain compounds of the present invention may exist in multiple crystalline or amorphous forms. In general, all physical forms are equivalent for the uses contemplated by the present invention and are intended to be within the scope of the present invention.

[0037] The compounds of the present invention may also contain unnatural proportions of atomic isotopes at one or more of the atoms that constitute such compounds. For example, the compounds may be labeled with deuterium (^{2}H) or radiolabeled with radioactive isotopes, such as for example tritium (^{3}H), iodine-125 (^{125}I) or carbon-14 (^{14}C). All isotopic variations of the compounds of the present invention, whether radioactive or not, are intended to be encompassed within the scope of the present invention.

[0038] The symbol  , displayed perpendicular to a bond, indicates the point at which the displayed moiety is attached to the remainder of the molecule.

[0039] In some embodiments, the definition of terms used herein is according to IUPAC.

Compositions

[0040] The invention provides numerous compounds (ligands) and metal ion complexes thereof. Generally, a ligand comprises a plurality of chelating moieties that are linked together by way of a scaffold moiety.

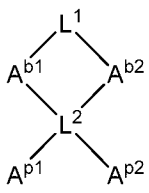
[0041] Compounds (ligands) of the present invention and metal ion complexes thereof are particularly useful for targeted radioisotope applications and sensitized luminescence applications (such as Eu sensitized luminescence immunoassays). As shown in the Examples, compounds (ligands) of the present invention stably coordinate metal cations, display facile complexation kinetics, and possess an exceptionally high aqueous quantum yield with Eu(III).

[0042] There are several factors to be considered in the design for an alpha chelating agent for anticancer therapy. Some of the key issues apart from the kinetics will be the high affinity for the target metal (such as Th) which at the same time needs to have a low exchange rate for other biologically significant metal ions. So, in our ligand design, the electronic properties of the target metal and ligand are considered and matched. The chelate should also be able to assume the appropriate coordination cavity size and geometry for the desired metal. In this case, Th, an actinide ion, is a "hard" cation and has a large charge-to-radius ratio. Hence, Th prefers "hard" electron donors and negatively charged oxygen donors. A coordination number of 8 or greater is generally preferred by actinide ions as they have a tendency to form stable complexes with ligands of high denticity; however, the selectivity towards the binding of the thorium will be determined by our design of the chelating unit. The effective but nonselective amino-carboxylic acid ligands such as DTPA can deplete essential biological metal ions from patients, thus causing serious health problems. Selecting the correct type of chelating unit, therefore, is an important factor in achieving high selectivity toward the specific metal ion.

[0043] A ligand can comprise numerous chelating moieties. Particularly useful ligands contain a number of chelating moieties sufficient to provide, for example, 6, 8 or 10 heteroatoms such as oxygen that coordinate with a metal ion to form a complex. The heteroatoms such as oxygen provide electron density for forming coordinate bonds with a positively charged ion, and such heteroatoms can thus be considered "donors". In some embodiments, the plurality of chelating moieties of a ligand comprises a plurality of oxygen donors and a metal ion (such as a radionuclide) is chelated to the ligand via at least one of the oxygen donors. In some embodiments, a ligand comprises a plurality of oxygen donors and a metal ion (such as a radionuclide) is chelated to the ligand via a plurality or all of the oxygen donors.

Ligands

[0044] In one aspect, the invention provides a compound (ligand) having the structure:



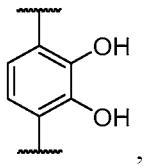
wherein L^1 and L^2 are independently selected scaffold moieties; A^{b1} and A^{b2} are independently selected bridging chelating moieties; and A^{p1} and A^{p2} are independently selected pendant chelating moieties.. Scaffold moieties, bridging chelating moieties and pendant chelating moieties are as defined herein.

[0045] Any of the combinations of L^1, L^2, A^{b1}, A^{b2}, A^{p1}, and A^{p2} are encompassed by this disclosure and specifically provided by the invention.

[0046] In some embodiments, the compound (ligand) comprises a linker to a reactive functional group, or a linker to a targeting moiety. In some embodiments, at least one of L^1, L^2, A^{p1} and A^{p2} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety. The linker to a reactive functional group and the linker to a targeting moiety are as defined herein. In some embodiments, the functional moiety is a reactive functional group or a protected functional group.

[0047] In some embodiments, the compound (ligand) comprises one or more modifying moieties. The modifying moieties can be the same or different.

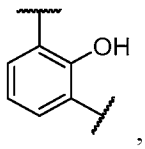
[0048] In some embodiments, when A^{b1} and A^{b2} are each:



,

the compound comprises a linker to a reactive functional group, or a linker to a targeting moiety.

[0049] In some embodiments, when A^{b1} and A^{b2} are each:

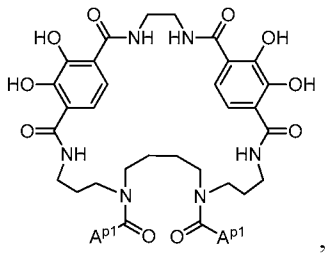


,

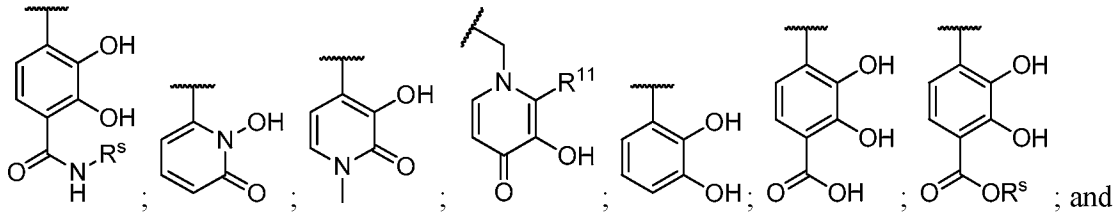
the compound comprises a linker to a reactive functional group, or a linker to a targeting moiety.

[0050] In some embodiments, the compounds (ligands) disclosed in WO 2013/187971 A2 are excluded.

[0051] In some embodiments, the compound (ligand) does not have the structure:



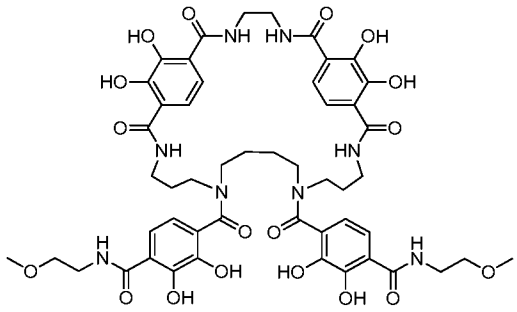
wherein each A^{p1} is as defined in WO 2013/187971 A2, paragraph [0078]; i.e., wherein each A^{p1} is independently selected from



wherein R^s comprises a solubilizing group; and

R^{11} is unsubstituted C_1, C_2, C_3, C_4, C_5 or C_6 alkyl.

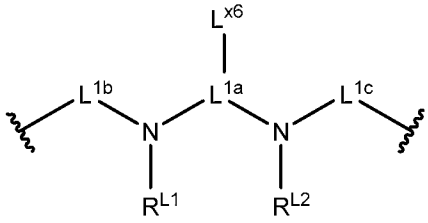
[0052] In some embodiments, the compound (ligand) does not have the structure:



(cf. WO 2013/187971 A2, paragraph [0080]).

Scaffold Moieties

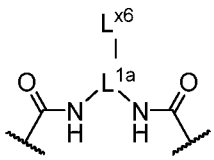
[0053] In some embodiments, L^1 has the structure:



wherein L^{1a}, L^{1b}, L^{1c}, L^{x6}, R^{L1}, and R^{L2} are as defined herein. Any of the combinations of L^{1a}, L^{1b}, L^{1c}, L^{x6}, R^{L1}, and R^{L2} are encompassed by this disclosure and specifically provided by the invention.

[0054] In some embodiments, L^1 is substituted with a linker to a reactive functional group, or a linker to a targeting moiety.

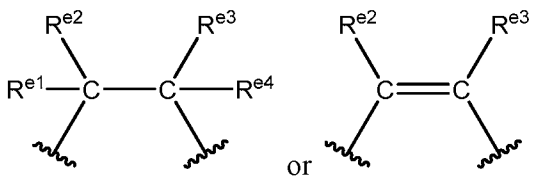
[0055] In some embodiments, L^1 has the structure:



wherein L^{1a} is as defined herein.

[0056] In some embodiments, L^{1a} is selected from substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylalkyl, substituted or unsubstituted biaryl, substituted or unsubstituted heteroaryl, and a substituted or unsubstituted polycyclic ring system.

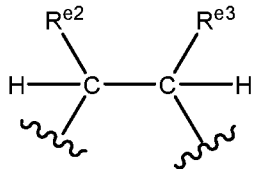
[0057] In some embodiments, L^{1a} has the structure:



wherein R^{e1}, R^{e2}, R^{e3}, and R^{e4} are independently selected from H, cyano, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkenyl, substituted

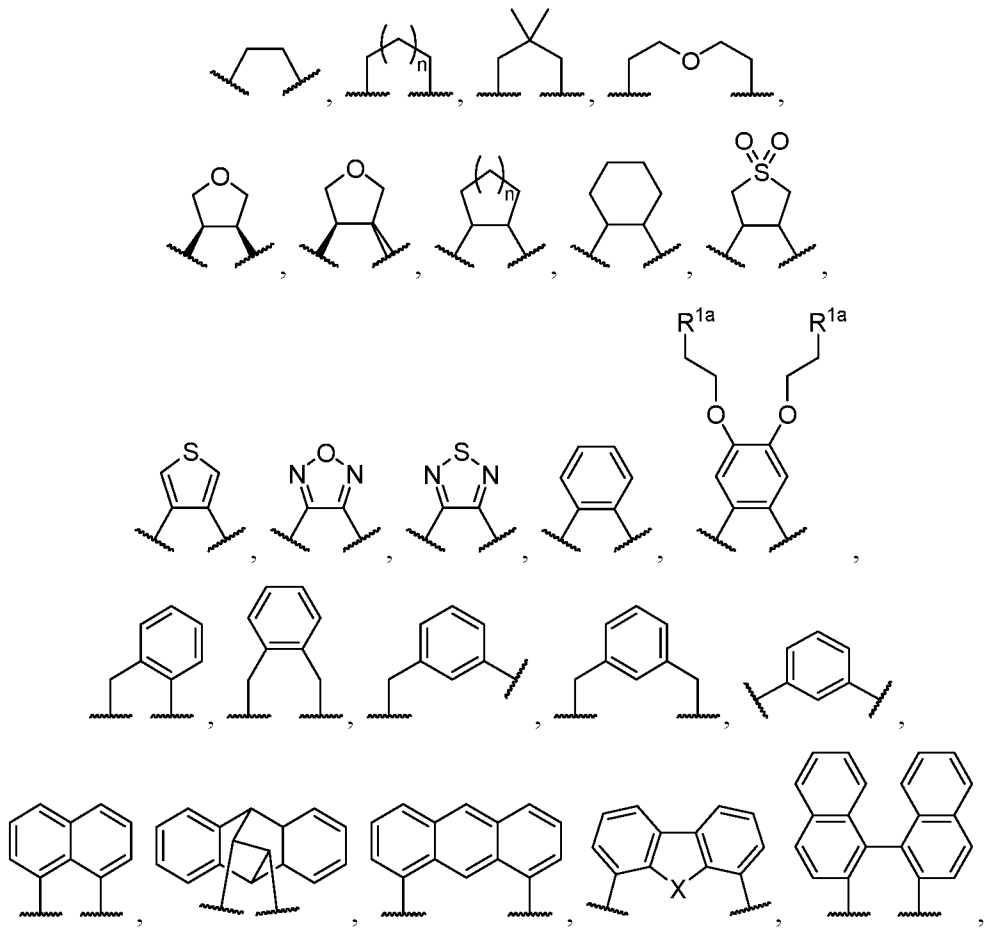
or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl; and two members selected from R^{e1}, R^{e2}, R^{e3}, and R^{e4}, together with the atom to which they are attached, are optionally joined, to form a substituted or unsubstituted ring (or ring system) selected from substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl. In some embodiments, R^{e1} or R^{e2} and R^{e3} or R^{e4} are hydrogen.

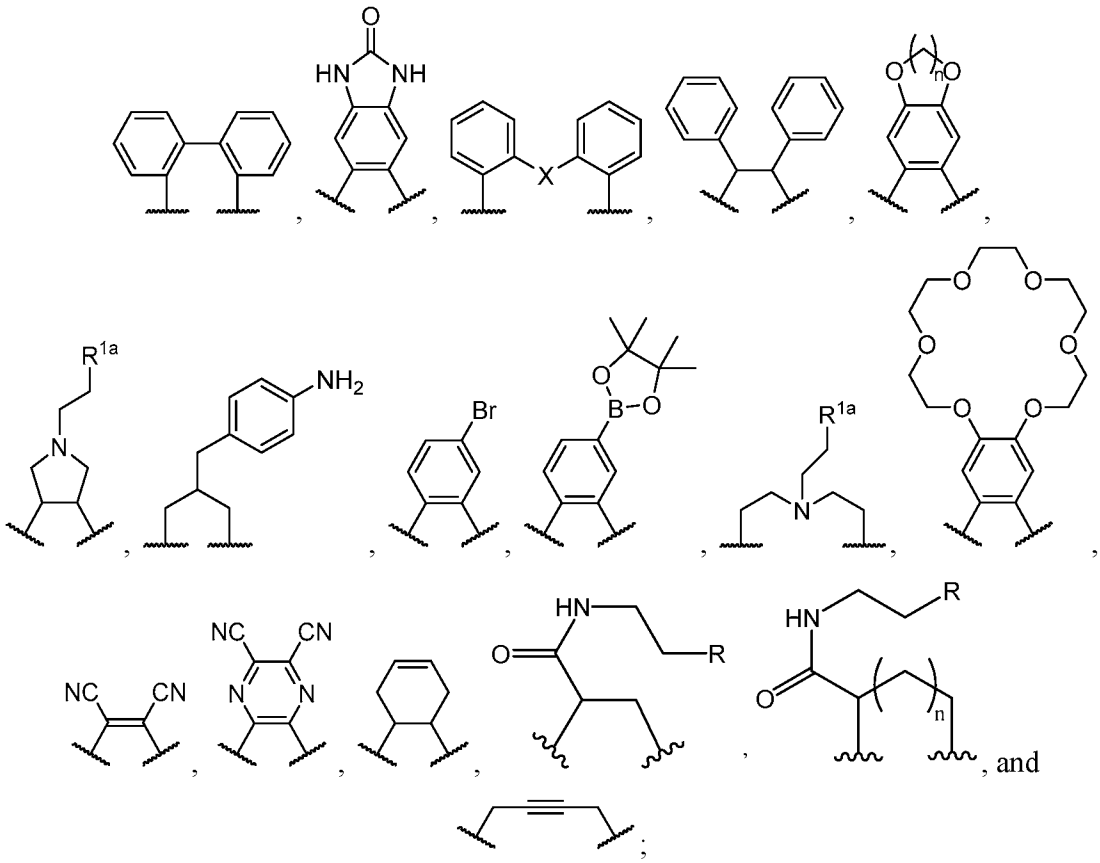
[0058] In a preferred embodiment, L^{1a} has the structure:



wherein R^{e2} and R^{e3} are as defined herein.

[0059] In some embodiments, L^{1a} is selected from:





wherein n is an integer selected from 0, 1, 2, 3, 4, 5, and 6. Each R^{1a} is independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, and a modifying moiety; X is O, S, or CH_2; and R is as defined herein.

[0060] In a preferred embodiment, L^{1a} is a member selected from 5 membered ring moieties and 6 membered ring moieties.

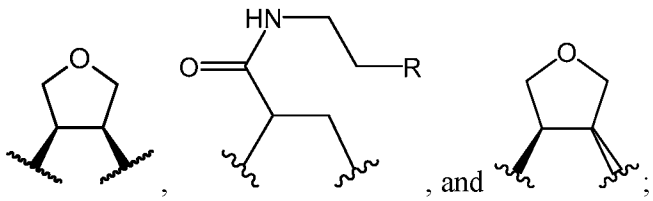
[0061] In another preferred embodiment, L^{1a} is a member selected from 5 membered ring moieties and 6 membered ring moieties, wherein the 5 membered ring moiety or the 6 membered ring moiety is part of a fused ring system.

[0062] In another preferred embodiment according to paragraph [0058], any implied hydrogens can be selected from substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl of 1, 2, 3, 4, 5, 6, 7, 8, 9 members selected from C or a heteroatom.

[0063] In some embodiments, any implicit hydrogen atom in the L^{1a} moieties shown above is optionally replaced by substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, or a modifying moiety.

[0064] In some embodiments, L^{1a} is not unsubstituted C_1, C_2, or linear C_3 alkyl. In some embodiments, L^{1a} is not an unsubstituted, linear alkyl. In some embodiments, L^{1a} is not unsubstituted alkyl.

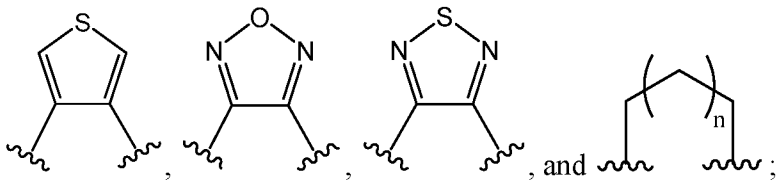
[0065] In a preferred embodiment, L^{1a} is selected from:



, , and ;

wherein R is as defined herein.

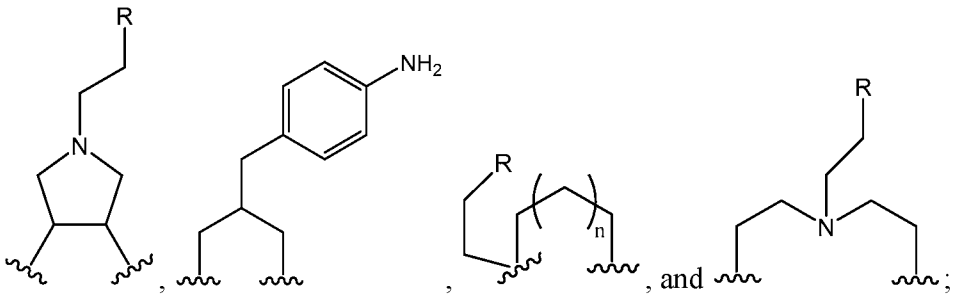
[0066] In another preferred embodiment, L^{1a} is selected from:



, , , , and ;

wherein n=0, 1, 2 or 3.

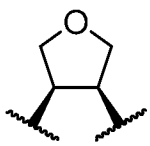
[0067] In another preferred embodiment, L^{1a} is selected from:



, , , and ;

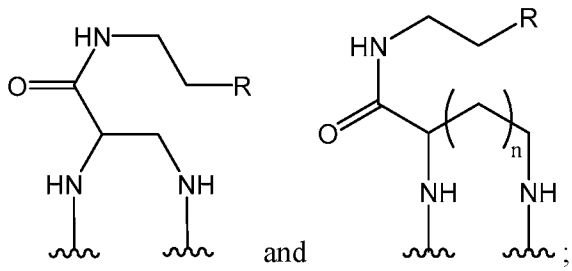
wherein n=0, 1, 2 or 3; and R is as defined herein.

[0068] In another preferred embodiment, L^{1a} has the structure:



.

[0069] In some embodiments, L^{1a} is selected from:



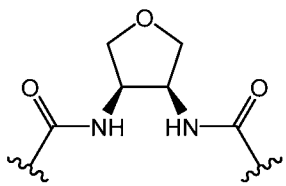
and ;

wherein n is an integer selected from 0, 1, 2, 3, 4, 5, and 6; and R is as defined herein.

[0070] In some embodiments, L^{1a} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety.

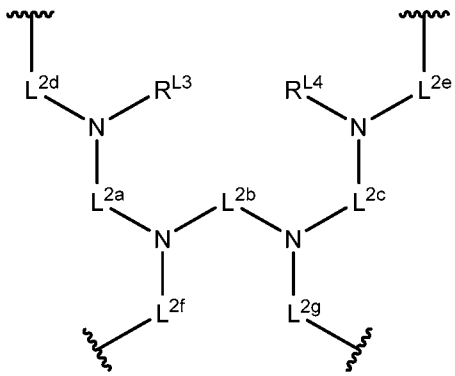
[0071] In some embodiments, L^{1b} and L^{1c} are independently selected from a bond, −C(O)−, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl.
In some embodiments, L^{1b} and L^{1c} are independently selected from a bond, −C(O)−, $-(CH_2)_aC(O)-$, and $-O(CH_2)_aC(O)-$; wherein a is an integer selected from 1, 2, 3, 4, 5, and 6.
In some embodiments, L^{1b} and L^{1c} are each −C(O)−.

[0072] In some embodiments, L^1 has the structure:



[0073] In some embodiments, R^{L1} and R^{L2} are independently selected from H, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl.
In some embodiments, R^{L1} and R^{L2} are each H.

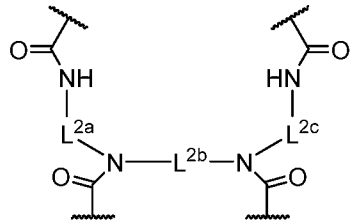
[0074] In some embodiments, L^2 has the structure:



wherein L^{2a}, L^{2b}, L^{2c}, L^{2d}, L^{2e}, L^{2f}, L^{2g}, R^{L3}, and R^{L4} are as defined herein. Any of the combinations of L^{2a}, L^{2b}, L^{2c}, L^{2d}, L^{2e}, L^{2f}, L^{2g}, R^{L3}, and R^{L4} are encompassed by this disclosure and specifically provided by the invention.

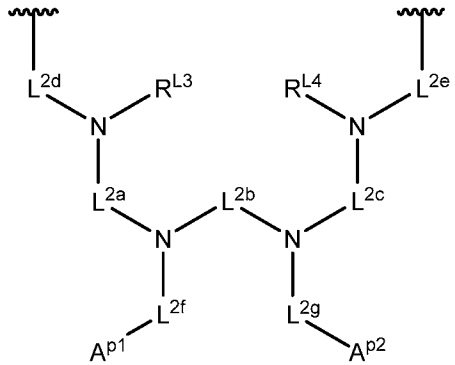
[0075] In some embodiments, L^2 is substituted with a linker to a reactive functional group, or a linker to a targeting moiety.

[0076] In some embodiments, L^2 has the structure:



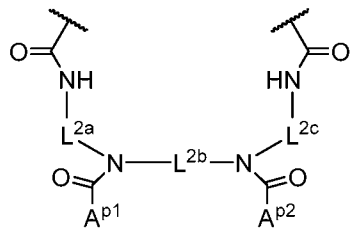
wherein L^{2a}, L^{2b}, and L^{2c} are as defined herein. Any of the combinations of L^{2a}, L^{2b}, and L^{2c} are encompassed by this disclosure and specifically provided by the invention.

[0077] In some embodiments, A^{p1}–L^2–A^{p2} has the structure:



wherein L^{2a}, L^{2b}, L^{2c}, L^{2d}, L^{2e}, L^{2f}, L^{2g}, R^{L3}, R^{L4}, A^{p1}, and A^{p2} are as defined herein. Any of the combinations of L^{2a}, L^{2b}, L^{2c}, L^{2d}, L^{2e}, L^{2f}, L^{2g}, R^{L3}, R^{L4}, A^{p1}, and A^{p2} are encompassed by this disclosure and specifically provided by the invention.

[0078] In some embodiments, A^{p1}–L^2–A^{p2} has the structure:



wherein L^{2a}, L^{2b}, L^{2c}, A^{p1}, and A^{p2} are as defined herein. Any of the combinations of L^{2a}, L^{2b}, L^{2c}, A^{p1}, and A^{p2} are encompassed by this disclosure and specifically provided by the invention.

[0079] In some embodiments, L^{2a}, L^{2b} and L^{2c} are independently selected from substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.
In some embodiments, L^{2a}, L^{2b} and L^{2c} are independently selected from substituted or unsubstituted C_1-C_8 alkyl.

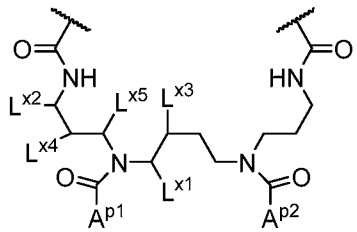
[0080] In some embodiments, L^{2a} and L^{2c} are independently selected from substituted or unsubstituted C_2, C_3 and C_4 alkyl; and L^{2b} is selected from substituted or unsubstituted C_2, C_3, C_4, and C_5 alkyl.

[0081] In some embodiments, one or more of L^{2a}, L^{2b} and L^{2c} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety. In some embodiments, L^{2a} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety. In some embodiments, L^{2b} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety. In some embodiments, L^{2c} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety.

[0082] In some embodiments, L^{2d}, L^{2e}, L^{2f} and L^{2g} are independently selected from a bond, $-C(O)-$, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl.
In some embodiments, L^{2d}, L^{2e}, L^{2f} and L^{2g} are independently selected from a bond, $-C(O)-$, $-(CH_2)_aC(O)-$, and $-O(CH_2)_aC(O)-$; wherein a is an integer selected from 1, 2, 3, 4, 5, and 6.
In some embodiments, L^{2d}, L^{2e}, L^{2f} and L^{2g} are each $-C(O)-$.

[0083] In some embodiments, R^{L3} and R^{L4} are independently selected from hydrogen, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl.
In some embodiments, R^{L3} and R^{L4} are each H.

[0084] In some embodiments, $A^{p1}-L^2-A^{p2}$ has the structure:

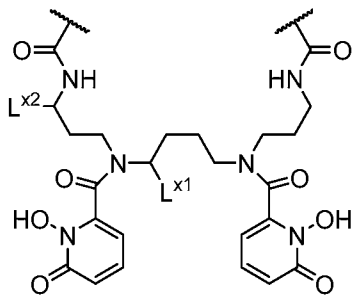


wherein L^{x1}, L^{x2}, L^{x3}, L^{x4}, and L^{x5} are as defined herein.

[0085] In some embodiments, L^{x1}, L^{x2}, L^{x3}, L^{x4}, L^{x5}, and L^{x6} are independently selected from H, a linker to a reactive functional group, and a linker to a targeting moiety. In some embodiments, at least one of L^{x1}, L^{x2}, L^{x3}, L^{x4}, L^{x5}, and L^{x6} is a linker to a reactive functional group, or a linker to a targeting moiety.

[0086] In some embodiments, L^{x1} is a linker to a reactive functional group, or a linker to a targeting moiety; and L^{x2}, L^{x3}, L^{x4}, L^{x5}, and L^{x6} are each H.
In some embodiments, L^{x2} is a linker to a reactive functional group, or a linker to a targeting moiety; and L^{x1}, L^{x3}, L^{x4}, L^{x5}, and L^{x6} are each H. In some embodiments, L^{x3} is a linker to a reactive functional group, or a linker to a targeting moiety; and L^{x1}, L^{x2}, L^{x4}, L^{x5}, and L^{x6} are each H. In some embodiments, L^{x4} is a linker to a reactive functional group, or a linker to a targeting moiety; and L^{x1}, L^{x2}, L^{x3}, L^{x5}, and L^{x6} are each H. In some embodiments, L^{x5} is a linker to a reactive functional group, or a linker to a targeting moiety; and L^{x1}, L^{x2}, L^{x3}, L^{x4}, and L^{x6} are each H. In some embodiments, L^{x6} is a linker to a reactive functional group, or a linker to a targeting moiety; and L^{x1}, L^{x2}, L^{x3}, L^{x4}, and L^{x5} are each H.

[0087] In some embodiments, A^{p1}–L^{2}–A^{p2} has the structure:

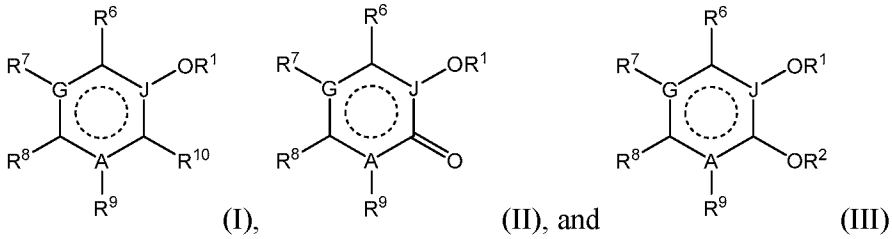


wherein L^{x1} and L^{x2} are as defined herein.

[0088] Precursors for the scaffold moeities, particularly L^{2}, can be synthesized as disclosed in WO 2016/106241 A1. The disclosure of which is incorporated herein by reference in its entirely.

Chelating Moieties

[0089] In some embodiments, A^{b1}, A^{b2}, A^{p1}, and A^{p2} are independently selected from:

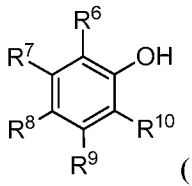
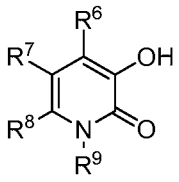
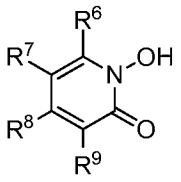
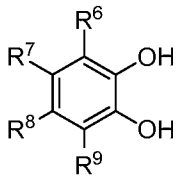
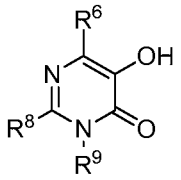
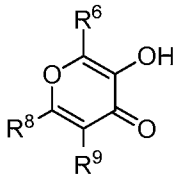


(I), (II), and (III)

wherein A and G are independently selected from carbon, nitrogen and oxygen; J is selected from carbon and nitrogen. Each R^1 and R^2 is independently selected from H, an enzymatically labile group, a hydrolytically labile group, a metabolically labile group, a photolytically labile group and a single negative charge. Each R^6, R^7, R^8, R^9, and R^{10} is independently selected from a bond to L^1 or L^2, alkanediyl attached to L^1 or L^2, H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, halogen, CN, $-CF_3$, $-C(O)R^{17}$, $-SO_2NR^{17}R^{18}$, $-NR^{17}R^{18}$, $-OR^{17}$, $-S(O)_2R^{17}$, $-COOR^{17}$, $-S(O)_2OR^{17}$, $-OC(O)R^{17}$, $-C(O)NR^{17}R^{18}$, $-NR^{17}C(O)R^{18}$, $-NR^{17}SO_2R^{18}$, and $-NO_2$, wherein at least two of R^6, R^7, R^8, R^9, and R^{10} are optionally joined to form a ring system selected from substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. R^{17} and R^{18} are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl; and R^{17} and R^{18}, together with the atoms to which they are attached, are optionally joined to form a 5-, 6- or 7-membered ring. When A is oxygen, R^9 is not present; and when G is oxygen, R^7 is not present. A^{b1} and A^{b2} are attached to L^1 and L^2 through two members selected from R^6, R^7, R^8, R^9 and R^{10}; and A^{p1} and A^{p2} are attached to L^2 through a member selected from R^6, R^7, R^8, R^9 and R^{10}.

[0090] In some embodiments, when A^{b1} has a structure according to formula (I), A^{b1} is attached to L^1 and L^2 through R^6 and R^{10}; when A^{b1} has a structure according to formula (II) or (III), A^{b1} is attached to L^1 and L^2 through R^6 and R^9; when A^{b2} has a structure according to formula (I), A^{b2} is attached to L^1 and L^2 through R^6 and R^{10}; when A^{b2} has a structure according to formula (II) or (III), A^{b2} is attached to L^1 and L^2 through R^6 and R^9; when A^{p1} has a structure according to formula (I), A^{p1} is attached to L^2 through R^6 or R^{10}; when A^{p1} has a structure according to formula (II) or (III), A^{p1} is attached to L^2 through R^6 or R^9; when A^{p2} has a structure according to formula (I), A^{p2} is attached to L^2 through R^6 or R^{10}; and when A^{p2} has a structure according to formula (II) or (III), A^{p2} is attached to L^2 through R^6 or R^9.

[0091] In some embodiments, A^{b1}, A^{b2}, A^{p1} and A^{p2} are each independently selected from:


(1);

(2a);

(2b);

(3);

(4); and

(5).

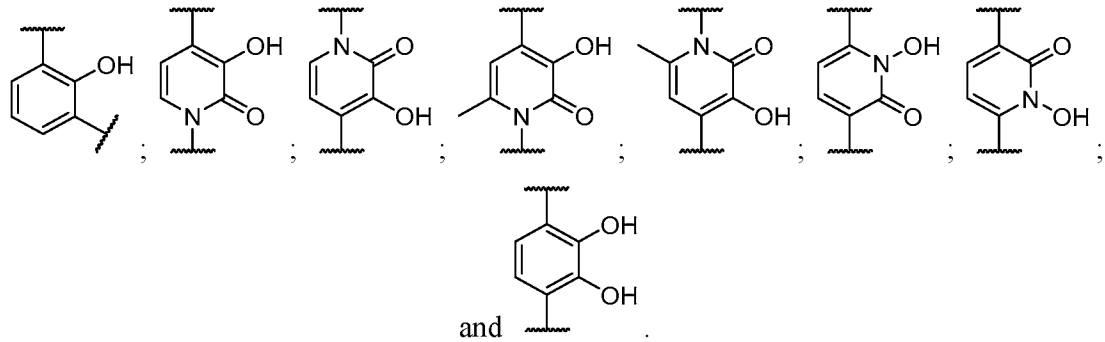
[0092] In some embodiments, when A^{b1} has a structure according to formula (1), A^{b1} is attached to L^1 and L^2 through R^6 and R^{10}; when A^{b1} has a structure according to formula (2a), (2b), (3), (4) or (5), A^{b1} is attached to L^1 and L^2 through R^6 and R^9; when A^{b2} has a structure according to formula (1), A^{b2} is attached to L^1 and L^2 through R^6 and R^{10}; when A^{b2} has a structure according to formula (2a), (2b), (3), (4) or (5), A^{b2} is attached to L^1 and L^2 through R^6 and R^9; when A^{p1} has a structure according to formula (1), A^{p1} is attached to L^2 through R^6 or R^{10}; and when A^{p1} has a structure according to formula (2a), (2b), (3), (4) or (5), A^{p1} is attached to L^2 through R^6 or R^9; when A^{p2} has a structure according to formula (1), A^{p2} is attached to L^2 through R^6 or R^{10}; and when A^{p2} has a structure according to formula (2a), (2b), (3), (4) or (5), A^{p2} is attached to L^2 through R^6 or R^9.

[0093] In a preferred embodiment, each A^{b1}, A^{b2}, A^{p1} and A^{p2} has a structure according to formula (2b).

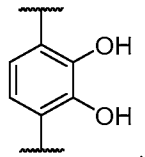
[0094] In another preferred embodiment, each A^{b1}, A^{b2}, A^{p1} and A^{p2} has a structure according to formula (2a).

[0095] In another preferred embodiment, each A^{b1}, A^{b2}, A^{p1} and A^{p2} has a structure according to formula (1).

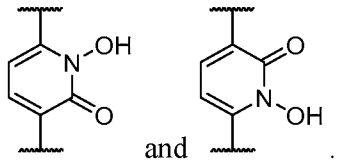
[0096] In some embodiments, A^{b1} and A^{b2} are each independently selected from:



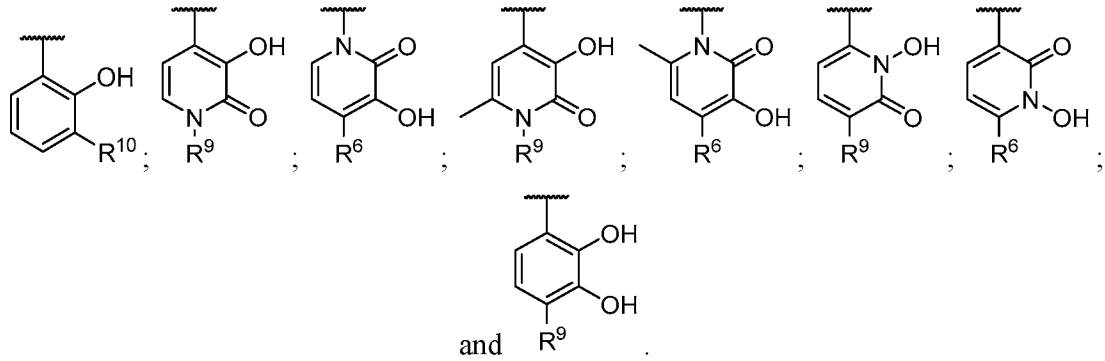
[0097] In some embodiments, a member selected from A^{b1} and A^{b2} and a combination thereof is not:



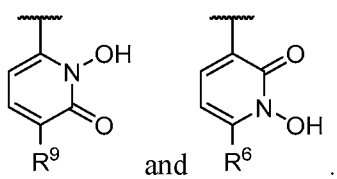
[0098] In some embodiments, A^{b1} and A^{b2} are each independently selected from:



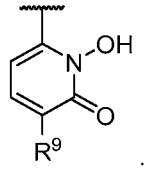
[0099] In some embodiments, A^{p1} and A^{p2} are each independently selected from:



[00100] In some embodiments, A^{p1} and A^{p2} are each independently selected from:



[00101] In some embodiments, A^{p1} and A^{p2} each are:



[00102] In some embodiments, one or both of A^{p1} and A^{p2} comprise a modifying moiety. Modifying moieties are as defined herein. In some embodiments, R^9 of A^{p1}, A^{p2}, or A^{p1} and A^{p2} comprises a modifying moiety. In some embodiments, R^9 of A^{p1}, A^{p2}, or A^{p1} and A^{p2} is $-C(O)NR^{17}R^{18}$, wherein R^{17} is H and R^{18} is a modifying moiety. In some embodiments, R^6 of A^{p1}, A^{p2}, or A^{p1} and A^{p2} comprises a modifying moiety. In some embodiments, R^6 of A^{p1}, A^{p2}, or A^{p1} and A^{p2} is $-C(O)NR^{17}R^{18}$, wherein R^{17} is H and R^{18} is a modifying moiety.

Linker to Functional / Targeting Moiety

[00103] A "linker", "linking member", or "linking moiety" as used herein is a moiety that joins or potentially joins, covalently or noncovalently, a first moiety to a second moiety. In particular, a linker attaches or could potentially attach a ligand described herein to another molecule, such as a targeting moiety. In some embodiments, a linker attaches or could potentially attach a ligand described herein to a solid support. A linker comprising a reactive functional group that can be further reacted with a reactive functional group on a structure of interest in order to attach the structure of interest to the linker is referred to as a "functionalized linker". In exemplary embodiments, a linker is a functionalized linker. In exemplary embodiments, a ligand comprises one or more functionalized linkers. In some embodiments, a linker comprises a targeting moiety. In some embodiments, a linker to a targeting moiety comprises a bond to the targeting moiety.
In some embodiments, the linker is a linker to a functional moiety, or a linker to a targeting moiety. In some embodiments, the functional moiety is a reactive functional group or a protected functional group. In some embodiments, the linker is a linker to a reactive functional group, or a linker to a targeting moiety.

[00104] A linker can be any useful structure for that joins a ligand to a reactive functional group or a targeting moiety, such as an antibody. Examples of a linker include 0-order linkers (i.e., a bond), substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl. Further

exemplary linkers include substituted or unsubstituted (C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9 or C_{10}) alkyl, substituted or unsubstituted heteroalkyl, -C(O)NR'-, -C(O)O-, -C(O)S-, and -C(O)CR'R'', wherein R' and R'' are members independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl. In some embodiments, a linker includes at least one heteroatom. Exemplary linkers also include -C(O)NH-, -C(O), -NH-, -S-, -O-, and the like. In an exemplary embodiment, a linker is a heteroalkyl substituted with a reactive functional group.

Reactive Functional Groups

[00105] In one embodiment, a linker comprises a reactive functional group (or a "reactive functional moiety", used synonymously), which can be further reacted to covalently attach the linker to a targeting moiety. Reactive functional groups and classes of reactions useful in practicing the present invention are generally those that are well known in the art of bioconjugate chemistry. Currently favored classes of reactions available with reactive functional groups of the invention are those which proceed under relatively mild conditions. These include, but are not limited to nucleophilic substitutions (e.g., reactions of amines and alcohols with acyl halides and activated esters), electrophilic substitutions (e.g., enamine reactions) and additions to carbon-carbon and carbon-heteroatom multiple bonds (e.g., Michael reactions and Diels-Alder reactions). These and other useful reactions are discussed, for example, in March, Advanced Organic Chemistry (3rd Ed., John Wiley & Sons, New York, 1985); Hermanson, Bioconjugate Techniques (Academic Press, San Diego, 1996); and Feeney et al., Modification of Proteins, Advances in Chemistry Series, Vol. 198 (American Chemical Society, Washington, D.C., 1982).

[00106] In some embodiments, a reactive functional group refers to a group selected from olefins, acetylenes, alcohols, phenols, ethers, oxides, halides, aldehydes, ketones, carboxylic acids, esters, amides, cyanates, isocyanates, thiocyanates, isothiocyanates, amines, hydrazines, hydrazones, hydrazides, diazo, diazonium, nitro, nitriles, mercaptans, sulfides, disulfides, sulfoxides, sulfones, sulfonic acids, sulfinic acids, acetals, ketals, anhydrides, sulfates, sulfenic acids isonitriles, amidines, imides, imidates, nitrones, hydroxylamines, oximes, hydroxamic acids thiohydroxamic acids, allenes, ortho esters, sulfites, enamines, ynamines, ureas, pseudoureas, semicarbazides, carbodiimides, carbamates, imines, azides, azo compounds, azoxy compounds, and nitroso compounds. Reactive functional groups also

include those used to prepare bioconjugates, e.g., N-hydroxysuccinimide esters, maleimides and the like. Methods to prepare each of these functional groups are well known in the art and their application or modification for a particular purpose is within the ability of one of skill in the art (*see*, for example, Sandler and Karo, eds., Organic Functional Group Preparations, (Academic Press, San Diego, 1989)).

[00107] A reactive functional group can be chosen according to a selected reaction partner. As an example, an activated ester, such as an NHS ester will be useful to label a protein *via* lysine residues. Sulfhydryl reactive groups, such as maleimides can be used to label proteins *via* amino acid residues carrying an SH-group (e.g., cystein). Antibodies may be labeled by first oxidizing their carbohydrate moieties (e.g., with periodate) and reacting resulting aldehyde groups with a hydrazine containing ligand.

[00108] The reactive functional groups can be chosen such that they do not participate in, or interfere with, the reactions necessary to assemble the reactive ligand. Alternatively, a reactive functional group can be protected from participating in the reaction by means of a protecting group. Those of skill in the art understand how to protect a particular functional group so that it does not interfere with a chosen set of reaction conditions. For examples of useful protecting groups, see, for example, Greene et al., PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York, 1991.

Amines and Amino-Reactive Groups

[00109] In one embodiment, a reactive functional group is selected from an amine, (such as a primary or secondary amine), hydrazine, hydrazide and sulfonylhydrazide. Amines can, for example, be acylated, alkylated or oxidized. Useful non-limiting examples of amino-reactive groups include N-hydroxysuccinimide (NHS) esters, sulfur-NHS esters, imidoesters, isocyanates, isothiocyanates, acylhalides, arylazides, p-nitrophenyl esters, aldehydes, sulfonyl chlorides, thiazolides and carboxyl groups.

[00110] NHS esters and sulfo-NHS esters react preferentially with primary (including aromatic) amino groups of a reaction partner. The imidazole groups of histidines are known to compete with primary amines for reaction, but the reaction products are unstable and readily hydrolyzed. The reaction involves the nucleophilic attack of an amine on the acid carboxyl of an NHS ester to form an amide, releasing the N-hydroxysuccinimide.

[00111] Imidoesters are the most specific acylating reagents for reaction with amine groups of a molecule such as a protein. At a pH between 7 and 10, imidoesters react only with primary amines. Primary amines attack imidates nucleophilically to produce an intermediate that breaks down to amidine at high pH or to a new imidate at low pH. The new imidate can react with another primary amine, thus crosslinking two amino groups, a case of a putatively monofunctional imidate reacting bifunctionally. The principal product of reaction with primary amines is an amidine that is a stronger base than the original amine. The positive charge of the original amino group is therefore retained. As a result, imidoesters do not affect the overall charge of the conjugate.

[00112] Isocyanates (and isothiocyanates) react with the primary amines of the conjugate components to form stable bonds. Their reactions with sulfhydryl, imidazole, and tyrosyl groups give relatively unstable products.

[00113] Acylazides are also used as amino-specific reagents in which nucleophilic amines of the reaction partner attack acidic carboxyl groups under slightly alkaline conditions, *e.g.* pH 8.5.

[00114] Arylhalides such as 1,5-difluoro-2,4-dinitrobenzene react preferentially with the amino groups and tyrosine phenolic groups of the conjugate components, but also with its sulfhydryl and imidazole groups.

[00115] p-Nitrophenyl esters of carboxylic acids are also useful amino-reactive groups. Although the reagent specificity is not very high, α- and ε-amino groups appear to react most rapidly.

[00116] Aldehydes react with primary amines of the conjugate components (*e.g.*, ε-amino group of lysine residues). Although unstable, Schiff bases are formed upon reaction of the protein amino groups with the aldehyde. Schiff bases, however, are stable, when conjugated to another double bond. The resonant interaction of both double bonds prevents hydrolysis of the Schiff linkage. Furthermore, amines at high local concentrations can attack the ethylenic double bond to form a stable Michael addition product. Alternatively, a stable bond may be formed by reductive amination.

[00117] Aromatic sulfonyl chlorides react with a variety of sites of the conjugate components, but reaction with the amino groups is the most important, resulting in a stable sulfonamide linkage.

[00118] Free carboxyl groups react with carbodiimides, soluble in both water and organic solvents, forming pseudoureas that can then couple to available amines yielding an amide linkage. Yamada et al., *Biochemistry*, 1981, 20: 4836-4842, e.g., teach how to modify a protein with carbodiimides.

Sulfhydryl and Sulfhydryl-Reactive Groups

[00119] In another embodiment, a reactive functional group is selected from a sulfhydryl group (which can be converted to disulfides) and sulfhydryl-reactive group. Useful non-limiting examples of sulfhydryl-reactive groups include maleimides, alkyl halides, acyl halides (including bromoacetamide or chloroacetamide), pyridyl disulfides, and thiophthalimides.

[00120] Maleimides react preferentially with the sulfhydryl group of the conjugate components to form stable thioether bonds. They also react at a much slower rate with primary amino groups and the imidazole groups of histidines. However, at pH 7 the maleimide group can be considered a sulfhydryl-specific group, since at this pH the reaction rate of simple thiols is 1000-fold greater than that of the corresponding amine.

[00121] Alkyl halides react with sulfhydryl groups, sulfides, imidazoles, and amino groups. At neutral to slightly alkaline pH, however, alkyl halides react primarily with sulfhydryl groups to form stable thioether bonds. At higher pH, reaction with amino groups is favored.

[00122] Pyridyl disulfides react with free sulfhydryl groups via disulfide exchange to give mixed disulfides. As a result, pyridyl disulfides are relatively specific sulfhydryl-reactive groups.

[00123] Thiophthalimides react with free sulfhydryl groups to also form disulfides.

Other Reactive Functional Groups

[00124] Other exemplary reactive functional groups include:

(i) carboxyl groups and various derivatives thereof including, but not limited to, N-hydroxybenztriazole esters, acid halides, acyl imidazoles, thioesters, p-nitrophenyl esters, alkyl, alkenyl, alkynyl and aromatic esters;

(ii) hydroxyl groups, which can be converted to esters, ethers, aldehydes, etc.;

(iii) haloalkyl groups, wherein the halide can be displaced with a nucleophilic group such as, for example, an amine, a carboxylate anion, thiol anion, carbanion, or an alkoxide ion, thereby resulting in the covalent attachment of a new group at the site of the halogen atom;

(iv) dienophile groups, which are capable of participating in Diels-Alder reactions such as, for example, maleimido groups;

(v) aldehyde or ketone groups, such that subsequent derivatization is possible via formation of carbonyl derivatives such as, for example, imines, hydrazones, semicarbazones or oximes, or via such mechanisms as Grignard addition or alkyllithium addition;

(vi) alkenes, which can undergo, for example, cycloadditions, acylation, Michael addition, etc;

(vii) epoxides, which can react with, for example, amines and hydroxyl groups;

(ix) phosphoramidites and other standard functional groups useful in nucleic acid synthesis and

(x) any other functional group useful to form a covalent bond between the functionalized ligand and a molecular entity or a surface.

Functional Groups with Non-specific Reactivities

[00125] In addition to the use of site-specific reactive moieties, the present invention contemplates the use of non-specific reactive groups to link a ligand to a targeting moiety. Non-specific groups include photoactivatable groups, for example.

[00126] Photoactivatable groups are ideally inert in the dark and are converted to reactive species in the presence of light. In one embodiment, photoactivatable groups are selected from precursors of nitrenes generated upon heating or photolysis of azides. Electron-deficient nitrenes are extremely reactive and can react with a variety of chemical bonds including N-H, O-H, C-H, and C=C. Although three types of azides (aryl, alkyl, and acyl derivatives) may be employed, arylazides are presently preferrred. The reactivity of arylazides upon photolysis is better with N-H and O-H than C-H bonds. Electron-deficient arylnitrenes rapidly ring-expand to form dehydroazepines, which tend to react with nucleophiles, rather than form C-H insertion products. The reactivity of arylazides can be

increased by the presence of electron-withdrawing substituents such as nitro or hydroxyl groups in the ring. Such substituents push the absorption maximum of arylazides to longer wavelength. Unsubstituted arylazides have an absorption maximum in the range of 260-280 nm, while hydroxy and nitroarylazides absorb significant light beyond 305 nm. Therefore, hydroxy and nitroarylazides are most preferable since they allow to employ less harmful photolysis conditions for the affinity component than unsubstituted arylazides.

[00127] In another preferred embodiment, photoactivatable groups are selected from fluorinated arylazides. The photolysis products of fluorinated arylazides are arylnitrenes, all of which undergo the characteristic reactions of this group, including C-H bond insertion, with high efficiency (Keana *et al., J. Org. Chem.* 55: 3640-3647, 1990).

[00128] In another embodiment, photoactivatable groups are selected from benzophenone residues. Benzophenone reagents generally give higher crosslinking yields than arylazide reagents.

[00129] In another embodiment, photoactivatable groups are selected from diazo compounds, which form an electron-deficient carbene upon photolysis. These carbenes undergo a variety of reactions including insertion into C-H bonds, addition to double bonds (including aromatic systems), hydrogen attraction and coordination to nucleophilic centers to give carbon ions.

[00130] In still another embodiment, photoactivatable groups are selected from diazopyruvates. For example, the p-nitrophenyl ester of p-nitrophenyl diazopyruvate reacts with aliphatic amines to give diazopyruvic acid amides that undergo ultraviolet photolysis to form aldehydes. The photolyzed diazopyruvate-modified affinity component will react like formaldehyde or glutaraldehyde forming intraprotein crosslinks.

[00131] In some embodiments, a linker joins a ligand to a reactive functional group. In exemplary embodiments, a linker joins a ligand to a targeting moiety. That is, in exemplary embodiments, a linker comprises a targeting moiety. In some embodiments, a ligand comprises a linker to a targeting moiety. Any linker described herein may be a linker comprising a reactive functional group that could react with a reactive functional group on a targeting moiety to join the linker to the targeting moiety. Any linker described herein may be a linker comprising a bond to a targeting moiety. The term "targeting moiety" refers to a moiety serves to target or direct the molecule to which it is attached (e.g., a ligand or a ligand complexed to a metal ion (such as a radionuclide)) to a particular location or molecule. Thus,

for example, a targeting moiety may be used to target a molecule to a specific target protein or enzyme, or to a particular cellular location, to a particular cell type or to a diseased tissue. As will be appreciated by those in the art, the localization of proteins within a cell is a simple method for increasing effective concentration. For example, shuttling an imaging agent and/or therapeutic into the nucleus confines them to a smaller space thereby increasing concentration. Finally, the physiological target may simply be localized to a specific compartment, and the agents must be localized appropriately.

[00132] The targeting moiety can be a small molecule (e.g., MW < 500D), which includes both non-peptides and peptides. Examples of a targeting moiety also include peptides, polypeptides (including proteins, and in particular antibodies, which includes antibody fragments), nucleic acids, oligonucleotides, carbohydrates, lipids, hormones (including proteinaceous and steroid hormones (for instance, estradiol)), growth factors, lectins, receptors, receptor ligands, cofactors and the like. Targets of a targeting moiety can include a complementary nucleic acid, a receptor, an antibody, an antigen or a lectin, for example.

[00133] In exemplary embodiments, a targeting moiety can bind to a target with high binding affinity. In other words, a targeting moiety with high binding affinity to a target has a high specificity for or specifically binds to the target. In some embodiments, a high binding affinity is given by a dissociation constant K_d of about 10^{-7} M or less. In exemplary embodiments, a high binding affinity is given by a dissociation constant K_d of about 10^{-8} M or less, about 10^{-9} M or less, about 10^{-10} M or less, about 10^{-11} M or less, about 10^{-12} M or less, about 10^{-13} M or less, about 10^{-14} M or less or about 10^{-15} M or less. A compound may have a high binding affinity for a target if the compound comprises a portion, such as a targeting moiety, that has a high binding affinity for the target.

[00134] In exemplary embodiments, a targeting moiety is an antibody. An "antibody" refers to a protein comprising one or more polypeptides substantially encoded by all or part of the recognized immunoglobulin genes. The recognized immunoglobulin genes, for example in humans, include the kappa (κ), lambda (λ) and heavy chain genetic loci, which together compose the myriad variable region genes, and the constant region genes mu (μ), delta (δ), gamma (γ), epsilon (ε) and alpha (α), which encode the IgM, IgD, IgG, IgE, and IgA isotypes respectively. Antibody herein is meant to include full length antibodies and antibody fragments, and may refer to a natural antibody from any organism, an engineered antibody or an antibody generated recombinantly for experimental, therapeutic or other purposes as

further defined below. Antibody fragments include Fab, Fab', $F(ab')_2$, Fv, scFv or other antigen-binding subsequences of antibodies and can include those produced by the modification of whole antibodies or those synthesized de novo using recombinant DNA technologies. The term "antibody" refers to both monoclonal and polyclonal antibodies. Antibodies can be antagonists, agonists, neutralizing, inhibitory or stimulatory.

[00135] While a targeting moiety may be appended to a ligand in order to localize the compound to a specific region in an animal, certain ligands have a natural affinity for cells, tissue, organs or some other part of the animal. For example, a ligand disclosed herein might have a natural or intrinsic affinity for bone. Thus, in some embodiments, a ligand does not comprise a targeting moiety or a linker to a targeting moiety. A ligand lacking a targeting moiety can be used in any method that does not require specific targeting.

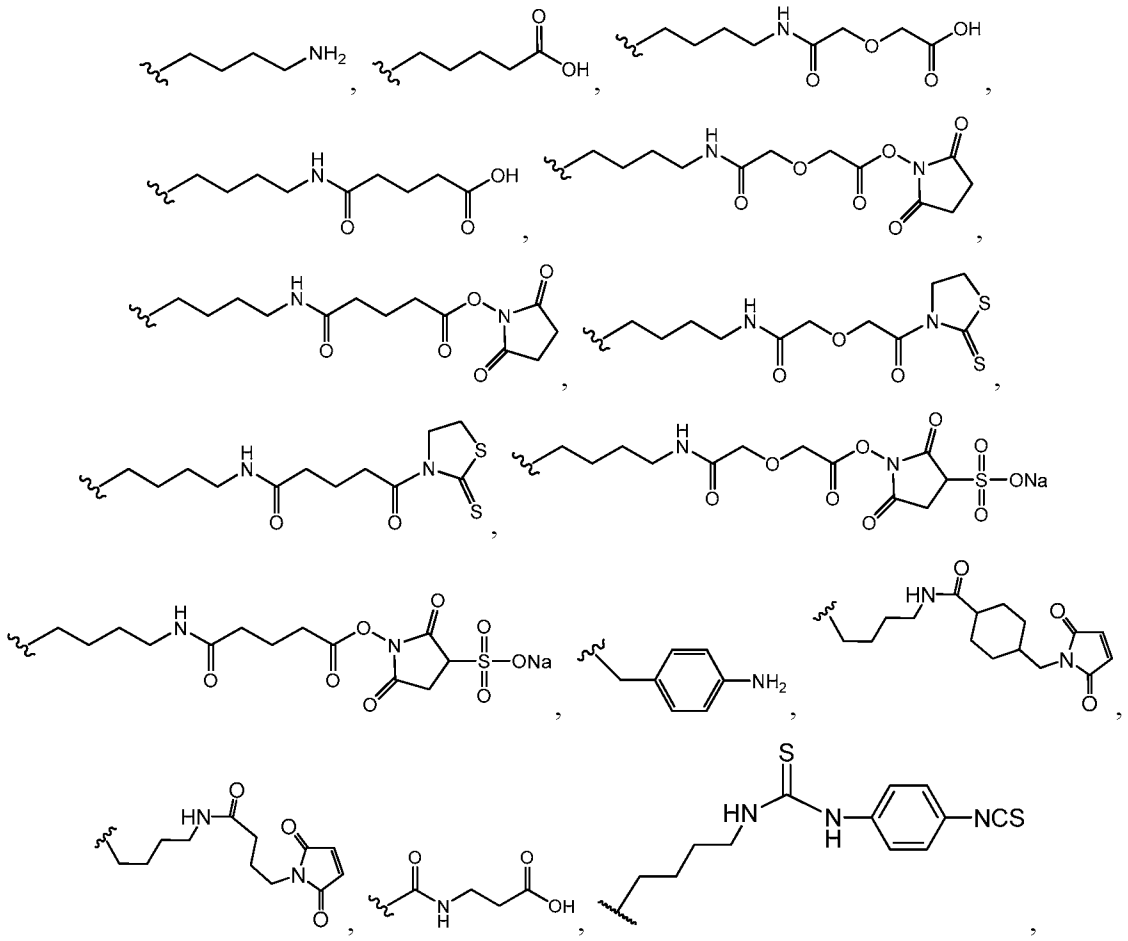
[00136] In some embodiments, a ligand comprises a linker to a solid support. That is, any linker described herein may be a linker comprising a reactive functional group that could react with a reactive functional group on a solid support to join the linker to the solid support. Any linker described herein may be a linker comprising a bond to a solid support. A "solid support" is any material that can be modified to contain discrete individual sites suitable for the attachment or association of a ligand. Suitable substrates include biodegradable beads, non-biodegradable beads, silica beads, magnetic beads, latex beads, glass beads, quartz beads, metal beads, gold beads, mica beads, plastic beads, ceramic beads, or combinations thereof. Of particular use are biocompatible polymers, including biodegradable polymers that are slowly removed from the system by enzymatic degradation. Example biodegradable materials include starch, cross-linked starch, poly(ethylene glycol), polyvinylpyrrolidine, polylactides (PLA), polyglycolides (PGA), poly(lactide-co-glycolides) (PLGA), polyanhydrides, polyorthoesters, poly(DTH iminocarbonate), poly(bisphenol A iminocarbonate), polycyanoacrylate, polyphosphazene, mixtures thereof and combinations thereof. Other suitable substances for forming the particles exist and can be used. In some embodiments, a solid support is a bead comprising a cross-linked starch, for example, cross-linked potato starch. Beads made from starch are completely biodegradable in the body, typically by serum amylase, a naturally occurring enzyme found in the body. In these embodiments, the ligand optionally further comprises a targeting moiety or a linker to a targeting moeity. In cases where a ligand that is attached to a solid support does not comprise a targeting moiety, the ligand can be localized directly by the practitioner, for example, by direct surgical implantation.

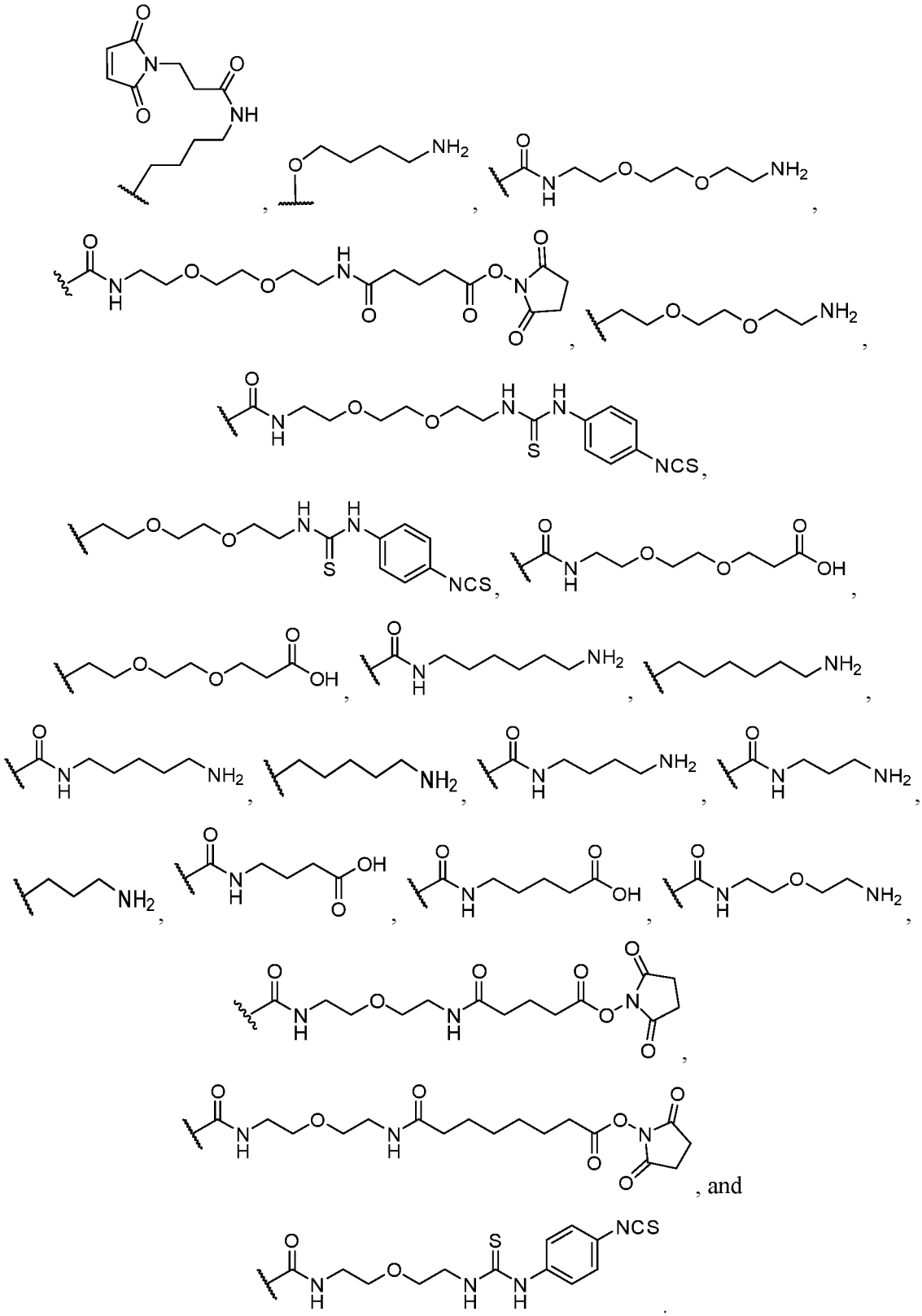
[00137] In some embodiments, a linker has the structure $-L^{11}-F^x$, wherein L^{11} is selected from a bond, acyl, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl; and F^x is selected from a reactive functional group, a protected functional group, or a targeting moiety.

[00138] In some embodiments, L^{11} is selected from substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl. In some embodiments, L^{11} is heteroalkyl. In some embodiments, L^{11} is (C_1, C_2, C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19} or C_{20}) alkyl in which 1, 2 or 3 atoms are replaced with a heteroatom, such as nitrogen or oxygen. In some embodiments, L^{11} comprises a modifying moiety.

[00139] In some embodiments, F^x is selected from $-NH_2$, -C(O)OH, alkyl ester (*e.g.*, methyl ester), N-hydroxysuccinimide (NHS) ester, sulfo-NHS ester, isothiocyanate, and maleimide. In some embodiments, F^x is selected from $-NH_2$ and -C(O)OH.

[00140] In some embodiments, $-L^{11}-F^x$ is selected from:

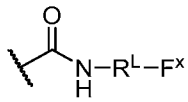




, and

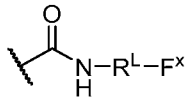
[00141] In a preferred embodiment according to paragraph [00139], any implied hydrogens can be selected from substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl of 1, 2, 3, 4, 5, 6, 7, 8, 9 members selected from C or a heteroatom.

[00142] In some embodiments, a linker has the structure:

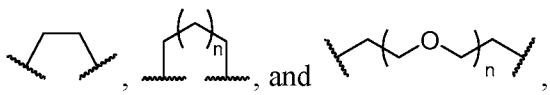


wherein R^L is seleceted from substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl; and F^x is as defined herein. In some embodiments, R^L is a substituted or unsubstituted alkoxyalkyl. In some embodiments, R^L is a substituted or unsubstituted monoether. In some embodiments, R^L is a substituted or unsubstituted polyether. In some embodiments, the polyether has from 2 to 10 (*i.e.*, 2, 3, 4, 5, 6, 7, 8, 9, or 10) ether groups. In some embodiments, R^L comprises a modifying moiety.

[00143] In some embodiments, a linker has the structure:

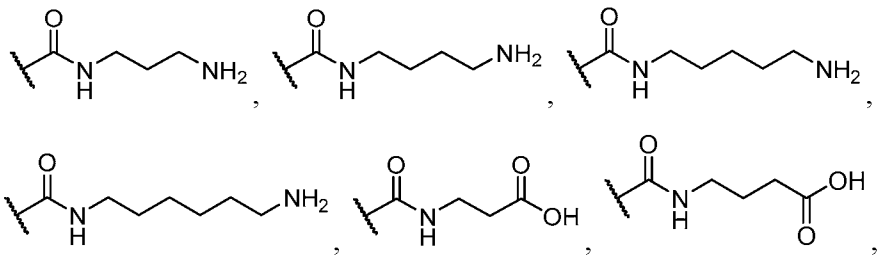


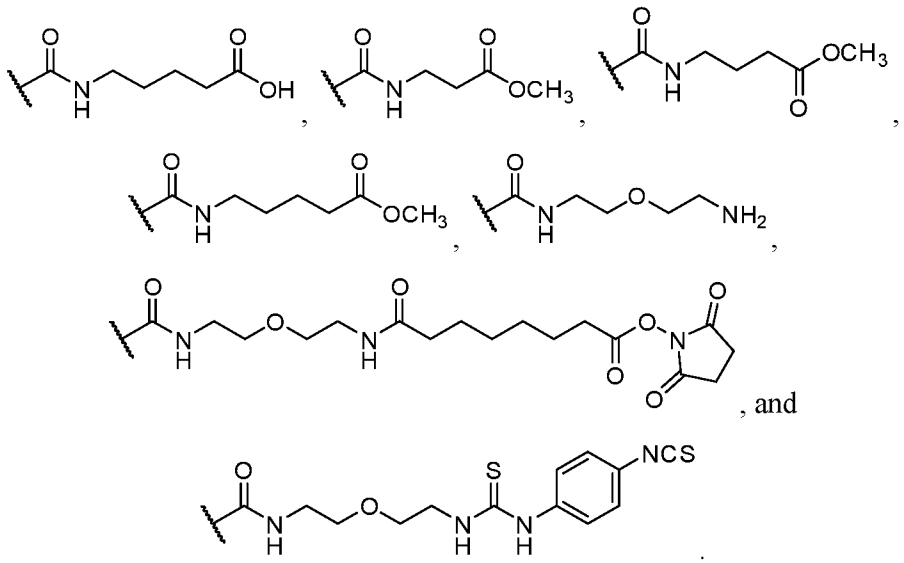
wherein R^L is selected from:



wherein n is an integer selected from 1, 2, 3, 4, 5, and 6; and F^x is a reactive functional group (such as NH_2) or a protected functional group.

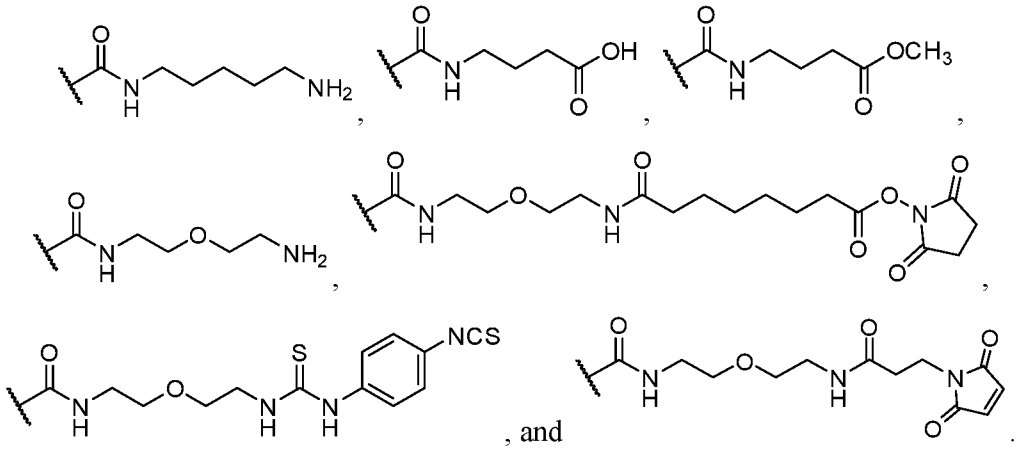
[00144] In some embodiments, a linker has a structure selected from:





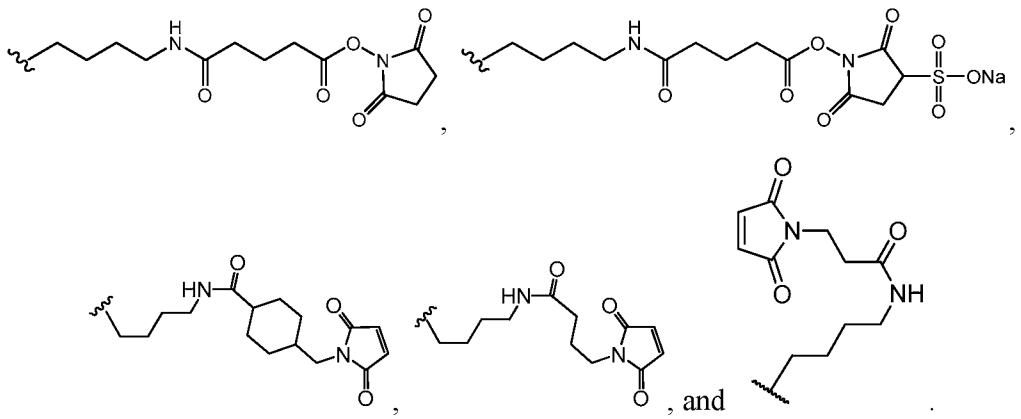
, and

[00145] In a preferred embodiment, a linker has a structure selected from:



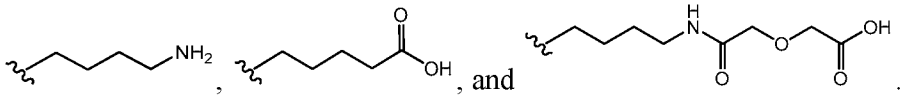
, and

[00146] In another preferred embodiment, a linker has a structure selected from:



, and

[00147] In another preferred embodiment, a linker has a structure selected from:



, , and .

[00148] In exemplary embodiments, F^x is a targeting moiety.

[00149] In exemplary embodiments, a linker is a linker to a targeting moiety. In some embodiments, the targeting moiety is selected from a polypeptide, a nucleic acid, a lipid, a polysaccharide, a small molecule, a cofactor and a hormone. In exemplary embodiments, the targeting moiety is an antibody or antibody fragment.

[00150] In a linker with multiple reactive functional groups, a particular functional group can be chosen such that it does not participate in, or interfere with, the reaction controlling the attachment of the functionalized spacer component to another ligand component. Alternatively, the reactive functional group can be protected from participating in the reaction by the presence of a protecting group. Those of skill in the art understand how to protect a particular functional group from interfering with a chosen set of reaction conditions. For examples of useful protecting groups, *See* Greene *et al.*, PROTECTIVE GROUPS IN ORGANIC SYNTHESIS, John Wiley & Sons, New York, 1991.

Modifying Moiety

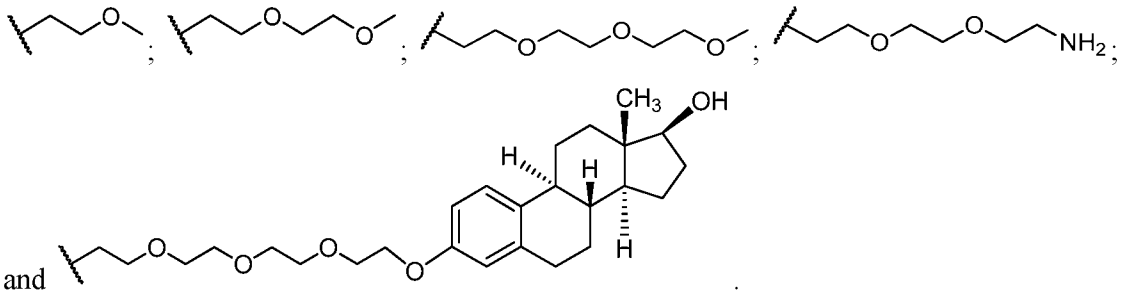
[00151] In some embodiments, the compound (ligand) comprises one or more a modifying moieties. In some embodiments, one or more of L^1, L^2, A^{b1}, A^{b2}, A^{p1}, and A^{p2} comprise(s) a modifying moiety. In some embodiments, one or more of L^{1a}, L^{1b}, L^{1c}, R^{L1}, and R^{L2}, L^{2a}, L^{2b}, L^{2c}, L^{2d}, L^{2e}, L^{2f}, L^{2g}, R^{L3}, R^{L4}, A^{b1}, A^{b2}, A^{p1}, and A^{p2} comprise(s) a modifying moiety. In some embodiments, a linker to a reactive functional group, or a linker to a targeting moiety comprises a modifying moiety. Each of the modifying moieties can be the same or different.

[00152] The modifying moiety modifies various properties of the ligand and/or a complex formed between the ligand and a metal ion, such as solubility, charge, or affinity. In some embodiments, the modifying moiety does not interact with the metal when the ligand is complexed to a metal. In some embodiments, the modifying moiety is a solubilizing group, a hormone-derived moiety, a prodrug moiety (for example, with a cleavable moiety), an oligonucleotide, ssDNA, dsDNA, RNA, or a peptide. The solubilizing group improves solubility of the ligand and/or a complex formed between the ligand and a metal ion in aqueous media. In some embodiments, the hormone (of the homone-derived moiety) is a steroid. In some embodiments, the steroid is estradiol. In some embodiments, the modifying

moiety is an estradiol-derived moiety. Peptides of a hydrophilic and hydrophobic nature by virtue of their amino acid composition may be used to tune solubility of the ligand and/or a complex formed between the ligand and a metal ion.

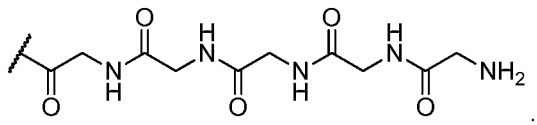
[00153] In some embodiments, the modifying moiety is substituted or unsubstituted heteroalkyl. In some embodiments, the modifying moiety is a substituted or unsubstituted alkoxyalkyl. In some embodiments, the modifying moiety is a substituted or unsubstituted monoether. In some embodiments, the modifying moiety is a substituted or unsubstituted polyether. In some embodiments, the modifying moiety comprises an estradiol-derived moiety. In some embodiments, the modifying moiety is a polyether substituted with an estradiol-derived moiety.

[00154] In some embodiments, the modifying moiety is selected from:



and .

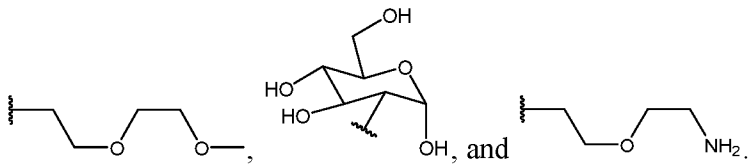
[00155] In some embodiments, the modifying moiety is a peptide. In some embodiments, the modifying moiety is:



.

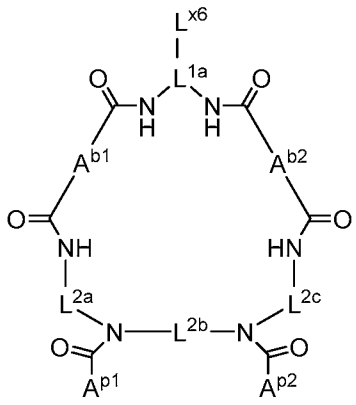
[00156] In some embodiments, the modifying moiety comprises an oligunucleotide.

[00157] In some embodiments, the modifying moiety is selected from:



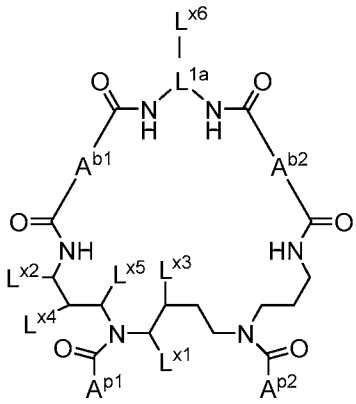
Exemplary Ligands

[00158] In some embodiments, the invention provides a ligand having the structure:



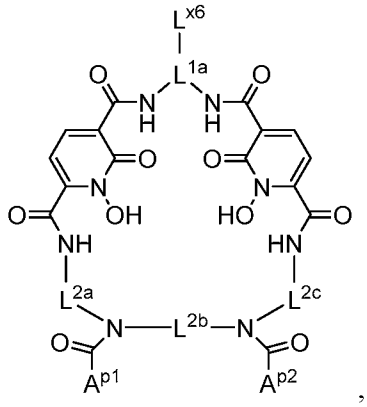
wherein L^{1a}, L^{2a}, L^{2b}, L^{2c}, L^{x6}, A^{b1}, A^{b2}, A^{p1}, and A^{p2} are as defined herein.

[00159] In some embodiments, the invention provides a ligand having the structure:



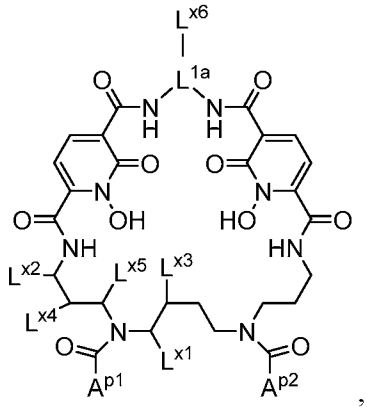
wherein L^{1a}, L^{x1}, L^{x2}, L^{x3}, L^{x4}, L^{x5}, L^{x6}, A^{b1}, A^{b2}, A^{p1}, and A^{p2} are as defined herein.

[00160] In some embodiments, the invention provides a ligand having the structure:



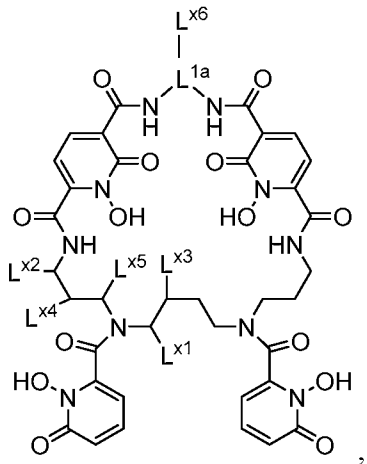
wherein L^{1a}, L^{2a}, L^{2b}, L^{2c}, L^{x6}, A^{p1}, and A^{p2} are as defined herein.

[00161] In some embodiments, the invention provides a ligand having the structure:



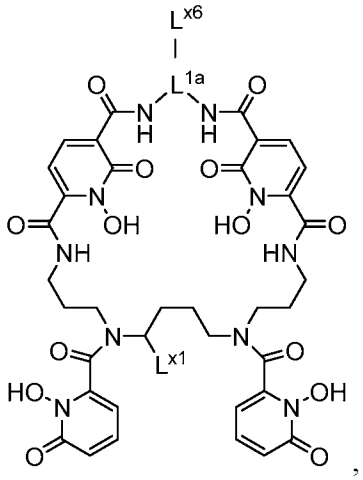
wherein L^{1a}, L^{x1}, L^{x2}, L^{x3}, L^{x4}, L^{x5}, L^{x6}, A^{p1}, and A^{p2} are as defined herein.

[00162] In some embodiments, the invention provides a ligand having the structure:



wherein L^{1a}, L^{x1}, L^{x2}, L^{x3}, L^{x4}, L^{x5} and L^{x6} are as defined herein.

[00163] In some embodiments, the invention provides a ligand having the structure:



wherein L^{1a}, L^{x6}, and L^{x1} are as defined herein.

[00164] Additional exemplary ligands are shown in the Examples.

Complexes

[00165] In one aspect, the invention provides a complex of a compound (ligand) disclosed herein with a metal ion.

[00166] Any of the combinations of compounds (ligands) disclosed herein and a metal ion disclosed herein are encompassed by this disclosure and specifically provided by the invention.

[00167] In some embodiments, the complex is luminescent.

[00168] Exemplary complexes are shown in the Examples.

[00169] In another aspect, the invention provides a complex of a compound (ligand) disclosed herein with an element, or ion thereof, from periods 4, 5, 6 and 7 and/or from groups 13, 14, 15, 16. In another aspect, the invention provides a complex of a compound (ligand) disclosed herein with an element, or ion thereof, from periods 3, 4, 7, 8, 9, 10, 11, 13,

14, and 15. In some embodiments, the invention provides a complex of a compound (ligand) disclosed herein with an element, or ion thereof, from periods 3, 4, and 13.

[00170] In some embodiments, complexes disclosed in WO 2013/187971 A2 are excluded.

Metals

[00171] In some embodiments, the metal is an actinide. In some embodiments, the actinide is thorium (Th). In some embodiments, the metal is a lanthanide. In some embodiments, the lanthanide is terbium (Tb). In some embodiments, the lanthanide is europium (Eu). In some embodiments, the lanthanide is dysprosium (Dy). In some embodiments, the lanthanide is lutetium (Lu). In some embodiments, the lanthanide is gadolinium (Gd). In some embodiments the metal is yttrium (Y). In some embodiments, the metal is zirconium (Zr). In some embodiments, the metal ion is yttrium(III). In some embodiments, the metal ion is europium(III). In some embodiments, the metal ion is terbium(III). In some embodiments, the metal ion is zirconium(IV). In some embodiments, the metal ion is thorium(IV). In some embodiments, the metal ion is selected from Th^{4+}, Zr^{4+}, Eu^{3+}, Dy^{3+}, Tb^{3+}, Lu^{3+}, and Y^{3+}. In some embodiments, the metal (ion) is a radionuclide. In some embodiments, the metal ion is ^{227}Th(IV). In some embodiments, the metal ion is ^{89}Zr(IV).

[00172] In some embodiments, the metal is ^{177}Lu. In some embodiments, the metal is ^{166}Ho. In some embodiments, the metal is ^{153}Sm. In some embodiments, the metal is ^{90}Y. In some embodiments, the metal is ^{86}Y. In some embodiments, the metal is ^{166}Dy. In some embodiments, the metal is ^{165}Dy. In some embodiments, the metal is ^{169}Er. In some embodiments, the metal is ^{175}Yb. In some embodiments, the metal is ^{225}Ac. In some embodiments, the metal is ^{149}Tb. In some embodiments, the metal is ^{153}Gd. In some embodiments, the metal is ^{230}U.

[00173] In some embodiments, the metal is ^{111}In. In some embodiments, the metal is ^{67}Ga. In some embodiments, the metal is ^{67}Cu. In some embodiments, the metal is ^{64}Cu. In some embodiments, the metal is ^{186}Re. In some embodiments, the metal is ^{188}Re. In some embodiments, the metal is ^{111}Ag. In some embodiments, the metal is ^{109}Pd. In some embodiments, the metal is ^{212}Pb. In some embodiments, the metal is ^{203}Pb. In some embodiments, the metal is ^{212}Bi. In some embodiments, the metal is ^{213}Bi. In some embodiments, the metal is ^{195m}Pt. In some embodiments, the metal is ^{201}Tl. In some embodiments, the metal is ^{55}Co. In some embodiments, the metal is ^{99m}Tc.

[00174] In some embodiments, the metal is selected from yttrium (Y), a lanthanoid, an actinoid, zirconium (Zr), iron (Fe), and indium (In). In some embodiments, the metal is selected from zirconium (Zr), iron (Fe), indium (In), europium (Eu), holmium (Ho), lutetium (Lu), yttrium (Y), terbium (Tb), ytterbium (Yb), gadolinium (Gd), samarium (Sm), dysprosium (Dy), erbium (Er), and thorium (Th). In some embodiments, the metal is selected from Eu, Tb, Sm, and Dy. In some embodiments, the metal is Gd.

[00175] In some embodiments, the metal ion is selected from Zr(IV), Fe(III), Ga(III), In(III), Eu(III), Ho(III), Lu(III), Y(III), Tb(III), Yb(III), Gd(III), Sm(III), Dy(III), Er(III), and Th(IV). In some embodiments, the metal ion is selected from ^{227}Th(IV), ^{89}Zr(IV), and ^{177}Lu(III).

[00176] In some embodiments, the metal is a radionuclide.

Radionuclides

[00177] The chelating moieties disclosed herein can be used to bind metal ions, in particular, a radionuclide. The term "radionuclide" or "radioisotope" refers to a radioactive isotope or element with an unstable nucleus that tends to undergo radioactive decay. Numerous decay modes are known in the art and include alpha decay, proton emission, neutron emission, double proton emission, spontaneous fission, cluster decay, β^- decay, positron emission (β^+ decay), electron capture, bound state beta decay, double beta decay, double electron capture, electron capture with positron emission, double positron emission, isomeric transition and internal conversion.

[00178] Exemplary radionuclides include alpha-emitters, which emit alpha particles during decay. In some embodiments, a radionuclide is an emitter of a gamma ray or a particle selected from an alpha particle, an electron and a positron.

[00179] In some embodiments, the radionuclide is an actinide. In some embodiments, the radionuclide is a lanthanide. In some embodiments, the radionuclide is a 3^+ ion. In some embodiments, the radionuclide is a 4^+ ion. In some embodiements the radionuclide is a 2^+ ion.

[00180] Of particular use in the complexes provided herein are radionuclides selected from isotopes of U, Pu, Fe, Cu, Sm, Gd, Tb, Dy, Ho, Er, Yb, Lu, Y, Th, Zr, In, Ga, Bi, Ra, At and Ac. In some embodiments, a radionuclide is selected form radium-223, thorium-227, astatine-211, bismuth-213, Lutetium-177, and actinium-225. Other useful radioisotopes include

bismuth-212, iodine-123, copper-64, iridium-192, osmium-194, rhodium-105, samarium-153, and yttrium-88, yttrium-90, and yttrium-91. In exemplary embodiments, the radionuclide is thorium, particularly selected from thorium-227 and thorium-232. In some embodiments, thorium-226 is excluded. In some embodiments, U is excluded. In some embodiments, uranium-230 is excluded. That is, in some embodiments, a radionuclide is not U, or a radionuclide is not uranium-230 or a radionuclide is not thorium-226.

[00181] In a preferred embodiment, the radionuclide is selected from Th(IV)-227, Zr(IV)-89, Lu(III)-177, Y(III)-90, Y(III)-86, and In(III)-111.

[00182] In another preferred embodiment, the radionuclide is Ac(III)-225.

[00183] In some embodiments, the radionuclide is selected from Tb(III)-149, Sc(III)-47, Dy(III)-166, Er(III)-169, Gd(III)-153, Ho(III)-166, Sm(III)-153, Yb(III)-175, Ac(III)-225, Bi(III)-212 and Bi(III)-213.

[00184] In a preferred embodiment, the complex is luminescent and comprises a metal ion which is selected from Tb(III), Eu(III), Sm(III), Dy(III), and Yb(III).

[00185] ^{232}Th exists in nature as an α-emitter with a half life of 1.4×10^{10} yr. In aqueous solution, Th(IV) is the only oxidation state. Thorium(IV) ion is bigger than Pu(IV) and usually forms complexes with 9 or higher coordination number. For example, the crystal structure of both Th(IV) complexes of simple bidentate 1,2-HOPO and Me-3,2-HOPO have been determined as nine coordinated species.

[00186] Similar to other actinide ions, thorium(IV) prefers forming complexes with oxygen, especially negative oxygen donor ligands. Thorium(IV) also prefers octadentate or higher multidentate ligands:

Ligand	**Acac**	**NTA**	**HEDTA***	**EDTA****	**DTPA**	**TTHA**
Ligand Type	Bi-dentate	Tetra-	Hexa-	Hexa-	Octa-	Deca-
Log K_1	**7.85**	**16.9**	**18.5**	**25.3**	**30.34**	**31.9**

*with one alcoholic oxygen and three carboxyl groups; **with four carboxyl groups.

[00187] Other radionuclides with diagnostic and therapeutic value that can be used with the compounds disclosed herein can be found, for example, in U.S. Patent Nos. 5,482,698 and 5,601,800; and Boswell and Brechbiel, Nuclear Medicine and Biology, 2007 October, 34(7): 757-778 and the manuscript thereof made available in PMC 2008 October 1.

Uses

[00188] The ligands and complexes disclosed herein can be used in a wide variety of therapeutic and diagnostic settings.

[00189] In one aspect, the invention provides a method of treating a disease in an animal comprising administering a complex disclosed herein to the animal, whereby the disease is ameliorated or eliminated.

[00190] In one aspect, the invention provides a method of diagnosing a disease in an animal comprising (a) administering a complex disclosed herein to the animal and (b) detecting the presence or absence of a signal emitted by the complex. In some embodiments, the detecting step comprises obtaining an image based on the signal.

[00191] In some embodiments, the disease is cancer.

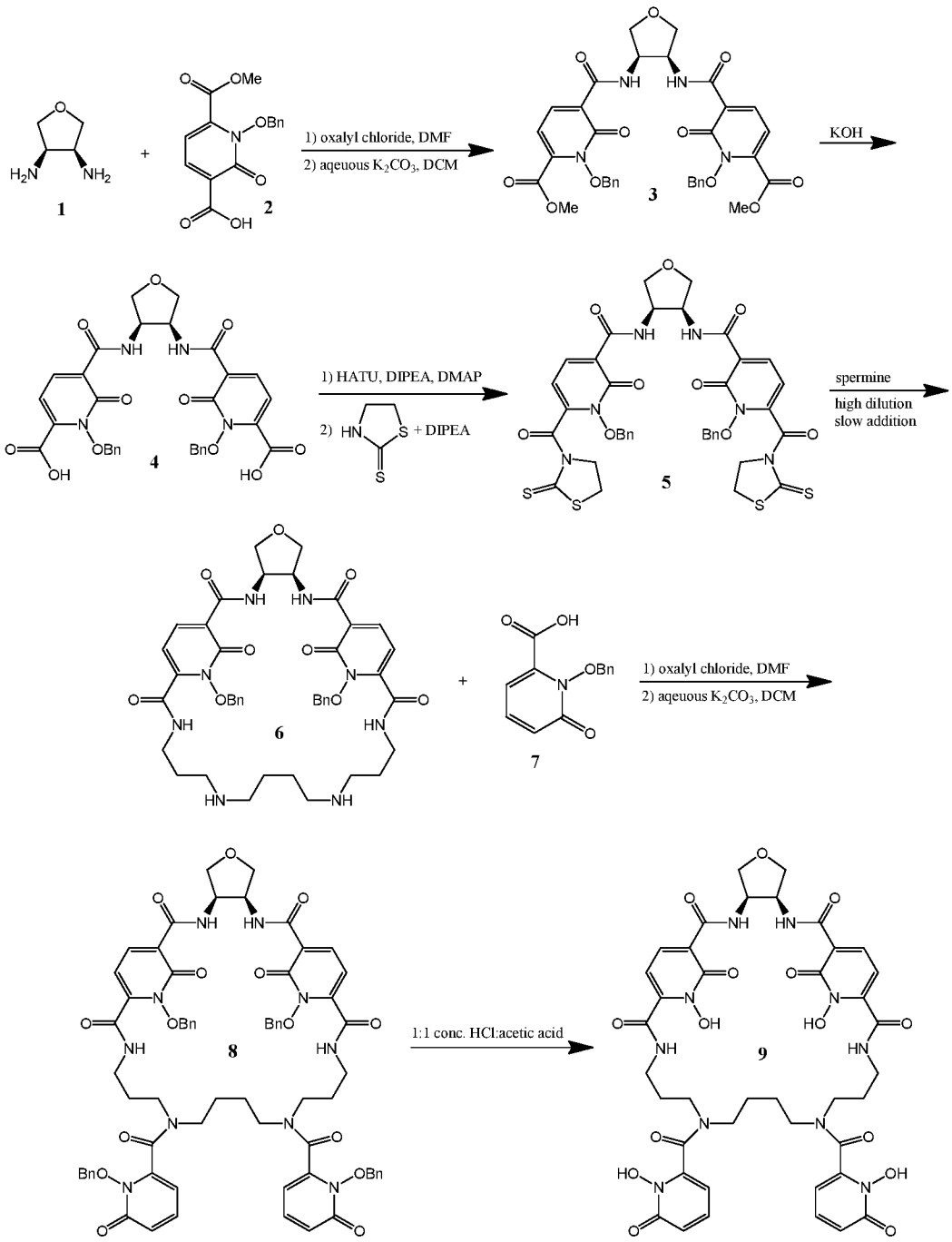
[00192] In some embodiments, the complex comprises a linker to a targeting moiety and the method further comprises localizing the complex to a targeting site in the animal by binding the targeting moiety to the targeting site.

[00193] The compounds disclosed herein are particularly well suited for the preparation of stable, pre-labeled antibodies for use in the diagnosis and treatment of cancer and other diseases. For example, antibodies expressing affinity for specific tumors or tumor-associated antigens are labeled with a diagnostic radionuclide-complexed chelate, and the labeled antibodies can be further stabilized through lyophilization. Where a chelate is used, it generally is covalently attached to the antibody. The antibodies used can be polyclonal or monoclonal, and the radionuclide-labeled antibodies can be prepared according to methods known in the art. The method of preparation will depend upon the type of radionuclide and antibody used. A stable, lyophilized, radiolabeled antibody can be reconstituted with suitable diluent at the time of intended use, thus greatly simplifying the on site preparation process. The methods of the invention can be applied to stabilize many types of pre-labeled antibodies, including, but not limited to, polyclonal and monoclonal antibodies to tumors associated with melanoma, colon cancer, breast cancer, prostate cancer, etc. Such antibodies are known in the art and are readily available.

EXAMPLES

[00194] The compounds and complexes of the invention are synthesized by an appropriate combination of generally well-known synthetic methods. Techniques useful in synthesizing the compounds of the invention are both readily apparent and accessible to those of skill in the relevant art. The discussion below is offered to illustrate certain of the diverse methods available for use in assembling the compounds of the invention, but it is not intended to limit the scope of reactions or reaction sequences that are useful in preparing the compounds of the present invention.

EXAMPLE 1. Synthesis of an exemplary parent ligand:



Scheme 1. Synthetic scheme for parent compound **9**

General Methods.

[00195] The precursors (3R,4S)-tetrahydrofuran-3,4-diamine (**1**), 1-(benzyloxy)-6-(methoxycarbonyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid (**2**), 1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxylic acid (**7**), and tert-butyl (2-(2-(2,5-bis((3-

aminopropyl)amino)pentanamido)ethoxy)ethyl)carbamate (**10**) were synthesized according to previously reported methods. All other solvents and reagents were purchased from commercial sources and used as received unless otherwise noted. ^{1}H-NMR and ^{13}C-NMR spectra were obtained at 600/150 MHz or 500/125 MHz using either a Bruker AV-600 or DRX-500 spectrometers as noted below. ^{1}H (or ^{13}C) chemical shifts are reported relative to residual solvent signals, taken as 7.24 (77.23) and 2.50 (39.51) ppm for $CDCl_3$ and DMSO-d_6 respectively. High resolution electrospray ionization mass spectra (HRMS-ESI) were performed by the Microanalytical Laboratory at the University of California, Berkeley.

[00196] Dimethyl 5,5'-((((3R,4S)-tetrahydrofuran-3,4-diyl)bis(azanediyl))bis(carbonyl))bis(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxylate) (**3**). Oxalyl chloride (4.10 g, 32.3 mmol) was added to a suspension of **2** (4.10 g, 13.5 mmol) in dichloromethane (40 mL), followed by 1 drop of dry DMF. The solution became homogenous within 30 min, and the reaction was stirred at room temperature for 3 hours total. The solvent was then removed under vacuum overnight. The residue was dissolved into dichloromethane (20 mL) and added dropwise to a solution of **1** (580 mg, 5.7 mmol) dissolved in dichloromethane (20 mL) and 40% aqueous K_2CO_3 (20 mL) at 0 °C with vigorous stirring. The reaction was stirred with warming to room temperature overnight. The dichloromethane layer was loaded directly onto a 4 inch tall x 1 inch wide silica gel column. Following a methanol/dichloromethane gradient elution, the desired product was collected using 2.5% methanol in dichloromethane. The solvent was removed under vacuum to yield the desired product as a hardened glass. Recrystallization from methanol followed by filtration and washing with 2-propanol yielded a free flowing white powder of **3**. Yield: 3.2 g, 83%. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.16 (s, 2H), 7.73 (d, J = 7.3 Hz, 2H), 7.36 – 7.29 (m, 6H), 7.28 – 7.23 (m, 4H), 6.07 (d, J = 7.2 Hz, 2H), 5.22 (d, J = 8.2 Hz, 2H), 5.02 (d, J = 7.8 Hz, 2H), 4.95 – 4.85 (m, 2H), 4.09 (dd, J = 9.0, 6.3 Hz, 2H), 3.73 (dd, J = 8.5, 4.2 Hz, 2H), 3.67 (s, 6H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 164.03, 160.01, 155.80, 147.39, 143.25, 132.88, 130.30, 129.42, 128.54, 121.43, 103.67, 79.80, 71.22, 52.43, 51.13. HRMS-ESI (m/z, $[M+H]^+$) Calcd for $C_{34}H_{33}N_4O_{11}$: 673.2140, Found: 673.2148.

[00197] 5,5'-((((3R,4S)-tetrahydrofuran-3,4-diyl)bis(azanediyl))bis(carbonyl))bis(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxylic acid) (**4**). Potassium hydroxide (934 mg, 16.6 mmol) was added to a suspension of **3** (2.80 g, 4.16 mmol) in 2:2:1 THF:MeOH:water (50 mL), and the reaction was heated to 50 °C overnight with stirring. The solvent was removed under vacuum and the resulting residue was dissolved into water (250

mL). Dilute HCl was added dropwise with stirring until the solution was acidic by litmus test. The desired product was collected by filtration, washed with dilute HCl and dried under vacuum overnight to yield a white powder of **4**. Yield: 2.3 g, 86%. ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.53 (s, 2H), 9.19 (d, J = 7.0 Hz, 2H), 8.28 (d, J = 7.4 Hz, 2H), 7.53 – 7.47 (m, 4H), 7.47 – 7.39 (m, 6H), 6.59 (d, J = 7.4 Hz, 2H), 5.37 (d, J = 8.6 Hz, 2H), 5.27 (d, J = 8.6 Hz, 2H), 4.80 – 4.70 (m, 2H), 3.99 (dd, J = 8.8, 6.3 Hz, 2H), 3.60 (dd, J = 9.0, 4.8 Hz, 2H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 163.98, 159.36, 158.61, 147.09, 143.63, 133.23, 129.73, 129.31, 128.55, 120.56, 105.44, 79.52, 69.53, 51.29. HRMS-ESI (m/z, $[M-H]^-$) Calcd for $C_{32}H_{27}N_4O_{11}$: 643.1682, Found: 643.1684.

[00198] N,N'-((3R,4S)-tetrahydrofuran-3,4-diyl)bis(1-(benzyloxy)-2-oxo-6-(2-thioxothiazolidine-3-carbonyl)-1,2-dihydropyridine-3-carboxamide) (**5**). HATU (2.40 g, 6.3 mmol), **4** (1.93 g, 3.00 mmol), and DMAP (73.3 mg, 0.6 mmol) were suspended in dichloromethane (75 mL). DIPEA (776 mg, 6 mmol) was added dropwise to the suspension, and the reaction was stirred at room temperature for 2 hours. Upon completion 2-mercaptothiazoline (894 mg, 7.5 mmol) was added to the homogenous solution, followed by DIPEA (1.5 g, 11.6 mmol). The reaction was stirred for an additional 1.5 hours at room temperature. The reaction mixture was then washed with water 3x75 mL to remove the bulk of the urea byproduct, concentrated, and then loaded onto a 6 inch tall x 1 inch wide silica gel column. Following a 2-propanol/dichloromethane gradient elution, the desired product was collected using 5% 2-propanol in dichloromethane. The solvent was removed under vacuum, and the residue was dissolved into 100 mL dichloromethane. The organic solution was again washed with water 3x75 mL to remove final traces of the urea byproduct, and the organic solution was concentrated to a volume of 10 mL. Addition of 2-propanol caused precipitation of the desired product, which was collected by evaporation of the solvent to give a yellow powder of **5**. Yield: 1.83 g, 72%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (d, J = 5.0 Hz, 2H), 7.44 – 7.37 (m, 4H), 7.37 – 7.28 (m, 6H), 7.25 (d, J = 7.0 Hz, 2H), 6.20 (d, J = 7.0 Hz, 2H), 5.26 (d, J = 8.7 Hz, 2H), 5.08 (d, J = 8.7 Hz, 2H), 4.79 – 4.68 (m, 2H), 4.57 – 4.41 (m, 4H), 3.98 (dd, J = 9.1, 6.0 Hz, 2H), 3.62 (dd, J = 9.2, 3.8 Hz, 2H), 3.49 – 3.33 (m, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 202.05, 165.47, 159.87, 155.36, 144.03, 138.67, 132.91, 130.27, 130.07, 129.61, 128.70, 105.73, 79.43, 70.99, 55.60, 51.75, 29.38. HRMS-ESI (m/z, $[M+H]^+$) Calcd for $C_{38}H_{35}N_6O_9{}^{32}S_4$: 847.1343, Found: 847.1338.

[00199] *Compound **6***. Spermine (229 mg, 1.13 mmol) was dissolved into 2-propanol (50 mL) and **5** (957 mg, 1.13 mmol) was separately dissolved into dichloromethane (50 mL).

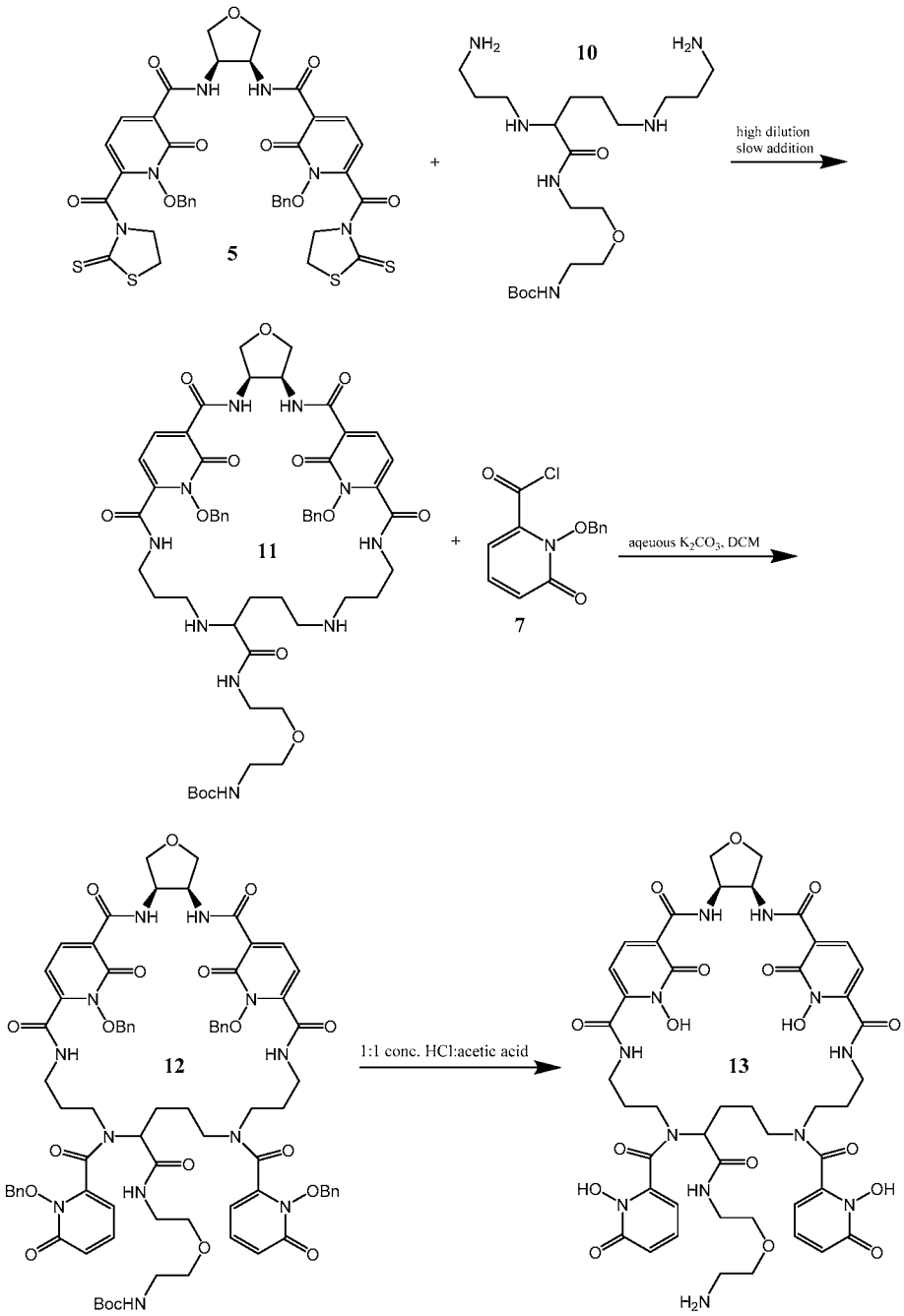
Using syringe pumps, the two solutions were dripped (0.5 mL/hour) into a large flask containing 1:1 dichloromethane:2-propanol (1 L) over four days. The reaction was stirred for an additional day at room temperature, followed by removal of the solvent under vacuum. The residue was dissolved into dichloromethane (200 mL) and extracted with aqueous potassium hydroxide to remove the 2-mercaptothiazoline byproduct. Removal of the solvent afforded the desired product as a viscous oil of **6**, which was used without further purification in the next reaction. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.60 (s, 2H), 8.08 (s, 2H), 7.64 – 7.11 (m, 12H), 6.05 (s, 2H), 5.35 – 5.08 (m, 4H), 4.81 (s, 2H), 4.09 (s, 2H), 3.85 (s, 2H), 3.69 – 3.22 (m, 4H), 2.96 – 2.37 (m, 8H), 1.97 – 1.39 (m, 8H). ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.97, 160.18, 158.11, 144.85, 141.33, 132.93, 130.44, 129.73, 128.73, 123.83, 104.66, 79.89, 71.46, 51.59, 49.65, 47.25, 37.26, 29.53, 27.30. HRMS-ESI (*m/z*, $[M+H]^+$) Calcd for $C_{42}H_{51}N_8O_9$: 811.3774, Found: 811.3768.

[00200] *Compound **8***. Oxalyl chloride (1.1 g, 8.7 mmol) was added to a suspension of **7** (1.11 g, 4.52 mmol) in dichloromethane (40 mL), followed by 1 drop of dry DMF. The solution became homogenous within 30 min, and the reaction was stirred at room temperature for 3 hours total. The solvent was then removed under vacuum overnight. The residue was dissolved into dichloromethane (20 mL) and added dropwise to a solution of **6** (1.13 mmol) dissolved in dichloromethane (20 mL) and 40% aqueous K_2CO_3 (20 mL) at 0 °C with vigorous stirring. The reaction was stirred with warming to room temperature overnight. The dichloromethane layer was loaded directly onto a 4 inch tall x 1 inch wide silica gel column. Following a methanol/dichloromethane gradient elution, the desired product was collected using 4% methanol in dichloromethane. The solvent was removed under vacuum to yield the desired product as a hardened glass of **7**. Yield: 520 mg, 36% over two steps. ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.60 – 8.98 (m, 2H), 8.43 – 7.83 (m, 4H), 7.80 – 7.05 (m, 20H), 7.05 – 5.54 (m, 8H), 5.49 – 4.98 (m, 6H), 4.98 – 4.69 (m, 2H), 4.28 – 3.98 (m, 2H), 3.93 – 2.75 (m, 14H), 2.05 – 1.16 (m, 10H). HRMS-ESI (*m/z*, $[M+H]^+$) Calcd for $C_{68}H_{69}N_{10}O_{15}$: 1265.4938, Found: 1265.4901.

[00201] *Compound **9***. Compound **8** (63 mg, 0.070 mmol) was dissolved into a 1:1 mixture of concentrated HCl and glacial acetic acid. The homogenous solution was stirred at room temperature for 3 weeks in the dark. Upon reaction completion, the solvent was removed under vacuum. Residual solvent was removed by co-evaporation with water, followed by methanol, and finally diethyl ether to yield a beige solid of **9**. Purity was assessed by HPLC by first adding a 5-fold molar excess of $EuCl_3$ to the sample dissolved in methanol.

Quantitative yield and > 90% purity. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.82 – 9.33 (m, 2H), 9.07 – 7.94 (m, 4H), 7.52 – 7.15 (m, 2H), 6.86 – 6.44 (m, 4H), 6.40 – 6.05 (m, 2H), 4.74 (s, 2H), 4.03 (s, 2H), 3.86 – 2.76 (m, 14H), 1.97 – 1.17 (m, 8H). HRMS-ESI (*m/z*, $[M+H]^+$) Calcd for $C_{40}H_{45}N_{10}O_{15}$: 905.3060, Found: 905.3053.

EXAMPLE 2. Synthesis of Exemplary Bifunctional Chelator



Scheme 2. Synthetic scheme for attachment point containing compound 13

[00202] *Compound 11*. Following the same procedure as used for compound **6**, using **5** (1.30 g, 1.54 mmol) and **10** (668 mg, 1.54 mmol) as starting materials. Similarly, the residue was used washed with base and used without further purification in the next reaction. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.89 – 9.45 (m, 2H), 8.43 – 7.62 (m, 6H), 7.62 – 7.09 (m, 10H), 6.49 – 5.92 (m, 2H), 5.59 – 4.51 (m, 6H), 4.11 – 2.24 (m, 23H), 2.10 – 1.03 (m, 17H). HRMS-ESI (*m/z*, $[M+H]^+$) Calcd for $C_{52}H_{69}N_{10}O_{13}$: 1041.5040, Found: 1041.5031.

[00203] *Compound 12*. Following the same procedure as used for compound **8**, using **11** (1.54 mmol) and **7** (1.51 g, 6.16 mmol) as starting materials. Yield: 1.08 g, 47 % over two steps. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.69 – 8.96 (m, 2H), 8.61 – 6.93 (m, 28H), 6.89 – 6.01 (m, 4H), 5.98 – 4.66 (m, 12H), 4.29 – 2.65 (m, 23H), 2.56 – 1.04 (m, 17H). HRMS-ESI (*m/z*, $[M+Na]^+$) Calcd for $C_{78}H_{86}N_{12}O_{19}Na$: 1517.6024, Found: 1517.6063.

[00204] *Compound 13*. Following the same procedure as used for compound **9**, using **12** (92 mg, 0.062 mmol) as the starting material. Quantitative yield and > 95% purity. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.79 – 9.26 (m, 2H), 8.85 – 7.79 (m, 5H), 7.61 – 7.19 (m, 2H), 6.75 – 6.40 (m, 4H), 6.37 – 6.02 (m, 2H), 4.74 (s, 2H), 4.04 (s, 2H), 3.75 – 2.85 (m, 21H), 1.94 – 1.29 (m, 8H). HRMS-ESI (*m/z*, $[M-H]^-$) Calcd for $C_{45}H_{53}N_{12}O_{17}$: 1033.3657, Found: 1033.3646.

EXAMPLE 3. Characterization of metal complexes for ligand **13**

[00205] Metal complexes of compound **13** may be prepared readily, for example, by treatment with an appropriate metal salt dissolved in methanol and a tertiary amine as described below. A stock solution of compound **13** (5.2 mg, 4.9 μmol) was suspended in methanol (1 mL), and triethylamine (9.8 mg, 98 μmol, 20 eq.) was added resulting in a homogenous yellow solution. Aliquots (34 μL each, 0.165 μmol) were taken from this stock and placed into separate sample tubes. Stock solutions of the metal salts were prepared in methanol (1 mL) at concentrations ranging from 5 to 20 mM. For each metal salt solution, a volume corresponding to 1.25 equivalents (0.206 μmol) was added to one of compound **13** containing sample tubes. A precipitate formed immediately for all samples. The solvent was removed by evaporation in a stream of compressed air, and the samples were analyzed in methanol or 10% DMSO in methanol by mass spectrometry, with results reported below. The europium(III) complex was noted to be luminescent when viewed using a long wavelength (365 nm) UV lamp. Metal cation salts tested include zirconium acetylacetonate, iron(III) nitrate nonahydrate, indium(III) chloride tetrahydrate, europium(III) chloride hexahydrate,

holmium(III) chloride hexahydrate, lutetium(III) chloride hexahydrate, yttrium(III) chloride hexahydrate, terbium(III) chloride hexahydrate, ytterbium trifluoromethanesulfonate, gadolinium(III) chloride hexahydrate, samarium(III) chloride hexahydrate, dysprosium(III) chloride hexahydrate, erbium(III) chloride hexahydrate, and thorium nitrate hydrate (99.8%).

[00206] **13**-Zr: FTMS +pESI: calculated for $C_{45}H_{51}N_{12}O_{17}{}^{90}Zr$ $[M+H]^+$ 1121.2537, found 1121.2554.

[00207] **13**-Fe: FTMS +pESI: calculated for $C_{45}H_{52}N_{12}O_{17}{}^{56}Fe$ $[M+2H]^+$ 1088.2917, found 1088.2921.

[00208] **13**-In: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{115}In$ $[M]^-$ 1145.2461, found 1145.2462.

[00209] **13**-Eu: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{151}Eu$ $[M]^-$ 1181.2621, found 1181.2619.

[00210] **13**-Ho: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{165}Ho$ $[M]^-$ 1195.2726, found 1195.2730.

[00211] **13**-Lu: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{175}Lu$ $[M]^-$ 1205.2830, found 1205.2836.

[00212] **13**-Y: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{89}Y$ $[M]^-$ 1119.2481, found 1119.2476.

[00213] **13**-Tb: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{159}Tb$ $[M]^-$ 1189.2676, found 1189.2677.

[00214] **13**-Yb: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{174}Yb$ $[M]^-$ 1204.2811, found 1204.2812.

[00215] **13**-Gd: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{158}Gd$ $[M]^-$ 1188.2663, found 1188.2677.

[00216] **13**-Sm: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{152}Sm$ $[M]^-$ 1182.2620, found 1182.2647.

[00217] **13**-Dy: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{164}Dy$ $[M]^-$ 1194.2714, found 1194.2737.

[00218] **13**·Er: FTMS -pESI: calculated for $C_{45}H_{50}N_{12}O_{17}{}^{166}Er$ $[M]^-$ 1196.2725, found 1196.2737.

[00219] A small sample (ca. 0.1 mg) of the free ligand **13**, and portion of the **13**·Eu and **13**·Th complexes were separately dissolved in ca. 50 µL DMF. These solutions were analyzed by HPLC (5 µL injection volume) on an Agilent 1260 Infinity device, using an Eclipse XDB-C18 reverse phase column (4.6x100 mm). The mobile phase consisted of 0.1% trifluoroacetic acid (TFA) in water and acetonitrile. Elution was achieved along a solvent gradient, starting at 90/10 water/acetonitrile and ending (20 min) at 40/60 water/acetonitrile. The chromatograms are plotted in Figure 1.

[00220] The shift in retention times of the Eu and Th complexes, relative to the free ligand, demonstrates that both Eu(III) and Th(IV) are successfully bound by ligand **13**. It is notable that the complexes remain intact during the separation, despite the low pH mobile phase. In order to investigate the metal binding in more detail, the absorption spectrum of each sample recorded on the HPLC is shown in **Figure 2**.

[00221] It is clear from the absorption spectra (**Figure 2**) that the Eu and Th complexes absorb at wavelengths distinct from that of the free ligand. It is also notable that the Eu and Th spectra are similar in shape. We take the chromatograms and the absorption spectra as evidence that ligand **13** successfully binds thorium(IV).

[00222] The Mass Spectrometry data of ligand 13 with chelated ^{232}Th(IV) stable isotope is consistent with the molecular weight of the Th complexes $C_{45}H_{50}N_{12}O_{17}{}^{232}$Th(IV) of about 1243 (e.g., 1243.3624).

EXAMPLE 4. Photophysical characterization of **9**·Eu and **13**·Eu

General Procedure.

[00223] For both **9** and **13**, stock solutions were prepared by separately dissolving 1.0 mg of each compound into 1 mL of MeOH, containing 10 equivalents of pyridine as a base. To each solution, 1.1 equivalents of europium chloride hexahydrate (dissolved in methanol) were added, yielding methanolic solutions (ca. 1 mM) of the desired **9**·Eu and **13**·Eu complexes. A few drops of these methanolic solutions were diluted to ca. 3 µM in TBS buffer (Tris-buffered saline, 50 mM Tris, 150 mM NaCl, pH = 7.6) to make aqueous stock solutions (20 mL) with an absorbance of 0.05 (1 cm path length) at 340 nm. Five dilutions (1/6, 2/6, 3/6, 4/6, and 5/6) were made from these aqueous stock solutions, to yield six samples with evenly

spaced concentrations (3 mL volume each). Quinine sulfate dissolved in 0.05 M sulfuric acid was similarly diluted to a final absorbance of 0.05 at 340 nm, and six 3 mL samples of evenly spaced concentration were similarly prepared. Quinine sulfate in 0.05 M sulfuric acid was used as the quantum yield standard ($\Phi = 0.508$). By measuring the absorbance and luminescence of these dilute solutions, the quantum yields of the new Eu complexes were determined as shown below. A 5 cm path-length quartz cell was used to measure the absorbance of the solutions in order to improve signal-to-noise. Steady state photoluminescence measurements were collected at 340 nm excitation wavelength, 1 nm resolution, 10 nm excitation slit width, 1 nm emission slit width, and 0.2 sec integration time. Absorption spectra were measured at 1 nm resolution, and the absorbance of each sample was taken as the average absorbance measured for 335-345 nm to reflect the 10 nm excitation slit width used for luminescence measurements. The UV-vis spectrum (top) and photoluminescence spectrum (bottom) of **9**·Eu measured in TBS buffer, pH 7.6 is shown in Figure 3.

[00224] The absorption spectrum for **9**·Eu shows two maxima at 318 nm and 358 nm, corresponding to the two distinct organic chromophores bound to the metal. The photoluminescent lifetime of **9**·Eu measured at 612 nm was found to be 745 +/- 2 μs, consistent with a coordinatively saturated 8-coordinate Eu complex.

[00225] The quantum yield of **9**·Eu was found to be 0.216 at an excitation wavelength of 340 nm (**Figure 4**).

[00226] Similar to **9**·Eu, the absorption spectrum for **13**·Eu shows two maxima at 318 nm and 357 nm (**Figure 5**). The photoluminescent lifetime of **13**·Eu measured at 612 nm was found to be 745 +/- 1 μs (**Figure 5**).

[00227] The quantum yield of **13**·Eu was found to be 0.272 at an excitation wavelength of 340 nm (**Figure 6**). The same samples were also measured at an excitation wavelength of 358 nm, and the quantum yield was found to be 0.295 at that wavelength.

EXAMPLE 5. Europium titration experiments for determining extinction coefficients

[00228] In order to determine accurate extinction coefficients for **13** and **13**·Eu, titration experiments using europium chloride as the titrant were performed. Europium chloride hexahydrate (47.58 mg, 0.1299 mmol) was dissolved into 50 mM citrate buffer (pH = 4) using a 10 mL volumetric flask to give a 12.99 mM europium chloride solution, which was

then transferred to a 50 mL volumetric flask and diluted 1:10 with water. The resulting 1.299 mM europium chloride solution (in 5 mM citrate buffer) was then titrated into a 1 mL solution of **13** in TBS buffer using 5 µL aliquots. The UV-vis spectra were adjusted for the increase in solvent volume due to each injection. The results of the UV-vis titration are plotted in Figure 7.

[00229] From the UV-vis titration (**Figure 7**), it was found that 36.55 µL of the 1.299 mM europium chloride solution was required to reach the equivalence point. The volume of europium chloride corresponds to 47.46 nmol of europium, meaning the starting concentration of ligand **13** was 47.46 µM. The lambda max absorbances of **13** and **13**·Eu were found to be 0.618 at 383 nm and 0.607 at 357 nm for the ligand and complex respectively. These absorbance and concentration values yield extinction coefficients of 13,000 $M^{-1}cm^{-1}$ and 12,800 $M^{-1}cm^{-1}$ for the ligand and complex respectively.

[00230] The photoluminescent titration of ligand **13** with europium chloride is shown in **Figure 8**. Titration of ligand **13** was also performed by luminescence, monitoring the europium signal at 612 nm upon photoexcitation of the solution at 340 nm. The ligand solution used for the UV-vis titration was first diluted 1:10 in TBS buffer. The stock europium solution in 5 mM citrate was separately diluted 1:10 with water. The results of the luminescence titration are shown in the above plot. It was found that 32.99 µL of the 0.1299 mM europium chloride solution was required to reach the equivalence point. The 32.99 µL equivalence point gives extinction coefficients of 14,400 $M^{-1}cm^{-1}$ at 383 nm and 14,200 $M^{-1}cm^{-1}$ at 357 nm for **13** and **13**·Eu respectively. Averaging the values from both titration experiments yields extinction coefficients of 13,700 $M^{-1}cm^{-1}$ at 383 nm and 13,500 $M^{-1}cm^{-1}$ at 357 nm for **13** and **13**·Eu respectively. Using these averaged values, extinction coefficients of 17,800 $M^{-1}cm^{-1}$ at 280 nm and 27,900 $M^{-1}cm^{-1}$ at 260 nm were determined for the **13** ligand. Similarly, extinction coefficients of 16,600 $M^{-1}cm^{-1}$ at 280 nm and 22,400 $M^{-1}cm^{-1}$ at 260 nm were determined for the **13**·Eu complex.

EXAMPLE 6. Stability of **13**·Eu in the presence of various competitors

[00231] In order for a luminescent metal complex to be useful in a practical sense, it must be stable in the presence of common competitive metal cations and chelating ligands. We measured the stability of **13**·Eu (ca. 7 µM) dissolved in TBS buffer, with various competitors present at ca. 25 mM concentration. Specifically, manganese dichloride tetrahydrate (14.8 mg, 74.8 µmol) was dissolved into TBS buffer (100 µL), and added to an aliquot of **13**·Eu (3

mL), making the final concentration of Mn(II) 24.1 mM. Magnesium chloride hexahydrate (33.6 mg, 165 µmol) was dissolved into TBS buffer (200 µL), and 100 µL was added to an aliquot of **13**·Eu (3 mL), making the final concentration of Mg(II) 26.7 mM. Calcium chloride hexahydrate (55.8 mg, 255 µmol) was dissolved into TBS buffer (300 µL), and 100 µL was added to an aliquot of **13**·Eu (3 mL), making the final concentration of Ca(II) 27.4 mM. For DTPA, first KOH (95.9 mg, 1.71 mmol) was dissolved into 1 mL of water, and 389 µL of this basic solution (5 equivalents) was used to dissolve DTPA (52.3 mg, 133 µmol). Then, 219 µL of this DTPA solution was added to an aliquot of **13**·Eu (3 mL), making the final concentration of DTPA 23.4 mM. EDTA (0.5 M, pH = 8, 150 µL) was added to an aliquot of **13**·Eu (3 mL), making the final concentration of EDTA 23.8 mM. For phosphate competition, the methanolic stock of **13**·Eu was diluted to ca. 7 µM in 50 mM phosphate buffer (pH = 8). Two control solutions were measured at every time point, labeled C1 and C2, which were measured at the start and the end of the series of samples respectively. Samples were normalized to T = 0 using the average brightness of the control solutions at T=1 hour, after adjusting for differences in **13**·Eu concentration.

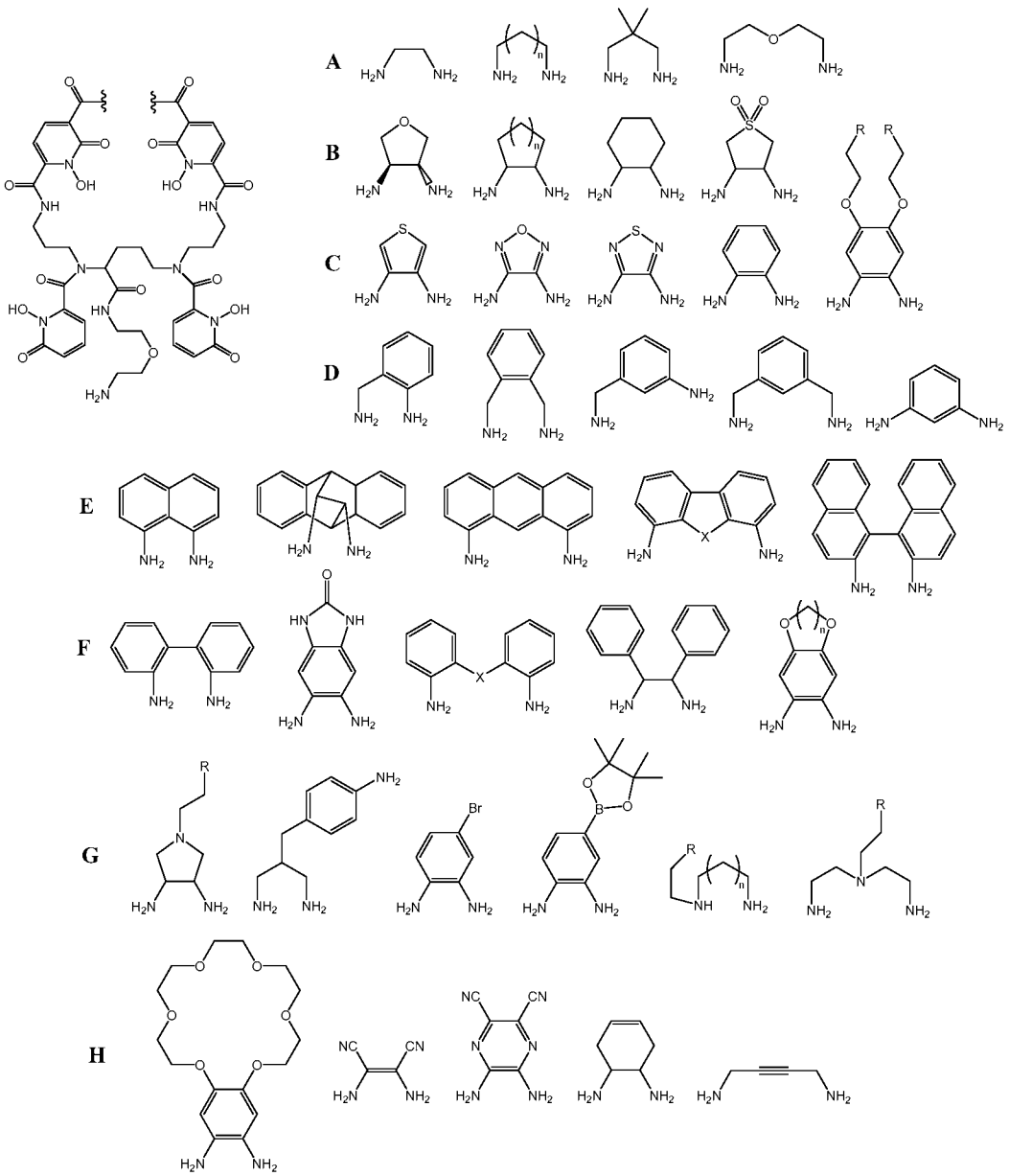
[00232] From the above plot (**Figure 9**), it is clear that most of the competitors have no significant effect on the brightness of **13**·Eu over the course of 26 hours. Specifically, magnesium, calcium, and phosphate cause no detectable change in sample brightness. A small drop in brightness was noted for the EDTA containing solution, but after 5 hours no further change was observed. Manganese caused a gradual decline in luminescence, but like the EDTA solution maintained ca. 90% of the original brightness after 26 hours. The DTPA solution decreased in luminescence steadily, reaching 73% the original brightness after 26 hours.

[00233] The absorbance was measured for each sample after sitting for five days at room temperature. The spectra confirm the findings of the luminescence study performed on the first day. Specifically, magnesium, calcium, and phosphate containing solutions show no difference in absorption compared to the control samples. The EDTA sample showed a very slight red shift towards the spectrum of the free ligand, consistent with the small initial change in brightness. The absorption spectrum of the manganese sample looks significantly different than the others, although the shape suggests that **13**·Eu remains mostly intact. It is clear that DTPA has continued to remove Eu from the **13**·Eu complex, as the absorption spectrum mostly resembles that of the free ligand after 5 days incubation. However, Eu removal is not complete even after 5 days, as significant red luminescence remains when the

sample is exposed to long-wave UV radiation. From these experiments, it is clear that **13**·Eu is remarkably stable to a wide range of competitive metal ions and chelators.

EXAMPLE 7

[00234] There are several diamines one could envision using within the existing synthetic scheme rather than the (3R,4S)-tetrahydrofuran-3,4-diamine (**1**) reported here. Representative examples of these new diamines for the formation of new monomacrocyclic ligands (n = 0, 1, 2 or 3; X = O, S, or $CH_{2)}$ are tabulated below, sorted into rows:

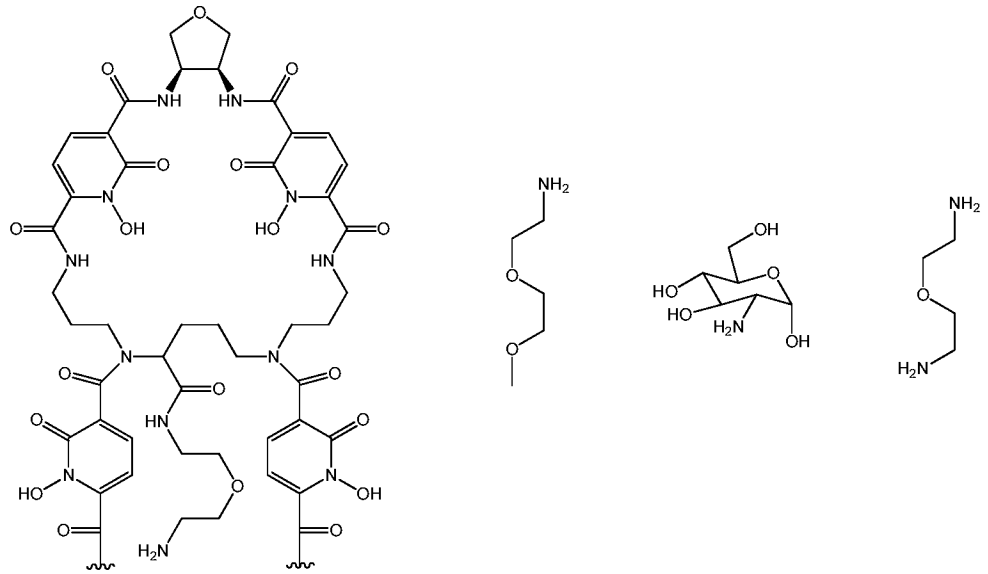


[00235] Row **A** consists of several acyclic, aliphatic linkers that may enhance or alter the photophysical properties of the analogous **13**·Eu complex, by affecting the geometry of the ligand around the metal ion. Similarly, the binding of other radiologically important metals ions may be altered or enhanced. Row **B** contains other cyclic, aliphatic diamine linkers. The cyclic structure of these examples may enhance the rigidity of the ligand structure, which may enhance the stability of the metal complexes formed. Row **C** contains cyclic, aromatic diamine linkers, which all have 2-carbon bridges much like the (3R,4S)-tetrahydrofuran-3,4-diamine 2-carbon bridge reported here. These aromatic diamines might extend the electronic conjugation of the 1,2-HOPO units, which can affect the photophysical characteristics of the

ligand. The final entry in row **C** can also include polyethylene glycol units for enhanced solubility. Rows **D**, **E**, and **F** contain additional examples of cyclic, aromatic systems that may enhance the stability of the metal complexes by altering the ligand geometry. Row **G** contains a variety of diamine bridges, which all have a functional handle that can be used for linking **13**·Eu-type complexes to a species of interest. The linkers on each of these diamines may be used to give **13**·Eu-type complexes an additional functional handle, or they may be used as the sole functional group linker in **9**·Eu-type complexes. Row **H** contains diamine bridges that may offer a way to sense or react with species of interest. The first entry contains an 18-crown-6 ether functionality, which should bind potassium ions. The cyano and cyclohexene examples may bind certain transition metals, while the butyne diamine might facilitate reaction with an organic azide.

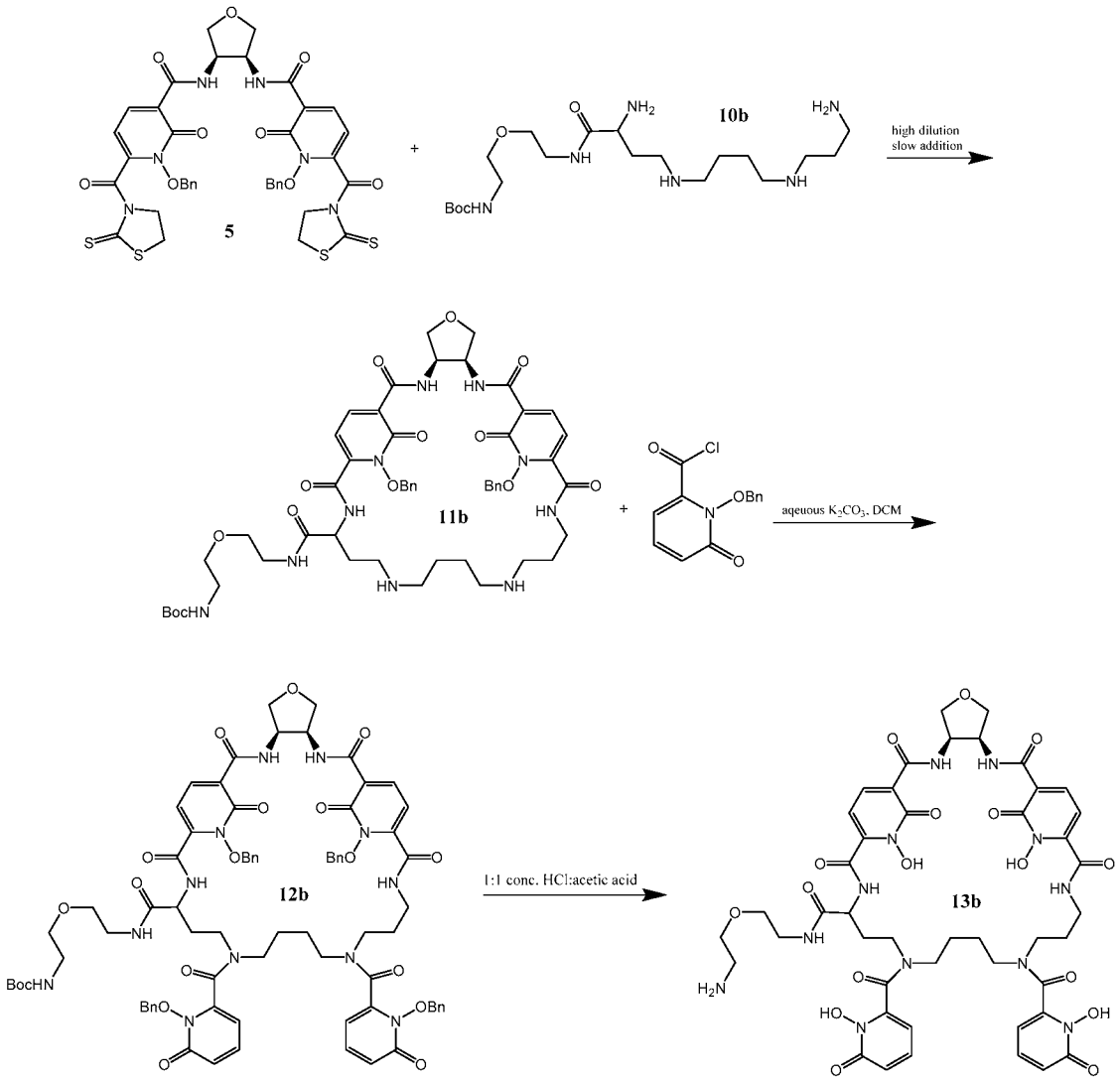
EXAMPLE 8

[00236] Representative new functional groups for attachedment to pendant 1,2-HOPO units for solubility and additional points of attachment are shown below:



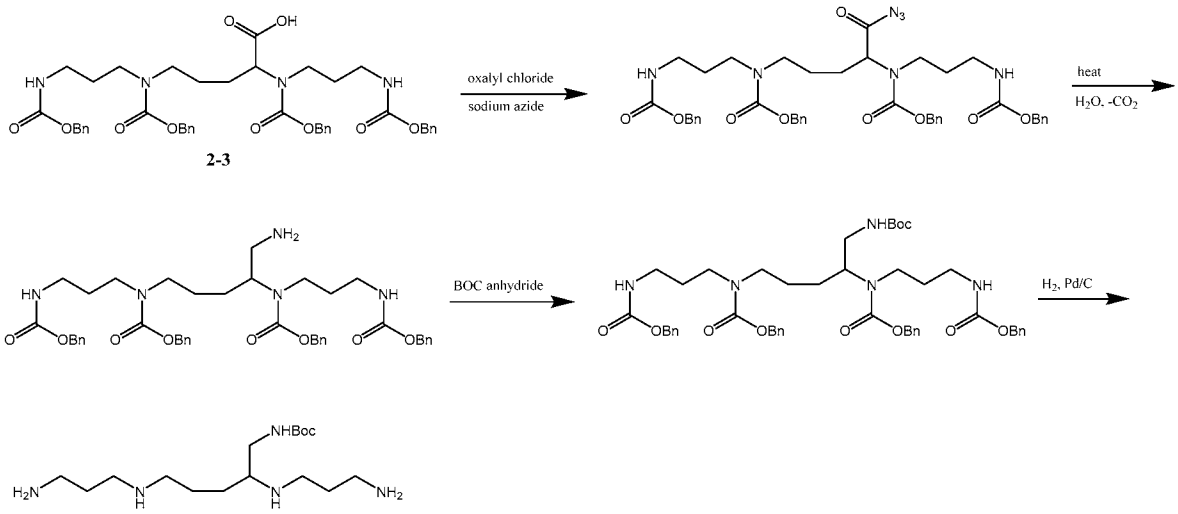
[00237] Reacting **2** (instead of **7**) with **6** or **11**, produces the structure shown above. Such a species allows for solubilizing groups such as aminopolyethylene glycol and glucosamine (shown above) to be added onto the ligand, enhancing the solubility of the metal complexes in water. Adding diamines (or similar bifunctional moieties) engenders ligands with additional attachment points.

EXAMPLE 9



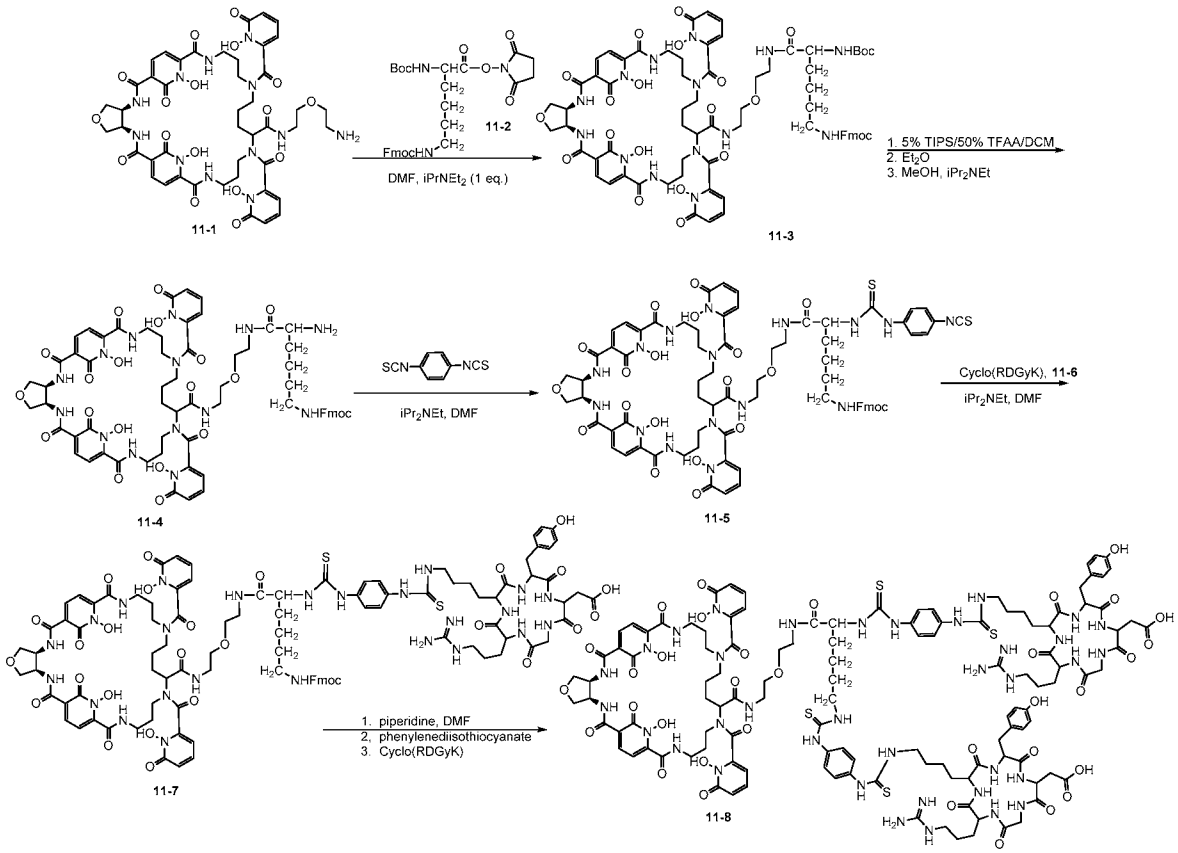
Scheme 3. Representative synthetic scheme of compound **13b** of the invention and precursors thereof.

EXAMPLE 10



Scheme 4. Representative synthetic scheme of an exemplary scaffold precursor.

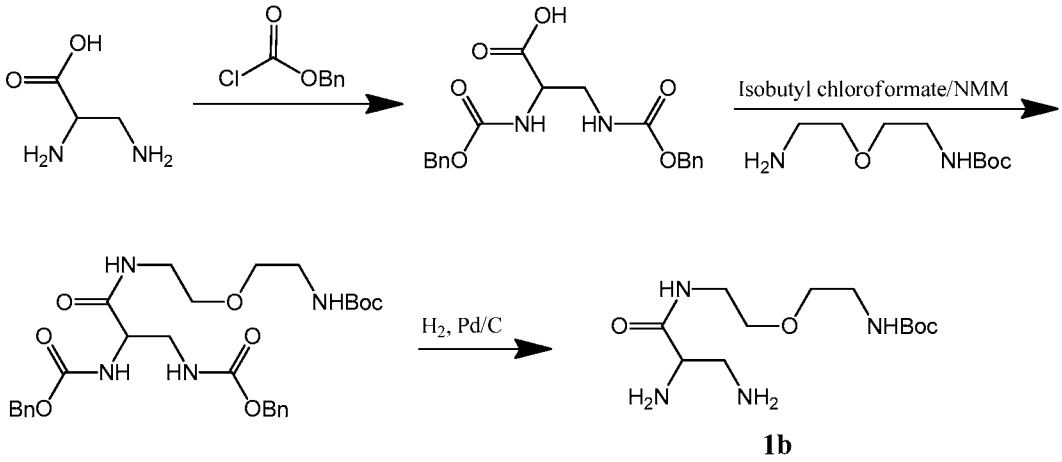
EXAMPLE 11



Scheme 5. Representative synthetic scheme of HOPO-Lys-cyclic-RDGyK.

[00238] The divalent peptide conjugate HOPO-Lys-cyclic-RDGyK can be synthesized according as outlined in Scheme 5. Cyclo(RDGyK) is a peptide that binds αvβ3 integrin, overexpressed in pancreatic cancer, available from Anaspec.

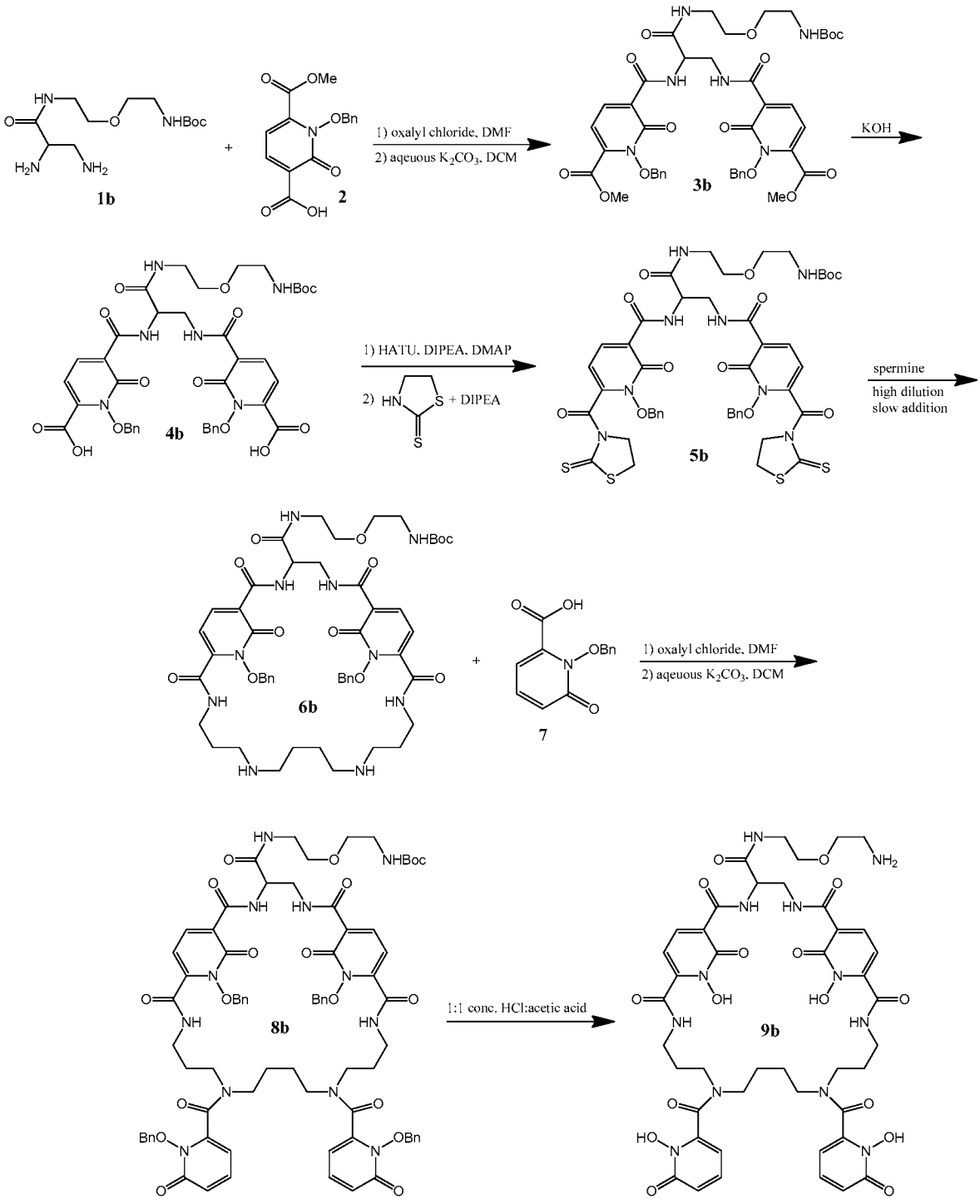
EXAMPLE 12



1b

Scheme 6. Representative synthetic scheme of compound **1b** .

EXAMPLE 13

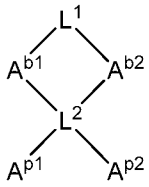


Scheme 7. Representative synthetic scheme of compound **9b** of the invention and precusors thereof.

[00239] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

WE CLAIM:

1. A compound having a structure selected from:



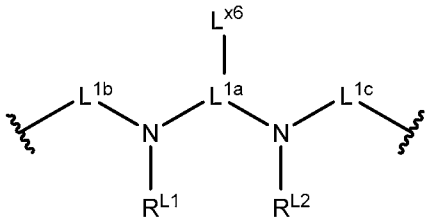
wherein

L^1 and L^2 are independently selected scaffold moieties;

A^{b1} and A^{b2} are independently selected bridging chelating moieties; and

A^{p1} and A^{p2} are independently selected pendant chelating moieties.

2. The compound according to claim **1**, wherein L^1 has the structure:



wherein

L^{1a} is selected from substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylalkyl, substituted or unsubstituted biaryl, substituted or unsubstituted heteroaryl, and a substituted or unsubstituted polycyclic ring system;

L^{x6} is independently selected from H, a linker to a reactive functional group, and a linker to a targeting moiety;

L^{1b} and L^{1c} are independently selected from a bond, −C(O)−, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl; and

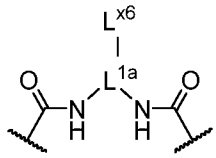
R^{L1} and R^{L2} are independently selected from H, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl.

3. The compound according to claim **2**, wherein L^{1b} and L^{1c} are independently selected from a bond, $-C(O)-$, $-(CH_2)_aC(O)-$, and $-O(CH_2)_aC(O)-$; wherein a is an integer selected from 1, 2, 3, 4, 5, and 6.

4. The compound according to claim **2**, wherein L^{1b} and L^{1c} are each $-C(O)-$.

5. The compound according to any one of claims **2-4**, wherein R^{L1} and R^{L2} are each H.

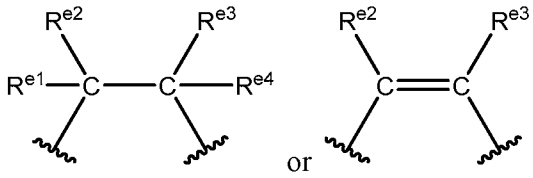
6. The compound according to any preceding claim, wherein L^1 has the structure:



wherein

L^{x6} is independently selected from H, a linker to a reactive functional group, and a linker to a targeting moiety.

7. The compound according to any one of claims **2-6**, wherein L^{1a} has the structure:

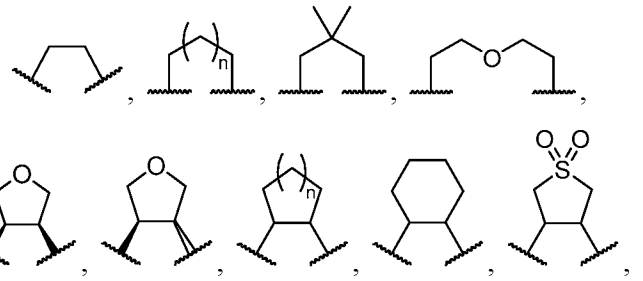


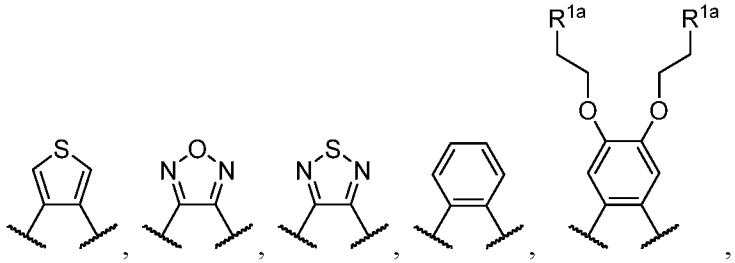
wherein

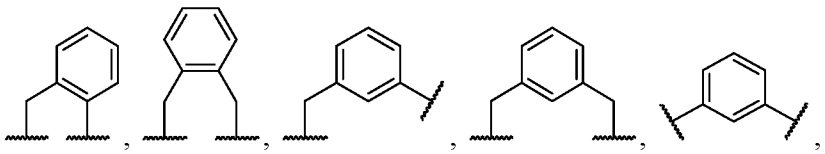
R^{e1}, R^{e2}, R^{e3}, and R^{e4} are independently selected from H, cyano, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, and substituted or unsubstituted heteroaryl; and

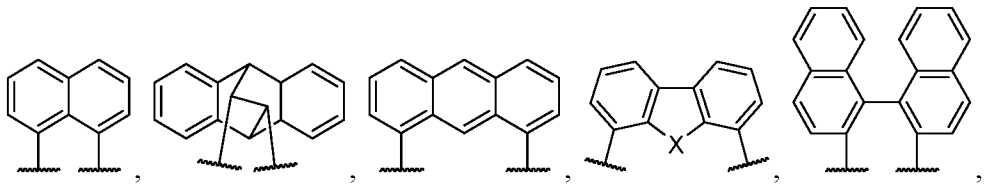
two members selected from R^{e1}, R^{e2}, R^{e3}, and R^{e4}, together with the atom to which they are attached, are optionally joined, to form a substituted or unsubstituted ring (or ring system) selected from substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl.

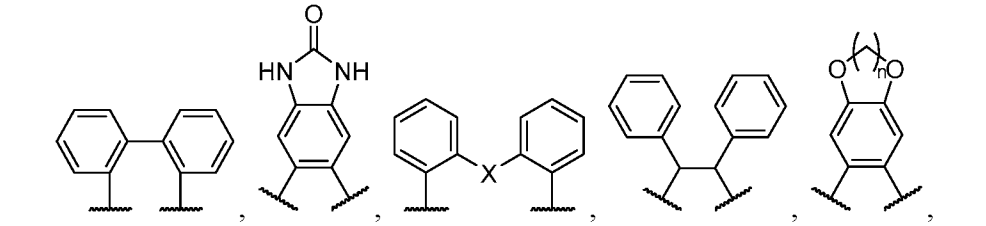
8. The compound according to any one of claims **2-6**, wherein L^{1a} is selected from:

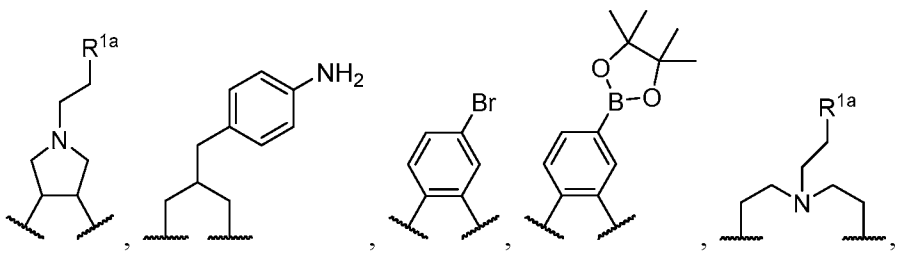


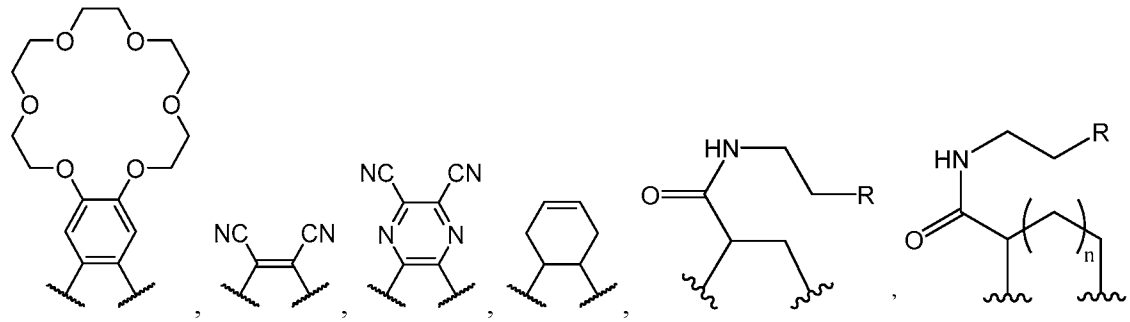


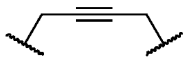










and  ;

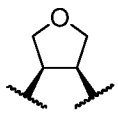
wherein

n is an integer selected from 0, 1, 2, 3, 4, 5, and 6;

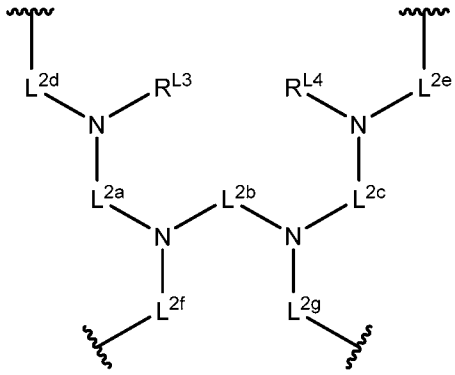
each R^{1a} is independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, and a modifying moiety; and

X is O, S, or CH_2.

9. The compound according to any one of claims **2-8**, wherein L^{1a} has the structure:



10. The compound according to any preceding claim, wherein L^2 has the structure:



wherein

L^{2a}, L^{2b} and L^{2c} are independently selected from substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

L^{2d}, L^{2e}, L^{2f} and L^{2g} are independently selected from a bond, −C(O)−, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl; and

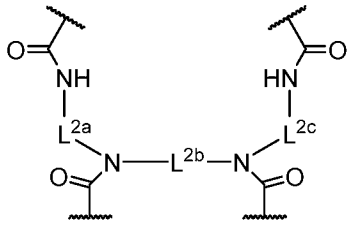
R^{L3} and R^{L4} are independently selected from hydrogen, substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl.

11. The compound according to claim **10**, wherein L^{2d}, L^{2e}, L^{2f} and L^{2g} are independently selected from a bond, $-C(O)-$, $-(CH_2)_aC(O)-$, and $-O(CH_2)_aC(O)-$; wherein a is an integer selected from 1, 2, 3, 4, 5, and 6.

12. The compound according to claim **10**, wherein L^{2d}, L^{2e}, L^{2f} and L^{2g} are each $-C(O)-$.

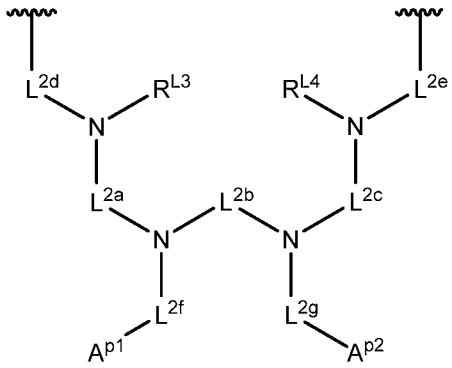
13. The compound according to any one of claims **10-12**, wherein R^{L3} and R^{L4} are each H.

14. The compound according to any one of claims **10-13**, wherein L^2 has the structure:



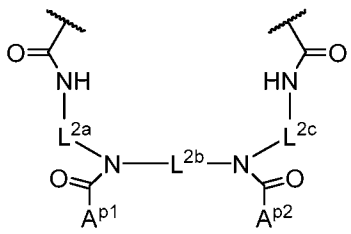
.

15. The compound according to any one of claims **10-14**, wherein $A^{p1}-L^2-A^{p2}$ has the structure:



.

16. The compound according to any one of claims **10-15**, wherein $A^{p1}-L^2-A^{p2}$ has the structure:



.

17. The compound according to any one of claims **10-16**, wherein L^{2a}, L^{2b} and L^{2c} are independently selected from substituted or unsubstituted C_1-C_8 alkyl.

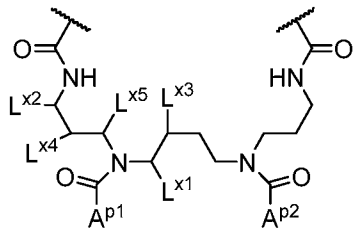
18. The compound according to claim **17**,

wherein

L^{2a} and L^{2c} are independently selected from substituted or unsubstituted C_2, C_3 and C_4 alkyl; and

L^{2b} is selected from substituted or unsubstituted C_2, C_3, C_4, and C_5 alkyl.

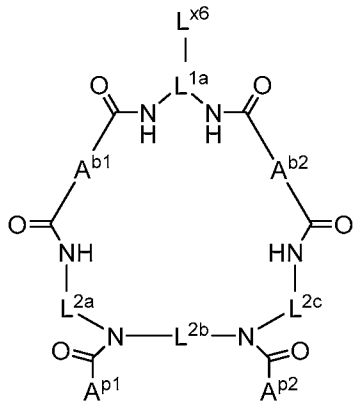
19. The compound according to any one of claims **10-15**, wherein L^2 has the structure:



wherein

L^{x1}, L^{x2}, L^{x3}, L^{x4}, and L^{x5} are independently selected from H, a linker to a reactive functional group, and a linker to a targeting moiety.

20. The compound according to claim **1**, having the structure:



wherein

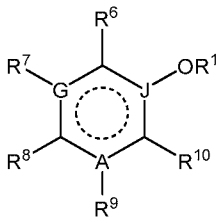
L^{1a} is selected from substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylalkyl,

substituted or unsubstituted biaryl, substituted or unsubstituted heteroaryl, and a substituted or unsubstituted polycyclic ring system;

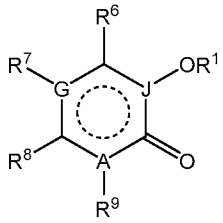
L^{x6} is independently selected from H, a linker to a reactive functional group, and a linker to a targeting moiety; and

L^{2a}, L^{2b} and L^{2c} are independently selected from substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

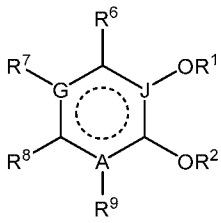
21. The compound according to any preceding claim, wherein A^{b1}, A^{b2}, A^{p1}, and A^{p2} are independently selected from:



(I),



(II), and



(III)

wherein

A and G are independently selected from carbon, nitrogen and oxygen;

J is selected from carbon and nitrogen;

each R^1 and R^2 is independently selected from H, an enzymatically labile group, a hydrolytically labile group, a metabolically labile group, a photolytically labile group and a single negative charge;

each R^6, R^7, R^8, R^9, and R^{10} is independently selected from a bond to L^1 or L^2, alkanediyl attached to L^1 or L^2, H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, halogen, CN, $-CF_3$, $-C(O)R^{17}$, $-SO_2NR^{17}R^{18}$, $-NR^{17}R^{18}$, $-OR^{17}$, $-S(O)_2R^{17}$, $-COOR^{17}$, $-S(O)_2OR^{17}$, $-OC(O)R^{17}$, $-C(O)NR^{17}R^{18}$, $-NR^{17}C(O)R^{18}$, $-NR^{17}SO_2R^{18}$, and $-NO_2$,

wherein

at least two of R^6, R^7, R^8, R^9, and R^{10} are optionally joined to form a ring system selected from substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl;

R^{17} and R^{18} are independently selected from H, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl and substituted or unsubstituted heterocycloalkyl; and

R^{17} and R^{18}, together with the atoms to which they are attached, are optionally joined to form a 5-, 6- or 7-membered ring;

when A is oxygen, R^9 is not present; and

when G is oxygen, R^7 is not present;

A^{b1} and A^{b2} are attached to L^1 and L^2 through two members selected from R^6, R^7, R^8, R^9 and R^{10}; and

A^{p1} and A^{p2} are attached to L^2 through a member selected from R^6, R^7, R^8, R^9 and R^{10}.

22. The compound according to claim **20**, wherein

when A^{b1} has a structure according to formula (I), A^{b1} is attached to L^1 and L^2 through R^6 and R^{10};

when A^{b1} has a structure according to formula (II) or (III), A^{b1} is attached to L^1 and L^2 through R^6 and R^9;

when A^{b2} has a structure according to formula (I), A^{b2} is attached to L^1 and L^2 through R^6 and R^{10};

when A^{b2} has a structure according to formula (II) or (III), A^{b2} is attached to L^1 and L^2 through R^6 and R^9;

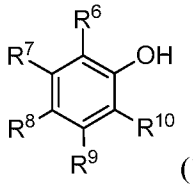
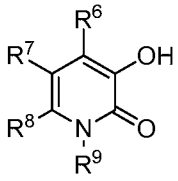
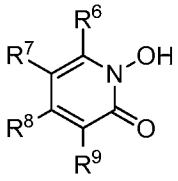
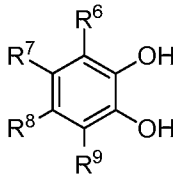
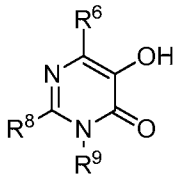
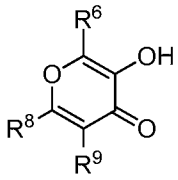
when A^{p1} has a structure according to formula (I), A^{p1} is attached to L^2 through R^6 or R^{10};

when A^{p1} has a structure according to formula (II) or (III), A^{p1} is attached to L^2 through R^6 or R^9;

when A^{p2} has a structure according to formula (I), A^{p2} is attached to L^2 through R^6 or R^{10}; and

when A^{p2} has a structure according to formula (II) or (III), A^{p2} is attached to L^2 through R^6 or R^9.

23. The compound according to claim **21**, wherein A^{b1}, A^{b2}, A^{p1} and A^{p2} are each independently selected from:


(1);

(2a);

(2b);

(3);

(4); and

(5).

24. The compound according to claim **23**, wherein

when A^{b1} has a structure according to formula (1), A^{b1} is attached to L^1 and L^2 through R^6 and R^{10};

when A^{b1} has a structure according to formula (2a), (2b), (3), (4) or (5), A^{b1} is attached to L^1 and L^2 through R^6 and R^9;

when A^{b2} has a structure according to formula (1), A^{b2} is attached to L^1 and L^2 through R^6 and R^{10};

when A^{b2} has a structure according to formula (2a), (2b), (3), (4) or (5), A^{b2} is attached to L^1 and L^2 through R^6 and R^9;

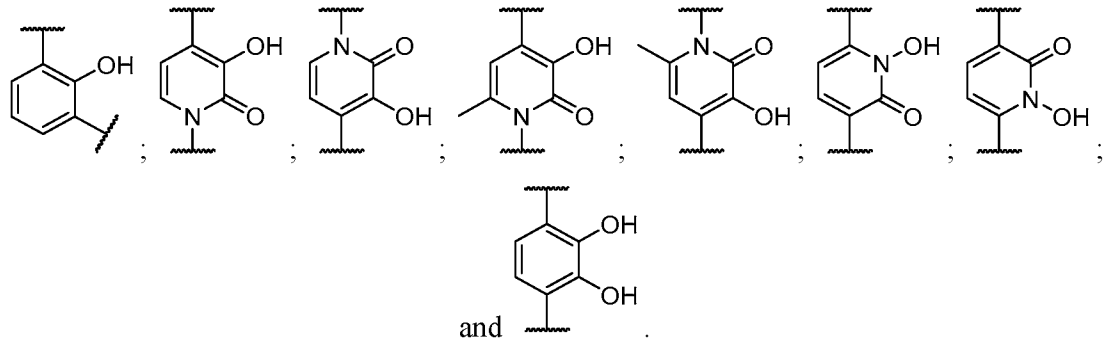
when A^{p1} has a structure according to formula (1), A^{p1} is attached to L^2 through R^6 or R^{10}; and

when A^{p1} has a structure according to formula (2a), (2b), (3), (4) or (5), A^{p1} is attached to L^2 through R^6 or R^9;

when A^{p2} has a structure according to formula (1), A^{p2} is attached to L^2 through R^6 or R^{10}; and

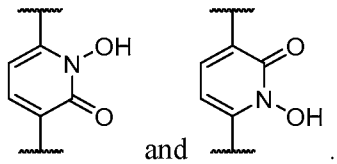
when A^{p2} has a structure according to formula (2a), (2b), (3), (4) or (5), A^{p2} is attached to L^2 through R^6 or R^9.

25. The compound according to any preceding claim, wherein A^{b1} and A^{b2} are each independently selected from:



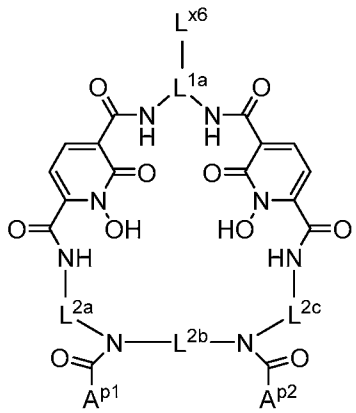
and .

26. The compound according to any preceding claim, wherein A^{b1} and A^{b2} are each independently selected from:



and .

27. The compound according to claim **1**, having the structure:



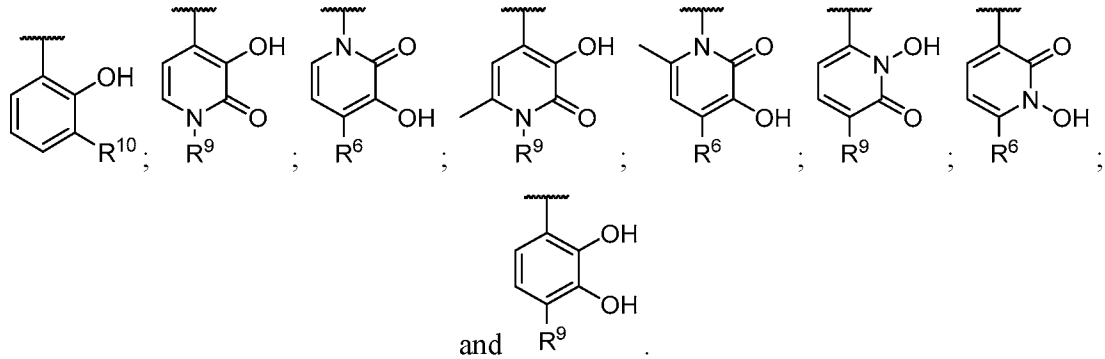
wherein

L^{1a} is selected from substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted cycloalkenyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl, substituted or unsubstituted arylalkyl, substituted or unsubstituted biaryl, substituted or unsubstituted heteroaryl, and a substituted or unsubstituted polycyclic ring system;

L^{x6} is independently selected from H, a linker to a reactive functional group, and a linker to a targeting moiety; and

L^{2a}, L^{2b} and L^{2c} are independently selected from substituted or unsubstituted alkyl and substituted or unsubstituted heteroalkyl.

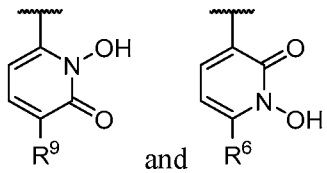
28. The compound according to any preceding claim, wherein A^{p1} and A^{p2} are each independently selected from:



and

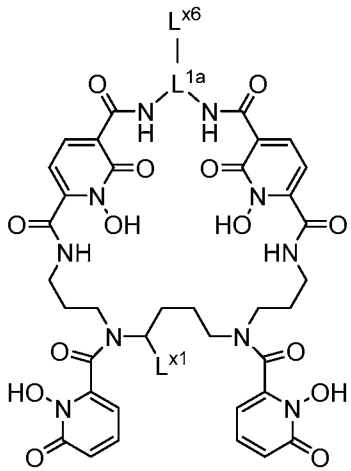
.

29. The compound according to any preceding claim, wherein A^{p1} and A^{p2} are each independently selected from:



.

30. The compound according to any preceding claim, having the structure:



wherein

L^{x1} and L^{x6} are independently selected from H, a linker to a reactive functional group, and a linker to a targeting moiety.

31. The compound according to any preceding claim, wherein the compound comprises a linker to a reactive functional group, or a linker to a targeting moiety.

32. The compound according to any preceding claim, wherein at least one of L^1, L^2, A^{p1} and A^{p2} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety.

33. The compound according to any preceding claim, wherein L^{1a} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety.

34. The compound according to any preceding claim, wherein at least one of L^{2a}, L^{2b} and L^{2c} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety.

35. The compound according to any preceding claim, wherein L^{2b} is substituted with a linker to a reactive functional group, or a linker to a targeting moiety.

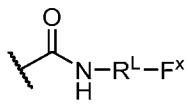
36. The compound according to any preceding claim, wherein the linker to the reactive functional group, or the linker to the targeting moiety has the structure:

$$-L^{11}-F^x,$$

wherein L^{11} is selected from a bond, acyl, substituted or unsubstituted alkyl, substituted or unsubstituted heteroalkyl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted heterocycloalkyl, substituted or unsubstituted aryl and substituted or unsubstituted heteroaryl; and

F^x is selected from a reactive functional group, a protected functional group, and a targeting moiety.

37. The compound according to any preceding claim, wherein the linker to the reactive functional group, or the linker to the targeting moiety has the structure:

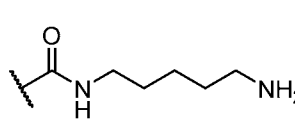
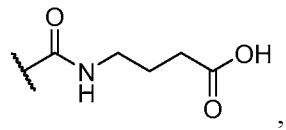
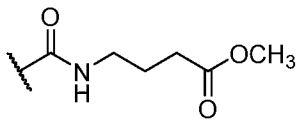
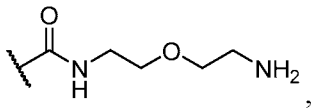
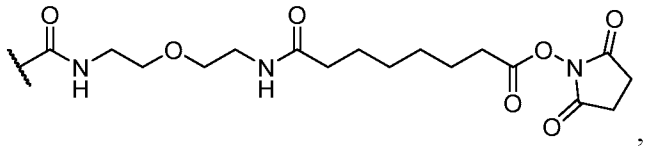
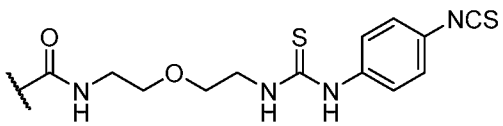
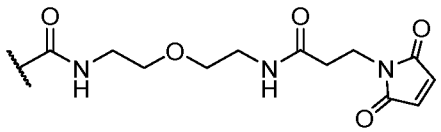


wherein

R^L is selected from substituted or unsubstituted alkyl, and substituted or unsubstituted heteroalkyl; and

F^x is selected from a reactive functional group, a protected functional group, and a targeting moiety.

38. The compound according to any preceding claim, wherein the linker to the reactive functional group has the structure:


,

,

,

,

,

, and

.

39. A complex comprising a compound according to any preceding claim and a metal ion.

40. The complex according to claim **39**, wherein the metal is selected from yttrium (Y), a lanthanide, an actinide, zirconium (Zr), iron (Fe), and indium (In).

41. The complex according to claim **39**, wherein the metal is selected from zirconium (Zr), iron (Fe), indium (In), europium (Eu), holmium (Ho), lutetium (Lu), yttrium (Y), terbium (Tb), ytterbium (Yb), gadolinium (Gd), samarium (Sm), dysprosium (Dy), erbium (Er), and thorium (Th).

42. The complex according to claim **39**, wherein the complex is luminescent.

43. The complex according to claim **39**, wherein the metal is selected from Eu, Tb, Sm, and Dy.

44. The complex according to claim **39**, wherein the metal is Gd.

45. The complex according to claim **39**, wherein the metal is a radionuclide.

46. The complex according to claim **39**, wherein the metal ion is selected from Zr(IV), Fe(III), Ga(III), In(III), Eu(III), Ho(III), Lu(III), Y(III), Tb(III), Yb(III), Gd(III), Sm(III), Dy(III), Er(III), and Th(IV).

47. The complex according to claim **39**, wherein the metal ion is selected from ^{227}Th(IV), ^{89}Zr(IV), and ^{177}Lu(III).

FIG. 1

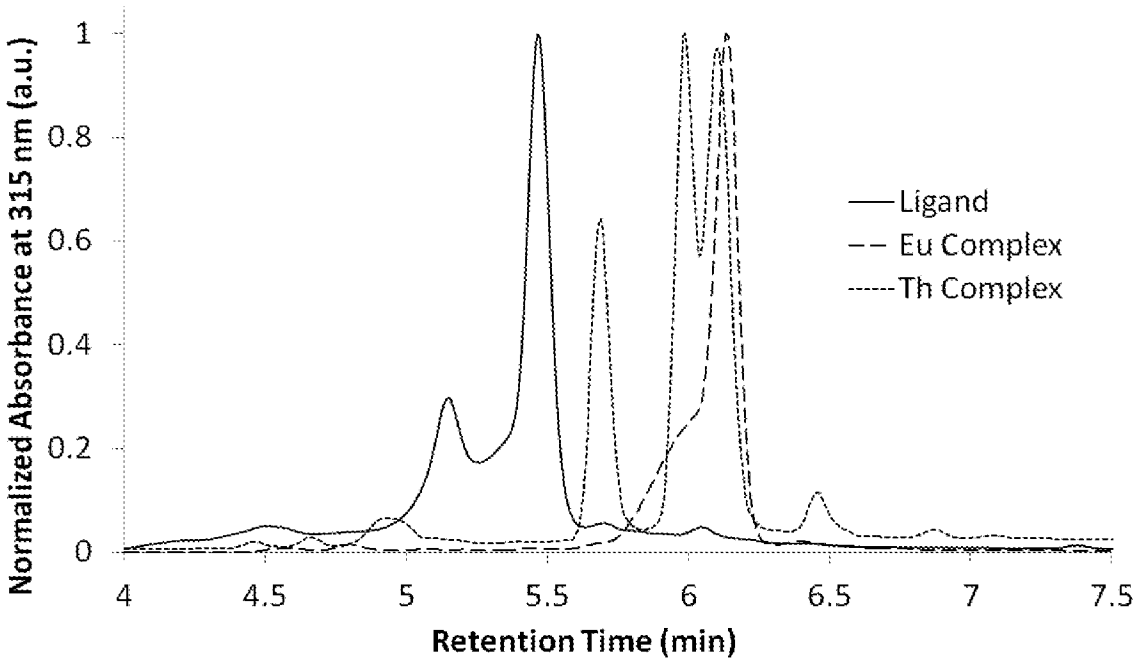

Normalized Absorbance at 315 nm (a.u.)
1
0.8
0.6
0.4
0.2
0
4
4.5
5
5.5
6
6.5
7
7.5
Retention Time (min)
Ligand
Eu Complex
Th Complex

FIG. 2

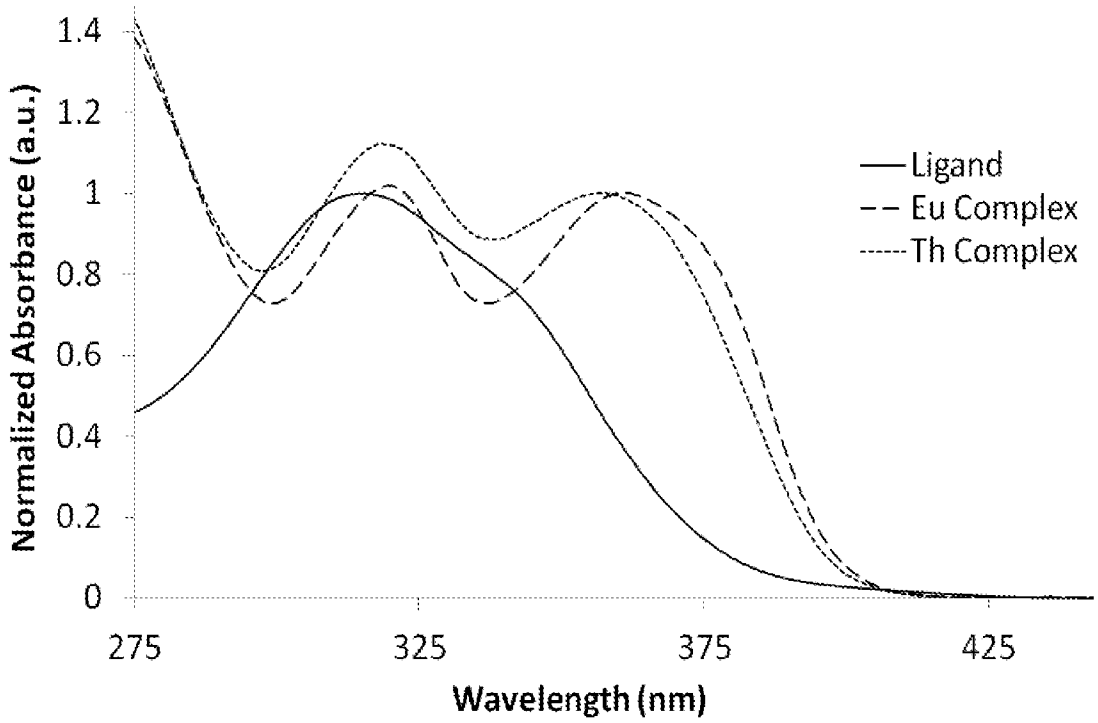

Normalized Absorbance (a.u.)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
275
325
375
425
Wavelength (nm)
Ligand
Eu Complex
Th Complex

FIG. 3A

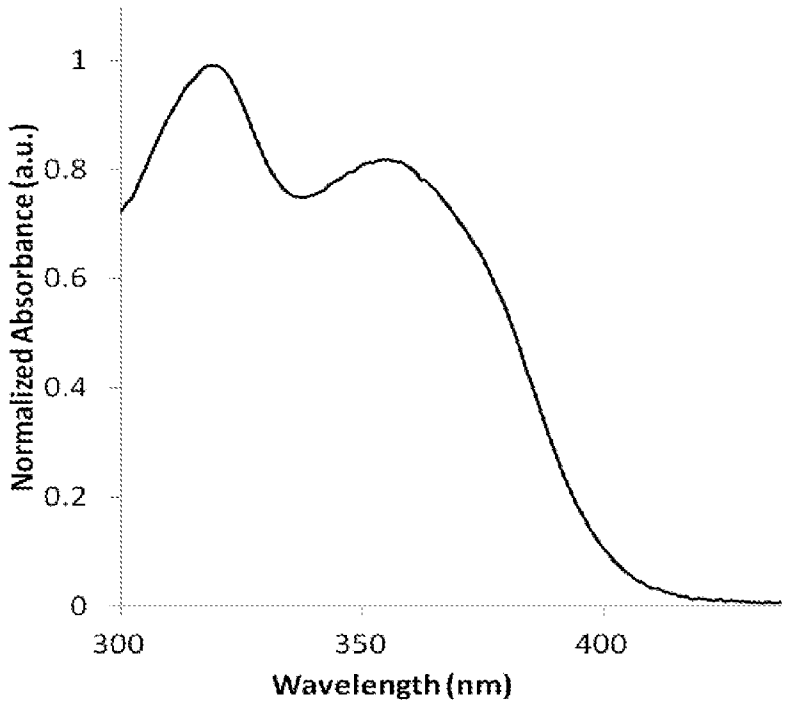

Normalized Absorbance (a.u.)
1
0.8
0.6
0.4
0.2
0
300
350
400
Wavelength (nm)

FIG. 3B

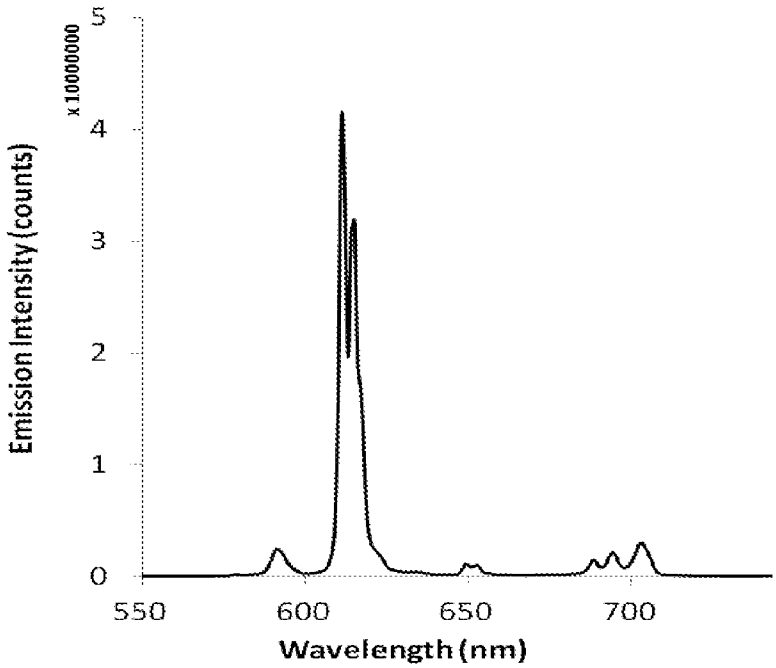

x 10000000
5
4
3
2
1
0
Emission Intensity (counts)
550
600
650
700
Wavelength (nm)

FIG. 4

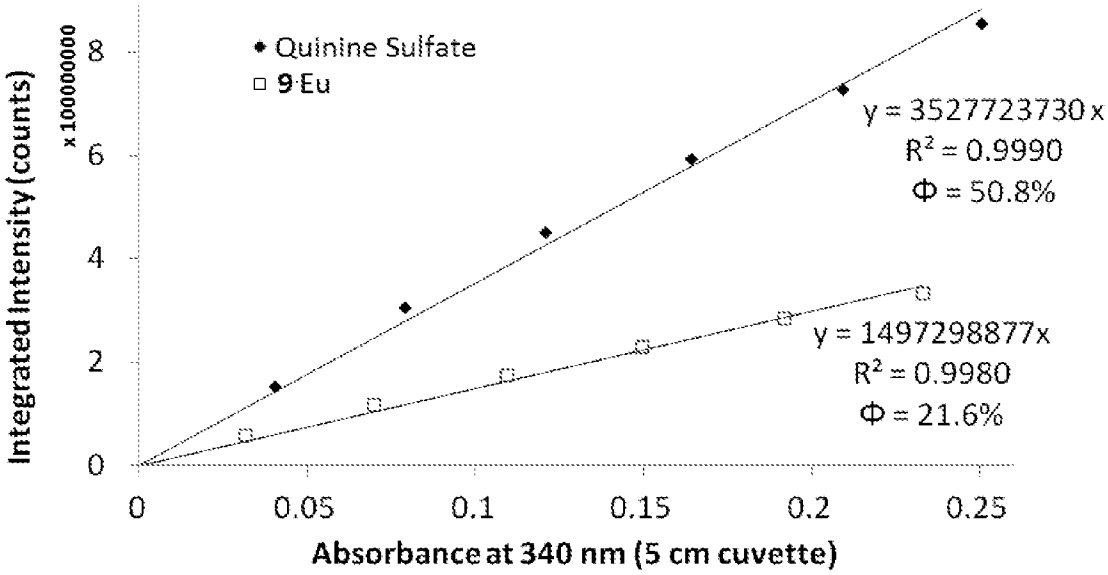

Integrated Intensity (counts)
x 100000000
8
6
4
2
0
Quinine Sulfate
9·Eu
y = 3527723730 x
R² = 0.9990
Φ = 50.8%
y = 1497298877x
R² = 0.9980
Φ = 21.6%
0
0.05
0.1
0.15
0.2
0.25
Absorbance at 340 nm (5 cm cuvette)

FIG. 5A

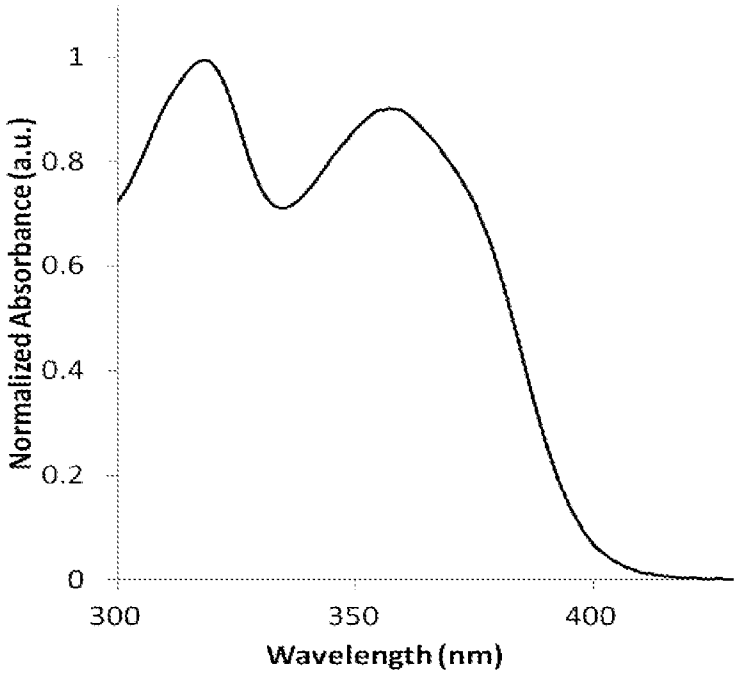

Normalized Absorbance (a.u.)
1
0.8
0.6
0.4
0.2
0
300
350
400
Wavelength (nm)

FIG. 5B

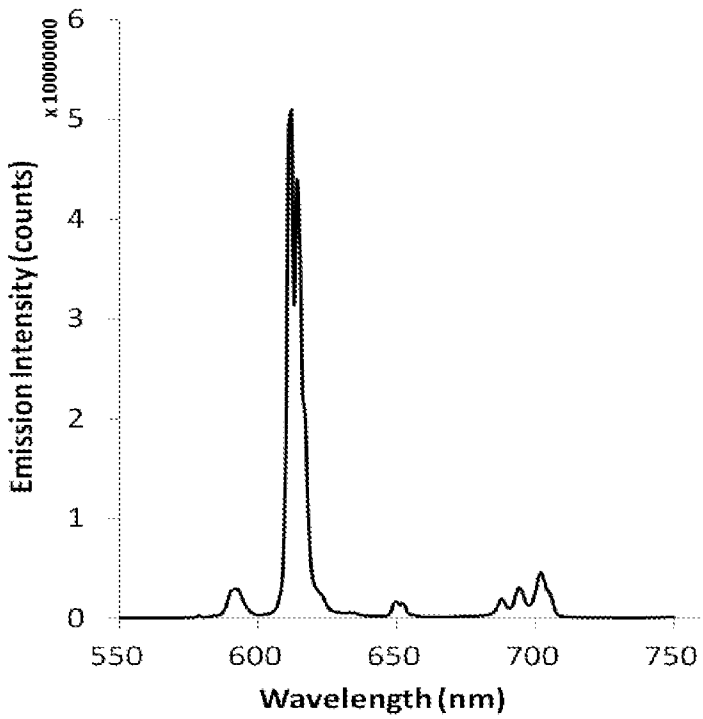

x 10000000
Emission Intensity (counts)
6
5
4
3
2
1
0
550
600
650
700
750
Wavelength (nm)

FIG. 6

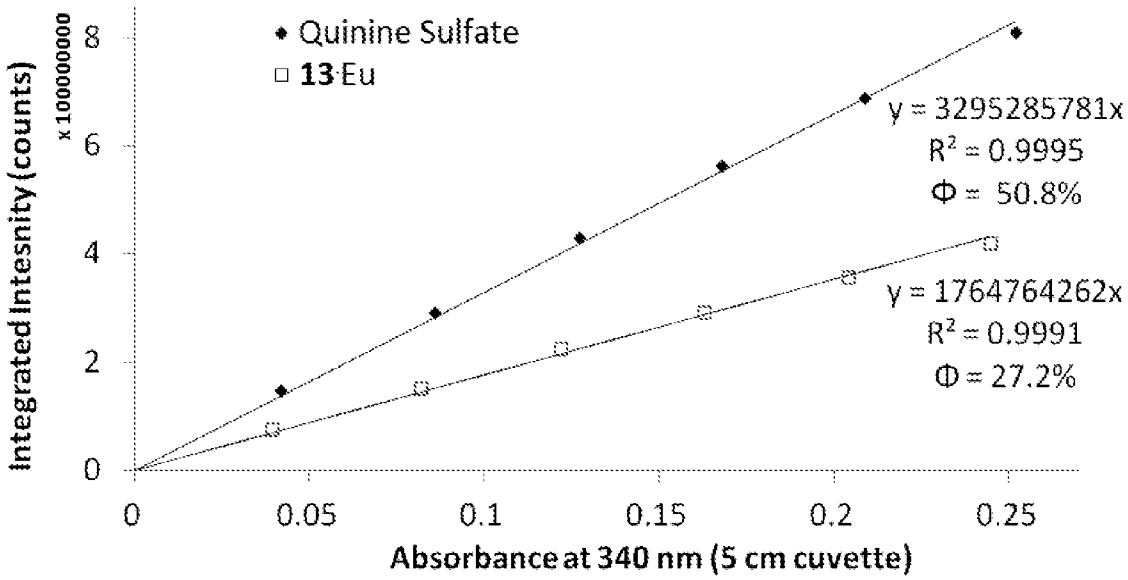

Integrated Intesnity (counts)
x 100000000
8
6
4
2
0
0
0.05
0.1
0.15
0.2
0.25
Absorbance at 340 nm (5 cm cuvette)
Quinine Sulfate
13-Eu
y = 3295285781x
R² = 0.9995
Φ = 50.8%
y = 1764764262x
R² = 0.9991
Φ = 27.2%

FIG. 7A

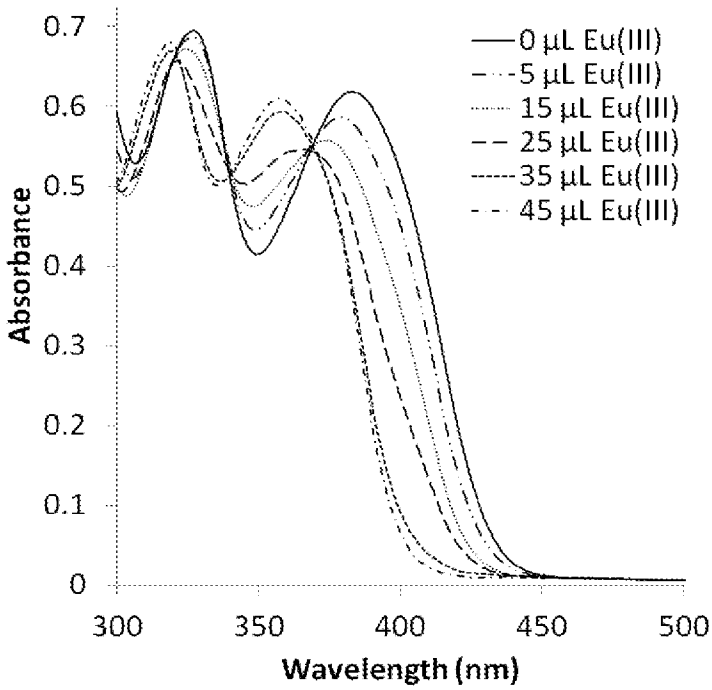

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Absorbance
300
350
400
450
500
Wavelength (nm)
0 µL Eu(III)
5 µL Eu(III)
15 µL Eu(III)
25 µL Eu(III)
35 µL Eu(III)
45 µL Eu(III)

FIG. 7B

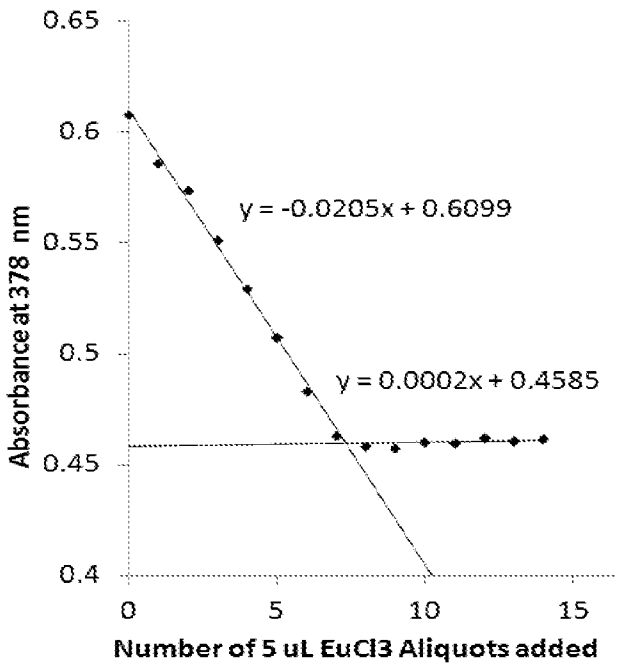

0.65
0.6
0.55
0.5
0.45
0.4
Absorbance at 378 nm
y = -0.0205x + 0.6099
y = 0.0002x + 0.4585
0
5
10
15
Number of 5 uL EuCl3 Aliquots added

FIG. 8

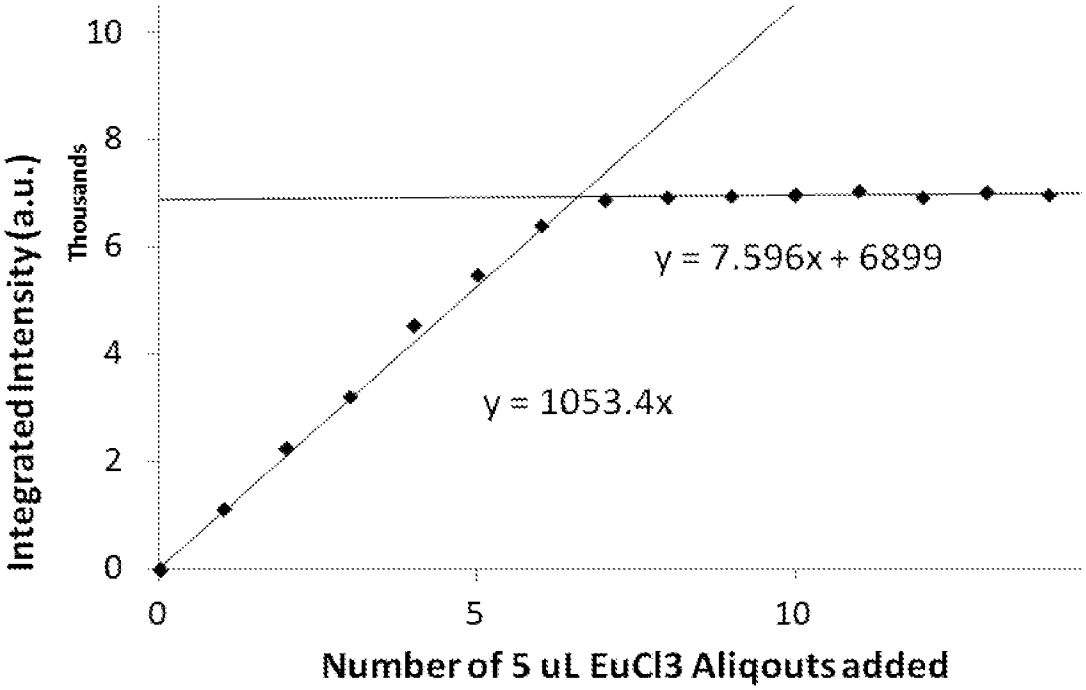

Integrated Intensity (a.u.)
Thousands
10
8
6
4
2
0
0
5
10
Number of 5 uL EuCl3 Aliqouts added
y = 7.596x + 6899
y = 1053.4x

FIG. 9

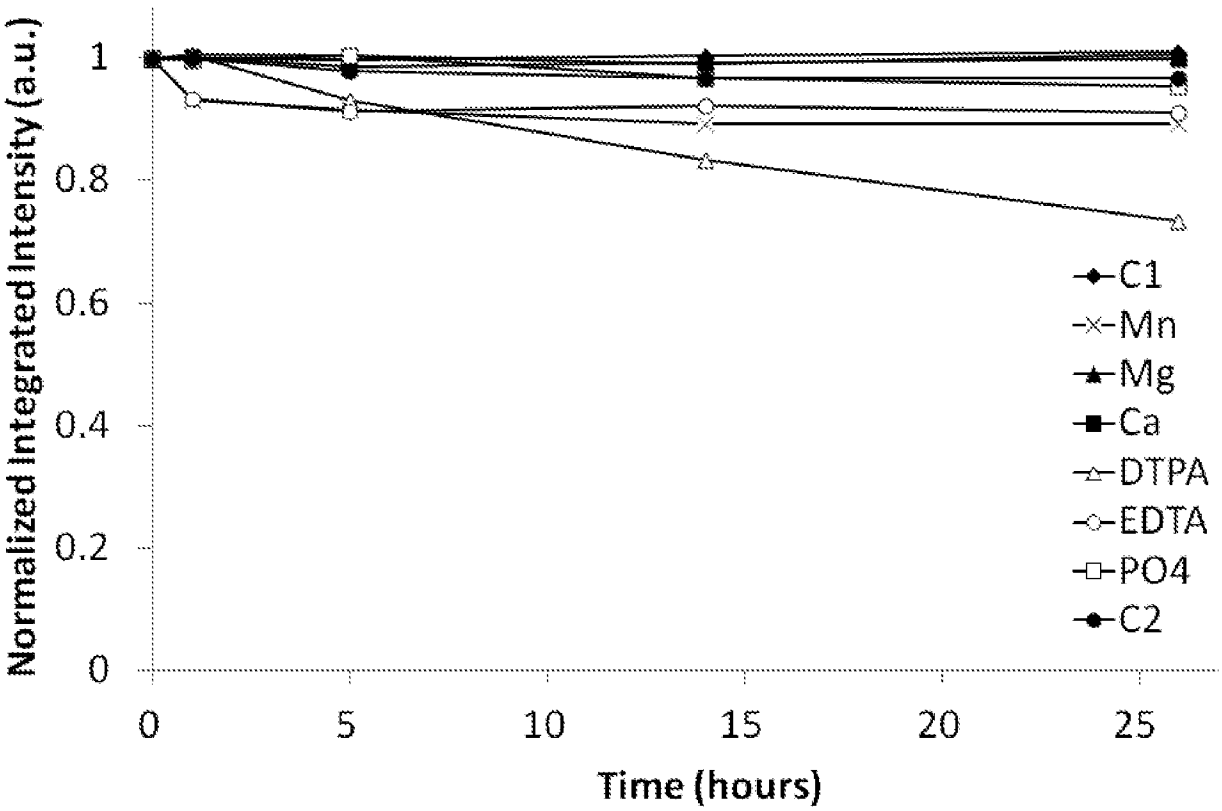
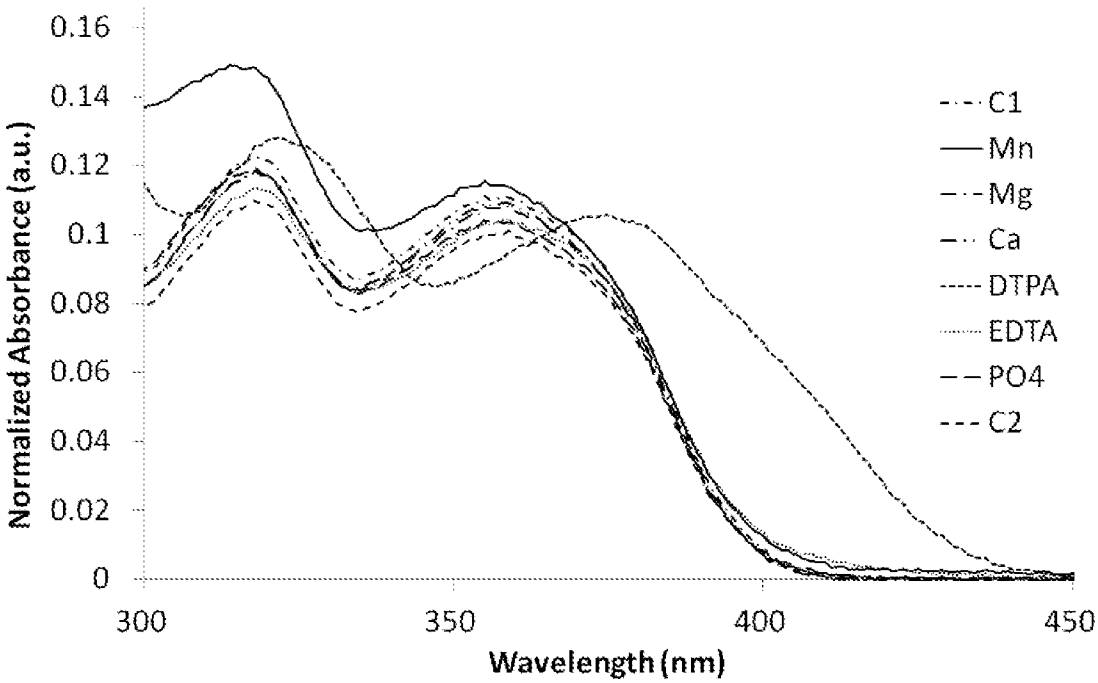

Normalized Integrated Intensity (a.u.)
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
Time (hours)
C1
Mn
Mg
Ca
DTPA
EDTA
PO4
C2

FIG. 10


0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Normalized Absorbance (a.u.)
300
350
400
450
Wavelength (nm)
C1
Mn
Mg
Ca
DTPA
EDTA
PO4
C2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/50118

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-19, 21, 23-26 and 28-47
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/50118

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 21/76; C07D 401/14; C07D 471/22 (2017.01)
CPC - G01N 21/76; C07D 401/14; C07D 471/22

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History Document

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2015/0157746 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 11 June 2015 (11.06.2015) para [0035];[0038];[0078]	1-5; 20-; 22 ---------- 27
Y	WO 2015/157057 A1 (LUMIPHORE INC.) 15 October 2015 (15.10.2015) para [0029];[0038];[0039]	27
A	US 2012/0214253 A1 (BUTLIN et al.) 23 August 2012 (23.08.2012) ENTIRE DOCUMENT	1-5; 20; 22; 27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 OCTOBER 2017	13 NOV 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)